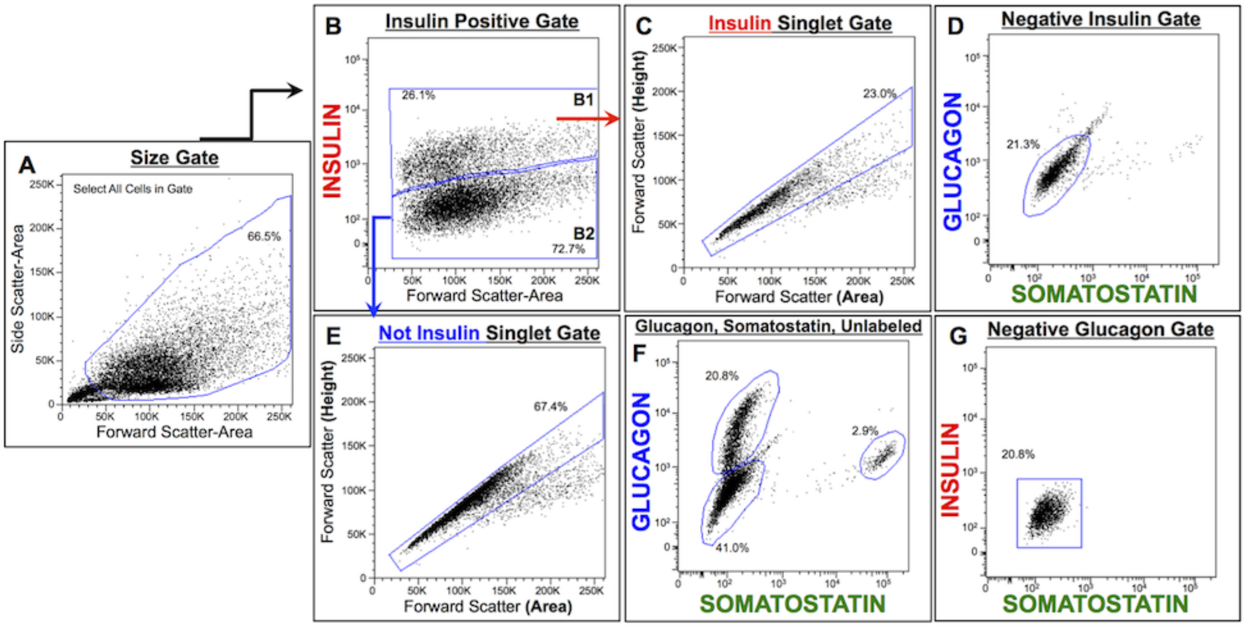
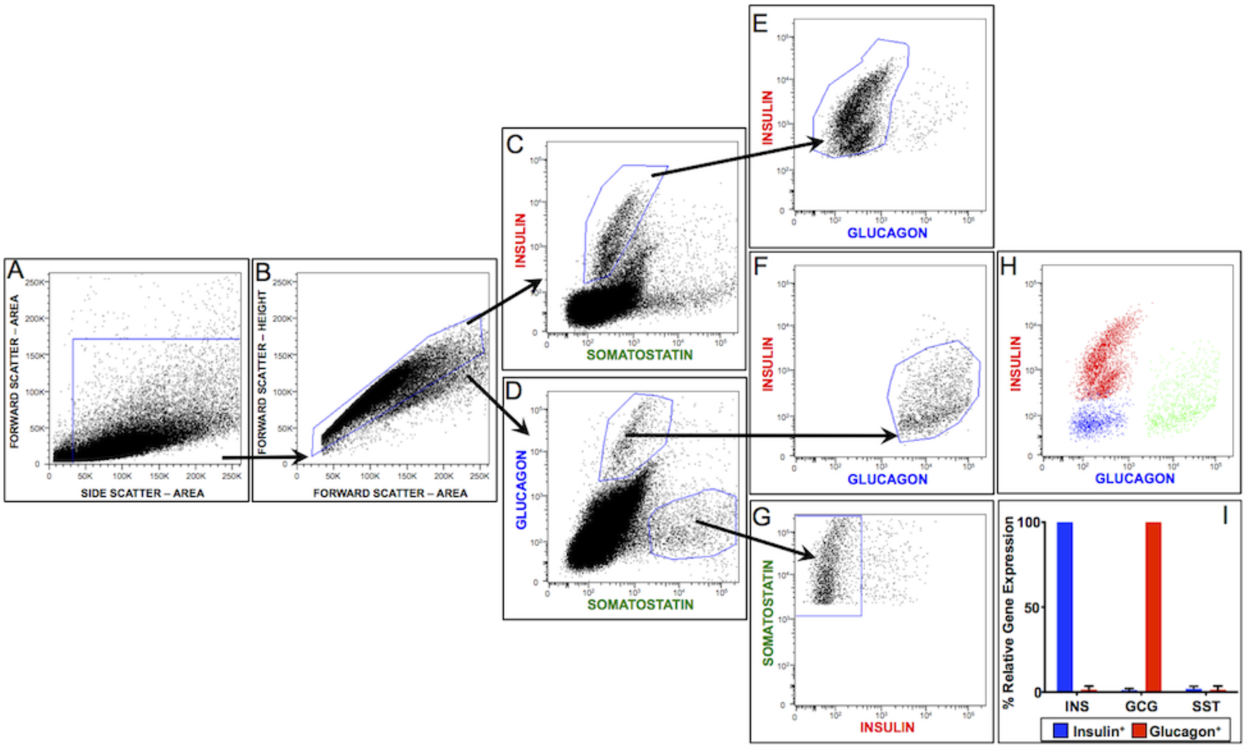
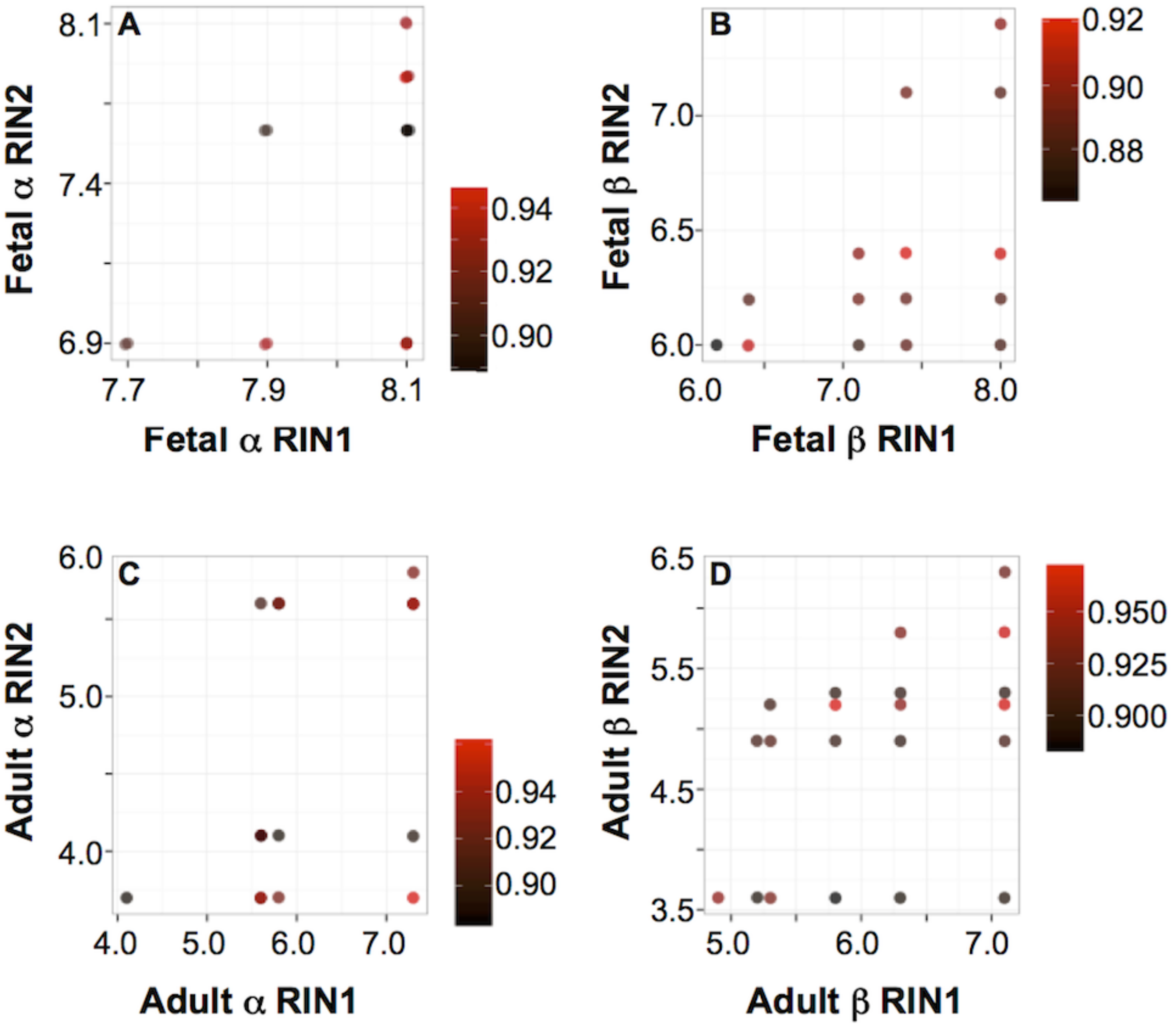
Supplementary Figure 1. Sorting adult α, β, and δ Cells – Gating Strategy. Viable cells are selected by gating out debris (A). Insulin positive cells (B1 gate in B) are selected, doublets are excluded (C), and insulin-positive cells are negatively selected (D). Similarly, insulin negative cells (B2 gate in B) are selected, doublets are excluded (E), glucagon-positive, somatostatin-positive, and unlabeled cells are gated (F), and glucagon-positive cells are negatively selected (G).



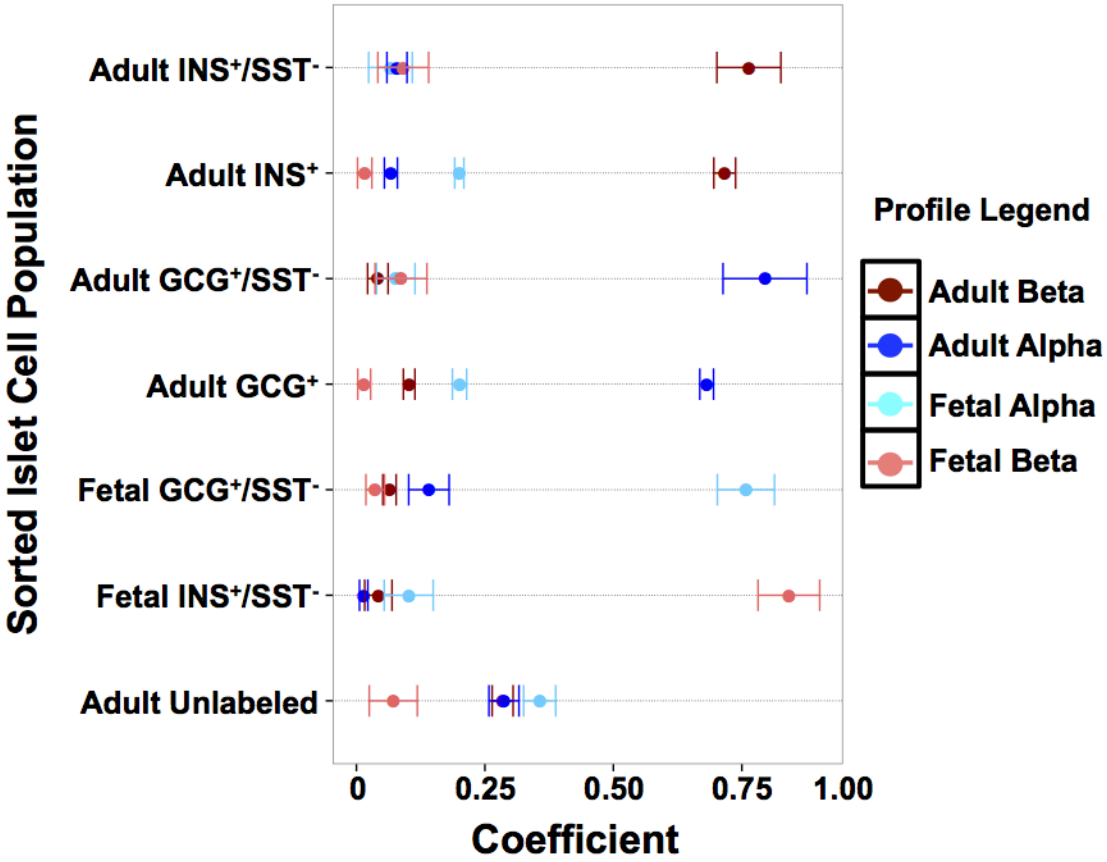
Supplementary Figure 2. Sorting fetal α and β Cells – Gating Strategy. Viable cells are selected by gating out debris (A) and doublets are immediately excluded (B). Insulin (C), Glucagon, and Somatostatin positive cells (D) are positively selected by plotting against somatostatin. Each