A

+vehicle   +sst

SSTR3 (Ab)
DNA ACIII

B

Ciliary SSTR3 (antibody, RFU)

IMCD3 +vehicle | +vehicle | 2h sst | 4h sst | 6h sst | +ACQ090 | +L796,778

Hippocampal neurons (top 20%)

C

Ciliary SSTR3 (% of t_0)

L796,778   ACQ090

D

IMCD3-[pEF1α$^\Delta$-^{AP}SSTR3^{NG}]

+vehicle   +sst

SSTR3 (Ab) DNA AcTub
SSTR3 (NG)

E

pEF1α$^\Delta$-driven expression

Ciliary GPCR (RFU)

^{AP}SSTR3^{NG} NPY2R^{NG} MCHR1^{NG}

F

pEF1α$^\Delta$-SSTR3^{NG}

Frequency (counts)

3129

Number of molecules in cilia

G

pCrys-GPR161^{NG3}

Number of cilia quantified

2175

Number of molecules in cilia

H

Signal-to-noise ratio

neuron | Ab
IMCD3-[^{AP}SSTR3^{NG}] | Ab | NG | SA647

I

Ciliary SSTR3 (antibody, RFU)

pEF1α$^\Delta$ pCrys parent

J

Ciliary SSTR3 (NG RFU, % of t_0)

NG+vehicle
mSA+vehicle
NG+sst
mSA+sst

Time (h)

Figure S1. Low-expression promoters recapitulate the physiological exit kinetics of SSTR3

