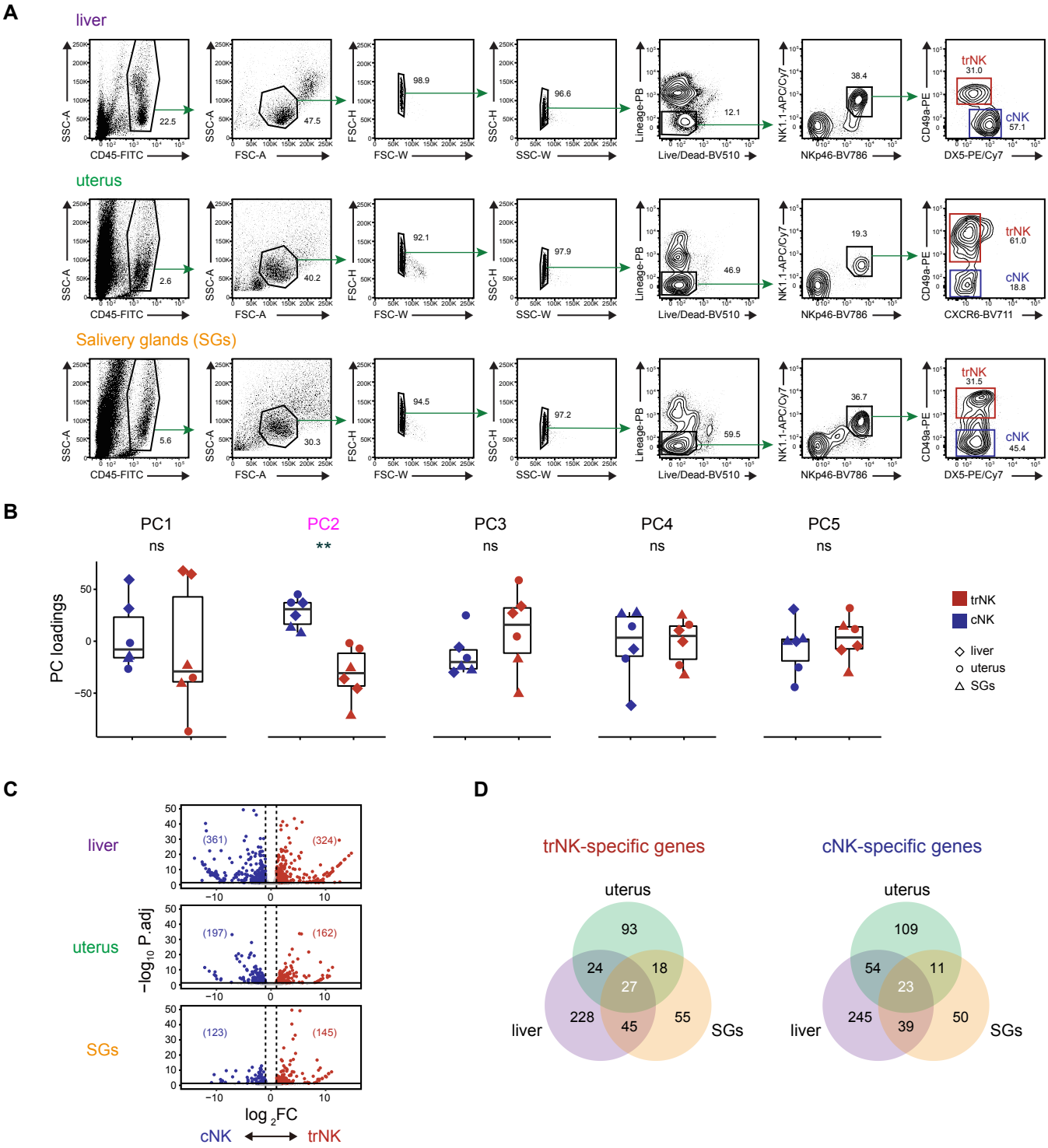
iScience, Volume *26*

Supplemental information

Genetic distinction between functional tissue-resident and conventional natural killer cells

Luni Hu, Mengwei Han, Yichen Deng, Jingjing Gong, Zhiyuan Hou, Yanyu Zeng, Yime Zhang, Jing He, and Chao Zhong

Supplementary Figure 1



Supplementary Figure 1. Transcriptome comparison of trNK and cNK in different tissues. Related to Figure 1.