positive selection is plotted against the third hormone to remove double positive cells and Insulin only (E), Glucagon only (F), and Somatostatin only (G) cells are collected. (G) A post-sorting plot shows the purity of insulin positive (red), glucagon positive (blue), and somatostatin positive (green) cells (H) and purity is further shown by qPCR for INS, GCG, and SST on RNA isolated from Insulin and Glucagon positive cells (I).



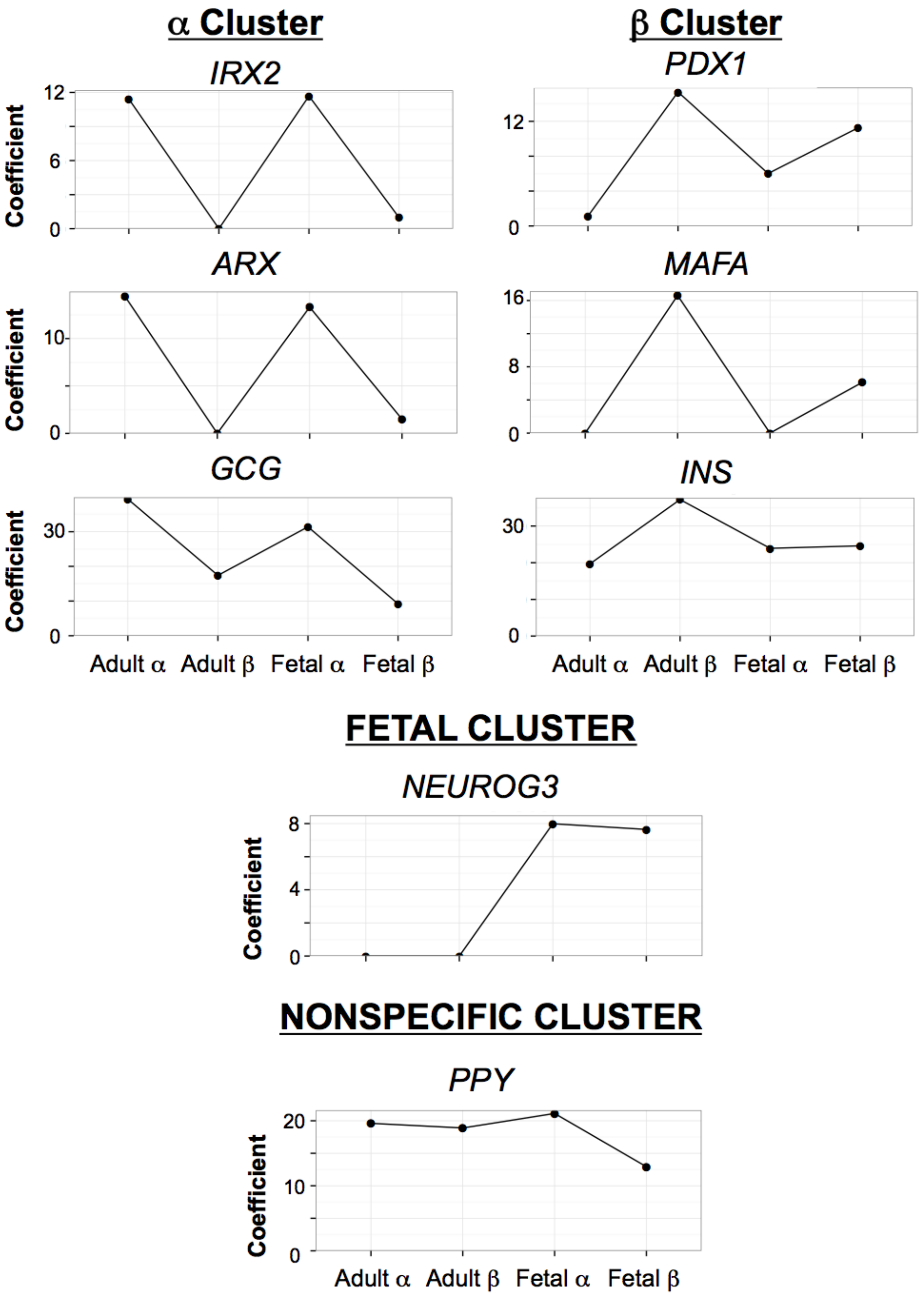
