Cell Reports

Supplemental Information

Systemic Akt1 Deletion after Tumor Onset in *p53*$^{-/-}$ Mice Increases Lifespan and Regresses Thymic Lymphoma Emulating p53 Restoration

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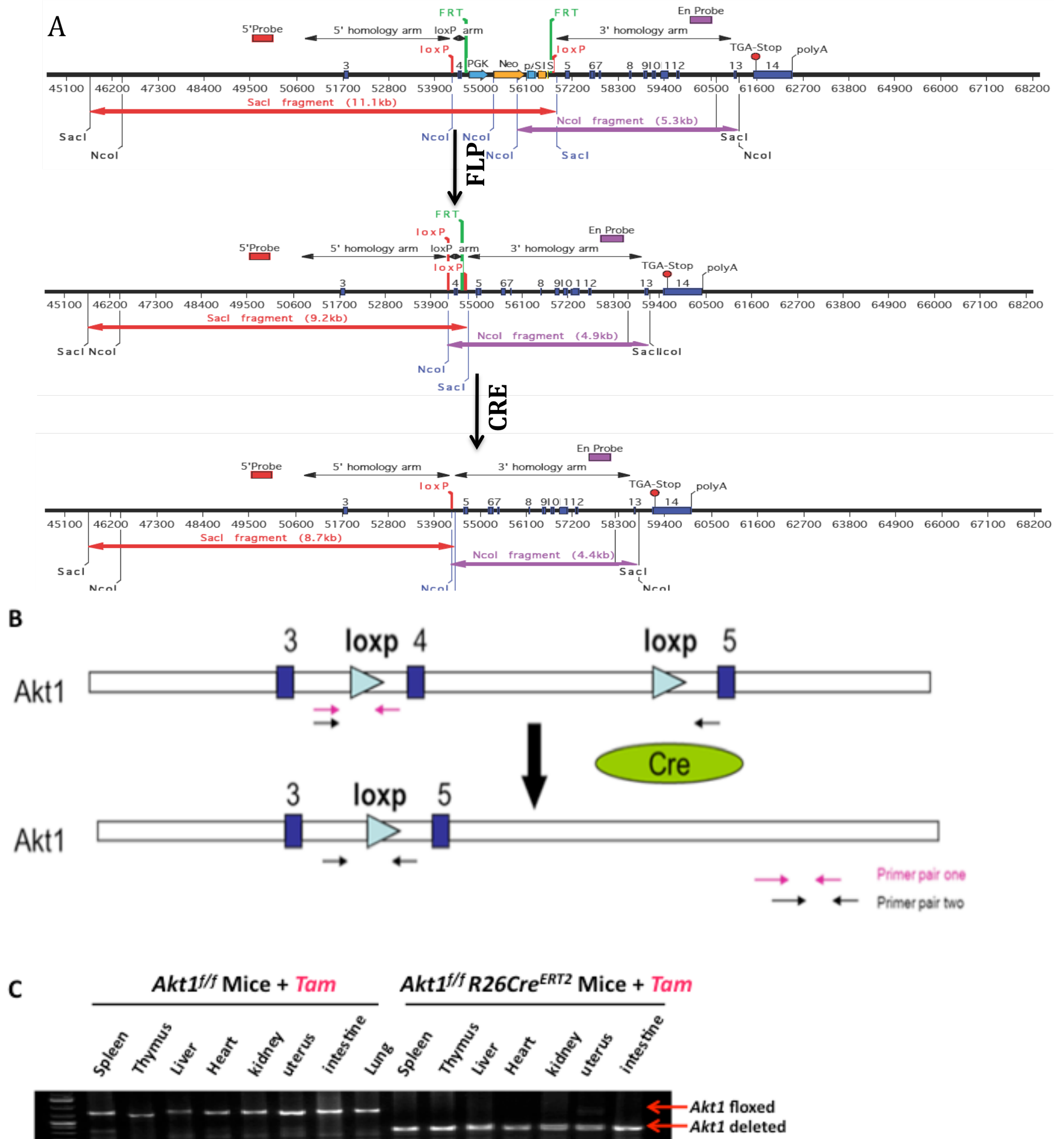


