Supplementary Materials

Regulation of YAP promotor Accessibility in Endothelial Mechanotransduction

Authors

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This file includes:

Supplemental Figure 1

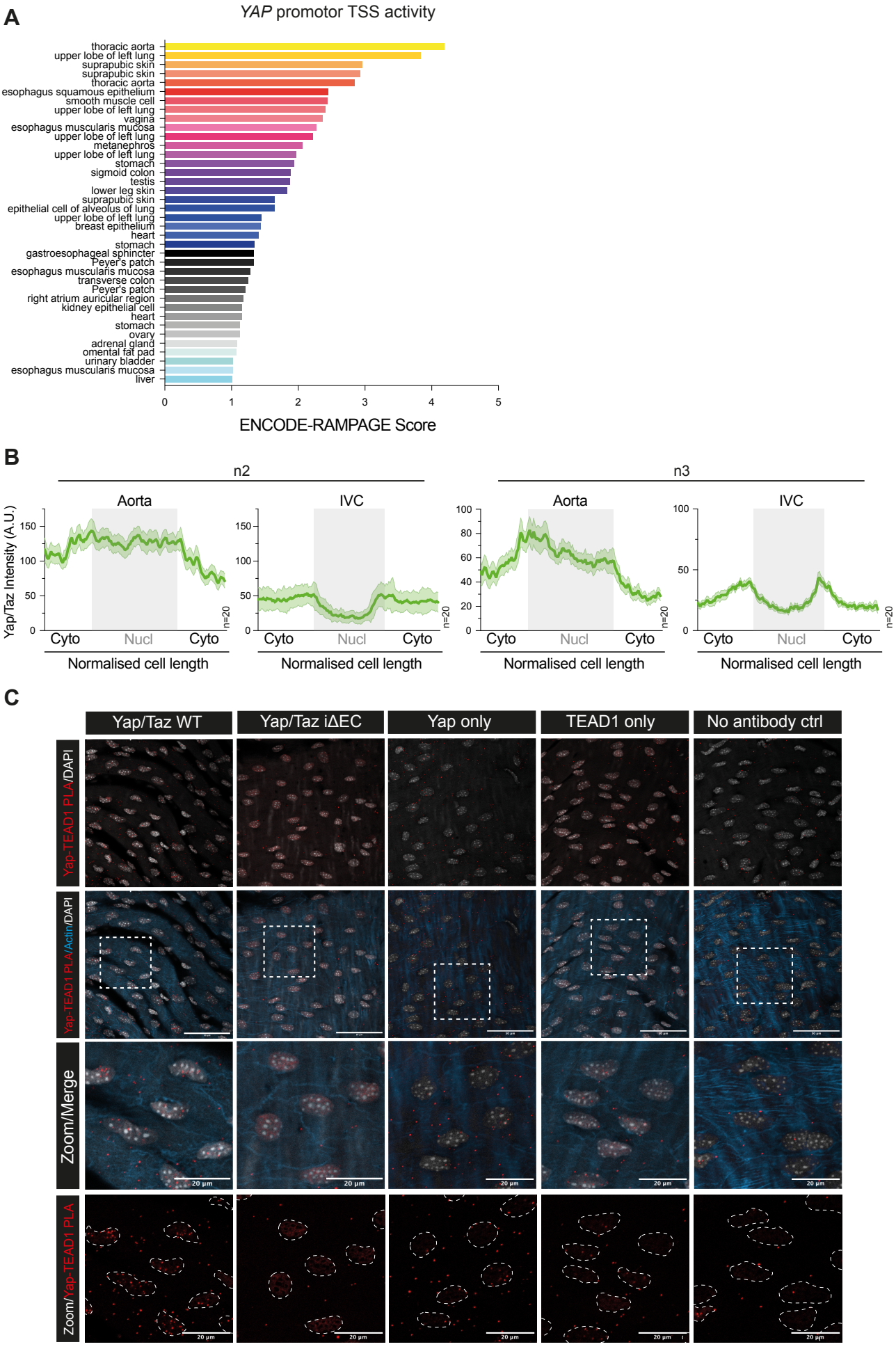
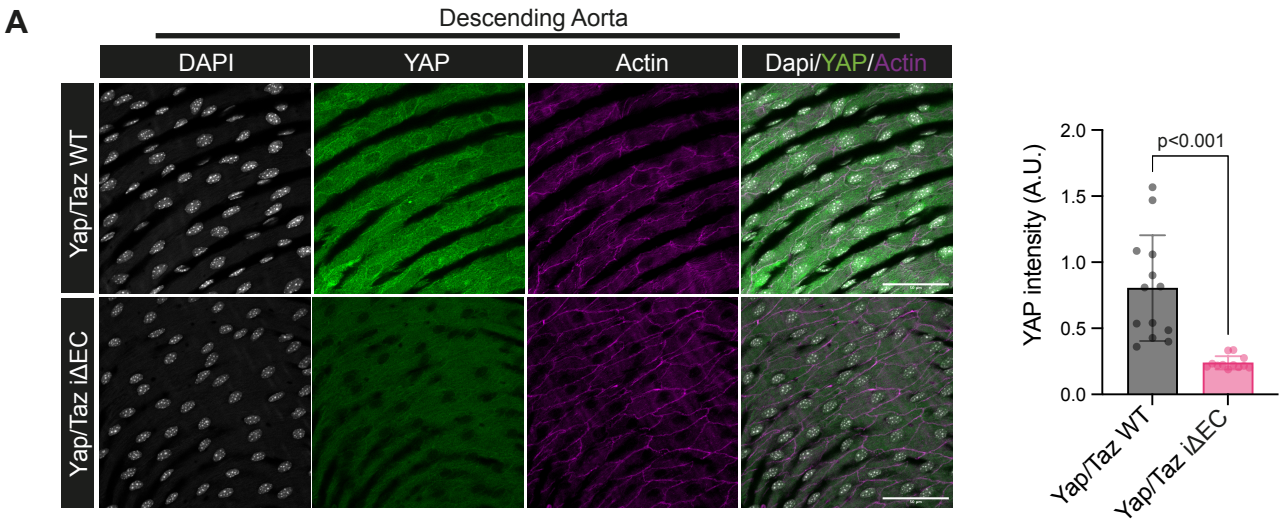


Fig. S1.

A, RNA Annotation and Mapping of Promoters for the analysis of Gene Expression (RAMPAGE) analysis of the transcriptional start sites (TSSs) of the YAP promotor, scored and ranked in descending order using SCREEN: Search Candidate cis-Regulatory Elements by ENCODE; https://screen.wenglab.org/. (*19, 20*) **B,** Quantification of nuclear and cytoplasmic staining of YAP/TAZ in the aorta and IVC. Histogram graph depicts the average intensity (mean± s.e.m.) of *n*=20 cells each from two independent animals labelled n2 and n3 (n1 is shown in Fig1b). A total of 60 cells per aorta or IVC were measured from *n*=3 mice. **C,** Representative images of *ex vivo en face* aortic tissue of both YAP/TAZ WT (*n*=5) and Yap/Taz iΔEC (*n*=5) inducible knockout mice, subjected to PLA showing the interaction between YAP and TEAD1 (indicated by red dots) and single antibody and no antibody controls. Quantification is shown in Fig1c. Nuclei are shown in grey and actin cytoskeleton in blue. In the bottom row, PLA dots (red) are shown with nuclei outlined by dashed white line. Scale bar, 50µm and 20µm, for 40x and indicated zoomed regions, respectively.

Supplemental Figure 2



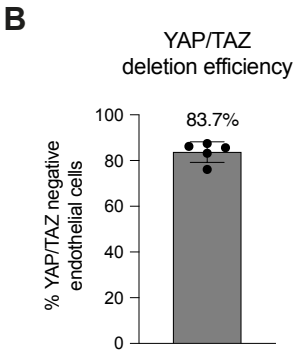


Fig. S2.

A, Representative images of *en face* staining of YAP in the descending aorta of both Yap/Taz WT and Yap/Taz iΔEC mice, *n*=3 per group. Nucleus (grey), Yap (green), Actin (magenta). Scale bar, 50μm. Bar graph indicates quantification of Yap fluorescent intensity, where each data point represents intensity profile from one image, 4-5 images/aorta (n=3 mice/group) were analysed, mean± s.d., Mann-Whitney). **B**, Quantification of YAP/TAZ efficiency of endothelial knockout by quantification of immunofluorescent staining of YAP/TAZ positive, VE-cadherin positive cells from images shown in Fig2A-B. Quantification were derived from n=5 Yap/Taz iΔEC mice.

