Mutational inactivation of mTORC1 repressor gene *DEPDC5* in human gastrointestinal stromal tumors

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Gastrointestinal stromal tumors (GISTs) are the most common human sarcoma and are initiated by activating mutations in the *KIT* or *PDGFRA* receptor tyrosine kinases. Chromosome 22q deletions are well-recognized frequent abnormalities in GISTs, occurring in ∼50% of GISTs. These deletions are thought to contribute to the pathogenesis of this disease via currently unidentified tumor suppressor mechanisms. Using whole exome sequencing, we report recurrent genomic inactivated *DEPDC5* gene mutations in GISTs (16.4%, 9 of 55 patients). The demonstration of clonal *DEPDC5* inactivation mutations in longitudinal specimens and in multiple metastases from individual patients suggests that these mutations have tumorigenic roles in GIST progression. DEPDC5 inactivation promotes GIST tumor growth in vitro and in nude mice. DEPDC5 reduces cell proliferation through the mTORC1-signaling pathway and subsequently induces cell-cycle arrest. Furthermore, DEPDC5 modulates the sensitivity of GIST to KIT inhibitors, and the combination therapy with mTOR inhibitor and KIT inhibitor may work better in GIST patients with DEPDC5 inactivation. These findings of recurrent genomic alterations, together with functional data, validate the DEPDC5 as a bona fide tumor suppressor contributing to GIST progression and a biologically relevant target of the frequent chromosome 22q deletions.

sarcoma | GIST | KIT tyrosine kinase inhibitors | DEPDC5

Sarcomas are diverse mesenchymal malignancies that account for ∼20% of pediatric and 1% of adult cancers (1). Gastrointestinal stromal tumors (GISTs) are the most common human sarcoma (2), which are mostly initiated by activating mutations of the receptor tyrosine kinase *KIT* (75–80%) or *PDGFRA* (5–10%) (3, 4). Although sharing the same *KIT*/*PDGFRA* mutations, micro-GISTs have a limited growth potential and hence are restrained at the subcentimeter level. The fact that micro-GISTs are common in general individuals (found in one-third of the general population) without clinical symptoms (5–7) indicates that additional genetic alterations contribute to the progression of clinical GISTs. Chromosome 22q deletions are frequent chromosomal abnormalities in human GISTs, occurring in ∼50% of GISTs (2, 8–11), and are thought to contribute to the pathogenesis of this disease by yet-unidentified tumor suppressor mechanisms (2, 8–11).

Most GISTs with activating mutations in *KIT* often respond to treatment with KIT tyrosine kinase inhibitors (TKIs), such as first-line imatinib, second-line sunitinib and third-line regorafenib, but the magnitude of tumor regression is variable (12–14). This heterogeneity in TKI response could result from genetic modifiers that regulate the degree to which tumor cells are dependent upon the driver kinase and the response to TKI treatment. Here we demonstrate that chromosome 22q-targeting DEPDC5, silenced by somatic mutations, is a GIST specific tumor suppressor and a TKI treatment response modifier.

Results and Discussion

Whole Exome Sequencing Identifies Recurrent Inactivating *DEPDC5* Aberrations in GISTs. To identify the causative tumor suppressor genes at chromosome 22q in GISTs, we performed whole exome sequencing in 40 GIST patients (Dataset S1). These studies confirmed reported GIST genes, such as *KIT* (3), *PDGFRA* (4), *RB1* (15), *CDKN2A* (15), *DMD* (16), *MAX* (17), and *SETD2* (18) (Datasets S2 and S3). Notably, these studies revealed somatic homozygous inactivating genomic *DEPDC5* (encoding Dishevelled, Egl-10 and Pleckstrin [DEP] domain-containing protein 5) aberrations,

Significance

Activating mutations of *KIT* or *PDGFRA* are initiating events in most gastrointestinal stromal tumors (GISTs) and indeed are present in micro-GISTs, which are asymptomatic subcentimeter lesions found in one-third of the general population. The biological underpinnings of GIST progression are poorly understood. Chromosome 22q deletions are well-recognized abnormalities in GISTs. However, the crucial gene has been unknown. We report recurrent genomic inactivated *DEPDC5* mutations in GISTs. The *DEPDC5* inactivated mutations are prognostic in that they are associated with aggressive GISTs in which they promote GIST progression and reduce sensitivity to KIT inhibitors.

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The authors declare no competing interest.

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Data deposition: Whole exome sequencing and RNA sequencing data reported in this study have been deposited in the National Omics Data Encyclopedia (accession no. OEP000478).

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including nonsense mutation, frameshift mutation, and deletions in 7 of 40 (17.5%) GIST patients (Figs. 1 *A* and *B* and Dataset S1). Homozygous *DEPDC5* mutations were confirmed by Sanger sequencing (*SI Appendix*, Fig. S1*A*), single-nucleotide polymorphism (SNP) arrays (*SI Appendix*, Fig. S1*B*), quantitative PCR (*SI Appendix*, Fig. S1*C*), and fluorescence in situ hybridization (*SI Appendix*, Fig. S1*D*). Somatic inactivating genomic *DEPDC5* aberrations were validated in 2 of 15 (13.3%) additional GIST patients (cases 41 to 55, Dataset S4). This total set of 55 GIST patients was shown to have somatic homozygous *DEPDC5*-inactivating aberrations in 9 GIST patients (16.4%). Of 55 patients, 31 (~56%) harbored chromosome 22 loss (Dataset S4). All of the 9 patients harboring genomic *DEPDC5* aberrations contain chromosome 22 loss (Dataset S4). Therefore, *DEPDC5* aberrations are significantly more frequent in GISTs with chromosome 22 loss compared to chromosome 22 normal copy number (29 vs. 0%, $P = 0.01177$, 2-tailed Fisher's test) (Dataset S4). All of the 9 patients with genomic *DEPDC5* aberrations have both copies of *DEPCD5* inactivated

