

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Deconvolution analysis of NL and NXG bulk RNA-seq data.

Supplemental Figure 2. Immunohistochemistry (IHC) analysis of NL lesional and control healthy skin. IHC analysis was performed on paraffin embedded tissue obtained from the University of Michigan. NL patients were compared to healthy skin controls. IHC results for CCL4, CCL5, IL32, and CXCL9 are shown.

Supplemental Figure 3. Immunohistochemistry (IHC) analysis of NL and NXG lesional skin reveals strong SPP1 expression. IHC of SPP1 (aka OSTP or Osteopontin) was performed on paraffin embedded tissue obtained from the University of Michigan.

Supplemental Figure 4. Cluster 8 fibroblasts have higher expression of myofibroblast markers ACTA2, CTGF, and PLOD2. Additional analysis of fibroblast Clusters 6 and 8 from Figure 3A are shown. Violin plot of gene expression for the corresponding scRNA-seq clusters are presented for the indicated genes. Each dot represents a single cell.

Supplemental Figure 5. Single-cell gene expression flex fixed RNA profiling (FRP) of NL and NXG fibroblasts; NL and NXG lesional fibroblasts compared to healthy skin control fibroblasts. FRP pseudobulk analysis is presented as box-and-whisker plots. Each dot represents a single sample. The upper and lower bars connected to each box indicate the boundaries of the normal distribution and the upper and lower box edges mark the first and third quartile boundaries within each distribution. The bold line within the box indicates the median value of the distribution. Below each box-and-whisker plot is a violin plot for the corresponding scRNA-seq data in which each dot represents a single cell. For this analysis, all fibroblast clusters were binned together. Significance was determined by the Student's t-test.

Supplemental Figure 6. Single-cell RNA-seq of lesional and non-lesional NL skin samples. A) Scatter plot of intracellular expression of CCL5 and CXCL9. Each dot represents a single fibroblast cell in NL lesional skin. B) Cultured fibroblast gene expression data. Primary fibroblasts were isolated from healthy skin and cultured with noted cytokines. Gene expression analysis was performed by bulk RNA-seq. *IL32* (upper plot), *CXCL9* (middle plot), and *CCL5* (lower plot) expression are shown as Log2 reads. C) The UMAP method was used to visualize scRNA-seq data of dermal cells isolated from systemic sclerosis and healthy skin. Each dot represents a single cell. Clusters containing fibroblast cells are colored. D) Violin plots representing each fibroblast cluster from “C” demonstrate that none of the fibroblast subclusters highly expressed *CCL5*. Other genes of interest are also shown. Numbers in red represent clusters with a greater percentage of systemic sclerosis fibroblasts.

Supplemental Figure 7. scRNA-seq of systemic sclerosis and sarcoidosis fibroblasts. A) Systemic sclerosis scRNA-seq gene expression data for all fibroblast clusters binned together are presented. For each violin plot each dot represents a single cell. Systemic sclerosis fibroblasts are compared to healthy skin fibroblasts. B) *Sarcoidosis* scRNA-seq gene expression data for all fibroblast clusters binned together. *Sarcoidosis* fibroblasts are compared to healthy skin fibroblasts. Plots in which gene expression has the same directionality as in NL are shown. The upper box-and-whisker plots represent scRNA-seq pseudobulk analysis. Each dot represents a single sample. The upper and lower bars connected to each box indicate the boundaries of the normal distribution and the upper and lower box edges mark the first and third quartile boundaries within each distribution. The bold line within the box indicates the median value of the distribution. Below each box-and-whisker plot is a violin plot for the corresponding scRNA-seq data in which each dot represents a single cell. Significance was determined by the Student's T-test. C) *Sarcoidosis* scRNA-seq gene expression data as described in “B” except plots are for gene expression data with opposite directionality as NL or for comparisons that do not reach significance in the pseudobulk analysis.

Supplemental Figure 8. scRNA-seq of NL myeloid cells. A) Violin plots for indicated differentially expressed genes across seven myeloid clusters from UMAP shown in Figure 4. Each dot represents a single cell. B) NL macrophages from *all myeloid cell clusters in Figure 4* were binned together and compared to control macrophages (healthy skin macrophages and non-lesional macrophages). Differentially expressed gene expression data is presented as violin plots where each dot represents a single cell.

Supplemental Figure 9. Single-cell gene expression flex fixed RNA profiling (FRP) reveals high expression of CCL5 and SPP1 in NL and NXG lesional macrophages compared to healthy skin control macrophages. FRP pseudobulk analysis is presented as box-and-whisker plots. Each dot represents a single sample. The upper and lower bars connected to each box indicate the boundaries of the normal distribution and the upper and lower box edges mark the first and third quartile boundaries within each distribution. The bold line within the box indicates the median value of the distribution. Below each box-and-whisker plot is a violin plot for the corresponding scRNA-seq data in which each dot represents a single cell. For this analysis all macrophage clusters were binned together. Significance was determined by the Student's T-test.

Supplemental Figure 10. scRNA-seq of sarcoidosis macrophages A) Sarcoidosis scRNA-seq - Plots in which gene expression has the same directionality as in NL are shown. scRNA-seq gene expression data for all sarcoidosis macrophage clusters were binned together and compared to healthy skin macrophages. scRNA-seq pseudobulk analysis is presented as box-and-whisker plots. Each dot represents a single sample. The upper and lower bars connected to each box indicate the boundaries of the normal distribution and the upper and lower box edges mark the first and third quartile boundaries within each distribution. The bold line within the box indicates the median value of the distribution. Below each box-and-whisker plot is a violin plot for the corresponding scRNA-seq data in which each dot represents a single cell. B) Sarcoidosis scRNA-seq expression data as described in “A” except plots are for gene expression data with opposite directionality as NL or for comparisons that do not reach significance in the pseudobulk analysis.

Supplemental Figure 11. Single-cell gene expression flex fixed RNA profiling (FRP) reveals increased CCR7, JAK3, SELL, and CCR7 in NL and NXG lesional T cells compared to healthy skin control T cells. A) The upper violin plots represent additional scRNA-seq gene expression data for NL lesional skin versus control (healthy skin and non-lesional skin). The samples analyzed were fresh biopsy specimens. All scRNA-seq T cell clusters were binned together for the analysis. Each dot represents a single cell. Below the scRNA-seq plots are results from the FRP analysis. FRP was performed on formalin fixed archived specimens. NL FRP pseudobulk results are presented as box-and-whisker plots. For this analysis all FRP T cell clusters were binned together. NL lesional skin is compared to healthy control skin. Each dot represents a single sample. The upper and lower bars connected to each box indicate the boundaries of the normal distribution and the upper and lower box edges mark the first and third quartile boundaries within each distribution. The bold line within the box indicates the median value of the distribution. Below each box-and-whisker plot is a violin plot for the corresponding scRNA-seq data in which each dot represents a single cell. Significance was determined by the Student's t-test. B) FRP gene expression data as described in “A” except for NXG samples. C) FRP gene expression analysis of NKG7-expressing NL lesional and healthy control skin T cells. This analysis differs from “A” and “B” in that only T cells expressing NKG7 were analyzed.

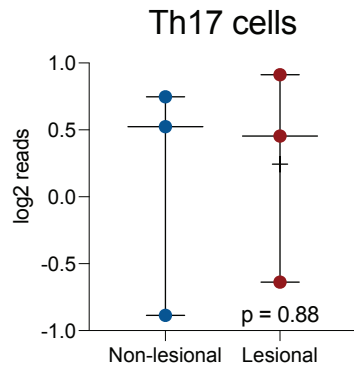
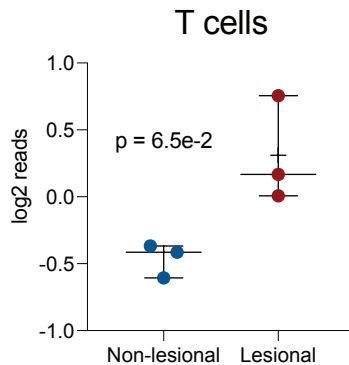
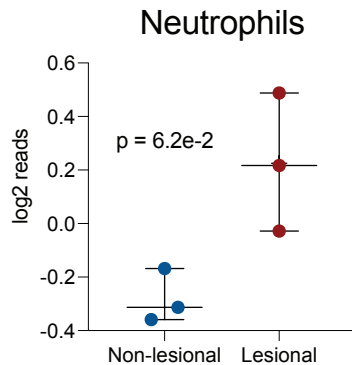
Supplemental Figure 12. ScRNA-seq gene expression profiling of sarcoidosis T cells highlights strong upregulation of STAT1 and IFNG. Upper violin plots- Additional scRNA-seq gene expression data for NL lesional skin versus control (healthy skin and non-lesional skin) is shown for comparison. All scRNA-seq T cell clusters were binned together for the analysis. Data is presented as violin plots where each dot represents a single cell. Below the NL scRNA-seq plots are the scRNA-seq plots for sarcoidosis T cells. Results of pseudobulk analysis are represented as box-and-whisker plots. For this analysis all sarcoidosis T cell clusters binned together. Each dot represents a single sample. The upper and lower bars connected to each box indicate the boundaries of the normal distribution and the upper and lower box edges mark the first and third quartile boundaries within each distribution. The bold line within the box indicates the median value of the distribution. Below each box-and-

whisker plot is a violin plot for the corresponding sarcoidosis scRNA-seq data in which each dot represents a single cell.

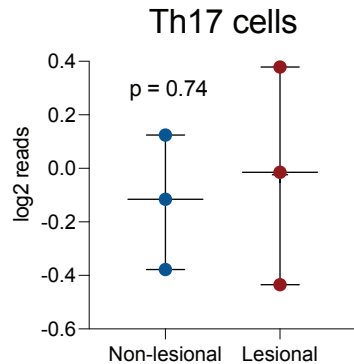
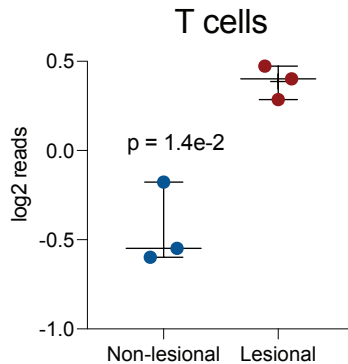
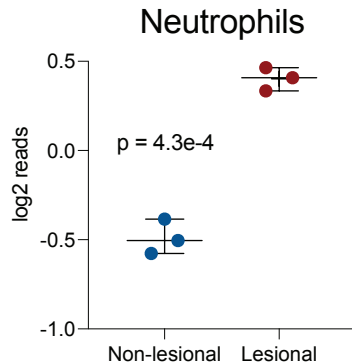
Supplemental Figure 13. A large percentage of IGHM-expressing B cells in NL lesional skin are plasma cells. A) Comparison of plasma cell markers expressed by IGHM B cells in NL and systemic sclerosis (Ssc) lesional skin. For this analysis IGHM-expressing B cells identified by scRNA-seq were analyzed. From within this population, plasma cells can be identified by their lack of *MS4A1* (encodes CD20) expression and the positive expression of one of the plasma cell markers- *PRDM1* (encodes BLIMP1), *SDC1* (encodes CD138), and *TNFRSF17* (encodes CD269). Venn diagrams are used for visual comparison of systemic sclerosis and NL IGHM-expressing B cells. Note that no IGHM-expressing B cells in systemic sclerosis (Ssc) are plasma cells, i.e. nearly all of the Ssc IGHM-expressing B cells express *MS4A1* and none express any of the plasma cell markers (*PRDM1*, *SDC1*, or *TNFRSF17*). B) Similar analysis to that described in “A” but for NL and NXG FRP datasets. Although the FRP technique is not as sensitive as scRNA-seq, FRP analysis was able to detect plasma cell markers in NL IGHM-expressing B cells. IGHM-expressing B cells in NXG have a similar phenotype to those in NL. C) scRNA-seq gene expression analysis of NL plasma cells demonstrates that most plasma cells in NL are either *IGHG1* or *IGHM* expressing. For this analysis all NL plasma cells were binned together and then *IGH* gene expression was assessed. In the violin plots each dot represents an individual plasma cell. D) Same scRNA-seq analysis described in “C” was performed for sarcoidosis. Note that practically no plasma cells were found in sarcoidosis lesional skin.

Supplemental Figure 14. Single-cell T cell receptor gene sequencing (scTCR) identifies clonal expansions of NKG7-expressing T cells. Top, Rank order plots illustrate the frequency of individual T cell expansions in NL lesional skin. Arrows indicate the most abundant expanded clones based on TCR alpha and beta hypervariable CDR3 sequence frequencies. Violin plots depict expression of genes relevant to NL pathophysiology or T cell differentiation. Note the top T cell expansion expresses low *ICOS*, *IL7R*, and *LTB* and high *IFNG*, *CCL5*, *NKG7*, *TNFRSF9*, and *LAG3*. Comparison of genes expressed by the most highly expanded T cells versus other NL lesional T cells is also presented as a volcano plot.

