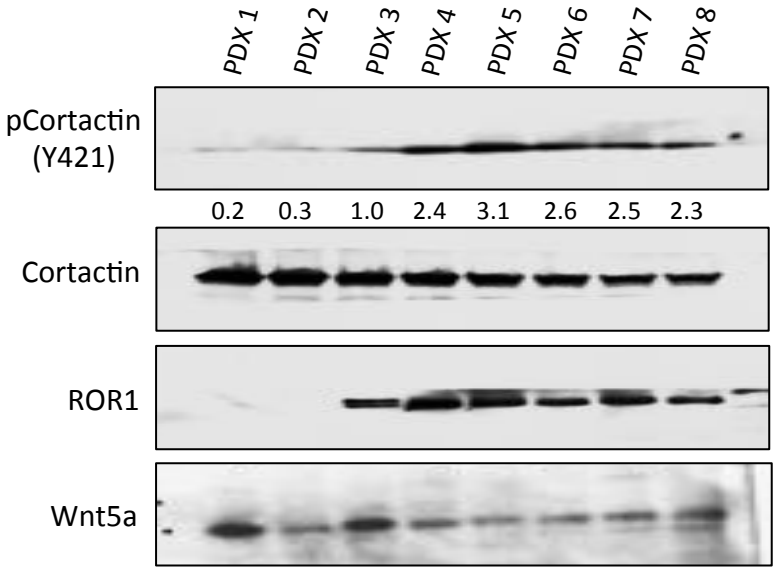
SUPPLEMENTARY INFORMATION

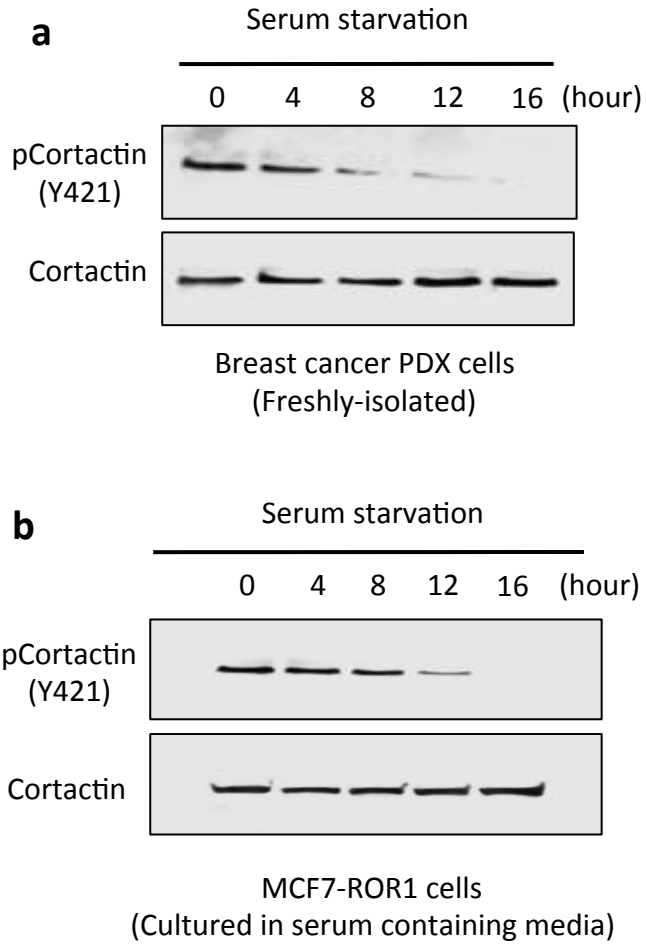
Wnt5a Induces ROR1 To Recruit Cortactin To Promote Breast Cancer Migration And Metastasis

Supplementary Figure 1



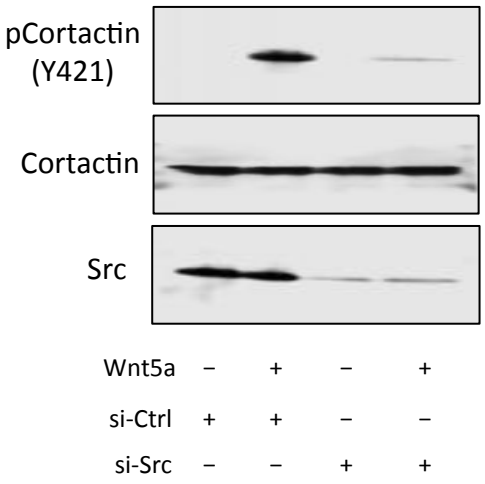
Supplementary Figure 1. Immunoblot analysis of lysates prepared from 8-different breast cancer PDX tumors; the membranes were probed with anti-phospho-cortactin (Y421), anti-cortactin, anti-ROR1, or anti-Wnt5a antibody, as indicated on the left. The numbers between two upper lanes are ratios of band IOD (integrated optical density) of pCortactin (Y421) versus total Cortactin.

Supplementary Figure 2



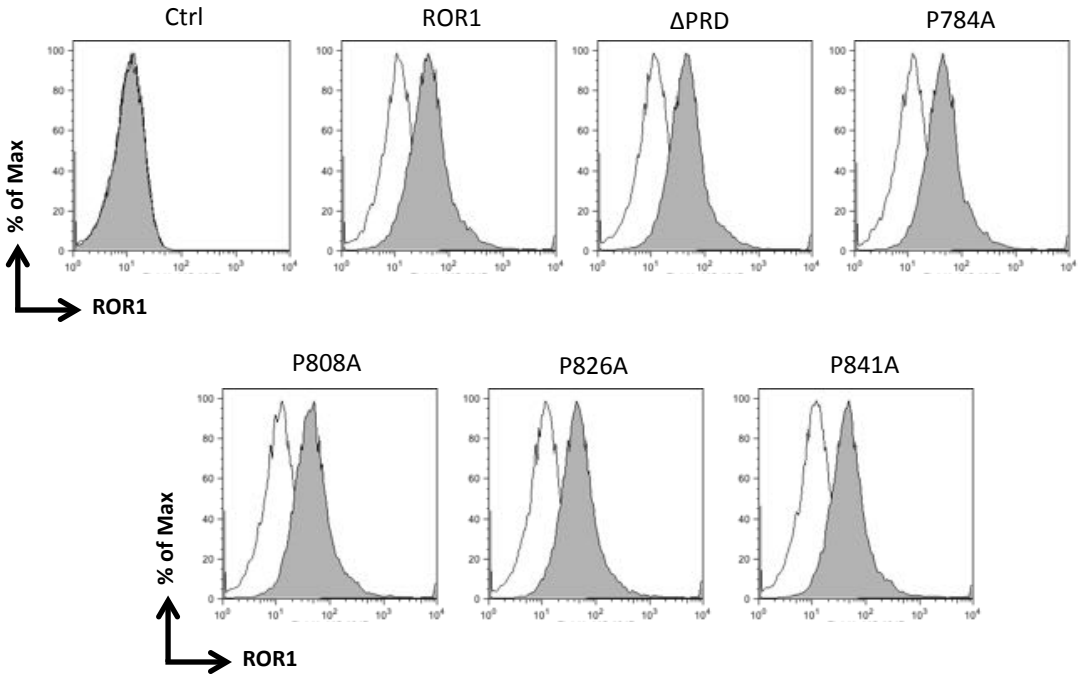
Supplementary Figure 2. a, Immunoblot analysis of lysates prepared from freshly-isolated breast cancer PDX5 cells that were serum-starved for the times indicated on the top (in hours); the membranes were probed with anti-cortactin, or anti-phospho-cortactin (Y421) antibody, as indicated on the left. **b**, Immunoblot analysis of lysates prepared from MCF7-ROR1 cells cultured in 10% serum containing DMEM media that were serum-starved for the times indicated on the top (in hours); the membranes were probed with anti-cortactin, or anti-phospho-cortactin (Y421) antibody, as indicated on the left.

