Supplemental material

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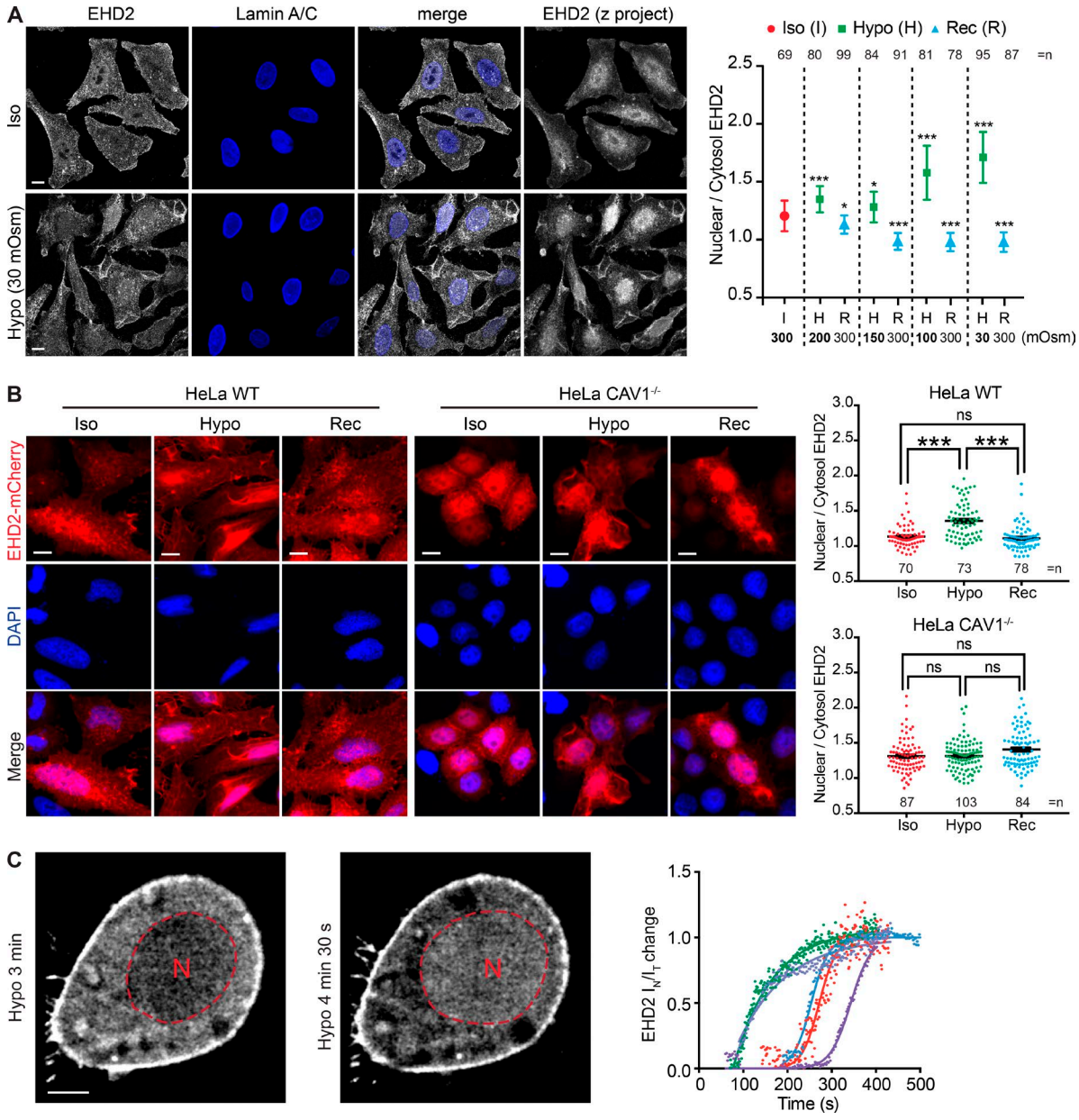


