

# Implantable Niche Identifies the Immuno-metabolic Drivers of Type 1 Diabetes

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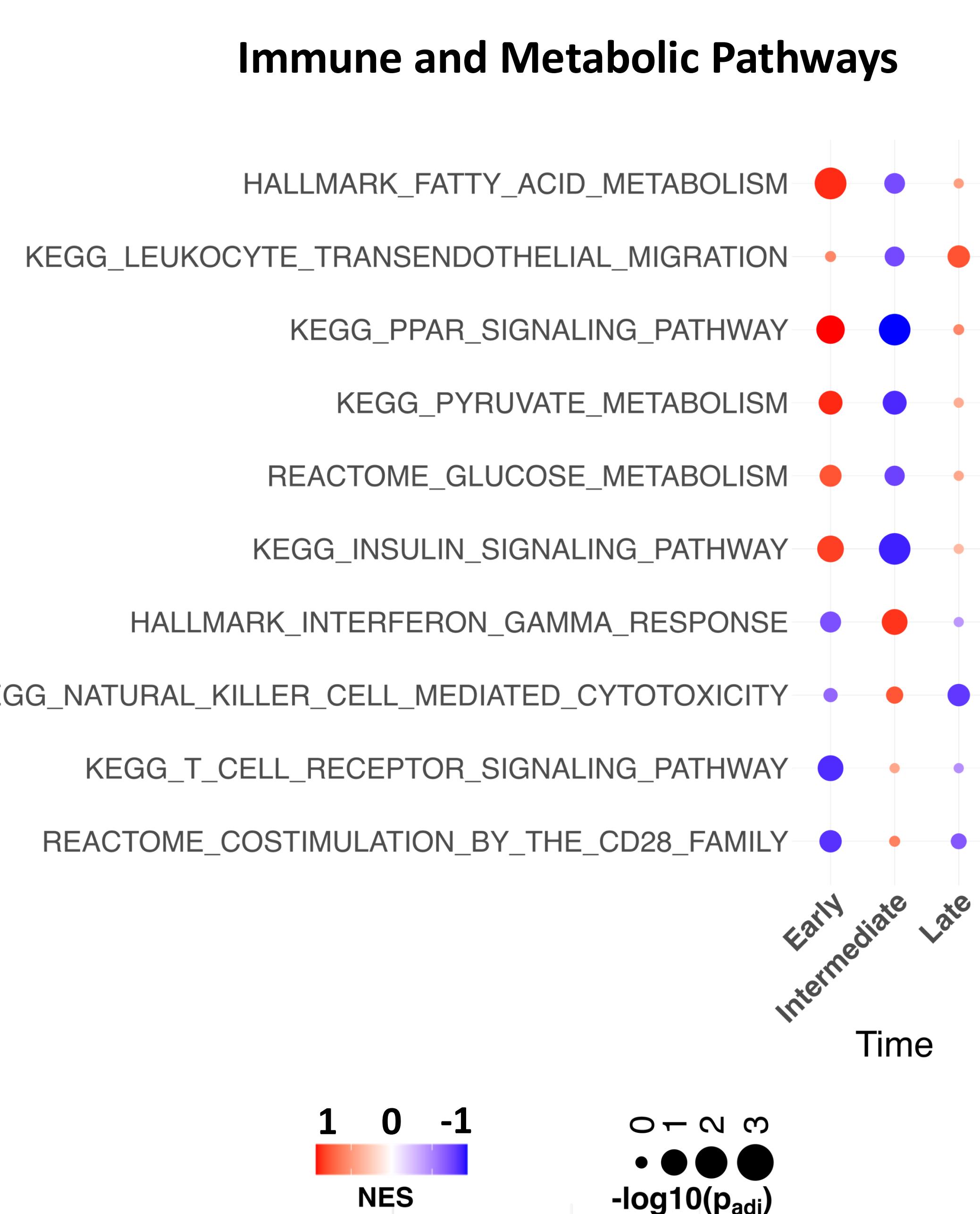
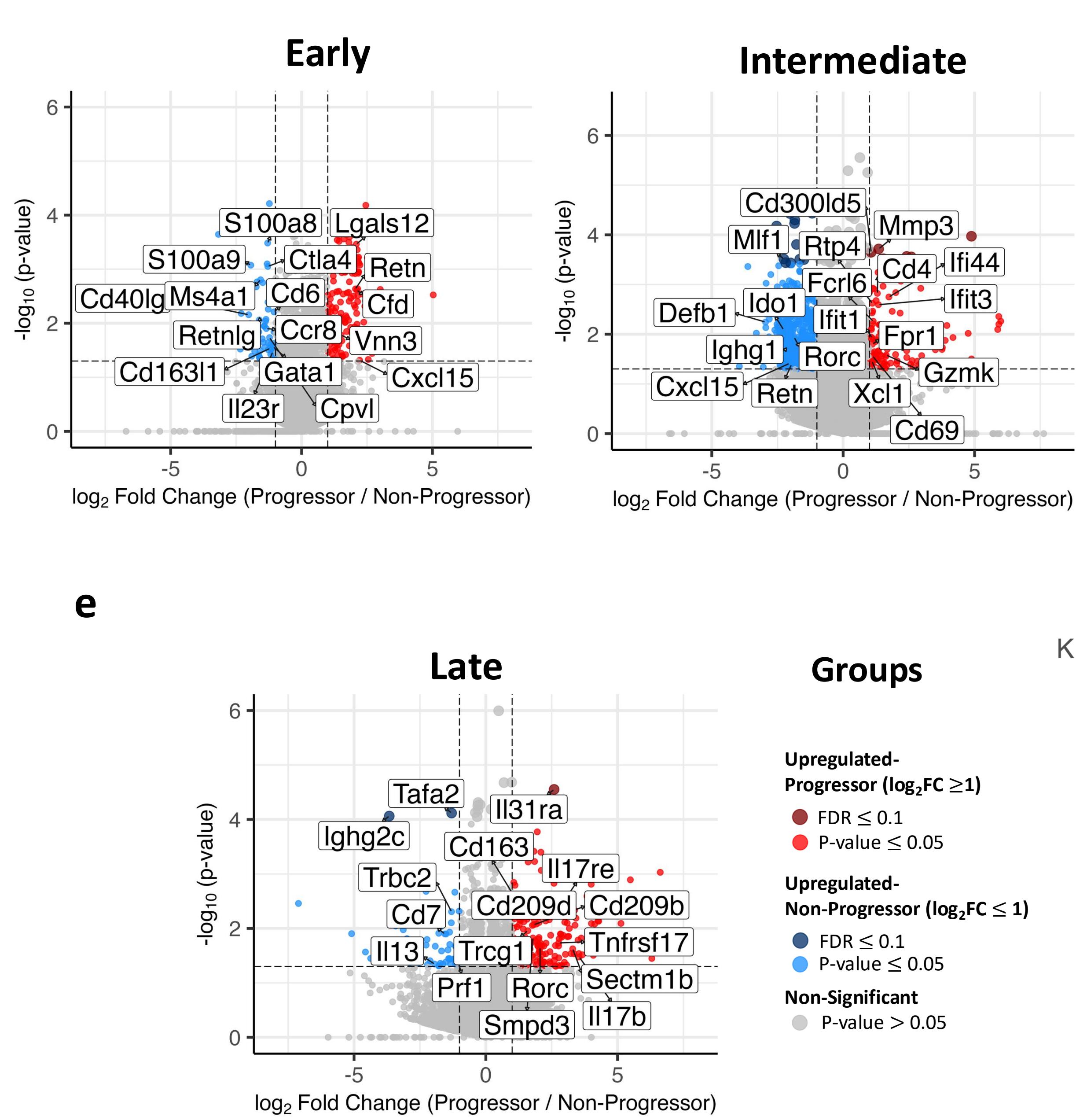
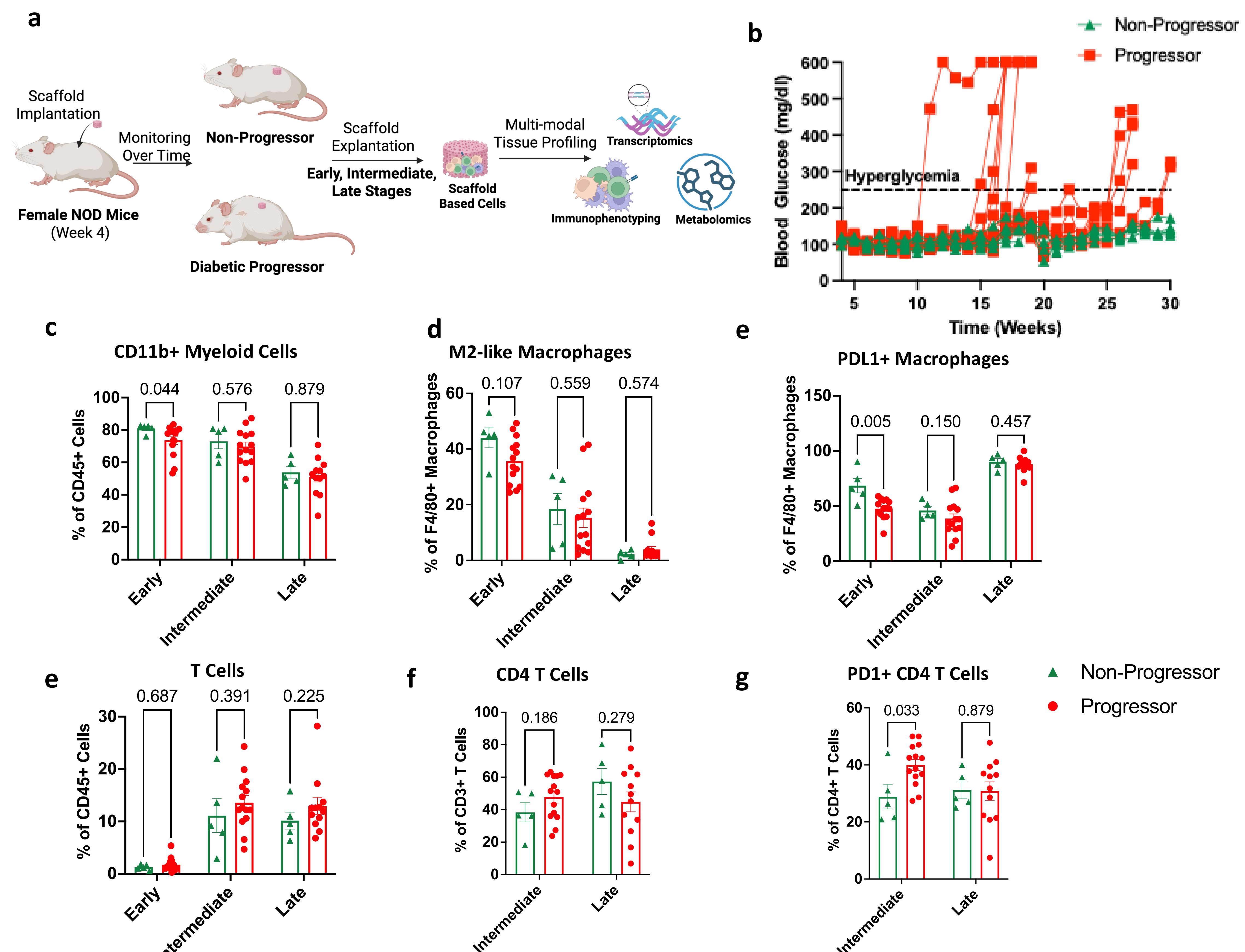
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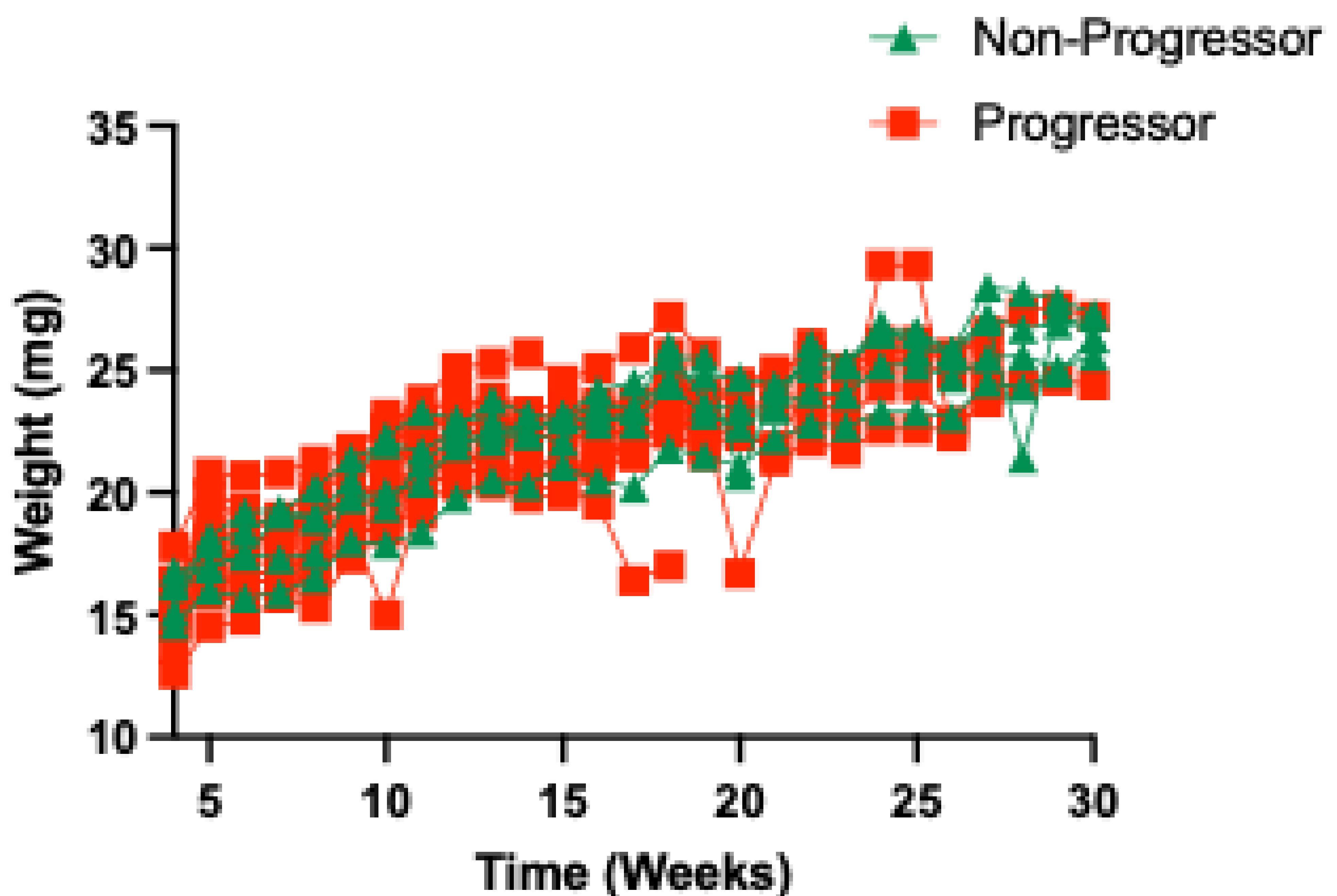
# Figure 1: Implantable Niche Based Multi-Omics Analysis Identifies Different Immuno-metabolic Profile Dynamics Between Diabetic Progressor vs Non-Progressor

