SUPPLEMENTARY MATERIAL 1

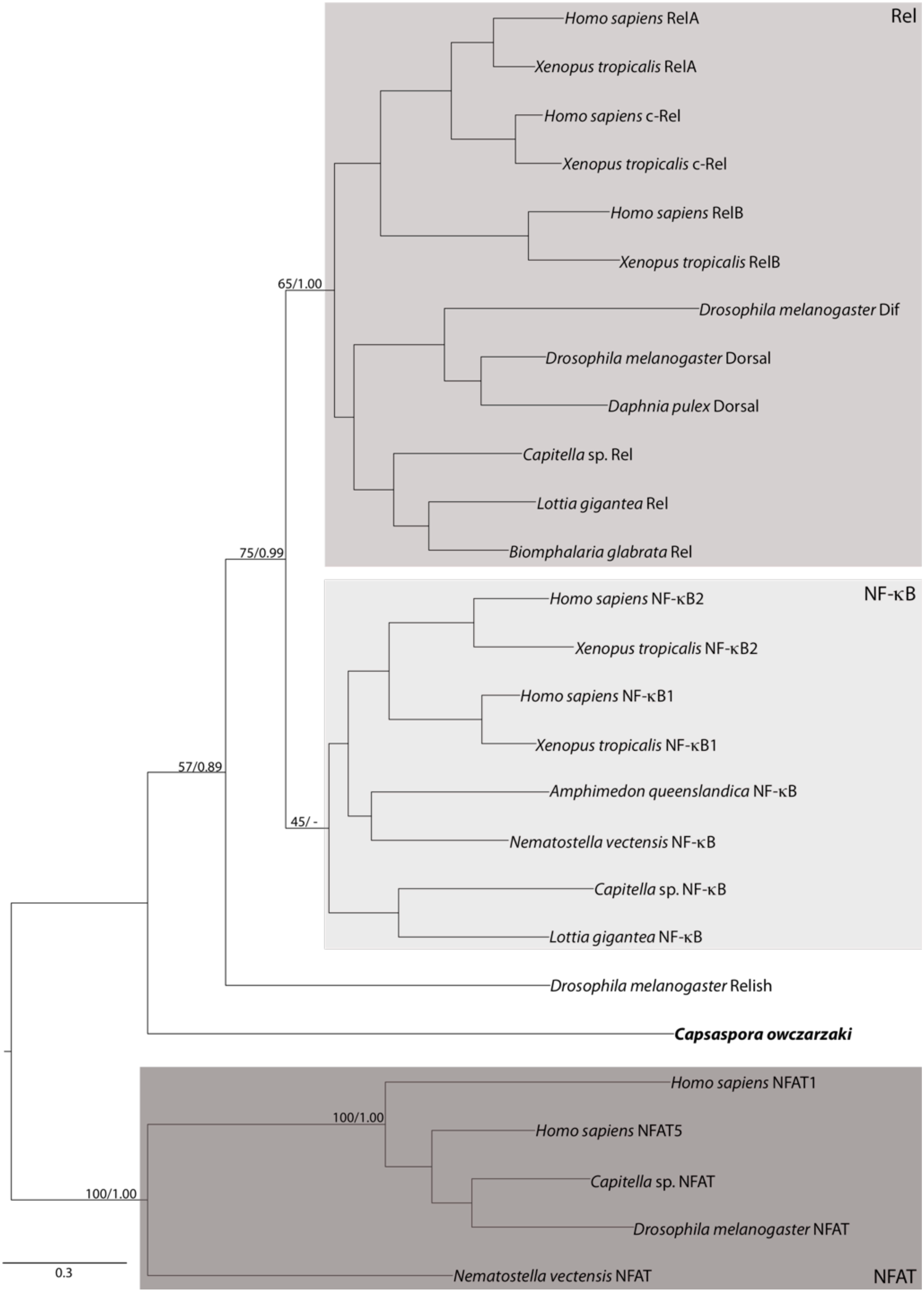
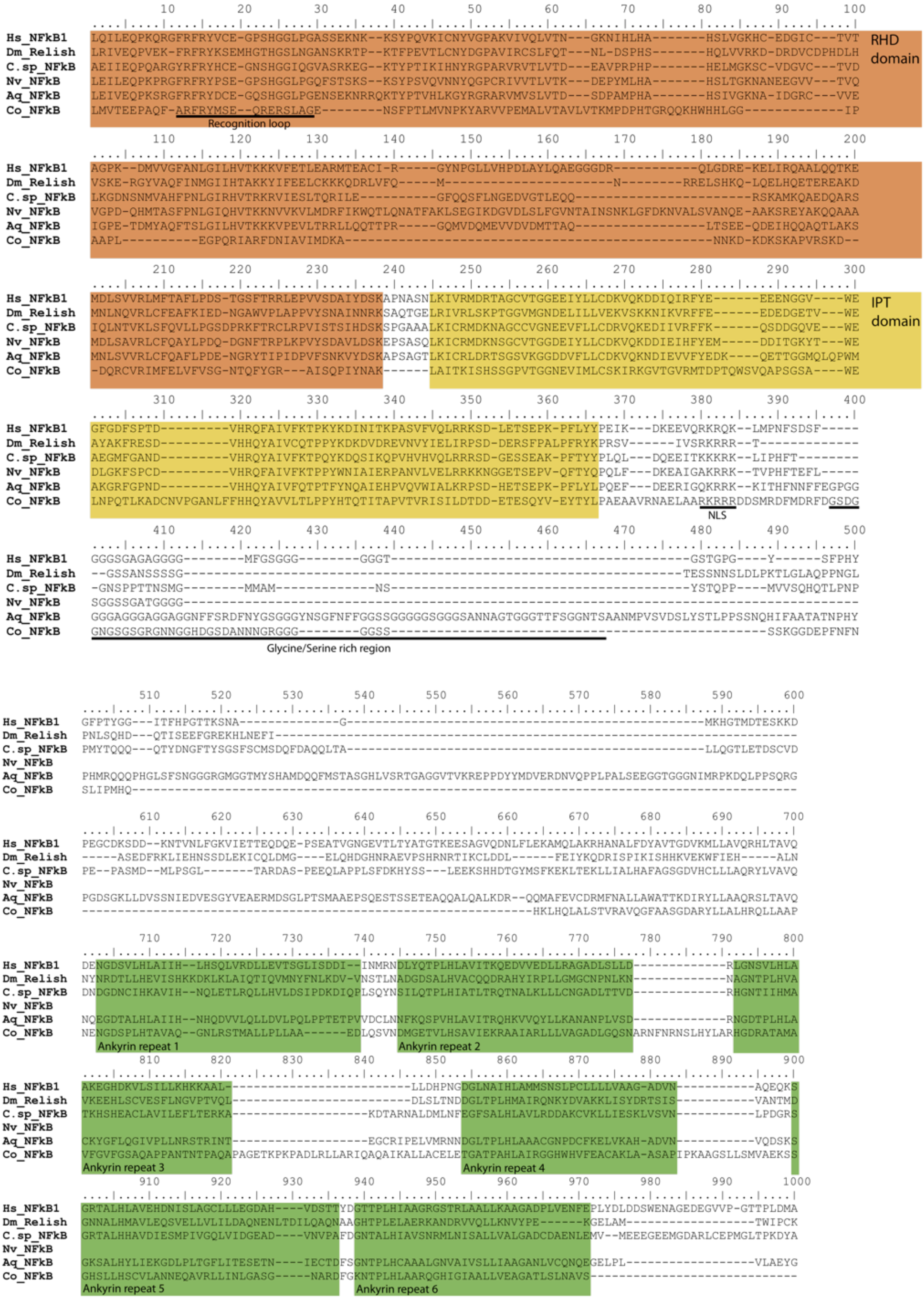
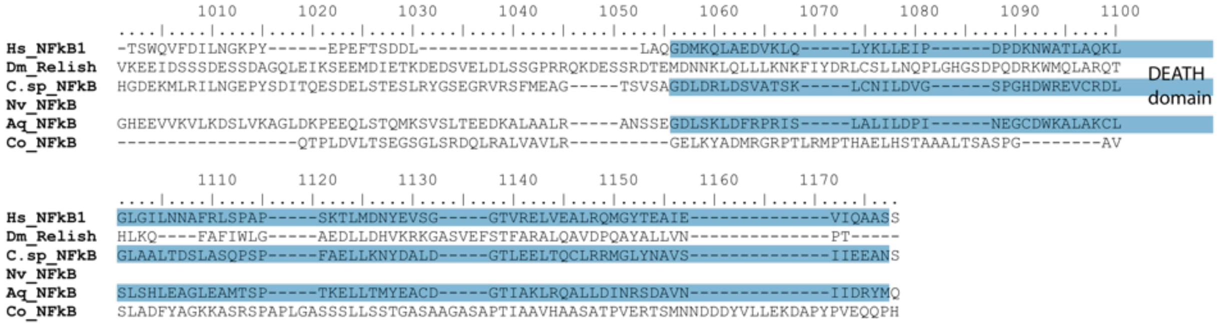


Figure S1. Maximum likelihood tree of RHD domain containing proteins NFAT, NF-kappaB and Rel using the common RHD domain. The tree is rooted using NFAT proteins. Statistical support was obtained by RaxML 100-bootstrap replicates (BV) and Bayes Posterior Probabilities (BPP). Both values are shown on key branches.

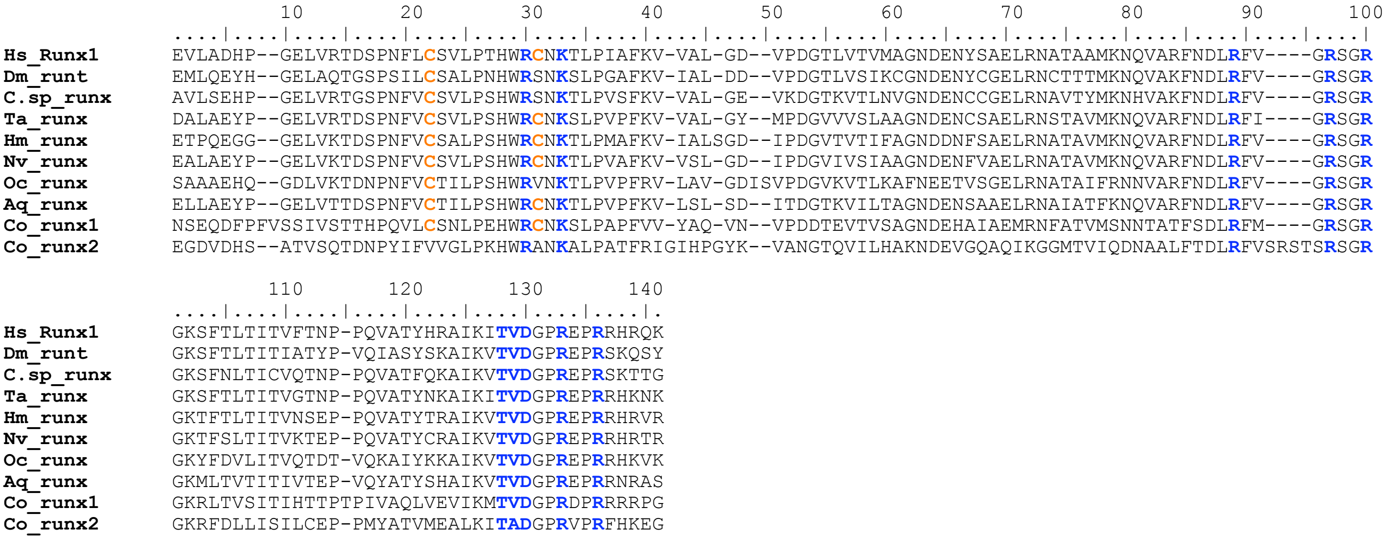


```
                   10        20        30        40        50        60        70        80        90       100
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Hs_NFkB1   LQILEQPKQRGFRFRYVCE-GPSHGGLPGASSEKNK--KSYPQVKICNYVGPAKVIVQLVTN---GKNIHLHA-------HSLVGKHC-EDGIC---TVT
Dm_Relish  LRIVEQPVEK-FRFRYKSEMHGTHGSLNGANSKRTP--KTFPEVTLCNYDGPAVIRCSLFQT---NL-DSPHS-------HQLVVRKD-DRDVCDPHDLH
C.sp_NFkB  AEIIEQPQARGYRFRYHCE-GNSHGGIQGVASRKEG--KTYPTIKIHNYRGPARVRVTLVTD---EAVPRPHP-------HELMGKSC-VDGVC---TVD
Nv_NFkB    LEILEQPKPRGFRFRYPSE-GPSHGGLPGQFSTSKS--KSYPSVQVNNYQGPCRIVVTLVTK---DEPYMLHA-------HSLTGKNANEEGVV---TVQ
Aq_NFkB    LEIVEQPKSRGFRFRYDCE-GQSHGGLPGENSEKNRRQKTYPTVHLKGYRGRARVMSLVTD---SDPAMPHA-------HSIVGKNA-IDGRC---VVE
Co_NFkB    LMVTEEPAQF-ARFRYMSE--QRERSLAGE--------NSFPTLMVNPKYARVVPEMALVTAVLVTKMPDPHTGRQQKHWHHLGG-------------IP

                  110       120       130       140       150       160       170       180       190       200
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Hs_NFkB1   AGPK--DMVVGFANLGILHVTKKKVFETLEARMTEACI-R-----GYNPGLLVHPDLAYLQAEGGGDR-----------QLGDRE-KELIRQAALQQTKE
Dm_Relish  VSKE-RGYVAQFINMGIIHTAKKYIFEELCKKKQDRLVFQ-----M----------------------N--------RRELSHKQ-LQELHQETEREAKD
C.sp_NFkB  LKGDNSNMVAHFPNLGIRHVTRKRVIESLTQRILE----------GFQQSFLNGEDVGTLEQQ-----------------------RSKAMKQAEDQARS
Nv_NFkB    VGPD-QHMTASFPNLGIQHVTKKNVVKVLMDRFIKWQTLQNATFAKLSEGIKDGVDLSLFGVNTAINSNKLGFDKNVALSVANQE-AAKSREYAKQQAAA
Aq_NFkB    IGPE-TDMYAQFTSLGILHVTKKKVPEVLTRRLLQQTTPR-----GQMVDQMEVVDVDMTTAQ-----------------LTSEE-QDEIHQQAQTLAKS
Co_NFkB    AAPL----------EGPQRIARFDNIAVIMDKA------------------------------------------------NNKD-KDKSKAPVRSKD--

                  210       220       230       240       250       260       270       280       290       300
