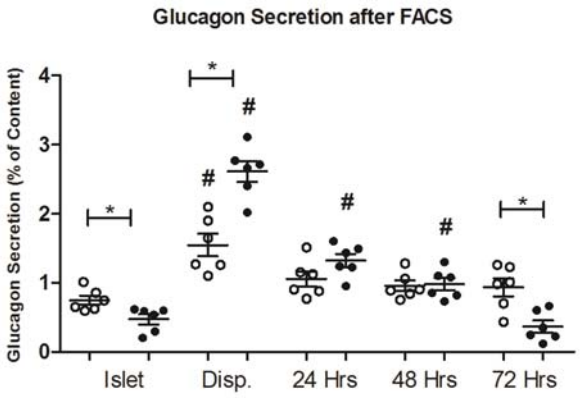
SUPPLEMENTARY DATA

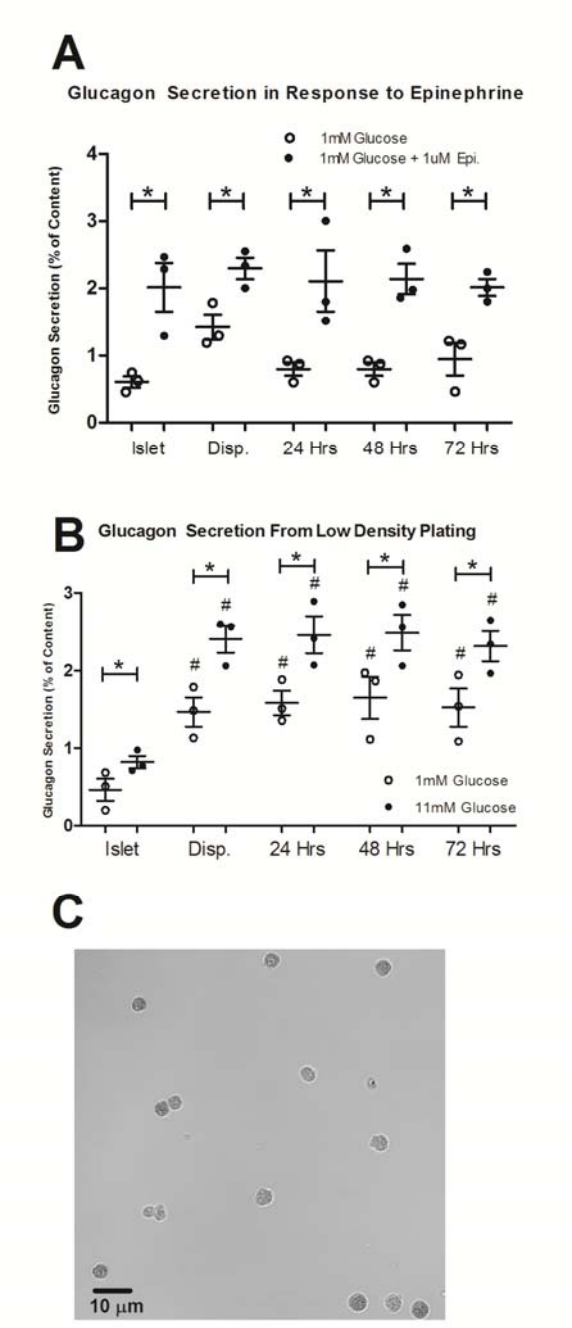
Supplementary Figure 1. Glucagon Secretion in Pseudo-Islets after FACS
Glucagon secretion from intact, dispersed, and forming pseudo-islets from all cells recovered from FACS. Removal of the small percentage (<5%) of undispersed doublet cells or any remaining cell clusters by FACS does not affect the temporal profile or amplitude of reestablished glucagon secretion compared to non-sorted samples (Figure 1). Data represent samples collected from 6 mice. Error bars represent standard error of the mean. Asterisks (*) label differences between 1 mM and 11 mM glucose; hash marks (#) label differences between control and conditions at the same glucose concentration ($P < 0.05$ by one-way ANOVA).



SUPPLEMENTARY DATA

Supplementary Figure 2. Glucagon Secretion in Response to Epinephrine Stimulation and Sparse Plating

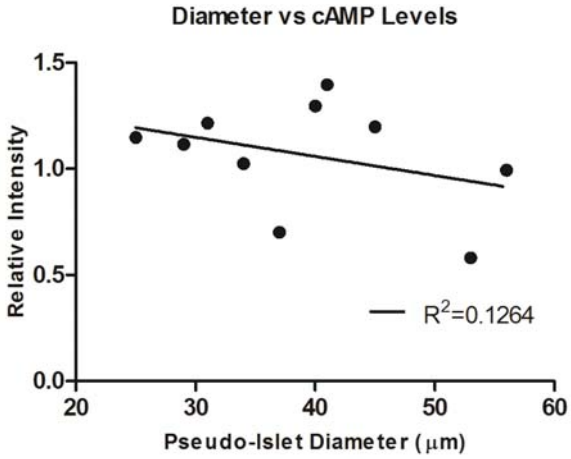
(A) Glucagon secretion from intact, dispersed, and pseudo-islets treated with 1 mM glucose or 1 mM glucose with 1 μM epinephrine. (B) Glucagon secretion from intact islets, dispersed islet cells, and dispersed islet cells plated at low density at 24, 48, and 72 hours after dispersion. (C) Representative image of sparsely plated cells after 72 hours in culture (~1/10 density described in methods). Majority of cells remain in single cell state, with occasional doublets present (possibly the result of cell division). Data represent samples collected from 3 mice. Error bars represent standard error of the mean. Asterisks (*) label differences between 1 mM and 11 mM glucose; hash marks (#) label differences between control and conditions at the same glucose concentration ($P < 0.05$ by one-way ANOVA).



