Supplemental Table 1. Driver mutations in PDX models.

PDX model	Mutations
WM4351	$BRAF^{V600E}$, TERT, MAP3K9, MAP3K9
WM4298	$BRAF^{V600E}$, BRCA2, KIT
WM4070	$BRAF^{V600K}$, MAP2K1, ERBB4, TERT

Related to Figure 6 and Figure S6.

Supplemental Table 2. Primers

Oligonucleotides		
Wnt5A forward primer ATT CTT GGT GGT CGC TAG GTA	This paper	N/A
Wnt5A reverse primer CGC CTT CTC CGA TGT ACT GC	This paper	N/A
TP53 forward 1 primer 1 GTG GAA GGA AAT TTG CGT GT	This paper	N/A
TP53 reverse 1 primer CCA GTG TGA TGA TGG TGA GG3	This paper	N/A
KDM5B forward 1 primer GGA TAC GTG GCG TAA AAT GAA	This paper	N/A
KDM5B forward 2 primer CAA TGC TGT GGA CCT GTA TGT	This paper	N/A
KDM5B reverse 2 primer TAC GGA GGG TAT AGT CCC TGG3	This paper	N/A
PUMA forward primer GTC CCC TGC CAG ATT TGT G	This paper	N/A
PUMA reverse primer AGA GGC CGC AGG ACA CTG	This paper	N/A
TP53 forward mouse primer AAG ATC CGC GGG CGT AA	This paper	N/A
TP53 reverse mouse primer CAT CCT TTA ACT CTA AGG CCT CAT TC	This paper	N/A
Cyclophilin A forward GGG TTC CTC CTT TCA CAG AA	This paper	N/A
Cyclophilin A reverse GAT GCC AGG ACC TGT ATG CT	This paper	N/A

Related to Star Methods.

