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Quantitative proteomics of MDCK cells identify unrecognized roles of clathrin adaptor AP-1 in polarized distribution of surface proteins

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The current model of polarized plasma membrane protein sorting in epithelial cells has been largely generated on the basis of experiments characterizing the polarized distribution of a relatively small number of overexpressed model proteins under various experimental conditions. Thus, the possibility exists that alternative roles of various types of sorting machinery may have been underestimated or missed. Here, we utilize domain-selective surface biotinylation combined with stable isotope labeling with amino acids in cell culture (SILAC) and mass spectrometry to quantitatively define large populations of apical and basolateral surface proteins in Madin-Darby canine kidney (MDCK) cells. We identified 313 plasma membrane proteins, of which 38% were apical, 51% were basolateral, and 11% were nonpolar. Silencing of clathrin adaptor proteins (AP) AP-1A, AP-1B, or both caused redistribution of basolateral proteins as expected but also, of a large population of apical proteins. Consistent with their previously reported ability to compensate for one another, the strongest loss of polarity was observed when we silenced AP-1A and AP-1B simultaneously. We found stronger evidence of compensation in the apical pathway compared with the basolateral pathway. Surprisingly, we also found subgroups of proteins that were affected after silencing just one adaptor, indicating previously unrecognized independent roles for AP-1A and AP-1B. While AP-1B silencing mainly affected basolateral polarity, AP-1A silencing seemed to cause comparable loss of apical and basolateral polarity. Our results uncover previously overlooked roles of AP-1 in polarized distribution of apical and basolateral proteins and introduce surface proteomics as a method to examine mechanisms of polarization with a depth not possible until now.

epithelial polarity | apical basolateral polarity | SILAC mass spectrometry | AP-1A | AP-1B

pithelial cells perform key vectorial functions in secretion and absorption that depend on the accurate localization of transporters, channels, and receptors to apical and basolateral surface domains. Polarized sorting of plasma membrane proteins is believed to occur via recognition of apical and basolateral signals by the intracellular sorting machinery (1, 2). The current model postulates that apical and basolateral sorting is mediated by separate machineries. While apical sorting signals are poorly understood, they seem to involve glycans and other determinants that engage lectins, lipid rafts, and cytoplasmic motors (3). On the other hand, basolateral sorting is believed to be mediated by discrete tyrosine or dileucine motifs, which engage clathrin (4) and the clathrin adaptor protein 1 (AP-1). AP-1 is a heterotetramer of different β -, γ -, μ -, and σ -subunits. The μ -subunit has two isoforms (μ 1A and μ 1B), which define the tissue-specific expression of the AP-1 complex, being AP-1A ubiquitous and AP-1B specific of epithelia (5).

Our understanding of the current epithelial sorting model and the role of AP-1 in polarized protein distribution in epithelial cells still has many gaps. (*i*) Some epithelia lack μ 1B, including liver (5), retinal pigment epithelium (6), kidney proximal tubule (7), and choroid plexus (8), and they still manage to direct proteins to the basolateral domain. (*ii*) Do AP-1A and AP-1B complement each other or have independent roles? Studies in Madin-Darby canine kidney (MDCK) cells show that, while loss of μ 1B decreases polarization of several basolateral proteins (9– 13), the combined silencing of μ 1A plus μ 1B has a more dramatic effect (14–16), indicating some degree of compensation. However, to our knowledge, a totally independent role of AP-1A in basolateral protein distribution has not been demonstrated. (*iii*) Do apical proteins also rely on AP-1 for polarized trafficking? In recent years, the role of AP-1 in determining the localization of basolateral proteins exclusively has been expanded to also include apical proteins in *Caenorhabditis elegans* (17, 18) and mice (19) and glycosylphosphatidylinositol (GPI)-anchored proteins in MDCK cells (20).

Many important aspects of the polarity mechanisms in epithelial cells remain unsolved, because conclusions have been drawn largely by observing the behavior of a very small set of representative proteins, which are often overexpressed via transfection. This approach poses the risk of overlooking the behavior of a less well-characterized repertoire of endogenous proteins under more physiological conditions. Mass spectrometry-based proteomics techniques have the potential of overcoming these limitations. The surface proteome of MDCK cells may encompass hundreds of proteins (21). Hence, a quantitative global surface proteomics approach would be highly advantageous to accurately measure the

Significance

Epithelial cells perform critical protective, secretory, absorptive, and sensory functions, for which they require plasma membrane polarization into apical and basolateral domains. Impaired polarity causes cancer and developmental and degenerative disorders. Research on fundamental polarity mechanisms has been hindered by the paucity of model proteins and by the use of overexpression systems. Here, we introduce a high-throughput surface proteomics approach based on domain-selective biotinylation and quantitative mass spectrometry that provides candidate proteins to study polarity under normal expression levels. Using this approach, we described that clathrin adaptors mediate apical and basolateral distribution of surface proteins, expanding the traditional notion that clathrin adaptors mediate only basolateral polarity. Our results establish quantitative surface proteomics as a powerful tool to study epithelial polarity.

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polarized distribution of surface proteins and its changes in response to deletion of potential components of the polarity machinery. Here, we applied domain-selective biotinylation of MDCK cells, a technique originally introduced by our laboratory (22), in combination with stable isotope labeling with amino acids in cell culture (SILAC) (23) and mass spectrometry to quantitatively define apical and basolateral plasma membrane protein populations in MDCK cells. This approach was recently performed successfully in nonpolarized cells to characterize the role of the retromer in plasma membrane protein recycling (24) and to define a newly identified retriever complex (25). Adapting this approach to polarized epithelial cells, here we identified previously overlooked roles of AP-1A and AP-1B in the polarized distribution of apical and basolateral proteins.

Results

Polarized Surface Proteome of MDCK Cells. To study the polarized surface proteome of MDCK cells, we followed the approach depicted in Fig. 1/4. We cultured MDCK cells in SILAC medium

and allowed them to polarize for 4 d. Then, we performed surface biotinylation on the apical or basolateral surface domains and analyzed the surface samples by mass spectrometry. We included a control sample without biotinylation to account for the background signal (Fig. 1B). We detected the majority of the signal at the surface protein fraction (Fig. 1 B and C), indicating very little background. We identified a total of 1,737 proteins in three or more replicates in six separate experiments. Of these proteins, 313 were transmembrane, GPI-anchored, or peripheral extracellular proteins that had a plasma membrane annotation in the UniProt knowledgebase; the rest (~80% of the proteins) were annotated as cytoplasmic. However, despite the higher abundance of cytoplasmic proteins, quantitative analysis showed that they contributed less than 10% of the total peptide signal in the mass spectrometry (Fig. 1D). It is reasonable to expect a certain degree of intracellular biotinylation due to endogenous biotinylation and to biotin leakage through the plasma membrane combined with the higher abundance of cytoplasmic proteins. Any nonspecific



Fig. 1. Polarized surface proteome in MDCK cells via surface biotinylation followed by SILAC. (A) Flow chart illustrating the procedure for obtaining a polarized surface proteome in MDCK cells. (*B*) Representative silver-stained gel showing apical and basolateral proteins retrieved after surface biotinylation and background from nonbiotinylated samples. Dividing line indicates noncontiguous gel lanes. (C) SILAC ratios of surface vs. nonbiotinylated proteins. Each data point represents a single protein. Gray datapoints represent proteins from the no biotin sample. Blue datapoints are proteins from the biotinylated sample. Dotted line indicates the $-\log(10)$ of a *P* value = 0.05. (*D*) Quantification of signal obtained from proteins annotated as plasma membrane or cytoplasmic (dark gray) and membrane membrane and cytoplasmic (dark gray) and membrane membrane domains. Ap, apical; BL, basolateral.

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biotinylation is expected to occur indiscriminately from the apical or basolateral domain. Therefore, when we analyzed the percentage frequency (density) distribution of proteins annotated as cytoplasmic or plasma membrane, we observed that most of the signal from cytoplasmic proteins is not polarized (Fig. 1*E*), ruling out any bias that may be associated with residual nonspecific biotinylation. Altogether, these results highlight the suitability of the surface biotinylation method.

Because we wished to study the surface proteome of MDCK cells, we focused our analysis on plasma membrane proteins only. The quantitative nature of our approach allowed us to determine the relative proportion of each protein at the apical or basolateral domain. Fig. 24 shows the location annotation for all 1,737 proteins detected in MDCK cells (Fig. 24, *Left*) and the polarized surface distribution of the subset of 313 proteins annotated as

plasma membrane proteins (Fig. 24, *Right*). Among these, 38% were apical, 51% were basolateral, and 11% were nonpolar, defined as being enriched by more than 60% in the respective membrane domain. The apical/basolateral ratio is easily visualized in the volcano plot in Fig. 2*B*, where we indicated the relative enrichment in each membrane domain and highlighted proteins of previously known distribution. Our proteomics data are in good agreement with the published literature. To further validate our data, we studied a subset of surface proteins by Western blot (Fig. 2*C*), and we found their polarity assessed with this procedure to be consistent with the proteomics results.

Functional classification of the surface proteins identified by global proteomics showed distinct groups at the apical and basolateral membranes (Table 1). Certain proteins, like adhesion molecules, tight junction proteins, and membrane-bound proteases, were enriched at



Fig. 2. Apical and basolateral proteome of MDCK. (*A*) Subcellular localization of all 1,737 proteins identified by mass spectrometry (*Left*), of which 313 were plasma membrane proteins and were classified as apical, basolateral, or not polarized based on the SILAC ratios (*Right*). (*B*) Volcano plot of log(2) SILAC ratios for apical vs. basolateral proteins in MDCK cells (horizontal axis) against –log(10) *P* value (vertical axis). Each data point represents a single protein. Dotted line indicates the –log(10) of a *P* value = 0.05. Labels indicate proteins of previously known polarized distribution. (*C*) Validation of a subset of proteins via surface biotinylation followed by Western blot (*Left*) compared with the percentage detected at the apical and basolateral membranes in proteomics (*Right*). Ap, apical; BL, basolateral.

the basolateral domain, while GPI-anchored proteins and proteins involved in purine metabolism among others were enriched apically. Surface receptors, ion channels, and transporters populate both membranes in comparable proportions (a complete list is in Dataset S1).

Altogether, these results indicate that the combination of domain-specific surface biotinylation and quantitative SILACbased proteomics provides a robust assessment of the steadystate global surface proteome in polarized MDCK cells.

AP-1A and AP-1B Maintain the Polarized Proteome of MDCK Cells. To study how AP-1A and AP-1B contribute to generating the polarized surface proteome of MDCK cells, we silenced µ1A and µ1B independently or simultaneously using small interfering RNA (siRNA) and measured the resulting changes in distribution of apical and basolateral plasma membrane proteins. To rule out artifacts due to biotin leakage through compromised tight junctions during gene silencing, we measured transepithelial resistance as indicative of functional tight junctions. In agreement with a previous report showing selective apical and basolateral surface biotinylation after AP-1 silencing (15), we did not detect any statistical difference in transepithelial resistance among silencing treatments (luciferase siRNA = $238 \pm 5 \Omega \cdot \text{cm}^2$; μ 1A-siRNA = 240 \pm 3 Ω ·cm²; μ 1B-siRNA = 232 \pm 4 Ω ·cm²; μ 1A + μ 1B-siRNA = 231 \pm 8 Ω ·cm²; ANOVA: P = 0.1805), indicating that AP-1 silencing does not compromise the epithelial monolayer. Plots in Fig. 3 represent the relative polarity of all detected plasma membrane proteins in control MDCK cells (luciferase siRNA) compared with each of the silencing conditions (siRNA for µ1A, μ 1B, or both). We performed a linear regression analysis to detect changes in protein polarity as deviations from the straight line that represents no change. In all cases, we observed basolateral proteins with decreased polarity as expected, but surprisingly, we also observed apical proteins with decreased polarity, suggesting that AP-1 may also determine apical protein localization. In addition, a large number of surface proteins did not have their polarity affected, indicating that a separate mechanism, mediated by a different adaptor or a clathrinindependent polarization mechanism, operates in epithelia.

From these data, we conclude that there is a previously underestimated role of AP-1 in apical protein localization in MDCK cells and that a nonidentified trafficking mechanism independent of AP-1 may operate in apical-basolateral distribution of plasma membrane proteins.

