Cell Stem Cell, Volume *6*

Supplemental Information

p53-Mediated Hematopoietic Stem and Progenitor Cell Competition

Tanya Bondar and Ruslan Medzhitov

INVENTORY OF SUPPLEMENTARY INFORMATION

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Generation of R26-mp53 mice. Mouse R172H p53 cDNA was cloned into Rosa26 CAGGs vector. Linearized construct was electroporated into C57BL/6-derived Bruce4 ES cells, and knock-in mice were generated by Yale Animal Genomics Services using standard protocols. Homologous integration was confirmed by Southern blotting of the genomic DNA digested with XbaI using a probe mapping to the EcoRV-PacI fragment at the 5' end.

Western blotting. Splenocytes were lysed in TNG buffer [50 mM Tris·HCl (pH 7.5), 200 mM NaCl, 50 mM β-glycerol phosphate, 1% Tween-20, and 0.2% Nonidet P-40] supplemented with protease and phosphatase inhibitors. Lysates were cleared by centrifugation at 13,000 × *g* for 10 min at 4°C, and protein concentration was assayed in the supernatants. Samples were normalized by the protein concentration and resolved on a 4–12% gradient Tris-Glycine NuPAGE gel (Invitrogen). After standard transfer, Western blotting was performed with mouse p53 antibody (Cell Signaling, clone 1C12, catalog #2524).

Microarray analysis. RNA was isolated from cells using Trizol (Invitrogen) from 500 sorted HSPCs (combined from at least 3 animals in each of the two experiments), amplified with RiboAmp^{HS} kit from Arcturus and labeled using 11-UTP-biotin during the second round of amplification. Hybridization to Mouse Illumina Expression Chip V6.1.1. was performed at the Yale W.M. Keck facility. Results were analyzed using GeneSpring software.

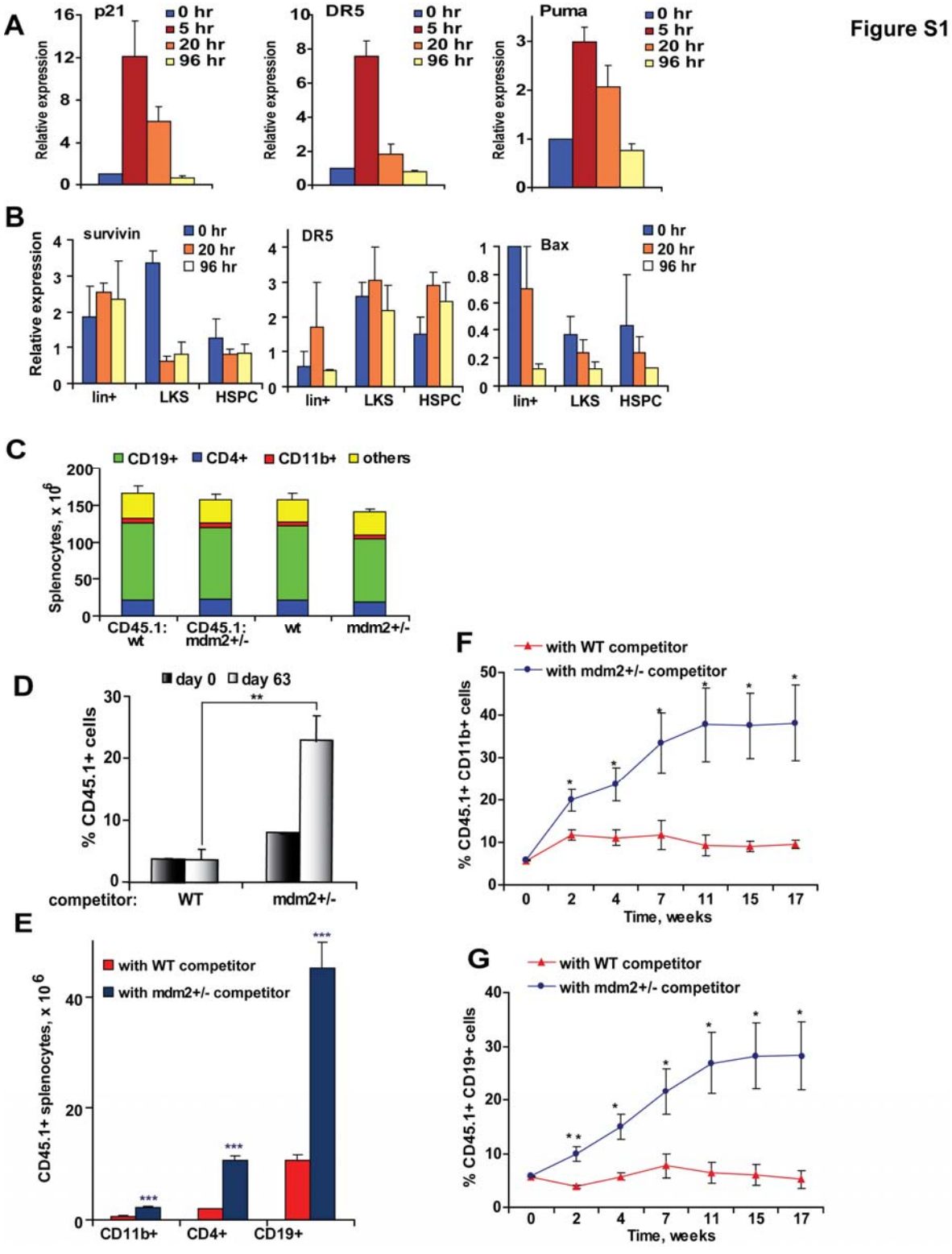
A
p21
DR5
Puma
0 hr
5 hr
20 hr
96 hr
Relative expression
B
survivin
DR5
Bax
0 hr
20 hr
96 hr
Relative expression
lin+
LKS
HSPC
C
CD19+
CD4+
CD11b+
others
Splenocytes, x 10^6
CD45.1: wt
CD45.1: mdm2+/-
wt
mdm2+/-
D
day 0
day 63
% CD45.1+ cells
competitor:
WT
mdm2+/-
E
with WT competitor
with mdm2+/- competitor
CD45.1+ splenocytes, x 10^6
CD11b+
CD4+
CD19+
F
with WT competitor
with mdm2+/- competitor
% CD45.1+ CD11b+ cells
Time, weeks
G
with WT competitor
with mdm2+/- competitor
% CD45.1+ CD19+ cells
Time, weeks

