Supplementary Material

Supplementary Methods

Primary tumor cell and mature fibroblasts isolation, culture, and retroviral infection

Tumors (T-cell lymphoma and sarcoma) that developed in *Mdm2*$^{fl/fl}$*p53*$^{-/-}$ mice were harvested and placed in short-term culture. *Mdm2*$^{fl/fl}$*p53*$^{-/-}$ lymphoma was grown in RPMI 1640 with 10% FBS, 1% L-glutamine, and penicillin/streptomycin. *Mdm2*$^{fl/fl}$*p53*$^{-/-}$ sarcoma was grown in DMEM with 10% FBS, 1% L-glutamine, penicillin/streptomycin, and MEM non-essential amino acids. Adult mouse fibroblasts were derived from ear punches of *Mdm2*$^{fl/fl}$*p53*$^{-/-}$ mice and subjected to short-term culture using the same media in which the sarcoma cells were grown. *p53*$^{-/-}$ status was confirmed by PCR in all three cell types evaluated (Supplementary Figure S1). All cell cultures tested negative for mycoplasma. Malignant cells and fibroblasts were infected with a bicistronic retrovirus expressing CreER^{T2} and GFP or GFP alone. Only cells with >90% GFP were used in experiments.

p53 genotyping

Genomic DNA was isolated from the *Mdm2*$^{fl/fl}$*p53*$^{-/-}$ lymphoma, sarcoma, and fibroblast cell cultures using the REDExtract-N-Amp Tissue PCR Kit (Sigma). PCR was performed with primers specific for the wild-type and knockout alleles of *p53* as previously published (1).

Bioinformatic analysis

RNA-seq: RNA-sequence (RNA-seq) profiles of high-quality total RNA from 4-hydroxytamoxifen (4-OHT) or vehicle control-treated *Mdm2*$^{fl/fl}$*p53*$^{-/-}$ lymphoma (6 hr), sarcoma

(48 hr), and fibroblast (48 hr) cells were obtained using the Illumina NextSeq 500 platform. Quality control was done using FastQC (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/). Maximum likelihood estimates of transcript read count for each sample were computed with Kallisto v0.43.0 (2). Transcriptome quantification was performed based on murine transcript definitions in Ensembl release 85. Both coding and noncoding RNA sequences, obtained from Ensembl in fasta format, were used to create a Kallisto index. The Kallisto quantification algorithm was then run on paired fastq files for each sample to estimate transcript abundance. The transcript level abundance was then summarized into gene level using tximport (3). Differentially expressed genes were identified from a pool of three 4-OHT treated and matched vehicle treated samples. Differential gene expression using edgeR (4) was performed following the procedure mentioned in (3). Genes were identified as differentially expressed based on the following criteria: 2 fold-change and $p<0.05$ after being adjusted by Benjamini-Hochberg multiple testing correction method (5).

GSEA, Gene Ontology and Pathway enrichment analyses: Gene set enrichment analysis (GSEA) (6) was performed using Kyoto Encyclopedia of Genes and Genomes (KEGG) suite embedded in GSEA to identify signaling pathways that are differentially activated in 4-OHT treated cells compared to vehicle treated cells. GSEA was run on a pre-ranked list of genes based on fold-change induction in the 4-OHT treated cells compared to vehicle treated cells. Mouse genes were converted to human gene symbols using NCBI's Homologene database before conducting GSEA pre-ranked analysis. Biological process terms of Gene Ontology (7) and Kyoto Encyclopedia of Genes and Genomes (KEGG) suite, which were embedded into the software WebGestalt (8), were used to conduct Gene Ontology and Pathway enrichment analysis.

qRT-PCR Primers

Primers for qRT-PCR were obtained from Operon:

