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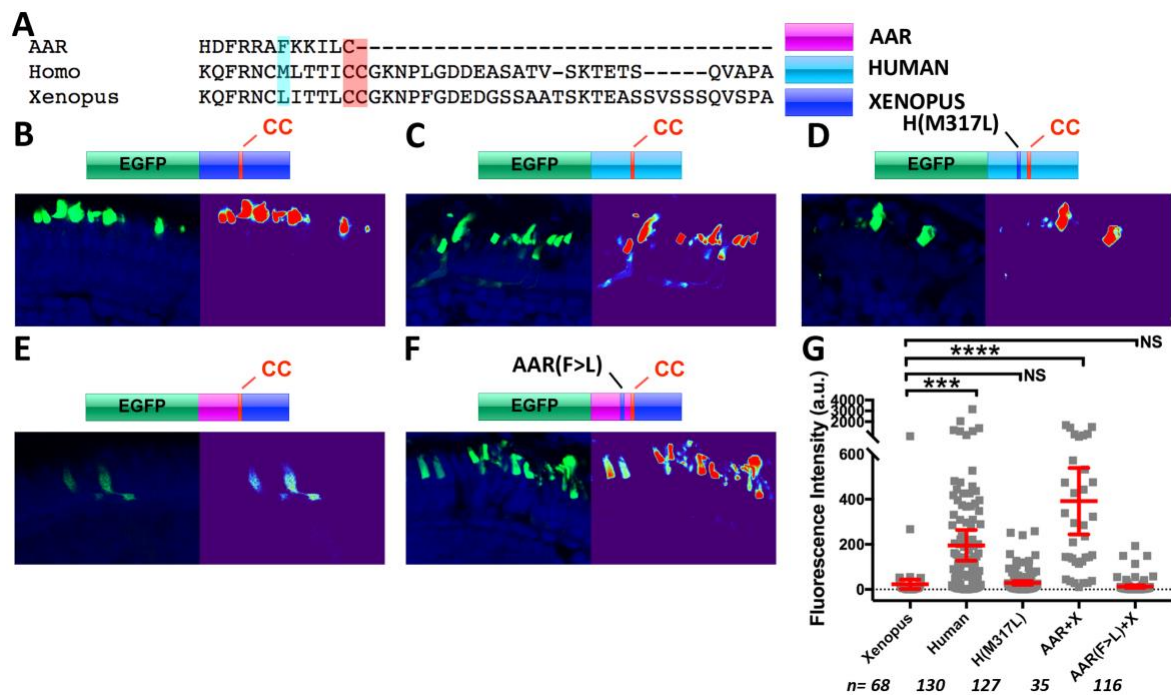
1 Supplementary Figures

