SUPPLEMENTAL MATERIAL

Ferris et al., https://doi.org/10.1084/jem.20170074

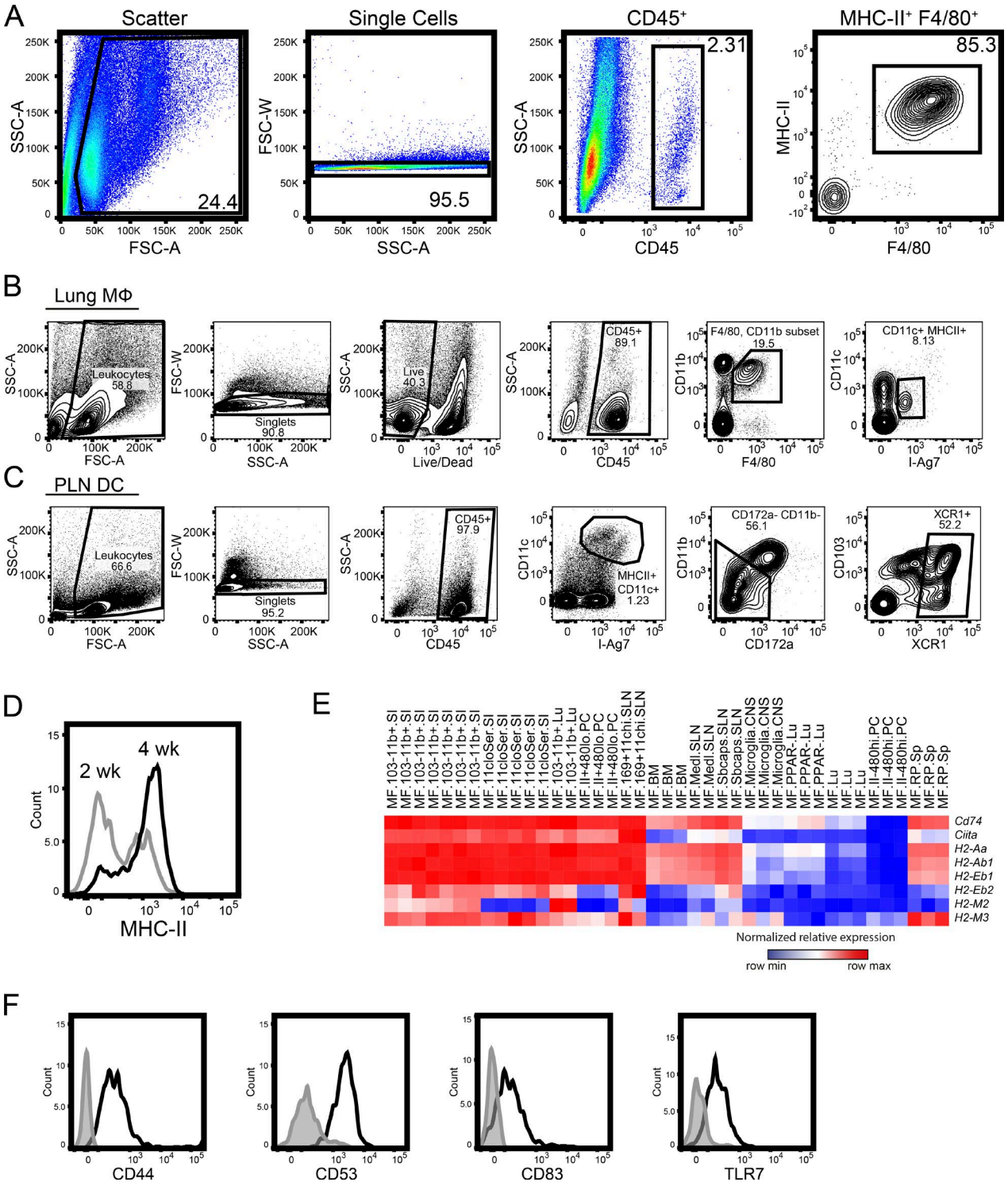
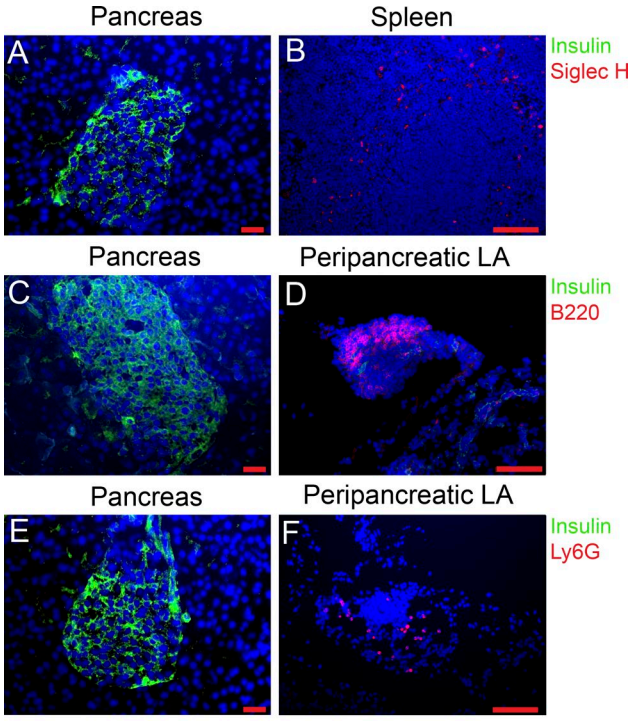


