Supplementary Figures

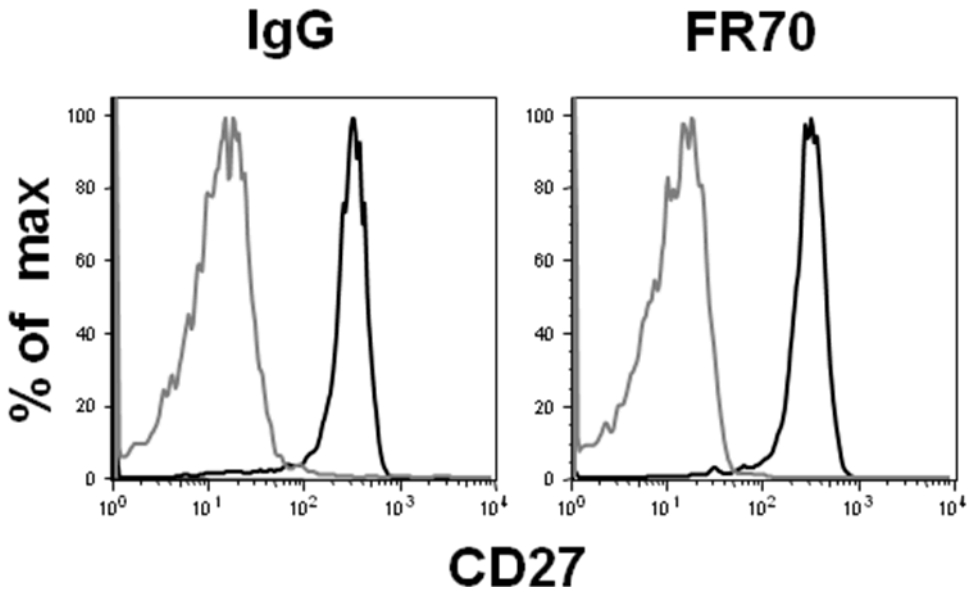


Figure S1: Expression of CD27 on HSCs of naïve BL/6 animals after FR70 treatment. Naïve BL/6 mice were either treated i.p. with 300µg IgG from rat serum or 300µg FR70. CD27 was stained on HSCs 12h after treatment. Isotype control (gray line), CD27 staining (black line).

