

Supplementary Table1: Accession number of the sequences used in this study

Species	Accession	Taxonomic Group
<i>Gallus gallus</i>	CAG30998.1	Aves/Chordata
<i>Mus musculus</i>	NP_001074944.1	Mammalia/Chordata
<i>Salmo salar</i>	NP_001167378.1	Actinopterygii / Chordata
<i>Xenopus laevis</i>	AAI70018.1	Amphibia/Chordata
<i>Xenopus laevis (V-2)</i>	AAH77440.1	Amphibia/Chordata
<i>Xenopus laevis (V-3)</i>	CAA28419.1	Amphibia/Chordata
<i>Xenopus tropicalis</i>	NP_001164988.1	Amphibia/Chordata
<i>Homo sapiens</i>	NP_689511.2	Mammalia/Chordata
<i>Bos taurus</i>	XP_005204766.1	Mammalia/Chordata
<i>Orcinus orca</i>	XP_004285798.1	Mammalia/Chordata
<i>Maylandia zebra</i>	XP_004562165.1	Actinopterygii / Chordata
<i>Taeniopygia guttata</i>	XP_004174360.1	Aves/Chordata
<i>Homo sapiens (tNASP)</i>	NM_001195193.1	Mammalia/Chordata
<i>Homo sapiens (tNASP-V2)</i>	NM_002482.3	Mammalia/Chordata
<i>Danio rerio</i>	NP_956076.1	Actinopterygii / Chordata
<i>Xiphophorus maculatus (NASP2)</i>	ENSXMAG00000011059	Actinopterygii / Chordata
<i>Xiphophorus maculatus (NASP1)</i>	ENSXMAG00000018420	Actinopterygii / Chordata
<i>Taeniopygia guttata</i>	ENSTGUT00000008366	Aves/Chordata
<i>Taeniopygia guttata (NASP2)</i>	ENSTGUT00000017166	Aves/Chordata
<i>Felis catus</i>	XM_003990037.1	Mammalia/Chordata
<i>Oryzias latipes (NASP2)</i>	ENSORLG00000011095	Actinopterygii / Chordata
<i>Felis catus (NASP2)</i>	ENSFCAT00000023663	Mammalia/Chordata
<i>Gorilla gorilla (tNASP)</i>	ENSGGOT00000033308	Mammalia/Chordata
<i>Gorilla gorilla (NASP2)</i>	ENSGGOT00000028193	Mammalia/Chordata
<i>Oryzias latipes (NASP1)</i>	ENSORLG00000010548	Actinopterygii / Chordata
<i>Gasterosteus aculeatus (NASP2)</i>	ENSGACG00000006499	Actinopterygii / Chordata
<i>Gasterosteus aculeatus (NASP1)</i>	ENSGACG00000016167	Actinopterygii / Chordata
<i>Oreochromis niloticus (NASP2)</i>	ENSONIG00000008932	Actinopterygii / Chordata
<i>Oreochromis niloticus (NASP1)</i>	XP_003453360.1	Actinopterygii / Chordata
<i>Takifugu rubripes (NASP2)</i>	XP_003974435.1	Actinopterygii / Chordata
<i>Takifugu rubripes (NASP1)</i>	ENSTRUG00000012389	Actinopterygii / Chordata
<i>Tetraodon nigroviridis (NASP2)</i>	ENSTNIG00000018075	Actinopterygii / Chordata
<i>Tetraodon nigroviridis (NASP1)</i>	ENSTNIG00000006947	Actinopterygii / Chordata
<i>Gadus morhua (Atlantic cod)(NASP2)</i>	ENSGMOG00000008773	Actinopterygii / Chordata
<i>Gadus morhua (Atlantic cod)(NASP1)</i>	ENSGMOG00000011853	Actinopterygii / Chordata
<i>Ictidomys tridecemlineatus (NASP1)</i>	I3MSG5	Mammalia/Chordata
<i>Ictidomys tridecemlineatus (NASP2)</i>	I3NEY0	Mammalia/Chordata
<i>Halocynthia roretzi</i>	Q02508.1	Tunicates
<i>Ciona intestinalis</i>	XP_002120883.2	Tunicates
<i>Tribolium castaneum</i>	XP_970862.2	Arthropods
<i>Megachile rotundata</i>	XP_003708323.1	Arthropods
<i>Danaus plexippus</i>	EJH78545.1	Arthropods
<i>Apis florea</i>	XP_003694981.1	Arthropods
<i>Bombus terrestris</i>	XP_003400722.1	Arthropods
<i>Drosophila melanogaster</i>	NP_649828.1	Arthropods

