Supplemental Materials for the MS by Michael Johnston et al

Olaparib Inhibits Tumor Growth of Hepatoblastoma in Patient Derived Xenograft Models

List of probe mixtures for QRT-PCR

TaqMan probe mixtures were purchased from Thermo Fisher: 18s, Hs03003631_g1; POU5F1, Hs04260367_gH; THY-1, Hs00264235_m1; DLK1, Hs00171584_m1; CYP3A4, Hs00604506_m1; CYP2A6, Hs00868409_s1; CYP2B6, Hs041838409_s1; CYP2C8, Hs02383390_s1; CYP1A2, Hs00167972_m1; CYP2E1, Hs00559367_m1; PCK1, Hs01572978_g1; SCL22A7, Hs00198527_m1; SCL22A1, Hs00427552_m1; SCL10A1, Hs00161820_m1; SLC22A18, Hs00180039_m1; PSM10, Hs01100439_g1; C/EBPα, Hs00269972_s1; GPC3, Hs01018936_m1; PARP1, Hs 00242302_m1; and REG3a, Hs-1-55563_gH.

Supplemental Information for Experimental Procedures.

PDX Establishment

For surgical preparation, fresh tumor fragments were briefly maintained in cell culture medium in combination with penicillin/streptomycin with/without 5% BSA on ice. Samples were fractioned into 4x3 mm fragments. These fragments were placed within an interscapular subcutaneous flap in athymic nude mice which were 6-8 weeks of age at surgery (Athymic Nude-Foxn1nu, ENVIGO-Harlan Laboratories, Gannat, France). Tumor growth for specimens within our studies had confirmed growth 1 to 2 months. HBL tumors with successful were serially transplanted and subsequently confirmed for preservation by comparative examination histologically.

In-vivo studies

Two arms of five well-established PDX HBL lines were utilized (Supplemental Figure 4). Three mice were within each arm. All mice for each tumor were implanted on the same day. Following the latency period, once tumors were 100 mm^3, mice were stratified into each treatment arm, with homogenous mean and median tumor volumes. All treatment protocols were based on internal guidelines on treatment toxicity and administration schedules. Animals were weighed and tumor volume was recorded three to four days out of the week and observed for physical symptoms including appearance, behavior, and clinical changes.

Antibodies Utilized

The following antibodies were used: PARP1 (Santa Cruz), ph-S6-p53 (Santa Cruz; sc-61), Ku70 (Santa Cruz; sc-1487), NRF2 (Santa Cruz; sc-81342), PGAP1 (Invitrogen: sc-PA5 72340), RUNDC1 (Abcam, sc-AB 151583), Hace1 (Abcam; sc-133637), JNK1/2 (Cell Signaling Technologies; 9252S), ph-JNK1/2 (Cell Signaling Technologies; 4668S), cyclin D1 (Santa Cruz; sc-753), Gank (Cell Signaling Technologies; 12985S), cdc2 (Santa Cruz; sc-954), CY3A4 (Santa Cruz; sc-30621), HDAC1 (EMD Millipore; 05-100-I), C/EBP-alpha (Santa Cruz; sc-61), p21 (Santa Cruz; sc-6246), HNF4-alpha (Perseus Proteomics; PP-K9218-00) and b-actin (Sigma; A5316). Whole gel images of Western blots are shown in Supplemental Figures S3-S19.

Protein Isolation and Western Blotting.

In this study, we have used nuclear extracts and whole cell extracts (WCE).

Nuclear extracts were isolated by following procedure. Tissues were homogenized in buffer **A** containing 10mM Tris-HCL pH 7.5, 50mM NaCl, 5 mM MgCl2, 5% beta-mercaptoethanol and 10% glycerol. After centrifugation at 10,000 rpm for 10 min, the supernatant (cytoplasm) was saved. For extraction of nuclear proteins, we have used Buffer **B** which contains the same components plus 0.42M NaCl and 25% Sucrose. The pellets were re-suspended in 4 volumes of buffer B and incubated on ice for 20 min. After centrifugation at 10,000 rpm for 10 min, the supernatant (nuclear extract) was immediately used Western blotting, Co-IPs or for SEC.

Whole Cell Extracts were isolated by homogenization of liver tissues in buffer B and subsequent steps described above for isolation of nuclear extracts. To protect protein integrity, we mainly used protein extracts without freezing.

Co-Immunoprecipitations were performed using an improved True blot protocol. Since the main problem for Co-IP is the presence of the added IgG which provide very strong signals on Western blots, we used True blot system that utilizes secondary antibodies that do not recognize antibody added during IP step (after their destruction by boiling of samples in the modified loading buffer). Therefore, we boiled IP samples in provided loading buffer for 30 min, which in many cases completely destroys IgGs. After that, samples were loaded on 4-20% gradient gels (Bio-Rad), transferred to nitrocellulose membranes (Bio-Rad) and incubated with primary and secondary antibodies according to protocols. Dilution of each primary antibodies was determined by preliminary experiments and varied between 500 and 10,000 (beta-actin) depending on abundance of proteins.

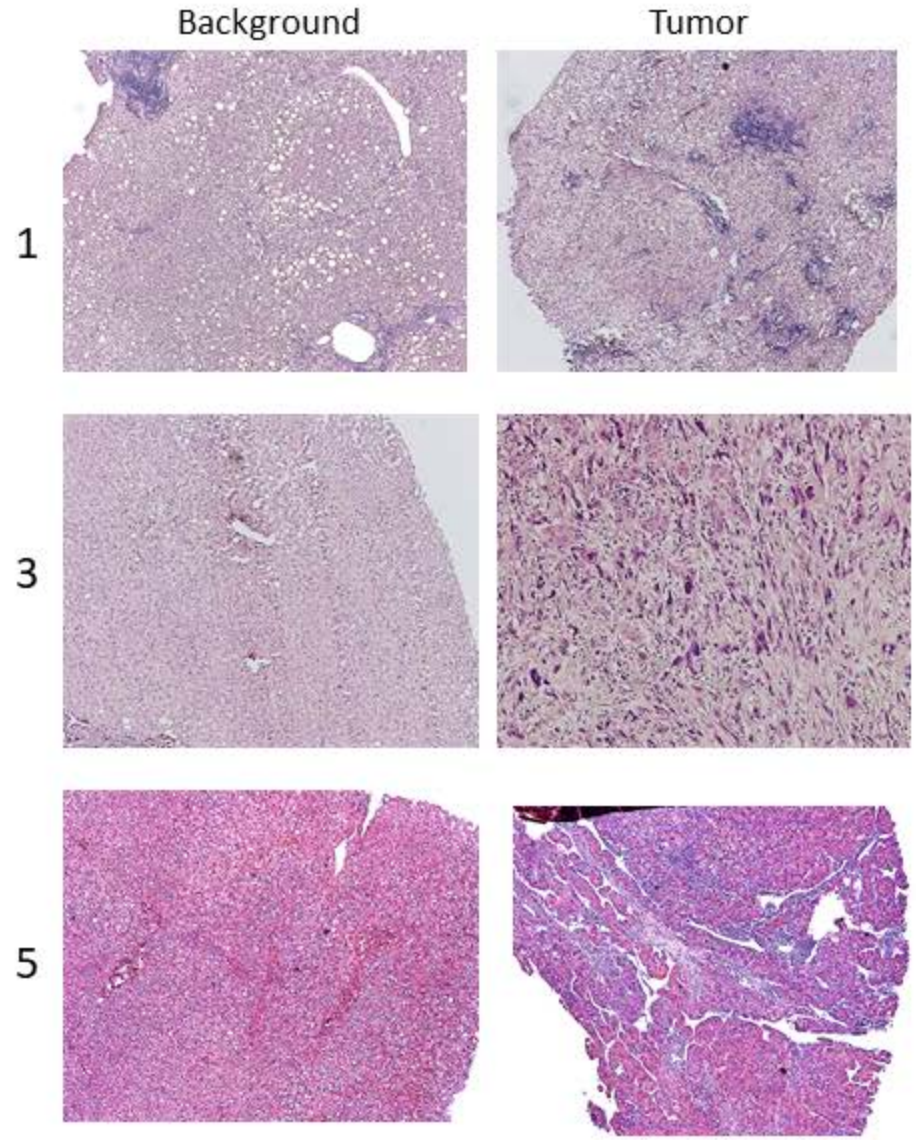
Supplemental Table 1: PDX HBL Patient Characteristics

Tumor Cod	Sex	Age (Mo)	Type of Sample	Surgery	Metastatic at Diagnosis	Main epithelial component	Vascular Invasion	Nodules	PRETEXT stage	Treatment protocol	AFP at diagnosis (ng/mL)	AFP post-chemotherapy (ng/mL)	AFP % Decrease	B-catenin status	NFE2L2 Status	PDX Growth Latency (Days)	
																60-200 mm3	1764 mm3
HB-295	F	26	Primary	R	Y	Fetal	Y	Mulitple	II	SIOPEL4	585,350	1,400	99.76%	Per Xentech pdf, CTNNB1 mutation, doesn't list specific type though	-	~12	~28
HB-268	F	12	Primary	R	N	Embryonal+crowded fetal+macrotrabecular	-	-	II	SIOPEL6	620,000	200,000	67.74%	Exon 3 Deletion	WT	~21	~29
HB-303	F	69	Primary	R	N	Fetal	N	Mulitple	II	SIOPEL6	158,645	26,000	83.61%	-	-	~19	~28
HB-243-RED-225	M	52	Intrahepatic relapse	LT	N	Embryonal	Y	Multiple	Relapse	CARBOPLATIN + VEPESIDE	6,000	5,000	16.67%	Exon 3 Deletion	WT	~16	~28
HB-283	M	24	Primary	R	N	Embryonal+crowded fetal	-	-	II	SIOPEL6	95,000	2,475	97.39%	Exon 3 Deletion	WT	~18	~28

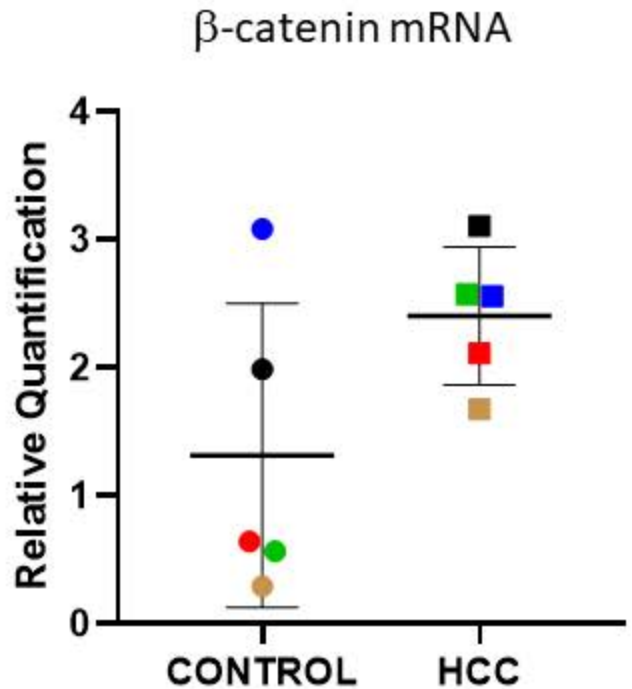
Liver resection=R; Liver transplant=LT, F=Female, M=Male

Supplemental Table 2: Hepatocellular Carcinoma Bio Bank

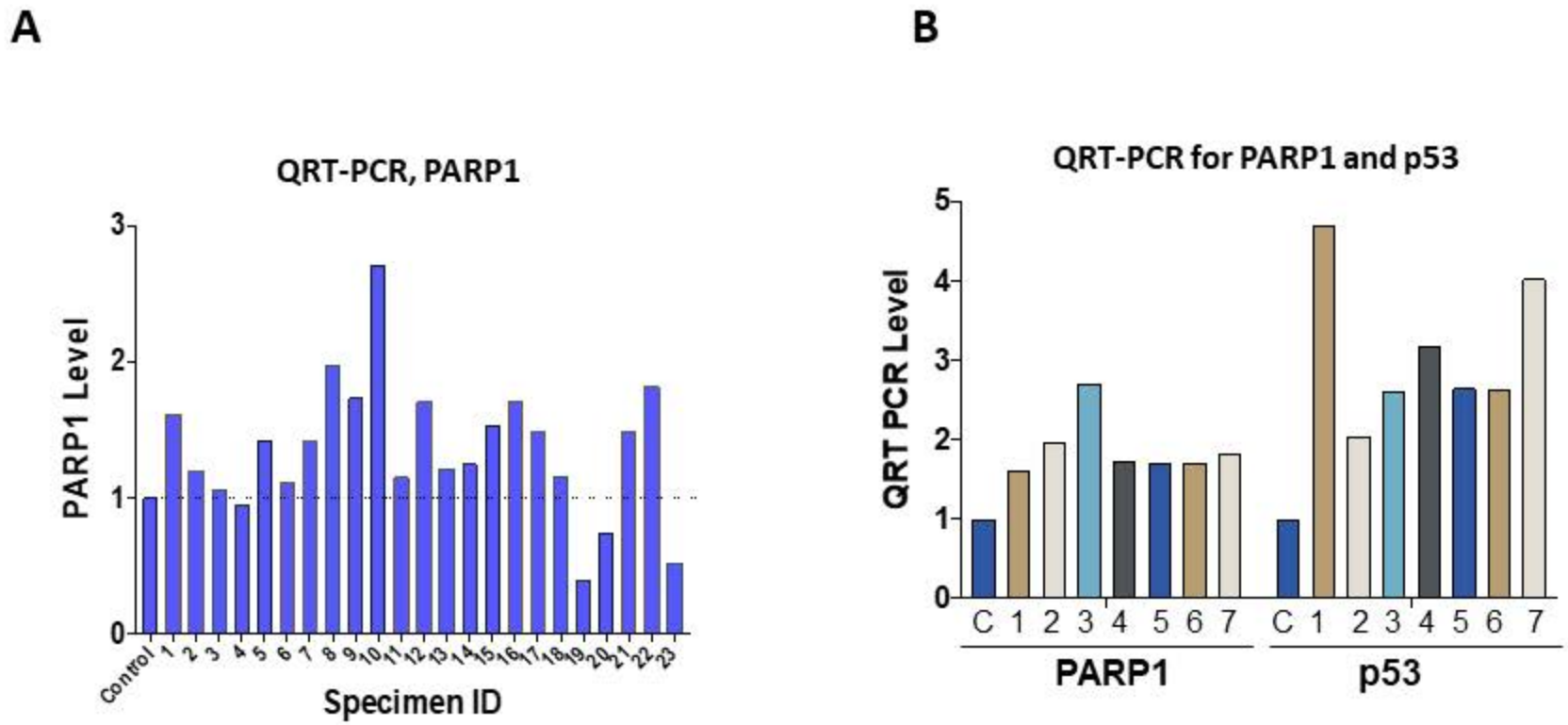
Tumor Code	Age (Years)	Sex	Etiology	Metastatic at Diagnosis	Pathology	AFP at Diagnosis	AFP Post-Surgery
HCC1	78	M	Hepatitis B	No	Sarcomatoid HCC, +Vascular invasion	6300	343
HCC2	50	M	Hepatitis B	No	Moderately differentiated HCC, +Vascular invasion	2	2
HCC3	68	M	NAFLD	No	Well to moderately differentiated, -Vascular invasion	2.5	2
HCC4	72	M	NAFLD	No	Moderately differentiated HCC, +Vascular invasion	31	1
HCC5	57	M	Hepatitis C	No	Moderately differentiated HCC, +Vascular invasion	123	2



