1-1-2017

Protein Phosphatase 2a Interactions In Islet And Human Skeletal Muscle In Diabetes

Divyasri Damacharla
Wayne State University,

PROTEIN PHOSPHATASE 2A INTERACTIONS IN ISLET AND HUMAN SKELETAL MUSCLE IN DIABETES

by

DIVYASRI DAMACHARLA

DISSERTATION

Submitted to the Graduate School

of Wayne State University,

Detroit, Michigan

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

2017

MAJOR: PHARMACEUTICAL SCIENCES

Approved By:

_____

Advisor Date

_____

_____

_____

DEDICATION

This work is dedicated to my father

Dr. Srinivas Rao Damacharla, who has been selflessly working very hard for the past 26 years and dreaming about our future (my brother and me). I am forever indebted to him.

ACKNOWLEDGEMENTS

I would like to first thank the department of pharmaceutical sciences for accepting me into this program and giving me a chance to discover my strengths through this program. I would also like to thank my advisor Dr.Zhengping Yi for believing in me by accepting me into his lab and assigning me a great project that contributes to the progress in science.

I am grateful to work under Dr.Anjan Kowluru, my co-mentor. His confidence in me has always been a huge support. Working with his lab members has broadened my exposure and that will always be a added strength to my career.

I will also cherish the support from my committee members Dr.Fei Chen and Dr.Kyle Burghardt for their constant support throughout the program, for their suggestions that helped strengthen my project.

This project wouldn't have been possible without the support of my lab members Dr.Xiangmin Zhang, Dr.Danjun Ma, Dr.Michael Caruso, Yue Qi, and many others. Their suggestions and guidance throughout helped me progress one step at a time. I would like to specially thank Dr.Xiangmin Zhang who has always been my mentor for the past 5 years and for inspiring me to work hard every day.

I am very much indebted for the love and affection of my mother, Padmavathy Damacharla and my brother, Sivaram Damacharla

Lastly, my heartfelt thanks to all my friends who helped. Achieving this degree wouldn't have been possible without my seniors, Anusha Mannepalli and Rahul Gururaj. They have supported me in all possible ways ever since I came to USA and continue to do so even now. I would also like to specially thank my friend Ratna Karthik who has been the pillar of my strength during the tough times I had and for just standing with me no matter what.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1 INTRODUCTION

1.1 INTRODUCTION TO DIABETES AND INSULIN SIGNALING PATHWAY

1.1.1 INTRODUCTION TO DIABETES

Diabetes is a metabolic disorder characterized by high blood glucose. According to 'Global diabetes report' presented by World Health Organization in 2014, the total number of people with diabetes is 422 million[1]. Data collected by Center for Disease Control and Prevention show that 23.5 million people have diagnosed diabetes by 2015 in United States of America itself. This accounts to about 7.5% of its population[2]. The number of diabetes cases has risen from 108 million in 1980 to 422 million in 2014 globally[1] and from 5.53 million in 1980 to 23.35 million in 2015 in United states[2]. This increase over the past few decades is alarming and requires immediate attention of the researchers, healthcare providers, and general public worldwide. There are many complications associated with diabetes due to high blood glucose levels. Eventually, if not controlled, they can have impaired functioning of heart, kidneys, nerves, eyes and blood vessels[1]. The high blood glucose levels can lead to life threatening conditions like diabetic ketoacidosis, condition where high amounts of ketones are found in blood and urine, due to utilization of fatty acids as a source of energy and hyperosmolar coma[1]. Diabetic retinopathy associated blindness accounts for 1% of the global blindness[3]. Analysis of 'causes of vision loss from 1990-2010' showed that 2.6% of the blindness and 1.9% of the visual impairment is caused due to diabetic retinopathy[4]. The risk of cardiovascular disease and stroke is higher in patients with high blood glucose[5]. Diabetes can also lead to kidney failure[6]. End Stage Renal Disease (ESRD) is seen more often in patients with diabetes compared to normal population. Diabetes is responsible for 12-55% cases of the ESRD[7]. Neuropathy (nerve damage) is another common effect and when combined with reduced blood flow can lead to other severe complications.

These combined effects increase the incidence of foot ulcers, infections, and can lead to limb amputations. The incidence of amputations is 10-20 times higher in patients with diabetes than those without[8]. Other complications of diabetes include periodontal disease, depression, erectile dysfuction, hearing loss, non-alcoholic fatty liver disease, pregnancy complications and more[2]. All these complications reduce the quality of life and they can lead to death as well. According to the WHO statistics, 1.5 million deaths were directly caused by diabetes globally in 2012[1]. Their projections show that Diabetes will be the 7th leading cause of death by 2030[9].

There are two major types of diabetes: type I and type II. Type II Diabetes accounts for 90-95% of all diabetic cases. Type I Diabetes is seen in children and young adults due to the lack of insulin production caused by the loss of pancreatic beta cells. This is also called insulin dependent diabetes mellitus. Type II Diabetes occurs in relatively older people, which is the consequence of a combination of insulin resistance and relative insulin deficiency[10].

1.1.2 NORMAL GLUCOSE HOMEOSTASIS

Majority of the cells in the body require glucose as a fuel. In the absorptive state after a meal, the blood glucose levels rise. To control the plasma glucose levels, insulin is released from pancreas. This hyperinsulinemia and hyperglycemia lead to the following:

1. Glucose uptake in the peripheral (muscle, adipocyte) and splanchnic (liver and gut) tissues
2. Suppression of the glucose production by the liver and kidneys

Majority of the insulin stimulated glucose disposal in the peripheral tissues takes place in the muscle (about 85%)[11].

In post-absorptive state, after an 8-12 hour overnight fast, about 85% of the endogenous glucose production takes place in the liver and the rest in kidneys[11]. The glucose production in the liver is either by gluconeogenesis or glycogenolysis. During this phase, 50% of the glucose is utilized by the brain and another 25% is used by the liver and gastro intestinal tissues. The rest 25% is utilized in insulin dependent manner majorly in the muscle[11].

In this manner, many tissues contribute to maintain an optimum level of glucose in the body[12] represented in figure 1.

1.1.3 INSULIN PRODUCTION AND RELEASE IN PANCREAS

Insulin is a hormone produced by the pancreatic beta cells in response to the rise in blood glucose levels. Translation of insulin mRNA leads to the production of 110-aminoacid large sized pre-proinsulin whose amino terminal signal tag directs it to endoplasmic reticulum. In the endoplasmic reticulum, signal tag is cleaved to form pro-insulin. This Pro-insulin then undergoes modifications such as formation of disulfide bonds and folding followed by its transport to the Golgi complex. In the golgi complex, pro-insulin is cleaved to form insulin and C peptide and are stored within the secretory granules[13].

When the blood glucose levels rise, this glucose enter the pancreatic cells through diffusion. Beta cells contain glucose sensors, one of them being Glucose transporter 2 (GLUT2), that can transport glucose into the cells through facilitated diffusion. Glucose then undergoes glycolysis where it is converted to glucose 6 phosphate by glucokinase which then leads to formation of pyruvate, end product of glycolysis. Pyruvate is further oxidized in mitochondria to produce ATP through tricarboxylic acid cycle. This increased ATP: ADP ratio causes ATP dependent potassium channels to close. The increased positive charge inside the cell due to potassium leads to opening

of voltage dependent calcium channels and thereby influx of calcium ions. This facilitates release of insulin stored in the Golgi complex into the surrounding blood vessels. This is the first phase of insulin release, which is rapid. The second phase is prolonged where the insulin needs to be translated, modified and then released. This insulin released from the beta cell enters the blood stream and reaches other tissues where it performs specific actions.

Glucose uptake in these insulin dependent tissues is facilitated through various glucose transporters present in the cell which upon insulin stimulation locate themselves at the plasma membrane. The translocation of glucose transporters occurs when insulin binds to the insulin receptors on the surface of the cell. Glucose that entered the cell is phosphorylated and then metabolized further depending on the tissue. The pathway through which insulin enters the cells and regulates various cellular functions is regulated by numerous signaling molecules and is described further in detail below as insulin signaling pathway, also represented in Figure 2.

1.1.4 INSULIN SIGNALING PATHWAYS

Insulin is an anabolic hormone and thereby increases glucose uptake, synthesis of proteins, lipids and glycogen through activation of various pathways in skeletal muscle cells[12,14]. In the PI3K-dependent signaling pathway, insulin binds to tyrosine kinase insulin receptor present on the cell membrane. This insulin receptor is composed of two extracellular alpha subunits and two transmembrane beta subunits. Binding of the insulin to the alpha subunits leads to conformational change induced activation of the receptor kinase activity in the beta subunits. This leads to the transphosphorylation of the beta subunits, further activating the kinase. This phosphorylation allows binding of other substrates. The various substrates for the insulin receptor include insulin receptor

substrate 1 (IRS1), APS (SHB2), Gab proteins, cbl and shc proteins. The phosphorylated tyrosines on these substrates allow them to bind to various downstream molecules, which include P85 subunit of the phosphatidylinositide 3 kinase (PI3K), Grb2, crk II, etc. The major pathway connecting actions of insulin and the IRS proteins is the **PI3K and AKT signaling pathway**. PI3K is a heterodimer with a regulatory and catalytic subunit. Binding of the regulatory subunit to the IRS proteins leads to the activation of catalytic subunit, which phosphorylates phosphatidylinositol 4,5-biphosphate (PIP_2) to form phosphatidylinositol(3,4,5)-triphosphate (PIP_3), a second messenger. To this membrane bound PIP3, PDK1(3-phosphoinositide-dependent protein kinase 1) binds and is activated. PDK1 then phosphorylates and activates AGC protein kinase family proteins, which are responsible for PI3K-PIP3 downstream effects. This AGC protein kinase family members include Akt/PKB, p70 ribosomal S6 kinase, serum and glucocorticoid induced protein kinase (SGK), and protein kinase C (PKC). Akt2 is the major isoform involved in the insulin metabolic actions. Akt is phosphorylated at Thr-308 by PDK-1 and at Ser-473 by mammalian target of rapamycin complex 2 (Mtorc2). Akt acts on substrates (TSC-2, FOXO, AS160, GSK3, PGC-1α) and leads to various physiological functions including GLUT4 translocation to the plasma membrane and glucose uptake, glycogen synthesis, and protein synthesis. Insulin also activates mitogen-activated protein kinases (MAPK) to increase gene expression and differentiation through the **Grb2-SOS-Ras-MAPK pathway**. This pathway is activated independently of the Akt pathway. The insulin receptor and IRS proteins bind to adaptor molecules like Grb2 and shc. Grb2 binds to Gab-1 with the carboxyterminal domain and to SOS with amino terminal domain. SOS is a Guanine nucleotide exchange factor, activates Ras-GDP to Ras-GTP. Activated Ras interacts and activates a series of down-

stream signaling molecules Raf-MEK1/2-ERK1/2. ERK is directly involved in regulating gene expression, cell proliferation or differentiation, cytoskeletal reorganization. Other insulin receptor substrates include APS (SHB2) and Cbl, which bind to proteins such as the Cbl-associated protein (CAP). Cbl-associated protein is involved in control of insulin-stimulated glucose uptake[14-16].

Insulin signaling is tightly regulated to control the physiological effects. There are several negative regulators of insulin signaling. They are important because the uncontrolled/abnormal activity in this regulation can lead to insulin resistance. The pathway is inhibited by the activity of some protein phosphatases including tyrosine phosphatases, such as PTP1B, transmembrane phosphatases, such as LAR[17], serine/threonine phosphatases including PP1[18], PP2A[19], PP2B[20], and some members of PP2C[21]. It is also inhibited by lipid phosphatases such as PTEN[22] and SHIP1[23]. Other negative regulators include Grb[24], Proteins of the suppressor of cytokine signaling (SOCS) family[25], Tribbles homolog 3 (Trb3)[26], inositol phosphate (IP7)[27]. These negative regulators are summarized in Figure 3. Phosphorylation on inhibitory serine/threonine sites on the insulin receptor can turn down the insulin signaling as well (Figure 4). This can be influenced by many factors such as hyperglycemia, fatty acids, cytokines, mitochondrial dysfunction, ER stress, increased cAMP concentration[14]. Insulin can also cause the inhibitory serine/threonine phosphorylation through activation of MAPK, JNK, IKK, Mtorc1/S6K[14].

1.1.5 PATHOGENESIS OF TYPE 2 DIABETES

In Type 2 diabetes, glucose homeostasis is disrupted. The two major defects involved in the pathogenesis of type 2 diabetes are

1. Abnormal insulin production/release
2. Impaired sensitivity of tissue to insulin[28]

As 80% of the insulin dependent blood glucose clearance takes place in skeletal muscle, insulin resistance in skeletal muscle is one of the major drawback in Type II diabetes[10].

1.1.1.1 β-CELL FAILURE IN T2D

Abnormalities in insulin secretion and release in β-cell are seen in type 2 diabetes. To compensate the insulin resistance, β-cell produces large amounts of insulin in the early stages of T2D. It is observed that in the stages of impaired glucose tolerance(IGT), there is impaired first phase of insulin release. This prandial hyperglycemia is the characterisitic feature of IGT. However, with a normal prolonged second phase[29], the hyperinsulinemia keeps the glucose under control or mildly impaired. As the disease progresses, the β-cell fails. Studies in human subjects have shown that β-cell mass is decreased in type 2 diabetes. This reduced mass is associated with cell death by apoptosis[30]. The mechanisms involved in β-cell failure involve glucotoxicity and lipotoxicity, a condition where the cells are exposed to high levels of glucose and fatty acids respectively for longer terms[31]. This can cause high burden on the mitochondria and ER in the β-cells which leads to increased ROS production and upregulated UPR response respectively, which cause cell apoptosis. Research has shown many signaling molecules involved in the cell dysfunction. High glucose induced increased ca^{+2} levels in the cells is also shown to cause β-cell dysfunction[32]. Chronic exposure of cells to high glucose can lead to production of IL-1β which activates NF_KB signaling, cell apoptosis, and β-cell dysfunction[33]. Hyperglycemia is known to cause glycation of intra or extracellular proteins and the Advanced Gycation End prodcuts (AGEs) that can cause cell damage. β-cells are vulnerable to oxidative stress, and under glucotoxic conditions, they can lead to activation of JNK and NF_KB. Activated JNK phosphorylate IRS-1 at ser-307[34], which attenuate the IRS-PI3K-AKT signaling, thereby increase in FOXO1

gene expression and decreased nuclear PDX-1, transcription factor important for β-cell survival[35]. mTOR signaling is important for cell growth and survival. Continuous activation of this pathway by glucose can lead to IRS2 phosphorylation and degradation, thereby cell apoptosis[36]. Lipotoxicity is shown to decrease glucose stimulated insulin secretion. In addition, there is reduced nuclear transclocation of PDX-1 (cell survival) , downregulated MafA expression (responsible for insulin expression), upregulated UCP2[37] (mitochondrial inner membrane protein uncoupling protein 2), activation of PLC-ε[38] (lipid-induced protein kinase), variations in machinery required for insulin secretion from its granules[39], and detachment of insulin secretory granules from Ca2+channels[40].

1.1.1.2 MECHANISMS OF INSULIN RESISTANCE

Many factors influence insulin sensitivity. Defects in any step along the insulin signaling pathway can cause insulin resistance. In addition, activation/abnormal function of the negative regulators of the pathway can also lead to this condition. Research over the years identified several causes for insulin resistance, which include genetic mutations, hyperglycemia, lipotoxicity, ER stress, and mitochondrial dysfunction[14]. Gene mutations in the IRS-1, PI3K, PTEN, AKT2, Trb3, and AS160 are linked to insulin resistance as seen in diabetic patients. Chronic hyperglycemic condition is known to change insulin sensitivity through mechanisms of oxidative stress in tissues like muscle, fat, and also reduce insulin secretion from beta cells. Accumulation of free fatty acids also leads to this metabolic dysfunction through activation of ser-307 IRS1 phosphorylation, JNK, IKK, PKC. Ceramides, a class of biologically-active sphingolipids act via activation of JNK, PKC, and by inhibiting Akt activation. Fatty acid Palmitate is shown to cause insulin resistance through induction of NF-κB signaling, cytokine

production, ER stress, and activation of JNK. Inflammatory cytokines also cause insulin resistance by various mechanisms.

1.2 KINASES AND PHOSPHATASES

Phosphorylation is one of the most important post translational modifications which regulates most signaling molecules in the cell. This process is carried out by kinases contrary to the phosphatases which carry out dephosphorylation. Of all the proteins in the eukaryotic cell, one third portions undergo reversible phosphorylation[41]. In 1950's, Edmond Fischer and Edwin Krebs discovered the idea of reversible phosphorylation using proteins isolated from rabbit skeletal muscle. They identified that this process required transfer of phosphate group from ATP to phosphorylase b to form phosphorylase a, a phosphoprotein using a 'converting enzyme'[42]. However, the concept of dephosphorylation was discovered about a decade earlier without the knowledge of phosphate group being its product until identified later by Drs.Krebs and Fischer. Phosphorylation and dephosphorylation are mostly carried out on amino acids containing OH group such as serine, threonine and tyrosine. Among these, serine undergoes almost 86.4% of the total phosphorylation followed by threonine (11.8%) and tyrosine (1.8%)[43]. Therefore 98% of the phosphorylation sites happen on serine/threonine[43]. Human genome sequence is expected to contain a total of 518 kinases with 90 Tyrosine and 428 Serine/Threonine kinases and about 130 total protein phosphatases with 107 Tyrosine and about 30 Serine/Threonine phosphatases[44]. Protein Tyrosine Phosphatases are almost similar in number to tyrosine kinases. However, Protein serine/threonine kinases are about 10fold higher than serine/threonine phosphatases. Combined, the number of genes coding kinases approximately 3 fold higher than that of phosphatases[45]. The large number of kinases is balanced through formation of holoenzyme com-

plexes of phosphatases, generally composed of a catalytic subunit and one or more regulatory subunits. Each subunit has been shown to exist in various isoforms. These various subunit isoforms produce numerous possible combinations for each phosphatase and thereby match the kinases. The catalytic subunit itself is relatively non-specific and can dephosphorylate numerous substrates. Therefore, the interaction between the catalytic subunit and regulatory subunits is required to regulate the specificity and activity of the phosphatases[45]. Phosphatases remove phosphate group via S_N2 mechanism with water as a nucleophile[46]. Characterization of these phosphatases based on their substrate is as follows.

i. Protein Tyrosine Phosphatases (PTP): remove the phosphate group from phosphorylated tyrosine residues.

ii. Protein Serine/Threonine Phosphatases: remove the phosphate group from phosphorylated serine/threonine residues. This group is further classified into

    a. Phospho Protein Phosphatase (PPP) which includes PP1, PP2A, PP2B, PP4, PP5, PP6, PP7.

       All the proteins in this group have a structurally conserved active site configuration, a catalytic water molecule and six conserved residues [two aspartate (D), one asparagine (N) and three histidine (H) residues] with two metal ions.

    b. Metal ion (Mg^{+2} and Mn^{+2}) dependent phosphatase (PPM) which contains PP2C.

       This family of proteins does not possess regulatory subunits to determine the substrate specificity. Instead they have conserved sequence motifs and additional domains. Both PPP and PPM class of proteins require metal ions to play catalytic role.

c. Aspartate bases based phosphatase comprising Fcp1 and Scp1.

As the name indicates, these phosphatases use aspartate based catalysis.

RNA polymerase II is the only substrate for this family of phosphatase.

iii. Dual specificity protein serine/threonine/tyrosine phosphatase.

iv. Histidine phosphatase.

v. Lipid phosphatase.

As their names suggest, their substrates involve phosphorylated tyrosine, serine/threonine, serine/threonine/tyrosine, or histidine residues and lipids, respectively.

1.3 PROTEIN PHOSPHATASE 2A (PP2A), REGULATION AND EFFECT OF INSULIN

PP2A is a one of the major serine-threonine protein phosphatases that belongs to the phosphoprotein phosphatase family. This phosphatase constitutes for about 1% of the total protein in the cell[47]. It is a hetero-trimeric complex with a dimeric core enzyme (see Figure 5), composed of a 65kda A subunit (PP2Aa), a 55kda B regulatory subunit (PP2Ab), and a 36kda catalytic subunit C (PP2Ac). Subunits A and C form the dimeric core[48]. PP2A can exert its activity as a dimer ($PP2A_d$) or as a trimer complex[49]. PP2Ac by itself can also act on substrates.

1.3.1 SUBUNITS OF PP2A

PP2Aa (A regulatory subunit) is ubiquitous and has two isoforms, alpha and beta, which are encoded by two different genes PPP2R1A and PPP2R1B. There is 86% similarity between these two isoforms. The dimer core, in most cases (90%), is composed of A alpha isoform[47]. Both the isoforms are located in the cytoplasm. The structure of the A subunit is unveiled in 1999[50]. It is composed of 15 non-identical repeats (HEAT sequence) containing 39 amino acids each. The repeats are arranged as two antiparallel alpha helices which are connected to each other by intra and inter repeat

loops forming a horse-shoe shape. B subunit binds to loops 1-10 whereas C subunit binds to loops 11-15[51]. Because of its flexibility, B subunits and other substrates can be incorporated easily[47]. PP2Aa guides PP2Ac in the interaction with PP2Ab and other substrates and regulates the specificity of PP2Ac[52].

PP2Ab (B regulatory subunit) regulates localization, activity and substrates for the complex. This regulatory subunit is encoded by 15 different genes which are transcribed to a minimum of 26 transcripts and splice variants. They are expressed variably depending on the tissue type. They are classified into four families B (B55/PR55), B' (B56/PR61), B'' (PR48/PR72/PR130), and B''' (PR93/PR110). They require ATP and Mg^{+2} to be active. B has four different isoforms and has a tryptophan-aspartate repeat which helps in its identification. B' has five isoforms which are all identical in the center region but different in the C and N terminals. B'' has three isoforms. Different regulatory subunits direct the holoenzyme to perform varied functions. For example, binding of B subunit to the PP2A complex prevents simian virus40 replication whereas binding of B'' does the opposite. Not all subunits bind at the same region on the A subunit.

PP2Ac (catalytic subunit) is in globular structure**,** ubiquitously expressed in almost all the tissues and is abundant in heart and brain. PP2Ac is conserved from eukaryotes to mammals, with 86% sequence match between yeast and humans. It is responsible for the catalytic activity of the enzyme. PP2Ac has two isoforms, alpha and beta, which are 97% identical encoded by two different genes. Both are composed of 309 amino acids and differ only by 8 amino acids at the N terminal. PP2Ac alpha is found mainly in plasma membrane whereas beta isoform is in cytoplasm and nucleus. PP2Ac alpha is more abundant than PP2Ac beta because of the high degree of mRNA translation[53]. Unique feature of PP2Ac is that C terminal tail is highly conserved

(^{304}TPDYEL^{309}). This tail binds to the A and B subunits of the complex. All the other phosphatases involved in PPP family cannot bind to A subunit even though they share sequence similarity with PP2Ac. This is because most of the amino acids required for specific interactions with A subunit are replaced[46].

All the subunits, their isoforms, tissue and subcellular distribution is summarized in Table 1.

1.3.2 REGULATION OF PP2A

Given the presence of large number of A, B and C subunit isoforms, various PP2A complexes are possible. The combination of the A, B and C subunit isoforms affects the activity and specificity of PP2A complexes against a particular substrate. Binding and the presence of other regulators can also influence PP2A activity and specificity[46,54]. One such example is the binding of α4 protein. Binding of PP2A to α4 is important to stabilize PP2Ac in its inactive conformation. Besides stabilization, it also hinders ubiquitination site on PP2Ac thereby preventing its degradation[55]. Phospho tyrosyl phosphatase activator (PTPA) is also shown to be an important regulator. It acts by stabilizing PP2A in an active conformation, which facilitates acquirement of its Serine/Threonine phosphatase activity[56].

PP2A activity is also regulated by post-translational modifications on PP2Ac[57]. Several experiments in vivo as well as in vitro showed that phosphorylation on Tyr^{307} on PP2A$_c$[57] deactivates PP2A$_c$, by preventing its interaction with the regulatory subunit. Phosphorylation is also reported in a few PP2A regulatory subunits[58], which altered their activity and also substrate specificity. In addition, PP2A undergoes carboxyl methylation on the carboxyl group of the C-terminal residue of Leu^{309}. Leucine Carboxyl Methyl Transferase (LCMT), also known as PP2A-Methyl transferase (PPMT), is re-

sponsible for methylation of PP2Ac, while PP2A Methyl Esterase (PPME) is responsible for PP2Ac de-methylation. Unlike phosphorylation, effect of methylation on activity is controversial. There are reports with an increase/decrease/no effect in the catalytic activity associated with carboxymethylation. It is however shown that PP2Ac methylation is required for binding of B subunit not the A/B$^{'}$/B$^{''}$B$^{'''}$ subunits[59,60]. Recent studies have shown additional functions of PME-1 in addition to affecting the activity. It is shown to be important for maintaining normal PP2A levels by preventing it from proteasome degradation[61].

1.3.3 INHIBITORS OF PP2A

I_1^{PP2A} and I_2^{PP2A} are two inhibitors which are found to inhibit PP2A through in vitro and in vivo experiments [57]. Many small compounds found naturally inhibit PP2A. One of them being okadaic acid which is being used in laboratory practices. It also inhibits other phosphatases like PP1 but at relatively higher concentrations. Other commercially available inhibitors include calyculin a, tautomycin, microcystins, cantharidin and endothall. Various inhibitors of PP2A, their specificity over other phosphatases and their origin is seen in Table 2.

1.3.4 ROLE OF PP2A

PP2A is found to be involved in many cell signaling pathways, cell cycle regulation and various other pathways. Experiments conducted by employing phosphatase inhibitor okadaic acid showed that PP2A plays a role in cell cycle regulation (G2/M transition). Using Yeast, they presented the role of various B subunit analogues in cell cycle, stress response, cytoskeleton organization and morphogenesis. Experiments in *drosophila* showed the importance of PP2A in early embryogenesis and the changes in the tissue distribution during its development. Several viral antigens are found to interact with PP2A and prevent the inhibitory role of PP2A in those signaling pathways and

promote cell proliferation. It is also shown in *Xenopus* eggs that it involves in initiation of DNA replication. Several studies showed the involvement of PP2A in termination of DNA replication, apoptosis, DNA damage response and heat shock response[53]. PP2A plays a role in numerous signaling pathways, including MAPK, mTOR, and Wnt signaling pathways, that initiate the cell cycle.

1.3.5 PP2A IN DIABETES

Our lab has shown that IRS1 interacts with PP2Ac using human skeletal muscle biopsies. Further, its interaction is increased in obese insulin resistant nondiabetic controls and type 2 diabetic subjects when compared to lean controls[62]. Its interaction with IRS-1 also shown in murine HL-1 cardiomyocytes[63].

There is evidence to indicate that insulin inactivates PP2A through in vitro and in vivo experiments. Also, published evidence shows interaction of PP2A with many signaling molecules, some of which are involved in insulin signaling pathway. Jian Chen et al showed that PP2A is phosphorylated in vitro by the tyrosine kinases which included insulin receptors. It is phosphorylated on Tyr^{307} and this inactivated PP2A[57] .

The effect of insulin on PP2A during myogenesis in rat L6 cells is shown by Srinivasan and Begum. They showed that insulin inactivated PP2A in the differentiated cells .They also showed that the phosphatase activity decreased relatively with the increased concentrations of insulin and also the incubation time[64].

One of the effects of insulin in skeletal muscle cells is the glycogen synthesis through the INS/IRS-1/AKT pathway. Rosanna Cazzolli and associates showed that ceramide treatment of C2C12 skeletal myotubes reduced the glycogen synthesis through inhibition of phosphorylation on PKB upon insulin stimulation. Their results indicated that this inhibition is mediated through activated PP2A via ceramide and thereby effecting the glycogen synthesis in the skeletal muscle cells[65].

It is also shown that PP2A has a positive effect on the insulin signaling pathway by preventing the excessive serine phosphorylation on the IRS-1 which will otherwise negatively regulate the pathway. One such serine kinases is ribosomal protein P70 S6K-1 which is an effector of mTOR. Madavia et al showed direct Interaction of PP2A with IRS-1 in cardiomyocytes protecting IRS-1 from excessive serine phosphorylation. They inferred from their results that PP2A interacts with IRS-1 via mTOR competing for serine residues on IRS-1 and thereby deciding the phosphorylation status of IRS-1. Many factors affect the association, one being the insulin stimulation[63].

One group has reported experiments on PP2Ac abundance in human skeletal muscle where they compared ten type II diabetics with ten lean controls. They showed that upon insulin stimulation, PP2Ac protein levels in control subjects reduced when compared to the basal levels but not in type II diabetics. They also showed corresponding reduction in glucose disposal, glucose oxidation and increase in lipid oxidation[66].

Saturated fatty acids like palmitate negatively regulate insulin signaling pathway by activating PP2A, which dephosphorylates Akt and ERK1/2. Opposite effect is seen with unsaturated fatty acids like oleic acid or linoleic acid[67].

Chronic exposure of pancreatic β-cells to high glucose (glucotoxicity) leads to metabolic dysfunction in these cells with reported beta cell death[31]. Experiments done by Arora., *et al.* showed that sustained activation of PP2Ac in insulin-secreting INS-1 832/13 cells and normal rat islets under these hyperglycemic conditions. They also showed an increased PP2A activity under similar glucotoxic conditions with a corresponding increase in carboxymethylation of PP2Ac[68]

1.4 INSULIN SENSITIVITY; PROTEIN INTERACTIONS AND MASS SPECTROMETRY

1.4.1 METHODS TO MEASURE INSULIN SENSITIVITY

There are several methods to measure insulin sensitivity in humans. Hyperinsulinemic euglycemic clamp and insulin suppression test are used for direct measurement of insulin sensitivity whereas Oral glucose tolerance test and minimal model analysis of frequently sampled intravenous glucose tolerance test are considered for indirect measurement. There are several other Indices used for quick measurement of insulin sensitivity in cases where feasibility is an issue.[69]

For settings where insulin sensitivity measurement and maintenance of steady state conditions is crucial, hyperinsulinemic euglycemic clamp should be the first choice. This technique is also mentioned as a gold standard to assess the action of insulin in vivo[70]. The action of insulin on the body is measured by the rate of exogenous glucose infused to maintain a constant blood glucose concentration. Under conditions of hyperinsulinemia, most (>70%) of the infused glucose is used by skeletal muscle. This implies that the index measured during the clamp mainly reflects the skeletal muscle sensitivity to insulin[10].

1.4.2 IMPORTANCE OF PROTEIN-PROTEIN INTERACTIONS

Most of the proteins in vivo act in the form of complexes. Protein-protein interactions play a very crucial role in various functions of the cell, such as gene transcription, signal transduction[71], cell cycle regulation, etc. Correct formation of these complexes is important for the normal body function. Abnormalities in protein-protein interactions cause aberrant cell signals and thereby cause diseases. Many protein complexes have been targeted to treat diseases[72]. Studying the interactions will help us to find out the function of the particular target protein which is specifically useful in cases

of any unidentified protein interaction partners. It will also enable us to analyze the signaling pathways. Protein-protein Interactions have been classified as homo oligomeric /hetero oligomeric based on interaction surface; obligate/non obligate based on stability and transient/permanent depending on persistence[73]

Protein-protein interactions may result in changes in

1. Kinetic characteristics of the complexes
2. Substrate channeling
3. A new binding site on the complex for other effector molecules
4. Substrate specificity
5. Activity of the complex
6. Downstream events

Methods to determine protein-protein interactions. Biophysical methods determine these interactions using the structural information of the proteins. These include X-ray crystallography, NMR Spectroscopy, fluorescence and atomic force microscopy. Direct high throughput methods include yeast two hybrid, affinity purification and mass spectrometry[74,75,76]. Indirect high throughput methods include gene co-expression and synthetic lethality. Computational predictions of the protein-protein interactions have also been reported[74]. Affinity purification coupled with mass spectrometry (AP-MS) is widely used for identification of interaction networks[75]. Affinity purification allows to enrich the target protein of interest and its co-interaction partners in a single step, and mass spectrometry offers supreme ability to identify proteins from a complex mixture in a high throughout fashion[75,76].

1.4.3 MASS SPECTROMETRY

Mass spectrometry is the most sensitive approach for global identification and quantification of proteins, protein-protein interactions, and protein post translational

modifications[77]. The main components of a mass spectrometry instruments, a mass spectrometer, include an ion source, a mass analyzer, and a detector[77]:

1. Ion source: a device to generate charged particles. Electrospray ionization (ESI) and matrix-assisted laser desorption ionization (MALDI) are two commonly used ion sources for proteomics studies[77].

2. Mass analyzer: a device to separate the ions based on their mass-to-charge ratio, m/z[77]. Four common types of analyzers for the proteomic analysis include quadruple, Ion trap (quadruple ion trap, linear ion trap), time of flight, and orbitrap analyzers[77].

3. Detector: A detector is a device to record either the charge induced when an ion hits a surface or the current produced when an ion passes by [77]. Two main detectors are electron multiplier (charge induced when ions hit a plate) and image current detector (current produced when ions pass) [77].

As every wet lab experiment, proteomics studies begin with collecting starting material (e.g., tissue, body fluid, cell lysates, etc.) and followed by protein separation (e.g., affinity capture, electrophoresis, liquid chromatography, etc.). Proteins are cleaved into peptides by enzymatic digestion. The most commonly used protease/enzyme for this purpose is trypsin due to its well-defined specificity, which hydrolyzes proteins at the carboxyl side (or "C-terminal side") of the amino acids lysine and arginine. Since one protein may generate many peptides after trypsin digestion, a tryptic digest of a complex mixture of proteins may contain thousands or even millions of peptides. Therefore, the resulting peptides are further separated using a variety of techniques (e.g. affinity capture, liquid chromatography, etc.). The separated peptides are analyzed by mass spectrometry for peptide/protein identification and quantification. These steps are summarized in Figure 6.

In the present work, the proteomic approach developed in our laboratory[78] was applied to investigate PP2Ac interaction partners in islet cells and human skeletal muscle biopsies from human participants.

1.5 SPECIFIC AIMS

This project aims to by study the activity of PP2A and to determine the interaction partners of PP2Ac in (i) clonal islet β-islet cells (INS-1 832/13) under basal and hyperglycemic conditions (ii) human skeletal muscle under basal and hyperinsulenemic conditions in in lean, obese/overweight non-diabetics and type II diabetics

1.5.1 SPECIFIC AIM 1: INS-1 832/13 CELLS

Our aim is to determine the activity and interaction partners of pp2ac in β-islet cells under basal and hyperglycemic conditions. We hypothesized that chronic exposure of insulin-secreting β cells to hyperglycemic conditions leads to increased interaction of PP2Ac with its regulatory and scaffolding subunits resulting in its catalytic activation with subsequent dephosphorylation and inactivation of key survival proteins.

1.5.2 SPECIFIC AIM 2: HUMAN SKELETAL MUSCLE BIOPSY

Here, our aim is to determine the activity, post translational modifications and interaction partners of pp2a in human skeletal muscle under basal and hyperinsulenemic conditions in lean, obese/overweight non-diabetics and type II diabetic subjects. PP2Ac activity is increased under hyperglycemic conditions and its regulation varies under this condition

CHAPTER 2 RESEARCH DESIGN AND METHODS

2.1 REAGENTS

Reagents are from these suppliers; protein A sepharose and iodoacetamide (Sigma, St Louis, MO); C18 ZipTip (Millipore, Billerica, MA). RPM1640 medium, normal fetal bovine serum (FBS) and penicillin-streptomycin-glutamine mixture (PSG) were purchased from Life Technologies. HPLC grade acetonitrile (ACN), trifluoroacetic acid (TFA) and formic acid (FA) were from Sigma. Sequence grade trypsin was from Promega. The normal mouse IgG (NIgG) and PP2Ac mouse monoclonal antibody (Cat. 05-421) were from Millipore.

2.2 SPECIFIC AIM 1: INS-1 832/13 CELLS

2.2.1 CELL CULTURE AND HIGH GLUCOSE TREATMENT

INS-1 832/13 cells (provided by Dr.Aris Newgard) were grown in RPMI1640 medium containing 2.5 mM glucose, 10% FBS and 1% PSG. In order to treat the cells with high glucose for 48 hours, same medium was supplemented with glucose to obtain a final concentration of 20 mM. Cells treated with low and high glucose were harvested after the treatment. The cells homogenized in lysis buffer containing 2mM EDTA, 2mM EGTA, 20mM imidazole-HCl, pH 7.0 with protease inhibitors aprotinin, leupeptin, and PMSF. The cells are centrifuged at about 14000rpm for 15min followed by protein quantification using bradford method. 4 mg of protein was used for each sample and was first incubated with 30 µl of protein A beads conjugated to 4 µg of mouse NIgG for three hours. Treating with NIgG beads served as control to detect non-specific interactions. The supernatant from NIgG beads was incubated with 30 µl of protein A beads conjugated to 4 µl of anti-PP2Ac antibody overnight. Next day, both NIgG and PP2Ac beads were harvested.

2.2.2 PROTEOMICS SAMPLE PREPARATION AND ANALYSIS

NIgG and PP2Ac beads were washed the next day with PBS for three times. Then, the beads were treated with 30 µl of 2 x SDS buffer comprising 50 mM DTT at 95ºC for 5 min. subsequently, the samples are treated with iodoacetamide (IAA) forabout 30min. The eluates were resolved on 4-15% SDS-PAGE. Five slices were excised from each lane (one sample) followed by in-gel trypsin digestion, peptide purification and HPLC-ESI-MS/MS. The analysis is done on an LTQ Orbitrap Elite as described[78-80]. Maxquant is used for the Peptide/protein identification and quantification[81]. The different steps involved are shown in Figure 7.

2.2.3 STATISTICAL ANALYSIS

Proteins are obtained from Maxquant with peak areas for each which is utilized for analysis (Figure 8). Proteins with atleast two unique peptides are considered for analysis. For it to be categorized as PP2Ac interaction partner, a protein has to meet the following criteria: 1) should be identified with label-free quantification PAs in more than half of the PP2Ac immunoprecipitates (4 IPs); and 2) should have an enrichment ratio (PP2Ac/NIgG) greater than 10, or should not be identified in any of the eight NIgG samples. The calculation of enrichment ratio is explained later in 2.3.5. The proteins which pass the above criteria are considered as potential interaction partners. Further identification of glucose responsive interaction partners (low glucose vs high glucose, n=4) from these PP2A interaction partners, is done as follows: 1) they should have fold change >0.05 of normalized peak areas; 3) and have significantly altered normalized peak areas (P <0.05 calculated by independent t-test).

Ingenuity Pathway Analysis (Ingenuity Systems, Inc., Redwood City, CA),a bioinformatics analysis software package[82-84] is used for pathway analysis on both interaction partners as well as glucose-responsive PP2Ac interaction partners. The software

contains chemical, biological interactions, and functional annotations formed by manual curation of the scientific literature[85,86].

2.2.4 VALIDATION THROUGH WESTERN BLOT ANALYSIS

To Validate a glucose responsive interaction partner, western blot technique is used. INS-1 832/13 cells were treated with low (2.5 mM) and high (25 mM) glucose for 24 hours followed by collecting cell lysates and protein concentration estimation using the bradford assay. Samples were then treated with SDS sample buffer and resolved on 10% SDS-PAGE. The gel is then transferred onto nitrocellulose membranes (Bio-Rad), and analyzed by Western blotting (WB) with the specific antibodies. enhanced chemiluminescence kit[87-89] is used to detect the protein complexes further.

2.3 SPECIFIC AIM 2: HUMAN SKELETAL MUSCLE BIOPSIES

2.3.1 SUBJECTS

A total of 24 participants including 8 lean, 8 overweight/obese non-diabetic and 8 type 2 diabetic volunteers were recruited and took part in the study at the Clinical Research Center at Wayne State University. Written consent was attained from all participants and the study was explained in detail including the indirect benefits and risks. No one had any significant medical problems except for type 2 diabetic participants who have type 2 diabetes, and none engaged in any heavy exercise, and they were directed to stop all kinds of exercise for at least 2 days prior to the study. Institutional Review Board of Wayne State University approved this protocol.