Supplemental Figure 3

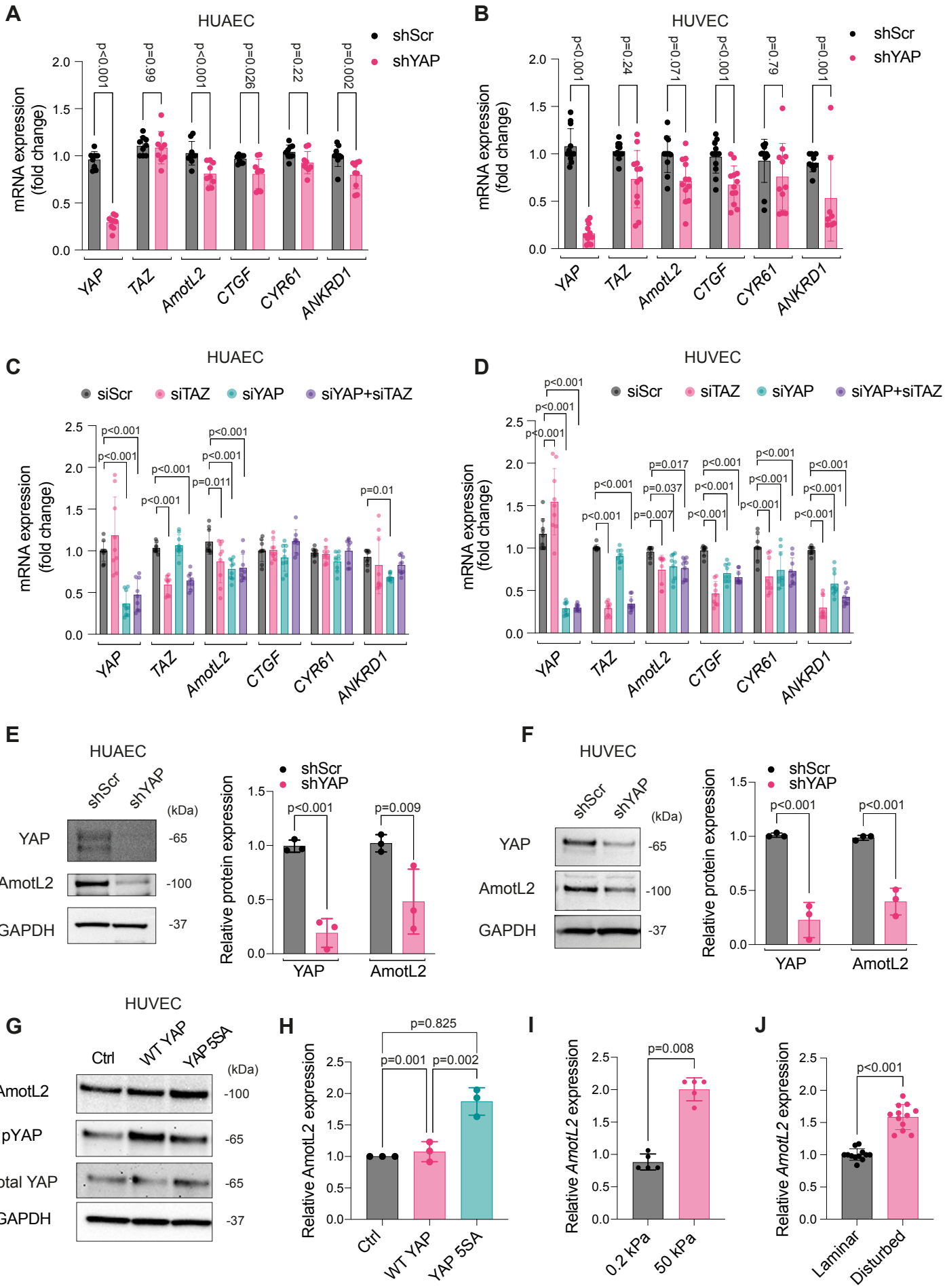


Fig. S3.

AmotL2 is transcriptionally regulated by YAP. **A,** Fold change in mRNA expression of *AmotL2* and known YAP target genes (*CTGF, CYR61* and *ANKRD1*) in HUAEC cells and HUVEC (**B**) transduced with shScr or shYAP lentivirus, analysed by qPCR and normalised to GAPDH. *n*=3 independent experiments, each with 3 technical replicates. (mean± s.d., 2way ANOVA with Dunnett's multiple comparisons). **C,** Fold change in mRNA expression of AmotL2 and known YAP target genes (*CTGF*, *CYR61* and *ANKRD1*) in HUAEC and HUVEC (**D**) cells transfected with scrambled siRNA (siScr), siTAZ, siYAP or codepletion of YAP and TAZ (siYAP+siTAZ) analysed by qPCR and normalised to GAPDH. *n*=3 independent experiments, each with 3 technical replicates. (mean± s.d., 2way ANOVA with Dunnett's multiple comparisons). **E,** Representative western blot showing YAP and AmotL2 expression in HUAEC cells and HUVEC (**F**) transduced with shScr or shYAP lentivirus for 96h prior to immunoblot analysis. GAPDH was used as a loading control and normalisation for respective quantification shown in the right-hand panel, *n*=3 independent experiments. (mean± s.d., 2way ANOVA with Dunnett's multiple comparisons). **G,** Representative western blot and quantification (**H**), showing AmotL2, total and phosphorylated YAP expression in none infected, WT YAP and YAP 5SA-overexpressing HUVEC, *n*=3 independent experiments. (mean± s.d., 2way ANOVA with Dunnett's multiple comparisons). **I,** RT-qPCR of AmotL2 from HUVEC plated to 0.2 or 50 kPa hydrogels, (*n*=5 independent experiments, mean± s.d., Mann-Whitney). **J,** RT-qPCR of AmotL2 from HUVEC plated to 6 well plates at confluency before being subject to orbital flow for 48 h before lysates were harvested as described in the materials and methods so as to obtain laminar and disturbed flow transcriptional responses (*n*=4 independent experiments, mean± s.d., Mann-Whitney).

