SUPPLEMENTARY INFORMATION

a. **Supplementary Fig S1** related to Fig. 1 and 2
b. **Supplementary Fig S2** related to Fig. 3
c. **Supplementary Fig S3** related to Fig. 4
d. **Supplementary Fig S4** related to Fig. 5
e. **Supplementary Fig S5** related to Fig. 6
f. **Supplementary Fig S6** related to Fig. 7
g. **Supplementary Fig S7** related to Fig. 8
h. **Supplementary Table SI** Oligonucleotide sequences for gene expression analysis by Real Time qRT-PCR
i. **Supplementary Table SII** Oligonucleotide sequences for Real Time qPCR analysis after p53-ChIP
j. **Supplementary Methods**

FIGURE S1

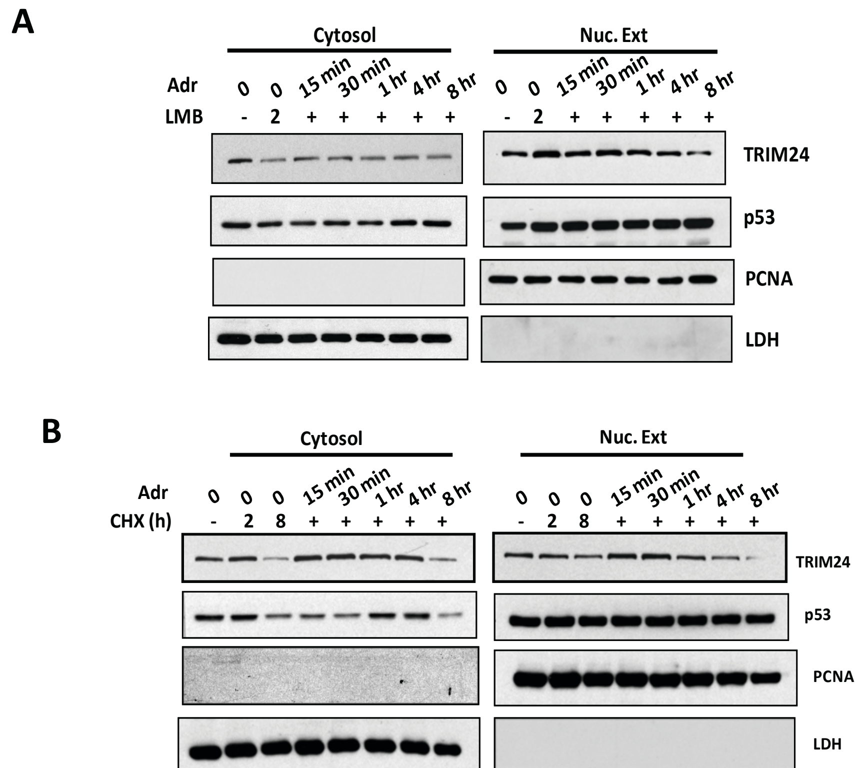


FIGURE S1. DNA-damage induces degradation of TRIM24 in nucleus. A-B) TRIM24 nuclear localization. Nuclear extracts prepared from HEK293T cells treated with Adr for different time points in presence of LMB (A) or protein synthesis inhibitor, Cycloheximide (CHX) (B) were analyzed by western blotting. Blots were quantitated and fold changes in nuclear protein levels are plotted in Figure 2.