Supplementary Figure 3. No Relationship between RIN Value and Sample Variability. Each plot shows the correlation coefficients that compare each sample to all samples from each group (Fetal α (A), Fetal β (B), Adult α (C), and Adult β (D)) by RIN value. The correlations for each sample range from 0.87-0.96 (less black, more red) and show no relationship to sample RIN value (e.g. in C, RIN value 3.7 has a higher correlation with RIN value 7.3 than with RIN value 4.1 - this highlights human sample to human sample variation as the largest contributor to differences among donors).



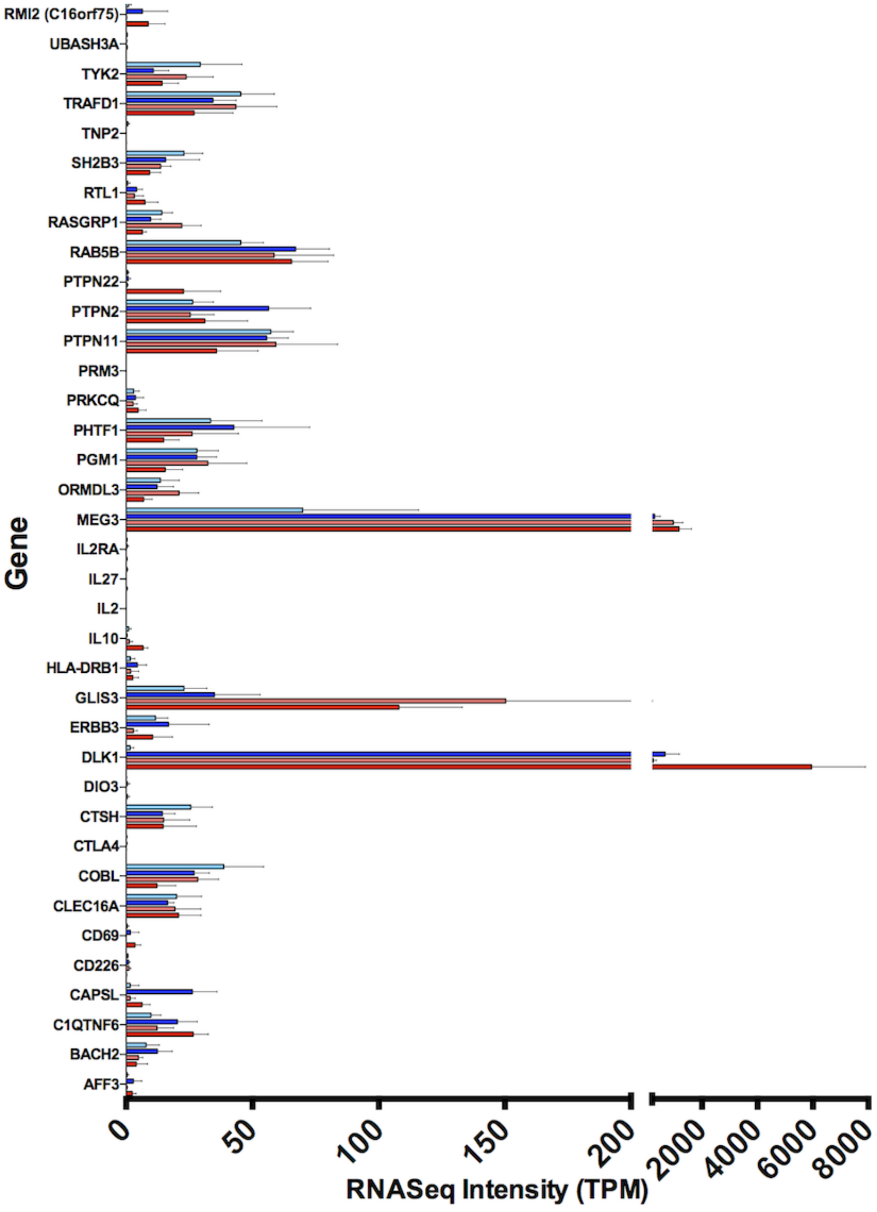
Supplementary Figure 4. Homogeneous Islet Cell Profiles. Homogeneous Islet Cell Profiles. Non-negative matrix factorization (NMF) method was used to define four expression profiles, corresponding to four cell populations (adult and fetal alpha and beta cells). Each sample is represented as a linear combination of the four profiles created by the NMF. The plot depicts the mean (points) and standard deviation (error bars) for each population. SST- indicates that the three hormone staining protocol was used so that SST+ cells were removed from the heterogeneous mixture of cells (as described in Figure 4C versus 4A). Non-negative least square was used to fit each sample to the four profiles, normalizing the coefficients so they would sum up to 1.