Supplemental Figure 4

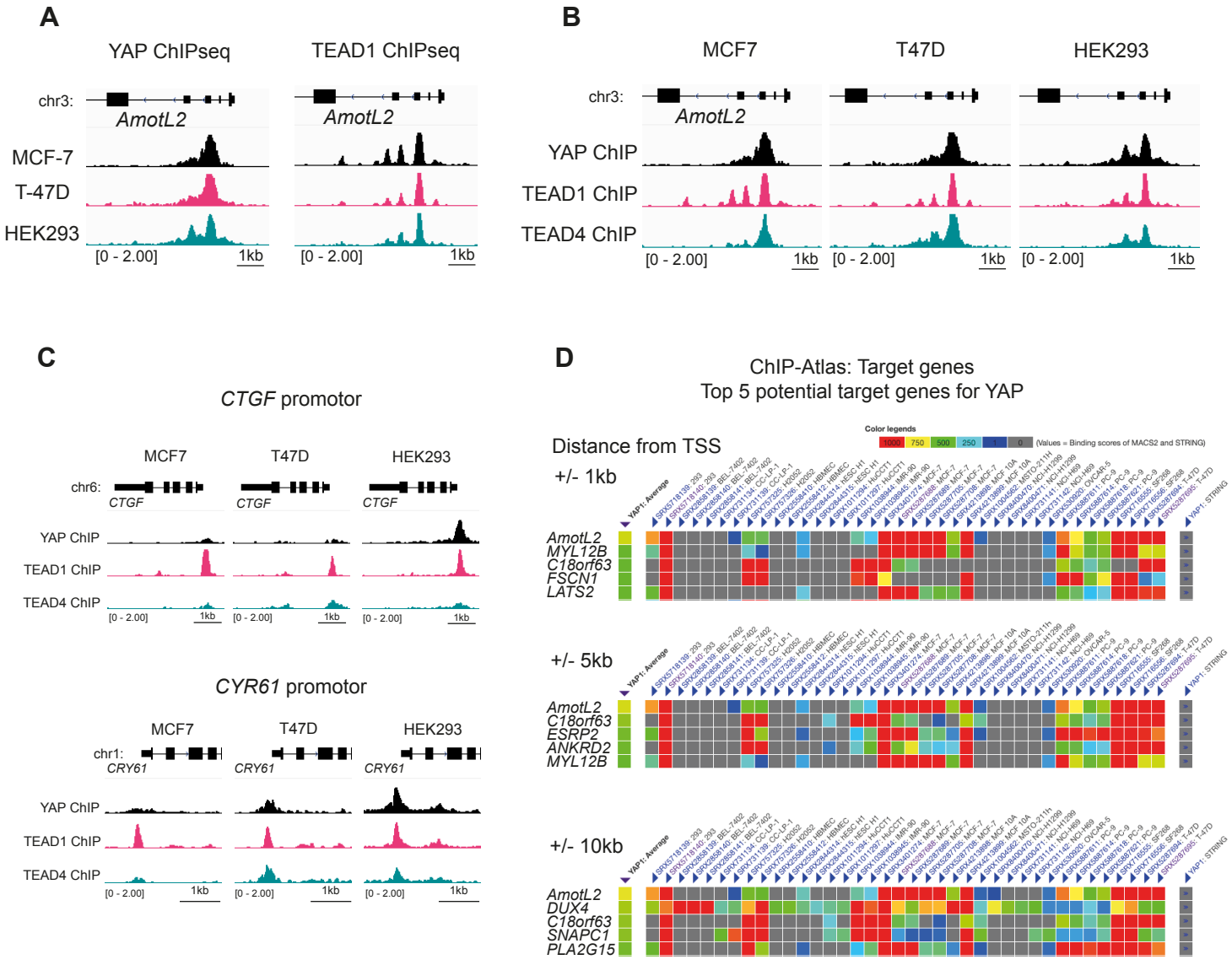


Fig. S4.

A, Genomic tracks displaying ChIP-Atlas (https://chip-atlas.org/) data of YAP (left panel) and TEAD1 (right panel) ChIP-seq data across MCF7, T47D, and HEK293, within the AmotL2 promotor (Data sources are referenced in the methods). **B**, Genomic tracks displaying overlayed YAP, TEAD1 and TEAD4 ChIP-seq enrichment at the AmotL2 promotor of indicated cell lines. **C**, Genomic tracks displaying overlayed YAP, TEAD1 and TEAD4 ChIP-seq enrichment at the CTGF and CYR61 promotor of indicated cell lines. **D**, Top 5 hits of ChIP-Atlas predicted target genes bound by YAP in indicated datasets at 1, 5 and 10 kb from the transcriptional start site (TSS) of indicated target genes.

Supplemental Figure 5

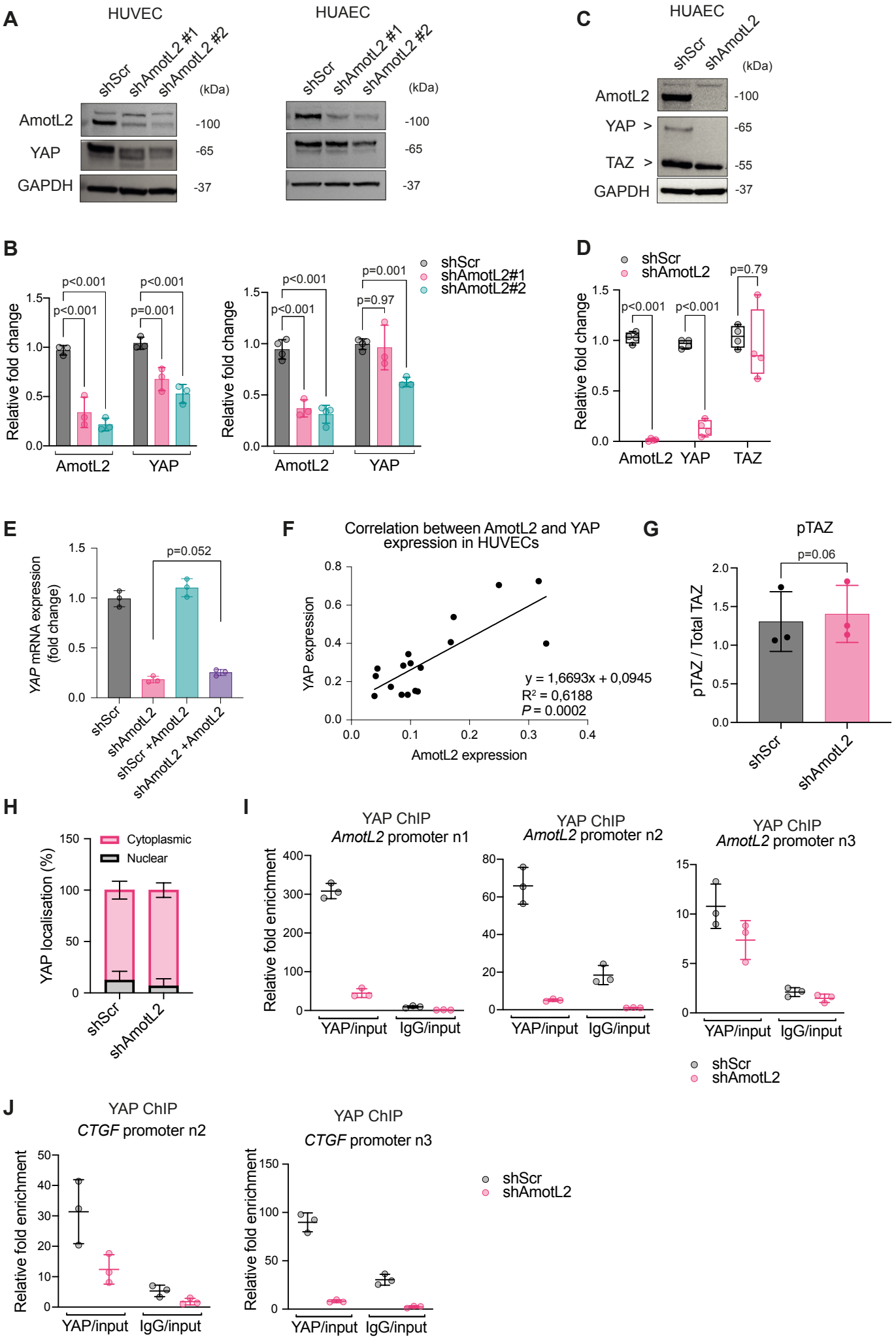
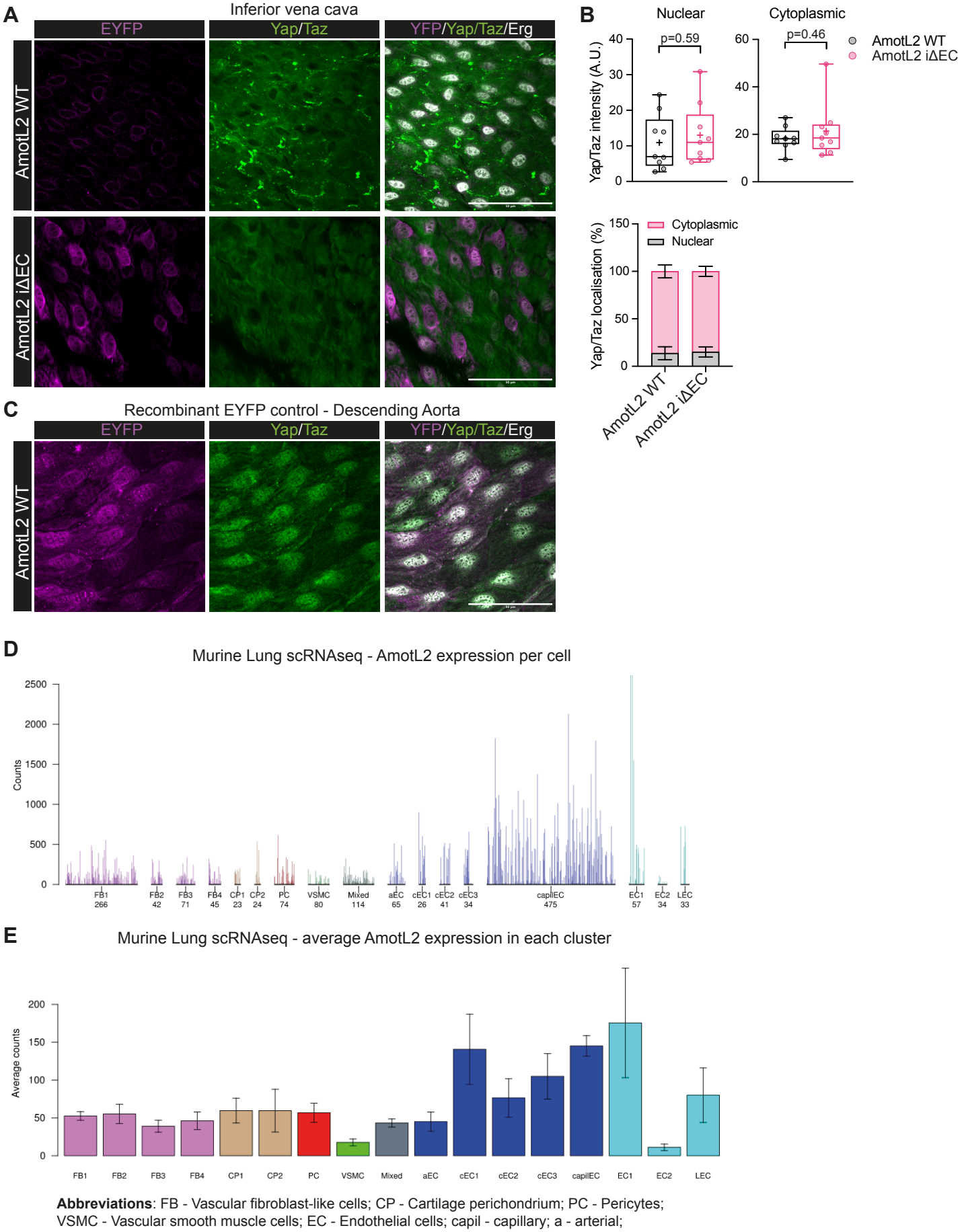


