

| | Nucleotide sequence | InDels | Protein sequence | Size |
|---------------------|---|--------|--|------|
| wt- <i>rho</i> .L | TACGAAGCCCATTTCGATTAC CCCTCAGTATTACTTAGCAGAGCC ATG | | ...VVRSPFDY PQYYLAEP WQYSALAA... | 354 |
| wt- <i>rho</i> .S | TGCGAAGCCCCTTTGATTAC CCCTCAGTATTACTTAGCAGAGCC ATG | | | |
| wt- <i>rho</i> .2.L | TACGAAGCCCATTTCGATTAC CCCTCAGTATTACTTAGCAGAGCC ATG | | | |
| Mix1-seq1 | TACGAAGCCCATTTCGATTAC CCC ---TATTACTTAGCAGAGCCATGG | -4 | ...VVRSPFDY PIT * | 29 |
| Mix1-seq2 | TACGAAGCCC---GATTAC CCCTCA -----TAGCAGAGCCATGG | -12 | ...VVRSP--ITLI-- AEP WQYSALAA... | 350 |
| Mix1-seq3 | TACGAAGCCCATTTCGATTAC C -----TTAGCAGAGCCATGG | -12 | ...VVRSPFDY--- LAEP WQYSALAA... | 350 |
| Embryo1-seq1 | TACGAAGCCCATTTCGATTAC CCCTCA-T -TTACTTAGCAGAGCCATG | -2 | ...VVRSPFDY PHLLSRAMAIF...WLLH * | 111 |
| Embryo1-seq2 | TACGAAGCCCATTTCGATTAC CCC-CA ---TTACTTAGCAGAGCCATG | -4 | ...VVRSPFDY PIT * | 29 |
| Embryo1-seq3 | TGCGAAGCCCCTTTGATTAC CCCTCAG ----- -----CACT | -31 | ...VVRSPFDY PQHLLTSCSSCLGYQSTS * | 46 |
| Embryo1-seq4 | TGCGAAGCCCCTTTGATTAC CCCTTTTTT ATTACTTAGCAGAGCCATG | -3;+3 | ...VVRSPFDY PFYYLAEP WQYSALAA... | 354 |
| Embryo2-seq1 | TACGAAGCCCATTTCGATTAC CCCT ---ATTACTTAGCAGAGCCATG | -4 | ...VVRSPFDY PIT * | 29 |
| Embryo2-seq2 | TACGAAGCCCATTTCGATTAC CCCT ---ATTACTTAGCAGAGCCATG | -4 | ...VVRSPFDY PIT * | 29 |
| Embryo2-seq3 | TACGAAGCCCATTTCGATTAC CCCTCAGTATTACTTAGCAGAGCC ATG | wt | ...VVRSPFDY PQYYLAEP WQYSALAA... | 354 |
| Embryo2-seq4 | TACGAAGCCCATTTCGATTAC CCCT -----TACTTAGCAGAGCCATG | -6 | ...VVRSPFDY P --- YLAEP WQYSALAA...VSPA* | 352 |
| Embryo3-seq1 | TGCGAAGCCCCTTTGATTAC CCCT-GGT ATTACTTAGCAGAGCCATG | -2;+1 | ...VVRSPFDY PGIT * | 30 |
| Embryo3-seq2 | TGCGAAGCCCCTTTGATTAC CCCT ---ATTACTTAGCAGAGCCATG | -4 | ...VVRSPFDY PIT * | 29 |
| Embryo3-seq3 | TACGAAGCCCATTTCGATTAC C -----TTAGCAGAGCCATG | -12 | ...VVRSPFDY--- LAEP WQYSALAA...VSPA* | 350 |
| Embryo3-seq4 | TACGAAGCCCATTTCGATTAC CCC -----TTAGCAGAGCCATG | -10 | ...VVRSPFDY P * | 27 |
| Embryo3-seq5 | TGCGAAGCCCCTTTGATTAC CCCTCAG -----AGCCATG | -13 | ...VVRSPFDY PQSHGNIQHLL...GYQSTS * | 52 |

Figure S1. Genomic analysis of *X. laevis rho* crispants. (A) Sequencing chromatograms showing the *rho* sequences of one *X. laevis* wild type embryo and two *rho* crispants. The bracket delineates the *rho* sgRNA target region. The PAM is in yellow. (B) TIDE analysis of the sequences presented in A. Note the presence of multiple Indels in these crispant individuals. (C) Sequences obtained from individual clones of PCR products amplified from genomic DNA. One mixture of *Xenopus laevis* F0 embryos (Mix1) or 3 single embryos were used. For each clone, the targeted sequence is shown in pink with PAM in bold, while indels are in blue. The right column indicates the corresponding protein sequence and its size (number of amino acids).