Figure S1

Figure S1, related to Figure 1. A, B, kinetics of DDR in the BM. Gene expression measured by qPCR in total BM (A) or sorted lineage+ (lin+), lin- c-kit+ Sca1+ (LKS), and lin- c-kit+ Sca-1+ CD48- CD150+ (HSPC) cells (B), isolated from wild type mice at indicated time points (hours) after 2.5 Gy. n = 4. (**C-G**), data supporting WT:mdm2 +/- HSPC competition experiment shown on Figure 1F. **C,** total number and splenic subset composition of the BM chimeras 8 weeks after reconstitution with WT and/or *mdm2*+/- BM. **D**, percent chimerism was determined in the BM of the recipient mice 8 weeks after BM transfer by staining for CD45.1 and HSPC markers. **E**, relative contribution of CD45.1+ wild type competitor cells to splenic subsets in mixed *WT:WT* or *WT:mdm2*+/- chimeras 8 weeks after BM transfer. **F**, **G,** percent chimerism was determined in the CD11b+ (F) and CD19+ (G) cells in the PB at the indicated time points after BM transfer. Error bars represent s.e.m. n =6 for wt:mdm2+/-; n=3 for wt:wt chimeras. * $p \leq 0.05$; ** $p \leq 0.01$.

Figure S2

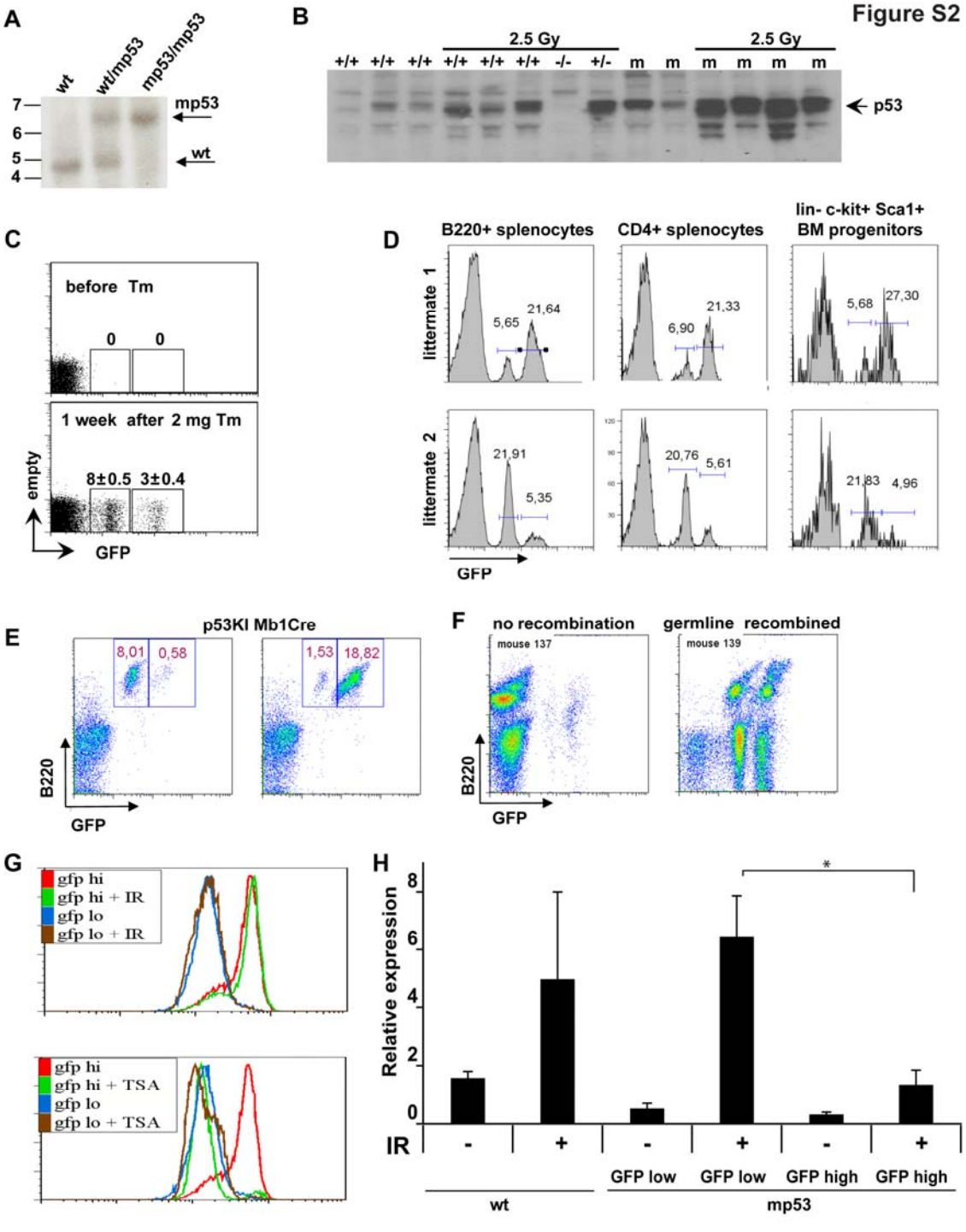
A
wt
wt/mp53
mp53/mp53
7
6
5
4
mp53
wt
B
2.5 Gy
2.5 Gy
+/+ +/+ +/+ +/+ +/+ +/+ -/- +/- m m m m m m
p53
C
before Tm
0 0
1 week after 2 mg Tm
8±0.5 3±0.4
empty
GFP
D
B220+ splenocytes
CD4+ splenocytes
lin- c-kit+ Sca1+ BM progenitors
littermate 1
littermate 2
5,65 21,64
6,90 21,33
5,68 27,30
21,91 5,35
20,76 5,61
21,83 4,96
GFP
E
p53KI Mb1Cre
8,01 0,58
1,53 18,82
B220
GFP
F
no recombination
germline recombined
mouse 137
mouse 139
B220
GFP
G
gfp hi
gfp hi + IR
gfp lo
gfp lo + IR
gfp hi
gfp hi + TSA
gfp lo
gfp lo + TSA
H
Relative expression
8
6
4
2
0
*
IR
- + - + - +
GFP low GFP low GFP high GFP high
wt
mp53