FIGURE S2

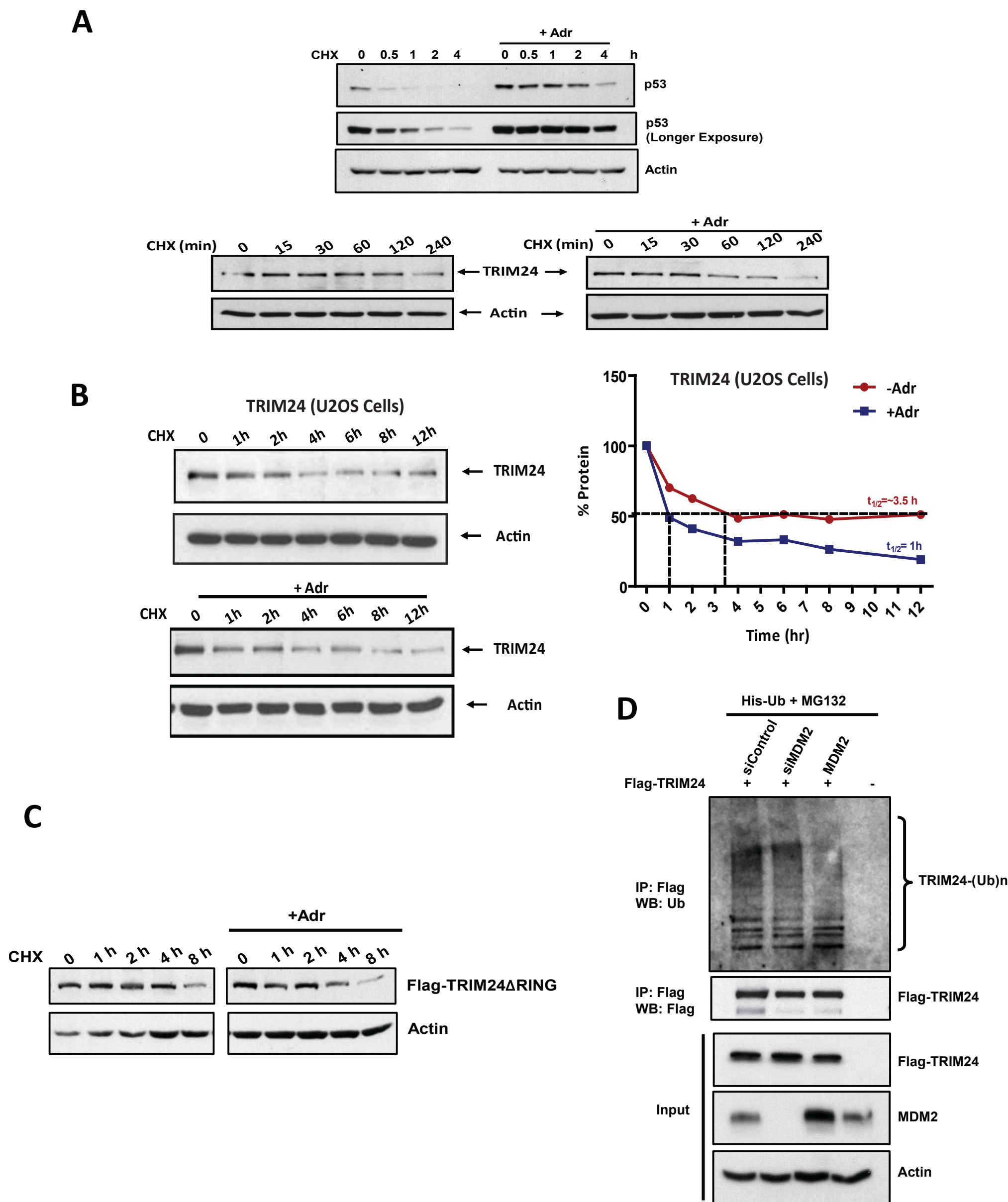


FIGURE S2. TRIM24 undergoes ubiquitination-mediated degradation in response to DNA-damage A-B) TRIM24 half-life. MCF7 cells (in A) and U2OS cells (in B) were treated with Cycloheximide (CHX) for different time points without (–Adr) or with (+Adr) DNA damage. TRIM24 protein levels were analyzed by immunoblotting, quantified by densitometry and plotted against time to determine TRIM24 half-life. C) TRIM24-ΔRING half-life. MCF7 cells were transfected with Flag TRIM24-ΔRING and treated as in A. Flag TRIM24-ΔRING protein levels were analyzed by immunoblotting, quantified by densitometry and plotted against time to determine TRIM24 half-life. C) TRIM24 auto-ubiquitination. MCF7 cells transiently transfected with Flag-TRIM24, siMDM2, siControl or CMV-MDM2 + His-Ub were treated with MG132 for 8 h. Total cell lysates were subjected to Flag-immunoprecipitation followed by western blotting with anti-Ubiquitin antibody to detect ubiquitinated Flag-TRIM24. The blot was reprobed with Flag antibody to show equal Flag-TRIM24 pull down.

