

Supplementary Materials for

The p130 Isoform of Angiotensin II Receptor Type 1 Is Required for Yap-Mediated Hepatic Epithelial Cell Proliferation and Tumorigenesis

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This PDF file includes:

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- Fig. S4. Amot-p130 specifically blocks Yap-Lats1 interaction.
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Other Supplementary Material for this manuscript includes the following:

(available at www.sciencesignaling.org/cgi/content/full/6/291/ra77/DC1)

- Table S1 (Microsoft Excel format). Commonly regulated genes by Yap and Amot.
- Table S2 (Microsoft Excel format). Pathways predicted to be regulated by Amot or Yap by GSEA.
- Table S3 (Microsoft Excel format). Primer sequences.

Supplementary Materials

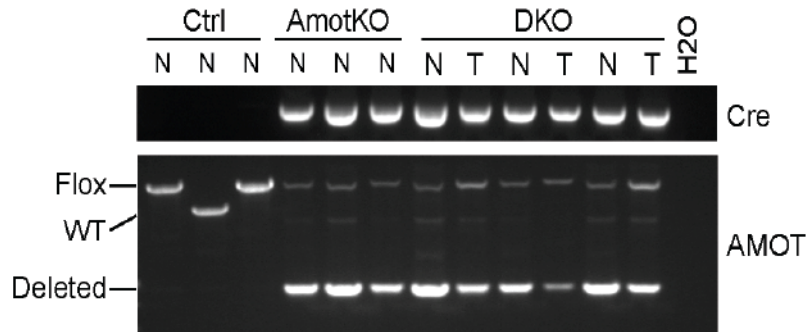


Figure S1: The *Amot*^{flox} allele incompletely recombines in the tumor regions of livers in DKO mice.

Genomic PCR analysis of *Cre* and *Amot* alleles in non-tumor (N) and tumor (T) regions of the livers from 3 wild-type (Ctrl), 3 *Amot*KO, and 3 DKO mice used for data presented in Fig. 2. Equal amount of genomic DNA was used for the analysis.

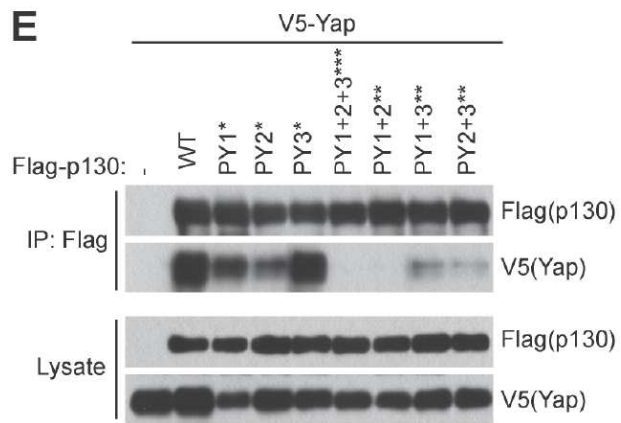
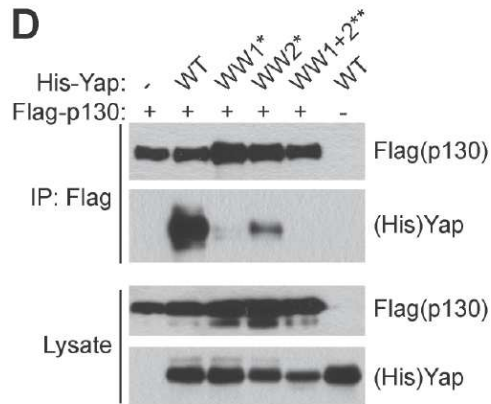
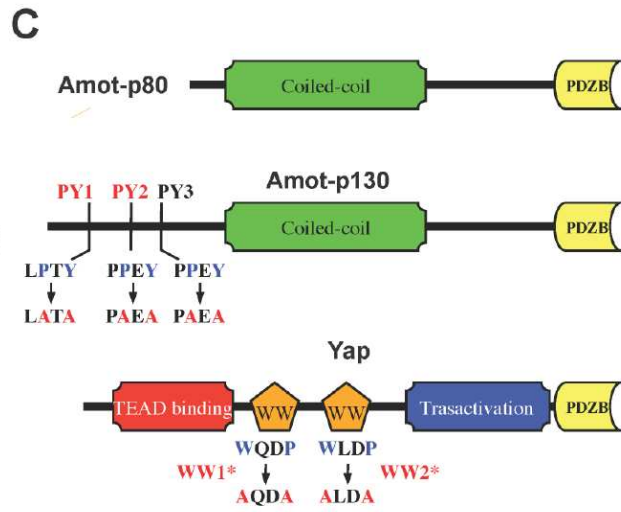
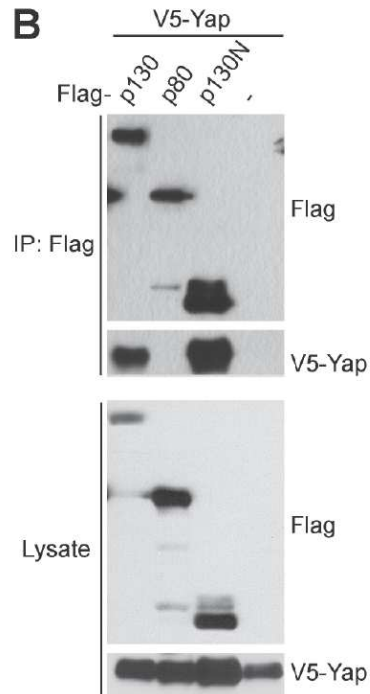
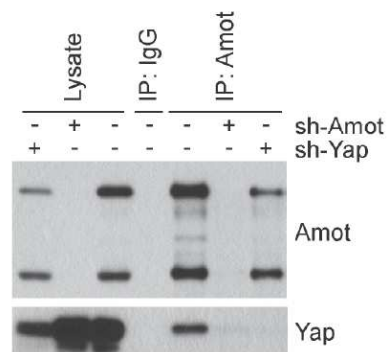
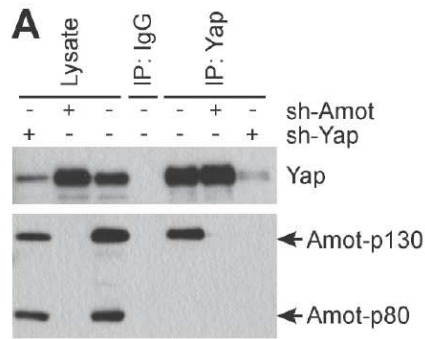


