Supplemental Data

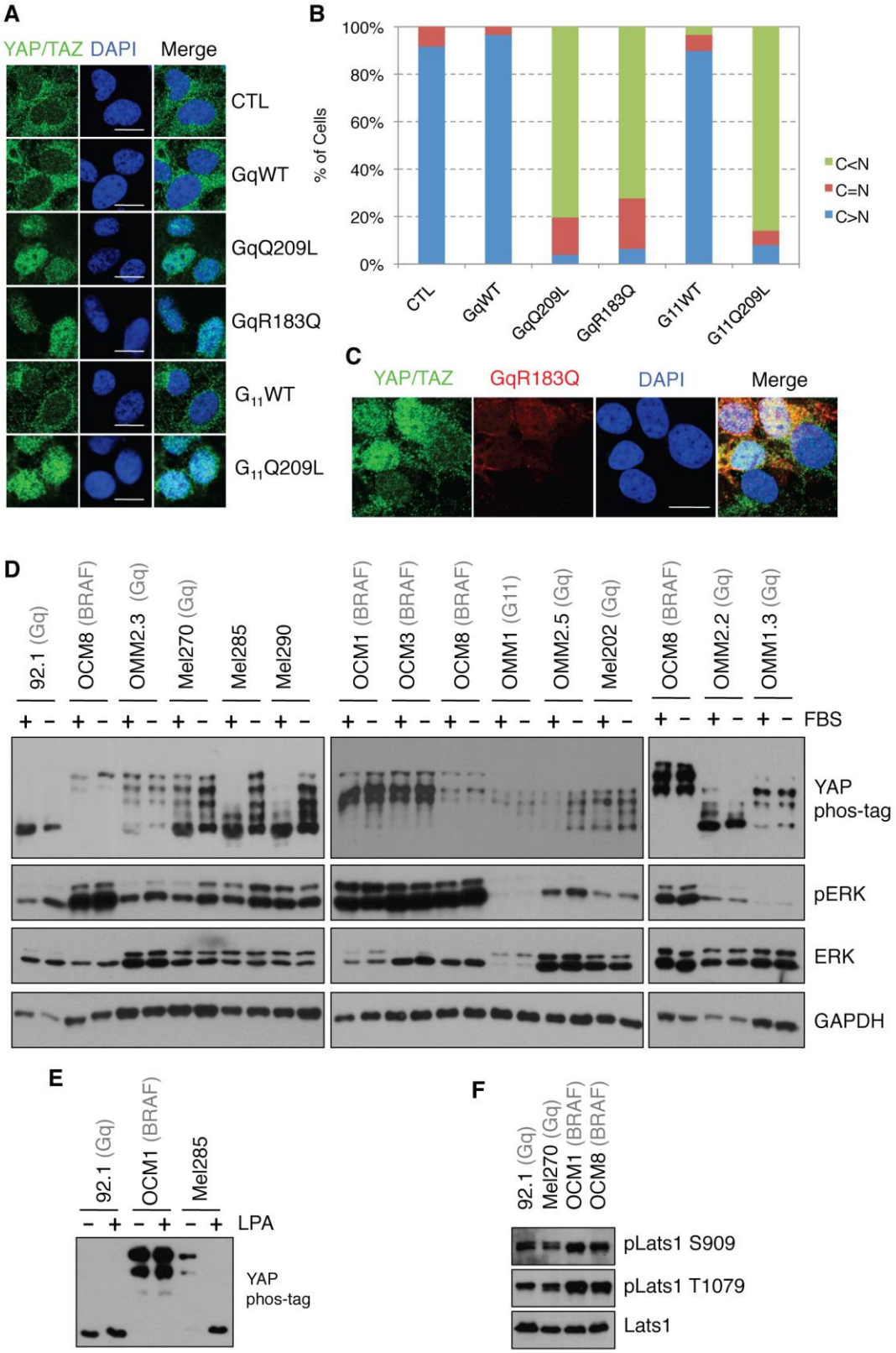

Figure S1. Related to Figure 1.