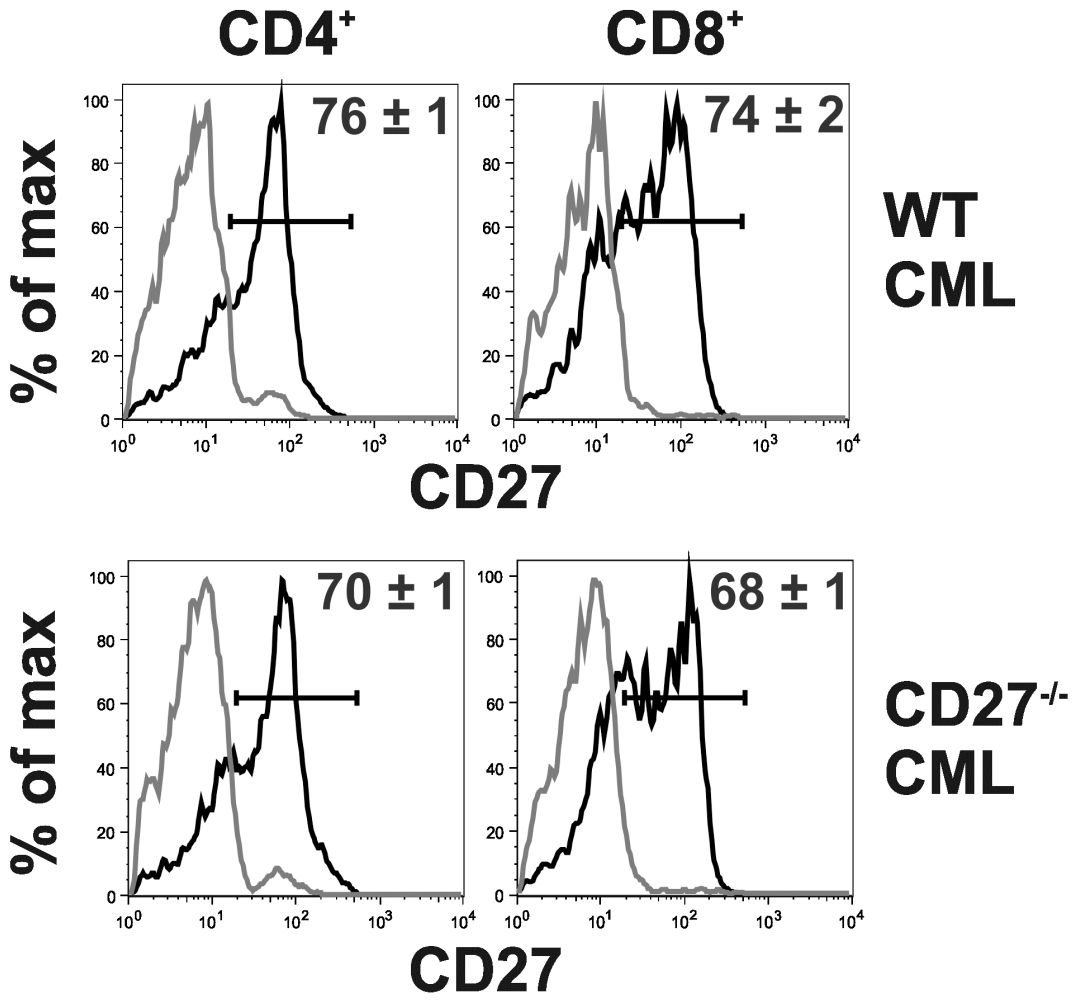


Figure S2: CD27-expression on splenic T lymphocytes in WT and CD27$^{-/-}$ CML mice. WT and CD27$^{-/-}$ CML mice were sacrificed at day 20 after transplantation. CD4^{+} and CD8^{+} T cells in the spleen were analyzed for the expression of CD27 by FACS (n=5 animals per group). Data are displayed as mean ± s.e.m. Isotype controls (gray lines), CD27 stainings (black lines).

