Expanded View Figures

Figure EV1. YAP and TEAD directly promote Skp2 transcription in response to mechanical cues.

A Immunoblots with the indicated antibodies. At the indicated time points, whole-cell lysates were taken from MCF10A cells transfected with the indicated siRNAs and treated with MG132 (10 μM).

B Immunoblots of MDA-MB-468 cells infected with retroviruses encoding an empty vector (Vec) or the indicated YAP mutant clones.

C Immunoblots of HeLa cells transduced with flag 5SA-YAP via retroviral infection.

D Immunoblots of RPE1 (above) or MCF10A cells (below) stably expressing vector or 5SA-YAP and incubated with cyclohexamide (CHX) for the indicated times. Relative Skp2 immunoblot band intensity normalized to β-actin is also shown for MCF10A cells.

E Relative mRNA levels of the indicated genes, as determined by qPCR, from MDA-MB-231 cells transfected with control siRNAs or TEAD1/3/4-specific siRNAs. Data were collected from seven independent experiments ($N = 7$).

F MDA-MB-231 cells were transfected with control siRNAs or TEAD1/3/4-specific siRNAs. After 48 h, the cells were treated with MG132 (10 μM). Shown are immunoblots from whole-cell lysates prepared at the indicated times with the indicated antibodies.

G Using the UCSC genome browser, we compared our own ChIP-seq results with those of two published reports (Galli *et al*, 2015; Stein *et al*, 2015). We looked specifically at well-known YAP targets including AMOTL2, CTGF, ANKRD1, Cyr61, KIBRA, TEAD4, and LATS2.

H Relative Skp2 and CTGF mRNA levels, as measured by qPCR, in MCF10A cells stably expressing vector or 5SA-YAP and treated with Y27632 (20 μM, 14 h) or not ($N = 3$).

I Enrichment of endogenous YAP at TB2 in the Skp2 promoter in MCF10A cells cultured as sparse or highly dense monolayers, as determined by ChIP-qPCR. The CTGF promoter (prmt) and the genomic region 3′ of the CTGF gene were used as positive (pos.ctl) and negative controls (neg.ctl), respectively ($N = 2$).

Data information: All error bars indicate s.e.m. Statistical significance, as determined by a two-tailed *t*-test, is indicated above each bar.