(A-C) Mutant Gq/11 induce YAP/TAZ nuclear localization. Different Gq/11 plasmids were transfected into HEK293A cells cultured on coverslips. Following 16 hr serum starvation, cells were fixed and subjected to immunostaining using a YAP/TAZ antibody (Green), and nuclear DNA was visualized by DAPI staining (blue) (A). YAP/TAZ localization of HEK293 cells transfected with various Gq or G11 mutants was determined by immunostanning. The percentage of cells with cytoplasmic (C>N), nuclear (C<N), or ubiquitous (C=N) YAP/TAZ localization was summarized in (B). In another experiment, over-expressed Gq^{R183Q} was stained using a Gq antibody (Red), and compared with YAP/TAZ staining (Green). Gq^{R183Q}-expressing cells showed nuclear localization of YAP/TAZ, whereas cells not transfected with Gq^{R183Q} showed more cytoplasmic localization of YAP/TAZ (C). Scale bars represent 5 μm.

(D) YAP and ERK phosphorylation status in UM cell lines. Different cell lines were cultured in the presence or absence of 10% FBS for 16 hr and harvested for immunoblotting with the indicated antibodies.

(E) Response of UM cells to LPA. UM cells were serum starved for 16 hr, and then treated with or without of 1 μM LPA for 1 hr.

(F) Lats1 phosphorylation status in UM cells. Lats1 was immunoprecipitated from different UM cells and subjected to immunoblotting using antibodies that recognize the phosphorylated activation loop (S909) or hydrophobic motif (T1079) of Lats1. Phosphorylation of S909 and T1079 positively correlate with Lats1 kinase activity. Therefore, Lats1 is less active in the Gq/11 mutant 92.1 and Mel270 cells than the BRAF mutant OCM-1 and OCM-8 cells.

Table S1. Related to Figure 2.

Sample ID	Gq/11 Mutation	YAP	YAP Score	YAP/TAZ
S10-6060	GNA11 (Q209L)	NH	2	NH
E11-216	WT	C	5	C
E10-459	GNA11 (Q209L)	N	1	N
E10-445	GNAQ (Q209P)	N	1	N
E10-368	WT	C	5	C
E09-219	GNAQ (Q209P)	N	1	N
E06-287	WT	CH	4	CH
E06-57	GNA11 (Q209P)	NH	2	NH
E06-35	GNA11 (Q209L)	N	1	N
11-283	WT	H	3	H
11-277	GNA11 (Q209L)	N	1	N
11-151	GNA11 (Q209L)	N	1	N
11-121	GNA11 (Q209L)	N	1	N
10-556	GNA11(Q209L)	N	1	N
10-549	GNAQ (Q209P)	H	3	H
10-419	WT	C	5	C
10-364	GNA11 (Q209H)	H	3	H
10-107	WT	NH	2	N
09-383	WT	NH	2	NH
09-361	WT	C	5	C
09-337	WT	C	5	C
09-203	WT	CH	4	CH
08-233	GNAQ (Q209P)	N	1	N

A summary of Gq/11 mutation and YAP localization in UM patient samples was tabulated. N: exclusively nuclear; NH: largely nuclear; H: heterogeneous; CH: largely cytoplasmic; C: exclusively cytoplasmic.