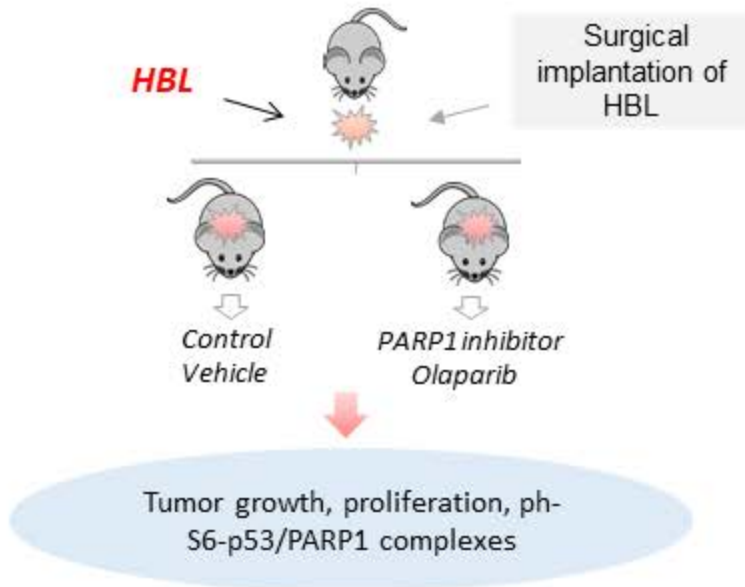
Supplemental Figure 1. Examples of H&E staining of livers of HCC patients



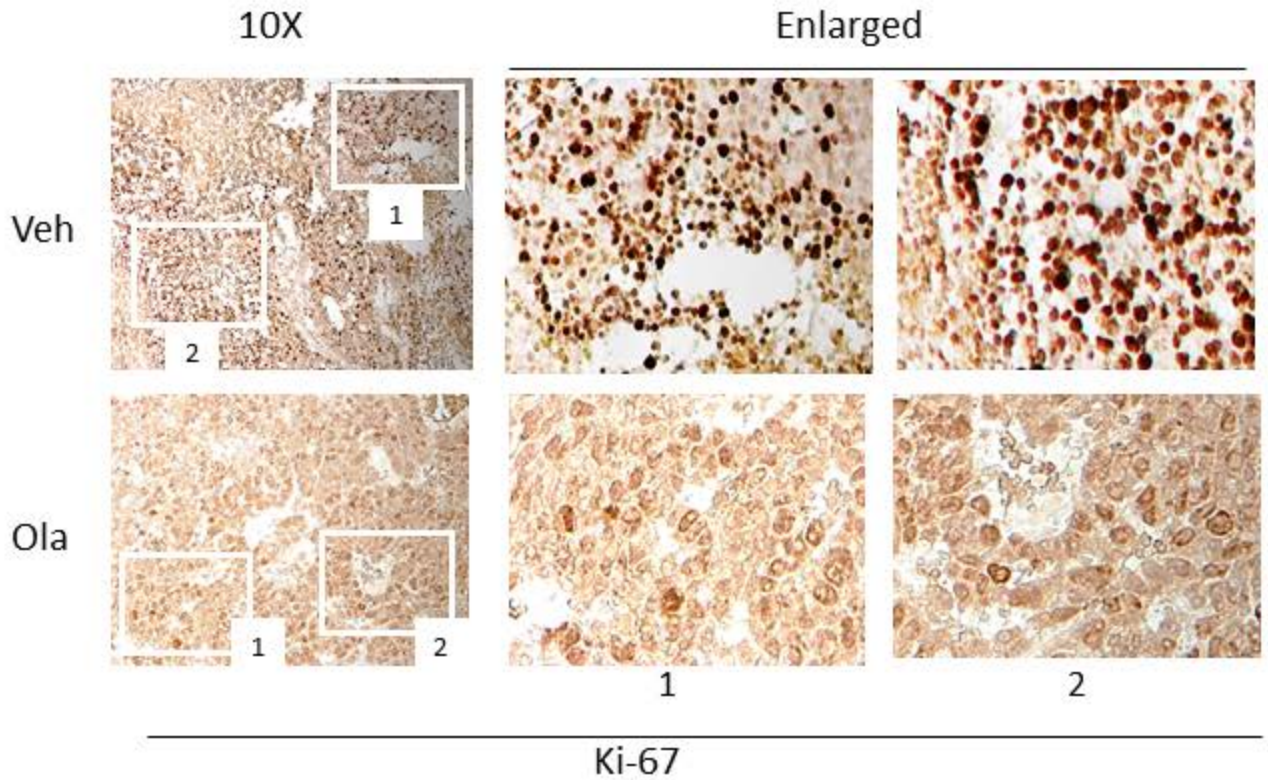
Supplemental Figure 2. Levels of β-catenin mRNA in HCC samples was determined by QRT-PCR.



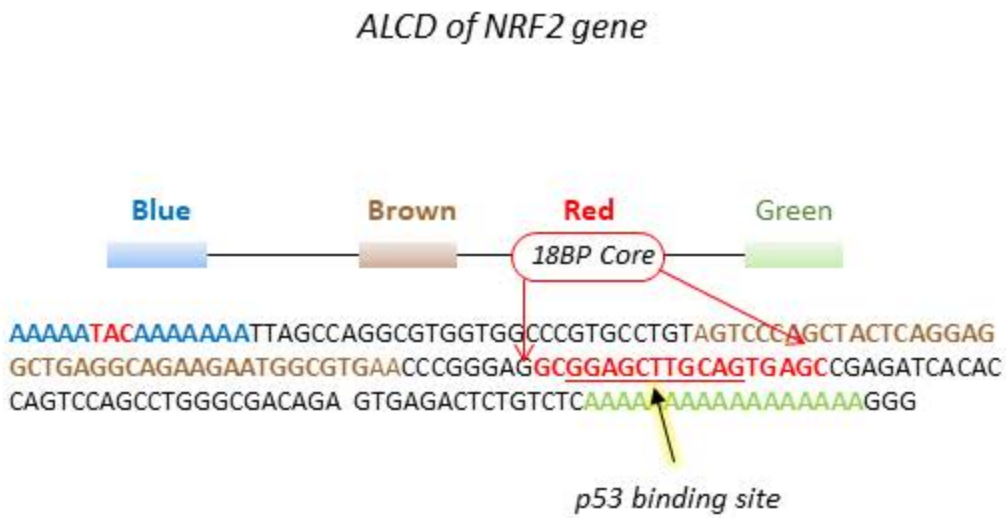
Supplemental Figure 3. Selection of PDXs for the treatments with inhibitor of PAR1 Olaparib. Examination of PARP1 and p53 expression in PDXs that were previously generated and characterized [21]. A) QRT-PCR-detected PARP1 in 23 generated PDXs. B) QRT-PCR for PARP1 and p53 in PDXs with high levels of PARP1. Control is RNA from a patients without liver disorders.



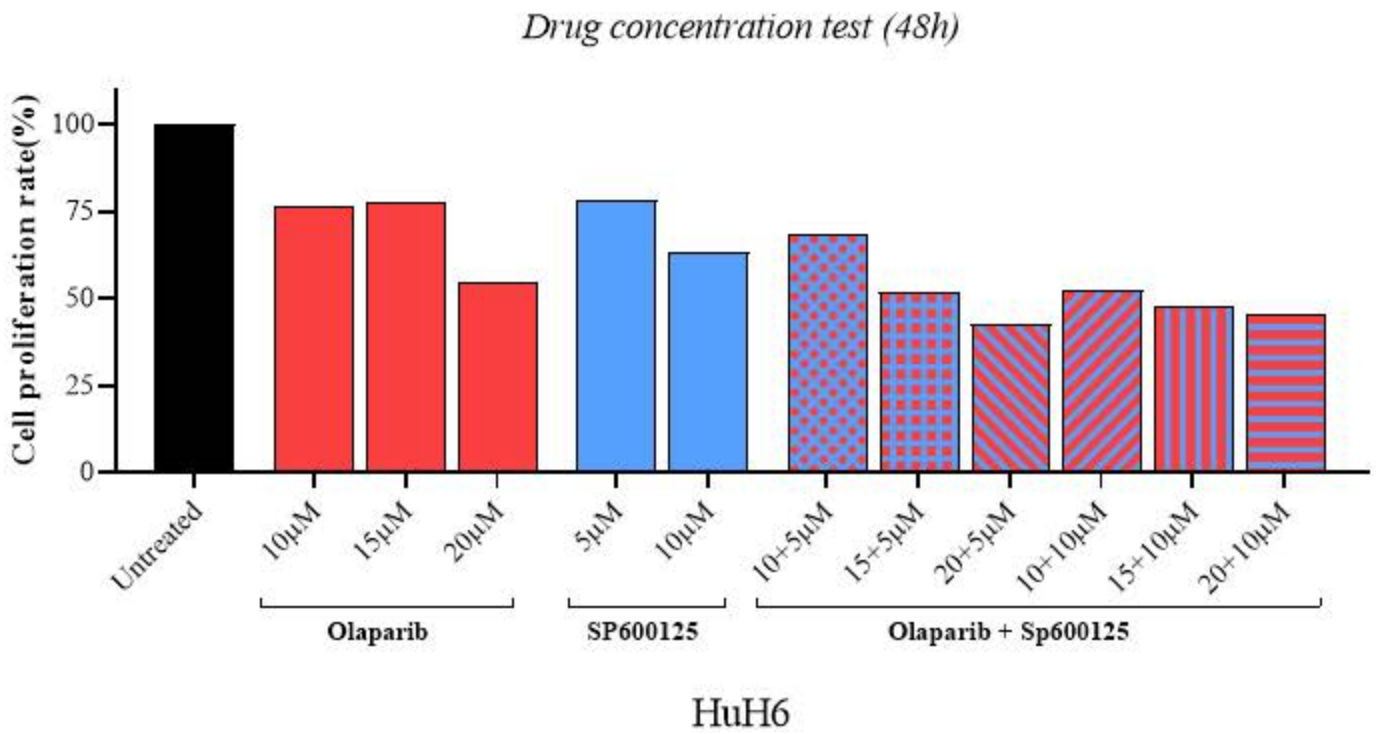
Supplemental Figure 4. A design for the treatments of PDXs with Olaparib.



Supplemental Figure 5. An example of ki67 staining of the PDXs treated with vehicle and Olaparib. Boxes show regions of the PDXs which were enlarged on the right images.



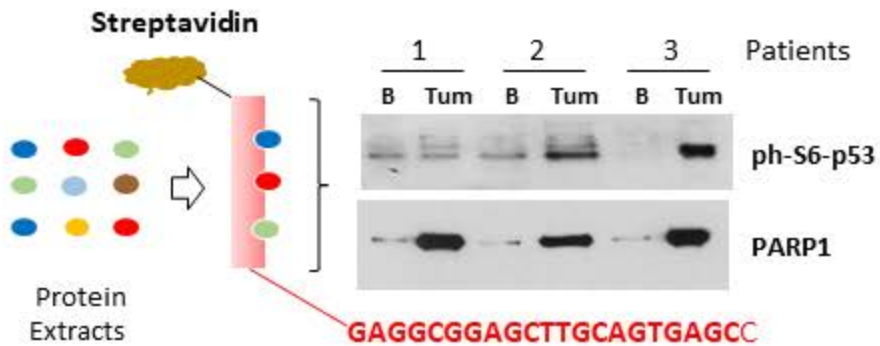
Supplemental Figure 6. Nucleotide sequence of ALCD in the NRF2 gene. 100% homological regions are shown by color boxes. P53 binding site within 18S core sequence is underlined.