<i>Drosophila sechellia</i>	XP_002031992.1	Arthropods
<i>Drosophila erecta</i>	XP_001980917.1	Arthropods
<i>Caenorhabditis elegans (NASP1)</i>	NP_496380.1	Nematoda
<i>Caenorhabditis elegans (NASP2)</i>	NP_506298.1	Nematoda
<i>Caenorhabditis remanei</i>	XP_003117223.1	Nematoda
<i>Caenorhabditis brenneri</i>	EGT44801.1	Nematoda
<i>Caenorhabditis briggsae (NASP1)</i>	XP_002631180.1	Nematoda
<i>Caenorhabditis briggsae (NASP2)</i>	XP_002637622.1	Nematoda
<i>Caenorhabditis brenneri (NASP2)**</i>	EGT53840.1	Nematoda
<i>Caenorhabditis remanei (NASP2)**</i>	XP_003104429.1	Nematoda
<i>Cryptococcus gattii</i>	XP_003191095.1	Fungi
<i>Trichosporon asahii var. asahii</i>	EKD02263.1	Fungi
<i>Tetrapisispora phaffii</i>	XP_003687774.1	Fungi
<i>Neurospora crassa</i>	XP_961229.1	Fungi
<i>Schizosaccharomyces pombe</i>	NP_595313.1	Fungi
<i>Coccidioides immitis</i>	XP_001243327.1	Fungi
<i>Saccharomyces cerevisiae</i>	NP_013078.1	Fungi
<i>Candida albicans</i>	XP_712231.1	Fungi
<i>Schizosaccharomyces japonicus</i>	XP_002175640.1	Fungi
<i>Tremella fuciformis**</i>	ADE10090.1	Fungi
<i>Candida glabrata**</i>	XP_446527.1	Fungi
<i>Arabidopsis thaliana</i>	NP_568019.1	Plants
<i>Capsella rubella</i>	EOA15483.1	Plants
<i>Theobroma cacao</i>	EOX91255.1	Plants
<i>Cicer arietinum</i>	XP_004511989.1	Plants
<i>Tetrahymena thermophila</i>	XP_001030823.3	Ciliates
<i>Ichthyophthirius multifiliis</i>	XP_004027727.1	Ciliates
<i>Paramecium tetraurelia</i>	XP_001456110.1	Ciliates
<i>Plasmodium yoelii</i>	XP_729565.1	Apicomplexans
<i>Neospora caninum</i>	XP_003882797.1	Apicomplexans
<i>Toxoplasma gondii</i>	XP_002365332.1	Apicomplexans
<i>Babesia bovis**</i>	XP_001609366.1	Apicomplexans
<i>Albugo laibachii</i>	CCA18837.1	Oomycete
<i>Thalassiosira pseudonana</i>	XP_002294415.1	Diatom
<i>Choanoflagellida_Salpingoecidae</i>	XP_004991759.1	<i>Choanoflagellates</i>
<i>Dictyostelium fasciculatum</i>	XP_004362161.1	Amoebozoa
<i>Dictyostelium discoideum**</i>	XM_629896.1	Amoebozoa
<i>Acanthamoeba castellanii**</i>	XP_004341330.1	Amoebozoa
<i>Acanthamoeba castellanii**</i>	XM_004341282.1	Amoebozoa
<i>Nannochloropsis gaditana**</i>	EKU20698.1	Alga
<i>Guillardia theta**</i>	EKX34129.1	Cryptophyta
<i>Trypanosoma cruzi</i>	XP_810178.1	Kinetoplastids/Excavata
<i>Trypanosoma brucei</i>	CBH18038.1	Kinetoplastids/Excavata
<i>Trichomonas vaginalis**</i>	A2DW29	Parabasalia/Trichomonadida /Excavata

** Not included in the phylogeny reconstruction; V stands for variant

Supplementary Table2: Average number of amino acid and nucleotide variations among different regions of the NASP proteins discriminating various taxonomic groups