1. Experiment Schematic
2. Volcano Showing Genes Changing Progressor Vs Non-Progressor-Early Timepoint
3. Volcano Showing Genes Changing Progressor Vs Non-Progressor-Intermediate Timepoint
4. Volcano Showing Genes Changing Progressor Vs Non-Progressor-Late Timepoint
5. GSEA showing Immune Related pathways are different between Progressor vs Non-Progressors
6. GSEA showing Metabolic pathways are different between Progressor vs Non-Progressor
7. Subtypes of macrophages and CD4 T Cell differences between progressor vs non-progressor
8. RainDrop Plot-Differentially Expressed Pathway and their Metabolites For Three Timepoints
9. DotPlot- Fold Change For Each Pathway For Three Timepoints

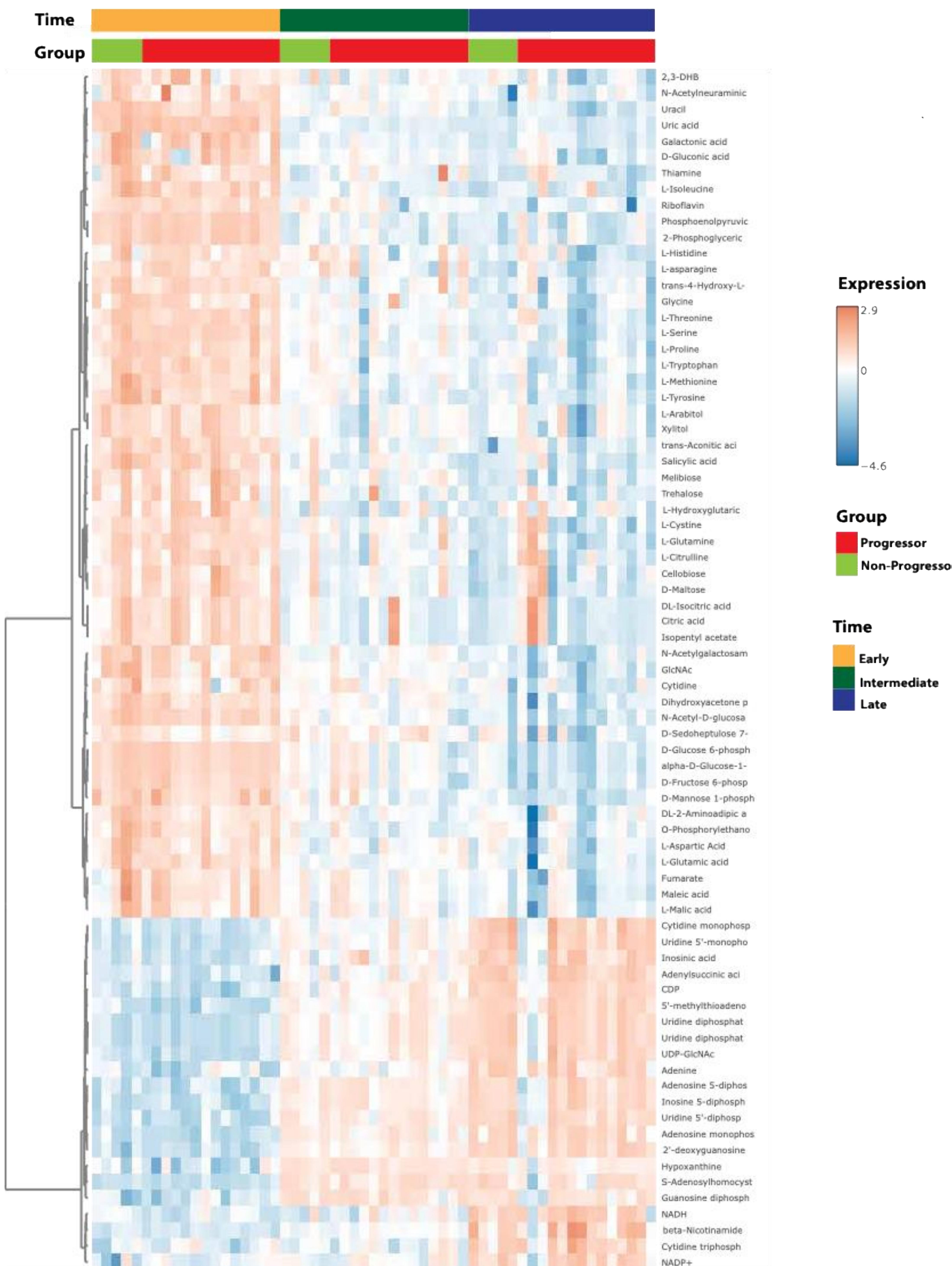


## Figure 1: Implantable Niche Based Multi-Omics Analysis Identifies Distinct Immuno-metabolic Profile Dynamics in Diabetic Progressor vs Non-Progressor:

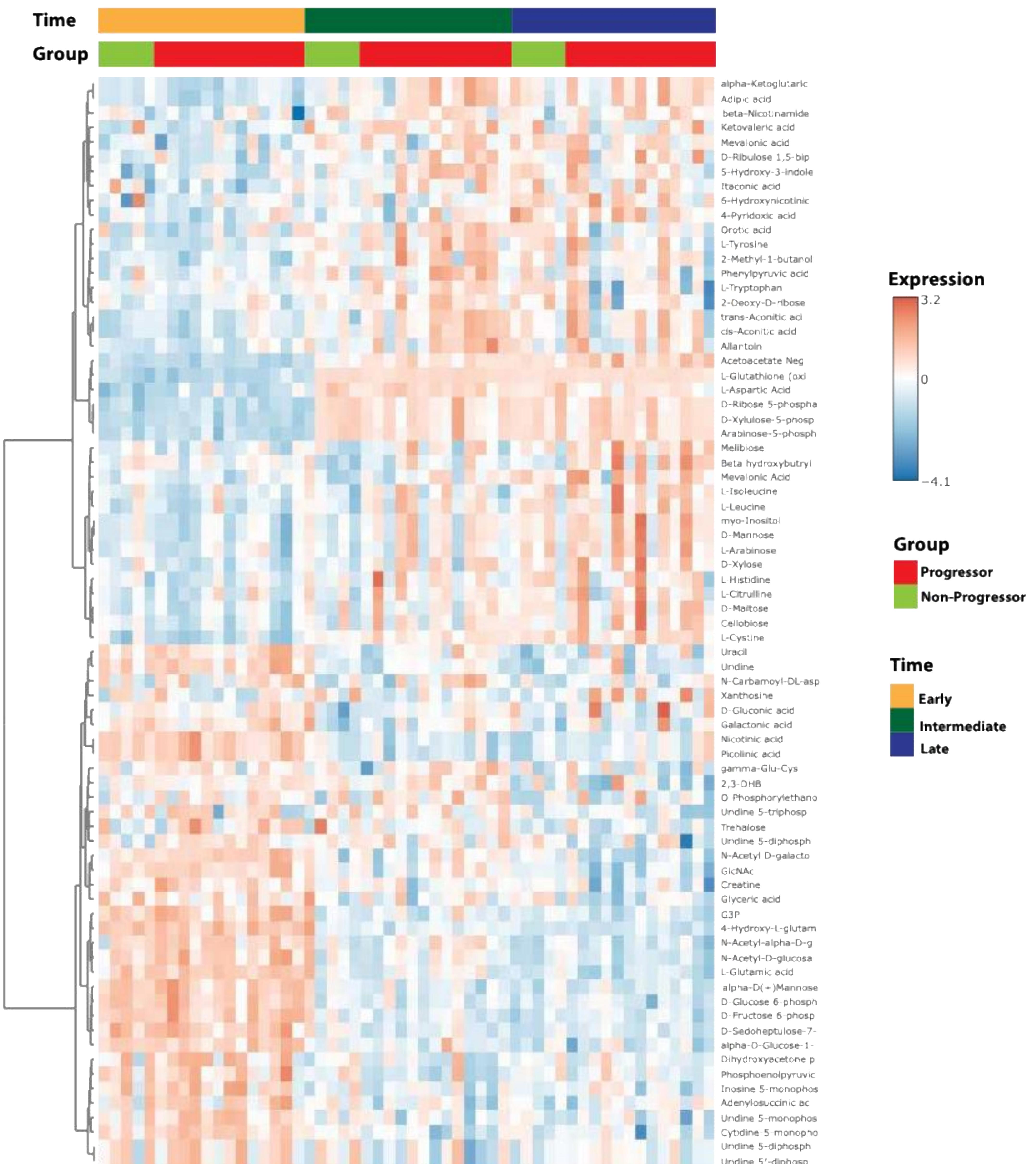
**a**, Schematic representation of experimental design for the study for characterization of immunological niche-based transcriptomics, metabolomics and immunological differences between diabetic progressors and non-progressors in NOD mouse model. **b-d**, Volcano plots showing differentially expressed genes ( $p\text{-value} < 0.05$  and  $\text{Log}_2\text{FC} > 1$  or  $< -1$ ) in progressors vs non-progressors over time with samples from three independent cohorts. **b**, Early Stage ( $n=16-24$  per group). **c**, Intermediate Stage ( $n=10$  per group). **d**, Late Stage ( $n=4-10$  per group). **e,f**, Geneset Enrichment Analysis(GSEA) identified changes immune and metabolic pathways in progressors vs non-progressors over time ( $p\text{-value} < 0.05$  and  $\text{NES} > 1$  or  $< -1$  for atleast one timepoint). **e**, Differentially Expressed Immune signaling related pathways. **f**, Differentially Expressed Metabolic pathways. **g-m**, Differences in frequency of niche based myeloid and lymphoid cells in progressor vs non-progressor over time **g**, Frequency of myeloid immune cells. **h**, Proportion of M2-like macrophages. **i**, Proportion of PD-L1+ macrophages. **j**, Frequency of T Cells. **k**, Proportion of CD4+ T Cells. **l**, Proportion of PD1+ CD4 T Cells. Data represent mean  $\pm$  SEM ( $n = 5-14$  per group). Comparisons between groups were calculated using unpaired Student's *t*-test. **m**, Pathway enrichment analysis based on metabolic pathways in the KEGG database quantifies metabolic changes progressor and non-progressor in early, intermediate and late stage. Pathway impact and results of statistical tests using MetaboAnalyst 5.0 are reported ( $n = 9-14$  per group). **n**, Raindrop plot representing fold-change (FC) of individual metabolites present in some of the significantly dysregulated metabolic pathways as defined by the KEGG knowledge base. Circle colours represent  $\log(\text{FC})$  of metabolites in cells expressing progressor with respect to non-progressor in early(red), intermediate(green) and late (blue) stage. Statistical analysis performed using two-sample *t*-tests built into MetaboAnalyst 5.0.



