Supplemental Figure Legends

Figure S1, related to Figure 1. *Gcgr-/-* mice have severe α-cell hyperplasia. (A-B) Representative images of pancreatic α-cell staining in *Gcgr*+/+ (left) and *Gcgr-/-* (right) 5 month old mice. Glucagon staining (green), amylase staining (red), and DAPI staining (blue); White boxes indicate regions for insets. White scale bars indicate 1mm. Islets form Gcgr-/- mice are often associated with ductal structures and even envelop ductal structures (nesidioblastosis- see white asterisks). (C) Schematic for *in vitro* α-cell proliferation assay with algorithm building to measure proliferation rates. (D) Dose responsiveness of α-cell proliferation in mouse to *Gcgr-/-* mouse serum-supplemented media. Control media with no mouse serum added (grey, n=3) and increasing doses of *Gcgr-/-* whole mouse serum-supplemented media (red, n=2-8) are shown. **p<0.01 vs control media, $^{\#\#}$p<0.01 vs. 0.1% *Gcgr-/-* mouse serum-supplemented media, and $^{\$}$p<0.05 vs. 1% *Gcgr-/-* mouse serum-supplemented media. (E-F) Representative images of (E) somatostatin and (F) insulin staining (green) in dispersed cytospin islets. Ki67 and DAPI staining are shown in red and blue, respectively. Data are mean ± SEM.

Figure S2, related to Figure 2. Acute and chronic models of interrupted glucagon receptor signaling have common alterations in liver gene. (A-B) Representative images of pancreatic islet α-cell proliferation in mice treated with PBS or GCGR mAb for 10 days. Glucagon staining (green), Ki67 staining (red), and DAPI staining (blue) are shown. White arrows indicate proliferating α-cells. (C) α-cell proliferation in mice treated with PBS or GCGR mAb for 10 days. (D) Principle component analysis of liver RNA-Seq from mice with interrupted glucagon signaling. (E) Spearman correlation of each treatment group from RNA-Seq analyses. (F) Venn diagram of hepatic gene changes in mice. Volcano plots of genes altered in RNA-Seq analyses of livers from (G) “acute” GCGR mAb treatment and (H) “chronic” *Gcgr-/-* mice versus *Gcgr*+/+ mice. Red dots are genes that are significantly downregulated in either GCGR mAb or *Gcgr-/-* mice (p<0.05). Green dots are genes that are significantly upregulated in either GCGR

mAb or *Gcgr-/-* mice ($p<0.05$). (I) Plot of top gene changes in each model. Log2 fold gene changes observed in both *Gcgr+/+* vs. *Gcgr-/-* and *Gcgr+/+* vs. GCGR mAb-treated mice are shown with green circles. Log_2 fold gene changes observed in both *Gcgr+/+* vs. *Gcgr-/-* and WT vs. *Gcg+* mice (see Song et al., 2014 for details on WT vs. *Gcg+* mice) are shown with blue triangles. Black arrows indicate gene expression changes in three secreted factors. (J) Log_2 fold changes in liver gene expression of predicted secreted proteins. (K) Log_2 fold changes in liver gene expression related to lipid and bile metabolism. GCGR mAb-treated *Gcgr+/+* versus PBS-treated *Gcgr+/+* mice (blue bars, n=3) and PBS-treated *Gcgr-/-* versus PBS-treated *Gcgr+/+* mice (red bars, n=3) are shown. (L) Mouse serum bile acid levels in PBS-treated *Gcgr+/+* (white bars), GCGR mAb-treated *Gcgr+/+* (blue bars), and PBS-treated *Gcgr-/-* (red bars) mice. One-way ANOVA with Tukey posthoc analyses; *p<0.05, ***p<0.001 vs Gcgr-/- mouse serum. (M) α-cell proliferation in response to delipidated *Gcgr-/-* mouse serum. Control media with no mouse serum added (grey bars, n=2), 10% *Gcgr-/-* whole mouse serum -supplemented media (red bar, n=2), 10% *Gcgr-/-* delipidated mouse serum -supplemented media (red left hashed bar, n=2) islet cultures are shown. One-way ANOVA with Tukey posthoc analyses; *p<0.05 vs control media. (N) α-cell proliferation in response to human low density lipoprotein (hLDL), Angptl-4 and palmitate. One-way ANOVA with Tukey posthoc analyses; ***p<0.001 vs all other conditions accepted Low AA versus High AA Low Q comparisons (not significant). Fatty acid-free bovine serum albumin (BSA-EtOH) was used as control for palmitate complexed with fatty acid-free BSA. Angptl-4 was complexed with hLDL. This experiment was performed as close as possible to the experiment described in Ben-Zvi et al., 2015 with the exception that here islets were cultured for 72 hours in the respective media. Data are mean ± SEM.

Figure S3, related to Figure 2. High levels of serum L-glutamine and other amino acids in mice with interrupted glucagon signaling stimulate selective α-cell proliferation. (A) Mouse serum AA levels measured by MS in *Gcgr-/-* (red), GCGR mAb-treated for 10 days (blue), and PBS-treated (white) *p<0.05, **p<0.01, ***p<0.001 vs PBS-treated *Gcgr+/+* mice.