Supplementary Figure 3



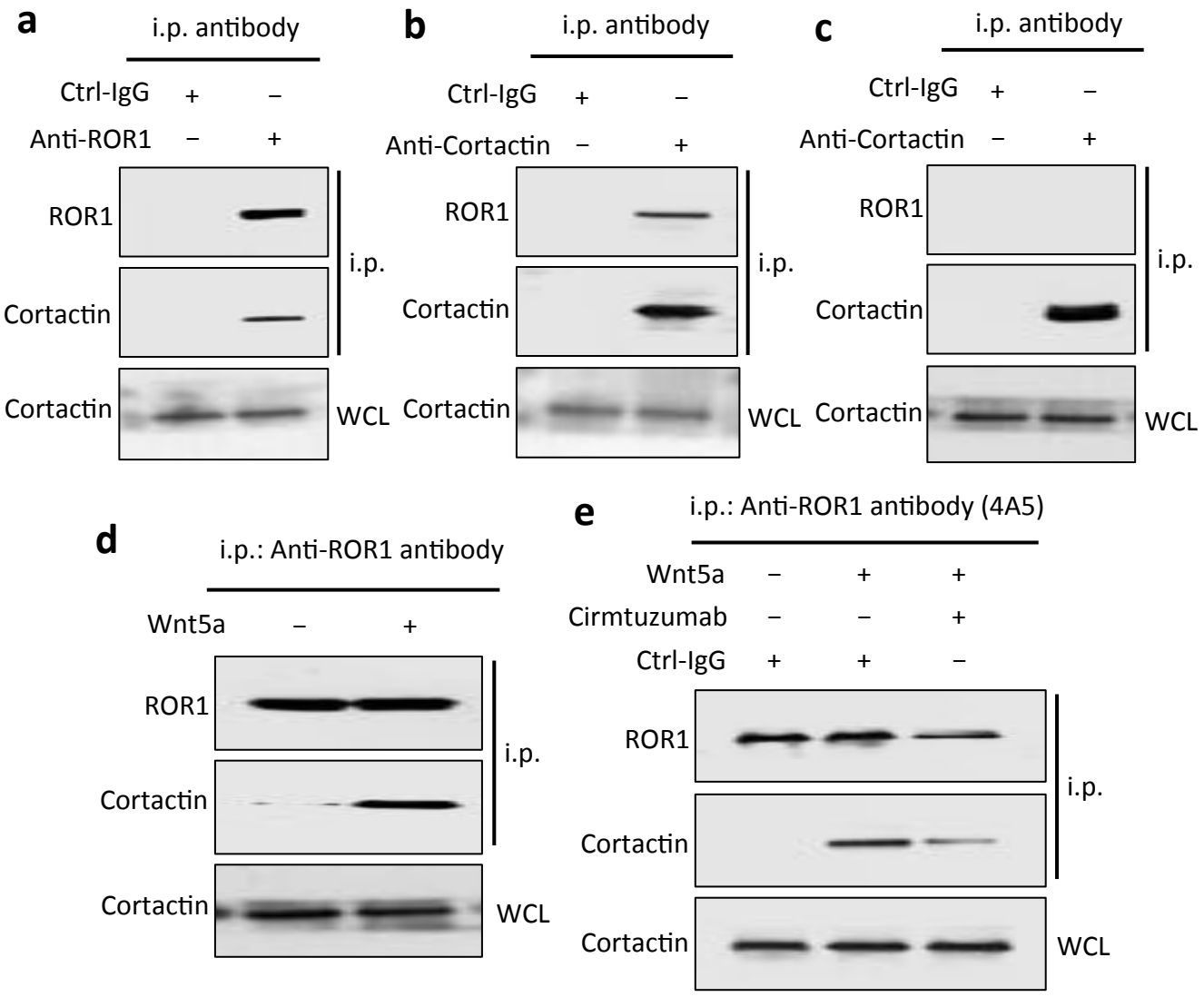
Supplementary Figure 3. Immunoblot analysis of lysates prepared from PDX5 cells transfected 72-hours previously with control siRNA or siRNA targeting Src; that subsequently were treated without (-) or with (+) Wnt5a (100 ng/ml), as indicated at the bottom; the membranes were probed with anti-phospho-cortactin (Y421), anti-cortactin, or anti-Src antibody, as indicated on the left.

Supplementary Figure 4



Supplementary Figure 4. Fluorescence of MCF7-Ctrl cells, MCF7-ROR1 (W/T), MCF7-ΔPRD, or MCF7 cells transfected with each of the various mutated forms of ROR1; after staining with a fluorochrome-labeled isotype control mAb (open histograms) or 4A5- Alexa-647 (shaded histograms).

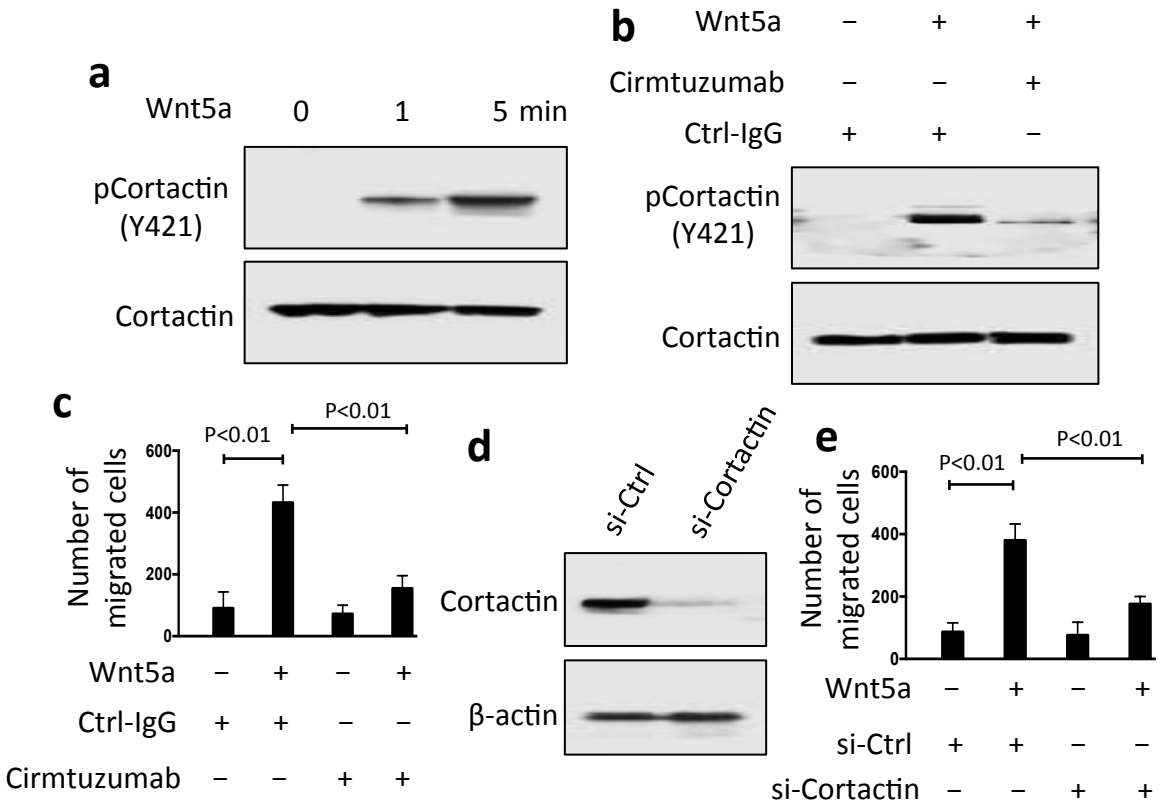
Supplementary Figure 5



Supplementary Figure 5. Association Of ROR1 With Cortactin In MCF7-ROR1 Cells

a, Immunoblot analysis of anti-ROR1 i.p. or control IgG (Ctrl-IgG) i.p., as indicated at the top, using lysates prepared from MCF7-ROR1 cells; the membranes were probed with anti-ROR1 or anti-cortactin antibody, as indicated on the left. An immunoblot of the whole-cell lysates probed with anti-cortactin mAb is provided in the bottom panel. **b**, Immunoblot analysis of anti-cortactin i.p. or Ctrl-IgG i.p., as indicated at the top, using lysates prepared from MCF7-ROR1 cells; the membranes were probed with anti-ROR1 or anti-cortactin antibody, as indicated on the left. An immunoblot of the whole-cell lysates probed with anti-cortactin mAb is provided in the bottom panel. **c**, Immunoblot analysis of anti-ROR1 i.p. or control IgG (Ctrl-IgG) i.p., as indicated at the top, using lysates prepared from ROR1 negative MCF7 cells; the membranes were probed with anti-ROR1 or anti-cortactin antibody, as indicated on the left. An immunoblot of the whole-cell lysates probed with anti-cortactin mAb is provided in the bottom panel. **d**, Immunoblot analysis of anti-ROR1 i.p. using lysates prepared from overnight, serum-starved MCF7-ROR1 cells that subsequently were treated for 30 minutes without (-) or with (+) Wnt5a (100 ng/ml), as indicated on the top; the membranes were probed with anti-ROR1 or anti-cortactin antibody, as indicated on the left. An immunoblot of the whole-cell lysates probed with anti-cortactin mAb is provided in the bottom panel. **e**, Immunoblot analysis of anti-ROR1 (4A5) i.p. using lysates prepared from overnight, serum-starved MCF7-ROR1 cells that had been treated with Ctrl-IgG or cirmtuzumab (10 μg/ml) for two hours, and subsequently treated for 30 minutes without (-) or with (+) Wnt5a (100 ng/ml), as indicated on the top; the membranes were probed with anti-ROR1 or anti-cortactin antibody, as indicated on the left. An immunoblot of the whole-cell lysates probed with anti-cortactin mAb is provided in the bottom panel.

