Breslow et al., http://www.jcb.org/cgi/content/full/jcb.201212024/DC1

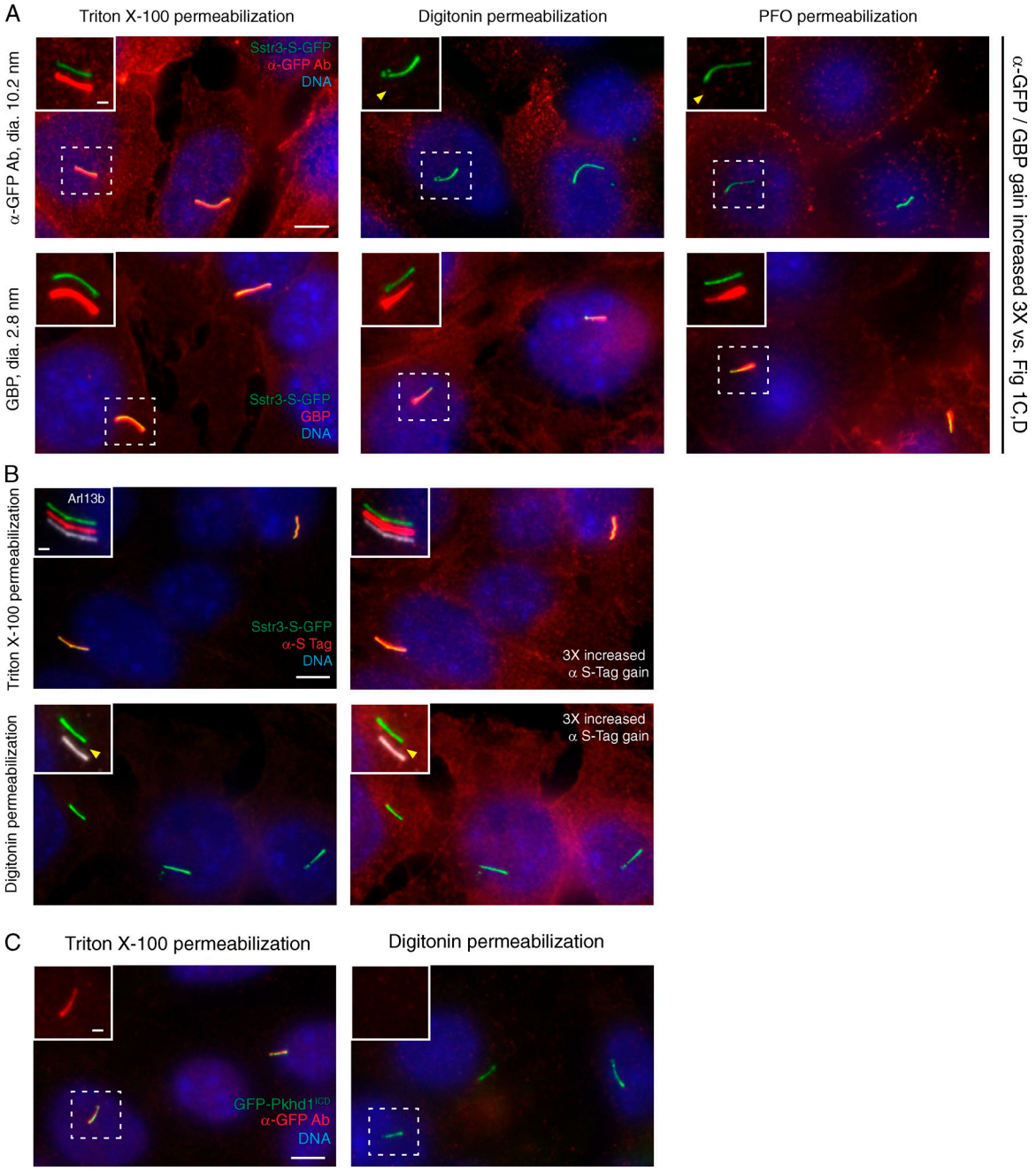


Figure S1. **Characterization of a permeabilized cell system for ciliary trafficking.** (A) The same images are shown here as in Fig. 1 (C and D) except that the gain is increased threefold for the anti-GFP/GBP channel to show staining of plasma membrane Sstr3-S-GFP. Insets show anti-GFP/GBP and GBP channels offset for clarity. Arrowheads point to the base of the cilium in the anti-GFP/GBP channel. (B) IMCD3 cells expressing Sstr3-S-GFP were stained with an anti–S tag antibody following a conventional immunofluorescence protocol using 0.1% Triton X-100 for permeabilization (top) or in live cells permeabilized with 30 µg/ml digitonin (bottom). After incubation of digitonin-permeabilized cells with the anti–S tag antibody, cells were washed, permeabilized with Triton X-100, and stained with anti-Arl13b antibody (purple). Insets show enlarged views of primary cilia for all channels except DNA, with channels shifted for clarity. Arrowheads point to the base of the cilium in the anti–S tag channel. Images on the right have increased anti–S tag channel gain. (C) IMCD3 cells expressing the Pkhd1 cytoplasmic tail fused to GFP were stained with an anti-GFP antibody following a conventional immunofluorescence protocol using 0.1% Triton X-100 for permeabilization (left) or in live cells permeabilized with 30 µg/ml digitonin (right). Insets show the anti-GFP channel only. Ab, antibody; dia., diameter. Bars: (main images) 5 µm; (insets) 1 µm.