HELIX 8

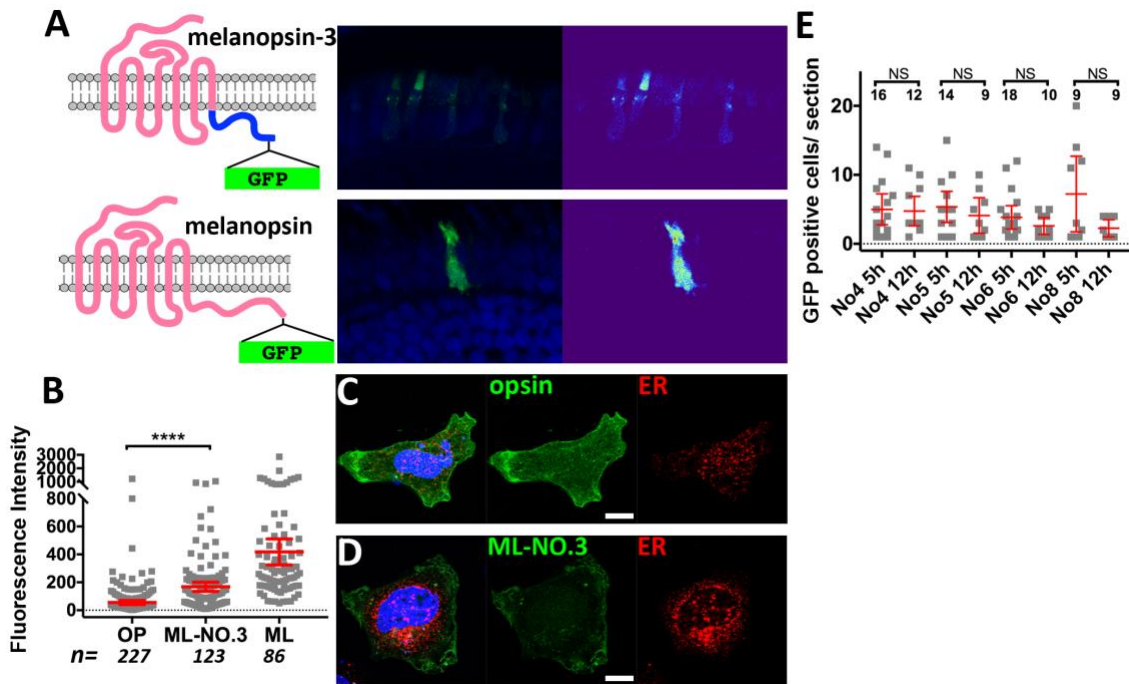
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Frog	YNPVIYIMLNKQFRNCMITTLCCGKNPFGDDDASS-AATSKTEATSVSTSQVSPA
Shark	YNPLIYILLNKQFRNCMITTLCCGKNPFEEDESTS-AAASKTEASSVSSSQVSPA
Pufferfish	YNPLIYICMNKQFRHCMITTLCCGKNPFEEEEEGAS-TT-SKTEASSVSSSVSPA
Ayu	YNPLIYVCMNKQFRHCMITTLCCGKNPFEEEEEGAS-TTASKTEASSVSSSVSPA
Carp	YNPCIYICMNKQFRHCMITTLCCGKNPFEEEEEGAS-TTASKTEASSVSSSVSPA
Zebrafish	YNPCIYICMNKQFRHCMITTLCCGKNPFEEEEEGAS-TTASKTEASSVSSSVSPA
Guppy	YNPLIYICMNKQFRHCMITTLCCGKNPFEEEEEGAS-TTASKTEASSVSSSVSPA
Medaka	YNPAIYICMNKQFRNCMITTLCCGKNPFEEEEEGAS-TTASKTEASSVSSSVSPA
Turtle	YNPIIYVLMNKQFRNCMITTICCGKNPFGDDVSSSTVSQSKTEVSSVSSSQVSPA
Alligator	YNPVIYIVMNKQFRNCMITTLCCGKNPLGDDETA-T--GSKTETSSVSTSQVSPA
Chick	YNPVIYIVMNKQFRNCMITTLCCGKNPLGDDETS-A--G-KTETSSVSTSQVSPA
Lamprey	YNPVIYILMNKQFRNCMITTLCCGKNPLGDDESGAS--TSKTEVSSVSTSPVSPA
Platypus	YNPVIYIMMNKQFRNCMLTTICCGKNPLGDDEASAT--ASKTEQSSVSTSQVSPA
Guinea Pig	YNPVIYIMMNKQFRNCMLTTICCGKNPLGDDEASTT--VSKTE-----TSQVAPA
Human	YNPVIYIMMNKQFRNCMLTTICCGKNPLGDDEASAT--VSKTE-----TSQVAPA
Cat	YNPVIYIMMNKQFRNCMLTTLCGKNPLGDDEASTT--GSKTE-----TSQVAPA
Pig	YNPVIYIMMNKQFRNCMLTTLCGKNPLGDDEASTT--TSKTE-----TSQVAPA
Bovine	YNPVIYIMMNKQFRNCMLTTLCGKNPLGDDEASTT--VSKTE-----TSQVAPA
Dog	YNPVIYIMMNKQFRNCMLTTLCGKNPLGDDEASAS--ASKTE-----TSQVAPA
Mouse	YNPVIYIMLNKQFRNCMLTTLCGKNPLGDDEASAT--ASKTE-----TSQVAPA
Rat	YNPIIYIMMNKQFRNCMLTTLCGKNPLGDDEASAT--ASKTE-----TSQVAPA
	*** **: :*****:*::**:*:*:*: ::: *** :***:**