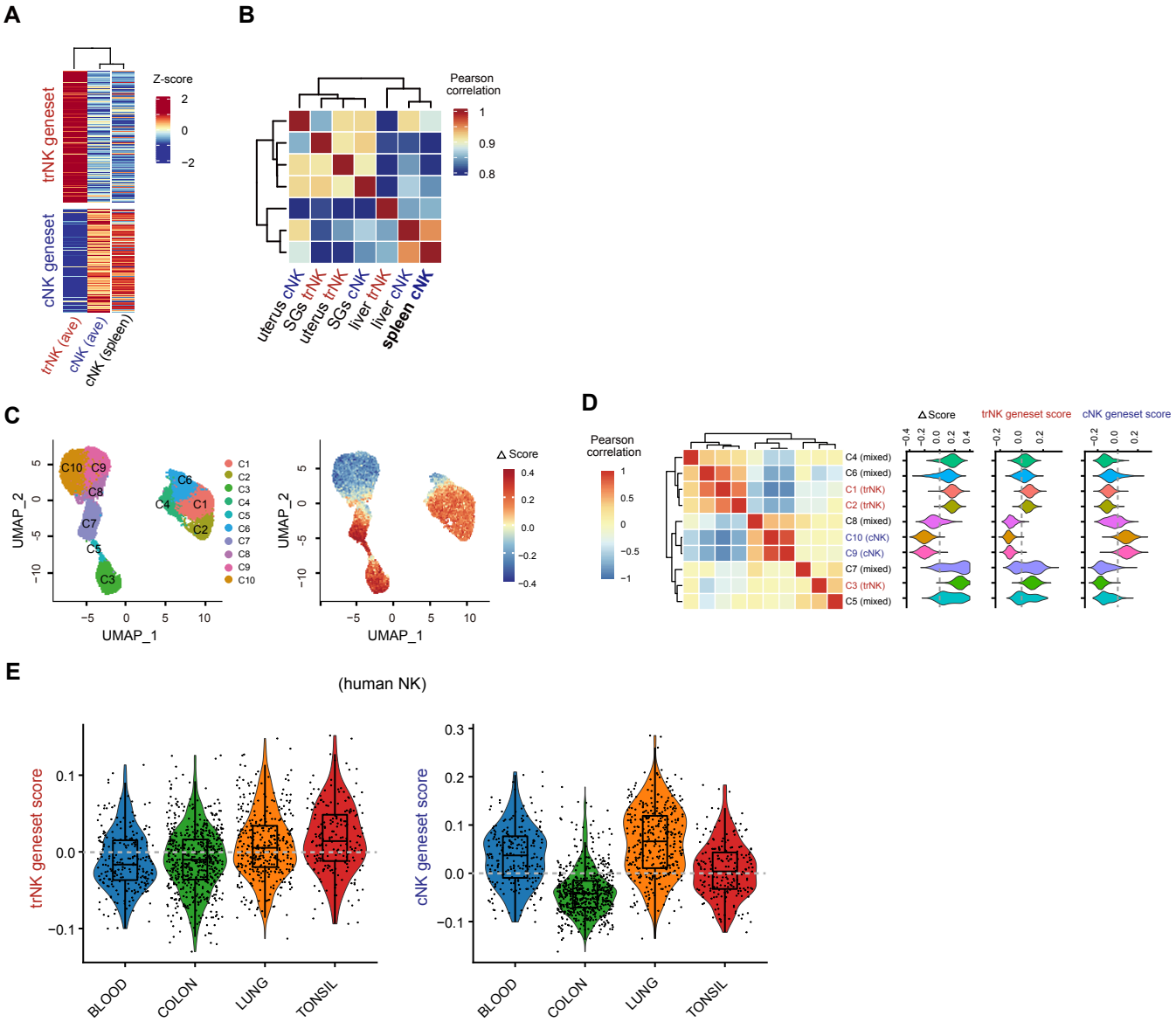
(A) Gating strategy for trNK and cNK in liver, uterus and SGs.

(B) Transcriptome difference between trNK and cNK from liver, uterus and SGs in the first five principal components (PCs).

