Figures and figure supplements

Fluorescein-based sensors to purify human α-cells for functional and transcriptomic analyses

Sevim Kahraman *et al*.

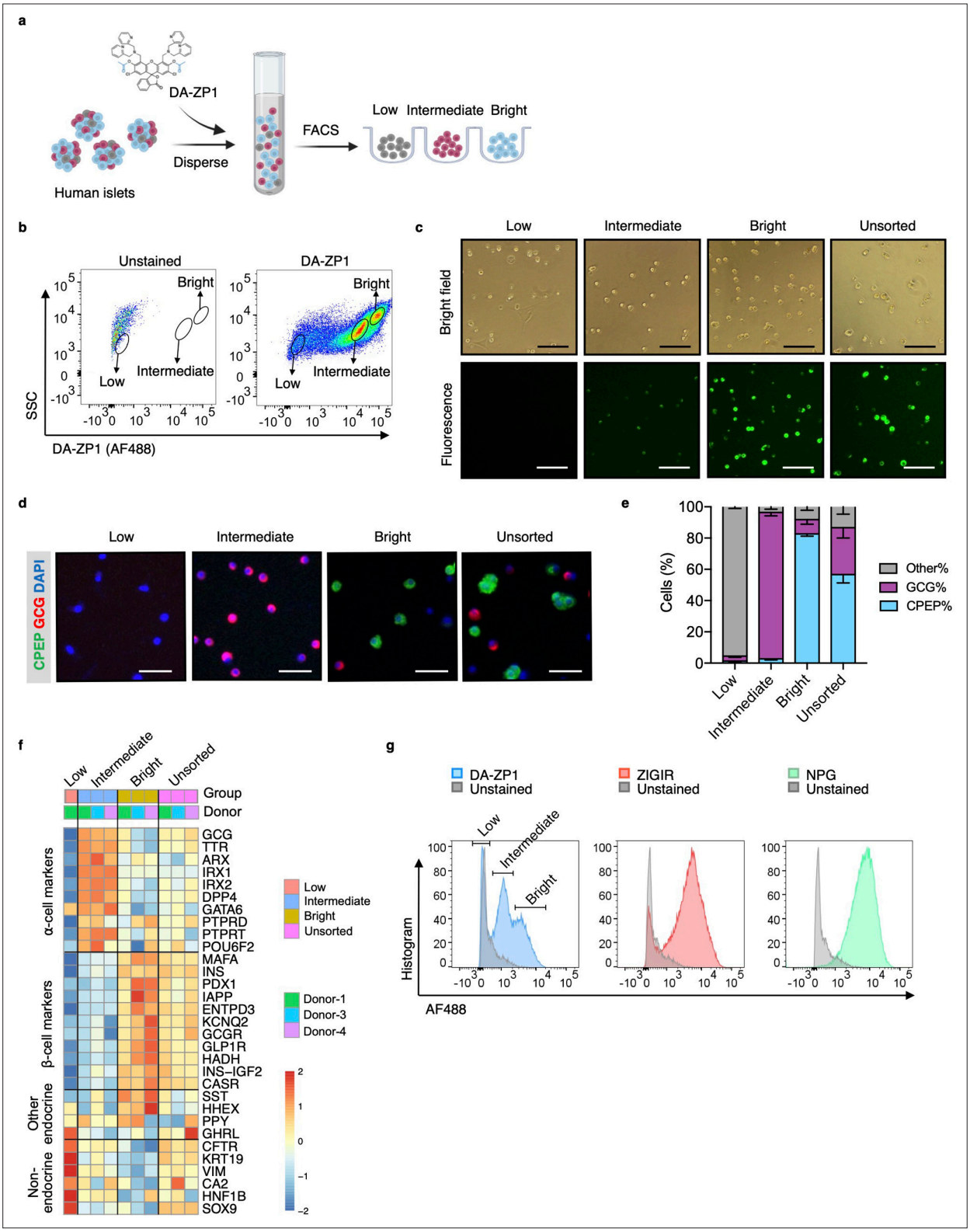
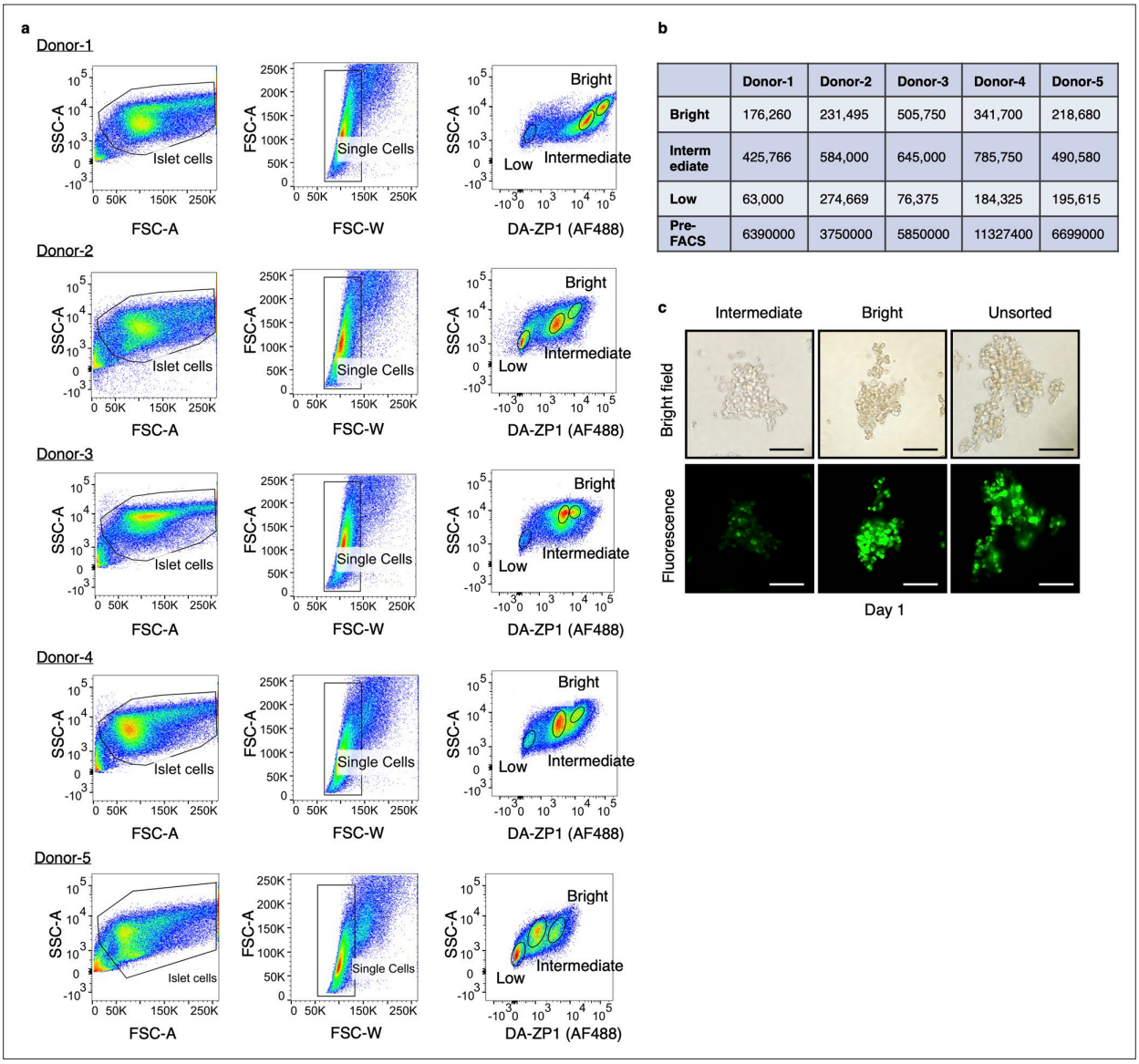