		$p_{AA}(SE)$	$p_{NT}(SE)$	$p_S(SE)$	$p_N(SE)$	R^a	Z-test ^b
Vertebrate	TPRs only	0.308±0.023	0.305±0.013	0.595±0.018	0.200±0.015	1.14	16.06 ^{***}
	Acidic only	0.525±0.038	0.394±0.015	0.598±0.018	0.334±0.024	1.02 [#]	7.216 ^{***}
	Other Regions	0.439±0.023	0.388±0.011	1.670±0.078	0.403±0.032	0.92	14.75 ^{***}
Tunicata	TPRs only	0.309±0.039	0.353±0.023	0.740±0.048	0.217±0.028	0.92	9.387 ^{***}
	Acidic only	0.750±0.036	0.466±0.021	0.630±0.052	0.416±0.031	1.07 [#]	3.381 ^{***}
	Other Regions	0.507±0.042	0.480±0.023	0.796±0.056	0.385±0.033	0.69	5.938 ^{***}
Arthropoda	TPRs only	0.511±0.022	0.426±0.013	0.60±0.018	0.361±0.019	1.19	7.541 ^{***}
	Acidic only	0.648±0.021	0.524±0.011	0.639±0.017	0.490±0.016	0.89 [#]	6.373 ^{***}
	Other Regions	0.499±0.023	0.440±0.014	0.645±0.019	0.372±0.020	1.02	9.054 ^{***}
Nematoda	TPRs only	0.350±0.029	0.337±0.017	0.630±0.032	0.230±0.023	1.13	9.583 ^{***}
	Acidic only	0.728±0.043	0.531±0.029	0.757±0.054	0.473±0.037	0.65 [#]	3.627 ^{***}
	Other Regions	0.425±0.030	0.371±0.015	0.686±0.032	0.273±0.021	1.07	11.24 ^{***}
Fungi	TPRs only	0.696±0.019	0.557±0.011	0.758±0.013	0.494±0.016	0.58	11.75 ^{***}
	Acidic only	0.833±0.021	0.634±0.011	0.770±0.016	0.599±0.017	0.54 [#]	6.551 ^{***}
	Other Regions	0.768±0.024	0.605±0.013	0.779±0.018	0.556±0.021	0.58	7.472 ^{***}
Plants	TPRs only	0.251±0.027	0.311±0.022	0.621±0.053	0.206±0.025	0.86	7.047 ^{***}
	Acidic only	0.609±0.036	0.571±0.031	0.663±0.075	0.547±0.039	0.45	1.366 [*]
	Other Regions	0.506±0.022	0.400±0.013	0.620±0.027	0.327±0.016	0.80	9.218 ^{***}
Ciliates	TPRs only	0.593±0.030	0.451±0.017	0.661±0.039	0.395±0.024	0.69	5.116 ^{***}
	Acidic only	0.862±0.033	0.444±0.026	0.546±0.058	0.422±0.038	0.53 [#]	1.835 ^{**}
	Other Regions	0.633±0.027	0.479±0.015	0.654±0.036	0.440±0.020	0.55	4.866 ^{***}
Apicomplexa	TPRs only	0.685±0.022	0.519±0.013	0.686±0.023	0.467±0.019	0.71	7.066 ^{***}
	Acidic only	0.779±0.039	0.529±0.032	0.729±0.051	0.461±0.042	0.69	3.358 ^{**}
	Other Regions	0.646±0.019	0.528±0.012	0.687±0.022	0.476±0.017	0.72	6.887 ^{***}
Euglenozoa	TPRs only	0.265±0.038	0.289±0.022	0.646±0.051	0.174±0.026	0.76	8.156 ^{***}
	Other Regions	0.444±0.029	0.410±0.020	0.745±0.037	0.300±0.023	0.92	9.998 ^{***}

p_{AA} , p_{NT} , p_S , and p_N , represent average number of amino acid, nucleotide, synonymous and non-synonymous nucleotide differences per site along with Z-test of selection. SE indicates standard error based on 1000 bootstrap replicates.