Figure S2, related to Figure 2. A, Southern blot analysis of XbaI-digested spenocyte genomic DNA of *R26-mp53* or wild type mice. The probe location is shown in Figure 2A. **B**, Protein levels of mutant p53. Total p53 levels were assayed 6 hours after irradiation in splenocytes of germline-recombined *R26-mp53Δ* mice ("m"), along with p53 +/+, +/- and -/- controls. Spleen extracts from individual mice are loaded on each lane. The data represent two independent experiments. **C**, induction of recombination results in mosaic expression of the knock-in allele, evidenced by GFP-positive PB lymphocytes (PBL). Numbers represent an average (n=55) ± s.e.m. **D**, All hematopoietic cell of *R26-mp53* mosaic mice display two levels of knock-in allele expression; the ratio of GFP^{high} to GFP^{low} cells is constant within one animal, regardless of age and cell type, but varies significantly among individual mice. Data from two *R26-mp53 Balancer* mosaic littermates are shown; the data is representative of at least 10 independent experiments with mp53 crossed to Balancer-Cre, Mx1-Cre, and CreER strains; total n ≥30. **E**, B cells from *R26-mp53 Mb1Cre* mosaic mice express the knock-in allele at two levels. Data from two *R26-mp53 Mb1Cre* littermates are shown. **F**, PBLs from Cre-negative, germline-recombined *R26-mp53Δ* mouse (right) also express GFP at two levels. A Cre-negative, unrecombined *R26-mp53* littermate control is shown on the left. **G**, GFP expression levels of the *R26-mp53* allele are not affected by differentiation or DNA damage. Whole BM cells from germline-recombined *R26-mp53Δ* mice were sorted by GFP levels, and macrophages were derived by culturing for 7 days in presence of MCSF. Cells were then either irradiated with 1 Gy, or incubated with trichostatin A, or left untreated. GFP levels were assayed by FACS 16 hours later. **G**, Effect of mp53 expression on p21 induction by DNA damage. Germline-recombined *R26-mp53Δ* mice and wild type littermate controls were exposed to 1 Gy. Thymocytes were isolated and sorted based on GFP levels, and RNA was prepared 8 hours after irradiation. p21 expression was measured by qPCR and normalized to HPRT levels. Error bars represent s.d.; n=3. *, $p \leq 0.05$.

