Article:

Fang, X., Peden, A.A. orcid.org/0000-0003-0144-7712, van Eeden, F.J.M. et al. (1 more author) (2021) Identification of additional outer segment targeting signals in zebrafish rod opsin. Journal of Cell Science, 134 (6). jcs.254995. ISSN 0021-9533

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Supplementary Figures

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                                    HELIX 8
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Xenopus            YNPVIYIVLNKQFRNCLITTLCCGKNPFGDEDGSS-AATSKTEASSVSSSQVSPA
Frog               YNPVIYIMLNKQFRNCMITTLCCGKNPFGDDDASS-AATSKTEATSVSTSQVSPA
Shark              YNPLIYILLNKQFRNCMITTLCCGKHPFEEDESTS-AAASKTEASSVSSSQVSPA
Pufferfish         YNPLIYICMNKQFRHCMITTLCCGKNPFEEEEGAS-TT-SKTEASSVSSSSVSPA
Ayu                YNPLIYVCMNKQFRHCMITTLCCGKNPFEEEEGAS-TTASKTEASSVSSSSVAPA
Carp               YNPCIYICMNKQFRHCMITTLCCGKNPFEEEEGAS-TTASKTEASSVSSSSVSPA
Zebrafish          YNPCIYICMNKQFRHCMITTLCCGKNPFEEEEGAS-TTASKTEASSVSSSSVSPA
Guppy              YNPLIYICMNKQFRHCMITTLCCGKNPFEEEEGAS-TTASKTEASSVSSSSVSPA
Medaka             YNPAIYICMNKQFRNCMITTLCCGKNPFEEEEGAS-TTASKTEASSVSSSSVSPA
Turtle             YNPIIYVLMNKQFRNCMITTICCGKNPFGDDDVSSTVSQSKTEVSSVSSSQVSPA
Alligator          YNPVIYIVMNKQFRNCMITTLCCGKNPLGDDETA-T--GSKTETSSVSTSQVSPA
Chick              YNPVIYIVMNKQFRNCMITTLCCGKNPLGDEDTS-A--G-KTETSSVSTSQVSPA
Lamprey            YNPVIYILMNKQFRNCMITTLCCGKNPLGDDESGAS--TSKTEVSSVSTSPVSPA
Platypus           YNPVIYIMMNKQFRNCMLTTICCGKNPLGDDEASAT--ASKTEQSSVSTSQVSPA
Guinea Pig         YNPVIYIMMNKQFRNCMLTTICCGKNPLGDDEASTT--VSKTE-----TSQVAPA
Human              YNPVIYIMMNKQFRNCMLTTICCGKNPLGDDEASAT--VSKTE-----TSQVAPA
Cat                YNPVIYIMMNKQFRNCMLTTLCCGKNPLGDDEASTT--GSKTE-----TSQVAPA
Pig                YNPVIYIMMNKQFRNCMLTTLCCGKNPLGDDEASTT--TSKTE-----TSQVAPA
Bovine             YNPVIYIMMNKQFRNCMVTTLCCGKNPLGDDEASTT--VSKTE-----TSQVAPA
Dog                YNPVIYIMMNKQFRNCMITTLCCGKNPLGDDEASAS--ASKTE-----TSQVAPA
Mouse              YNPVIYIMLNKQFRNCMLTTLCCGKNPLGDDDASAT--ASKTE-----TSQVAPA
Rat                YNPIIYIMMNKQFRNCMLTTLCCGKNPLGDDEASAT--ASKTE-----TSQVAPA
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Fig. S1 The alignment of the CT44 C-terminal sequences from vertebrate species as indicated. The methionine at position 317 is highlighted in red. In Xenopus, it is substituted by leucine. Alligator, *Alligator mississippiensis*; Ayu, *Plecoglossus altivelis*; Bovine, *Bos taurus*; Carp, *Cyprinus carpio*; Cat, *Felis catus*; Chick, *Gallus gallus*; Dog, *Canis lupus familiaris*; Frog, *Lithobates pipiens*; Guinea pig, *Cavia porcellus*; Guppy, *Poecilia reticulate*; Human, *Homo sapiens*; Lamprey, *Lethenteron camtschaticum*; Medaka, *Oryzias latipes*; Mouse, *Mus musculus*; Platypus, *Ornithorhynchus anatinus*; Pig, *Sus scrofa*; Pufferfish, *Tetraodon nigroviridis*; Rat, *Rattus norvegicus*; Shark, *Callorhinchus milii*; Turtle, *Chelonia mydas*; Xenopus, *Xenopus laevis*; Zebrafish, *Danio rerio*.

