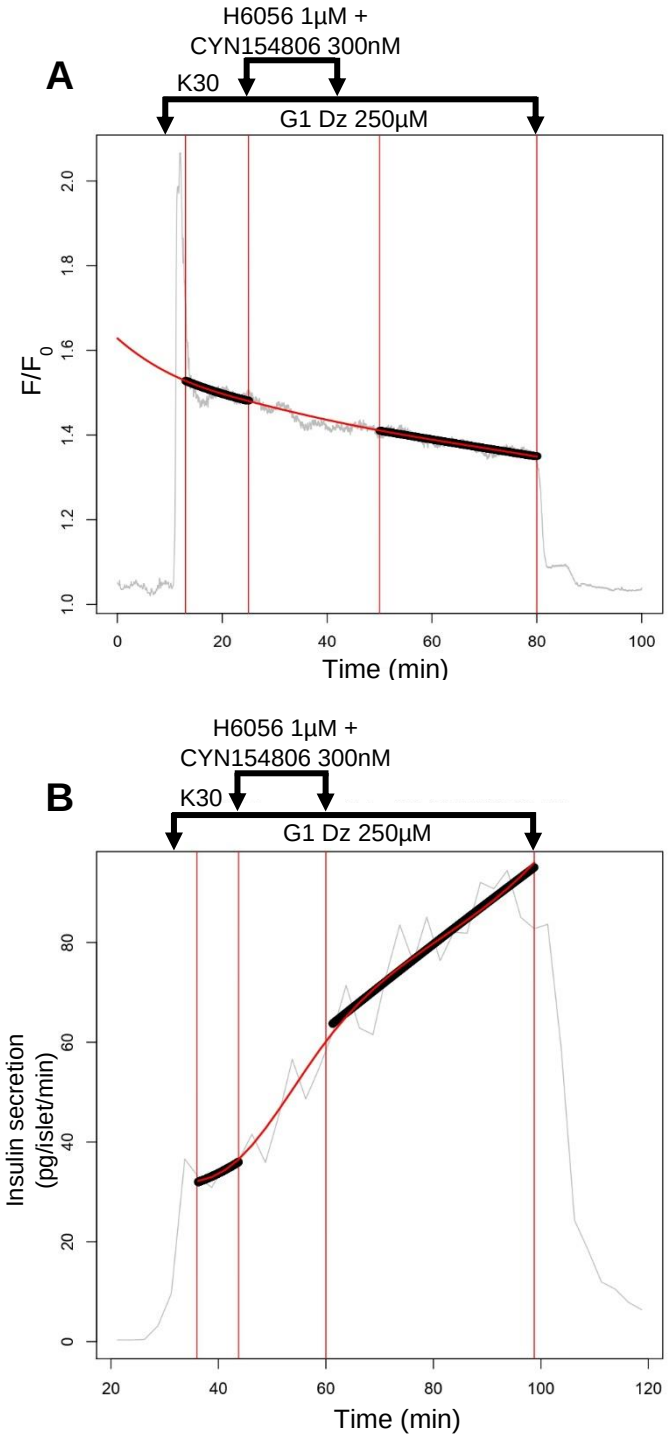
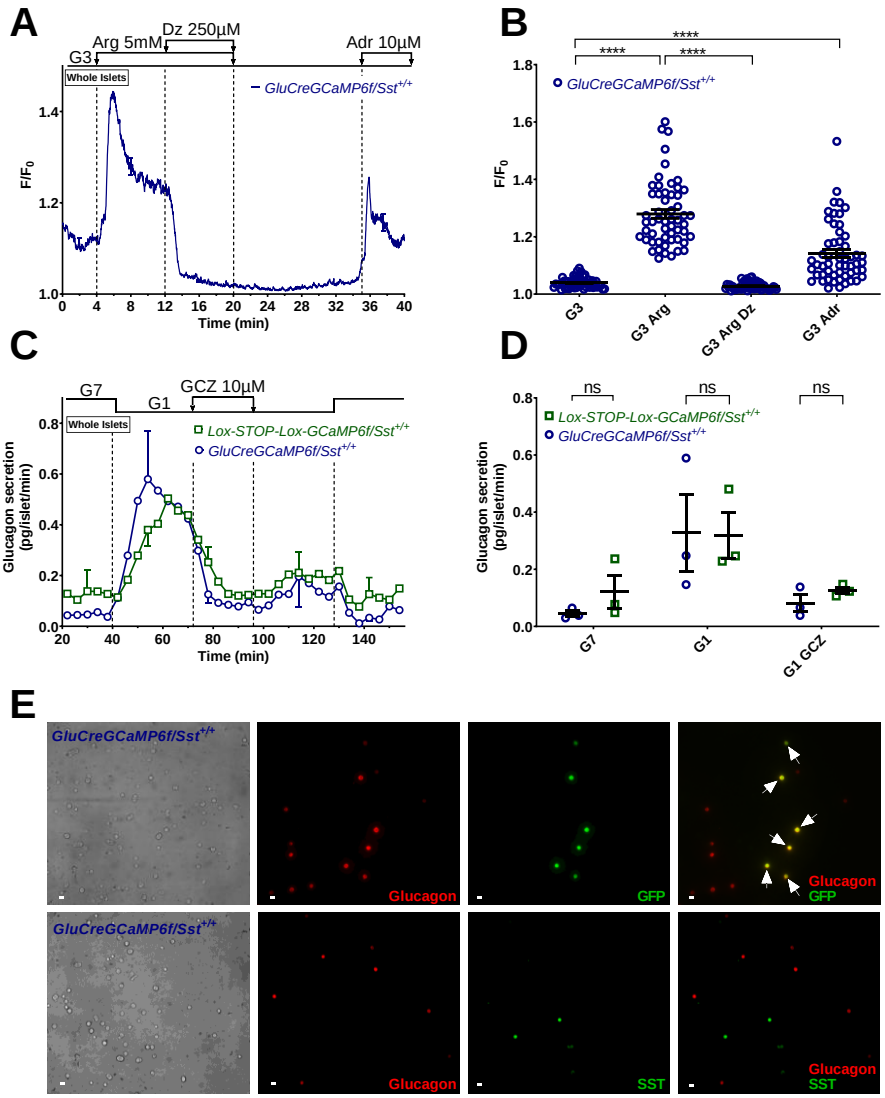
Online supplementary materials

