



Supplementary Fig. S1

**Supplementary Figure S1. IL3R $\alpha$ / $\beta$ c transcript and protein expression ratio in AML patient samples.** **A**, IL3R $\alpha$ / $\beta$ c transcript ratio in AML patient samples (Beat AML cohort) after stratification based on top and bottom 10% and 25% of IL3R $\alpha$  (left panel) and  $\beta$ c (right panel) expression. **B**, Kaplan-Meier overall survival curve for normal karyotype AML patients comparing those with higher or lower than 1.1 ratio of IL3R $\alpha$ / $\beta$ c gene expression in the Beat AML cohort (n=102) (26). **C**, Multivariate meta-analysis of patient survival combining five patient cohorts (25-28). **D, E**, Kaplan-Meier overall survival curve for AML patients (TCGA) (25), comparing those with higher or lower than 1.12 ratio of *CSF2RA/CSF2RB* (**D**) and 3.5 ratio of *IL6R/IL6ST* (**E**). Ratio cut-offs were determined based on the highest hazard ratio for survival from the log-rank statistical analysis considering all possible ratio cut-offs. **F**, IL3R $\alpha$ / $\beta$ c gene expression ratio in leukemic cells from *de novo* AML patients carrying different driver mutations (mutations found in  $\geq 5$  patients shown) from Beat AML (26) and TCGA (25) cohorts. **G**, GSEA plots showing enrichment of hematopoietic and leukemic stem and progenitor cell gene sets in differentially expressed genes between AML patients with high vs. low IL3R $\alpha$ / $\beta$ c transcript ratio. **H, I** Flow cytometric analysis (**H**) and quantification (**I**) for sub-fractionation into 4 populations based on cell surface IL3R $\alpha$  and  $\beta$ c expression profiles (%IL3R $\alpha$ / $\beta$ c) in high (n=5, blue) and low (n=5, red) IL3R $\alpha$ / $\beta$ c ratio (as determined by RNA-seq) AML samples (CD3-CD19-CD45+) in the Toronto cohort. CB mononuclear cells (n=3) and mobilized peripheral blood (mPB) MNC (n=1) served as controls (CTRLS). **J**, Flow cytometric analysis for %IL3R $\alpha$ <sup>hi</sup> $\beta$ c<sup>lo</sup> population of high (n=5, blue) and low (n=6, red) IL3R $\alpha$ / $\beta$ c transcript ratio AML samples in the Adelaide cohort based on cell surface IL3R $\alpha$  and  $\beta$ c protein expression profiles. **K**, IL3R $\alpha$ / $\beta$ c cell surface ratio in high (n=5, blue) and low (n=6, red) IL3R $\alpha$ / $\beta$ c transcript ratio AML samples in the Toronto cohort based on cell surface IL3R $\alpha$  and  $\beta$ c protein expression profiles. **L**, IL3R $\alpha$ / $\beta$ c cell surface ratio in CD34+ and CD34- subpopulations from high (n=4) and low (n=5) IL3R $\alpha$ / $\beta$ c transcript ratio patient samples in the

Adelaide cohort.  $\Delta$ MFI: stained minus unstained median fluorescence intensity. **M**, IL3R $\alpha$ / $\beta$ c cell surface ratio in CD34<sup>+</sup> and CD34<sup>-</sup> subpopulations from high IL3R $\alpha$ / $\beta$ c transcript ratio patient samples (n=3) in the Toronto cohort.  $\Delta$ MFI as for **(L)**. **N-P**, Correlation of relative abundance of cells with quiescent **(N)** and primed **(O)** LSPC and monocyte-like **(P)** transcriptional phenotypes with %IL3R $\alpha$ <sup>hi</sup> $\beta$ c<sup>lo</sup> population in CD3-CD19-CD45<sup>+</sup> cells with high and low IL3R $\alpha$ / $\beta$ c ratio (by RNA-seq) in Toronto cohort by Pearson analysis. **Q**, Engraftment data (CD45<sup>+</sup>CD33<sup>+</sup>) for limiting dilution assays xenotransplanting high/med/low IL3R $\alpha$ / $\beta$ c ratio fractions from AML#140005 and AML#130578 into NSG-SGM3 and NSG mice at the indicated cell doses is shown for injected and non-injected femora at 7-8 weeks. Positive engraftment was scored at >0.1% CD45<sup>+</sup>CD33<sup>+</sup> and is indicated by a solid horizontal line. At high cell doses sample AML#140005 engrafted aggressively and either lead to mortality or required euthanization between 4-7 weeks post-transplantation and those cases are represented by open circles. Due to low cell numbers retrieved the low ratio fraction of AML#130578 was only transplanted into NSG-SGM3.