Supplemental Information

Figure S1. Subcellular localization of select identified proteins, Related to Figure 3.

(A) Immunofluorescence imaging of IMCD3 cell cilia (ARL13B or TUB^{ac}, red) and transfected fusions of GFP with randomly selected proteins from the human proteome. The GFP fusions localized to cilia or the ciliary base (green). The distal basal body is marked by CEP164 (white). Nuclei were stained with Hoescht (blue). **(B)** Immunofluorescence imaging of IMCD3 cells stained as in (A). GFP fusions of the human homologs of randomly selected high-confidence ciliary proteins. **(C)** Immunofluorescence imaging of three human homologs of randomly selected high-confidence ciliary proteins fused to GFP (green) in the *D. rerio* embryo (somite stage 6-10). Motile and primary cilia are labeled by TUB^{ac} (red) and DNA stained with Hoescht is shown in blue. All scale bars, 5 μm.

Figure S2. Phylogenetic distribution of ciliome proteins, Related to Figure 4.

CLIME analysis of 171 ciliome members that display a phylogenetic profile closely reflecting the distribution of cilia (listed in Table S6). The species tree includes ciliated species in black (*G. lamblia, T. vaginalis, T. brucei, T. cruzi, L. infantum, L. major, L. braziliensis, P. knowlesi, P. vivax, P. falciparum, P. chabaudi, P. berghei, P. yoelii, P. tetraurelia, T. thermophila, P. infestans, T. pseudonana, N. gruberi, C. reinhardtii, V. carteri, P. patens, S. moellendorffii, T. trahens, A. macrogynus, S. punctatus, S. arctica, C. owczarzaki, M. brevicollis, S. rosetta, S. mansoni, C. briggsae, C. elegans, D. pulex, A. pisum, P. humanus, A. mellifera, N. vitripennis, B. mori, T. castaneum, D. melanogaster, D. pseudoobscura, A. gambiae, A. aegypti, C. quinquefasciatus, B. floridae, T. adhaerens, S. purpuratus, H. magnipapillata, N. vectensis, C. intestinalis, D. rerio, O. latipes, F. rubripes, T. nigroviridis, X. tropicalis, G. gallus, M. gallopavo, O. anatinus, M. domestica, S. scrofa, M. musculus, C. familiaris, B. taurus,* and *H. sapiens*). Unciliated species are in gray (prokaryotes, *E. cuniculi, E. histolytica, E. dispar, C. hominis, C. parvum, B. bovis, T. annulata, T. parva, P. tricornutum, C. merolae, O. lucimarinus, O. tauri, S. bicolor, Z. mays, O. sativa, B. distachyon, A. lyrata, A. thaliana, L. japonicus, M. truncatula, V. vinifera, P. trichocarpa, R. communis, D. discoideum, M. globosa, U. maydis, C. neoformans, P. chrysosporium, S. commune, C. cinerea, L. bicolor, S. pombe, B. fuckeliana, S. sclerotiorum, F. graminearum, M. grisea, N. crassa, P. anserine, P. chrysogenum, A. clavatus, A. fumigatus, N. fischeri, A. flavus, A. oryzae, A. niger, A. nidulans, U. reesii, C. immitis, C.*

posadasii, P. nodorum, T. melanosporum, Y. lipolytica, P. pastoris, C. lusitaniae, D. hansenii, M. guilliermondii, S. stipitis, L. elongisporus, C. tropicalis, C. albicans, C. dubliniensis, K. lactis, A. gossypii, K. waltii, L. thermotolerans, Z. rouxii, V. polyspora, C. glabrata, S. bayanus, S. mikatae, S. cerevisiae, and *S. paradoxus*). Ciliome gene symbols (left) are in gray if they are paralogs of another gene within the Evolutionary Conserved Module (ECM). Gold indicates the presence of a homolog of a SYSCILIA Gold Standard ciliary component in the corresponding species, whereas blue indicates the presence of a homolog not found in the SYSCILIA Gold Standard set. Gray indicates its absence.

Figure S3. Sea urchin signaling protein homologs localize to mammalian cilia, Related to Figure 3.

