Supplement Material

YAP1/TEAD1 Upregulate Platelet-derived Growth Factor Receptor Beta to Promote Vascular Smooth Muscle Cell Proliferation and Neointima Formation

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Supplemental Methods

Mouse carotid artery ligation injury. Mouse left carotid artery (LCA) ligation was performed as previously described [1, 2]. Briefly, to examine YAP1 and PDGFRβ expression following mouse carotid artery ligation injury, C57BL/6J male mice (Jackson Laboratory, 12-14 weeks old) were anesthetized by isoflurane *via* inhalation. The LCA was dissected and completely ligated just proximal to the carotid bifurcation. The right carotid artery (RCA) served as an uninjured contralateral control. The RCAs and LCAs were harvested at day 7 post-injury for Western blot analysis as indicated in the figures. To determine the effects of SM-specific ablation of *Yap1* on neointima formation following carotid artery ligation injury, we generated *Myh11*-Cre ER^{T2}/$Yap1^{F/F}$ mice by crossing *Yap1* flox (F) female mice [3, 4] with male mice expressing tamoxifen-inducible Cre driven by the SM-specific *Myh11* gene promoter (*Myh11*-$CreER^{T2}$) [5]. 10-week-old *Myh11*-Cre ER^{T2}/$Yap1^{F/F}$ male mice were randomly divided into 2 groups, intraperitoneally injected with either sunflower oil (control) or tamoxifen (iKO) for 10 times within 2 weeks. Following a 2-week washout time after the last tamoxifen injection, control or iKO mice were subjected to carotid artery ligation injury as described above. At day 7 or day 28 post-injury, carotid arteries were harvested for Western blotting or histological analysis, respectively. Only male mice were used in this study because *Myh11*-$CreER^{T2}$ transgene is localized only in the Y chromosome [5]. Primers for genotyping were listed in the **Online Table II**.

Sections, Hematoxylin and Eosin (HE) staining, morphometric analysis of carotid artery, and immunofluorescence (IF) staining. Mice were euthanized by an overdose of 4% Isoflurane *via* inhalation, then systemically perfused with PBS *via* the left ventricle. Isolated carotid arteries were fixed with 4% paraformaldehyde in PBS over-night at 4°C, washed 3 times with PBS, then kept in 30% sucrose in PBS over-night at 4°C. Fixed tissues were embedded in optimal cutting temperature compound and kept at -80°C until cryo-sectioning. Cross-sections of carotid arteries (8-μm thickness) were prepared from proximal to the ligature to the aortic arch. Morphometric analysis was performed using 6 sections from each artery that were located at around 250 μm proximal to the ligature. HE staining was performed following a standard protocol as previously described [6]. HE-stained images were captured using an Olympus BX51 inverted microscope. Sections were analyzed blindly by an independent investigator for neointimal areas and neointima-to-media ratios using Image J software. The neointimal area was calculated by subtraction of the luminal area from the area enclosed by the internal elastic lamina. The medial area was calculated by subtraction of the area enclosed by the internal elastic lamina from the area enclosed by the external elastic lamina. For IF, cryo-sections were air-dried for 15 minutes and antigen retrieval was performed by heating at 98°C for 10 minutes in citric acid buffer (10 mM, pH 6.0). Sections were blocked and permeabilized with goat serum (10%, Thermo Fisher Scientific) plus 0.1% Tween for 30 minutes, then incubated with MKI67 (Thermo Scientific, RM-9106, rabbit, 1:30) or ACTA2 (Abcam, Ab7817, mouse, 1:80) antibodies over-night at 4°C. After washing with PBS, sections were incubated with secondary antibodies (488 nm-conjugated anti-rabbit secondary antibody or 594 nm-conjugated anti-mouse secondary antibody, 1:250 dilution, Thermo Fisher Scientific) diluted in blocking buffer for 1 hour at room temperature. Following washing with PBS for three times, sections were mounted with ProLong Gold antifade reagent with DAPI (Thermo Fisher Scientific) and imaged using a confocal microscopy (780 upright, Zeiss) at the imaging core facility of Augusta University.

Cell culture. HCASMCs (Gibco, cat. #: C-017-5C; Lot #: 1130140 and Lot #: 1689414), vascular cell basal medium (cat. #: M231500), and smooth muscle growth supplement with 5% FBS (SMGS, cat. #: S00725) were purchased from Thermo Fisher Scientific. HCASMCs (passages 3-5) were incubated in vascular cell basal medium containing SMGS (complete medium) and 5.5

mM D-glucose along with antibiotic/antimycotic solution in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. Sub-confluent VSMCs were trypsinized, centrifuged, and seeded onto Petri-dishes or multi-well plates. In some experiments, VSMC quiescence was induced by substituting the complete medium with vascular cell basal medium without SMGS for 48 hours before treatments.

Adenoviral construction and cell infection. Adenovirus encoding YAP1 or TEAD1 were generated as previously described [1, 6, 7]. As these vectors contain an independent cytomegalovirus promoter-driven transcription cassette for green fluorescent protein (GFP), the efficiency of transduction was directly monitored by visualization of GFP expression. The adenovirus expressing GFP alone served as control.

siRNA transfection. Scrambled control siRNA duplex (#4390843), human *YAP1* siRNA duplex (#s20367), human *PDGFRB* siRNA duplex (#s10242), and human *TEAD1* siRNA duplex (#s13961) were purchased from Thermo Fisher Scientific (Ambion). The siRNA duplex sequences are listed in **Online Table II**. Delivery of siRNA into HCASMCs was done using Neon transfection system (Thermo Fisher Scientific) essentially following the manufacturer's protocol and as described in our previous report [6]. After transfection, VSMCs were equally plated on 6-well plates for different time points, as indicated in the figure legends, for cell count assays, qRT-PCR, or Western blot analysis. For WST-1 proliferation assays, equal numbers of VSMCs were plated on 96-well plates.

Cell counts. Sub-confluent HCASMCs were plated onto 6-well plates in complete medium with or without treatments as described in the figure legends. During the treatment period, the media was replaced every 48 hours with a fresh complete medium containing the indicated concentrations of treatments. VSMCs were then trypsinized and the changes in cell counts were determined using a hemocytometer.

WST-1 proliferation assay. The assay was done according to the manufacturer's instructions as we previously reported [8, 9]. Briefly, sub-confluent HCASMCs were plated onto 96-well plates in complete medium with or without the respective treatments for the indicated periods of time as described in the figure legends. WST-1 reagent (Sigma-Aldrich, cat. #: 5015944001, 10 μl/100 μl medium) was added to the culture medium and cells were kept at 37°C for 2 hours. Subsequently, the absorbance at 450nm, as an indirect measurement of cell proliferation, was measured using a microplate reader.

5-ethynyl-20-deoxyuridine (EdU) incorporation assay. EdU incorporation assay was performed using the Click-iT EdU imaging kit (Thermo Fisher Scientific, cat. #: C10339) according to the manufacturer's instructions and as described in our previous report [6]. Briefly, HCASMCs were incubated with EdU (10 mM) for the indicated period of time as described in the figure legends. EdU-positive nuclei were detected following the manufacturer's protocol and imaged using a confocal microscopy (780 upright, Zeiss). Cell nuclei were co-stained with DAPI.

Quantitative real-time PCR (qRT-PCR) analysis. For aortic tissue samples, mice were euthanized by 4% Isoflurane *via* inhalation, then systemically perfused with PBS. Dorsal aortae were rapidly dissected and cleared of perivascular fat and connective tissues under a stereoscope. Then, aortic tissues were rapidly frozen in liquid nitrogen and stored at -80°C until subsequent analysis. Total RNA from aortic tissues or HCASMCs was extracted with TRIzol reagent (Thermo Fisher Scientific). 1 μg of RNA was reverse transcribed to cDNA using the High-Capacity RNA-to-cDNA Kit with random hexamer primers (Thermo Fisher Scientific). qRT-PCR was performed using the respective gene-specific primers as listed in **Online Table II**. All samples were amplified

in duplicate and all experiments were repeated at least 3 independent times. Relative gene expression was determined using the $2^{-\triangle\triangle CT}$ method (CT, comparative threshold cycle). CT values were normalized to the internal control hypoxanthine phosphor ribosyl transferase 1 (*Hprt1*) for mouse tissue samples and β2 microglobulin (*B2M*) for human SMC samples. $\triangle\triangle CT = (CT_{experimental\ gene} - CT_{experimental\ Hprt1\ or\ B2M}) - (CT_{control\ gene} - CT_{control\ Hprt1\ or\ B2M})$. PCR array was performed to screen cell proliferation-related gene expression profile using PCR Array kit (Qiagen, cat. #: PAHS-507Z) as described in our previous report [3].

Protein extraction and Western blotting. Isolated mouse tissues or HCASMCs were homogenized in RIPA buffer (Thermo Fisher Scientific) plus 1% protease/phosphatase inhibitor cocktail (Thermo Fisher Scientific). After sonication and centrifugation of the tissue or cell lysates, protein in the supernatant was quantified using BCA assay (Thermo Fisher Scientific) and resolved on a 7.5%, 10%, or 12.5% SDS-PAGE gel using 5-20 μg of protein per lane as appropriate. Antibodies used in this study are: YAP1 (Sigma, WH0010413M1, mouse, 1:1000), PDGFRβ (Cell signaling, #3169, rabbit, 1:1000), PCNA (Santa Cruz, sc-56, mouse, 1:100), CCND1 (Cell signaling, #2978, rabbit, 1:1000), ACTA2 (Abcam, Ab7817, mouse, 1:2000), CNN1 (Sigma, C2687, mouse, 1:1000), TAGLN (Abcam, ab10135, goat, 1:1000) , CCN1 (Cell signaling, #39382, rabbit, 1:1000), MYC (Cell signaling, #5605, rabbit, 1:1000), SLC1A5 (Cell signaling, #5345, rabbit, 1:1000), VCL (Sigma, V4505, mouse,1:5000), pPDGFRβ (Cell signaling, #4549, rabbit, 1:1000), pMAPK1/3 (Cell signaling, #4370, rabbit, 1:1000), MAPK1/3 (Cell signaling, #4695, rabbit, 1:1000), pAKT (Cell signaling, #2965, rabbit, 1:1000), AKT (Cell signaling, #4691, rabbit, 1:1000), pRPS6 (Cell signaling, #4858, rabbit, 1:1000), TUBA1A (Cell signaling, #2144, rabbit, 1:1000), TEAD1 (Abcam, ab133533, rabbit, 1:1000), HSP90AA1 (Cell Signaling, #4874, rabbit, 1:1000), pHIST1H3A (Millipore, 06-570, rabbit, 1;1000), ACTB (Sigma, A5316, mouse, 1:2000), MYLK (Sigma, #M7905, mouse, 1:2000) and TGFβ1I1 (BD, 611164, mouse, 1:5000). Images were acquired by ImageQuant LAS 4000 Imaging Station (GE) and band densities were quantified using the Image J software.

