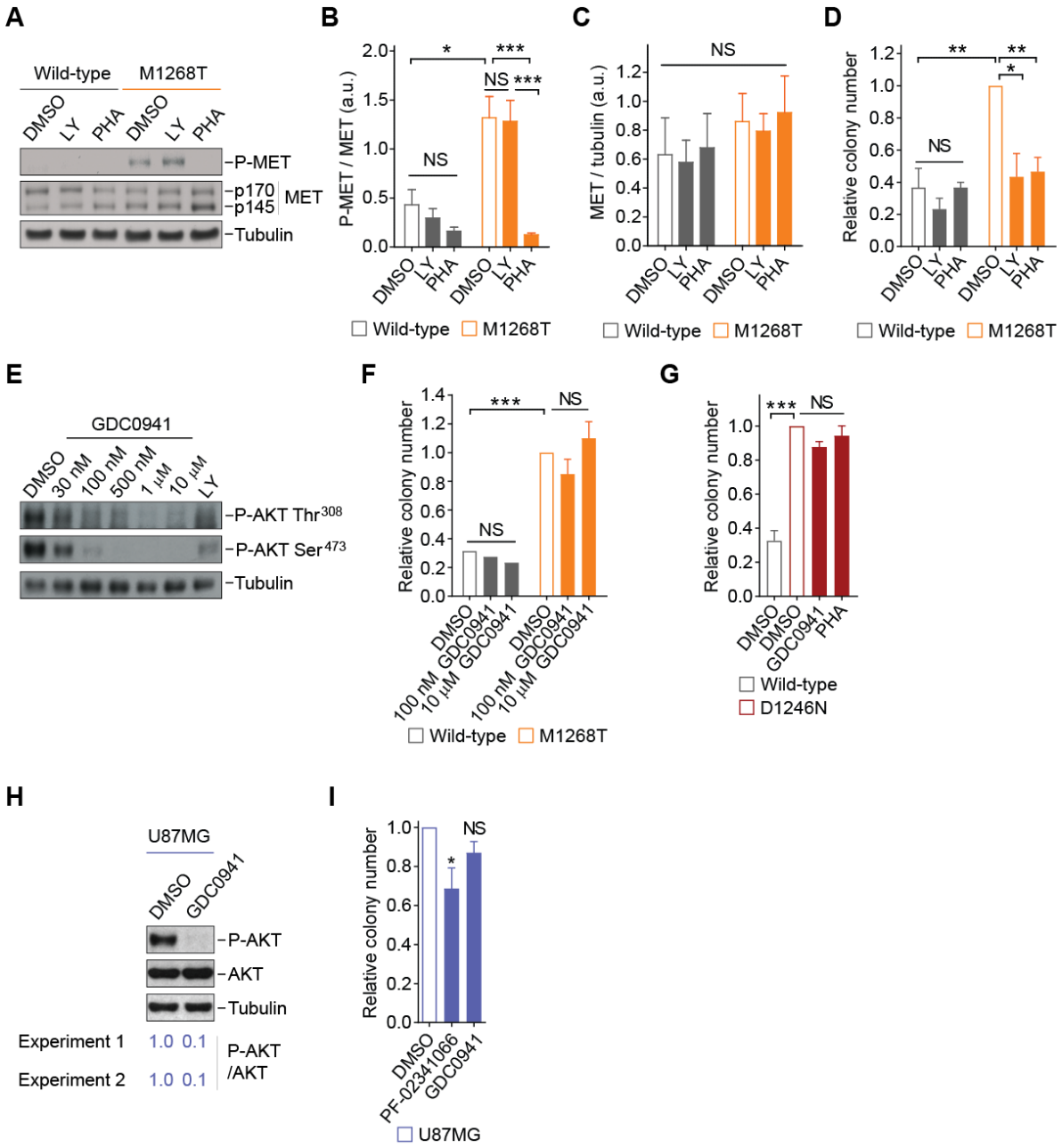

Figure S1: Class I PI3K inhibition reduces MET-dependent cell migration but not anchorage-independent growth. (A to C) Western blotting analysis for MET (p170 and p145, the precursor and mature forms, respectively, of the beta chain), phosphorylated MET [$Tyr^{1234/1235}$; "P-MET"], and tubulin were performed on lysates from wild-type and MET^{M1268T}-expressing cells treated with DMSO, LY294002 (LY, 10 μM) or PHA-665752 (PHA, 100 nM). Densitometry for the abundance of phosphorylated MET ($Tyr^{1234/5}$; B) and the mature form of MET (C) was each normalized to tubulin. Data are means ± SEM from N=3 independent biological replicates. a.u.: arbitrary units. **(D)** Relative colony number of