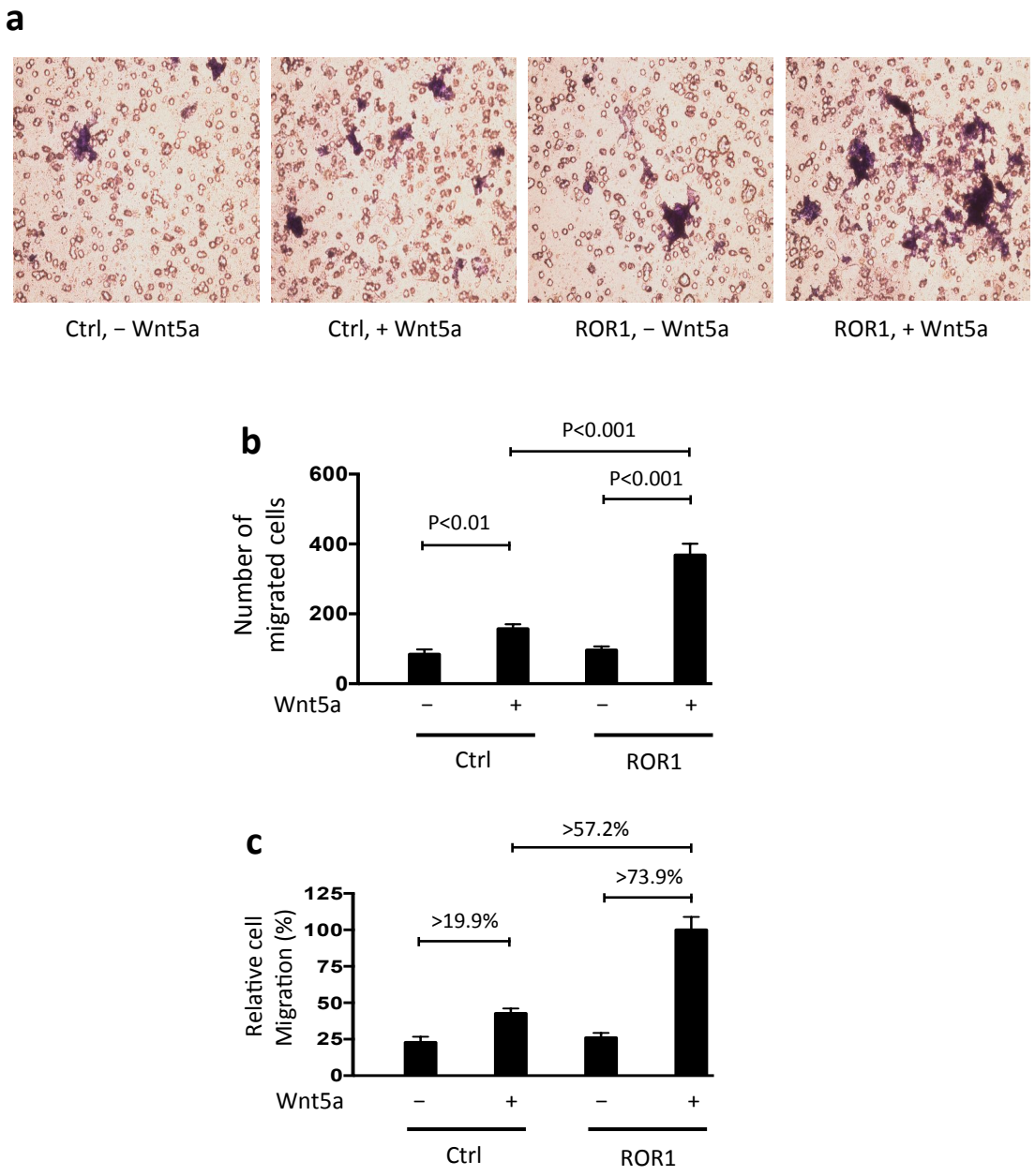
Supplementary Figure 6



Supplementary Figure 6. Wnt5a Induces ROR1-Dependent Phosphorylation Of Cortactin And Enhances Breast Cancer MCF7 Cell Migration

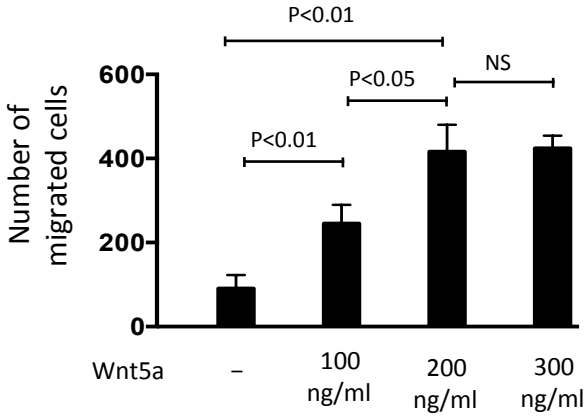
a, Immunoblot analysis of lysates prepared from overnight, serum-starved MCF7-ROR1 cells that subsequently were treated with Wnt5a (100 ng/ml) for the times indicated on the top (in minutes); the membranes were probed with anti-cortactin or anti-phospho-cortactin (Y421) antibody, as indicated on the left. **b**, Immunoblot analysis of lysates prepared from overnight, serum-starved MCF7-ROR1 cells that subsequently were treated with Ctrl-IgG or cirmtuzumab (10 μg/ml), without (-) or with (+) Wnt5a (100 ng/ml), as indicated on the top; the membranes were probed with anti-cortactin or anti-phospho-cortactin (Y421) antibody, as indicated on the left. **c**, MCF7-ROR1 cells were serum-starved overnight, subsequently treated with Ctrl-IgG or cirmtuzumab (10 μg/ml), and then cell migration assay was performed for 10 hours in the absence (-) or presence (+) exogenous Wnt5a (200 ng/ml), as indicated at the bottom. Data are shown as mean ± S.D. from 3 independent experiments (n=3). $P < 0.01$, as assessed by 2-tailed Student's *t* test. **d**, Immunoblot analysis of lysates prepared from MCF7-ROR1 cells transfected 72-hours previously with control siRNA or siRNA targeting cortactin; membranes were probed with anti-cortactin or anti-β-actin antibody, as indicated on the left. Cell viability was over 85% both in control or cortactin-siRNA transfected cells. **e,** MCF7-ROR1 cell migration was assessed for 10 hours in the absence (–) or presence (+) of exogenous Wnt5a (200 ng/ml), as indicated at the bottom. Data are shown as mean ± S.D. from 3 independent experiments (n=3). $P < 0.01$, as assessed by 2-tailed Student's *t* test.