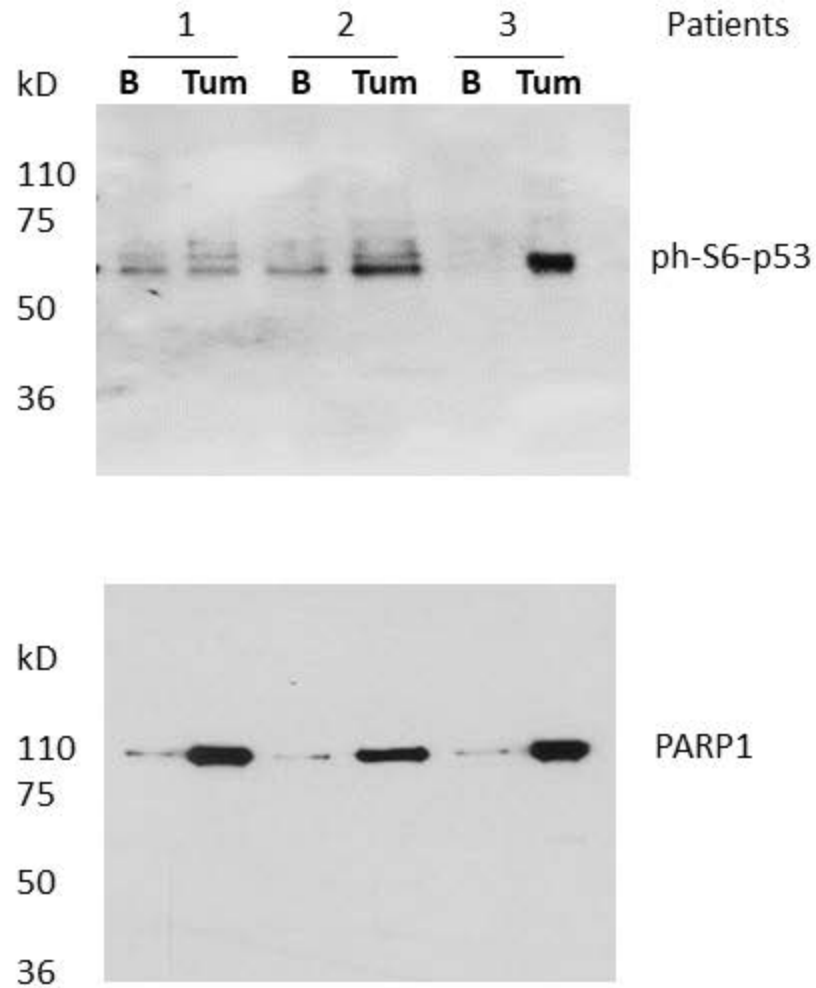


Supplemental Figure 7. Drug concentration test to optimize treatments of Huh6 cells with Olaparib and SP600125. Cells were treated for 48 hours with concentrations shown on the bottom. The assay was performed as described in Experimental Procedures.