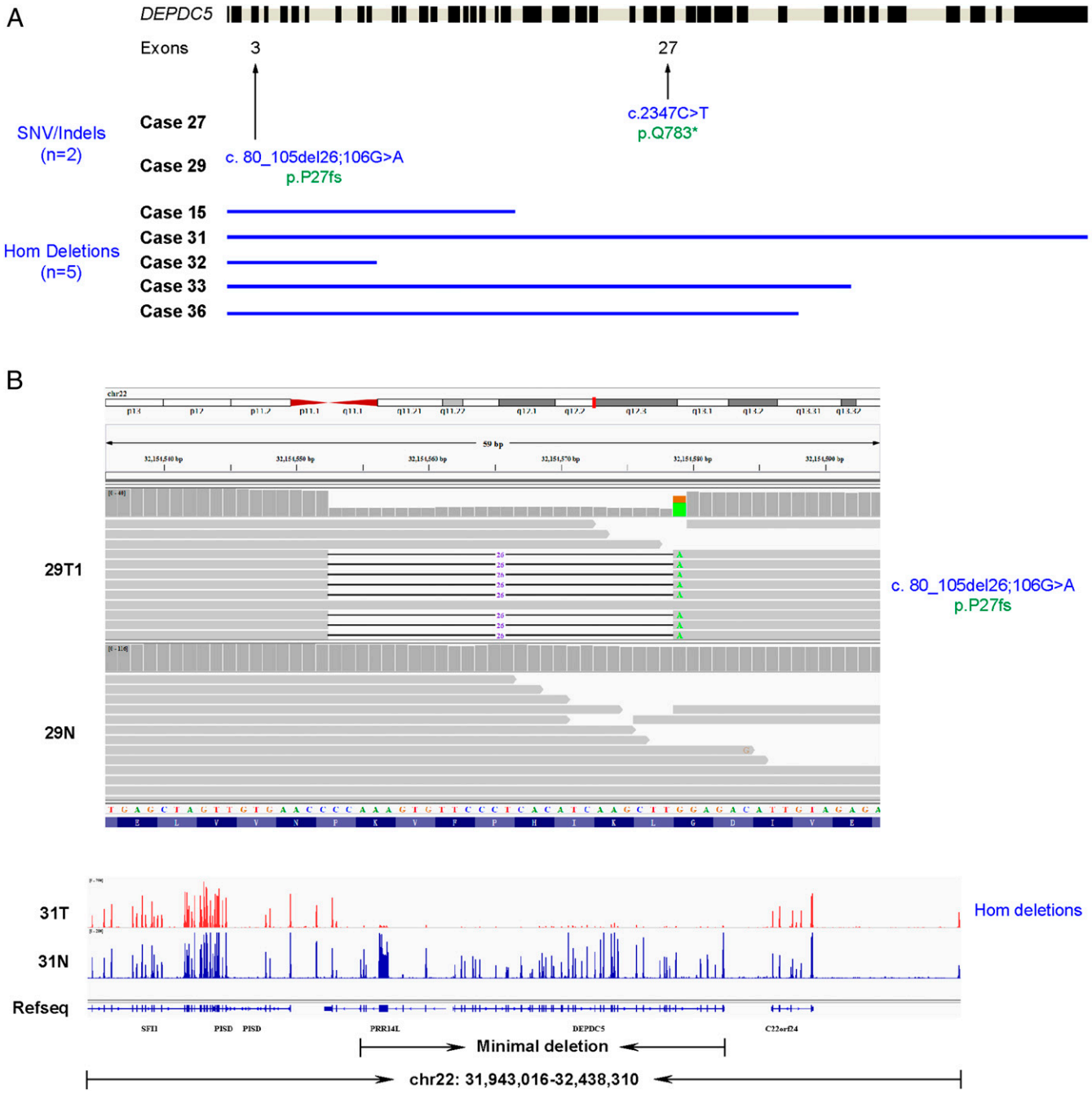

Fig. 1. Genomic *DEPDC5* aberrations in 40 GIST patients. (*A*) Whole exome sequencing identifies genomic *DEPDC5* aberrations in 7 of 40 (17.5%) GIST patients. Inactivating *DEPDC5* mutations were intragenic homozygous deletions (blue lines indicate deleted exons) and hemizygous nucleotide alterations. Mutations are described according to international guidelines for sequence variant nomenclature provided by the Human Genome Variation Society (http://varnomen.hgvs.org). Annotations in blue represent the nucleotide coding sequence mutations (indicated by "c.") whereas annotations in green represent the resultant protein sequence mutations (indicated by "p."). (*B*) Integrative genome viewer images of part of chromosome 22q from matched tumor and nonneoplastic cell DNAs from the same patients, demonstrating the tumor-restricted nature of *DEPDC5* mutations.

