# Supplementary Information

**Evolutionary and Structural analyses of the NADPH Oxidase Family in Eukaryotes reveal an initial calcium dependency**

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**Supplemental Files**
- *Supplementary_Eukaryota sequence list*: text file listing all sequences used for the analysis in fasta format.
- *Supplementary_SIAS*: excel file listing the pairwise sequence identities from the raw multiple sequence alignment.
- *Supplementary Eukarya phylogenetic analysis*: Newick tree format file picturing the phylogenetic analysis of eukaryotic NOXs.

**Supplementary Figures and Table**
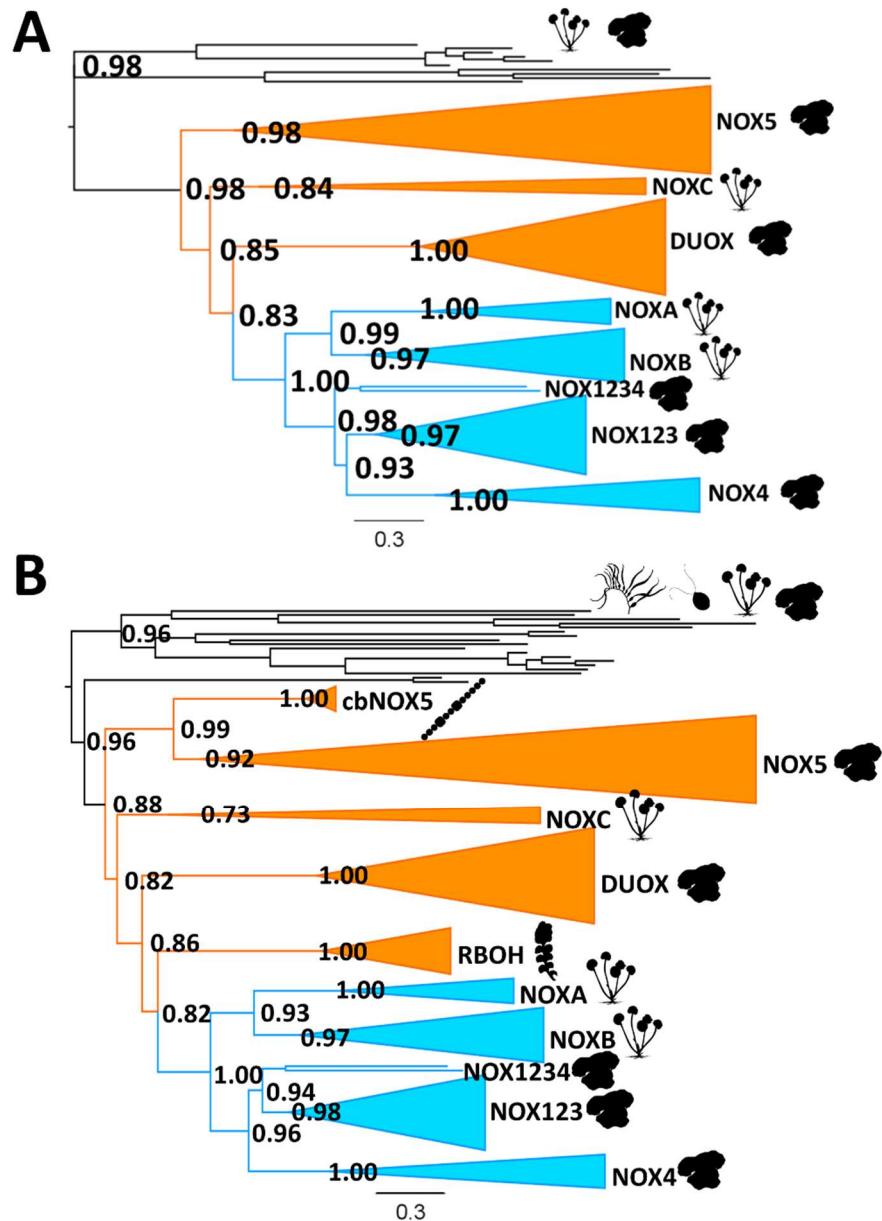


**Figure S1. (A)** Phylogenetic analysis of *Opisthokonta* NOXs by ML inference method**. (B)** Phylogenetic analysis of *Eukarya* NOXs and cyanobacterial NOX5-like sequences by ML inference method. The evolution of the NOX family is shown with orange clades corresponding to calcium-dependent NOXs and cyan clades representing calcium-independent NOXs. Each clade is labelled with their respective type NOX and the organisms (shown as silhouettes) that possess them. Branch supports are shown at key diverging points as transfer bootstrap expectations (TBE). Silhouettes represents: early divergent eukaryotes, Cyanobacteria, Chlorophyta, Streptophyta, Fungi and Metazoa.
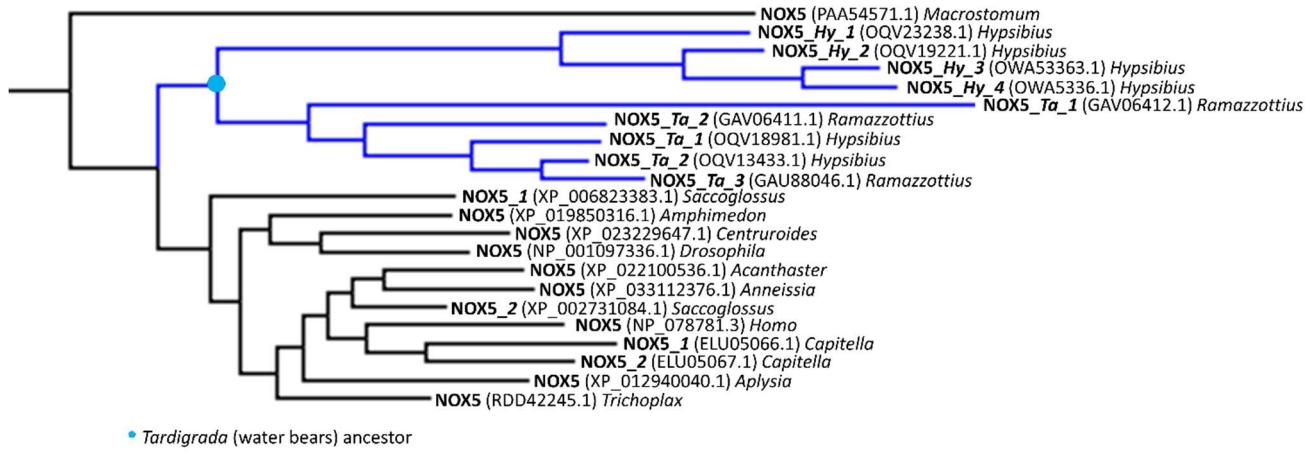
**Figure S2.** *Eukaryota* phylogenetic tree section focused on NOX5.
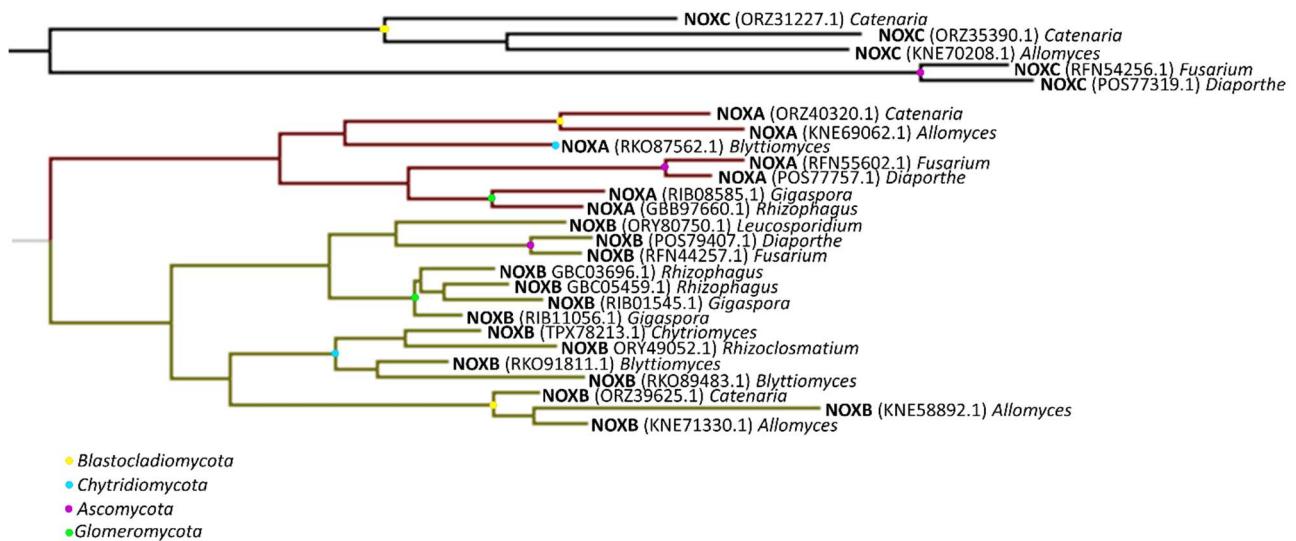