Whole images of Western blots

Fig 1D

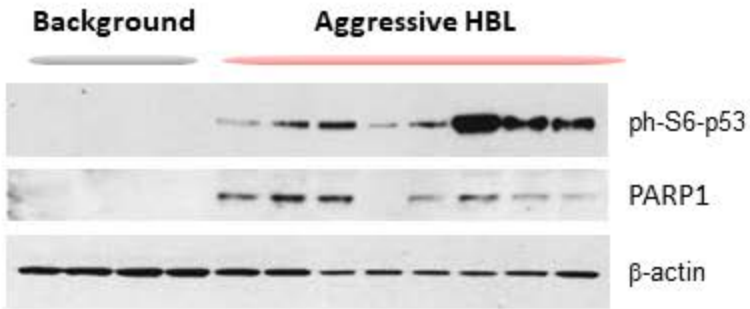


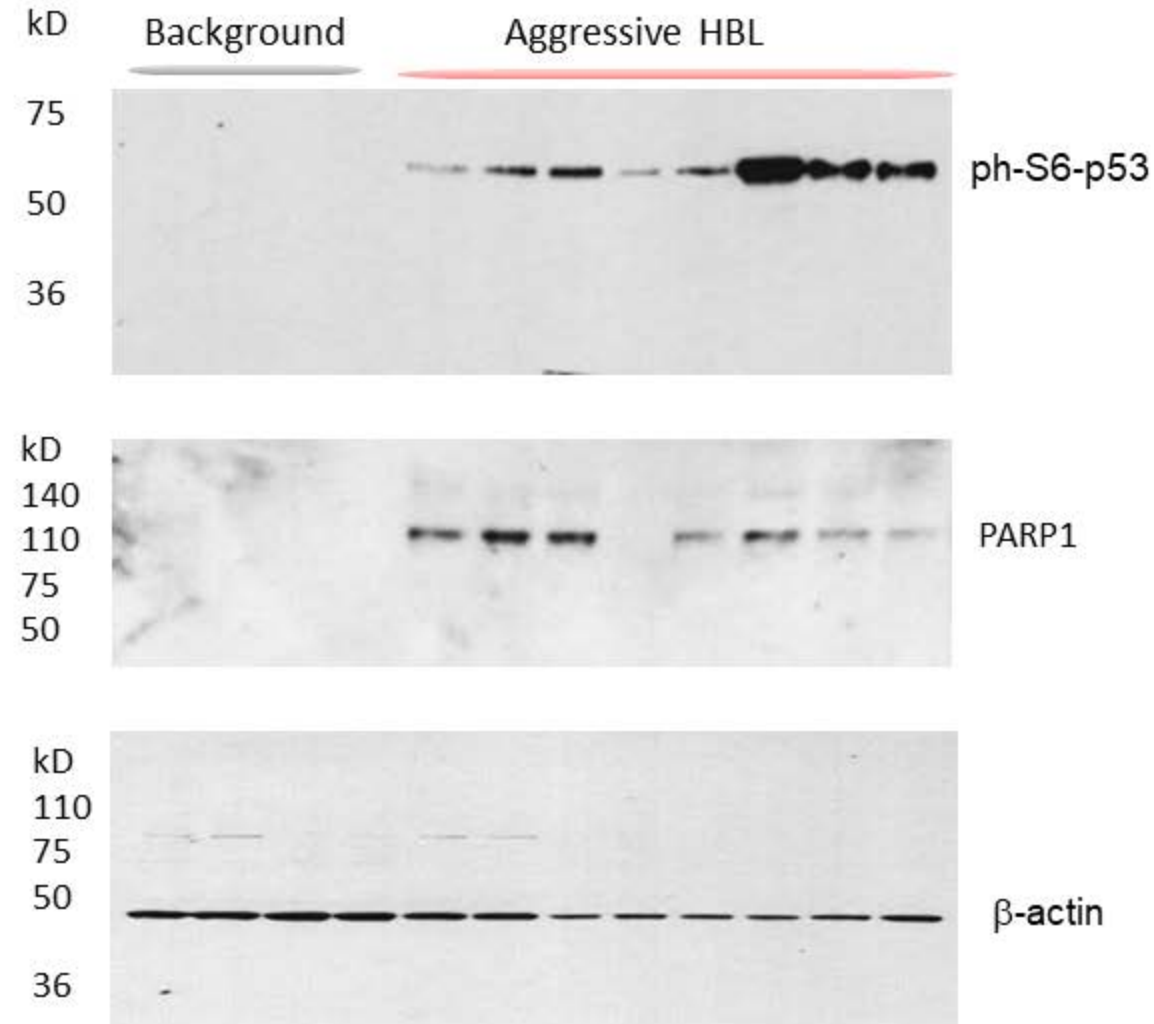


Supplemental Figure 8

Whole images

Fig 2B

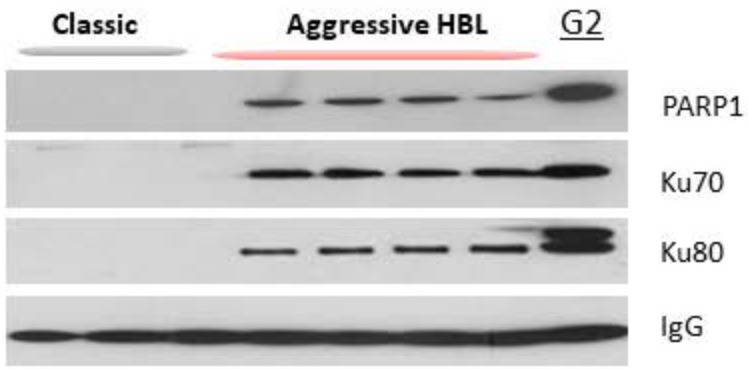




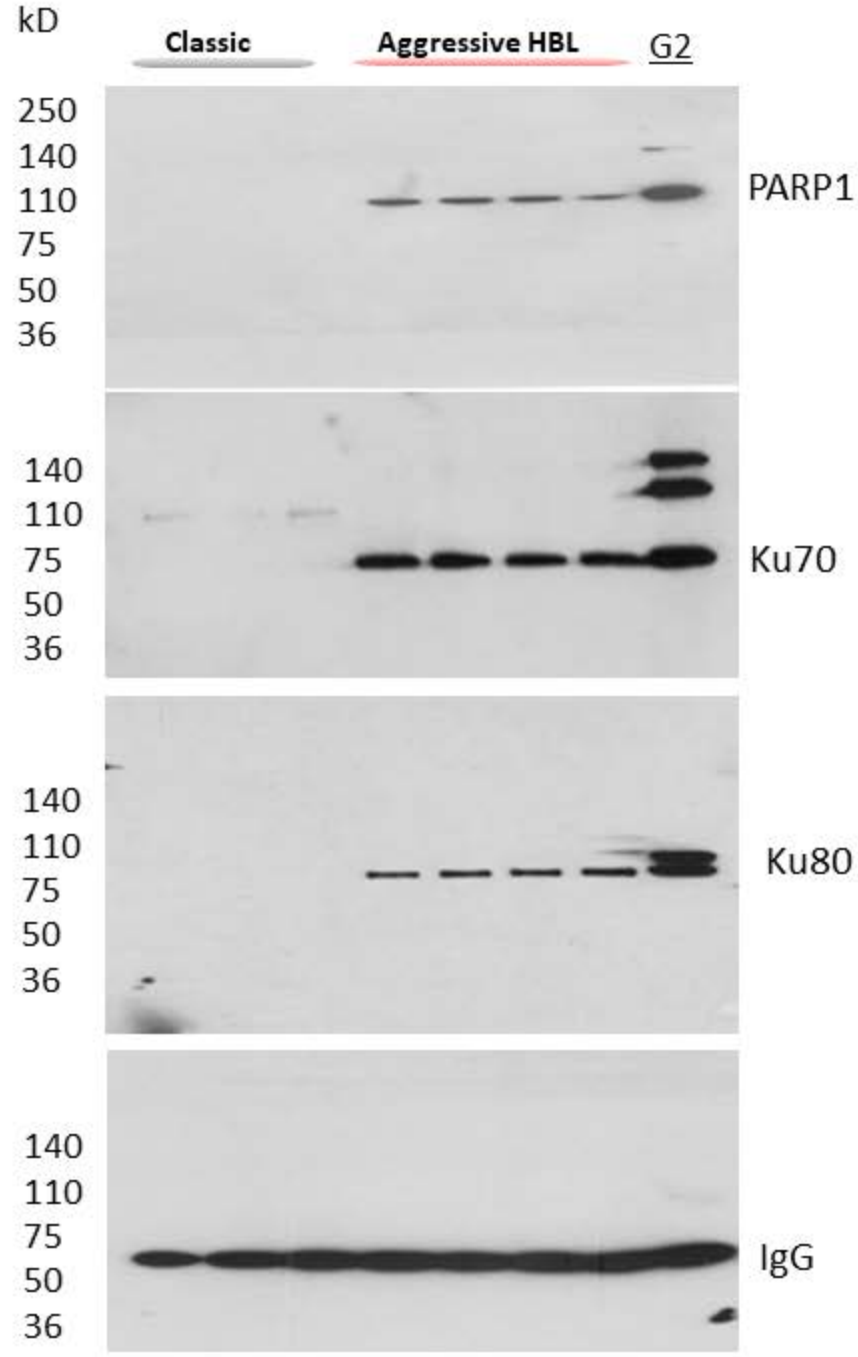
Whole images

Supplemental Figure 9

Fig 2C



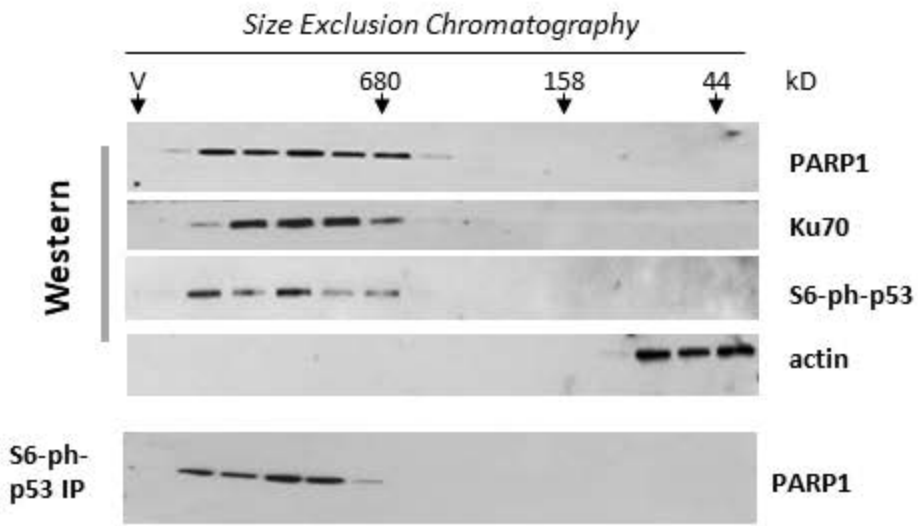
S6-ph-p53 IP

