Supplementary Data

Title

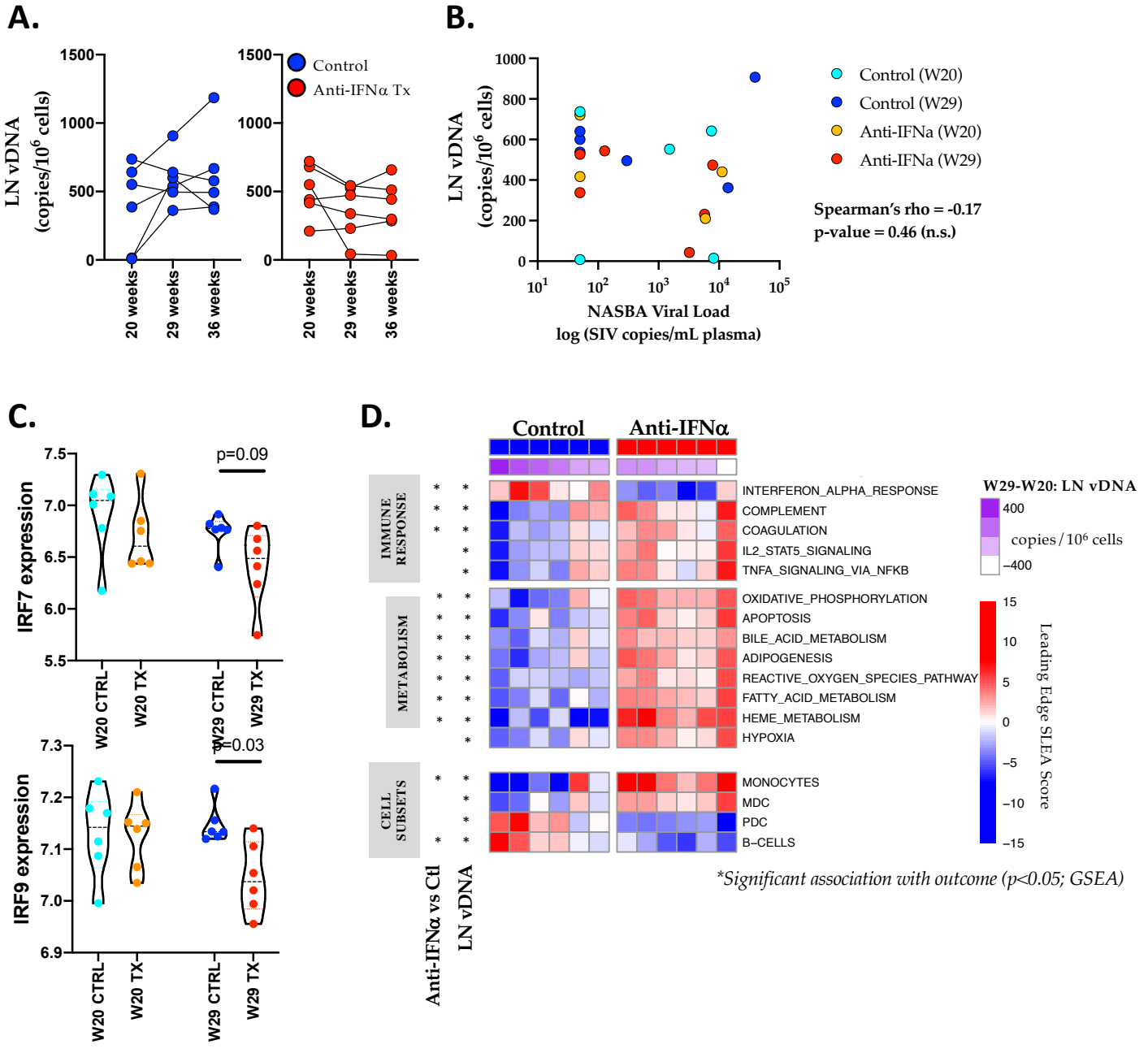
IFNα-blockade during ART-treated SIV infection lowers tissue vDNA, rescues immune function, and improves overall health

