

Expanded View Figures

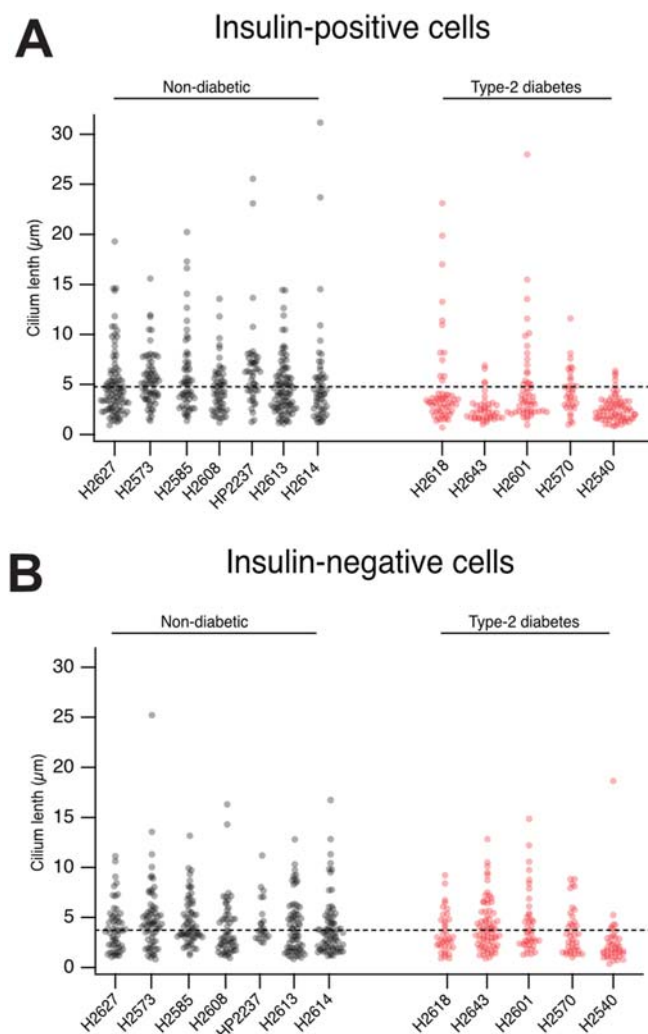


Figure EV1. Cilia length in human islets from non-diabetic and type-2 diabetic donors.

(A) Quantification of cilium length in insulin-positive β -cells in islets from 7 non-diabetic (black; $n = 94, 66, 57, 56, 45, 92$ and 54 cilia) and 5 type-2 diabetic (red; $n = 57, 42, 53, 33$ and 71 cilia) human organ donors. Dashed line shows average for all non-diabetic donors. (B) Quantification of cilium length in insulin-negative cells in islets from 7 non-diabetic (black; $n = 54, 65, 63, 54, 22, 66$ and 74 cilia) and 5 type-2 diabetic (red; $n = 37, 70, 40, 38$ and 40 cilia) human organ donors. Dashed line shows average for all non-diabetic donors. Source data are available online for this figure.

