Diverse Cell Type-Specific Mechanisms Localize G Protein-Coupled Receptors to *Caenorhabditis elegans* Sensory Cilia

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ABSTRACT The localization of signaling molecules such as G protein-coupled receptors (GPCRs) to primary cilia is essential for correct signal transduction. Detailed studies over the past decade have begun to elucidate the diverse sequences and trafficking mechanisms that sort and transport GPCRs to the ciliary compartment. However, a systematic analysis of the pathways required for ciliary targeting of multiple GPCRs in different cell types *in vivo* has not been reported. Here we describe the sequences and proteins required to localize GPCRs to the cilia of the AWB and ASK sensory neuron types in *Caenorhabditis elegans*. We find that GPCRs expressed in AWB or ASK utilize conserved and novel sequences for ciliary localization, and that the requirement for a ciliary targeting sequence in a given GPCR is different in different neuron types. Consistent with the presence of multiple ciliary targeting sequences, we identify diverse proteins required for ciliary localization of individual GPCRs in AWB and ASK. In particular, we show that the TUB-1 Tubby protein is required for ciliary localization of a subset of GPCRs, implying that defects in GPCR localization may be causal to the metabolic phenotypes of *tub-1* mutants. Together, our results describe a remarkable complexity of mechanisms that act in a protein- and cell-specific manner to localize GPCRs to cilia, and suggest that this diversity allows for precise regulation of GPCR-mediated signaling as a function of external and internal context.

SIGNALING molecules must be precisely localized to specific subcellular domains to optimize detection and transduction of external stimuli. G protein-coupled receptors (GPCRs) comprise a large family of transmembrane signaling proteins that directly bind and transduce a range of cues including photons, odorants, neurotransmitters, and peptides (Pierce *et al.* 2002; Kato and Touhara 2009; Demaria and Ngai 2010; Sung and Chuang 2010; Chamero *et al.* 2012; Frooninckx *et al.* 2012; Montell 2012; Bathgate *et al.* 2013). Regulation of GPCR function, including regulation of membrane targeting and trafficking to specific subcellular regions, is a major contributor to the tuning of signaling efficacy and fidelity (*e.g.*, Deretic *et al.* 1995; Dwyer *et al.* 1998; Ango *et al.* 2000; Xia *et al.* 2003; Esseltine *et al.* 2012; Loktev and Jackson 2013). However, much remains to be understood regarding the mechanisms by which GPCR trafficking and membrane localization are regulated.

GPCR-mediated signal transduction in many cellular contexts requires these proteins to be localized to specialized microtubule-based primary cilia. For example, in photoreceptors and olfactory neurons, efficient sensory signal transduction is mediated via localization of rhodopsin and olfactory receptors, together with other signaling molecules, to photoreceptor outer segments and olfactory neuron cilia, respectively (Insinna and Besharse 2008; Berbari *et al.* 2009; Pifferi *et al.* 2010; Deretic and Wang 2012). Receptors in the Hedgehog (Hh) morphogen signaling pathway such as the Smoothened and Patched transmembrane proteins, and the Gpr161 putative GPCR, are dynamically localized in the cilium as a function of the presence or absence of the Hh cue; failure to correctly localize these receptors results in altered Hh signaling and severe developmental consequences (Goetz *et al.* 2009; Mukhopadhyay *et al.* 2013; Nozawa *et al.* 2013). However, within a given cell

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type, closely related members of a GPCR family can be differentially targeted to different cell compartment membranes. For instance, in mammalian neurons, only the somatostatin receptor subtype 3 (Sstr3) GPCR is localized to cilia, whereas the five related Sstr GPCRs are instead targeted to membranes of neuronal soma, axons, or dendrites (Handel *et al.* 1999; Schulz *et al.* 2000; Berbari *et al.* 2008a). Thus, ciliary localization of GPCRs is tightly regulated as a function of cell type, signaling conditions, as well as GPCR identity.

Mechanisms required for GPCR targeting to cilia have been studied intensively. Ciliary GPCRs contain sequences (termed ciliary targeting sequences, CTSs) that are recognized by specific adaptor proteins that promote trafficking to cilia (Rosenbaum and Witman 2002; Pazour and Bloodgood 2008; Emmer *et al.* 2010; Nachury *et al.* 2010; Loktev and Jackson 2013; Sung and Leroux 2013). Both CTSs and the required adaptor proteins are quite diverse. Sequences in the third intracellular loop, as well as in the C-terminal tail, have been implicated in ciliary targeting of individual GPCRs (Deretic *et al.* 1998; Tam *et al.* 2000; Dwyer *et al.* 2001; Corbit *et al.* 2005; Berbari *et al.* 2008a; Loktev and Jackson 2013; Mukhopadhyay *et al.* 2013). Similarly, multiple trafficking mechanisms including vesicle-mediated transport and lateral diffusion both to and within the cilium have been shown to play a role in regulating ciliary GPCR composition in different cell types (Pazour and Bloodgood 2008; Emmer *et al.* 2010; Nachury *et al.* 2010; Ye *et al.* 2013; Sung and Leroux 2013). These results imply that GPCR trafficking may employ both protein- and cell-specific mechanisms. Thus, a detailed analysis of GPCR ciliary trafficking within and across defined cell types *in vivo* may greatly inform our knowledge of the underlying pathways.

GPCR-mediated signaling also plays a critical role in the lifecycle of *Caenorhabditis elegans*. As in the corresponding mammalian chemosensory cells, GPCRs required for sensory signaling are concentrated in cilia present at the dendritic endings of a subset of sensory neurons in *C. elegans* (Troemel *et al.* 1995; Sengupta *et al.* 1996; Kim *et al.* 2009; McGrath *et al.* 2011; Park *et al.* 2012). In the head, 12 pairs of ciliated sensory neuron types are contained in the bilateral amphid organs (Ward *et al.* 1975; Perkins *et al.* 1986; Doroquez *et al.* 2014). Many of the amphid sensory neurons are multimodal; even within a modality such as chemosensation, each neuron responds to many chemicals (Bargmann and Horvitz 1991; Bargmann *et al.* 1993; Hart *et al.* 1995; Maricq *et al.* 1995; Wes and Bargmann 2001; Biron *et al.* 2008; Kuhara *et al.* 2008; Ortiz *et al.* 2009; Bretscher *et al.* 2011). Reflecting this diversity of function, each sensory neuron expresses multiple putative sensory GPCRs from different subfamilies (Troemel *et al.* 1995; Colosimo *et al.* 2004; McCarroll *et al.* 2005; Nokes *et al.* 2009), providing an excellent system in which to begin to describe the diversity of mechanisms by which defined subsets of GPCRs are localized to cilia in individual cell types.

Here we systematically characterize the *cis*- and *trans*-acting mechanisms required to localize GPCRs to the cilia of the AWB and ASK amphid chemosensory neuron types in *C. elegans*. We identify ciliary localized GPCRs in both neuron types and define conserved and new sequence motifs required for targeting these proteins to cilia. We show that different GPCRs use diverse sequences for ciliary localization within a given cell type, and that sequences within an individual GPCR mediate ciliary localization via different mechanisms across cell types. We further characterize the roles of multiple trafficking pathways in ciliary localization of GPCRs and uncover both receptor- and cell type-specific diversity in ciliary trafficking mechanisms. In particular, we find that the *tubby*-like protein TUB-1 is required for correct ciliary localization of a subset of sensory GPCRs in AWB and ASK, possibly linking to its previously described metabolic and sensory phenotypes (Ashrafi *et al.* 2003; Mukhopadhyay *et al.* 2005; Mak *et al.* 2006). Together, our results provide a detailed description of the range of mechanisms by which the appropriate set of GPCRs is localized to the cilia of individual cell types, thereby ensuring optimal cell-specific functions.

Materials and Methods

Strains

Animals were grown on *Escherichia coli* OP50 bacteria at 20°. Transgenic strains were generated by microinjecting plasmids driving expression in AWB or ASK at 5 and 10 ng/μl, respectively, unless indicated otherwise. *unc-122*p::*dsRed* or *unc-122*p::*gfp* injected at 50 ng/μl was used as the co-injection marker. The presence of specific mutations in generated strains was verified by genotyping or visible phenotypes. Alleles analyzed in this study and associated references are provided in Supporting Information, Table S1. A complete list of all strains used in this study is provided in Table S2.

Molecular biology

Cell-specific expression constructs were generated by fusing relevant cDNAs in frame to *gfp* containing either 2.1 kb *srbc-66* (ASK specific) (Kim *et al.* 2009), or 3.0 kb *str-1* (AWB specific) (Troemel *et al.* 1995) upstream regulatory sequences. *srbc-64*::*gfp* (Kim *et al.* 2009) expression was driven under its own promoter. Truncated *str-163* and *srbc-64* sequences were generated by PCR using iProof high-fidelity DNA polymerase (Bio-Rad). Point mutations were made using the QuikChange Lightning Site-Directed Mutagenesis kit (Agilent). All mutations were verified by sequencing.

Microscopy

Animals were mounted on 10% agarose pads set on microscope slides and anesthetized using 10 mM tetramisole. Imaging was performed on an inverted spinning disk microscope (Zeiss Axiovert with a Yokogawa CSU22 spinning disk confocal head). Optical sections were acquired at

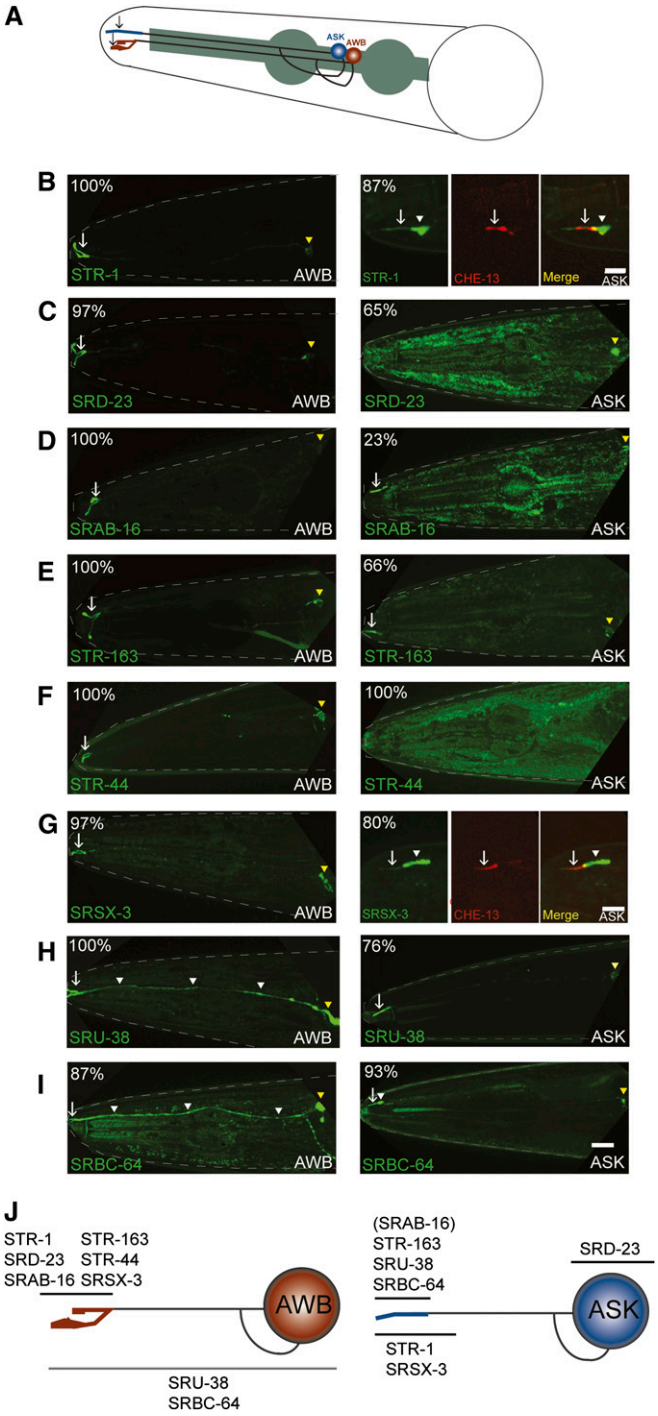

Figure 1 Localization of GPCR::GFP fusion proteins in the AWB and ASK chemosensory neurons. (A) Cartoon of AWB and ASK sensory neurons in the head of *C. elegans*. Cell bodies are indicated in blue (ASK) or red (AWB). Shaded structure indicates the pharynx. Arrows indicate cilia. Only one of each bilateral pair of neurons is seen in this lateral view. Anterior is at left. (B–I) Representative examples of the localization pattern of indicated GPCR fusion proteins in AWB (left panels) or ASK (right panels) in adult hermaphrodites. Numbers in top left corners indicate the percentage of examined animals exhibiting the phenotype (see Table 1). White arrows indicate cilia; white arrowheads indicate dendrites and dendritic domains proximal to cilia; yellow arrowheads indicate cell bodies. Expression was driven in AWB and ASK under the *str-1* and *srbc-66* promoters, respectively. (B and G, right panels) Cilia were visualized via cell-specific expression of CHE-13::TagRFP. Lateral views. Bars, 10 μm (B–I, left panels and C–F, H, and I, right panels); 5 μm (B and G right panels). (J) Summary of GPCR localization patterns in AWB and ASK. Horizontal lines indicate subcellular localization patterns of indicated fusion proteins.

