Supplementary material for:

Evolution of Tre-2/Bub2/Cdc16 (TBC) Rab GTPase-activating proteins

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Methods

Trypanosome in vitro *culture:* Bloodstream form *Trypanosoma brucei* MITat 1.2 (Lister 427) were grown at 37ºC in HMI-9 with supplements as previously described. Cell numbers in cultures were monitored using a Z1 Coulter Counter as previously described (Gabernet-Castello et al., 2009).

Recombinant DNA manipulations: All primer sequences used in the study are given in Table S4. RNAi in BSF was performed using a recombinant p2T7 plasmid containing a ~250bp long fragment of the relevant TbTBC gene. The TbTBC ORF sequence was obtained from geneDB, and analyzed using RNAit (Redmond et al., 2003); a 250bp region that would ensure specificity for down-regulation was identified and was PCR amplified from BSF genomic DNA and cloned into p2T7^{TAblu} using the *Eam1105*I sites. p2T7^{TAblu} RNAi plasmids were digested with *Not*I before transfection into BSF SMB cells. All constructs were sequence verified prior to use in transfections.

Yeast two hybrid analysis: TBC and Rab proteins were expressed in *S. cerevisiae* using the Matchmaker III system (Clontech), with TBCs as the target constructs and Rabs as baits. Sequences were PCR amplified from genomic DNA isolated from *T. brucei brucei* strain 427 (note: trypanosomes lack conventional *cis*-acting introns, primer sequences are given in Table S4). For Rabs the entire ORF was used and cloned into pGBKT7. For TBCs only the TBC domain was used, as predicted by pfam and CCDB searches, together with alignments of the relevant regions (Figure S8), and cloned into pGADT7-AD. An initial analysis using wild type sequences failed to identify any significant interactions. To increase affinity of interactions between the TBC and Rab constructs, the glutamine of the 'Q-finger' was mutated to alanine for predicted catalytically-active

trypanosome TBCs as described (Pan et al., 2006), while Rabs were mutagenised to the GTP-locked form, i.e. glutamine to leucine substitution in the DTAGQE sequence. Mutagenesis was performed using PCR of the entire plasmid, and the critical insert regions of all constructs were sequenced and validated using dye-deoxy Sanger sequencing prior to use. *S. cerevisiae* were transformed and selected on triple dropout (TDO) SD plates (–Leu/–Trp/-His) and allowed to grow for three days as described by the manufacturer (Clontech).

Quantitative real-time PCR: Total RNA from *T. brucei* cells was extracted using the Qiagen RNeasy mini kit. Synthesis of cDNA was performed in a 25 µl reaction volume with 2 µg RNA and oligo dT primers using the superscript II reverse transcriptse kit (Stratagene). Further, PCR amplification was performed either under standard PCR conditions or in a reaction mixture containing cDNA and IQ-SyBr-green supermix using a mini-opticon instrument (BioRad). Typically telomerase and/or ß-tubulin were included as controls for data normalization.

Protein electrophoresis and Western blotting: SDS lysates from $1x10^6$ – $1x10^7$ cells were separated on 12% SDS-polyacrylamide gels and wet-blotted onto PVDF membrane (Immobilon, Millipore, Bedford, MA), blocked with 5% milk in TBS-T (Tris-buffered saline, 0.5% Tween 20) for two hours at room temperature and probed with antibody to TbRab5A at 1:1000, TbRab11 at 1:1000 and BiP at 1:10000 in 1% milk followed by HRP-conjugated goat anti-rabbit IgG (Sigma) at 1:10 000 dilution in 1% milk in TBS-T. Detection was by chemiluminescence and exposure to X-ray film (Kodak BioMax MR).

Immunofluorescence: Trypanosomes were harvested by centrifugation, washed with PBS and fixed with 4% PFA in ice-cold vPBS. Immunofluorescence was performed as described with modifications (Field et al., 2004). Staining was with primary antibody concentrations of anti-TbRab5A at 1:200 and anti-TbRab11 at 1:200 and secondary antibody anti-rabbit Cy3 (Sigma) at 1:1000. Specimens were analyzed on a Nikon Eclipse epifluorescence microscope equipped with a Hammamatsu CCD camera and data collected in Metamorph under non-saturating conditions (Molecular Devices). For presentation only, acquired gray scale images were false-colored, enhanced and assembled in Adobe Photoshop CS (Adobe Systems Inc); quantitative analysis was

performed on the raw data. Cells were counterstained with 4',6-diamidino-2-phenylindole (DAPI) to visualise the nucleus and kinetoplast.

Supplementary references

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Supplementary figure legends

Figures S1 to S5: Supergroup-specific phylogenetic reconstructions of TBC evolution. Phylogenetic reconstructions were generated by Bayesian inference and maximum likelihood analysis using a multiple alignment of the TBC domains found in each supergroup. Reconstructed clades are shown with gray shading and systematically named using a controlled nomenclature. Ribbons to the right show the domain architectures of the overall proteins containing the TBC domains using the same symbolic conventions as in Figures 2 and 3 and tick marks above the TBC domain represent the positions of R and Q catalytic residues. Domains are drawn approximately to scale and proteins are shown N-terminal leftmost. Figures are S1; Opisthokonta, S2; Amoebozoa, S3; Archaplastida, S4; SAR and S5; Excavata. Bootstrap and posterior probability support values are given for MrBayes/PhyML/RAxML and iconised by varying shades of gray-scale circles using the key at top left. Bar is genetic distance.

Figure S6: Sequence comparisons of the putative R and Q catalytic sites and surrounding residues in those TBC classes where it was possible to predict a putative catalytic site. The graphic was generated with Easy Sequencing in Postcript (ESPRIPT) (Gouet et al. 1999), based on a ClustalW sequence alignment of those TBC-classes containing R and Q catalytic sites. Red boxes depict residues strictly conserved; bold residues in yellow boxes depict residues conserved in more than 70% of the aligned sequences. Black triangles indicate predicted R and Q catalytic residues.

Figure S7: TBC and Rab family interactions and expression in *Trypanosoma brucei*. Panel A: Q to L GTP-locked mutants of all sixteen Rab and Rab-like proteins predicted in the *T. brucei* genome were screened against Q to A mutants of the fourteen TBC ORFs in the genome using standard yeast two hybrid approaches and triple dropout selection. Positive interactions were scored after three days growth. Systematic names (derived herein) for the trypanosome TBCs are used, while the nomenclature of Ackers (Ackers et al., 2005) is used for the GTPases. Lozenges indicate increased expression in procyclic culture form (insect stage, or PCF) (green), increased expression in bloodstream form (BSF) (red), predicted catalytically inactive TBC (black center) or predicted catalytically

active (gray center). At right are data from multiple studies for *S. cerevisiae* were retrieved from online databases and literature representing recorded interactions between each partner. The principal interaction is highlighted in the table in bold. Note that the level of promiscuity appears to be quite high, and the discrimination between a principal and secondary interaction is poorly defined or quantitated, so that true multiple interactions may be overlooked. After excluding TbRab2 and TbRabX3 as probable transcriptional activators, the screen identified 23 interactions out of 196 pairs, ~12%. TbRab5B, 6, 21, 23 and X1and TbTBC-D3, E, L, ExC and RootA do not interact, suggesting that the interactome was significantly underpopulated. Comparisons between *H. sapiens*, *S. cerevisiae* data and *T. brucei* revealed that; trypanosomes and yeast possess members of TBC clades B, D, M and Q, which interact with Rab1/Ypt1 and are conserved, as is interaction between TBC-Q1 and Rab11/Sec4. Only a TBC-B clade member interaction is common between trypanosomes and humans; while there was mutual recognition of Rab5, we note that TBC-B from *S. cerevisiae* does not. Many additional interactions between these TBC proteins and Rabs are also apparent from the three interactomes but are not consistently observed between the ortholog pairs studied here, Panel B: qRT-PCR analysis of mRNA levels for the entire cohort of Rab and TBC ORFs in *Trypanosoma brucei* 427. Data are plotted with ORFs exhibiting increased expression in the BSF leftmost, followed by ORFs upregulated in the PCF stage and finally those ORFs where there is no significant difference in detectable mRNA abundance between life stages. A cutoff of two-fold was applied. BSF are shown as black bars and PCF as gray. Ten of sixteen Rabs and six of fourteen TBCs exhibit statistically different mRNA levels between life stages. There is sparse evidence for coexpression within the detected interactions; only TbTBC-M and interactors TbRab1B, 7 and 11 exhibit coexpression. Data are the mean of three determinations with the standard error shown. * is statistically significant difference, $p<0.01$ by Student's t-test. All data are consistent with RNAseq analysis at TriTrypDB (Aslett et al., 2010).