Figure 1. Isolation of live human pancreatic α-cells after staining with diacetylated Zinpyr1 (DA-ZP1) by fluorescence activated cell sorting (FACS). (**a**) Experimental outline. (**b**) Representative FACS plot showing three cell populations with low, intermediate, or bright fluorescence. The plot represents the data collected from Donor-1 islets. Unstained (left) vs DA-ZP1-treated (right) human islets. Gating strategy and the data collected from the other donors (n=4) are given in ***Figure 1—figure supplement 1***. (**c**) The DA-ZP1 derived green fluorescence is maintained in the next day of sorting in the sorted islet cells. The cells were plated in Matrigel-coated flat-bottom plates. Scale bar, 100 μm. See also ***Figure 1—figure supplement 1***. (**d**)

Figure 1 continued on next page

Figure 1 continued

Representative images of human islet cells after FACS showing C-peptide (green) and glucagon (red) expressing islet cells. Nuclei were stained with DAPI (blue). Scale bar, 50 μm. (**e**) Quantification of percentage of CPEP+, GCG+, and other cells (CPEP- GCG-) in each cell population. Data are presented as mean values ± s.e.m. n=3 donors. (**f**) Heatmap showing expression of genes in different cell subsets. n=3 donors. (**g**) Comparison of other zinc-based dyes with DA-ZP1 by FACS.