Fig. S5.

A, Western blot analysis of AmotL2 and YAP in HUVEC and HUAEC cells 96h post-treatment with lentivirus encoding shScr or two additional shRNA constructs targeting AmotL2 (shAmotL2#1 and shAmotL2#2). GAPDH was used as a loading control. Membranes are representative of n=3 independent experiments. **B,** Quantification of AmotL2 and YAP protein levels from panel a, relative to GAPDH loading control. *n*=3 independent experiments for both HUVEC and HUAEC, mean± s.d., 2way ANOVA with Dunnett's multiple comparisons. **C,** Western blot analysis of shAmotL2 treated HUAEC using an antibody with specificity for both YAP and TAZ. Box plots shown in **D,** indicate quantification of AmotL2, YAP, and TAZ relative to GAPDH loading control, *n*=4, mean± s.d., 2way ANOVA with Dunnett's multiple comparisons. **E,** SYBR green RT-qPCR of *YAP* expression relative to housekeeping gene *GAPDH*, in shScr and AmotL2 +/- AmotL2 overexpression. (*n*=3 independent experiments, mean± s.d., 2way ANOVA with Dunnett's multiple comparisons). **F,** Correlation between *AmotL2* and *YAP* expression normalized to *GAPDH* from *n*=17 AmotL2 HUVEC knockdown samples. **G,** Quantification of pTAZ ser89 levels, relative to total TAZ shown in Fig4e. (*n*=3 independent experiments, mean± s.d., Mann-Whitney). **H,** Quantification of nuclear:cytoplasmic fractionation and probing of YAP localisation, shown in Fig4g. HUVEC cells 96h post-treatment with shScr or shAmotL2 lentivirus. GAPDH and lamin A/C were used as positive and negative controls and were used for normalisation for quantification. *n=3* independent experiments. ChIP showing YAP binding to *AmotL2* promotor (**I,**) and *CTGF* promotor (**J,**) of shScr or shAmotL2 treated HUVEC. ChIP qPCR was performed using SYBR green reagents and quantification was normalised to an IgG control antibody. Plot shown is a representative experiment from n=3 independent experiments (Fig3h shows *n*=1 for the *CTGF* promotor). Each data point represents a technical repeat within one independent experiment (performed in triplicate). Graphs display (mean± s.d.).

Supplemental Figure 6



Abbreviations: FB - Vascular fibroblast-like cells; CP - Cartilage perichondrium; PC - Pericytes; VSMC - Vascular smooth muscle cells; EC - Endothelial cells; capil - capillary; a - arterial; c - continuum; L - Lymphatic; 1,2,3,4 - subtypes.

Fig. S6.

A, Representative images of en face staining of EYFP (magenta), Yap/Taz (green), and ERG (grey) in the inferior vena cava of both AmotL2 WT and AmotL2 iΔEC mice. Scale bar, 50µm. Images are representative of n=3 mice/group. **B,** Quantification of Yap/Taz nuclear:cytoplasmic localisation and immunofluorescent intensity of the inferior vena cava of both AmotL2 WT (n=3) and AmotL2 iΔEC (n=3) mice. **C,** Staining as in a, of recombinant EYFP control murine aorta indicating that induction of EYFP expression in WT AmotL2 cells does not affect Yap/Taz expression. Scale bar, 50µm. Images are representative of n=3 mice/group. **D,** Raw counts of AmotL2 expression across cell types of adult lung from scRNAseq data accessed through (https://betsholtzlab.org/VascularSingleCells/database.html) (*30, 31*). **E**, As in d, but displaying average counts.

Supplemental Figure 7

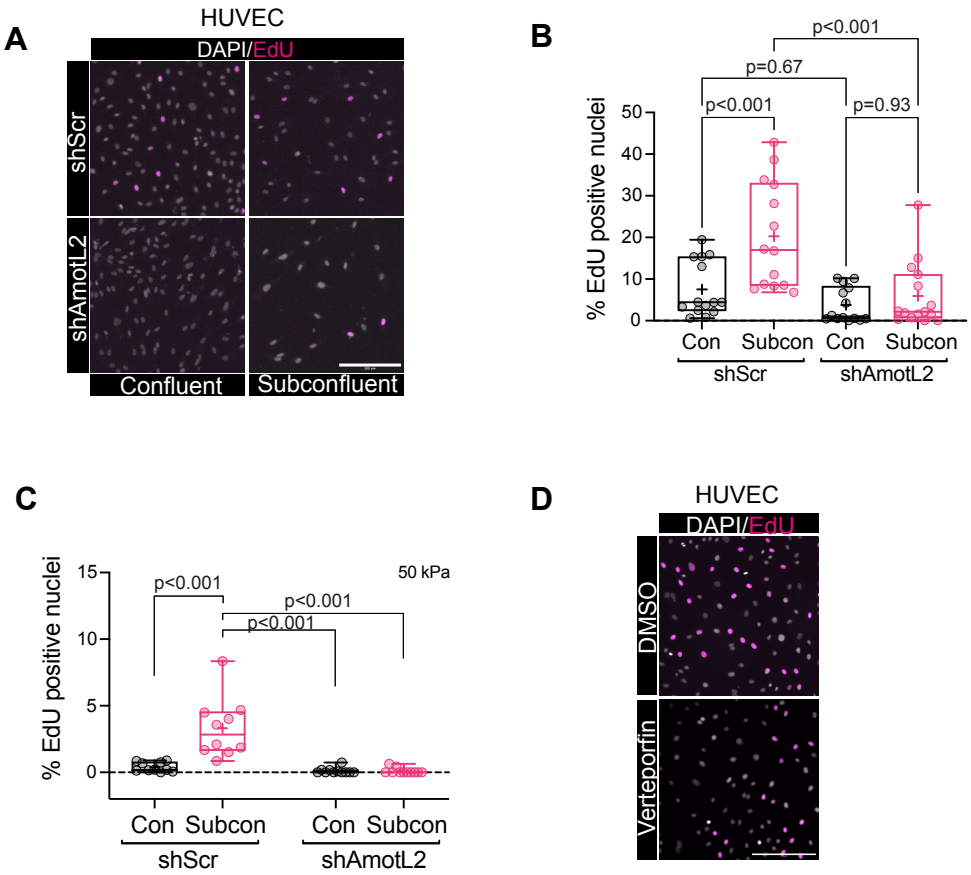


Fig. S7.

