Genotoxic stresses promote clonal expansion of hematopoietic stem cells expressing mutant p53

Sisi Chen[1], Rui Gao[2], Chonghua Yao[2,3], Michihiro Kobayashi[2], Stephen Z. Liu[2], Mervin C. Yoder[2], Hal Broxmeyer[4], Reuben Kapur[2], H. Scott Boswell[5], Lindsey D. Mayo[1,2], Yan Liu[1,2]

[1]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN 46202; [2]Department of Pediatrics, Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN 46202; [3]Department of Rheumatism, Shanghai Municipal Hospital of Traditional Chinese Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, China; [4]Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN 46202; [5]Department of Medicine, Indiana University School of Medicine, Indianapolis, IN 46202.

Correspondence: Yan Liu, Ph.D., Department of Pediatrics, Herman B Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, IN 46202, USA. Email: liu219@iu.edu.

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Clonal hematopoiesis increases with age, where a single mutant hematopoietic stem or progenitor cell contributes to a significant, measurable clonal proportion of mature blood lineages.[1-5] Evolution of mutant clonal hematopoiesis with age predisposes the elderly to myelodysplastic syndromes (MDS), acute myeloid leukemia (AML), and other aging-associated diseases, suggesting that mutations identified in hematopoietic stem cells contribute to disease development.[1-5] However, the mechanisms by which age-induced stem cell mutations cause clonal expansion of hematopoietic stem cell are largely unknown.

Recently, acquired somatic mutations in *TP53* gene were identified in the blood of aged healthy individuals and the frequency of *TP53* mutations increases with age.[3-5] *TP53* mutations rank top five among mutations identified in aged healthy individuals. [3-5] The *TP53* gene, which encodes the tumor suppressor p53, is the most frequent target for mutation in human cancer, including hematological malignancies.[6] *TP53* mutations are present in 10% of MDS cases and 20 to 30% in secondary MDS arising after exposure to radiation or alkylating agents. [1, 7] The frequency of *TP53* mutations in AML is approximately 10%. However, in AML with complex karyotype, the frequency of p53 mutations and/or deletions is almost 70%. [8] Further, *TP53* mutations are associated with poor prognosis and decreased survival in MDS and AML. [7-9] However, the role of *TP53* mutations in clonal hematopoiesis and the pathogenesis of hematological malignancies is largely unknown.

To date, no studies have investigated the *in vivo* effect of *TP53* mutations expressed from the endogenous locus in hematopoietic cells, which might allow delineation of how these mutations contribute to the pathogenesis of hematological malignancies. [10-13] The vast majority of missense *TP53* mutations are mapped to the DNA-binding domain (DBD) of p53 protein, and usually abrogate its sequence-specific DNA-binding activity.[6] Previous studies show that codon 248 of the p53 protein is most frequently mutated in MDS and AML.[7-8] $p53^{R248W}$ mutant has also been identified in aged healthy individuals.[3-5] To better understand the role of mutant p53 in hematopoiesis when expressed physiologically from its endogenous promoter, we utilized the humanized $p53^{R248W}$ knock-in mice, expressing human p53 mutant protein from the endogenous murine *Trp53* promoter.[14] Given that most *TP53* mutations in clonal hematopoiesis and hematological malignancies are monoallelic missense mutations,[3-9] we examined HSC

function in the heterozygous $p53^{R248W/+}$ mice to recapitulate clinical conditions. We first analyzed the peripheral blood and bone marrow of $p53^{+/+}$ and $p53^{R248W/+}$ mice (8 to 12 week-old). Peripheral blood cell counts and bone marrow cellularity were comparable among these mice (Figures S1a, S1b, S1c, S1d, S1e, and S1f). In p53 knockout mice, there is a dramatic increase of LT-HSCs;[11] however, we found that $p53^{+/+}$ and $p53^{R248W/+}$ mice have a similar number of LT-HSCs (Figures 1a and S1g). In addition, expression of $p53^{R248W}$ did not affect the frequency of myeloid progenitors (Figures 1a, 1b, and S1g). To determine the role of $p53^{R248W}$ in hematopoiesis *in vivo*, we performed competitive bone marrow transplantation assays as shown in Figure S1h. We transplanted 5 x 10^5 donor bone marrow cells ($p53^{+/+}$ or $p53^{R248W/+}$, $CD45.2^+$) into lethally irradiated (9.5 Gy) recipient mice ($CD45.1^+CD45.2^+$) along with 5 x 10^5 competitor marrow cells ($CD45.1^+$). At 16 weeks post transplantation, the repopulating ability of $p53^{R248W/+}$ cells was significantly higher than that of the wild type cells (Figure 1c). We also observed increased frequency of donor-derived hematopoietic stem and progenitor cells in the bone marrow of recipient mice repopulated with $p53^{R248W/+}$ cells (Figures 1d, 1e, and S1i). $p53^{R248W}$ did not affect myeloid and lymphoid differentiation in the peripheral blood and the bone marrow of the primary recipient mice (Figures 1f and 1g). We then transplanted 3 x 10^6 bone marrow cells isolated from the primary recipient mice into lethally irradiated secondary recipients. Sixteen weeks after transplantation, $p53^{R248W/+}$ cells continued to show increased repopulating ability (Figure 1h). $p53^{R248W}$ did not affect myeloid and lymphoid differentiation in the peripheral blood of secondary recipients (Figure 1i). These findings suggest that expression of mutant p53 in normal HSCs does not cause leukemic transformation, but rather generates a premalignant state.[15]

HSCs in aged mice have decreased per-cell repopulating activity, self-renewal and homing abilities, myeloid skewing of differentiation, and increased apoptosis with stress.[1,16] To determine the role of mutant p53 in hematopoiesis during aging, we maintained the secondary transplantation recipient mice shown in Figure 1h for more than 12 months. We observed enhanced repopulating potential of $p53^{R248W/+}$ bone marrow cells compared to $p53^{+/+}$ cells 48 weeks after transplantation (Figure 1j). We found

decreased frequency of donor-derived myeloid cells and increased frequency of lymphoid cells in the PB of the recipients repopulated with $p53^{R248W/+}$ cells (Figure 1k).

