Appendix - additional supporting results

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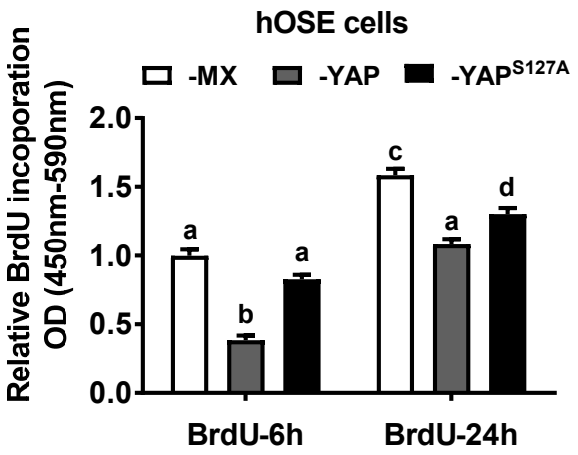
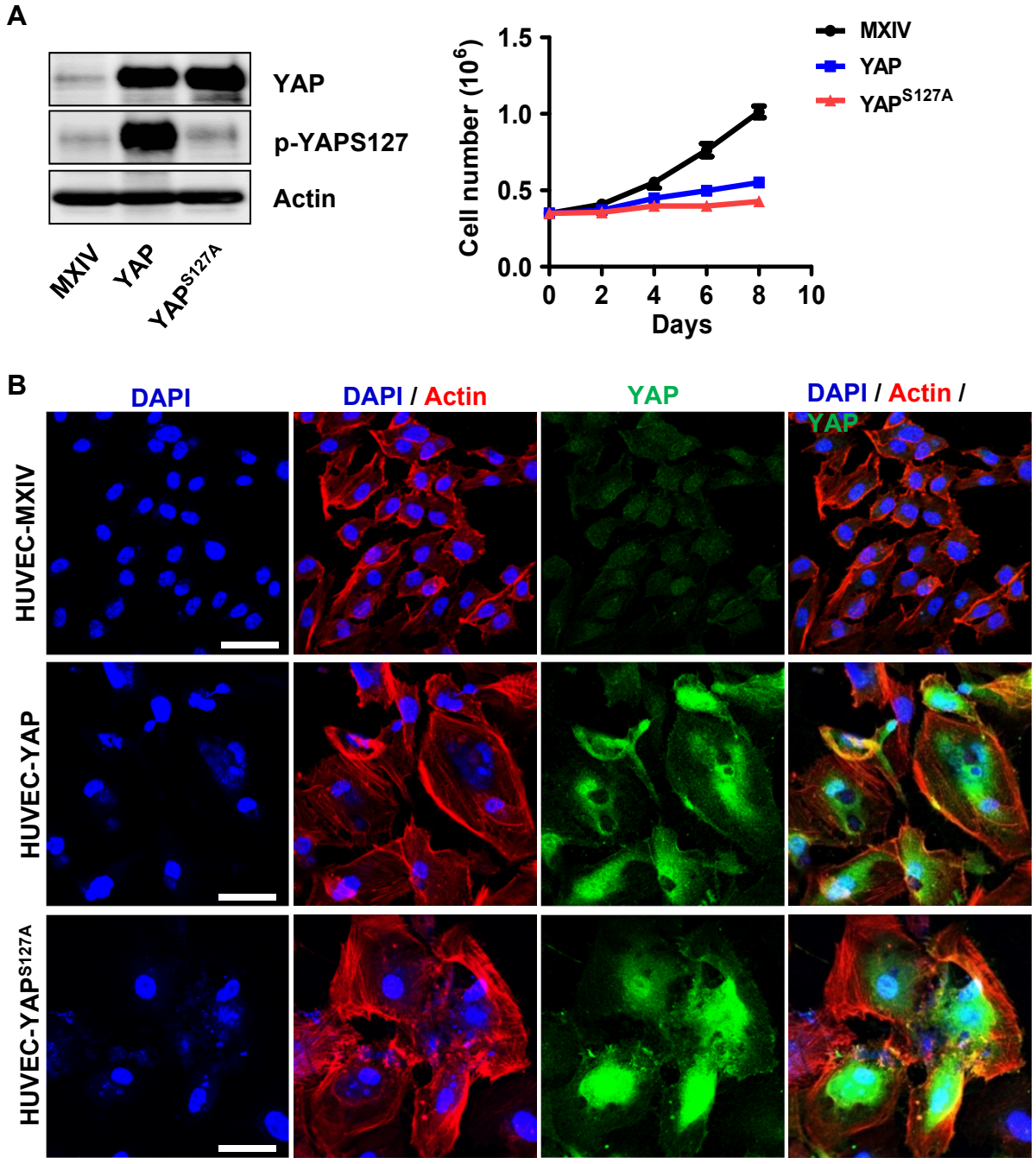
Appendix Figure S1



Appendix Figure S1. YAP1 inhibits BrdU incorporation in the late passage hOSEs.

The 7th passage hOSE-MX cells (hOSEs transfected with empty vector as control), hOSE-YAP cells (hOSEs transfected with vectors expressing wild type YAP1), and hOSE-YAP^{S127A} cells (hOSEs transfected with vectors expressing YAP^{S127A} , a constitutively active YAP1) were seeded in a 96-well plate (n=6) at 5000 cells / well and incubated for 48 hours. The cells were then incubated in BrdU-containing media for 6 or 24 hours. The relative BrdU incorporation rates (indicated by OD450/OD590) in each group were calculated following the instruction provided in the BrdU cell proliferation assay kit (Cell Signaling Technologies, inc. #6813). Each bar represents mean ± SEM (n = 6). Bars with different letters are significantly different from each other ($P < 0.05$).

