Supplementary Materials

for

Prominin-1 Localizes to the Open Rims of Outer Segment Lamellae in *Xenopus laevis* Rod and Cone Photoreceptors

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Figure S1

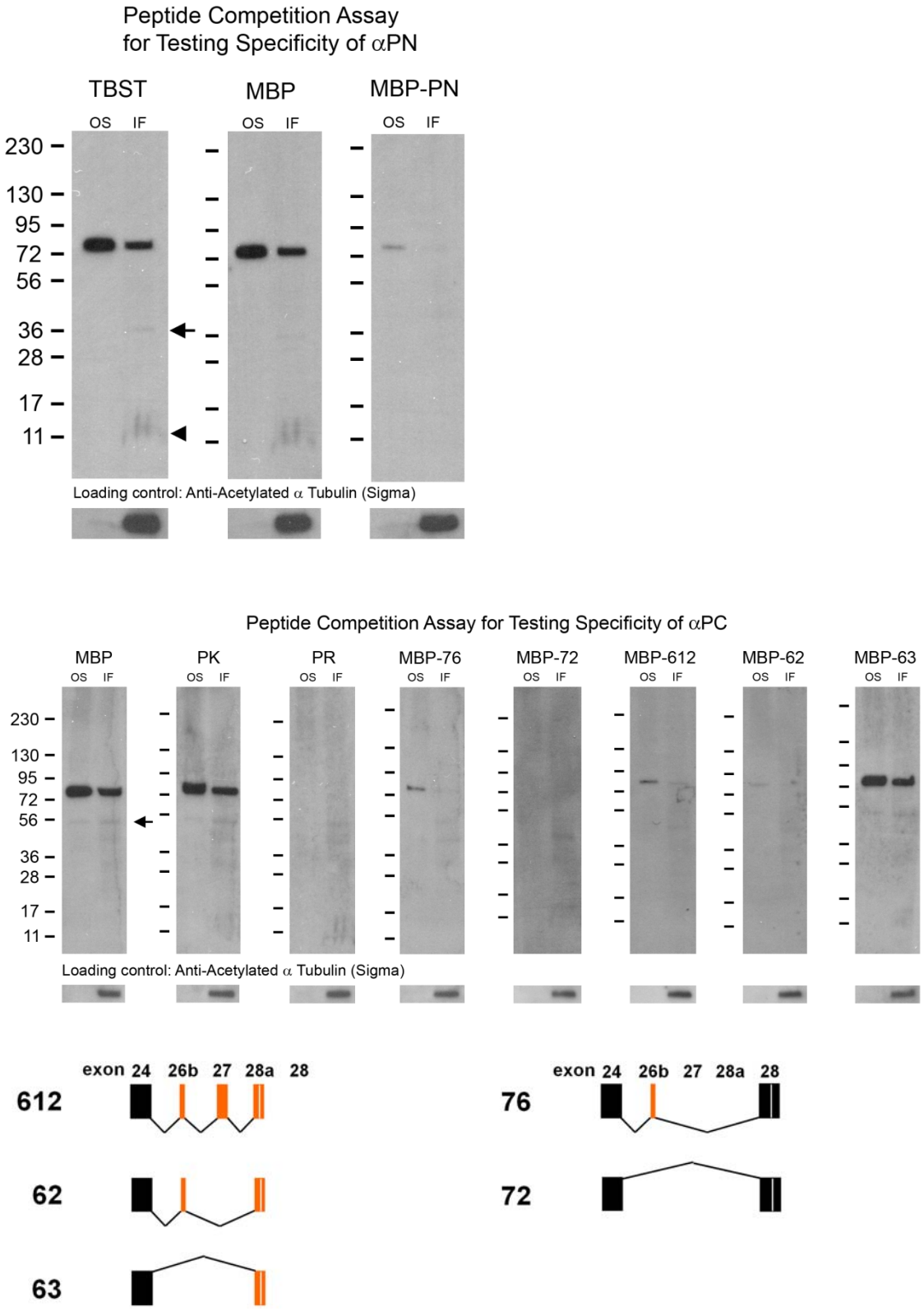
Peptide Competition Assay
for Testing Specificity of αPN
TBST
MBP
MBP-PN
OS IF
230
130
95
72
56
36
28
17
11
Loading control: Anti-Acetylated α Tubulin (Sigma)
Peptide Competition Assay for Testing Specificity of αPC
MBP
PK
PR
MBP-76
MBP-72
MBP-612
MBP-62
MBP-63
exon 24 26b 27 28a 28
612
62
63
76
72

Figure S1. Verification of antibody specificities by peptide competition assay. Membrane preparation of OS (outer segments) and IF (intermediate fraction) fractions of retina were treated with PNGase F, separated on an SDS-PAGE gel and electrophoretically transferred to a PVDF membrane for immunoblotting. PNGase F treatment removes the oligosaccharide chains, lowering the apparent MWs of the proteins and sharpens the bands. Membrane preparations not treated with PNGase F were not used in this assay, since the target epitopes of both antibodies are predicted not to be glycosylated. Arrows indicate minor bands detected by the immunoblotting. These minor bands could represent proteolytic products of xlProminin-1, products from alternative splicing of the xlProminin-1 gene or cross reaction with other proteins. Arrowhead indicates a band that might represent a degradation product of xlProminin-1. α-tubulin detected with anti-acetylated α-tubulin antibody (Sigma, Cat. No. T6793) was used as loading control.

