Expanded View Figures

Figure EV1. Osmotic stress induces YAP phosphorylation but not cytoplasmic retention.

A Osmotic stress-induced YAP phosphorylation is dose-dependent. HEK293A cells were treated with different doses of sorbitol for 30 and 60 min. YAP phosphorylation is determined by mobility shift on phos-tag gel and a S127 phosphospecific antibody.

B Sorbitol stimulates YAP phosphorylation in MCF10A cells. MCF10A cells were cultured in the presence or absence of serum, and were treated with 0.2 M sorbitol for the indicated time points. A phos-tag gel was used to assess total YAP phosphorylation.

C NaCl stimulates YAP and LATS phosphorylation. HEK293A cells were cultured in the presence of serum and were treated with 0.1 M NaCl for the indicated time points. A phos-tag gel was used to assess YAP phosphorylation.

D NaCl does not induce YAP cytoplasmic localization. HEK293A cells were treated with 0.1 M NaCl for 1 h in the presence of serum. YAP/TAZ subcellular localization was determined by immunofluorescence staining with an antibody that recognizes both YAP and TAZ (red). DAPI (blue) was used to stain for DNA (cell nuclei). Scale bars: 20 μm. Quantification of YAP/TAZ more nuclear (N > C) or more cytosolic (N < C) is determined with randomly chosen fields, each with approximately 100 cells.

E Sustained osmotic stress induces YAP cytoplasmic localization in the presence of serum. HEK293A cells were treated with 0.2 M sorbitol from 30 min to 8 h in the presence of serum. YAP/TAZ subcellular localization was determined by immunofluorescence staining. Scale bars: 20 μm.

Source data are available online for this figure.

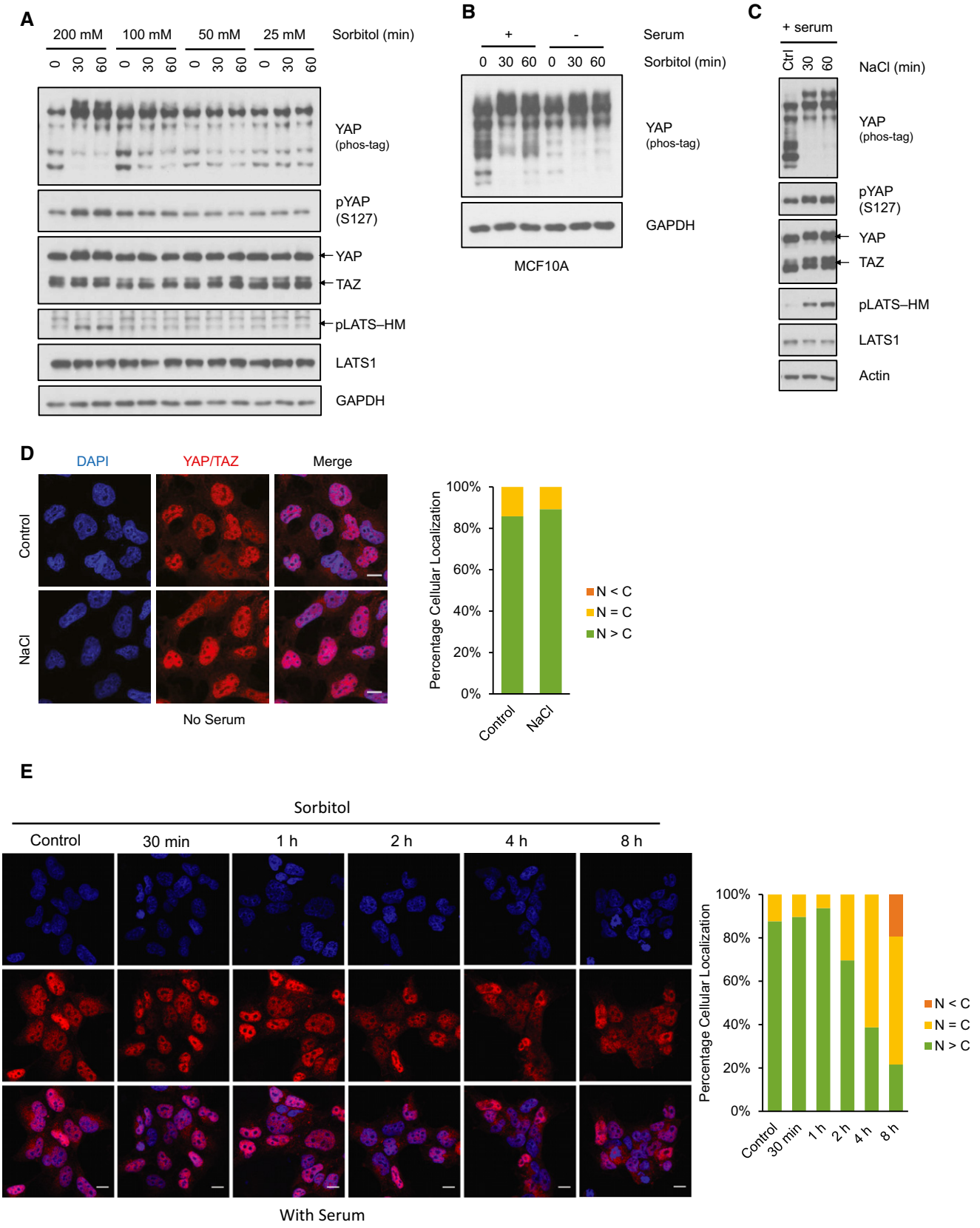
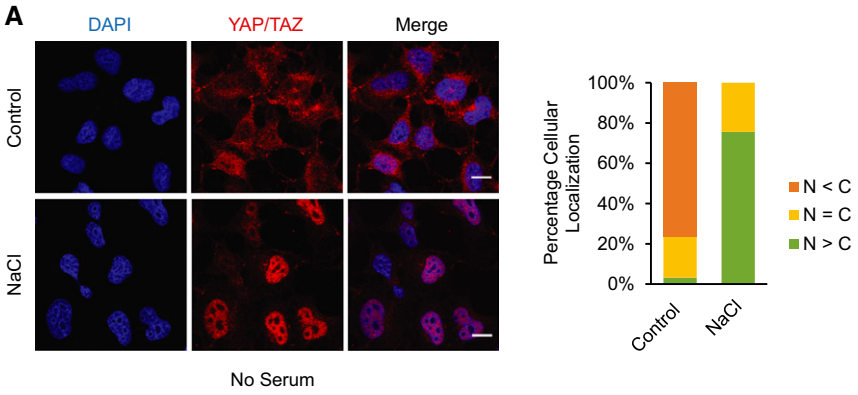