Figure S1 (related to Figure 1). Systemic conditional targeting of Akt1. **A.** Schematics depicting the generation of *Akt1$^{F/F}$* mice. *Akt1$^{F/F}$* mice were generated by flanking exon 4 of the mouse Akt1 gene with LoxP sites. Splicing of exon 3 to 5 should cause a frame shift mutation with introduction of early stop codon. The targeting vector, which includes the PGK-neo selection cassette flanked by FRT sites was used to generate C57Bl/6 ES cells with targeted alleles of Akt1. Mice generated from the ES cells were then crossed with FLP recombinase expressing mice to excise the PGK-neo cassette. **B.** Schematic depicting primers used to determine deletion by Cre recombinase. **C.** Systemic deletion of Akt1 after tamoxifen (Tam) injection into Akt1f/f/R26CreERT2 mice as determined by PCR in the indicated tissues.

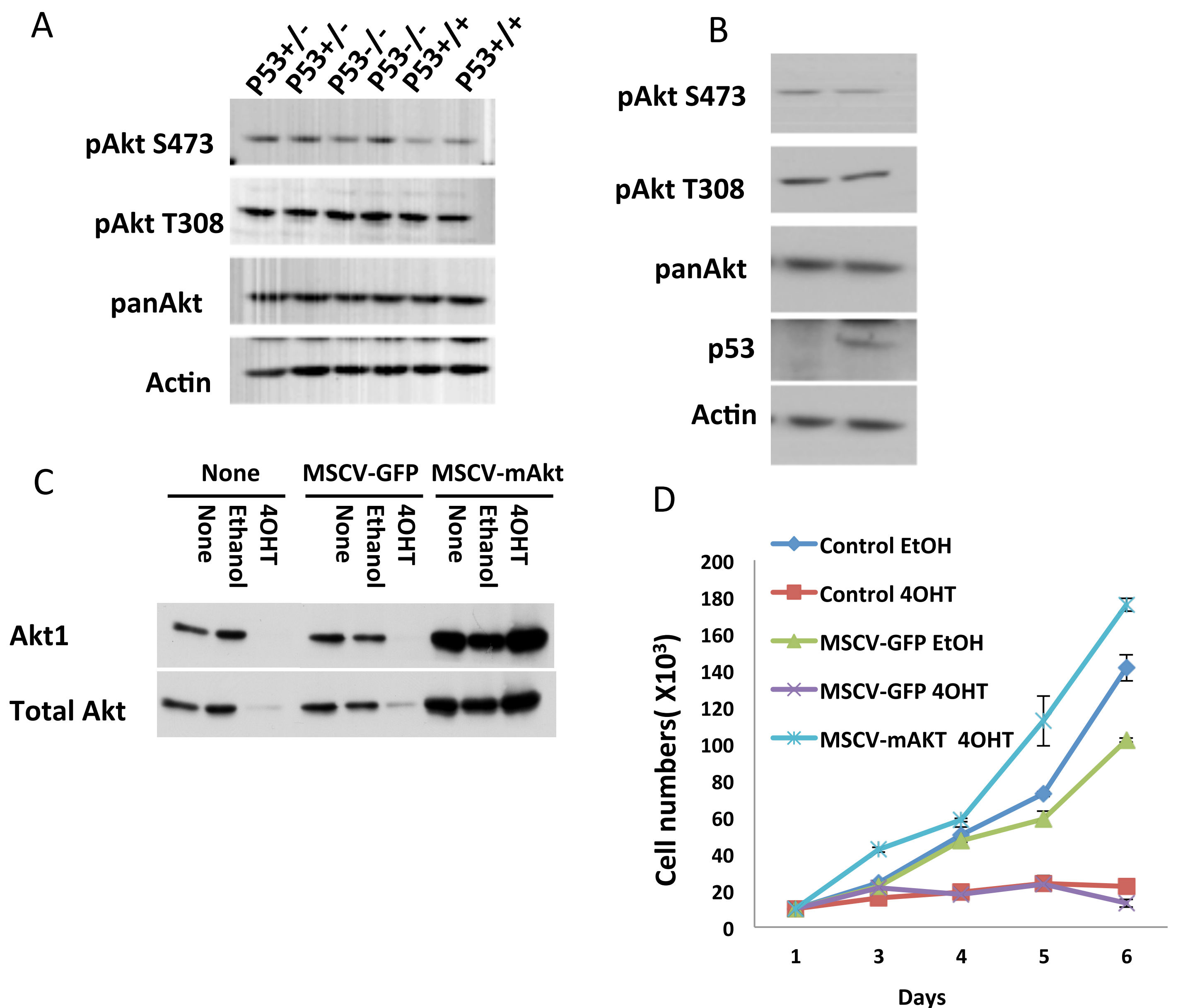


