Supplemental Figures

Figure S1

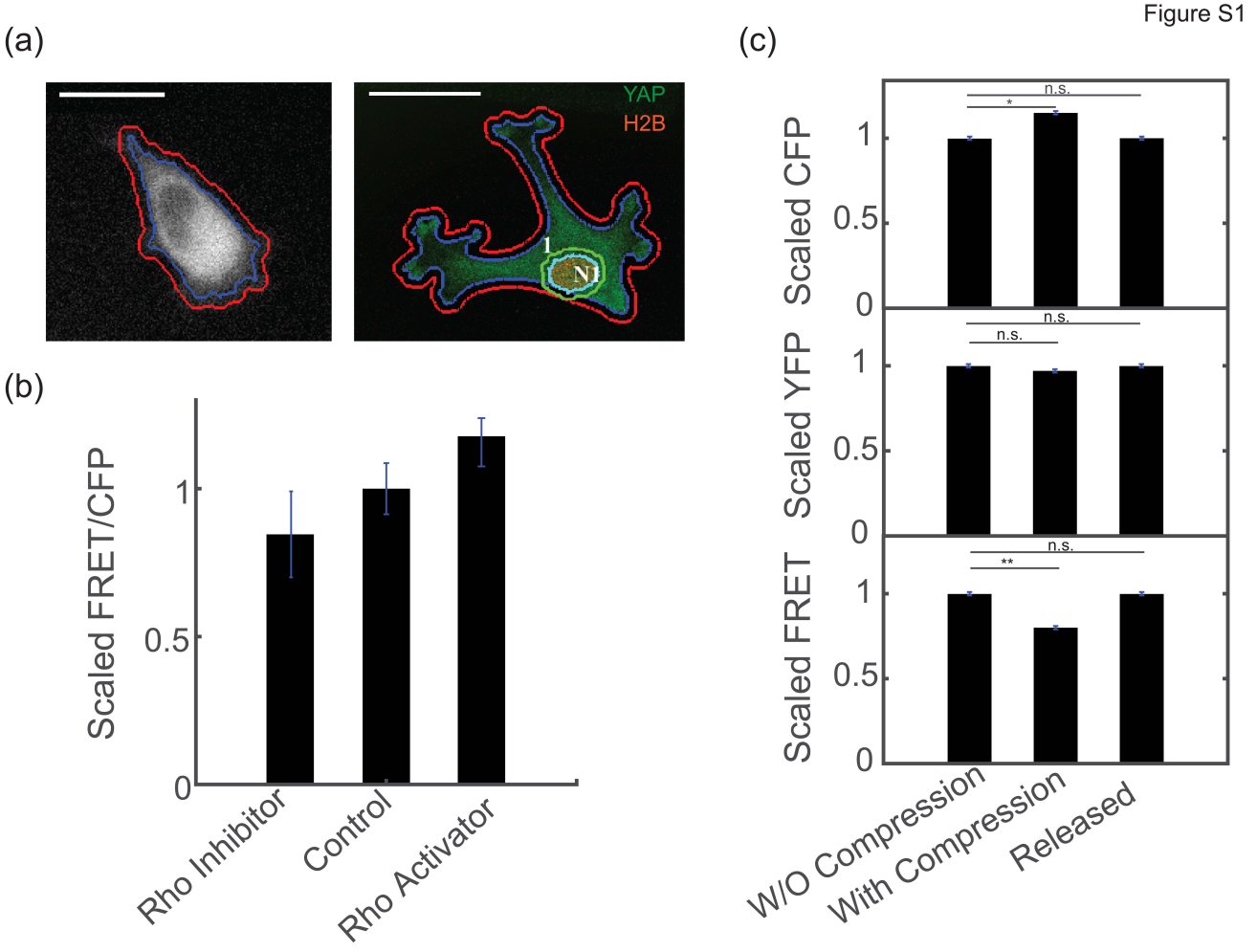

Figure S1: (a) Computational tracing of cell boundary from fluorescence images of cells stably express FRET sensor (YFP Channel) and fix and stain images; **(b)** HT1080 cells stably expressing RhoA FRET sensor were treated with 1 unit/ml Rho activator (Cytoskeleton, CN01) for 30 minutes or 2 μg/ml inhibitor (Cytoskeleton, CT04) for 4 hours, respectively. RhoA inhibitor and activator significantly increases or decreases the activity of the RhoA sensor after respective treatment. **(c)** Intensity changes in three fluorescence channels when cell is under compression: fluorescence intensities are scaled with precompression intensity value. The overall CFP intensity increased by 10 to 15% when compression is applied; overall YFP intensity remains more or less a constant (within 1% variation); while FRET intensity decreases about 15 to 20 % when compression took place. (Scale Bar = 20 micrometer; P Values: *P<0.05; ** P<0.01. Number of cells and biological and technical repeats are consistent with description in Fig. 1 in the main text. Error bar stands for standard error).

