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Supplementary Materials for

Core-binding factor β and Runx transcription factors promote adaptive natural killer cell responses

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:
(available at immunology.sciencemag.org/cgi/content/full/2/18/eaan3796/DC1)

Supplementary Figures:

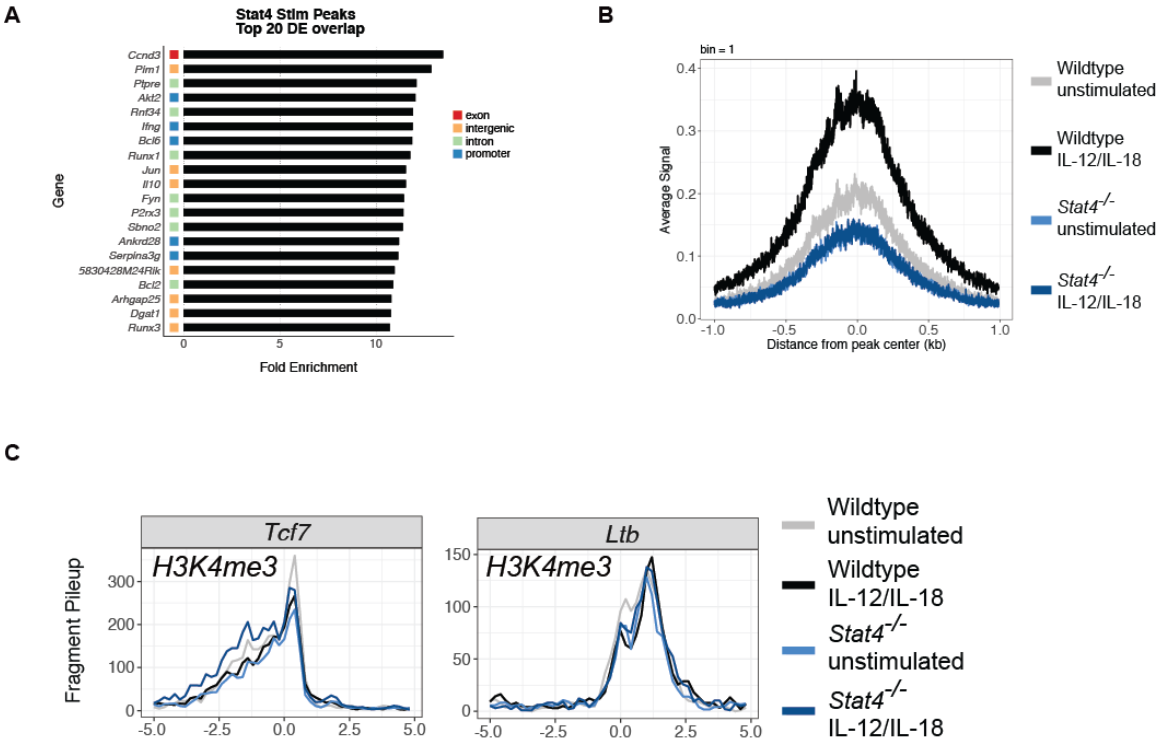

Fig. S1. Epigenetic regulation by STAT4 in cytokine-stimulated NK cells. Splenic NK cells (TCRβ$^-$CD19$^-$CD3ε$^-$Ly6G$^-$TER119$^-$TCRγδ$^-$NK1.1$^+$) were isolated from either WT or *Stat4*$^{-/-}$ mice and stimulated with IL-12 and IL-18, or media alone as a control (unstimulated). STAT4 and H3K4me3 chromatin immunoprecipitation were performed, followed by high-throughput DNA sequencing. RNA-seq was performed on splenic Ly49H$^+$ WT NK cells and *Stat4*$^{-/-}$ NK cells sorted from mixed chimeras two days following MCMV infection. **(A)** Top 20 genes bound by STAT4 and differentially expressed in RNA-seq data (padj < 0.05), ranked on fold change over input calculated by MACS2. **(B)** Meta-peak of all H3K4me3 non-promoter regions. Overlap of midpoints of ChIP fragments (defined as regions between properly paired sequence reads) for each

peak region were counted for each base pair +/- 1kb from the annotated peak center. Line plot depicts average signal for all regions for each base pair. **(C)** H3K4me3 signals from *Tcf7* and *Ltb* loci plotted as normalized fragment counts binned at 200bp across a 10kb window centered on the transcriptional start site.