Figure S8: Gene silencing analysis of select trypanosome TBCs. TbTBC-Q1, Q2 and B were selected on account of a differing number of predicted connections (TbTBC-Q1 with six, TbTBC-Q2 with one), and developmental expression (TbTBC-Q1 and TbTBC-

B constitutive and TbTBC-Q2 down-regulated in bloodstream stage). Further, we wished to compare TbTBC-B with TbTBC-Q1 and TbTBC-Q2 as TbTBC-Q1 interactions are, except for its interaction with TbRab1A, restricted to endocytic Rabs and TbTBC-Q2 interactions are restricted to a non-essential Golgi complex Rab-like protein TbRabX2, while TbTBC-B interacts with endocytic and exocytic Rabs as well as TbRabX2 (Natesan et al., 2009). The selection also addresses the potential functional similarities and/or redundancy between TbTBC-Q1 and TbTBC-Q2, which are paralogs. Panel A: Predicted domain architecture for trypanosome TBC-domain proteins. Panel B: Proliferative defects induced by RNAi targeted against select trypanosome TBCs. In the lower right plot a chimeric construct that targeted both Q1 and Q2 was used. Data are the mean of triplicate cultures and error bars are the standard deviations. Panel C: Knockdown of trypanosome TBCs Q1, Q2 and B. Bar graphs show quantitation of residual levels of mRNA following induction for 48 hours as detected by qRT-PCR. Data are normalized to β-tubulin, set at 100% for both induced (Inc) and uninduced (Un) cells (white and black bars respectively). The experiment was performed on two distinct inductions with essentially identical results and error bars are the standard error for triplicate assays from the same mRNA sample. Lower panels show Western blots for endosomal compartment markers Rab5 and Rab11, with the ER chaperone, BiP, used as a loading control. TbTBC-B is predicted to interact with TbRab5A and TbTBC-Q1 is predicted to interact with both TbRab5A and TbRab11. Surprisingly we found no evidence of alterations to expression level or location of either Rab protein. Nor did we observe an enlarged flagellar pocket, a hallmark of inhibition of endocytosis in trypanosomes and a direct result of knockdown of TbRab5A or TbRab11 (Hall et al., 2004; Hall et al., 2005). Panel D: Indirect immunofluorescence micrographs showing locations of endosomal Rab proteins in induced and uninduced cells (induction of 48 hours in all cases). Nuclei were counterstained with DAPI (blue) and antibody fluorescence is shown in green. Right panels of each pair show the fluorescence and the left panel is a merge with phase contrast. Scale bar = 2um.

Table S1: Accession numbers for sources of sequence data for construction of pan-eukaryote predicted proteome database.

Table S2: TBC ORF counts and paralogs from selected taxa. SG; supergroup. Two letter abbreviations of Linnean names are defined in Table S3. 1; In first round of SG phylogenies (before removal of organism redundant TBCs), statistical support for inclusion >0.8 PP/50% BS. 2; TBC-C, TBC-W/GYP6 counted as singletons. 3; TBC subfamilies recovered from the Unikonta tree are also included.

Table S3: Systematic clade assignments for TBC-domain proteins. Clade membership is given at left based on the phylogenetic reconstructions, and the nonsystematic names and/or accession numbers are given at right.

Table S4: Sequences of oligonucleotides used in this study. All sequences are written with the 5‘ end to the left.