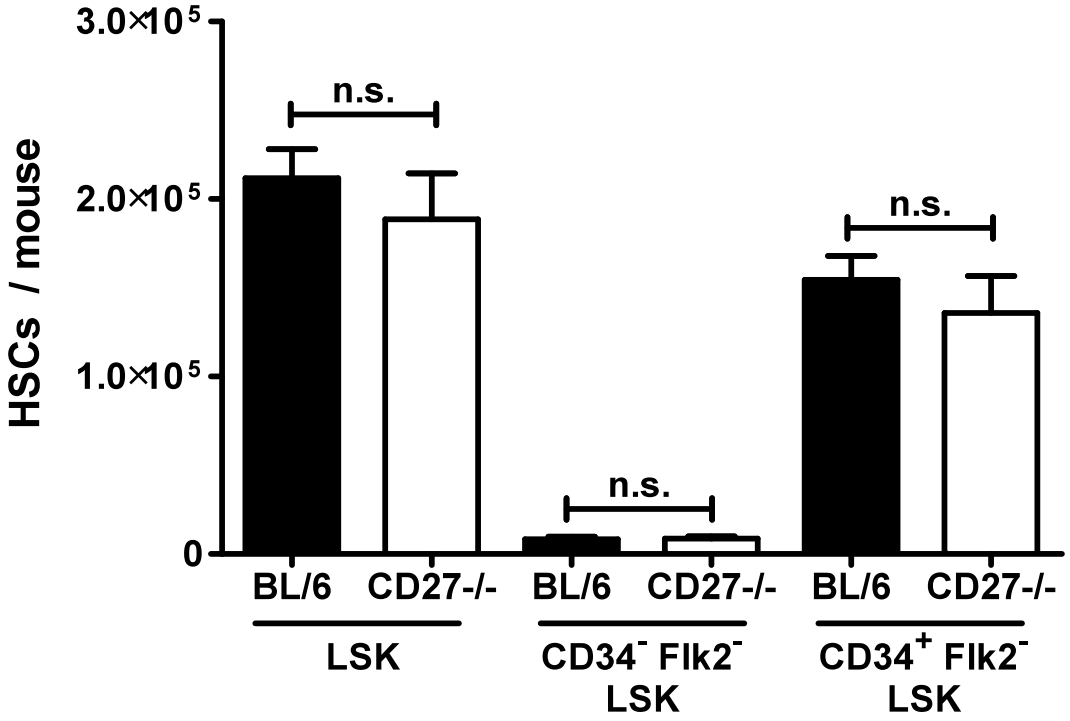
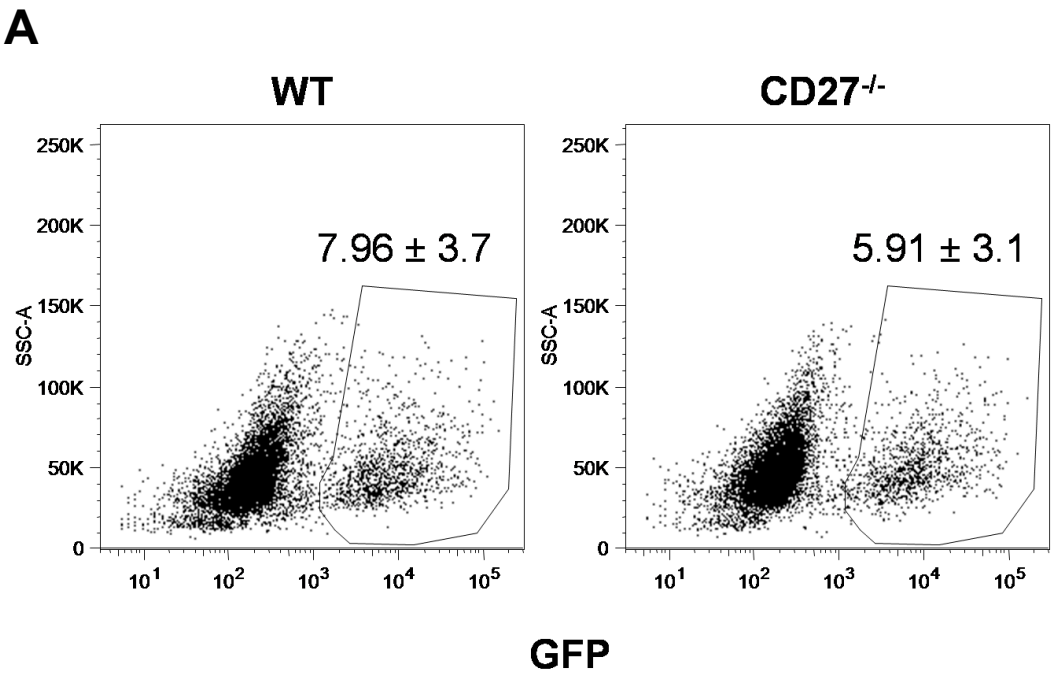


Figure S3: HSC compartments in naïve BL/6 and CD27$^{-/-}$ mice. BM cells of n=8 mice per group were lineage depleted and stained for c-kit, Sca-1, CD34 and Flk2 (CD135). Pooled data (mean ± s.e.m.) from 2 independent experiments are shown. (HSCs/mouse ≡ HSCs from both femora, tibiae and humeri). Statistics: student's t-test. $p<0.05$ was considered significant.



