Expanded View Figures

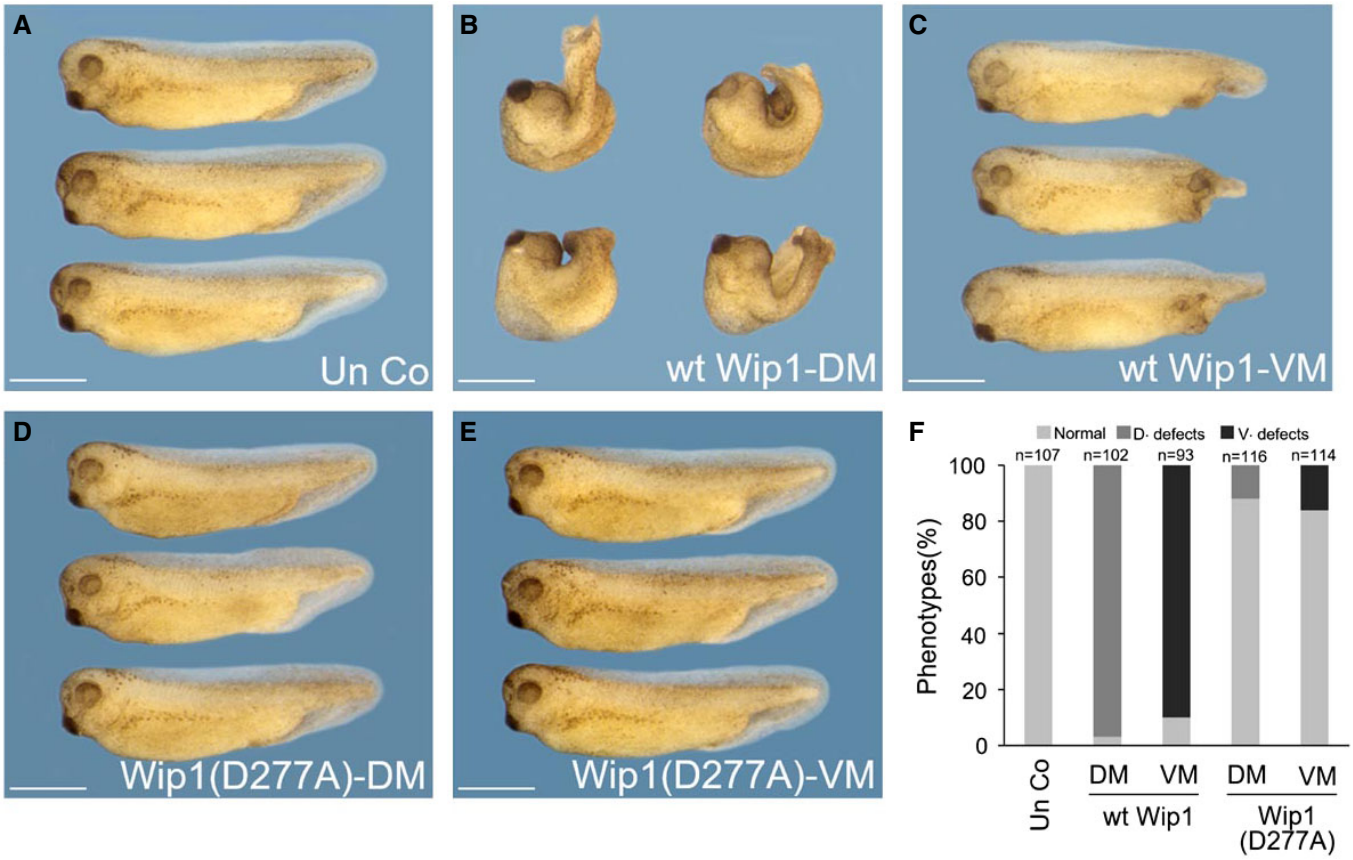

Figure EV1. Morphological phenotypes of embryos overexpressing *Wip1*.