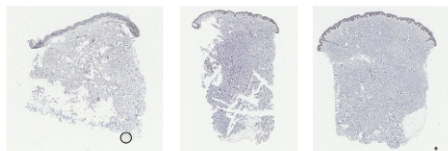
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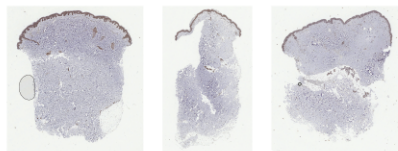
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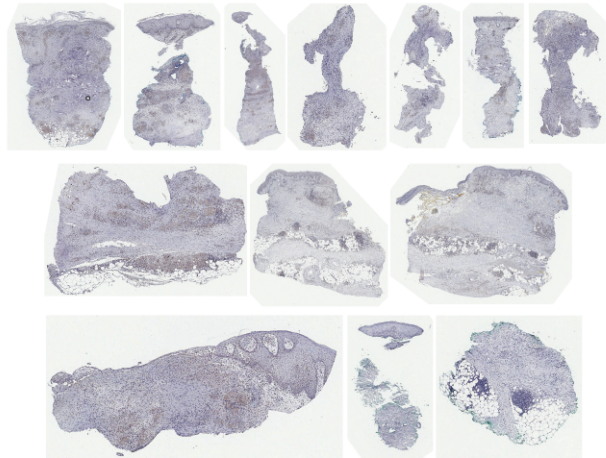
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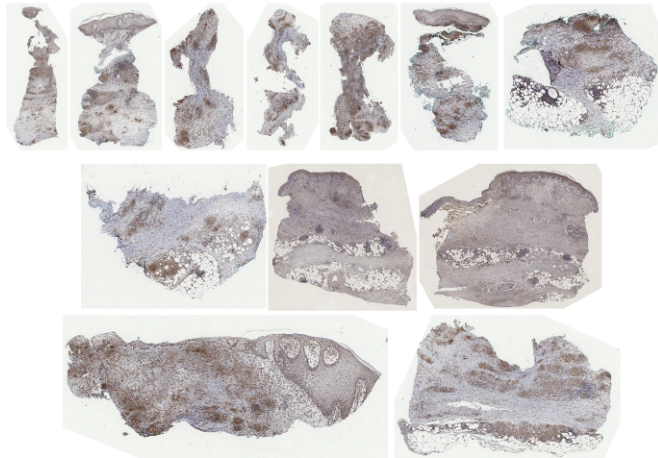
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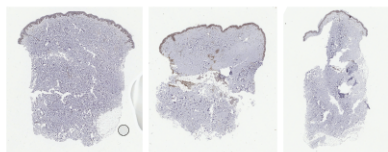
CCL4 NL



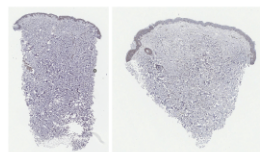
CCL5 NL



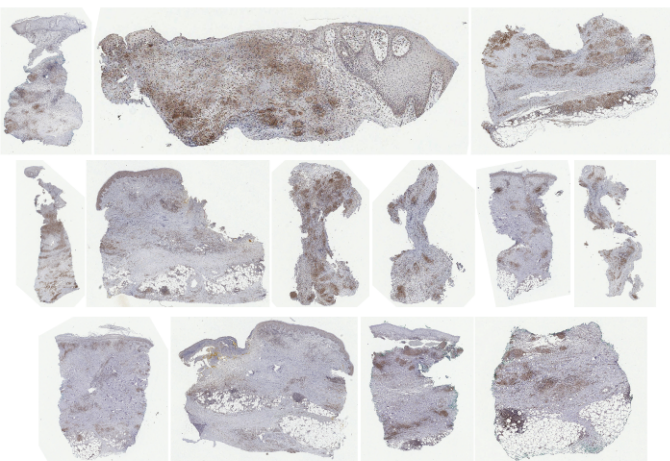
CXCL9 Healthy



IL32 Healthy



CXCL9 NL



IL32 NL

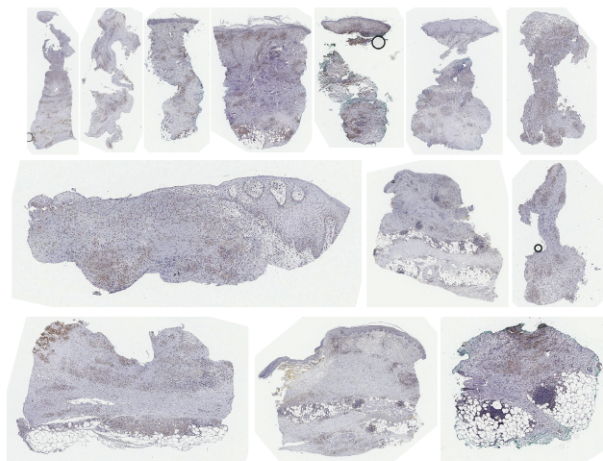
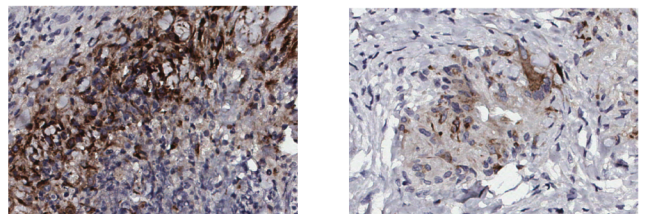
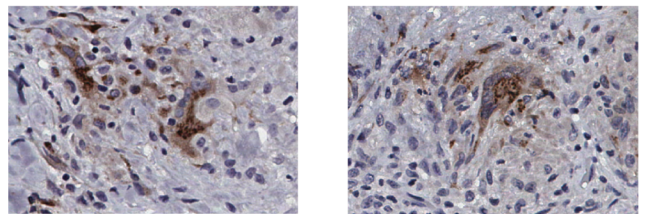
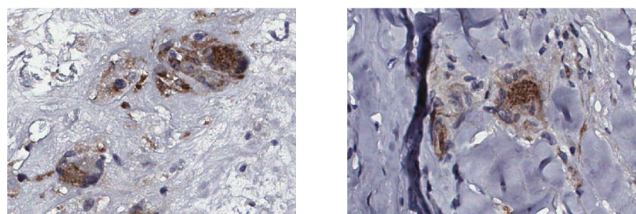
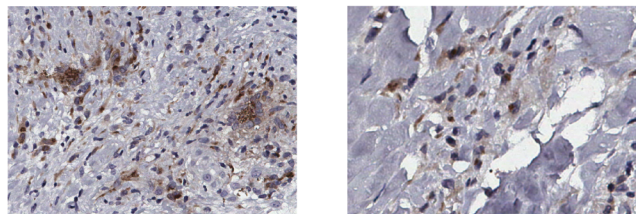
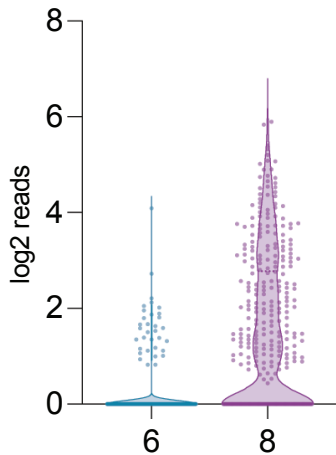


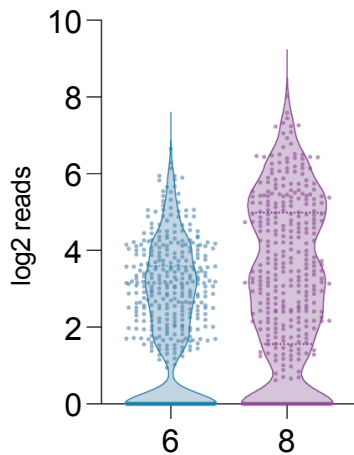
Figure 1 consists of six histological sections of the placenta, labeled (a) through (f), showing various types of hemorrhage. The sections are stained with hematoxylin and eosin (H&E). Red arrows indicate areas of hemorrhage. The hemorrhages are categorized as: (a) subchorionic, (b) chorionic, (c) deciduar, (d) fetal, (e) maternal, and (f) mixed.



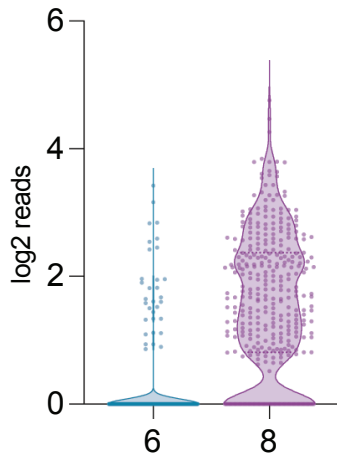
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CTGF

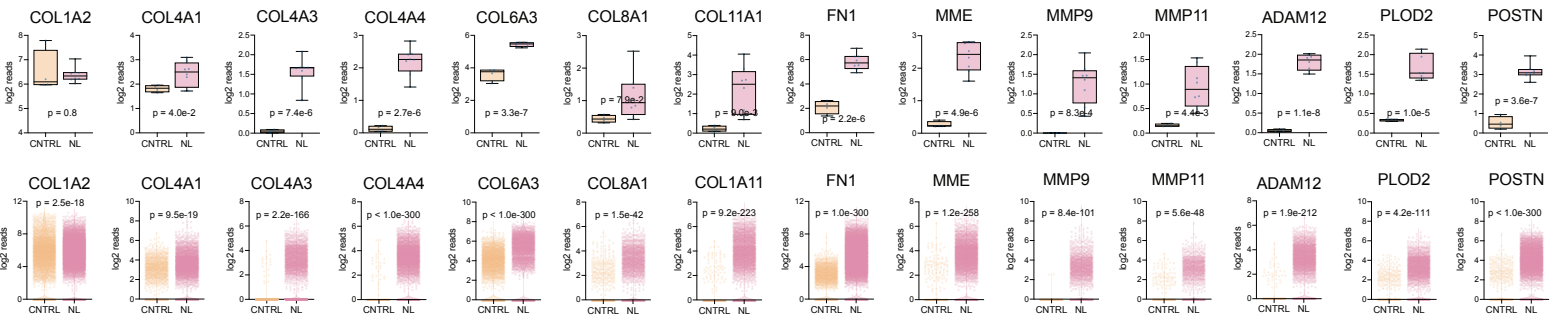


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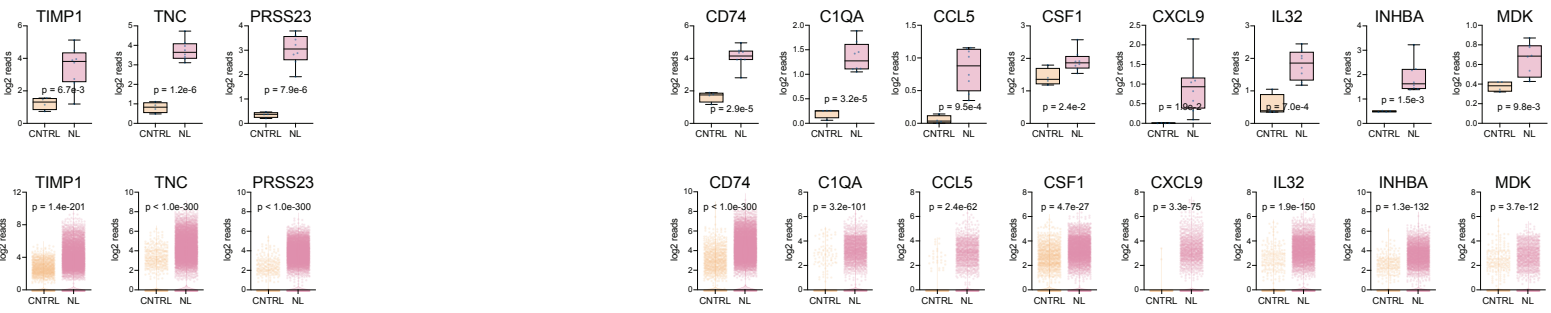