Supplemental Figure 1

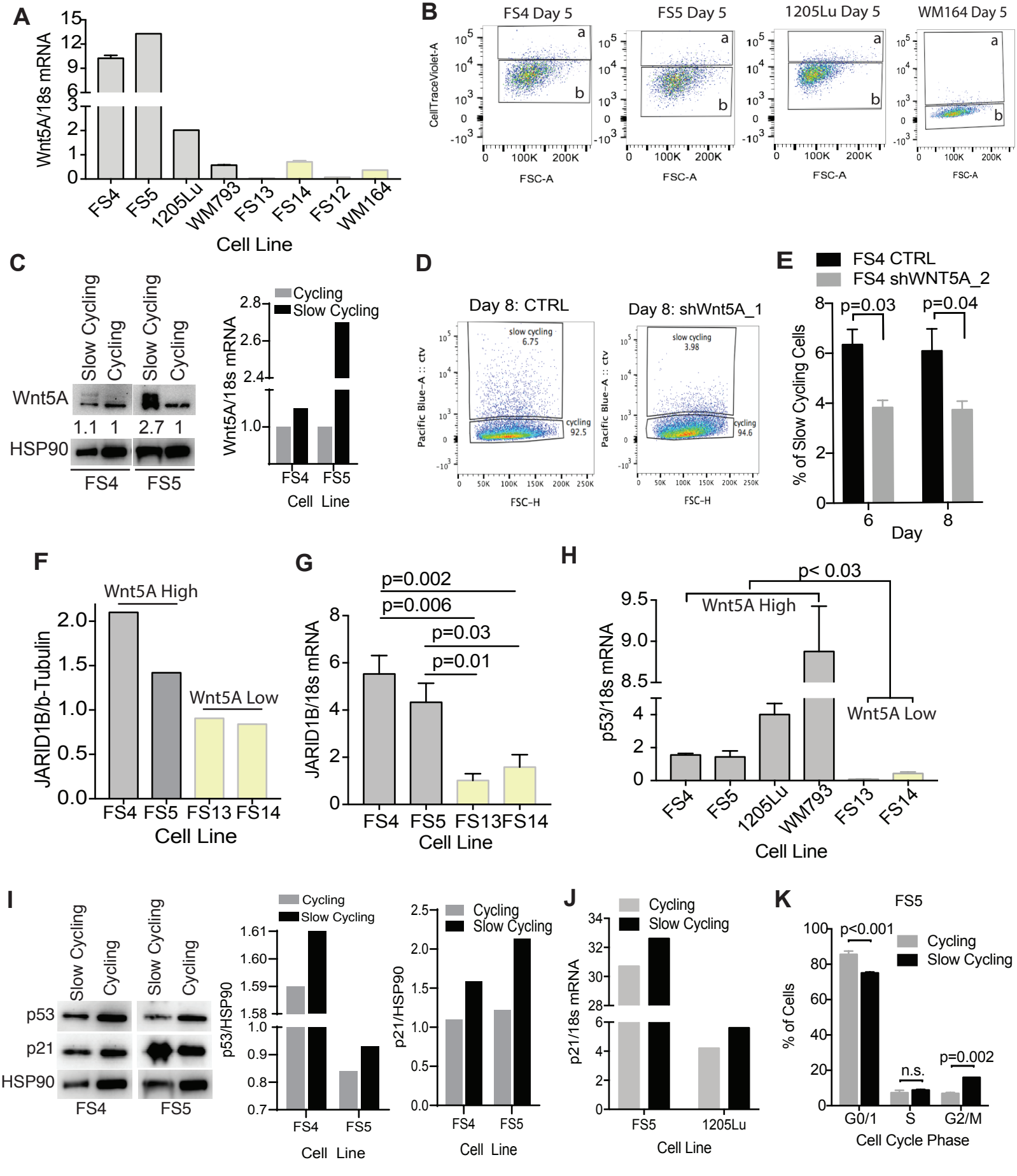