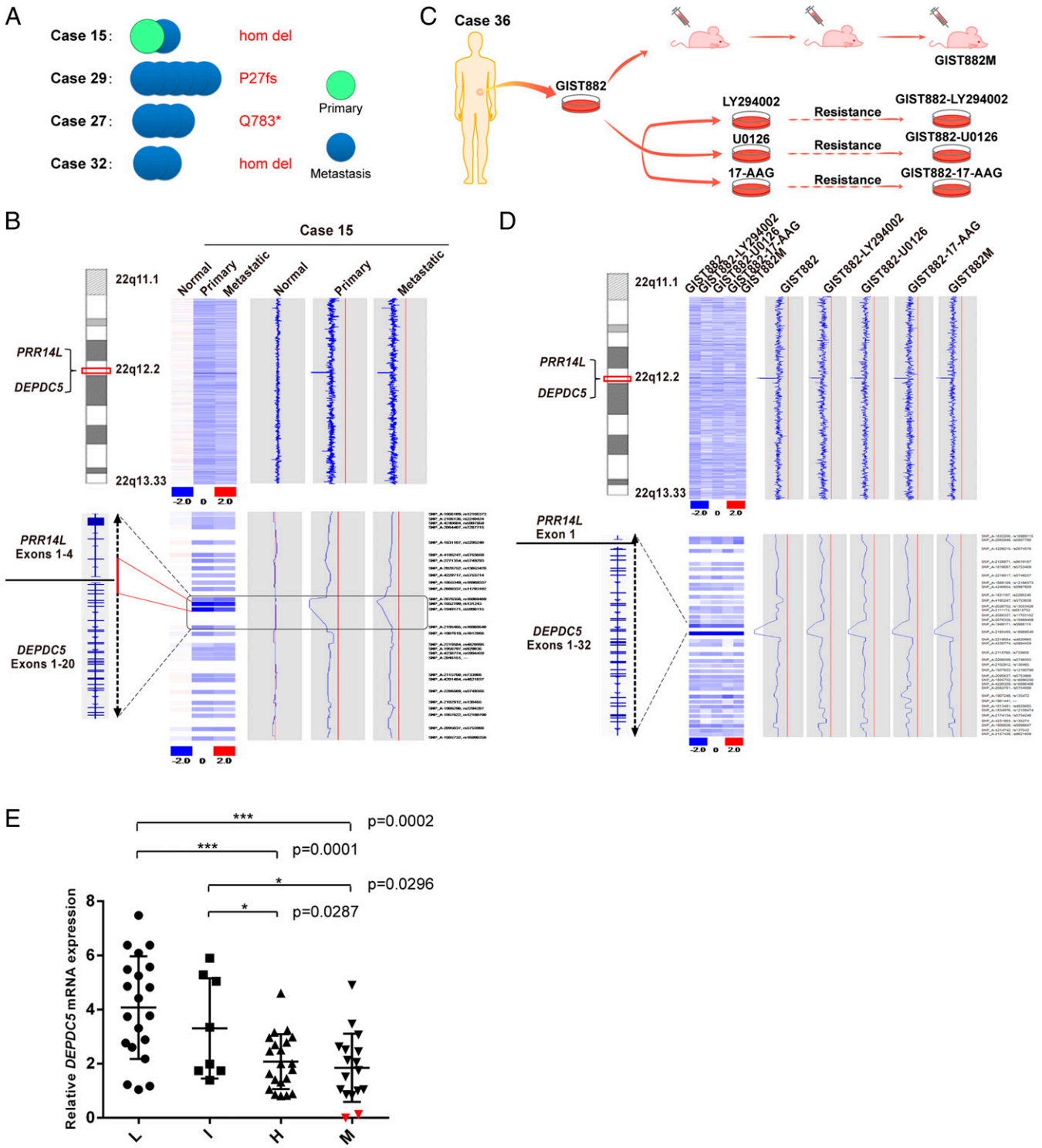

Fig. 2. Genomic *DEPDC5* aberrations are a clonal genetic event in GIST progression. (*A*) Multiple tumors from the same patients share the same mutation. Identical *DEPDC5* deletions in primary gastric GIST and a subsequent metastasis, diagnosed 1 y later, from case 15. Identical genomic *DEPDC5* aberrations in multiple anatomically distinct metastases from the same patients (cases 29, 27, and 32). (*B*) Longitudinal monitoring of GIST patient progression in the natural history of the disease. SNP profiles of nonneoplastic DNA from the patient, the primary gastric GIST, and the subsequent metastasis are shown. Identical *DEPDC5* deletions in primary gastric GIST and a subsequent metastasis from case 15. (*Top*) The entire chromosome 22q. (*Bottom*) The *DEPDC5* locus. Data are shown as dChip SNP log2 ratio copy number. (*C* and *D*) Longitudinal monitoring of the xenografted GIST progression and the GIST cell-line progression in response to small-molecule inhibitors. (*D*) Identical *DEPDC5* deletions in the various GIST882 sublines and the xenografted lesion (GIST882M). (*E*) qRT-PCR analysis indicates that *DEPDC5* mRNA expression is negatively correlated with clinical stages of GIST progression. The red inverted triangles indicate 2 GISTs with homozygous *DEPDC5* deletions. L, low-risk; I, intermediate-risk; H, high-risk; M, metastatic. $*P < 0.05$, $***P < 0.001$, 2-tailed Student's *t* test.

(Dataset S4). The GISTs with genomic *DEPDC5* aberrations have loss of heterozygosity of chromosome 22 (Dataset S4). These data show that *DEPDC5* is a classical tumor suppressor gene in GIST. RNA sequencing (RNA-seq) data and DNA methylation studies indicate that dysregulation of DNA methylation is not common in regulation of *DEPDC5* expression in GIST (*SI Appendix*, Fig. S2).