#p<0.05, ##p<0.01 vs. GCGR mAb-treated 10 day mice; n=3-8. (B) Quantification of L-alanine, L-citrulline, and L-ornithine dose response effects alone or in combination on α-cell proliferation in cultured mouse islets treated for 3 days. One-way ANOVA with Tukey posthoc analyses; ###p<0.001 vs *Gcgr-/-* mouse serum-treated islets (red), ***p<0.001 vs control media-treated islets (first gray bar on left). (B) Quantification of total islet cell proliferation, (C) percentage of non α-cells proliferation and (D) percentage of cells proliferating under each AA condition that are α-cells in response to media with increasing AA concentration media. This is the total number of glucagon$^+$ Ki67$^+$ double positive cells per the total number of glucagon$^+$ cells. White (low) to gray (intermediate) to black (high) color indicates combined concentration of all AA in each media condition (see Table S4 for combined and individual AA media concentrations); n=3-8. The red bar indicates 10% *Gcgr-/-* mouse serum -supplemented media similar to Figure 1E. One-way ANOVA with Tukey posthoc analyses; ***p<0.001 vs *Gcgr-/-* mouse serum -supplemented media (red), ###p<0.01 vs. highest AA media-treated islets (black), and $p<0.05 vs. highest AA media-treated islets (dark gray). in response to media with increasing amino acid concentration containing media for 3 days. (F) Quantification of islet cell proliferation and (G) non-α-cell proliferation in response to altering individual amino acid levels in cultured mouse islets. One-way ANOVA with Tukey posthoc analyses; n=3, There were no statistical differences observed between high L-glutamate, L-leucine, and L-glutamine media (High ELQ)-treated islets, low L-glutamate, L-leucine, and L-glutamine media (Low ELQ)-treated islets, low L-glutamine (Low Q)- treated islets, low L-glutamate (Low E)- treated islets, or low L-leucine (Low L)-treated islets. (H) Quantification of L-glutamine dose response stimulated islet cell proliferation and (I) non-α-cell proliferation in cultured mouse islets. One-way ANOVA with Tukey posthoc analyses; n=3, ***p<0.001 vs 3250 μM L-glutamine media-treated islets, #p<0.05, ##p<0.01 vs 2055 μM L-glutamine media-treated islets. Data are mean ± SEM.

Figure S4, related to Figure 3. mTOR signaling and FoxP transcription factor are essential for α-cell proliferation in response to interrupted glucagon signaling. (A)

Random blood glucose (mg/dl), (B) glucose/arginine-stimulated blood glucose (mg/dl), and (C) glucose/arginine-stimulated serum glucagon (pg/ml) in mice after cotreatment with GCGR mAb and rapamycin. Saline/PBS treated (white), Saline/GCGR mAb treated (blue), Rapamycin/PBS-treated (white left hashed) and rapamycin/GCGR mAb treated (blue left hashed) are shown. Two-way ANOVA with Bonferroni posthoc analyses; *p<0.05, **p<0.01, and ***p<0.001 vs PBS treated and $^{\#}$p<0.05 and $^{\#\#\#}$p<0.001 vs. Saline treated. (D-E) Representative images of pancreatic islet α-cell proliferation in Saline/PBS- and Rapamycin/PBS- treated mice. Glucagon staining (green), Ki67 staining (red), and DAPI staining (blue) are shown. White scale bars indicate 100μm. White dashed boxes indicate region selected for insets (D'-E'). (F-G) Representative images of pancreatic islet α-cell expression of pS6 protein in Saline/PBS- and Rapamycin/PBS-treated mice. Glucagon staining (green), pS6(pS235/S236) staining (red), and DAPI staining (blue) are shown. White scale bars indicate 100μm. White dashed boxes indicate region selected for insets (F'-G'). (H) Body mass, (I) random blood glucose (mg/dl), and (J) pancreatic mass in Gcgr mAb-treated *FoxP1/2/4*$^{-/-}$ mice. Wildtype/PBS-treated (white), Wildtype/GCGR mAb-treated (blue), *FoxP1/2/4*$^{-/-}$/PBS-treated (white left hashed) and *FoxP1/2/4*$^{-/-}$/GCGR mAb-treated (blue left hashed) are shown. Two-way ANOVA with Bonferroni posthoc analyses; *p<0.05 and ***p<0.001 vs PBS-treated and $^{\#\#}$p<0.01 vs. Saline-treated. Representative images of pancreatic islet (K) α- and (L) β-cell expression of Cre Recombinase protein in *FoxP1/2/4*$^{-/-}$ mice. Glucagon and insulin staining (green) respectively, Cre staining (red), and DAPI staining (blue) are shown (n=3). (M-P) Representative images of pancreatic islet α-cell expression of pS6 protein in (K) Wildtype/PBS-, (L) *FoxP1/2/4*$^{-/-}$/PBS-, (M) Wildtype/GCGR mAb-, and (N) *FoxP1/2/4*$^{-/-}$/GCGR mAb-treated mice. Glucagon staining (green), pS6(pS235/S236) staining (red), and DAPI staining (blue) are shown (n=3). White scale bars indicate 50μm. White dashed boxes indicate region selected for insets. (O) Quantification of rapamycin effects on amino acid-stimulated islet cell and (P) non-α-cell proliferation in cultured mouse islets treated for 3 days. Highest amino acid media with DMSO added (black,

n=3), highest amino acid media with 30nM rapamycin added for the last 24 hours of culture (black left hashed, n=2), highest amino acid media with 30nM rapamycin added for the full 72 hours of culture (black right hashed, n=3); one-way ANOVA with Tukey posthoc analyses; *p<0.05 and **p<0.01 vs DMSO-treated. Data are mean ± SEM.