FIGURE S3

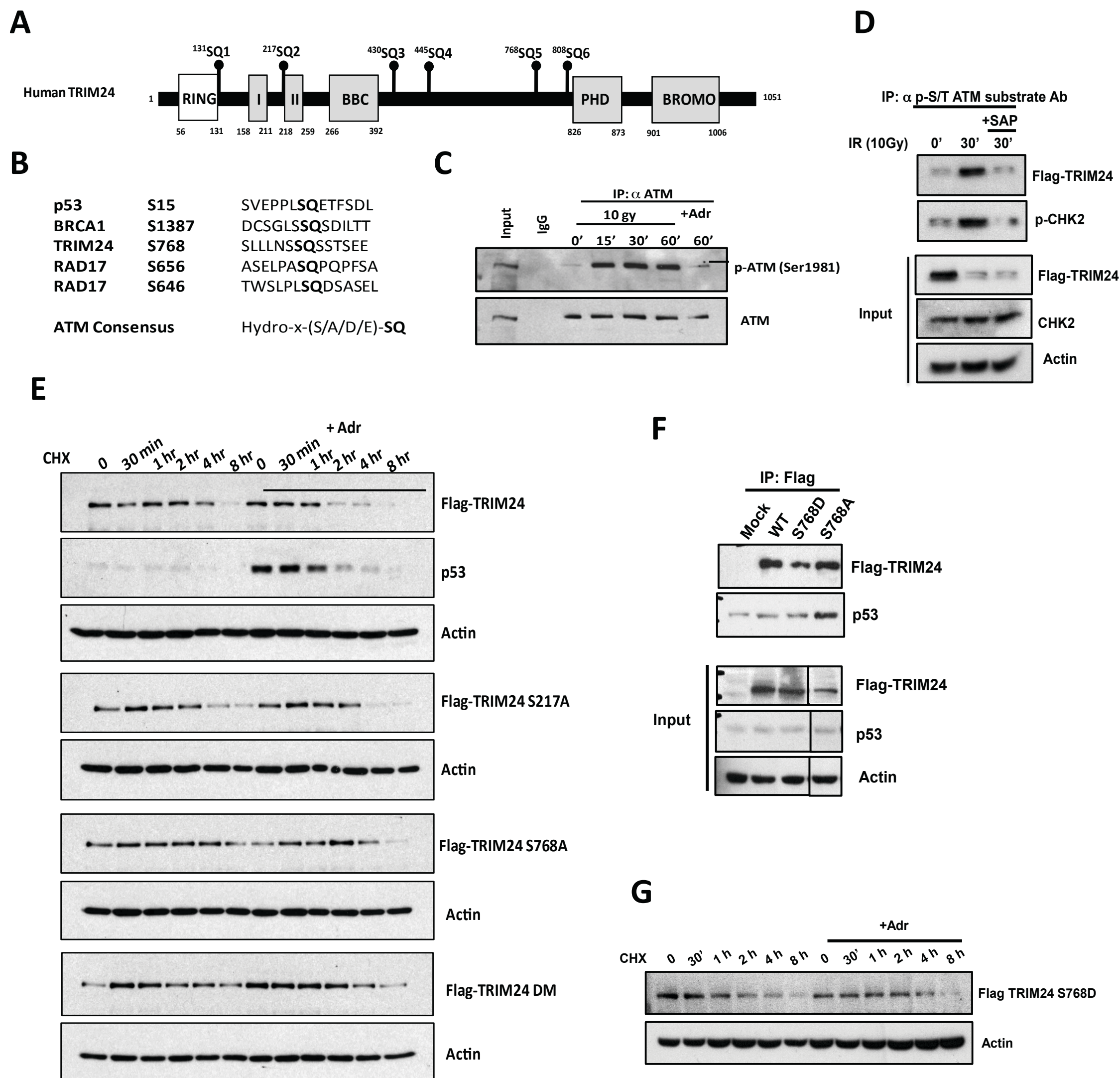


FIGURE S3. Phosphorylation at S786 by ATM-kinase induces TRIM24 degradation during DNA damage A) ATM phosphorylation sites on TRIM24. Scheme representing six ATM-signature SQ-motifs on human TRIM24 protein. The location of probable ATM target Serine residue on human TRIM24 is marked as superscript. B) Comparisons of Ser768 ATM phosphorylation site on TRIM24 with other consensus ATM-sites on substrate proteins. C) ATM autophosphorylation. MCF7 cells were exposed to IR and Adriamycin (Adr) for different time points. Total lysates were immunoprecipitated by anti-ATM antibody and probed with anti-pATM (Ser1981) antibody. D) TRIM24 phosphorylation. MCF7 cells transfected with Flag-TRIM24 were collected at 30 min post-IR. Total lysates were subjected to SAP treatment in some cases and immunoprecipitated with anti-pS/T ATM substrate antibody and blotted with Flag and pChk2. Inputs are shown in bottom panels. E) TRIM24 half-life. MCF7 cells transfected with wild type or mutants of Flag-TRIM24 were treated with CHX for 0, 0.5, 1, 2, 4, and 8 h without (–Adr) or with DNA damage (+Adr). TRIM24 protein levels were analyzed by immunoblotting with Flag antibody, to determine TRIM24-half-lives shown in Figure 4. F) TRIM24-p53 interaction. Lysates from MCF7 cells transfected with Flag-TRIM24 mutants were immunoprecipitated with Flag antibody and immunoblotted for Flag, and p53. G) TRIM24 S768D half-life determined as in D.

