Supplementary Methods

Immunoblotting

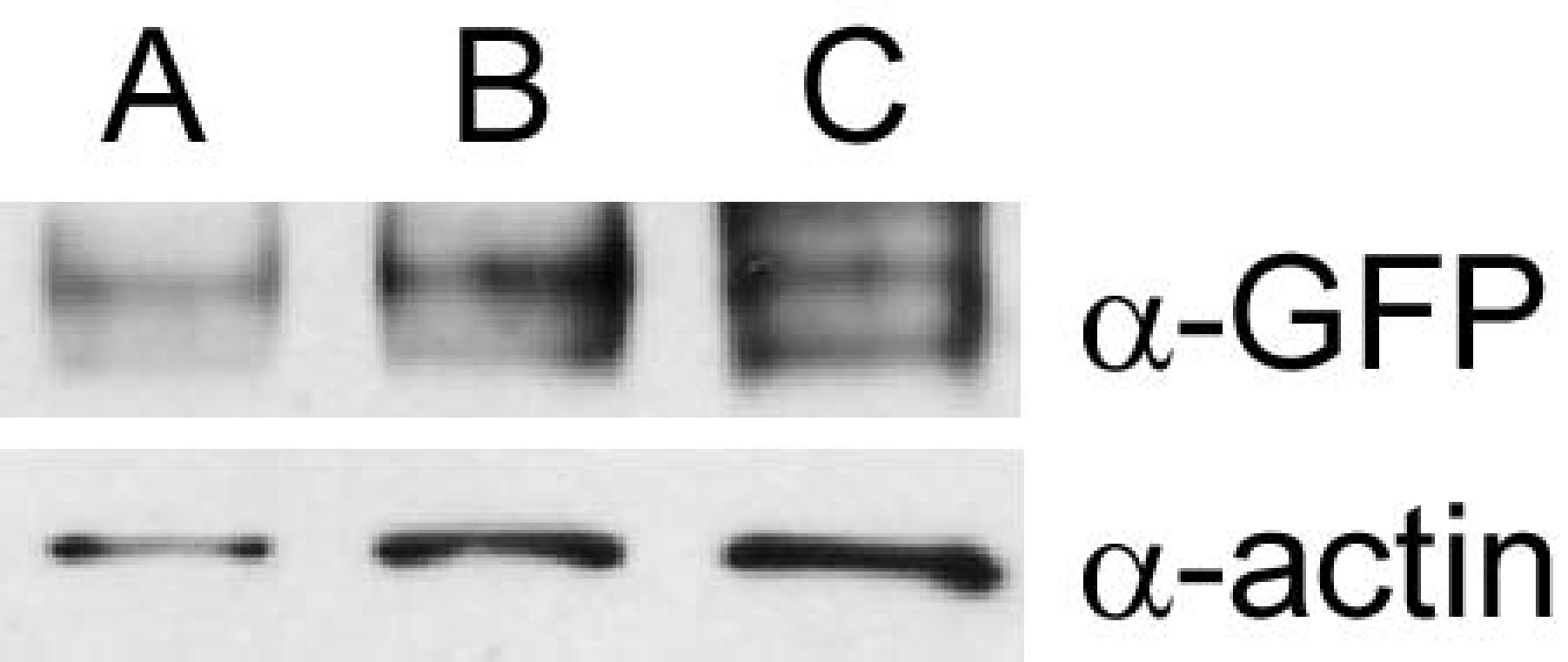
48 hours post-transfection, cells were washed twice with cold PBS and then scraped in 1ml of cold PBS. Cells were transferred to a microfuge tube and spun at 900 x g for 1 minute. PBS was aspirated off and cells were resuspended in 300µl ice-cold lysis buffer (137 mM NaCl, 20 mM Tris pH 8.0, 1% Triton X-100, 10% glycerol, complete EDTA-free protease inhibitor cocktail (Roche Diagnostics), 1mM sodium orthovanadate, 1mM PMSF, 1µg/ml aprotinin, and 1µg/ml leupeptin). After a 5 second sonication the cell lysate was incubated on ice for 30 minutes and then vortexed briefly before centrifugation at 10,000 x g at 4°C for 10 minutes. The supernatant was collected and the soluble protein concentrations were determined by the Bradford assay (Bio-Rad Laboratories). 40 µg of each protein sample was run on a denaturing 4-15% gradient polyacrylamide gel (Bio-Rad Laboratories) and transferred to Immobilon-Psq transfer membrane (Millipore). Membranes were blocked in TBS-T (10mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1% Tween-20) with 5% dried milk for 1 hour and incubated with anti-GFP mouse monoclonal antibody (sc-9996; Santa Cruz Biotechnology) diluted 1:200 in TBS-T with 5% dried milk for 16-24 hours at 4°C. Membranes were probed with goat anti-mouse horse radish peroxidase (HRP) conjugated secondary antibody (sc-2031; Santa Cruz Biotechnology) diluted 1:1000 in TBS-T with 5% dried milk for 1 hour at room temperature. Secondary antibodies were detected using SuperSignal West Pico Chemiluminescent Substrate (Pierce) and bands were visualized using Blue Ultra Autorad Film (ISC Bioexpress). Blots were then stripped with Re-Blot Plus (Chemicon