Figure S5, related to Figure 4. *Slc38a5* expression is upregulated in α-cells and required for expansion of α-cells in response to interrupted glucagon signaling. (A-D) Representative images of pancreatic islet α-cell SLC38A5 expression in (A) Saline/PBS, (B) Rapamycin/PBS-, (C) Saline/GCGR mAb-, and (D) Rapamycin/GCGR mAb-treated NSG^{TM} mice (n=3). Glucagon staining (green), SLC38A5 staining (red), and insulin staining (blue) are shown. White scale bars indicate 50 μm. White dashed boxes indicate region selected for insets. (E-H) Representative images of pancreatic islet α-cell expression of SLC38A5 protein in (E) Wildtype/PBS-, (F) *FoxP1/2/4*$^{-/-}$/PBS-, (G) Wildtype/GCGR mAb-, and (H) *FoxP1/2/4*$^{-/-}$/GCGR mAb-treated mice (n=3). Glucagon staining (green), SLC38A5 staining (red), and insulin staining (blue) are shown. White scale bars indicate 50μm. White dashed boxes indicate region selected for insets. (I) Quantification of SLC38A5+ β-cells in *Gcgr*$^{Hep+/+}$ (white) and *Gcgr*$^{Hep-/-}$ (red) mouse pancreas. Students t-test - not significant. Quantification of (J) total SLC38A5^{+} islet-cell proliferation and (K) SLC38A5^{+} non-α-cells in isolated islets cultured in Low AA, High AA Low Q, or High AA media for four days. *p<0.05 and **p<0.01 vs High-AA treated; n=3. Data are mean ± SEM.

Figure S6, related to Figure 5. Human pancreatic islet α-cells proliferate when glucagon signaling is interrupted. (A) Perifusion of donor human islets used in these experiments. Insulin secretion in response to 5.6mM to 16.7mM glucose and 100μM IBMX is shown in blue. (B) Body mass over treatment (n=9-46), (C) random blood glucose over treatment (n=9-39), (D) glucose/arginine stimulated blood glucose (n=49-50), (E) glucose/arginine stimulated serum glucagon (n=49-50), and (F) glucose/arginine stimulated serum GLP-1 levels (n=10) in NSG mice treated with PBS (grey circles) or GCGR mAb (grey triangles) for 14 days. (Student's t-

test, *** p<0.0001; N.D. not detected). Note: Serum GLP-1 levels of all PBS-treated mice (n=10) were below the detection limit of the GLP-1 assay (41 pg/ml). The GLP-1 level for all glucose/arginine-stimulated Gcgr mAb mouse sample (n=10) were within the assay's detection limit. Therefore, no statistical analyses were performed. (G) Mouse pancreas mass (n=43-45) after 2 weeks of treatment with GCGR mAb; Student's t-test, *** p<0.0001. Representative images of α-cell proliferation in PBS and GCGR mAb-treated (H-I) mouse pancreas and (J-K) human islet donor grafts at 2 weeks treatment. Glucagon staining (green), Ki67 staining (red), and DAPI staining (blue) are shown. Proliferating α-cells ($Ki67^+$ $glucagon^+$) are indicated by white arrows. (L) Quantification of mouse α-cell proliferation (n=3) from non-responder human islet donors after 2 weeks of treatment with GCGR mAb. Student's T-test analyses; *p<0.05 vs PBS-treated. (M) Human α-cell proliferation in donor islets pooled from 7 different experiments of NSG mice treated with PBS or GCGR mAb and separated by responders (n=23) or non-responders (n=11). Student's T-test analyses; ***p<0.001 vs PBS-treated. Representative images of α-cell proliferation in PBS and GCGR mAb-treated (N-O) human islet donor grafts at 2 weeks treatment. Glucagon staining (green), SLC38A5 staining (red), and Insulin staining (blue) are shown. White asterisk indicates mouse kidney tissue. Data are mean ± SEM.

Table S1, related to Figure 2. Fold Change ≥1.5X in hepatic gene expression in PBS-treated *Gcgr-/-* versus PBS-treated *Gcgr+/+* mice, GCGR mAb-treated *Gcgr+/+* versus PBS-treated *Gcgr+/+* mice, and common to both models.

Table S2, related to Figure 2. IPA analyses of hepatic gene expression in PBS-treated *Gcgr-/-* versus PBS-treated *Gcgr+/+* mice and GCGR mAb-treated *Gcgr+/+* versus PBS-treated *Gcgr+/+* mice.

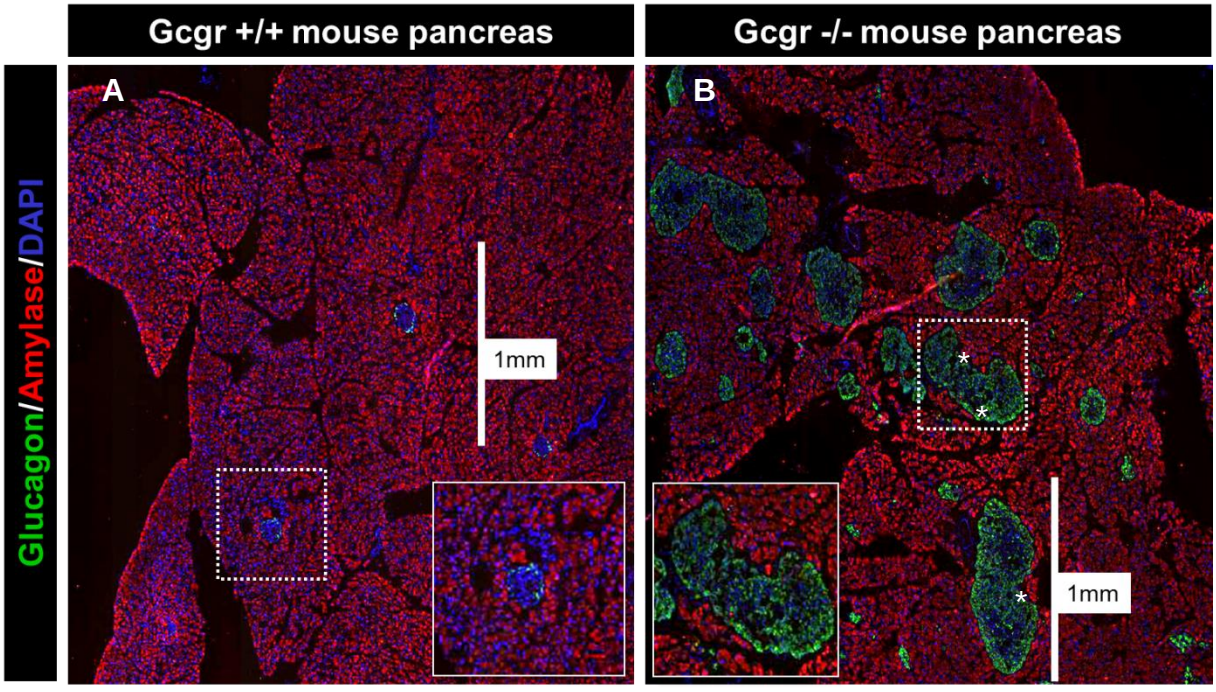
Table S3, related to Figure 2. Aptamer screening of serum proteome in mice with interrupted glucagon signaling.

