Supplemental Figure 1. Recombination of the Brca1 floxed allele in hematopoietic tissue following pIpC treatment. RT-PCR genotyping analysis of Brca1 allele status in spleen and brain of control Brca1<sup>F/F</sup>;Trp53<sup>+/−</sup> (n=3) and diseased Mx1-Cre;Brca1<sup>F/F</sup>;Trp53<sup>+/−</sup> (n=8) mice. Values represent mean ± SEM. Statistical significance was assessed using a two-tailed Student’s t test (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001). All mice carry Mx1-Cre except controls.
Supplemental Figure 2 – related to Figures 1 and 2

A

WBC reads

K/U/L

Control (average)

Brcal+/-(average)

Brcal+/+;Trp53+/-(individual)

Weeks post initial pylpC

Peripheral Blood

B

B220+ cells

% Live Cells

*** ****

n.s.

C

CD3+ cells

% Live Cells

** n.s.

D

Gr1+ cells

% Live Cells

n.s. n.s.

E

Bone marrow

% c-Kir/CD71+ cells

*** ****

dKIt

cKit

CD71+/c-Kit+ CD71+

Spleen

% c-Kir/CD71+ cells

**** ****

dKIt

cKit

CD71+/c-Kit+ CD71+
Supplemental Figure 2, related to Figures 1 and 2. Expansion of erythroid lineage in *Mx1-Cre;Brca1^{F/F};Trp53^{+/−}* mice. (A) Individual peripheral blood white blood cell (WBC) reads* of *Mx1-Cre;Brca1^{F/F};Trp53^{+/−}* mice (n=9) and average WBC reads* of control (n=13) and *Mx1-Cre;Brca1^{F/F}* (n=11) mice before (prebleed) and after plpC treatment. * Indicate erythroid blast cells. (B-D) No increase of B220⁺ B cell (B), CD3⁺ T cell (C), or Gr1⁺ cell (D) frequencies in peripheral blood of diseased *Mx1-Cre;Brca1^{F/F};Trp53^{+/−}* mice (n=9) compared to control (n=8) or *Mx1-Cre;Brca1^{F/F}* (n=6) mice. (E) Frequencies of c-kit⁺, CD71⁺/c-kit⁺, and CD71⁺ cells in bone marrow and spleen in diseased *Mx1-Cre;Brca1^{F/F};Trp53^{+/−}* mice (n=5) show the high abundance of CD71 and c-kit double positive cells. Values represent mean ±SEM. Statistical significance was assessed using one-way ANOVA followed by Bonferroni correction (*p < 0.0167, **p < 0.003, ***p < 0.0003, ****p < 0.00003). Controls were without *Mx1-Cre*, all other mice carry *Mx1-Cre.*
**Supplemental Figure 3**

**Supplemental Figure 3.** *Vav1-iCre; Brca1^{F/F}; Trp53^{+/−}* mice recapitulate hematopoietic phenotypes of Mx1-Cre driven Brca1/Trp53 deficiency. (A) *Vav1-iCre; Brca1^{F/F}; Trp53^{+/−}* mice develop pIpC-independent splenomegaly. Increased spleen weights of *Vav1-iCre; Brca1^{F/F}; Trp53^{+/−}* (n=6) mice compared to control (n=7) and *Vav1-iCre; Brca1^{F/F}* (n=4) mice. (B-C) H&E stains of spleen sections show that compared to controls (B), *Vav1-iCre; Brca1^{F/F}; Trp53^{+/−}* spleens (C) are effaced with monomorphic cells. (D-E) Compared to cytopenic *Vav1-iCre; Brca1^{F/F}* mice, *Vav1-iCre; Brca1^{F/F}; Trp53^{+/−}* mice have higher white blood cell (WBC) reads (D) and bone marrow megakaryocyte/erythroid progenitor (MEP)(E) frequencies. WBC and flow cytometry numbers: Control (n=20), *Vav1-iCre; Brca1^{F/F}* (n=11), *Vav1-iCre; Brca1^{F/F}; Trp53^{+/−}* (n=18). Values represent means ±SEM. Statistical significance was assessed using one-way ANOVA followed by Bonferroni correction (*p<0.0167, **p<0.003, ***p<0.0003, ****p<0.00003). Controls were either wildtype or without *Vav1-iCre.*
Supplemental Figure 4 – related to Figure 3