Tumor suppressor regulates HSC quiescence and response to genotoxic stress.[11] Given that $p53^{-/-}$ mice are resistant to 5-FU treatment,[10] we hypothesized that $p53^{R248W/+}$ mice may be resistant to chemotherapy treatment. We treated $p53^{+/+}$ and $p53^{R248W/+}$ mice with 5-FU weekly and then monitored their survival. While most wild-type mice were died 4 weeks following 5-FU treatment, all $p53^{R248W/+}$ mice were still alive (Figure 2a). To measure the kinetics of hematopoietic recovery, we administered a single dose of 5-FU (200mg/kg) and serially followed peripheral blood cell counts. 5-FU-treated $p53^{R248W/+}$ mice had less severe leukopenia than $p53^{+/+}$ mice, with more rapid recovery (Figures 2b, S2a, S2b, S2c, and S2d). We treated $p53^{+/+}$ and $p53^{R248W/+}$ mice with one dose of 5-FU (200mg/kg) and then examined bone marrow cellularity, HSC frequency, and apoptosis one week later. We found that $p53^{R248W/+}$ mice exhibited higher bone marrow cellularity compared to $p53^{+/+}$ mice following 5-FU treatment (Figure S2e). There were more hematopoietic stem and progenitor cells in the bone marrow of 5-FU treated $p53^{R248W/+}$ mice compared to that of the $p53^{+/+}$ mice (Figures 2c, S2f, and S2g). Moreover, $p53^{R248W/+}$ hematopoietic stem and progenitor cells were less apoptotic compared to $p53^{+/+}$ HSPCs following 5-FU treatment (Figures 2d and S2h).

To determine the impact of 5-FU treatment on $p53^{R248W/+}$ hematopoietic cells *in vivo*, we performed serial competitive transplantation assays using live bone marrow cells isolated from $p53^{+/+}$ and $p53^{R248W/+}$ mice treated with one dose of 5-FU (Figure S2i). We found that 5-FU treated $p53^{R248W/+}$ bone marrow cells showed enhanced repopulation potential compared to 5-FU treated $p53^{+/+}$ cells at 16 weeks following primary transplantation (Figure 2e). Multi-lineage differentiation of hematopoietic stem and progenitor cells in the peripheral blood was comparable in $p53^{+/+}$ and $p53^{R248W/+}$ mice (Figure 2f). We then transplanted 3×10^6 BM cells isolated from primary recipient mice into lethally irradiated secondary recipient mice. $p53^{R248W/+}$ bone marrow cells continued to show enhanced engraftment compared to $p53^{+/+}$ cells at 16 weeks following secondary transplantation (Figure 2g). Mutant p53 did not affect differentiation in the peripheral blood and the bone marrow of secondary recipient mice

(Figures 2h and S2j). The frequency of donor-derived hematopoietic stem and progenitor cells was significantly increased in the BM of secondary recipient mice repopulated with mutant BM cells (Figure 2i). To determine the impact of mutant p53 on DNA damage response, we treated wild type and mutant p53 mice with DMSO or 5-FU and then examined the expression of p53 target gene *p21* in HSPCs. While the mRNA levels of *p21* were significantly increased in wild-type cells following 5-FU treatment, the expression of *p21* in mutant cells was not induced by 5-FU treatment (Figure 2j).

To determine whether functional *TP53* mutations promote clonal expansion of HSCs following chemotherapy treatment, we generated mixed bone marrow chimaeras containing both $p53^{+/+}$ and $p53^{R248W/+}$ cells (the ratio of $p53^{+/+}$ to $p53^{R248W/+}$ cells is 10:1). 8 weeks following transplantation, recipient mice were treated with DMSO or chemotherapy drug *N*-ethyl-*N*-nitrosourea (ENU), respectively (Figure S3a). While DMSO treatment did not affect the repopulating ability of $p53^{R248W/+}$ HSCs, $p53^{R248W/+}$ HSCs outcompeted $p53^{+/+}$ cells and become clonal dominance upon ENU treatment (Figure 2k). ENU treatment did not affect the differentiation of $p53^{+/+}$ and $p53^{R248W/+}$ HSCs in the peripheral blood of recipient mice (Figure S3b). The frequency of mutant LSKs was significantly increased in the BM of recipient mice at 16 weeks following ENU treatment (Figures 2i and S3c). We sacrificed the recipient mice at 16 weeks following DMSO or ENU treatment and then performed pathological analysis to detect tumor formation. While there were no tumors in recipient mice treated with DMSO, 4 out of 7 recipient mice treated with ENU developed lymphoma and thymoma (data not shown).

Given p53 null hematopoietic stem and progenitor cells are less sensitive to irradiation, [11-13] we examined the impact of mutant p53 on HSC function flowing irradiation. We found that HSCs expressing mutant p53 show decreased apoptosis and increased repopulating potential compared to wild type HSCs following irradiation. Furthermore, $p53^{R248W/+}$ HSCs show competitive advantage over $p53^{+/+}$ cells and underwent clonal expansion following total body irradiation (SC and YL, unpublished data).

Thus, we have identified a critical role for mutant p53 in regulating the response of HSCs to genotoxic stresses. Further, we discovered that chemotherapy and radiotherapy cause the expansion of HSCs expressing mutant p53. While mutant p53 proteins accumulate in cancer cells, the levels of mutant p53 proteins in normal cells are very low. [14, 17] Genotoxic stresses stabilize mutant p53 in hematopoietic cells.[14, 17-18] Given that the $p53^{R248W}$ mutant has both dominant-negative (DN) and gain-of-function (GOF) roles in human cancer,[6, 14] stabilized mutant p53 may inhibit the wild type p53 function or gain new oncogenic functions through protein-protein interactions to promote leukemic transformation. In addition, the expansion of mutant HSCs following genotoxic stresses may increase the possibility of acquiring additional genetic and/or epigenetic changes that facilitate leukemic transformation.

CONFLICT OF INTEREST

The authors declared that no conflict interest exists.

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AUTHOR CONTRIBUTIONS

SC and YL designed the research. SC, RG, CY, MK, and SZL performed the research. SC and YL analyzed the data and performed the statistical analysis. MCY, HB, RK, HSB, and LDM provided reagents and/or input to the study. SC and YL wrote the manuscript. All authors read, comment on, and approved the manuscript.