Figure S2 (related to Figure 3): Akt is not hyperactivated in p53$^{-/-}$ thymocytes or p53$^{-/-}$ thymic Lymphoma, while the expression of activated Akt exerts resistance to Akt1 deletion.

A. Thymocytes isolated from one month old mice with the indicated genotypes. Protein extracts from the cells were subjected to immunoblotting using anti-phospho Akt antibodies.

B. Thymic lymphoma cells isolated from p53-/-mice were infected with with retrovirus expressing p53 and then subjected to immunoblotting as in A.

C,D. P53-/- thymic lymphoma. Cells were infected with a control GFP expressing retrovirus or retrovirus co-expressing myristoylated Akt1(mAkt). Cells were then treated with either ethanol of 300nM 4-OHT. . Immunoblot showing Akt1 deletion and total phospho-serine 473 of Akt (**C**). Proliferation curves after exposure to 4-OHT (**D**).

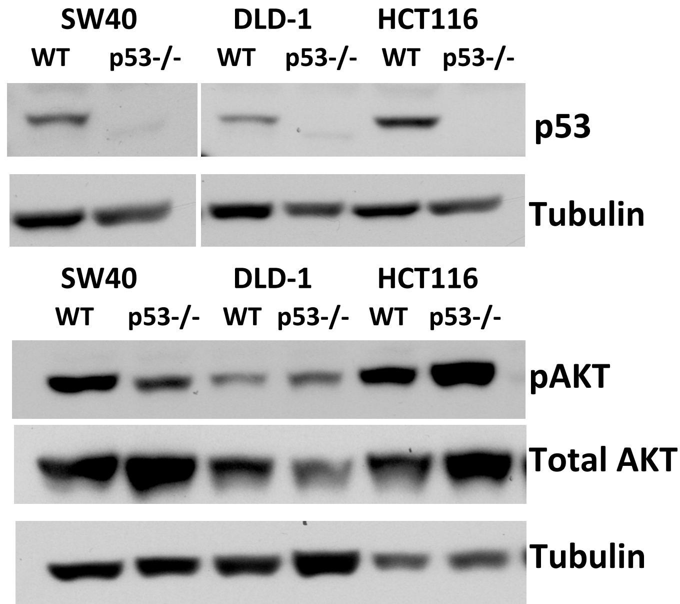
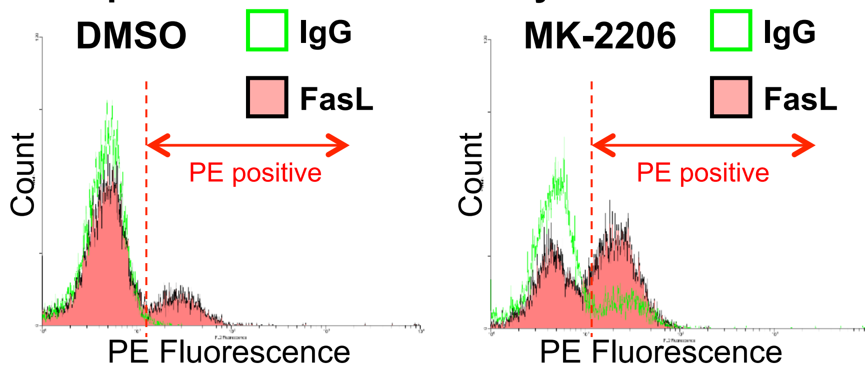