A

B

C

D

E

F

G

H

I

J

K

B cell

Spleen

T cell

Gr+Mac1

% Live Cells

% Live Cells

% Live Cells
Supplemental Figure 4, related to Figure 3. Mx1-Cre;Brca1<sup>F/insC</sup>;Trp53<sup>+/−</sup> mice develop an erythroproliferative disease similar to that of Mx1-Cre;Brca1<sup>F/F</sup>;Trp53<sup>+/−</sup> mice. (A) Individual mouse white blood cell (WBC) reads* show that Mx1-Cre;Brca1<sup>F/insC</sup>;Trp53<sup>+/−</sup> mice (black, n=5) develop high WBC reads* earlier than Mx1-Cre;Brca1<sup>F/F</sup>;Trp53<sup>+/−</sup> mice (red, n=4). (B) Time to elevated WBC read* in Mx1-Cre;Brca1<sup>F/insC</sup>;Trp53<sup>+/−</sup> (black, n=5) and Mx1-Cre;Brca1<sup>F/F</sup>;Trp53<sup>+/−</sup> (red, n=4) mice. (C-D) Spleen (C) and liver (D) weights 8-12 weeks post initial plpC treatment. The spleens of Mx1-Cre;Brca1<sup>F/insC</sup>;Trp53<sup>+/−</sup> and Mx1-Cre;Brca1<sup>F/F</sup>;Trp53<sup>+/−</sup> mice were on average 14-fold larger (5.8% vs. 0.4% spleen/body weight) and 12-fold larger (4.9% vs. 0.4% spleen/body weight) than control mice. Livers were on average 2- (9.0% vs. 4.3% liver/body weight) and 1.9-fold (9.3% vs. 5.0% liver/body weight) larger. Numbers of mice: Controls (n=6), Mx1-Cre;Brca1<sup>F/insC</sup>; Trp53<sup>+/−</sup> (n=9), and Mx1-Cre;Brca1<sup>F/F</sup>; Trp53<sup>+/−</sup> (n=13). (E-J) Representative H&E stained sections of effaced spleens (F,G) and infiltrated liver (I,J) of Mx1-Cre;Brca1<sup>F/insC</sup>; Trp53<sup>+/−</sup> and Mx1-Cre;Brca1<sup>F/F</sup>; Trp53<sup>+/−</sup> mice compared to control (E,H). (K) Flow cytometric analysis of spleen B cells, T cells, and granulocyte/monocyte late progenitors (Gr<sup>+</sup>Mac1<sup>+</sup>) in control (n=5), Mx1-Cre;Brca1<sup>F/insC</sup>; Trp53<sup>+/−</sup> (n=2) and Mx1-Cre;Brca1<sup>F/F</sup>; Trp53<sup>+/−</sup> (n=5) mice. Values represent mean ± SD. Statistical significance was assessed using a log rank test or one-way ANOVA followed by Bonferroni correction (*p < 0.0167, **p < 0.003, ***p < 0.0003, ****p < 0.00003). All mice carry Mx1-Cre except controls.
Supplemental Figure 5. Enlarged spleens of diseased Mx1-Cre; Brca1<sup>1F/F</sup>; Trp53<sup>+/-</sup> mice show Trp53 LOH. (A) Whole exome analysis show specific loss of heterozygosity in the spleens of diseased Mx1-Cre;Brca1<sup>1F/F</sup>;Trp53<sup>+/-</sup> mice compared to brains and tissue of control Brca1<sup>1F/F</sup>;Trp53<sup>+/-</sup> mice. Signal intensity of the genomic region corresponding to the Trp53 deletion is reduced in Mx1Cre;Brca1<sup>1F/F</sup>;Trp53<sup>+/-</sup> spleens compared to control Brca1<sup>1F/F</sup>;Trp53<sup>+/-</sup> spleens. No difference in signal between control
Brca1<sup>F/F</sup>;Trp53<sup>+/−</sup> and Mx1-Cre;Brca1<sup>F/F</sup>;Trp53<sup>+/−</sup> brains. (B) RT-PCR genotyping analysis show decreased wildtype (WT) probe and increased knockout (KO) probe in enlarged Mx1-Cre;Brca1<sup>F/F</sup>;Trp53<sup>+/−</sup> spleens compared to control Brca1<sup>F/F</sup>;Trp53<sup>+/−</sup> spleens. No difference in brain tissue. Control Brca1<sup>F/F</sup>;Trp53<sup>+/−</sup> (n=2) and Mx1-Cre;Brca1<sup>F/F</sup>;Trp53<sup>+/−</sup> (n=3).
Supplemental Figure 6