Figure S2: An LPxY motif on Amot-p130 mediates its binding to Yap.

(A) Western blot and co-immunoprecipitation (co-IP) analysis of Amot and Yap abundance and binding in total cell lysates from Amot-KD and Yap-KD HEK293 cells. (B) Western blot of Amot and Yap abundance and reciprocal co-IP analysis of Amot-Yap binding in total cell lysates from HEK293 cells transfected with V5-Yap and Flag-tagged Amot-p80, Amot-p130 or Amot-p130N as indicated. (C) Schematic representation of the domains in the Amot and Yap proteins. (D) Western blot and co-IP analysis of total cell lysates from HEK293 cells transfected with Flag-Amot-p130 and His-tagged wild-type Yap (WT), single WW domain mutant YAP (WW1* or WW2*), or double WW domain mutant YAP (WW1+2**). (E) Western blot and co-IP analysis of total cell lysates from HEK293 cells transfected with V5-Yap and Flag-tagged wild-type Amot-p130 (WT), single PY motif mutant Amot-p130 (PY1*, PY2* or PY3*), double PY motif mutant Amot-p130 (PY1+2**, PY1+3** and PY2+3**), or triple PY motif mutant Amot-p130 (PY1+2+3***). “*” indicates number of PY mutations. Westerns are representative of 3 independent experiments.