**Figure S3.** *Eukaryota* phylogenetic tree section focused on fungal NOXC (top; black clade) and fungal calcium-independent sequences (bottom; brown clade for NOXA and gold clade for NOXB). *Leucosporidium creatinivorum* NOXB (ORY80750.1, *Basidiomycota*) and *Chytriomyces confervae* NOXB (TPX78213.1) sequences were added to enrich the original dataset.

**A**



**Figure S4.**

**B**
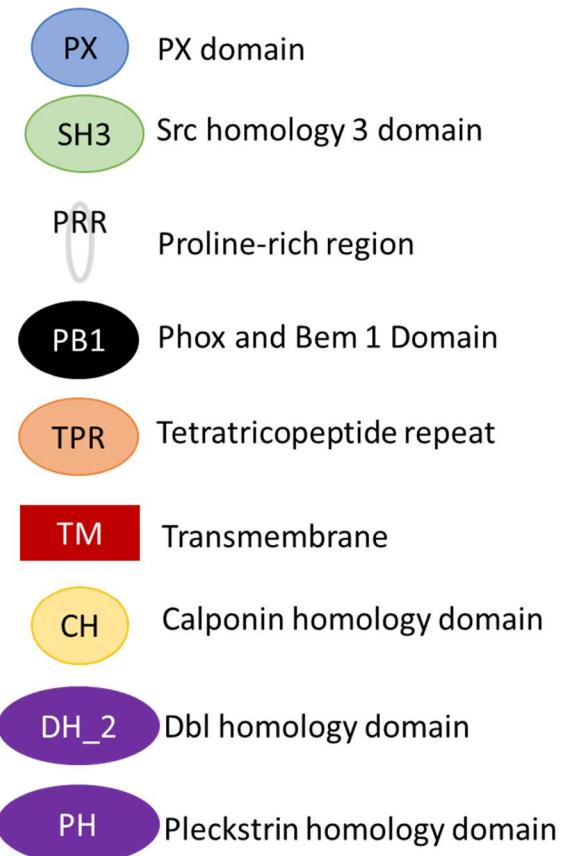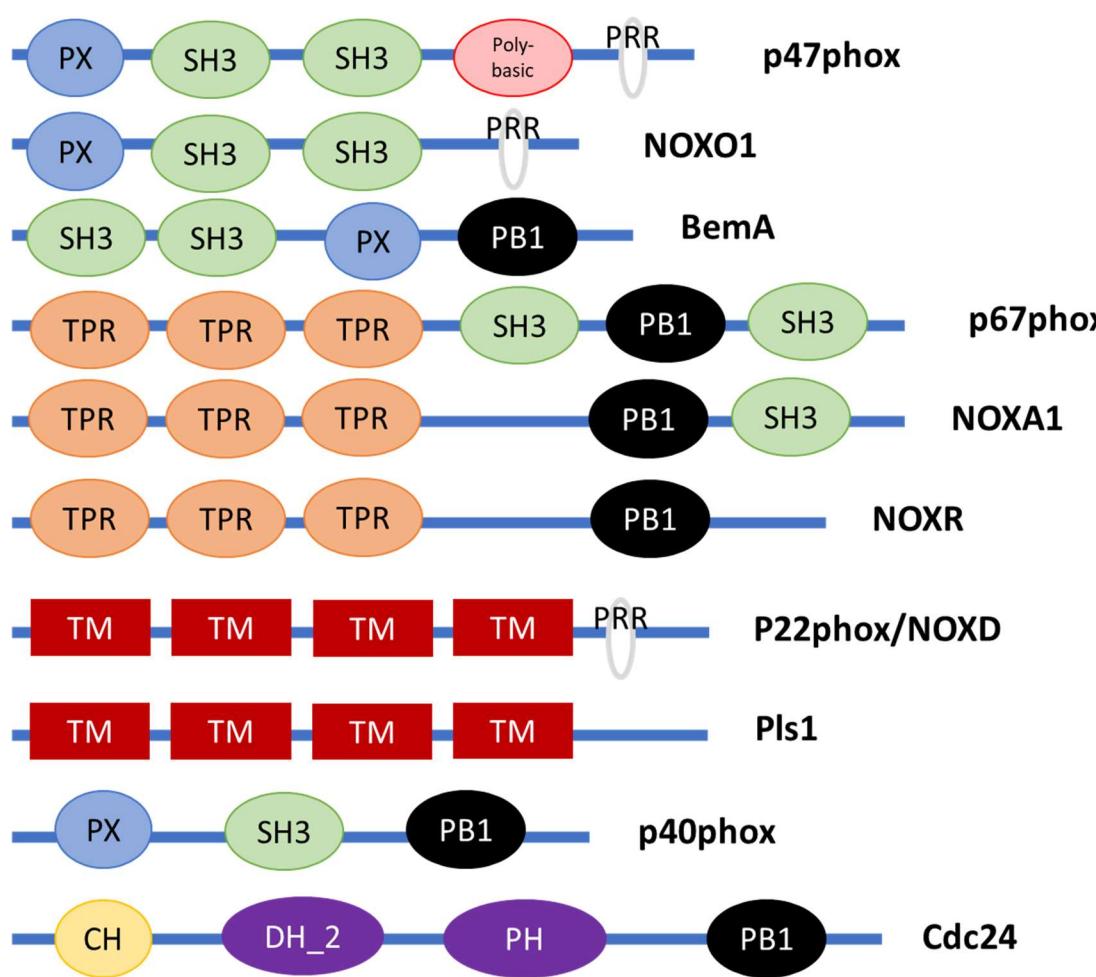
| Phylum | Species | ORGANIZERS | | | ACTIVATORS | | | TRANS-MEMEBRANE SUBUNIT | | | OTHER REGULATORS | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NOXO1-like | p47phox-like | BemA-like | NOXA1-like | p67phox-like | NOXR-like | p22phox-like | NOXD-like | Pls1-like | p40phox-like | Cdc24-like |
| **Early divergent eukaryotes** | | | | | | | | | | | | |
| *Phaeophyceae* | *Ectocarpus siliculosus* | | | | | | | | | | | |
| *Bacillariophyta* | *Thalassiosira pseudonana CCMP1335* | | | | | | | | | | | |
| | *Phaeodactylum tricornutum CCAP 1055/1* | | | | | | | | | | | |
| **Viridiplantae** | | | | | | | | | | | | |
| *Streptophyta* | *Arabidopsis thaliana* | | | | | | | | | | | |
| | *Nicotiana tabacum* | | | | | | | | | | | |
| *Chlorophyta* | *Micromonas commoda* | | | | | | | | | | | |
| | *Chlamydomonas reinhardtii* | | | | | | | | | | | |
| **Fungi** | | | | | | | | | | | | |
| *Blastocladiomycota* | *Allomyces macrogynus ATCC 38327* | | | ✓ (no PB1 domain) | | | ✓ (+ SH3 domain) | | ✓ | | | ✓ (no CH and PH domains) |
| | *Catenaria anguillulae PL171* | | | ✓ (no PB1 domain) | | | ✓ (no TPR domain) | | | | | ✓ (no CH, PH and PB1 domains) |
| *Chytridiomycota* | *Blyttiomyces helicus* | | | ✓ (no PX and PB1 domains) | ✓ (no SH3 and PB1 domains) | | ✓ (no PB1 domain) | | ✓ | | | ✓ (no CH and PB1 domains) |