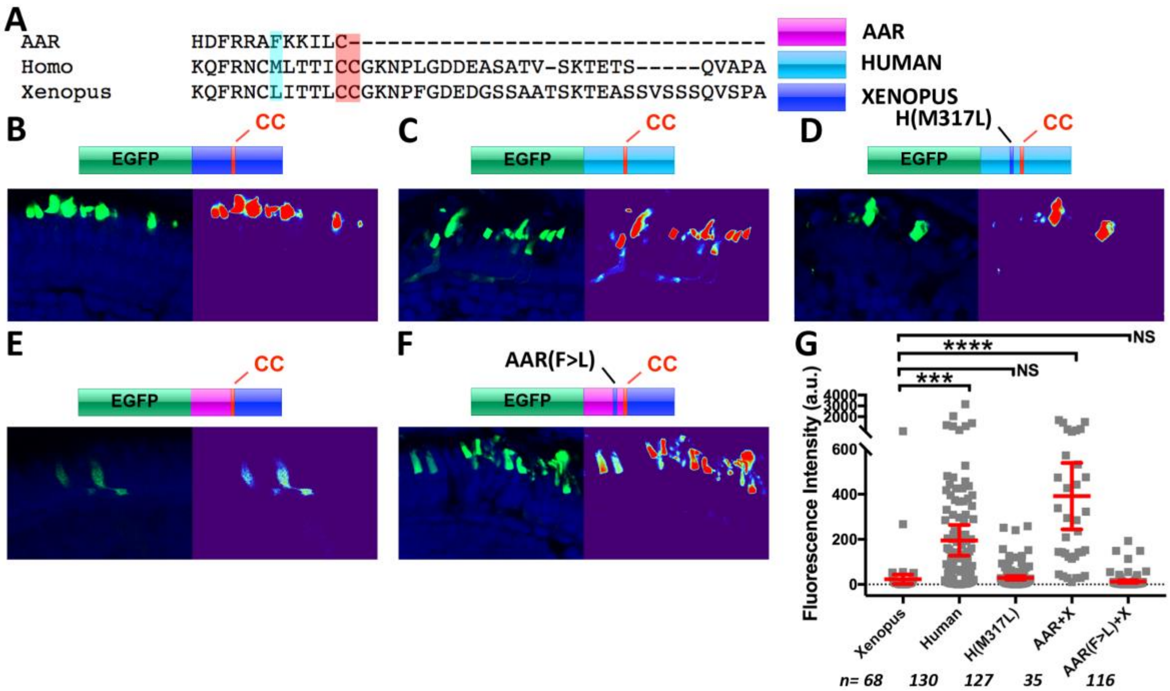

Fig. S2 Comparison of OS-targeting efficiency of Xenopus and human opsin C-terminal tails (CT44). (A) Alignment of rod opsin C terminal amino acids from human and Xenopus and the AAR sequence applied in this assay. Palmitoylated cysteines are highlighted in red. (B-F) Confocal images of cryosections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

