Supplementary Methods

Cell lines

Established human extrahepatic cholangiocarcinoma cell lines EGI-1 and WITT were used for the experiments. The EGI-1 cell line was cultured in Roswell Park Memorial Institute 1640 (RPMI1640; Gibco) supplemented with 10% fetal bovine serum and 0.1% primocin (Invitrogen); WITT was cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 10% FBS and 0.1% primocin (Invitrogen). Both cell lines were grown at 37°C in a 5% CO2 incubator.

Plasmids and transfection

To examine the effects of suppression of SULF2 expression, HuCCT1 cells that constitutively express high SULF2 were stably transfected with a lentivirus expressing shRNA targeting SULF2 mRNA. The target sequence, CATCAATGAGACTCACAATTT, was cloned into the pLKO.1 vector that harbors the puromycin-resistance gene. Lentiviral particles were then produced by transfecting the plasmids into HEK 293T cells. HuCCT1 cells were infected with lentiviral particles and treated with puromycin to select and maintain the cells. Lentivirus expressing a scrambled shRNA sequence was used as a control. By contrast, to examine the effects of forced expression of SULF2, SULF2-negative CCLP1 cells were transfected with a pcDNA3.1 plasmid (Invitrogen, Carlsbad, CA) harboring full-length SULF2 complementary DNA (cDNA). These cells were treated with 500 μg/mL of geneticin (Invitrogen) for 14-21 days to select geneticin-resistant clones. The isolated clones were tested for SULF2 expression and maintained with 200 μg/mL of geneticin. CCLP1 cells transfected with empty pcDNA3.1 were used as controls. Lipofectamine LTX with Plus Reagent (15338100; Invitrogen) and Fugene 6

Transfection Reagent (11814443001; Roche Diagnostics, Mannheim, Germany) were used for transfecting HEK 293T and CCLP1 cells, respectively.

Western immunoblotting

Tumor xenografts or whole cell pellets were homogenized in RIPA buffer (#sc-24948, Dallas, TX) according to the manufacturer's manual. Protein extracts were quantified using Protein Assay reagent from Bio-Rad (#5000114, Hercules, CA). Equal amounts of protein (15ug) were loaded onto an SDS-PAGE gel, resolved and transferred to nitrocellulose membranes from BioRad (#1620115, Hercules, CA). Specific primary antibodies (Supplementary Table 1) and horseradish peroxidase-conjugated secondary antibodies (Cell Signaling #7074, #7076 or Santa Cruz #sc-2354) were used for chemiluminescent detection (GE Healthcare #RPN2106 or ThermoFisher scientific #34095). Band intensity was measured using ImageJ software.

Immunofluorescence and confocal microscopy

Tumor xenograft sections or cells were fixed with 4% paraformaldehyde and then permeabilized in 0.1% Triton-X-100 in phosphate-buffered saline (PBS) for 5 minutes. After washing, the samples were blocked with 5% normal goat serum in PBS at room temperature for 1 hour. The blocking buffer was washed out and the samples were incubated with primary antibodies (Supplementary Table 1) diluted in 5% BSA overnight at 4°C. The samples were then washed and incubated with appropriate secondary antibodies diluted 1:500 in 5% BSA (Alexa Fluor 488 goat anti-mouse [A11032] and Alexa Fluor 568 goat anti-rabbit [A11034]) (Invitrogen) at room temperature for 1 hour, protected from light. The prepared slides were examined by confocal microscopy (Zeiss LSM-710).

RNA extraction, reverse transcription PCR and quantitative real time PCR

Total RNA was isolated by RNeasy Plus mini kit from Qiagen (#74134, Germantown, MD) and reverse-transcribed into complementary DNA using High capacity Reverse Transcription Kit from Applied Biosystems by Thermofisher Scientific (#4368814, Waltham, MA). Quantitative real time PCR (qRT-PCR) was performed using gene specific primers (Supplementary Table 2) and Light Cycler 480 SYBR Green I Master Mix from Roche Diagnostics (#04707516001, Indianapolis, IN). All datasets are expressed as fold change relative to control as $\Delta\Delta$Ct utilizing β-actin as the housekeeping gene.

Histology and immunohistochemistry

H&E staining was performed by the Mayo Clinic Histology Core. For immunohistochemistry (IHC), formalin-fixed paraffin embedded tissue sections were deparaffinized, hydrated and stained with specific antibodies (Supplementary Table 1). Bound antibody was detected using the Mouse and Rabbit Specific HRP/DAB (ABC) Detection IHC kit (Abcam #ab64264, Cambridge, MA). The tissue slices were counterstained with hematoxylin. Slides were quantified by measuring the percentage of Ki-67 or Cleaved Caspase 3 positive cells in total cells per high power field for at least 10 fields per sample.

TUNEL Assay

Tumor tissues were embedded in Tissue-Tek O.C.T. and frozen at -80ºC to be used to make frozen section tissue slides. In Situ Cell Death Detection Kit Fluorescein (Roche) was used to detect DNA strand breaks by the TUNEL assay. Tumor apoptosis was

quantified by measuring the percentage of TUNEL positive nuclei in total nuclei per high power field for at least 10 fields per sample.