Quantitative chromatin immunoprecipitation (ChIP) assay. Assays were performed as described by the manufacturer (Active Motif) and in our previous reports [1, 6, 10]. Briefly, after transducing with TEAD1 adenovirus for 48 hours, HCASMCs were washed, fixed with formaldehyde, and equal amounts of chromatin were immunoprecipitated using 2 different anti-TEAD1 antibodies (Abcam, ab133533; or BD Biosciences, 610923), or rabbit IgG control. The genomic DNA purified from the precipitated genomic DNA or from input was amplified by qPCR. Primers for quantitative evaluation of enrichment of TEAD1 at different regions of a gene are listed in **Online Table II**. Data were expressed as relative binding by using the $2^{-\triangle\triangle CT}$ method against the IgG control samples (set to 1) where $\triangle\triangle CT = (CT_{IP\ TEAD1} - CT_{input\ TEAD1}) - (CT_{IP\ control\ IgG} - CT_{input\ control\ IgG})$.

Luciferase reporter assay. Cloning of WT or mutant *PDGFRB* enhancer-driven luciferase reporters and dual luciferase assays were performed as described in our recent reports [1, 6]. Briefly, fragments spanning MCAT sequences identified in the human *PDGFRB* enhancer were amplified by PCR with primers harboring KpnI and XhoI restriction enzyme sites (primer sequences are listed in the **Online Table II**) by using human genomic DNA (Promega) as the template. The PCR products were first cloned into pSC-B blunt vector (Stratagene), then sub-cloned into pGL2E luciferase reporter vector (Promega). All plasmids were sequenced to verify the integrity of the insert (Genewiz). Mammalian expression plasmids for *YAP1* and *TEAD1* were generously provided by Dr. Kun-liang Guan, UCSD [11]. Transfection was carried out with X-tremeGENE 9 transfection reagent (Roche) essentially following the manufacturer's protocol. The enhancer activity was evaluated by measurement of the firefly luciferase activity relative to the

internal control TK-renilla luciferase activity using the Dual-Luciferase Assay System as described by the manufacturer (Promega).

Statistical analysis. All data are expressed as means ± SE of at least three independent experiments. For *in vivo* LCA injury experiments, the sample size was calculated based on power analysis to achieve a Power of 0.8 at a p=0.05. Statistical analysis of data involving more than two groups was performed using one-way or two-way analysis of variance (ANOVA), where appropriate, followed by Bonferroni t-test. Statistical analysis of data involving two groups was performed using unpaired two-tailed t-test for samples with a normal distribution (Shapiro-Wilk test was initially used to test for Normality). Otherwise, unpaired non-parametric Mann Whitney test was performed (GraphPad). Values of $p \leq 0.05$ were considered statistically significant.

Supplemental References

[1] X. Wang, G. Hu, C. Betts, E.Y. Harmon, R.S. Keller, L. Van De Water, J. Zhou, Transforming growth factor-beta1-induced transcript 1 protein, a novel marker for smooth muscle contractile phenotype, is regulated by serum response factor/myocardin protein, The Journal of biological chemistry 286(48) (2011) 41589-99.

[2] W. Zhang, K.E. Halligan, X. Zhang, J.M. Bisaillon, J.C. Gonzalez-Cobos, R.K. Motiani, G. Hu, P.A. Vincent, J. Zhou, M. Barroso, H.A. Singer, K. Matrougui, M. Trebak, Orai1-mediated I (CRAC) is essential for neointima formation after vascular injury, Circ Res 109(5) (2011) 534-42.

[3] Y. Wang, G. Hu, F. Liu, X. Wang, M. Wu, J.J. Schwarz, J. Zhou, Deletion of yes-associated protein (YAP) specifically in cardiac and vascular smooth muscle cells reveals a crucial role for YAP in mouse cardiovascular development, Circ Res 114(6) (2014) 957-65.

[4] N. Zhang, H. Bai, K.K. David, J. Dong, Y. Zheng, J. Cai, M. Giovannini, P. Liu, R.A. Anders, D. Pan, The Merlin/NF2 tumor suppressor functions through the YAP oncoprotein to regulate tissue homeostasis in mammals, Developmental cell 19(1) (2010) 27-38.

[5] A. Wirth, Z. Benyo, M. Lukasova, B. Leutgeb, N. Wettschureck, S. Gorbey, P. Orsy, B. Horvath, C. Maser-Gluth, E. Greiner, B. Lemmer, G. Schutz, J.S. Gutkind, S. Offermanns, G12-G13-LARG-mediated signaling in vascular smooth muscle is required for salt-induced hypertension, Nat Med 14(1) (2008) 64-68.

[6] I. Osman, X. He, J. Liu, K. Dong, T. Wen, F. Zhang, L. Yu, G. Hu, H. Xin, W. Zhang, J. Zhou, TEAD1 (TEA Domain Transcription Factor 1) Promotes Smooth Muscle Cell Proliferation Through Upregulating SLC1A5 (Solute Carrier Family 1 Member 5)-Mediated Glutamine Uptake, Circ Res 124(9) (2019) 1309-1322.

[7] X. Wang, G. Hu, J. Zhou, Repression of versican expression by microRNA-143, The Journal of biological chemistry 285(30) (2010) 23241-50.

[8] F. Xu, A.S. Ahmed, X. Kang, G. Hu, F. Liu, W. Zhang, J. Zhou, MicroRNA-15b/16 Attenuates Vascular Neointima Formation by Promoting the Contractile Phenotype of Vascular Smooth Muscle Through Targeting YAP, Arteriosclerosis, thrombosis, and vascular biology 35(10) (2015) 2145-52.

[9] X. Wang, G. Hu, X. Gao, Y. Wang, W. Zhang, E.Y. Harmon, X. Zhi, Z. Xu, M.R. Lennartz, M. Barroso, M. Trebak, C. Chen, J. Zhou, The induction of yes-associated protein expression after arterial injury is crucial for smooth muscle phenotypic modulation and neointima formation, Arterioscler Thromb Vasc Biol 32(11) (2012) 2662-9.

[10] J. Zhou, G. Hu, X. Wang, Repression of smooth muscle differentiation by a novel high mobility group box-containing protein, HMG2L1, The Journal of biological chemistry 285(30) (2010) 23177-85.

[11] B. Zhao, X. Ye, J. Yu, L. Li, W. Li, S. Li, J.D. Lin, C.Y. Wang, A.M. Chinnaiyan, Z.C. Lai, K.L. Guan, TEAD mediates YAP-dependent gene induction and growth control, Genes & development 22(14) (2008) 1962-71.

Online Table I. PCR array results demonstrating the relative changes in gene expression in HCASMCs after transduction with YAP1 adenovirus vs GFP control.

Gene Symbol	Fold Change
ABCC1	4.5345
AKT1	1.4898
AKT2	1.4682
ATF2	-3.1181
AURKA	1.4446
AURKB	1.4576
AURKC	1.8355
BCL2	-1.012
BIRC5	2.1637
CDC25A	4.6798
CDK1	1.9017
CDK2	1.6753
CDK4	-1.1537
CDK5	2.5211
CDK7	1.3683
CDK8	-2.5427
CDK9	1.1375
CTSB	-1.1023
CTSD	1.1278
CTSL	1.3666
CTSS	-1.0532
EGFR	1.3521
ERBB2	1.7633
ERBB3	16.0848
ERBB4	-1.1436
ESR1	-2.1889
ESR2	-1.9137
FIGF	-1.565
FLT1	3.2405
FLT4	-2.8194
GRB2	2.5021
GSTP1	1.1791
HDAC1	1.8544
HDAC11	1.1424
HDAC2	1.291
HDAC3	1.7899
HDAC4	-1.0342
HDAC6	1.1761

HDAC7	-1.1924
HDAC8	1.1552
HIF1A	-3.1913
HRAS	1.607
HSP90AA1	2.6558
HSP90B1	1.0001
IGF1	-2.3338
IGF1R	1.3789
IGF2	1.0786
IRF5	-3.2047
KDR	14.9219
KIT	-1.4763
KRAS	-1.5934
MDM2	-1.5853
MDM4	-1.6443
MTOR	2.4664
NFKB1	-1.4085
NRAS	1.0558
NTN3	-1.3508
PARP1	1.6214
PARP2	1.8232
PARP4	1.2897
PDGFRA	-2.1837
PDGFRB	2.8083
PGR	-3.9064
PIK3C2A	1.1652
PIK3C3	-1.1613
PIK3CA	-1.0453
PLK1	6.1131
PLK2	2.156
PLK3	-1.2635
PLK4	-1.2329
PRKCA	-1.6018
PRKCB	-2.1808
PRKCD	1.2341
PRKCE	1.6806
PTGS2	-1.3105
RHOA	1.2835
RHOB	-1.1381
TERT	1.8202
TNKS	-2.2143

TOP2A	2.9894
TOP2B	-1.3295
TP53	1.9035
TXN	1.0541
TXNRD1	-1.7617

Online Table II. List of oligonucleotides used in the study.

