SUPPLEMENTARY FIGURE LEGENDS

Figure S1 Hydrophaty plots of human (*Hs*) and zebrafish (*Dr*) sialidase proteins.
The amino acid position is plotted as a function of the average hydrophobicity (window size: 11 amino acids) following the Kyte and Doolittle methods. The profile was obtained using the DNA Strider 1.4 program. The shaded area present in *Hs* NEU1 plot indicates the leader peptide. The hydrophaty plots have been arranged based on the level of sequence identity between human and zebrafish sialidases.

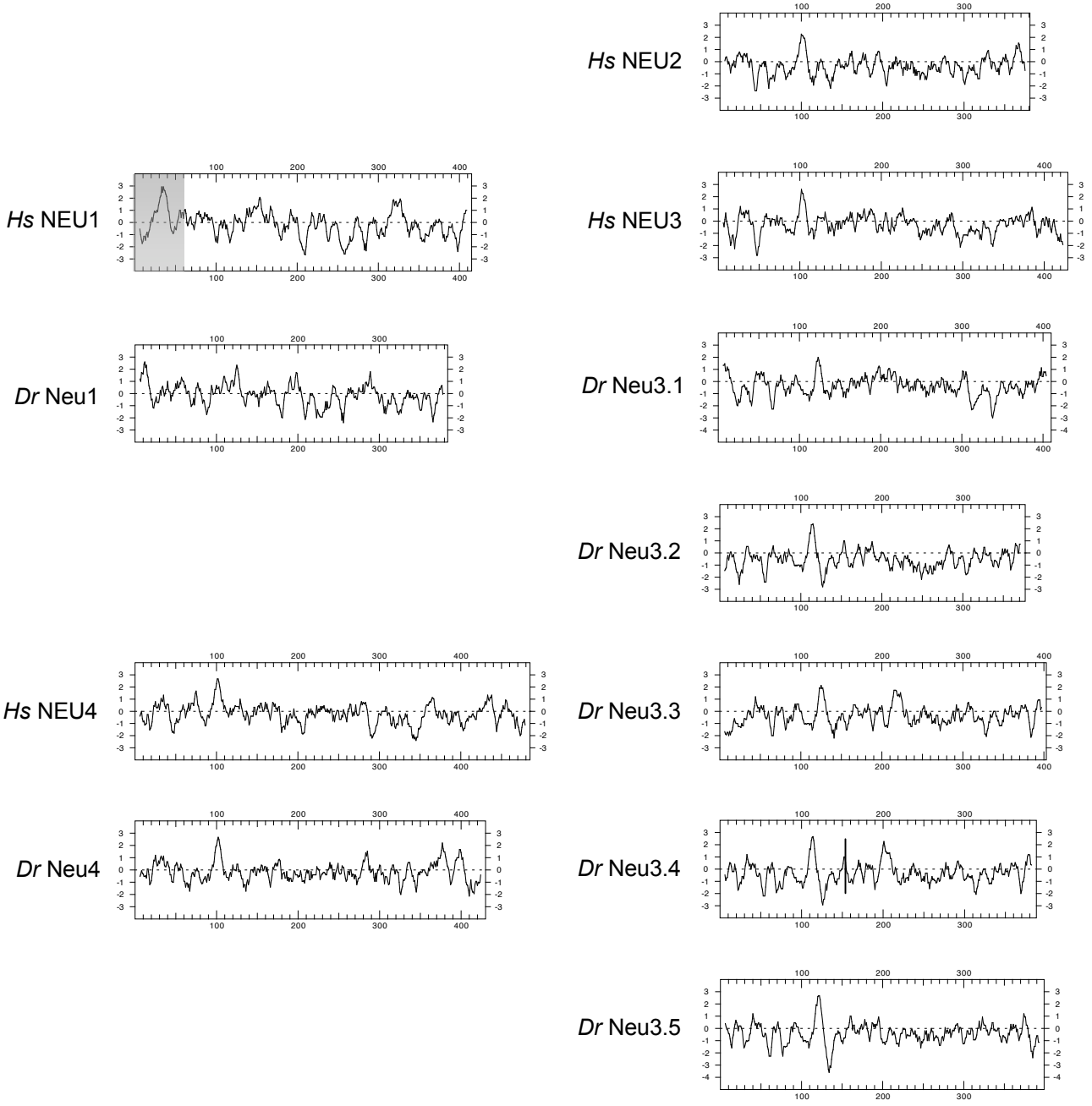
Figure S2 *In situ* hybridization of sense probes for *neu3.1* and *neu4* (s-neu3.1 and s-neu4).
A, sense probe for *neu3.1* in a 3 dpf embryo. B-D, sense probe for *neu4* in 21 hpf, 2 dpf and 3 dpf embryos. Embryos are in lateral (A, D) and dorsal (B, C) views with anterior to the left.

