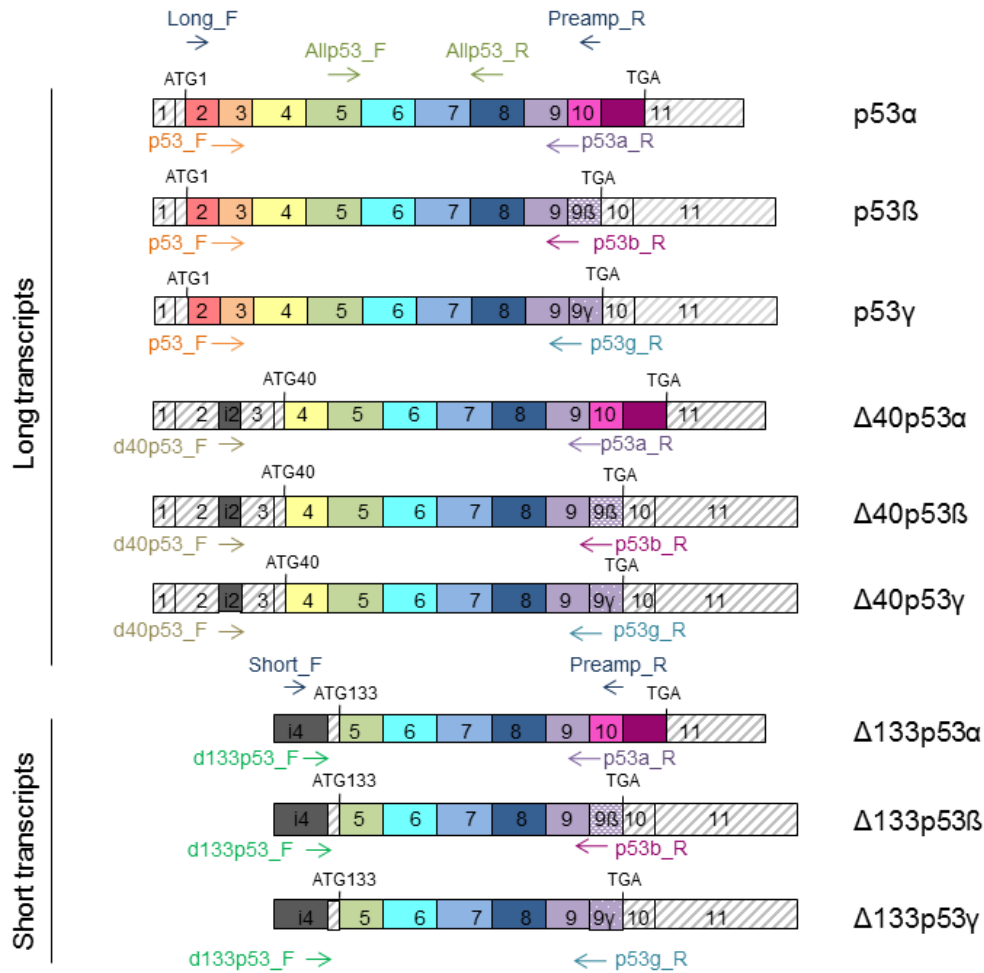
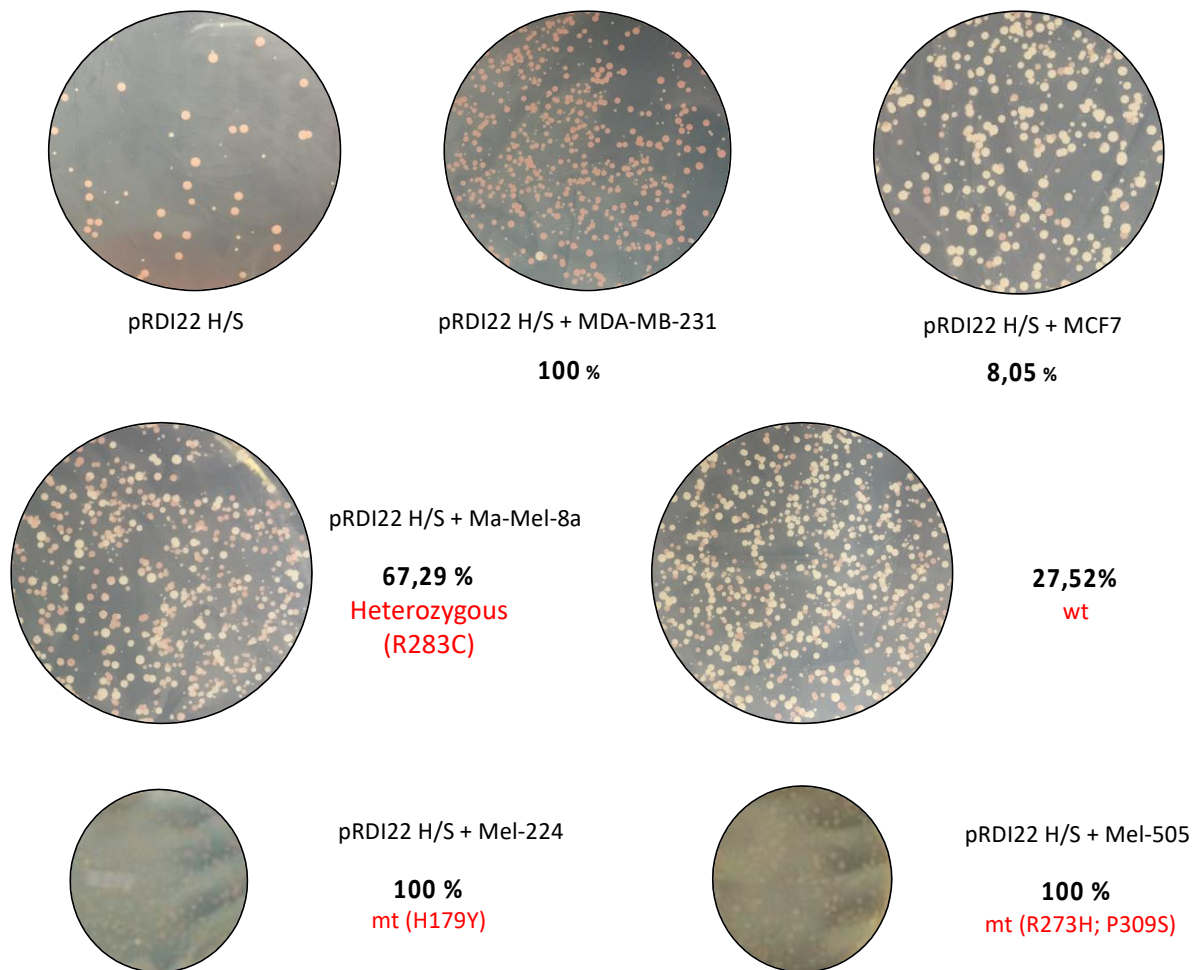


	CELL LINES	Abbreviation*	MELAMOMA STAGE	STATUS p53	ClinVar p53	STATUS BRAF
PRIMARY	WM793B	CRL-2806	primary	wt	-	p.V600E
	WM278	CVCL_6473	primary	wt	-	p.V600E
	LM6 (4405P)	CVCL_WM23	primary	Null	-	wt
	Mel-505	CVCL_3976	primary	p.R273H p.P309S	12366 P 458575 UC	wt
	Mel-224	CVCL_U915	primary	p.H179Y	127815 P/LiP	wt
	G-361	CVCL_122	primary	wt	-	p. V600E
METASTATIC	A375	CRL-7904	metastatic	wt	-	p.V600E
	A375M	CVCL_B222	metastatic	wt	-	p.V600E
	Mel-501	CVCL_4633	metastatic	wt	-	wt
	CHL-1	CRL-9446	metastatic	p.H193R	184979 LiP	wt
	SK-MEL-2	CVCL_0069	metastatic	p.G245S	12365 P	wt
	SK-MEL-3	CVCL_0550	metastatic	p.R267W	141764 LiP	p.V600E
	SK-MEL-5	CVCL_0527	metastatic	wt	-	p.V600E
	SK-MEL-24	CVCL_0599	metastatic	wt	-	p.V600E
	SK-MEL-28	CVCL_0526	metastatic	p.L145R	1027110 UC	p.V600E
	MeWo	CVCL_0445	metastatic	p.E258K p.Q317*	12348 P/LiP 450344 P/LiP	wt
	RPMI-7951	CVCL_1666	metastatic	p.S166*	528239 UC	p.V600E
	WM266-4	CVCL_2765	metastatic	wt	-	p.V600D
	Ma-Mel-8a	Univ. Essen	metastatic	Het R283C	127824 UC	wt
	Ma-Mel-35	CVCL_A170	metastatic	wt	-	wt
	Ma-Mel-54a	CVCL_A189	metastatic	wt	-	p.V600E
	Ma-Mel-55	CVCL_A190	metastatic	wt	-	p.V600E
	Ma-Mel-61b	CVCL_C292	metastatic	wt	-	p.V600E
	Ma-Mel-61f	CVCL_S668	metastatic	wt	-	p.V600E
	Ma-Mel-86a	CVCL_A221	metastatic	p.R248W	12347 P	p.V600E
	Ma-Mel-86c	Univ. Essen	metastatic	p.R248W	12347 P	p.V600E
	<ul style="list-style-type: none"> <li>Abbreviations from ATCC or Cellosaurus database</li> <li>P, Pathogenic; LiP, Likely pathogenic; UC, Uncertain</li> <li>p53 data from <a href="http://p53.iarc.fr/CellLines.aspx">http://p53.iarc.fr/CellLines.aspx</a></li> </ul>					