(**A**) Immunofluorescence of rat hippocampal neurons after 6 h treatment with 10 μM sst or vehicle. Cells were stained with antibodies against adenylate cyclase 3 (ACIII) and SSTR3. Channels within insets are shifted. Scale bars: 4 μm. (**B**) Immunofluorescence measurements of SSTR3 levels in individual cilia from rat hippocampal neurons or IMCD3-[pEF1α$^{\Delta}$-^{AP}SSTR3^{NG}] cells. In neuronal cultures, only 20% of cells appeared to express SSTR3 as judged by immunostaining for SSTR3. Therefore, only the 20% brightest cilia in the SSTR3 channel were plotted for each subsequent time point. In IMCD3-[pEF1α$^{\Delta}$-^{AP}SSTR3^{NG}] cells, all cilia were positive for SSTR3 by immunostaining. A horizontal line marks the average for each condition. Red dots correspond to the cells shown in Fig. **S1A**. N = 52-424 cilia. (**C**) The amount of SSTR3 remaining in cilia following 6h treatment with the SSTR3-specific agonist L796,778 or SSTR3-specific antagonist ACQ090 was quantified as in Fig. **S1B**. Error bars: 95% CI. N = 280-424 cilia. (**D**) IMCD3-[pEF1α$^{\Delta}$-^{AP}SSTR3^{NG}] were treated for 6 h with either sst or vehicle. Cells were stained with antibodies against acetylated tubulin (AcTub) and SSTR3 (Ab). In addition, ^{AP}SSTR3^{NG} was visualized by direct fluorescence of NeonGreen (NG). Cells were pre-treated with the translation inhibitor emetine to eliminate signals from new protein synthesis. Scale bars: 4 μm. Channels are shifted. (**E**) pEF1α$^{\Delta}$-driven expression produces low levels of ciliary GPCRs. ^{AP}SSTR3^{NG}, NPY2R^{NG}, and MCHR1^{NG} were stably expressed in IMCD3 Flp-In cells. The box plots show the NeonGreen intensity measured by live-cell TIRF imaging. The numbers of NPY2R^{NG} and MCHR1^{NG} molecules per cilia are calculated to be 295 and 43 molecules respectively. N = 15-20 cilia. (**F**) Distribution of the absolute number of ^{AP}SSTR3^{NG} per cilia. IMCD3-[pEF1α$^{\Delta}$-^{AP}SSTR3^{NG}] cells were serum starved for 18 h, the ciliary fluorescence intensities measured and background subtracted and molecules counted as detailed in Methods. N = 57 cilia. (**G**) Distribution of the absolute number of ^{AP}GPR161^{NG3} per cilia. IMCD3-[pCrys-^{AP}GPR161^{NG3}] cells were serum starved for 18 h, the ciliary fluorescence intensities measured and background subtracted and molecules counted as detailed in Methods. N = 53 cilia. (**H**) Limited sensitivity of SSTR3 immunostaining. Bars represent measurements of signal to noise ratio of SSTR3 ciliary levels by different modalities. Signal to noise ratio is defined as [average(signal) - average(background)]/standard deviation(background). "Ab" indicates antibody (Ab)-based immunofluorescence measurements in fixed cells quantified as in Fig. **S1B**. NeonGreen and mSA647 measurements were conducted by live cell imaging of IMCD3-[pEF1α$^{\Delta}$-^{AP}SSTR3^{NG}] as in Fig. **1F**. Error bars represent the propagated error. (**I**) Immunostaining fails to detect low concentrations of SSTR3. IMCD3[pEF1α$^{\Delta}$-^{AP}SSTR3^{GFP}], IMCD3[pCrys-^{AP}SSTR3^{GFP}], and parental IMCD3 cells (which do not express SSTR3 endogenously) were immunostained for SSTR3 and ciliary levels of SSTR3 measured, N = 36-45 cilia. (**J**) Kinetics of ^{AP}SSTR3^{NG} removal as assessed by NeonGreen fluorescence. IMCD3-[pEF1α$^{\Delta}$-^{AP}SSTR3^{NG}] cells were pulse-labeled with mSA647 and imaged every hour in the NG and mSA647 channels following addition of vehicle or sst. Imaging NeonGreen resulted in a slower apparent removal rate than imaging the same cilia by a mSA647 pulse as the ciliary NG signal is influenced by both import of newly synthesized ^{AP}SSTR3^{NG} and export of ^{AP}SSTR3^{NG} out of cilia; meanwhile mSA647 pulse-labeled ^{AP}SSTR3^{NG} only reports the ciliary export. Error bars represent SEM. N = 10-13 cilia.
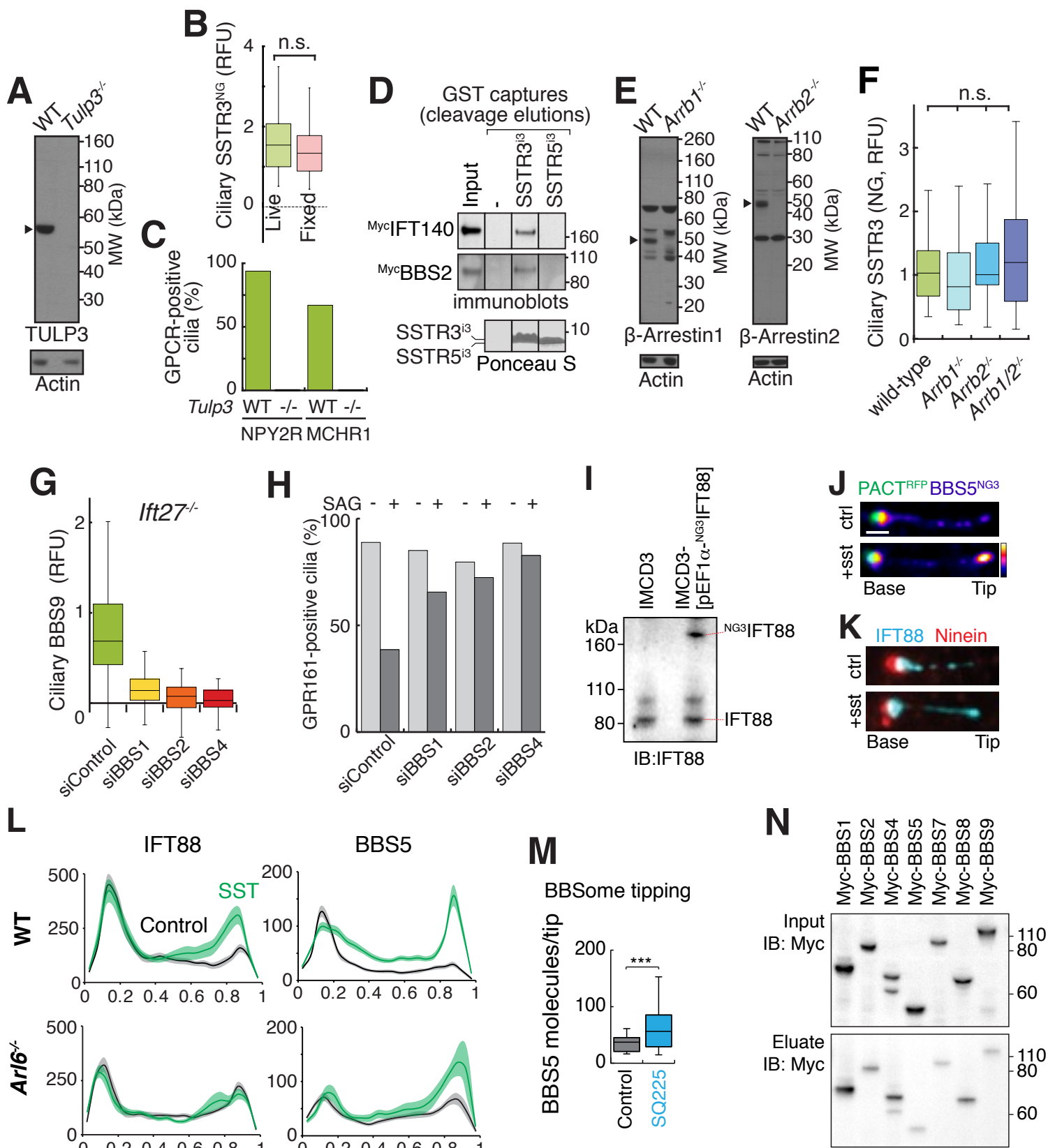
A

WT | *Tulp3⁻/⁻*

160
110
80
60 ◄
50
40
30

MW (kDa)

TULP3

Actin

B

Ciliary SSTR3^NG (RFU)

n.s.

Live | Fixed

C

GPCR-positive cilia (%)

100
50
0

Tulp3 WT -/- WT -/-

NPY2R | MCHR1

D

GST captures (cleavage elutions)

Input | - | SSTR3^i3 | SSTR5^i3

^Myc IFT140 — 160
^Myc BBS2 — 110 / 80

immunoblots

SSTR3^i3 / SSTR5^i3 — 10

Ponceau S

E

WT | *Arrb1⁻/⁻*   WT | *Arrb2⁻/⁻*

260   110
160   80
110
80   60
60 ◄   50 ◄
50   40
40
30   30
20   20

MW (kDa)   MW (kDa)

β-Arrestin1   β-Arrestin2

Actin   Actin

F

Ciliary SSTR3 (NG, RFU)

n.s.

