Supplementary Materials
Molecular Biology of the Cell
Collins *et al.*

Table S1. Cell lines used in this study.

Cell line	Classification	Hormone Receptor Presentation	Oncogenic Ras
MDAMB231	Basal-like breast cancer	Triple negative	Yes
MDAMB453*	Basal-like breast cancer	Triple negative	No
MCF7	Luminal A breast cancer	ER+, PR+	No
SKBR3	HER2-enriched breast cancer	HER2+	No
MCF10A	Non-transformed breast epithelial	N/A	No
HER2/MCF10A	HER2-transformed	HER2+	No

* MDAMB453 cells express some HER2 and, thus, are sometimes classified as HER2+ [31]. However, because HER2 is not amplified in these cells, they are often considered more like triple negative breast cancer cells [32,33].

ER+, Estrogen Receptor positive
PR+, Progesteron Receptor positive
HER2+, Human Epidermal growth factor Receptor 2 positive

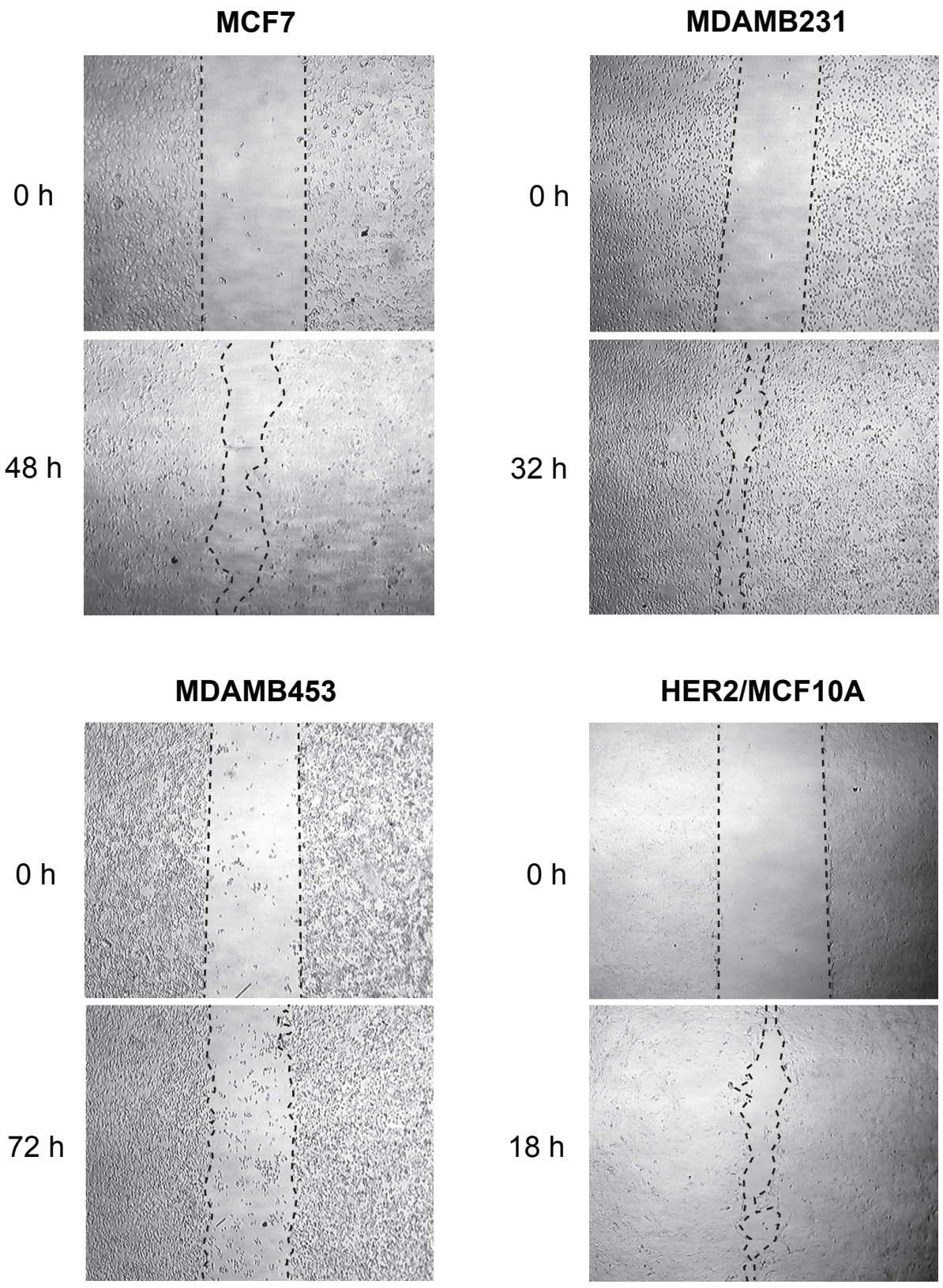

Fig. S1. 2D breast cancer cell migration.
Representative images of MCF7, MDAMB231, MDAMB453, and HER2/MCF10A cells in the wound closure assay are shown, taken immediately after wounding (0 h) and after the indicated time.Data are representative of at least 3 independent experiments.