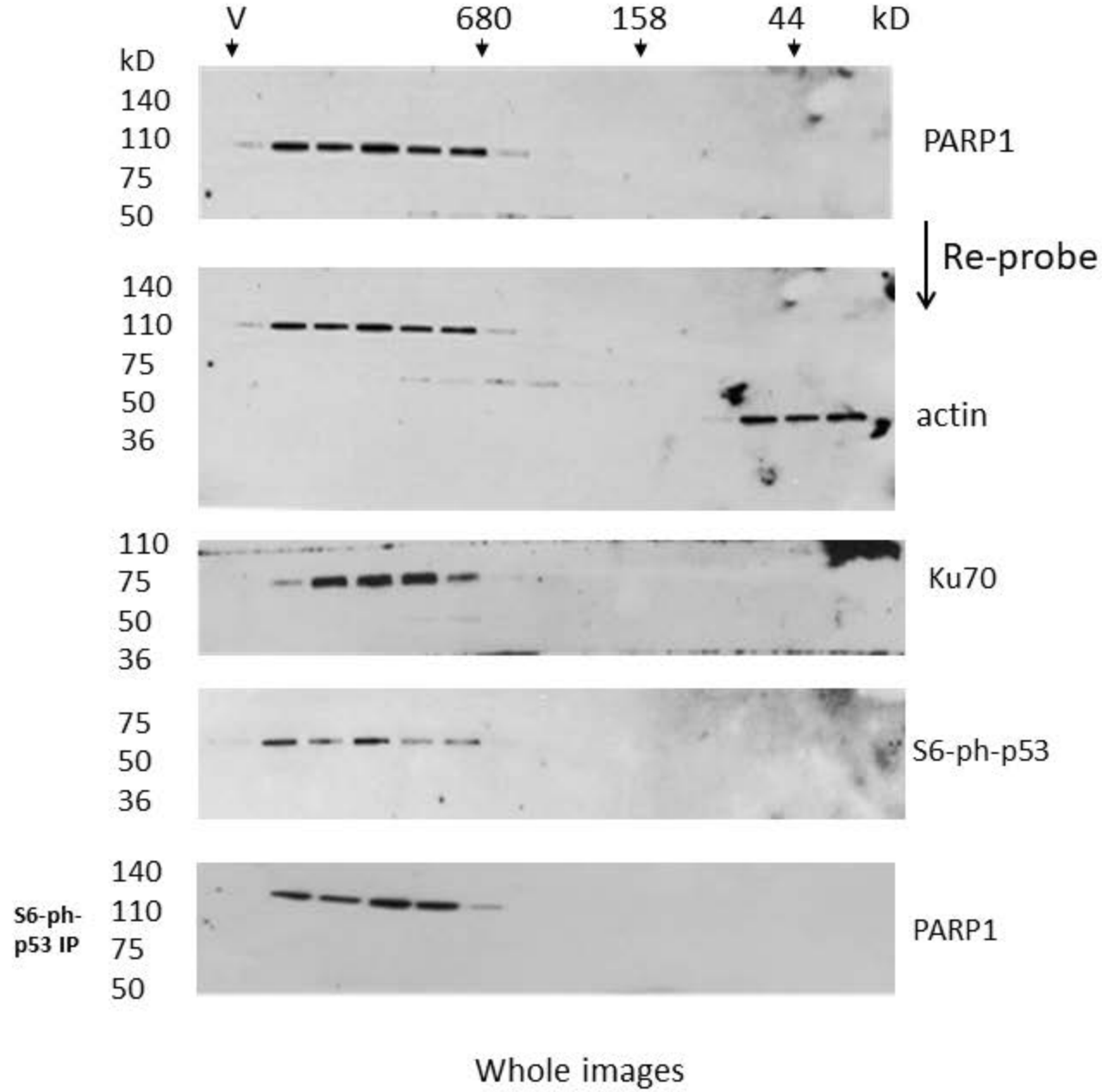


Whole images

Supplemental Figure 10

Fig 2D

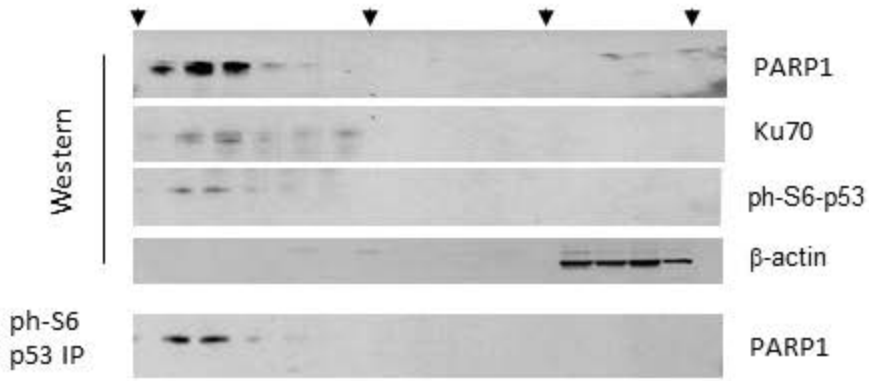




Whole images

Supplemental Figure 11

Fig 2D



Supplemental Figure 12

Whole Images

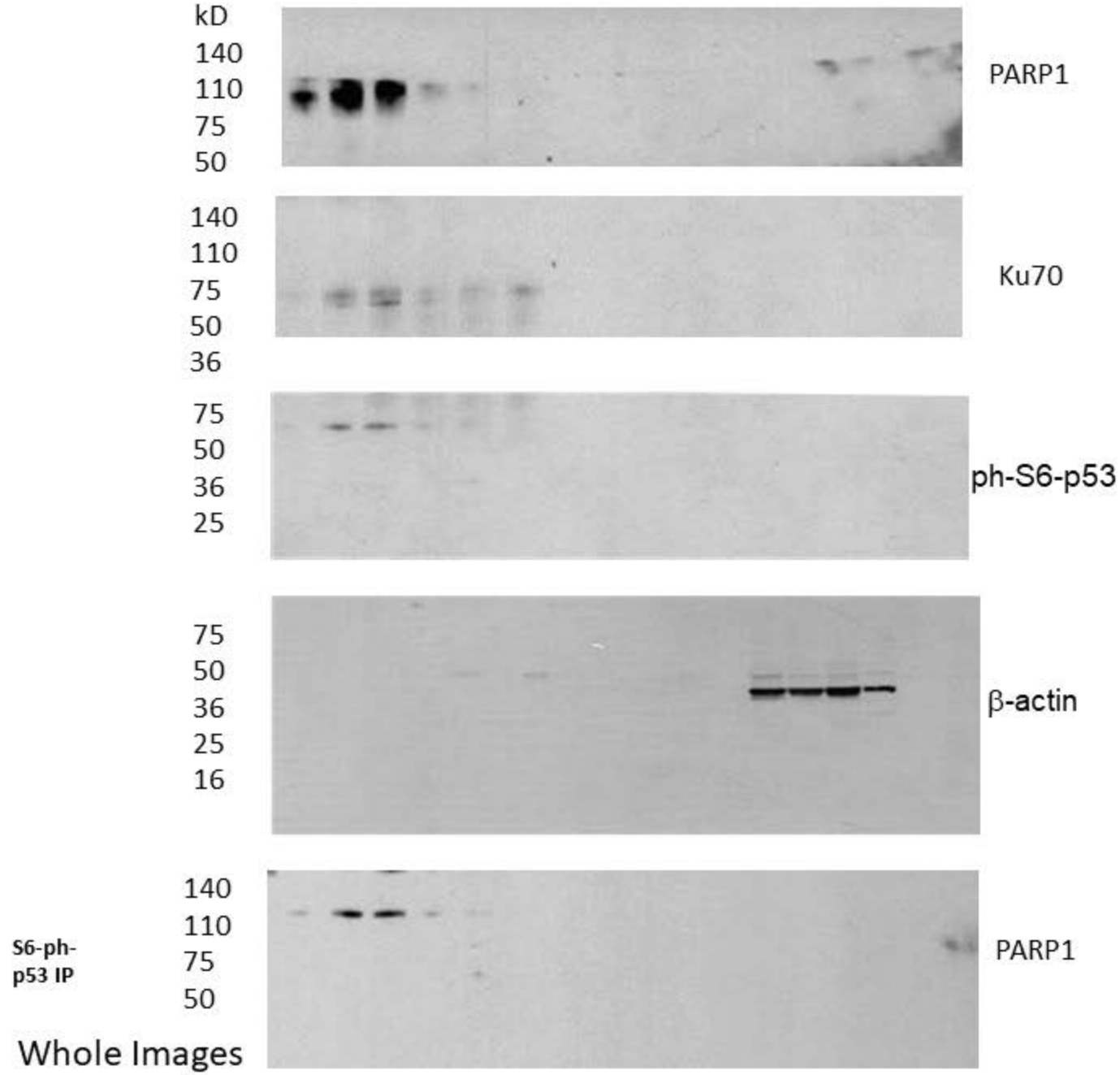
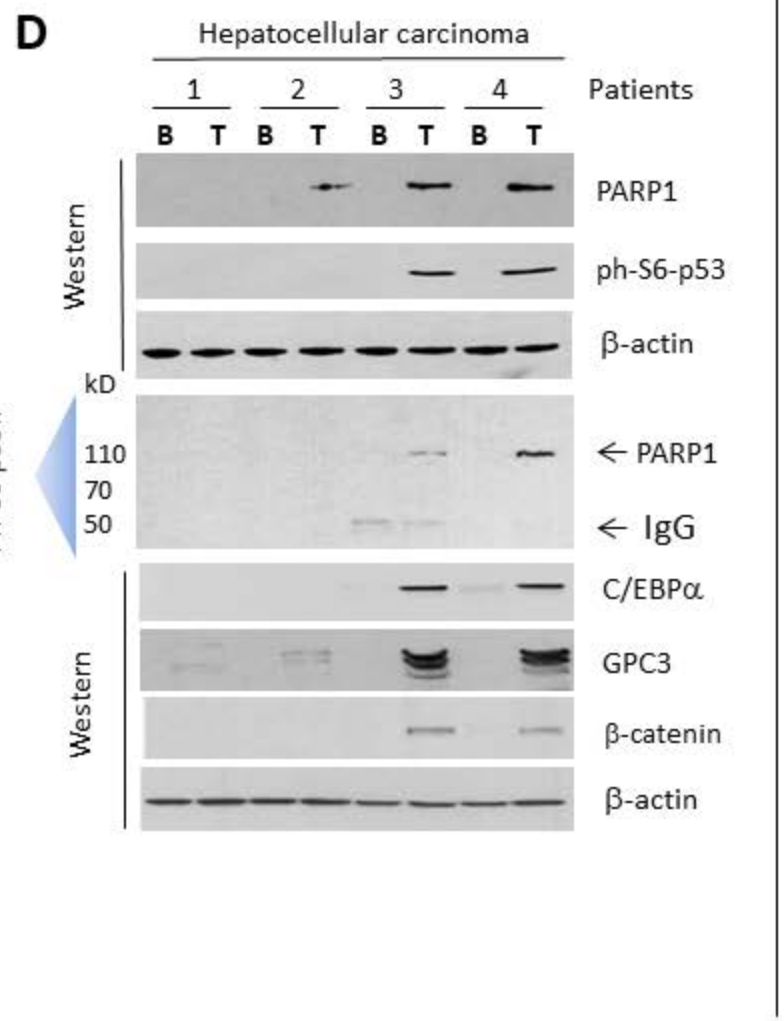
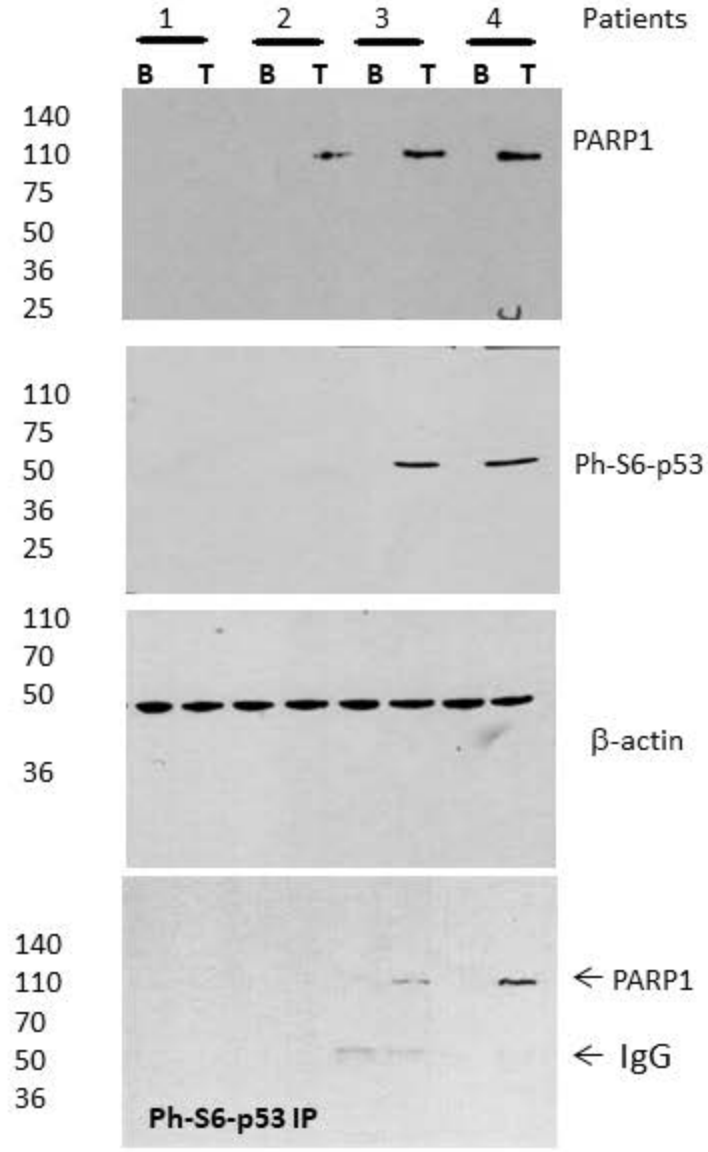
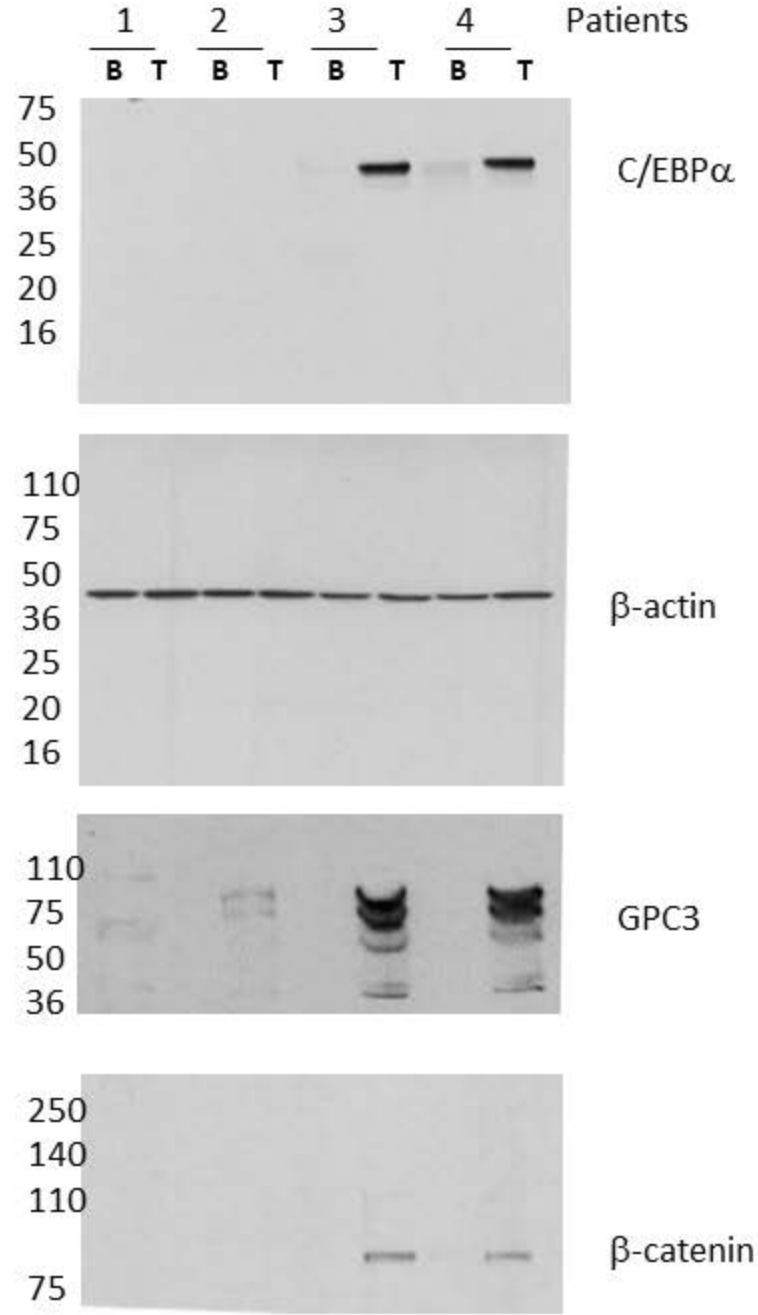


