Expanded View Figures

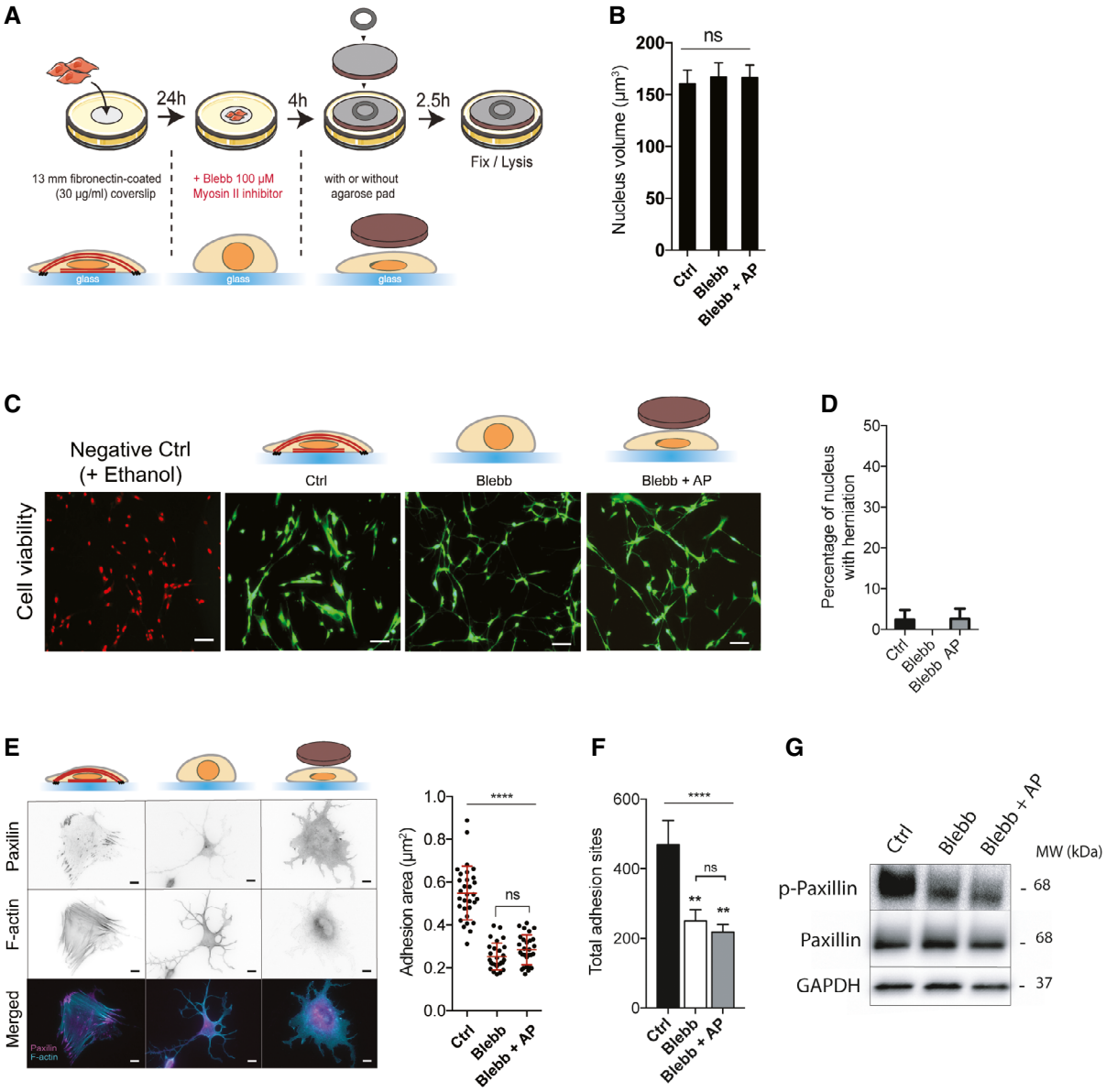


Figure EV1. Agarose pad-mediated nuclear flattening.

A MRC5 cells were cultured on a 13-mm fibronectin-coated glass coverslip (for IF) or a 35-mm fibronectin- coated dish (for biochemistry) during 24 h. The medium is replaced with a fresh medium containing blebbistatin (100 µM) for 4 h. Then, an agarose pad (AP) is gently applied on cells. A 32-mm coverslip with a 425 mg washer is used to maintain the agarose pad. 2.5 h later, cells are fixed using 3.7% PFA (IF) or directly lysed with a Laemmli lysis buffer.

