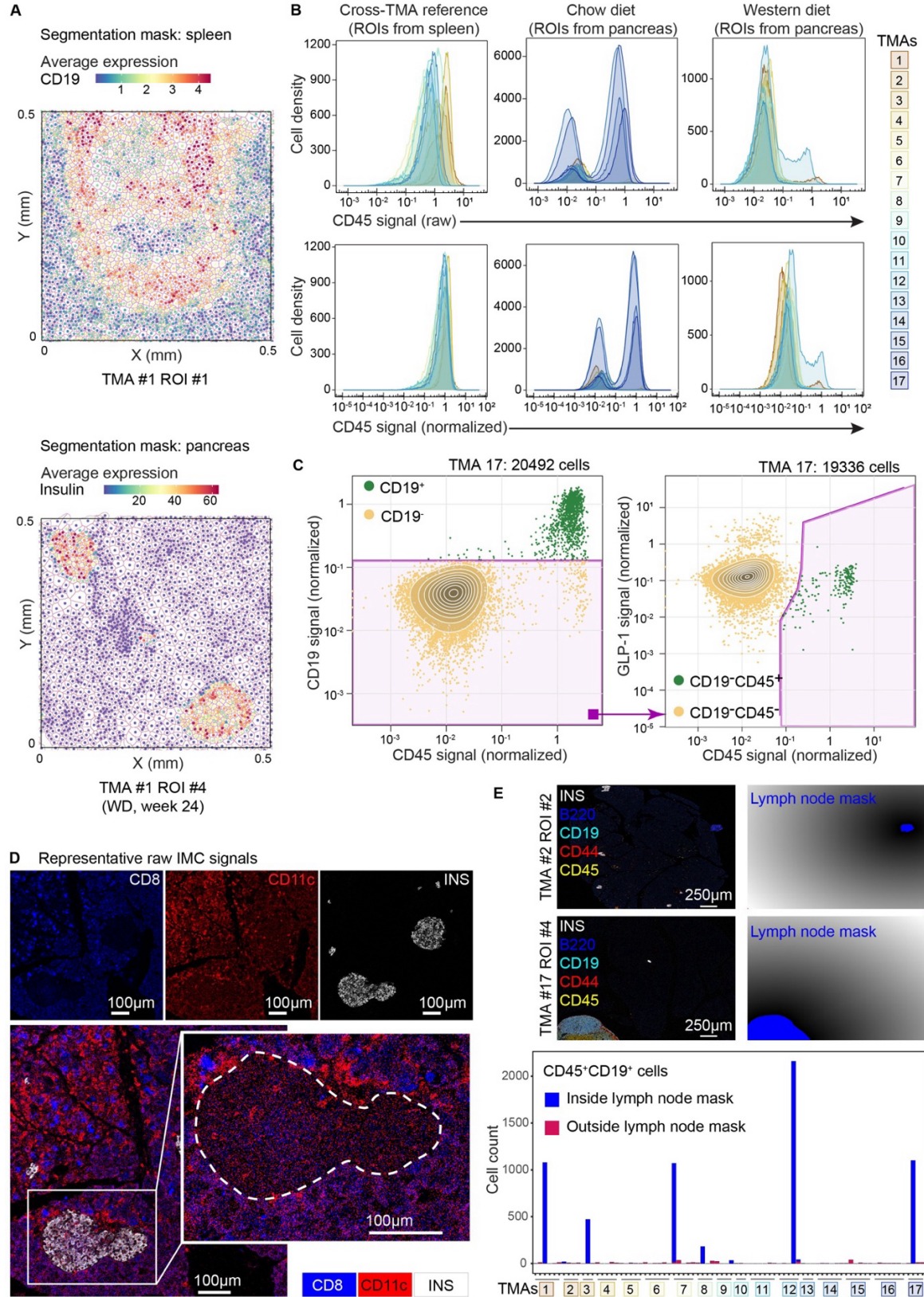




Supplementary Figure 1: Quality control of IMC data.