	Donor-1	Donor-2	Donor-3	Donor-4	Donor-5
Bright	176,260	231,495	505,750	341,700	218,680
Intermediate	425,766	584,000	645,000	785,750	490,580
Low	63,000	274,669	76,375	184,325	195,615
Pre-FACS	6390000	3750000	5850000	11327400	6699000

Figure 1—figure supplement 1. Gating strategy for isolation of live human pancreatic α-cells. (**a**) Starting cell population was determined by SSC-A/FSC-A gating. Single human islet cells were gated according to FSC-A/FSC-W gating. Treatment of the single cells with diacetylated Zinpyr1 (DA-ZP1) resulted in three cell populations with different fluorescence intensity (low, intermediate, and bright). n=5 human islet donors. Donor information is given in ***Supplementary file 9***. (**b**) Number of live cells collected by fluorescence activated cell sorting (FACS) using 15,000 islet equivalent determined by trypan blue staining. (**c**) The DA-ZP1 derived green fluorescence is maintained in the next day of sorting in the sorted islet cells. Scale bar, 100 µm.

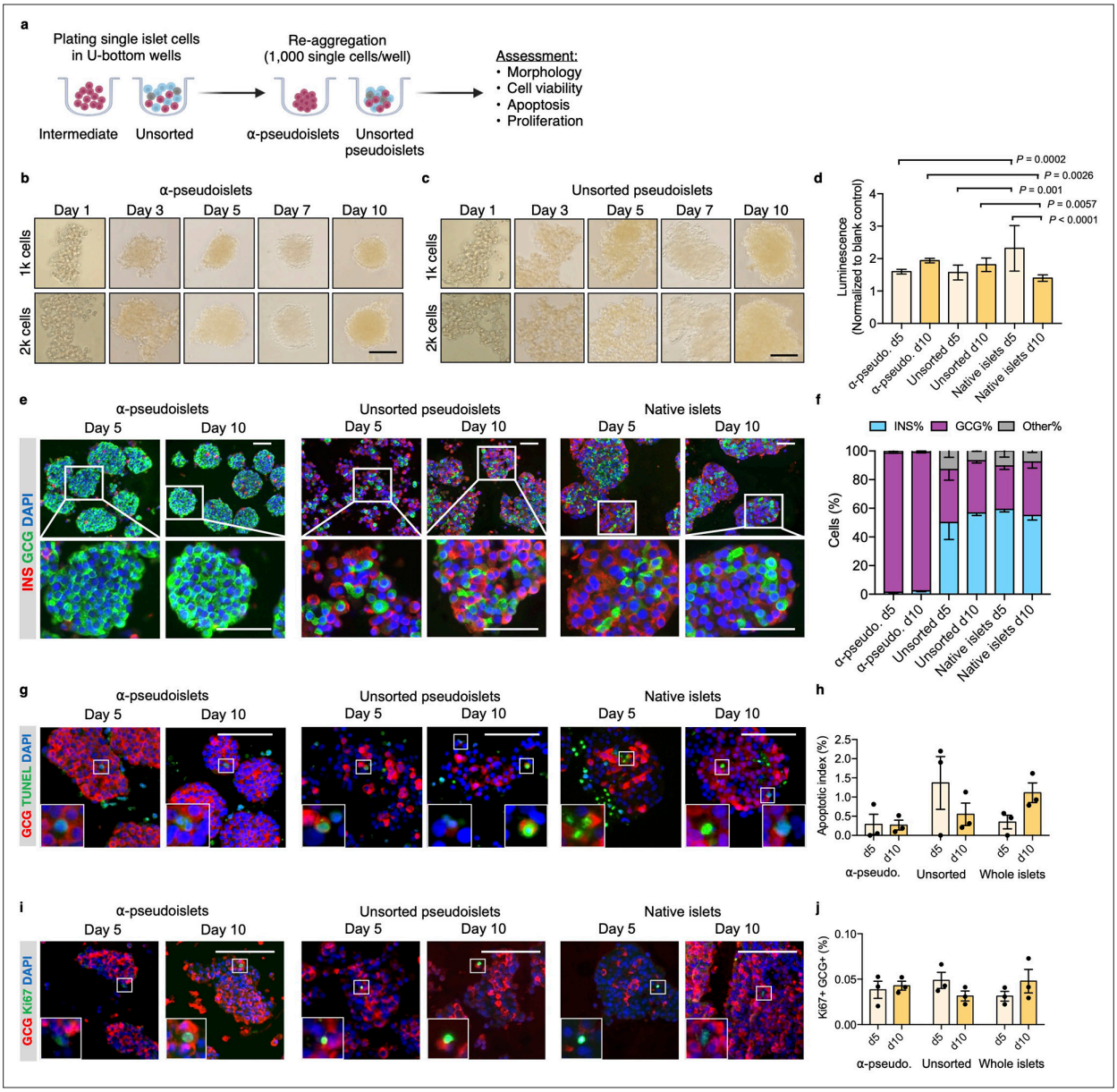


Figure 2. α-Pseudoislets are viable and able to proliferate in vitro post-sorting. (**a**) The single islet cells were seeded in round-bottom wells (1k cells per well) after sorting to allow re-aggregation. (**b, c**) Bright-field images of intermediate (sorted α-cells) (**b**) and unsorted pseudoislets (**c**) post-sorting. 