Bax-forward-TGGAGCTGCAGAGGATGATTG

Bax-reverse-GATCAGCTCGGGCACTTTAG

Noxa-forward-CACTCGCGATTCATCTTGCG

Noxa-reverse-GTTATGTCCGGTGCACTCCA

Puma-forward-CCCAGCAGCACTTAGAGTCG

Puma-reverse-AGTTGGGCTCCATTTCTGGG

p21-forward-GGACCACGTGGCCTTGTC

p21-reverse-TCTCTTGCAGAAGACCAATCTG

14-3-3-σ-forward-AGAACTGGACTGTGGCAAGG

14-3-3-σ-reverse-CATGGTCACACCCAGCAAAC

Gadd45a-forward-TGCAGAGCAGAAGACCGAAA

Gadd45a-reverse-CACGTTATCGGGGTCTACGTT

β-actin-forward-CCTGAACCCTAAGGCCAACC

β-actin-reverse-ATGGCGTGAGGGAGAGCATA

Supplementary References

1. Jacks T*, et al.* (1994) Tumor spectrum analysis in p53-mutant mice. *Curr Biol* 4(1):1-7.

2. Bray NL, Pimentel H, Melsted P, & Pachter L (2016) Near-optimal probabilistic RNA-seq quantification. *Nat Biotechnol* 34(5):525-527.

3. Soneson C, Love MI, & Robinson MD (2015) Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences. *F1000Res* 4:1521.

4. Robinson MD, McCarthy DJ, & Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26(1):139-140.

5. Benjamini Y & Hochberg Y (1995) Controlling the False Discovery Rate - a Practical and Powerful Approach to Multiple Testing. *J Roy Stat Soc B Met* 57(1):289-300.

6. Subramanian A*, et al.* (2005) Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proceedings of the National Academy of Sciences of the United States of America* 102(43):15545-15550.

7. Ashburner M*, et al.* (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat. Genet.* 25(1):25-29.

8. Zhang B, Kirov S, & Snoddy J (2005) WebGestalt: an integrated system for exploring gene sets in various biological contexts. *Nucleic Acids Res.* 33(Web Server issue):W741-748.

Supplementary Figure Legends

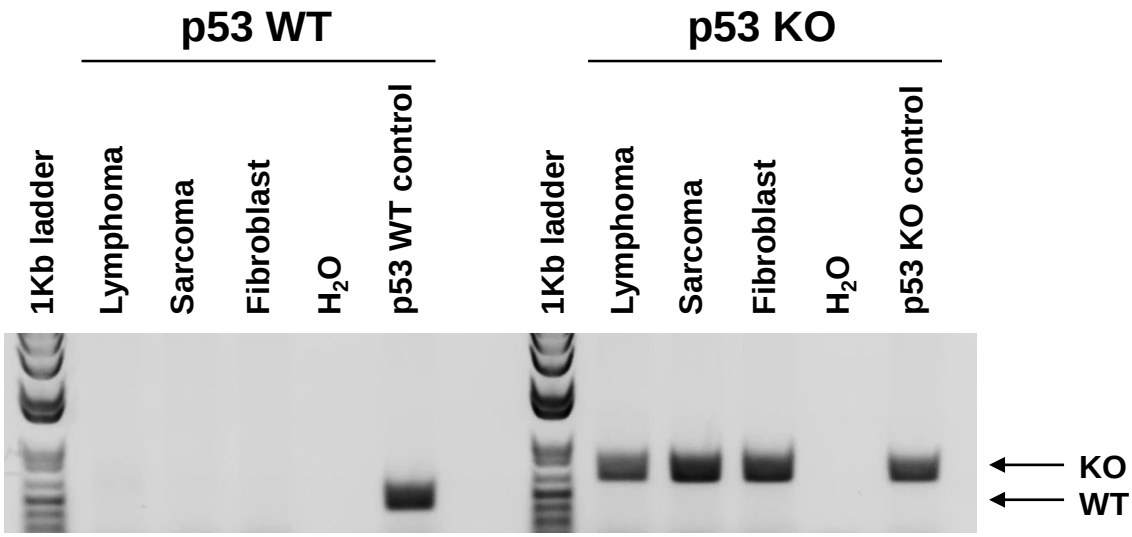
Supplementary Figure S1. Confirmation of *p53*-null status. PCR was used to perform genotyping analysis on the T-cell lymphoma, sarcoma, and fibroblast cells isolated from *Mdm2*$^{fl/fl}$*p53*$^{-/-}$ mice. Two primer sets were used to distinguish between the *p53* wild-type (WT, left) and knockout (KO, right) alleles. Water (H_20), instead of DNA, and a DNA known to contain WT or KO alleles of p53 were used as negative and positive controls, respectively.