3
2
1
0

wild-type | *Arrb1⁻/⁻* | *Arrb2⁻/⁻* | *Arrb1/2⁻/⁻*

G

Ift27⁻/⁻

Ciliary BBS9 (RFU)

2
1
0

siControl | siBBS1 | siBBS2 | siBBS4

H

GPR161-positive cilia (%)

SAG - + - + - + - +

100
50
0

siControl | siBBS1 | siBBS2 | siBBS4

I

IMCD3 | IMCD3-[pEF1α-^NG3 IFT88]

kDa

160 ..... ^NG3 IFT88
110
80 ..... IFT88

IB:IFT88

J

PACT^RFP BBS5^NG3

ctrl

+sst

Base | Tip

K

IFT88 Ninein

ctrl

+sst

Base | Tip

L

IFT88 | BBS5

WT

SST
Control

500 / 250 / 0
0 0.2 0.4 0.6 0.8 1

200 / 100 / 0
0 0.2 0.4 0.6 0.8 1

Arl6⁻/⁻

500 / 250 / 0
0 0.2 0.4 0.6 0.8 1

200 / 100 / 0
0 0.2 0.4 0.6 0.8 1

M

BBSome tipping

BBS5 molecules/tip

200
100
0

***

Control | SQ225

N

Myc-BBS1 | Myc-BBS2 | Myc-BBS4 | Myc-BBS5 | Myc-BBS7 | Myc-BBS8 | Myc-BBS9

Input IB: Myc

110
80
60

Eluate IB: Myc

110
80
60

Figure S2. Molecular requirement for ciliary entry and exit

(**A**) Immunoblots of IMCD3 clones knocked out for *Tulp3* by CRISPR/Cas9. Actin was used as a loading control. The immunoblotted antigen is shown below the blot. (**B**) Box plots of ciliary NeonGreen fluorescence intensities for IMCD3-[pEF1α$^\Delta$-^{AP}SSTR3^{NG}] cells imaged live or after fixation. No significant difference was detected by a t test. N = 27-59 cilia. (**C**) Ciliary localization of NPY2R^{NG} and MCHR1^{NG} requires Tulp3. The number of NeonGreen-positive cilia was counted for wild-type and *Tulp3$^{-/-}$* cells by TIRF imaging of fixed cells. Cilia were identified by immunostaining for acetylated tubulin N = 63-91 cilia. (**D**) Capture of IFT-A and BBSome subunits by recombinant GST-SSTR3^{i3}. HEK293 cells were transfected with either ^{Myc}IFT140 or ^{Myc}BBS2. GST-SSTR3^{i3} and GST-SSTR5^{i3} were expressed and purified from *E.coli*. HEK293 extracts were applied to GST-SSTR3^{i3}- and GST-SSTR5^{i3}-coated beads, captured material was eluted by cleavage with Precission protease and the resulting eluates were analyzed by Ponceau S and immunoblotting for Myc-tagged subunits. Molecular weights (kDa) are indicated on the right.10 input equivalents were loaded in the eluate lanes. (**E**) Immunoblots of IMCD3 clones knocked out for β-Arrestin 1 (*Arrb1*) or β-Arrestin 2 (*Arrb2*) by CRISPR/Cas9. Actin was used as a loading control. The immunoblotted antigen is shown below the blot. (**F**) β-Arrestin 1 and 2 are not required for the accumulation of SSTR3 in cilia. Ciliary ^{AP}SSTR3^{NG} intensities were measured by NG fluorescence (Relative Fluorescent Unit, RFU) for various IMCD3 lines as in Fig. **2A**. All cells were imaged live as cilia were readily identified in the NG channel. n.s indicate ANOVA significance value P > 0.05. N = 31-59 cilia. (**G**) siRNA knockdown of BBSome subunits was assessed by measuring ciliary BBSome intensities in *Ift27$^{-/-}$* IMCD3 cells. Ift27 deletion greatly increases the ciliary abundance of endogenous BBSome (Eguether et al., 2014; Liew et al., 2014) thus facilitating detection of the ciliary BBSome by immunostaining. Cells were treated with either Control siRNA or siRNA against BBS1, BBS2, or BBS4 for 48 h then cells were serum starved for 24 h, fixed, and stained for endogenous BBS9. N = 50-67 cilia. (**H**) The BBSome is necessary for retrieval of GPR161 following Hedgehog pathway activation. BBSome subunits were depleted by siRNA as in panel **G**. Cells were then treated with blebbistatin to block ectocytosis-mediated exit (Nager et al., 2017) and with either SAG or vehicle for 2 h, fixed, and immunostained for GPR161. N = 29-96 cilia. (**I**) Relative expression levels of ^{NG3}IFT88. Lysates of IMCD3 and IMCD3-[pEF1α-^{NG3}IFT88] cells were blotted for IFT88. Molecular weights (kDa) are indicated on the right. Asterisks indicate the band intensities measured by Image Lab (Bio-Rad). Measurement of band intensities with Image Lab (Bio-Rad) indicates that the molar ratios between ^{NG3}IFT88 and endogenous IFT88 is 1.04. (**J**) The signal-dependent BBSome accumulation at the ciliary tip depicted in Fig. **3B** is shown with the centrosome marker PACT^{RFP} that was transiently expressed to mark the ciliary base (green). Scale bar: 2 μm. (**K**) Signal-dependent accumulation of IFT-B at the ciliary tip. IMCD3-[pEF1α$^\Delta$-^{AP}SSTR3; pEF1α-^{NG3}IFT88] were treated with sst or vehicle for 1 h. Cells were fixed and stained for ninein to mark the basal body and ^{NG3}IFT88 was imaged by direct NG fluorescence. (**L**) Arl6 is required for the signal-dependent accumulation of IFT-B but not BBSome at the tip of cilia. Line scans of ^{NG3}BBS5 or ^{NG3}IFT88 fluorescence intensities along cilia of live cells. The line marks the average intensity along length-normalized cilia. The shaded area shows the 95% confidence interval. N = 16-32 length-normalized cilia. (**M**) Quantitation of ^{NG3}BBS5 tip fluorescence. ^{NG3}BBS5 tip fluorescence was measured in live IMCD3-[pEF1α$^\Delta$-^{AP}SSTR3, pEF1α-^{NG3}BBS5] cells after the indicated drug treatments (as described in Methods). The total number of BBS5 molecules at the tip was calculated using the NG3 calibrator and the measured ratio of ^{NG3}BBS5 to total BBS5. SQ225 is an AC6 inhibitor. Asterisks indicate Mann