NLD

Extracellular Matrix-Related Genes

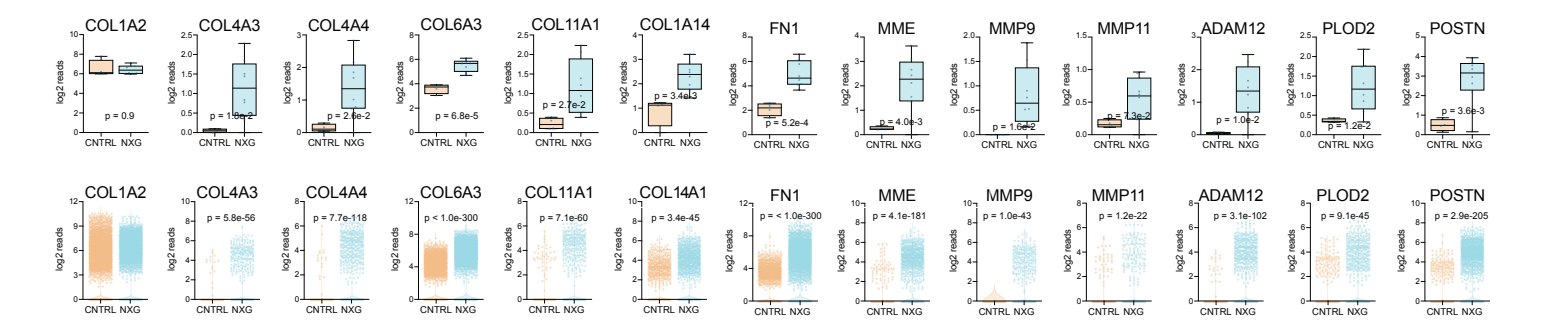


Immune-Related Genes

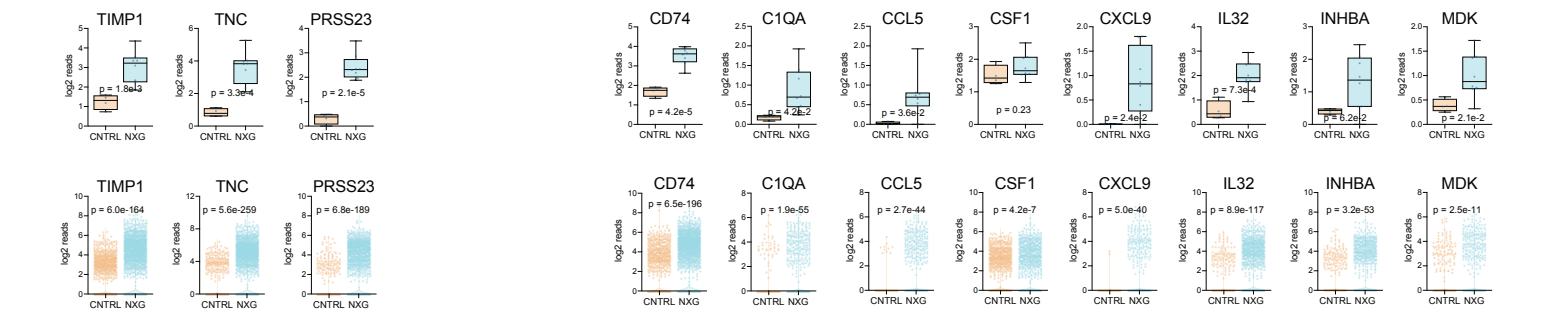


NXG

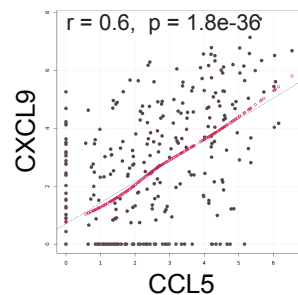
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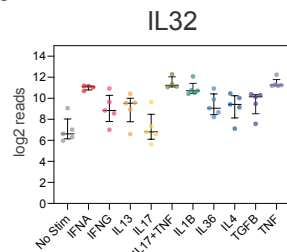
Immune-Related Genes



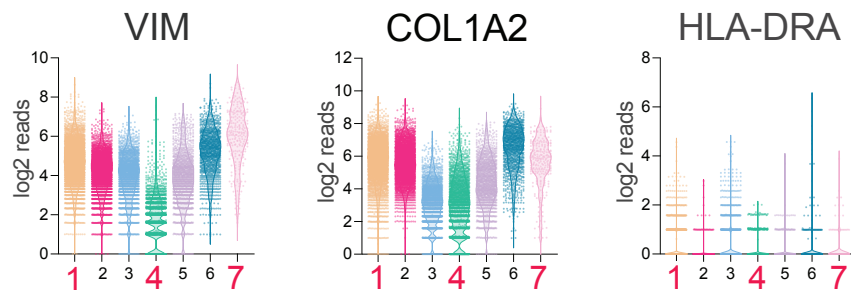
a



b



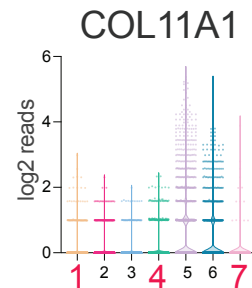
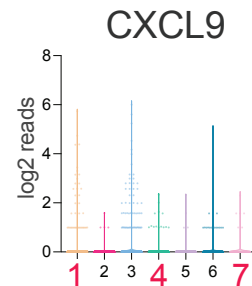
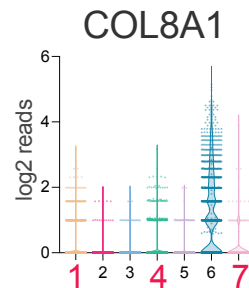
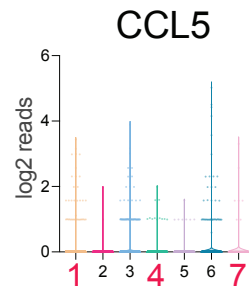
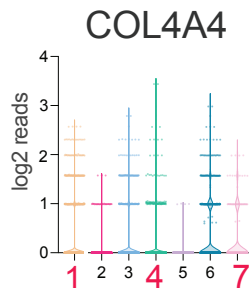
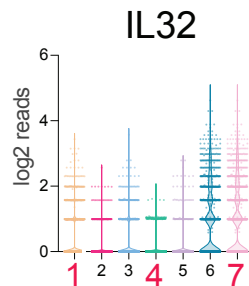
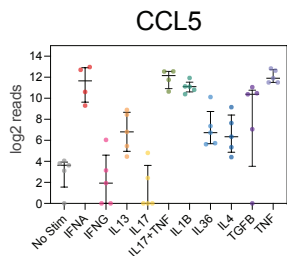
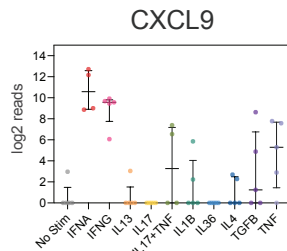
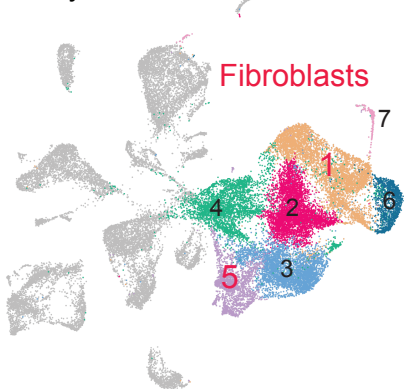
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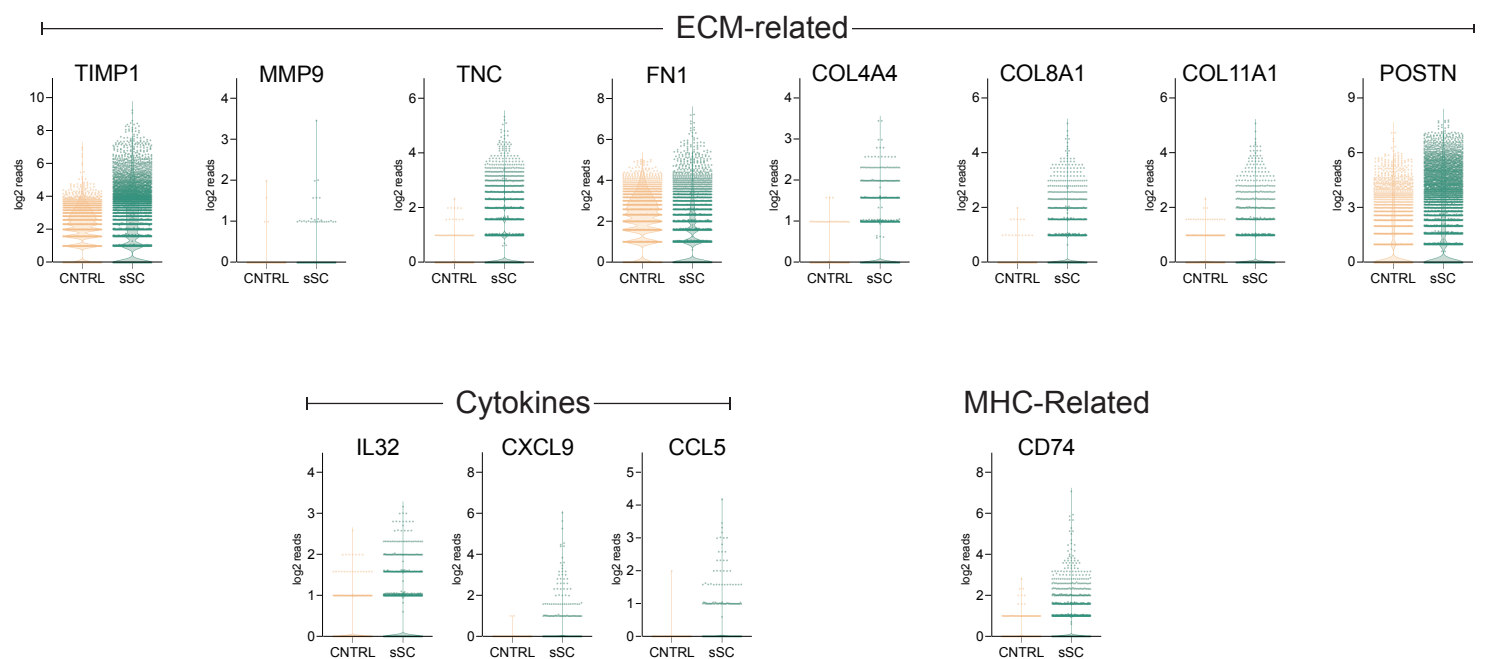
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Systemic Sclerosis