2.3.2 HYPERINSULINEMIC-EUGLYCEMIC CLAMP WITH MUSCLE BIOPSIES

A hyperinsulinemic-euglycemic clamp was used to assess insulin sensitivity and expose skeletal muscle to insulin *in vivo*, as previously described[78]. Followed by a ten hour overnight fast, the study began at approximately 08:30 hours (time -60 min).

Two catheters were placed, one in an antecubital vein, maintained throughout the study for infusions of insulin and glucose. The second in a vein in the contra lateral arm, which was covered with a heating pad (60°C). The purpose of heating pad is to arterialize the venous blood being collected. Blood samples were collected for determination of plasma glucose concentrations. At approximately 09:00 hours (time −30 min), under local anesthesia, a percutaneous needle biopsy of the vastus lateralis muscle was performed. These biopsy samples were blotted free of blood, cleaned of connective tissue and fat (~30 sec), and then frozen in liquid nitrogen. At 09:30 hours (time 0 min), continuous human insulin (Humulin R; Eli Lilly, Indianapolis, IN) infusion was begun at a rate of 80 mU m^{-2} $minute^{-1}$, and continued for 120 min. Plasma glucose was measured at 5-min intervals throughout the clamp. Euglycemia was maintained at 90 mg/dl by variable infusion of 20% d-glucose. Another biopsy is taken at 11:30 hours (time 120 minutes) in the contralateral leg.

Plasma insulin concentration was calculated using the ALPCO Insulin ELISA Jumbo (Alpco Diagnostics, Salem, NH).

2.3.3 OUTLINE

Clinical and proteomics studies were carried out similar to those describe[78], which reported the discovery of new IRS1 interaction partners in human skeletal muscle. The main difference was that PP2Ac Co-immunoprecipitation was used to enrich PP2Ac interaction partners in the present work instead of IRS1 Co-immunoprecipitation used in the publication.

As illustrated in Figure 11, the approach we used included extensive clinical and proteomics data acquisition and data analysis. We first recruited subjects which was followed by comprehensive tests to screen them for eligibility. This is followed by

hyperinsulinemic-euglycemic clamp, procedure to measure insulin sensitivity and muscle biopsies are collected. The proteomics study was performed in the following order: biopsy homogenization; immunoprecipitation of the "bait" protein (PP2Ac), at the endogenous level; followed by one dimensional SDS-PAGE to separate co-interaction proteins; in-gel trypsin digestion to generate peptide fragments; and HPLC-ESI-MS/MS analysis to identify co-immunoprecipitating proteins. Multiple biological comparisons and immunoprecipitation of NIgG (as non-specific control) were used for false positive minimization. Extensive literature searches as well as bioinformatics were used to integrate clinical and proteomics data and to identify pathways and functional categories in which identified PP2Ac interaction partners were involved.

2.3.4 PROTEOMIC SAMPLE PREPARATION

Biopsies were homogenized and processed as described[78,79,90] . The lysate proteins were precleared with NIgG followed by PP2AC immunoprecipitation. The co-immunoprecipitates were resolved on one dimensional SDS-PAGE, which is followed by in-gel trypsin digestion, peptide enrichment, and HPLC-ESI-MS/MS analysis using a LTQ-Orbitrap Elite as described[78]. Peptides/protein identification and quantification were performed using the MaxQuant software. It is one of the most prevalent quantitative proteomics software[81]. Using this, peak areas for each protein were obtained by selecting the option for label-free quantification (LFQ). Only those proteins with a minimum of 2 unique peptides and with false discovery rate (FDR) at 0.01 were considered. In total, 2057 proteins were identified in the 48 muscle biopsies using HPLC-ESI-MS/MS.

To be considered as a PP2Ac interaction partner, a protein has to additionally pass these following criteria: 1). with an enrichment ratio >10; 2). Identified with LFQ

peak area (PA) in more than half of the PP2Ac IP (i.e. >24 biopsies used). The enrichment ratio was calculated as follows: 1st, PA for a protein identified in a gel lane was normalized against the sum of the peak areas for all proteins identified in the same gel lane to obtain normalized ratio for individual protein, Norm:*i*,

$$\text{Norm: } i = \frac{\text{PA}i}{\sum_1^n \text{PA}i}$$

Then, the average of normalized ratio for each protein in the PP2Ac co-immunoprecipitates, Average_Norm:*i*_IRS1, as well as the average of normalized ratio for the same protein in the NIgG co-immunoprecipitates, Average_Norm:*i*_NIgG, were obtained. Finally, Average_Norm:*i*_PP2Ac was divided by Average_Norm:*i*_NIgG, which gives the enrichment ratio for each protein.

$$\text{Enrichment\_Ratio: } i = \frac{\text{Average\_Norm: } i\text{\_PP2Ac}}{\text{Average\_Norm: } i\text{\_NIgG}}$$

Proteins exclusively detected in the PP2Ac immunoprecipitates were identified as PP2Ac interaction partners as we used NIgG as a control. Nevertheless, this will give rise to false negatives since our high sensitivity method would identified trace amounts of a protein non-specifically absorbed on the NIgG beads. However, if a protein is true component of the PP2Ac complex, higher peak area will be assigned to this protein in the PP2Ac sample than in the NIgG sample.

To determine the relative quantities of PP2Ac interaction partners in human skeletal muscle biopsies among lean controls, obese insulin resistant non-diabetic controls, and type 2 diabetic participants, the PA for each protein identified in a specific biopsy was normalized against the PA for PP2Ac identified in the same biopsy, which results in Norm:*j*.

$$\text{Norm: } j = \frac{\text{PA}j}{\text{PA\_PP2Ac}}$$

The normalization strategy is widely used in proteomics studies involving protein-protein interactions[91], and uses similar concept as in western blotting, where the signal for an interaction protein is normalized against that for the protein serving as the "bait." The normalized peak area for each PP2Ac interaction partner, Norm:*j*, was converted to log2 form and compared within the group to assess effects of insulin or across the 3 groups to determine effects of obese insulin resistance and type 2 diabetes on protein-protein interactions involving PP2Ac.

2.3.5 STATISTICAL ANALYSIS FOR PP2A

To be considered as a PP2Ac interaction partner, a protein has to further satisfy the following criteria: 1) the protein is identified with label-free quantification PAs in more than 4 of the PP2Ac immunoprecipitates (IPs); and 2) those proteins have an enrichment ratio larger than 10, or not identified in all of the eight NIgG control samples. The calculation of enrichment ratio was described in our previous publication[81]. To be considered as glucose responsive PP2Ac interaction partners, has to: 1) be an identified PP2Ac interaction partner; 2) has >1.5fold change of normalized peak areas (low glucose vs high glucose, n=4); 3) and has significantly changed normalized peak areas (P <0.05 assessed by independent *t*-test). Although a large number of proteins were assigned in at least one of 48 biopsies that were studied, various filters narrowed the number of proteins that were used in comparisons among groups as described above. This approach is diagrammed in Figure 12. To assess the effects of insulin within a group, statistical significance was calculated by paired *t* tests. For across group comparisons, statistical significance was assessed using ANOVA with post hoc independent *t* tests. Differences were considered statistically significant at $p<0.01$.

Pathway analysis on PP2Ac interaction partners was performed using Ingenuity Pathway Analysis (Ingenuity Systems, Inc., Redwood City, CA), which is widely used

and contain biological and chemical interactions and functional annotations created by manual curation of the scientific literature[84]. A pathway was considered significantly enriched if the p-value for that pathway was less than 0.01 and contained at least 4 identified PP2Ac partners.

CHAPTER 3 RESULTS

3.1 SPECIFIC AIM 1: DETERMINE THE INTERACTION PARTNERS OF PP2AC IN β-ISLET CELLS UNDER BASAL AND HYPERGLYCEMIC CONDITIONS

3.1.1 PP2AC INTERACTION PARTNERS IN INS-1 832/13 CELLS

All these results are published in[92]. Using the proteomics approach developed in our lab[78], a total of 1131 proteins with FDR at 0.01 were identified from PP2Ac coimmunoprecipitations which have a minimum of 2 unique peptides. Among the 1131 proteins, 606 proteins had enrichment ratio larger than 10. Out of these 606 proteins, 514 proteins were identified with a peak area (PA) in more than half (e.g., >4 out of 8) PP2Ac coimmunoprecipitates. These 514 proteins are considered as potential interaction partners of PP2Ac listed in Table 5. These 514 proteins are then compared with the PP2A interactions obtained from BioGRID3.2 database, which came upto 38 proteins (Table 7). Thus, excluding 38 previously known PP2A partners, 476 proteins from this study were considered novel PP2Ac interaction partners. The previously reported PP2Ac interaction partners include the α and β isoforms of PP2A 65 kDa regulatory subunit A, α and δ isoforms of PP2A 55 kDa regulatory subunit B (PPP2R2A and PPP2R2D), the α isoform of PP2A 72/130 kDa regulatory subunit B (PPP2R3A), and the γ isoform of PP2 56 kDa regulatory subunit B (PPP2R5C). Most of these 38 interaction partners were identified in human cells. These known partners are symbolic to the effectiveness of our proteomic approach. However, most of these proteins were first identified in rat β-cells through our study. Among the 476 novel PP2Ac interaction partners, there were more than 15 different kinases, such as dual specificity mitogen-activated protein kinase kinase 2 (MAP2K2), mitogen-activated protein kinase 1 (MAPK1), CRA_a isoform of LIM motif-containing protein kinase 1 (LIMK1), and

calcium/calmodulin-dependent protein kinase type 1 (CAMK1). There were some protein phosphatases (regulatory/catalytic subunits) as well. Examples include serine/ threonine-protein phosphatase 4 regulatory subunit 1 (PPP4R1), serine/threonine-protein phosphatase 6 catalytic subunit (PPP6C), protein phosphatase 1 regulatory subunit 12A (PPP1R12A), and the α isoform of protein phosphatase 3 catalytic subunit (PPP3CA). We also identified insulin-degrading enzyme (IDE), UDP-glucose:glycoprotein glucosyltransferase 1 (UGGT1) and voltage-dependent anion-selective channel protein 1 (VDAC1) as PP2A partners. There were a number of ribosomal proteins, translation initiation factors as well as Ras related proteins identified in the current study.

3.1.2 GLUCOSE RESPONSIVE PP2AC INTERACTION PARTNERS

Out of the 514 PP2Ac interaction partners, 265 are identified with fold change greater than 1.5 (i.e., 1.5fold increase) or less than 0.67 (i.e., 1.5fold decrease) by comparing high low glucose treated samples. Among these 265, 89 proteins showed a significant change in response to the high glucose treatment ($P < 0.05$). These 89 PP2Ac partners were considered as glucose responsive interaction partners. All these 89 partners are mentioned in Table 6. Among them, seven proteins are known to interact with PP2Ac previously in other cell models. They include regulatory subunits of PP2A such as PPP2R1B[93-97] and PPP2R2A[10-12,35,43]. The interaction of PPP2R1B and PPP2R2A with PP2Ac was increased by 1.83 and 2.32 folds, respectively in response to high glucose treatment. The other three PP2Ac partners, sarcolemmal membrane-associated protein (SLMAP)[94], cortactin binding protein 2 (CTTNBP2)[95], and Ints5 protein[98], also presented an increased interaction with PP2Ac with fold change 4.95, 11.03 and 2.47, respectively. Conversely, protein phosphatase 1B (PPM1B)[99], a known PP2Ac partner, displayed a decreased interaction with PP2Ac (0.27 fold change) in response to the high glucose treatment. Out of the thirteen PP2Ac interaction partners with a fold

change higher than 5 or lower than 0.2 ($P < 0.01$) in response to high glucose treatment, only protein peripherin presented a reduced association with PP2Ac (0.19fold change), while others showed an increase in association with PP2Ac. For example, association of CRA_a isoform of LIM motif-containing protein kinase 1 (LIMK1), LIM domain-containing protein 1 (LIMD1) increased 9.85 fold and 12.94 fold, respectively with PP2Ac.

3.1.3 GLUCOSE RESPONSIVE PP2AC INTERACTION PARTNERS RELATED TO INSULIN SECRETION

Ingenuity Pathway Analysis of the 514 PP2Ac interaction partners showed 59 significantly enriched pathways (with a minimum of four interaction partners in a specific pathway and $P < 0.01$; Table 8). Most of the pathways are related to AMPK signaling, cytoskeleton dynamics, and protein synthesis and degradation. On the other hand, very few glucose responsive PP2Ac partners were recognized in these pathways including PPP2R2A, PPP2R1B, ARPC4, LIMK1 and RhoA. Through this IPA analysis, we did not find any enrichment in the insulin secretion pathway. We further did a manual literature search for the proteins involved in insulin secretion. Through this methos, we identified several proteins involved in insulin secretion and other related cellular functions. RHOA, PLA2G6, APPL1, EIF2C2, PFKFB2 and RAB10 have been shown to regulate insulin secretion (Figure 9). Protein CIAPIN1 and PPP4R1 are promote anti-apoptosis, thus retaining islet survival and function. We identified few proteins involved in vesicle trafficking that include VPS52, VPS37A, TSG101, RAB5C, RAB10 and EEA1. There were few components of ribosomes such as RPL9, RPL4, RPL30, RPL18A and MRPL35. All these ribosomal components were found with increased PP2Ac association in response to high glucose treatment. LIMD1, VGLL4,

STAT6, PHF5A, DDX17, NCOR1, ILF3, HIST1H1C and TRIP11 are all involved in regulation of transcription.

3.1.4 EXPERIMENTAL VALIDATION OF PPP2R1B AS A GLUCOSE RESPONSIVE PP2AC INTERACTION PARTNER

PPP2R1B, β isoform of PP2A A subunit was validated by co-IP and western blot. It was identified as a glucose responsive PP2Ac interaction partner. INS-1 832/13 cells were incubated with low (2.5 mM) and high glucose (25 mM). Through western blot, we showed an increased association of PPP2R1B with (1.57 fold) under glucotoxic/high glucose condition ($n = 4$, $P < 0.05$) (shown in Figure 10). These findings are consistent with our proteomics results where there was 1.83fold change in response to high glucose treatment ($n = 4$, $P < 0.05$). Furthermore, we quantified abundance of this PPP2R1B, normalized to β-actin level. This presented only 1.13-fold change in high glucose over the basal conditions ($P > 0.05$). However, when PPP2R1B is normalized with levels of PP2Ac PPP2R1B/PP2Ac, a significantly increase is seen upon high glucose treatment ($n = 4$, 1.47 folds, $P < 0.01$).

3.2 SPECIFIC AIM 2: DETERMINE INTERACTION PARTNERS OF PP2A IN HUMAN SKELETAL MUSCLE UNDER BASAL AND HYPERINSULENEMIC CONDITIONS IN LEAN, OBESE/OVERWEIGHT NON-DIABETICS AND TYPE 2 DIABETIC SUBJECTS.

3.2.1 PP2AC INTERACTION PARTNERS IN SKELETAL MUSCLE FROM LEAN, OVERWEIGHT/OBESE, AND TYPE 2 DIABETIC HUMAN PARTICIPANTS

Clinical characteristics of all the 24 human subjects (8 lean, 8 obese/overweight, and 8 type 2 diabetic) is listed in Table 3 and Table 4.

PP2Acα and PP2Acβ were detected in PP2Ac immunoprecipitates from all 48 biopsies used for the study. After performing statistical analysis as mentioned in the Figure 12, 211 proteins met the criteria for classification as PP2Ac interaction partners. These 211 partners may interact with PP2Ac directly or indirectly.

Table 9 lists the 211 PP2Ac interaction partners with their enrichment ratio. PP2A interaction partners were pooled from various databases including BioGrid, SPIKE, IntAct, and STRING. By comparing these partners from databases with the 211 partners identified in our study (human skeletal muscle), 21 were found in common (Table 11). Further comparison with 514 partners previously identified in beta cells (in our study) yielded 38 proteins (listed in Table 12) while 9 out of these 38 are redundant. Altogether, a total of 50 partners are previously identified while 161 were novel. The 50 known PP2Ac interaction partners included AMPK, CAV1, CCDC6, CCT2, CCT6A, CUL1, IGBP1, PPME1, PPP2R1A, PPP2R2A, PPP2R3A, PPP2R5D, PPP4C, PSMC6, PSMD1, RAC1, SOD1, STRN, STRN3, TIPRL, USP7 from the databases and AKR1B1, APPL1, ARCN1, ASNA1, NTPCR, CAND1, ***CCDC6***, DARS, EIF2B1, FAHD1, FLNA, GFPT1, GSN, IDH3B, ***IGBP1***, MYH14, NAP1L4, PDIA6, ***PPME1***, ***PPP2R1A***, ***PPP2R2A***, ***PPP2R3A***, ***PPP4C***, PPP4R2, PSMC2, PSMC3, PSMD12, PSMD13, PSMD14, RAB1B, ***RAC1***, RPS15A, RPS25, S100A11, ***STRN***, TALDO1, TSN, TUBB2A from the beta cells (the ones in bold italics are redundant).

Ingenuity pathway analysis on the 211 PP2Ac interaction partners and PP2Ac suggested various pathways significantly enriched compared to the whole genome background, such as IRS, Mtor, and MAPK signaling. Two of the significantly enriched pathway, IRS and mTOR signaling, are illustrated in Figure 13 and Figure 14 respectively.

We also performed network analysis using Ingenuity pathway analysis for the 211 PP2Ac interaction partners and PP2Ac to illustrate how these partners can be interrelated. Figure 15 shows the network with the highest score and highest number of interaction partners identified in this study.

3.2.2 PARTNERS WITH SIGNIFICANT DIFFERENCE AMONG LEAN CONTROL, OBESE/OVERWEIGHT CONTROL, AND TYPE 2 DIABETIC GROUPS

As mentioned in the Figure 12, by comparing the normalized peak areas of the 211 proteins, 69 interaction partners exhibited significant difference among the three groups. All the 69 partners are listed in Table 10.

Upon insulin stimulation, in lean control group, 4 proteins showed significant difference which included ACO1, IRP1, PPME1, and PPP4R2 whereas insulin stimulation in obese control significantly changed CCDC6 and LUM and in type 2 diabetic group, it seemed to significantly change one protein, ACOT9.

63 proteins showed a significant change in obese/overweight insulin resistant controls when compared with lean controls while 37 proteins exhibited a significant change in type 2 diabetics compared to lean controls. When the 63 proteins between lean and obese, 37 proteins between lean and T2D are compared, 32 proteins are in common i.e., they are seen with a change in both type 2 diabetics and obese group when compared to lean. When type 2 diabetic group is compared to obese non-diabetic insulin resistant group, 47 proteins presented with a significant change. These partners showed either a significant increase or decrease. Out of 63 proteins difference between lean and obese/overweight, interaction of only PDE4D and SCPEP1 is increased in obese/overweight compared to lean while the rest presented with an increase. CCT2, COPS2, PDE4D, ACO1/IRP1, CA1, GSTM3, BLVRB showed an increased interaction in T2D

compared lean among 37 proteins different between these two groups. 43 out of 47 significant proteins between T2D and obese had an increased interaction with PP2A in T2D while the remaining four, EIF2B1, LAP3, LUM, and SCPEP1 exhibited a decrease. For easy access and understanding, all the 69 proteins are divided per their function and are color coded based on their difference between groups in Figure 16A and Figure 16B. One protein can show difference between groups in more than one case (for example, AKT2 protein show difference between lean and obese group, lean and type 2 diabetic group; hence you can see AKT2 coded in two different colors). It is to be noted that one protein can be involved in more than one function mentioned but, to simplify, a protein is grouped only under one function.

CHAPTER 4 DISCUSSION

4.1 SPECIFIC AIM 1: DETERMINE THE INTERACTION PARTNERS OF PP2AC IN HUMAN B-ISLET CELLS UNDER BASAL AND HYPERGLYCEMIC CONDITIONS

4.1.1 PP2AC INTERACTION WITH SIGNALING PROTEINS IMPORTANT FOR PHYSIOLOGICAL INSULIN SECRETION

We discovered several PP2Ac interactions that have been involved in islet function and insulin secretion. They include proteins involved in protein sorting and trafficking, SRP72 and GGA2, and vesicle trafficking (VPS52, VPS37A, Rab10, Rab5C etc.). They also include small G proteins Rac1, Rho A, Rab5c. These findings suggest a close interaction between these signaling proteins and PP2Ac. These small G-proteins (Rac1, Cdc42, Rho A and Rab) play a pivotal role in glucose stimulated insulin secretion[100,101]. They also play an important role to traffic insulin stored vesicles to the cell membrane and cytoskeletal remodeling to allow fusion of these secretory granules with the plasma membrane in order for insulin secretion[100,101].

We identified an important interaction, PP2Ac with LIMK1 because LIMK1 is a serine/threonine-protein kinase which plays a vital role in the regulation of dynamics of actin filament at the cell membrane. Activation of kinases like ROCK1, PAK1 and PAK4 cause phosphorylation and activation of LIMK1, which then phosphorylates and thereby inactivates the actin binding/depolymerizing factors[102,103]. This inactivation of the depolymerizing factors result in the prevention of breakdown of F-actin and thereby actin cytoskeleton stabilization. Besides, LIMK1 has shown to regulate quite a few actin-dependent biological processes including cell cycle progression, cell motility, and

cell differentiation[104]. This finding has huge significance considering that a glucose responsive PP2Ac partner is involved in vesicle trafficking and actin cytoskeletal remodeling, essential for glucose stimulated insulin secretion.

An othe important interaction partner to be noted is the immunogloblin-binding protein [Igbp1]. Igbp1, also known as α4, is a non-canonical adaptor subunit of PP2A[60]. In addition to binding to its regulatory subunits, PP2Ac is also shown to interact with other substrates, including α4 that regulate its localization, abundance, and activity. In this case, α4 is known to involve in PP2A biogenesis, stability and activation[60,105]. PP2AC-IGBP1 complex protects the catalytic subunit from proteasomal degradation[106]. Other such regulators are found later in this study. α4 is also shown to interact with other phosphatases like PP4 and PP6[105]. It is worthwhile to note that we also identified PP4 as an interacting partner of PP2Ac. This is the first evidence for regulation of protein phosphatase 4 in beta cells its involvement in the induction of defects in nuclear lamin processing stimulated by cytokines[107].

4.1.2 PP2AC INTERACTION WITH KEY PROTEINS THAT REGULATE CELL DYSFUNCTION AND APOPTOSIS

We also identified protein methyl esterase-1 as an interaction partner. As discussed earlier, PP2Ac undergoes methylation at the carboxyterminal leucine (Leu-309) residue. As already mentioned, PP2A activity is increased under glucotoxic conditions with corresponding increase in C-terminal methylation of PP2Ac[68]. LCMT-1/leucine carboxy methyl tranferase, involved in transferring methyl onto leucine -309 of PP2Ac. siRNA-mediated knockdown of LCMT-1 significantly decreased the carboxylmethylation of PP2Ac and hyperactivation of PP2A under high glucose/glucotoxic conditions. This implies that the carboxylmethylation leads to a sustained activation of PP2A[89,108]. However, potential regulatory roles of PME-1 in islet function are yet to be defined.

We also noted another key protein with a significant increase [~3.6 fold] in the interaction with PP2Ac, PPP4R1, a regulatory subunit of PP4. High levels of expression of protein phosphatase 4 catalytic subunit (PP4c) in the nuclear fraction is found in β-cells[107]. Additionally, exposing β-cells to IL-1β, a proinflammatory cytokine, lead to a marked increase in nitric oxide release with a corresponding decrease in carboxyl-methylation of PP4C. IP studies indicated a potential interaction of PP4c with nuclear lamin-B, a vital regulatory protein important in the nuclear envelope assembly[107].

We also identified a significant [2.3-fold] increase in the interaction between PP2Ac and its regulatory subunit, B55α. Yan et al presented involvement of a B55α-containing PP2A holoenzyme in the dephosphorylation of FOXO1 in islet β-cells under H_2O_2-induced oxidative stress conditions[109]. They also reported increased expression of B55α subunits in islets obtained from *db/db* mouse, a diabetic mouse kodel[109]. Significant increase in the abundance of B55α subunit under hyperglycemic conditions in INS-1 832/13 cells has been reported recently[109].

4.2 SPECIFIC AIM 2: DETERMINE INTERACTION PARTNERS OF PP2A IN HUMAN SKELETAL MUSCLE UNDER BASAL AND HYPERINSULENEMIC CONDITIONS IN LEAN, OBESE/OVERWEIGHT NON-DIABETICS AND TYPE 2 DIABETIC SUBJECTS

4.2.1 PP2AC INTERACTION PARTNERS IN SKELETAL MUSCLE

Using the proteomics approach for protein-protein interactions developed in our laboratory[78], we have identified 211 PP2Ac interaction partners in skeletal muscle from 8 lean, 8 overweight/obese, and 8 human participants, which represents the largest PP2Ac interaction network in humans to date. Among them, 50 were known PP2Ac interaction partners while 161 were novel (Table 9).

4.2.2 KNOWN PARTNERS

Among these 50 PP2Ac interaction partners are some regulatory and a scaffold subunit of PP2A. They include PPP2R1A, 'A' subunit alpha isoform, PPP2R2A, 'B' subunit alpha isoform, PPP2R3A, B'' subunit alpha isoform, PPP2R5D, B' subunit delta isoform, STRN, B''' subunit alpha isoform, and STRN3, B''' subunit beta isoform. It is also known to bind to catalytic subunit of protein phosphatase 4 (PPP4C)[110]. Other important known partners include PPME1 and IGBP1. PPME1 is protein methylesterase, which catalyzes the demethylation of PP2A on leucine309. As mentioned in the introduction, regulation of PP2A through methylation is controversial. However, PPME-1 is shown to protect PP2A from degradation[61] and so does IGBP1. It is also known as alpha4, binds to catalytic subunit thereby stabilizing and preventing it from the degradation[105].

Caveolin-1 is a scaffolding protein which is found in most cell types as a prime component of the caveolae plasma membranes. This protein is involved in promoting cell cycle progression. Protein expression of Insulin Receptor Substrate (IRS)-1 is reduced in caveolin knock out cells[111]. In addition, our lab has shown interaction of IRS1 with CAV1 in human skeletal muscle biopsies[112]. It's interaction with PP2A is also shown in human prostate cancer cells where cav-1 acts as a positive regulator in the Akt signaling pathway via inhibition of PP1 and PP2A[113]. Mechanism of inhibition involves binding of cav-1 to the catalytic subunits of both PP1 and PP2A (have a consensus cav-1 binding motif). Thus, the lowered activities of PP1 and PP2A lead to increased phosphorylation levels of their specific substrates like PDK1, Akt, and ERK1/2[113].

Coiled-coil domain containing 6 (CCDC6) translates to a protein that is ubiquitously expressed and its gene re-arrangements is seen in many malignancies[114]. Its interaction with PP2Ac is seen in high throughput experiments as an attempt to understand phosphatase interactions using human cell lines[110,115].

Protein levels are regulated in many ways. Various cell signals regulate the translation of proteins through mRNA. In this process of translation, proteins have to synthesized, folded, and localized specifically. In contrast, protein degradation can occur through proteasome machinery where unneeded/misfolded proteins are tagged with ubiquitin and are degraded through E1, E2, and E3 enzymes. PP2A is known to interact with molecules involved in these processes. Here, we identified few such proteins like CCCT2, CCT61, CUL-1, PSMC6, PSMD1, and USP7. CCT2 and CCT6A are chaperone proteins. All the proteins after translation require proper folding to achieve the tertiary structure. The function of these chaperones is to correct the partially folded or misfolded proteins which otherwise can aggregate to form lethal complexes using ATP as source of energy[116]. Cullin-1 protein is a core component of a E-3 Ubiquitin protein ligase complex, Cullin-RING ubiquitin ligases (CRLs), involved in the ubiquitination of proteins in cell cycle and signal transduction. It's interaction with PP2A is seen while elucidating the structure of the cullin-RING ubiquitin ligase (CRL) network using human 293T cell lines[117]. PSMC6 and PSMD1 are subunits of a proteasome complex machinery. This machinery degrades proteins tagged with ubiquitin. Interaction of PP2A with PSMC6, PSMD1 and superoxide dismutase-1 is identified using high throughput quantitative tandem mass spectrometry[93] in human HeLa S3 and HEK 293 cells. Ubiquitin specific peptidase 7 (USP7) deubiquitinates proteins, including p53, FOXO4, MDM2, PTEN and others, thereby controlling important cellular functions such as cell proliferation, apoptosis, and signal transduction[118]. Using two-dimensional SDS-PAGE

analysis and other proteomics-based experiments, USP7 is shown to interact with PP2A along with other substrates in HeLa cells[118]

Small G-protein Rac1 is known to involve in insulin signaling pathway. It is also shown to play a role in actin cytoskeleton remodeling and insulin-stimulated GLUT4 translocation in L6 myotubes[119,120]. In addition, experiments on rat and human muscle indicated the activation of Rac1 after exercise and its role in contraction induced glucose uptake[121]. Using western blot, PP2A is shown to bind to c-terminus of Rac1 in cell culture models[122].

Target of rapamycin (TOR) is a serine/threonine protein kinase which belongs to the phosphatidylinositol kinase-related kinase family, plays key roles in cellular processes such as proliferation and cell growth. Yeast ortholog of TOR signaling pathway regulator (TIPRL), Tip41 is shown to negatively regulate TOR signaling[123] In an attempt to study the role of TIPRL in TOR signaling, it was found that TIPRL facilitates TOR signaling via its association with PP2Ac in human cell lines in contrast to the findings in yeast[123]. We found TIPRL as PP2A partner in muscle.

AMPK is a protein kinase, composed of alpha beta and gamma subunits, and is activated in response to altered energy levels in the cell. Higher ATP levels reduce the activity of AMPK. When the AMP levels rise, ATP is exchanged for AMP and activates AMPK. Isoforms identified here are PRKAG1 (gamma 1) and PRKAB2 (beta 2). PRKAG1 is ubiquitously expressed whereas PRKAB2 is found abundant in skeletal muscle cells. It is known to have important role in skeletal muscle insulin sensitivity[124]. In skeletal muscle, activation of AMPK will cause fatty acid and glucose oxidation. It also plays a role in activation of GLUT4 transporters, for uptake of glucose and in glycogen metabolism[125]. PP2A has been shown to be able to dephosphorylate AMPK[126] Activation of AMPK is achieved by phosphorylation of AMPK at various serine and

threonine sites. Its binding to PP2Ac may change the phosphorylation status of this kinase and thereby its activation or inactivation. Since *de novo* AMP synthesis will activate AMPK, experiments were conducted using rat hepatocytes to see if altering the activity of enzymes involved in purine biosynthesis will improve insulin sensitivity. They found that abundant adenosuccinate lyase (ADSL) can lead to increased AMP production, thereby AMPK activation and improved insulin sensitivity[127]. Both ADSL and AMPK are found as PP2Ac interaction partners in our study.

4.2.3 PROTEINS INVOLVED IN INSULIN RECEPTOR AND mTOR SIGNALING

Binding of insulin to the insulin receptor on the cell membrane leads activates a cascade of signaling molecules. The pathways activated are PI3K-AKT signaling pathway and Grb2-SOS-Ras-MAPK pathway. These result in various physiological functions such as GLUT4 translocation to the plasma membrane, glucose uptake, glycogen synthesis, and protein synthesis[12,14]. Here we see seven molecules associated with Insulin signaling pathway as PP2A interaction partners. These include AKT2, eukaryotic translation initiation factor 2B subunit alpha (ELF2B1), MAP2K1/MEK1, protein phosphatase 1 regulatory subunit 7, AMPK subunit gamma isoform (PRKAG1), protein tyrosine phosphatase, non-receptor type 11, and Ras. Akt2 isoform is found to be crucial for insulin action in vivo[128]. PP2A is shown to negatively regulate Akt2 in fibroblast cells[129]. PP2A hyperactivation associated with insulin resistance in response to saturated fatty acids like ceramide is seen with an associated Akt deactivation[130]. However, experiments on liver hepatocytes *in vitro* and *in vivo* showed that PP2A activity is essential for insulin-stimulated glycogen storage[131]. This is supported by our data where its interaction is significantly decreased in obese insulin resistant non-diabetic control basal and insulin stimulated biopsies when compared to lean control bas

and insulin stimulated biopsy respectively. Similar pattern is seen with type 2 diabetic group. Interaction is significantly decreased in type 2 diabetic basal and insulin stimulated biopsies when compared to lean control basal and insulin stimulated biopsies respectively. PP2A might play a protective role in terms of its interaction with Akt2 in a normal lean person while this interaction may be disrupted in cases of obese insulin resistance and type 2 diabetes. PTPN11, also known as SHP2, encodes a protein tyrosine phosphatase containing SHP binding domain. It is shown to bind with a variety of intermediate signaling molecules such as Grb2, p85 subunit of PI3 kinase, IRS-1, and Gab1 and 2. Being a protein tyrosine phosphatase, SHP-2 is believed to act by dephosphorylating these molecules, thereby lessening the signal[132]. PP2A, being a phosphatase itself can be regulated through phosphorylation and dephosphorylation on its Tyr307 site. This interaction between PTPN11 and PP2A is a novel. Further, its interaction is significantly decreased in obese insulin stimulated biopsy when compared to lean insulin stimulated biopsy. eukaryotic translation initiation factor 2B subunit alpha (ELF2B1), as the name indicates is involved in protein synthesis. Elf2B is regulated through phosphorylation. GSK3 inhibits the activity of elf2B by phosphorylating it under basal conditions. Upon insulin stimulation, GSK3 is inactivated by Akt which leads to elf2B dephosphorylation and activation, thereby increasing protein synthesis[133]. PP2A is shown to interact with elf2B in our study. In addition, its interaction is 1) decreased in obese basal and insulin stimulated biopsy compared to basal and insulin stimulated biopsy in lean respectively, 2) decreased in type 2 diabetic basal and insulin stimulated biopsy when compared to obese basal and insulin stimulated biopsy respectively and 3) decreased in type 2 diabetic insulin stimulated biopsy compared to lean insulin stimulated biopsy. PP2A is also seen to interact with regulatory subunit of protein phosphatase 1. PP1 is known to dephosphorylate and activate glycogen synthase,

promoting glycogen synthesis. Insulin is shown to activate PP1 in L6 rat skeletal muscle cells[134]. Its interaction with PP2A among three groups varied significantly specifically between type 2 diabetic, obese and obese, lean groups. Interaction is decreased in

1) obese bas and insulin stimulated biopsy when compared to lean basal and insulin stimulated biopsy and 2) type 2 diabetic basal and insulin stimulated biopsy when compared to obese basal and insulin stimulated biopsy correspondingly. Through the Grb2-SOS-Ras-MAPK pathway, Insulin activates mitogen-activated protein kinases (MAPK) to increase gene expression and differentiation. Activation of Insulin receptor and IRS proteins activates signaling cascade that promotes activation of Ras-GDP to Ras-GTP. Activated Ras interacts and activates a series of downstream signaling molecules Raf-MEK1/2-ERK1/2. ERK is directly involved in regulating gene expression, cell proliferation or differentiation, cytoskeletal reorganization. Ras and MAP2K1/MEK1 are identified as PP2A interaction partners. Nevertheless, these two molecules did not show any significant difference among groups.

mTOR pathway has great impact on the cell growth and metabolism. It regulates protein biosynthesis, lipid synthesis, mitochondrial biogenesis and metabolism. Previous reports show that PP2A is down regulated by mTOR, and degradation of IRS1 by mTOR is achieved through inhibition of PP2A[135]. Growth factors like insulin stimulate mTOR by increased phosphorylation of TSC2 protein by kinases like PKB, ERK1/2 and RSK1. This TSC2 phosphorylation leads to inactivation of TSC1/2 and there by activation of mTOR. AMPK is activated in response to low energy levels. This activated AMPK phosphorylates and reduce the activity of TSC2 and thereby reduce mTOR activation[136]. RSK1 and AMPK regulate the mTOR pathway through phosphorylation and dephosphorylation. In the present work, we detected many PP2A interaction partners involved in the mTORpathway. These include small G proteins Ras and Rac,

transcription factors elf3 and elf4A, PRKAG1 (gamma 1) and PRKAB2 (beta 2) subunits of AMPK, Ribosomal protein S6 kinase alpha-1, and ribosomal protein S15A that regulate protein synthesis.

4.2.4 INTERACTION PARTNERS WITH SIGNIFICANT CHANGES IN THEIR INTERACTION TO PP2AC IN SKELETAL MUSCLE IN LEAN, OVERWEIGHT/OBESE, AND TYPE 2 DIABETIC HUMAN PARTICIPANTS

Upon insulin stimulation, in lean control group, 4 proteins showed significant difference which included ACO1/IRP1- Cytoplasmic aconitate hydratase, protein methylesterease-1, and PPP4R2. Aconitate hydratase is an enzyme involved in tricarboxylic acid cycle. Besides, it acts as an iron-sulfur protein, maintaining levels of iron inside the cell. PPME-1, serves two functions, demethylates PP2Ac and helps maintain levels of PP2A. PPP4R2 is a regulatory subunit of protein phosphatase 4. All the three proteins, protein methylesterase-1, PP4 regulatory subunit, and aconitate hydratase displayed a decreased interaction with PP2Ac upon insulin stimulation.

Insulin stimulation in obese control significantly changed CCDC6 and LUM. CCDC6 is a well-known tumor suppressor and its chromosomal re-arrangement is seen in thyroid papillary carcinoma. It is a known PP2Ac interaction partner identified in HeLa and other human cell lines[110,115] using different proteomic approaches. Here, we saw an increased interaction of PP2Ac with CCDC6 upon insulin stimulation in obese/overweight insulin resistant non-diabetic group. LUM encodes for protein Lumican, which belongs to the family of comparatively small leucine-rich proteoglycans. Proteoglycans are a major component in the extracellular matrix of many tissues. This is a major proteoglycan found in cornea but high levels of expression of this protein is found in skeletal muscle[137]. It binds to collagen fibrils in the tissue spaces, thus regulating collagen fibril organization in addition to corneal transparency, epithelial cell

migration, apoptosis, tissue repair, angiogenesis and cell growth[138]. Interaction of lumican with PP2Ac is decreased upon insulin stimulation in obese subjects. The significance of this interactions is yet to be elucidated.

In type 2 diabetic group, upon insulin stimulation, only one protein showed significant change, ACOT9. Acyl-CoA thioesterase 9 is a mitochondrial protein which catalyzes hydrolysis of acyl-CoAs to form coenzyme A and free fatty acid. It is well documented that mitochondrial dysfunction and free fatty acid induce skeletal muscle insulin resistance[139]. The major pathway for oxidation of fatty acids is the mitochondrial fatty acid oxidation (β-oxidation), producing majority of ATP required for the cells where Coenzyme A (CoA) is an important co-factor[140]. Hence, this enzyme is one of the factors that regulate levels of coenzyme A. It is interesting to observe that the interaction of PP2Ac with ACOT9 is decreased significantly upon insulin stimulation given the fact that insulin decreases fatty acid oxidation in the skeletal muscle.

4.2.5 PARTNERS WITH SIGNIFICANT CHANGE BETWEEN TYPE 2 DIABETIC AND LEAN SUBJECTS

37 proteins exhibited a significant change in type 2 diabetics compared to lean controls out of which seven proteins showed an increased interaction and rest with a decreased interaction in type 2 diabetic compared to lean. The seven proteins included BLVRB, CCT2, COPS2, PDE4D, ACO1/IRP1, CA1, GSTM3 which are explained in detail below. While CCT2, COPS2, and PDE4D are seen with a difference in both basal and insulin stimulated biopsies, change in BLVRB is seen only in basal and change in ACO1/IRP1, CA1, GSTM3 are seen in only insulin stimulated biopsies. Out of these 37, 25 proteins showed a difference in basal biopsies and 32 in insulin stimulated biopsies with 20 in common. Akt2 is one among them. As discussed earlier, Akt2 is an

important signaling molecule in insulin signaling pathway in skeletal muscle. The decreased interaction of Akt2 with PP2A in T2D biopsies compared to lean is an important observation. PP2A might positively regulate Akt2 considering that decreased interaction is seen in type 2 diabetics where insulin signaling is impaired. Nevertheless, further studies are required to conclude this.

