# Supplemental material

Alshafie et al., https://doi.org/10.1083/jcb.201904054

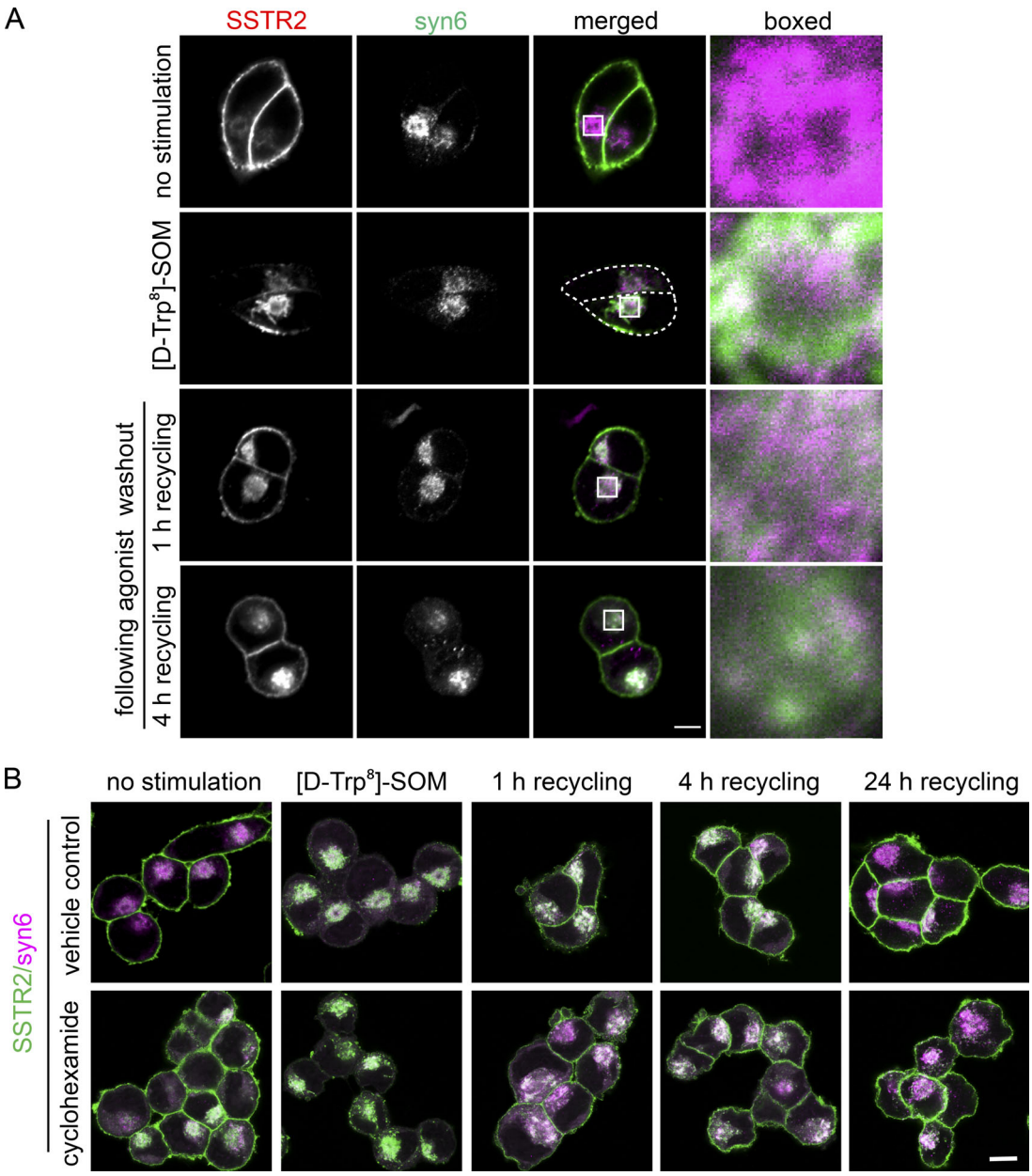



Figure S1. **SSTR2 displays slow recycling dynamics in the presence of Brefeldin A and cycloheximide. (A)** AtT20 cells were incubated for 40 min without (no stimulation) or with 100 nM [D-Trp$^8$]-SOM in the presence of 10 µM Brefeldin A and then fixed and processed for immunofluorescence with antibodies recognizing SSTR2 or syntaxin-6 (top two rows of panels). In parallel, cells treated with 100 nM [D-Trp$^8$]-SOM for 40 min in the presence of Brefeldin A were subsequently transferred to 4°C, surface-bound agonist was stripped by brief acid wash, and the cells were returned to 37°C for 1 or 4 h before being processed for immunofluorescence with antibodies recognizing SSTR2 or syntaxin-6 (bottom two rows of panels). The boxes in the merged images are shown at higher magnification on the far right. Scale bars represent 5 µm and 1 µm for the low- and high-magnification images, respectively. Dashed line indicates cell boundaries. **(B)** AtT20 cells were incubated with DMSO or 100 µg/ml cycloheximide 2 h before the start of experiment. Th cells were then incubated for 40 min without (no stimulation) or with 100 nM [D-Trp$^8$]-SOM in the presence of vehicle control or cycloheximide (left 4 most panels). In parallel, cells treated with [D-Trp$^8$]-SOM were subsequently transferred to 4°C, surface-bound agonist was stripped by a brief acid wash, and the cells were returned to 37°C for 1, 4, and 24 h (right six most panels). In all cases, at the end of the incubations cells were processed for immunofluorescence with antibodies recognizing SSTR2 or syntaxin-6. Scale bar represents 5 µm.