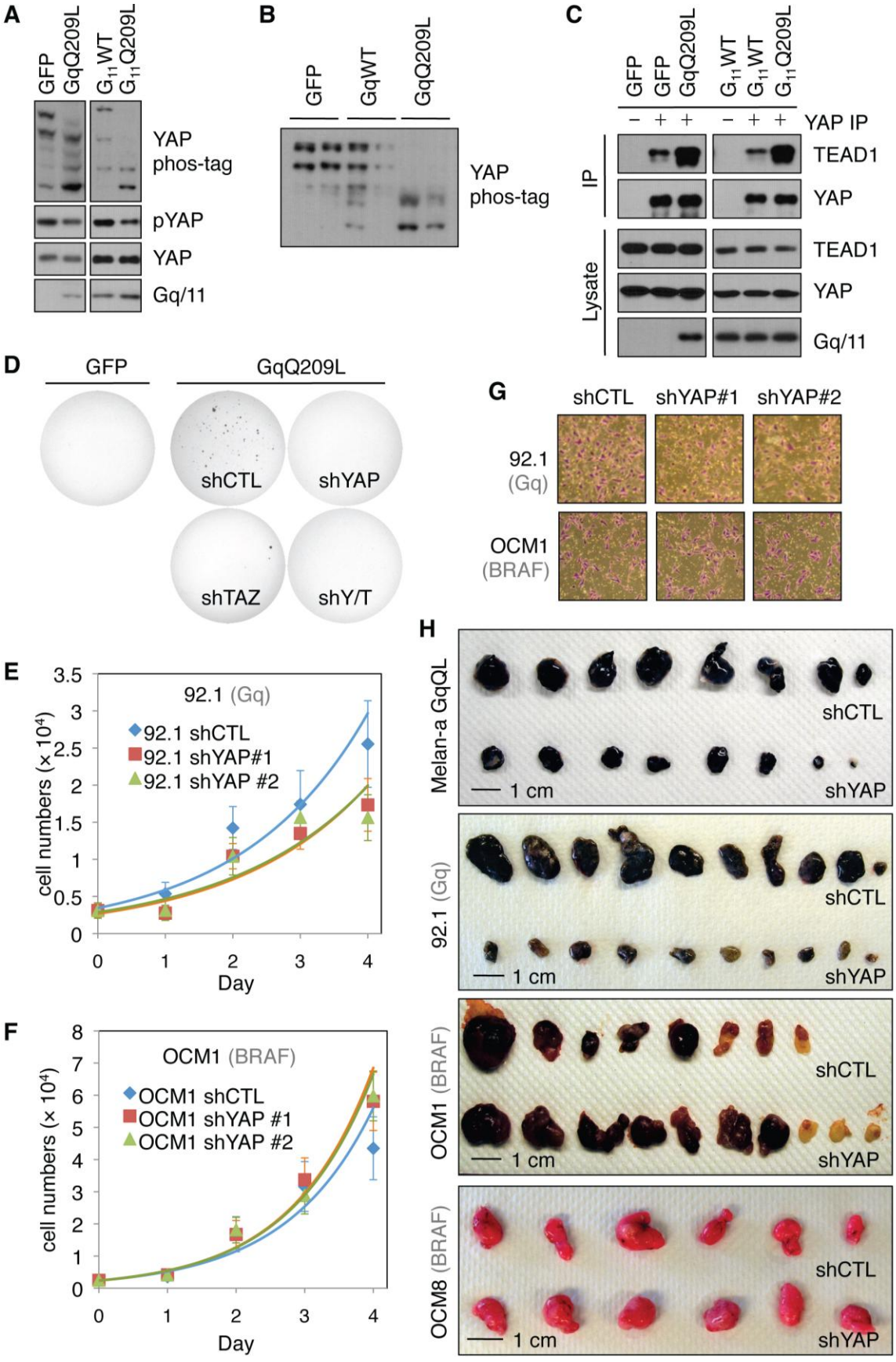
A
GFP
GqQ209L
G11WT
G11Q209L
YAP phos-tag
pYAP
YAP
Gq/11
B
GFP
GqWT
GqQ209L
YAP phos-tag
C
GFP
GFP
GqQ209L
G11WT
G11WT
G11Q209L
YAP IP
IP
TEAD1
YAP
Lysate
TEAD1
YAP
Gq/11
D
GFP
GqQ209L
shCTL
shYAP
shTAZ
shY/T
G
shCTL
shYAP#1
shYAP#2
92.1 (Gq)
OCM1 (BRAF)
E
92.1 (Gq)
92.1 shCTL
92.1 shYAP#1
92.1 shYAP #2
cell numbers (× 10⁴)
Day
F
OCM1 (BRAF)
OCM1 shCTL
OCM1 shYAP #1
OCM1 shYAP #2
cell numbers (× 10⁴)
Day
H
Melan-a GqQL
92.1 (Gq)
OCM1 (BRAF)
OCM8 (BRAF)
shCTL
shYAP
1 cm

Figure S2. Related to Figure 4.
(A and B) Mutant Gq/11 activate YAP in melan-a cells. Melan-a cell lines expressing GFP, wild type (WT) Gq/11, or Q209L Gq/11 were established, and YAP phosphorylation in these cell lines was analyzed.
(C) Expression of Gq/11 in melan-a cells enhances endogenous YAP-TEAD interaction. YAP was immunoprecipitated from melan-a cell lines (as in A), and TEAD1 co-immunoprecipitated was assessed by immunoblotting.
(D) YAP and/or TAZ are important for Gq^{Q209L}-induced anchorage independent growth. Melan-a cells with YAP and/or TAZ knockdown were cultured in soft agar, and colony formation was determined.
(E and F) Knockdown of YAP slightly reduces proliferation of 92.1 cells but not OCM1 cells. Error bars represent SD.
(G) Knockdown of YAP reduces cell migration of 92.1 but not OCM1 cells. Results of trans-well cell migration assays were photographed. (H) Tumors were harvested from xenograft experiments using melan-a (Gq^{Q209L}) cells, 92.1, OCM1, and OCM8 cells.

