Supplemental Data

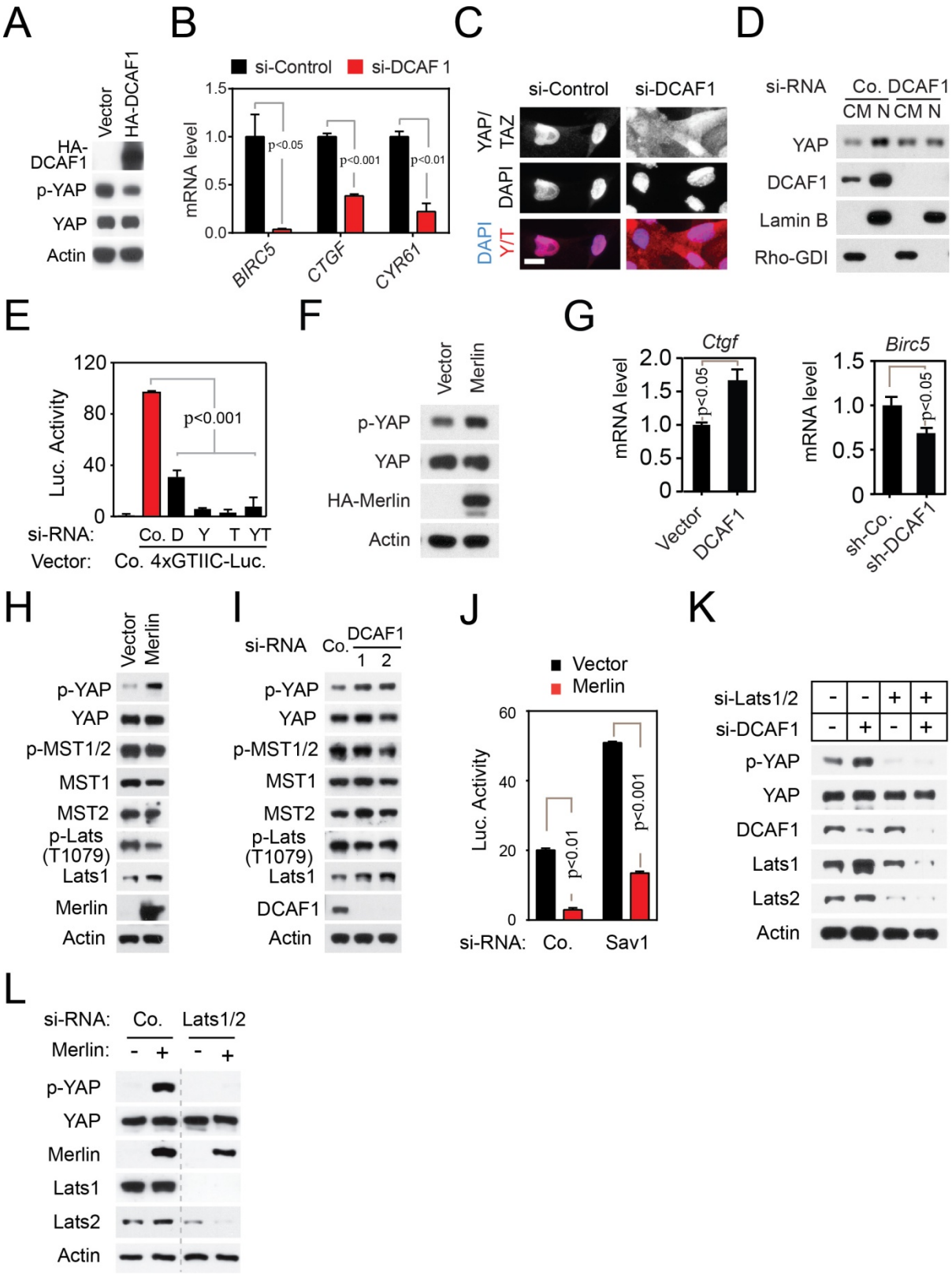

Figure S1. Related to Figure 1. (**A**) Meso-33 cells expressing control vector or HA-DCAF1 were serum deprived for 24 hours and followed by full medium treatment for 4hrs. Cell lysates were analyzed for YAP S127 phosphorylation by immunoblotting as indicated. **(B)** Meso-33 cells were transfected with SMARTpool si-RNAs targeting DCAF1 or a control si-RNA and subjected to qPCR for *BIRC5*, *CTGF*, and *CYR61* mRNA levels. (**C**) Meso-33 cells were transfected with SMARTpool si-RNAs targeting DCAF1 or a control si-RNA and subjected to immunofluorescent staining with an antibody that recognizes YAP and TAZ. Nuclei were stained with DAPI. Scale bar, 20 μm. **(D)** Meso-33 cells were transfected with SMARTpool si-RNAs targeting DCAF1 or a control si-RNA. Cytosolic plus crude membrane fractions (CM) and nuclear fractions (N) from these cells were immunoblotted as indicated. **(E)** Meso-33 cells were transfected with a control si-RNA SMART pool or with SMART pools targeting DCAF1, YAP, TAZ, or both YAP and TAZ, transduced with a TEAD reporter (4xGTIIC-luc) or a promoter-less luciferase vector (Co.), and subjected to luciferase assay. **(F)** FC-1801 cells were transduced with either a control vector or Merlin. Total cell lysates were immunoblotted as indicated. (**G**) FC-1801 cells were transduced with either a control sh-RNA or a sh-RNA targeting DCAF1 (Left), or were transduced with either a control vector or DCAF1 (Right), and subjected to qPCR for *Ctgf* (Left) or *Birc5* (Right) mRNA levels. (**H**) Meso-33 cells were transduced with empty vector or Merlin, and subjected to immunoblotting. (**I**) Meso-33 cells were transduced with a control si-RNA or two si-RNAs targeting DCAF1 and subjected to immunoblotting. (**J**) Meso-33 cells were transfected with either a control si-RNA or a SMARTpool si-RNA targeting Sav1, and then transduced with empty vector or Merlin followed by a TEAD reporter (4xGTIIC-luc), and subjected to luciferase assay. (**K**) Meso-33 cells were transfected with the indicated si-RNAs and subjected to immunoblotting. (**L**) Meso-33 cells were transfected with a control si-RNA or a si-RNA targeting Lats1/2 and then with empty vector or Merlin and were subjected to immunoblotting. Error bar, +/-SEM.