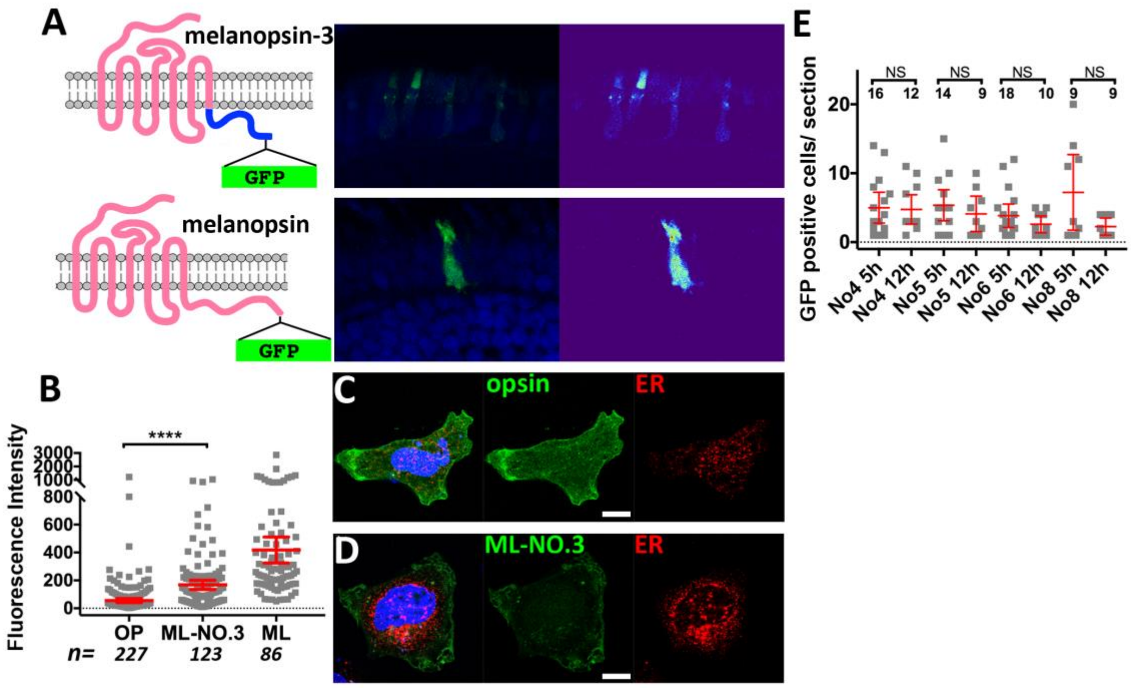

Fig. S3 Transport efficiency of opsin/melanopsin hybrid GPCRs into the photoreceptor ciliary compartment**.** (A) Images of cryosections through zebrafish retinae, expressing wild-type or hybrid GPCRs, schematically shown to the left of each image. In each panel, a confocal image (left) and a heat map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

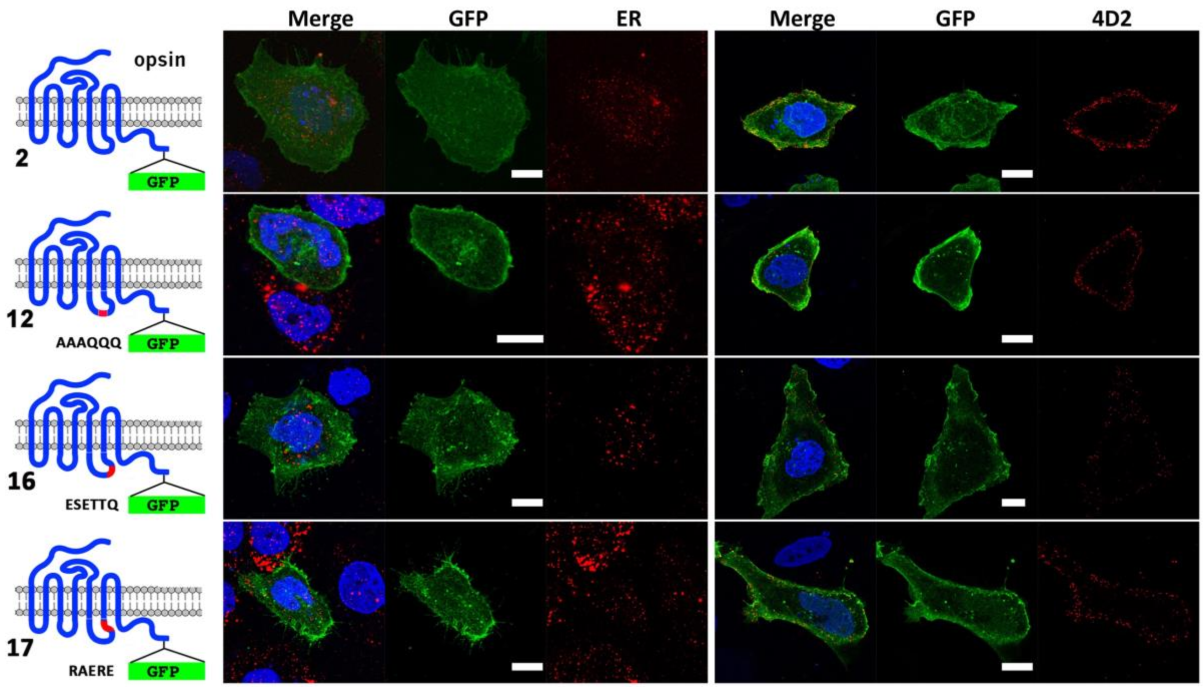

Fig. S4 Confocal images of PAC2 cells showing the expression of wild-type opsin and deletion variants (green) in relation to the ER (GRP78/Bip) (left, red) and the plasma membrane (4D2) (right, red). Nuclei are stained with DAPI (blue). Scale bars represent 10μm.

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