B DNA staining was used to quantify nuclear volume in the indicated conditions. Data are presented as mean ± s.e.m. (n = 37 minimum from two independent experiments, ns: not significant, one-way ANOVA).

C Representative images of MRC5 cells assessed for cell viability using calcein/AM (green) and ethidium homodimer (Red) in Ctrl, Blebb, and Blebb + AP conditions. Ethanol was used to induce cell apoptosis (negative Ctrl).

D Percentage of cells with nuclear herniation. Data are presented as mean ± s.e.m. (n = 40 minimum from two independent experiments).

E, F Representative cells stained for actin-F (cyan) and focal adhesions (paxillin—magenta) in Ctrl, Blebb, and Blebb + AP conditions. Scale bar = 10 µm. Quantifications of (e) adhesion areas using paxillin staining (n = 28 from three independent experiments, ****P < 0.001 one-way ANOVA—Tukey's multiple comparisons post-test) and (f) total adhesion sites using p-Tyr antibody (n = 10, **P < 0.01, ****P < 0.001 one-way ANOVA—Tukey's multiple comparisons post-test). Data are presented as mean ± s.e.m.

G Immunoblots of p-paxillin (Y118), paxillin, and GAPDH for Ctrl, Blebb, and Blebb + AP conditions.

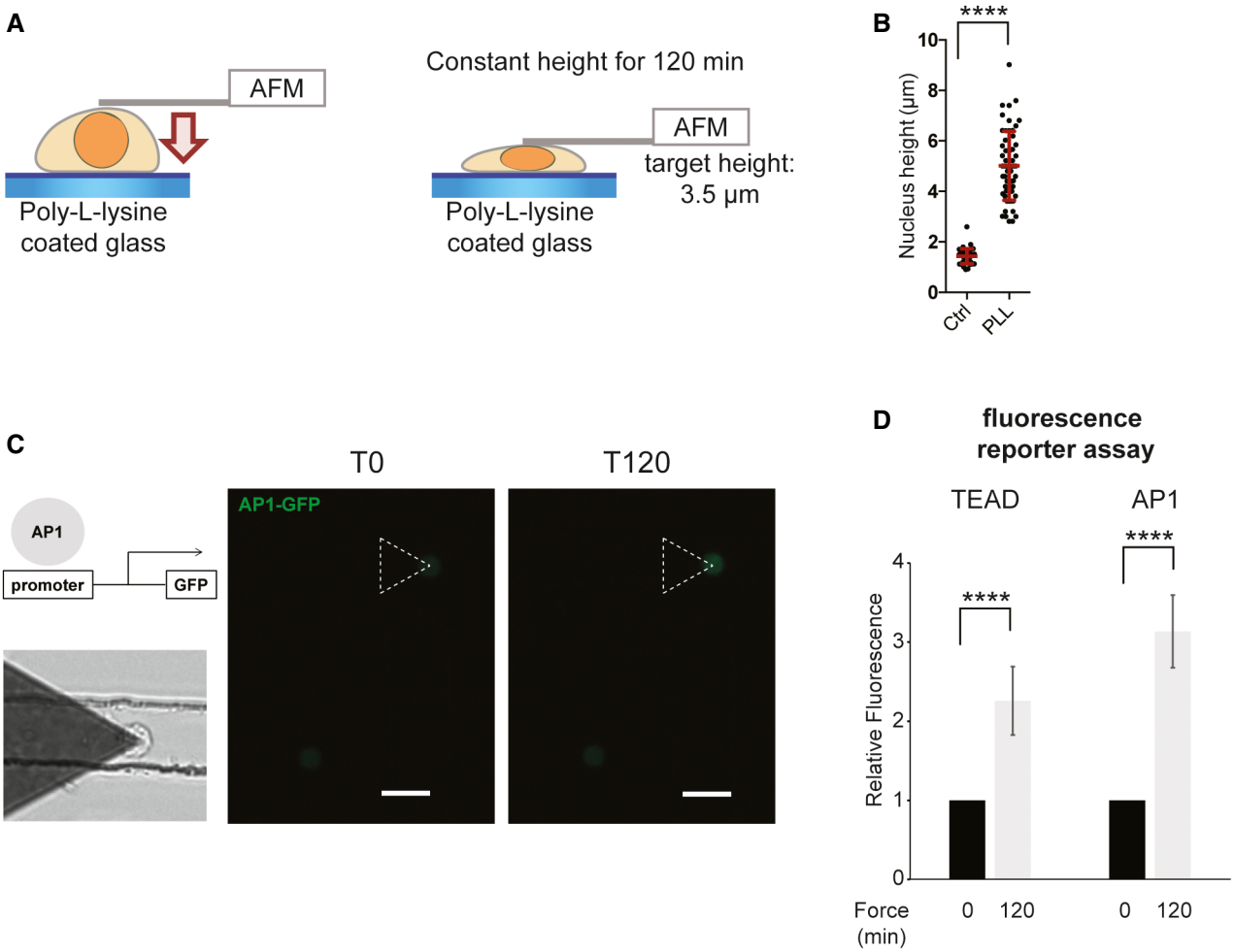


Figure EV2. TEAD and AP1 activation in response to nuclear compression by AFM.

A–D HeLa cells were transfected with AP1 GFP reporter or TEAD GFP reporter and plated on poly-L-lysine surface for 1 h. Then, atomic force microscopy was used to apply compressive force on the nucleus. After positioning the cantilever above the nucleus, constant height mode was used (target height of 3 µm below the surface) and maintained for 120 min. (c) Scale bar = 20 µm. Data are presented as mean ± s.e.m. (n = 12 minimum, ****P < 0.001 t-test).

Figure EV3. Nuclear shape regulates transcription factor activity.

A Membrane spot localization of the 15 TFs regulated by nuclear shape.

B Raw membranes of Affymetrix protein–DNA combo array kit after exposition to the ChemiDoc (Abcam) apparatus.

