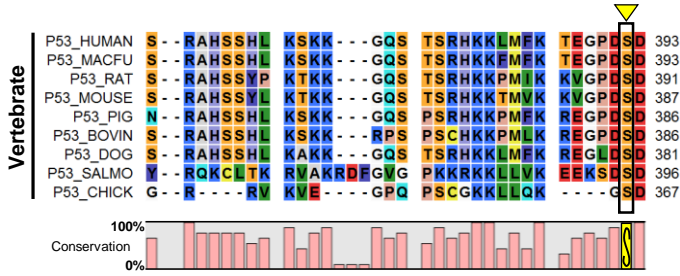


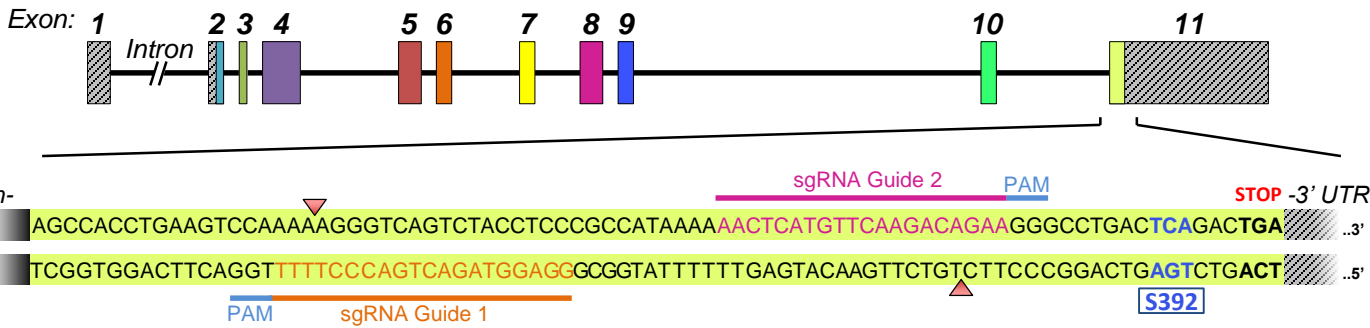
# Supplemental data :

S1



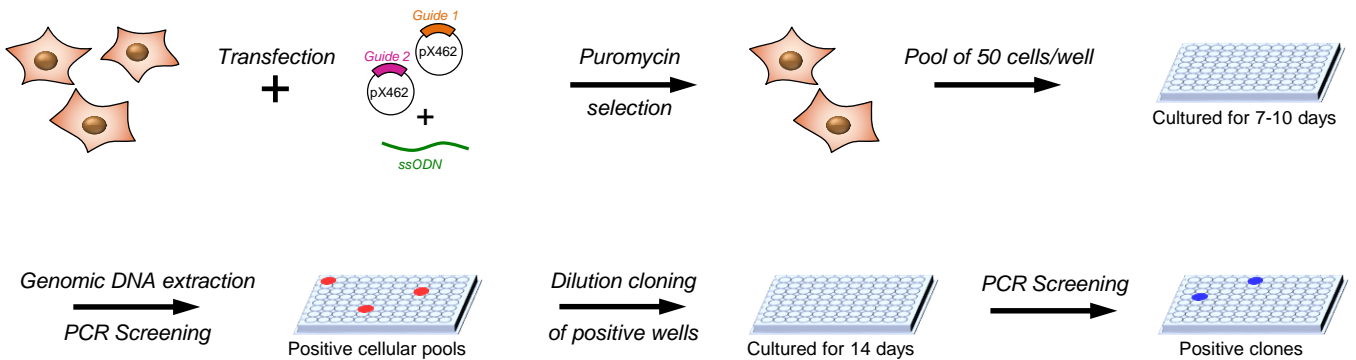
S2. A

## Strategy - STEP 1:



## Repair template – 200 bases:

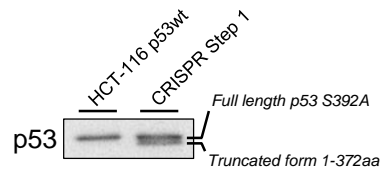
Single-stranded DNA oligonucleotide (ssODN)



