Supporting Information

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SI Text

Generation of $p53^{TAP}$ Mouse ES Cells and Mice. Linearized targeting vector was electroporated into a B6/129 hybrid ES cell line RJ2.2 (generated from blastocysts derived from mating 129SvEvTac male with C57BL/6 albino females) and correctly targeted clones identified by Southern hybridization with an external 3′ probe. The *neo* cassette was removed by electroporating targeted ES cells with the cre-expressing plasmid pOG231 (1), followed by a second round of Southern hybridization using an internal probe. Chimeric mice were generated by aggregation of diploid morula stage CD1 embryos with targeted ES cells by standard procedures (2), and high percentage male chimeras were mated with CD1 females to assay for germline transmission. Pups containing the targeted allele were identified by Southern hybridization.

siRNA Knockdown in ES Cells. Trim24 (L-047483–00) and control (D-001210–01-20) siRNAs were obtained from Dharmacon RNA Technologies and transfected using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions at a concentration of 100 nM siRNA. mES cells were treated with 0.5 μg/mL doxorubicin for the indicated timepoints after 48 h siRNA knockdown.

Size-Exclusion Chromatography. The column was calibrated with a high molecular weight gel filtration calibration kit (GE Healthcare, 28–4038-42). Chromatography was performed at 0.4 mL/min, collecting 0.5 mL fractions at 4°C. The fractions were analyzed by SDS/PAGE gels followed by immunoblotting with an anti-p53 (CM5) antibody.

Quantitative Real-Time PCR Analysis. RNA was isolated using TRIzol Reagent (Invitrogen) as per the manufacturer's specifications. cDNA was synthesized as previously described (3, 4). The average threshold (Ct) was determined for each gene and normalized to 18S rRNA as an internal control. Primer sequences are available upon request.

ChIP Assays. Cells were crossed linked with 1% formaldehyde for 10 min at room temperature, then sonicated 10 times for 20 s in an ice bath. The chromatin lysate was further digested with micrococcal nuclease (0.25 U/μL, Worthington Biochemicals) to obtain DNA fragments of average length under 500 bp. The following antibodies were used: p53 (OP03, Oncogene Ab1), Protein A (P2921, Sigma), histone H3 (Ab1791, Abcam), normal sheep IgG (Millipore), and TRIM24 (TIF1-α, A300–815A, Bethyl). To analyze specific antibody-bound DNA fractions, PCR was performed (primer sequences available upon request).

Human Cell Lines, siRNA, and Protein Analysis. MCF7, U2OS, and HEK293T cells were obtained from ATCC and cultured in under suggested conditions. Human full-length and N-terminal Ring domain truncated TRIM24 were amplified from pCMV6-XL4-TRIM24 plasmid (Origene Technologies) and subcloned into a Flag-vector. Cells were transfected with plasmids encoding human MDM2 (from the laboratory of G. Lozano), Flag-TRIM24, or Flag-TRIM24ΔRing using Effectene (Qiagen) following the manufacturer's recommendations. Oligonucleotide pools of siRNA targeting human TRIM24, p53 and nontarget (control) RNAs were purchased from Dharmacon and were transfected cells. Then, 48 h after siRNA transfection (Lipofectamine2000, Invitrogen), MCF7 cells were treated with doxorubicin (500 ng/mL) or MG132 (20 μM) and lysed in RIPA buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 1% Nonidet P-40, 0.5% Deoxycholic acid, 0.1% SDS, 1 mM PMSF, and 1 mM Na-Vanadate) supplemented with protease inhibitor mixture (Calbiochem), and phosphatase inhibitor cocktails I and II (Sigma). Fifty micrograms cell lysate was analyzed by immunoblottting with anti-p53 (DO1, Santa Cruz), anti-TRIM24 (Novus), anti-Flag (Sigma) and anti-actin (Sigma) antibodies. Protein bands on immunoblots were quantitated using ImageQuant software, normalized to actin and plotted.

ES Cell Culture. Mouse ES cells (mES) were maintained on 0.1% gelatin-coated dishes in Dulbecco's modified Eagle's medium (DMEM, HyClone) supplemented with 15% FBS (FBS, Gemini), 2 mM L-glutamine (HyClone), 100 IU/mL penicillin, 100 μg/mL streptomycin (Mediatech), 0.1 mM β-mercaptoethanol, and 10 ng/mL recombinant LIF (5).