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Hs_NFkB1   MDLSVVRLMFTAFLPDS-TGSFTRRLEPVVSDAIYDSKAPNASNLKIVRMDRTAGCVTGGEEIYLLCDKVQKDDIQIRFYE------EEENGGV----WE
Dm_Relish  MNLNQVRLCFEAFKIED-NGAWVPLAPPVYSNAINNRKSAQTGELRIVRLSKPTGGVMGNDELILLVEKVSKKNIKVRFFE------EDEDGETV---WE
C.sp_NFkB  IQLNTVKLSFQVLLPGSDPRKFTRCLRPVISTSIHDSKSPGAAALKICRMDKNAGCCVGNEEVFLLCDRVQKEDIIVRFFK------QSDDGQVE---WE
Nv_NFkB    MDLSAVRLCFQAYLPDQ-DGNFTRPLKPVYSDAVLDSKEPSASQLKICRMDKNSGCVTGGDEIYLLCDKVQKDDIEIHFYEM-----DDITGKYT---WE
Aq_NFkB    MNLSVVRLCFQAFLPDE-NGRYTIPIDPVFSNKVYDSKAPSAGTLKICRLDRTSGSVKGGDDVFLLCDKVQKNDIEVVFYEDK----QETTGGMQLQPWM
Co_NFkB    -DQRCVRIMFELVFVSG-NTQFYGR---AISQPIYNAK------LAITKISHSSGPVTGGNEVIMLCSKIRKGVTGVRMTDPTQWSVQAPSGSA----WE

                  310       320       330       340       350       360       370       380       390       400
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Hs_NFkB1   GFGDFSPTD---------VHRQFAIVFKTPKYKDINITKPASVFVQLRRKSD-LETSEPK-PFLYYPEIK---DKEEVQRKRQK--LMPNFSDSF-----
Dm_Relish  AYAKFRESD---------VHHQYAIVCQTPPYKDKDVDREVNVYIELIRPSD-DERSFPALPFRYKPRSV------IVSRKRRR--T-------------
C.sp_NFkB  AEGMFGAND---------VHRQYAIVFKTPQYKDQSIKQPVHVHVQLRRRSD-GESSEAK-PFTYYPLQL---DQEEITKKKRK--LIPHFT--------
Nv_NFkB    DLGKFSPCD---------VHRQFAIVFKTPPYWNIAIERPANVLVELRRKKNGGETSEPV-QFTYQPQLF---DKEAIGAKRRK--TVPHFTEFL-----
Aq_NFkB    AKGRFGPND---------VHHQYAIVFQTPTFYNQAIEHPVQVWIALKRPSD-HETSEPK-PFLYLPQEF---DEERIGQKRRK--KITHFNNFFEGPGG
Co_NFkB    LNPQTLKADCNVPGANLFFHHQYAVVLTLPPYHTQTITAPVTVRISILDTDD-ETESQYV-EYTYLPAEAAVRNAELAARKRRRDDSMRDFMDRFDGSDG

                  410       420       430       440       450       460       470       480       490       500
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Hs_NFkB1   GGGSGAGAGGGG--------MFGSGGG--------GGGT---------------------------------------GSTGPG----Y------SFPHY
Dm_Relish  --GSSANSSSSG-----------------------------------------------------------------TESSNNSLDLPKTLGLAQPPNGL
C.sp_NFkB  -GNSPPTTNSMG--------MMAM-------------NS---------------------------------------YSTQPP----MVVSQHQTLPNP
Nv_NFkB    SGGSSGATGGGG----------------------------------------------------------------------------------------
Aq_NFkB    GGGAGGGAGGAGGNFFSRDFNYGSGGGYNSGFNFFGGSSGGGGGSGGGSANNAGTGGGTTFSGGNTSAANMPVSVDSLYSTLPPSSNQHIFAATATNPHY
Co_NFkB    GNGSGSGRGNNGGHDGSDANNNGRGGG--------GGSS-------------------------------------------------SSKGGDEPFNFN

