LETTER TO THE EDITOR

TNF-α signaling drives myeloid skewing and clonal expansion of stem and progenitor cells in *RUNX1*-familial platelet disorder

Authors

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SUPPLEMENTAL INFORMATION

Supplemental Figures

- Figure S1: TNFR2 (TNFRSF1B) is upregulated in *RUNX1*-mutated AML samples.
- Figure S2: Etanercept treatment can reverse *RUNX1*-FPD phenotype without causing toxicity.
- Table S1: Information on FPD patient samples and HD controls used in the manuscript.

Supplemental Figure 1

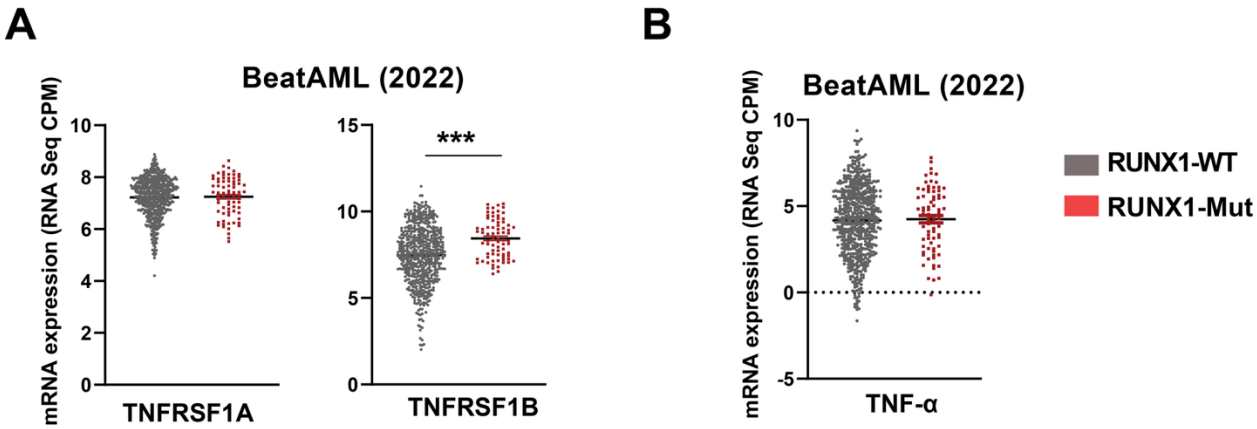

Figure S1: TNFR2 (TNFRSF1B) is upregulated in *RUNX1*-mutated AML samples. Using BeatAML databases, the expression of TNF-α and its receptors was evaluated among patient samples with (n=75) and without (n=560) *RUNX1* mutations using RNAseq data analysis. Panel **A** is the TNF-α receptors data from BeatAML 2022, and panel **B** is TNF-α expression. *P*-value significance was determined using an unpaired t-test between the unaffected and affected groups as *** $p \leq 0.001$.