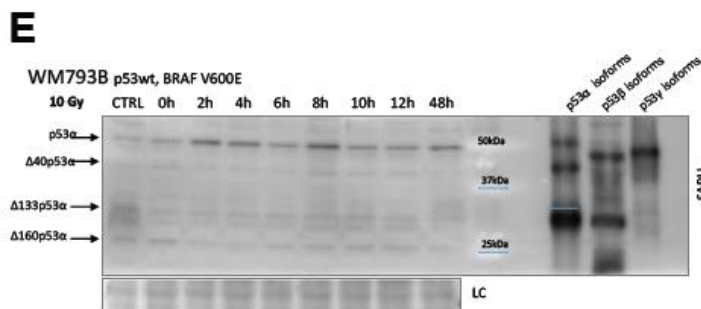
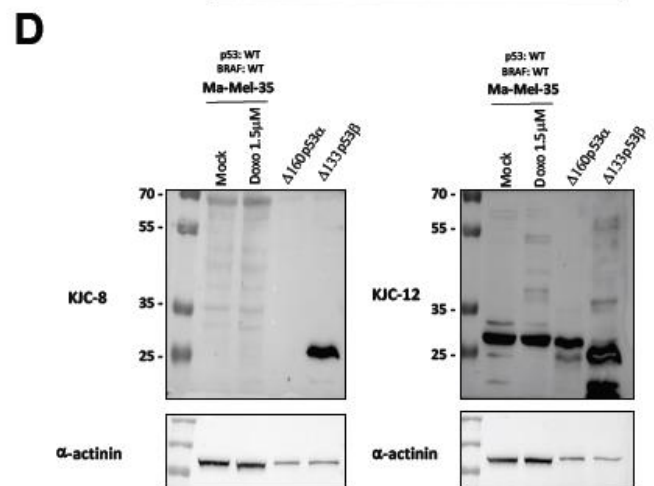
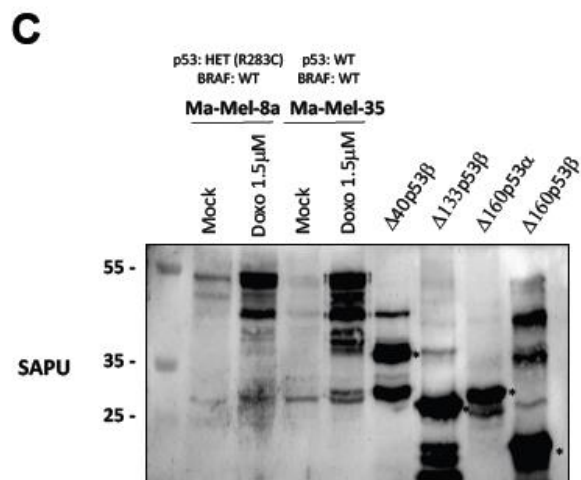
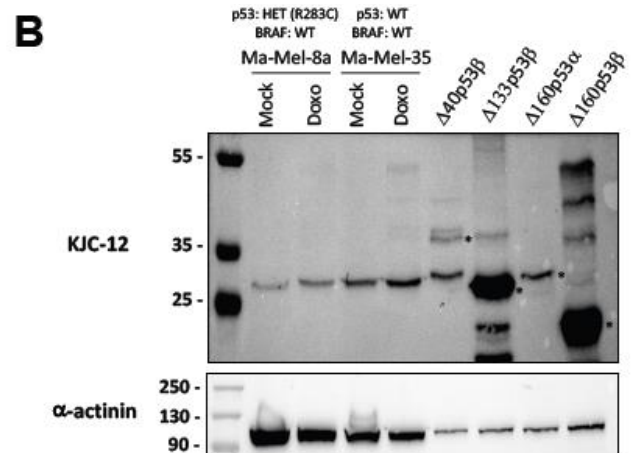
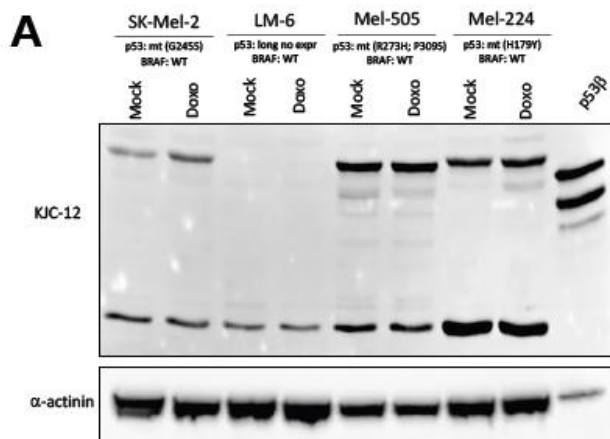


**Figure S1. Position of primers used to quantify the gene expression of TP53 isoforms.** First, two separate PCR reactions were performed to distinguish “long” full-length and Δ40 from “short” Δ133 isoforms. Generated pre-amplified cDNA templates were used for nested qPCR with combinations of three forward and three reverse isoform-specific primers.

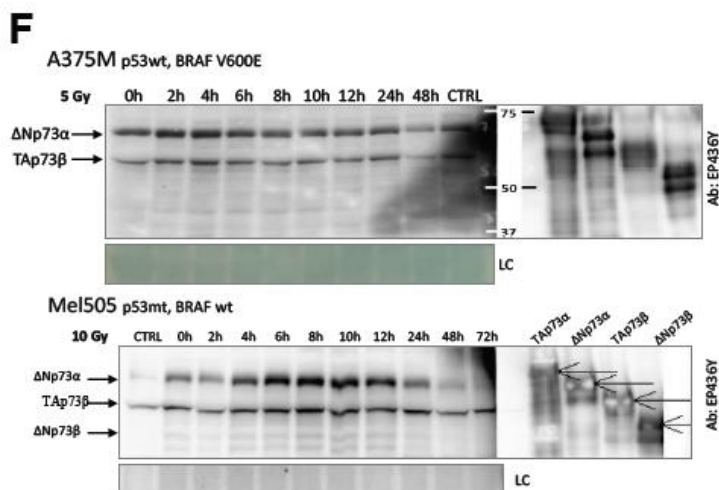
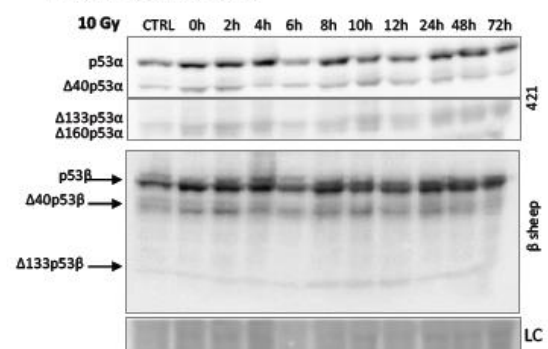
**FASAY**  
Functional Analysis of Separated Alleles in Yeast



**Figure S2. FASAY assays on melanoma cell lines with uncertain *TP53* status.** FASAY (Functional Analysis of Separate Alleles in Yeast) assay was done for all the cell lines with a doubtful/unconfirmed *TP53* status. PCR on the cDNA from melanoma-derived cell lines was performed on *TP53* coding region with p53-P3 and p53-P4 primers. PCR products and linearized pRDI22 vector were co-transformed in yeast cells to allow for homologous recombination in vivo. The sample pRDI22 digested with Hind III and Stu I restriction endonucleases (pRDI22 H/S) was used as negative control and represented the background of red colonies. The analysis of MDA-MB-231 and MCF7 cells served as positive and negative controls for the presence of a mutant or wild-type p53, respectively. Indicated are the percentages of red colonies and the *TP53* status.



