***Mice*.** Female IFN-γ knockout (GKO) mice on the BALB/c (BALB/c-*Ifngtm1Ts*, H-2^d, $K^dI^dD^d$) background and IFN-γ receptor knockout (GRKO) mice on the C57BL/6 (C57BL/6-*Ifngtm1Ts*, H-2^b, $K^bI^bD^b$) background were purchased from The Jackson Laboratory (Bar Harbor, ME). Wild-type (WT) C57BL/6 (B6), BALB/c, and BALB/c x B6 F1 (CB6F1; H-$2^{b/d}$) mice were purchased from The Jackson Laboratory or Frederick Cancer Research Facility (National Institutes of Health, Frederick, MD). Mice were used in experiments at 8 to 12 weeks of age and housed in a specific pathogen-free microisolator environment. Protocols involving animals used in this study were approved by the Massachusetts General Hospital Subcommittee of Research Animal Care.

***Development of IFN-γR-deficient T cell leukemia*.** Hematopoietic stem cell-enriched Lin^-Sca1^+ cell population was prepared from GRKO B6 mouse bone marrow cells (BMCs) and transduced with retroviruses containing cDNA encoding the intracellular domain of Notch-1 (ICN1) (MSCV-ICN1-IRES-GFP) as previously described [1; 2]. The plasmid MSCV-ICN1-IRES-GFP (kindly provided by Dr. David Scadden, Harvard University, Boston, MA) was cotransfected into the packaging cell line 293T with pCMV-VSVG and pKAT using lipofectamine 2000 (Invitrogen; Carlsbad, CA). Supernatant harvested at 48 and 72 hours after transfection was used to transduce $Lin^-Sca\text{-}1^+$ cells. Injection i.v. of the Notch-1-transduced BMCs ($4x10^5$/mouse) plus non-transduced BMCs ($2x10^5$/mouse) into lethally-irradiated (9.5Gy) syngeneic GRKO mice led to development of T cell leukemia in approximately 2-3 months. We then expanded the leukemia cells by adoptive cell transfer into multiple WT syngeneic mice, and preserved leukemia cells (i.e., bone marrow and spleen cells) in liquid nitrogen. In each experiment, we prepared two leukemia

mice by injection i.v. of the cryopreserved leukemia cells into naïve B6 mice, and harvested the spleens from these mice when leukemia had been identified (by measuring GFP+ cells in the blood and monitoring clinical appearance). The spleen cells were injected i.v. into the recipient mice for assessing GVL effects. The leukemic spleen cells used in this study contained >95% of leukemia (i.e., GFP^+) cells.

Allogeneic hematopoietic cell transplantation (allo-HCT) in freshly irradiated recipients. Recipient mice were lethally irradiated (9.25 Gy) and reconstituted within 4 to 8 hours with BMCs (7.5×10^6) and splenocytes ($7.5\text{-}20\times10^6$) from allogeneic or syngeneic donors. In some experiments, lethally-irradiated recipient mice received T cell-depleted (TCD) syngeneic BMCs plus allogeneic TCD BMCs and $CD4^+$ cell-depleted splenocytes. T cell depletion was performed by using the MACS system (Miltenyi Biotec, Auburn, CA) according to the manufacturer's instructions, and completeness of depletion (<0.2% cells of the depleted phenotype remaining) was verified by FACS in each experiment. Leukemic recipients were additionally injected i.v. with GRKO leukemia cells (ranging from $3x10^3$ to $3x10^5$ per mouse) at the time of HCT. To avoid bias from cage-related effects, animals were randomized before and after HCT. Carcasses were saved in 10% formalin after death or euthanasia for autopsy. Necropsies were performed on randomly chosen samples by observers who were unaware of which treatment group the carcasses belonged to.

Preparation of mixed allogeneic chimeras and administration of delayed donor leukocyte infusion. Mixed chimeras were prepared by injection of a mixture of $4x10^6$ T cell-depleted (TCD) syngeneic CB6F1 and $8x10^6$ TCD allogeneic WT or GKO BALB/c BMCs into lethally irradiated (9.25 Gy) CB6F1 mice. TCD BMCs were prepared by depleting CD4+ and CD8+ cells with anti-

CD4 (L3T4) and CD8a (Ly-2) microbeads using the MACS separation system. TCD was analyzed by FACS and completeness of depletion (<0.2% cells of the depleted phenotype remaining) was verified in each experiment. Donor lymphocyte infusion (DLI) was performed using spleen cells ($3.5x10^7$ to $4.5x10^7$) from WT or GKO BALB/c donors 56 days after initial TCD BMC injection, as previously described [3]. In leukemia studies, the recipient mice received intravenous injection of GRKO leukemia cells (ranging from $1x10^6$ to $1.5x10^6$ per mouse) at the time of DLI. Animals were randomized between cages before and after transplantation to avoid cage-related bias. The levels of donor chimerism in PBMCs were followed up by flow cytometric analysis, in which biotinylated anti-H-$2D^b$ mAb KH95 developed with APC streptavidin was used to detect recipient cells.

