Distinct transcriptomic profiles in children prior to the appearance of type 1 diabetes-linked islet autoantibodies and following enterovirus infection

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Supplementary Information

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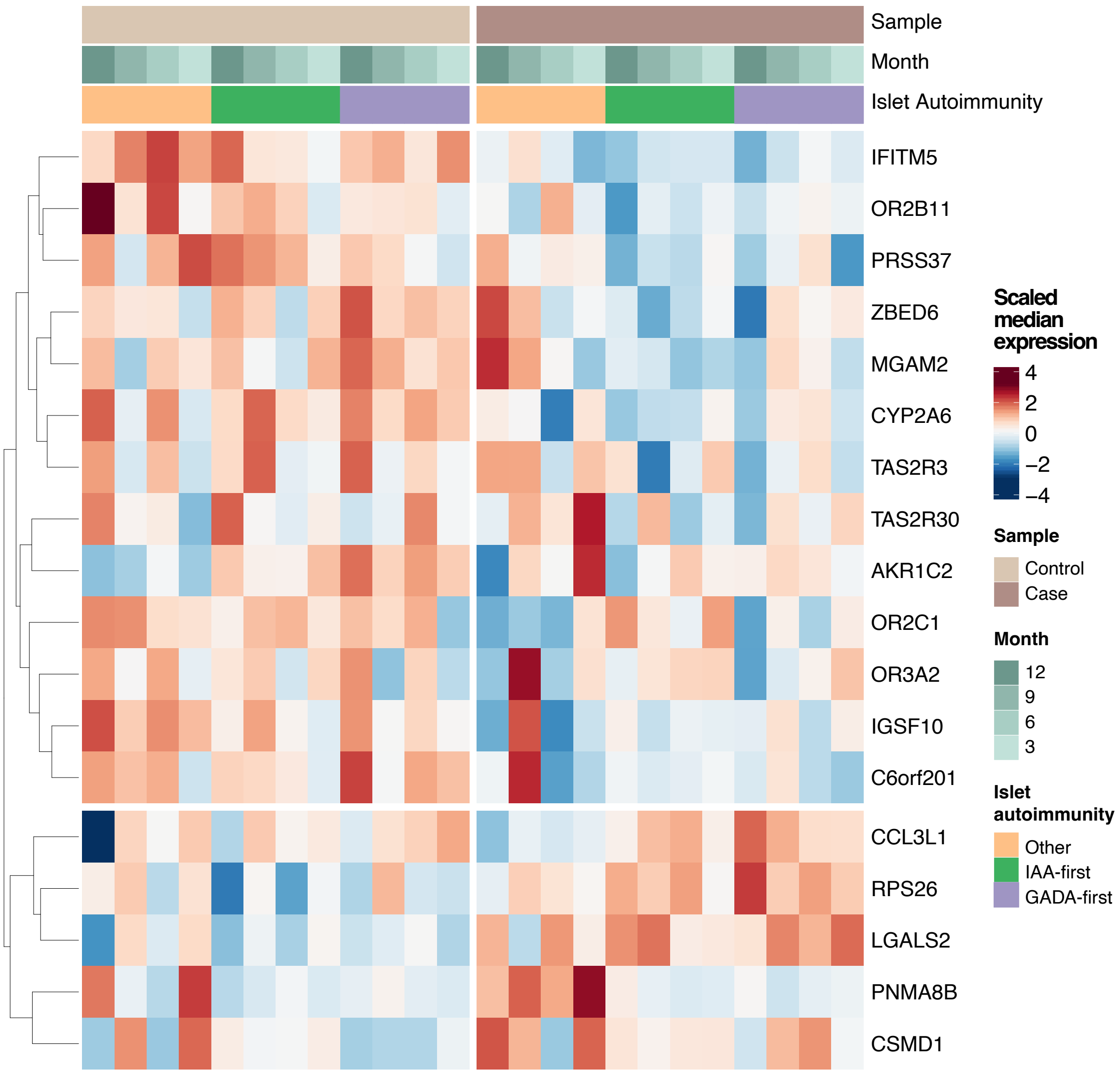
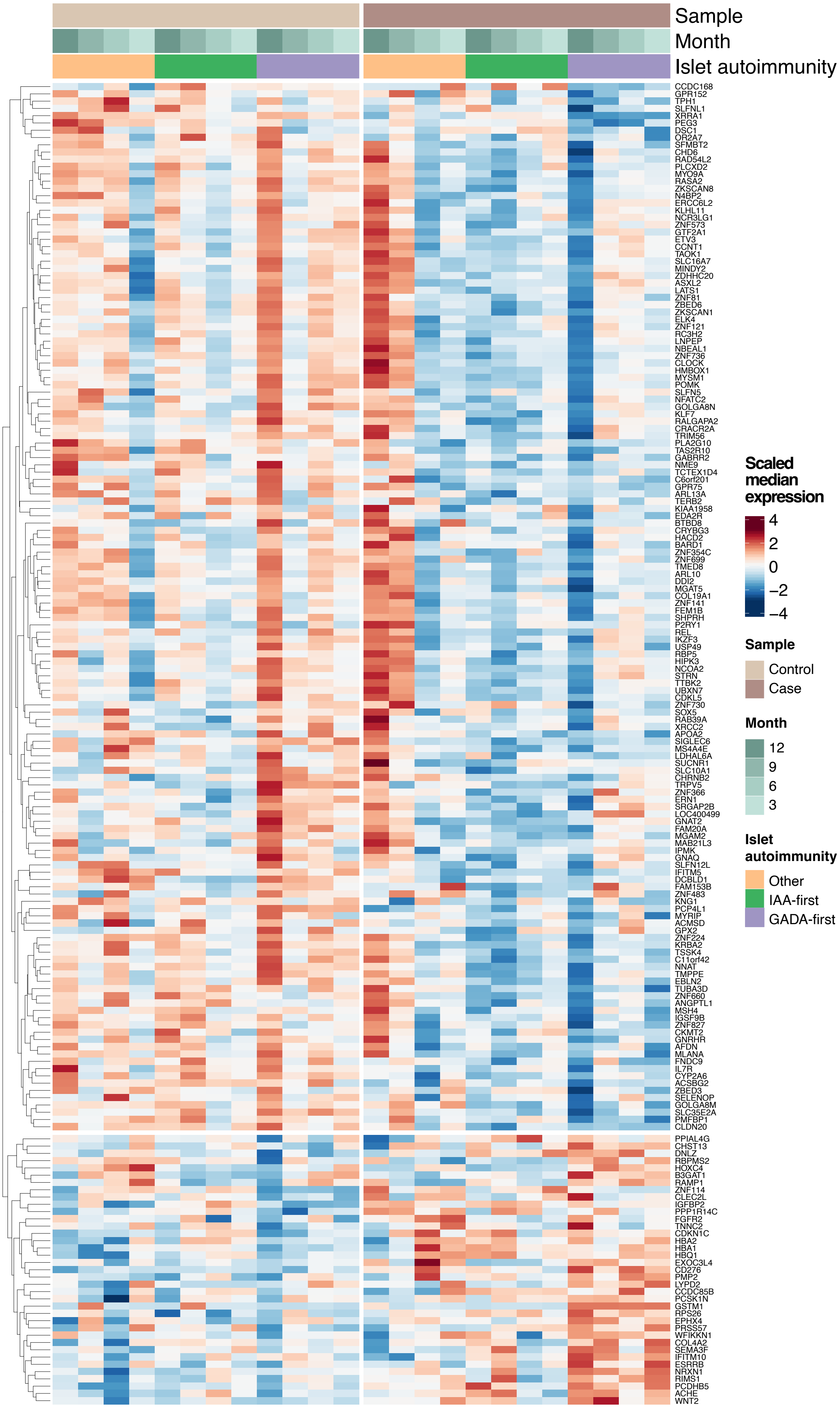
Supplementary Figures 1–17
Supplementary Note 1: Members of the TEDDY Study Group

