Supplemental information

Single-cell RNA sequencing

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dysregulation in diet-induced obesity

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SUPPLEMENTARY FOR

Single-cell RNA sequencing identifies hippocampal microglial dysregulation in diet-induced obesity

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[1]Department of Neurology, University of Michigan, Ann Arbor, MI USA

[2]NeuroNetwork for Emerging Therapies, University of Michigan, Ann Arbor, MI USA

[3]Department of Biomedical Sciences, University of North Dakota, Grand Forks, ND USA
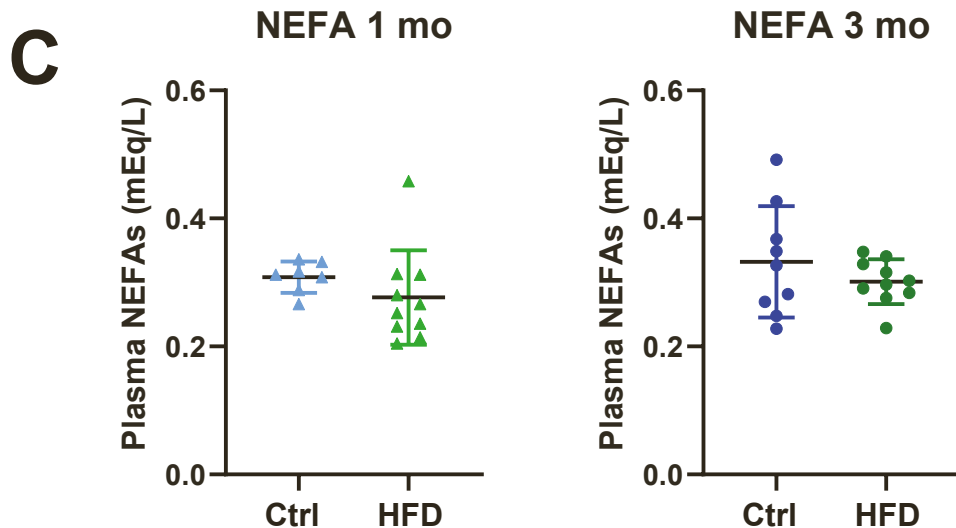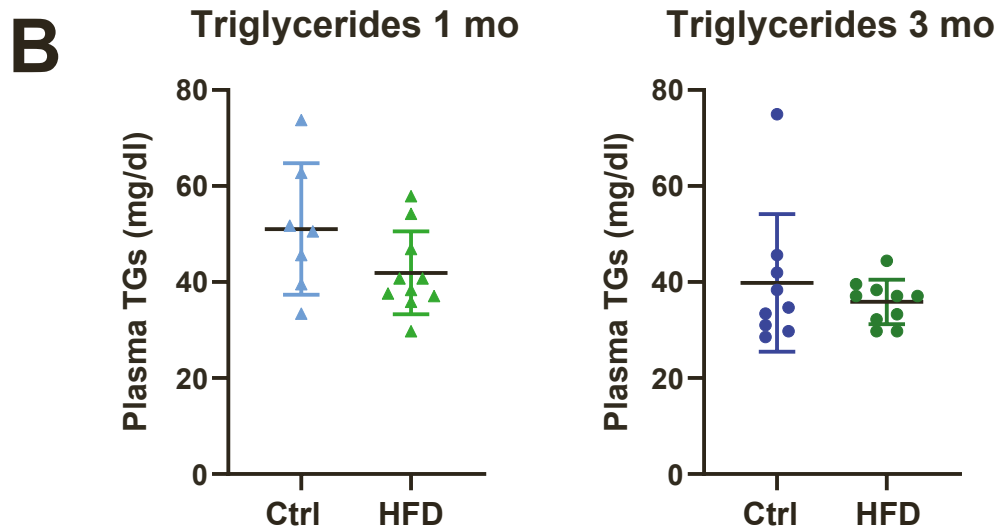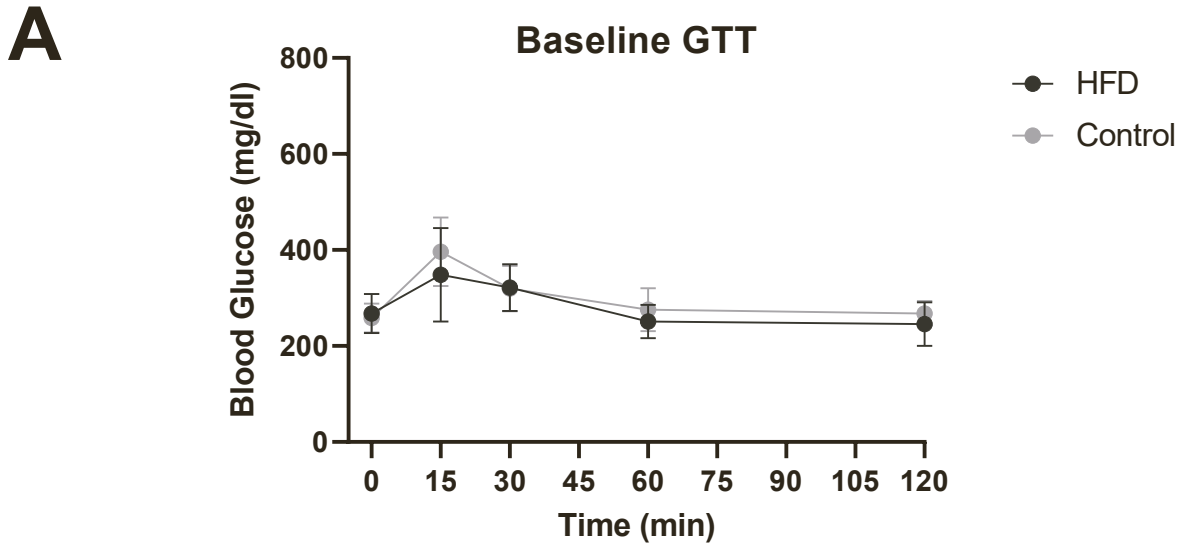
[†]Corresponding author

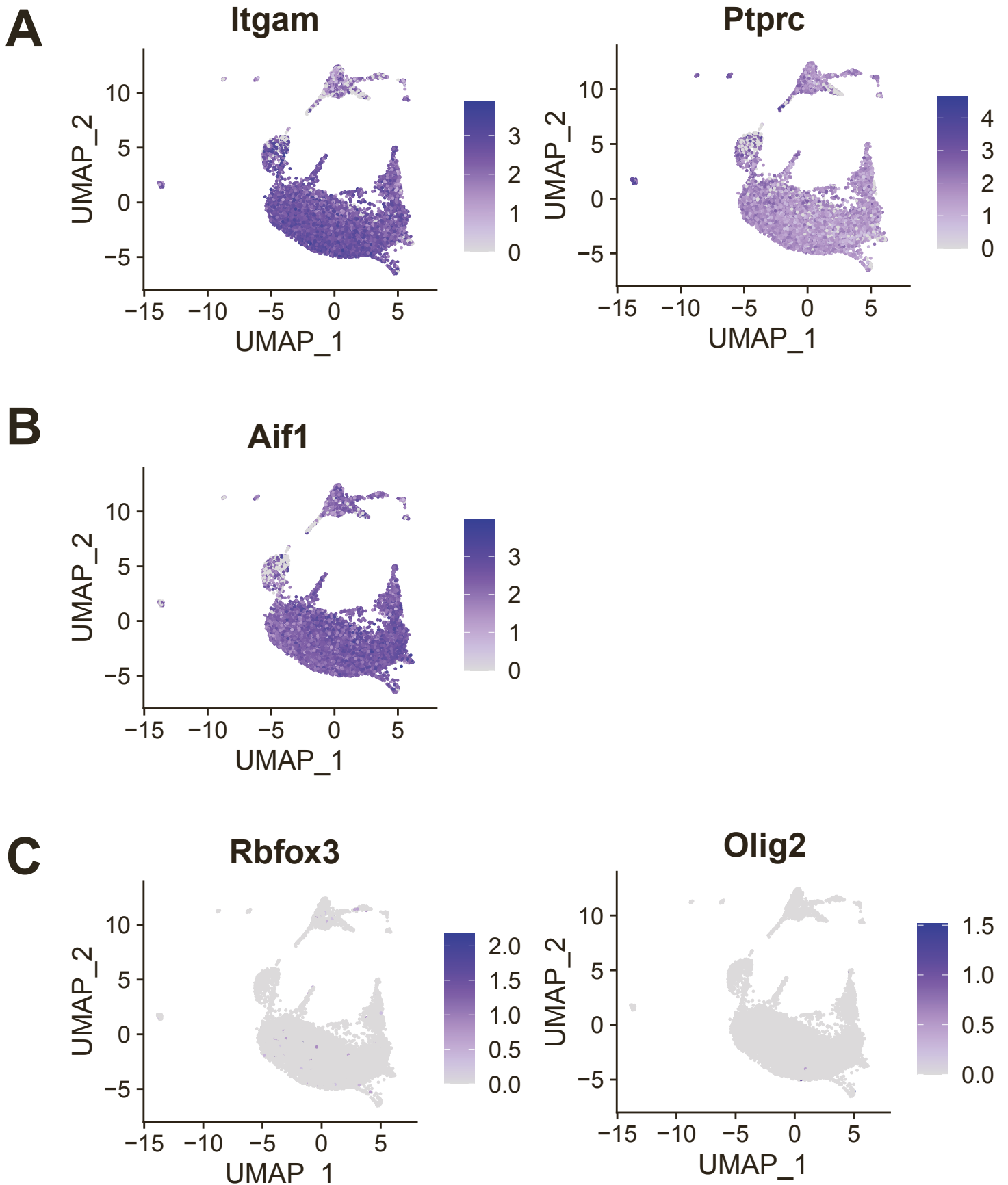Eva L. Feldman, MD, PhD,

Russell N. DeJong Professor of Neurology

5017 AAT-BSRB, 109 Zina Pitcher Place, Ann Arbor, Michigan 48109, United States

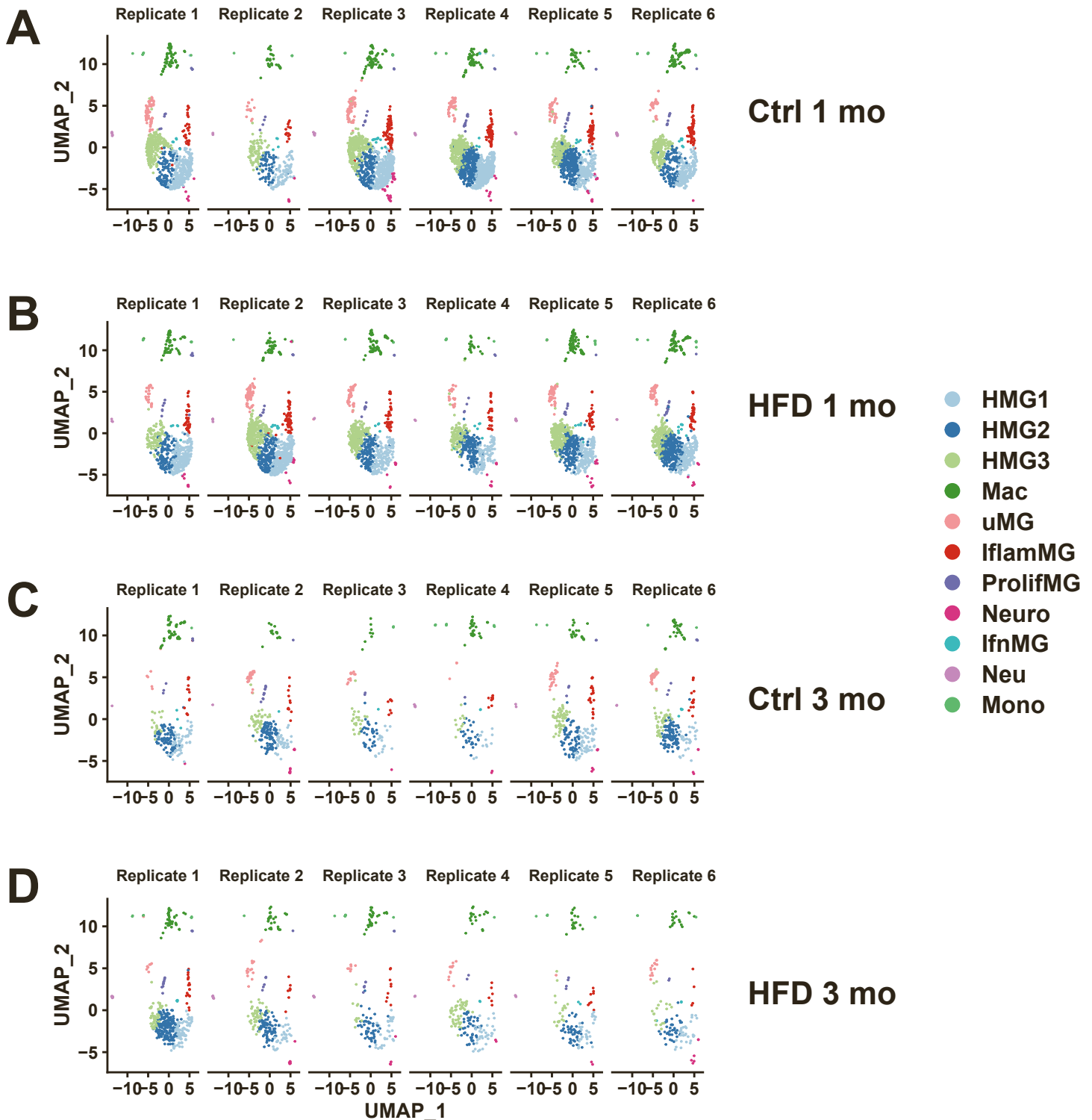Email: efeldman@umich.edu, Phone: (734) 763-7274 / Fax: (734) 763-7275

Figure S1. Metabolic parameters in HFD and control mice. Related to Figure 1. (**A**) Baseline glucose tolerance test (GTT) for HFD (1 mo and 3 mo combined; n=20; dark grey) versus control (ctrl; 1 mo and 3 mo com-bined; n=20; light grey) mice. Plasma (**B**) triglycerides (TGs) and (**C**) non-esterified fatty acids (NEFAs); left panels for HFD (n=10; light green) versus control (n=7; light blue) at 1 mo (triangles); right panels for HFD (n=10; dark green) versus control (n=9; dark blue) at 3 mo (circles). No significant difference between HFD and control in (A) by repeated measures two-way ANOVA with Sidak's multiple comparisons test for GTT, and in (B,C) by Welch's t-test for TG 1 mo and NEFA 3 mo and by Mann-Whitney test for TG 3 mo and NEFA 1 mo since data were not normally distributed; data represented as mean ± standard deviation.
