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## Appendix Figure Legends

**Appendix Figure S1.** Effect of USP9X over-expression on YAP target genes.

A. Densely cultured stable RPE clones integrated with empty vector (Vec), USP9X-V5 WT or CS were analyzed by Western blotting for the indicated proteins.

B. Densely cultured RPE cells transduced with USP9X CRISPR-SAM were analyzed by Western blotting for the indicated proteins.

C. USP9X siRNA transfected CRISPR-SAM transduced cells were analyzed by Western blotting for the indicated proteins.

**Appendix Figure S2.** MST1/2 does not mediate the effect of USP9X depletion.

A. Control or USP9X shRNA-expressing RPE cells were transfected with the indicated siRNAs and re-seeded to the sparse density. Cell extracts were analyzed by Western blotting for the indicated proteins.

B. The cells described in (A) were analyzed by RT-qPCR for the indicated target genes (n=3). Error bars indicate the S.E.M. (\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001; paired Student's t-test).

**Appendix Figure S3.** USP9X de-ubiquitinates AMOTL2 *in vitro*.

WT or catalytically inactive (CS) USP9X-V5 were immunoprecipitated with an anti-V5 antibody and incubated with ubiquitinated AMOTL2 CC (coiled-coil domain). Samples were fractionated by SDS-PAGE and analyzed by Western blotting for the indicated proteins. Myc (Ub-AMOTL2) refers to ubiquitinated full-length AMOTL2 CC and thus reduces by USP9X. Myc (Free Ub) refers to Ub monomer and thus appears by USP9X.

**Appendix Figure S4.** Both WT and K0 Ub activate LATS2.

293T cells were transfected as indicated and immunoprecipitated with an anti-Myc antibody .

LATS2 immune complexes were subjected to a kinase assay with His-YAP and cold ATP.

**Appendix Figure S5.** Evolutionarily conserved LATS2 target sequence in USP9X.

The amino acid sequences of USP9X in the indicated species were aligned. Red colored S/T residues correspond to the predicted target site. The amino acid number of the predicted target site in human USP9X is indicated (1019).

