**Supplementary Information**

**Supplementary Methods Table**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Anti-mouse CD3      | Thermo Fisher Scientific | Cat#13-0031-85; RRID:AB_466320 |
| Anti-mouse CD4      | Thermo Fisher Scientific | Cat#13-0041-85; RRID:AB_466326 |
| Anti-mouse CD8      | Thermo Fisher Scientific | Cat#13-0083-85; RRID:AB_657763 |
| Anti-mouse B220     | Thermo Fisher Scientific | Cat#13-0452-85; RRID:AB_466450 |
| Anti-mouse CD19     | Thermo Fisher Scientific | Cat#13-0193-85; RRID:AB_657658 |
| Anti-mouse IgM      | Thermo Fisher Scientific | Cat#13-5790-85; RRID:AB_466676 |
| Anti-mouse Gr-1     | Thermo Fisher Scientific | Cat#13-5931-85; RRID:AB_466801 |
| Anti-mouse CD11b    | Thermo Fisher Scientific | Cat#13-0112-85; RRID:AB_466360 |
| Anti-mouse NK1.1    | Thermo Fisher Scientific | Cat#13-5941-85; RRID:AB_466805 |
| Anti-mouse Ter119   | Thermo Fisher Scientific | Cat#13-5921-85; RRID:AB_466798 |
| Anti-mouse CD45.1   | Thermo Fisher Scientific | Cat#25-0453-82; RRID:AB_469629 |
| Anti-mouse CD45.2   | Thermo Fisher Scientific | Cat#11-0454-85; RRID:AB_465062 |
| Anti-mouse CD3      | Thermo Fisher Scientific | Cat#100218; RRID:AB_1595492 |
| Anti-mouse CD19     | Thermo Fisher Scientific | Cat#17-0193-82; RRID:AB_1659676 |
| Anti-mouse Ter119   | Thermo Fisher Scientific | Cat#116220; RRID:AB_528963 |
| Anti-mouse B220     | Thermo Fisher Scientific | Cat#56-0452-82; RRID:AB_891458 |
| Anti-mouse CD11b    | Thermo Fisher Scientific | Cat#47-0112-82; RRID:AB_1603193 |
| Anti-mouse Gr-1     | Thermo Fisher Scientific | Cat#12-5931-83; RRID:AB_466046 |
| Streptavidin        | BioLegend | Cat#405229 |
| Anti-mouse CD45     | Thermo Fisher Scientific | Cat#56-0451-82; RRID:AB_891454 |
| Anti-mouse CD16/32  | Thermo Fisher Scientific | Cat#56-0161-82; RRID:AB_493994 |
| Anti-mouse CD117    | Biolegend | Cat#105826; RRID:AB_1626278 |
| Anti-mouse Sca-1    | Biolegend | Cat#12-5981-82; RRID:AB_493596 |
| Anti-mouse CD48     | Thermo Fisher Scientific | Cat#17-0481-82; RRID:AB_469408 |
| Anti-mouse CD150    | BioLegend | Cat#115922; RRID:AB_2303663 |
| Anti-mouse CD150    | Thermo Fisher Scientific | Cat#12-1502-82; RRID:AB_1548765 |
| Anti-mouse CD34     | Thermo Fisher Scientific | Cat#50-0341-82; RRID:AB_10596826 |
| Anti-mouse Fli3     | Thermo Fisher Scientific | Cat# 15-1351-82; RRID:AB_494219 |
| Anti-mouse CD127    | BD Biosciences | Cat# 562419; RRID:AB_1115131 |
| Anti-mouse CD105    | Biolegend | Cat# 120411, RRID:AB_1877185 |
| Anti-mouse CD41     | BD Biosciences | Cat# 740136, RRID:AB_2739892 |
| Anti-mouse CD71     | BD Biosciences | Cat# 553266, RRID:AB_394743 |
| Anti-mouse Ter119   | Thermo Fisher Scientific | Cat# 15-5921-82, RRID:AB_468810 |
| Anti-mouse CD45.1   | BioLegend | Cat# 110727, RRID:AB_893348 |
| Anti-mouse CD45.2   | Thermo Fisher Scientific | Cat# 17-0454-82; RRID:AB_469400 |
| Anti-mouse CD45.1   | BioLegend | Cat# 110723, RRID:AB_493732 |
| Anti-Ki67           | Thermo Fisher Scientific | Cat#11-5698-80; RRID:AB_11151689 |
| Phospho-Stat5 (Tyr694) | Cell Signaling Technology (CST) | Cat # 5387 |
| Anti-human CD45     | Thermo Fisher Scientific | Cat # 47-0459-42; RRID:AB_1944368 |
| Anti-human CD34     | Thermo Fisher Scientific | Cat# 17-0349-42; RRID:AB_2016672 |
| Anti-human CD38     | Thermo Fisher Scientific | Cat# 12-0388-42; RRID:AB_1518748 |
| Anti-human CD90     | Thermo Fisher Scientific | Cat # 45-0909-42; RRID:AB_10718245 |
| **Anti-human CD45RA** | Thermo Fisher Scientific | Cat # 25-0458-42; RRID:AB_1548774 |
|----------------------|-------------------------|----------------------------------|
| **Anti-human CD117 (c-Kit)** | Thermo Fisher Scientific | Cat # 11-1178-42; RRID:AB_2572472 |