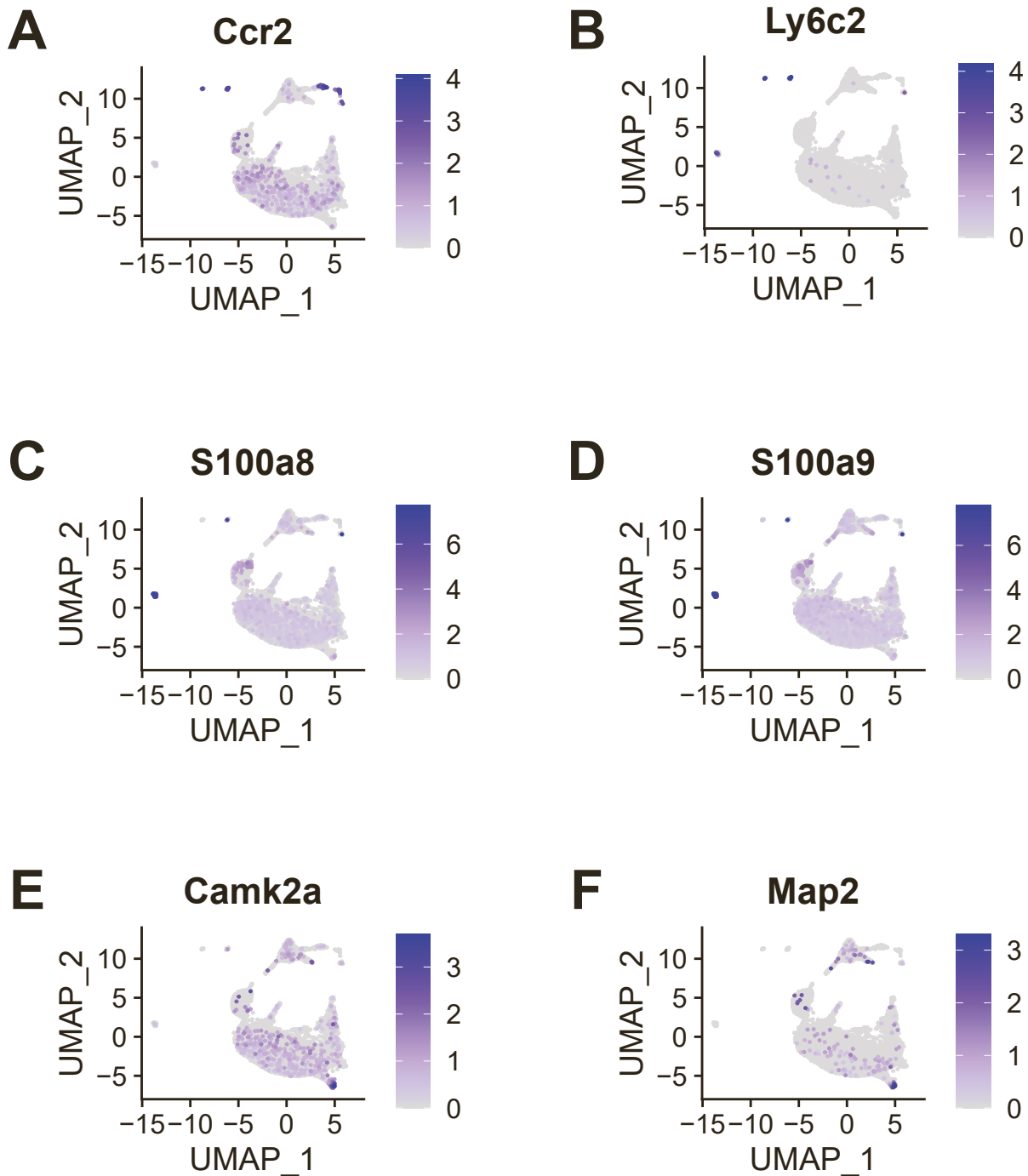
Figure S2. UMAP shows purity of microglial isolation and successful FACS sort. Related to Figure 3. (**A**) UMAP plots of gene expression for antigens used in FACS sorting, CD11b and CD45. Almost all cells express *Itgam* (integrin subunit alpha M; left panel), the gene encoding CD11b (cluster of differentia-tion 11b), and *Ptprc* (protein tyrosine phosphatase receptor type C; right panel), the gene encoding CD45. (**B**) UMAP plot with expression of *Aif1* (allograft inflammatory factor 1), the gene encoding Iba1 protein, a microglial marker. (**C**) UMAP plot with expression of *Rbfox3* (RNA binding fox-1 homolog 3; left panel), the gene encoding NeuN protein, a neuronal marker, and for *Olig2* (oligodendrocyte tran-scription factor 2; right panel), an oligodendrocyte marker.