A, Representative images of shScr and shAmotL2 treated HUVEC 72h post infection, replated to gelatin coated plastic in confluent or subconfluent conditions. Incorporated EdU was detected with secondary antibodies and counterstained with Hoechst. Scale bar, 250µm. **B,** Box plots show quantification of a, where % of EdU positive cells was calculated against total number of cells stained with Hoechst. Each data point represents one field of view from *n*=3 independent experiments. (mean± s.d., 2way ANOVA with Dunnet's multiple comparisons). **C,** Box plots show quantification of EdU incorporation of HUVEC replated to gelatin coated 50kPa hydrogels following 48h post-lentiviral transduction with shScr or shAmotL2 lentivirus, where % of EdU positive cells was calculated against total number of cells stained with Hoechst. Each data point represents one field of view from *n*=3 independent experiments. (mean± s.d., 2way ANOVA with Dunnet's multiple comparisons). **D,** Representative images of EdU positive HUVEC treated for 48h with 0.2µg/ml Verteporfin or DMSO vehicle. Scale bar, 50µm. Cells were counterstained with Hoechst. Scale bar, 50µm.

Supplemental Figure 8

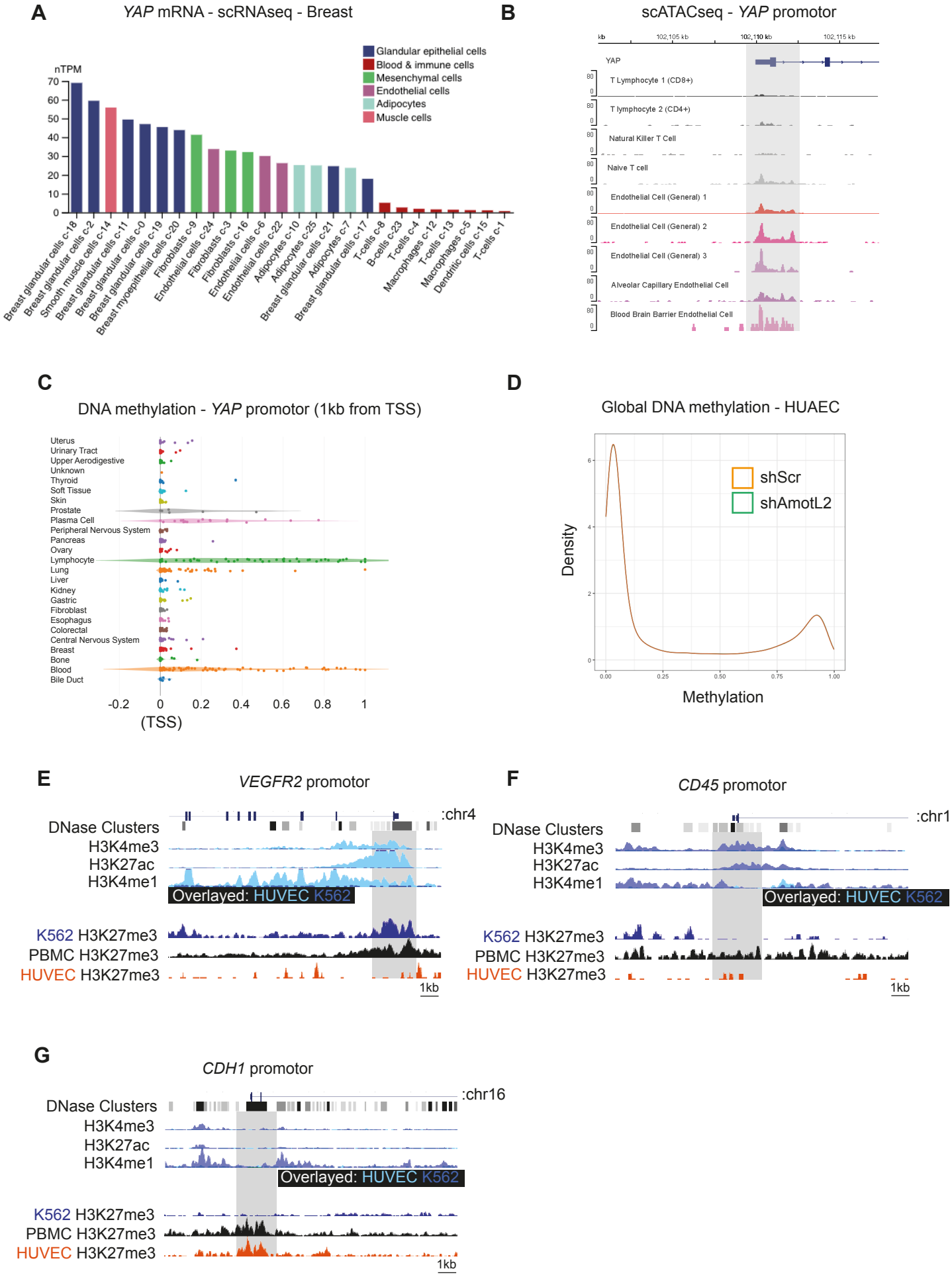
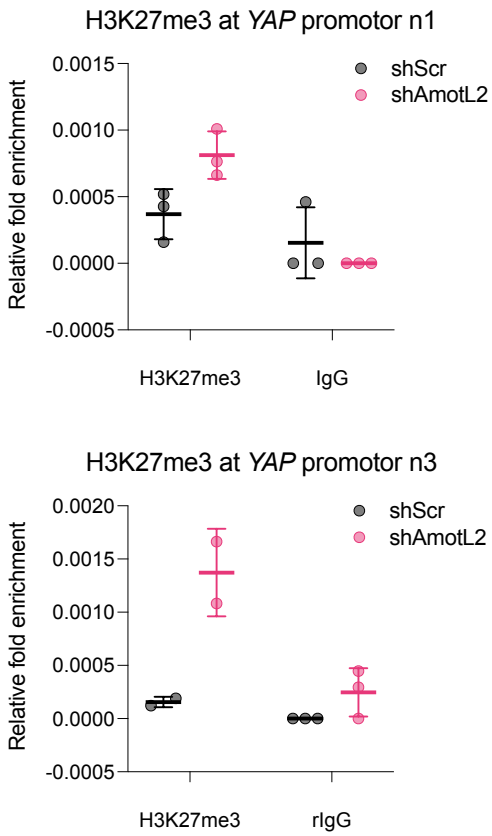


Fig. S8.

A, Screengrab of the Human protein atlas showing *YAP* mRNA expression from scRNAseq data of human breast cancer samples. **B,** scATACseq data indicating chromatin conformation around the *YAP* promotor using (http://catlas.org/catlas_hub/). Histograms indicate chromatin accessibility from specific cell types. **C,** publicly available DNA methylation data from the Dependency Map (Depmap.org) portal, showing DNA methylation of indicated cell types derived from human samples. **D,** Infinium EPIC array of 850,000 CpG sites across the genome. Data are averages of *n=4* for both shScr and shAmotL2 HUAEC samples. Histograms show the average distribution of DNA methylation profile of shScr (orange) and shAmotL2 (green). Screengrab of the UCSC browser displaying genomic tracks of ENCODE data of H3K4me3, H3K27ac and H3K4me1 ChIP-seq data across HUVEC and K562 (overlayed)(Data sources are referenced in the methods) and H3K27me3 of ChIP-seq data across K562, PBMC and HUVEC (Data sources are referenced in the methods), within the *VEGFR2* promotor (**E**), *CD45* promotor (**F**), *CHD1* promotor (**G**).

Supplemental Figure 9



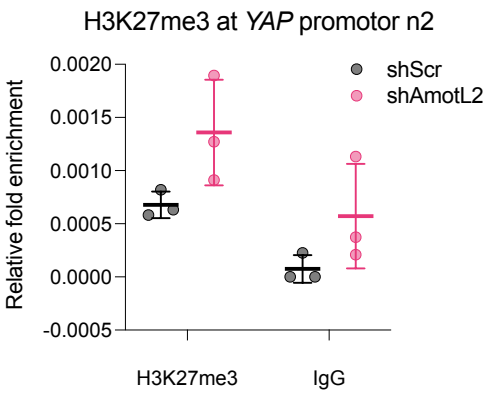


Fig. S9.