Other Supplementary Material Includes:

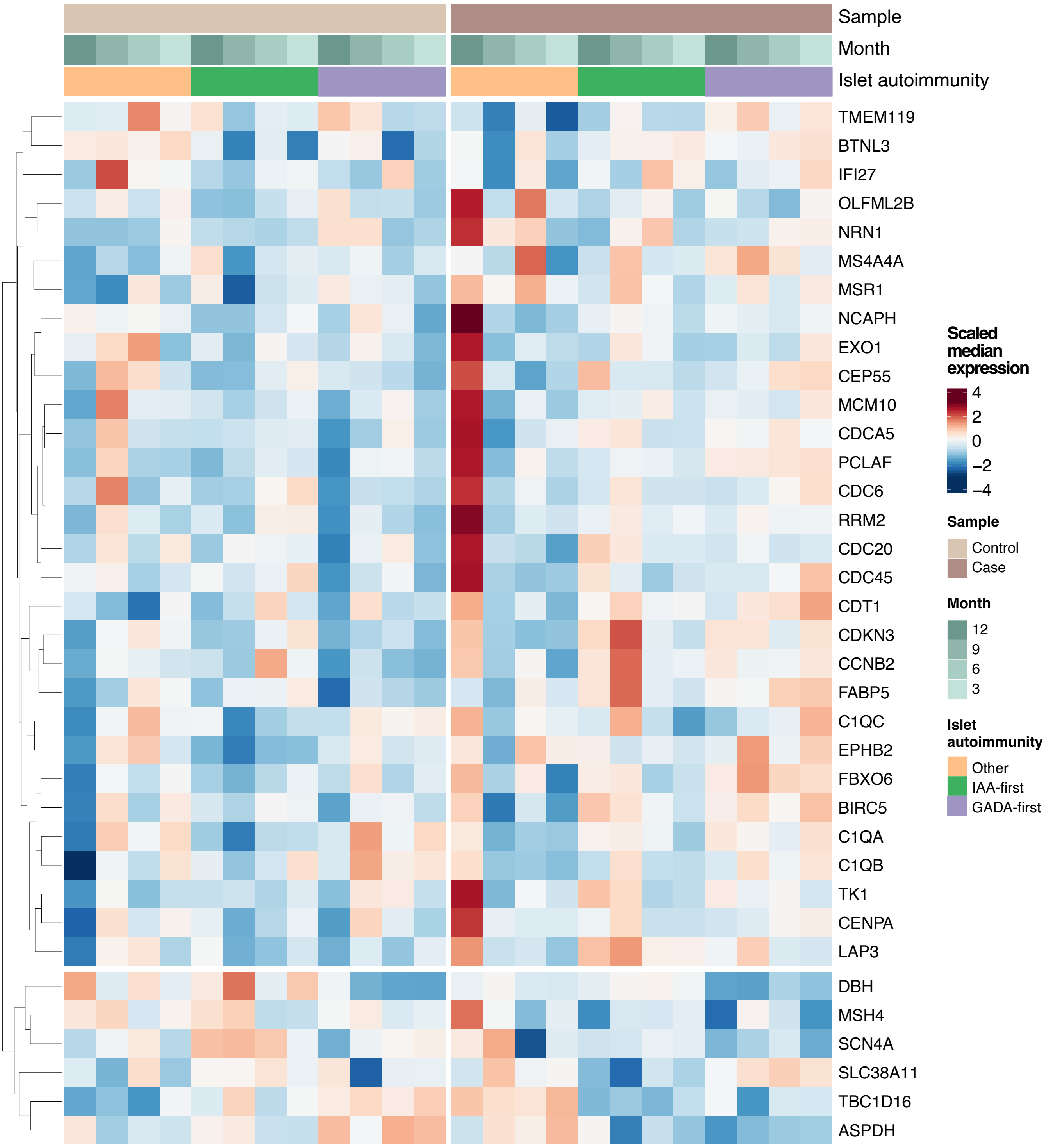
Supplementary Data 1–15



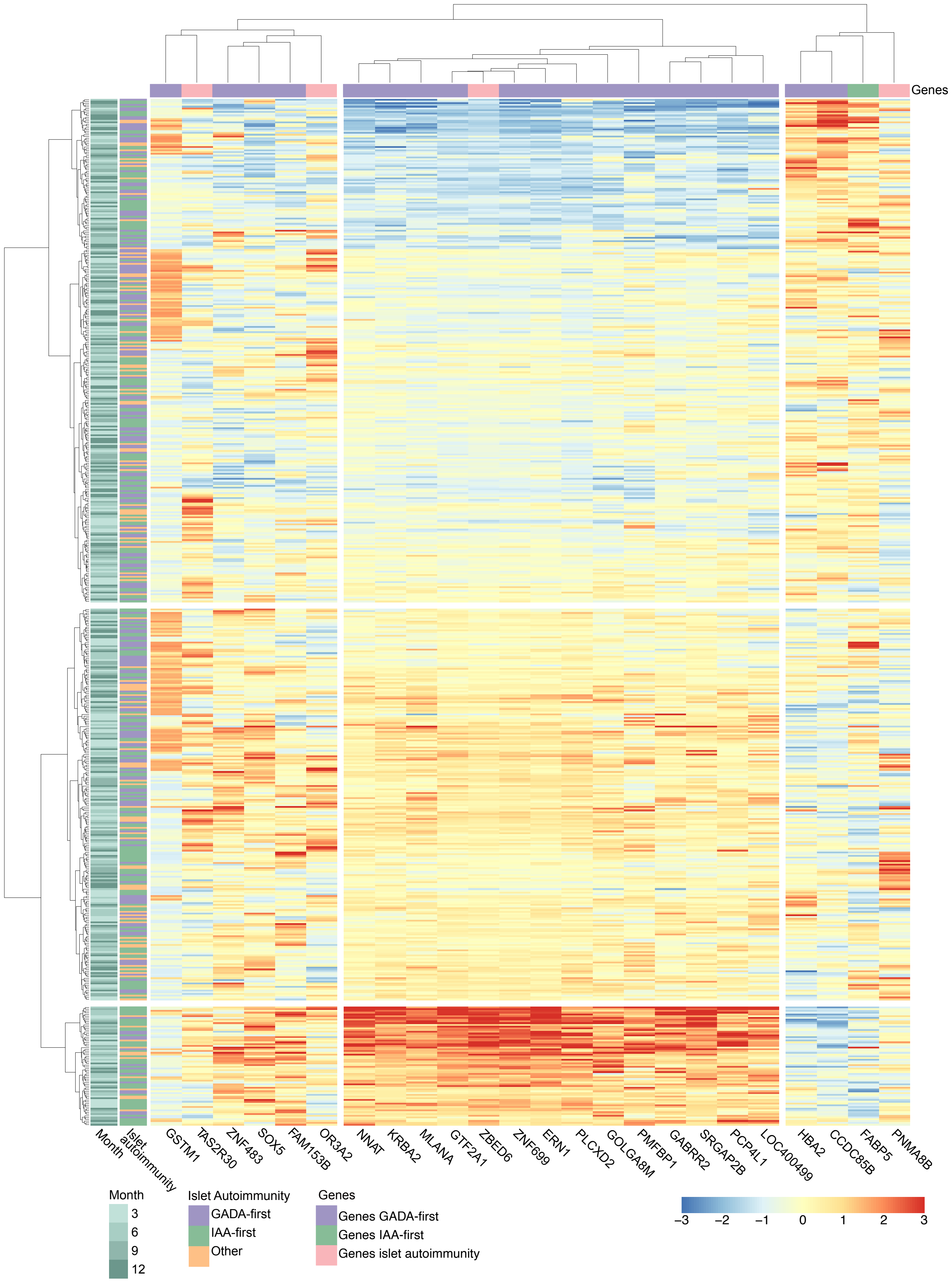
Supplementary Fig. 1: Differential gene expression in Islet Autoimmunity
Heatmap of the differentially expressed genes selected in the islet autoimmunity NCC1 cohort shows their expression across GADA-first, IAA-first and other T1D islet autoimmunity patterns (Supplementary Data 2). Heatmap value is the scaled median expression level at each timepoint.