Figure S1. **Analysis of islet macrophages.** (A) Flow cytometry gating strategy for sorting of macrophages from 3-wk-old mice. Cells were gated on the basis of forward and side scatter, single cells, CD45 expression, MHC-II, and F4/80 expression. Flow cytometry gating strategy of lung macrophages (B) and PLN XCR1$^+$ DC (C) populations. Lung macrophages were gated on FSC/SSC, singlets, live dead, CD45, CD11c, F4/80, and MHC-II expression. XCR1$^+$ DCs were gated on FSC/SSC, singlets, CD45, CD11c, MHC-II, CD11b, CD172a, CD103, and XCR1. (D) Expression of I-A^{g7} on CD45$^+$ CD11c$^+$ F4/80$^+$ macrophages isolated from islets of 2-wk-old and 4-wk-old NOD female mice. (E) Expression of MHC-II complex genes, including the master regulator *Ciita* by different macrophage populations, on the basis of the ImmGen dataset (GSE15907). (F) Flow cytometry of CD45$^+$ CD11c$^+$ I-A^{g7+} NOD macrophages examining for CD44, CD53, CD83, and TLR7.



G

	Islets Positive for:		
	B220+	1A8+	Siglec H+
NOD 3wk Females	0/408	0/380	0/370
B6 8wk Females	1/197	0/183	0/224

Figure S2. **Immunofluorescence of pancreas and lymphoid tissue.** (A, C, and E) Pancreatic sections and (B) splenic sections or (D and F) peripancreatic lymphoid aggregates (LA) were stained with anti-insulin (β cells) and (A and B) Siglec H (pDCs), (C and D) B220 (B cells), or (E and F) Ly6G (neutrophils). All sections were nuclear-stained with bisbenzimide. (G) The indicated number of islets were scored for the presence of either $B220^+$, 1A8 $(Ly6G)^+$, or $SiglecH^+$ cells. Results were obtained from NOD mice at 3 wk and C57BL/6 mice at 8 wk of age (three mice for each).