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0.95/70/60
0.80/50
0.80

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0.8/-/27
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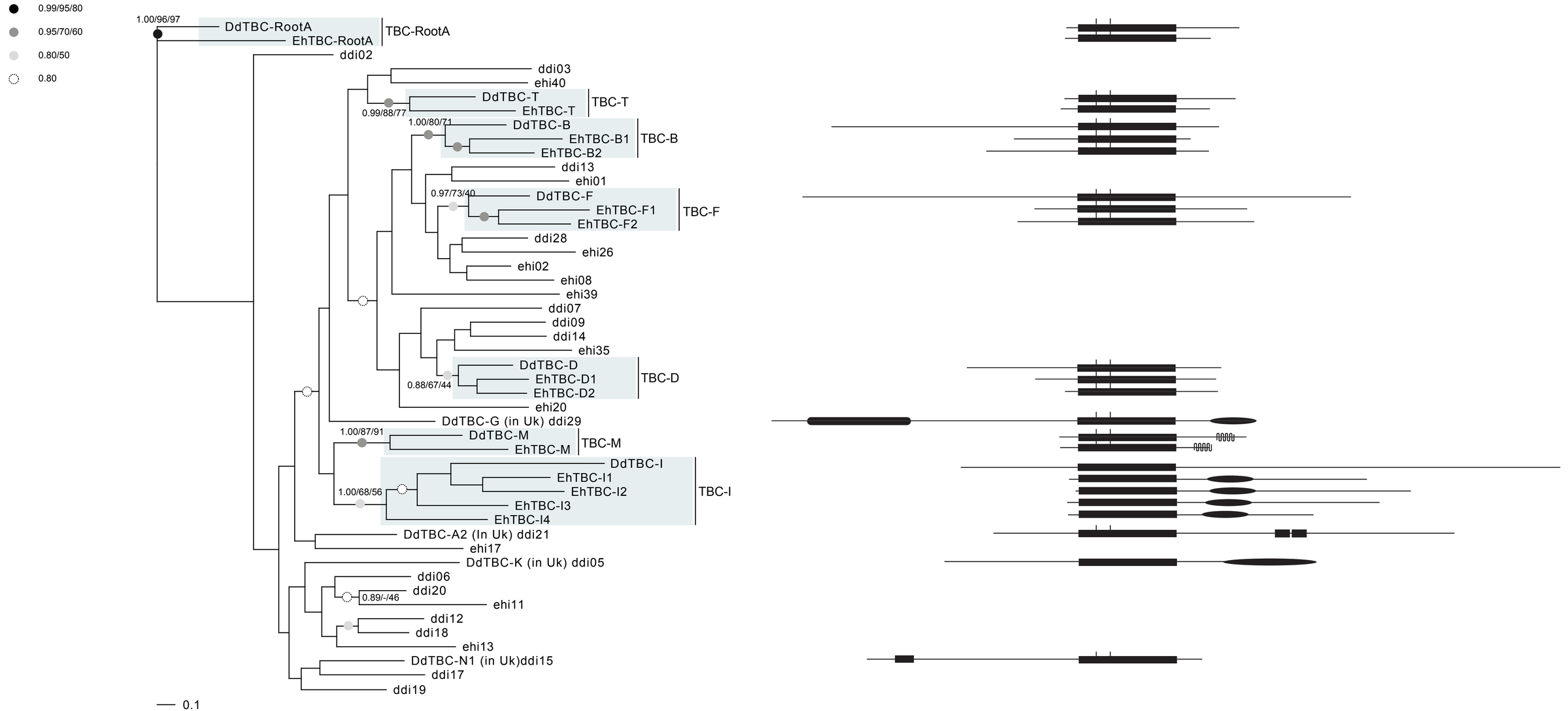
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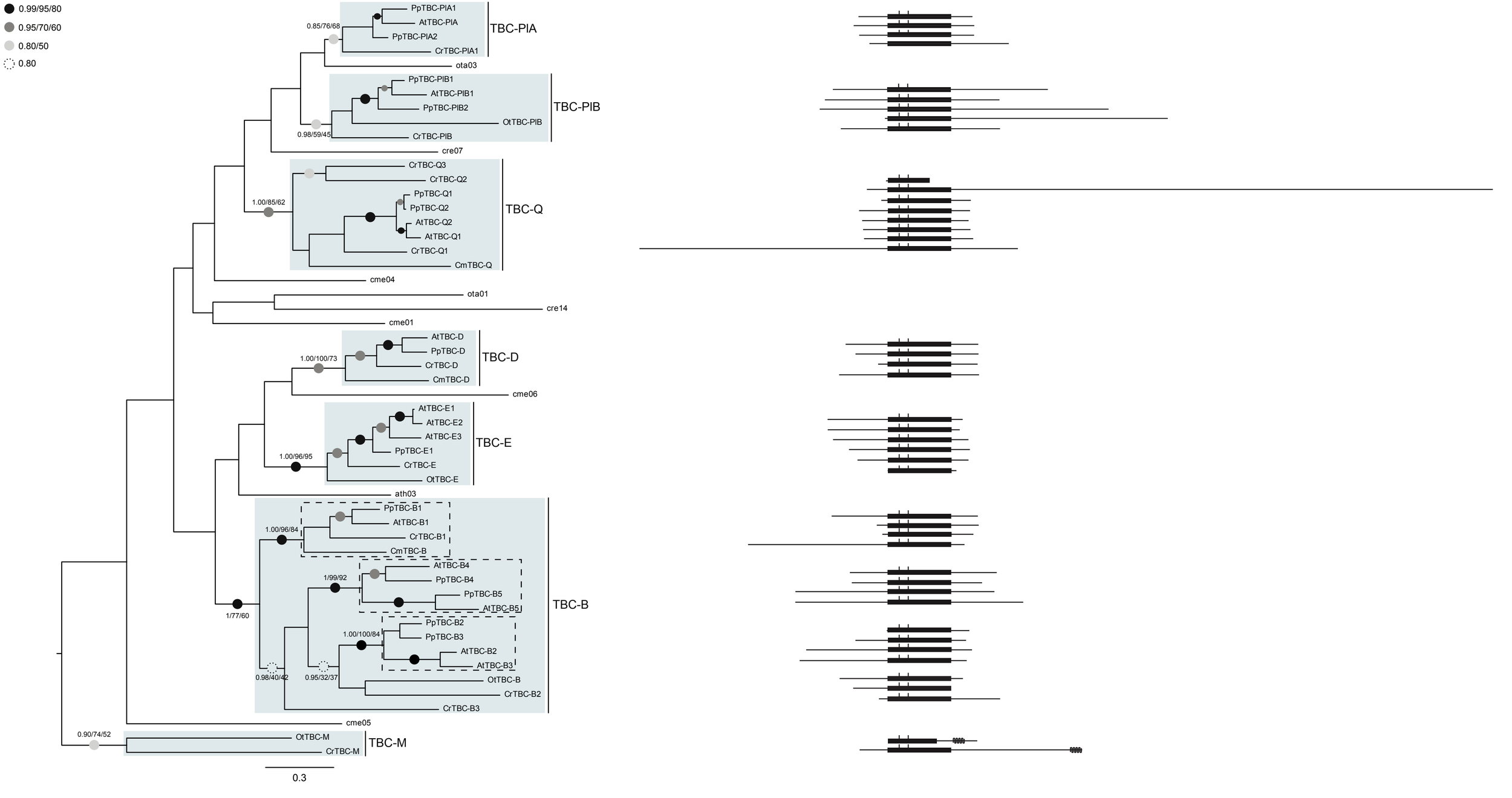
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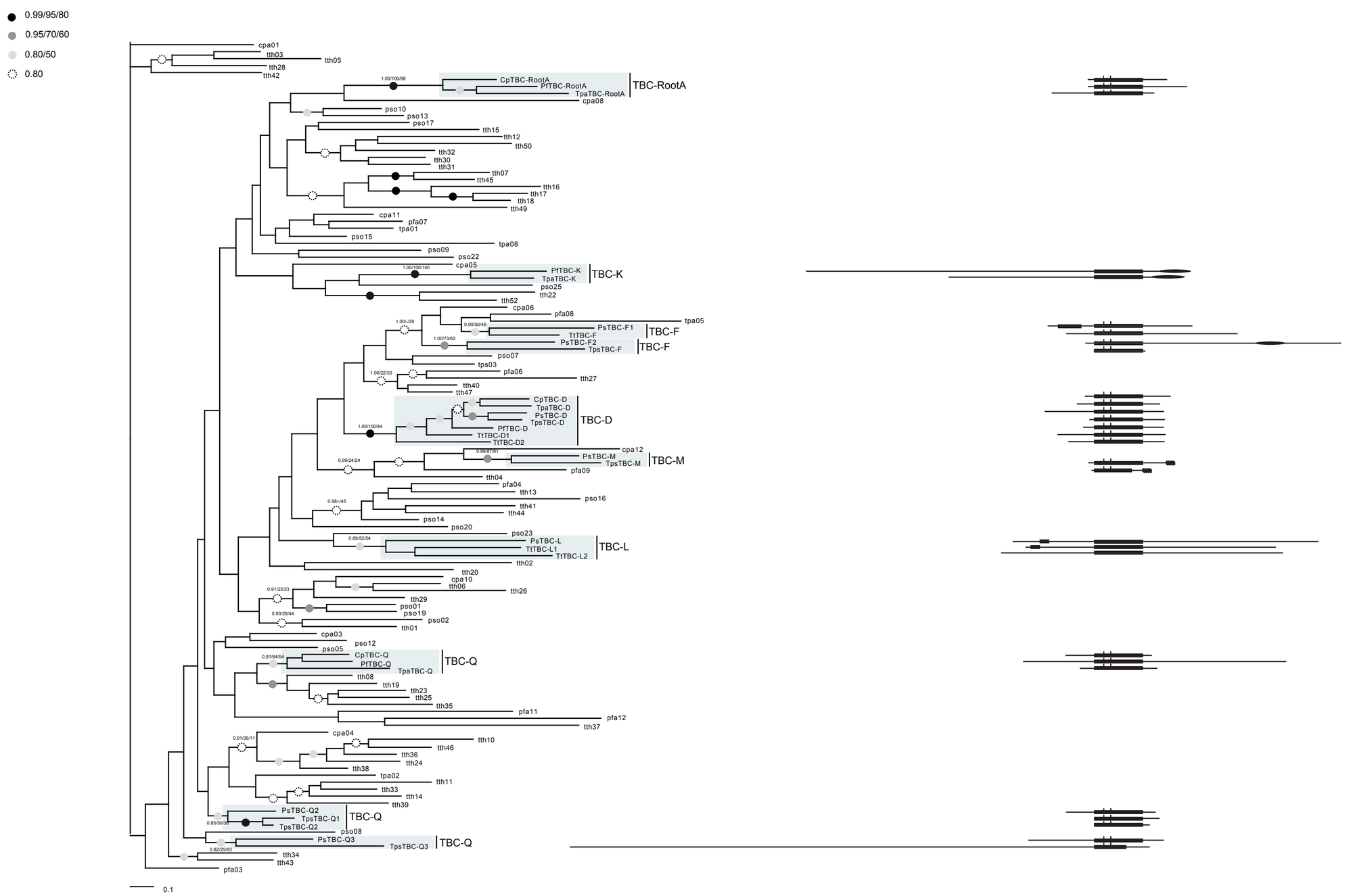
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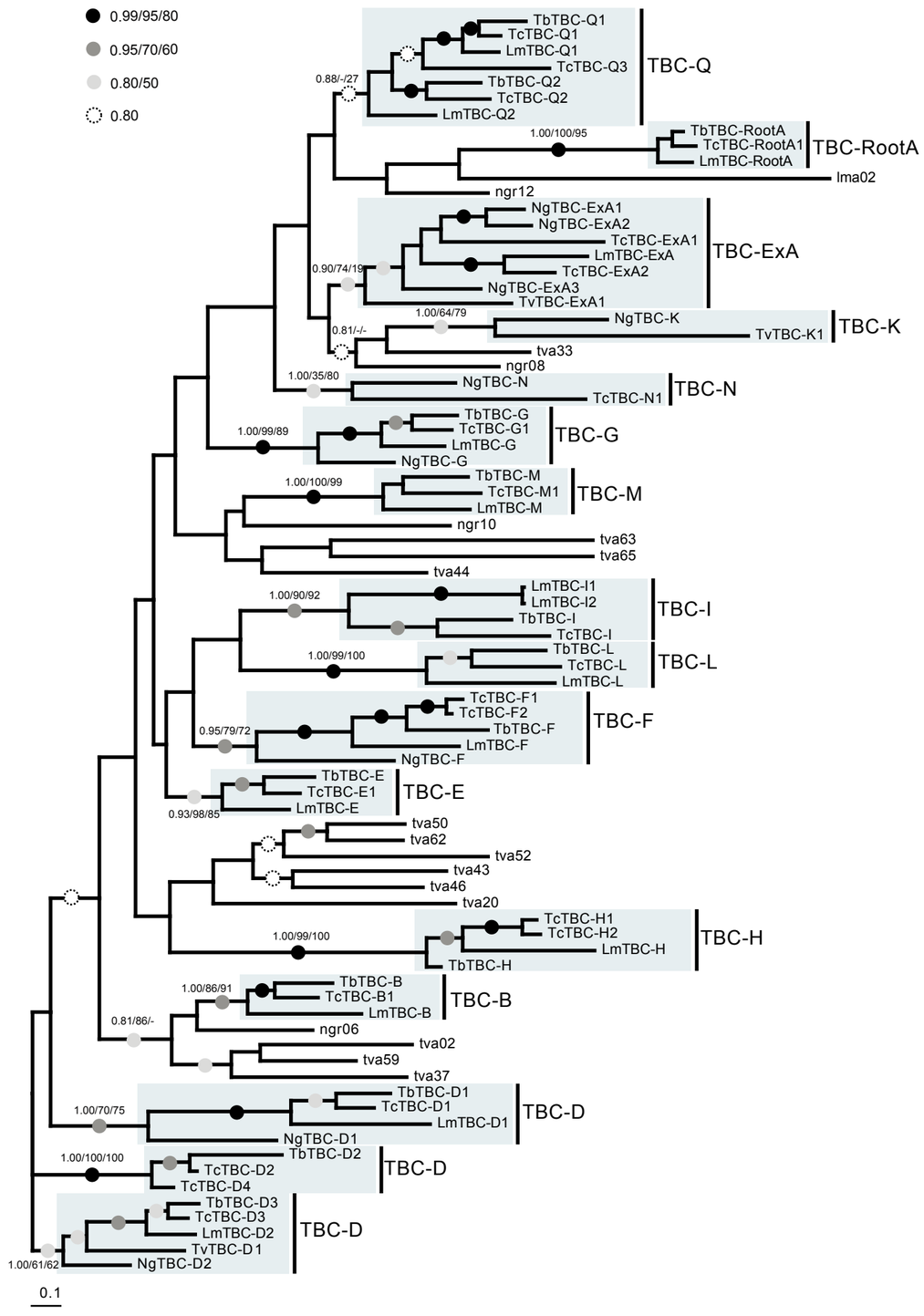
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tva44
LmTBC-I1
LmTBC-I2
TbTBC-I
TcTBC-I
TBC-I
1.00/90/92
TbTBC-L
TcTBC-L
LmTBC-L
TBC-L
1.00/99/100
TcTBC-F1
TcTBC-F2
TbTBC-F
LmTBC-F
NgTBC-F
TBC-F
0.95/79/72
TbTBC-E
TcTBC-E1
LmTBC-E
TBC-E
0.93/98/85
tva50
tva62
tva52
tva43
tva46
tva20
TcTBC-H1
TcTBC-H2
LmTBC-H
TbTBC-H
TBC-H
1.00/99/100
TbTBC-B
TcTBC-B1
LmTBC-B
TBC-B
1.00/86/91
ngr06
tva02
tva59
tva37
0.81/86/-
TbTBC-D1
TcTBC-D1
LmTBC-D1
NgTBC-D1
TBC-D
1.00/70/75
TbTBC-D2
TcTBC-D2
TcTBC-D4
TBC-D
1.00/100/100
TbTBC-D3
TcTBC-D3
LmTBC-D2
TvTBC-D1
NgTBC-D2
TBC-D
1.00/61/62
0.1

