Figure S1

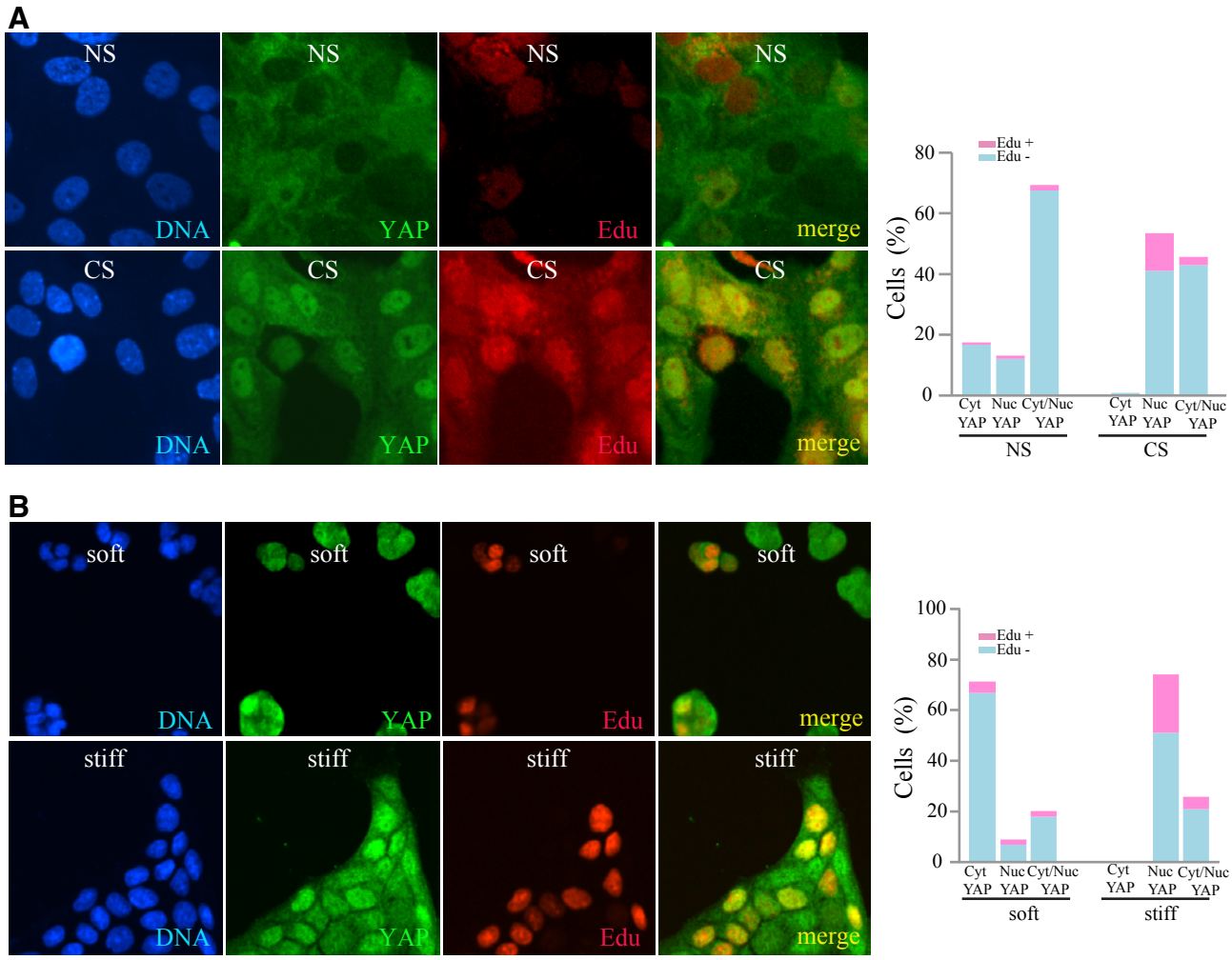

A
NS
DNA
YAP
Edu
merge
CS
B
soft
stiff
Edu +
Edu -
Cells (%)
Cyt YAP
Nuc YAP
Cyt/Nuc YAP
NS
CS
soft
stiff