| Group | Species | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Rhizoclosmatium globosum* | | | | | | ✓ (no PB1 domain) | | ✓ | | | | ✓ (no CH, PH and PB1 domains) |
| *Ascomycota* | *Fusarium fasciculatum* | | | | | | | | | | | | |
| | *Diaporthe helianthi* | | | ✓ | | | | | | | ✓ | | ✓ (no CH and PH domains) |
| *Glomeromycota* | *Rhizophagus clarus* | | | ✓ (no PB1 domain) | | | ✓ | | | | ✓ | | ✓ (no CH domain) |
| | *Gigaspora rosea* | | | ✓ | | | ✓ | | | | ✓ | | ✓ (no CH domain) |
| **Metazoa** | | | | | | | | | | | | |
| *Porifera* | *Amphimedon queenslandica* | | ✓ | | | ✓ (no SH3 domain) | | ✓ | | | ✓ (no PB1 domain) | |
| *Placozoa* | *Trichoplax adhaerens* | | ✓ (no SH3 domain) | | | | ✓ (no PB1 domain) | | | | ✓ (no SH3 and PB1 domains) | |
| | *Trichoplax sp. H2* | | ✓ | | | | ✓ (no PB1 domain) | ✓ | | | ✓ (no SH3 and PB1 domains) | |
| *Cnidaria* | *Stylophora pistillata* | | ✓ | | | | | ✓ | | | ✓ (no PB1 domain) | |
| | *Nematostella vectensis* | | ✓ | | | ✓ (no SH3 and PB1 domains) | | ✓ | | | ✓ (no PB1 domain) | |
| *Platyhelminthes* | *Macrostomum lignano* | | ✓ | | | | | ✓ | | | ✓ (no PB1 domain) | |

| Phylum | Species | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Chordata | *Ciona intestinalis* | | ✓ | | | ✓ (no SH3 and PB1 domains) | | ✓ | | | ✓ (no PB1 domain) | |
| | *Homo sapiens* | ✓ | ✓ | | ✓ | ✓ | | ✓ | | | ✓ | |
| Hemichordata | *Saccoglossus kowalevskii* | | ✓ | | | ✓ (no PB1 domain) | | ✓ | | | ✓ | |
| Echinodermata | *Acanthaster planci* | | ✓ | | | ✓ (no PB1 domain) | | ✓ | | | ✓ (no PB1 domain) | |
| | *Anneissia japonica* | | ✓ | | | ✓ (no SH3 and PB1 domains) | ✓ (no PB1 domain) | ✓ | | | ✓ (no PB1 domain) | |
| Arthtopoda | *Drosophila melanogaster* | | | | | | | | | | | |
| | *Centruroides sculpturatus* | | | | | | | | | | | |
| Tardigrada | *Hypsibius dujardini* | | | | | | | | | | | |
| | *Ramazzottius varieornatus* | | | | | | | | | | | |
| Nematoda | *Caenorhabditis elegans* | | | | | | | | | | | |
| | *Trichinella nativa* | | | | | | | | | | | |
| Annelida | *Helobdella robusta* | | | | | | | | | | | |
| | *Capitella teleta* | | ✓ | | | | | | | | ✓ (no PB1 domain) | |
| Mollusca | *Octopus vulgaris* | | | | | | | | | | | |
| | *Aplysia californica* | | ✓ | | | | | ✓ | | | ✓ (no PB1 domain) | |