Supplementary Figure 1. Anti-IFNα treatment and the associated decline in LN vDNA is seen in conjunction with down-regulation of pDC/TI-IFN genesets. A. LN vDNA (normalized to LN CD4+ T cell frequency) in control and anti-IFNα treated arms during ART (i.e., week 20-36). **B**. Lack of association in LN vDNA levels and incomplete plasma viral suppression during ART. **C**. Reduced IFNα-inducible genes post-anti-IFNα treatment (i.e., lower expression of Hallmark TI-IFN geneset in **Figure 1F**) is characterized by a downregulation of master regulators of TI-IFN responses, IRF7 (upper plot) and IRF9 (lower plot) (p-values computed using Mann-Whitney U-test) (**Table S3**). **D.** Heatmap showing the sample level leading-edge scores of immune/metabolic genesets (extracted from MSigDB's Hallmark module) and cell subset signatures significantly altered with anti-IFNα treatment (compared to the control arm; week 29) and/or associated with decline in LN vDNA (column annotations shown in purple) (**Table S3**). Gene Set Enrichment Analysis (GSEA) was used to determine significance with each outcome and significant associations (p-value < 0.05) were marked (*). Sample Level Enrichment Analyses (SLEA) intersecting leading-edge genes (obtained from GSEA results against both outcomes) are shown in the heatmap.

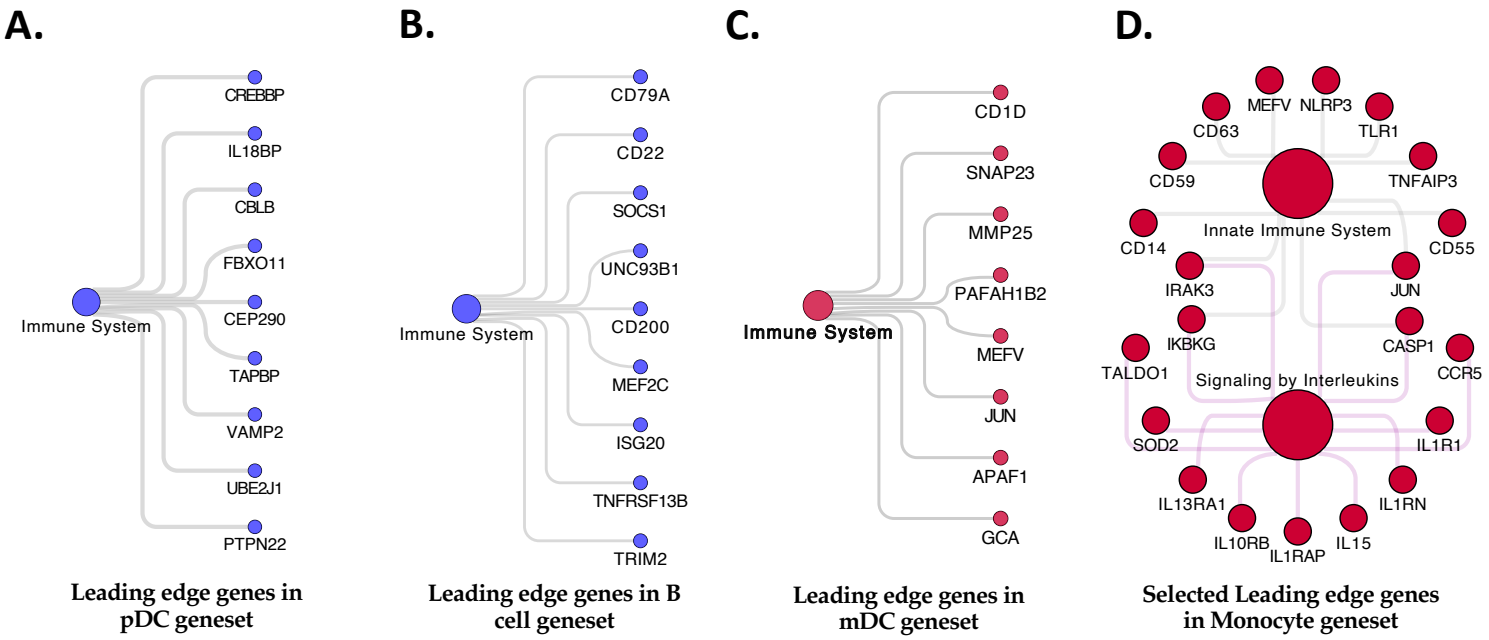
Supplementary Figure 2. Induction of pro-inflammatory genesets post-IFNα blockade in rectal biopsies and whole blood. A-B. Leading edge genes in pDC and B-cell signatures (i.e., genes identified post-GSEA – Figure 1F - that define significant differences in cell subset genesets post-anti-IFNα treatment or that showed significant association with LN vDNA). Only the genes that are members of ReactomeDB Immune System sub-category are represented in the networks. Blue node denotes leading-edge genes that are members of pathways downregulated post-anti-IFN treatment or associated with higher vDNA levels. **C-D.** Leading edge genes in mDC and monocyte signatures (i.e., genes identified post-GSEA – **Supplementary Figure 1D** - that define significant differences in cell subset genesets post-anti-IFNα treatment or that showed significant association with LN vDNA). Only the genes that are members of ReactomeDB Immune System sub-category are represented in the networks. Red nodes indicate leading-edge genes that are members of pathways upregulated post-anti-IFNα treatment or associated with lower vDNA. **E.** Leading-edge genes from two significantly altered genesets (Hallmark's Type I IFN response was reduced and TNFα via NF-κB signaling geneset was induced) in rectal biopsies 9 weeks post-IFNα blockade. Gene Set Enrichment Analysis (GSEA) was used to determine significance with each outcome and significant associations (p-value < 0.05) were marked(*). Sample Level Enrichment