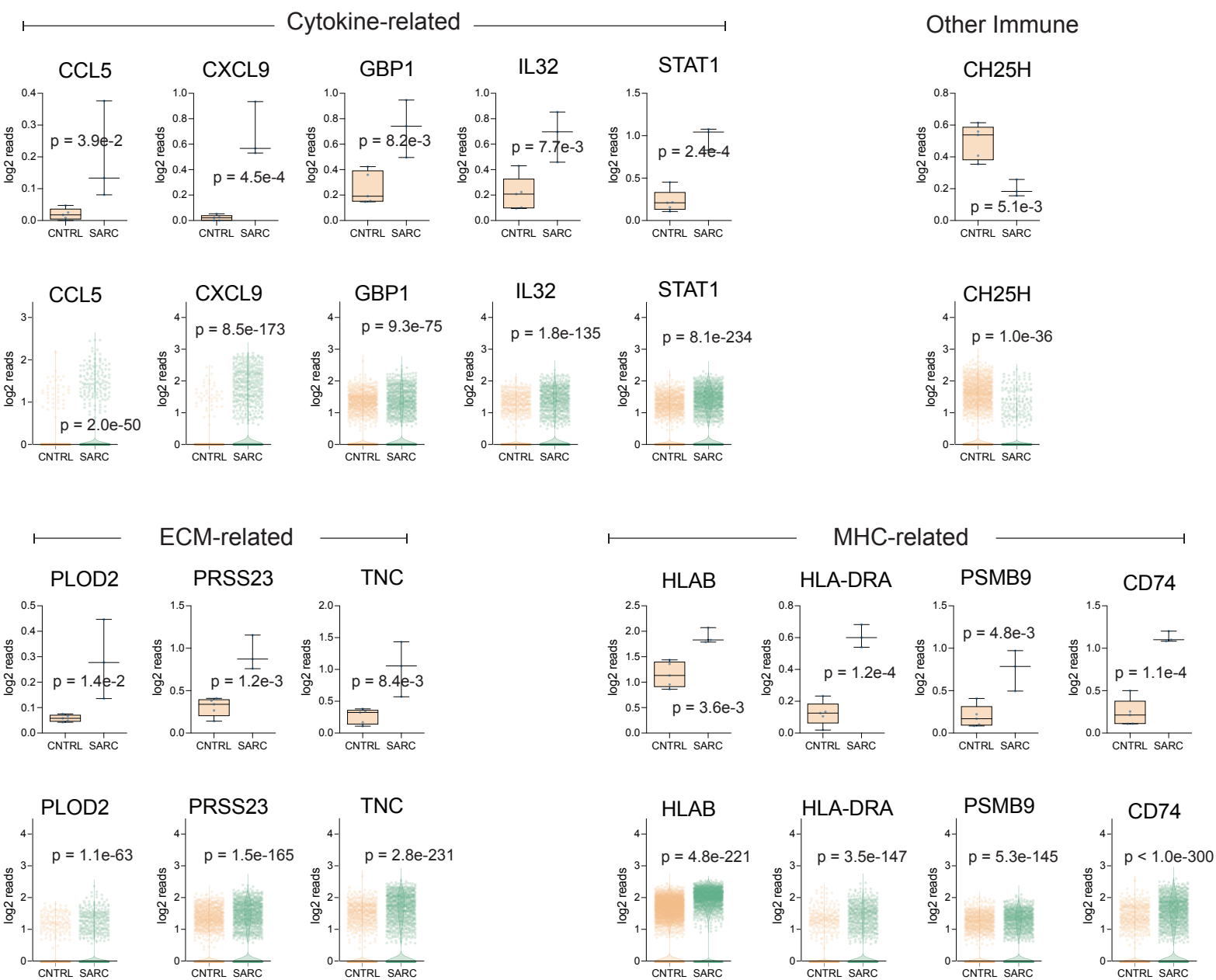
Fibroblasts



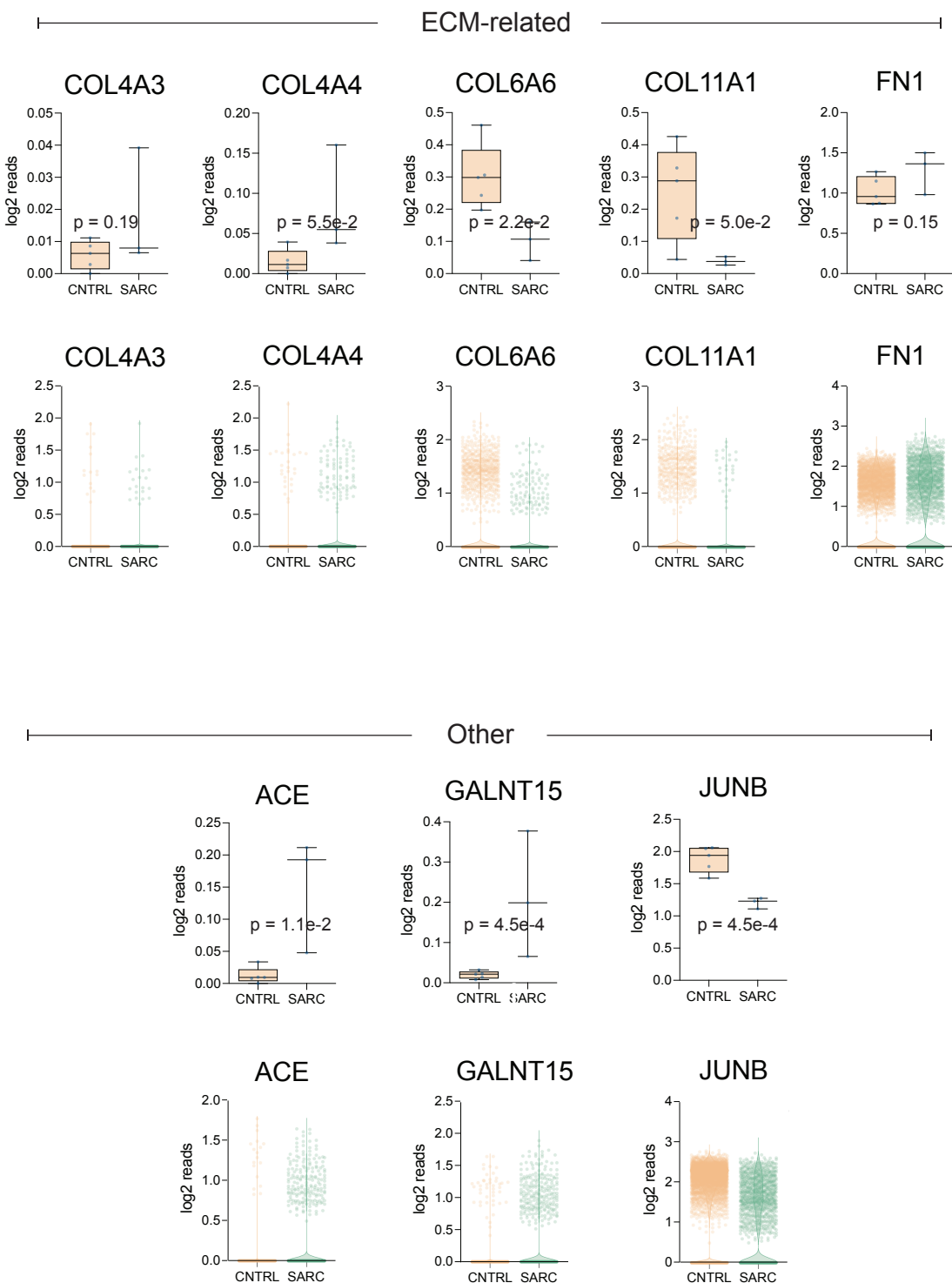
a Systemic Sclerosis



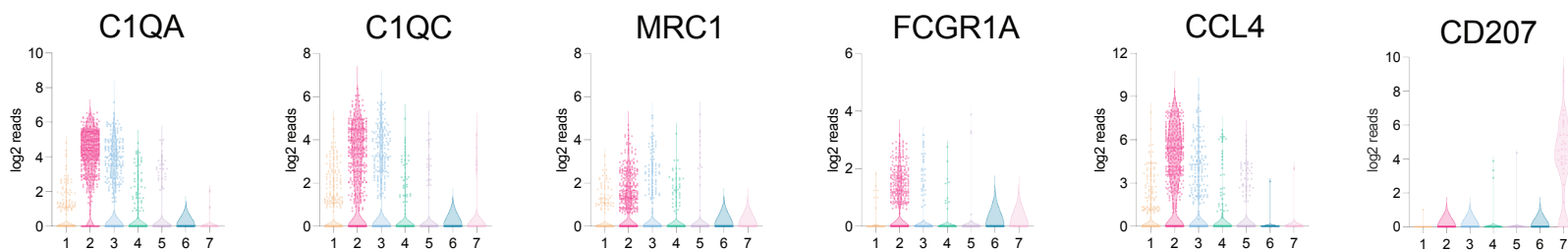
b Sarcoidosis (same direction as NL)



c Sarcoidosis (opposite direction to NL or no significant difference)



a

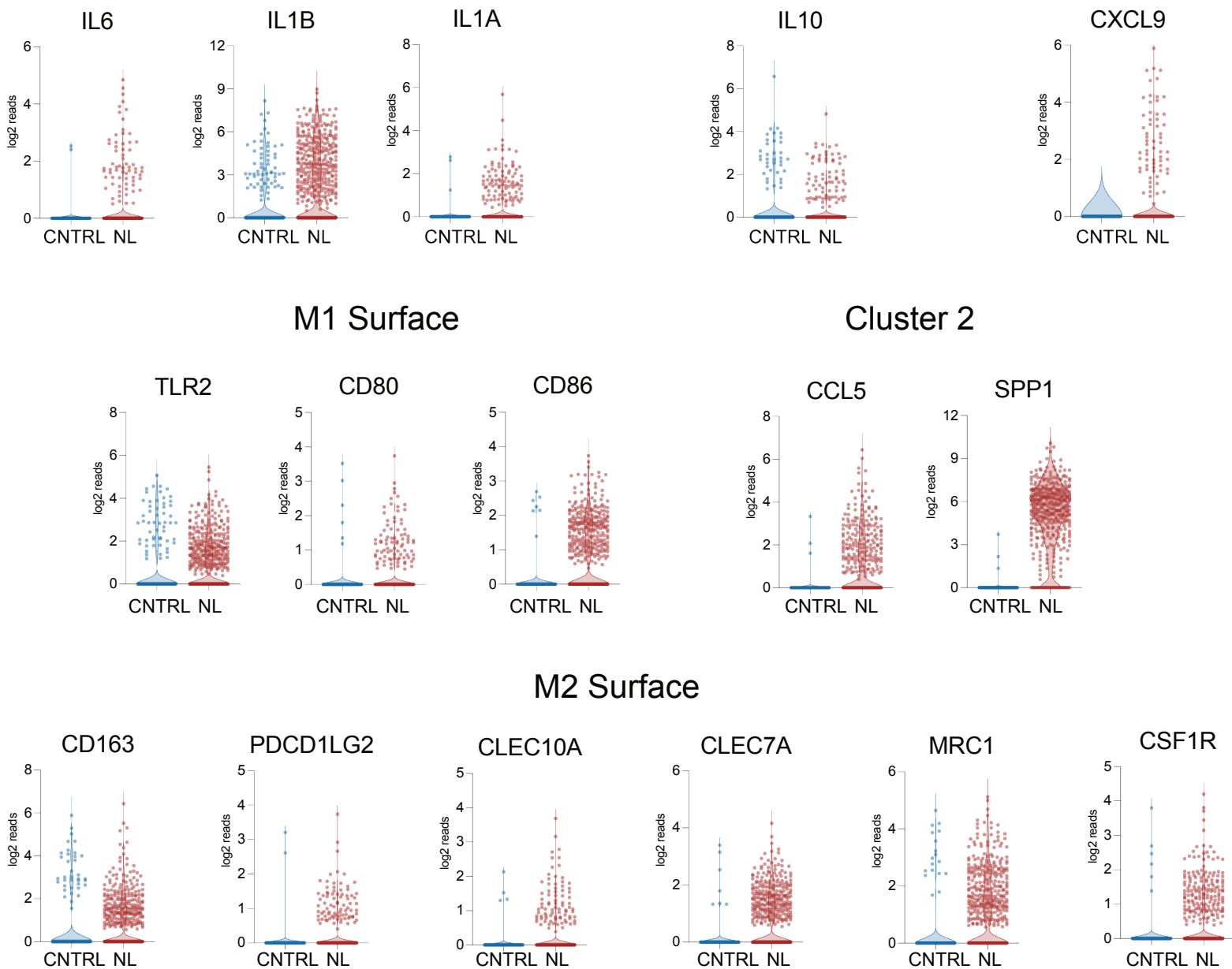


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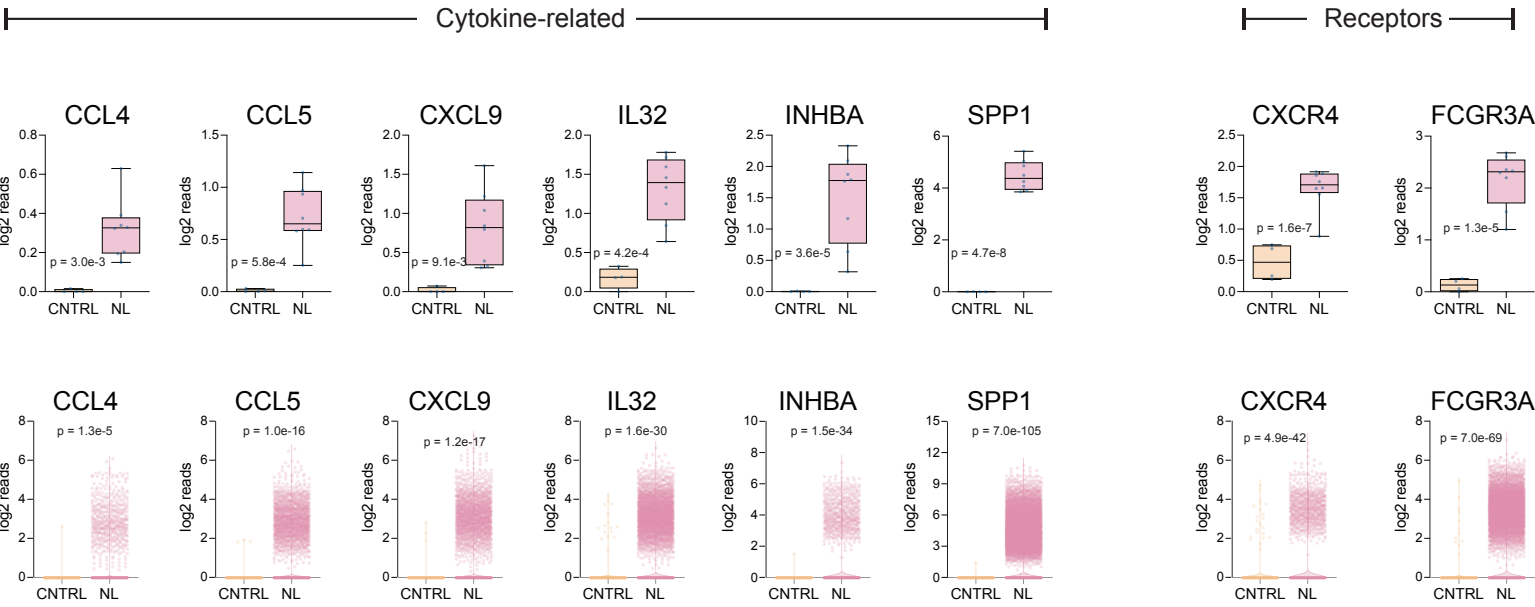
↑ M1 Effector

