Expanded View Figures

Figure EV1. YAP/TAZ regulate cell responses to hydrostatic pressure.

A PhosTag-based Western blot probed for YAP reveals no changes in YAP phosphorylation levels in HEK293A and 143B cells in response to static 500 mbar hydrostatic pressure (+) compared to control (−).
B Western blot showing LAT51 and LAT52 levels in WT and LAT51/2 DKO cells.
C Western blot showing NF2 expression level in WT and NF2 KO cells.
D Western blot showing TEAD expression level in WT and TEAD KO cells.
E Western blot showing YAP, TAZ and CYR61 levels in 143B YAP KO and TAZ KO cells relative to WT.
F 143B WT, YAP KO and TAZ KO response to 100 mbar cyclic hydrostatic pressure as measured by DHM. Each dot represents a single cell from three independent experiments and error bars represent mean ± 95% CI. Mann-Whitney U test. ***P < 0.001 (WT vs. YAP KO), ***P = 0.006 (WT vs. TAZ KO).
G Steady-state volume of Y/T DKO relative to WT YAP and S94A mutant YAP re-expressing Y/T DKO cells as measured by DHM. Each dot represents a single cell from three independent experiments and error bars represent 95% CI. Kruskal–Wallis test with Dunn’s post-hoc. P > 0.999 (vector vs. WT YAP), P = 0.2173 (vector vs. S94A YAP) and, P = 0.9837 (WT YAP vs. S94A YAP).
H Western blot showing total YAP, TAZ and CYR61 levels in WT TAZ re-expressing cells compared to WT and Y/T DKO vector control.
I Steady-state cell volume WT TAZ re-expressing cells relative to Y/T DKO as imaged and analysed by DHM. Each dot represents a single cell from three independent experiments. Error bars represent mean ± 95% CI. Mann-Whitney U test. ***P < 0.001.
J Average change in cell volume in response to hydrostatic pressure in WT TAZ re-expressing cells relative to Y/T DKO as imaged and analysed by DHM. Each dot represents a single cell from three independent experiments. Error bars represent mean ± 95% CI. Mann-Whitney U test. ***P < 0.001.
K Candidate genes conferring YAP/TAZ-mediated cellular response to hydrostatic pressure. Gene expression levels of HEK293A WT cells in response to 0.1 Hz, 200 mbar cyclic hydrostatic pressure (4 h) compared with steady-state levels within each genotype and analysed by RT-qPCR. Graphs show data obtained from four independent experiments. Error bars represent mean ± SD. Kruskal–Wallis test with Dunn’s post-hoc.
L Gene expression level induced by hydrostatic pressure in Y/T DKO#1 cells normalized to WT levels. Graphs show data obtained from four independent experiments. Error bars represent mean ± SD. Kruskal–Wallis test with Dunn’s post-hoc.
M Gene expression level induced by hydrostatic pressure in Y/T DKO#2 cells normalized to WT levels. Graphs show data obtained from four independent experiments. Error bars represent mean ± SD. Kruskal–Wallis test with Dunn’s post-hoc.

Source data are available online for this figure.
Figure EV1.
Figure EV2. Cytoskeletal implications in the cellular response to hydrostatic pressure.

A Average change in cell volume in response to 100 mbar cyclic hydrostatic pressure in WT and LATS1/2 DKO clones. Each dot represents a single cell. Data pooled from three independent experiments. Error bars represent mean ± 95% CI. Kruskal–Wallis test with Dunn’s post-hoc. \( P > 0.9999 \) (for all comparisons).

B Average change in cell volume in response to 100 mbar cyclic hydrostatic pressure in WT and MST1/2 DKO. Each dot represents a single cell. Data pooled from three independent experiments. Error bars represent mean ± 95% CI. Mann–Whitney test. \( P = 0.0781 \).

C HEK293A NF2 KO steady-state volume compared to WT cells. Each dot represents a single cell and Error bars represent mean ± 95% CI. Data pooled from three independent experiments. Mann–Whitney U test. *** \( P < 0.001 \).

D PhosTag-based Western blot probing for YAP reveals dephosphorylation of YAP in response to 0.5 \( \mu \)M latrunculin B (LB) and 2 \( \mu \)M cytochalasin D (CD) treatment in WT cells.

E Comparison of average change in cell volume measured by DHM in response to 100 mbar cyclic hydrostatic pressure with 2 \( \mu \)M latrunculin B treatment in WT, Y/T DKO and LATS1/2 DKO cells. Each cell represents a single cell and error bars represent mean ± 95% CI. Data pooled from four independent experiments. Mann–Whitney U test. *** \( P < 0.001 \) (WT vs. Y/T DKO) and * \( P = 0.0723 \) (WT vs. LATS1/2 DKO).