                  510       520       530       540       550       560       570       580       590       600
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Hs_NFkB1   GFPTYGG---ITFHPGTTKSNA--------------G--------------------------------------------------MKHGTMDTESKKD
Dm_Relish  PNLSQHD---QTISEEFGREKHLNEFI-------------------------------------------------------------------------
C.sp_NFkB  PMYTQQQ---QTYDNGFTYSGSFSCMSDQFDAQQLTA--------------------------------------------------LLQGTLETDSCVD
Nv_NFkB    
Aq_NFkB    PHMRQQQPHGLSFSNGGGRGMGGTMYSHAMDQQFMSTASGHLVSRTGAGGVTVKREPPDYYMDVERDNVQPPLPALSEEGGTGGGNIMRPKDQLPPSQRG
Co_NFkB    SLIPMHQ---------------------------------------------------------------------------------------------

                  610       620       630       640       650       660       670       680       690       700
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Hs_NFkB1   PEGCDKSDD--KNTVNLFGKVIETTEQDQE-PSEATVGNGEVTLTYATGTKEESAGVQDNLFLEKAMQLAKRHANALFDYAVTGDVKMLLAVQRHLTAVQ
Dm_Relish  -----ASEDFRKLIEHNSSDLEKICQLDMG----ELQHDGHNRAEVPSHRNRTIKCLDDL------FEIYKQDRISPIKISHHKVEKWFIEH-----ALN
C.sp_NFkB  PE--PASMD--MLPSGL-------TARDAS-PEEQLAPPLSFDKHYSS---LEEKSHHDTGYMSFKEKLTEKLLIALHAFAGSGDVHCLLLAQRYLVAVQ
Nv_NFkB    
Aq_NFkB    PGDSGKLLDVSSNIEDVESGYVEAERMDSGLPTSMAAEPSQESTSSETEAQQALQALKDR--QQMAFEVCDRMFNALLAWATTKDIRYLLAAQRSLTAVQ
Co_NFkB    ---------------------------------------------------------------HKLHQLALSTVRAVQGFAASGDARYLLALHRQLLAAP

                  710       720       730       740       750       760       770       780       790       800
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Hs_NFkB1   DENGDSVLHLAIIH--LHSQLVRDLLEVTSGLISDDI--INMRNDLYQTPLHLAVITKQEDVVEDLLRAGADLSLLD-------------RLGNSVLHLA
Dm_Relish  NYNRDTLLHEVISHKKDKLKLAIQTIQVMNYFNLKDV-VNSTLNADGDSALHVACQQDRAHYIRPLLGMGCNPNLKN-------------NAGNTPLHVA
C.sp_NFkB  DNDGDNCIHKAVIH--NQLETLRQLLHVLDSIPDKDIQPLSQYNSILQTPLHIATLTRQTNALKLLLCNGADLTTVD-------------RHGNTIIHMA
Nv_NFkB    
Aq_NFkB    NQEGDTALHLAIIH--NHQDVVLQLLDVLPQLPPTETPVVDCLNNFKQSPVHLAVITRQHKVVQYLLKANANPLVSD-------------RNGDTPLHLA
Co_NFkB    NENGDSPLHTAVAQ--GNLRSTMALLPLLAA------EDLQSVNDMGETVLHSAVIEKRAAIARLLLVAGADLGQSNARNFNRNSLHYLARHGDRATAMA

                  810       820       830       840       850       860       870       880       890       900