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FIGURE LEGENDS

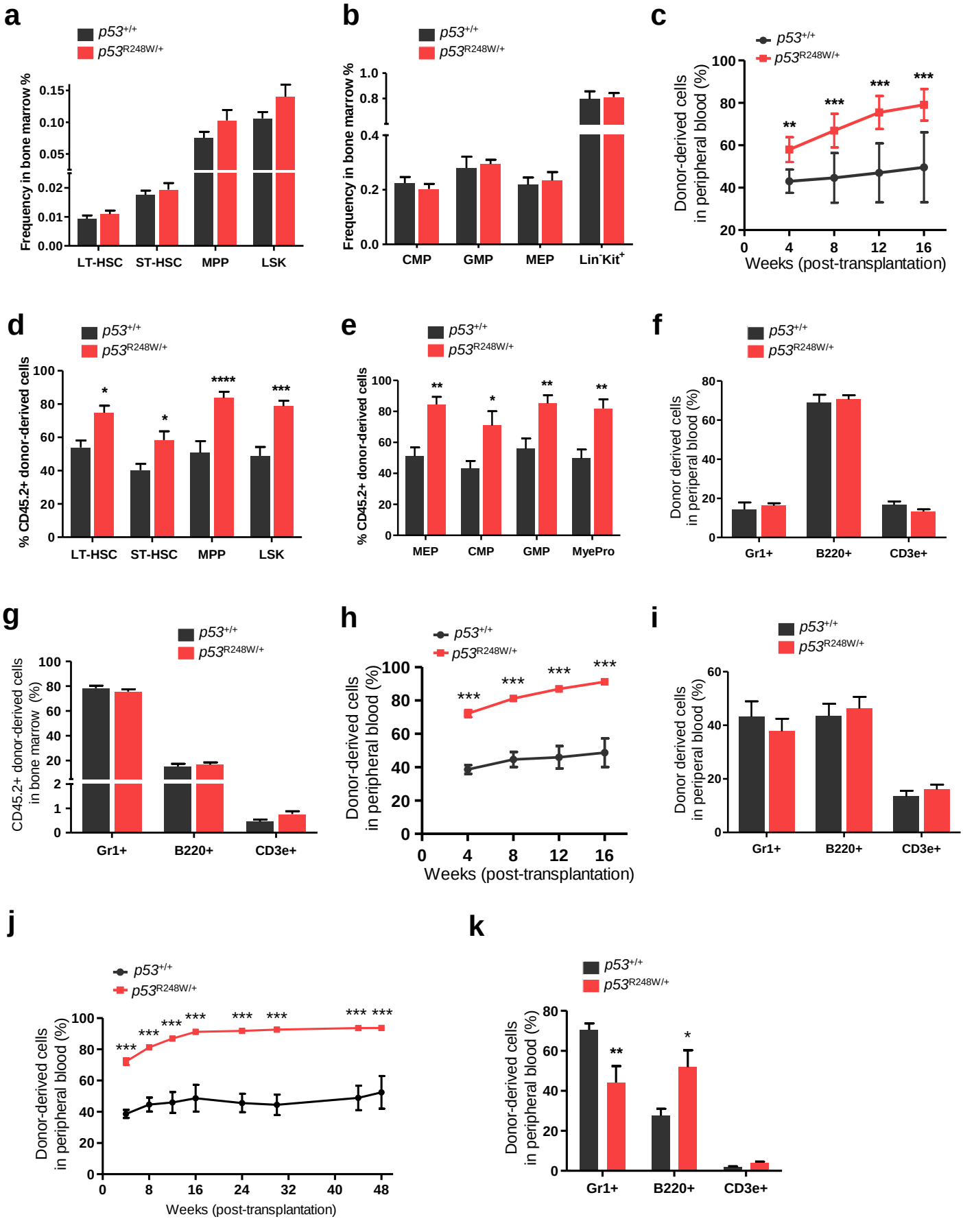
Figure 1. Mutant p53 enhances the repopulating potential of bone marrow cells. (**a**) The frequency of LT-HSCs, ST-HSCs, MPPs, and LSKs in the BM of young $p53^{+/+}$ and $p53^{R248W/+}$ mice. n=10 mice per genotype. (**b**) The frequency of CMPs, MEPs, GMPs, and $Lin^{-}Kit^{+}$ cells in the bone marrow of young $p53^{+/+}$ and $p53^{R248W/+}$ mice. n=10 mice per genotype. (**c**) Percentage of donor-derived ($CD45.2^{+}$) cells in the peripheral blood of primary recipient mice post-transplantation, measured at 4-week intervals. **p<0.01, ***p<0.001, n=7 mice per group. (**d**) The frequency of donor-derived LT-HSCs, ST-HSCs, MPPs, and LSKs in the bone marrow of primary recipient mice 16 weeks following transplantation. *p<0.05, ***p<0.001, n = 7 mice per group. (**e**) The frequency of donor-derived MEPs, CMPs, GMPs, and myeloid progenitors ($Lin^{-}Kit^{+}$) in the bone marrow of primary recipient mice 16 weeks following transplantation. *p<0.05, **p<0.01, n = 7 mice per group. (**f**) The percentage of donor-derived myeloid cells ($Gr1^{+}$), B cells ($B220^{+}$), and T cells ($CD3e^{+}$) in the peripheral blood of primary recipient mice 16 weeks following transplantation. n=7 mice per group. (**g**) The percentage of donor-derived myeloid cells, B cells, and T cells in the bone marrow of primary recipient mice 16 weeks following transplantation. n = 7 mice per group. (**h**) The percentage of donor-derived cells in the peripheral blood of secondary recipient mice. ***p<0.001, n=7 mice per group. (**i**) The percentage of donor-derived myeloid cells, B cells, and T cells in the peripheral blood of secondary recipient mice 16 weeks following transplantation. n=7 mice per group. (**j**) The percentage of donor-derived cells in the peripheral blood of secondary recipients 48 weeks after transplantation. ***p<0.001, n=7 mice per group. (**k**) The percentage of donor-derived myeloid cells, B cells, and T cells in the peripheral blood of secondary recipient mice 48 weeks after transplantation. *p<0.05, **p<0.01, n=7 mice per group.

