# Supporting Information

## Kim et al. 10.1073/pnas.1103345108

### SI Methods

**Cell Culture and Antibodies.** MCF-7 cells obtained from ATCC were maintained in DMEM/F12 medium with 10% of FBS. MCF10A cells (a gift from Joan S. Brugge, Harvard Medical School, Boston, MA) were cultured in DMEM/F12 medium supplemented with 5% of horse serum, 0.5 μg/mL hydrocortisone, 100 ng/mL cholera toxin, 10 μg/mL insulin, and 20 ng/mL recombinant human EGF. Parental MDA-MB-231 cells and E-cadherin inducible MDA-MB-231 cell lines were maintained in DMEM with 10% FBS (1). Expression of full-length or mutant E-cadherin was induced by 2 μg/mL doxycycline for 2 d. Induction of E-cadherin expression was confirmed by Western blot. β-Catenin #3 siRNA in our previous study (2) was used to knockdown endogenous β-catenin. For knockdown of α-catenin, two different siRNAs (α-catenin siRNA #1, GUAAAGGGCCCUCUAAUAAUU; α-catenin siRNA #2, GAAGAGAGGUCGUUCUAAGUU) were used. Smart pool siGENOME duplexes (Dharmacon RNA Technologies) were used to knockdown the expression of other genes. siRNA duplex oligonucleotides were transfected using RNAiMax (Invitrogen). Antibodies used for immunofluorescence staining or Western blot include BrdU (Millipore; BU-1), β-catenin (BD), α-catenin (BD), E-cadherin (BD), Flag (Sigma; M2), Lats1 (Santa Cruz), Mst1/2 (Bethyl Laboratories), Phospho-YAP (Yes-associated protein) (S127, Cell Signaling), and YAP (Santa Cruz; 63.7).

**Immunofluorescence Staining.** For immunofluorescence staining, cells were cultured on fibronectin-coated coverslips. Cells were fixed with 4% paraformaldehyde in PBS for 15 min and washed three times for 5 min each in 100 mM glycine containing PBS, followed by permeabilization with 0.1% Triton X-100 in PBS for 10 min. After blocking in 2% BSA, 5% normal goat serum for 30 min, coverslips were incubated with primary antibody diluted in 2% BSA overnight at 4 °C. After washing with PBS, coverslips were incubated with Alexa Fluor 488- or 594-conjugated secondary antibodies for 1 h. For quantification of immunofluorescence staining, the BlobFinder software supplied by Olink (http://www.cb.uu.se/~amin/BlobFinder/) was used.


1. Wong AS, Gumbiner BM (2003) Adhesion-independent mechanism for suppression of tumor cell invasion by E-cadherin. *J Cell Biol* 161:1191–1203.

2. Perrais M, Chen X, Perez-Moreno M, Gumbiner BM (2007) E-cadherin homophilic ligation inhibits cell growth and epidermal growth factor receptor signaling independently of other cell interactions. *Mol Biol Cell* 18:2013–2025.


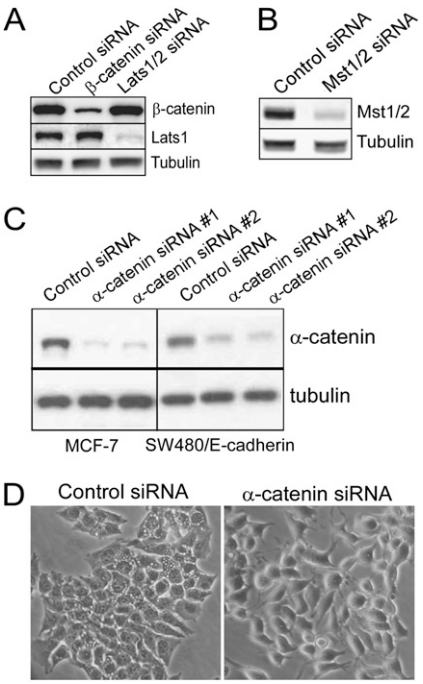


**Fig. S1.** Verification of protein knockdown by siRNA transfection. (*A*) Knockdown of β-catenin or Lats1 in MCF-7 cells was verified by Western blot. (*B*) Knockdown of Mst1/2 in MCF10A cells was verified by antibodies against Mst1/2. (*C*) Knockdown efficiency of α-catenin siRNAs were monitored by Western blot. (*D*) Depletion of α-catenin in MCF-7 cells disrupts cell–cell adhesion. (Magnification, 200×.)

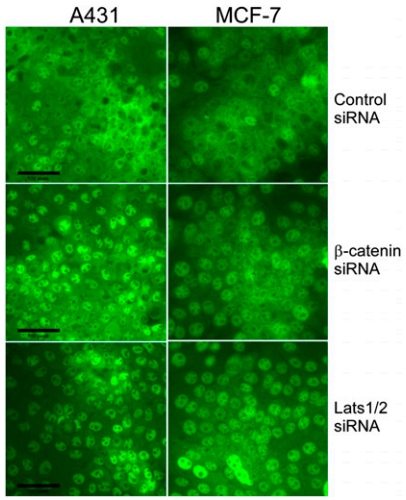


**Fig. S2.** Depletion of β-catenin or Lats1/2 leads to the nuclear accumulation of YAP in densely cultured A431 or MCF-7 cells. (Scale bars, 50 μm.)

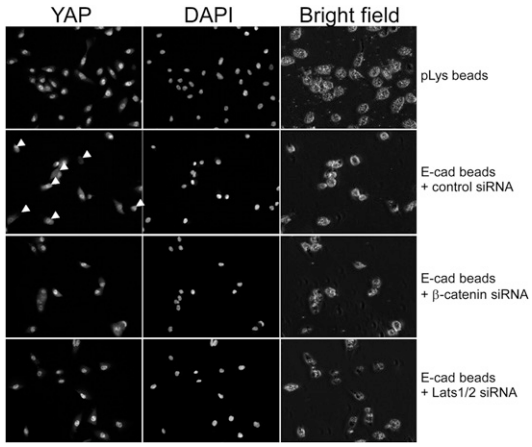


**Fig. S3.** E-cadherin homophilic ligation changes the subcellular localization of YAP. As represented by arrowheads, binding of E-cadherin-IgG Fc domain chimera (Fc-hE)-coated microspheres to the surface of MCF10A cells decreases the nuclear YAP relative to cytoplasmic YAP. Depletion of β-catenin or Lats1/2 siRNA in MCF10A cells, however, blocked this effect. The count of isolated cells with a higher ratio (>3) of nuclear to cytoplasmic YAP staining is represented in Fig. 4*B*. (Magnification, 100×.)