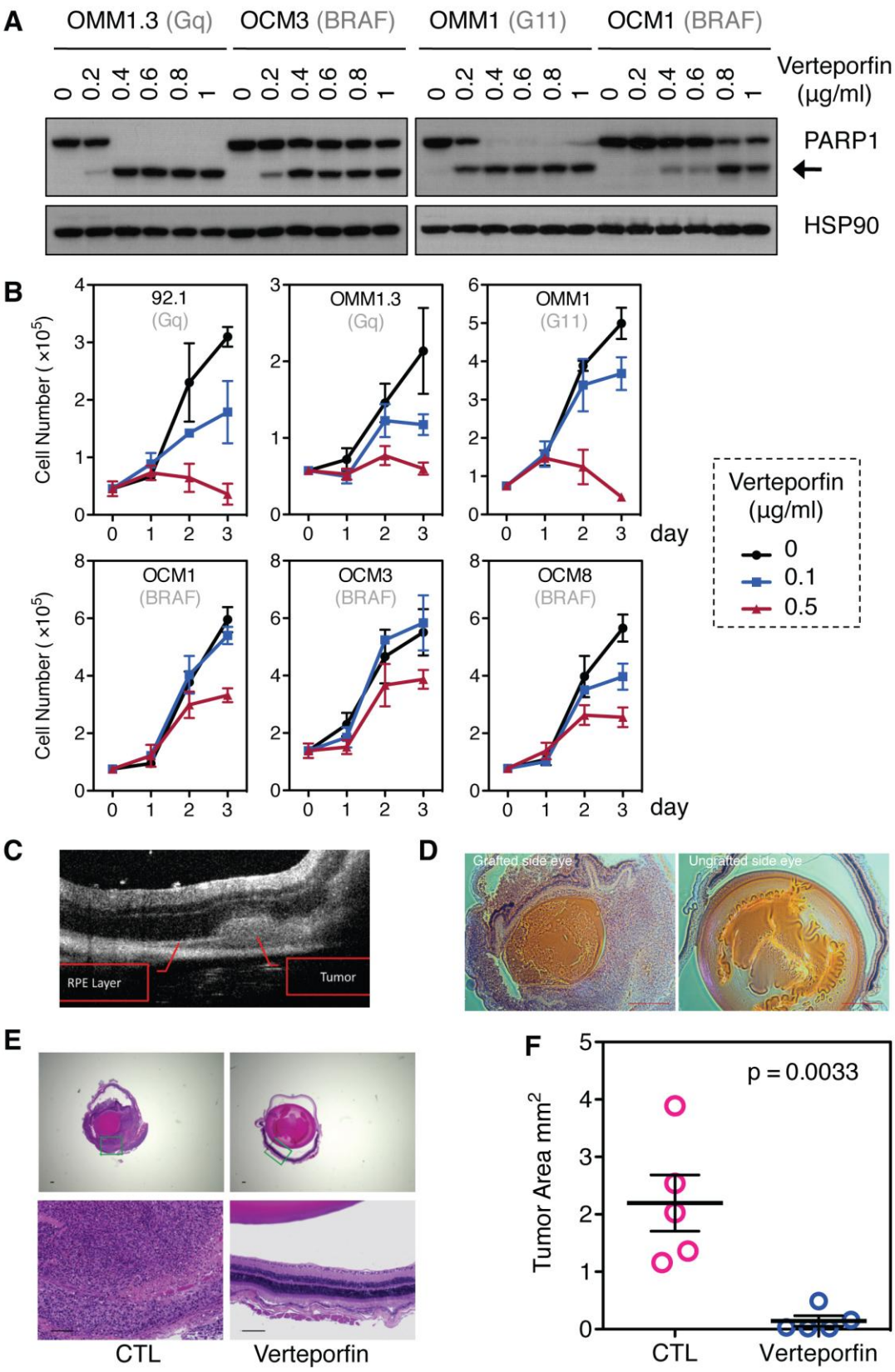
A
OMM1.3 (Gq)
OCM3 (BRAF)
OMM1 (G11)
OCM1 (BRAF)
0 0.2 0.4 0.6 0.8 1
Verteporfin (μg/ml)
PARP1
HSP90
B
92.1 (Gq)
OMM1.3 (Gq)
OMM1 (G11)
OCM1 (BRAF)
OCM3 (BRAF)
OCM8 (BRAF)
Cell Number (×10^5)
day
Verteporfin (μg/ml)
0
0.1
0.5
C
RPE Layer
Tumor
D
Grafted side eye
Ungrafted side eye
E
CTL
Verteporfin
F
p = 0.0033
Tumor Area mm^2
CTL
Verteporfin