Figure 2. Chemotherapy treatment promotes clonal expansion of HSCs expressing mutant p53. (**a**) Kaplan-Meier survival curve of $p53^{+/+}$ and $p53^{R248W/+}$ mice following weekly 5-FU treatment. 5-FU was administered intraperitoneally weekly (the initial dose was 125 mg/kg, with subsequent doses of 90 mg/kg) for 3 weeks and the survival rates of 5-FU treated mice were measured. Results were analyzed

with a log-rank nonparametric test and expressed as Kaplan-Meier survival curves (***p<0.001, n = 10 mice per group). (**b**) Hematopoietic recovery of *p53*$^{+/+}$ and *p53*$^{R248W/+}$ mice following a single dose of 5-FU treatment (200 mg/kg intraperitoneally). WBC counts are shown at each point after 5-FU administration as a percentage of the initial values for each group of mice. Mean ± SEM values are shown (*p<0.05, **p<0.01, ***p<0.001, n = 5 mice per group for each time point). (**c**) Absolute number of HSCs (Lin$^-$Sca1$^+$CD48$^-$CD150$^+$ cells) in the bone marrow of *p53*$^{+/+}$ and *p53*$^{R248W/+}$ mice seven days after a single dose of 5-FU treatment (200 mg/kg intraperitoneally). Mean ± SEM values are shown (**p<0.01, n = 5 mice per group). (**d**) The percentage of early apoptotic (AnnexinV$^+$DAPI$^-$) HSCs (Lin$^-$Sca1$^+$CD48$^-$CD150$^+$ cells) in the bone marrow of *p53*$^{+/+}$ and *p53*$^{R248W/+}$ mice seven days after a single dose of 5-FU treatment (200 mg/kg intraperitoneally). Mean ± SEM values are shown (**p<0.01, n = 5 mice per group). (**e**) The percentage of donor-derived cells in the peripheral blood of primary recipient mice reconstituted with bone marrow cells from 5-FU treated *p53*$^{+/+}$ and *p53*$^{R248W/+}$ mice. Mean ± SEM values are shown (***p<0.001, n = 7-8 mice per group). (**f**) The percentage of donor-derived myeloid cells (Gr1$^+$), B cells (B220$^+$), and T cells (CD3e$^+$) in the peripheral blood of secondary recipient mice 16-weeks after transplantation. Mean ± SEM values are shown (n.s, p>0.05, n = 7-8 mice per group). (**g**) The percentage of donor-derived cells in the peripheral blood of secondary recipient mice. Mean ± SEM values are shown (***p<0.001, n = 7-8 mice per group). (**h**) The percentage of donor-derived myeloid cells, B cells, and T cells in the peripheral blood of secondary recipient mice 16-weeks after transplantation. Mean ± SEM values are shown (n.s, p>0.05, n = 7-8 mice per group). (**i**) The chimerism of donor-derived LT-HSCs, ST-HSCs, and MPPs in the bone marrow of secondary recipient mice at 16 weeks following transplantation. Mean ± SEM values are shown (*p<0.05, **p<0.01, ***p<0.001, n = 7-8 mice per group). (**j**) The mRNA levels of p53 target gene *p21* in hematopoietic stem and progenitor cells isolated from *p53*$^{+/+}$ and *p53*$^{R248W/+}$ mice treated with DMSO or 5-FU were determined by quantitative real-time PCR analysis. **p<0.01, n = 3. **(K**) Mutant p53 promotes clonal expansion of HSCs following chemotherapy treatment. The percentage of *p53*$^{R248W/+}$ cells (CD45.2$^+$) in the peripheral blood of recipient mice following ENU or DMSO treatment was determined by flow cytometry analysis at monthly intervals.

Mean ± SEM values are shown (***p<0.001, n = 7 mice per group). (**l**) The frequency of *p53*$^{R248W/+}$ LSK cells (CD45.2^{+}) in the bone marrow of recipient mice at 16 weeks following ENU or DMSO treatment. Mean ± SEM values are shown (*p<0.05, n = 7 mice per group).