1. O'Gorman S, Fox DT, Wahl GM (1991) Recombinase-mediated gene activation and site-specific integration in mammalian cells. *Science* 251:1351–1355.
2. Eakin GS, Hadjantonakis AK (2006) Production of chimeras by aggregation of embryonic stem cells with diploid or tetraploid mouse embryos. *Nat Protoc* 1:1145–1153.
3. Wilkinson DS, Tsai WW, Schumacher MA, Barton MC (2008) Chromatin-bound p53 anchors activated Smads and the mSin3A corepressor to confer transforming-growth-factor-beta-mediated transcription repression. *Mol Cell Biol* 28:1988–1998.
4. Chao C, et al. (2003) Cell type- and promoter-specific roles of Ser18 phosphorylation in regulating p53 responses. *J Biol Chem* 278:41028–41033.
5. Mereau A, Grey L, Piquet-Pellorce C, Heath JK (1993) Characterization of a binding protein for leukemia inhibitory factor localized in extracellular matrix. *J Cell Biol* 122:713–719.

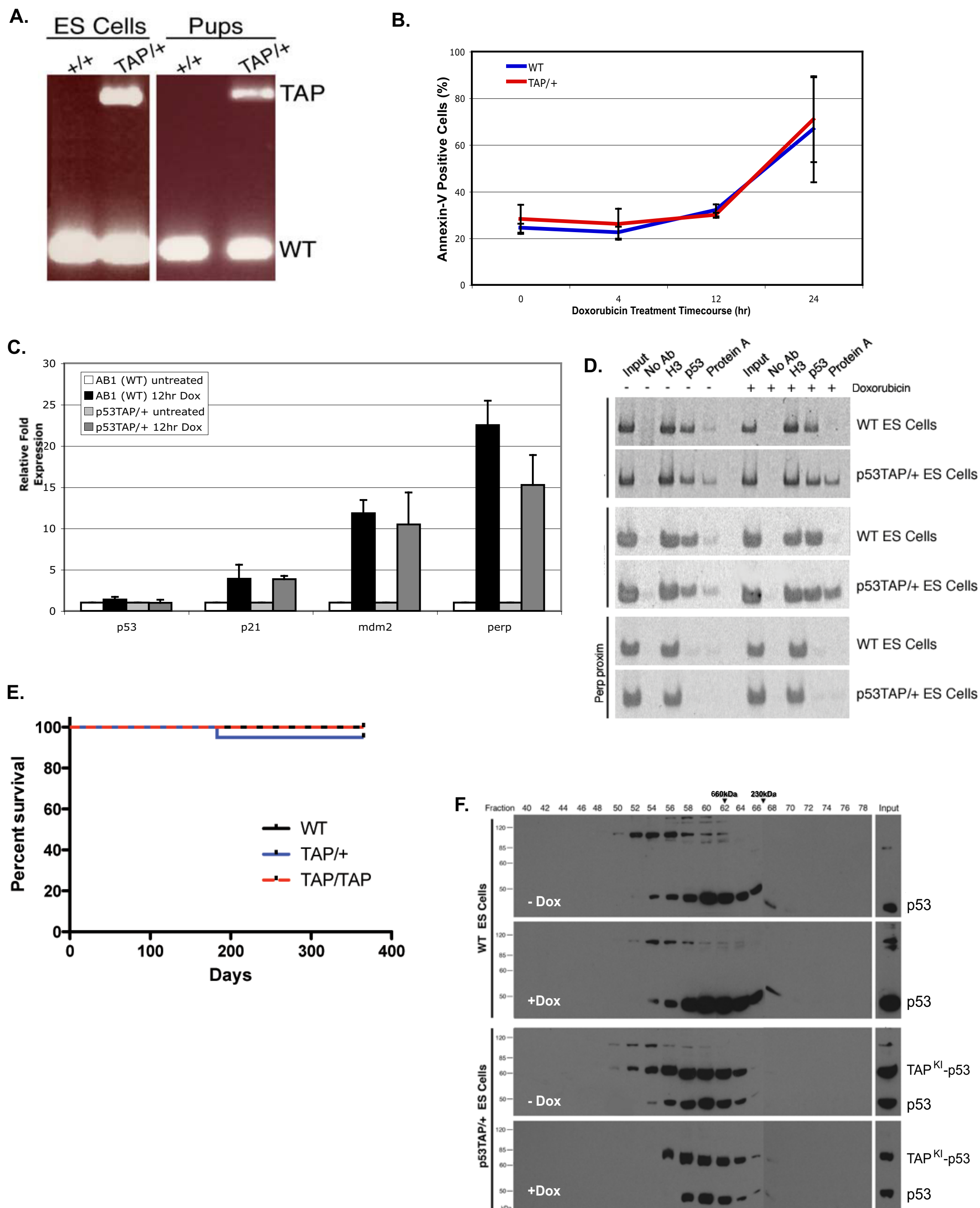

Fig. S1. TAP-KI does not affect p53 functions. (*A*) Screening for correctly targeted ES and pups was conducted by PCR genotyping. (*B*) Apoptosis of ES cells: Heterozygous p53$^{TAP\text{-}KI/+}$ and wild-type (WT) ES cells were collected after 0, 4, 12, and 24 h of exposure to doxorubicin (Dox, 0.5 μg/mL), stained with annexin V-FITC and sorted. (*C*) ES cells, both