Competition experiments were performed using either peptides or purified fusion proteins containing the epitope to verify the specificity of antibodies. MBP-PN is a maltose binding protein (MBP) fusion protein that containing the peptide epitope for antibody αPN (see Table 1 in the main text). PR is a peptide containing the αPC epitope (see Table 1 in the main text). PK is a peptide encoded by exon 28a of xlProminin-1, but does not contain the αPC epitope. MBP fusion protein-72 contains the translated sequence from exon 24 and 28, whereas MBP-76 is from exons 24, 26b and 28, MBP-612 is from exons 24, 26b, 27 and 28a, MBP-62 is from exons 24, 26b and 28a, and MBP-63 is from exons 24 and 28a. See Figure S10 in the supplementary material for a diagram and sequences of the engineered fusion proteins. They differ from each other so as to represent the various isoforms generated by alternative splicing events on xlProminin-1's C-terminus but all contain the αPC epitope. The reactive bands are blocked by pre-incubation of the affinity purified antibodies with peptides or fusion proteins containing specific epitopes, except for MBP-63. MBP-63 represents the alternatively spliced isoforms of xlProminin-1 that contain exon 24 and 28a. These isoforms only account for a small portion of the total xlProminin-1 protein in *X. laevis* retina.[1] Tris buffered saline supplemented with 1% Tween (TBST) is used to dissolve all the proteins used in this assay.

Figure S2

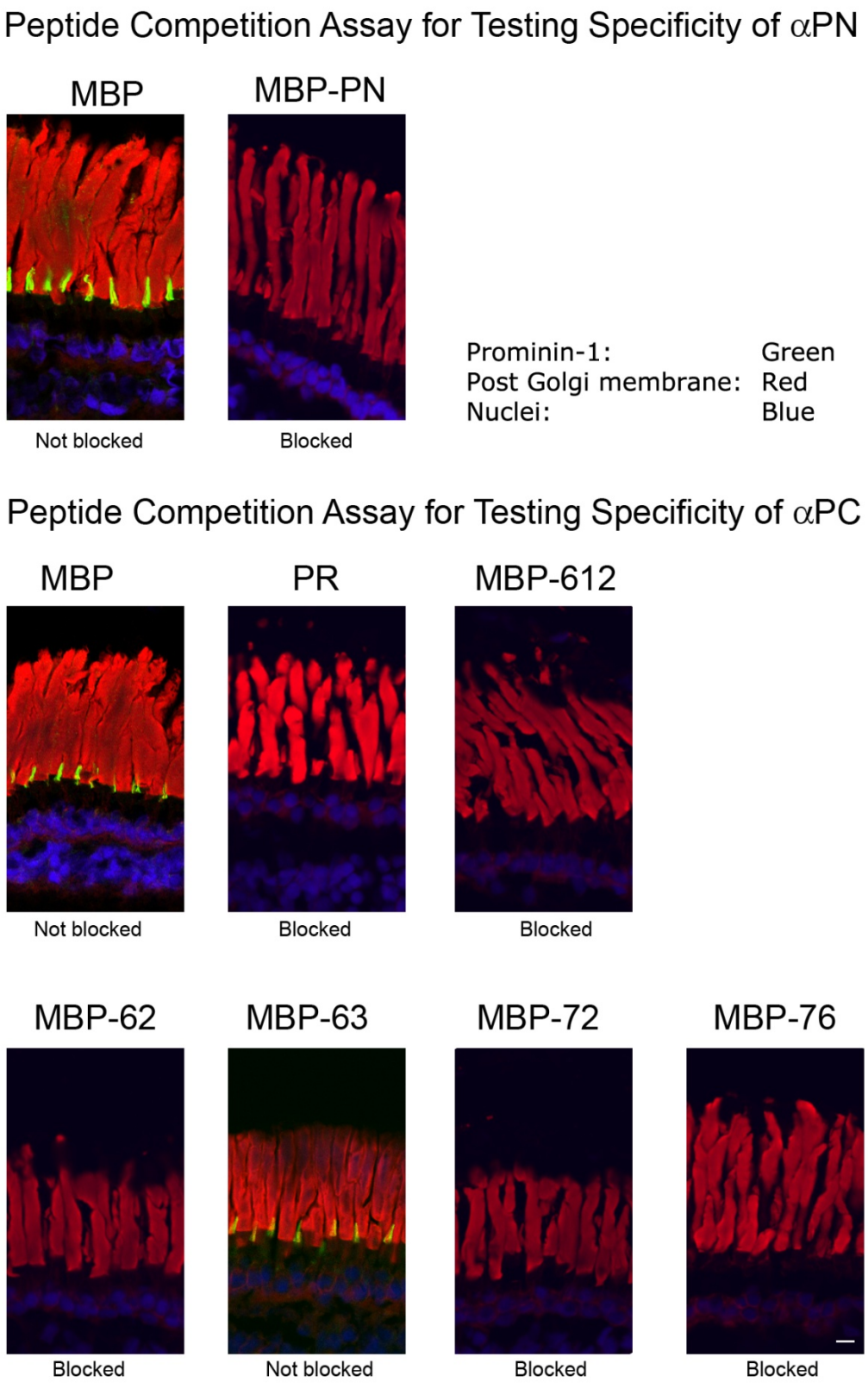
Peptide Competition Assay for Testing Specificity of αPN
MBP
MBP-PN
Not blocked
Blocked
Prominin-1: Green
Post Golgi membrane: Red
Nuclei: Blue
Peptide Competition Assay for Testing Specificity of αPC
MBP
PR
MBP-612
Not blocked
Blocked
Blocked
MBP-62
MBP-63
MBP-72
MBP-76
Blocked
Not blocked
Blocked
Blocked

Figure S2. Peptide competition assay to test specificities of αPN and αPC on sections of *X. laevis* retina. Antibodies αPN and αPC were pre-incubated with one of the purified proteins (MBP, MBP-PN, 612, 62, 63, 72 or 76) or synthetic peptide PR, before being used to label sections of *X. laevis* tadpole retinas. Sections treated with antibodies that were blocked by pre-incubation with specific competitors do not show labeling on the sections, while the COS on sections treated with antibodies pre-incubated with non-specific competitors are clearly labeled. The labeling of αPN was blocked by pre-incubation of the antibody with MBP-PN, but not by pre-incubation with MBP. The labeling of αPC was blocked by pre-incubation of the antibody with MBP-612, 62, 72, 76 or peptide PR, but not by pre-incubation with MBP, MBP-63 or peptide PK. This result is in accordance with those from the competition assay performed with immunoblotting (Figure S1 in the supplementary material). Scale bar = 5μm.