Analyses (SLEA) intersecting leading-edge genes (obtained from GSEA results against both outcomes) are shown in the heatmap.

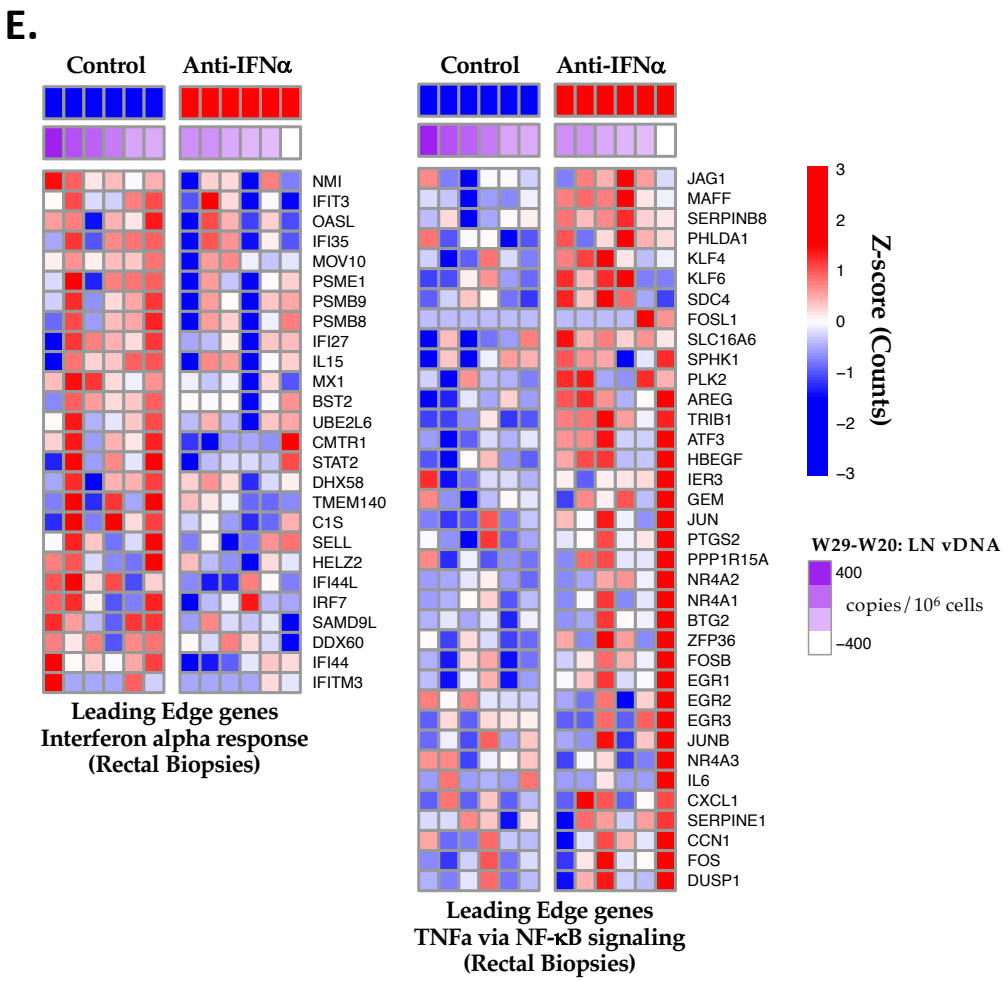
Supplementary Figure 3. Anti-IFNα treatment and the associated decline in LN vDNA are correlated with increased expression of transcription factors (and targets) responsible for T cell proliferation/differentiation, and with decreased expression of transcription factors (and targets) that drive T cell quiescence and stemness cascades. A. Heatmap showing leading-edge genes that define the association of the decline in LN vDNA with expression of transcription factor targets that regulate T cell effector function (i.e., increased STAT5A targets and reduced TCF7 targets). Targets were extracted from CHEA and ENCODE databases and enrichment was assessed using GSEA (p-value<0.05; see Table S4 for details). **B.** Correlation networks showing the interaction between gene-sets associated with decline in LN vDNA (SLEA scores computed from leading-edge genes post-GSEA; GSEA p-value<0.05), C-**D.** Cytokine Clusters 4/5 **(C)** and effector CD8 T cell signatures **(D)** identified in Figure 3A. Edges represent correlations (Spearman's rho; p-value<0.05) and node color represents change in SLEA scores between anti-IFNα treated and untreated groups. **E.** Transcription factors (with known targets extracted from CHEA and ENCODE databases) that drive effector differentiation of T cells (i.e., STAT4, ID2) were associated with a decline in plasma TGFβ1, TGFβ2, and LN vDNA levels at week 29. Correlation network edges based on rho values obtained from Spearman's correlation test (p-value<0.05).