Supplementary Figure 7



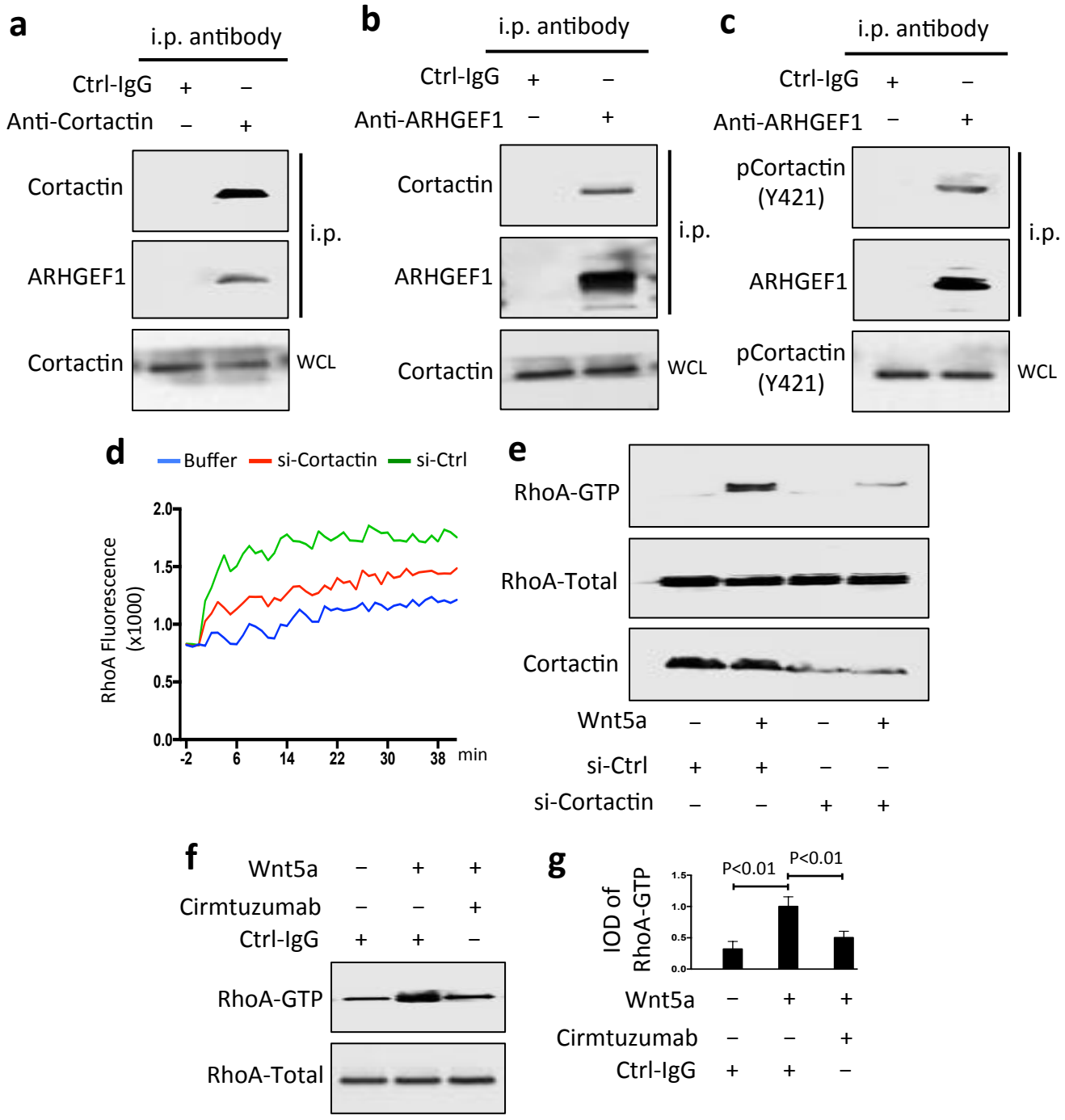
Supplementary Figure 7. a, MCF7-Ctrl or MCF7-ROR1 cells were serum-starved overnight, and cell migration assay was performed for 10 hours in the absence (-) or presence (+) exogenous Wnt5a (200 ng/ml), as indicated at the bottom. Representative photomicrographs of MCF7-Ctrl or MCF7-ROR1 cells in assays for cell-migration. **b**, The histograms depict the relative cell migration as normalized to control. Data are shown as mean ± S.D. from 3 independent experiments (n=3). $P < 0.01$; $P < 0.001$, as assessed by 2-tailed Student's *t* test. **c**, The histograms represent the normalized percentage (%) of cell migration of panel B.

Supplementary Figure 8



Supplementary Figure 8. MCF7-ROR1 cells were serum-starved overnight, and cell migration assay was performed for 10 hours in the absence (-) or presence (+) of exogenous Wnt5a (100, 200, or 300 ng/ml), as indicated at the bottom. The histograms depict the number of cells migrated. Data are shown as mean ± S.D. from 3 independent experiments (n=3). $P < 0.05$; $P < 0.01$, as assessed by 2-tailed Student's *t* test. 'NS' indicates not significant.

Supplementary Figure 9

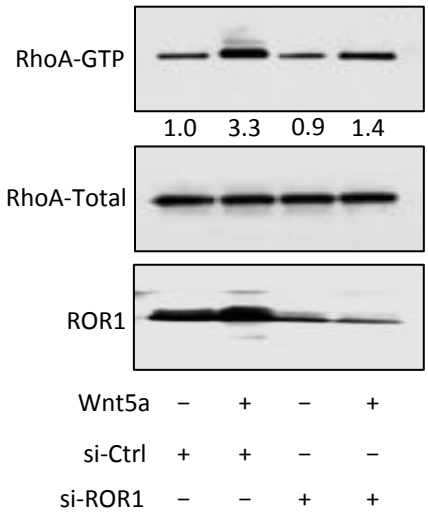


Supplementary Figure 9. Cortactin Associates With ARHGEF1, Which Undergoes Cortactin-dependent Activation To Enhance Activation Of RhoA

a, Immunoblot analysis of anti-cortactin i.p. or Ctrl-IgG i.p., as indicated at the top, using lysates prepared from PDX3 tumor; the membranes were probed with anti-cortactin or anti-ARHGEF1 antibody, as indicated on the left. An immunoblot of the whole-cell lysates probed with anti-cortactin mAb is provided in the bottom panel. **b**, Immunoblot analysis of anti-ARHGEF1 i.p. or Ctrl-IgG i.p., as indicated at the top, using lysates prepared from PDX4 tumor; membranes were probed with anti-cortactin or anti-ARHGEF1 antibody, as indicated on the left. An immunoblot of the whole-cell lysates probed with anti-cortactin mAb is provided in the bottom panel.

c, Immunoblot analysis of anti-ARHGEF1 i.p. or Ctrl-IgG i.p., as indicated at the top, using lysates prepared from PDX5 tumor; membranes were probed with anti-phospho-cortactin (Y421) or anti-ARHGEF1 antibody, as indicated on the left. An immunoblot of the whole-cell lysates probed with anti-phospho-cortactin (Y421) mAb is provided in the bottom panel. **d**, *In vitro* exchange assay on RhoA of anti-ARHGEF1 i.p. from lysates of PDX5 cells transfected with Ctrl-siRNA (green line) or siRNA specific for cortactin (red line) in the presence of Wnt5a. The blue line depicts GTPase-activation using buffer alone. **e**, Immunoblot analysis of lysates prepared from MCF7-ROR1 cells transfected 72-hours previously with control siRNA or siRNA targeting cortactin, that subsequently were treated without (-) or with (+) Wnt5a (100 ng/ml), as indicated at the bottom; expression of cortactin, total RhoA, and activated RhoA was measured, as indicated on the left. **f**, Immunoblot analysis of lysates prepared from overnight, serum-starved breast cancer PDX5 cells (representative of 3 PDXs) that subsequently were treated with Ctrl-IgG or cirmtuzumab (10 μg/ml), without (-) or with (+) Wnt5a (100 ng/ml), as indicated on the top; expression of total RhoA, and activated RhoA was measured, as indicated on the left. **g**, Bars indicate the relative activation of RhoA in breast cancer PDX4, 5, and 6 cells that that had been treated with Ctrl-IgG or cirmtuzumab (10 μg/ml) for two hours, and subsequently treated for 30 minutes without (-) or with (+) Wnt5a (100 ng/ml), as indicated at the bottom. Data are shown as mean ± S.D. of PDX cells from each of 3 different patients. $P < 0.01$, as assessed by 2-tailed Student's *t* test.

Supplementary Figure 10



Supplementary Figure 10. Immunoblot analysis of lysates prepared from MDA-MB-231 cells transfected 72-hours previously with control siRNA or siRNA targeting ROR1, that subsequently were treated without (-) or with (+) Wnt5a (100 ng/ml), as indicated at the bottom; expression of ROR1, total RhoA, and activated RhoA was measured, as indicated on the left. The numbers between two lanes are ratios of band IOD (integrated optical density) of GTP-RhoA versus total RhoA.