Table S1. Summary of peptide competition assay

	MBP	***MBP-PN***	***PR***	***PK***	***MBP-612***	***MBP-62***	***MBP-63***	***MBP-72***	***MBP-76***
αPN	N	Y	nt	nt	nt	nt	nt	nt	nt
αPC	N	nt	Y	N	Y	Y	N	Y	Y

Y: antibody competed by the specific competitor

N: antibody not competed by the competitor

nt: not tested

Figure S3

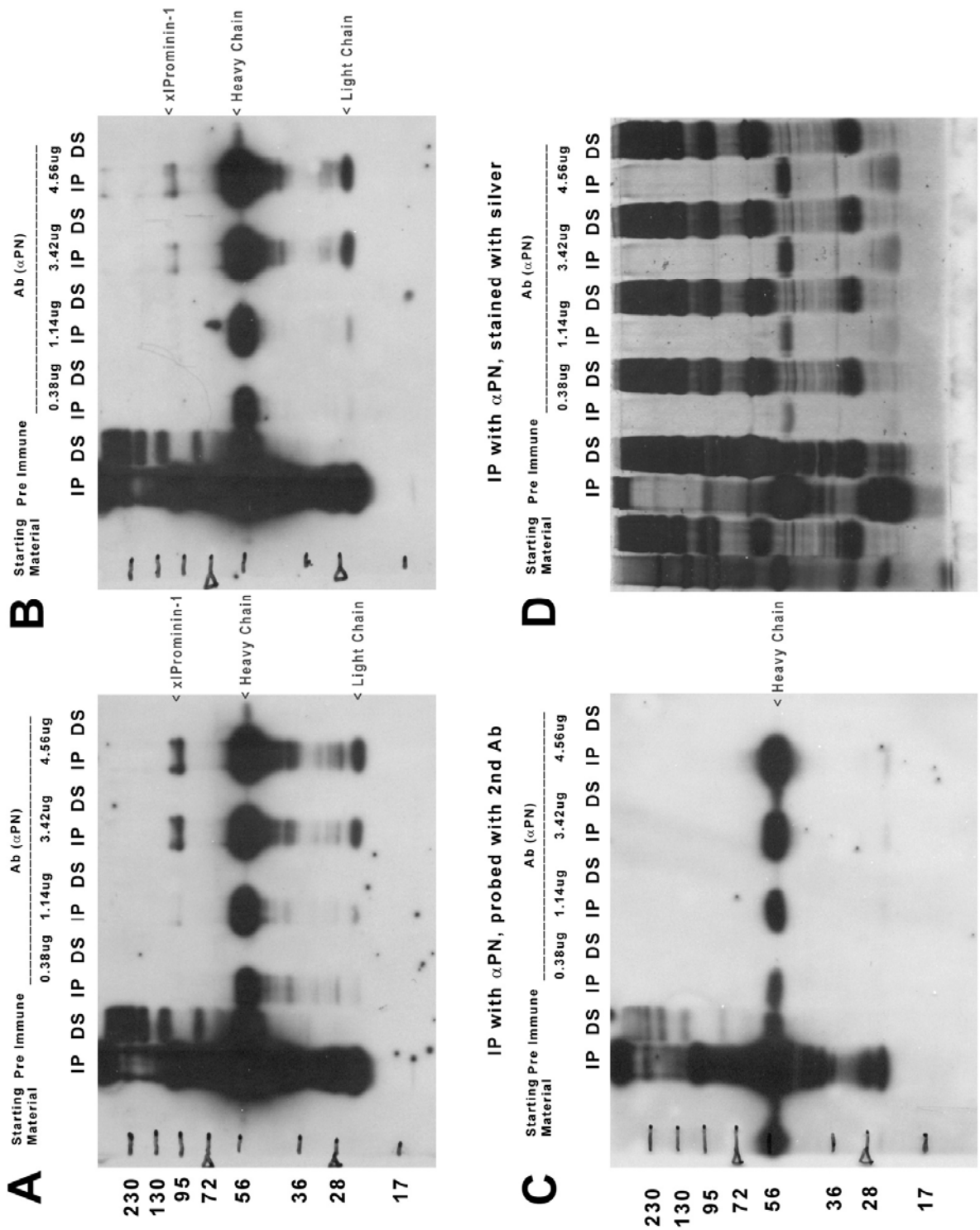
A
IP with αPN, probed with αPN
Starting Material
Pre Immune
Ab (αPN)
0.38ug 1.14ug 3.42ug 4.56ug
IP DS IP DS IP DS IP DS IP DS
230
130
95
72
56
36
28
17
< xIProminin-1
< Heavy Chain
< Light Chain
B
IP with αPN, probed with αPC
Starting Material
Pre Immune
Ab (αPN)
0.38ug 1.14ug 3.42ug 4.56ug
IP DS IP DS IP DS IP DS IP DS
< xIProminin-1
< Heavy Chain
< Light Chain
C
IP with αPN, probed with 2nd Ab
Starting Material
Pre Immune
Ab (αPN)
0.38ug 1.14ug 3.42ug 4.56ug
IP DS IP DS IP DS IP DS IP DS
230
130
95
72
56
36
28
17
< Heavy Chain
D
IP with αPN, stained with silver
Starting Material
Pre Immune
Ab (αPN)
0.38ug 1.14ug 3.42ug 4.56ug
IP DS IP DS IP DS IP DS IP DS

Figure S3. Verification of specificities of antibodies by immunoprecipitation. Two eyes from *X. laevis* tadpole were homogenized and used as starting material for immunoprecipitation. **A.** Proteins immunoprecipitated (IP) with different amount (0.38μg, 1.14μg, 3.42μg and 4.56μg) of anti-xlProminin-1 N- terminus antibody αPN and depleted supernatant (DS) were separated on an SDS-PAGE gel, transferred to a PVDF membrane and detected with αPN. Pre-immune serum of the rabbit was used as control. A doublet of approximately 95 kDa bands is readily detected with αPN in the IP fractions precipitated with 3.42μg or 4.56μg of the same antibody. The doublet may represent the full length xlProminin-1 with different glycosylation. **B.** Proteins immunoprecipitated (IP) with αPN and depleted supernatant (DS) were separated on an SDS-PAGE gel, transferred to PVDF membrane and detected with anti-xlProminin-1 C- terminus antibody αPC. A doublet of approximately 95 kDa bands is readily detected with αPC in the IP fractions precipitated with 3.42μg or 4.56μg of αPN. **C.** Proteins immunoprecipitated (IP) with αPN and depleted supernatant (DS) were separated on an SDS-PAGE gel, transferred to a PVDF membrane and detected with secondary antibody only. **D.** SDS-PAGE gel after silver staining. Each of the 4 images were from a different gel, membranes were not stripped in order to prevent false results coming from incomplete removal of antibodies. We showed here that antibodies αPN and αPC specifically detect a same protein, logically xlProminin-1, in *X. laevis* retina.