WT and p53$^{TAP\text{-}KI/+}$, respond to Dox-induced stress by activation of p53-target genes. Real-time RT-PCR was done to determine levels of RNA expression for each gene, and expressed relative to $t = 0$ levels of expression in each case. (*D*) ChIP analysis of WT and p53$^{TAP\text{-}KI/+}$ ES cells showed that p53 and p53-TAP^{KI} exhibit the same ability to bind to p53 response elements in the presence or absence of Dox-induced stress. Protein-A antibody was used to precipitate p53-TAP^{KI}-bound chromatin. Anti-p53 antibody (AB1) was used for p53 lacking a TAP-tag (WT). Histone H3 antibody was used as a positive control. Samples were separated by PAGE and stained with Sybr-Green. Input is 10% of total used in ChIP reaction. (*E*) Survival of p53-TAP^{KI} mice: Cohorts of mice, with indicated p53 status: WT, TAP-homozygous and TAP-heterozygous, were aged for more than a year. WT and TAP/TAP lines overlap. (*F*) Size-exclusion chromatography: Extracts of p53$^{TAP\text{-}KI/+}$ and WT ES cells, -Dox or +Dox for 5 h, were fractionated by Superose-6, analyzed by SDS/PAGE, and immunoblotted for p53 protein. Migration of p53 and TAP^{KI}-p53 proteins are indicated next to Input. Slower migrating, unlabeled bands are likely post-translationally modified forms of p53.

