SUPPLEMENTAL DATA

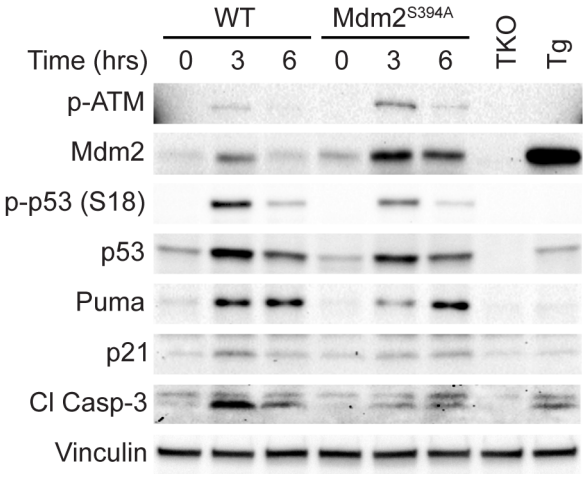

Figure S1. ATM phosphorylation of Mdm2-S394 regulates Mdm2 levels and p53 activity in spleen, Related to Figure 1
WT and *Mdm2*^{S394A} mice were left untreated or exposed to 1.75 Gy ionizing radiation (IR) and whole spleens were harvested at 3 and 6 hours. Protein levels were analyzed by western blotting. TKO indicates *Mdm2*$^{-/-}$, *MdmX*$^{-/-}$, *p53*$^{-/-}$ control; Tg indicates *Mdm2*$^{Tg/+}$ Mdm2 overexpressing control.

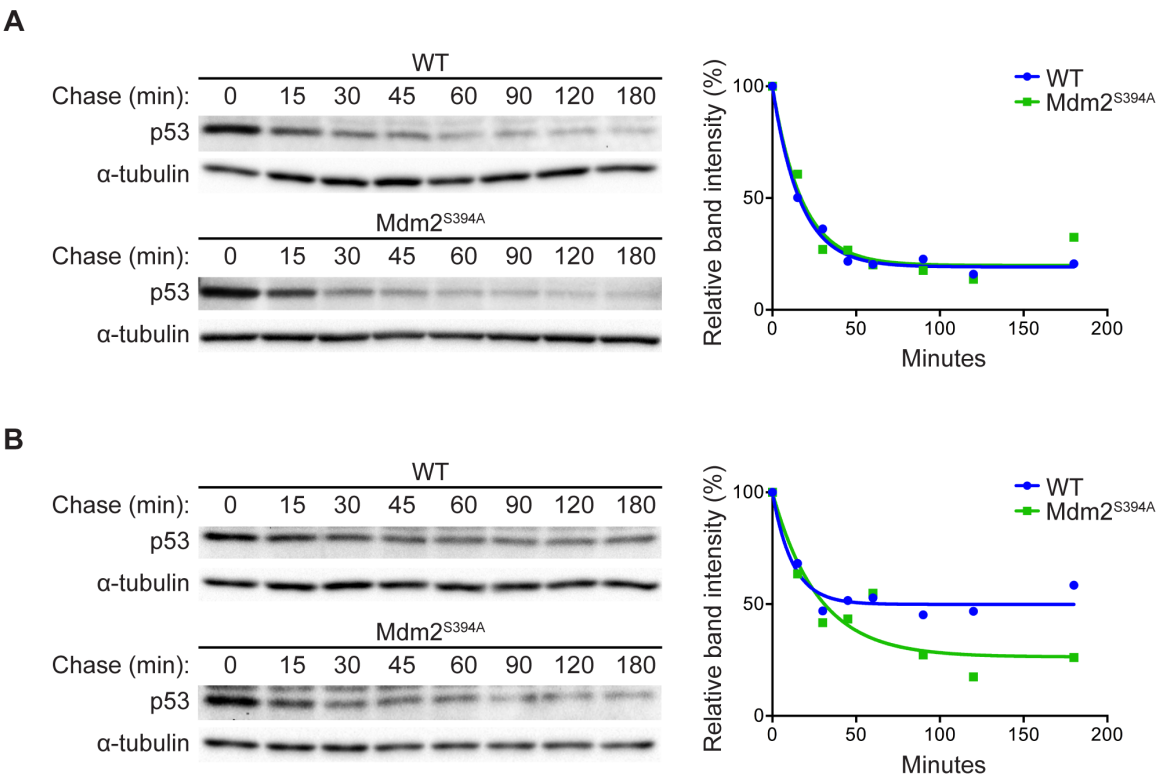

Figure S2. ATM phosphorylation of Mdm2-S394 impacts p53 destabilization after DNA damage, Related to Figure 2

(A) Thymocytes harvested from WT and *Mdm2*^{S394A} mice (*n* = 6) were treated with 100 μg/ml cycloheximide and harvested at the indicated time points. The levels of p53 and α-tubulin were analyzed by western blotting. Band intensities were determined by densitometry and p53 levels normalized to α-tubulin were plotted. One-phase decay curves were fitted using GraphPad Prism software.