A–E Four-cell stage embryos were injected in the dorsal or ventral marginal region of two blastomeres with wt *Wip1* (1 ng) or *Wip1* (*D277A*) (1 ng) mRNA and cultured to tadpole stages. Embryos are shown in lateral views with anterior to the left. Scale bar, 1 mm.

F Quantification of the phenotypes shown in (A–E). D. defects (dorsal defects) include shortened and kinked body axis, *spina bifida*, and gastrulation defects. V. defects (ventral defects) denote malformed posterior structures. DM, dorsal marginal zone; VM, ventral marginal zone.

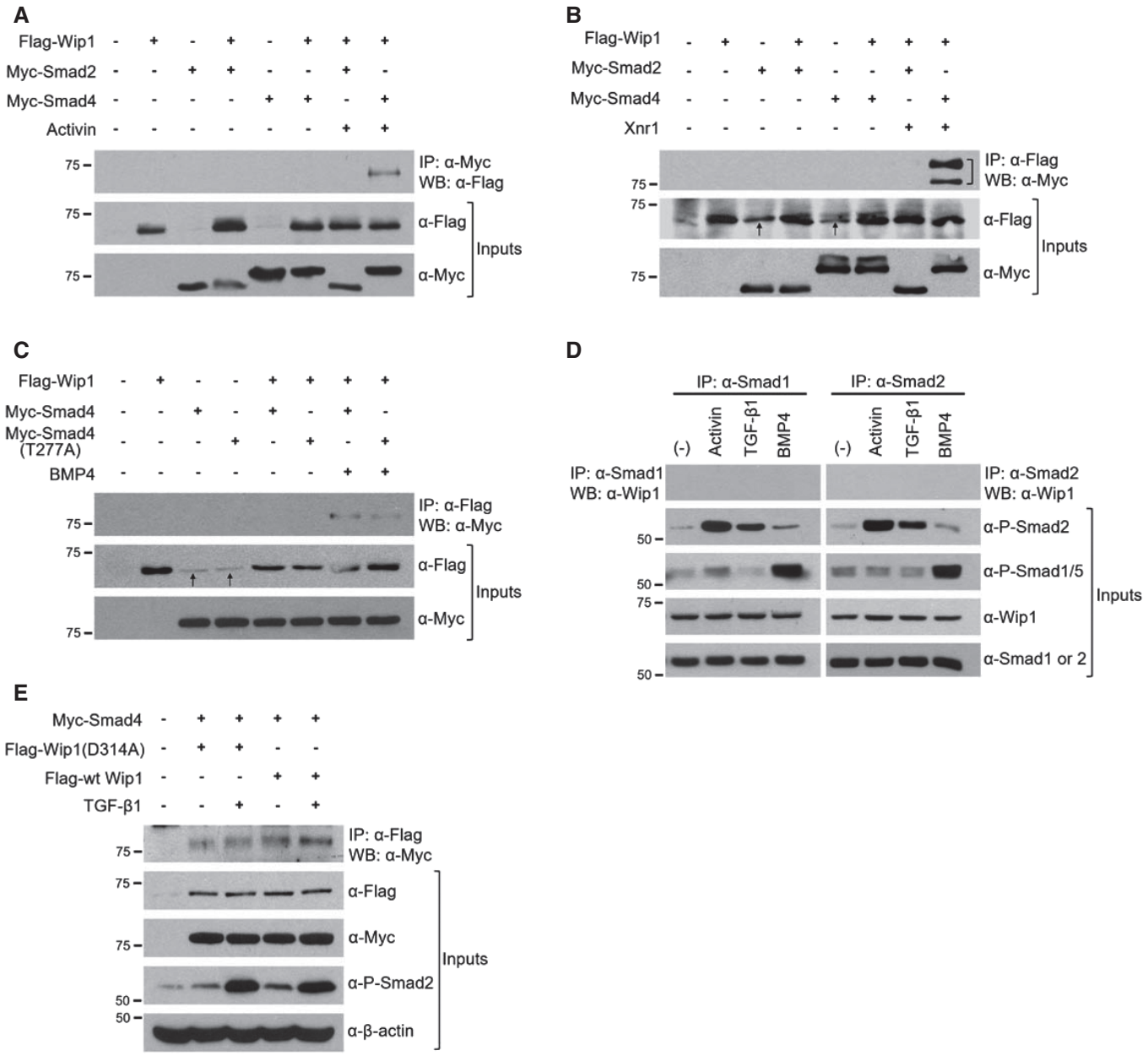

Figure EV2. Wip1 binds to Smad4 in a signal-dependent manner.

A–C Animal caps from embryos injected with *Flag-Wip1* (1 ng), *Myc-Smad4* (1 ng), *Myc-Smad4* (*T277A*) (1 ng), *Myc-Smad2* (1 ng), *Xnr1* (100 pg), and *BMP4* (200 pg) as indicated were cultured in the presence (A) or absence (B, C) of activin protein (10 ng/ml) and harvested for co-immunoprecipitation analysis. Note that Smad4 (T277A) mutant interacts with Wip1, albeit more weakly than wt Smad4, as shown in (C). A bracket in (B) denotes Myc-Smad4. Anti-Flag antibody often produced non-specific bands at the size of Flag-Wip1 protein as indicated by arrows in (B, C).

D No interaction between Wip1 and Smad1 or Smad2. The cell lysates were subjected to immunoprecipitation with anti-Smad1 or anti-Smad2 antibody and subsequent Western blotting with anti-Wip1 antibody.

E Phosphatase-inactive Wip1 mutant weakly binds to Smad4. HEK293T cells transfected with *Myc-Smad4*, *Flag-wt Wip1*, and phosphatase-dead *Flag-Wip1 (D314A)* as indicated were treated or not with TGF-β1 (20 ng/ml) for 1 h and subjected to immunoprecipitation followed by Western blotting.

Source data are available online for this figure.