Whitney test significance values; n.s. $P > 0.1$, *** $P < 0.0005$. $N = 20\text{-}29$ cilia from 3 independent experiments. (**N**) Kif7 interacts with BBSome. Cell lysates of HEK293 cells co-transfected with Kif7^{GFP} and individual Myc-tagged BBSome subunits were subjected to immunoprecipitation with anti-GFP antibodies. Lysates and eluates were blotted for Myc. Molecular weights (kDa) are indicated on the right.
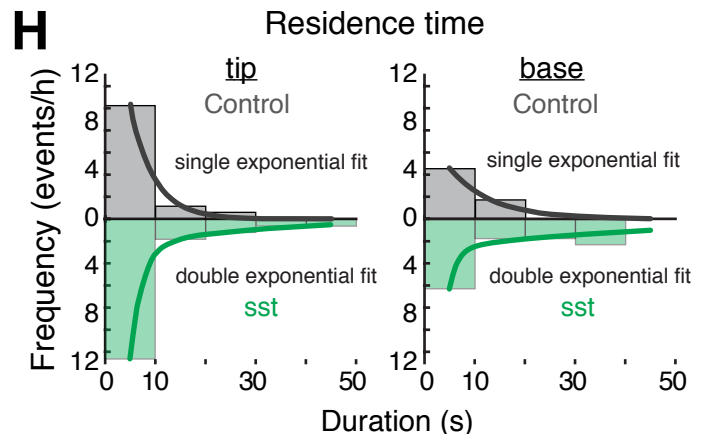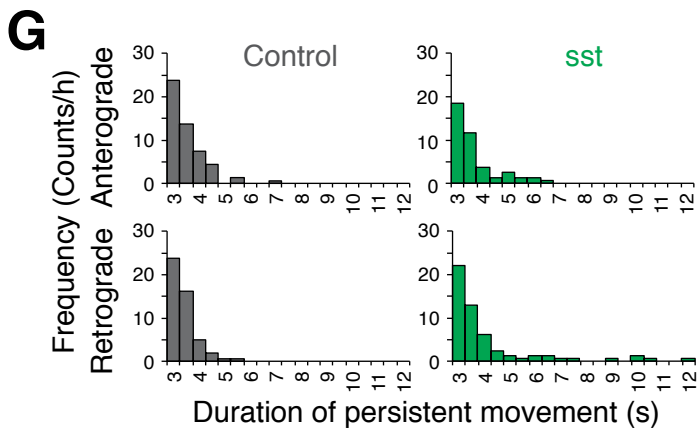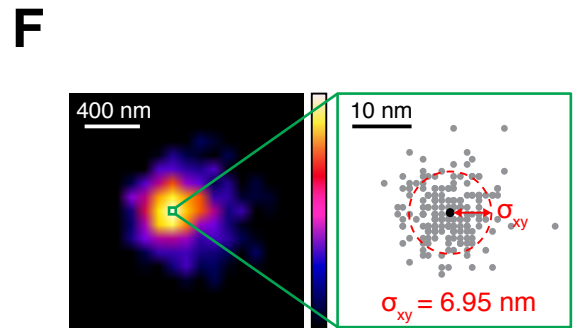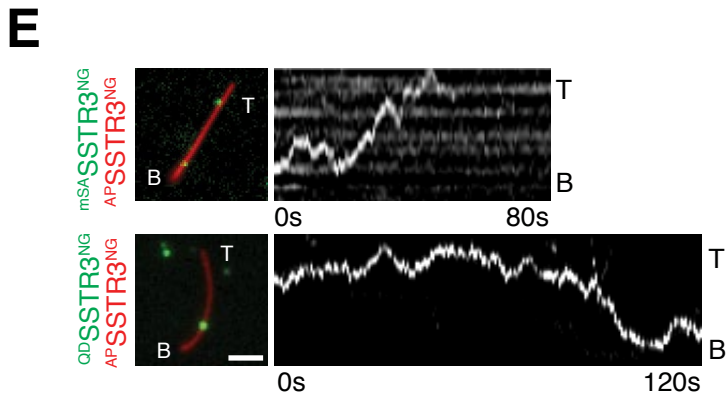
A IFT88 Train Procesivity

Anterograde / Retrograde

B BBS5 Train Procesivity

Anterograde / Retrograde

C *Ift27⁻/⁻*

BBS1-Anterograde

BBS1-Retrograde

D Arl6⁻/⁻ + sst

E

F

σ_xy = 6.95 nm

G Control / sst

Anterograde / Retrograde

Duration of persistent movement (s)