Protein synthesis and degradation

Here, we found many proteins involved in protein synthesis as well as is degradation. STAT proteins, as mentioned above, are transcription factors that regulate protein synthesis. Transcription factors STAT3 andSTAT5 show a significant difference between lean and T2D. In inactive state, STAT's exist as cytosolic proteins in an unphosphorylated state. Stimulation by cytokine or growth factors induce tyrosine phosphorylation of STATs and its translocation to the nucleus. In addition to tyrosine phosphorylation, all STAT's including STAT3 and STAT5, are regulated by serine phosphorylation (PP2A being a serine/threonine phosphatase). This serine phosphorylation is facilitated by several serine/threonine kinases including, but not restricted to, ERK, p38, JNK, mTOR, CaMKII, IKKε, and PKCδ[141]. We also saw gamma subunit of calcium/calmodulin-dependent protein kinase type II (CAMK2G) as PP2A interaction partner. It is noteworthy that both STAT proteins and CaMK-II subunit gamma showed a decreased interaction in type 2 diabetic (basal and insulin stimulated). These STAT proteins in addition to eukaryotic translation initiation factor 2B subunit alpha(EIF2B1), glycyl-tRNA synthetase (GARS), and C-terminal-binding protein 1 (CTBP1) regulate protein synthesis at the transcription and translation level. All of them showed a decreased interaction in T2D. C-terminal-binding protein 1 is a transcriptional repressor involved in physiological and pathological functions like apoptosis (antagonist) and tumorigenesis (suppress tumor suppressor genes)[142]. CTBP1 dimerizes with a

second closely related gene, CTBP2. It is shown in human hepatic cell line that CTBP2 over-expression improved insulin sensitivity by augmenting phosphorylation of (AKT) and glycogen synthase kinase 3β (GSK3β)[143]. It also showed to reverse the effects of palmitate on ROS level, gluconeogenesis, lipid accumulation, and hepatic glucose uptake[143]. In other human tumor cell lines, under hypoxia conditions, overexpression of CtBP2 resulted in reduction of PTEN levels with corresponding increase in the levels of PI3K and pAkt[144]. Though such role in skeletal muscle is not shown, it will be interesting to study the role of this protein in skeletal muscle and the function of pp2a-CtBP1 interaction in insulin resistance and type 2 diabetes. Protein levels can also be regulated post-translation. In this context, we see T-complex protein 1 subunit beta (CCT2) and proteasome activator subunit 2(PSME2) as PP2A partners. T-complex protein 1 subunit beta, a chaperone protein, as mentioned earlier, corrects the partially folded or misfolded proteins[116] showed an increased interaction in T2D whereas proteasome activator subunit 2 presented with an decreased interaction. PSME-2 is involved in the degradation of proteins through ubiquitin-proteasome system.

Protein modifications

We also saw proteins associated with protein modifications. ADP ribosylation is a post translational modification, where a ADP-ribosyl group is transferred onto protein from nicotinamide adenine dinucleotide (NAD^+)[145]. This reversible modification is involved in many cellular processes such as apoptosis, DNA damage repair, cell proliferation, gene transcription and others. The transfer of ADP-ribosly group is aided by group of enzymes called ADP-ribosyl transferase[145]. Here, we have found arginine specific ribosyl transferase (ART3) as PP2A interaction partner, with a reduced interaction in T2D. Sumoylation is another post translational modification that regulates protein structure and intracellular localization through addition of small protein SUMO.

SUMO-activating enzyme subunit 2 (UBA2) is found in our study, with a decreased interaction in T2D. This protein forms a heterodimer with another protein SME-1 that acts as a SUMO-activating enzyme[146]. Ubiquitination is also an important PTM which leads to protein degradation through proteasome complex. COP9 signalosome complex subunit 2 (COPS2), subunit of the COP9 signalosome complex, is involved in the ubiquitin-proteasome pathway through regulation of a E3ubiquitin -protein ligase complex family, culin-RING ubiquitin ligase (CRLs). One of CRLs include cullin-RING-based SCF (SKP1-CUL1-F-box protein), which mediate the ubiquitination of proteins involved in cell cycle progression, signal transduction and transcription. These complexes require neddylation, the attachment of a ubiquitin-like NEDD8 molecule, for its function and this neddylation process is closely regulated by the COP9 signalosome[147,148]. Cullin-1 is a known interaction partner of PP2A. CUL-1 and COP9 interact with each other shown through various high throughput and low throughput experiments[117,149-151]. Here, we found both COP9 and CUL-1 as PP2A interactions in our study. However, Cullin-1 (CUL1) showed no significant difference between groups while COP9 is presented with an increased interaction in T2D. Other protein involved in protein modification is leucine aminopeptidase (LAP3), an exopeptidase, catalyzes the hydrolysis of leucine residues from the amino-terminus of protein. Its interaction with PP2A is decreased significantly in T2D. Being an exopeptidase, changes in LAP abundance and/or its activation result can alter a protein activation.

Membrane proteins

Insulin stimulation leads to a rapid actin filament reorganization that corresponds with recruitment PI 3-kinase subunits and glucose transporter proteins to regions of reorganized actin in culture muscle cells[152-154]. Initiation of these effects of insulin

requires an intact actin cytoskeleton and activation of PI 3-kinase[152-154]. It is my speculation that these changes might alter the proteins involved in cell membrane organization and also the proteins to which they are connected to in the extracellular matrix. Extracellular matrix is made of **proteoglycans** and fibrous proteins, including collagen, elastin, fibronectin, and laminin[155]. The extracellular matrix is linked to the cell via transmembrane cell adhesion proteins that connect the matrix to the cell's cytoskeleton[155]. The principal cell adhesion proteins are the integrins that act as cell surface receptors. **Fermitin family homolog 2** (FERMT2) is scaffolding protein that enhances this integrin activation. While the integrins are connected to extracellular matrix through their extracellular domains, their intracellular domains are anchored to the actin filaments via intracellular anchor proteins, **filamin**, talin, actinin, and vinculin[155]. In our study, we saw proteins Lumican (LUM), a proteoglycan, Fermitin (FERMT2), and filamin A (FLNA) as interactors of PP2A with a decreased interaction in T2D. Besides, Fermitin is also shown to be a key protein required for muscle differentiation[156]. During development and regeneration, for the growth of skeletal muscles, muscle precursor cells (*or* satellite cells) proliferate, known as myoblasts, and consequently differentiate into myofibers[156]. Another interesting protein involved in the actin cytoskeleton organization, cellular adhesion is the Ras GTPase-activating-like protein (IQGAP1)[157]. It is also known to regulate MAPK and Wnt/β-catenin signaling pathways. IQGAP1 is a downstream effector of Rac and Cdc42, small GTPases that regulate actin assembly[157]. As mentioned above, integrin family of cell surface receptors mediate cell adhesion by anchoring to actin assembly. This cell adhesive function of integrins is regulated by its phosphorylation or dephosphorylation modulated by Ca^{2+}/calmodulin-dependent protein kinase II (CaMK) or protein phosphatase 2A[158]. In human mammillary epithelial cells, β1 integrin is immunoprecipitated with Rac and vice versa[159]. Given the role of

IQGAP in actin assembly, further studies showed that IQGAP1 and PP2A coimmunoprecipitated with Rac and β1 integrin[159]. Combining the results, formation of a quaternary complex that consists of IQGAP1, PP2A, Rac, and β1 integrin is possible[159]. Additional experiments were conducted to show that PP2A functions by recruitment of IQGAP1 to Rac-β1 integrin[159]. Subsequently, by stimulating human mammillary epithelial cells with Epidermal Growth Factor (EGF), they presented a mechanism to explain the regulation by PP2A: activated PP2A promotes IQGAP1 recruitment to β1 integrin-Rac under basal conditions but activation by a growth factor causes dissociation of IQGAP1 from β1 integrin-Rac through activation of CaMKII and formation of PP2A-IQGAP1-CaMKII complex[160]. EGF stimulation is thus shown to abolish the PP2A function[160]. IQGAP1 can also directly recruit and sequentially activate B-Raf, Mek1/2(MAPK1/2) and Erk1/2 as a part of MAPK signaling pathway[161]. Its direct interaction with MAP2K1 is shown[162]. It is also shown to interact directly with β-catenin under basal conditions (as part of degradation complex along with axin, GSK3, CK1α, and APC) and after activation, β-catenin is rescued from degradation through IQGAP1-PP2A-mediated dephosphorylation with its subsequent nuclear translocation and specific gene transcriptions[161]. It is very significant to note that we showed interaction of PP2A with IQGAP1, MAP2K1, Rac 1, and CAM2KG in our study. IQGAP1 is seen with a decreased interaction in T2D while MAP2K1, Rac1 or CAM2KG exhibited no significant difference.

Other proteins

ATP synthase subunit S (ATP5S) is one of the subunits of the catalytic core of ATP synthase enzyme. This enzyme catalyzes the formation of ATP from ADP in the mitochondria[163]. Experiments were conducted on human skeletal muscle comparing the abundance and phosphorylation of ATP synthase between basal and insulin stimulated

biopsies of lean, obese and T2D. The amount of ATP synthase in basal biopsies is found to be decreased in obese and T2D compared to lean. They found abnormal phosphorylation sites in obese and T2D[164]. In our study, we saw a decreased interaction of PP2Ac with ATP5S in type 2 diabetic insulin stimulated biopsy compared to lean insulin stimulated biopsy. The interaction in basal biopsies of T2D also dampened but it's not significant (p=0.05).

cAMP-specific 3,5-cyclic phosphodiesterase 4D (PDE4D), involved in hydrolysis of cAMP is seen as a PP2A partner with increased interaction in T2D. PDE4D regulates cAMP levels in the cell which is an important second messenger that regulates various cellular processes. In skeletal muscle, acute cAMP signaling has been implicated in regulation of muscle contraction, glycogenolysis, and sarcoplasmic calcium dynamics[165]. In adipocytes, cAMP effects lipid metabolism through cAMP dependent protein kinase (PKA)[112]. It is known that activation of cells by insulin inhibits lipolysis. Insulin mediates this process through activation of phosphodiesterases thereby reduction in cAMP levels and reduced PKA activity[112].

SAM domain and HD domain-containing protein 1 (SAMHD1) plays a role in regulation of the innate immune response, upregulated in response to viral infection and may be involved in mediation of tumor necrosis factor-alpha proinflammatory responses. Its interaction with PP2A is decreased in T2D. The exact role of this interaction or this protein in skeletal muscle and diabetes is unknown and yet to be explored.

4.2.6 PARTNERS WITH SIGNIFICANT CHANGE BETWEEN OBESE AND LEAN

63 proteins showed a significant change in obese/overweight insulin resistant controls when compared with lean controls. Among these 63, 59 showed a significant difference between lean basal and obese basal biopsies whereas 56 proteins showed a

significant difference between lean insulin stimulated and obese insulin stimulated biopsies with an overlap of about 50 proteins. Among these 63, only two proteins showed an increased interaction with PP2A while the rest showed a decrease. The two proteins are PDE4D and SCPEP1. The difference in PDE4D is seen in both basal and insulin stimulated biopsies but SCPEP1 presented with an increase only in basal biopsy.

Protein synthesis and degradation

Among these 50 are the proteins involved in proteasome complex machinery. These include proteasome 26S subunit, ATPase 2 (PSMC2), proteasome 26S subunit, ATPase 3 (PSMC3), proteasome 26S subunit, non-ATPase 1(PSMD1), proteasome 26S subunit, non-ATPase 11(PSMD11), proteasome 26S subunit, non-ATPase 12(PSMD12), proteasome 26S subunit, non-ATPase 13(PSMD13), proteasome 26S subunit, non-ATPase 14(PSMD14), and proteasome activator subunit 2(PSME2). It is worthwile to note that the interactions of all these proteins with PP2Ac is decreased in obese group compared to lean (both basal and insulin stimulated biopsies).

chaperonin containing TCP1 subunit 2(CCT2) and chaperonin containing TCP1 subunit 6A(CCT6A) are chaperone proteins. These chaperones correct the partially folded or misfolded proteins using ATP as source of energy[116].

Ribosomal Protein S25 (RPS25), eukaryotic translation initiation factor 2B subunit alpha(EIF2B1), eukaryotic translation initiation factor 3 subunit M (EIF3M), glycyl-tRNA synthetase (GARS), valyl-tRNA synthetase (VARS), and STAT3 are involved in protein synthesis and their interaction with PP2Ac is decreased as well (in obese). STAT3 is a transcription factor which upon activation by cytokines or growth factors will promote transcription of appropriate genes. It was reported that phosphorylated STAT3 amounts are increased by two-fold in overweight T2D compared to over-

weight controls. STAT3 is also shown to contribute to insulin resistance in various tissues like liver and smooth muscle[166]. In our study its interaction with PP2Ac is decreased in both basal and insulin stimulated biopsies of obese insulin resistant group when compared to the corresponding lean biopsies.

Mitochondrial proteins

Among these are mitochondrial proteins which include acyl-CoA dehydrogenase (ACADS & ACADM), acyl-CoA thioesterase 9(ACOT9), glycyl-tRNA synthetase(GARS), hydroxysteroid 17-beta dehydrogenase 8(HSD17B8), and superoxide dismutase 1, soluble(SOD1). Among these Acycl dehydrogenase and hydroxysteroid 17-beta dehydrogenase 8 are involved in fatty acid metabolism.

Membrane proteins

Extracellular proteins like fermitin family homolog 2 (FERMT2), filamin A (FLNA), and lumican (LUM) (explained in 4.2.6) also show significant change in their interactions. Both showed decreased interaction in obese subjects (both basal and insulin stimulated biopsies).

Other proteins

Other important proteins that showed significant change between obese and lean are AKT2 (involved in insulin signaling), PPME1 (demethylation of PP2Ac), CAV1 (caveolae plasma membrane protein), CCDC6. All these proteins are explained in 4.2.2 and show a decreased interaction in obese.

Arginine specific ribosyl transferase (ART3; involved in post translational modification[145]), leucine aminopeptidase (LAP3; an exopeptidase that catalyzes the hydrolysis of leucine residues from the amino-terminus of protein), SUMO-activating enzyme subunit 2 (UBA2; involved in sumoylation, a post translational modification), cAMP-specific 3,5-cyclic phosphodiesterase 4D (PDE4D; regulates cAMP levels in the cell

which is an important second messenger that regulates various cellular processes). All these proteins are explained in detail in 4.2.5. Interaction of ART3, LAP3, and UBA2 with PP2A is decreased in obese whereas interaction of PDE4D is increased.

Aldo-keto reductase family 7 member A2 (AKR7A2; catalyze redox transformations of various substrates including glucose), X-ray repair cross complementing 5 (XRCC5; involved in DNA damage repair), and dipeptidyl peptidase 9 (DPP9; post-proline dipeptidyl peptidase that cleaves dipeptides) are seen with a decreased interaction and are explained later in 4.2.7.

PP2Ac also seems to bind to protein phosphatase 1 regulatory subunit 7 (PPP1R7) and protein phosphatase 4 regulatory subunit 2 (PPP4R2) with a decreased interaction.

4.2.7 PARTNERS WITH SIGNIFICANT CHANGE BETWEEN TYPE 2 DIABETIC AND OBESE

47 proteins presented with a significant change when type 2 diabetic group is compared to obese non-diabetic insulin resistant group. Among 47, 33 proteins are seen with a change in basal biopsies while 40 in insulin stimulated biopsies with 26 shared. Among these 47 proteins, only four proteins showed a decreased interaction in T2D while the rest presented with an increased interaction. These four include translation initiation factor eIF-2B subunit alpha (EIF2B1), cytosol aminopeptidase (LAP3), lumican (LUM), and serine carboxypeptidase 1 (SCPEP1). While the change in interaction with ElF2B1 and LAP3 are seen in only basal biopsies, LUM and SCPEP1 are seen in both basal and insulin stimulated biopsies.

PP2A regulatory subunit A alpha isoform (PPP2R1A) is the only PP2A subunit that showed significant difference among groups and it is only seen to change between

T2D and obese groups. Its interaction with PP2Ac is increased in type 2 diabetes subjects compared to obese/overweight in basal and insulin stimulated biopsies correspondingly.

Protein degradation and synthesis

When compared between obese and T2D, we saw some proteins involved in protein synthesis and degradation as PP2A partners with significant change between those groups. They include, proteins involved in proteasome complex machinery, proteasome 26S subunit, ATPase 2 (PSMC2), proteasome 26S subunit, non-ATPase 1(PSMD1), proteasome 26S subunit, proteasome 26S subunit, non-ATPase 14(PSMD14). In addition to proteasome complexes, we also identified COP9 signalosome complex subunit 2 (COPS2), subunit of the COP9 signalosome complex, involved in the ubiquitin-proteasome pathways. All of them showed an increased interaction in T2D. glycyl-tRNA synthetase(GARS) and valyl-tRNA synthetase (VARS) are involved in protein synthesis which also exhibited increased interaction in T2D.

ATP-dependent Clp protease ATP-binding subunit (CLPX), chaperonin containing TCP1 subunit 2(CCT2) and chaperonin containing TCP1 subunit 6A(CCT6A) are chaperone proteins. CLPX is involved in mitochondrial unfolded protein response (UPR^{mt}). It identifies any unfolded proteins, utilizes cycles of ATP hydrolysis to disrupt its innate structure, and translocates the unfolded protein into ClpP protease for irreversible proteolysis[167]. Experiments were conducted on *in vitro* muscle cells to determine the role of ClpP knock down on mitochondrial function and cellular changes[168]. In addition to the reduced UPR^{mt}, dampened mitochondrial respiration, increased production of reactive oxygen species, and transformed mitochondrial morphology at the level of mitochondria besides the other changes were observed[168]. At the cellular level,

translation inhibition, impaired myoblast differentiation, and cell proliferation were detected[168]. Since CLPX is directly associated with this protease machinery, it would be interesting to know the role of CLPX in the muscle. Because its association with PP2A in T2D is increased compared to obese, it might play a prominent role in the metabolic dysfunction associated with type 2 diabetes. It is also important to note that mitochondrial dysfunction is associated with T2D.

Protein modifications

Proteins involved in protein modifications are as follows: Dipeptidyl peptidase 9(DPP9) belongs to the family of serine peptidases, dipeptidyl peptidase IV. DPP9 acts as a post-proline dipeptidyl peptidase that cleaves Xaa-Pro (Xaa is any amino acid except proline) dipeptides from the N-terminus of proteins[169]. It is highly expressed in skeletal muscle[170] but its role in skeletal muscle or diabetes is unknown. It would be interesting to learn because DPP4 (other protein that belongs to this dipeptidyl peptidase IV family) inhibitors are used to treat type 2 diabetes[171]. Substrates of DPP4 include glucagon-like-peptides 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP)13[169]. These two proteins promote almost 60% of postprandial insulin secretion[172]. DPP4 inhibition thereby extends the activity of GLP and GIP with improved insulin secretion and blood glucose regulation. Another protein involved in protein modification is the serine carboxypeptidase 1(SCPEP1), that can break the peptide bond at c-terminal residues of proteins. SCPEP1 showed a decreased interaction with PP2Ac in T2D.

Metabolic processes

Proteins involved in various metabolic processes like pentose phosphate pathway, polyol pathway, and anti-oxidant mechanisms are seen as PP2A partners and with

a change between obese and T2D. Glucose is converted to glucose-6-phosphate in glycolysis. This glucose-6-phosphate, undergoes pentose phosphate pathway besides citric acid acid cycle and electron transport chain. Pentose phosphate pathway yields NADPH (maintain glutathione at a reduced state) and pentoses (precursor for synthesis of DNA nucleotides). Transaldolase 1 (TALDO1) is an enzyme involved in this pentose phosphate pathway and is seen as a partner of PP2A with increased interaction in T2D. We saw TALDO1 as PP2A partner in beta cells as well[92].

Aldo-keto reductase family 1 member B (AKR1B1) and aldo-keto reductase family 7 member A2 (AKR7A2) belong to aldo-reductase family, catalyze redox transformations of various substrates including glucose[173]. The role of AKR1B1 (aldose reductase) in hyperglycemia associated injury is widely studied[174-177] considering the fact that it catalyzes the reduction of glucose to sorbitol in a NADPH + H^+ dependent manner. Glucose, in addition to glycolysis is also metabolized through polyol pathway. The polyol pathway involves conversion to glucose to sorbitol by aldose reductase and subsequent conversion to fructose by sorbitol dehydrogenase. Under hyperglycemic conditions associated with diabetes, the high amounts of the glucose are converted to sorbitol. In some tissues like retina, nerve cells, and kidney, which lack enzyme sorbitol dehydrogenase, sorbitol gets accumulated. This accumulation of sorbitol is shown to be responsible for diabetic complications like retinopathy[177], neuropathy[174,176], and others. Though skeletal muscle has the enzyme sorbitol dehydrogenase, use of aldose reductase inhibitors is shown to improve contractile function in skeletal muscle of diabetic rats[178]. We are the first to show an interaction of PP2Ac with AKR1B1 and its increased interaction in type 2 diabetes compared to obese/overweight. We also identified this protein in beta cells however with no change under hyperglycemic conditions. Other aldo-keto reductase, AKR7A2, is a aflatoxin reductase which is mainly involved in conversion of

succinic semialdehyde (SSA) to γ-hydroxybutyrate (GHB)[173]. However, its role in skeletal muscle is not known.

Oxidative damage in the cells is caused by imbalance between production of reactive oxygen species and the capacity of the cell to neutralize these. This antioxidant mechanisms include enzymes such as superoxide dismutase and glutathione S-transferase among others. Here, in our study we saw superoxide dismutase 1 (SOD1) and glutathione S-transferase mu 3 (GSTM3) as PP2A partners with an increased interaction in T2D. Superoxide dismutase 1 (SOD1), converts superoxide anions to hydrogen peroxide and oxygen, reducing reactive oxygen species in the cell. This is mainly found in the cytoplasm of the cell. Studies have shown that deletion of SOD1 gene in mice led to significant, age-dependent loss of muscle mass which was specific to skeletal muscle[179]. These mice also presented with high amounts of oxidative damage in skeletal muscle, particularly in older animals[179]. However, knockout of SOD from only skeletal muscle showed no significant changes in the muscle mass or reactive oxygen species (ROS) production[180]. But, there was enhanced Akt-mTOR signaling and increased number of muscle fibers with centrally located nuclei in skeletal muscle, which suggests elevated regenerative pathways or muscle weakness[180]. This is important because it is clearly indicated that in patients with diabetes, oxidative stress (due to high glucose concentrations) is evident and that some complications of diabetes involve oxidative stress among other reasons[181]. Reciprocally, oxidative stress is also shown to be one of the causes of insulin resistance in type 2 diabetes[181]. Macromolecules such as molecules of extracellular matrix, lipoproteins and deoxyribonucleic acid are also damaged by free radicals in diabetes mellitus[181]. To counteract this damage to cell membrane proteins, especially lipids, Glutathione is present in the cells. glutathione S-transferase functions by conjugating glutathione to detoxify these compounds[182]. Further studying

the role of these antioxidant enzymes, glutathione S-transferase mu 3 (GSTM3) and Superoxide dismutase 1 (SOD1) in association with PP2A in skeletal muscle might be significant considering their importance in diabetes.

Other proteins

Other proteins involved in other important cellular processes are also seen which are as follows:

We saw increased interaction of PP2A with X-ray repair cross complementing 5 (XRCC5), a 80-kilodalton subunit of the Ku heterodimer protein (ATP-dependant DNA helicase II) which is involved in DNA damage repair[183].

Interaction of Lumican (LUM), a proteoglycan (component in the extracellular matrix; explained in 4.2.6), is decreased in T2D.

cAMP-specific 3,5-cyclic phosphodiesterase 4D (PDE4D), involved in hydrolysis of cAMP is seen with increased interaction in T2D. PDE4D regulates cAMP levels[165] (explained in 4.2.6).

4.3 SUMMARY AND FUTURE DIRECTIONS

PP2A is one of the important serine/threonine phosphatase involved in many cellular functions. It's localization and function is regulated by different ways. They include, binding of different regulatory B subunits, post translational modifications, and binding to different substrates. The activity of PP2A is altered in glucotoxic conditions in beta cells and it is also shown to be effected by insulin in skeletal muscle cells thus playing a vital role in diabetes. Considering its complex regulation and its prime role in diabetes, we studied the interactions of PP2A in beta islet cells and skeletal muscle, tissues that significantly contribute to glucose metabolism. Using high throughput proteomics approach, we identified 516 interaction partners of PP2Ac in INS-1 832/13

beta cells and 211 interactions in human skeletal muscle biopsies. In beta cells, 89 proteins showed a significant change in interaction with PP2Ac under hyperglycemic conditions. Similarly, 69 proteins showed a significant difference in interacting with PP2Ac when compared among lean, obese/overweight and type 2 diabetic group. To be more precise, 63 proteins presented a significant change between obese/overweight and lean group, 37 proteins between type 2 diabetics and lean, and 47 proteins between type 2 diabetic and obese group. This is the largest PP2Ac interactome till date. These interactions helped us understand the role and regulation of PP2A in beta cells and skeletal muscle. In addition, the 37 proteins that showed a significant difference between lean and type 2 diabetic human skeletal muscle biopsies further advances our understanding of the role of PP2A in type 2 diabetes in humans. Similarly, analyzing 89 glucose stimulated interactions unveiled on the function of PP2A in insulin secretion and production. In depth analysis of the the altered interactions can provide with a target to correct either hyperglycemia induced beta cell death or metabolic dysfunctions in type 2 diabetes.

FIGURES

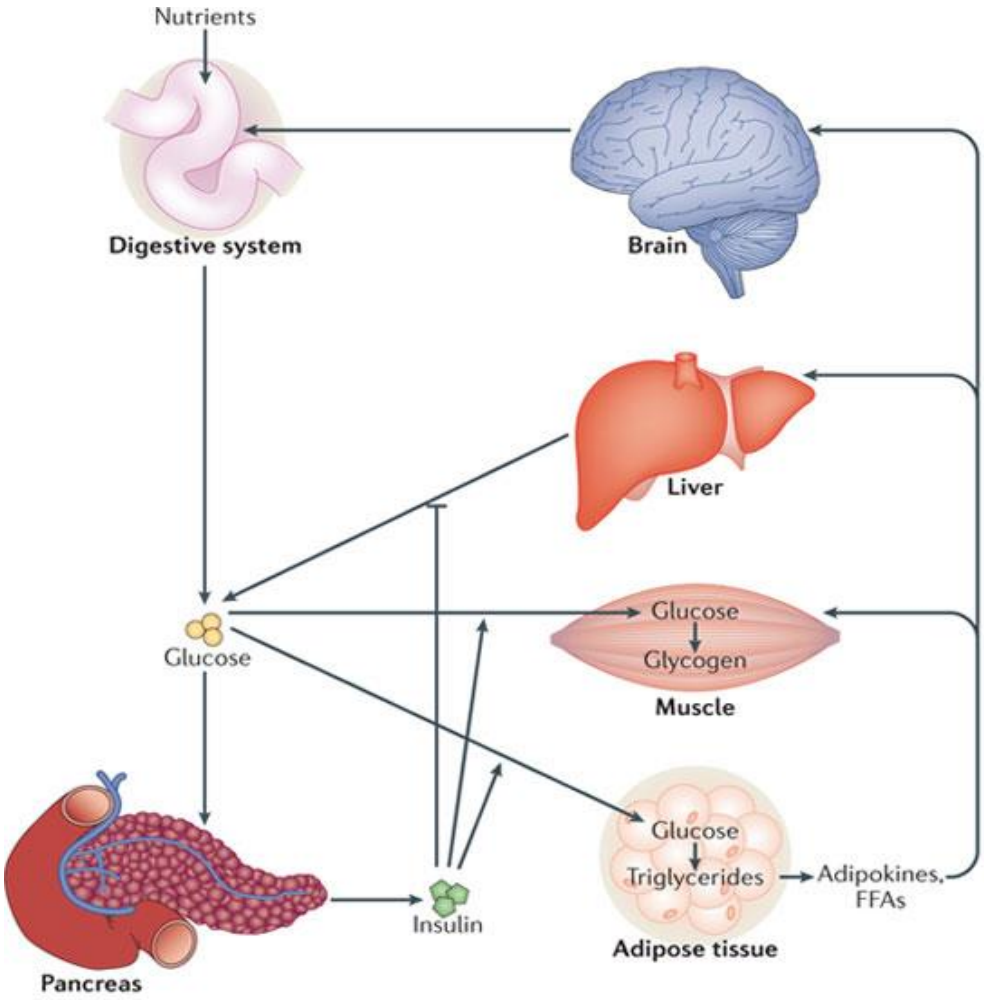

Figure 1. Glucose homeostasis involving major tissues[12]

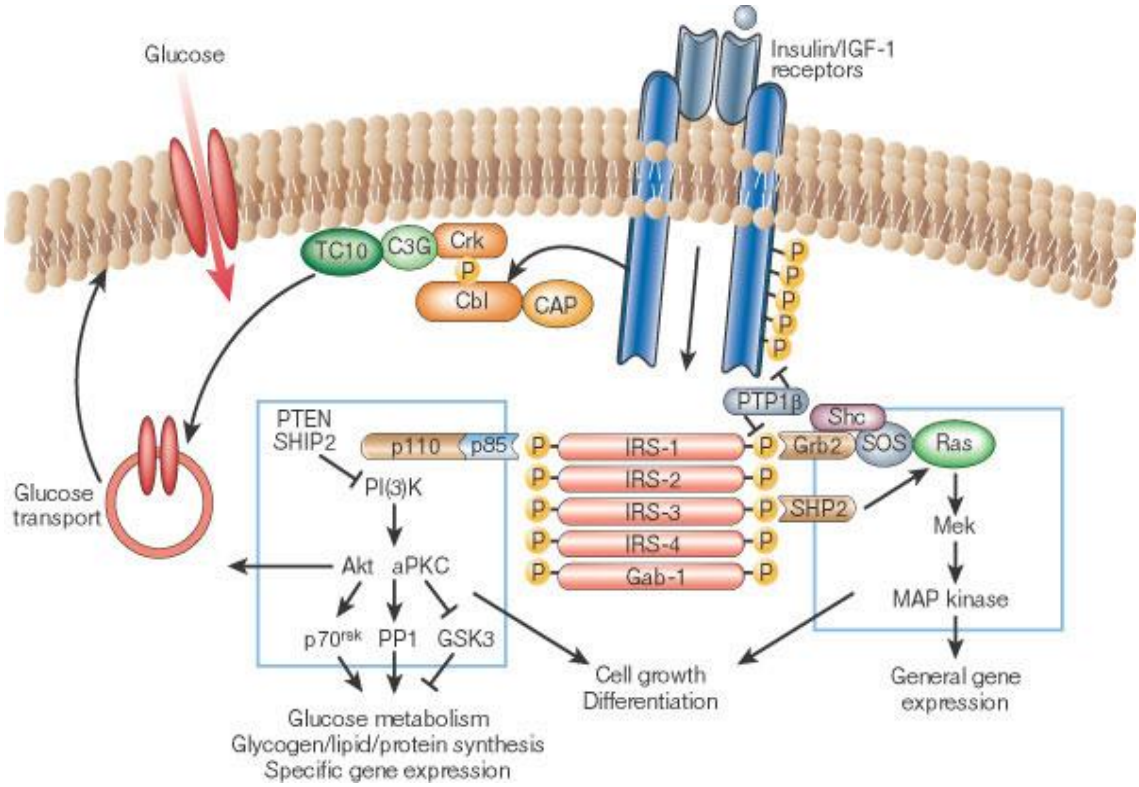

Figure 2. Insulin signaling pathway showing the signaling molecules involved and various effects seen[16]

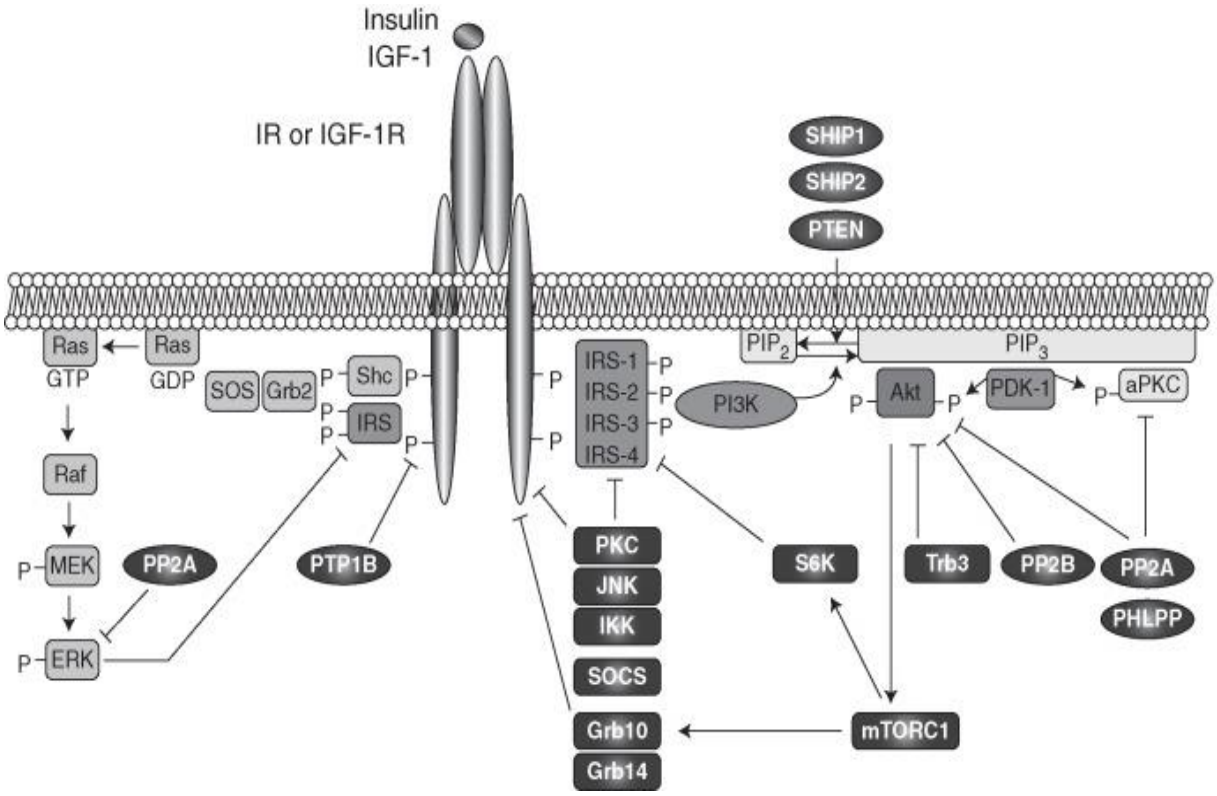

Figure 3. Negative regulators of insulin signaling pathway[14]

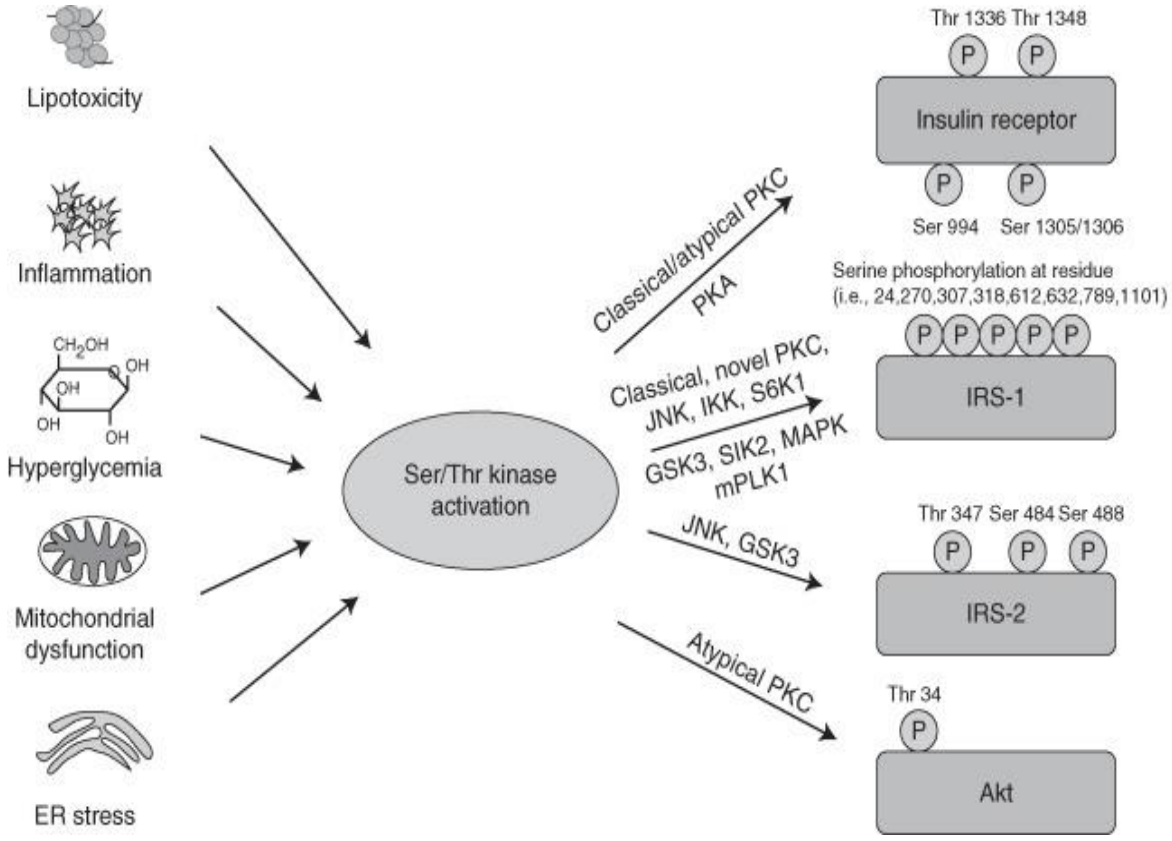

Figure 4. Insulin signaling regulation by inhibitory serine/threonine phosphorylation[14]