Figure 1


a
p53+/+
p53R248W/+
Frequency in bone marrow %
0.15
0.10
0.05
0.02
0.01
0.00
LT-HSC
ST-HSC
MPP
LSK
b
p53+/+
p53R248W/+
Frequency in bone marrow %
1.0
0.8
0.4
0.2
0.0
CMP
GMP
MEP
Lin-Kit+
c
p53+/+
p53R248W/+
Donor-derived cells in peripheral blood (%)
100
80
60
40
20
**
***
***
***
0
4
8
12
16
Weeks (post-transplantation)
d
p53+/+
p53R248W/+
% CD45.2+ donor-derived cells
100
80
60
40
20
0
*
*
****
***
LT-HSC
ST-HSC
MPP
LSK
e
p53+/+
p53R248W/+
% CD45.2+ donor-derived cells
100
80
60
40
20
0
**
*
**
**
MEP
CMP
GMP
MyePro
f
p53+/+
p53R248W/+
Donor derived cells in peripheral blood (%)
80
60
40
20
0
Gr1+
B220+
CD3e+
g
p53+/+
p53R248W/+
CD45.2+ donor-derived cells in bone marrow (%)
100
80
60
40
20
2
1
0
Gr1+
B220+
CD3e+
h
p53+/+
p53R248W/+
Donor-derived cells in peripheral blood (%)
100
80
60
40
20
0
***
***
***
***
0
4
8
12
16
Weeks (post-transplantation)
i
p53+/+
p53R248W/+
Donor derived cells in peripheral blood (%)
60
40
20
0
Gr1+
B220+
CD3e+
j
p53+/+
p53R248W/+
Donor-derived cells in peripheral blood (%)
100
80
60
40
20
0
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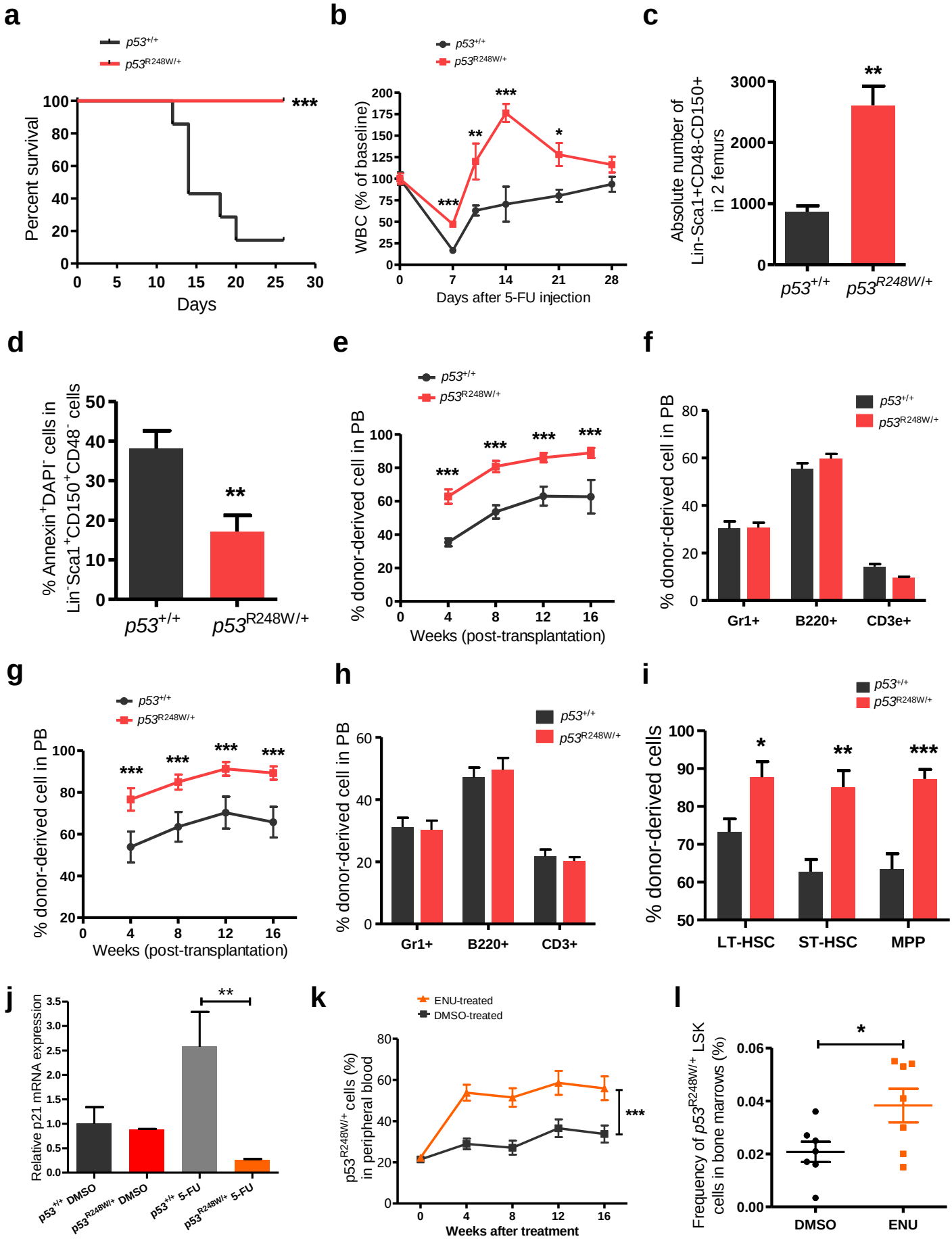
0
8
16
24
32
40
48
Weeks (post-transplantation)
k
p53+/+
p53R248W/+
Donor-derived cells in peripheral blood (%)
80
60
40
20
0
**
*
Gr1+
B220+
CD3e+

Figure 2


a
p53+/+
p53R248W/+
Percent survival
Days
***
b
WBC (% of baseline)
Days after 5-FU injection
c
Absolute number of Lin-Sca1+CD48-CD150+ in 2 femurs
p53+/+
p53R248W/+
d
% Annexin+DAPI- cells in Lin-Sca1+CD150+CD48- cells
e
% donor-derived cell in PB
Weeks (post-transplantation)
f
% donor-derived cell in PB
Gr1+
B220+
CD3e+
g
% donor-derived cell in PB
Weeks (post-transplantation)
h
% donor-derived cell in PB
Gr1+
B220+
CD3+
i
% donor-derived cells
LT-HSC
ST-HSC
MPP
j
Relative p21 mRNA expression
p53+/+ DMSO
p53R248W/+ DMSO
p53+/+ 5-FU
p53R248W/+ 5-FU
k
ENU-treated
DMSO-treated
p53R248W/+ cells (%) in peripheral blood
Weeks after treatment
l
Frequency of p53R248W/+ LSK cells in bone marrows (%)
DMSO
ENU

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Supplemental Methods

Mice

The humanized *p53* knock-in mice (*HUPKI*, $p53^{+/+}$) and $p53^{R248W/+}$ mice have been backcrossed to the *C57BL6* background for at least 8 generations (Song et al., 2007). All $p53^{+/+}$ and $p53^{R248W/+}$ mice used in these studies are 8 to 12 weeks old and are tumor free. Wild type C57BL/6 ($CD45.2^+$), B6.SJL ($CD45.1^+$) and F1 mice ($CD45.2^+$ $CD45.1^+$) mice were obtained from an on-site core breeding colony. All mice were maintained in the Indiana University Animal Facility according to IACUC-approved protocols.

Flow cytometry

Flow cytometry analysis of hematopoietic stem and progenitor cells was performed as described previously (Liu et al., 2009). Murine hematopoietic stem and progenitor cells were identified and evaluated by flow cytometry using a single cell suspension of bone marrow mononuclear cells (BMMCs). Hematopoietic stem and progenitors are purified based upon the expression of surface markers. Bone marrow (BM) cells were obtained from femurs by flushing cells out of the bone using a syringe and phosphate-buffered saline (PBS) with 2mM EDTA. Red blood cells (RBCs) were lysed by RBC lysis buffer (eBioscience) prior to staining. Experiments were performed on FACS LSR IV cytometers (BD Biosciences) and analyzed by using the FlowJo Version 9.3.3 software (TreeStar).

Transplantation

For the competitive repopulation assays, we injected 5 x 10^5 BM cells from $p53^{+/+}$ and $p53^{R248W/+}$ mice ($CD45.2^+$) plus 5 x 10^5 competitor BM cells ($CD45.1^+$) into 9.5Gy lethally irradiated F1 mice ($CD45.1^+CD45.2^+$). Peripheral blood was obtained by tail vein bleeding every 4-week after transplantation, RBC lysed, and the PB mononuclear cells stained with anti-CD45.2 FITC and anti-

CD45.1 PE, and analyzed by flow cytometry. 16 weeks following transplantation, bone marrow cells from recipient mice were analyzed to evaluate donor chimerism in bone marrows. For secondary transplantation, $3X10^6$ BM cells from mice reconstituted with $p53^{+/+}$ or $p53^{R248W/+}$ BM cells were injected into 9.5Gy lethally irradiated F1 mice ($CD45.1^+CD45.2^+$).