C mRNA expression for corresponding TF's target genes. Using the TRRUST database, we selected genes known to be positively regulated by these 15 TFs and we analyzed their mRNA expression: SPP1 gene for FOXD3; SLC4A9 gene for FOXI1; PCSK9 gene for HNF-4; AHRR gene for AHR; SOD2 gene for NFE2L2; BAMB1 gene for FOXF2; HBB gene for NFE2L2; PPAR gene for HIF1A; TGFB1 gene for USF1; and FAM89B gene for NFIA. Only genes with increase in mRNA expression superior to 1.5-fold change in response to nuclear flattening were selected. Data are presented as mean ± s.e.m. (n = 4 minimum, t-test *P < 0.5; ns: not significant).

D Protein–Protein Interaction Network reconstruction and analysis of the AP1, TEAD, SP1, and PPAR.

E Cell height was measured using AFM in cells cultured on soft or stiff matrix. Data are presented as mean ± s.e.m. (n = 15 minimum, ****P < 0.001 t-test).

F AP1 activity was measured in cells depleted or not for c-Jun in Ctrl, Blebb, and Blebb + AP conditions. Data are presented as mean ± s.e.m. (n = 5, ****P < 0.001 one-way ANOVA—Tukey's multiple comparisons post-test)

G Percentage of EdU-positive cells for cells depleted or not for c-Jun and YAP. Data are presented as mean ± s.e.m. (n = 4 independent experiments with at least 60,000 events for each condition). Data have been generated from two independent experiments using two different siRNAs targeting c-Jun and YAP. ***P < 0.01 one-way ANOVA—Tukey's multiple comparisons post-test.

H TEAD activity was analyzed in HeLa cells co-transfected with a Renilla plasmid as a luciferase reporter plasmid controlled by the TEAD-responsive promoter, and with a Renilla plasmid as a gene reporter; HeLa cells were depleted or not for SUN1 and SUN2. Data are presented as mean ± s.e.m. (n = 6, t-test *P < 0.5)

I AP1 activity was measured in cells depleted or not for SUN1 and SUN2. Data are presented as mean ± s.e.m. (n = 5, ****P < 0.001 t-test)

J mRNA expression for corresponding TF's target genes for HeLa cells depleted or not for SUN1 and SUN2. Data are presented as mean ± s.e.m. (n = 4, t-test *P < 0.5; **P < 0.1; ***P < 0.01).