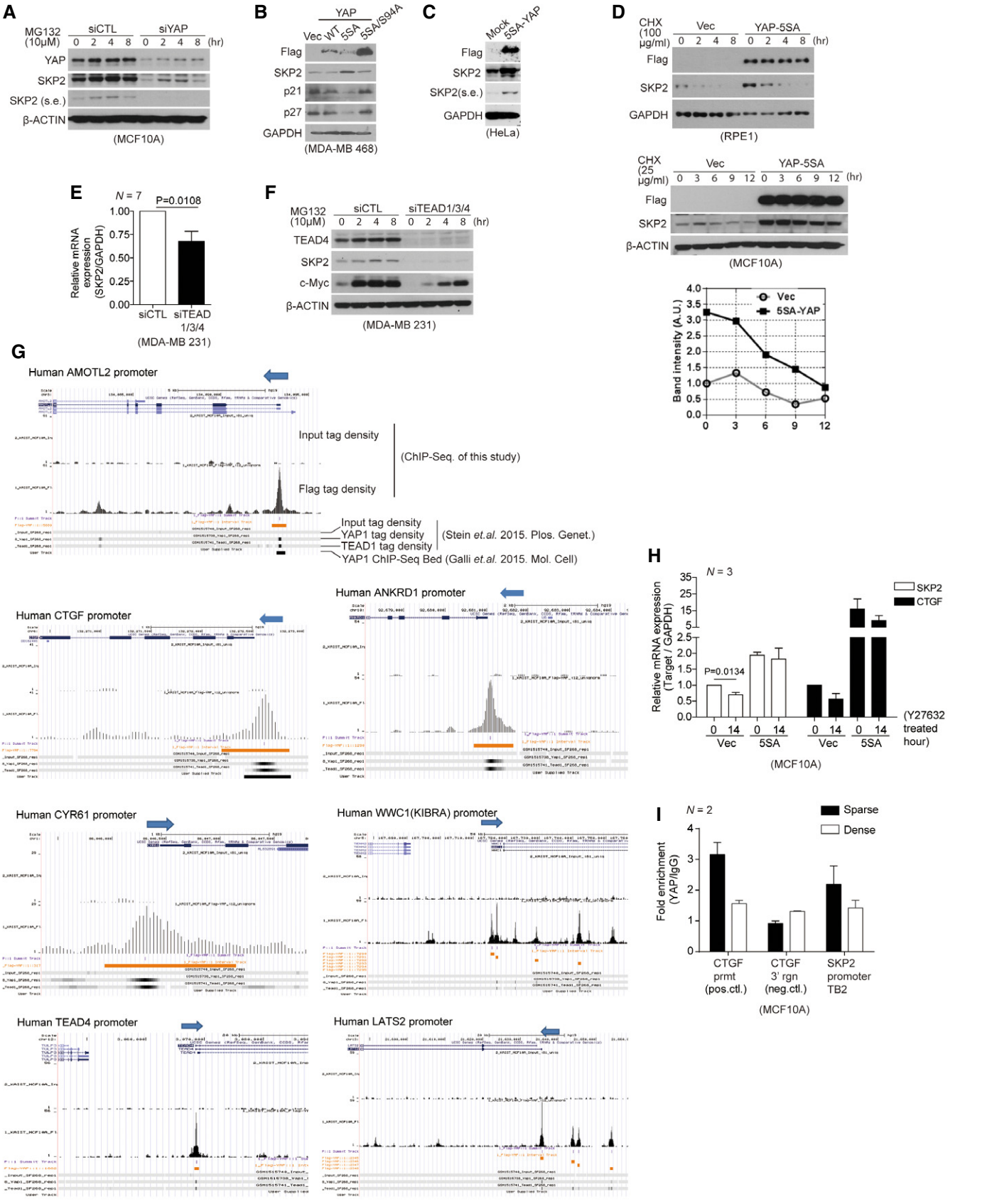

A
MG132 (10μM)
siCTL
siYAP
0 2 4 8 0 2 4 8 (hr)
YAP
SKP2
SKP2 (s.e.)
β-ACTIN
(MCF10A)
B
YAP
Vec WT 5SA 5SA/S94A
Flag
SKP2
p21
p27
GAPDH
(MDA-MB 468)
C
Mock 5SA-YAP
Flag
SKP2
SKP2(s.e.)
GAPDH
(HeLa)
D
CHX (100 μg/ml)
Vec
YAP-5SA
0 2 4 8 0 2 4 8 (hr)
Flag
SKP2
GAPDH
(RPE1)
CHX (25 μg/ml)
Vec
YAP-5SA
0 3 6 9 12 0 3 6 9 12 (hr)
Flag
SKP2
β-ACTIN
(MCF10A)
Band intensity (A.U.)
Vec
5SA-YAP
E
N = 7
P=0.0108
Relative mRNA expression (SKP2/GAPDH)
siCTL
siTEAD 1/3/4
(MDA-MB 231)
F
MG132 (10μM)
siCTL
siTEAD1/3/4
0 2 4 8 0 2 4 8 (hr)
TEAD4
SKP2
c-Myc
β-ACTIN
(MDA-MB 231)
G
Human AMOTL2 promoter
Input tag density
Flag tag density
(ChIP-Seq. of this study)
Input tag density
YAP1 tag density
TEAD1 tag density
(Stein et.al. 2015. Plos. Genet.)
YAP1 ChIP-Seq Bed (Galli et.al. 2015. Mol. Cell)
Human CTGF promoter
Human ANKRD1 promoter
Human CYR61 promoter
Human WWC1(KIBRA) promoter
Human TEAD4 promoter
Human LATS2 promoter
H
N = 3
SKP2
CTGF
Relative mRNA expression (Target / GAPDH)
P=0.0134
0 14 0 14 0 14 0 14
Vec 5SA Vec 5SA
(Y27632 treated hour)
(MCF10A)
I
N = 2
Sparse
Dense
Fold enrichment (YAP/IgG)
CTGF prmt (pos.ctl.)
CTGF 3' rgn (neg.ctl.)
SKP2 promoter TB2
(MCF10A)