ChIP showing H3K27me3 pulldown at the *YAP* promotor of shScr or shAmotL2 treated HUAEC. ChIP qPCR was performed using SYBR green reagents and quantification was normalised to input and IgG control. Plots shown are representative of n=3 independent experiments. Each data point represents a technical repeat within one independent experiment (performed in triplicate). Graphs display (mean± s.d.).

Supplemental Figure 10

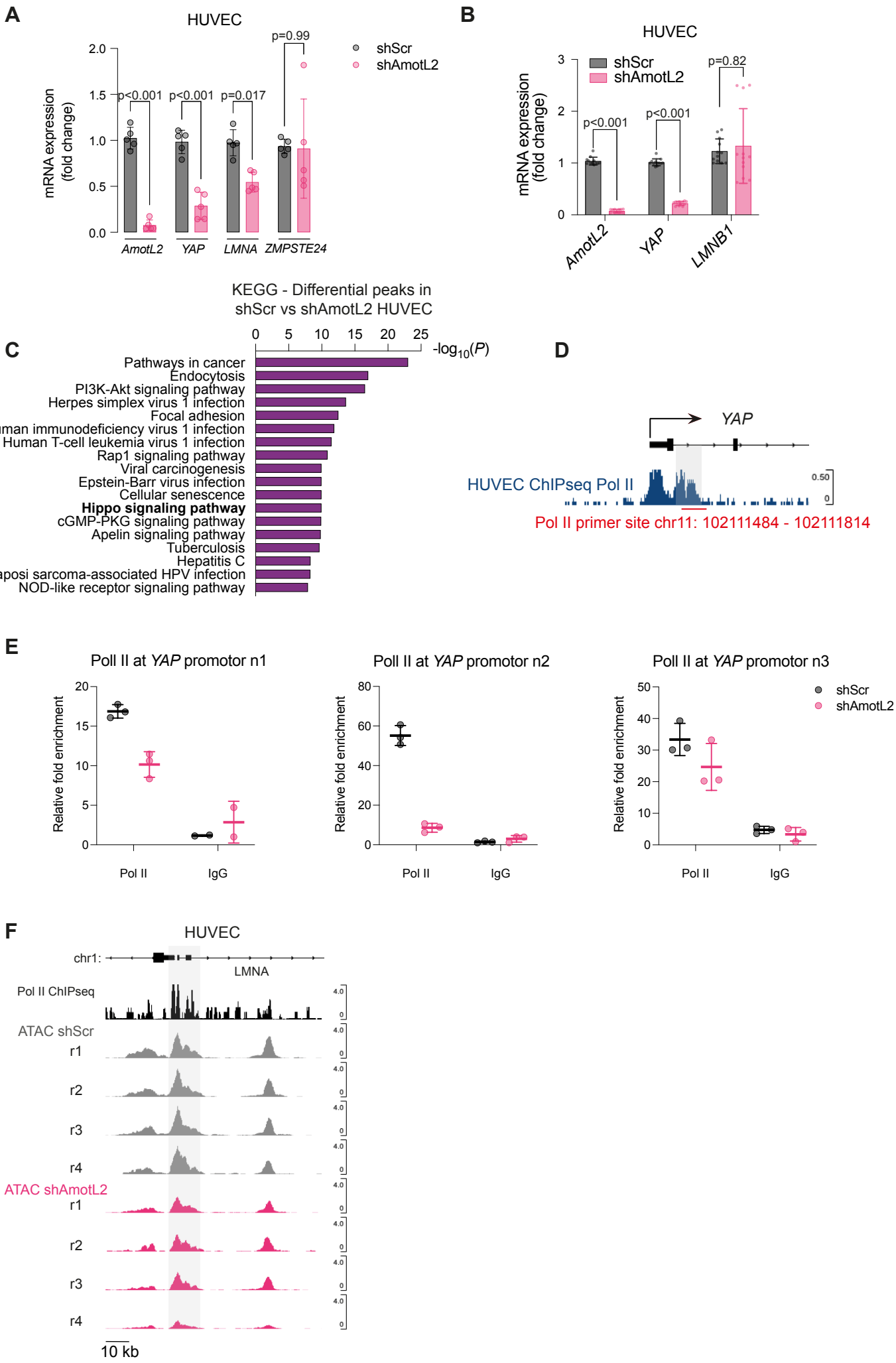


Fig. S10.

A, SYBR green RT-qPCR of *AmotL2*, *YAP*, *LMNA* and *ZMPSTE24* relative to housekeeping gene *GAPDH*, in AmotL2 knockdown HUVEC cells. (*n*=5 independent experiments, mean± s.d., 2way ANOVA with Šidák's multiple comparisons). **B,** SYBR green RT-qPCR of *AmotL2*, *YAP*, and *LMNB1* relative to housekeeping gene *GAPDH*, in AmotL2 knockdown HUVEC cells. (*n*=4 independent experiments, mean± s.d., 2way ANOVA with Šidák's multiple comparisons). **C**, Top 20 differential KEGG pathway analysis of shScr, shAmotL2 transduced HUVEC indicating pathways with differential peak accessibility. Note 'Hippo signalling pathway' in bold. **D**, Schematic of primers designed for ChIP analysis of region highlighted in the second peak of bimodal accessibility within the *YAP* promotor where differential accessibility was observed from ATAC-seq data shown in Fig7i-j. **E**, ChIP showing Pol II pulldown at the region of the *YAP* promotor show in (d) of shScr or shAmotL2 treated HUVEC. ChIP qPCR was performed using SYBR green reagents and quantification was normalised to input and IgG control. Plots shown are representative of *n*=3 independent experiments. Each data point represents a technical repeat within one independent experiment (performed in triplicate). Graphs display (mean± s.d.). **F,** IGV browser view of the *LMNA* promotor showing publicly available ChIPseq data of Pol II, alongside ATACseq data of HUVEC treated with shScr or shAmotL2. r indicates independent biological replicates, of which there are *n*=4 per condition.