(C) Volcano plots displaying gene expression difference between trNK and cNK from liver, uterus and SGs. Genes labelled in red or blue respectively indicate higher expression in trNK or cNK (TPM > 10 in either group, FC > 2 and P adj. < 0.05).

(D) Venn diagrams showing genes with consistent expression changes in trNK or cNK from liver, uterus and SGs.

Supplementary Figure 2



Supplementary Figure 2. trNK and cNK cannot be efficiently distinguished by their whole transcriptomes. Related to Figure 2.

(A) Heatmap showing expression of trNK and cNK genesets in trNK and cNK from liver, uterus and SGs on average and in splenic cNK.

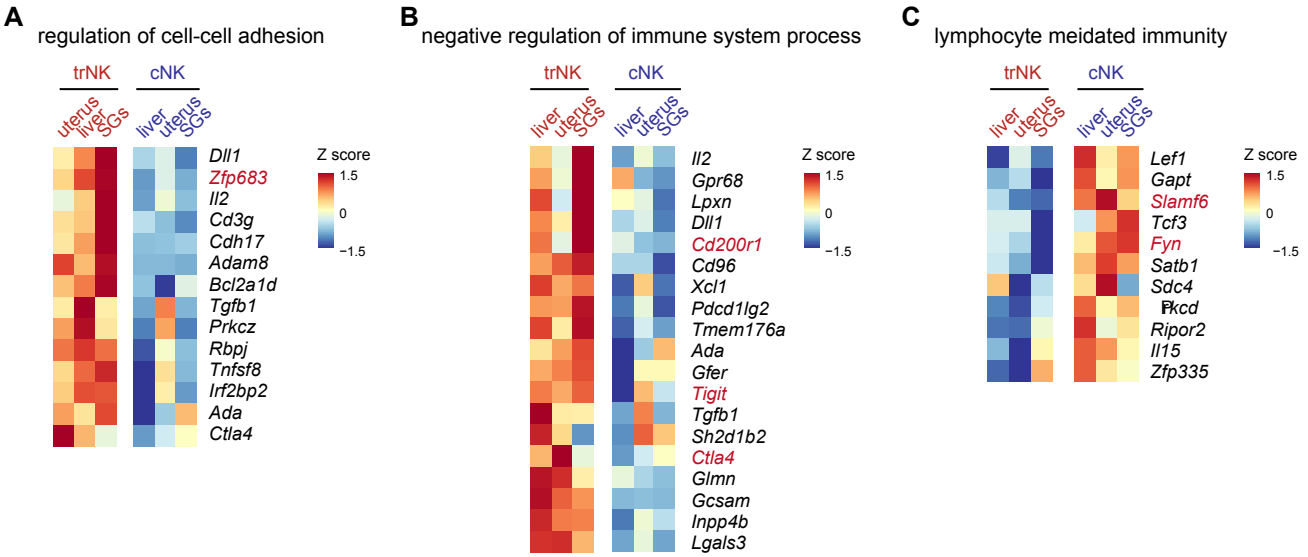
(B) Pearson correlation of trNK and cNK from liver, uterus, SGs and spleen based on their whole transcriptomes.

(C) UMAP visualization of classification of NK cells from liver, SGs, gut and spleen (left), using single-cell transcriptome (scRNA-Seq, GSE189807). ΔScore of each cell representing the difference between trNK and cNK geneset scores is calculated (right).

(D) Pearson correlation between all NK clusters in (C) (left) and assessment of each cluster's trNK and cNK genesets, as well as their ΔScore.

(E) Assessment of trNK and cNK genesets in human NK cells (GSE70580, GSE150050).