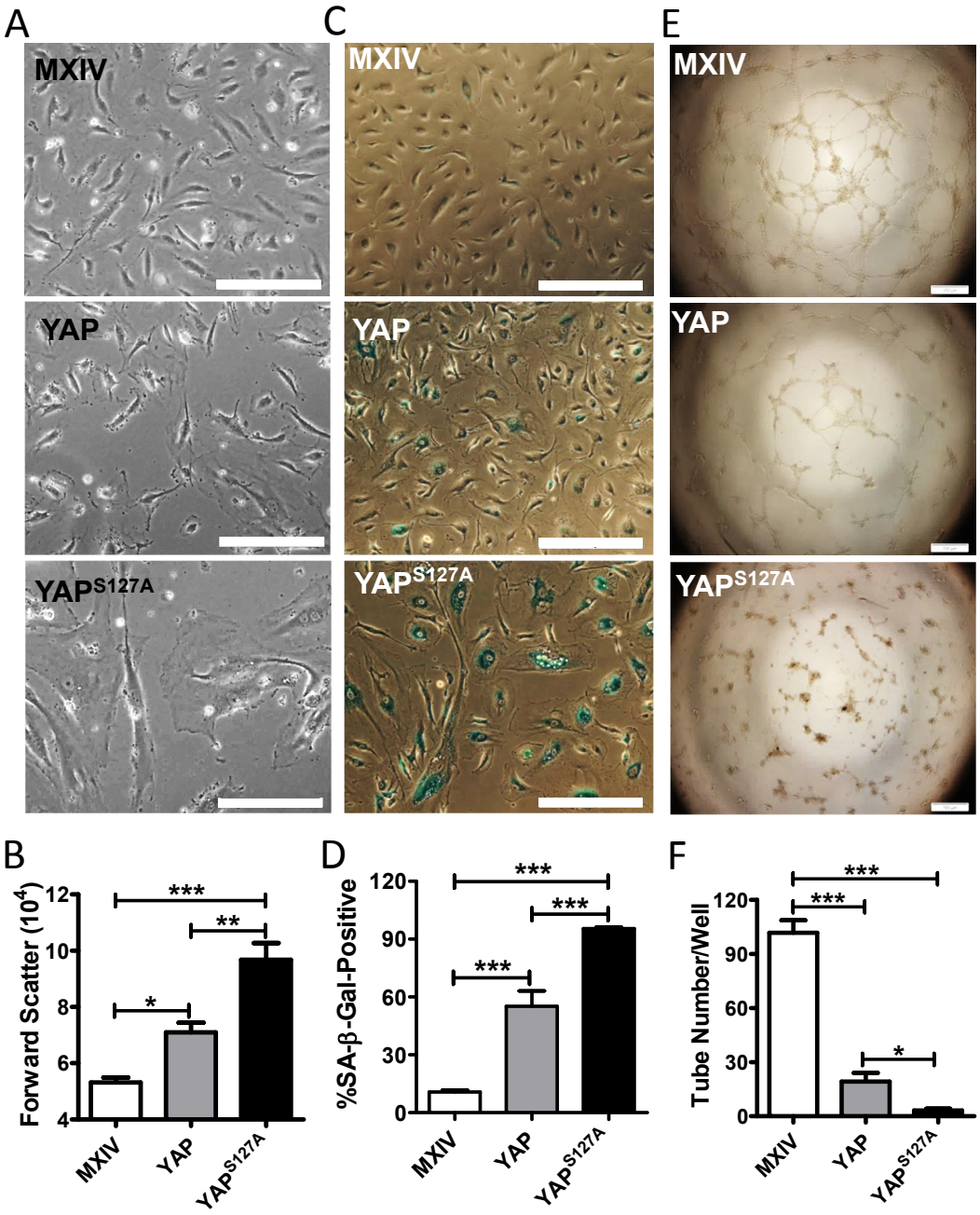
Appendix Figure S2



Appendix Figure S2. hyperactivation of YAP1 induced senescence in HUVEC cells.

A) YAP overexpression suppressed HUVEC Cell Proliferation. Left panel: representative images showing that the YAP and constitutively activated YAP^{S127A} were successfully expressed in HUVEC cells. Right panel: expression of YAP and YAP^{S127A} significantly suppressed HUVEC cells (passage four) proliferation. **B**) YAP induces cellular morphologic change in HUVEC cells**.** Representative images showed the expression and location of YAP in the forth passage of HUVEC-MXIV, HUVEC-YAP and HUVEC-YAP^{S127A} cells. YAP was visualized using an Alexa-488 (Green) conjugated secondary antibody. Nuclei were stained with DAPI. Subculture for 14 days. Scale bar = 50μm.

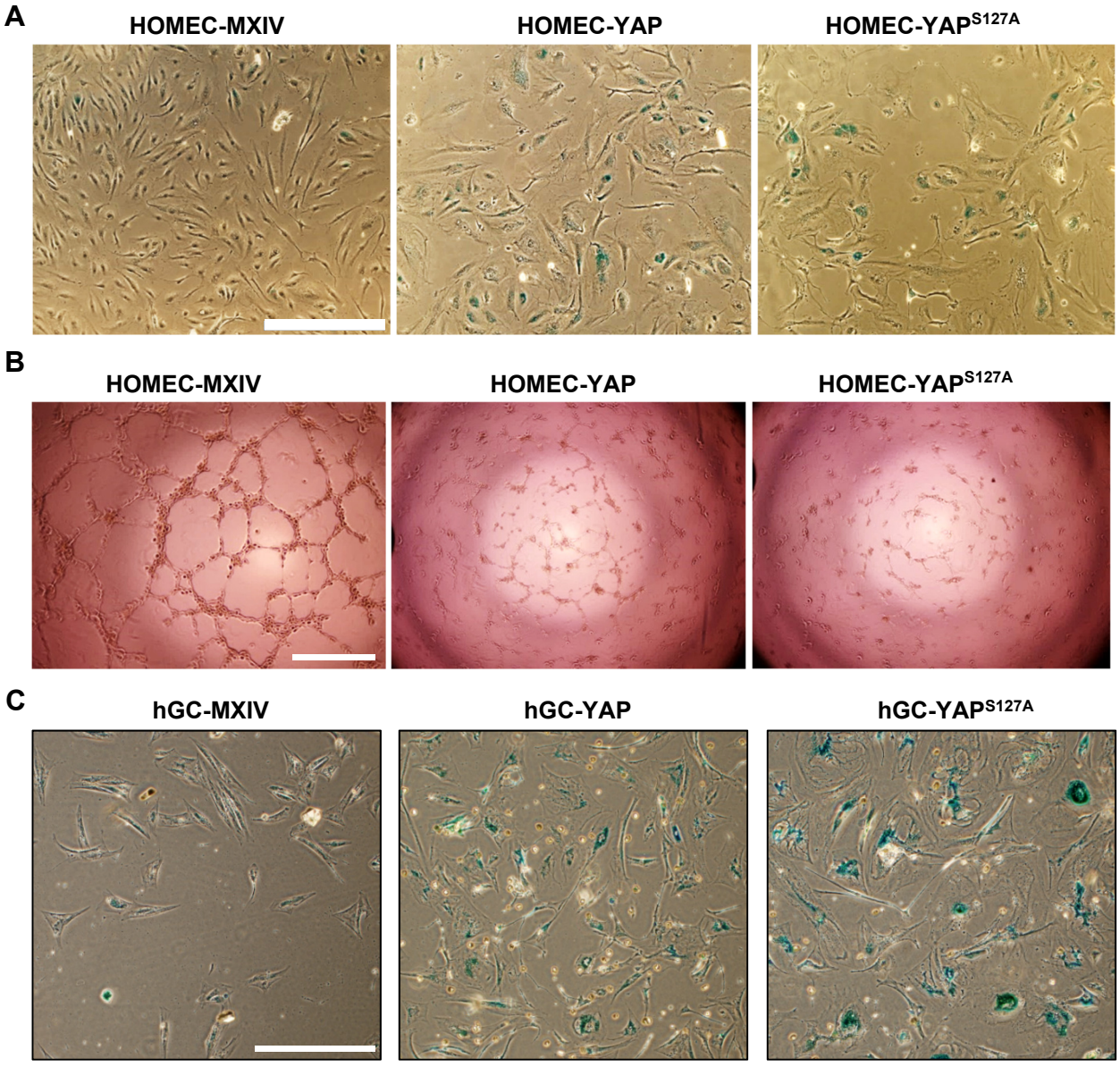
Appendix Figure S3



Appendix Figure S3. YAP induces cellular senescence in human endothelial cells.

A) Representative images showing morphologic changes of HUVEC cells with ectopic expression of YAP or YAP^{S127A}. scale bar: 200μm. **B)** Quantitative data showing the Forward Scatter (FS: proportional to cell size) of HUVEC-MXIV, HUVEC-YAP and HUVEC-YAP^{S127A} cells. **C**) Representative images showing SA-β-gal staining in HUVEC-MXIV, HUVEC-YAP and HUVEC-YAP^{S127A} cells. scale bar: 200μm. **D**) Quatitative data of C). **E)** Representative images showing tubule formation ability of HUVEC-MXIV, HUVEC-YAP and HUVEC-YAP^{S127A} cells. Scale bar: 1mm. **F)** Quantitative data of (E) to show the ratio of SA-β-gal positive cells in HUVEC-MXIV, HUVEC-YAP and HUVEC-YAP^{S127A} cells. All cells were passaged for three times. All experiments were repeated at least three times. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$, compared to the control groups (MXIV).