hAMOT p80	-----	-----	-----	-----	-----	-----	-----	-----	80
hAMOT p130	-----	-----	-----	-----	-----	-----	-----	-----	
hAMOT L1	MWRAKLRRG	CEPAVKGSPS	ACYSPSSPVQ	VLEDSTYFSP	DFQLYSGRHE	TSALTVEATS	SIREKVVEDP	LCNFHSPNFI	
hAMOT L2	-----	-----	-----	-----	-----	-----	-----	-----	
hAMOT p80	-----	-----	-----	-----	-----	-----	-----	-----	160
hAMOT p130	-----MRNS	EEQPSGGTTV	LQRLLEQQLR	YGNPSENRS	LAIHQQATGN	GPPFPSSGSGN	PGPQSDVLSF	QDHHQQLVAH	
hAMOT L1	RISEVEMRGS	EDAAAG--TV	LQRLIQEQLR	YGTPTENMNL	LAIQHQATGS	AGPAHPPTNN	---FSSTENL	TQEDPQMVYQ	
hAMOT L2	-----MRTL	ED-SSG--TV	LHRLIQEQLR	YGNLTETRTL	LAIQQQALRG	GAGTGGTGS-	--PQASLEIL	APEDSQVLQQ	
				PY1					
hAMOT p80	-----	-----	-----	-----	-----	-----	-----	-----	240
hAMOT p130	AARQEPQGE	IQ--SENLM	EKQLSPR--M	QNNEELPTYE	EAKVQSQYFR	GQQHAS---	-VGAAFYVTG	VTNQKMRTEG	
hAMOT L1	SARQEPQGE	HQ--VDNTVM	EKQVRSTQPQ	QNNEELPTYE	EAKAQSQFFR	GQQQQQQQQG	AVGHGYMAG	GTSQKSRTEG	
hAMOT L2	ATRQEPQGE	HQGGENHLAE	NTRYRLCPQF	SKGEEELPTYE	EAKAHSQYYA	AQQAGTR---	-----	-----PHAG	
hAMOT p80	-----	-----	-----	-----	-----	-----	-----	-----	320
hAMOT p130	RPSVQRLNPG	KMHQDEGLRD	LKQGHVRSLS	ERLMQMSLAT	SGVKAHPVPT	SAPLSPFPQN	DLYKNPTSSS	EFYKAQGFLP	
hAMOT L1	RPTVNRANGS	QAHKDEALKE	LKQGHVRSLS	ERIMQLSLER	NGAKQLPGS	GN-----	-----GK	GFKVGGGSPS	
hAMOT L2	DRDRPAGPAG	SRRQDEALRE	LRHGHVRSLS	ERLLQLSLER	NGARAPSHMS	SS-----	-----HSFPQ	LARNQQGPPL	
		PY2						PY3	
hAMOT p80	-----	-----	-----	-----	-----	-----	-----	-----	400
hAMOT p130	NQHSKLGMEH	RGPPEEYFFK	GMPPQSVVCK	QPEPGHFYSE	HR-----	---LNQFGR	TEGQLMRYQH	PEEYGAARPA	
hAMOT L1	AQPAGKVLDP	RGPPEEYFFK	TKQMSSPVSK	TQEHGLPYGD	QHPGMLHEMV	KPYAPAPVVR	TDVAVLRYQP	PEEYGHVSRP	
hAMOT L2	RGPPEEYFFK	RGPPEEYFFK	VLAHETTTAV	TDPD---YRA	R-----	---GSPHFQH	AEVRLQAQV	PFVFLQQQQQ	
hAMOT p80	-----	-----	-----	-----	-----	-----	-----	-----	480