wild-type or MET^{M1268T}-expressing cells grown in soft agar and treated with DMSO, LY294002 (LY, 10 μM) or PHA-665752 (PHA, 100 nM). Data are means ± SEM from N= 3 independent biological replicates. **(E)** Western blotting for phosphorylated AKT (Thr^{308} and Ser^{473}; "P-AKT") and tubulin were performed on unstarved MET^{M1268T}-expressing cells treated with DMSO, LY294002 (LY, 10 μM) or GDC0941 (30 nM to 10 μM). Blots are representative of at least N=2 experiments. **(F and G)** Relative colony number of wild-type or MET^{M1268T}-expressing cells grown in soft agar and treated with DMSO, GDC0941 (100 nM or 10 μM; F) or PHA-665752 (PHA, 100 nM; G) as indicated, shown as means ± SEM from N=3 independent biological replicates. One-way ANOVA test followed by Tukey's multiple comparisons test: NS: non-significant, ***P<0.005. **(H)** Western blots for phosphorylated AKT (Ser^{473}; "P-AKT"), total AKT, and tubulin were performed on U87MG cells treated with DMSO or GDC0941 (100 nM). Below, mean densitometry of phosphorylated AKT relative to total in N=2 independent biological replicates. **(I)** Relative colony number of U87MG cells grown in soft agar and treated with DMSO, PF-02341066 (100 nM, N=3) or GDC0941 (100 nM, N=4). Results are means ± SEM of independent biological replicates. Student's *t* test comparing to DMSO: NS, non-significant; *P<0.05, **P<0.01, ***P<0.005. The figure is related to Figure 1.