↓ M2 Effector

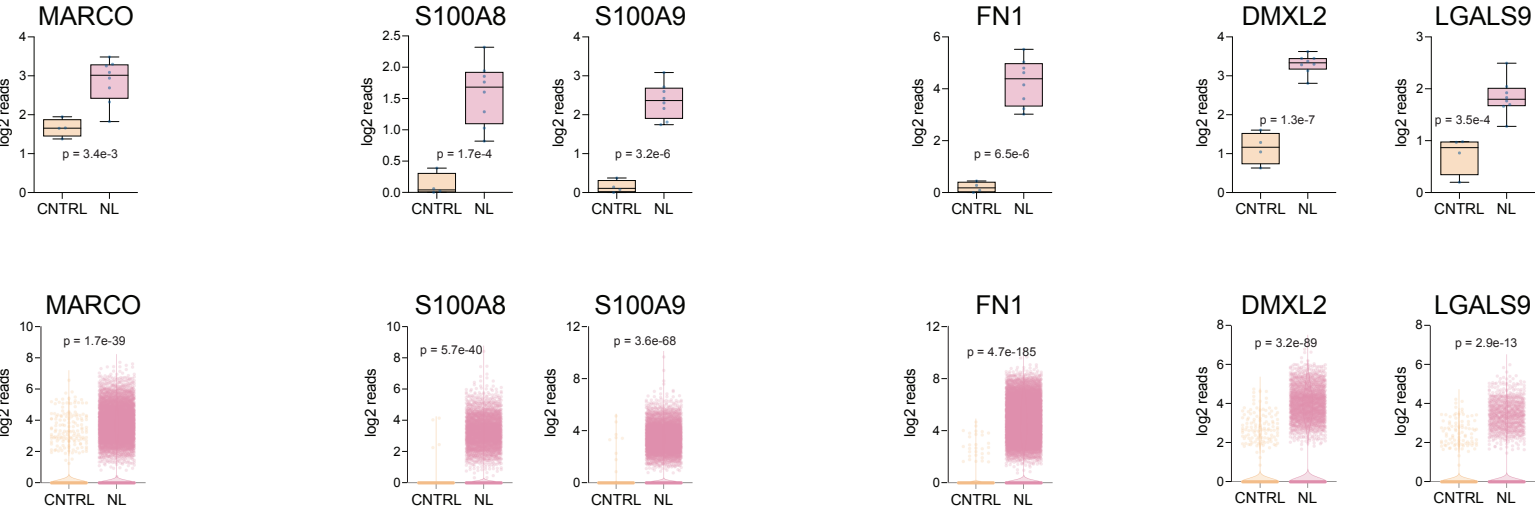
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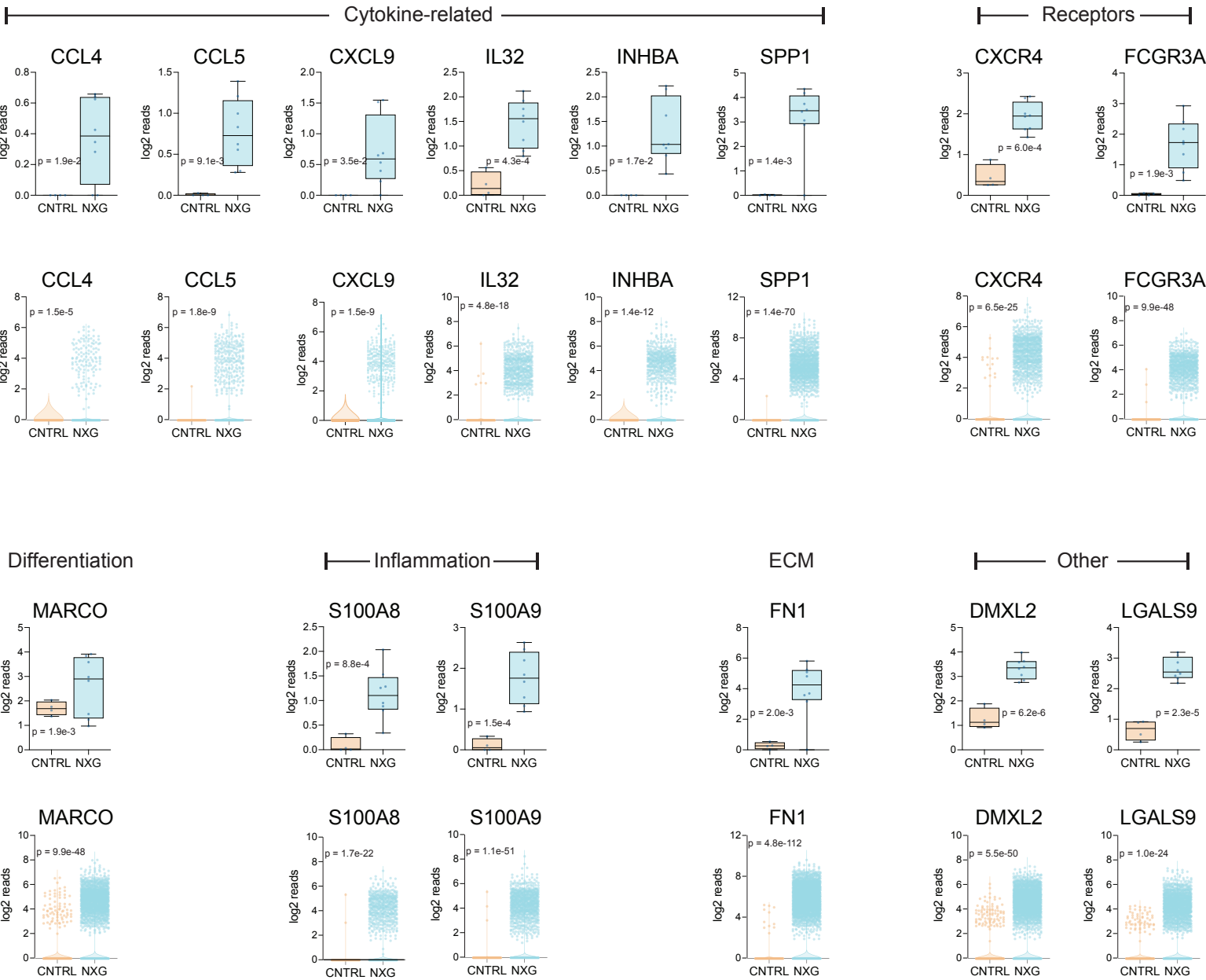
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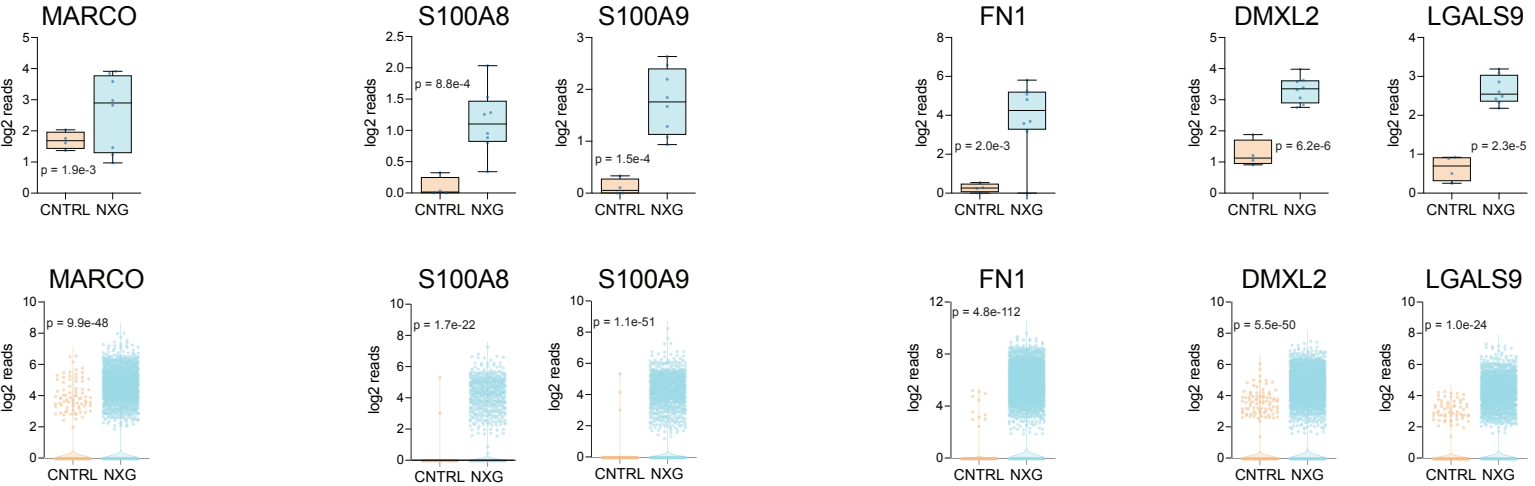
Differentiation



NXG

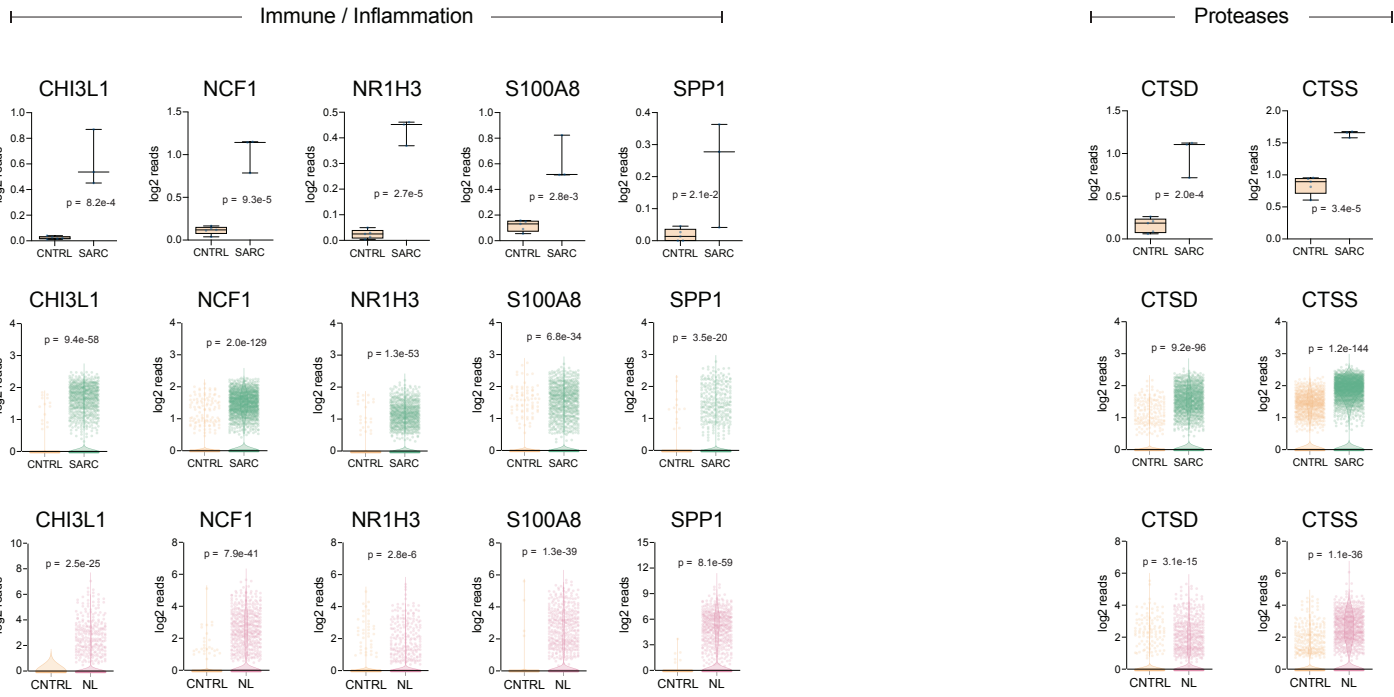
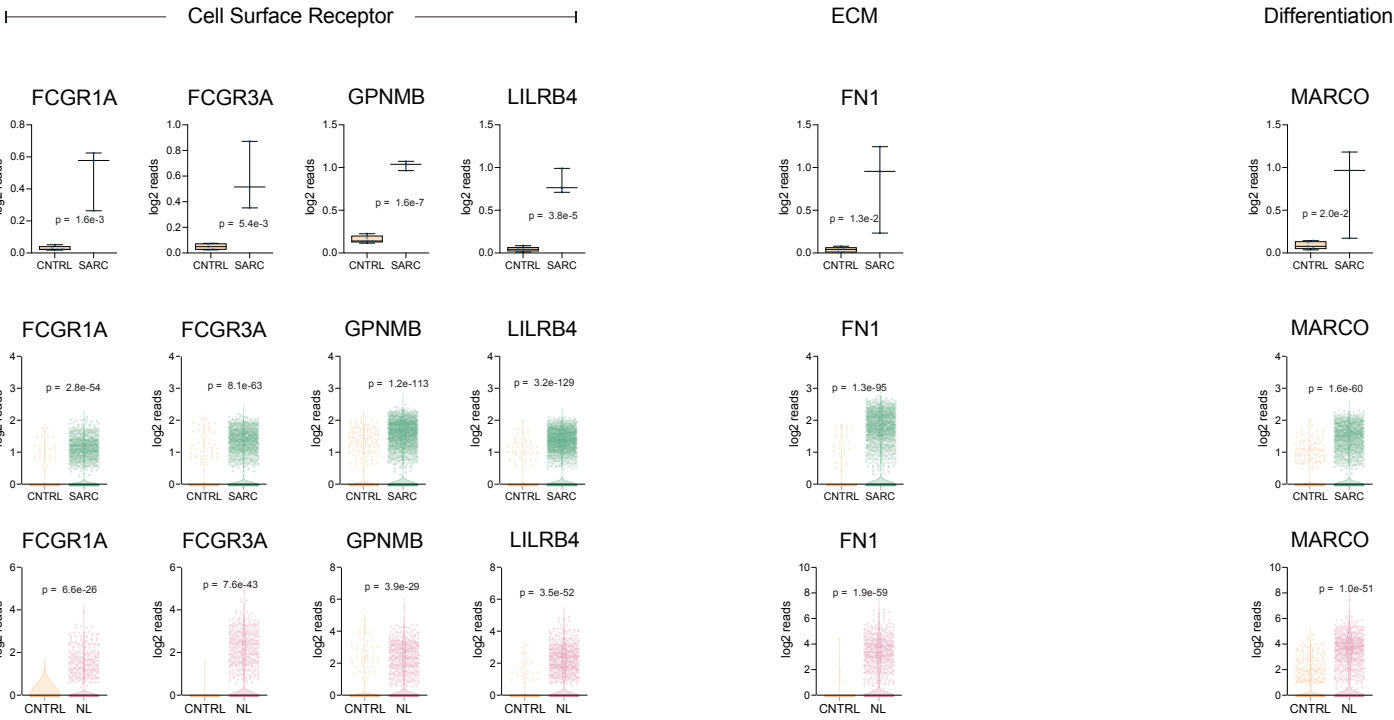
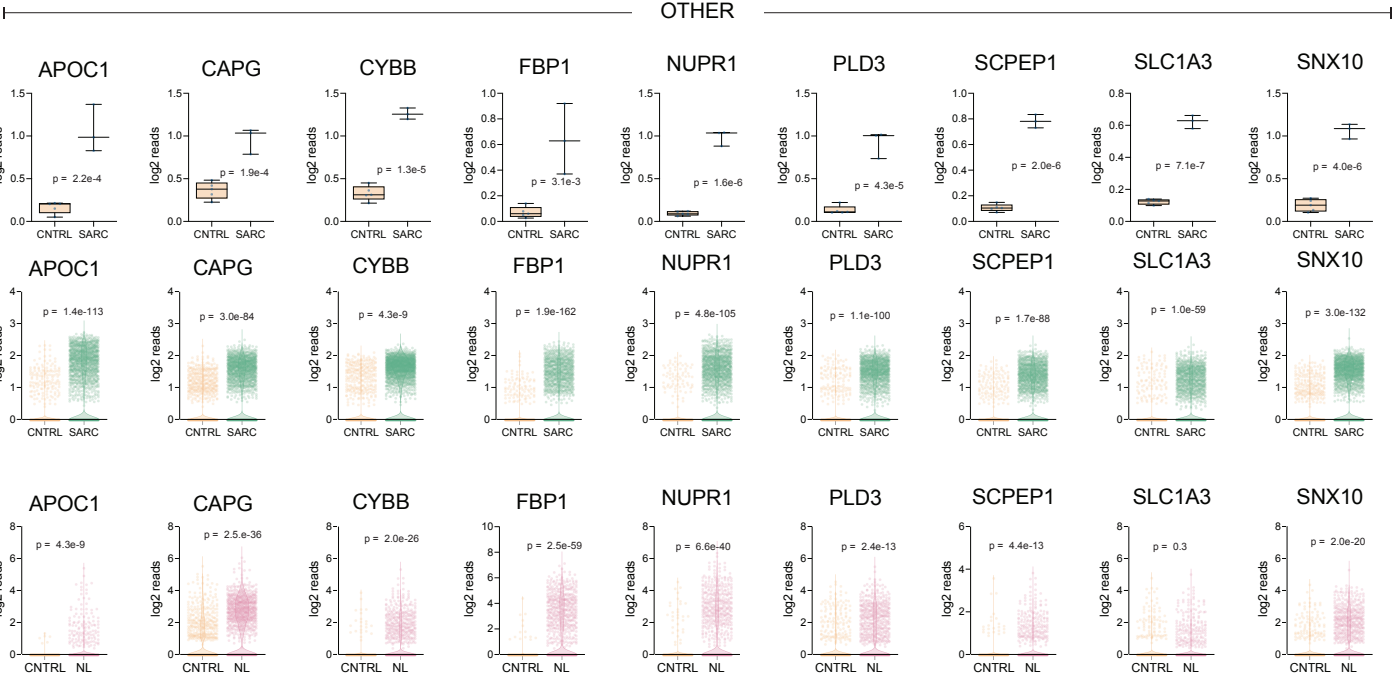