H Residence time

tip / base

single exponential fit

double exponential fit

Duration (s)
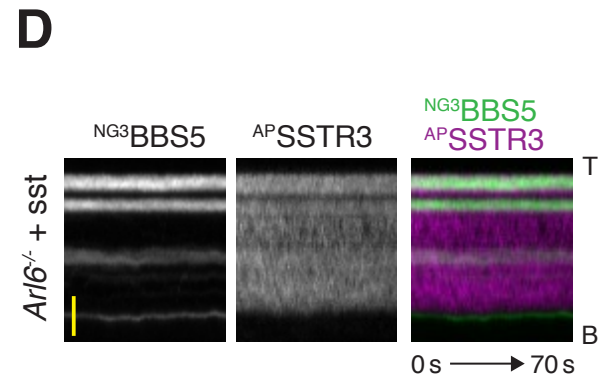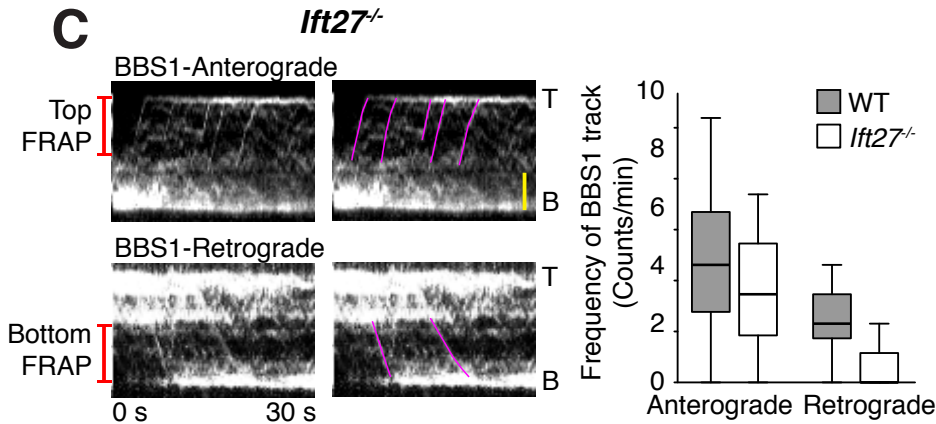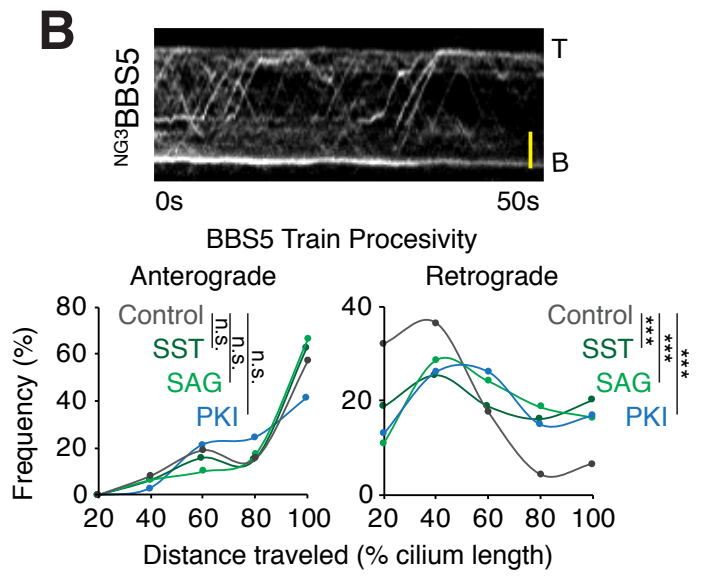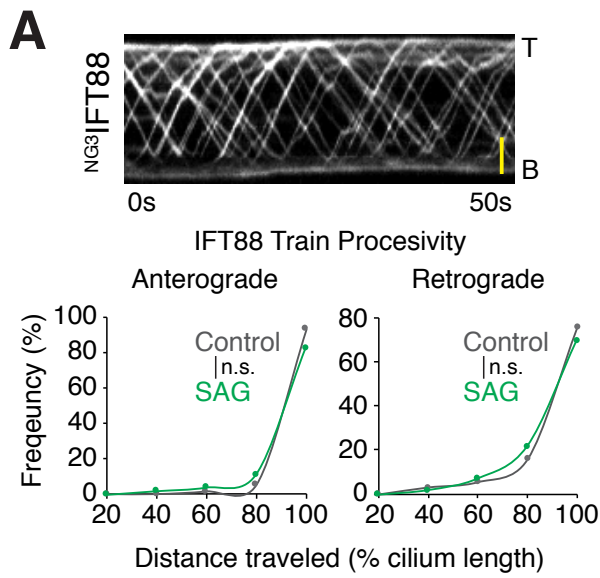
Figure S3. BBS5/IFT88 train procesivity and single molecule quantitation