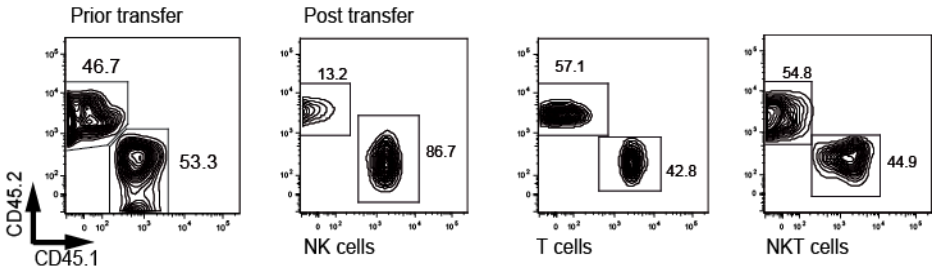

Fig. S2. Cell-intrinsic requirement of CBF-β for the development of mature NK cells. *Rag2*$^{-/-}$ *x Il2rg*$^{-/-}$ mice were lethally irradiated and reconstituted with an equal number of bone marrow cells from WT (CD45.1) and *Cbfb*$^{fl/fl}$ x *NKp46*^{iCre} (CD45.2) mice. Representative flow cytometric plots show ratio of CD45.1 and CD45.2 cells before and 28 days post transfer in the blood of recipient mice. Indicated cell types are identified as CD8^{+} T cells (TCRβ^{+}CD3ε^{+}CD8^{+}), NKT cells (TCRβ^{+}CD3ε^{+}NK1.1^{+}) and NK cells (TCRβ^{-}CD3ε^{-}NK1.1^{+}). Data are representative of two independent experiments, with n=5 mice.

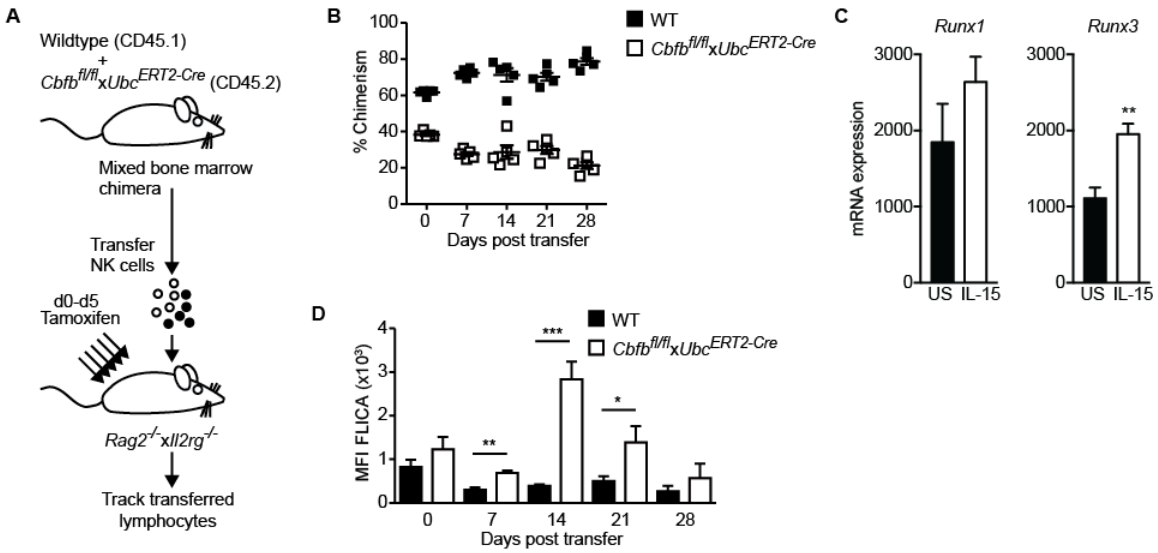

Fig. S3. CBF-β is critical for NK cell survival following homeostatic proliferation. **(A)** NK cells of WT:*Cbfb*$^{fl/fl}$ x *Ubc*$^{ERT2\text{-}Cre}$ mixed bone marrow chimeras were adoptively transferred into *Rag2*$^{-/-}$ x *Il2rg*$^{-/-}$ hosts which were treated with 1 mg of tamoxifen daily for 5 days. **(B)** Percentages of adoptively transferred WT or *Cbfb*$^{fl/fl}$ x *Ubc*$^{ERT2\text{-}Cre}$ NK cells in the blood are shown for indicated time points after transfer. **(C)** Normalized counts of *Runx1* and *Runx3* in unstimulated or IL-15 treated splenic NK cells (16 hour stimulation), as assessed by RNA sequencing (n=2 biological replicates per group). **(D)** FLICA incorporation is shown as mean fluorescence intensity (MFI) of FLICA^{+} WT and *Cbfb*$^{fl/fl}$ x *Ubc*$^{ERT2\text{-}Cre}$ NK cells at indicated time points after transfer. Data are mean ± s.e.m. and representative of two independent experiments with n=5-6 mice per group (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

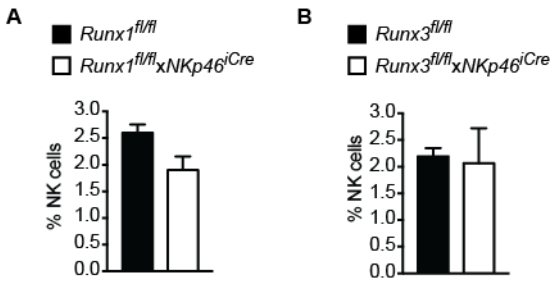

Fig. S4. Runx1- or Runx3-deficient mice have normal NK cell numbers. *Runx1*$^{fl/fl}$ x *NKp46*^{iCre} and *Runx3*$^{fl/fl}$ x *NKp46*^{iCre} mice were generated. Percentages of NK cells in mice with specific deletion of Runx1 or Runx3 are compared to Cre-negative littermate controls.