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Figure S2

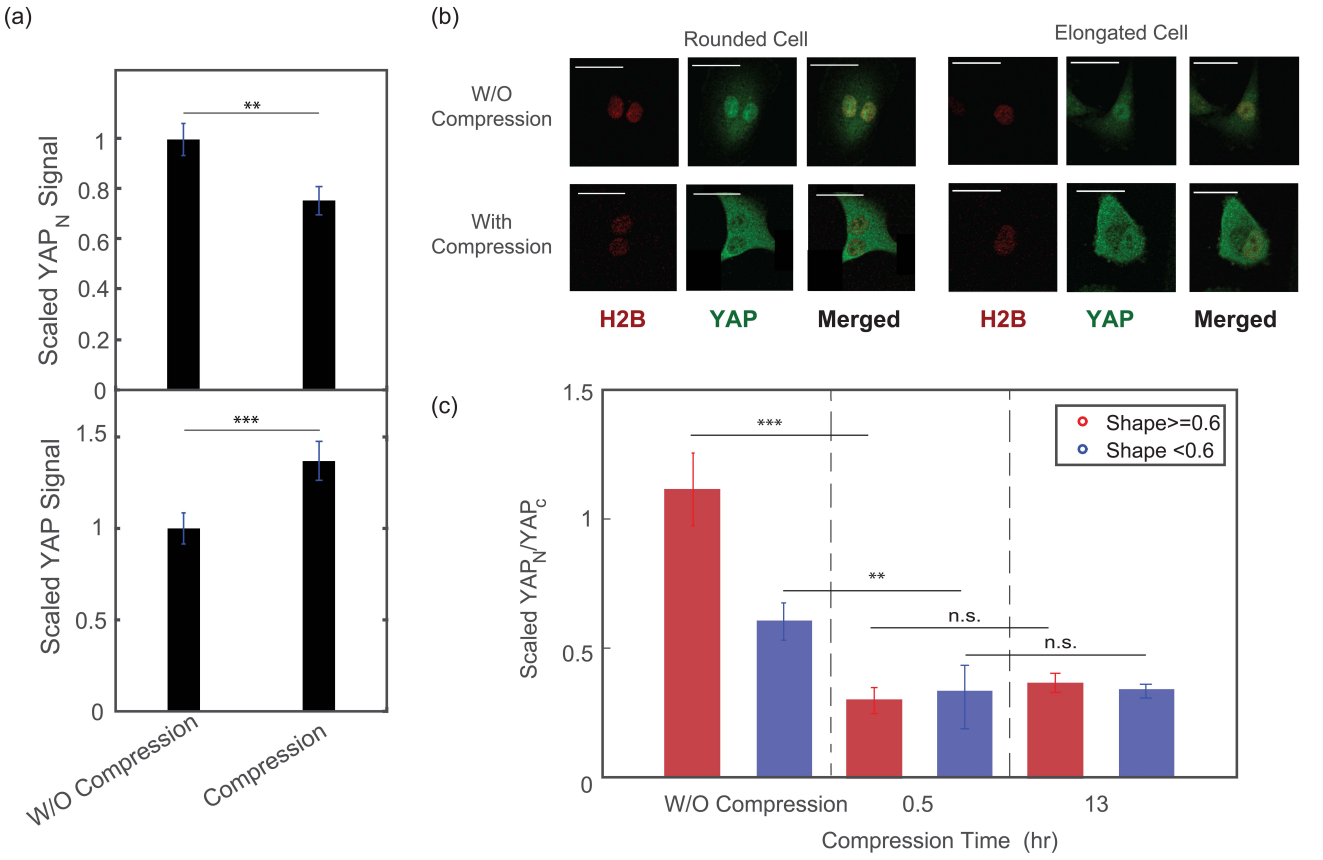

Figure S2: YAP/TAZ expression within a cell with and without compression and the adhesion shape dependence of mechanosensation of YAP. (a) YAP/TAZ transcription factor is labeled using immunofluorescence with and without compression. When the cell is under compression, the nuclear portion of YAP decreases, but the total YAP expression significantly increases**. (b)** Examples of rounded cell and elongated cell before and after compression; **(c)** Ratio of nuclear vs. cytoplasmic YAP in response to mechanical compression. For elongated cells, YAP is less sensitive to mechanical compression. (P values: ** P<0.01, *** P<0.001. Scale Bar = 20 micrometer. Here, the shape factor is defined as: $\frac{4\pi \times \text{Adhesion Area}}{\text{Adhesion Perimeter}^2}$; cells are more rounded if the shape factor is closer to 1 and are more elongated if the shape factor is closer to zero. Number of cells and biological and technical repeats are consistant with the description at Fig. 5. Error bar stands for standard error.)