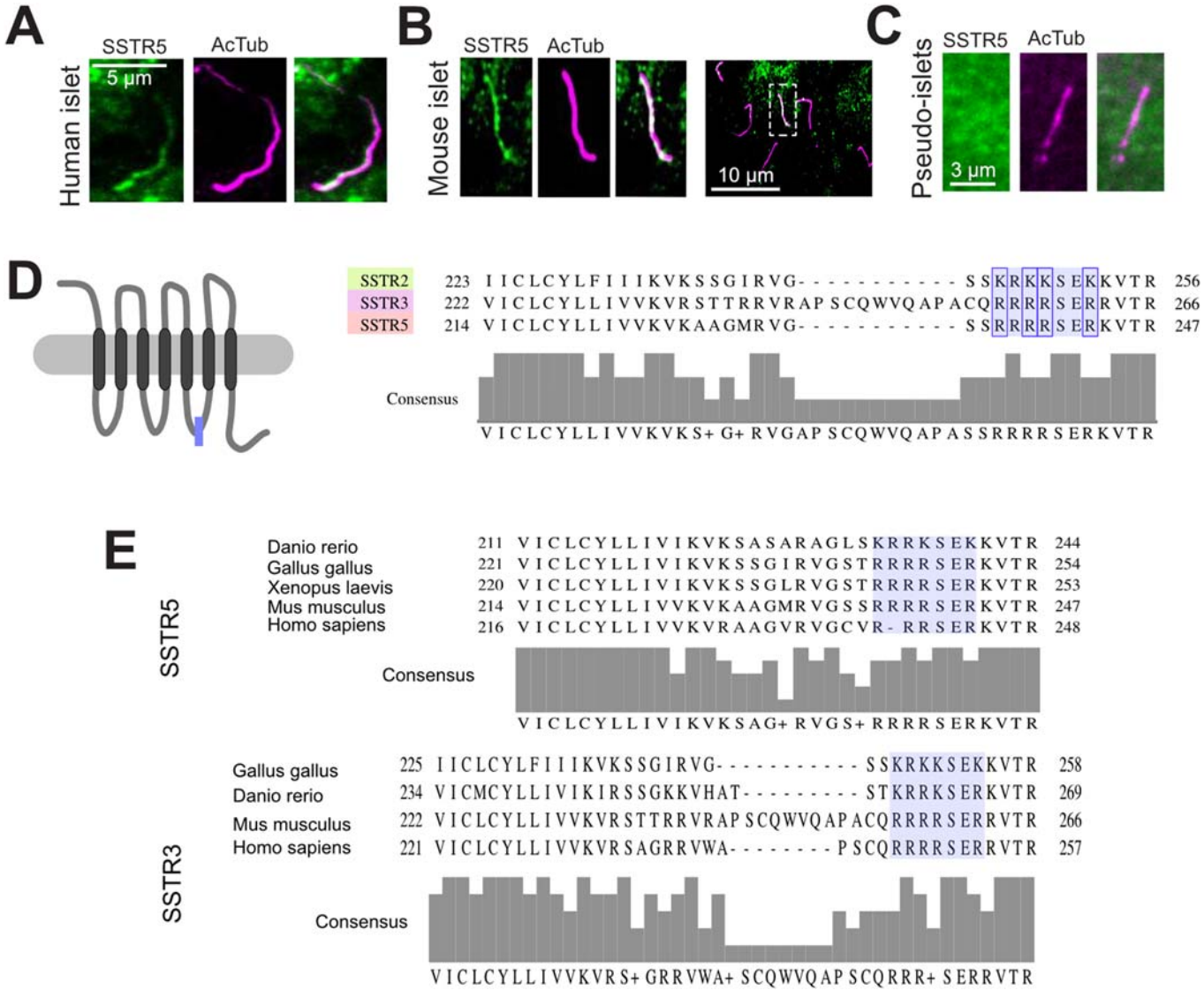


Figure EV2. SSTR5 localized to primary cilia.

(A) Confocal microscopy image of a primary cilium (acetylated tubulin; magenta) in a human islet that expresses SSTR5 (green). (B) Confocal microscopy image of primary cilia (acetylated tubulin; magenta) in a mouse islet that expresses SSTR5 (green). (C) Confocal microscopy image of a primary cilium (acetylated tubulin; magenta) in a MIN6 pseudoislet that lack expression of SSTR5 (green). (D) An illustration shows the membrane localization of SSTRs, with IC3 loop indicated by the purple box. Sequences of mSSTR2, mSSTR3 and mSSTR5. Conserved motif RxRxxR is highlighted. (E) Sequences of SSTR5 and SSTR3 showing the evolutionary conservation of a stretch of amino acids in purple. Source data are available online for this figure.