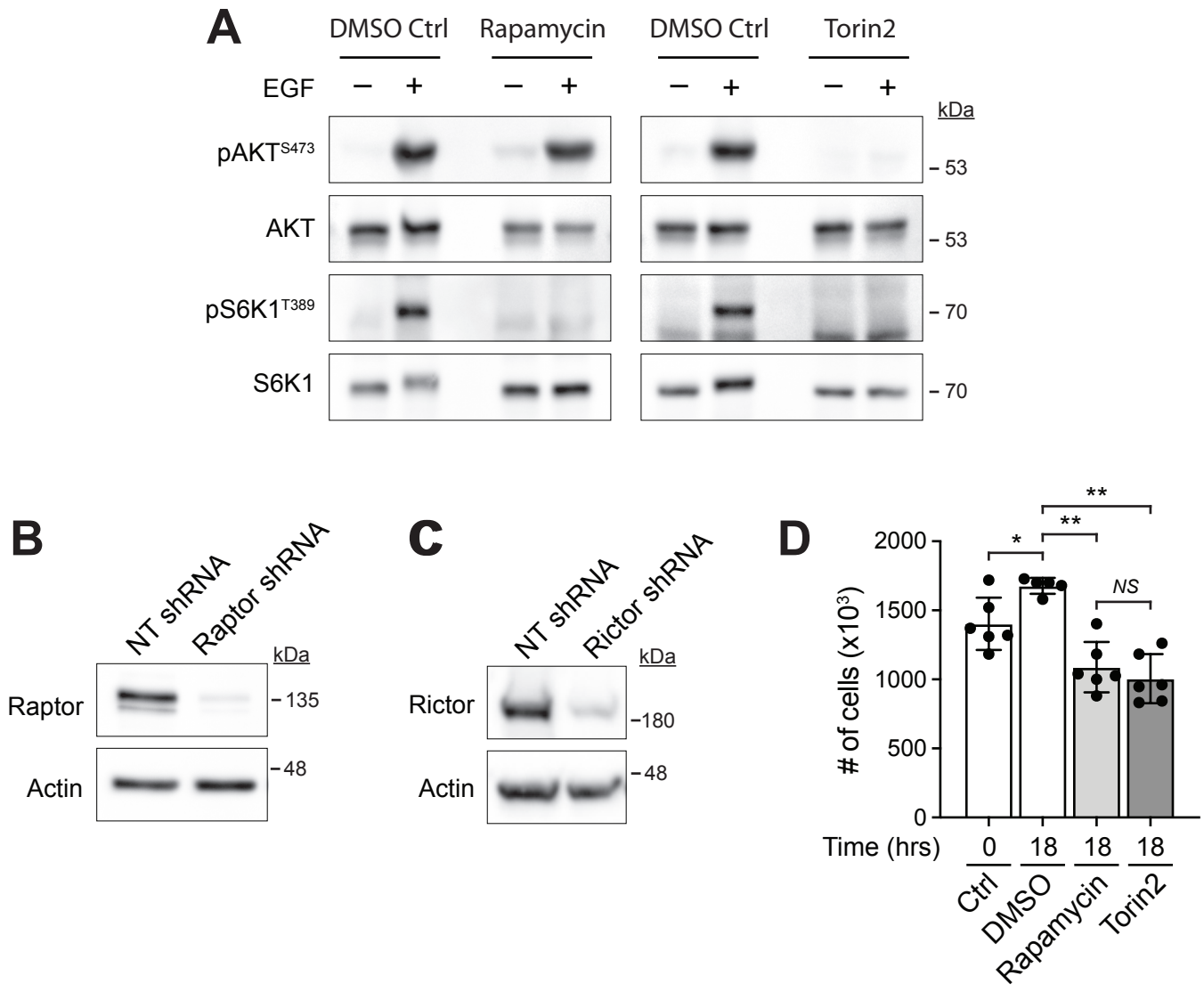

Fig. S2. Inhibition of mTORC1 and mTORC2 function.
A) MCF10A cells were used and mTORC1 and mTORC2 activities were monitored through detection of their substrate phoshorylation, S6K1 at threonine 389 (pS6K1^{T389}) and AKT at serine 473 (pAKT^{S473}), respectively, by immunoblot. Where indicated, cells were pretreated with 0.1% DMSO (control), 10 μM Rapamycin, or 100 nM Torin2 for 1 h, and stimulated with 100 ng/mL EGF for 5 min at 37ºC. Phosphorylated S6K1 and total S6K1 proteins were detected by immunoblot using phospho-p70 S6 Kinase (Thr389) (1A5) mouse mAb and rabbit p70 S6 Kinase antibody; and phosphorylated AKT and total AKT proteins were detected by immunoblot using phospho-AKT (Ser473) (13H12) rabbit mAb and AKT (pan) (40D4) mouse mAb, all from Cell Signaling Technology, Inc. (Danvers, MA) **B) and C)** Where indicated, cells were treated with non-targeting shRNA, Raptor shRNA, or Rictor shRNA as described in the Methods section. Cell lysates were subjected to immunoblotting using rabbit Raptor and rabbit Rictor antibodies (Bethyl Laboratories Inc., Montgomery, TX). As a loading control, actin was immunoblotted using Beta-Actin (C4) antibody (Santa Cruz Biotechnology, Dallas, TX). Data presented in A, B, and C are representative of at least 3 independent experiments. **D)** Wound closure cell migration assays were performed with MCF10A cells in the presence of Rapamycin, Torin2, or 0.1% DMSO control (Ctrl) as described in the Methods section. At 18 h after wounding, cells were resuspended and counted to assess proliferation during the assay. Graph shows the number of cells ± S.D. in at least 6 wounds from at least 3 independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