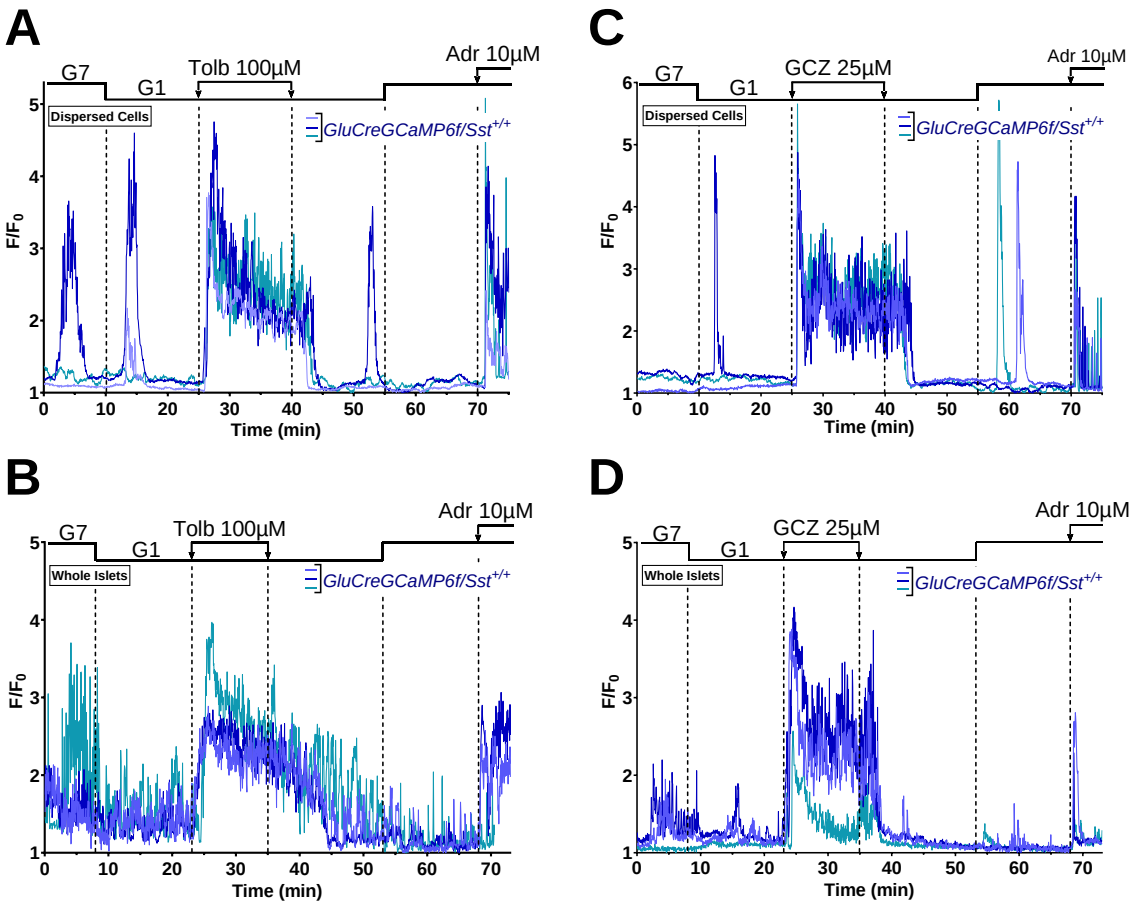


Supplementary Figure S1. Examples of smoothing curves for $[Ca^{2+}]_c$ measurements and insulin secretion experiments.

Panel A shows a representative mean of $[Ca^{2+}]_c$ measurements with a spontaneous decrease of the signal whereas panels B shows representative mean of insulin secretion experiment with a spontaneous increase of the signal. The red line represents the smoothing curve calculated based on the signal before (13-25 min for A; 36-44 min for B) and after (50-80 min for A; 60-99 min for B) the treatment condition (here H6056 1 µM + CYN154806 300 nM : 25-40 min for A; 45-60 min for B). The difference between the signal and the smoothing curve is then calculated for each cell (A) or each mouse (B) at each time point. The appropriate statistical test is then used to compare the control condition with the treatment condition from the mean of these subtracted values. This method has been developed by our statistician.

