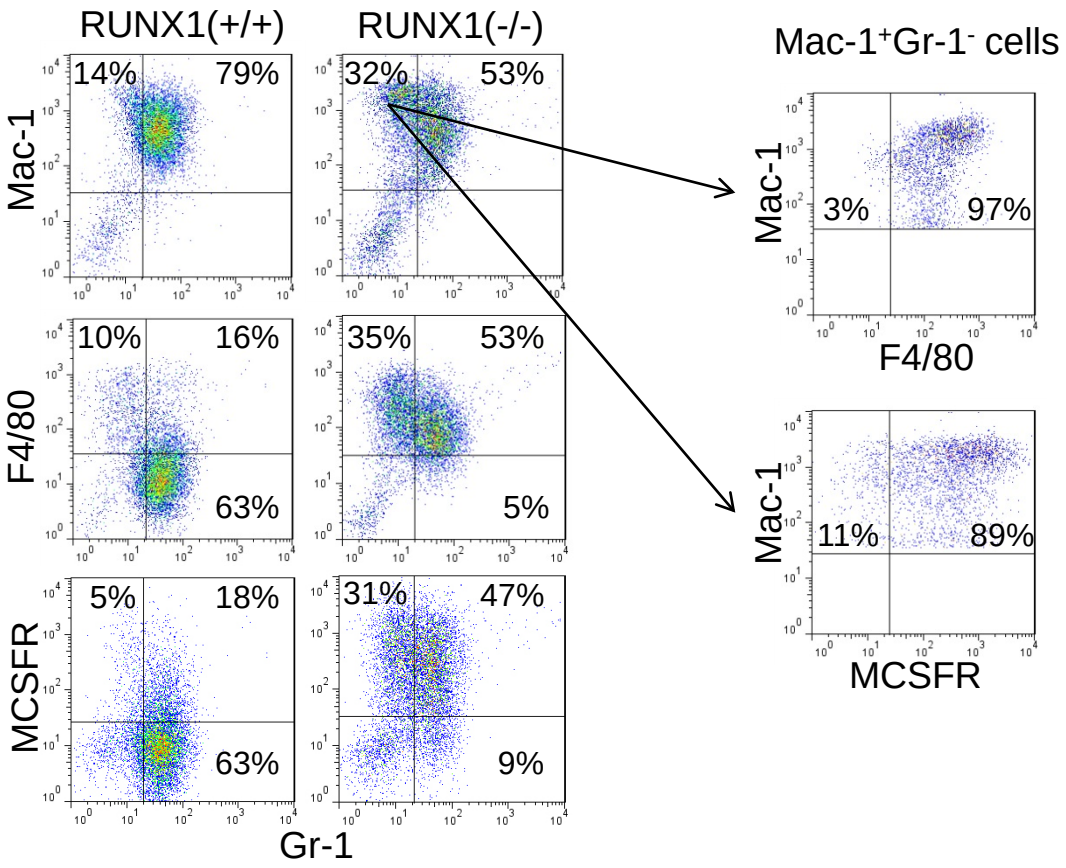

Figure S1. CFUs derived from wild-type or RUNX1-deleted marrow cells were stained with FITC-anti-Gr-1, PerCP-Cy5.5-anti-Mac-1, PE-anti-F4/80, biotin-anti-CD115/MCSFR, and streptavidin-APC. The left two columns depict Mac-1;Gr-1, F4/80;Gr-1, and MCSFR;Gr-1 analyses. In response to RUNX1-deletion, the large majority of cells are F4/80$^+$ and MCSFR$^+$. The right two panels were obtained by gating on the Mac-1$^+$Gr-1$^-$ cells within the RUNX1(-/-) cell population. The large majority of these are F4/80$^+$ and MCSFR$^+$, consistent with their monocyte/macrophage morphology and absence of immature granulocytic cells evident upon Wright-Giemsa staining of the RUNX1(-/-) CFU population.