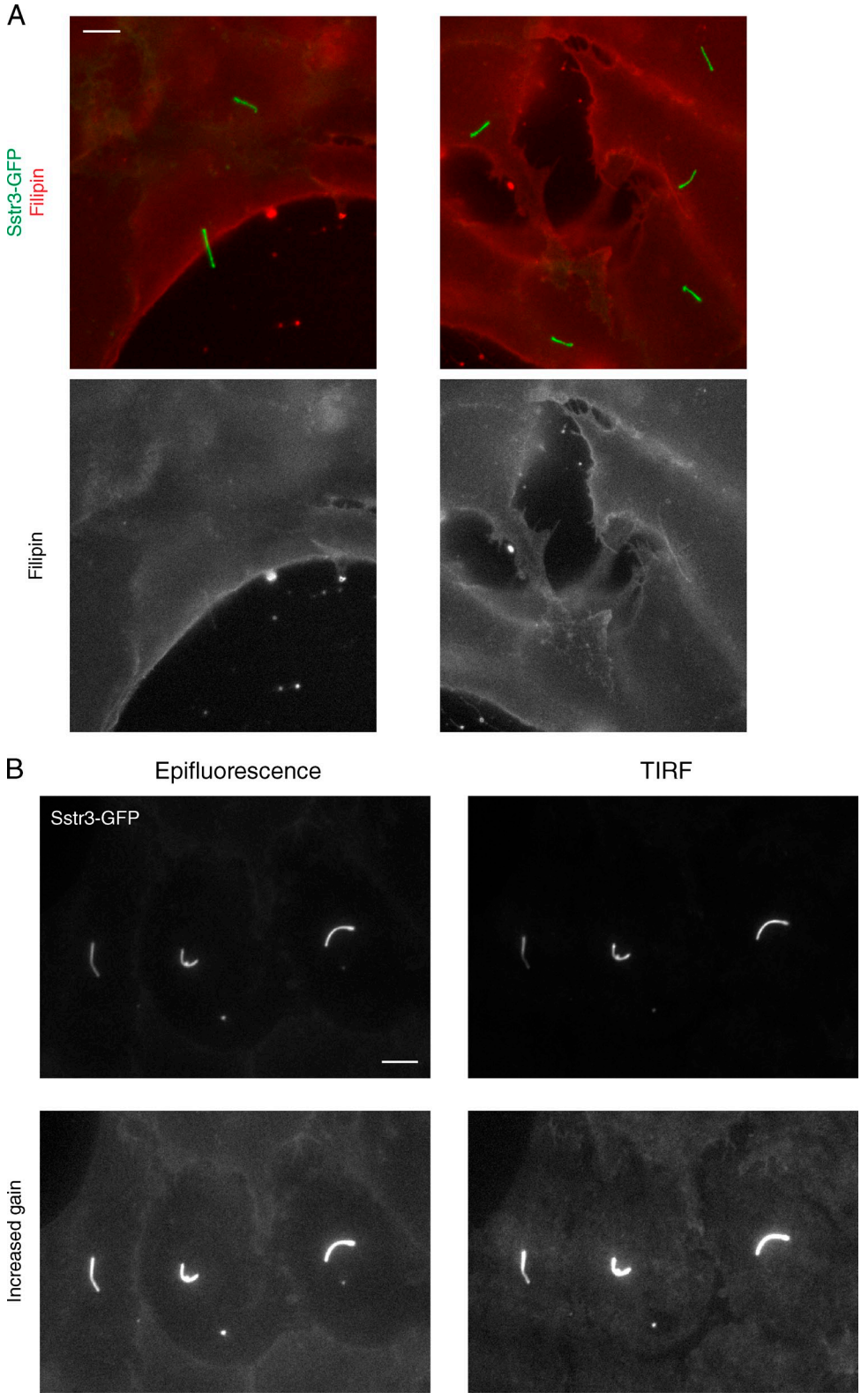


Figure S2. **Filipin staining and ventral localization of primary cilia of IMCD3 cells.** (A) IMCD3 cells expressing Sstr3-GFP were stained with 50 µg/ml filipin to visualize cholesterol under conditions matching those used for digitonin permeabilization. (top) Filipin and Sstr3-GFP are shown. (bottom) Filipin alone is shown. Left and right images show two different fields of cells. (B) Comparison of epifluorescence micrographs (left) and total internal reflection fluorescence (TIRF) micrographs of Sstr3-GFP in IMCD3 cells grown on glass coverslips confirms that primary cilia are on the ventral surface of cells that faces the coverslip. Images with higher gain are shown at the bottom for clarity. Bars, 5 µm.