**Fig S1:** Body weights are not significantly different between diabetic progressor vs non-progressor



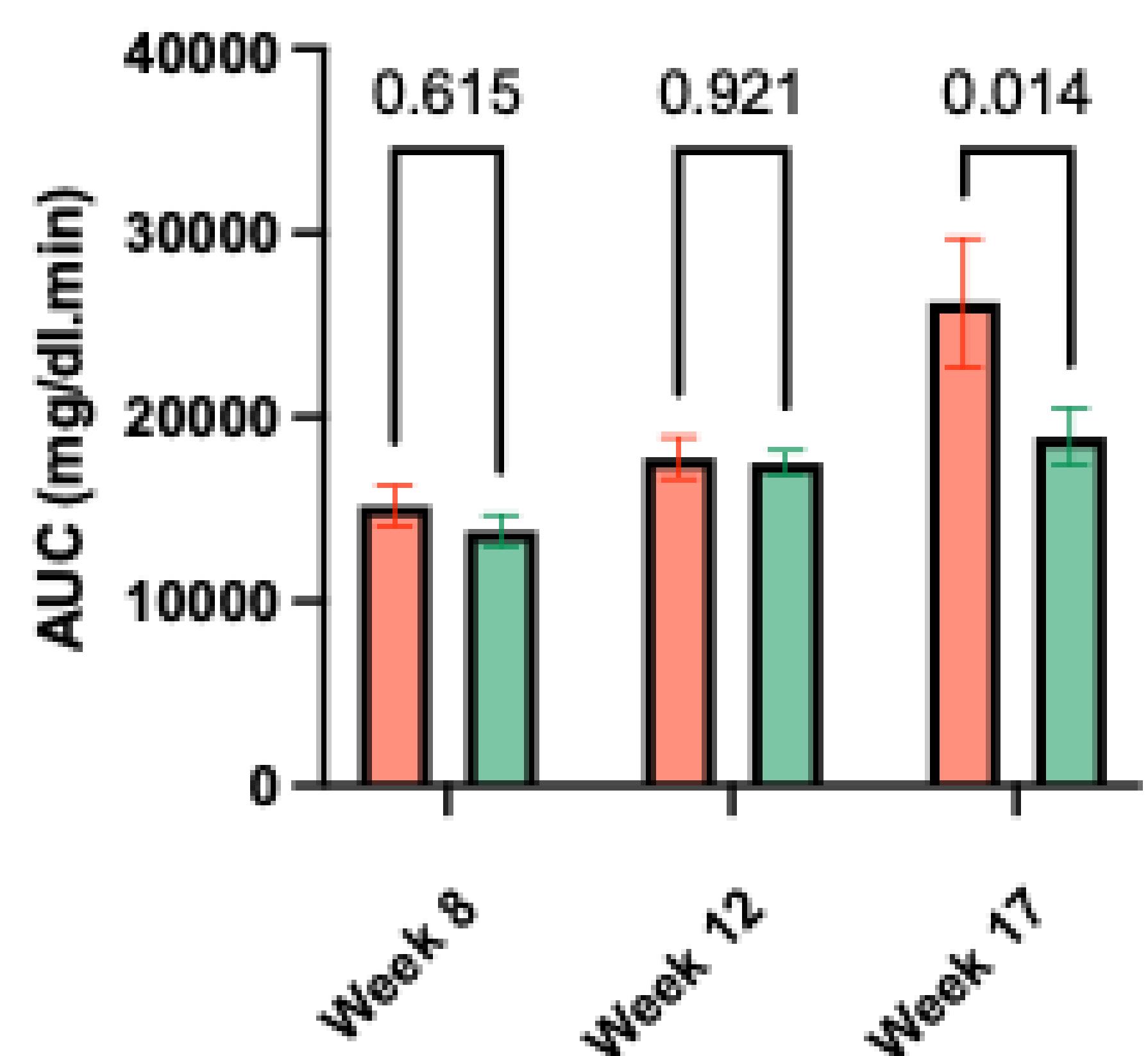
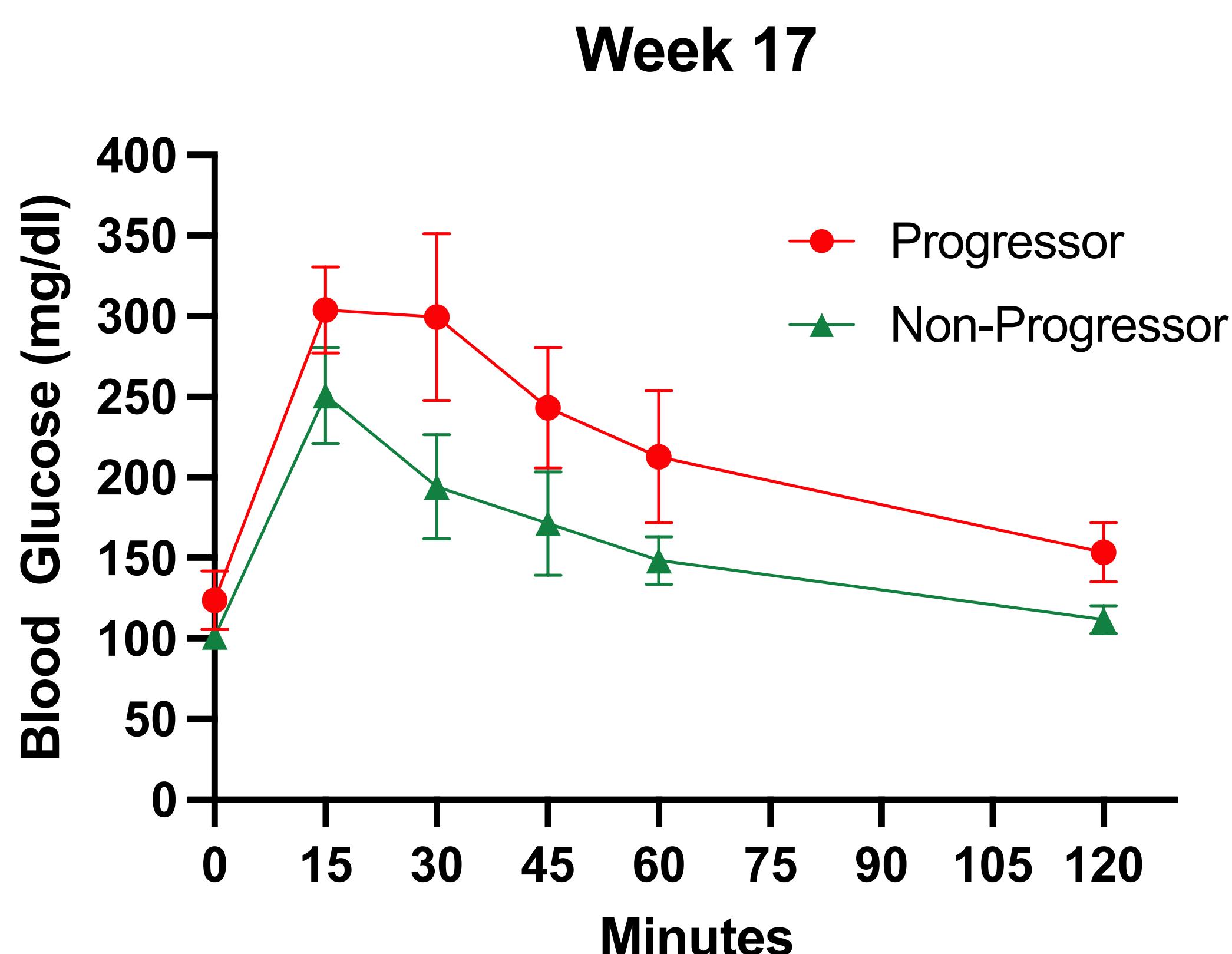
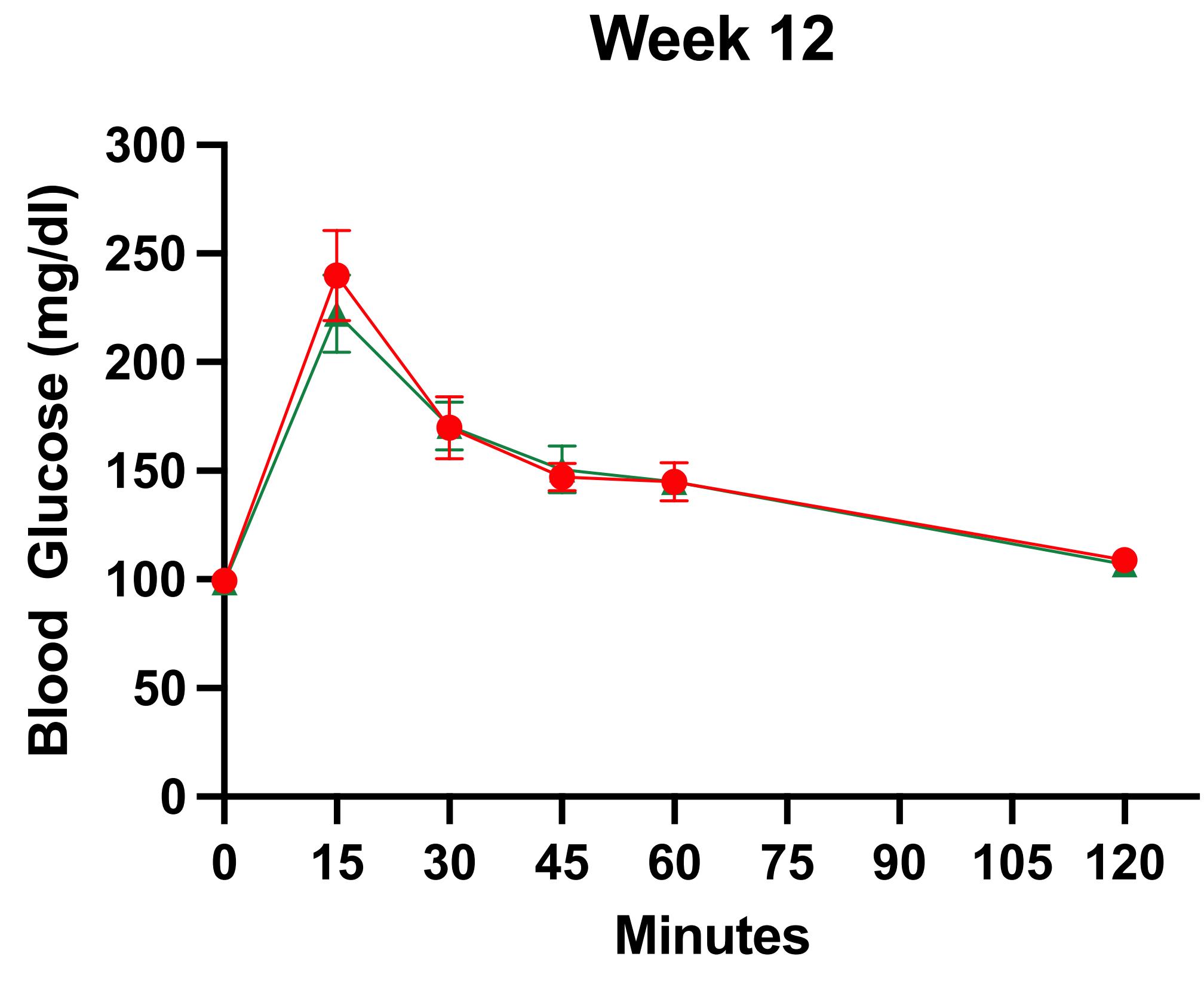