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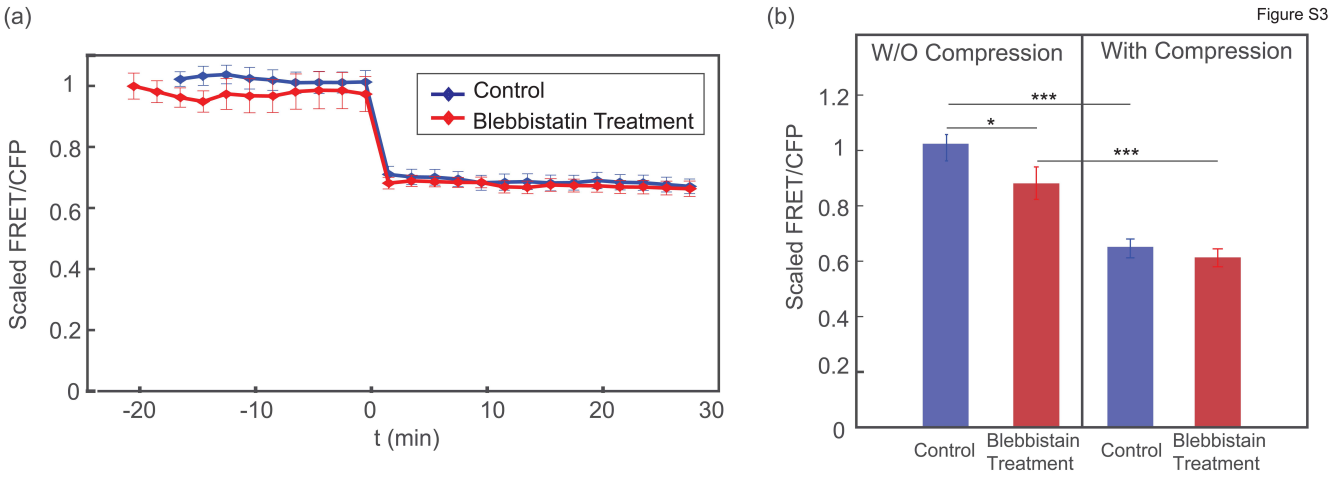

Figure S3: Blebbistatin treatment does not significantly affect mechanosensation of RhoA when cells are subject to mechanical compression. HT1080 cells stably expressing RhoA FRET sensor was incubated in 25 μM blebbistatin for one hour before imaging took place. **(a)** Overall FRET/CFP ratio over time. Compression takes place at t = 0 min. **(b)** Time average FRET/CFP in terms of mechanical compression. Before compression, blebbistatin does slightly decrease overall RhoA activity, but it does not affect the change in RhoA when compression takes place. (P Values: *P <0.01*** P<0.000 001 . n = 34 cells for control data and n = 28 cells for blebbistatin data. Biological Repeats = 3. For each biological repeat, technical repeat = 1)

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