We treated $p53^{+/+}$ and $p53^{R248W/+}$ mice ($CD45.2^+$) with a single dose of 5-FU (200 mg/kg intraperitoneally). 7 days later, we injected 5 x 10^5 live BM cells from 5-FU treated $p53^{+/+}$ and $p53^{R248W/+}$ mice ($CD45.2^+$) plus 5 x 10^5 competitor BM cells ($CD45.1^+$) into lethally irradiated (9.5Gy) F1 mice ($CD45.1^+CD45.2^+$). Peripheral blood was obtained by tail vein bleeding every 4-week after transplantation, RBC lysed, and the PB mononuclear cells stained with anti-CD45.2 FITC and anti-CD45.1 PE, and analyzed by flow cytometry. For secondary transplantation, $3x10^6$ BM cells from primary recipient mice reconstituted with $p53^{+/+}$ or $p53^{R248W/+}$ BM cells were injected into lethally irradiated (9.5Gy) F1 mice ($CD45.1^+CD45.2^+$).

Apoptosis assays

Bone marrows were stained with cell surface markers as described above. After staining, cells were washed with 0.2% BSA in PBS, and suspended in 1 x Annexin V binding buffer (eBiosciences) and then incubated with FITC-conjugated-AnnexinV (eBiosciences) and 4′,6-diamidino-2-phenylindole (DAPI) (Sigma) at room temperature for 30 mines. Cells were acquired using LSR IV flow cytometer. Data analysis was performed using FlowJo software. Early apoptotic cells were defined as Annexin V^+ $DAPI^-$ cells.

Statistical Analysis

Statistical analysis was performed with GraphPad Prism 6 software (GraphPad software, Inc). All data are presented as mean ± standard error of the mean (SEM). The sample size for each experiment are included in the figure legends. Statistical analyses were performed using unpaired, two-tailed Student's t

test where applicable for comparison between two groups, and a One-way ANOVA test or Two-way ANOVA was used for experiments involving more than two groups. Statistical significance was defined as *p < 0.05; **p < 0.01; ***p < 0.001; ns, not significant.

Supplementary Figure Legends

Figure S1. (**a**) White blood cell, (**b**) Neutrophil, (**c**) lymphocyte, (**d**) Red blood cell, and (**e**) Platelet counts in the peripheral blood of *p53*$^{+/+}$ and *p53*$^{R248W/+}$ mice (8 to 12 weeks old). n=5 mice per group. (**f**) Bone marrow cell numbers from *p53*$^{+/+}$ and *p53*$^{R248W/+}$ mice. n=10 mice per group. (**g**) The frequency of LT-HSCs (CD48^{-}CD150^{+}LSKs), ST-HSCs (CD48^{-}CD150^{-}LSKs), MPPs (CD48^{+}CD150^{-}LSKs), CMPs (Lin^{-}Sca1^{-}Kit^{+}FcγRII/III^{low}CD34^{high}), GMPs (Lin^{-}Sca1^{-}Kit^{+}FcγRII/III^{high}CD34^{high}) and MEPs (Lin^{-}Sca1^{-}Kit^{+}FcγRII/III^{low}CD34^{low}) in the bone marrow of *p53*$^{+/+}$ and *p53*$^{R248W/+}$ mice was determined by flow cytometry analysis of cell surface markers. Representative flow cytometry plots of hematopoietic stem and progenitor cells are shown. (**h**) Experimental schema of competitive bone marrow transplantation assays. (**i**) The percentage of donor-derived cells (CD45.2^{+}) in the bone marrow of primary recipient mice 16 weeks following transplantation was determined by flow cytometry analysis. n = 7 mice per group, *p<0.05.

Figure S2. (**a**) Lymphocyte, (**b**) Neutrophil, (**c**) Red blood cell, and (**d**) Platelet counts in the peripheral blood of *p53*$^{+/+}$ and *p53*$^{R248W/+}$ mice following a single dose of 5-FU treatment (200 mg/kg intraperitoneally). Cell counts are shown at each point after 5-FU administration as a percentage of the initial values for each group of mice. Mean ± SEM values are shown (*p<0.05, **p<0.01, ***p<0.001, n = 5 mice per group for each time point). (**e**) Bone marrow cell counts of *p53*$^{+/+}$ and *p53*$^{R248W/+}$ mice seven days after a single dose of 5-FU treatment (200 mg/kg intraperitoneally). Mean ± SEM values are shown (***p<0.001, n = 5 mice per group)**.** (**f**) The frequency of HSCs (Lin^{-}Sca1^{+}CD48^{-}CD150^{+} cells) in the

bone marrow of *p53*$^{+/+}$ and *p53*$^{R248W/+}$ mice seven days after a single dose of 5-FU treatment (200 mg/kg intraperitoneally). Mean ± SEM values are shown (*p<0.05, n = 5 mice per group). (**g**) The frequency of MPPs (Lin$^-$Sca1$^+$CD48$^+$CD150$^-$ cells) in the bone marrow of *p53*$^{+/+}$ and *p53*$^{R248W/+}$ mice seven days after a single dose of 5-FU treatment (200 mg/kg intraperitoneally). Mean ± SEM values are shown (*p<0.05, n = 5 mice per group). (**h**) The percentage of early apoptotic (AnnexinV$^+$DAPI$^-$) MPPs (Lin$^-$ Sca1$^+$CD48$^+$CD150$^-$ cells) in the bone marrow of *p53*$^{+/+}$ and *p53*$^{R248W/+}$ mice seven days after a single dose of 5-FU treatment (200 mg/kg intraperitoneally). Mean ± SEM values are shown (**p<0.01, n = 5 mice per group). (**i**) Experimental schema of competitive bone marrow transplantation assays using live bone marrow cells isolated from *p53*$^{+/+}$ and *p53*$^{R248W/+}$ mice treated with 5-FU. (**j**) The percentage of donor-derived myeloid cells, B cells, and T cells in the BM of secondary recipient mice 16-weeks after transplantation. Mean ± SEM values are shown (n.s, p>0.05, n = 7-8 mice per group).