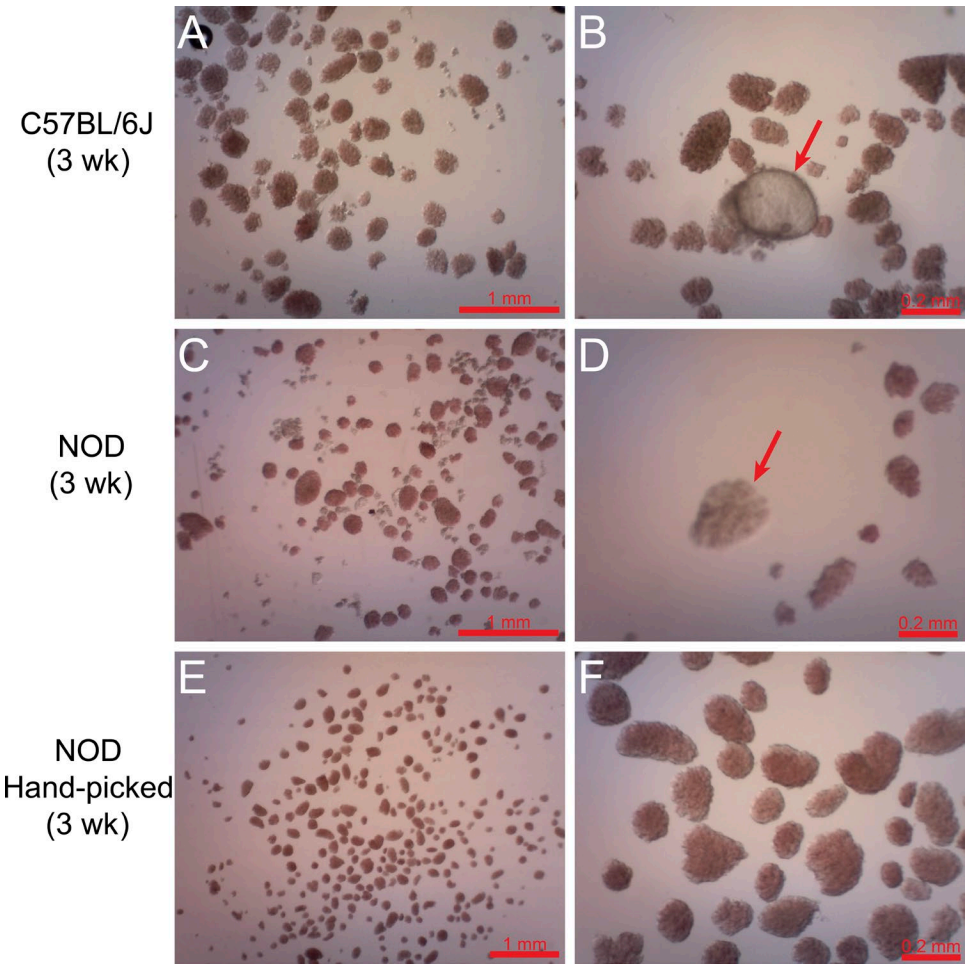


Figure S3. **Imaging of isolated islet preparations.** (A and B) C57BL/6J and (C and D) NOD pancreata were digested without hand picking and imaged. (E and F) NOD pancreata were digested, hand-picked under a dissection microscope, and imaged. All islet preparations were stained with dithizone to allow visual separation of islets from the acinar or lymphoid aggregate structures that associate with pancreas digestion preparations. Arrows indicate contaminating acinar or leukocytic aggregate structures.