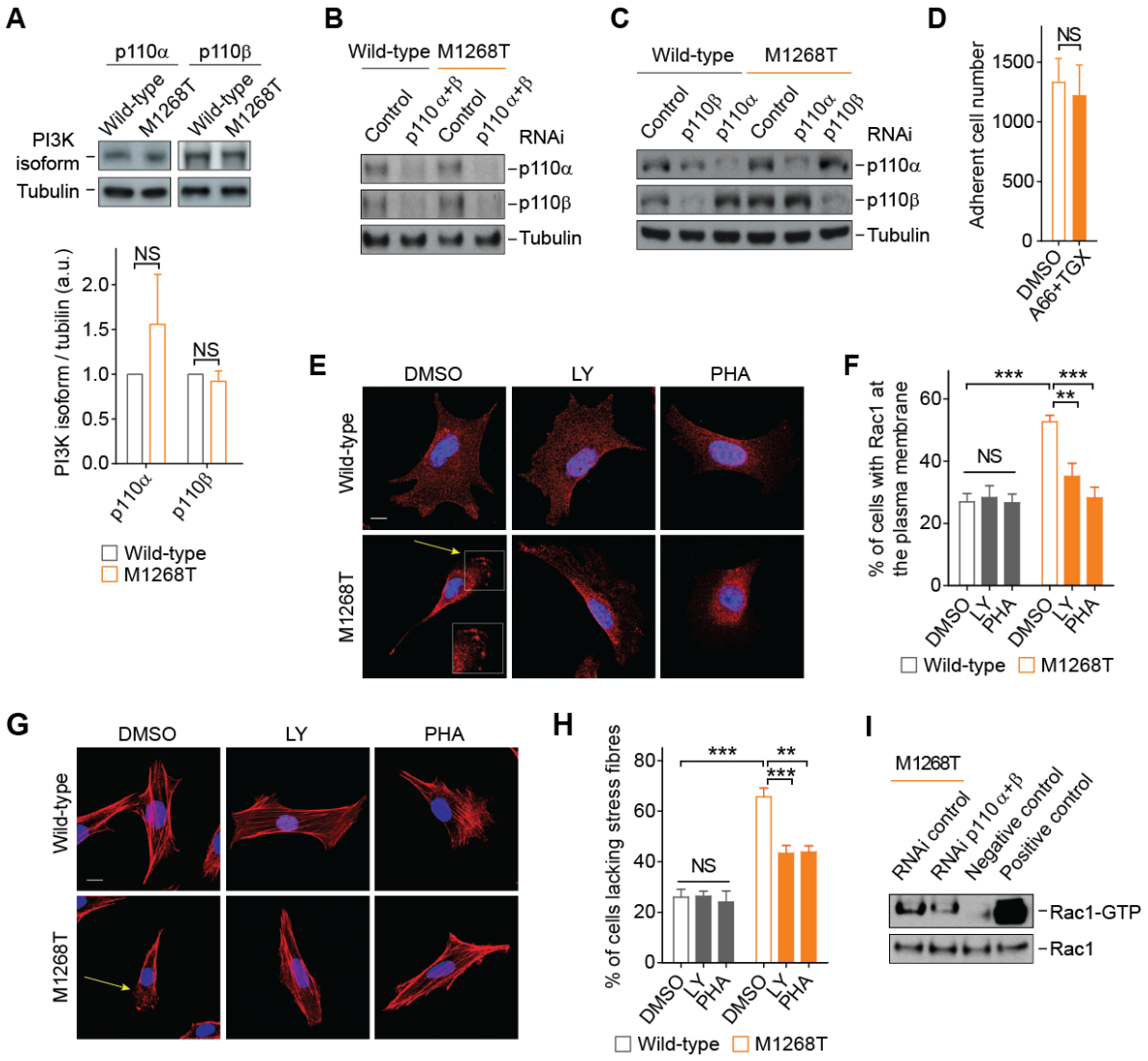

Figure S2: Class I PI3K promotes MET-dependent cell migration through Rac1 activation. (A) Western blots for class I PI3K isoforms p110α and p110β and for tubulin, in unstarved wild-type or MET^{M1268T}-expressing cells. The graph displays the average abundance of each PI3K isoform normalized to tubulin, with the values for wild-type cells set as 1. Data (below) mean ± SEM densitometry from 3 independent blots from N=3 independent biological replicates. **(B and C)** Western blots for p110α, p110β and tubulin in wild-type and MET^{M1268T}-expressing cells transfected with either a negative control siRNA (B; "Control") or siRNA targeting p110α and p110β, either simultaneously (B; "p110α+β") or separately (C). Blots are representative of N=3 (B) or 4 (C) independent biological replicates. **(D)** Number of adherent MET^{M1268T}-expressing cells cultured in the presence of DMSO or A66 (500 nM) and TGX221 (TGX, 40 nM) combined. Data are means ± SEM from N=4 independent biological replicates. **(E and F)** Wild-type and MET^{M1268T}-expressing cells were grown on coverslips and treated with DMSO, LY294002 (LY, 10 μM) or PHA-665752 (PHA, 100 nM) for 1 hour then stained for Rac1 (red) and counterstained with DAPI

(blue). Confocal microscopy images (E) are representative and data (F) are mean (± SEM) percentage of cells with Rac1 at the plasma membrane, quatified from N=11, 7 and 8 independent biological replicates for DMSO, LYand PHA respectively. Scale bar: 10μm. **(G and H)** Cells described and treated as in (E and F) were stained with DAPI (blue) and rhodamin-phalloidin (red). Scale bar: 10μm. Data are mean (± SEM) percentage of cells lacking stress fibres, quantified from N=11 (DMSO) and 6 (LY and PHA) independent biological replicates. **(I)** GST-CRIB assay to detect Rac1-GTP in MET^{M1268T}-expressing cells transfected with negative control siRNA ("RNAi control") or combined siRNAs targeting p110α and p110β ("RNAi p110α+β"). Negative and positive controls Rac1-GTP detection were also included (see Methods). Data are mean ± SEM. Student's *t* test: NS: non-significant, **P<0.01, ***P<0.005. The figure is related to Figure 2.