A
200 mM
100 mM
50 mM
25 mM
Sorbitol (min)
0 30 60 0 30 60 0 30 60 0 30 60
YAP (phos-tag)
pYAP (S127)
YAP
TAZ
pLATS–HM
LATS1
GAPDH
B
+
-
Serum
0 30 60 0 30 60
Sorbitol (min)
YAP (phos-tag)
GAPDH
MCF10A
C
+ serum
Ctrl 30 60
NaCl (min)
YAP (phos-tag)
pYAP (S127)
YAP
TAZ
pLATS–HM
LATS1
Actin
D
DAPI
YAP/TAZ
Merge
Control
NaCl
No Serum
Percentage Cellular Localization
100%
80%
60%
40%
20%
0%
Control
NaCl
N < C
N = C
N > C
E
Sorbitol
Control
30 min
1 h
2 h
4 h
8 h
DAPI
YAP/TAZ
Merge
With Serum
Percentage Cellular Localization
100%
80%
60%
40%
20%
0%
Control
30 min
1 h
2 h
4 h
8 h
N < C
N = C
N > C

Figure EV1.



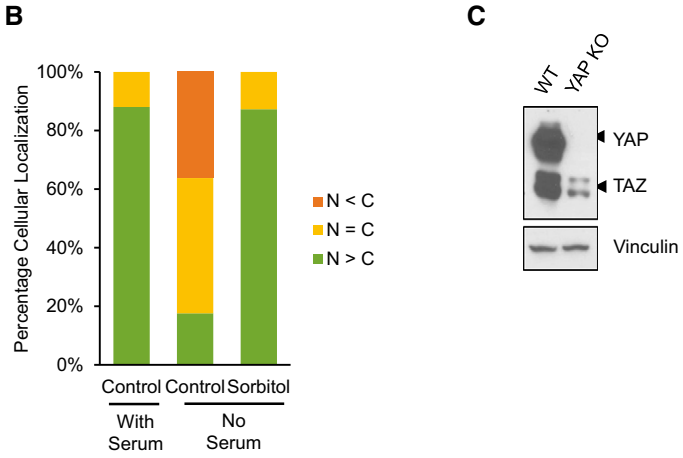


Figure EV2. Osmotic stress induces YAP and TAZ nuclear translocation in serum-free conditions.

