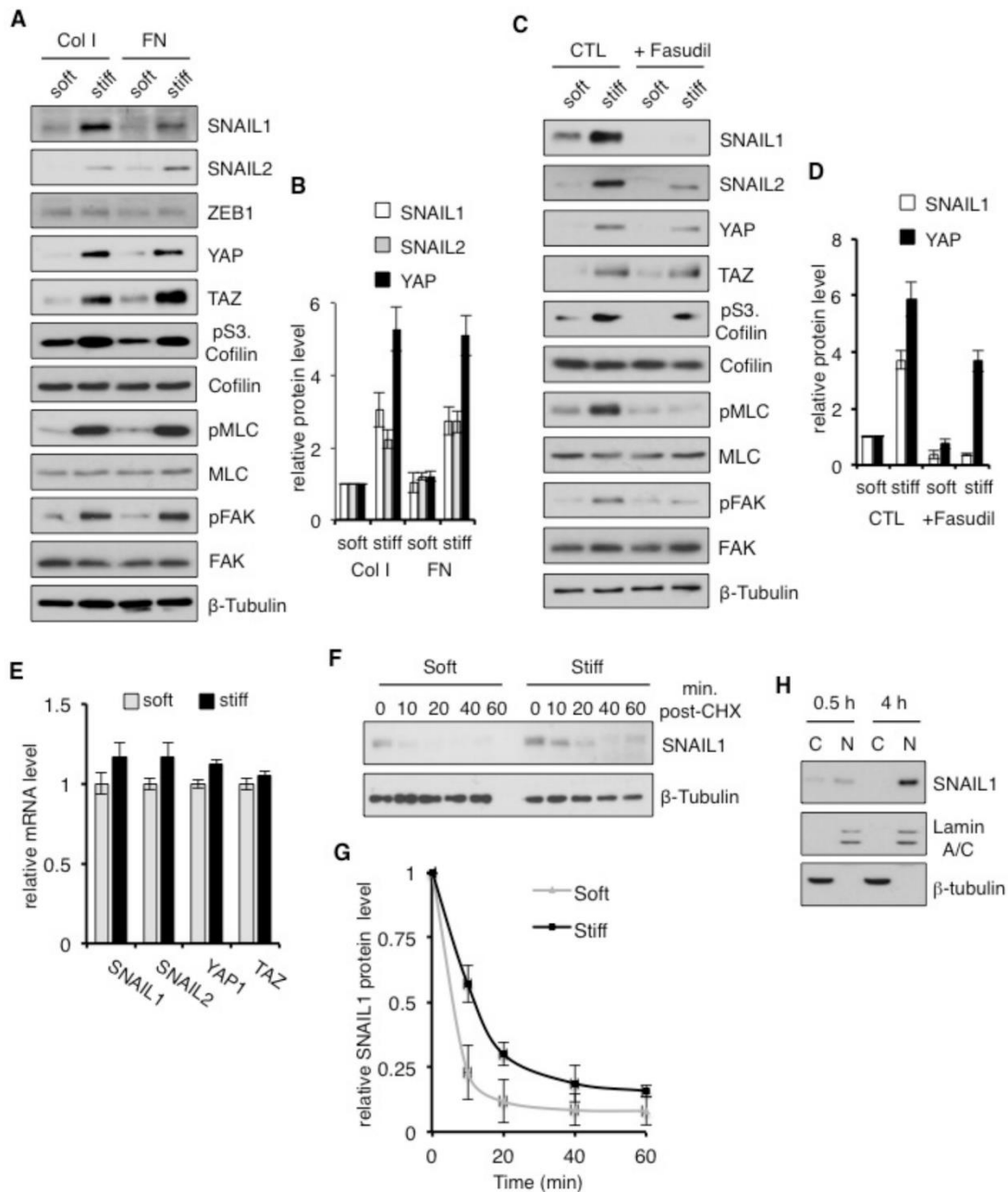


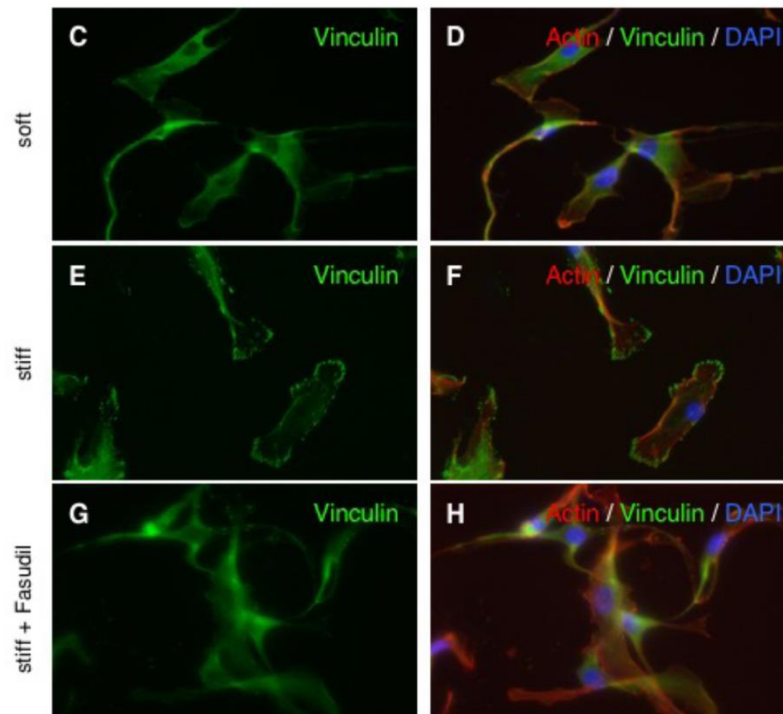
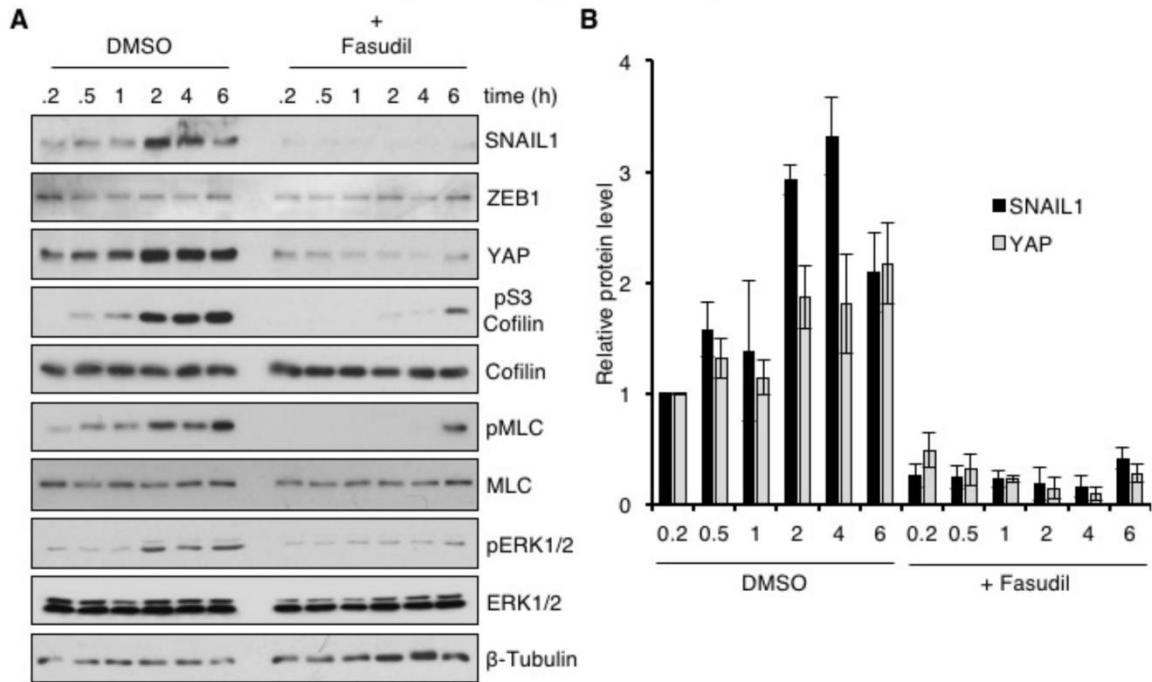
**Supplemental Figure1. The presence of ROCK1 or ROCK2 influences SNAIL1 protein levels in breast tumor cells. (A)** ROCK1 or ROCK2 were shRNAi-depleted (two different RNAi each) or CTL (scrambled shRNAi) in MDA-MB-231 human breast cancer cells. Western blots were performed on cell extracts with the indicated antibodies. **(B)**

Quantification of SNAI1, SNAIL2, and YAP relative protein levels from experiments in (A). CTL values were arbitrarily set = 1. (C) Q-PCR analysis for level of indicated mRNAs in MDA-MB-231 tumor cells or CAFs transduced with CTL (scrambled) (black columns), or ROCK1/2 shRNAi expressing lentiviruses (gray columns). Results are presented as mRNA levels relative to control cells, in which mRNA level was set as 1. (D) Human MDA-MB-231 breast cancer cells were treated with Fasudil or Y27632 at the indicated concentration and for the indicated times. Cell extracts were then Western blotted with the indicated antibodies. This experiment was repeated twice and a representative result shown (E) Quantification of relative level of SNAIL1 protein level in cells described in (D). Protein level in untreated cells was arbitrarily set = 1.