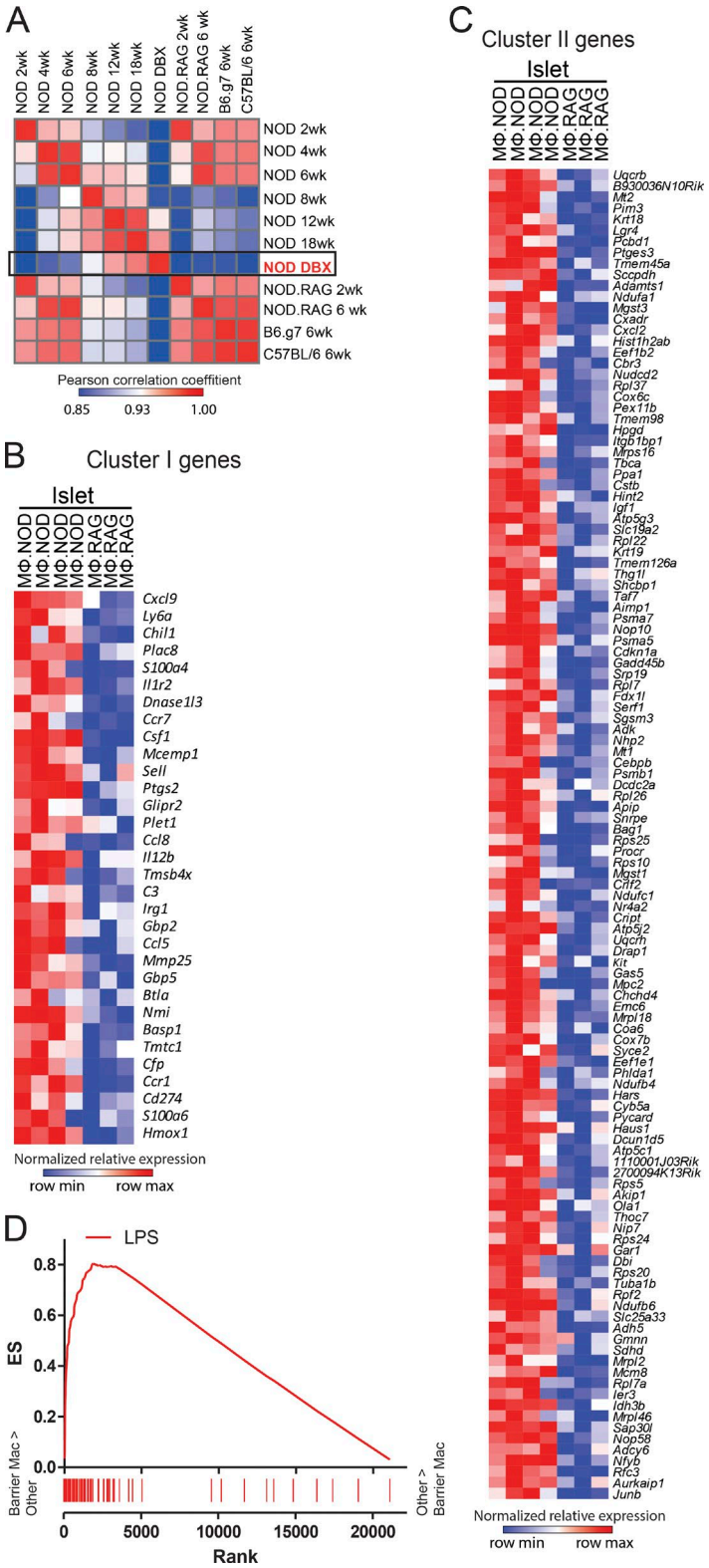


Figure S4. **There are two clusters of genes up-regulated in islet NOD macrophages compared with islet NOD.*Rag1*$^{-/-}$ macrophages.** (A) Pearson's correlation between all samples on the basis of genes, differentially up-regulated in NOD versus NOD.*Rag1*$^{-/-}$ islet macrophages with $P \leq 0.05$, and maximum likelihood estimate–moderated fold change ≥2. Heat maps of (B) cluster I and (C) cluster II genes. (D) GSEA plots of lamina propria, serosal, and CD11b^{+}CD103^{neg} lung macrophages comparing with other macrophages from ImmGen dataset, on the basis of LPS signature. ES, enrichment score.