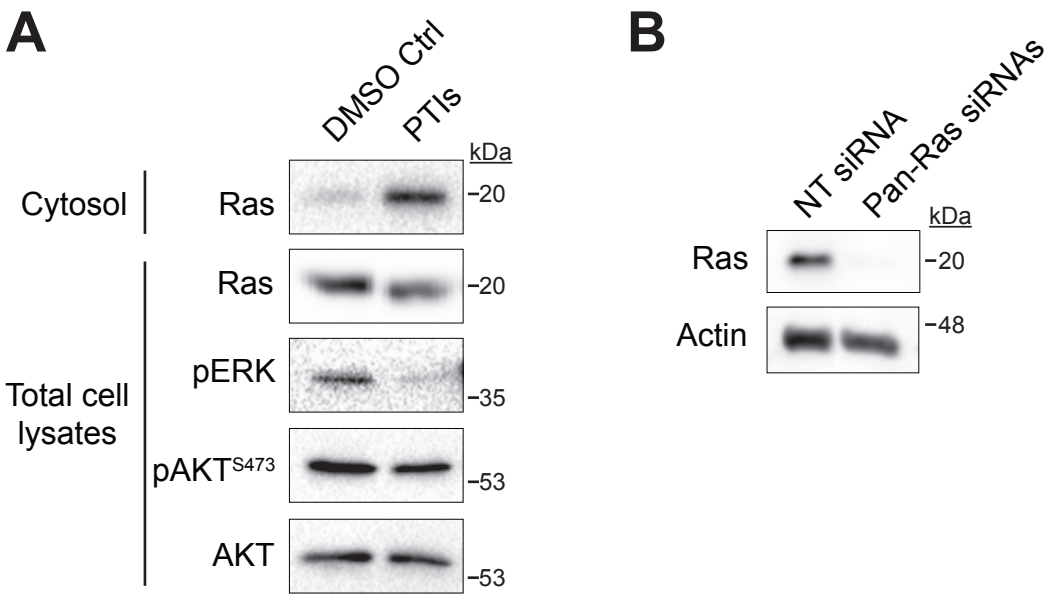

Fig. S3. Inhibition of Ras function.
A) MCF10A cells were treated with 0.1% DMSO (control) or 50 μM prenyltransferase inhibitors (PTIs; 25 μM farnesyltransferase inhibitor and 25 μM geranylgeranyltransferase inhibitor) for 16 h at 37°C. Cells were lysed in 20 mM Tris pH 7.5, 5 mM EDTA ,150 mM NaCl, and 1% NP-40 supplemented with protease inhibitors. To seperate membrane from cytosolic Ras, the lysates were subjected to sonication and the membranes were pelleted by centrifugation. The supernatant fraction was collected to assess the presence of Ras in the cytosol. Phospho-ERK (pERK) was used as a control for Ras inhibition. The proteins were immunoblotted using anti-pan-Ras (Ab-3) mouse mAb (RAS 10; Millipore Sigma Calbiochem, Burlington, MA), and rabbit phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) antibody (Cell Signaling Technology, Inc., Danvers, MA). $pAKT^{473}$and AKT immunoblot are described in the Methods section. **B)** Where indicated, cells were treated with either