Figure S4

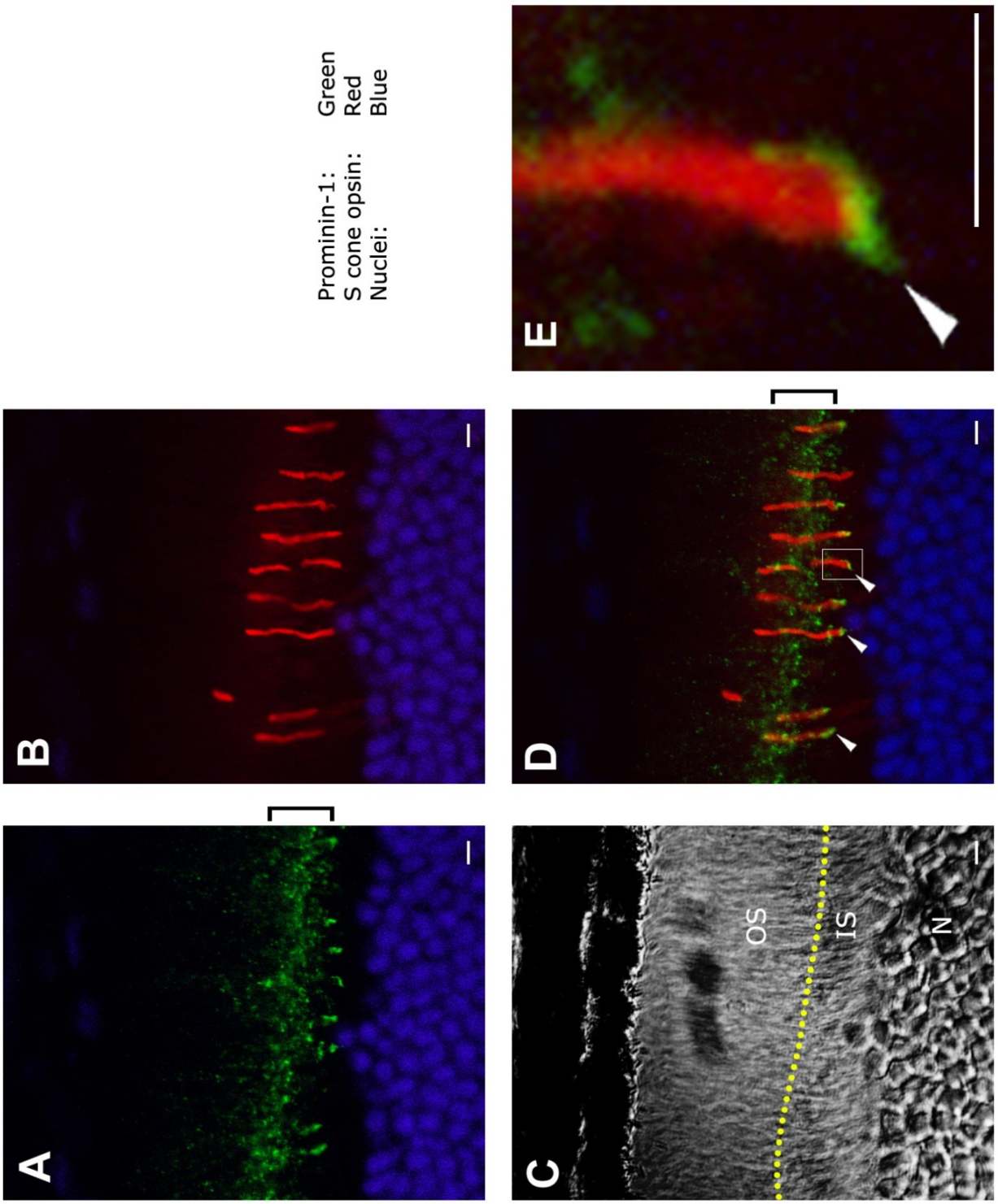

Figure S4. Double immunolabeling of a mouse retina with anti prominin-1 antibody 13A4 and anti S cone opsin antibody. A. Monoclonal antibody 13A4 (eBioscience, Cat. No. 11-1331-80, rat anti mouse prominin-1, FITC conjugated) labels the mouse retina as numerous bright dots at the junction of the inner and outer segments (green). This junction is marked with a bracket. Some labeled dots are larger in size and lower in position. **B.** Outer segments of S cones labeled with the anti S cone opsin antibody[2] (A gift from Dr. Cheryl Craft, University of Southern California) (red). **C.** Nomarski view of the same retina section. Mouse photoreceptors are much thinner than frog's. **D.** Superimposed images show the relative position of 13A4 and anti-S cone opsin immunolabeling. The larger, lower dots labeled by 13A4 (arrowheads) are at the base of the S cone outer segments. The numerous small dots labeled with 13A4 (bracket) likely represent the localization of prominin-1 at the proximal portion of the ROS. **E.** Enlarged image of the boxed area illustrating that prominin-1 in the murine cone is largely confined to the base, as it is in the ROS. The nuclei are labeled with Hoechst 33342 dye (Invitrogen) (blue). Scale bars = 5μm.