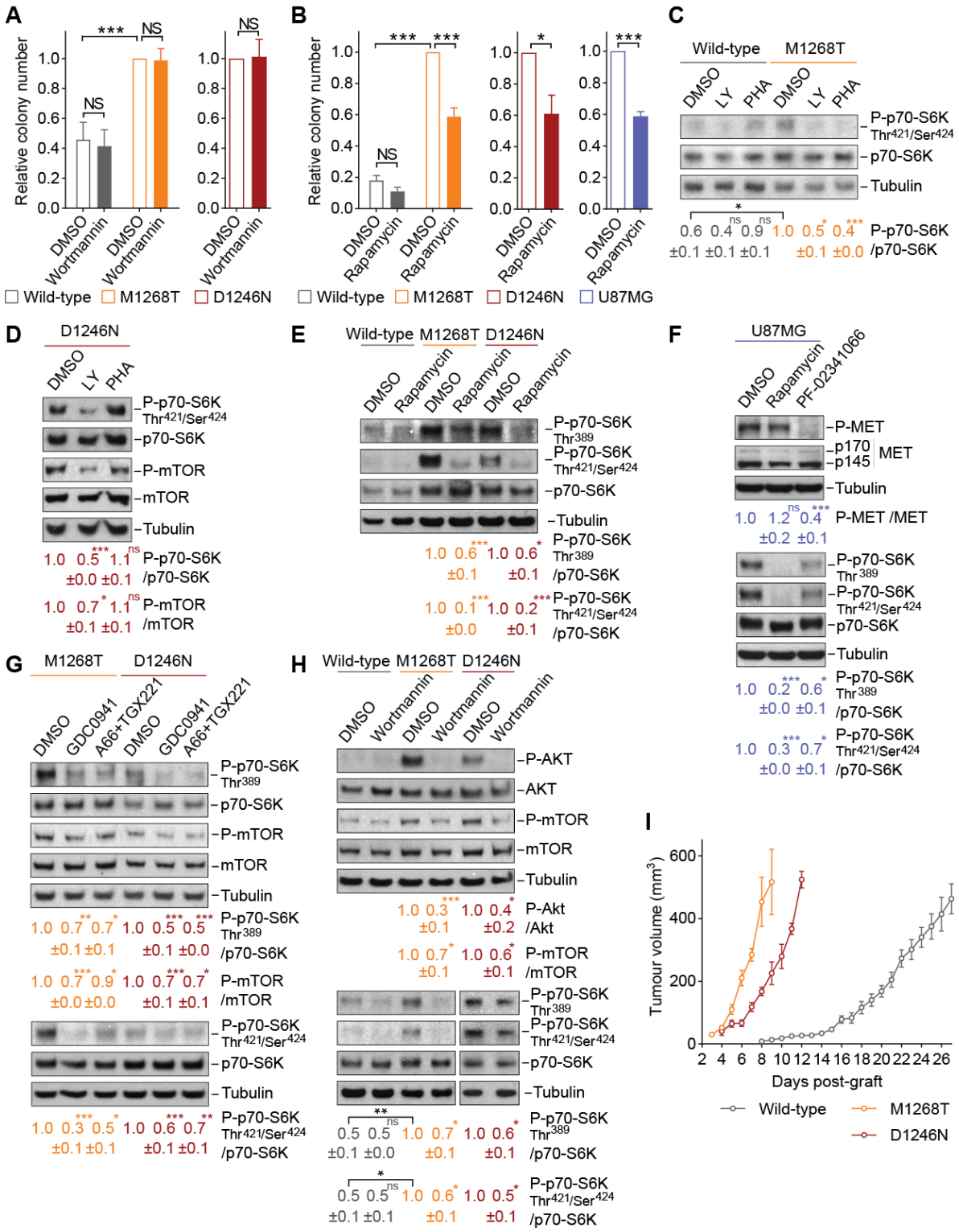

Figure S3: Oncogenic MET-induced anchorage-independent cell and tumor growth is mediated by mTORC1. (A) Relative colony number of wild-type MET-, MET^{M1268T}- (N=6), and MET^{D1246N}-expressing NIH3T3 cells (N=3 independent biological replicates)

grown in soft agar and treated with DMSO or wortmannin (100 nM; A). Data are means ± SEM of independent biological replicates. **(B)** Relative colony number of the cells described in (A) (N=3) and U87MG cells (N=4) grown in soft agar and treated with DMSO or rapamycin (2 nM). Data are means ± SEM of independent biological replicates. **(C to H)** Western blotting for total and phosphorylated ("P-") forms of the indicated proteins in lysates from the indicated cells (wild-type MET-, MET^{M1268T}- or MET^{D1246N}-expressing NIH3T3 cells or U87MG glioblastoma cells) and treated with DMSO or the indicated inhibitor: LY294002 (LY, 10 μM), PHA-665752 (PHA, 100 nM), rapamycin (2 nM), PF-02341066 (100 nM), GDC0941 (100 nM), A66 (500 nM) and TGX221 (40 nM), or wortmannin (100 nM). Densitometries of phosphorylated protein indicated relative to total were quantified from at least 3 independent biological replicates and shown as means ± SEM. Student's *t* test (unless indicated, compared to DMSO condition): NS, non-significant; *P<0.05, **P<0.01, ***P<0.005. **(I)** Tumor growth curves in nude mice, measured daily after subcutaneous injection of $5x10^5$ wild-type MET-, MET^{M1268T}- or MET^{D1246N}-expressing NIH3T3 cells. Data are means ± SEM of 5 mice per group. The figure is related to Figure 3.