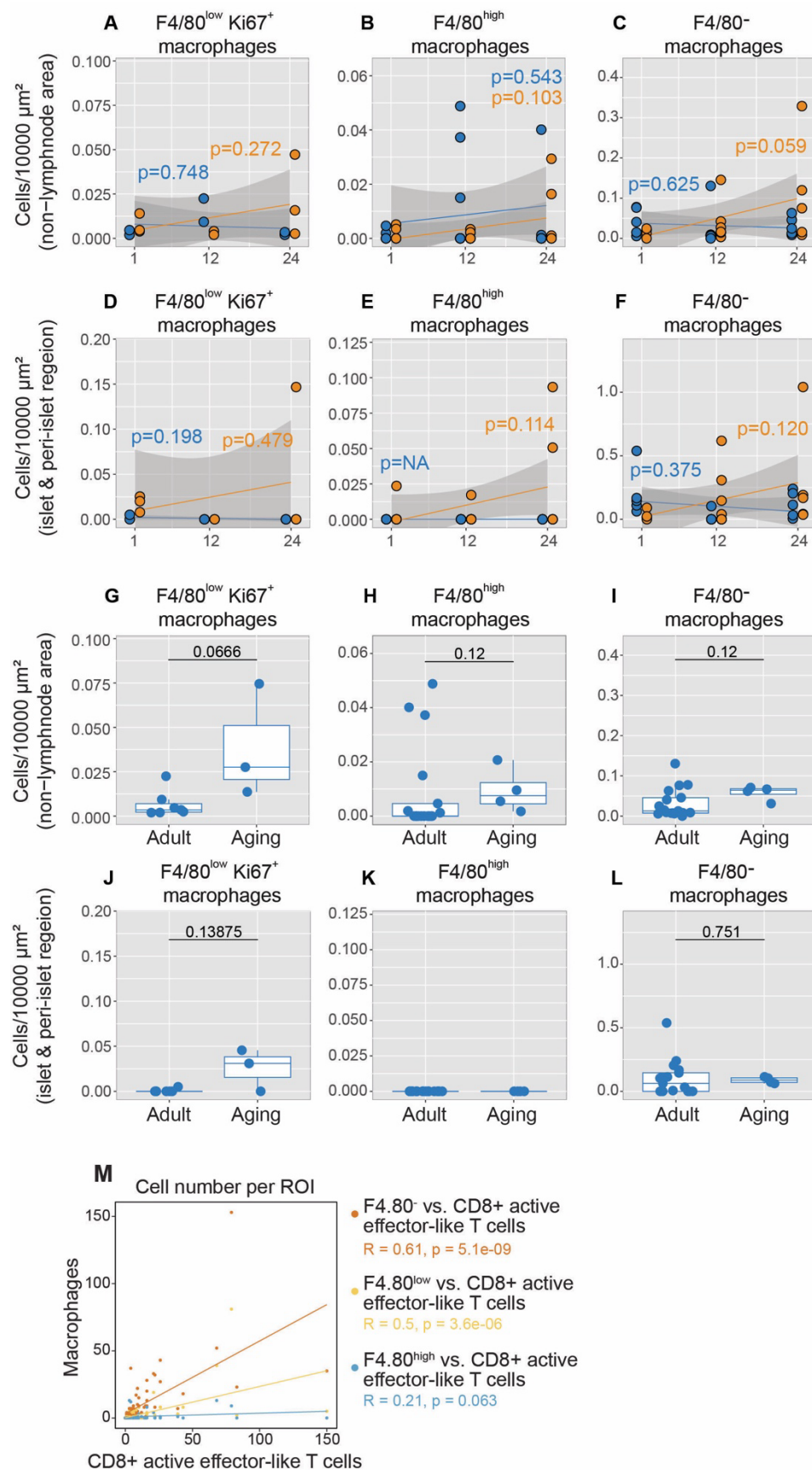
(A) Representative images showing cell segmentations and the expression of marker proteins on spleen (CD19, top) and pancreas (insulin, bottom) sections. The color of the dot at the center of each cell segmentation reflects the average expression of CD19 (top) and insulin (bottom). The dimensions of each region of interest (ROI) are indicated along both axes.

(B) Histograms showing raw (top) and reference-ROI-normalized (bottom) IMC signal intensity from the CD45 channel across all TMAs. ROIs from different TMAs are distinguished by varying colors.

(C) Representative scatter plots depicting the Boolean gating strategy employed to identify pancreatic non-B cell immune cells (CD19-CD45⁺GLP1R⁻) within each TMA.

(D) Representative ROI showing raw signals from the CD8 (blue), CD11c (red), and insulin (white) channels. Scale bar, 100 μm .