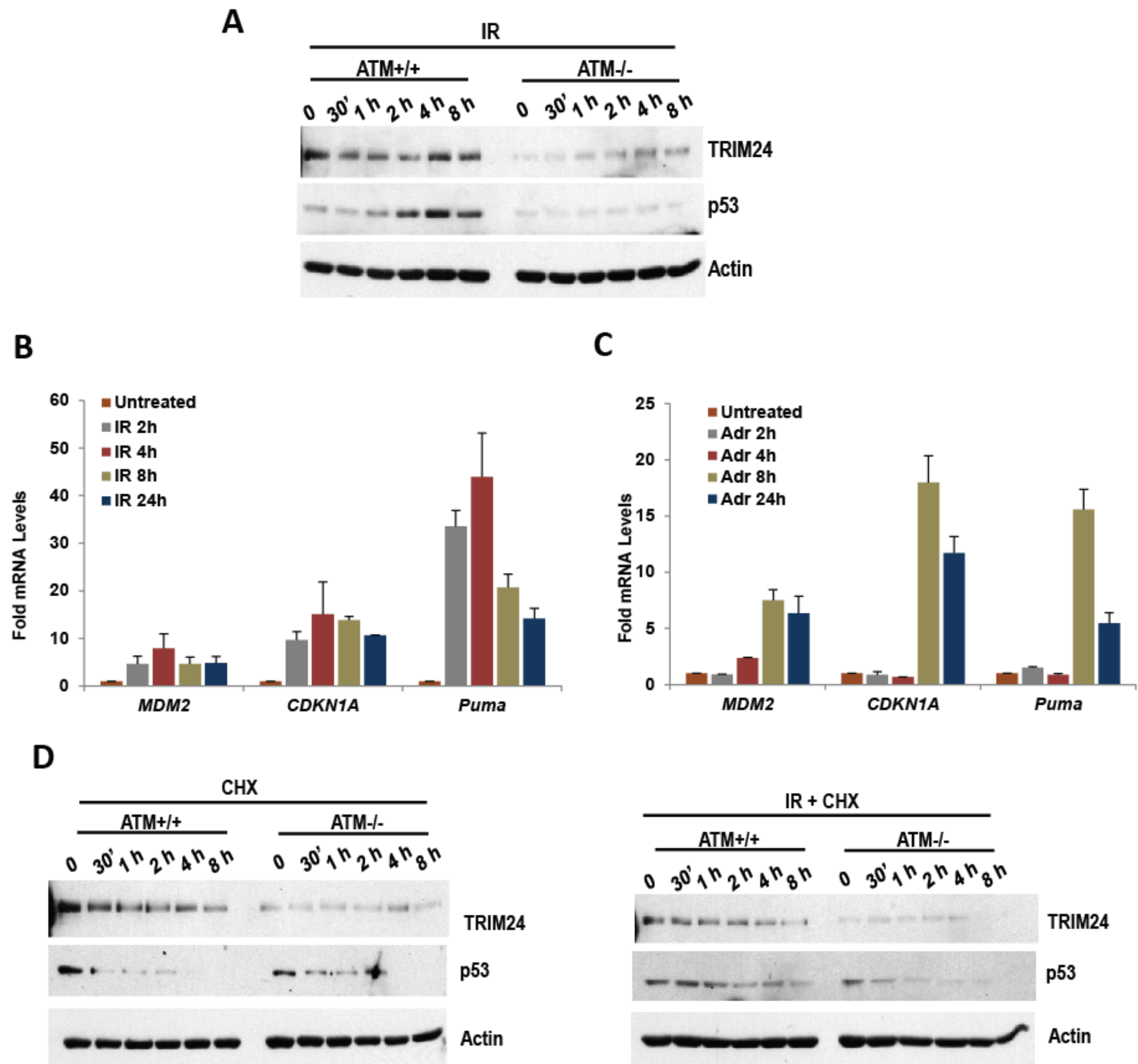


FIGURE S4. DNA damage induced degradation of TRIM24 is dependent on ATM. A) TRIM24 protein levels. Wild type (ATM+/+) and ATM-null (ATM-/-) fibroblasts were exposed to IR for different time points and TRIM24 and p53 protein were analyzed by western blotting. B-C) qRT-PCR assay. MCF7 cells were treated with IR (B) or Adr (C) and harvested at different time points. RNA prepared from these cells was subjected to qRT-PCR assay with primers specific for *MDM2, CDKN1A*, *Puma*, and *Actin*. Fold mRNA levels were calculated using actin expression as internal control. D) TRIM24 half-life. Wild type (ATM+/+) and ATM-null (ATM-/-) fibroblasts were exposed to CHX or IR followed by CHX (IR + CHX) for different time points and TRIM24 and p53 protein were analyzed by western blotting. Blots were quantitated and fold changes in nuclear protein levels are plotted in Figure 5.