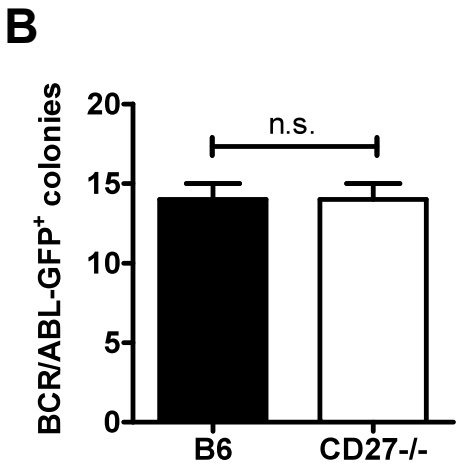


Figure S4: Retroviral transduction control. (A) Retroviral transduction rates of BL/6 and CD27$^{-/-}$ BM cells. 4x10^6 BL/6 or CD27$^{-/-}$ BM cells were transduced twice on 2 consecutive days with 1x10^5 retroviral particles. 5x10^5 transduced BM cells were incubated for 3 days in transplant medium and GFP expression was assessed by FACS. Pooled data (mean ± s.e.m.) of 5 independent experiments are shown. **(B)** Colony forming capacity of BCR/ABL-GFP transduced BL/6 and CD27$^{-/-}$ BM cells. 1x10^3 transduced BM cells were plated in methylcellulose and BCR/ABL-GFP$^+$ colonies were enumerated after 7 days. Pooled data from 2 independent experiments (mean ± s.e.m.). Statistics: student's t-test. $p<0.05$ was considered significant.

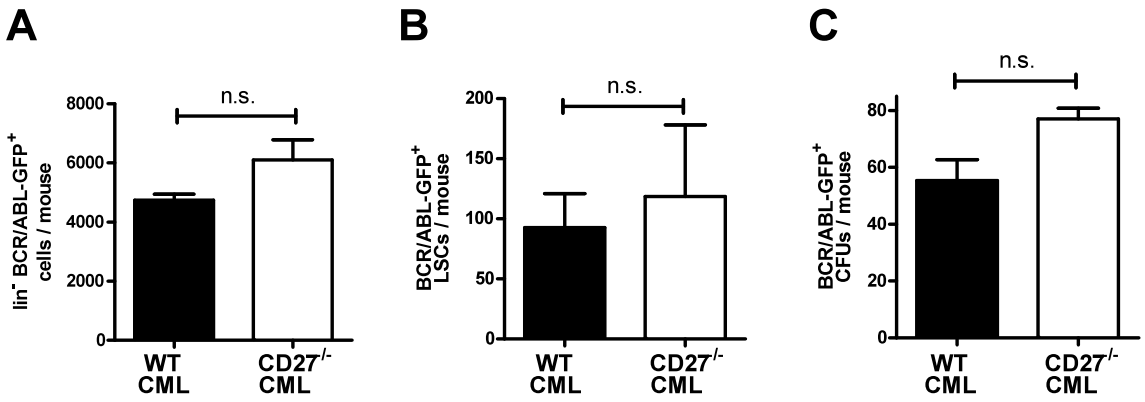


Figure S5: Homing of BCR/ABL-expressing BL/6 and CD27$^{-/-}$ BM cells to the BM. 2x10^6 transduced BL/6 or CD27$^{-/-}$ BM cells were injected i.v. into irradiated (4.5 Gy) BL/6 recipient mice. 3 days later, BM was harvested, lineage depleted and analyzed by FACS. **(A)** Lin$^-$ BCR/ABL-GFP$^+$ cell numbers per mouse and **(B)** BCR/ABL-GFP$^+$ LSC numbers per mouse. **(C)** 1x10^5 lin$^-$ cells were plated into methylcellulose, BCR/ABL-GFP$^+$ colonies were enumerated after 7 days and total BCR/ABL-GFP$^+$ CFUs per mouse were calculated. n=3 mice per group. Data are displayed as mean ± s.e.m. Statistics: student's t-test. $p<0.05$ was considered significant. CFUs: Colony forming units. (Cells; LSCs/mouse ≡ cells; LSCs from both femora, tibiae and humeri).