(B) Thymocytes harvested from WT and *Mdm2*^{S394A} mice (*n* = 6-8) were exposed to 2.5 Gy IR before being treated with 100 μg/ml cycloheximide and harvested at the indicated time points. The levels of p53 and α-tubulin were analyzed by western blotting. Band intensities were determined by densitometry and p53 levels normalized to α-tubulin were plotted. One-phase decay curves were fitted using GraphPad Prism software.

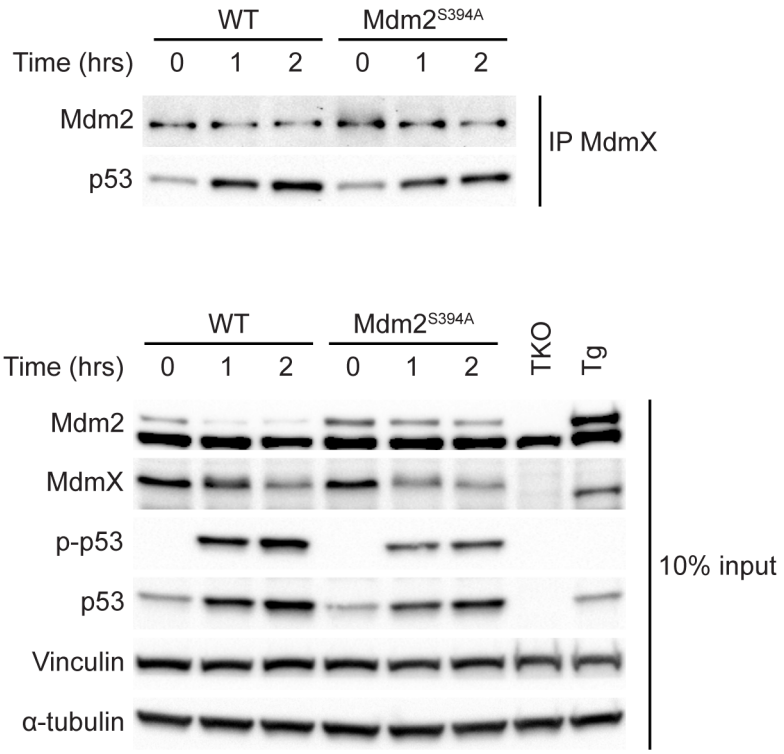

Figure S3. Relative amounts of Mdm2-bound MdmX or p53-bound MdmX before or after DNA damage are not affected in *Mdm2*^{S394A} mice, Related to Figure 2

Whole thymus protein extracts were generated from mice that were untreated and 1 and 2 hours after exposure to 5 Gy IR. Protein extracts (500 µg) were immunoprecipitated with an antibody for MdmX (MDMX-82; Sigma). Immunoprecipitates were analyzed by western blotting for Mdm2 (NBP1-02158; Novus) and p53 (CM5; Novocastra). Total lysate (10% input) was analyzed by western blotting with antibodies for Mdm2, MdmX, p53, phospho-p53(S18), vinculin and α-tubulin.