0.13-μm intervals using a ×63 or ×100 objective with SlideBook 5.0 software (Intelligent Imaging Innovations, 3i). Images were *z*-projected at maximum intensity values. Images were linear adjusted for brightness and contrast using Photoshop (Adobe Systems) to optimize visualization of cilia. Animals from two independent lines were examined for each transgenic strain. Expression was quantified on at least two independent days for each data point.

Results

GPCRs are localized to sensory cilia in a cell-specific manner

The AWB and ASK amphid chemosensory neurons (Figure 1A) respond largely to volatile and aqueous compounds, including lipophilic pheromones, respectively (Bargmann and Horvitz 1991; Troemel *et al.* 1997; Kim *et al.* 2009; Macosko *et al.* 2009; Wakabayashi *et al.* 2009). We and others previously showed that the AWB neurons express a subset of predicted GPCRs encoded by the *C. elegans* genome (Troemel *et al.* 1995; Colosimo *et al.* 2004; Nokes *et al.* 2009; C. Bargmann, personal communication; www.wormbase.org). Of these, *str-1* and *str-44* are expressed exclusively or primarily in AWB, whereas *srsx-3*, *srd-23*, *sru-38*, *srab-16*, and *str-163* are expressed in AWB as well as in additional chemosensory neurons (Troemel *et al.* 1995; Colosimo *et al.* 2004; Nokes *et al.* 2009; C. Bargmann, personal communication; www.wormbase.org). In particular, *srd-23* has been reported to be expressed in both the AWB and ASK chemosensory neuron types (Colosimo *et al.* 2004). Although fewer GPCRs expressed in ASK have been identified, this neuron type also expresses the *srbc-64* and *srbc-66* pheromone receptors (Kim *et al.* 2009). Only STR-1, and the SRBC-64 and SRBC-66 pheromone receptors, have been shown to be localized to the sensory cilia of AWB and ASK, respectively (Dwyer *et al.* 2001; Kim *et al.* 2009; Olivier-Mason *et al.* 2013). The characterized expression pattern of multiple receptors in these neuron types, together with our previous analyses showing distinct mechanisms of ciliary membrane protein localization in AWB and ASK (Wojtyniak *et al.* 2013), prompted us to analyze GPCR localization mechanisms in these two sensory neurons in detail.

We first investigated whether other identified AWB-expressed GPCRs also localize to sensory cilia. We found that similar to the localization pattern of STR-1, five of six examined receptor proteins tagged with GFP were also restricted to, or enriched throughout, the cilia of the AWB neurons when expressed specifically in this cell type (Figure 1, B–G, Table 1). Although the ligands for these GPCRs are unknown, their ciliary localization pattern implies that these receptors may directly recognize environmental chemicals.

Table 1 GPCRs exhibit cell-specific subcellular localization patterns

		% localized to:[a]				
Fusion protein[b]	Neuron	Cilium	Cilium, PCMC/distal dendrite	Cilium, dendrite, cell body	Cell body	No expression
STR-1	AWB	100	0	0	0	0
STR-1[c]	ASK	0	87	0	0	13
SRD-23	AWB	97	0	0	0	3
SRD-23	ASK	0	0	0	65	35
SRAB-16	AWB	100	0	0	0	0
SRAB-16	ASK	23	0	0	0	77
STR-163	AWB	100	0	0	0	0
STR-163	ASK	66	0	0	12	22
STR-44	AWB	100	0	0	0	0
STR-44	ASK	0	0	0	0	100
SRSX-3	AWB	97[d]	0	0	0	3
SRSX-3[c]	ASK	0	80	0	0	20
SRU-38	AWB	0	0	100[e]	0	0
SRU-38	ASK	76	0	0	6	18
SRBC-64	AWB	0	0	87[e]	0	13
SRBC-64	ASK	0	93	0	0	7

Adult animals grown at 20° were examined; n = 30–75 animals each.

[a] Fusion protein expression was enriched in the indicated subcellular locations, although weaker expression was occasionally detected elsewhere in the cell.

[b] Proteins were expressed in AWB under the *str-1* promoter, and in ASK under the *srbc-66* promoter with the exception of SRBC-64, which was expressed under its own promoter. Under endogenous conditions, *str-1*, *srab-16*, *str-163*, *str-44*, *srsx-3*, and *sru-38* are expressed in AWB, *srd-23* is expressed in AWB and ASK, and *srbc-64* is expressed in ASK.

[c] Cilia in these strains were visualized via expression of *srbc-66*p::*che-13*::*TagRFP*.

[d] Weak expression was also observed in dendrites.

[e] Expression was also observed in axons.

In contrast, SRU-38::GFP expression was detected in the cilia as well as in the dendrites, cell bodies, and axons of the AWB neurons (Figure 1H, Table 1). The broader localization pattern of this protein was not simply an artifact of overexpression, since this fusion protein exhibited a similar localization pattern when expressed at low levels (Figure S1). Similarly, the cilia-targeted STR-163::GFP fusion protein remained cilia-enriched when injected at high concentrations into wild-type animals (Figure S1). In all cases, receptor::GFP localization was observed throughout the cilia in contrast to the ciliary subdomain-specific localization of molecules such as cyclic nucleotide-gated channels (Mukhopadhyay *et al.* 2008; Wojtyniak *et al.* 2013).

Since SRD-23 is expressed both in AWB and ASK, we asked whether a SRD-23::GFP fusion protein also localized to the cilia of ASK; however, SRD-23::GFP was present largely in the cell bodies of the ASK neurons and was excluded from the ASK sensory cilia (Figure 1C, Table 1). This observation suggests that individual GPCRs may exhibit distinct subcellular localization patterns in expressing cells. To further examine this issue, we misexpressed AWB-expressed GPCRs in ASK and *vice versa*. We reasoned that although AWB (ASK)-expressed receptors are not endogenously expressed in ASK (AWB), these misexpression studies would nevertheless provide insights into GPCR-specific ciliary trafficking mechanisms employed by individual cell types. Indeed, similar approaches utilizing misexpressed GPCRs have been instrumental in the identification of required ciliary sorting and transport mechanisms in multiple experimental systems (Dwyer *et al.* 2001; Berbari *et al.* 2008a; Kaplan *et al.* 2010, 2012; Loktev and Jackson 2013).

A subset of AWB-expressed and ciliary localized GPCRs such as SRAB-16 and STR-163 retained ciliary localization in animals in which expression could be detected in ASK (Figure 1, D and E, Table 1); we did not observe expression of STR-44::GFP in ASK (Figure 1F, Table 1). However, STR-1::GFP and SRSX-3::GFP exhibited a distinct subcellular localization pattern upon misexpression in ASK, such that these fusion proteins accumulated in a distal dendritic domain corresponding to the periciliary membrane compartment (PCMC) (Kaplan *et al.* 2012) with weaker expression in the cilium (Figure 1, B and G, Table 1). Accumulation of these GPCR fusion proteins at the ciliary base was confirmed via coexpression with a CHE-13/IFT57::TagRFP fusion protein, which is present in the ciliary shaft as well as in the putative basal body region (Williams *et al.* 2011; Kaplan *et al.* 2012) (Figure 1, B and G, Table 1). These observations suggest that while STR-1::GFP and SRSX-3::GFP are sorted to the ciliary base, mechanisms required for ciliary membrane targeting or ciliary retention of these proteins are absent in ASK. Unexpectedly, although SRU-38::GFP was not cilia-enriched in AWB, this protein was primarily localized to the cilia of the ASK neurons (Figure 1H, Table 1). We also misexpressed the ASK-expressed SRBC-64 pheromone receptor in AWB. In ASK, SRBC-64::GFP was localized to the cilia with significant accumulation at the PCMC (Figure 1I, Table 1). However, in AWB, SRBC-64::GFP expression was observed in the cilia, dendrites, cell bodies, and axons (Figure 1I, Table 1). Together, these results (summarized in Figure 1J) indicate that distinct mechanisms operate in AWB and ASK to target individual GPCRs to sensory cilia.

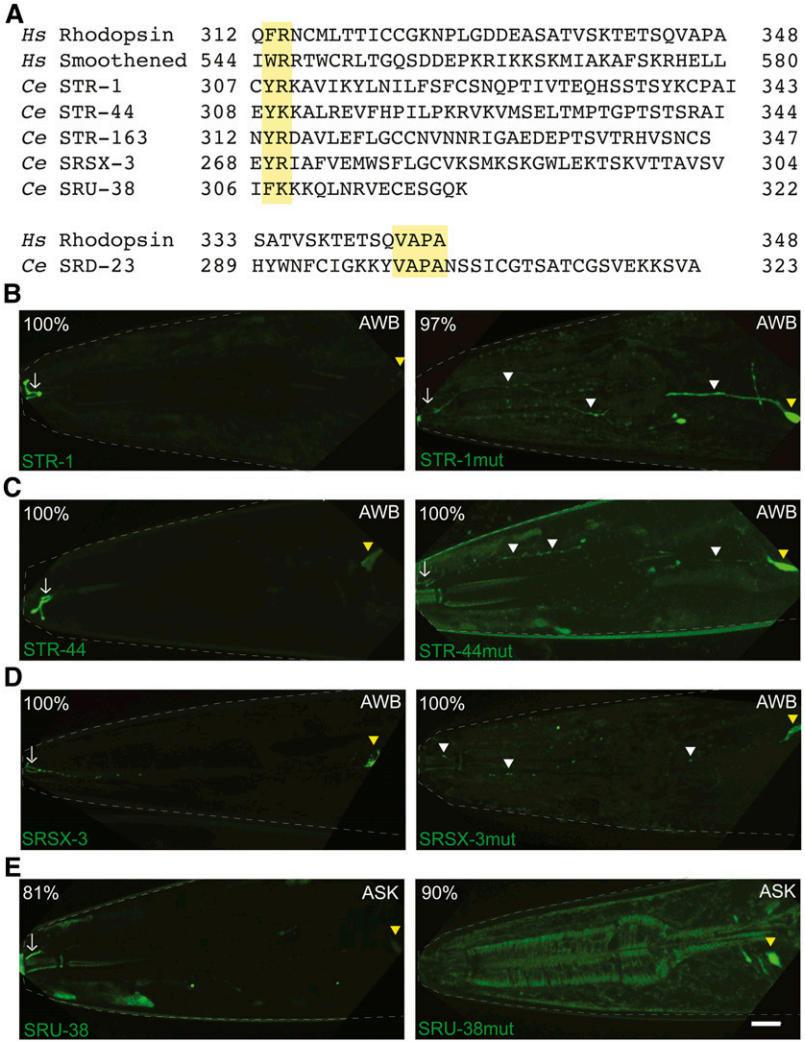

Figure 2 Conserved pair of hydrophobic/basic residues mediate ciliary targeting of a subset of GPCRs in AWB and ASK. (A) Alignment of C-terminal sequences of Rhodopsin and Smoothened with STR-1, STR-44, STR-163, SRSX-3, SRU-38, and SRD-23. The hydrophobic/basic residue pairs and the VxPx motif mutated to alanines are shaded. (B–E) Localization patterns of indicated GPCR::GFP fusion proteins in AWB (B–D) or ASK (E). Note cell membrane localization of SRSX-3mut::GFP in D. White arrows indicate cilia; white arrowheads indicate dendrites; yellow arrowheads indicate cell bodies. Lateral views. Bar, 10 μm.

Conserved C-terminal motifs are required for ciliary localization of a subset of GPCRs

Similar to other ciliary targeted transmembrane proteins, GPCRs contain specific CTSs that interact with adaptor or other trafficking proteins to ensure sorting and transport to cilia (Pazour and Bloodgood 2008; Emmer *et al.* 2010). To date, CTSs have been identified in the third intracellular loops of GPCRs, as well as in their C termini (C termini in this context include all sequences C terminal to the seventh transmembrane segment) (Olsson *et al.* 1992; Deretic *et al.* 1998; Tam *et al.* 2000; Corbit *et al.* 2005; Berbari *et al.* 2008a, b; Loktev and Jackson 2013; Mukhopadhyay *et al.* 2013). We examined the AWB- and ASK-expressed GPCRs to determine whether related sequences might mediate targeting of these proteins to cilia in *C. elegans*.