**Supplemental Figure 2. Mechanical signals increase SNAIL1 protein level in breast tumor cells, post-transcriptionally** (A) Human invasive breast tumor cells MDA-MB-231 were cultured on soft (80-120 Pa) or stiff (120 kPa) polyacrylamide hydrogels coated with Collagen I or Fibronectin for 12 hours. Western blots of cell extracts were performed with the indicated antibodies. (B) Quantification of relative SNAIL1, SNAIL2, and YAP protein level in experiment in (A). Amounts in cells on soft substrate were arbitrarily set = 1. (C)

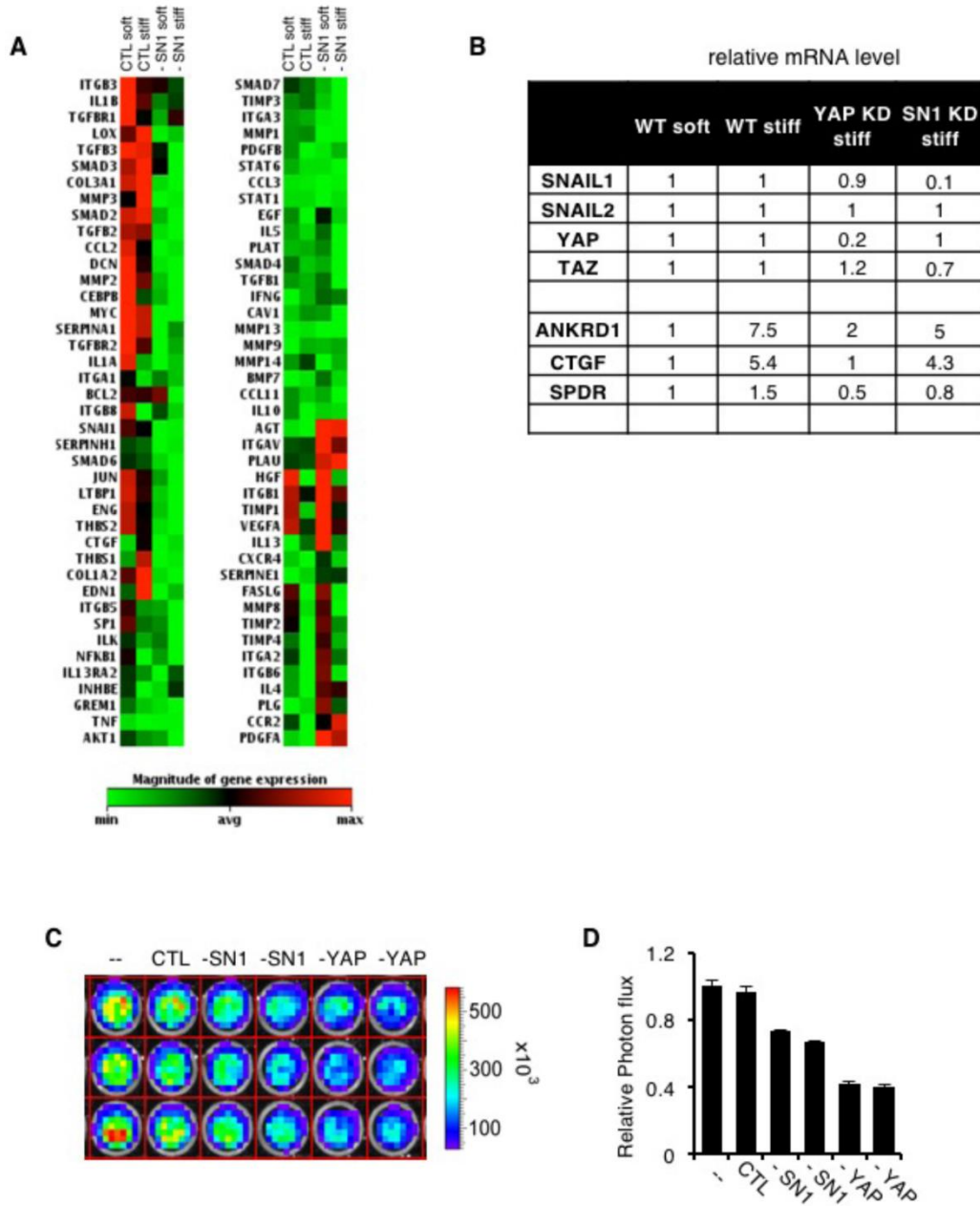
Human breast tumor cells MDA-MB-231 were cultured on fibronectin-coated soft (80-120 Pa) or stiff (120 kPa) polyacrylamide hydrogels coated for 12 hours in the presence or absence (CTL) of the ROCK inhibitor Fasudil (10  $\mu$ M). Western blots of cell extracts were performed with the indicated antibodies. **(D)** Quantification of relative SNAIL1 and YAP protein level in experiment in (C). Amount in cells on soft substrate were arbitrarily set = 1. **(E)** Q-PCR determination of relative SNAIL1, SNAIL2, YAP and TAZ mRNA level in MDA-MB-231 cells cultured on Fibronectin coated soft (80-120 Pa) or stiff (120 kPa) hydrogels. **(F)** Human breast tumor CAFs were treated with cycloheximide (100  $\mu$ g/ml) and then plated on soft or stiff FN-coated polyacrylamide hydrogels for increasing times, cells lysed, and Western blots performed with the indicated antibodies. **(G)** Quantification of relative SNAIL1 protein level in each experimental setting in (F) was quantified and plotted as level relative to time = 0, which was arbitrarily set to equal 1. **(H)** CAFs were dissociated from tissue culture plates and maintained in suspension with gentle rocking for 30 minutes and then added to plastic tissue culture plates for 4 hours. Cells were lysed and nuclear (N) and cytosolic (C) fractions isolated. Western blots were performed on cell fraction extracts with the indicated antibodies. Lamin A/C served as a nuclear marker while  $\beta$ -tubulin served as a cytosolic marker.