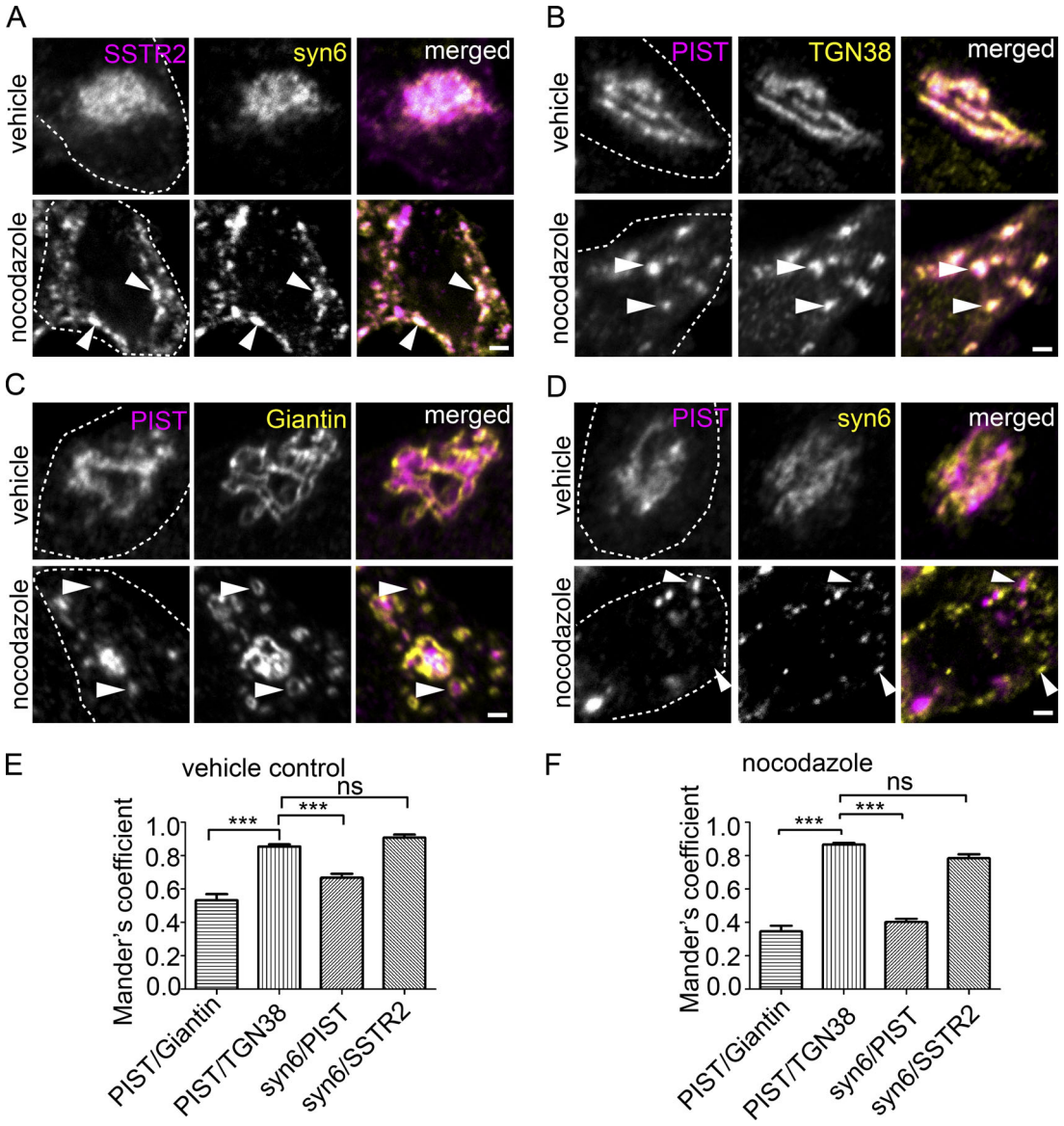



Figure S2. **SSTR2 and syntaxin-6 remain colocalized under nocodazole treatment. (A)** AtT20 cells were treated with vehicle or 20 µM nocodazole for 2 h in DMEM and for the last 40 min of the nocodazole treatment with 100 nM [D-Trp$^{8}$]-SOM before being fixed and processed for immunofluorescence with antibodies against the indicated proteins. **(B–D)** AtT20 cells were treated with vehicle or 20 µM nocodazole for 2 h in DMEM before being fixed and processed for immunofluorescence with antibodies against the indicated proteins. The arrowheads in A and B point to colocalizing structures, while the arrowheads in C and D point to noncolocalizing structures. Scale bars represent 1 µm. Dashed lines indicate cell boundaries. **(E and F)** Mander's correlation coefficient measurements of at least 30–40 cells from two or three independent experiments for cells treated with vehicle (E) or nocodazole (F). Data were analyzed by one-factor ANOVA and Tukey comparison test (procedures implemented in the GraphPad Prism 5 statistical package). Data are presented as least-square means ± SEMs with treatment effects significant at ***, $P < 0.001$.