Previous work identified a C-terminal hydrophobic/basic residue pair required for ciliary localization of the rhodopsin GPCR and the Smoothened seven transmembrane protein in mammalian cells (Corbit *et al.* 2005; Wang *et al.* 2012) (Figure 2A), as well as the ODR-10 GPCR in *C. elegans* sensory neurons (Dwyer *et al.* 2001). A similar hydrophobic/basic residue pair was also identified in the C terminus of STR-1 (Figure 2A) but its requirement for ciliary targeting has not been examined experimentally (Dwyer *et al.* 2001). We identified a hydrophobic/basic residue pair in the C termini of STR-44, STR-163, SRSX-3, and SRU-38 (Figure 2A), but not in SRAB-16, SRD-23, or SRBC-64. To determine whether these residues were required for ciliary localization, we mutated these residues to alanines and examined the subcellular localization of the corresponding mutant fusion proteins. Mutating the hydrophobic/basic residue pair resulted in altered subcellular localization of STR-1, STR-44, and SRSX-3, but not STR-163, in AWB (Figure 2, B–D, Table 2). Mutating these residues also did not affect STR-163 localization in ASK (Table 2). In the cases of affected GPCRs, the proteins were mistargeted since we observed fusion proteins localized to the dendrites and cell bodies (Figure 2, B–D). In particular, we observed SRSX-3mut::GFP localization to the AWB cell body membrane as well as in punctae in dendrites (Figure 2D) suggesting that this fusion protein was sorted to the membrane but not targeted to cilia. Despite containing a hydrophobic/basic residue pair

Table 2 C-terminal hydrophobic/basic residue pair is required for ciliary localization of a subset of GPCRs

		% localized to:[a]					
Fusion protein[b]	Neuron	Cilium	Dendrite, cell body	Cilium, dendrite, cell body, axons	Cell body	No expression	*P*-value[c]
STR-1	AWB	100	0	0	0	0	
STR-1mut	AWB	0	0	97	0	3	<0.001
STR-44	AWB	100	0	0	0	0	
STR-44mut	AWB	0	0	100	0	0	<0.001
STR-163	AWB	100	0	0	0	0	
STR-163mut	AWB	100	0	0	0	0	
STR-163	ASK	70	0	0	9	21	
STR-163mut	ASK	52	0	0	15	33	
SRSX-3	AWB	100	0	0	0	0	
SRSX-3mut	AWB	0	100[d]	0	0	0	<0.001
SRU-38	ASK	81	0	0	3	16	
SRU-38mut	ASK	0	0	0	90	10	<0.001
SRD-23	AWB	100	0	0	0	0	
SRD-23mut	AWB	100	0	0	0	0	

Adult animals grown at 20° were examined; $n > 30$ animals each.

[a] Fusion protein expression was enriched in the indicated subcellular locations, although weaker expression was occasionally detected elsewhere in the cell.

[b] See Figure 2A for sequences mutated in the corresponding receptor proteins.

[c] Differences among proportions in different categories were compared with respective wild-type values in the relevant neuron type for statistical significance. *P*-values were determined using a χ^2 test of independence. Only differences at $P < 0.05$ or lower are indicated.

[d] The fusion protein was localized to the membrane.

at the C terminus, SRU-38 is localized to the cilia of ASK, but not AWB neurons (Figure 1H), indicating that these residues are not sufficient to drive ciliary localization of GPCRs in AWB. Replacing these residues with alanines resulted in loss of ciliary localization of SRU-38 in ASK (Figure 2E, Table 2), indicating a requirement of this motif for ciliary localization of GPCRs in different cell types. Intriguingly, although the FR ciliary targeting motif in rhodopsin interacts directly with the ASAP1 Arf4 GAP protein (Wang *et al.* 2012), the *C. elegans* genome does not encode an ASAP1 ortholog, implying that this motif may also be recognized by alternate ciliary trafficking mechanisms.

In addition to the hydrophobic/basic residue, the C terminus of rhodopsin also contains a VxPx motif required for localization to the photoreceptor outer segment via interaction with the Arf GTPase (Deretic *et al.* 1998, 2005; Wang *et al.* 2012). A VxPx motif was identified in the C terminus of SRD-23 (Figure 2A); however, mutating this motif did not affect SRD-23 localization to the AWB cilia (Table 2). We did not identify a VxPx motif in the C termini of SRBC-64 or SRAB-16. We also did not detect CTSs found in the third intracellular loops of GPCRs such as Sstr3 and Mchr1 (melanin-concentrating hormone receptor 1) (Berbari *et al.* 2008a; Nagata *et al.* 2013), in equivalent domains of any of the examined GPCRs in *C. elegans*. However, we noted that the third intracellular loops of both STR-163 and SRAB-16 contain residues homologous to those shown to be necessary and/or sufficient for ciliary localization of the NPY2R neuropeptide receptor in mammalian hypothalamic neurons (Loktev and Jackson 2013) (Figure S2). Replacing these residues in STR-163 with alanines did not affect ciliary localization of this fusion protein in AWB (Figure 3A), indicating that these sequences are not necessary to target STR-163 to cilia in this neuron type. Since CTSs in this region can be divergent (Loktev and Jackson 2013; Mukhopadhyay *et al.* 2013), we next asked whether these sequences are sufficient to target GPCRs to cilia. An SRBC-64/SRAB-16 chimeric protein, in which all sequences including, and C-terminal to, the third intracellular loop of SRBC-64 were replaced with sequences from SRAB-16, remained localized throughout the AWB cell and its processes similar to the full-length SRBC-64::GFP fusion protein (Figure 3C), suggesting that the SRAB-16 sequences are not sufficient to target SRBC-64 to AWB cilia. Taken together, these results indicate that *C. elegans* may deploy both conserved and novel mechanisms to traffic GPCRs to cilia in specific cell types.

Novel C-terminal sequences are required for ciliary localization of STR-163 and SRBC-64 in AWB and ASK

We next performed deletion and mutagenesis experiments to identify sequences required for ciliary localization of STR-163 and SRBC-64 in AWB and ASK. We focused primarily on the C termini of these proteins since both the hydrophobic/basic and VxPx CTSs are present in this region in other GPCRs, and sequences in this domain have been shown to be both necessary and sufficient to direct GPCRs to cilia (Deretic *et al.* 1998, 2005; Dwyer *et al.* 2001; Corbit *et al.* 2005; Wang *et al.* 2012; Loktev and Jackson 2013).

Deletion of the C-terminal 36 amino acids immediately following the last transmembrane domain (STR-163Δ311–347) resulted in mislocalization of STR-163 to the membranes of all cellular compartments in AWB but to the cell bodies in ASK (Figure 3A). Thus, residues within this region are required for ciliary localization of this fusion protein although we cannot rule out effects on protein folding. Deletion of the C-terminal 18 amino acids (STR-163Δ329–347) also resulted in a similar mislocalization pattern in both neuron types, whereas deletion of the C-terminal 9

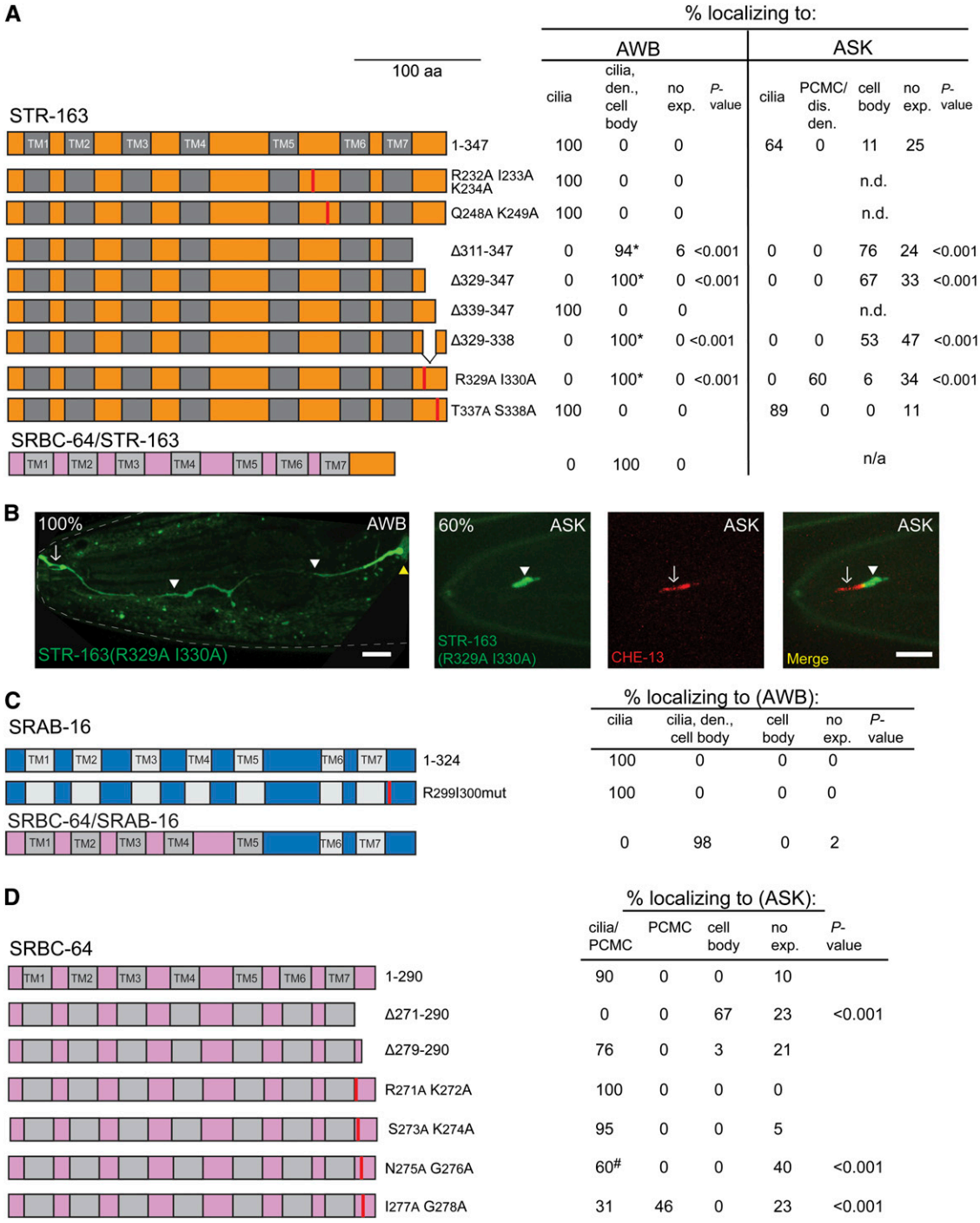

Figure 3 Identification of novel sequences required for ciliary localization of GPCRs in AWB or ASK. (A) Localization patterns of full-length and indicated mutant STR-163::GFP fusion proteins in AWB and ASK. The localization pattern of a SRBC-64/STR-163::GFP chimeric fusion protein in AWB is also shown. GFP coding sequences were fused to the C termini of the indicated proteins. Sequences mutated in the third intracellular loop are shown in Figure S2. *, localization to the membranes of the indicated cellular compartments; vertical red lines, position of mutated residues; TM, transmembrane domain; den., dendrite; dis. den., (includes ciliary base) dendrite; PCMC, periciliary membrane compartment; no exp., no expression; n.d., not done; n/a, not applicable. *n* = 30–60 animals each; two independent transgenic lines were examined for each construct. (B) Localization of mutant STR-163::GFP fusion proteins in AWB (left panel) and ASK (right panels). ASK cilia were visualized via cell-specific expression of CHE-13::TagRFP. White arrows indicate cilia; white arrowheads indicate dendritic domains/PCMC; yellow arrowheads indicate cell bodies. Bars, 10 μm (left panel), 5 μm (right panels). (C) Localization pattern of full-length, chimeric, and mutant SRAB-16::GFP in AWB. Vertical red lines indicate position of mutated residues. *n* = 20–35 animals each; two independent transgenic lines were examined for each construct. (D) Localization of full-length and mutant SRBC-64::GFP fusion proteins in ASK. Vertical red lines indicate position of mutated residues. #, weak expression. *n* = 20–35 animals each; two independent transgenic lines were examined for each construct.

amino acids (STR-163Δ339–347) did not affect ciliary localization (Figure 3A). We note that the conserved YR residues in the C terminus of STR-163 (Figure 2A) are retained in STR-163Δ329–347, further confirming that this motif is neither necessary nor sufficient to localize STR-163 to cilia. Deletion of residues 329–338 in an otherwise wild-type protein (STR-163Δ329–338) disrupted ciliary localization in both neuron types (Figure 3A), supporting the notion that residues within this region are necessary for ciliary localization in both AWB and ASK. However, these sequences are not sufficient for ciliary restriction since a chimeric SRBC-64 fusion protein containing the C-terminal 36 amino acids of STR-163 was not restricted to the cilia of AWB neurons (Figure 3A).