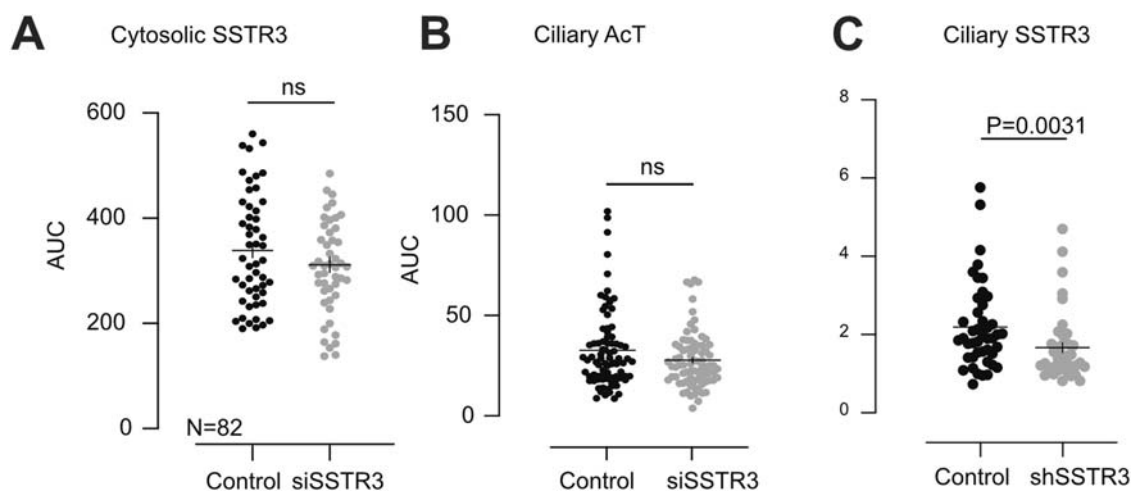


Figure EV3. SSTR3 is a ciliary somatostatin receptor.

(A) Quantification of cytosolic area selected in MIN6 pseudoislets. Fluorescence intensity is from control (black) and SSTR3 KD (gray) cells immunostained for SSTR3. (means $\pm$ SEM; $n_{\text{ctrl}} = 50$ and $n_{\text{KD}} = 48$, 3 different preparations, no statistical difference by Mann-Whitney *U* test, unpaired.). (B) Quantifications of line profiles drawn along cilia of MIN6 pseudoislets for acetylated tubulin. Acetylated tubulin signal is unaffected on the left. (means $\pm$ SEM; $n_{\text{ctrl}} = 83$ and $n_{\text{KD}} = 86$, three different preparations, no statistical difference by Mann-Whitney *U* test, unpaired.). (C) Quantifications of line profiles drawn along cilia of MIN6 pseudoislets positive for SSTR3. Ciliary SSTR3 signal is significantly reduced in shSSTR3 expressing cells (means $\pm$ SEM; $n_{\text{ctrl}} = 44$ and $n_{\text{KD}} = 40$ cilia, 1 preparation; Mann-Whitney *U* test, unpaired). Source data are available online for this figure.