Mel505 p53mt, BRAF wt



G

Figure 3C - upper panel A375M

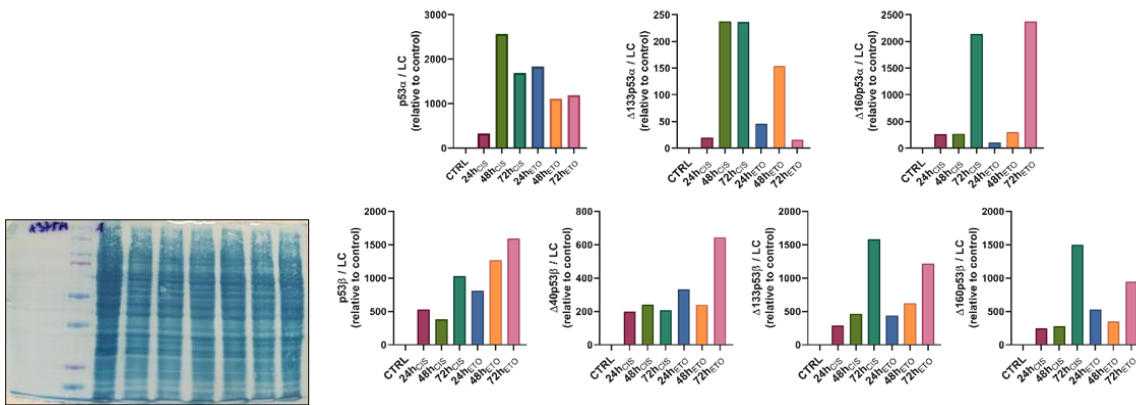


Figure 3C - lower panel WM793B

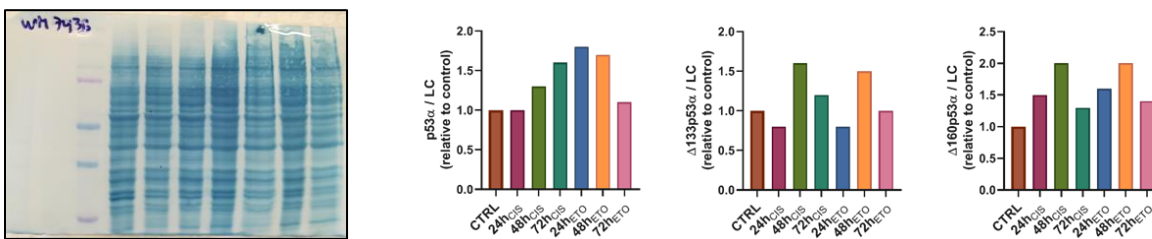


Figure 3D - upper panel A375M 5Gy

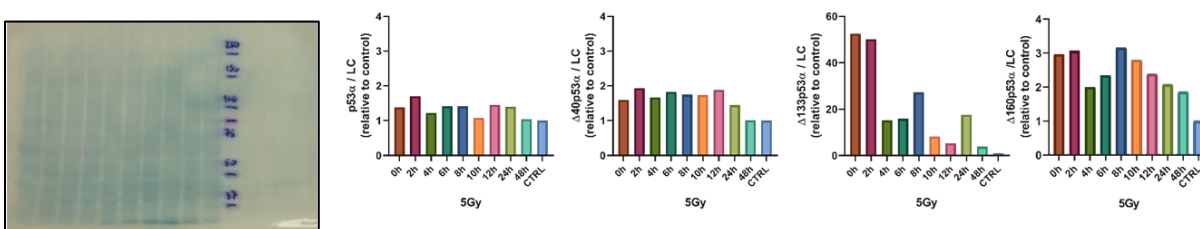


Figure 3D - lower panel A375M 10Gy

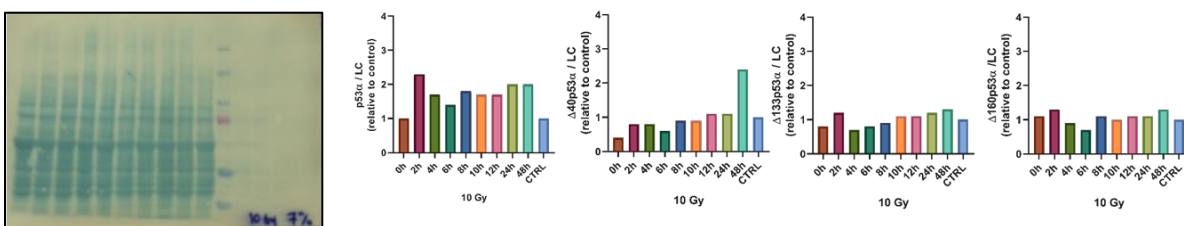
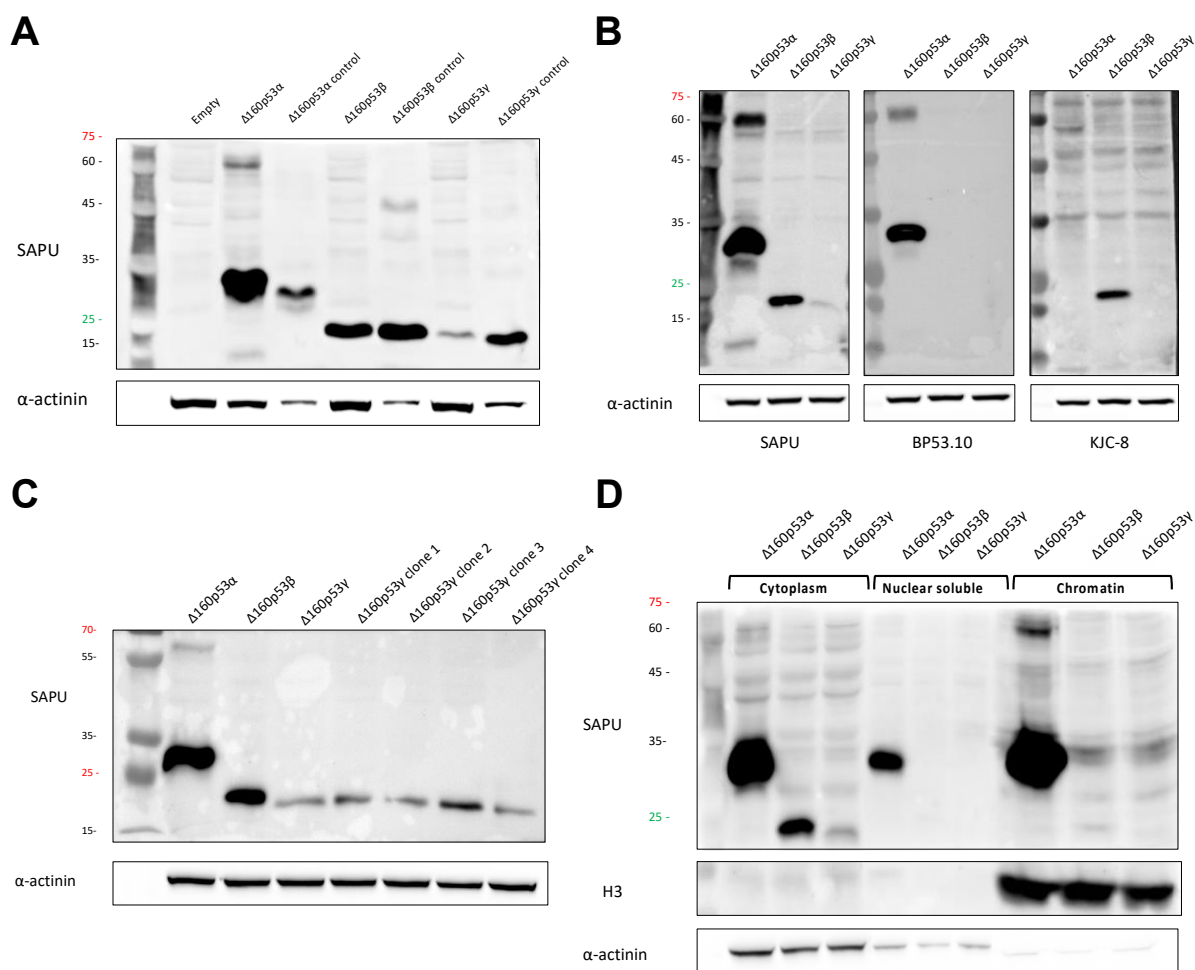


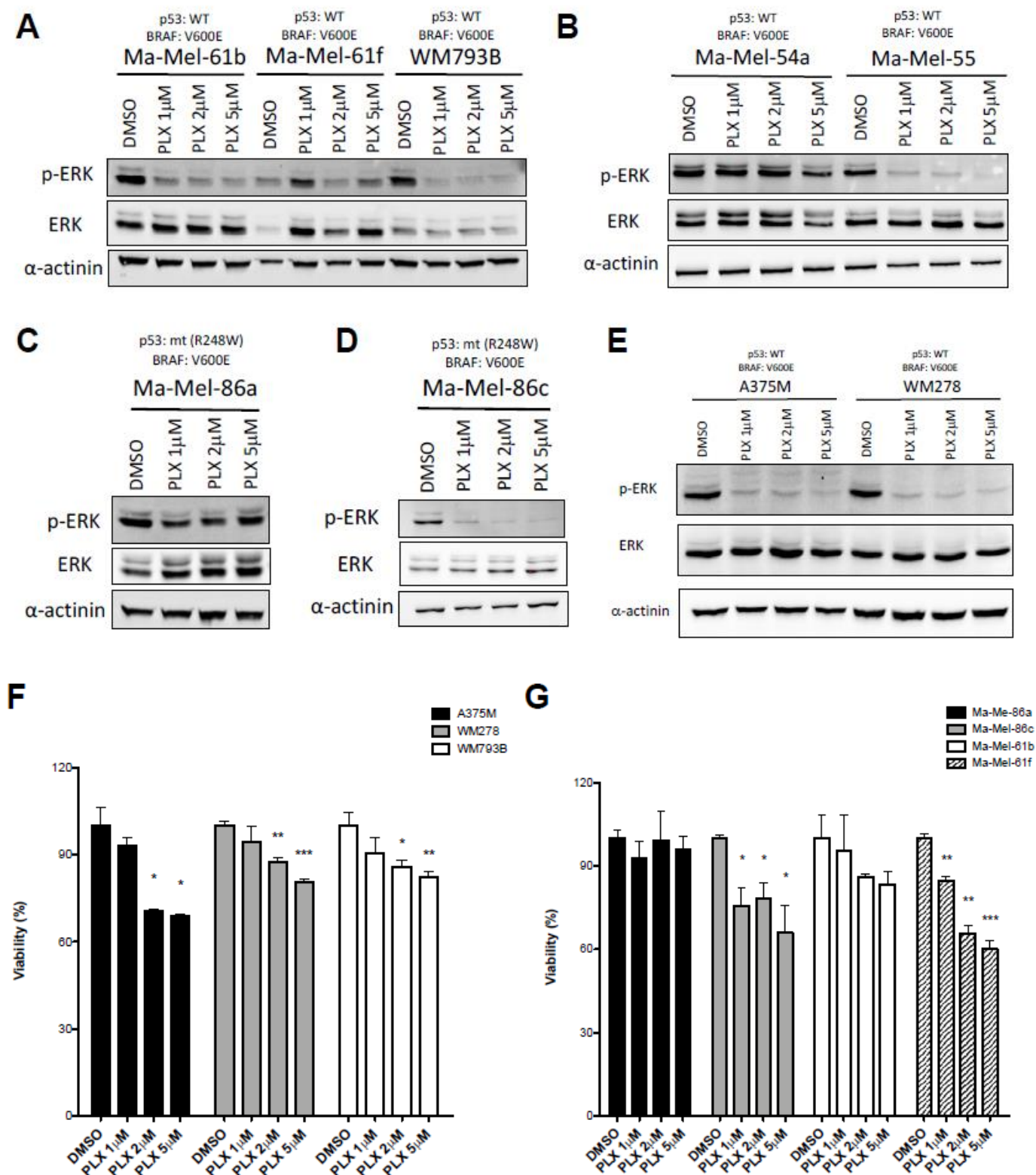
Figure S3. p53 isoforms are differentially expressed in melanoma cell lines in response to DNA damage. (A) An additional group of melanoma-derived cell lines was analyzed to determine the p53 isoforms expression levels upon treatment with 1.5  $\mu$ M doxorubicin for 24 h. (B-D) Given the highly variability in expression of a low molecular weight p53 isoform among the different melanoma-derived cell lines, to pinpoint the nature of that p53 isoform, we performed western blot