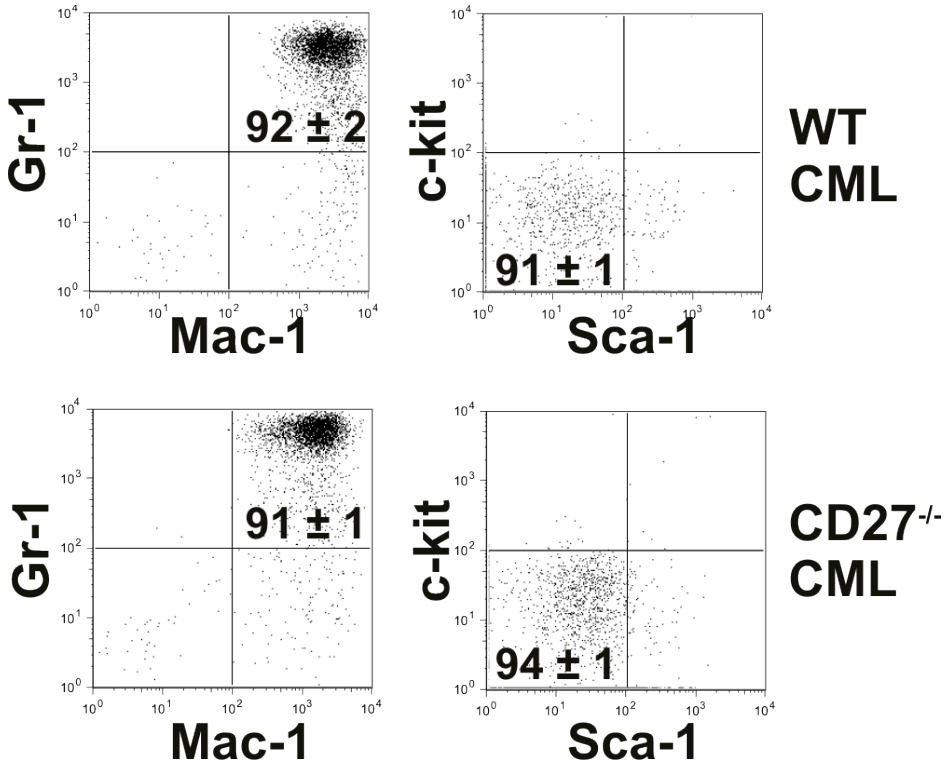


Figure S6: Maturation status of WT and CD27$^{-/-}$ BCR/ABL-GFP^{+} CML granulocytes in peripheral blood. WT and CD27$^{-/-}$ CML mice were sacrificed at day 20 after transplantation and BCR/ABL-GFP^{+} granulocytes were analyzed by FACS for the expression of Gr-1, Mac-1, c-kit and Sca-1 in peripheral blood (n=5 animals per group)**.** Data are displayed as mean ± s.e.m.