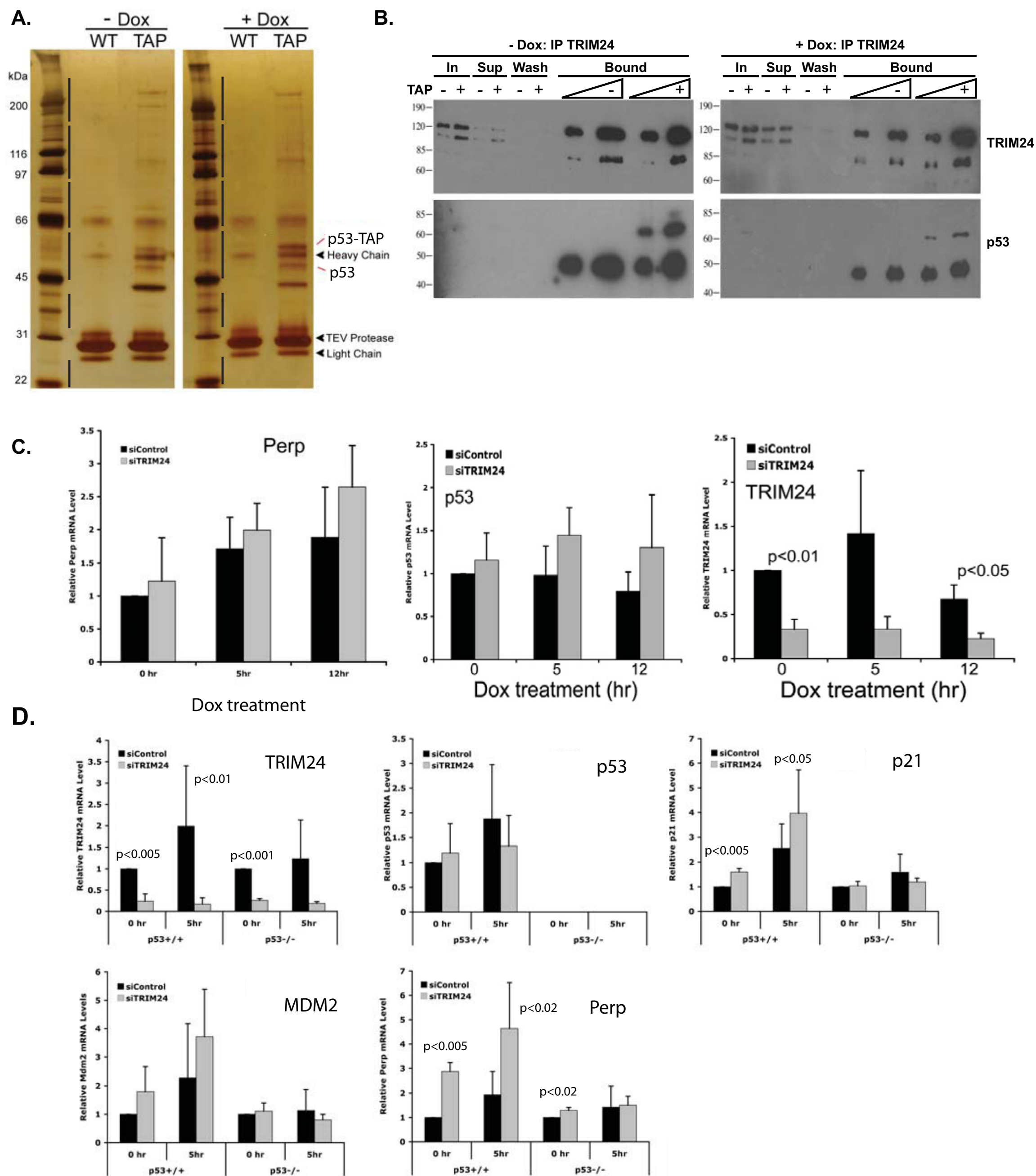

Fig. S2. Trim24 interacts with p53. (*A*) TAP-purified proteins of $p53^{TAP-KI/+}$ (TAP) and control (WT) ES cell extracts were separated by SDS/PAGE and stained, before excision of gel bands and regions (demarcated by vertical lines) from each lane. After in-gel digestion, peptides were separated by an LTQ mass spectrometer and considered specific when present only in TAP samples. (*B*) IP-verification of Trim24-p53 interaction. Ten milligrams total Input protein of WT (- TAP) or $p53^{TAP-KI/+}$ were used for IP of Trim24 from ES cell extracts prepared after Dox (0.5 μg/mL) or without Dox induction of stress for 5 h. Immunoblotting was conducted as indicated after SDS/PAGE. (*C*) siRNA-depletion of *Trim24* led to increased activation of specific p53-target genes, but not *Perp* or *Trp-53*, in ES cells. Cells were treated with siControl (black bars) or siTRIM24 oligos (gray bars). RNA expression values, determined by real-time RT-PCR, are expressed as fold-change in comparison to siControl-treated cells at $t = 0$. (*D*) siRNA-depletion of *Trim24* caused p53-dependent activation of specific p53-target genes in MEFs. e13.5 MEFs, isolated from WT and p53-null embryos, were treated with 0.5 μg/mL Dox for 0 or 5 h as indicated. siRNA treatment and determination of RNA expression levels were performed as described above. Significantly altered RNA expression levels are indicated by *P* values less than 0.05.