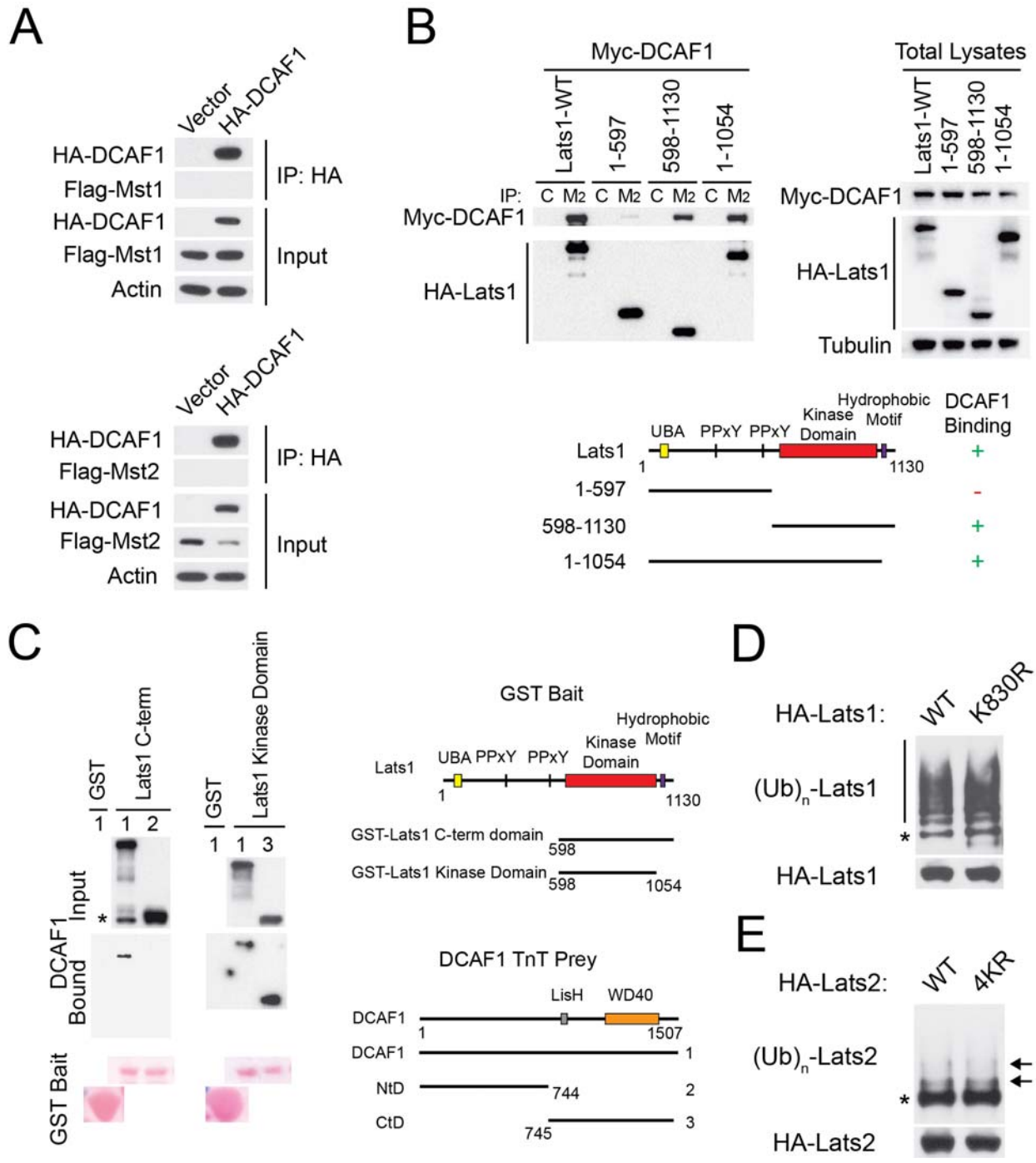

Figure S2. Related to Figure 2. (**A**) HA immunoprecipitates and total lysates from 293T cells expressing HA-DCAF1 in combination with Flag-MST1 (Top) or Flag-MST2 (bottom) were immunoblotted as indicated. (**B**) DCAF1 binds to the kinase domain of Lats1. 293T cells expressing Myc-DCAF1 in combination with Flag-HA-Lats1 or the indicated mutants were lysed in RIPA buffer. Flag immunoprecipitates (M2) and total lysates were immunoblotted as indicated. Mouse IgG agarose was used for control (C) precipitations. (**C**) The C-terminus of DCAF1 interacts with the kinase domain of Lats1 *in vitro*. Purified GST-Lats1-Cterm (residues

598-1130) precipitates *in vitro* translated and biotinylated (TnT) full-length DCAF1 but not the N-terminal half of DCAF1. Purified GST-Lats1-Kinase domain (residues 598-1054) precipitates full-length and C-terminal TnT-DCAF1. As a control, TnT-DCAF1 was subjected to pull down with GST. The GST-Lats1 precipitates and aliquots of TnT-DCAF1 along with respective mutants were blotted with peroxidase-conjugated Streptavidin. (**D**) and (**E**) Denatured lysates of 293T cells expressing HA-tagged Lats1 wild-type or K830R mutant (D) and HA-tagged Lats2 wild-type or K527/633/383/968R (4KR) (E) in combination with His-Ubiquitin were subjected to nickel precipitation. Total lysates (bottom) and nickel precipitates were immunoblotted with anti-HA to analyze ubiquitylated species.

Table S1. Related to Figure 2 Ubiquitylation sites identified in Lats1 and Lats2

Lats1			**LATS1Lower**	**LATS1Upper**
*Amino Acid #:	Amino Acid Sequence	Ub site:	Number of Assigned Spectra(Spectral counts):	
828-838	DI**K**PDNILIDR	K830	1	1
Lats2			**LATS2Lower**	**LATS2Upper**
*Amino Acid #:	Amino Acid Sequence	Ub site:	Number of Assigned Spectra(Spectral counts):	
526-545	S**K**SEQYDLDSLCAGMEQSLR	K527	1	1
624-645	RLQLEQEMA**K**AGLCEAEQEQMR	K633	2	1
378-391	RDSLQ**K**PGLEAPPR	K383	not detected	2
962-983	NGADDL**K**AHPFFSAIDFSSDIR	K968	not detected	1

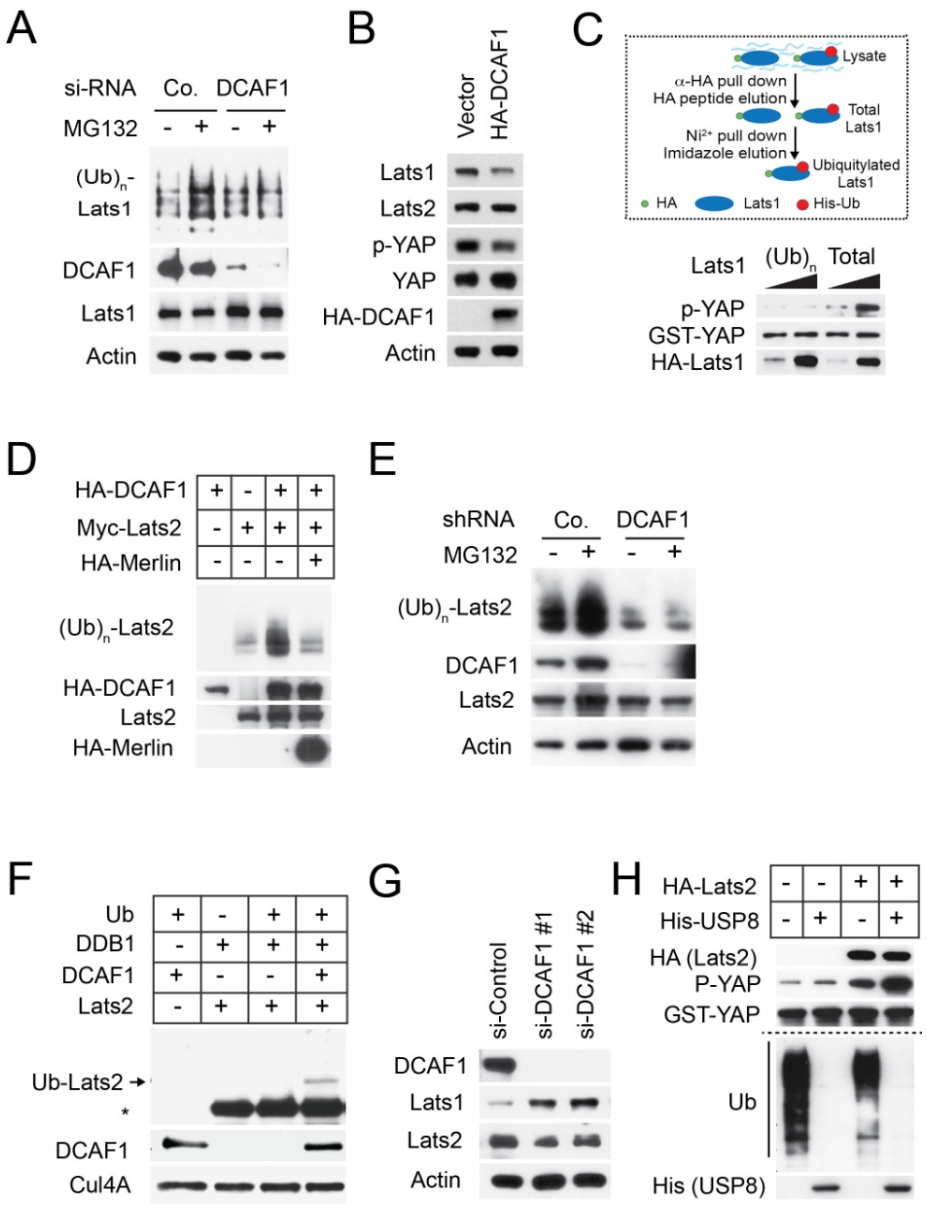

Figure S3. Related to Figure 3. (**A**) Control and DCAF1-depleted 293T cells expressing His-Ubiquitin were treated with or without MG132. Nickel-purified ubiquitylated proteins and total lysates were immunoblotted as indicated. **(B)** FC-1801 cells were transduced with either a control vector or HA-DCAF1. Total cell lysates were immunoblotted as indicated. (**C**) Total and His-ubiquitylated HA-Lats were isolated by sequential affinity steps (top) and subjected to in vitro kinase assay with GST-YAP as a substrate. Glutathione-precipitated YAP was analyzed for S127 phosphorylation by immunoblotting. (**D**) 293T cells were transfected with Myc-Lats2 in combination with His-Ubiquitin and the indicated recombinant proteins. Ubiquitylated proteins were nickel purified and immunoblotted with anti-Lats2. Total lysates were immunoblotted as indicated. (**E**) Control and DCAF1-depleted 293T cells expressing Myc-Lats2 and His-Ubiquitin were treated with or without MG132. Nickel-purified ubiquitylated proteins and total lysates were immunoblotted as indicated. (**F**) $CRL4^{DCAF1}$ was reconstituted in vitro using purified

recombinant Cul4A/Rbx1, DDB1, and DCAF1 and incubated with recombinant HA-Lats2 as indicated. Reaction mixtures were immunoblotted as indicated. (**G**) Meso-33 cells were transfected with a control si-RNA or 2 si-RNAs targeting DCAF1 and subjected to immunoblotting. (**H**) Crude lysates from 293T cells transfected with HA-Lats2 were treated with or without recombinant His-USP8. Lysates were subjected to in vitro kinase assay using GST-YAP as a substrate. Immunoblotting was used to assess phosphorylation of GST-YAP and global ubiquitylation.