(**A**) Top panel: representative kymograph from IMCD3-[pEF1α-^{NG3}IFT88] cells showing the highly processive movement of IFT-B trains. Bottom panel: distribution of the processivity of IFT-B trains. The processivity of IFT-B trains was measured in IMCD3-[pEF1α-^{NG3}IFT88] cells as described in Fig. **5A**. Significance value of Mann Whitney test applied to the entire distribution: n.s. *P > 0.05*. (**B**) Top panel: representative kymograph from IMCD3-[pEF1α-^{NG3}BBS5] cells showing the movement of BBSome trains. Bottom panel: distribution of the processivity of BBSome trains. IMCD3-[pEF1α-^{NG3}BBS5] cells were treated with vehicle, sst, SAG or PKI for 40 min before imaging. Processivity was scored as described in Fig. **5A**. Asterisks indicate the significance values of Mann Whitney tests applied to the entire distribution; *** $P < 0.0005$, n.s. $P > 0.1$. (**C**) Ift27 is required for the assembly of processive retrograde BBSome trains. Left panel: representative kymographs from *Ift27$^{-/-}$* IMCD3-[pEF1α-^{NG3}BBS1] cilia. To overcome the difficulties in visualizing BBSome trains in cilia of *Ift27$^{-/-}$* cells where the levels of BBSome are 4-fold elevated compared to WT cells (Liew et al., 2014), one half of the ciliary ^{3NG}BBS1 fluorescent signal was photobleached and the trains entering the bleached area were imaged and counted. The red lines mark areas photobleached at t=0. The anterograde or retrograde BBSome trains entering the photobleached areas are highlighted with magenta lines. Right panel: The frequency of highly processive BBSome trains in WT or *Ift27$^{-/-}$* cells was graphed. Error bars: SEM, Scale bar: 2 μm. (**D**) Arl6 is required for the assembly of cargo-laden BBSome retrograde trains. Kymographs showing BBSome and SSTR3 foci in cilia of *Arl6$^{-/-}$* cells. mSA647-labeled IMCD3-[pEF1α^{Δ}-^{AP}SSTR3, pEF1α-^{NG3}BBS5] cells were treated with sst for 1 h before imaging at 1.21 Hz. The cells stably expressed an ER-localized biotin ligase BirA to enable visualization of ^{AP}SSTR3 by mSA647 labeling. Channels are shown individually and merged. B is for base and T is for tip. In this kymograph, BBSome and SSTR3 stay in immobile foci near the tip of cilia. In some *Arl6$^{-/-}$* cilia such as the one shown in Fig. **5G**, the large accumulations of BBSome at the tip occasionally gave rise to retrograde trains. In neither kymograph are retrograde tracks of SSTR3 detected. (**E**) Representative kymographs showing the diffusive behavior of mSA647-labeled ^{AP}SSTR3^{NG} (top panel) and QDot655-labeled ^{AP}SSTR3^{NG} (bottom panel) in the absence of sst. (**F**) Localization precision of Qdot655. Left panel: a representative fluorescence image from a single Qdot655 emitter immobilized on the glass coverslip. Intensities are presented in fire scale. Right panel: repeated acquisitions of the same Qdot655 over time were used to map the centroid of fluorescence (grey dots) and to calculate the lateral localization precision σ_{xy} as described in (Deschout et al., 2014). (**G**) Durations of persistent movement events of single molecules of ^{QD}SSTR3 in anterograde and retrograde directions. (**H**) Durations of residence events for ^{QD}SSTR3 at ciliary tip or base. For visualization, data was fitted as indicated to either a single or a double exponential.
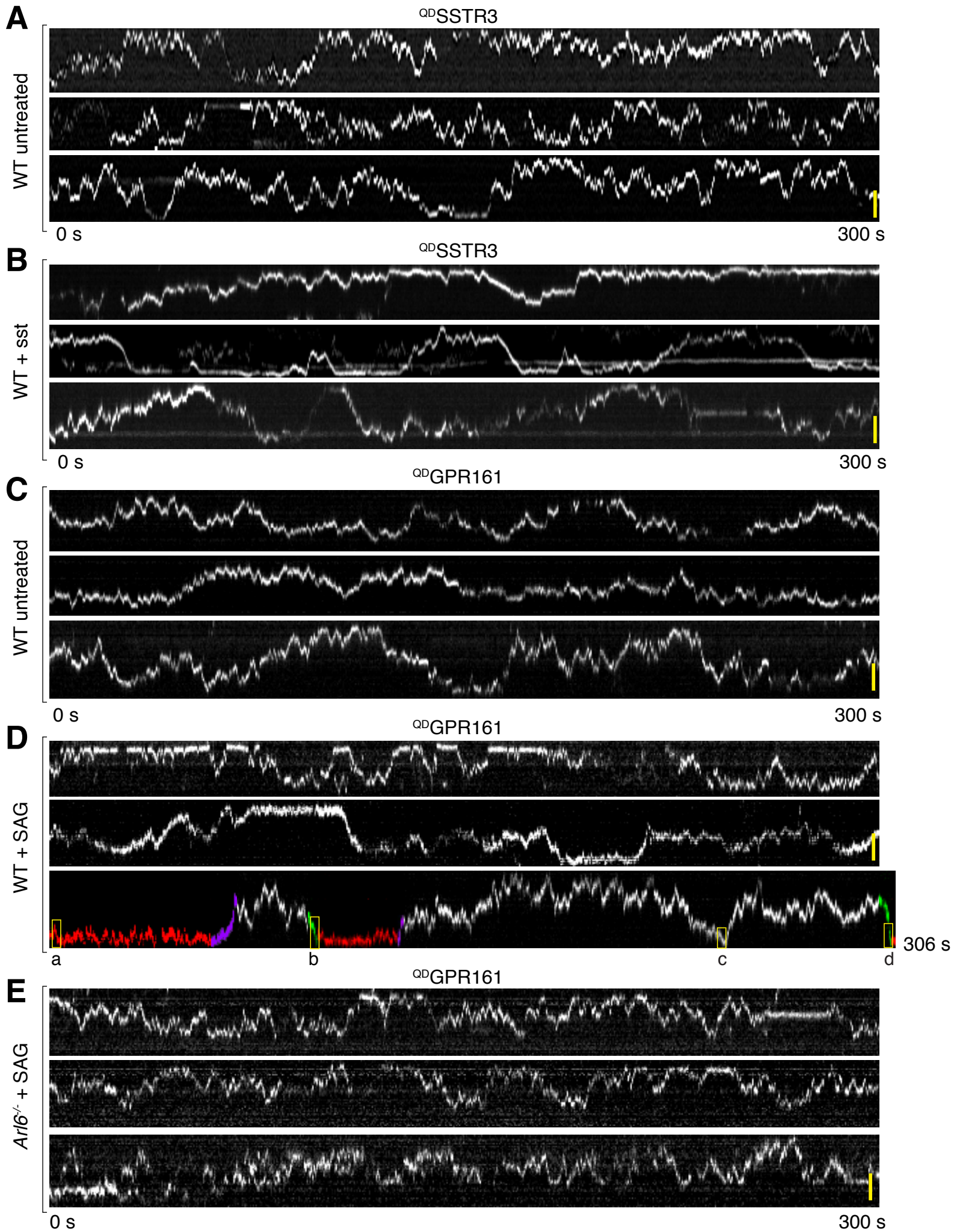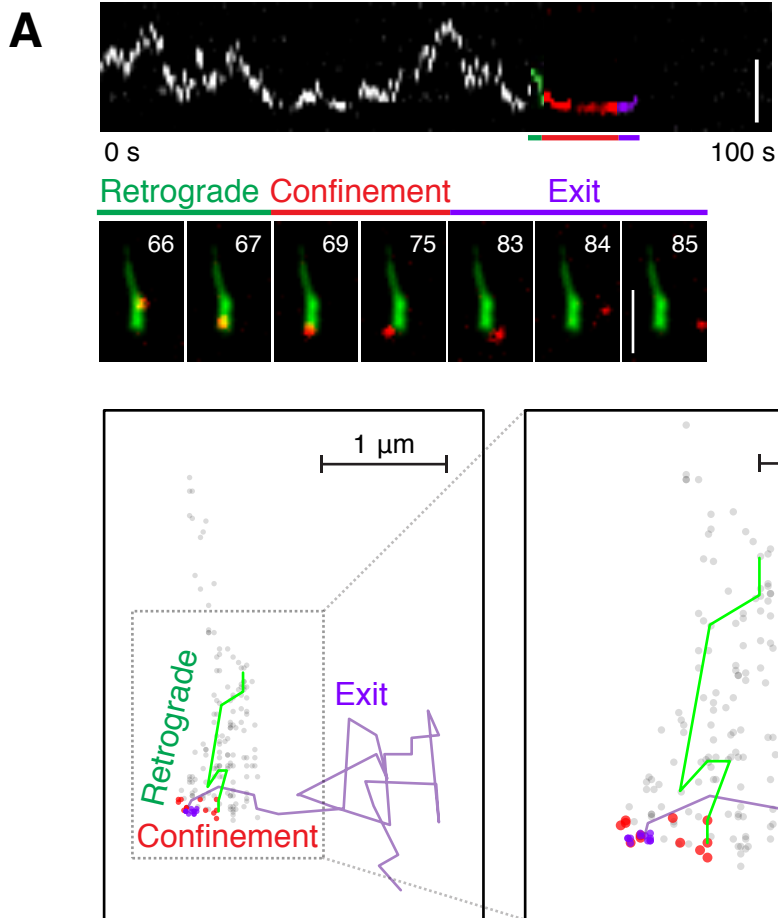
4