**Supplemental Figure 3. Mechanical signals increase SNAIL1 protein level and focal adhesion size**

(A) CAFs were dissociated from tissue culture plates and maintained in suspension with

gentle rocking for 30 min with DMSO (CTL) or 10  $\mu$ M Fasudil, then added to plastic tissue culture plates. At the indicated times after addition to TC plates, cells were lysed and Western blots performed with the indicated antibodies. All experiments were performed 2-3 times and a representative example shown. Quantification is plotted as the mean  $\pm$  SD. **(B)** Quantification of the relative protein level of SNAIL1 and YAP in experiment described in **(A)**. Level of protein in control cells (+DMSO) at t=0 was arbitrarily set = 1. **(C-H)** CAFs were added to soft (80-120 Pa) **(C, D)** or stiff (120 kPa) **(E, F)** polyacrylamide hydrogels coated with Fibronectin, or stiff (120 kPa) polyacrylamide hydrogels coated with Fibronectin in the presence of Fasudil **(G, H)** for 8 hours. Immunofluorescence with Vinculin antibody to detect focal adhesions (green) **(C, E, G)**, phalloidin to detect actin fibers (red), and DAPI to identify nuclei (blue) was then performed. **(D, F, H)** are merged images.



**Supplemental Figure 4. SNAIL1 influences fibrogenic gene expression in CAFs when exposed to a stiff matrix.**

(A) Control and SNAIL1 RNAi-depleted CAFs were plated on soft or stiff FN-coated

polyacrylamide hydrogels for 12 hours. mRNA was isolated and analyzed on RT<sup>2</sup> Profiler™ PCR Array (Human Fibrosis) from Qiagen. Data were analyzed by web-based software (Qiagen) using the comparative cycle threshold method normalized to expression of housekeeping genes. **(B)** Q-PCR analysis for YAP-regulated gene expression (ANKRD1, CTGF, SPDR) in control (CTL), SNAIL1-depleted, or YAP-depleted CAFs exposed to soft or stiff FN-coated polyacrylamide hydrogels for 12 hours. **(C)** STBS-Luc activity (YAP/TEAD activity) was determined in human CAFs cells following SNAIL1, YAP, or control scrambled shRNAi infection. **(D)** Relative photon flux was calculated. Control cells were arbitrarily set to 1. All experiments were performed 2-3 times and a representative example shown. Quantification is plotted as the mean  $\pm$  SD.



**Table S1.**

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**Table S2.**

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**Table S3.**

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