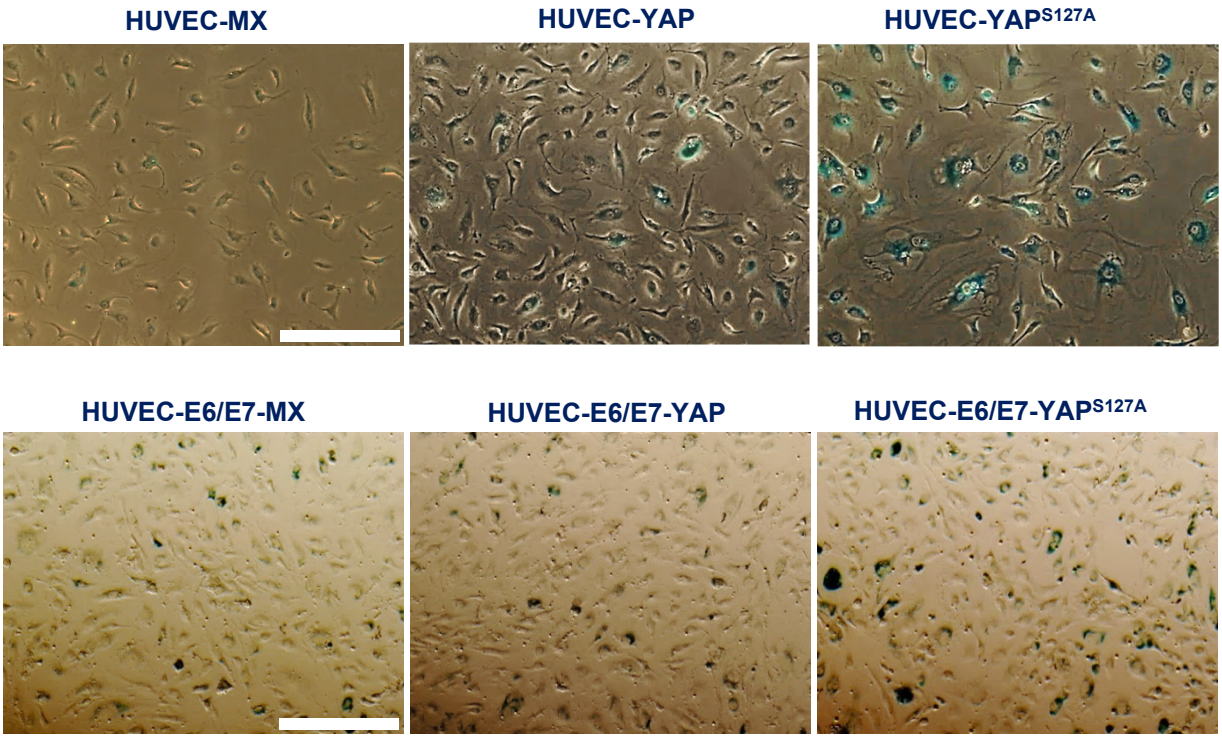
Appendix Figure S4



Appendix Figure S4. YAP1 induced senescent phenotype in cultured human ovarian endothelial cells (HOMEC) and Ovarian granulosa cells.

A) YAP1 induced cellular senescence in Human Ovarian Microvascular Endothelial Cells (HOMEC). Scale bar = 200 µm. **B)** tube formation in HOMEC cells transfected with or without vectors expression wild-type YAP or Constitutively active YAP (YAP^{S127A}). Scale Bar = 1.0 mm. **C)** Representative images showed SA-b-gal staining in the two months cultured primary human ovarian granulosa cells (hGC) hGC-MXIV, hGC-YAP and hGC-YAP^{S127A} cells; Scale bar = 100 µm.

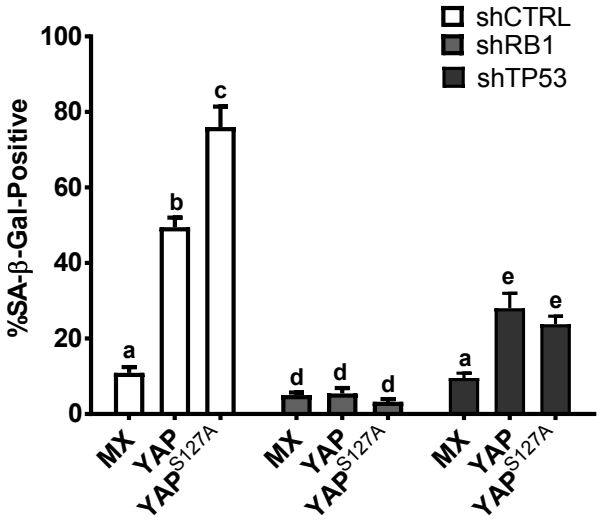
Appendix Figure S5



Appendix Figure S5. HPVE6/E7 inhibits YAP-induced senescence in HUVEC cells.

Representative images showed SA-β-gal staining in the fourth passage of HUVEC-MXIV, HUVEC-YAP and HUVEC-YAP^{S127A} cells with or without E6/E7 expression. Expression of HPV E6/E7 in HUVEC cells prevent these cells from YAP-induced senescence. All experiments are repeated at least three times. Scale bar: 200 μm.

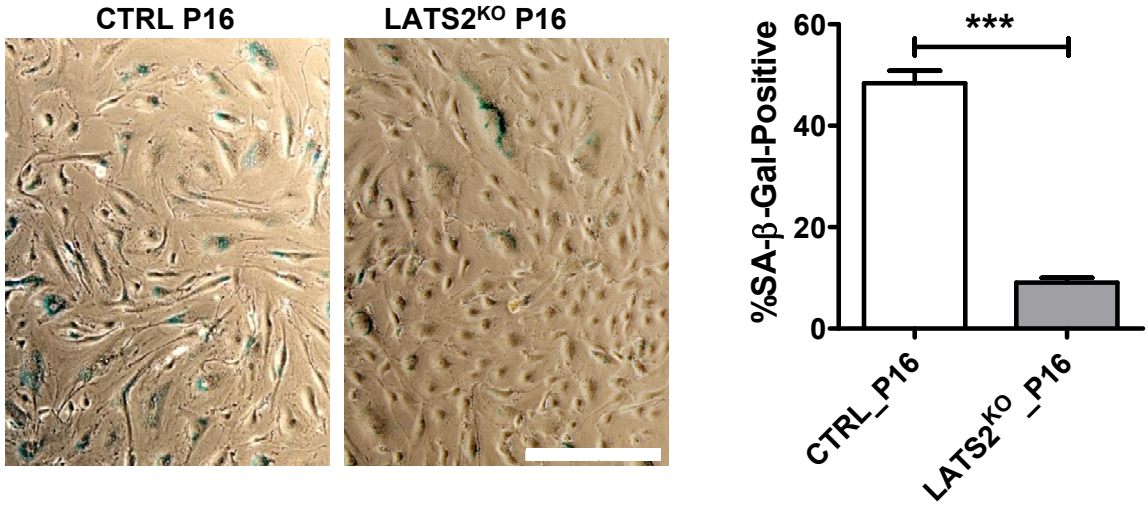
Appendix Figure S6



Appendix Figure. S6. Effects of RB1 and TP53 on YAP1-induced senescence of hOSE cells.

Quantitative data of Figure 4C showing the ratio of SA-β-gal positive cells in hOSE-MX, hOSE-YAP, and hOSE-YAP^{S127A} cells with or without knockdown of RB1 and TP53 with lentivirus-based shRNAs against RB1 (shRB1) and TP53 (shTP53). Non-targeting shRNA was used as a control (shCTRL). Each bar represents mean ± SEM (n = 4). Bars with different letters are significantly different from each other ($P < 0.01$).