Figure S2

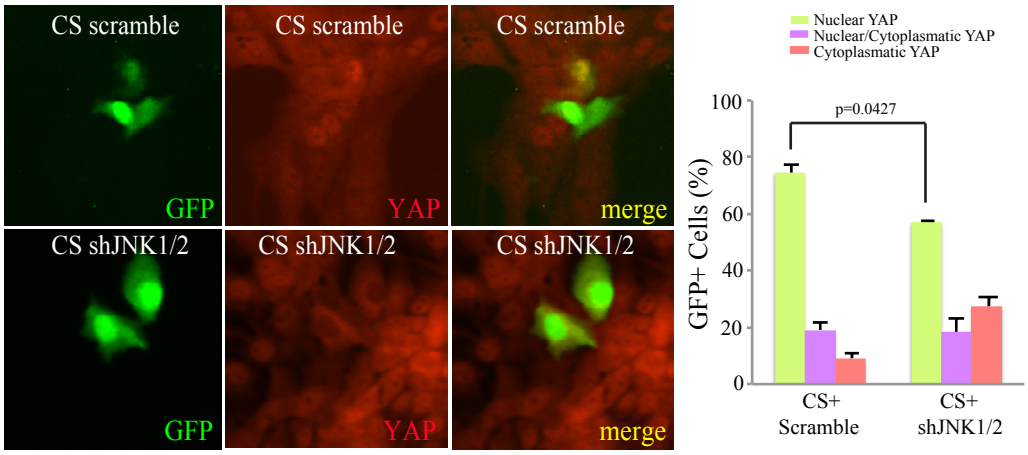

CS scramble
GFP
CS scramble
YAP
CS scramble
merge
CS shJNK1/2
GFP
CS shJNK1/2
YAP
CS shJNK1/2
merge
Nuclear YAP
Nuclear/Cytoplasmatic YAP
Cytoplasmatic YAP
p=0.0427
GFP+ Cells (%)
100
80
60
40
20
0
CS+
Scramble
CS+
shJNK1/2