**Figure S4.** Domain organization (**A**) and distribution among eukaryotic species (**B**) of the proteins regulating the NOXs that do not directly bind calcium. All the species containing NOX1-4, NOXA-B are highlighted in light blue. *Capitella teleta* is exceptional in that it does not own any NOX1-3 or NOX4 but contains a p47/40phox-like sequence. The search was performed using the following reference sequences: human NOXO1 (NP_751908.1), human p47phox (NP_000256.4), *Gigaspora rosea* BemA (RIB26013.1), human NOXA1 (NP_001242996.1), human p67phox (NP_000424.2), *Gigaspora rosea* NOXR (RIB16941.1), human p22phox (NP_000092.2), *Allomyces macrogynus* NOXD (KNE56820.1), *Gigaspora rosea* Pls1 (RIB25271.1), human p40phox (NP_000622.2), *Gigaspora rosea* Cdc24 (RIB18608.1). The listed sequences have at least 30% query cover on BLASTp with one of the reference sequences. The presence of SH3, PB1, PX, TPR, CH, DH_2, PH domains was verified with the PROSITE tool from Expasy (https://www.expasy.org).

**Table S1. Sequences and posterior probabilities (PP) of ancestral reconstructed proteins.**

>Node 151
MAKALNNDWGSVGSPLTRKVPPSYADGVYKPAGAKRPNPRSASNDSRGGRLLPLLRTRHDKNTSLLFRLGDPRGNENPAIRALGLLFYRYHNYLAELVARQVFSKDRRWTIA
TLQKVILHEPSTSQLFQLAAMEEELILGMASQLTERDDNVIVEDLRGRETPGLLERFKLLHNNDASNLGLLERFEFLKGHGKGVELKEVGQKELLLSAETDEKWLKWIEKRF
KEVAAEDAQLDREEFAEALGMKPSSLFAERLFSLVDKDRSGSISFRELLDALNMLTHGSSDDKLKLLFDMYDVDGDGRIDREELKQVLRSCMEANGLSLSPEQLDELVNAIL
ESLDADGDGEISFEDFKVLLRKYPEELANLSLSAASWLKPPEKRRKSVPARARKTLLKAYAERKKSRTLTESLKPEKEKSRVRTPWRRLRRYLQNHGPKIVFLVLFLLINLG
LFVWSFYQYKNSPEYYSLRKIMGYSVAIARGCGSVLNFNCAFILLPMCRNLLTWLRSTRLNRYIPFDKSITFHKLIGWVIVFFSLVHTVCHLFNFSHLAEDPSADLESLLRR
LPNLTYYADALPPKFGYGLFAKSIAGLTGWILVVVLVIMFVTSLPFVRRSHYFEVFWYTHLLFIPFIVLLILHGSSGALVKPPTFWYYLVVPAVLYVLERILRVKCLRLSRYR
KVTIVKVELLPSNVVELVMKRPPGFKYKPGQYVFINVPAISRFEWHPFTISSCPEQDDHLSLHIRAVGDWTKKLYELFDENEGEEKPLLGDPDDNLQEETARKLPTKPTLSR
KTRRPGKLGTLLRLVHVEVYIDGPYGAPSQDFFKYEHAVLIGAGIGVTPFASILKDLVHRHKSGRQSCPSANTDWSDDVPGSKMKLRKVDFFWVTRDQKSFEWFADLLNELE
EEQAEEGNLDDFLEIHIYLTSARGKSDLKSVMLHMALELAHHKKNGRDLITGLRTRTHYGRPDWDKIFEKIAEEHNGSKVGVFYCGPPALAKTLRKACQKYNKKEGTRFEFHK
ENF
PP = 0.60

>Node 152
MAKAVPPSYADGVPNPRSASNGLPSLRNRTTLFVYFGEILPATGPGCPPDSRGGRLLPLLRTRHDKNTGLSPIYSTSEAWADALRSFKGGKLAADDGAEFPPENTIGLPLAN
PPPPATAKLRGNENPAIRASRPLGLLFYRYHNYLAERIARQHPDKDRRWTIATLQKIILHEWLPAFLGHLQPSTSTLVPPGTFLRNGACHFRGTLPALRLCRDDNVIVEDLR
GRGLPDYPPLTNWEDINPLLHNNDASNLDLYPGGLLETESDPPGPLEFLKGHGKGVELKEVGQKELLLSAETDEKWLKWIEKRFKEVAAEEELKDVLDAQLDREEFAEALGM
KPSSLFAERLFSLVDKDRSGSISFRELLDALNMLTHGSSDDKLKLLFDMYDTDGDGRIDREELKQVLRSAMEANGLSLSPEQLDELVVKPSAHHVLHTDHDNDAGNAILESL
DADGDGEISFEDFKVLLRKYPEELANLSLSAAWLKPPEKRRKSVPARARKTLLKAYAERKKSRTLTESLKPEKEKSRVRTPWRRLRRYLQNHGPIIFLVLFLLINLGLFV
WSFYQYKNSPEYSLRKIMGYSVAIARGCAAVLNFNCAFILLPMCRNLLTWLRSTRLNRYIPFDKSITFHKLIGWVIVFFSLVHTVCHLCFNFSHLAEDPTADLESLLRRTYY
SDALPPKFGYLLFAKSIPGLTGWILVIVLVIMFVTSTPAVRRSHFEVFWYTHHLFIPFIVLLILHGSSGALIKPPTFWYYLVVPAVLYVLERLLRVKCFRSRYRKVTIVKVEL
LPSNVVELVMKRPPGFKYKPGQYVFINVPAISRFEWHPFTITSCPSEDDYLSVHIRAVGDWTRKLYELFDENEGEEKPLLGDPDDNLQEALVEPPAGGKSGRETARKLPTKP
TALLRLVNVEVYIDGPYGAPSQDFFKYEHAVLIGAGIGVTPFASILKDLVHRHKSGRQSCPSANTDWSDDTSEEPPKKDESGDGAPGSKMKLRKVDFFWVTRDQKSFEWFAD
LLRELEEEQAEEGNLDDFLEIHIYLTSARGKSDLRSVMLHMALELHAHKKNGRDLITGLRTSRTHYGRPDWDKIFEKIVEEVLKLRAEEHPNGSKVGVFYCGPPALAKTLRK
ACQKYNKKERRGEGGTRFEFHKENF
PP = 0.59