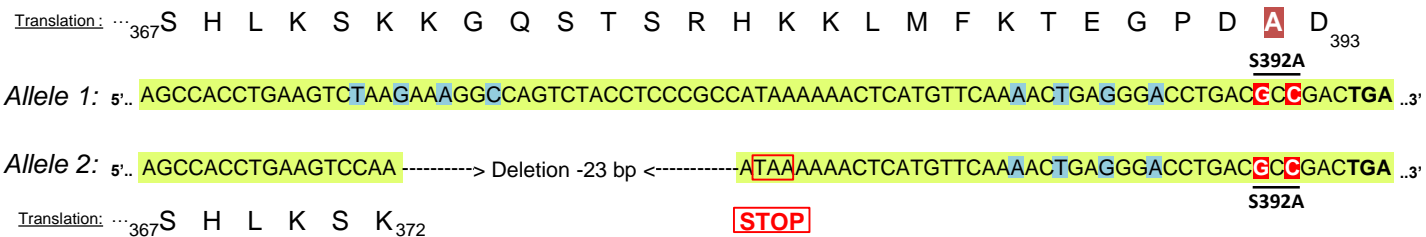
Characterization of the positive clones

- Exon sequencing
- cDNA sequencing
- Protein expression

## Protein expression:



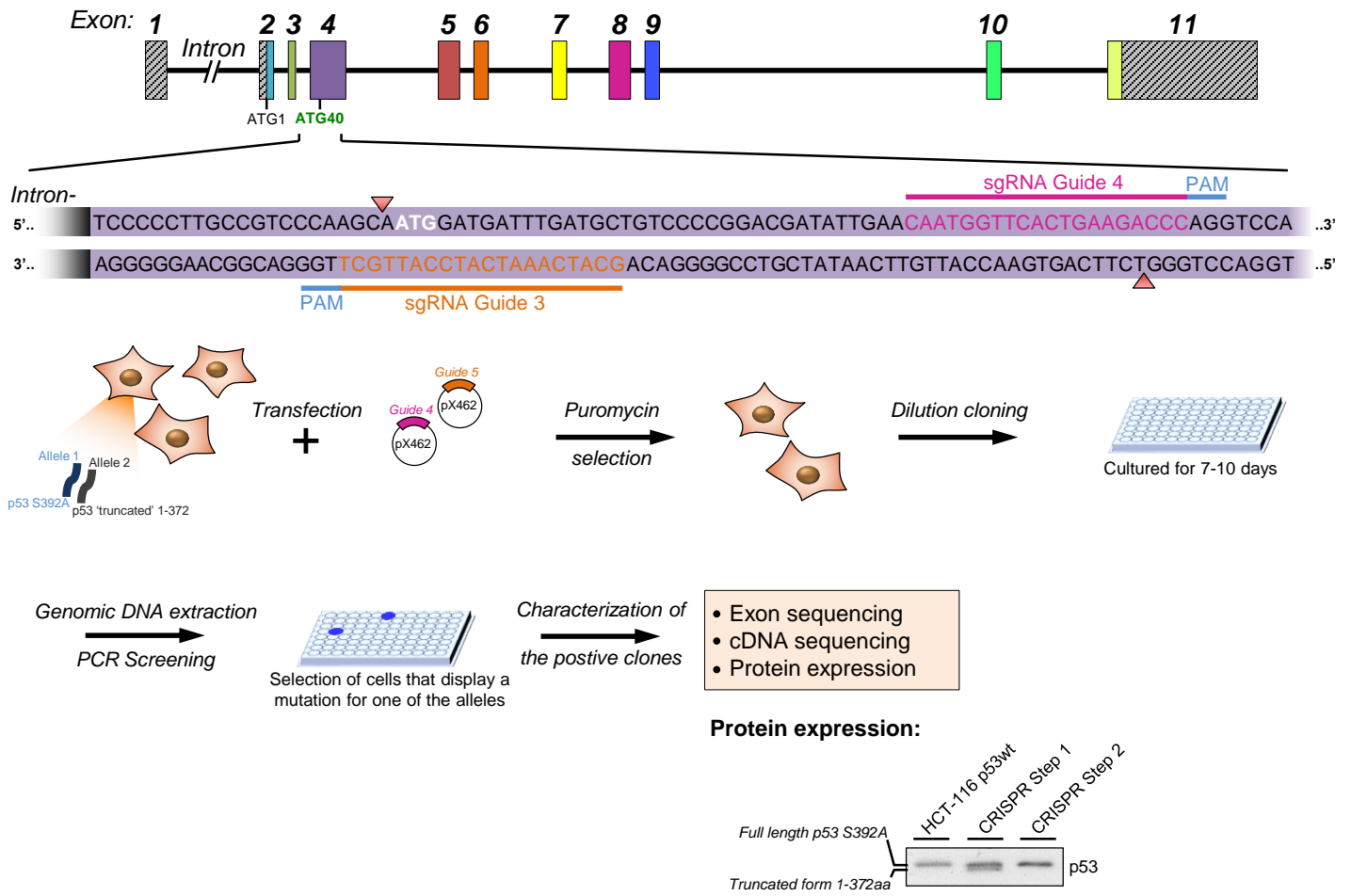
## Result after the first p53 CRISPR editing:



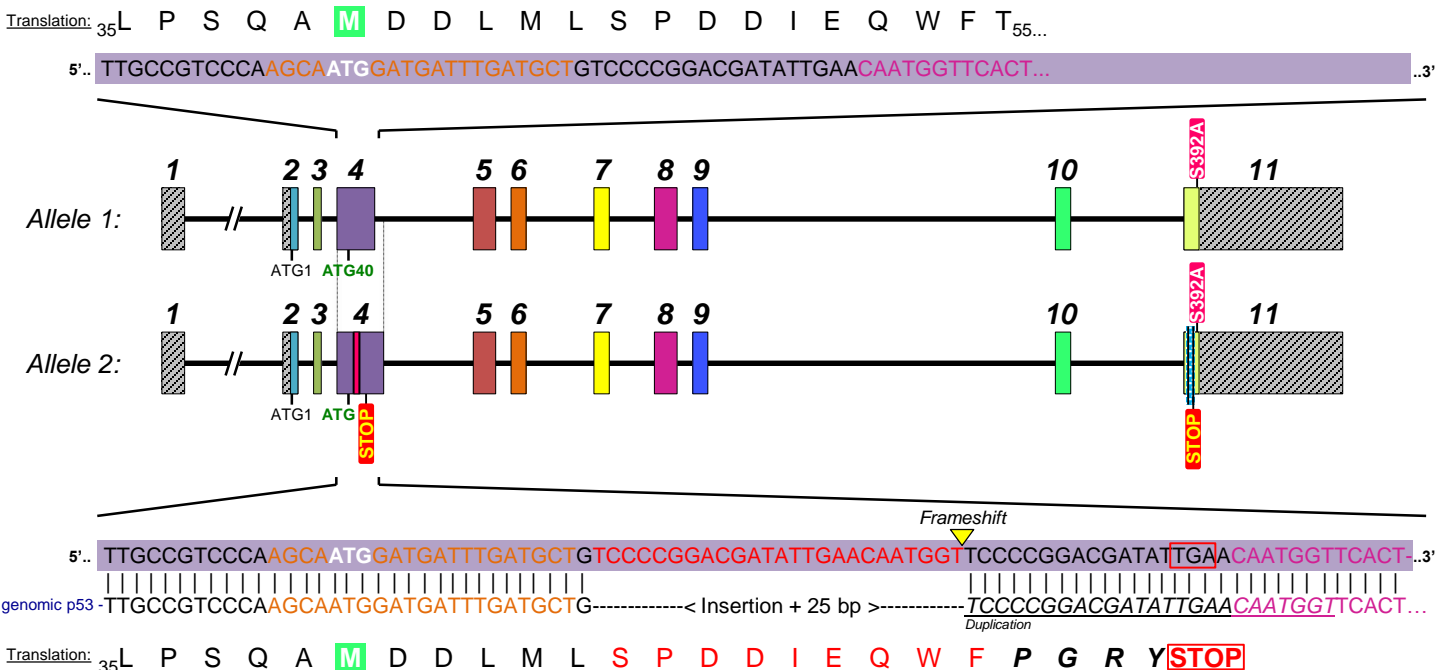
# Supplemental data :