Xenograft model

The care and use of mice for experimental purposes were carried out in accordance with the requirements set out by Mayo Clinic IACUC committee. Female athymic Nude mice, NU/J (002019) ages 6-8 months from Jackson Laboratory were used to develop the mouse xenografts. HuCCT1 cells were cultured on a $75cm^3$ flask and then injected aseptically into left flank of each mouse using a syringe. For each mouse $1x10^6$ cells re-suspended in 200 uL matrigel (Corning #354230, Tewksbury, MA) were injected. Tumors were measured weekly using calipers with the formula $V=0.5*Length*Width^2$ to determine the size of the tumor. Mice were then split randomly into two treatment groups using the randomization tool on the Studylog software: Group 1 mice were treated with the anti-human SULF2 antibody 5D5 at a concentration of 40 mg/kg diluted in 0.2 ml PBS; Group 2 mice treated with mouse IgG antibody at a concentration of 40mg/kg diluted in 0.2 ml PBS served as the control group. Antibody treatments were administered intraperitoneally 3 times a week for 5 weeks. Mouse weight and tumor size were assessed at each treatment time. At the end of the study, tumor weight was measured and the tumor xenografts were saved for further analysis.

Supplementary Figure legends

Supplementary Figure 1: *Sulf2* mRNA relative expression in HuCCT1 scrRNA and HuCCT1 shSULF2 cells (A), or CCLP1 Vector and CCLP1 SULF2 cells (B).

Supplementary Figure 2: Representative images of apoptosis assays for HuCCT1 cells without cisplatin treatment. (A) HuCCT1 scrRNA or HuCCT1 shSULF2 cells were stained using Fluorochrome-labeled Annexin V (green), Propidium Iodide (PI, red), and Hoechst 33342 (blue) (B) Densitometry of western blots in Figure 2D.

Supplementary Figure 3: Representative images of apoptosis assays for HuCCT1 cells with cisplatin treatment at concentrations of 1uM, 5uM. HuCCT1 scrRNA or HuCCT1 shSULF2 cells were treated with cisplatin at concentrations of 1uM (A), 5uM (B) for 24h.

Supplementary Figure 4: Representative images of apoptosis assays for HuCCT1 cells with cisplatin treatment at concentration of 10uM. HuCCT1 scrRNA or HuCCT1 shSULF2 cells were treated with cisplatin at concentrations of 10uM for 24h (A) or 48h (B).

Supplementary Figure 5: Densitometry of western blots in Figure 4A, B (A) and IF images in Figure 4D (B).

Supplementary Figure 6: (A) K-M curves showed mouse survival as indicated by the percentage of mice with a tumor volume less than 500 mm^3 was significantly higher in the 5D5 group than in the IgG group (n=10). (B) Body weights of the 5D5 group and the IgG group showed no significant differences.

Supplementary Figure 7: (A) In the Mayo Clinic RNA sequence dataset, SULF2 gene expression in extrahepatic CCA was compared to adjacent normal tissue. Gene expression is reported in units of RPKM (B) Western immunoblotting shows that the extrahepatic CCA cell line WITT expresses a higher level of SULF2 protein compared to normal human cholangiocytes (NHC). In contrast, the extrahepatic CCA cell line EGI-1 expresses a lower level of SULF2 protein (C) Forced expression of SULF2 in EGI-1 cells increased the levels of phospho-YAP^{Y357} and Cyclin D1.

Supplementary Figure 8: (A) Western blotting of the PDX protein extracts showed that compared with NHC, CCA PDX showed no difference in the level of VEGFR1, encoded by the FLT1 gene, a non-significant 40% decrease in EGFR protein level, and a significant 70% decrease in heparanase protein level. (B) Western blotting of HuCCT1 xenograft protein extracts showed that compared to IgG treatment, 5D5 treatment significantly reduced the levels of VEGFR1, EGFR, and heparanase.

Supplementary Table 1. Antibodies used for western blotting, immunofluorescence and immunohistochemistry.

Antibody	Company	Reference	Use
SULF2	BIO-RAD	MCA5692GA	Western blot 1:1000
GAPDH	Invitrogen	#AM4300	Western blot 1:10000
Ki-67	Novus Biologicals	NB500-170	Western blot 1:500, Immunohistochemistry 1:100
Caspase 9	Santa Cruz Biotechnology	sc-17784	Western blot 1:1000
Caspase 8	Santa Cruz Biotechnology	sc-7890	Western blot 1:500
Caspase 3	Santa Cruz Biotechnology	sc-1225	Western blot 1:200
p-PDGFRβ	Cell Signaling	#4549	Western blot 1:500
PDGFRβ	Cell Signaling	#3169	Western blot 1:500
p-YAP^{Y357}	Abcam	ab62751	Western blot 1:2000, Immunofluorescence 1:200
p-YAP^{S127}	Cell Signaling	#4911	Western blot 1:1000
YAP	Santa Cruz Biotechnology	sc-101199	Western blot 1:500, Immunofluorescence 1:50

p-ERK1/2	Cell Signaling	#4370	Western blot 1:1000
ERK1/2	Cell Signaling	#9102	Western blot 1:1000
Cyclin D1	Santa Cruz Biotechnology	sc-20044	Western blot 1:1000
Cleaved Caspase 3	Cell Signaling	#9661	Immunohistochemistry 1:200