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Hs_NFkB1   AKEGHDKVLSILLKHKKAAL-------------------------LLDHPNGDGLNAIHLAMMSNSLPCLLLLVAAG-ADVN-----------AQEQKS
Dm_Relish  VKEEHLSCVESFLNGVPTVQL------------------------DLSLTNDDGLTPLHMAIRQNKYDVAKKLISYDRTSIS-----------VANTMD
C.sp_NFkB  TKHSHEACLAVILEFLTERKA-------------------KDTARNALDMLNFEGFSALHLAVLRDDAKCVKLLIESKLVSVN-----------LPDGRS
Nv_NFkB    
Aq_NFkB    CKYGFLQGIVPLLNRSTRINT-------------------EGCRIPELVMRNNDGLTPLHLAAACGNPDCFKELVKAH-ADVN-----------VQDSKS
Co_NFkB    VFGVFGSAQAPPANTNTPAQAPAGETKPKPADLRLLARIQAQAIKALLACELETGATPAHLAIRGGHWHVFEACAKLA-ASAPIPKAAGSLLSMVAEKSS

                  910       920       930       940       950       960       970       980       990      1000
           ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Hs_NFkB1   GRTALHLAVEHDNISLAGCLLLEGDAH----VDSTTYDGTTPLHIAAGRGSTRLAALLKAAGADPLVENFEPLYDLDDSWENAGEDEGVVP-GTTPLDMA
Dm_Relish  GNNALHMAVLEQSVELLVLILDAQNENLTDILQAQNAAGHTPLELAERKANDRVVQLLKNVYPE------KGELAM------------------TWIPCK
C.sp_NFkB  GRTALHHAVDIESMPIVGQLVIDGEAD----VNVPAFDGNTALHIAVSNRMLNISALLVALGADCDAENLEMV--MEEEGEEMGDARLCEPMGLTPKDYA
Nv_NFkB    
Aq_NFkB    GKSALHYLIEKGDLPLTGFLITESETN----IECTDFSGNTPLHCAAALGNVAIVSLLIAAGANLVCQNQEGELPL------------------VLAEYG
Co_NFkB    GHSLLHSCVLANNEQAVRLLINLGASG-----NARDFGKNTPLHLAARQGHIGIAALLVEAGATLSLNAVS-----------------------------
```



```
                  1010      1020      1030      1040      1050      1060      1070      1080      1090      1100
            ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Hs_NFkB1    -TSWQVFDILNGKPY------EPEFTSDDL----------------------LAQGDMKQLAEDVKLQ-----LYKLLEIP------DPDKNWATLAQKL
Dm_Relish   VKEEIDSSSDESSDAGQLEIKSEEMDIETKDEDSVELDLSSGPRRQKDESSRDTEMDNNKLQLLLKNKFIYDRLCSLLNQPLGHGSDPQDRKWMQLARQT
C.sp_NFkB   HGDEKMLRILNGEPYSDITQESDELSTESLRYGSEGRVRSFMEAG-----TSVSAGDLDRLDSVATSK-----LCNILDVG------SPGHDWREVCRDL
Nv_NFkB
Aq_NFkB     GHEEVVKVLKDSLVKAGLDKPEEQLSTQMKSVSLTEEDKALAALR-----ANSSEGDLSKLDFRPRIS-----LALILDPI------NEGCDWKALAKCL
Co_NFkB     ------------------QTPLDVLTSEGSGLSRDQLRALVAVLR----------GELKYADMRGRPTLRMPTHAELHSTAAALTSASPG--------AV

                  1110      1120      1130      1140      1150      1160      1170
            ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|...