A) Immunofluorescence imaging of RPE-1 cell cilia (ARL13B, red) and a transfected fusion of sea urchin OPRM1L and GFP (green). Nuclei were stained with Hoescht (blue). Sea urchin OPRM1L-GFP localizes to cilia. **(B)** RPE-1 cells stained as above except for ARRB1-GFP, a fusion of the sea urchin ortholog of ARRB1 with GFP (green). Scale bar for whole cell images, 5 μm. Scale bar for cilia only images, 2.5 μm.

Figure S4. ENKUR alignment, Related to Figure 5.

An alignment of ENKUR orthologs from diverse eukaryotic species. Asterisks indicate amino acids that are fully conserved. Colons indicate amino acids that have strongly similar properties. Periods indicate amino acids that have weakly similar properties. Color scheme indicates residues with similar chemical properties. Genus and species names: Trypanosome, *Trypanosoma rangeli;* Chytrid*, Batrachochytrium dendrobatidis; Chlamydomonas reinhardtii;* Oomycete, *Phytopthora parasitica*; *Paramecium tetraurelia*; *Tetrahymena thermophila;* Choanoflagellate, *Salpingoeca rosetta*; Human, *Homo sapiens*; Mouse, *Mus musculus*; Zebrafish, *Danio rerio*; Sea urchin, *Strongylocenrotus purpuratus; Nematostella vectensis.*

Figure S5. ENKUR supplemental data, Related to Figure 5 and 6.

(A) Whole mount in situ hybridization of *N. vectensis* embryos at the indicated developmental stages for *Enkur*. Surface view indicates the heterogeneous expression of *Enkur*. Arrowhead marks aboral pole. Scale bar, 50μm. **(B)** In situ hybridization of sea urchin embryos at mesenchyme blastula (MB), early gastrula (EG), late gastrula (LG) and prism (PM) stage using

the sense probe for *Enkur*. Scale bar, 50 μm. **(C)** Immunofluorescence imaging of primary cultured mouse tracheal epithelial cells for cilia (TUB^{ac}, red) and ENKUR (green). Nuclei are labeled with Hoechst (blue). Cells transfected with 4 different shRNAs against *Enkur* display reduced ENKUR immunofluorescence at cilia. Scale bar, 5μm. (**D**) Immunofluorescent staining of cilia (ARL13B, red) and ENKD1 fused to GFP (green) expressed in IMCD3 cells. ENKD1-GFP co-localizes with the basal body component CEP164 (blue). Scale bar for whole cell image, 5 μm. Scale bar for cilia only image, 2.5 μm. **(E)** A schematic diagram of the wild type mouse *Enkur* allele (+) and the targeted allele (-). The targeting vector was designed to replace part of exon 2 with a neomycin-resistance and thymidine kinase fusion. **(F)** Southern blot confirming homologous recombination at the mouse *Enkur* locus (wild type, +/+; heterozygote, +/-; homozygous mutant, -/-). **(G)** RT-PCR of *X. laevis* control embryos and those injected with 10 or 20 ng *Enkur* morpholino. *Enkur* expression is reduced in morphants. (**H)** Quantification of *Pitx2c* expression patterns in control embryos, embryos injected with *Enkur* MO and embryos injected with *Enkur* MO plus plasmid encoding sea urchin *Enkur-GFP*. **(I)** Immunofluorescence image of the *Xenopus* GRP depicting ACTIN (red) and cilia (TUB^{ac}, green). Ciliation of the GRP in embryos with KD of *Enkur* expression is similar to control embryos. Scale bar, 100μm. **(J)** Representative images of *Pitx2* in situ hybridization in which *Pitx2c* expressed is on the right side, left side, both sides or is absent in *Xenopus* embryos (ventral view). (**K)** In situ hybridization of 1-4 somite stage mouse embryos using the sense probe for *Enkur*. Asterisk marks the node. Scale bar, 100μm. **(L)** A chest X-ray of affected individual OP 1605-II2 demonstrating dextracardia. The heart is indicated with an "H", right (R) and left (L) side of the body are indicated.

Figure S6. Mutation of human *ENKUR* does not affect localization of diverse proteins involved in ciliary motility, Related to Figure 7.