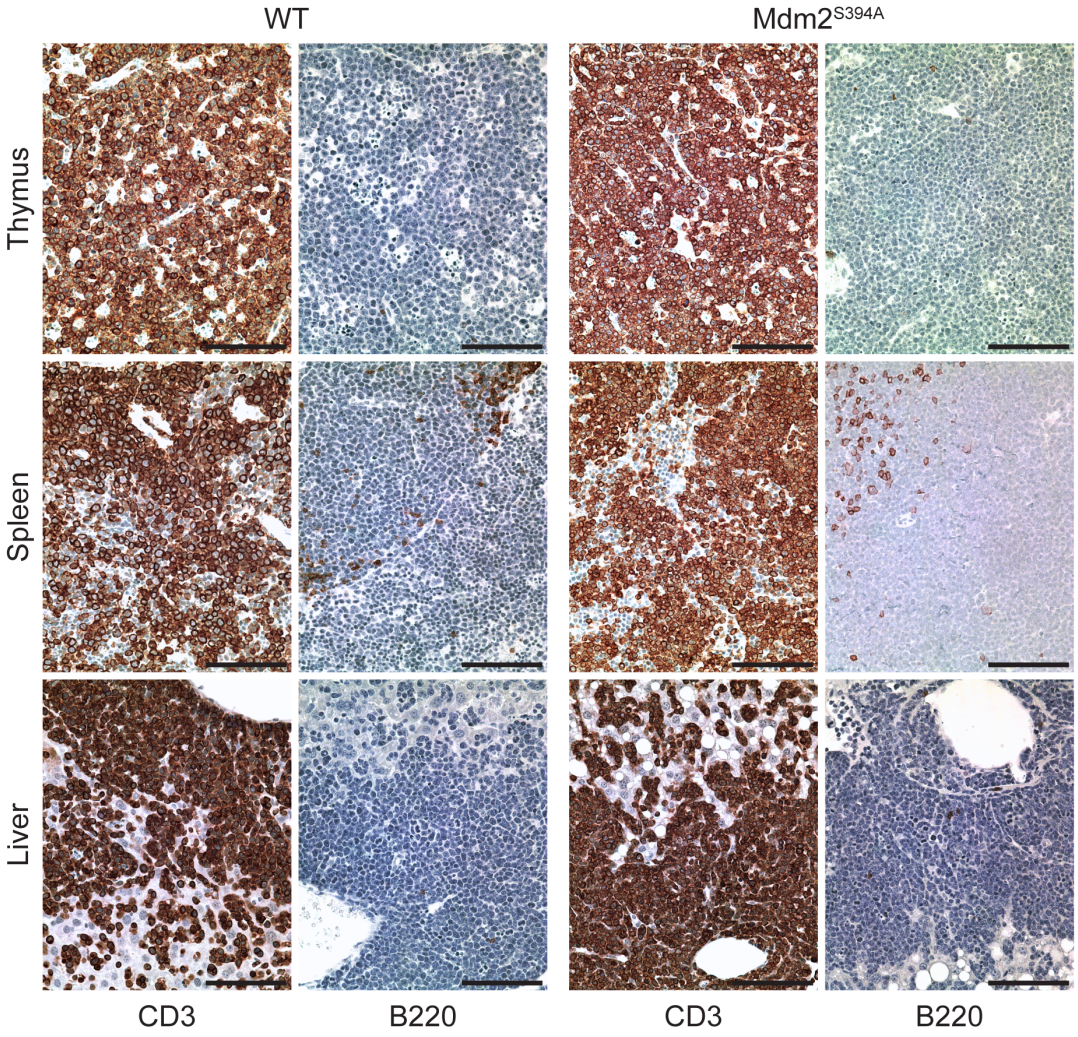

Figure S4. Immunohistochemical staining of representative tissue sections of IR-induced lymphomas, Related to Figure 4

Higher magnification images of representative tissue sections of IR-induced lymphomas that developed in the thymus, spleen and liver of WT and *Mdm2*^{S394A} mice stained with antibodies specific for CD3 (left) and B220 (right) as in Figure 4C. Scale bars represent 100 μm.

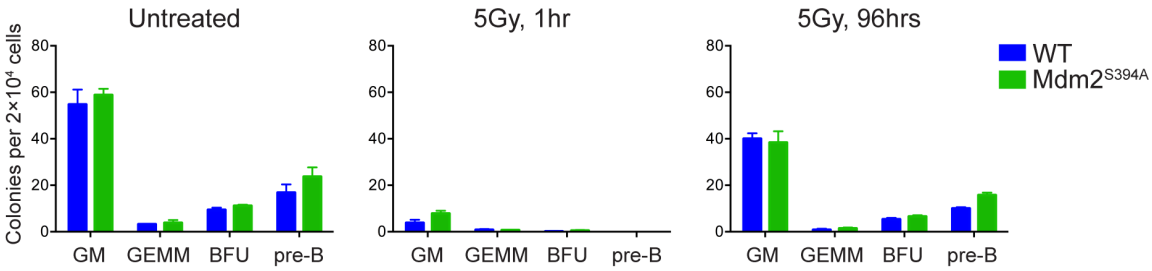

Figure S5. Colony-forming cell numbers in bone marrow before or after IR are not affected in *Mdm2*^{S394A} mice, Related to Figure 6

Total bone marrow was harvested from WT and *Mdm2*^{S394A} mice that were left untreated, and 1 and 96 hours after exposure to 5 Gy IR. Colony-forming cell (CFC) assays (MethoCult 3434 and MethoCult 3630; StemCell Technologies) were carried out per manufacturer's instructions (n = 3-6, ±SEM).