***Statistical analysis*.** Survival data are presented as Kaplan–Meier survival curves and differences between groups were analyzed by the log-rank test using GraphPad Prism (version 4; San Diego, CA). Differences between group means were tested using Student's t test by Microsoft Excel software. A probit transformation was applied to the percentage of donor chimerism using the inverse function of a standard Normal cumulative distribution. A linear mixed effects model was used to fit an overall trend in time to the transformed data, with a random intercept and slope assumed for each mouse and an unstructured covariance matrix. Comparisons were based on hypothesis tests of the fixed-effect contrasts between the DLI groups, using the F-test for inference. The statistical analysis was conducted using SAS 9.2 (SAS Inst., Cary, NC). A p value of <0.05 was considered to be significant.

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Table S1. Gross evidence for tumor at autopsy

Group (n) [a]	Death [b]	Tumor at autopsy [c]	Tumor-free survival [d]
Syn-HCT (6)	0	0	6
WT Allo-HCT (16)	6	0	10
GKO Allo-HCT (16)	16	0	0
Syn-HCT/GRKO leukemia (15)	15	15	0
WT Allo-HCT/GRKO leukemia (21)	7	0	14
GKO Allo-HCT/GRKO leukemia (21)	21	0	0

[a] HCT was performed as described in Figure S1A-C, and combined results from the 3 experiments shown in Figure S1A-C are presented.

[b] Number of mice died by 100 days after HCT

[c] Number of mice with tumor at autopsy

[d] Number of long-term (>100 days) surviving mice without tumor at autopsy.