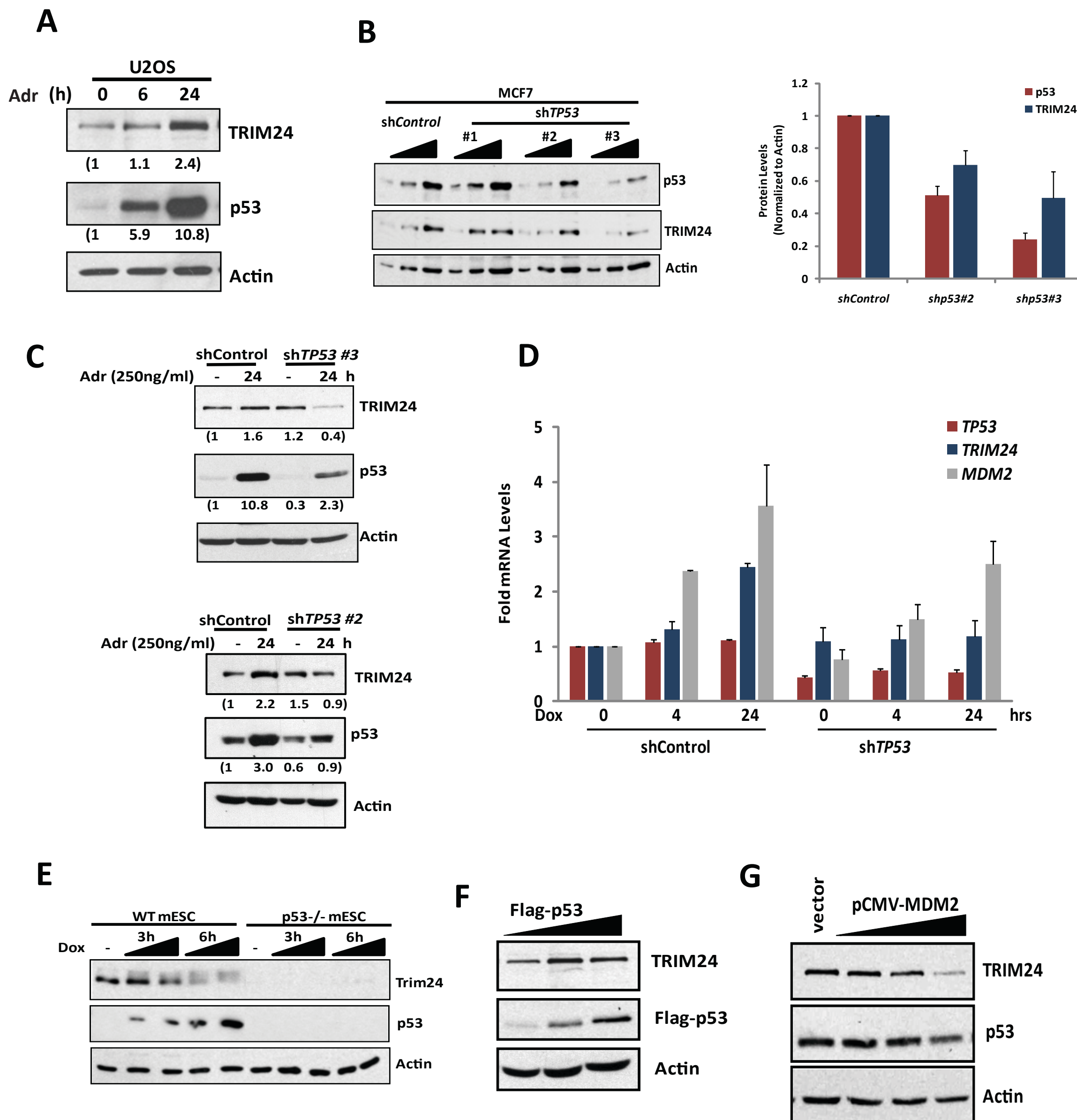


FIGURE S5. p53 activates *TRIM24* transcription after DNA damage A) Total cell lysates from U2OS cells treated with low dose Adr (250 ng/ml) for different time points were immunoblotted to detect TRIM24 and p53 protein levels. B) MCF7 cells stably expressing shRNA against non-target (shControl) or three different shRNAs specific to p53 (*shTP53*) were lysed and different amounts of lysates analyzed by immunoblotting to detect TRIM24 and p53. The blots were quantitated and fold changes in protein levels are plotted [right panel]. C-D) MCF7 cells stably expressing shRNA against