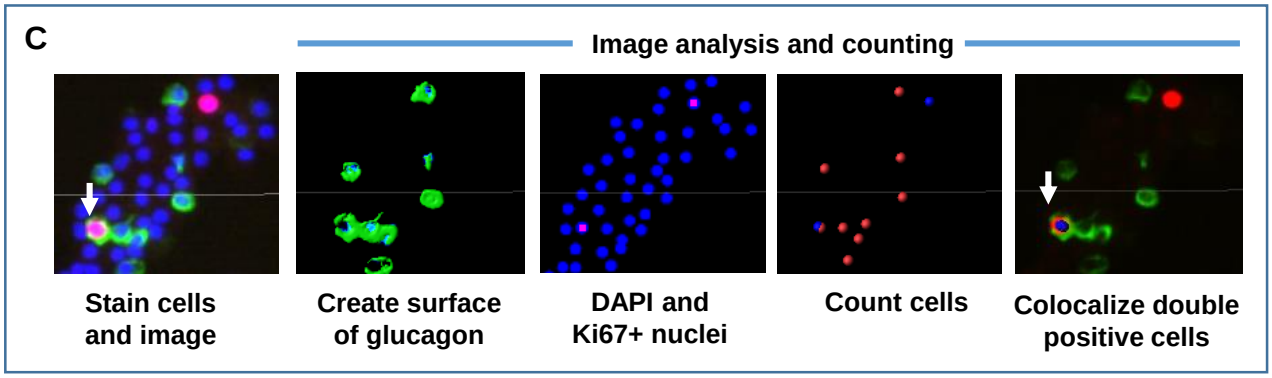
Table S4, related to Figure 4. Amino acid media concentrations used for islet culture model.

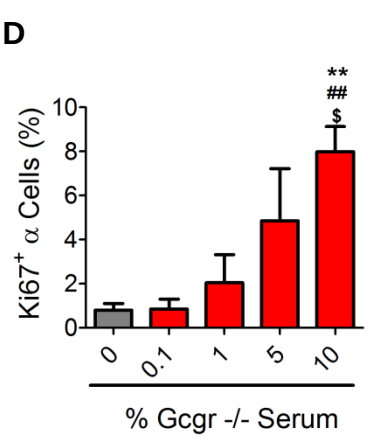
Table S5, related to Figure 4. Linear regression analyses of media amino acid concentration versus α-cell proliferation rate in mouse islet cultures.

Table S6, related to Figure 5. Donor information for human islet experiments.

Figure S1, Dean et al.







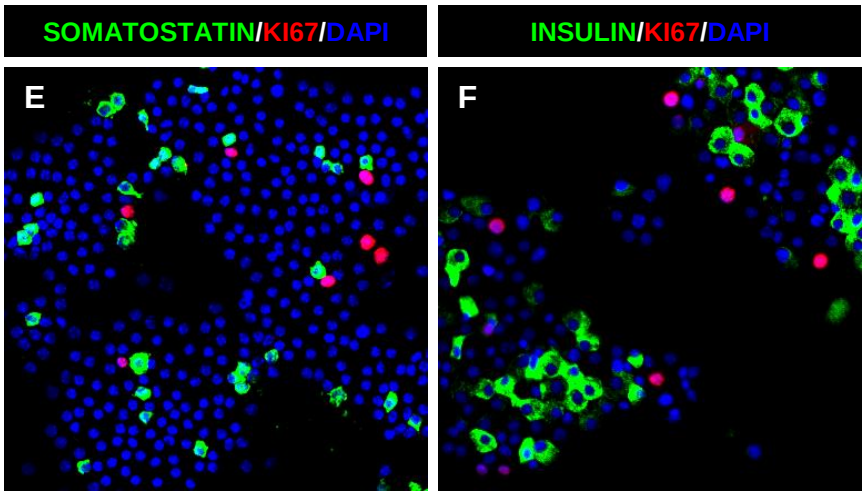


Figure S2 continued, Dean et al.