To further identify the required residues within amino acids 329–338 of STR-163, we replaced residues with alanines in a pairwise manner and examined the localization of the corresponding fusion proteins. We found that mutating R329 and I330, but not T337 and S338, to alanines affected ciliary localization of STR-163::GFP in both neuron types (Figure 3, A and B). Interestingly, the mislocalization pattern was distinct in AWB and ASK. In AWB, STR-163(R329A I330A)::GFP was mislocalized to the AWB cell membrane similar to the mislocalization pattern of the STR-163Δ329–338 fusion protein (Figure 3, A and B). In contrast, STR-163(R329A I330A)::GFP accumulated at the PCMC region in ASK (Figure 3, A and B). Thus, in AWB, the RI motif is required to restrict STR-163 to the cilia; in the absence of this motif, the fusion protein is localized at the cell membrane perhaps via unmasking of a nonciliary membrane targeting signal as described previously in rhodopsin (Lodowski *et al.* 2013). However, in ASK, the RI motif may specifically mediate trafficking of STR-163 into the cilium from the ciliary base or be required to retain STR-163 within the cilium, while additional sequences within the 329–338 region of STR-163 may enable membrane targeting of STR-163 as well as trafficking to the ASK ciliary base. We asked whether a similar RI motif is required to target other GPCRs to cilia in *C. elegans*. We did not identify an RI sequence in SRD-23 or SRBC-64, but noted an RI sequence in the C terminus of SRAB-16 (R299 I300). Mutating this residue pair did not affect localization of SRAB-16 in AWB (Figure 3C), indicating that alternative sequences or context are important for ciliary trafficking of this GPCR.

We performed similar truncation and mutational analyses to identify sequences required for ciliary targeting of the SRBC-64 pheromone receptor in ASK. Required sequences are likely present within amino acids 271–278 in the C terminus of SRBC-64 since, while a SRBC-64Δ271–290 fusion protein was mislocalized, a SRBC-64Δ279–290 fusion protein was localized to the cilia in ASK neurons (Figure 3D). To further identify required sequences, we replaced residues within amino acids 271–278 of SRBC-64 with alanines. We found that mutating several residues within this region in a pairwise manner did not affect SRBC-64 ciliary localization in ASK (Figure 3D). However, although SRBC-64(N275A G276A)::GFP continued to be localized to ASK cilia, expression of this fusion protein was decreased, and a significantly larger fraction of animals failed to exhibit GFP expression (Figure 3D). Effects on ciliary localization were also observed upon mutating the I277 and G278 residues; localization of the SRBC-64(I277A G278A) fusion protein was restricted to the ASK ciliary base in a significant percentage of animals (Figure 3D). We conclude that multiple residues within amino acids 271–278 of SRBC-64 contribute to correct ciliary localization of this GPCR in ASK.

Since sequences in the C termini of GPCRs can interact with G proteins (Oldham and Hamm 2006; Qin *et al.* 2011), we asked whether mutations or truncation of these C-terminal residues alter ciliary localization indirectly via altered coupling to G proteins. We previously showed that SRBC-64 acts via the GPA-2 and GPA-3 Gα subunits to transduce pheromone signals in the ASK neurons (Kim *et al.* 2009). We found that SRBC-64::GFP localization was unaltered in *gpa-2 gpa-3* double mutants (Figure S3), suggesting that association with these Gα proteins is not necessary for localization of SRBC-64 to cilia. However, since each sensory neuron type in *C. elegans* expresses multiple Gα protein subunits Jansen *et al.* 1999 we cannot exclude the possibility that association with other Gα proteins contributes to ciliary targeting of GPCRs.

Distinct trans-acting mechanisms act in a cell- and receptor-specific manner to localize GPCRs to sensory cilia

The observation that the requirement of the RI motif in localizing STR-163 to the cilia is distinct in AWB and ASK provided us with an opportunity to compare ciliary trafficking mechanisms of an individual GPCR across two distinct sensory neuron types. Moreover, since distinct motifs are required for ciliary localization of STR-44 and STR-163 in AWB, and similarly, distinct residues are employed for ciliary localization of STR-163 and SRBC-64 in ASK, we reasoned that comparison of the *trans*-acting mechanisms required for ciliary localization of different GPCRs within a given cell type would also allow us to describe the diversity of required mechanisms that act in a GPCR- and cell-specific manner to traffic and localize these proteins to cilia. We examined whether pathways and molecules previously shown to be required for ciliary localization of subsets of GPCRs and other ciliary transmembrane molecules in both *C. elegans* and mammals also operate to localize STR-44, STR-163, and SRBC-64 to the cilia of AWB and/or ASK.

Vesicular transport pathways: BBS proteins comprising the BBSome have been implicated in the ciliary localization of GPCRs including Sstr3, MchR1, rhodopsin, and the neuropeptide receptor NPY2R in mammalian cells (Nishimura *et al.* 2004; Abd-El-Barr *et al.* 2007; Berbari *et al.* 2008b; Jin *et al.* 2010; Domire *et al.* 2011; Loktev and Jackson 2013). However, STR-44 retained ciliary localization in AWB, and STR-163 remained localized to the cilia of both

Table 3 Vesicular trafficking and chaperone/adaptor proteins required for localization of GPCR::GFP fusion proteins to AWB and ASK cilia

			% localized to:[a]					
Strain[b]	Fusion protein	Neuron	Cilium	Cilium, PCMC/distal dendrite	Cilium, dendrite, cell body	Cell body	No expression	*P*-value[c]
Wild type	STR-44	AWB	100	0	0	0	0	
	STR-163	AWB	100	0	0	0	0	
	STR-163	ASK	67	0	0	8	25	
	SRBC-64	ASK	0	100	0	0	0	
Vesicular transport								
bbs-8(nx77)	STR-44	AWB	95	0	0	0	5	
	STR-163	AWB	100	0	0	0	0	
	STR-163	ASK	67	0	0	24	9	
bbs-1(ok1111)	STR-44	AWB	100	0	0	0	0	
	STR-163	AWB	100	0	0	0	0	
	STR-163	ASK	48	0	0	19	33	
rab-8(tm2526)	STR-44	AWB	100	0	0	0	0	
	STR-163	AWB	100	0	0	0	0	
	STR-163	ASK	67	0	0	9	24	
arl-3(tm1703)	STR-44	AWB	96	0	0	0	4	
	STR-163	AWB	100	0	0	0	0	
	STR-163	ASK	64	0	0	4	32	
arl-13(tm2322)	STR-44	AWB	100	0	0	0	0	
	STR-163	AWB	100	0	0	0	0	
	STR-163[d]	ASK	0	90	0	0	10	<0.001
	SRBC-64	ASK	0	100	0	0	0	
arl-13(gk513)	STR-44	AWB	100	0	0	0	0	
	STR-163	AWB	100	0	0	0	0	
	STR-163	ASK	0	89	0	0	11	<0.001
	SRBC-64	ASK	0	100	0	0	0	
Chaperone/adaptor								
odr-4(n2144)	STR-44	AWB	0	0	0	76	24	<0.001
	STR-163	AWB	100	0	0	0	0	
	STR-163	ASK	80	0	0	20	0	
	SRBC-64	ASK	0	100	0	0	0	
unc-101(m1)	STR-44	AWB	0	0	0	65	35	<0.001
	STR-163[d]	AWB	100	0	0	0	0	
	STR-163	ASK	0	0	0	12	88	<0.001
	SRBC-64	ASK	0	0	57	0	43	<0.001
daf-25(m98)	STR-44	AWB	0	0	97	3	0	<0.001
	STR-163	AWB	47[e]	0	6	0	47	<0.001
	STR-163	ASK	0	0	35	30	35	<0.001
	SRBC-64	ASK	0	0	78	0	22	<0.001
daf-25(m362)	STR-44	AWB	0	0	100	0	0	<0.001

Adult animals grown at 20° were examined. $n \geq 20$ animals. Expression from the same array was examined in wild-type and mutant strains.

[a] Localization patterns were examined in animals exhibiting relatively wild-type ciliary morphology with the exception of *unc-101* mutants. AWB and ASK cilia were either truncated or lacked a cilium in AWB in the majority of animals in *unc-101* mutants (Dwyer *et al.* 2001; Kaplan *et al.* 2010).

[b] Mutant strains were examined together with wild type in each experiment.

[c] Differences among proportions in different categories were compared with respective wild-type values in the relevant neuron type for statistical significance. *P*-values were determined using a χ^2 test of independence. Only differences at $P < 0.05$ or lower are indicated.

[d] Cilia in these strains were visualized via expression of *str-1*p::*mCherry* (AWB) or *srbc-66*p::*mCherry* (ASK).

[e] Expression was weak.

AWB and ASK in *bbs-1* and *bbs-8* mutants (Table 3), indicating that ciliary localization of these GPCRs is independent of the two examined BBSome components in AWB and ASK.

The Rab8 small GTPase has been shown to be required for docking and fusion of rhodopsin-containing post-Golgi vesicles at the base of the photoreceptor outer segment and is also required for ciliary localization of other GPCRs (Deretic *et al.* 1995; Moritz *et al.* 2001; Kaplan *et al.* 2010; Mukhopadhyay *et al.* 2010). Similarly, the Arl13 and Arl3 Arf-family small GTPases (Zhang *et al.* 2013) play roles in ciliary transmembrane protein targeting and localization in *C. elegans* sensory neurons and in mammalian cells (Schrick *et al.* 2006; Cevik *et al.* 2010; Larkins *et al.* 2011; Humbert *et al.* 2012; Li *et al.* 2012; Schwarz *et al.* 2012). No effects were found on STR-44 or STR-163 localization in *rab-8* mutants in either AWB or ASK (Table 3). Similarly, we did not observe an effect of loss of *arl-3* function on the localization of these proteins in either cell type (Table 3). However, mutations in *arl-13* affected ciliary localization of STR-163 only in ASK such that the STR-163::GFP fusion protein exhibited increased accumulation at the ASK ciliary base and distal dendrite with reduced expression in the cilium

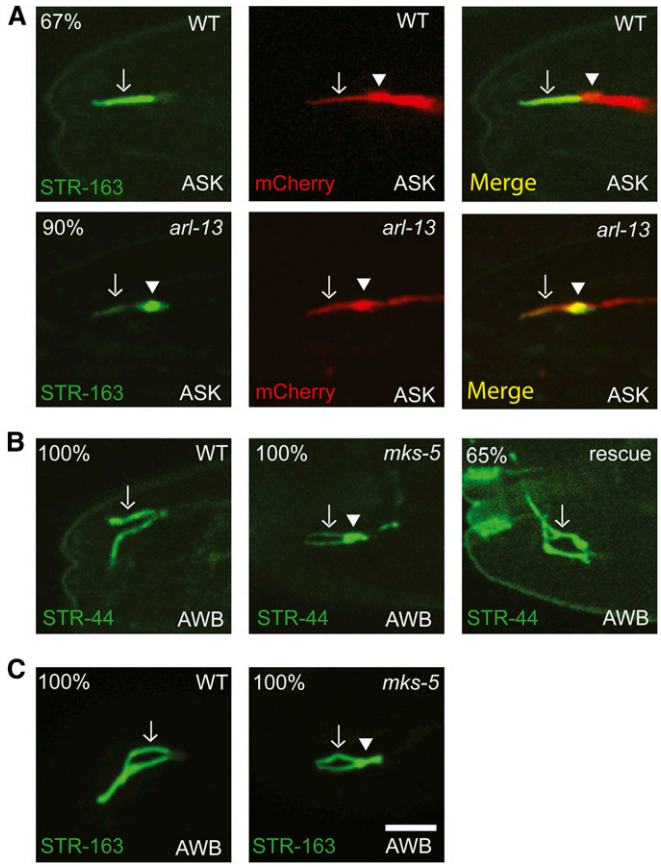

Figure 4 ARL-13 and MKS-5 are required for ciliary localization of GPCRs in AWB and ASK. (A–C) Localization patterns of GPCR::GFP fusion proteins in ASK (A) or AWB (B and C) in the indicated genetic backgrounds. Numbers in top left corners indicate the percentage of examined animals exhibiting the phenotype (see Table 3 and Table 4). Wild-type *mks-5* sequences were expressed specifically in AWB under the *str-1* promoter to assess rescue of the STR-44::GFP localization and ciliary morphological defects (B). White arrows indicate cilia; white arrowheads indicate dendritic domains. ASK cilia and dendrites were visualized via expression of mCherry under the *srbc-66* promoter (A). Alleles used here were *arl-13 (tm2322)* and *mks-5(tm3100)*. Bar, 5 μm.

as compared to the wild-type expression pattern (Figure 4A, Table 3). However, SRBC-64::GFP localization in ASK was unaffected in *arl-13* mutants (Table 3), indicating that ARL-13 does not regulate ciliary localization of all ASK-expressed GPCRs.