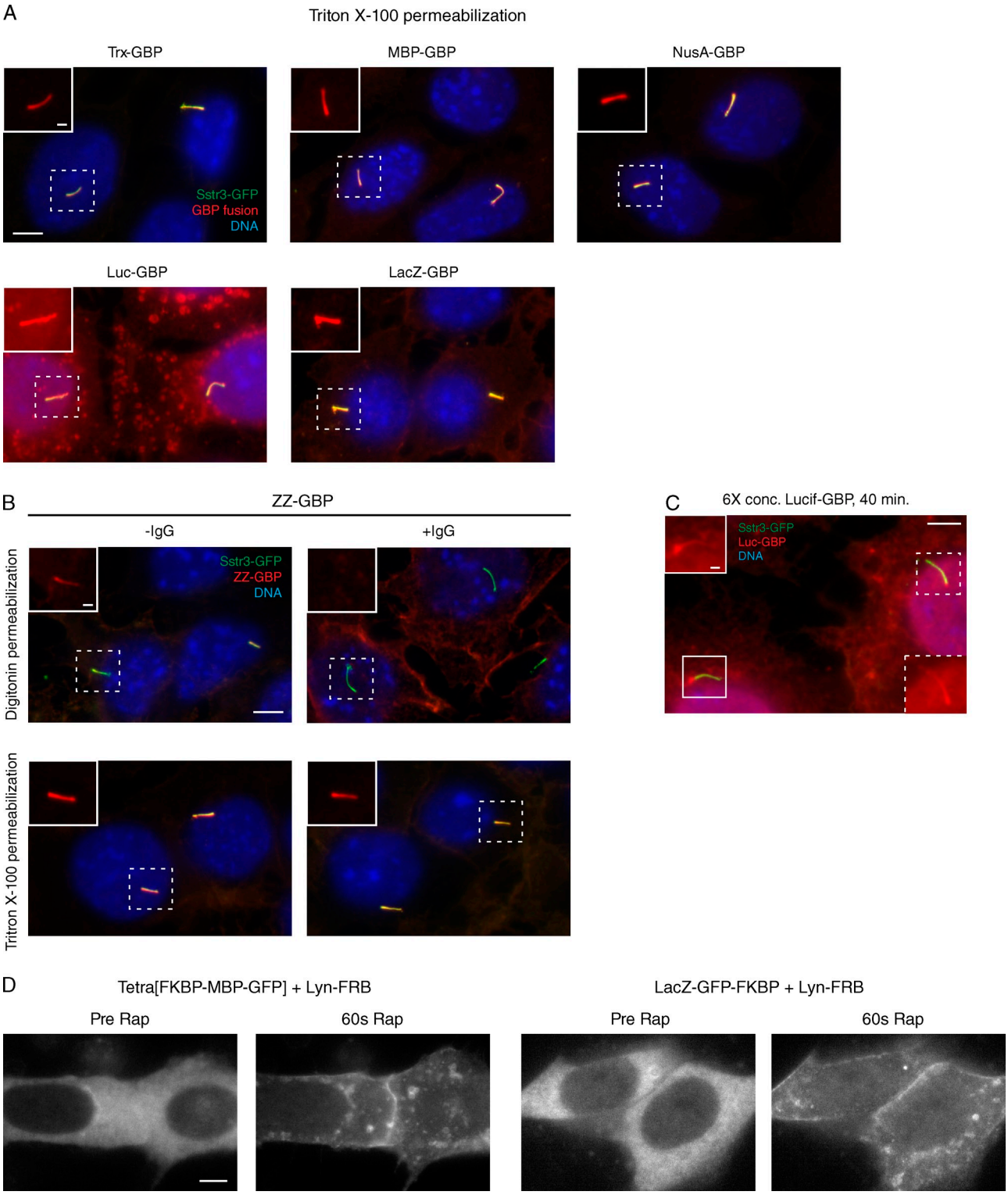


Figure S3. **Size-dependent access of GBP fusion proteins to ciliary GFP after permeabilization with digitonin but not Triton X-100.** (A) IMCD3 cells expressing Sstr3-GFP were permeabilized with digitonin, mock stained, fixed, permeabilized with 0.1% Triton-X-100, and then incubated with the indicated GBP fusion proteins. Insets show enlarged views of cilia with GBP channel alone. (B) IMCD3 cells expressing Sstr3-GFP were stained with a GBP fusion to two tandem copies of the IgG-binding Z domain from protein A. Before staining, the ZZ-GBP fusion protein was incubated with excess rabbit IgG or left untreated. Cells were stained immediately after digitonin permeabilization (top) or after Triton X-100 permeabilization as in A. Insets show enlarged views of cilia with GBP channel alone. (C) Diffusion-to-capture assays were conducted with permeabilized cells in the presence of 330 nM Luc-GBP (a sixfold increase compared with Fig. 3 A) for 40 min (fourfold increase compared with Fig. 3 A). The detection of weak Luc-GBP signals in cilia indicates that this fusion is able to enter cilia at a very slow rate. Insets show enlarged views of cilia with GBP channel alone. conc., concentration. (D) Large FKBP fusions are competent for rapamycin-induced heterodimerization. The plasma membrane–targeted Lyn-FRB was cotransfected with Tetra[FKBP-MBP-GFP] or LacZ-GFP-FKBP in IMCD3 cells, and rapamycin (Rap) was added 48 h after transfection. The rapid recruitment of these FKBP fusions to plasma membrane Lyn-FRB indicates that they can be efficiently captured by FRB when no diffusion barrier is present. Bars: (main images) 5 µm; (insets) 1 µm.