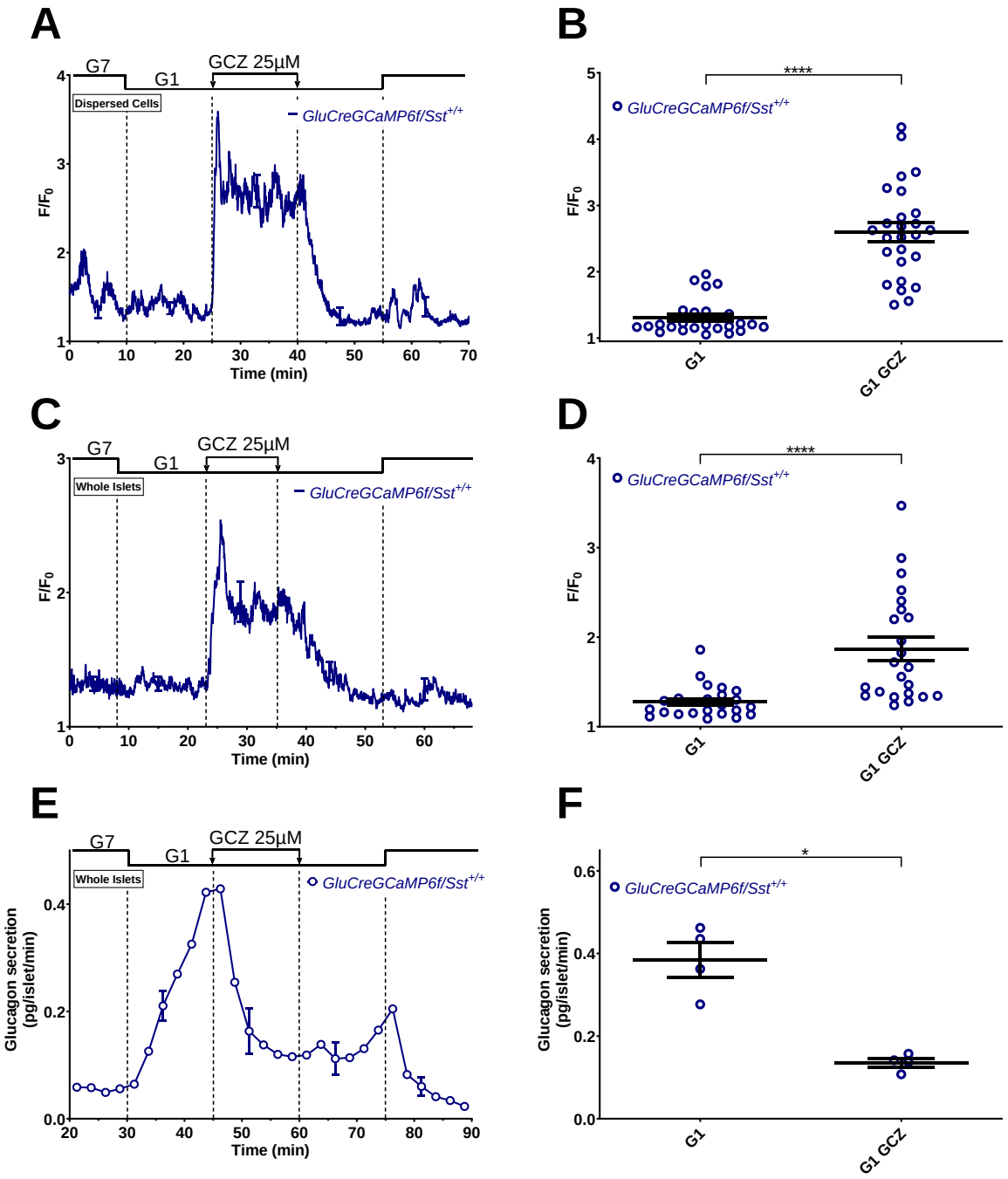


Supplementary Figure S2. Validation experiments of the *GluCreGCaMP6f/Sst*$^{+/+}$ mouse model.
(A and B) GCaMP6f reliability was assessed by measuring $[Ca^{2+}]_c$ in α-cells within islets of *GluCreGCaMP6f/Sst*$^{+/+}$ mice perifused with 3 mM glucose (G3) in response to arginine (Arg 5 mM) and adrenaline (Adr 10 µM). Diazoxide (Dz 250 µM), a K_{ATP} channel opener, was added as indicated to inhibit the rise in $[Ca^{2+}]_c$ elicited by arginine. Panel A represents the mean trace ± SEM of 3 experiments and panel B shows the scatter plot of individual cells (n = 55 cells/5 islets/3 mice; one-way RM ANOVA with Sidak correction) with the means ± SEM of the average $[Ca^{2+}]_c$ calculated from panel A (G3, mean of 0-4 min and 20-35 min; G3 Arg, mean of 6-12 min; G3 Arg Dz, mean of 14-20 min; G3 Adr, mean of 36-40min). ****P < 0.0001. (C and D) Potential interference of GCaMP6f expression on glucagon secretion was assessed by measuring glucagon secretion from *GluCreGCaMP6f/Sst*$^{+/+}$ (blue trace) and *Lox-STOP-Lox-GcaMP6f/Sst*$^{+/+}$ isolated islets (green trace) perifused with 7 mM (G7) and 1 mM glucose (G1) as indicated. Gliclazide (GCZ 10 µM), a K_{ATP} channel closer, was added as indicated. It inhibited the rise in glucagon secretion elicited by G1. Panel C represents the mean traces ± SEM of 3 experiments and panel D shows the scatter plot of individual experiments with the means ± SEM (Ordinary two-way ANOVA with Sidak correction) of the average glucagon secretion calculated from panel C (G7, mean of 22-40 min and 138-156 min; G1, mean of 54-72 min and 114-128 min; G1 GCZ, mean of 86-100 min). ns, not significant. (E) GCaMP6f specificity of expression was assessed by immunodetection of dispersed islet cells using a combination of either an anti-glucagon antibody in red and an anti-GFP (to label GcaMP6f) in green (top panels) or an anti-glucagon in red and an anti-SST in green (lower panels). 96.4% of GFP-positive cells were positive for glucagon (white arrows). Scale bars: 10 µm.