>Node 153
MARAVPPSYADGVQPAGANRPNPRSVSNELFKGPDGLPSLRNRTTLFVFFGEILPATGPGCPDSRGGRLMPLLRTRYDRNTGLSPNNPDEQLNEVTLWIIYSTSEAWADALR
SFKGGRLAADDGAEFPPENTIGLPLANPPPPATAKLRGNENPALLAPLGLLFYRWHNYLAERIARQHPDWSDKDRRWTIATLQNIILHEWLPAFLGHLHPSTSTLVPPGTYL
RNGQCHFRGTLPALRLCRDDNVIVEDLRGHVFGPLSFSRRDLTLGLPDYPPLTNWEDINPLHNNDLNNIDLYPGGLLETESNGPGTIIRDQFLRLRDTEEEIEEIRSLTLLA
YLLARRRERKQKKLDRARKGAKRSLRKLKKESTEEFAALEWLGHKEASRPVEVKLGPEATIRVLNRKGELLRTIDLKSLEAVSLLLSANKDRPLLLLRIPKEYDLVLRFDNE
EERNKFIEELEEFLKGHGKGVELRELGQKELLLSAETKEKRLKWIERYFKEVAAEAFNLDEDEDSKKLENAEELKDVLDAQLSREEFAEALGMKPSSLFAERLFSLVKDDRN
GSISFRELLDALNLLTHGSTDDKLKLLFDMYDTDGDGRISREELKQVLRGLMEANGLSLSEEQLDELVNAILKSLDADGNGEISFEDFKVLLRKYPEELANLSLSAASLLKP
PEKRRKSVPARARKTLLKAYAERKKSRTLTESLKPKKERSPVRSPWRRLRRYLQNHGPRIFFLVLFLVINIGLFVWSFYQYKNSPEYYSLRKIMGYSVAIARGAAAVLNFNC
ALILLPMCRNLLTWLRSTYLNRYIPFDKHITFHKVIAWVIVFFTVVHTVCHLCFNFSHLAESPSSDLESLLRRVYYSDTLPPKFSYLLFAKSIPGLTGVILVIVLVIMFVTA
TPAVRRSGFEVFWYTHHLFIVFYVLLILHGSGALIKKPTFWYYLVVPAVLYVLERLLRVKRSRRKVTIVKVELLPSNVVELQMKRPPGFKYKPGQYVFINCPAISRFEWHPF
TITSAPSEDYLSVHIRAVGDWTRKLRELFDEDEGEEQPAAVEPPVGGKSGRETARKFPTIYIDGPYGAPSQDFFKYEVAVLVGAGIGVTPFASILKDLVHRHKSNRQSKMKL
RKVYFFWVTRDQKSFEWFADLLRELEEQQAEENLDDFLEIHIYLTSAYDKSDLRSVMLHMAQEHAHKKNGRDLITGLRSRTHYGRPDWDKIFEKIREEHPNGSKVGVFYCGP
PALAKTLRKACQKFNKKEGEDGTRFEFHKENF
PP = 0.57

>Node 154
MSPLTKPVGDKRPNPRSASNELFEGPDGLGSSGPGCPGGKLLPLLRTRYDRNTGLSPNNPDEQLNEVTLWLRDDNVIVEDLEGKVFGPLSFSRRDLTLLNIMRGINPDLYKE
NPELGLLETEANGPGPLFTSIIRDQFLRLRDTEEELEEIRSLTGLAYLLARRRERKQKKLDRARKGAKRALRGLRLRFLLSAETKEKRLKWIEKYFKEVAAEDYLDAQLSR
EEFAEALGMKPSSLFAERLFSLLDKDRNIKTGSISFEELLDALELLTHGSTDDKLKLLFDMYDKNGDGRISREELKEVLLSAMEANGLSLSEEQLDELVNAILESLDADGSG
KISFEEFKVLLRKYPEELTNLSAYEERKKSRTLTESLKPKKERSPVRSPWRRLRRYLQNHGPRIFFLVLFTVVHTVAHLCFNFSRLAEAPSSDLESLLVYYSDTQPPKFTYLLFVKTVPGLTGVIMVIVLVIMFT
TATPAVRRSHFEVFWYTHHLFIVFYILLILHGAGAFIKKQWGGPTTWMYLVVPAVLYVLERLLRVLSRYRKVTIVKVLHPSNVIELQMKKPGFKYKPGQYVFINCPAISRF
EWHPFTITSAPEDDYLSVHIRAVGDWTEKLRELFDEDEGEEQELAVEPPVGGKSGRETARKFPEIYIDGPYGAPSQDFFKYEVAVLVGAGIGVTPFASILKDLLHRHKNDKS
PMKLRKVYFFWVTRDQNSFEWFADLLQELEEQQAEENLDDFLEIHIYLTSAYDESDARSVMLHMAQELAHTKNGRDLITGLRSRTHYGRPNWDKIFEKIAEKHPGTKVGVFY
CGPPALSKTLRKACQKFSKKGEDGTRFYFHKENF
PP = 0.61

>Node 155 – Ancestral Ca$^{2+}$-independent NOX
MAKRPSKAAKRVKSPWRRFRSWLVNEGPRLFFLIIWLVINIGLFVYTFYQYKNSPEYYTLRTLLGYSVAIARGAAAVLNFNCALILLPVCRNLLSWLRSTFLNRYIPFDKNI
TFHKVIAWTIVFFTLVHTVAHCFNFYRLAEAYSSDADSYSNTDPHLLFVKTVPGLTGVIMVIVLILMVTSATEAVRRSHFEVFWYTHHLFIVFFILLILHGAGCFIKSQTNP
VCRGPTTWMYLIAPAVLYVLERILRVFRSRRKVTIVKVVMHPSNVIEIQMKKPGFKYKPGQYVFINCPSISRFEWHPFTLTSAPEDDYLSVHIRVVGDWTEKLAKLLGADEG
ERETARKLPEIYIDGPYGAPSEDVFKYEVAILVGAGIGVTPFASILKSIWYRHNQDNSPMKLRKVYFFWVCRDTQSFEWFADLLQELEEQNLPDFLEIHIYLTGGWDENEAR
NIMLHETKNDRDAITGLRSRTHYGRPNWDKIFKKIAESHPGTNVGVFFCGPKALSKTLHKACNKYSKAEDGTRFYYHKENF
PP = 0.75