A. Primers used for quantitative RT-PCR (F: forward; R: reverse)

Gene Name	*Species*	*Sequence (5'-3')*
Yap1	Mouse	F: CAGCATGTTCGAGCTCACTC
	Mouse	R: CAGGAACGTTCAGTTGCGAA
Pdgfrb	Mouse	F: AGGACAACCGTACCTTGGGTGACT
	Mouse	R: CAGTTCTGACACGTACCGGGTCTC
Myh11	Mouse	F: CACAGGAAACTTCGCAGTGA
	Mouse	R: TTCTGTTTTCCCTGACATGGT
Acta2	Mouse	F: ATGCTCCCAGGGCTGTTTTCCCAT
	Mouse	R: GTGGTGCCAGATCTTTTCCATGTCG
Cnn1	Mouse	F: AACTGGCACCAGCTGGAGAACATAG
	Mouse	R: GAGTGGACTGAACTTGTGTATGGTTG
Tagln	Mouse	F: TGACATGTTCCAGACTGTTGACCTCT
	Mouse	R: CTTCATAAACCAGTTGGGATCTCCAC
Hprt1	Mouse	F: TCTTTGCTGACCTGCTGGATTACA
	Mouse	R: AGTTGAGAGATCATCTCCACCAAT
YAP1	Human	F: TAGCCCTGCGTAGCCAGTTA
	Human	R: TCATGCTTAGTCCACTGTCTGT
PDGFRB	Human	F: GTGGTGATCTCAGCCATCCT
	Human	R: CCGACATAAGGGCTTGCTT
CCND1	Human	F: GAACACTTCCTCTCCAAAATGCCAGA
	Human	R: GAAATGAACTTCACATCTGTGGCAC
TEAD1	Human	F: TTTGTGCAGCAGGCCTACCCCATC
	Human	R: GGCGAAGCTTGGTTGTGCCAATGGA
B2M	Human	F: GGGTTTCATCCATCCGACA
	Human	R: ACACGGCAGGCATACTCAT

B. siRNA duplexes

siRNA duplex Name	*Sequence (5'-3')*
Human *YAP1*	F: AGAGAUACUUCUUAAAUCAtt
	R: UGAUUUAAGAAGUAUCUCUga
Human *PDGFRB*	F: GGAACGUGCUCAUCUGUGAtt
	R: UCACAGAUGAGCACGUUCCta
human *TEAD1*	F: GGACAUUCGUCAGAUUUAUtt
	R: AUAAAUCUGACGAAUGUCCac

C. Primers for quantitative ChIP assay

Primer Name	*Sequence (5'-3')*
human *TEAD1* promoter	CTCCGGAGCGAGTTCGG
	CGCGAGCGATGCTAAGAATG
Human *TEAD1* Exon 3	AGTGACTCTGCAGATAAGCCAA
	AAAGCTTTGCTCGATGTCGG
Human *PDGFRB* MCAT 1/2	TCATGACATGGGTCCCTCCC
	CTTTCAGGGCTGACATGCTG
Human *PDGFRB* MCAT 3	GCTGCAGTGACCTCCCTTCT
	TGGCTGGCCACACTGTTTATT
Human *PDGFRB* MCAT 4	TCCCTGTATGCTCTGGGCT
	CAACAGGAAATGGAGGAGGCT

Human *PDGFRB* Exon 12	ATTGAGTCTGTGAGCTCTGACG CTCCCACGTGGAGTCATAGGG

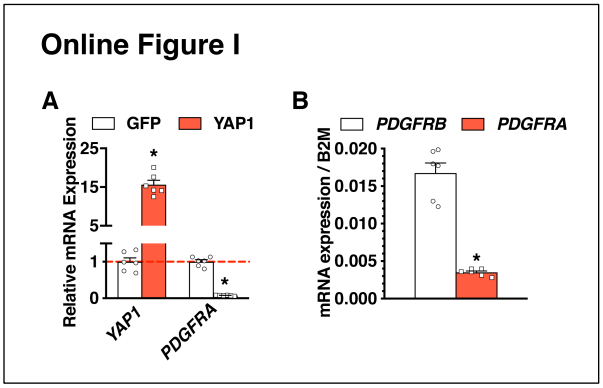
D. Primers for cloning human *PDGFRB* gene to generate the luciferase reporters (F: forward; R: reverse)

Primer Name	*Sequence (5'-3')*
PDGFRB MCAT 1/2 F (KpnI)	AGGTACCTGTAGCCCTTAGAGTGATCCCGAGG
PDGFRB MCAT 1/2 R (XhoI)	ACTCGAGGGTTGGGGTGGGAAGTAAGAAGACC
PDGFRB MCAT 3/4 F (KpnI)	AGGTACCCAGAAGAGAGCCTGAAGGGGAGAGG
PDGFRB MCAT 3/4 R (XhoI)	ACTCGAGTGTGATGAAAGGGATGGGAGGAGGA
PDGFRB Del 1 F (KpnI)	TCTGGTACCTGCCTTCCCAGAGTGGAGCCCTG
PDGFRB Del 1/2 F (KpnI)	TCTGGTACCCCAAAGTTTCAGGCATCTACTGGGGCC
PDGFRB Del 3 F (KpnI)	TCTGGTACCGAAGGGCTGGGCTTTGAGTCAGGAGCA
PDGFRB Del 3/4 F (KpnI)	TCTGGTACCTTCCCTTCCCACAGCCCACAGCCTCC

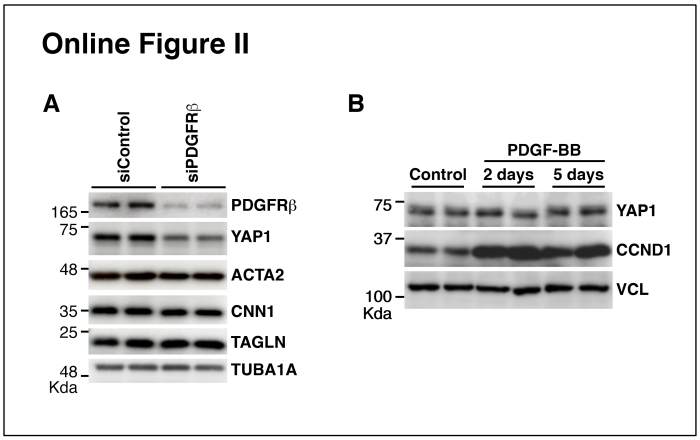
E. Primers for *Yap1* knockout mouse genotyping

Primer Name	*Sequence (5'-3')*
Yap1 P1 forward	CCATTTGTCCTCATCTCTTACTAAC
Yap1 P2 reverse	GATTGGGCACTGTCAATTAATGGGCTT
Yap1 P3 reverse	CAGTCTGTAACAACCAGTCAGGGATAC

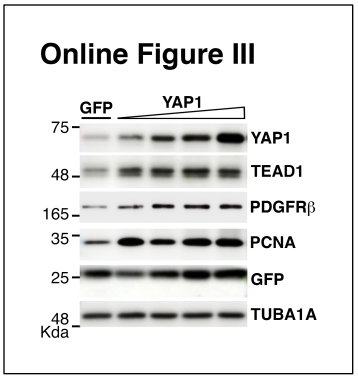
Supplemental Figures and Figure Legends



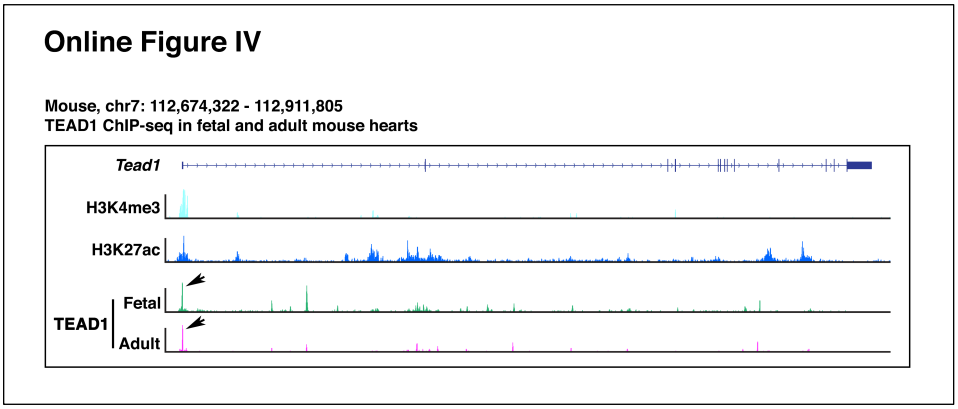
Online Figure I. YAP1 downregulates PDGFRα expression in VSMCs. A. HCASMCs were transduced with GFP or YAP1 adenovirus for qRT-PCR analysis. **B.** qRT-PCR analysis of basal *PDGFRB* or *PDGFRA* expression in HCASMCs relative to the housekeeping gene β2 microglobulin (*B2M*). *p<0.05. N=6 per group.



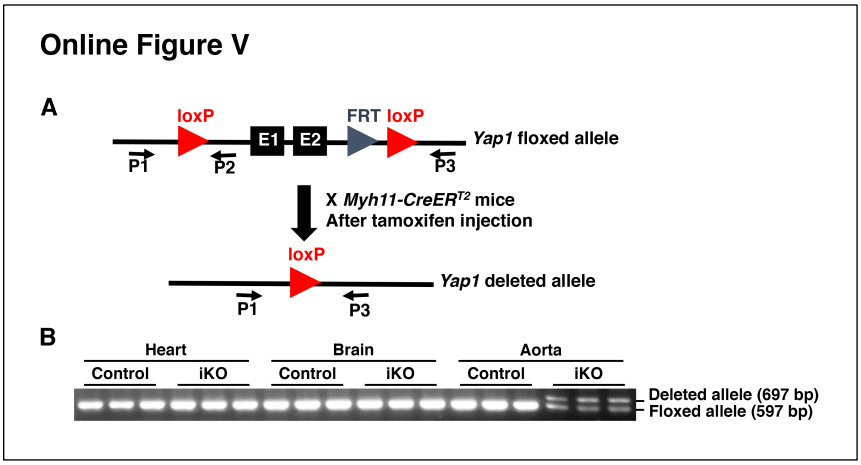
Online Figure II. Effects of silencing PDGFRβ or treatment of PDGF-BB on YAP1 expression in HCASMCs. A. HCASMCs were transfected with control scrambled silencing RNA duplex (siControl) or silencing RNA duplex against PDGFRβ (siPDGFRβ) for 48 hours. Cells were then harvested for Western blot analysis. **B.** HCASMCs were incubated in the absence (control) or presence of PDGF-BB (50 ng/ml) for 2 or 5 days and then harvested for Western blotting.