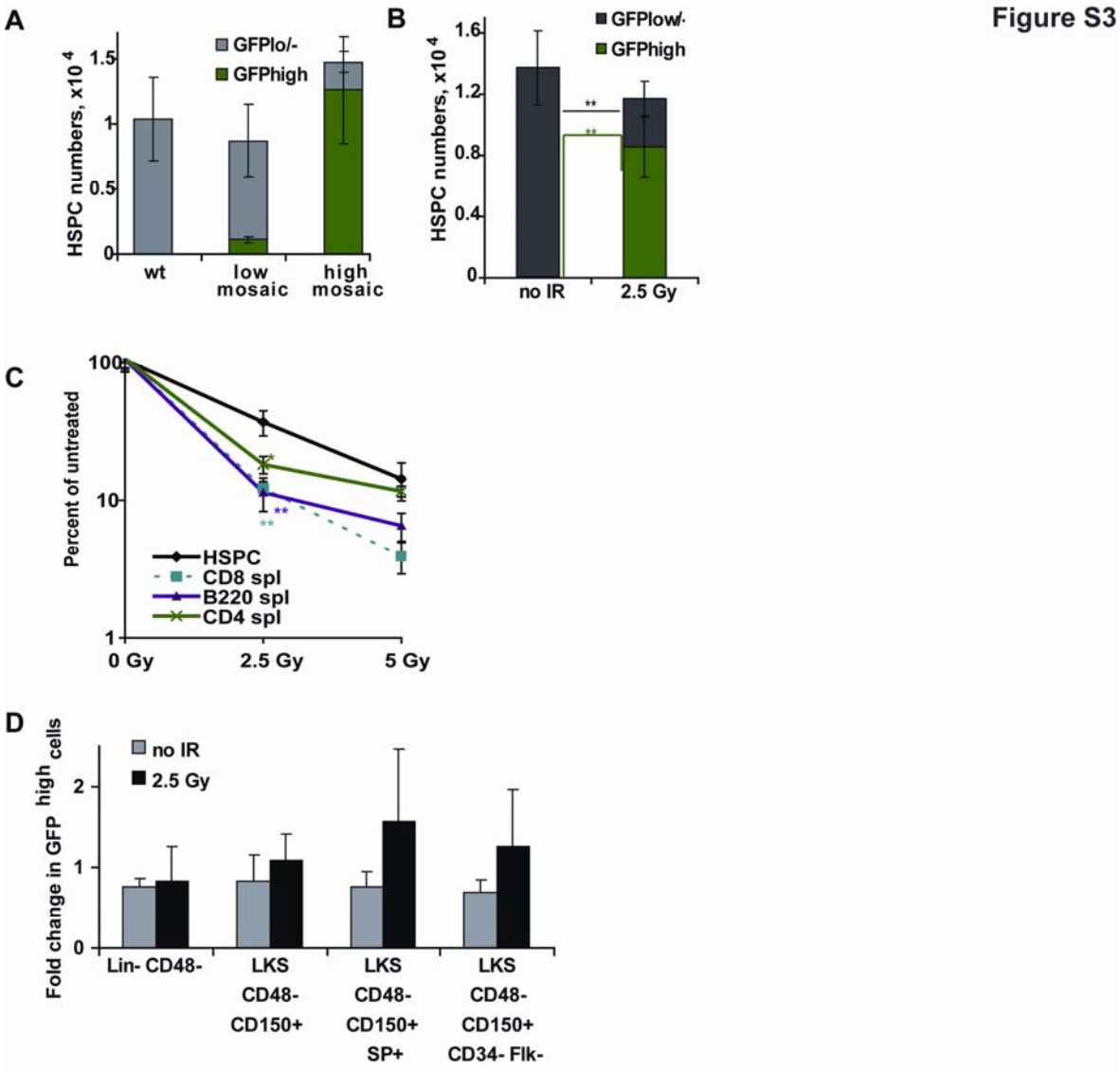

Figure S3, related to Figure 3. A, absolute numbers of HSPCs in *R26-mp53 CreER* mice with different mosaicism level 3 weeks after 2.5 Gy. **B**, absolute numbers of HSPCs in *R26-mp53 CreER* mice 200 days after one group has been treated with 2.5 Gy (same group as in Figure 3A). **C**, Relative radiosensitivity of hematopoietic cell types. Wild type mice were euthanized 20 hours after exposure to the indicated doses. Total BM and spleens cell numbers were counted, and percent HSPC in the BM and indicated cell types in the spleen were analyzed by FACS. Number of cells of each type per organ was determined by multiplying total number of cells per organ by the percent of each cell type in the organ. **D**, Changes in GFP^{high} cells (normalized to prebleed) within different stem/progenitor populations in the BM of *R26-mp53 CreER* mice 4 days after 2.5 Gy exposure. LKS stands for lineage- c-kit+ Sca-1+. Data of 2 independent experiments is combined, each with n≥3. Error bars represent s.e.m. *, p≤0.05; **, p≤0.01.

