

**Figure S1: CRISPR-Cas9 loss-of-function mutants of zebrafish *ly96* exon 1.**

Exon 1 coordinates (GRCz11)Chr2: 30,281,444-30,281,710

Genotype: *ly96* +/+

Exon 1 coding sequence:

ATGGCGCTTTGGTGCCCGTCAGCTTTTCTTTGTTTTATTGCATTATCTTGTATGGCATCTGAGA  
AAGCAGAAAGAAAATCCTTATGCTCATCAGAACAAGTTAATTTTTGGTACACCTTTGAAG

Predicted Md-2 sequence:

MALWCPSAFLCFIALSCMASEKAERKSLCSSEQVNFWYTFEGPLHYLSMTVSPCSLGVSLYFINL  
TLIPMFTITRFGFHGVVYYKGMWLEHEVPNVLCEDYKICHIIKGETLRETFPVIIRKIDGPEGEYQ  
IHLTVNITDDLEMKETIHFNMTVEVKTTGGNKSFFSPQIGRL\*

Genotype: *ly96* A/A

Fish line number: b1364

Exon 1 coding sequence:

TTTGGTGCCCGTCAGCTTTTCTTTGTTTTATTGCATTATCTTGTATGGCATCTGAGAAAGCAG  
AAAGAAAATCCTTATGCTCATCAGAACAAGTTAATTTTTGGTACACCTTTGAAG

Predicted Md-2 sequence:

No start codon

Genotype: *ly96* B/B

Fish line number: b1365

Exon 1 coding sequence:

ATG-----

GTGCCCGTCAGCTTTTCTTTGTTTTATTGCATTATCTTGTATGGCATCTGAGAAAGCAGAAAG  
AAAATCCTTATGCTCATCAGAACAAGTTAATTTTTGGTACACCTTTGAAG

Predicted Md-2 sequence:

MVPVSFSLFYCIILYGI\*

Genotype: *ly96* C/C

Fish line number: b1366

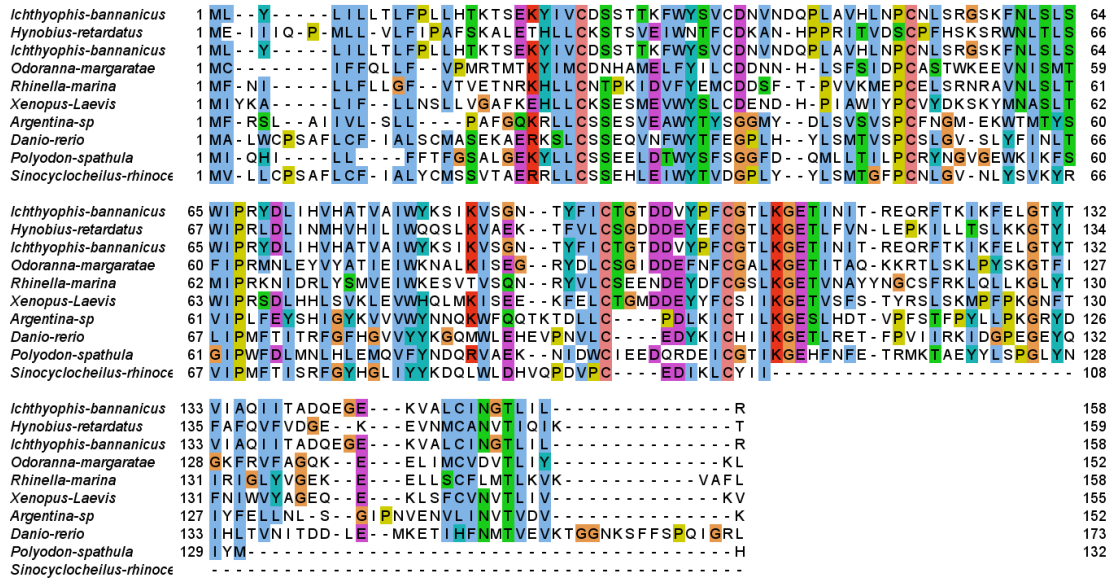
Exon 1 coding sequence:

ATG-----

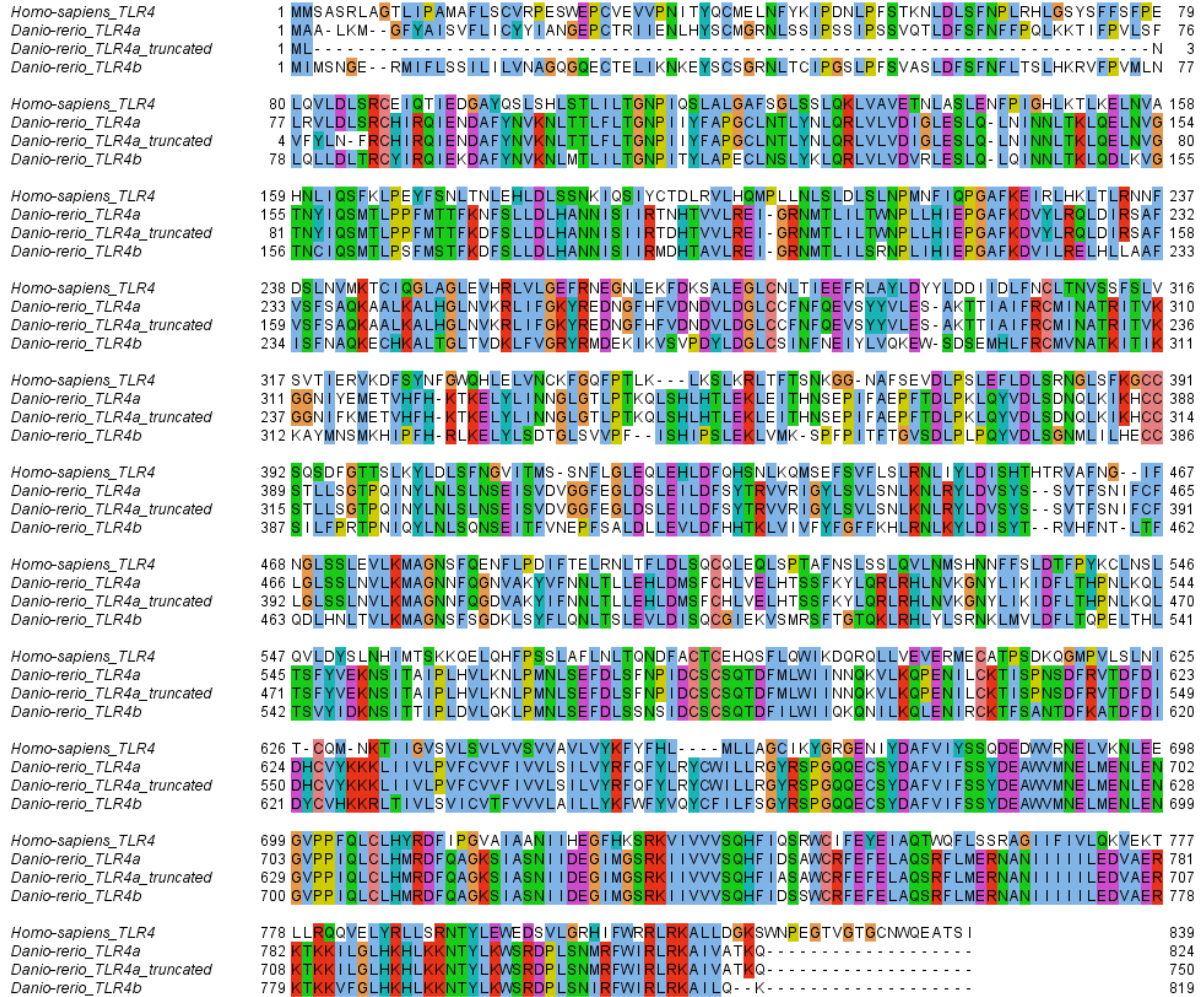
GCCCGTCAGCTTTTCTTTGTTTTATTGCATTATCTTGTATGGCATCTGAGAAAGCAGAAAGAA  
AATCCTTATGCTCATCAGAACAAGTTAATTTTTGGTACACCTTTGAAG

Predicted Md-2 sequence:

MARQLFFVLLHYLVWHLRKQKENPYAHQNKLIFGTPLKDHCIQC\*



**Figure S2. Alignment of newly identified fish and amphibian Md-2 proteins.** Md-2 proteins were identified using transcriptomic data from amphibians (dryad) and fish (Fish-T1K). Sequences were aligned with MSAPROBS. Colors are used to denote chemically similar residues and conservation. Alignment was constructed with Jalview.



**Figure S3: Comparison of zebrafish Tlr4a sequence used in this paper versus previous work.** Sequences were aligned to human TLR4 with T-coffee. Colors are used to denote chemically similar residues and conservation. Alignment was constructed with Jalview.