Fig 3D



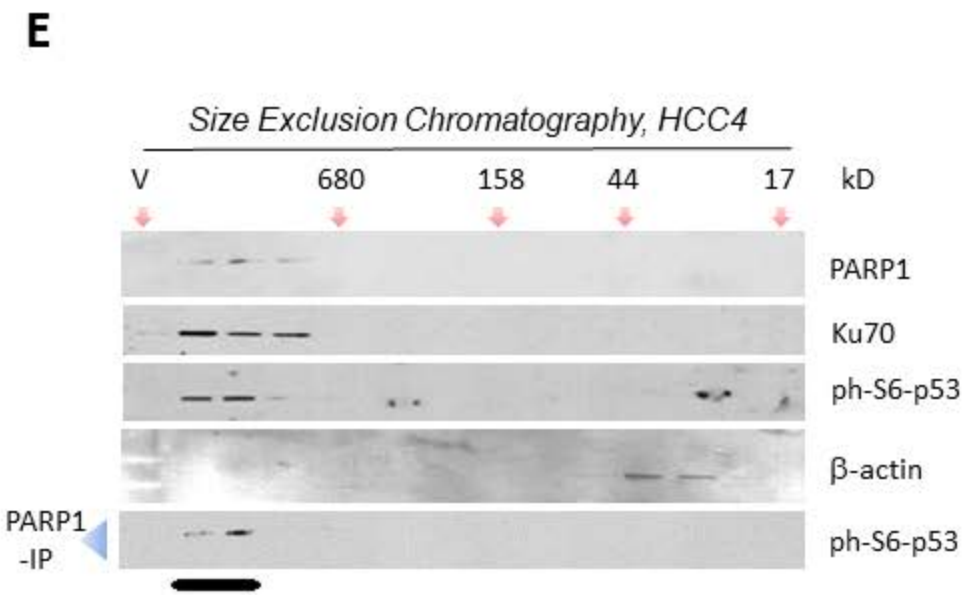


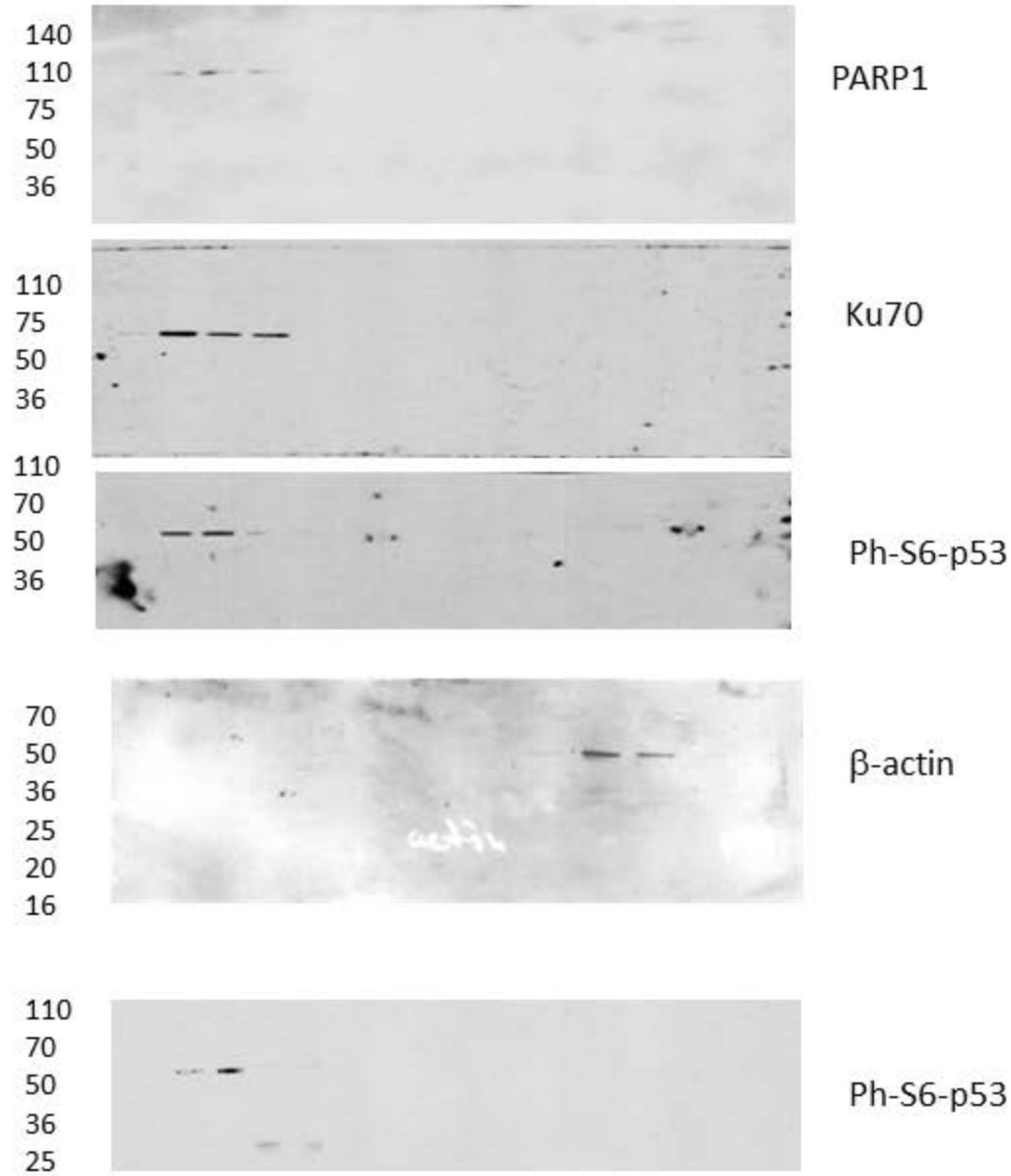


Whole images

Supplemental Figure 13

Fig 3E

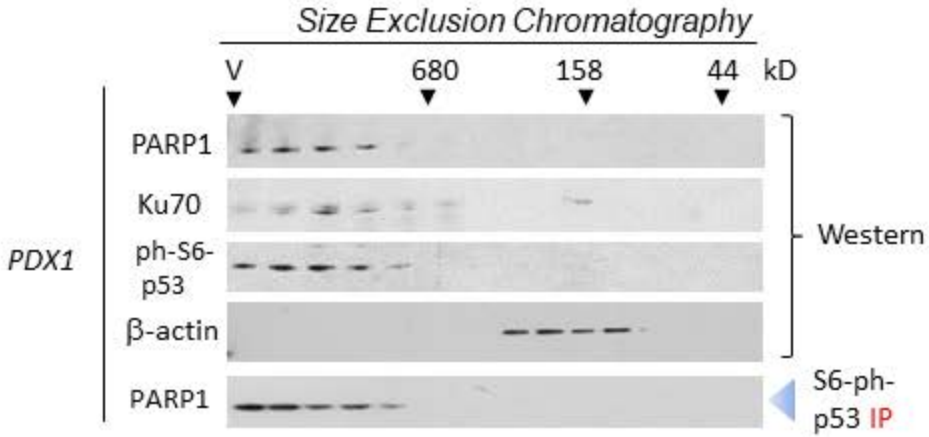


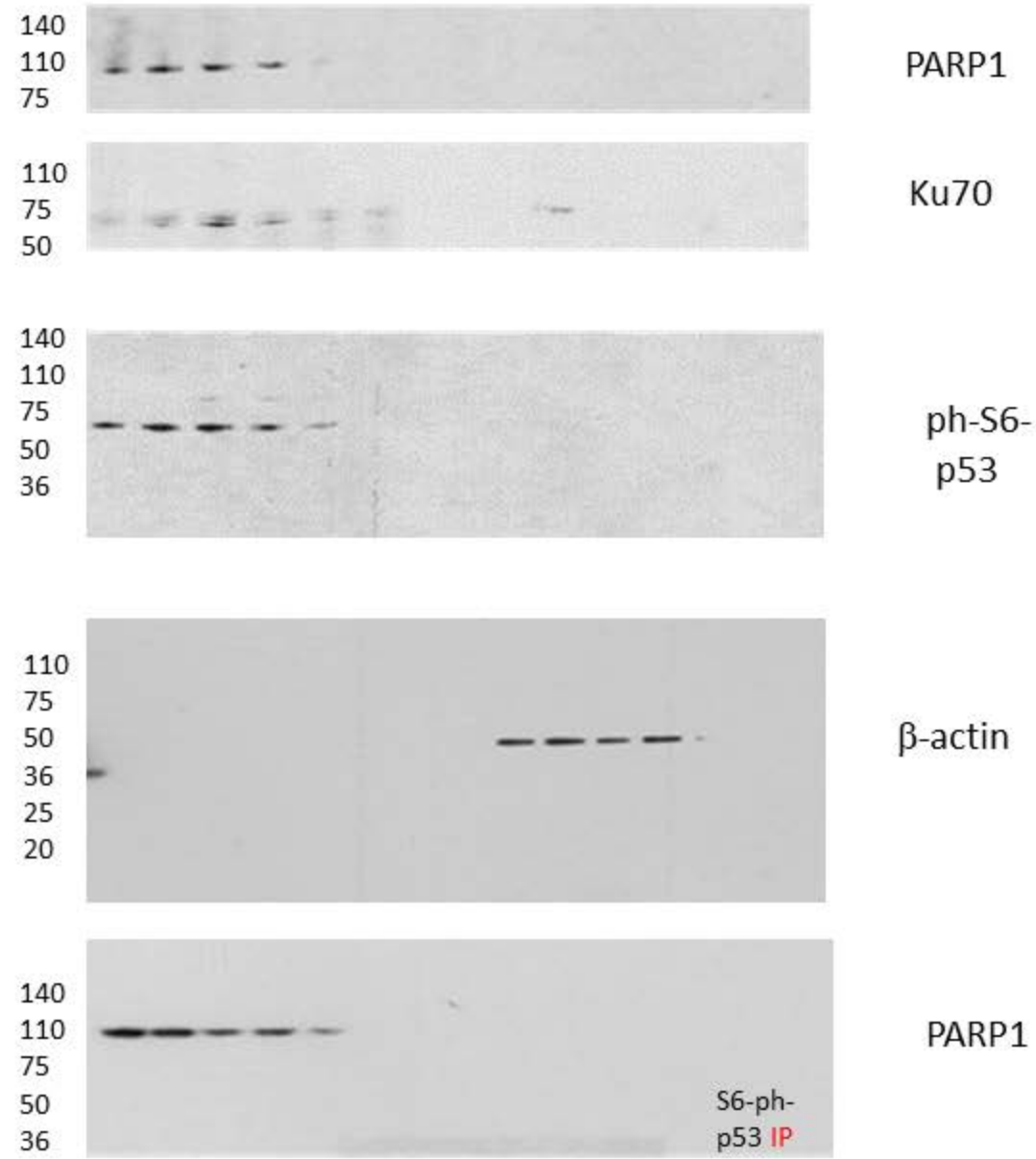


Supplemental Figure 14

Whole images

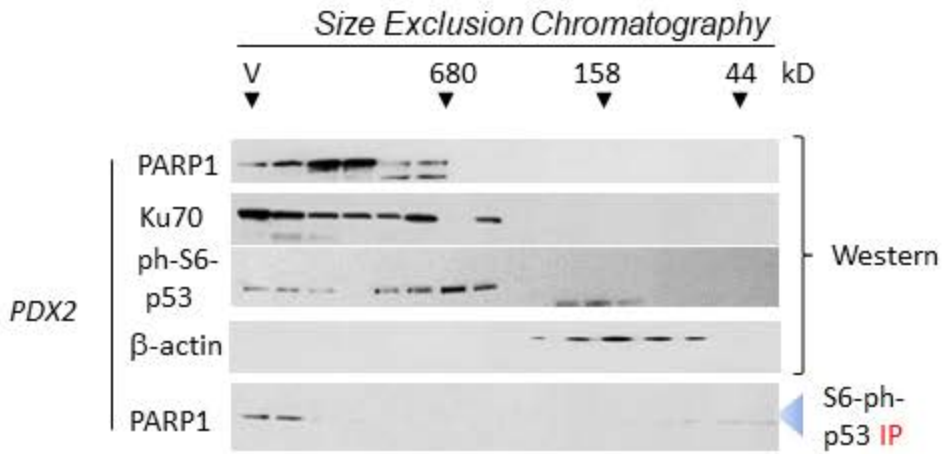
Fig 4A

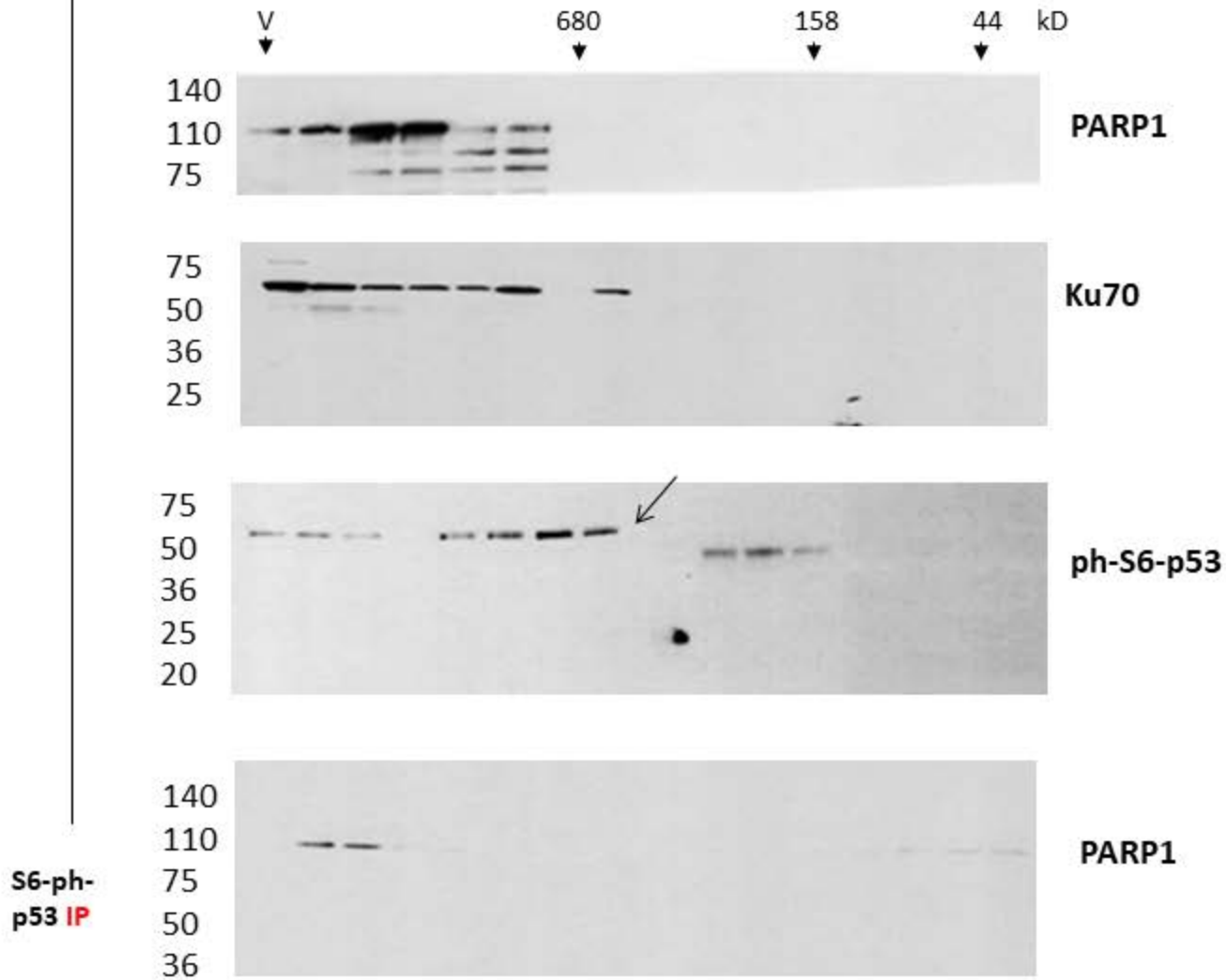