**Chemicals, Peptides, and Recombinant Proteins**

| Chemical | Supplier | Catalog Number | RRID |
|----------|----------|----------------|-------|
| Collagenase II | Sigma-Aldrich | Cat# C1764 | |
| Dispase II | Sigma-Aldrich | Cat#4942078001 | |
| DNase I | Sigma-Aldrich | Cat#D4263 | |
| BSA | Sigma-Aldrich | Cat#A9418 | |
| DAPI | Thermo Fisher Scientific | Cat#D1306; RRID:AB_2629482 | |
| Fixable Viability Dye eFluor™ 450 | Thermo Fisher Scientific | Cat#65-0863-14 | |

**Critical Commercial Assays**

| Assay | Supplier | Catalog Number | RRID |
|-------|----------|----------------|-------|
| Quantikine SCF Elisa Kit | R&D | cat# MCK00 | |
| RNeasy Micro Kit | QIAGEN | Cat#74004 | |
| Direct Lineage Cell Depletion Kit | Miltenyi Biotech | Cat#130-110-470 | |
| Nextera XT DNA Library Preparation Kit | Illumina | Cat#FC-131-1096 | |
| SMART-Seq v4 Ultra Low Input RNA Kit | Takara Bio | Cat#634891 | |

**Deposited Data**

| Data Type | Repository | Accession Number |
|-----------|------------|------------------|
| RNA Seq data | UAB Bioinformatics Core | GSE180496 | |

**Experimental Models: Organisms/Strains**

| Model | Supplier/Stock Number | Information |
|-------|----------------------|-------------|
| Mouse: SCL-tTA-BCR-ABL | Daniel Tenen, BIDMC, Boston | N/A |
| Mouse: B6.SJL-PtprcPepcb/BoyCrCrl | Charles River Laboratory | Cat#564 |
| Mouse: C57BL/6Ncr | Charles River Laboratory | Cat#556 |
| Mouse: ScfΔ/Δ (Kitltm2.1Sjm/J) | Jackson Laboratory | Stock No: 017861 |
| Mouse: Scfgef/+ (Kitltm1.1Sjm/J) | Jackson Laboratory | Stock No: 017860 |

**Oligonucleotides**

| Probe | Supplier | Catalog Number |
|-------|----------|----------------|
| mKit qPCR probe | Thermo Fisher Scientific | Cat#Mm004442972_m1 |
| mKit qPCR probe | Thermo Fisher Scientific | Cat#Mm00445212_m1 |
| mGapdh qPCR probe | Thermo Fisher Scientific | Cat#Mm99999915_g1 |