Figure S3. Related to Figure 5.
(A) Gq/11 mutant cells or BRAF mutant cells were treated with different doses of verteporfin for 48 hr, and cell lysates were assessed for PARP1 cleavage (the black arrow indicate the position of cleaved PARP1).
(B) Gq/11 mutant cells or BRAF mutant cells were treated with different doses of verteporfin, cell proliferation were monitored by counting cell numbers everyday. Error bars represent SD.
(C, D) An orthotopic UM mouse model. An optical coherent tomography (OCT) image of a tumor formed by 92.1 cells (C). Following injection of UM cells into the right eye of SCID mice, tumor formation was monitored by OCT every week. When mice were euthanized, sections were prepared for histological analysis. Sections from an eye with tumor formation (left panel) and a control eye (right) were shown (D). Scale bars represent 1 mm.
(E, F) Verteporfin treatment effectively blocked tumor growth of Mel270 (Gq mutant) cells. Student t test (two-tailed, 95% confidence intervals) was used for statistical analysis, and error bars represent SD. Scale bars represent 100 μm.

Supplemental Experimental Procedures

Chemicals
The following chemicals were used in this study: LPA (Sigma-Aldrich), doxycycline (Sigma-Aldrich), verteporfin (US Pharmacopeial Convention, for in vitro use), Puromycine (Invivogen), hygromycin (Invitrogen), forskolin (Tocris), IBMX (Tocris), and U0126 (Cell Signaling Technology).

Antibodies

Antibodies used in this study: pYAP (S127), YAP (for WB), TAZ, pERK, ERK1/2, pLats1 (S909), pLats1 (T1079), Lats1, and PARP1 were from Cell Signaling; YAP (H-125, for IF), YAP/TAZ (63.7, for IF and WB), and Gq/11 were from Santa Cruz; TEAD1 and HSP90 were from BD biosciences; Flag antibody was from Sigma-Aldrich; YAP (for IP) was from Novus Biologicals. IF, IP, WB denote immunofluorescence, immunoprecipitation, and western blotting, respectively.

Transfection
Cells were transfected with plasmid DNA using PolyJet™ DNA *In Vitro* Transfection Reagent (Signagen Laboratories) according to manufacturer's instruction. Gq/11 and YAP expression plasmids have been described previously (Yu et al., 2012), and Gq^{R183Q} were made by quick-change mutagenesis.

Virus and stable cell lines

Lentivirus was produced using psPAX2 and pMD2.g as packaging vector in HEK293T cells. Flag-G11 (WT and Q209L) ORFs were subcloned into pLVX-puro lentiviral vector. ShRNA plasmids were from Sigma-Aldrich, the ID for human *GNAQ* sequence #1 and #2 are TRCN0000036763 and TRCN0000036759 respectively. For inducible knockdown of YAP, pTRIPz plasmids containing the same targeting sequences used previously (Zhao et al., 2008) were constructed. Retrovirus was produced in HEK293P cells. Gq^{Q209L} was cloned into the pQCXIH vector and used for retrovirus production. Medium containing virus was harvested and concentrated by centrifugation and stored at -80^{o}C. Cells were infected with virus in the presence of polybrene (10 μg/ml, Sigma-Aldrich), and infected cells were selected using puromycin (Invivogen) or hygromycin (Invotrogen). GFP, Gq, and Gq^{Q209L} expressing melan-a cells were described previously (Van Raamsdonk et al., 2009).