Chaperone/adaptor proteins: Ciliary trafficking and localization of subsets of odorant receptors in a subset of *C. elegans* sensory neurons including the AWB neurons require chaperones such as ODR-4, the UNC-101, and APS-1 μ- and σ1-subunits of the AP1 clathrin adaptor complex and the CHC-1 clathrin heavy chain (Dwyer *et al.* 1998, 2001; Kaplan *et al.* 2010). We found that ODR-4 and UNC-101 regulate ciliary localization of different GPCRs in a cell-specific manner. Specifically, in AWB, ciliary localization of STR-44 required ODR-4 and UNC-101, whereas neither protein is required for ciliary localization of STR-163 in this neuron type (Table 3). In contrast, in ASK, ciliary localization of both STR-163 and SRBC-64 require UNC-101 but not ODR-4 (Table 3). Only a small number of animals expressed STR-163::GFP in the ASK cell bodies in *unc-101* mutants (Table 3). We confirmed that expression driven by the *srbc-66* promoter used to misexpress STR-163::GFP in ASK was not affected by mutations in *unc-101* (Figure S4), suggesting that the protein may be instead degraded upon mistargeting and/or mislocalization.

We and others have shown that the DAF-25 Ankmy2 protein is required for ciliary trafficking of proteins such as receptor guanylyl cyclases and cyclic nucleotide-gated channels implicated in sensory signal transduction in *C. elegans* sensory cilia (Fujiwara *et al.* 2010; Jensen *et al.* 2010; Wojtyniak *et al.* 2013). We examined whether this protein also regulates ciliary localization of GPCRs. In *daf-25* mutants, all examined GPCRs were mislocalized and/or failed to be expressed in both AWB and ASK (Table 3). Although a fraction of STR-163::GFP fusion proteins retained ciliary localization in AWB in *daf-25* mutants, expression levels were markedly decreased (Table 3). Thus, DAF-25 regulates the trafficking of multiple GPCRs in different cell types.

Transition zone proteins: The transition zone at the base of the cilium is thought to comprise a ciliary gate restricting access of nonciliary proteins to the cilium proper (Nachury *et al.* 2010; Garcia-Gonzalo *et al.* 2011; Hu and Nelson 2011; Czarnecki and Shah 2012; Reiter *et al.* 2012; Shiba and Yokoyama 2012; Szymanska and Johnson 2012). This gate is composed of a large multiprotein complex including MKS and NPHP protein modules, which interact genetically and physically, although the exact interactions may be organism and/or cell type-specific (Jauregui and Barr 2005; Bialas *et al.* 2009; Hu *et al.* 2010; Sang *et al.* 2011; Williams *et al.* 2011; Chih *et al.* 2012). Mutations in one or more of these proteins can result in defects in ciliary membrane protein composition (McEwen *et al.* 2007; Craige *et al.* 2010; Williams *et al.* 2011; Chih *et al.* 2012; Lechtreck *et al.* 2013). We found that loss of multiple components of the MKS module in *mks-1*; *mksr-2*; *mksr-1* triple mutants did not affect GPCR localization in either AWB or ASK (Table 4). We also did not observe effects on GPCR localization in *nphp-4* mutants in either cell type (Table 4).

MKS-5 has been suggested to act as a scaffold for the placement of MKS and NPHP proteins at ciliary transition zones in *C. elegans*, such that localization of components of these modules are altered in *mks-5* mutants (Williams *et al.* 2011). We found that loss of MKS-5 altered GPCR localization specifically in AWB (Figure 4, B and C, Table 4). STR-44::GFP and STR-163::GFP accumulated both in the cilia as well as at the ciliary base of AWB (Figure 4, B and C, Table 4); both cilia morphological and STR-44::GFP localization defects were rescued upon cell-specific expression of wild-type *mks-5* (Figure 4B, Table 4). Since AWB, but not ASK cilia, are significantly truncated in *mks-5* mutants (Figure 4,

Table 4 Transition zone and IFT components required for trafficking and localization of GPCR::GFP fusion proteins to AWB and ASK cilia

			% localized to:[a]						
Strain[b]	Fusion protein	Neuron	Cilium	Cilium, PCMC/distal dendrite	PCMC/Distal dendrite[c]	Cilium, dendrite, cell body	Cell body	No expression	*P*-value[d]
Wild type	STR-44	AWB	100	0	0	0	0	0	
	STR-163	AWB	100	0	0	0	0	0	
	STR-163	ASK	68	0	0	0	11	21	
	SRBC-64	ASK	0	95	0	0	0	5	
TZ + inversin compartment									
mks-1(tm2705); mksr-2(tm2452); mksr-1(ok2092)	STR-44	AWB	100	0	0	0	0	0	
	STR-163	AWB	100	0	0	0	0	0	
	STR-163	ASK	48	0	0	0	19	33	
nphp-4(tm925)	STR-44	AWB	100	0	0	0	0	0	
	STR-163	AWB	100	0	0	0	0	0	
	STR-163	ASK	85	0	0	0	0	15	
mks-5(tm3100)	STR-44[e]	AWB	0	100	0	0	0	0	<0.001
	STR-163[e]	AWB	0	100	0	0	0	0	<0.001
	STR-163	ASK	52	0	0	0	15	33	
Ex[*str-1*p::*mks-5*]	STR-44	AWB	65[e]	35	0	0	0	0	<0.001[f]
nphp-2(gk653)	STR-44	AWB	100	0	0	0	0	0	
	STR-163	AWB	100	0	0	0	0	0	
	STR-163	ASK	68	0	0	0	9	23	
	SRBC-64	ASK	0	95	0	0	0	5	
IFT related									
osm-3(p802)	STR-44	AWB	100	0	0	0	0	0	
	STR-163	AWB	100	0	0	0	0	0	
	STR-163	ASK	72	0	0	0	14	14	
kap-1(ok676)	STR-44	AWB	100	0	0	0	0	0	
	STR-163	AWB	100	0	0	0	0	0	
	STR-163	ASK	67	0	0	0	14	19	
tub-1(nr2004)	STR-44[e]	AWB	0	100	0	0	0	0	<0.001
	STR-163[e]	AWB	0	100	0	0	0	0	<0.001
	STR-163[e]	ASK	0	0	81	0	0	19	<0.001
	SRBC-64	ASK	0	100	0	0	0	0	
tub-1(nr2044)	STR-163[e]	AWB	0	100	0	0	0	0	<0.001
daf-10(e1387)	STR-44[e]	AWB	0	100	0	0	0	0	<0.001
	STR-163[e]	AWB	0	100	0	0	0	0	<0.001
	STR-163	ASK	62	0	0	0	24	14	
	SRBC-64	ASK	0	0	100	0	0	0	<0.001
daf-10(p821)	STR-44[e]	AWB	0	100	0	0	0	0	<0.001
	SRBC-64	ASK	0	0	100	0	0	0	<0.001

Adult animals grown at 20° were examined. $n \geq 20$ animals each. Expression from the same array was examined in wild-type and mutant strains except for the *mks-1*; *mksr-2*; *mksr-1* triple mutant strain, which was injected with the indicated fusion constructs. TZ, transition zone.

[a] Localization patterns were examined in animals exhibiting relatively wild-type ciliary morphology with the exception of *mks-5*, *tub-1*, *daf-10*, and *osm-3* mutants. AWB cilia are truncated in *mks-5* and *tub-1* mutants; AWB and ASK cilia are truncated in *daf-10* mutants; ASK cilia are truncated in *osm-3* mutants.

[b] Mutant strains were examined together with wild type in each experiment.

[c] Weak expression was observed in cilia.

[d] Differences among proportions in different categories were compared with respective wild-type values unless indicated otherwise in the relevant neuron type for statistical significance. *P*-values were determined using a χ^2 test of independence. Only differences at $P < 0.05$ or lower are indicated.

[e] Cilia in these strains were visualized via expression of *str-1*p::mCherry (AWB) or *srbc-66*p::*che-13*::*TagRFP* (ASK).

[f] As compared to values in *mks-5* mutants. The AWB ciliary morphological defects were also rescued (see Figure 4B).

B and C, Figure S5), it is possible that the GPCR localization defect is a secondary consequence of the ciliary morphological defect. NPHP-2/Inversin is localized to a restricted proximal ciliary domain and required for correct transition zone placement in *C. elegans* cilia (Warburton-Pitt *et al.* 2012). Mutations in *nphp-2* alter ciliary localization of different cyclic nucleotide-gated channel subunits in AWB and ASK (Wojtyniak *et al.* 2013). However, mutations in NPHP-2 did not affect ciliary localization of any examined GPCR in either AWB or ASK (Table 4). Thus, loss of specific transition zone and associated proteins affect ciliary localization of GPCRs differentially in AWB and ASK.

IFT motor proteins: In vertebrates, members of the kinesin 2 family of anterograde IFT motors regulate the transport of opsin to the photoreceptor outer segments, and are also

required for movement of activated Smoothened receptor to cilia (Marszalek *et al.* 2000; Jimeno *et al.* 2006; Kovacs *et al.* 2008; Avasthi *et al.* 2009; Trivedi *et al.* 2012). We asked whether anterograde kinesin motors play a role in regulating ciliary trafficking of GPCRs in AWB and ASK. Mutations in either the *osm-3* homodimeric kinesin or the *kap-1* kinesin II subunit had no effect on localization of STR-44 and STR-163 in either AWB or ASK cilia (Table 4), although as expected, ASK cilia were truncated in *osm-3* mutants (Perkins *et al.* 1986; Snow *et al.* 2004). Taken together, these observations indicate that multiple pathways regulate ciliary trafficking and/or localization of examined GPCRs in AWB and ASK in both a cell- and receptor-specific manner (see Figure 5E for a summary).

Tubby-like proteins and the IFT-A complex are required for ciliary localization of GPCRs in AWB and ASK neurons

Tubby-like proteins are instrumental in trafficking GPCRs in the cilium in part by acting as a bridge between the GPCR molecule and the IFT-A complex (Hagstrom *et al.* 2001; Mukhopadhyay *et al.* 2010; Sun *et al.* 2012; Loktev and Jackson 2013). The *C. elegans* genome encodes a single tubby-like protein TUB-1, which has been shown to move in both sensory neuron dendrites and cilia; TUB-1 has been suggested, but not demonstrated, to play a role in GPCR recycling (Mukhopadhyay *et al.* 2005, 2007a; Mak *et al.* 2006). We found that ciliary localization of STR-44 and STR-163 in AWB, and STR-163 in ASK, was altered in *tub-1* mutants (Figure 5, A–C, Table 4). STR-44::GFP accumulated at the ciliary base and distal dendritic region of AWB in *tub-1* mutant animals (Figure 5A, Table 4). Similar accumulation of STR-163::GFP at the ciliary base and distal dendritic region in both AWB and ASK was also observed in *tub-1* animals (Figure 5, B and C, Table 4). AWB, but not ASK cilia, were severely truncated in *tub-1* mutants (Figure 5, A–D), suggesting that the altered localization pattern in AWB may not simply be a secondary effect of the cilia structural defects. However, loss of TUB-1 function had little effect on localization of SRBC-64 in ASK (Figure 5D, Table 4). These results indicate that TUB-1 regulates ciliary localization of subsets of GPCRs in both AWB and ASK.