A NaCl induces YAP nuclear translocation under serum-starved conditions. HEK293A cells were serum starved for 1 h followed by 0.1 M NaCl treatment for 1 h. YAP/TAZ subcellular localization was determined by immunofluorescence staining (red). Scale bars: 20 μm.

B Quantification of Fig 2B. YAP knockout (KO) HEK293A cells were serum starved for 1 h followed by 0.2 M sorbitol treatment for 1 h. Quantification of more nuclear (N > C) or more cytosolic (N < C) YAP signal was determined with randomly chosen fields.

C YAP expression is completely abolished in YAP knockout (KO) cells. Western blot shows that YAP is absent and TAZ is still present in the YAP KO HEK293A cells. Vinculin serves as a loading control.

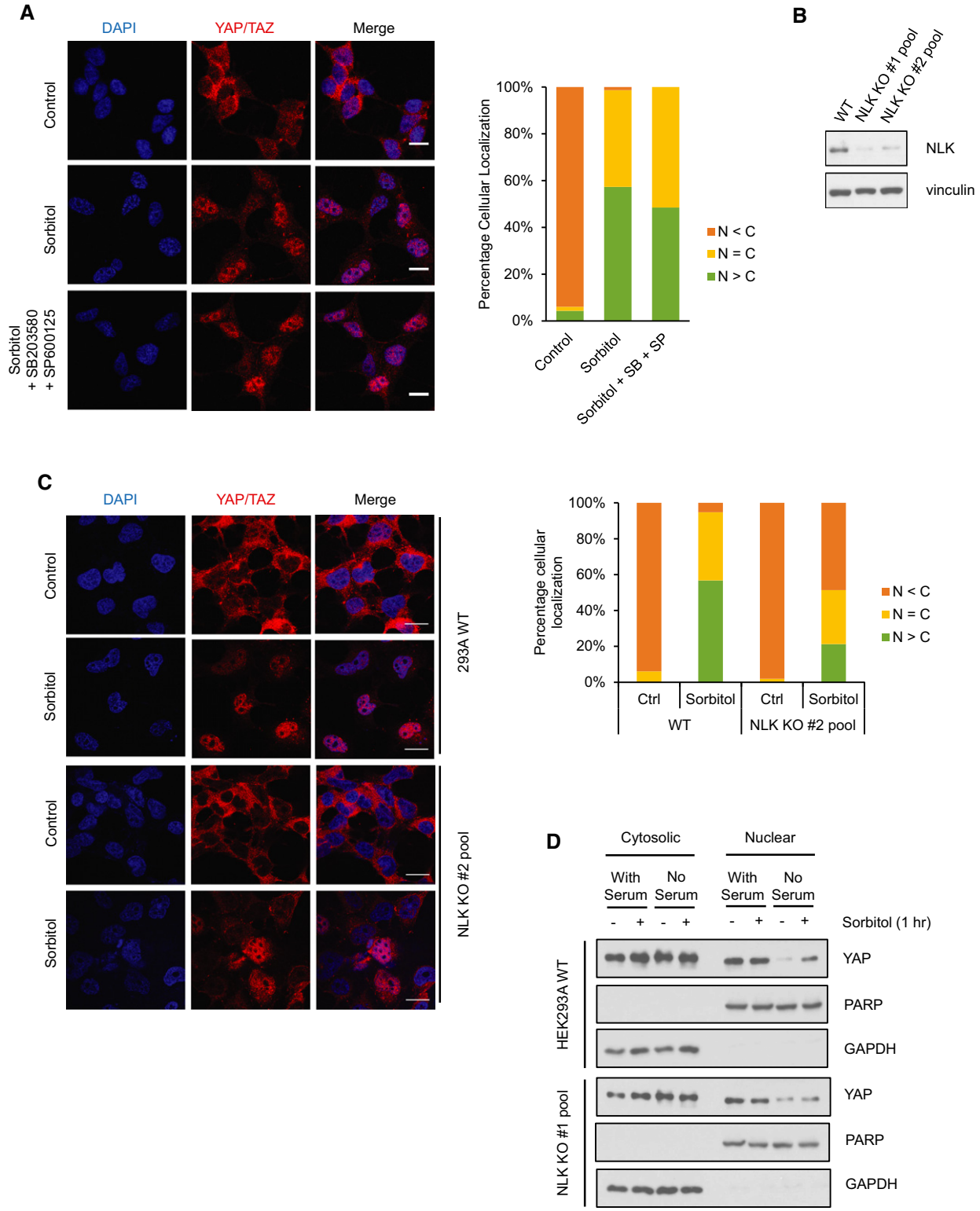

A
DAPI
YAP/TAZ
Merge
Control
Sorbitol
Sorbitol + SB203580 + SP600125
Percentage Cellular Localization
100%
80%
60%
40%
20%
0%
Control
Sorbitol
Sorbitol + SB + SP
N < C
N = C
N > C
B
WT
NLK KO #1 pool
NLK KO #2 pool
NLK
vinculin
C
DAPI
YAP/TAZ
Merge
Control
Sorbitol
Control
Sorbitol
293A WT
NLK KO #2 pool
Percentage cellular localization
100%
80%
60%
40%
20%
0%
Ctrl
Sorbitol
Ctrl
Sorbitol
WT
NLK KO #2 pool
N < C
N = C
N > C
D
Cytosolic
Nuclear
With Serum
No Serum
With Serum
No Serum
- + - + - + - +
Sorbitol (1 hr)
HEK293A WT
YAP
PARP
GAPDH
NLK KO #1 pool
YAP
PARP
GAPDH

Figure EV3.

Figure EV3. NLK mediates osmotic stress-induced YAP nuclear translocation.