Figure S3 (related to Figure 5). Immunoblot showing p53 expression, p-Akt and total Akt in the Isogenic p53 –proficient and p53-deficient colorectal cell lines.



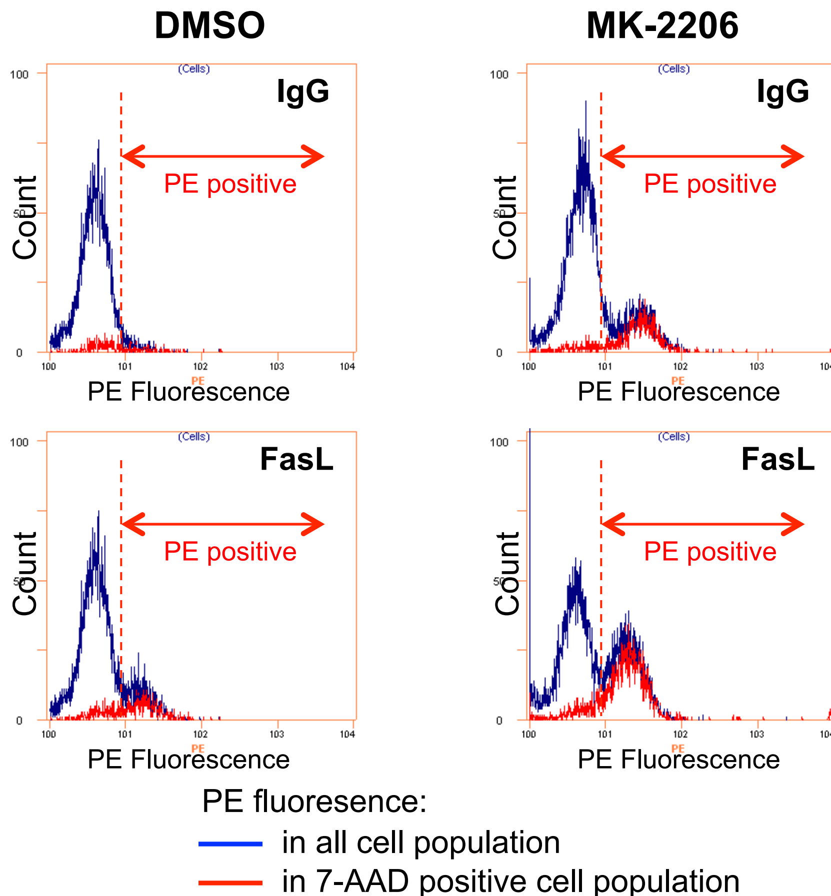


Figure S4 (related to Figure 6). Representative raw data of FACS to determine FasL expression.
Cells were incubated with DMSO or MK-2206 (1µM) for 18h before collection to assess FasL surface expression by FACS. For FasL staining, cell pellets wereresuspended for cell staining. Cells were stained with PE anti-mouse FasL antibody (BioLegend) and counterstained with 7-AADto assess viability. As a negative control, cells were stained with PE IgG isotype control antibody. Flow cytometry was then performed using Cell lab QuantaTM SC.
Panel A shows an overlay of representative data analyzed with WinMDI 2.8 in the different conditions, showing a shift of PE fluorescence after induction of cell death with MK-2206.
Panel B shows representative raw data extracted from Cell lab QuantaTM SC analysis program. Software collected PE fluorescence and7-AAD fluorescence concomitantly and data are showing PE fluorescence in the all cells (blue) or in the dead cells (7-AAD positive, red).