Online Figure III. Overexpression of YAP1 induces endogenous TEAD1 and PDGFRβ expression in VSMCs. HCASMCs were transduced with control GFP or increasing doses of YAP1 adenovirus for 48 hours and then cells were harvested for Western blotting. The YAP1 adenoviral construct contains an independent expression cassette for GFP, therefore GFP signal in YAP1 expressing cells was used to compare the viral transduction level to control cells transduced with GFP adenovirus.



Online Figure IV. ChIP-seq of TEAD1 in fetal and adult mouse hearts. Integrative Genomics Viewer (IGV) tracks depicting mouse *Tead1* gene locus with ChIP-seq of TEAD1 in fetal and adult mouse hearts (bottom panel), active transcription histone marks H3K4me3 and H3K27ac in adult mouse hearts (middle panel). Arrows point to a TEAD1 enrichment peak in *Tead1* gene promoter in fetal or adult mouse hearts, respectively.

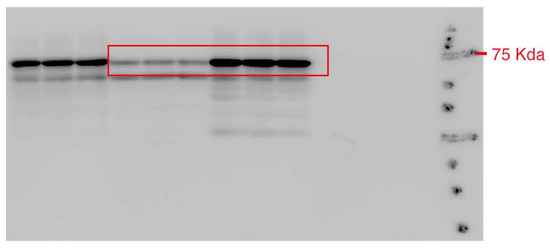


Online Figure V. Generation of inducible smooth muscle-specific *Yap1* KO mouse. A. Schematic depicting the strategy used to generate inducible smooth muscle-specific *Yap1* KO mouse model (iKO). Exon 1 and 2 of mouse *Yap1* gene that are flanked by 2 loxP sites will be deleted upon Cre-mediated recombination. **B.** 10-week-old male *Myh11*-CreER^{T2+}/*Yap1*$^{F/F}$ mice were intraperitoneally injected with sunflower oil (control) or tamoxifen (iKO; 1 mg/mouse) for 2 rounds of 5 days each, with 2 days' break in-between. 2 weeks after the last tamoxifen administration mice were sacrificed. DNA extracted from heart, brain, or aortic tissues was subjected to genotyping analysis using the primer (P) sets described in "**A**". The deleted *Yap1* allele can only be detected in aortic tissues from iKO mice. N=3 per group.

Figure 1, uncropped Western Blots

Figure 1G

YAP1



PDGFRβ

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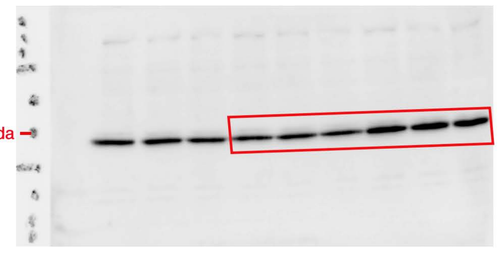
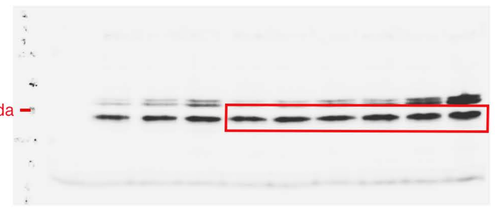
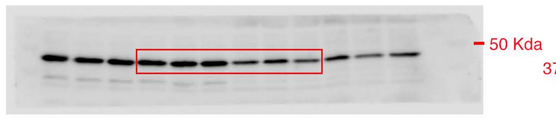


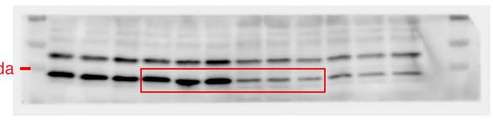
CCND1



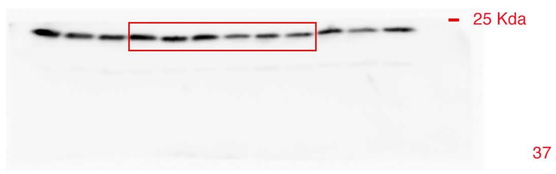
ACTA2



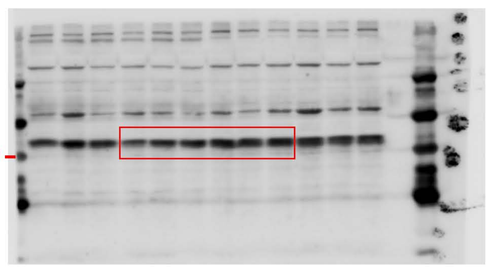
CNN1



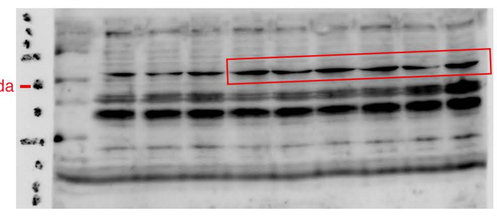
TAGLN



CCN1



MYC



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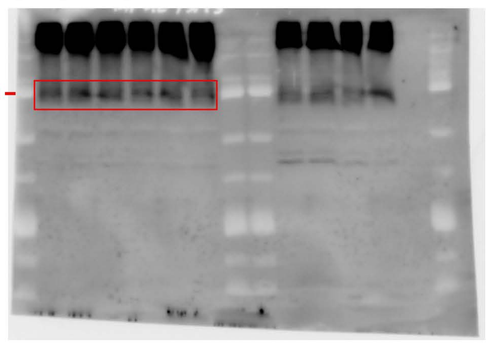

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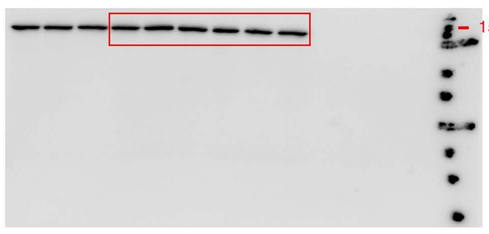
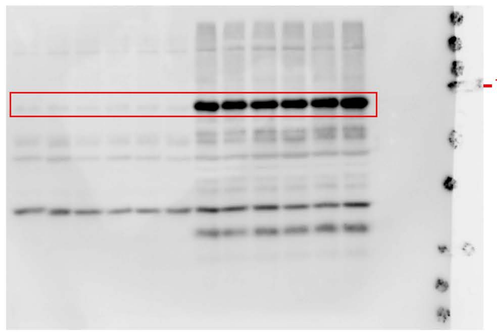


Figure 1I

YAP1



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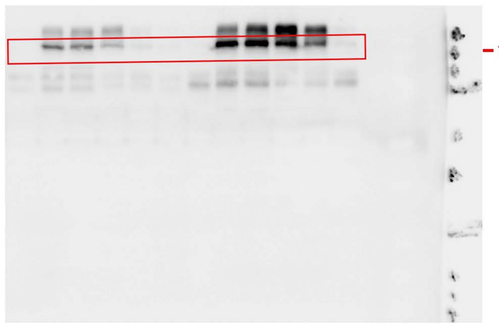

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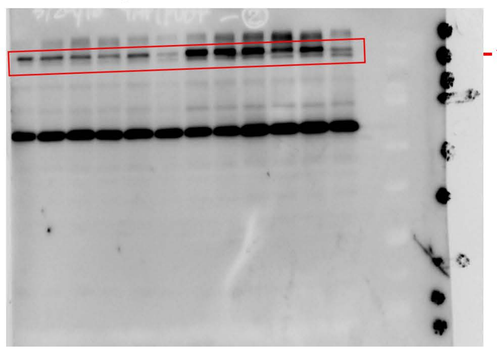
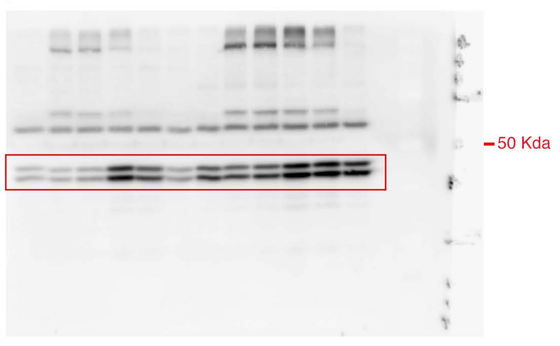


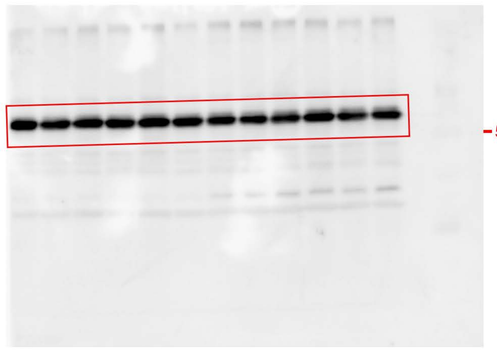
pMAPK1/3



MAPK1/3

pAKT

AKT



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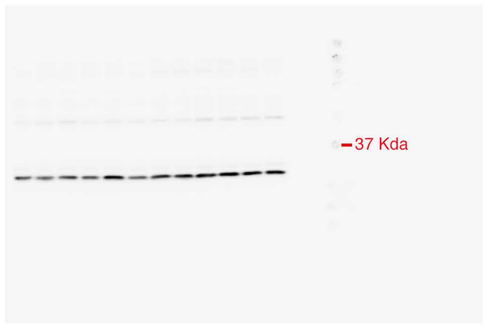