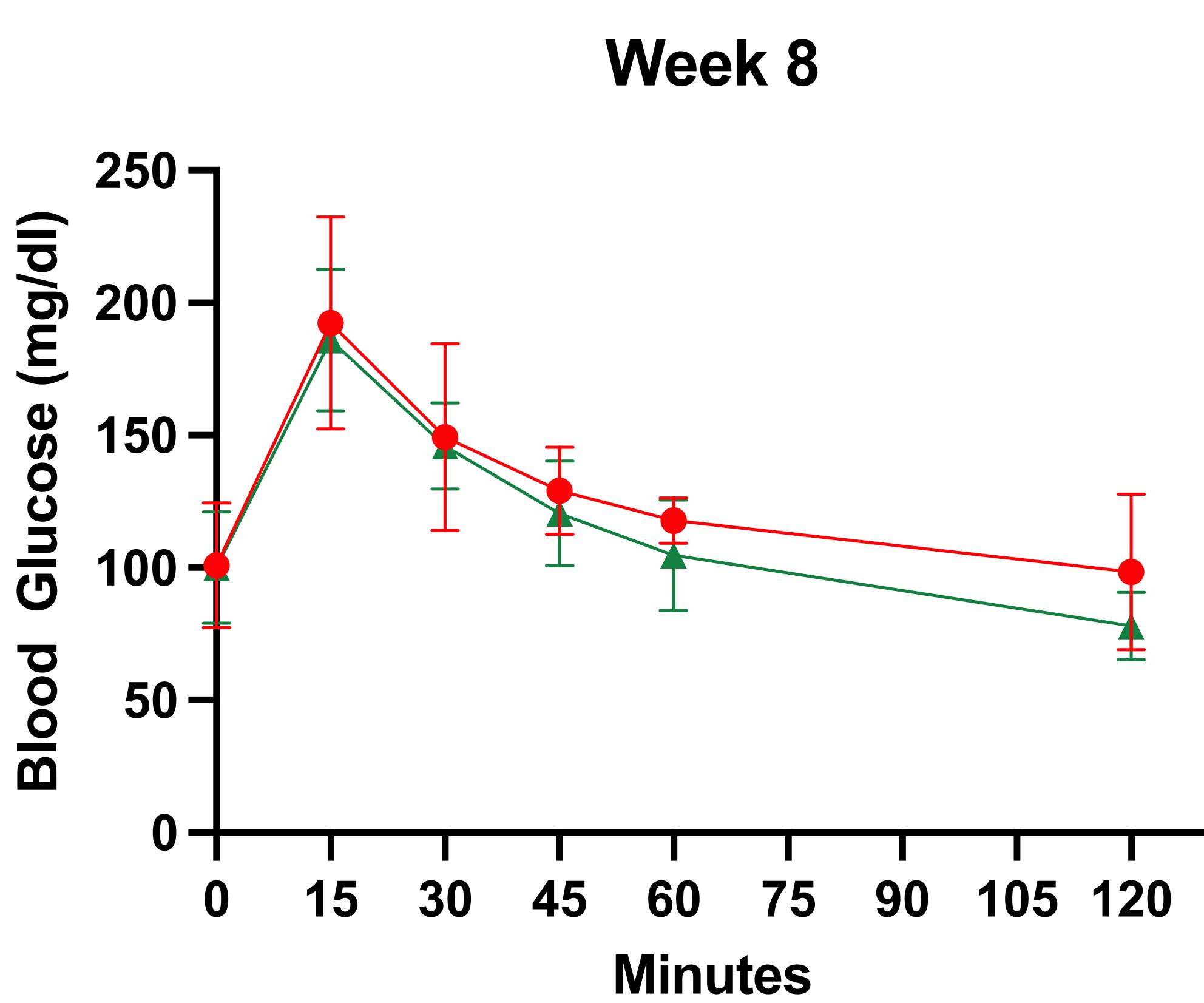
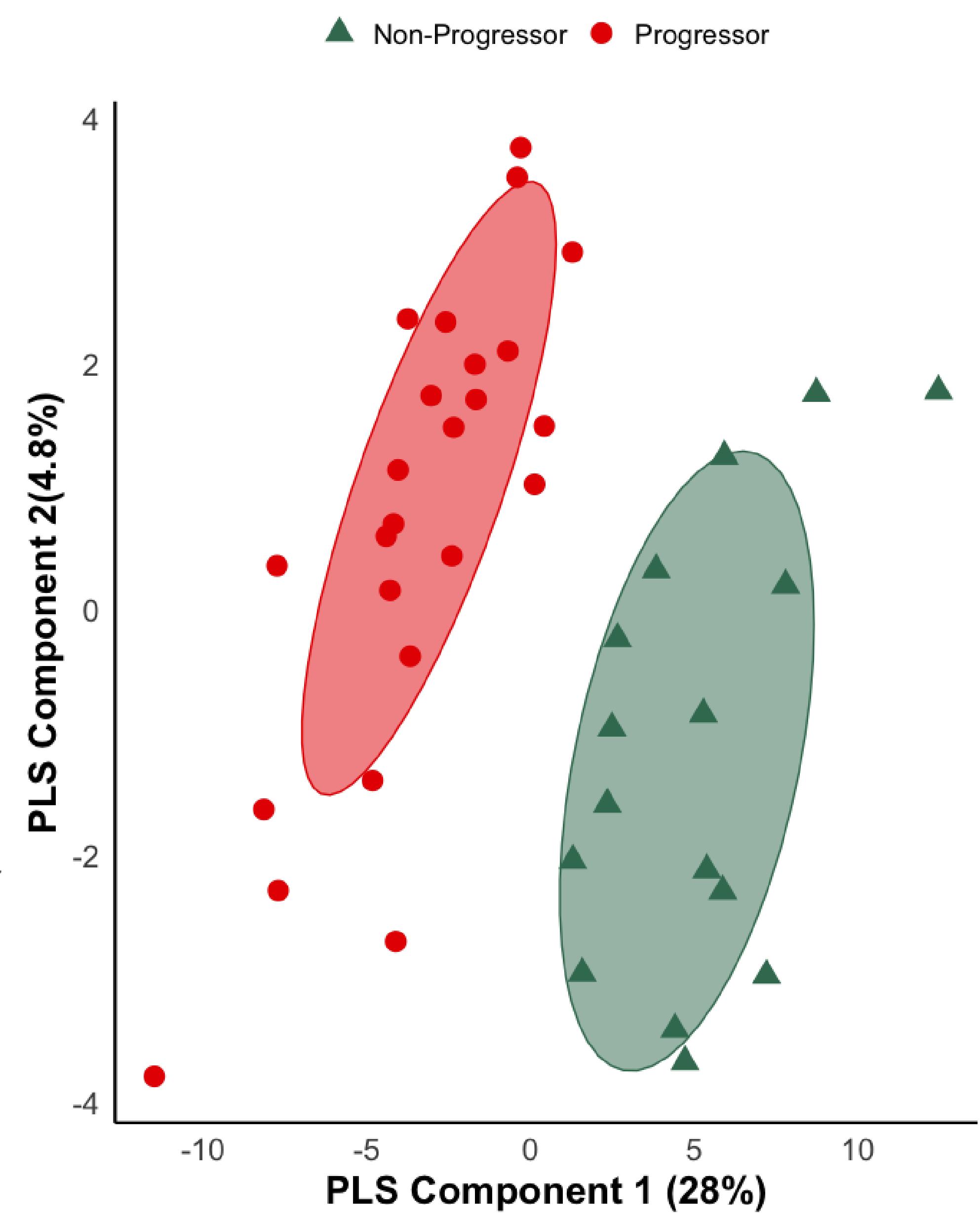
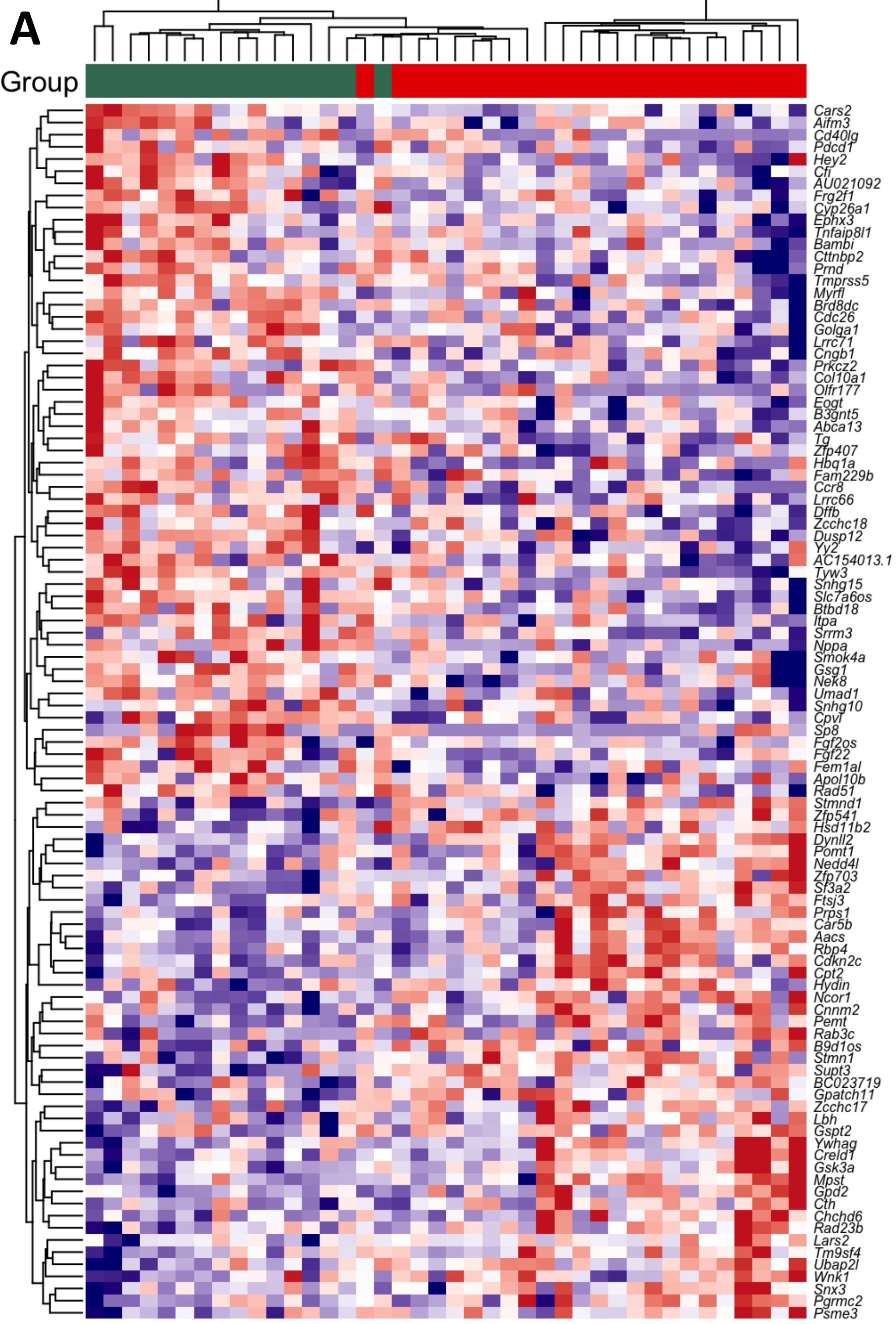
**Fig S3:** Plasma metabolomics profiling of Progressor vs Non-Progressor Over Time