A.
LN vDNA (copies/10^6 cells)
1500
1000
500
0
20 weeks
29 weeks
36 weeks
Control
Anti-IFNα Tx
B.
LN vDNA (copies/10^6 cells)
1000
800
600
400
200
0
10^1
10^2
10^3
10^4
10^5
NASBA Viral Load
log (SIV copies/mL plasma)
Control (W20)
Control (W29)
Anti-IFNa (W20)
Anti-IFNa (W29)
Spearman's rho = -0.17
p-value = 0.46 (n.s.)
C.
IRF7 expression
7.5
7.0
6.5
6.0
5.5
p=0.09
W20 CTRL
W20 TX
W29 CTRL
W29 TX
IRF9 expression
7.3
7.2
7.1
7.0
6.9
p=0.03
W20 CTRL
W20 TX
W29 CTRL
W29 TX
D.
Control
Anti-IFNα
IMMUNE RESPONSE
INTERFERON_ALPHA_RESPONSE
COMPLEMENT
COAGULATION
IL2_STAT5_SIGNALING
TNFA_SIGNALING_VIA_NFKB
METABOLISM
OXIDATIVE_PHOSPHORYLATION
APOPTOSIS
BILE_ACID_METABOLISM
ADIPOGENESIS
REACTIVE_OXYGEN_SPECIES_PATHWAY
FATTY_ACID_METABOLISM
HEME_METABOLISM
HYPOXIA
CELL SUBSETS
MONOCYTES
MDC
PDC
B-CELLS
Anti-IFNα vs Ctl LN vDNA
W29-W20: LN vDNA
400
copies / 10^6 cells
-400
15
10
5
0
-5
-10
-15
Leading Edge SLEA Score
*Significant association with outcome (p<0.05; GSEA)