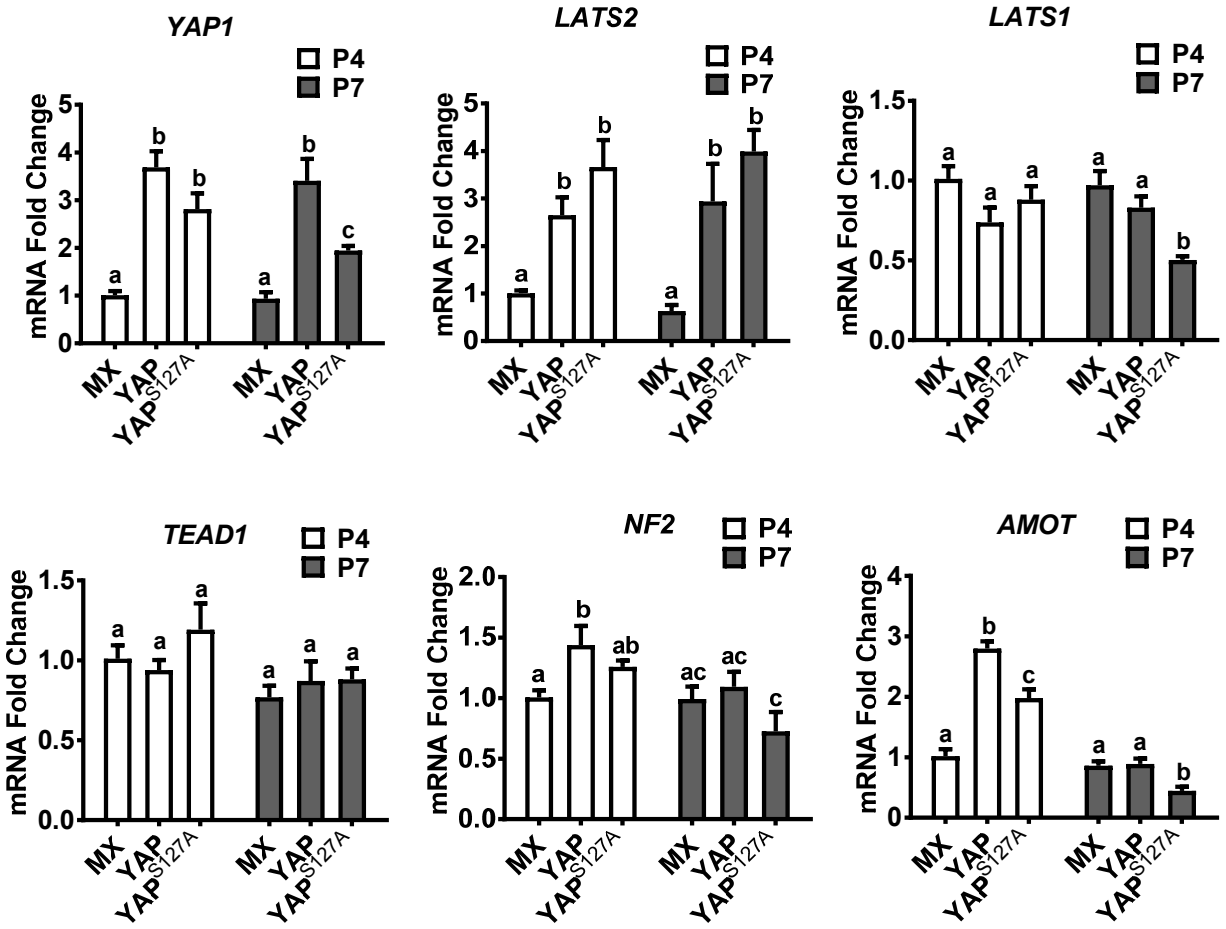
Appendix Figure S7



Appendix Figure S7. LATS2 knockout inhibits natural replicative senescence in HUVEC cells.

Left panel: Representative images showing SA-β-gal staining in HUVEC cells with (LATS2^{KO} P16) or without (CTRL P16) LATS2 knockout. P16: Cells were passaged for 16 times before β-gal staining. Scale bar: 200μm. Right panel: Quantitative data (n=5) showing the percentage of SA-β-gal positive cells in control HUVEC cells and LATS2-knockout HUVEC cells (LATS2^{KO} P16). Each bar represents mean ± SEM (n=5). ***: $P < 0.001$, compared to the control (CTRL_P16).

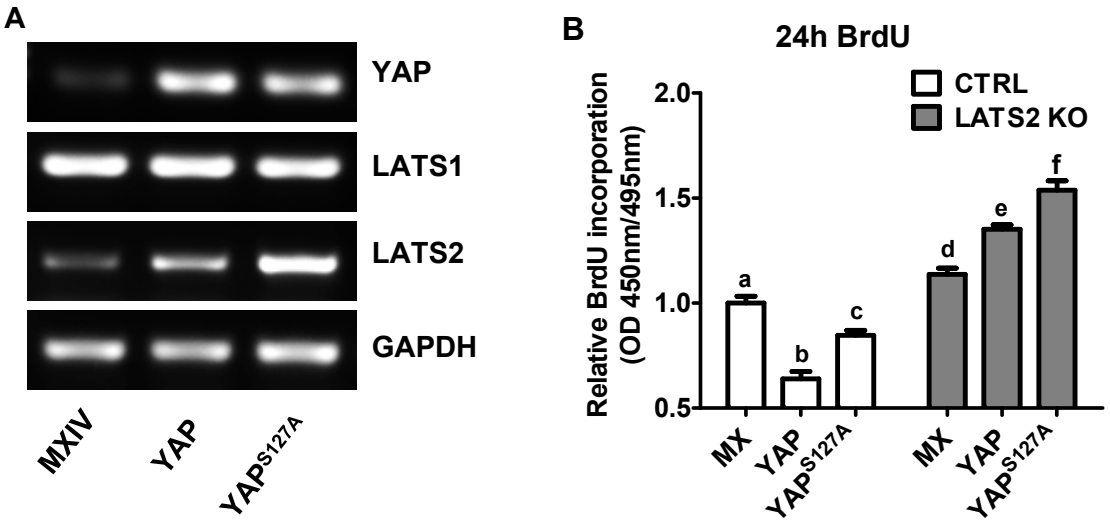
Appendix Figure S8



Appendix Figure S8. LATS2 is up-regulated in YAP1-induced senescent cells.

mRNA expressions of YAP1, LATS1, LATS2 (upstream suppressor of YAP1), TEAD1(binding transcript factor of YAP1), NF2 and AMOT (two known negative-feedback regulators of YAP1) in hOSE-MX cells (hOSEs transfected with empty MXIV vectors), hOSE-YAP cells (hOSEs transfected with vectors expressing wild-type YAP), and hOES-YAPS127A cells (hOSEs transfected with vectors expression constitutively active YAP, indicated as YAPS127A) at their 4th and 7th passages. Data was normalized with mRNA levels of GAPDH. Each bar represent mean ± SEM (n = 4). Bars with different letters are significantly different from each other.

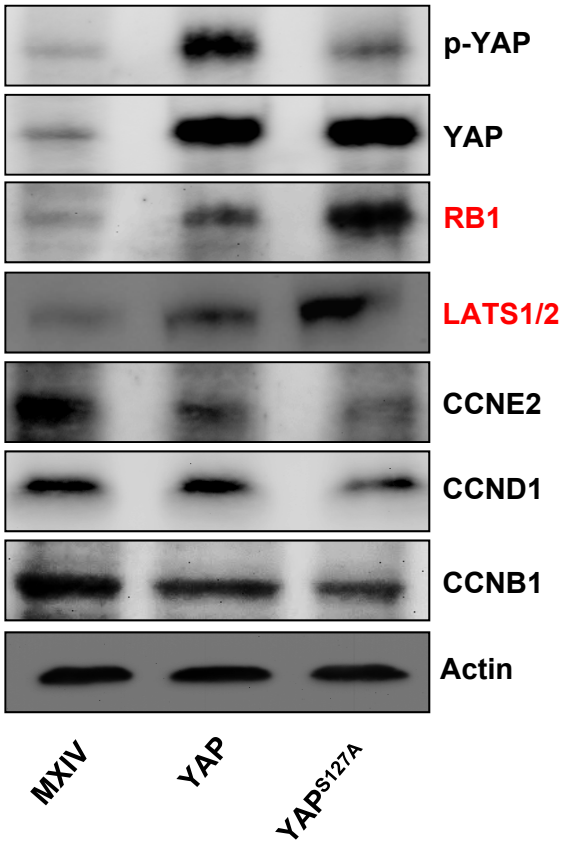
Appendix Figure S9



Appendix Figure S9. Deletion of LATS2 prevents YAP-induced senescence in hOSE cells.