>Node 156 – Ancestral fungal Ca$^{2+}$-independent NOX
MANKFKNWMVNEGPRLFFFILWLVIHVGLFVYSFFNYKNSPELTTLRTLLGYSVAIARGAAAVLNFDCALILLPVCRNLISWLRSTFLNRFIPFDKNITFHKCIAWAIVFFT
LVHTVAHYFNFYKVAEALSGPATALQLHFVTWPGLTGHIMLIVLFLMVTSATEKVRRSHFEVFWYTHHLFIVFFLCLLFHGAGCFIKSQTNPLCRGGPTTWKYLIASGVLYL
LERILREKKGRRKTTITKVVMHPSKVIEIQMKKPSFKYKPGQYIFINCPEISRFQWHPFTLTSAPEEDYISVHIRVVGDWTKALAKLLGCDEGDEEELENPRKLPEIMIDGP
YGAPSEDVFKYEVAILVGAGIGVTPFASILKSIWYRHNNPNSPMKLRKVYFFWVCRDTESFEWFQSLLQTLEEQNLDDFLEIHIYLTGGLDEDEIKNIMLNDTGNDKDAITG
LRSRTHYGRPNWDKIFKKIRDSHPGTNVGVFFCGPKALSKTLHKACNKYSSAGTRFYYHKENF
PP = 0.77

>Node 157 – Ancestral NOXA
MAASLFRKEFAPLFFWIFWLAFHIGLFVYGFFKQRDDPELRTLNSLGFSVWISRGAGLVLAFDSALILLPVCRNIISWLRSTFLNRFIPFDENIFFHKCVAYSMLFFTLVHT
TAHYVNFFKVEEQLPAPAWQIHYTTWAGITGHIMLILMFLMYTSATHKIRQQSFETFWYTHHLFIVFFLCLFFHGVGCFVKTTENGRCKGYFSWRYTIWSGVIYLLERVLRE
KCIRQPTTITKVVMHPSKAIEIQFDKPSFKYKPGQYLFINVPEISSFQWHPFTITSCPEEPYVSVHIRLVGDWTKALAKVLGCDEGDGVDPLENPKELPKLRIDGPYGAPAE
DVFKYEVAILVGAGIGVTPFASILKSIWYRNRKAPMKLRKVYFFWVCRDTESFEWFQSLLKTLEEQQAEAAPSGFLEIHIYLTGKLDEDEIQNIVLNDVGNDYDPLTDLRS
RTHYGRPNWDRIFSKIRDAVLSGRYLPGAEAHQKTNVGVFFCGPAALAKTLKKACNKASTEGVKFHFHKEHF
PP = 0.79

>Node 163 – Ancestral NOXB
MSNSYNLQKKTSQKIRLIKKPESLWEKFENWMVNEGPKRFFFVLWILLHVGLFAYSFYNYQTSPNLTTLRTVLGYSLAIARSAAAVLNLDCALILFPVCRNLISLLRTTFLN
RFIPFDKNITFHKCIAWAIVFFTLVHTVAHYYNFYKVALALNGGVVAWLQLNFVTGPGLTGHVMLIALFLMVTSATEKVRRAHFEIFWYTHHLFIVFFAALLFHGAFCFIKA
DKNPLCRGGPTFWKYWIASGVLYLVERILREKKGRRKTYISKVVMHPSKVIEIQIKKPSCTTRAGQYIFINCPEISPFQWHPFTLTSAPEEDYISVHIRVVGDWTKAFAKAL
GCNFGDEDEEANKDRENPKSLPEIMIDGPYGSASEDVFKYEVAILVGAGIGVTPFASILKSIWYRMNHPNSKMKLRKVYFFWVCRDTDSFEWFQSLLMTIEEENLDDFLEIHT
YLTGGLKPDEIKNIMINDAGGGKDAITGLRSRTHFGRPNWDKIFKGIREAHPGTDVGVFFCGPKALSKTLHKACNKWTDAGEDGTRFYYGKENF
PP = 0.78

>Node 176 – Ancestral Metazoan Ca$^{2+}$-independent NOX
MAKRFRSWLVNEGPRYLVLIIWLVINVVLFVYTFYNYKTSPEYYLRTMLGYSLAIARGSAAVLNFNCALILLPVCRNLLSFLRGSCQVCSRNVRRLLDKNITFHKVIAWMI
CFHTAIHVVAHCFNFERFAESYNSSADELNPVRYSNTDPHLLFVKTVPGLTGVIMTLVLILMVTSSTEAVRRSHFEVFWYTHHLFIIFFILLVVHGMGGVIKRQTNLDEHNP
PVCHNNKTRWGNAKECKPVPQFAPSGPTTWMWVVAPMVLYVLERLLRFRKHYRKVTIVKVVQHPSNVIEIQMKKKGFKAKPGQYVFINCPSISRFEWHPFTLTSAPEDDYFS
VHIRIVGDWTERLAKLLGADEGEEQERETARKFPEIYIDGPFGSPSEDVFKYEVAILIGAGIGVTPFASILKSIWYRHNQDNQGMKLKKVYFFWICRDTQSFEWFADLLQEL
EQQMQEQNMPDFLEIHIYLTGGWDENQARNIMLHEDDDKDAITGLRSRTHYGRPNWDKIFKKIAESHPGTNVGVFFCGPKALSKTLHKMCNKYSSAEDGTRFYYNKENF
PP = 0.78

**>Node 177 – Ancestral NOX4**

MATAGLKRTARLFRSWLVNEGLKYLLLIIWLGINVLLFWRTFYQYKNGPEYYYLHQMLGYSLCWSRGSAAVLNFNCALILLPMCRNLLSFLRGSHKVASRRVRRLLDKNITF
HKVCAVTICLAAAVHVVAHLVNAKRFSENYNSDYPELNVARYRNEDPLKLLTKTVPGLTGILMMLVLFLMITSSTKAVRRSNYEIFWYTHHLFIVFYLLLLVHGMGGVIKHQ
TNLDAHTPGCISLNKTSSTNISLPEPEPEPEHAPNNTRWGTVKPCKEEPQFAPNPPQTWMWVVAPLVLYVVERLLRLLRKYQKVTIIKVVQHPCDVIEIRMKKKGFKAKPGQ
YVFINCPSISRFEWHPFTLTSCPSEKDDTFSVHIRIVGDWTERLADLLEEDSSEDSEHTQCRKFPELYIDGPFGSPSEDVFKYEVSICIAAGIGVTPFASILNSLRNKYIQD
DKPMKLRRLYFIWVCRDVQSFQWFADLLRELHRQLWEENRPDFLNIQLYLTGSDQKDAISSLNSRLHYGRPDWKQIFEEIAKEHPGTNVGVFYCGPRSLSKTLHKRCNRFNS
HKGTRFYYNKESFS