Differentiation



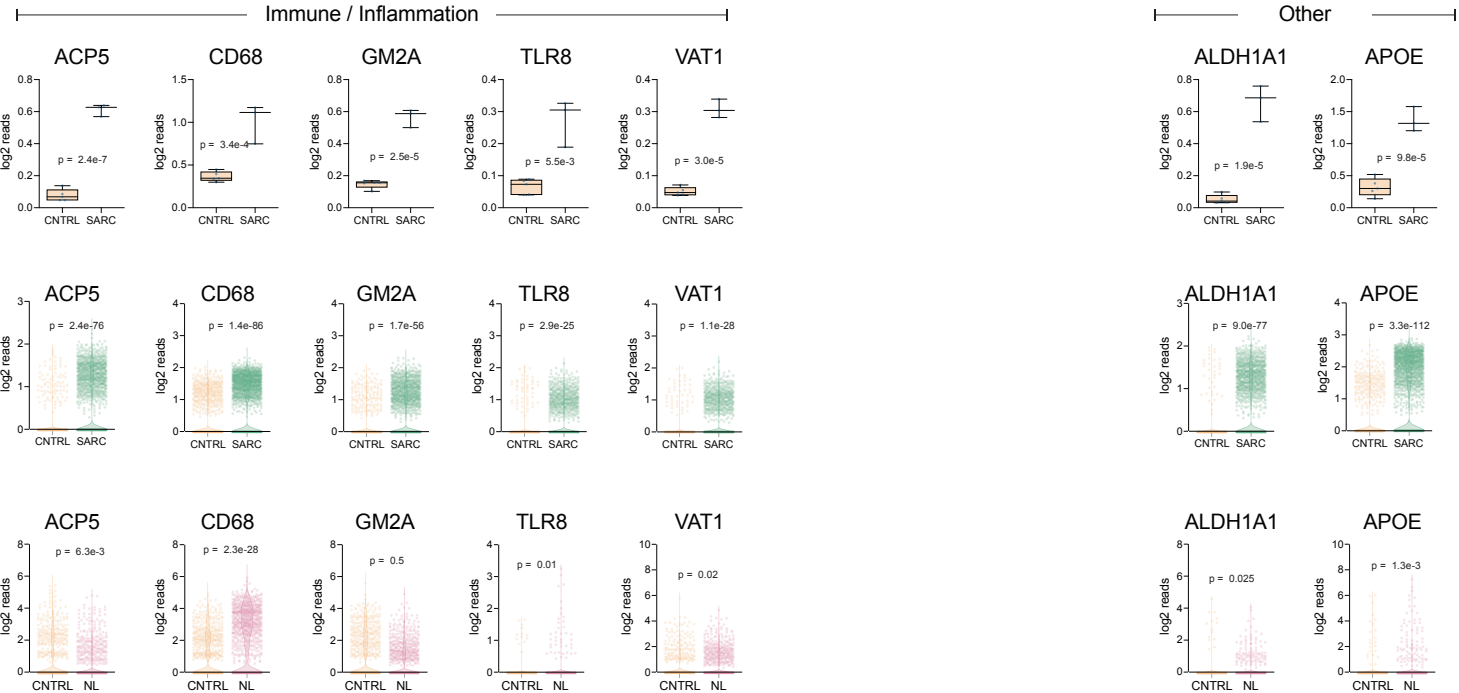
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SAME DIRECTIONALITY



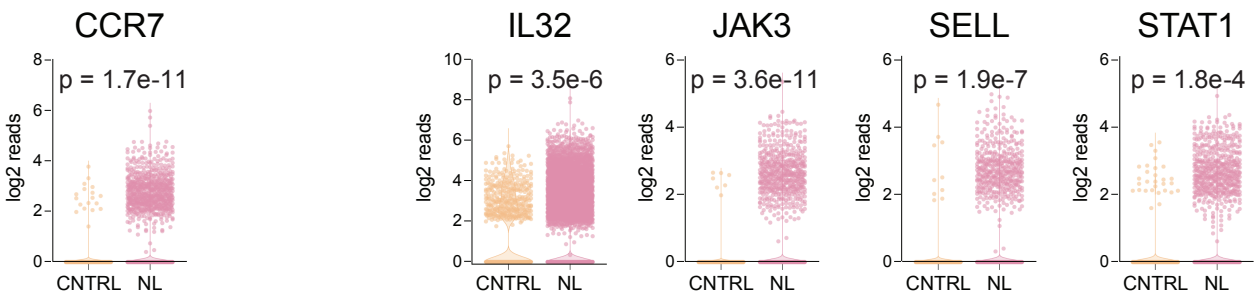
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MODEST TO NO UPREGULATION OR OPPOSITE DIRECTIONALITY