Supplementary Table 2. Human qPCR Primers

Gene	Forward primer	Reverse primer
β-actin	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT
Sulf2	TCGACCACGAGATTGAAACC	CTGGGTGTGGTAGCTGATTT
Ctgf	CAGTGTCTGACTTCGACAACGC	CCATCGGCGTGTTTGGAGTA
Mcl1	GAGGGCGACTTTTGGCTAC	GTACCCGTCCAGCTCCTCTT
Pdgfb	CTG GCA TGC AAG TGT GAG AC	CGA ATG GTC ACC CGA GTT T
Cyr61	GAG TGG GTC TGT GAC GAG GAT	GGT TGT ATA GGA TGC GAGGCT

Supplementary Table 3. Clinical features of South Korea and Mayo Clinic CCA Cohorts

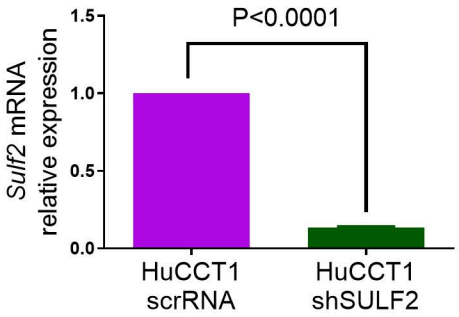
	South Korea Cohort (N=29)	Mayo Clinic Cohort (N=48)
Age, mean (SD)	65.1 (8.5)	64.1 (11.7)
Gender (female/male)	6 / 23	24 / 24
Anatomic site, no. (%)		
Intrahepatic	29 (100%)	37 (77.1%)
Perihilar	0	10 (20.8%)
Distal	0	1 (2.1%)
AJCC stage, no. (%)		
I	15 (51.7%)	12 (25%)
II	5 (17.3%)	11 (22.9%)
III	1 (3.4%)	6 (12.5%)
IV	8 (27.6%)	19 (39.6%)
Comorbidities, no. (%)		
Hepatitis B or C	4 (13.8%)	5 (10.4%)
Cholangitis	7 (24.1%)	3 (6.3%)
Parasite infection	3 (10.3%)	1 (2.1%)
Sample Use	RNA-seq	RNA-seq

Supplementary Table 4. Clinical features of PDXs

	PAX165	LIV27	LIV31	LIV61	LIV63
Age	67	67	55	63	61
Gender	Male	Female	Female	Male	Male
Anatomic site	Intrahepatic CCA	Intrahepatic CCA	Intrahepatic CCA	Intrahepatic CCA	Intrahepatic CCA
AJCC stage	I	III	I	II	II
Comorbidities	Mild steatohepatitis	PSC Cirrhosis Ulcerative colitis	Uterine leiomyomata	Chronic hepatitis B	Obstructive jaundice
Sample Use	Western blotting	Western blotting	Western blotting	Western blotting	Western blotting