Immunofluorescence imaging of nasal epithelial cells from healthy, unaffected controls and an affected *ENKUR* homozygous mutant individual (OP-1605 II3) for (**A**) DNAH5 (green) and GAS8 (red), (**B**) DNAH11 (green) and RSPH9 (red), (**C**) CCDC151 (red), (**D**) DNALI1 (red), (**E**) CCDC39 (red), (**F**) CCDC11 (red), and (**C-F**) TUB^{ac} (green). Nuclei are stained with Hoechst (blue). DIC image also included. Mutation of human *ENKUR* does not disrupt the axonemal localization of these proteins involved in ciliary motility. Scale bar, 5 μm.

Figure S7. ENKUR localizes to cilia independently of DNAAF2, CCDC11 and CCDC40, Related to Figure 7.

Immunofluorescence imaging of human nasal epithelial cells for cilia (TUB^{ac}, green) and ENKUR (red) from an unaffected control individual and three individuals with PCD with the following genotypes: *DNAAF2*$^{-/-}$ (OP-146 II1), *CCDC11*$^{-/-}$ (OP-1096 II1) and *CCDC40*$^{-/-}$ (OP-799). Nuclei are stained with Hoechst (blue). DIC image also included. Scale bar, 5 μm.

Figure S1

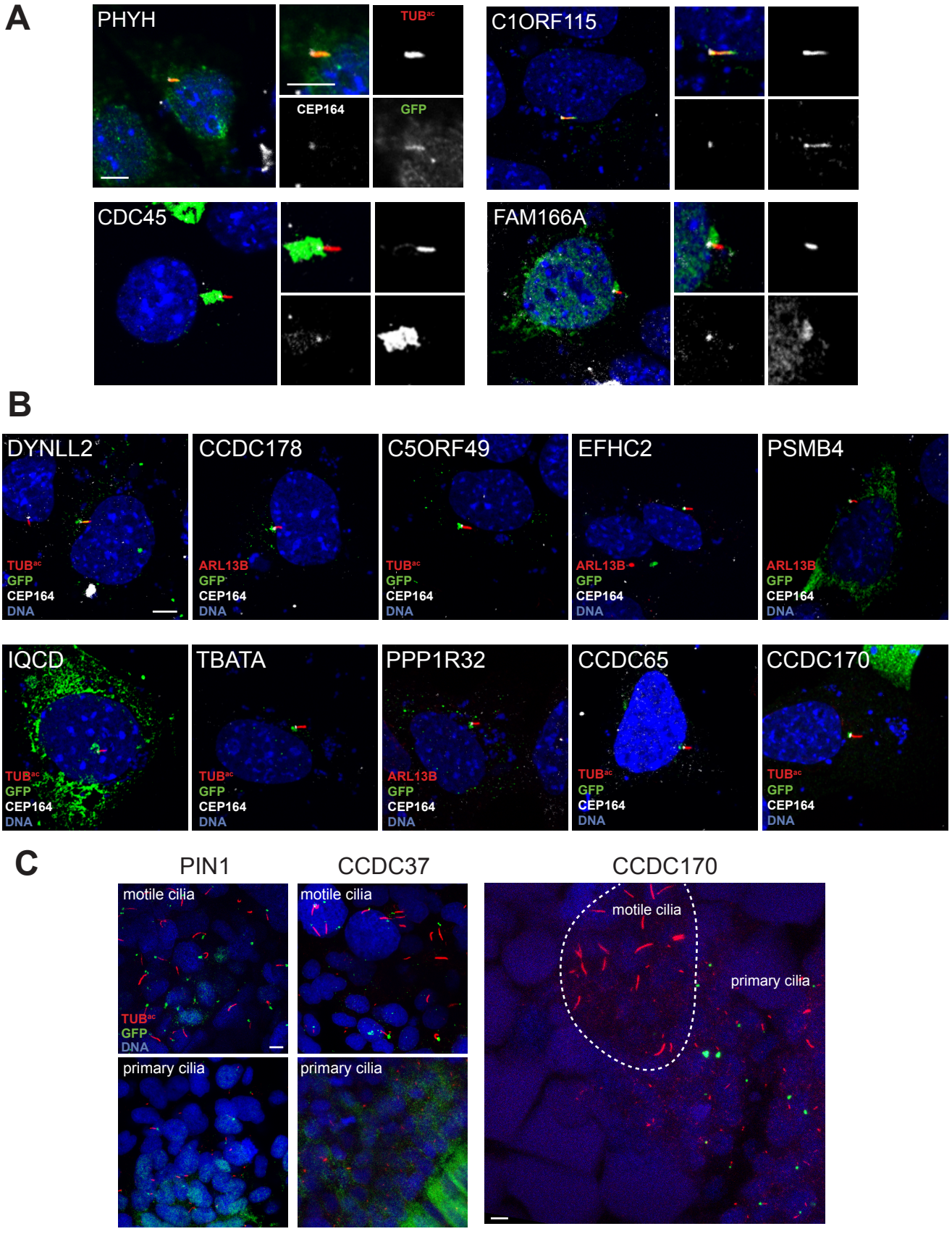
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Figure S2