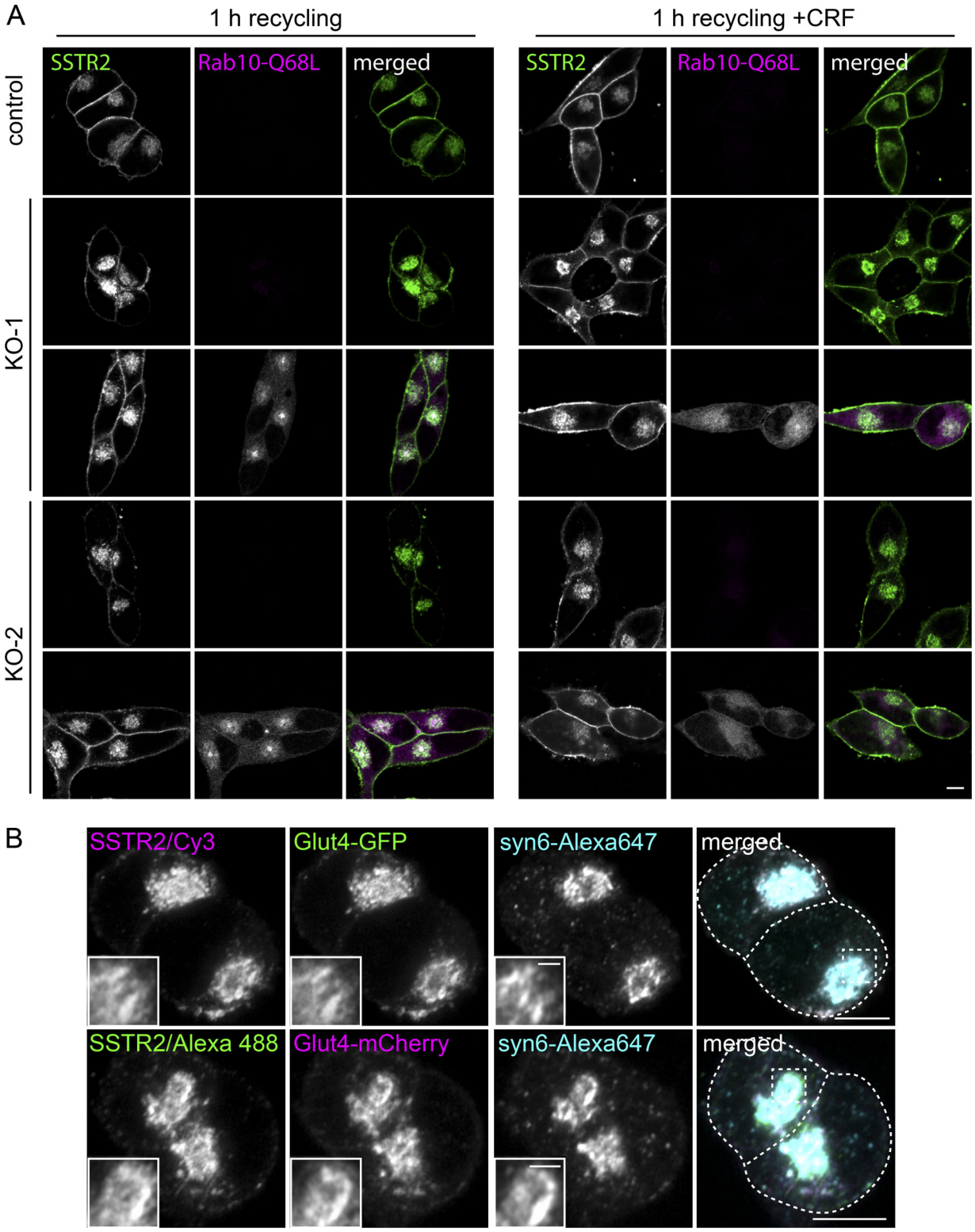



Figure S3. **SSTR2 is in a vesicle that requires Rab10 for recycling and is positive for transfected GLUT4. (A)** Control AtT20 cells or the two Rab10 knockout lines (KO-1 and KO-2) were electroporated with 5 µg EGFP-Rab10Q68L. At 72 h after transfection, cells were treated for 60 min with [D-Trp$^8$]-SOM to induce maximal SSTR2 internalization followed by brief acid wash and incubation in ligand-free media for 1 h at 37°C without or with 100 nM CRF. After the treatments, cells were fixed and processed for immunofluorescence with antibody recognizing SSTR2. Scale bar represents 5 µm. **(B)** GLUT4 is sorted to GLSVs in AtT20 cells. AtT20 cells were electroporated with 5 µg GLUT4-GFP or GLUT4-mCherry. 24 h after transfection, cells were treated for 40 min with [D-Trp$^8$]-SOM and then fixed and processed for immunofluorescence with antibody recognizing SSTR2 and syntaxin-6. Scale bars represent 5 µm and 1 µm for the low- and high-magnification images, respectively.