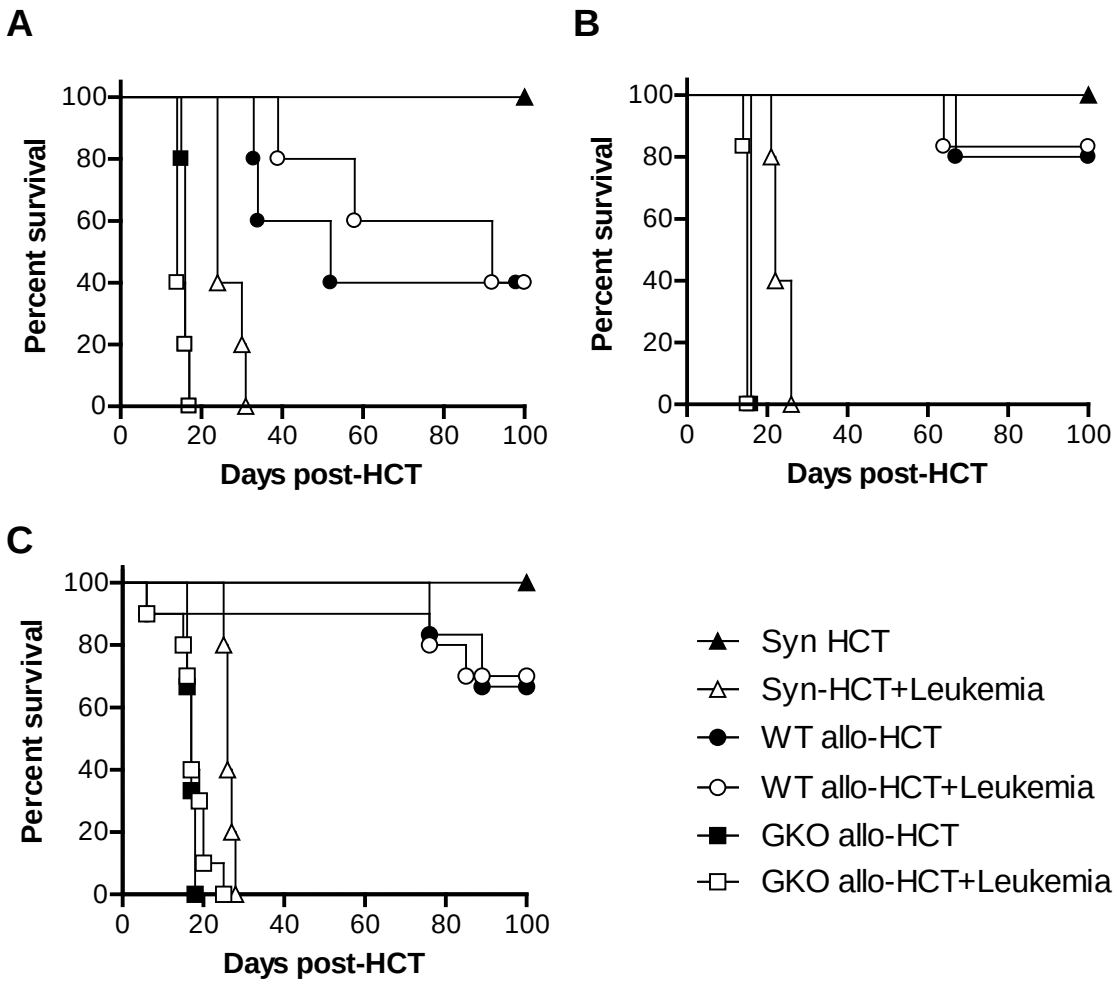

Figure S1. GVL effects against IFN-γ-unresponsive leukemia in freshly-irradiated recipients. CBF1 mice were irradiated and transplanted with $7.5x10^6$ CBF1 BMCs (Syn-HCT; ▲/△), or $7.5x10^6$ BMCs and splenocytes (*A*, $20x10^6$; *B*, $12.5x10^6$; *C*, $7.5x10^6$) from WT (WT allo-HCT; ●/○) or GKO (GKO allo-HCT; ■/□) BALB/c donors. Leukemic recipients were also injected with $2x10^4$ GRKO leukemia cells along with the HCT inoculum. Survival curves of non-leukemic (▲,●,■) and leukemic (△,○,□) recipients are shown. The numbers of mice per group in these experiments are: syn-HCT (▲), n=2, syn-HCT+leukemia (△), n=5; WT allo-HCT (●) and GKO allo-HCT (■), n=5-6; WT allo-HCT+leukemia (○) and GKO allo-HCT+leukemia (□), n=5-10.

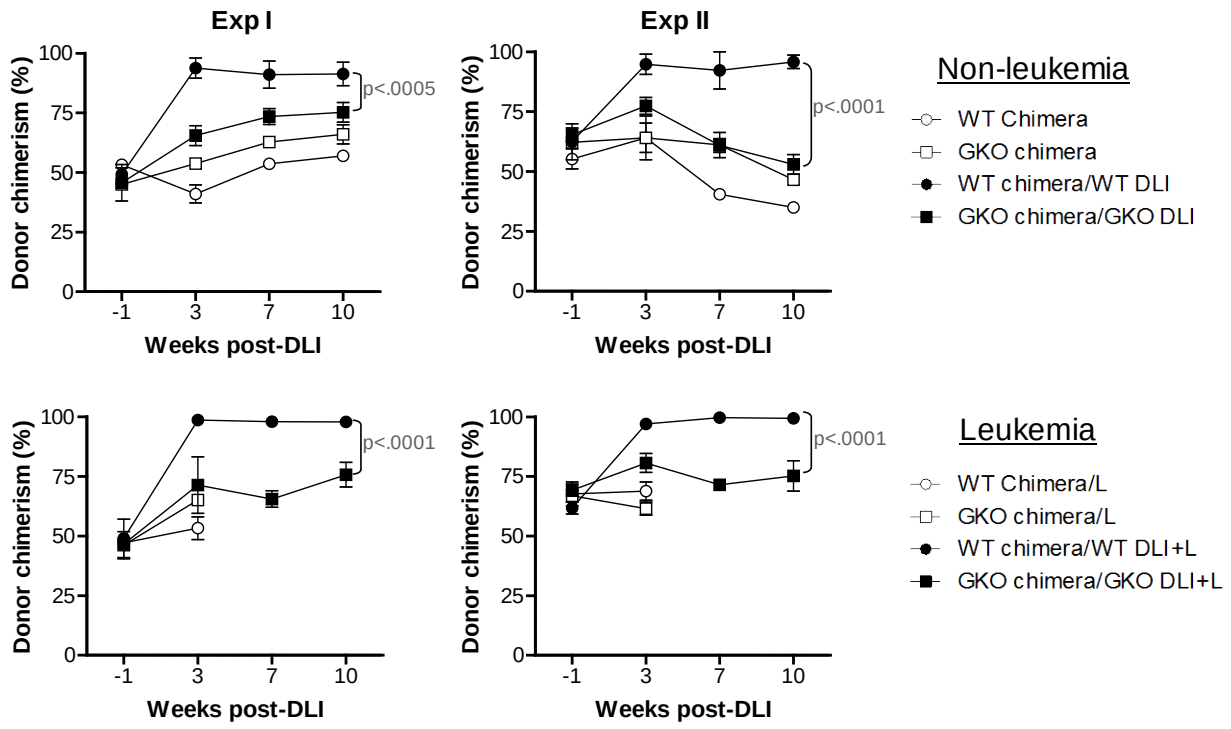

Figure S2. Lethally-irradiated CBF1 mice were reconstituted with a mixture of TCD CBF1 plus WT or GKO BALB/c mouse BMCs. Eight weeks later, these BM chimeras were administered either GRKO leukemia cells ($1x10^6$ or $1.5x10^6$ cells per mouse for Exp I and Exp II, respectively) alone or along with DLI. DLI was performed by injection of $3.5x10^7$ (Exp I) or $4.5x10^7$ (Exp II) splenocytes from WT or GKO B6 mice into the WT and GKO BM chimeras, respectively. Shown is kinetics of donor chimerism in peripheral blood of WT (○/●) and GKO (□/■) chimeras (open and solid symbols denote the chimeras that did not and did receive DLI, respectively). Non-leukemic and leukemic recipients are shown in the top and bottom panels, respectively. Because leukemic recipients that did not receive DLI (○/□) all died before 7 weeks, chimerism data are not available for these mice at weeks 7 and 10. Note: These mice are the same mice shown in Figure 2D-E.