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                  1       10        20        30        40        50
HsTBC-B1/TBC1D16  TVDKDVVRTDRNNQFFR.....................GEDNPNVESMRRILLNYAVYNPAVGYSQQCMSDLVAPILAE
HsTBC-B2/TBC1D15  LIEKDVNRTDRTNKFYEGQD...................NPGLILLHDILMTYCMYDFDLGYVQQCMSDLLSPLLYV
HsTBC-B3/TBC1D17  LIERDVSRTDRTNKFYEGPE...................NPGLGLLNDILLTYCMYHFDLGYVQQCMSDLLSPILYV
HsTBC-B4/SGSM2    RIDKDVQRCDRNYWYFTP.....................PNLERLRDVMCSYVWEHLDVGYVQQCMCDLLAPLLVT
HsTBC-B5/SGSM1    RIEKDVQRCDRNYWYFTP.....................ANLEKLRNIMCSYIWQHIEIGYVQQCMCDLLAPLLVI
HsTBC-B6/TBC1D25  TVLKDVLRTDRAHPYYAG.....................PEDGPHLRALHDLTTYAVTHPQVSYCQQCMSDLASPILAV
DmTBC-B1          VVEKDVVRTDRTNPFFC.....................GDDNPNTEVMKNILLNFAVYNTGMSYSQQCMSDLLAPVLCE
DmTBC-B2          QIEKDVKRTDRSLQFFAGED..................NPNLTLLQGILMTYVMYNFDLGYVQQCMSDLLAPILEI
DmTBC-B3          RIEKDVQRCDRNYWYFAN.....................ENLDKLRNVISTYVWEHLDVGYMQQCMCDLVAPLLVI
DmTBC-Ba4         MVKKDVLRTDRLHPFYAG.....................SDDNQNIAALFNILTTYALNHPSVSYCQQCMSDIASPLLVT
DmTBC-B5          RIEKDVQRCDRNYWYFAN.....................ENLDKLRNVISTYVWEHLDVGYMQQCMCDLVAPLLVI
NvTBC-B1          TVDKDVPRTDRSHPYFA......................GEGNPNIEVMRSILLNYAIHNPEIGYSQQCMSDLLSPVLAA
NvTBC-B2          LVDKDVTRTDRTHPYYVEKET..................ENDNVRKLYDVLMTYCMYNFDLGYVQQCMSDLLSPVLFL
NvTBC-B3          RIDKDVQRCDRNYWYFTQ.....................ENLLKLRNVISSYVWTTLNVGYVQQCMCDLVAPLLVI
NvTBC-B4          MVQKDVVRTDRVHPYFDV.....................TDDHPHIRSLFNILVTYALANPDVSYVQQCMSDLASPILVV
NvTBC-B5          SIDKDVPRTDRAHPFFK......................GQGNPNLIVLRDILITYAAYHQDVGYAQQCMNDILSRFLVV
MbTBC-B2          TIIKDAQRTDRHFPMFA......................RRDSEWLNALLDILATFVN.HHNVDYVQQCMSDILAPLVAV
MbTBC-B3          IVKKDVPRTDRQHEYFE......................KDDSEHLVWLHDILVTYAVFHQEVGYVQQCMNDVLAIILFV
MbTBC-B4          SIDKDVPRTDRHLPEFKYED...................SAGLTAVRELLLAYLMLNFDLGYVQQCMNDIASALWLV
ScTBC-B/GYP7      RISKDVRRCDRNLEIFQYNTIDGLPPPPQQLPANENNSTSPESANDESDDADDGVRNPHLIHLQNILITYNVYNTNLGYVQQCMTDLLSPIYVI
RoTBC-B2          RIQKDVQRTDRTHEAFVEENN..................NPKMETMKDILLSYNFHNTNLGYVQQCMSDLLAPLLVV
RoTBC-B3          RIDKDVHRTDRTQEAFAGEDMPNPDPDMVV.........GTNPNLETMKDILVTYNFYNTELGYVQQCMSDLLAPLFVV
CnTBC-B           RIDVDCRRTDRNQPIYAVPTTPTVPRALGEEENGRKSEKGEWEDDEEEGGM..ASLNPHIAALRTILMTYHTFSPELGYVQQCMSDLLSPIYVV
DdTBC-B           LIQKDVIRTDRLHPMFIQGEDD.................IDSNENLRLMRDVLLTYSFFNFDIGYVQQCMSDLLSPIISV
EhTBC-B1          IICKDVQRTDRDNVLFKDLT...................STTLKVMFDVLVSMSIT.SECGYGQQCMRILELVKEFY
EhTBC-B2          QIDKDVRRTDNKHEKFFNE....................KNVVMLRDVLRTYALYNWRIGYGQQCMNDICSLIMEI
EhTBC-B3          QIKKDVIRTDREDTKFEKDG...................CQNLKTLTNVLMSSSMFNMKIGYGQQCMNDIVAVLMRI
EhTBC-B4          QIENDIGRTHISTACSKNWN...................LSKKEIKSIVERLLKCWTLYNFDGGYQQCMSDILIGLMEY
EhTBC-B5          QIEKDLNRTVLNNVQWTD.....................KPVEALKRILMCYGIYDMETMYVQQCMNEICALCMEY
EhTBC-B7          QIDKDVRRTDRNDSKFVDLE...................GKNCKILKNVLMTYSFFNMRVGYGQQCMNDICALLMDI
EhTBC-B8          QILKDVKRTTVNKSVLEYWN...................CPIEKIKTCIELLMKCWIIYDLDKGYQQCMSDILVGIMEC
EhTBC-B9          QIDKDVRRTDNKHEKFFNE....................KNVVMLRDVLRTYALYNWRIGYGQQCMNDICSLIMEI
AtTBC-B1          LIDKDVVRTDRAFEYYEGDD...................NLHVNSMRDILLTYSFYNFDLGYCQQCMSDYLSPILFV
AtTBC-B2          IIRLDALRADSEWANYSPYSTAITESKARRLAESV.....GLKDYDHLESCRLYHAARLVAILEAYAMYDPEIGYCQQCMSDLLSPILAV
AtTBC-B3          IIRLDAVRADSEWTSYSPSQAVISEDRACRAAEAV.....GLKDYNHLEPYKIFQAARLVAVLEAYALYDPDIGYCQQCMSDLLSPILSV
AtTBC-B4          QIGLDVNRTDRALVFYEK.....................KENLSKLWDILSVYAWIDNDVGYCQQCMSDLCSPMIIL
AtTBC-B5          RIVVDVVRTDSHLEFYED.....................PGNLGRMSDILAVYAWVDPATGYCQQCMSDLVSPFVVL
AtTBC-B6          IIRLDAVRANDEWVPYSPTQAAVSETKARGIAIQV.....GLNDYDHLEPCRIFHAARLVGILEAYAVYDPEIGYCQQCMSDLLSPLIAV
AtTBC-B7          IIRLDALRADTEWTPYSQSQALVSENRARRAAEAV.....GLKDYSYLEPSKIFHAARLVAVLEAYALHDPEIGYCQQCMSDLLSPILSV
AtTBC-B8          RIVVDVVRTDSHLEFYED.....................PGNLGRMSDILAVYAWVDPATGYCQQCMSDLVSPFVFL
AtTBC-B9          QIGLDVARTDRYLCFYEN.....................DRNQSKLWDVLAIYTWLNLDIGYVQQCMNDICSPMIIL
AtTBC-B10         SISLDVVRTDRYLCFYES.....................ESNQARLWDILSIYTWLNPDIGYVQGKSTLVLILNLQ
PpTBC-B1          RVEKDVVRTDRTIPFYEGDD...................NKNVDILRDILVTYSFYNFDLGYCQQCMSDLLSPILHV
PpTBC-B2          IIRLDAVRMNAEWIPYAETQAHVTFEEAARLSKVA.....GLQDDEHLEPPRRHHAARLVLILEAYTIYDPKTGYCQQCMSDLLSPFVAL
PpTBC-B3          IIKLDAVRMNAEWIPYAATQASVTSQEAERLSKEA.....GLMDDEHLEPPMRHHAARVVLILEAYTMYDPETGYCQQCMSDLLSPFVAL
PpTBC-B4          QIGLDVVRTDRMLQYYES.....................QEHMSKLWDILAVYCWLDPAIGYCQQCMSDFCSPLVLM
PpTBC-B5          RIVVDVVRTDRHLEFYNE.....................GKNSARMSDILAVYAWVDPDTGYCQQCMSDLLSPFIVL
CrTBC-B1          AVDKDVRRTDRRHPFFAREG...................GAGLRALRAVLLSHVTYDADLGYCQQCMSDLASPLLVV
CrTBC-B3          RIAMDAERISHEGQVLR......................DASRPAGIKRLRRILTAYALMDCEVGYCQQCMTDLLAPFLEV
OtTBC-B           VIANDAPRTPLAYGLFARDWEAG................TLPGENDEADWQAAQRKRLTRVEAYSILDPAIGYTQQCMSDLAAVFLQN
CmTBC-B           QIEKDVVRTDRNVDLFRNDD...................SVALSQLFNILLTHAFFNFDLGYCQQCMSDLAAPIVYV
TbTBC-B           AVEKDVLRTDRFLPAYADEC...................GEKLCMLRNVLLSRVMLNLDLGYCQQCMSDILSPIALL
TcTBC-B1          SIEKDVIRTDRSHEAYVDAD...................GVKQRMLYNVLMTHGMLNFDLGYCQQCMSDVLSPIAIL
LmTBC-B           SIVKDVQRTDRAHPAFREDD...................SDMLRVLQELLLAHVMLDMDLGYSQQCMSDVAAVVLLA
                        ▲                                                       ▲