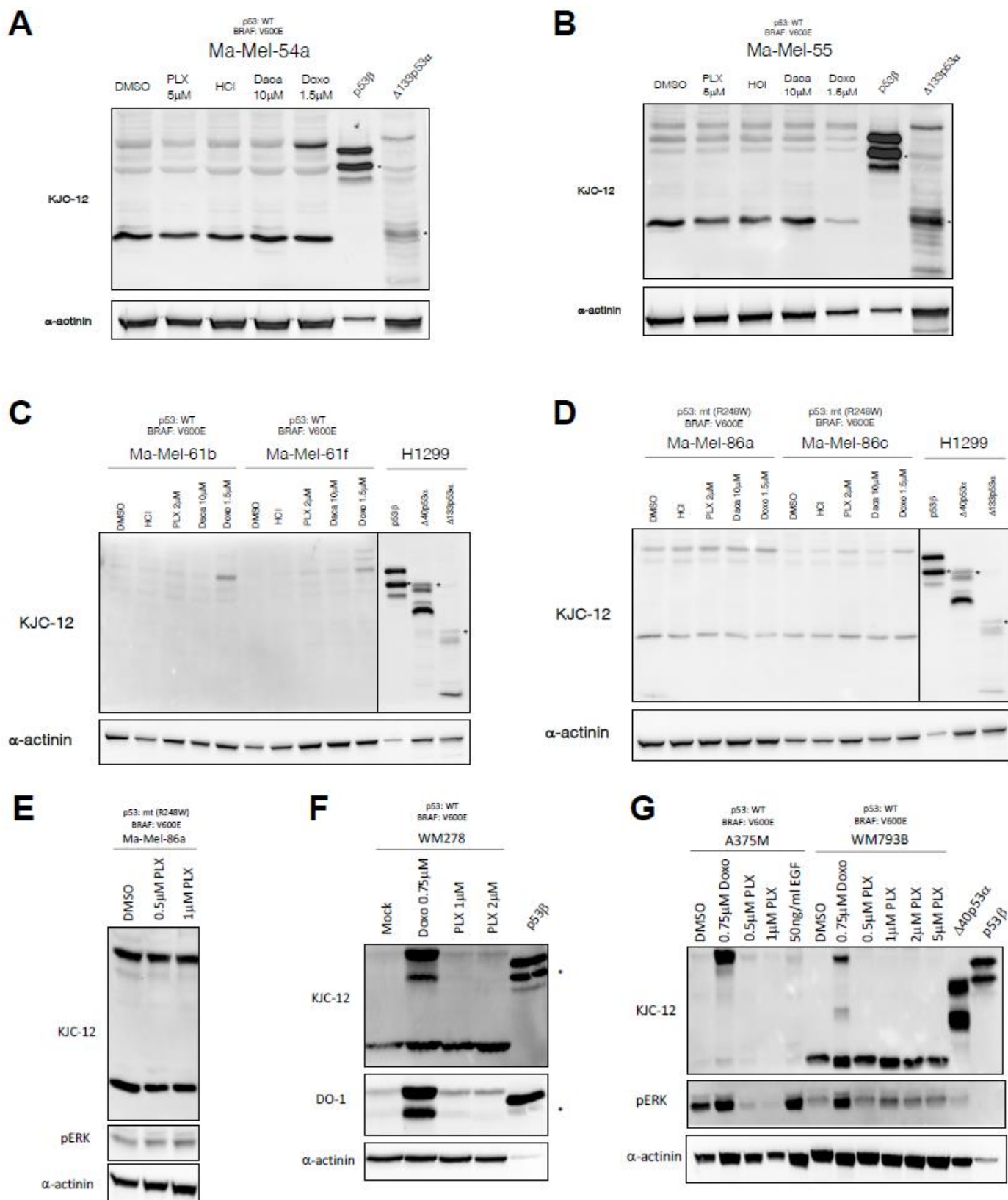
using different pantropic (SAPU and KJC-12) as well as isoform-specific antibodies (KJC-8,  $\beta$ -specific) in additional cell lines Ma-Mel-8a and Ma-Mel-35 cells. To better visualize full length p53 isoforms cells were treated with 1.5  $\mu$ M doxorubicin for 24 h. As a control we used transient over-expression of specific p53 isoforms in H1299 cells. (E) Two additional melanoma cell lines, WM793B, a p53 wt cell line, and Mel505, a p53 mut, were analyzed after  $\gamma$ -irradiation to determine p53 isoforms expression. To detect p53 isoforms in WM793B cells pantropic anti-p53 antibody (SAPU) was used. To distinguish  $\alpha$  and  $\beta$  p53 isoforms after  $\gamma$ -irradiation in Mel505 cells,  $\alpha$ - and  $\beta$ -specific anti-p53 antibodies (421 and  $\beta$  sheep, respectively) were used. (F) To detect the expression of p73 isoforms after  $\gamma$ -irradiation in two melanoma cell lines, we used pantropic anti-p73 antibody (EP436Y). (G) Naphthol blue stained membranes and quantification of the expression of p53/p73 proteins shown in Figure 3C-D using Image Lab software 6. The expression of p53/p73 proteins was normalized to the intensity of the naphthol blue membrane staining of the same samples. Three random strips of naphthol blue staining (a representative one is shown in the Figure 3C,D) were quantified and their sum was used for normalization of the expression of a protein of interest.



**Figure S4. Generation of H1299 cell lines stably over-expressing  $\Delta$ 160p53 $\alpha$ ,  $\Delta$ 160p53 $\beta$  and  $\Delta$ 133p53 $\gamma$ .** (A) Western blot analysis with SAPU antibody on protein extracts from H1299 cells stably transfected with the indicated  $\Delta$ 160p53 isoforms. As a control we used transient transfection in H1299 cells. (B) To confirm  $\alpha$  or  $\beta$  specificity, protein extracts from panel A were also detected with  $\alpha$ -specific (BP53.10) and  $\beta$ -specific (KJC-8) antibodies. (C) Western blot analysis with SAPU antibody on different single cell H1299 clones stably over-expressing  $\Delta$ 160p53 $\gamma$  isoform to check putative variability in expression among clones.  $\alpha$ -actinin was used as loading control in all the blots. (D) Cellular fractionation of H1299 cells stably over-expressing  $\Delta$ 160p53 isoforms. SAPU antibody was used to detect the  $\Delta$ 160p53 isoforms;  $\alpha$ -actinin was used as loading control for the cytoplasmic protein fraction whereas Histone H3 for the chromatin-enriched protein fraction.



**Figure S5. Evaluation of the sensitivity to vemurafenib in several melanoma cell lines.** In order to evaluate the sensitivity of different melanoma cell lines to PLX-4032, cells were treated with increasing concentration of PLX-4032 (1, 2, and 5 μM) for 24 hours. (A-E) As a readout of the sensitivity to PLX-4032, we detected by Western blotting the phosphorylated form of ERK in comparison with total ERK protein. DMSO was used as a control and α-actinin as reference protein. (F, G) Alternatively, the viability of most of the cell lines analyzed with Western blotting was measured with MTT assay upon the treatment with PLX-4032 (1, 2, and 5 μM) for 48 hours. Presented are the averages and the standard deviations of 3 biological experiments. \* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$ , t-student test.





H

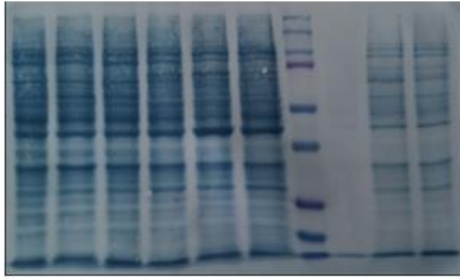


Figure 6B upper panel

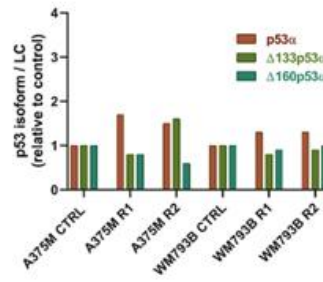


Figure 6D lower panel

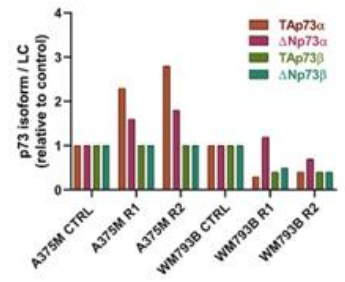


Figure 6B lower panel

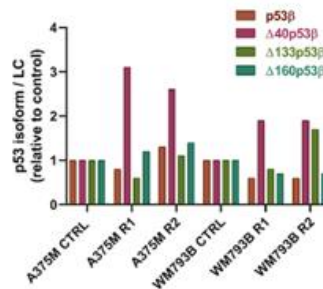
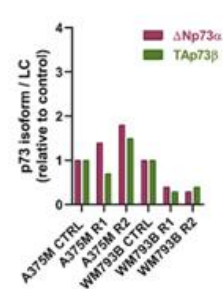


Figure 6D lower panel



**Figure S6. Analysis of the effect of different anti-cancer agents on p53 isoforms' expression.** Several melanoma-derived cell lines with different status of *TP53* were treated with doxorubicin, dacarbazine, and vemurafenib (PLX-4032) anti-cancer agents at the indicated doses for 24 h. DMSO was used as control for vemurafenib and doxorubicin, while HCl for dacarbazine. (A-G) Endogenous p53 isoforms expression was determined by western blotting using the p53 pantropic antibody KJC-12.  $\alpha$ -actinin was used as loading control. To better identify the different p53 isoforms, transient transfections in the p53 null cells H1299 with single isoforms were included (asterisks indicated the position on the membrane of the over-expressed p53 isoform). (E, G) The detection of phosphorylated form of ERK was included as a control for the sensitivity to vemurafenib in BRAF mutated cell lines Ma-Mel-86a, A375M, and WM793B. (F) To better evaluate the accumulation of full length p53 isoforms in response to doxorubicin and vemurafenib in WM278 cells (p53 wild-type and BRAF mutant) cell extracts were incubated with DO-1 antibody able to recognize only p53 $\alpha$ ,  $\beta$ , and  $\gamma$  isoforms. (G) The treatment with 50 ng/ml EGF was included as a positive control for the detection of phospho-ERK protein. (H) Naphthol blue stained membrane used for quantification in Figure 6B (upper panel) and quantification of the expression of p53/p73 proteins shown in Figure 6B and D using Image Lab software 6. The expression of p53/p73 proteins was normalized to the expression level of  $\beta$ -actin of the same samples.

Original uncropped blots

Figure 2A

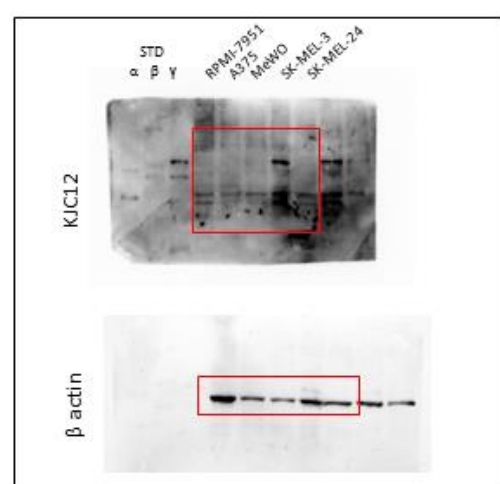
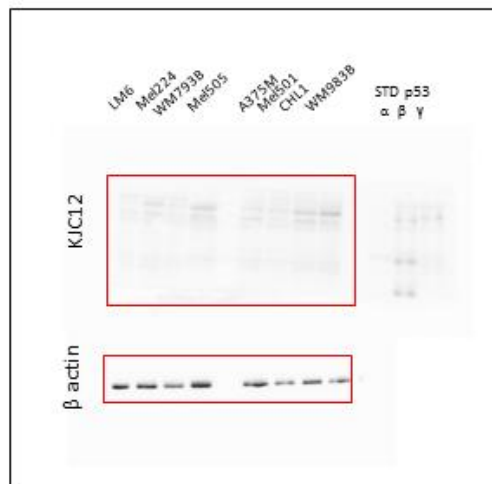
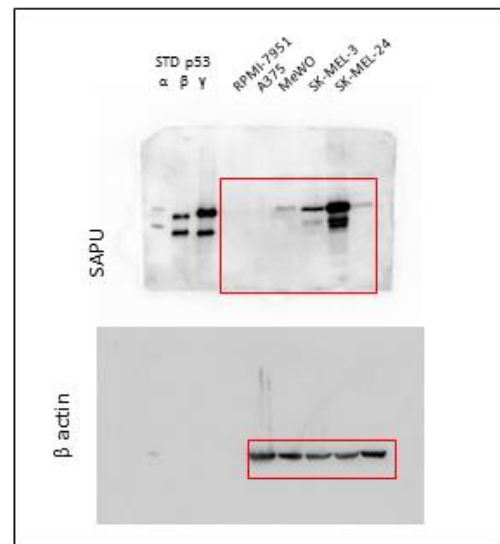
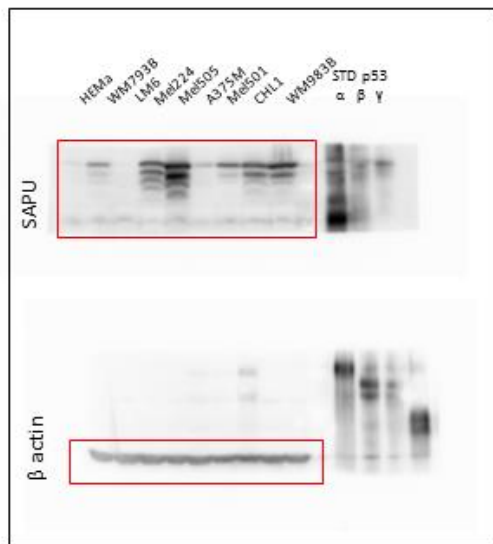