Figure S5

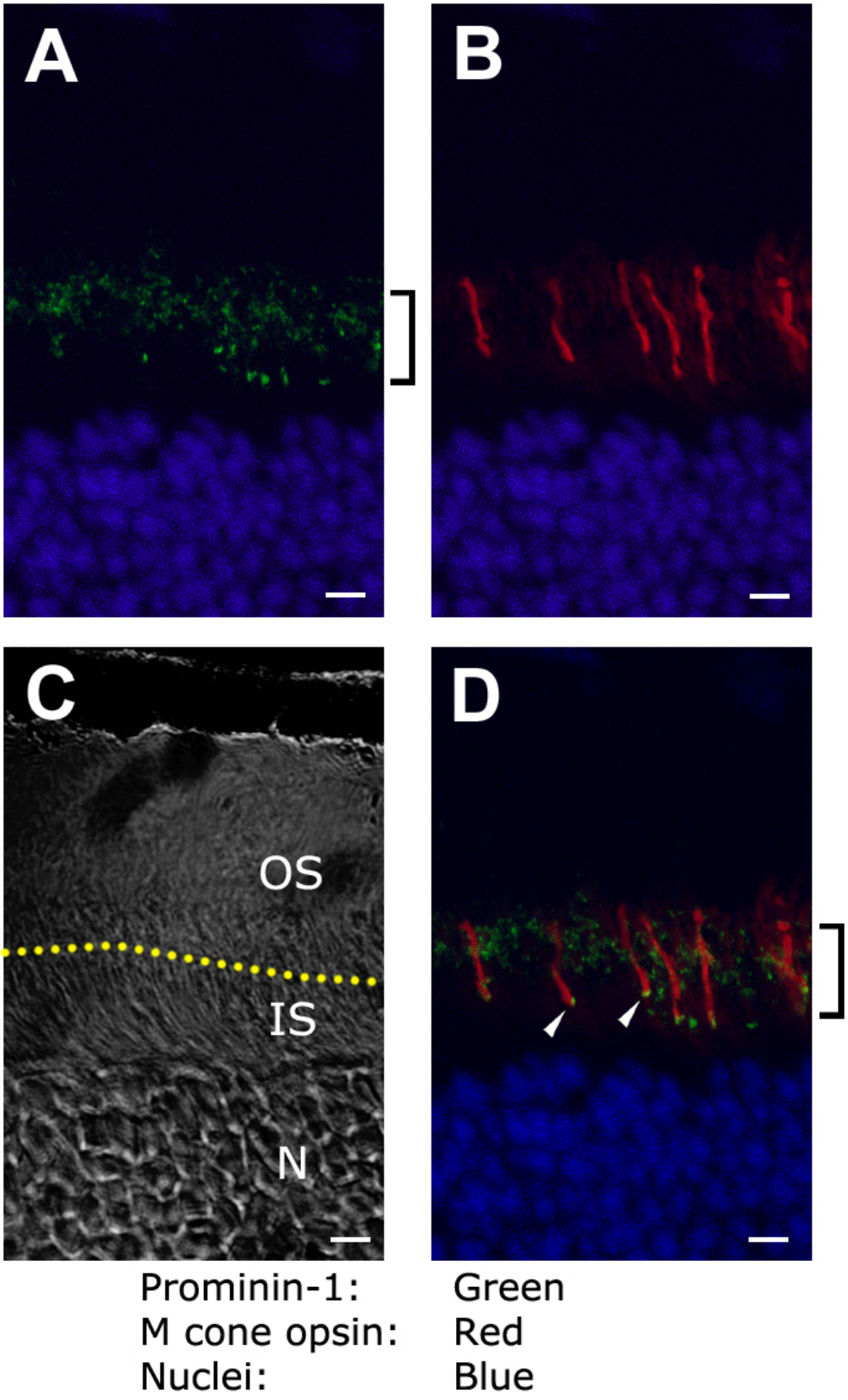

Figure S5. Double immunolabeling of mouse retina with anti prominin-1 antibody 13A4 and anti M cone opsin antibody. A. Monoclonal antibody 13A4 (eBioscience, Cat. No. 11-1331-80, rat anti mouse prominin-1, FITC conjugated) labels the mouse retina as numerous bright dots at the junction of the inner and outer segments (green). This region is marked with a bracket. Some dots are larger in size and lower in position. **B.** Outer segments of M cones were labeled with the anti M cone opsin antibody [2] (A gift from Dr. Cheryl Craft, University of Southern California) (red). **C.** Nomarski view of the same retina section to show the morphology of cells. The junction of the inner and outer segments is marked with a yellow dashed line. **D.** Superimposed images show the relative position of 13A4 and anti M cone opsin immunolabeling. The larger, lower dots labeled by 13A4 (arrowheads) were positioned at the base of the M cone outer segments. The numerous small dots labeled with 13A4 (bracket) presumably represent the localization of prominin-1 at the proximal portion of the ROS. The nuclei are labeled with Hoechst 33342 dye (Invitrogen) (blue). Scale bars = 5μm.