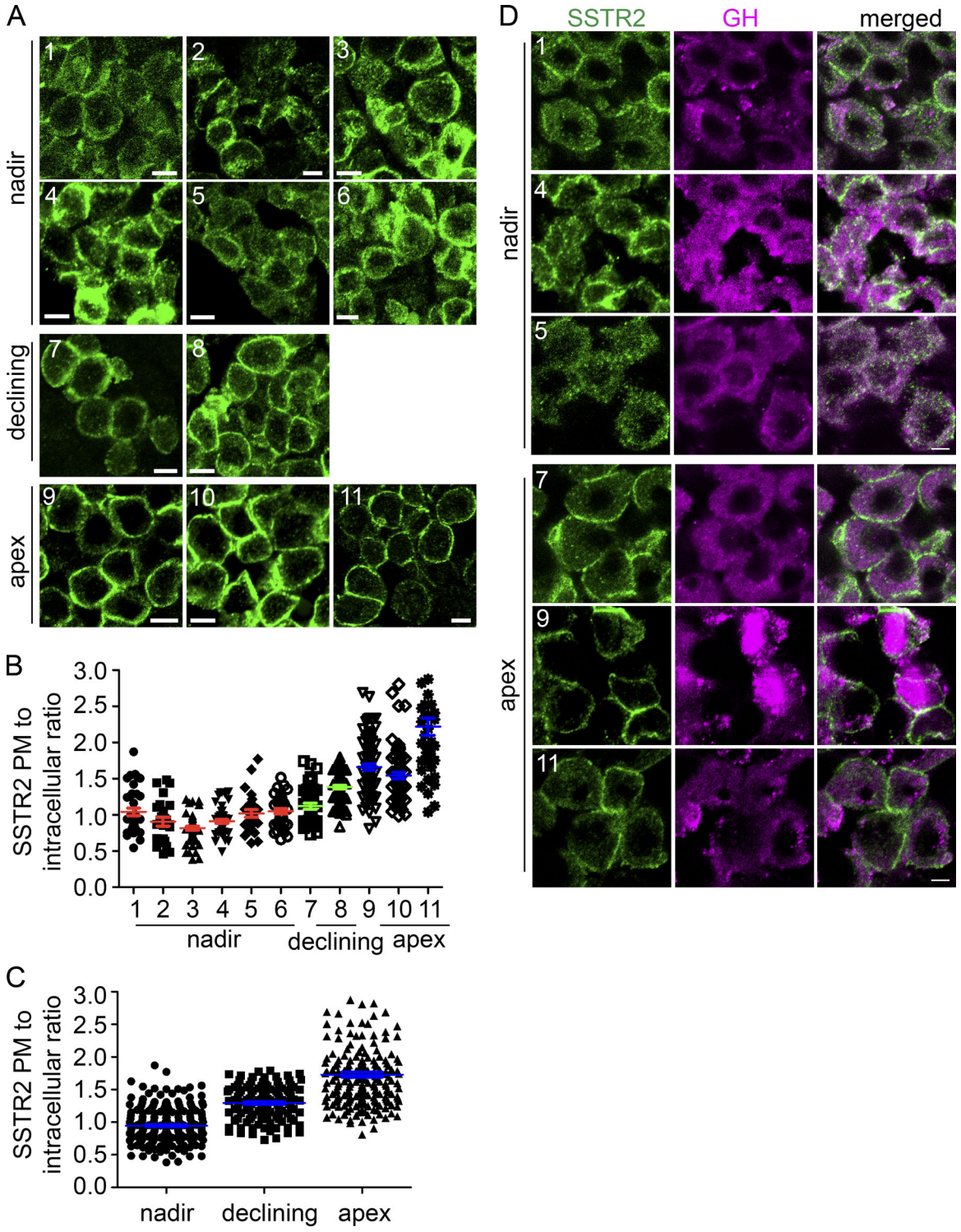



Figure S4. **Analysis of the SSTR2 surface to intracellular ratio in mouse pituitary. (A)** SSTR2 staining in sections of pituitary with blood GH levels at the nadir, declining or at the apex as indicated. The mouse number is stated on each panel and corresponds to the GH profiles shown in Fig. 10 C. Scale bars represent 5 µm. **(B and C)** The cell surface to intracellular ratio of SSTR2 immunofluorescent signal from individual cells from individual animals (B) or grouped depending on the GH status (C). PM, plasma membrane. **(D)** Sections of pituitary glands from selected mice as indicated were immunostained with antibodies recognizing SSTR2 and GH. The mice were selected from the animals that were sacrificed at the apex or nadir of blood GH levels. Scale bars represent 5 µm.