**Software and Algorithms**

| Software | Supplier | RRID |
|----------|----------|------|
| FlowJo software (version 8.5.2) | FlowJo | RRID:SCR_008520 |
| BD FACSDiva | BD Biosciences | RRID:SCR_001456 |
| GraphPad Prism 7 | GraphPad | RRID:SCR_002798 |
**Supplementary Table 1.** GSEA analysis using the C2 gene set

| CML c-Kit<sup>−/−</sup> vs c-Kit<sup>+/+</sup> (positive correlation) | NES         | FDR q-val          |
|--------------------------------------------------|------------|--------------------|
| **TNFA/NF-KB pathway**                           |            |                    |
| PID_TNF_PATHWAY                                  | 2.116324   | 0                  |
| BIOCARTA_NFKB_PATHWAY                            | 1.907848   | 0.006071145        |
| SCHEIDEREIT_IIK_INTERACTING_PROTEINS            | 1.849322   | 0.007142454        |
| BIOCARTA_TNFR2_PATHWAY                           | 1.747719   | 0.015076728        |
| REACTOME_NFKB_AND_MAP_KINASES_ACTIVATION_MEDIATED_BY_TLR4_SIGNALING_REPERTOIRE | 1.741105   | 0.0157799          |
| REACTOME_TRAF6_MEDIATED_INDUCTION_OF_NFKB_AND_MAP_KINASES_UPON_TLR7_8_OR | 1.720655   | 0.018181676        |
| ZHANG_RESPONSE_TO_IIK_INHIBITOR_AND_TNF_UP       | 1.694828   | 0.022621538        |
| **IL1/IL2/IL6 signaling**                       |            |                    |
| REACTOME_SIGNALING_BY_ILS                        | 1.929466   | 0.004898063        |
| BIOCARTA_IL22BP_PATHWAY                          | 1.886121   | 0.006695281        |
| BIOCARTA_IL1R_PATHWAY                            | 1.876359   | 0.006694058        |
| PID_IL2_1PATHWAY                                 | 1.872696   | 0.006694001        |
| REACTOME_IL_3_5_AND_GM_CSF_SIGNALING             | 1.863667   | 0.007030382        |
| BYSTROEM_CORRELATED_WITH_ILS_DN                  | 1.86354    | 0.006870613        |
| PID_IL6_7_PATHWAY                                | 1.826958   | 0.008023258        |
| PID_IL1_PATHWAY                                  | 1.792247   | 0.010340108        |
| SIG_IL4RECEPTOR_IN_B_LYPHOCYTES                  | 1.773158   | 0.011945327        |
| REACTOME_IL1_SIGNALING                           | 1.72922    | 0.016938413        |
| ST_INTERLEUKIN_4_PATHWAY                         | 1.717131   | 0.018580658        |
| BIOCARTA_IL2_PATHWAY                             | 1.709963   | 0.019836994        |
| BIOCARTA_IL6_PATHWAY                             | 1.708532   | 0.020122023        |
| WIERENGA_STATSA_TARGETS_DN                       | 1.604818   | 0.04359974         |
| **WNT signaling**                                |            |                    |
| BIOCARTA_WNT_PATHWAY                             | 1.84486    | 0.006766611        |
| PID_WNT_CANONICAL_PATHWAY                        | 1.844276   | 0.006712065        |
| KEGG_WNT_SIGNALING_PATHWAY                       | 1.655055   | 0.031312972        |
| ST_WNT_BETA_CATENIN_PATHWAY                      | 1.601411   | 0.044222824        |
### CML c-Kit\textsuperscript{low} vs c-Kit\textsuperscript{high} (negative correlation)