Supplementary Figure. 1

A



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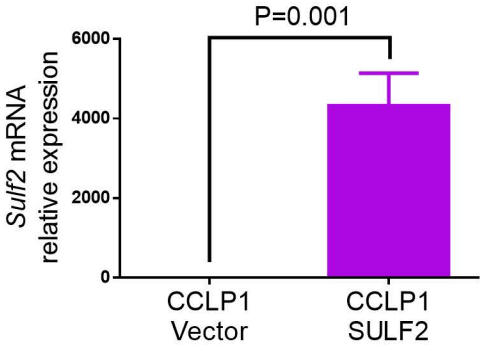
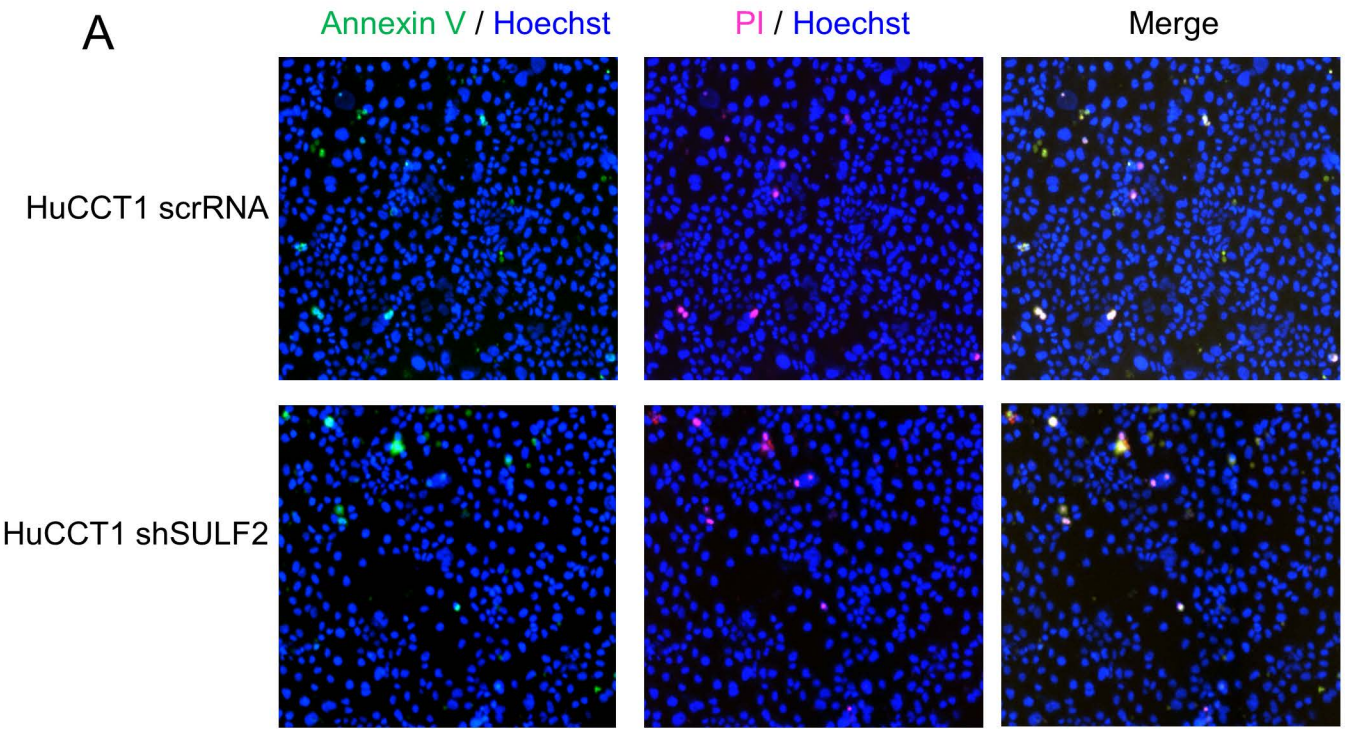
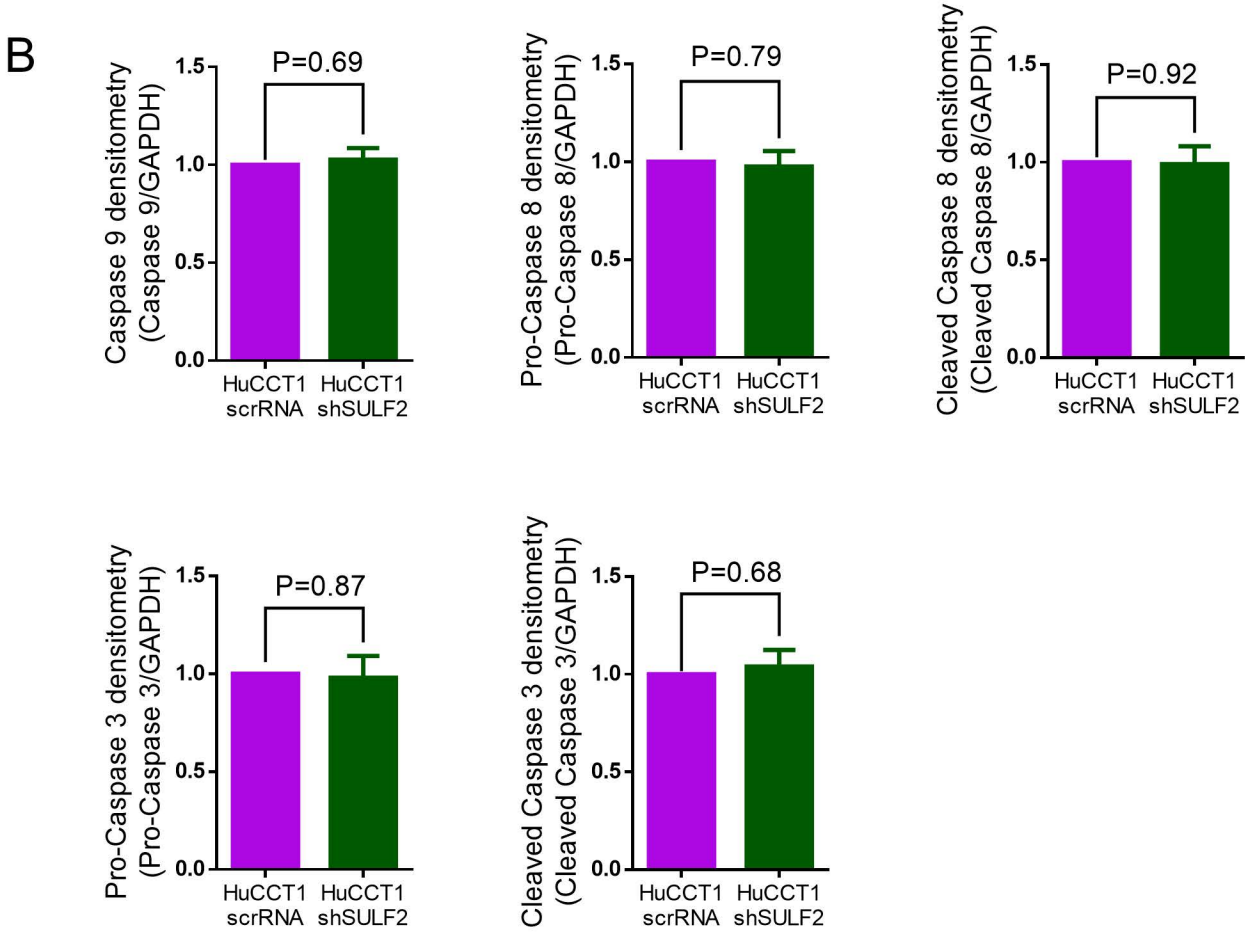
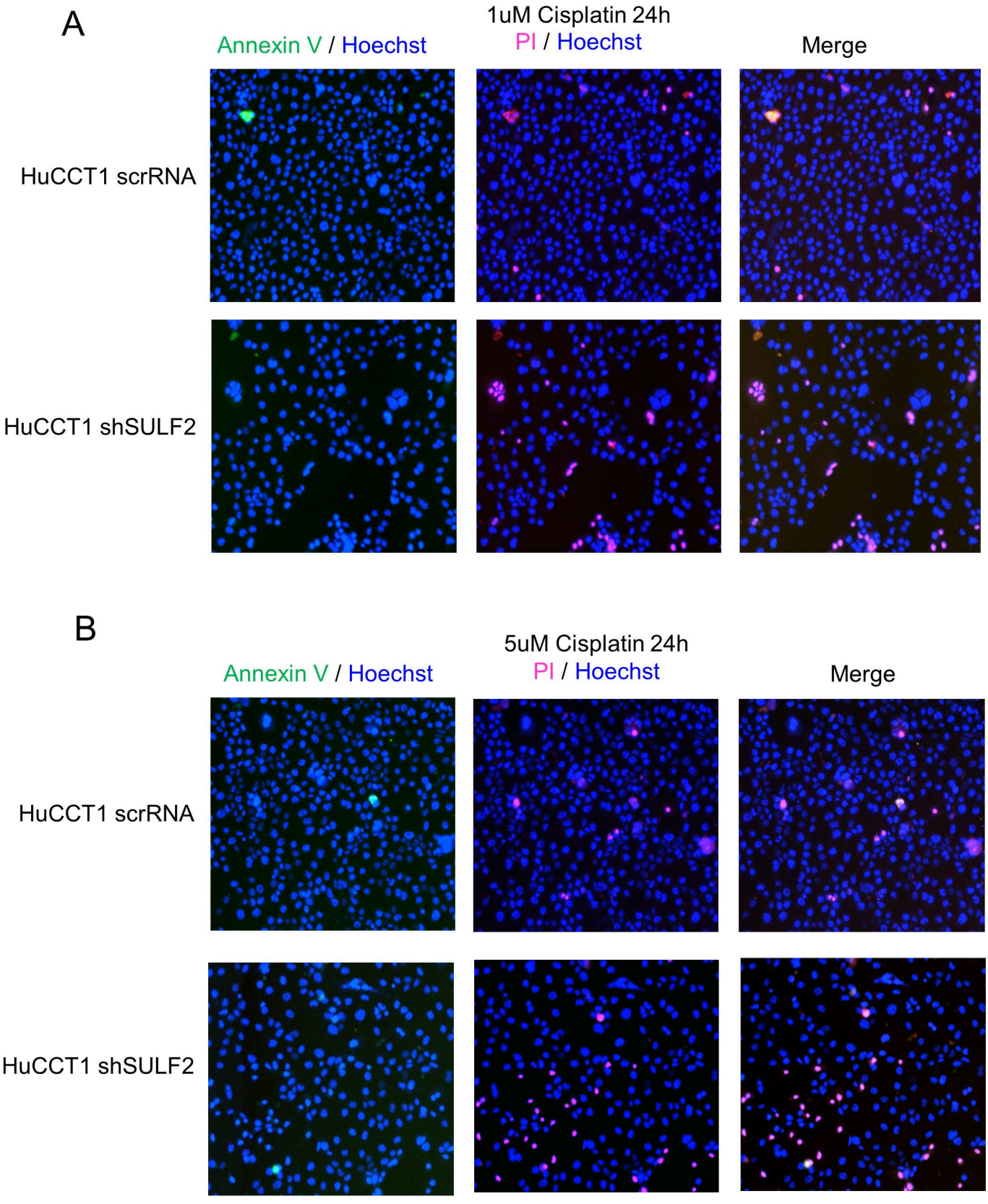