Figure 2B

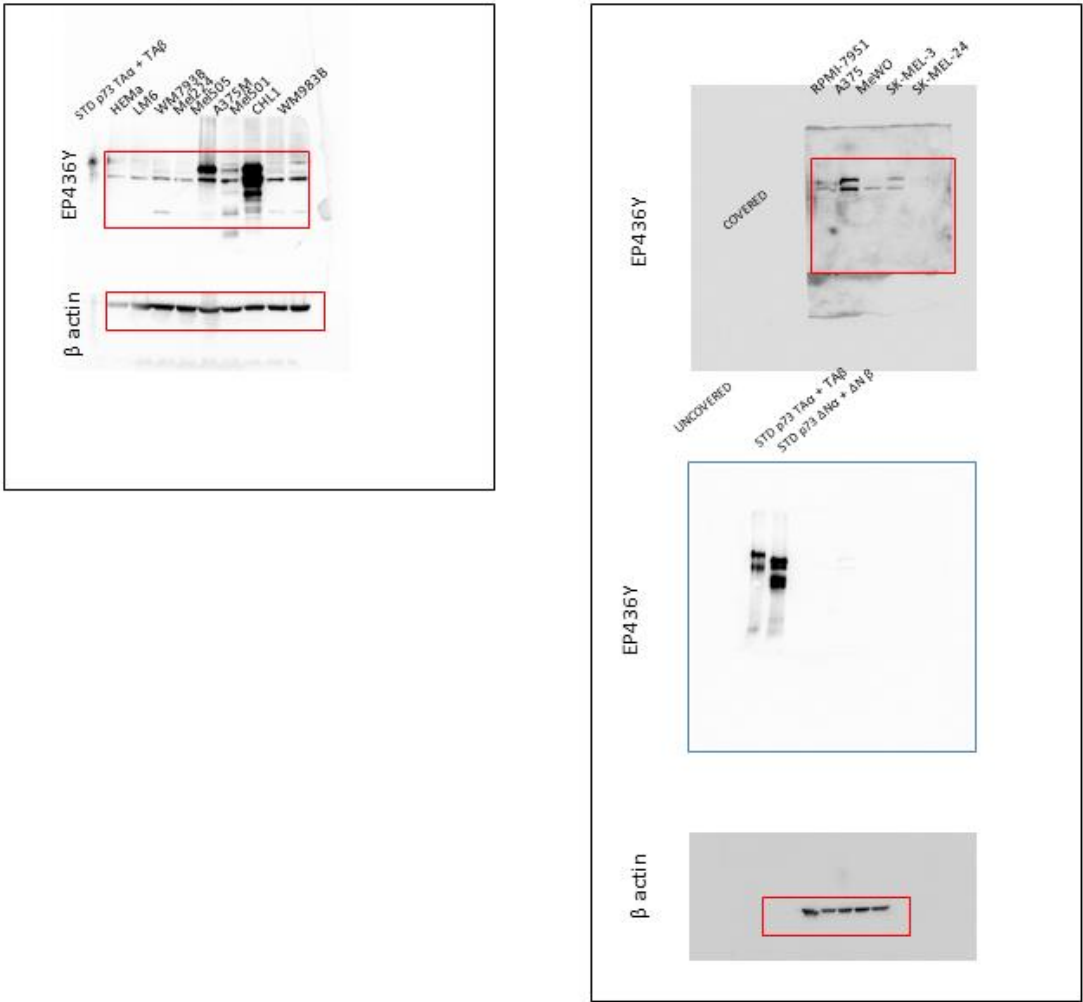
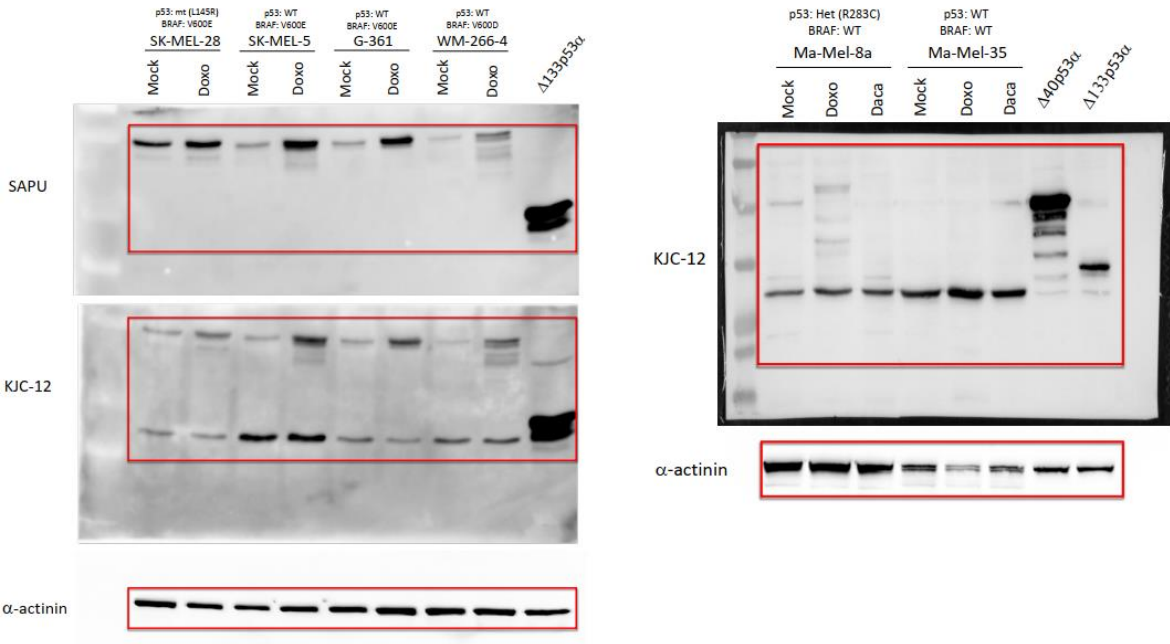
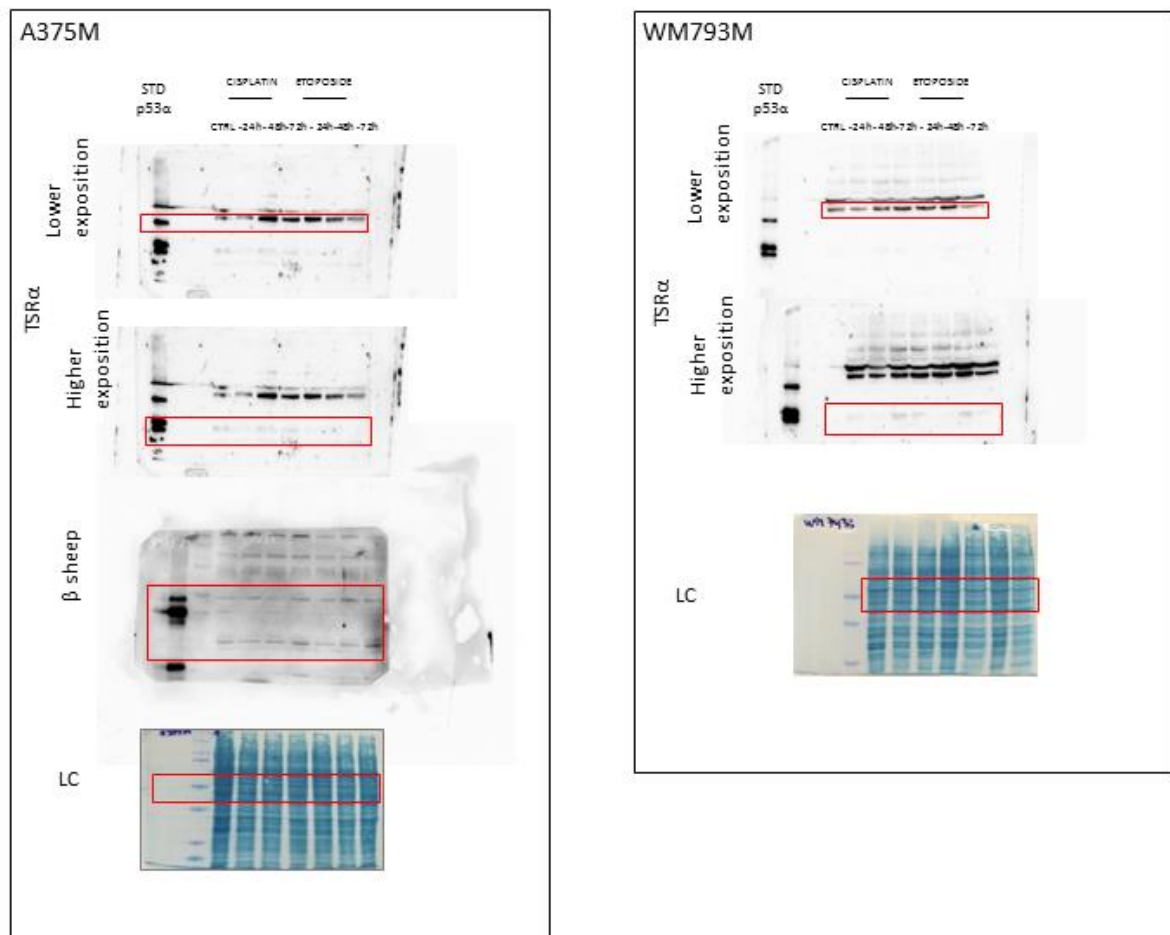