Evolutionarily conserved modules (ECMs) of ciliome members

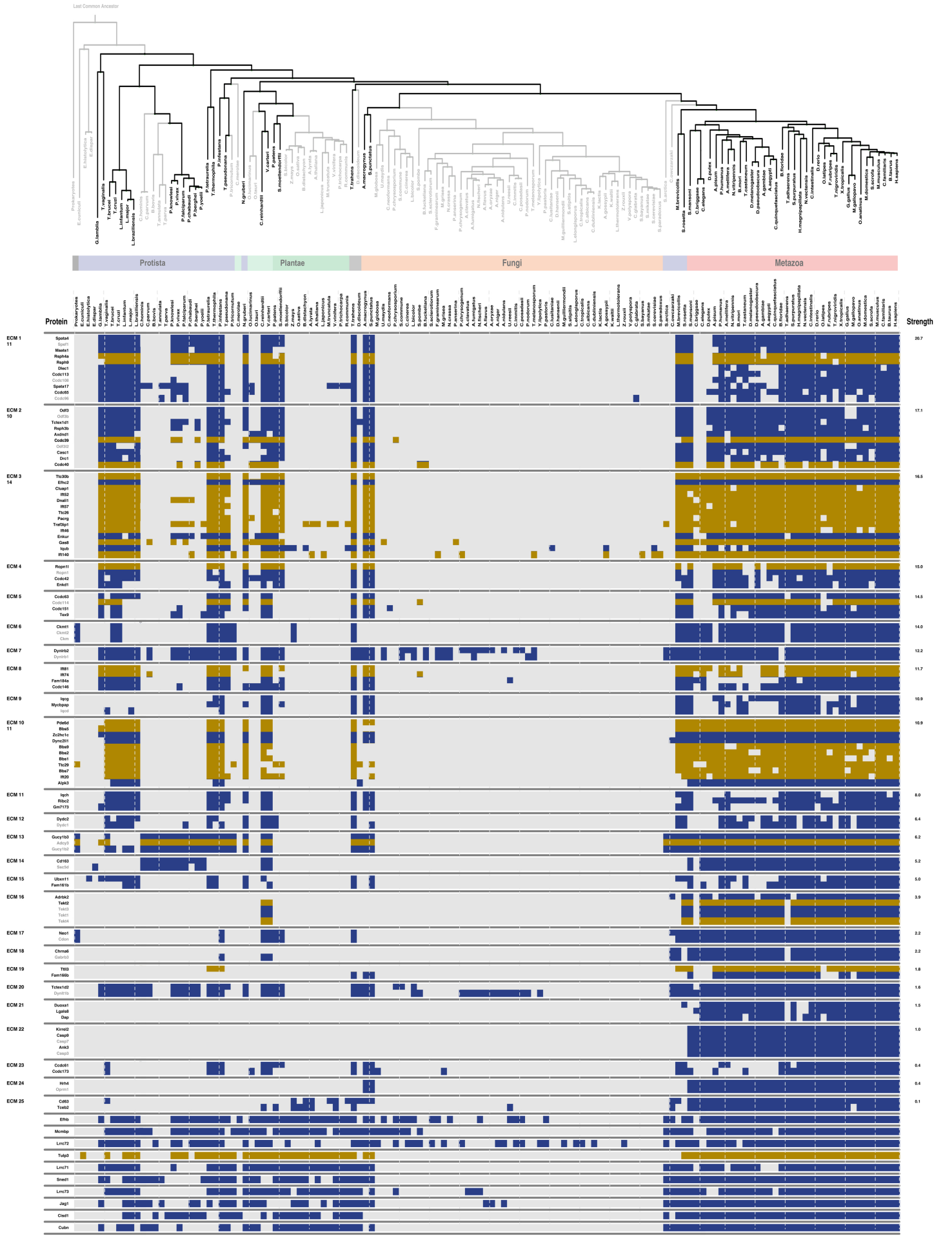