Hs_NFkB1    GLGILNNAFRLSPAP-----SKTLMDNYEVSG-----GTVRELVEALRQMGYTEAIE--------------VIQAASS
Dm_Relish   HLKQ----FAFIWLG-----AEDLLDHVKRKGASVEFSTFARALQAVDPQAYALLVN--------------PT-----
C.sp_NFkB   GLAALTDSLASQPSP-----FAELLKNYDALD-----GTLEELTQCLRRMGLYNAVS--------------IIEEANS
Nv_NFkB
Aq_NFkB     SLSHLEAGLEAMTSP-----TKELLTMYEACD-----GTIAKLRQALLDINRSDAVN--------------IIDRYMQ
Co_NFkB     SLADFYAGKKASRSPAPLGASSSLLSSTGASAAGASAPTIAAVHAASATPVERTSMNNDDDYVLLEKDAPYPVEQQPH
```

Figure S2. Alignment of NF-kappaB. Taxa include Hs (*Homo sapiens*), Dm (*Drosophila melanogaster*), C.sp (*Capitella sp.*), Nv (*Nematostella vectensis*), Aq (*Amphimedon queenslandica*) and Co (*Capsaspora owczarzaki*). Different Pfam domains (colored) and functional motifs (underlined) are shown. NLS: nuclear localization signal. See main text for further details.



```
                        10        20        30        40        50        60        70        80        90       100
                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Hs_Runx1        EVLADHP--GELVRTDSPNFLCSVLPTHWRCNKTLPIAFKV-VAL-GD--VPDGTLVTVMAGNDENYSAELRNATAAMKNQVARFNDLRFV----GRSGR
Dm_runt         EMLQEYH--GELAQTGSPSILCSALPNHWRSNKSLPGAFKV-IAL-DD--VPDGTLVSIKCGNDENYCGELRNCTTTMKNQVAKFNDLRFV----GRSGR
C.sp_runx       AVLSEHP--GELVRTGSPNFVCSVLPSHWRSNKTLPVSFKV-VAL-GE--VKDGTKVTLNVGNDENCCGELRNAVTYMKNHVAKFNDLRFV----GRSGR
Ta_runx         DALAEYP--GELVRTDSPNFVCSVLPSHWRCNKSLPVPFKV-VAL-GY--MPDGVVVSLAAGNDENCSAELRNSTAVMKNQVARFNDLRFI----GRSGR
Hm_runx         ETPQEGG--GELVKTDSPNFVCSALPSHWRCNKTLPMAFKV-IALSGD--IPDGVTVTIFAGNDDNFSAELRNATAVMKNQVARFNDLRFV----GRSGR
Nv_runx         EALAEYP--GELVKTDSPNFVCSVLPSHWRCNKTLPVAFKV-VSL-GD--IPDGVIVSIAAGNDENFVAELRNATAVMKNQVARFNDLRFV----GRSGR
Oc_runx         SAAAEHQ--GDLVKTDNPNFVCTILPSHWRVNKTLPVPFRV-LAV-GDISVPDGVKVTLKAFNEETVSGELRNATAIFRNNVARFNDLRFV----GRSGR
Aq_runx         ELLAEYP--GELVTTDSPNFVCTILPSHWRCNKTLPVPFKV-LSL-SD--ITDGTKVILTAGNDENSAAELRNAIATFKNQVARFNDLRFV----GRSGR
Co_runx1        NSEQDFPFVSSIVSTTHPQVLCSNLPEHWRCNKSLPAPFVV-YAQ-VN--VPDDTEVTVSAGNDEHAIAEMRNFATVMSNNTATFSDLRFM----GRSGR
Co_runx2        EGDVDHS--ATVSQTDNPYIFVVGLPKHWRANKALPATFRIGIHPGYK--VANGTQVILHAKNDEVGQAQIKGGMTVIQDNAALFTDLRFVSRSTSRSGR

                       110       120       130       140
                ....|....|....|....|....|....|....|....|.
Hs_Runx1        GKSFTLTITVFTNP-PQVATYHRAIKITVDGPREPRRHRQK
Dm_runt         GKSFTLTITIATYP-VQIASYSKAIKVTVDGPREPRSKQSY
C.sp_runx       GKSFNLTICVQTNP-PQVATFQKAIKVTVDGPREPRSKTTG
Ta_runx         GKSFTLTITVGTNP-PQVATYNKAIKITVDGPREPRRHKNK
Hm_runx         GKTFTLTITVNSEP-PQVATYTRAIKVTVDGPREPRRHRVR
Nv_runx         GKTFSLTITVKTEP-PQVATYCRAIKVTVDGPREPRRHRTR
Oc_runx         GKYFDVLITVQTDT-VQKAIYKKAIKVTVDGPREPRRHKVK
Aq_runx         GKMLTVTITIVTEP-VQYATYSHAIKVTVDGPREPRRNRAS
Co_runx1        GKRLTVSITIHTTPTPIVAQLVEVIKMTVDGPRDPRRRRPG
Co_runx2        GKRFDLLISILCEP-PMYATVMEALKITADGPRVPRFHKEG
```

Figure S3. Alignment of the Runt domain. Taxa include Hs (*Homo sapiens*), Dm (*Drosophila melanogaster*), C.sp (*Capitella sp.*), Ta (*Trichoplax adhaerens*), Hm (*Hydra magnipapillata*), Nv (*Nematostella vectensis*), Oc (*Oscarella carmela*), Aq (*Amphimedon queenslandica*) and Co (*Capsaspora owczarzaki*). Key DNA binding aminoacids are highlighted in blue and the two Cys residues involved in redox binding affinity regulation are highlighted in orange.