A) Representative gel photographs showing mRNA levels of *LATS1*, *LATS2*, and *YAP1* in hOSE-MX cells (hOSEs transfected with empty vector as control), hOSE-YAP cells (hOSEs transfected with vectors expressing wild type YAP1), and hOSE-YAP^{S127A} cells (hOSEs transfected with vectors expressing YAP^{S127A} , a constitutively active YAP1). mRNA levels were examined using semi-quantitative RT-PCR at passage seven. **B**) Silencing of LATS2 aided YAP1 to promote cell proliferation as detected by BrdU cell proliferation assay. hOSE-MX cells, hOSE-YAP cells, and hOSE-YAP^{S127A} cells with or without LATS2 knockout (LATS2^{KO}) were seeded in a 96-well plat (n=6) at 5000 cells/well. After incubating for 48 hours, BrdU was added into culture media and cells were incubated for another 24 hours. The relative BrdU incorporation was calculated using OD450-OD590 values following the instruction provided in the BrdU cell proliferation Assay kit (Cell Signaling Technologies, Inc. #6813). Each bar represents mean ± SEM (n = 6). Bars with different letters are significantly different from each other ($P < 0.05$).

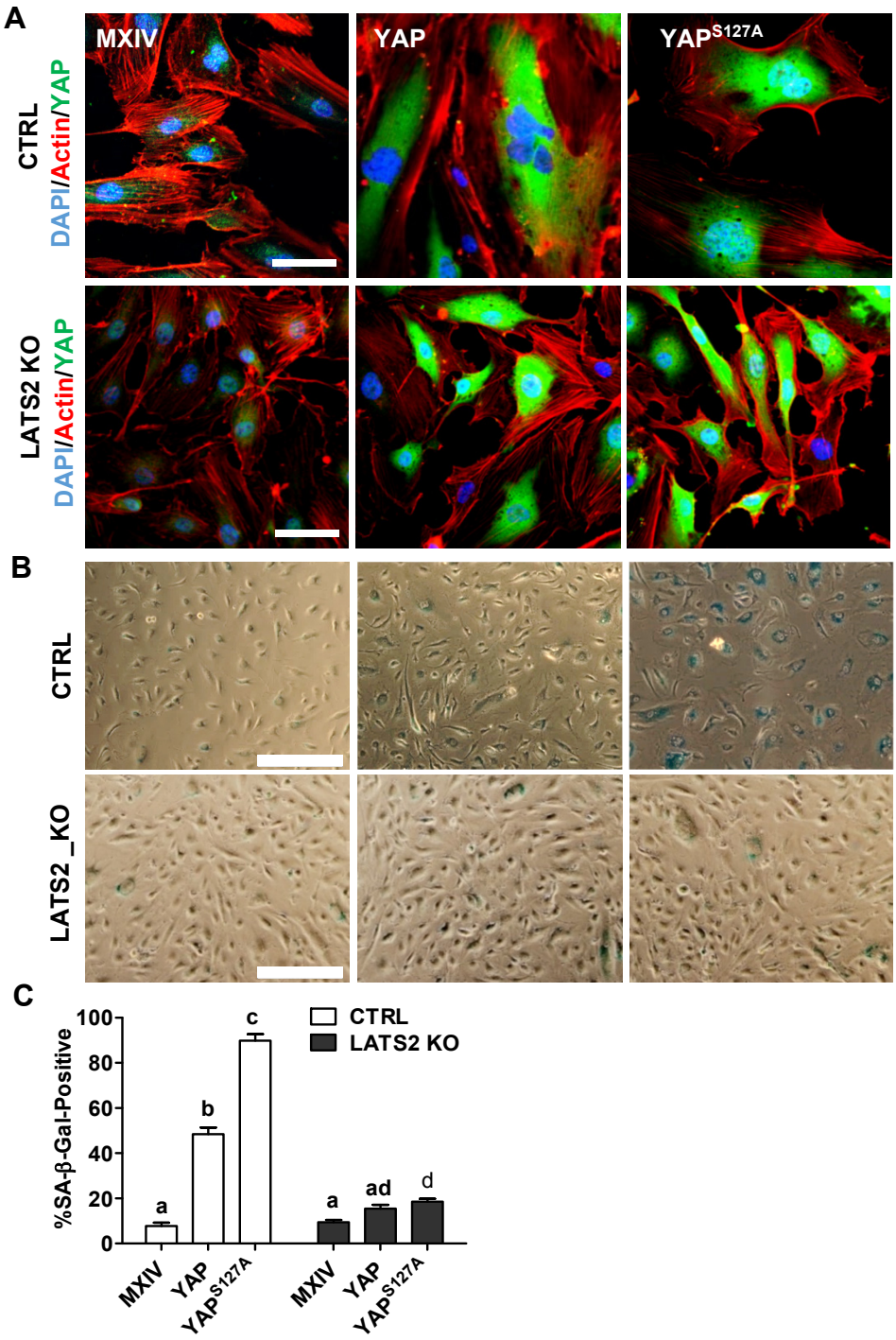
Appendix Figure S10



Appendix Figure S10. YAP induces up-regulation of RB1 and LATS2 in HUVEC cells.

Cells were collected at the 7th passage (5 passages after transfection with control or YAP-expressing vectors) and homogenized for analyzing the relative levels of YAP, LATS2, RB1 and other cell cycle-associated proteins. The experiments were repeated at least three times. Representative Western blotting images were presented to show the expressions of YAP, pRB and LATS2 in HUVEC-MXIV, HUVEC-YAP and HUVEC-YAP^{S127A} cells.

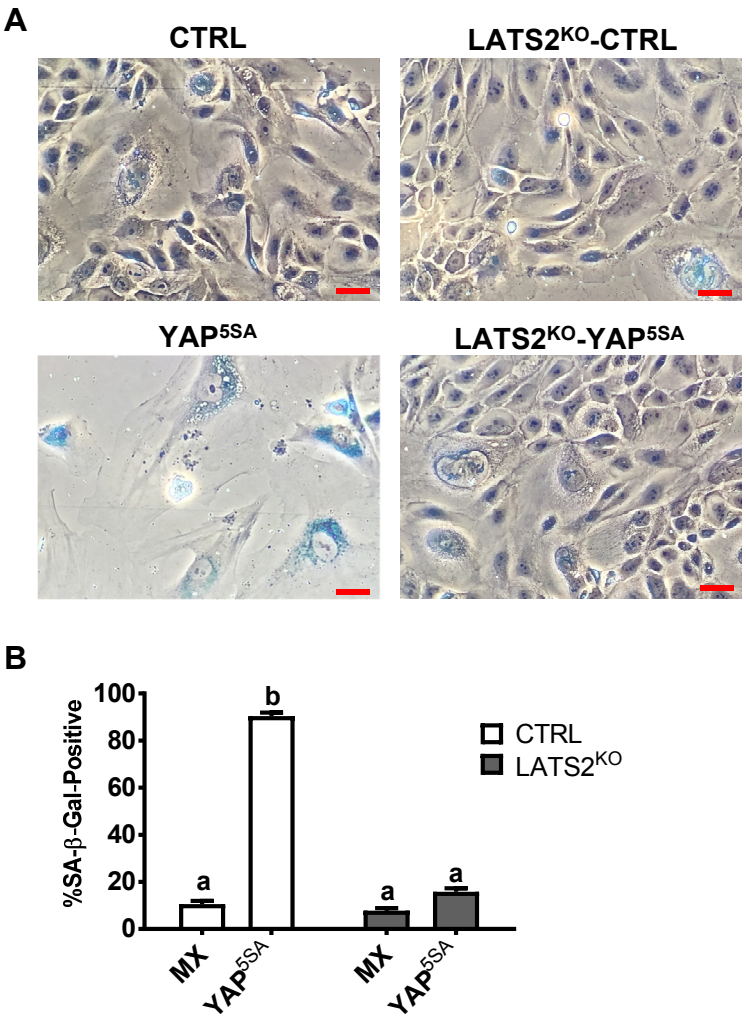
Appendix Figure S11



Appendix Figure S11. LATS2 knockout inhibits YAP-induced cell senescence in HUVEC cells.