Figure EV1.

Figure EV2. Inactivation of Skp2 does not effectively suppress YAP-driven cell cycle control in a 2D culture system.

A Using the CRISPR-Cas9 system, we generated control or Skp2 knockout MCF10A clones. These were further manipulated to generate the doxycycline-inducible 5SA-YAP expression system (Tet-ON: 5SA-YAP). Immunoblots confirmed both the Skp2 knockout and the function of the inducible 5SA-YAP expression system.

B (Above) Experimental protocol schematic. (Below) Cells prepared as in (A) were treated with or without doxycycline for 2 days. Then, cells were either serum-starved or not in the absence or presence of 2 days of doxycycline. After 1 h of BrdU incorporation, the BrdU-positive cells were counted. > 500 cells were analyzed in each of three independent experiments.

C (Above) Experimental protocol schematic. Doxycycline-inducible 5SA-YAP-expressing MCF10A cells were either serum-starved or not. After 2 days, some cells were fixed and others were treated with doxycycline. After an additional 2 days, the cells were fixed and stained with a Ki67-specific antibody and DAPI.

D Immunoblots of cells treated as in (C).

E Quantification of Ki67 positivity among cells treated as in (C). > 300 cells were analyzed for each of two independent experiments.

F Doxycycline-inducible 5SA-YAP-expressing MDA-MB-231 cells treated as in (C) except for an additional 1 h of BrdU incorporation. > 300 cells were analyzed for each of two independent experiments.

G MCF10A cells were infected with lentiviruses encoding either vector or Skp2. The cells were then serum-starved for 2 days to induce cell cycle exit. After 1 h BrdU incorporation, the cells were fixed and stained with BrdU- or Ki67-specific antibodies. (Left) Representative cells stained for BrdU and counterstained with DAPI. Scale bars: 50 μm. (Right) Quantification of BrdU- or Ki67-positive cells. > 800 cells were analyzed in each of three independent experiments.

H RPE1 cells were treated with DMSO or SZL P1-41. After 48 h, cells were fixed and stained with a p27-specific antibody and DAPI. Scale bars: 20 μm.

I (Left) Experimental protocol schematic. Doxycycline-inducible 5SA-YAP-expressing cells were grown on 3D Matrigel for acinus formation. After arresting acinar growth 8 days later, one group was fixed and the other two were treated with or without doxycycline for 3 days before also being fixed. (Right) All fixed samples were visualized and quantified using phase contrast microscopy and the ImageJ program. Orange bars indicate the median. > 200 acini were analyzed for each of two independent experiments.

Data information: All error bars indicate s.e.m. Statistical significance, as determined by a two-tailed *t*-test, is indicated in the graph. N.S. indicates non-significance.