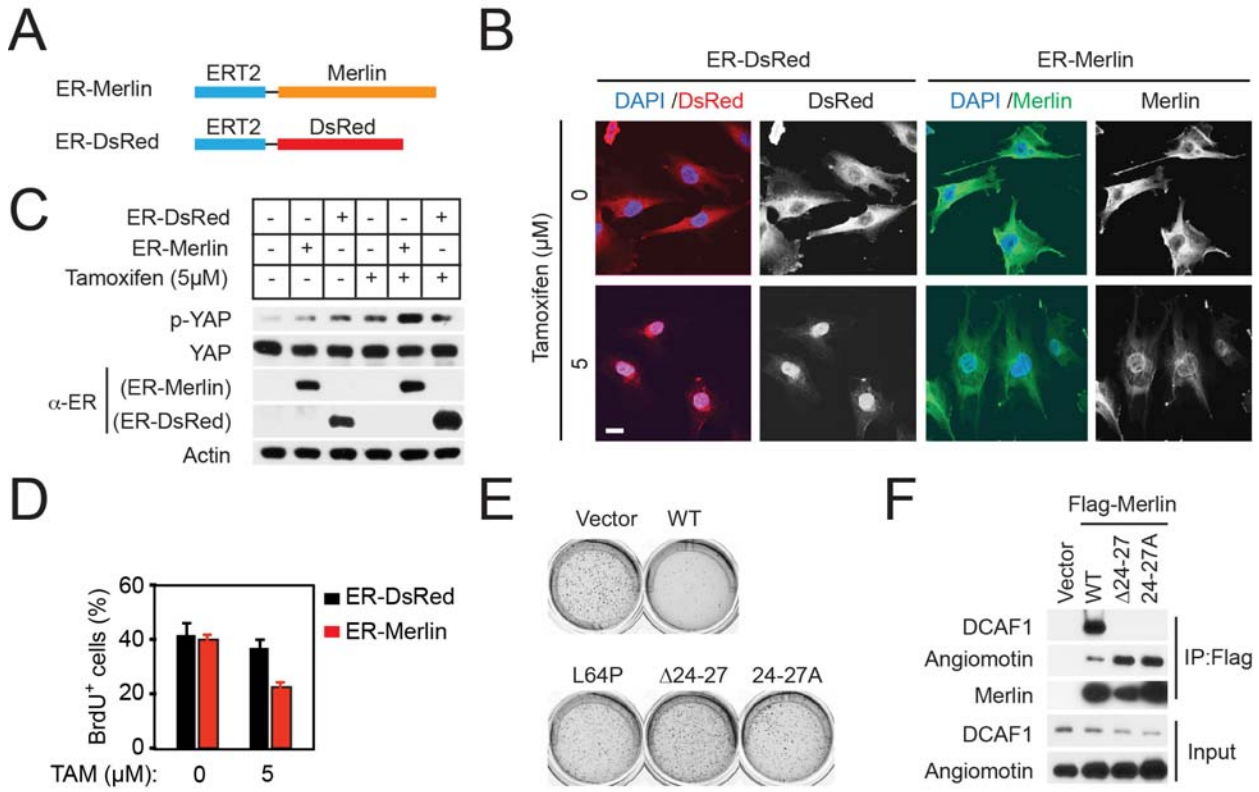

Figure S4. Related to Figure 4. (A) Schematic of tamoxifen-inducible nuclear translocation fusion proteins of Merlin and DsRed (control). (**B**) Tamoxifen induces robust nuclear localization of the ERT2 fusion proteins. Meso-33 cells expressing ERT2-Merlin or ERT2-DsRed were treated with tamoxifen at the indicated concentrations for 24 hours. Fixed cells were immunostained as indicated to analyze nuclear transport of the fusion proteins. Scale bar, 20 μm. (**C**) Translocation of Merlin to the nucleus induces YAP phosphorylation. Meso-33 cells expressing ERT2-Merlin or ERT2-DsRed were treated with or without 5 μM tamoxifen for 24 hours and immunoblotted as indicated to analyze YAP S127 phosphorylation. (**D**) Induced Merlin nuclear translocation significantly inhibits growth. Meso-33 cells treated as in (B) were subjected to BrdU assay. The graph indicates the percentage of BrdU-positive cells. Error bar, +/-SEM. (**E**) FC-1801 cells transduced with wild-type Merlin or its mutants were subjected to soft agar assay. Representative wells are shown. (**F**) Flag-HA-tagged Merlin or the indicated mutants were expressed in 293T cells. Total lysates and Flag immunoprecipitates were immunoblotted as indicated.

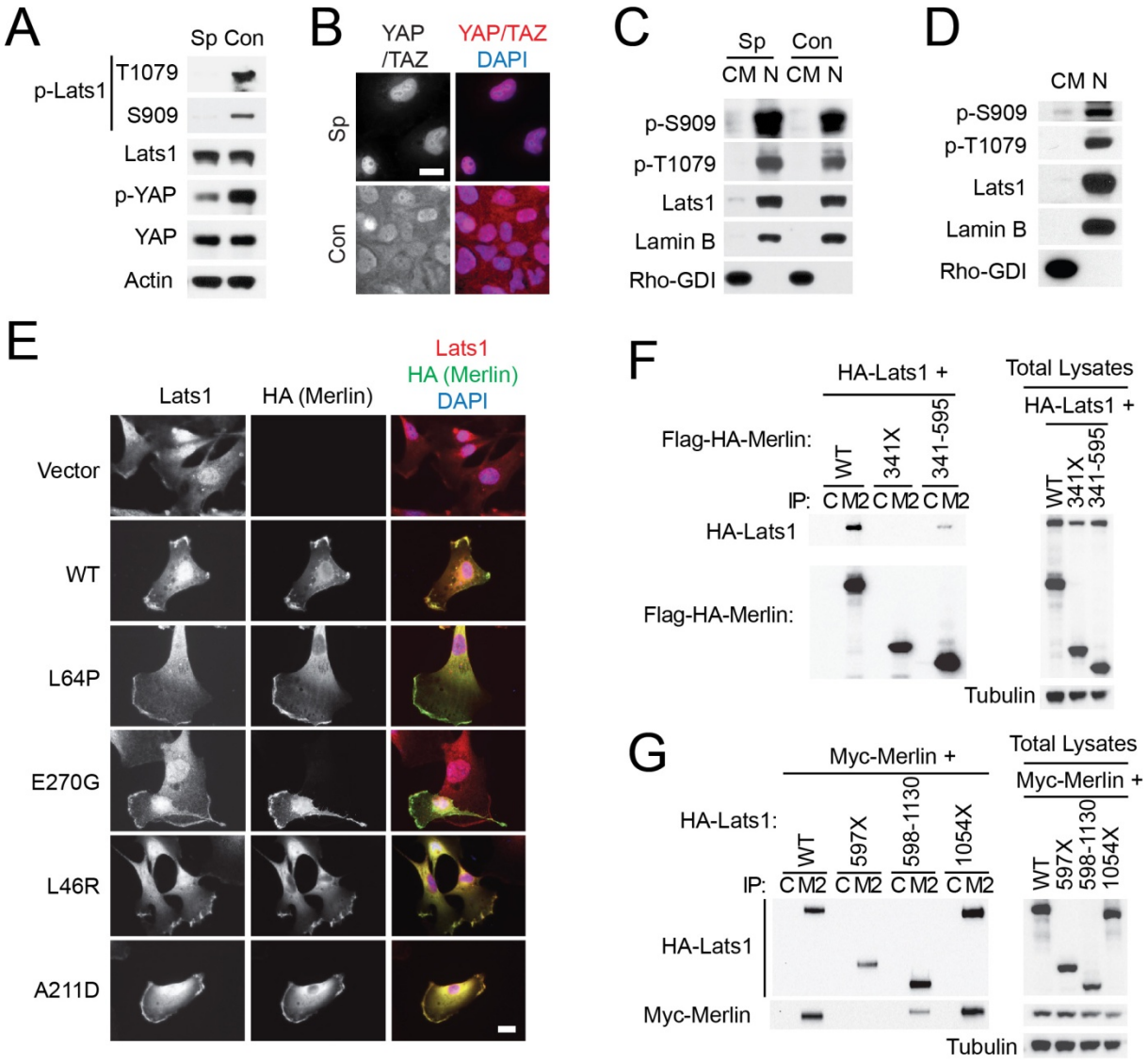
A
Sp Con
p-Lats1
T1079
S909
Lats1
p-YAP
YAP
Actin
B
YAP /TAZ
YAP/TAZ DAPI
Sp
Con
C
Sp Con
CM N CM N
p-S909
p-T1079
Lats1
Lamin B
Rho-GDI
D
CM N
p-S909
p-T1079
Lats1
Lamin B
Rho-GDI
E
Lats1
HA (Merlin)
Lats1 HA (Merlin) DAPI
Vector
WT
L64P
E270G
L46R
A211D
F
HA-Lats1 +
Total Lysates
HA-Lats1 +
Flag-HA-Merlin:
WT
341X
341-595
IP: C M2 C M2 C M2
HA-Lats1
Flag-HA-Merlin:
Tubulin
G
Myc-Merlin +
Total Lysates
Myc-Merlin +
HA-Lats1:
WT
597X
598-1130
1054X
IP: C M2 C M2 C M2 C M2
HA-Lats1
Myc-Merlin
Tubulin
H
HA Ctrl
WT
24-27A
Δ24-27
L46R
L64P
L141P
A211D
E270G
DCAF1
Lats1
HA (Merlin)
IP: FLAG
DCAF1
Lats1
HA (Merlin)
Tubulin
Rho-GDI
Input