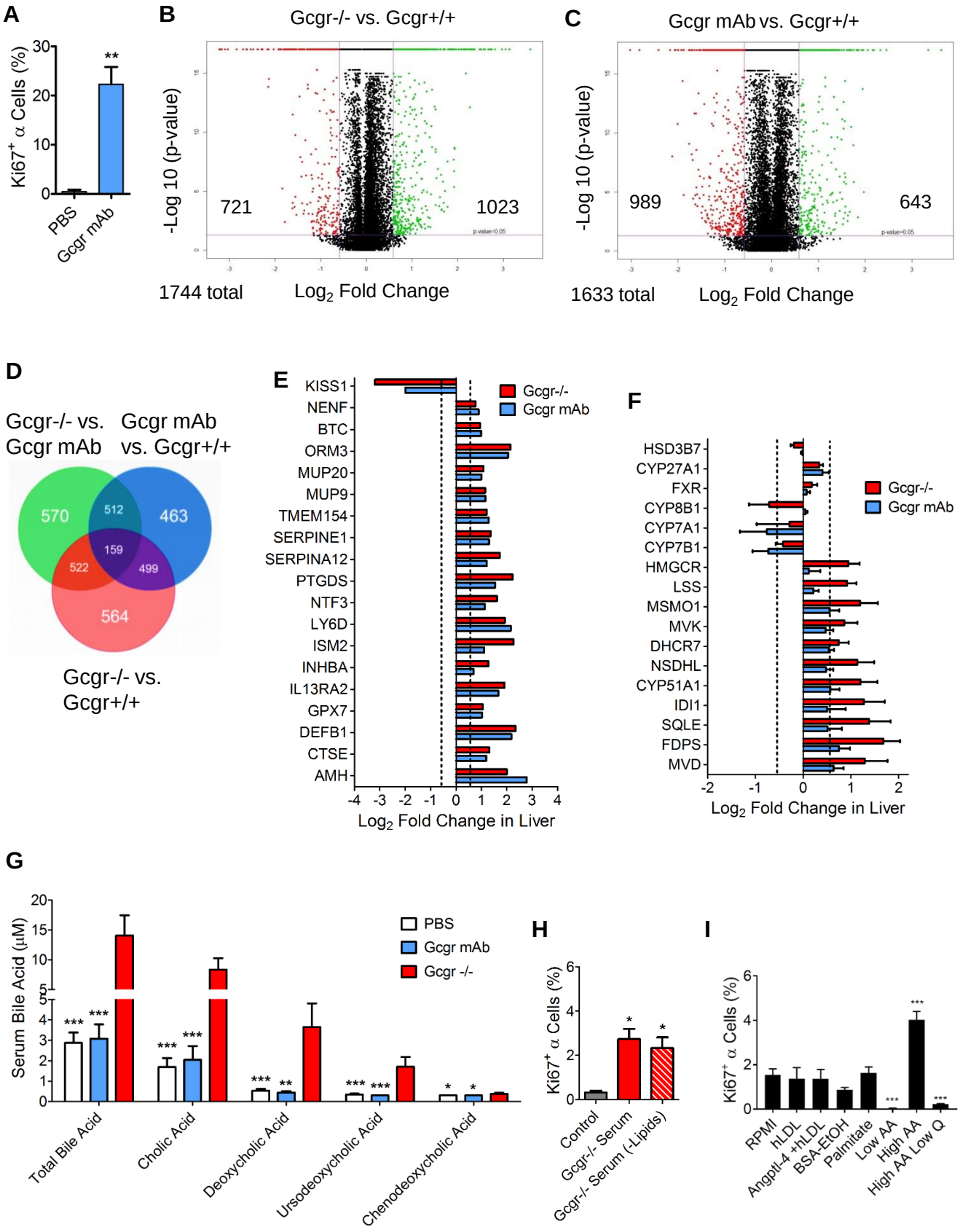


Figure S3, Dean et al.