SUPPLEMENTARY DATA

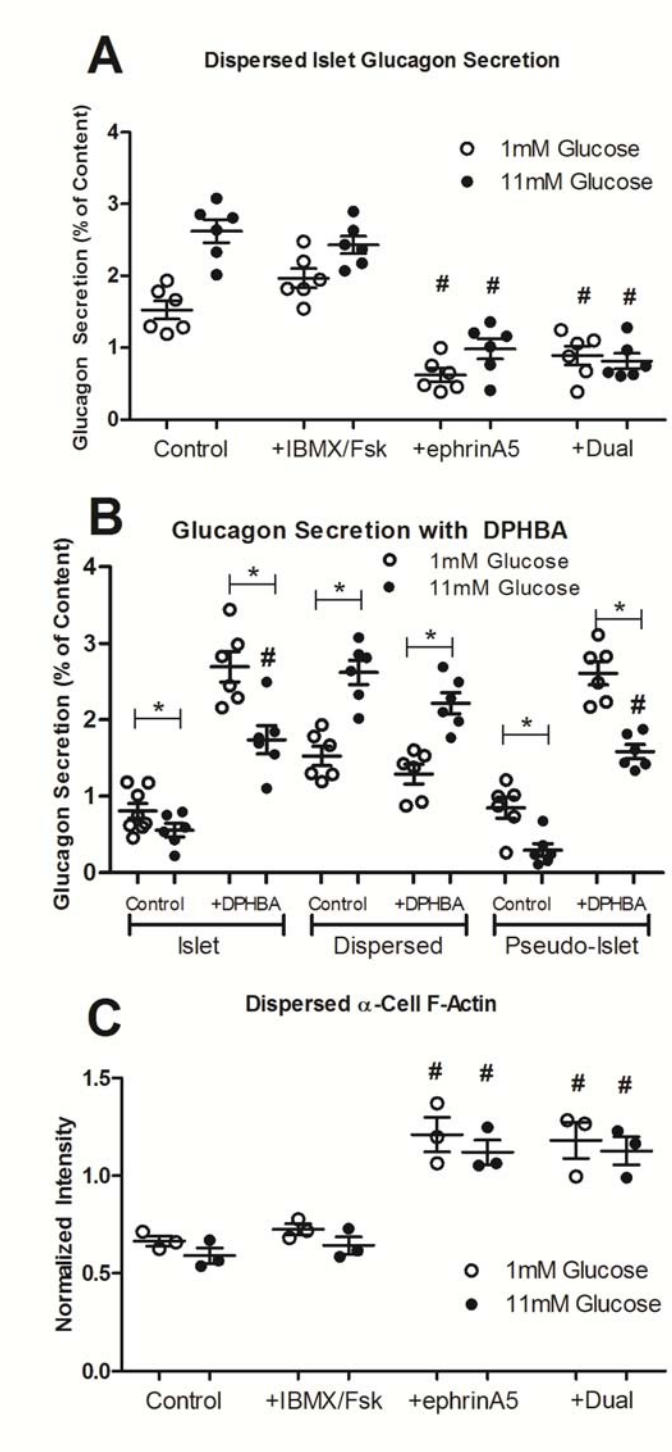
Supplementary Figure 3. Changes in F-Actin and Glucagon Secretion in Dispersed α-Cells

(A) Glucagon secretion from dispersed α-cells at 1 mM and 11 mM glucose treated with 100 μM IMBX and 50 μM Fsk, 4 μg/mL ephrinA5-Fc, or both. (B) Quantification of glucagon secretion from islets, dispersed cells, and pseudo-islets at 1 mM and 11 mM glucose treated with 12.5 μM DPHBA. Data represent samples collected from 6 mice. (C) Quantification of α-cell specific F-actin levels from dispersed α-cells at 1 mM and 11 mM glucose treated with 100 μM IMBX and 50 μM Fsk, 4 μg/mL ephrinA5-Fc, or both. Data represent samples collected from 3 mice. Error bars represent standard error of the mean. Asterisks (*) label differences between 1 mM and 11 mM glucose; hash marks (#) label differences between control and conditions at the same glucose concentration ($P < 0.05$ by one-way ANOVA).



SUPPLEMENTARY DATA

Supplementary Figure 4. Comparison of Pseudo-Islet Diameter versus α-Cell cAMP Levels
The relative fluorescence level of cAMP measured in α-cells at 1 mM glucose from 10 pseudo-islets were plotted against the diameter (μm). A line of best fit was found to have a slope of -0.01 ± 0.01, with $R^2 = 0.1264$ ($p = 0.3$).



SUPPLEMENTARY DATA

Supplementary Table 1. Human Islet Donor Info Chart
Age, body mass index (BMI), and gender of human islet donors. Averages are listed in bold.

Donor	Age	BMI	Gender
A	38	34.4	F
B	35	24.2	M
C	45	30.5	M
D	47	31	M
E	59	28.3	M
F	32	24.9	F
	42.7	**28.9**	