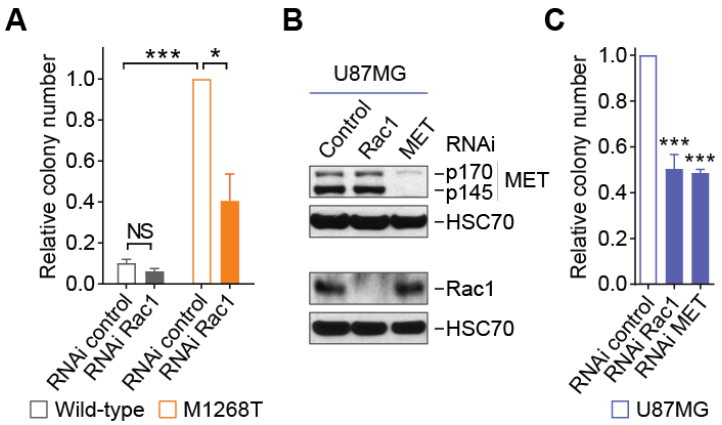

Figure S4: Oncogenic MET promotes anchorage-independent growth through a Rac1-mTOR pathway. (A) Relative colony number of wild-type MET- and MET^{M1268T}-expressing NIH3T3 cells transfected with negative control (RNAi control) or Rac1 siRNAs (RNAi Rac1) and grown in soft agar. Data are means ± SEM of 3 independent biological replicates. Student's *t* test: NS, non-significant; *P<0.05, ***P<0.005. **(B)** Western blotting for MET (p170 indicates the precursor form and p145indicates the mature form of the beta chain), Rac1 and HSC70 were performed on U87MG cells transfected with negative control (Control), Rac1 or MET siRNAs. Blots are representative of N=3 independent biological replicates. **(C)** Relative colony number of U87MG cells transfected with negative control (RNAi control), Rac1 (RNAi Rac1) or MET (RNAi Met) siRNAs and grown in soft agar. Data are means ± SEM of 3 independent biological replicates. Student's *t* test, compared to DMSO: ***P<0.005. The figure is related to Figure 4.