The Tubby-like protein TULP3 has been shown to interact directly with core components of the IFT-A complex to mediate ciliary trafficking of GPCRs (Mukhopadhyay *et al.* 2010). Thus, mutations in IFT-A proteins also result in defects in ciliary localization of GPCRs such as Sstr3 (Mukhopadhyay *et al.* 2010). Although many of the N-terminal residues required for TULP3 interaction with IFT-A components are not conserved in *C. elegans* TUB-1 (Mukhopadhyay *et al.* 2010), we asked whether IFT-A core proteins such as DAF-10/IFT122 play a role in regulating GPCR ciliary localization in *C. elegans*. We found that in AWB, STR-44 and STR-163 fusion proteins exhibited a localization pattern in *daf-10* mutants that resembled the pattern observed in *tub-1* mutant animals (Figure 5, A and B, Table 4); both fusion

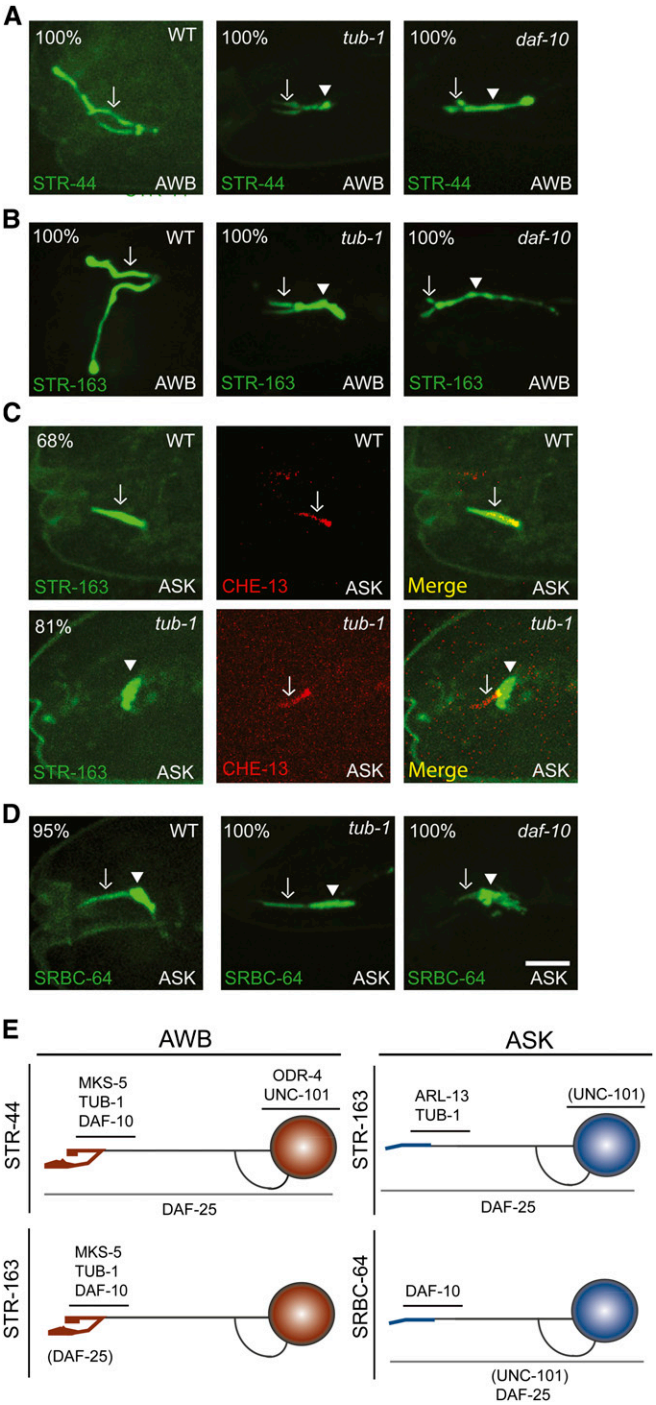

Figure 5 The TUB-1 Tubby homolog regulates ciliary localization of GPCRs. (A–D) Localization patterns of STR-44::GFP (A), STR-163::GFP (B and C), and SRBC-64::GFP (D) fusion proteins in AWB and/or ASK in the indicated genetic backgrounds. Numbers in top left corners indicate the percentage of examined animals exhibiting the phenotype (see Table 4). White arrows indicate cilia; white arrowheads indicate dendritic domains. AWB cilia are truncated in *tub-1* and *daf-10* mutants (A and B); ASK cilia are truncated in *daf-10* mutants (D). ASK cilia were visualized via cell-specific expression of CHE-13::TagRFP (C). Bar, 5 μm. (E) Cartoon summarizing effects of mutations in the indicated trafficking proteins on ciliary localization of STR-44 in AWB, STR-163 in AWB and ASK, and SRBC-64 in ASK. Horizontal lines above or below protein names indicate the site of GPCR mislocalization in the relevant mutant background.

proteins accumulated in the cilia and the distal dendritic region in AWB (Figure 5, A and B, Table 4). However, TUB-1 and DAF-10 regulate ciliary localization of different GPCRs in ASK, such that TUB-1 but not DAF-10 was required for ciliary localization of STR-163 (Figure 5C, Table 4), whereas the converse was true for ciliary localization of SRBC-64 (Figure 5D, Table 4). These results suggest that TUB-1 and IFT-A complex proteins may act in the same pathway to localize a subset of GPCRs to cilia in AWB, but that these proteins act in distinct pathways for ciliary localization of GPCRs in ASK (summarized in Figure 5E).

Discussion

Our results indicate that *C. elegans* sensory neurons employ a remarkable diversity of *cis*-acting sequences and *trans*-acting mechanisms to localize GPCRs to their cilia. We find that multiple mechanisms operate to not only target different GPCRs to cilia within an individual cell type, but to also mediate ciliary targeting of a specific GPCR across cell types. These mechanisms could operate at different levels including regulation of protein sorting, dendritic or ciliary trafficking, and/or tethering to localize GPCRs to cilia. Our observations demonstrate that ciliary localization of GPCRs is a highly regulated process that likely ensures the generation of defined cellular responses to external cues in a context-dependent manner.

A conclusion both from this work, as well as from previous studies, is that *C. elegans* sensory neurons do not appear to contain a mechanism that acts generally and broadly to sort all cilia-destined membrane proteins (*e.g.*, Dwyer *et al.* 1998, 2001; Bae *et al.* 2006; Cevik *et al.* 2010; Jensen *et al.* 2010; Wojtyniak *et al.* 2013). For instance, although the DAF-25 Ankmy2 protein regulates ciliary localization of all GPCRs examined in this work, previous studies have shown that mutations in *daf-25* affect localization of only a subset of other ciliary membrane proteins, including GPCRs, in different cell types (Fujiwara *et al.* 2010; Jensen *et al.* 2010; Wojtyniak *et al.* 2013). The clathrin adaptor AP-1 complex has been suggested to act broadly to retrieve cilia-targeted membrane proteins from a default trafficking pathway that would otherwise sort these proteins to the general cellular membrane compartment (Kaplan *et al.* 2010). Indeed, in AP-1 complex mutants, multiple ciliary membrane proteins including channels and GPCRs are mistargeted to the nonciliary plasma membrane (Dwyer *et al.* 2001; Bae *et al.* 2006; Kaplan *et al.* 2010). However, our results indicate that the AP-1 complex acts in a protein- and cell-specific manner to target GPCRs to cilia such that localization of STR-44, but not STR-163, to AWB cilia is affected in *unc-101* mutants, whereas UNC-101 is required for ciliary localization of both STR-163 and SRBC-64 in ASK. Since the AP-1 complex acts in the *trans*-Golgi network and endosomes to mediate early steps in protein sorting (Folsch *et al.* 2001; Owen *et al.* 2004; Robinson 2004), these observations suggest that diverse mechanisms operate even at these early steps to target GPCRs to cilia. Consistent with this hypothesis, distinct *cis*-acting residues are also required to localize the examined GPCRs both within and across cell types.

We speculate that differences in cilia structure may in part contribute to the observed diversity in protein trafficking and localization pathways. As an example, mutations in the *mks-5* TZ gene affect cilia structure (Williams *et al.* 2011) as well as both STR-44 and STR-163 ciliary localization in AWB, but have no effect in ASK. MKS-5 may play a more critical role in regulating TZ structure and/or function in AWB than in ASK. In turn, defects in ciliary gate function of the TZ in AWB may alter ciliary transport of STR-44 and STR-163 into or out of the AWB cilia leading to protein accumulation at the ciliary base. Conversely, mutations in the *arl-13* small GTPase gene result in accumulation of STR-163 in the distal dendrites of ASK, but have no effect on ciliary localization of examined GPCRs in AWB. Loss of *arl-13* function also leads to abnormal accumulation of other ciliary proteins in either the distal dendritic regions or in the cilium proper in cell types other than AWB (Cevik *et al.* 2010; Li *et al.* 2012). Interestingly, although ARL-13 localization is restricted to the membrane of only the proximal ciliary domain in the majority of examined cilia (Cevik *et al.* 2010; Li *et al.* 2012), ARL-13 is present throughout the cilia in AWB (Cevik *et al.* 2010). The cell-specific subciliary localization pattern of ARL-13 may reflect distinct membrane composition of AWB and ASK cilia, leading in turn to different effects of ARL-13 on ciliary GPCR localization. Alternatively, since ARL-13 has been shown to coordinate the IFT-A and IFT-B complexes in *C. elegans* cilia (Cevik *et al.* 2010; Li *et al.* 2010), the cell-specific roles of this protein in localizing GPCRs in AWB and ASK cilia may be a consequence of the cell-specific IFT mechanisms reported in these neuron types (Snow *et al.* 2004; Mukhopadhyay *et al.* 2007b).

A particularly intriguing observation was our finding that mutations in the *tub-1 tubby*-like gene result in defects in GPCR localization in both AWB and ASK such that proteins accumulate at the ciliary base and distal dendrite. Loss of *tub-1* function results in metabolic defects and increased fat storage in *C. elegans* similar to phenotypes observed in *tubby* mice (Coleman and Eicher 1990; Ashrafi *et al.* 2003; Mukhopadhyay *et al.* 2005, 2007a; Mak *et al.* 2006; Wang *et al.* 2006). Moreover, *tub-1* mutant worms exhibit chemosensory deficits and extended lifespan (Mukhopadhyay *et al.* 2005, 2007a). Taken together with the observation that TUB-1 is expressed in chemosensory neurons and is present in their dendrites and cilia (Mukhopadhyay *et al.* 2005; Mak *et al.* 2006), these results have led to the hypothesis that the metabolic defects in *tub-1* mutants arise from defective endocrine signaling as a consequence of sensory deficits (Mukhopadhyay *et al.* 2005, 2007a; Mak *et al.* 2006). Tubby and Tubby-like proteins have previously been shown to be required to traffic GPCRs and channels to cilia in mammals and *Drosophila* (Hagstrom *et al.* 2001; Mukhopadhyay *et al.*

2010, 2013; Sun *et al.* 2012; Loktev and Jackson 2013; Park *et al.* 2013). In *tubby* mutant mice, defects in ciliary localization of the anorexigenic NPY2R GPCR in arcuate nucleus neurons of the hypothalamus have been suggested to partly underlie the metabolic defects in these animals (Loktev and Jackson 2013). Our results suggest that defects in ciliary localization of specific sensory GPCRs may also be a major underlying cause of the sensory and metabolic deficits in *tub-1* mutants in *C. elegans*.

How does TUB-1 facilitate ciliary GPCR localization? Although the residues required for interaction of Tubby with IFT-A proteins are not present in *C. elegans* TUB-1 (Mukhopadhyay and Jackson 2011), mutations in the *daf-10*/IFT122 IFT-A protein result in GPCR mislocalization phenotypes in AWB, similar to those in *tub-1* mutants. However, phenotypes in *tub-1* and *daf-10* mutants are distinct in ASK, suggesting that possible interactions between TUB-1 and IFT-A proteins may be complex and cell specific. Interaction of Tubby proteins with phosphoinositides via highly conserved KR residues is also important for ciliary membrane protein trafficking (Santagata *et al.* 2001; Mukhopadhyay *et al.* 2010; Park *et al.* 2013). The KR motif is conserved in TUB-1, suggesting that interaction with membrane lipids may represent a conserved mechanism by which TUB-1 localizes transmembrane proteins to cilia. Tubby proteins may also regulate endocytosis of signaling proteins (Mukhopadhyay *et al.* 2005, 2007a; Chen *et al.* 2012). We and others previously showed that defects in endocytosis result in ciliary protein localization defects as well as characteristic ciliary morphological defects (Hu *et al.* 2007; Kaplan *et al.* 2012). Although phenotypes in *tub-1* mutants are not identical to those in endocytic mutants, we cannot exclude the possibility that the GPCR mislocalization phenotypes in *tub-1* mutants arise from defects in endocytosis. In the future, it will be important to further define the mechanisms by which TUB-1 localizes GPCRs to the cilia of specific sensory neuron types in *C. elegans* and determine whether mislocalization of ciliary signaling proteins is causal to the metabolic phenotypes of *tub-1* mutants.