(E) Representative ROIs showing two pancreatic lymph nodes (highlighted in blue). Pancreatic lymph node masks were generated based on a composite overlay of signals from B220, CD19, CD44, and CD45 (top right, see SI), scale bar, 250 μm . Bottom: Bar plot with counts of CD19⁺ B cells within and outside of these lymph node masks.



Supplementary Figure 2: Cell density quantification and spatial correlation analysis.

(A-C) Scatter plots depicting the cell counts of F4/80^{low} Ki67⁺ **(A)**, F4/80^{high} **(B)** and F4/80⁻ **(C)** macrophages per 10000 μm^2 of the pancreas (excluding lymph nodes) across different time points and feeding conditions. Solid lines represent linear regression fits, with shaded areas indicating 95% confidence intervals calculated from the standard error. Each condition includes data from $n \geq 3$ mice. P-values for the regression coefficient slope were determined using a linear model and t-tests.

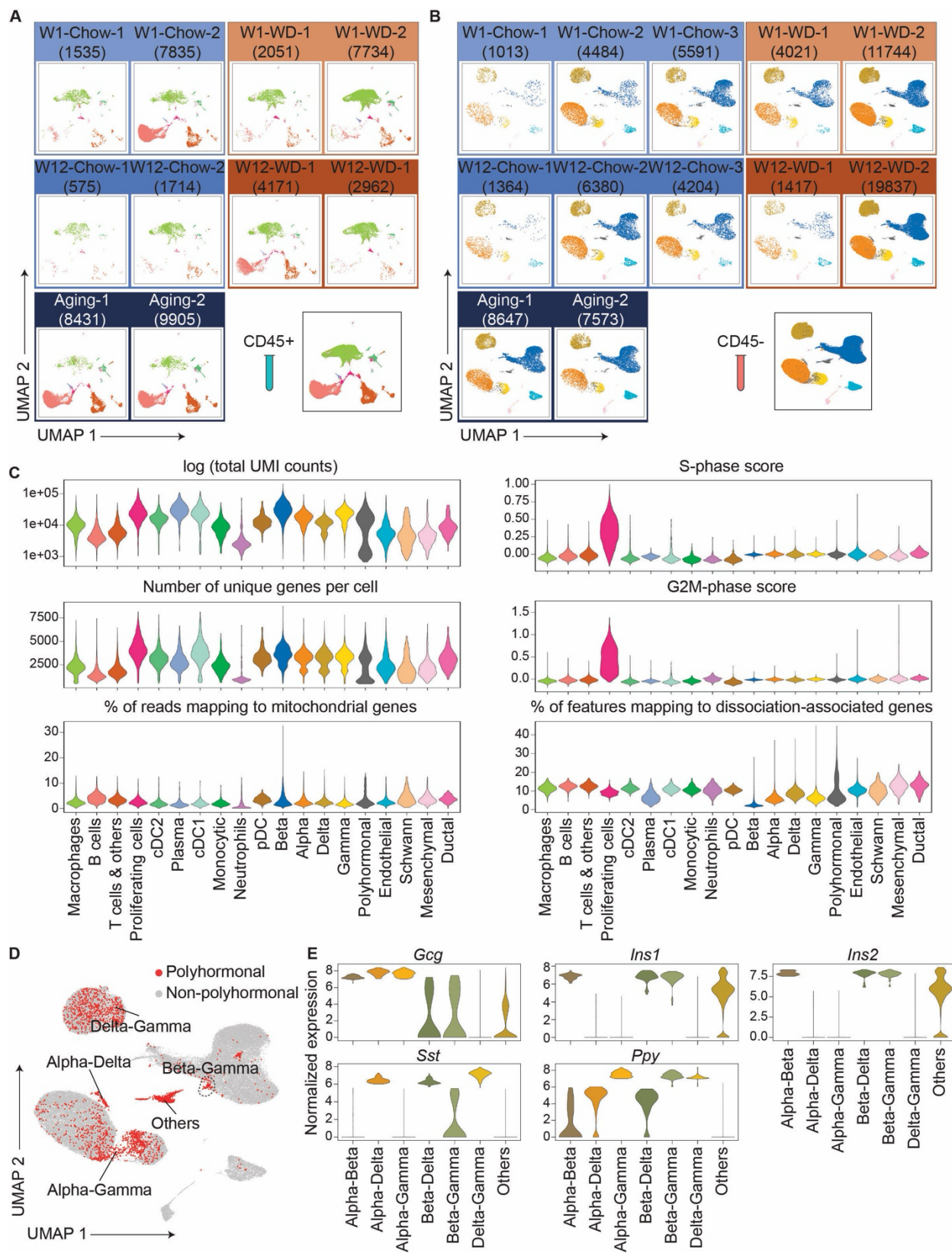
(D-F) Scatter plots showing the cell counts of F4/80^{low} Ki67⁺ **(D)**, F4/80^{high} **(E)** and F4/80⁻ **(F)** macrophages per 10000 μm^2 of the islets and peri-islet regions over time under feeding conditions. Linear regression fits (solid lines) and 95% confidence intervals (shaded areas) are displayed. Each condition includes data from $n \geq 3$ mice. P-values for the regression coefficient slope were determined using a linear model and t-tests.

