Protective *Renalase*-deficiency in beta cells shapes immune metabolism and function in autoimmune diabetes

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Supplemental Figure Legends

Supplemental Figure 1: Comparison of immune cells in WT NIT-1 grafts, spleen and pancreas after adoptive transfer of diabetogenic splenocytes.

NIT-1 WT cells ($1.5x10^7$) were injected s.c. in the left flank of NOD.scid mice, followed by i.v. injection of diabetogenic splenocytes 4 days (d) later. Grafts, spleen and pancreas were digested with collagenase D and DNAse I. Single cell suspensions were stained for the indicated surface markers and analyzed by flow cytometry. (**a, b**) Immune cell populations from the indicated tissue obtained at d10 (**a**) or d17 (**b**) after splenocyte transfer. (**c-d**) Analysis of T cell activation markers. Summary data is shown is in **c**, representative contour plots are shown in **d**. Results represent the mean ± SD of four biological replicates.

Supplemental Figure 2: Characterization of immune cells isolated from WT or *Rnls* NIT-1 grafts, from their respective draining lymph nodes (dLN) and from the pancreas at different time points after adoptive transfer of diabetogenic splenocytes.

WT or *Rnls* mutant NIT-1 cells ($1.5x10^7$) were injected *s.c.* in opposite flanks of NOD.scid mice, followed by *i.v.* injection of diabetogenic splenocytes 4 days later. Grafts, whole pancreas or inguinal dLN were analysed by flow cytometry at d10 (early) or d17 (late) (**a**) Quantification of graft weight from paired WT and RNLS-deficient grafts isolated at d10. Note that graft destruction in this particular experiment was not as pronounced at d10 as in the experiment shown in Figure 2. (**b**) Quantification of the indicated immune cell subpopulations within 6 paired grafts at d10. (**c**) Quantification of the indicated immune cell subpopulations within dLN at d17 d. (**d**) Comparison of the indicated immune cell subpopulations in paired WT and *Rnls*-deficient NIT-1 grafts and pancreas at d17. (**e-f**) Mean fluorescence intensity (MFI) of MHC class II surface expression on antigen presenting cells (APC, **e**) or macrophages (**f**) in NIT-1 grafts at d10 and d17. Results represent the mean of six (**a**, **b**, **e** and **f**) or four (**c** and **d**) paired biological replicates. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ (multiple paired or unpaired, two-tailed t-test (**a,b,c,e,f**), or one-way ANOVA analysis (**d**)).

Supplemental Figure 3: *Rnls*-deficient beta cell grafts harbor a similar composition of infiltrating immune cells but are enriched for adipose-tissue (AT)-like macrophages (Mφ cluster 2) compared with WT grafts. (**a**) Representative contour plot of FACS-sorted CD45 positive and propidium iodide (PI) negative immune cells used in 3‘ gene expression single cell RNA sequencing (scRNAseq) approach. (**b**) UMAP showing the distribution of late graft-infiltrated immune cell subpopulations within all RNLS-deficient (top) or WT (bottom) grafts.

(**c**) Immunological Genome Project (ImmGen ULI RNA-Seq data base) geneset enrichment analysis of the 20 most upregulated genes in Mφ cluster 2 compared to indicated MF subsets. Squares show low (blue), intermediate (white to pink) or high (yellow to orange) gene expression of indicated subsets. (**d**) Reactome analysis of AT-Mφ cluster 2 identified by scRNAseq analysis at d17 showing the 10 most significantly upregulated pathways (-log10(FDR) ≥ 2).

Supplemental Figure 4: $CD4^+$ T cell cluster 0 is characterized by a distinct anti-inflammatory gene expression profile. Gene expression profile analysis was performed by geneset enrichment analysis of the 30 most upregulated genes in $CD4^+$ T cell cluster 0 compared to the indicated αβ T cell populations from the Immunological Genome Project (ImmGen ULI RNA-Seq data base). Squares denote low (blue), intermediate (white to pink) or high (yellow to orange) gene expression.

Supplemental Figure 5: Dendritic cell (DC) clusters 17 and 25 are characterized by an inflammatory gene expression profile. Hallmark gene expression analysis of (**a**) DC cluster 17 identified as conventional DC subtype 2 (cDC2) and (**b**) DC cluster 25 identified as plasmatoid DC (pDC) subset derived from single cell RNA sequencing (scRNAseq) showing the 10 most significantly upregulated pathways.