Supplementary Figure 11: Uncropped blots

Figure 1b

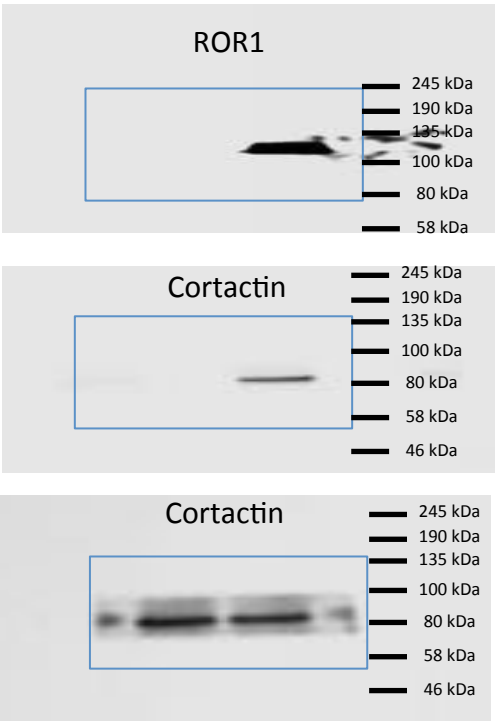


Figure 1d

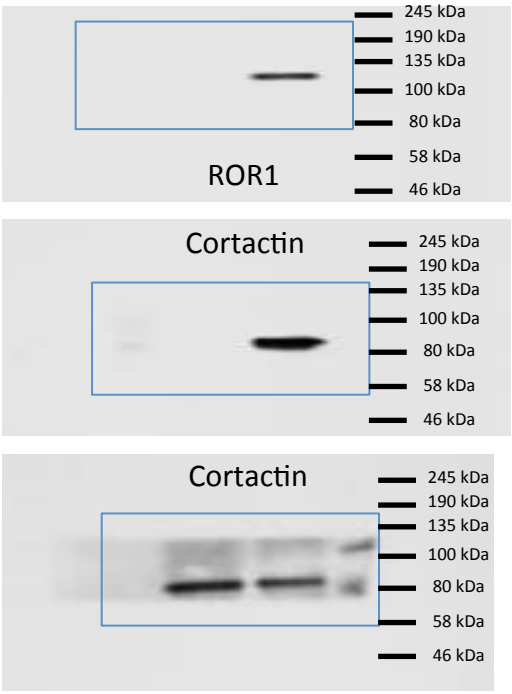


Figure 1f

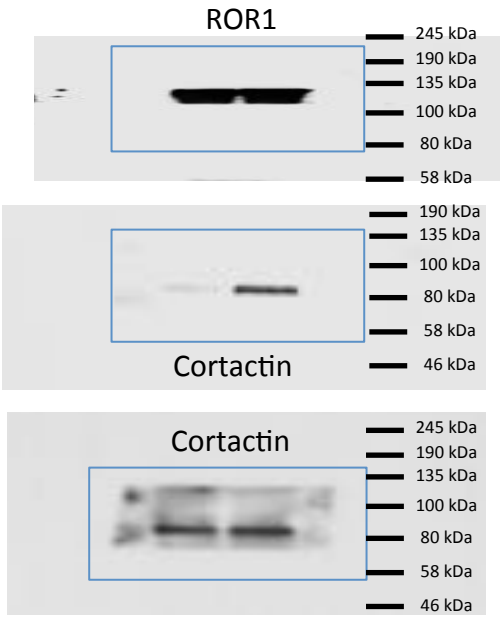


Figure 1h

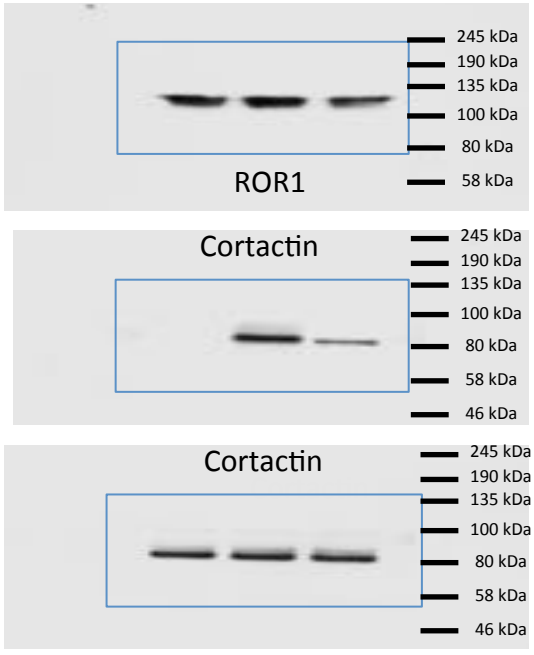


Figure 2a

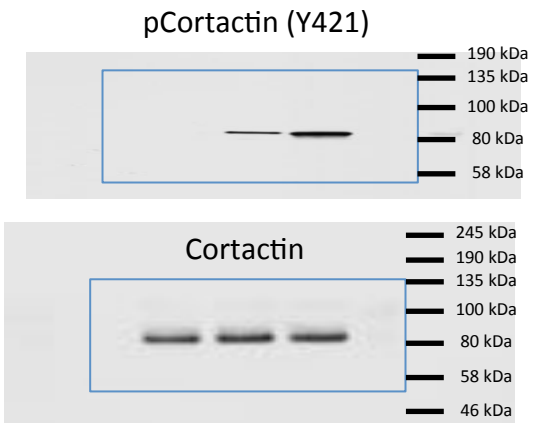


Figure 2c