Supplemental Figure 11

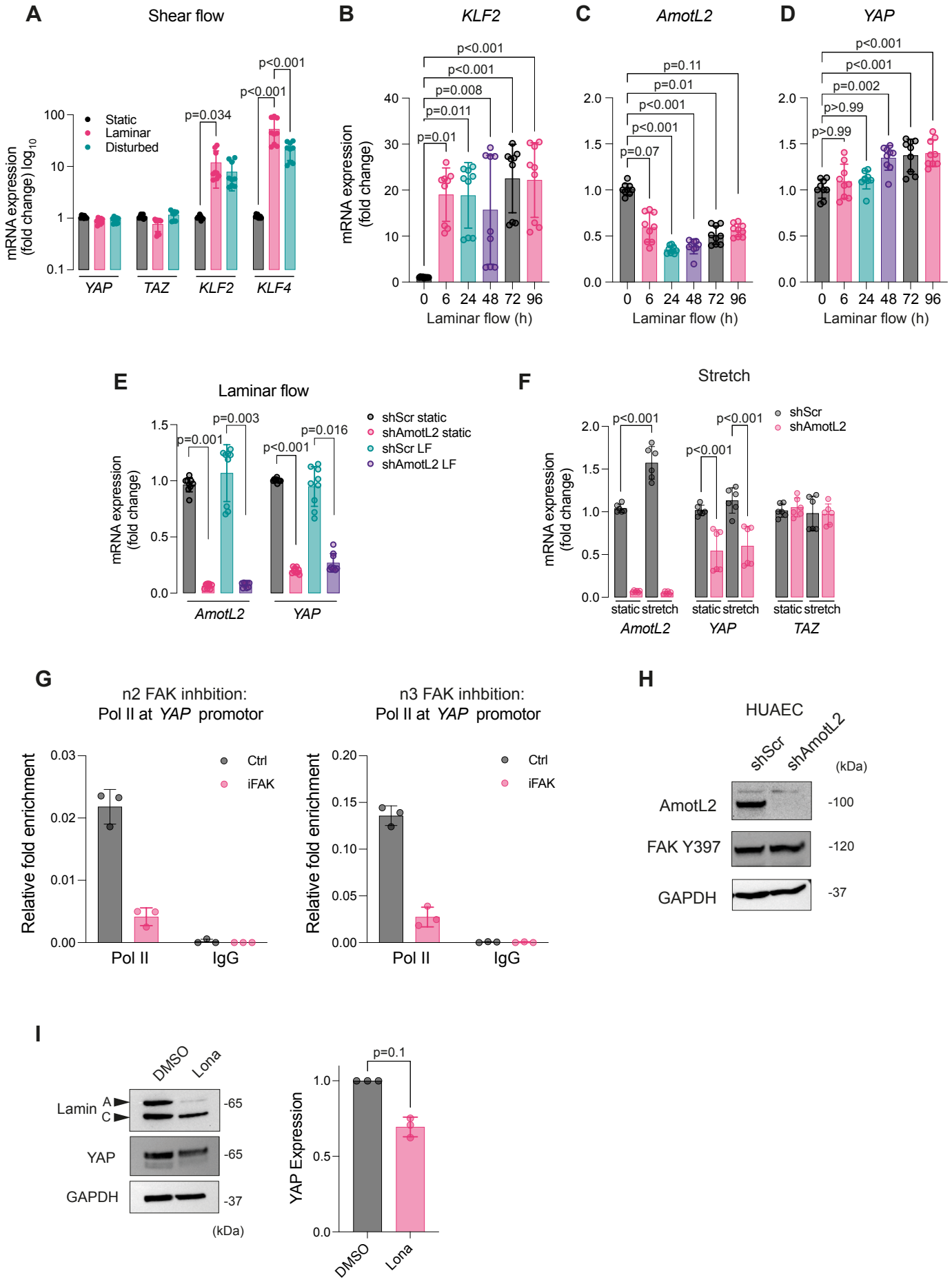


Fig. S11.

A, SYBR green RT-qPCR of YAP, TAZ, KLF2 and KLF4 relative to housekeeping gene GAPDH, in HUVEC cells exposed to static, laminar or disturbed flow conditions. (n=3 independent experiments, mean± s.d., 2way ANOVA with Dunnett's multiple comparisons). RT-qPCR of *KLF2* (**B**), *AmotL2* (**C**) and *YAP* (**D**) from HUVEC plated to 6 well plates at confluency before being subject to orbital flow for indicated time points before lysates were harvested as described in the materials and methods so as to obtain laminar flow transcriptional responses (*n*=3 independent experiments, mean± s.d., Kruskal-Wallis with Dunn's multiple comparisons). **E,** RT-qPCR of *AmotL2* and *YAP* from HUVEC following 72 h shScr or shAmotL2 knockdowna and either static or exposure to 48 h orbital flow (*n*=3 independent experiments, mean± s.d., Kruskal-Wallis with Dunn's multiple comparisons). **F,** RT-qPCR of YAP, TAZ, and AmotL2 relative to housekeeping gene GAPDH, of shScr and shAmotL2 treated HUVEC exposed to uniaxial 19% stretch for 24 h. (n=2 independent experiments, mean± s.d., 2way ANOVA with Dunnett's multiple comparisons). **G,** ChIP showing Pol II binding to YAP promotor of control or FAK inhibitor treated HUVEC. ChIP qPCR was performed using SYBR green reagents and quantification was normalised to an IgG control antibody. Plots shown are representative experiments from n=3 independent experiments (Fig8c shows n=1). Each data point represents a technical repeat within one independent experiment (performed in triplicate). Graphs display (mean± s.d.). **H,** Western blot analysis of AmotL2 and phospho FAK in HUAEC cells 96h post-treatment with shScr or shAmotL2 lentivirus. Membranes shown in Supplemental figure 5C were re-probed for FAK Y397. GAPDH was used as a loading control. Data are representative of n=2 independent experiments. **I,** Western blot analysis of YAP and Lamin A/C in HUVEC cells 48 h post-Lonafarnib 10 μM treatment. GAPDH was used as a loading control. Bar graph indicates quantification of YAP, relative to GAPDH. (*n*=3 independent experiments, mean± s.d., Mann-Whitney).

Table S1.

List of primers used for RT-PCR analysis.

Target	Forward sequence (5'-3')	Reverse sequence (5'-3')
YAP	AATTGAGAACAATGACGACC	AGTATCACCTGTATCCATCTC
TAZ	TTTTCCAGAAGATGAATCCG	CAGGCTCCTTAAAGAAAGAG
AMOTL2	GCAGAAGTATTTGGAGGAAC	CCTTTAACCTGCTTTCCATC
CTGF	TTAAGAAGGGCAAAAAGTGC	CATACTCCACAGAATTTAGCTC
CYR61	TTGATTGCAGTTGGAAAAGG	GCCTTGTAAAGGGTTGTATAG
ANKRD1	TGAGTATAAACGGACAGCTC	TATCACGGAATTCGATCTGG
EZH2	AAGAAATCTGAGAAGGGACC	CTCTTTACTTCATCAGCTCG
GAPDH	TCGGAGTCAACGGATTTC	CAACAATATCCACTTTACCAGAG
KLF2	CCAAGAGTTCGCATCTGAAGGC	CCGTGTGCTTTCGGTAGTGGC
KLF4	CATCTCAAGGCACACCTGCGAA	TCGGTCGCATTTTTGGCACTGG
LMNA	AGAACATCTACAGTGAGGAG	CAGAATAAGTCTTCTCCAGC
LMNB	AAAATTCTCAGGGAGAGGAG	TGGAAAAGTTCTTCCTCAAC
ZMPSTE24	ACTCAGTGTATTTTGTTGCC	AACCAGAGACACAACTAATG

Table S2.

Software used to process and perform statistical analysis of ATAC-seq datasets.

Analysis	Software	Version	Parameters	Remarks
Trimming	skewer	0.2.2	-m pe	Filter rawdata
QC	fastqc	v0.11.5		
mapping	BWA	0.7.12-r1039	-T 25 -k 18	Mapped to the reference genome
correlation between samples	deepTools	3.0.2	--corMethod pearson	
peak calling	MACS2	2.1.2	-q 0.05 --call-summits --nomodel --shift -100 --extsize 200 --keep-dup all	
Identification of motif	homer findMotifsGenome.pl	v4.9.1	-gc -len 8,10,12,14	
GO enrichment	Goseq, topGO, Bioconductor (2.13)	4.10.2	corrected pvalue<0.05	
KEGG enrichment	KOBAS	3	corrected pvalue<0.05	

Data S1. (separate file) - (GEO accession: GSE253761) ATAC-seq excel file HUVEC shScr vs. shAmotL2 peak comparison

Data S2. (separate file) - (GEO accession: GSE253761) ATAC-seq excel file HUVEC shScr vs. shAmotL2 peak down related genes

Major Resources Table

In order to allow validation and replication of experiments, all essential research materials listed in the Methods should be included in the Major Resources Table below. Authors are encouraged to use public repositories for protocols, data, code, and other materials and provide persistent identifiers and/or links to repositories when available. Authors may add or delete rows as needed.

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Persistent ID / URL
Mus musculus	Jackson Laboratory, Taconics Inc. or as otherwise specified below	C57BL/6J	M and F	

Genetically Modified Animals

	Species	Vendor or Source	Background Strain	Other Information	Persistent ID / URL
Wwtr1 flox/flox; Yap flox/flox x *Cdh5(BAC)-CreERT2*	Mus musculus	*Wwtr1 flox/flox; Yap flox/flox* - Jackson Laboratory *Cdh5(BAC)-CreERT2* – Ref.(18)	C57BL/6J	*Wwtr1 flox/flox; Yap flox/flox* mice (Jackson Laboratory) were crossed to *Cdh5(BAC)-CreERT2* transgenic mice	
amotl2 flox/flox x Cdh5(PAC)-CreERT2 x ROSA26-EYFP	Mus musculus	*amotl2 flox/flox* – Taconics Inc *Cdh5(PAC)-CreERT2* – Ref. (19) *ROSA26-EYFP* Jackson Laboratory	C57BL/6J	*amotl2 flox/flox* mice with loxP-flanked amotl2 gene, were crossed with *Cdh5(PAC)-CreERT* and ROSA26-EYFP transgenic mice	