Supplementary Figure. 2





Supplementary Figure. 3



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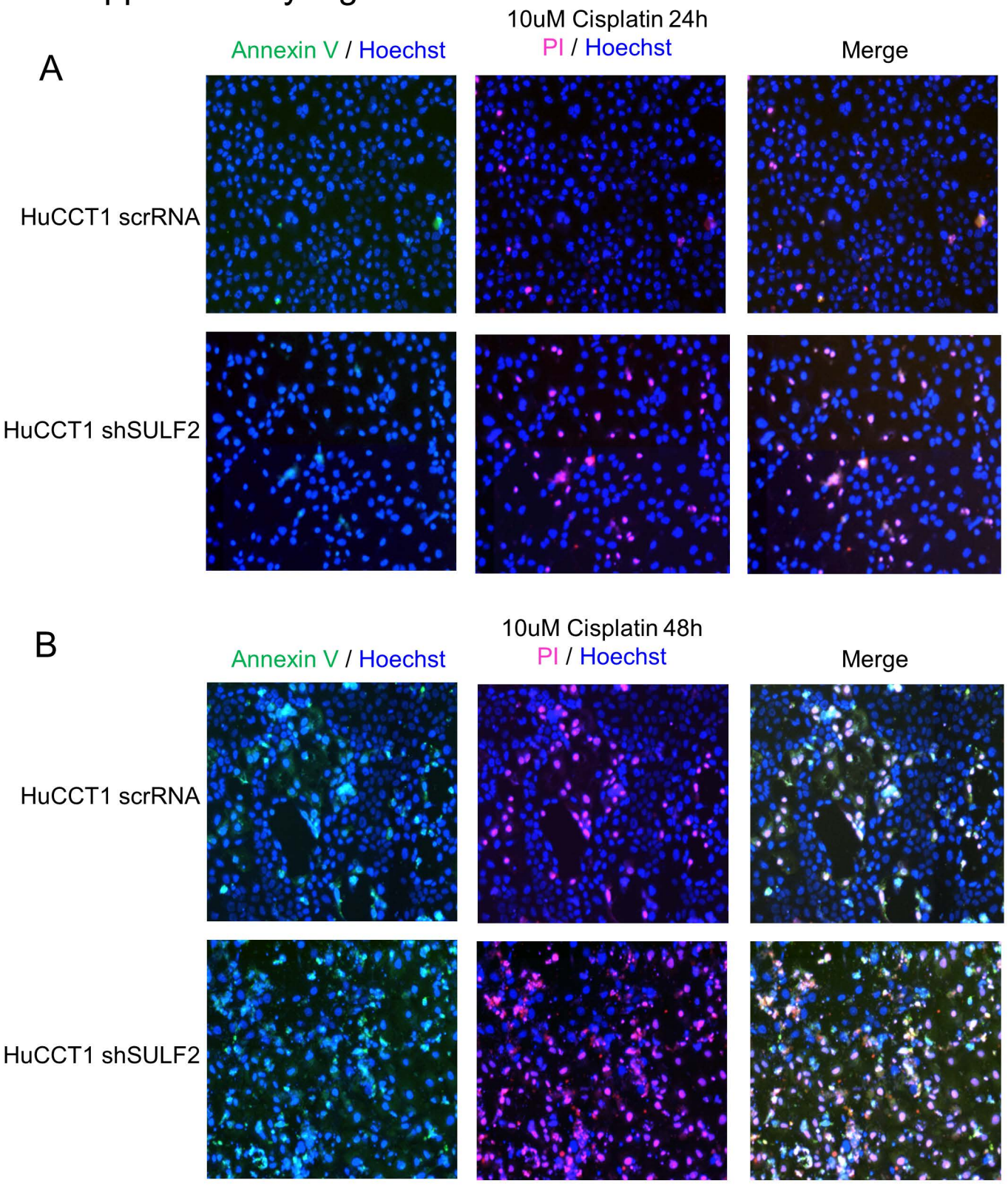