hAMOT p130	QDISLPLSAR	NSQPHSPPTSS	LTSGGSLPLL	QSPSTRSLP	ARHPLVNPQG	DHSAHLPRPQ	QHFLPNQAHQ	GDHYRLSQPG	
hAMOT L1	CQLPFPS---	TMQQHSPMSS	QTSSASGPLH	SVSLPLPLPM	ALG-----	-----	-----	-----	
hAMOT L2	YQYLQQS---	--QEHPPPH	PAALGHGPLS	SLSFP---	-----	-----	-----	-----	
hAMOT p80	-----	-----	-----	-----MPRA	QPSSASYQPV	PADPFAIVSR	AQQMVEILSD	ENRNLRQELE	560
hAMOT p130	LSQQQQQQQQ	QHSHHHHHHQ	QQQQQQQQQP	GEAYSAMPRA	QPSSASYQPV	PADPFAIVSR	AQQMVEILSD	ENRNLRQELE	
hAMOT L1	-----	-----	-----	-----AFQP	PPAASPSQQL	GPDAFAIVSR	AQQMVEILTE	ENRVLHQELQ	
hAMOT L2	-----	-----	-----	-----AVEGP	VSAQASSATS	GSAHLAQMEA	VLRNARLQR	DNERLQRELE	
hAMOT p80	GCYEKVARLQ	KVETEIQRVS	EAYENLVKSS	SKREALEKAM	RNKLEGEIRR	MHDFNRDLRE	RLETANKQLA	EKEYEGSEDT	640
hAMOT p130	GCYEKVARLQ	KVETEIQRVS	EAYENLVKSS	SKREALEKAM	RNKLEGEIRR	MHDFNRDLRE	RLETANKQLA	EKEYEGSEDT	
hAMOT L1	KYEDNADKLH	KFEKELQRIS	EAYESLVKST	TKRESLNGKAM	RNKLEGEIRR	LHDFNRDLRD	RLETANRQLS	SREYEGHED-	
hAMOT L2	SSAEKAGRIE	KLESEIQRIS	EAHESLTRAS	SKREALEKTM	RNKMDSEMRR	LQDFNRDLRE	RLESANRRLA	SKTQEAGAGS	
hAMOT p80	RKTISQLFAK	NKESQREKEK	LEAELATARS	TNEDQRRHIE	IRDQALSNAQ	AKVVKLEEEEL	KKKQVYVDKV	EKMQQALVQL	720
hAMOT p130	RKTISQLFAK	NKESQREKEK	LEAELATARS	TNEDQRRHIE	IRDQALSNAQ	AKVVKLEEEEL	KKKQVYVDKV	EKMQQALVQL	
hAMOT L1	KAABEGHYASQ	NKEFLKEKEK	LEMELAAVRT	ASEDHRRHIE	ILDQALSNAQ	ARVIKLEEEEL	REKQAYVEKV	EKLQALTLQL	
hAMOT L2	QDMVAKLLAQ	SYEQQQEQEK	LEREMALLRG	ATDQRRRAE	LLEQALGNAQ	GRAARAEEL	RKKQAYVEKV	ERLQALGQL	
hAMOT p80	QAACEKREQL	EHRLRTRLER	ELESRIQQR	Q-----GNCQ	PTNVSEYNAA	ALMELLREKE	ERILALEADM	TKWEQKYLEE	800
hAMOT p130	QAACEKREQL	EHRLRTRLER	ELESRIQQR	Q-----GNCQ	PTNVSEYNAA	ALMELLREKE	ERILALEADM	TKWEQKYLEE	
hAMOT L1	QSACEKREQM	ERRLRTWLER	ELDALRTQQK	H-----GNCQ	PANMPEYNAP	ALLELVREKE	ERILALEADM	TKWEQKYLEE	
hAMOT L2	QAACEKREQL	ELRLRTRLEQ	ELKALRAQQR	QAGAPGGSSG	SGGSPELSAL	RLSEQLREKE	EQTILALEADM	TKWEQKYLEE	