Endogenous Markers to Study AP-1A- and AP-1B-Mediated Polarized Protein Distribution in MDCK Cells. To confirm the roles of AP-1A and AP-1B in polarized protein distribution that we observed by proteomics, we silenced µ1A and µ1B and measured the polarized localization of a select group of endogenous proteins by surface biotinylation and Western blot (Fig. 4). We found a high degree of agreement between our proteomics data and the subset assessed by Western blot. We observed that some basolateral proteins, including Hephaestin (HEPH), CD44, SLIT and NTRK Like Family Member 4 (SLITRK4), and Anion Exchanger 2 (AE2), required the presence of both adaptors, since basolateral polarity was decreased only after simultaneous silencing of µ1A and µ1B (Fig. 4 A-D). Some basolateral proteins, like the Retinol-Binding Protein Receptor STRA6, and the membrane protease ADAM17 were only dependent on AP-1B (Fig. 4 E and F). Conversely, apical proteins, like gp114 (CEACAM1), the aminopeptidase ENPEP, the intercellular adhesion molecule 1 (ICAM1), and the G proteincoupled receptor C5A (GPRC5A) also required AP-1 for apical localization (Fig. 4 G-J). In this last set, CEACAM1, ENPEP, and ICAM1 had reduced apical polarity after simultaneous silencing of μ 1A and μ 1B. However, individual silencing of either μ 1A or μ 1B was enough to decrease apical polarity of GPRC5A. This suggests that, while AP-1A and AP-1B are individually sufficient for apical localization of GPRC5A, they require the presence of each other

to determine the distribution of other apical proteins, like CEACAM1, ENPEP, and ICAM1. Finally, two other proteins that we tested, the apical Podocalyxin (PODXL) and the basolateral β 1-subunit of the Na/K ATPase (ATP1B1), had no major change in their polarized distribution after silencing either of the adaptors alone or together (Fig. 4 *K* and *L*) (similar to the proteomics results), indicating that some apical and basolateral proteins rely on an AP-1–independent mechanism(s) for polarized localization.

Altogether, we report here a set of endogenous basolateral and apical proteins previously unknown to be dependent on AP-1 for apical-basolateral distribution and describe various polarity patterns that AP-1 supports. The reporter proteins that we identified will be useful to study not only roles of AP-1 in basolateral polarity but also, the role of AP-1 in apical polarized trafficking that we describe here. As we also uncovered reporter proteins that depend differently for their polarized distribution from individual adaptors or both, it will be possible in the future to dissect more precisely the specific functions of AP-1A and AP-1B. Last but not least, using endogenous proteins to study AP-1 function has the appeal of eliminating the confounding effects resulting from overexpression of exogenous proteins.

Independent and Compensatory Roles of AP-1A and AP-1B in Polarized Distribution Analyzed by Empirical Bayes Method. Our data so far suggest that AP-1A and AP-1B may have independent roles in protein polarity, and they also compensate for each other in some cases. The compensatory mechanism is evident during simultaneous silencing of µ1A and µ1B, which had a larger effect than each individual silencing. The proteomics approach that we introduce here provides an excellent tool to analyze the global effects of silencing a component of the polarization machinery. Accordingly, statistical analysis was conducted through the limma package in R, wherein an empirical Bayes procedure was implemented to shrink protein sample variances toward mean protein sample variance (26, 27). Thereafter, differential protein expression was determined through moderated t tests between experimental conditions. We applied this kind of analysis to detect global patterns when silencing μ 1A and μ 1B in MDCK cells. To further reduce variability, we also minimized potential batch effects by performing a set of triplicate experiments in parallel, thus minimizing variation associated with sample processing. Fig. 5 summarizes the magnitude of changes toward the apical or basolateral directions when we silenced µ1A or µ1B. While similar numbers of proteins experienced an apical or basolateral shift during μ 1A silencing (Fig. 5A), more proteins shifted toward the apical membrane when we silenced μ 1B (Fig. 5B). These shifts in both directions involved more proteins when we silenced µ1A and µ1B simultaneously (Fig. 5C). To assess the magnitude of changes in each direction, we constructed vectors to quantify the global effect of silencing by compounding the count, the direction, and the degree of the shift for all proteins affected beyond the significance threshold computed by the empirical Bayes approach. The vectors obtained in this way are summarized in Fig. 5D. We observed that μ 1A silencing had the smaller effect in magnitude, but the shift leaned toward the basolateral direction. Silencing µ1B had the largest effect but in the opposite direction toward the apical membrane. Simultaneous silencing of µ1A and µ1B had the largest effect and still leaned toward an apical shift, most likely due to the relatively larger contribution of µ1B. This analysis indicates that AP-1A and AP-1B may have independent roles. Since this was previously unrecognized, we analyzed directly this possibility by comparing the effects of silencing µ1A and µ1B on the polarized distribution of all proteins detected. We observed a significant bias toward the basolateral direction after silencing μ 1A and toward the apical direction when silencing μ 1B (µ1A: 43.8% toward apical, 56.2% toward basolateral; µ1B: 59.9% toward apical, 40.1% toward basolateral; P < 0.05, Fisher's exact test). Altogether, these data indicate that µ1A and µ1B are independent and that µ1A had a predominant influence in maintaining

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Table 1. Apical and basolateral distribution of surface proteins in MDCK