Figure S3

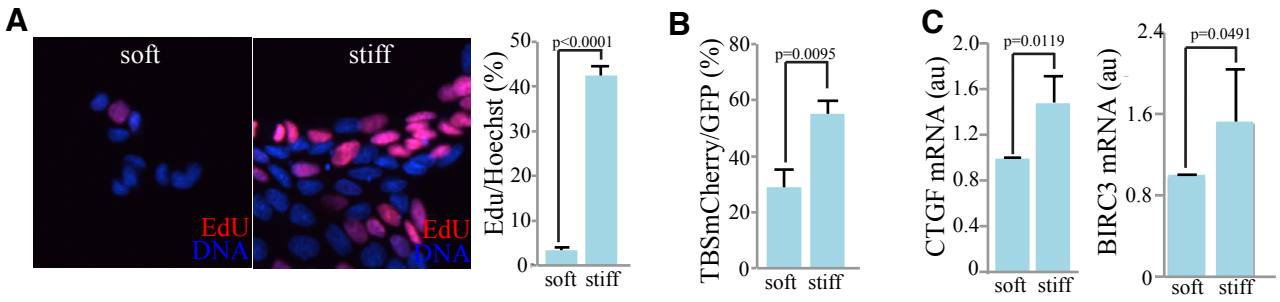
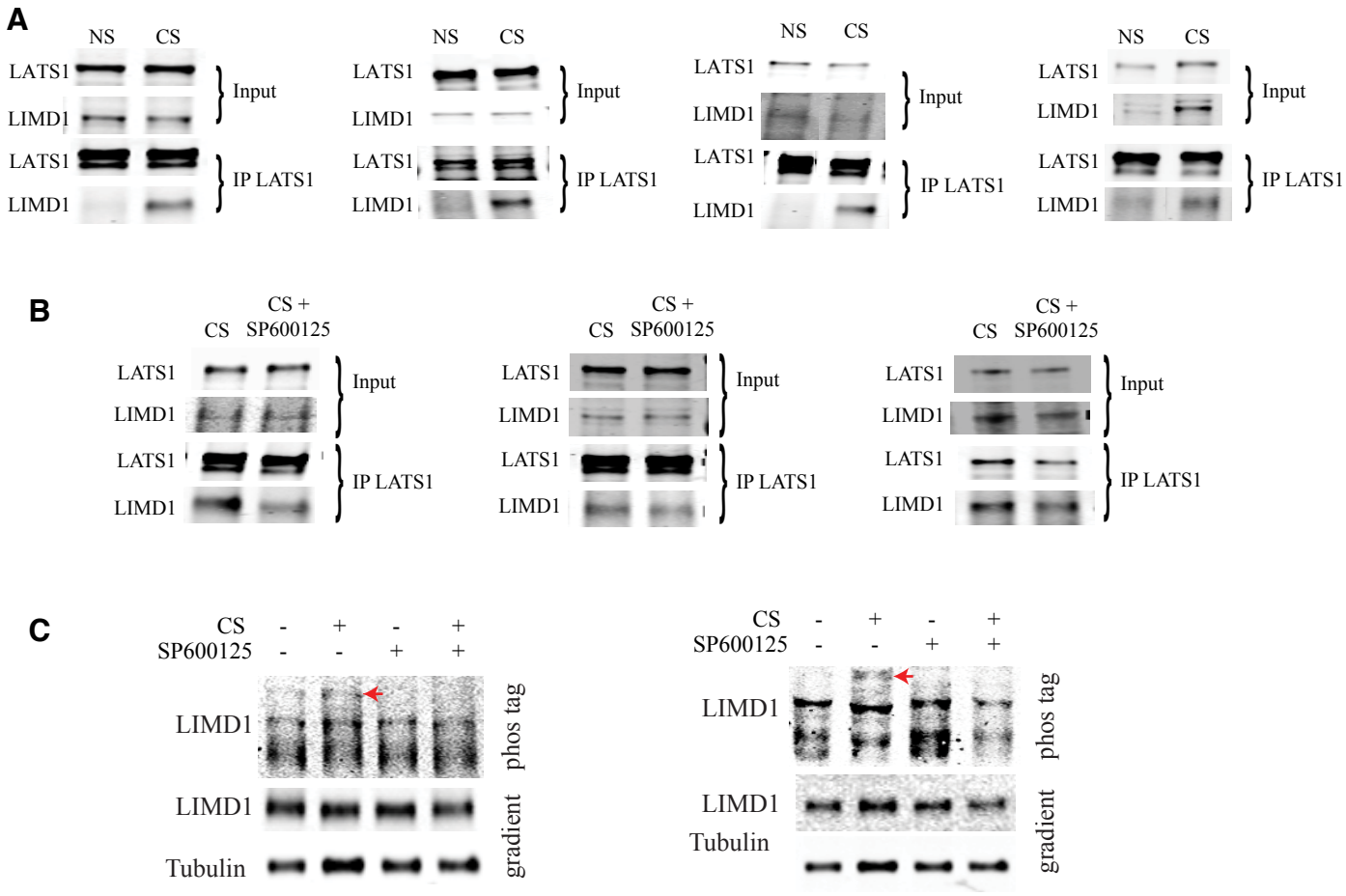

A
soft
stiff
EdU
DNA
EdU
DNA
Edu/Hoechst (%)
p<0.0001
soft
stiff
B
TBSmCherry/GFP (%)
p=0.0095
soft
stiff
C
CTGF mRNA (au)
p=0.0119
soft
stiff
BIRC3 mRNA (au)
p=0.0491
soft
stiff

Figure S4


A
NS
CS
LATS1
LIMD1
Input
IP LATS1
B
CS
CS + SP600125
C
SP600125
phos tag
gradient
Tubulin

Supplementary Figure S1: Correlation between YAP activity and cell proliferation, related to Figure 1

Immunolocalization of YAP (green) and Edu labeling (red) with nuclei labeled by Hoechst (DNA, blue). Histograms at right shows quantitation of the mean percent of cells EdU labeled cells (pink bars) or EdU negative cells (blue bars) according to their YAP localization profile, from three biological replicates comparing cells subject to CS or NS controls (A) or cells plated on soft versus stiff substrates (B).