non-target (shControl) or specific to p53 (*shTP53*) were treated 500ng/ml Adr and analyzed by western blotting (C) and RNA by qRT-PCR (D). The blots were quantitated and fold changes in protein levels is indicated below each blot. E) Different amount of total lysates from wild type (WT) or p53-null (p53-/-) mouse ES cells untreated or treated with Adr were analyzed by western blotting. F-G) MCF7 cells transiently transfected with increasing amounts of either Flag-p53 (F) or pCMV-MDM2 (G) plasmids were analyzed by western blotting.

FIGURE S6

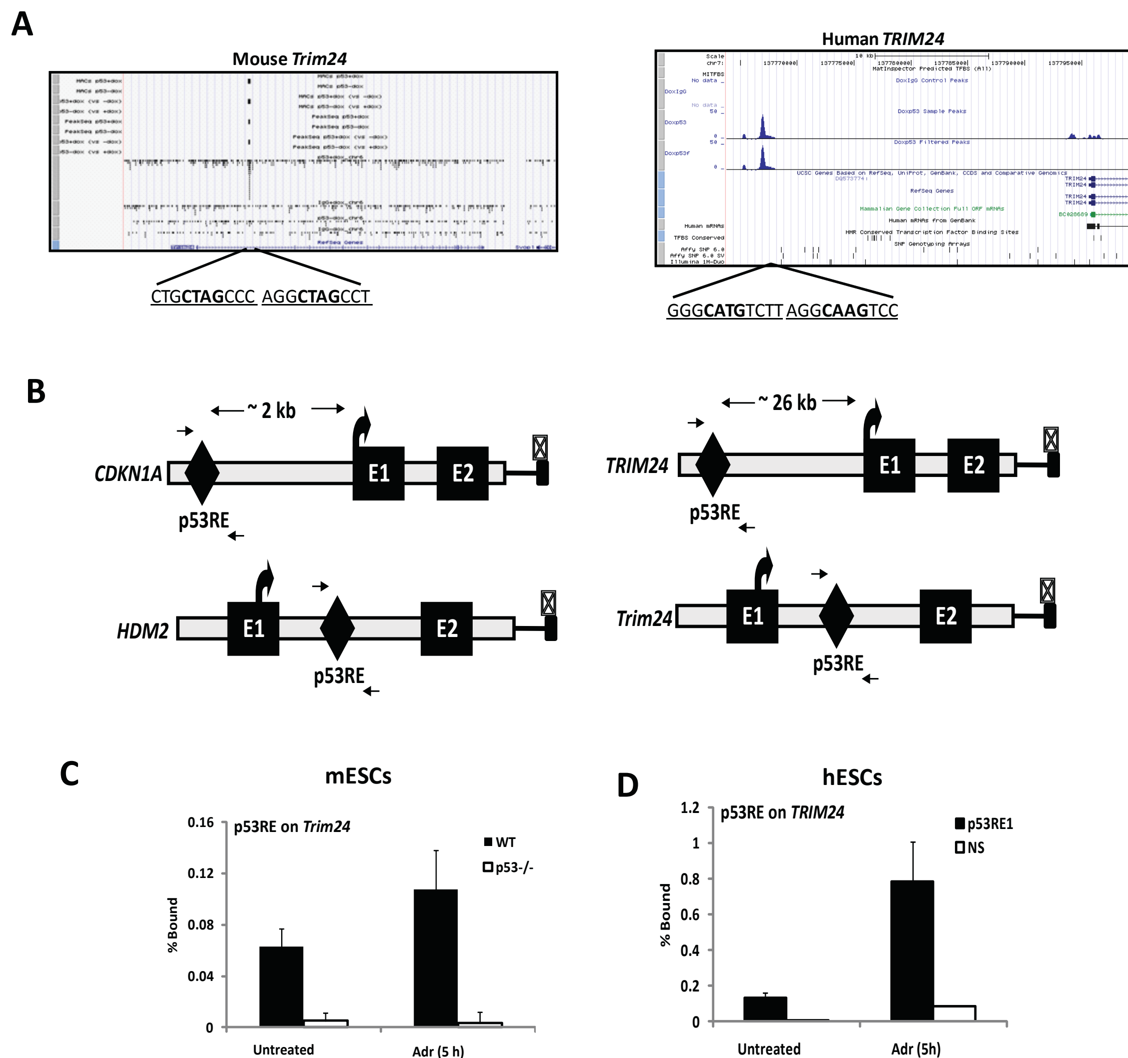
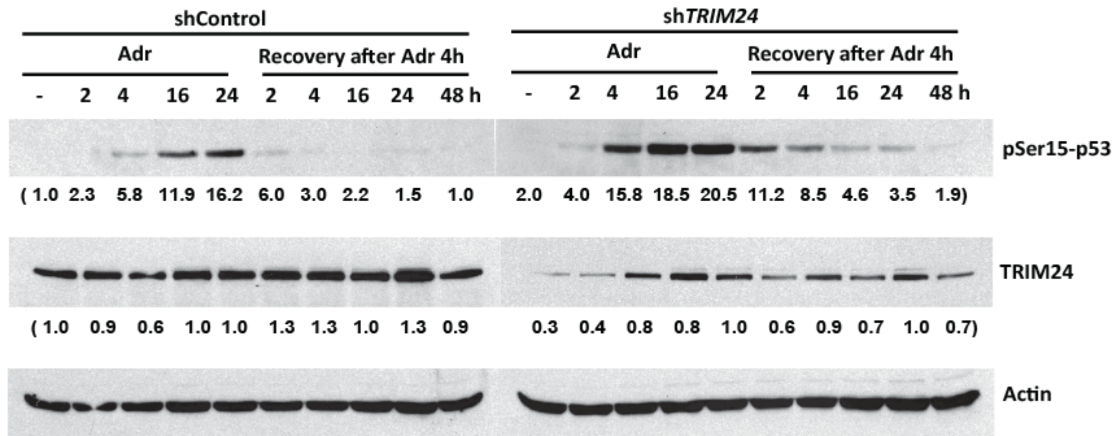