A Inhibition of both p38 and JNK does not block osmotic stress-induced YAP nuclear translocation. HEK293A cells were pretreated with 2 μM p38 inhibitor (SB203580) and 20 μM JNK inhibitor (SP600125) before treatment followed by 0.2 M sorbitol for 1 h in the absence of serum. Endogenous YAP/TAZ subcellular localization was determined by immunofluorescence staining (red). Scale bars: 20 μm.

B Transient NLK CRISPR/Cas9 transfection reduces NLK expression levels. HEK293A cells were transiently transfected with CRISPR/Cas9 and guide RNAs to knock out NLK. Two NLK guide RNAs were used. NLK protein levels were measured by Western blot with vinculin as a loading control.

C NLK knockout blocks osmotic stress-induced YAP/TAZ nuclear localization. HEK293A cells were transiently transfected with CRISPR/Cas9 to knock out NLK with gRNA #2. Wild-type (WT) cells and the NLK KO cell pool were treated with 0.2 M sorbitol for 1 h in the absence of serum. YAP/TAZ subcellular localization was determined by immunofluorescence staining (red). Scale bars: 20 μm.

D Sorbitol-induced YAP nuclear accumulation requires NLK. Both WT and NLK KO HEK293A cells were cultured in the presence or absence and with or without sorbitol as indicated. Cytosolic and nuclear fractions were collected by differential fractionation. PARP and GAPDH were used as nuclear and cytosolic markers, respectively.

Source data are available online for this figure.