Guo et al,
Fig. S2

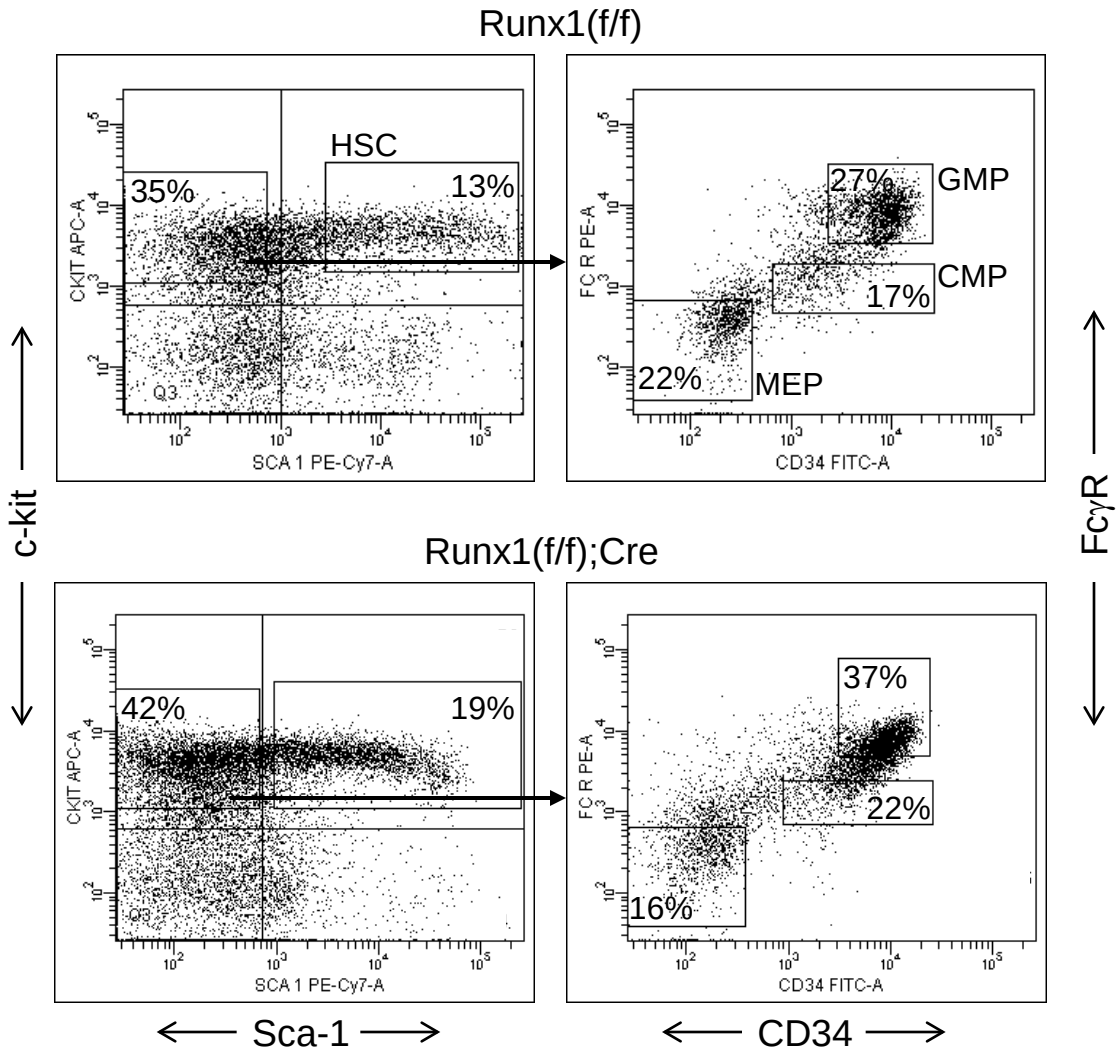

Figure S2. Marrow mononuclear cells were lineage-depleted and then stained for Sca-1, c-kit, CD34, and FcγR and subjected to FACS analysis and cell sorting. Left panels indicate HSC and progenitors (left box) that were further gated in the right panels for CMP, GMP, and MEP. Note increased HSC, CMP, and GMP and reduced MEP upon *Runx1* gene deletion.