Figure S2

Evolutionarily conserved modules (ECMs) of ciliome members

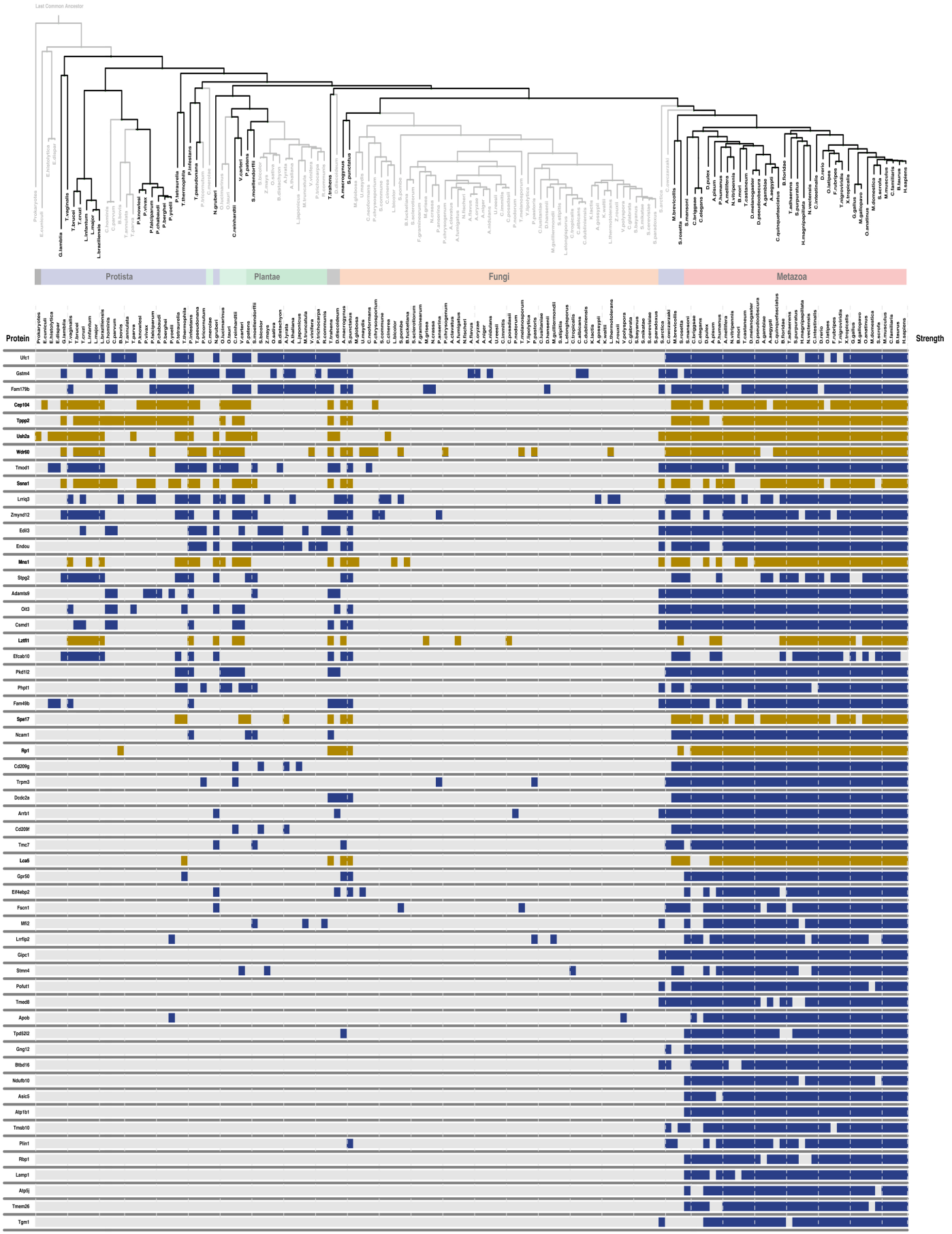


Figure S3

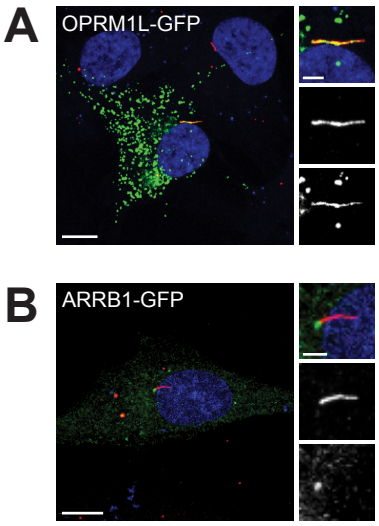


Figure S4