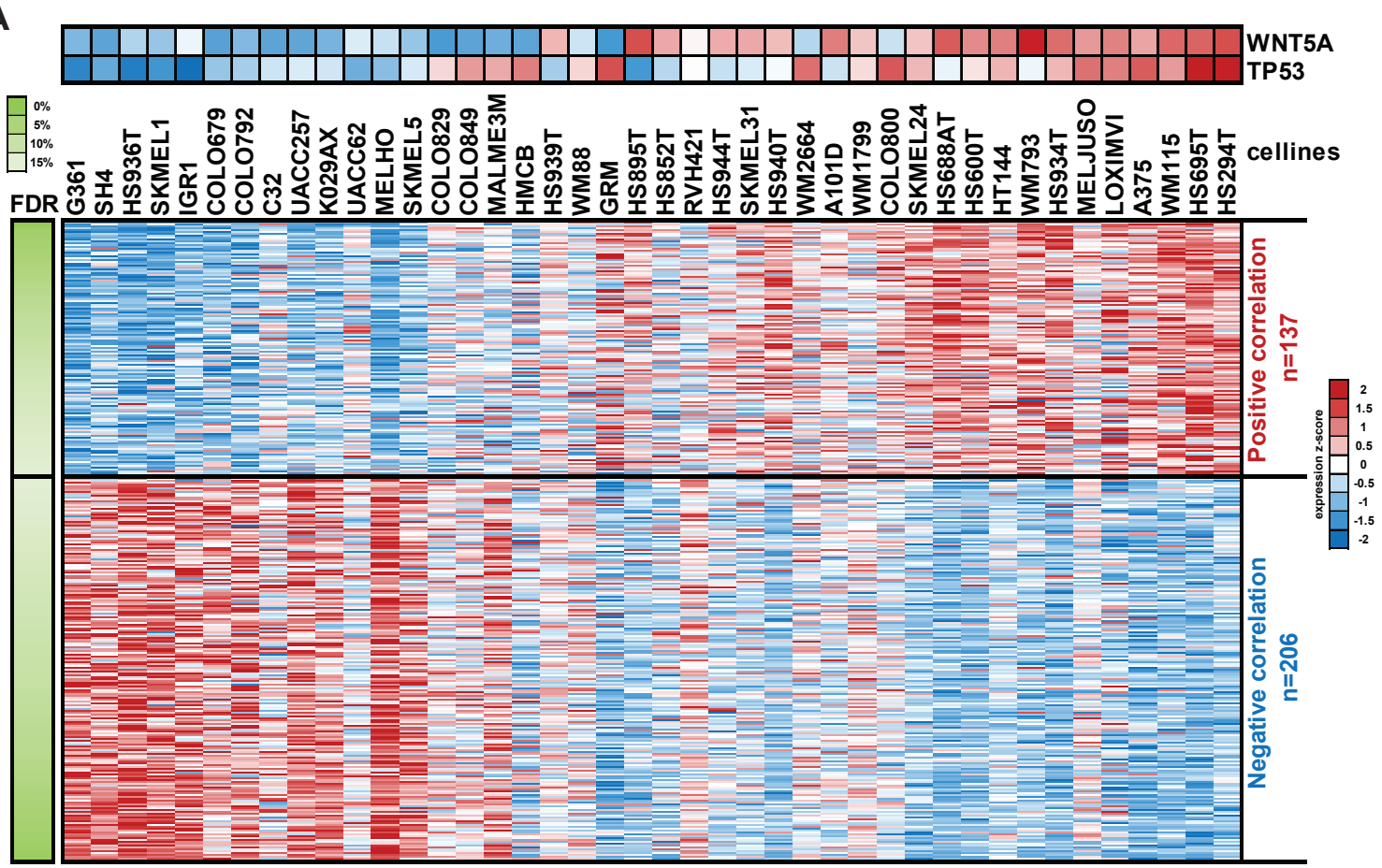