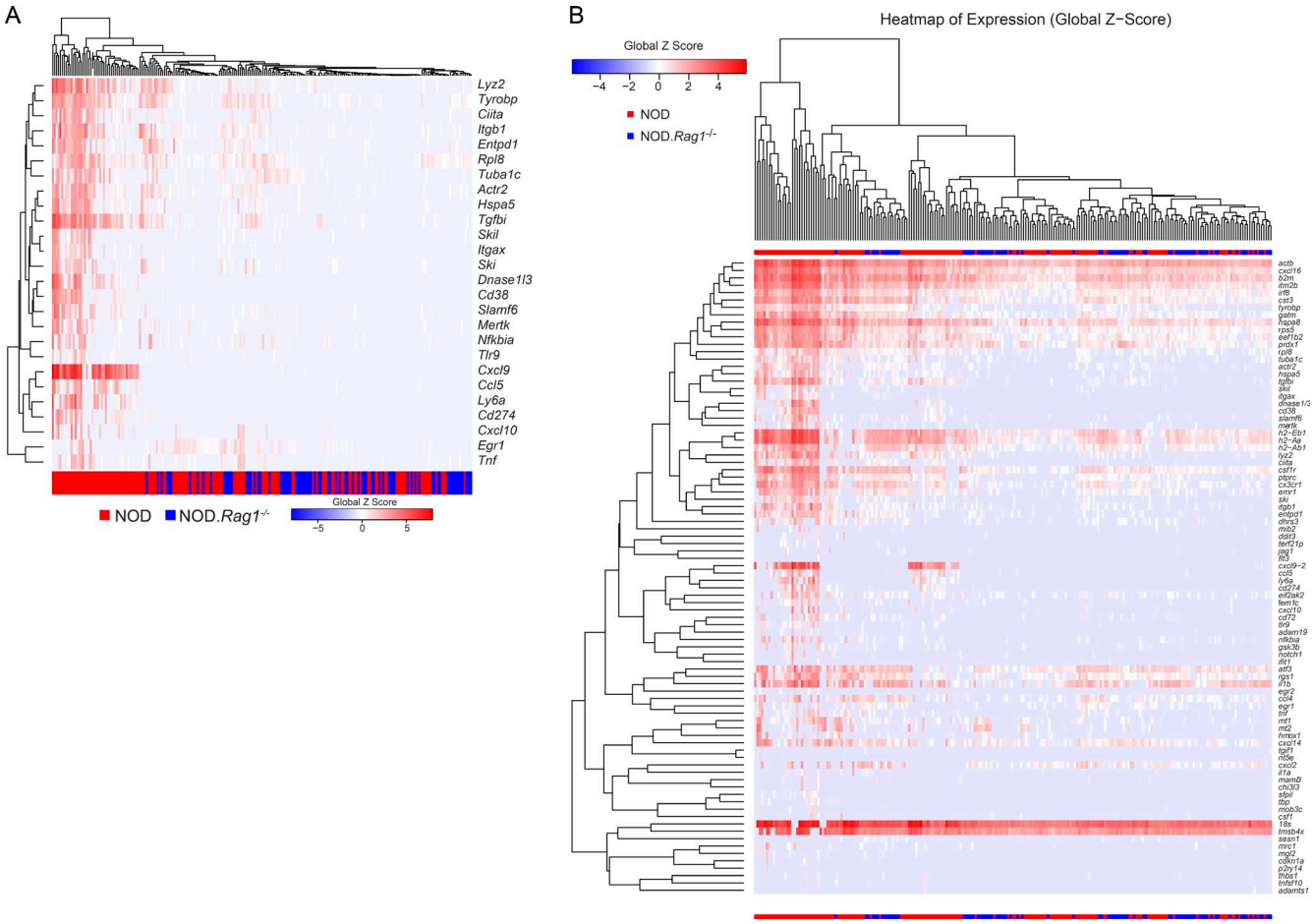


Figure S5. **Heat map of single islet macrophage qRT-PCR data.** Single-cell qRT-PCR data for NOD and NOD.*Rag1*$^{-/-}$ macrophages were hierarchically clustered using Pearson's correlation. (A) Genes selected as significant in Fig. 7 B and (B) all genes were plotted by hierarchical clustering using Pearson's correlation for grouping and global Z score for scaling. The key on the bottom of the heat map indicates the cell type examined.

Table S1, included in a separate Excel file, is a gene expression table of islet and lung macrophages and NOD PLN DCs.