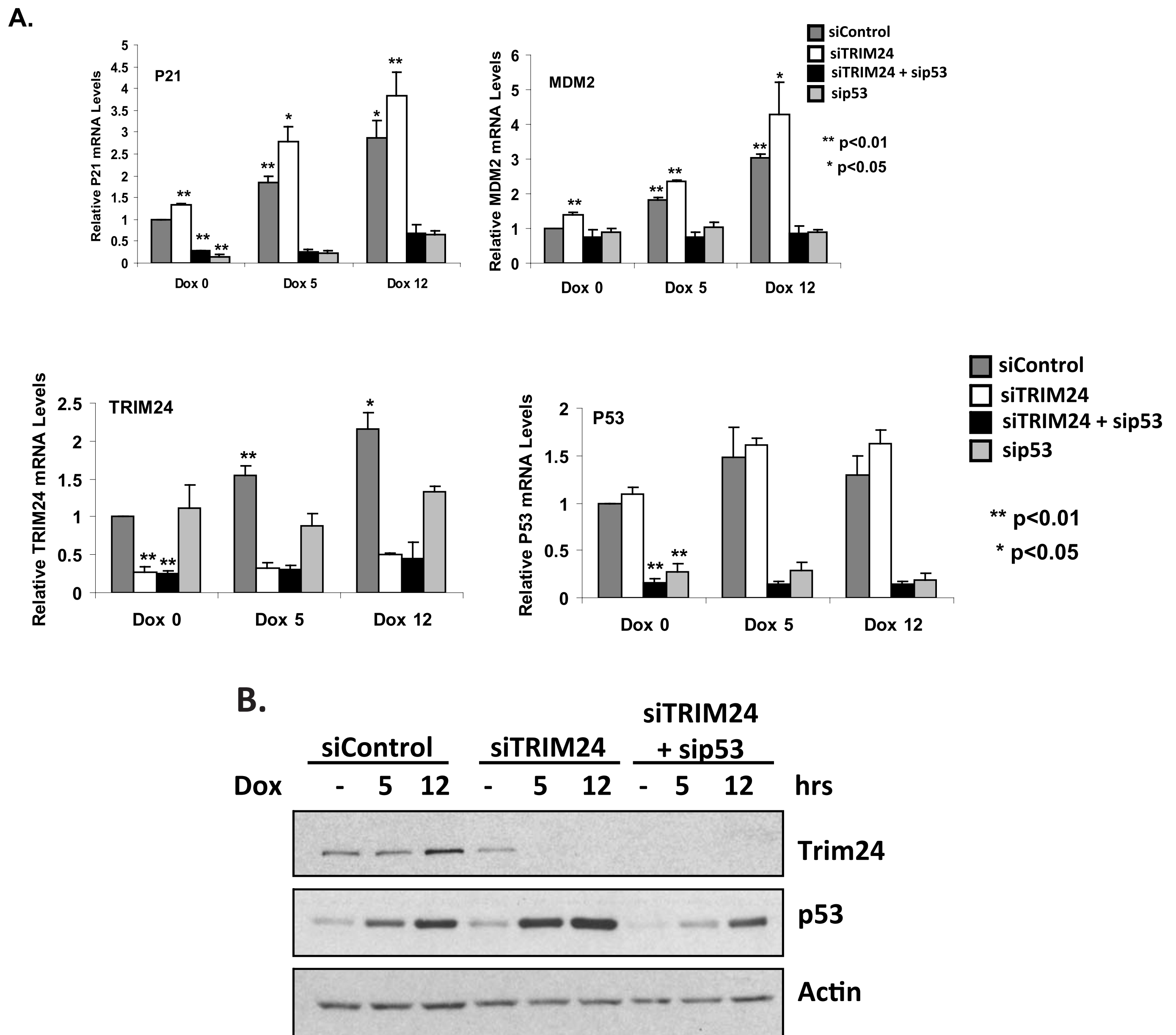

Fig. S3. Trim24 and p53 depletion in MCF7 cells. (*A*) MCF7 cells were treated with siControl, siTrim24, sip53, or siTrim24/p53 oligos, as indicated. RNA levels of MDM2 and p21 were assessed at $t = 0$ or after treatment with 0.5 μg/mL Dox for 5 or 12 h as indicated. RNA expression values, determined by real-time RT-PCR, are expressed as fold-change in comparison to siControl-treated cells at $t = 0$. (*B*) Protein analysis of si-depleted MCF7 cells as in *A*.