Figure S1. Slow cycling cells express increased Wnt5A, p53 and p21. A) Quantification of Wnt5A mRNA in human melanoma cell lines by qPCR. B) Representative day 5 flow cytometry analysis of cycling and slow-cycling cell populations in Wnt5A high (FS4, FS5, 1205Lu) and Wnt5A low (WM164) cell lines. C) Western quantification of Wnt5A in slow-cycling and cycling cells sorted by flow cytometry. D) Representative flow cytometry analysis of slow-cycling and cycling cells on day 8 in Wnt5A high FS5 control and Wnt5A knockdown cells (shWnt5A_1). E) Quantification of changes in slow-cycling populations at day 6 and 8 upon knockdown of Wnt5A in FS4 human melanoma cell line. F) Quantification of JARID1B expression by western (Wnt5A low cell lines, yellow). G) qPCR analysis of JARID1B mRNA expression in Wnt5A high (FS4 and FS5, gray) and Wnt5A low (FS13 and FS14, yellow) cells. H) qPCR analysis of p53 mRNA expression in Wnt5A high (gray) and low (yellow) cell lines. I) Western of p21 expression in cycling and slow cycling cells sorted by flow cytometry. J) qPCR analysis of p21 expression in cycling and slow cycling melanoma cells. K) Cell cycle analysis of slow-cycling and cycling cell populations in FS5 (Wnt5A high) human melanoma cells. Related to Figure 1.

Supplemental Figure 2

A



B

Regulator	N	*pvalue*	Z	
TGFB1	58	$4x10^{-8}$	4.8	Activated
TWIST1	10	0.0002	3.0	
NFATC2	10	0.0002	2.9	
F2	13	0.0002	2.5	
TNF	52	$5x10^{-6}$	2.2	
HIF1A	17	0.0002	2.0	
SNAI1	10	$2x10^{-5}$	2.0	
ERG	13	10^{-5}	2.0	
EDN1	14	$3x10^{-6}$	1.7	
IFNG	40	$4x10^{-5}$	1.5	
KRAS	20	0.0001	1.4	
MYC	32	0.0005	1.3	
SREBF1	14	$4x10^{-5}$	-2.6	Inh
MITF	38	$2x10^{-27}$	-5.0	

Figure S2. Analysis of genes that are positively and negatively enriched in melanoma cells expressing wild-type p53 and Wnt5A. A) Global expression heatmap of genes significantly correlated with both WNT5A and TP53. B) Enriched regulators. Inh = Inhibited. N=number of regulator's target genes. Z=activation z-score predicted by IPA. Related to Figure 1.