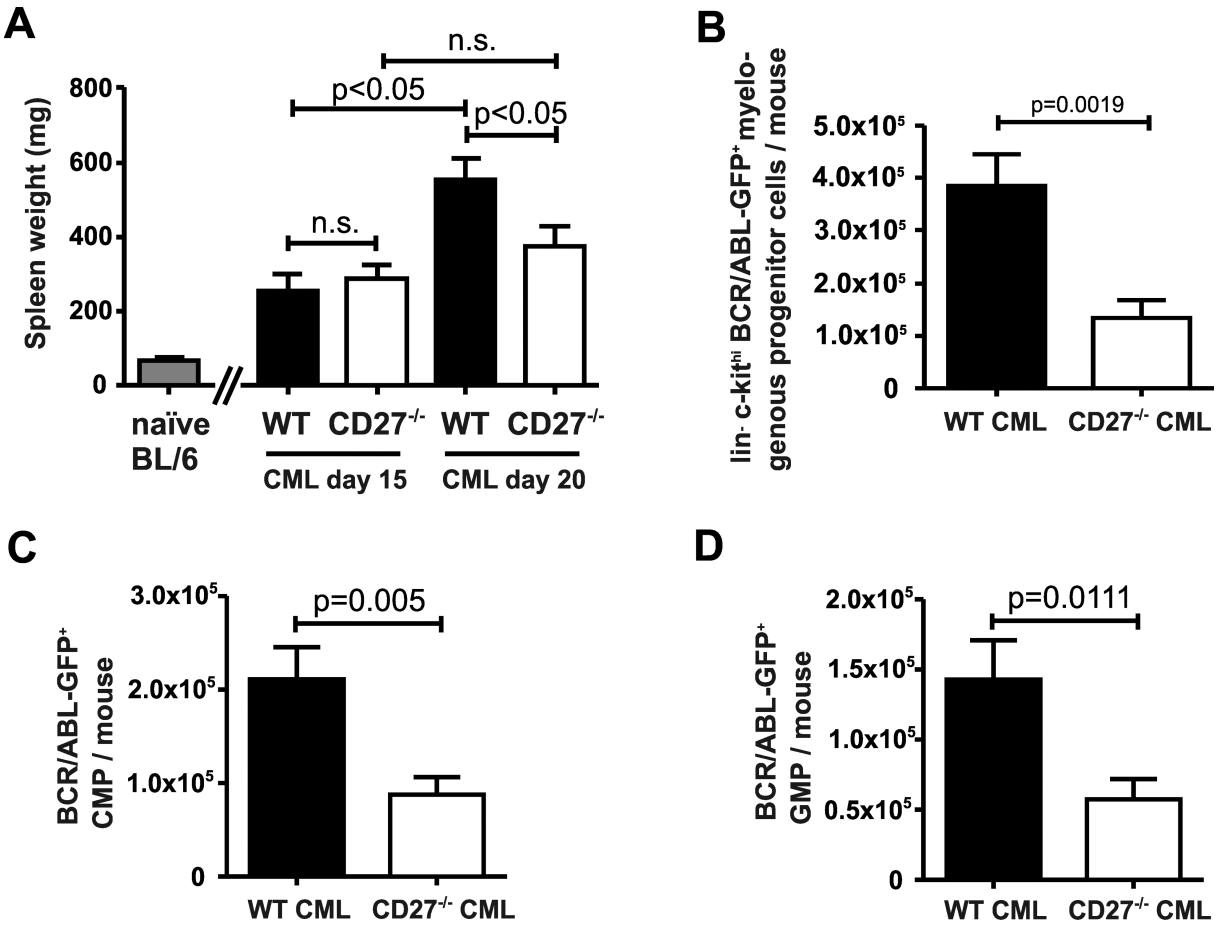


Figure S7: Analysis of spleens weights and myelogenous leukemia progenitor cells from WT CML and CD27$^{-/-}$ CML mice. (A) Spleen weights of naïve BL/6 mice (n=5), WT CML and CD27$^{-/-}$ CML mice 15 days (n=5 mice per group) and 20 days (n=15 mice per group) after transplantation. **(B)** Numbers of lin^{-}, c-kit^{hi}, BCR/ABL-GFP^{+} myelogenous progenitor cells; **(C)** BCR/ABL-GFP^{+} CMPs and **(D)** BCR/ABL-GFP^{+} GMPs per mouse 20 days after transplantation (n=15 mice per group). Data are displayed as mean ± s.e.m. Statistics: (A) one-way ANOVA, (B-D) student's t-test. $p<0.05$ was considered significant. lin^{-}, lineage negative cells; CMPs, common myeloid progenitors; GMPs, granulocyte-monocyte progenitors. (Cells/mouse = cells from both femora, tibiae and humeri).