hAMOT p80	NVMRHFALDA	AATVAAQRDT	TVISHSPNTS	Y-DTALEARI	QKEEEEILMA	NKRCLDMEGR	IKTLHAQIIE	KDAMIKVLQQ	880
hAMOT p130	NVMRHFALDA	AATVAAQRDT	TVISHSPNTS	Y-DTALEARI	QKEEEEILMA	NKRCLDMEGR	IKTLHAQIIE	KDAMIKVLQQ	
hAMOT L1	STIRHFAMNA	AATAAAERDT	TIINHSPQPS	YGESSLEAHI	WQEEEEVVQA	NRRCQDMEYT	IKNLHAKIIE	KDAMIKVLQQ	
hAMOT L2	RAMRQFAMDA	AATAAAQRDT	TLIRHSPQPS	P-SSSFN---	---EGLLTG	GHRHQEMESR	LKVLHAQILE	KDAVIKVLQQ	
hAMOT p80	RSRKEPSKTE	QLSCMRPAKS	LMSISNAGSG	LLSHSSTLTG	SPIMEEKRDD	KSWKGSGLGIL	LGGDYRAEYV	PSTPSPVPPS	960
hAMOT p130	RSRKEPSKTE	QLSCMRPAKS	LMSISNAGSG	LLSHSSTLTG	SPIMEEKRDD	KSWKGSGLGIL	LGGDYRAEYV	PSTPSPVPPS	
hAMOT L1	RSRKEGAKTD	S-SSLRPARS	VPSIA-AATG	THSRQTSLTS	SQLAEEKKEE	KTWKGSIGLL	LG-----	-----	
hAMOT L2	RSRRDPGKAT	Q-GSLRPAKS	VPSVFAAAAA	GTQGWQGLSS	S-----	-----	-----	-----	
hAMOT p80	TPLLSAHSKT	GSRDCSTQTE	RGTESNKTAA	VAPISVPAPV	AAAATAAAIT	ATAATITTTM	VAAAPVAVAA	AAAPAAAAAP	1040
hAMOT p130	TPLLSAHSKT	GSRDCSTQTE	RGTESNKTAA	VAPISVPAPV	AAAATAAAIT	ATAATITTTM	VAAAPVAVAA	AAAPAAAAAP	
hAMOT L1	-----	---KEHHEHA	SAPLLPPPTT	SALSSIASTT	A-----	-----	---A	SSAHAKTGSK	
hAMOT L2	-----	-----ERQTA	DAPARLTTDR	APTEPVTVA	P-----	-----	-----	PAAHAKHGSR	
hAMOT p80	SPATAAATAA	AVSPAAAGQI	PAAASVASAA	AVAPSAAAAA	AVQVAPAAPA	PVPAPALVPV	PAPAAAQASA	PAQTQAPTSA	1120
hAMOT p130	SPATAAATAA	AVSPAAAGQI	PAAASVASAA	AVAPSAAAAA	AVQVAPAAPA	PVPAPALVPV	PAPAAAQASA	PAQTQAPTSA	
hAMOT L1	DSSTQTDKSA	ELFWPSMASL	PSRGLSTTP	AHSP-----	-VLKHFAAKG	-----	---TAEKLENS	PGHGKSPD--	
hAMOT L2	DGSTQTEGPP	DSTSTCLPPE	PDS-----	-----	-LLGSSSSQR	-----	---AASLDSV	ATS-----	
hAMOT p80	PAVAPTAPPT	PTPAVAQAEV	PASPATGPGP	HRLSIPSLTC	NPKDKTGPFV	HSNTLERKTP	IQILGQEPDA	EMVEYLI	1197
hAMOT p130	PAVAPTAPPT	PTPAVAQAEV	PASPATGPGP	HRLSIPSLTC	NPKDKTGPFV	HSNTLERKTP	IQILGQEPDA	EMVEYLI	
hAMOT L1	-----	-----	-----	HRGRVSSLH	KP-----	-----	-----EFPDG	EMMEVLI	
hAMOT L2	-----	-----	-----RVQDL--	-----	-----	-----	-----S	DMVEILI	