Supplemental Figure 3

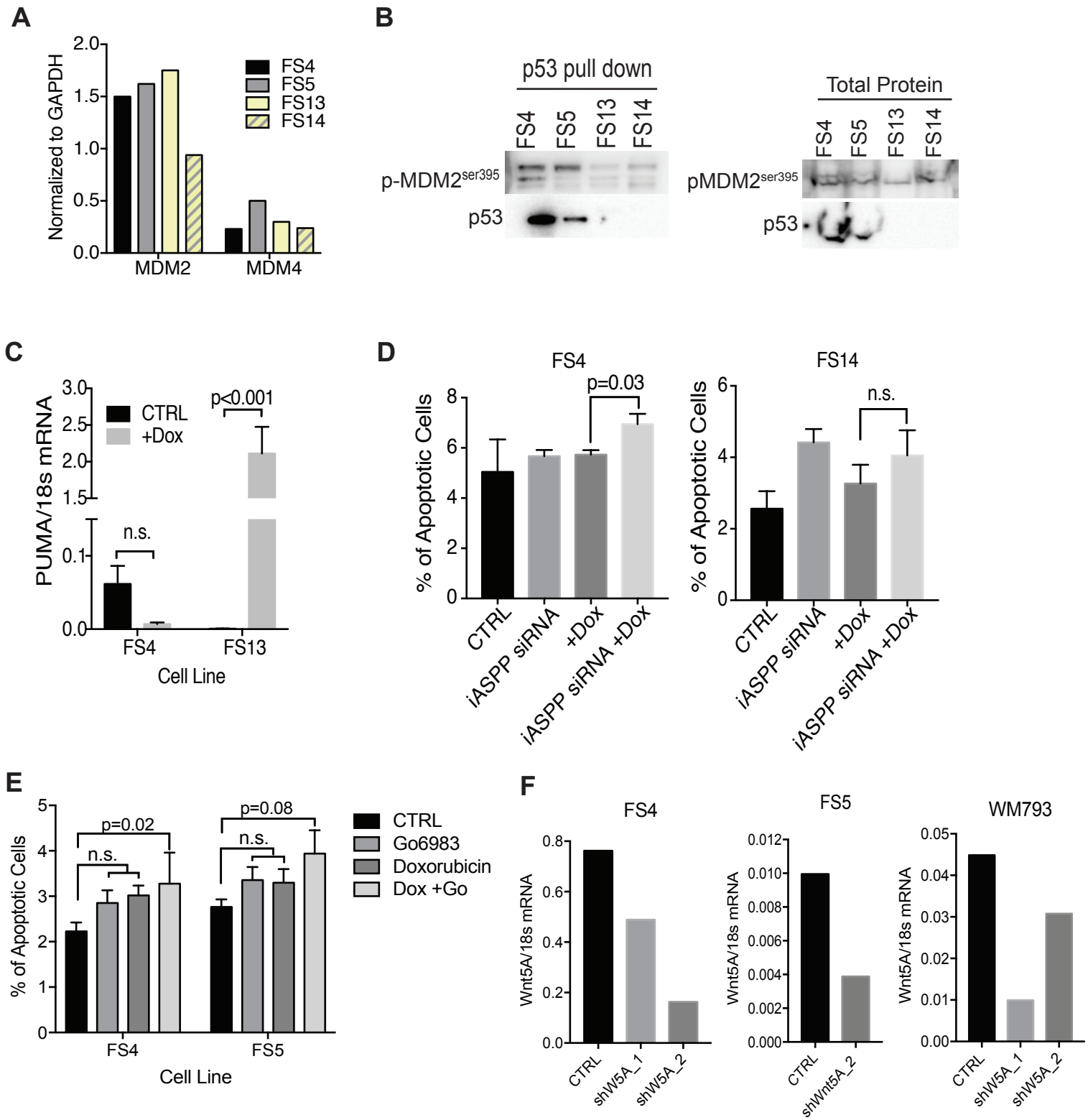


Figure S3. p53 interacts with p-MDM2ser395. (A) Quantification of MDM2 and MDM2 expression in Wnt5A high (FS4 and FS5) and Wnt5A low (FS13 and FS14) expressing cells (also see Fig. 2D). (B)Western blot analysis of p-MDM2ser395 pulled down with p53 antibody in Wnt5A high (FS4, FS5) and Wnt5A low (FS13, FS14) expressing cell lines. (C). qPCR analysis of PUMA mRNA in FS4 (Wnt5A high) and FS13 (Wnt5A low) cells following DNA damage (1μg/mL doxorubicin). D) Quantification of apoptosis following knockdown of iASPP by siRNA using AnnexinV and propidium iodide in Wnt5A high (FS4) and Wnt5A low (FS14) cells following doxorubicin 1 ug/mL treatment. E) Quantification of apoptosis in Wnt5A high cell lines following treatment with a PKC inhibitor (1μM Go6983) and 1 μg/mL doxorubicin for 8 h. Related to Figure 2 and Figure 3.