Figure S1. **Mechanical stress induces EHD2 nuclear translocation. (A)** Representative confocal images (left) and quantification (right) of the nuclear translocation of endogenous EHD2 in HeLa cells under resting (Iso), under hypo-osmotic shocks of decreasing osmolarity (Hypo), and 5 min after return to iso-osmotic conditions (Rec). Ratios are compared with Iso condition; *n* = 3 independent experiments; data are mean ± SD. **(B)** Representative wide-field images (left) and quantification (right) of EHD2-mCherry nuclear translocation in HeLa WT but not in HeLa Cav1$^{-/-}$ cells under Iso, after 5 min of 30 mOsm hypo-osmotic shock (Hypo), and Rec; *n* = 3 independent experiments; data are mean ± SEM. **(C)** Representative lattice light sheet microscopy images (left) and quantification of EHD2 nuclear (I_N)/total cellular (I_T) signal ratio (right, *n* = 5 cells) illustrating EHD2 nuclear translocation in EHD2-mEmerald–expressing HeLa cells during the indicated times of hypo-osmotic shock; nucleus (N) is delineated by dashes. Scale bar = 10 µm; data are representative of three experiments; *, P < 0.05; ***, P < 0.001; in A, one-way ANOVA with Tukey's multiple comparisons test; in B, two-tailed *t* test; numbers of cells are indicated on the graphs.