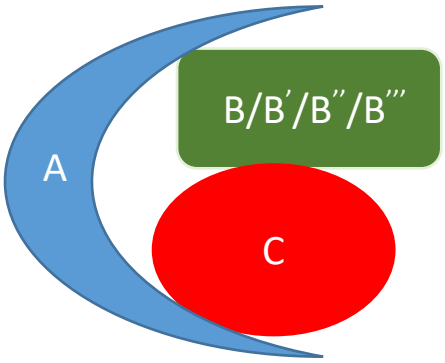

Figure 5. Diagrammatic representation of heterotrimeric PP2A complex

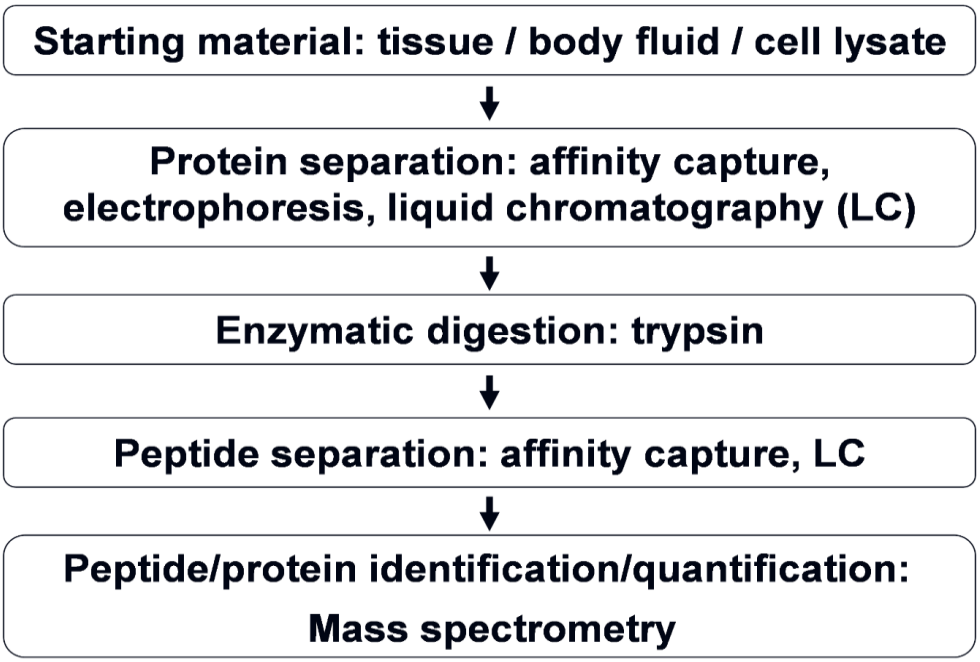

Figure 6. Main steps in mass spectrometry-based proteomics studies

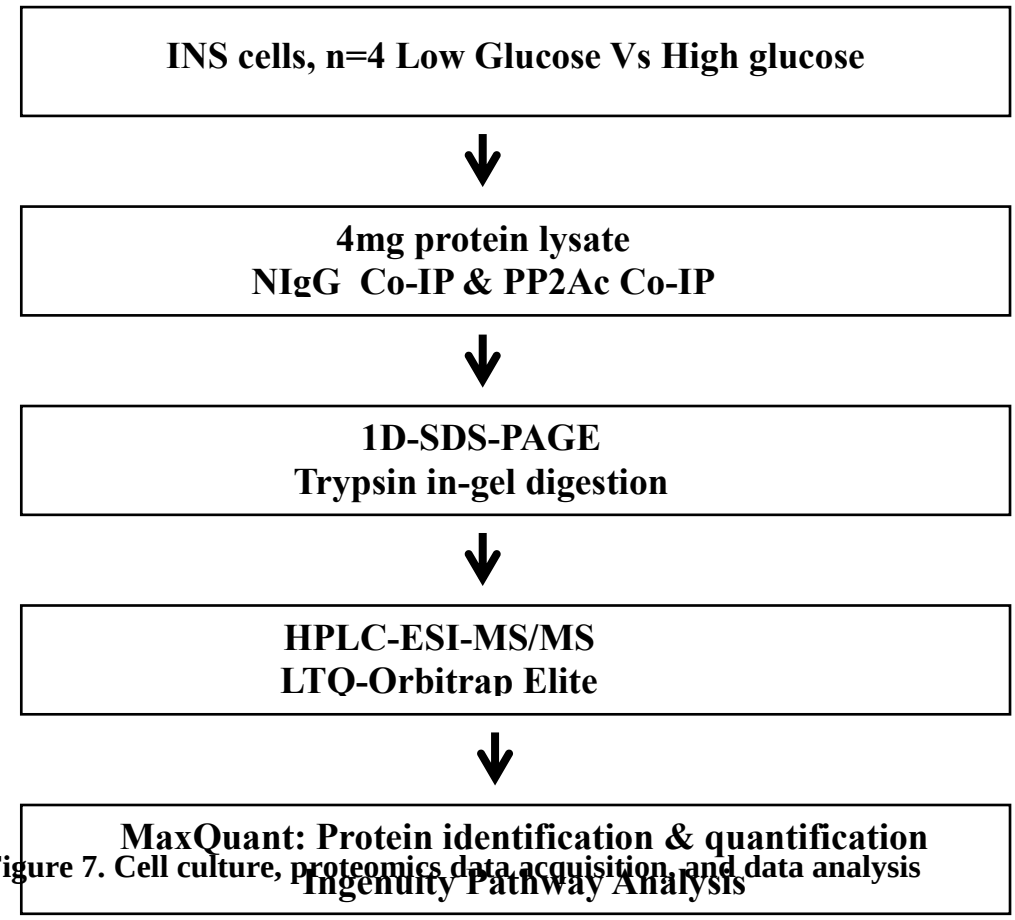

Figure 7. Cell culture, proteomics data acquisition, and data analysis

Proteins identified with minimum 2 unique peptides with FDR at 0.01 in at least one PP2Ac IP?
(1131 proteins)

Figure 8. Proteomic data analysis (INS-1 832/13 CELLS)

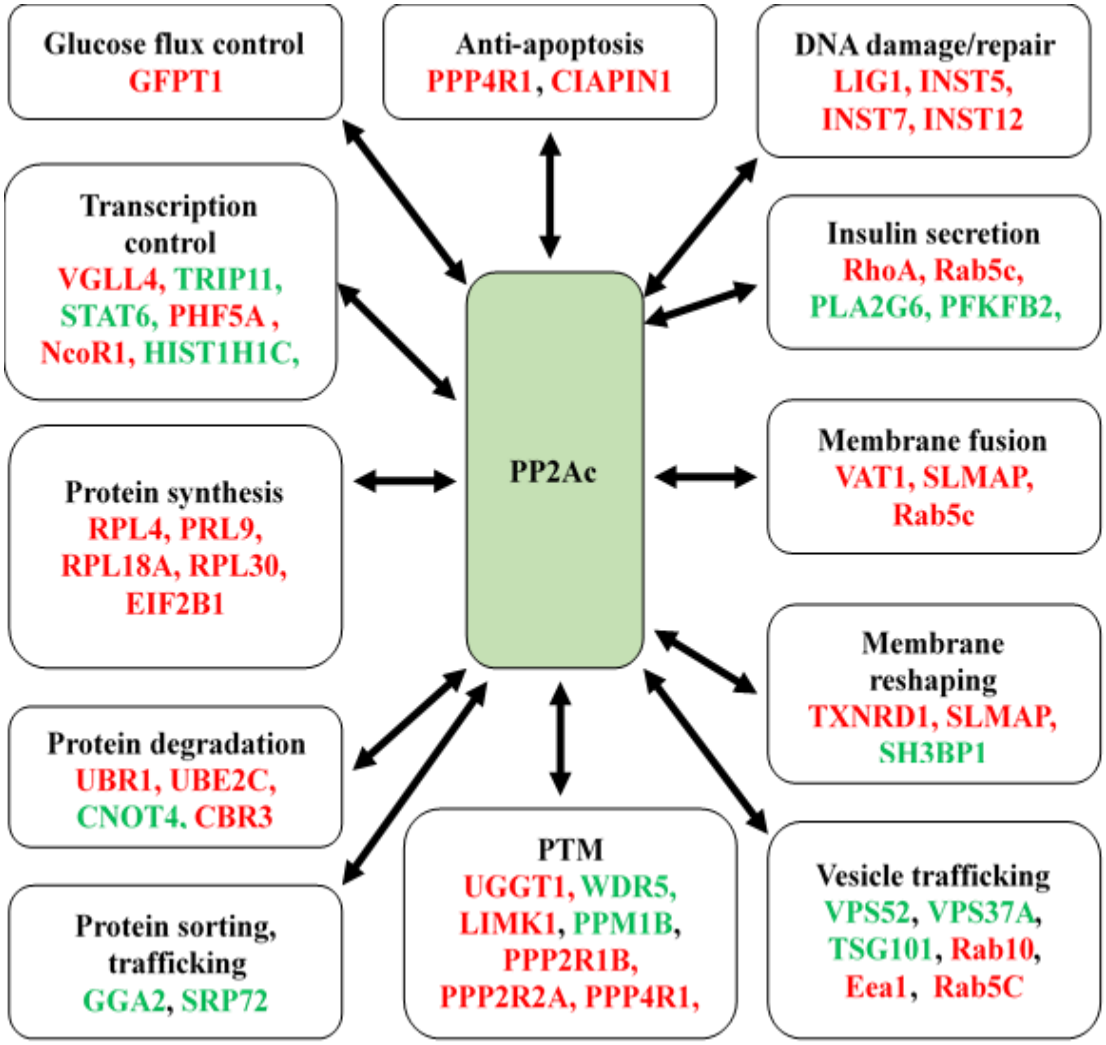

Figure 9. Summary of glucose-responsive PP2Ac interaction partners. In response to high glucose treatment, the proteins with increased PP2Ac association are highlighted in red, and the ones with decreased association are highlighted in green

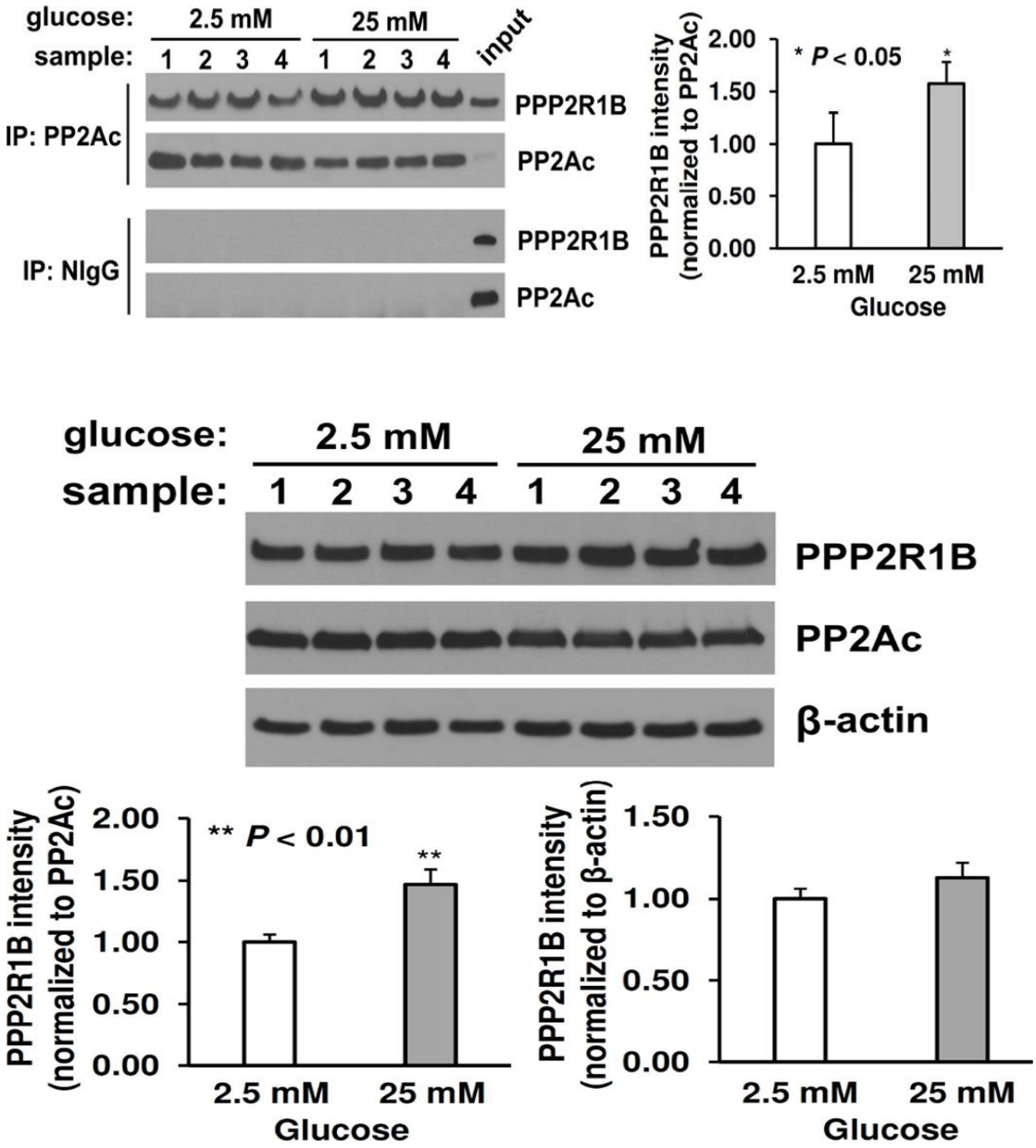

Figure 10. Experimental validation of PPP2R1B as a glucose responsive PP2Ac interaction partner

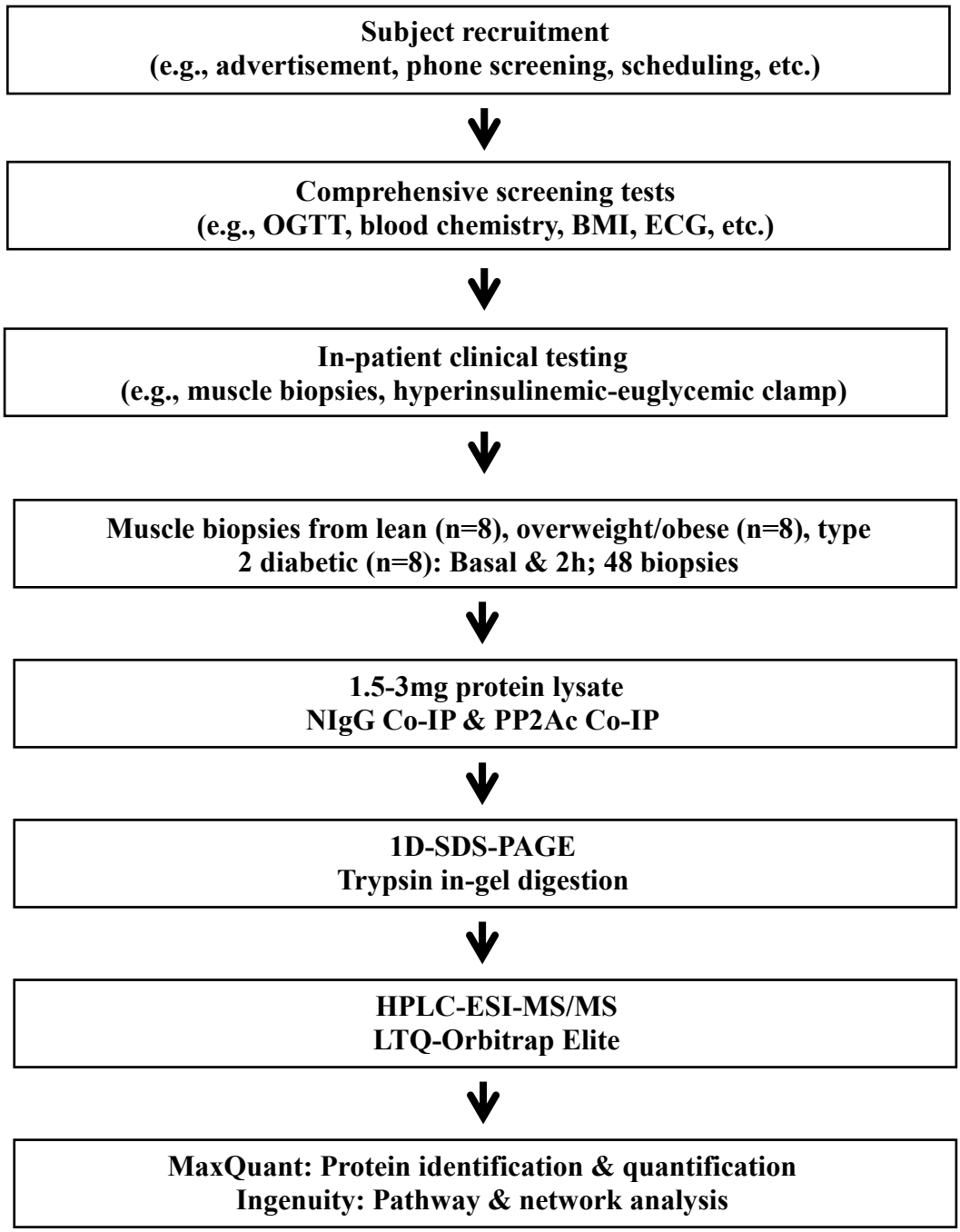

Figure 11. Clinical and proteomics data acquisition and data analysis

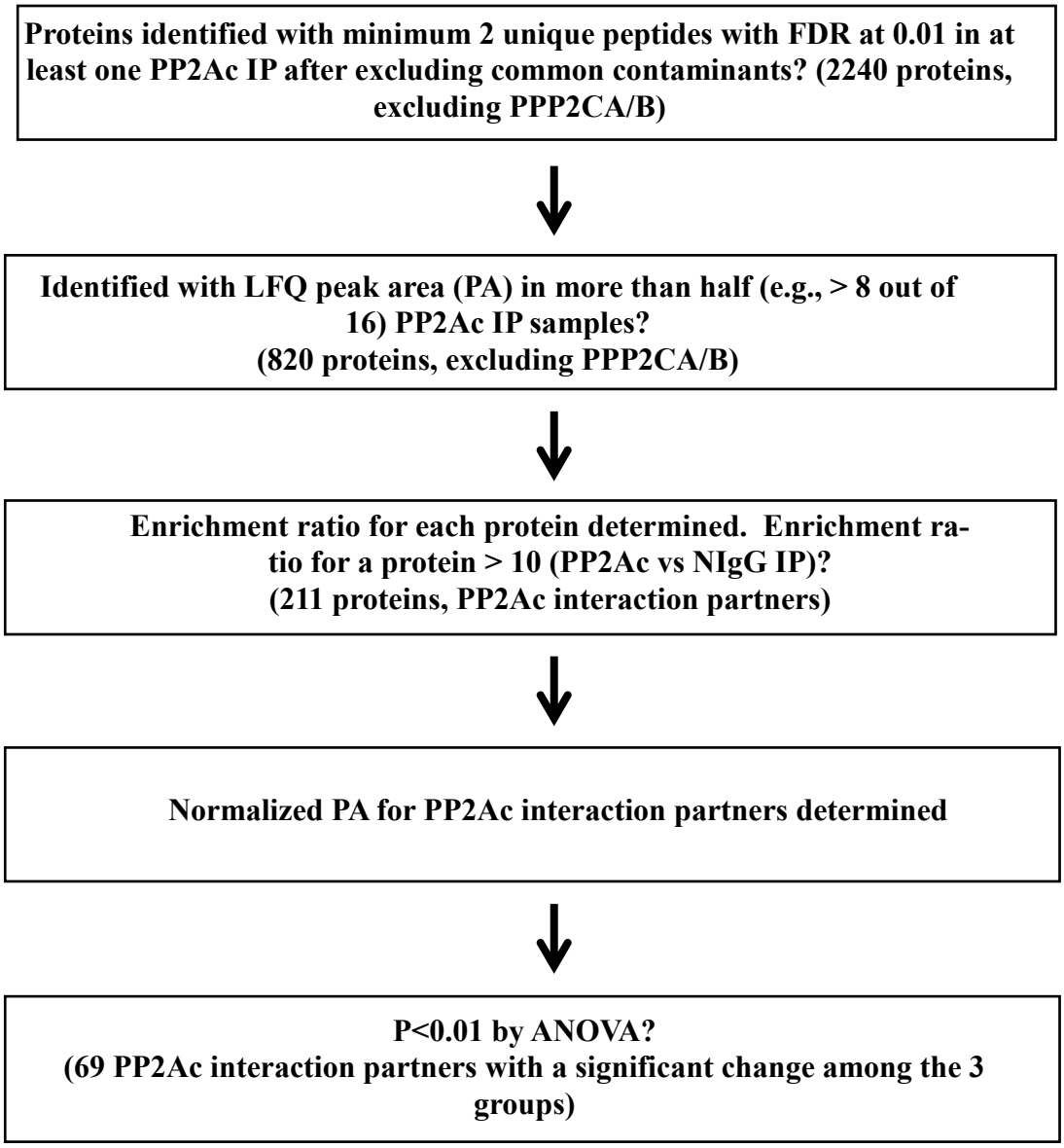

Figure 12. Proteomic data analysis (Human skeletal muscle)

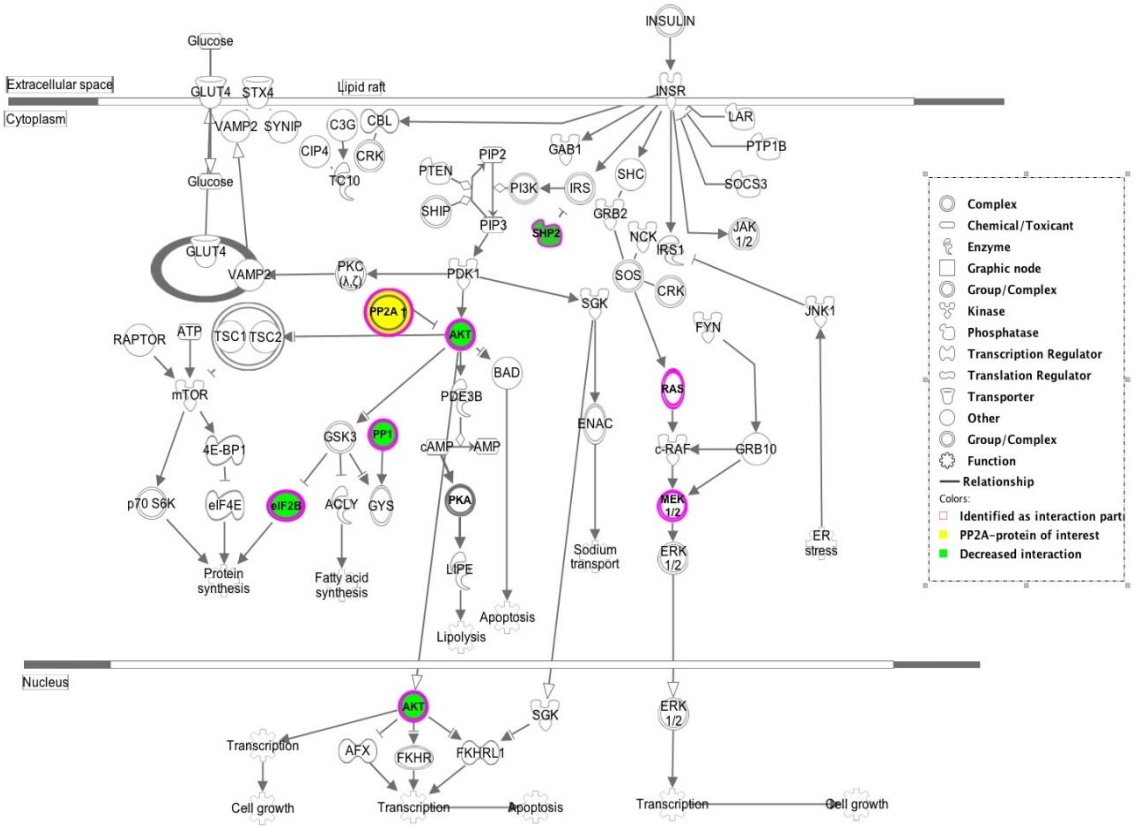

Figure 13. The significantly enriched pathway, Insulin Receptor signaling, for the 211 PP2Ac interaction partners and PP2Ac in human skeletal muscle. PP2Ac interaction partners were highlighted in purple; partners with increased interaction between groups was indicated in green; PP2A was highlighted in yellow

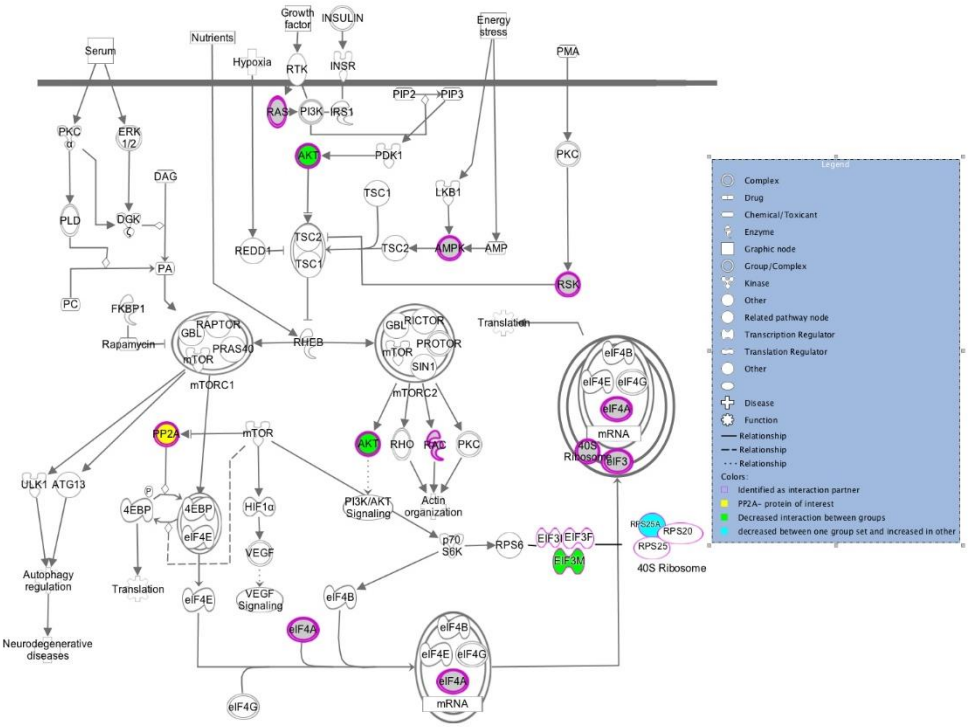

Figure 14. The significantly enriched pathway, mTOR signaling, for the 211 PP2Ac interaction partners and PP2Ac in human skeletal muscle. PP2Ac interaction partners were highlighted in purple; partners with increased interaction between groups was indicated in green; partners with increased interaction between one group set and decreased in other group set was indicated in blue; PP2A was highlighted in yellow

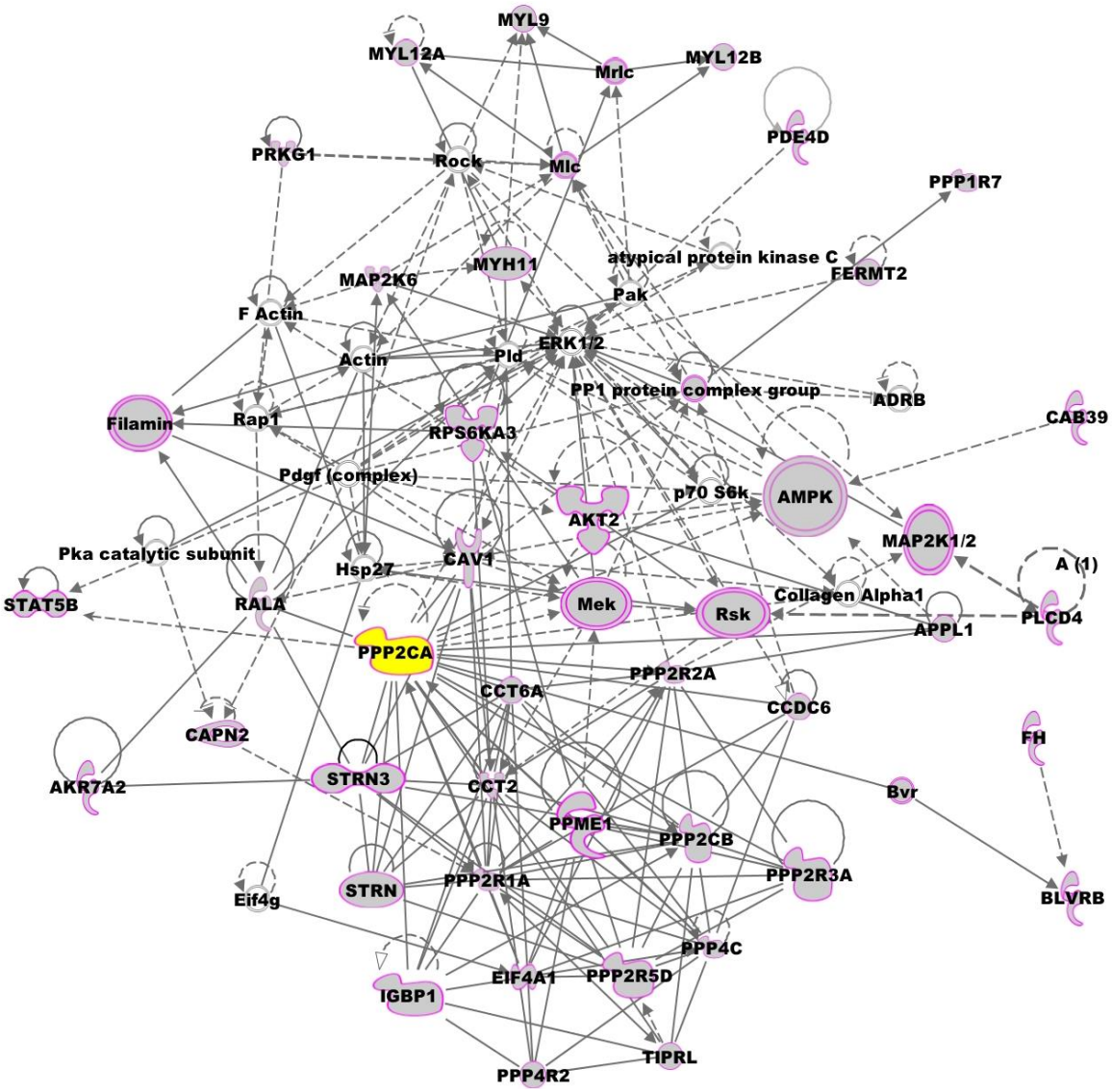

Figure 15. Network pathway obtained from Ingenuity Pathway Analysis. Pathway obtained using 70 molecules per network and the one assigned with highest score is taken; shows 45 interaction partners in human skeletal muscle; target protein PP2Ac (in yellow) and its interaction partners (in purple)

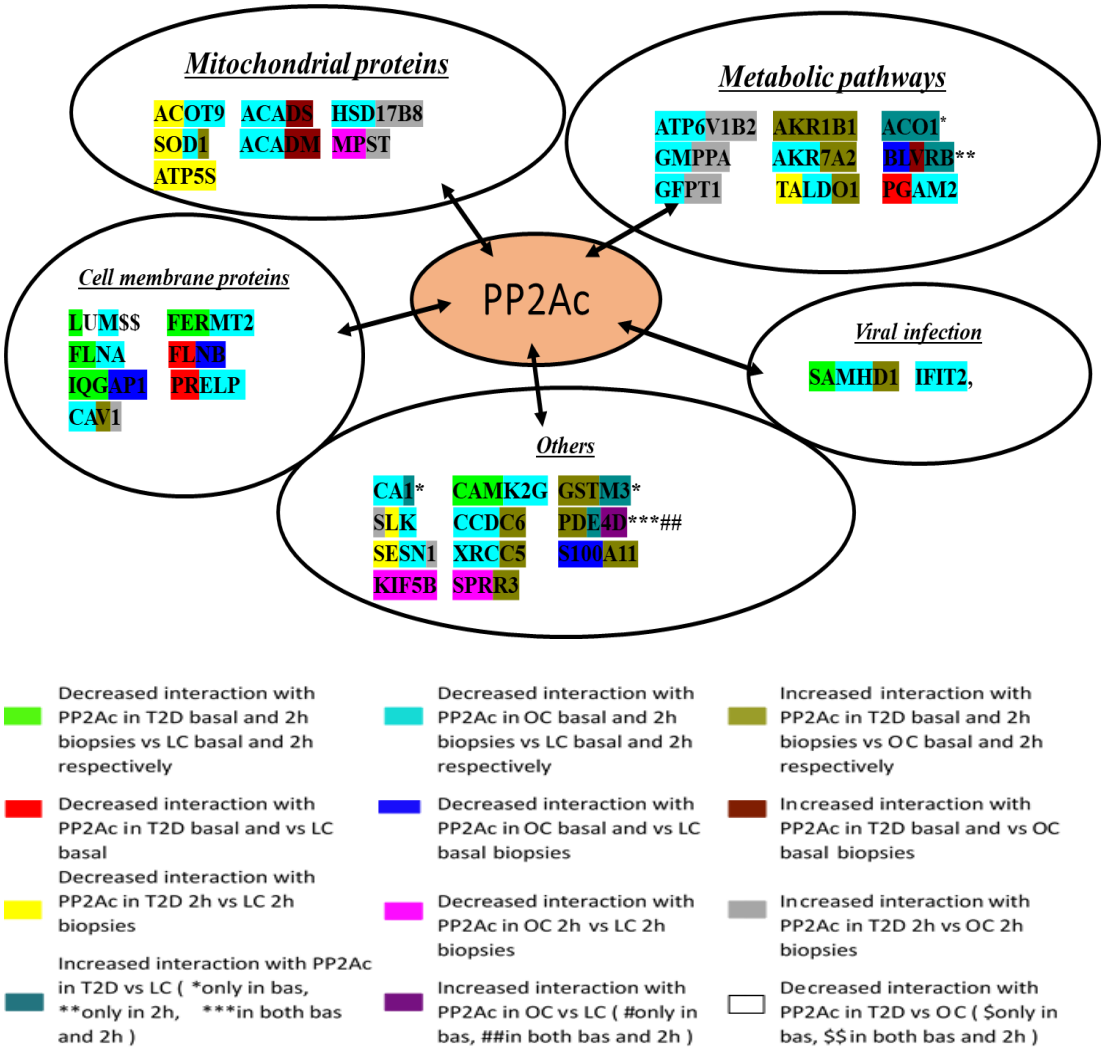

Figure 16A. 69 proteins PP2Ac partners in human skeletal muscle with significant change among different groups (color coded)

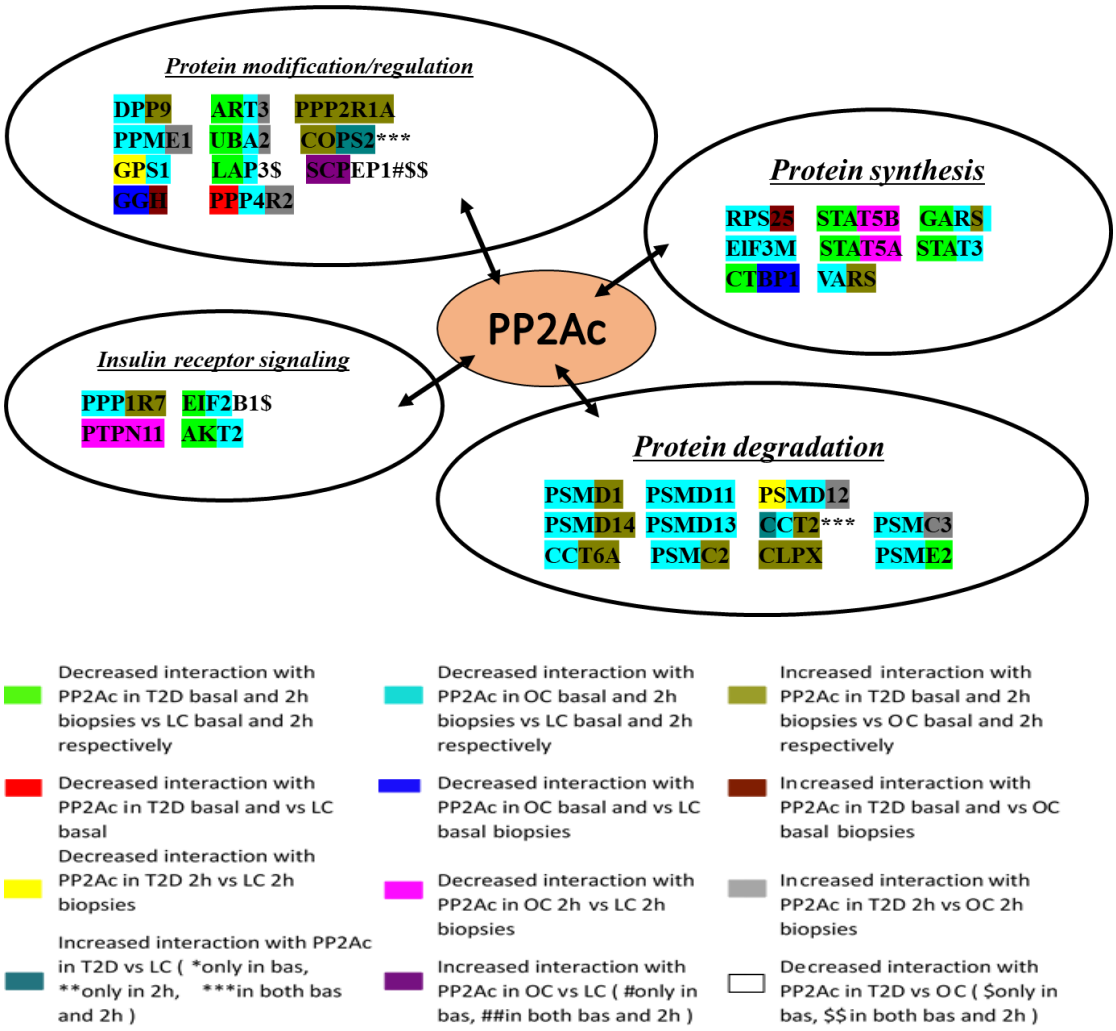

Figure 16B. 69 proteins PP2Ac partners in human skeletal muscle with significant change among different groups (color coded)

TABLES

Table 1. Various isoforms of PP2A subunits, their cellular and sub-cellular distribution[45]. HE: high expression

Subunit	Gene	Isoform	Other name	Normal tissue distribution	Subcellular distribution
Scaffold (A)	PPP2R1A	A	PR65α, PP2A-Aα	Ubiquitously expressed and highly expressed in ovary (oogenesis)	Cytosol
	PPP2R1B	B	PR65β, PP2A-Aβ	Ubiquitously expressed and highly expressed in ovary (oogenesis)	Cytosol
Catalytic (C)	PPP2CA	A	PP2Acα	Brain and heart (HE). Present in skeletal muscle[66]	Cytoplasm and nucleus
	PPP2CB	B	PP2Acβ	Brain and heart (HE)	Cytoplasm and nucleus
Regulatory (B)	PPP2R2A	A	PR55α, PP2ABα	Widely distributed in all tissues	Membranes, cytoplasm, microtubules nucleus.

					Golgi complex, endoplasmic reticulum and neurofilaments
	PPP2R2B	B	PR55β, PP2ABβ	Brain and testis (HE)	Cytosol
	PPP2R2C	Γ	PR55γ, PP2ABγ	Brain (SE)	Mainly in Cytoskeletal fraction
	PPP2R2D	Δ	PR55δ, PP2ABδ	Wide spread distribution in tissues, Testis (HE)	Cytosol
Regulatory (B′)	PPP2R5A	A	PR56/61α, PP2AB′α	Cardiac tissues and skeletal muscles[184] (HE)	Cytoplasm
	PPP2R5B	B	PR56/61β, PP2AB′β	Brain (HE)	Cytoplasm
	PPP2R5C	Γ1, 2,3	PR56/61γ, PP2AB′γ	Cardiac tissues and skeletal muscles[185] (HE)	Cytoplasm and nucleus

	PPP2R5D	Δ	PR56/61δ, PP2AB′δ	Primarily exist in brain	Cytoplasm, nucleus, mitochondria, microsomes
	PPP2R5E	E	PR56/61ε, PP2AB′ε	Primarily exist in brain	Cytoplasm
Regulatory (B″)	PPP2R3A	A	PR130, B″α1	Brain (HE), heart, lung, kidney and muscle[186]	Centrosome and Golgi complex
	PPP2R3A	A	PR72, B″α2	Heart (HE) and skeletal muscle[186]	Cytosol and nucleus
	PPP2R3B	B	PR70, PR48, B″β	Placenta	Nucleus
	PPP2R3C	Γ	G5PR, G4-1	During developmental process expressed in fetal brain	Nucleus
	PPP2R3D	Δ	PR59, B″δ	Cardiac tissue, kidney and lungs	Nucleus
Regulatory (B‴)	STRN		Striatin, PR110	Brain	Membrane and cytoplasm
	STRN3		SG2NA	Neurons	Nucleus
	PPP2R4		PTPA, PR53	Widely expressed	Cytosol, nucleus

Table 2. Various inhibitors of PP2A including their sources and specificity to different phosphatases _ENREF_41[41].

Inhibitor	Source	Inhibitory potency
Okadaic acid	*Dinoflagellates*	PP2A ~ PP4 > PP1 ~ PP5 >>> PP2B*
Dinophysistoxin-1	*Dinoflagellates*	PP2A > PP1 >>> PP2B
Microcystins	Cyanobacteria	PP2A ~ PP1 >>> PP2B
Nodularins/Motuporin	Cyanobacteria	PP2A ~ PP1 >>> PP2B
Calyculin A	Isolated from marine sponges	PP2A > PP1 >>> PP2B
Tautomycin	*Streptomyces spiroventricillatus*	PP1 > PP2A >>> PP2B
Cantharidin	Blister beetles	PP2A > PP1 >>> PP2B
Endothall	Synthetic compound	PP2A > PP1 >>> PP2B
Fostriecin	*Streptomyces pulveraceus*subsp. *Fostreus*	PP2A ~ PP4*
TF-23A	Isolated from marine red alga	PP2A
Cytostatin	*Streptomyces sp*. MJ654-NF4	PP2A
I_1^{PP2A}	Cellular inhibitor	PP2A
I_2^{PP2A} (SET, PHAP-II, TAF-1β)	Cellular inhibitor	PP2A

Table 3. Clinical characteristics for the 8 lean, 8 overweight/obese, and 8 type 2 diabetic participants in the PP2Ac interaction partner study. Results were shown as mean ± SEM.