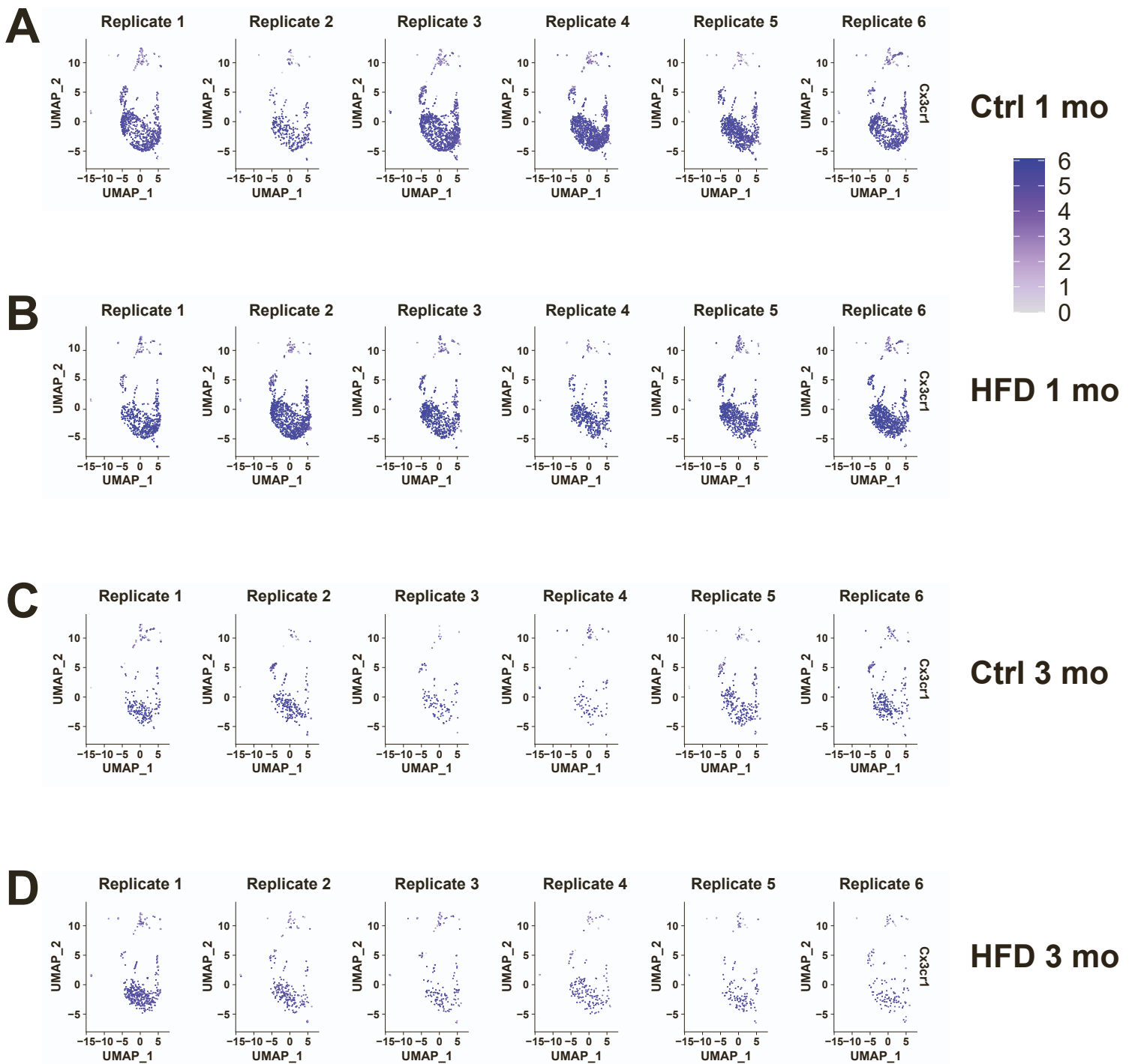
Figure S3. UMAP plots per mouse. Related to Figure 3. Uniform Manifold Approximation and Projection (UMAP) for cells from each of the 6 replicates per condition (Ctrl 1 mo (**A**), HFD 1 mo (**B**), Ctrl 3 mo (**C**), HFD 3 mo (**D**)) shows 11 clusters, which represent homeostatic microglia 1 (HMG1), HMG2, HMG3, macro-phages (Mac), uMG (unknown, functionally undescribed), inflammatory microglia (InflamMG), prolifer-ating microglia (ProlifMG), neurons (Neuro), interferon-related microglia (IfnMG), neutrophils (Neu), and monocytes (Mono).