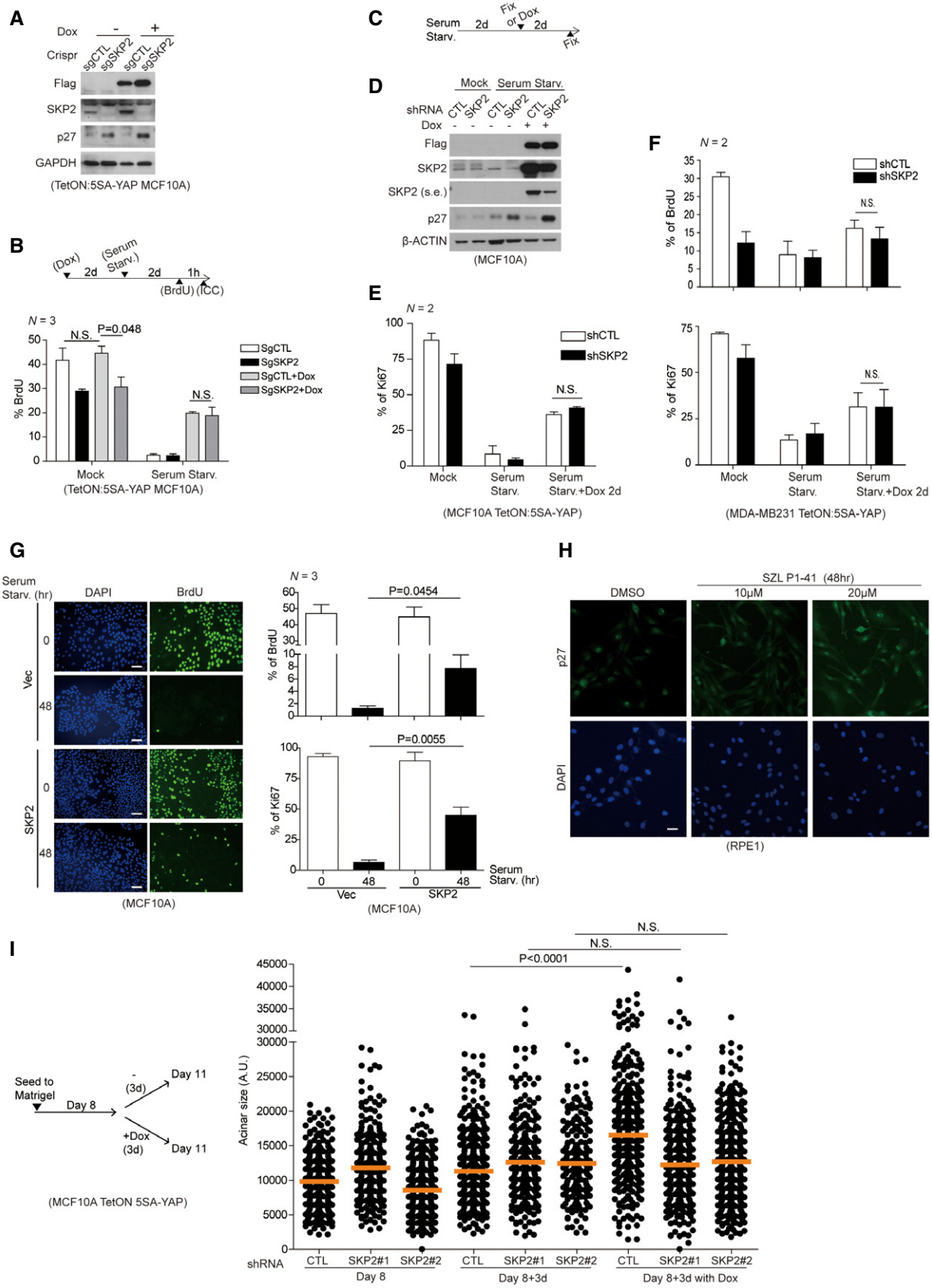

A
Dox
Crispr
sgCTL
sgSKP2
Flag
SKP2
p27
GAPDH
(TetON:5SA-YAP MCF10A)
B
(Dox)
(Serum Starv.)
2d
1h
(BrdU) (ICC)
N = 3
N.S.
P=0.048
% BrdU
SgCTL
SgSKP2
SgCTL+Dox
SgSKP2+Dox
Mock
Serum Starv.
(TetON:5SA-YAP MCF10A)
C
Serum Starv.
Fix or Dox
Fix
D
Mock
Serum Starv.
shRNA
Dox
Flag
SKP2
SKP2 (s.e.)
p27
β-ACTIN
(MCF10A)
E
N = 2
% of Ki67
shCTL
shSKP2
Mock
Serum Starv.
Serum Starv.+Dox 2d
(MCF10A TetON:5SA-YAP)
F
N = 2
% of BrdU
(MDA-MB231 TetON:5SA-YAP)
G
Serum Starv. (hr)
DAPI
BrdU
Vec
SKP2
(MCF10A)
P=0.0454
P=0.0055
H
DMSO
SZL P1-41 (48hr)
10μM
20μM
p27
DAPI
(RPE1)
I
Seed to Matrigel