### **Appendix Table Legends**

**Appendix Table S1.** Proteins that interact with SBP-YAP in RPE cells, as identified by mass spectrometry.

**Appendix Table S2.** List of primers and siRNA/shRNA/sgRNA sequences used in this study.

Figure S1

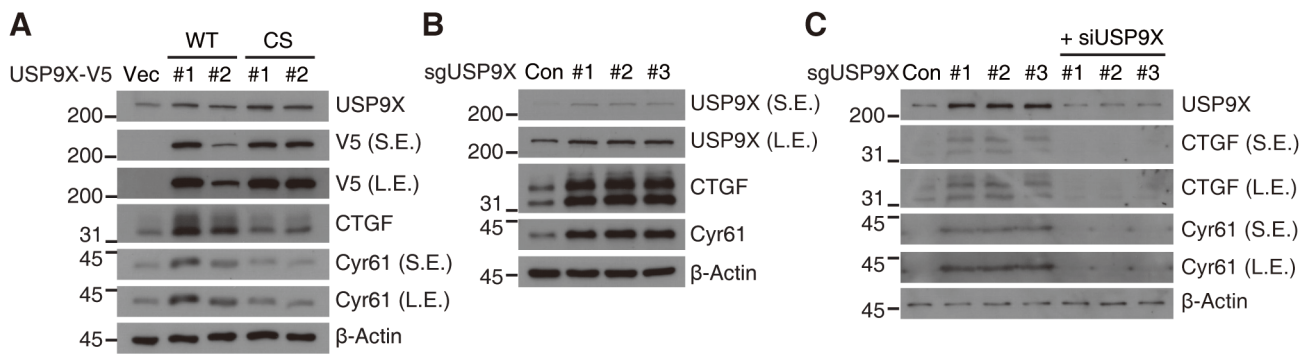


Figure S2

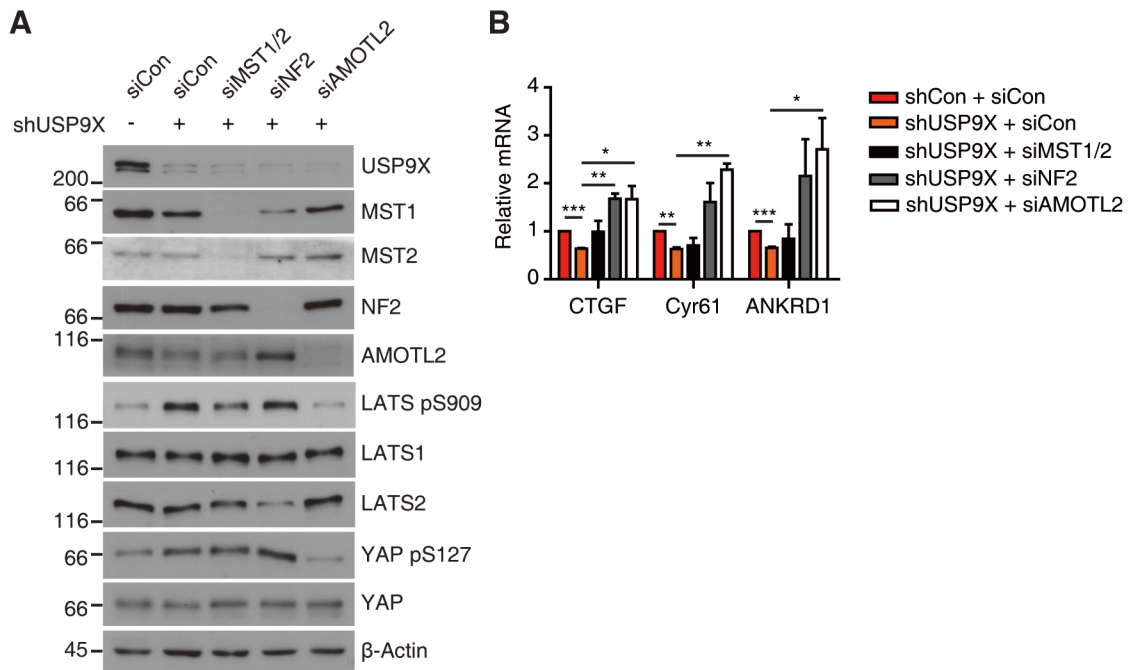


Figure S3

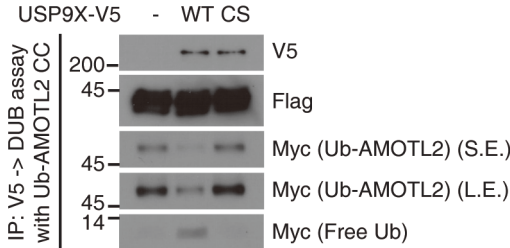


Figure S4

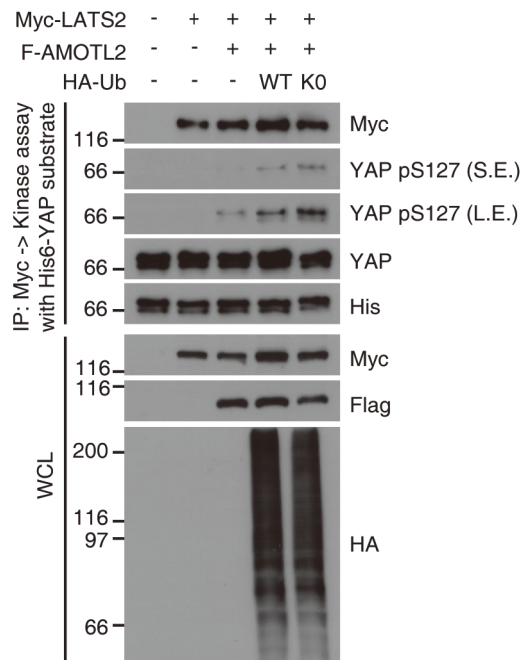


Figure S5

	1019
Homo sapiens	M S L H P R Y I S F L W
Mus musculus	M S L H P R Y I S F L W
Rattus norvegicus	M S L H P R Y I S F L W
Canis lupus	M S L H P R Y I S F L W
Gallus gallus	M S L H P R Y I S F L W
Danio Rerio	M S L H V R Y I S F L W
Drosophila	I S Q N Y Q Y T E F F L
LATS consensus	H x R x x S/T