## S2. B

### Strategy - STEP 2:



### Result after STEP 2 – HCT-116 p53 S392A +/- :

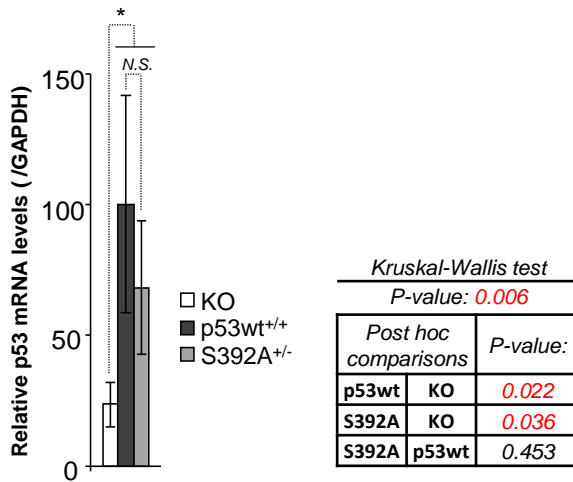


# Supplemental data :

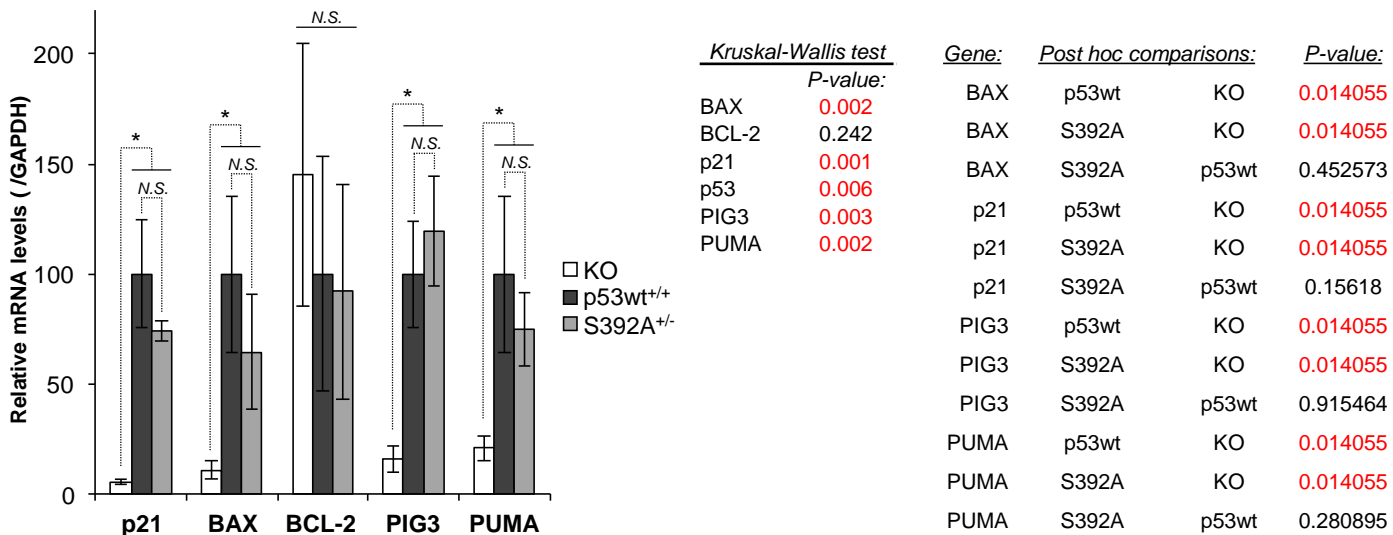
## S3



## S4

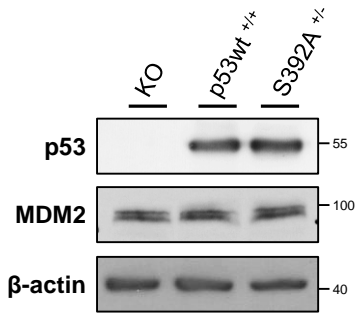