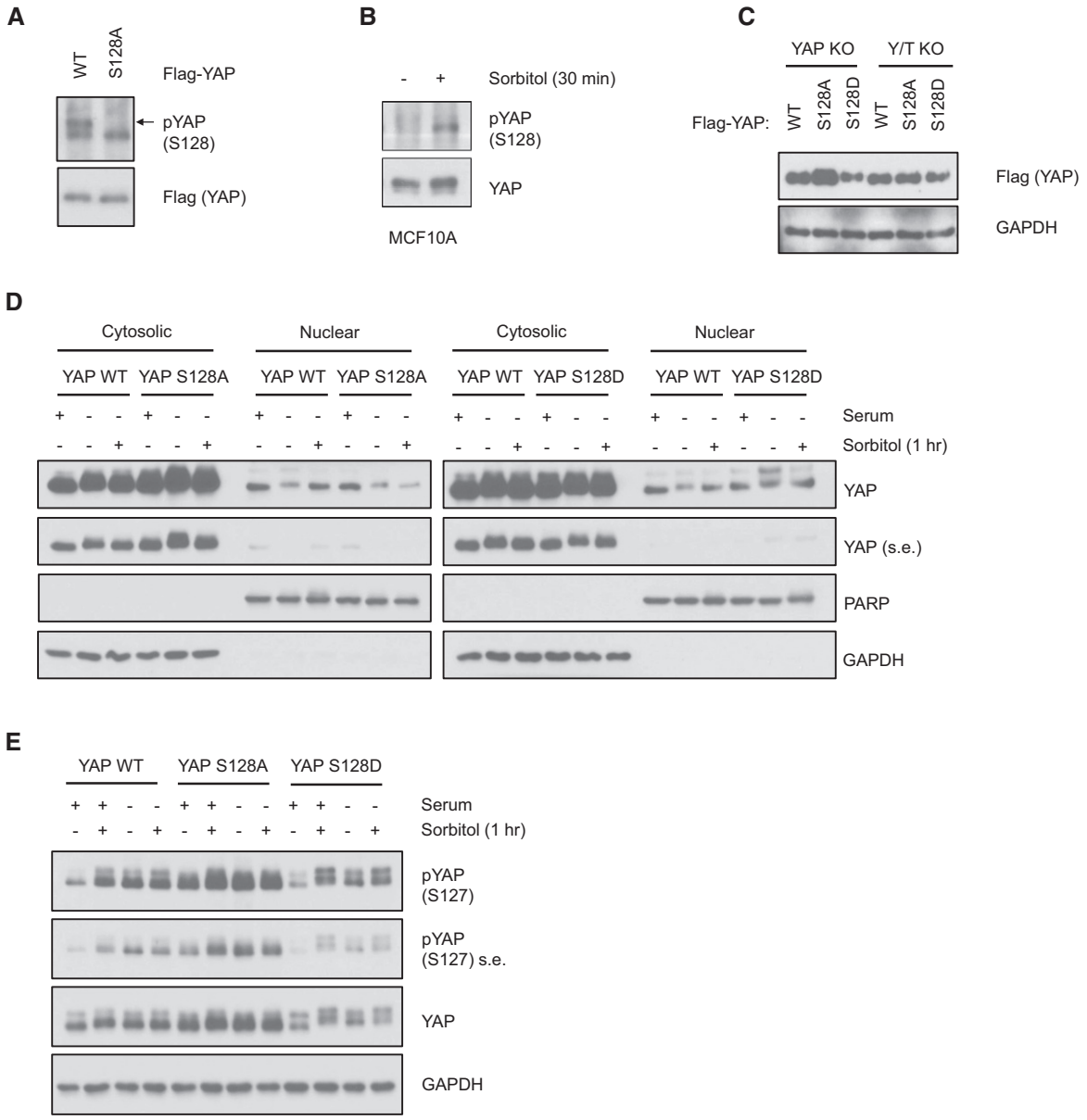


Figure EV4. Osmotic stress induces YAP Ser128 phosphorylation to determine its subcellular localization.

A pYAP S128 antibody is specific to YAP S128 site. Flag-YAP wild-type (WT) and S128A mutant constructs were transfected into HEK293A cells. Cell lysates were collected for Western blot analysis by indicated antibodies.

B Osmotic stress induces endogenous YAP Ser128 phosphorylation in MCF10A cells. MCF10A cells were treated with sorbitol and endogenous YAP was immunoprecipitated. YAP Ser128 phosphorylation and protein levels were determined by Western blot.

C Expressions of Flag-YAP WT, S128A, and S128D mutant stable cell lines are at similar levels. Stable cell lines were generated with retroviral infection of YAP WT and mutant constructs into YAP KO or YAP/TAZ dKO HEK293A cells. Cell lysates were collected for Western blot analysis. YAP expression level was detected by Flag antibody, with GAPDH as a loading control.

D Subcellular fractionation of YAP WT-, S128D-, and S128A-reconstituted cells. YAP KO HEK293A cells were stably reconstituted with YAP WT, S128D, or S128A. Cytosolic and nuclear fractions were collected by differential fractionation. PARP and GAPDH were used as nuclear and cytosolic markers, respectively. s.e. denotes short exposure of the YAP Western blot.

E Osmotic stress induces YAP Ser127 phosphorylation despite Ser128 phosphorylation status. YAP KO HEK293A cells were stably reconstituted with YAP WT, S128D, or S128A mutants and were treated with sorbitol in the presence or absence of serum. YAP Ser127 phosphorylation and protein levels were determined by Western blot. s.e. denotes short exposure.

Source data are available online for this figure.