                  1       10        20        30        40        50
HsTBC-D1/TBC1D22A QIHIDIPRMSPEA.LILQPKVTEIFERILFIWAIRHPASGYVQGINDLVTPFFVV
HsTBC-D2/TBC1D22B QIHIDIPRTNPLIPLFQQPLVQEIFERILFIWAIRHPASGYVQGINDLVTPFFVV
DmTBC-D           QIHIDVPRMNPQIPLFQQQLVQEMFERILFIWAIRHPASGYVQGINDLVTPFFIV
NvTBC-D           QIHIDIPRMNPLIPLFQQILVQEIFERILYIWAIRHPASGYVQGMNDLVTPFFVV
MbTBC-D           QIQIDVLRTSPAIRTFQQPVMKEMLERVLYIWAIRHPASGYVQGMNDLATPFFAV
ScTBC-D/GYP1      QIEIDIPRTNPHIPLYQFKSVQNSLQRILYLWAIRHPASGYVQGINDLVTPFFET
CnTBC-D1          QIEIDVPRTRPGVPLWSCEKTQRSLERILYVWAIRHPASGYVQGINDLVTPFFEV
RoTBC-D1          QIHIDIPRTNPGIPLYQNEATQLCLERILYQWAIRHPASGYVQGINDLVTPIFEV
DdTBC-D           QIQMDVPRTNPGVPFFQQPLIQDILERILYLWGIRHPSTGYVQGINDLATPFIWV
EhTBC-D1          QIKKDLKRSNKEIPFLFNSKIQTMMENVLLVWALRHPACGYVQGMNDLLVPLIYV
EhTBC-D2          QIKLDLVRSTTEVPFLFHDKAQEIMERVLFLWALRHPASGYVQGINDLIVPLFVV
AtTBC-D           QIAVDCPRTVPDVSFFQQEQVQKSLERILYTWAIRHPASGYVQGINDLVTPFLVI
PpTBC-D           QIGVDAPRTLPEVPFFQDPIVQATLKRILYIWAIRHPASGYVQGINDLATPFIVV
CrTBC-D           QVAVDVPRTTAPNVPFFHEPIIQESLQRLLYIWGIRHPASGYVQGMNDLVTPFLAV
CmTBC-D           QIDLDVPRTTAPEFPLFHTGAVQQAMTRILHLWSVRHPAAGYVQGLNDILVPLLYV
CpTBC-D           QIRVDLPRTTSIEYESLKNRIILDLMERVLFLWAIRNPASGYVQGINDLLPPFLVV
PfTBC-D           QIKVDIPRTKSCYNIFINKKIQQLSEHVLFIYSVRHPACGYVQGINDLITPFLIV
PsTBC-D           QILVDIPRTNADVKLFQNERIHQLQYRSL....VAIVSSGYVQGINDLMTPFLVV
TpaTBC-D          QIRVDLPRTNPSFKIFKYKRLQDCMERILFVWSCLNPDSGYVQGINDLLTLFVIV
TpsTBC-D          QVLVDVPRTTAPETPLFHNDRVRRCLSRLLYIWAMRHPASSYVQGINDLATPLIAV
TtTBC-D1          VVDTDVPRTQPLYEIYKAPSIQNMLKRILVIWGLRHPACGYVQGINEIATPFILV
TtTBC-D2          QVIKDVETTVPNSKLFRNNKIKEILRILFIWNVRHPASGYVQGMNDVLSTFIIV
NgTBC-D2          QIHIDVIRTNPDLQLYQNPRIQQALERILYIWSIRHPASGYVQGLNDLVTPFMSV
TvTBC-D1          QIDRDLPRTTKII..LLRNKKVQDLFERVLFVWSVRHPASGYVQGMNDLLQPFFFA
TvTBC-D2          QIDIDLPRTTPNK..LLKDPRVTLLFQHILFVWAVRHPASGYVQGMNDILLPFFIV
TvTBC-D3          QIQKDLPRTTSVA..ILRNLRVKNLFERVLFVYSVRHPASGYVQGMNDVLQPFFFA
TbTBC-D2          QIASDIPRHTQA..VFRHTKTVSALARCLFFWSRRYPAVGYVQGIDDIMVVFFSV
TbTBC-D3          QIRKDIPRMAAGLAFLSNRRVVMLSVERCLYIWALRHPACGYVQGMDDFTIPFISV
TcTBC-D3          QIRKDVPRMSAGVAYLHHKRVMLSIERILYIWALRHPACGYVQGINDLLIPFISV
TcTBC-D4          QIALDLPRHSYA..LFHFSRTASALARCLFLWSRRYPAVGYVQGIDDIIAVFFFV
LmTBC-D2          QIRKDIPRMSGGHCYLRHPRVQGSIERILFIWSLRHPACGYVQGMNDLVVPFMGV

                  1       10        20        30        40        50
HsTBC-E/TBC1D13   QIDKDVRRLCPDISFFQRATDYPCLLILDP.........
DmTBC-E           QIDKDVRRLCPDISFFQQPTDYPCEIVVHS.........
NvTBC-E           QIDKDCRRLCPDISFFQVATKYPCEDVIGE.........
MbTBC-E           QINKDVRRLNPDFSFFQQPTGRP................
RoTBC-E1          QIDKDVRRTLPDFAFFQLHVPANPLNPLSPPTKHYTIE
RoTBC-E2          QIDKDVRRTLPDFAFFQLPINSQQQEQQKDDYITDPLS
AtTBC-E1          QIDRDVKRTHPDIPFFSG.....................
AtTBC-E2          QIDRDVKRTHPDIPFFSG.....................
AtTBC-E3          QIERDVMRTHPDMHFFSG.....................
PpTBC-E1          QINRDVKRTHPDMQFFCG.....................
PpTBC-E2          QINRDVKRTHPDMPFFCG.....................
CrTBC-E           QVERDVMRTHPDMHFFTG.....................
OtTBC-E           QIDRDVMRVHPDMHFFNDEGEDGRR..............
TbTBC-E           EIDIDIPRTMPTMHFFNLER...................
TcTBC-E1          AVDLDIPRTMPSMHFFKSGE...................
TcTBC-E2          AVDLDIPRTMPSMHFFKSGE...................
LmTBC-E           FIDVDVPRTMPSLNFFLADESRL................
                        ▲

                  1       10        20        30                                90        100       110       120
HsTBC-E/TBC1D13   QIDKDVRRLCPDISFFQRATDYPCLLILDP.........                ...GCEAHWEVVERILFIYAKLNPGIAYVQGMNEIVGPLYYT
DmTBC-E           QIDKDVRRLCPDISFFQQPTDYPCEIVVHS.........                ...GQEAHWEVVQRILFIYAKLNPGQGYVQGMNEIVGPIYYV
NvTBC-E           QIDKDCRRLCPDISFFQVATKYPCEDVIGE.........                ...GQEAHWEVVERILFVYAKLNPGIAYVQGMNEILGPLYYV
MbTBC-E           QINKDVRRLNPDFSFFQQPTGRP................                .ASGDERHWEVIERILFIYAKLNPGIKYVQGMNEILGPIYFC
RoTBC-E1          QIDKDVRRTLPDFAFFQLHVPANPLNPLSPPTKHYTIE                 SDHSVDYHWEVIERILFIYAKLNPGVGYVQGMNELLAPIYYV
RoTBC-E2          QIDKDVRRTLPDFAFFQLPINSQQQEQQKDDYITDPLS                 DDDNDAYHWQVIQRILFIYAKLNPGVGYVQGMNELLAPIYYV
AtTBC-E1          QIDRDVKRTHPDIPFFSG.....................                .........ESMKNILLVFAKLNQGIRYVQGMNEILAPIFYV
AtTBC-E2          QIDRDVKRTHPDIPFFSG.....................                .........ESMKNILLVFAKLNQGIRYVQGMNEILAPIFYV
AtTBC-E3          QIERDVMRTHPDMHFFSG.....................                .........DALKNILTIFAKLNPGIRYVQGMNEILAPIFYI
PpTBC-E1          QINRDVKRTHPDMQFFCG.....................                .........EALKRILFIFAKLNPGIRYVQGMNEVLAPLYYV
PpTBC-E2          QINRDVKRTHPDMPFFCG.....................                .........EALKRILFIFAKLNPGIRYVQGMNEVLAPLYYV
CrTBC-E           QVERDVMRTHPDMHFFTG.....................                .........EDMKRALFMYAKLNPGLRYIQGMNELIAPLYYL
OtTBC-E           QIDRDVMRVHPDMHFFNDEGEDGRR..............                ..........LYYAKLNPGVGYVQGMHEMFGCLYYV
TbTBC-E           EIDIDIPRTMPTMHFFNLER...................                .........QCLRRILHTFAGVNKGFGYVQGMNELVGHLLFA
TcTBC-E1          AVDLDIPRTMPSMHFFKSGE...................                .........QSLRRIIHTLAGVNKGLGYVQGMNELVGHLLYA
TcTBC-E2          AVDLDIPRTMPSMHFFKSGE...................                .........QSLRRIIHTLAGVNKGLGYVQGMNELVGHLLYA
LmTBC-E           FIDVDVPRTMPSLNFFLADESRL................                .........HALRRILISTAMANKSLEYVQGMNEYVAHLLYA
                        ▲                                                                 ▲

                  1       10        20        30        40        50
HsTBC-F/TBC1D5    MIEQDVKRTTFPE..MQFFQQENVRKILTDVLFCYARENEQLLYKQQCMHELLAPIVFV
DmTBC-F           VIRQDVVRTTFPG..VDFFRKPLVQNAMVNILFYYAREHPYMCYRQQCMHEILAPIIFV
NvTBC-F           IILRDLERLYPE..NPYFHTERVRDMMLNILFCHAKKNETLGYKQQCMHELLAPLIHV
DdTBC-F           EISHDISRTYPG..LGFFERLDIQDIMIRILFIFSKQYPKIKYLQQCMNEILAPILYS
EhTBC-F1          EIKKDARRTHFDN..DILKK...RFLLHKLLLLHQLDT.QFEYVQQCMNEIISIIIEV
EhTBC-F2          DIKKDIRRTKLEKKFQTCEN...RQMLHRILFLFAIKHPELNYTQQCMNELIAVIFNI
PsTBC-F1          SIYKDVRRTRGD..MPYFRDPVVQCLLIRVLYVYSATHSEISYNQQCMGELLATLVYL
PsTBC-F2          EINTDLDRLFPAGNEGFFQNEIYLSTLRHVLFVWCRLHPDVAYRQQCMHDVVAVVLYA
TtTBC-F           DIKKDVERTYQD..KQFFVNLKIKNMLTNVLFVFCKKNSDVSYKQQCMNEVAASFIIV
NgTBC-F           VIVQDLKRLYPE..YPFFRTEEIQNYLKRMLFIWSKENDDISYRQQCMHELLSPILLV
TcTBC-F1          TAQKDLDRLHWD..FPLFEKSTTRKALLDIIVNYCLRHDY.GYKQQCMHEIAAFIFYL
TbTBC-F           IIALDVDRIHWD..IPLFELRTTRDALLKILSVYCVQHSC.EYKQQCMHEVAAFVFYL
LmTBC-F           TVAKDMSRLSWD..VPPFHDVRAKETVADILLKYSLMENE.DYMQQCFHEIVAFLYYA
                        ▲                                                 ▲