**PP = 0.77**

**>Node 185 – Ancestral NOX123+NOX1234**

MRSWLVNEGPRWLVLIIWLVINVVLFVYTFYNYQTSKEYYYLRVMLGYSLAFARGSAAVLNFNCALILLPVCRNLLSFLRGSCQVCSRNVRRLLDKNITFHKVIAWMICFHT
AIHVVAHLFNFERFVESYSSSNDSLLSRLSQLPNNGSDTWLNPIRYSNTDPHELFVKTVPGITGVIMTLVLILMVTSSTEAVRRSHFEVFWYTHHLFIIFFILLLVVHGVGGV
IRRQTNLDEHNPPVCHNNKTRWGNPSDCPVPQFAPSGPTTWMWVIGPMVLYVLERLLRFRKSHQKVTIVKVVQHPSNVIEIQMKKKGFKAKPGQYVFINCPSISRFEWHPFT
LTSAPEDDYFSVHIRIVGDWTEKLAKLCGADEGEDQEARKFPEIYIDGPFGTASEDVFKYEVAILIGAGIGVTPFASILKSIWYKHNQDNSPMKLKKVYFFWICPDTQAFEW
FADLLQELEQQMQEQNMPDFLEIHIYLTGGWDENQARNIMLHEDDDVDAITGLRSRTHYGRPNWDKIFKKIAESHPNTNIGVFFCGPKALSKTLHKMCNKYSSAPDGTRFYY
NKENF

**PP = 0.81**

**>Node 186 – Ancestral NOX123**

MRSWLVNEGPRWLVLIIWLVINVILFVYTFFNYQTSKEYYYLRVLLKSALAFARGSAAVLNFNCALILLPVCRNLLSFIRGSCQVCSRNIRRLLDKNITFHKAIAWMICLHT
AIHVVAHLFNFERFVESYSSSNDELLSRLSQLPNNGSDTWLNPIRYSNTDPHELFVKTVPGITGVIITLVLILMITSSTELIRRSYFEVFWYTHHLFIIFFIGLVVHGVGGI
IRRQTNLDEHNPPVCHNKHSRWGNPSDCPVPQFAPSGPTTWMWVIGPMVLYVLERLLRFRKSHQKVVITKVVQHPSNVIEIQMKKKGFKAEPGQYVFINCPSISRFEWHPFT
LTSAPSDDYFSVHIRIVGDWTEALAKACGADEGEFQEAWKMPEIYIDGPFGTASEDVFKYEVAILIGAGIGVTPFASILKSIWYKYNQDNQGLKLKKVYFFWICPDTNAFEW
FADLLQELEQQMVEQGMADFLEYHIYLTRGWDENQARNIMLHEDDEVDAITGLRSKTHYGRPNWDKIFKDIAESHPGTNIGVFFCGPKALSKTLHKMCNKYSSAPDGTRFYY
NKENF

**PP = 0.83**

**>Node 205 – Ancestral RBOH**

MQPLSNRHTSSSETFEGKDAAGESGPGTGGKLLPIFRNPASRNSGNSSGNADEEFVEVTLDLRDDSVILQEIESASAGPSAASPELTLLEKNLSKNSTLGASIIRSSSVRLR
QVSQELKRLRSFNARAGRKQRAKLDRTKSSAARALRGLRFISKTTTGDGWREVEKRFDQLAADGLLPREKFGECIGMKESKEFAAELFDALARRRRIKTENINKDELREFWE
QITDQSFDARLQIFFDMVDKNGDGRITEEEVKEIIMLSASANKLSKLKEQADEYAALIMEELDPDNLGYIELWQLETLLRQMPTHSVNLTNESRKTSQTLSQNLKPTRERNP
IKRCYCKLKYLLQDNWKRIWVLTLWIMINVGLFIWKFLQYKRKAAFQVMGYCVCVAKGAAETLKFNMALILLPVCRNTLTWLRSTRLGRIVPFDDNINFHKVIAWAIAIGTL
LHAVSHLACNFPRLTHASEEEFQTLSSYFGNTQPKTYLNLVKSVPGITGIIMVVLMAIAFTLATPWFRRNRIKLPKPFNRLTGFNAFWYSHHLFVIVYILLIIHGYFLYLTK
KWYKKTTWMYLAVPVILYACERLLRVFRSHYYSVKILKVAVYPGNVLALHMSKPPGFKYKSGQYMFVNCPAISPFEWHPFSITSAPGDDYLSVHIRTLGDWTQELKRVFSEA
CEPPTAGKSGLLRADTKESKKNFPEIFIDGPYGAPAQDYKKYDVLLLVGLGIGATPFISILKDLLNNIKEQEQGADSGNSDISNGTSESPKTKKSTLRTRRAYFYWVTREQG
SFDWFKGVMNEVAEMDHKGVIEMHNYLTSVYEEGDARSALITMVQSLHHAKNGVDIVSGTRVRTHFARPNWKKVFSRIATKHPNSRIGVFYCGAPALTKELRKLCQEFSQKT
STRFEFHKENF