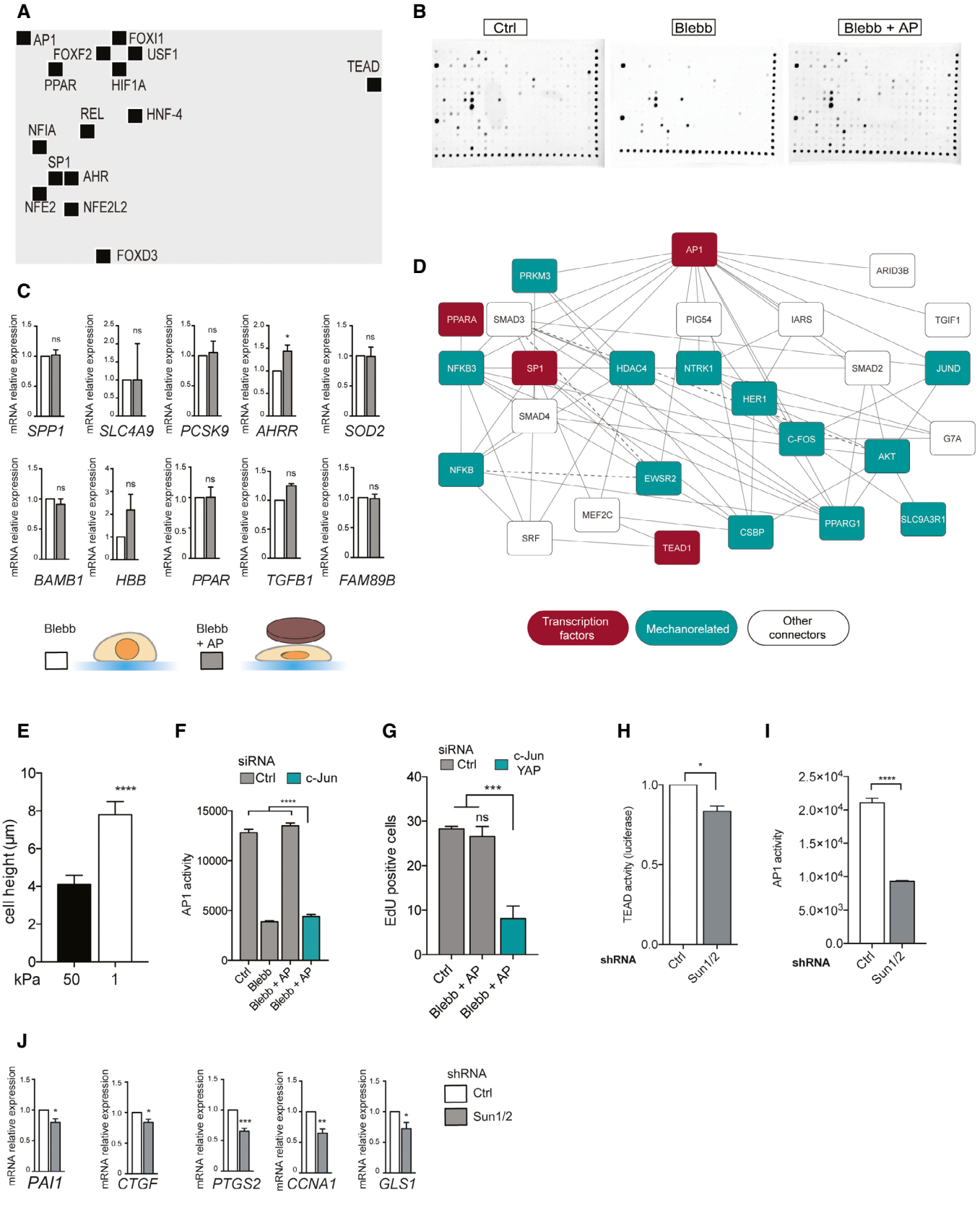


Figure EV3.