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Chioride intracellular channel 1 CLC1 5 27.4 6 89 11 0.2417082 Anoctamin 6 Leuriner ich repeat containing 8 VRAC subunit A ANO6 25 26.6 6 6 84 4 74988E 7 1 Cell migration-inducing PVRAC subunit A LRRCA 1 1 2.5 4 29 7 1 0.139840212 Cell migration-inducing PVRAC subunit A LRRCA 1 1 2.5 4 29 7 7 3 0.00485444 7 7 7 3 0.00485444 7 7 7 3 0.0048544 7 7 7 3 0.0048544 7 7 7 3 0 0.0049544 7 7 7 7 7 7 7 0 0.0049544 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7	Transmembrane and coiled-coil domains 1	TMCO1	2	12.2	4	74	26	0.170385728
Solute carrier family 9 member A1 SLC9A1 9 B.7 4 5 95 0.016457311 Anotamin 6 CPI 10 Anotamin 6 Anotamin 6 Anotamin 6 CPI 10 Anotamin 6 CPI 10 Anotamin 6 Anotamin 6 CPI 10 Anotamin 6 CPI 10 CPI 10 <t< td=""><td>Chloride intracellular channel 1</td><td>CLIC1</td><td>5</td><td>27.4</td><td>6</td><td>89</td><td>11</td><td>0.234170658</td></t<>	Chloride intracellular channel 1	CLIC1	5	27.4	6	89	11	0.234170658
Anoctamin 6 ANO6 25 26.6 6 6 84 4.74988E.02 Cell migration-inducing plautronidase 2 TMEMZ 8 5.1 6 27 73 0.004854642 Cell migration-inducing plautronidase 2 TMEMZ 8 5.1 6 86 14 0.000350922 2,3'-Cyclic nucleotide 3' phosphodiesterase CNP 20 46.4 6 87 13 0.000350922 Endonuclease domain containing 1 ENDOD1 7 33.7 6 83 17 0.004033892 Lamin B receptor LBR 13 29.1 6 71 29 0.00493892 Cabherin 16 CDH16 26 46.7 4 8 92 0.0438835 Lipolysis stimulated lipoprotein receptor LSR 20 2.93 6 70 00 0.00122693 Signal transducer and activator of transcription 3 STA3 18 25.3 4 22 5 42 59 0.997935 Protein tyrosine phosphatase, receptor type M PTPRM 26 28.3 4 22 <t< td=""><td>Solute carrier family 9 member A1</td><td>SLC9A1</td><td>9</td><td>8.7</td><td>4</td><td>5</td><td>95</td><td>0.016457311</td></t<>	Solute carrier family 9 member A1	SLC9A1	9	8.7	4	5	95	0.016457311
Leucine-rich repeat containing 8 VRAC subunit A RRCSA 11 12.5 4 829 71 0.138940212 Cell migration-inducing hysthemidding 2 TMEM2 8 5.1 6 27 73 0.00845648 Pyrophosphatase (norganic) 1 PPA1 5 33.1 6 86 14 0.00339825 23.°Cyclic nucleotide 3' phosphodiestrase CNP 20 46.4 6 87 13 0.00955522 Premylysterine oxidase 1 PCYOX1 8 6.5 4 100 0 0.0101195 Catherin 16 CDH16 26 45.7 4 8 92 0.026995224 Lamin B receptor LBR 13 29.1 6 71 29 0.09695044 Catherin 16 CDH16 26 45.7 4 8 92 0.024358355 Ectonucleoside triphosphate diphosphohydrolase 3 ENTPD3 17 34 6 72 28 0.23271607 Tripartite motif-containing 25 TRIM25 14 27.9 6 70 30 0.004216133 Prenzoxonase 2 PON2 4 18.1 6 77 23 0.002126897 Tripartite motif-containing 25 TRIM25 14 27.9 6 3 97 5.49045-06 Signal transducer and activator of transcription 3 STAT3 18 35.3 5 42 58 0.1969393671 Protein tyrosine phosphatae, receptor type F PTPRF 79 64.5 6 6 73 87 5.49045-06 Calpain 2 CAPN2 40 64.9 6 64 64 36 0.131432840 Transmembrane serine protease 4 TMPRS54 6 18.6 6 113 87 1.52653E00 ADAM metallopeptidase domain 10 ADAM10 20 36.7 6 13 87 1.52653E00 ADAM metallopeptidase domain 10 ADAM17 13 21.9 6 77 93 9.63391E-05 ADAM metallopeptidase domain 10 ADAM17 13 21.9 6 71 93 9.63391E-05 ADAM metallopeptidase domain 10 ADAM17 11 5 23 77 0.02635564 Carboxypeptidase Momain 10 ADAM17 11 5 23 77 0.02635564 Carboxypeptidase Momain 10 ADAM17 13 21.9 6 191 0.005019663 ADAM metallopeptidase domain 10 ADAM10 20 36.7 6 100 0 2.70435564 Carboxypeptidase Momain 10 ADAM10 20 36.7 6 100 0.274045705 Xenotropic and polytopic retrovirus receptor 2 CLES2 25 12.3 6 194 0.6 0.1383571 Frizield cluss sectors of 6 72 05 8 4 24 76 0.03437433 Carboxypeptidase Momain 10 ADAM10 29 36.7 6 100 0.274045705 Xenotropic and polytopic retrovirus receptor 2 CLES2 25 12.3 6 194 0.005019663 AdHesion G protein-coupled receptor G1 ADGRG1 4 2.5 4 2.4 76 0.13836571 Frizield cluss sectors of 6 72 73 0.01745781 Frizield cluss sectors of 6 72 73 0.01745981 ATP binding cassetts subfamily F member 1 AGCE1 2 2.2 6	Anoctamin 6	ANO6	25	26.6	6	16	84	4.74988E-05
Cell migration-inducing hyslaronidase 2 TMEM2 8 5.1 6 27 73 0.004854648 Prophosphates (inorganic) 1 PPA1 5 33.1 6 86 14 0.00330922 2,3'-Cyclic nucleotide 3' phosphodiestrase CNP 20 46.4 6 87 13 0.000530922 2,3'-Cyclic nucleotide 3' phosphodiestrase CNP 20 46.4 6 87 13 0.000330922 Endonuclease domain containing 1 ENDCOI 7 33.7 6 83 17 0.004033892 Cadherin 16 ECONTO LBR 13 29.1 6 71 29 0.004938925 Econucleoside triphosphate diphosphohydrolase 3 ENTPOB 17 34 6 72 28 2.3237-607 Paraoxonase 2 PON2 4 18.1 6 77 28 0.00425895 Signal transducer and activator of transcription 3 STAT3 18 35.3 5 42 27 8 0.00245697 Protein tyrosine phosphates, receptor type M PTPRK 26 28.3 4 22 78 0.00245697 Protein tyrosine phosphates, receptor type M PTPRK 26 28.3 4 22 78 0.078938671 Protein tyrosine phosphates, receptor type M PTPRK 26 28.3 4 22 78 0.078938671 Protein tyrosine phosphates, receptor type M PTPRK 26 28.3 4 22 78 0.078938671 Protein tyrosine phosphates, receptor type M CAPN2 40 64.9 6 64 36 0.13419204 Transmembrane serine protease 4 TMPR54 6 18.6 6 13 87 1.226538-05 ADAM metallopeptidase domain 10 ADAM17 13 2.1,9 6 7 93 9.53381620 ADAM metallopeptidase domain 10 ADAM17 11 5 23 77 0.02435545 ADAM metallopeptidase domain 10 ADAM17 11 5 23 77 0.02435545 Carboxypeptidase M Cpm 5 12.2 5 74 26 0.02747236 Carboxypeptidase M Cpm 5 12.2 6 6 13 0.005016634 ATP binding casette subfamily F member 1 ABCF1 12 5 8 92 0.022747236 Carboxypeptidase M Cpm 5 12.2 6 13 0.000516634 ATP binding casette subfamily F member 1 ABCF1 12 4 5 8 4 24 76 0.1388571 ATP binding casette subfamily F member 1 ABCF1 12 5 8 92 0.00276173 ATP binding casette subfamily F member 1 ABCF1 12 5 8 92 0.00276173 ATP binding casette subfamily F member 1 ABCF1 12 5 8 92 0.00276173 ATP binding casette subfamily F member 1 ABCF1 12 5 8 92 0.00276173 ATP aba/N*t-transporting subunit_13 ATP181 71 52.3 5 8 14 0.00061633 ATP binding c	Leucine-rich repeat containing 8 VRAC subunit A	LRRC8A	11	12.5	4	29	71	0.139840212
Pyrophosphatae (norganic) 1 PPA1 5 33.1 6 86 14 0.00309922 2.3°-Cyclic nucleotide 3 ⁻ phospholositerase CNP 2/0 46.4 6 87 13 0.00095522 Lamin B receptor LBR 13 29.1 6 83 17 0.004033925 Lamin B receptor LBR 13 29.1 6 71 29 0.00999004 Catherin 16 CDH16 26 46.7 4 8 92 0.024383855 Ectonucleoside triphosphate diphospholydrolase 3 ENTPD3 17 34 6 77 23 0.00122697 Triphartite moff-containing 25 TRIM25 14 27.9 6 70 30 0.04216133 Lipolysis stimulated lipoprotein receptor TSTA3 18 35.3 5 42 78 0.07993871 Protein tyrosine phosphatase, receptor type F PTRF 9 64.5 6 18 7 12.55554.5 Calpan 2 CAPN2 40	Cell migration-inducing hyaluronidase 2	TMEM2	8	5.1	6	27	73	0.004854648
2,3'-Cyclic nucleaties is phosphodiesterase CNP 20 46.4 6 87 13 0.00595224 Prenylcysteine oxidase 1 PCYOX1 8 6.5 4 100 0 0.001011956 Endonuclease domain containing 1 ENDOD1 7 33.7 6 83 17 0.004938925 Ectonucleoside triphosphate diphosphohydrolase 3 ENTPD3 17 34 6 72 28 2.2327607 Tripartite motif-containing 25 TRIMZ5 14 2.7.9 6 70 30 0.004216133 Lipolysis stimulated lipoprotein receptor LSR 20 2.9.3 6 3 97 549804-00 6 64 36 0.139172604 Protein tyrosine phosphatase, receptor type F PTPRM 26 2.8.3 4 22 78 0.139172604 Calpain 2 CAPN2 40 6.4.9 6 64 36 0.134122604 Calpain 2 Capmot 3 12.2 5 7.4 2.6 0.239767207 <td>Pyrophosphatase (inorganic) 1</td> <td>PPA1</td> <td>5</td> <td>33.1</td> <td>6</td> <td>86</td> <td>14</td> <td>0.005309926</td>	Pyrophosphatase (inorganic) 1	PPA1	5	33.1	6	86	14	0.005309926
Prencylcysteine oxidase 1 PCVOXI 8 6.5 4 100 0 0.0101195 Lamin B receptor LBR 13 29.1 6 71 29 0.004693892 Lamin B receptor LBR 13 29.1 6 71 29 0.0042389355 Ectonucleoside triphosphate diphosphotydrolase 3 ENTPD3 17 34 6 72 28 2.23276:07 Paraxonase 2 PON2 4 181 6 77 23 0.001226897 Signal transducer and activator of transcription 3 STAT3 18 35.3 5 42 58 0.16979355 Protein tyrosine phosphatase, receptor type M PTPRM 26 28.3 4 22 6.964566:05 Calpain 2 CaPAN2 40 6.49 6 64 36 0.134152804 ADAM metallopeptidase domain 17 ADAM17 13 21.9 5 74 26 0.024747236 Suppression of tumorigenicity 14 ST14 7 11.2 <	2',3'-Cyclic nucleotide 3' phosphodiesterase	CNP	20	46.4	6	87	13	0.005965224
Endonuclease domain containing 1 ENDOD1 7 33.7 6 83 17 0.00405382 Lamin B receptor LBR 13 2.91 6 71 2.9 0.06699004 Cadherin 16 CDH16 26 46.7 8 8 92 0.02435835 Ectonucleoside triphosphate diphosphohydrolas 3 ENTPD3 17 34 6 72 28 2.3237E-07 Paraoxonase 2 PON2 4 18.1 6 77 2.3 0.00122697 Tripartite motif-containing 25 TRIM25 14 2.7.9 6 70 30 0.004216133 Lipplysi stimulated lipportoein receptor LSR 20 2.9.3 6 3 97 5.496042-06 Signal transducer and activator of transcription 3 STAT3 18 35.3 5 42 58 0.0169979357 Protein tryosine phosphatase, receptor type F PTPRF 79 64.5 6 2.8 72 6.59458E-0 Calpain 2 CAPN2 40 64.9 6 64 36 0.13192204 Transmembrane serine protease 4 TMPR54 6 18.6 13 87 9.5306FeC 7 Suppression of tumorigenicity 14 ST14 7 11 5 23 77 0.026355564 Carboxypeptidase domain 10 ADAM10 20 36.7 6 13 87 9.5306FEC 7 Suppression of tumorigenicity 14 ST14 7 11 5 23 77 0.026355564 Carboxypeptidase M Cpm 5 12.2 5 7.4 26 0.02474732 Glutamy 1 aminopeptidase comain 10 ADAM10 7 11 5 2.3 77 0.026355564 Carboxypeptidase M Cpm 5 12.2 6 10 0 0.270445E0 Carboxypeptidase M Cpm 5 12.2 6 61 39 0.002019459 Carboxipeptione conjust receptor 1 XPR1 7 11.2 6 14 86 8.781317E-05 Carboxipeptione oplotropic retreovirus receptor 1 XPR1 7 11.2 6 14 86 8.781317E-05 Carboxipeptical coloris orceptor 6 F2D6 8 8.6 5 36 64 0.1380571 Frizzled class receptor 6 F2D6 8 8.6 5 36 64 0.1380571 Frizzled class receptor 6 F2D6 8 8.6 5 36 64 0.022070751 APP binding casette subfamily E member 1 ABCE1 29 52 6 61 39 0.0027076157 APP binding casette subfamily E member 1 ABCE1 29 52 6 61 39 0.00270751 APP binding casette subfamily E member 1 ABCE1 29 52 6 61 0.02214915 Solute carrier family 34 member 2 SLC3AA1 4 10.7 6 82 9.5 9.54655-06 Solute carrier family 34 member 1 SLC3A1 4 10.7 6 82 9.5 9.54655-06 Solute carrier family 34 member 1 SLC3A1 4 10.7 6 82 9.5 9.54655-06 Solute carrier family 34 member 1 SLC3A1 4 10.7 6 82 9.5 9.54655-06 Solute carrier family 34 member 1 SLC3A1 4 10.7 6 82 9.5 9.54655-06 Solute carrier family 34 membe	Prenylcysteine oxidase 1	PCYOX1	8	6.5	4	100	0	0.01011956
Lamin B receptor LBR 13 29.1 6 71 29 0.096990045 Carberin 15 CDH 16 26 46.7 4 8 92 0.02438355 Ectorucleoside triphosphate diphosphotydrolase 3 ENTPD3 17 34 6 72 28 2.3237E-07 Paraoxonase 2 PON2 4 18.1 6 77 23 0.001226897 Tipartite motif-containing 25 TRIM25 14 2.79 6 70 30 0.004216133 Lipolysis stimulated lipoprotein receptor LSR 20 29.3 6 3 97 5.498042 6 Signal transducer and activator of transcription 3 STAT3 18 35.3 5 42 58 0.169979355 Protein tyrosine phosphatase, receptor type M PTPRM 26 28.3 4 22 78 0.078938671 Transmembrane serine protease 4 TMPRS54 6 18.6 6 13 87 1.52553E-05 ADAM metallopeptidase domain 17 ADAM17 13 21.9 6 7 93 9.53067E-07 Suppression of tumorigenicity 14 ST14 7 11 5 23 77 0.02635554 Carbony metallopeptidase domain 10 ADAM10 20 36.7 6 13 87 9.53067E-07 Suppression of tumorigenicity 14 ST14 7 11 5 23 77 0.02635554 Carbonypeptidase M Cpm 5 12.2 5 74 26 0.024747236 Carbonypeptidase M Cpm 5 12.2 5 74 26 0.024747236 Carbonypeptidase Grower 1 XPR1 7 11.2 6 14 86 8.78517E-05 Carbonypeptidase Grower 1 XPR1 7 11.2 6 14 86 8.78517E-05 Carbonypeptidase Grower 1 XPR1 7 11.2 6 14 86 0.024747236 Carbonypeptidase Grower 1 XPR1 7 11.2 6 14 86 0.024747236 Adherin GF Lofs even pass G-type receptor 2 CELSR2 25 12.3 6 19 81 0.000519663 Adheris GF Lofs even pass G-type receptor 2 CELSR2 25 12.3 6 19 81 0.000519663 ATP binding cassette subfamily F member 1 ABCE1 29 52 6 61 39 0.000519663 ATP binding cassette subfamily F member 1 ABCE1 29 52 6 61 39 0.000519663 ATP binding cassette subfamily F member 1 ABCE1 29 52 6 71 0.02231591 Soluce carrier family 33 member 1 ABCE1 29 52 6 71 0.002315915 Soluce carrier family 33 member 1 ABCE1 29 52 6 71 0.002315915 Soluce carrier family 33 member 1 SLC3AA1 4 10.7 6 82 7.7 3 0.00136391 G protein-coupled receptor G1 SLC3AA1 4 10.7 6 82 9.5 9.50652-65 Soluce carrier family 34 member 1 SLC3AA1 4 10.7 6 82 7.7 3 0.000516632 ATP binding cassette subfamily F member 1 ABCE1 29 52 6 75 0.007675232 ATPs har %."-transporting subunit+13 ATP1A1 71 56.7 6 16 84 2.21092F-11 Scavenger r	Endonuclease domain containing 1	ENDOD1	7	33.7	6	83	17	0.004053892