(G-I) Box plots showing the cell counts of F4/80^{low} Ki67⁺ **(G)**, F4/80^{high} **(H)** and F4/80⁻ **(I)** macrophages per 10000 μm^2 of the pancreas (excluding lymph nodes) in adult and aging mice. Each condition includes data from $n \geq 3$ mice. P-values were determined by Wilcoxon signed-rank test with Benjamini & Hochberg adjustment.

(J-L) Box plots showing the cell counts of F4/80^{low} Ki67⁺ **(J)**, F4/80^{high} **(K)** and F4/80⁻ **(L)** macrophages per 10000 μm^2 of islets and peri-islet regions in adult and aging mice. Each condition includes data from $n \geq 3$ mice. P-values were determined by Wilcoxon signed-rank test with Benjamini & Hochberg adjustment.

(M) Scatter plot showing the Pearson correlation between the counts of CD8⁺ activated effector-like T cells and three macrophage subpopulations (F4/80⁻, F4/80^{low} and F4/80^{high}) within the same

regions of interest (ROIs). Solid lines indicate the best fit for each correlation analysis, with Pearson correlation coefficients and p-values provided for the corresponding macrophage subsets.



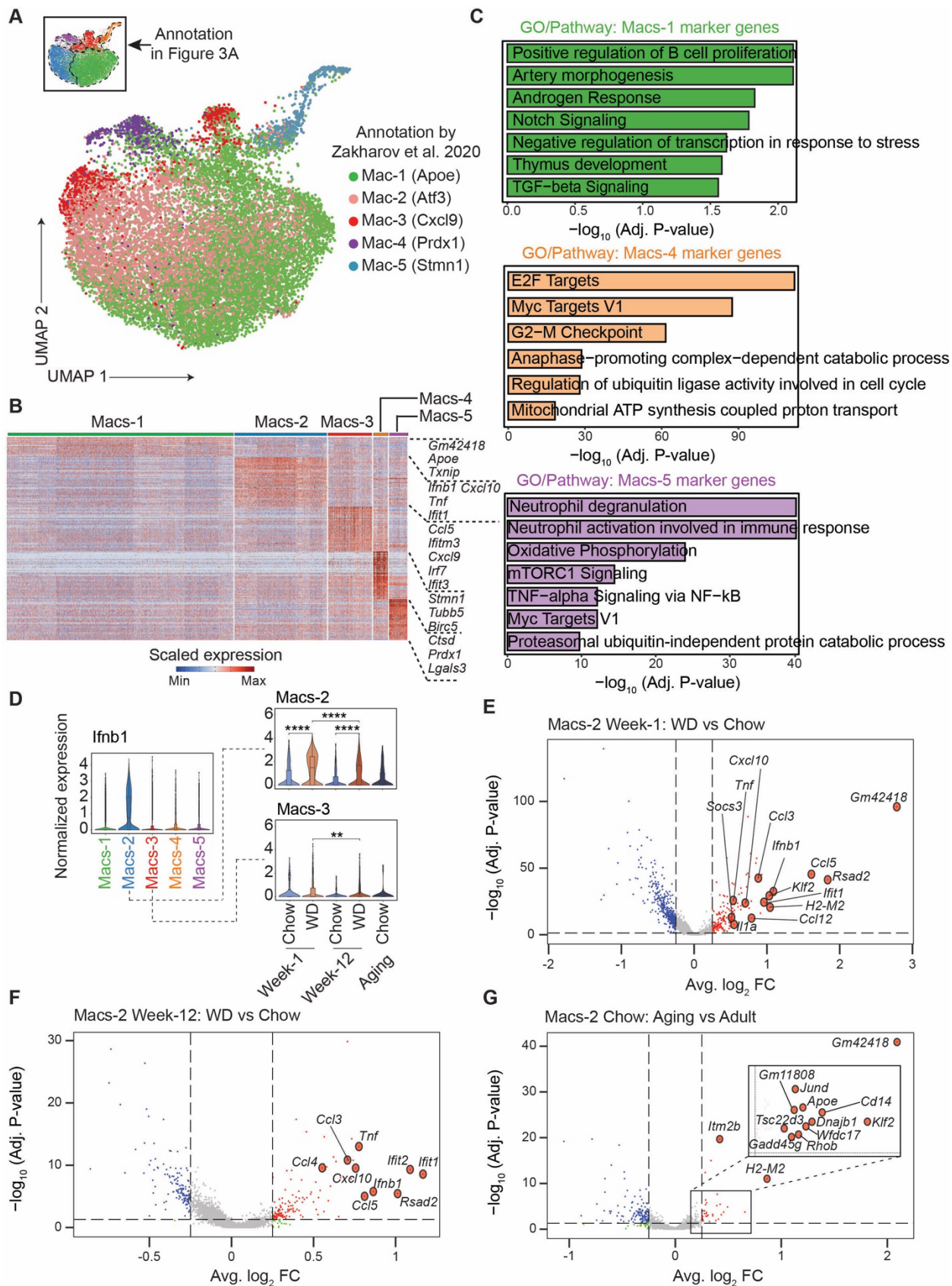
Supplementary Figure 3: Quality control of scRNA-seq data.