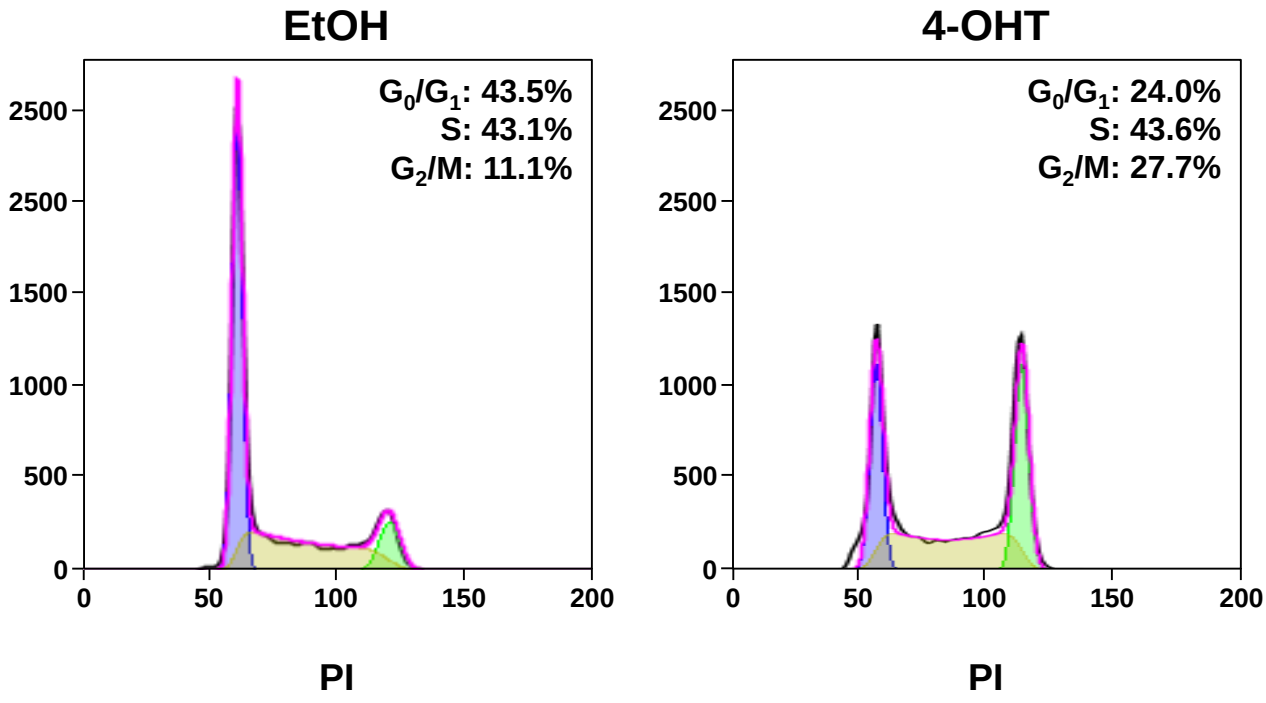
Supplementary Figure S2. Loss of *Mdm2* induces a G_2/M cell cycle arrest. To cultures of *Mdm2*$^{fl/fl}$*p53*$^{-/-}$ sarcoma cells expressing CreER^{T2}, 4-OHT or vehicle control (EtOH) was added, and 36 hours later DNA was stained with propidium iodide (PI) and cells subjected to flow cytometry. G_2/M DNA content was evaluated using the Dean-Jett-Fox cell cycle model. Representative cell cycle plots are shown for the mean values depicted in Fig. 2H.