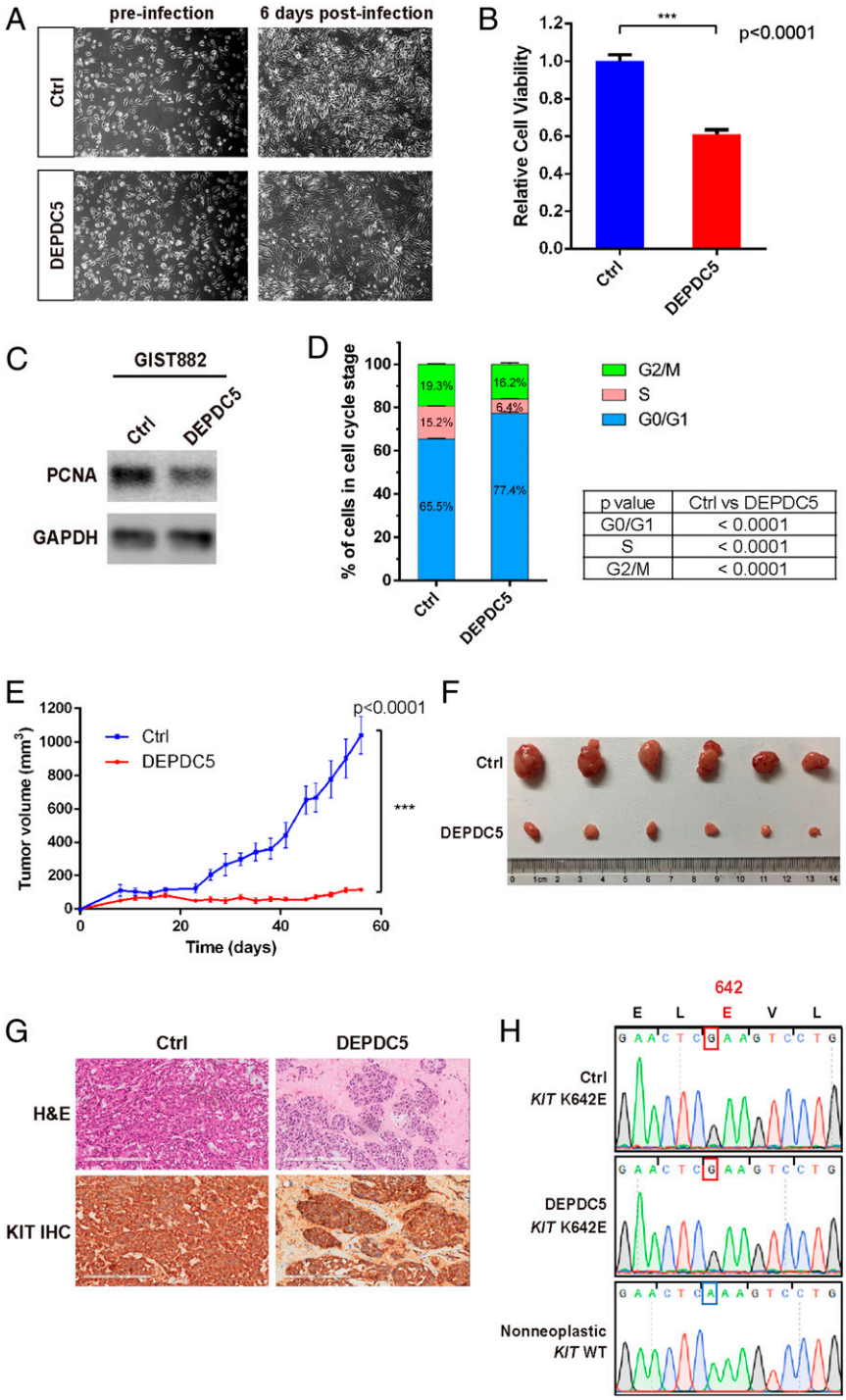

Fig. 3. Restoration of DEPDC5 expression inhibits tumor growth in *DEPDC5*-inactivated GISTs. (*A* and *B*) DEPDC5 restoration reduces cell viability in GIST882, as assessed by representative bright-field microscopy images (*A*) and by CellTiter-Glo viability assay (*B*). ***$P < 0.001$ by 2-tailed Student's *t* test. (*C*) Western blotting with proliferating cell nuclear antigen antibody demonstrates that DEPDC5 restoration represses cell proliferation. (*D*) Cell-cycle analyses demonstrating that DEPDC5 restoration decreases the cell cycle in GIST882, reducing the proportion of cells in S phase and increasing the proportion of cells in G0/G1 phase. Experiments were performed in triplicate. (*E* and *F*) DEPDC5 restoration suppresses growth of GIST882 xenografts in nude mice. Growth curves (*E*) and representative photo images (*F*) of transplanted tumors are shown. Error bars are the mean ± SEM of 6 replicates. ***$P < 0.001$ by 2-tailed Student's *t* test. (*G*) Histologic evaluation of formalin-fixed and paraffin-embedded samples after hematoxylin and eosin staining demonstrates sparsely cellular areas with no mitotic activity in DEPDC5-restored GIST xenografts, in comparison to highly cellular and mitotically active xenografts of control lentiviral infected cells (*Top*). KIT immunohistochemistry shows KIT expression in DEPDC5-restored GIST xenografts (*Bottom*). (*H*) Sanger sequencing demonstrates *KIT* gain-of-function mutation (K642E) in DEPDC5-restored GIST xenografts. E, glutamate; L, leucine; V, valine.