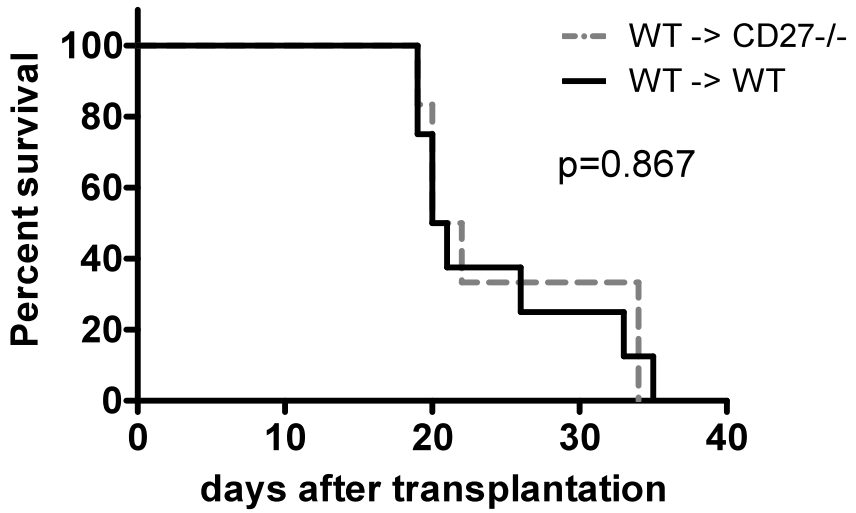


Figure S8: CML development is independent of CD70-CD27-signaling to host T cells. WT BM cells were retrovirally transduced with BCR/ABL-GFP and 10^5 cells were injected i.v. into irradiated (4.5 Gy) BL/6 (WT -> WT, n=8) or $CD27^{-/-}$ (WT -> $CD27^{-/-}$, n=6) animals. Data are displayed as Kaplan-Meier-survival curve. Statistics: log-rank test. $p<0.05$ was considered significant.

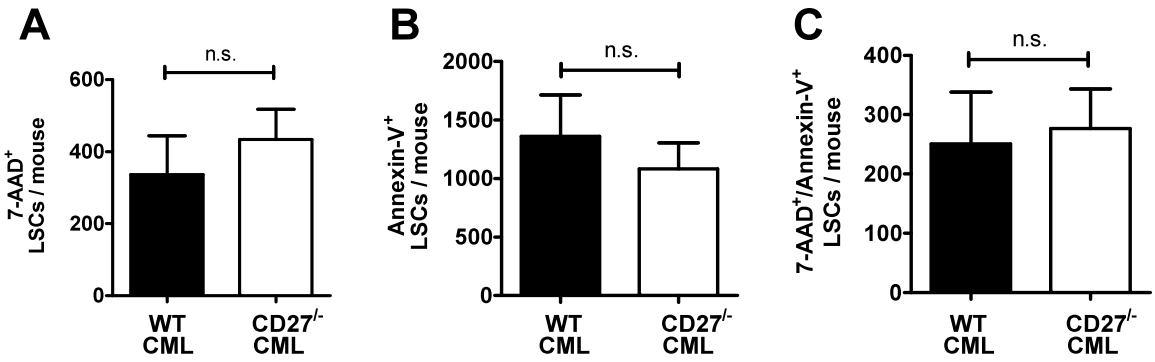


Figure S9: Necrosis and apoptosis of LSCs from WT CML and CD27$^{-/-}$ CML animals. (A) 7-AAD^{+} LSC numbers, **(B)** Annexin-V^{+} LSC numbers and **(C)** 7-AAD^{+}/Annexin-V^{+} LSC numbers per mouse 20 days after transplantation. n=5 mice per group. Data are displayed as mean ± s.e.m. Statistics: student's t-test. $p<0.05$ was considered significant. 7-AAD: 7-amino-actinomycin D. (LSCs/mouse ≡ LSCs from both femora, tibiae and humeri).

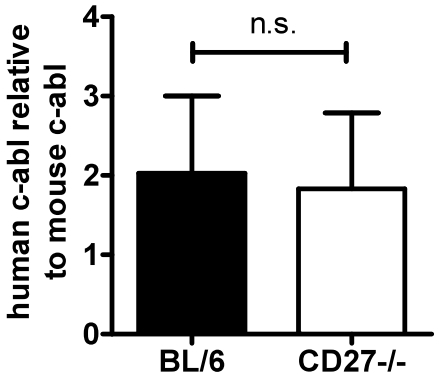


Figure S10: Genomic real-time PCR of BCR/ABL proviral integration in secondary CML. Genomic DNA was isolated from spleens of secondary WT (n= 5) and CD27$^{-/-}$ (n= 3) CML mice and analyzed by real-time PCR. $\Delta\Delta C_t$ values of human c-abl were normalized to $\Delta\Delta C_t$ values of murine c-abl. Data are displayed as mean ± s.e.m. Statistics: student's t-test. $p<0.05$ was considered significant.