1k (top panel) or 2k (bottom panel) single cells were seeded per well. Scale bar, 100 µm. See also ***Figure 2—figure supplement 1***. (**d**) Cell viability was quantified by luminescence reflecting intracellular ATP levels on days 5 and 10 following fluorescence activated cell sorting (FACS). Fold-change relative to blank control. n=7–9 replicates using islet cells from two donors. (**e**) Representative immunostaining images of α-pseudoislets, unsorted pseudoislets, and native islets on days 5 and day 10 showing INS (red), GCG (green). Nuclei stained with DAPI are blue. For top and bottom images, scale bar, 100 µm. (**f**) Percentage of INS+, GCG+, and other (INS-GCG-) islet cells. n=3 donors. (**g**) Representative immunostaining images of α-pseudoislets, unsorted pseudoislets, and native islets on day 5 and day 10 showing GCG (red), TUNEL (green). Nuclei stained with DAPI are blue. Scale bar, 100 µm. Boxes show apoptotic α-cells. (**h**) Percentage of TUNEL+GCG+ cells. n=3 donors. (**i**) Representative immunostaining images of α-pseudoislets, unsorted pseudoislets, and native islets on day 5 and day 10 showing GCG (red), Ki67 (green). Nuclei stained with DAPI are blue. Scale bar, 50 µm. Boxes show proliferating α-cells. (**j**) Percentage of Ki67+GCG+ cells. n=3 donors. Data are presented as mean values ± s.e.m (**b–j**). n=3 donors. Two-way ANOVA followed by Sidak's multiple comparison test (**d, h, j**).

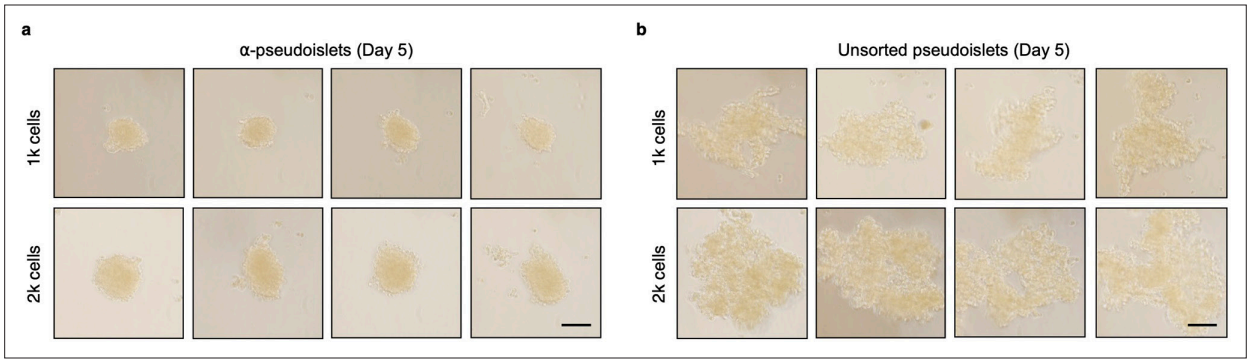


Figure 2—figure supplement 1. α-Pseudoislets tended to form tighter clusters. Bright-field images of α-pseudoislets (**a**) and unsorted pseudoislets (**b**) 5 days post-sorting. 1k (top panel) or 2k (bottom panel) single cells were seeded per well. Scale bar, 100 μm. n=3 biological replicates using islet cells from a single donor (**a, b**).