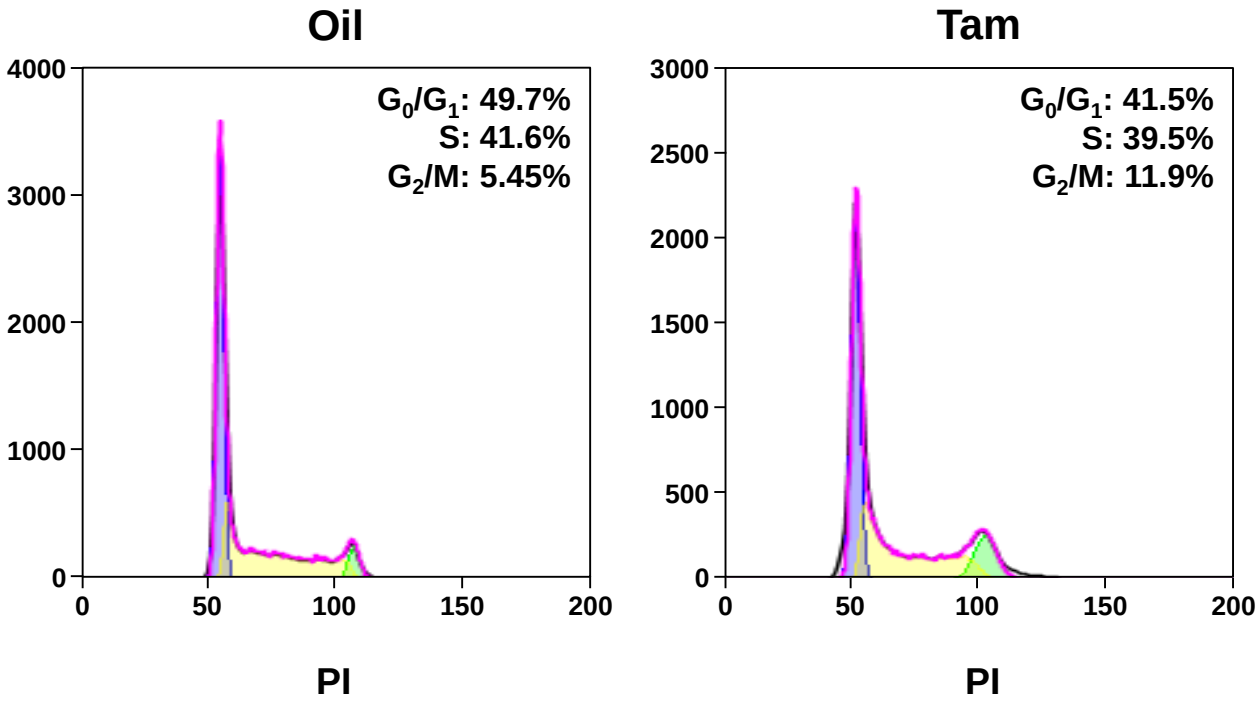
Supplementary Figure S3. Loss of *Mdm2* induces a G_2/M cell cycle arrest *in vivo*. Nude mice were injected (subcutaneously) with CreER^{T2}-expressing *Mdm2*$^{fl/fl}$*p53*-null lymphoma cells and administered tamoxifen (Tam) or vehicle (corn oil, Oil) control. After 48 hours, tumors were harvested, DNA was stained with propidium iodide (PI), and lymphoma cells subjected to flow cytometry. G_2/M DNA content of the lymphoma cells was evaluated using the Dean-Jett-Fox cell cycle model. Representative cell cycle plots are shown for the mean values depicted in Fig. 3E.