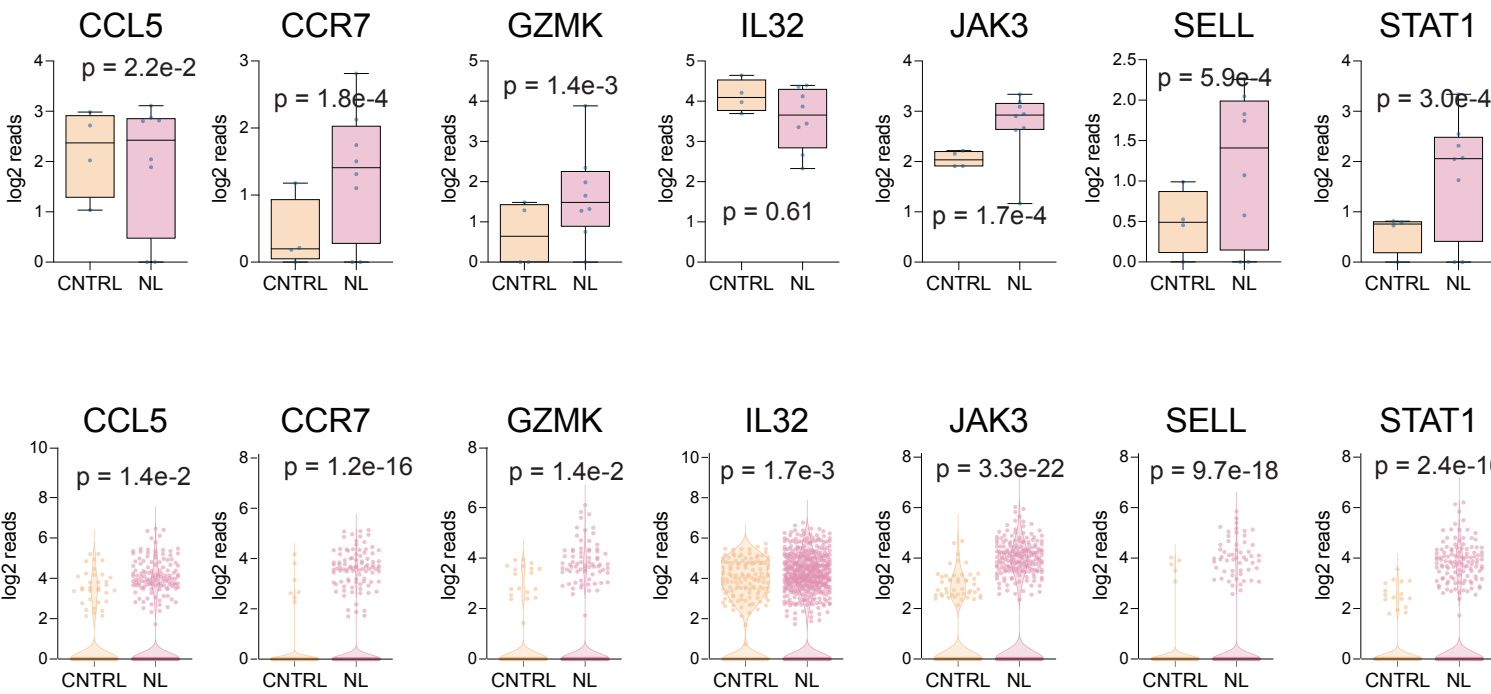


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FRESH NL T cells

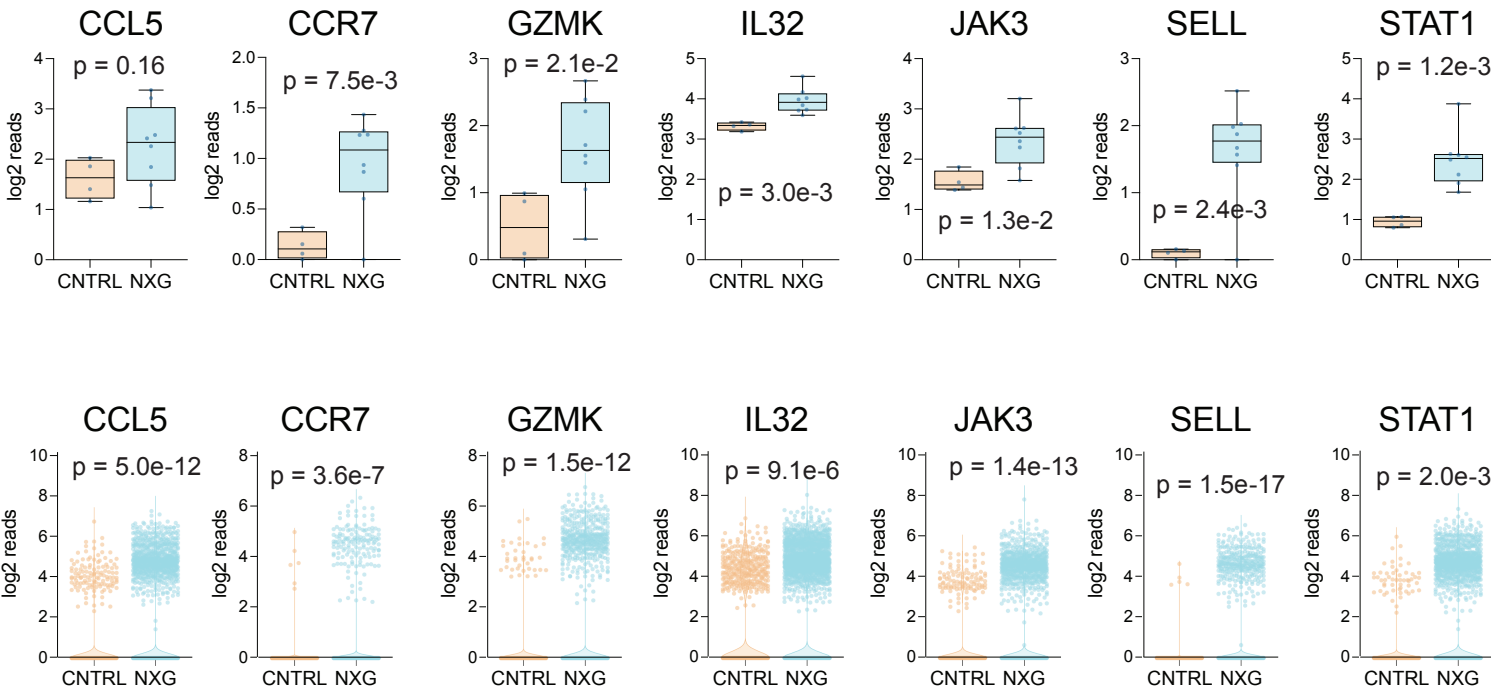


FRP NL T cells



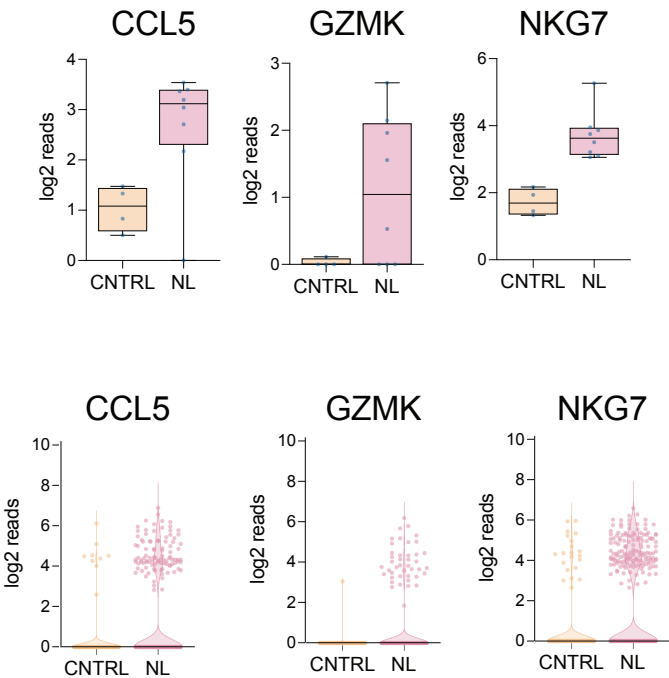
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FRP NXG T cells

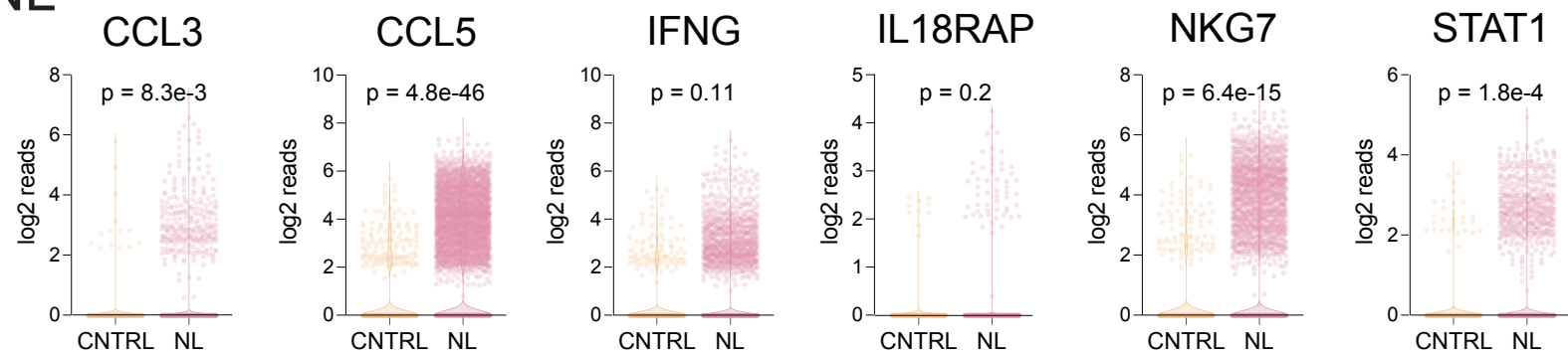


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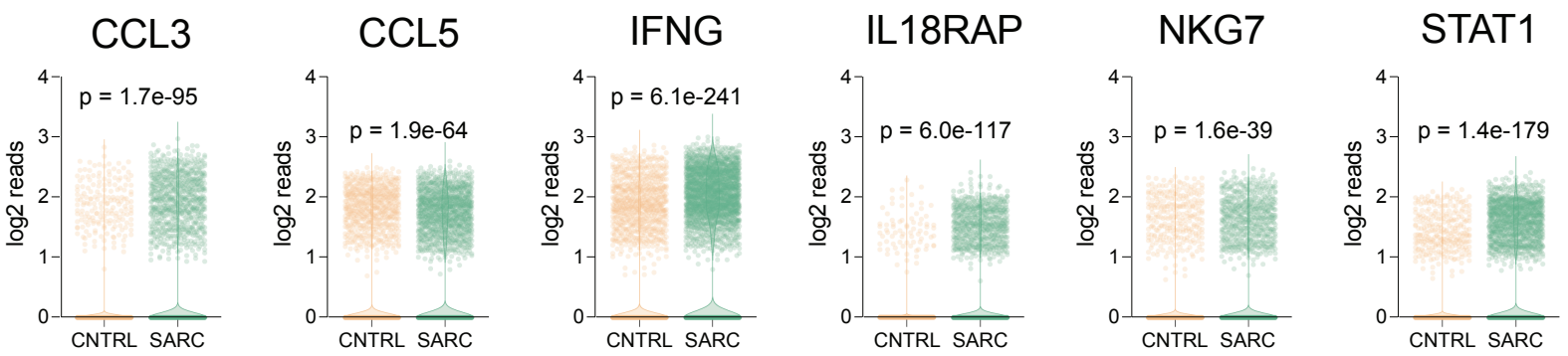
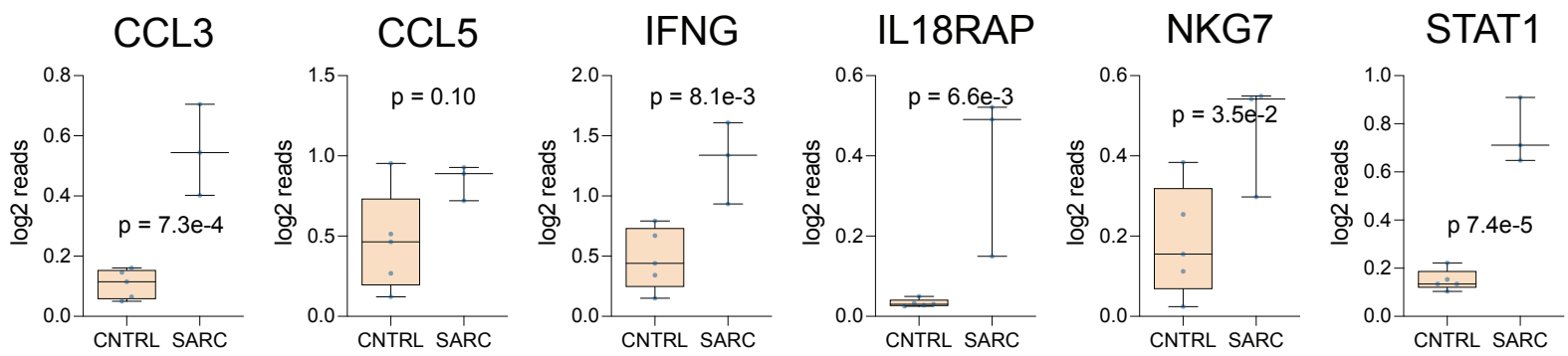
FRP NXG7-expressing T cells