A): Representative images showing the expression and location of YAP in HUVEC-MXIV, HUVEC-YAP and HUVEC-YAP^{S127A} cells with or without LATS2 knockout. YAP is visualized by a Alexa 488-conjugated second antibody (green). Actin was stained by rhodamine-phalloidin (red). Nuclei wad stained by DAPI (blue). Scale bar: 50μm **B)** Representative images showing SA-β-gal staining in the fourth passage of HUVEC-MXIV, HUVEC-YAP and HUVEC-YAP^{S127A} cells with or without LATS2 knockout. 200 μm. **C**) quantitative data showing percentages of SA-β-gal positive cells in HUVEC-MXIV, HUVEC-YAP and HUVEC-YAP^{S127A} cells with or without LATS2 knockout. Each bar represents mean ± SEM (n=5). Bars with different letters are significantly different from each other ($P < 0.05$).

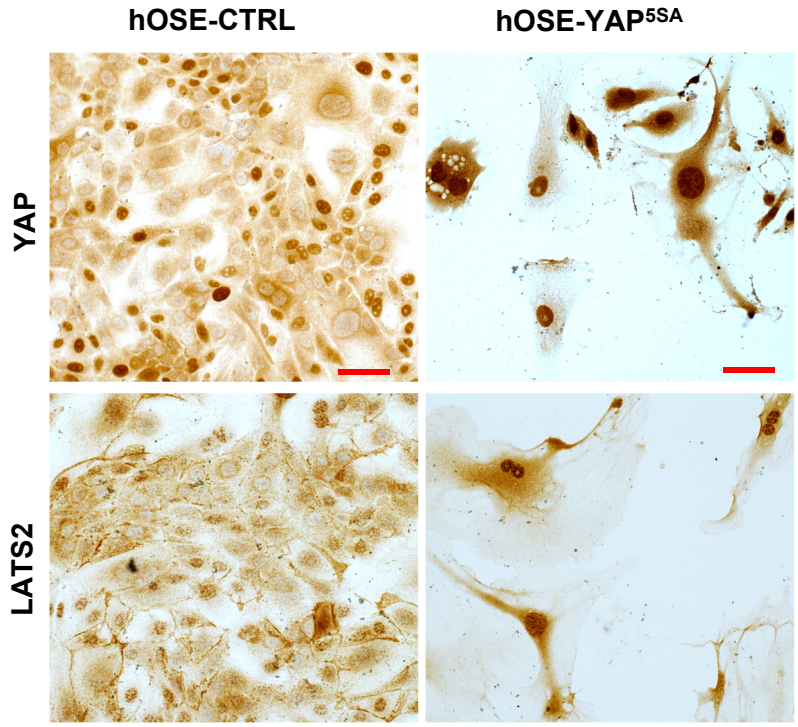
Appendix Figure S12



Appendix Figure S12. Knockout of LATS2 diminishes senescent phenotype induced by YAP^{S5A} in hOSE cells.

A) Representative images showing the activity of senescence β-galactosidase in hOSE control and hOSE-YAP^{5SA} cells with or without LATS2 deletion. The primary hOSE cells were transfected with modified retrovirus expressing control and YAP^{5SA} at their 2nd passage. Positive cells were selected under 150 μg/ml hygromycin for 6 days. IHC and β-gal staining were performed 12-15 days (2 more subcultures) selection. after another culture. Scale bar: 15μm. **B**) quantitative data showing percentages of SA-β-gal positive cells in HUVEC-MXIV, HUVEC-YAP and HUVEC-YAP^{S127A} cells with or without LATS2 knockout. Each bar represents mean ± SEM (n=5). Bars with different letters are significantly different from each other ($P < 0.05$)

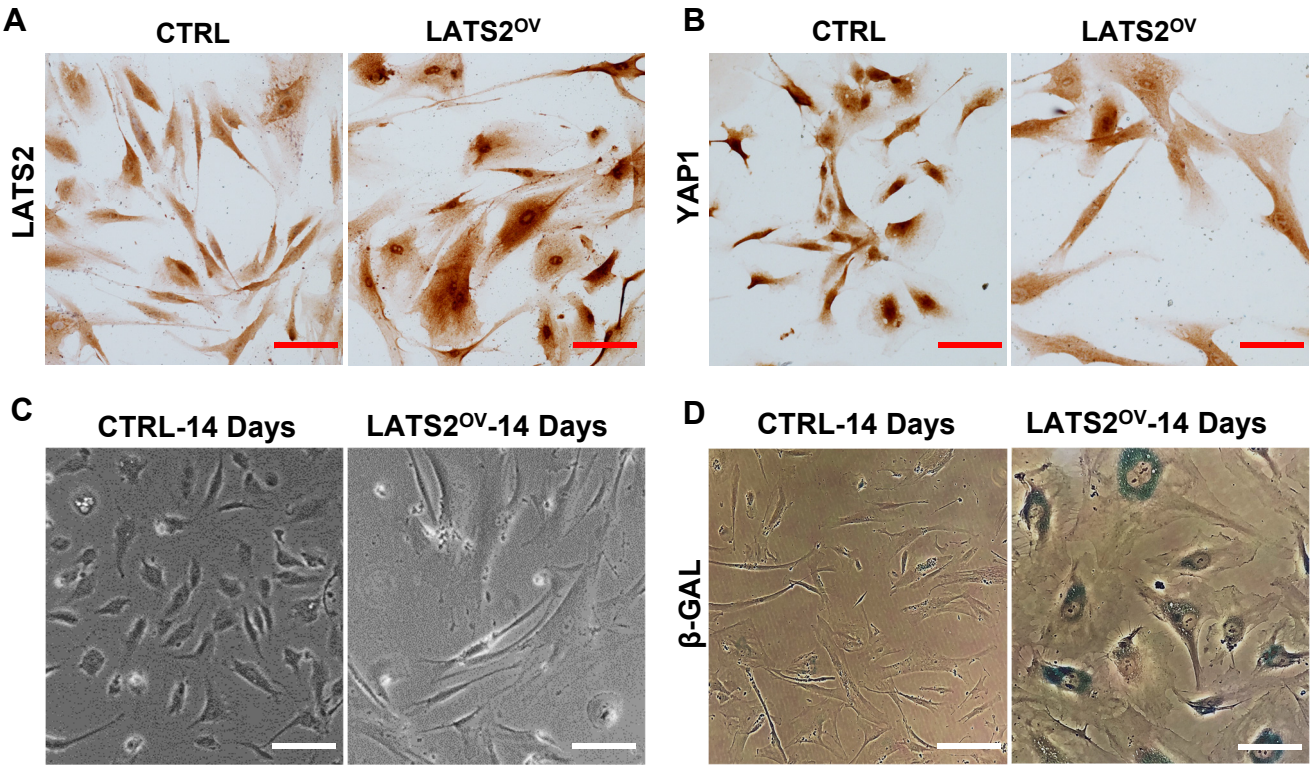
Appendix Figure S13



Appendix Figure S13. Expression of YAP1 and LATS2 in hOSE-CTRL and hOSE-YAP^{5SA} cells.