FIGURE S6. TRIM24 is a direct gene-target of p53. A) Mouse and human ES cells treated with Adr for 6 h were subjected to Chromatin-immunoprecipitation (ChIP) either with IgG (control) or p53 antibody. Data obtained after sequencing the chromatin fragments (ChIP-Seq) enriched for p53 were uploaded on UCSC genome browser and chromosomal location of p53 binding on mouse *Trim24* and human *TRIM24* were identified. The screenshot represents the peaks where p53-pulled down tags were enriched. Peak height indicates the intensity of p53-enrichment on these locations. Resulting p53REs identified are in promoter region of *TRIM24* and intron-1 of *Trim24*. B) Schematic representation of p53REs on *MDM2*, *CDKN1A*, *TRIM24* and *Trim24* with the location of primers used for ChIP-qPCR. *Represent the end of the gene. C-D) p53-ChIP. p53-bound chromatin was immunoprecipitated from wild type (WT) and p53-null (p53-/-) mES cells treated with Adr and p53 enrichment on *Trim24* was analyzed by q-PCR using primers encompassing p53REs and plotted as fold p53-enrichment compared to input. In a similar experiment (D), p53-bound chromatin was immunoprecipitated from hES cells treated with Adr and p53 enrichment on *TRIM24* analyzed by q-PCR using primers encompassing p53REs and plotted as fold p53-enrichment compared to input. A primer amplifying a region in human genome that does not contain p53RE was used as a negative control (NS).