## S5

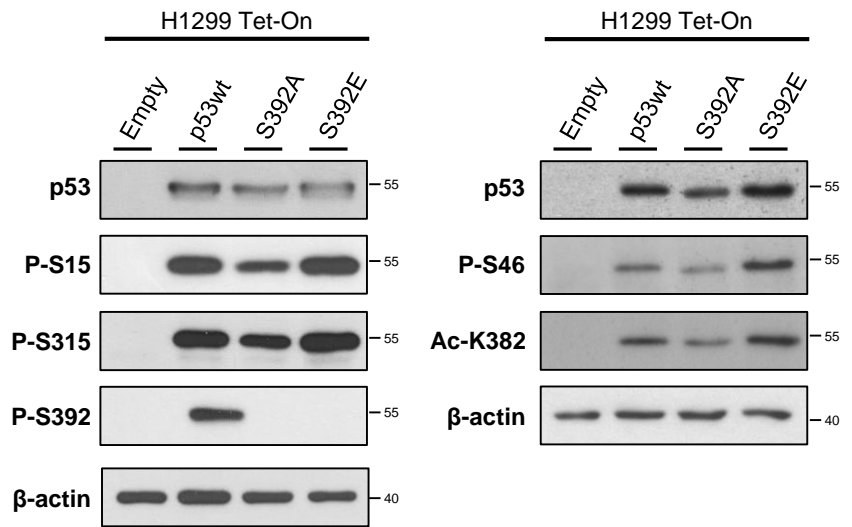


# Supplemental data :

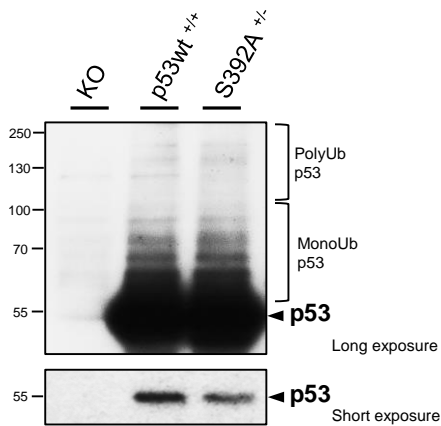
**S6**



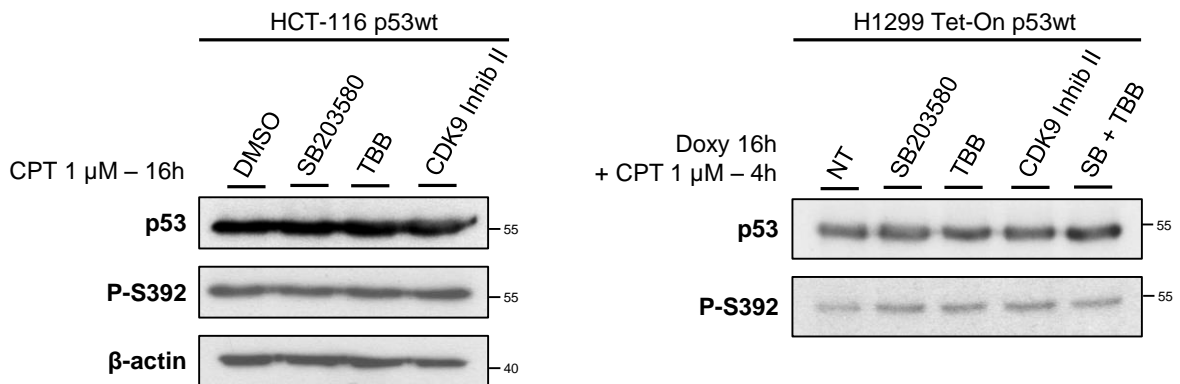
**S7**



**S8**



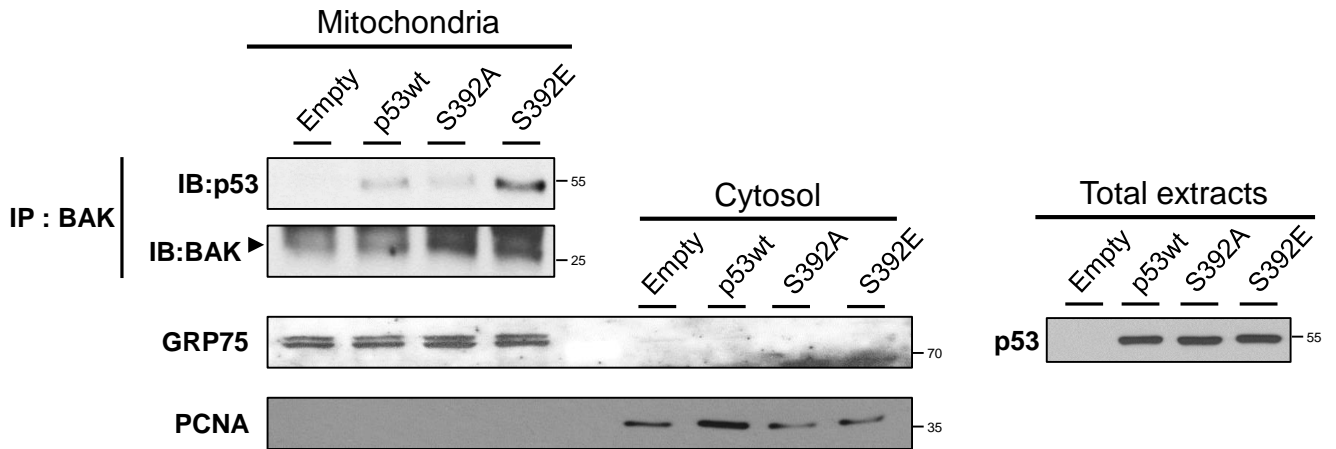