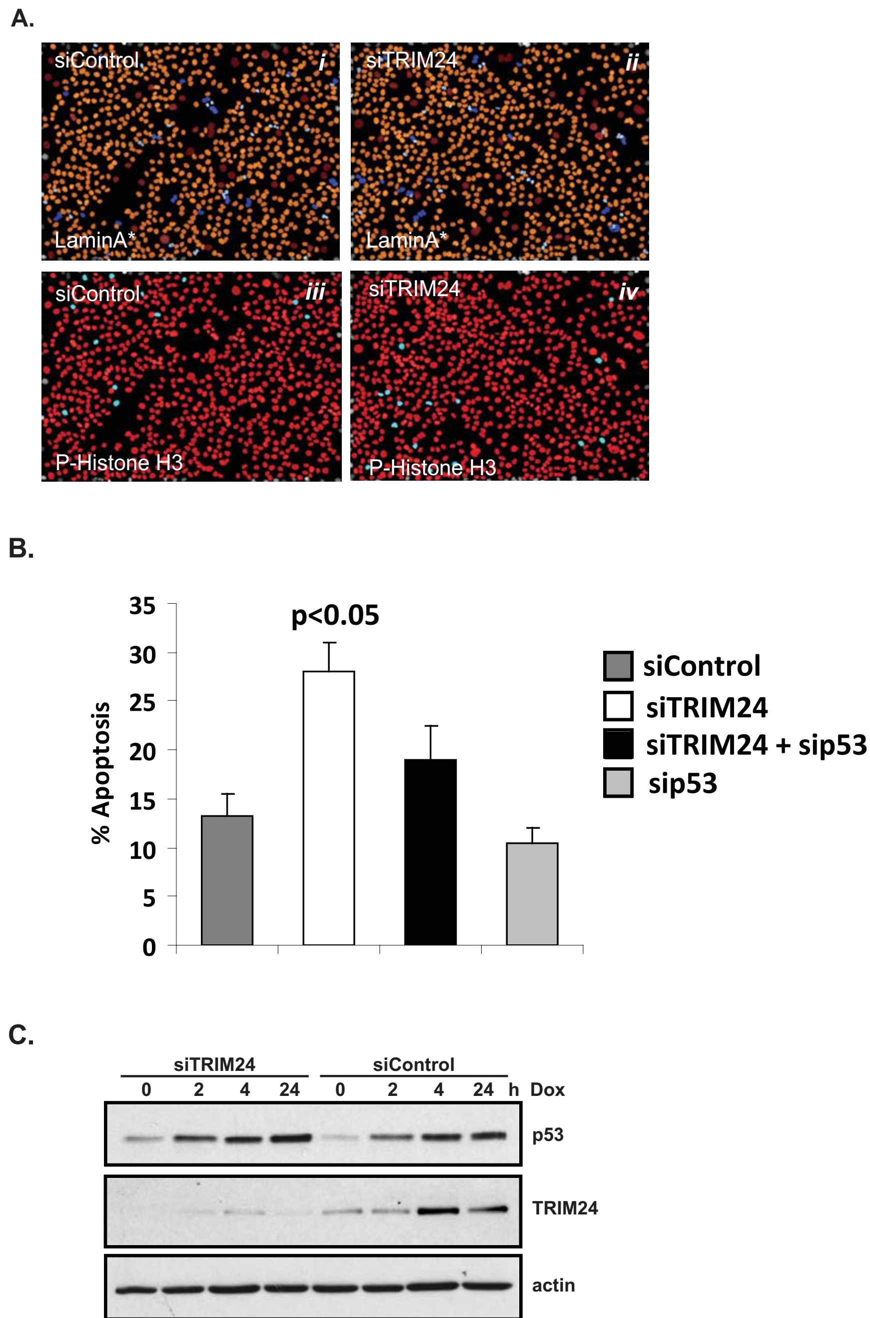

Fig. S4. TRIM24 depletion induces apoptosis by increased p53-protein levels. (*A*) Depletion of TRIM24 in T47D cells (p53-mutant) does not increase apoptosis or decrease cell number and mitotic status. Antibodies recognizing cleaved lamin-A (top) to detect apoptosis (green), or phospho-histone H3 (bottom) to detect cells in mitosis (blue), were used after transfection with siControl (left) and siTRIM24 (right) oligos. Additionally, cells were stained with Hoechst followed by automated image acquisition and analysis. (*B*) Depletion of TRIM24 in MCF7 cells induces significant levels of apoptosis that is p53-dependent. Replicate annexin-V-staining and FACS analyses were performed in triplicate on siRNA-treated cells, and %-apoptotic cells graphed in comparison to each other. Only siTRIM24 exhibited significant increases in apoptosis ($P = 0.02$), compared to si-p53 ($P = 0.11$) and siTRIM24/p53 ($P = 0.11$), as assessed by Student's *t* test. (*C*) Levels of p53 protein increase when TRIM24 is depleted. MCF7 cells were transfected with *TRIM24* siRNA or nontarget (control) siRNA and treated with Dox for 0, 2, 4, and 24 h. Actin, p53 and TRIM24 proteins were analyzed by immunoblotting whole-cell lysates.

