



Supplementary Materials:

Table S1. Oligonucleotide sequences used for sgRNA synthesis.

| Oligo Name | Sequence (5' > 3') |
|--|--|
| constant oligo | AAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGAC TAGCCTTATTTAACTTGCTATTTCTAGCTCTAAAC |
| target-specific oligo 5' <i>eyS</i> ^{<i>Δexon40-44</i>} | CCGCTAGCTAATACGACTCACTATAGGCAAAACAAGCCAAACC TTGTTTATAGAGCTAGAAATAGCAAG |
| target-specific oligo 3' <i>eyS</i> ^{<i>Δexon40-44</i>} | CCGCTAGCTAATACGACTCACTATAGGTCTTCTGCTCTAAAAAG GGTTTATAGAGCTAGAAATAGCAAG |

T7 promoter in bold. Gene specific region in italics. Overlapping regions of the constant and target-specific oligonucleotides are underlined.

Table S2. Predicted off-target loci containing one mismatch compared to the on-target sequence for selected sgRNAs (Assembly: GRCz11/danRer11).

| Location | Strand | Sequence (5' > 3') |
|----------------------------|--------|--------------------------|
| chr20: 35406954 - 35406976 | - | CCTCCTTTTATAGAGCAGAACACC |
| chr22: 1380801 - 1380823 | + | GGTGTCTGCTCTAAAAAGGAGG |
| chr24: 15475502 -15475524 | + | GGTGTCTGCTCTAAAAAGGAGG |

Mismatches in bold.

Table S3. Primers used for RT-PCR analysis.

| | Primer name | Primer sequence (5' > 3') |
|--|---|-------------------------------|
| <i>eys</i> ^{Δexon40-44} genotyping | <i>Eys</i> wild-type or Δ exon 40-44 forward | AGGACGATTTTCTGCCTCTG |
| | <i>Eys</i> Δ exon 40-44 reverse | GGGTAAATTCCACATACATAT TGAC |
| | <i>Eys</i> wild-type reverse | GACAGACTAAAACCAGGCCA AA |
| CRISPR/Cas9 Off-target genotyping (for off-target sequences containing 1 mismatch compared to on-target sgR- NAs) | chr20: 35406954 – 35406976 forward | TCGACCAGGTTTTGATTGGT |
| | chr20: 35406954 – 35406976 reverse | GGACTTTCGACCAGGTTTTG |
| | chr22: 1380801 – 1380823 forward | GTTTCAGCTTGGCGTAGGAG |
| | chr22: 1380801 – 1380823 reverse | CCGGCTTTGTTTTGGTCTAA |
| | chr24: 15475502 – 15475524 forward | TGCACCACGTTGTTTACAGG |
| | chr24: 15475502 – 15475524 reverse | ATAACCCGTTCTGCACTGGA |
| <i>eys</i> ^{Δexon40-44} transcript analysis | <i>Eys</i> exon 38-47 forward | GGGGCCAAAATATCCAAAAC |
| | <i>Eys</i> exon 38-47 reverse | AAAGCCAGCAGACAGGGATA |
| Long-range PCR | <i>Eys</i> exon 1-20 forward | ATCCTCATCACAGTCCAGGC |
| | <i>Eys</i> exon 1-20 reverse | AGAACACCTGCAGCAGTTTG |
| | <i>Eys</i> exon 13-29 forward | TTTTGTGCACCTGGGTTTCG |
| | <i>Eys</i> exon 13-29 reverse | GCTGATGTCTGTGCTTCTCC |
| | <i>Eys</i> exon 27-46 forward | TGGAGATGAATGAGTGCTGC |
| | <i>Eys</i> exon 27-46 reverse | AGCTGTTTGGTGTCTGATAC |
| | <i>Eys</i> exon 1-46 forward | TCATTGTCTTCCTGCTCAGC |
| | <i>Eys</i> exon 1-46 reverse | AGCTGTTTGGTGTCTGATAC |
| | <i>Eys</i> exon 1-46 forward - nested | AGCCAAAGAACATCAGCGTG |
| | <i>Eys</i> exon 1-46 reverse - nested | CCAGATGTAACCGATGCCAC |

