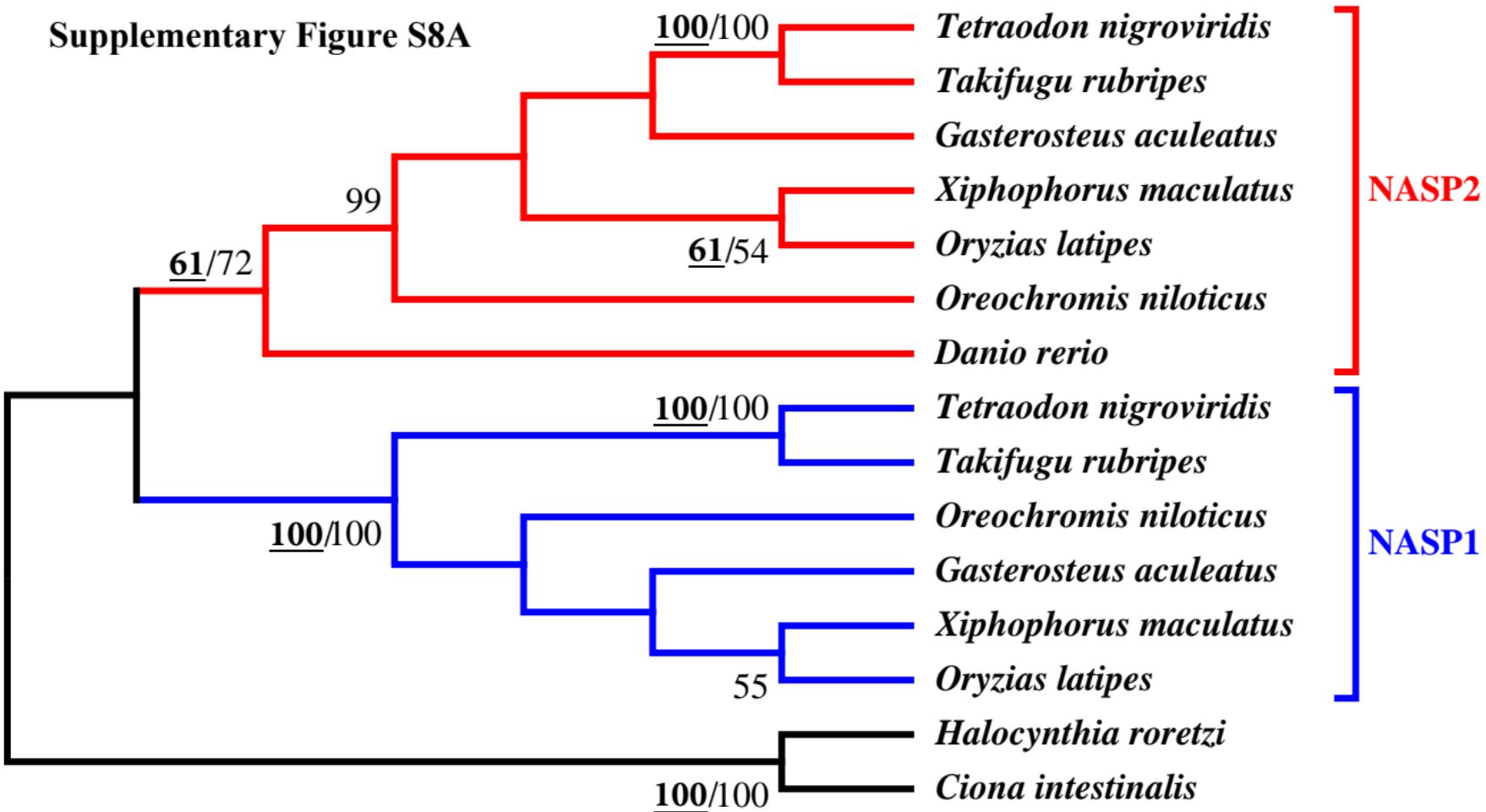
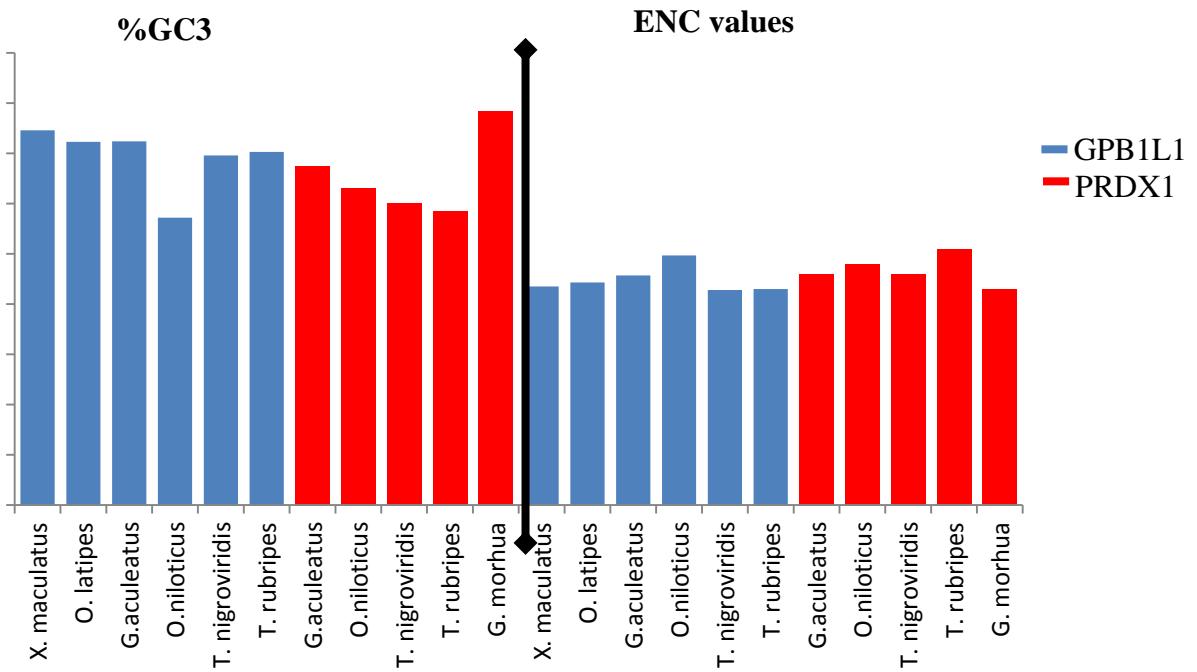