Supplemental Figure 15

Fig 4A

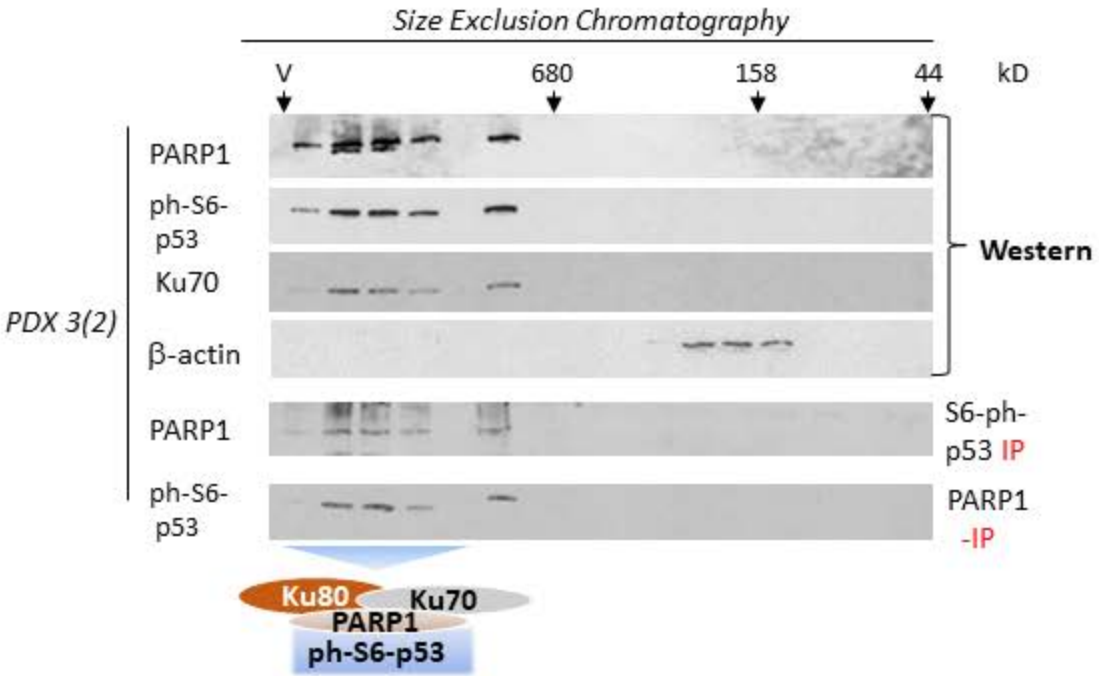


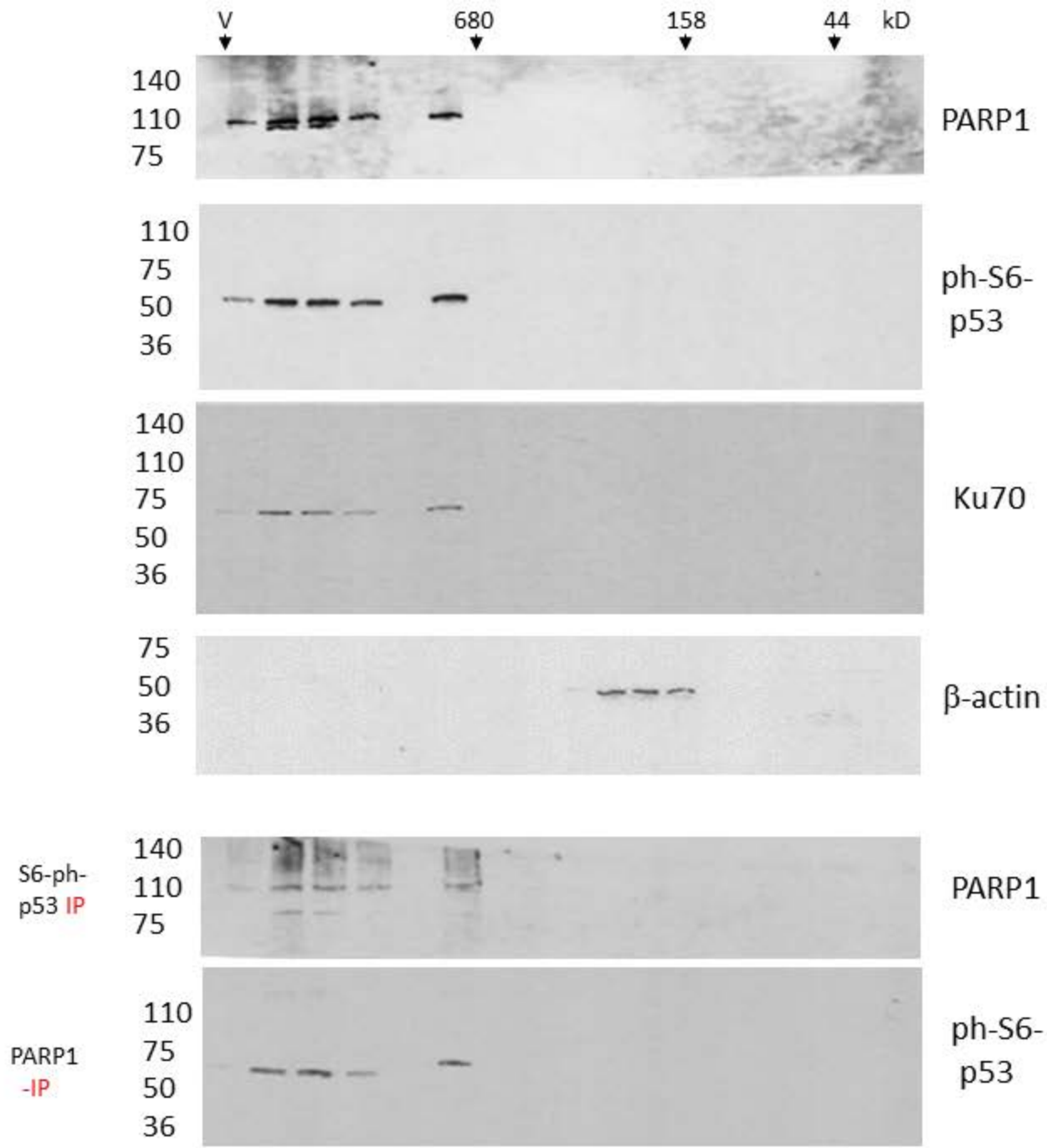


Whole images

Supplemental Figure 16

Fig 4A





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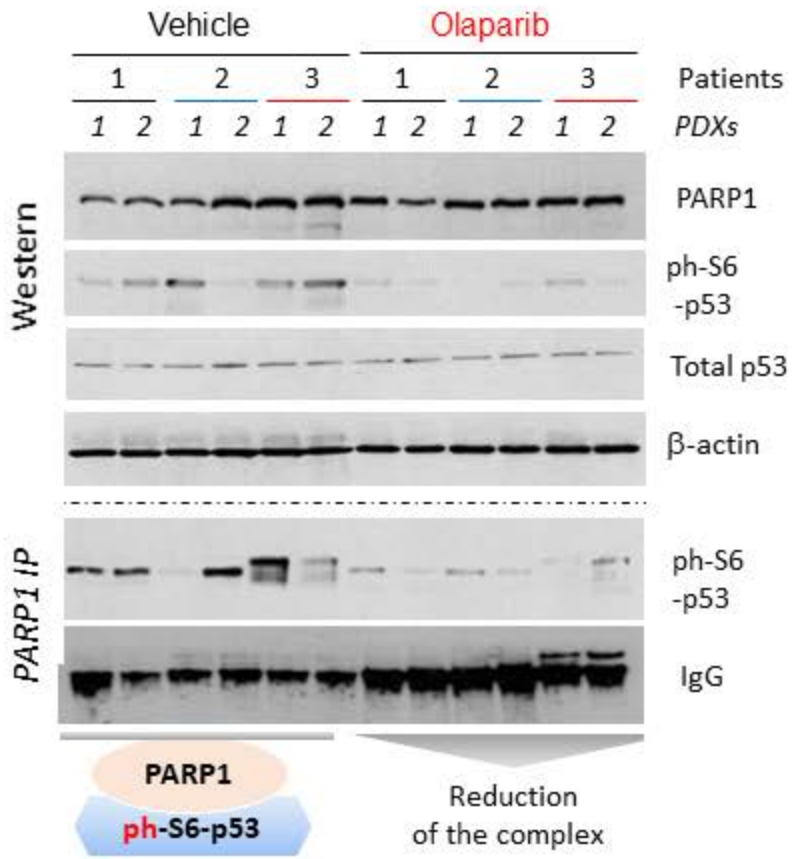
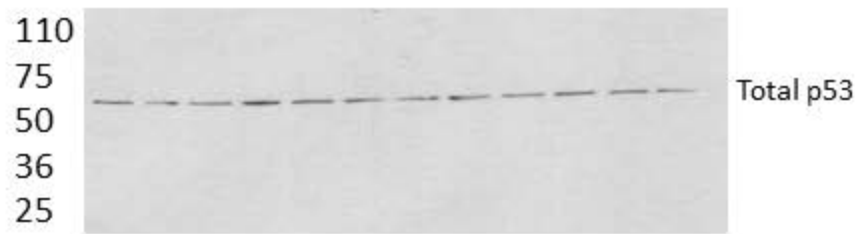
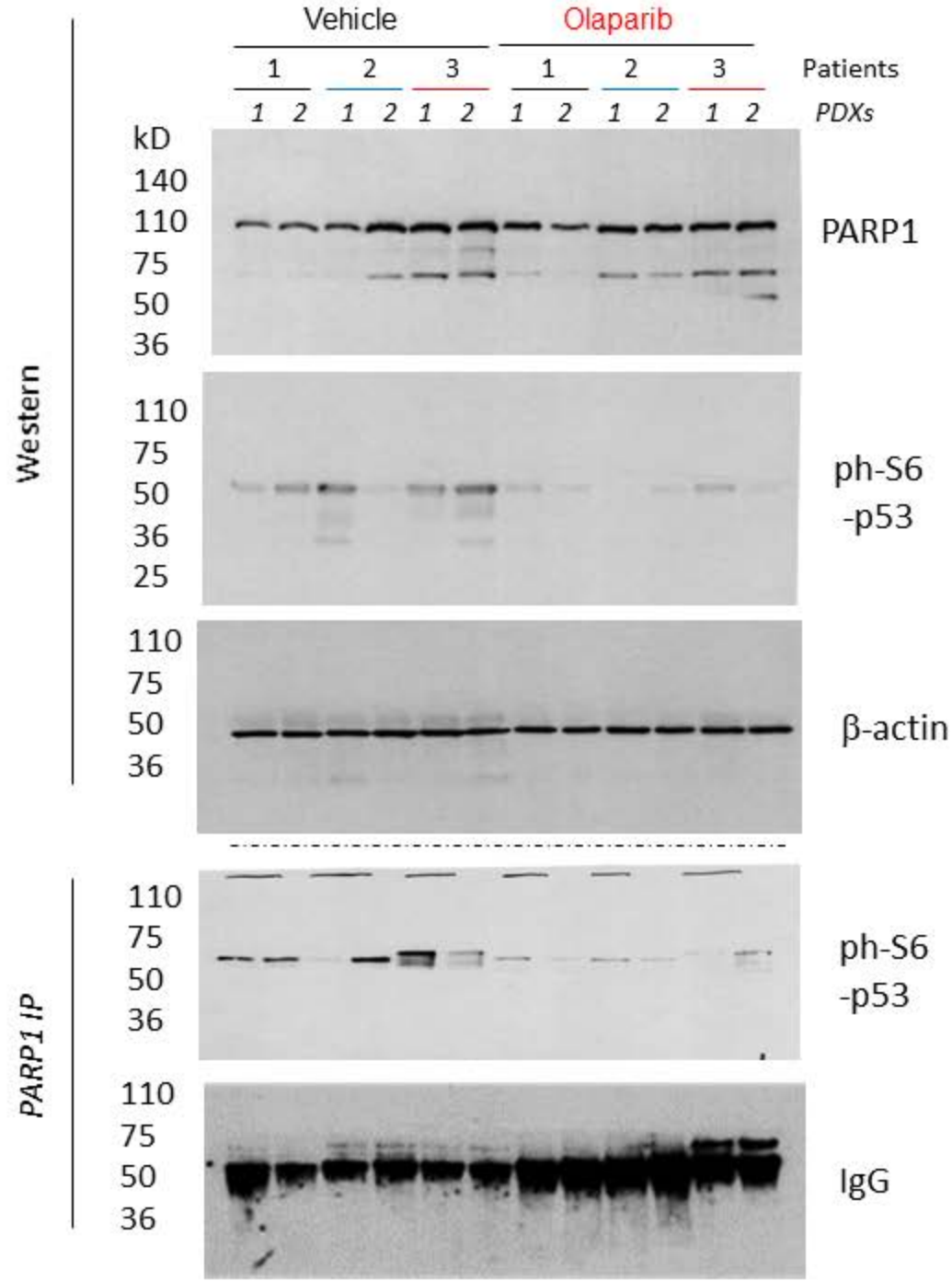
Supplemental Figure 17