Day 8
Day 11
+Dox (3d)
(MCF10A TetON 5SA-YAP)
N.S.
P<0.0001
Acinar size (A.U.)
shRNA
CTL
SKP2#1
SKP2#2
Day 8
Day 8+3d
Day 8+3d with Dox

Figure EV2.

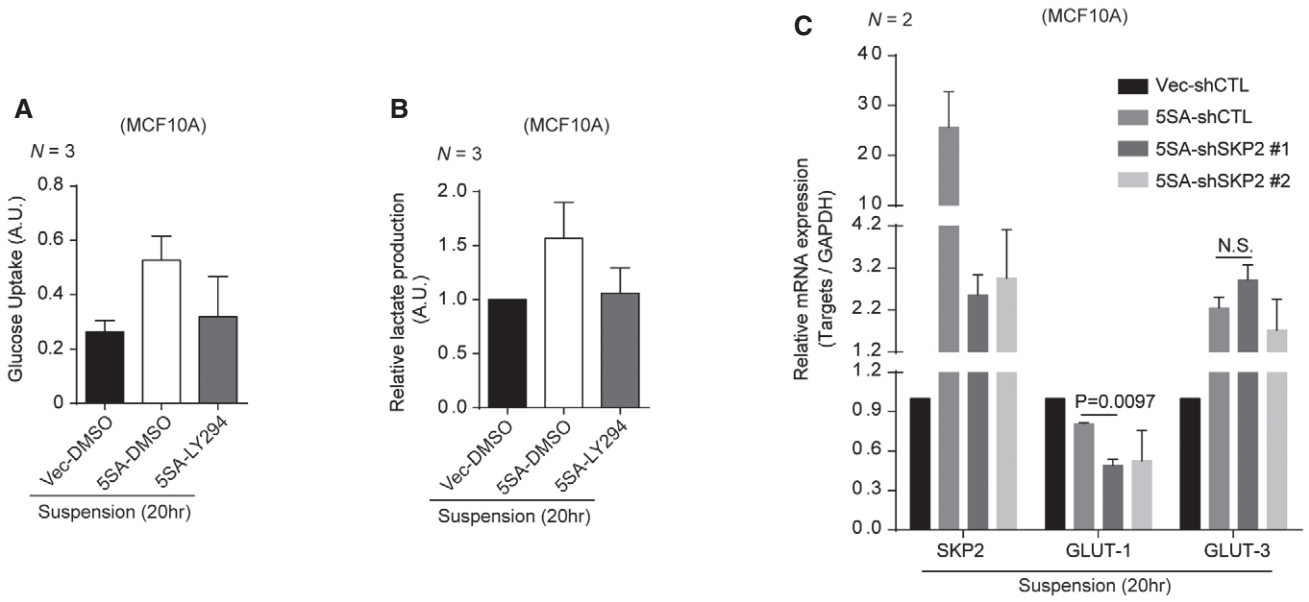


Figure EV3. YAP-Skp2 axis affects aerobic glycolysis.

A, B MCF10A cells expressing vector or 5SA-YAP were suspended with DMSO or LY294002 (50 μM) treatment. After 20 h, glucose uptake (A) and lactate production (B) were measured in the indicated cells.

C Relative mRNA levels for the indicated genes in suspended MCF10A cells expressing control and 5SA-YAP with the indicated shRNA lentiviruses, as measured by qPCR.