^a average transition/transversion ratio

[#] Calculation performed using pair wise deletion

^b $H_1: p_N < p_S$ and $H_0: p_N = p_S$

^{***} $P < 0.001$; ^{**} $P < 0.05$; ^{*} $P < 0.1$

NOTE: Remaining region encompasses all sites excluding TPRs 1-4 and acidic interruption region of TPR2

Supplementary Table3: Average number of amino acid among different TPRs discriminating various taxonomic groups

	TPR1(SE) <i>pAA</i>	TPR2(SE) <i>pAA</i>	TPR3(SE) <i>pAA</i>	TPR4(SE) <i>pAA</i>
Vertebrate	0.313±0.043	0.392±0.03	0.273±0.049	0.428±0.048
Tunicata	0.559±0.086	0.400±0.047	0.206±0.070	0.235±0.069
Arthropoda	0.505±0.038	0.567±0.023	0.567±0.046	0.577±0.040
Nematoda	0.377±0.054	0.452±0.038	0.294±0.059	0.456±0.064
Fungi	0.761±0.027	0.741±0.022	0.646±0.041	0.663±0.043
Plants	0.284±0.055	0.404±0.034	0.206±0.048	0.284±0.062
Chromalveolata	0.824±0.021	0.669±0.034	0.694±0.037	0.805±0.022
Euglenozoa	0.412±0.084	0.180±0.054	0.294±0.076	0.235±0.073

pAA represents average number of amino acid variation and SE denotes standard error calculated based on 1000 bootstrap replicates

Supplementary Table 4: Number of acidic residues (Asp + Glu) and theoretical iso-electric points distinguishing between the entire protein and acidic regions for various NASP proteins

Species	Whole protein (Asp + Glu)/ Theoretical pI	TPR2-Acidic region (Asp + Glu)/ Theoretical pI
<i>Tetrahymena thermophila</i>	110/ 4.58	40/4.12
<i>Saccharomyces cerevisiae</i>	78/ 4.64	45/3.71
<i>Coccidioides immitis</i>	84/ 4.64	31/4.24
<i>Schizosaccharomyces pombe</i>	76/ 4.76	23/4.28
<i>Neurospora crassa</i>	105/ 4.58	41/4.49
<i>Tetrapisispora phaffii</i>	105/ 4.07	56/3.38
<i>Trichosporon asahii</i>	82/ 4.63	30/3.84
<i>Cryptococcus gattii</i>	79/ 4.40	28/3.63
<i>Tribolium castaneum</i>	79/ 4.42	38/4.09
<i>Megachile rotundata</i>	106/ 4.54	61/4.27
<i>Danaus plexippus</i>	86/ 4.46	42/3.85
<i>Apis florea</i>	113/ 4.50	68/4.22
<i>Bombus terrestris</i>	115/ 4.44	70/4.13
<i>Drosophila melanogaster</i>	102/ 4.30	48/3.89
<i>Drosophila sechellia</i>	103/ 4.30	49/3.88
<i>Drosophila erecta</i>	102/ 4.32	48/3.94
<i>Danio rerio</i>	164/ 4.40	111/4.14
<i>Salmo salar</i>	98/ 4.53	55/4.08
<i>Oreochromis niloticus</i>	139/ 4.37	88/4.12
<i>Takifugu rubripes</i>	105/ 4.37	52/4.19
<i>Xenopus laevis</i>	152/ 4.42	89/4.15
<i>Xenopus tropicalis</i>	155/ 4.38	93/4.11
<i>Homo sapiens</i>	99/ 4.35	35/3.95
<i>Caenorhabditis elegans</i>	95 /4.34	30/3.87
<i>Caenorhabditis remanei</i>	95 /4.31	30/3.93
<i>Caenorhabditis brenneri</i>	85 /4.30	31/3.99
<i>Caenorhabditis briggsae</i>	89 /4.38	23/3.98
<i>Ichthyophthirius multifiliis</i>	91 /4.81	24/4.26
<i>Paramecium tetraurelia</i>	86 /4.60	27/4.11
<i>Halocynthia roretzi</i>	119/ 4.67	54/4.40
<i>Ciona intestinalis</i>	117 /4.57	74/4.46
<i>Mus musculus</i>	98 /4.42	35/4.04
<i>Gallus gallus</i>	82 /4.79	36/3.94
<i>Plasmodium yoelii</i>	75 /4.59	19/4.41
<i>Toxoplasma gondii</i>	95 /4.59	17/4.50
<i>Arabidopsis thaliana</i>	96 /4.28	28/3.89
<i>Capsella rubella</i>	96 /4.47	28/4.04
<i>Theobroma cacao</i>	95 /4.37	33/4.12
<i>Cicer arietinum</i>	90 /4.46	27/4.22
<i>Trypanosoma cruzi</i>	100/ 4.48	-
<i>Trypanosoma brucei</i>	76 /4.60	-
<i>Bos taurus</i>	101/ 4.36	35/3.98
<i>Orcinus orca</i>	101 /4.36	35/3.98