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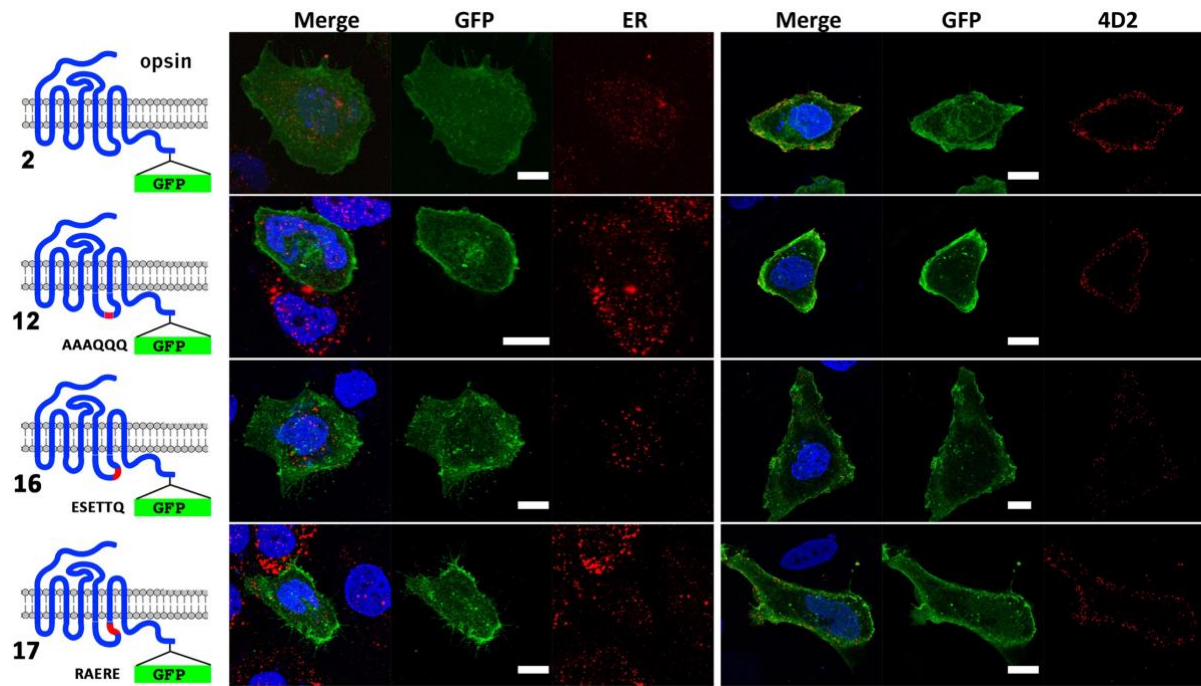
3 **Fig. S1** The alignment of the CT44 C-terminal sequences from vertebrate species as
 4 indicated. The methionine at position 317 is highlighted in red. In Xenopus, it is substituted
 5 by leucine. Alligator, *Alligator mississippiensis*; Ayu, *Plecoglossus altivelis*; Bovine, *Bos*
 6 *taurus*; Carp, *Cyprinus carpio*; Cat, *Felis catus*; Chick, *Gallus gallus*; Dog, *Canis lupus*
 7 *familiaris*; Frog, *Lithobates pipiens*; Guinea pig, *Cavia porcellus*; Guppy, *Poecilia*
 8 *reticulata*; Human, *Homo sapiens*; Lamprey, *Lethenteron camtschaticum*; Medaka, *Oryzias*
 9 *latipes*; Mouse, *Mus musculus*; Platypus, *Ornithorhynchus anatinus*; Pig, *Sus scrofa*;
 10 Pufferfish, *Tetraodon nigroviridis*; Rat, *Rattus norvegicus*; Shark, *Callorhynchus milii*;
 11 Turtle, *Chelonia mydas*; Xenopus, *Xenopus laevis*; Zebrafish, *Danio rerio*.