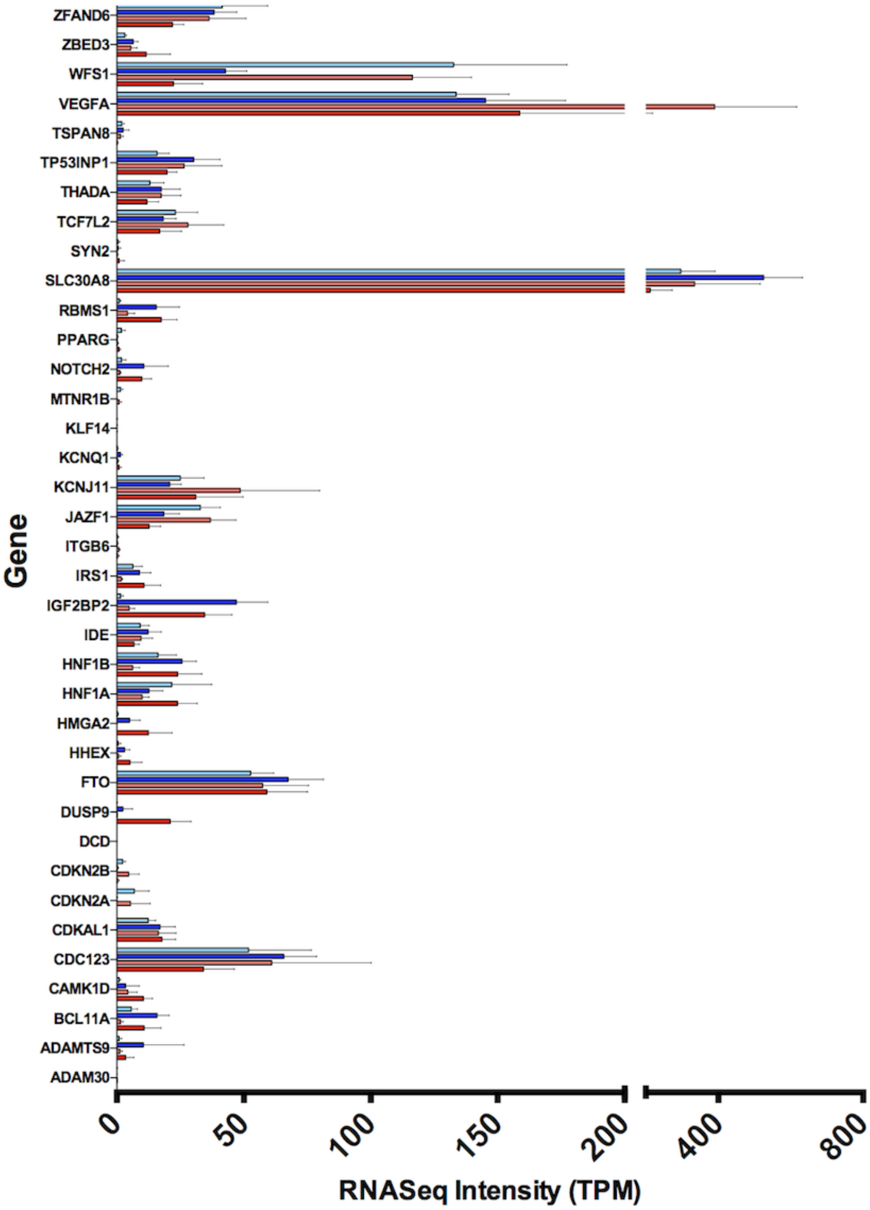
Supplementary Figure 5. Profile expression cluster patterns. Plots of the coefficient value for selected genes for the four purified cell clusters. The larger the coefficient value, the greater the contribution of the gene is to the specific profile.



Supplementary Figure 6. Type 1 Diabetes GWAS Gene Expression Levels. Gene expression levels (in TPM) for 37 genes associated with type 1 diabetes for fetal α (dark blue), adult α (light blue), fetal β (red), adult β (light red). The populations show 22, 19, 24, and 18 genes with expression above 5 TPM, respectively.



Supplementary Figure 7. Type 2 Diabetes GWAS Gene Expression Levels. Gene expression levels (in TPM) for 37 genes associated with type 2 diabetes for fetal α (dark blue), adult α (light blue), fetal β (red), adult β (light red). The populations show 23, 18, 25, and 17 genes with expression above 5 TPM, respectively.



Supplementary Figure 8. Insulin and Glucagon Related GWAS Gene Expression Levels. Gene expression levels (in TPM) for 19 genes associated with Insulin and Glucagon expression for fetal α (dark blue), adult α (light blue), fetal β (red), adult β (light red). The populations show 11, 13, 10, and 11 genes with expression above 5 TPM, respectively.