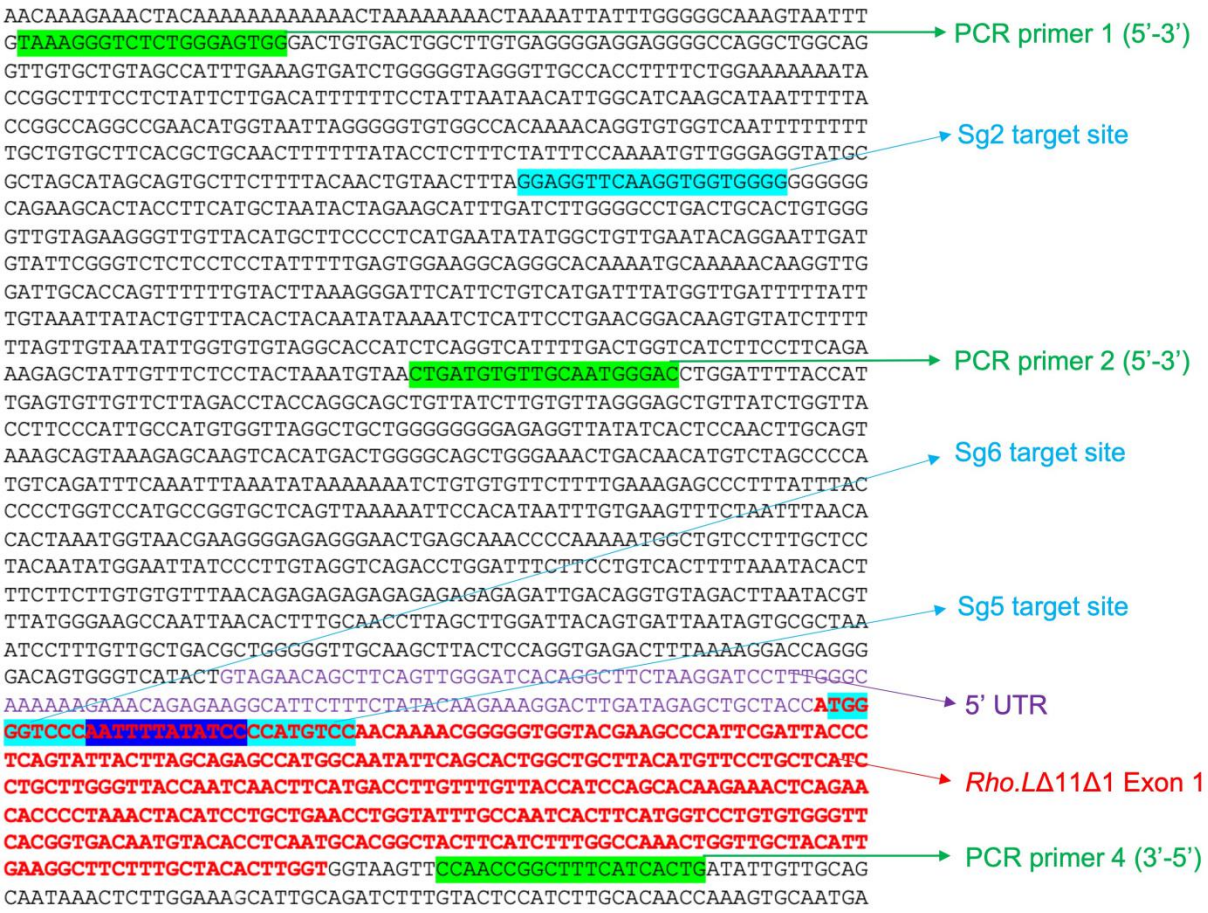
Supplementary Material 1

***X. laevis Rho.L*Δ11Δ1 sequence upstream and downstream from the start codon.**



Highlighted in green are the PCR primers used to validate the edits via fragment analysis as well as Sanger sequencing. Highlighted in light blue are the selected unique target sites for the designed sgRNAs, with the overlap between the sg5 and sg6 sequences indicated in dark blue.