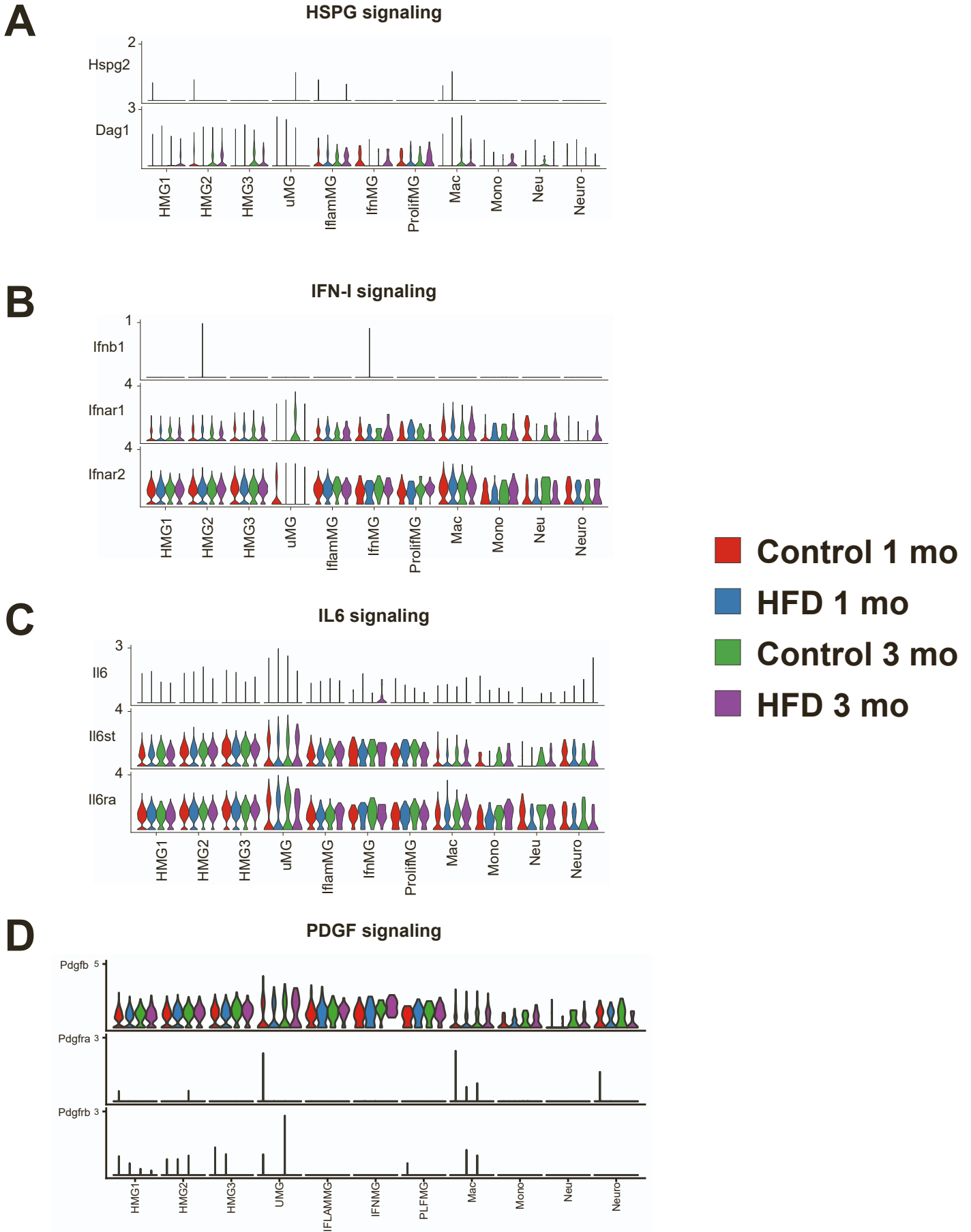
Figure S4. UMAP plots of markers for monocyte, neutrophil, and neuron clusters. Related to Figure 3. UMAP plot with expression of markers for monocytes expressing (**A**) C-C motif chemokine receptor 2 (Ccr2) and (**B**) lymphocyte antigen 6 complex, locus C2 (Ly6c2); neutrophils expressing (**C**) S100 calcium binding protein A8 (S100a8) and (**D**) S100a9; neurons expressing (**E**) calcium/calmodulin dependent protein kinase II alpha (Camk2a) and (**F**) microtubule associated protein 2 (Map2).

Figure S5. UMAP plots per mouse shows purity of microglial isolation. Related to Figure 3.
UMAP plot with expres-sion of *Cx3cr1* (C-X3-C motif chemokine receptor 1), a microglial marker, for cells from each of the 6 replicates per condition (Ctrl 1 mo (**A**), HFD 1 mo (**B**), Ctrl 3 mo (**C**), HFD 3 mo (**D**)). Scale the same for all panels.