GROUP	ID	Gender	BMI at V2	Age	2h OGTT	M value mg/kgBW/min average of last 30 m	HBA1c	Fasting plasma glucose (mmol/l)
OC/OW	106PAT	M	27.5	37.0	141.5	7.7	5.6	89.0
OC/OW	237MWS	M	29.5	36.0	81.5	6.9	5.7	105.0
OC/OW	281TEJ	F	29.2	42.0	91.0	4.6	5.4	90.4
OC/OW	274GRD	M	26.7	40.0	141.0	4.0	5.5	90.1
OC/OW	115D-G	F	36.7	48.0	132.0	3.9	5.4	90.0
OC/OW	199JMA	F	33.1	29.0	109.5	5.5	5.2	77.8
OC/OW	330GDS	F	35.7	57.0	113.5	5.4	6.0	86.7
OC/OW	354JMG	M	32.5	47.0	111.5	8.0	5.3	108.0
LC	217MCB	M	22.7	30.0	97.7	7.4	5.4	84.4
LC	242DDR	M	24.7	28.0	128.0	9.0	4.7	87.7
LC	341S-D	M	25.0	59.0	87.8	6.6	5.6	92.4
LC	407NJZ	F	24.0	30.0	126.5	13.0	5.4	91.2
LC	418KMN	F	23.6	26.0	134.5	9.6	5.2	91.1
LC	482D-S	F	23.1	65.0	114.0	10.0	5.3	93.2
LC	506E-J	M	24.5	44.0	102.0	13.1	5.4	90.9
LC	360JMS	F	24.2	33.0	95.1	15.4	4.7	83.6
T2D	292TJ	F	25.4	59.0	NA	6.5	9.0	201.2
T2D	423I_C	M	34.8	37.0	NA	0.5	7.3	143.0
T2D	640 YAN	M	32.5	49.0	NA	3.6	6.9	134.6
T2D	699N-M	F	36.2	19.0	NA	2.5	0.0	113.6
T2D	304-Rb	F	28.0	57.0	NA	2.0	7.6	153.0
T2D	337NMS	F	32.3	41.0	NA	6.1	10.6	83.6
T2D	359-EBK	M	39.1	44.0	NA	2.0	6.4	100.6
T2D	322BRM	F	29.2	51.0	NA	5.0	6.5	165.6

Table 4. Clinical characteristics for the 8 lean, 8 overweight/obese, and 8 type 2 diabetic participants in the PP2Ac interaction partner study. Results were shown as mean ± SEM.

	LC	OC/OW	T2D
Gender (M/F)	(4/4)	(4/4)	(3/5)
Age (years)	39.3±5.32	42.0 ± 3.0	44.6±4.5
BMI (kg/m^2)	23.9±0.26(<25)	31.3 ± 1.3 (>25)	32.1±1.5
2h OGTT Glucose (mg/dl)	110.6±5.78	115.2 ± 7.8 (<140)	N/A
HBA1c (%)	5.2±0.1	5.5 ± 0.1 (<5.7)	6.7±1.0(>6.5)
Fasting plasma glucose (mg/dl)	89.3±1.20	92.1 ± 3.5 (<100)	136.8±13.3(>126)
M-value (mg/kg/min)	10.5±1.0	5.8 ± 0.6	3.5±0.7

Table 5. The 516 proteins identified as interaction partners in INS-1 832/13 cells.

Gene name	Protein ID	Protein name	MW [kDa]	Sequence length	Number of unique peptides detected in the PP2Ac IP	Enrichment ratio
Acta2	P62738	Actin, aortic smooth muscle	42.009	377	2	Infinite[a]
Actr2	Q5M7U6	Actin-related protein 2	44.733	394	3	Infinite
Actr3	F1LRA3	Actin-related protein 3	47.285	418	2	Infinite
Add3	Q62847	Gamma-adducin	78.803	705	2	Infinite
Ahcy	P10760	Adenosylhomocysteinase	47.538	432	3	Infinite
Ahcyl2	D3ZWL6	Adenosylhomocysteinase	66.498	613	2	Infinite
Aimp2	Q32PX2	Aminoacyl tRNA synthase complex-interacting multifunctional protein 2	35.442	320	3	12.2
Aip	Q5FWY5	AH receptor-interacting protein	37.598	330	10	Infinite
Akap10	F1LNB3	Protein Akap10	73.773	662	4	Infinite
Akr1b1	P07943	Aldose reductase	35.797	316	2	Infinite
Aldoa	G3V900	Fructose-bisphosphate aldolase	45.086	418	3	Infinite
Amz2	F1LPX7	Archaemetzincin-2	41.703	362	5	Infinite
Ankhd1	E9PTK9	Protein Ankhd1	269.26	2540	2	Infinite
Ankle2	Q7TP65	Ankyrin repeat and LEM do-	106.44	964	31	Infinite

		main-containing protein 2				
Anks1a	D4AC12	Ankyrin repeat and SAM domain containing 1 (Predicted)	122.1	1125	16	12.4
Anxa1	D3ZVZ4	Annexin	43.592	385	7	Infinite
Anxa4	P55260	Annexin A4	35.848	319	2	Infinite
Api5	B1WC49	Api5 protein	56.784	504	9	Infinite
Apip	D3ZUI1	APAF1 interacting protein (Predicted), isoform CRA_a	27.053	241	8	Infinite
Appl1	D3ZWA8	Protein Appl1	79.363	707	3	Infinite
Arcn1	Q66H80	Coatomer subunit delta	57.199	511	2	Infinite
Arfip1	D3ZNX6	Arfaptin-1	38.322	341	4	Infinite
Arfip2	Q6AY65	Arfaptin-2	37.772	341	3	Infinite
Arg1	P07824	Arginase-1	34.973	323	2	Infinite
Arhgap1	D3ZLP8	Protein Arhgap1	54.606	478	5	Infinite
Arhgef11	D3ZZE7	Rho guanine nucleotide exchange factor 11	173.04	1565	3	Infinite
Arhgef7	F1LNB0	Rho guanine nucleotide exchange factor 7	97.165	862	4	Infinite
Arl6	D3ZIB8	Protein Arl6	21.786	193	2	Infinite
Armcx3	Q5XID7	Armadillo repeat-containing X-linked protein 3	42.552	379	3	Infinite
Arpc4	B2RZ72	Actin related protein 2/3 complex, subunit 4 (Predicted), isoform CRA_a	19.667	168	2	Infinite

Asap2	F1M7E9	Protein Asap2 (Fragment)	107.52	967	2	Infinite
Ascc3	F1LPQ2	Activating signal cointegrator 1 complex subunit 3	250.22	2197	3	Infinite
Asna1	D3ZD98	Protein Asna1	38.993	350	3	Infinite
Atox1	Q9WUC4	Copper transport protein ATOX1	7.2924	68	3	Infinite
Atp5a1	F1LP05	ATP synthase subunit alpha	59.812	553	4	Infinite
Atp5b	G3V6D3	ATP synthase subunit beta	56.344	529	8	20.0
Atp6v1a	D4A133	Protein Atp6v1a	68.264	617	3	Infinite
Atp6v1h	D3ZW96	Protein Atp6v1h	55.868	483	2	Infinite
Atxn10	Q9ER24	Ataxin-10	53.726	475	13	43.0
Atxn2	F1M049	Protein Atxn2 (Fragment)	103.98	966	6	Infinite
Bax	G3V8T9	Apoptosis regulator BAX	21.444	192	3	Infinite
Blmh	A1A5L1	Bleomycin hydrolase	52.451	455	3	Infinite
Btaf1	F1LW16	Protein Btaf1 (Fragment)	207.12	1848	2	Infinite
Calm2	D4A5H3	Uncharacterized protein	16.827	149	2	Infinite
Calr	P18418	Calreticulin	47.995	416	3	Infinite
Camk1	Q63450	Calcium/calmodulin-dependent protein kinase type 1	41.638	374	2	Infinite
Camk2b	F1LNI8	Calcium/calmodulin-dependent protein kinase type II subunit beta	65.034	589	11	Infinite

Cand1	P97536	Cullin-associated NEDD8-dissociated protein 1	136.36	1230	8	Infinite
Cand2	G3V7E8	Cullin-associated NEDD8-dissociated protein 2	139.72	1273	6	Infinite
Capn1	F1LS29	Calpain-1 catalytic subunit	82.099	713	2	Infinite
Capza1	F8WFI5	F-actin-capping protein subunit alpha-1	33.197	289	3	Infinite
Capza2	Q3T1K5	F-actin-capping protein subunit alpha-2	32.967	286	2	Infinite
Cat	P04762	Catalase	59.756	527	3	Infinite
Cbr3	B2GV72	Carbonyl reductase 3	30.841	277	3	Infinite
Cc2d1a	F1LQC6	Coiled-coil and C2 domain-containing protein 1A	103.71	942	3	Infinite
Ccdc6	D4AEK9	Protein Ccdc6	52.973	470	38	Infinite
Ccdc88a	D3ZYD7	Protein Ccdc88a	215.86	1874	4	Infinite
Ccdc88b	D3ZSB7	Ribosomal protein S6 kinase	85.307	771	3	Infinite
Cdc14a	E9PSZ9	Protein Cdc14a	66.902	597	2	226.9
Cdc16	Q4V884	CDC16 cell division cycle 16 homolog (S. cerevisiae)	71.36	620	2	Infinite
Cdc42bpg	D3Z837	Protein Cdc42bpg	172.42	1552	11	14.8
Cdk2	D3ZJC8	Cyclin-dependent kinase 2	39.034	346	2	Infinite
Cdk5	Q03114	Cyclin-dependent kinase 5	33.254	292	4	Infinite
Celf1	G3V7F9	CUG triplet repeat, RNA	55.142	513	4	Infinite

		binding protein 1, isoform CRA_a				
Cep290	D4A5F2	Protein Cep290	289.68	2479	2	Infinite
Cfl1	F1M510	Cofilin-1 (Fragment)	24.457	226	8	Infinite
Ciapin1	Q5XID1	Anamorsin	33.041	309	4	Infinite
Ckap5	F1M949	Protein Ckap5 (Fragment)	196.83	1778	24	22.8
Ckb	P07335	Creatine kinase B-type	42.725	381	6	Infinite
Cltb	P08082	Clathrin light chain B	25.117	229	2	Infinite
Cmpk1	Q4KM73	UMP-CMP kinase	22.169	196	3	Infinite
Cnbp	P62634	Cellular nucleic acid-binding protein	19.463	177	22	12.6
Cndp2	Q6Q0N1	Cytosolic non-specific dipeptidase	52.693	475	3	Infinite
Cnot3	D3ZXE8	Protein Cnot3	81.88	751	2	Infinite
Cnot4	F1MAD6	Protein Cnot4	78.211	713	3	Infinite
Cnot6l	F1M642	Protein Cnot6l (Fragment)	62.735	553	4	Infinite
Copb1	P23514	Coatomer subunit beta	107.01	953	3	Infinite
Copb2	O35142	Coatomer subunit beta	102.55	905	2	Infinite
Copg1	Q4AEF8	Coatomer subunit gamma-1	97.613	874	7	Infinite
Cpped1	Q66H71	Calcineurin-like phosphoesterase domain-containing protein 1	35.26	312	2	Infinite
Crip1	P63255	Cysteine-rich protein 1	8.5497	77	3	Infinite
Crip2	P36201	Cysteine-rich protein 2	22.696	208	4	Infinite
Crkl	Q5U2U2	Crk-like protein	33.865	303	5	Infinite

Csnk1a1	D3ZRE3	Casein kinase I isoform alpha	41.933	365	3	Infinite
Csrp1	P47875	Cysteine and glycine-rich protein 1	20.613	193	9	Infinite
Csrp2	G3V9V9	Cysteine and glycine-rich protein 2, isoform CRA_b	20.926	193	10	31.6
Cstb	P01041	Cystatin-B	11.196	98	2	Infinite
Ctnna1	Q5U302	Catenin (Cadherin associated protein), alpha 1	100.24	908	4	Infinite
Cttnbp2	Q2IBD4	Cortactin binding protein 2	178.77	1649	7	Infinite
Cttnbp2nl	D4A8X8	CTTNBP2 N-terminal like (Predicted), isoform CRA_a	70.076	638	32	Infinite
Cwf19l1	D3Z863	CWF19-like 1, cell cycle control (S. pombe) (Predicted)	60.377	537	3	Infinite
Cyld	Q66H62	Ubiquitin carboxyl-terminal hydrolase CYLD	106.71	953	2	Infinite
Dars	P15178	Aspartate--tRNA ligase, cytoplasmic	57.126	501	7	Infinite
Dctn2	Q6AYH5	Dynactin subunit 2	44.147	402	4	Infinite
Ddx17	E9PT29	Protein Ddx17	72.827	651	2	Infinite
Ddx41	B2RYL8	DEAD (Asp-Glu-Ala-Asp) box polypeptide 41	69.798	622	2	Infinite
Ddx5	Q6AYI1	DEAD (Asp-Glu-Ala-Asp) box polypeptide 5	69.238	615	6	Infinite

Ddx6	D3ZD73	Protein RGD1564560	54.244	483	6	Infinite
Dhx38	D4A321	Protein Dhx38	140.54	1228	23	Infinite
Dnaja1	P63036	DnaJ homolog subfamily A member 1	44.868	397	8	13.7
Dnaja2	O35824	DnaJ homolog subfamily A member 2	45.765	412	5	Infinite
Dnm1	D3ZNS3	Dynamin-1	97.497	866	2	Infinite
Dstn	Q7M0E3	Destrin	18.533	165	10	Infinite
Dtd1	B0K014	Dtd1 protein	23.394	209	2	Infinite
Dync1h1	P38650	Cytoplasmic dynein 1 heavy chain 1	532.25	4644	3	Infinite
Dync1li2	Q62698	Cytoplasmic dynein 1 light intermediate chain 2	54.744	497	3	Infinite
Dynlrb1	P62628	Dynein light chain roadblock-type 1	10.99	96	2	Infinite
Dyrk1a	Q63470	Dual specificity tyrosine-phosphorylation-regulated kinase 1A	85.54	763	2	Infinite
Edc4	Q3ZAV8	Enhancer of mRNA-decapping protein 4	152.59	1407	10	Infinite
Eea1	F1LUA1	Protein Eea1 (Fragment)	161.1	1411	20	Infinite
Eef1a2	P62632	Elongation factor 1-alpha 2	50.454	463	5	56.5
Eftud2	F1LM66	Protein Eftud2	109.48	972	2	Infinite
Eif2b1	Q64270	Translation initiation factor eIF-2B subunit alpha	33.678	305	2	Infinite

Eif2b4	Q63186	Translation initiation factor eIF-2B subunit delta	57.809	524	3	Infinite
Eif2c2	Q9QZ81	Protein argonaute-2	97.317	860	5	Infinite
Eif3d	Q6AYK8	Eukaryotic translation initiation factor 3 subunit D	63.988	548	2	Infinite
Eif3g	Q5RK09	Eukaryotic translation initiation factor 3 subunit G	35.651	320	5	Infinite
Eif4g1	D3ZU13	Protein Eif4g1	175.7	1598	4	Infinite
Eif5a	Q3T1J1	Eukaryotic translation initiation factor 5A-1	16.832	154	4	Infinite
Eif6	Q3KRD8	Eukaryotic translation initiation factor 6	26.571	245	2	Infinite
Erlin2	B5DEH2	Erlin-2	37.71	339	2	Infinite
Erp29	G8JLQ4	Endoplasmic reticulum resident protein 29 (Fragment)	28.674	261	2	Infinite
Ewsr1	F1MA60	Protein Ewsr1	68.742	661	3	Infinite
Exoc2	F1LMB9	Uncharacterized protein	104.02	924	2	Infinite
Exoc3	Q62825	Exocyst complex component 3	86.496	755	2	Infinite
Exoc6	O54923	Exocyst complex component 6	93.177	804	2	Infinite
Fahd1	F1M7U1	Acylpyruvase FAHD1, mitochondrial (Fragment)	24.581	222	2	Infinite
Fam40a	G3V8E2	Protein Fam40a	95.609	837	26	Infinite
Fam83h	D3ZRK0	Protein Fam83h	131.16	1209	2	Infinite

Fam98a	Q5FWT1	Protein FAM98A	55.07	515	2	Infinite
Fblim1	D3Z8E7	Filamin binding LIM protein 1, isoform CRA_a	41.479	376	5	Infinite
Fbxl15	D4ABB4	F-box/LRR-repeat protein 15	33.179	300	2	Infinite
Fbxo7	Q68FS3	F-box only protein 7	57.56	522	2	Infinite
Fgfr1op	Q4V7C1	FGFR1 oncogene partner	43.011	399	3	Infinite
Fgfr1op2	Q6TA25	FGFR1 oncogene partner 2 homolog	29.374	253	11	Infinite
Fhdc1	D3ZL83	Protein Fhdc1	125.6	1148	3	Infinite
Fhl2	O35115	Four and a half LIM domains protein 2	32.086	279	6	Infinite
Filip1	Q8K4T4	Filamin-A-interacting protein 1	137.75	1212	11	Infinite
Flna	C0JPT7	Filamin alpha	280.49	2639	16	Infinite
Fn3krp	B2RYN1	Fructosamine-3-kinase-related protein	34.169	309	10	61.5
Fubp3	G3V829	Protein Fubp3	61.436	569	6	Infinite
Fus	Q5PQK2	Fusion, derived from t(12;16) malignant liposarcoma (Human)	52.673	518	2	Infinite
Fxr1	Q5XI81	Fragile X mental retardation syndrome-related protein 1	63.947	568	2	Infinite
Fxr2	F1M3Y6	Protein Fxr2 (Fragment)	70.645	654	24	42.6
Fzd7	D4ADM3	Protein Fzd7	48.677	434	2	Infinite
Ganab	D4A0W9	Protein Ganab	106.9	944	4	Infinite

Gapvd1	D4A022	Protein Gapvd1	162.76	1463	3	Infinite
Gart	G3V918	Phosphoribosylglycinamide formyltransferase, isoform CRA_a	107.58	1010	2	Infinite
Gbf1	F1M8X9	Protein Gbf1 (Fragment)	200.37	1807	6	11.9
Gcn1l1	F1LRI5	Protein Gcn1l1 (Fragment)	293.17	2672	45	356.3
Gemin5	D3ZGD0	Uncharacterized protein	167.1	1501	3	Infinite
Gfpt1	D3ZZH8	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] 1	78.918	699	6	15.4
Gga1	F1LPF4	Protein Gga1	70.008	635	2	Infinite
Gga2	G3V8F7	Golgi associated, gamma adaptin ear containing, ARF binding protein 2, isoform	66.214	604	5	Infinite
Git1	Q9Z272	ARF GTPase-activating protein GIT1	85.23	770	5	Infinite
Glod4	Q5I0D1	Glyoxalase domain-containing protein 4	33.267	298	4	Infinite
Gmds	Q3MHS7	GDP-mannose 4, 6-dehydratase	42.094	372	4	Infinite
Gmps	F1LS80	GMP synthase [glutamine-hydrolyzing]	77.762	699	3	Infinite
Golga1	D4A6K4	Golgi autoantigen, golgin subfamily a, 1 (Predicted)	87.384	758	2	Infinite

Gpd1	O35077	Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic	37.452	349	6	Infinite
Gprasp2	D4A542	Protein Gprasp2	92.936	827	3	Infinite
Gpsm1	G3V8F6	G-protein signalling modulator 1 (AGS3-like, C. elegans), isoform CRA_b	78.235	705	2	Infinite
Gsdma	D4A2V1	Protein Gsdma	50.787	454	3	Infinite
Gsn	F8WFK3	Gelsolin	86.285	781	2	Infinite
Gstz1	P57113	Maleylacetoacetate isomerase	23.961	216	4	Infinite
Hadha	Q64428	Trifunctional enzyme subunit alpha, mitochondrial	82.664	763	2	Infinite
Hal	P21213	Histidine ammonia-lyase	72.283	657	5	Infinite
Hcn1	Q9JKB0	Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 1	102.42	910	2	Infinite
Hist1h1c	P15865	Histone H1.4	21.987	219	4	Infinite
Hist1h2ail	D3ZJ08	Histone H3	15.388	136	2	Infinite
Hist2h2aa3	P02262	Histone H2A type 1	14.077	130	4	Infinite
Hmmr	D4A2M9	Hyaluronan mediated motility receptor (RHAMM)	82.935	715	3	Infinite
Hnrnpa2b1	A7VJC2	Heterogeneous nuclear	37.477	353	8	Infinite

		ribonucleo-proteins A2/B1				
Hnrnpf	Q794E4	Heterogene-ous nuclear ribonucleo-protein F	45.729	415	2	Infinite
Hnrnpl	F1LPP9	Protein Hnrnpl	67.902	623	2	Infinite
Hnrnpul2	D4ABT8	Protein Hnrnpul2	84.858	745	2	Infinite
Hspa4l	B4F772	Heat shock 70 kDa pro-tein 4L	94.22	838	5	Infinite
Hspb1	G3V913	Heat shock 27kDa pro-tein 1	22.807	206	2	Infinite
Hsph1	Q66HA8	Heat shock protein 105 kDa	96.417	858	8	Infinite
Ica1	Q63054	Islet cell au-toantigen 1	54.662	480	2	Infinite
Ide	P35559	Insulin-de-grading en-zyme	117.71	1019	4	Infinite
Idh3B	Q68FX0	Isocitrate de-hydrogenase [NAD] sub-unit beta, mitochon-drial	42.353	385	2	Infinite
Igbp1	O08836	Immuno-globulin-binding pro-tein 1	39.135	340	22	Infinite
Ilf3	F1LNJ4	Interleukin enhancer-binding fac-tor 3	98.079	915	4	Infinite
Ilk	Q99J82	Integrin-linked pro-tein kinase	51.373	452	3	Infinite
Ints10	E9PTE0	Protein Ints10	82.135	710	12	Infinite
Ints12	Q68FR3	Integrator complex subunit 12	48.477	461	5	Infinite
Ints3	D3ZUT9	Protein Ints3	117.89	1041	18	Infinite
Ints4	D3ZZQ6	Protein Ints4	108.25	964	17	21.6
Ints5	D3ZTW1	Protein Ints5	108.4	1019	9	Infinite

Ints7	D4ADS6	Protein Ints7	106.91	967	20	Infinite
Ipo4	D3ZQZ8	Protein Ipo4	90.527	817	2	Infinite
Irf2bpl	Q5EIC4	Interferon regulatory factor 2-binding protein-like	81.495	783	6	Infinite
Itpk1	D3ZQM7	Protein Itpk1	46.204	421	2	Infinite
Kab	D3ZET9	Protein Kab	174.99	1588	4	Infinite
Kalrn	P97924-4	Isoform 4 of Kalirin	337.57	2968	2	Infinite
Kti12	Q5I0L7	Protein KTI12 homolog	38.357	350	2	Infinite
Lancl1	Q9QX69	LanC-like protein 1	45.239	399	2	Infinite
Larp1	F1M062	Protein Larp1 (Fragment)	116.21	1024	7	Infinite
Lgals2	Q9Z144	Galectin-2	14.732	130	2	Infinite
Lig1	Q9JHY8	DNA ligase 1	102.48	918	14	48.3
Limch1	F1M392	Protein Limch1 (Fragment)	110.11	979	3	Infinite
Limd1	B5DEH0	LIM domain-containing protein 1	71.392	663	3	Infinite
Limk1	G3V663	LIM motif-containing protein kinase 1, isoform CRA_a	72.607	647	4	Infinite
Lin7c	Q792I0	Protein lin-7 homolog C	21.834	197	2	Infinite
Lmna	P48679	Prelamin-A/C	74.323	665	28	16.2
LOC100359593	Q6PDW1	40S ribosomal protein S12	14.515	132	7	Infinite
LOC100359636	D3ZRV7	Protein LOC100359636	12.49	110	4	Infinite
LOC100360491	D3ZRM9	60S ribosomal protein L13	24.202	211	9	Infinite
LOC100360722	F1LW33	Protein LOC100360	29.716	270	2	Infinite

		722 (Fragment)				
LOC100361025	D3Z9I6	Heterogeneous nuclear ribonucleoproteins methyltransferase-like 2	42.435	371	2	Infinite
LOC100361517	D3ZIU2	Protein RGD1305593	13.346	122	2	Infinite
LOC100361915	F1M4I4	Uncharacterized protein (Fragment)	73.376	663	2	Infinite
LOC100362366	D3ZFA8	Protein LOC100364909	15.468	135	12	Infinite
LOC100362464	F1LR65	Cold shock domain-containing protein E1	88.882	798	4	Infinite
LOC100364240	D4A0X3	Lysophosphatidylcholine acyltransferase 2B	103.59	967	3	Infinite
LOC100365869	G3V6W6	Protein LOC100365869	45.796	403	2	Infinite
LOC100365889	F1M8D7	Protein LOC100365889 (Fragment)	90.003	806	2	Infinite
LOC100365889	D3ZIT7	Protein LOC100365889	83.784	750	14	Infinite
LOC500726	D4A1G8	Uncharacterized protein	171.69	1575	11	Infinite
LOC681718	F1MA29	Protein LOC681718	24.908	215	3	Infinite
LOC686548	D3ZE63	Protein LOC679748	12.605	115	2	Infinite
LOC687994	D3ZC82	Protein LOC687994	75.435	689	12	16.9
LOC688393	D3ZKM5	Protein LOC688393	41.563	373	2	Infinite
LOC689899	D3ZTH8	Uncharacterized protein	17.753	156	4	Infinite
LOC690416	D3ZQ62	Uncharacterized protein	65.587	600	2	Infinite

LOC690728	D4ABT1	Protein LOC690728	82.795	732	8	Infinite
Lpin1	Q5XIM8	Lipin 1	101.9	924	5	Infinite
Lpp	F1LSB9	Lipoma-preferred partner homolog	68.389	633	4	Infinite
Lsm12	D4A8G0	Protein Lsm12	21.701	195	6	Infinite
Maged2	Q3B7U1	Melanoma antigen, family D, 2	65.753	618	3	Infinite
Map1s	P0C5W1	Microtubule-associated protein 1S	102.8	972	3	Infinite
Map2k2	F1LMI4	Dual-specificity mitogen-activated protein kinase kinase 2	44.38	401	3	Infinite
Map3k7	P0C8E4	Mitogen-activated protein kinase kinase kinase 7	67.199	606	2	Infinite
Map3k7ip1	D4A6C6	Protein Map3k7ip1	54.6	502	3	Infinite
Map7	F1MA82	Protein Map7 (Fragment)	80.41	713	5	Infinite
Mapk1	P63086	Mitogen-activated protein kinase 1	41.275	358	4	Infinite
Mapk3	P21708-2	Isoform 2 of Mitogen-activated protein kinase 3	45.769	406	4	Infinite
Mark2	D3ZZQ3	Serine/threonine-protein kinase MARK2	85.779	773	3	Infinite
Mark3	F1M836	Uncharacterized protein	88.72	797	2	Infinite
Mast1	Q810W7	Microtubule-associated serine/threonine-protein kinase 1	171.03	1570	3	Infinite
Mat2a	P18298	S-adenosylmethionine	43.715	395	6	Infinite

		synthase isoform type-2				
Mb21d2	D4ACS3	Protein RGD1559643	55.761	491	7	Infinite
Mbnl2	F2Z3T4	Muscleblind-like protein 2	40.156	373	6	Infinite
Mcm7	Q6AYN8	Minichromosome maintenance deficient 7 (S. cerevisiae)	81.062	719	2	Infinite
Mcmbp	B1H268	Mini-chromosome maintenance complex-binding protein	73.006	642	6	Infinite
Mdh2	P04636	Malate dehydrogenase, mitochondrial	35.683	338	2	Infinite
Memo1	Q4QQR9	Protein MEMO1	33.679	297	2	Infinite
Metap1	D3ZE72	Methionine aminopeptidase	43.205	386	5	Infinite
Mettl11a	Q5BJX0	N-terminal Xaa-Pro-Lys N-methyltransferase 1	25.464	223	2	Infinite
Micall2	D3ZEN0	Protein Micall2	107.79	1003	17	Infinite
Mob4	Q9QYW3	MOB-like protein phocein	26.032	225	11	Infinite
Mobkl1a	D4A1V7	MOB1, Mps One Binder kinase activator-like 1A (Yeast) (Predicted)	25.091	216	3	Infinite
Mpo	D4A856	Protein Mpo	80.882	718	4	Infinite
Mrpl35	D3ZE10	Mitochondrial ribosomal protein L35 (Predicted), isoform CRA_a	21.479	188	2	Infinite
Msh2	B1WBQ7	DNA mismatch repair	104.15	933	3	Infinite

		protein Msh2				
Msn	F1LP60	Uncharac-terized pro-tein (Frag-ment)	67.651	576	6	Infinite
Mt3	P37361	Metallothi-onein-3	6.809	66	2	Infinite
Mtpn	P62775	Myotrophin	12.861	118	2	Infinite
Mvp	F1LM41	Uncharac-terized pro-tein (Frag-ment)	96.692	870	4	Infinite
Myh10	F1LQ02	Myosin-10	233.61	2013	4	Infinite
Myh14	F1LMN2	Protein Myh14	232.24	2032	6	Infinite
Myl6	B2GV99	Myl6 protein	17.013	152	6	Infinite
Myo18a	D3ZR07	Protein Myo18a	234.36	2064	2	Infinite
NA	Q66H58	UPF0464 protein C15orf44 homolog	57.143	515	3	Infinite
NA	Q5U2Q3	Ester hydro-lase C11orf54 homolog	34.993	315	2	Infinite
NA	Q5I034	Uncharac-terized pro-tein C12orf43 homolog	30.056	277	6	Infinite
NA	Q497C3	UPF0585 protein C16orf13 homolog	22.612	204	3	Infinite
NA	P56571	ES1 protein homolog, mitochon-drial	28.172	266	2	Infinite
NA	F1MAA3	Uncharac-terized pro-tein	69.311	596	11	Infinite
NA	F1M7S9	Uncharac-terized pro-tein	1409	12659	3	Infinite
NA	F1M3H8	Uncharac-terized pro-tein (Frag-ment)	20.329	194	2	Infinite

NA	F1M2M4	Uncharacterized protein (Fragment)	32.179	285	2	Infinite
NA	F1M1H0	Protein Dera (Fragment)	34.054	308	3	Infinite
NA	F1LZD9	Uncharacterized protein (Fragment)	37.667	354	2	Infinite
NA	F1LXS1	Uncharacterized protein (Fragment)	205.02	1849	12	Infinite
NA	F1LTJ4	Uncharacterized protein (Fragment)	29.427	267	4	Infinite
NA	F1LNQ9	Uncharacterized protein	168.9	1536	5	Infinite
NA	D4AE73	Uncharacterized protein	116.07	1036	2	Infinite
NA	D4ACH3	Uncharacterized protein	30.624	282	3	Infinite
NA	D4ABC4	Uncharacterized protein	92.436	820	34	Infinite
NA	D3ZKL0	Uncharacterized protein	14.3	133	3	Infinite
NA	D3ZIL8	Uncharacterized protein	14.723	134	2	Infinite
NA	D3ZFF2	Uncharacterized protein	99.531	883	19	Infinite
NA	B0BNA9	UPF0760 protein C2orf29 homolog	54.835	504	2	Infinite
Naa50	F1M8I2	Protein Nat13 (Fragment)	19.46	169	4	Infinite
Nampt	Q80Z29	Nicotinamide phosphoribosyltransferase	55.437	491	3	Infinite
Nans	B1WC26	N-acetylneuraminic acid synthase	40.051	359	2	Infinite

Nap1l4	D3ZE23	Uncharacterized protein	47.303	421	3	Infinite
Nav2	F1LR12	Protein Nav2 (Fragment)	252.51	2342	4	Infinite
Ncbp1	Q56A27	Nuclear cap-binding protein subunit 1	91.91	790	4	Infinite
Nccrp1	D3ZQ18	Protein Nccrp1	33.004	291	3	Infinite
Ncoa1	D4ADD6	Protein Ncoa1	156.43	1443	4	15.1
Ncor1	F1LSA0	Nuclear receptor corepressor 1	271.19	2456	2	Infinite
Ndel1	Q78PB6	Nuclear distribution protein nudE-like 1	38.365	345	2	Infinite
Nme2	P19804	Nucleoside diphosphate kinase B	17.283	152	3	Infinite
Nploc4	Q9ES54	Nuclear protein localization protein 4 homolog	68.055	608	4	Infinite
Npm3	D3ZYK9	Protein Npm3	18.917	173	2	Infinite
Ntpcr	D4A478	Protein RGD1306192	20.813	192	2	Infinite
Nudt10	D3ZYH3	Protein Nudt11	18.593	164	2	Infinite
Nudt5	Q6AY63	ADP-sugar pyrophosphatase	24.117	219	2	Infinite
Nup93	Q66HC5	Nuclear pore complex protein Nup93	93.301	819	2	Infinite
Otud4	F1M7Q7	Protein Otud4	122.85	1106	2	Infinite
P4hb	P04785	Protein disulfide-isomerase	56.951	509	4	Infinite
Pabpc4	G3V9N0	Protein Pabpc4	72.411	660	6	20.8
Patl1	B5DF93	Protein PAT1 homolog 1	86.868	770	2	Infinite

Pdcd10	Q6NX65	Programmed cell death protein 10	24.355	210	2	Infinite
Pdia6	Q63081	Protein disulfide-isomerase A6	48.173	440	3	Infinite
Pfkfb2	Q9JJH5	6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2	64.155	557	5	Infinite
Pfkl	P30835	6-phosphofructokinase, liver type	85.338	780	2	Infinite
Pfkm	Q52KS1	6-phosphofructokinase	85.342	780	11	Infinite
Pfkp	P47860	6-phosphofructokinase type C	85.719	788	4	Infinite
Pgk1	P16617	Phosphoglycerate kinase 1	44.538	417	4	Infinite
Phf5a	P83871	PHD finger-like domain-containing protein 5A	12.405	110	3	Infinite
Pias1	F1LTZ9	Protein Pias1 (Fragment)	71.638	651	2	Infinite
Pick1	Q6GQQ2	PRKCA-binding protein	46.705	416	3	14.0
Pklr	P12928-2	Isoform L-type of Pyruvate kinase isozymes R/L	58.793	543	17	Infinite
Pla2g6	P97570-2	Isoform Short of 85/88 kDa calcium-independent phospholipase A2	83.561	752	16	Infinite
Plec	D4A323	Uncharacterized protein	534.37	4692	26	Infinite
Plekha1	D3Z8M0	Protein Plekha1	39.705	357	3	Infinite
Pola1	F1LRJ6	DNA polymerase	166.85	1464	3	Infinite

Pold1	G3V8M1	DNA polymerase	123.57	1103	6	Infinite
Polr2a	D4A5A6	DNA-directed RNA polymerase	217.2	1970	22	Infinite
Polr2b	G3V8Y5	DNA-directed RNA polymerase	133.9	1174	9	Infinite
Polr2c	D4A8A8	Protein Polr2c	37.622	330	3	Infinite
Polr2e	B0BNE2	DNA-directed RNA polymerases I, II, and III subunit RPABC1	24.57	210	4	Infinite
Polr2h	G3V678	Protein Polr2h	17.143	150	3	Infinite
Ppat	P35433	Amidophosphoribosyltransferase	57.436	517	2	Infinite
Ppfia1	D3ZXH0	Protein Ppfia1	142.65	1266	2	Infinite
Ppm1b	Q99ND8	Ppm1b protein	51.009	465	2	Infinite
Ppm1h	Q5M821	Protein phosphatase 1H	56.379	513	5	Infinite
Ppme1	Q4FZT2	Protein phosphatase methylesterase 1	42.316	386	25	Infinite
Ppp1r12a	F1LMS2	Protein phosphatase 1 regulatory subunit 12A	114.67	1032	2	Infinite
Ppp2ca	P63331	Serine/threonine-protein phosphatase 2A catalytic subunit α isoform	35.608	309	6	Infinite
Ppp2cb	P62716	Serine/threonine-protein phosphatase 2A catalytic subunit β isoform	35.575	309	4	Infinite
Ppp2r1a	Q5XI34	Protein Ppp2r1a	65.322	589	46	176.7

Ppp2r1b	D4A1Y3	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform	76.104	694	23	Infinite
Ppp2r2a	P36876	Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform	51.677	447	13	Infinite
Ppp2r2d	P56932	Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B delta isoform	51.982	453	14	Infinite
Ppp2r3a	D3ZLD7	Protein Ppp2r3a	129.9	1150	4	Infinite
Ppp2r5c	D4A1A5	Protein Ppp2r5c	59.909	514	5	Infinite
Ppp3ca	F1LR85	Serine/threonine-protein phosphatase	58.53	521	5	Infinite
Ppp4c	G3V8M5	Serine/threonine-protein phosphatase	35.08	307	22	Infinite
Ppp4r1	Q8VI02	Serine/threonine-protein phosphatase 4 regulatory subunit 1	105.61	951	12	Infinite
Ppp4r2	D4A8H5	Protein Ppp4r2	46.271	416	3	Infinite
Ppp6c	Q64620	Serine/threonine-protein phosphatase 6 catalytic subunit	35.159	305	2	Infinite
Prkaa1	P54645	5-AMP-activated protein	63.973	559	4	Infinite

		kinase catalytic subunit alpha-1				
Prph	P21807	Peripherin	53.549	468	5	Infinite
Prps1	P60892	Ribose-phosphate pyrophosphokinase 1	34.834	318	2	Infinite
Prrc2a	Q6MG48	Protein PRRC2A	229.04	2161	16	68.2
Psma2	P17220	Proteasome subunit alpha type-2	25.926	234	3	Infinite
Psma3	P18422	Proteasome subunit alpha type-3	28.419	255	2	Infinite
Psma4	P21670	Proteasome subunit alpha type-4	29.497	261	2	Infinite
Psmb1	P18421	Proteasome subunit beta type-1	26.479	240	4	Infinite
Psmc2	G3V7L6	26S protease regulatory subunit 7	48.633	433	5	Infinite
Psmc3	D3ZF94	26S protease regulatory subunit 6A	50.341	451	2	Infinite
Psmd12	Q5XIC6	Proteasome (Prosome, macropain) 26S subunit, non-ATPase, 12	52.936	456	5	29.7
Psmd13	B0BN93	26S proteasome non-ATPase regulatory subunit 13	42.817	376	2	Infinite
Psmd14	F1LMW6	Protein Psmd14 (Fragment)	32.924	294	2	Infinite
Psmd6	Q6PCT9	Proteasome (Prosome, macropain) 26S subunit, non-ATPase, 6	45.598	389	2	Infinite
Psmg1	D4AAH6	Down syndrome critical region homolog 2 (Human)	33.238	289	3	Infinite

		(Predicted), isoform CRA_b				
Psmg2	D3ZAQ4	Protein Psmg2	29.653	264	3	Infinite
Rab10	P35281	Ras-related protein Rab-10	22.858	200	2	Infinite
Rab1b	G3V6H0	RCG48149, isoform CRA_b	22.177	201	8	37.0
Rab22a	B0BN19	Protein Rab22a	21.775	194	2	Infinite
Rab2a	F1LP82	Ras-related protein Rab-2A	23.517	212	4	Infinite
Rab3c	P62824	Ras-related protein Rab-3C	25.872	227	3	Infinite
Rab3gap2	F1LMT8	Rab3 GTPase-activating protein non-catalytic subunit	154.08	1386	3	Infinite
Rab43	Q53B90	Ras-related protein Rab-43	23.229	210	2	Infinite
Rab4a	D4ADS8	Ras-related protein Rab-4A	23.962	214	2	Infinite
Rab5c	D4ABV4	Protein Rab5c	25.178	232	4	Infinite
Rac1	D4ADX3	Ras-related C3 botulinum toxin substrate 1, isoform CRA_b	23.436	211	4	Infinite
Racgap1	B2GV02	Protein Racgap1	69.919	626	2	Infinite
Rassf6	Q4QR82	Ras association domain-containing protein 6	39.793	341	2	Infinite
Rbbp4	B5DFB2	Protein Rbbp4	47.655	425	3	Infinite
Rbm4	E9PTZ4	Protein Rbm4	69.373	667	2	Infinite
Rem2	Q9WTY2	GTP-binding protein REM 2	37.274	341	2	13.5