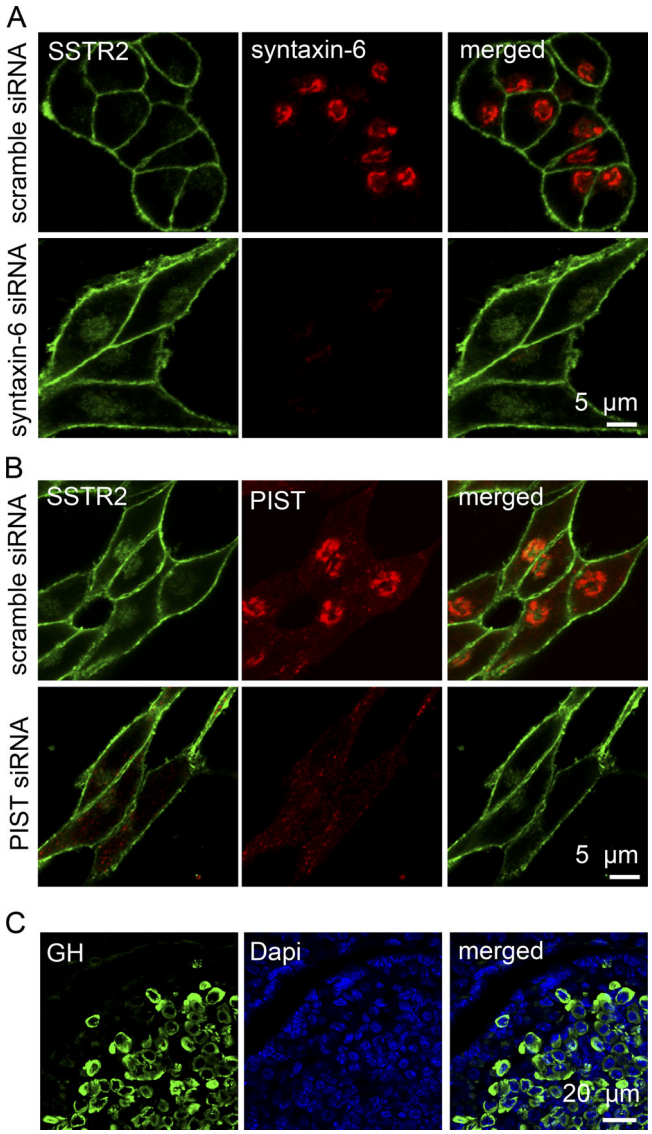
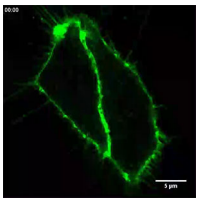
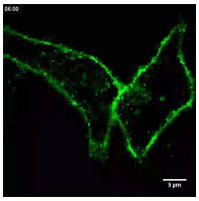


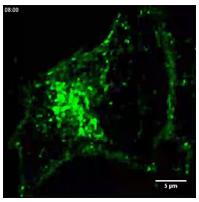

Figure S5. **Antibody validation.** Validation of syntaxin-6 (A) and PIST (B) antibodies was done using predesigned siRNA directed against mouse syntaxin-6 (FlexiTube siRNA, NM-021433, #SI02674231, and #S102717729; Qiagen) or PIST (FlexiTube siRNA, NM-001199272, #SI01054900; Qiagen). AtT20 cells were transfected with 50 nM of siRNA 48 h before immunostaining for the indicating proteins. Note the disappearance of the immunofluorescent signal in the siRNA-transfected cells, but not in the cells transfected with a scrambled siRNA. **(C)** GH antibody was tested on a pituitary section showing selective staining of a subset of pituitary cells.