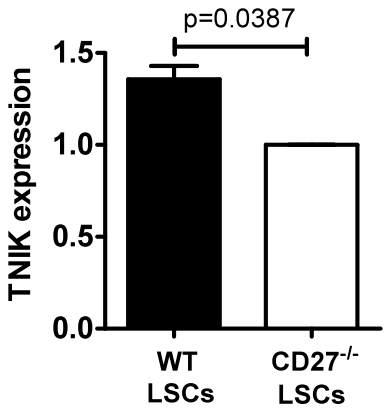


Figure S11: TNIK mRNA expression in WT and CD27$^{-/-}$ LSC. mRNA was extracted from FACS-purified LSCs from pooled WT or CD27$^{-/-}$ CML mice (n=5-7 animals/group), reversely transcribed into cDNA and analyzed by quantitative real-time RT-PCR. Pooled data from 2 independent experiments are shown. Statistics: one-sample t-test (hypothetical value: CD27$^{-/-}$ LSCs = 1.0). $p<0.05$ was considered significant.

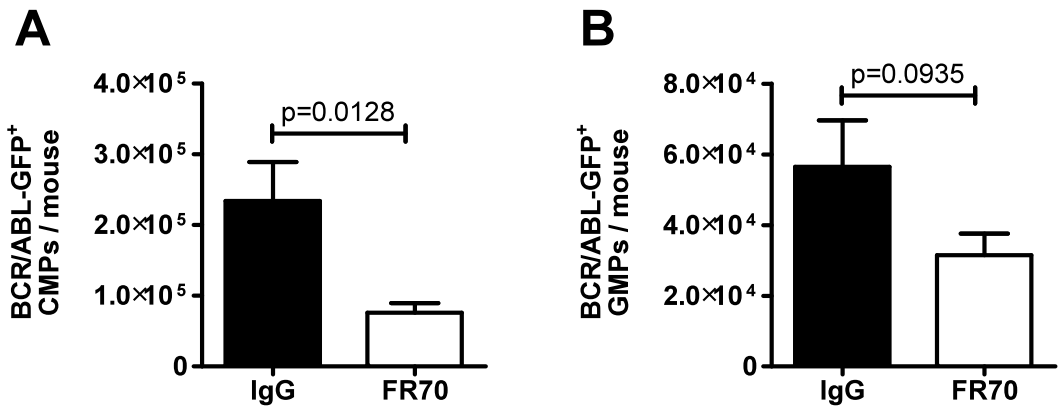


Figure S12: Analysis of myelogenous leukemia progenitor cells from WT CML mice treated with FR70 or control IgG. WT CML mice were treated i.p. every other day with 300µg FR70 or control IgG from rat serum starting at the day of transplantation. **(A)** BCR/ABL-GFP$^+$ CMPs and **(B)** BCR/ABL-GFP$^+$ GMPs per mouse 20 days after transplantation (n=18 mice per group, pooled data from 2 independent experiments). Data are displayed as mean ± s.e.m. Statistics: Student's t-test. $p<0.05$ was considered significant. CMPs, common myeloid progenitors; GMPs, granulocyte-monocyte progenitors. (Cells/mouse = cells from both femora, tibiae and humeri).

Supplementary Table

Table S1: Induced and repressed genes in LSCs from WT CML mice compared to CD27$^{-/-}$ CML mice[a].

	Fold change
Induced genes	
Runx1	+86.3
Wnt3a	+62.1
Inha	+50.7
Gata1	+39.5
Pax5	+23.0
Lef1	+19.0
Nos2	+12.3
Il2	+12.2
Vegfa	+9.9
Dll1	+4.5
Cd27	+4.2
Hdac5	+4.1
Repressed genes	
Cd3g	-109.2
Ets1	-89.1
Kdr	-34.9
Kit-ligand (Scf)	-33.9
Tek	-24.1
Blnk	-16.2
Cd14	-15.0
Trim10	-13.6
Cd8a	-13.5
Hdac9	-13.0
Cd4	-12.4
Csf2	-12.4
Il12b	-7.4
Socs5	-6.0
Ccr1	-5.6
Fut10	-5.2
Notch2	-4.95
Il25	-4.4
Mal	-4.4

[a] LSCs from WT and CD27$^{-/-}$ CML mice (n=8 animals each) were sorted and pooled. RNA was isolated and cDNA was probed on a Hematopoietic Stem Cells and Hematopoiesis RT-PCR Array. $\Delta\Delta C_t$-based fold changes represent changes from CD27$^{-/-}$ CML LSCs. Only genes that showed up-regulation or down-regulation ≥ 4-fold are shown.