Figure S5. Related to Figure 5. (**A**) Met5-A cells grown under sparse (Sp) or confluent (Con) conditions were analyzed for Lats1-T1079 or -S909 and YAP S127 phosphorylation by immunoblotting as indicated. (**B**) Met5-A cells grown under sparse (Sp) or confluent (Con) conditions were subjected to immunofluorescent staining with an antibody that recognizes YAP and TAZ. Nuclei were stained with DAPI. Scale bar, 20 μm. (**C**) and (**D**) Cytosolic and crude membrane fractions (CM), and nuclear fractions (N) from human liver epithelial HepG2 cells (C) and primary mouse liver progenitor cells (D) were immunoblotted as indicated. (**E**) Meso-33 cells expressing empty vector, wild-type HA-Merlin, or the indicated HA-Merlin mutants were fixed and analyzed for endogenous Lats1 by immunostaining as indicated. Scale bar, 20 μm. (**F**) Merlin's coiled-coil/C-terminal domain binds to the Lats1 kinase domain. 293T cells expressing HA-Lats1 in combination with Flag-HA-Merlin or the indicated mutants were lysed in RIPA buffer. Flag immunoprecipitates (M2) and total lysates were immunoblotted as indicated. Mouse IgG agarose was used for control (C) precipitations. (**G**) Merlin binds to the kinase domain of Lats1. 293T cells expressing Myc-Merlin in combination with the indicated Flag-HA-tagged Lats1 fusion proteins were lysed in RIPA buffer. Flag immunoprecipitates (M2) and total lysates were immunoblotted as indicated. Mouse IgG agarose was used for control (C) precipitations. (**H**) 293T cells were transduced with empty vector (HA Ctrl) or Flag-HA-tagged Merlin (wild type or its mutant). Flag immunoprecipitates and input were immunoblotted as indicated.

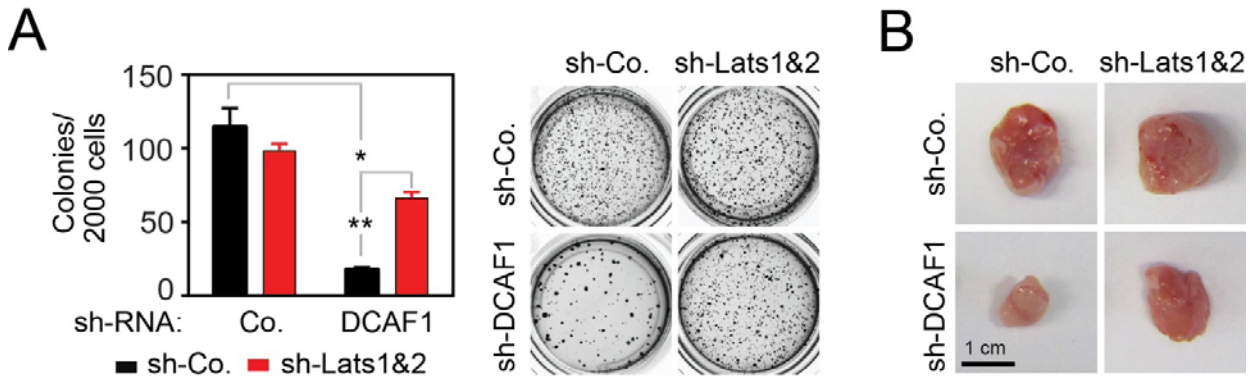

Figure S6. Related to Figure 6. (**A**) FC-1801 cells transduced with a control sh-RNA (left) or sh-RNAs targeting Lats1/2 (right) were subsequently transduced with a control sh-RNA or a sh-RNA targeting DCAF1 and were subjected to soft agar assay. Typical cell colonies in individual culture wells are shown (right) and the graph (left) illustrates the quantification of colonies. Error bar, +/-SEM. (**B**) Typical tumors raised from the experiment described in Figure 5C.

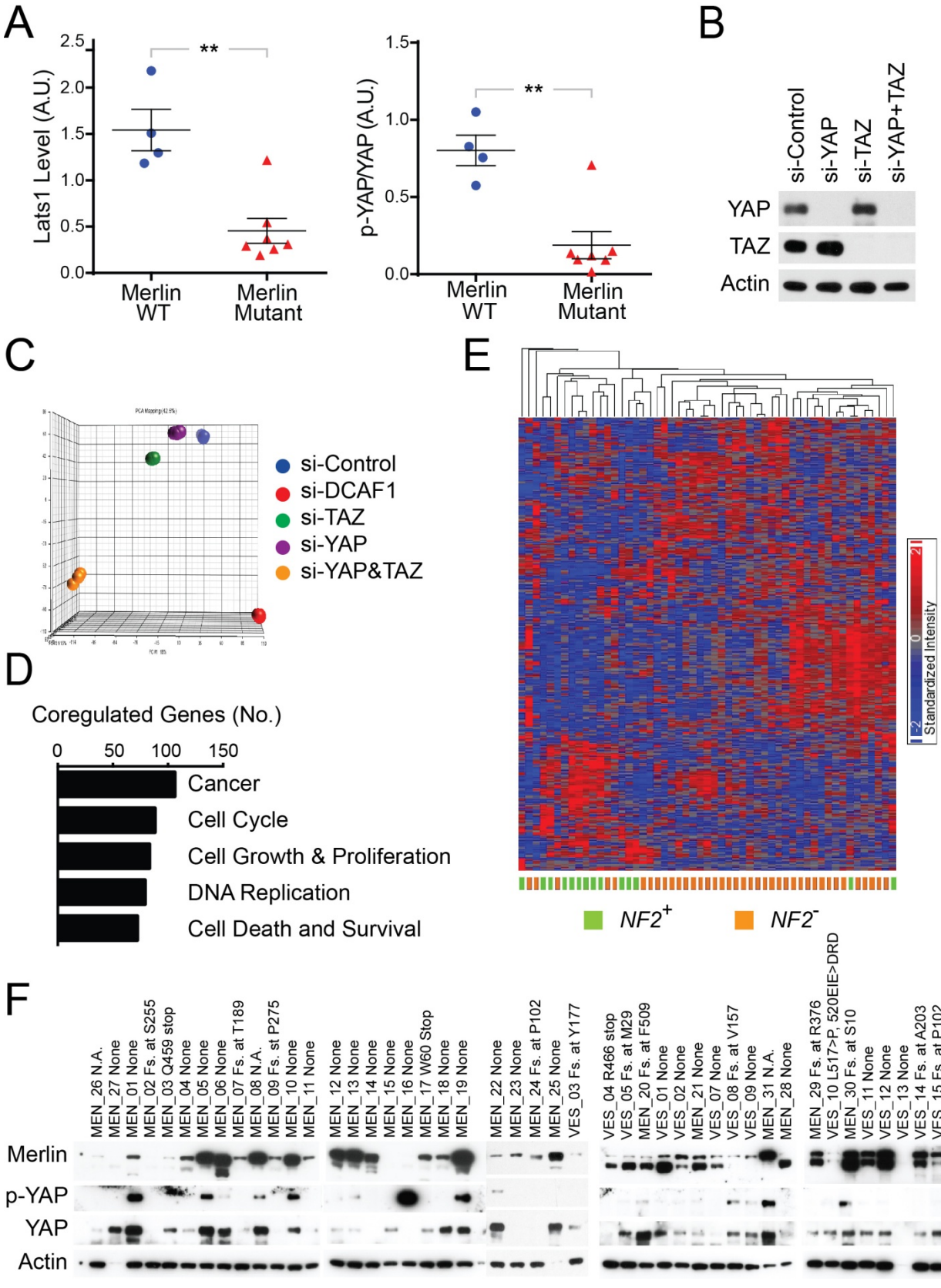
A
Lats1 Level (A.U.)
2.5
2.0
1.5
1.0
0.5
0.0
**
Merlin WT
Merlin Mutant
p-YAP/YAP (A.U.)
1.5
1.0
0.5
0.0
**
Merlin WT
Merlin Mutant
B
si-Control
si-YAP
si-TAZ
si-YAP+TAZ
YAP
TAZ
Actin
C
si-Control
si-DCAF1
si-TAZ
si-YAP
si-YAP&TAZ
D
Coregulated Genes (No.)
0
50
100
150
Cancer
Cell Cycle
Cell Growth & Proliferation
DNA Replication
Cell Death and Survival
E
Standardized Intensity
2
0
-2
NF2+
NF2-
F
MEN_26 N.A.
MEN_27 None
MEN_01 None
MEN_02 Fs. at S255
MEN_03 Q459 stop
MEN_04 None
MEN_05 None
MEN_06 None
MEN_07 Fs. at T189
MEN_08 N.A.
MEN_09 Fs. st P275
MEN_10 None
MEN_11 None
MEN_12 None
MEN_13 None
MEN_14 None
MEN_15 None
MEN_16 None
MEN_17 W60 Stop
MEN_18 None
MEN_19 None
MEN_22 None
MEN_23 None
MEN_24 Fs. at P102
MEN_25 None
VES_03 Fs. at Y177
VES_04 R466 stop
VES_05 Fs. at M29
MEN_20 Fs. at F509
VES_01 None
VES_02 None
MEN_21 None
VES_07 None
VES_08 Fs. at V157
VES_09 None
MEN_31 N.A.
MEN_28 None
MEN_29 Fs. at R376
VES_10 L517>P, 520EIE>DRD
MEN_30 Fs. at S10
VES_11 None
VES_12 None
VES_13 None
VES_14 Fs. at A203
VES_15 Fs. at P102
VES_16 None
VES_17 Fs. at L397
VES_06 None
Merlin
p-YAP
YAP
Actin