**S9**



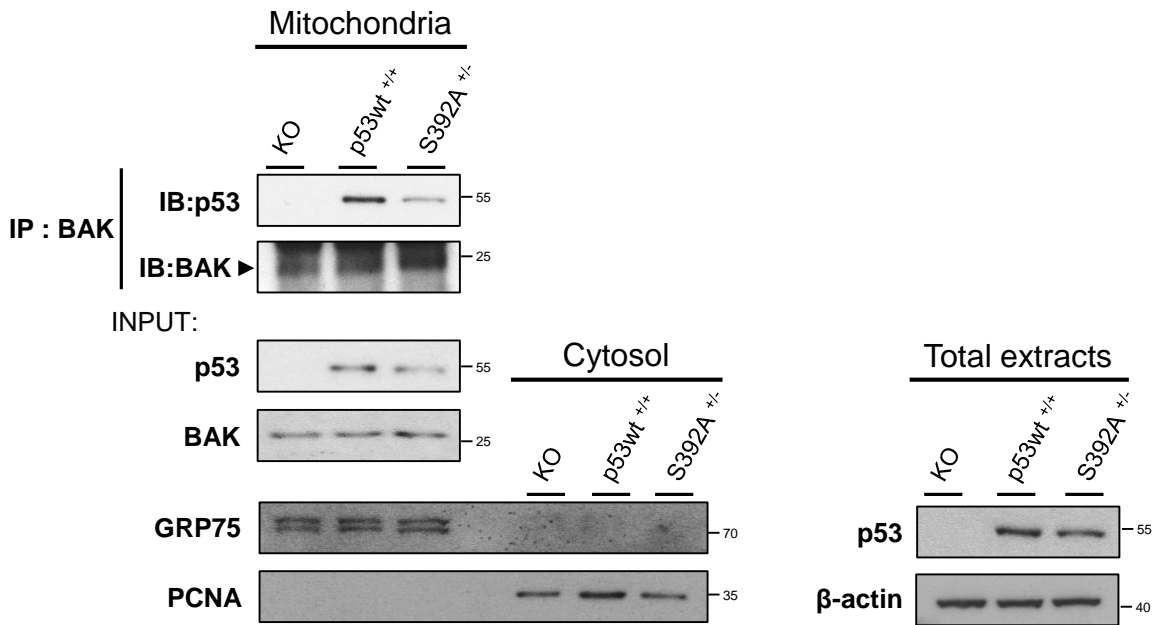


# Supplemental data :

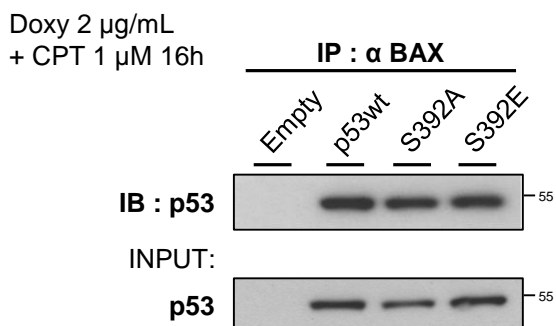
## S12



## S13



## S14



# Supplemental data :

## Table S1

Detailed statistical analyses of the data from figure 2F with the exact P-values obtained.