non-targeting (NT) shRNA or Pan-Ras siRNA and the samples prepared as described in the Methods section. Ras was immunoblotted as described in A and actin was immunobloted as loading control using Beta-Actin (C4) antibody (Santa Cruz Biotechnology, Dallas, TX). Data are representative of at least 3 independent experiments.

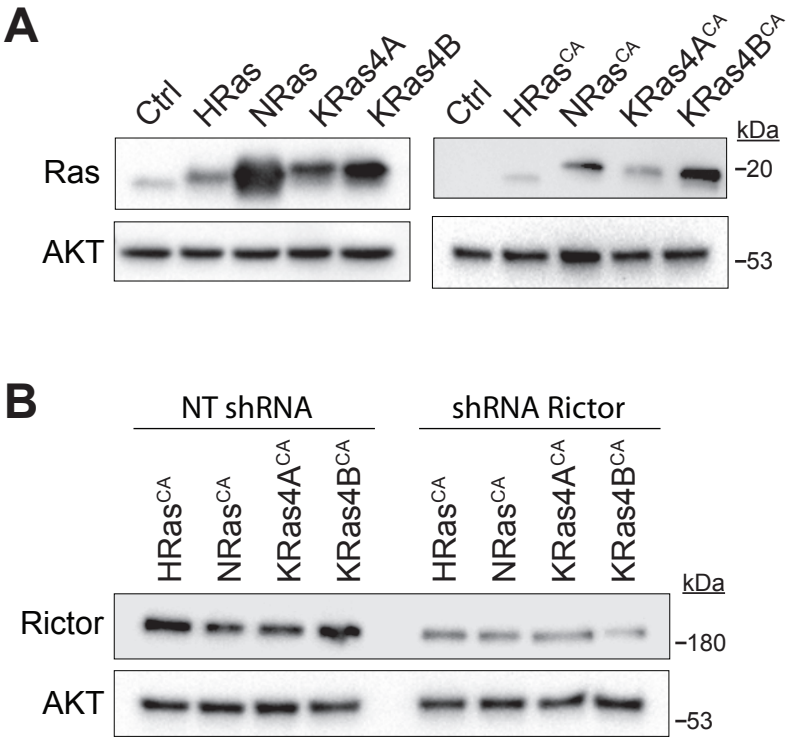

Fig. S4. Expression of Ras and silencing of Rictor.
A) Cell lysates from MCF10A cells expressing the indicated wild-type or constitutively active (CA; G12V) Ras isoforms were subjected to immunoblotting using anti-pan-Ras (Ab-3) mouse mAb (RAS 10; Millipore Sigma Calbiochem, Burlington, MA) and AKT antibody as loading control, as described in the Methods section. **B)** Ras^{CA}-expressing MCF10A cells were treated with non-targeting (NT) shRNA or Rictor shRNA and samples prepared as described in the Methods section. Lysates were subjcted to immunoblotting using rabbit Rictor antibodies (Bethyl Laboratories Inc., Montgomery, TX) and AKT antibody (loading control).