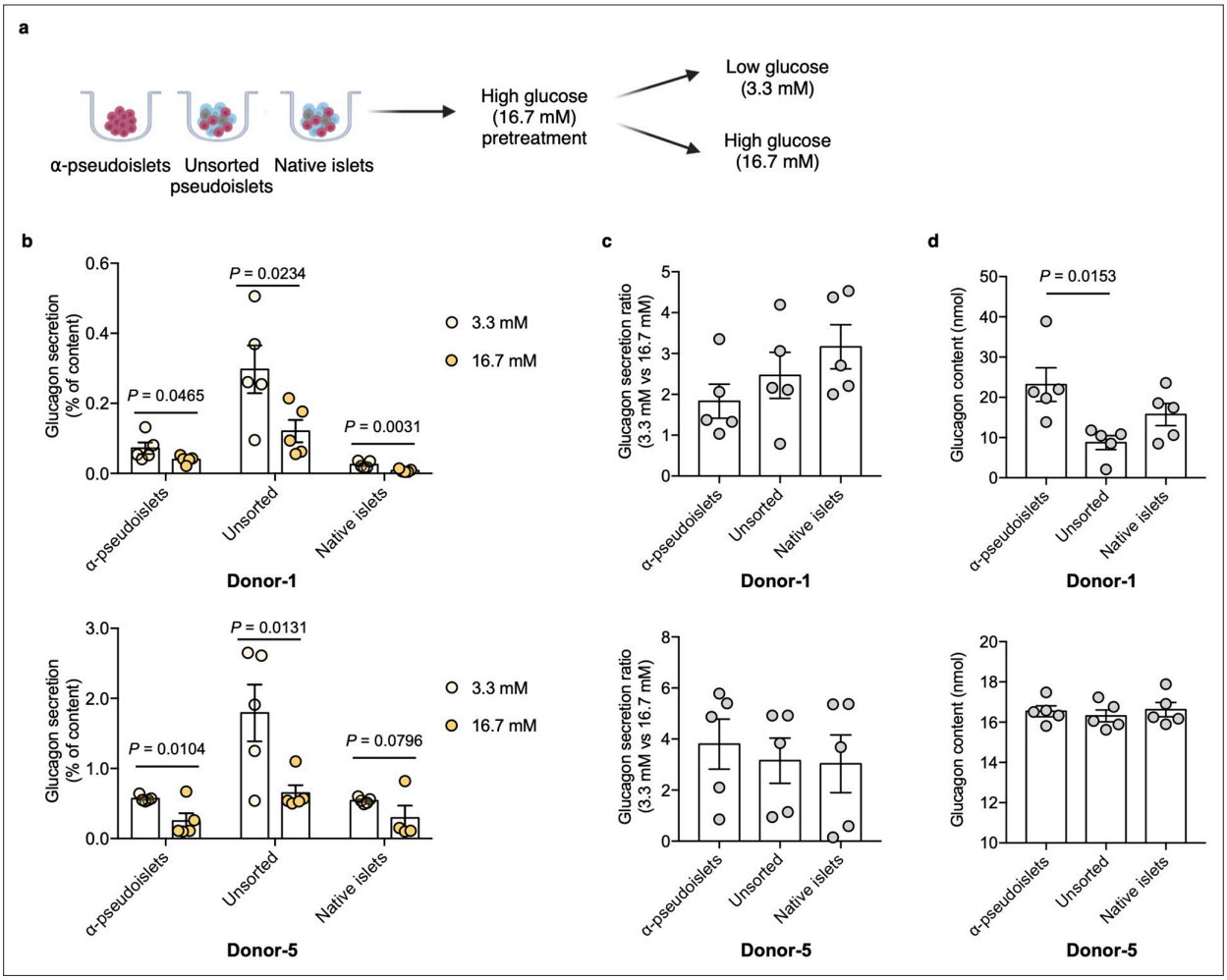


Figure 3. Glucagon secretion in response to glucose challenge. (**a**) α-Pseudoislets, unsorted pseudoislets, or native islets were preincubated in Krebs-Ringer bicarbonate (KRB) buffer with 16.7 mM glucose followed by the incubation in KRB buffer with 3.3 mM glucose and 16.7 mM glucose on day 5 post-sorting. (**b**) Glucagon secretion in response to glucose challenge (3.3 mM vs 16.7 mM). One-tailed Student's t-test. (**c**) Ratio of glucagon released by each groups of cells at 16.7 mM glucose versus that at 3.3 mM glucose. (**d**) Glucagon content measured in each well containing ~8000 cells (eight α-pseudoislets, eight unsorted pseudoislets, and eight native islets). Data are presented as mean values ± s.e.m. (**b–d**). n=5 replicates using islet cells from two donors (**b–d**). One-way ANOVA corrected for Tukey applied to (**c, d**).