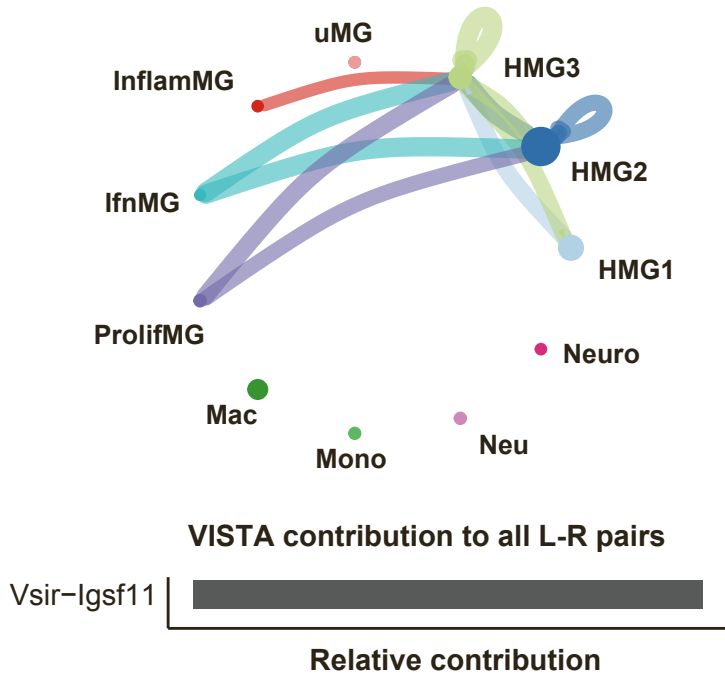
Figure S6. Ligand/receptor expression for the information flow in cell-to-cell communication. Related to Figure 5. Violin plots for the expression level of signaling ligand/receptor pairs from Figure 5, for (**A**) HSPG, (**B**) IFN-I, (**C**) IL6, and (**D**) PDGF signaling.
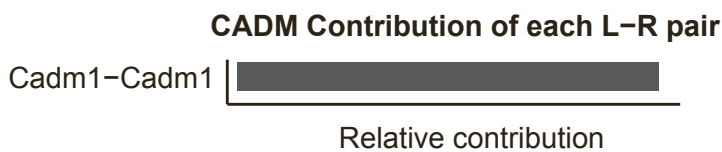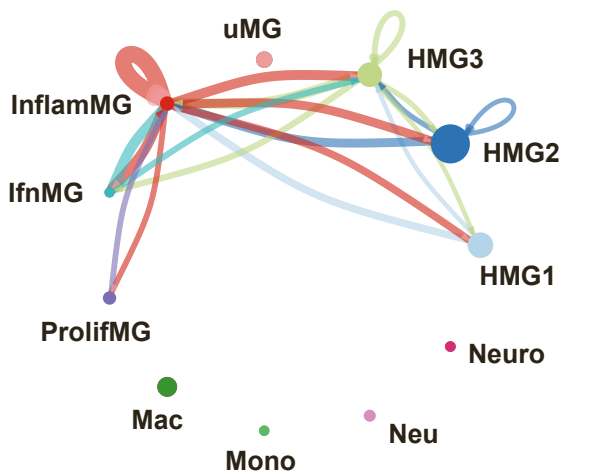
Figure S7. Cell-to-cell communication analyses. Related to Figure 5. Circle plots of cellular signaling interactions (top) and their top contributing ligand-receptor (L-R) pairs (bottom) for pathway networks involving (**A**) VISTA for HFD at 3 mo, (**B**) CADM for control (Ctrl) at 3 mo, (**C**) CADM for HFD at 3 mo. Dots in circle plots represent cell populations with color codes matching UMAP clusters; strokes represent commu-nication between distinct cell populations and loops represent signaling within cell populations. Stroke and loop colors reflect the cluster sending the signal, thickness reflects strength of the signaling pair.
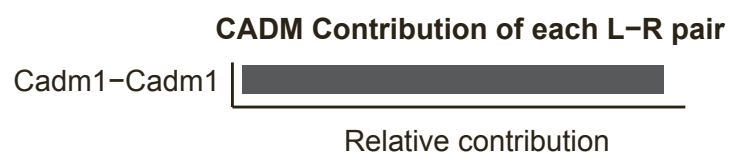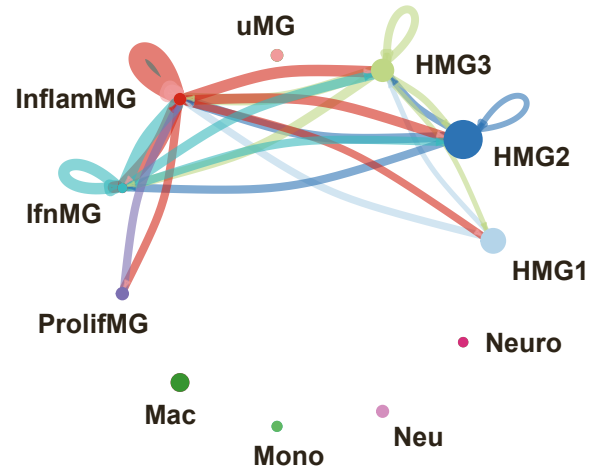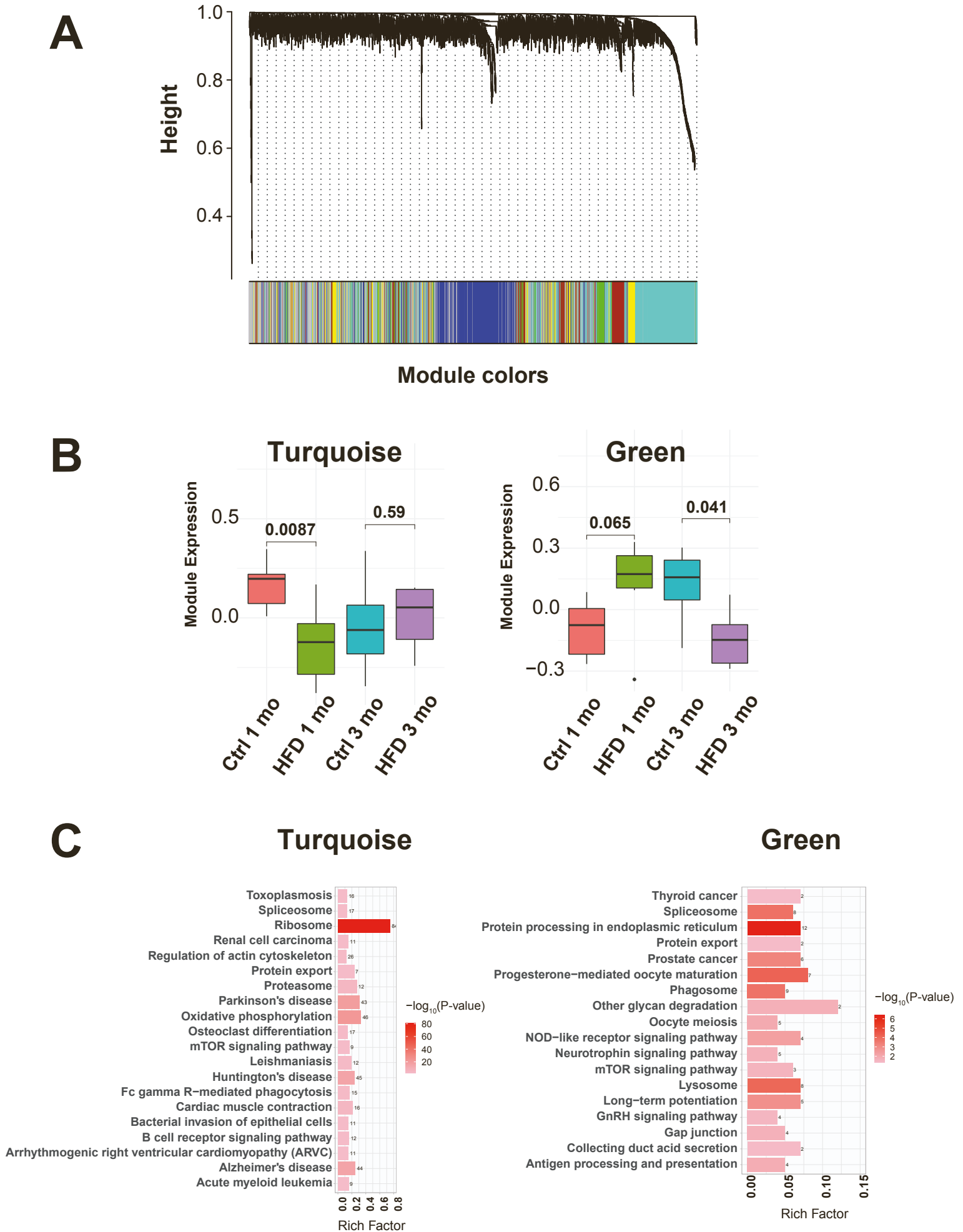