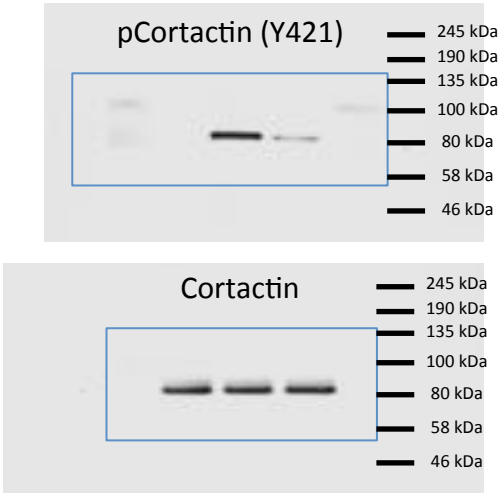


Figure 2e

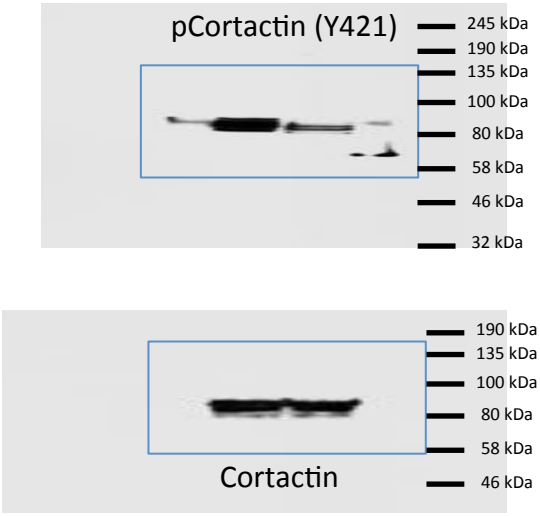


Figure 3d

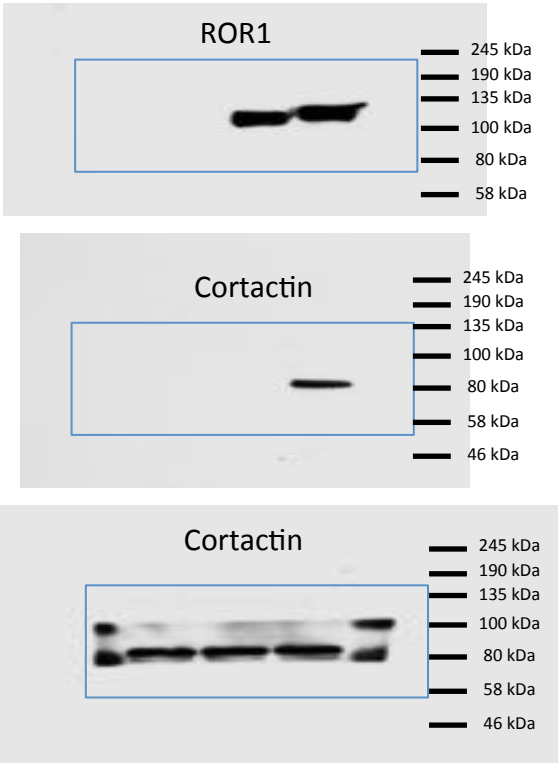


Figure 3f

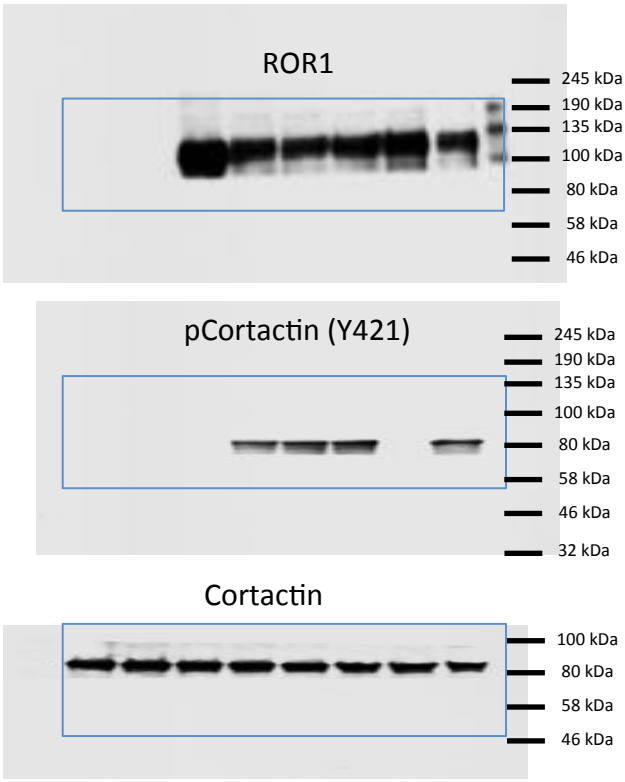


Figure 3e

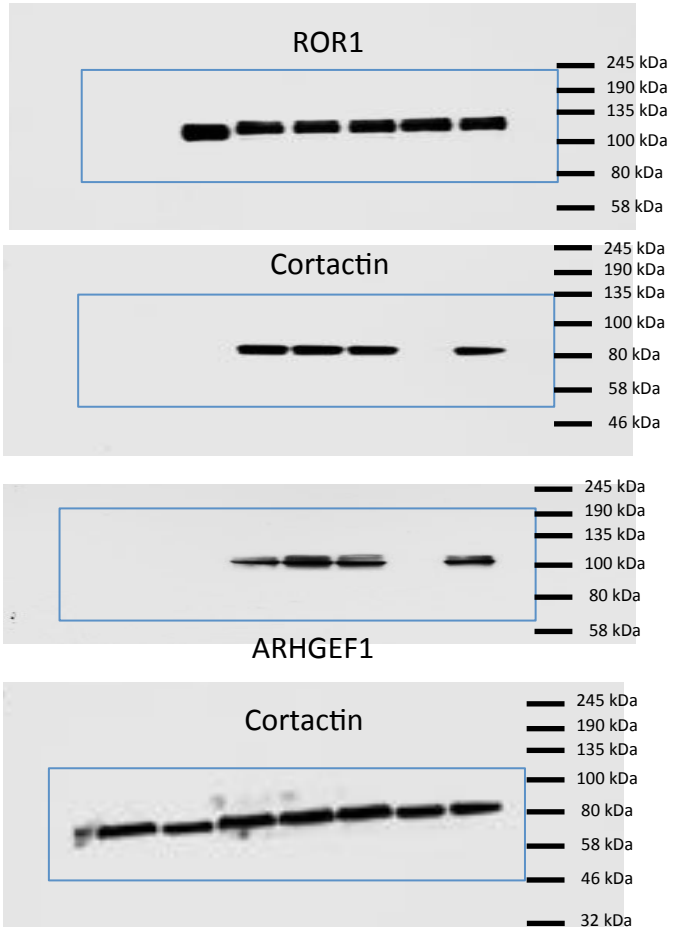
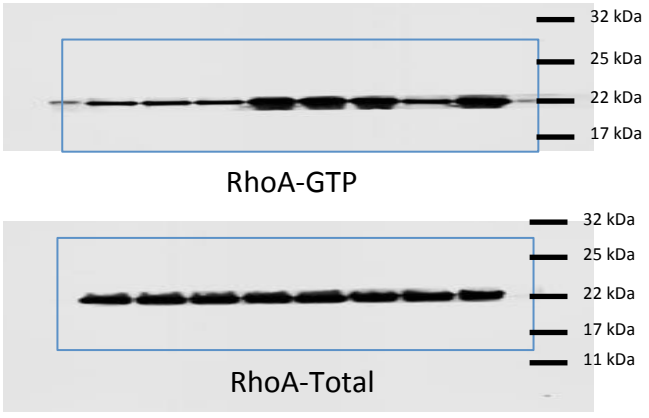
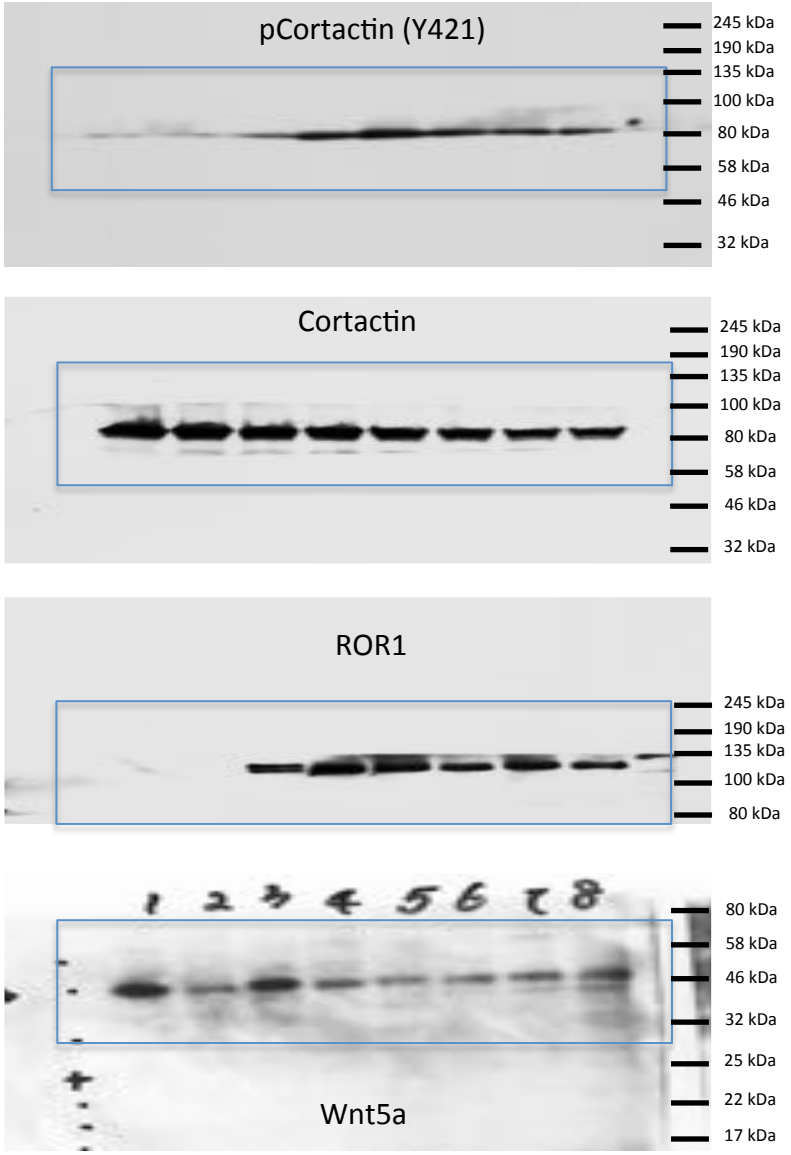