(A and B) Split UMAPs depicting the distribution of the annotated immune (**A**, CD45⁺) and non-immune (**B**, CD45⁻) cell types across experimental cohorts.

(C) Violin plots depicting quality control metrics for scRNA-seq data. In addition to standard quality control metrics, we also examined genes induced by enzymatic dissociation (**Supplementary Table 1D**).

(D) UMAP embedding of non-immune cells in grey. Polyhormonal cells are highlighted in red.

(E) Violin plots depicting the expression of five endocrine hormone genes across annotated polyhormonal cells.



Supplementary Figure 4: Annotation of islet-associated macrophages based on single-cell analysis.

(A) UMAP embedding of islet-associated macrophages from this study overlaid with cell type annotations from Zakharov et al. [29] following the label transfer workflow.

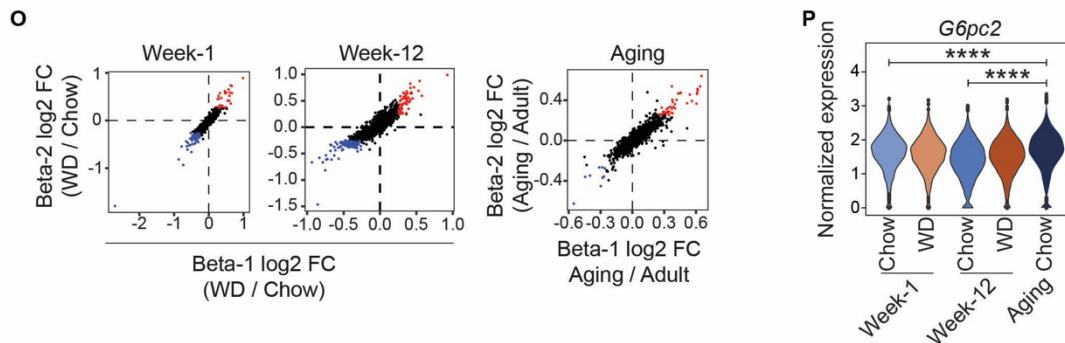
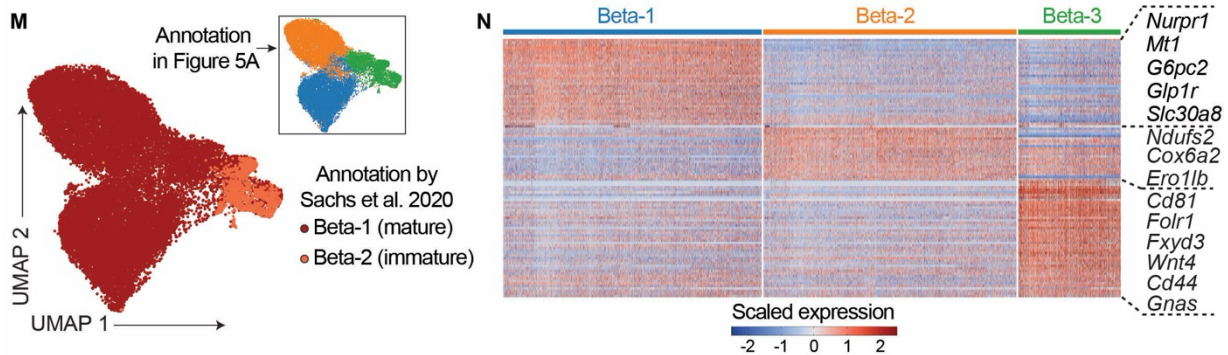
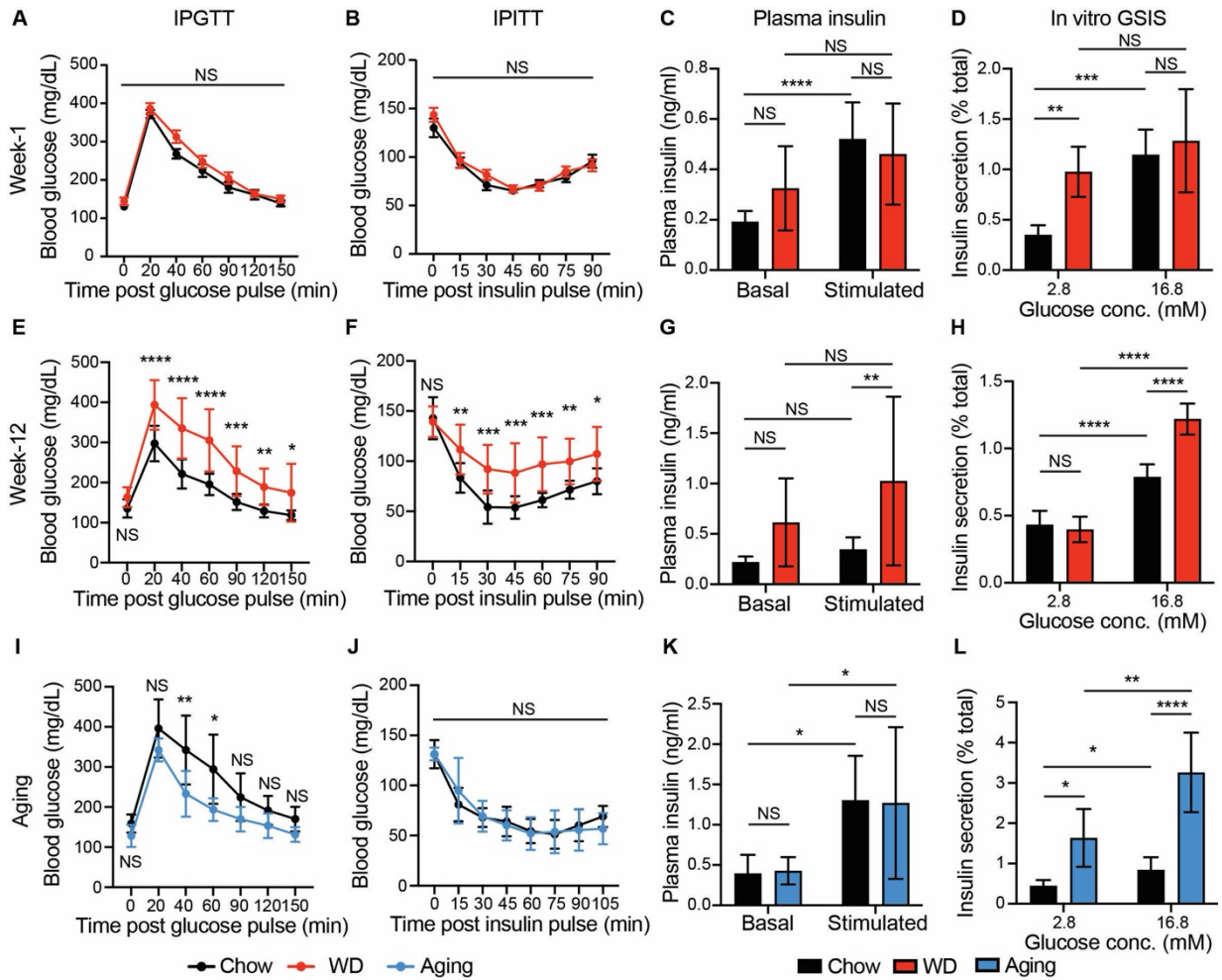
(B) Heatmap showing scaled expression levels of marker genes in each macrophage subpopulation from **Figure 4A**.