RGD1304694	F1LN82	Protein RGD1304694 (Fragment)	48.675	430	3	Infinite
RGD1305547_predicted	G3V6G2	Similar to RIKEN cDNA 2810417D08 (Predicted)	133.54	1198	6	Infinite
RGD1306215	G3V7Z0	Protein RGD1306215	22.087	205	2	Infinite
RGD1306487	F1LP59	Uncharacterized protein (Fragment)	95.957	860	3	Infinite
RGD1310592	D3ZLQ6	Protein RGD1310592	112.48	994	5	Infinite
RGD1560341	F1LRI8	Methionine aminopeptidase	52.978	479	2	Infinite
RGD1560501	D3ZU80	Ribosomal protein L15	24.176	204	2	Infinite
RGD1562502	G3V6C3	Protein RGD1562502	22.423	198	2	Infinite
RGD1562601	D3ZLH3	Protein RGD1562601	18.766	160	2	Infinite
RGD1564370	F1M2Q3	Protein RGD1564370 (Fragment)	30.233	270	2	Infinite
Rgl2	Q6MGC5	Protein Rgl2	83.726	778	3	Infinite
Rhoa	P61589	Transforming protein RhoA	21.782	193	2	Infinite
Ric8a	B1H241	Resistance to inhibitors of cholinesterase 8 homolog A (C. elegans)	59.826	530	2	Infinite
Rlc-a	P13832	Myosin regulatory light chain RLC-A	19.895	172	2	Infinite
Rnf20	D3ZYQ9	Protein Rnf20	113.46	973	6	Infinite
Rpl10a	P62907	60S ribosomal protein L10a	24.831	217	8	Infinite

Rpl12	F8WFW0	Protein LOC685320 (Fragment)	20.91	191	3	Infinite
Rpl13a	P35427	60S ribosomal protein L13a	23.476	203	2	Infinite
Rpl18a	F1LQL3	60S ribosomal protein L18a (Fragment)	22.319	191	5	Infinite
Rpl19	P84100	60S ribosomal protein L19	23.466	196	8	16.3
Rpl26	G3V6I9	60S ribosomal protein L26	17.258	145	5	Infinite
Rpl27	P61354	60S ribosomal protein L27	15.798	136	3	Infinite
Rpl30	P62890	60S ribosomal protein L30	12.784	115	4	Infinite
Rpl37a-ps1	F1LNS9	60S ribosomal protein L37a	10.335	92	2	Infinite
Rpl38	P63174	60S ribosomal protein L38	8.2178	70	3	Infinite
Rpl4	P50878	60S ribosomal protein L4	47.256	421	8	Infinite
Rpl6	F1LQS3	60S ribosomal protein L6	33.545	298	2	Infinite
Rpl9	P17077	60S ribosomal protein L9	21.893	192	5	Infinite
Rplp1	P19944	60S acidic ribosomal protein P1	11.498	114	2	Infinite
Rprd1a	D4AAU4	Protein Rprd1a	35.73	312	3	10.4
Rps10	P63326	40S ribosomal protein S10	18.916	165	6	Infinite
Rps15	P62845	40S ribosomal protein S15	17.04	145	4	Infinite
Rps15a	P62246	40S ribosomal protein S15a	14.839	130	9	Infinite
Rps19	P17074	40S ribosomal protein S19	16.085	145	11	Infinite

Rps2	P27952	40S ribosomal protein S2	31.231	293	6	Infinite
Rps21	P05765	40S ribosomal protein S21	9.1272	83	4	Infinite
Rps25	P62853	40S ribosomal protein S25	13.742	125	5	Infinite
Rps28	P62859	40S ribosomal protein S28	7.8409	69	4	Infinite
Rps5	B0BN81	Ribosomal protein S5, isoform CRA_b	22.906	204	6	Infinite
Rps6	P62755	40S ribosomal protein S6	28.68	249	3	Infinite
Rps7	P62083	40S ribosomal protein S7	22.127	194	6	30.6
Rps9	P29314	40S ribosomal protein S9	22.591	194	5	Infinite
Rtcd1	Q68FS8	Protein Rtcd1	39.313	366	2	Infinite
Rufy1	F1LR42	Protein Rufy1	80.376	711	6	Infinite
Ruvbl2	G3V8T5	Protein Ruvbl2	51.112	463	5	Infinite
S100a11	Q6B345	Protein S100-A11	11.065	98	2	Infinite
Saps3-ps1	F1MAH5	Uncharacterized protein	94.677	844	2	Infinite
Scg2	G3V7X2	Secretogranin 2, isoform CRA_a	71	619	2	Infinite
Scyl2	D4A1Y0	Protein Scyl2	103.36	930	9	Infinite
Serpinb13	D3ZKA0	Protein Serpinb13	44.249	389	3	Infinite
Serpinb6	Q6P9U0	Protein Serpinb6a	43.018	379	2	Infinite
Sf1	F1LSC3	Protein Sf1	70.149	653	3	Infinite
Sf3a1	D3ZQM0	Protein Sf3a1	88.587	791	5	Infinite
Sf3b1	G3V7T6	Protein Sf3b1	145.83	1304	6	Infinite
Sf3b2	D3ZJX7	Protein Sf3b2	98.359	878	6	Infinite

Sf3b3	E9PT66	Protein Sf3b3	102.62	920	6	53.4
Sh3bp1	D3ZFJ3	Protein Sh3bp1	74.851	689	4	Infinite
Sh3glb1	D4A8Q6	Endophilin-B1	43.17	386	2	Infinite
Sike1	Q5FWT9	Suppressor of IKBKE 1	23.578	207	12	Infinite
Sirt5	Q68FX9	NAD-dependent protein deacylase sirtuin-5, mitochondrial	34.098	310	2	Infinite
Slc25a5	Q09073	ADP/ATP translocase 2	32.901	298	3	Infinite
Slc9a3r1	Q9JJ19	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	38.83	356	5	Infinite
Slmap	F1LM85	Sarcolemmal membrane-associated protein	98.238	858	7	Infinite
Smc2	D4AB57	Structural maintenance of chromosomes protein	139.1	1230	4	Infinite
Smc3	P97690	Structural maintenance of chromosomes protein 3	138.45	1191	7	Infinite
Smek2	D3ZCR4	Protein Smek2	93.969	820	27	Infinite
Sorbs2	F1LPM3	Sorbin and SH3 domain-containing protein 2	134.07	1196	5	12.4
Spast	D4A0I3	Uncharacterized protein	66.772	613	3	Infinite
Spata5	D4A6T1	Protein Spata5	91.161	838	2	Infinite
Sphkap	F1LNS0	A-kinase anchor protein SPHKAP	187.09	1708	5	Infinite
Srp72	D4A7R0	Protein Srp72	75.002	671	2	Infinite

Srr	Q76EQ0	Serine racemase	35.693	333	4	Infinite
Stat6	Q1KQ07	Protein Stat6	93.868	841	2	Infinite
Stmn2	D3ZW73	Stathmin-2	23.334	204	2	Infinite
Strap	Q5XIG8	Serine-threonine kinase receptor-associated protein	38.456	350	4	Infinite
Strn	G3V6L8	RCG61894, isoform CRA_a	86.14	780	28	Infinite
Strn4	F1M6V8	Protein Strn4	81.436	759	23	Infinite
Svil	D3ZEZ9	Uncharacterized protein	241.94	2167	3	Infinite
Synj1	F1LPS0	Synaptojanin-1	172.87	1574	3	Infinite
Taf9	Q5BKE0	Transcription initiation factor TFIID subunit 9	28.994	264	4	Infinite
Taldo1	Q9EQS0	Transaldolase	37.46	337	5	Infinite
Tbc1d1	D4AC16	Protein Tbc1d1	142.33	1257	5	Infinite
Tbc1d9b	F1LRL4	Protein Tbc1d9b (Fragment)	137.62	1224	4	Infinite
Tbk1	D4A7D3	Protein Tbk1	83.387	729	6	Infinite
Tbl1x	G3V6G5	Protein Tbl1x	56.802	527	5	Infinite
Tceb1	P83941	Transcription elongation factor B polypeptide 1	12.473	112	9	Infinite
Tcf25	D3ZC46	Protein Tcf25	76.804	677	3	Infinite
Tes	Q2LAP6	Testin	47.632	419	11	Infinite
Tf	F1LMP2	Serotransferrin (Fragment)	107.41	979	2	Infinite
Tfip11	Q5U2Y6	Tuftelin-interacting protein 11	96.151	837	2	Infinite
Tgm1	P23606	Protein-glutamine	90.769	824	4	Infinite

		gamma-glutamyltransferase K				
Tgm2	Q9WVJ6	Protein Tgm2	76.934	686	8	Infinite
Thop1	P24155	Thimet oligopeptidase	78.385	687	3	Infinite
Tkt	G3V826	Transketolase, isoform CRA_a	71.158	655	5	Infinite
Tmed10	Q63584	Transmembrane emp24 domain-containing protein 10	24.857	219	2	Infinite
Tnpo3	D4AAM0	Protein Tnpo3	104.86	929	2	Infinite
Tpd52l1	Q499Q2	Protein Tpd52l1	18.348	163	3	Infinite
Tpm3	Q63610	Tropomyosin alpha-3 chain	29.006	248	7	Infinite
Tpm4	P09495	Tropomyosin alpha-4 chain	28.509	248	3	Infinite
Trappc3	Q5U1Z2	Trafficking protein particle complex subunit 3	20.302	180	3	Infinite
Trim3	G3V8D6	Tripartite motif protein 3, isoform CRA_a	80.76	744	6	51.5
Trim33	D3ZUK4	Protein Trim33	124.13	1144	2	Infinite
Trip11	D4ABD7	Protein Trip11	226.06	1976	3	Infinite
Tsg101	F1LRB7	Tumor susceptibility gene 101 protein	44.242	391	3	Infinite
Tsn	E9PT79	Protein Tsn	31.473	278	3	Infinite
Ttc9c	Q6P5P3	Tetratricopeptide repeat protein 9C	20.067	171	2	Infinite
Tubb2a	P85108	Tubulin beta-2A chain	49.906	445	6	Infinite
Tubb3	Q4QRB4	Tubulin beta-3 chain	50.418	450	8	255.6

Tubgcp2	B2RYP8	A disintegrin and metallo-protease do-main 8 (Pre-dicted), iso-form CRA_b	103.06	905	3	Infinite
Txnrd1	O89049	Thioredoxin reductase 1, cytoplasmic	54.688	499	11	Infinite
Uba5	Q5M7A4	Ubiquitin-like modi-fier-activat-ing enzyme 5	44.895	403	2	Infinite
Uba52	P62986	Ubiquitin-60S riboso-mal protein L40	14.728	128	4	Infinite
Ube2c	D3ZUW6	Protein Ube2c	19.679	179	2	Infinite
Ubr1	D3ZQC6	Protein Ubr1	199.76	1756	4	Infinite
Ubxn1	Q499N6	UBX do-main-con-taining pro-tein 1	33.581	297	3	Infinite
Uggt1	Q9JLA3	UDP-glu-cose:glyco-protein glu-cosyltrans-ferase 1	176.43	1551	2	Infinite
Umps	Q4QQS7	Protein Umps	52.378	481	7	Infinite
Usp14	Q5U2N2	Ubiquitin carboxyl-terminal hy-drolase	55.976	493	3	Infinite
Vat1	Q3MIE4	Synaptic vesicle membrane protein VAT-1 hom-olog	43.118	404	4	Infinite
Vcpip1	Q8CF97	Deubiqui-tinating pro-tein VCIP135	134.56	1221	4	Infinite
Vdac1	Q9Z2L0	Voltage-de-pendent an-ion-selective channel pro-tein 1	30.755	283	2	Infinite
Vgll4	Q5BJP0	Protein Vgll4	31.02	287	4	Infinite

Vps13a	D3Z8N6	Protein Vps13a	360.05	3167	19	Infinite
Vps37a	Q4V794	Protein Vps37a	44.487	398	2	Infinite
Vps52	O55166	Vacuolar protein sorting-associated protein 52 homolog	82.102	723	4	Infinite
Vps53	D3ZPE5	Protein Vps53	94.428	832	2	Infinite
Vwa5b2	D4A7U5	Protein Vwa5b2	133.3	1248	4	Infinite
Wdr37	D3ZLR5	Protein Wdr37	53.9	492	2	Infinite
Wdr5	Q498M4	WD repeat-containing protein 5	36.588	334	3	Infinite
Wdr81	D4A929	WD repeat-containing protein 81	212.26	1933	7	Infinite
Wdr91	B2RYI0	WD repeat-containing protein 91	83.145	747	10	Infinite
Xpo5	D3ZQE8	Protein Xpo5	136.88	1204	2	Infinite
Xpot	D3ZSC0	Protein Xpot	109.68	963	2	Infinite
Yars	G3V713	Tyrosine--tRNA ligase, cytoplasmic	63.025	564	3	Infinite
Ybx2	D4A3P0	Protein Ybx2	38.094	359	4	Infinite
Zc3h14	Q7TMD5	Zinc finger CCCH domain-containing protein 14	82.631	736	2	Infinite
Zfand5	B5DF11	AN1-type zinc finger protein 5	23.088	213	6	Infinite
Zfp655	Q5RKG8	Protein Znf655	63.599	541	2	Infinite
Zyg11b	F1M8P2	Protein Zyg11b (Fragment)	83.804	743	5	Infinite

Table 6. 89 Glucose responsive interaction partners in INS-1 832/13 cells.

Gene name	Protein ID	Protein name	MW [kDa]	Sequence length	Number of unique peptides detected in the PP2A c IP	Enrichment ratio
Apip	D3ZUI1	APAF1 interacting protein (Predicted), isoform CRA_a	27.053	241	8	Infinite[a]
Appl1	D3ZWA8	Protein Appl1	79.363	707	3	Infinite
Arpc4	B2RZ72	Actin related protein 2/3 complex, subunit 4 (Predicted), isoform CRA_a	19.667	168	2	Infinite
Cbr3	B2GV72	Carbonyl reductase 3	30.841	277	3	Infinite
Cc2d1a	F1LQC6	Coiled-coil and C2 domain-containing protein 1A	103.71	942	3	Infinite
Ciapin1	Q5XID1	Anamorsin	33.041	309	4	Infinite
Cnbp	P62634	Cellular nucleic acid-binding protein	19.463	177	22	12.6
Cnot4	F1MAD6	Protein Cnot4	78.211	713	3	Infinite
Csrp1	P47875	Cysteine and glycine-rich protein 1	20.613	193	9	Infinite
Cttnbp2	Q2IBD4	Cortactin binding protein 2	178.77	1649	7	Infinite
Dctn2	Q6AYH5	Dynactin subunit 2	44.147	402	4	Infinite
Ddx17	E9PT29	Protein Ddx17	72.827	651	2	Infinite

Eea1	F1LUA1	Protein Eea1 (Fragment)	161.1	1411	20	Infinite
Eif2b1	Q64270	Translation initiation factor eIF-2B subunit alpha	33.678	305	2	Infinite
Eif2c2	Q9QZ81	Protein argonaute-2	97.317	860	5	Infinite
Fhl2	O35115	Four and a half LIM domains protein 2	32.086	279	6	Infinite
Filip1	Q8K4T4	Filamin-A-interacting protein 1	137.75	1212	11	Infinite
Gfpt1	D3ZZH8	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] 1	78.918	699	6	15.4
Gga2	G3V8F7	Golgi associated, gamma adaptin ear containing, ARF binding protein 2, isoform	66.214	604	5	Infinite
Gmds	Q3MHS7	GDP-mannose 4, 6-dehydratase	42.094	372	4	Infinite
Gmps	F1LS80	GMP synthase [glutamine-hydrolyzing]	77.762	699	3	Infinite
Gpd1	O35077	Glycerol-3-phosphate dehydrogenase [NAD(+)], cytoplasmic	37.452	349	6	Infinite
Gprasp2	D4A542	Protein Gprasp2	92.936	827	3	Infinite
Hist1h1c	P15865	Histone H1.4	21.987	219	4	Infinite
Hmmr	D4A2M9	Hyaluronan mediated motility receptor (RHAMM)	82.935	715	3	Infinite
Hnrnpf	Q794E4	Heterogeneous nuclear ribonucleoprotein F	45.729	415	2	Infinite

Ilf3	F1LNJ4	Interleukin enhancer-binding factor 3	98.079	915	4	Infinite
Ints12	Q68FR3	Integrator complex subunit 12	48.477	461	5	Infinite
Ints5	D3ZTW1	Protein Ints5	108.4	1019	9	Infinite
Ints7	D4ADS6	Protein Ints7	106.91	967	20	Infinite
Itpk1	D3ZQM7	Protein Itpk1	46.204	421	2	Infinite
Kalrn	P97924-4	Isoform 4 of Kalirin	337.57	2968	2	Infinite
Lig1	Q9JHY8	DNA ligase 1	102.48	918	14	48.3
Limd1	B5DEH0	LIM domain-containing protein 1	71.392	663	3	Infinite
Limk1	G3V663	LIM motif-containing protein kinase 1, isoform CRA_a	72.607	647	4	Infinite
LOC100364240	D4A0X3	Lysophosphatidylcholine acyltransferase 2B	103.59	967	3	Infinite
LOC100365889	D3ZIT7	Protein LOC100365889	83.784	750	14	Infinite
Lpin1	Q5XIM8	Lipin 1	101.9	924	5	Infinite
Lsm12	D4A8G0	Protein Lsm12	21.701	195	6	Infinite
Map7	F1MA82	Protein Map7 (Fragment)	80.41	713	5	Infinite
Mcmbp	B1H268	Mini-chromosome maintenance complex-binding protein	73.006	642	6	Infinite
Mettl11a	Q5BJX0	N-terminal Xaa-Pro-Lys N-methyltransferase 1	25.464	223	2	Infinite
Micall2	D3ZEN0	Protein Micall2	107.79	1003	17	Infinite

Mrpl35	D3ZE10	Mitochondrial ribosomal protein L35 (Predicted), isoform CRA_a	21.479	188	2	Infinite
NA	D3ZFF2	Uncharacterized protein	99.531	883	19	Infinite
NA	F1LTJ4	Uncharacterized protein (Fragment)	29.427	267	4	Infinite
NA	F1LZD9	Uncharacterized protein (Fragment)	37.667	354	2	Infinite
Naa50	F1M8I2	Protein Nat13 (Fragment)	19.46	169	4	Infinite
Nav2	F1LR12	Protein Nav2 (Fragment)	252.51	2342	4	Infinite
Ncor1	F1LSA0	Nuclear receptor corepressor 1	271.19	2456	2	Infinite
Ntpcr	D4A478	Protein RGD1306192	20.813	192	2	Infinite
Nudt10	D3ZYH3	Protein Nudt11	18.593	164	2	Infinite
Pfkfb2	Q9JJH5	6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 2	64.155	557	5	Infinite
Phf5a	P83871	PHD finger-like domain-containing protein 5A	12.405	110	3	Infinite
Pla2g6	P97570-2	Isoform Short of 85/88 kDa calcium-independent phospholipase A2	83.561	752	16	Infinite
Pold1	G3V8M1	DNA polymerase	123.57	1103	6	Infinite
Ppat	P35433	Amidophosphoribosyltransferase	57.436	517	2	Infinite
Ppm1b	Q99ND8	Ppm1b protein	51.009	465	2	Infinite

Ppp2r1b	D4A1Y3	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A beta isoform	76.104	694	23	Infinite
Ppp2r2a	P36876	Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform	51.677	447	13	Infinite
Ppp4r1	Q8VI02	Serine/threonine-protein phosphatase 4 regulatory subunit 1	105.61	951	12	Infinite
Ppp6c	Q64620	Serine/threonine-protein phosphatase 6 catalytic subunit	35.159	305	2	Infinite
Prph	P21807	Peripherin	53.549	468	5	Infinite
Rab10	P35281	Ras-related protein Rab-10	22.858	200	2	Infinite
Rab5c	D4ABV4	Protein Rab5c	25.178	232	4	Infinite
Rhoa	P61589	Transforming protein RhoA	21.782	193	2	Infinite
Rpl18a	F1LQL3	60S ribosomal protein L18a (Fragment)	22.319	191	5	Infinite
Rpl30	P62890	60S ribosomal protein L30	12.784	115	4	Infinite
Rpl4	P50878	60S ribosomal protein L4	47.256	421	8	Infinite
Rpl9	P17077	60S ribosomal protein L9	21.893	192	5	Infinite
Scyl2	D4A1Y0	Protein Scyl2	103.36	930	9	Infinite
Serpinb6	Q6P9U0	Protein Serpinb6a	43.018	379	2	Infinite
Sh3bp1	D3ZFJ3	Protein Sh3bp1	74.851	689	4	Infinite

Slmap	F1LM85	Sarcolemmal membrane-associated protein	98.238	858	7	Infinite
Sorbs2	F1LPM3	Sorbin and SH3 domain-containing protein 2	134.07	1196	5	12.4
Srp72	D4A7R0	Protein Srp72	75.002	671	2	Infinite
Stat6	Q1KQ07	Protein Stat6	93.868	841	2	Infinite
Tceb1	P83941	Transcription elongation factor B polypeptide 1	12.473	112	9	Infinite
Trip11	D4ABD7	Protein Trip11	226.06	1976	3	Infinite
Tsg101	F1LRB7	Tumor susceptibility gene 101 protein	44.242	391	3	Infinite
Txnrd1	O89049	Thioredoxin reductase 1, cytoplasmic	54.688	499	11	Infinite
Ube2c	D3ZUW6	Protein Ube2c	19.679	179	2	Infinite
Ubr1	D3ZQC6	Protein Ubr1	199.76	1756	4	Infinite
Uggt1	Q9JLA3	UDP-glucose:glycoprotein glucosyltransferase 1	176.43	1551	2	Infinite
Vat1	Q3MIE4	Synaptic vesicle membrane protein VAT-1 homolog	43.118	404	4	Infinite
Vgll4	Q5BJP0	Protein Vgll4	31.02	287	4	Infinite
Vps37a	Q4V794	Protein Vps37a	44.487	398	2	Infinite
Vps52	O55166	Vacuolar protein sorting-associated protein 52 homolog	82.102	723	4	Infinite
Wdr5	Q498M4	WD repeat-containing protein 5	36.588	334	3	Infinite

Table 7. Thirty-eight previously reported PP2Ac interaction partners were identified in this study

Gene name	Protein ID	Protein name	MW [kDa]	Enrichment ratio	Reference	Species*
Ankle2	Q7TP65	Ankyrin repeat and LEM domain-containing protein 2	106.4	1000#	[94]	H
Arpc4	B2RZ72	Actin related protein 2/3 complex, subunit 4 (Predicted), isoform CRA_a	19.7	1000	[187]	H
Cdk2	D3ZJC8	Cyclin-dependent kinase 2	39.0	1000	[99,188]	H
Csnk1a1	D3ZRE3	Casein kinase I isoform α	41.9	1000	[189]	H
Cttnbp2	Q2IBD4	Cortactin binding protein 2	178.8	1000	[95]	H
Cttnbp2nl	D4A8X8	CTTNBP2 N-terminal like (Predicted), isoform CRA_a	70.1	1000	[95]	H
Fam40a	G3V8E2	Protein Fam40a	95.6	1000	[95]	H
Fgfr1op	Q4V7C1	FGFR1 oncogene partner	43.0	1000	[95,190]	H, M
Fgfr1op2	Q6TA25	FGFR1 oncogene partner 2 homolog	29.4	1000	[94]	H
Gga1	F1LPF4	Protein Gga1	70.0	1000	[191]	H
Hnrnpa2b1	A7VJC2	Heterogeneous nuclear ribonucleoproteins A2/B1	37.5	1000	[192]	M
Igbp1	O08836	Immunoglobulin-binding protein 1	39.1	1000	[94,95,106,193-201]	H, M, R
Ints3	D3ZUT9	Protein Ints3	117.9	1000	[98]	H
Ints5	D3ZTW1	Protein Ints5	108.4	1000	[98]	H
Map3k7ip1	D4A6C6	Protein Map3k7ip1	54.6	1000	[194]	H
Mapk3	P21708-2	Isoform 2 of Mitogen-activated protein kinase 3	45.8	1000	[202]	H
Mob4	Q9QYW3	MOB-like protein phocein	26.0	1000	[94,95]	H
Myh10	F1LQ02	Myosin-10	233.6	1000	[94]	H
Pdcd10	Q6NX65	Programmed cell death protein 10	24.4	1000	[95]	H
Pola1	F1LRJ6	DNA polymerase	166.9	1000	[203]	H
Ppfia1	D3ZXH0	Protein Ppfia1	142.7	1000	[94,95]	H
Ppm1b	Q99ND8	Ppm1b protein	51.0	1000	[99]	H
Ppme1	Q4FZT2	Protein phosphatase methylesterase 1	42.3	1000	[94,95]	H
Ppp2r1a	Q5XI34	PP2 65 kDa regulatory subunit A, α isoform	65.3	176.7	[93-97,105,187,190,204-210]	H, M
Ppp2r1b	D4A1Y3	PP2 65 kDa regulatory subunit A, β isoform	76.1	1000	[93-97]	H

Ppp2r2a	P36876	PP2 55 kDa regulatory subunit B, α isoform	51.7	1000	93-95,204,211	H, M
Ppp2r2d	P56932	PP2 55 kDa regulatory subunit B, δ isoform	52.0	1000	94,95,212	H
Ppp2r3a	D3ZLD7	PP2 72/130 kDa regulatory subunit B, α isoform	129.9	1000	213,214	H, M
Ppp4c	G3V8M5	PP4 catalitic subunit	35.1	1000	215	H
Ppp2r5c	D4A1A5	PP2 56 kDa regulatory subunit B, γ isoform	59.9	1000	94,95,184,216-218	H
Prkaa1	P54645	5-AMP-activated protein kinase catalytic subunit α-1	64.0	1000	219,220	H
Rplp1	P19944	60S acidic ribosomal protein P1	11.5	1000	221	H
Sike1	Q5FWT9	Suppressor of IKBKE 1	23.6	1000	94	H
Slmap	F1LM85	Sarcolemmal membrane-associated protein	98.2	1000	94	H
Strn	G3V6L8	RCG61894, isoform CRA_a	86.1	1000	94,95	H
Strn4	F1M6V8	Protein Strn4	81.4	1000	94,95	H
Tnpo3	D4AAM0	Protein Tnpo3	104.9	1000	187	H
Uba52	P62986	Ubiquitin-60S ribosomal protein L40	14.7	1000	195	H
Ankle2	Q7TP65	Ankyrin repeat and LEM domain-containing protein 2	106.4	1000#	94	H
Arpc4	B2RZ72	Actin related protein 2/3 complex, subunit 4 (Predicted), isoform CRA_a	19.7	1000	187	H
Cdk2	D3ZJC8	Cyclin-dependent kinase 2	39.0	1000	99,188	H
Csnk1a1	D3ZRE3	Casein kinase I isoform α	41.9	1000	189	H
Cttnbp2	Q2IBD4	Cortactin binding protein 2	178.8	1000	95	H
Cttnbp2nl	D4A8X8	CTTNBP2 N-terminal like (Predicted), isoform CRA_a	70.1	1000	95	H
Fam40a	G3V8E2	Protein Fam40a	95.6	1000	95	H
Fgfr1op	Q4V7C1	FGFR1 oncogene partner	43.0	1000	95,190	H, M
Fgfr1op2	Q6TA25	FGFR1 oncogene partner 2 homolog	29.4	1000	94	H
Gga1	F1LPF4	Protein Gga1	70.0	1000	191	H
Hnrnpa2b1	A7VJC2	Heterogeneous nuclear ribonucleoproteins A2/B1	37.5	1000	192	M
Igbp1	O08836	Immunoglobulin-binding protein 1	39.1	1000	94,95,106,19	H, M, R

					3-201	
Ints3	D3ZUT9	Protein Ints3	117.9	1000	98	H
Ints5	D3ZTW1	Protein Ints5	108.4	1000	98	H
Map3k7ip1	D4A6C6	Protein Map3k7ip1	54.6	1000	194	H
Mapk3	P21708-2	Isoform 2 of Mitogen-activated protein kinase 3	45.8	1000	202	H
Mob4	Q9QYW3	MOB-like protein phocein	26.0	1000	94,95	H
Myh10	F1LQ02	Myosin-10	233.6	1000	94	H
Pdcd10	Q6NX65	Programmed cell death protein 10	24.4	1000	95	H
Pola1	F1LRJ6	DNA polymerase	166.9	1000	203	H
Ppfia1	D3ZXH0	Protein Ppfia1	142.7	1000	94,95	H
Ppm1b	Q99ND8	Ppm1b protein	51.0	1000	99	H
Ppme1	Q4FZT2	Protein phosphatase methylesterase 1	42.3	1000	94,95	H
Ppp2r1a	Q5XI34	PP2 65 kDa regulatory subunit A, α isoform	65.3	176.7	93-97,105,187,190,204-210	H, M
Ppp2r1b	D4A1Y3	PP2 65 kDa regulatory subunit A, β isoform	76.1	1000	93-97	H
Ppp2r2a	P36876	PP2 55 kDa regulatory subunit B, α isoform	51.7	1000	93-95,204,211	H, M
Ppp2r2d	P56932	PP2 55 kDa regulatory subunit B, δ isoform	52.0	1000	94,95,212	H
Ppp2r3a	D3ZLD7	PP2 72/130 kDa regulatory subunit B, α isoform	129.9	1000	213, 214	H, M
Ppp4c	G3V8M5	PP4 catalitic subunit	35.1	1000	215	H
Ppp2r5c	D4A1A5	PP2 56 kDa regulatory subunit B, γ isoform	59.9	1000	94,95,184,216-218	H

Prkaa1	P54645	5-AMP-activated protein kinase catalytic subunit α-1	64.0	1000	[219, 220]	H
Rplp1	P19944	60S acidic ribosomal protein P1	11.5	1000	[221]	H
Sike1	Q5FWT9	Suppressor of IKBKE 1	23.6	1000	[94]	H
Slmap	F1LM85	Sarcolemmal membrane-associated protein	98.2	1000	[94]	H
Strn	G3V6L8	RCG61894, isoform CRA_a	86.1	1000	[94,95]	H
Strn4	F1M6V8	Protein Strn4	81.4	1000	[94,95]	H
Tnpo3	D4AAM0	Protein Tnpo3	104.9	1000	[187]	H
Uba52	P62986	Ubiquitin-60S ribosomal protein L40	14.7	1000	[195]	H

Table 8. IPA analysis of the 516 partners showing the 39 enriched pathways

Ingenuity Canonical Pathways	P value	Glucose responsive PP2Ac partners identified in the study	Glucose nonresponsive PP2Ac partners identified in the study
EIF2 Signaling	1.26E-18	RPL4, RPL18A, RPL30, EIF2B1, RPL9	EIF2B4, MAPK1, RPL26, RPS21, EIF4G1, RPS7, RPL6, MAP2K2, EIF3D, MAPK3, RPS9, RPL19, RPL12, RPL37A, RPL27, RPS2, RPS19, RPL10A, EIF3G, RPS6, UBA52, RPS15, RPS25, RPS15A, LOC100360491, RPL38, RPL13A
Regulation of eIF4 and p70S6K Signaling	2.51E-13	PPP2R2A, EIF2B1, PPP2R1B	EIF2B4, MAPK1, , RPS2, RPS19, RPS21, EIF4G1, EIF3G, RPS7, RPS6, , PPP2R1A, MAP2K2, PPP2R3A, EIF3D, MAPK3, RPS9, RPS15, RPS15A, RPS25
mTOR Signaling	5.01E-11	PPP2R2A, RHOA, PPP2R1B	MAPK1, , RPS2, RPS19, RAC1, RPS21, EIF4G1, EIF3G, RPS7, RPS6, , PPP2R1A, PPP2R3A, EIF3D, MAPK3, RPS9, PRKAA1, RPS15, RPS15A, RPS25
AMPK Signaling	3.63E-07	PPM1B, PPP2R2A, PPP2R1B, PPAT, PFKFB2	TAF9, MAPK1, , PFKP, PFKL, PFKM, , PPP2R1A, PPP2R3A, PRKAA1
CDK5 Signaling	5.89E-07	PPP2R2A, PPP2R1B	PPP2R1A, MAP2K2, CDK5, MAPK1, , PPP2R3A, MAPK3, CAPN1, PPP1R12A
ILK Signaling	1.07E-06	PPP2R2A, RHOA, PPP2R1B	MYH10, FBLIM1, MYL6, MAPK1, CFL1, , MYH14, ILK, , PPP2R1A, PPP2R3A, FLNA, MAPK3, PPP1R12A
Actin Cytoskeleton Signaling	1.62E-06	RHOA, ARPC4, LIMK1	ACTR2, MYH10, MYL6, CFL1, MAPK1, CRKL, MYH14, RAC1, GSN, GIT1, ACTR3, MAP2K2, FLNA, MAPK3, PPP1R12A, MSN
Salvage Pathways of Pyrimidine Ribonucleotides	8.71E-06	LIMK1	MAP2K2, CDK5, MAPK1, MAPK3, PRKAA1, NME2, CSNK1A1, CMPK1, CDK2, DYRK1A

Cell Cycle Regulation by BTG Family Proteins	1.02E-05	PPP2R2A, PPP2R1B	PPP2R1A, , PPP2R3A, CDK2
Glycolysis I	1.15E-05		PGK1, PKLR, ALDOA, PFKP, PFKL, PFKM
Pyridoxal 5'-phosphate Salvage Pathway	1.51E-05	LIMK1	MAP2K2, CDK5, MAPK1, MAPK3, PRKAA1, CSNK1A1, CDK2, DYRK1A
Mitotic Roles of Polo-Like Kinase	1.74E-05	PPP2R2A, PPP2R1B	SMC3, PPP2R1A, , PPP2R3A, CAPN1, CDC16
Protein Ubiquitination Pathway	1.86E-05	UBR1, TCEB1, UBE2C	USP14, PSMA3, HSPH1, PSMD13, PSMD6, THOP1, DNAJA1, PSMD12, PSMA4, PSMB1, PSMD14, PSMA2, PSMC2, PSMC3, HSPA4L, HSPB1
Chemokine Signaling	2.19E-05	RHOA, LIMK1	Calm1 (includes others), CAMK1, MAP2K2, MAPK1, CFL1, MAPK3, PPP1R12A
Estrogen Receptor Signaling	2.82E-05	NCOR1	TAF9, DDX5, POLR2A, POLR2C, MAP2K2, MAPK1, POLR2E, MAPK3, NCOA1, POLR2H, POLR2B
Tight Junction Signaling	4.07E-05	PPP2R2A, RHOA, PPP2R1B	MYH10, MYL6, , MYH14, MARK2, RAC1, CTNNA1, , PPP2R1A, PPP2R3A
Glucocorticoid Receptor Signaling	8.51E-05	NCOR1, TSG101	TAF9, MAPK1, RAC1, POLR2B, POLR2C, POLR2A, MAP2K2, MAP3K7, POLR2E, ANXA1, MAPK3, NCOA1, PRKAA1, POLR2H, PPP3CA
ERK/MAPK Signaling	8.71E-05	PLA2G6, PPP2R2A, PPP2R1B	MAPK1, , CRKL, RAC1, , PPP2R1A, MAP2K2, PPP2R3A, MAPK3, PPP1R12A, HSPB1
Regulation of Actin-based Motility by Rho	9.77E-05	RHOA, ARPC4, LIMK1	ACTR2, ACTR3, CFL1, MYL6, RAC1, PPP1R12A, GSN
D-myo-inositol (1, 4, 5, 6)-Tetrakisphosphate Biosynthesis	1.00E-04	ITPK1, NUDT11, PPP4R1	PPFIA1, NUDT5, SYNJ1, PPP2R3A, PPM1H, PPP1R12A, IGBP1, PPP3CA
D-myo-inositol (3, 4, 5, 6)-tetrakisphosphate Biosynthesis	1.00E-04	ITPK1, NUDT11, PPP4R1	PPFIA1, NUDT5, SYNJ1, PPP2R3A, PPM1H, PPP1R12A, IGBP1, PPP3CA

PI3K/AKT Signaling	1.00E-04	PPP2R2A, PPP2R1B	PPP2R1A, SYNJ1, MAP2K2, MAPK1, , PPP2R3A, MAPK3, ILK
Rac Signaling	1.12E-04	RHOA, ARPC4, LIMK1	ACTR2, ACTR3, ARFIP2, MAP2K2, MAPK1, CFL1, MAPK3, RAC1
Androgen Signaling	2.09E-04		CALR, POLR2A, Calm1 (includes others), POLR2C, MAPK1, POLR2E, MAPK3, NCOA1, POLR2H, POLR2B
Role of CHK Proteins in Cell Cycle Checkpoint Control	2.14E-04	PPP2R2A, PPP2R1B	PPP2R1A, , PPP2R3A, CDK2
Calcium Signaling	2.14E-04		MYH10, CALR, Calm1 (includes others), CAMK1, MAPK1, MYL6, MYH14, Acta2, Camk2b, Tpm4, MAPK3, PPP3CA, Tpm3
3-phosphoinositide Degradation	3.09E-04	ITPK1, NUDT11, PPP4R1	PPFIA1, NUDT5, SYNJ1, PPP2R3A, PPM1H, PPP1R12A, IGBP1, PPP3CA
D-myo-inositol-5-phosphate Metabolism	3.09E-04	ITPK1, NUDT11, PPP4R1	PPFIA1, NUDT5, SYNJ1, PPP2R3A, PPM1H, PPP1R12A, IGBP1, PPP3CA
p70S6K Signaling	3.63E-04	PPP2R2A, PPP2R1B	RPS6, , PPP2R1A, MAP2K2, MAPK1, , PPP2R3A, MAPK3
RhoA Signaling	4.47E-04	RHOA, ARPC4, LIMK1	ACTR2, ACTR3, CFL1, MYL6, PPP1R12A, ARHGEF11, ARHGAP1, MSN
Wnt/Î²-catenin Signaling	4.79E-04	APPL1, PPP2R2A, PPP2R1B	PPP2R1A, , MAP3K7, PPP2R3A, MARK2, CSNK1A1, ILK, RUVBL2
3-phosphoinositide Biosynthesis	6.31E-04	ITPK1, NUDT11, PPP4R1	PPFIA1, NUDT5, SYNJ1, PPP2R3A, PPM1H, PPP1R12A, IGBP1, PPP3CA
PAK Signaling	7.76E-04	LIMK1	MAP2K2, MAPK1, CFL1, MYL6, MAPK3, RAC1, GIT1
Remodeling of Epithelial Adherens Junctions	7.94E-04	RAB5C, ARPC4	DNM1, ACTR2, TUBB3, ACTR3, TUBB2A, CTNNA1, EXOC2
Assembly of RNA Polymerase II Complex	8.32E-04		TAF9, POLR2A, POLR2C, POLR2E, POLR2H, POLR2B
Signaling by Rho Family GTPases	8.91E-04	RHOA, ARPC4, LIMK1	ACTR2, MAPK1, CFL1, MYL6, RAC1, ACTR3, ARFIP2, MAP2K2, MAPK3, PPP1R12A, ARHGEF11, MSN

IL-3 Signaling	1.02E-03	STAT6	MAP2K2, MAPK1, CRKL, MAPK3, RAC1, PPP3CA
Nucleotide Excision Repair Pathway	1.02E-03		POLR2A, POLR2C, POLR2E, POLR2H, POLR2B
Aryl Hydrocarbon Receptor Signaling	1.20E-03	TRIP11	TGM2, MAPK1, POLA1, MAPK3, BAX, CDK2, HSPB1, MCM7, AIP
Ephrin B Signaling	1.32E-03	KALRN, RHOA, LIMK1	MAPK1, CFL1, MAPK3, RAC1
Integrin Signaling	1.62E-03	RHOA, ARPC4	ACTR2, ACTR3, MAP2K2, MAPK1, CRKL, MAPK3, CAPN1, RAC1, ILK, PPP1R12A, GIT1
Epithelial Adherens Junction Signaling	1.66E-03	RHOA, ARPC4	MYH10, ACTR2, TUBB3, ACTR3, MYL6, MYH14, TUBB2A, RAC1, CTNNA1
RhoGDI Signaling	1.91E-03	RHOA, ARPC4, LIMK1	ACTR2, ACTR3, CFL1, MYL6, RAC1, PPP1R12A, ARHGEF11, ARHGAP1, MSN
Ephrin Receptor Signaling	2.00E-03	KALRN, RHOA, ARPC4, LIMK1	ACTR2, ACTR3, MAP2K2, MAPK1, CFL1, CRKL, MAPK3, RAC1
Ceramide Signaling	2.04E-03	PPP2R2A, PPP2R1B	PPP2R1A, , PPP2R3A, MAPK3
Cyclins and Cell Cycle Regulation	2.04E-03	PPP2R2A, PPP2R1B	PPP2R1A, PPP2R3A, CDK2
Clathrin-mediated Endocytosis Signaling	3.02E-03	RAB5C, TSG101, ARPC4	DNM1, ACTR2, ACTR3, RAB4A, TF, SYNJ1, CLTB, RAC1, SH3GLB1, PPP3CA
FAK Signaling	3.31E-03	HMMR	MAP2K2, MAPK1, MAPK3, CAPN1, RAC1, Acta2
Cdc42 Signaling	3.39E-03	ARPC4, LIMK1	ACTR2, ACTR3, MAPK1, CFL1, MYL6, EXOC2, PPP1R12A, EXOC6, EXOC3
Superpathway of Inositol Phosphate Compounds	3.72E-03	ITPK1, NUDT11, PPP4R1	PPFIA1, NUDT5, SYNJ1, PPP2R3A, PPM1H, PPP1R12A, IGBP1, PPP3CA
Pyrimidine Ribonucleotides De Novo Biosynthesis	4.27E-03		NUDT5, NME2, CMPK1, UMPS
PPAR Signaling	5.13E-03	NCOR1	MAP2K2, MAPK1, MAP3K7, MAPK3, NCOA1, AIP
Amyloid Processing	5.50E-03		CDK5, MAPK1, MAPK3, CAPN1, CSNK1A1

Phospholipase C Signaling	6.61E-03	PLA2G6, RHOA	TGM2, Calm1 (includes others), MAP2K2, MAPK1, MYL6, MAPK3, RAC1, PPP1R12A, ARHGEF11, PPP3CA
PPARÎ±/RXRÎ± Activation	6.61E-03	GPD1, NCOR1	CAND1, MAP2K2, MAPK1, MAP3K7, MAPK3, CKAP5, PRKAA1, AIP
NRF2-mediated Oxidative Stress Response	7.41E-03	TXNRD1	USP14, ERP29, MAP2K2, MAPK1, MAP3K7, MAPK3, CAT, DNAJA2, DNAJA1,
Actin Nucleation by ARP-WASP Complex	8.13E-03	RHOA, ARPC4	ACTR2, ACTR3, RAC1, PPP1R12A
Insulin Receptor Signaling	9.77E-03	EIF2B1	EIF2B4, SYNJ1, MAP2K2, MAPK1, CRKL, MAPK3, PPP1R12A

Table 9. The 211 proteins/ protein groups met the 2 rigorous criteria (See Methods for details) for classification as PP2Ac interaction partners in human skeletal muscle. # indicating previously identified PP2A partners.