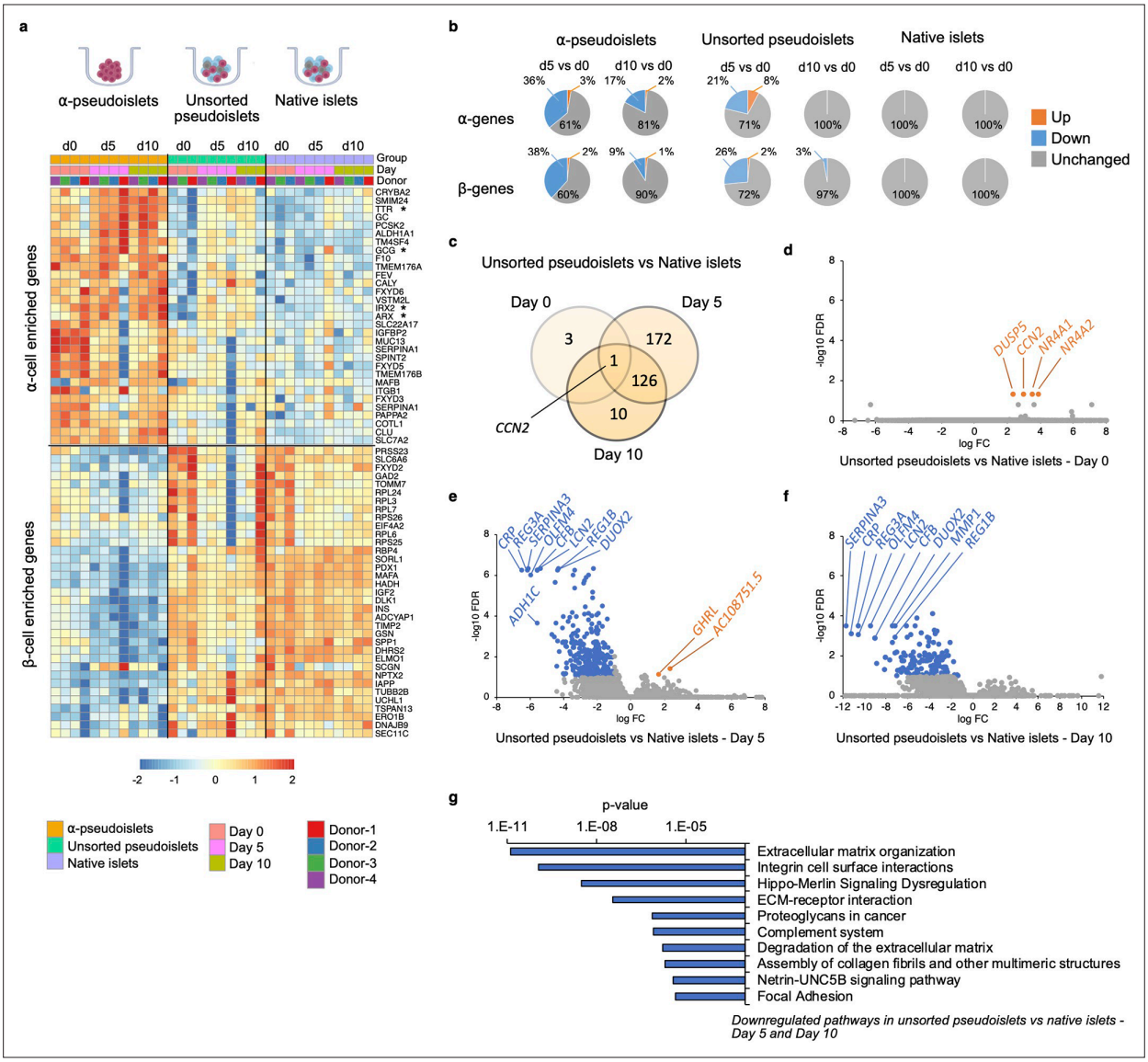


Figure 4. Changes in gene expression levels driven by dissociation and re-aggregation of human islet cells. (**a**) Heatmap showing expression levels of α-cell enriched and β-cell enriched genes in α-pseudoislets, unsorted pseudoislets, and native islets on days 0, 5, and 10. Asterisks show genes associated with α-cell identity and function (GCG, ARX, IRX2, TTR). (**b**) Pie charts showing percentage of α-cell enriched (top panel) and β-cell enriched (bottom panel) genes that alter in α-pseudoislets, unsorted pseudoislets, and native islets on day 5 or day 10 compared to day 0. See also ***Figure 4—figure supplement 1***. (**c**) Transcriptome of unsorted pseudoislets was compared with native islets on days 0, 5, 10. Venn diagram shows number of differentially expressed genes (DEGs) between unsorted pseudoislets and native islets on different days. (**d–f**) Volcano plots showing genes downregulated (blue) or upregulated (orange) significantly (FC < –2 or FC > 2, respectively, FDR < 0.1) on day 0 (**d**), day 5 (**e**), and day 10 (**f**). Gray shows non-significant genes with FDR > 0.1 and –2 < FC < 2. (**g**) Top 10 pathways downregulated in unsorted pseudoislets on day 5 and day 10 compared to native islets. n=4 donors; α-pseudoislets d0, d5, d10, unsorted pseudoislets d5, native islets d5, d10, and n=3 donors; unsorted pseudoislets d0, d10, native islets d0 (**a–g**).