Stage 60 *X. laevis*

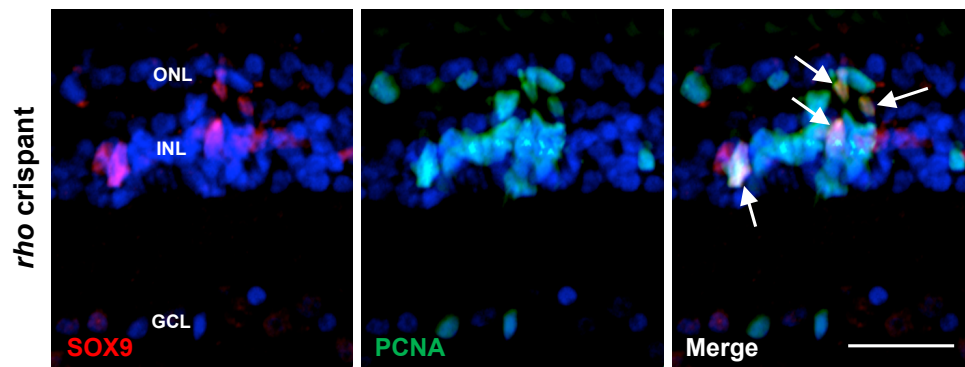


Figure S2. Analysis of proliferative cells in *X. laevis rho* crispants. Typical sections from stage 60 crispant *X. laevis* tadpoles, immunostained for PCNA (a marker of proliferative cells) and SOX9 (a marker of Müller cells). Arrows point to double positive cells. Cell nuclei are counterstained with Hoechst. GCL: Ganglion Cell Layer, INL: Inner Nuclear Layer, ONL: Outer Nuclear Layer. Scale bar: 25 μ m.

Supplementary Table S1. Primers

| Primers for genotyping in <i>X. tropicalis</i> | Primers for genotyping in <i>X. laevis</i> |
|---|---|
| F: TGGCAGCTTACTTCAGGTGG | F: TGGGATCACAGGCTTCTAGG |
| R: TGTAGCAACCAGTTTGGCCA | R: TGTAGCAACCAGTTTGGCCA |

Supplementary Table S2. List of antibodies

| | Species | Reference | Dilution |
|--|----------------|----------------------------------|-----------------|
| Primary Antibody | | | |
| Anti- Rhodopsin | Mouse | MABN15, Millipore | 1:1000 |
| Anti- S-opsin | Rabbit | AB5407, Millipore | 1:500 |
| Anti- M-opsin | Rabbit | AB5405, Millipore | 1:500 |
| Anti- Calbindin | Rabbit | PC253L, Calbiochem | 1:100 |
| Anti- BrdU | Rat | Ab6326, Abcam | 1:400 |
| Anti- Recoverin | Rabbit | AB5585, Millipore | 1:1000 |
| Anti- Sox9 | Rabbit | AB5535, Millipore | 1:100 |
| Anti-cleaved Caspase3 | Rabbit | 9661S, Cell signaling | 1:300 |
| Anti -YAP | Rabbit | Ab3961, Abcam | 1:50 |
| Anti PCNA | Mouse | M0879, Dako | 1:500 |
| Secondary Antibody | | | |
| Alexa Fluor 594 anti-mouse | | A11005, Thermo Fisher Scientific | 1:1000 |
| Alexa Fluor 488 anti-mouse | | A11001, Thermo Fisher Scientific | 1:1000 |
| Alexa Fluor 488 anti-mouse highly cross-adsorbed (used in combination with anti-rat) | | A11029, Thermo Fisher Scientific | 1:1000 |
| Alexa Fluor 594 anti-rabbit | | A11012, Thermo Fisher Scientific | 1:1000 |
| Alexa Fluor 488 anti-rabbit | | A11008, Thermo Fisher Scientific | 1:1000 |
| Alexa Fluor 594 anti-rat | | A11007, Thermo Fisher Scientific | 1:1000 |