Gene name	Score	Coverage	# Unique Peptides	# Peptides	# PSMs
YWHAE	3827.06	81.57%	32	34	1385
YWHAQ	2295.51	83.27%	27	31	841
YWHAZ	1842.09	80.82%	23	29	709
YWHAG	1321.28	96.76%	19	28	529
WBP2	1135.25	65.76%	18	18	407
MPDZ	1073.63	57.44%	91	93	366
YWHAB	889.45	78.28%	13	22	385
YWHAH	879.57	73.98%	19	26	357
TUBB	537.03	53.99%	5	24	190
TUBB4B	514.62	55.96%	2	26	186
TUBB2A	470.55	47.42%	2	22	172
DSP	399.3	23.72%	63	63	147
TUBB4A	392.55	46.85%	1	19	138
TEAD1	388.26	36.85%	10	21	134
PPP1CA	348.11	65.76%	9	23	124
TUBA1B	346.55	65.19%	3	26	123
TUBA1C	327.69	65.48%	3	26	119
LIN7C	283.76	73.60%	18	18	130
TUBB3	278.93	36.89%	3	18	97
TUBB8	243.15	24.55%	2	11	89
PPP1CB	240.4	42.81%	2	16	88
TUBB6	234.99	50.00%	9	20	85
ACTB	187.05	53.60%	17	17	74
TEAD4	184.1	38.71%	8	18	71
TEAD3	147.54	26.21%	3	14	61
DSG1	133.92	24.02%	16	16	48
ANXA2	128.61	51.33%	17	17	34
PRDX1	123.85	45.23%	15	15	63
PTPN14	113.33	20.64%	20	20	47
CIT	107.72	14.31%	25	25	45
SLC25A6	104.96	40.94%	6	11	46
FBXW11	100.62	35.43%	17	17	38
FHL2	95.01	50.18%	13	13	37
PDLIM7	80.97	40.92%	17	17	37
RASSF8	77.76	51.31%	20	20	33
DUSP14	77.14	41.92%	5	5	20
SMAD3	60.51	26.77%	9	9	22
CAPNS1	57.68	41.04%	7	7	20

CCDC85C	53.45	39.38%	14	14	24
SLC25A5	53.19	30.20%	5	10	27
MPP5	49.41	17.47%	11	11	20
CSNK1E	41.88	18.75%	6	6	15
SLC25A3	40.59	22.53%	6	6	15
PPP2R2A	39.2	23.27%	7	7	14
ARG1	37.56	31.99%	8	8	14
ATP5A1	36.6	18.09%	7	7	13
USP9X	35.93	4.42%	10	10	15
SERPINB12	33.34	20.49%	7	7	12
SLC25A11	32.56	27.76%	5	5	12
EEF1A1	32.28	27.06%	7	7	10
SKP1	32.19	48.47%	5	5	11
HSPA8	28.73	52.94%	6	7	13
PAICS	28.41	28.24%	10	10	14
TRIP6	28.04	19.12%	7	7	12
SLC25A10	25.31	26.64%	4	4	8
LRRC15	25.02	2.75%	2	2	9
FASN	24.83	2.43%	5	5	8
HSD17B12	24.62	24.68%	6	6	10
PKP1	22.34	9.92%	7	7	10
GAPDH	21.06	23.08%	4	4	8
SLC25A5	20.82	21.14%	2	6	8
MYH9	20.23	3.83%	7	7	9
SLC25A22	19.46	28.30%	3	3	8
RPS3	19.1	41.03%	4	4	7
DSG4	18.86	5.10%	4	4	6
SLC25A1	18.65	13.83%	4	4	9
CDSN	18.17	6.43%	3	3	6
DSC1	17.89	6.67%	4	4	6
UBC	17.7	45.64%	3	3	7
PRPSAP1	16.87	24.04%	2	2	6
PKP1	16.67	6.20%	4	4	6
SLC9A3R2	16.13	18.71%	6	6	8
WWC3	16.09	1.83%	2	2	6
SAPCD2	15.98	19.04%	6	6	7
FLG2	15.23	3.85%	4	4	6
ANXA11	15.07	15.89%	6	6	6
HIST1H1C	15.07	10.80%	2	2	5
TMEM33	14.59	18.62%	5	5	6