Data information: All error bars indicate s.e.m. Statistical significance, as determined by a two-tailed *t*-test, is indicated in the graph.

Figure EV4. Skp2 inactivation effectively suppreses YAP-driven aberrant stiff 3D matrix-evoked epithelial tissue behaviors.

A MCF10A cells were embedded in soft or stiff 3D BM/COL1 gels. After 8 days of growth, the cells were fixed and stained with phalloidin and a YAP-specific antibody. DAPI was used as a nuclear counterstain. Images were taken by confocal microscopy. Scale bars: 50 μm.

B Relative mRNA levels for the indicated genes in MCF10A cells treated as in (A), as measured by qPCR. Error bars indicate standard error of the mean (s.e.m.) for four independent experiments. Statistical significance, as determined by a two-tailed *t*-test, is indicated in the graph. N.S. indicates non-significance.

C Vector or 5SA-YAP-expressing MCF10A cells infected by the indicated shRNA lentiviruses were embedded in soft or stiff 3D BM/COL1 gels. After 7–8 days of growth, the cells were imaged by phase contrast (gray) or confocal microscopy (blue: DAPI). Scale bars: black, 100 μm; white, 50 μm.

D Empty vector- or 5SA-YAP-expressing MCF10A cells were embedded in stiff 3D BM/COL1 gels. After 7–8 days of growth, the cells were treated as in (A). Scale bars: 50 μm.

E Vector- or 5SA-YAP-expressing MCF10A cells were embedded with either DMSO or the Skp2 inhibitor SZL P1-41 (20 μM) in soft or stiff 3D BM/COL1 gels. After 7–8 days of growth, the cells were fixed and imaged by phase contrast microscopy. Images are representative of three independent experiments. Scale bars: 100 μm.