Figure S3: Sequence alignment of human Amot-p80, Amot-p130, AmotL1, and AmotL2 proteins. Three conserved PY motifs of human Amot proteins are highlighted

in yellow. Sequences were obtained from National Center for Biotechnology Information (NCBI) gene database.

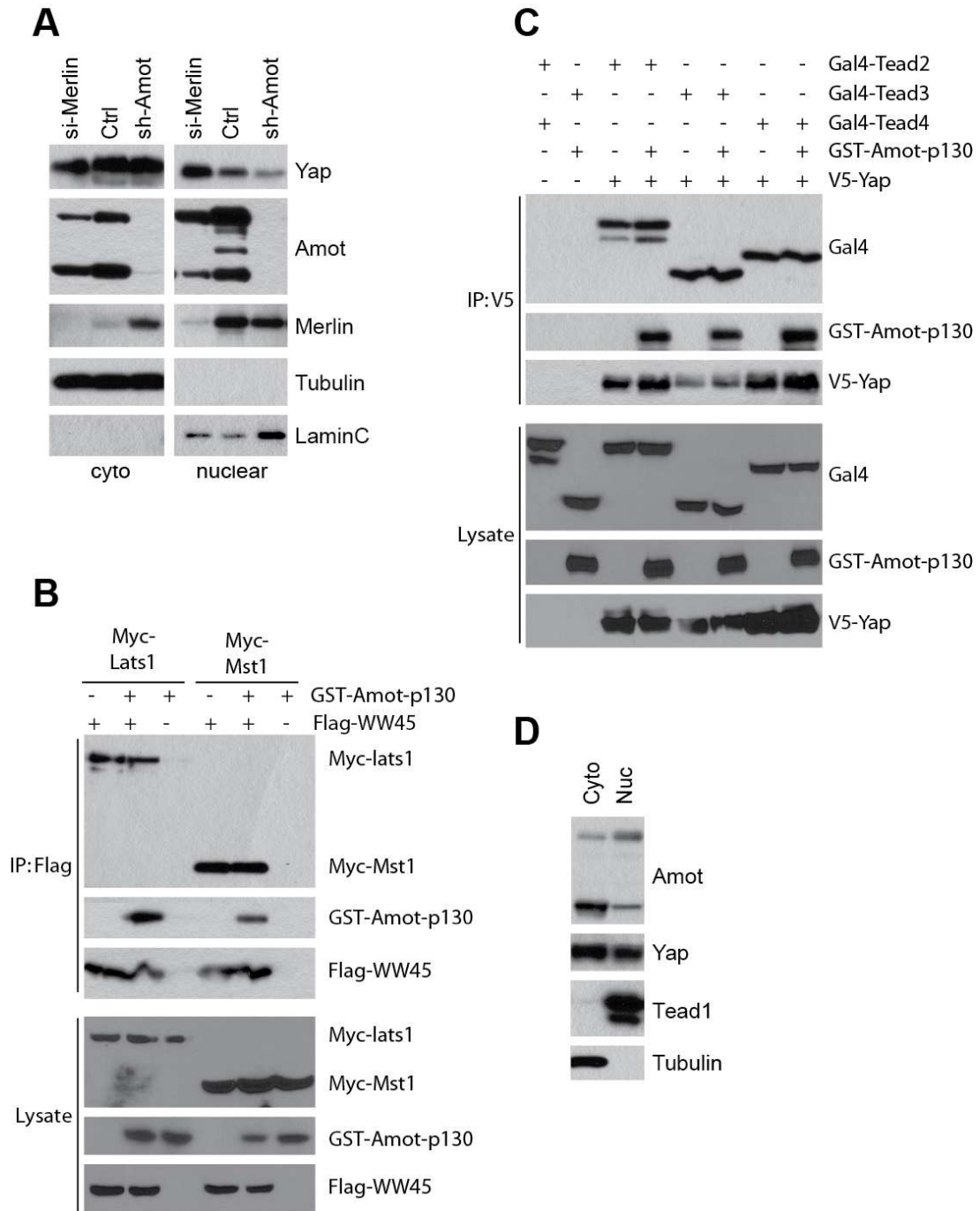


Figure S4: Amot-p130 specifically blocks Yap-Lats1 interaction.

(A) Western blotting analysis for Yap, Amot, and Merlin in cytoplasmic and nuclear fractions of HEK293 cells transfected with smartpool siRNAs against Merlin (si-Merlin), a vector control (Ctrl), or shRNAs against Amot (sh-Amot). Tubulin was used as the cytoplasmic marker and Lamin C the nuclear marker. **(B)** Western analysis with Myc, GST and Flag antibodies of Flag-IP and cell lysate from HEK293 cells transfected with Flag-WW45 in combination of Myc-tagged Mst1 or Lats1 in the presence or absence of GST-Amot-p130 as indicated. **(C)** Western analysis with Gal4, GST, and V5 antibodies of V5-IP and cell lysate from HEK293 cells transfected with V5-Yap and Gal4-tagged Tead2, 3, or 4 in the presence or absence of GST-Amot-p130 as indicated. **(D)** Western analysis with Amot, Yap, Tead and Tubulin antibodies of 293T cytoplasmic and nuclear lysates used for IP in Fig 6A. All data are representative of 3 independent experiments.