ENKUR

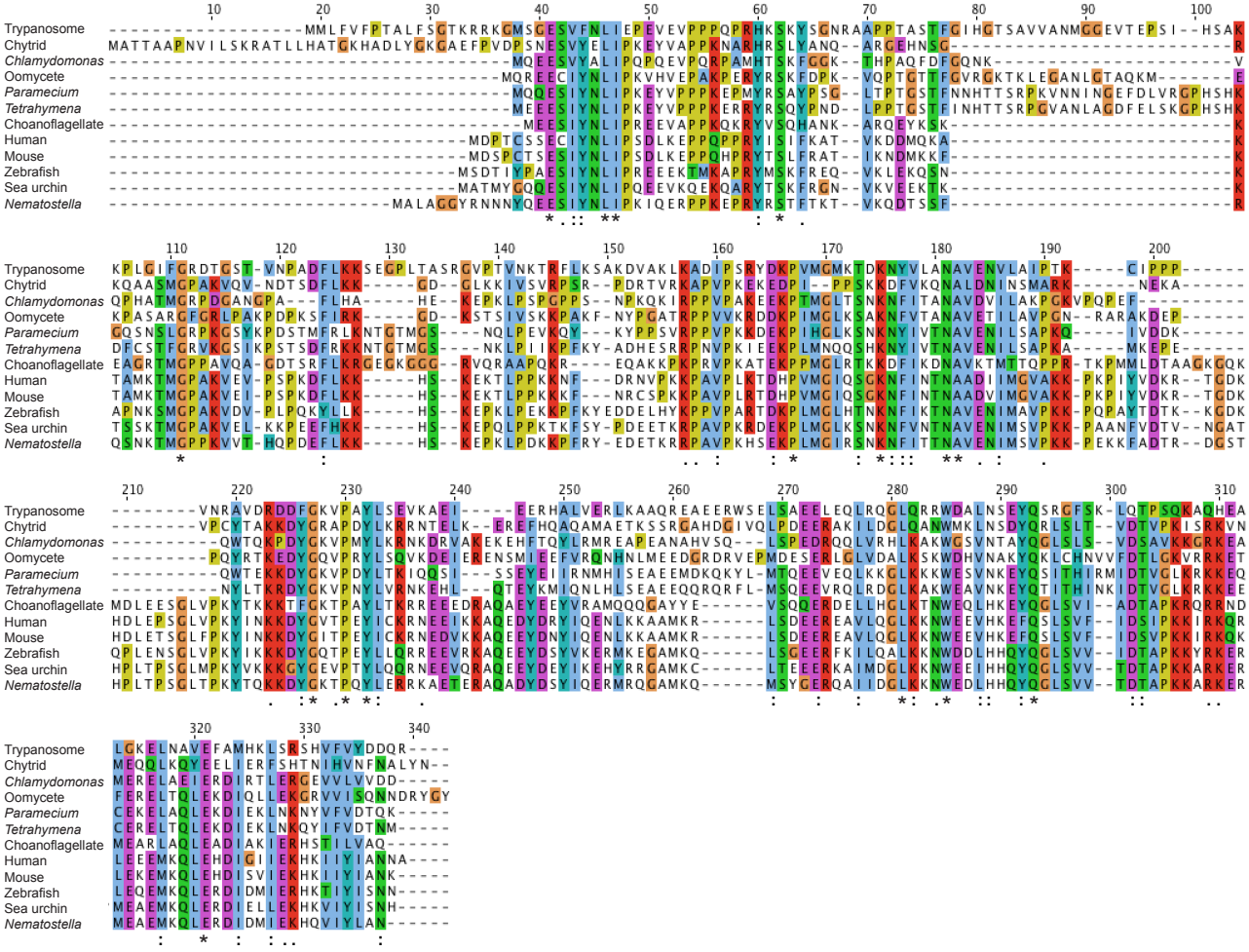
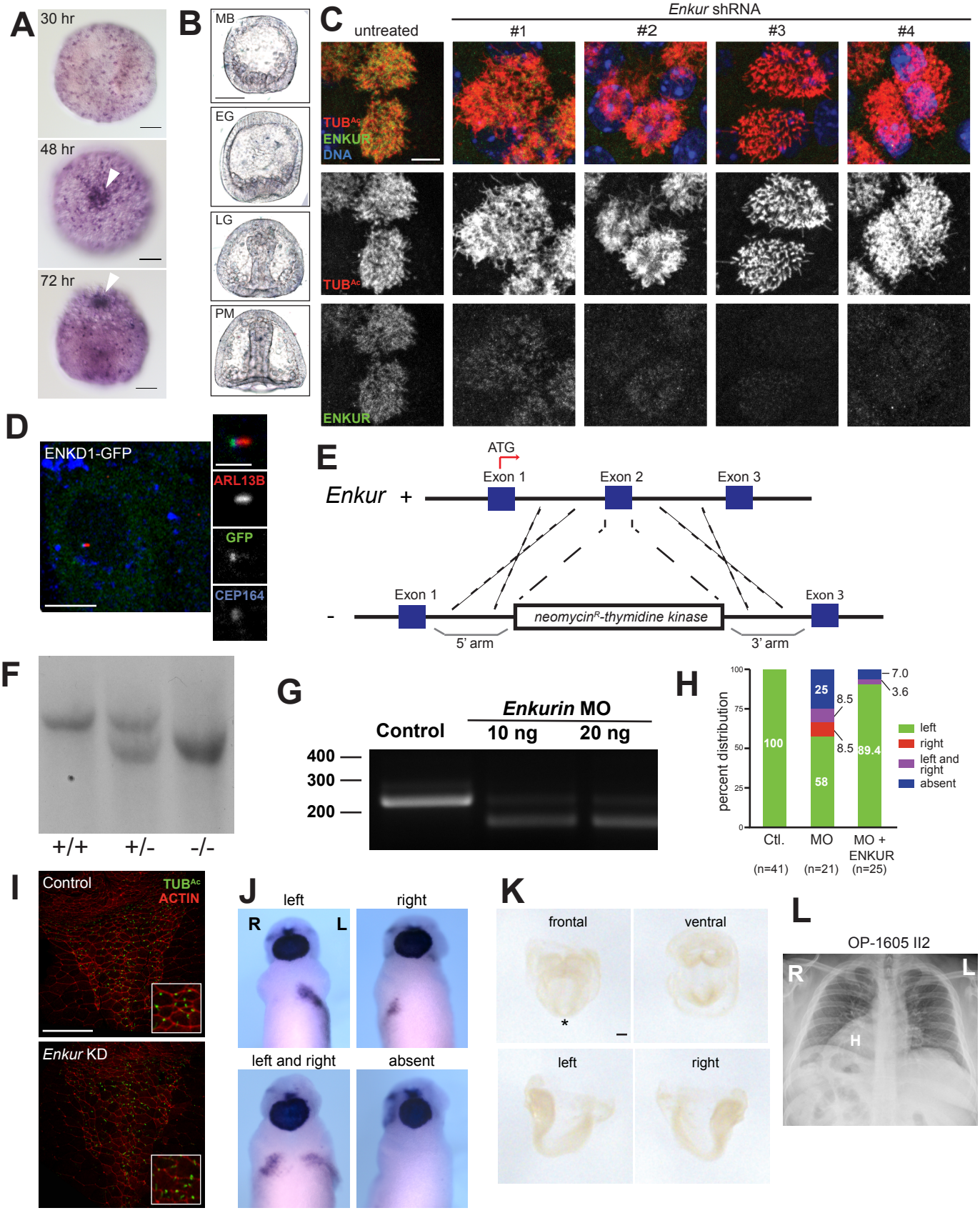