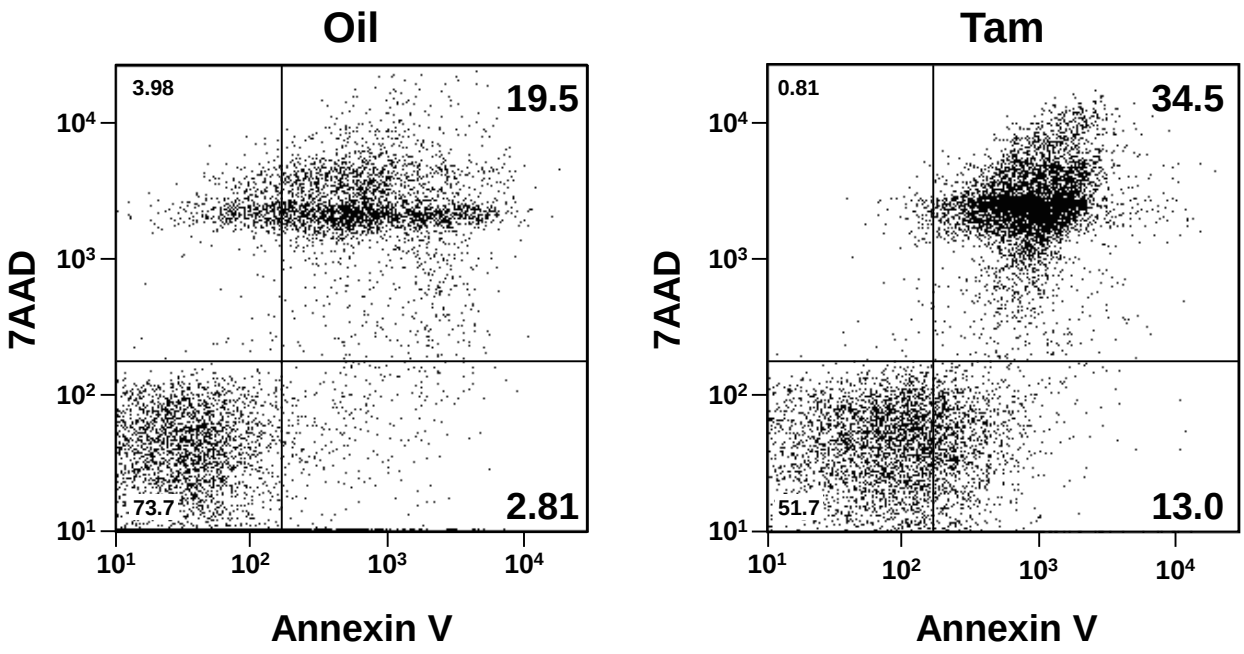
Supplementary Figure S4. *Mdm2* deletion induces apoptosis *in vivo*. Nude mice were injected (subcutaneously) with CreER^{T2}-expressing *Mdm2*$^{fl/fl}$*p53*-null sarcoma cells. Once tumors were palpable, mice were administered tamoxifen (Tam) or vehicle corn oil (Oil) control. After 72 hours, tumors were harvested and Annexin-V/7AAD was evaluated by flow cytometry. Representative dot plots are shown for the mean values depicted in Fig. 4C.

Supplementary Figure S5. Gene set enrichment analyses for apoptosis and p53 pathway genes. Using RNA-sequencing data generated from CreER^{T2}-expressing *Mdm2*$^{fl/fl}$*p53*$^{-/-}$ lymphoma, sarcoma, and mature fibroblasts following addition of 4-OHT or vehicle control (EtOH) for 6 hours (lymphoma) or 48 hours (sarcoma and fibroblasts), gene set enrichment analysis was performed. The distribution of genes (black lines) is presented as a function of the fold-change in gene expression. Genes are distributed from highly up-regulated (red) to highly down-regulated (blue); normalized enrichment score (NES), nominal *P*-value (Nom.), and false discovery rate (FDR).