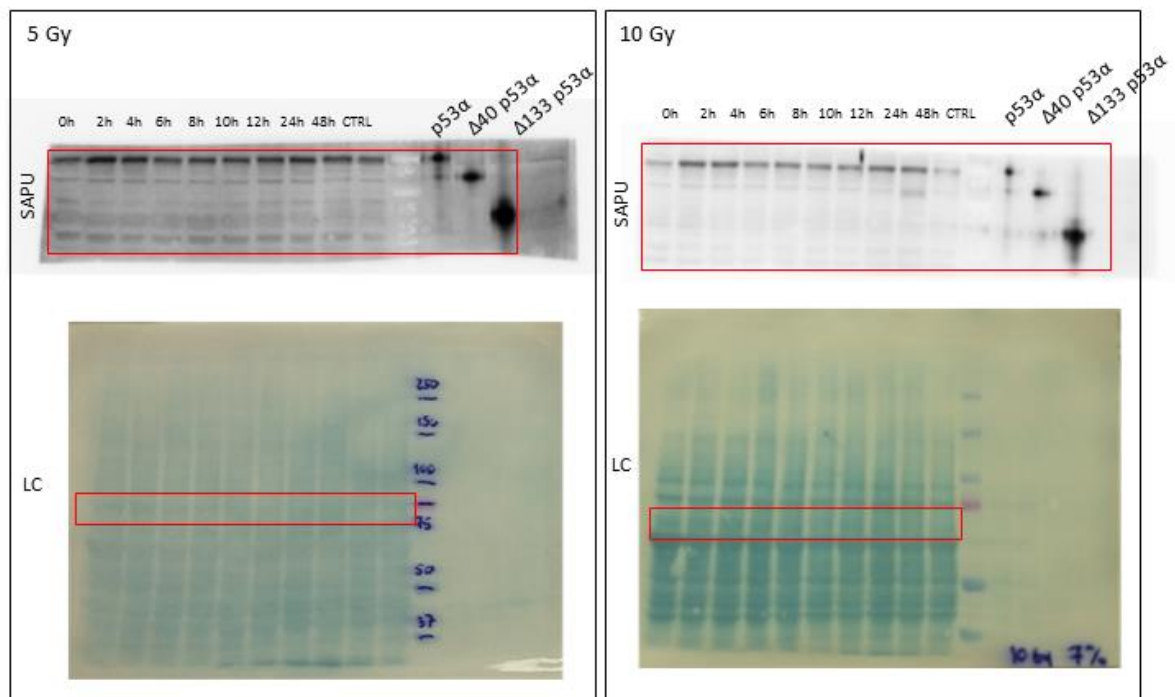
Figure 3A-B



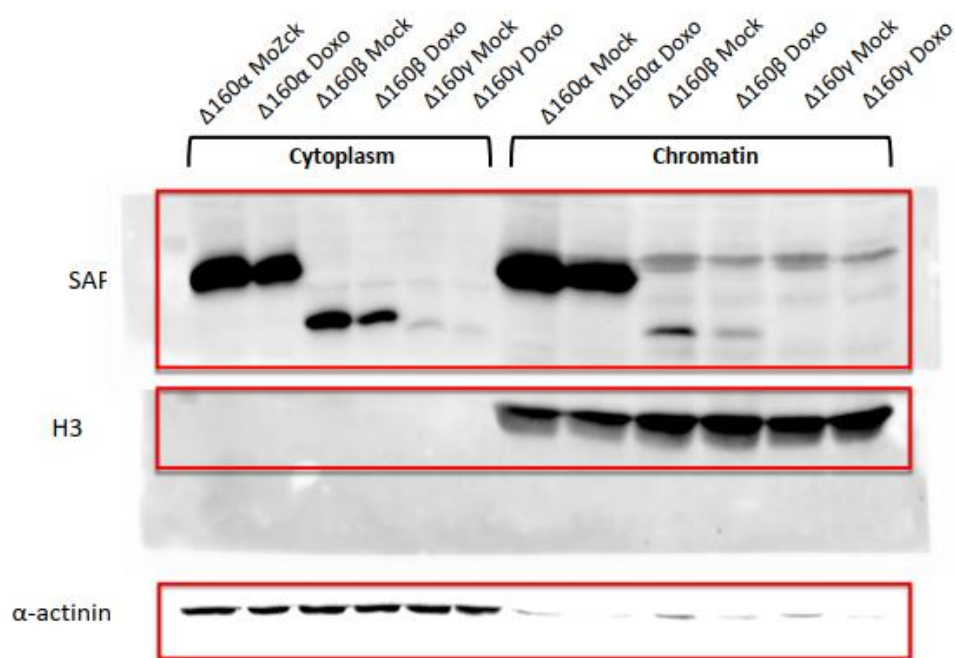
**Figure 3C**



**Figure 3D**



# Figure 4



## Figure 5B

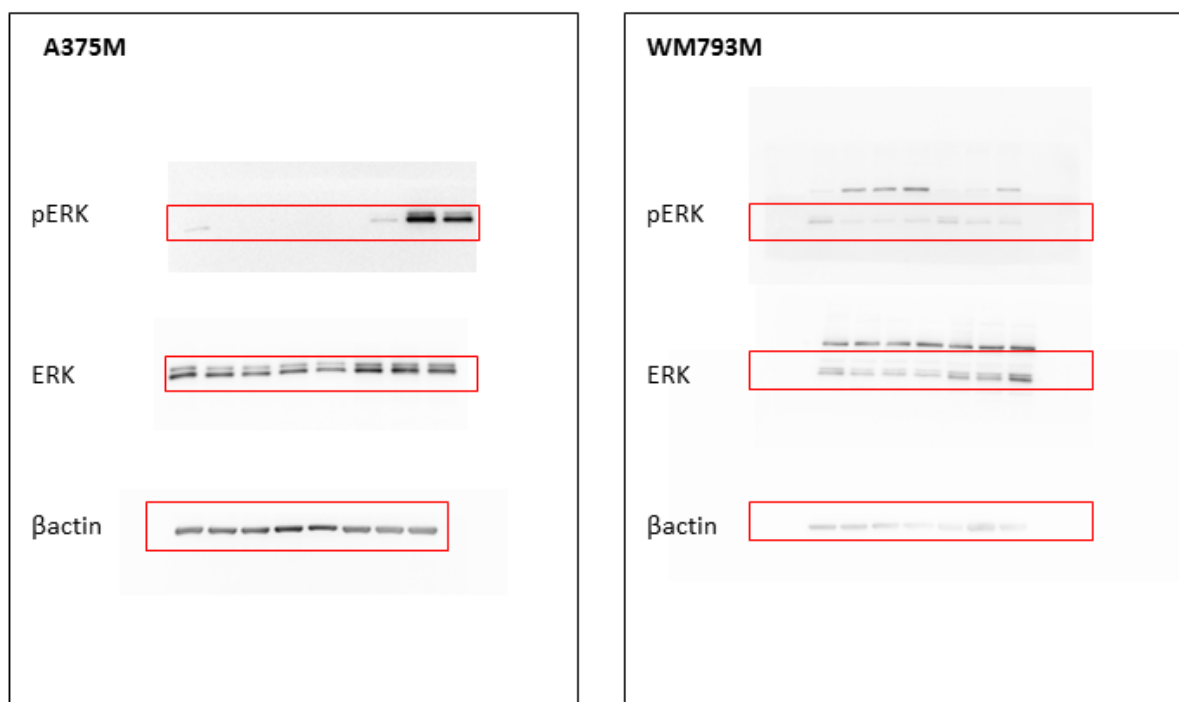
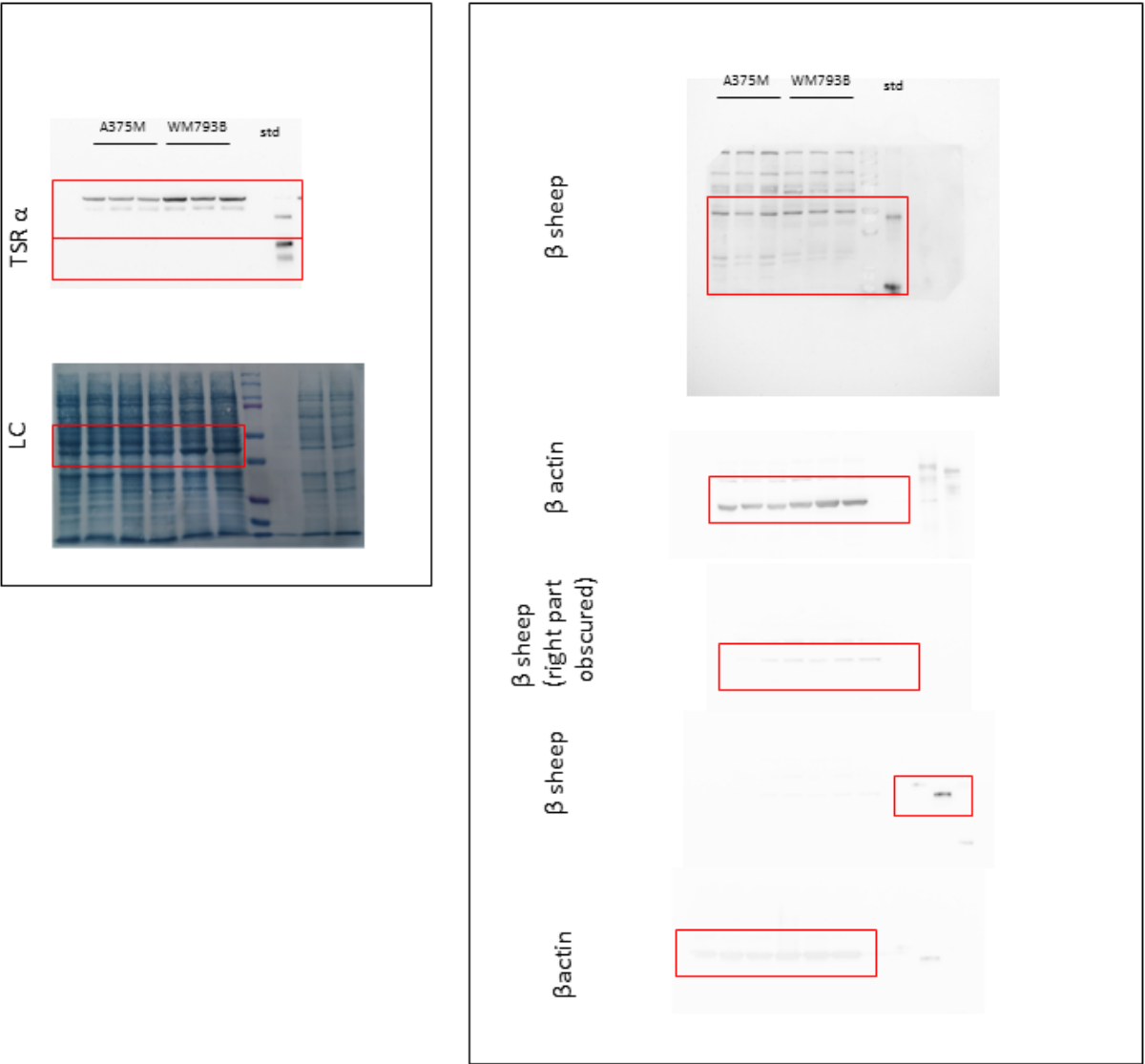