Table S1. Antibodies Used for Flow Cytometry, Related to Figure 6

Antibody	Clone	Fluorophore	Source
CD3	145-2C11	Biotin, APC	BD Bioscience
B220	RA3-6B2	Biotin, PE	BD Bioscience
Ter119	TER-119	Biotin	Biolegend
Gr-1	RB6-8C5	Biotin, APC, PE	Biolegend
Mac-1	M1/70	Biotin, FITC	Biolegend
Sca-1	D7	APC/Cy7	Biolegend
CD117	2B8	APC	BD Bioscience
CD34	RAM34	FITC	BD Bioscience
Flk2	A2F10	PE	Biolegend
CD45.2	104	FITC, PE/Cy7	Biolegend
CD45.1	A20	PercP/Cy5.5	Biolegend

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Protein Analysis

Tissues and cells were lysed in NP-40 lysis buffer (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 0.5% NP-40) or in CelLytic MT Cell Lysis Reagent (Sigma), supplemented in both cases with protease and phosphatase inhibitor cocktail tablets (Roche). Protein extracts were analyzed by direct western blotting or immunoprecipitation/western blotting. Immunoprecipitations were performed on 500 µg whole thymus lysate with antibodies specific for Mdm2 (NBP1-02158; Novus (Ab-I) and Ab-5; Calbiochem (Ab-II)), p53 (CM5; Novocastra) and MdmX (MDMX-82; Sigma), using PureProteome Protein A/G Mix Magnetic Beads (Millipore). Mdm2 Ab-I immunoprecipitates were counterblotted with Mdm2 (Ab-5; Calbiochem (Ab-II)) and p53 (IMX25; Novocastra) antibodies. Mdm2 Ab-II immunoprecipitates were counterblotted with Mdm2 (NBP1-02158; Novus (Ab-I)) and p53 (CM5; Novocastra) antibodies. Western blotting was performed with antibodies specific for Mdm2 (NBP1-02158; Novus and Ab-5; Calbiochem), p53 (CM5; Novocastra), MdmX (MDMX-82; Sigma), p-ATM(S1981) (Cell Signaling), p-p53(S15) (#9284 or #12571; Cell Signaling), α-tubulin (B-5-1-2; Sigma), p21 (SXM30; BD Pharmingen), Puma (#7467; Cell Signaling), Arf (5-C3-1; Santa Cruz) and Cleaved Caspase-3(Asp175) (#9661; Cell Signaling). For cycloheximide experiments, single cell suspensions of thymocytes from 6-8 thymi were generated maintained in DMEM supplemented with 10% FBS. Thymocyte pools were untreated or irradiated with 2.5 Gy IR and immediately treated with cycloheximide (100 µg/ml; Sigma). Pools were aliquoted and maintained in a 37°C incubator with 5% CO_2 and collected for protein analysis at the indicated time points. Blots were imaged on a Chemidoc MP (Bio-Rad) and relative band intensities determined by densitometry using Image Lab software (v4.1, Bio-Rad).

Gene Expression Analysis and Sequencing

Total RNA was isolated from tissues by RNeasy mini kit (QIAGEN) and cDNA synthesized by Superscript III First Strand Synthesis System (oligo-dT priming) (Invitrogen). Quantitative PCR was performed using SYBR Select Master Mix (Applied Biosystems) in conjunction with a 7300 Real-Time PCR System (Applied Biosystems). Thymus cDNA input was 10 ng and bone marrow cDNA input was 100 ng. Fold expression was calculated using the ΔΔCt method relative to untreated WT samples using *Rplp0* as internal reference. Primers used were as follows: *Puma*, 5'ACGACCTCAACGCGCAGTACG3' and 5'GAGGAGTCCCATGAAGAGATTG3'; *Noxa*, 5'CTCAGGAAGATCGGAGACAAAG3' and 5'GCACACTCGTCCTTCAAGT3'; *Bax*, 5'GTGGTTGCCCTCTTCTACTTT3' and 5'CAGCCCATGATGGTTCTGAT3'; *p21*, 5'CTGAGCGGCCTGAAGATT3' and 5'ATCTGCGCTTGGAGTGATAG3'; *Mdm2*, 5'AGTCTCTGGACTCGGAAGATTA3' and 5'CTGTATCGCTTTCTCCTGTCTG3'; *Rplp0*, 5'CTGAGTACACCTTCCCACTTAC3' and 5'CTCTTCCTTTGCTTCAGCTTTG3'. Arf message was amplified from tumor sample cDNA using the following primer pair: 5'TCGCTTGTCACAGTGAGG3' and 5'CCGGATTTAGCTCTGCTCTT3'. p53 message was amplified from tumor sample cDNA in overlapping fragments using three separate primer pairs: p53seq1, 5'TTCATTGGGACCATCCTGGCTGTA3' and 5'AGGCACAAACACGAACCTCAAAGC3'; p53seq2, 5'CTTATCCGGGTGGAAGGAAAT3' and 5'GAAGTAGACTGGCCCTTCTTG3'; p53seq3, 5'AGCTTTGAGGTTCGTGTTTGTGCC3' and 5'ATGCAGACAGGCTTTGCAGAATGG3'. Amplified fragments were gel extracted (QIAGEN) and sequenced by Sequegen, Inc (Worcester, MA).