Figure S4

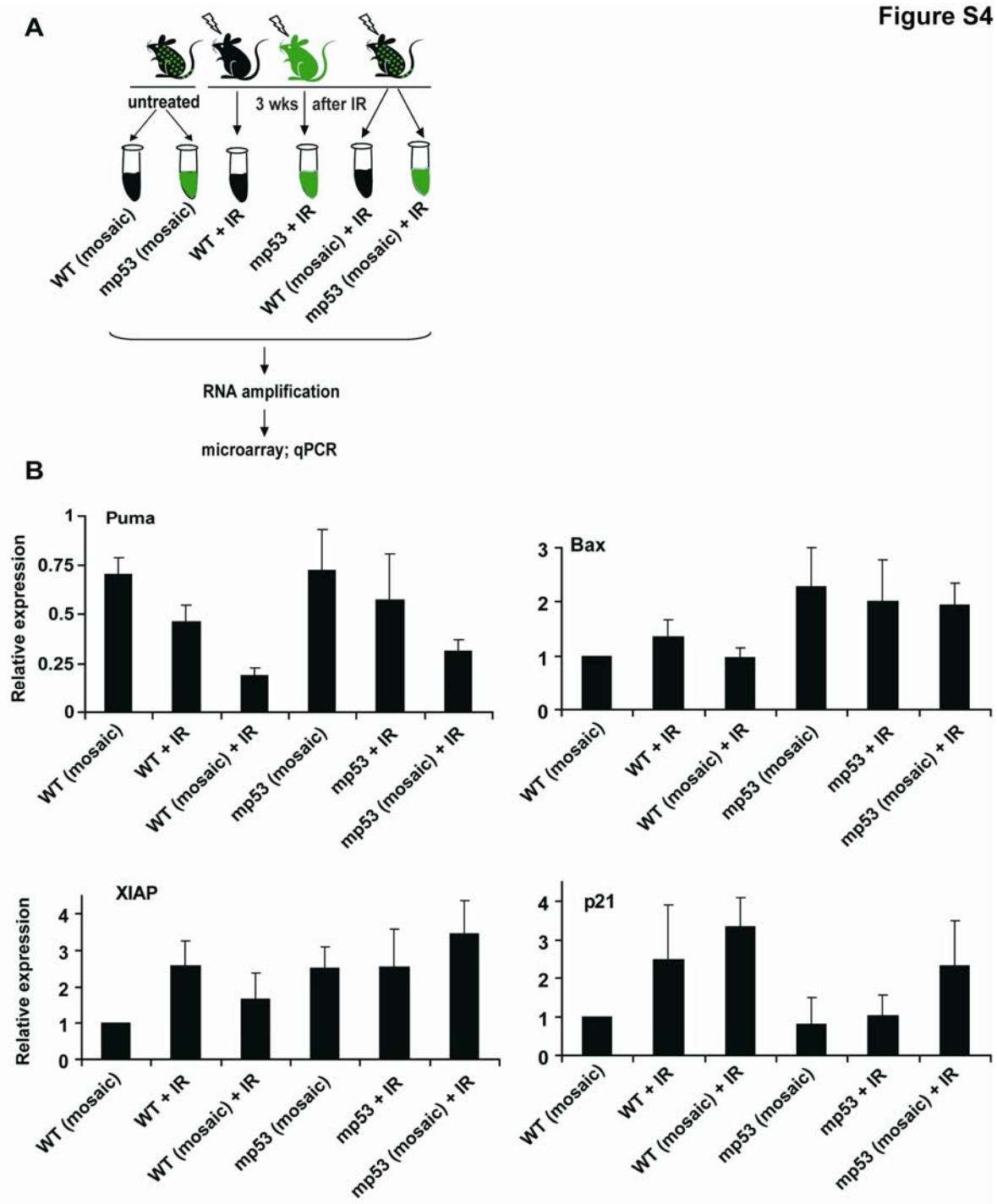

Figure S4, related to Figure 5. A, B, HSPCs were sorted from untreated *R26-mp53* mice, or from WT, *R26-mp53Δ* or *R26-mp53* mosaic mice 3 weeks after 2.5 Gy exposure. mRNA was amplified and analysed by microarray (not shown) and qPCR (B). Data is representative of 2 independent experiments, each with n≥3. Error bars represent SEM.

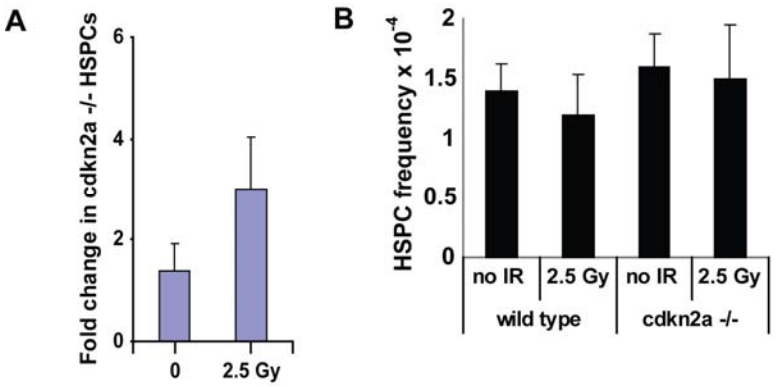

Figure S5

Figure S5, related to Figure 6. **A**, proportion of *cdkn2a -/-* HSPCs in mixed *WT:cdkn2a -/-* BM chimeras of indicated genotypes, with or without 2.5 Gy treatment on week 4, was assayed 16 weeks after BM transfer and normalized to prebleed. **B**, HSPC frequency of the experimental mice shown in Figure 6E. Error bars represent SEM.

Figure S6

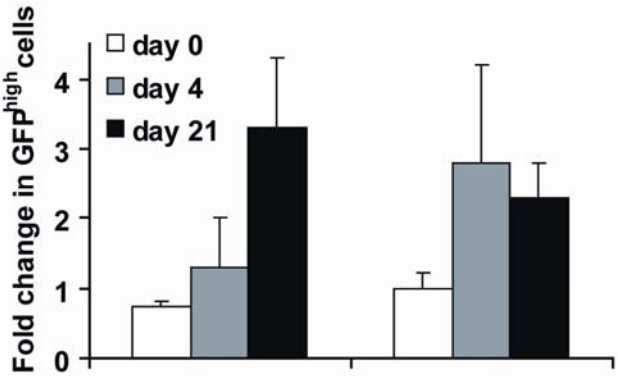

Figure S6, related to Figure 7. Percent of GFP^{high} HSPCs in the BM and spleen of *R26-mp53; CreER* mosaic mice was determined at the indicated times after 2.5 Gy exposure, and normalized to prebleed. n≥3. Error bars represent SEM.