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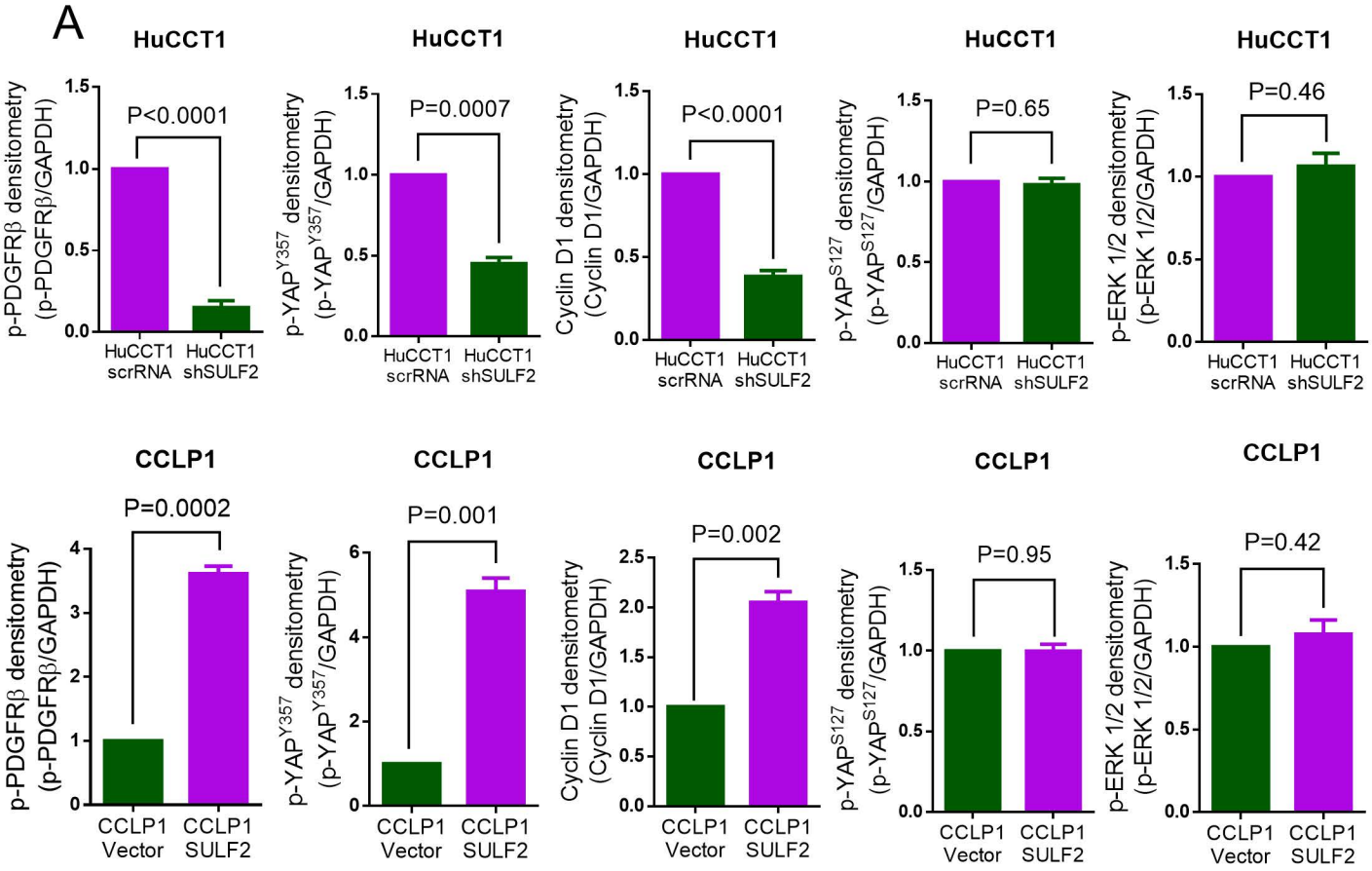