A. WBC reads

B. Erythroblasts (CD71^+\text{c-kit}^+)

C. WBC reads

D. RBCs

E. Erythroblasts (CD71^+\text{c-kit}^+)

F. UF Spleen cells

G. CD71^+\text{c-kit}^+ Spleen cells

H. Spleen

I. % Body Weight
Supplemental Figure 6. Erythroid neoplasia of *Brca1* and *Trp53* double deficiency is transplantable through diseased bone marrow and spleen. (A) Terminal white blood cell (WBC) reads of primary and secondary recipients of control or *Mx1-Cre;Brca1<sup>F/F</sup>;Trp53<sup>+/-</sup>* bone marrow (BM) cells. WBC reads* of CD71+ recipient mice with prolonged survival are marked by open circles. Control UF (n=4) and *Mx1-Cre;Brca1<sup>F/F</sup>;Trp53<sup>+/-</sup>* primary UF (n=7), primary CD71+/c-kit+ (n=12), primary CD71+ (n=7), primary c-kit+ (n=1), secondary UF (n=5), secondary CD71+c-kit+ (n=5). (B) Flow cytometric analysis for CD71+/c-kit+ erythroblasts in recipients of control UF (n=4) or *Mx1-Cre;Brca1<sup>F/F</sup>;Trp53<sup>+/-</sup>* bone marrow – primary (1<sup>o</sup>) UF (n=4), 1<sup>o</sup> CD71+/c-kit+ (n=3), secondary (2<sup>o</sup>) CD71+/c-kit+ (n=4), 1<sup>o</sup> CD71+(n=6), 1<sup>o</sup> c-kit+(n=1). (C-D) Terminal white blood cell reads* (WBCs) (C) and red blood cell counts (RBCs) of recipients of *Mx1-Cre;Brca1<sup>F/F</sup>;Trp53<sup>+/-</sup>* spleen cells - UF (n=4-5), CD71+/c-kit+ (n=3), CD71+ (n=4-5), and c-kit+ (n=2). (E) Flow cytometric analysis for CD71+/c-kit+ erythroblasts in spleen and bone marrow of mice that received spleen cells. UF (n=4), CD71+/c-kit+ (n=5), CD71+ (n=3), and c-kit+ (n=2). (F) Kaplan-Meier curves of overall survival for recipients of 2.0x10<sup>6</sup> (bold solid line, n=3), 0.2x10<sup>6</sup> (solid line, n=6), or 0.02x10<sup>6</sup> (dotted line, n=4) of unfractionated (UF) *Mx1-Cre;Brca1<sup>F/F</sup>;Trp53<sup>+/-</sup>* spleen (SP) cells. Average time to disease is 3.14 weeks, 4.07 weeks, and 5.57 weeks respectively. (G) Kaplan-Meier survival curves for recipients of 20,000 (solid line, n=7) or 1,000 (dotted line, n=4) CD71+/c-kit+ spleen cells from *Mx1-Cre;Brca1<sup>F/F</sup>;Trp53<sup>+/-</sup>* mice. (H) Elevated terminal spleen weights of recipient mice in (F). (I) Elevated terminal spleen weights of recipient mice in (G). * Indicate erythroid blast cells. Values represent mean ± SEM.
**Supplemental Figure 7**