VCL

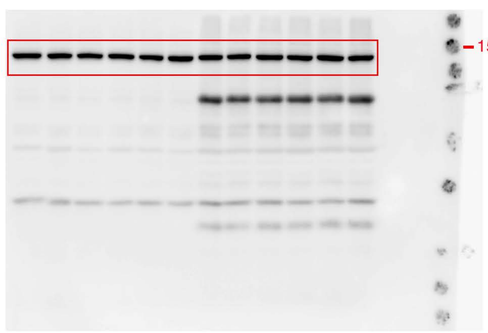
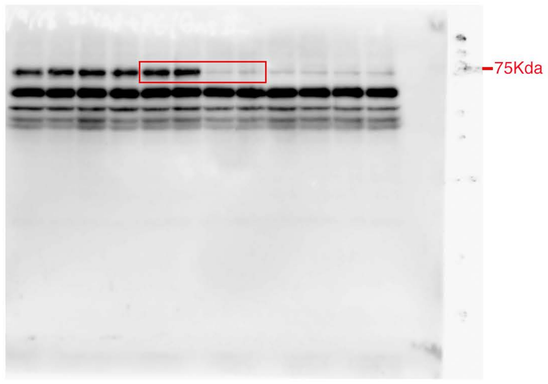


Figure 2, uncropped Western Blots

Figure 2F

YAP1



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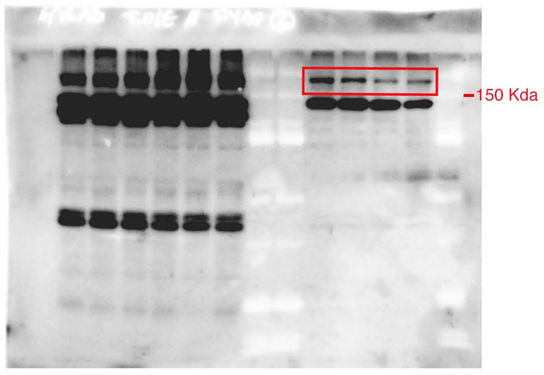
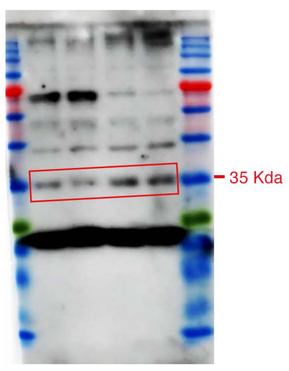


PCNA

ACTA2

CNN1



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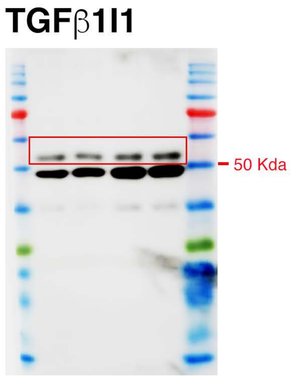


VCL

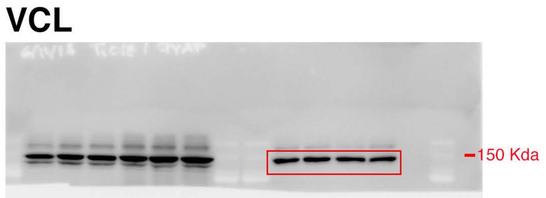
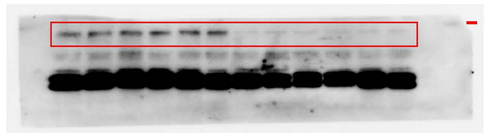


Figure 2H

YAP1



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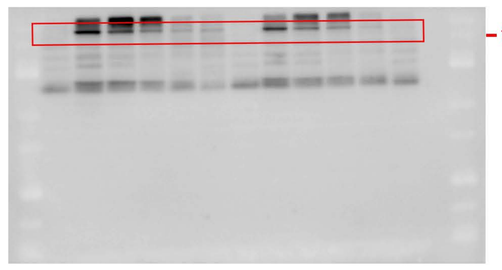

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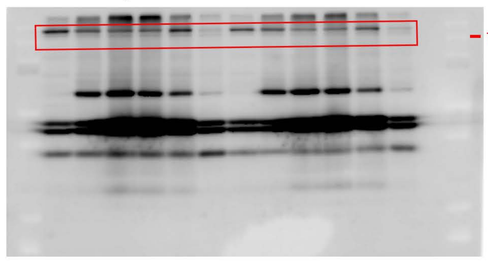
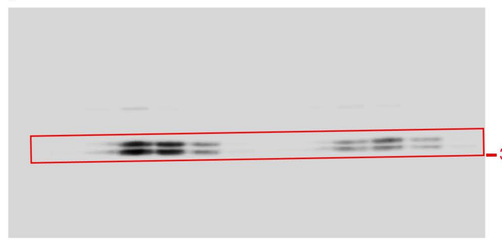
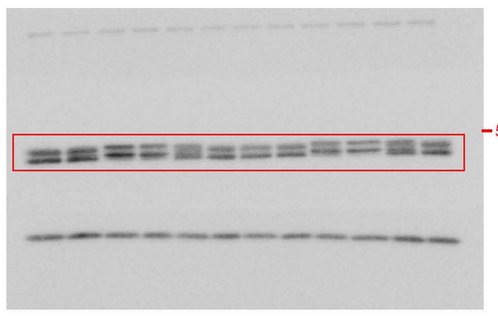


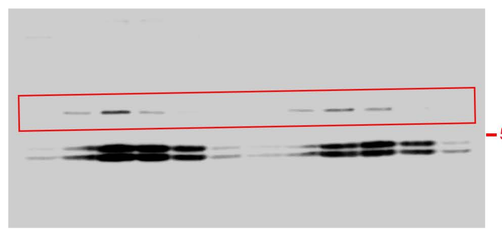
pMAPK1/3



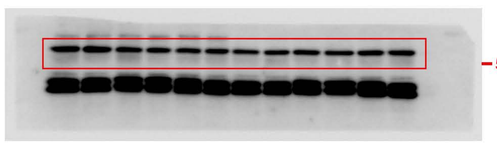
MAPK1/3



pAKT



AKT



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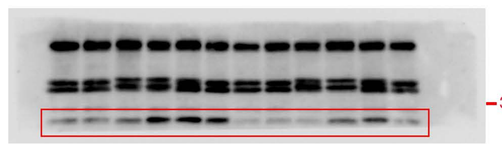
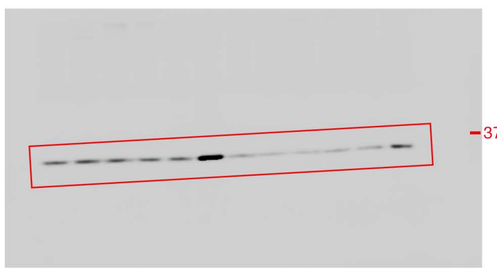


CCND1



VCL

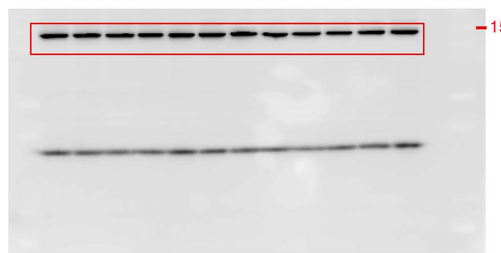
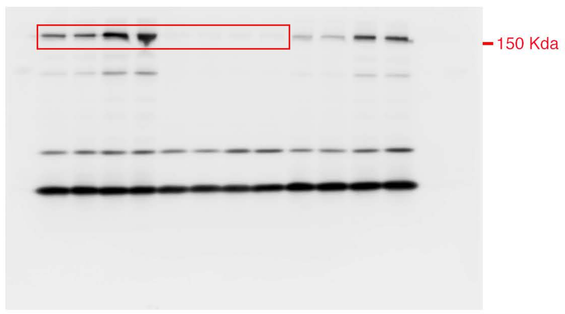


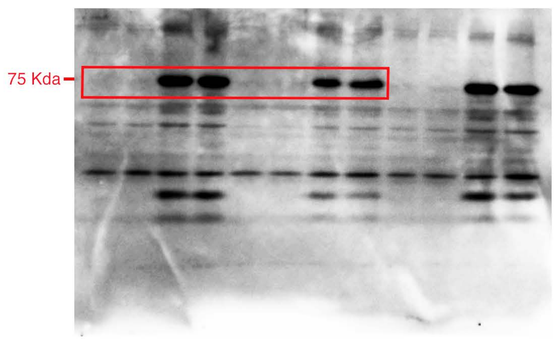
Figure 3, uncropped Western Blots

Figure 3A

PDGFRβ



YAP1



CCND1

TUBA1B

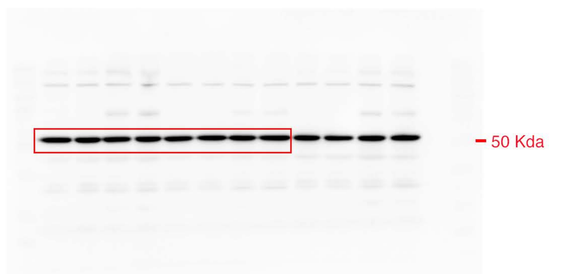
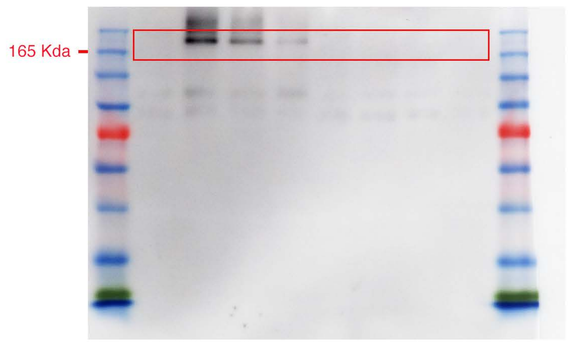