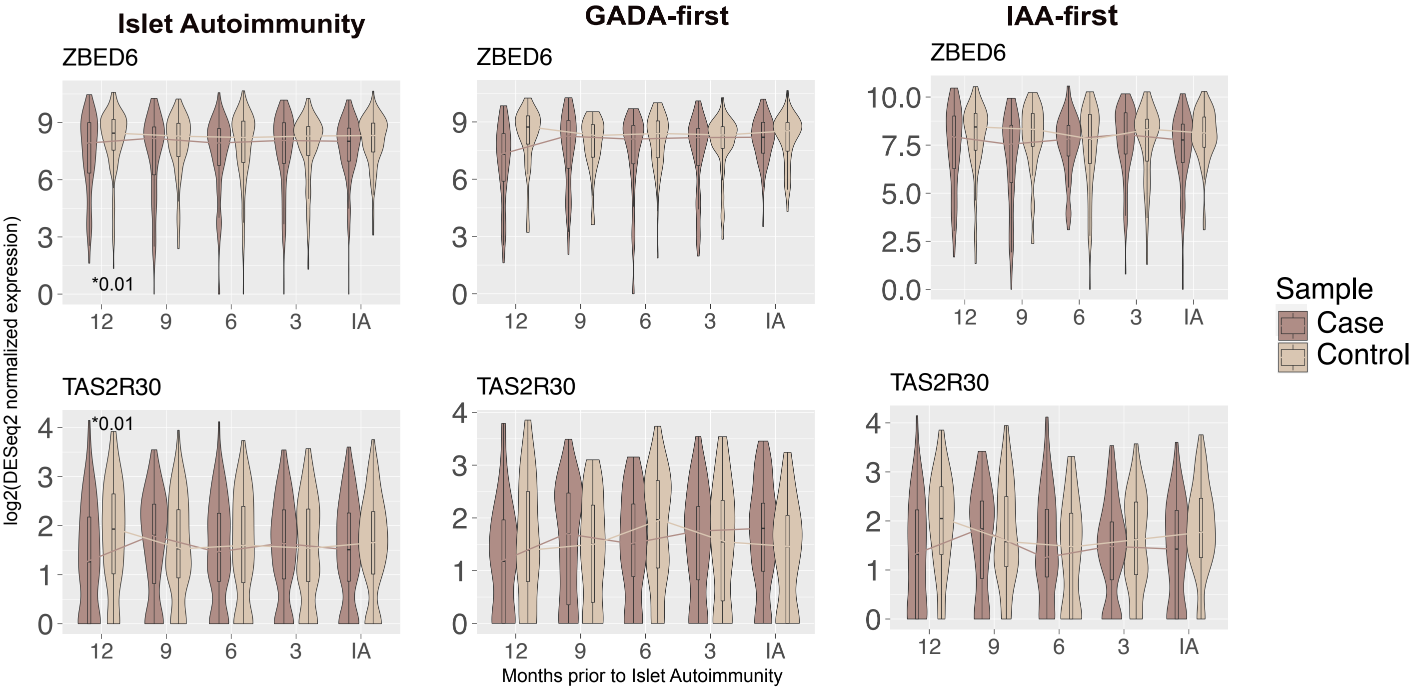
Supplementary Fig. 2: Differential gene expression in GADA-first
Heatmap of the differentially expressed genes selected in GADA-first T1D islet autoimmunity NCC1 cohort shows their expression across GADA-first, IAA-first and other T1D islet autoimmunity patterns (Supplementary Data 2). Heatmap value is the scaled median expression level at each timepoint.