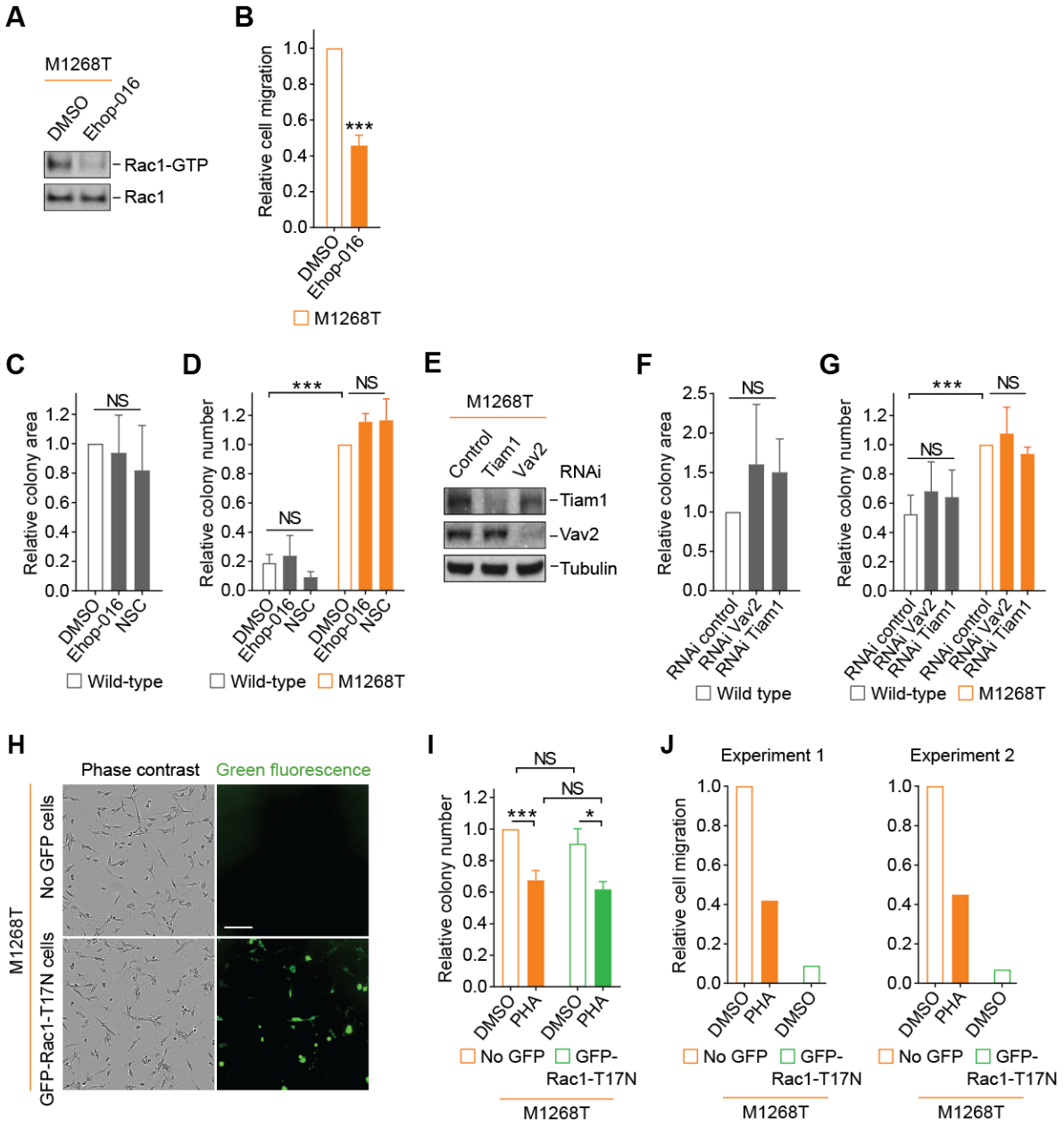

Figure S5: Rac1 promotes anchorage-independent growth in MET-mutant cells independently of its GTPase activity. (A and B) GST-CRIB assay to detect Rac1-GTP (A) and Transwell migration assay (B) performed on M1268T MET-expressing cells treated with DMSO or Ehop-016 (4 µM). N=3 (B) independent biological replicates. **(C, D)** Relative colony area and colony number of wild-type and M1268T MET-expressing cells grown in soft agar and treated with DMSO, Ehop-016 (4 µM) or NSC23766 (NSC, 100 µM). N=3 independent biological replicates. **(E to G)** Western blotting (E) and relative colony area (F) and colony number (G) formed in soft agar by wild-type and M1268T MET-expressing NIH3T3 cells transfected with control, *Vav2*-targeted or *Tiam1*-targeted siRNAs. N= 2 (E) or 4 (F and G) independent biological replicates. **(H to J)** M1268T MET-expressing cells

were transiently transfected with the construct GFP-Rac1-T17N and separated by FACS (GFP negative (No GFP cells) and GFP positive cells (GFP-Rac1-T17N cells) were collected separately), then imaged by IncuCyte (Sartorius) in phase contrast and in the green fluorescence channel (H; scale bar, 150 μm). Relative colony number formed by the cells in soft agar (I) and cell migration through Transwells (J) was assessed after the cells were treated with DMSO or PHA-665752 (PHA, 100 nM). In (I), data are means ± SEM of 3 independent biological replicates. In (J), data are the results of 2 independent biological replicates. Student's *t* test, compared to DMSO: NS: non-significant, *P<0.05, ***P<0.005. In (J), results of two independent biological replicates is shown. The figure is related to Figure 5.