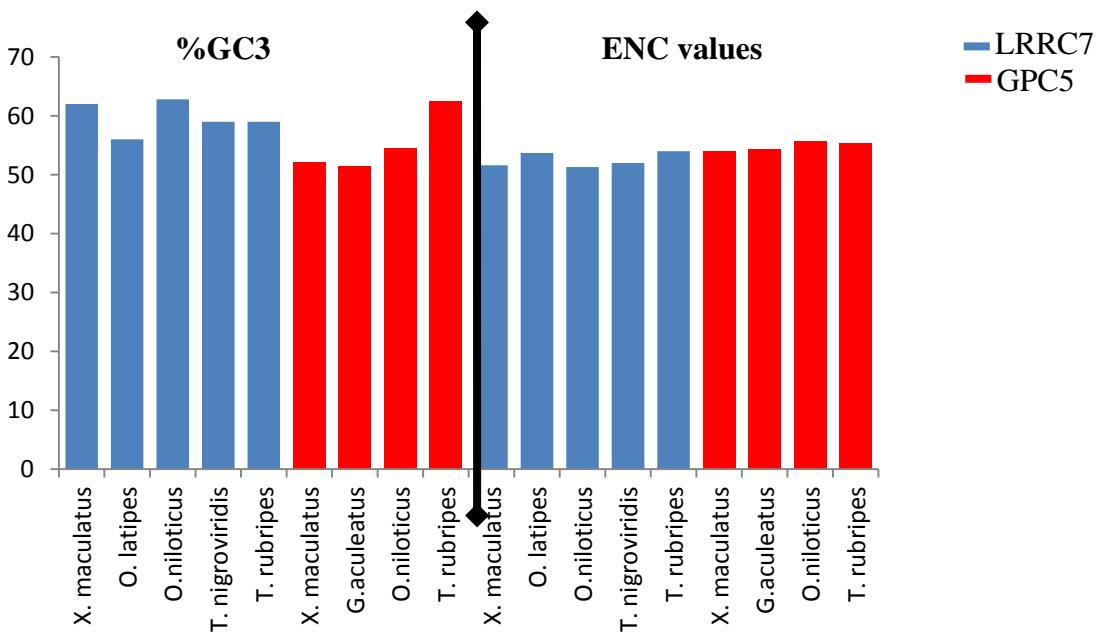