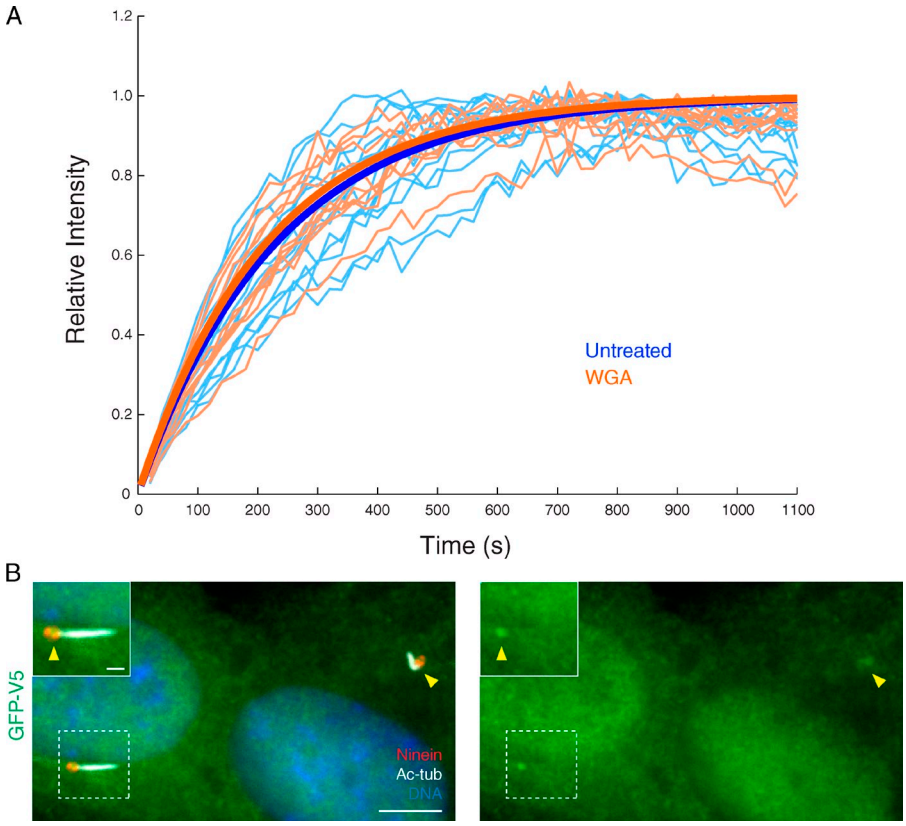


Figure S4. **Effect of reagents that block or loosen the NPC hydrogel on MBP-GBP entry into primary cilia.** (A) The MBP-GBP signal at the most proximal region of the cilium is plotted against time for digitonin-permeabilized IMCD3 cells stained in the presence and absence of 75 µg/ml WGA. Traces for individual cilia are shown in thin, light lines ($n \geq 10$). Fitted exponential curves corresponding to the mean entry rate are shown in thick, dark lines. (B) GFP alone is mildly enriched in the vicinity of the ciliary base and centrosome upon transient transfection. GFP with a C-terminal V5 tag was transfected into IMCD3 cells, and cells were processed for immunofluorescence after 48 h. The centrioles and basal bodies were visualized with ninein, and cilia were stained with acetylated tubulin (Ac-tub). Arrowheads point to the base of cilia. Bar, 5 µm.