                  1       10        20        30        40        50
HsTBC-G/TBCK      QIEVDIPRCHQYDELLSSPEGHAKFRRVLKAWVVSHPD...LVYWQGLDSLCAPFLYL
DmTBC-G           QIEVDIPRCHQYDELLSSPDGHRKLRRLLKAWVTAHPQ...YVYWQGLDSLTAPFLYL
NvTBC-G           QIEVDIPRCHQYDQLLSSPTAHAKFKRVLKAWVVSHPN...LGYWQGLDSLCAPFLSQ
MbTBC-G           QLDVDIPRCHQYDQLLSSPEGHRKLKRVLKAWVNANPD...LVYWQGIDSLTAPFLKL
RoTBC-G           QIEVDVPRCHQYNQLLASSAGHEKLRRLLKAWVAANPN...LVYWQDNTPVLQEYLAV
DdTBC-G           QFELDIPRCHQYHPLLSSRQGHVQLFKILKAWSLLNLEKG..CYWQGLDNVASPFLVH
NgTBC-G           QIGVDVPRCHQYNPLLHSKIGHEKLRKVLEAWVYYHKNE.GLVYWQGLDSLCAPLLVL
TbTBC-G           QLSVDIPRCHQYHPLLASSDGHERMWRIIKAWLLLNPEL...SYWQGLDSVCAVLLAA
TcTBC-G1          QLSVDIPRCHQYHPLLVTAEGHERMRRVIKAWLLMNPDL...TYWQGIDSVCAVLLTV
LmTBC-G           QLSVDIPRCHAYHPLLNSSSGSAQLKRILRAWLYLRPEH...AYWQGLDSVCAVLLTV
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                  1       10        20        30        40        50
HsTBC-M/TBC1D20   QVLLDVRRSLRRFPP.GMPEEQ.REGLQEELIDIILLILERNP.QLHYYQGYIDIVVTFLLV
DmTBC-M           QVVLDVNRSLKRFPP.GIPYEQ.RIALQDQLTVLILRVIQKYP.NLRYYQGYIDVAVTFLLV
NvTBC-M           QVNLDVDRSHRRFPK.EMRVSR.RKVLQSQLTNVIMRVICKHQ.NLHYYQGYIDIAVTLLLV
MbTBC-M           QLEVDVARSLWKYPD.NINADQ.REVLRTRLLNIMLNILHEYP.DLHYYQGFIDVTAVIMLT
ScTBC-M/GYP8      QVILDAERSFG.....GIVDKNLKLQLRKLLVELITRVLRKYP.TLNYYQGYIDIVSVFIMC
CnTBC-M           QVKLDTDRSFVTYPK.EITSEN.KLAMQEDLYDLIVNVLRKYP.TLSYFQGYIDILSVFYLT
DdTBC-M           QVTKDVERSMWRFT..KGNSQ.LRNRKKPELTRIVNAVLSIHP.QLYYFQGYIEIASVLLLI
EhTBC-M           QITLDVKRTYGRLLN.....EEDSTRQKERLLKILDGIFVLHP.EFKYYQGFIDIVSYLLFT
CrTBC-M           VVEVDVARSLWSFAA.DDDREA.RRAQLKRLLNAVVG.AHAG..DVYYYQGLIDVAGVLLLS
PsTBC-M           QVEKDVERSLWHYDVLQGLKESERRVKRRALTHIILGVLGAND.ELFYFQGYIDIVSVFLLT
TpsTBC-M          QIDRDVARVTWHLLT..GNQRALLKKKQRRLGDFLNLVLLEEEDRLRYYQGFIDIATNTAAS
TbTBC-M           VIVADVKRSLWKLYP....NETVREKKRKMLCNILAQILSNNP.ERHYYQGLIEMVGFVMYV
TcTBC-M1          VIQVDVQRSLWLLYR....DHSKREIMRDVLKNLLQHVLLNNS.ERHYYQGLIELMGFVMYV
LmTBC-M           VVEADTERSLWTLYP....HSAERTEQRRRLKNMILRVLLHNP.ERFYYQGLIELMGYMMYM
                        ▲                                                 ▲

                  1       10        20        30        40        50
HsTBC-N1/TBC1D12  LIKLDISRTFPSLYIFQK.GGPYHDVLHSILGAYTCYRPDVGYVQGMSFIAAVLILN
HsTBC-N2/TBC1D14  LIKLDISRTFPNLCIFQQ.GGPYHDMLHSILGAYTCYRPDVGYVQGMSFIAAVLILN
NvTBC-N           LIMLDVSRTFPSLCIFQE.GGPFHDVLHSILGAYTCYRPDVGYVQGMSFIAAVLLLN
DdTBC-N1          LIHLDLPRTFPMLSIFQD.EGPLHQSLANVLEAYVCYRPDVGYVQGMSYLAAVFLLI
DdTBC-N2          MIKVDLPRTFPQLRLFNP.AGALHEQLRTTLLLFALYTPQIGYVQGMSYLAAVFCLF
NgTBC-N           LISVDLSRTFPALQFFQYPDGPYYQPFKTILECYSKFRPDIGYVQGMSYMVATLLLY
                        ▲                                                 ▲

                  1       10        20        30        40
DdTBC-RootA       IFVLDAERTFSNDINKKKLVKILTHLKKEFGDYQQGLSFVASFLMLT
EhTBC-RootA       IIYLDVFRTLKSPVYQNPLIKLLTKCWKDFGDYAQPMCLVAAFLRLT
CpTBC-RootA       IFKLDAERTFSDEENRIKMITVLSTIYQEIKDYHQGLGFVVAFFLLQ
PfTBC-RootA       IIKLDAERTFNKEENRSLLIQVLQSIYPITNDYHQGISFISSFLLLF
TpaTBC-RootA      LFRLDSERTFKTPQYRDQFYNNLCVVYERLGDYHQGEGFVIALLSIY
TbTBC-RootA       VIIKDADRTFFHPDHRKKFVSFLNAMSHEFKAYGQAMSYLAGICLLV
TcTBC-RootA1      VIIKDADRTFFHPEHRNKFVNFLTAMHNEFNAYGQAMSYLAGLCLLV
LmTBC-RootA       VIIKDADRTFFHPDHRRKMVAFLNAMYHEFHAYGQAMSYLAGLCMLV
                        ▲                 ▲