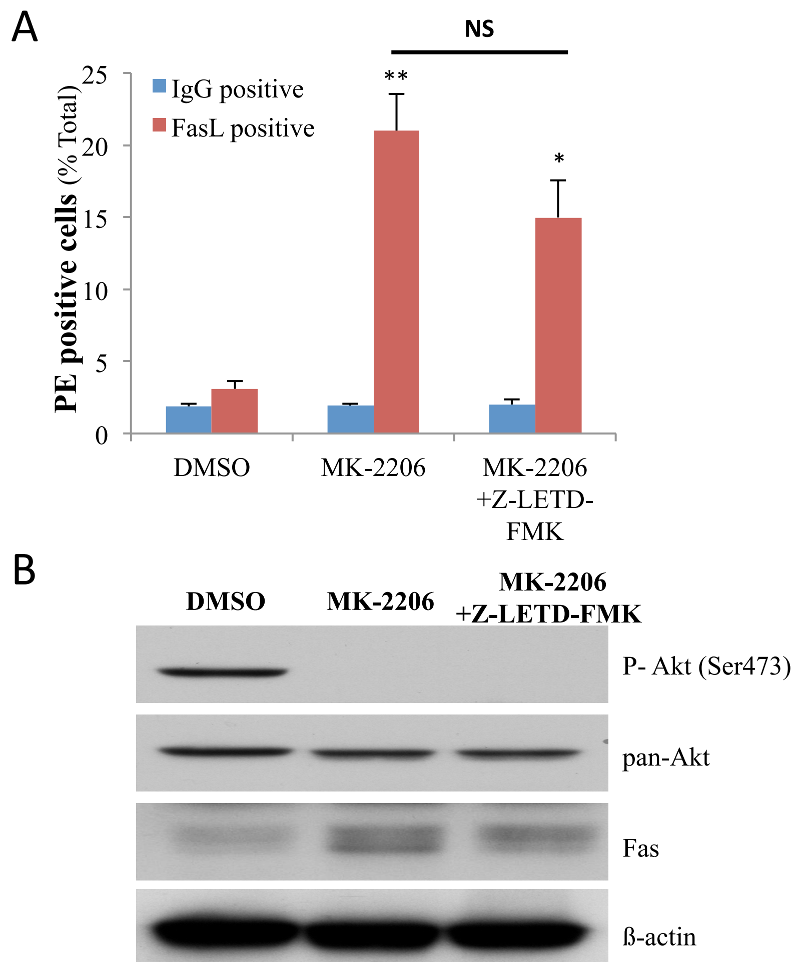
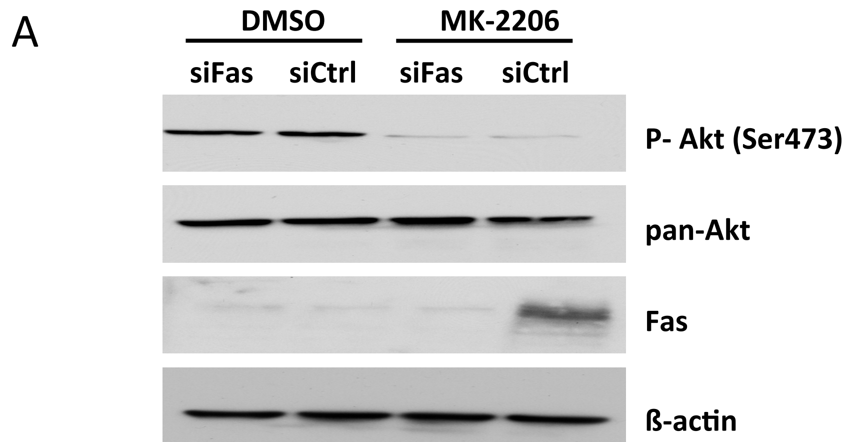