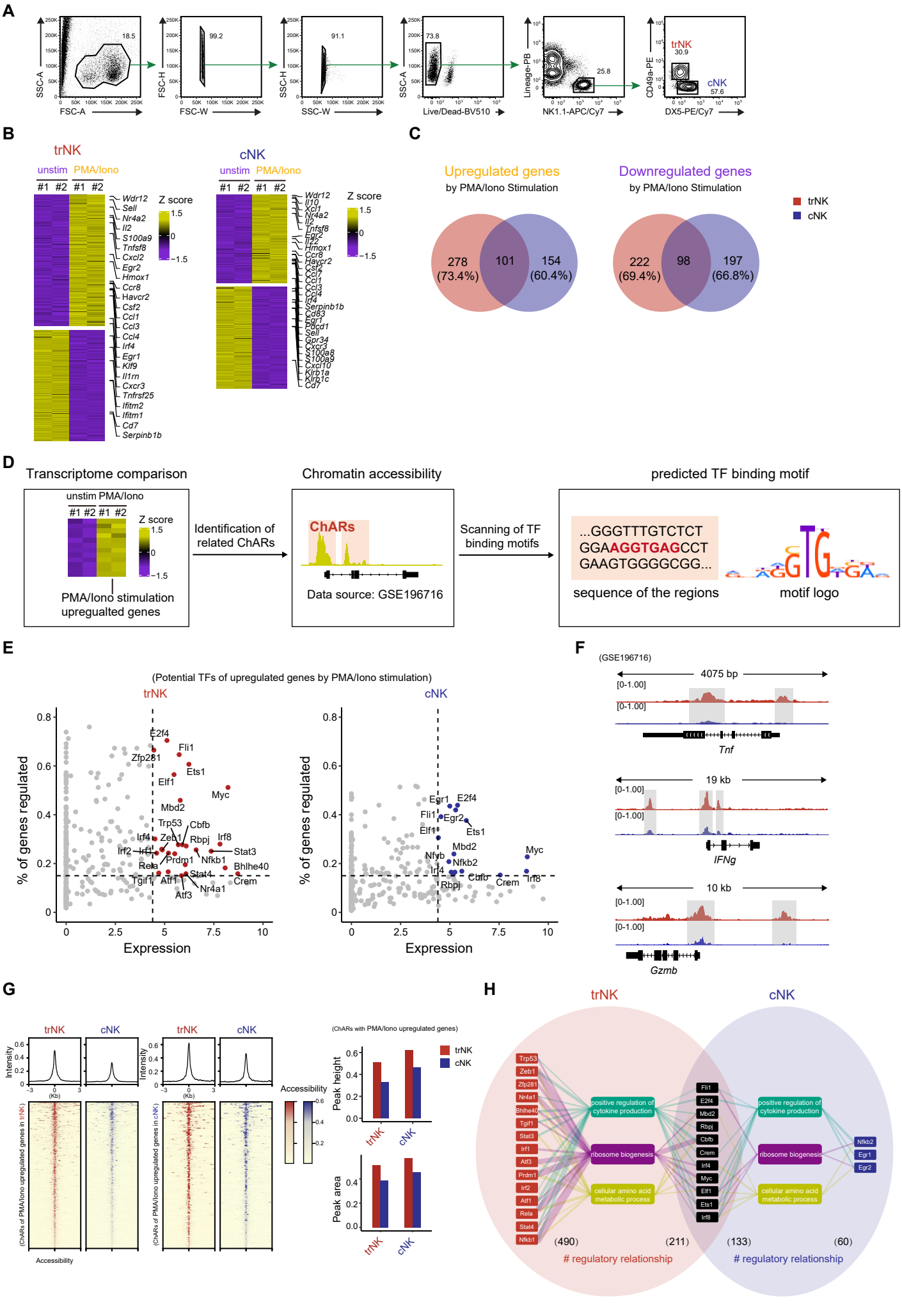
Supplementary Figure 3



Supplementary Figure 3. trNK and cNK are distinguished in activation-related features. Related to Figure 3.

(A-C) Heatmap profiling of genes enriched in ‘regulation of cell-cell adhesion’ (A), ‘negative regulation of immune system process’ (B) and ‘lymphocyte mediated immunity’ (C) in trNK and cNK genesets (related to 3A).

Supplementary Figure 4



Supplementary Figure 4. trNK and cNK activation are differentially regulated. Related to Figure 4.

(A) Gating strategy for *in vitro* stimulated liver trNK and cNK.

(B) Heatmap profiling of the differentially expressed genes in trNK (left) and cNK (right) after PMA/ionomycin stimulation (4A).

(C) Venn diagram showing difference between trNK and cNK in PMA/ionomycin stimulation upregulated (left) and downregulated (right) genes.