Figure S3 Expression of Neu1, Neu3.1, Neu3.2, Neu3.3 and Neu4 sialidases in COS7 cells.
COS-7 cells were transfected overnight with the constructs encoding Neu3.1-, Neu3.2-, Neu3.3- and Neu4-Myc epitope-tagged enzymes. Sialidase-specific activity toward the artificial substrate 4MU-NeuAc was determined as described in Materials and Methods using as enzymatic source aliquots of the crude cell extracts derived from mock and Neus expressing cells. Variations in the observed activity are indicated by the error bar (n = 3).

Figure S4 Subcellular localization of zebrafish sialidase Neu3.1.
COS7 cells expressing the Myc-tagged Neu3.1 sialidase were treated for double indirect immunofluorescence. *Left*: subcellular distribution of Neu3.1; *middle*: labeling of PDI (endoplasmic reticulum marker); *right*: merge of the previous staining patterns. The different primary antibodies were detected using isotype specific secondary antibodies conjugated with Alexa fluor 488 (green) and 555 (red). Speciments were analysed using a Zeiss Axiovert 200 microscope equipped with Confocal Laser System LSM 510 META.

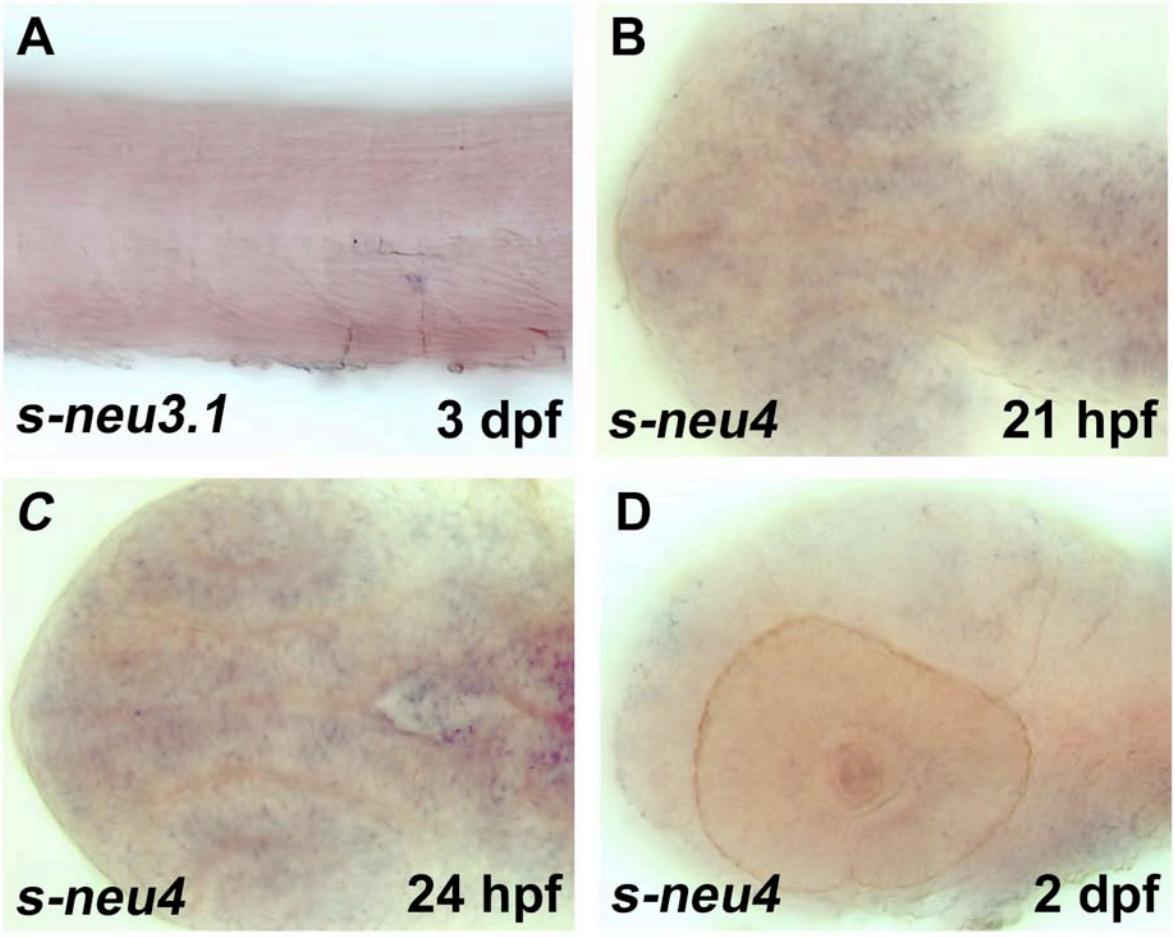
Supplementary Figure

Figure S1



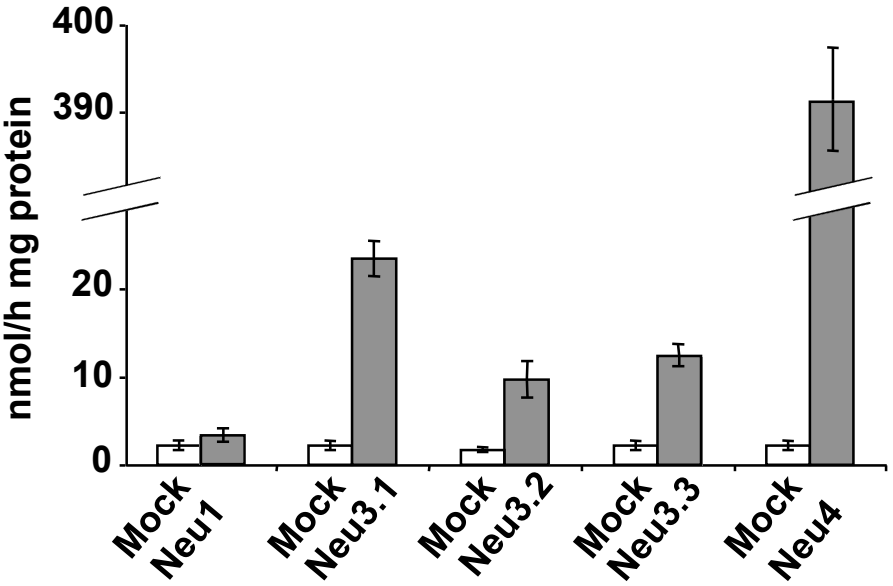
Supplementary Figure

Figure S2



Supplementary Figure

Figure S3



Supplementary Figure

Figure S4

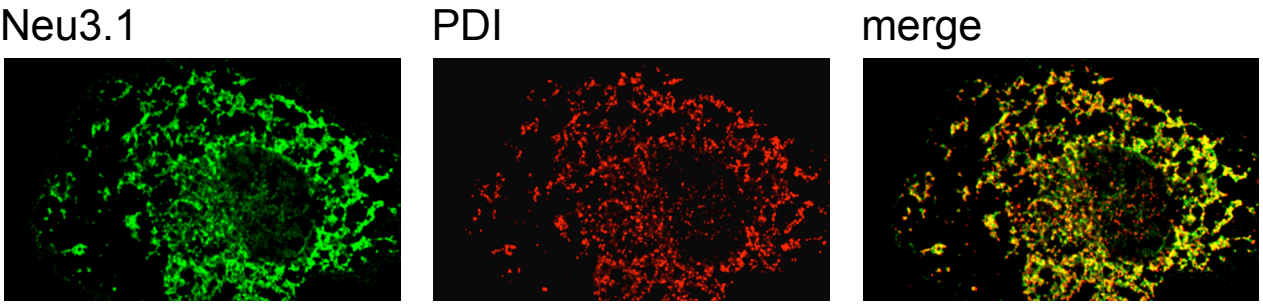


Table S1
General features of zebrafish sialidase genes

Gene name	Encoded polypeptide	Position on Zv6 assembly	Size (bp)
neu1	383	chr19:21918225-21931324	13100