ANOVA 1								
CPT conc. ( $\mu\text{M}$ ):	0.01	0.02	0.04	0.06	0.08	0.1	0.15	0.2
P-value:	0.0409	0.0004	0.0001	0.0012	<.0001	0.0014	<.0001	<.0001

Comparisons with p53wt using Dunnett's Method (P-values)								
CPT conc. ( $\mu\text{M}$ ):	0.01	0.02	0.04	0.06	0.08	0.1	0.15	0.2
Empty	0.0254	0.0002	0.0001	0.0015	<.0001	0.0021	<.0001	0.0001
S392A	0.0466	0.0063	0.0023	0.0788	0.0015	0.0703	0.0033	0.0105
S392E	0.1920	0.2756	0.9624	0.9277	0.1645	0.8301	0.2671	0.4241

## Table S2

Detailed statistical analyses of the data from figure 3A with the exact P-values obtained.

### p21

ANOVA 1					Comparisons with p53wt using Dunnett's Method (P-values)				
Time (h):	0	4	8	16	Time (h):	0	4	8	16
P-value:	0.0003	0.0013	<.0001	<.0001	KO	0.0005	0.0008	<.0001	<.0001
					S392A <sup>+/-</sup>	0.6275	0.0822	0.3482	0.1149

### PUMA

ANOVA 1					Comparisons with p53wt using Dunnett's Method (P-values)				
Time (h):	0	4	8	16	Time (h):	0	4	8	16
P-value:	0.0011	0.003	0.0002	0.0015	KO	0.0025	0.0017	0.0003	0.0026
					S392A <sup>+/-</sup>	0.4229	0.1252	0.9286	0.7703

### BAX

ANOVA 1			Comparisons with p53wt using Dunnett's Method (P-values)		
Time (h):	8	16	Time (h):	8	16
P-value:	<.0001	0.0004	KO	<.0001	<.0001
			S392A <sup>+/-</sup>	0.0058	0.0771

# Supplemental data :

## Table S3

*Sequences of the ssODN and the DNA-Oligos used for CRISPR/Cas9n genome editing.*

<b>ssODN sequence 5'-3'</b>	
CACAGACCCTCTCACTCATGTGATGTCATCTCTCCTCCCTGCTTCTGTCTCCTACAGCC ACCTGAAGTCTAAGAAAGGCCAGTCTACCTCCC GCCATAAAAACTCATGTTCAA AACT GAGGGACCTGACGCCGACTGACATTCTCCACTTCTTGTTC CCACTGACAGCCTCCCA CCCCATCTCTCCCTCCCCTGCCA	

<b>DNA-Oligos guide sequences cloned into PX462 v2.0</b>	
S392_step1.sgRNA_1.UP	CACCGGAGGTAGACTGACCCTTTT
S392_step1.sgRNA_1.DOWN	AAACAAAAGGGTCAGTCTACCTCC
S392_step1.sgRNA_2.UP	CACCGAACTCATGTTCAAGACAGAA
S392_step1.sgRNA_2.DOWN	AAACTTCTGTCTTGAACATGAGTTC
ATG40_step2.sgRNA_3.UP	CACCGCATCAAATCATCCATTGCT
ATG40_step2.sgRNA_3.DOWN	AAACAGCAATGGATGATTTGATGC
ATG40_step2.sgRNA_4.UP	CACCGCAATGGTTCCTGACTGAAGACCC
ATG40_step2.sgRNA_4.DOWN	AAACGGGTCTTCAGTGAACCATTGC

## Table S4

*Primers used for quantitative real-time PCR analysis.*

Targets	Primers (5'-3')	Product length	Ref:
PUMA	For: CGACCTCAACGCACAGTACGA	151	54
	Rev: AGGCACCTAATTGGGCTCCAT		
p21	For: ACAGCAGAGGAAGACCATGTGGACC	113	55
	Rev: CGTTTTCGACCCTGAGAGTCTCCAG		
PIG3	For: CCATGCAGGACTGAGTGGTG	103	56
	Rev: CTGCTCCAAGCTTTTCTGCC		
GAPDH	For: CTGCCAACGTGTCAGTGGTG	104	56
	Rev: TCAGTGTAGCCCAGGATGCC		
BAX	For: GAGAGGTCTTTTTCCGAGTGG	85	57
	Rev: CCTTGAGCACCAGTTTGCTG		
BCL-2	For: CTGCACCTGACGCCCTTCACC	119	58
	Rev: CACATGACCCACCGAACTCAAAGA		