Figure S5 (related to Fig. 7E). Thymic lymphoma cells cells were treated with DMSO or 1μM MK-2206 for 18h in the presence or absence of caspase-8 inhibitor (Z-LETD-FMK). Following treatment, (A) FasL surface expression was assessed by FACS analysis and (B) cell lysates were subjected to immunoblotting using the indicated antibodies Data are presented as an average of 3 independent experiments in triplicates ± SEM. **$p<0.005$ and *$p<0.05$ compared to DMSO. NS-not significant.



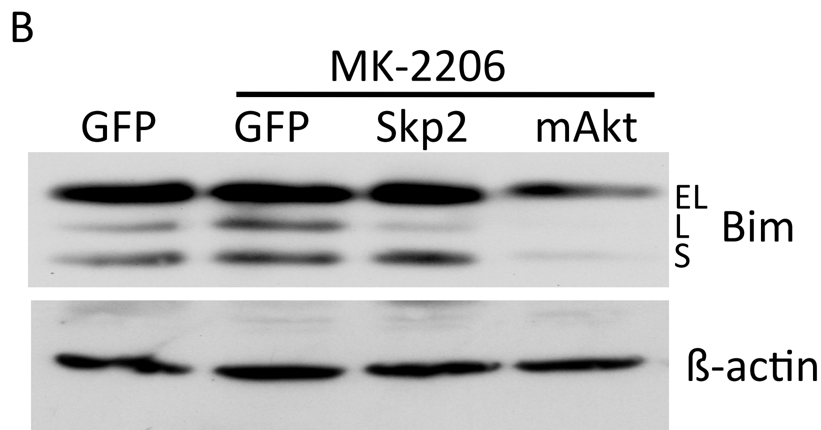
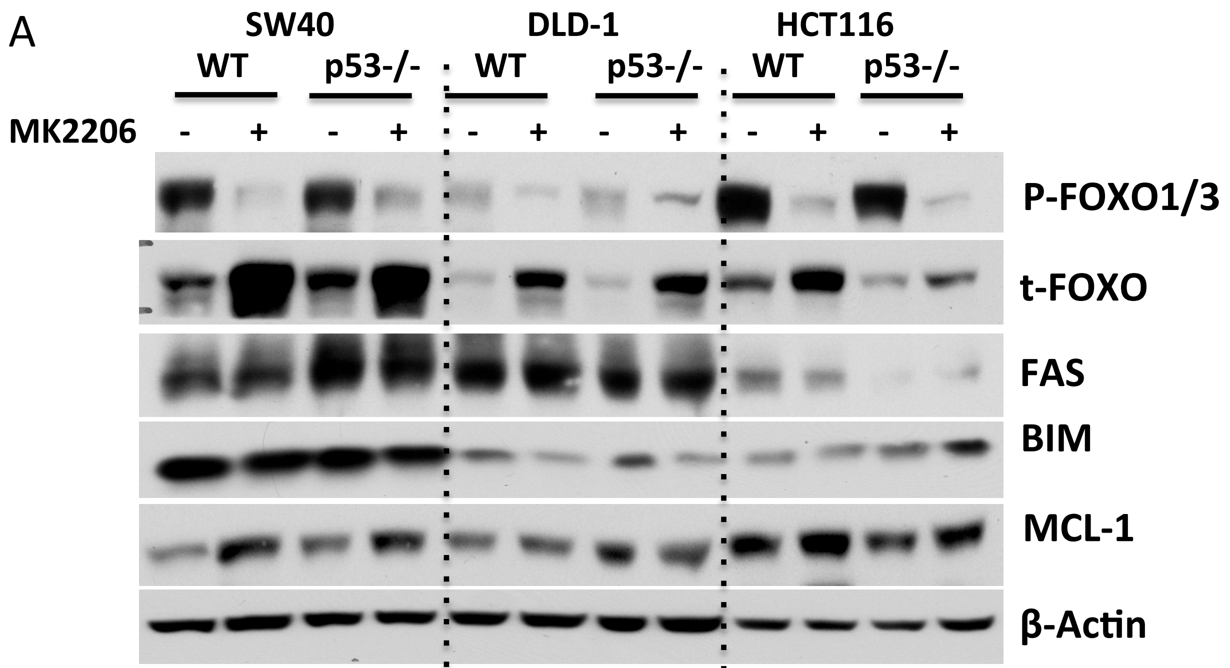
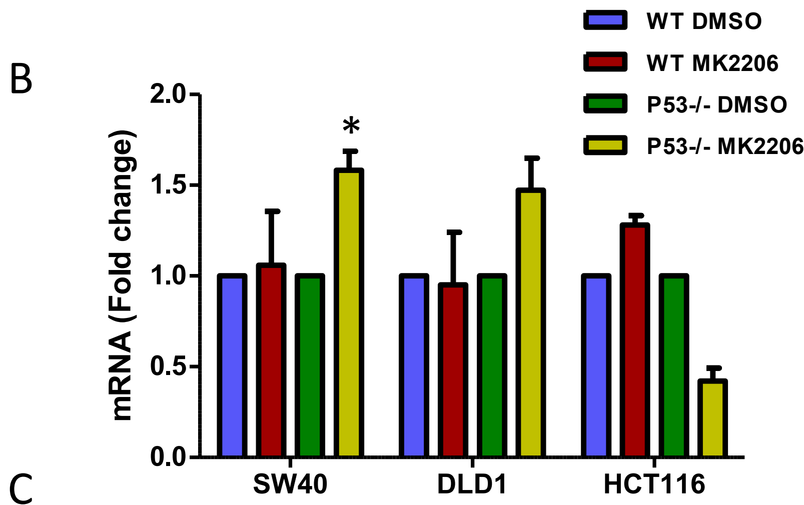