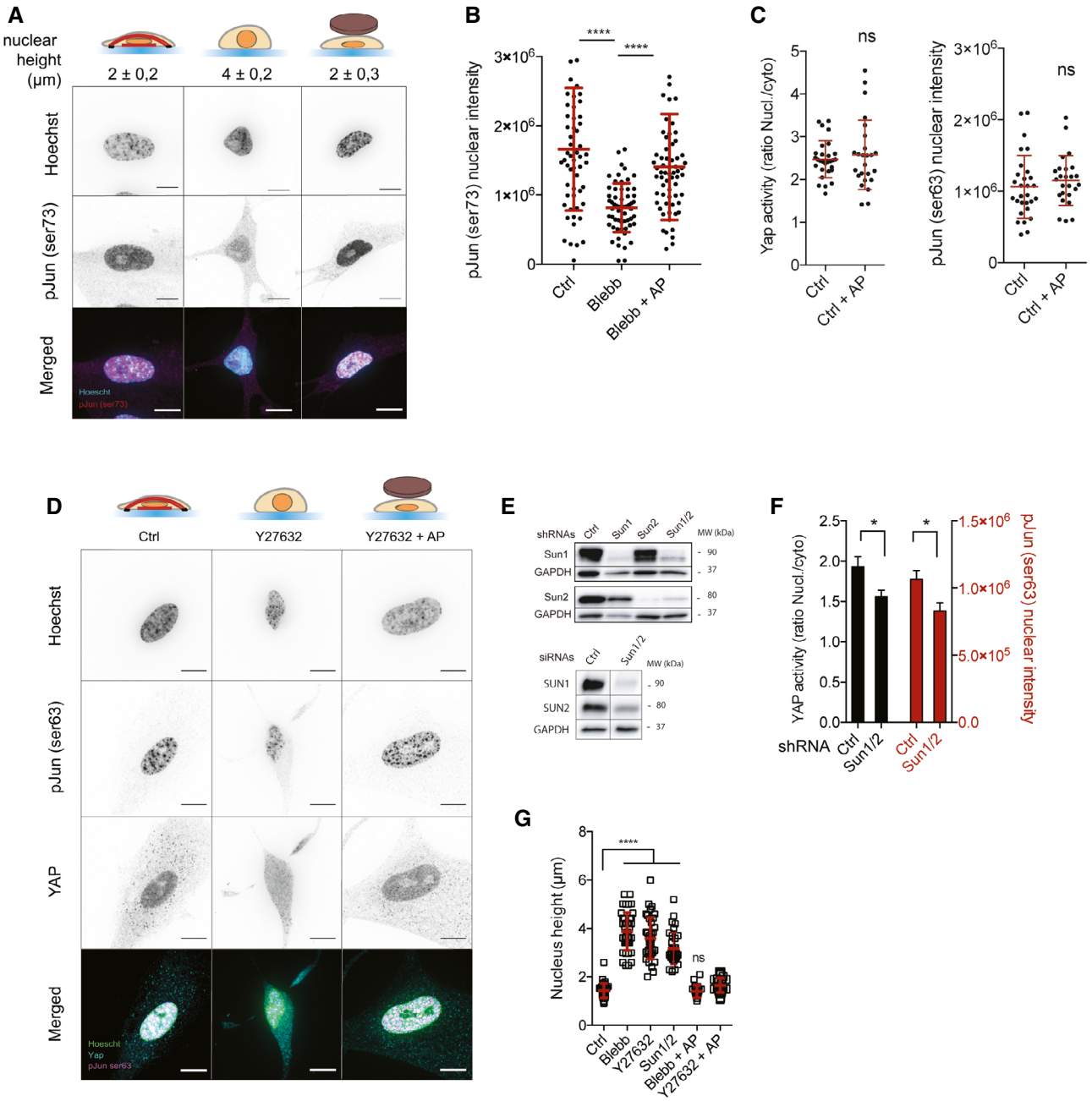


Figure EV4. Nuclear flattening is sufficient to activate c-Jun and YAP independently of actomyosin contractility.

A Representative cells stained for p-Jun Ser73 (magenta) and for nucleus (cyan) in Ctrl, Blebb, and Blebb + AP conditions. Scale bar = 10 µm. Nuclear heights were measured using Hoechst staining.

B Corresponding quantifications of p-Jun Ser73 nuclear intensity. Data are presented as mean ± s.e.m. (n = 58 minimum from two independent experiments, ****P < 0.001 one-way ANOVA—Tukey's multiple comparisons post-test).

C Quantifications of YAP activity (nucleo-cytoplasmic ratio) and p-Jun Ser63 nuclear intensity in Ctrl and Ctrl + AP conditions. Data are presented as mean ± s.e.m. (n = 25 minimum, ns: not significant, t-test).

D Representative cells stained for p-Jun Ser63 (magenta) and for DNA (cyan) in Ctrl, Y27632, and Y27632 + AP conditions. Scale bar = 10 µm.