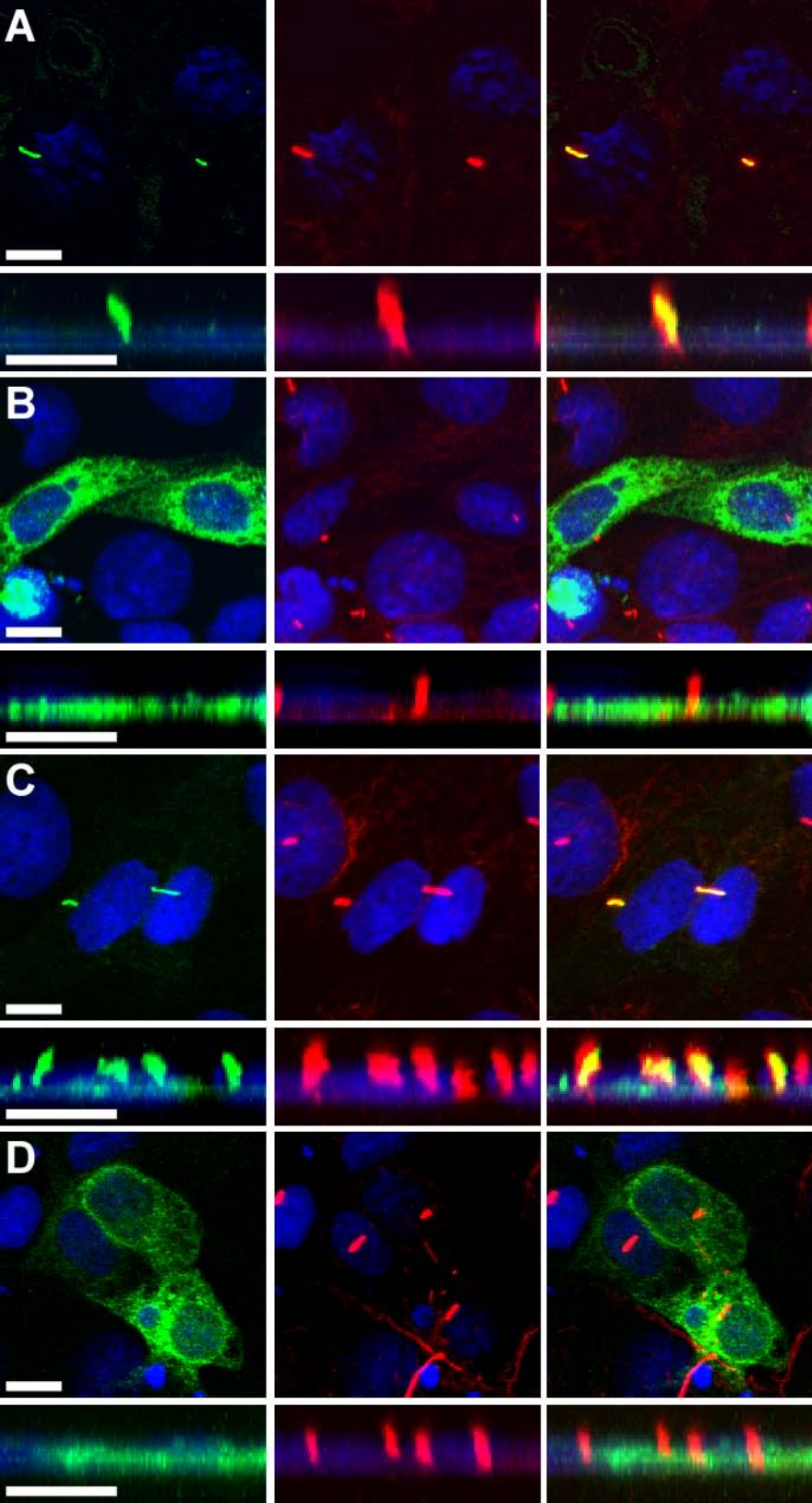
International), probed with anti-actin mouse monoclonal antibody (sc-8432; Santa Cruz Biotechnology) diluted at 1:1000, and detected as described above.

Supplementary Figure Legends

Figure 1. Immunoblot of whole protein lysates from IMCD cells transiently transfected with Sstr5[TM4-5Sstr3] (A), Sstr5[TM5-6Sstr3] (B), and Sstr5[TM5-6Sstr3mut2] (C) reveals similar expression of each chimeric receptor. Proteins were probed with antibodies to GFP (upper panel) and actin (lower panel), as a loading control.

Figure 2. Mutational analysis indicates that the A and Q are important for ciliary localization of chimeric receptors Sstr5[TM5-6Sstr3] and Htr7[TM5-V241Htr6]. Representative images of transiently transfected IMCD cells expressing Sstr5[TM5-6Sstr3] (A), or Sstr5[TM5-6Sstr3mut2] (B), in which the A and Q in both consensus sequences have been mutated to F, fused at the C-terminus to EGFP. (Left panels) EGFP fluorescence (green); (middle panels) acetylated -tubulin (red); (right panels) merged images. Ciliary localization is rarely seen in cells expressing Sstr5[TM5-6Sstr3mut2] (B). Representative images of transiently transfected IMCD cells expressing Htr7[TM5-V241Htr6] (C), or Htr7[TM5-V241Htr6mut] (D), in which the A and Q have been mutated to F, fused at the C-terminus to EGFP. (Left panels) EGFP fluorescence (green); (middle panels) acetylated -tubulin (red); (right panels) merged images. Ciliary localization is rarely seen in cells expressing Htr7[TM5-V241Htr6mut] (D). Nuclei are labeled with DRAQ5 (blue). All scale bars, 10 μm.


A
B
C
α-GFP
α-actin


A
B
C
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