Figure S6

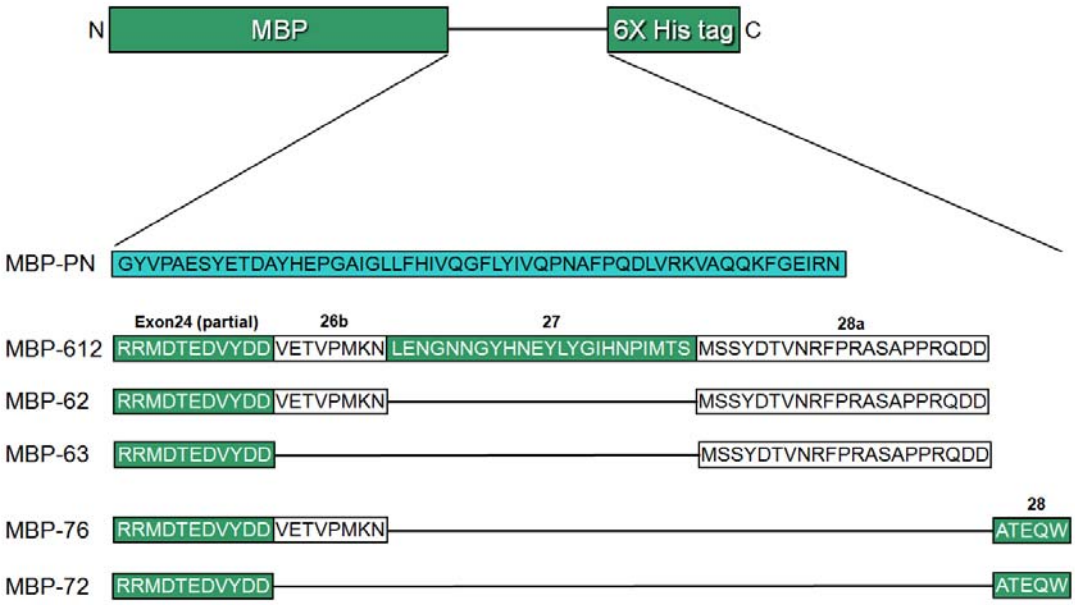

Figure S6. Engineering of the double tagged fusion proteins. Fragments of xlProminin-1 were inserted in between the MBP and 6X His tags. MBP-PN carries a fragment from the N-terminus of xlProminin-1. MBP-612, 62, 63, 72 and 76 carry fragments from the C-terminus of xlProminin-1. They differ from each other by inclusion or exclusion of exons 24, 26b and 28a, resulting from alternative splicing. Peptide GYVPAESYETDAYHEPGAIGLLFHIVQGFLYIVQPNAFPQDLVRKVAQQKFGEIRN in MBP-PN and Peptides RRMDTEDVYDDATEQW in MBP-72 were also fused to Glutathione S-transferase (GST) and used as antigens to generate polyclonal antibodies αPN and αPC, respectively.

Figure S7

GAAGCTTGAGGAGGACTACCCCCTGCTCCGAGGCTGCTAAACATGAAAATACCCAATATATAATGAGGC
CACACTGGAGCTCCCTCAATATACTACTTGTTTAAATACAGGCCAAAGCTAGCCTTACAGAGAGTGATA
TTTTAAAGAAGTGAGGGGGAGACAGTATATAAAGGGAGCACTGAATTTCTAGGCTGCTGATAGCATCAC
TGATCCAGATACAAAAAAACTAAATCTTTTTTGTTTCAGTGCTCTCATATGCTTTCTAAATTGAAGTCC
CAAATCATAAGCTATTTTACCACTGTAACACCACTTTAATAATGCAGGTAGATTAGATATTTCAAATAA
AACAAATGACCCTATGCCTTACTATTAATTTGGCATTGATTCTAATTAGTTACTGCCACATACACATGT
TAAATATATTGAAGCACCCTTTTGTGACCCAAGATTTATTCAGGACCCCCTTCTCATGAAACAAGATTA
AATTAAATGAAAACATGATCATATCCTTCACCCTGCTTTAGTTTTGAGAATCTCTAAAAACAAATTAGC
ATAAAAGACCCAGATCACCTGACCTGAAGGCTGTGCCCCATCTATTTCCATGTACCCCCTAAAAGAGTT
GTTTCACATTGTGGCATCCTCTGGTCTAAATATTAGTCCAAGTCAGTGTGAATGAATAAATAGTTGAAC
TTGCTCTCTATTCCATATGTAAGTTGTGAGTGAACCCCTGGGGGCATGGGTAAAGACTTGCCTGTTAAT
GCTGATTGCTGCAGGGTCCTCACGTTGTGTTGTGTGAGCTGTTGCAGAGAAACTAGTGAAGCAGAGTGA
GTAGGAAGGAAACTGTGTCTCATCACCCACAGAACTGTGGCTTCTACAGAAGAATAAAAGCGTTGGACA
AAAAGCATATTAAATGTGAATTTATTCAGAGATATCAAATCCAGTTGCTCAGGGAAAAACTTAAAGAGC
CAAACTGAGGGAACAATGGGCTAACAAATTCCTTTTTCTGTCAGTTCAAGTCACCTTATGGGGTTGTGC
GAGGTTGTGTAAATCTCTCTCCCACTCCAAGGAATTTCCACTAAATTAATTATGGGAAGTTCATTTTTA
ATCTTGTTTCTGTTCATCTCTGAGCATTTAGTAGGCAGAAAAAGCTAACCCTGGTCTCCAGGTAATTAG
AAGATGGACAGGGGTTATAATGTGGCAGACCCATTTTTCCCTCCTACCTCTAGGGCTGGTACCACACGG
GGTTATTTCTTGGCCTTAACATGAATCCACTATGCAAACAACTAAGTAACCTTCCTGTCCCTGCACAAT
TTCCCATAGGAAAATATTCTGTCAATAATAATGGCAAGTTGACTCACAGACTTCTGAGACTGAAAATTG
GAAACGTAAAAAGTTAAATAATCTGTATATATAGCACTCAGTCTTCGGCTTTGTAGATTATAAGGTCAT
GGAAATGCTCATTTTAGAATCTCTGCAAAATGCTGCATTACATGTTGACACTATATAAATAAAGAACTC
AAACAATAATTCTGAAAACTTGTGTCAAGTGTATATAACTGTAGGGCAGACAATCTGTACAAATGCACA
AACGTTTTGCTATGCAGCCAGATGCCCCTGGCAGTGGGGATGCAGAGTCTATTACCTCTTGAGCAGTAA
TGTCAGAAAGTTAAGACCTCTGTACTCTTCTCCCTCATCCTGTAATCCACTTGAAGCCTTTTCTTTAAC
CTAAGCTTCTTCAGACAGCTAATAATAAGTCACTGTTTCTGCTGCCTCCCCCACCCTCCCTAATAGGAT
TTCGGATTTCTCTCCCATCCACTTTTTTTTCATGAATTGGGGGGTCAGACATATATAAGCGGTAGTGAC
AAGGGTCACCAAAACCATCAGTGCAACTGAAGTCCAAGAGAACTGATACAGGTA