**Figure S8A:** Phylogenetic relationship among different fish specific NASP paralogs. Tree topology corresponds to the ML method under the JTT+G model as predicted by MEGA. Branch lengths do not reflect genetic distance. Alternatively, the same topology was also recovered by the neighbour joining method using p-distances. Confidence values for ML and NJ trees are based on 1000 bootstrap replicates and are indicated ( $\geq 50\%$ ) in light-face and boldface (underlined), respectively. The tree is rooted with *Halocynthia roretzi* and *Ciona intestinalis*.

**Figure S8, B-C:** %GC3 content and ENC values are shown in chart diagrams for the fish-specific NASP1 and NASP2 neighbouring genes, respectively. Black line separates GC3 and ENC whereas blue and red colors represent different neighbouring genes as indicated in the chart legends. **Figure S8, D-G:** Predicted fish specific NASP1/2 TPRs 1-4 sequence alignments are shown in figures B to E, respectively. Reconstructed ancestral sequence using the ML method is shown in black background whereas different amino acid substitutions in each TPR motif for two paralogous proteins are shown in blue. **Figure S8, H-K:** Predicted NASP1/2 TPRs 1-4 sequence alignments are shown in figures H to K, respectively, for various lineages.

Supplementary Figure S8A



**B****C**

**D**

|                            |   |          |                |      |    |         |          |       |      |  |
|----------------------------|---|----------|----------------|------|----|---------|----------|-------|------|--|
| <i>X.maculatus/1-34</i>    | A | KKLI GTG | KRHLVMGDVVS    | AVN  | V  | FQEACS  | M        | LAAK  |      |  |
| <i>O.latipes/1-34</i>      | A | KKLI GTG | KRHLVMGDVVS    | AVN  | V  | FQDACS  | I        | LASK  |      |  |
| <i>G.aculeatus/1-34</i>    | A | NKLI GTG | NRHLVMGDVVS    | AVR  | V  | FQDACS  | M        | LAAR  |      |  |
| <i>O.niloticus/1-34</i>    | A | KKLI GTG | KRHLVMGDVVS    | AVS  | V  | FQEACG  | M        | LAEK  |      |  |
| <i>T.nigroviridis/1-34</i> | A | KKLVGAG  | NKYLVL GDVVS   | AVG  | V  | FQDACS  | M        | LAAK  |      |  |
| <i>T.rubripes/1-34</i>     | A | KKLVGAG  | NKYLVL GDVVS   | AVA  | V  | FQDACS  | M        | LAAK  |      |  |
| <i>D.rerio/1-34</i>        | A | KKLI GTG | S RHLVMGDVVS   | AVS  | V  | FQEAC   | A        | MLAEK |      |  |
| <b>Ancestral/1-34</b>      | A | AKKL?    | G? GKKHLVMGDV? | SAVN | A  | FQEACS? | LAEK     |       |      |  |
| <i>X.maculatus/1-34</i>    | A | NKLVGTG  | KKFLVT GKVV    | EA   | V  | ALQEACG | M        | LAKK  |      |  |
| <i>O.latipes/1-34</i>      | A | NKLI GAG | KKFLVMGKAVE    | AVS  | AL | QEACG   | M        | LAKQ  |      |  |
| <i>G.aculeatus/1-34</i>    | A | NKLI GS  | GKKHLVMGKV     | V    | EA | V       | ALQEACG  | M     | LAQK |  |
| <i>O.niloticus/1-34</i>    | A | NKLI GTG | KKHLVMGKV      | V    | EA | V       | TLQEACG  | M     | LAKK |  |
| <i>T.nigroviridis/1-34</i> | A | NKLVGAG  | KKHLVMGKV      | V    | EA | VNS     | SLQEACGI | LAKT  |      |  |
| <i>T.rubripes/1-34</i>     | A | NKLI GAG | KKHLVMGKV      | V    | EA | VNS     | SLQEACG  | M     | LAKT |  |