(C) Enriched gene ontology (GO) terms for marker genes in Macs-1 (top), Macs-4 (middle) and Macs-5 (bottom). Significance ($-\log_{10}$ adjusted p-value) of the enrichments is shown.

(D) Violin plot showing normalized expression level of *Ifnb1* across macrophage subpopulations and across different feeding and age groups. **FDR < 0.01, ****FDR < 0.0001; FDR was calculated using the Wilcoxon rank-sum test.

(E-G) Volcano plots showing differentially expressed genes in Macs-2 under different conditions:

(E) WD versus chow at week-1, **(F)** WD versus chow at week-12, and **(G)** aging versus adult (combined week-1 and week-12 chow data). Genes with significant upregulation and downregulation are highlighted in red and blue, respectively. Dashed vertical lines indicate an absolute average $\log_2$ -fold change threshold of 0.25, while the dashed horizontal line represents a significance threshold of $-\log_{10}(\text{adjusted p-value}) = 1.3$ (corresponding to adjusted p-value of 0.05).



Supplementary Figure 5: Functional and transcriptional changes in beta cells in response to western diet and aging.

(A, E, I) Glucose tolerance tests in mice fed chow or WD for 1 week (**A**, n=10 both groups), 12 weeks (**E**, n=10 both groups) or with aging (**I**, adult <3-month-old versus 2-year-old, n=10 both groups). NS, not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ by multiple comparisons test post two-way ANOVA.

(B, F, J) Insulin tolerance tests in mice fed chow or WD for 1 week (**B**, n=10 both groups), 12 weeks (**F**, n=10 both groups) or with aging (**J**, adult <3-month-old versus 2-year-old, n=10 both groups). NS, not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by multiple comparisons test post two-way ANOVA.

(C, G, K) Plasma insulin levels in mice pre- and post-glucose injection in mice fed chow or WD for 1 week (**C**, n=10 both groups), 12 weeks (**G**, n=10 both groups) or with aging (**K**, adult <3-month-old versus 2-year-old, n=10 both groups). NS, not significant, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ by multiple comparisons test post two-way ANOVA.