neu3.1	409	chr21:19849217-19854475	5259
neu3.2	376	chr21:19844531-19847289	2759
neu3.3	402	chr21: 19835936-19840817	4882
neu3.4	387	chr21:19827188-19828973	1786
neu3.5	398	chr21:19821489-19822841	1353
neu4	429	chrNA_random:108563257-108570506	7250

Note
chrNA: Whole Genome Shotgun (WGS) contigs that could not be related to any FingerPrint Contig (FPC). These are unmapped scaffolds in random order separated by 1000 Ns between scaffolds. In the Zv7 assembly the *neu4* gene maps on chr 15.

Table S2
Amino acid sequence comparison between human (*Hs*) and zebrafish (*Dr*) sialidases

	Hs NEU1	*Hs* NEU2	*Hs* NEU3	*Hs* NEU4
Hs NEU1				
Hs NEU2	18			
Hs NEU3	18	40		
Hs NEU4	20	43	44	
Dr Neu1	**58**	17	20	21
Dr Neu3.1	15	38	**49**	41
Dr Neu3.2	15	37	**46**	42
Dr Neu3.3	17	41	**42**	40
Dr Neu3.4	17	39	**47**	40
Dr Neu3.5	15	39	**43**	39
Dr Neu4	15	42	40	**48**

Numbers represent amino acid percentage identity calculated with the ClustalW algorithm.
The highest values of identity in pairwise alignements are highlighted in bold.

Table S3
Amino acid sequence comparison among zebrafish (*Dr*) sialidases

	Dr Neu1	*Dr* Neu3.1	*Dr* Neu3.2	*Dr* Neu3.3	*Dr* Neu3.4	*Dr* Neu3.5
Dr Neu3.1	17					
Dr Neu3.2	20	**54**				
Dr Neu3.3	18	48	**59**			
Dr Neu3.4	18	55	63	**79**		
Dr Neu3.5	18	55	**73**	57	67	
Dr Neu4	21	44	**45**	41	**45**	43

Numbers represent amino acid percentage identity calculated with the ClustalW algorithm.
The highest values of identity in pairwise alignements are highlighted in bold.