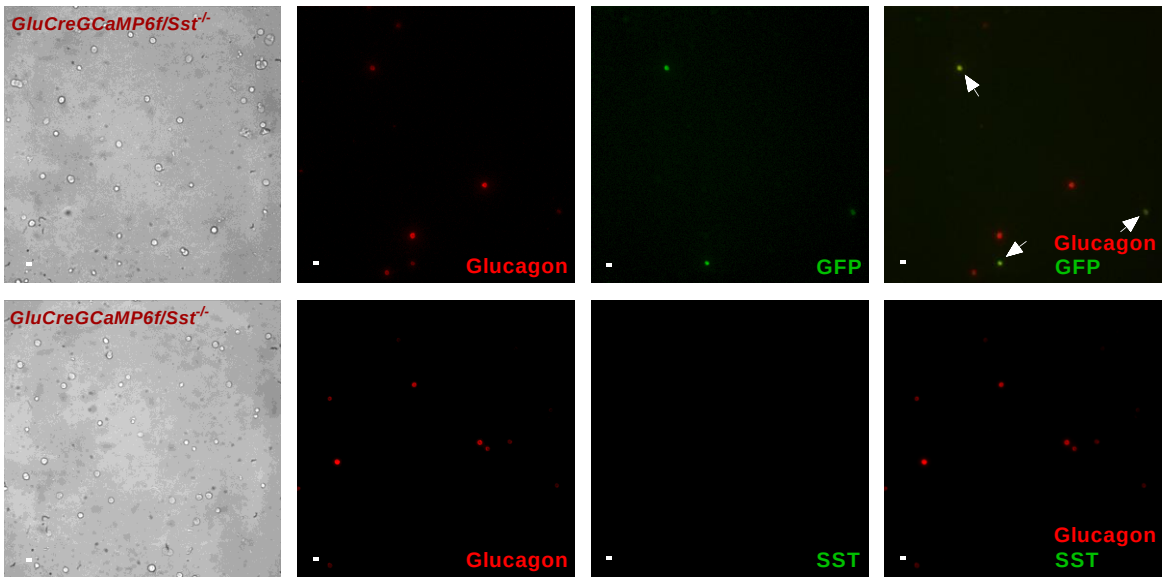


Supplementary Figure S3. Sulfonylureas increase $[Ca^{2+}]_c$ in dispersed α-cells and in α-cells within islets in G1.

Each panel shows 3 representative traces of $[Ca^{2+}]_c$ measurements in α-cells from 3 different *GluCreGCaMP6f/Sst*$^{+/+}$ islets. Dispersed islet cells (A and C) and isolated islets (B and D) were perifused sequentially with 7 mM (G7) and 1 mM glucose (G1). Tolbutamide (Tolb 100 µM) (A and B) or gliclazide (GCZ 25 µM) (C and D), two K_{ATP} channel inhibitors, were added in G1 as indicated. At the end of each experiment, adrenaline (Adr 10 µM) was added to make sure that only α-cells are selected. They are representative traces of experiments shown in Figure 1A, C and Supplementary Figure S4A, C.