Supplemental Figure 4

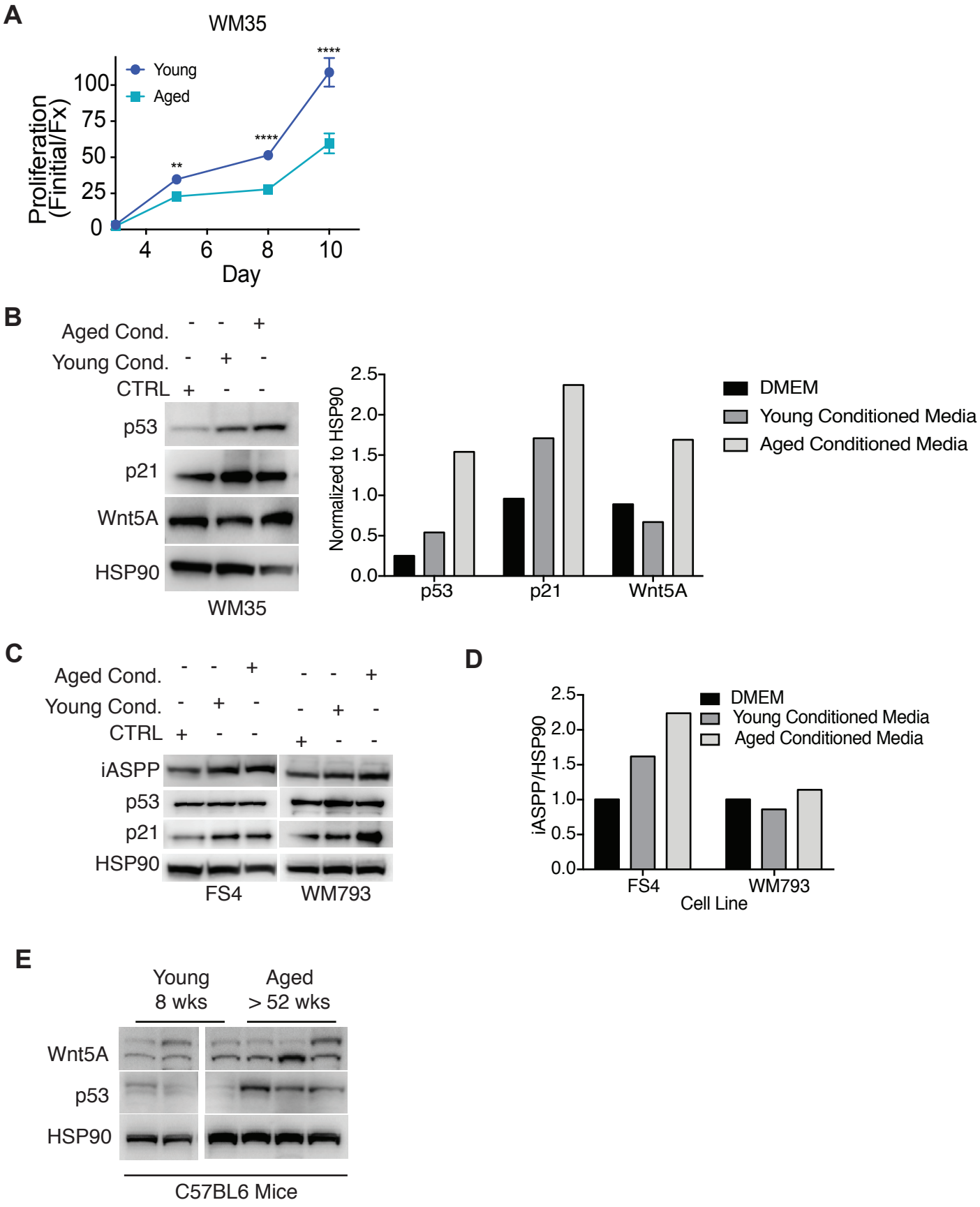


Figure S4. The aged microenvironment decreases proliferation and increases wild type p53. A) proliferation of WM35 human melanoma cells grown in conditioned media from young or aged fibroblasts. (ANOVA, ** p= 0.0013; **** p <0.0001). B) Western analysis of Wnt5A, p53 and p21 expression in lysates from melanoma cells treated with DMEM control, conditioned media from young or aged dermal fibroblasts. C) Western blot analysis of iASPP expression in melanoma cells treated with conditioned media from young and aged fibroblasts. D) Quantification of iASPP protein in melanoma cells treated with conditioned media from young or aged dermal fibroblasts. E) Western of Wnt5A and p53 expression in tumor lysates from tumors grown in young and aged mice shown in Fig. 4B. Related to Figure 4.

Supplemental Figure 5

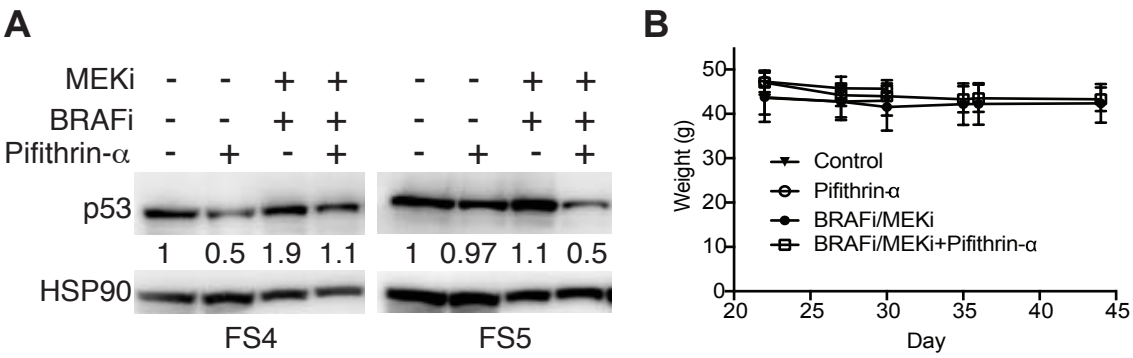


Figure S5. Wild type p53 and Wnt5A promote slow cycling and therapy resistance. A) Western of Wnt5A high (FS4, FS5) melanoma cells with +/- 5 μM pifithrin-α +/- BRAF/MEKi (5μM PLX4720/100 nM PD0325901). Changes in p53 were quantified using densitometry and normalized to HSP90. B) Weight (g) of aged C57Bl6 mice during treatment with BRAF/MEKi chow (Day 22-44), BRAF/MEKi chow with a single dose of 2mg/kg pifithrin-α on day 23, a single dose of 2mg/kg pifithrin-α on day 23, or vehicle control. Related to Figure 5.