Figure S3. (**a**) Bone marrow chimaeras were generated by transplanting a 10:1 ratio of *p53*$^{+/+}$ cells (CD45.1$^+$) to *p53*$^{R248W/+}$ cells (CD45.2$^+$) into irradiated recipient mice (CD45.1$^+$CD45.2$^+$). After hematopoietic reconstitution (8-weeks after transplantation), mice were treated with DMSO or ENU (100mg/kg two doses, 9 days apart). (**b**) The percentage of *p53*$^{R248W/+}$ (CD45.2$^+$) myeloid cells (Gr1$^+$), B cells (B220$^+$) and T cells (CD3e$^+$) in the peripheral blood of recipient mice 16-weeks following ENU or DMSO treatment. Mean ± SEM values are shown (n.s, p>0.05, n = 7 mice per group). (**c**) The frequency of *p53*$^{R248W/+}$ cells (CD45.2$^+$) in the bone marrow of recipient mice 16-weeks following ENU or DMSO treatment. Mean ± SEM values are shown (*p<0.05, n = 7 mice per group).

Figure S1

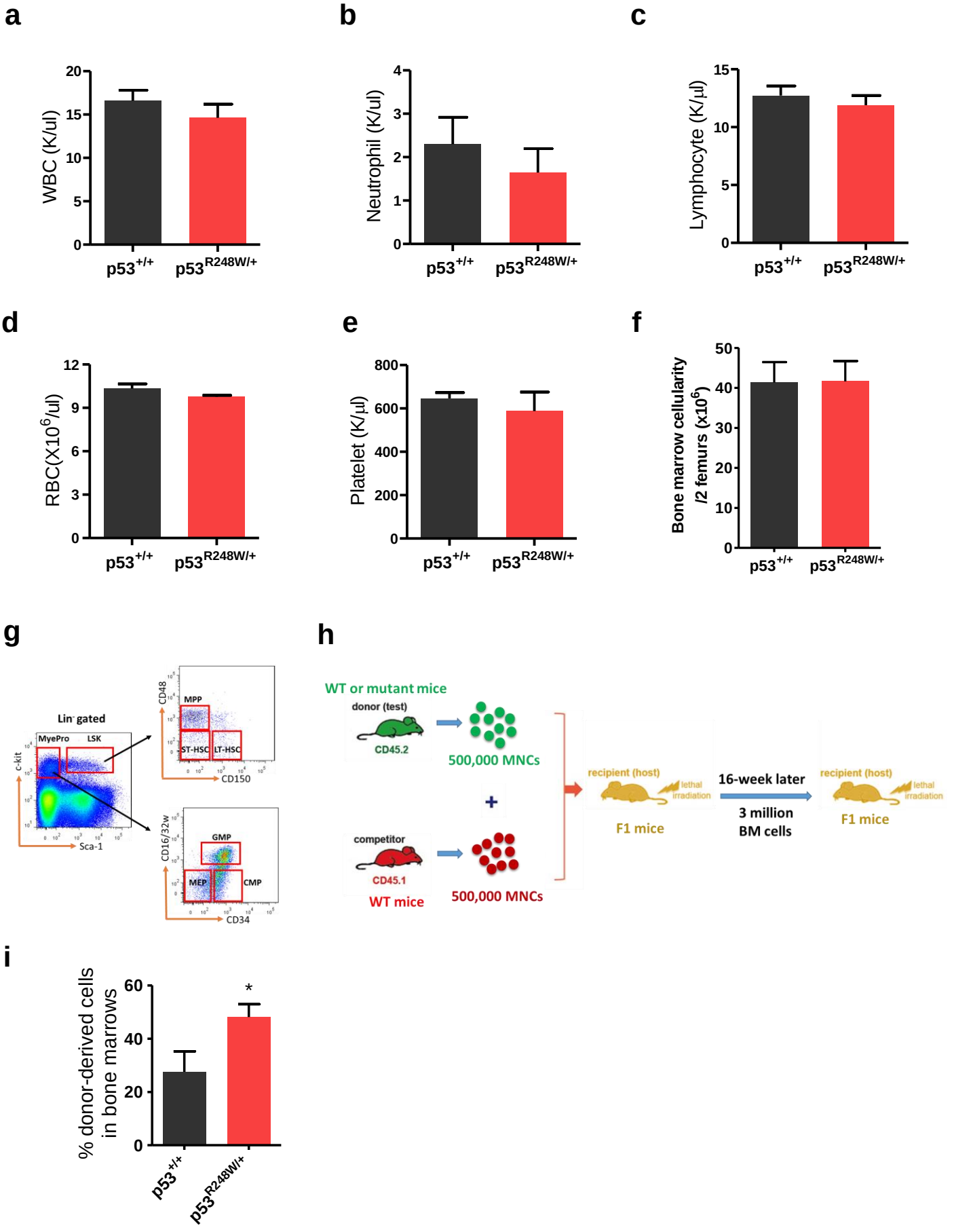

a
WBC (K/ul)
20
15
10
5
0
p53+/+
p53R248W/+
b
Neutrophil (K/ul)
4
3
2
1
0
p53+/+
p53R248W/+
c
Lymphocyte (K/μl)
15
10
5
0
p53+/+
p53R248W/+
d
RBC(X10^6/ul)
12
9
6
3
0
p53+/+
p53R248W/+
e
Platelet (K/μl)
800
600
400
200
0
p53+/+
p53R248W/+
f
Bone marrow cellularity /2 femurs (x10^6)
50
40
30
20
10
0
p53+/+
p53R248W/+
g
Lin- gated
MyePro
LSK
c-kit
Sca-1
CD48
MPP
ST-HSC
LT-HSC
CD150
CD16/32w
GMP
MEP
CMP
CD34
h
WT or mutant mice
donor (test)
CD45.2
500,000 MNCs
+
competitor
CD45.1
WT mice
500,000 MNCs
recipient (host)
lethal irradiation
F1 mice
16-week later
3 million BM cells
recipient (host)
lethal irradiation
F1 mice
i
% donor-derived cells in bone marrows
60
40
20
0
*
p53+/+
p53R248W/+