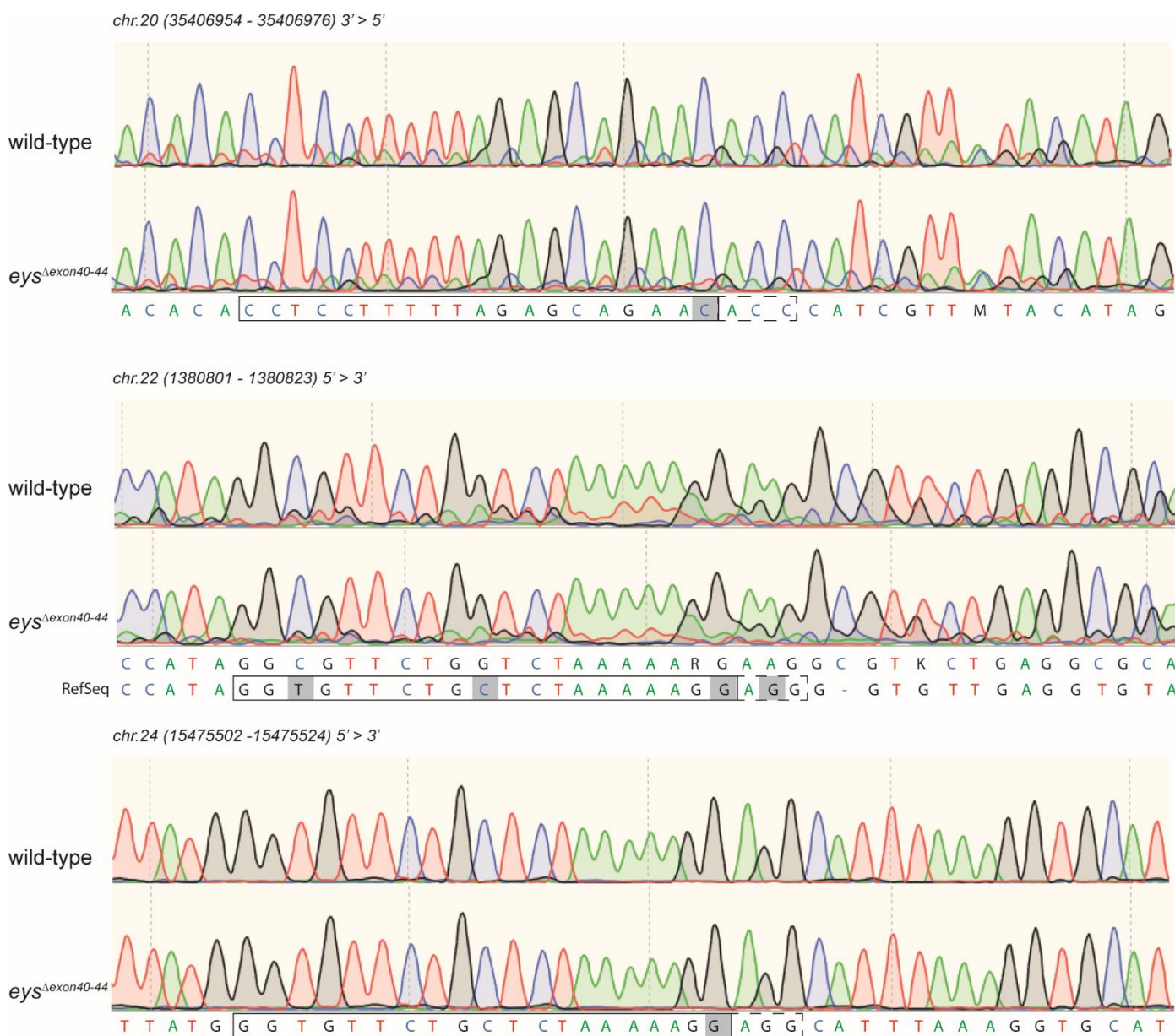


Figure S1. Screening of the predicted off-target loci containing one nucleotide mismatch with the gRNAs used. Sanger sequencing confirmed the absence of any off-target editing events at the three predicted 1-nucleotide mismatch off-target sites. sgRNAs are marked by a box, PAM-sites are marked by a dashed box, mismatches are marked in gray.