A ᵠᴰSSTR3 — WT untreated — 0 s to 300 s

B ᵠᴰSSTR3 — WT + sst — 0 s to 300 s

C ᵠᴰGPR161 — WT untreated — 0 s to 300 s

D ᵠᴰGPR161 — WT + SAG — a, b, c, d — 306 s

E ᵠᴰGPR161 — Arl6⁻/⁻ + SAG — 0 s to 300 s

Figure S4. Kymographs of single molecules of ^{QD}SSTR3 and ^{QD}GPR161

(**A-B**) Additional examples of ^{QD}SSTR3 tracking in wild-type cells treated with either vehicle or somatostatin (sst). Each kymograph shows a single cilium that was tracked for 5 min. (**C-E**) Additional examples of ^{QD}GPR161 tracking in either wild-type or *Arl6*$^{-/-}$ cells treated with either vehicle or SAG. Each kymograph shows a single cilium that was tracked for 5 min. The annotated kymograph for the WT + SAG condition corresponds to the kymograph shown in Fig. **7A** with the addition of boxes highlighting the 4 events analyzed in Fig. **8E**.

A

0 s   100 s

Retrograde Confinement   Exit

66 67 69 75 83 84 85

1 µm   0.5 µm

Retrograde   Exit

Confinement

B

^{QD}GPR161 single molecule imaging data set	Theoritical probability of witnessing one or more exit event	Number of exit events imaged
#1: 26 movies x 20min =520 min	90.3%	1
#2: 78 movies x 5min =390 min	81.4%	1
#3: 17 movies x 20min =340 min (NG channel captured at 1 min interval)	78.3%	1

C

GPR161^{NG3}
live
fixed

Normalized linescan intensity

Relative distance at ciliary base (µm)

D

Airlock visits by ^{QD}GPR161

Total: 40 events/h
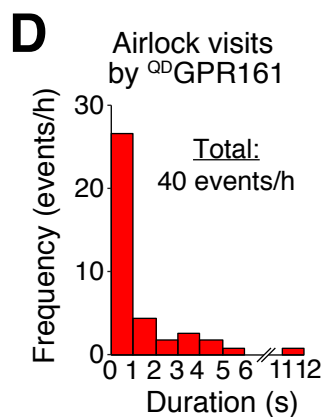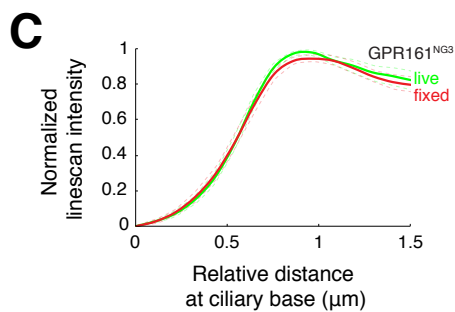
Frequency (events/h)

Duration (s)