Supplemental Figure 6

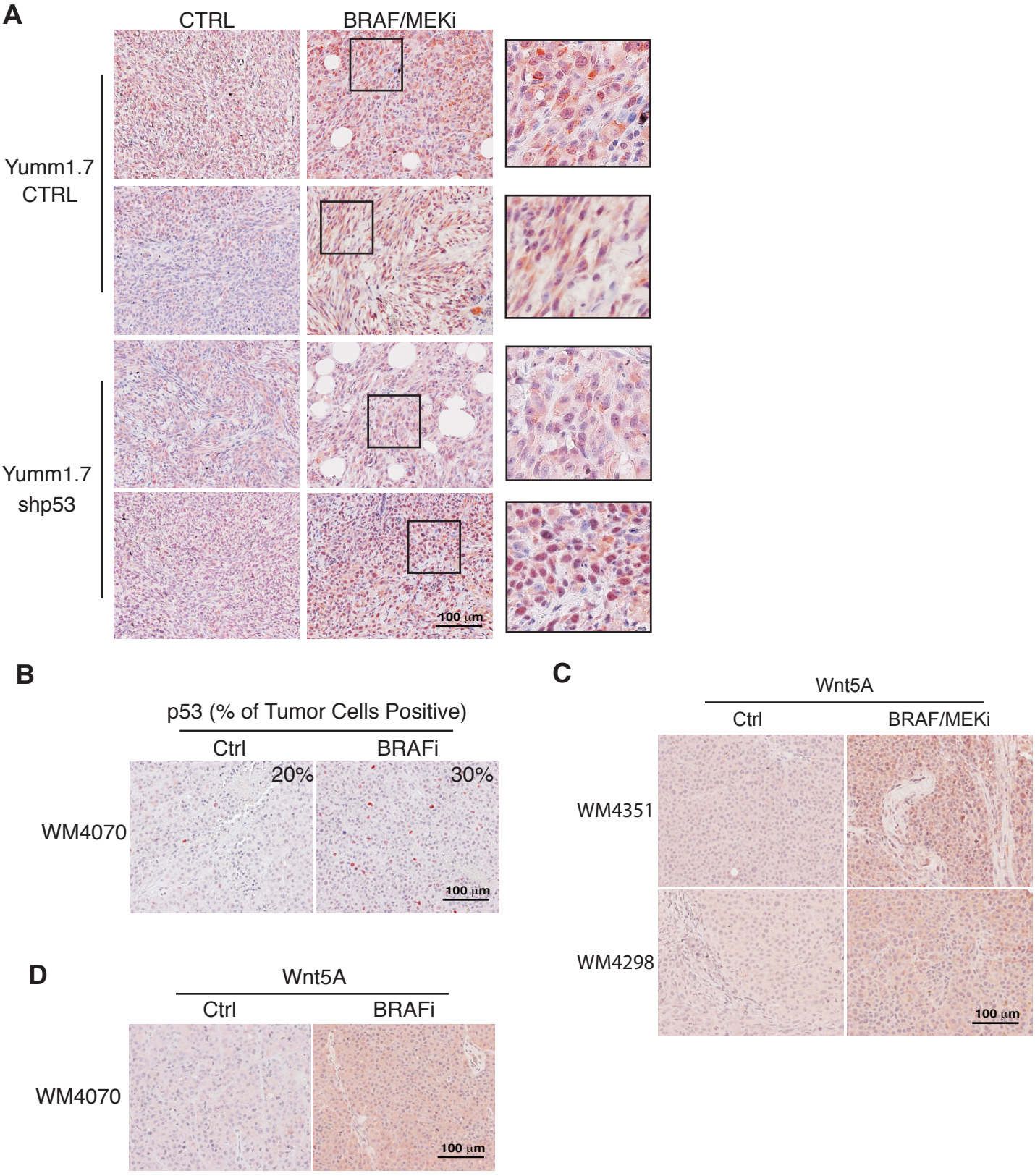


Figure S6. WT p53 and Wnt5A are increased following therapy. A) IHC for p53 expression in tumors from control and shp53 KD tumors given control or BRAF/MEKi chow. (B) IHC staining for p53 in PDX tumors from control and BRAFi treated mice. H) IHC for Wnt5A expression in PDX tumors from control and BRAF/MEKi treated mice. I) IHC for Wnt5A expression in PDX tumors from control and BRAFi treated mice. Related to Figure 6.