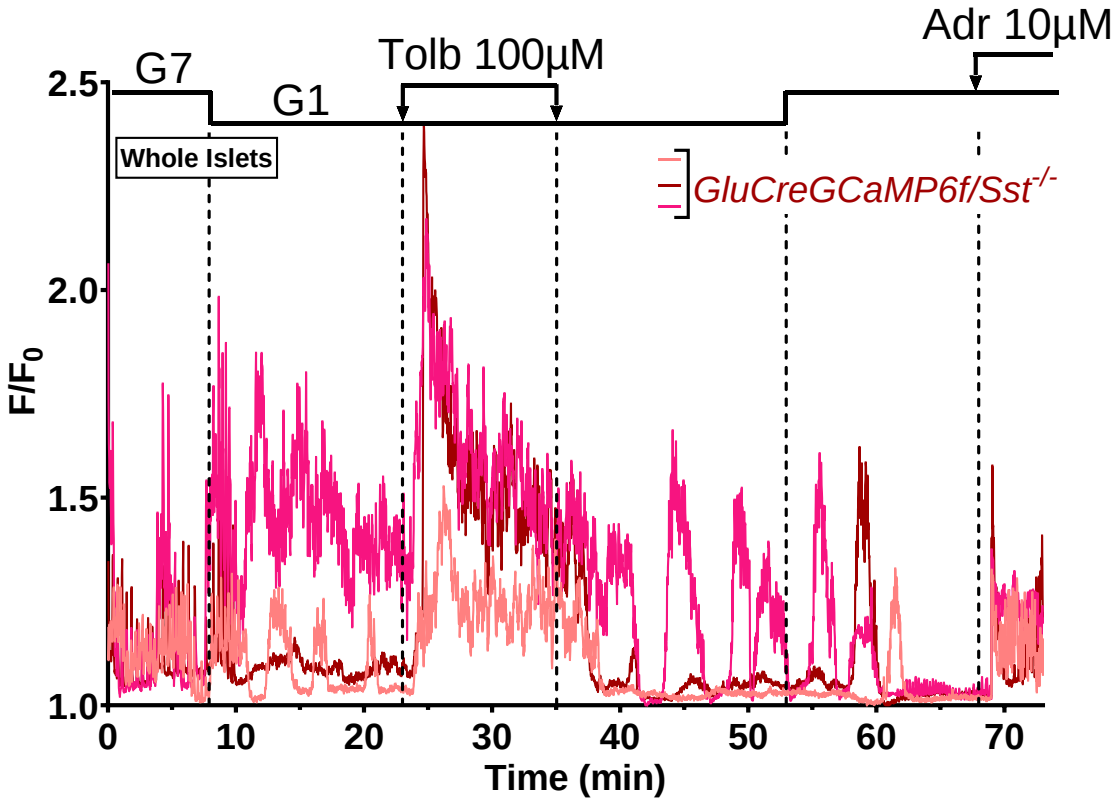


Supplementary Figure S4. Gliclazide increases α-cell $[Ca^{2+}]_c$ but inhibits glucagon secretion in G1. α-cell $[Ca^{2+}]_c$ was measured in dispersed islet cells (A and B) and isolated islets (C and D) whereas glucagon secretion was only measured in isolated islets (E and F). Dispersed islet cells and isolated islets from *GluCreGCaMP6f/Sst*$^{+/+}$ mice were both perifused with 7 mM (G7) and 1 mM glucose (G1). Gliclazide (GCZ 25 µM), a K_{ATP} channel closer, was added in G1 as indicated. Panels A, C and E represent the mean traces ± SEM of 5, 5 and 4 experiments, respectively. Panels B and D represent the scatter plots of individual cells (B, n = 26 cells/3 mice; D, n = 23 cells/5 islets/4 mice; two-tailed paired t-test) with the means ± SEM of the average $[Ca^{2+}]_c$ calculated from panel A (G1, mean of 17-25 min and 47-55 min; G1 GCZ, mean of 32-40 min) and C (G1, mean of 15-23 min and 45-53 min; G1 GCZ, mean of 27-35 min), respectively. Panel F represents the scatter plot of individual experiments with the means ± SEM (two-tailed paired t-test) of the average glucagon secretion calculated from panel E (G1, mean of 37-45 min; G1 GCZ, mean of 55-60 min). *P < 0.05; ****P < 0.0001.

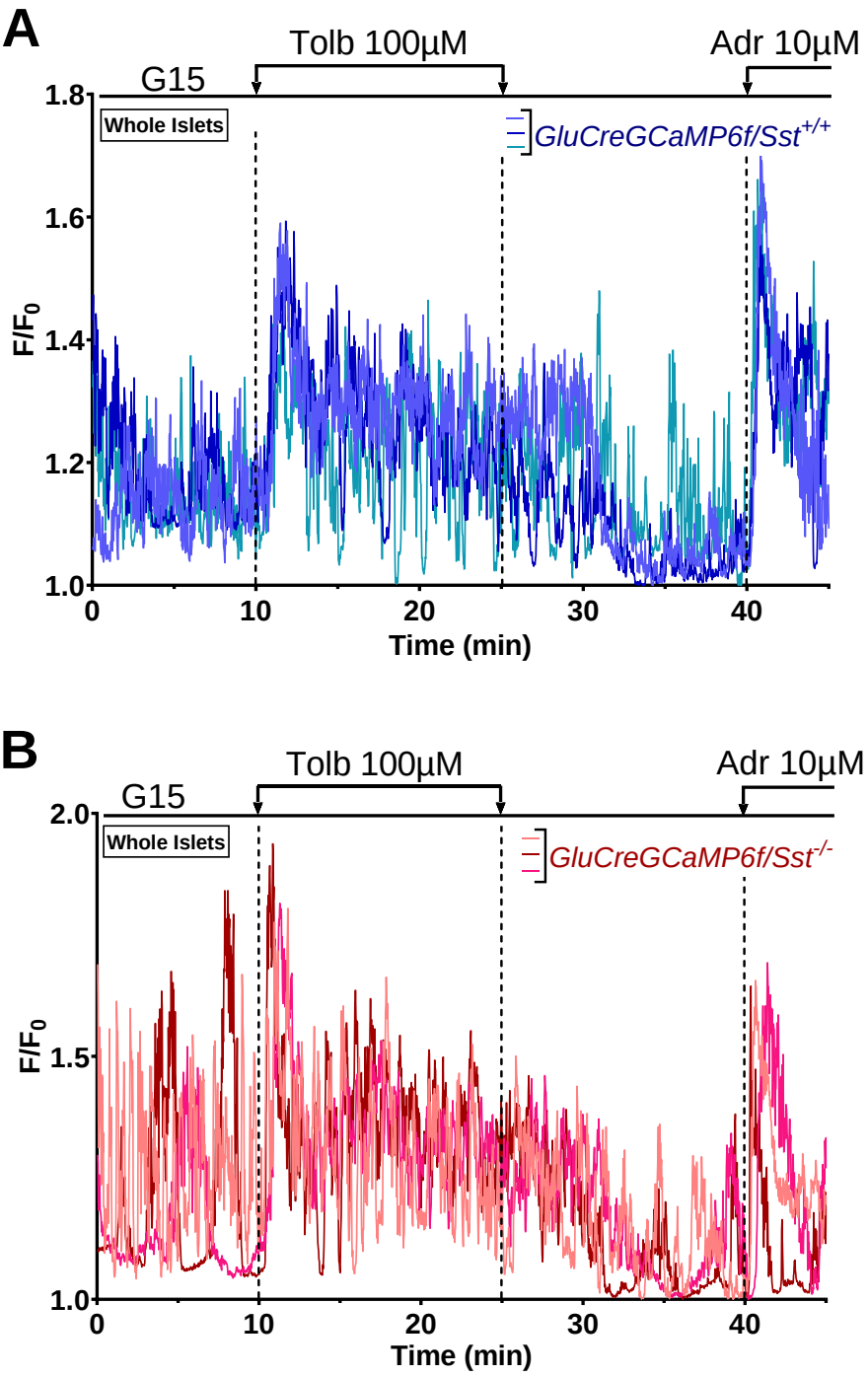


Supplementary Figure S5. GCaMP6f is specifically expressed in α-cells from *GluCreGCaMP6f/Sst*$^{-/-}$ mice which lack SST expression.