Cadherin 16 CDH16 26 46.7 4 8 92 0.02438323 Echanclesoite triphosphate diphosphohydrolas 3 ENTPD3 17 34 6 77 23 0.00126897 Tripartite motif-containing 25 TRIM25 14 27.9 6 70 30 0.004216133 Lipolysis stimulated lipoprotein receptor LSR 20 22.3 6 3 97 5.489042-06 Signal transducer and activator of transcription 3 STAT3 18 25.3 4 22 78 0.0769338671 Protein tyrosine phosphatase, receptor type F PTPRF 79 64.5 6 13 87 1.526538-05 ADAM metallopeptidase domain 17 ADAM11 13 21.9 6 7 33 9530F6-07 Suppression of tumorigenicity 14 ST14 7 11 5 23 77 0.02635564 Carboxypertidase ENPEP 30 22.6 6 100 0 2.7448E0.5 Carboxypertidase Gportein-coupled receptor 61 ADAG61 4 2.5 4 2.4	Lamin B receptor	LBR	13	29.1	6	71	29	0.096990048
Ectonucleoside triphosphate diphospholydrolase 3 ENTPD3 17 34 6 72 28 2.3237E-07 Porazonoase 2 PON2 4 18.1 6 77 23 0.000226897 Tripartite motif-containing 25 TRIM25 14 27.9 6 70 30 0.000216133 Lipolysis stimulated lipoprotein receptor LSR 20 29.3 6 3 97 5.49804E-06 Signal transducer and activator of transcription 3 STAT3 18 35.3 5 42 58 0.169979355 Protein tyrosine phosphatase, receptor type M PTPRM 26 28.3 4 22 78 0.076938677 Transmembrane serine protease 4 TMPRS4 6 18.6 6 13 37 1.5263E-05 ADAM metallopeptidase domain 17 ADAM17 13 21.9 6 7 93 9.63391E-00 ADAM metallopeptidase domain 17 ADAM17 13 21.9 6 7 93 9.63391E-00 ADAM metallopeptidase domain 17 ADAM17 13 21.9 6 7 93 9.63391E-00 Suppression of tumorigenicity 14 ST14 7 11 5 23 77 0.026355564 Carbony petidase M Cpm 5 12.2 5 74 26 0.022474223 Gutantyl aminopeptidase domain 10 ADAM10 20 36.7 6 10 0 2.70449E-05 Xenotropic and polytropic retrovirus receptor 1 XPR1 7 11.2 5 14 86 8.781517E-05 Carbony petidase M Cpm 5 12.2 5 74 26 0.022474223 Gutantyl aminopeptidase Grupe Scype receptor 2 CELSE2 25 12.3 6 19 81 0.002619663 Adhesion G protein-coupled receptor G1 ADCRG1 4 2.5 4 24 76 0.13836571 Frizzled dass receptor 6 FZD6 8 8.6 5 6 61 39 0.027707251 ATP binding cassette subfamily F member 1 ABCF1 12 21.2 6 61 39 0.0027107253 ATP binding cassette subfamily F member 1 ABCF1 12 21.2 6 61 39 0.0027107255 ADTP sinding cassette subfamily F member 1 ABCF1 12 21.2 6 61 39 0.002710725 ATP binding cassette subfamily F member 1 ABCF1 12 21.2 6 61 39 0.002710725 ATP binding cassette subfamily F member 1 SLC34A1 4 10.7 6 82 18 0.002315915 Solute carrier family 39 member 1 SLC34A1 4 10.7 6 82 18 0.002315915 Solute carrier family 39 member 1 SLC34A1 4 10.7 6 82 18 0.002315915 Solute carrier family 39 member 1 SLC34A1 4 10.7 6 82 5 75 0.07072531 ATP short M*C+transporting subunit;11 ATP181 19 46.9 6 12 88 1.28265E-10 Solute carrier family 39 member 1 SLC34A2 4 2.5 75 0.07072532 ATPase Na*K'-transporting subunit;13 ATP181 77 53.8 6 27 73 0.04166374 Polymeri Ig receptor 4 PEPHA 16	Cadherin 16	CDH16	26	46.7	4	8	92	0.024358355
Parasonase 2 PON2 4 18.1 6 77 23 0.001226897 Tripartite motif-containing 25 TRIM25 14 27.9 6 70 30 0.001226897 Signal transducer and activator of transcription 3 STAT3 18 35.3 5 42 58 0.169979357 Protein tyrosine phosphatase, receptor type F PTPRF 79 64.5 6 28 72 5.646458-00 Calpain 2 CAPN2 40 64.9 6 64 36 0.13192804 Transmembrane serine protease 4 TMPRS54 6 18.6 6 13 87 9.53067E-07 Suppression of tumorigenicity 14 ST14 7 11 5 23 77 0.02835564 Carboxypetidase domain 10 ADAM 17 17 12.2 5 74 26 0.024747236 Glutamyl aminopetidase domain 10 ADAM 17 17 11.2 5 6 100 0 2.70448E-06 2.02.6 10.000519663 <td>Ectonucleoside triphosphate diphosphohydrolase 3</td> <td>ENTPD3</td> <td>17</td> <td>34</td> <td>6</td> <td>72</td> <td>28</td> <td>2.3237E-07</td>	Ectonucleoside triphosphate diphosphohydrolase 3	ENTPD3	17	34	6	72	28	2.3237E-07
Tripartite motif-containing 25 TRIM25 14 27.9 6 70 30 0.004216133 Lipolysis stimulated lipoprotein receptor LSR 20 23.3 6 3 97 5.49804E-06 Signal transducer and activator of transcription 3 STAT3 18 35.3 5 42 78 0.016997355 Protein tyrosine phosphatase, receptor type F PTPRF 79 64.5 6 28 72 6.56458E-05 Calpain 2 CAPN2 40 64.9 6 64 36 0.134192804 Transmembrane serine protease 4 TMPRSA 6 18.6 6 13 87 9.53391E-05 ADAM metallopeptidase domain 10 ADAM10 20 36.7 6 13 87 9.5307E-07 Suppression of tumorigenicity 14 ST14 7 11 5 23 77 0.26 6 100 0 2.27447236 Cathory petidase M Cpm 5 12.2 6 19 81 0.0001663 Adhesion G protein-couple teceptor 1 XPR1 7 11.2	Paraoxonase 2	PON2	4	18.1	6	77	23	0.001226897
Lipolysis stimulated lipoprotein receptor LSR 20 29.3 6 3 97 5.49804-06 Signal transducer and activator of transcription 3 STAT3 18 35.3 5 42 58 0.169979355 Protein tyrosine phosphatase, receptor type M PTRM 26 28.3 4 22 78 0.07893867 Protein tyrosine phosphatase, receptor type F PTRF 79 64.5 6 28 72 6.56458E-05 Calpain 2 CAPN2 40 64.9 6 64 36 0.131415204 Transmembrane serine protease 4 TMPR554 6 18.6 6 13 87 1.5253E-05 ADAM metallopeptidase domain 10 ADAM10 20 36.7 6 13 87 9.53067E-07 Suppression of tumorigenicity 14 ST14 7 11 5 23 77 0.02635564 Carboxypeptidase M Cpm 5 12.2 5 74 26 0.02474723 Glutamyl aminopeptidase domain 10 ADAM10 20 36.7 6 113 87 9.53067E-07 Suppression of tumorigenicity 14 ST14 7 11.2 6 14 86 8.78517E-05 Carboxypetidase M Cpm 5 12.2 5 74 26 0.02474723 Glutamyl aminopeptidase domain 2 ADAM474 13 12 5 74 26 0.02474723 Glutamyl aminopeptidase domain 2 ADAM474 13 12 6 14 86 8.78517E-05 Carboxypetidase M Cpm 5 12.2 5 74 26 0.02474723 Glutamyl aminopeptidase domain 2 ADAM474 13 12 6 14 86 8.78151E-05 Carboxypetim-coupled receptor 1 XPR1 7 11.2 6 14 86 8.78151E-05 Carboxip certerin-coupled receptor G ADCGRG 4 20.5 4 24 76 0.13386571 Frizzled class receptor 6 FZD6 8 8.6 5 36 64 0.12801500 G protein-coupled receptor G ADCGRG 4 20.5 4 24 76 0.13386571 ATP binding cassette subfamily F member 1 ABCE1 29 52 6 61 39 0.022770251 ATP binding cassette subfamily F member 1 ABCE1 29 52 6 61 39 0.022770251 ATP binding cassette subfamily F member 1 ABCE1 29 52 6 74 0.02315915 Solute carrier family 34 member 2 SLC3A0 4 20 28.6 6 88 92 5.95465E-06 Solute carrier family 34 member 3 ACGRB 11 22.2 6 75 0.076726321 ATP aba MarK*transporting subunit f1 ATP1B1 19 46.9 6 12 88 1.82805E-01 Solute carrier family 44 member 2 SLC3A1 4 11 22.2 6 75 0.076726321 ATP3e NarK*transporting subunit f1 ATP1B1 19 45.9 6 12 88 1.82805E-01 Solute carrier family 35 member 1 SLC3A1 4 11 22.4 6 73 0.01463934 Tarasferin (receptor FA EPMB4 16 22.4 6 11 99 0.000376316 EPh2 receptor J ATP1B1 19 45.9 6 12 88 6 27 73 0.01776251 Folate recepto	Tripartite motif-containing 25	TRIM25	14	27.9	6	70	30	0.004216133
Signal transducer and activator of transcription 3 STAT3 18 35.3 5 42 58 0.16979355 Protein tyrosine phosphatase, receptor type M PTRM 26 28.3 4 22 78 0.078938671 Protein tyrosine phosphatase, receptor type F PTRF 79 64.5 6 28 72 6.56458E05 Calpain 2 CAPN2 40 64.9 6 64 36 0.13419204 Transmembrane serine protease 4 TMPRS54 6 13 87 9.53067E07 ADAM metallopeptidase domain 10 ADAM10 20 36.7 6 13 87 9.53067E07 Suppression of tumorigenicity 14 ST14 7 11 5 23 77 0.02635564 Carboxypeptidase M Cpm 5 12.2 5 74 26 0.02474226 Carboxypeptidase ENPEP 30 2.25 6 100 0 2.74494E0 Carboxypeptidase ENPEP 30 0.27707251	Lipolysis stimulated lipoprotein receptor	LSR	20	29.3	6	3	97	5.49804E-06
Protein tyrosine phosphatase, receptor type M PTPRM 26 28.3 4 22 78 0.078938671 Protein tyrosine phosphatase, receptor type F PTPRF 79 64.5 6 28 72 6.96458E.05 Calpain 2 CAPN2 40 64.9 6 64 36 0.134192804 Transmembrane serine protease 4 TMPR554 6 18.6 6 13 87 1.52658E.05 ADAM metallopeptidase domain 10 ADAM10 20 36.7 6 13 87 9.53067E.07 Suppression of tumorigenicity 14 ST14 7 11 5 23 77 0.02635554 Carboxyperbidase ENPEP 30 22.6 6 100 0 2.70449E:05 Cadherin EGF LAG seven-pass G-type receptor 2 CELSR2 25 12.3 6 19 81 0.005019663 ATP binding casset subfamily F member 1 ADCRG1 4 2.5 4 24 76 0.13836571 Frizzled class receptor	Signal transducer and activator of transcription 3	STAT3	18	35.3	5	42	58	0.169979355
Protein tyrosine phosphatase, receptor type F PTRFF 79 64.5 6 28 72 6.96458E-05 Calpain 2 CAPN2 40 64.9 6 64 36 0.134192844 Transmembrane serine protease 4 TMPRS54 6 18.6 6 13 87 15.5263E-05 ADAM metallopeptidase domain 10 ADAM17 13 21.9 6 7 93 9.63391E-07 Suppression of tumorigenicity 14 ST14 7 11 5 23 77 0.02635564 Carboxypeptidase M Cpm 5 12.2 5 74 26 0.02747235 Carboxypeptidase ENPEP 30 2.6 6 100 0 2.7449E-05 Achesion G protein-couple receptor G1 ADGG1 4 2.5 4 24 76 0.13836571 Frizzled class receptor 6 FZD6 8 8.6 5 36 64 0.128045605 Solute carrier family 35 member 1 ABCF1 29	Protein tyrosine phosphatase, receptor type M	PTPRM	26	28.3	4	22	78	0.078938671
Calpain 2 CAPN2 40 64.9 6 64 36 0.134192804 Transmembrane serine protease 4 TMPRSS4 6 18.6 6 13 87 1.52633E05 ADAM metallopeptidase domain 10 ADAM10 20 36.7 6 13 87 9.53067E-07 Suppression of tumorigenicity 14 ST14 7 11 5 23 77 0.02635554 Carboxypeptidase M Cpm 5 12.2 5 74 26 0.024747236 Glutamyl aminopeptidase M Cpm 5 12.2 5 74 26 0.024747236 Cadherin EGF LAG seven-pass G-type receptor 2 CELSR2 25 12.3 6 19 81 0.005019663 Adhesion G protein-coupled receptor G1 ADGRG1 4 2.5 4 24 76 0.13836571 Frizzled Class receptor 6 FZD6 8 8.6 5 36 64 0.128016301 Gaterin-coupled receptor class C group 5 member A GPRCS	Protein tyrosine phosphatase, receptor type F	PTPRF	79	64.5	6	28	72	6.96458E-05
Transmembrane serine protease 4TMPRS54618.6613871.52653E-05ADAM metallopeptidase domain 10ADAM171321.967939.63391E-05Suppression of tumorigenicity 14ST14711523770.026355564Carboxypeptidase MCpm512.2574260.024747236Carboxypeptidase MCpm512.2574260.024747236Carboxypeptidase MCpm512.2610002.70448E-05Cadherin EGF LAG seven-pass G-type receptor 2CELSR 22512.3619810.005019663Cadherin EGF LAG seven-pass G-type receptor 2CELSR 22512.3619810.005019663G protein-coupled receptor G1ADGRG142.5424760.13836571Frizzled class receptor 6FZD688.6536640.128016301G protein-coupled receptor 13ADGR142.5424760.13836571Solute carrier family 57 member AGPRC5A410.1687130.0027707251ATP binding cassette subfamily F member 1ABCF11221.266180.22707251Solute carrier family 39 member 10SLC39A1049.9626742.85037E-08Solute carrier family 39 member 11ATP18117156.7616	Calpain 2	CAPN2	40	64.9	6	64	36	0.134192804