Figure S5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DNA
TUBAc
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D
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ARL13B
GFP
CEP164
E
ATG
Enkur +
Exon 1
Exon 2
Exon 3
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Exon 3
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8.5
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7.0
3.6
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Control
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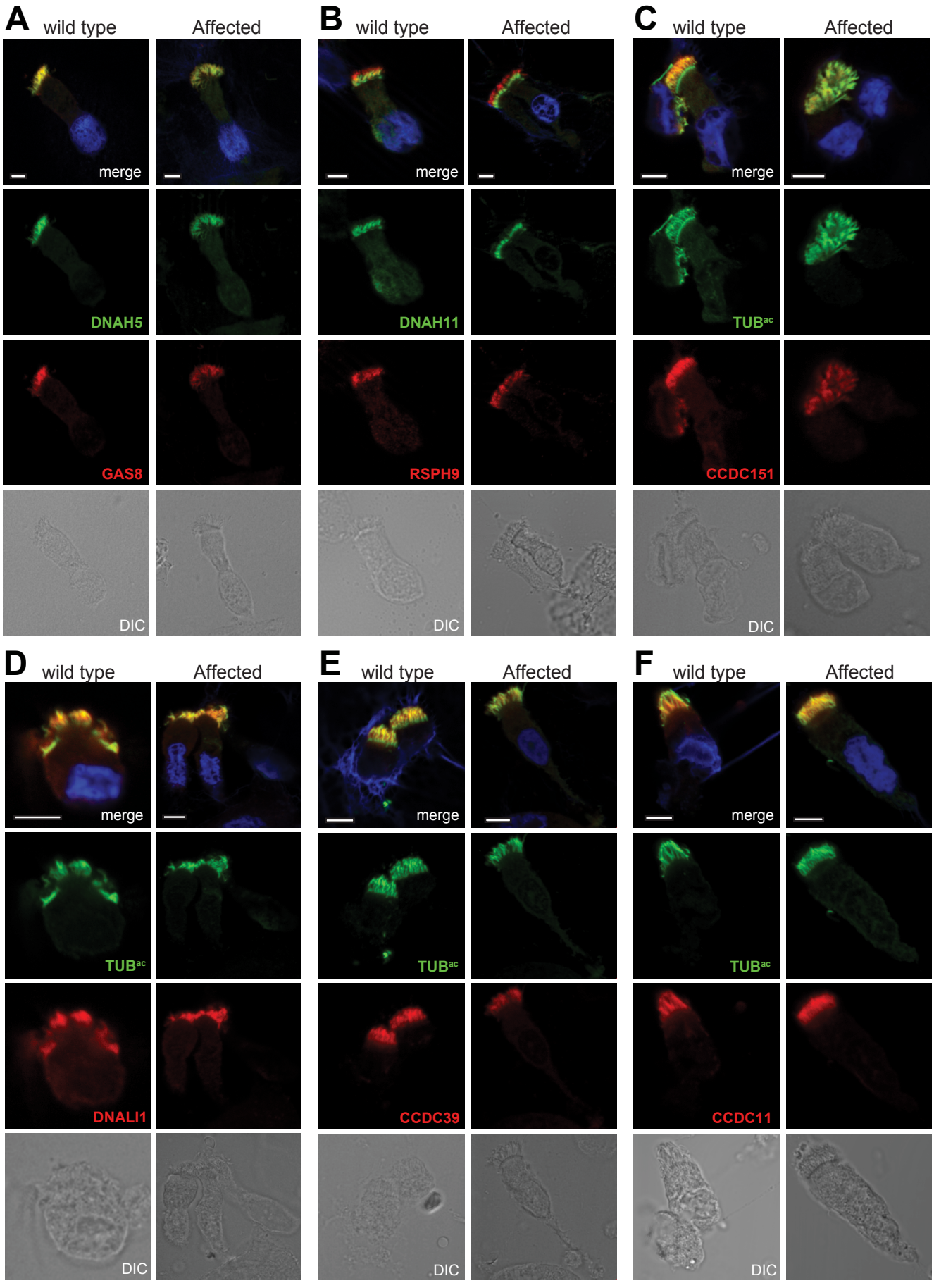
Figure S6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DNALI1
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Figure S7