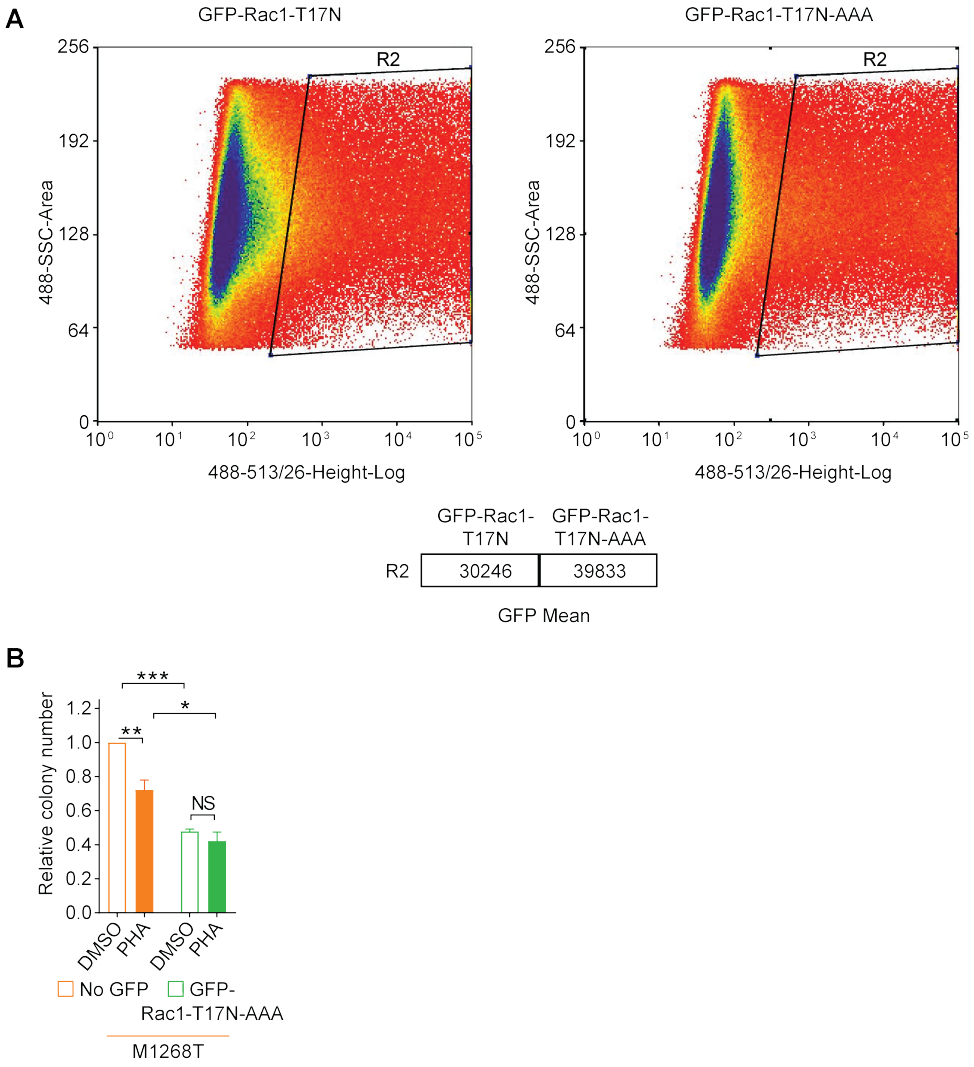

Figure S6: In mutant METexpressing cells, Rac1 associates with mTOR and promotes mTOR plasma membrane localization and anchorage-independent cell growth through its RKR C-terminal motif.

(A) Representative FACS plot illustrating GFP expression in M1268T MET-expressing cells transiently transfected with the indicated construct. Below, the GFP mean in R2-gated cells. **(B)** Relative colony number formed by M1268T MET-expressing cells transiently transfected with the construct GFP-Rac1-T17N-AAA and not ("No GFP"), grown in soft agar, and treated with DMSO or PHA-665752 (PHA, 100 nM). N=3 independent biological replicates. Results are mean ± SEM. Student's *t* test: NS, non-significant; *P<0.05, **P<0.01, ***P<0.005. The figure is related to Figure 7.

siRNAs from Qiagen:

Negative control: AllStars Neg (no sequence provided)

Mouse Tiam1, pool of two oligos; sequences:
CACGATGACTTTATATTTATA (oligo 2)
TAGGTGTAACTCAGTCTGAAA (oligo 4)

Mouse *Vav2*, pool of two oligos. Target sequences:
CTGCTGCTTGTTAGAGATTCA (oligo 8)
CCAGATGTACACATTCGACAA (oligo 9)

siRNAs from Dharmacon:

Mouse *PI3K* - p110 alpha, ON-TARGET, sequence:
GGAGAACCCUUAUGUGACA (oligo 8)

Mouse *PI3K* - p110 beta, ON-TARGET, sequence:
GAACGAAAUGGACCUUAUU (oligo 7)

Mouse *Rac1*, siGENOME, pool of four oligos; sequences:
GGACGAAGCTTGATCTTAG (oligo 1)
AGACGGAGCTGTTGGTAAA (oligo 2)
GATCGGTGCTGTCAAATAC (oligo 3)
GCAAAGTGGTATCCTGAAG (oligo 4)

Human *MET*: siGENOME: D-003156-10

Human *RAC1*, pool of two oligos: siGENOME: D-003560-08 and D-003560-09

Rac1-T17N-AAA sequence (GeneArt, Thermo Scientific):