| Pathway                                             | NES    | FDR q-val |
|-----------------------------------------------------|--------|-----------|
| **Oxidative Phosphorylation**                       |        |           |
| MOOTHA_VOXPHOS                                      | -2.865 | 0         |
| KEGG_OXIDATIVE_PHOSPHORYLATION                      | -2.830 | 0         |
| REACTOME_TCA_CYCLE_AND_RESPIRATORY_ELECTRON_TRANSPORT | -2.330 | 0         |
| MOOTHA_MITOCHONDRIA                                 | -1.825 | 0.013205438 |
| **Cell proliferation**                              |        |           |
| REACTOME_REGULATION_OF_MITOTIC_CELL_CYCLE           | -2.241 | 2.14E-05  |
| REACTOME_CYCLIN_E_ASSOCIATED_EVENTS DURING_G1_S_TRANSITION | -2.236 | 4.35E-05  |
| GRAHAM_CML_DIVIDING_VS_NORMALQUIESCENT_UP           | -1.909 | 0.006530864 |
| ZHOU_CELL_CYCLE_GENES_IN_IR_RESPONSE_24HR           | -1.882 | 0.008336465 |
| REACTOME_S_PHASE                                    | -1.856 | 0.010300171 |
| REACTOME_M_G1_TRANSITION                            | -1.798 | 0.016241971 |
| REACTOME_G1_S_TRANSITION                            | -1.790 | 0.017485164 |
| GRAHAM_NORMALQUIESCENT_VSNORMALDIVIDINGDN           | -1.765 | 0.020875871 |
| REACTOME_DNA_REPLICATION                             | -1.750 | 0.023024708 |
| REACTOME_CELL_CYCLE_CHECKPOINTS                     | -1.732 | 0.025807915 |
| REACTOME_MITOTIC_M_M_G1_PHASES                      | -1.703 | 0.031561214 |
| REACTOME_CELL_CYCLE                                 | -1.702 | 0.031628624 |
| TANG_SENESCENCE_TP53_TARGETS_DN                     | -1.692 | 0.034107182 |
| ISHIDA_E2F_TARGETS                                  | -1.692 | 0.03388545 |
| REACTOME_CELL_CYCLE_MITOTIC                          | -1.651 | 0.043265298 |
| KONG_E2F3_TARGETS                                   | -1.640 | 0.04547825 |
| WHITFIELD_CELL_CYCLE_LITERATURE                     | -1.587 | 0.060423955 |
| CML c-Kit<sup>low</sup> vs Normal c-Kit<sup>low</sup> (positive correlation) | NES       | FDR q-val |
|---------------------------------------------------------------|----------|-----------|
| **Cell cycle**                                                |          |           |
| EGUCHI_CELL_CYCLE_RB1_TARGETS                                | 2.041842 | 0.00312   |
| PID_E2F_PATHWAY                                               | 2.00988  | 0.00378   |
| KONG_E2F3_TARGETS                                            | 1.999559 | 0.004074  |
| CHANG_CYCLING_GENES                                          | 1.953056 | 0.006972  |
| ROSTY_CERVICAL_CANCER_PROLIFERATION_CLUSTER                   | 1.870724 | 0.016825  |
| REICHERT_MUTATION_LIN9_TARGETS                                | 1.832064 | 0.022796  |
| WHITFIELD_CELL_CYCLE_LITERATURE                               | 1.809162 | 0.02946   |
| TANG_SENESCENCE_TP53_TARGETS_DN                               | 1.716452 | 0.055583  |
| ISHIDA_E2F_TARGETS                                           | 1.710974 | 0.06271   |
| FISCHER_G2_M CELL_CYCLE                                      | 1.671032 | 0.068208  |
| SCIAN_CELL_CYCLE_TARGETS_OF_TP53_AND_TP73_DN                  | 1.642611 | 0.077356  |
| REN_BOUND_BY_E2F                                             | 1.635987 | 0.076771  |
| **Inflammation**                                             |          |           |
| BIOCARTA_IL1R_PATHWAY                                         | 1.877218 | 0.016093  |
| MANTOVANI_NFKB_TARGETS_UP                                     | 1.850237 | 0.020109  |
| PHONG_TNF_TARGETS_UP                                         | 1.800883 | 0.032767  |
| GALINDO_IMMUNE_RESPONSE_TO.ENTEROTOXIN                       | 1.776825 | 0.038194  |
| BIOCARTA_TNFR1_PATHWAY                                        | 1.765925 | 0.03964   |
| SCHÖNEN_NFKB_SIGNALING                                       | 1.719057 | 0.055504  |
| CROONQUIST_IL6_DEPRIVATION_DN                                | 1.717999 | 0.055546  |
| DASU_IL6_SIGNALING_SCAR_UP                                    | 1.706593 | 0.057743  |
| BIOCARTA_NFKB_PATHWAY                                         | 1.687181 | 0.06174   |
| MAHAJAN_RESPONSE_TO_IL1A_UP                                   | 1.664432 | 0.070661  |
| TIAN_TNF_SIGNALING_NOT_VIA_NFKB                               | 1.654689 | 0.077293  |
| ZHOU_INFLAMMATORY_RESPONSE_FIMA_UP                            | 1.636054 | 0.077534  |