Figure 6B



**Figure 6D**

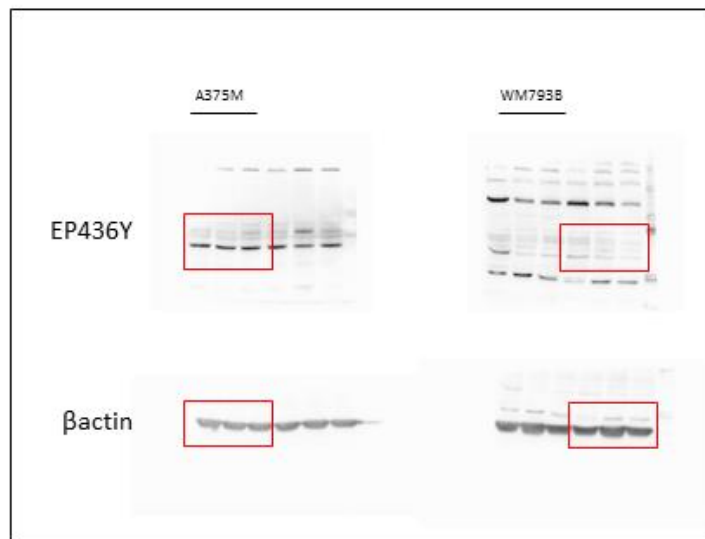


Figure S3A-D

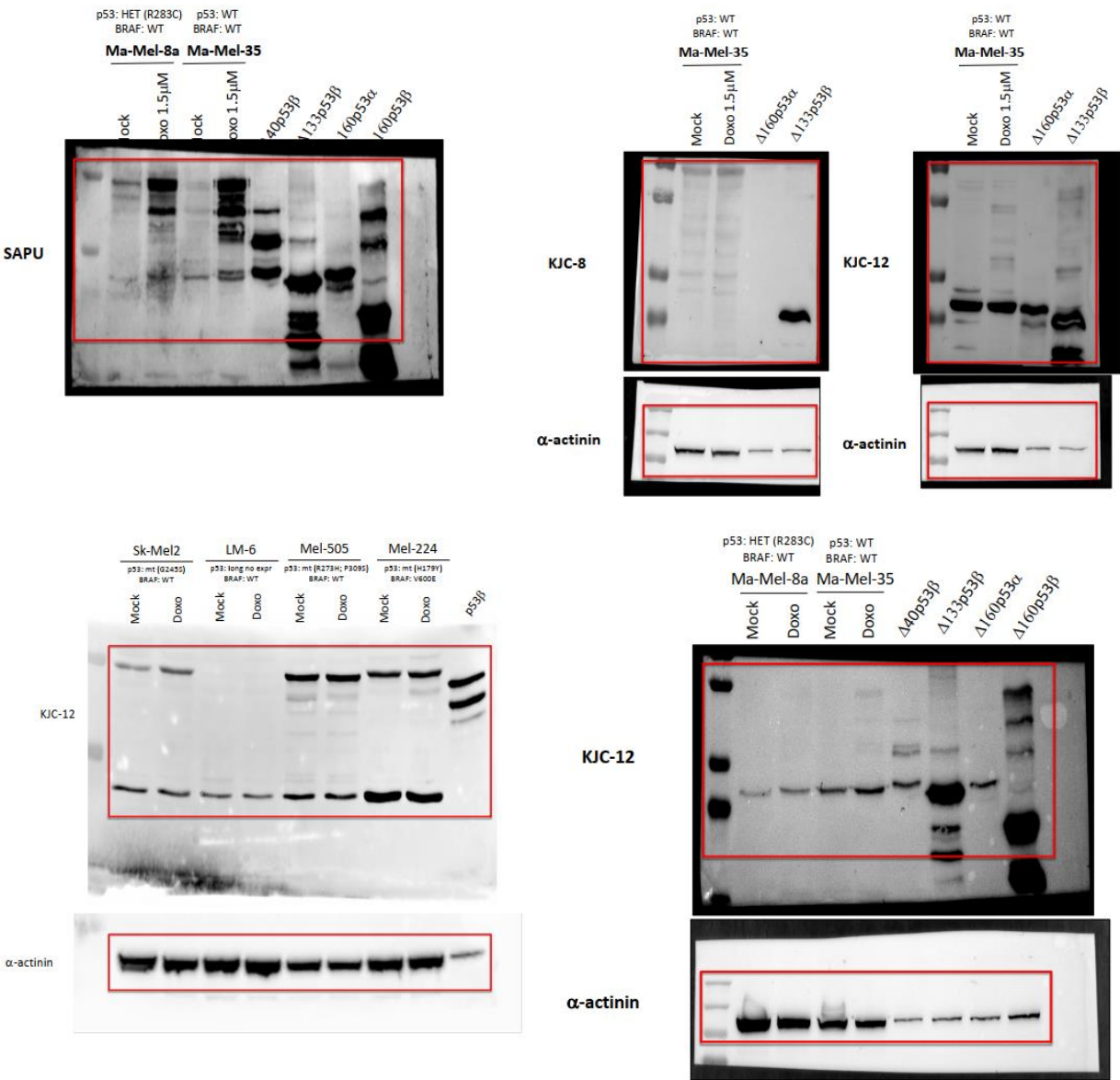
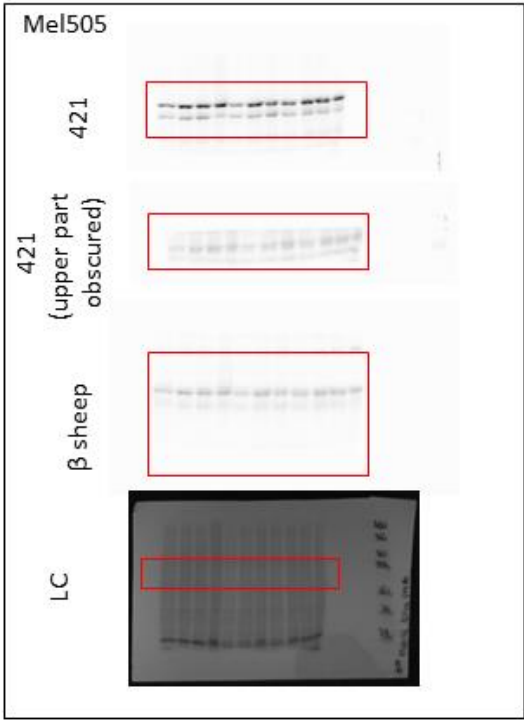
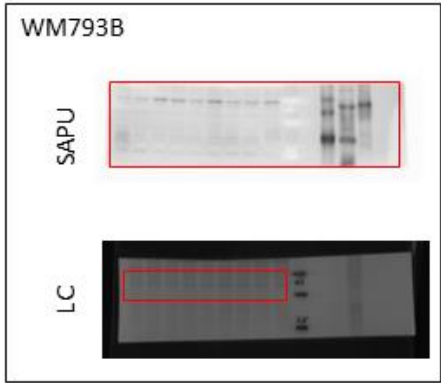


Figure S3

E



F

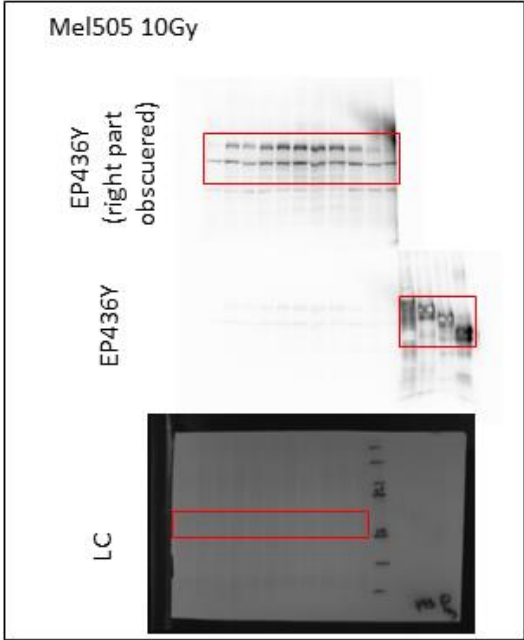
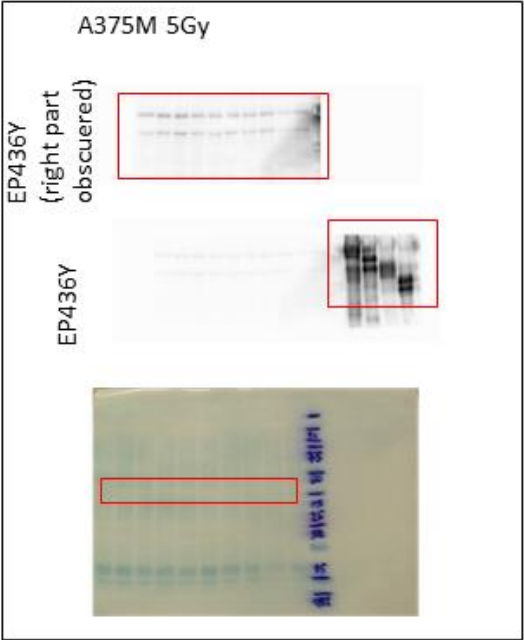