Figure S8. WGCNA modules. Related to Figure 6. (**A**) WGCNA cluster dendrogram arranges genes from all cell types into 6 modules: grey, turquoise, yellow, brown, green, blue. (**B**) Significant differences in expression of genes in turquoise and green modules between experimental groups by ANOVA, *P<0.05, **P<0.01. (**C**) KEGG pathway analysis of genes in HFD versus control from the turquoise (left) and green (right) modules. Bar color represents -log10(P-value) from least significant (light pink) to most significant (red); bar length represents the number of genes in the KEGG pathway, annotated with a number; rich factor represents the fraction of genes among all genes in the KEGG pathway.

Figure S9. Correlation analysis of body weight to transcript levels in various cell types. Related to Figure 7. Spearman correlation analysis of body weights at 1 mo and 3 mo to transcriptomic profiles at 1 mo and 3 mo in all cell types (x-axis) represented by heatmap. Scale represents P-value of positive (orange) and negative (green) body weights correlations with transcripts (y-axis). Homeostatic microglia 1 (HMG1), HMG2, HMG3, inflammatory microglia (InflamMG), interferon-related microglia (IfnMG), macrophages (Mac), monocytes (Mono), neutrophils (Neu), neurons (Neuro), proliferating microglia (ProlifMG), uMG (unknown, functionally undescribed).