**PP = 0.8**

**>Node216 – Ancestral DUOX**

MLRFLVCVLLLVLSTGTLGNEPTTEETIFSHNVEYQRYDGWYNNLAHPDWGSVGSPLTRRVPPAYADGVYQPAGSNRPNPRTISNALFRGPTGLPSTRRNRTTLFAFFGQLVA
YEILQASEPGCPAEILNIEIPECDPTFDKDCRGEKFMPFLRSRYDKNTGQSPNNPREQLNEVTSWIDGSFIYSTSEAWANALRSFKGGRLASDDGSNFPPYNTIGLPLANPP
PPATHKLRDPERLFKLGDPRSNENPALLAFGILFFRWHNYLADRIQRQHPDWSDEKIFQRARRWVIATLQNIVLYEWLPAFLGEEVPPYTGYKPHIHPSISHVFQAAAMRFG
HTMVPPGIYLRNAQCHFRKTVAGYPALRLCNTYWNSQDVLQETGIEELLMGMASQIAEREDNVIVEDLRGHVFGPLHFSRRDLVALNIMRGRDHGLPDYNTARKAFGLPPIT
NWQDINPDLYKTNPEILERLKELYNNDINKIDIYPGGLLETTSNGPGELFTTIIKDQFLRIRDGDRFWFENRQNGLFTEEEIBEEIRNITLYDVIIAATNINPNQIQKNVFFW
REGDPCPQPHQLNESDLEPCTPLHGYDYFSGSEVSYIFTFIALGLIPLVCVLVAYILARRRKRKLNKARKRTRQNVRKEKKESTDEFHALEWLGHKEHSRPVQVKLGPDATI
RVTNRKGEVLRTIDLKHLQAVTIFVSANKNRPFMLLRIPKEYDLVLRFDNEEERNKFINELEDFLKGHGKGVEIREMKQKELLTSAETKEKRQKLLERFFREAFAQAFNLDE
DEDSKQLDNSEDCKDVLDCELSKEEFAEALGMKPNSLFVEQMFSLVDKDHNGYISFREFLDVVVLFAKGSAEDKLKLLFNMYDVDGNGQISREELKEMLKSLMEMANSSLSE
EQIDELVNSMFKSAGLEDKEELTFEDFKVLLKDHKEELSNVSLDLKGVNQNEQTRRQNVPSRARKTIVKAYAERRQTRERVETVKSKYQSTPLKAKWNRFKRFLENYRQHIF
YLVLFYAICLGLFVERFYYYSFESEHAGLRRIMGYGIAITRGAAAAMSFTFSLLLLTMCRNLITWLRETFLNRYIPFDSAISFHKIVAWTALFFTVIHTIGHCINFYHVSTQ
PLSHLKCLFRDIFFRSDYLPKFHYWLFQTITGLTGVLLVLVMCIMYVFATPTVRRHAFNAFWLTHHLYILLYILTILHGSARLVKKPTFWYYFIGPAIIFVLDKLISLKKKY
KELSVIKAELLPSDVTYIEFKRPPNFEYKSGQWVRISCPALGSNEYHPFTLTSAPHEDTLSVHIRAVGPWTWKLRNIFDPNNVKDQPYPTLYLDGPYGAGNQDWYKYEVAVL
VGAGIGVTPYASILKDLVHRTSTNKHSRIKCKKVYFIWVTRSQKHFEWFVDILREVEEKDTTDLLEVHIFITQFFQKFDLRTTMLYICEKHFQKLSGRSMFTGLKAITHFGR
PDFESFFESVQEKHPQVSKVGVFSCGPPGLTKSVEKACQEVNKQEKAHFIHHFENF

**PP = 0.78**

**>Node 239 – Ancestral NOXC**

MAENAVSLELVPPSYADGVGLPSLRNRTTLFVYFGEILPATGPGCPPDGRGGRLLPLLRTRHDKNTSLSPIYSTSEAWADLLRSFKGGKLASDEGLEFPPENSIGLPTANPP
PPATAKLRGNENPAVRASRPLGLLFYRYHNALAEAIARAHPDRDRRWTIATLQKIILHEWLPAFLGHLQPSTSTLVPPGTLTRSGACHFRGTLLKSPALRLCGLPDYPPLTN
WEDINPLLHDDDLNDLDLFPGGLLETEADPPGPEELKDVLDAQLDREEFAEALGMKPSSLFAERLFSLVDKDRTGSISFKELLDALNMLTHGSSDDKLKLLFDMYDTDGDGR
IDYEELKQVLRSAMEANGLSLSPEQLDELVPKPSAHHVLHTDHDNDARNAILKSLDADGDGKISFEDFKLLLRKYPEELANLSLSAASLKAYEERKKSRTLTESLKPEEEKS
RVRTPWRRLRRYLQNHGPKIIFLVLFLLINLGLFVWSFFQYKNSPEYSLRKIMGYSVAIARGCAAVLNFNCAFILLPMCRNLLTWLRSTRLNRYIPFDKSITFHKLIGWVIV
FFSLVHTVCHLCFNFSHLAEDPAAVYYADALPPKFAYLLFVKSIPGLTGWILVVVLVIMFVTSTPAVRRSGFEVFWYTHHLFIPIIVLLILHGSGALIKPPTFWYYLVVPAV
LYVLERLLRVKCFRLKVTIVKVLLPSDTVELVMKRPPGFKYKPGQYVFINVPAISRFQWHPFTITSCPSEEDYLSVHIKAVGDWTGKLYELFDENEDEEKPLLGDADDNALV
EPPPGGKSGTARKLPTKPTALLKLVNIEVYIDGPYGAPSQDFFKYEHAVLIGAGIGVTPFASILKDLVHRHKSGRQSCPSANTDWSDDTSEEPPKRDESGDGAPGSKMKLRR
VDFFWITRDQKSFEWFADLLRELEEEQAEEGNLDDFLEIHISLTSARGKSDLKSVMLHMALELHAHKKNGRSLITGLRTSRTHYGRPDWDKIFEKIREEVLKLRAEEHPNGS
KVGVFYCGPPALAKTLRKACRKYNKKERKGEGGTRFEFHKENF

**PP = 0.57**

**>Node 243 – Ancestral NOX5**

MTTARENNEWGSVGSPLSLEVPPSYSDGPYKPAPAQQPNPRSASNLSRGAANLPEKRTRGDKNTSLLDRLGSPKGNENPAIRLFGLLFYRYNNCLAELVAREVFSKDRRLTI
ATLQKVILHEPSSSQLFQLTQAEIIGLASQLTENDDQNIEHDLEGKGFLERFEFLKGKGKGVELKEVGSLELLLSADEDEKWLKWIEKRFKEVAGEDRQIDLEEFKKALGMK
KSFFAERLFSLFDKDRSGSISLNELLDALNMLTHGSSTDKLKFLFDMYDVDGNGLIDHEELRTVLRSCMEENALSLSPEQLDELTNALFESADADGSGTISFEEFKVLLEKY
PEVLENLTISAASWLKPPEKKRKKNRKRTPYYRLWRYLRNNRKQVVFLVLFLLINIGLFVWAFYQYRDSYSVAIARGCGQCLNFNCAFILIFMLRKCLTWLRSTRLGRYLPL
DQSITFHKLIGWVIVFFSLVHTVCHLLNFSSLAENPSNNFTYWEYLFPTKTGIGWVGGIASLTGWILVIILIIMFVTSLPFVRRSHYFEVFYYTHLLYIPFLVLLILHGPNF
WYFFIVPAVIYILERILRVKCIRLARYGKTTIKEVNLLPSNVVELVMKRPPGFKYRPGQYVFINIPAISKFEWHPFTISSCPEQQDTLSLHIRAVGNWTKRLYEYFEEKEKK
EKALAEKSPTKSTLSRKTRRPGKLGTLLREVHVEVYIDGPYGAPSQDFFKYEHAVLIGAGIGVTPFASILQSLMHRHRSGRQTCPKCNHSWTDDVPGSKMLKKVDFFWITR
DQKSFEWFADLLQELEEEQAEEGNLDDFLDMHIYLTSARGKSDLKSVGLQMALDLLHKKHNRDLITGLRTRTQYGRPDWDEIFEKIVEEKGGKVGVFYCGPPALAKTLRKAC
QKYNFEFHKENF

**PP = 0.63**