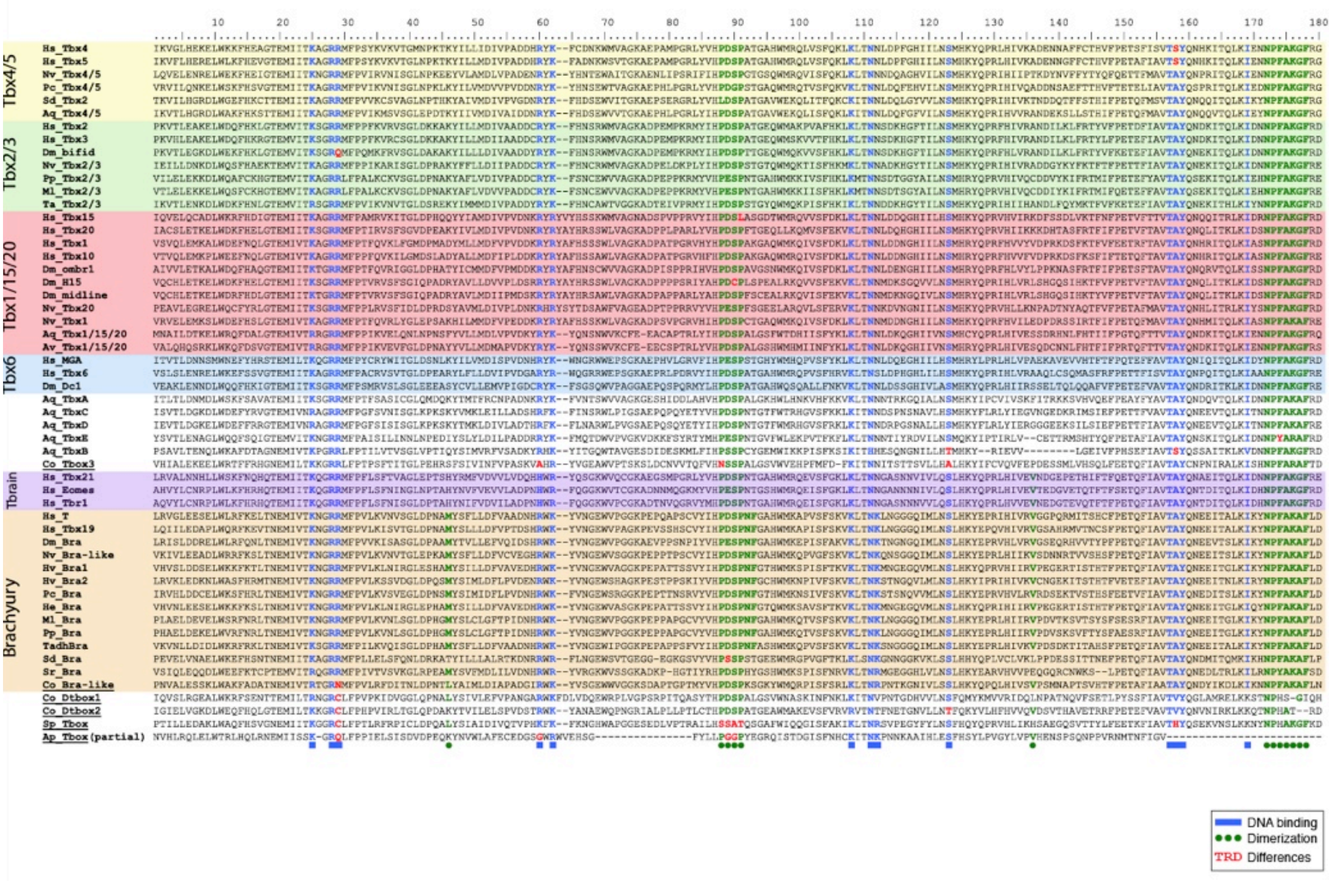


Figure S4. Alignment of the T-box domain with the different families shown in distinct colors. Taxa include Aq (*Amphimedon queenslandica*), Av (*Axinella verrucosa*), Co (*Capsaspora owczarzaki*), Dm (*Drosophila melanogaster*), He (*Hydractinia echinata*), Hs (*Homo sapiens*), Hv (*Hydra vulgaris*), Ml (*Mnemiopsis leydi*), Nv (*Nematostella vectensis*), Pc (*Podocoryne carnea*), Pp (*Pleurobrachia pileus*), Sd (*Suberites domuncula*), Sp (*Spizellomyces punctatus*), Sr (*Sycon raphanus*), and Ta (*Trichoplax adhaerens*). Key DNA binding amino acids are highlighted in blue and dimerization aminoacids in green. Non-conservative amino acidic changes are depicted in red.