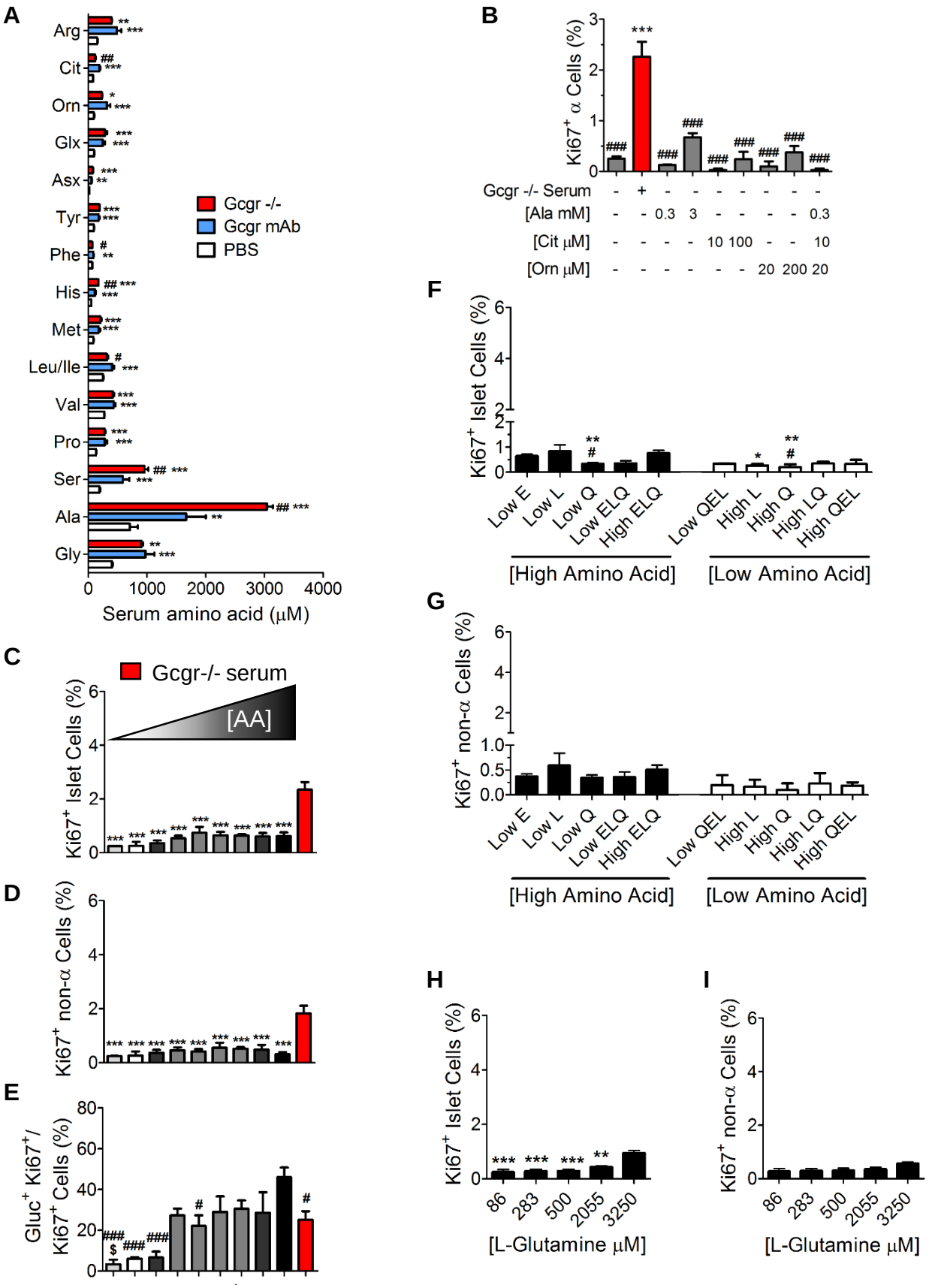


Figure S4, Dean et al.

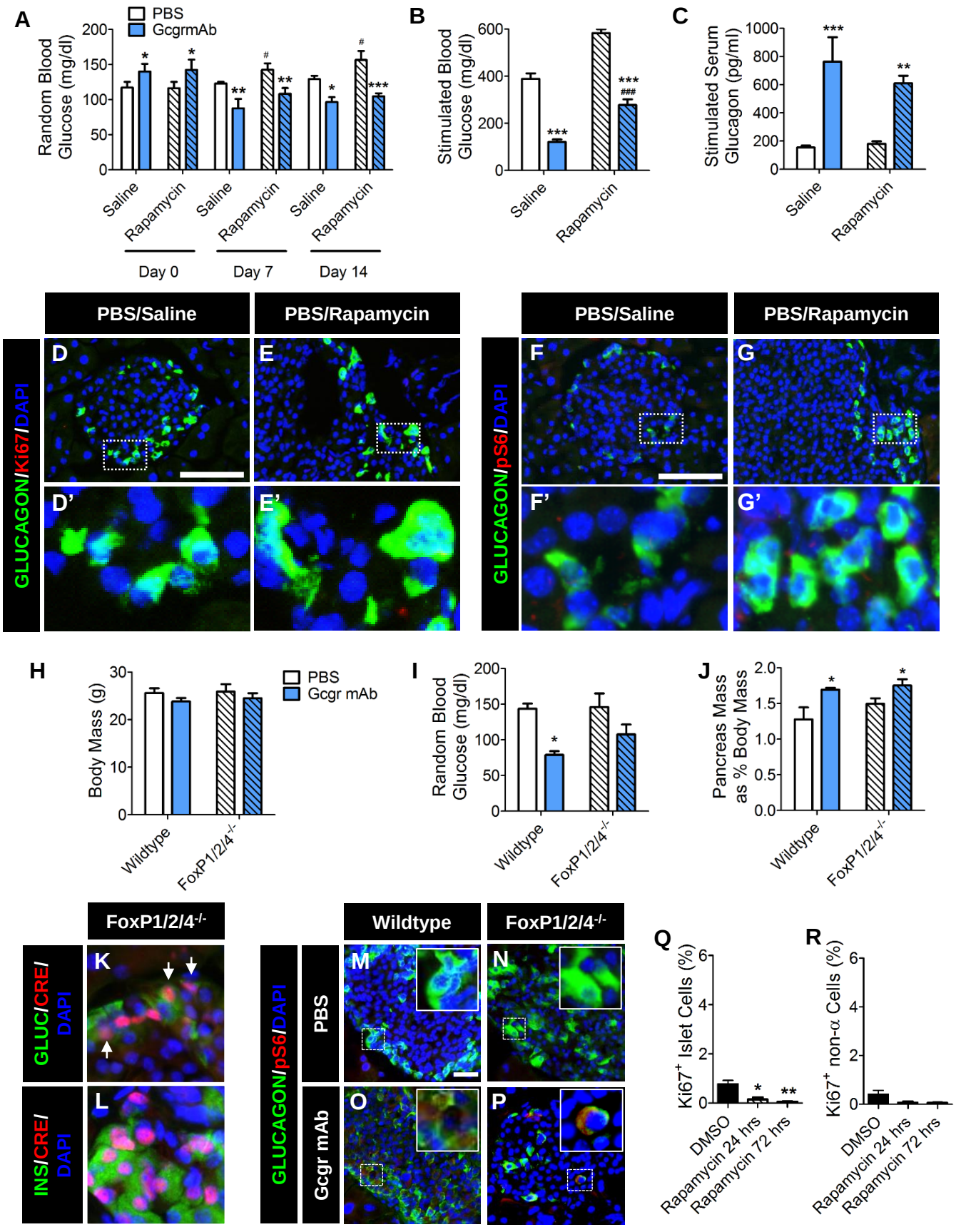


Figure S5, Dean et al.