Immunoprecipitation

Cells were lysed using mild lysis buffer (50 mM HEPES pH 7.5, 150 mM NaCl, 1 mM EDTA, 1% NP-40, 10 mM pyrophosphate, 10 mM glycerophosphate, 50 mM NaF, 1.5 mM Na_3VO_4, protease inhibitor cocktail [EDTA-free, Roche], 1 mM PMSF). Cell lysates were centrifuged at top speed in a microfuge for 10 min at 4^{o}C, and supernatants were used for immunoprecipitation. YAP antibody (Novus Biologicals) was mixed with the supernatant for 1 hr, and protein A–agarose beads were added in for another hr. Beads were washed four times with lysis buffer, and proteins were eluted with SDS-PAGE sample buffer and used for immunoblotting.

Immunoblotting

Immunoblotting was performed using a standard protocol. The phos-tag reagents were purchased from Wako Chemicals, and gels containing phos-tag were prepared according to the manufacture's instructions. YAP proteins can be separated into multiple bands in the presence of phos-tag depending on differential phosphorylation levels, with phosphorylated proteins migrating more slowly.

Immunofluorescence staining

HEK293A or melan-a cells were seeded on coverslips. After serum starvation, cells were fixed with 4% paraformaldehyde-phosphate buffered saline (PBS) for 15 min and permeabilized with 0.1% Triton X-100 in Tris buffered saline (TBST). After blocking in 5% goat serum in TBST for 30 min, cells were incubated with primary antibodies overnight at 4^{o}C. After three washes with TBST, cells were incubated with Alexa Fluor 488- or 555-conjugated secondary antibodies (Invitrogen, 1:1000 dilution) for 1 hr at room temperature. Slides were then washed three times and mounted. Immunofluorescence was detected using Olympus confocal microscopy. Primary antibodies used were: YAP (H-125) and YAP/TAZ (63.7) from Santa Cruz, Gq/11 antibody from Santa Cruz. For paraffin-embedded tissues from UM patients, 5 μm sections were subjected to immunostaining. Following deparaffinization and hydration, slides were heated in

sub-boiling buffer (10 mM sodium citrate, pH 6.0) for 30 min for antigen retrieval and used for staining.

Cell proliferation Assay

OCM1 or 92.1 cells (expressing control shRNA or YAP shRNA) were cultured in the presence of Dox for 3 days and then 0.3×10^4 cells were seeded into each well (24-well plate) in the presence of Dox, and cell numbers were counted every day using a cell counter (Bio-Rad).

Colony formation assay

Each 6-well plate (ultra low attachment) was coated with 2 ml of bottom agar (RPMI containing 10% FBS and 0.7% Difco agar noble). Melan-a cell lines were trypsinized, and 1×10^4 cells were suspended in 1.5 ml of top agar (RPMI containing 10% FBS and 0.4% Difco agar noble) into each well. Cells were incubated for 4 weeks, with fresh medium added every three days. Colonies were stained using 0.05% crystal violet.

Genomic DNA sequencing

DNA was extracted from paraffin-embedded uveal melanoma biopsies with QIAamp DNA FFPE Tissue Kit (Qiagen) according to the manufacturer's instructions. Direct (Sanger) sequencing analysis was completed for both *GNAQ* and *GNA11*. The exon 4 and exon 5 of *GNAQ* and *GNA11* were amplified by PCR and sequenced on Genetic Analyzer 3130 (Applied Biosystems). The primers used to amplify the exons in *GNAQ* and *GNA11* are shown below

Gene	Sequence (5'-3')
GNAQ-Exon4-outer-F	GACTCCTCTACCACTTTCTGAT
GNAQ-Exon4-outer-R	GAAGCCTACACATGATTCCAGT
GNAQ-Exon4-inner-F	GTCCTTCCCTTTCCGTAGACAGCT
GNAQ-Exon5-outer-F	GATCATCGTCATTCAAGAGAAT
GNAQ-Exon5-outer-R	GACAGAAGAGCTTACCACAGGATT
GNAQ-Exon5-inner-F	CCTAAGTTTGTAAGTAGTGCTAT
GNA11-Exon4-outer-F	GGTCCACCCCCTCCTGGTGGCT
GNA11-Exon4-outer-R	GATATGAGGTCTGGCTATGTT
GNA11-Exon4-inner-R	GTTGCCCAGGGTGGTCTCAAACT
GNA11-Exon5-outer-F	GCCGTCCTGGGATTGCAGATT
GNA11-Exon5-outer-R	GAGTTCTGGAACCAGGGGTAGGT
GNA11-Exon5-inner-R	GCTTGGCAGGTGGGGAAGGC