                  1       10        20        30        40        50
HsTBC-Q1/TBC1D4   AILVDLGRTFPTHPYFSVQ....................LGPGQQLSLFNLLKAYSLLDKEVGYCQGISFVAGVLLLH
HsTBC-Q2/TBC1D1   AILIDLGRTFPTHPYFSAQ....................LGAGQQLSLYNILKAYSLLDQEVGYCQGLSFVAGILLLH
HsTBC-Q3/TBC1D18  VITRDIHRTFPAHDYFKDT....................GGDGQESLYKICKAYSVYDEDIGYCQGQSFLAAVLLLH
HsTBC-Q4/TBC1D11  AITRDINRTFPAHDYFKDT....................GGDGQDSLYKICKAYSVYDEEIGYCQGQSFLAAVLLLH
HsTBC-Q5/EVI5L    LIRRDIARTYPEHEFFKGQ....................DSLGQEVLFNVMKAYSLVDREVGYCQGSAFIVGLLLMQ
HsTBC-Q6/EVI5     LIRRDIARTYPEHNFFKEK....................DSLGQEVLFNVMKAYSLVDREVGYCQGSAFIVGLLLMQ
DmTBC-Q1          VIRRDIARTYPEVEFFKEK....................DGPGQEALFNVIKAYSLHDREVGYCQGSGFIVGLLLMQ
DmTBC-Q2          AIFIDLGRTFPNHQFYKDP....................LGLGQLSLFNLLKAYSILDPELGYCQGLGFICGVLLLH
DmTBC-Q3          VIQRDIHRTFPAHKCFKEI....................GGSGQDALFKVSKAYAVHDSEVGYCQGLSFIAASLLLH
NvTBC-Q1          MIKRDIARTFPDHSFFKDK....................DGIGQGTLFNVIKAYSIYDKEVGYCQGSAFIVGLLLMQ
NvTBC-Q2          SIFIDLGRTFPRHPYFTSQ....................FGPGQLSLFNLLKAYSVLDTDVGYCQGLSFVAGILLMH
NvTBC-Q3          VIVWDIHRTFPAHDYFKDS....................GGEGQEALYKISKAYSVYDEEVGYCQGLSFFIAVLLLH
MbTBC-Q1          LIRLDIARTFPEHEMFASN....................EGLGQEVLYNVVKAYSIYDNVVGYCQGIPFLVGLLLMH
MbTBC-Q2          AIKWDLDRTFPGHERFRDK....................EGEGQKQLYRINSAYSVYDEEIGYVQGLSFITAVLLLH
ScTBC-Q1/GYP5     TIRRDLRRTKFVAEDKMES....................LYKVIKVYSVYDPDVGYTQGMGFIAAPLLIN
CnTBC-Q           AIMKDLNRTFPNHKYFKEG....................GGVGQEGLFMVVKAYSLYDQEVGYTQGLAFIVAALLLN
RoTBC-Q1          MIQRDLARTFPGHNYFKES....................DGQGQEGLYNVVRAYSVYDKDVGYCQGLAFIVGPMLLN
RoTBC-Q4          AITRDLHRTFPHHPYFQS.....................HQGQESLFNVVKAYSLYDPEVGYCQGLAFVAGPLLLN
RoTBC-Q6          IIQRDLSRTFPQLEMFKAD....................GGDGQLALGRLLKAYSIYDLHVGYCQGLAFLAGPLLMT
AtTBC-Q1          DIIRDISRTFPSHVFFQKR....................HGPGQRSLYNVLKAYSVYDRDVGYVQGMGFIAGLLLLY
AtTBC-Q2          DIIRDISRTFPSHVFFQKR....................HGPGQRSLYNVLKAYSVYDRDVGYVQGMGFIAGLLLLY
PpTBC-Q1          DIIRDISRTFPSHVFFQQR....................HGPGQRALYNVLKAYSVYDRDVGYVQGMGFLAGLLLLY
PpTBC-Q2          DIIRDISRTFPSHVFFQQR....................HGPGQRALYNVLKAYSVYDRDVGYVQGMGFLAGLLLLY
CrTBC-Q1          AIMRDLNRTYPTHIYFTQR....................QGPGQRALYSVLRAYAVYDSKVGYVQGMGFLAAVLLLY
CrTBC-Q3          .ILRDIHRTFPEHPYFG......................LEAGQRSLFRVLKAYSLHDLEVAYCQGMAFMAGVLLMY
CmTBC-Q           IIDRDVTRTFCNHRLFWRGG..................AALGLGLGALRNILRAYACLDPAVGYCQGMSSITGALYIY
CpTBC-Q           LISIDVPRTFPELNIFR......................DQASQECLFRVLNATANHIPDVGYCQGMNFIAALLLIT
PfTBC-Q           LILIDISRTFPELLIF.......................DKYAQQQLYRVLNAYSNYEPSVGYCQGMNFLVGLLLII
PsTBC-Q1          TLDRDIDRTFQQLQARMANNKRPNFLVKSQHSSSEFMAWKSSTHQLLSVFMMLKPQIGYCQGMTSVAAVLVQE
PsTBC-Q2          TIARDIGRTFPRHSLFRDRS..........SLG....QCALMNVLKAYSLHDPEVGYCQGMGFLSAMFLCY
PsTBC-Q3          LIMTDVRRTYGRVAPHKRAANHKD.....EVSEEDLTTQLSEILHALAGRFPAVGYCQGMDYIAAHVLYK
TpaTBC-Q          IINIDVPRTFPELKVF.......................NKDSQNQLHRILSAYGNYQPEIGYCQGMNFVAGLLLLV
TpsTBC-Q1         VIERDLHRTFPRHILFAGNN..................DINHLEGPAALRRLLYAYNVYDEEVGYCQGMNFIAAMFLTF
TpsTBC-Q2         TIERDIHRTFPRHYLFHNGL..................DE....DERALRRVLRAYSVYDSEVGYCQGMNFIAAMFLTF
TbTBC-Q1          TISRDLPRTFPKHVLFREE...................GGI....GQTFLRNVLHAYANIDPEVGYVQGMAFVVGALYTQ
TbTBC-Q2          IIERDLGRTFPTHRLFNKP...................GST....GQMGLRSILRAYANLNPETGYVQGMGFLVGTLLIQ
TcTBC-Q1          TIGRDLARTFPTHILFRDE...................GGV....GQTFLRNVLHAYAAVDPEVGYVQGMGFVVGVLSTQ
TcTBC-Q2          VIERDLGRTFPAHQWFARA...................DGV....GQTKLRGILRAYANIHPEVGYVQGMAFLASTLLLH
TcTBC-Q3          AIQRDLSRTFPTHCLFVGE...................GSV....GQVELRRILSVYSRLDPAVGYCQGMAFVVAMLLLH
LmTBC-Q1          IISRDLARTFPTHVLFQDP...................GGV....GQTFLRNILHAYAGCDPEVGYVQGMGFLVAALSTQ
LmTBC-Q2          VIARDLDRTFPTNRLFSVK...................SGQ....GQQILRRLLHAYANYNPGVGYCQGMGFLAATLILQ

                  1       10        20        30        40        50
HsTBC-A1/TBC1D8B  EIERDLRRSLPEHPAFQ.SDTG.ISALRRVLTAYAYRNPKIGYCQAMNILTSVLLLY
HsTBC-A2/TBC1D8   EIERDLHRSLPEHPAFQ.NETG.IAALRRVLTAYAHRNPKIGYCQSMNILTSVLLLY
HsTBC-A3/TBC1D9B  EIERDLHRSMPEHPAFQ.NELG.IAALRRVLTAYAFRNPTIGYCQAMNIVTSVLLLY
HsTBC-A4/TBC1D9   EIERDLHRSLPEHPAFQ.NEMG.IAALRRVLTAYAFRNPNIGYCQAMNIVTSVLLLY
DmTBC-A           EIDRDLPRSLPEHPAFQ.STDG.IGALRRVLQAYALRNPQVGYCQAMNIVSSVFLLF
NvTBC-A           EIERDLHRSLPEHPAFQ.SDVG.IGALRRVLTAYAWRNPSIGYCQAMNIVASVLLLY
MbTBC-A           EIERDLHRSLPEHPAFQ.SSVG.IDALRRVLTAYSFRTPEIGYCQAMNIVSSVLLIY
ScTBC-A/GYP2      EIEKDLKRSLPEYSAYQ.TEEG.IQRLRNVLTAYSWKNPDVGYCQAMNIVVAGFLIF
CnTBC-A           EIEKDLNRSLPEYKAYQ.SEEG.LARLRRVLVAYSFRNPELGYCQALNIVVAGLLIY
RoTBC-A1          EIEKDLNRSLPEYAGYQ.SSEG.IDRLRRVLTAYAWKNPELGYCQAMNIVTSALLIY
RoTBC-A2          EIEKDLNRSLPEYKAYQ.QPEG.IDRLRRVLVAYSWKDPELGYCQAMNIVTSAILIY
RoTBC-A3          EIEKDLNRSLPEYAGYQ.SPEG.IDRLRRVLTAYAWKNPELGYCQAMNIATSAFLIY
DdTBC-A2          EIDKDISRTFPGHPFFE.SDEG.KRKLSRVLQAYSIRNRKVGYCQSMNIVAGFVLFV
DdTBC-A1          EILSDLERTFPTHPKSLDPEFK..DKLKRILFVFS.ETTTVGYCQSLNYITFFLLMI
DdTBC-A3          QILMDVDRTFPDHKFLN.TKEK.MESLTNVLVAYSWRNPKVGYCQCMNFIAGYLLIY
DdTBC-A4          DIEKDIHRSFPKHPWFREKEAGGQDALKNILQAYSWRNPSIGYCQSMNIVAAVLLIY
DdTBC-A5          DIEKDVRRTFSHHPYFRDNGAG.VDSLRRILIAYSRRNPTIGYCQGMNNVAGIMLLY
                        ▲                 ▲

                  1       10        20        30        40        50
HsTBC-O1/USP6NL   QIDLDVNRTFRDHIMFRDRYGVKQQSLFHVLAAYSIYNTEVGYCQGMSQITALLLMY
DmTBC-O           QIDADVNRQFRDNLAFRERYSVKQCSLFNVLNAYSIYNSELGYCQGMACVAGVLLLY
NvTBC-O           QIDLDVNRTYRDHIMFRERYGIKQQALFHVLAAYSMYNVEVGYCQGMSGIAALLLMY
CnTBC-O           QIDLDVPRTISGHTMFVTRYGTGQRNLWHVLHAFSQRCPTCGYVQGMGPIVATLLCY
RoTBC-O           QIDLDIPRTLRDHIMFKQRYGSGQRALFNVLRAFANYDQEVGYCQGMTNIVATILMY
DdTBC-O           YIDLDVNRAYRNHIFFRERYGMGQVSLFNVLKVYSLYDQGVGYTQGMSSIASLLVMY
                        ▲                 ▲

                  1       10        20        30        40        50
HsTBC-P1/TBC1D10B VIEKDLHRQFPFHEMFAARGGHGQQDLYRILKAYTIYRPDEGYCQAQAPVAAVLLMH
HsTBC-P2/TBC1D10A VIERDLHRQFPFHEMFVSRGGHGQQDLFRVLKAYTLYRPEEGYCQAQAPIAAVLLMH
HsTBC-P3/TBC1D10C TIGRDLHRQFPLHEMFVSPQGHGQQGLLQVLKAYTLYRPEQGYCQAQGPVAAVLLMH
DmTBC-P           EIKKDKHRQFPFHEMFLDEQKVGQIELFNVLKAYSIYNPKVGFCQAQAPIAAFLLMH
NvTBC-P           TIEKDLCRVFPYHEQFTDTGGQGQKDLFRVLKAYSLYDSHTGYCQAMAPVVAVLLMH
RoTBC-P           VIERDIERCYPDHTQFMEKDGLGQQNLRNILKAYAQYNSDLEYCQGMGRLAGLMLMQ
                        ▲                 ▲