Gene name	Protein ID	Protein name	enrichment ratio
PPP2R1A	B3KQV6	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	213.3
PPP2R2A	E5RFR9	Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform	155.7
PPP2R3A	F6URX5	Serine/threonine-protein phosphatase 2A regulatory subunit B subunit alpha	10435.3
PPP2R5D	E9PFR3	Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit delta isoform	Infinite
AASDHPPT	B4DDW7	L-aminoadipate-semialdehyde dehydrogenase-phosphopantetheinyl transferase	Infinite
ACAD8	B7Z5W4	Isobutyryl-CoA dehydrogenase, mitochondrial	715.6
ACADM	B4DJE7	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial	20.0
ACADS	E9PE82	Short-chain specific acyl-CoA dehydrogenase, mitochondrial	58.5
ACADSB	B4DQ51	Short/branched chain specific acyl-CoA dehydrogenase, mitochondrial	28.9
ACO1,IRP1	P21399	Cytoplasmic aconitate hydratase	Infinite
ACOT9	C9J7L8	Acyl-coenzyme A thioesterase 9, mitochondrial	11.0
ACTR1A	B4DXP9	Alpha-centractin	Infinite
ACTR1B	P42025	Beta-centractin	Infinite
ADSSL1	G3V232	Adenylosuccinate synthetase isozyme 1	10.7
AKR1B1	E9PCX2	Aldose reductase	Infinite
AKR7A2	H3BLU7	Aflatoxin B1 aldehyde reductase member 2	Infinite

AKT2	A8MX96	RAC-beta serine/threonine-protein kinase	Infinite
ALDH4A1	P30038	Delta-1-pyrroline-5-carboxylate dehydrogenase, mitochondrial	Infinite
ALDH6A1	G3V4Z4	Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial	Infinite
ANXA5	D6RBE9	Annexin A5;Annexin	Infinite
AP1G1	B3KXW5	AP-1 complex subunit gamma-1	Infinite
APPL1	C9JAB0	DCC-interacting protein 13-alpha	Infinite
APRT	H3BQB1	Adenine phosphoribosyltransferase	Infinite
ARCN1	B0YIW6	Coatomer subunit delta	Infinite
ARHGDIA	J3KRE2	Rho GDP-dissociation inhibitor 1	23.1
ART3	E7ER42	Ecto-ADP-ribosyltransferase 3	18.6
ASNA1	K7ERW9	ATPase ASNA1	Infinite
ATP1B1	A6NGH2	Sodium/potassium-transporting ATPase subunit beta-1	Infinite
ATP5J2,PTCD1	C9JJT5	ATP synthase subunit f, mitochondrial	10.6
ATP5S	Q8WXQ4	ATP synthase subunit s, mitochondrial	Infinite
ATP6V1B2	C9J5E3	V-type proton ATPase subunit B, brain isoform	Infinite
ATP6V1E1	C9J8H1	V-type proton ATPase subunit E 1	22.0
BLVRB	M0QZL1	Flavin reductase (NADPH)	10.1
BPNT1	A6NF51	3(2),5-bisphosphate nucleotidase 1	Infinite
BZW2	B5MCE7	Basic leucine zipper and W2 domain-containing protein 2	112.6
C1ORF57,NTPCR	Q5TDF0	Cancer-related nucleoside-triphosphatase	50.4
C21ORF33	F2Z2Q0	ES1 protein homolog, mitochondrial	Infinite
CA1	E5RFE7	Carbonic anhydrase 1	Infinite
CAB39	B7ZBJ4	Calcium-binding protein 39	34.3
CAMK2G	B4DVQ3	Calcium/calmodulin-dependent protein kinase type II subunit gamma	46.8
CAND1	H0YH27	Cullin-associated NEDD8-dissociated protein 1	96.8
CAPN2	P17655	Calpain-2 catalytic subunit	11.1

CARM1	K7EK20	Histone-arginine methyltransferase CARM1	13.3
CARNS1	A5YM72	Carnosine synthase 1	12.7
CAV1	E9PCT5	Caveolin-1;Caveolin	Infinite
CBR1	A8MTM1	Carbonyl reductase [NADPH] 1	Infinite
CCDC6	Q16204	Coiled-coil domain-containing protein 6	Infinite
CCT2	F5GWF6	T-complex protein 1 subunit beta	97.7
CCT6A	B4DPJ8	T-complex protein 1 subunit zeta	11.6
CDC37	K7EIU0	Hsp90 co-chaperone Cdc37	27.3
CECR5	A8MYZ9	Cat eye syndrome critical region protein 5	Infinite
CLPX			Infinite
COPA	P53621	Coatomer subunit alpha;Xenin;Proxenin	16.9
COPS2	B4DIH5	COP9 signalosome complex subunit 2	115.6
COPS3	C9JLV5	COP9 signalosome complex subunit 3	17.9
COPS6	E7EM64	COP9 signalosome complex subunit 6	18.2
COPS7A	F5GXT7	COP9 signalosome complex subunit 7a	14.5
COQ3	Q5T063	Hexaprenyldihydroxybenzoate methyltransferase, mitochondrial	14.1
CTBP1	D6RAX2	C-terminal-binding protein 1	Infinite
CUL1	Q13616	Cullin-1	13.9
DARS,DKFZP781B11202	C9J7S3	Aspartate--tRNA ligase, cytoplasmic	10.6
DDX19A,DDX19B	B4DRZ7	ATP-dependent RNA helicase DDX19A;ATP-dependent RNA helicase DDX19B	15.5
DNAJA4	C9JDE6	DnaJ homolog subfamily A member 4	12.3
DPP9	M0QXA6	Dipeptidyl peptidase 9	Infinite
ECI2	C9J000	Enoyl-CoA delta isomerase 2, mitochondrial	14.9
EHD1	C9J2Z4	EH domain-containing protein 1	Infinite
EIF2B1	B4DGX0	Translation initiation factor eIF-2B subunit alpha	240.6
EIF3F	B3KSH1	Eukaryotic translation initiation factor 3 subunit F	56.5

EIF3I	Q13347	Eukaryotic translation initiation factor 3 subunit I	Infinite
EIF3M	B4E2Q4	Eukaryotic translation initiation factor 3 subunit M	586.7
EIF4A1	B4E102	Eukaryotic initiation factor 4A-I	Infinite
EIF4A2	E7EQG2	Eukaryotic initiation factor 4A-II	Infinite
ENDOG	Q14249	Endonuclease G, mitochondrial	35.2
FAF1	B1ANM7	FAS-associated factor 1	Infinite
FAHD1	Q6P587	Acylpyruvase FAHD1, mitochondrial	Infinite
FAM49B	E5RFS4	Protein FAM49B	Infinite
FBP2	O00757	Fructose-1,6-bisphosphatase isozyme 2	166.1
FERMT2	G3V1L6	Fermitin family homolog 2	7502.3
FH	P07954	Fumarate hydratase, mitochondrial	12.3
FLNA	E9PHF0	Filamin-A	19.6
FLNB	E7EN95	Filamin-B	32.9
GALK1	B4E1G6	Galactokinase	Infinite
GARS	H7C443	Glycine--tRNA ligase	Infinite
GCDH	B4DK85	Glutaryl-CoA dehydrogenase, mitochondrial	32.0
GDI2	E7EU23	Rab GDP dissociation inhibitor beta	12.5
GFPT1	E5RJP4	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] 1	Infinite
GGH	Q92820	Gamma-glutamyl hydrolase	Infinite
GMPPA	C9J255	Mannose-1-phosphate guanyltransferase alpha	Infinite
GMPPB	Q9Y5P6	Mannose-1-phosphate guanyltransferase beta	Infinite
GPD1L	C9JFA7	Glycerol-3-phosphate dehydrogenase 1-like protein	17.2
GPS1	C9JFE4	COP9 signalosome complex subunit 1	692.0
GSN	P06396	Gelsolin;Isoform 4 of Gelsolin	22.1
GSTM3	P21266	Glutathione S-transferase Mu 3	63.2
HAGH	E7EN93	Hydroxyacylglutathione hydrolase, mitochondrial	26.5
HDDC2	Q7Z4H3	HD domain-containing protein 2	Infinite
HMGCL	B1AK13	Hydroxymethylglutaryl-CoA lyase, mitochondrial	17.9

HMGCS2	P54868	Hydroxymethylglutaryl-CoA synthase, mitochondrial	Infinite
HSD17B8	Q92506	Estradiol 17-beta-dehydrogenase 8	101.7
IDH3B	O43837	Isocitrate dehydrogenase [NAD] subunit beta, mitochondrial	23.9
IDH3G	E7EQB8	Isocitrate dehydrogenase [NAD] subunit gamma, mitochondrial	17.7
IFIT2	P09913	Interferon-induced protein with tetratricopeptide repeats 2	Infinite
IGBP1	P78318	Immunoglobulin-binding protein 1	61343.7
IMPDH2	C9J381	Inosine-5-monophosphate dehydrogenase 2	Infinite
IQGAP1	E9PDT6	Ras GTPase-activating-like protein IQGAP1	64.3
IVD	H0YKV0	Isovaleryl-CoA dehydrogenase, mitochondrial	Infinite
KIF5B	C9JWB9	Kinesin-1 heavy chain	Infinite
KPNA3	H0Y4S9	Importin subunit alpha-3	Infinite
KPNA4	H7C4F6	Importin subunit alpha-4	56.9
LAMP1	B4DWL3	Lysosome-associated membrane glycoprotein 1	Infinite
LAP3	H0Y983	Cytosol aminopeptidase	Infinite
LARS	B4DER1	Leucine--tRNA ligase, cytoplasmic	98.0
LUM	P51884	Lumican	72.8
LYPLAL1	Q5VWZ2	Lysophospholipase-like protein 1	11.8
MAML3	E7EVW8	Mastermind-like protein 3	Infinite
MAOB	B7Z242	Amine oxidase [flavin-containing] B	13.8
MAP2K1	G5E9C7	Dual specificity mitogen-activated protein kinase kinase 1	87.8
MAP2K6	K7EIW3	Dual specificity mitogen-activated protein kinase kinase 6	118.7
MARCKS	P29966	Myristoylated alanine-rich C-kinase substrate	Infinite
MPST	B1AH49	3-mercaptopyruvate sulfurtransferase;Sulfurtransferase	14.5
MUSTN1,TMEM110			Infinite
MYH11	E7ERA5	Myosin-11	Infinite
MYH14	F2Z2U8	Myosin-14	72.7

MYL12A,MYL12B,MYL9	J3KTJ1	Myosin regulatory light chain 12B;Myosin regulatory light chain 12A;Myosin regulatory light polypeptide 9	Infinite
NAP1L4	A8MXH2	Nucleosome assembly protein 1-like 4	14.4
NEK7	C9J1H8	Serine/threonine-protein kinase Nek7	357.1
NT5C3	B9A035	Cytosolic 5-nucleotidase 3	Infinite
OTUB1	F5GYJ8	Ubiquitin thioesterase OTUB1	27.4
PACSIN3	E9PIY1	Protein kinase C and casein kinase substrate in neurons protein 3	26.5
PCTP	I3L2M9	Phosphatidylcholine transfer protein	Infinite
PCYOX1	B7Z3Y2	Prenylcysteine oxidase 1	12.2
PDE4D	D6RHE0	cAMP-specific 3,5-cyclic phosphodiesterase 4D	Infinite
PDIA3	G5EA52	Protein disulfide-isomerase A3;Thioredoxin	39.9
PDIA6	B5MCQ5	Protein disulfide-isomerase A6	59.1
PDK2	D6R983	[Pyruvate dehydrogenase [lipoamide]] kinase isozyme 2, mitochondrial	181.5
PGAM2	P15259	Phosphoglycerate mutase 2	36.1
PLCD4	C9JAE4	1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase delta-4	2677.5
PLIN5	K7EIX1	Perilipin-5	15.3
PPME1	F5H2D4	Protein phosphatase methylesterase 1	Infinite
PPP1R7	B5MBZ8	Protein phosphatase 1 regulatory subunit 7	Infinite
PPP4C	H3BTA2	Serine/threonine-protein phosphatase 4 catalytic subunit;Serine/threonine-protein phosphatase	Infinite
PPP4R2	C9IZ04	Serine/threonine-protein phosphatase 4 regulatory subunit 2	Infinite
PRELP	P51888	Prolargin	Infinite
PRKAB2	B4DH06	5-AMP-activated protein kinase subunit beta-2	17.4
PRKAG1	E9PGP6	5-AMP-activated protein kinase subunit gamma-1	27.0
PRKG1	B1ALS0	cGMP-dependent protein kinase 1	49.1

PSMA7,PSMA8	H0Y586	Proteasome subunit alpha type-7;Proteasome subunit alpha type-7-like	Infinite
PSMC1	B4DR63	26S protease regulatory subunit 4	17.6
PSMC2	B7Z5E2	26S protease regulatory subunit 7	33.9
PSMC3	E9PKD5	26S protease regulatory subunit 6A	13.7
PSMC5	J3KRP2	26S protease regulatory subunit 8	12.1
PSMC6	H0YJC0	26S protease regulatory subunit 10B	19.5
PSMD1	C9J9M4	26S proteasome non-ATPase regulatory subunit 1	13.9
PSMD11	J3KSW3	26S proteasome non-ATPase regulatory subunit 11	28.2
PSMD12	J3KSK1	26S proteasome non-ATPase regulatory subunit 12	24.5
PSMD13	E9PL38	26S proteasome non-ATPase regulatory subunit 13	40.0
PSMD14	C9JW37	26S proteasome non-ATPase regulatory subunit 14	11.9
PSME2	H0YKU2	Proteasome activator complex subunit 2	27.9
PTGR1	F2Z3J9	Prostaglandin reductase 1	Infinite
PTPN11	H0YF12	Tyrosine-protein phosphatase non-receptor type 11	Infinite
RAB12	Q6IQ22	Ras-related protein Rab-12	12.7
RAB1B,RAB1C	E9PLD0	Ras-related protein Rab-1B;Putative Ras-related protein Rab-1C	32.3
RAC1			11.0
RALA,RALB	B4E040	Ras-related protein Ral-A;Ras-related protein Ral-B	61.9
RBX1	P62877	E3 ubiquitin-protein ligase RBX1	40.0
RNF114	Q9Y508	RING finger protein 114	10.2
RNF123	C9J266	E3 ubiquitin-protein ligase RNF123	20.4
RPLP0,RPLP0P6	F8VPE8	60S acidic ribosomal protein P0;60S acidic ribosomal protein P0-like	13.2
RPS15A	H3BN98	40S ribosomal protein S15a	Infinite
RPS20	E5RIP1	40S ribosomal protein S20	91.4
RPS25	P62851	40S ribosomal protein S25	328.0
RPS6KA3	B1AXG1	Ribosomal protein S6 kinase alpha-3	10.3

RRAS2	B7Z5Z2	Ras-related protein R-Ras2	134.5
S100A11	P31949	Protein S100-A11	13.9
S100A7	P31151	Protein S100-A7	Infinite
SAMHD1	A6NDZ3	SAM domain and HD domain-containing protein 1	Infinite
SCFD1	B7Z5N7	Sec1 family domain-containing protein 1	164.5
SCPEP1	Q9HB40	Retinoid-inducible serine carboxypeptidase	Infinite
SEMA6C	Q9H3T2	Semaphorin-6C	Infinite
SESN1	P58005	Sestrin-1	148.6
SLK	Q9H2G2	STE20-like serine/threonine-protein kinase	Infinite
SNX1	A6NKH4	Sorting nexin-1	575.2
SNX2	D6RC15	Sorting nexin-2	Infinite
SOD1	H7BYH4	Superoxide dismutase [Cu-Zn]	21.5
SPR	P35270	Sepiapterin reductase	11.2
SPRR3	B1AN48	Small proline-rich protein 3	11.2
SPTAN1	A6NG51	Spectrin alpha chain, brain	249.0
STARD7	C9JTD3	StAR-related lipid transfer protein 7, mitochondrial	94.3
STAT3	G8JLH9	Signal transducer and activator of transcription 3	11.3
STAT5A,STAT5B	C9J4I3	Signal transducer and activator of transcription 5B;Signal transducer and activator of transcription 5A	32.0
STRN	O43815	Striatin;Isoform 2 of Striatin	Infinite
STRN3	G3V340	Striatin-3	Infinite
SUGT1	F5H5A9	Suppressor of G2 allele of SKP1 homolog	Infinite
SYCP1	Q15431	Synaptonemal complex protein 1	Infinite
TALDO1	E9PKI8	Transaldolase	Infinite
TIMM44	M0QXU7	Mitochondrial import inner membrane translocase subunit TIM44	11.3
TIPRL	O75663	TIP41-like protein	Infinite
TLN2	H0YMT1	Talin-2	635.0
TPD52L2	O43399	Tumor protein D54	13.7
TRIM28	M0R0K9	Transcription intermediary factor 1-beta	81.2
TRIM54	Q969Q1	Tripartite motif-containing protein 54	17.7
TSN	E9PGT1	Translin	82.0
TUBB2A,TUBB2B			15.2

UBA2	B3KWB9	SUMO-activating enzyme subunit 2	Infinite
UQCRFS1,UQCRFS1P1	P0C7P4	Cytochrome b-c1 complex subunit Rieske, mitochondrial;Cytochrome b-c1 complex subunit 11;Putative cytochrome b-c1 complex subunit Rieske-like protein 1	17.5
USP7	F5H2X1	Ubiquitin carboxyl-terminal hydrolase 7;Ubiquitin carboxyl-terminal hydrolase	61.1
USP9X	O00507	Probable ubiquitin carboxyl-terminal hydrolase FAF-X	Infinite
VARS	A2ABF4	Valine--tRNA ligase	42.8
VPS28	E9PI55	Vacuolar protein sorting-associated protein 28 homolog	68.0
VPS4A	I3L4J1	Vacuolar protein sorting-associated protein 4A	Infinite
WARS	G3V227	Tryptophan--tRNA ligase, cytoplasmic;T1-TrpRS;T2-TrpRS	Infinite
XRCC5	C9JZ81	X-ray repair cross-complementing protein 5	199.5

Table 10. 69 proteins PP2Ac partners in human skeletal muscle with significant change among different groups

Gene name	Protein ID	Protein name	enrich-ment ratio
PPP2R1A	B3KQV6	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform	213.3
ACADM	B4DJE7	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial	20.0
ACADS	E9PE82	Short-chain specific acyl-CoA dehydrogenase, mitochondrial	58.5
ACO1, IRP1	P21399	Cytoplasmic aconitate hydratase	Infinite
ACOT9	C9J7L8	Acyl-coenzyme A thioesterase 9, mitochondrial	11.0
AKR1B1	E9PCX2	Aldose reductase	Infinite
AKR7A2	H3BLU7	Aflatoxin B1 aldehyde reductase member 2	Infinite
AKT2	A8MX96	RAC-beta serine/threonine-protein kinase	Infinite
ART3	E7ER42	Ecto-ADP-ribosyltransferase 3	18.6
ATP5S	Q8WXQ4	ATP synthase subunit s, mitochondrial	Infinite
ATP6V1B2	C9J5E3	V-type proton ATPase subunit B, brain isoform	Infinite
BLVRB	M0QZL1	Flavin reductase (NADPH)	10.1
CA1	E5RFE7	Carbonic anhydrase 1	Infinite
CAMK2G	B4DVQ3	Calcium/calmodulin-dependent protein kinase type II subunit gamma	46.8
CAV1	E9PCT5	Caveolin-1;Caveolin	Infinite
CCDC6	Q16204	Coiled-coil domain-containing protein 6	Infinite
CCT2	F5GWF6	T-complex protein 1 subunit beta	97.7
CCT6A	B4DPJ8	T-complex protein 1 subunit zeta	11.6
CLPX			Infinite
COPS2	B4DIH5	COP9 signalosome complex subunit 2	115.6
CTBP1	D6RAX2	C-terminal-binding protein 1	Infinite
DPP9	M0QXA6	Dipeptidyl peptidase 9	Infinite
EIF2B1	B4DGX0	Translation initiation factor eIF-2B subunit alpha	240.6
EIF3M	B4E2Q4	Eukaryotic translation initiation factor 3 subunit M	586.7
FERMT2	G3V1L6	Fermitin family homolog 2	7502.3
FLNA	E9PHF0	Filamin-A	19.6

FLNB	E7EN95	Filamin-B	32.9
GARS	H7C443	Glycine--tRNA ligase	Infinite
GFPT1	E5RJP4	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] 1	Infinite
GGH	Q92820	Gamma-glutamyl hydrolase	Infinite
GMPPA	C9J255	Mannose-1-phosphate guanyltransferase alpha	Infinite
GPS1	C9JFE4	COP9 signalosome complex subunit 1	692.0
GSTM3	P21266	Glutathione S-transferase Mu 3	63.2
HSD17B8	Q92506	Estradiol 17-beta-dehydrogenase 8	101.7
IFIT2	P09913	Interferon-induced protein with tetratricopeptide repeats 2	Infinite
IQGAP1	E9PDT6	Ras GTPase-activating-like protein IQGAP1	64.3
KIF5B	C9JWB9	Kinesin-1 heavy chain	Infinite
LAP3	H0Y983	Cytosol aminopeptidase	Infinite
LUM	P51884	Lumican	72.8
MPST	B1AH49	3-mercaptopyruvate sulfurtransferase;Sulfurtransferase	14.5
PDE4D	D6RHE0	cAMP-specific 3,5-cyclic phosphodiesterase 4D	Infinite
PGAM2	P15259	Phosphoglycerate mutase 2	36.1
PPME1	F5H2D4	Protein phosphatase methylesterase 1	Infinite
PPP1R7	B5MBZ8	Protein phosphatase 1 regulatory subunit 7	Infinite
PPP4R2	C9IZ04	Serine/threonine-protein phosphatase 4 regulatory subunit 2	Infinite
PRELP	P51888	Prolargin	Infinite
PSMC2	B7Z5E2	26S protease regulatory subunit 7	33.9
PSMC3	E9PKD5	26S protease regulatory subunit 6A	13.7
PSMD1	C9J9M4	26S proteasome non-ATPase regulatory subunit 1	13.9
PSMD11	J3KSW3	26S proteasome non-ATPase regulatory subunit 11	28.2
PSMD12	J3KSK1	26S proteasome non-ATPase regulatory subunit 12	24.5
PSMD13	E9PL38	26S proteasome non-ATPase regulatory subunit 13	40.0
PSMD14	C9JW37	26S proteasome non-ATPase regulatory subunit 14	11.9
PSME2	H0YKU2	Proteasome activator complex subunit 2	27.9
PTPN11	H0YF12	Tyrosine-protein phosphatase non-receptor type 11	Infinite
RPS25	P62851	40S ribosomal protein S25	328.0

S100A11	P31949	Protein S100-A11	13.9
SAMHD1	A6NDZ3	SAM domain and HD domain-containing protein 1	Infinite
SCPEP1	Q9HB40	Retinoid-inducible serine carboxypeptidase	Infinite
SESN1	P58005	Sestrin-1	148.6
SLK	Q9H2G2	STE20-like serine/threonine-protein kinase	Infinite
SOD1	H7BYH4	Superoxide dismutase [Cu-Zn]	21.5
SPRR3	B1AN48	Small proline-rich protein 3	11.2
STAT3	G8JLH9	Signal transducer and activator of transcription 3	11.3
STAT5A,STAT5B	C9J4I3	Signal transducer and activator of transcription 5B;Signal transducer and activator of transcription 5A	32.0
TALDO1	E9PKI8	Transaldolase	Infinite
UBA2	B3KWB9	SUMO-activating enzyme subunit 2	Infinite
VARS	A2ABF4	Valine--tRNA ligase	42.8
XRCC5	C9JZ81	X-ray repair cross-complementing protein 5	199.5

Table 11. Known partners from databases

Gene name	Protein name
PPP2R1A	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform
PPP2R2A	Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform
PPP2R3A	Serine/threonine-protein phosphatase 2A regulatory subunit B subunit alpha
PPP2R5D	Serine/threonine-protein phosphatase 2A 56 kDa regulatory subunit delta isoform
STRN	Striatin;Isoform 2 of Striatin
STRN3	Striatin-3
CCDC6	Coiled-coil domain-containing protein 6
CCT2	T-complex protein 1 subunit beta
CCT6A	T-complex protein 1 subunit zeta
CUL1	Cullin-1
IGBP1	Immunoglobulin-binding protein 1
PPME1	Protein phosphatase methylesterase 1
PSMC6	26S protease regulatory subunit 10B
PSMD1	26S proteasome non-ATPase regulatory subunit 1
RAC1	
SOD1	Superoxide dismutase [Cu-Zn]
TIPRL	TIP41-like protein
USP7	Ubiquitin carboxyl-terminal hydrolase 7;Ubiquitin carboxyl-terminal hydrolase
PPP4C	Serine/threonine-protein phosphatase 4 catalytic subunit;Serine/threonine-protein phosphatase
CAV1	Caveolin-1;Caveolin
AMPK	AMP-activated protein kinase

Table 12. 38 proteins; Comparing partners from both INS-1 cells and human skeletal muscle biopsies (bold italics are in common with the database proteins)

Gene name	Protein name
PPP2R1A	Serine/threonine-protein phosphatase 2A 65 kDa regulatory subunit A alpha isoform
PPP2R2A	Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B alpha isoform
PPP2R3A	Protein phosphatase 2A regulatory subunit B subunit alpha
STRN	Striatin;Isoform 2 of Striatin
CCDC6	Coiled-coil domain-containing protein 6
IGBP1	Immunoglobulin-binding protein 1
PPME1	Protein phosphatase methylesterase 1
RAC1	
AKR1B1	Aldose reductase
APPL1	DCC-interacting protein 13-alpha
ARCN1	Coatomer subunit delta
ASNA1	ATPase ASNA1
NTPCR	Cancer-related nucleoside-triphosphatase
CAND1	Cullin-associated NEDD8-dissociated protein 1
DARS	Aspartate--tRNA ligase, cytoplasmic
EIF2B1	Translation initiation factor eIF-2B subunit alpha
FAHD1	Acylpyruvase FAHD1, mitochondrial
FLNA	Filamin-A
GFPT1	Glucosamine--fructose-6-phosphate aminotransferase [isomerizing] 1
GSN	Gelsolin;Isoform 4 of Gelsolin
IDH3B	Isocitrate dehydrogenase [NAD] subunit beta, mitochondrial
MYH14	Myosin-14
NAP1L4	Nucleosome assembly protein 1-like 4
PDIA6	Protein disulfide-isomerase A6
PPP4C	Serine/threonine-protein phosphatase 4 catalytic subunit;
PPP4R2	Serine/threonine-protein phosphatase 4 regulatory subunit 2
PSMC2	26S protease regulatory subunit 7
PSMC3	26S protease regulatory subunit 6A
PSMD12	26S proteasome non-ATPase regulatory subunit 12
PSMD13	26S proteasome non-ATPase regulatory subunit 13
PSMD14	26S proteasome non-ATPase regulatory subunit 14
RAB1B	Ras-related protein Rab-1B;Putative Ras-related protein Rab-1C
RPS15A	40S ribosomal protein S15a
RPS25	40S ribosomal protein S25
S100A11	Protein S100-A11
TALDO1	Transaldolase
TSN	Translin
TUBB2A	

REFERENCES

1. World Health Organization. Global report on diabetes. Vol. 2017 (2016).
2. Center for Disease Control. Long-term Trends in Diabetes Vol. 2017 (2017).
3. Pascolini, D. & Mariotti, S.P. Global estimates of visual impairment: 2010. *The British journal of ophthalmology* **96**, 614-618 (2012).
4. Bourne, R.R., *et al.* Causes of vision loss worldwide, 1990-2010: a systematic analysis. *The Lancet. Global health* **1**, e339-349 (2013).
5. Sarwar, N., *et al.* Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet (London, England)* **375**, 2215-2222 (2010).
6. Global status report on noncommunicable diseases 2010 Geneva: World Health Organization; x. (Global status report on noncommunicable diseases 2010 Geneva: World Health Organization; 2011).
7. System, U.S.R.D. USRDS annual data report: Epidemiology of kidney disease in the United States. National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD, 2014. (2014).
8. Moxey, P.W., *et al.* Lower extremity amputations--a review of global variability in incidence. *Diabetic medicine : a journal of the British Diabetic Association* **28**, 1144-1153 (2011).
9. Mathers, C.D. & Loncar, D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS medicine* **3**, e442 (2006).
10. DeFronzo, R.A. & Tripathy, D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes care* **32 Suppl 2**, S157-163 (2009).

11. Cersosimo, E., Triplitt, C., Mandarino, L.J. & DeFronzo, R.A. Pathogenesis of Type 2 Diabetes Mellitus. in *Endotext* (eds. De Groot, L.J., *et al.*) (MDText.com, Inc., South Dartmouth (MA), 2000).

12. Leto, D. & Saltiel, A.R. Regulation of glucose transport by insulin: traffic control of GLUT4. *Nature reviews. Molecular cell biology* **13**, 383-396 (2012).

13. Fu, Z., Gilbert, E.R. & Liu, D. Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes. *Current diabetes reviews* **9**, 25-53 (2013).

14. Boucher, J., Kleinridders, A. & Kahn, C.R. Insulin receptor signaling in normal and insulin-resistant states. *Cold Spring Harbor perspectives in biology* **6**(2014).

15. Siddle, K. Signalling by insulin and IGF receptors: supporting acts and new players. *Journal of molecular endocrinology* **47**, R1-10 (2011).

16. Saltiel, A.R. & Kahn, C.R. Insulin signalling and the regulation of glucose and lipid metabolism. *Nature* **414**, 799-806 (2001).

17. Goldstein, B.J., Ahmad, F., Ding, W., Li, P.M. & Zhang, W.R. Regulation of the insulin signalling pathway by cellular protein-tyrosine phosphatases. *Molecular and cellular biochemistry* **182**, 91-99 (1998).

18. Brady, M.J. & Saltiel, A.R. The role of protein phosphatase-1 in insulin action. *Recent progress in hormone research* **56**, 157-173 (2001).

19. Millward, T.A., Zolnierowicz, S. & Hemmings, B.A. Regulation of protein kinase cascades by protein phosphatase 2A. *Trends in biochemical sciences* **24**, 186-191 (1999).

20. Ni, Y.G., *et al.* FoxO transcription factors activate Akt and attenuate insulin signaling in heart by inhibiting protein phosphatases. *Proceedings of the*

National Academy of Sciences of the United States of America **104**, 20517-20522 (2007).

21. Brognard, J. & Newton, A.C. PHLiPPing the switch on Akt and protein kinase C signaling. *Trends in endocrinology and metabolism: TEM* **19**, 223-230 (2008).

22. Chagpar, R.B., *et al.* Direct positive regulation of PTEN by the p85 subunit of phosphatidylinositol 3-kinase. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 5471-5476 (2010).

23. Suwa, A., Kurama, T. & Shimokawa, T. SHIP2 and its involvement in various diseases. *Expert opinion on therapeutic targets* **14**, 727-737 (2010).

24. Holt, L.J., *et al.* Dual ablation of Grb10 and Grb14 in mice reveals their combined role in regulation of insulin signaling and glucose homeostasis. *Molecular endocrinology (Baltimore, Md.)* **23**, 1406-1414 (2009).

25. Ueki, K., Kondo, T. & Kahn, C.R. Suppressor of cytokine signaling 1 (SOCS-1) and SOCS-3 cause insulin resistance through inhibition of tyrosine phosphorylation of insulin receptor substrate proteins by discrete mechanisms. *Molecular and cellular biology* **24**, 5434-5446 (2004).

26. Du, K., Herzig, S., Kulkarni, R.N. & Montminy, M. TRB3: a tribbles homolog that inhibits Akt/PKB activation by insulin in liver. *Science (New York, N.Y.)* **300**, 1574-1577 (2003).

27. Chakraborty, A., *et al.* Inositol pyrophosphates inhibit Akt signaling, thereby regulating insulin sensitivity and weight gain. *Cell* **143**, 897-910 (2010).

28. DeFronzo, R.A. Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes* **37**, 667-687 (1988).

29. Leahy, J.L. Pathogenesis of type 2 diabetes mellitus. *Archives of medical research* **36**, 197-209 (2005).

30. Butler, A.E., *et al.* Beta-cell deficit and increased beta-cell apoptosis in humans with type 2 diabetes. *Diabetes* **52**, 102-110 (2003).

31. Poitout, V. & Robertson, R.P. Glucolipotoxicity: fuel excess and beta-cell dysfunction. *Endocrine reviews* **29**, 351-366 (2008).

32. Khaldi, M.Z., Guiot, Y., Gilon, P., Henquin, J.C. & Jonas, J.C. Increased glucose sensitivity of both triggering and amplifying pathways of insulin secretion in rat islets cultured for 1 wk in high glucose. *American journal of physiology. Endocrinology and metabolism* **287**, E207-217 (2004).

33. Maedler, K., *et al.* Glucose-induced beta cell production of IL-1beta contributes to glucotoxicity in human pancreatic islets. *The Journal of clinical investigation* **110**, 851-860 (2002).

34. Aguirre, V., Uchida, T., Yenush, L., Davis, R. & White, M.F. The c-Jun NH(2)-terminal kinase promotes insulin resistance during association with insulin receptor substrate-1 and phosphorylation of Ser(307). *The Journal of biological chemistry* **275**, 9047-9054 (2000).

35. Martinez, S.C., *et al.* Inhibition of Foxo1 protects pancreatic islet beta-cells against fatty acid and endoplasmic reticulum stress-induced apoptosis. *Diabetes* **57**, 846-859 (2008).

36. Briaud, I., *et al.* Insulin receptor substrate-2 proteasomal degradation mediated by a mammalian target of rapamycin (mTOR)-induced negative feedback down-regulates protein kinase B-mediated signaling pathway in beta-cells. *The Journal of biological chemistry* **280**, 2282-2293 (2005).

37. Chan, C.B., *et al.* Increased uncoupling protein-2 levels in beta-cells are associated with impaired glucose-stimulated insulin secretion: mechanism of action. *Diabetes* **50**, 1302-1310 (2001).

38. Schmitz-Peiffer, C., *et al.* Inhibition of PKCepsilon improves glucose-stimulated insulin secretion and reduces insulin clearance. *Cell metabolism* **6**, 320-328 (2007).

39. Olofsson, C.S., *et al.* Long-term exposure to glucose and lipids inhibits glucose-induced insulin secretion downstream of granule fusion with plasma membrane. *Diabetes* **56**, 1888-1897 (2007).

40. Hoppa, M.B., *et al.* Chronic palmitate exposure inhibits insulin secretion by dissociation of Ca(2+) channels from secretory granules. *Cell metabolism* **10**, 455-465 (2009).

41. Zolnierowicz, S. Type 2A protein phosphatase, the complex regulator of numerous signaling pathways. *Biochemical Pharmacology* **60**, 1225-1235 (2000).

42. Fischer, E.H. & Krebs, E.G. Conversion of phosphorylase b to phosphorylase a in muscle extracts. *The Journal of biological chemistry* **216**, 121-132 (1955).

43. Olsen, J.V., *et al.* Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. *Cell* **127**, 635-648 (2006).

44. Sun, X.J., Goldberg, J.L., Qiao, L.Y. & Mitchell, J.J. Insulin-induced insulin receptor substrate-1 degradation is mediated by the proteasome degradation pathway. *Diabetes* **48**, 1359-1364 (1999).

45. Seshacharyulu, P., Pandey, P., Datta, K. & Batra, S.K. Phosphatase: PP2A structural importance, regulation and its aberrant expression in cancer. *Cancer letters* **335**, 9-18 (2013).

46. Shi, Y. Serine/Threonine Phosphatases: Mechanism through Structure. *Cell* **139**, 468-484 (2009).

47. Cho, U.S. & Xu, W. Crystal structure of a protein phosphatase 2A heterotrimeric holoenzyme. *Nature* **445**, 53-57 (2007).

48. Lin, X.H., *et al.* Protein phosphatase 2A is required for the initiation of chromosomal DNA replication. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 14693-14698 (1998).

49. Kremmer, E., Ohst, K., Kiefer, J., Brewis, N. & Walter, G. Separation of PP2A core enzyme and holoenzyme with monoclonal antibodies against the regulatory A subunit: abundant expression of both forms in cells. *Molecular and cellular biology* **17**, 1692-1701 (1997).

50. Groves, M.R., Hanlon, N., Turowski, P., Hemmings, B.A. & Barford, D. The structure of the protein phosphatase 2A PR65/A subunit reveals the conformation of its 15 tandemly repeated HEAT motifs. *Cell* **96**, 99-110 (1999).

51. Berridge, Michael J. Cell Signalling Biology: Module 5 - Off Mechanisms. *Biochemical Journal* (2012).

52. Price, N.E. & Mumby, M.C. Effects of regulatory subunits on the kinetics of protein phosphatase 2A. *Biochemistry* **39**, 11312-11318 (2000).

53. Janssens, V. & Goris, J. Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. *The Biochemical journal* **353**, 417-439 (2001).

54. Silverstein, A.M., Barrow, C.A., Davis, A.J. & Mumby, M.C. Actions of PP2A on the MAP kinase pathway and apoptosis are mediated by distinct regulatory subunits. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 4221-4226 (2002).