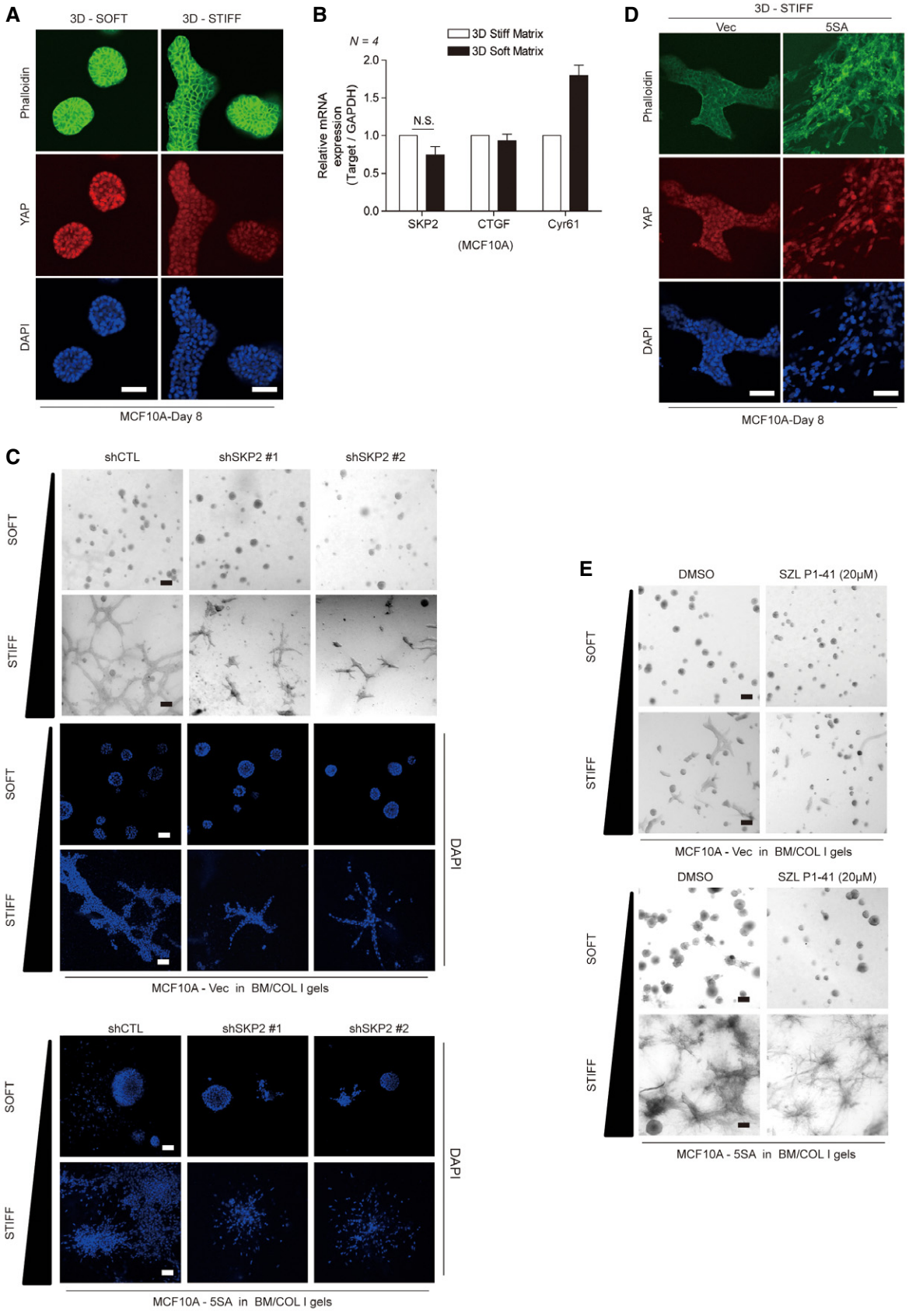
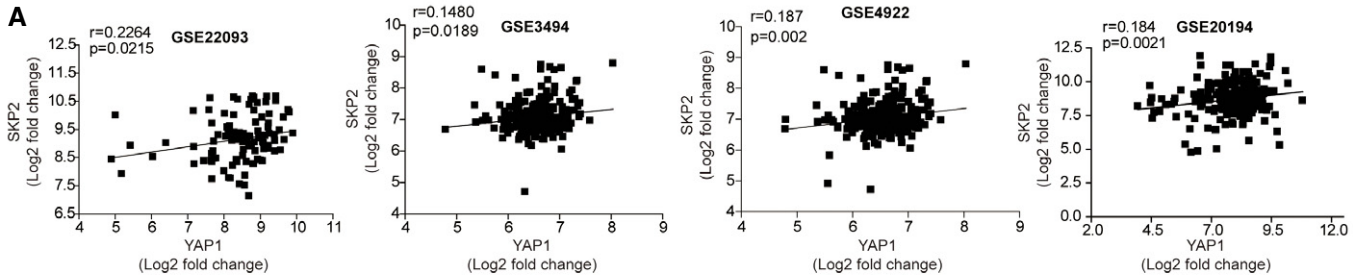


Figure EV4.

Figure EV5. Positive correlation of YAP and Skp2 expression in breast cancer patients.

A Scatter plot comparing Skp2 and YAP1 mRNA expression in breast cancer patient microarray data. r = Pearson's correlation coefficient.
B Summary and statistical analysis of the correlation between YAP and Skp2 mRNA expression in various cancer patients using cBioPortal.
C Heatmap analysis using cBioPortal showing Skp2 and YAP target gene sets in kidney renal clear cell carcinoma and brain lower-grade glioma.