Supplementary Figure S2: Activation of YAP by cyclic stretch is JNK-dependent, related to Figure 2

Quantitation of YAP localization among cells subject to 6h CS or NS controls, and treated either with a shJNK specific for JNK or a negative control shRNA (scramble) from three biological replicates. Immunolocalization, at the left, shows cells expressing shRNAs marked by GFP (green) and YAP localization (red). Error bars indicate standard error of the mean.

Supplementary Figure S3: Cell proliferation and YAP activation induced by ECM stiffness, related to Figure 3

Comparisons of MCF10A cells plated on soft (0.2 kPa) versus stiff (35 kPa) substrates. A) Cell proliferation, visualized by Edu labeling (red), with nuclei labeled by Hoechst (blue). Histogram at right shows quantitation of the mean percent labeled cells, from three biological replicates. B) YAP activity. Histogram shows mean percentage of transfected cells (GFP-expressing) that are positive for TBS-mCherry, from three biological replicates. C) Histogram shows result of quantitative RT-PCR on BIRC3 and CTGF mRNA level in cells plated on soft and stiff substrates from three biological replicates. The mRNA over GAPDH ratios were normalized to the ratio under soft conditions. Error bars indicate standard error of the mean.

Supplementary Figure S4: Cyclic Stretch increases LIMD1-LATS1 binding to activate YAP , related to Figure 4

Comparisons of MCF10A cells subject to 6h cyclic stretch (CS) and non-stretched controls (NS) or cells treated with SP600125, as indicated. This figure shows multiple examples of the blots supporting the results depicted in Fig. 4, from independent biological replicates. In all panels, error bars show standard error. A) Western blots showing the results of co-immunoprecipitation experiments on cells subject to CS or NS controls. Upper two blots (input) show relative amounts of endogenous LATS1 and LIMD1 in cell lysates, lower two panels show relative amounts immunoprecipitated using anti-LATS1 sera. B) Western blots showing the results of co-immunoprecipitation experiments on cells subject to CS and treated with SP600125 or vehicle control. Upper two blots (input) show relative amounts of endogenous LATS1 and LIMD1 in cell lysates, lower two panels show relative amounts immunoprecipitated using anti-LATS1 sera. C) Western blots on lysates of MCF10A cells treated with DMSO or SP600125, and with or without CS, as indicated. The lower two blots show a standard 4-15% gradient gel, and the upper blot shows a Phos-tag gel. The arrow indicates a band of slow mobility (highly phosphorylated) LIMD1 induced by CS, and suppressed by SP600125.

Supplemental Experimental Procedures

Cell culture, transfection and treatment.

MCF10A cells were cultured in DMEN/F12 medium (Life Technologies) supplemented with 5% horse serum, 20 mg/ml EGF, 10 mg/ml insulin, 0.1mg/ml cholera toxin, 1 mg/ml hydrocortisone and antibiotic-antimicotic at 37^0C and 5%CO_2. For cyclic stretch experiments, MCF10 cells were seeded over silicon chambers previously sterilized with 20 min of UV radiation and coated with collagen (Sigma). Cells were allowed to spread on the membranes in complete media, and then serum starved overnight prior to stretching.

For substrate stiffness experiments, polyacrylamide gel was prepared on glass cover slips to provide soft matrix for cell culture as described previously[S1]. Brielfy, the cover slips surface were smeared trwith a drop of 0.1N NaOH and air-dried. Thereafter, the cover slips were treated with 3-aminopropyltrimethoxysilane and then with 0.5% glutaraldehyde. Polyacrylamide gels of different stiffness were polymerized in between the treated cover slip at the bottom and an untreated cover slip on the top[S1]. After polymerization, the untreated cover slip was removed to expose the ~100 μm thin layer of gel to be used as cell culture matrix. Previous studies have shown that the stiffness of the polyacrylamide gel matrix is directly proportional to the concentration of *bis*-acrylamide cross-linker. After polimerization the expected modulus of elasticity was 0.2 kPa for soft condition and 35 kPa for stiff condition[S1]. Finally, covers were coated with 0.2 mg/ml collagen (sigma) overnight in the cold room, then rinsed with PBS andexposed to UV for 15 min before use.