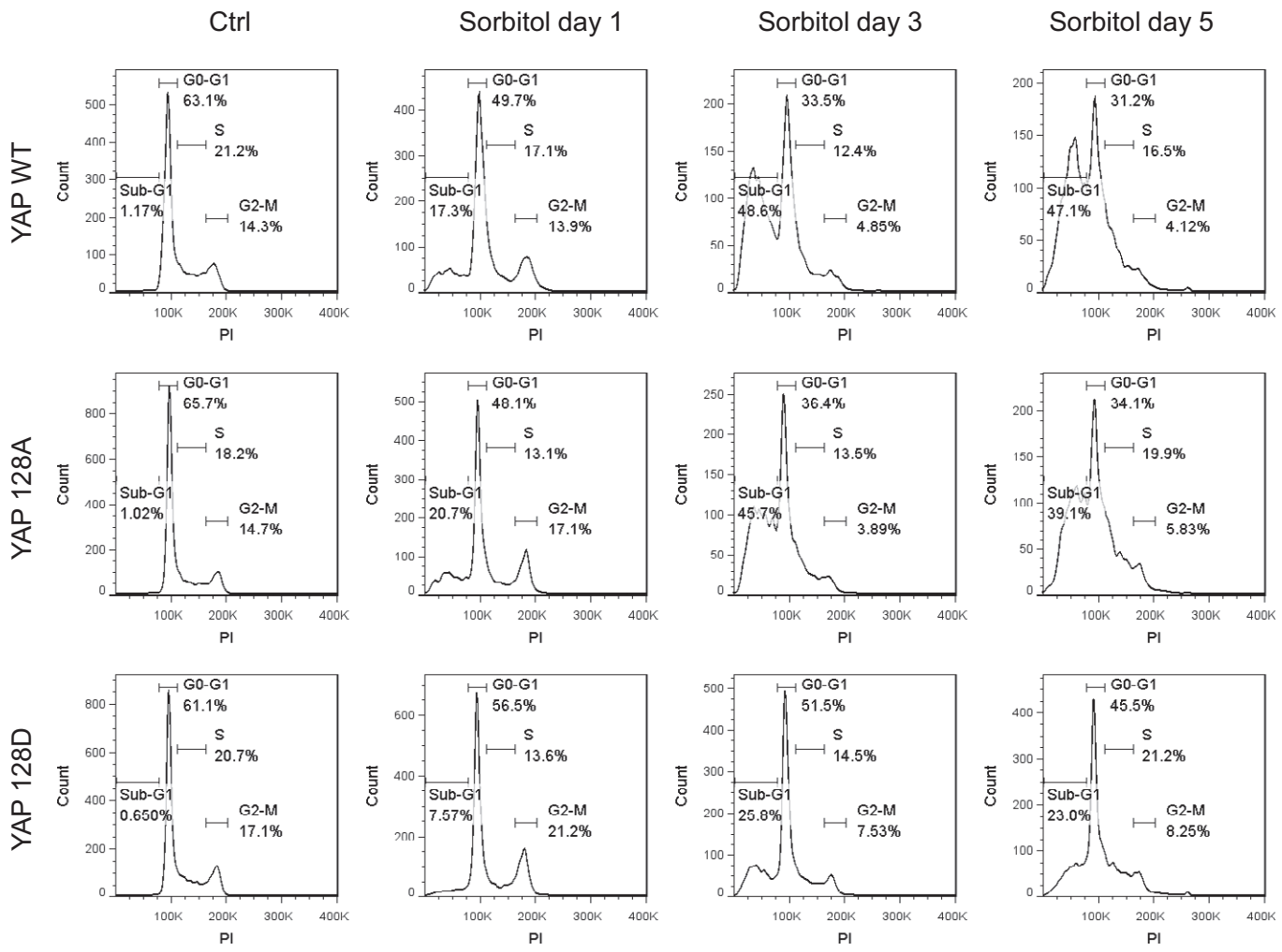


Figure EV5. YAP Ser128 phosphorylation prevents cell death under osmotic stress.
Cell cycle analysis of YAP-reconstituted cells in hyperosmotic environment. Cell cycle analyses of YAP/TAZ dKO HEK293A cells with stable expression of YAP WT, S128A, or S128D after 0.2 M sorbitol treatment were determined using flow cytometry. Propidium iodine (PI) was used for DNA staining. Quantification of sub-G1 phase is shown in Fig 6B.