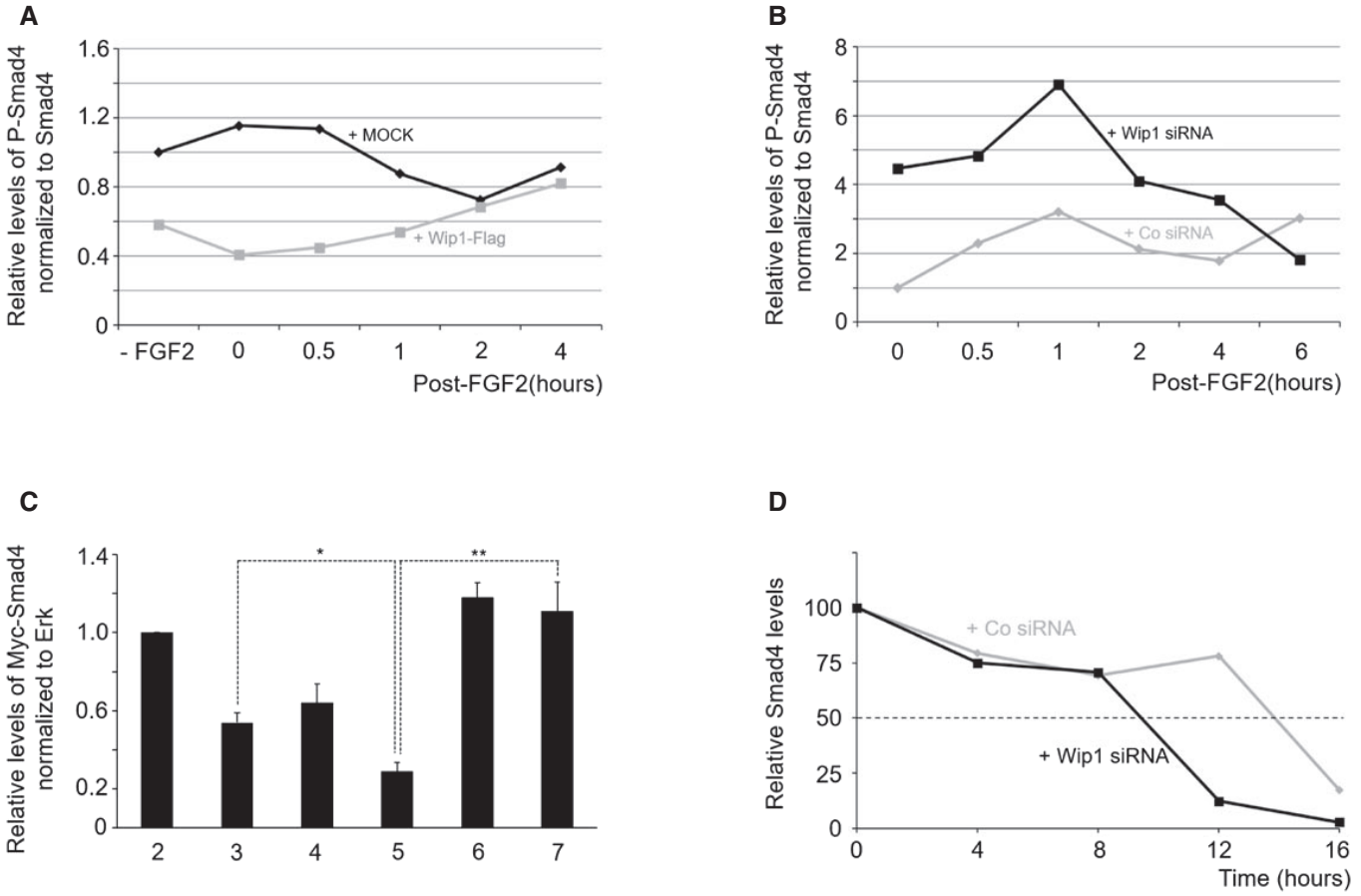

Figure EV3. Quantification of the results shown in Fig 6A, B, D and F.

A Quantification of the levels of phosphorylated Smad4 (normalized to Smad4) in Fig 6A.

B Quantification of Western blotting results shown in Fig 6B.

C Quantification of the levels of Myc-Smad4 (normalized to ERK) in Fig 6D. Data are expressed as the mean ± SEM (n = 3 biological replicates). *$P < 0.05$, **$P < 0.01$ by unpaired Student's t-test.

D Quantification of Western blotting results shown in Fig 6F.