A



B

www.cBioportal.org			
Type of tissue	**Source**	**# of samples**	**Tendency towards co-occurrence (mRNA data; YAP1 vs SKP2)**
Breast invasive carcinoma	TCGA, Provisional	1105	p-value=0.014
Liver Hepatocellular carcinoma	TCGA, Provisional	360	p-value=0.040
Brain Lower Grade Glioma	TCGA, Provisional	530	p-value<0.001
Glioblastoma	TCGA, Cell 2013	154	p-value=0.007
Stomach Adenocarcinoma	TCGA, Provisional	415	p-value=0.037
Head and Neck Squamous Cell Carcinoma	TCGA, Provisional	522	p-value=0.023
Kidney Renal Clear Cell Carcinoma	TCGA, Provisional	534	p-value<0.001
Kidney Renal Papillary Cell Carcinoma	TCGA, Provisional	291	p-value=0.029
Lung Adenocarcinoma	TCGA, Provisional	517	p-value=0.026
Lung Squamous Cell Carcinoma	TCGA, Provisional	501	p-value=0.042
Pancreatic Adenocarcinoma	TCGA, Provisional	179	p-value=0.012
Prostate Adenocarcinoma	Broad/Cornell, Nat Genet 2012	31	p-value=0.032
Prostate Adenocarcinoma	MSKCC, Cancer Cell 2010	216	p-value<0.001
Sarcoma	MSKCC/Broad, Nat Genet 2010	207	p-value=0.017

C

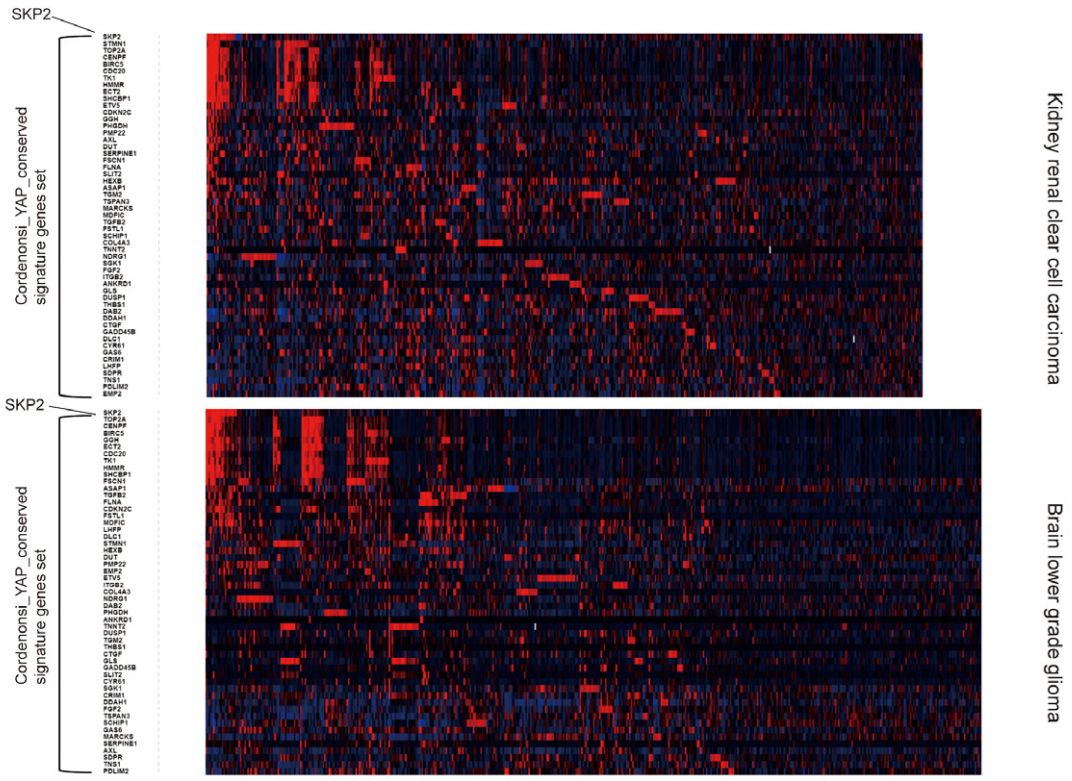


Figure EV5.