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Supplementary Materials for

Microtubules tune mechanotransduction through NOX2 and TRPV4 to decrease sclerostin abundance in osteocytes

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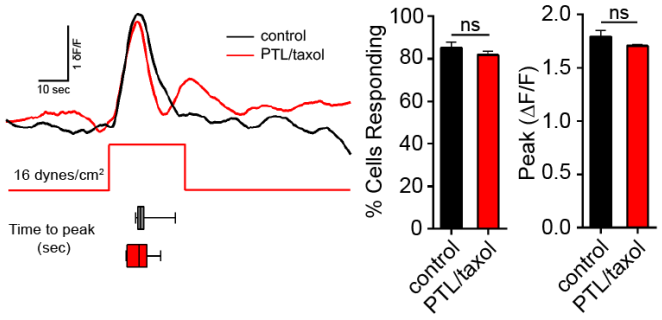

Fig. S1. Control and PTL/Taxol-treated Ocy454 cells show indistinguishable FSS-induced Ca^{2+} responses at 16 dynes/cm^2. FSS-induced Ca^{2+} response of Fluo-4 loaded Ocy454 cells treated with combination of PTL and Taxol, and subjected to 16 dynes/cm^2 FSS. Ca^{2+} traces are aggregated from >200 cells per treatment from n=3 independent experiments. Data were analyzed by a two tailed Mann-Whitney test. ns, not significantly different.

Figure 6a - AFM

Comparison	P value
control vs. taxol	P=0.0019
control vs. PTL	P<0.0001
control vs. PTL/taxol	P=0.0026
taxol vs. PTL	P<0.0001
taxol vs. PTL/taxol	P<0.0001
PTL vs. PTL/taxol	P=0.0189

Figure 6b - western blot

Comparison	P value
control vs. taxol	P=0.0466
control vs. PTL	P=0.0014
control vs. PTL/taxol	P=0.0113
taxol vs. PTL	P=0.0004
taxol vs. PTL/taxol	P=0.0017
PTL vs. PTL/taxol	P=0.0320

Fig. S2. Statistical significance of treatment groups in Fig. 6. (**a**) p values of indicated comparisons of different groups in Fig 5D. (**b**) p values of indicated comparisons of different groups in Fig 5E.

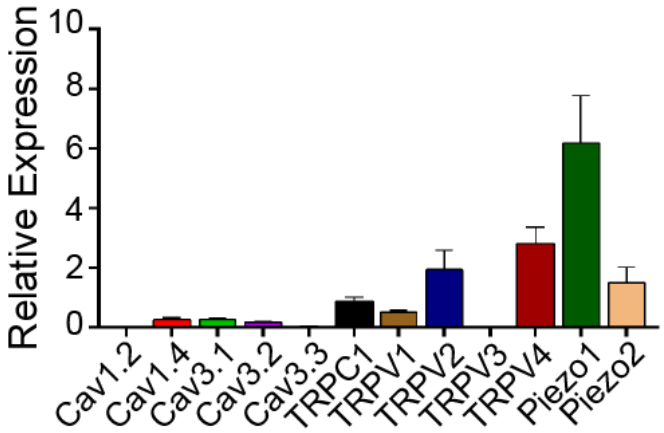

Fig. S3. The Ca^{2+} channel TRPV4 is abundant at the mRNA level in Ocy454 cells. Ca^{2+} channel expression was analyzed in Ocy454 cells by qRT-PCR. Graphs show mean ± sem. n=6 biological replicates.

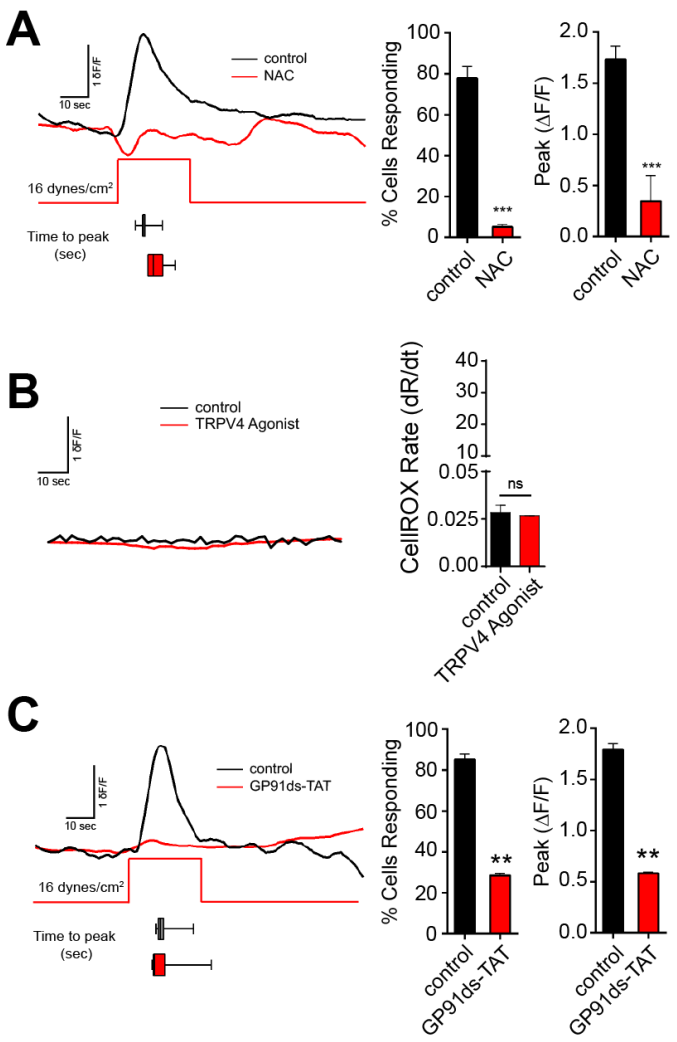

Fig. S4. Increased FSS does not rescue FSS-induced Ca^{2+} influx in Ocy454 cells treated with α-NAC or GP91ds-TAT, and TRPV4 activation does not affect ROS production. (**A**) Ca^{2+} response of Fluo-4 loaded Ocy454 cells treated with α-N-acetyl cysteine (NAC) and subjected to 16 dynes/cm^2 FSS. Ca^{2+} traces are aggregated data from >200 cells per treatment from n=3 independent experiments. (**B**) ROS response in Ocy454 cells treated with or without (control) the TRPV4 agonist GSK-1016790A, loaded with CellROX and subjected to 4 dynes/cm^2 FSS. ROS traces are aggregated data from >200 cells per treatment from n=3 independent experiments. (**C**) Ca^{2+} response of Fluo-4 loaded Ocy454 cells treated with GP91ds-TAT and subjected to 16 dynes/cm^2 FSS. Ca^{2+} traces are aggregated data from >200 cells per treatment from n=3 independent experiments. Ca^{2+} data for controls at 16 dynes/cm^2 FSS are same traces shown in Fig 2D and Supplementary Fig 1, respectively, because these data were collected in parallel to the indicated interventions. Graphs show mean ± sem. ** p<0.001, *** p<0.0001 compared to control by two tailed Mann-Whitney test. ns, not significantly different.