Finally, it is interesting to speculate on the necessity for the observed diversity in GPCR ciliary localization mechanisms in *C. elegans* sensory neurons. Each *C. elegans* sensory neuron expresses multiple sensory GPCRs, likely allowing the neuron to respond to a range of sensory cues (Troemel *et al.* 1995; Bargmann 2006). Coordinated regulation of all neuron-specific GPCRs would, therefore, coordinately alter responses to all cues sensed by that neuron type. Instead, we and others have previously shown that the expression of different GPCRs in a neuron type is regulated by distinct mechanisms under different external and internal conditions, allowing animals to more precisely modulate their sensory behaviors (Troemel *et al.* 1999; Peckol *et al.* 2001; Lanjuin and Sengupta 2002; Nolan *et al.* 2002; Van Der Linden *et al.* 2007). Diversity and cell specificity in ciliary localization mechanisms may represent yet another layer by which GPCR function and neuronal sensory responses are regulated in *C. elegans*. Diversity in *trans*-acting factors predicts that the sequences in GPCRs that these proteins interact with are also diverse (Emmer *et al.* 2010). Indeed, our results indicate that different residues are required for correct ciliary trafficking of GPCRs both within, as well as between, AWB and ASK. Related systematic studies in other organisms may uncover a similarly broad range of mechanisms that ensure that the correct complement of GPCRs is localized to the cilia of specific cell types as a function of internal and external conditions.

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Diverse Cell Type-Specific Mechanisms Localize G Protein-Coupled Receptors to *Caenorhabditis elegans* Sensory Cilia

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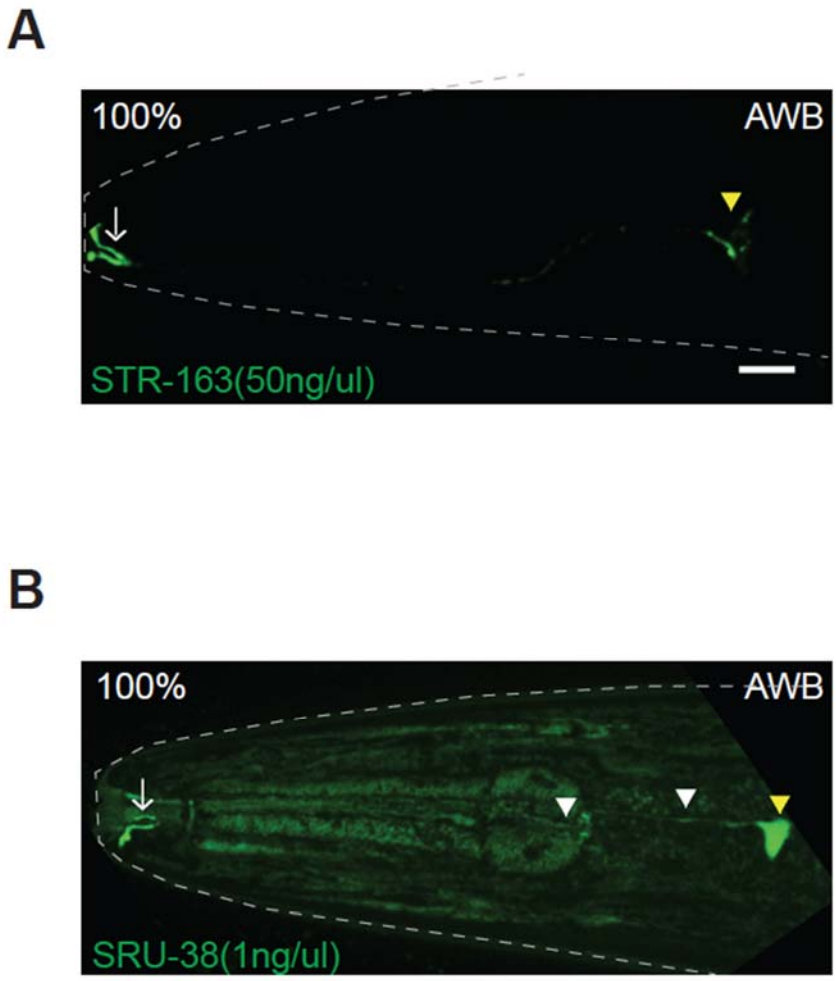

Figure S1 Changes in expression levels do not affect localization pattern of GPCRs.
A-B) The subcellular localization patterns of STR-163::GFP **(A)** and SRU-38::GFP **(B)** in AWB are unaltered when expressed at higher or lower concentrations, respectively (compare with Figures 1E and 1H). Yellow and white arrowheads indicate cell bodies and dendrites; white arrows indicate cilia. Numbers in top left corners indicate the percentage of examined animals exhibiting the phenotype. n= >30 animals each. Scale bar: 10 μm.

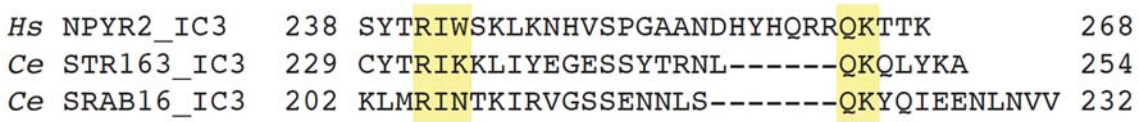

Figure S2 A subset of GPCRs contain conserved ciliary targeting sequences in their third intracellular loops. Alignment of sequences in the third intracellular loops of NPY2R, STR-163 and SRAB-16. Conserved residues shown to be necessary and/or sufficient for ciliary targeting in NPY2R (Loktev and Jackson, 2013) are shaded.

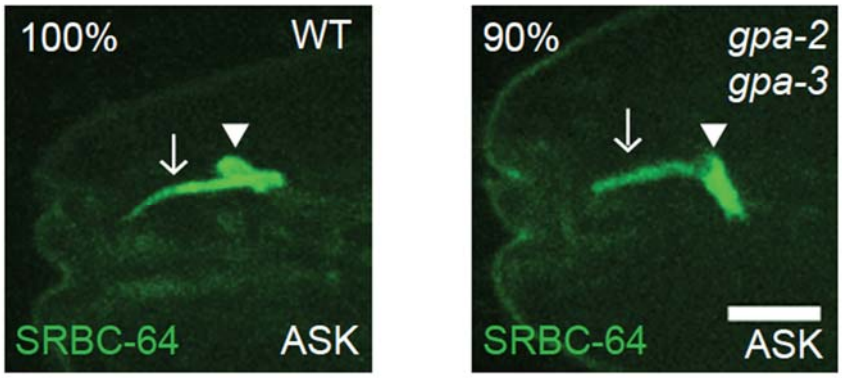

Figure S3 Ciliary localization of SRBC-64::GFP is unaltered in *gpa-2 gpa-3* mutants. Shown is the localization pattern of SRBC-64::GFP in the ASK neurons of wild-type animals, or animals doubly mutant for the *gpa-2* and *gpa-3* Gα protein subunit genes. Alleles used were *gpa-2(pk16)* and *gpa-3(pk35)*. Number in top left corner indicates the percentage of animals exhibiting the shown phenotype. n≥30 each. Scale bar: 5 μm.

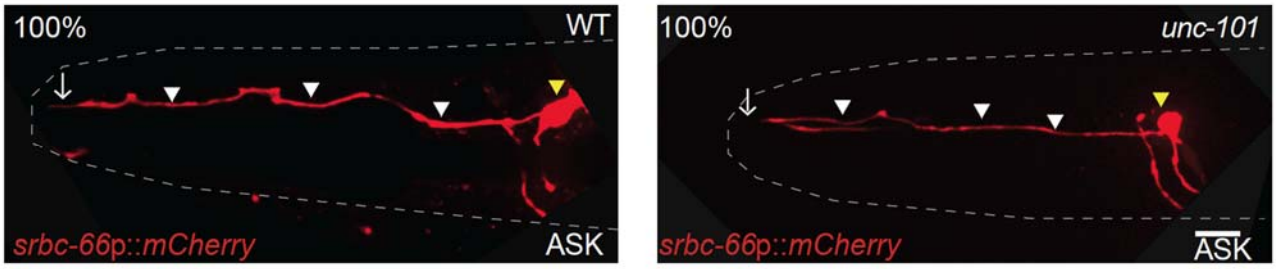

Figure S4 Mutations in *unc-101* do not affect expression driven under the *srbc-66* promoter. Shown is the expression of *srbc-66*p*::mCherry* in ASK in wild-type and *unc-101(m1)* mutants. White and yellow arrowheads indicate dendrites and cell bodies; white arrows indicate cilia. The upstream regulatory sequences of *srbc-66* were also used to misexpress *str-163* in ASK. n= 30 each. Scale bar: 10 μm.

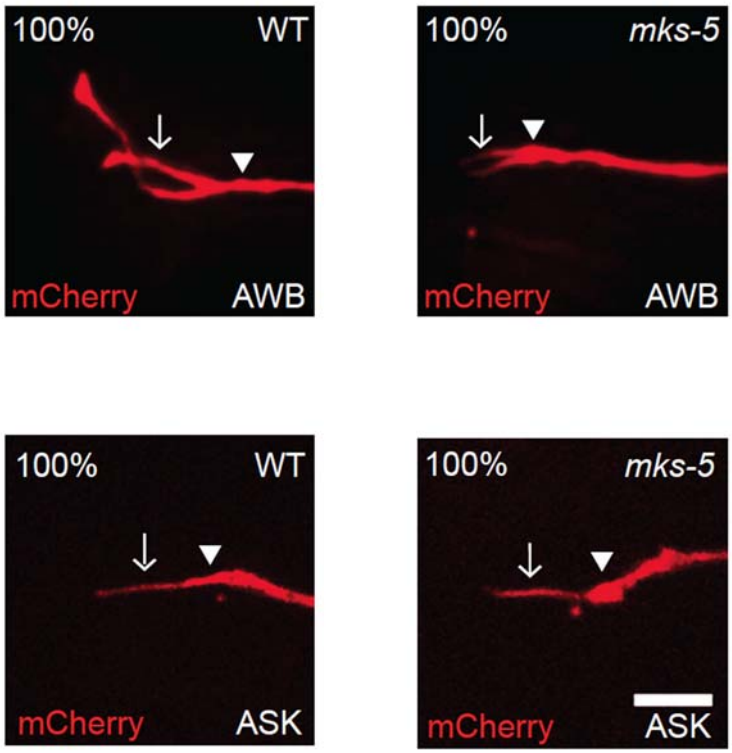

Figure S5 AWB, but not ASK, cilia are truncated in *mks-5(tm3100)* mutants. Shown are the morphologies of AWB and ASK cilia visualized in the indicated genetic backgrounds using *str-1*p*::gfp* and *srbc-66*p*::gfp* transcriptional reporter fusions, respectively. Numbers in top left corner indicate percentage of examined animals exhibiting the phenotype. Arrows and arrowheads indicate cilia and dendrites, respectively. n= 30 each. Scale bar: 5 μm.

Table S1 Alleles examined in this study.

Gene (allele)	Nature of allele[a]	References
bbs-8(nx77)	deletion	(Blacque *et al.* 2004)
bbs-1(ok1111)	deletion	(Ou *et al.* 2007)
rab-8(tm2526)	deletion	(Mukhopadhyay *et al.* 2008)
arl-3(tm1703)	deletion	(Li *et al.* 2010)
arl-13(tm2322)	deletion	(Cevik *et al.* 2010; Li *et al.* 2010)
arl-13(gk513)	deletion	(Li *et al.* 2010)
odr-4(n2144)	missense	(Dwyer *et al.* 1998)
unc-101(m1)	nonsense	(Dwyer *et al.* 2001)
daf-25(m98)	deletion	(Jensen *et al.* 2010)
daf-25(m362)	deletion	(Jensen *et al.* 2010)
mks-1(tm2705)	deletion	(Bialas *et al.* 2009)
mksr-2(tm2452)	deletion	(Bialas *et al.* 2009)
mksr-1(ok2092)	deletion	(Williams *et al.* 2011)
nphp-4(tm925)	deletion	(Jauregui and Barr 2005)
mks-5(tm3100)	deletion	(Williams *et al.* 2011)
nphp-2(gk653)	deletion	(Warburton-Pitt *et al.* 2012)
osm-3(p802)	nonsense	(Shakir *et al.* 1993)
kap-1(ok676)	insertion	(Snow *et al.* 2004)
tub-1(nr2004)	deletion	(Mak *et al.* 2006)
tub-1(nr2044)	deletion	(Mak *et al.* 2006)
daf-10(e1387)	nonsense	(Bell *et al.* 2006)
daf-10(p821)	Splice site mutation	(Bell *et al.* 2006)

[a]Information from Wormbase (www.wormbase.org) or from indicated references.

Table S2 Strains used in this work.