ADAM metallopeptidase domain 17ADAM171321.967939.63391E-05ADAM metallopeptidase domain 10ADAM102036.7613879.53067E-07Suppression of tumorigenicity 14ST14711523770.026355546Carboxypeptidase MCpm512.257.42.60.024747236Glutamyl aminopeptidaseENPEP3022.6610002.70449E-05Xenotropic and polytropic retrovirus receptor 1XPR1711.2614868.78517E-05Cadherin EGE LAG seven-pass G-type receptor 2CELSR22512.3619810.005019663Adhesion G protein-coupled receptor G1ADGRG142.5424760.13836571Grizzle dass receptor 6FZD688.6536640.128016301G protein-coupled receptor class C group 5 member AGPRC5A410.1687130.002707251ATP binding cassette subfamily F member 1ABCF11221.2669310.00370451Solute carrier family 35 member A1SLC35A1410.7682180.022707251Solute carrier family 39 member 10SLC39A1049.9626742.85037E-06Solute carrier family 39 member 10SLC39A1049.9626742.85037E-05Solute carrier family 39 member 1S	Transmembrane serine protease 4	TMPRSS4	6	18.6	6	13	87	1.52653E-05
ADAM metallopeptidase domain 10 ADAM10 20 36.7 6 13 87 9.53067E-07 Suppression of tumorigenicity 14 ST14 7 11 5 23 77 0.02635564 Carboxypeptidase M Cpm 5 12.2 5 74 26 0.02474223 Glutamyl aminopeptidase ENPEP 30 22.6 6 100 0 2.70449E-05 Xenotropic and polytropic retrovirus receptor 1 XPR1 7 11.2 6 14 86 8.78517E-05 Cadherin EGF LAG seven-pass G-type receptor 2 CELSR2 25 12.3 6 19 81 0.005019663 G protein-coupled receptor G1 ADGRG1 4 2.5 4 2.4 7.6 0.13836571 Frizzled class receptor 6 Group 5 Group 5 2.2 6 61 39 0.02707251 ATP binding cassette subfamily F member 1 ABCF1 29 52 6 61 39 0.027107251 Solute carrier family 35 member A1 SLC3A21 4 10.7 6 82 18.80761	ADAM metallopeptidase domain 17	ADAM17	13	21.9	6	7	93	9.63391E-05
Suppression of tumorigenicity 14 ST14 7 11 5 23 77 0.026355564 Carboxypeptidase M Cpm 5 12.2 5 74 26 0.024742236 Glutamyl aminopeptidase ENPEP 30 22.6 6 10 0 2.70449E-05 Xenotropic and polytropic retrovirus receptor 1 XPR1 7 11.2 6 14 86 8.78517E-05 Cadherin EGF LAG seven-pass G-type receptor 2 CELSR2 25 12.3 6 19 81 0.005019663 Adhesion G protein-coupled receptor G1 ADGRG1 4 2.5 4 24 76 0.13836571 ATP binding cassett subfamily F member 1 ABCE1 29 52 6 61 39 0.027707251 ATP binding cassett subfamily F member 1 ABCF1 12 21.2 6 69 31 0.08017044 Solute carrier family 3 member 10 SLC39A10 4 9.9 6 26 74 2.85037E-08 Solute carrier family 4 member 2 SLC44A2 37 11.2 5 8 92	ADAM metallopeptidase domain 10	ADAM10	20	36.7	6	13	87	9.53067E-07
Carboxypetidase MCpm51.2.2574260.024747236Glutamyl aminopeptidaseENPEP3022.6610002.70449E.05Xenotropic and polytropic retrovirus receptor 1XPR1711.2614868.78517E.05Cadherin EGF LAG seven-pass G-type receptor 2CELSR22512.3619810.00501963Adhesion G protein-coupled receptor G1ADGRG142.5424760.13836571Frizzled class receptor 6FZD688.6536640.128016301G protein-coupled receptor class C group 5 member AGPRC5A410.1687130.000616634ATP binding cassette subfamily E member 1ABCE12952661390.027707251ATP binding cassette subfamily F member 1ABCE11221.2669310.0080170041Solute carrier family 35 member A1SLC35A1410.7682180.02315915Solute carrier family 34 member 2SLC4A22028.668925.95465E-06Solute carrier family 39 member 1SLC4A23711.258920.002769117ATPase Na*/K*transporting subunit β1ATP1B11946.9612881.82896E-10ATPase Na*/K*transporting subunit β3ATP1B3753.8425750.102593542ATPase Na*/K*transporting	Suppression of tumorigenicity 14	ST14	7	11	5	23	77	0.026355564
Glutamyl aminopeptidase ENPEP 30 22.6 6 100 0 2.70449E-05 Xenotropic and polytropic retrovirus receptor 1 XPR1 7 11.2 6 14 86 8.78517E-05 Cadherin EG LAG seven-pass G-type receptor 2 CELSR2 25 12.3 6 19 81 0.005019663 Adhesion G protein-coupled receptor G1 ADGRG1 4 2.5 4 24 76 0.13836571 Gi protein-coupled receptor dass C group 5 member A GPRCSA 4 10.1 6 87 13 0.000616634 ATP binding cassette subfamily F member 1 ABCE1 29 52 6 61 39 0.027707251 ATP binding cassette subfamily F member 1 ABCE1 29 52 6 61 39 0.027707251 ATP binding cassette subfamily F member 1 SLC34A1 4 10.7 6 82 18 0.02315915 Solute carrier family 39 member 10 SLC34A2 20 28.6 6 8 92 0.002769173 ATPase Na*/K*-transporting subunit-01 ATPIA1 71 5	Carboxypeptidase M	Cpm	5	12.2	5	74	26	0.024747236
Xenotropic and polytropic retrovirus receptor 1 XPR1 7 11.2 6 14 86 8.78517E-05 Cadherin EGF LAG seven-pass G-type receptor 2 CELSR2 25 12.3 6 19 81 0.005019663 Adhesion G protein-coupled receptor G1 ADGRG1 4 2.5 4 24 76 0.13836571 Frizzled class receptor 6 FZD6 8 8.6 5 36 64 0.128016301 G protein-coupled receptor class C group 5 member A GPRCSA 4 10.1 6 87 13 0.000616634 ATP binding cassette subfamily E member 1 ABCF1 12 21.2 6 69 31 0.00070251 Solute carrier family 35 member A1 SLC35A1 4 10.7 6 82 18 0.02315915 Solute carrier family 9 member 10 SLC39A10 4 9.9 6 26 74 2.850371-08 Solute carrier family 9 member 10 SLC39A10 4 9.9 6 12 88 1.82896E-101 ATPase Na*/K*-transporting subunit β1 ATP181 71 56.7	Glutamyl aminopeptidase	ENPEP	30	22.6	6	100	0	2.70449E-05
Cadherin EGF LAG seven-pas G-type receptor 2CELSR22512.3619810.005019663Adhesion G protein-coupled receptor G1ADGRG142.5424760.13836571Frizzled class receptor 6FZD688.6536640.128016301G protein-coupled receptor class C group 5 member AGPRC5A410.1687130.000616634ATP binding cassette subfamily F member 1ABCF11221.2669310.080170041Solute carrier family 35 member A1SLC35A1410.7682180.02315915Solute carrier family 39 member 10SLC35A1049.9626742.85037E-08Solute carrier family 39 member 10SLC39A1049.9626742.85037E-08Solute carrier family 4 member 2SLC4A23711.258920.002769117ATPase Na ⁺ /K ⁺ -transporting subunit β 1ATP1A17156.7616842.21093E-11Scavenger receptor class B member 1SCARB11122.2625750.076726321ATPase Na ⁺ /K ⁺ -transporting subunit- β 3ATP1B373.8425750.102593542HephaestinHEPH2325626740.00961634Polymeric Ig receptorFIRC2443.5686140.000183635Gracrionembryonic antigen-rel	Xenotropic and polytropic retrovirus receptor 1	XPR1	7	11.2	6	14	86	8.78517E-05
Adhesion G protein-coupled receptor G1ADGRG142.5424760.13836571Frizzled class receptor 6FZD688.6536640.128016301G protein-coupled receptor class C group 5 member AGPRC5A410.1687130.000616634ATP binding cassette subfamily E member 1ABCE12952661390.027707251ATP binding cassette subfamily F member 1ABCF11221.2669310.080170041Solute carrier family 44 member 2SLC4A422028.668925.95465E-06Solute carrier family 44 member 2SLC4A22028.668920.00276917ATPase Na*/K*-transporting subunit β 1ATP1B11946.9612881.82896E-10ATPase Na*/K*-transporting subunit- α 1ATP1B1753.8425750.076726321ATPase Na*/K*-transporting subunit- β 3ATP1B3753.8425750.01293542Polymeric Ig receptorPIGR1018.8627730.04168744Transferrin receptor- β FOLR2423.5686140.00183835Polymeric Ig receptor- β FOLR2423.5686140.00183836Polymeric Ig receptor- β FOLR2423.5686140.00183836Polymeric Ig receptor- β FOLR24	Cadherin FGE LAG seven-pass G-type receptor 2	CELSR2	25	12.3	6	19	81	0.005019663
Frizzled class receptor 6FIZD688.6536640.128016301G protein-coupled receptor class C group 5 member AGPRC5A410.1687130.000616634ATP binding cassette subfamily F member 1ABCF11221.2669310.000710245Solute carrier family 35 member A1SLC35A1410.7682180.02315915Solute carrier family 35 member A1SLC35A1410.7682180.02315915Solute carrier family 44 member 2SLC44A22028.668925.95465E-06Solute carrier family 39 member 10SLC39A1049.9626742.85037E-08Solute carrier family 4 member 2SLC4A23711.258920.002769117ATPase Na*/K+*transporting subunit β1ATP1811946.9612881.82896E-10ATPase Na*/K+*transporting subunit-α1ATP1A17156.7616842.21093E-11Scavenger receptor class B member 1SCARB11122.2625750.102593542HephaestinHEPH2325626740.019696101Polymeric Ig receptorPIGR1018.8627730.004168334Carcinoembryonic antigen-related cell adhesion molecule 1CEACAM1162969370.000146391 <trr<tr>EPH receptor A1EPHB4<td>Adhesion G protein-coupled receptor G1</td><td>ADGRG1</td><td>4</td><td>2.5</td><td>4</td><td>24</td><td>76</td><td>0 13836571</td></trr<tr>	Adhesion G protein-coupled receptor G1	ADGRG1	4	2.5	4	24	76	0 13836571
$\begin{array}{c} \mbox{TransPict cuts} receptor class C group 5 member A & GPRC5A & 4 & 10.1 & 6 & 87 & 13 & 0.00616634 \\ \mbox{ATP binding cassette subfamily E member 1 & ABCE1 & 29 & 52 & 6 & 61 & 39 & 0.027707251 \\ \mbox{ATP binding cassette subfamily F member 1 & ABCF1 & 12 & 21.2 & 6 & 69 & 31 & 0.080170041 \\ \mbox{Solute carrier family 35 member A1 & SLC35A1 & 4 & 10.7 & 6 & 82 & 18 & 0.02315915 \\ \mbox{Solute carrier family 44 member 2 & SLC4A2 & 20 & 28.6 & 6 & 8 & 92 & 9.59465E-06 \\ \mbox{Solute carrier family 44 member 2 & SLC4A2 & 37 & 11.2 & 5 & 8 & 92 & 0.002769117 \\ \mbox{ATPase Na^+/K^+-transporting subunit $\beta1 & ATP181 & 19 & 46.9 & 6 & 12 & 88 & 1.82896E-10 \\ \mbox{ATPase Na^+/K^+-transporting subunit-$\alpha1 & ATP181 & 71 & 56.7 & 6 & 16 & 84 & 2.21093E-11 \\ \mbox{Scavenger receptor class B member 1 & SCARB1 & 11 & 22.2 & 6 & 25 & 75 & 0.076726321 \\ \mbox{ATPase Na^+/K^+-transporting subunit-$\beta3 & ATP183 & 7 & 53.8 & 4 & 25 & 75 & 0.102593542 \\ \mbox{Hephaestin } & HEPH & 23 & 25 & 6 & 26 & 74 & 0.019696101 \\ \mbox{Polymeric Ig receptor } & FIRC & 32 & 45.3 & 6 & 27 & 73 & 0.041687344 \\ \mbox{Franspertin receptor } & FIRC & 32 & 45.3 & 6 & 27 & 73 & 0.001648345 \\ Franspertin receptor A1 & EPHB4 & 16 & 22.4 & 6 & 1 & 99 & 0.000591366 \\ \mbox{EPH receptor B4 & EPHB4 & 16 & 22.4 & 6 & 1 & 99 & 0.000591366 \\ \mbox{EPH receptor A1 & EPHB4 & 16 & 22.4 & 6 & 1 & 99 & 0.000591366 \\ \mbox{EPH receptor A1 & EPHB4 & 16 & 22.4 & 6 & 1 & 99 & 0.000591366 \\ \mbox{EPH receptor A1 & EPHB4 & 16 & 22.4 & 6 & 1 & 99 & 0.000591366 \\ \mbox{EPH receptor A1 & EPHB4 & 16 & 22.4 & 6 & 1 & 99 & 0.000591366 \\ \mbox{EPH receptor A1 & EPHB4 & 16 & 22.4 & 6 & 1 & 99 & 0.000591366 \\ \mbox{EPH receptor A1 & EPHB4 & 16 & 22.4 & 6 & 13 & 87 & 2.46446E-05 \\ \mbox{Ephrin B1 & FNR & 73.7 & 6 & 28 & 72 & 0.012405291 \\ \mbox{Foldermal growth factor receptor tyrosine kinase & MET & 38 & 36.8 & 6 & 27 & 73 & 0.0111350932 \\ \mbox{Ephrin B1 & PODXL & 13 & 24.7 & 6 & 99 & 1 & 9.85873E-10 \\ \mbox{Foldermal growth factor receptor tyr$	Frizzled class recentor 6	FZD6	8	8.6	5	36	64	0 128016301
Op DefinitionCase (Correction)CorrectorC	G protein-coupled receptor class C group 5 member A	GPRC5A	4	10.1	6	87	13	0.000616634
ATP binding classette subfamily F member 1ABCF11221661510.080170041Solute carrier family 35 member A1SLC35A1410.7682180.02315915Solute carrier family 35 member A1SLC39A1049.9626742.80037E-08Solute carrier family 39 member 10SLC39A1049.9626742.80037E-08Solute carrier family 39 member 2SLC4A23711.258920.002769117ATPase Na*/K*-transporting subunit β 1ATP1B11946.9612881.82896E-10ATPase Na*/K*-transporting subunit- α 1ATP1A17156.7616842.21093E-11Scavenger receptor class B member 1SCARB11122.2625750.076726321ATPase Na*/K*-transporting subunit- β 3ATP1B3753.8425750.012693542HephaestinHEPH2325626740.001696101Polymeric Ig receptorPIGR1018.8627730.041687344Transferrin receptor- β FOLR2423.5686140.000183835Carcinoembryonic antigen-related cell adhesion molecule 1CEACAM1162969370.000581366EPH receptor A1EPHB41622.461990.000581366EPH receptor A1EPHB41622.46 </td <td>ATP hinding cassette subfamily E member 1</td> <td>ABCE1</td> <td>29</td> <td>52</td> <td>6</td> <td>61</td> <td>39</td> <td>0.027707251</td>	ATP hinding cassette subfamily E member 1	ABCE1	29	52	6	61	39	0.027707251
ATT binding clastice subseties solute carrier family 35 member A1LC35A1410.7682180.030170041Solute carrier family 35 member A1SLC35A1410.7682180.02315915Solute carrier family 39 member 10SLC39A1049.9626742.85037E-08Solute carrier family 4 member 2SLC4A23711.258920.002769117ATPase Na ⁺ /K ⁺ -transporting subunit β1ATP1A17156.7616842.21093E-11Scavenger receptor class B member 1SCARB11122.2625750.076726321ATPase Na ⁺ /K ⁺ -transporting subunit-β3ATP1B3753.8425750.102593542HephaestinHEPH2325626740.019696101Polymeric Ig receptorPIGR1018.8627730.041687344Transferrin receptor-βFOLR2423.5686140.000183835Carcinoembryonic antigen-related cell adhesion molecule 1CEACAM1162969370.003683916EPH receptor A1EPHA11725.358920.003683916EPH-b2 receptor tyrosine kinase 2ERB23841.2613872.46446E-05Epidermal growth factor receptorEGFR2931.9618822.48095E-05Epidermal growth factor receptorEGFR<	ATP binding cassette subfamily E member 1	ABCE1	12	21.2	6	69	31	0.027707231