Supplementary Material
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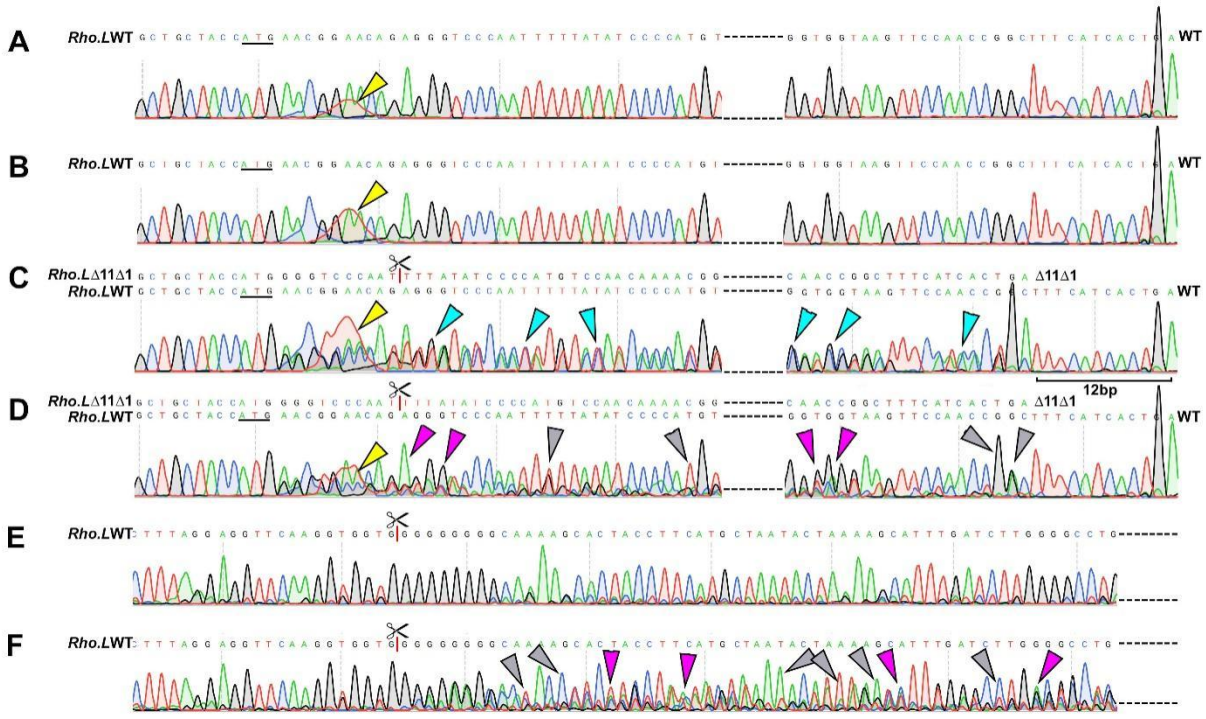

Illustration of typical Sanger sequencing results. Regions of the *Rho.L* gene were amplified from genomic DNA by PCR as described under Methods and in Appendix A, and analyzed by direct Sanger sequencing. Several scenarios are represented. **A-D** show sequencing of PCR products derived from the region of exon1 in which the *Rho.L*Δ11Δ1 deletion is located, and edited using the Sg5 guide RNA. Similar regions from the beginning and end of the sequencing traces are shown in each case. **A:** Sequencing trace from an untreated WT animal. Only one set of peaks is observed, matching the previously described sequence of the *Rho.L* gene. The large red peak indicated by the yellow arrowhead is an artifact. **B:** Sequencing trace from a WT animal treated using the Sg5 guide RNA. The trace looks identical to that in A, indicating no editing by Sg5. **C:** Sequencing trace from an animal heterozygous for the *Rho.L*Δ11Δ1 deletion. Following the "ATG" start codon (underlined), the sequence diverges into two overlapping traces that are easily deconvolved into the superimposed *Rho.L*WT and *Rho.L*Δ11Δ1 sequences. For reference, both sequences are shown above the trace. The superimposed peaks are mostly of roughly equal height (blue arrowheads show examples), indicating that the two DNA sequences are present in equal amounts. However, as the *Rho.L*WT sequence is 12bp longer, only single peaks appear in the last 12 bp of the trace. **D:** Sequencing trace from an animal originally heterozygous for the *Rho.L*Δ11Δ1 deletion, treated with Sg5. Again, the trace diverges into multiple peaks following the ATG start codon, initially resembling the trace shown in C. However, following the predicted cut site for Sg5 (indicated by the scissors icon and the vertical red line), peaks derived from the WT sequence become more dominant, indicating that the *Rho.L*Δ11Δ1 sequence has diverged into multiple sequences. From this point, peaks that are not

derived from the WT sequence no longer consistently match the *Rho.L*Δ11Δ1 sequence (grey arrowheads show examples that clearly diverge from C above), and triple or quadruple peaks sometimes occur (magenta arrowheads show examples). The end of the trace no longer has a clear sequence of 12 single peaks; in the example shown, 13 are present. This result indicates chimeric editing that alters the length of the *Rho.L*Δ11Δ1 allele in the presence of an unedited wildtype allele. **E-F** show sequencing of a PCR product derived from an upstream region of *Rho.L* that is targeted by Sg2 in both the *Rho.L*WT and *Rho.L*Δ11Δ1 alleles. **E** shows a sequencing trace for an untreated animal. A single set of peaks occurs, except for a few instances that likely represent polymorphisms. F shows a sequencing trace for an animal treated with Sg2. The sequence initially resembles the untreated sequence above, but following the predicted cut site for Sg2 (indicated by the scissors icon and the vertical red line) the trace diverges into multiple superimposed peaks, including peaks that no longer match the *Rho.L* sequence (examples indicated by grey arrowheads) (the unedited *Rho.L* sequence is shown above the trace for reference) and triple or quadruple peaks (examples indicated by magenta arrowheads). This indicates chimeric editing that alters the length of the *Rho.L* gene at the predicted cleavage site for Sg2. As the end of this PCR product is considerably farther from the sequencing primer used (817 bp vs 472 bp), the sequence at the end of the trace is of low quality (not shown) and not easily interpreted as in **A-E**.