Figure 3D

pPDGFRβ



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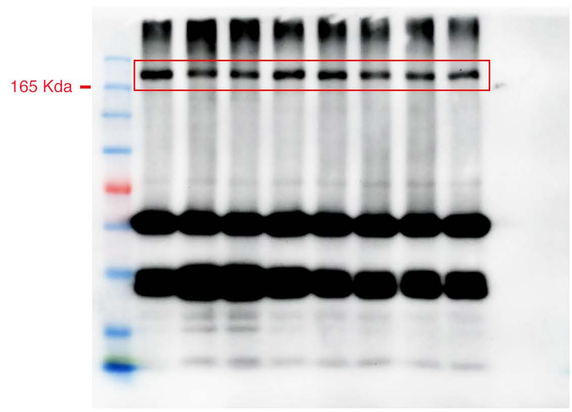
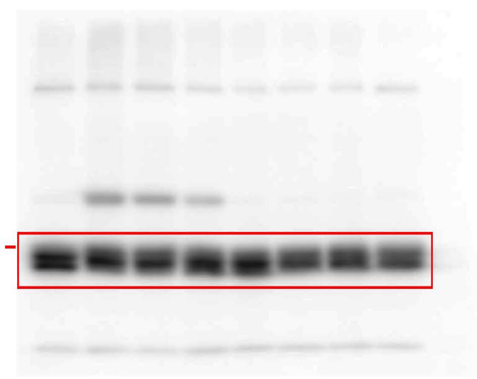
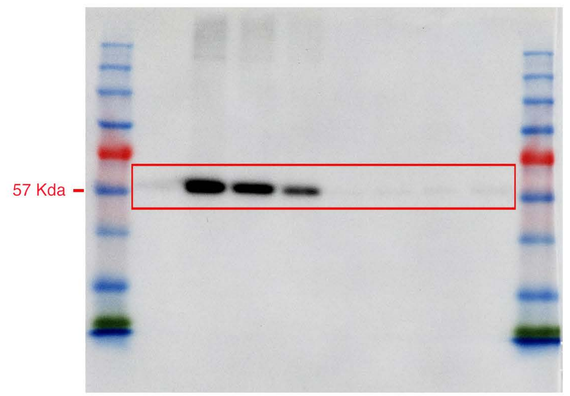


pMAPK1/3

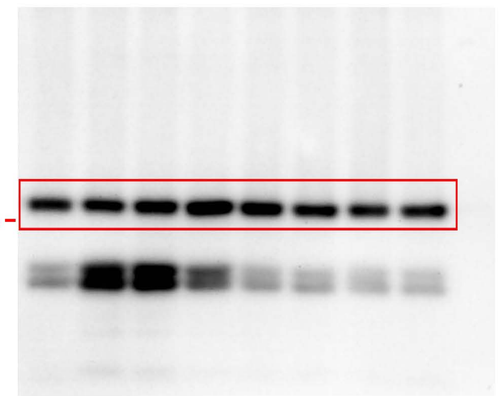
MAPK1/3



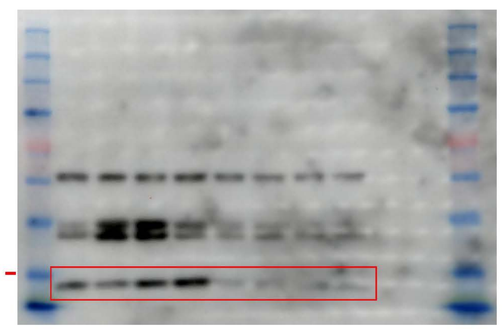
pAKT



AKT



pRPS6



VCL

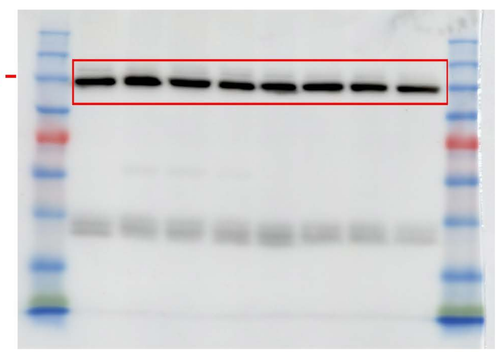
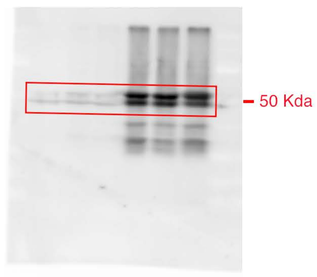


Figure 4, uncropped Western Blots

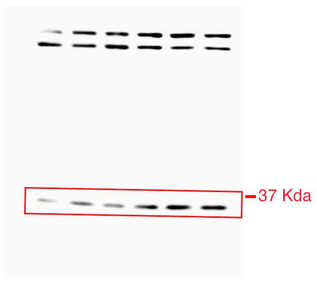
Figure 4B

TEAD1

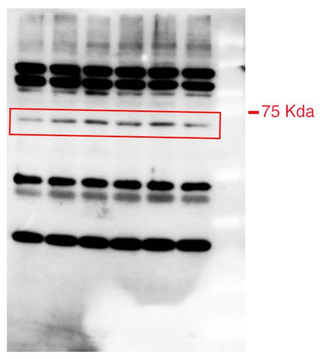


PDGFRβ

PCNA



YAP1



VCL

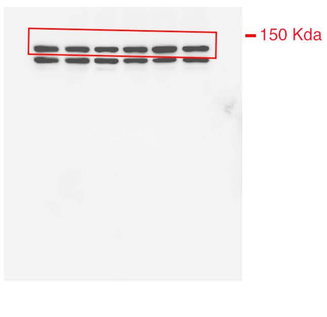
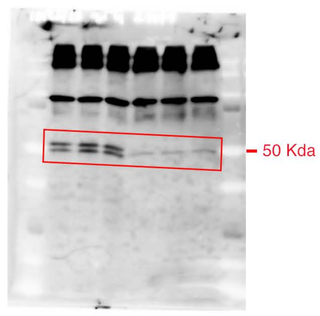


Figure 4D

TEAD1



PDGFRβ

HSP90AA1

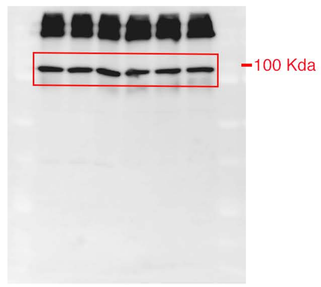
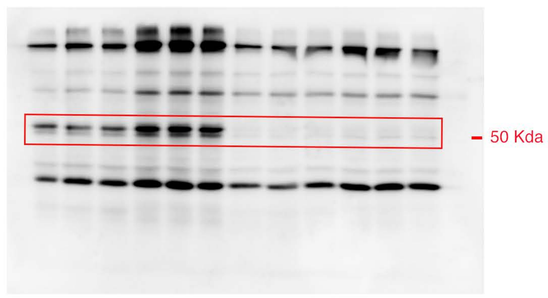
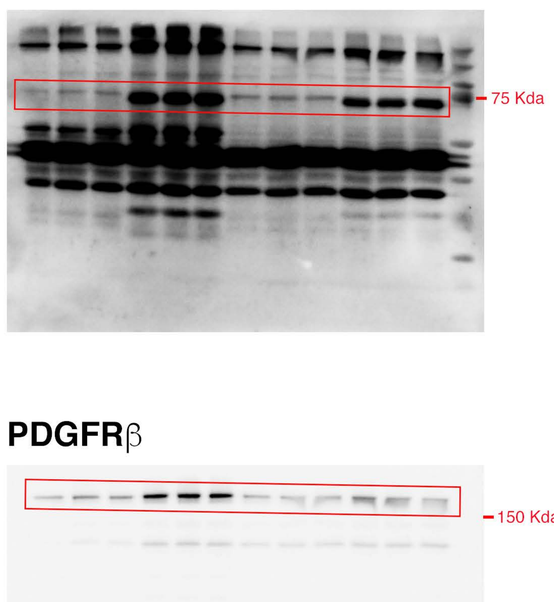


Figure 4F

TEAD1



YAP1



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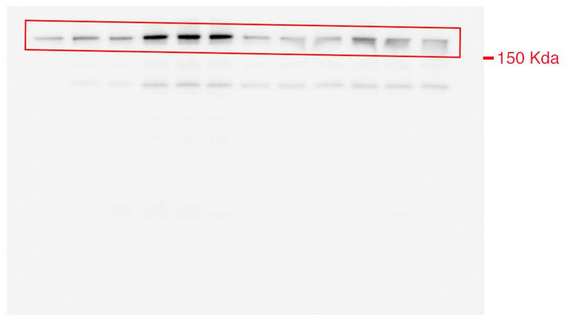
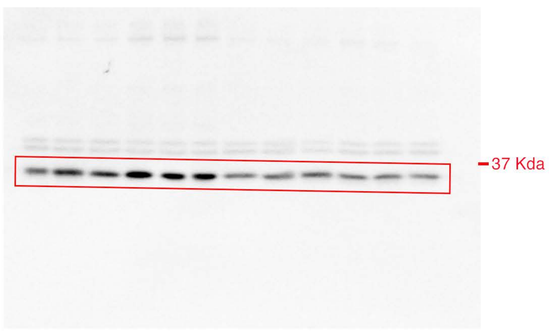