Figure S7. Related to Figure 7. (**A**) A panel of human mesothelioma cell lines, including Merlin-wild-type (WT) and Merlin-mutant, were cultured under the same condition. Cell lysates were immunoblotted as indicated in Figure 7A. Densitometry of p-YAP/YAP and Lats1 was performed and plotted. Error bar, +/-SEM. ***P*<0.01. (**B**) Meso-33 cells were transfected with the indicated si-RNAs and subjected to immunoblotting as indicated. (**C**) Analysis of gene expression responding to DCAF1 or YAP combined with TAZ depletion. After normalization, expression data were evaluated by Principal Component Analysis (PCA). (**D**) Ingenuity pathway analysis of genes coregulated by depletion of DCAF1 or both YAP and TAZ. Gene numbers include both upregulated and downregulated genes. (**E**) Human malignant mesothelioma samples were subjected to DNA microarray analysis. Unsupervised hierarchical clustering indicates *NF2* mutant (*NF2*-) tumors segregate from *NF2* wild-type (*NF2*+) tumors. (**F**) Human meningioma and vestibular schwannoma samples were subjected to targeted genomic sequencing of the NF2 gene and immunoblotting. Identified *NF2* mutations are indicated (None=no mutation detected; N.A.=not determined).

Table S2, related to Figure 7. Provided as an Excel file.

Table S3. Related to Figure 7. Top upstream regulators derived from co-downregulated (A) or co-upregulated (B) genes responding to knockdown of DCAF1 or Yap and Taz

A		
Upstream Regulator	**p-value of overlap**	**Predicted Activation State**
E2F4	1.62E-39	N.A.
TP53 (includes EG:22059)	3.92E-30	Activated
RB1	3.72E-25	Activated
FOXM1	2.29E-24	Inhibited
E2F1	8.18E-24	Inhibited
B		
Upstream Regulator	**p-value of overlap**	**Predicted Activation State**
SMAD3	1.03E-06	Activated
HIF1A	1.28E-06	Activated
SMAD7	4.82E-05	N.A.
MYC	6.27E-05	N.A.
SMAD2	1.98E-04	N.A.

Table S4. Related to Figure 7. Top canonical pathways derived from co-downregulated genes responding to knockdown of DCAF1 or Yap and Taz

Name	**p-value**	**Ratio**
Mitotic Roles of Polo-Like Kinase	2.39E-09	9/69 (0.13)
Cyclins and Cell Cycle Regulation	2.80E-07	8/89 (0.09)
Estrogen-mediated S-phase Entry	1.54E-06	5/28 (0.179)
GADD45 Signaling	1.76E-05	4/22 (0.182)
Cell Cycle: G2/M DNA Damage Checkpoint Regulation	3.06E-05	5/48 (0.104)

Table S5. Related to Figure 7. Co-upregulated genes responding to knockdown of DCAF1 or Yap and Taz

SYMBOL	DEFINITION
TXNIP	Homo sapiens thioredoxin interacting protein (TXNIP), mRNA.
SYT11	Homo sapiens synaptotagmin XI (SYT11), mRNA.
SAT1	Homo sapiens spermidine/spermine N1-acetyltransferase 1 (SAT1), mRNA.
SPOCD1	Homo sapiens SPOC domain containing 1 (SPOCD1), mRNA.
TMEM200A	Homo sapiens transmembrane protein 200A (TMEM200A), mRNA.
TNC	Homo sapiens tenascin C (hexabrachion) (TNC), mRNA.
LOC730994	PREDICTED: Homo sapiens similar to NACHT, leucine rich repeat and PYD (pyrin dom
IL11	Homo sapiens interleukin 11 (IL11), mRNA.
ANTXR2	Homo sapiens anthrax toxin receptor 2 (ANTXR2), mRNA.
CAMK2N1	Homo sapiens calcium/calmodulin-dependent protein kinase II inhibitor 1 (CAMK2N1
ARHGAP22	Homo sapiens Rho GTPase activating protein 22 (ARHGAP22), mRNA.
LOC399959	Homo sapiens hypothetical LOC399959 (LOC399959), non-coding RNA.
ANGPTL4	Homo sapiens angiopoietin-like 4 (ANGPTL4), transcript variant 3, mRNA.
LUM	Homo sapiens lumican (LUM), mRNA.
TP53INP1	Homo sapiens tumor protein p53 inducible nuclear protein 1 (TP53INP1), mRNA.
GLIPR2	Homo sapiens GLI pathogenesis-related 2 (GLIPR2), mRNA.
SH3BGRL3	Homo sapiens SH3 domain binding glutamic acid-rich protein like 3 (SH3BGRL3), mR
ACTA2	Homo sapiens actin, alpha 2, smooth muscle, aorta (ACTA2), mRNA.
PCDH18	Homo sapiens protocadherin 18 (PCDH18), mRNA.
TIMP3	Homo sapiens TIMP metallopeptidase inhibitor 3 (TIMP3), mRNA.
BNIP3L	Homo sapiens BCL2/adenovirus E1B 19kDa interacting protein 3-like (BNIP3L), mRNA
PLAUR	Homo sapiens plasminogen activator, urokinase receptor (PLAUR), transcript varia
SRPX2	Homo sapiens sushi-repeat-containing protein, X-linked 2 (SRPX2), mRNA.
CLIP3	Homo sapiens CAP-GLY domain containing linker protein 3 (CLIP3), mRNA.
VAMP5	Homo sapiens vesicle-associated membrane protein 5 (myobrevin) (VAMP5), mRNA.
HMOX1	Homo sapiens heme oxygenase (decycling) 1 (HMOX1), mRNA.
RAC2	Homo sapiens ras-related C3 botulinum toxin substrate 2 (rho family, small GTP b
ECM1	Homo sapiens extracellular matrix protein 1 (ECM1), transcript variant 1, mRNA.
NT5E	Homo sapiens 5'-nucleotidase, ecto (CD73) (NT5E), mRNA.
ATP2B4	Homo sapiens ATPase, Ca++ transporting, plasma membrane 4 (ATP2B4), transcript v
TOX2	Homo sapiens TOX high mobility group box family member 2 (TOX2), transcript vari
COL6A3	Homo sapiens collagen, type VI, alpha 3 (COL6A3), transcript variant 1, mRNA.
CXXC5	Homo sapiens CXXC finger 5 (CXXC5), mRNA.
KIAA1539	Homo sapiens KIAA1539 (KIAA1539), mRNA.
P4HA2	Homo sapiens prolyl 4-hydroxylase, alpha polypeptide II (P4HA2), transcript vari
RGL1	Homo sapiens ral guanine nucleotide dissociation stimulator-like 1 (RGL1), mRNA.
DNER	Homo sapiens delta/notch-like EGF repeat containing (DNER), mRNA.
SLC2A3	Homo sapiens solute carrier family 2 (facilitated glucose transporter), member 3
DYNLT3	Homo sapiens dynein, light chain, Tctex-type 3 (DYNLT3), mRNA.