DEPDC5-inactivated mutations, when present within a primary GIST, were perpetuated in subsequent metastatic lesions (Figs. 2 *A* and *B* and *SI Appendix*, Fig. S3*A*) and, when present in any GIST metastasis, were also found in other metastases from the same patient (Fig. 2*A* and *SI Appendix*, Fig. S4). A cell line (GIST882) was identified with a homozygous *DEPDC5* exons 1 to 32 deletion (Fig. 2*C*). After extensively culturing with small-molecule inhibitors in vitro, or being extensively xenografted to nude mice in vivo, homozygous *DEPDC5* deletions were always present (Fig. 2*D* and *SI Appendix*, Fig. S3*B*). These results demonstrate that the *DEPDC5* alterations are a clonal event either in the natural history or in the inhibitor-induced stress condition of the disease. Genomic *DEPDC5* aberrations were observed only infrequently (1.2%) in 255 non-GIST sarcomas in the The Cancer Genome Atlas Pan-Cancer Atlas program (19–21) (*SI Appendix*, Fig. S5). These data show that the frequency of inactivating *DEPDC5* aberrations is higher in GISTs compared to non-GIST sarcomas ($P < 0.0001$). No mutations were identified in the DEPDC5 pathway (including components of the DEPDC5 complex, such as DEPDC5, NPRL3, and NPRL2) (22) in GISTs without genomic *DEPDC5* aberrations. Quantitative reverse transcriptase PCR (qRT-PCR) analysis followed by correlation studies in 66 GISTs indicated that DEPDC5 messenger RNA (mRNA) expression in tumor was inversely associated with the stages of GIST progression (Fig. 2*E*). These genomic data reveal recurrent inactivating *DEPDC5* aberrations in GISTs.

DEPDC5 Inactivation Promotes GIST Progression In Vitro and In Vivo. The biologic function of DEPDC5 was investigated using various GIST models. Re-expression of DEPDC5 in *DEPDC5*-inactivated GIST882 cells reduced the number of viable cells (Figs. 3 *A* and *B*) and proliferative properties (Fig. 3*C*), but not the cell apoptosis (*SI Appendix*, Fig. S6). Exogenous DEPDC5 was also introduced into the GIST-T1 cell line that retained DEPDC5 expression. Cell viability and proliferation generally remained the same, arguing that the differential viability and proliferation in GIST882 was not caused by the cytotoxicity of the large DEPDC5 construct (*SI Appendix*, Fig. S7). To determine whether the inhibition of cell proliferation manifests in vivo, we generated both control and DEPDC5-restored GIST882 xenografts in nude mice. DEPDC5 restoration markedly attenuated tumor growth although the tumor contained the *KIT* gain-of-function mutation (Fig. 3 *E–H*). To further test the role of DEPDC5 in GISTs, we established DEPDC5 knockout (KO) cells from GIST430 and GIST-T1 cells that retained DEPDC5 expression using a CRISPR system (*SI Appendix*, Fig. S8 and Table S4). DEPDC5 knockout facilitated the cell growth and proliferation (*SI Appendix*, Fig. S9 *A* and *B*). Collectively, these results demonstrate that DEPDC5 inactivation promotes GIST progression.

DEPDC5 Inhibits GIST Cell Proliferation through the mTORC1 Signaling Pathway and Subsequently Induces Cell Cycle Arrest. To gain insight into the mechanism by which DEPDC5 inactivation promotes GIST progression, we measured the changes in gene expression of GIST882 with or without DEPDC5 restoration using RNA-seq. Gene set enrichment analysis (GSEA) revealed that cell-cycle–related genes, including E2F targets (23, 24), G2M checkpoint (24), and mitotic spindle genes, were down-regulated in DEPDC5-restored GIST882 (Fig. 4*A*). GSEA also revealed significant enrichment in mTORC1 signaling (Fig. 4*A*), which is consistent with reports that suggest that DEPDC5 is a negative regulator of the mTORC1 signaling pathway as a component of the GATOR1 complex (22). Based on the link between mTOR and the cell cycle (25–28), it is rational to hypothesize that DEPDC5 represses the mTORC1-signaling pathway and mediates cell-cycle progression. Consistent with this notion, DEPDC5 restoration in GIST strongly suppressed signaling downstream of