Note that in addition to the analysis shown above, the exact sequence of *Rho.L*Δ11Δ1 was also previously determined by sequencing the cloned PCR product (Feehan et al. 2017).

Supplementary Material 3

***Rho.L*WT:**

GTAGAACAGCTTCAGTTGGGATCACAGGCTTCTAAGGATCCTTTGGGCAAAAAAGA
AACAGAGAAGGCATTCTTTCTATACAAGAAAGGACTTGATAGAGCTGCTACCATGA
ACGGAACAGAGGGTCCCAATTTTTATATCCCCATGTCCAACAAAACTGGGGTGGTAC
GAAGCCCATTCGATTACCCTCAGTATTACTTAGCAGAGCCATGGCAATATTCAGCAC
TGGCTGCTTACAT

11+1 deleted bases shown:

GTAGAACAGCTTCAGTTGGGATCACAGGCTTCTAAGGATCCTTTGGGCAAAAAAGA
AACAGAGAAGGCATTCTTTCTATACAAGAAAGGACTTGATAGAGCTGCTACCATGA~~A~~
~~ACGGAACAGA~~GGGTCCCAA~~T~~TTTTATATCCCCATGTCCAACAAAACTGGGGTGGTAC

GAAGCCCATTCGATTACCCTCAGTATTACTTAGCAGAGCCATGGCAATATTCAGCAC
TGGCTGCTTACAT

***Rho.L*Δ11Δ1:**

GTAGAACAGCTTCAGTTGGGATCACAGGCTTCTAAGGATCCTTTGGGCAAAAAAGA
AACAGAGAAGGCATTCTTTCTATACAAGAAAGGACTTGATAGAGCTGCTACCATGG
GGTCCCAATTTTATATCCCCATGTCCAACAAAACTGGGGTGGTACGAAGCCCATTCG
ATTACCCTCAGTATTACTTAGCAGAGCCATGGCAATATTCAGCACTGGCTGCTTACA
T

HDR Repair Template (120nt):

TTCTTTCTATACAAGAAAGGACTTGATAGAGCTGCTACCATGAACGGAACAGAGGG
TCCAAATTTTTATATCCCCTTTTCCAACAAAACTGGGGTGGTACGAAGCCCATTCGA
TTACCCT

Start codon

11+1 deletion

Sg5 target sequence

Insert = 36nt

M13F mutation: ATG → TTT

*Apo*I restriction site silent mutation: CAATTT → AAATTT

WT and *Rho.L*Δ12 N-terminal amino acid sequences:

```
      WT: MNGTEGPNFYIPMS…
          |      ||||||
Rho.LΔ12: MGSQ----FYIPMS…
          *      *
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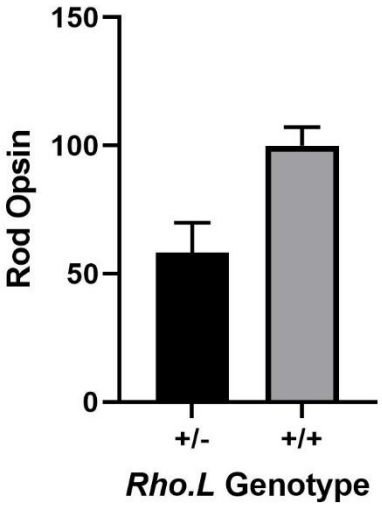
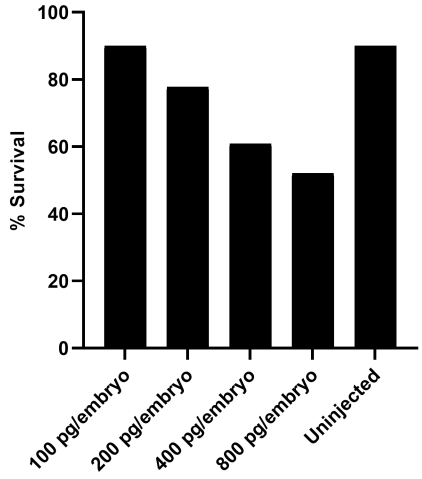
Supplementary Material 4



***Rho.L* is the highest expressing *X. laevis* rod opsin gene.** Using an experimental paradigm identical to Figure 1B, we compared rod opsin levels between animals heterozygous for a 7 bp deletion in exon 1 that creates a frameshift and premature termination consistent with a null allele, and their wildtype siblings. The heterozygous animals (n = 5) had a 42% reduction in rod opsin levels relative to wildtype (n = 6), indicating *Rho.L* produces 84% of all *X. laevis* rod opsin. As previously described (Feehan et al., 2017), these animals had little or no retinal degeneration.

Supplementary Material 5



Toxicity assessment of the HDR repair template. Varying amounts of ssDNA repair template were injected into single-cell WT *X. laevis* embryos to assess the optimal amount to use in the experiment shown in Figure 5. Survival was monitored for 15 days. The amount of repair template injected was inversely correlated with survival. The starting value was 66 embryos for each category.