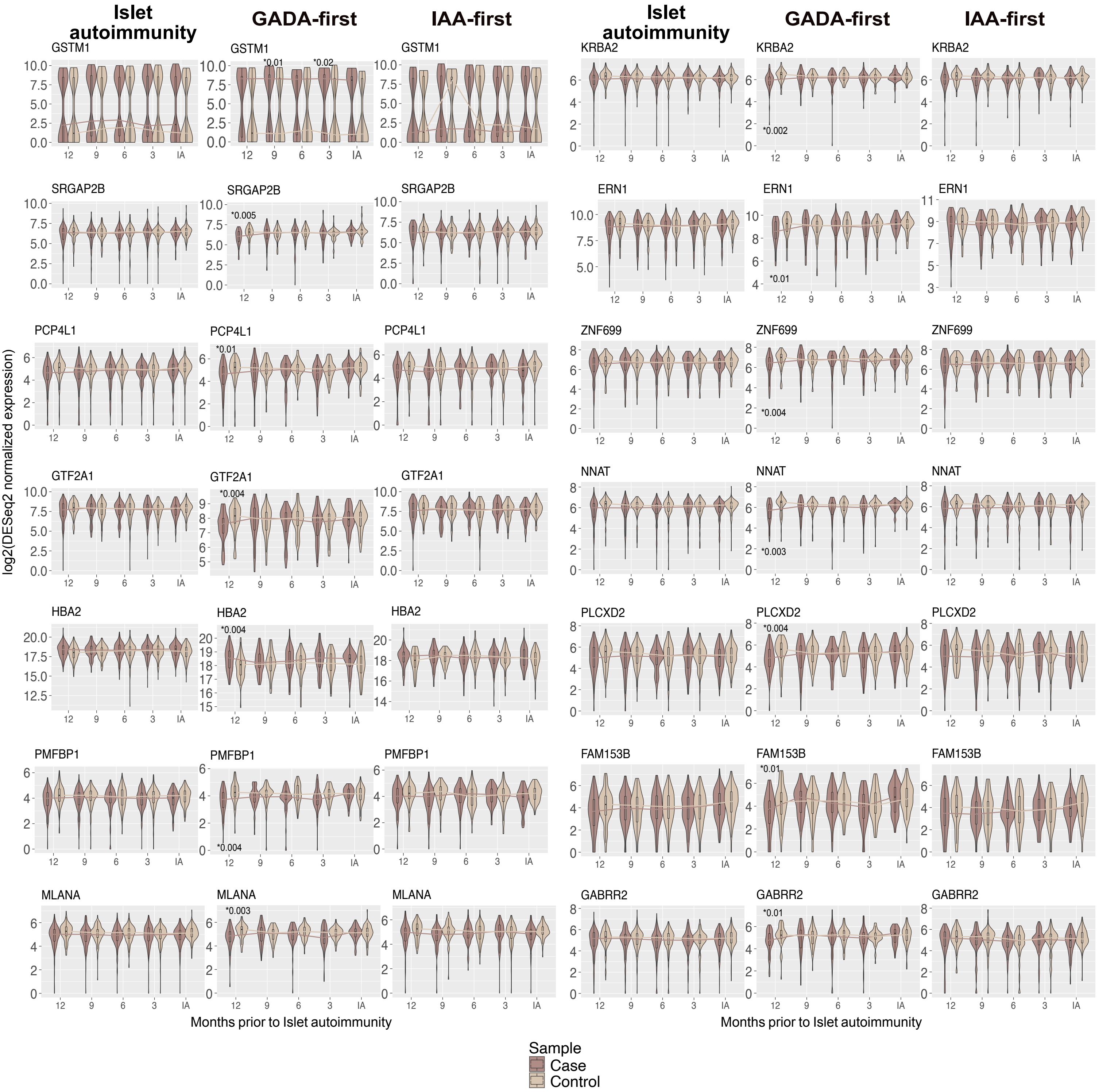
Supplementary Fig. 3: Differential gene expression in IAA-first
Heatmap of the differentially expressed genes selected in IAA-first T1D islet autoimmunity pattern shows their expression across GADA-first, IAA-first and other T1D islet autoimmunity patterns (Supplementary Data 2). Heatmap value is the scaled median expression level at each timepoint.



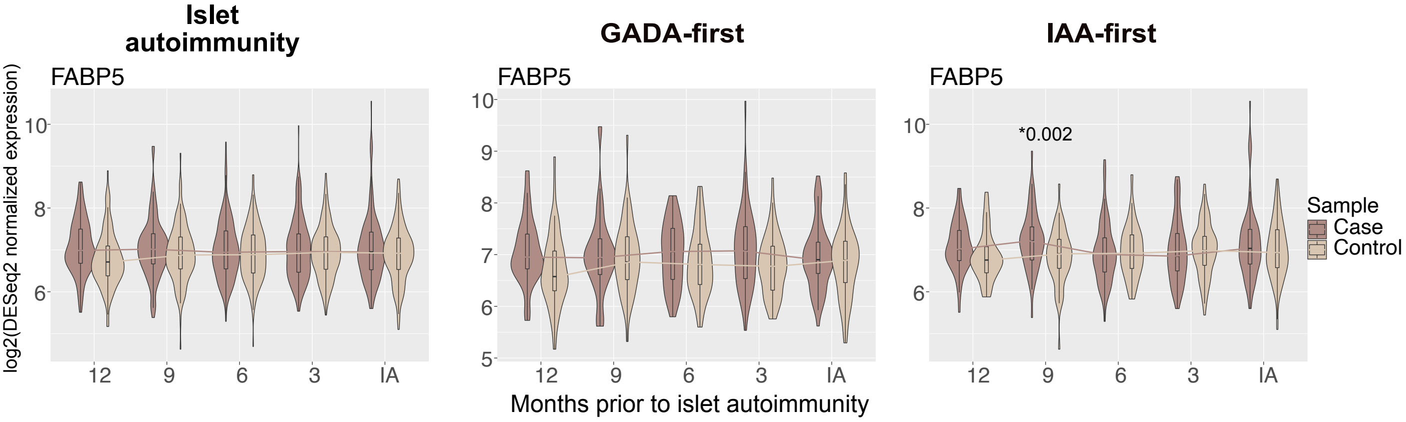
Supplementary Fig. 4: Differential gene expression in genes selected with additional criteria of temporal changes
Distinct endotype GADA-first and IAA-first clusters are formed using the genes selected from the temporal analysis with inclusion of slope changes between samples prior to seroconversion. These genes and their statistical details are shown in Supplementary Data 4. Heatmap value is the scaled ratio between matched case and control.