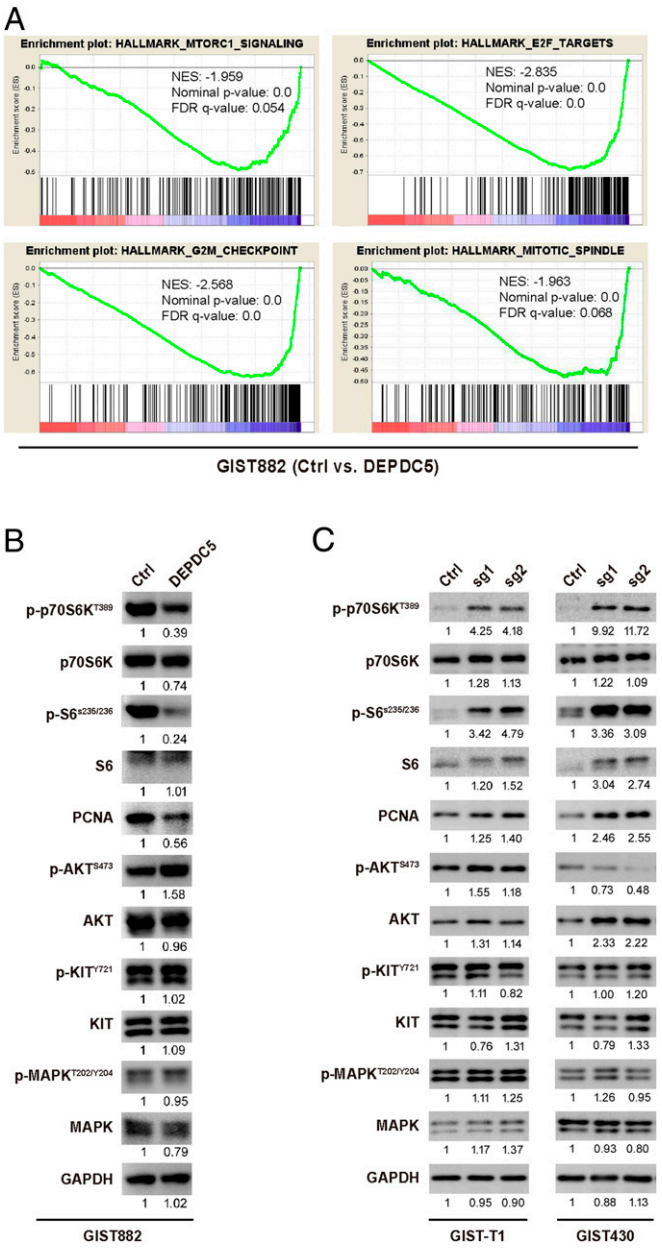

Fig. 4. DEPDC5 represses the mTORC1-signaling pathway in GISTs. (*A*) GSEA of differentially expressed genes demonstrates that DEPDC5 restoration regulates genes involved in the cell cycle (including E2F targets, G2M checkpoint, and mitotic spindle genes) and the mTORC1-signaling pathway. NES, normalized enrichment score. (*B*) Western blotting reveals that DEPDC5 restoration represses the mTORC1 pathway as indicated by reduced phosphorylation of p70S6K and S6 but not KIT and MAPK phosphorylation levels. Corresponding relative quantitation of indicated protein level normalized to control GIST882 is shown. (*C*) Western blotting reveals that DEPDC5 KO activates the mTORC1 pathway as indicated by increased phosphorylation of p70S6K and S6. Corresponding relative quantitation of the indicated protein level normalized to control GIST-T1 or GIST430 is shown.

mTORC1 as indicated by reduced phosphorylation of p70S6K and S6 (Fig. 4*B*). In contrast, DEPDC5 knockout in GIST430 and GIST-T1 increases the phosphorylation of p70S6K and S6 (Fig. 4*C*). In addition, DEPDC5 did not influence KIT and MAPK phosphorylation levels (Fig. 4 *B* and *C*). Flow cytometric analysis showed that DEPDC5 restoration inhibited cell-cycle progression at the G1S checkpoint, reducing the proportion of

cells in S phase (15.2 ± 0.08% vs. 6.4 ± 0.33%, $P < 0.0001$) and increasing the proportion of cells in G0/G1 phase (65.5 ± 0.40% vs. 77.4 ± 0.54%, $P < 0.0001$) (Fig. 3*D*). In contrast, DEPDC5 KO in GIST430 accelerated cell-cycle progression with a lower proportion of G0/G1 phase cells (DEPDC5 KO by single-guide RNA 1 [sg1] vs. control [ctrl]: 42.2 ± 0.46% vs. 47.9 ± 1.15%, $P < 0.0001$; DEPDC5 KO by single-guide RNA 2 [sg2] vs. ctrl: 43.7 ± 0.57% vs. 47.9 ± 1.15%, $P < 0.0001$) and a higher proportion of S-phase cells (sg1 vs. ctrl: 21.7 ± 1.06% vs. 19.3 ± 0.46%, $P = 0.0101$; sg2 vs. ctrl: 22.9 ± 0.86% vs. 19.3 ± 0.46%, $P < 0.0001$) (*SI Appendix*, Fig. S9*C*), which is consistent with cell proliferation analysis (*SI Appendix*, Fig. S9 *A* and *B*). Thus, our results indicate that DEPDC5 inhibits GIST cell proliferation through the mTORC1-signaling pathway and subsequently induces cell-cycle arrest.

DEPDC5 Modulates the Sensitivity of GISTs to KIT Inhibitors. Given that imatinib, a first-line treatment in advanced/metastatic GISTs (2, 12), inhibits KIT receptor tyrosine kinase and its downstream pathway, including the mTORC1-signaling pathway, we hypothesized that DEPDC5 impacts the sensitivity of GISTs to imatinib. Indeed, DEPDC5-restored GIST882 exhibited increased sensitivity to imatinib, as indicated by reduced IC_{50} (Fig. 5*A*).