                  1       10        20        30        40        50        60
ScTBC-T/BUB2      KIKNDTSRTFQTDP
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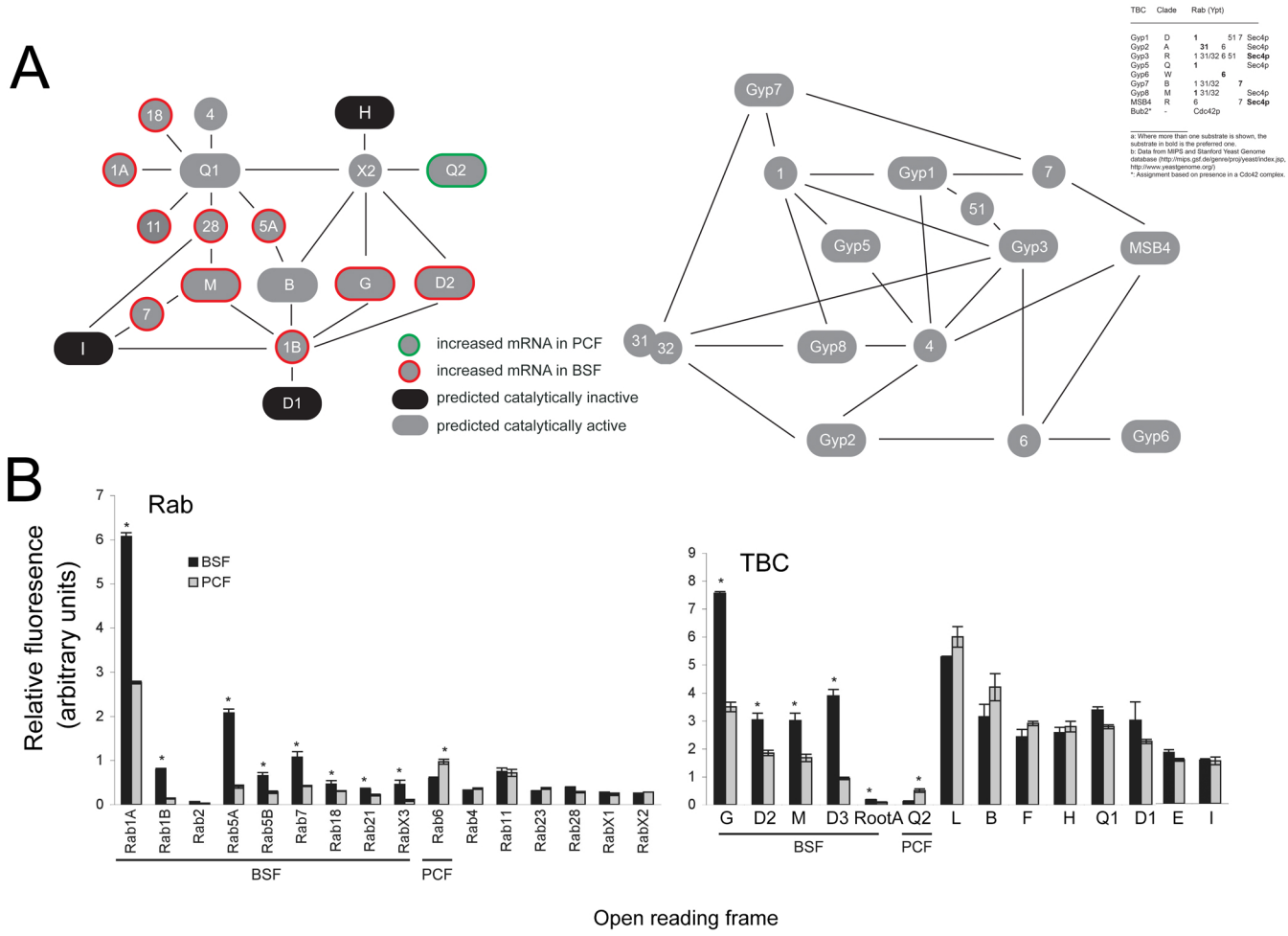
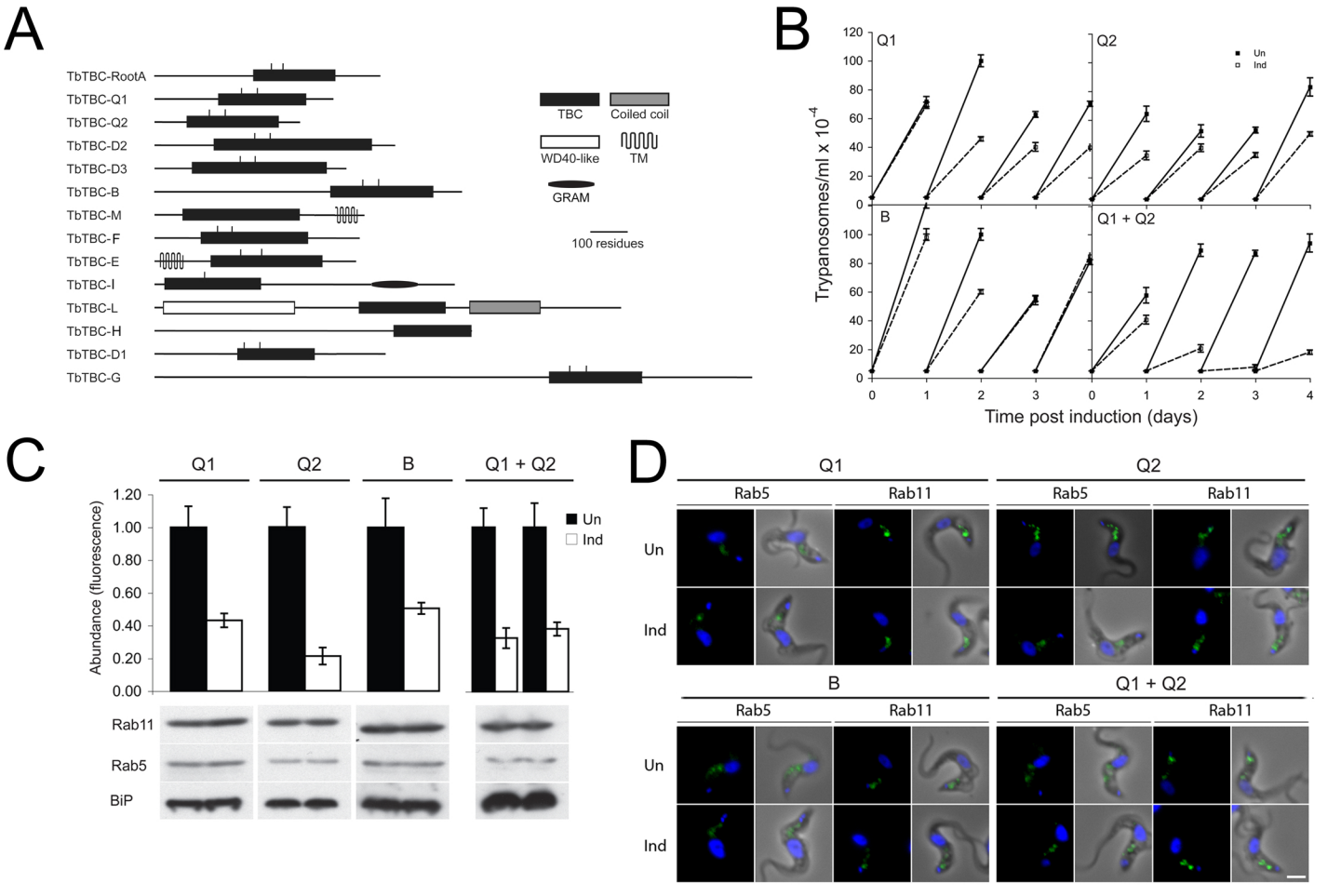

A
18
4
H
1A
Q1
X2
Q2
11
28
5A
M
B
G
D2
7
I
1B
D1
increased mRNA in PCF
increased mRNA in BSF
predicted catalytically inactive
predicted catalytically active
Gyp7
1
Gyp1
7
51
Gyp5
Gyp3
MSB4
31
32
Gyp8
4
Gyp2
6
Gyp6
TBC Clade Rab (Ypt)
Gyp1 D 1 51 7 Sec4p
Gyp2 A 31 6 Sec4p
Gyp3 R 1 31/32 6 51 Sec4p
Gyp5 Q 1 Sec4p
Gyp6 W 6
Gyp7 B 1 31/32 7
Gyp8 M 1 31/32 Sec4p
MSB4 R 6 7 Sec4p
Bub2* - Cdc42p
a: Where more than one substrate is shown, the substrate in bold is the preferred one.
b: Data from MIPS and Stanford Yeast Genome database (http://mips.gsf.de/genre/proj/yeast/index.jsp, http://www.yeastgenome.org/)
*: Assignment based on presence in a Cdc42 complex.
B
Relative fluorescence (arbitrary units)
Rab
BSF
PCF
Rab1A
Rab1B
Rab2
Rab5A
Rab5B
Rab7
Rab18
Rab21
RabX3
Rab6
Rab4
Rab11
Rab23
Rab28
RabX1
RabX2
BSF
PCF
TBC
G
D2
M
D3
RootA
Q2
L
B
F
H
Q1
D1
E
I
BSF
PCF
Open reading frame


A
TbTBC-RootA
TbTBC-Q1
TbTBC-Q2
TbTBC-D2
TbTBC-D3
TbTBC-B
TbTBC-M
TbTBC-F
TbTBC-E
TbTBC-I
TbTBC-L
TbTBC-H
TbTBC-D1
TbTBC-G
TBC
Coiled coil
WD40-like
TM
GRAM
100 residues
B
Q1
Q2
B
Q1 + Q2
Un
Ind
Trypanosomes/ml x 10^-4
Time post induction (days)
C
Q1
Q2
B
Q1 + Q2
Un
Ind
Abundance (fluorescence)
Rab11
Rab5
BiP
D
Q1
Q2
B
Q1 + Q2
Rab5
Rab11
Un
Ind