Figure S7. Sequence of the *X. tropicalis* cone arrestin (ARR3) promoter (XtCAP1.9). The boxed region indicates partial sequence of the predicted first exon (exon1). Putative TATA boxes are highlighted with yellow and putative CRX binding elements (CBEs) are highlighted with blue. These *cis*-elements were previously identified in murine cone arrestin (ARR3) promoter and were found to be responsible for the specific expression of ARR3 gene in cone photoreceptors.[3, 4]

Figure S8

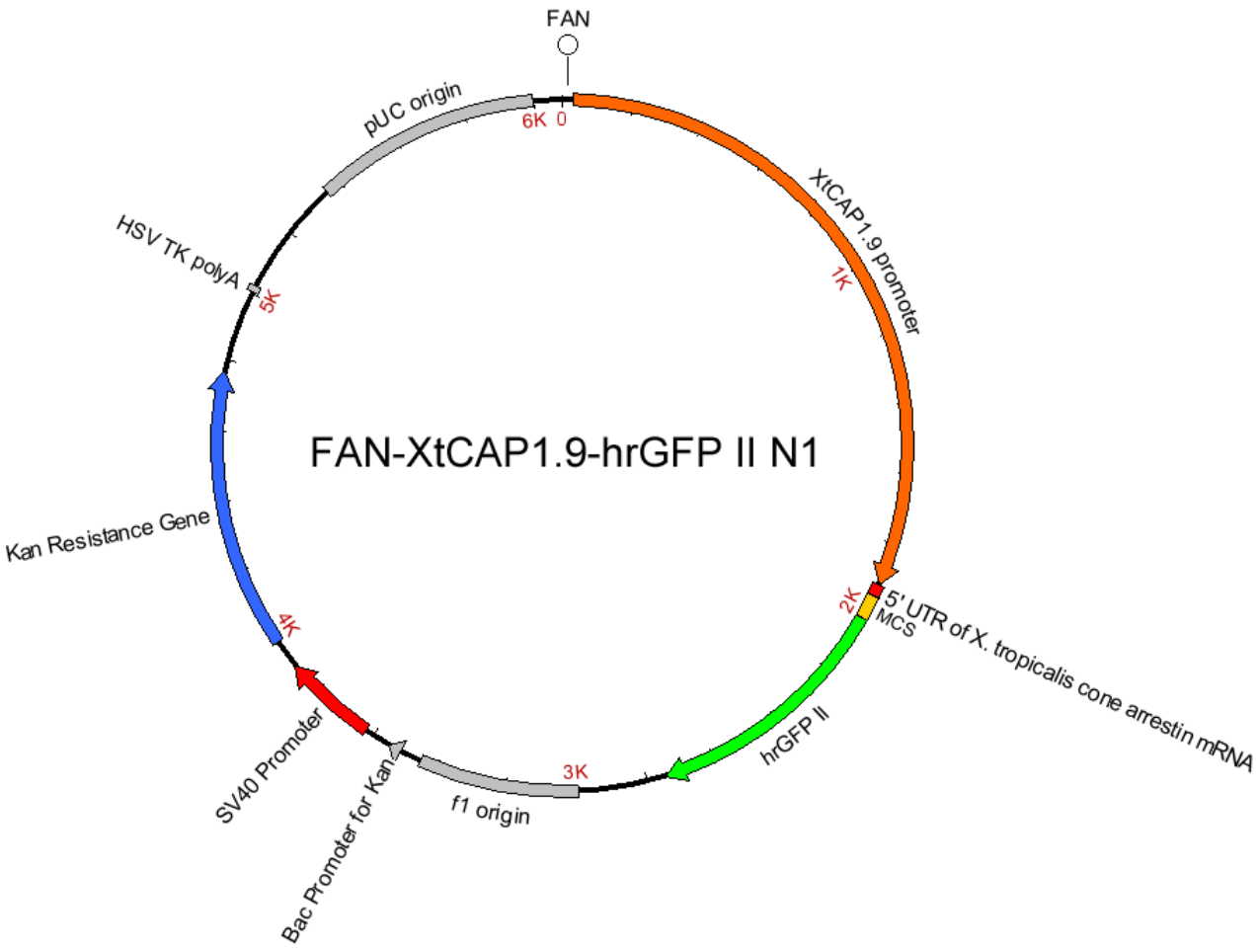

Figure S8. Map of the FAN-XtCAP1.9-hrGFP II N1 vector. The XtCAP1.9 promoter is 1.9 kbp, including partial sequence of the predicted first exon (exon 1) of the *X. tropicalis* cone arrestin (ARR3) gene. This partial sequence of exon 1 encodes for part of the 5'UTR (untranslated region) of the *X. tropicalis* cone arrestin mRNA. The hrGFP II coding region is downstream of the XtCAP1.9 promoter. The vector carries the kanamycin resistance gene. (FAN: three restriction sites used to linearize the plasmid: Fse I, Asc I and Not I; MCS: multiple cloning sites.)