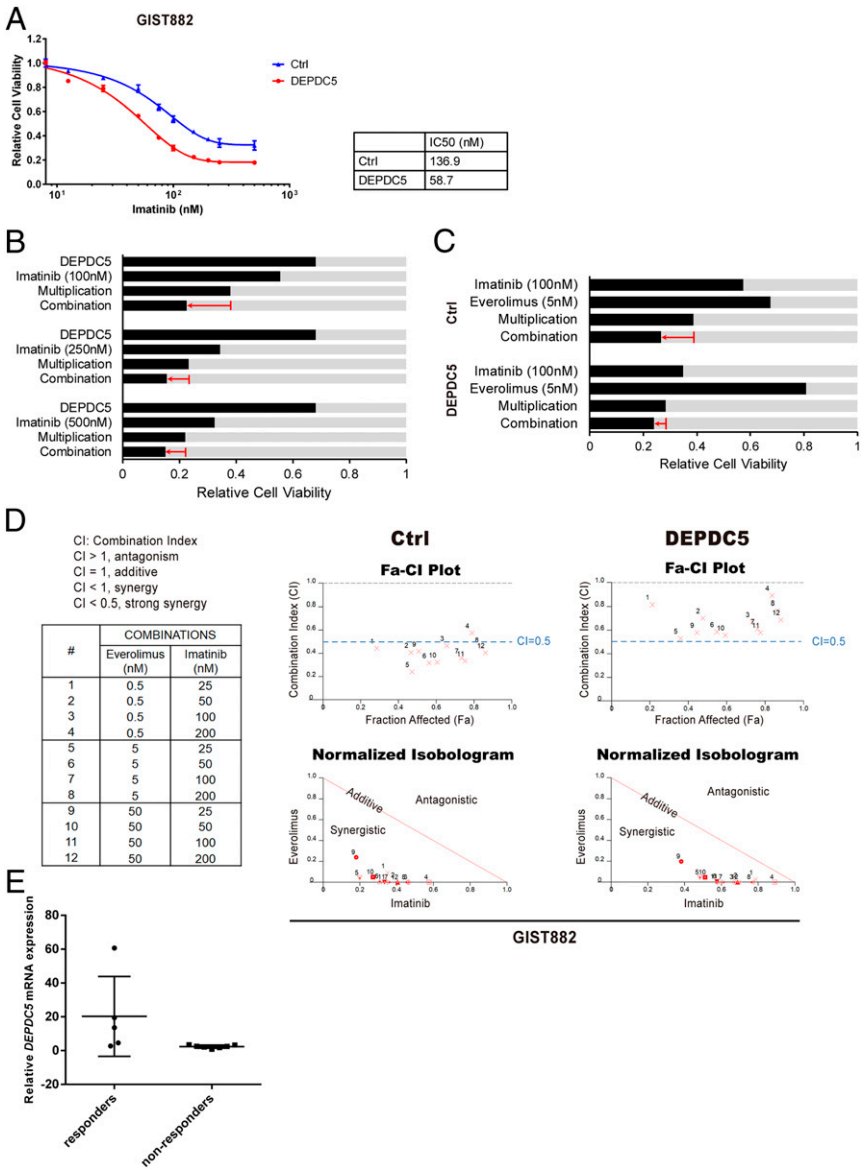

Fig. 5. DEPDC5 modulates the sensitivity of GISTs to KIT inhibitors. (*A*) DEPDC5 restoration sensitizes the GIST882 cells to KIT inhibitor (imatinib). IC_{50} values of GIST882 with or without DEPDC5 restoration are shown. (*B*) Cell viability assay reveals the synergistic effect of DEPDC5 restoration and imatinib in GIST882. Light gray bars indicate control values. "Multiplication" indicates expected effect of combined treatment if single-treatment effects (DEPDC5 restoration and imatinib) are multiplied; red arrow indicates actual effect of combination. The effect of DEPDC5 restoration and imatinib combined was even stronger than would be expected if the individual effects were multiplied. (*C*) Cell viability assay reveals the synergistic effect of mTOR inhibitor (everolimus) and KIT inhibitor in GIST882. The synergistic interaction was also observed in the case of DEPDC5 restoration but to a lesser degree. (*D*) Combination index (CI) (*Top Left* and *Top Right*) and isobologram (*Bottom Left* and *Bottom Right*) analyses reveal the synergistic effect of mTOR inhibitor and KIT inhibitor in GIST882. DEPDC5 restoration compromises the synergistic effect, increasing the CI values (*Top Right*) and shifting toward antagonistic state (*Bottom Right*) for each combination. Representative fraction affected (Fa)-CI plots (*Top Left* and *Top Right*) and normalized isobolograms (*Bottom Left* and *Bottom Right*) are shown. (*E*) *DEPDC5* quantitative RT-PCR analysis of a set of GIST biopsies from patients treated with KIT inhibitors (responders, $n = 5$; nonresponders, $n = 7$). TKI nonresponded GISTs were correlated with decreased *DEPDC5* expression compared to responded GISTs.

Conversely, DEPDC5 KO in GIST430 diminished the sensitivity to imatinib (*SI Appendix*, Fig. S11*A*). Western blotting verified that both DEPDC5 restoration and imatinib repress the mTORC1-signaling pathway (*SI Appendix*, Fig. S10). Furthermore, the combination of DEPDC5 restoration and imatinib had a greater effect than either one alone (Fig. 5*B*). We next investigated how pharmacologic inhibition of mTOR with everolimus (29) affects the sensitivity to imatinib. To this end, we treated GIST882 (control vs. DEPDC5-restored, Figs. 5 *C* and *D*) and GIST430 (control vs. DEPDC5-KO, *SI Appendix*, Fig. S11*B*) with a wide range of imatinib/everolimus combinations. Isobologram and combination index (CI) analyses revealed that the combined treatment synergistically inhibited GIST882 cell growth with a CI < 0.5 for most concentration pairings (Fig. 5*D*). The synergistic interaction was also observed in the case of DEPDC5 restoration in GIST882 but to a lesser degree (Figs. 5 *C* and *D*, and *SI Appendix*, Table S1). In other words, DEPDC5 restoration compromises the synergistic effect, increasing the CI values and shifting toward an antagonistic state for each combination (Fig. 5*D*). In contrast, DEPDC5 KO increases the synergistic effect of the mTOR inhibitor and KIT inhibitor in GIST430, decreasing the CI values and shifting toward a synergistic state for each combination (*SI Appendix*, Fig. S11*B* and Table S2). Together, these data reveal that inhibition of mTOR with either DEPDC5 or pharmacologic inhibitors increases the sensitivity of GISTs to imatinib.