The ablation of SST in *GluCreGCaMP6f/Sst*$^{-/-}$ mouse model was assessed by immunodetection of dispersed islet cells using a combination of either an anti-glucagon antibody in red and an anti-GFP (to label GCaMP6f) in green (top panels) or an anti-glucagon in red and an anti-SST in green (lower panels). Whereas 94% of GFP-positive cells were positive for glucagon (white arrows), none of the cells expressed SST. Scale bars: 10 µm.

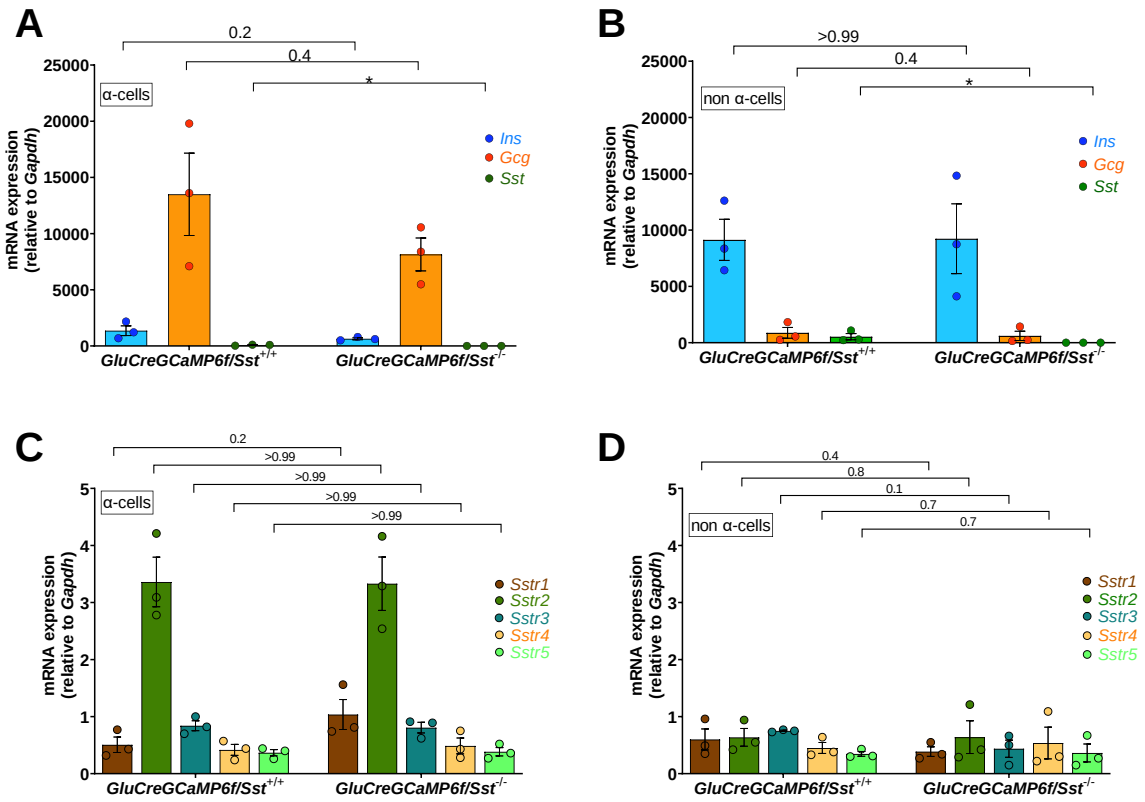


Supplementary Figure S6. Tolbutamide increases $[Ca^{2+}]_c$ in α-cells within islets lacking SST in G1. This panel shows 3 representative traces of $[Ca^{2+}]_c$ measurements in α-cells from 3 different *GluCreGCaMP6f/Sst*$^{-/-}$ islets. Tolbutamide (Tolb 100 µM) was added in 1 mM glucose (G1) as indicated. At the end of each experiment, adrenaline (Adr 10 µM) was added to make sure that only α-cells are selected. They are representative traces of the experiment shown in Figure 2A.