AAGGGCGGCCGCGGCGGCATGCAGGCCATCAAGTGTGTGGTGGTGGGAGACGGA
GCTGTAGGTAAAAATTGCCTACTGATCAGTTACACAACCAATGCATTTCCTGGAGAA
TATATCCCTACTGTCTTTGACAATTATTCTGCCAATGTTATGGTAGATGGAAAACCG
GTGAATCTGGGCTTATGGGATACAGCTGGACAAGAAGATTATGACAGATTACGCCC
CCTATCCTATCCGCAAACAGATGTGTTCTTAATTTGCTTTTCCCTTGTGAGTCCTGC
ATCATTTGAAAATGTCCGTGCAAAGTGGTATCCTGAGGTGCGGCACCACTGTCCCA
ACACTCCCATCATCCTAGTGGGAACTAAACTTGATCTTAGGGATGATAAAGACACG
ATCGAGAAACTGAAGGAGAAGAAGCTGACTCCCATCACCTATCCGCAGGGTCTAG
CCATGGCTAAGGAGATTGGTGCCGTAAAATACCTGGAGTGCTCGGCGCTCACACA
GCGAGGCCTCAAGACAGTGTTTGACGAAGCGATCCGAGCAGTCCTCTGCCCGCCT
CCCGTGAAGAAGGCCGCAGCTAAATGCCTGCTGTTGTAATGAATTCCTG

Table S1: RNAi oligonucleotides and Rac1-T17N-AAA plasmid. Listed in the table are the sources and sequences of siRNA oligonucleotides and the dominant-negative, RKR motif-mutant Rac1 expression plasmid that were used in this study.

Rac1-T17N-AAA sequence:

AAGGGCGGCCGCGGCGGCATGCAGGCCATCAAGTGTGTGGTGGTGGGAGACGGAG

CTGTAGGTAAAAATTGCCTACTGATCAGTTACACAACCAATGCATTTCCTGGAGAATAT

ATCCCTACTGTCTTTGACAATTATTCTGCCAATGTTATGGTAGATGGAAAACCGGTGA

ATCTGGGCTTATGGGATACAGCTGGACAAGAAGATTATGACAGATTACGCCCCCTATC

CTATCCGCAAACAGATGTGTTCTTAATTTGCTTTTCCCTTGTGAGTCCTGCATCATTTG

AAAATGTCCGTGCAAAGTGGTATCCTGAGGTGCGGCACCACTGTCCCAACACTCCCA

TCATCCTAGTGGGAACTAAACTTGATCTTAGGGATGATAAAGACACGATCGAGAAACT

GAAGGAGAAGAAGCTGACTCCCATCACCTATCCGCAGGGTCTAGCCATGGCTAAGGA

GATTGGTGCCGTAAAATACCTGGAGTGCTCGGCGCTCACACAGCGAGGCCTCAAGA

CAGTGTTTGACGAAGCGATCCGAGCAGTCCTCTGCCCGCCTCCCGTGAAGAAGGCC

GCAGCTAAATGCCTGCTGTTGTAATGAATTCCTG.

Rac1-T17N-AAA sequence:

AAGGGCGGCCGCGGCGGCATGCAGGCCATCAAGTGTGTGGTGGTGGGAGACGGA
GCTGTAGGTAAAAATTGCCTACTGATCAGTTACACAACCAATGCATTTCCTGGAGAA
TATATCCCTACTGTCTTTGACAATTATTCTGCCAATGTTATGGTAGATGGAAAACCG
GTGAATCTGGGCTTATGGGATACAGCTGGACAAGAAGATTATGACAGATTACGCCC
CCTATCCTATCCGCAAACAGATGTGTTCTTAATTTGCTTTTCCCTTGTGAGTCCTGC
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ACACTCCCATCATCCTAGTGGGAACTAAACTTGATCTTAGGGATGATAAAGACACG
ATCGAGAAACTGAAGGAGAAGAAGCTGACTCCCATCACCTATCCGCAGGGTCTAG
CCATGGCTAAGGAGATTGGTGCCGTAAAATACCTGGAGTGCTCGGCGCTCACACA
GCGAGGCCTCAAGACAGTGTTTGACGAAGCGATCCGAGCAGTCCTCTGCCCGCCT
CCCGTGAAGAAGGCCGCAGCTAAATGCCTGCTGTTGTAATGAATTCCTG