TJP1	14.51	3.60%	5	5	6
VDAC2	14.5	16.86%	4	4	6
HSPA6	13.19	6.22%	2	3	5
VPS26A	12.57	13.87%	3	3	5
SELENBP1	12.5	6.78%	3	3	5
HNRNPH1	12.07	13.99%	4	4	5
UQCRC2	11.36	16.26%	5	5	6
PRKDC	11.18	1.39%	4	4	4
PYCRL	10.97	8.43%	2	2	4
GSR	10.86	13.95%	3	3	5
PCBP2	10.71	25.00%	3	3	5
ALB	10.51	3.60%	2	2	4
DPM1	10.37	10.59%	2	2	5

**Appendix Table S1.** Proteins that interact with SBP-YAP in RPE cells, as identified by mass spectrometry.

<b>siRNA</b>		
<b>Gene</b>	<b>Target sequence</b>	
Control (GL2)	CGT ACG CGG AAT ACT TCG A	
USP9X #1	GAG AGT TTA TTC ACT GTC TTA	
USP9X #2	CGC CTG ATT CTT CCA ATG AAA	
MST1	GGG CAC TGT CCG AGT AGC AGC	
MST2	CGG TCA AGT TGT CGC AAT T	
SAV1	GCA CAT GAA GAC TAC AGA TAT	
NF2	GAG GAA GCA ACC CAA GAC GTT	
AMOTL2	GAA CAA GAT GGA CAG TGA AAT	
LATS1	GTC TGC TTC ATA CAT TCC TAA	
LATS2	AAA GGC GTA TGG CGA GTA G	
<b>shRNA</b>		
<b>Gene</b>	<b>Target sequence</b>	
Control (GFP)	CAA CAA GAT GAA GAG CAC CAA	
USP9X	CGC CTG ATT CTT CCA ATG AAA	
AMOTL2	GAA CAA GAT GGA CAG TGA AAT	
<b>sgRNA</b>		
<b>Gene</b>	<b>Target sequence</b>	
Control (CAG)	GTT CCG CGT TAC ATA ACT TA	
USP9X #1	AGG ACG GGC ATC CGC GGC TG	
USP9X #2	AGG CGC GAC AGA GGC CCT GA	
USP9X #3	GGC CGG GTG CGG AGA GGT TG	
<b>qRT PCR</b>		
<b>Gene</b>	<b>Forward sequence</b>	<b>Reverse sequence</b>
$\beta$ -Actin	CAT GTA CGT TGC TAT CCA GGC	CTC CTT AAT GTC ACG CAC GAT
CTGF	CCA ATG ACA ACG CCT CCT G	TGG TGC AGC CAG AAA GCT C
Cyr61	GGT CAA AGT TAC CGG GCA GT	GGA GGC ATC GAA TCC CAG C
ANKRD1	CAC TTC TAG CCC ACC CTG TGA	CCA CAG GTT CCG TAA TGA TTT
E-cadherin	CGA GAG CTA CAC GTT CAC GG	GGG TGT CGA GGG AAA AAT AGG
N-cadherin	TCA GGC GTC TGT AGA GGC TT	ATG CAC ATC CTT CGA TAA GAC TG
Vimentin	AGT CCA CTG AGT ACC GGA GAC	CAT TTC ACG CAT CTG GCG TTC
Fibronectin	CGG TGG CTG TCA GTC AAA G	AAA CCT CGG CTT CCT CCA TAA
Col8a1	AAA GGG GAA ATT GGG CCT ATG	CTG GTT GCC CTG GTA ACC C

**Appendix Table S2.** List of primers and siRNA/shRNA/sgRNA sequences used in this study.