For cyclic stretch experiments, cells were incubated with DMEN/F12 starvation medium (supplemented only with antibiotic-antimicotic) 12 h prior to stretching. Cells were transfected with Lipofectamine 2000 (Life Techonologies) according to manufacturer's protocols, and

harvested 24 h after transfection. For the inhibition of JNK, cells were treated with 50 μM SP600125 in DMSO for 6h, with DMSO only used as a vehicle control in non-treated cells.

Plasmids.

TBSmCherry plasmid was a gift from Dr. Fernando Camargo[S2] while pBABE-GFP was adquired from Addgene. pFLRu shLIMD1 and scramble plasmid were a gift from Dr. Dan Foxler[S3]. shJNK1/2 plasmid was a gift from Dr. Stelios Andreadis [S4].

Stretch Experiments.

MCF10A were stretched using a mechanical cell stretching instrument (STREX) at a frequency of 1 Hz with 20% of linear stretch. The device was kept in a humidified 5% CO_2 incubator at 37^0C.

Inmunoblotting and Immunoprecipitation

Cells were lysed in RIPA buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA , 1% NP-40) supplemented with protease inhibitor cocktail (Roche) and phosphatase inhibitor cocktail (Calbiochem). Samples were run on a 4-15% gradient gels (Bio-Rad). Transferred onto nitrocellulose membranes (Bior-Rad) and blocked with Blocking Buffer (Li-Cor Biosciences). For immunoprecipitation, protein samples were incubated with rabbit anti-LATS1 (Cell Signaling Technologies) overnight followed by incubation with protein G sepharose (GE Healthcare) for 1h at 4^0C. Blots were incubated (1:1000) with rabbit anti-YAP (Abcam), rabbit anti-phospho (Ser127)YAP (Cell Signaling Technologies), rabbit anti-TAZ (Cell Signaling Technologies), goat anti-LATS (Santa Cruz Biotechnologies), rabbit anti-phospho

(Thr1079)LATS1 (Cell Signaling Technologies), rabbit anti-MST1/MST2 (Cell Signaling Technologies), rabbit anti-phospho (Thr183)MST1/(Thr180)MST2 (Cell Signaling Technologies), rabbit anti-LIMD1 (Bethyl Laboratories). Blots were visualized and quantified using fluorescent-conjugated secondary antibodies (Li-Cor Biosciences) and Odyssey Imaging System (Li-Cor Biosciences).

Phos-tag gel

Cells were lysed in 50mM Tris·HCl pH7.5, 150mM NaCl, 1% Triton X-100, 0.1% NP-40. Lysates were applied to 6% SDS-PAGE containing 25mM Phos-tag Acrylamide AAL-107 (Wako pure chemical industries, Ltd) and 50mM $MnCl_2$, followed by western blotting.

Stadistical Analysis

Stadistical differences were determined using t-student or ANOVA, with $p<0.05$ set as criteria for significance.

Quantitative RT-PCR

RNA was extracted from MCF10A cells treated with different drugs using Trizol reagent (Life Technologies). SuperScript III reverse transcriptase (Life Technologies) was used for reverse transcription. Quantitative PCR was conducted using QuantiTect SYBR green PCR kit (Qiagen).

Inmunostaining and Proliferation Assay.

Cells were fixed with 4% paraformaldehide for 20 min at Room Temperature, then washed with PBS and permeabilized with PBS containing 0.3% TritonX-100 for 30 min, and blocked by 5%

BSA. Antibodies for immunostaining (1:100) included mouse anti-YAP (Santa Cruz Biotechnologies) and rabbit anti-TAZ (Cell signaling Techonologies). For evaluating cell proliferation we used Click-it Edu Cell Proliferation kit (Life Technologies) according to the manufacturer's protocols. Cells were visualized on a confocal microscope (PerkinElmer) and anayzed with Volocity Software (PerkinElmer).

Supplemental References

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