Solute carrier family 39 member 1SLC34A22028.668925.9465E.06Solute carrier family 49 member 2SLC34A22028.668925.9465E.06Solute carrier family 49 member 2SLC34A23711.258920.002769117ATPase Na ⁺ /K ⁺ -transporting subunit β 1ATP1B11946.9612881.82896E.10ATPase Na ⁺ /K ⁺ -transporting subunit- α 1ATP117156.7616842.21093E.11Scavenger receptor class B member 1SCARB11122.2625750.076726321ATPase Na ⁺ /K ⁺ -transporting subunit- β 3ATP1B3753.8425750.01259354HephaestinHEPH2325626740.019696101Polymeric Ig receptorPIGR1018.8627730.041687344Transferrin receptor- β FOL2423.5686140.000183835Carcinoembryonic antigen-related cell adhesion molecule 1CEACAM1162969370.0001463916EPH receptor A1EPHA11725.358920.003683916Erb-b2 receptor tyrosine kinase 2ERB23841.2613872.46446E-05Epidermal growth factor receptorEGFR2931.9618822.48095E-05MET protooncogene, receptor tyrosine kinaseMET38 <td>Solute carrier family 35 member A1</td> <td></td> <td>12</td> <td>10.7</td> <td>6</td> <td>82</td> <td>18</td> <td>0.000170041</td>	Solute carrier family 35 member A1		12	10.7	6	82	18	0.000170041
Solute carrier family 44 member 2SLC39A1049.9626742.85037E-08Solute carrier family 39 member 10SLC39A1049.9626742.85037E-08Solute carrier family 4 member 2SLC4A23711.258920.002769117ATPase Na ⁺ /K ⁺ -transporting subunit β 1ATP1B11946.9612881.82896E-10ATPase Na ⁺ /K ⁺ -transporting subunit- α 1ATP1A17156.7616842.21093E-11Scavenger receptor class B member 1SCARB11122.2625750.076726321ATPase Na ⁺ /K ⁺ -transporting subunit- β 3ATP1B3753.8425750.102593542HephaestinHEPH2325626740.019696101Polymeric Ig receptorPIGR1018.8627730.041687344Folate receptor- β FOLR2423.5686140.000183835Carcinoembryonic antigen-related cell adhesion molecule 1CEACAM1162969370.000146391EPH receptor B4EPHB41622.461990.002591366EPH receptor tyrosine kinase 2ERB23841.2613872.46406E-05Epidermal growth factor receptorEGFR2931.9618822.480369-03EPhrin B1PODXL1324.7699<	Solute carrier family 33 member 2	SLCAAAA	20	78.6	6	02	07	5 95/655 06
Solute carrier family 35 member 10SLC3A1043.9626742.50376-06Solute carrier family 4 member 2SLC3A23711.258920.002769117ATPase Na*/K*-transporting subunit β 1ATP1811946.9612881.82896E-10ATPase Na*/K*-transporting subunit- α 1ATP1A17156.7616842.21093E-11Scavenger receptor class B member 1SCARB11122.2625750.076726321ATPase Na*/K*-transporting subunit- β 3ATP1B3753.8425750.102593542HephaestinHEPH2325626740.019696101Polymeric Ig receptorPIGR1018.8627730.041687344Transferrin receptor- β FOLR2423.5686140.00183835Carcinoembryonic antigen-related cell adhesion molecule 1CEACAM1162969370.000146391EPH receptor A1EPHB41622.461990.003683916Erb-b2 receptor tyrosine kinase 2ERB23841.2613872.46446E-05Epidermal growth factor receptorEGFR2931.9618822.48095E-05MET protooncogene, receptor tyrosine kinaseMET3836.8627730.111350932PodocalyxinPODXL1324.7699 <td>Solute carrier family 20 member 10</td> <td>SLC44AZ</td> <td>20</td> <td>20.0</td> <td>6</td> <td>0 26</td> <td>92 74</td> <td>3.95405E-00</td>	Solute carrier family 20 member 10	SLC44AZ	20	20.0	6	0 26	92 74	3.95405E-00
Solute Carlier family 4 member 251515652 (0.027917) ATPase Na ⁺ /K ⁺ -transporting subunit β 1ATP1811946.9612881.82896E-10ATPase Na ⁺ /K ⁺ -transporting subunit- α 1ATP1A17156.7616842.21093E-11Scavenger receptor class B member 1SCARB11122.2625750.102593542ATPase Na ⁺ /K ⁺ -transporting subunit- β 3ATP1B3753.8425750.102593542HephaestinHEPH2325626740.019696101Polymeric Ig receptorPIGR1018.8627730.041687344Transferrin receptor- β FOLR2423.5686140.000183835Carcinoembryonic antigen-related cell adhesion molecule 1CEACAM1162969370.000146391EPH receptor B4EPHB41622.461990.000591366Erb-b2 receptor tyrosine kinase 2ERBB23841.2613872.46446E-05Epidermal growth factor receptorEGFR2931.9618822.48095E-05Epirtmal B1FODAL1324.769919.85873E-10	Solute carrier family 4 member 2	SLCAAD	4	3.5 11 C	5	20	74	2.030371-00
ATPase Na /K -transporting subunit α 1ATP is 1is 346.5612861.2.25362-10ATPase Na */K +transporting subunit α 1ATP is 17156.7616842.21093E-11Scavenger receptor class B member 1SCARB11122.2625750.076726321ATPase Na */K +transporting subunit- β 3ATP iB3753.8425750.102593542HephaestinHEPH2325626740.019696101Polymeric Ig receptorPIGR1018.8627730.041687344Transferrin receptorTFRC3245.3627730.041687344Transferrin receptor- β FOLR2423.5686140.000183835Carcinoembryonic antigen-related cell adhesion molecule 1CEACAM1162969370.000146391EPH receptor B4EPHB41622.461990.000591366Erb-b2 receptor tyrosine kinase 2ERBB23841.2613872.46446E-05Epidermal growth factor receptorEGFR2931.9618822.48095E-05MET3836.8627730.111350932Ephrin B1FFNB1737.2628720.012405291PodocalyxinPODXL1324.769919.85873E-10	Solute carrier family 4 member 2 ATPase Na^{+}/V^{+} transporting subunit 01	ATD1D1	57 10	11.2	5	0 12	92	1 929065 10
A IP A17156.7616842.21093E-11Scavenger receptor class B member 1SCARB11122.2625750.076726321ATPase Na ⁺ /K ⁺ -transporting subuit- β 3ATP1B3753.8425750.102593542HephaestinHEPH2325626740.019696101Polymeric Ig receptorPIGR1018.8627730.041687344Transferrin receptorTFRC3245.3627737.07717E-08Folate receptor- β FOLR2423.5686140.000183835Carcinoembryonic antigen-related cell adhesion molecule 1CEACAM1162969370.000146391EPH receptor B4EPHB41622.461990.000591366EPH receptor tyrosine kinase 2ERBB23841.2613872.46446E-05Epidermal growth factor receptorEGFR2931.9618822.48095E-05MET protooncogene, receptor tyrosine kinaseMET3836.8627730.111350932Ephrin B1PODXL1324.769919.85873E-10	ATPase Na /K -transporting subunit p_1	ATPIBI	19	46.9	0	12	00	1.82896E-10
Scavenger receptor class B member 1SCARB 11122.262575 0.076726321 ATP ase Na ⁺ /K ⁺ -transporting subunit- β 3ATP 1B3753.842575 0.102593542 HephaestinHEPH232562674 0.019696101 Polymeric Ig receptorPIGR1018.862773 0.041687344 Transferrin receptorTFRC3245.362773 $7.07717E.08$ Folate receptor- β FOLR2423.568614 0.000183835 Carcinoembryonic antigen-related cell adhesion molecule 1CEACAM116296937 0.000146391 EPH receptor B4EPHB41622.46199 0.000591366 EPH receptor tyrosine kinase 2ERBB23841.2613872.46446E.05Epidermal growth factor receptorEGFR2931.9618822.48095E.05MET protooncogene, receptor tyrosine kinaseMET3836.862773 0.111350932 Ephrin B1PODXL1324.76991 $9.85873E-10$	A Pase Na $/K$ -transporting subunit- α i	AIPIAI	/1	56.7	6	16	84	2.21093E-11
A I Pase Na '/K'-transporting subunit-β3A I P1B3753.8425750.102593542HephaestinHEPH2325626740.019696101Polymeric Ig receptorPIGR1018.8627730.041687344Transferrin receptorTFRC3245.3627737.07717E-08Folate receptor-βFOLR2423.5686140.000183835Carcinoembryonic antigen-related cell adhesion molecule 1CEACAM1162969370.000146391EPH receptor B4EPHB41622.461990.000591366EPH receptor A1EPHA11725.358920.003683916Erb-b2 receptor tyrosine kinase 2ERBB23841.2613872.46446E-05Epidermal growth factor receptorEGFR2931.9618822.48095E-05MET protooncogene, receptor tyrosine kinaseMET3836.8627730.111350932Ephrin B1FODXL1324.769919.85873E-10	Scavenger receptor class B member 1	SCARBI	11	22.2	6	25	75	0.076726321
HephaestinHEPH2325626740.019696101Polymeric Ig receptorPIGR1018.8627730.041687344Transferrin receptorTFRC3245.3627737.07717E-08Folate receptor-βFOLR2423.5686140.000183835Carcinoembryonic antigen-related cell adhesion molecule 1CEACAM1162969370.000146391EPH receptor B4EPHB41622.461990.000591366EPH receptor tyrosine kinase 2ERB23841.2613872.46446E-05Epidermal growth factor receptorEGFR2931.9618822.48095E-05MET7337.2628720.0111350932Ephrin B1PODXL1324.769919.85873E-10	AlPase Na ⁺ /K ⁺ -transporting subunit-β3	ATP1B3	/	53.8	4	25	/5	0.102593542
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Transferrin receptorTFRC 32 45.3 6 27 73 $7.07717E-08$ Folate receptor- β FOLR2 4 23.5 6 86 14 0.000183835 Carcinoembryonic antigen-related cell adhesion molecule 1CEACAM1 16 29 6 93 7 0.000146391 EPH receptor B4EPHB4 16 22.4 6 1 99 0.000591366 EPH receptor A1EPHB4 17 25.3 5 8 92 0.003683916 Erb-b2 receptor tyrosine kinase 2ERBB2 38 41.2 6 13 87 $2.46446E-05$ Epidermal growth factor receptorEGFR 29 31.9 6 18 82 $2.48095E-05$ MET protooncogene, receptor tyrosine kinaseMET 38 36.8 6 27 73 0.111350932 Ephrin B1FODXL 13 24.7 6 99 1 $9.85873E-10$	Polymeric Ig receptor	PIGR	10	18.8	6	27	73	0.041687344
Folate receptor-βFOLR2423.5686140.000183835Carcinoembryonic antigen-related cell adhesion molecule 1CEACAM1162969370.000146391EPH receptor B4EPHB41622.461990.000591366EPH receptor A1EPHA11725.358920.003683916Erb-b2 receptor tyrosine kinase 2ERBB23841.2613872.46446E-05Epidermal growth factor receptorEGFR2931.9618822.48095E-05MET protooncogene, receptor tyrosine kinaseMET3836.8627730.111350932Ephrin B1EFNB1737.2628720.012405291PodocalyxinPODXL1324.769919.85873E-10	Transferrin receptor	TFRC	32	45.3	6	27	73	7.07717E-08
Carcinoembryonic antigen-related cell adhesion molecule 1CEACAM1162969370.000146391EPH receptor B4EPHB41622.461990.000591366EPH receptor A1EPHA11725.358920.003683916Erb-b2 receptor tyrosine kinase 2ERBB23841.2613872.46446E-05Epidermal growth factor receptorEGFR2931.9618822.48095E-05MET protooncogene, receptor tyrosine kinaseMET3836.8627730.111350932Ephrin B1EFNB1737.2628720.012405291PodocalyxinPODXL1324.769919.85873E-10	Folate receptor-β	FOLR2	4	23.5	6	86	14	0.000183835
EPH receptor B4EPHB41622.461990.000591366EPH receptor A1EPHA11725.358920.003683916Erb-b2 receptor tyrosine kinase 2ERBB23841.2613872.46446E-05Epidermal growth factor receptorEGFR2931.9618822.48095E-05MET protooncogene, receptor tyrosine kinaseMET3836.8627730.111350932Ephrin B1FNB1737.2628720.012405291PodocalyxinPODXL1324.769919.85873E-10	Carcinoembryonic antigen-related cell adhesion molecule 1	CEACAM1	16	29	6	93	7	0.000146391
EPH receptor A1EPHA11725.358920.003683916Erb-b2 receptor tyrosine kinase 2ERBB23841.2613872.46446E-05Epidermal growth factor receptorEGFR2931.9618822.48095E-05MET protooncogene, receptor tyrosine kinaseMET3836.8627730.111350932Ephrin B1FNB1737.2628720.012405291PodocalyxinPODXL1324.769919.85873E-10	EPH receptor B4	EPHB4	16	22.4	6	1	99	0.000591366
Erb-b2 receptor tyrosine kinase 2ERBB23841.2613872.46446E-05Epidermal growth factor receptorEGFR2931.9618822.48095E-05MET protooncogene, receptor tyrosine kinaseMET3836.8627730.111350932Ephrin B1FNB1737.2628720.012405291PodocalyxinPODXL1324.769919.85873E-10	EPH receptor A1	EPHA1	17	25.3	5	8	92	0.003683916
Epidermal growth factor receptor EGFR 29 31.9 6 18 82 2.48095E-05 MET protooncogene, receptor tyrosine kinase MET 38 36.8 6 27 73 0.111350932 Ephrin B1 EFNB1 7 37.2 6 28 72 0.012405291 Podocalyxin PODXL 13 24.7 6 99 1 9.85873E-10	Erb-b2 receptor tyrosine kinase 2	ERBB2	38	41.2	6	13	87	2.46446E-05
MET 38 36.8 6 27 73 0.111350932 Ephrin B1 EFNB1 7 37.2 6 28 72 0.012405291 Podocalyxin PODXL 13 24.7 6 99 1 9.85873E-10	Epidermal growth factor receptor	EGFR	29	31.9	6	18	82	2.48095E-05
Ephrin B1 EFNB1 7 37.2 6 28 72 0.012405291 Podocalyxin PODXL 13 24.7 6 99 1 9.85873E-10	MET protooncogene, receptor tyrosine kinase	MET	38	36.8	6	27	73	0.111350932
Podocalyxin PODXL 13 24.7 6 99 1 9.85873E-10	Ephrin B1	EFNB1	7	37.2	6	28	72	0.012405291
	Podocalyxin	PODXL	13	24.7	6	99	1	9.85873E-10