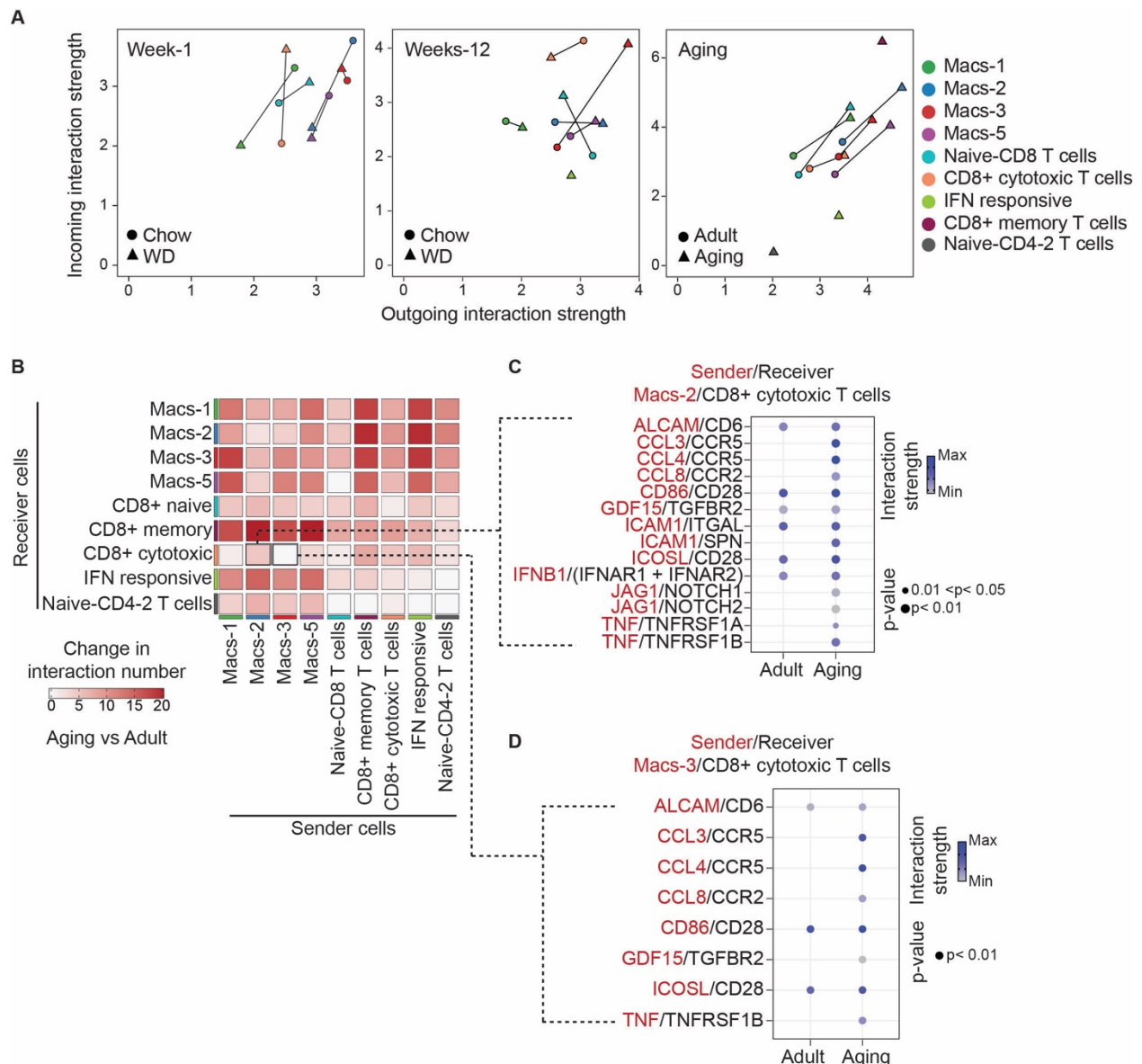
(D, H, L) Glucose stimulated insulin secretion (GSIS) assay in mouse islets from mice fed chow or WD for 1 week (**D**, 6 mice, with 10 islets collected per mouse for each data point), 12 weeks (**H**, n=6 with 10 islets per data point) or with aging (**L**, adult <3-month-old versus 2-year-old, n=6 with 10 islets per data point). NS, not significant, * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ by multiple comparisons test post two-way ANOVA.

(M) UMAP embedding of beta cells in this study depicting the cell type annotations from Sachs et al. [39] following the label transfer workflow.

(N) Heatmap depicting scaled expression levels of marker genes in each beta cell subtypes from **Figure 5A**.

(O) Scatter plots showing average log₂-fold changes in gene expression for Beta-1 and Beta-2 cells after 1-week and 12-week WD feeding compared to chow-fed mice, and in aged versus adult mice.

(P) Violin plot showing normalized expression level of *G6pc2* in beta cells across western diet feeding conditions and age. ****FDR < 0.0001; FDR was calculated using the Wilcoxon rank-sum test.



Supplementary Figure 6: Ligand-receptor analysis in islet-resident immune cells.

(A) Scatter plot showing the outgoing (X-axis) and incoming (Y-axis) interaction strength for the indicated cell populations across various conditions: week-1 chow (n=3 cohorts), week-1 WD (n=2 cohorts), week-12 chow (n=3 cohorts), week-12 WD (n=2 cohorts), aging (n=2 cohorts), and adult (week-1 and week-12 chow, n=6 cohorts).

(B) Heatmap showing changes in the number of ligand-receptor pairs among indicated cell populations, comparing adult to aging (n=6 cohorts for adult, n=2 cohorts for aging).

(C and D) Dot plots showing the strength of ligand-receptor interactions from Macs-2 to CD8⁺ cytotoxic T cells **(C)** and from Macs-3 to CD8⁺ cytotoxic T cells **(D)** in adult and aging conditions. Dot color and size represent the communication probability and p-values, calculated using a one-sided permutation test in CellChat.