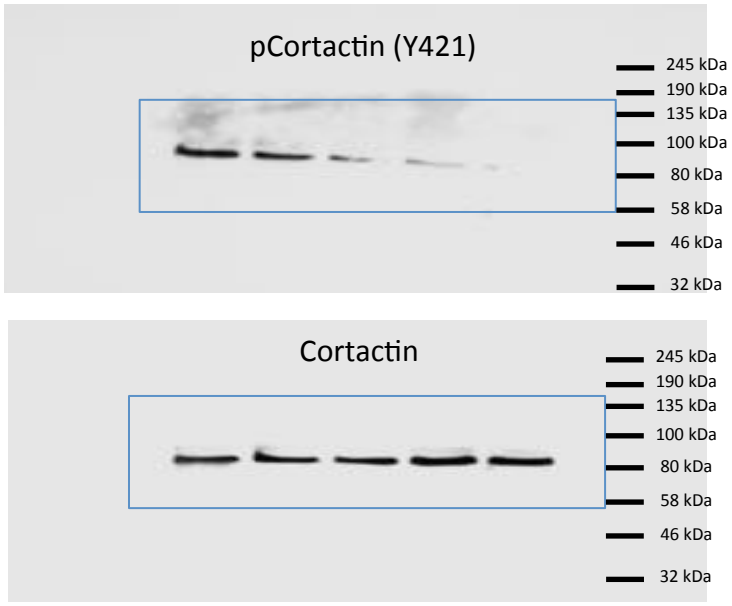
Figure 3g



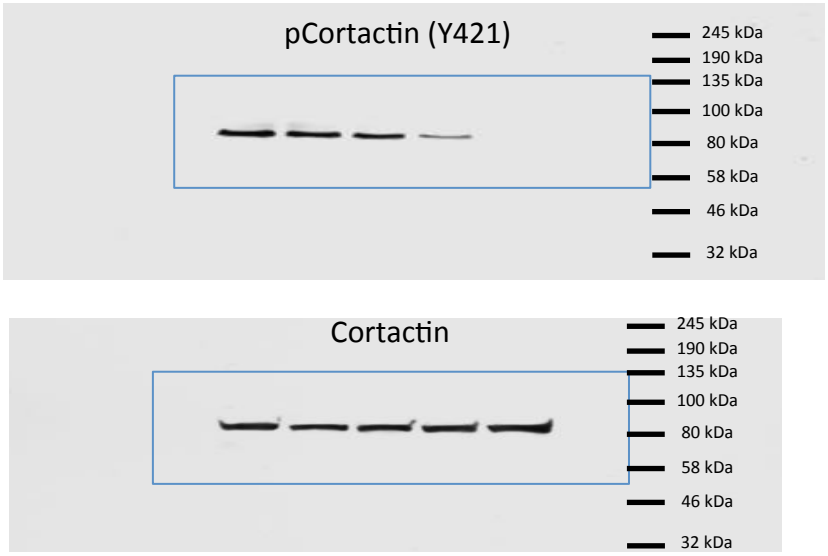
Supplementary Figure 1



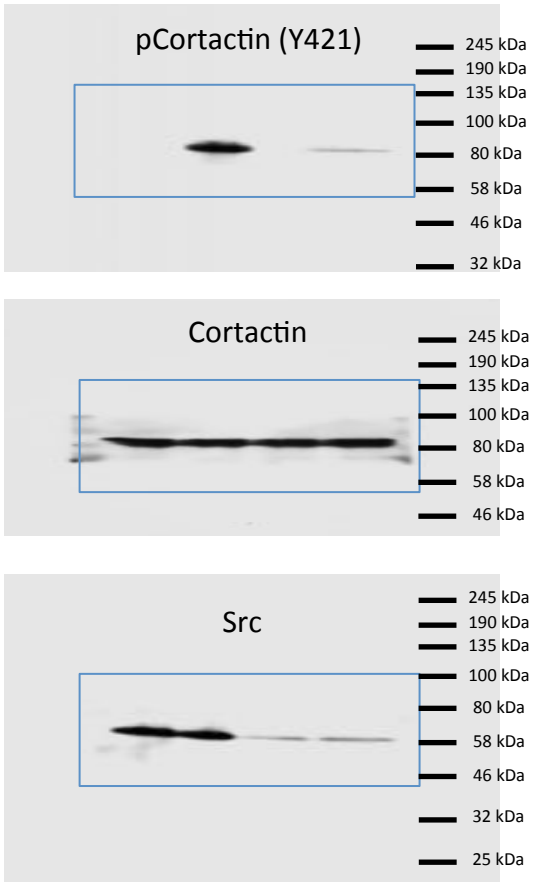
Supplementary Figure 2a



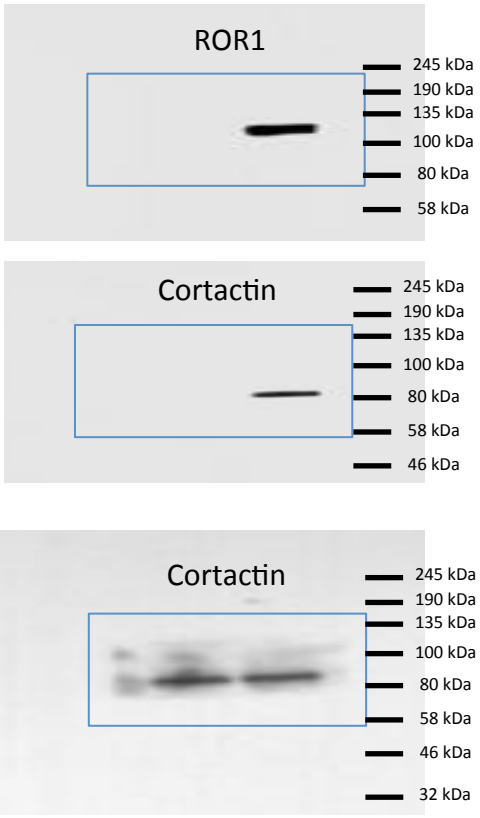
Supplementary Figure S2b



Supplementary Figure 3



Supplementary Figure 5a



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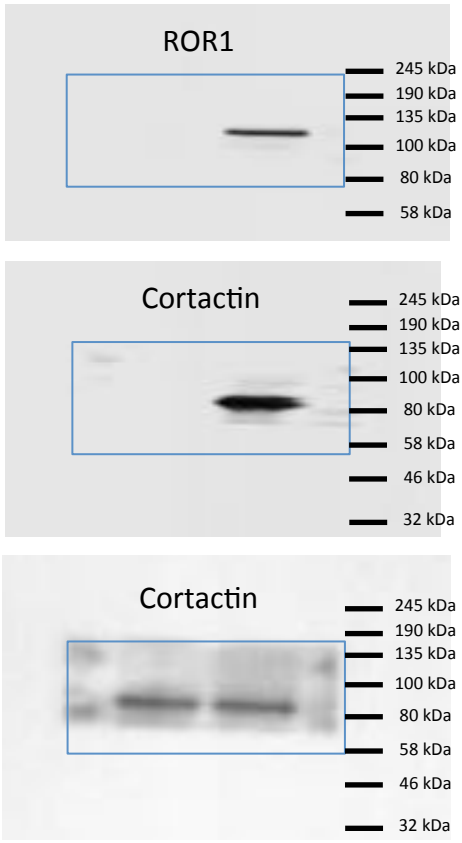
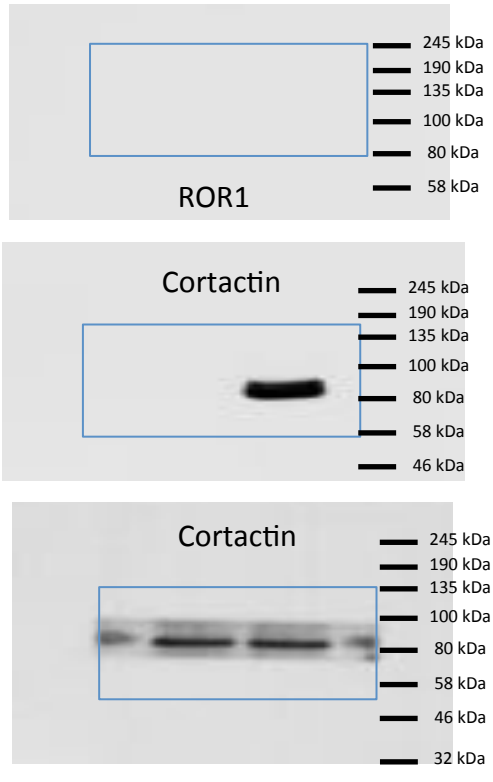
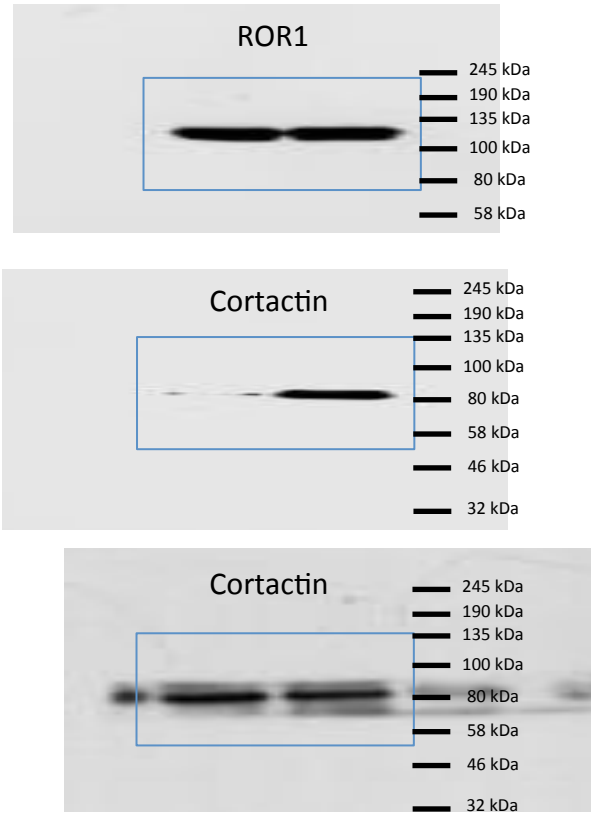


