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Supplementary Materials for

The p130 Isoform of Angiomotin Is Required for Yap-Mediated Hepatic Epithelial Cell Proliferation and Tumorigenesis

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Supplementary Materials

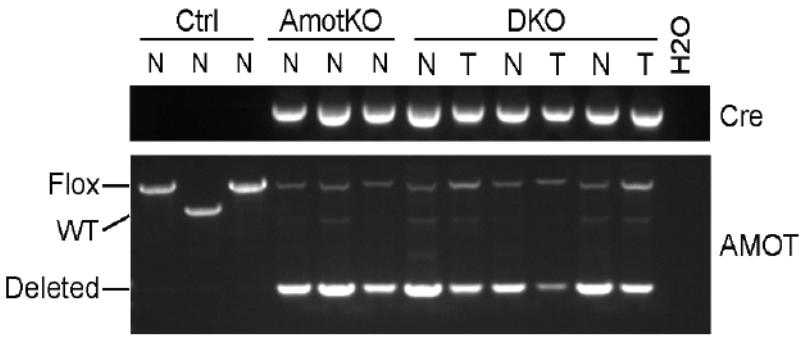

Figure S1: The *Amot*^{flox} allele incompletely recombines in the tumor regions of livers in DKO mice.

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.

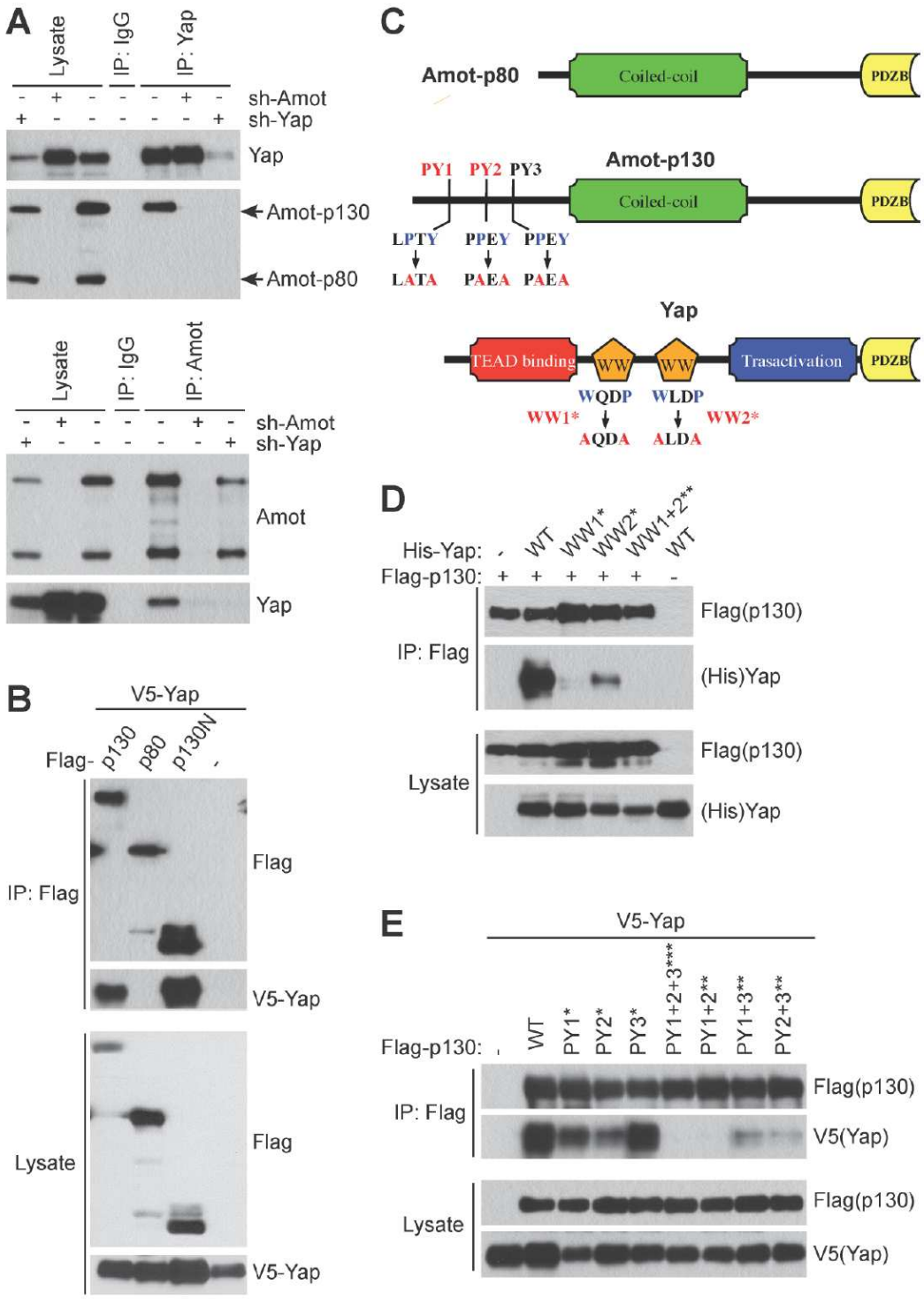
A
Lysate
IP: IgG
IP: Yap
sh-Amot
sh-Yap
Yap
Amot-p130
Amot-p80
Lysate
IP: IgG
IP: Amot
sh-Amot
sh-Yap
Amot
Yap
B
V5-Yap
Flag-
p130
p80
p130N
IP: Flag
Flag
V5-Yap
Lysate
Flag
V5-Yap
C
Amot-p80
Coiled-coil
PDZB
Amot-p130
PY1
PY2
PY3
Coiled-coil
PDZB
LPTY
PPEY
PPEY
LATA
PAEA
PAEA
Yap
TEAD binding
WW
WW
Trasactivation
PDZB
WQDP
WLDP
WW1*
WW2*
AQDA
ALDA
D
His-Yap:
WT
WW1*
WW2*
WW1+2**
WT
Flag-p130:
IP: Flag
Flag(p130)
(His)Yap
Lysate
Flag(p130)
(His)Yap
E
V5-Yap
Flag-p130:
WT
PY1*
PY2*
PY3*
PY1+2+3***
PY1+2**
PY1+3**
PY2+3**
IP: Flag
Flag(p130)
V5(Yap)
Lysate
Flag(p130)
V5(Yap)