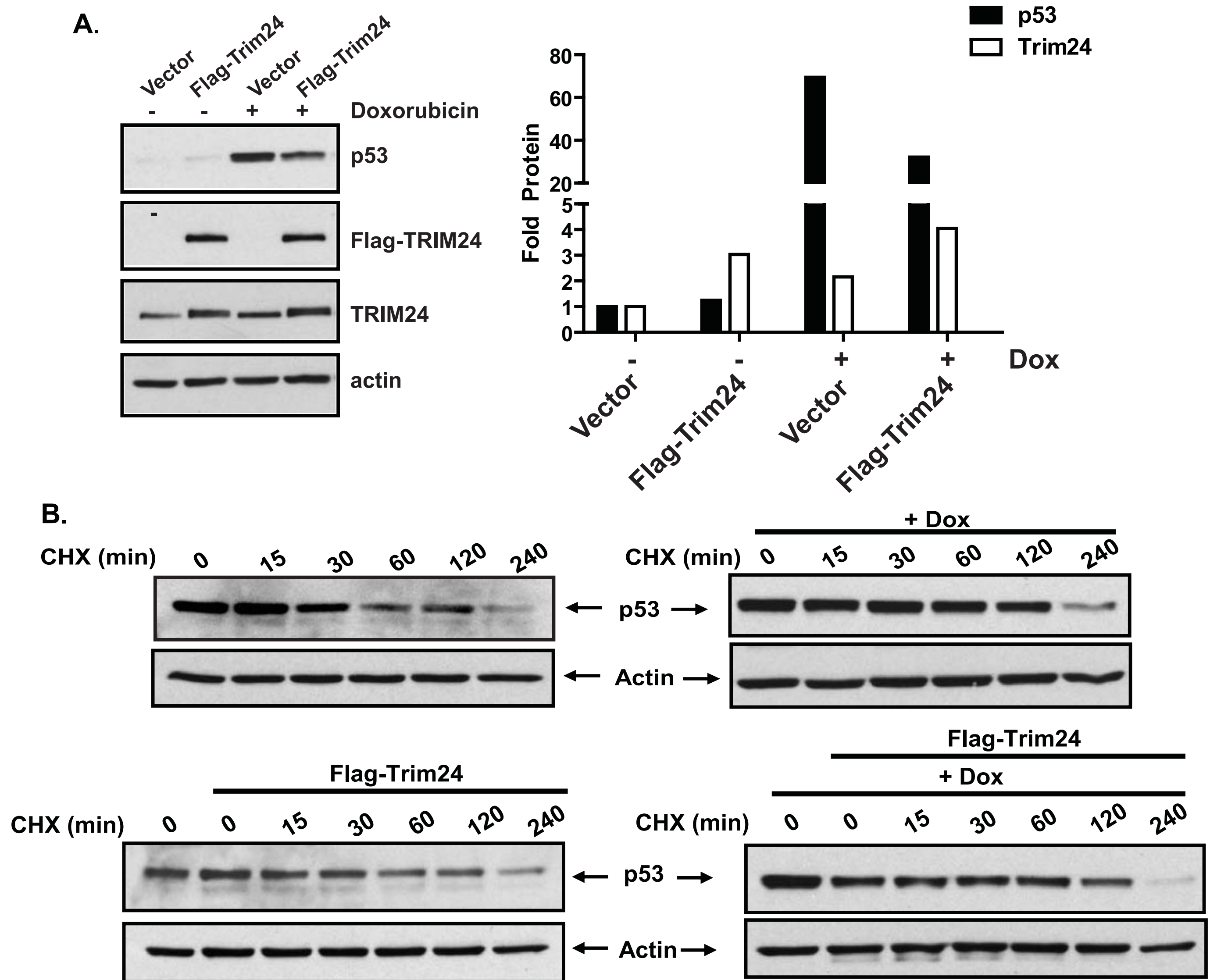

Fig. S5. TRIM24 negatively regulates p53 protein stability. (*A*) MCF7 cells transiently transfected with vector or Flag-TRIM24 were treated with Dox for 0 (-) or 8 h (+). Actin, p53, and TRIM24 protein levels were analyzed by immunoblotting and quantified by densitometry. (*B*) p53 half life is decreased by TRIM24 expression. MCF7 cells were treated with cycloheximide for 0, 15, 30, 60, 120, and 240 min or transfected with Flag- TRIM24 followed by similar treatment (Left panels: –Dox, Right panels: +Dox). p53 protein levels were analyzed by SDS/PAGE of lysates and immunoblotting with the indicated antibodies (p53 and actin).