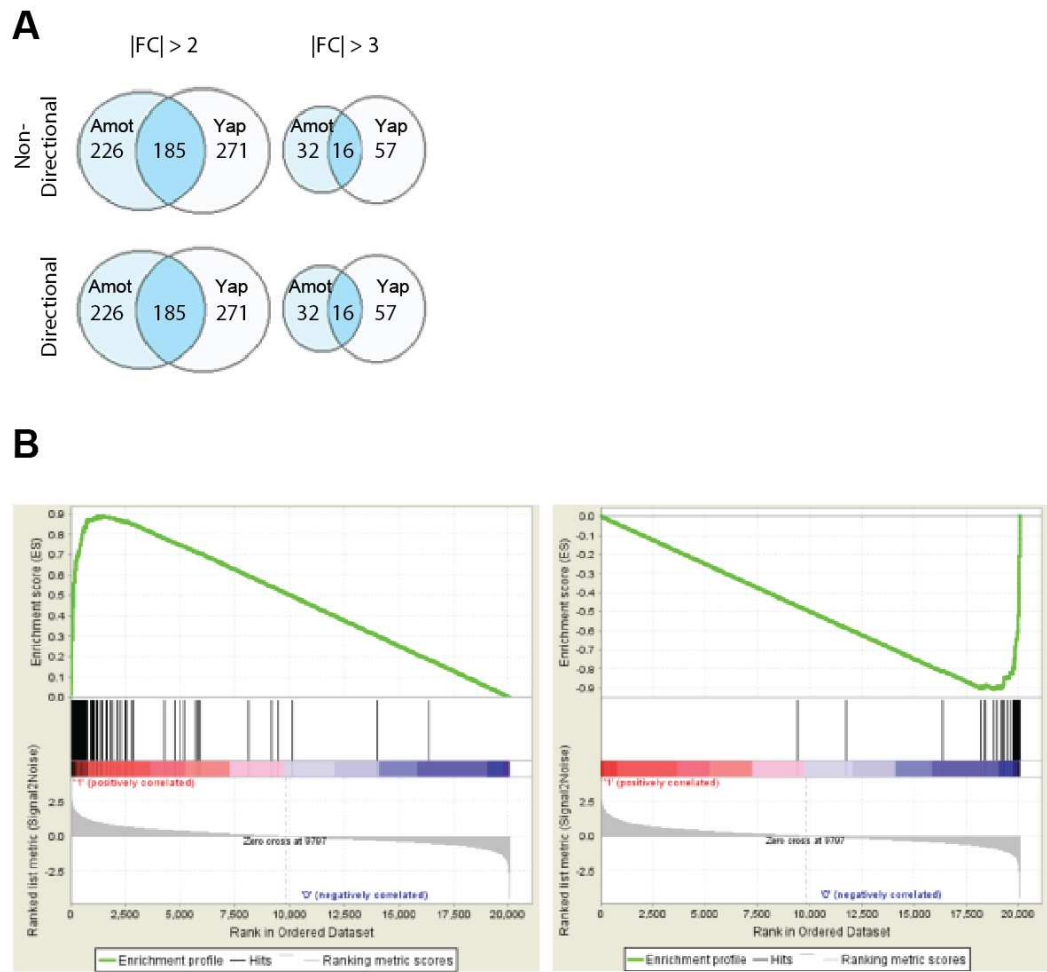


Figure S5: Yap and Amot co-regulate a large set of genes.

(A) Venn diagram of the number of genes whose expression was changed more than 2-fold by knockdown of Amot (light blue) or Yap (white); the number of genes in common are in blue. Expression was assessed by microarray analysis in control, Amot-KD, and Yap-KD HEK293 cell lines. Nondirectional comparison included genes differentially expressed in both Amot-KD and Yap-KD lines compared to the control line regardless of whether their expression increased or decreased. Directional comparison only included genes that were either increased or decreased in both Amot-KD and Yap-KD lines

compared with the control line. $|\text{FC}|$ = absolute fold change. **(B)** Gene Set Enrichment Analysis (GSEA) between top-ranked Yap and Amot target genes that were increased or decreased after Yap or Amot knockdown in HEK293 cells.