E Immunoblots of SUN1, SUN2, and GADPH for MRC5 cells depleted or not for SUN1, SUN2, and SUN1 and SUN2 using siRNA or shRNA approaches.

F Quantifications of YAP activity (nucleo-cytoplasmic ratio) and p-Jun Ser63 nuclear intensity in cells depleted or not for SUN1 and SUN2. Data are presented as mean ± s.e.m. (n = 20 minimum, t-test *P < 0.5).

G Nucleus heights were measured using Hoechst staining in the indicated conditions. Data are presented as mean ± s.e.m. (n = 18 minimum, ****P < 0.001 one-way ANOVA—Tukey's multiple comparisons post-test).

Figure EV5. Molecular mechanisms mediating YAP and c-Jun activation in response to nuclear flattening.

A Representative cells stained for p-Jun Ser63 (magenta) and for DNA (cyan) in Ctrl, Blebb, and Blebb + AP conditions, treated or not with importazole. Scale bar = 10 μm.

B Quantifications of p- Jun Ser63 nuclear intensity in Ctrl, Blebb, and Blebb + AP conditions, treated or not with importazole. Data are presented as mean ± s.e.m. (n = 44 minimum from two independent experiments, ****$P < 0.001$ one-way ANOVA—Tukey's multiple comparisons post-test).

C Immunoblots of p-YAP (Ser127), YAP, and GFP for HeLa cells transfected with these following plasmids: Ctrl-GFP, LATS1 ^{WT}-GFP, LATS1T^{1079E}-GFP, LATS1^{T1079D}-GFP.

D Quantifications of YAP activity (nucleo-cytoplasmic ratio) in Ctrl, Blebb, and Blebb + AP conditions for cells transfected with GFP, LATS1-GFP, LATS1^{T1079E}-GFP, or LATS1^{T1079D}-GFP. Data are presented as mean ± s.e.m. (n = 18 minimum ****$P < 0.001$ one-way ANOVA—Tukey's multiple comparisons post-test).

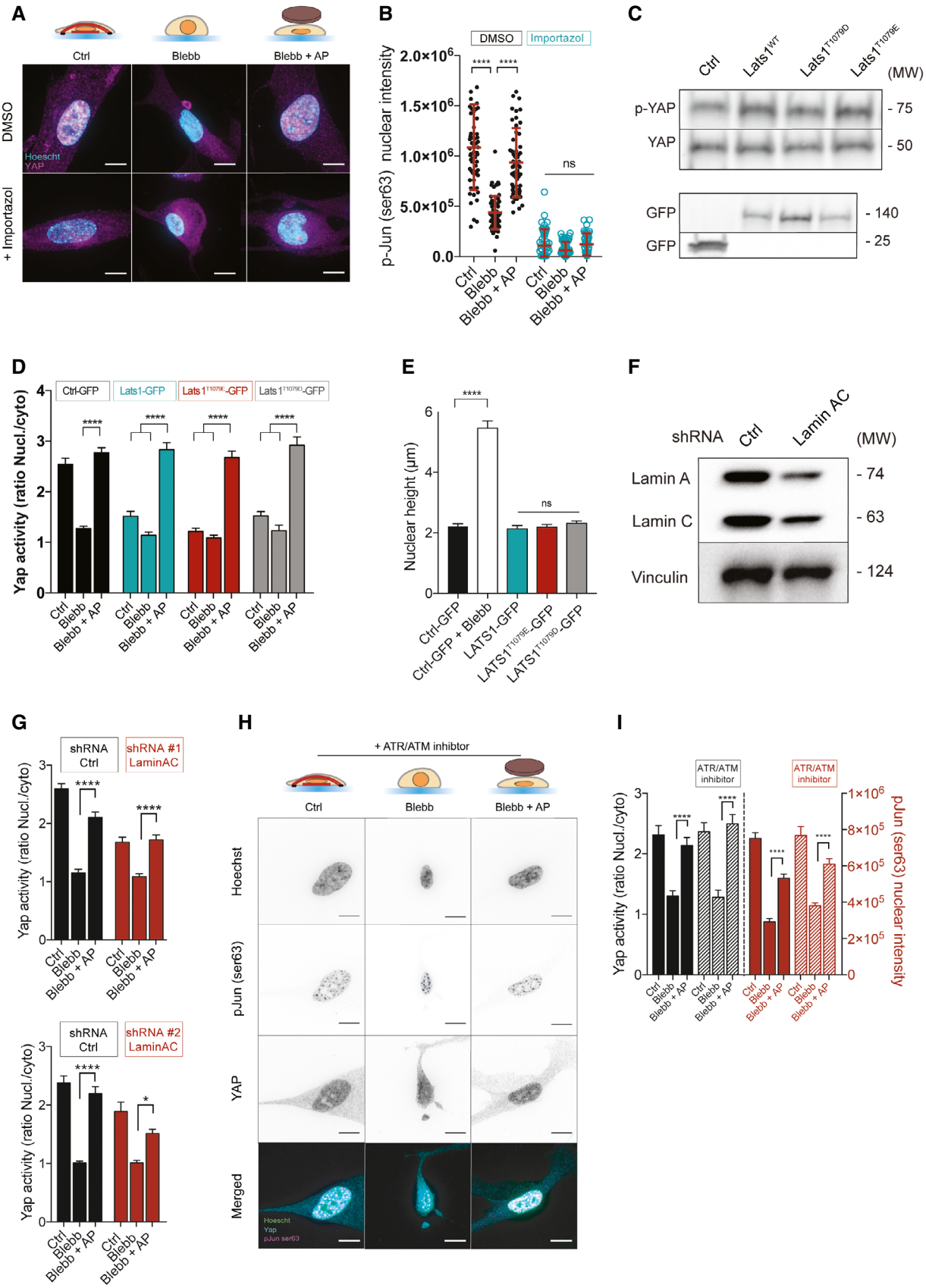
E Nuclear heights were measured for cells transfected with GFP in Ctrl and Blebb conditions, and for those transfected with LATS1-GFP, LATS1^{T1079E}-GFP, LATS1^{T1079D}-GFP in Ctrl condition. Data are presented as mean ± s.e.m. (n = 18 minimum ****$P < 0.001$ one-way ANOVA—Tukey's multiple comparisons post-test).

