Novel Ovarian Cancer Maintenance Therapy Targeted at Mortalin and Mutant p53

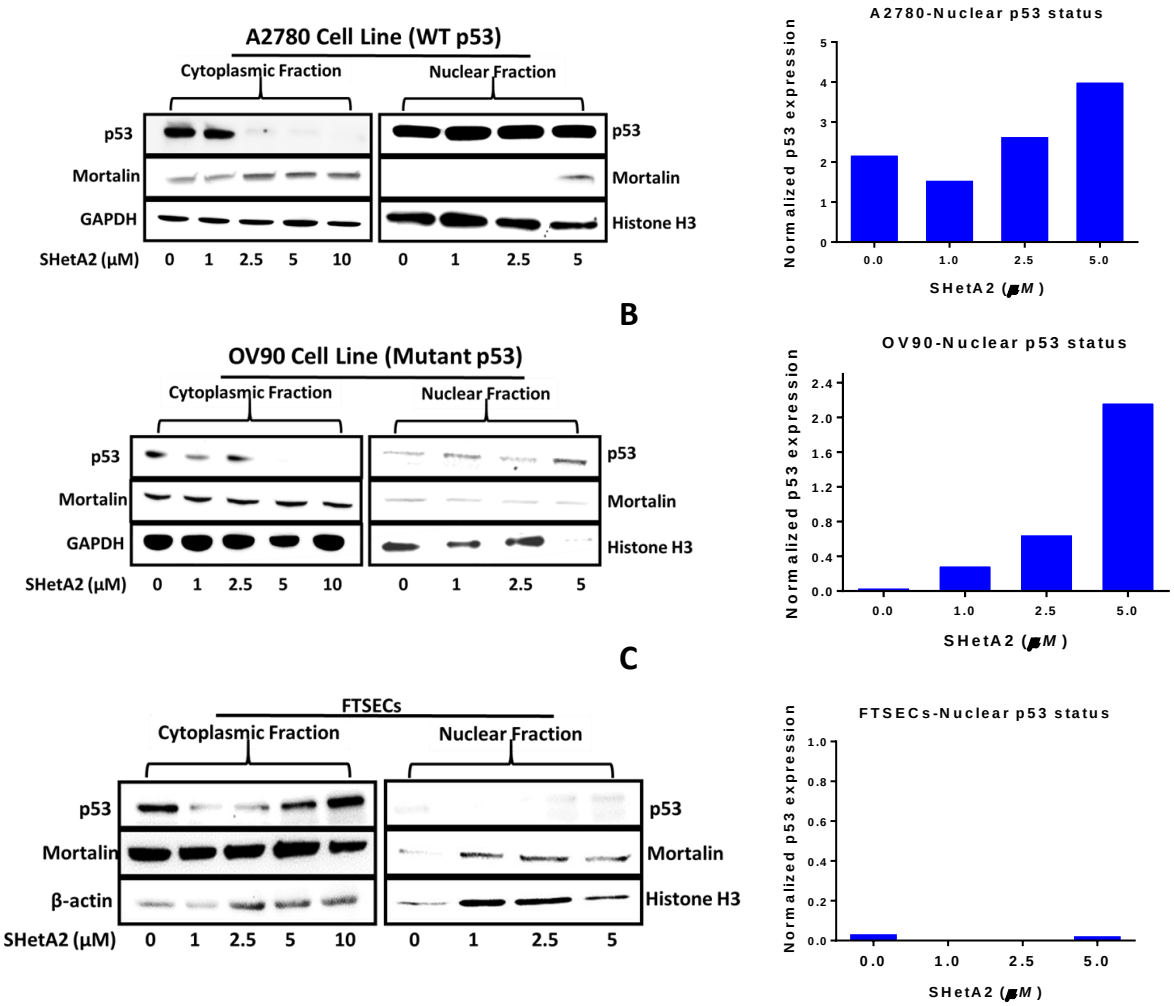
Satish Kumar Ramraj, Sugantha Priya Elayapillai, Richard C. Pelikan, Yan Daniel Zhao, Zitha Redempta Isingizwe, Amy L. Kennedy, Stanley A. Lightfoot, Doris Mangiaracina Benbrook