**Fig S2:** Scaffold metabolomics profiling of Progressor vs Non-Progressor Over Time

# Figure 2: Machine Learning Model Using Implantable Niche Omics Predicts Diabetic Progressors From Non-Progressors Before Clinical Symptoms

1. Heatmap of Top 100 Genes
2. PLSDA – Top 100 Genes
3. Enrichment analysis- Top 100 Genes -?
4. ROC-AUC
5. BoxPlot Showing Accuracy, Sensitivity and Specificity for Scaffold Transcriptomics,
6. IPGTT--AUC
7. Blood Based Elisa on autoantibodies



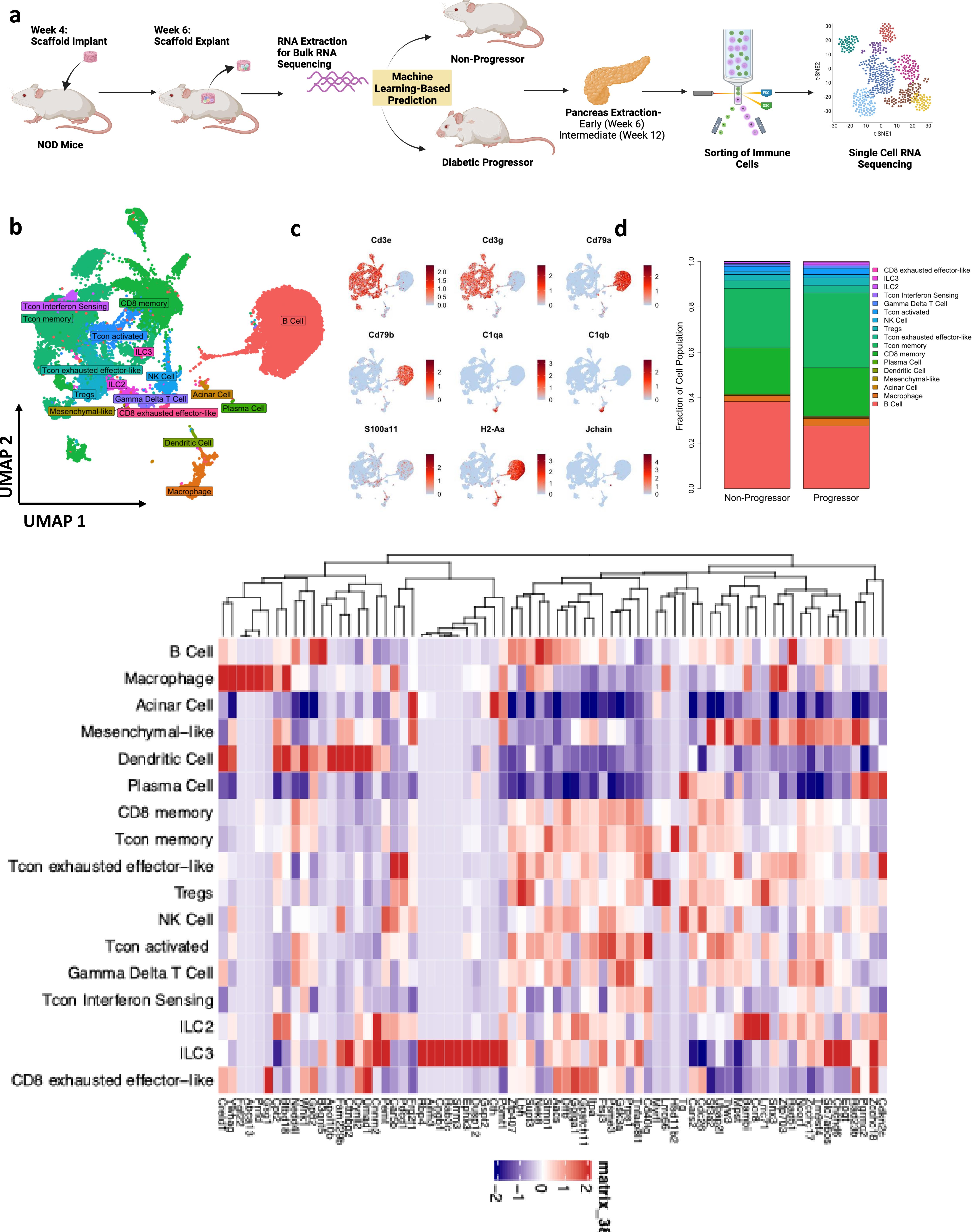
## **Figure 2: Machine Learning Model Using Implantable Niche based Transcriptomics Predicts Diabetic Progressors From Non-Progressors Before Clinical Symptoms :**

**a**, Schematic representation of the computational workflow, outlining preprocessing and normalization of transcriptomics and metabolomics data for training the Support Vector Classifier (SVC) model. **b-d**, Principal Component Analysis (PCA) illustrating the separation between progressors and non-progressors at the early stage (Week 6): **b**, Scaffold Transcriptomics ( $n = 19-24$  per group), **c**, Scaffold Metabolomics ( $n = 9-14$  per group), **d**, Plasma Metabolomics ( $n = 5-14$  per group). Ellipse represent 65% (SEM) confidence interval **e-h** Box plots showing the performance of the SVC model at Week 6 in terms of **e**, Accuracy, **f**, Specificity, **g**, Sensitivity for Scaffold Transcriptomics ( $n = 19-20$  per group), Scaffold Metabolomics ( $n = 9-14$  per group), and Plasma Metabolomics ( $n = 5-14$  per group). **h-j**, Intra-Peritoneal Glucose Tolerance Test (IPGTT) comparing progressors and non-progressors ( $n = 4-6$  per group) at different disease stages: **h**, Early (8 weeks), **i**, Intermediate (12 weeks), **j**, Late (17 weeks)

- **(k-l)** ELISA analysis.

# Figure 3: : Early-Stage Pancreatic Immune Microenvironment are Different Between Non-Progressors and Progressors

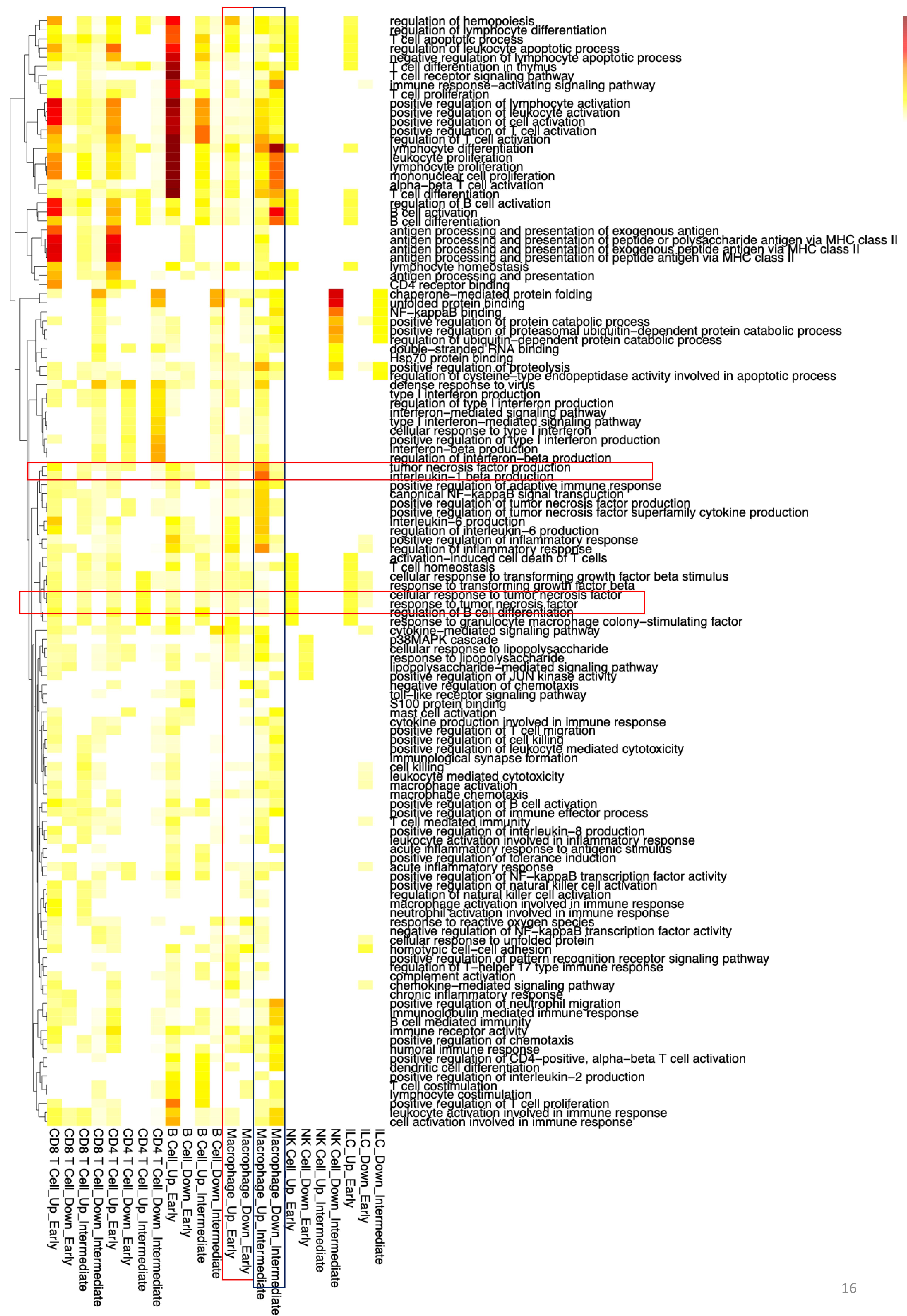
1. Experimental workflow for Identifying the Progressor and Non-Progressor using the SVC Model Trained on Immunological Niche Based Transcriptomics
2. Pancreas Microenvironment Composition-Early Stage
3. Changes in Progressor vs Non-Progressor–BarPlot–Early Stage
4. Signatures for Cell Type Identification
5. Scaffold Based Early Stage DE Genes are Expressed on in the Pancreas Immune Microenvironment by both Innate and Adaptive Immune Cells