12
 13 **Fig. S2** Comparison of OS-targeting efficiency of Xenopus and human opsin C-terminal tails
 14 (CT44). (A) Alignment of rod opsin C terminal amino acids from human and Xenopus and the
 15 AAR sequence applied in this assay. Palmitoylated cysteines are highlighted in red. (B-F)
 16 Confocal images of cryosections through the photoreceptor cell layer of wild-type zebrafish
 17 retinæ that transiently express the following variants of the EGFP-CT44 construct at 5 dpf: (B)
 18 XCT44; (C) HCT38; (D) H-M317L; (E) AAR-X; (F) AAR(F>L)-X. Confocal image (left) and
 19 a heat map (right) of GFP signal intensity are juxtaposed. (G) Quantification of fluorescence
 20 intensity in photoreceptor cell bodies. Each dot represents a measurement from a single
 21 photoreceptor cell. Data are from 3 independent experiments. Mean and 95% confidence
 22 interval are indicated. Sample sizes are provided in italics below the horizontal axis. In
 23 schematic drawings above panels (B-F), human, Xenopus and AAR sequences are color-coded
 24 in light blue, dark blue and magenta, respectively. Red bars indicate conserved cysteine
 25 residues in opsin C terminus. Data are log transformed for statistical analysis as described in
 26 methods.



27
 28 **Fig. S3** Transport efficiency of opsin/melanopsin hybrid GPCRs into the photoreceptor ciliary
 29 compartment. (A) Images of cryosections through zebrafish retinæ, expressing wild-type or
 30 hybrid GPCRs, schematically shown to the left of each image. In each panel, a confocal image
 31 (left) and a heat map (right) of GFP signal intensity are shown. (B) Quantification of the GFP
 32 signal intensity in photoreceptor cell bodies for wild-type and hybrid GPCRs shown in (A).
 33 Data from 4 independent experiments are shown. Sample sizes are provided in italics below
 34 the horizontal axis. (C-D) Confocal images of PAC2 cells showing the expression of wild-type
 35 opsin (C) and melanopsin-No3 (ML-NO.3) (D) (green) in relation to the ER marker GRP78/Bip
 36 (red). Merged (left), GFP (middle) and ER (right) images are provided. Nuclei are stained with
 37 DAPI (blue). (E) Counts of GFP-positive cells in retinæ expressing four opsin/Sstr5 hybrid
 38 constructs at 5 and 12 hours after heat-shock as indicated. Each data point represents a single
 39 section. [Data are from 4 independent experiments](#). Mean and 95% confidence interval are
 40 shown. Mann-Whitney test is used. Scale bars represent 10µm.



41
 42 **Fig. S4** Confocal images of PAC2 cells showing the expression of wild-type opsin and deletion
 43 variants (green) in relation to the ER (GRP78/Bip) (left, red) and the plasma membrane (4D2)
 44 (right, red). Nuclei are stained with DAPI (blue). Scale bars represent 10µm.

45
 46 **Table S1** Amino acid sequences of Sstr5, rod opsin, opsin/Sstr5 hybrids, melanopsin and
 47 opsin/melanopsin hybrid appended with GFP. The Sstr5 sequence is in pink, rod opsin
 48 sequence is in blue, melanopsin sequence is in purple and GFP sequence is in black. For Sstr5
 49 and opsins, letters in upper case indicate transmembrane regions. Cytoplasmic and extracellular
 50 sequences are in lower case. Cytoplasmic sequences are underlined.