55. Jiang, L., *et al.* Structural basis of protein phosphatase 2A stable latency. *Nature communications* **4**, 1699 (2013).

56. Guo, F., *et al.* Structural basis of PP2A activation by PTPA, an ATP-dependent activation chaperone. *Cell research* **24**, 190-203 (2014).

57. Chen, J., Martin, B.L. & Brautigan, D.L. Regulation of protein serine-threonine phosphatase type-2A by tyrosine phosphorylation. *Science (New York, N.Y.)* **257**, 1261-1264 (1992).

58. Kirchhefer, U., *et al.* Protein phosphatase 2A is regulated by protein kinase Calpha (PKCalpha)-dependent phosphorylation of its targeting subunit B56alpha at Ser41. *The Journal of biological chemistry* **289**, 163-176 (2014).

59. Janssens, V., Longin, S. & Goris, J. PP2A holoenzyme assembly: in cauda venenum (the sting is in the tail). *Trends in biochemical sciences* **33**, 113-121 (2008).

60. Sents, W., Ivanova, E., Lambrecht, C., Haesen, D. & Janssens, V. The biogenesis of active protein phosphatase 2A holoenzymes: a tightly regulated process creating phosphatase specificity. *The FEBS journal* **280**, 644-661 (2013).

61. Yabe, R., *et al.* Protein Phosphatase Methyl-Esterase PME-1 Protects Protein Phosphatase 2A from Ubiquitin/Proteasome Degradation. *PloS one* **10**, e0145226 (2015).

62. Caruso, M., *et al.* Increased Interaction With Insulin Receptor Substrate 1, a Novel Abnormality in Insulin Resistance and Type 2 Diabetes. *Diabetes* **63**, 1933-1947 (2014).

63. Mandavia, C. & Sowers, J.R. Phosphoprotein Phosphatase PP2A Regulation of Insulin Receptor Substrate 1 and Insulin Metabolic Signaling. *Cardiorenal medicine* **2**, 308-313 (2012).

64. Srinivasan, M. & Begum, N. Regulation of protein phosphatase 1 and 2A activities by insulin during myogenesis in rat skeletal muscle cells in culture. *The Journal of biological chemistry* **269**, 12514-12520 (1994).

65. Cazzolli, R., Carpenter, L., Biden, T.J. & Schmitz-Peiffer, C. A role for protein phosphatase 2A-like activity, but not atypical protein kinase Czeta, in the inhibition of protein kinase B/Akt and glycogen synthesis by palmitate. *Diabetes* **50**, 2210-2218 (2001).

66. Hojlund, K., Poulsen, M., Staehr, P., Brusgaard, K. & Beck-Nielsen, H. Effect of insulin on protein phosphatase 2A expression in muscle in type 2 diabetes. *European journal of clinical investigation* **32**, 918-923 (2002).

67. Nardi, F., *et al.* Enhanced insulin sensitivity associated with provision of mono and polyunsaturated fatty acids in skeletal muscle cells involves counter modulation of PP2A. *PloS one* **9**, e92255 (2014).

68. Kowluru, A., Seavey, S.E., Rabaglia, M.E., Nesher, R. & Metz, S.A. Carboxylmethylation of the catalytic subunit of protein phosphatase 2A in insulin-secreting cells: evidence for functional consequences on enzyme activity and insulin secretion. *Endocrinology* **137**, 2315-2323 (1996).

69. Muniyappa, R., Lee, S., Chen, H. & Quon, M.J. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. *American journal of physiology. Endocrinology and metabolism* **294**, E15-26 (2008).

70. Kim, J.K. Hyperinsulinemic-euglycemic clamp to assess insulin sensitivity in vivo. *Methods in molecular biology (Clifton, N.J.)* **560**, 221-238 (2009).

71. Pawson, T. & Nash, P. Protein-protein interactions define specificity in signal transduction. *Genes & development* **14**, 1027-1047 (2000).

72. Miller, J.P. & Hughes, R.E. Frontiers in Neuroscience Protein Interactions and Target Discovery in Huntington's Disease. in *Neurobiology of Huntington's Disease: Applications to Drug Discovery* (eds. Lo, D.C. & Hughes, R.E.) (CRC Press Llc., Boca Raton (FL), 2011).

73. Rao, V.S., Srinivas, K., Sujini, G.N. & Kumar, G.N. Protein-Protein Interaction Detection: Methods and Analysis. *International journal of proteomics* **2014**, 147648 (2014).

74. Gonzalez, M.W. & Kann, M.G. Chapter 4: Protein Interactions and Disease. *PLoS Comput Biol* **8**, e1002819 (2012).

75. Vermeulen, M., Hubner, N.C. & Mann, M. High confidence determination of specific protein-protein interactions using quantitative mass spectrometry. *Current opinion in biotechnology* **19**, 331-337 (2008).

76. Han, X., Aslanian, A. & Yates, J.R., 3rd. Mass spectrometry for proteomics. *Current opinion in chemical biology* **12**, 483-490 (2008).

77. Angel, T.E., *et al.* Mass spectrometry-based proteomics: existing capabilities and future directions. *Chemical Society reviews* **41**, 3912-3928 (2012).

78. Caruso, M., *et al.* Increased interaction with insulin receptor substrate 1, a novel abnormality in insulin resistance and type 2 diabetes. *Diabetes* **63**, 1933-1947 (2014).

79. Geetha, T., *et al.* Label-free proteomic identification of endogenous, insulin-stimulated interaction partners of insulin receptor substrate-1. *Journal of the American Society for Mass Spectrometry* **22**, 457-466 (2011).

80. Chen, X.W., *et al.* SEC24A deficiency lowers plasma cholesterol through reduced PCSK9 secretion. *eLife* **2**, e00444 (2013).

81. Cox, J. & Mann, M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nature biotechnology* **26**, 1367-1372 (2008).

82. Martinez, E., *et al.* Myocardium proteome remodelling after nutritional deprivation of methyl donors. *The Journal of nutritional biochemistry* **24**, 1241-1250 (2013).

83. Ye, Y., *et al.* Quantitative proteomics by amino acid labeling in foot-and-mouth disease virus (FMDV)-infected cells. *Journal of proteome research* **12**, 363-377 (2013).

84. Thomas, S. & Bonchev, D. A survey of current software for network analysis in molecular biology. *Human genomics* **4**, 353-360 (2010).

85. Jimenez-Marin, A., Collado-Romero, M., Ramirez-Boo, M., Arce, C. & Garrido, J.J. Biological pathway analysis by ArrayUnlock and Ingenuity Pathway Analysis. *BMC Proc* **3 Suppl 4**, S6 (2009).

86. Werner, T. Bioinformatics applications for pathway analysis of microarray data. *Curr Opin Biotechnol* **19**, 50-54 (2008).

87. Syeda, K., Mohammed, A.M., Arora, D.K. & Kowluru, A. Glucotoxic conditions induce endoplasmic reticulum stress to cause caspase 3 mediated lamin B degradation in pancreatic beta-cells: protection by nifedipine. *Biochem Pharmacol* **86**, 1338-1346 (2013).

88. Khadija, S., Veluthakal, R., Sidarala, V. & Kowluru, A. Glucotoxic and diabetic conditions induce caspase 6-mediated degradation of nuclear lamin A in human islets, rodent islets and INS-1 832/13 cells. *Apoptosis : an international journal on programmed cell death* **19**, 1691-1701 (2014).

89. Arora, D.K., *et al.* High glucose exposure promotes activation of protein phosphatase 2A in rodent islets and INS-1 832/13 beta-cells by increasing the posttranslational carboxylmethylation of its catalytic subunit. *Endocrinology* **155**, 380-391 (2014).

90. Yi, Z., *et al.* Global assessment of regulation of phosphorylation of insulin receptor substrate-1 by insulin in vivo in human muscle. *Diabetes* **56**, 1508-1516 (2007).

91. Wepf, A., Glatter, T., Schmidt, A., Aebersold, R. & Gstaiger, M. Quantitative interaction proteomics using mass spectrometry. *Nature methods* **6**, 203-205 (2009).

92. Zhang, X., *et al.* Quantitative proteomics reveals novel protein interaction partners of PP2A catalytic subunit in pancreatic beta-cells. *Molecular and cellular endocrinology* **424**, 1-11 (2016).

93. Havugimana, P.C., *et al.* A census of human soluble protein complexes. *Cell* **150**, 1068-1081 (2012).

94. Glatter, T., Wepf, A., Aebersold, R. & Gstaiger, M. An integrated workflow for charting the human interaction proteome: insights into the PP2A system. *Molecular systems biology* **5**, 237 (2009).

95. Goudreault, M., *et al.* A PP2A phosphatase high density interaction network identifies a novel striatin-interacting phosphatase and kinase complex linked to

the cerebral cavernous malformation 3 (CCM3) protein. *Molecular & cellular proteomics : MCP* **8**, 157-171 (2009).

96. Sablina, A.A., *et al.* The tumor suppressor PP2A Abeta regulates the RalA GTPase. *Cell* **129**, 969-982 (2007).

97. Zhou, J., Pham, H.T., Ruediger, R. & Walter, G. Characterization of the Aalpha and Abeta subunit isoforms of protein phosphatase 2A: differences in expression, subunit interaction, and evolution. *The Biochemical journal* **369**, 387-398 (2003).

98. Malovannaya, A., *et al.* Streamlined analysis schema for high-throughput identification of endogenous protein complexes. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 2431-2436 (2010).

99. Cheng, A., Kaldis, P. & Solomon, M.J. Dephosphorylation of human cyclin-dependent kinases by protein phosphatase type 2C alpha and beta 2 isoforms. *The Journal of biological chemistry* **275**, 34744-34749 (2000).

100. Wang, Z. & Thurmond, D.C. Mechanisms of biphasic insulin-granule exocytosis - roles of the cytoskeleton, small GTPases and SNARE proteins. *Journal of cell science* **122**, 893-903 (2009).

101. Kowluru, A. Small G proteins in islet beta-cell function. *Endocrine reviews* **31**, 52-78 (2010).

102. Bernard, O. Lim kinases, regulators of actin dynamics. *The international journal of biochemistry & cell biology* **39**, 1071-1076 (2007).

103. Manetti, F. LIM kinases are attractive targets with many macromolecular partners and only a few small molecule regulators. *Medicinal research reviews* **32**, 968-998 (2012).

104. Scott, R.W. & Olson, M.F. LIM kinases: function, regulation and association with human disease. *Journal of molecular medicine (Berlin, Germany)* **85**, 555-568 (2007).

105. Kong, M., Ditsworth, D., Lindsten, T. & Thompson, C.B. Alpha4 is an essential regulator of PP2A phosphatase activity. *Molecular cell* **36**, 51-60 (2009).

106. McConnell, J.L., *et al.* Alpha4 is a ubiquitin-binding protein that regulates protein serine/threonine phosphatase 2A ubiquitination. *Biochemistry* **49**, 1713-1718 (2010).

107. Veluthakal, R., Wadzinski, B.E. & Kowluru, A. Localization of a nuclear serine/threonine protein phosphatase in insulin-secreting INS-1 cells: potential regulation by IL-1beta. *Apoptosis : an international journal on programmed cell death* **11**, 1401-1411 (2006).

108. Kowluru, A. & Matti, A. Hyperactivation of protein phosphatase 2A in models of glucolipotoxicity and diabetes: potential mechanisms and functional consequences. *Biochem Pharmacol* **84**, 591-597 (2012).

109. Yan, L., *et al.* The B55alpha-containing PP2A holoenzyme dephosphorylates FOXO1 in islet beta-cells under oxidative stress. *The Biochemical journal* **444**, 239-247 (2012).

110. Hein, M.Y., *et al.* A human interactome in three quantitative dimensions organized by stoichiometries and abundances. *Cell* **163**, 712-723 (2015).

111. Chen, J., *et al.* Regulation of insulin receptor substrate-1 expression levels by caveolin-1. *Journal of cellular physiology* **217**, 281-289 (2008).

112. Choi, S.M., *et al.* Insulin regulates adipocyte lipolysis via an Akt-independent signaling pathway. *Molecular and cellular biology* **30**, 5009-5020 (2010).

113. Li, L., Ren, C.H., Tahir, S.A., Ren, C. & Thompson, T.C. Caveolin-1 maintains activated Akt in prostate cancer cells through scaffolding domain binding site interactions with and inhibition of serine/threonine protein phosphatases PP1 and PP2A. *Molecular and cellular biology* **23**, 9389-9404 (2003).

114. Thanasopoulou, A., Stravopodis, D.J., Dimas, K.S., Schwaller, J. & Anastasiadou, E. Loss of CCDC6 affects cell cycle through impaired intra-S-phase checkpoint control. *PloS one* **7**, e31007 (2012).

115. St-Denis, N., *et al.* Phenotypic and Interaction Profiling of the Human Phosphatases Identifies Diverse Mitotic Regulators. *Cell reports* **17**, 2488-2501 (2016).

116. Kubota, H. Function and regulation of cytosolic molecular chaperone CCT. *Vitamins and hormones* **65**, 313-331 (2002).

117. Bennett, E.J., Rush, J., Gygi, S.P. & Harper, J.W. Dynamics of cullin-RING ubiquitin ligase network revealed by systematic quantitative proteomics. *Cell* **143**, 951-965 (2010).

118. Park, J.J., Lim, K.H. & Baek, K.H. Annexin-1 regulated by HAUSP is essential for UV-induced damage response. *Cell death & disease* **6**, e1654 (2015).

119. JeBailey, L., *et al.* Skeletal muscle cells and adipocytes differ in their reliance on TC10 and Rac for insulin-induced actin remodeling. *Molecular endocrinology (Baltimore, Md.)* **18**, 359-372 (2004).

120. Ueda, S., Kataoka, T. & Satoh, T. Activation of the small GTPase Rac1 by a specific guanine-nucleotide-exchange factor suffices to induce glucose uptake into skeletal-muscle cells. *Biology of the cell* **100**, 645-657 (2008).

121. Sylow, L., *et al.* Rac1 is a novel regulator of contraction-stimulated glucose uptake in skeletal muscle. *Diabetes* **62**, 1139-1151 (2013).

122. ten Klooster, J.P., Leeuwen, I., Scheres, N., Anthony, E.C. & Hordijk, P.L. Rac1-induced cell migration requires membrane recruitment of the nuclear oncogene SET. *The EMBO journal* **26**, 336-345 (2007).

123. Nakashima, A., *et al.* A positive role of mammalian Tip41-like protein, TIPRL, in the amino-acid dependent mTORC1-signaling pathway through interaction with PP2A. *FEBS letters* **587**, 2924-2929 (2013).

124. Steinberg, G.R. & Jorgensen, S.B. The AMP-activated protein kinase: role in regulation of skeletal muscle metabolism and insulin sensitivity. *Mini reviews in medicinal chemistry* **7**, 519-526 (2007).

125. Steinberg, G.R. & Kemp, B.E. AMPK in Health and Disease. *Physiological reviews* **89**, 1025-1078 (2009).

126. Park, S., Scheffler, T.L., Rossie, S.S. & Gerrard, D.E. AMPK activity is regulated by calcium-mediated protein phosphatase 2A activity. *Cell calcium* **53**, 217-223 (2013).

127. Sadasivan, S.K., *et al.* Modulation of de novo purine biosynthesis leads to activation of AMPK and results in improved glucose handling and insulin sensitivity. *Journal of diabetes and metabolic disorders* **13**, 51 (2014).

128. Cho, H., *et al.* Insulin resistance and a diabetes mellitus-like syndrome in mice lacking the protein kinase Akt2 (PKB beta). *Science (New York, N.Y.)* **292**, 1728-1731 (2001).

129. Andjelkovic, M., *et al.* Activation and phosphorylation of a pleckstrin homology domain containing protein kinase (RAC-PK/PKB) promoted by serum and protein phosphatase inhibitors. *Proceedings of the National Academy of Sciences of the United States of America* **93**, 5699-5704 (1996).

130. Galbo, T., Olsen, G.S., Quistorff, B. & Nishimura, E. Free fatty acid-induced PP2A hyperactivity selectively impairs hepatic insulin action on glucose metabolism. *PloS one* **6**, e27424 (2011).

131. Galbo, T., *et al.* PP2A inhibition results in hepatic insulin resistance despite Akt2 activation. *Aging* **5**, 770-781 (2013).

132. Qu, C.K. The SHP-2 tyrosine phosphatase: signaling mechanisms and biological functions. *Cell research* **10**, 279-288 (2000).

133. Lizcano, J.M. & Alessi, D.R. The insulin signalling pathway. *Current biology : CB* **12**, R236-238 (2002).

134. Ragolia, L. & Begum, N. Protein phosphatase-1 and insulin action. *Molecular and cellular biochemistry* **182**, 49-58 (1998).

135. Hartley, D. & Cooper, G.M. Role of mTOR in the degradation of IRS-1: regulation of PP2A activity. *Journal of cellular biochemistry* **85**, 304-314 (2002).

136. Laplante, M. & Sabatini, D.M. mTOR signaling at a glance. *Journal of cell science* **122**, 3589-3594 (2009).

137. Grover, J., Chen, X.N., Korenberg, J.R. & Roughley, P.J. The human lumican gene. Organization, chromosomal location, and expression in articular cartilage. *The Journal of biological chemistry* **270**, 21942-21949 (1995).

138. Nikitovic, D., Papoutsidakis, A., Karamanos, N.K. & Tzanakakis, G.N. Lumican affects tumor cell functions, tumor-ECM interactions, angiogenesis and inflammatory response. *Matrix biology : journal of the International Society for Matrix Biology* **35**, 206-214 (2014).

139. Rachek, L.I. Free fatty acids and skeletal muscle insulin resistance. *Progress in molecular biology and translational science* **121**, 267-292 (2014).

140. Tillander, V., *et al.* Acyl-CoA thioesterase 9 (ACOT9) in mouse may provide a novel link between fatty acid and amino acid metabolism in mitochondria. *Cellular and molecular life sciences : CMLS* **71**, 933-948 (2014).

141. Jatiani, S.S., Baker, S.J., Silverman, L.R. & Reddy, E.P. Jak/STAT pathways in cytokine signaling and myeloproliferative disorders: approaches for targeted therapies. *Genes & cancer* **1**, 979-993 (2010).

142. Chinnadurai, G. The transcriptional corepressor CtBP: a foe of multiple tumor suppressors. *Cancer research* **69**, 731-734 (2009).

143. Liu, P., *et al.* CtBP2 ameliorates palmitate-induced insulin resistance in HepG2 cells through ROS mediated JNK pathway. *General and comparative endocrinology* **247**, 66-73 (2017).

144. Paliwal, S., *et al.* The alternative reading frame tumor suppressor antagonizes hypoxia-induced cancer cell migration via interaction with the COOH-terminal binding protein corepressor. *Cancer research* **67**, 9322-9329 (2007).

145. Liu, C. & Yu, X. ADP-ribosyltransferases and poly ADP-ribosylation. *Current protein & peptide science* **16**, 491-501 (2015).

146. Okuma, T., Honda, R., Ichikawa, G., Tsumagari, N. & Yasuda, H. In vitro SUMO-1 modification requires two enzymatic steps, E1 and E2. *Biochemical and biophysical research communications* **254**, 693-698 (1999).

147. Wei, N., Serino, G. & Deng, X.W. The COP9 signalosome: more than a protease. *Trends in biochemical sciences* **33**, 592-600 (2008).

148. Lyapina, S., *et al.* Promotion of NEDD-CUL1 conjugate cleavage by COP9 signalosome. *Science (New York, N.Y.)* **292**, 1382-1385 (2001).

149. Scherer, P.C., *et al.* Inositol hexakisphosphate (IP6) generated by IP5K mediates cullin-COP9 signalosome interactions and CRL function. *Proceedings*

of the National Academy of Sciences of the United States of America **113**, 3503-3508 (2016).

150. Min, K.W., *et al.* CAND1 enhances deneddylation of CUL1 by COP9 signalosome. *Biochemical and biophysical research communications* **334**, 867-874 (2005).

151. Yang, X., *et al.* The COP9 signalosome inhibits p27(kip1) degradation and impedes G1-S phase progression via deneddylation of SCF Cul1. *Current biology : CB* **12**, 667-672 (2002).

152. Tsakiridis, T., *et al.* Role of the actin cytoskeleton in insulin action. *Microscopy research and technique* **47**, 79-92 (1999).

153. Patel, N., Rudich, A., Khayat, Z.A., Garg, R. & Klip, A. Intracellular segregation of phosphatidylinositol-3,4,5-trisphosphate by insulin-dependent actin remodeling in L6 skeletal muscle cells. *Molecular and cellular biology* **23**, 4611-4626 (2003).

154. Bisht, B. & Dey, C.S. Focal Adhesion Kinase contributes to insulin-induced actin reorganization into a mesh harboring Glucose transporter-4 in insulin resistant skeletal muscle cells. *BMC cell biology* **9**, 48 (2008).

155. Alberts B, J.A., Lewis J, et al. *Molecular Biology of the Cell*, (2002).

156. Suzuki, A., Pelikan, R.C. & Iwata, J. WNT/beta-Catenin Signaling Regulates Multiple Steps of Myogenesis by Regulating Step-Specific Targets. *Molecular and cellular biology* **35**, 1763-1776 (2015).

157. Noritake, J., Watanabe, T., Sato, K., Wang, S. & Kaibuchi, K. IQGAP1: a key regulator of adhesion and migration. *Journal of cell science* **118**, 2085-2092 (2005).

158. Suzuki, K. & Takahashi, K. Reduced cell adhesion during mitosis by threonine phosphorylation of beta1 integrin. *Journal of cellular physiology* **197**, 297-305 (2003).

159. Suzuki, K., Chikamatsu, Y. & Takahashi, K. Requirement of protein phosphatase 2A for recruitment of IQGAP1 to Rac-bound beta1 integrin. *Journal of cellular physiology* **203**, 487-492 (2005).

160. Takahashi, K. & Suzuki, K. Regulation of protein phosphatase 2A-mediated recruitment of IQGAP1 to beta1 integrin by EGF through activation of Ca2+/calmodulin-dependent protein kinase II. *Journal of cellular physiology* **208**, 213-219 (2006).

161. Abel, A.M., *et al.* IQGAP1: insights into the function of a molecular puppeteer. *Molecular immunology* **65**, 336-349 (2015).

162. Carmon, K.S., Gong, X., Yi, J., Thomas, A. & Liu, Q. RSPO-LGR4 functions via IQGAP1 to potentiate Wnt signaling. *Proceedings of the National Academy of Sciences of the United States of America* **111**, E1221-1229 (2014).

163. Habersetzer, J., *et al.* ATP synthase oligomerization: from the enzyme models to the mitochondrial morphology. *The international journal of biochemistry & cell biology* **45**, 99-105 (2013).

164. Hojlund, K., *et al.* Human ATP synthase beta is phosphorylated at multiple sites and shows abnormal phosphorylation at specific sites in insulin-resistant muscle. *Diabetologia* **53**, 541-551 (2010).

165. Berdeaux, R. & Stewart, R. cAMP signaling in skeletal muscle adaptation: hypertrophy, metabolism, and regeneration. *American journal of physiology. Endocrinology and metabolism* **303**, E1-17 (2012).

166. Mashili, F., Chibalin, A.V., Krook, A. & Zierath, J.R. Constitutive STAT3 phosphorylation contributes to skeletal muscle insulin resistance in type 2 diabetes. *Diabetes* **62**, 457-465 (2013).

167. Shin, Y., *et al.* Single-molecule denaturation and degradation of proteins by the AAA+ ClpXP protease. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 19340-19345 (2009).

168. Deepa, S.S., *et al.* Down-regulation of the mitochondrial matrix peptidase ClpP in muscle cells causes mitochondrial dysfunction and decreases cell proliferation. *Free radical biology & medicine* **91**, 281-292 (2016).

169. Kirby, M., Yu, D.M., O'Connor, S. & Gorrell, M.D. Inhibitor selectivity in the clinical application of dipeptidyl peptidase-4 inhibition. *Clinical science (London, England : 1979)* **118**, 31-41 (2009).

170. Olsen, C. & Wagtmann, N. Identification and characterization of human DPP9, a novel homologue of dipeptidyl peptidase IV. *Gene* **299**, 185-193 (2002).

171. Drucker, D.J. Dipeptidyl peptidase-4 inhibition and the treatment of type 2 diabetes: preclinical biology and mechanisms of action. *Diabetes care* **30**, 1335-1343 (2007).

172. Baggio, L.L. & Drucker, D.J. Harnessing the therapeutic potential of glucagon-like peptide-1: a critical review. *Treatments in endocrinology* **1**, 117-125 (2002).

173. Barski, O.A., Tipparaju, S.M. & Bhatnagar, A. The aldo-keto reductase superfamily and its role in drug metabolism and detoxification. *Drug metabolism reviews* **40**, 553-624 (2008).

174. Bril, V. & Buchanan, R.A. Long-term effects of ranirestat (AS-3201) on peripheral nerve function in patients with diabetic sensorimotor polyneuropathy. *Diabetes care* **29**, 68-72 (2006).

175. Dvornik, E., *et al.* Polyol accumulation in galactosemic and diabetic rats: control by an aldose reductase inhibitor. *Science (New York, N.Y.)* **182**, 1146-1148 (1973).

176. Gabbay, K.H., Merola, L.O. & Field, R.A. Sorbitol pathway: presence in nerve and cord with substrate accumulation in diabetes. *Science (New York, N.Y.)* **151**, 209-210 (1966).

177. Lorenzi, M. The polyol pathway as a mechanism for diabetic retinopathy: attractive, elusive, and resilient. *Experimental diabetes research* **2007**, 61038 (2007).

178. Cotter, M.A., Cameron, N.E., Robertson, S. & Ewing, I. Polyol pathway-related skeletal muscle contractile and morphological abnormalities in diabetic rats. *Experimental physiology* **78**, 139-155 (1993).

179. Muller, F.L., *et al.* Absence of CuZn superoxide dismutase leads to elevated oxidative stress and acceleration of age-dependent skeletal muscle atrophy. *Free radical biology & medicine* **40**, 1993-2004 (2006).

180. Zhang, Y., *et al.* CuZnSOD gene deletion targeted to skeletal muscle leads to loss of contractile force but does not cause muscle atrophy in adult mice. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **27**, 3536-3548 (2013).

181. Bonnefont-Rousselot, D., Bastard, J.P., Jaudon, M.C. & Delattre, J. Consequences of the diabetic status on the oxidant/antioxidant balance. *Diabetes & metabolism* **26**, 163-176 (2000).

182. Hussey, A.J. & Hayes, J.D. Human Mu-class glutathione S-transferases present in liver, skeletal muscle and testicular tissue. *Biochimica et biophysica acta* **1203**, 131-141 (1993).

183. Fell, V.L. & Schild-Poulter, C. The Ku heterodimer: function in DNA repair and beyond. *Mutation research. Reviews in mutation research* **763**, 15-29 (2015).

184. McCright, B., Rivers, A.M., Audlin, S. & Virshup, D.M. The B56 family of protein phosphatase 2A (PP2A) regulatory subunits encodes differentiation-induced phosphoproteins that target PP2A to both nucleus and cytoplasm. *The Journal of biological chemistry* **271**, 22081-22089 (1996).

185. Tehrani, M.A., Mumby, M.C. & Kamibayashi, C. Identification of a novel protein phosphatase 2A regulatory subunit highly expressed in muscle. *The Journal of biological chemistry* **271**, 5164-5170 (1996).

186. Mayer-Jaekel, R.E. & Hemmings, B.A. Protein phosphatase 2A--a 'menage a trois'. *Trends in cell biology* **4**, 287-291 (1994).

187. Kristensen, A.R., Gsponer, J. & Foster, L.J. A high-throughput approach for measuring temporal changes in the interactome. *Nature methods* **9**, 907-909 (2012).

188. Cheng, A., Gerry, S., Kaldis, P. & Solomon, M.J. Biochemical characterization of Cdk2-Speedy/Ringo A2. *BMC biochemistry* **6**, 19 (2005).

189. Dubois, T., Howell, S., Zemlickova, E. & Aitken, A. Identification of casein kinase Ialpha interacting protein partners. *FEBS letters* **517**, 167-171 (2002).

190. Hutchins, J.R., *et al.* Systematic analysis of human protein complexes identifies chromosome segregation proteins. *Science* **328**, 593-599 (2010).

191. Ghosh, P. & Kornfeld, S. Phosphorylation-induced conformational changes regulate GGAs 1 and 3 function at the trans-Golgi network. *The Journal of biological chemistry* **278**, 14543-14549 (2003).

192. Vera, J., *et al.* Heterogeneous nuclear ribonucleoprotein A2 is a SET-binding protein and a PP2A inhibitor. *Oncogene* **25**, 260-270 (2006).

193. McDonald, W.J., Thomas, L.N., Koirala, S. & Too, C.K. Progestin-inducible EDD E3 ubiquitin ligase binds to alpha4 phosphoprotein to regulate ubiquitination and degradation of protein phosphatase PP2Ac. *Molecular and cellular endocrinology* **382**, 254-261 (2014).

194. Wang, J., *et al.* Toward an understanding of the protein interaction network of the human liver. *Molecular systems biology* **7**, 536 (2011).

195. LeNoue-Newton, M., *et al.* The E3 ubiquitin ligase- and protein phosphatase 2A (PP2A)-binding domains of the Alpha4 protein are both required for Alpha4 to inhibit PP2A degradation. *The Journal of biological chemistry* **286**, 17665-17671 (2011).

196. McDonald, W.J., Sangster, S.M., Moffat, L.D., Henderson, M.J. & Too, C.K. alpha4 phosphoprotein interacts with EDD E3 ubiquitin ligase and poly(A)-binding protein. *Journal of cellular biochemistry* **110**, 1123-1129 (2010).

197. Gingras, A.C., *et al.* A novel, evolutionarily conserved protein phosphatase complex involved in cisplatin sensitivity. *Molecular & cellular proteomics : MCP* **4**, 1725-1740 (2005).

198. Liu, J., Prickett, T.D., Elliott, E., Meroni, G. & Brautigan, D.L. Phosphorylation and microtubule association of the Opitz syndrome protein mid-1 is regulated by protein phosphatase 2A via binding to the regulatory subunit alpha 4.

Proceedings of the National Academy of Sciences of the United States of America **98**, 6650-6655 (2001).

199. Maeda, K., Inui, S., Tanaka, H. & Sakaguchi, N. A new member of the alpha4-related molecule (alpha4-b) that binds to the protein phosphatase 2A is expressed selectively in the brain and testis. *European journal of biochemistry / FEBS* **264**, 702-706 (1999).

200. Chung, H., Nairn, A.C., Murata, K. & Brautigan, D.L. Mutation of Tyr307 and Leu309 in the protein phosphatase 2A catalytic subunit favors association with the alpha 4 subunit which promotes dephosphorylation of elongation factor-2. *Biochemistry* **38**, 10371-10376 (1999).

201. Chen, J., Peterson, R.T. & Schreiber, S.L. Alpha 4 associates with protein phosphatases 2A, 4, and 6. *Biochemical and biophysical research communications* **247**, 827-832 (1998).

202. Quevedo, C., Salinas, M. & Alcazar, A. Initiation factor 2B activity is regulated by protein phosphatase 1, which is activated by the mitogen-activated protein kinase-dependent pathway in insulin-like growth factor 1-stimulated neuronal cells. *The Journal of biological chemistry* **278**, 16579-16586 (2003).

203. Dehde, S., *et al.* Two immunologically distinct human DNA polymerase alpha-primase subpopulations are involved in cellular DNA replication. *Molecular and cellular biology* **21**, 2581-2593 (2001).

204. Honarpour, N., *et al.* F-box protein FBXL16 binds PP2A-B55alpha and regulates differentiation of embryonic stem cells along the FLK1+ lineage. *Molecular & cellular proteomics : MCP* **13**, 780-791 (2014).

205. Fogeron, M.L., *et al.* LGALS3BP regulates centriole biogenesis and centrosome hypertrophy in cancer cells. *Nature communications* **4**, 1531 (2013).

206. Shamay, M., *et al.* A protein array screen for Kaposi's sarcoma-associated herpesvirus LANA interactors links LANA to TIP60, PP2A activity, and telomere shortening. *Journal of virology* **86**, 5179-5191 (2012).

207. Ohama, T. & Brautigan, D.L. Endotoxin conditioning induces VCP/p97-mediated and inducible nitric-oxide synthase-dependent Tyr284 nitration in protein phosphatase 2A. *The Journal of biological chemistry* **285**, 8711-8718 (2010).

208. Junttila, M.R., *et al.* CIP2A inhibits PP2A in human malignancies. *Cell* **130**, 51-62 (2007).

209. Nazarenko, I., Schafer, R. & Sers, C. Mechanisms of the HRSL3 tumor suppressor function in ovarian carcinoma cells. *Journal of cell science* **120**, 1393-1404 (2007).

210. Hsu, W., Zeng, L. & Costantini, F. Identification of a domain of Axin that binds to the serine/threonine protein phosphatase 2A and a self-binding domain. *The Journal of biological chemistry* **274**, 3439-3445 (1999).

211. Kamibayashi, C., Lickteig, R.L., Estes, R., Walter, G. & Mumby, M.C. Expression of the A subunit of protein phosphatase 2A and characterization of its interactions with the catalytic and regulatory subunits. *The Journal of biological chemistry* **267**, 21864-21872 (1992).

212. Oberg, E.A., Nifoussi, S.K., Gingras, A.C. & Strack, S. Selective proteasomal degradation of the B'beta subunit of protein phosphatase 2A by the E3 ubiquitin ligase adaptor Kelch-like 15. *The Journal of biological chemistry* **287**, 43378-43389 (2012).

213. Yan, Z., Fedorov, S.A., Mumby, M.C. & Williams, R.S. PR48, a novel regulatory subunit of protein phosphatase 2A, interacts with Cdc6 and

modulates DNA replication in human cells. *Molecular and cellular biology* **20**, 1021-1029 (2000).

214. Zwaenepoel, K., Goris, J., Erneux, C., Parker, P.J. & Janssens, V. Protein phosphatase 2A PR130/B"alpha1 subunit binds to the SH2 domain-containing inositol polyphosphate 5-phosphatase 2 and prevents epidermal growth factor (EGF)-induced EGF receptor degradation sustaining EGF-mediated signaling. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **24**, 538-547 (2010).

215. Ewing, R.M., *et al.* Large-scale mapping of human protein-protein interactions by mass spectrometry. *Molecular systems biology* **3**, 89 (2007).

216. Dozier, C., Bonyadi, M., Baricault, L., Tonasso, L. & Darbon, J.M. Regulation of Chk2 phosphorylation by interaction with protein phosphatase 2A via its B' regulatory subunit. *Biology of the cell / under the auspices of the European Cell Biology Organization* **96**, 509-517 (2004).

217. Ito, A., *et al.* A truncated isoform of the PP2A B56 subunit promotes cell motility through paxillin phosphorylation. *The EMBO journal* **19**, 562-571 (2000).

218. Letourneux, C., Rocher, G. & Porteu, F. B56-containing PP2A dephosphorylate ERK and their activity is controlled by the early gene IEX-1 and ERK. *The EMBO journal* **25**, 727-738 (2006).

219. Chandrashekarappa, D.G., McCartney, R.R. & Schmidt, M.C. Subunit and domain requirements for adenylate-mediated protection of Snf1 kinase activation loop from dephosphorylation. *The Journal of biological chemistry* **286**, 44532-44541 (2011).

220. Al-Hakim, A.K., *et al.* 14-3-3 cooperates with LKB1 to regulate the activity and localization of QSK and SIK. *Journal of cell science* **118**, 5661-5673 (2005).

221. Vinayagam, A., *et al.* A directed protein interaction network for investigating intracellular signal transduction. *Science signaling* **4**, rs8 (2011).

ABSTRACT

PROTEIN PHOSPHATASE 2A INTERACTIONS IN ISLET AND HUMAN SKELETAL MUSCLE IN DIABETES

by

DIVYASRI DAMACHARLA

August 2017

Advisor: Dr. Zhengping Yi

Major: Pharmaceutical Sciences (Pharmacology and Toxicology)

Degree: Doctor of Philosophy

Type 2 Diabetes is a metabolic disorder associated with insulin resistance and consequent high blood glucose levels. Under normal conditions, in response to high blood glucose levels, pancreatic beta cells produce insulin. The secreted insulin is distributed to tissues thereby stimulating insulin stimulated glucose uptake. However, maximum glucose disposal takes place in skeletal muscle. Thus, studying beta cells and skeletal muscle in respect to diabetes is crucial. Protein Phosphatase 2A (PP2A) is one of the major serine/threonine phosphatases belonging to PhosphoProteinPhosphatase (PPP) family. It constitutes about 80% of all serine/threonine phosphatases. It is regulated by numerous regulatory subunits as well as other substrate molecules and post translational modifications. This alters their localization, activity and its target molecules. Many evidences show the effect of insulin on PP2Ac and its abnormal regulation in conditions of glucolipotoxicity. Thus, studying PP2Ac interaction partners in respect to type 2 diabetes will give insight into its role in insulin resistance.

Here, we studied interaction partners of PP2Ac in both beta cells and human skeletal muscle. INS-1 832/13 insulin secreting cells are used to study beta cell which are treated with basal and high glucose for 48hrs which are then harvested and analyzed.

Skeletal muscle biopsies are collected from human subjects. Two biopsies are collected from each individual, basal and insulin stimulated using hyperinsulenemic euglycemic clamp technique. We collected biopsies from individuals characterized in three different groups, lean controls, obese/overweight insulin resistant, and type 2 diabetics. Both beta cells and human skeletal muscle biopsies are analyzed using a similar proteomics approach using ESI-HPLC-MS/MS. Using this technique, we identified 514 partners in INS-1 832/13 cells with 89 partners classified as glucose responsive. Similarly, 211 interaction partners are identified in human skeletal muscle biopsies and 69 proteins presented a significant difference among three gropus. Several important PP2Ac interaction partners were identified which included some known partners (identified in other cell types) as well. Many proteins involved in insulin secretion are found as PP2Ac partners in beta cells whereas several vital molecules involved in insulin signaling pathway are identified in skeletal muscle biopsies. Some important molecules like Rac1, Limk1, Akt2, MAPK are identified among others. Proteins that effect PP2Ac post translational modification, such as PPME-1, are also identified and presented with a significant change. Further validation of these partners will help with a better understanding of the role and regulation of PP2Ac in diabetes.

AUTOBIOGRAPHICAL STATEMENT

DIVYASRI DAMACHARLA

EDUCATION

2017 PhD in Pharmaceutical Sciences, Wayne State University, Detroit, Michigan, U.S.A**.**
2015 Masters in Pharmaceutical Sciences, Wayne State University, Detroit, Michigan, U.S.A.
2012 Bachelors in Pharmacy, Rajiv Gandhi University of Health sciences, India

PROFESSIONAL ASSOCIATIONS

American Society for Mass Spectrometry (ASMS)
American Association for Pharmaceutical Scientists (AAPS)

PRESENTATIONS

Divyasri Damacharla., et al. Identification of Protein Interaction Partners of Protein Phosphatase 2A Catalytic Subunit Using Quantitative Mass Spectrometry, the 62nd American Society for Mass Spectrometry Conference on Mass Spectrometry, June 15-19, 2014, Baltimore Convention Center, Baltimore, MD.

Divyasri Damacharla., et al. Protein Interaction Partners of Protein Phosphatase 2A Catalytic Subunit in Rat β-Islet cells Using Quantitative Mass Spectrometry, the 63rd ASMS Conference on Mass Spectrometry and Allied Topics, May 31 - June 4, 2015 - America's Center, St. Louis, Missouri

Divyasri Damacharla., et al. Identification of Interaction Partners of Protein Phosphatase 2A Catalytic Subunit in human skeletal muscle using Label free Mass Spectrometry, the 65th American Society for Mass Spectrometry Conference on Mass Spectrometry, June 4-8, 2017, Indianapolis Convention Center, Indianapolis, Indiana.

PUBLICATION

Zhang, X., *et al.* Quantitative proteomics reveals novel protein interaction partners of PP2A catalytic subunit in pancreatic beta-cells. *Molecular and cellular endocrinology* **424**, 1-11 (2016).