The complete table is in Dataset S1.

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Fig. 3. Silencing of the μ 1A or μ 1B subunits of the AP-1 complex in MDCK cells produces shifts in the polarity of surface proteins. Linear regressions obtained by comparing the apical distribution of control proteins (luciferase siRNA) with (A) μ 1A siRNA, (B) μ 1B siRNA, and (C) double silencing of μ 1A and μ 1B simultaneously (μ 1A + μ 1B). The fitted line indicating the region of no change is represented in red and crosses the origin at x = 0 and y = 0. Protein shifts resulting from loss of apical polarity fall in the quadrant indicated in green. A select set of proteins (indicated by name) was identified to verify the results by surface biotinylation and Western blot after silencing of μ 1A, μ 1B, or both in MDCK cells. Ap, apical; BL, basolateral.

polarity of both apical and basolateral proteins, while μ 1B predominated in maintenance of basolateral polarity.

These separate roles of AP-1A and AP-1B are evident when looking at the proportions of proteins affected by the single and double silencing (Fig. 5*E*). Of all of the proteins that experienced a shift in the apical-to-basolateral direction, the largest number of proteins was included in the double-silencing group. Individual µ1A or µ1B silencing affected a smaller proportion of proteins, but the predominant shift was due to silencing of µ1A. In contrast, among the proteins that experienced a shift in the basolateral-to-apical direction, the largest group was due to silencing of µ1B.

Altogether, our analysis indicates that $\mu 1B$ silencing favored shifts in the basolateral-to-apical direction, while $\mu 1A$ silencing had an independent effect that was comparable in both directions. This may indicate physiological differences between the two adaptors. While AP-1B function may be more relevant to basolateral polarity, AP-1A provides a mechanism for some proteins to achieve apical distribution. In certain cases, however, the two adaptors seem to cooperate in the polarized distribution of specific proteins.

Discussion

With the advent of high-throughput mass spectrometry-based techniques, the study of membrane surface proteins has advanced toward discovery approaches and a more comprehensive characterization of surface proteomes (28). However, the study of surface proteins presents unique challenges, such as their relative low abundance compared with intracellular proteins, solubility issues, and contamination with intracellular proteins. These limitations can be minimized to some extent by enriching the samples via membrane fractionation or biochemical methods as discussed elsewhere (29). In this work, we used a domainspecific surface biotinylation approach followed by quantitative mass spectrometry to characterize the polarized surface proteome of epithelial cells. These techniques are readily available in the toolkit of most laboratories, making it attractive for routine studies. However, it is important to pinpoint the limitations of our approach. One of them is inherent to mass spectrometry, which fails to detect proteins that might be underrepresented in the total protein pool. An example of this may be the LDL receptor, previously used as a model protein in epithelial polarity via overexpression methods (9, 12, 15). However, it lies below the limit of detection in our dataset of endogenous MDCK proteins. The Nhydroxysulfosuccinimide (NHS)-SS-Biotin reagent requires access to primary amines, therefore underestimating proteins with few or no exposed extracellular lysines or arginines. Alternative techniques used in proteomics target glycosylated proteins either by aminooxybiotinylation (30) or by cell surface capturing technique (31). These methods apply to the detection of glycosylated proteins and may be advantageous in minimizing intracellular labeling. However, the membrane-impermeant NHS-SS-Biotin reagent that we used here has been previously shown to have negligible intracellular labeling (32). In this work, we show that the signal ratio from plasma membrane/cytoplasmic proteins with NHS-SS-Biotin is at least 10:1 (Fig. 1), which indicates that intracellular biotinylation may not be a great concern. We propose that future applications taking advantage of combinations of surface-labeling methods as performed in nonpolarized cells (33) will expand the repertoire of surface proteins detected in epithelia.

To minimize confounding signals from cytoplasmic proteins, we considered only proteins to which a plasma membrane annotation could be assigned based on inclusion criteria from the UniProt knowledgebase. It is possible that, in so doing, we underestimated the number of plasma membrane proteins due to incomplete annotations, as the database is constantly updated based on new data. We expect this to improve as progress is made in efforts toward more complete "surfaceomes" (34, 35).



Fig. 4. Selected apical and basolateral proteins affected by silencing the μ 1A or μ 1B subunits of the AP-1 complex in MDCK cells as measured by surface biotinylation followed by Western blot. The examples provided illustrate basolateral proteins that had reduced polarity after silencing AP-1 subunits (*A*–*F*), apical proteins that had reduced polarity after silencing μ 1A and μ 1B simultaneously (*G*–*I*) or individually (*J*), and an apical (*K*) and a basolateral protein (*L*) that were not affected by silencing of μ 1A or μ 1B. For each protein, we calculated the total surface levels as indicated in *A*′–*L*′. Bars represent mean percentage surface distribution \pm SD. *n* = 3–4. Ap, apical; BL, basolateral. **P* < 0.05 vs. control (si-Luc) one-way ANOVA with Dunnett's correction for multiple comparisons against control.

To our knowledge, few attempts have been made to apply proteomics to study polarity in MDCK cells, mainly focused on secreted (36) and GPI-anchored proteins (37). A previous work by Mathias et al. (21) used an approach consisting of isolation of apical and basolateral membranes followed by label-free mass spectrometry. This technique highly enriches membrane proteins,

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Fig. 5. Global patterns of major shifts in polarity after silencing AP-1A, AP-1B, or both. Volcano plots showing the magnitude of (*A*) the shifts toward the apical and basolateral sides when silencing μ 1A, (*B*) the shifts when silencing μ 1B, and (*C*) the shifts with the double silencing. Dotted lines represent P = 0.05. Data points above the line are statistically significant. (*D*) Quantification of the magnitude of shifts toward the apical (positive values) or basolateral (negative values) domains after silencing μ 1A (blue), μ 1B, (red), or both (green). The lengths of the vectors (radii) are directly proportional to the compounded contribution of the number of proteins affected and the intensity of the shift. (*E*) Percentages of proteins not affected by silencing of AP-1 subunits or affected by experiencing a shift toward the basolateral or apical sides. Subcharts indicate the proportions of proteins that experienced a shift toward the apical or basolateral.

but intracellular proteins may still be overrepresented, as many of the highest-abundance proteins detected are cytoplasmic. Our approach selectively labels surface proteins and in addition, is quantitative, since we used SILAC to measure the relative distribution of each protein at the apical and basolateral membranes. This allowed us to determine the polarity of proteins even if there is a minimal presence in the opposite membrane. This is advantageous when performing measurements of polarity after different treatments or gene silencing, like we conducted here.

To study the role of AP-1A and AP-1B in polarity, we silenced µ1A and µ1B in MDCK cells, because there are several open questions regarding these adaptors. The general notion is that these two adaptors complement each other (15, 16). We found evidence of this cooperation, since many proteins required double silencing of µ1A and µ1B to have their polarity affected. This effect was more pronounced for a larger set of proteins that experienced a shift in the apical-to-basolateral direction, suggesting higher overlapping function in the apical pathway. This is intriguing, because the cooperation between AP-1A and AP-1B has only been recognized before in the basolateral pathway, although it is still debated whether they share the same subcellular location (16) or they act at sequential trafficking compartments (15). However, while any one of these scenarios entails overlap between AP-1A and AP-1B function, our current data indicate that each adaptor also has an independent trafficking role. We draw this conclusion from subsets of apical and basolateral proteins that had their polarity affected by silencing only one of the adaptors but not the other one and vice versa. Future research should address the complex trafficking behavior of AP-1 variants. An important point in our study is that we identified endogenous cargo proteins that showed altered polarity when AP-1 subunits are silenced. These cargo proteins expand the repertoire of model proteins to study the role of AP-1 in polarized trafficking and are expressed at physiological levels, thus minimizing confounding effects associated with overexpression (38, 39). In addition, because not all plasma membrane proteins were depolarized by AP-1 silencing, which is consistent with previous reports (15, 20), the decreased polarity of apical or basolateral proteins is likely not the result of a global indirect effect on epithelial polarity.