FIGURE S7



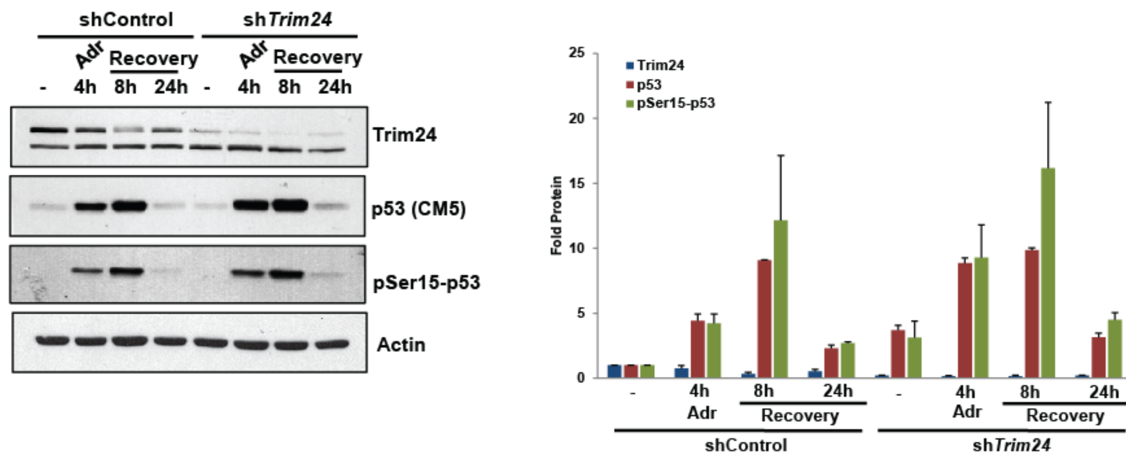


FIGURE S7. TRIM24 preferentially targets phosphorylated p53. A) Stability of pSer15-p53 after DNA damage. MCF7 cells stably expressing sh*Control* or sh*TRIM24* shRNAs were exposed to Adr for different time points upto 24 h. In a parallel set, cells were treated with Adr for 3 h followed by Adr removal and subsequent culture in regular medium for indicated time points. Total cell lysate were immunoblotted to detect pSer15-p53 and TRIM24 levels during the course of Adr treatment or after removal of Adr. B) Mouse ES cells stably expressing sh*Control* or sh*TRIM24* shRNAs were exposed to Adr for 4 h followed by Adr removal and subsequent culture in regular medium for indicated time points. Total cell lysate were immunoblotted to detect pSer15-p53, p53 and Trim24 levels and normalized to actin.

Supplementary Table SI. Oligonucleotide sequences for gene expression analysis by Real Time qRT-PCR

Gene	Primers
hTP53-5’	GCGAGCACTGCCCAACAACA
hTP53-3’	GGATCTGAAGGGTGAAATATTCT
hCDKN1A-5'	TACCCTTGTGCCTCGCTCAG
hCDKN1A-3'	CGGCGTTTGGAGTGGTAGA
hMDM2-5'	CTGGCTCTGTGTGTAATAAGGGAG
hMDM2-3'	CCTGATCCAACCAATCACCTG
hTRIM24-5'	GCGCCTACTTTTATTTCTTTACTG
hTRIM24-3'	AATGCTTTTGAGGCGTTTCTT
hPuma-5’	GTCCTGTACAATCTCATCA
hPuma-3’	CTAATTGGGCTCCATCTC
hActin-5'	GTGGATCAGCAAGCAGGAG
hActin-3'	TTTGTCAAGAAAGGGTGTAACG

Supplementary Table SII. Oligonucleotide sequences for Real Time qPCR analysis after p53-ChIP

p53RE on Gene	Primers
mTrim24 p53RE 5'	AGACTAGCGCTTACTCCCCC
mTrim24 p53RE 3'	TGTAATACTGGTGTCATCCCG
mTrim24-NS 5’	GTGCGTTCCGGGCTTTTTA
mTrim24-NS 3’	TCCGAAAGCAGCAGACAACA
mCdkn1a p53RE 5'	CCTTTCTATCAGCCCCAGAGGATACC
mCdkn1ap53RE 3'	GGGACGTCCTTAATTATCTGGGGTC
mMdm2 p53RE 5'	GGTCAAGTTGGGACACGTCC
mMdm2 p53RE 3'	AGCGTTTAAATAACCCCAGCTG
hTRIM24 p53RE 5’	AGACAGAATTTTCCATTGCAG
hTRIM24 p53RE 3’	TCAAGCAGCAGGCACAGAT
hTRIM24 NS 5’	CCGCCTCACCTACAACAATT
hTRIM24 NS 3’	CAGCGTGATCTTCCAAGTCA
hCDKN1A p53RE 5'	AGCAGGCTGTGGCTCTGATT
hCDKN1A p53RE 5'	CAAAATAGCCACCAGCCTCTTCT
hMDM2 p53RE 5’	GTGGGCAGGTTGACTCAGCTT
hMDM2 p53RE 3’	CCAATCGCCACTGAACACAGC