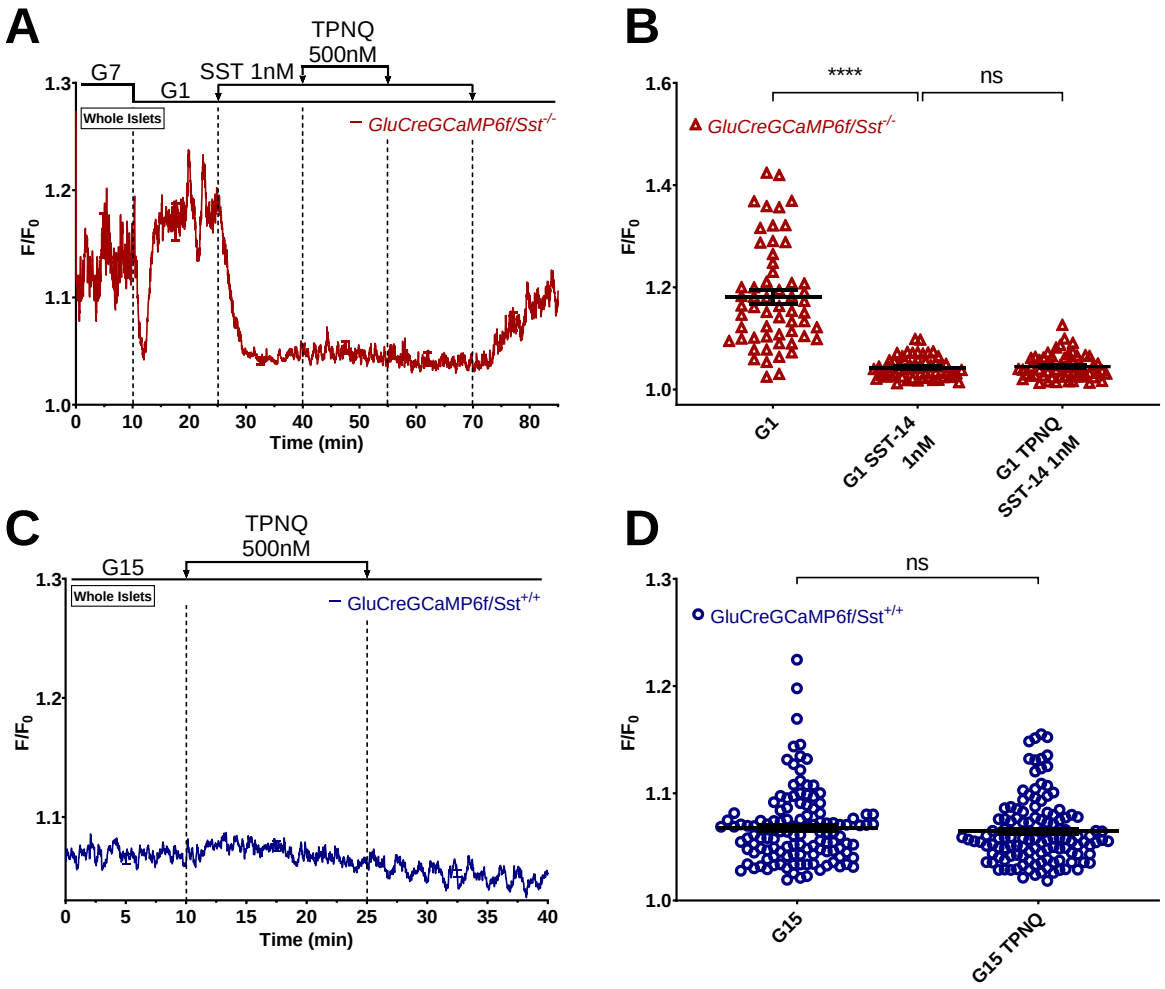
Supplementary Figure S7. Tolbutamide increases $[Ca^{2+}]_c$ in α-cells within islets from both SST-lacking and -expressing mice in G15.

Each panel shows 3 representative traces of $[Ca^{2+}]_c$ measurements in α-cells from 3 different *GluCreGCaMP6f/Sst*$^{+/+}$ (A) *and GluCreGCaMP6f/Sst*$^{-/-}$ (B) islets. Tolbutamide (Tolb 100 µM) was added in 15 mM glucose (G15) as indicated. At the end of each experiment, adrenaline (Adr 10 µM) was added to make sure that only α-cells are selected. They are representative traces of the experiment shown in Figure 3A and C.