NASP-2

**E**

|                            |   |      |   |   |      |     |     |     |        |           |           |       |     |
|----------------------------|---|------|---|---|------|-----|-----|-----|--------|-----------|-----------|-------|-----|
| <i>X.maculatus/1-34</i>    | G | EAFF | F | L | C    | GKS | LLE | LAR | ---    | LQLAWE    | MLEVAKVIF | KRK   |     |
| <i>O.latipes/1-34</i>      | G | EAFF | F | L | Y    | GKS | LLE | LAR | ---    | LQLAWE    | MLEVAKL   | IYKRK |     |
| <i>G.aculeatus/1-34</i>    | A | EAFF | F | L | C    | GKS | LLE | LAR | ---    | LQLAWE    | MLEVAKVIY | KRK   |     |
| <i>O.niloticus/1-34</i>    | G | EAFF | F | F | C    | GKS | LLE | LAR | ---    | LQLAWE    | MLEVAKVIY | KRK   |     |
| <i>T.nigroviridis/1-34</i> | G | EAFF | F | F | C    | GKS | LLE | LAR | ---    | LQLAWE    | MLEVAKVIY | KRK   |     |
| <i>T.rubripes/1-34</i>     | G | EAFF | F | F | C    | GKS | LLE | LAR | ---    | LQLAWE    | MLEVAKVIY | KRK   |     |
| <i>D.rerio/1-34</i>        | G | EAFF | F | F | C    | GKS | LLE | LAR | ---    | LQLAWE    | MLEVAKVIY | KRK   |     |
| <b>Ancestral/1-34</b>      | G | GEAF | F | F | C    | GKS | LLE | LAR | ---    | LQLAWEML? | LAK?      | I?    | LR? |
| <i>X.maculatus/1-34</i>    | G | GEAF | Y | W | C    | GKA | LLD | LAR | ---    | LQLAWE    | MLEVAKVIY | RRK   |     |
| <i>O.latipes/1-34</i>      | G | GEAF | L | W | C    | GKA | LLD | LAR | ---    | LQLAWE    | MLEVAKS   | IYKRK |     |
| <i>G.aculeatus/1-34</i>    | G | GEAF | F | W | C    | GKA | LLD | LAR | ---    | LQLAWE    | MLEVAKVIY | KRK   |     |
| <i>O.niloticus/1-34</i>    | G | GEAF | F | W | Y    | GKA | LLD | LAR | ---    | LQLAWE    | MLEVAKVIY | KRK   |     |
| <i>T.nigroviridis/1-34</i> | G | GEAF | F | W | C    | GKA | LLD | LAR | ---    | LQLAWE    | MLEVAKVIY | KRK   |     |
| <i>T.rubripes/1-34</i>     | G | GEAF | F | W | CGKA | LLD | LAR | --- | LQLAWE | MLEVAKVIY | KRK       |       |     |

NASP-2

**F**

|                            |   |               |
|----------------------------|---|---------------|
| <i>X.maculatus/1-34</i>    | AQAYLKLGEVS AETGNYP QALEDFQECLI I QL KL   | <b>NASP-2</b> |
| <i>O.latipes/1-34</i>      | AQAHLKLGEVGAETGNYP QALEDFQECLAI QL QH     |               |
| <i>G.aculeatus/1-34</i>    | AQAYLKLGEVS AESGNYS QALEDFQECLAL QL KH    |               |
| <i>O.niloticus/1-34</i>    | AQAYLKLGEVS AESGNYP QALDDFQECLAL QL KH    |               |
| <i>T.nigroviridis/1-34</i> | AQIYLKLGEVS AESGNYP QALEDFQECLCL QL KY    |               |
| <i>T.rubripes/1-34</i>     | AQIYLKLGEVS AESGNYP QALEDFQECLCL QL KH    |               |
| <i>D.rerio/1-34</i>        | AQIHLKLAEVGVESGNYS QALEDSNECLTL QL KH     |               |
| <b>Ancestral/1-34</b>      | AQAHLKLGEVS VESGNYP QA? EDFQ? CL? I QK? H |               |
| <i>X.maculatus/1-34</i>    | AQTHLKLGEVS AESGNYN QALEDFQECLKVQL KH     |               |
| <i>O.latipes/1-34</i>      | AQAHLKLGEVS AESGNYT QALEDFQECLKL QVKH     |               |
| <i>G.aculeatus/1-34</i>    | AQAHSKLGEVS S ESGNYT QALEDFQECLKL QVKH    |               |
| <i>O.niloticus/1-34</i>    | AQAHKLGEVS AESGNYP QALEDFQECLKL QVKH      |               |
| <i>T.nigroviridis/1-33</i> | AQAHKLGEVS VESGNYT QALDDFQECLKL QAK-      |               |
| <i>T.rubripes/1-34</i>     | AQAHKLGEVS VESGNYT QALDDFECLKL QL KH      |               |