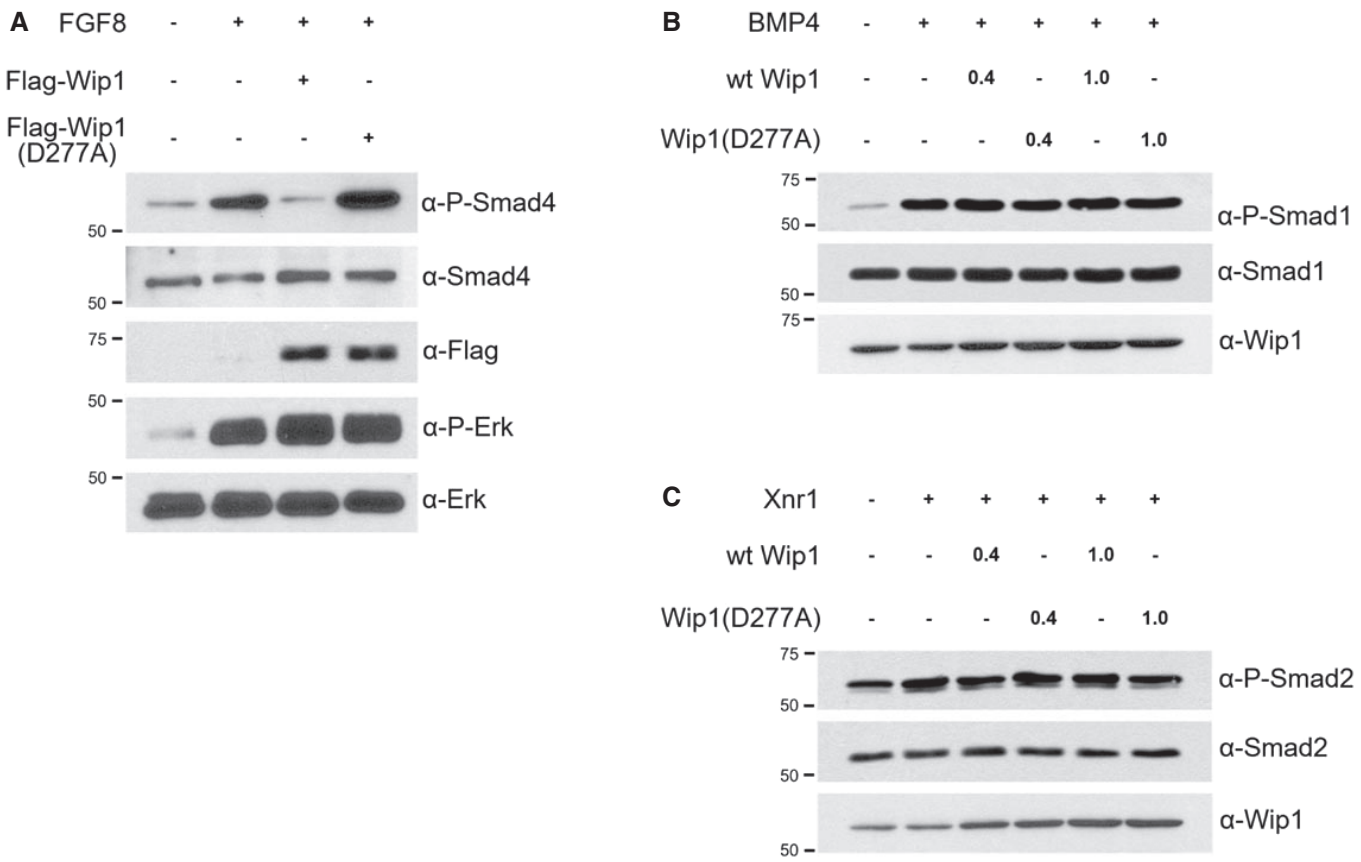

Figure EV4. Wip1 specifically dephosphorylates Smad4 in its linker region but not Smad1 and Smad2 at their C-termini.

A–C Animal caps from embryos injected with wt *Wip1* (0.4, 1 ng), *Wip1* (*D277A*) (0.4, 1 ng), *Flag-Wip1* (1 ng), *Flag-Wip1* (*D277A*) (1 ng), *Xnr1* (100 pg), *BMP4* (200 pg), and *FGF8b* (100 pg) as indicated were harvested at stage 15 (A) or 10.25 (B, C) for Western blotting analysis. Total Smad1, Smad2, and Erk serve as loading controls.

Source data are available online for this figure.