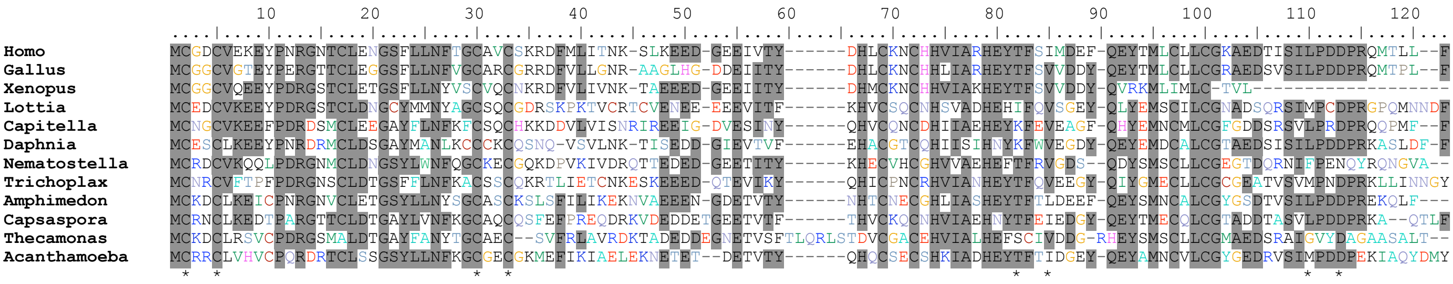


Figure S5. Alignment of Churchill domain. Asterisks indicate the CXXC motifs of the two C4-type zinc-fingers of the Churchill proteins (Sheng et al. 2003).

```
                  10        20        30        40        50        60        70        80        90       100
         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Hs_p53   GSYGFRLGFLHSGTAK--------------------------------------------SVTCTYSPALNKMFCQLAKTCPVQLWVDSTPP---PGTRV
Hs_p63   GPHSFDVSF--QQSST------------------------------------------AKSATWTYSTELKKLYCQIAKTCPIQIKV-MTPPP--QGAVI
Hs_p73   GPHHFEVTF--QQSST------------------------------------------AKSATWTYSPLLKKLYCQIAKTCPIQIKV-STPPP--PGTAI
Dm       GGYCFSMVLDEPPKSL-----------------------------------------------WMYSIPLNKLYIRMNKAFNVDVQFKSKMP--IQPLNL
C.sp.    GEFGFSISFQQQSKET-------------------------------------------KSTTWTYSEPIRKLFVRMATTCPVRFKTDLQPP---HGAVI
Nv1      GELGFCVSFGPPTESASK------------------------------------------SATWTYSEKCKKLYVNLASFCPIKFKTTVKPP---PGSYL
Nv2      GEYGFDVGFDKENGPTPK------------------------------------------SAPWTYSHQLQKLLCRMKCLVPVRLVFRSKVPP--EGFYI
Nv3      GEYSFKLTLETQPKKVA-------------------------------------------NPDWIYSTSQNKLYIKPQTPCPMKFSVTGCVP---PGTFI
Aq       GEYGFTLILNDDDNSKPPK-----------------------------------------TVPFTYSNLMKRAYIKRDSTVGMTFSFSKVPP---PNAVI
Mb       GPYDLQLDLIDENSLKPRNTSPWTVSTPGLDLTLLQNGSAPIPISSNSLHVPCRWPHFDVLLMIQYSPQLGRFFVHVNADVVLKIVLARAPP---KGTDL
Mb2      NPAGFRANLADSSVAAGPGA---------------------------------------RAIGWTYSPILNTLFTPMDYSCPIRFATNESVP---DLSRI
Co       GEAGFLLSVDVSNARHSAI-----------------------------------------SSAYSYSEALGTLFTNFDVGVPFVFRVAKPPPTVHP-LHI

                 110       120       130       140       150       160       170       180       190       200
         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Hs_p53   RAMAIYKQSQHMTEVVRRCPHHE--RCSDSDGLAPPQHLIRVEGNLRVEYLDDR----NTFRHSVVVPYEPPEVGSDC----------------------
Hs_p63   RAMPVYKKAEHVTEVVKRCPNHELSREFNEGQIAPPSHLIRVEGNSHAQYVEDP----ITGRQSVLVPYEPPQVGTEF----------------------
Hs_p73   RAMPVYKKAEHVTDVVKRCPNHELGRDFNEGQSAPASHLIRVEGNNLSQYVDDP----VTGRQSVVVPYEPPQVGTEF----------------------
Dm       RVFLCF--SNDVSAPVVRCQNHLSVEPLTANNAKMRESLLRSE-NPNSVYCGNAQGKGISERFSVVVPLNMSRSVTRSGL--------------------
C.sp.    RAMPIYMKPEHVQEVVTRCPNHATTKEHN-ENHPAPKHLVRCE-HKLAQYKDDH----YTLRQSVVIPHEPPQAGAEW----------------------
Nv1      RGVAVFKGSTNLHDIVKRCPNH---METSQDGQEKISHFMR-SNNPSARYNVCP----ESGRHSILIPYTGPQVGTE-----------------------
Nv2      RAVVVYKQPEHFREVVERCANH---ITRQDDGHTAPKHLLR-CENTKTLYRTCN----LTGRHELMFPTRKPDAGMD-----------------------
Nv3      RAIPIFKLPEHAKDVVRCCPNHTL-LEQSNRDHPAMAHFIR-SDNPRAEYERCA----QSGRLSVKIPFHVTQGSISEEI--------------------
Aq       RAMAIHKSPDLIGDILQCCPKHI--EDQKKRGHQFPKHFICGAAKTETIYCEDP----ASGRLSITMPISSLQAKSLTSG--------------------
Mb       VFRLRYALPEHRKTRVETCVTH---QQAGSHFFGAPHNHLMSINREHVTYDTD-----STGHHYARVALDQFPFTDN-----------------------
Mb2      VAHLEYTQTNQRNFVVNRCDMH---RQGDSG--PFAEHVLR-VNNPQANYHQ------RQERLAVSVPVASTRSGKV-----------------------
Co       RATLRYKQMQFMKEPVRRCPLH---LSIDSD-----LHLLRACDQDTVYSVD------YHGRASIAVPFTPTMQPLVPVLINTLKDAHVPLVTRHPSSTH

            210       220       230       240       250       260       270
         ....|....|....|....|....|....|....|....|....|....|....|....|....|....|.