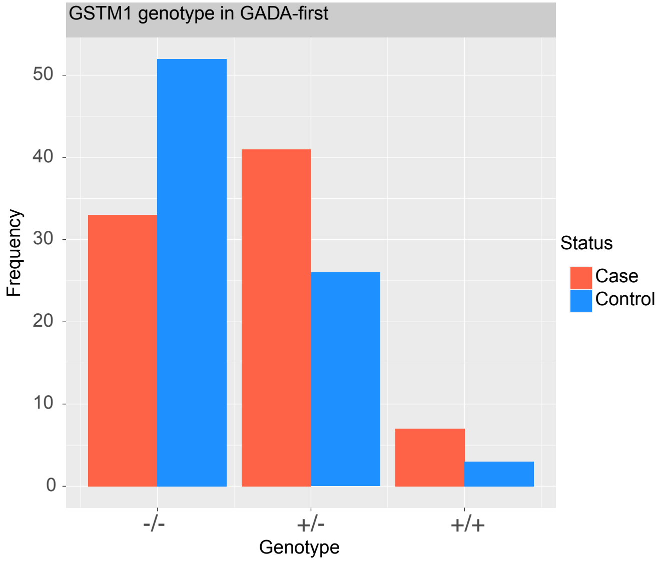
Supplementary Fig. 5: Temporal expression of the genes selected with additional criteria in Islet Autoimmunity
Violin plots of the selected differentially expressed genes with inclusion of temporal slope changes and adjustment for multiple timepoints from IAb conversion to 12 months prior relative to all 3 islet autoimmunity patterns. Figure shows two genes selected with the full islet autoimmunity in all three cohorts (full islet autoimmunity (n=168), GADA 1st (n=55) and IAA first (n=85)) and the corresponding significant p-values from conditional logistic regression (Supplementary Data 5) with line traversing the median. Boxes: center lines, median; box limits, upper and lower quartiles; whiskers, values within 1.5 × IQR of the top and bottom quartiles. X-axis IA = Islet Autoimmunity time of seroconversion.



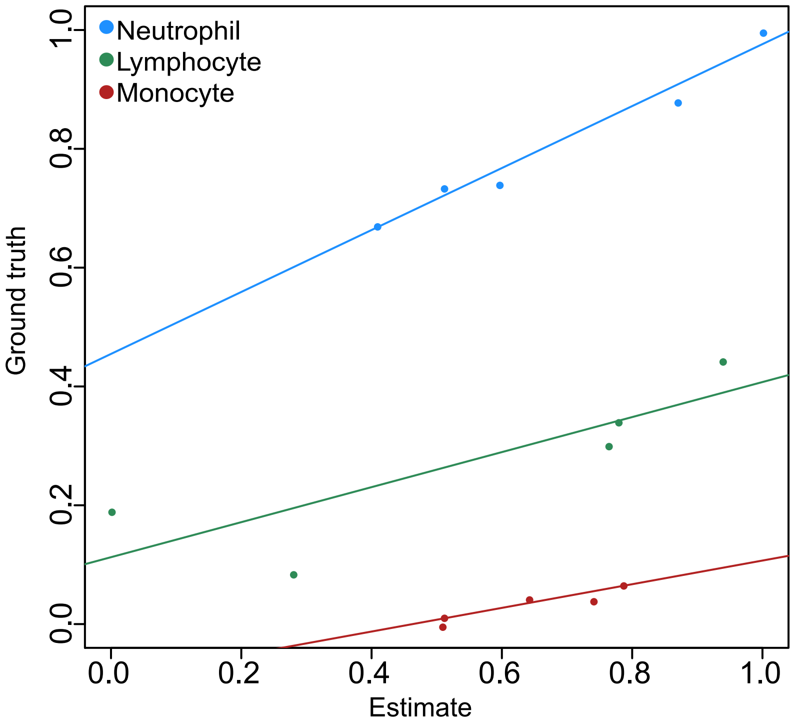
Supplementary Fig. 6: Temporal expression of the genes selected with additional criteria in GADA-first
Violin plots of the selected differentially expressed genes with inclusion of temporal slope changes and adjustment for multiple timepoints from IAb conversion to 12 months prior relative to all 3 islet autoimmunity patterns. Figure shows the 14 genes tested from GADA-first pattern in all three cohorts (full islet autoimmunity (n=168), GADA 1st (n=55) and IAA first (n=85)) and the corresponding significant p-values from conditional logistic regression (Supplementary Data 5) with line traversing the median. Boxes: center lines, median; box limits, upper and lower quartiles; whiskers, values within 1.5 × IQR of the top and bottom quartiles. X-axis IA = Islet Autoimmunity time of seroconversion.



Supplementary Fig. 7: Temporal expression of the genes selected with additional criteria in IAA-first
Violin plots of the selected differentially expressed genes with inclusion of temporal slope changes and adjustment for multiple timepoints from IAb conversion to 12 months prior relative to all 3 islet autoimmunity patterns. Figure has 1 gene selected from IAA-first shown in all three cohorts (full islet autoimmunity (n=168), GADA 1st (n=55) and IAA first (n=85)) and the corresponding significant p-value from conditional logistic regression (Supplementary Data 5) with line traversing the median. Boxes: center lines, median; box limits, upper and lower quartiles; whiskers, values within 1.5 × IQR of the top and bottom quartiles. X-axis IA = Islet Autoimmunity time of seroconversion.