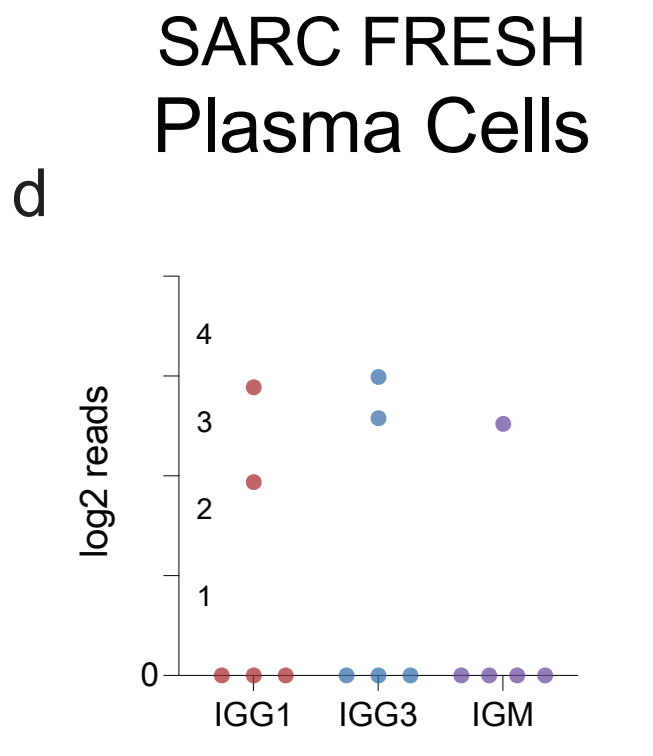
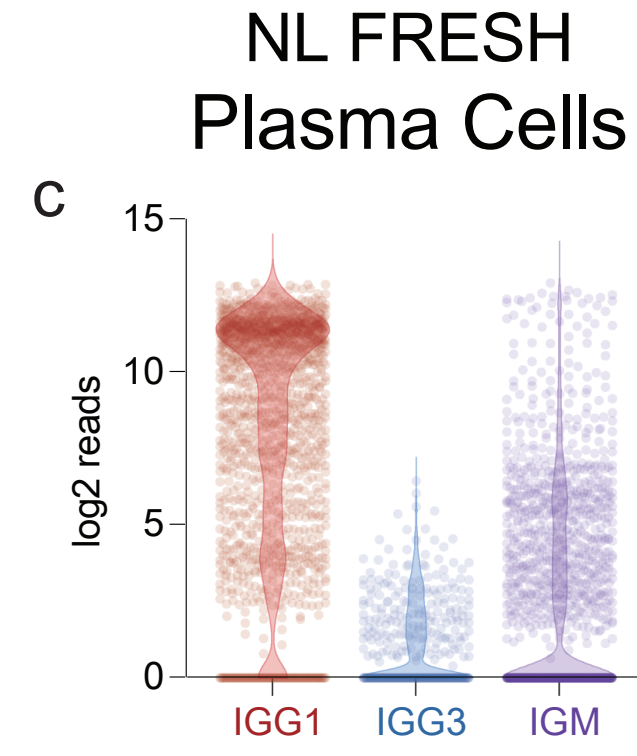
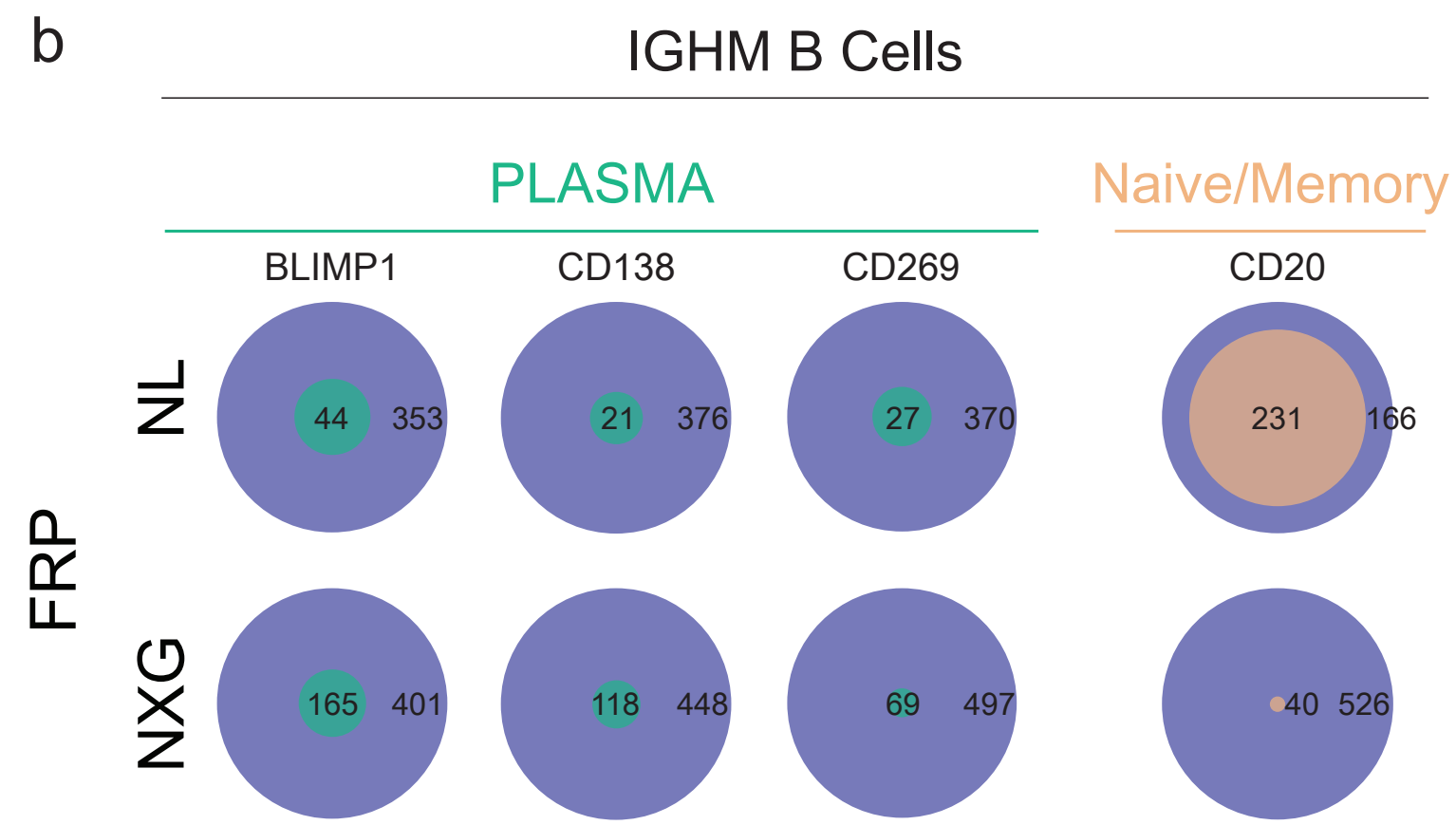
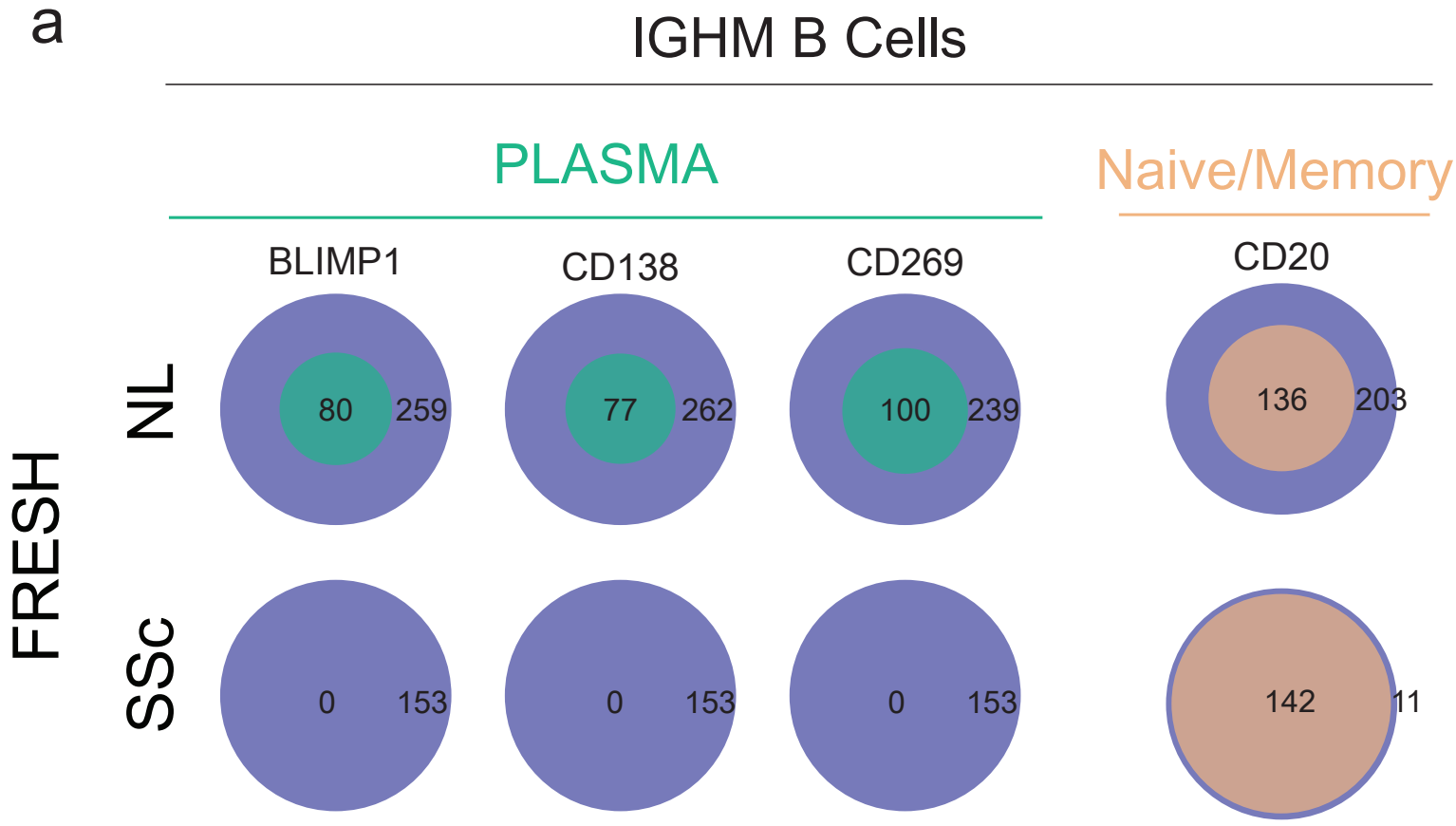


NL

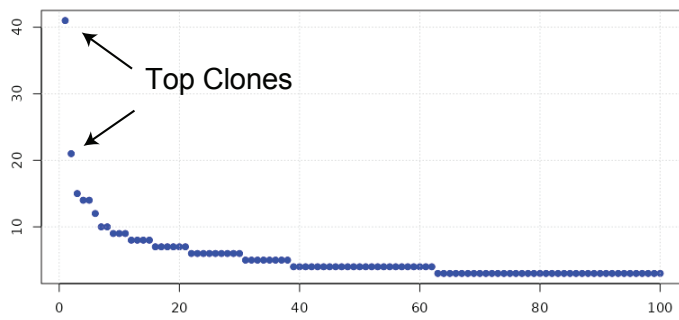


Sarcoid

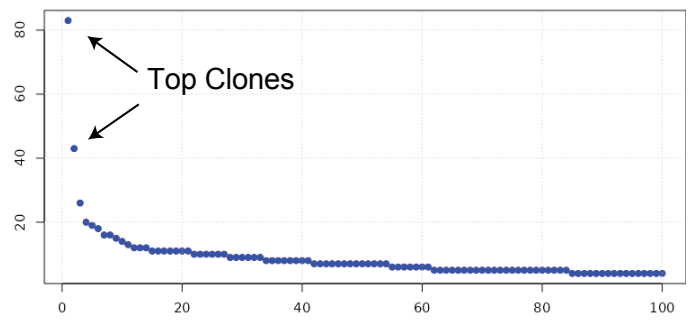




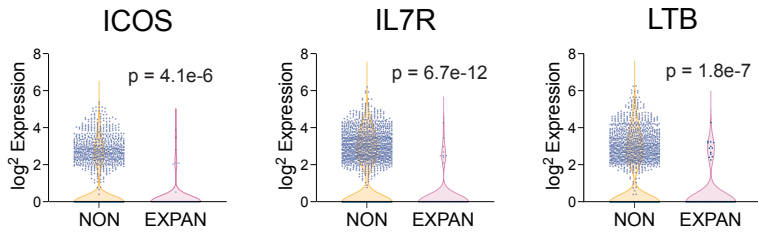
TRA



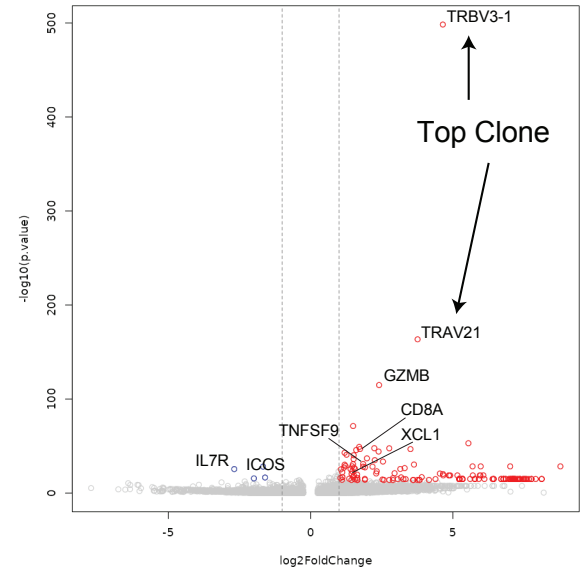
TRB



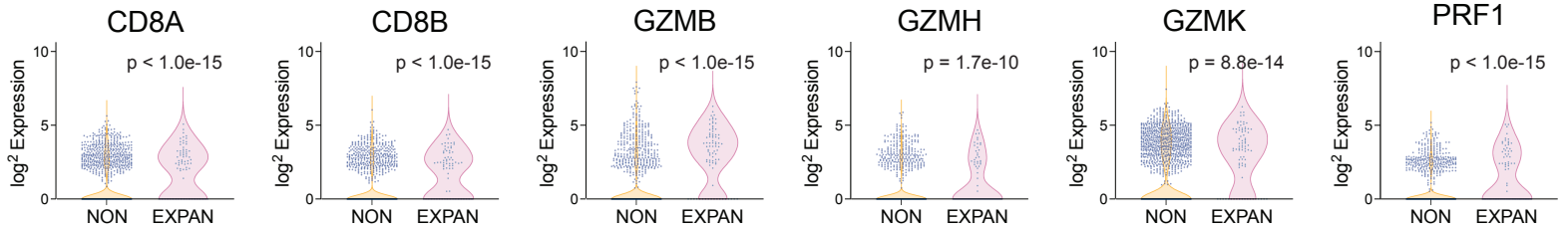
DOWNREGULATED GENES



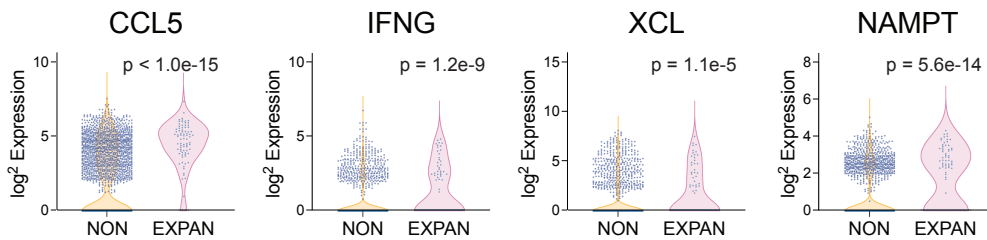
UPREGULATED GENES



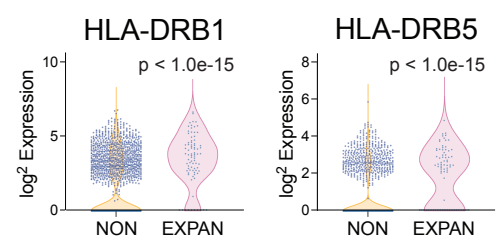
Cytotoxic T Cell Marker Genes



Effector Genes



Activation Markers



Cell Surface Receptors