Hs_p53   -------------TTIHYNYMCNSSCMGGMNRRPILTIITLEDSSG-NLLGRNSFEVRVCACPGRDRRTEE
Hs_p63   -------------TTVLYNFMCNSSCVGGMNRRPILIIVTLETRDG-QVLGRRCFEARICACPGRDRKADE
Hs_p73   -------------TTILYNFMCNSSCVGGMNRRPILIIITLEMRDG-QVLGRRSFEGRICACPGRDRKADE
Dm       -----------TRQTLAFKFVCQNSCIG---RKETSLVFCLEKACG-DIVGQHVIHVKICTCPKRDRIQDE
C.sp.    -------------VTNLFQFMCFSSCVGGLNRRPIQVIFTLEH-DG-RVLGRQAVEVRICACPGRDRRADE
Nv1      ------------FVTEMFAFMCFSSCPSGPSRRPVEIIFTLE-KDG-QTLGRQVVEIRVCACPGRDRKSDE
Nv2      ------------YFKDMFQFMCFNSCPGGLNRRPIIVIFTLELS-G-VVYGRKVLDVRVCACPGRDKADQE
Nv3      ------------IVHELFSFVCNNSC-GGLNRRAIQIVFTLETGGGCELLGRCSIETRVCACPGRDSKQDN
Aq       ------------SVQAFFVFPCFTSELHKGPGQVAQLIFTLEIGG--VLYGRAVVDIRVCASTGRDRDNDE
Mb       ------------VYSVPLRFHCFSSCPGSIARRMMQLMVYLEHSE--HILGITSVDCRCCACPGRDRLSAE
Mb2      ------------EQNELFEWHCLTSCAGGINRRKIRVVFRLIDPDQ-NVLGVQHINVRVCACPVRDRRTHE
Co       GEASRSNPYVCWSMTWFLKFMCYTSCTGGMNRRATEIVFTLEDSQG-LIYGAQALDFRTCASPSRDRKQLE
```

Figure S6. Alignment of the p53 DNA binding domain. Taxa include Hs (*Homo sapiens*), Dm (*Drosophila melanogaster*), C.sp (*Capitella sp.*), Nv (*Nematostella vectensis*), Aq (*Amphimedon queenslandica*), Mb (*Monosiga brevicollis*) and Co (*Capsaspora owczarzaki*). DNA binding aminoacidic motifs (Nedelcu and Tan 2007) are depicted in blue. Non-conservative aminoacidic changes are shown in red.

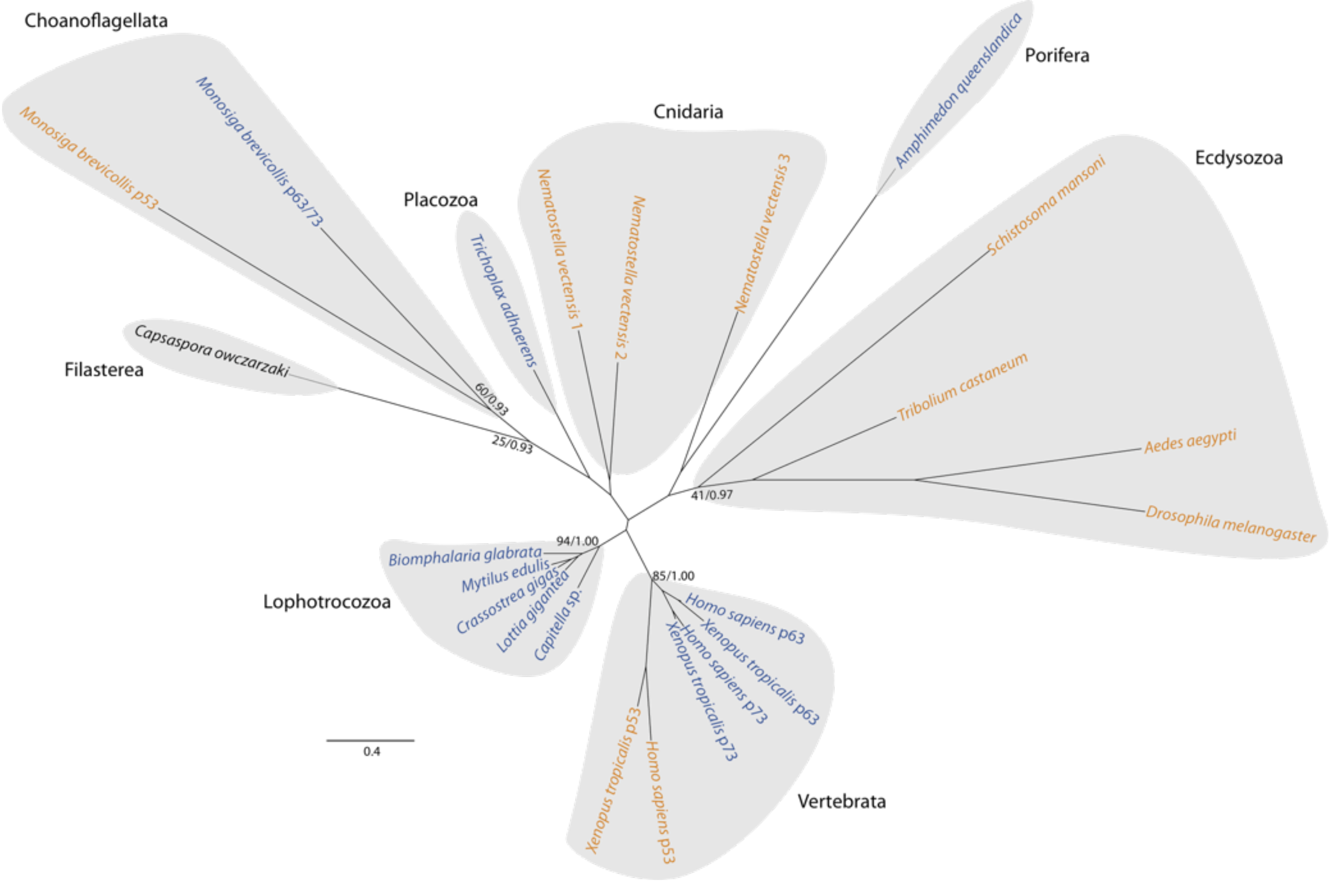


Figure S7. Maximum likelihood tree of p53 family. The tree is rooted using the midpoint-rooted tree option. Statistical support was obtained by RAxML with 100-bootstrap replicates (bootstrap value, BV) and by Bayesian Posterior Probabilities (BPP). Both values values are shown on key branches. p63/73 subfamily members are shown in blue and p53 subfamily members in orange.