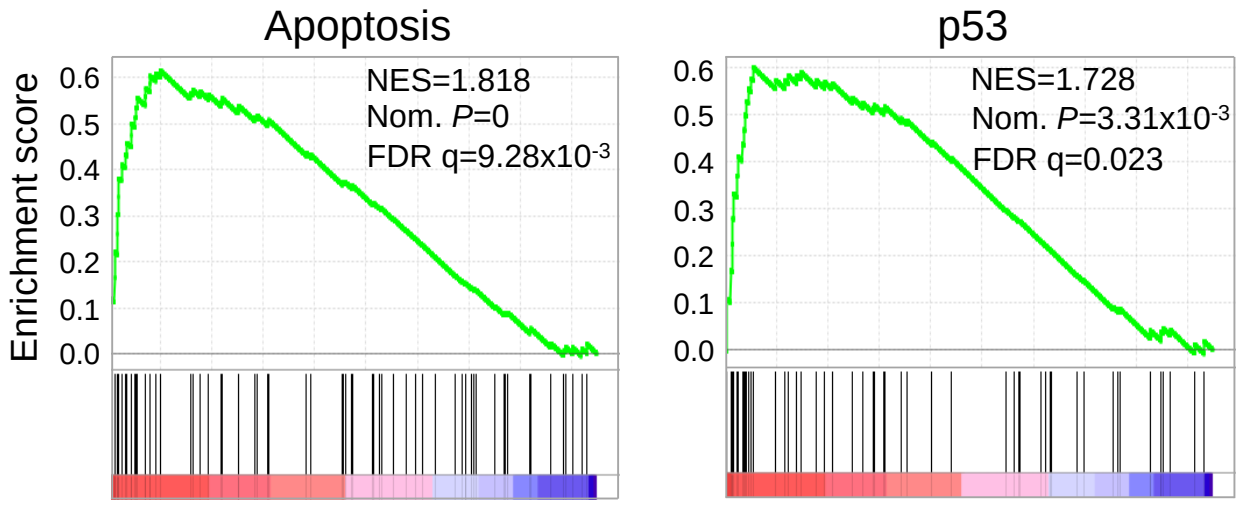

p53 WT
p53 KO
1Kb ladder
Lymphoma
Sarcoma
Fibroblast
H2O
p53 WT control
1Kb ladder
Lymphoma
Sarcoma
Fibroblast
H2O
p53 KO control
KO
WT


EtOH
G0/G1: 43.5%
S: 43.1%
G2/M: 11.1%
2500
2500
1500
1000
500
0
0
50
100
150
200
PI
4-OHT
G0/G1: 24.0%
S: 43.6%
G2/M: 27.7%
2500
2500
1500
1000
500
0
0
50
100
150
200
PI

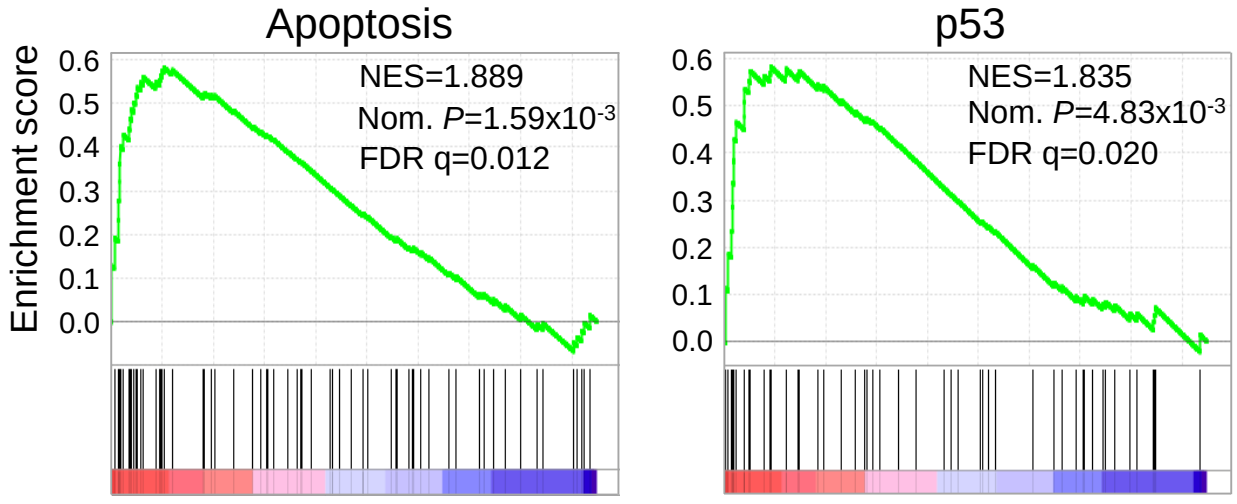

Oil
4000
3000
2000
1000
0
0
50
100
150
200
PI
G0/G1: 49.7%
S: 41.6%
G2/M: 5.45%
Tam
3000
2500
2000
1500
1000
500
0
0
50
100
150
200
PI
G0/G1: 41.5%
S: 39.5%
G2/M: 11.9%


Oil
3.98
19.5
73.7
2.81
7AAD
Annexin V
Tam
0.81
34.5
51.7
13.0
7AAD
Annexin V

Lymphoma



Sarcoma



Fibroblast