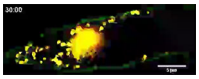
Video 1. **HeLa cells transfected with EGFP-SSTR2 were imaged for 5 min at the basal levels.** Frames were obtained using a Zeiss LSM 880 microscope with an airyscan at 10-s intervals.



Video 2. **HeLa cells transfected with EGFP-SSTR2 were imaged 6 min after applying 100 nM of [D-Trp$^8$]-SOM for 42 min at 10-s intervals using a Zeiss LSM 880 microscope with an airyscan.**

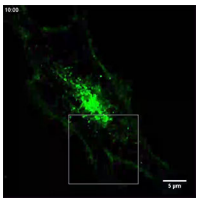


Video 3. **HeLa cells transfected with EGFP-SSTR2 were stimulated for 60 min with 100 nM of [D-Trp$^8$]-SOM.** Cells were imaged 8 min following ligand washout for 22 min at 10-s intervals using a Zeiss LSM 880 microscope with an airyscan.

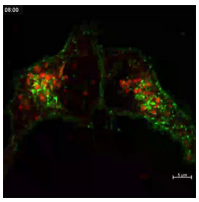


Video 4. **HeLa cells were transfected with EGFP-SSTR2 (green) and Ds-Red-syntaxin-6 (red).** Cells were stimulated for 60 min with 100 nM of [D-Trp$^8$]-SOM. Cells were imaged 30 min following ligand washout for 27 min using a Zeiss LSM 880 microscope with airyscan at 20-s intervals.

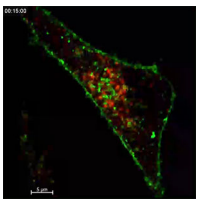
Video 5. **Cropped area from Video 4 showing SSTR2 fusion at the plasma membrane.** Upon reaching the plasma membrane, the syntaxin-6–positive vesicles of SSTR2 can be seen to come in contact with the surface followed by the insertion of SSTR2 into the plasma membrane, while syntaxin-6 remains on the cytoplasmic side of the plasma membrane. Cells were imaged as in Video 4.



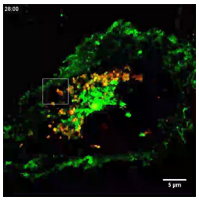
Video 6. **HeLa cells transfected with EGFP-SSTR2 were stimulated for 60 min with 100 nM of [D-Trp$^8$]-SOM.** Cells were imaged 10 min following ligand washout for 15 min at 20-s intervals using a Zeiss LSM 880 microscope with an airyscan. Note the recycling of SSTR2 on tubular carriers.



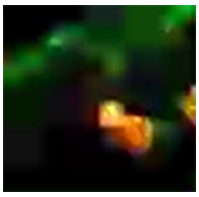
Video 7. **HeLa cells transfected with EGFP-SSTR2 and mCherry-Rab8 were stimulated for 60 min with 100 nM of [D-Trp$^8$]-SOM.** Cells were imaged 8 min following ligand washout for 28 min at 20-s intervals using a Zeiss LSM 880 microscope with an airyscan. Note the absence of colocalization of SSTR2 with Rab8.



Video 8. **HeLa cells transfected with EGFP-SSTR2 and mCherry-Rab13 were stimulated for 60 min with 100 nM of [D-Trp$^8$]-SOM.** Cells were imaged 12 min following ligand removal for 15 min at 10-s intervals using a Zeiss LSM 880 microscope with airyscan. Note the absence of colocalization of SSTR2 with Rab13.



Video 9. **HeLa cells transfected with EGFP-SSTR2 and mCherry-Rab10 were stimulated for 60 min with 100 nM of [D-Trp$^{8}$]-SOM.** Cells were imaged 28 min following ligand removal at 20-s intervals using a Zeiss LSM 880 microscope with airyscan. Note colocalization of SSTR2 with Rab10.



Video 10. **Cropped area from Video 9 showing SSTR2/Rab10 cotrafficking and fusion of SSTR2 at the plasma membrane.** Cells were imaged as in Video 9.