Figure S6 (related to Figure 7).

A. Cells were treated with DMSO or MK2206 for 18h after knockdown of Fas with siRNA cell lysates were prepared for immunoblotting. Immunoblot shows the phosphorylation of Akt, and expression of Fas.

B. Expression of BIM protein before and after treatment in control GFP expressing cells or in SKP2 and mAkt expressing,





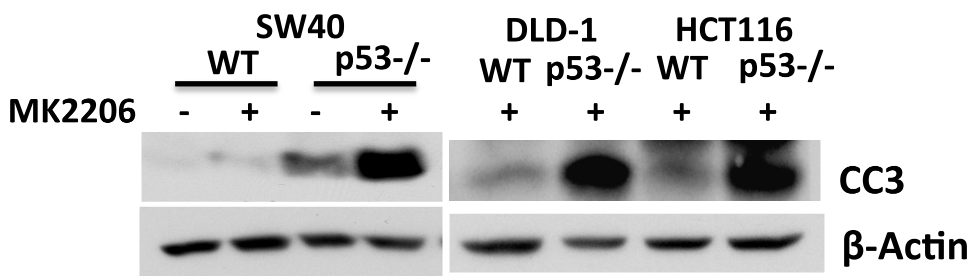


Figure S7 (related to Figures 5F and 7). A. Immunoblot showing FOXO phosphorylation, FAS, BIM and MCL-1 after exposure of p53-proficient and p53-deficient Isogenic human colorectal cancer cell lines after exposure to MK2206. Cells were treated with 10μM MK2206 for 48 h and cell extract were prepared for immunoblotting. **B.** Relative levels **of** FasL mRNA after exposure to MK2206 for 48h as measured by RT-qPCR. Results presented as an average +/-SEM. *p<0.05. . **C.** Cell extracts were isolated to determine caspase 3 cleavage (CC3) after exposure to 10μM MK2206 for 48 h (SW40 cells) or 72 h (DLD-1 and HCT116 cells).