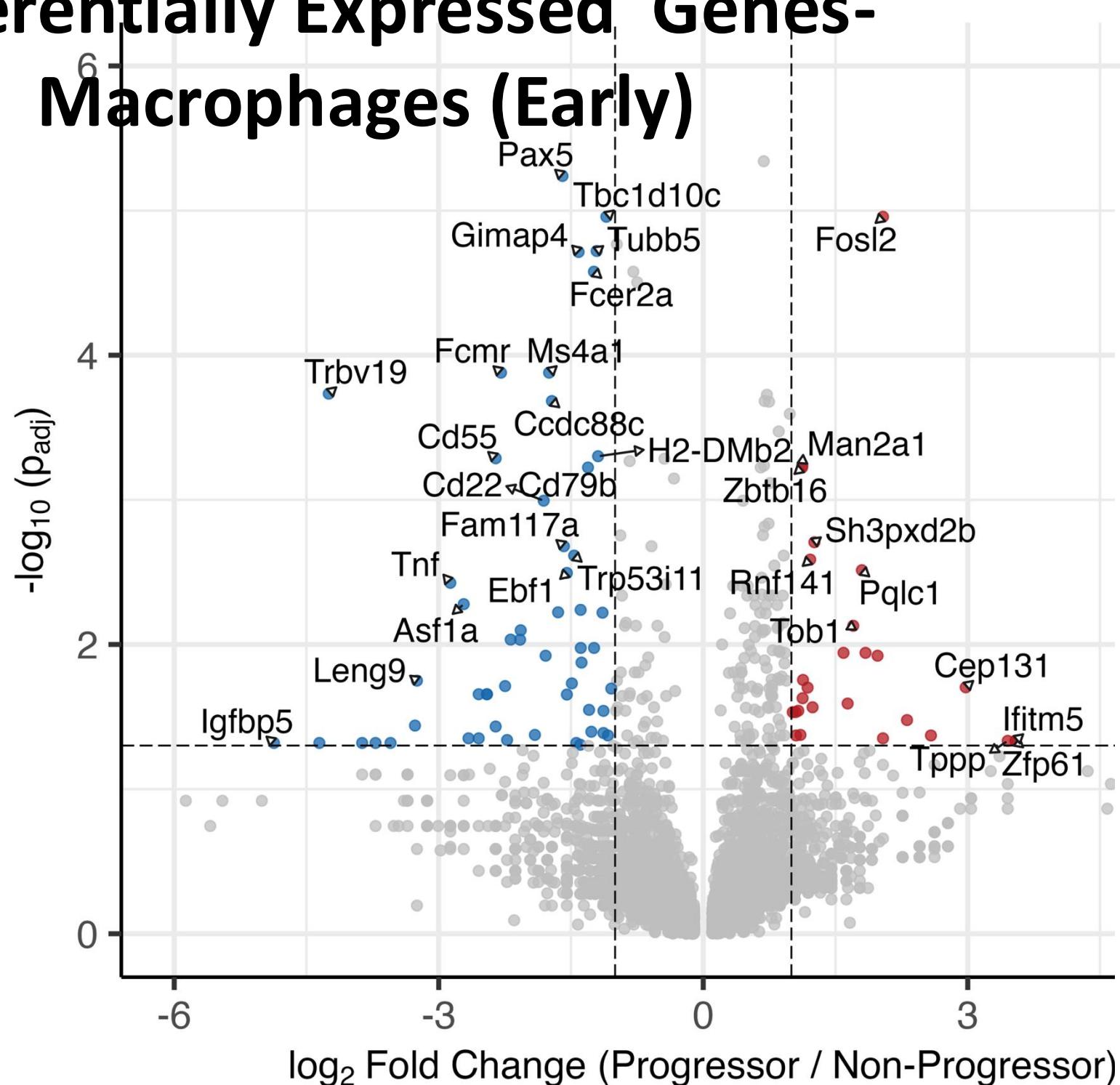
**Figure 3: Scaffold Signature Based Predicted Progressors and Non-Progressors Exhibit Distinct Immune Cell Infiltration at Early Stage:** **a**, Schematic representation of experimental workflow scaffold transcriptomic-based prediction of Progressor and Non-Progressor using Support Vector Classifier at Early Stage (6 Weeks) followed by pancreas extraction from Progressor and Non-Progressor at Early (n=3 per group), followed by Immune cell extraction using FACS and the single cell sequencing **b**, UMAP presenting the broad immune cell types present in the pancreas **c**, Barplot showing higher T Cell Infiltration in Progressor vs Non-Progressor at Early Stages **d**, Feature plot showing some of the convention Gene markers used for annotation. **e**, DotPlot showing differentially expressed genes in Scaffold for Progressor vs Non-Progressors are expressed by pancreas immune cell types highlighting the ability of scaffolds to capture pancreas immune specific transcriptomic changes