CCND1



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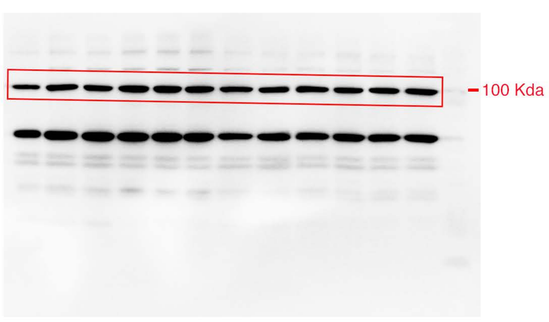
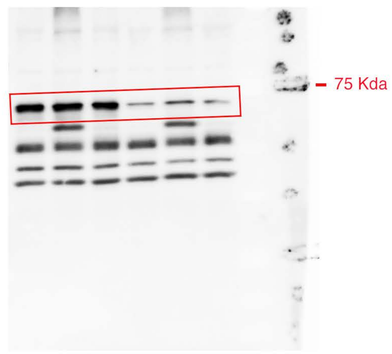


Figure 4I

YAP1



TEAD1

VCL

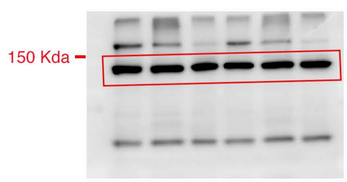
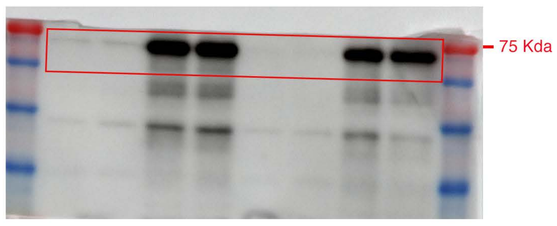


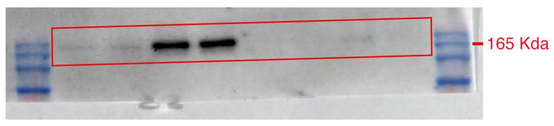
Figure 5A, uncropped Western Blots

YAP1

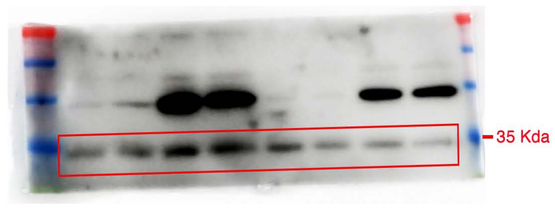


TEAD1

PDGFRβ



PCNA



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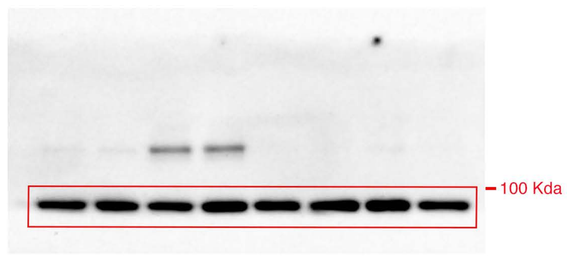
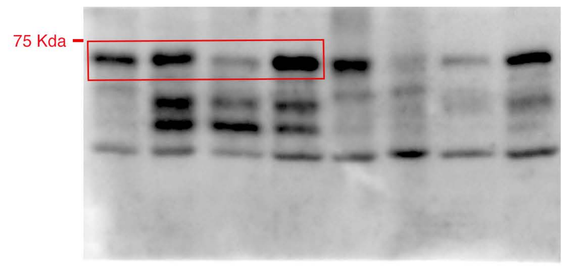


Figure 6, uncropped Western Blots

Figure 6A

YAP1



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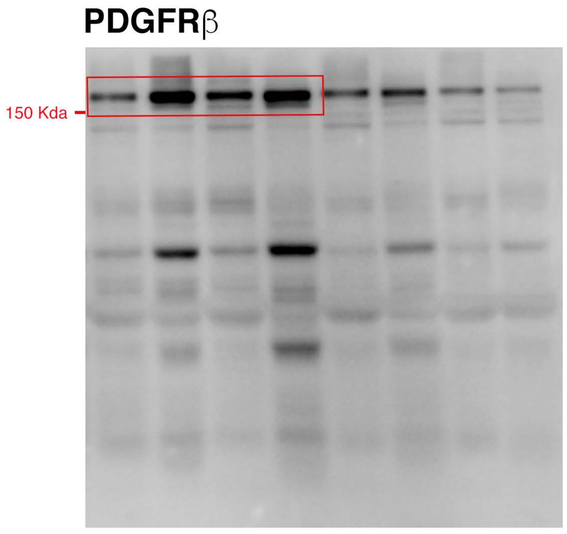

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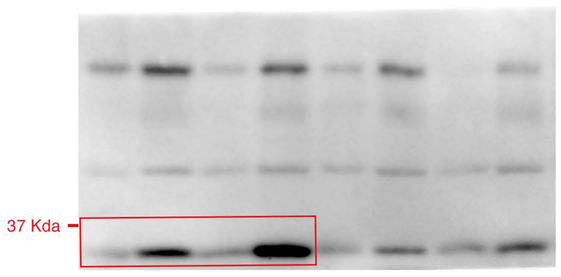

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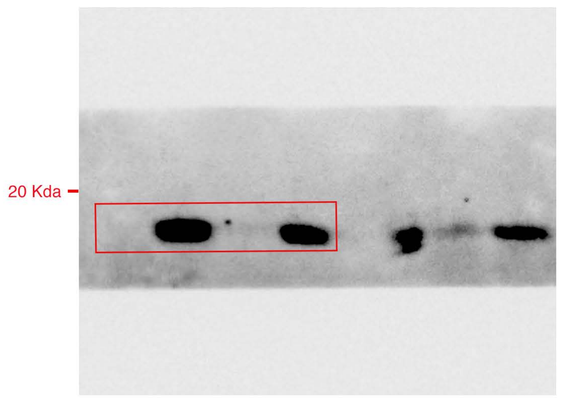


ACTB

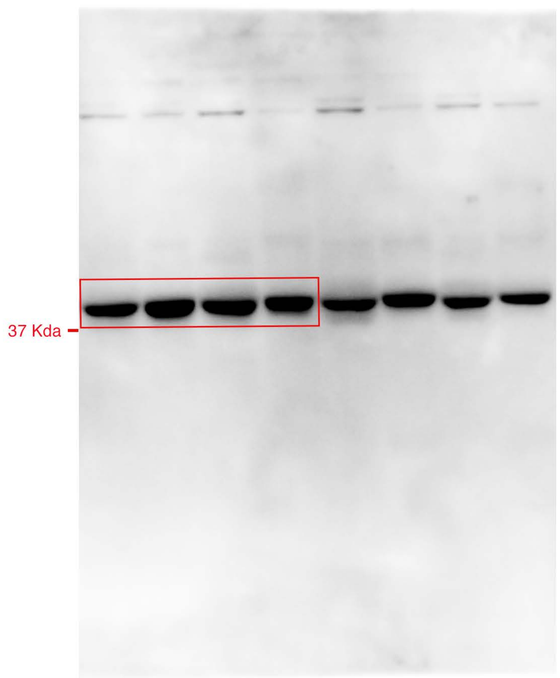
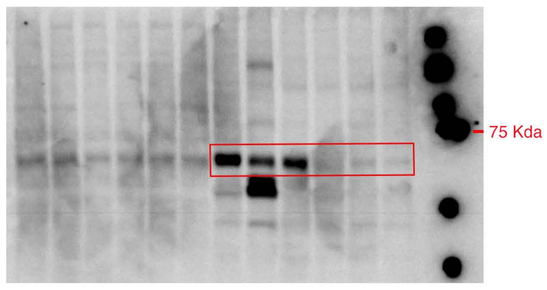
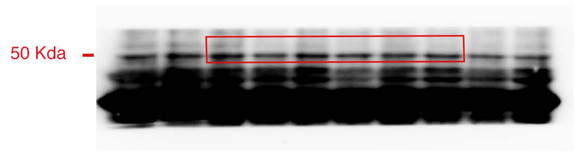


Figure 6D

YAP1



TEAD1



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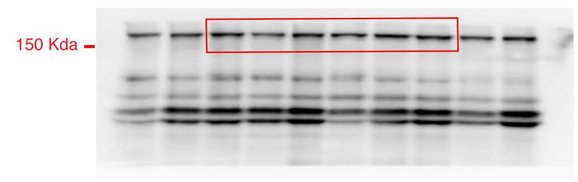
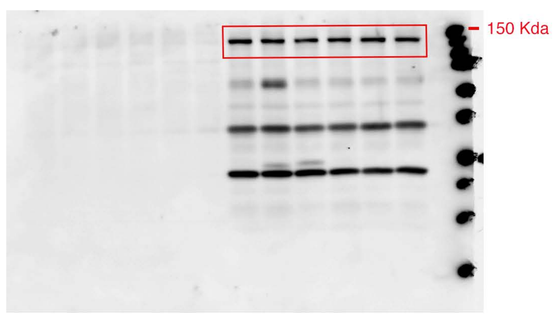


MYCK



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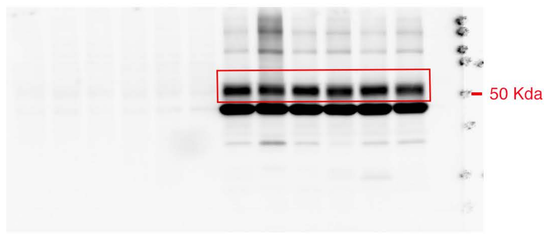
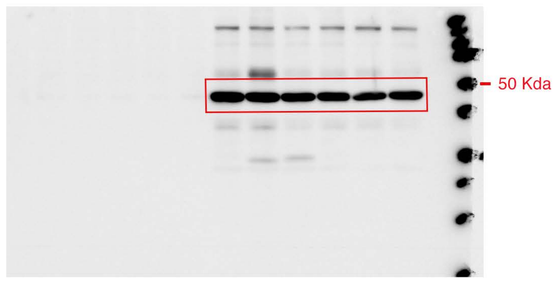
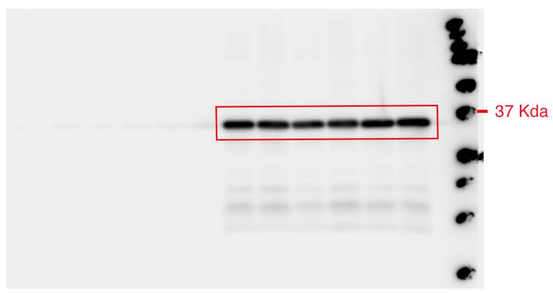