Table S6. Related to Figure 7. Summary of patient characteristics, tumor histology, grade, location, NF2 status, tumor *NF2* gene sequencing, Merlin expression by immunoblot and nuclear YAP/cytoplasmic phospho-YAP expression by immunohistochemistry

Specimen	Patient Age	Gender	Tumor Histology	WHO grade	Tumor Location	Patient NF2 status	Tumor *NF2* sequencing	Tumor Merlin IB	YAP IHC	p-YAP IHC
MEN_26	17	M	meningioma	WHO I	pineal	NF2	NA	-	1	1
MEN_27	10	M	meningioma	WHO I	posterior fossa	NF2	WT	NA	NA	NA
MEN_01	27	M	meningioma	WHO I	right frontal	NF2	WT	-	1	0
MEN_02	8	M	meningioma	WHO I	right frontal falx/parasagittal	NF2	Del, FS at S255	-	NA	NA
MEN_03	21	F	meningioma	WHO I	third ventricular	NF2	Nonsense Q459 stop	-	1	0
MEN_04	59	F	meningioma	WHO II	right frontal falx	sporadic	WT	-	1	0
MEN_05	55	F	meningioma	WHO I	sella	sporadic	WT	+	NA	NA
MEN_06	54	M	meningioma	WHO I	left frontal	sporadic	WT	+	0	1
MEN_07	56	F	meningioma	WHO I	left tentorial	sporadic	Ins, FS at T189	-	1	0
MEN_08	68	F	meningioma	WHO I	cribriform/sphenoid	sporadic	WT	+	NA	NA
MEN_09	63	F	meningioma	WHO II	right posterior. parafalcine	sporadic	Del, FS at P275	-	1	0
MEN_10	57	F	meningioma	WHO I	right sphenoid wing	sporadic	WT	+	0	1
MEN_11	80	M	meningioma	WHO II	left frontal	sporadic	WT	-	1	1
MEN_12	54	F	meningioma	WHO I	left sphenoid wing	sporadic	WT	+	0	1
MEN_13	43	M	meningioma	WHO I	planum sphenoidale/sella	sporadic	WT	+	0	1
MEN_14	58	M	meningioma	WHO I	olfactory groove	sporadic	WT	+	1	1
MEN_15	64	F	meningioma	WHO II	left posterior falx	sporadic	WT	-	NA	1
MEN_16	40	F	meningioma	WHO I	right cerebellopontine angle	sporadic	WT	-	1	0
MEN_17	51	F	meningioma	WHO II	right temporal	sporadic	Nonsense W60 stop	-	1	0
MEN_18	82	M	meningioma	WHO II	left frontal	sporadic	WT	-	1	0
MEN_19	67	M	meningioma	WHO II	left frontal convexity	sporadic	WT	+	0	1
VES_04	67	M	VS	WHO I	cerebellopontine angle	NF2	WT	+	NA	NA
VES_05	29	F	VS	WHO I	cerebellopontine angle	NF2	Del, FS at M29WT	-	NA	NA
MEN_20	23	F	meningioma	WHO II	fourth ventricle	sporadic	Del, FS at F509	-	1	1
VES_01	48	F	VS	WHO I	cerebellopontine angle	NF2	WT	-	NA	NA
VES_02	35	F	VS	WHO I	cerebellopontine angle	sporadic	WT	-	NA	NA
MEN_21	55	F	meningioma	WHO I	left parietal convexity	sporadic	WT	-	1	0
VES_07	50	F	VS	WHO I	cerebellopontine angle	sporadic	WT	+	NA	NA
VES_08	48	F	VS	WHO I	cerebellopontine angle	sporadic	WT	-	NA	NA
VES_09	35	F	VS	WHO I	cerebellopontine angle	sporadic	WT	-	NA	NA
MEN_31	83	F	meningioma	WHO II	left sphenoid wing	sporadic	NA	+	NA	NA
MEN_28	71	M	meningioma	WHO I	right posterior fossa	sporadic	WT	-	NA	NA
MEN_29	42	M	meningioma	WHO I	left tentorial	sporadic	FS at R376	+	NA	NA
VES_10	37	M	VS	WHO I	cerebellopontine angle	sporadic	Del, FS at Y177	-	NA	NA
MEN_30	57	F	meningioma	WHO III	parietal convexity	sporadic	FS at S10	+	NA	NA
MEN_22	12	M	meningioma	WHO I	right intraventricular	sporadic	WT	-	1	0
MEN_23	29	F	meningioma	WHO I	right posterior fossa	sporadic	WT	-	1	0
MEN_24	57	M	meningioma	WHO I	right superior parietal convexity	sporadic	Del, FS at P102	-	1	0
MEN_25	50	F	meningioma	WHO III	right tentorial	sporadic	WT	+	0	1
VES_03	37	M	VS	WHO I	cerebellopontine angle	NF2	Del, FS at Y177	-	NA	NA

(F) female, (M) male, (WHO) World Health Organization, (WT) wild-type (IB) immunoblot, (IHC) immunohistochemistry, (YAP) YES associated protein, (p-YAP) phospho-YAP, (NA) not available. For Merlin IB, (+) normal or strong Merlin signal, (-) weak or absent Merlin signal. For YAP IHC, nuclear staining (0) absent, (1) present. For p-YAP IHC, cytoplasmic staining (0) weak/absent (1) moderate/strong.

Supplemental Experimental Procedures

Patients and tumor samples

A total of 31 meningioma and 9 vestibular schwannoma frozen samples, including sporadic and NF2-related, were obtained from the NYU Brain Tumor Bank and subjected to immunoblotting. The clinical features of these patients are summarized in Table 1. Depending on frozen tissue quantity and availability of matching formalin-fixed paraffin-embedded tissue blocks, further studies including *NF2* gene sequencing and immunohistochemistry were performed. Patient age at time of tumor resection was 8–83 years (median 55 years) for meningiomas and 35–67 years (median 37 years) for vestibular schwannomas, respectively. Five of the meningioma samples and four of the vestibular schwannoma samples were derived from NF2 patients, the remainder were sporadic tumors.

Cells

Met5-A immortalized mesothelial cells, 293FT cells, FC-1801 $Nf2^{-/-}$ mouse Schwannoma cells, and primary human Schwannoma cells were cultured as previously described (Li *et al.*, 2010). Human mesothelioma cell lines were cultured as previously described (Lopez-Lago *et al.*, 2009). HepG2 human liver epithelial cells were from ATCC and cultured as recommended. Human schwannoma cells were kindly provided by NF2 patients after informed consent. E13.5 fetal liver progenitor cells were isolated from mice in a C57B6 background as described previously (Saborowski *et al.*, 2013). Unless otherwise indicated, experiments were performed on cells grown to 70%–90% confluence in complete medium.

Antibodies

Antibodies to p-YAP (#4911), YAP/TAZ (#8418), p-MST1/2 (#3681), MST1 (#3682), MST2 (#3952), Sav1 (#3507), Lats1 (#3477), p-Lats1-T1079 (# 8654), p-Lats1-S909 (#9157), Lats2 (#5888), Histone H3 (#9715) were purchased from Cell Signaling Technology. Antibodies to YAP (#SC-15407), Merlin (#SC-332), CTGF (#SC-14939), Cul4 (#SC-8557), ERα (#SC-8002) were purchased from Santa Cruz biotechnology. Antibodies to Merlin (#ab88957) and Lats2 (#ab70566) were purchased from Abcam. The antibody to DDB1 (#BL1999) was purchased from Bethyl. Antibodies to the HA tag (MMS-101) were obtained from Covance, to Myc (9B11, #2276) from Cell Signaling Technology, to β-Actin (#A3376) from Sigma, to Lamin B1 (#6581-1) and to YAP (#2060-1) from Epitomics, to DsRed (#632397) and to RhoGDI (#610255) from BD Biosciences. EZview Red Anti-FLAG M2 affinity gel, agarose-conjugated anti-HA monoclonal antibody, and agarose-conjugated normal mouse IgGs were purchased from Sigma. Ni-NTA Magnetic Agarose Beads were purchased from Qiagen.

Vectors

pRK5-Flag-HA-Merlin and its various mutants, pRK5-Flag-HA-DCAF1, pRK5-Flag-HA-DCAF1 (1-1417), pRK5-Myc-DCAF1, pXJ40-HA-Merlin and pRK5-Myc-Merlin were described previously (Li *et al.*, 2010). pcDNA3-Flag-MST1, pcDNA3-Flag-MST2, pcDNA3-Myc-Lats1, pcDNA3-Myc-Lats2, pcDNA3-TEAD2-DN, and pcDNA3-Myc-Sav1 were described previously (Dong *et al.*, 2007; Liu-Chittenden *et al.*, 2012) and generously provided by DJ Pan. pGEX-GST- YAP, pBabe-puro- YAP and pBabe-puro- YAP(5SA) were described previously (Zhao *et al.*, 2010; Zhao *et al.*, 2007) and generously provided by Kunliang Guan. pGL3 and pGL3-4xGTIIC were described previously (Mahoney *et al.*, 2005) and generously provided by Iain

Farrance. pHis-Myc-Ub was generously provided by Michele Pagano. pCAG-ERT2CreERT2 was described previously (Matsuda and Cepko, 2007) and was purchased from Addgene (#13777). To generate a vector encoding Lats1 or Lats2 bearing an N-terminal HA or Flag-HA tag, the cDNA encoding Lats1 or Lats2 from the above pcDNA3 constructs were subcloned downstream of the sequences encoding the HA or Flag-HA tag in pRK5-HA or pRK5-Flag-HA vector. Constructs encoding truncation mutants of DCAF1 and Lats1 were generated by subcloning PCR products of a cDNA encoding human DCAF1 or human Lats1 from the above vectors in pRK5-Myc, pRK5-FH, or pGEX vectors. pQCXIP-YAP or YAP (5SA) were generated by PCR subcloning the cDNA encoding human YAP or 5SA from the pBabe-puro-YAP or 5SA vector described above. To generate pRK5-ERT2-Merlin, the cDNA encoding ERT2 from pCAG-ERT2CreERT2 was subcloned upsteam of the sequences encoding Merlin in the pRK5 vector. To generate pERT2-DsRed, the cDNA encoding ERT2 from pCAG-ERT2CreERT2 was subcloned upstream of the sequences encoding DsRed in the pDsRed-N1 vector.