To investigate whether this modification of the TKI sensitivity could be recapitulated in a clinical setting, we performed *DEPDC5* qRT-PCR analysis on a set of GIST biopsies from patients treated with KIT inhibitors. Resistance to KIT inhibitors, such as imatinib, in GIST most commonly involves secondary point mutations in the KIT kinase domain that reduce or abrogate drug potency (2). We removed the GISTs with secondary *KIT* mutations from the analyses. We observed that TKI-nonresponded GISTs were correlated with decreased *DEPDC5* expression compared to responded GISTs (Fig. 5*E*), indicating that at least some features of the TKI response modification by DEPDC5 can also be observed in a clinical setting.

DEPDC5, but Not PRR14L, Is the Major Target at Chromosome 22q in GISTs. In human GISTs, including GIST882, 22q homozygous deletions simultaneously target the 5′ ends of *DEPDC5* and *PRR14L* genes due to their proximity (Fig. 1*B* and *SI Appendix*, Fig. S12*A*). We then asked whether *PRR14L* was another driver gene at chromosome 22q. PRR14L and/or DEPDC5 were restored in GIST882. PRR14L/DEPDC5 corestoration induced inhibition of cell viability comparable to DEPDC5 restoration alone (*SI Appendix*, Fig. S12*B*). In addition, PRR14L had no effect downstream of the mTORC1-signaling pathway (*SI Appendix*, Fig. S12*C*). Hence, these functional results imply that DEPDC5 is the major target at chromosome 22q. The 2 intragenic mutations (e.g., the frameshift and nonsense mutations) in *DEPDC5* further support *DEPDC5* over *PRR14L* as the target. The loss of PRR14L may represent a potential vulnerability specific to GISTs with *DEPDC5* homozygous deletions according to collateral lethality proposed by Muller et al. (30). Therefore, the role of PRR14L in GISTs merits further investigation.

The molecular mechanism underlying the progression of GISTs is not fully understood. Accumulation of chromosomal aberrations seen in conjunction with disease progression is considered to indicate the involvement of other yet-unidentified genes. Loss of the long arm of chromosome 22 is observed in ∼50% of GISTs. Our findings of recurrent genomic alterations, together with functional data, highlight the *DEPDC5* gene as a bona fide tumor suppressor contributing to GIST progression and a biologically relevant target of the frequent chromosome 22q deletions.

In addition to GIST, *DEPDC5*-inactivated mutations have been identified in other tumors at a low frequency, such as glioblastoma (22, 31) and ovarian cancer (22) (*SI Appendix*, Table S3). The association of *DEPDC5* in hepatitis C virus-related hepatocellular carcinoma has also been reported (32). However, it is unknown whether those genomic alterations had functional consequences. It is intriguing that *DEPDC5*-inactivated mutations are so frequent in GISTs given that DEPDC5 is ubiquitously expressed (33). Certainly, this is one limitation of our results. Nonetheless, it indicates that the DEPDC5-mTORC1 pathway plays a more prominent role in GIST pathogenesis. It is also striking that humans with germline *DEPDC5* mutations only develop an overt pathology within the central nervous system (33–39). From a pathophysiologic standpoint, it seems that inactivation of certain genes, such as *DEPDC5*, leads to distinct physiological outcomes depending on cellular context. Indeed, the *DMD* and *PARK2* genes function as tumor suppressors (16, 40), but their germline mutations result in muscular dystrophy and Parkinson's disease, respectively (41, 42). Notably, cohorts of individuals with epilepsy do reveal an increase in the risk of malignancies such as digestive organ cancers (43).

Our findings also explain, in part, the nonuniform response to KIT TKI treatment observed in KIT-mutant GIST patients and provide a rationale for testing an mTOR inhibitor in combination with a KIT TKI in KIT-mutant GIST patients. Several clinical or preclinical trials have been performed to test the efficacy of the combination of everolimus and imatinib in imatinib-resistant GISTs (44–46). Our studies demonstrate an enhanced synergistic effect of everolimus and imatinib in DEPDC5-deficient GISTs. These findings suggest that the combination therapy with mTOR and KIT inhibitors may work better in GISTs with DEPDC5 inactivation. DEPDC5 is an attractive therapeutic target in focal epilepsy (47, 48), as effects of DEPDC5 agonists would likely be anti-epileptogenic, and these DEPDC5 agonists warrant evaluation as potential therapeutic agents in oncology.

Materials and Methods

Tumor and Tissue Samples. Discarded, de-identified snap-frozen tumor biopsies and matched normal samples were from GIST patients at Brigham and Women's Hospital, Harvard Medical School, and Ren Ji Hospital, Shanghai Jiao Tong University School of Medicine. All samples were collected with institutional review board approval from Brigham and Women's Hospital and Ren Ji Hospital. Informed written consent was obtained from all human participants.

Xenograft Tumor Model. The animal experiments were approved by Institutional Animal Care and Use Committee (IACUC) of the Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.

Detailed methods for whole exome sequencing, bioinformatics analysis, transcriptome sequencing, bisulfite genomic sequencing, PCR, SNP arrays, fluorescence in situ hybridization, DEPDC5 restoration, cell lines, real-time quantitative RT-PCR and quantitative PCR, cell viability assays, isobologram, combination index analysis, Western blotting, cell cycle, apoptosis assays, xenograft tumor model, CRISPR knockout, gene expression profiling, GSEA, and statistical analysis are available in *SI Appendix*.

Data Availability Statement. Whole exome sequencing and RNA-seq datasets reported in this study have been deposited in the National Omics Data Encyclopedia (https://www.biosino.org/node/) under accession no. OEP000478 (49).

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