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 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.

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.

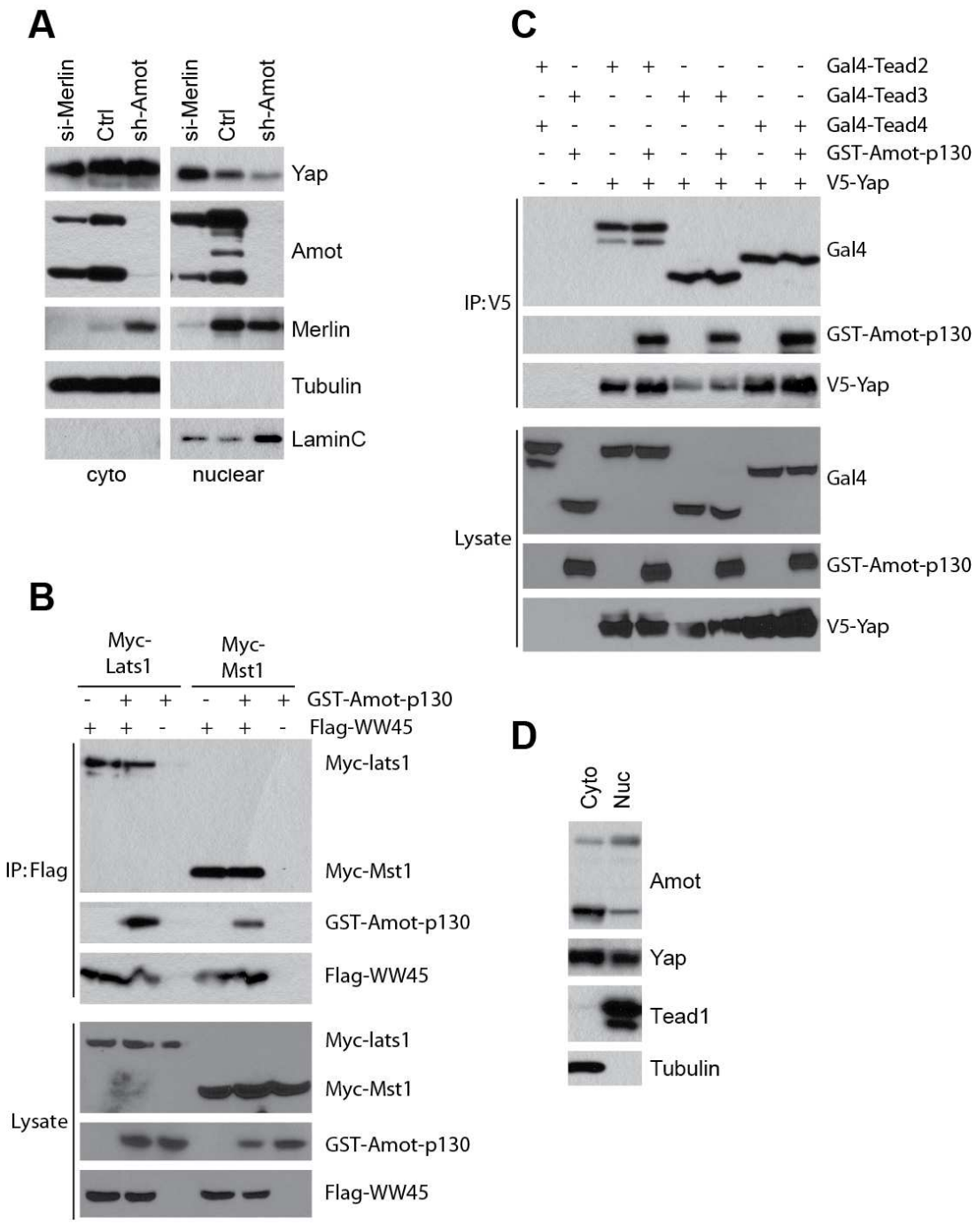