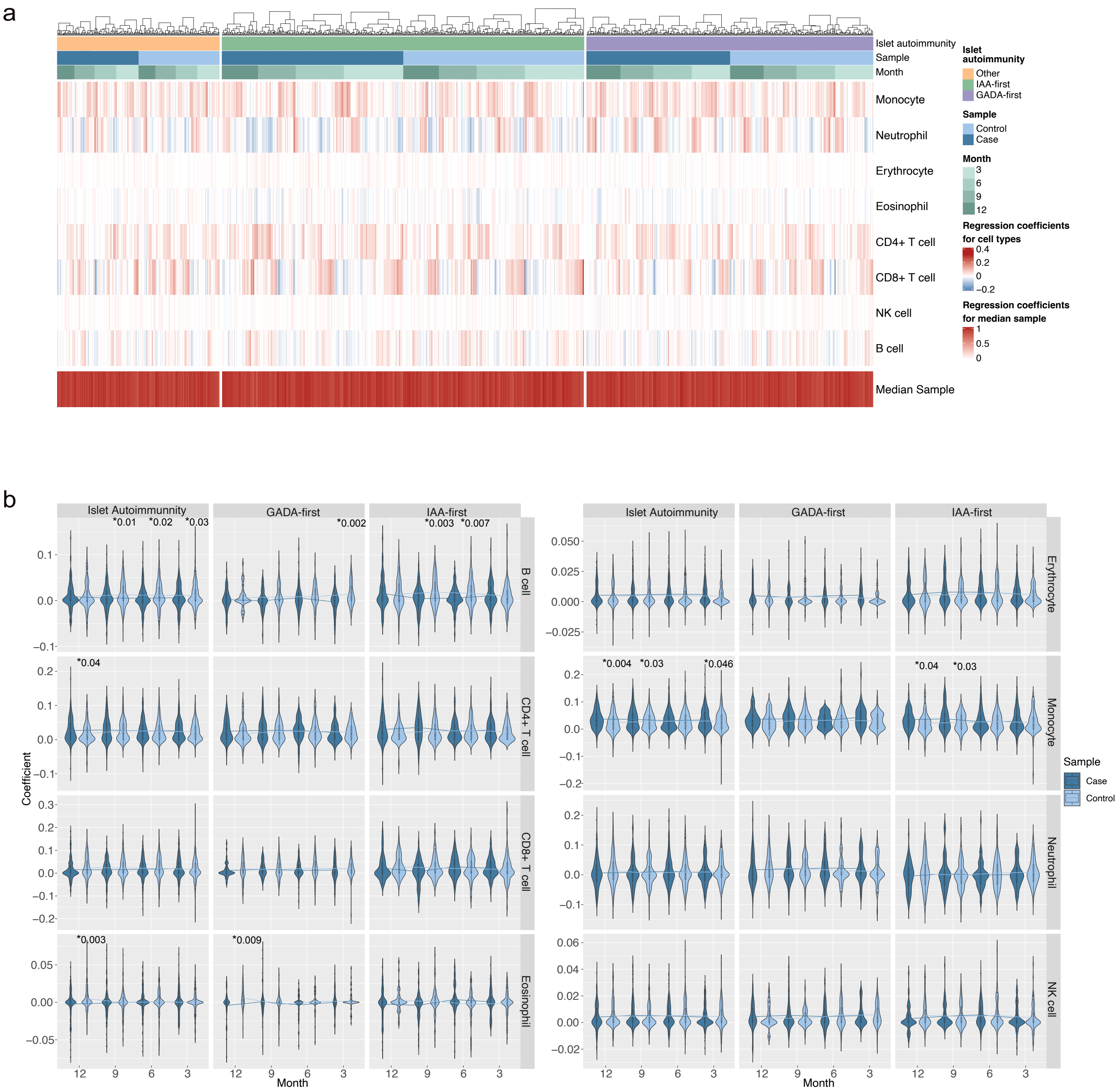


Supplementary Fig. 8: Frequencies of GSTM1 genotype in GADA-first case and control children
Barplot shows how the frequencies of genotypes in the GADA-first cohort differ for cases (red, n=81) and matched controls (blue, n=81). More controls harbor the homozygous deletion and diploid genotype was found more often in cases. Genotype is indicated as: +/+ is for diploid, +/- for hemizygous deletion, and -/- for homozygous deletion.



Supplementary Fig. 9: Validation of the cell-type deconvolution method

Assessment of the deconvolution method was performed using a validation reference consisting of five whole blood RNAseq samples with known cell type fractions from Gene Expression Omnibus (accession GSE60424). The deconvoluted cell type coefficients yielded 0.920 Pearson correlation with the ground truth cell type proportions.

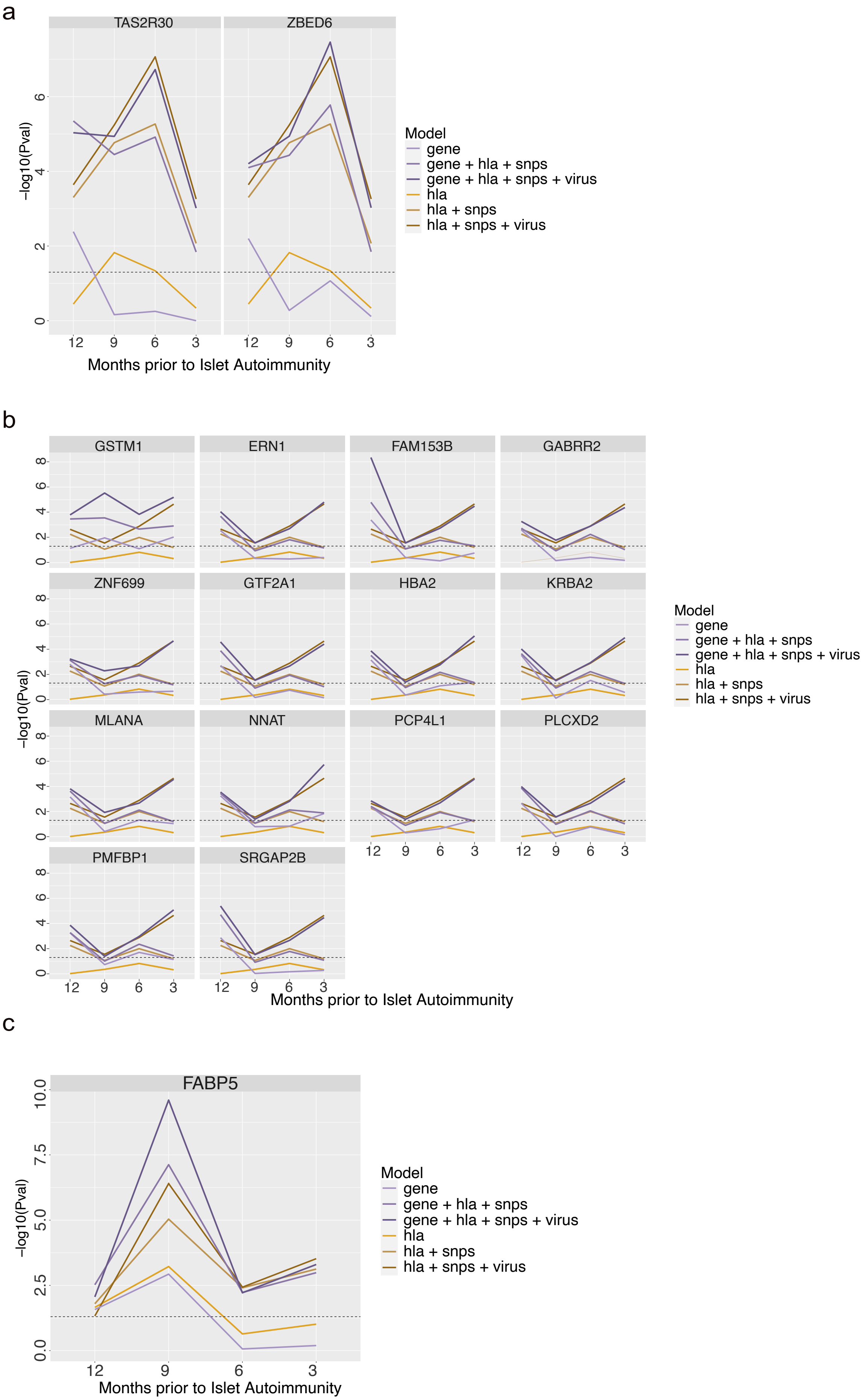


Supplementary Fig. 10: Cell type regression coefficients show differing temporal patterns between the different Islet Autoimmunity groups