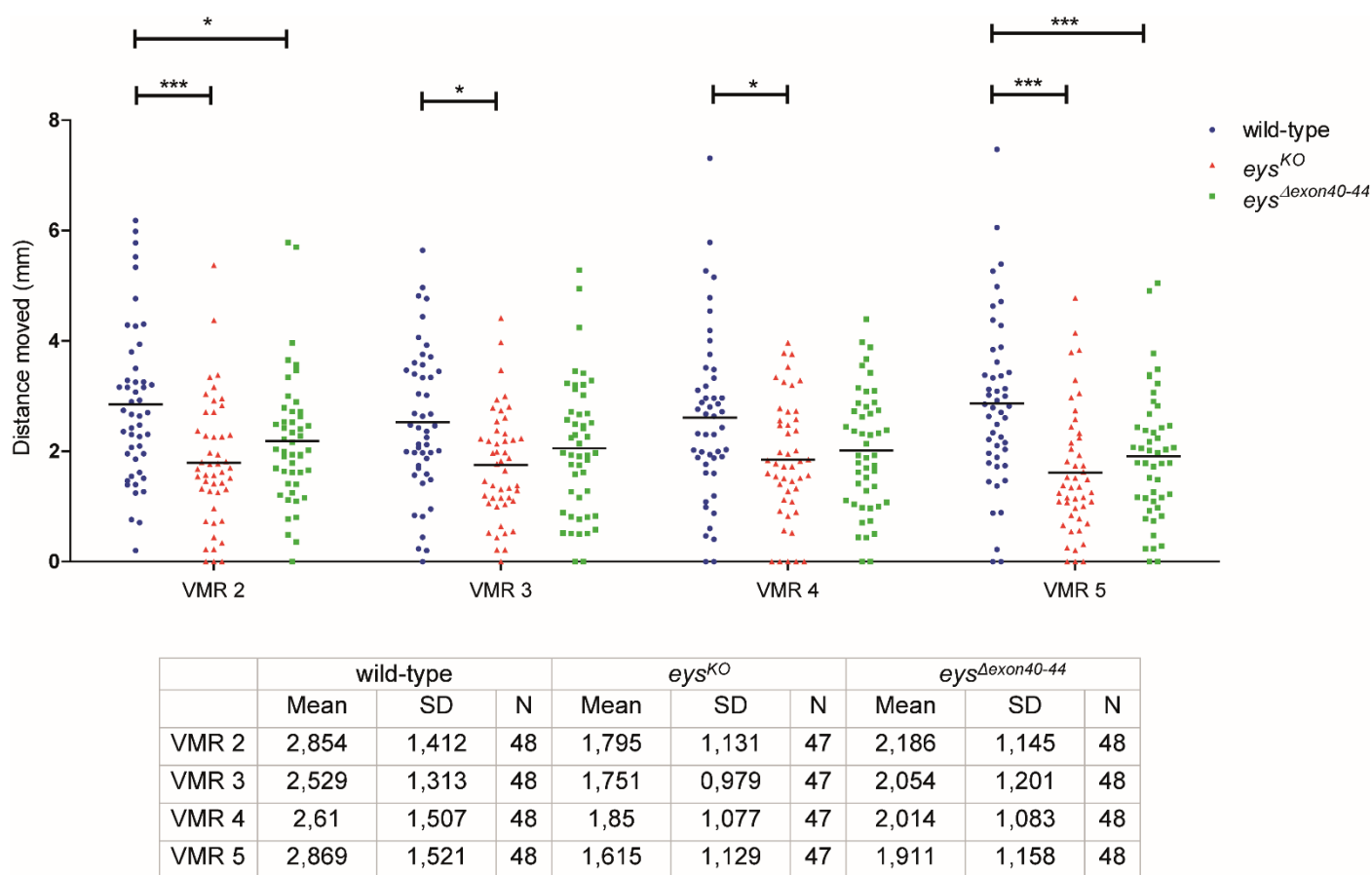


Figure S2. Visual Motor Responses in wild-type, *eys*^{KO} and *eys*^{Δexon40-44} zebrafish. The distance moved (mm) during the first second after the Light-ON transition for wild-type, *eys*^{KO} and *eys*^{Δexon40-44} larvae (5 dpf). The distance moved was significantly lower in *eys*^{Δexon40-44} larvae compared to wild-type larvae after two out of four Light-ON transitions, while in none of the transitions a significant difference was observed between *eys*^{KO} and *eys*^{Δexon40-44} larvae (5 dpf; n= 47 larvea, 3 biological replicates, 4 technical replicates). The means and standard deviations (SD) are shown in the table.