Supplemental Figure 1. Knock-in templates used for gene editing of the MLL gene.

(A) Schematic illustration of the knock-in templates containing the MLL fusion partners and the fluorescent marker gene NeonGreen.

(B) DNA sequence of the MLL AF9 template.

(C) DNA sequence of the MLL ENL2 cDNA.

(D) DNA sequence of the MLL ENL7 cDNA.
Supplemental Figure 2. Knock-in primary CD34<sup>+</sup> cells display survival advantage and clonal expansion in liquid culture.

(A) Growth curves chart the differences in proliferative capacity between control (template alone) and oncogene-induced cells.
(B) Representative phenotypes and morphologies are shown for control cells and oncogene-induced cells (MLL-AF9) on day 45 of liquid culture. Scale bar denotes 10 µm.
**Supplemental Figure 3.** Knock-in primary CD34\(^+\) cells display survival advantage and clonal expansion in colony-forming cell assays.

(A) Colony forming cell (CFC) assays were performed to assess the effect of oncogenes on the replating efficiency of CD34\(^+\) cells in semi-solid medium. Representative results shown for four of six independent experiments performed in triplicate. Bars represent mean number of colonies generated per 10\(^4\) seeded cells. Error bars indicate standard error of the mean (SEM). *, \(p<0.05\) was considered statistically significant.

(B) Representative morphology of secondary colonies generated by CD34\(^+\) cells nucleofected with MLL TALENs and the template (MLL-AF9) or control (template alone): very diffuse colony (left) and compact colony (right). Scale bars define 100 \(\mu\)m.
Supplemental Figure 4. Pathologic features of acute leukemias induced by genome editing of the *MLL* oncogene.

Representative cytospins of different tissues from leukemic mice and of a patient leukemia with MLL translocation show immature morphology of blast cells in comparison with control mice. Scale bars define 10 µm.
Supplemental Materials and Methods

Mice

NSG mice (purchased from the Jackson Laboratories) were housed in sterile cages, and maintained on sterilized acidified water and sterilized food containing antibiotics. Xenotransplants were performed by tail vein injection of sub-lethally irradiated (200 rad) 9-12 weeks old NSG mice. All experiments on mice in this study were performed with the approval of and in accordance with the Stanford University Administrative Panel on Laboratory Animal Care.

Flow cytometry

Analyses were performed using an LSR II flow cytometer (BD Biosciences) and data were analyzed using FlowJo (TreeStar, V10). Leukemic blasts were identified by expression of human CD45 and side scatter characteristics. For further analysis the following fluorochrome-conjugated monoclonal antibodies were used: CD34-APC (clone 4H11, eBioscience), CD38-PE/Cy7 (clone HIT2, BioLegend), CD45-PE (clone HI30, BioLegend) CD33-PerCP/Cy5.5 (clone WM53, BioLegend), CD33-brilliant violet 421 (clone WM53, BioLegend), CD14-Alexa Fluor 700 (clone HCD14, BioLegend), CD64-APC/Cy7 (clone 10.1, BioLegend), CD117-brilliant violet 421 (clone104D2, BioLegend), IgM PerCP/Cy5.5 (clone MHM88, BioLegend), CD19-Alexa Fluor 700 (clone HIB19, BioLegend), CD10-APC/Cy7 (clone HI10A, BioLegend), CD20-brilliant violet 421 (clone 2H7, BioLegend), CD15-PerCP/Cy5.5 (clone W6D3, BioLegend), CD11b (clone ICRF44, BioLegend).
Histopathology

Tissues were preserved in 10% formalin and bones in Cal-Ex II (Fisher Scientific) before being embedded in paraffin, sectioned and stained with hematoxylin and eosin by Histotec, Stanford. Stained sections were visualized using a Nikon ECLIPSE E1000 microscope and photographed with camera Nikon FDX-35. Cytospins were prepared using a Cytospin-2 centrifuge (Shandon) and stained by Wright-Giemsa (Sigma).

Western blots

200,000 cells for each sample were counted, lysed in 2X SDS-protein sample buffer and boiled for 5 minutes. WT MLL and MLL-AF9 fusion proteins were separated in 4-15% TGX gradient gel (Bio-Rad) and visualized by Western blot using anti-MLL antibody (Bethyl Laboratories, A300-086A). Anti-GAPDH antibody (SIGMA, G9545) was used for loading control. To quantify relative MLL-AF9 expression to WT MLL, band intensities were measured, normalized to GAPDH, and compared using ImageJ and Excel, respectively.

Statistical analysis

Differences in animal survival (Kaplan-Meier survival curves) were analyzed with the log-rank test. All other comparisons were performed with the Student’s t test. $p<0.05$ was considered statistically significant.