Figure S4

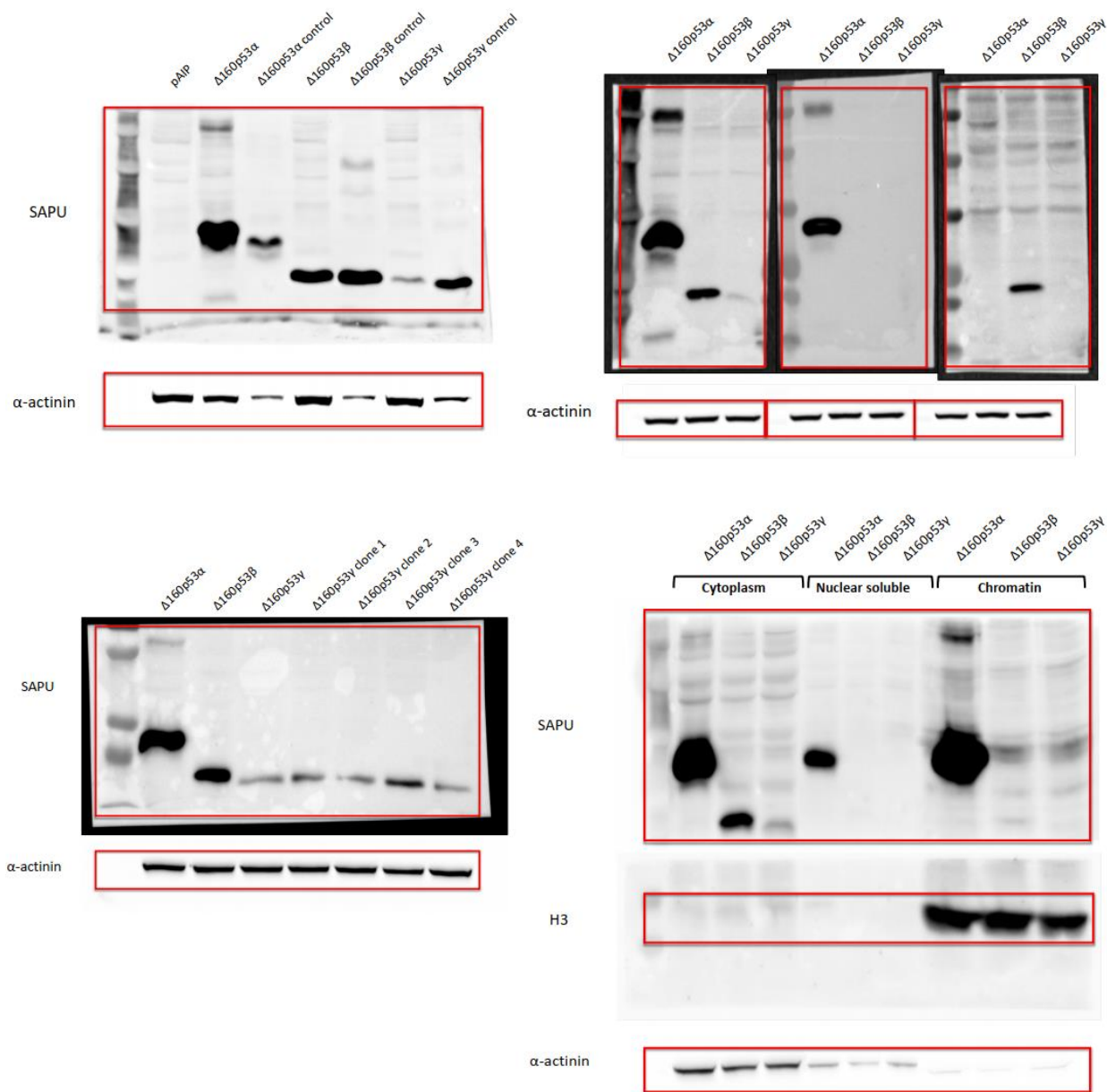
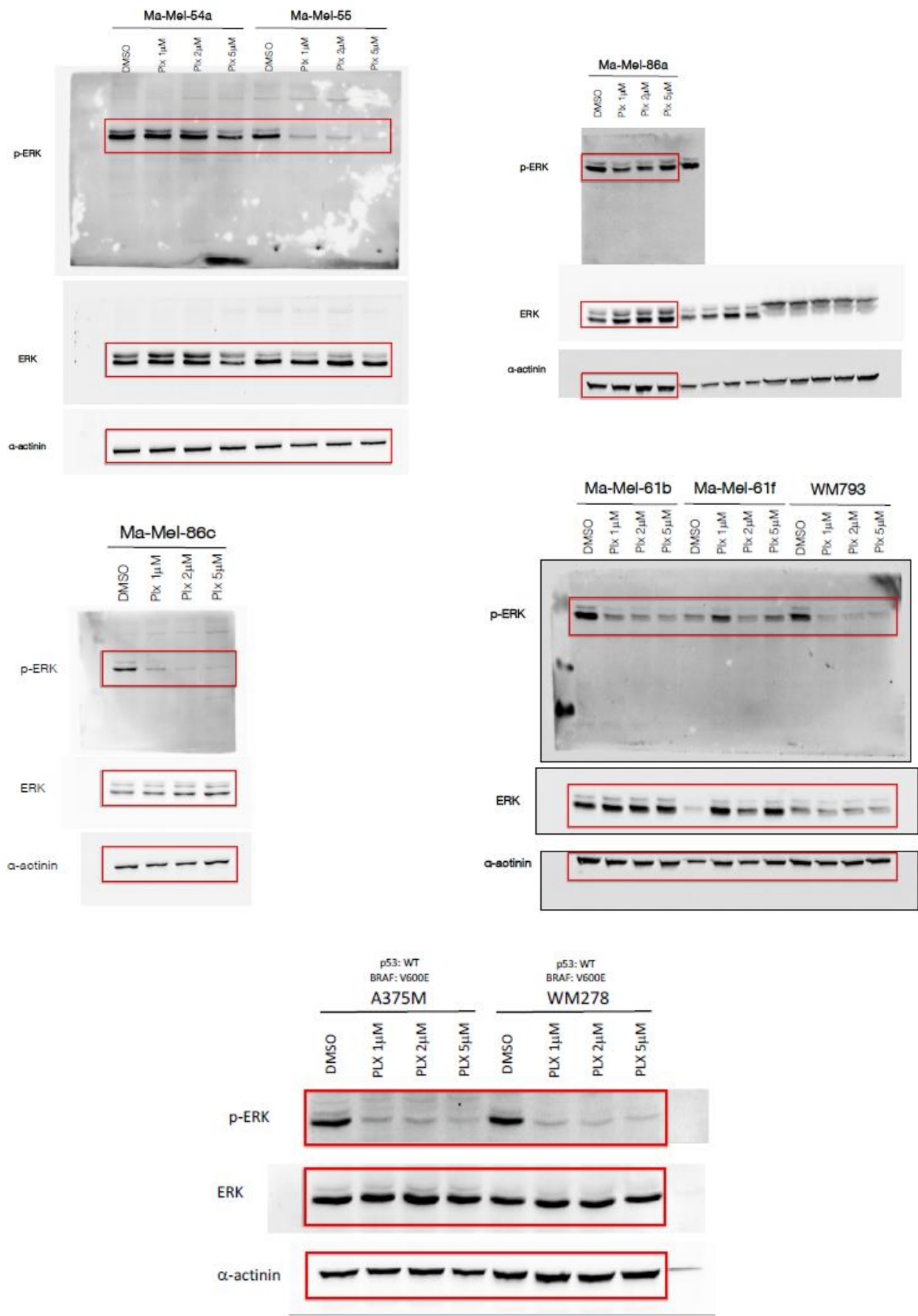
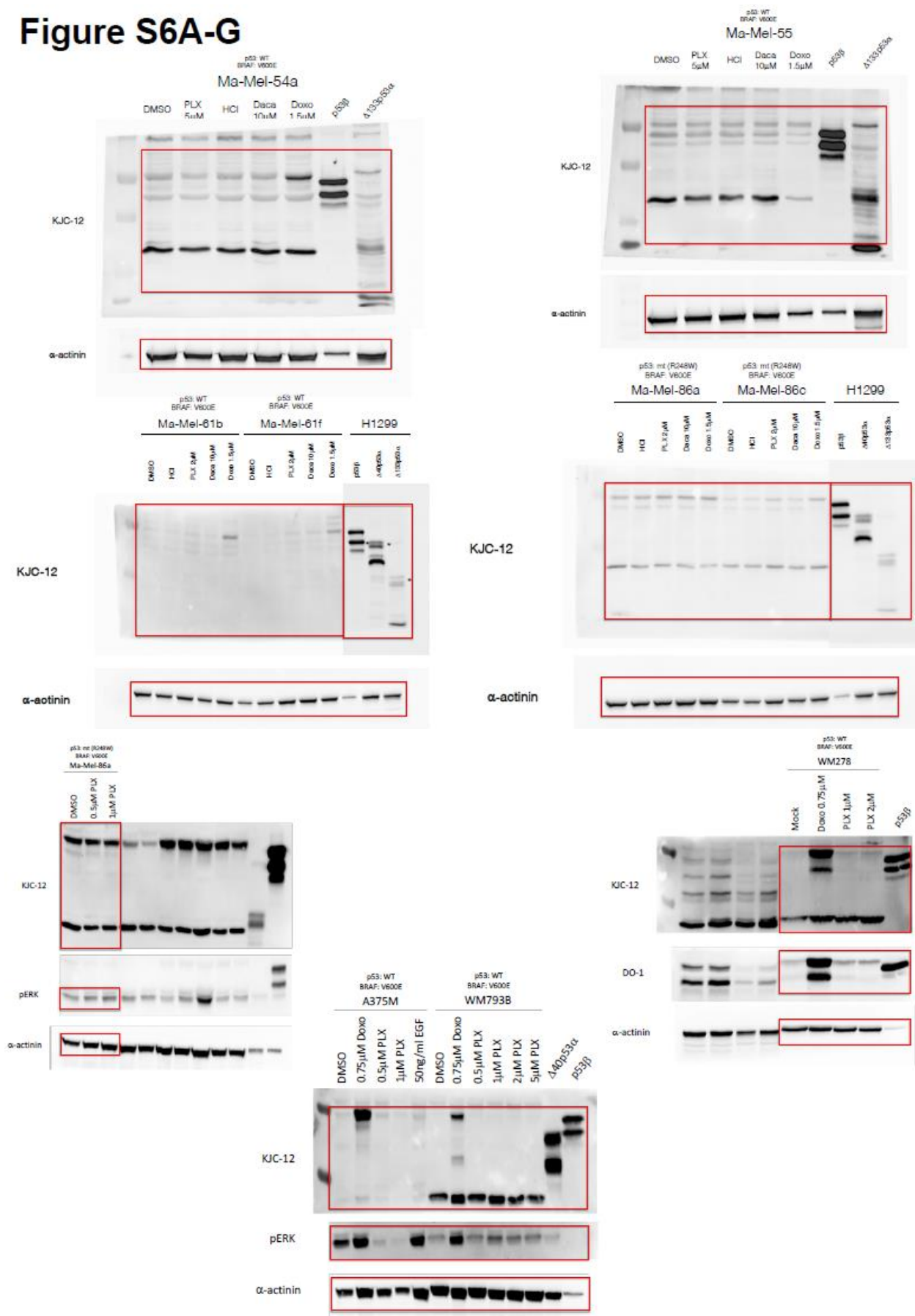




Figure S5A-E



### Figure S6A-G



**Figure S7.** Original uncropped blots.