Guo et al,
Fig. S3

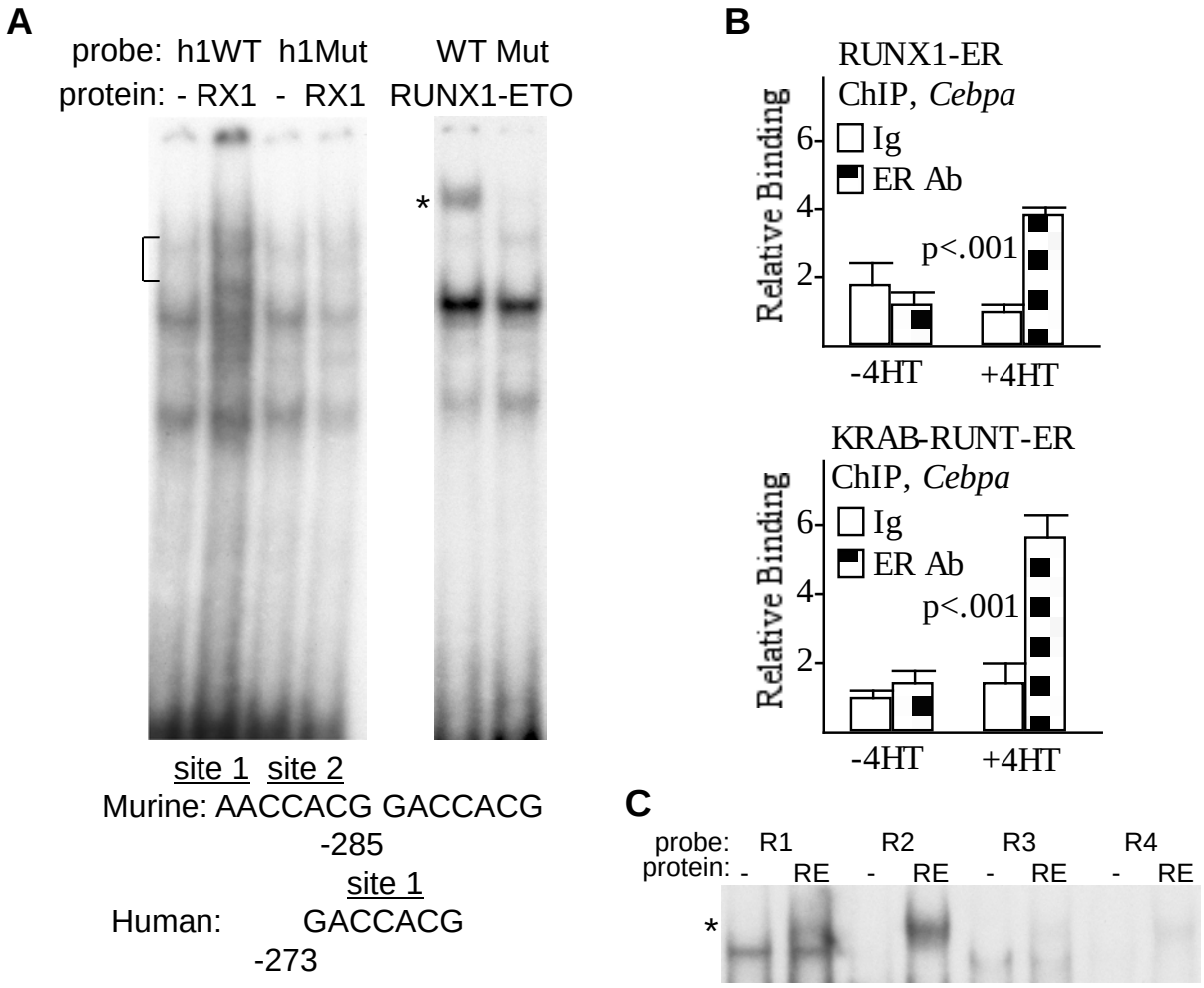

Figure S3. (A) Gel shift assay was conducted using radio-labeled probes from the human *CEBPA* promoter containing a Runx1 consensus binding site (h1WT), or with a mutant version of h1WT (h1Mut) and nuclear extracts from 293T cells transfected with empty CMV vector (-) or with CMV-CBFβ and CMV-RUNX1c (RX1). In h1Mut, GACCACG is changed to GTGCACG. Bracket denotes specific Runx1 gel shift complexes (left). Gel shift was also conducted using these two probes and an extract expressing RUNX1-ETO; * denotes the specific complex (right). Below are the two murine *Cebpa* promoter Runx1 consensus sites and the homologous site in the human promoter. (B) ChIP assays for promoter binding were conducted using ERα rabbit antiserum or rabbit IgG with 32Dcl3-RUNX1-ER (top) or with 32Dcl3-KRAB-RUNT-ER cells (bottom) cultured +/- 4HT for 24 hrs (mean and SE from 3 determinations). Binding was normalized to input in each sample. (C) Gel shift using radio-labelled probes R1, R2, R3, or R4 from the +37 kb *Cebpa* enhancer and nuclear extracts from 293T cells transfected with empty CMV vector (-) or with CMV-RUNX1-ETO (RE). *denotes the specific RUNX1-ETO DNA-binding complex.

Guo et al,
Figure S4

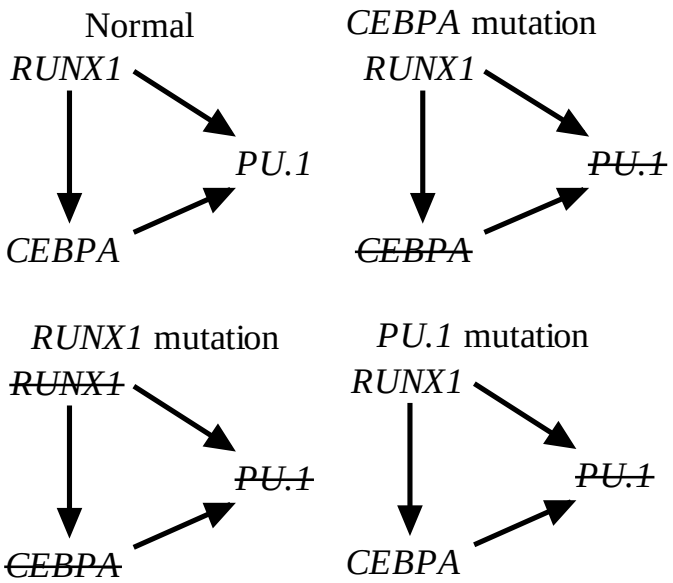

Figure S4. RUNX1 activates the *CEBPA* and *PU.*1 genes. *RUNX1* gene mutation, present in approximately 30% of AML cases, is predicted to reduce C/EBPα and PU.1 as well as RUNX1 expression. *CEBPA* mutation, present in about 10% of AML cases, is predicted to reduce C/EBPα and PU.1 expression. *PU.1* mutations, which occur only rarely in AML cases, may only reduce PU.1 expression.