(D) Schematics of predicting potential transcription factors for the upregulated genes in PMA/ionomycin stimulation activated trNK and cNK according to an ATAC-Seq analysis (GSE196716) of their chromatin accessibility.

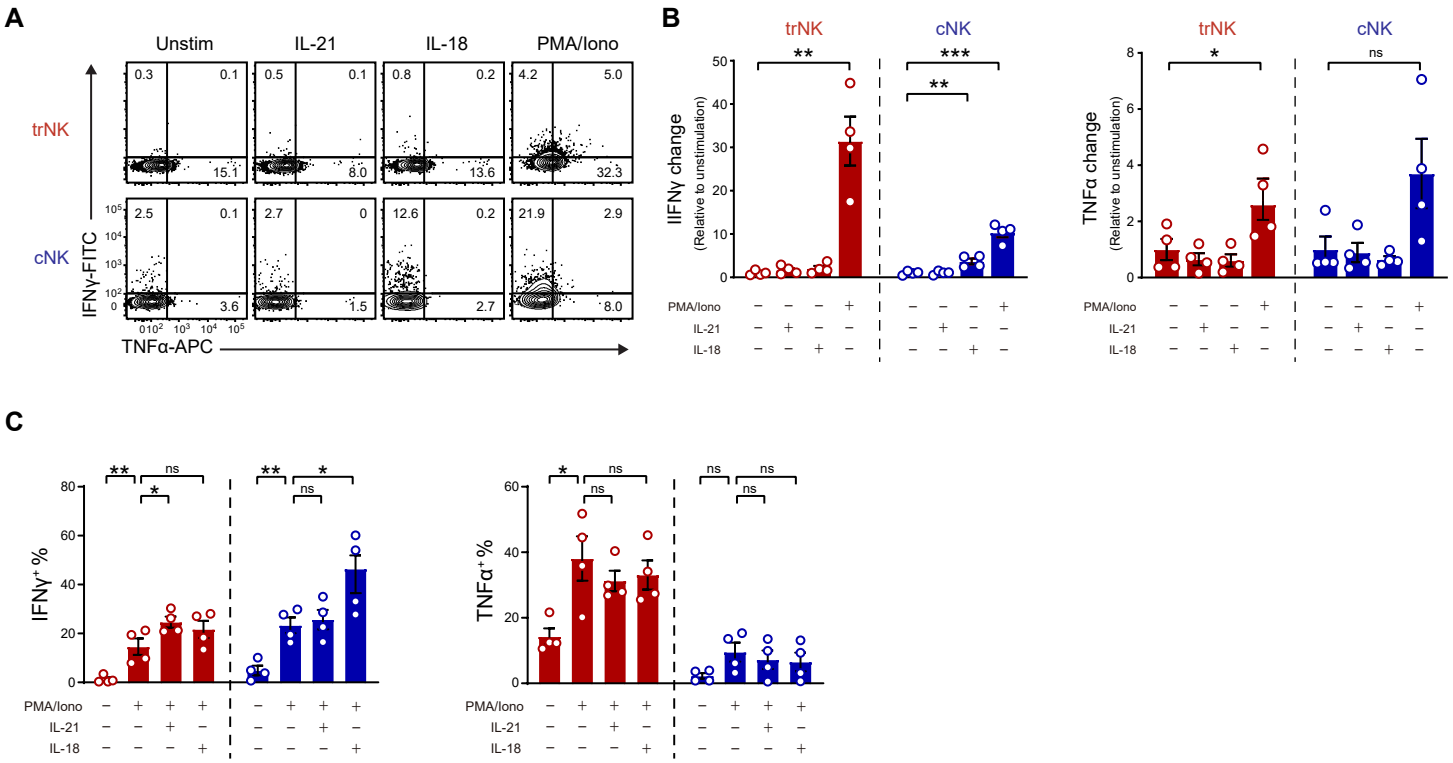
(E) Scatter plots of potential transcription factors in PMA/ionomycin stimulation activated trNK (left) and cNK (right) showing both sufficient expression and substantial regulatory roles for the upregulated genes.

(F) Chromatin accessibility comparison between trNK and cNK for the indicated effector molecules.