F Comparison of average change in cell volume in response to 100 mbar cyclic hydrostatic pressure with 0.5 \( \mu \)M cytochalasin D treatment in WT, Y/T DKO and LATS1/2 DKO cells. Each cell represents a single cell and error bars represent mean ± 95% CI. Data pooled from four independent experiments. Mann–Whitney U test. \( P = 0.2021 \) (WT), *** \( P < 0.0035 \) (Y/T DKO) and \( P = 0.9148 \) (LATS1/2 DKO).

G Fold difference in average change in cell volume in response to hydrostatic pressure from B normalized against cells not treated with 2 \( \mu \)M latrunculin B. Each dot represents a single cell and error bars are 95% CI. Mann–Whitney U test. \( P > 0.9999 \) (WT vs. Y/T DKO) and \( P = 0.0723 \) (WT vs. LATS1/2 DKO).

H Fold difference in average change in cell volume in response to hydrostatic pressure from C normalized against cells not treated with 0.5 \( \mu \)M cytochalasin D. Each dot represents a single cell and error bars represent mean ± 95% CI. Mann–Whitney U test. \( P = 0.4619 \) (WT vs. Y/T DKO) and \( P = 0.7087 \) (WT vs. LATS1/2 DKO).

I HEK293A YAP/TAZ DKO and LATS1/2 DKO clone #2’s responses to cyclic 0.1 Hz 200 mbar dynamic hydrostatic pressure with AP180C-mediated inhibition of clathrin-dependent endocytosis. Each dot represents a single cell and error bars represent mean ± 95% CI. Data pooled from four independent experiments. Kruskal–Wallis test with Dunn’s post-hoc. *** \( P < 0.001 \) (WT), \( P = 0.2522 \) (Y/T DKO) and \( P = 0.0632 \) (LATS1/2 DKO).

Source data are available online for this figure.
Figure EV2.
Figure EV3. Additional mechanistic insights into cell volume responses mediated by hydrostatic pressure.

A Lysates from cells treated for 40 min with Torin (1 μM) or Rapamycin (0.5 μM) compared to control cells were analysed by immunoblots for the levels of the mTORC1 substrate 56K, p56K and GAPDH (loading control). Note the levels of p56K are drastically decreased in Torin- and Rapamycin-treated HEK293A cells, highlighting that these drugs effectively inhibits 56K phosphorylation.

B Cells treated with Torin (1 μM) and Rapamycin (0.5 μM) as in (A) and analysed by DHM to obtain their optical cellular volume. Each dot represents a single cell from three independent experiments. Forty minutes Torin or Rapamycin treatment does not affect steady-state cell volume. Error bars represent mean ± 95% CI. Kruskal–Wallis test with Dunn’s post-hoc. P = 0.5709 (con vs. Torin), P > 0.9999 (con vs. Rapamycin) and P > 0.9999 (Torin vs. Rapamycin).

C WT cells treated with Torin (1 μM) and Rapamycin (0.5 μM) as in (A) and imaged using DHM while being subjected to cyclic 0.1 Hz 100 mbar fluid pressure. Forty minutes Torin (1 μM) and Rapamycin (0.5 μM) treatment has no effect on cell volume changes in response to hydrostatic pressure. Each dot represents a single cell from three independent experiments. Error bars represent mean ± 95% CI. Kruskal–Wallis test with Dunn’s post-hoc. P = 0.5321 (con vs. Torin), P > 0.9999 (con vs. Rapamycin) and P = 0.4860 (Torin vs. Rapamycin).

D Western blot confirming no CAV1 protein expression in CAV1 KO HEK293A cells.

E CAV1 KO cellular response to cyclic 0.1 Hz 200 mbar hydrostatic pressure compared to WT. Each dot represents a single cell from three independent experiments and error bars represent mean ± 95% CI. Mann–Whitney U test. Error bars represent mean ± SD. **P = 0.0079.

F Relative CAV1 expression levels of CAV1 knockdown clones #1 and #2 from four independent experiments. Mann–Whitney U test. Error bars represent mean ± SD.

G Western blot confirming reduction in total CAV1 protein levels in shCAV1 clones #1 and #2.

H shRNA CAV1 knockdown cell response to cyclic 0.1 Hz 100 mbar hydrostatic pressure compared to WT. Each dot represents a single cell and error bars represent mean ± 95% CI. Data pooled from four independent experiments. Kruskal–Wallis test with Dunn’s post-hoc. P > 0.9999 (vector vs. shCAV#1), P = 0.1892 (vector shCAV#2) and P = 0.0812 (shCAV#1 vs. shCAV#2).