Representative images showing expression of YAP1 and LATS2 in hOSE-CTEL cells (hOSE cells transfected with empty vectors) and hOSE-YAP ^{5SA} cells (hOSE cells transfected with vectors expressing YAP ^{5SA}). Cultured primary hOSE cells were transfected with retroviral vectors at their 2nd passage. Positive cells were selected by Hygromycin (150 µg/ml) for 6 days. Cells were fixed and processed for determining YAP1 and LATS2 protein levels 12-15 days (2 subcultures) after antibiotic selection. YAP1 and LATS2 expression were determined by immunohistochemistry (IHC). YAP ^{5SA} represents a constitutively active form of YAP1 with mutations in all five LATS1/2 phosphorylation sites. Scale bar: 25µm.

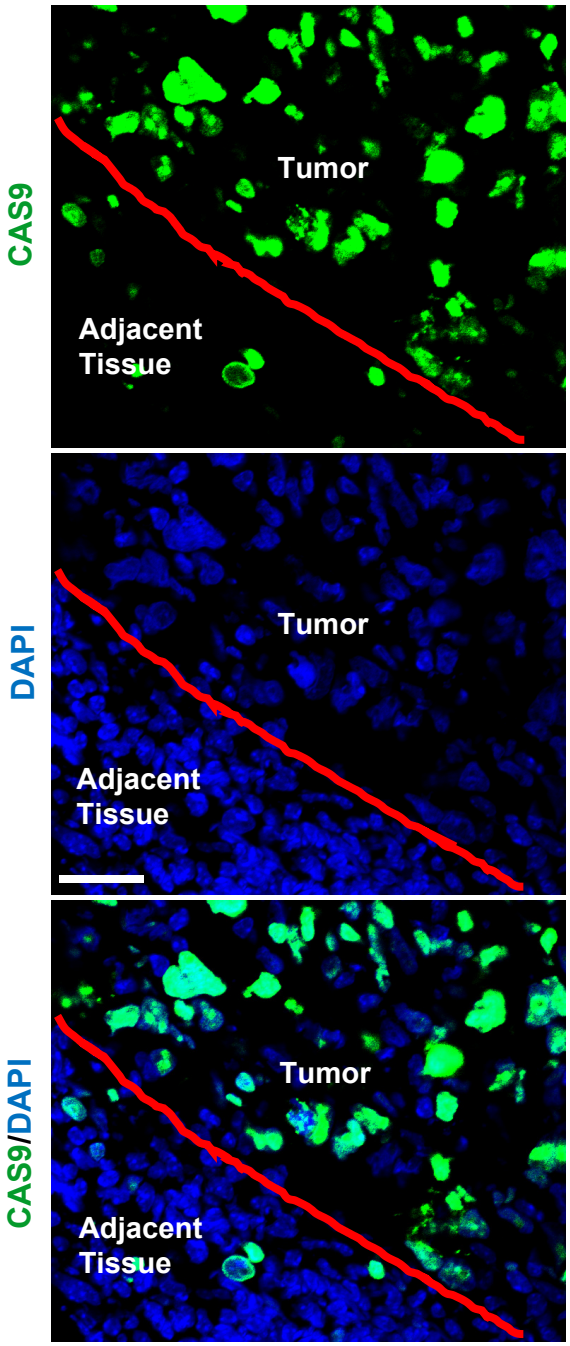
Appendix Figure S14



Appendix Figure S14. Ectopic expression of LATS2 in HUVEC cells induced senescence.

A & B) Representative images showing the expression of LATS2 (**A**) and YAP1 (**B**) protein examined by immunohistochemistry in control (CTRL) and LATS2 overexpressing ($LATS2^{OV}$) HUVEC cells. Scale bar: 50μm. **C**) Representative images showing morphology of control (CTRL) and LATS2 overexpressing ($LATS2^{OV}$) HUVEC cells. Scale bar: 50μm. **D**) Representative images showing senescence β-galactosidase activity (revealed by β-gal staining) in control (CTRL) and LATS2 overexpressing ($LATS2^{OV}$) HUVEC cells 14 days after transfection. The primary HUVEC cells were transfected with Lentiviral-based control and LATS2 expression virus at their 2^{nd} passage. Positive cells were selected by puromycin (3ng/ml) for 6 days. IHC and β-gal staining were performed 8 days after selection. Scale bar: 50μm.

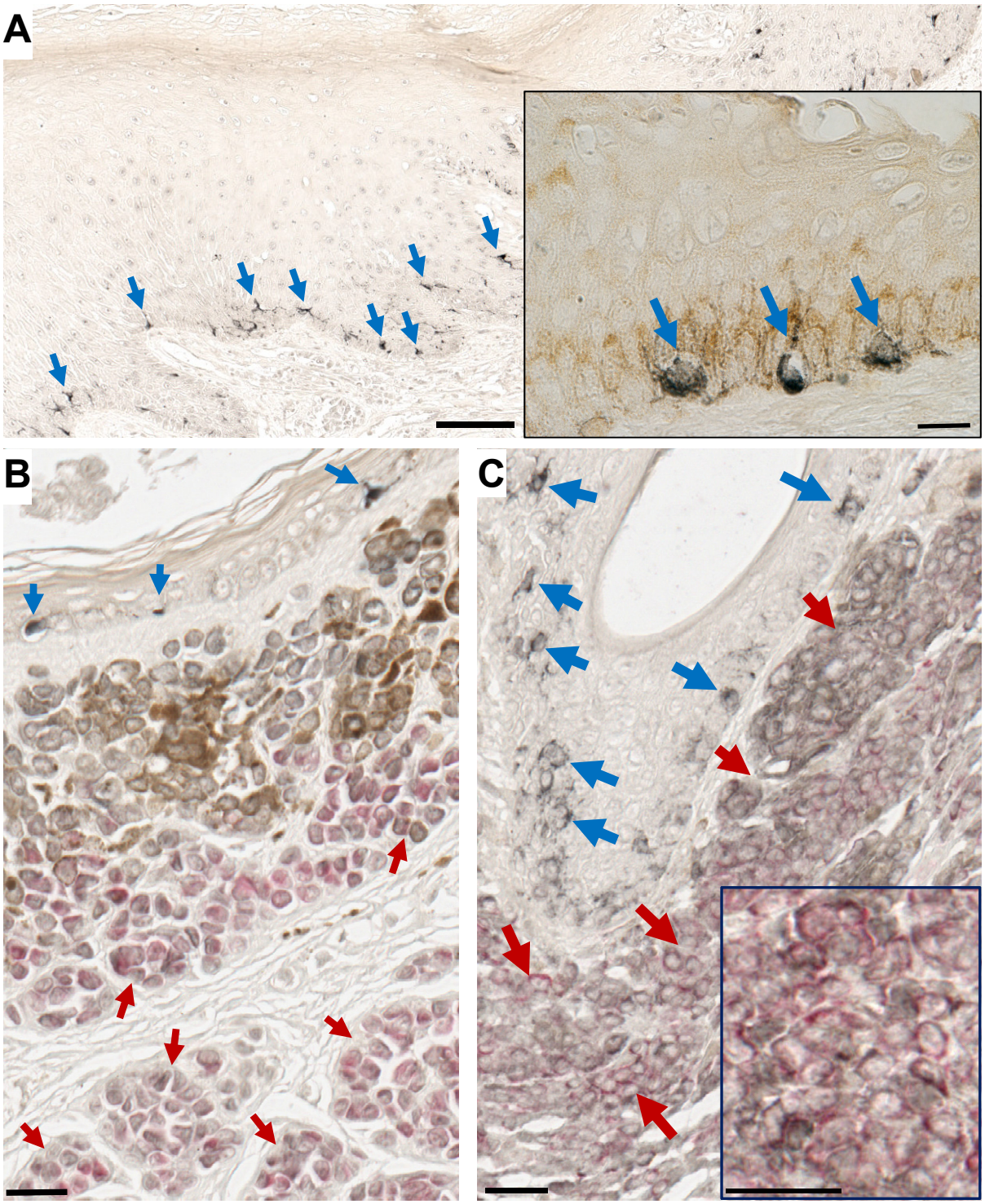
Appendix Figure S15



Appendix Figure S15. Knockout of LATS2 switched YAP-induced cellular senescence to malignant transformation in hOSE cells.