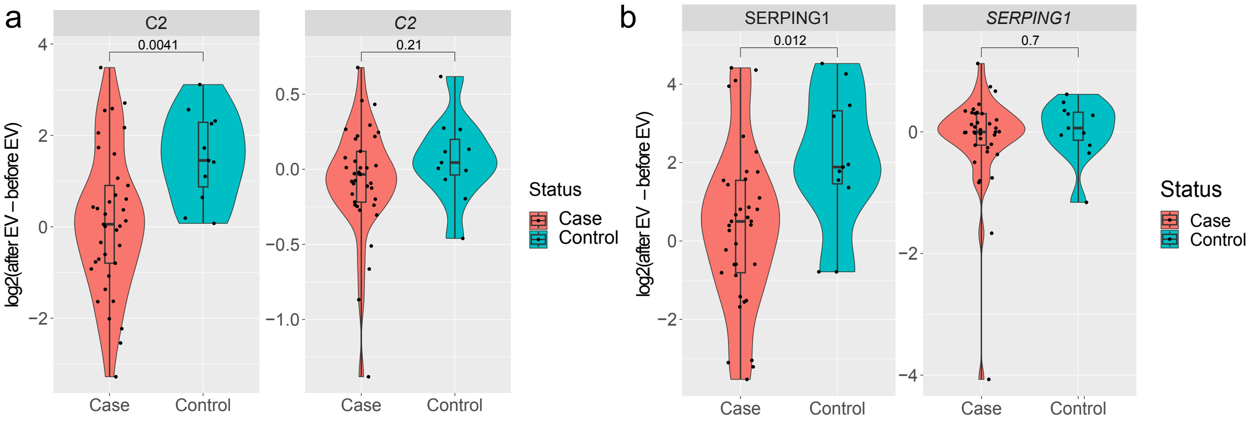
(a) Expression patterns are distinct within immune cell type across IAA-first and GADA-first islet autoimmunity groups. Supporting Fig. 2d, Monocytes have higher coefficient values in cases particularly in IAA-first (middle shown in light green). Interestingly, CD4 and CD8 mostly have alternating odds ratios from 9+ months prior to seroconversion as listed in Supplementary Data 6. (b) Temporal patterns of the cell type coefficients, results significant in conditional logistic regression with p-value < 0.05 are marked with an asterisk (*) followed by the corresponding p-value. The line indicates the median, with boxes center lines, median; box limits, upper and lower quartiles; whiskers, values within 1.5 × IQR of the top and bottom quartiles.

Supplementary Fig. 11: Pairwise associations between the cell type coefficients estimated from the transcriptome

Scatterplots to illustrate pairwise associations between the inferred cell type coefficients. Data is shown to support the interpretation of cell type coefficients as compositional nature of the deconvolution may introduce dependencies between cell types. Figure shows Pearson correlation coefficients (R) and their corresponding p-values (p).



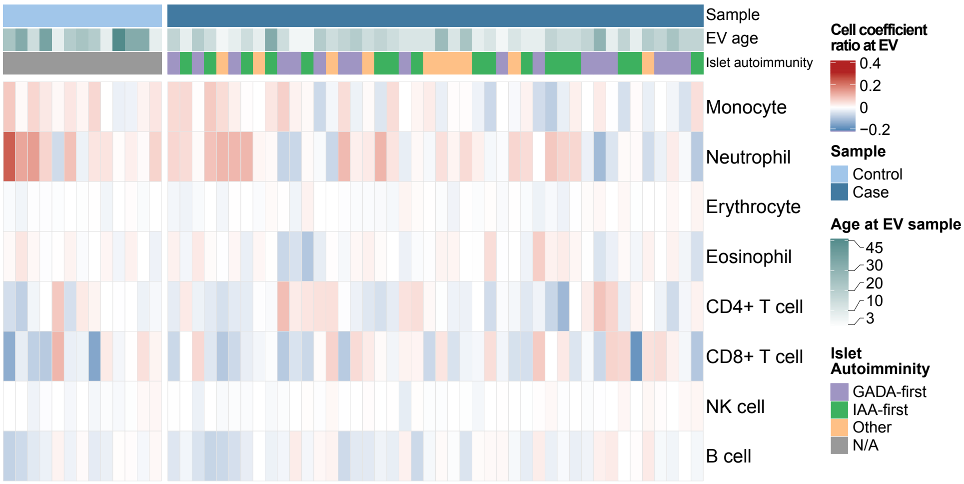
Supplementary Fig. 12: Results from Likelihood Ratio Test support the inclusion of transcriptomic data
Lineplot shows that HLA, SNP and Virus Likelihood ratio test p-values are consistently better with the inclusion of transcriptomic data for the selected genes in the (a) full islet autoimmunity (b) GADA-first and (c) IAA-first cohorts (Supplementary Data 8a-c).



Supplementary Fig. 13: Gene expression and protein abundance show similar trend in case and control children at EV infection
Violinplots of log fold change (LFC) of the EV genes with matched protein data show a similar trend in case and control children. Gene is shown on the left, and protein on the right. Boxplot center lines, median; box limits, upper and lower quartiles; whiskers, values within 1.5 × IQR of the top and bottom quartiles. Statistic is derived with two-sided Wilcoxon rank-sum test. (a) C2 LFC for gene in case (n=37) and control (n=11) is 0.056 and 1.45 and for protein -0.034 and 0.044, in the same subjects respectively. (b) SERPING1 LFC for gene in case (n=37) and control (n=11) samples is 0.50 and 1.88, and for protein -0.002 and 0.062, respectively.