Luciferase assay

pGL3 or pGL3-4xGTIIC vector was transfected into Meso-33 cells or FC-1801 cells. pCMV-Renilla Luciferase was co-transfected with each above vector (ratio of 20:1) as an internal control. After 24hrs incubation, firefly luciferase expression was assessed by using the Dual-Glo Luciferase Assay System (Promega). Luciferase substrate signals were read with the Synergy H1 hybrid reader from Biotek.

Immunoprecipitation and Immunoblotting

293FT cells in 6-well plates were transiently transfected with 1 μg of pRK5 plasmids expressing tagged DCAF1 and 1 μg of pRK5 plasmids expressing tagged Lats1 by using Lipofectamine 2000 (Invitrogen) or Polyethylenimine (PEI) and were lysed 24 hours later with RIPA buffer or RIPA buffer without SDS as indicated. To isolate Flag-HA-DCAF1, extracts were pre-cleared with agarose-mouse IgGs and incubated with anti-FLAG M2 Affinity Gel (Sigma) at 4 ^{0}C for 2 hours. The immunoprecipitates were washed four times with RIPA buffer or RIPA buffer without SDS as indicated, and bound proteins were dissociated in 20 μL 1 x SDS sample buffer (25 mM Tris pH 6.8, 4% SDS, 5% Glycerol, bromophenol blue). Eluted proteins were separated on 4-12% Bis-Tris SDS-PAGE gels (Invitrogen) and transferred to Immobilon-P membranes (Millipore). Membranes were incubated in blocking buffer (5% skim milk, 0.1% Tween, 10 mM Tris at pH 7.6, 100 mM NaCl) for 1 hour at room temperature and then with primary antibodies diluted in blocking buffer for another hour at the same temperature. After three washes, the membranes were incubated with goat anti Rabbit HRP-conjugated antibody (1:5,000; Santa Cruz sc-2054) or goat anti-mouse HRP-conjugated antibody (1:10,000; Santa Cruz sc-2005) at room temperature for 1 hour and subjected to chemiluminescence using ECL (Pierce #1856136). When indicated, cells were lysed in SDS-boiling buffer (10 mM Tris pH 7.5, 1% SDS, 50 mM NaF, 1 mM $NaVO_4$) and subjected to SDS-PAGE and immunoblotting.

BrdU Incorporation Assay

Unless indicated otherwise, Meso-33 cells were plated at 0.5 x 10^4 per cm^2 on coverslips and were deprived of growth factors for 24 hours. They were then incubated with complete medium

supplemented with BrdU for 24 hours. After fixation with 100% cold methanol, cells were stained with BrdU Labeling and Detection Kit I (Roche).

Immunohistochemistry

Immunohistochemistry was performed on formalin fixed, paraffin embedded tissues using monoclonal rabbit anti-human YAP (clone EP1674Y) and polyclonal rabbit anti-human phospho-YAP antibodies. In brief, sections were deparaffinized in xylene (3 changes), rehydrated through graded alcohols (3 changes 100% ethanol, 3 changes 95% ethanol) and rinsed in distilled water. Antigen retrieval was not necessary. Antibody incubation and detection were carried out at 37ºC on a NEXes instrument (Ventana Medical Systems Tucson, Arizona) using Ventana's reagent buffer and detection kits unless otherwise noted. Endogenous peroxidase activity was blocked with hydrogen peroxide. Primary antibodies were diluted 1:3,200 (YAP) and 1:400 (phospho-YAP) in SignalStain Antibody Diluent (Cell Signaling, Danvers, MA, USA), and incubated overnight at room temperature. Primary antibody was detected with Ventana's biotinylated goat anti-rabbit secondary antibody, followed by application of streptavidin-horseradish-peroxidase conjugate. The complex was visualized with 3,3 diaminobenzidene and enhanced with copper sulfate. Slides were washed in distilled water, counterstained with hematoxylin, dehydrated and mounted with permanent media. Appropriate positive controls were included with the study sections. Omission of the secondary antibody was used as negative control.

Scoring of YAP and phospho-YAP staining on whole tissue sections was performed by two experienced neuropathologists (DZ and MS). For YAP, a two-tiered scale was used based on the absence (0) or presence (1) of any nuclear staining in tumor cells. For phospho-YAP, a two-tiered scale was used based on the presence of weak to absent (0) or strong (1) cytoplasmic staining in tumor cells. Non-tumor cells, such as endothelial cells and reactive or inflammatory cells were excluded from scoring. For immunohistochemical analysis, variables were described using proportions, ranges and medians as appropriate. Statistical analysis was performed using SPSS version 20 software (IBM Corp., Armonk, NY), using the Mann-Whitney U test to compare proportions. Analyses were 2-sided, with p values <0.05 considered statistically significant.

In vivo Ubiquitylation Assay

293FT cells in 6-well plates were transfected using Lipofectamine 2000 (Invitrogen) with 1 µg of pHis-Myc-Ub and 0.5 µg of pRK5-HA-Lats1 or pRK5-HA-Lats2. When indicated cells were treated with 25 µM MG132 for 6 hrs before harvest. 48 hrs after transfection, cells were lysed in 1 ml of Guanidinium chloride lysis buffer (6 M Guanidinium-HCL, 0.1 M NaHPO4, 0.01 M Tris/HCL, pH 8.0, 20 mM Imidazole, 10 mM beta-mercaptoethanol). 500 ug of protein lysates were incubated with 150 ul Ni-NTA magnetic beads (slurry) for 3 hrs at room temperature. After incubation, Ni-NTA magnetic beads were washed once with each of Guanidinium washing buffer (6 M Guanidinium-HCL, 0.1 M NaHPO4, 0.01 M Tris/HCL, pH 8.0, 10 mM beta-mercaptoethanol, 0.2% Triton X-100), Urea washing buffer I (8 M Urea, 0.1 M NaHPO4, 0.01 M Tris/HCL, pH 8.0, 10 mM beta-mercaptoethanol, 0.2% Triton X-100), Urea washing buffer II (8 M Urea, 0.1 M NaHPO4, 0.01 M Tris/HCL, pH 6.3, 10 mM beta-mercaptoethanol, 0.2%

Triton X-100) and Urea washing buffer III (8 M Urea, 0.1 M NaHPO4, 0.01 M Tris/HCL, pH 6.3, 10 mM beta-mercaptoethanol, 0.1% Triton X-100). Bound proteins were dissociated in 20 μL SDS sample/elution buffer (25 mM Tris pH 6.8, 4% SDS, 5% Glycerol, 250 mM Imidazole, bromophenol blue). Eluted proteins were separated on 4-12% Bis-Tris SDS-PAGE gels (Invitrogen) and subjected to standard immunoblotting.

Mass Spectrometry

Ubiquitylated protein complexes containing Lats1 and Lats2 were resolved using SDS-polyacrylamide gel electrophoresis, followed by staining with Simply Blue (Life Technologies, CA) and excision of the separated protein bands. In situ trypsin digestion of polypeptides in each gel slice was performed as described (Sebastiaan Winkler *et al.*, 2002). The tryptic peptides were purified using a 2-μl bed volume of Poros 50 R2 (Applied Biosystems, CA) reversed-phase beads packed in Eppendorf gel-loading tips (Erdjument-Bromage *et al.*, 1998). The purified peptides were diluted to 0.1% formic acid and then subjected to nano-liquid chromatography coupled to tandem mass spectrometry (nanoLC-MS/MS) analysis as follows. Peptide mixtures (in 20 μl) were loaded onto a trapping guard column (0.3 x 5 mm Acclaim PepMap 100 C18 cartridge from LC Packings, Sunnyvale, CA) using an Eksigent nano MDLC system (Eksigent Technologies, Inc. Dublin, CA) at a flow rate of 20 μl/min. After washing, the flow was reversed through the guard column and the peptides eluted with a 5-45% acetonitrile gradient over 85 min at a flow rate of 200 nl/min, onto and over a 75-micron x 15-cm fused silica capillary PepMap 100 C18 column (LC Packings, Sunnyvale, CA). The eluent was directed to a 75-micron (with 10-micron orifice) fused silica nano-electrospray needle (New Objective, Woburn, MA). The electrospray ionization needle was set at 1800 V. A linear ion quadrupole trap-Orbitrap hybrid analyzer (LTQ-Orbitrap, ThermoFisher, San Jose, CA) was operated in automatic, data-dependent MS/MS acquisition mode with one MS full scan (450-2000 m/z) in the Orbitrap analyzer at 60,000 mass resolution and up to ten concurrent MS/MS scans in the LTQ for the ten most intense peaks selected from each survey scan. Survey scans were acquired in profile mode and MS/MS scans were acquired in centroid mode. The collision energy was automatically adjusted in accordance with the experimental mass (m/z) value of the precursor ions selected for MS/MS. Minimum ion intensity of 2000 counts was required to trigger an MS/MS spectrum; dynamic exclusion duration was set at 60 s. Initial protein/peptide identifications from the LC-MS/MS data were performed using the Mascot search engine (Matrix Science, version 2.3.02; www.matrixscience.com) with the human segment of Uniprot protein database (20,329 sequences; European Bioinformatics Institute, Swiss Institute of Bioinformatics and Protein Information Resource). The search parameters were as follows: (i) two missed cleavage tryptic sites were allowed; (ii) precursor ion mass tolerance = 10 ppm; (iii) fragment ion mass tolerance = 0.8 Da; and (iv) variable protein modifications were allowed for peptide ubiquitylation (internal Lysine residues that carry GlyGly or LeuArgGlyGly) methionine oxidation, cysteine acrylamide derivatization and protein N-terminal acetylation. MudPit scoring was typically applied using significance threshold score $p<0.01$. Decoy database search was always activated and, in general, for merged LS-MS/MS analysis of a gel lane with $p<0.01$, false discovery rate averaged around 1%. Scaffold (Proteome Software Inc., Portland, OR), version 3_6_1 was used to further validate and cross-