- **A:**
  - WBC reads
  - RBCs
  - Spleen
  - Time post initial plpC
  - Time post Initial plpC
  - Time post Initial plpC

- **B:**
  - 3.5 weeks
  - Percent survival
  - Time post transplant (weeks)

- **C:**
  - 6.5 weeks
  - Percent survival
  - Time post transplant (weeks)

- **D:**
  - 3.5 weeks
  - WBC reads
  - Spleen

- **E:**
  - 6.5 weeks
  - WBC reads
  - Spleen

Legend:
- Control
- Brca1^{+/+}; Trp53^{++}
- Control BM
- Control SP
- Brca1^{+/+}; Trp53^{++} BM
- Brca1^{+/+}; Trp53^{++} SP

Graphs showing data for WBC reads, RBCs, spleen, percent survival, and body weight with various time points and conditions.
Supplemental Figure 7. *Brca1/Trp53* deficiency-associated erythroleukemia can be transplanted from bone marrow or spleen prior to disease manifestation in peripheral blood (A) White blood cell reads* (WBCs), red blood cell counts (RBCs) and spleen weights of 3.5-, 6.5-m, and >10.5-week old *Mx1-Cre;Brca1^{F/F};Trp53^{+/−}* or control donors. (B-C) Kaplan-Meier survival curves of the recipients of 3.5- or 6.5-week bone marrow (BM) or spleen (SP) cells. Control BM (n=5), SP (n=4) and *Mx1-Cre;Brca1^{F/F};Trp53^{+/−}* BM (n=3), SP (n=5). (D-E) Terminal white blood cell reads* (WBCs) and spleen weights of recipient mice. * Indicate erythroid blast cells. Values represent mean ± SEM. Statistical significance was assessed using a two-tailed Student’s t test (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001). All mice carry *Mx1-Cre* except controls.
Supplemental Figure 8

A. Floxed Brca1 probe

B. Trp53 WT probe

C. Trp53 KO probe

D. Brain relative expression

E. Brain relative expression

F. Brain relative expression

G. WBC reads

H. RBCs

I. Spleen

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Legend:
- Control - Veh
- Control - Olap
- Vav-cre, Brca1fl/fl - Veh
- Vav-cre, Brca1fl/fl - Olap

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Supplemental Figure 8. *Brca1* deficient hematopoietic cells are sensitive to PARP inhibitor olaparib. (A-F) The signal levels of the probes that recognize unrecombined floxed *Brca1* (A) and wild-type *Trp53* (B) are significantly higher and the probe that recognize *Trp53* knockout (C) lower in olaparib-treated spleens (n=7) compared to vehicle-treated spleens (n=7). No differences seen between the two treatment groups in brain tissue (D-F). (G-H) Peripheral blood counts before (Pre Tx) and after (Post Tx) 10 daily treatments of olaparib (50mg/kg) or vehicle. Reduced white blood cell (WBC) reads* (2.55-fold) and red blood cell (RBC) (2.64-fold) counts in olaparib-treated Vavi-Cre;*Brca1*F/F mice (n=5) compared to vehicle-treated Vavi-Cre;*Brca1*F/F mice (n=7). No significant decrease of WBC reads* and modest decrease of RBC counts seen in olaparib-treated control mice (n=6) compared to vehicle-treated control mice (n=6). * Indicate erythroid blast cells. (I) Reduced spleen weights of olaparib-treated Vavi-Cre;*Brca1*F/F mice (n=6) compared to vehicle-treated Vavi-Cre;*Brca1*F/F mice (n=7). No difference in spleen weights between olaparib- and vehicle-treated control mice (n=6). Values represent mean ± SEM. Statistical significance was assessed using a two-tailed Student’s t test (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001). All mice carry Vav1- iCre except controls.
Supplemental Figure 9. BLG-cre;Brca1^{F/F};Trp53^{+/+} and BLG-cre;Brca1^{F/insC};Trp53^{+/+} mice develop mammary tumors following a long latency. (A-C) Histopathology of BLG-cre;Brca1^{F/F};Trp53^{+/+} tumors categorized as poorly differentiated (A), moderately differentiated (B), or well differentiated (C). (D) Kaplan-Meier curves for survival of control (dashed black, n=15), BLG-cre;Brca1^{F/F};Trp53^{+/+} (solid black, n=20), and BLG-cre;Brca1^{F/insC};Trp53^{+/+} (solid red, n=21) mice. Average time to maximum tumor BLG-cre;Brca1^{F/F};Trp53^{+/+} 11.5 months vs. BLG-cre;Brca1^{F/insC};Trp53^{+/+} 13.9 months, p=0.032). Statistical significance was assessed using a log-rank test. Controls were paired littermates of various genotypes, all without a BLG-Cre allele.
Supplemental Table 1. Antibodies used in flow cytometric analysis.