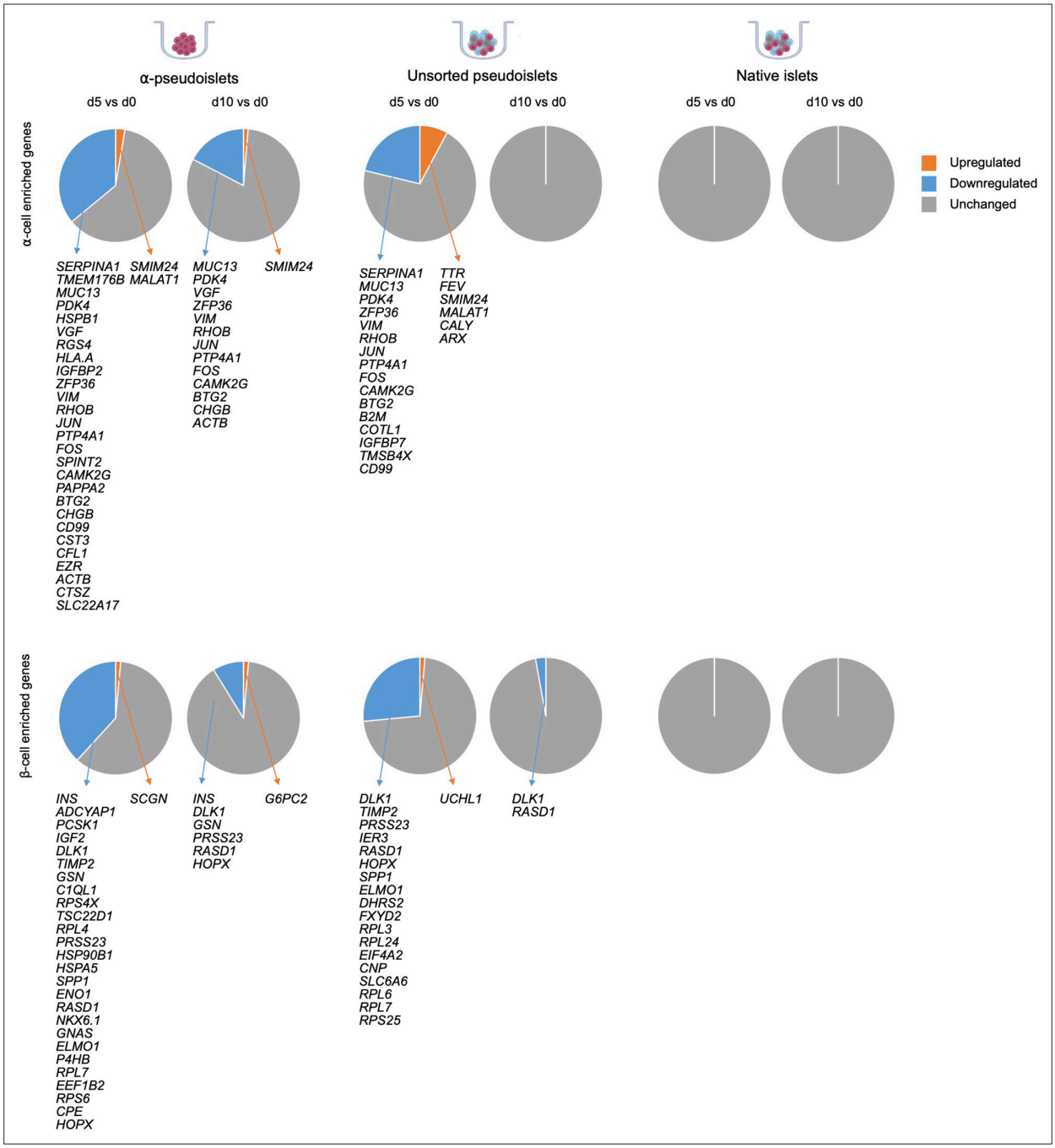


Figure 4—figure supplement 1. Changes in expression levels of α-cell enriched and β-cell enriched genes in α-pseudoislets, unsorted pseudoislets, and native islets on days 0, 5, 10. Pie charts show percentage of genes altered on day 5 or 10 compared to day 0 (FDR < 0.1; FC > 2 upregulated or FC < –2 downregulated). n=4; α-pseudoislets d0, d5, d10, unsorted pseudoislets d5, native islets d5, d10, and n=3; unsorted pseudoislets d0, d10, native islets d0.