**G**

|                            |  |               |
|----------------------------|--|---------------|
| <i>X.maculatus/1-34</i>    | AETHYHVATT L VFMDQYD QAI KHYNS SVK VIE T R     | <b>NASP-2</b> |
| <i>O.latipes/1-34</i>      | AETHYHVATT LCYMD E YR QAI QHYNS SI EVIE NR     |               |
| <i>G.aculeatus/1-34</i>    | AETHYHVATT LCYMD QYS QAI QHYNS SI KVIE TR      |               |
| <i>O.niloticus/1-34</i>    | AETHYHVATT LCYMD QYS QAI QHYNS SI KVIE TR      |               |
| <i>T.nigroviridis/1-34</i> | AETHYHVATT LCYMD KYS QAI QHYNS SI EVIE KR      |               |
| <i>T.rubripes/1-34</i>     | AETHYHVATT LCYMD KYS QAI QHYNS SI EVIE KR      |               |
| <i>D.rerio/1-34</i>        | TEHYQLGTTYSYT T QYN QAI EHF S NSI KVIES R      |               |
| <b>Ancestral/1-34</b>      | AETHYQLGLAYS YN ? QYD? AI ? HF N? SI ? VIE ? R |               |
| <i>X.maculatus/1-34</i>    | AETHYQLGVT HSLNL QYVPAI QALNN SI S VIK NR      |               |
| <i>O.latipes/1-34</i>      | AETHYQLGLTYSLN L QYS PAI EALNN SI S VIKS R     |               |
| <i>G.aculeatus/1-34</i>    | AETHYQLGLTYSLDVQYGRAI EELKS SI S VIKS R        |               |
| <i>O.niloticus/1-34</i>    | AETHYQLGVT YSLNT QYS EAI ESLKS SI S I IKNR     |               |
| <i>T.nigroviridis/1-34</i> | AETHYQLGLTHGLNL QHGAAVAELSRSI S VIKS R         |               |
| <i>T.rubripes/1-34</i>     | AETHYQLGLTYGLNL QYNN QAI AELNRSI S VIKS R      |               |

H

|                                  |                     |            |                 |              |
|----------------------------------|---------------------|------------|-----------------|--------------|
| <i>C.elegans</i> -NASP1          | LAEELLAAGRRRALKVNDI | DKASD      | SLSEATEL        | SSE I        |
| <i>C.elegans</i> -NASP2          | LSENI               | IAGRRFMRT  | DVFDKAVDVL      | SVAAS IAAEV  |
| <i>C.briggsae</i> -NASP1         | AAELLAAGRRALKV      | SDYETATEAL | SEASEM          | I VEL        |
| <i>C.briggsae</i> -NASP2         | YTEQL               | IAGRRFMKT  | NVFDFKATEAL     | SLAAAL GAE I |
| <i>I.tridecemlineatus</i> _NASP1 | AKRLLGLGQKHL        | VMDG       | I PAAVNAFQEAAAS | LLGKK        |
| <i>I.tridecemlineatus</i> _NASP2 | VKKLLGLGQKHL        | VVGDI      | PVA - - -       | EEAAS LLGKR  |
| <i>G.gorilla</i> _tNASP          | AKKLLGLGQKHL        | VMGD       | I PAAVNAFQEAAAS | LLGKK        |
| <i>G.gorilla</i> _NASP2          |                     |            |                 |              |