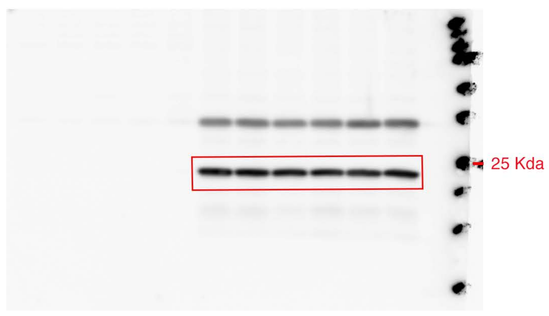
ACTA2



CNN1



TAGLN



TUBA1B

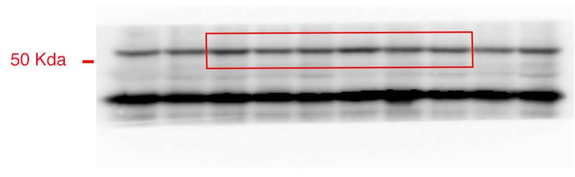
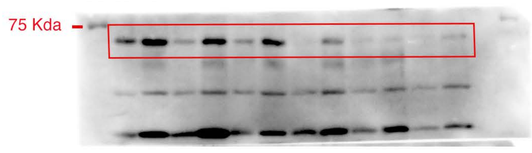


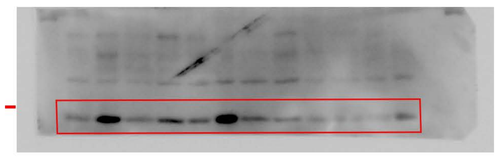
Figure 6F

YAP1

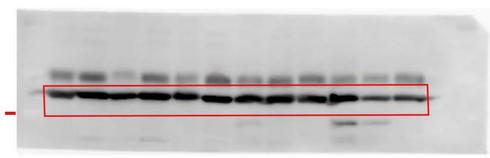


PDGFRβ

PCNA



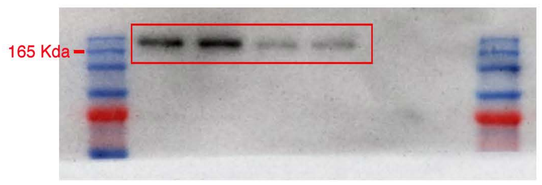
ACTB



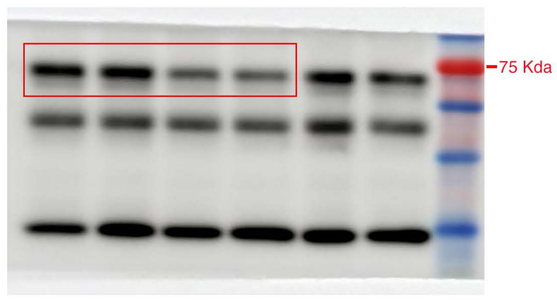
Online Figure II, Uncropped Western Blots

Online Figure IIA

PDGFRβ

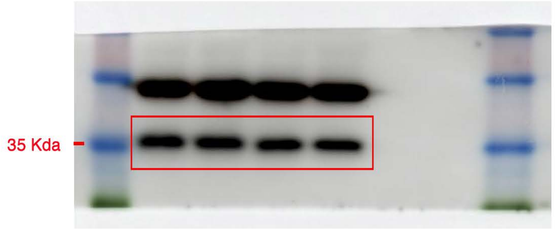


YAP1

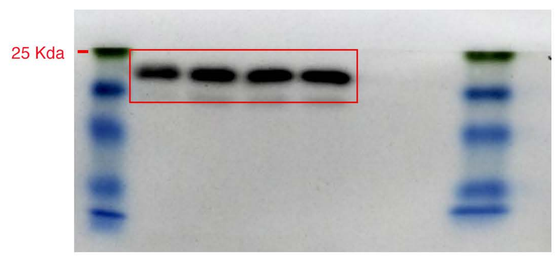


ACTA2

CNN1



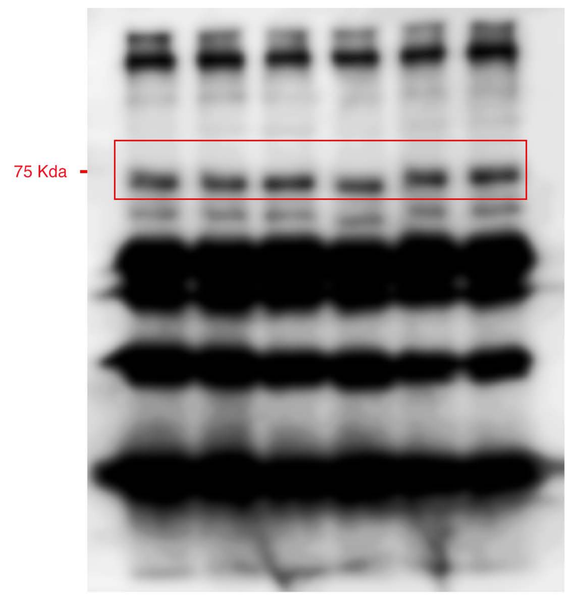
TAGLN



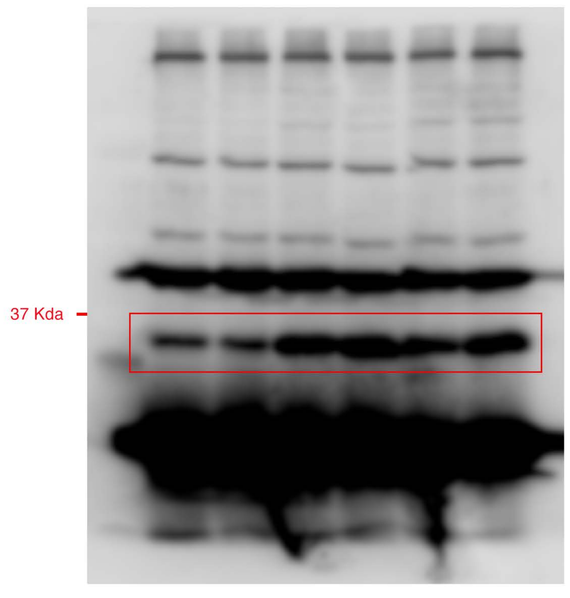
TUBA1A

Online Figure IIB

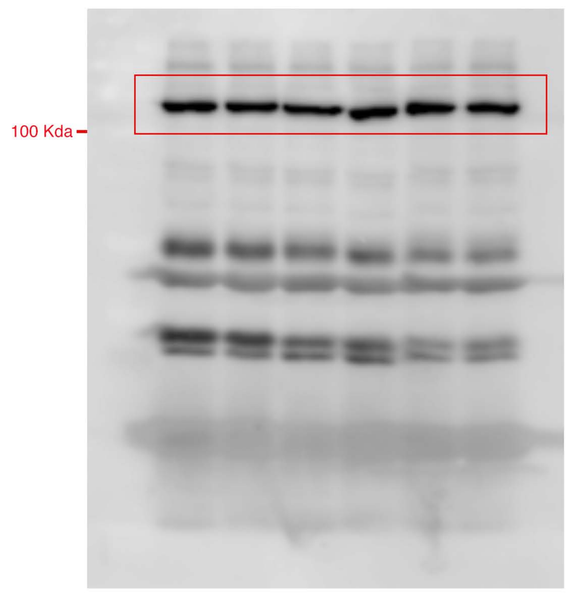
YAP1



CCND1

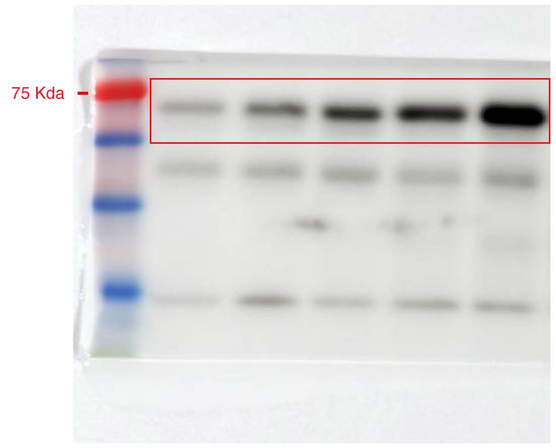


VCL



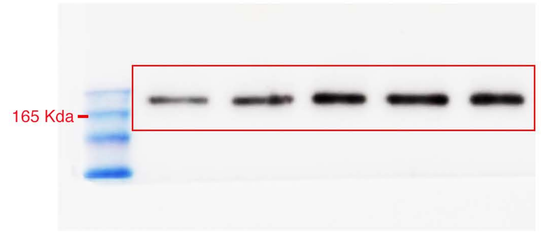
Online Figure III, Uncropped Western Blots

YAP1



TEAD1

PDGFRβ



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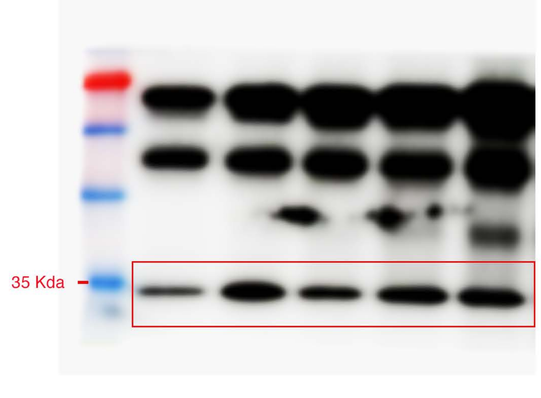

GFP

TUBA1A