<i>Albugo laibachii</i>	90 /4.84	18/4.40
<i>Thalassiosira pseudonana</i>	94 /4.51	23/3.66
<i>Maylandia zebra</i>	89 /4.26	37/3.75
<i>Taeniopygia guttata</i>	83 /4.86	36/3.92
<i>Candida albicans</i>	93 /4.41	36/3.84
<i>Schizosaccharomyces japonicus</i>	70 /4.69	22/4.38
<i>Choanoflagellida Salpingoecidae</i>	70 /4.85	32/3.98
<i>Dictyostelium fasciculatum</i>	87 /4.72	25/4.28
<i>Neospora caninum</i>	98/4.59	17/4.55

Supplementary Table5: Average number of amino acid and nucleotide variations among different regions of the NASP for various lineage specific paralogs

		$p_{AA}(SE)$	$p_{NT}(SE)$	$p_S(SE)$	$p_N(SE)$	R^a	Z-test ^b
Fish	TPRs only	0.232±0.023	0.253±0.011	0.525±0.019	0.152±0.016	1.03	13.68 ^{***}
	Acidic only	0.383±0.039	0.329±0.019	0.577±0.032	0.251±0.030	0.93	8.326 ^{***}
	Other Regions	0.344±0.030	0.309±0.014	0.555±0.022	0.225±0.020	0.93	12.05 ^{***}
Nematoda	TPRs only	0.461±0.031	0.403±0.016	0.673±0.026	0.311±0.022	0.87	10.66 ^{***}
	Acidic only	0.719±0.031	0.495±0.024	0.694±0.039	0.440±0.030	0.75	5.088 ^{***}
	Other Regions	0.579±0.027	0.448±0.016	0.674±0.026	0.377±0.020	0.84	9.048 ^{***}

p_{AA} , p_{NT} , p_S , and p_N , represent average number of amino acid, nucleotide, synonymous and non-synonymous nucleotide differences per site along with Z-test of selection. SE indicates standard error based on 1000 bootstrap replicates.

^a average transition/transversion ratio

^b $H_1: p_N < p_S$ and $H_0: p_N = p_S$

^{***} $P < 0.001$

Note: A pair wise comparison for each pair of sequences (NASP1/2) in these lineages was also carried out (not shown; See text for details).

Supplementary Table6: Average number of amino acid and nucleotide variations among different regions of the NASP for various lineage specific paralogs using pair wise comparisons

		$p_{AA}(SE)$	$p_{NT}(SE)$	$p_S(SE)$	$p_N(SE)$	R^a	Z-test ^b
<i>I. tridecemlineatus</i> Paralogs	TPRs only	0.187±0.035	0.117±0.016	0.169±0.035	0.096±0.018	1.68	N/A [†]
	Acidic only	0.178±0.045	0.132±0.022	0.207±0.060	0.111±0.029	0.93	1.488 [*]
	Other Regions	0.211±0.027	0.105±0.011	0.095±0.020	0.107±0.014	2.13	N/A [†]
<i>G. gorilla</i> Paralogs	TPRs only	0.105±0.033	0.050±0.013	0.033±0.020	0.058±0.019	2.25	N/A [†]
	Acidic only [‡]	0.096±0.017	0.038±0.007	0.016±0.008	0.046±0.008	1.82	2.59 ^{**}
	Other Regions	0.215±0.028	0.143±0.014	0.155±0.026	0.139±0.019	0.92	N/A [†]

$p_{AA}, p_{NT}, p_S,$ and p_N , represent average number of amino acid, nucleotide, synonymous and non-synonymous nucleotide differences per site along with Z-test of selection. SE indicates standard error based on 1000 bootstrap replicates.

^a average transition/transversion ratio

^b $H_1: p_N < p_S$ and $H_0: p_N = p_S$

** P<0.05; * P<0.1

† Null hypothesis of $p_N = p_S$ could not be rejected

‡ Test of Positive selection; $H_1: p_N > p_S$ and $H_0: p_N = p_S$

** P<0.05