Supplementary Materials

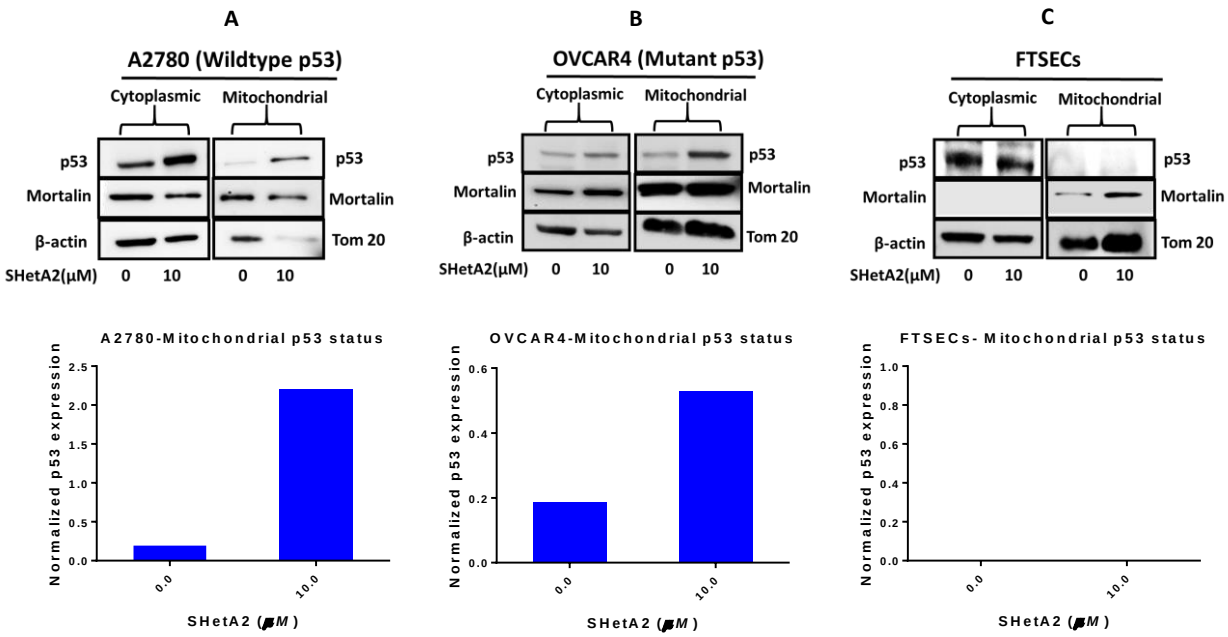
Supplementary Table S1: Dose reduction indices of SHetA2 and PRIMA-1/PRIMA-1^{MET} drug combination

	Combinations	Dose-reduction index (DRI)* at			
	SHetA2 + PRIMA-1	IC_{50}	IC_{75}	IC_{90}	IC_{95}
A2780 (WT p53)	SHetA2 +	3.84	3.72	3.6	3.53
	PRIMA-1	2.12	2.13	2.14	2.15
MES-OV (R282W p53)	SHetA2 +	1.79	2.21	2.73	3.15
	PRIMA-1	3.94	3.27	2.71	2.38
SKOV3 R248W p53	SHetA2 +	2.48	3.03	3.70	4.24
	PRIMA-1	1.79	2.38	3.15	3.81
CaOV3 (Null p53)	SHetA2 +	2.48	2.74	3.01	3.22
	PRIMA-1	1.56	1.48	1.40	1.35
FTSECs	SHetA2 +	1.62	2.37	3.48	4.5
	PRIMA-1	0.52	0.86	1.44	2.03
	SHetA2 + PRIMA-1Met				
MES-OV (R282W p53)	SHetA2 +	1.84	2.17	2.56	2.87
	PRIMA-1Met	2.10	2.76	3.64	4.38
FTSECs	SHetA2 +	2.3	2.59	2.9	3.14
	PRIMA-1Met	0.78	0.67	0.58	0.52

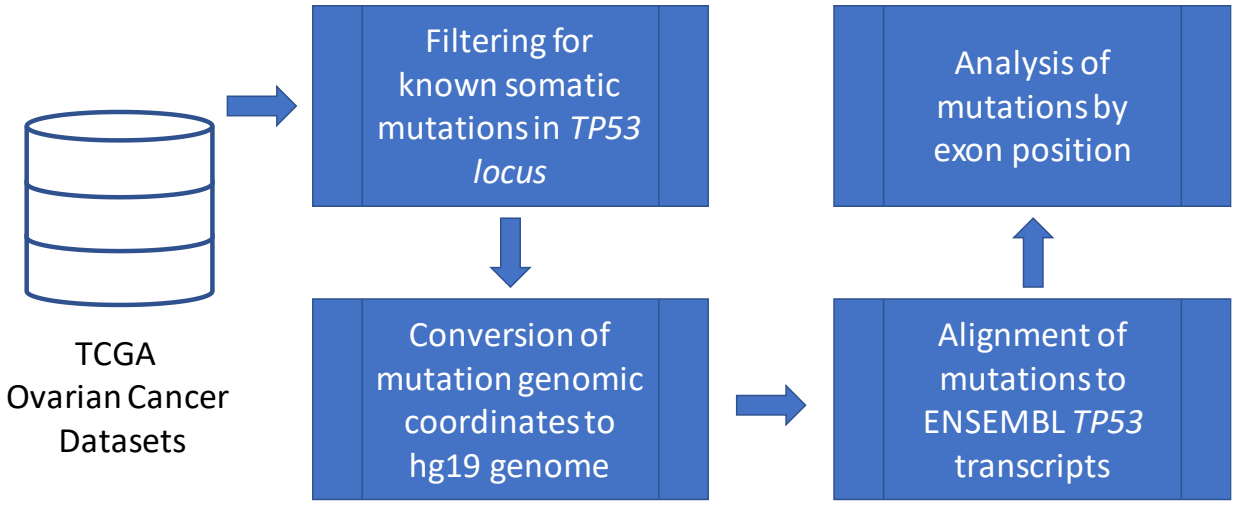
*Folds of dose reduction when compared with each drug alone