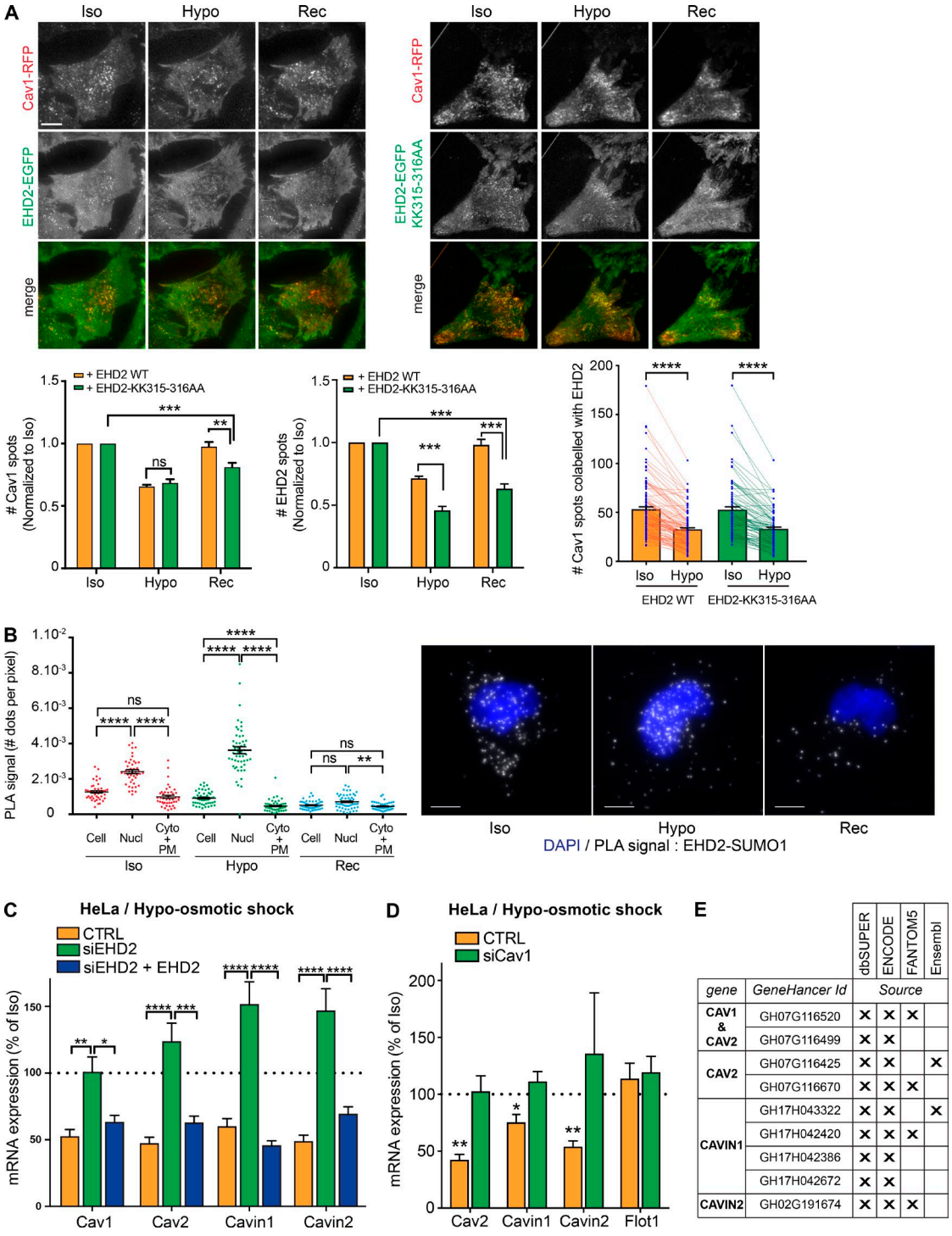


Figure S2. **EHD2 is SUMOylated by SUMO1 and controls gene transcription upon mechanical stress. (A)** Representative TIRF images and quantification of changes in Cav1 (left), EHD2 (middle), and Cav1/EHD2 (right) spot numbers at the cell surface of HeLa cells transfected with Cav1-RFP, EHD2-EGFP (n = 112), or EHD2-KK315-316AA-EGFP (n = 83) under resting (Iso), after 5 min of 30 mOsm hypo-osmotic shock (Hypo), and 5 min after return to iso-osmotic conditions (Rec). Scale bar = 10 µm. **(B)** Representative images (right) and quantification (left) of in situ PLA experiments monitoring the level of endogenous interaction between EHD2 and SUMO1 in the whole cell (Cell), the nucleus (Nucl), and the cell without the nucleus (Cyto + plasma membrane) of Hs578T cells under Iso (n = 44), Hypo (n = 51), and Rec (n = 51) conditions. **(C)** Quantification of Cav1, Cav2, cavin1, and cavin2 mRNA levels in HeLa cells transfected with control siRNA (CTRL) or siEHD2, harvested 1 h after 5 min of 30 mOsm hypo-osmotic shock. **(D)** Quantification of Cav2, cavin1, cavin2, and Flot1 mRNA levels under hypo-osmotic shock in HeLa cells depleted or not (CTRL) from Cav1, harvested 1 h after 5 min of 30 mOsm hypo-osmotic shock. **(E)** Data bank analysis indicating KLF7 binding to gene enhancers of different caveolae constituent genes. $n \geq 3$ independent experiments; mRNA levels are compared with resting conditions (dotted line); *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001; in A, two tailed t test; data are representative of two independent experiments; mean ± SEM; in B, data are representative of three independent experiments; ANOVA with Dunn's multiple comparisons test. In C and D, n = 3 (C) or n = 4 (D) independent experiments; Bonferroni's multiple comparison test; data are mean ± SEM.