tabulate the tandem mass spectrometry (MS/MS) based peptide and protein identifications. Protein and peptide probability was set at 95% with a minimum peptide requirement of 1.

Kinase Assays

GST-YAP was expressed in BL21 (DE3) competent cells (Invitrogen C6000-03) and purified from lysates using glutathione-Sepharose 4B (GE Healthcare 17-0756-01). Glutathione-conjugated GST-YAP was used as the substrate in the kinase assay system. To assess the kinase activity of Lats1 on phosphorylation of YAP, 293FT cells were co-transfected with HA-Lats1 and His-Ubiquitin. After 24 hrs, cells were lysed in ice cold HEPES lysis buffer (50 mM HEPES pH 7.4, 150 mM NaCl, 0.5% Triton X-100) containing 30 mg/ml RNase A, 30 mg/ml DNase and protease inhibitors (Protease Inhibitor Cocktail Set III from Calbiochem, 1:200) and phosphatase inhibitors (20 mM NaF, 20 mM glycerophosphate, 0.3 mM sodium vanadate). After preclear using Mouse-IgG agarose, HA-Lats1 from the total lysate was Immunoprecipitated by anti-HA agarose. Beads were washed using the HEPES lysis buffer 4 times followed by washing twice using kinase assay buffer (25 mM HEPES pH 7.4, 50 mM NaCl, 5 mM MgCl2 and 5 mM $MnCl_2$, 5 mM b-glycerophosphate). HA-Lats1 was eluted with 150 ug HA peptide. One aliquot (total HA-Lats1) was directly used in the kinase assay described below. In the rest of the eluate, 60 ul Ni-NTA beads were added (pre-equilibrated by kinase buffer (w/o DTT)) and incubated 30 min at 4 ^{o}C. This was followed by a wash with kinase buffer (w/o DTT) 3 times and eluted with 30 ul kinase buffer (w/o DTT) containing 200 mM imidazole at 4 ^{o}C for 20 min. This eluate was dialyzed against 500 ml kinase buffer (w/o DTT) at 4 ^{o}C, for 1 hr. The eluate after dialysis (ubiquitylated HA-Lats1) was applied to the following kinase assay. Total HA-Lats1 or ubiquitylated HA-Lats1 was added into the kinase assay system with GST-YAP, Kinase buffer (25 mM HEPES pH 7.4, 50 mM NaCl, 5 mM MgCl2 and 5 mM MnCl2, 5 mM b-glycerophosphate, 1 mM dithiothreitol, 10 uM ATP). Reactions were conducted at 30 ^{o}C for 30 min and stopped by addition of SDS sample buffer and boiling for 6 min. The mixture was subjected to SDS-PAGE and immunoblotting. To assess the kinase activity of Lats2 on phosphorylation of YAP, HA-Lats2 was transfected into 293FT cells. After 24 hrs, cells were lysed in ice cold HEPES lysis buffer. After pre-clear by centrifugation, lysate was treated with human recombinant USP8 (Bostonbiochem) at 37 ^{o}C for 1 hr. Lysate without USP8 was also subject to the same incubation. After treatment, samples were mixed with 2xSDS sample buffer, boiled and subjected to SDS-PAGE and immunoblotting.

GST Pull Down Assay

GST fusion proteins containing Lats1 residues 598-1054 (GST-Lats1-Kinase Domain) or 598-1130 (GST-Lats1-Cterm) were expressed in BL21 (DE3) competent cells (Invitrogen C6000-03) and purified from lysates using glutathione-Sepharose 4B (GE Healthcare 17-0756-01). A TnT SP6 High-Yield Expression System (Promega L3261) was used to transcribe and translate DCAF1 or Merlin in the presence of biotinylated lysine tRNA (Promega L5061). 10 µl of the TnT reaction, containing less than or equal to 1 µg of DCAF1 or Merlin, was diluted in 0.5 ml of Binding Buffer (20 mM Tris pH7.5, 100 mM NaCl, 10% Glycerol, 0.05% NP-40, 0.5 mM DTT, and protease inhibitors) and incubated with GST-Lats1 fragment fusion protein

immobilized on Sepharose-Glutathione for 2 hours at 4 °C. After washing with Binding Buffer, the samples were analyzed by SDS-PAGE and immunoblotting.

Gene Expression and Silencing

Transient transfections were performed using Lipofectamine 2000 (Invitrogen), Amaxa Nucleofector System (Lonza) or DharmaFECT 1 (Dharmacon). Retroviral and lentiviral vectors encoding recombinant proteins Merlin, YAP or YAP (5SA), shRNAs targeting DCAF1 (#1: TRCN0000129909; #2: TRCN0000129831; #3: V3LMM_460165; #4: V3LMM_460166; #5: V2LHS_255610; #6: V2LHS_74081 from Open Biosystems), Lats1 (for Human: TRCN0000001780; for Mouse: TRCN0000022941 from Open Biosystems), Lats2 (for Human: TRCN0000000884, for Mouse: TRCN0000022707 from Open Biosystems) were generated as previously described (Pylayeva *et al.*, 2007). Adenovirus containing GFP and wild type Merlin (recombinant adenovirus AdNF2) were gifts from J. Testa (Xiao *et al.*, 2005). Cells were incubated with infectious particles in the presence of 10 μg/ml hexadimethrine bromide (Sigma) overnight. After recovery in complete medium for 24 hours, Meso-33 cells and FC-1801 cells were placed under selection in 2 μg/ml puromycin or 500 μg/ml G418. Meso-33 cells were transfected with 50 pmol of ON-TARGETplus SMARTpool against DCAF1, YAP, TAZ, Lats1, Lats2, Sav1, or ON-TARGETplus siCONTROL. Where indicated, individual ON-TARGETplus si-RNAs (si-DCAF1 #1, J-021119-09, #2, J-021119-11, #3, J-021119-10, #4, J-021119-12 from Dharmacon) against DCAF1 were used. All si-RNAs were from Dharmacon. Efficiency of knock down was verified by immunoblotting. When indicated, cells were lysed in SDS-boiling buffer (10 mM Tris pH 7.5, 1% SDS, 50 mM NaF, 1 mM $NaVO_4$) and subjected to SDS-PAGE and immunoblotting.

DNA Microarray Analysis

Meso-33 cells were transfected with 50 pmol of ON-TARGETplus SMARTpool against DCAF1, YAP, TAZ or ON-TARGETplus si-CONTROL Non-targeting pool (Dharmacon). Each transfection was performed in triplicate. Cells were plated at subconfluent density in complete medium. Three days after transfection, cells were lysated in Trizol reagent and total RNA was extracted. cDNAs generated by retrotranscription were hybridized to *illumina HT-12 v4* array chips. Raw expression data were imported into Partek Genomics Suite. Expression data were normalized by quantile normalization to the median value of all samples. Principal Component Analysis (PCA) was used to verify the quality of data. Gene lists showing differential expression (>2 folds or <-2 folds, $p<0.05$) between si-DCAF1, or si-YAP/TAZ and non-targeting control were generated. A gene cluster was created based on differentially expressed genes between si-DCAF1 group and non-targeting control group identified above. Q-PCR was performed with Taqman Gene Expression Assay (Applied Biosystems). GSEA was performed using the web-based software provided by the Broad Institute (http://www.broadinstitute.org/gsea/index.jsp). The gene sets used for the analysis were generated from the above microarray analysis and the differential expression cutoff is <-2.5 fold. Affymetrix U133A gene expression data on human pleural mesotheliomas were from (Bott *et al.*, 2011) and are available at http://cbio.mskcc.org/Public/Ladanyi_lab_mesothelioma_datasets/.

Supplemental References

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