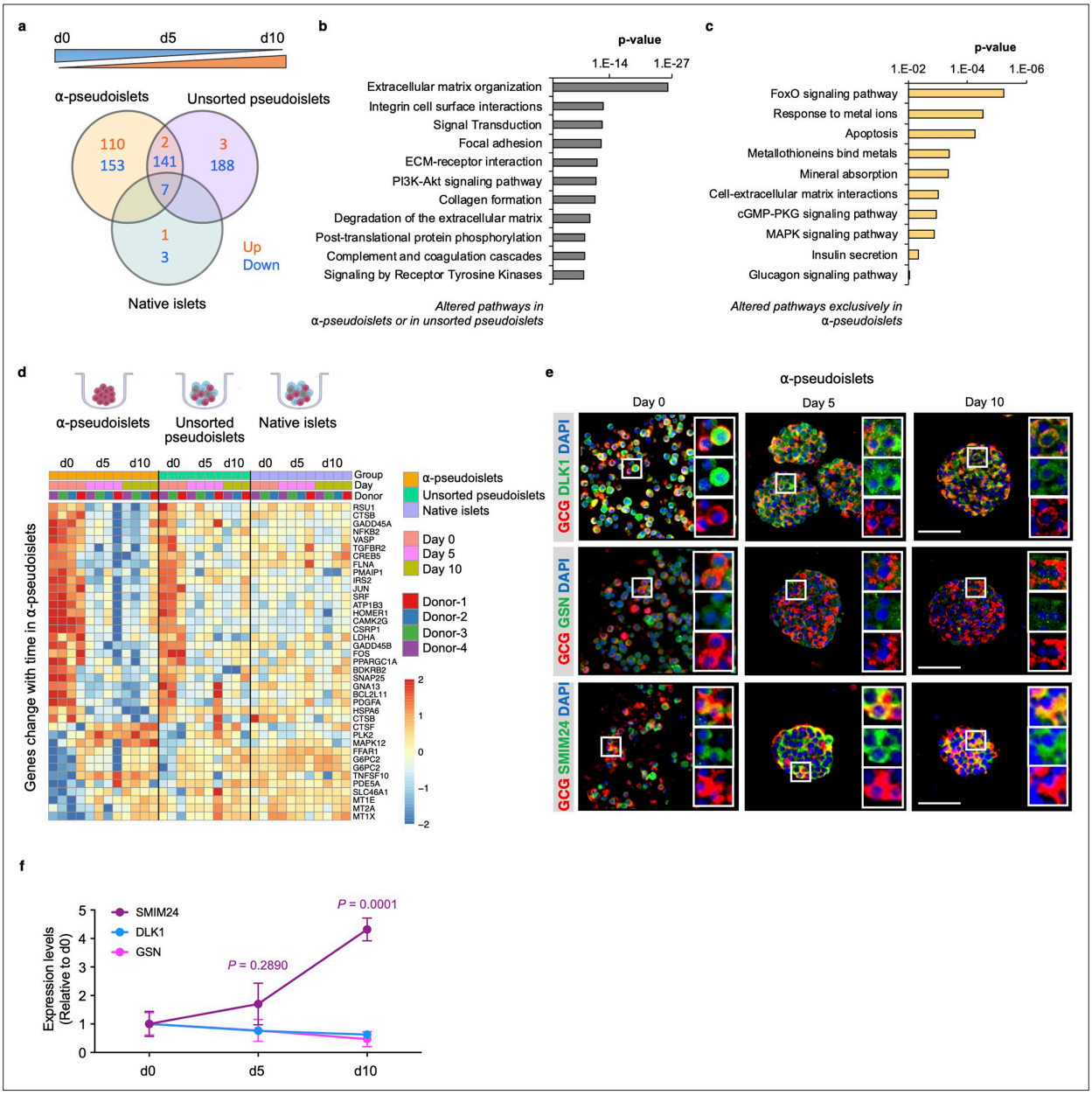


Figure 5. Time-dependent changes in transcriptome of α-pseudoislets. (**a**) Venn diagram shows number of genes that progressively up- or downregulated in α-pseudoislets, unsorted pseudoislets, and native islets in culture over the period from day 0 to day 5 to day 10. (**b, c**) Pathway analysis showing altered pathways in α-pseudoislets or in unsorted pseudoislets except native islets (**b**), and altered pathways only in α-pseudoislets with time (**c**). (**d**) Heatmap showing expression levels of genes significantly change with time only in α-pseudoislets. n=4 donors; α-pseudoislets d0, d5, d10, unsorted pseudoislets d5, native islets d5, d10, and n=3 donors; unsorted pseudoislets d0, d10, native islets d0 (**a–d**). (**e**) Representative immunostaining images of α-pseudoislets on day 0, 5, and 10 showing GCG (red), DLK1, GSN, and SMIM24 (green). Nuclei stained with DAPI are blue. Scale bar, 50 μm. (**f**) Expression level of each protein in α-pseudoislets on days 0, 5, 10. Data are presented as mean values ± s.e.m. Two-way ANOVA followed by Dunnett's multiple comparison test compared to d0. n=3 donors (**e, f**).