(G) Average plot (left-top) and heatmap (left-bottom) of chromatin accessibility in trNK and cNK, for regions related to the upregulated genes by PMA/ionomycin stimulation. Peak heights and peak areas of the average plots were calculated and compared between trNK and cNK.

(H) Transcriptional regulation between the potential transcription factors (S4D and 4E), grouped by trNK-specific, common and cNK-specific, and the features commonly enriched in trNK and cNK during PMA/ionomycin induced activation (4D).

Supplementary Figure 5

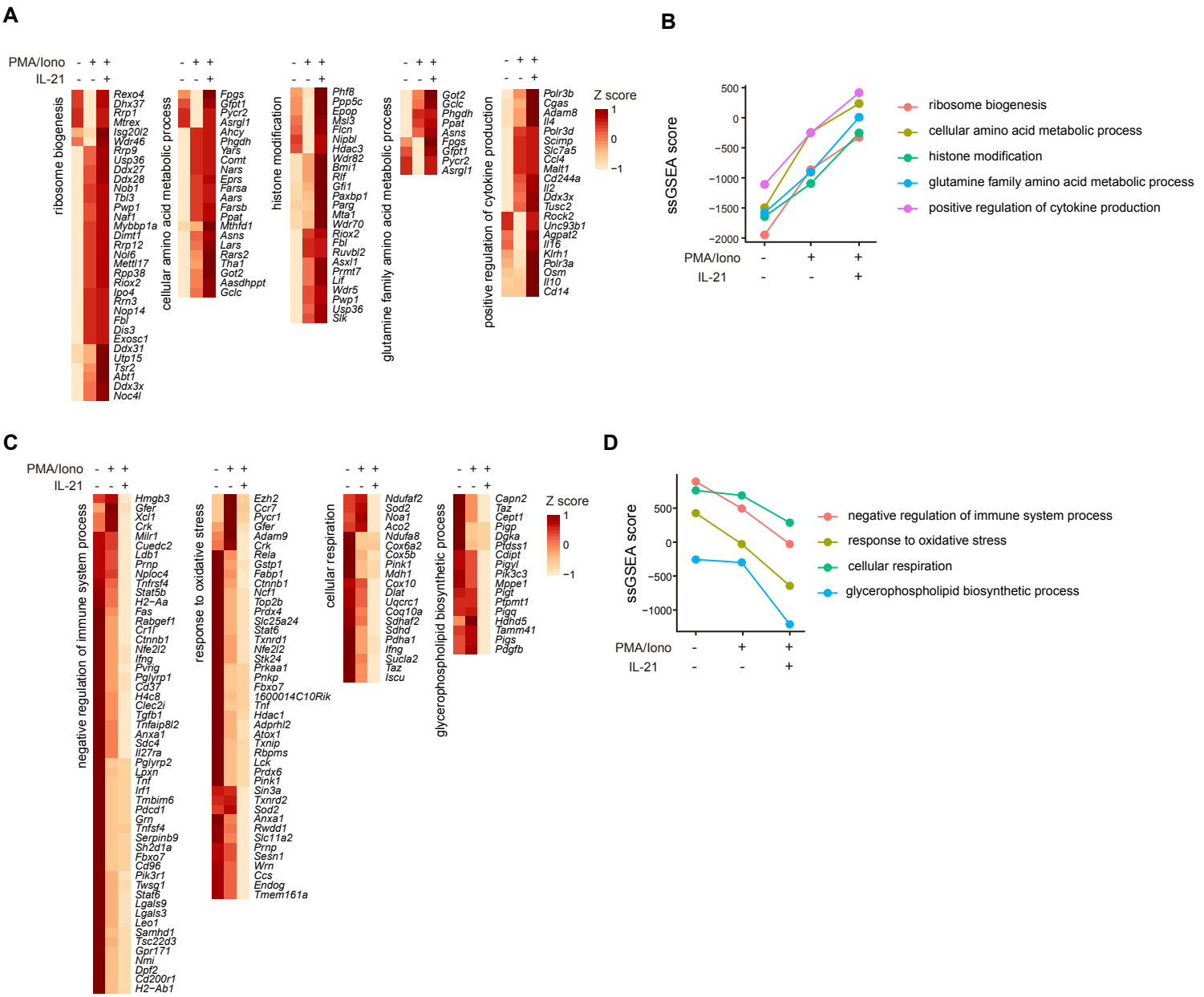


Supplementary Figure 5. IL-21 and IL-18 play accessory roles during trNK and cNK activation. Related to Figure 5.