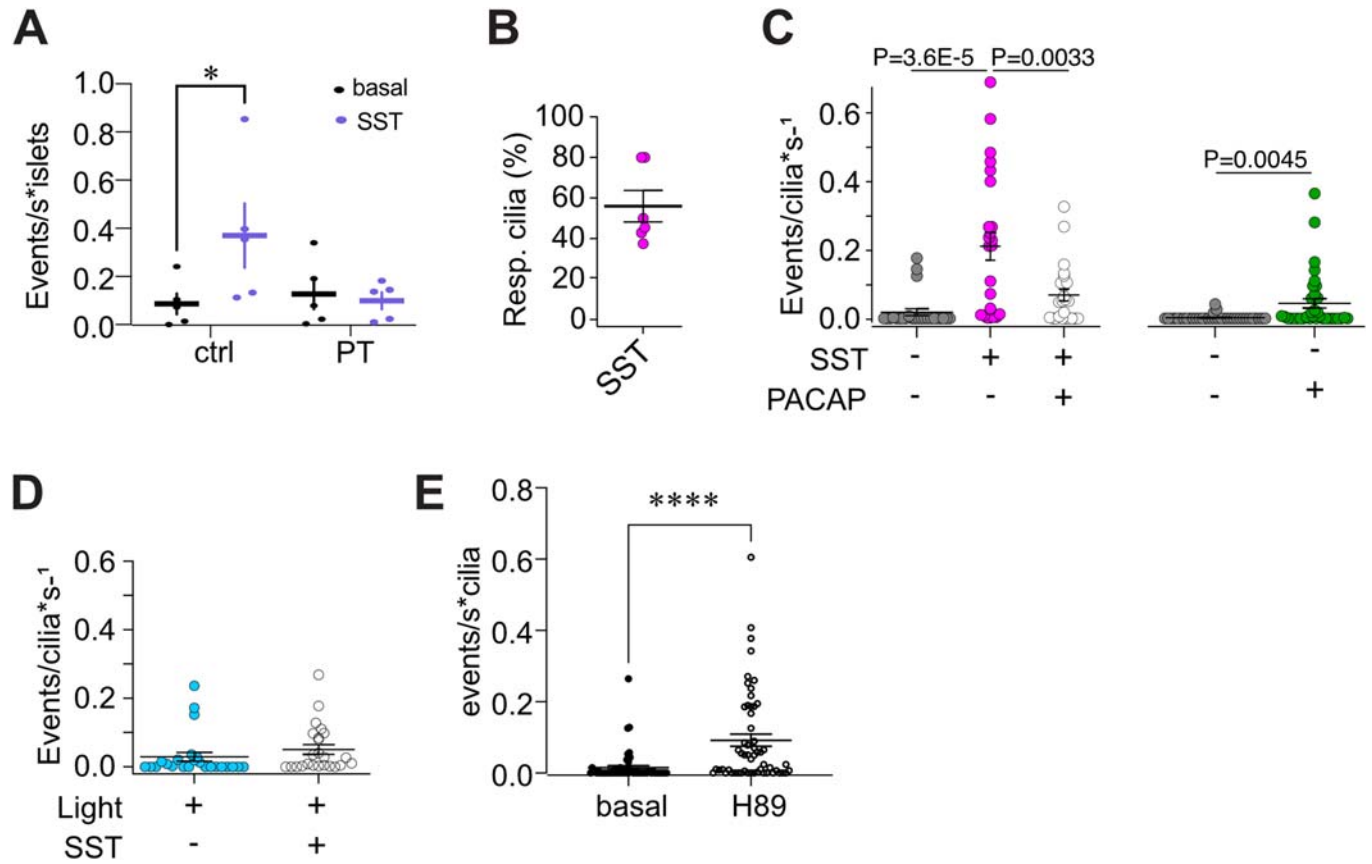


Figure EV4. Elevation of cAMP counteracts somatostatin-induced ciliary Ca^{2+} signaling.

(A) MIN6 pseudoislets were cultured under control condition (black) or in the presence of pertussis toxin (PT; purple) for 18 h. Quantifications of the Ca^{2+} responses to 100 nM somatostatin are shown to the right (means $\pm$ SEM; $n_{\text{ctrl}} = 5$ islets, $n_{\text{PT}} = 5$ islets; from three different preparations, SST response in control $P = 0.0425$, SST response in PT not significant, assessed by Sidak's multiple comparison). (B) Fraction of MIN6 cell cilia per pseudoislet that exhibit somatostatin-induced ciliary Ca^{2+} signaling (means $\pm$ SEM; $n = 6$ islets). (C) Ciliary Ca^{2+} responses under basal conditions (gray) or in the presence of 100 nM somatostatin (magenta), a combination of somatostatin and 100 nM PACAP (white) or 100 nM PACAP alone (green) in MIN6 pseudoislets (means $\pm$ SEM; left panel: $n = 25$ cilia from 6 islets; right panel: $n = 36$ cilia from 4 islets; Student's paired t test). (D) Ciliary Ca^{2+} responses to 100 nM somatostatin in MIN6 pseudoislets co-expressing cytosolic bPac. bPac activity was continuously stimulated during the experiment by 491-nm illumination (Light). (mean $\pm$ SEM; $n = 24$ cilia from 3 islets; Student's paired t test). (E) Event count of ciliary Ca^{2+} changes from mouse islets in response to 10 μM H89 (means $\pm$ SEM; 7 islets; 56 cilia; 3 different preparations. **** $P < 0.0001$, Wilcoxon-matched pair t test). Source data are available online for this figure.