Cell migration Assay

Cell migration assays were performed using BD Falcon™ Cell culture inserts for 24-well plates with 8.0 μm pores filter, and the filter was pre-coated with 20 μg/ml Fibronectin. OCM1 or 92.1 cells (expressing control shRNA or YAP shRNA)

were cultured in the presence of Dox for 3 days and then 2×10^5 cells were seeded into each upper chamber of the insert in serum-free media with Dox, and lower chamber was filled with complete medium with Dox. After 24 h, cells were fixed using 4% paraformaldehyde and stained using 0.05% crystal violet. Cells in the upper chamber were carefully removed, and cells that migrated through the filter were assessed by photography. For quantification, filters were removed from the insert and cells were stained with DAPI, and nuclei were counted under a fluorescence microscope.

Animal work

All animal procedures were carried out according to protocols approved by the Institutional Animal Care and Use Committee of University of California, San Diego (UCSD). For subcutaneous xenograft experiments, five or three (for OCM8) 12-week-old male nude mice (UCSD animal facility) were used for each group. Melan-a (1×10^6), 92.1 (2×10^6), OCM1 (2×10^6), or OCM8 (2×10^6) with manipulations of YAP or Gq expression were grafted subcutaneously into both flanks mice, and tumor growth was monitored three times a week. Growth factor reduced Matrigel matrix (BD biosciences, 50% of the injection volume) was used for 92.1, OCM1, and OCM8 cells. Mice were euthanized with CO2 after 6 weeks (for melan-a, OCM1, and OCM8) or 8 weeks (for 92.1) of cell injection. One mouse injected with OCM1 control cells died one day after injection. For the orthotopic UM mouse model, five 4-week-old male SCID mice (Jackson Laboratories) were used for each group. Mice were anesthetized with an intraperitoneal injection of a mixture of ketamine and xylazine (Sigma-Aldrich). UM Cells (5×10^4), mixed with nanoparticles containing 40 µg of verteporfin (QLT ophthalmics), were injected into the suprachoroidal space in the right eye using a 33 gauge needle. Veterpofin was administered by intraperitoneal route at a dose of 100 mg/kg every other day over a period of 14 days. Tumor formation was monitored every week by OCT. Mice were euthanized with CO_2 after 6 weeks because of the development of very large masses in the eyes of several mice in the no drug treatment group. Eyes harvested were analyzed microscopically for the presence of tumors. Horizontal sections (10µm) were cut and stained with H&E. Cross-sectional tumor area was measured using a BZ-9000 Biorevo camera and software (Keyence corp. of America).

Preparation of drug loaded nanoparticles

Verteporfin-loaded nanoparticles were prepared through a modified nanoprecipitation process. Briefly, the drug molecules were mixed with polycaprolactone (PCL) polymer in acetonitrile with a polymer concentration of 2.5 mg/mL. Lecithin and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-carboxy(polyethylene glycol)2000 (DSPE-PEG-COOH) (7.5:2.5, molar ratio) were dissolved in a 4% ethanol aqueous solution at 20% of the PCL polymer weight and heated to 65°C. The polymer/drug solution was then added into the preheated lipid aqueous solution drop-wise (1 ml/min) under gentle stirring followed by vortexing for 3 min. The nanoparticles were allowed to self-assemble for 2 hr with continuous stirring while the organic solvent was allowed to

evaporate. The remaining organic solvent and free molecules were removed by washing the nanoparticle solution three times using an Amicon Ultra-4 centrifugal filter (Millipore, Billerica, MA) with a molecular weight cut-off of 10 kDa and then re-suspended in PBS buffer to obtain a final desired concentration. The size, size distribution, surface charge of the resulting particles were characterized by dynamic light scattering. The drug loading yield was quantified by high performance liquid chromatography.