Figure S5. Visualization of ^{QD}GPR161 exit at single molecule resolution.
(**A**) Exit event observed from ^{QD}GPR161 single molecule tracking. Cells were treated with SAG for 40 min before the start of imaging. Green, red and purple labels in kymograph (top panel) and line coloring in time lapse images (middle panel) indicate retrograde movements, confinement and exit from cilia respectively. Bottom panel: centroid mapping of ^{QD}GPR161. Scale bars in the top and middle panel: 2 μm. (**B**) The frequency of GPR161 exit events captured by single molecule imaging is consistent with values predicted from bulk imaging of ^{AP}GPR161^{NG3}. Single molecule imaging movies are grouped into three data sets each with slightly different acquisition settings. The theoretical probability of witnessing one or more exit event is $P_{exit} = 1 - (1-(R_{exit}*N_{min}))^{\wedge}N_{movie}$, where R_{exit} is the exit rate of GPR161 (0.256/h = 0.0043/min, measured in Fig. **1G**), N_{min} is the length of each movie in minutes and N_{movie} is the number of movies captured. (**C**) Longitudinal scans of ^{AP}GPR161^{NG3} fluorescence in live cells (green line) and fixed cells (red line). Dotted lines represent 95% CI. $N = 20\text{-}28$ cilia. (**D**) Distribution of airlock visit durations for a single molecule of ^{QD}GPR161. IMCD3 cells were treated with SAG for 40 min before imaging each cilium for 5 to 10 min. Airlock visits are defined by the centroid of ^{QD}GPR161 crossing the 50th percentile of bulk NG fluorescence of ^{AP}GPR161^{NG3}. Combined imaging time: 70 min.

Movie S1. Somatostatin-dependent removal of SSTR3 from cilia

Live-cell imaging of SSTR3^{NG} following addition of somatostatin. IMCD3-[pEF1α^A-^{AP}SSTR3^{NG}] cells were pulse-labeled by SA647 and cilia tracked for 2 or 6 h following addition of somatostatin or vehicle. The fluorescence is displayed in fire color scale. Scale bars: 4 μm.

Movie S2. IFT-B foci movements in cilia

Live imaging of ^{NG3}IFT88 in IMCD3-[pEF1α-^{NG3}IFT88] cells. An arrow marks the tip of the cilium. Time stamp is in seconds. Scale bar: 2 μm.

Movie S3. Co-movement between BBSome and IFT-B

Live cell imaging showing BBSome (green) and IFT-B (red) foci co-moving in IMCD3-[pEF1α-^{NG3}BBS5, pCMV-^{mCherry}IFT88] cells. Channels are shifted to aid visualization. An arrow marks the tip of the cilium. Time stamp is in seconds. Scale bar: 2 μm

Movie S4. BBSome foci movement in cilia

Live imaging of ^{NG3}BBS5 in IMCD3-[pEF1α-^{NG3}BBS5] cells treated with vehicle or SAG for 40 min. Arrows mark the cilium tips. Time stamp is in seconds. A bright retrograde BBSome foci is seen in the SAG-treated cell between 12.0 and 15.0 s. Time stamp is in seconds. Scale bar: 2 μm.

Movie S5. Dynamics of BBSome-mediated retrieval

Live-cell imaging of BBSome foci and SSTR3 in wild-type and *Arl6$^{-/-}$* cells. IMCD3-[^{AP}SSTR3, ^{NG3}BBS5] were pulse-labeled with SA647 and treated with somatostatin for 1 h before imaging at 1.21 Hz. Channels are shifted to aid visualization. Yellow and red triangles point to the base and tip of cilia respectively. Scale bars: 4 μm.

Movie S6. Behavior of Qdot-labeled SSTR3 in control-treated cells

Single-molecule imaging of SSTR3 in IMCD3-[pEF1α^A-^{AP}SSTR3^{NG}] cell. SSTR3 was sparsely labeled with Qdot655 (^{QD}SSTR3, red channel) and tracked as detailed in the Methods. The bulk NeonGreen fluorescence from ^{AP}SSTR3^{NG} is shown in the green channel. An arrow marks the base of the cilium. Time stamp is in seconds. Scale bar: 2 μm.

Movie S7. Behavior of Qdot-labeled SSTR3 in somatostatin-treated cells

Single-molecule imaging of SSTR3 in a somatostatin-treated IMCD3-[pEF1α^A-^{AP}SSTR3^{NG}] cell. SSTR3 was sparsely labeled with Qdot655 (^{QD}SSTR3, red channel) and tracked as detailed in the Methods. The cell was treated with somatostatin for over an hour prior to imaging. The bulk NeonGreen fluorescence from ^{AP}SSTR3^{NG} is shown in the green channel. An arrow marks the base of the cilium. Scale bar: 2 μm.

Movie S8. Direct observation of ciliary exit of Qdot-labeled GPR161

Single-molecule imaging of GPR161 exit in a SAG-treated IMCD3-[pCrys-^{AP}GPR161^{NG3}] cell. GPR161 was sparsely labeled with Qdot655 (^{QD}GPR161, red channel) and tracked as detailed in Method. The cell was treated with SAG for over an hour prior to imaging. The bulk NeonGreen fluorescence from ^{AP}SSTR3^{NG} is shown in the green channel. An arrow marks the base of cilium. Scale bar: 2 μm.

Movie S9. Direct observation of ciliary exit of Qdot-labeled GPR161

Single-molecule imaging of GPR161 exit in a SAG-treated IMCD3-[pCrys-^{AP}GPR161^{NG3}] cell. GPR161 was sparsely labeled with Qdot655 (^{QD}GPR161, red channel) and tracked as detailed in Method. The cell was treated with SAG for over an hour prior to imaging. The bulk NeonGreen fluorescence from ^{AP}SSTR3^{NG} is shown in the green channel. An arrow marks the base of cilium. Scale bar: 2 μm.