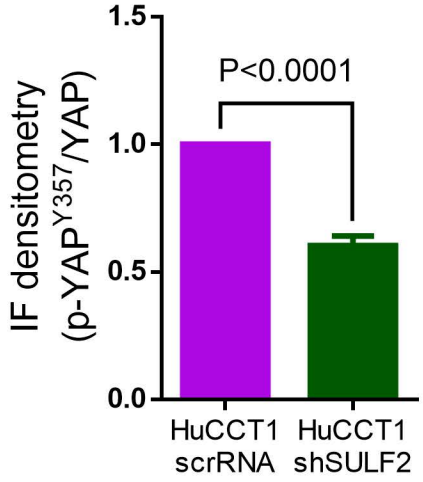

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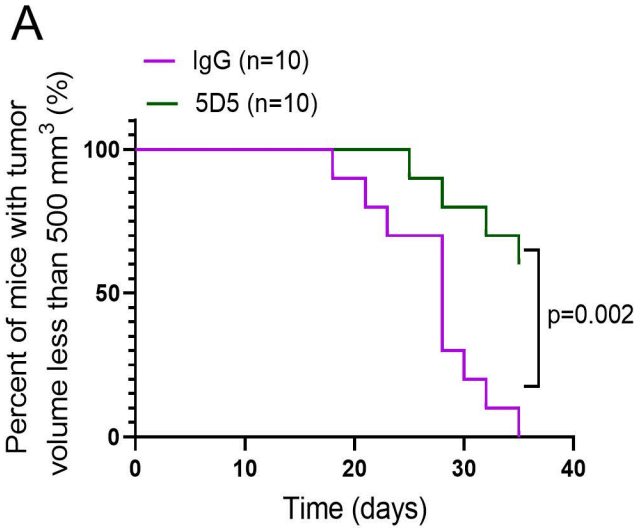
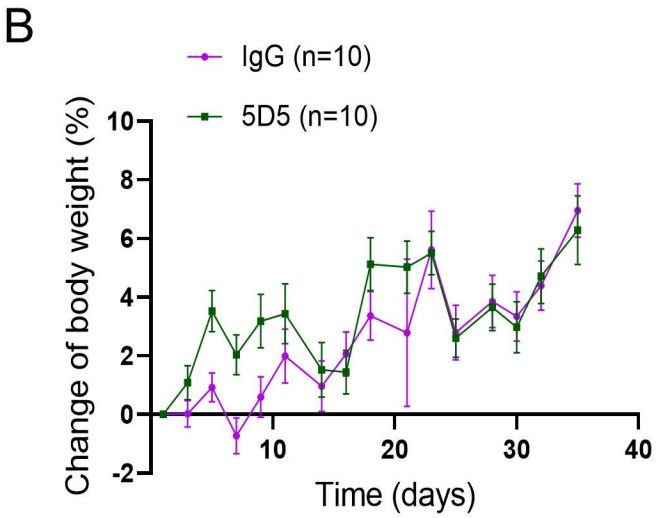
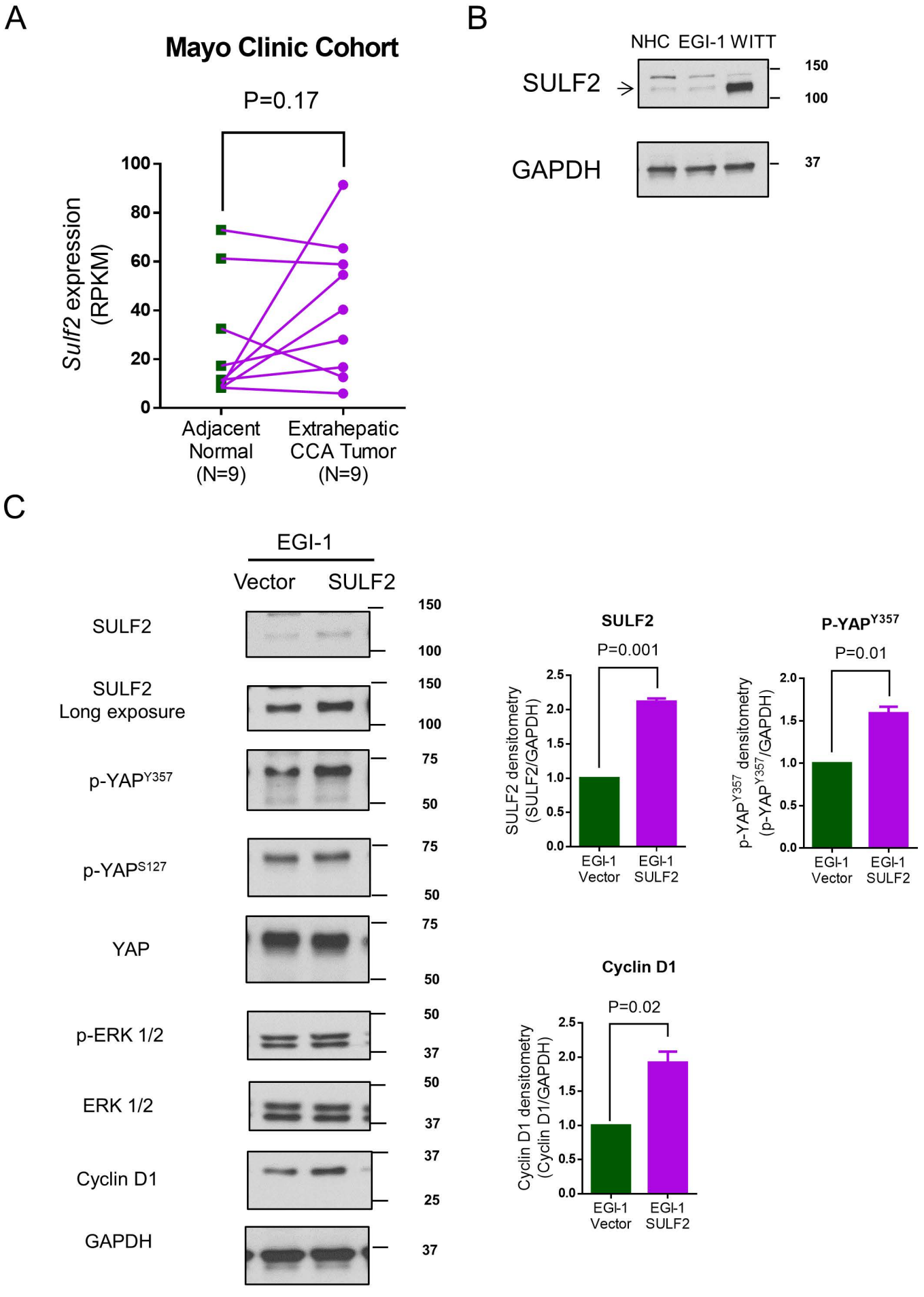




Supplementary Figure. 7



Supplementary Figure. 8