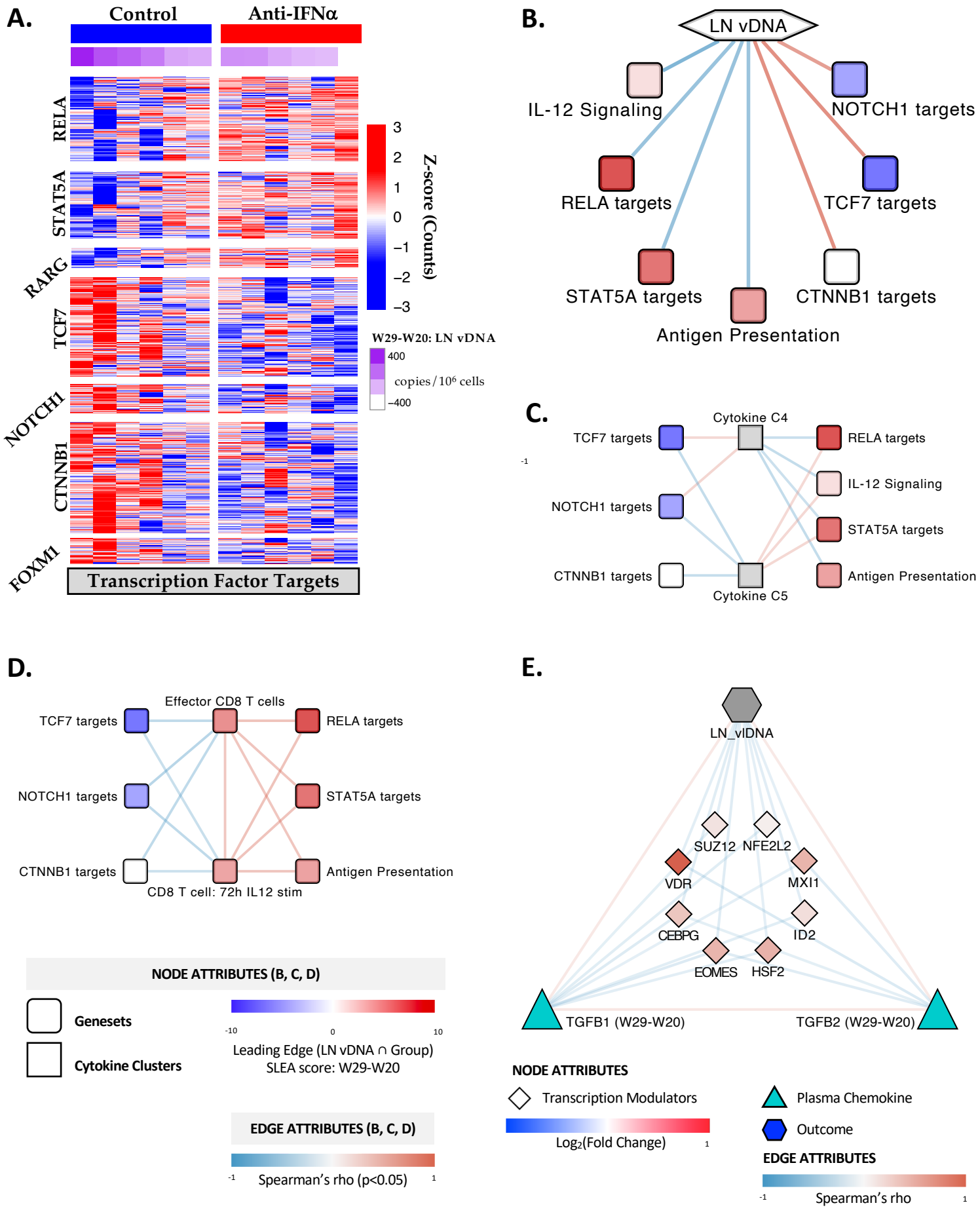
Supp Fig. 1



Genes that map to the "immune system" (Reactome database) within each enriched cell subset signatures



Supp Fig. 2


A.
Control
Anti-IFNα
RELA
STAT5A
RARG
TCF7
NOTCH1
CTNNB1
FOXM1
Transcription Factor Targets
Z-score (Counts)
3
2
1
0
−1
−2
−3
W29-W20: LN vDNA
400
copies / 10⁶ cells
−400
B.
LN vDNA
IL-12 Signaling
NOTCH1 targets
RELA targets
TCF7 targets
STAT5A targets
CTNNB1 targets
Antigen Presentation
C.
Cytokine C4
TCF7 targets
RELA targets
IL-12 Signaling
NOTCH1 targets
STAT5A targets
CTNNB1 targets
Antigen Presentation
Cytokine C5
D.
Effector CD8 T cells
TCF7 targets
RELA targets
NOTCH1 targets
STAT5A targets
CTNNB1 targets
Antigen Presentation
CD8 T cell: 72h IL12 stim
NODE ATTRIBUTES (B, C, D)
Genesets
Cytokine Clusters
-10
0
10
Leading Edge (LN vDNA ∩ Group)
SLEA score: W29-W20
EDGE ATTRIBUTES (B, C, D)
-1
Spearman's rho (p<0.05)
1
E.
LN_vIDNA
SUZ12
NFE2L2
VDR
MXI1
CEBPG
ID2
EOMES
HSF2
TGFB1 (W29-W20)
TGFB2 (W29-W20)
NODE ATTRIBUTES
Transcription Modulators
Log2(Fold Change)
1
Plasma Chemokine
Outcome
EDGE ATTRIBUTES
-1
Spearman's rho
1

Supp Fig. 3