Figure S9

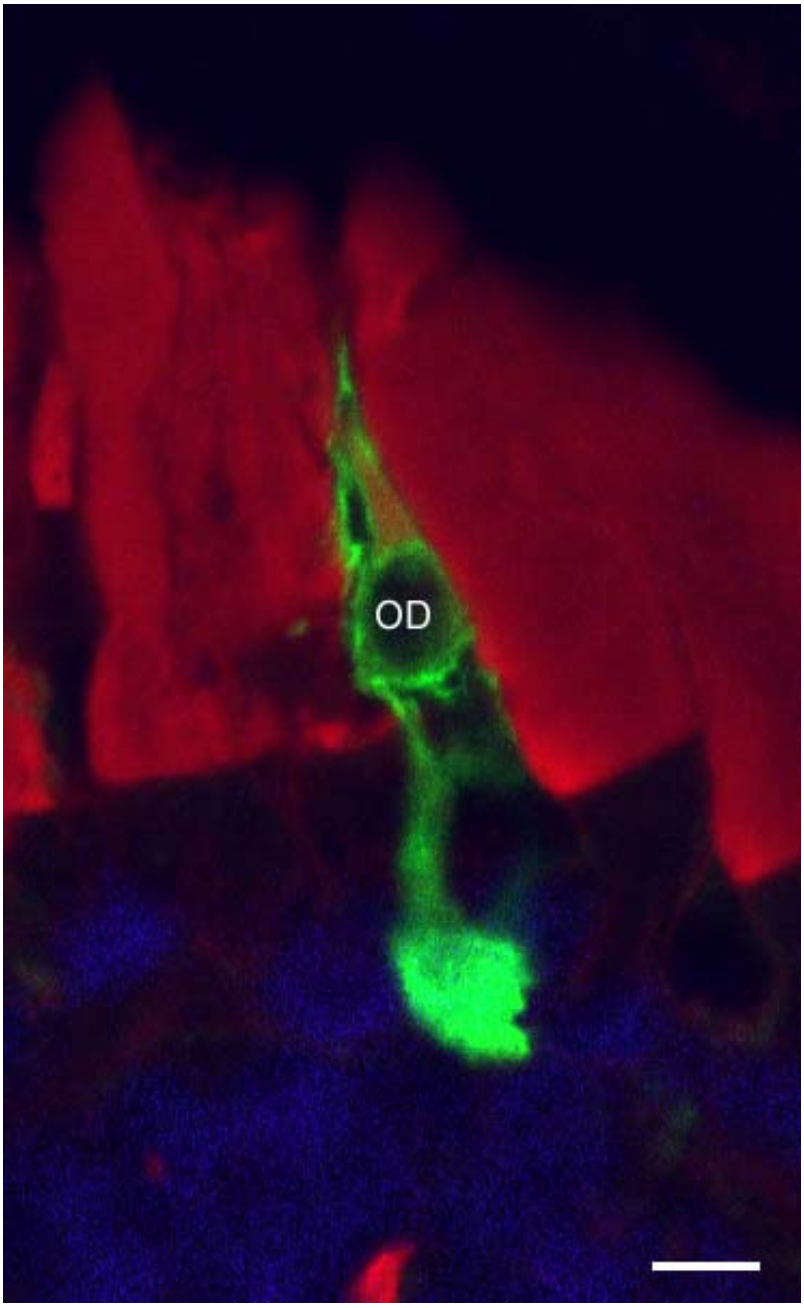

Figure S9. Expression of hrGFP II in *X. laevis* cone driven by XtCAP1.9 promoter. The expression of the hrGFP II is confined to cone photoreceptors, which can be verified by the presence of oil droplet (OD) in the inner segments and the conical shape of the outer segment. No fluorescence was observed in the rod photoreceptors. Scale bar = 5μm.

Figure S10

MAFKLYLVFLLFCGFTLSERTLPLNSAPARLDFGYVPAESYETDAYHEPGAIGLLFHIVQGFLYIVQPN
AFPQDLVRKVAQQKFGEIRNDYQKPENVVLTLQTIHYEIGFIIAAVLGLVFLLLMPLVGLCFCMCRCCD
NCGGEMHQRQKKNGDCQRGCYSTFLFVTTLLISVGVICAYAANQNLTNQIRGSKKLVQSNFKDLKTLLN
DAPTQINYVLSKFNVTKDKALSEMNNLGPLLGERVHERLGKDVRPAFDAVLNMAGAIKETKEALENVSL
CVEVLQEAIDRLNNNLTEAKLQLTSTLSDPACSANVALIPCNKIISSLNQLNTNANFSVLPDLSHNLIR
LNEVLRTDLSNLVQKGYAAFNSTPEMVQNQTRNIEGAAIPHMKNVLESIGANITSFSKTVPVLQIMANI
NNHISQSETYVREFYPVVEQYDFYRWLGSLVLCCMVSLIVVFYCLGLLCGTCGYDQHSSPTTRGCITNT
GGNFLMAGVGFSFIFSWILMLIVVVTFLAGGNVQKLVCEPFQERSLFKVLDTPYLINSQWKNYLSGILY
QNPNMNLTFEKVYSDCKENKGIFTALQIDNLLNLNEFLNINMYIDDISAKIETLNLDLSTIVLLDEKGK
RNLEDFSSTGLDEIDFDAFLSEVAKSNTKVDLLAFANELEENADLLPKGALGNALKGHANSIRTIHIQQ
VVPLEQSMKYVKARITLNQSIKLLQRTSFDIEGKVLDVITAVEAAQNLINNNASLIIKQESKKLMDEVI
GYFIQYVQWVKDSIAFDVAGCKPIANVVDTAVDVFLCSYIVDSVNSFWFGLGGATVLLIPAIIFAIRLA
KFYRRMDTEDVYDDATEQWRDPPVATMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKL
TLKFICTTGKLPVPWPTLVTTLTYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAE
VKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLAD
HYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK*

Shaded: xlProminin-1
Green: hrGFP II
Boxed area: Predicted signal peptide

AIP: Exon 11a
KYVKAR: Exon 19
RDPPVAT: peptide translated from the multiple cloning sites (MCS)

Figure S10. Sequence of xlProminin-1-hrGFP II protein. The profile of alternative splicing of xlProminin-1 used in this fusion protein is: 3a-, 8a-, 11a+, 19+, 26b-, 27-, 28a-. xlProminin-1 lacking exons 26b, 27 and 28a represents a major product resulting from alternative splicing of its gene in *X. laevis* retina.[1]

References

1. Han Z, Papermaster DS. Identification of three prominin homologs and characterization of their messenger RNA expression in Xenopus laevis tissues. *Mol Vis* 2011;17:1381-1396.
2. Zhu X, Brown B, Li A, Mears AJ, Swaroop A, Craft CM. GRK1-dependent phosphorylation of S and M opsins and their binding to cone arrestin during cone phototransduction in the mouse retina. *J Neurosci* 2003;23:6152-6160.
3. Pickrell SW, Zhu X, Wang X, Craft CM. Deciphering the contribution of known cis-elements in the mouse cone arrestin gene to its cone-specific expression. *Invest Ophthalmol Vis Sci* 2004;45:3877-3884.
4. Zhu X, Ma B, Babu S, Murage J, Knox BE, Craft CM. Mouse cone arrestin gene characterization: promoter targets expression to cone photoreceptors. *FEBS Lett* 2002;524:116-122.