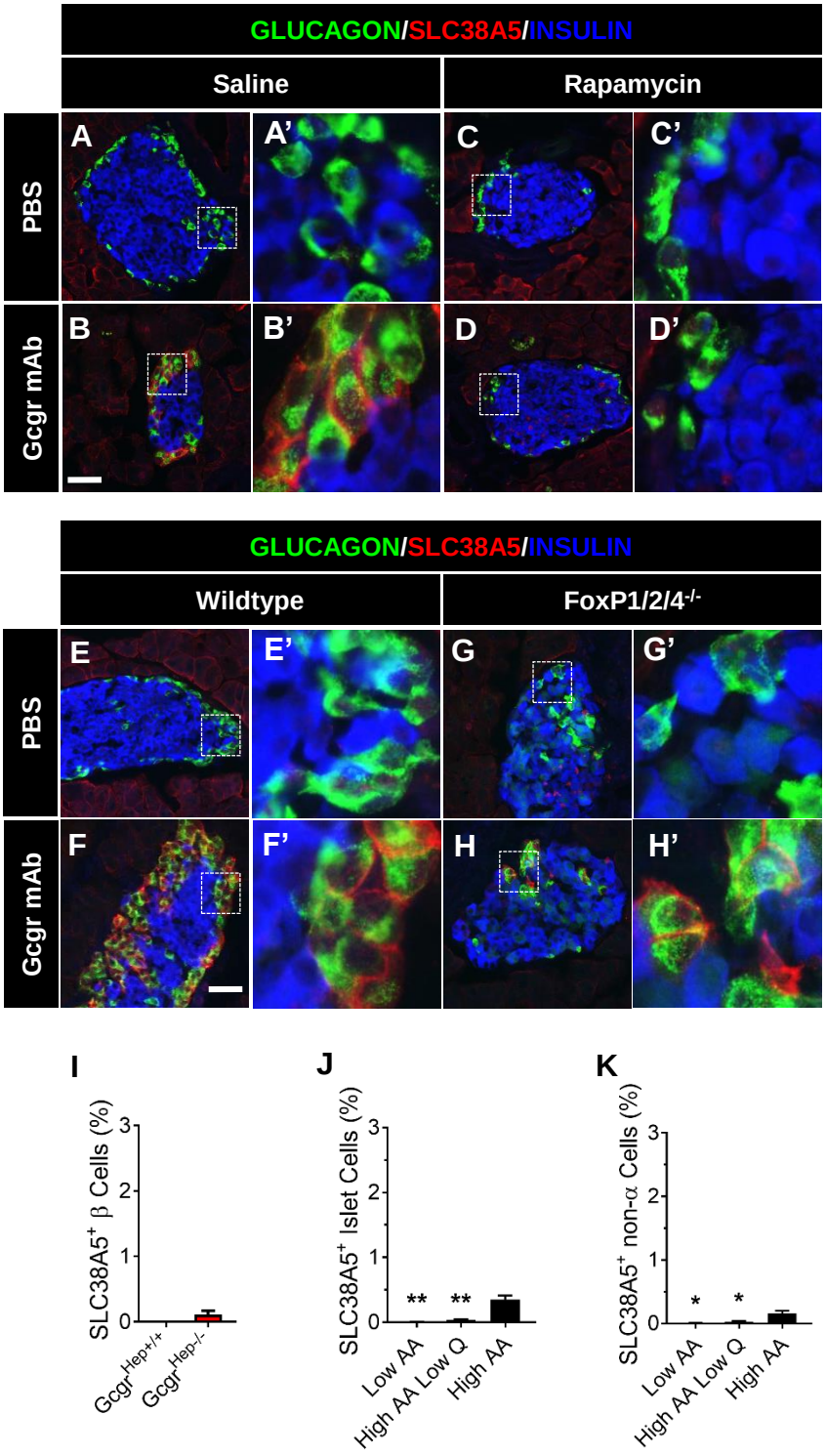


Figure S6, Dean et al.