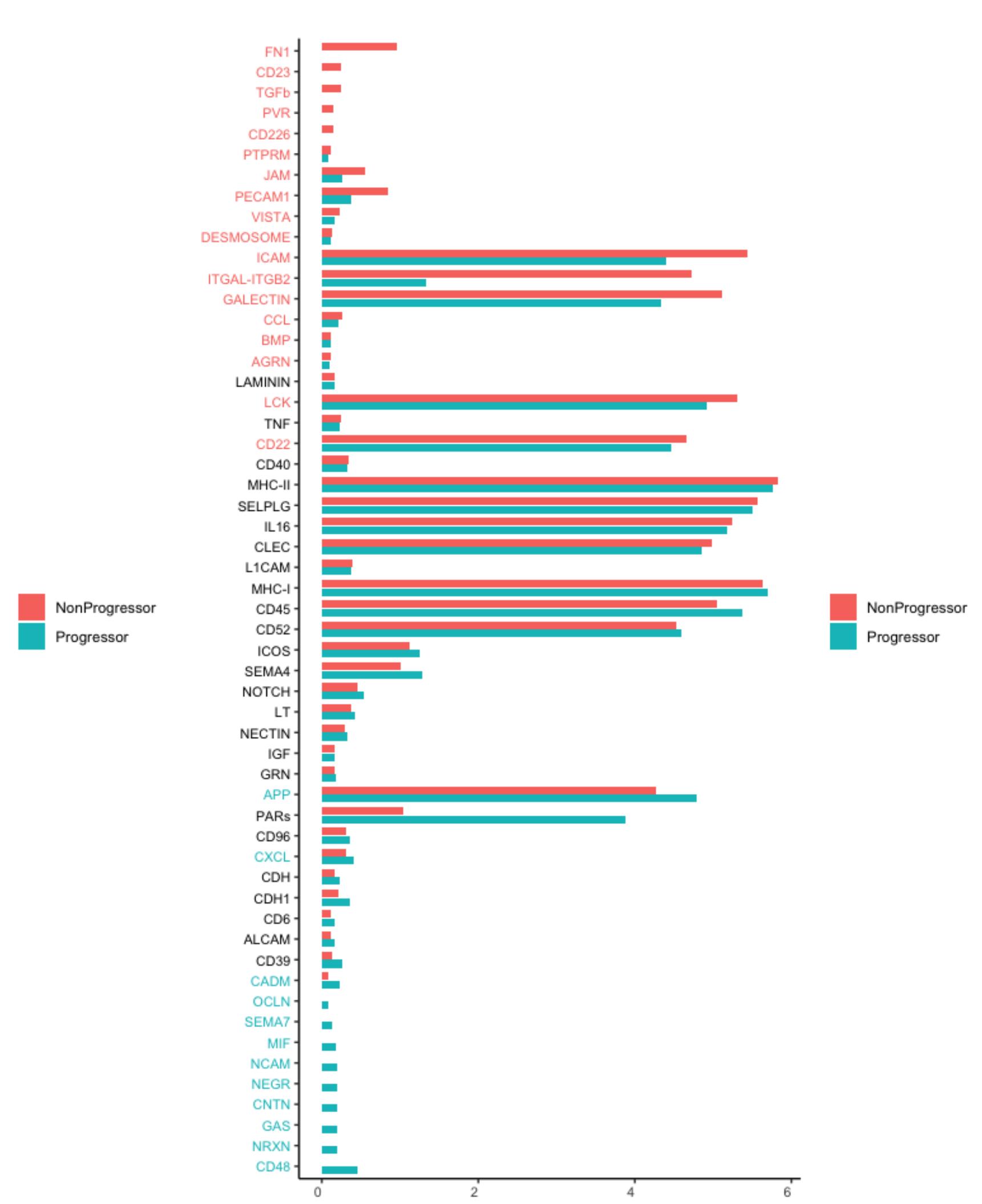
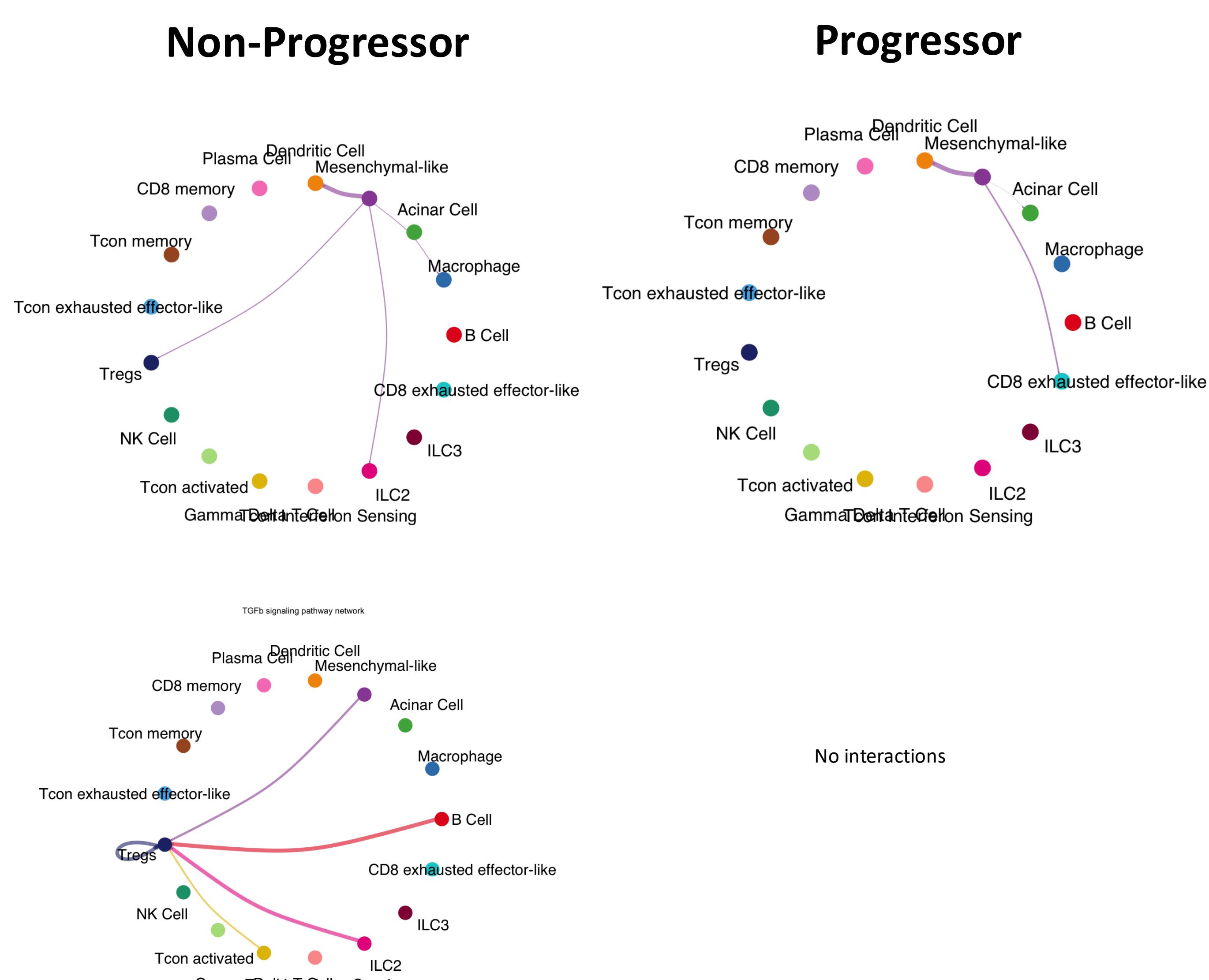
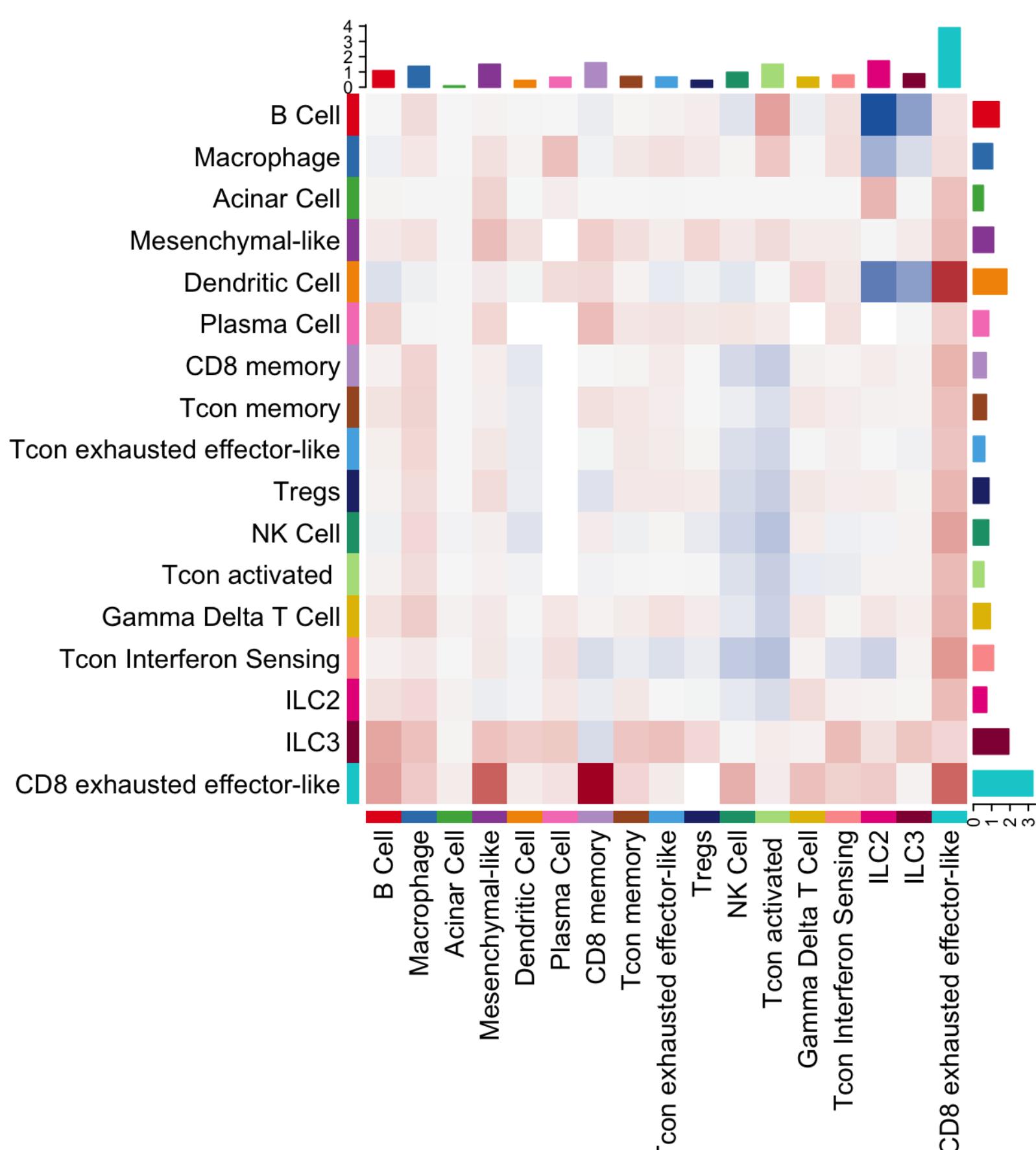
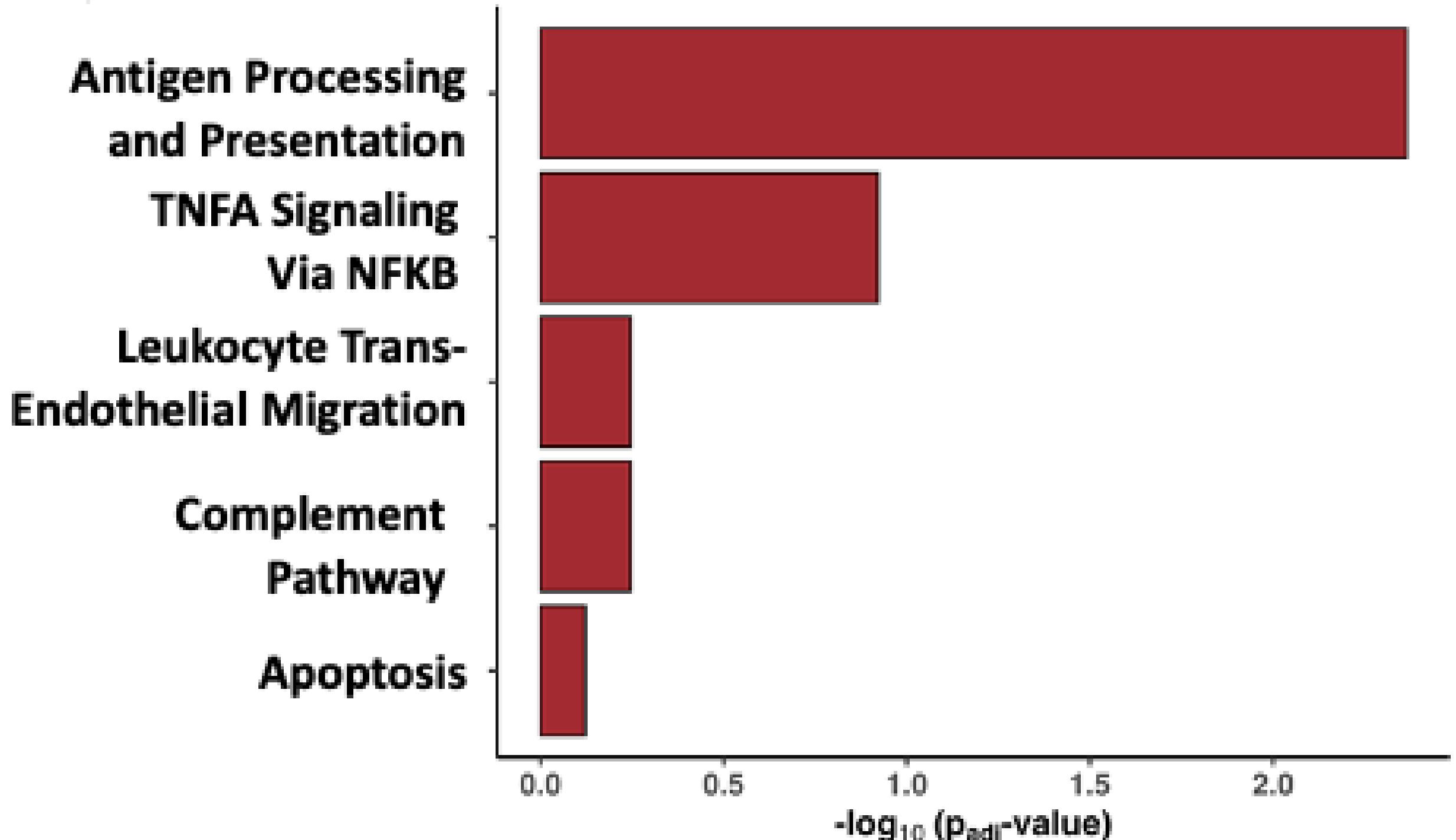
Figure 4: Early-stage Progressors exhibit heightened TNF- $\alpha$  mediated interactions in antigen-presenting cells, prominently captured within the immunological niche.



# Differentially Expressed Genes - Macrophages (Early)



# Upregulated Pathways- Macrophages (Early)



# Figure 5: Early TNF- $\alpha$ blockade reduces T1D incidence and reprograms immune profiles toward a non-progressor state as captured by the immunological niche

1. Reduced infiltration and insulitis in anti-TNF  $\alpha$  treated (early) vs untreated and TNF alpha treated (positive control)
2. Reduced capability of APCs to proliferate T cells upon anti-TNF  $\alpha$  treatment-in-vitro assay
3. Incidence rate of diabetes is less in anti-TNF  $\alpha$  treated (early) vs untreated and anti-TNF alpha treated (late)
4. Transcriptomics analysis of Immunological Niche highlights shift in TNF treated group from Progressor vs Non-Progressors (Before vs After Treatment Vs Progressor/Non-Progressor)- DEGs, Pathway
5. PCA Plots showing TNF alpha treated vs untreated
6. Histology to Focus on PD1+ T Cells/ PDL1+ Macrophages in anti-TNF  $\alpha$  treated vs progressor and non-progressor

## Anti-TNF $\alpha$ Treatment Cohort