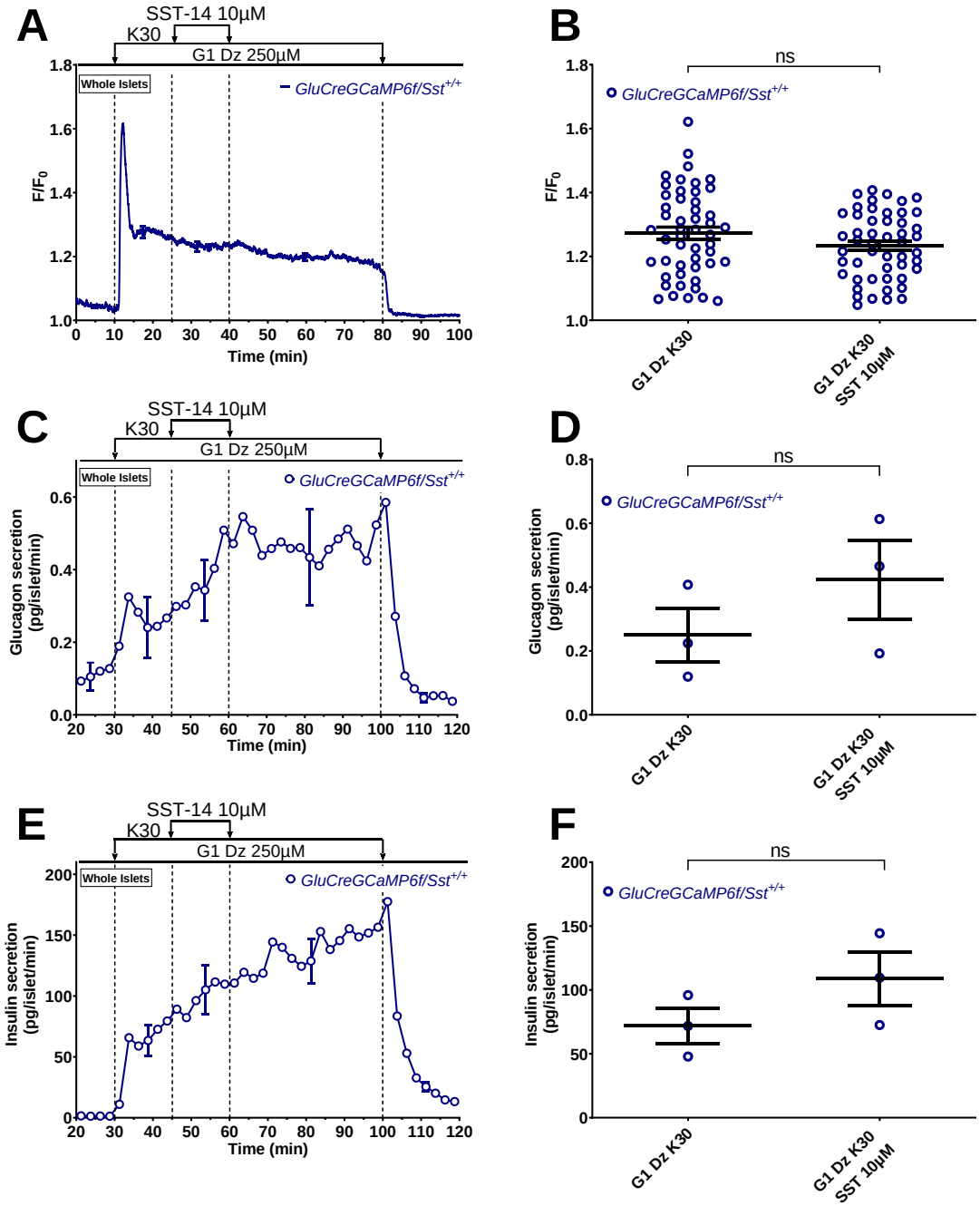
Supplementary Figure S8. *Sstr1-5* mRNA are equally expressed in α-cells between *GluCreGCaMP6f/Sst*$^{+/+}$ and *GluCreGCaMP6f/Sst*$^{-/-}$ mice.

mRNA expression of insulin (*Ins*), glucagon (*Gcg*), somatostatin (*Sst*) (A and B) and somatostatin receptors (*Sstr1-5*) (C and D) relative to *Gapdh* were assessed by qPCR in FACS-sorted α- and non α-cells from *GluCreGCaMP6f/Sst*$^{+/+}$ and *GluCreGCaMP6f/Sst*$^{-/-}$ mice. Panels represent the scatter plots with bars of 3 experiments from 3 different cell populations (two-tailed Mann-Whitney test except for *Sst* mRNA expression for which one-tailed Mann-Whitney test was used instead). *P < 0.05.



Supplementary Figure S9. GIRK channels inhibition does not reverse the inhibitory effect of exogenous and endogenous SST on α-cell $[Ca^{2+}]_c$.

α-cell $[Ca^{2+}]_c$ was measured in isolated islets from either *GluCreGCaMP6f/Sst*$^{-/-}$ (A and B) perifused sequentially with 7 mM (G7) and 1 mM glucose (G1) or *GluCreGCaMP6f/Sst*$^{+/+}$ (C and D) mice perifused with 15 mM glucose (G15). Tertiapin-Q (TPNQ 500 nM, from Alomone or Tocris), a GIRK channels inhibitor, was added in either G1 in the presence of 1 nM SST (A and B) or in G15 (C and D) as indicated. Panels A and C represent the mean traces ± SEM of 6 and 5 experiments, respectively. Panels B and D represent the scatter plots of individual cells (B, 58 cells/15 islets/2 mice; one-way RM ANOVA with Sidak correction; D, 124 cells/15 islets/2 mice; two-tailed paired t-test) with the means ± SEM of the average $[Ca^{2+}]_c$ calculated from panel A (G1, mean of 20-25 min; G1 SST-14 1 nM, mean of 35-40 min and 65-70 min; G1 TPNQ SST-14 1 nM, mean of 50-55 min) and C (G15, mean of 5-10 min; G15 TPNQ, mean of 20-25 min), respectively. ns, not significant; ****P < 0.0001.



Supplementary Figure S10. In SST-expressing islets, a high concentration of exogenous SST is unable to inhibit glucagon and insulin secretions in a depolarizing condition where K_{ATP} channels and $[Ca^{2+}]_c$ are clamped.

α-cell $[Ca^{2+}]_c$ (A and B), glucagon (C and D) and insulin secretions (E and F) were measured in isolated islets from *GluCreGCaMP6f/Sst*$^{+/+}$ mice perifused with 1 mM glucose (G1) and the K_{ATP} channel opener, diazoxide (Dz 250 µM). The K^+ concentration of the medium was increased from 4.8 to 30 mM (K30), and SST (SST-14 10 µM) was added in the presence of K30 as indicated. Panels A, C and E represent the mean traces ± SEM of 3 experiments. Panel B represents the scatter plots of individual cells (n = 50 cells/4 islets/3 mice; two-tailed paired t-test) with the means ± SEM of the average $[Ca^{2+}]_c$ calculated from panel A (G1 Dz K30, mean of 15-25 min; G1 Dz K30 SST 10 µM, mean of 30-40 min). Panels D and F represent the scatter plots of individual experiments with the means ± SEM (two-tailed paired t-test) of the average glucagon and insulin secretions calculated from panel C and E, respectively (G1 Dz K30, mean of 37-45 min; G1 Dz K30 SST 10 µM, mean of 52-60 min). ns, not significant.