Source data are available online for this figure.
Figure EV3.
Figure EV4. Endocytosis rates upon oscillating hydrostatic pressure.

A Confocal image of fluorescently labelled transferrin (red) uptake by 143B cells under steady-state conditions and in response to cyclic 0.1 Hz 200 mbar hydrostatic pressure. Cells labelled for Hoechst (blue). Scale bar = 20 μm.

B Quantification of transferrin uptake in 143B cells treated with hydrostatic pressure compared to steady state from images as in A. Each dot represents a single cell and error bars represent mean ± 95% CI. Data from four independent experiments. Mann–Whitney U test. *** P < 0.001.

C Confocal IF images of HEK293A cells. Cells are labelled with Dextran (green) and Hoechst (blue). HEK293A WT dextran uptake in response to 10 min (left) and 30 min (right) cyclic hydrostatic pressure compared to steady state (Con). Scale bar = 20 μm.

D Confocal images of Y/T DKO HEK293A cells. Cells are labelled with Dextran (green) and Hoechst (blue). Y/T DKO HEK293A dextran uptake in response to 10 min (left) and 30 min (right) cyclic hydrostatic pressure compared to steady state (Con). Scale bar = 20 μm.

E Confocal images of LATS1/2 DKO HEK293A cells. Cells are labelled with Dextran (green) and Hoechst (blue). LATS1/2 DKO HEK293A dextran uptake in response to 10 min (left) and 30 min (right) cyclic hydrostatic pressure compared to steady state (Con). Scale bar = 20 μm.

F Changes in Dextran signal (30 min uptake) in response to cyclic hydrostatic pressure compared to control in WT, Y/T DKO and LATS1/2 DKO. Data obtained from images as in A–C. Each dot represents a single cell from three independent experiments. Error bars represent mean ± 95% CI. Data points obtained from images as in A–C. Kruskal–Wallis test with Dunn’s post-hoc. P = 0.1406 (WT), P = 0.6850 (Y/T DKO) and P > 0.9999 (LATS1/2 DKO).

G Dextran uptake (30 min) at steady state in Y/T DKO and LATS1/2 DKO cells relative to WT steady state. Each dot represents a single cell from three independent experiments. Data points obtained from images as in A–C. Error bars represent mean ± 95% CI. Mann–Whitney U test. P > 0.9999.

H Dextran uptake (30 min) in response to hydrostatic pressure relative to steady state. Each dot represents a single cell from three independent experiments. Error bars represent mean ± 95% CI. Kruskal–Wallis test with Dunn’s post-hoc. P = 0.0542 (WT vs. Y/T DKO), P = 0.8633 (WT vs. LATS1/2 DKO) and P = 0.5795 (Y/T DKO vs. LATS1/2 DKO).
Transferrin uptake in 143B cells

WT

WT + H.P.

WT Dextran Uptake

Y/T DKO Dextran Uptake

LATS1/2 DKO Dextran Uptake

Dextran uptake (30min) at steady-state relative to WT

Dextran uptake (30min) at steady-state relative to WT

Fold-change in dextran uptake (30min) in response to H.P.
Figure EV5. AP180C’s effect on the Hippo pathway.

A–F PhosTag probing from HA-tagged YAP levels in AP180C transfection positive cells. Vector or AP180C plasmid was co-transfected with HA-YAP and YAP activation status in response to hydrostatic pressure is shown (PhosTag gel). Anti-HA tag antibody was used to probe for HA-tagged YAP. YAP phosphorylation levels in HEK293A (A) WT, (B) LATS1/2 DKO, (C) MST1/2 DKO, (D) M4K4/6/7 KO (M4K KO), (E) MST1/2-M4K4/6/7 KO (SKO) and (F) MST1/2-M4K1/2/3/4/6/7 KO (8KO) cells are shown.

G Immunofluorescence images show AP180C positive cells in green and YAP in red. Hoechst highlights the nucleus (shown in blue). AP180C promotes nuclear exclusion of YAP in M4K KO but not MST1/2 DKO cells. Cells were transfected with Myc-tagged AP180C and the subcellular localization of YAP was examined in response to hydrostatic pressure. Scale bar = 20 μm.

H Quantification of nuclear-to-cytoplasmic ratio (Nucl/Cyt) of YAP in AP180C transfection positive cells compared to control using images as shown in (G). Each dot represents a single cell and error bars are mean ± 95% CI. Graph shows data obtained from three independent experiments. Mann–Whitney U test. P = 0.3516 (MST1/2 DKO) and P = 0.0001 (M4K KO).

Source data are available online for this figure.
**Effect of inhibition of CDE on YAP localisation**

**Figure EV5.**