Supplementary Figure 5c



Supplementary Figure 5d



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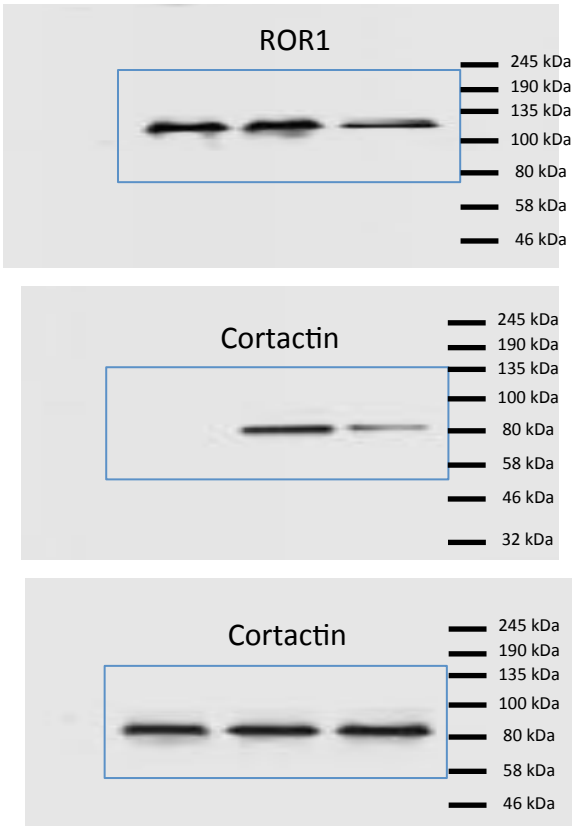
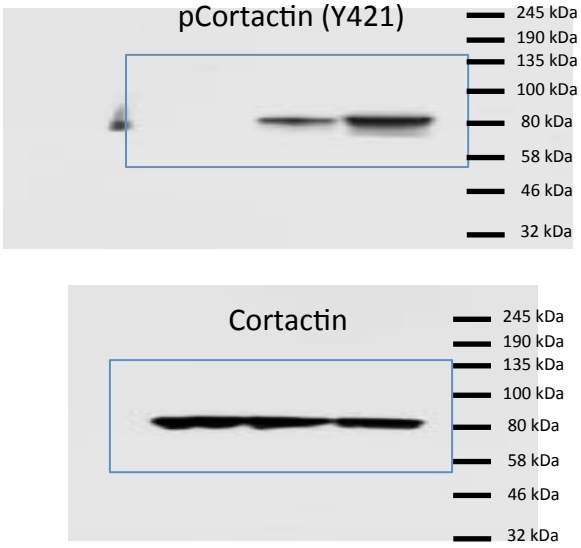
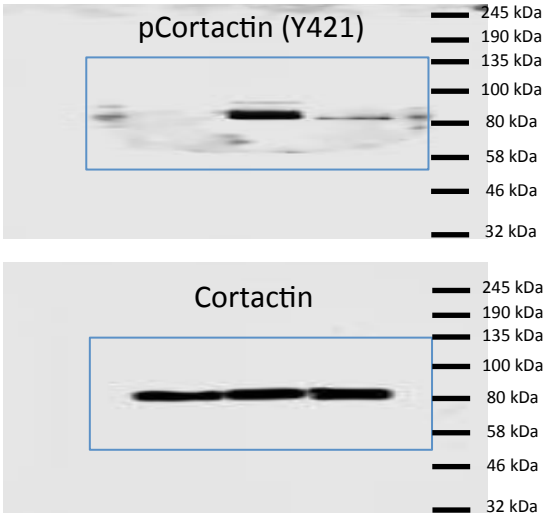


Supplementary Figure 6a



Supplementary Figure 6b



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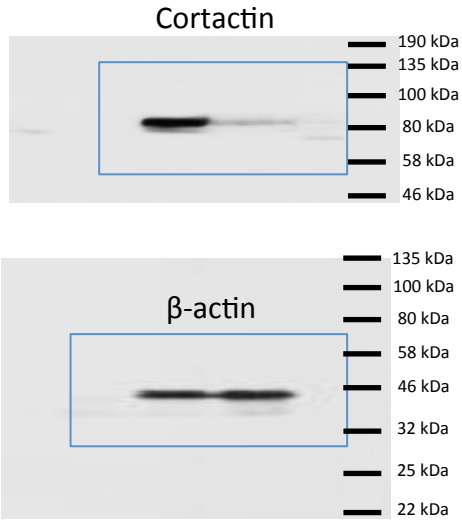
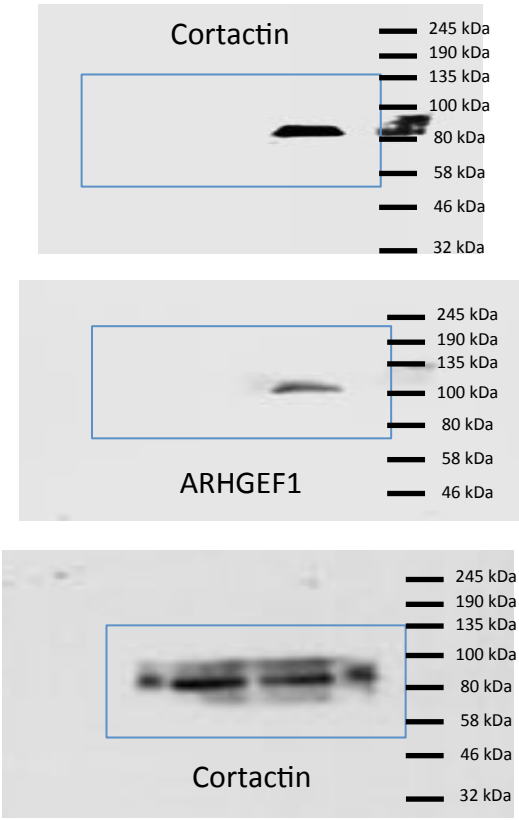


Supplementary Figure 9a



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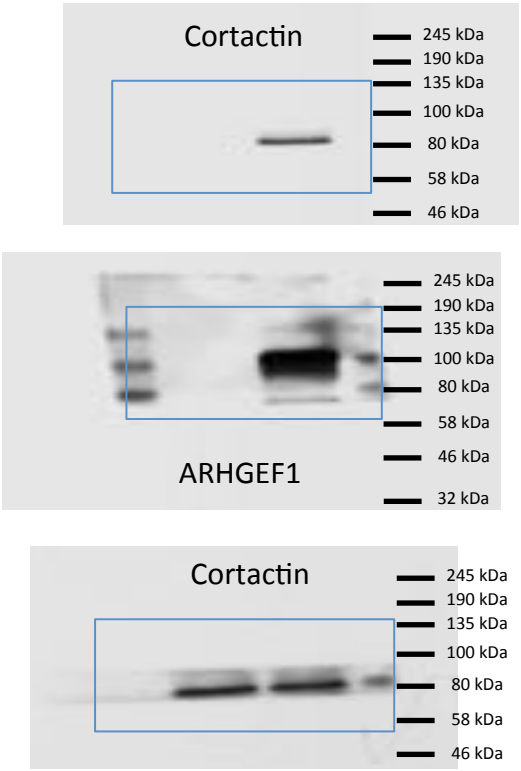

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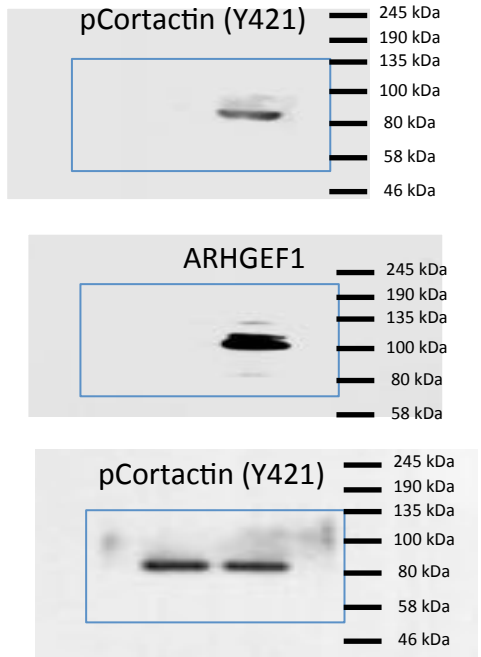

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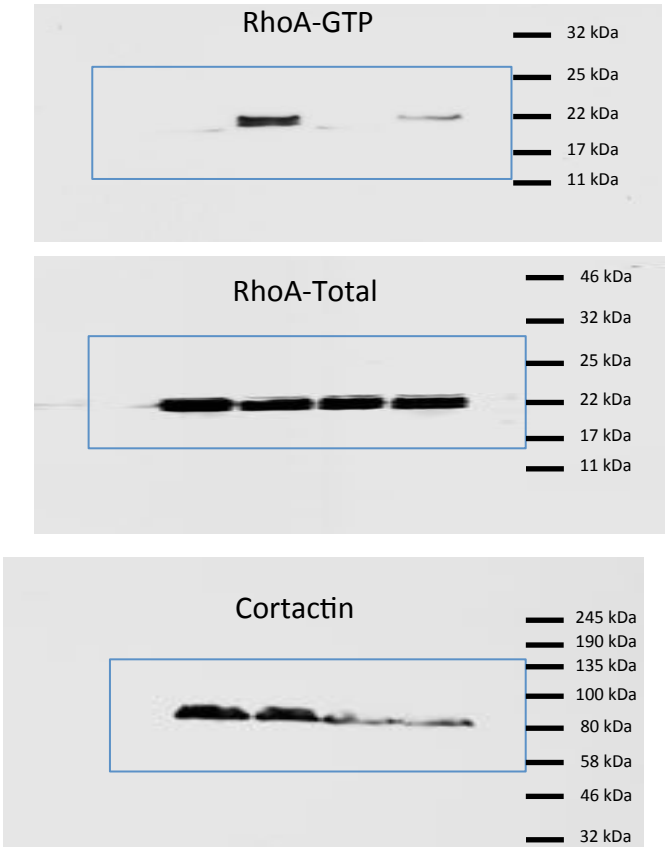
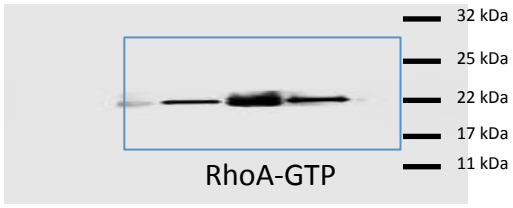


Supplementary Figure 9f



Supplementary Figure 10