| CML c-Kit<sup>low</sup> vs Normal c-Kit<sup>low</sup> (negative correlation) | NES       | FDR q-val |
|--------------------------------------------------------------------------|----------|-----------|
| **Oxidative phosphorylation**                                             |          |           |
| MOOTHA_VOXPHOS                                                            | -2.6092  | 0         |
| WONG_MITOCHONDRIA_GENE_MODULE                                             | -2.56294 | 0         |
| REACTOME_TCA_CYCLE_AND_RESPIRATORY_ELECTRON_TRANSPORT                    | -2.47558 | 0         |
| KEGG_OXIDATIVE_PHOSPHORYLATION                                           | -2.32705 | 7.89E-05  |
| MOOTHA_MITOCHONDRIA                                                      | -1.94712 | 0.006495  |
| MOOTHA_PGC                                                               | -1.55193 | 0.134756  |
| **DNA Damage**                                                            |          |           |
| REACTOME_P53_INDEPENDENT_G1_S_DNA_DAMAGE_CHECKPOINT                       | -2.22717 | 2.27E-04  |
| REACTOME_P53_DEPENDENT_G1_DNA_DAMAGE_RESPONSE                             | -2.19736 | 2.28E-04  |
| **Interferon**                                                            |          |           |
| ZHANG_INTERFERON_RESPONSE                                                 | -2.18809 | 2.82E-04  |
| REACTOME_INTERFERON_ALPHA_BETA_SIGNALING                                  | -1.99925 | 0.003644  |
| RADAeva_RESPONSE_TO_IFNA1_UP                                              | -1.8887  | 0.012022  |
| BROWNE_INTERFERON_RESPONNSIVE_GENES                                      | -1.88273 | 0.012581  |
| DER_IFN_ALPHA_RESPONSE_UP                                                | -1.79038 | 0.029329  |
| MOSERLE_IFNA_RESPONSE                                                    | -1.76144 | 0.036749  |
| REACTOME_INTERFERON_SIGNALING                                            | -1.61588 | 0.093382  |
| BOSCO_INTERFERON_INDUCED_ANTIVIRAL_MODULE                                 | -1.57393 | 0.1226    |
| DER_IFN_GAMMA_RESPONSE_UP                                                | -1.48814 | 0.170784  |
| SANA_RESPONSE_TO_IFNG_UP                                                 | -1.46686 | 0.186366  |
Supplementary Figure 1. C-Kit\textsuperscript{Low} LT-HSCs are increased in CML compared to normal bone marrow. (Related to Figure 1). Peripheral blood WBC [A], bone marrow cellularity [B] and spleen size [C] in normal control and CML-induced mice. Frequency of donor LT-HSCs of normal control and CML-induced mice [D]. Expression of c-Kit mRNA measured by Q-PCR (2-ΔΔcT values) in normal and CML c-Kit\textsuperscript{Low} and c-Kit\textsuperscript{High} LT-HSC. Data normalized to GAPDH (n=3) [E]. MFI of cell surface and internal c-Kit levels in normal and CML LT-HSCs [F]. Histograms of internal and external c-Kit expression from concatenated normal and CML LT-HSCs stratified as c-Kit\textsuperscript{Low} and c-Kit\textsuperscript{High} LT-HSCs (n=3 mice) [G]. Data represented as mean ± SEM, \*p<0.05; **p<0.01, ***p<0.001.