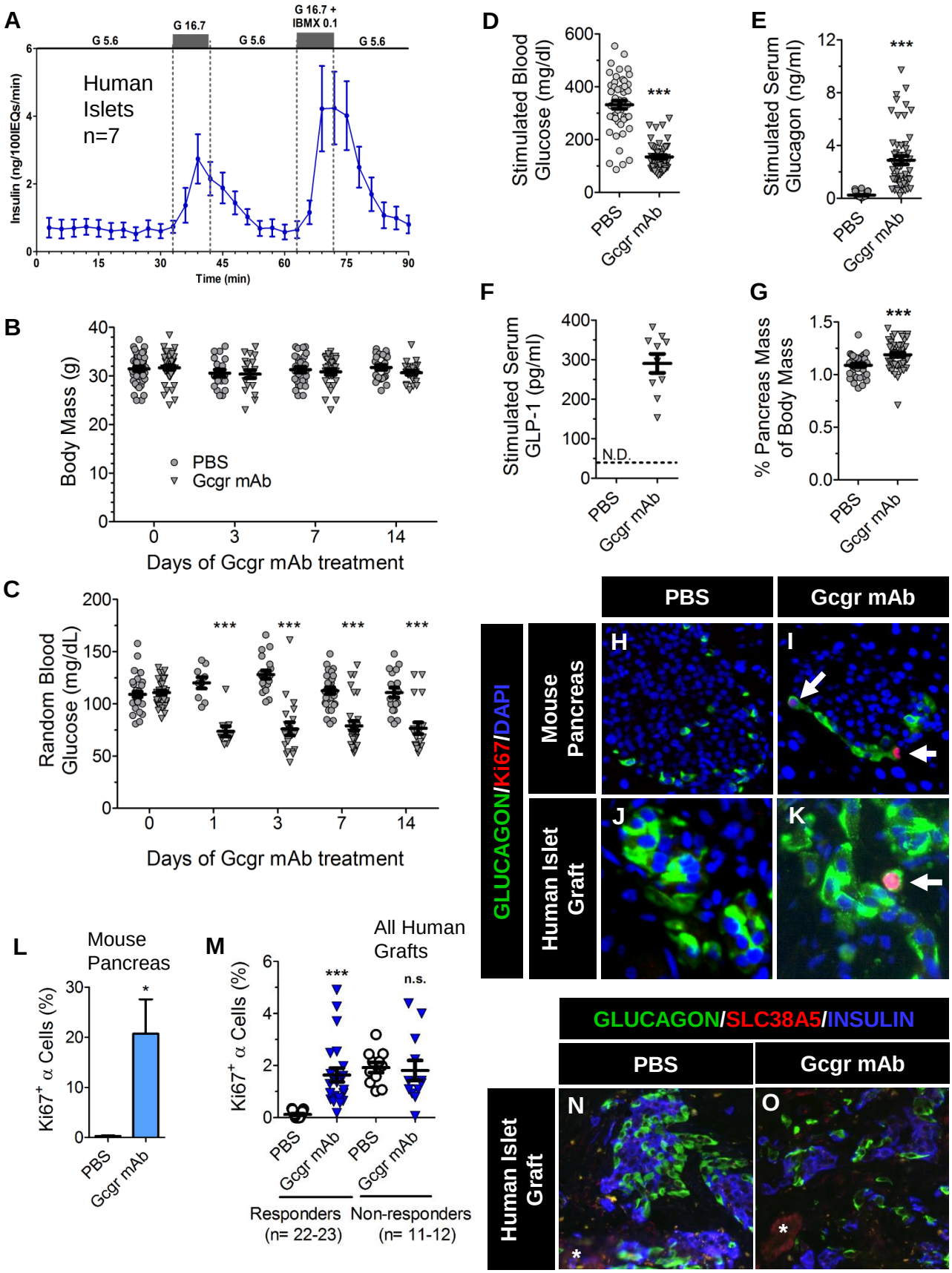


Table S4, related to Figure 4. Amino acid media concentrations used for islet culture model.

Media #	1	2	3	4	5	6	7	8	9	Gcgr+/+ serum	Gcgr-/- serum	Est. media plus 10% Gcgr+/+ serum	Est. media plus 10% Gcgr-/- serum	FC in Gcgr-/- versus Gcgr+/+ serum
Glycine	451	200	905	165	210	141	133	270	1500	297	1575	149	277	5.30
L-Arginine	158	40	397	1050	1074	1142	1149	1094	600	106	519	1045	1086	4.90
L-Asparagine	27	40	76	344	349	376	379	376	350	107	787	352	420	7.35
L-Aspartic acid	50	10	60	140	141	149	150	136	10	16	50	137	140	3.11
L-Cystine 2HCl	20	10	40	189	191	206	208	188	10	ND	ND	187	187	ND
L-Glutamic Acid	50	100	100	127	132	136	136	152	300	55	136	128	136	2.47
L-Glutamine	86	500	283	1858	1878	2037	2055	2174	3250	495	3102	1899	2160	6.26
L-Histidine	57	40	170	93	104	98	97	112	250	67	285	94	116	4.26
L-Hydroxyproline	40	0	108	40	108	152	153	137	0	ND	ND	138	138	ND
L-Isoleucine	273	125	317	371	375	381	382	369	250	135	218	357	366	1.62
L-Leucine	273	225	317	371	375	381	382	384	400	167	330	361	377	1.97
L-Lysine HCL	350	200	1000	282	347	281	274	367	1200	253	974	272	344	3.86
L-Methionine	106	60	209	101	111	102	101	119	280	66	213	98	112	3.21
L-Phenylalanine	69	75	67	89	89	91	91	89	75	67	75	89	89	1.11
L-Proline	154	85	279	172	184	175	174	197	400	118	313	168	188	2.67
L-Serine	239	100	959	281	353	292	286	382	1250	133	1296	271	387	9.75
L-Threonine	200	150	900	171	241	175	168	326	1750	166	1521	168	303	9.18
L-Tryptophan	40	65	45	26	27	25	25	29	65	78	99	30	32	1.26
L-Tyrosine	104	50	196	110	120	112	111	125	250	83	187	108	119	2.25
L-Valine	265	300	414	180	195	173	171	214	600	250	492	179	203	1.97
Alanine	1125	350	3040	113	304	30	0	225	2250	513	2736	51	274	5.34
Ornithine	86	100	234	9	23	2	0	40	400	86	418	9	42	4.84
Citrulline	65	0	118	7	12	1	0	0	0	70	144	7	14	2.06
Taurine	0	0	0	0	0	0	0	0	0	466	623	47	62	1.34
Total [AA] concentration in media	4288	2825	10234	6289	6943	6658	6625	7505	15440	3328	15469	6295	7509	4.65

Table S6, related to Figure 5. Donor information for human islet experiments.

Donor	Age	Gender (M/F)	BMI (kg/m^2)
1	32	M	29.5
2	47	M	22.3
3	55	M	28.4
4	43	M	29.6
5	46	M	28.8
6	41	F	31.1
7	47	F	25.6
8	52	M	33.2