I

|                                  |                 |                |          |                |           |
|----------------------------------|-----------------|----------------|----------|----------------|-----------|
| <i>C.elegans</i> -NASP1          | FDSL            | YYYGMATLELAK-- | MKL      | SWE I LET      | TARCIAAAK |
| <i>C.elegans</i> -NASP2          | FEANFL          | YGKALLEVGK--   | MKLAWE   | LLET           | SRCIADKK  |
| <i>C.briggsae</i> -NASP1         | YEYYYYYYGMATLEL | GK--           | MKLAWE   | VLENARCIAMAK   |           |
| <i>C.briggsae</i> -NASP2         | FDANFL          | YGMKALLELSK--  | I KLAWE  | LLET           | SRCICEKK  |
| <i>I.tridecemlineatus</i> _NASP1 | GEAFFFYGK       | SLL ELAR--     | LELAWDM  | DLAKI IFKRQ    |           |
| <i>I.tridecemlineatus</i> _NASP2 | GEDFFFYGK       | SQL - - -      | LEL - LD | I LDLAKI IFKSQ |           |
| <i>G.gorilla</i> _tNASP          | GEAFFFYGK       | SLL ELAR--     | LELAWDM  | DLAKI IFKRQ    |           |
| <i>G.gorilla</i> _NASP2          |                 |                | LELAWN   | TLDLAKIVFKRQ   |           |

J

|                                  |            |                 |                 |            |        |
|----------------------------------|------------|-----------------|-----------------|------------|--------|
| <i>C.elegans</i> -NASP1          | ADVLVLLLG  | EHG I SDGKY     | TQAF            | EDLDRALN   | IQRNV  |
| <i>C.elegans</i> -NASP2          | ADVL       | TSLGEHG I ADSKY | EQAQKDLTEA      | ISI        | QTvh   |
| <i>C.briggsae</i> -NASP1         | ADVLVVLGDH | SVSDGKY         | DQALE           | EDLEQALE   | IQKNV  |
| <i>C.briggsae</i> -NASP2         | ADVL       | TSLGEHG I VDSKY | EQAQKDLAEAVAI   | QSAL       |        |
| <i>I.tridecemlineatus</i> _NASP1 | AQAH       | LKLGEVSVE       | ENY IQA         | VEEFQACLN  | LQEQQY |
| <i>I.tridecemlineatus</i> _NASP2 | - - - H    | I KLGEV         | YVESEN Y IQAMEV | FQTFNLNL   | QEQC   |
| <i>G.gorilla</i> _tNASP          | AQAH       | LKLGEVSVE       | ENY VQA         | VEEFQSCLNL | LQEQQY |
| <i>G.gorilla</i> _NASP2          | AQARL      | LKLGEVG         | VESEN Y VQA     | VEEFQSCLKL | WEQQY  |

K

|                                  |       |            |          |          |                  |             |
|----------------------------------|-------|------------|----------|----------|------------------|-------------|
| <i>C.elegans</i> -NASP1          | AQTY  | I L I      | GNACASD  | ANYDE    | TVQYFGKTKDVL     | I AR        |
| <i>C.elegans</i> -NASP2          | ANT   | THL L      | AKAFSS   | DSL      | FEQAAAHFNDT      | KN I L I AK |
| <i>C.briggsae</i> -NASP1         | AQTY  | I LMADACT  | SGMNYDEA | I NYFEK  | TKE TLKSR        |             |
| <i>C.briggsae</i> -NASP2         | ANT   | THL L      | AKSFSLDS | I FEKAAT | HFCESKN I L I AK |             |
| <i>I.tridecemlineatus</i> _NASP1 | AETHY | YQL GLAYG  | YN S QYD | EAVAQFN  | KSI EVI EKR      |             |
| <i>I.tridecemlineatus</i> _NASP2 | AETHY | YQL - FAYG | YN S QC  | DEAVAQFN | RSEALEKR         |             |
| <i>G.gorilla</i> _tNASP          | AETHY | YQL GLAYG  | YN S QYD | EAVAQFS  | SKS I EVI ENR    |             |
| <i>G.gorilla</i> _NASP2          | AETHY | YQL GLAYG  | YN S QYD | DEAVAQFN | KS I EVI EKR     |             |