Supplementary Figure 2. CML c-Kit\textsuperscript{low} LT-HSC exhibit gene signatures characteristic of primitive drug resistant leukemia stem cells (Related to Figure 2) Normalized enrichment scores (NES) of Hallmark gene sets significantly enriched (FDR<0.05) in Normal c-Kit\textsuperscript{low} LT-HSCs compared to Normal c-Kit\textsuperscript{high} LT-HSCs [A]. Heatmap showing inflammatory gene expression in Normal and CML c-Kit\textsuperscript{low} LT-HSCs and c-Kit\textsuperscript{high} LT-HSCs [B].
Supplementary Figure 3. Differential response of Normal and CML LT-HSC to SCF. (Related to Figure 4)
A. Gating strategy of cultured mouse stem/progenitor cells.
Supplementary Figure 4. Deletion of SCF alters c-Kit\textsuperscript{low} LT-HSC numbers in vivo. (Related to Figure 6) ELISA for SCF protein in BM lysates [A], and q-RT-PCR for SCF mRNA expression normalized to GAPDH (2-ΔΔCT values) in whole murine bone marrow cells, from normal and SCL-tTA-BCR-ABL mice (n=4) [B]. Genomic PCR for Scf excision [C] and q-RT-PCR for Scf mRNA expression in BM cells [D] from cre- and cre+ Scf\textsuperscript{fl/fl}/Ubc-cre mice. Total number of bone marrow cells [E], LT-HSC [F], ST-HSCs [G], MPP2 [H], MPP3 [I], GMP [J], and MEP [K] cells in cre- (n=3-6) and cre+ (n=3-6) Scf\textsuperscript{fl/fl}/Ubc-cre mice. Total number of donor bone marrow MPP3 [L], and GMP [M], and donor spleen LT-HSCs [N], MPP3 [O], and GMP [P], 14 weeks post transplantation of CML bone marrow cells into lethally irradiated cre- (n=3-6) and cre+ (n=8-11) Scf\textsuperscript{fl/fl}/Ubc-cre mice. Data represented as mean ± SEM, *p<0.05; **p<0.01, ***p<0.001, ****p<0.0001
Supplementary Figure 5. Effect of TKI-treatment on murine leukemic LT-HSC (Related to Figure 7)
Peripheral blood WBC [A] and bone marrow cellularity [B] in CML mice with and without nilotinib (TKI)-treatment. The dotted line represents the upper limit of normal range. Frequency of CML LT-HSC [C], and number of CML LT-HSC [D] in CML mice with and without nilotinib (TKI) treatment. CML-induced mice were treated with SCF, Nilotinib, or Combo for 14 days. Percentage of Neutrophils [E] in the blood post treatment. Spleen size [F], and total number donor CML spleen LSK [G], LT-HSC [H], ST-HSC, [I], MPP3, [J] and GMP[K]. Total number of normal donor bone marrow LT-HSC [L], ST-HSCs [M], MPP3 [N], GMP [O], of control (CRTL), SCF-treated (SCF), Nilotinib-treated (NIL) or SCF and Nilotinib-treated (COMBO) mice. n=6-7. Data represented as mean ± SEM, *p<0.05, **p<0.01.