| Antibody              | Clone | Conjugate       | Catalog no. | Provider       |
|-----------------------|-------|-----------------|-------------|----------------|
| Sca1                  | D7    | PE-Cy7          | 108113      | BioLegend      |
| CD117 (c-kit)         | 2B8   | APC-Cy7         | 105825      | BioLegend      |
| CD117 (c-kit)         | 2B8   | APC-eFluor780   | 47-1171-82  | Invitrogen     |
| CD117 (c-kit)         | 2B8   | PE-Cy7          | 105813      | BioLegend      |
| CD48                  | HM48-1| APC             | 103411      | BioLegend      |
| CD150                 | TC15-12F12.2 | PE | 115903      | BioLegend      |
| CD16/32               | 93    | Alexa Fluor 700 | 56-0161-82  | Invitrogen     |
| CD34                  | RAM34 | FITC            | 11-0341-82  | Invitrogen     |
| CD3                   | 17A2  | Alexa Fluor 700 | 56-0032-82  | Invitrogen     |
| CD3                   | 17A2  | PE              | 100205      | BioLegend      |
| CD3ε                  | 142-2C11 | Biotin        | 100301      | BioLegend      |
| CD4                   | GK1.5 | PE              | 12-0041-82  | eBioscience    |
| CD4                   | GK1.5 | FITC            | 100405      | BioLegend      |
| CD4                   | 53-7.3| Biotin          | 100603      | BioLegend      |
| CD8a                  | 53-6.7| Biotin          | 100703      | BioLegend      |
| CD8a                  | 53-6.7| FITC            | 100705      | BioLegend      |
| Ter119                | TER-119 | APC          | 116211      | BioLegend      |
| Ter119                | TER-119 | Biotin       | 116203      | BioLegend      |
| CD45                  | 30-F11| Alexa Fluor 700 | 103127      | BioLegend      |
| CD45R (B220)          | RA3-6B2 | PE            | 103207      | BioLegend      |
| CD45R (B220)          | RA3-6B2 | PerCP-Cy5.5   | 65-0452  | TONBO          |
| CD45R (B220)          | RA3-6B2 | Biotin        | 103203      | BioLegend      |
| Gr-1 (Ly-6G)          | RB6-8C5 | PE            | 108407      | BioLegend      |
| Gr-1 (Ly-6G)          | RB6-8C5 | PE-Cy7        | 108415      | BioLegend      |
| Gr-1 (Ly-6G)          | RB6-8C5 | Biotin        | 108403      | BioLegend      |
| CD11b (Mac-1)         | M1/70 | APC-eFluor780  | 47-0112-82  | Invitrogen     |
| CD11b (Mac-1)         | M1/70 | PE              | 553311      | BD Pharmigen   |
| CD11b (Mac-1)         | M1/70 | Biotin          | 101230      | BioLegend      |
| CD71                  | RI7217 | BV421         | 113813      | BioLegend      |
| Streptavidin          |       | PE-CF594       | 562318      | BD Biosciences |

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