Figure S4: Amot-p130 specifically blocks Yap-Lats1 interaction.

(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.

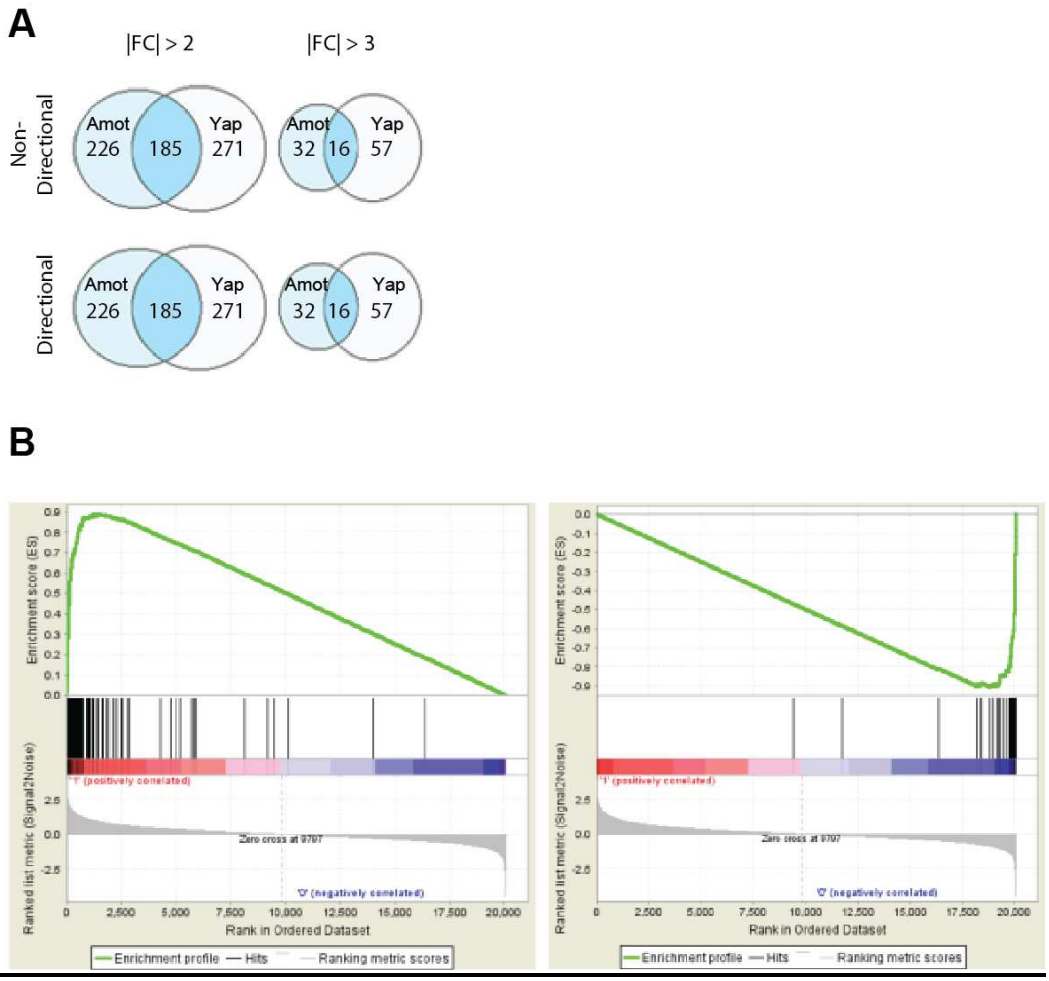

Figure S5: Yap and Amot co-regulate a large set of genes.