F Immunoblots of lamin A/C and vinculin for MRC-5 cells depleted or not for lamin A/C.

G Quantification of YAP activity in Ctrl, Blebb, and Blebb + AP conditions for cells depleted or not for lamin A/C. Data are presented as mean ± s.e.m. (n = 48 minimum (TOP), n = 12 minimum (BOTTOM), ****$P < 0.001$, *$P < 0.05$ one-way ANOVA—Tukey's multiple comparisons post-test).

H Representative cells stained for p-Jun Ser63 (magenta), for YAP (cyan), and for DNA (green) treated with ATR/ATM inhibitor. Scale bar = 10 μm.

I Quantifications of p-Jun Ser63 nuclear intensity in Ctrl, Blebb, and Blebb + AP conditions for cells treated with an ATR/ATM inhibitor. Data are presented as mean ± s.e.m. (n = 30, ****$P < 0.001$ one-way ANOVA—Tukey's multiple comparisons post-test.).


A
Ctrl
Blebb
Blebb + AP
DMSO
+ Importazol
Hoescht
YAP
B
DMSO
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p-Jun (ser63) nuclear intensity
2.0×10^6
1.5×10^6
1.0×10^6
5.0×10^5
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ns
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Blebb
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Ctrl
Lats1^WT
Lats1^T1079D
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p-YAP
YAP
- 75
- 50
GFP
GFP
- 140
- 25
D
Ctrl-GFP
Lats1-GFP
Lats1^T1079E-GFP
Lats1^T1079D-GFP
Yap activity (ratio Nucl./cyto)
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E
Nuclear height (µm)
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ns
Ctrl-GFP
Ctrl-GFP + Blebb
LATS1-GFP
LATS1^T1079E-GFP
LATS1^T1079D-GFP
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(MW)
Lamin A
Lamin C
Vinculin
- 74
- 63
- 124
G
shRNA Ctrl
shRNA #1 LaminAC
shRNA #2 LaminAC
Yap activity (ratio Nucl./cyto)
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Ctrl
Blebb
Blebb + AP
Hoechst
pJun (ser63)
YAP
Merged
Hoescht
Yap
pJun ser63
I
ATR/ATM inhibitor
Yap activity (ratio Nucl./cyto)
pJun (ser63) nuclear intensity
1×10^6
8×10^5
6×10^5
4×10^5
2×10^5
0
****

Figure EV5.