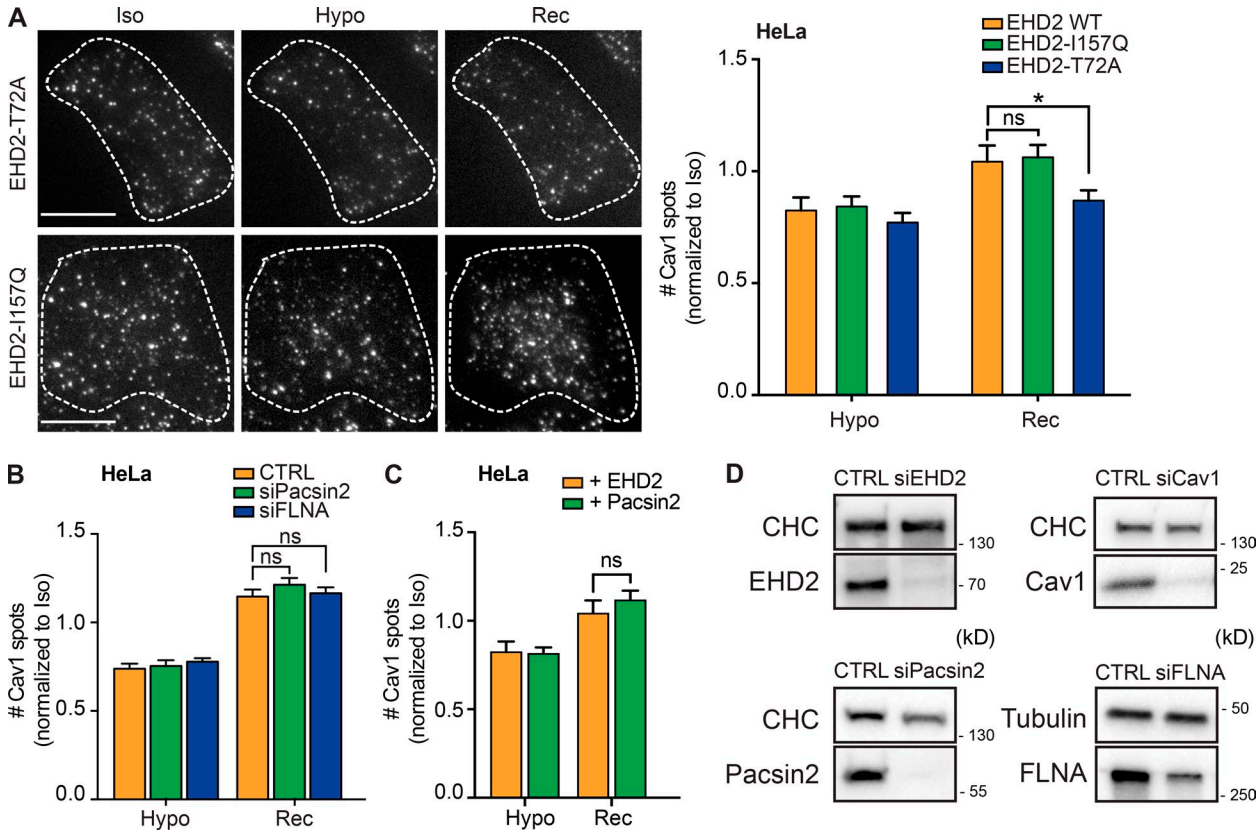
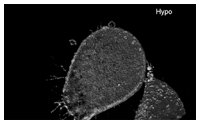


Figure S3. **EHD2 is required for the stabilization of caveolae during tension variations at the plasma membrane. (A)** Representative TIRF images (left) and quantification (right) showing changes of cell surface Cav1 spot numbers in HeLa cells expressing EHD2 WT (n = 28), EHD2-I157Q (n = 29), or EHD2-T72A (n = 30) under resting (Iso), under hypo-osmotic shock (Hypo), and 5 min after return to iso-osmotic conditions (Rec). Dashes delineate cells. Scale bar = 10 µm. **(B)** Quantification of cell surface Cav1 spot number changes observed by TIRF in HeLa cells depleted or not (CTRL, n = 42) for Pacsin2 (n = 37) or FilaminA (FLNA, n = 87) under Hypo and Rec conditions. **(C)** Quantification of Cav1 spot number changes observed by TIRF in HeLa cells overexpressing EHD2 (n = 28) or Pacsin2 (n = 24) under Iso, Hypo, and Rec conditions. **(D)** Representative Western blots showing the efficiency of EHD2, Cav1, Pacsin2, and FilaminA (FLNA) down-expression by siRNA in HeLa cell experiments. CHC or tubulin was used as loading control. For all panels, n = 3 independent experiments; *, P < 0.05; two tailed t test; data are mean ± SEM.



Video 1. **Hypo-osmotic stress results in rapid EHD2 nuclear translocation.** A HeLa cell expressing EHD2-mEmerald was imaged in hypo-osmotic medium (30 mOsm) at 37°C with lattice light sheet microscopy. EHD2 rapidly accumulated into the nucleus in response to hypo-osmotic shock.