(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. |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.

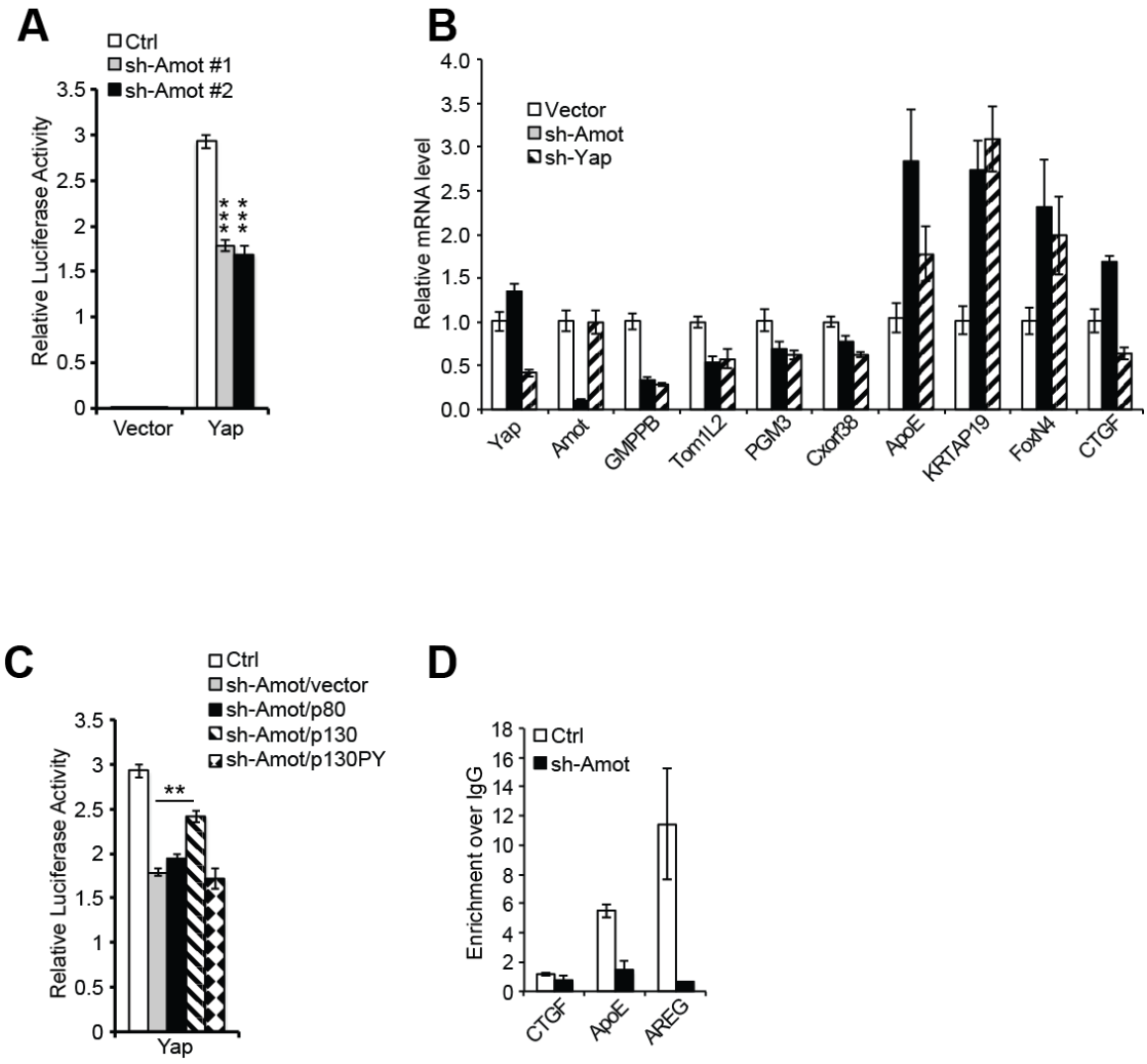

Figure S6: Yap requires Amot for its transcriptional regulatory activity. (A) Dual luciferase reporter assays of control and two independent Amot-KD HEK293 cell lines transfected with GTIIC-luc and phRL-CMV vectors in combination of either control vector or Yap. Data are means ± SEM from 3 independent experiments; ***$p < 1x\ 10^{-4}$. 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 ± 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.