Antibodies

Target antigen	Vendor or Source	Catalog #	Working concentration	Persistent ID / URL
Rabbit pAb anti-AmotL2	Innovagen, Lund, Sweden	Ref. 21	1:100 (IF) 1:1000 (WB)	Ref. 21
Rabbit mAb Anti-YAP	Cell Signalling Technologies	D8H1X; #14074	1:100 (IF) 1:1000 (WB)	https://www.cellsignal.com/products/primary-antibodies/yap-d8h1x-xp-rabbit-mab/14074
Rabbit mAb anti-pYAP Ser127	Cell Signalling Technologies	D9W2I; #13008	1:1000 (WB)	https://www.cellsignal.com/products/primary-antibodies/phospho-yap-ser127-d9w2i-rabbit-mab/13008
rabbit mAb anti-TAZ	Cell Signalling Technologies	E8E9G; #83669	1:1000 (WB)	https://www.cellsignal.com/products/primary-antibodies/taz-e8e9g-rabbit-mab/83669
Mouse anti-GAPDH	Abcam	ab181602	1:5000 (WB)	https://www.abcam.com/products/primary-antibodies/gapdh-antibody-epr16891-loading-control-ab181602.html
rabbit anti-FAK (phosphor Y397)	Abcam	ab81298	1:1000 (WB)	https://www.abcam.com/products/primary-antibodies/fak-phospho-y397-antibody-ep2160y-ab81298.html
Mouse anti-Lamin A/C	Santa Cruz Biotechnology	sc-7292; 636	1:1000 (WB) 1:200 (IF)	https://www.scbt.com/p/lamin-a-c-antibody-636?requestFrom=search
Mouse anti-YAP/TAZ	Santa Cruz Biotechnology	sc-101199; 63.7	1:100 (IF) 1:1000 (WB)	https://www.scbt.com/p/yap-antibody-63-7?requestFrom=search
rabbit pAb anti-ki67	Abcam	ab15580	1:100 (IF)	https://www.abcam.com/products/primary-antibodies/ki67-antibody-ab15580.html
rabbit pAb anti-VE-cadherin	Abcam	ab33168	1:250 (IF)	https://www.abcam.com/products/primary-antibodies/ve-cadherin-antibody-intercellular-junction-marker-ab33168.html
chicken pAb anti-GFP	Abcam	ab13970	1:200 (IF)	https://www.abcam.com/products/primary-antibodies/gfp-antibody-ab13970.html
goat pAb anti-GFP	Abcam	ab6673	1:200 (IF)	https://www.abcam.com/products/primary-antibodies/gfp-antibody-ab6673.html
rabbit anti-ERG mAb	Abcam	ab92513	1:200 (IF)	https://www.abcam.com/products/primary-antibodies/erg-antibody-epr3864-ab92513.html
Rat anti-Cd31	BD Biosciences	MEC 13.3; 553370	1:100 (IF)	https://www.bdbiosciences.com/en-de/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/purified-rat-anti-mouse-cd31.553370
Rat anti-Cd144/VEcadherin	BD Biosciences	1104.1; 555289	1:200 (IF)	https://www.bdbiosciences.com/en-de/products/reagents/functional-cell-based-reagents/purified-rat-anti-mouse-cd144.555289

DOI [to be added]

Mouse anti-prelamin A	Sigma	PL-1C7	1:100 (IF)	https://www.sigmaaldrich.com/DE/de/product/mm/mabt858
Mouse Anti-TEAD1 mAb	BD Biosciences	610923	1:100 (PLA)	https://www.bdbiosciences.com/en-de/products/reagents/microscopy-imaging-reagents/immunofluorescence-reagents/purified-mouse-anti-tef-1.610923
RNA pol II mAb	Active Motif	39097		https://www.activemotif.com/catalog/details/39097/rna-pol-ii-antibody-mab
Histone H3K27me3 pAb	Active Motif	39155		https://www.activemotif.com/catalog/details/39155/histone-h3-trimethyl-lys27-antibody-pab
Mouse IgG	Sigma	I8765		https://www.sigmaaldrich.com/DE/de/product/sigma/i8765
Rabbit IgG	Diagenode	C15410206		https://www.diagenode.com/en/p/rabbit-igg-250-ug-250-ul
Anti-Histone H3 (acetyl K27)	Abcam	ab4729		https://www.abcam.com/products/primary-antibodies/histone-h3-acetyl-k27-antibody-chip-grade-ab4729.html
anti-Histone H3 (tri methyl K4)	Abcam	ab8580		https://www.abcam.com/products/primary-antibodies/histone-h3-tri-methyl-k4-antibody-chip-grade-ab8580.html
TexasRed phalloidin	Invitrogen	T7471	1:200 (IF)	https://www.thermofisher.com/order/catalog/product/T7471?SID=srch-srp-T7471
phalloidin-Atto 647N	Sigma	65906	1:200 (IF)	https://www.sigmaaldrich.com/DE/de/product/sigma/65906

DOI [to be added]

Cultured Cells

Name	Vendor or Source	Sex (F, M, or unknown)	Persistent ID / URL
HUVEC	Promocell	Unknown	https://promocell.com/product/human-umbilical-vein-endothelial-cells-huvec/
HUAEC	Promocell	Unknown	https://promocell.com/product/human-umbilical-artery-endothelial-cells-huaec/

Data & Code Availability

Description	Source / Repository	Persistent ID / URL
Data S1. ATAC-seq excel file HUVEC shScr vs. shAmotL2 peak comparison	https://www.ncbi.nlm.nih.gov/geo/ GEO gene expression omnibus	(GEO accession: GSE253761)
Data S2. ATAC-seq excel file HUVEC shScr vs. shAmotL2 peak down related genes	https://www.ncbi.nlm.nih.gov/geo/ GEO gene expression omnibus	(GEO accession: GSE253761)
ATACseq raw data files for HUVEC and HUAEC shScr vs. shAmotL2	https://www.ncbi.nlm.nih.gov/geo/ GEO gene expression omnibus	(GEO accession: GSE253761)

ARRIVE GUIDELINES

The ARRIVE guidelines (https://arriveguidelines.org/) are a checklist of recommendations to improve the reporting of research involving animals. Key elements of the study design should be included below to better enable readers to scrutinize the research adequately, evaluate its methodological rigor, and reproduce the methods or findings.

Study Design

Groups	**Sex**	**Age**	**Number (prior to experiment)**	**Number (after termination)**	**Littermates (Yes/No)**	**Other description**
Yap/Taz WT *Wwtr1 flox/flox; Yap flox/flox* - *Cre*-negative	Male and female	8 weeks	Total: 21	Total: 21	Yes	To induce endothelial-specific Yap/Taz gene inactivation, tamoxifen (Sigma, T5648) in corn oil (Sigma, C8267) was administered by oral gavage for 5 continuous days in 8-week-old mice (2 mg/mouse/day).
Yap/Taz iΔEC- *Wwtr1 flox/flox; Yap flox/flox*; *Cdh5(BAC)-CreERT2 – Cre*-positive	Male and female	8 weeks	Total: 20	Total: 20	Yes	To induce endothelial-specific Yap/Taz gene inactivation, tamoxifen (Sigma, T5648) in corn oil (Sigma, C8267) was administered by oral gavage for 5 continuous days in 8-week-old mice (2 mg/mouse/day).
AmotL2 WT - *amotl2 flox/flox x Cdh5(PAC)-CreERT2 x ROSA26-EYFP (Cre-* negative)	Male and female	6 weeks	Total: 12	Total: 12	Yes	To induce endothelial-specific amotl2 deletion, tamoxifen was administered by intraperitoneal (IP) injection for 5 continuous days. For adult mice over 6 weeks old, 100µl of tamoxifen (20mg/ml) was administered and analysis of aortic samples was performed four weeks following injections.

AmotL2 iΔEC - *amotl2 flox/flox x Cdh5(PAC)-CreERT2 x ROSA26-EYFP-(Cre-* positive)	Male and female	6 weeks	Total: 14	Total:14	Yes	To induce endothelial-specific amotl2 deletion, tamoxifen was administered by intraperitoneal (IP) injection for 5 continuous days. For adult mice over 6 weeks old, 100µl of tamoxifen (20mg/ml) was administered and analysis of aortic samples was performed four weeks following injections.
CreERT2 x ROSA26-EYFP-(Cre- positive)	Male and female	6 weeks	Total: 3	Total: 3	Yes	Tamoxifen was administered by intraperitoneal (IP) injection for 5 continuous days. For adult mice over 6 weeks old, 100µl of tamoxifen (20mg/ml) was administered and analysis of aortic samples was performed four weeks following injections.

Sample Size: Sample size was determined by assessment of similar research in the literature and adopting similar n per sample group.

Inclusion Criteria – By genotyping, *Cre* positive or negative

Exclusion Criteria - By genotyping, *Cre* positive or negative

Randomization - None

Blinding - None