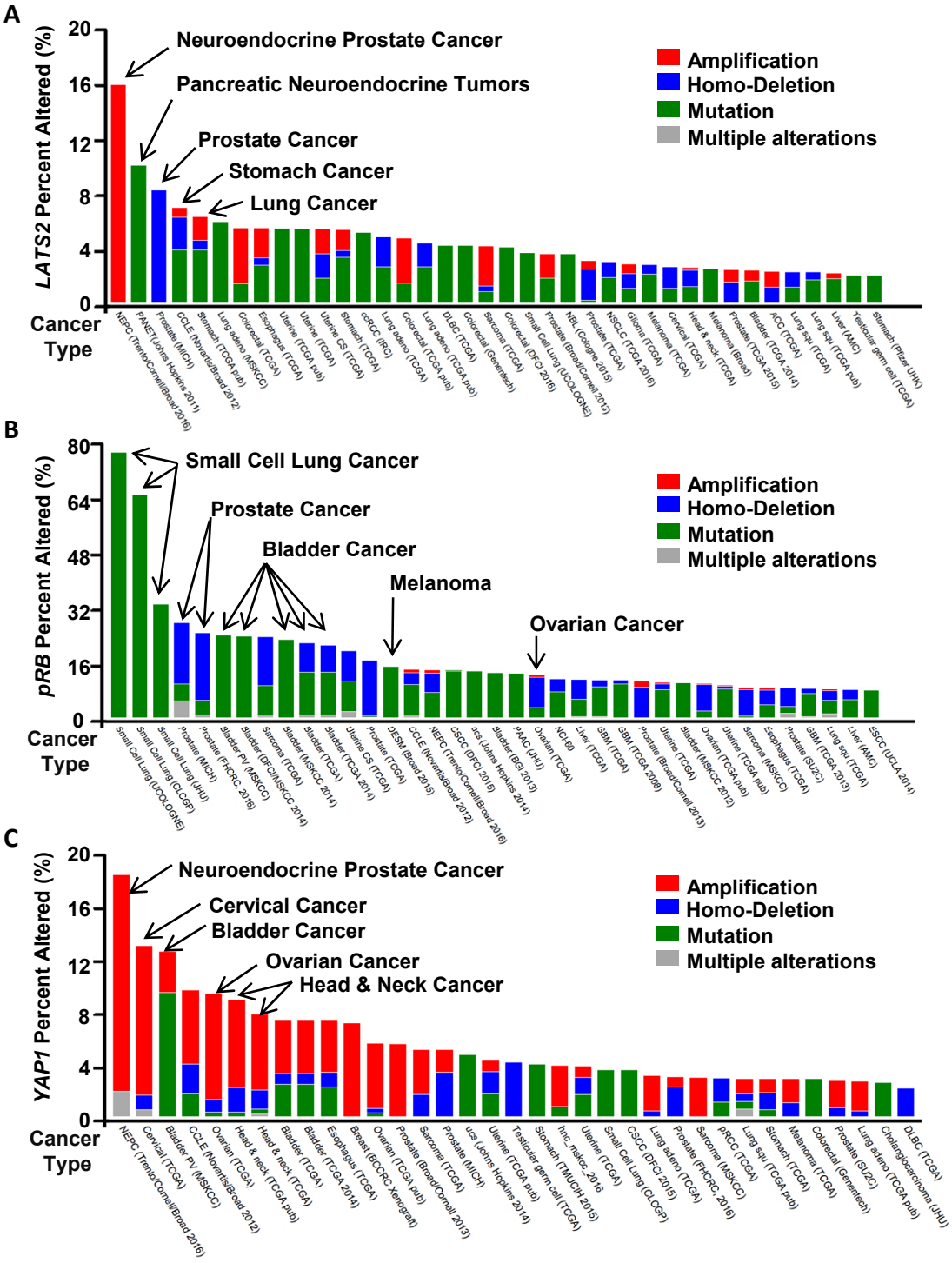
Representative images showing the expression and location of CAS9 in hOSE-LATS2^{KO}-YAP^{S127A}-derived tumor tissue. CAS9 is visualized by a Alexa 488-conjugated second antibody (green). Nuclei wad stained by DAPI (blue). Scale bar: 20μm

Appendix Figure S16



Appendix Figure S16. Expression of LATS2 in melanocytes of nevus tissues. A) LATS2 (in red) is undetectable in the melanocytes. Melanocytes are cells with gray blue color, which are identified with a melanocyte biomarker antibody cocktail (MART-1, Tyrosinase, and gp100) using a dual color IHC staining kit, see detail in method section). Scale bar: 25 μm. Insert in (A) is a representative high resolution image showing negative LATS2 staining in three melanocytes. The brown pigment are melanin. Scale bar: 10μm. **B** & **C**) Two representative images showing the colocalization of LATS2 (in red) and melanocyte biomarker (in gray blue). LATS2 protein in skin melanocytes (blue arrows) is undetectable, but it is highly expressed in melanocytes of the Nevi tissue (red arrows). High resolution Insert in (C) showing the colocalization of LATS2 (red) and melanocyte biomarker (Gray) in Nevi. Scale bar: 25 μm.

Appendix Figure S17



Appendix Figure S17. Cross-cancer alterations of *LATS2, RB1* & *YAP1 genes.*

The cross cancer alterations (frequencies of mutation, deletion and amplification) of *LATS2*, *RB1* and *YAP1* were analyzed using online TCGA datasets and the online data mining tool (the cBioPortal for Cancer Genomics and the datasets from the TCGA research Network) as described previously (Gao et al. *Sci. Sinal*, 2013). Data from a total of 146 available studies are selected for analyzing alterations of *LATS2*, *RB1* and *YAP1* genes .

Appendix Table S1. Information of primary antibodies used in this study

Antibody Name	Catalog Number	Vender
Ki67	ab15580	Abcam (Cambridge, MA)
RB1	#9309	Cell Signaling Technology Inc. (Danvers, MA)
p-RB1	#8516	Cell Signaling Technology Inc. (Danvers, MA)
p16INK4A	MA5-17054	Thermo Fisher (Rockford, IL)
P53	sc-126	Santa Cruz Biotechnology Inc (Dallas, TX)
YAP	#4912	Cell Signaling Technology Inc. (Danvers, MA)
phospho-YAP (Ser127)	#4911	Cell Signaling Technology Inc. (Danvers, MA)
CCND1	#2978	Cell Signaling Technology Inc. (Danvers, MA)
CCNA2	#4656	Cell Signaling Technology Inc. (Danvers, MA)
CCNB1	#4138	Cell Signaling Technology Inc. (Danvers, MA)
LATS1	#9153	Cell Signaling Technology Inc. (Danvers, MA)
LATS2	#5888	Cell Signaling Technology Inc. (Danvers, MA)
CAS9	#14697	Cell Signaling Technology Inc. (Danvers, MA)
ATG7	#2631	Cell Signaling Technology Inc. (Danvers, MA)
Beclin-1	#3495	Cell Signaling Technology Inc. (Danvers, MA)
MacroH2A	#4827	Cell Signaling Technology Inc. (Danvers, MA)
ATG3	#3415	Cell Signaling Technology Inc. (Danvers, MA)
TAZ	#70148	Cell Signaling Technology Inc. (Danvers, MA)
H3k9me3	#13969	Cell Signaling Technology Inc. (Danvers, MA)
β-actin	A5441	Sigma-Aldrich (St. Louis, MO)