Figure S2

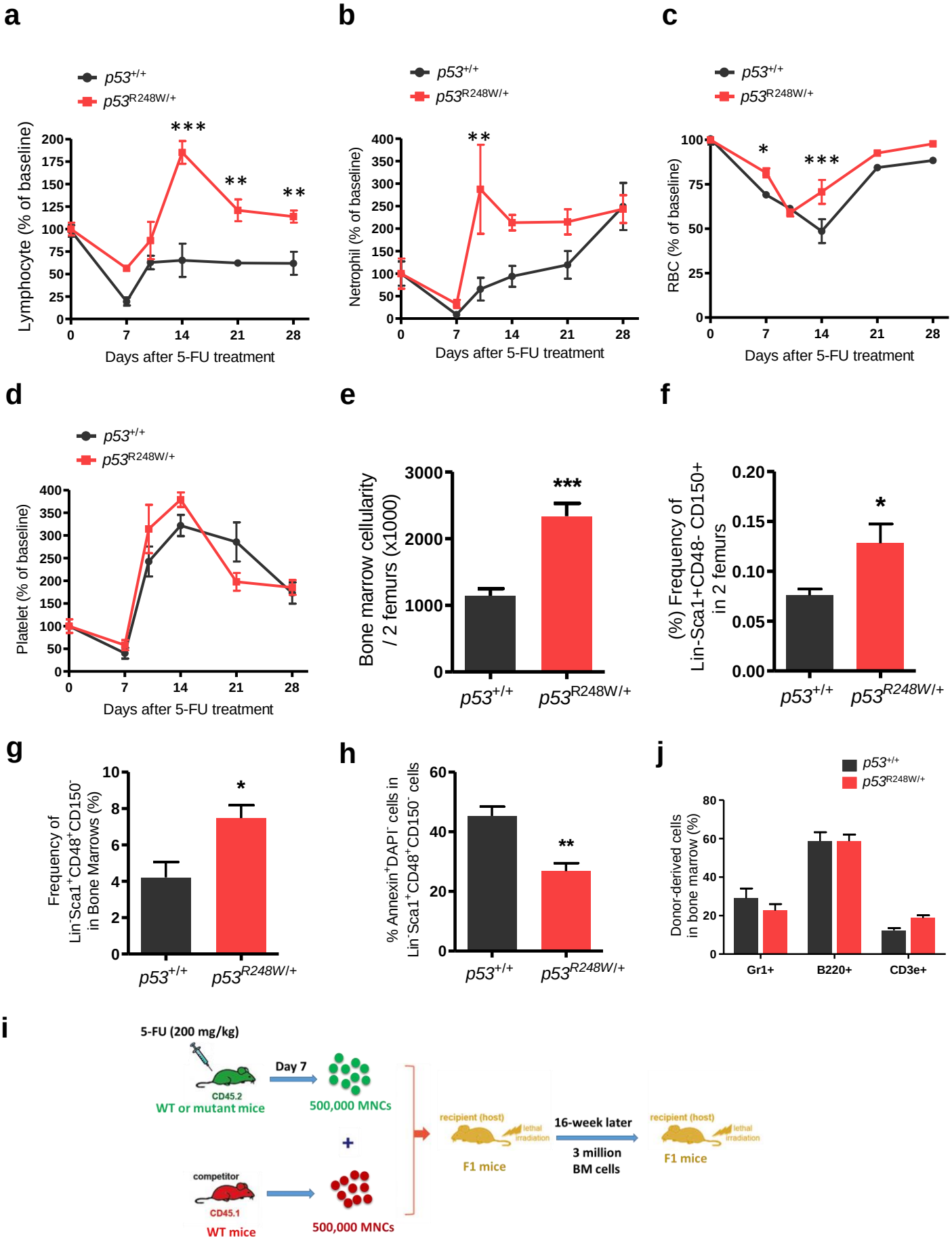
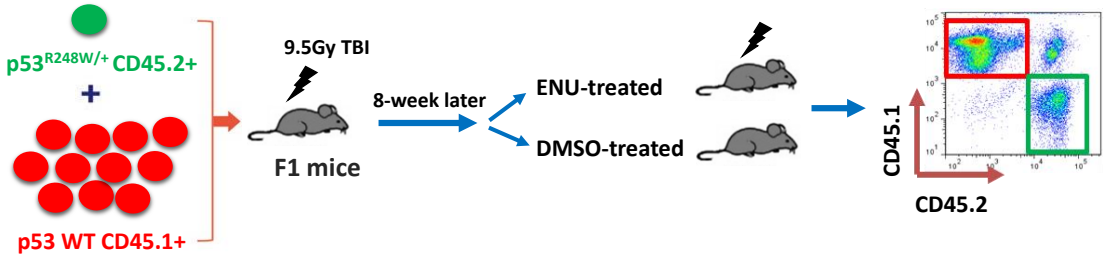
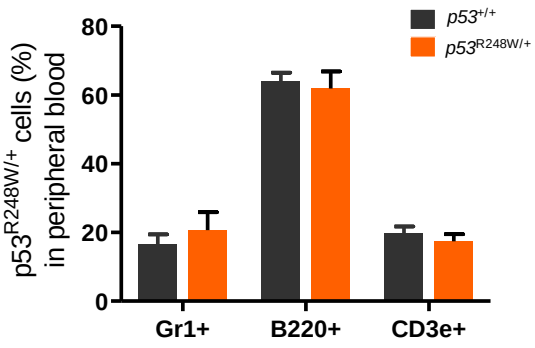

a
b
c
d
e
f
g
h
j
i
p53+/+
p53R248W/+
Lymphocyte (% of baseline)
Netrophil (% of baseline)
RBC (% of baseline)
Platelet (% of baseline)
Days after 5-FU treatment
Bone marrow cellularity / 2 femurs (x1000)
(%) Frequency of Lin-Sca1+CD48- CD150+ in 2 femurs
Frequency of Lin-Sca1+CD48+CD150- in Bone Marrows (%)
% AnnexinDAPI cells in Lin-Sca1+CD48+CD150- cells
Donor-derived cells in bone marrow (%)
Gr1+
B220+
CD3e+
5-FU (200 mg/kg)
Day 7
CD45.2
WT or mutant mice
500,000 MNCs
competitor
CD45.1
WT mice
500,000 MNCs
recipient (host)
lethal irradiation
F1 mice
16-week later
3 million BM cells
recipient (host)
lethal irradiation
F1 mice

Figure S3

a



b



c