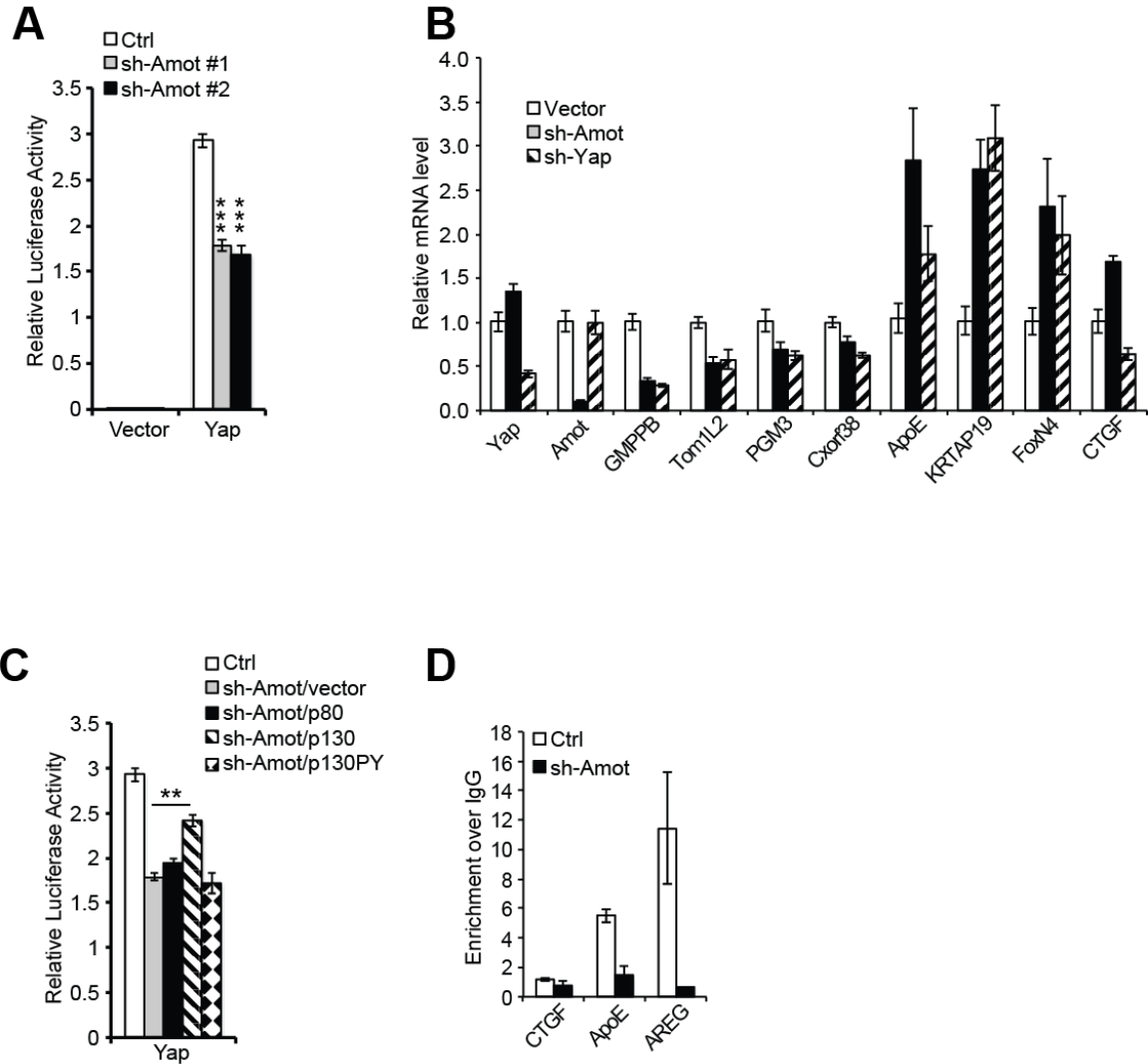


Figure S6: Yap requires Amot for its transcriptional regulatory activity. (A) Dual luciferase reporter assays of control and two independent Amot-KD HEK293 cell lines transfected with GTIIC-luc and phRL-CMV vectors in combination of either control vector or Yap. Data are means \pm SEM from 3 independent experiments; *** $p < 1 \times 10^{-4}$. The GTIIC-luc reading was below detection in vector-transfected cell lines, approximately 700-fold lower than in YAP-overexpressing cells. (B) Real-time qPCR validation of candidate Yap/Amot co-regulated genes identified from the microarray

study in Fig. 6C. **(C)** Dual luciferase reporter assays of control and Amot-KD HEK293 cells transfected with Yap, GTIIC-luc, and phRL-CMV vectors in combination with control vector, Amot-p80 (p80), wild-type Amot-p130 (p130), or the Amot-p130 PY1+2 mutant (p130PY). **p=0.001 **(D)** ChIP analysis with control IgG or Amot antibody of control of Amot-KD HEK293 cells. Real time qPCR analysis was performed with eluted DNA using primers targeting the promoter regions of *ApoE*, *AREG*, and *CTGF*. Fold enrichment of individual promoter with Amot antibody was calculated relative to IgG. All data are means \pm SEM from 3 independent experiments. P-values were calculated using two-tail Student's t-test.

Table S1: Commonly regulated genes by Yap and Amot. Listed are genes that exhibited an absolute fold change of 2 and more in Amot-KD and Yap-KD HEK293 cells, corresponding to overlapping areas in the Venn diagrams in fig. S6A.

Table S2: Pathways predicted to be regulated by Amot or Yap by GSEA. Genes affected by Amot or Yap knockdown were grouped by pathways identified through KEGG (Kyoto Encyclopedia of Genes and Genomes) and BioCarta, shows substantial enrichment of genes involved in proteasome pathways and several metabolic pathways. NOM, nominal (unadjusted) significance of the enrichment score; FDR, false discovery rate; FWER, family wise error rate.

Table S3: Primer sequences. The table shows the sequences of all primers used for qPCR and CHIP analysis.