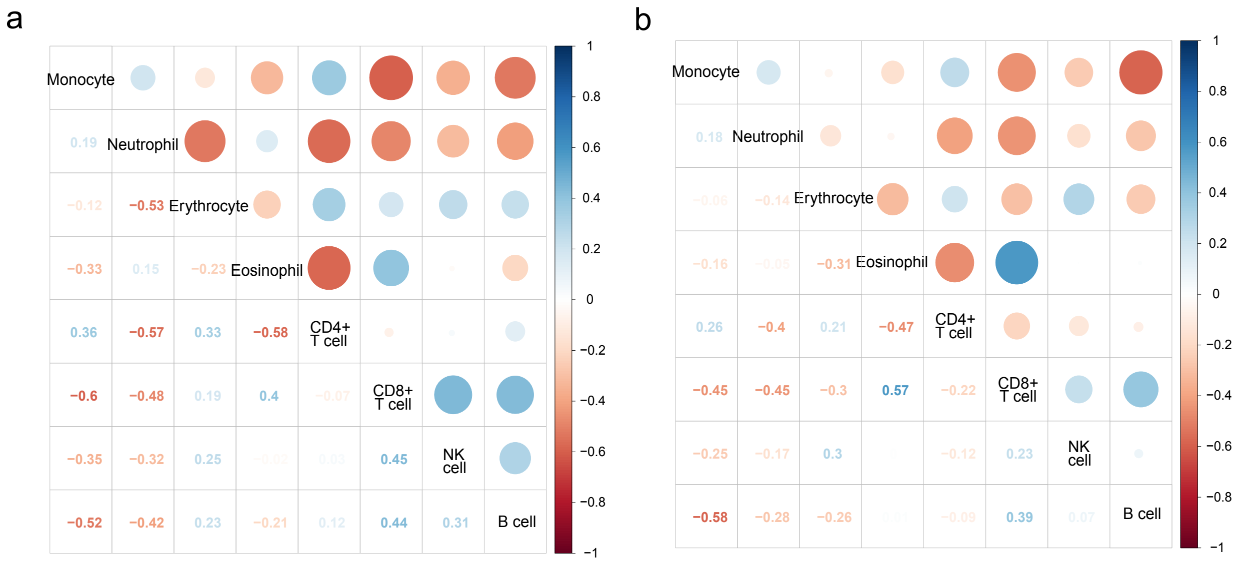
Supplementary Fig. 14: Heatmap of all the genes detected at EV in both case and control children

Distinct clusters are detected between after and prior to the initial EV infections of 483 selected genes (Supplementary Data 10) in case and control subjects. Samples with lowest gene profiles (ratio of after and prior to EV) are shown in blue and are clustered in the seroconverted children after infection.

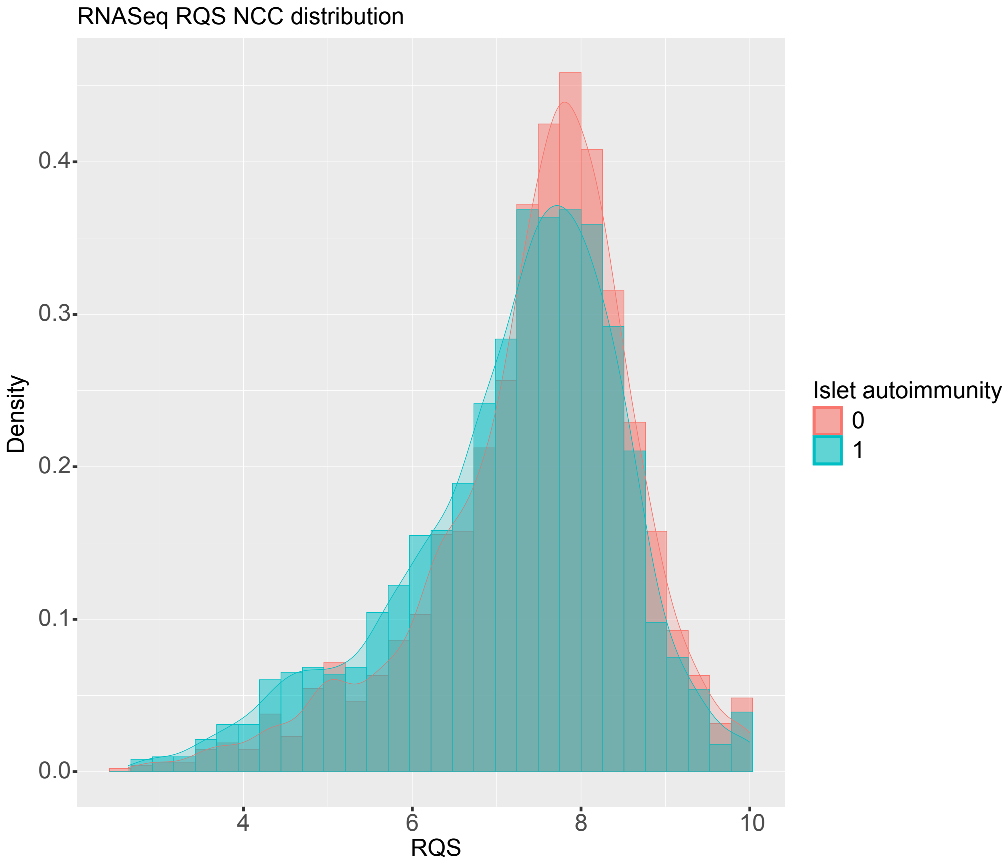


Supplementary Fig. 15: Heatmap shows immune cell type patterns before and after EV

Cell type coefficient patterns in case and control samples before and after the initial EV infection. The sample ratio patterns show negative correlation associations between monocyte and B cell as well as alternating values between CD4+ and CD8+ T cell types in seroconverted subjects. Sample order is the same as in Fig. 3a enabling comparison to gene expression patterns.



Supplementary Fig. 16: Correlation map of the cell types before and after HAdV
Correlation map between different immune cell types in control (n=74) (a) and case (n=88) (b) children based on the samples taken before and after the initial Human Adenovirus (HAdV) infection. Contrary to EV (Fig. 3d-e), the negative correlation between Neutrophils and CD8+ T cells was similar in both cases and controls (-0.48 in controls (a), -0.45 in cases (b)). Correlation coefficients were derived using the Pearson method



Supplementary Fig. 17: Histogram of RNAseq Quality Score distribution in the data
The histograms of the full islet autoimmunity NCC1 cohort RNAseq Quality Scores (RQS) show similar density distributions between islet autoimmunity cases and controls. Of all the samples >90% have RQS > 5.5 (median 7.54, IQR 6.67-8.16).

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