Strain	Genotype	Source
PY9012	Ex[*str-1*p::*srab-16*::*gfp*]	This work
PY9014	Ex[*str-1*p::*str-44*::*gfp*]	This work
PY9015	Ex[*str-1*p::*str-44*mut::*gfp*]	This work
PY9019	Ex[*str-1*p::*str-163*::*gfp*]	This work
PY9022	Ex[*str-1*p::*srd-23*::*gfp*]	This work
PY9023	Ex[*srbc-66*p::*str-163*::*gfp*]	This work
PY9025	Ex[*str-1*p::*srsx-3*::*gfp*]	This work
PY9026	Ex[*str-1*p::*srsx-3*mut::*gfp*]	This work
PY9029	*odr-4(n2144)*; Ex[*str-1*p::*str-44*::*gfp*]	This work
PY9030	Ex[*srbc-66*p::*str-163*(Δ311-347)::*gfp*]	This work
PY9031	Ex[*str-1*p::*str-163*(Δ311-347)::*gfp*]	This work
PY9033	Ex[*srbc-66*p::*sru-38*::*gfp*]	This work
PY9034	Ex[*srbc-66*p::*sru-38*mut::*gfp*]	This work
PY9035	Ex[*str-1*p::*str-1*mut::*gfp*]	This work
PY9036	*nphp-2(gk653);* Ex[*srbc-66*p::*str-163*::*gfp*]	This work
PY9041	*daf-25(m98);* Ex[*srbc-66*p::*str-163*::*gfp*]	This work
PY9043	*arl-3(tm1703);* Ex[*str-1*p::*str-163*::*gfp*]	This work
PY9044	*nphp-2(gk653);* Ex[*str-1*p::*str-163*::*gfp*]	This work
PY9046	*osm-3(p802);* Ex[*str-1*p::*str-163*::*gfp*]	This work
PY9047	*odr-4(n2144);* Ex[*str-1*p::*str-163*::*gfp*]	This work
PY9048	*nphp-4(tm925);* Ex[*str-1*p::*str-163*::*gfp*]	This work
PY0949	*osm-3(p802);* Ex[*srbc-66*p::*str-163*::*gfp*]	This work
PY9050	*rab-8(tm2526);* Ex[*str-1*p::*str-163*::*gfp*]	This work
PY9051	*tag-232(gk325);* Ex[*str-1*p::*str-44*::*gfp*]	This work
PY9052	*kap-1(ok676);* Ex[*srbc-66*p::*str-163*::*gfp*]	This work
PY9053	*osm-3(p802);* Ex[*str-1*p::*str-44*::*gfp*]	This work
PY9054	*bbs-1(ok1111);* Ex[*str-1*p::*str-44*::*gfp*]	This work
PY9055	*bbs-8(nx77);* Ex[*srbc-66*p::*str-163*::*gfp*]	This work
PY9056	*rab-8(tm2526);* Ex[*str-1*p::*str-44*::*gfp*]	This work
PY9057	*rab-8(tm2526);* Ex[*srbc-66*p::*str-163*::*gfp*]	This work
PY9058	*bbs-1(ok1111);* Ex[*srbc-66*p::*str-163*::*gfp*]	This work
PY9059	*arl-13(tm2322);* Ex[*str-1*p::*str-163*::*gfp*]	This work
PY9060	*tag-232(gk325);* Ex[*str-1*p::*str-163*::*gfp*]	This work
PY9061	*kap-1(ok676);* Ex[*str-1*p::*str-163*::*gfp*]	This work
PY9063	*daf-25(m98);* Ex[*str-1*p::*str-44*::*gfp*]	This work
PY9064	*bbs-1(ok1111);* Ex[*str-1*p::*str-163*::*gfp*]	This work
PY9065	*unc-101(m1);* Ex[*str-1*p::*str-44*::*gfp*]	This work
PY9066	*bbs-8(nx77);* Ex[*str-1*p::*str-163*::*gfp*]	This work
PY9067	*odr-4(n2144);* Ex[*srbc-66*p::*str-163*::*gfp*]	This work
PY9068	Ex[*srbc-64*p::*srbc-64*::*gfp*]	This work
PY9069	Ex[*srbc-64*p::*srbc-64*(Δ279-290)::*gfp*]	This work
PY9070	*kap-1(ok676);* Ex[*str-1*p::*str-44*::*gfp*]	This work
PY9071	*arl-3(tm1703);* Ex[*str-1*p::*str-44*::*gfp*]	This work
PY9072	*mks-1(tm2705), mksr-1(ok2092), mksr-2(tm2452);* Ex[*srbc-66*p::*str-163*::*gfp*]	This work
PY9073	*arl-13(tm2322);* Ex[*str-1*p::*str-44*::*gfp*]	This work
PY9074	*mks-5(tm3100);* Ex[*srbc-66*p::*str-163*::*gfp*]	This work
PY9075	Ex[*srbc-66*p::*str-163*(Δ311-347)::*gfp*]	This work
PY9076	Ex[*str-1*p::*srbc-64*::*gfp*]	This work
PY9077	Ex[*str-1*p::*srbc-64/str-163*chimera::*gfp*]	This work
PY9078	Ex[*srbc-66*p::*str-163*(Δ329-338)::*gfp*]	This work
PY9079	Ex[*srbc-66*p::*srd-23*::*gfp*]	This work
PY9081	*unc-101(m1);* Ex[*srb*c-64p::*srbc-64*::*gfp*]	This work

PY9082	*arl-3(tm1703);* Ex[*srbc-66*p*::str-163::gfp*]	This work
PY9083	*bbs-8(nx77);* Ex[*str-1*p*::str-44::gfp*]	This work
PY9084	Ex[*srbc-66*p*::str-44::gfp*]	This work
PY9085	Ex[*srbc-66*p*::str-*163mut*::gfp*]	This work
PY9086	*daf-25(m98);* Ex[*str-1*p*::str-163::gfp*]	This work
PY9087	Ex[*srbc-64*p*::srbc-64(*Δ271-290*)::gfp*]	This work
PY9089	Ex[*srbc-66*p*::srab-16::gfp*]	This work
PY9090	Ex[*str-1*p*::sru-38::gfp*]	This work
PY9091	*nphp-2(gk653*); Ex[*srbc-64*p*::srbc-64::gfp*]	This work
PY9092	*arl-13(tm2322);* Ex[*srbc-64*p*::srbc-64::gfp*]	This work
PY9093	*nphp-2(gk653);* Ex[*str-1*p*::str-44::gfp*]	This work
PY9094	*tub-1(nr2004);* Ex[*srbc-64*p*::srbc-64::gfp*]	This work
PY9095	Ex[*srbc-64*p*::srbc-64(*R271A K272A*)::gfp*]	This work
PY9096	Ex[*str-1*p*::str-163(*Δ339-347*)::gfp*]	This work
PY9097	*daf-10(e1387);* Ex[*srbc-66*p*::str-163::gfp*]	This work
PY9098	Ex[*srbc-66*p*::str-1::gfp*; *srbc-66*p*::che-13::TagRFP*]	This work
PY9099	*daf-10(e1387); oyIs65*[*str-1*p*::mCherry*]; Ex[*str-1p::str-163::gfp*]	This work
PY9100	Ex[*srbc-66*p*::srsx-3::gfp; srbc-66*p*::che-13::TagRFP*]	This work
PY9101	Ex[*srbc-66*p*::str-163::gfp*] Ex[*srbc-66*p*::che-13::tagrfp*]	This work
PY9102	Ex[*str-1*p*::str-1::gfp*]	This work
PY9103	*tub-1(nr2004);* Ex[*srbc-66p::str-163::gfp*] [*srbc-66*p*::str-163::gfp*]	This work
PY9104	*tub-1(nr2004); oyIs65*[*str-1*p*::mCherry*]; Ex[*str-1*p*::str-44::gfp*]	This work
PY9105	*mks-5(tm3100); oyIs65*[*str-1*p*::mCherry*]; Ex[*str-1*p*::str-163::gfp*]	This work
PY9106	Ex[*srbc-66*p*::str-1::gfp; srbc-66*p*::che-13::tagrfp*]	This work
PY9107	Ex[*srbc-*66p*::str-163(*R329A I330A)*::gfp; srbc-66*p*::che-13::TagRFP]*	This work
PY9108	Ex[*str-1*p*::str-163(*R329A I330A)*::gfp*]	This work
PY9109	*mks-5(tm3100); oyIs65(str-1*p*::mCherry);* Ex[*str-1*p*::str-44::gfp]*	This work
PY9110	*nphp-4(tm925);* Ex[*srbc-66*p*::str-163::gfp*]	This work
PY9111	*nphp-4(tm925);* Ex[*str-1*p*::str-163::gfp*]	This work
PY9112	*mks-5(tm3100); oyIs65*[*str-1*p*::mCherry*]*;* Ex[*str-1*p*::str-44::gfp*]	This work
PY9115	*daf-25(m98)* Ex[*srbc-64*p*::srbc-64::gfp*]	This work
PY9116	*tub-1(nr2004);* oyIs65[str-*1*p*::mCherry*]*;* Ex[*str-1*p*::str-163::gfp]*	This work
PY9117	*mks-1(tm2705), mksr-1(ok2092), mksr-2(tm2452) Ex*[*str-1*p::*str-44::gfp*]	This work
PY9118	Ex[*str-1*p*::srd-23*mut*::gfp*	This work
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PY9121	Ex[*str-1*p*::str-163(*Δ329-338)*::gfp*]	This work
PY9122	*unc-101(m1);* Ex[*str-1*p*::str-163::gfp*]	This work
PY9123	Ex[*srbc-64*p*::srbc-64(*N275A G276A)*::gfp*]	This work
PY9124	*daf-10(e1387);* Ex[*srbc-64*p*::srbc-64::gfp*]	This work
PY9125	Ex[*srbc-66*p::*str-163(*T337A S338A)*::gfp*]	This work
PY9126	Ex[*str-1*p*::str-163*(T337A S338A)*::gfp*]	This work
PY9127	*daf-10(p821);* Ex[*srbc-64*p*::srbc-64::gfp*]	This work
PY9128	*daf-10(p821); oyIs65*[*str-1*p*::mCherry*]*;* Ex[*str-1*p*::str-44::gfp*]	This work
PY9130	*arl-13(tm2322);* Ex[*srbc-66*p*::str-163::gfp*] Ex[*srbc-66*p*::che-13::tagrfp*]	This work
PY9131	*mks-5(tm3100); oyIs65*[*str-1p::mCherry*]*;* Ex[*str-1*p*::str-44::gfp*] Ex[*str-1*p*::mks-5*]	This work
PY9132	*odr-4(n2144);* Ex[*srbc-64*p*::srbc-64::gfp*]	This work
PY9133	*unc-101(m1);* Ex[*srbc-66*p*::str-163:;gfp*]	This work
PY9134	Ex[*srbc-64*p*::srbc-64*(I277A G278A)*::gfp*]	This work
PY9135	Ex[*str-1*p*::srab-16*(R299A I300A)*::gfp*]	This work
PY9136	Ex[*str-1*p*::str-163*mut*::gfp*]	This work
PY9137	*unc-101(m1);* Ex[*srbc-66*p*::str-163::gfp*]	This work
PY9138	*arl-13(tm1746);* Ex[*srbc-66*p*::str-163::gfp*] Ex[*srbc-66*p*::mCherry*]	This work

PY9139	*daf-25(m362)* Ex[*str-1*p::*str-44::gfp*]	This work
PY9140	*srbc-64(tm1946);* Ex[*srbc-64*p::*srbc-64(*Δ271-290*)*]	This work
PY9141	Ex[*str-1*p::*str-163::gfp*]	This work; plasmid injected at 50 ng/μl
PY9142	Ex[*str-1*p::*str-163*(Δ329-347)::*gfp*]	This work
PY9143	Ex[*srbc-64*p::*srbc-64*(S273A K274A)::*gfp*]	This work
PY9144	*tub-1(nr2044); oyIs65*[*str-1*p::*mCherry*]*;* Ex[*str-1*p::*str-163::gfp*]	This work
PY9145	Ex[*srbc-66*p::*mCherry*]	This work
PY9148	Ex[*str-1*p::*srbc-64*/*srab-16*chimera::*gfp*]	This work
PY9149	Ex[*str-1*p::*sru-38::gfp*]	This work; plasmid injected at 1 ng/μl
PY9150	Ex[*str-1*p::*str-163*(R232A I233A K234A)::*gfp*	This work
PY9151	Ex[*str-1*p::*str-163*(Q248A K249A)::*gfp*	This work
PY9152	*gpa-2(pk16), gpa-3(pk35)*; Ex[*srbc-64*p::*srbc-64::gfp*]	This work
PY9153	*arl-13(gk513);* Ex[*str-1*p::*str-44::gfp*]	This work
PY9154	*arl-13(gk513);* Ex[*str-1*p::*str-163::gfp*]	This work
PY9155	*arl-13(gk513);* Ex[*srbc-66*p::*str-163::gfp*] Ex[*srbc-66*p::*mCherry*]	This work
PY9156	*arl-13(gk513);* Ex[*srbc-64*p::*srbc-64::gfp*]	This work

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