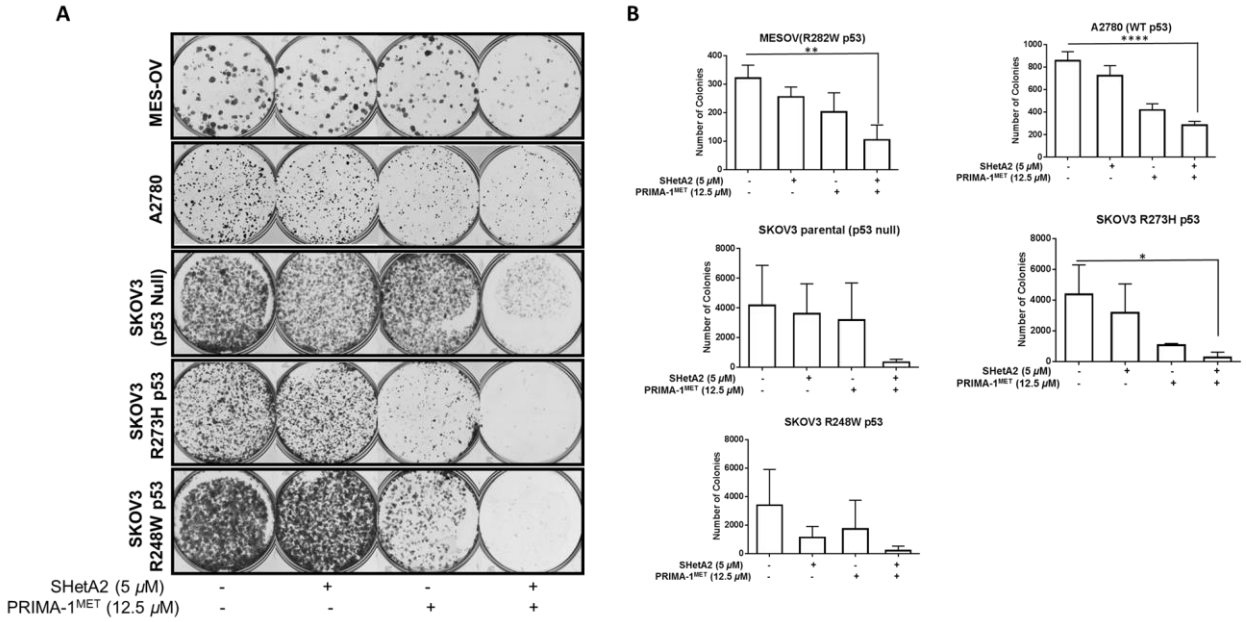
Supplementary Figure S1: SHetA2 causes p53 translocation from the cytoplasm to the nucleus. Immunoblots of p53 in cytoplasmic and nuclear protein fractions of **A**. A2780 cells, **B**. OV-90 cells and **C**. healthy control hFTSECs. GAPDH/ β-actin and Histone H3 served as loading controls for cytoplasmic and nuclear fractions respectively. In the bar graphs, p53 levels were normalized to histone H3 levels.



Supplementary Figure S2: Mitochondrial accumulation of p53 after SHetA2 treatment in cancer but not healthy cells. A. p53 levels were examined in cytoplasmic and mitochondrial fractions after SHetA2 treatment for 24 h in A2780 (Wild type p53) cells, **B.** OVCAR4 (L130V p53) cells and **C.** FTSECs (healthy human control cells). β-actin and Tom20 served as protein loading controls in cytoplasmic and mitochondrial fractions respectively. The corresponding bar graph plots p53 expression normalized with Tom20 in the mitochondrial extract of A2780, OVCAR4 and FTSECs.



Supplementary Figure S3: Flowchart of *p53* mutation analysis. Exome data from ovarian cancer samples were obtained from The Cancer Genome Atlas. To improve functional analysis of the discovered somatic mutations, their genomic coordinates were transferred to the hg19 build of the human genome and aligned to an updated set of transcript definitions for *p53*. The frequencies of mutations in *p53* functional domains were then calculated.



Supplementary Figure S4. Colony formation assay of ovarian cancer cells treated with drugs alone or in combination. A. Representative image of colony formation assay of ovarian cancer cells treated with mock, SHetA2, PRIMA-1^{MET} or SHetA2+PRIMA-1^{MET} . **B.** Quantification of colony formation assay of ovarian cancer cells run in triplicate using Optronix GelCount colony counter. *p≤ 0.05, **p ≤0.01, ****p ≤0.0001