FIG 5A



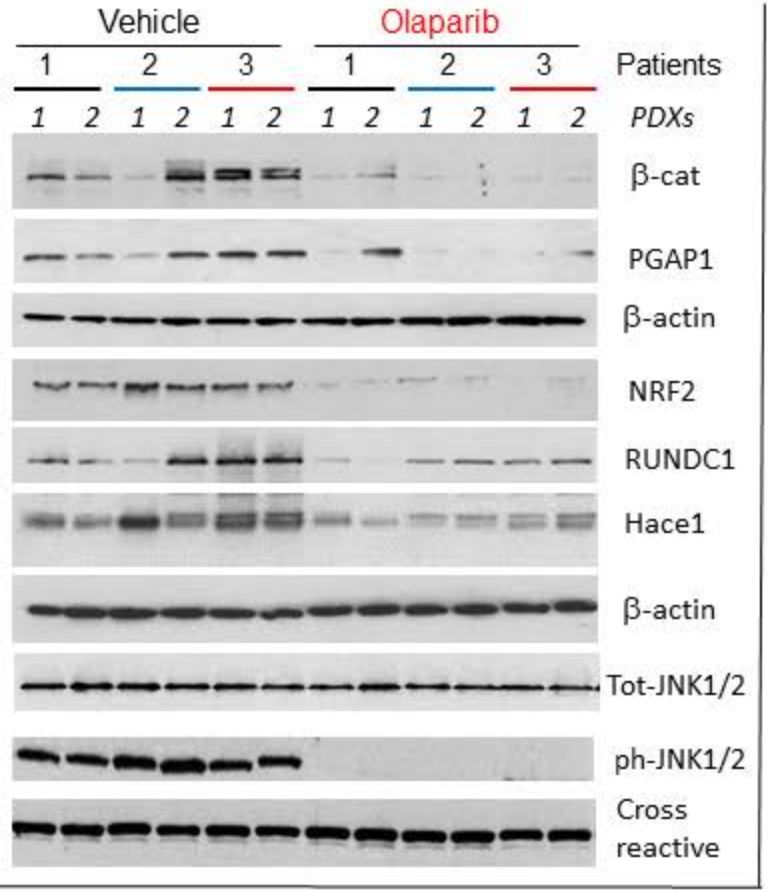


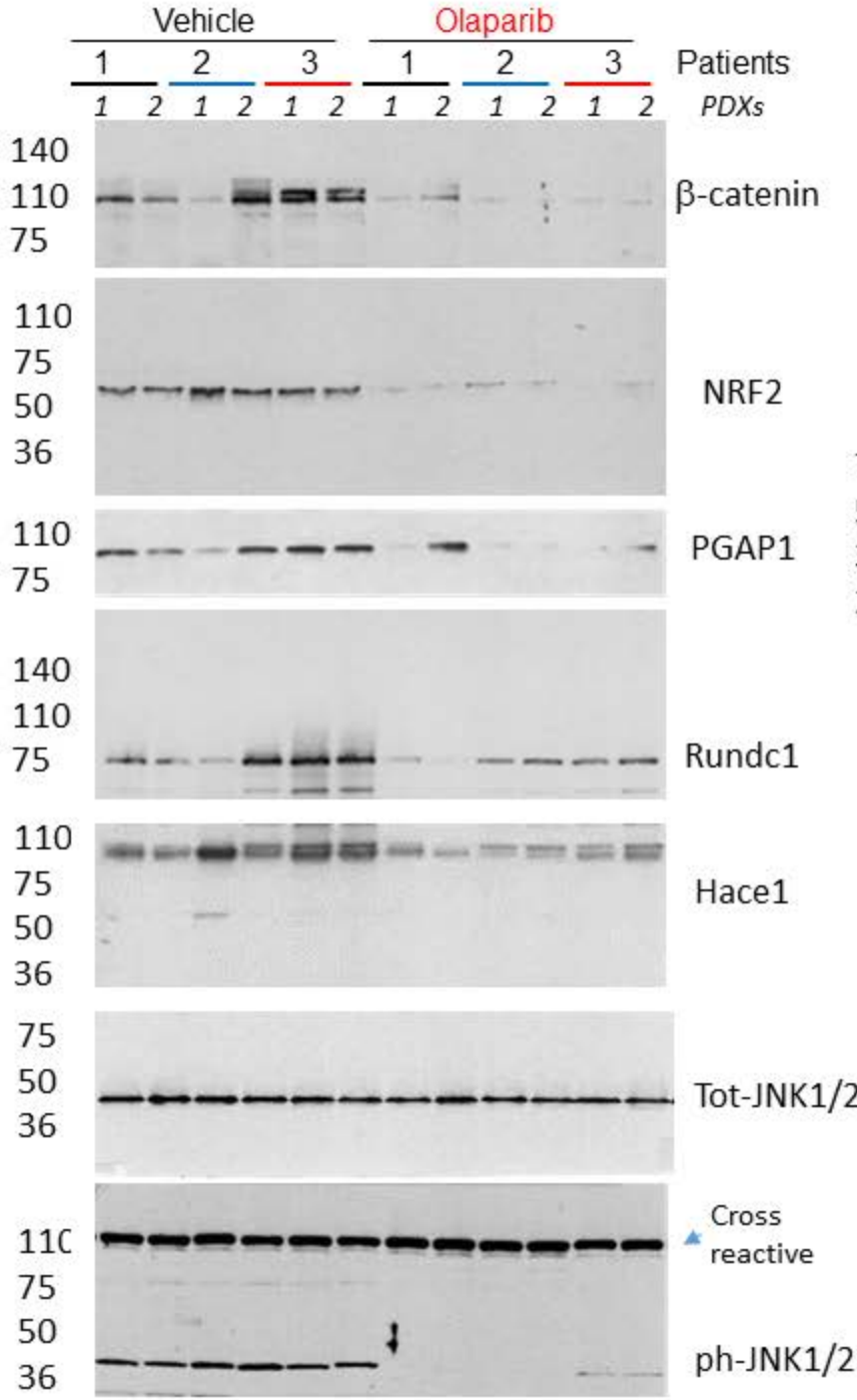


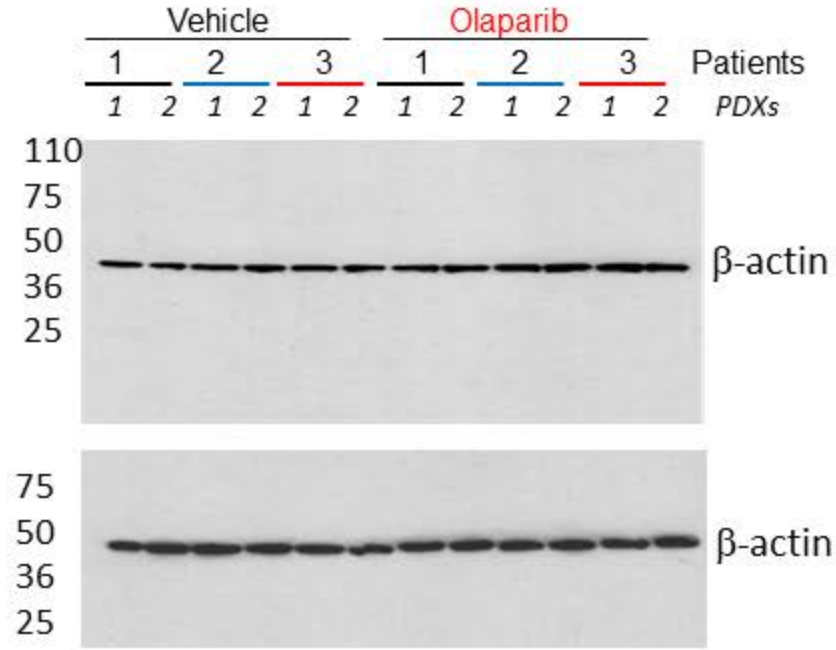
Supplemental Figure 18

Whole images

FIG 5C



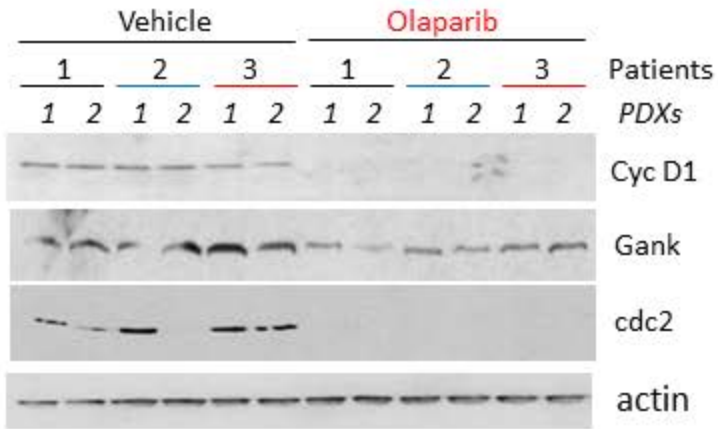


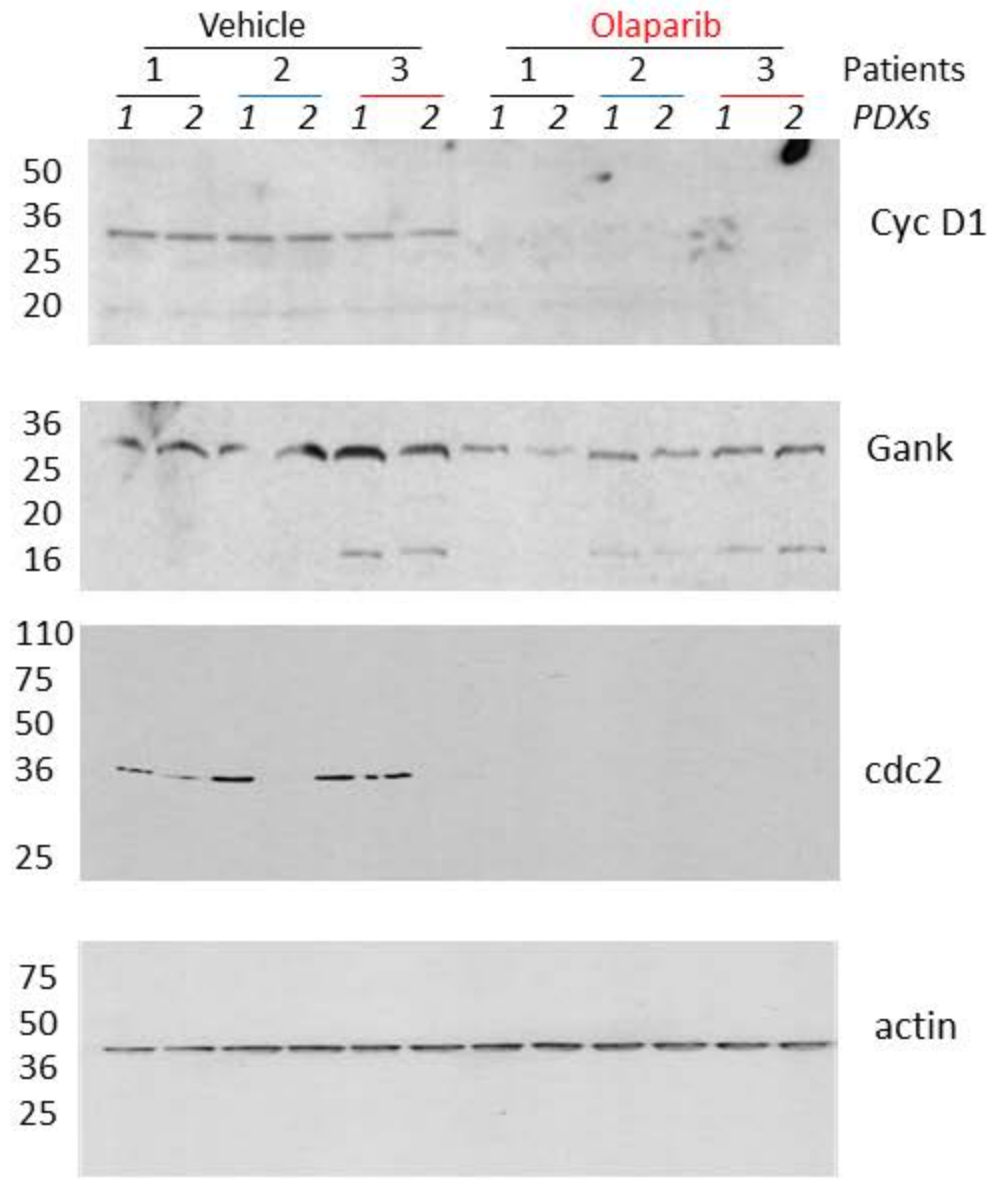


Whole images

Supplemental Figure 19

Johnston Fig 5E

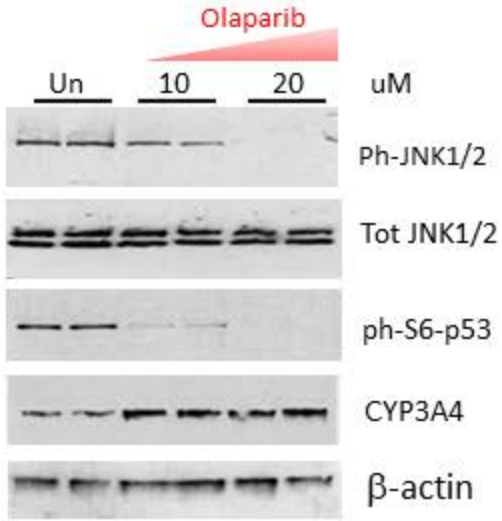


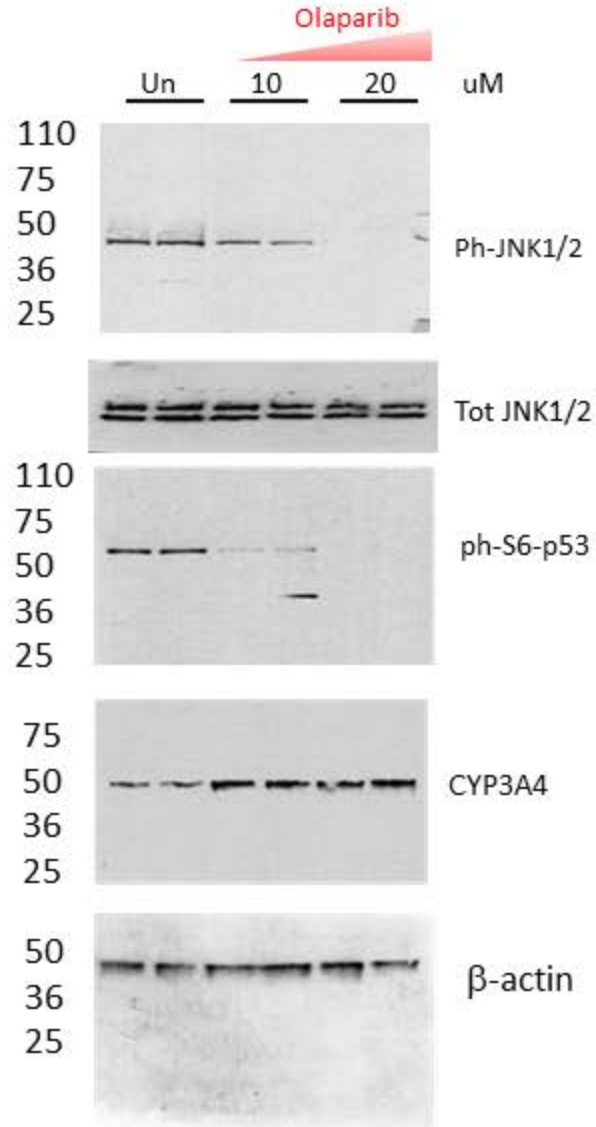


Supplemental Figure 20

Whole images

Fig 7A

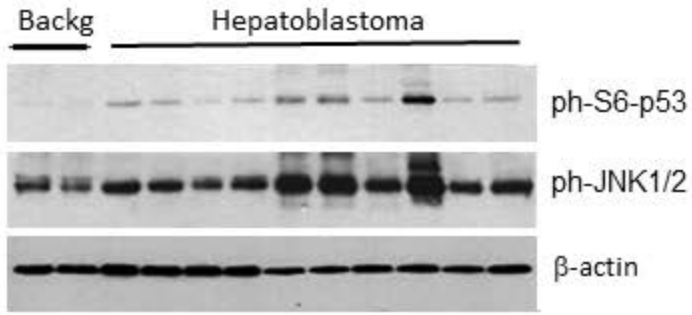


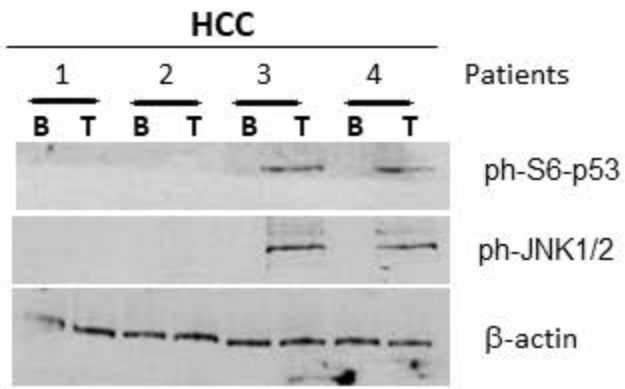


Full images

Supplemental Figure 21

Fig 7C





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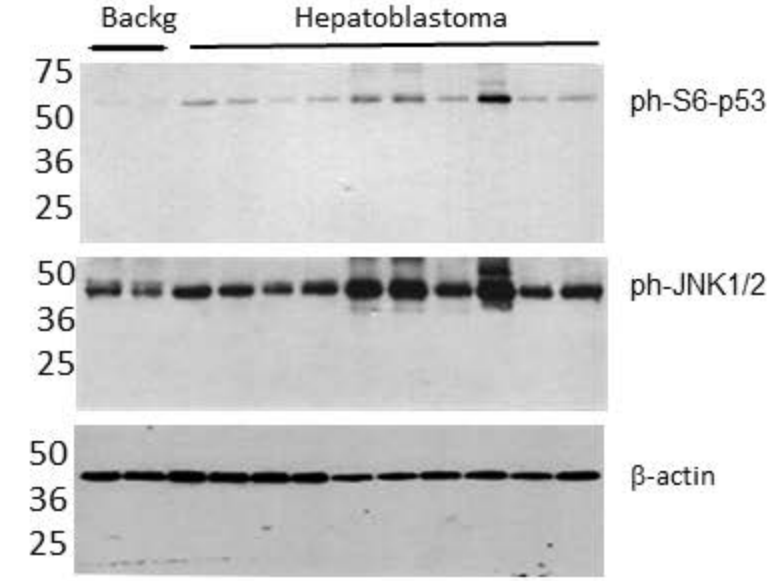

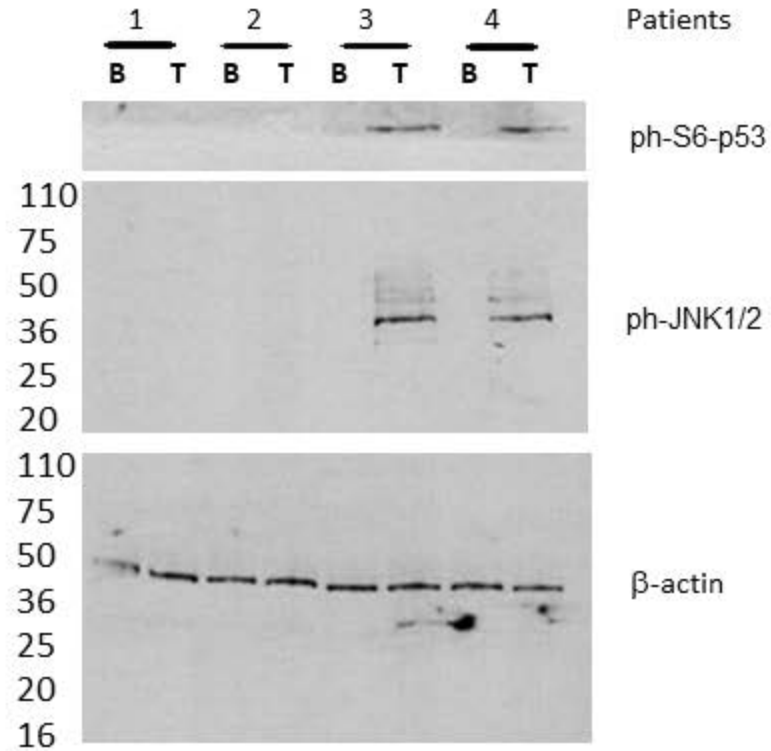

Supplemental Figure 22