Supplemental Figure 2

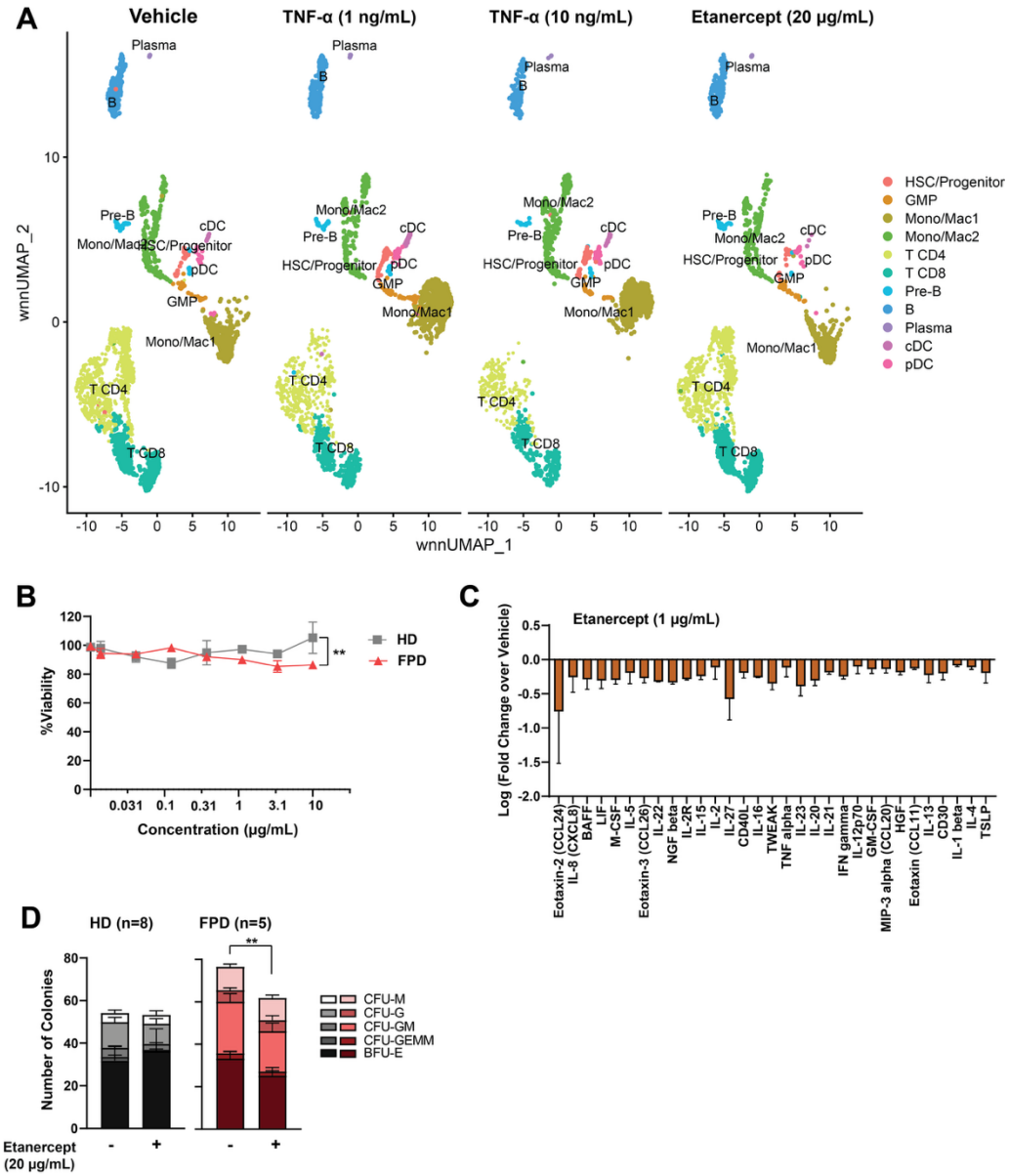

Figure S2: Etanercept treatment can reverse *RUNX1*-FPD phenotype without causing toxicity. A) *RUNX1*-FPD (n=2) bone marrow cells were cultured in IMDM medium supplemented with 20% FBS, 2mM L-glutamine, and 100U/mL penicillin-streptomycin, and treated overnight with TNF-α (1 ng/mL and 10ng/mL) and etanercept (20 µg/mL). Cells then were subjected to 10x chromium single cell RNA sequencing. FASTQ files for the CITE-seq libraries were aligned to the human genome (GRCh38) on Cell Ranger v7.1.0 (10x Genomics), and the feature-barcode matrices were analyzed using Seurat v5. Approximately 2,600 cells in the vehicle, 2,200 cells in TNF-α (1 ng/mL), 1,700 cells in TNF-α (10 ng/mL), and 2,100 cells in the etanercept-treated groups were analyzed. The UMAP illustrates the clustering of different cell populations within each treatment group. **B)** The viability of FPD and HD MNCs was measured using MTS assay at 6 days post culture in the presence of etanercept at different concentrations. The assay was

performed in triplicate for each concentration. **C)** The cultured media from FPD MNCs (n=2) treated with etanercept (1 μg/mL) and vehicle were collected 48 hrs post-treatment. A 65-plex Luminex assay was performed to measure cytokine levels. The bar graph shows the log of fold changes in etanercept treatment over vehicle. **D)** The results of colony formation ability of FPD (n=5) and HD (n=8) $CD34^+$ HSPCs in the presence of etanercept. Primary $CD34^+$ cells were seeded at a density of 1,000 cells/well (in duplicate plating) and treated with etanercept at 20 μg/mL in MethoCult (*StemCell*, H4434). Colonies were counted on day 14 post-seeding.