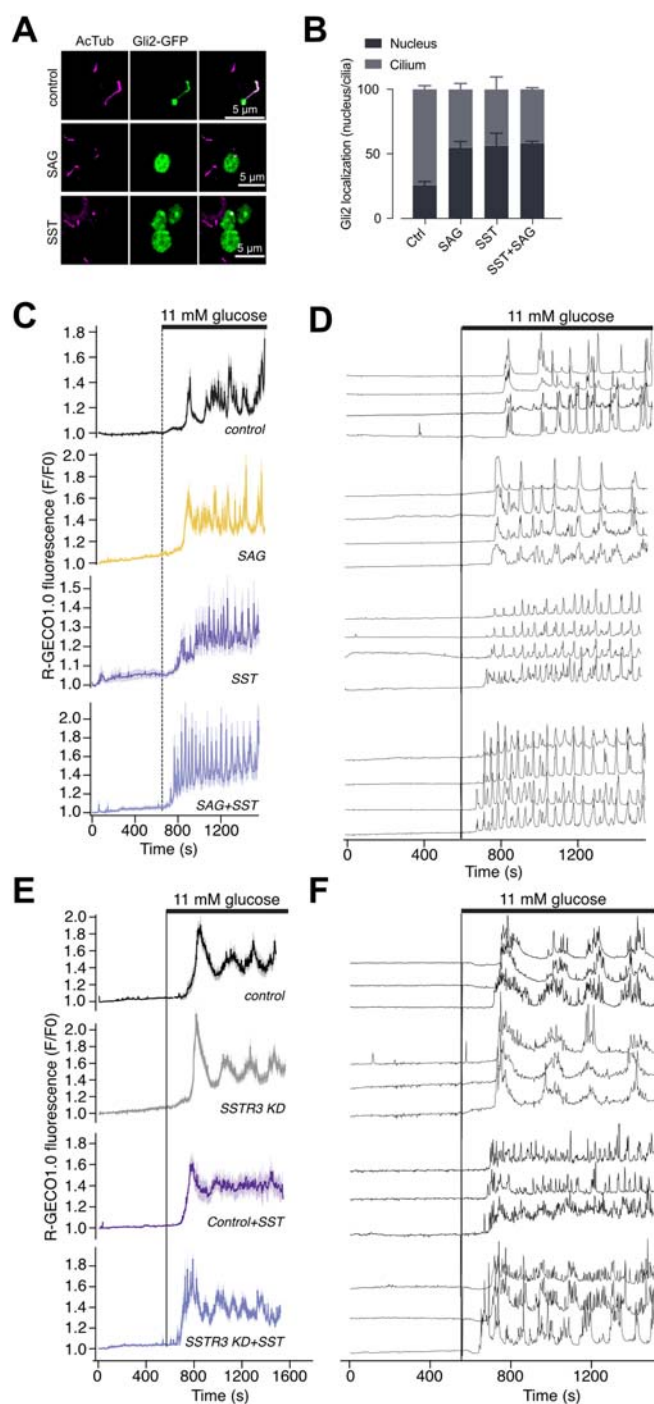


Figure EV5. Long-term somatostatin stimulation alters beta cell Ca^{2+} response to glucose.

(A) Confocal microscopy images of MIN6 cells expressing Gli2-GFP (green) and immunostained against acetylated tubulin (magenta). 18 h treatment with 100 nM SAG and 100 nM SST induced translocation of Gli2 to the nucleus. (B) Means $\pm$ SEM for the nucleus/cilia Gli2 ratio change in MIN6 cells ($P_{\text{SAG}} = 0.0345$, $P_{\text{SST}} = 0.0266$, $P_{\text{SAG+SST}} = 0.0186$ all compared to control and assessed by Sidak's multiple comparison test). (C, D) Islet averages (C) and example recordings (D) of glucose-induced R-GECO1 fluorescence changes in MIN6 pseudoislets treated for 18 h with DMSO (control), 100 nM SAG, 100 nM somatostatin or 100 nM SAG in combination with 100 nM somatostatin ($n = 3$ replicates). (E, F) Islet averages (E) and example recordings (F) of glucose-induced R-GECO1 fluorescence changes in control and SSTR3 knockdown MIN6 pseudoislets treated for 18 h with DMSO (control) or 100 nM somatostatin ($n = 3$ replicates). Source data are available online for this figure.