The role of the AP-1 adaptor as mediator of basolateral polarity has been extensively studied and involves recognition of well-defined sorting signals in basolateral cargo proteins (9–15). However, previous evidence indicates that AP-1 may also play a role in the distribution of apical proteins. For example, in µ1B knockout mice, the apical localization of sucrase and villin was disrupted in intestinal cells (19). More recently, the apical localization of GPI-anchored proteins has been shown to depend on AP-1 in MDCK cells (20). Here, we show that AP-1 also mediates the apical localization of a considerable fraction of transmembrane proteins. It is possible that this role of AP-1 has been previously overlooked, because the number of apical proteins studied in the context of AP-1 function so far has been relatively limited [i.e., p75, FcL receptor (9), TLR-2 (40), and gp135 (15)] and because apical sorting signals are less well defined than basolateral signals (3). Further characterization of apical signals is crucial to elucidating the trafficking mechanism mediated by AP-1. However, this is likely a complex issue, as this mechanism may not necessarily involve direct cargo-AP-1 interactions as described for basolateral proteins (13-16). For example, GPI-anchored proteins rely on AP-1 for apical localization, despite lacking a cytoplasmic tail that could be recognized by AP-1 (20).

The considerations discussed above highlight the need to search for additional partners of AP-1 that may provide directionality to the apical-basolateral trafficking mechanism mediated by AP-1. Candidate partners are vesicle fusion proteins, like syntaxin 3 and 4, which are apical and basolateral, respectively, in epithelia (41), and require AP-1 for their localization (20, 42). The search for additional partners of AP-1 may be facilitated by the identification of suitable cargoes, which should be empowered by the large-scale proteomics approach that we present here. For example, we noticed that some plasma membrane proteins displayed increased or decreased total surface expression levels on AP-1 silencing, whereas others did not (Fig. 4). Our identification via proteomics of such proteins provides an opportunity to study the underlying mechanism, which may entail changes in recycling affecting protein retrieval from the plasma

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membrane or intracellular compartments or alterations in protein turnover due to retromer activity. Additional research is required to clarify this point.

In summary, using a combined quantitative surface biotinylation/ mass spectrometry approach, we determined the surface proteome of the prototypical epithelial cell line MDCK. Using this approach in conjunction with silencing of AP-1A and AP-1B, we uncovered global aspects of the trafficking role of these adaptors, in particular, a previously unrecognized role in maintaining polarity of transmembrane apical plasma membrane proteins. We identified selected subsets of apical and basolateral proteins that require AP-1A, AP-1B, or both to maintain their apical-basolateral distribution, indicating that these adaptors may control different sets of proteins. Hence, the assay that we have introduced should constitute a useful tool to screen for additional members of the epithelial trafficking machinery and to investigate unknown roles of existing members of this machinery.

Methods

Cell Culture and SILAC Labeling. MDCK cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Corning,) supplemented with 5% fetal bovine serum (FBS) (Thermo Fisher Scientific). To obtain a polarized monolayer, cells were seeded at a density of 3×10^5 cells per 1 cm² in polycarbonate permeable support in Transwell inserts (Corning) and grown for 4 d. For SILAC labeling, unless indicated otherwise, all media and reagents were from Cambridge Isotope Laboratories. Cells were cultured for at least six cell divisions in DMEM free of Lysine and Arginine supplemented with 5% dialyzed FBS and 150 μ M Lysine and Arginine isotopes as follows: light (Lys0 + Arg0): L-Lysine2HCl + ¹³C₆-L-Arginine:HCl; heavy (Lys8 + Arg10): ¹³C₆ ¹⁵N₂-L-Lysine 2HCl + ¹³C₆ ¹⁵N₄-L-Arginine:HCl. After labeling, cells were either plated for polarization or transfected with siRNAs as needed while kept in the corresponding SILAC medium until the surface biotinylation procedure was performed.

Blotting Conditions and Antibodies. For Western blot, eluted surface proteins were run in gradient NuPAGE 4–12% Bis-Tris Protein gels (Thermo Fisher Scientific) and transferred to nitrocellulose membranes using the iBlot system according to the manufacturer's instructions (Thermo Fisher Scientific). Membranes were blocked with Odyssey Blocking Buffer (Li-Cor) for 1 h at room temperature in rocking platform. Next, membranes were incubated for 1 h at room temperature with the corresponding primary antibody, washed three times with Tris-buffered saline (TBS)-0.1% Tween-20 buffer, then incubated 1 h with the corresponding infrared dye-tagged secondary antibody IRDye-680 or IRDye-800 (Li-Cor), and washed again. Membranes were scanned and bands were quantified with Odyssey imager system (Li-Cor). The primary antibodies sources and dilutions were the following: ADAM17 [chicken (1:2,000; R&D Systems)]; AE2 [rabbit (1:1,000; GeneTex)]; CD44 [rabbit (1:500; Developmental Studies Hybridoma Bank Product 5D2-27); deposited by J. T. August]; CDH1 (E-cadherin) [mouse (1:20,000; BD Transduction Laboratories)]; ENPEP [goat (1:1,000; R&D Systems)]; EphB4 [mouse (1:500; Developmental Studies Hybridoma Bank Product CPTC-EPHB4-1); deposited by Clinical Proteomics Technologies for Cancer]; CEACAM1 (gp114) [rabbit (1:1,000; rabbit polyclonal generated previously) (43)]; PODXL (gp135) [mouse (1:500; hybridoma from G. K. Ojakian and R. Schwimmer (State University of New York Downstate Medical Center, Brooklyn, New York) (44)]; GPRC5A [mouse (1:1,000; R&D Systems)]; HEPH [rabbit (1:500; GeneTex)]; ICAM1 [mouse (1:500; Developmental Studies Hybridoma Bank Product P2A4); deposited by E. A. Wayner and G. Vercellotti]; LSR [rabbit (1:500; Cell Signaling)]; MET [rabbit (1:500; Cell Signaling)]; ATP1B1 (Na/K ATPase) [mouse (1:1,000; Millipore-Sigma)]; SLITRK4 [mouse (1:1,000; R&D Systems)]; and STRA6 [rabbit (1:1,000; ProSci)].

Gene Silencing. Silencing was performed as described previously using highly potent siRNAs against µ1A (GTGCTCATCTGCCGGAATT) (15) and µ1B (AACAAGCTGGTGACTGGCAAA) (12). Three rounds of silencing spaced by 3 d were performed in MDCK cells. Cells were suspended by trypsinization, and 4×10^6 cells were electroporated with 160 pmol of siRNA in Amaxa Nucleofector kit V (program T-23) following the manufacturer's instructions (Lonza). All siRNA oligos, including a control against luciferase, were custom synthesized by Dharmacon. After the final round of silencing, cells were seeded at a density of 3×10^5 cells per 1 cm² in Transwell inserts and cultured 4 d to achieve full polarization. Efficiency of silencing was corroborated by qRT-PCR as done routinely.

Surface Biotinylation. Polarized MDCK cells were washed three times with biotinylation buffer [10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 130 mM NaCl, 2 mM MgSO₄, 1 mM CaCl₂, 5.5 mM glucose, pH 7.8–8.0] at 4 °C and kept on ice for the rest of the biotinylation procedure to stop protein trafficking. Surface protein was biotinylated at the apical or basolateral membrane with cell-impermeant EZ-Link Sulfo-NHS-SS-Biotin (Thermo Fisher Scientific) at 1 mg/mL by two consecutive incubations of 15 min each. Biotin was washed twice and guenched with 100 mM glycine to remove nonreacted Sulfo-NHS-SS-Biotin. Cells were lysed in buffer containing 150 mm NaCl, 50 mm HEPES, pH 7.5, 5 mm ethylenediaminetetraacetic acid (EDTA), 1% Triton X-100, 0.1% sodium dodecyl sulfate (SDS), and Protease Inhibitor Mixture Set III (Thermo Fisher Scientific). Total protein was quantified by Bradford method, and 1 mg of protein (estimated to be contained in $\sim 1.2 \times 10^7$ cells at confluency) was incubated with high-capacity NeutrAvidin-coated agarose beads (Thermo Fisher Scientific) overnight at 4 °C to separate biotinylated proteins. Beads were washed twice in high-salt (1 M NaCl, 50 mm HEPES, 0.1% Triton X-100, pH 7.4) buffer and twice in no-salt (50 mm HEPES, pH 7.4) buffer. Proteins were eluted by 20-min incubations at room temperature in Laemmli buffer containing 5% SDS, 100 mM NaCl, 100 mM dithiothreitol (DTT), and 5% β -Mercapto-ethanol. Eluted surface proteins were loaded in polyacrylamide gels and run by electrophoresis for either Western blot or proteomics.

Mass Spectrometry. Purified surface proteins were briefly run into a polyacrylamide gel by electrophoresis for 10 min at 90 V and stained with SimplyBlue SafeStain (Thermo Fisher Scientific) for 1 h at room temperature. Gel bands were cut into small pieces and destained in 25 mM $\rm NH_4HCO_3$ and 50% acetonitrile. Proteins were reduced with DTT, then alkylated with iodoacetamide, and digested with 12.5 ng/µL trypsin in 25 mM NH₄HCO₃ overnight at 37 °C. Peptides were extracted twice with 5% formic acid and 50% acetonitrile followed by a final extraction with acetonitrile. Peptides were concentrated by vacuum centrifugation, desalted using C18 StageTips, and concentrated again. The liquid chromatography-mass spectrometry was performed in a Thermo Scientific EASY-nLC 1200 coupled to a Fusion Lumos Orbitrap mass spectrometer (Thermo Fisher Scientific). A self-packed 75- μ m imes 25-cm reversedphase column (Reprosil C18, 3 μ m) was used for peptide separation. Peptides were eluted by a gradient of 3-30% acetonitrile in 0.1% formic acid over 120 min at a flow rate of 300 nL/min at 45 °C. The Lumos was operated in datadependent mode with a cycle time of 1.5 s. Survey scans were acquired in the Orbitrap at a resolution of 120,000 at m/z 200. Ions with two to six charges from the survey scan were selected with an isolation window of 1.6 Thomsons and fragmented by higher-energy collisional dissociation with normalized collision energies of 35. Fragment ions were acquired in the ion trap. The maximum ion injection times for the survey scan and the tandem mass spectrometry scans were 100 and 60 ms, respectively. The ion target values were set to 1,000,000 and 10,000, respectively.

Protein Identification and Quantitation. The raw files were processed using the MaxQuant computational proteomics platform (version 1.5.5.1) for peptide identification and quantitation. The fragmentation spectra were searched against the UniProt canine database containing 25,539 protein sequences, allowing up to two missed tryptic cleavages. Carbamidomethylation of cysteine was set as a fixed modification, and oxidation of methionine, protein N-terminal acetylation, D₄-Lysine, ¹³C₆-Arginine, ¹³C₆ ¹⁵N₂-Lysine, and ¹³C₆ ¹⁵N₄-Arginine were used as variable modifications for database searching. The precursor and fragment mass tolerances were set to 5 ppm and 0.5 Da, respectively. Both peptide and protein identifications were filtered at 1% false discovery rate.

Bioinformatics Analysis. Proteomics data were analyzed in R via the limma package. Proteins were cross-referenced with the UniProt knowledgebase to assign annotated subcellular location, presence of transmembrane domains, and Gene Ontology annotation. Proteins were filtered out before analysis if they had less than three replicates or were not plasma membrane associated as defined by the UniProt knowledgebase. SILAC apical/basolateral ratios were log₂ transformed and median normalized before statistical analysis. Proteins were fit to linear models, and proteinwise sample variances were shrunk toward mean sample variance of all proteins in the sample. Pairwise moderated *t* tests were conducted between experimental conditions to determine differential protein expression. Pathway analysis was performed with Ingenuity Pathway Analysis software (Qiagen Bioinformatics).

Statistics. Student's *t* test for independent variables was used for comparisons between two means, and one-way ANOVA with Dunnett's correction was used for multiple comparisons against control when required. Fisher's exact test for small samples was utilized to compare the independent effects of μ 1A and μ 1B silencing in apical and basolateral shifts (2 × 2 contingency

table). Density plots were obtained by kernel smoothing of the percentage frequency of the datasets over a continuous interval spanning the basolateralapical distribution at the membrane. Linear regressions were performed with the least squares approach, setting the *y* and *x* intercepts at zero. Statistical analysis was performed with GraphPad Prism 7 Software. A significance level of 0.05 was used.

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