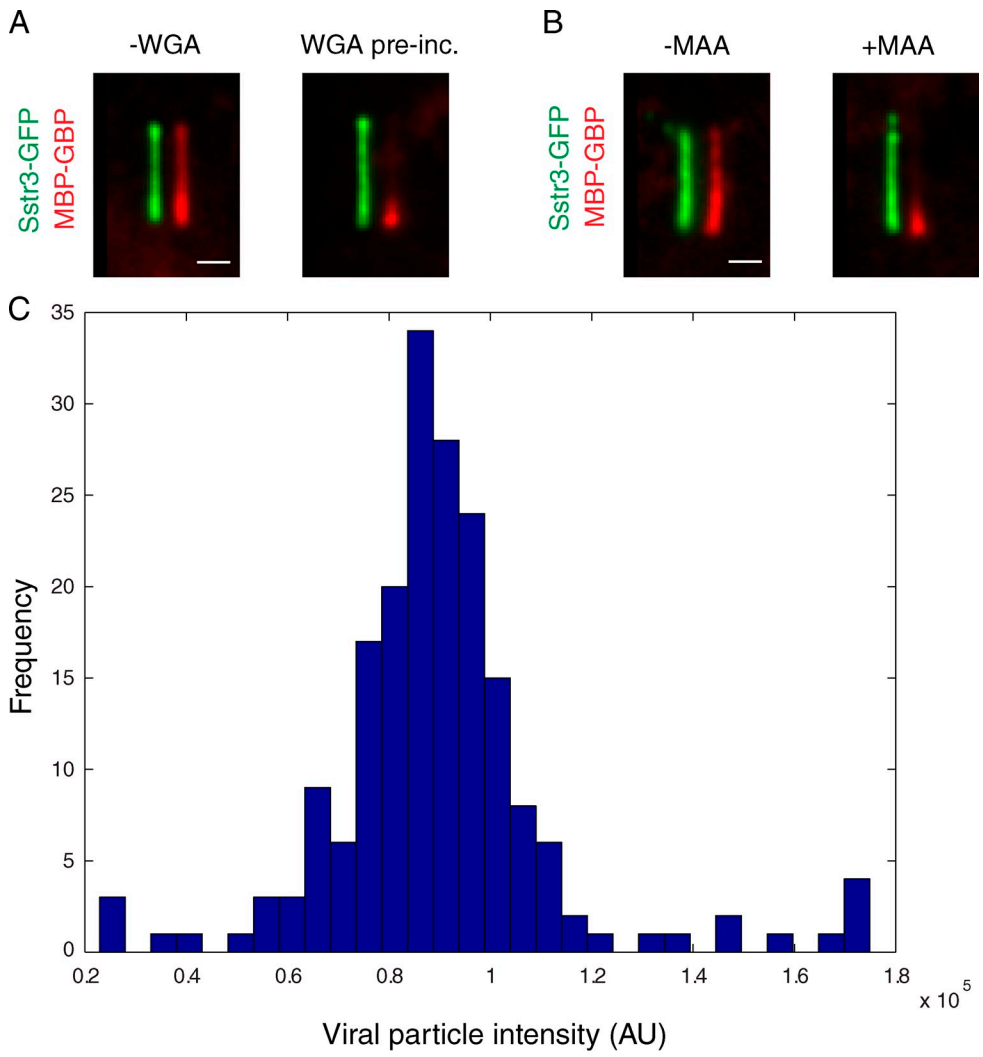
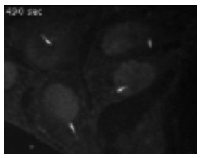


Figure S5. **Effect of lectins on GBP capture by ciliary Sstr3-GFP.** (A) Effect of 75 µg/ml WGA on MBP-GBP staining when the lectin is added to live cells and excess washed away before digitonin permeabilization. No WGA is added after permeabilization or during staining with MBP-GBP. inc., increase. (B) Effect of 75 µg/ml *M. amurensis* lectin (MAA), which binds sialic acid–containing glycans, on MBP-GPB staining of digitonin-permeabilized IMCD3 cells expressing Sstr3-GFP. GFP and MBP-GBP channels are offset for clarity. (C) Viral particles containing 120 GFP molecules were imaged and used as a calibrated reference to obtain the number of Sstr3-GFP molecules per cilium from the raw fluorescence intensities. The histogram shows the distribution of viral particle intensities (n = 192) obtained under the same imaging conditions that were used to image IMCD3-(Sstr3-GFP) cells. AU, arbitrary unit. Bars, 1 µm.



Video 1. **Entry of GBPs into primary cilia of permeabilized IMCD3 cells.** IMCD3 cells stably expressing Sstr3-GFP were permeabilized with digitonin and incubated with Alexa Fluor 647–labeled GBPs. Images were acquired by time-lapse spinning-disk confocal microscopy on a microscope (TE2000; Nikon). Frames were taken every 9–16 s for 9 min and 36 s as indicated in the video time stamps. Sstr3-GFP fluorescence is shown first; all subsequent frames show fluorescence of labeled GBPs. Entry is shown for GBP alone (left), Trx-GBP (middle), and MBP-GBP (right).