Supplementary information, Data S1 Materials and Methods

Mice and *in vivo* Tamoxifen Treatment. The DOT1L conditional mouse was previously described [8]. The R26-Cre-ER mice were generated by Tyler Jacks' lab and were obtained from the NCI Mouse Models of Human Cancers Consortium (Strain 01XAB). DOT1L$^{2lox/+}$ and DOT1L$^{1lox/+}$ mice were intercrossed to generate DOT1L$^{2lox/1lox}$/Cre-ER and DOT1L$^{+/+}$/Cre-ER mice. Mice were kept on a mixed 129Sv/Jae and C57BL/6 Ly5.2 background. Wild type C57BL/6 Ly5.1 mice were purchased from Jackson Laboratory. For *in vivo* Cre-recombination, tamoxifen (Sigma) was administered to 8-12 week old mice via intraperitoneal injection every 2 days (100 μl of 10 mg/ml in corn oil) for a period of 2 weeks. For all experiments, bone marrow aspirates were collected and analyzed 2 weeks after last tamoxifen injection. All animal protocols adhere to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the UNC Institutional Animal Care and Use Committee.

Bone Marrow Transplantation for Competition Assay. Recipient C57BL/6 Ly5.1 mice (6-10 weeks old) began prophylactic treatment to prevent gastro-intestinal infections through administration of sulfamethoxazole/trimethoprim (SMZ, TW Medical), 6 mg/1.2 mg per mL water, one week prior to bone marrow transplantation (BMT) and continued for 4 weeks post-transplantation. SMZ containing water was changed every other day. On the day of transplantation recipient mice received two doses, 4.8 Gy each, of total body irradiation (TBI) 4 hours apart using a cesium radiation source. Four hours after the second irradiation, recipient mice were anaesthetized through intraperitoneal injection of 300 ul per 10 g body weight of avertin (0.0125 g/ml 2,2,2, tribromoethanol and 1.25% tert-amyl alcohol in PBS). Once anaesthetized, 2.5x10^5 wild type, competitor Ly5.1 cells plus 2.5x10^5 cells DOT1L$^{2lox/1lox}$/Cre-ER or DOT1L$^{+/+}$/Cre-ER cells resuspended in 100 μl PBS were transplanted via retro-orbital injection. Six weeks post-transplantation, mice were injected with tamoxifen as described above.

FACS Analysis and Cell Sorting. Bone marrow cells were flushed from both femurs of mice using 25G needle and syringe. Red blood cells were lysed using ammonium chloride (StemCell

Technologies). Cells were resuspended in PBS + 2% FBS at a concentration of 10^6 cells per 100 μl. To analyze progenitor lineages, cells were stained for 30min at 4^0C with primary unconjugated differentiation lineage markers, washed twice with PBS, stained with secondary antibody PE-conjugated goat anti-rat IgG for 30min at 4^0C, washed three times with PBS, blocked with 10ug/ml rat IgG for 15min, then stained with conjugated progenitor markers for 30min., washed, and resuspended in PBS + 2% FBS for analysis. To analyze differentiated lineages, cells were stained for 30min at 4^0C, washed three times with PBS, and resuspended in PBS + 2% FBS. For lineage negative population, primary antibodies include rat anti-mouse Mac-1, Gr-1, CD8, CD3, B220, and TER119, and secondary antibody PE-conjugated goat anti-rat IgG (eBiosciences). For HSC, CMP, GMP, MEP populations antibodies include rat anti-mouse c-Kit-APC, Sca-1-FITC, CD34-Pacific Blue, CD16/32-PE-Cy7, CD48-PE-Cy7 (eBiosciences), and CD150-Pacific blue (Biolegend). For lineage markers, antibodies include rat anti-mouse Mac-1-FITC, Gr-1APC, B220-APC, and CD27-FITC (eBiosciences). For competition assay, cells were also simultaneously stained with Ly5.2-PerCP-Cy5.5. Corresponding rat IgG2a isotype controls were used to determine negative populations (eBiosciences). All analysis and sorting was performed using BD FACSAria II Flow Cytometer.

Statistics. All p-values were calculated using student t-test.