(A) Flow cytometric analysis of IFN-γ and TNF-α expression in trNK and cNK kept unstimulated or stimulated by the indicated conditions.

(B-C) Statistical calculation of IFN-γ and TNF-α expression in trNK and cNK under the indicated conditions (n = 4 per group). Data are shown as the mean ± SEM. P. values are calculated by unpaired t-test, *P < 0.05. Data are representative of at least three independent experiments.

Supplementary Figure 6



Supplementary Figure 6. IL-21 facilitates trNK activation. Related to Figure 6.

(A) Heatmap profiling of the genes in each GO term enriched by additional IL-21 stimulation upregulated genes in trNK (6E and 6F).

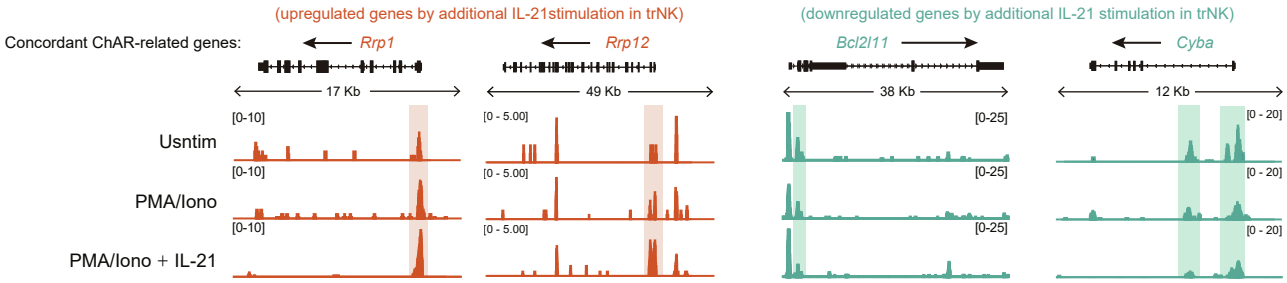
(B) ssGSEA scoring of trNK under the indicated stimulatory conditions, using the profiled genes in each GO term (S6A).

(C) Heatmap profiling of the genes in each GO term enriched by additional IL-21 stimulation downregulated genes in trNK (6E and 6F).

(D) ssGSEA scoring of trNK under the indicated stimulatory conditions, using the profiled genes in each GO term (S6C).

Supplementary Figure 7

A



B

Potential TFs	Transcriptional activation	Transcriptional repression
Zbtb17	Semin Immunol 23, 379-387. [50] Proc Natl Acad Sci U S A 111, E5411-5419. [51]	Nature Cell Biology 3, 392-399 [52]
Zbtb281	Cell Mol Immunol 17, 1222-1232. [45]	Stem Cells 29, 1705-1716. [44]
Sp1	J Lipid Res 41, 583-594. [41]	J Cell Biochem 112, 2089-2096. [57]
Sp2	J Lipid Res 41, 583-594. [41]	Cancer Res 64, 3072-3078 [54]
Sp3	J Lipid Res 41, 583-594. [41]	J Biol Chem 271, 8533-8536 [55]
Egr1	J Cell Biochem 111, 207-217 [47]; Am J Pathol 185, 513-523 [53]	Eur J Immunol 38, 528-536. [42] PLoS One 10, e0127641. [43]
Egr2	J Cell Biochem 111, 207-217 [56]	Eur J Immunol 38, 528-536. [42]
E2f4	Nat Commun 10, 2939. [46]	Cell Cycle 15, 3183-3190. [47]
Ets1	JCI Insight 4, e124202. [48]	Oncogene 19, 6524-6532. [49]

Supplementary Figure 7. Bifunctional transcription factors are involved in IL-21 mediated trNK activation. Related to Figure 7.

(A) Peak plots showing representative concordant ChARs for upregulated (left and middle) or downregulated (right) genes by additional IL-21 stimulation in trNK.

(B) Representative studies showing bifunctional features of the potential transcription factors (7G) involved in additional IL-21 mediated gene expression changes in trNK.