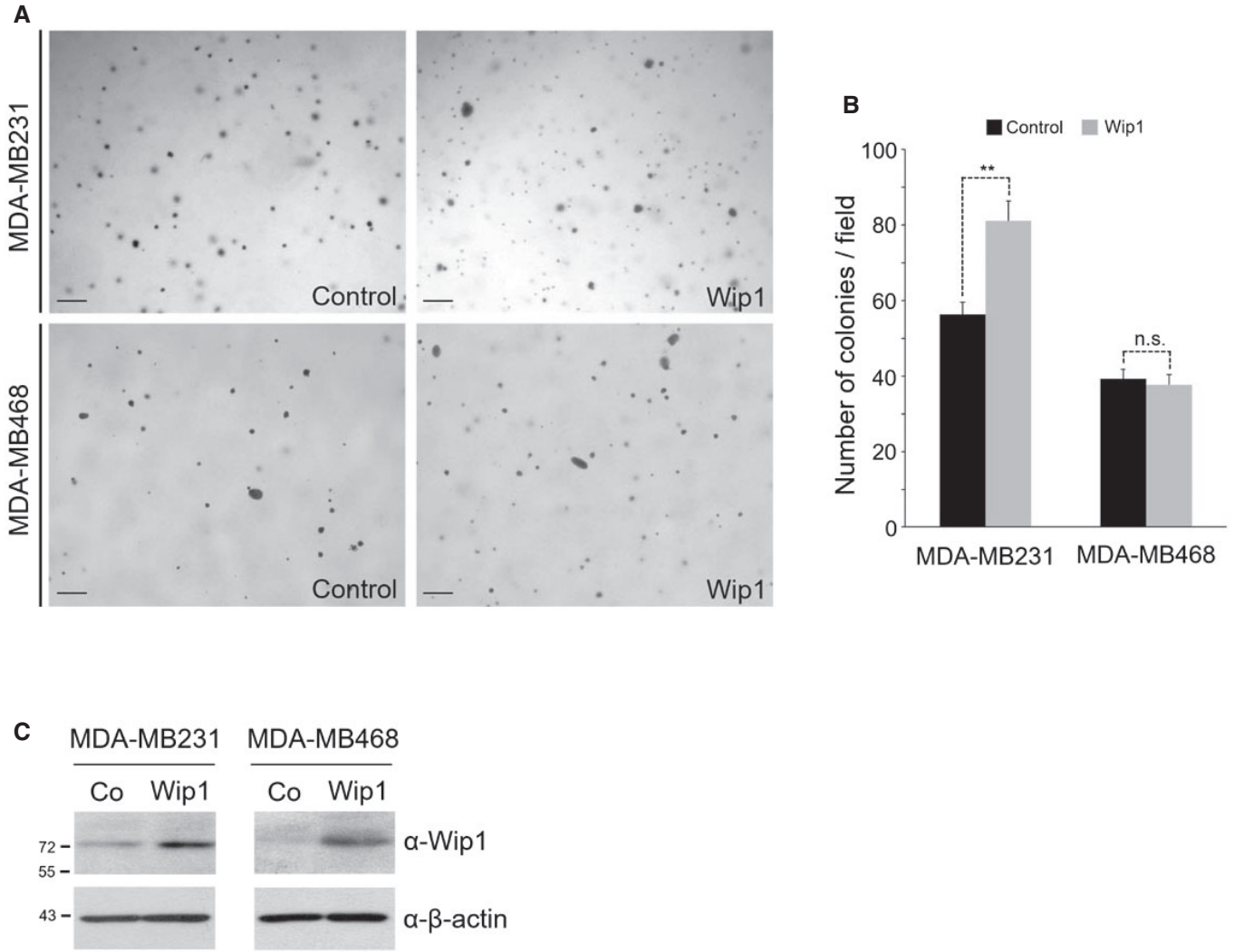

Figure EV5. Overexpression of Wip1 promotes the anchorage-independent growth of MDA-MB231 cells, but not of MDA-MB468 cells.

A Soft agar colony-formation assays were performed in MDA-MB231 and MDA-MB468 cells stably overexpressing Wip1 or negative control constructs. Scale bar, 200 μm.

B Quantitative analysis of colony formation shown in (A). Data are expressed as the mean ± SEM (n = 3 biological replicates). $^{**}P < 0.01$ by unpaired Student's t-test. n.s., not significant.

C Western blotting analysis of Wip1 expression in MDA-MB231 and MDA-MB468 cells stably overexpressing Wip1. Co, Vector control stable cell line; Wip1, Wip1 stable cell line.

Source data are available online for this figure.