

Supporting information for: “Structure of Trifunctional THI20 from Yeast” by Jarrod B. French, Tadhg P. Begley and Steven E. Ealick

Figure Legends

Figure S1. Sequence alignment of several TenAs with the conserved cysteine residue in the active site (marked with a blue arrow). The species name and PDB code are given. Red boxes with white lettering represent absolutely conserved residues, while boxes with blue outlines and red lettering represent highly conserved residues.

Figure S2. Sequence alignment of several TenAs lacking the conserved cysteine residue in the active site (marked with a blue arrow). The species name and PDB code are given. Red boxes with white lettering represent absolutely conserved residues, while boxes with blue outlines and red lettering represent highly conserved residues.

Figure S3. Phylogenetic trees showing the relationship between A) TenA sequences from ten different organisms and B) the same organisms organized by taxonomy. The phylogenetic tree in A) was generated using ClustalW and the tree in B) was generated using the NCBI taxonomy browser. In both cases, those TenAs that contain a conserved cysteine residue are annotated with 'Cys', while those containing a pair of conserved glutamate residues are annotated with 'Glu'.

Figure S4. Sequence alignment of *Saccharomyces cerevisiae* THI20, THI21 and THI22. A Thr to Ala mutation from THI20 to THI22 (indicated with a blue arrow) may contribute to the loss of HMP(P) kinase function in THI22. In the alignment, red boxes with white lettering represent absolutely conserved residues, while boxes with blue outlines and red lettering represent highly conserved residues.

| | | | | | | |
|-------------------------|---|---------------|----------------|---------|-------------|----------------------------|
| | | 1 | 10 | 20 | 30 | 40 |
| H_pylori_2RD3 | | GIDP..... | FTMQVSOYLY | QNA | QSIWGD | CISHP |
| B_subtilits_1T09 | | SSGVDLGTENLYF | QSNAMKFSEECR | SAA | AEWEGSFVHP | FVCGITGRCTLERD |
| S_epidermis_3N06 | | MGSDKIH | HHHHHENLYFQGMT | FSKELR | EAS | RPIIDDIYNDGFIQDLLAGKLSNQAV |
| S_solfataricus_2QZC | | GMS | IVGNVENL | INGV | GELWNKYVKHE | FILKMRDGSILPLDIF |
| B_thetaiotaomicron_2A2M | DFKNQWLRKRRTFAIPASRLTGRLLTTLKSDVPAADSLFWKLW | NGS | LDTAVQVQLQTD | YFKGLAA | CTLD | PNAY |

| | | | | | | | |
|-------------------------|-----|---------|----------|--------|------------------|------------|-----------------------|
| | 50 | 60 | 70 | 80 | 90 | 100 | 110 |
| H_pylori_2RD3 | RFY | TIQDYLF | LLEYAKVF | ALGVV | KACDEAVMREFS | NA | TQDILNNEMSIHNNHYIRELO |
| B_subtilits_1T09 | KYY | VLD | SYLTHFAK | VQSF | GAAYAKDLYTTGRMA | SHA | QGTYEAEAMALHREFAEELLE |
| S_epidermis_3N06 | RQY | LRA | ASYLKEF | TNIY | AMLIPKMSMEDVKFLV | EO | IEFMLEGEVEAEHVLADFINE |
| S_solfataricus_2QZC | RY | LIQ | DGKYVEDM | LRALL | IAS | SKG.PIDKVT | KILNLVFS |
| B_thetaiotaomicron_2A2M | GSL | MVQ | DGYCFRGR | DDYATA | ATCAQDET | LRFFKAK | AKS.....YDEYNETYH |

| | | | | | | | |
|-------------------------|---------|-------------|----------|--------|------------|---------|--------------------|
| | 120 | 130 | 140 | 150 | 160 | 170 | 180 |
| H_pylori_2RD3 | .ANKSYT | SYMLAEGFKGS | .IKEVA | AAVLS | CGWSYLV | TAQN | LSQIPNALEHAFYGH |
| B_subtilits_1T09 | .TAYSYT | SHMYRSVLSGN | .FAEIL | AALLFC | YWLYYEV | GEKLL | HCD..PGHPITYQK |
| S_epidermis_3N06 | PSGDHYI | KHMYFN | AFARENA | AFTIAA | MAPCPYVYAV | IGKRA | MEDPKLNKESVTSK |
| S_solfataricus_2QZC | .IN | YAYTRH | LY..YANL | DWNKFL | VAVTF | CMFGYSI | VGDYVIDSP...NEVYKT |
| B_thetaiotaomicron_2A2M | GHD | IKDYAD | YEAYVAGS | LSPYMC | VVMLE | CEYLWPW | HANFLDGYT..PTNSLYR |

| | | | | |
|-------------------------|--------|------|----------|-----------|
| | 190 | 200 | 210 | 220 |
| H_pylori_2RD3 | WNINL | DSLT | LASSKQEI | EKLKEIT |
| B_subtilits_1T09 | EQINRF | DELA | ENSTEEV | RAKMKENF |
| S_epidermis_3N06 | VFDQLM | DRLT | KHCSETE | KEIKENF |
| S_solfataricus_2QZC | AILYAL | DEVS | IT..... | EDLLNFIN |
| B_thetaiotaomicron_2A2M | GAYQMG | NMLE | QYRDKI | DEDKAVEIT |

Figure S1.

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          1      10      20      30      40      50
P_furiosis_1RTW      .....MFSEELK1ENENI10WRRF20PHKF30LIE40MAENT50TKK55ENF60E65KW70L75V80ND85Y90F95VKNALRFMAL
S_pneumoniae_1Z72    SNAMETQDYAFQPG1LVGEL10LK20SSQKDWQAA30INHRF40VKE50LFAGT60IEN70KV80LK90DY100L110Q120DY130HF140FD150AF160LS170MLGA
A_thaliana_2F2G      .....MEKRGVIDT1WID10K20HRSIY30TAA40TRHAF50V60VS70IR80DGS90V100DL110SS120FR130TW140L150G160Q170DY180L190F200VRRFV210PFVAS
T_thermophilus_1WWM .....MGLGLDL1L10K20EV30PG40...L50EE60IK70AL80FL90R...L100DE110ER120FR130FW140L150Q160DY170F180VEALY190RY200QV210G

          60      70      80      90      100      110
P_furiosis_1RTW      LMAK60AP...DDL70PPFAESI80YYI90SK100ELEM110F120EK130KA140Q150ELG160ISLNG...EIDWR...AK170SY180VNY190L200LS210VASLGS
S_pneumoniae_1Z72    CVAH60ADK...LESK70LRF80AK90QLG100FLE110AD120DGY130F140QK150AF160K170ELK180VAENDY190LEV200TL210LHP220VT230KA240FQ250DL260MY270SA280VASSD
A_thaliana_2F2G      VLI60RA70CKDSGESS80MEV90VLLGGI100ASL110N120DE130IEW140FR150EG160SK170WD180VDF190ST200VVP210QRAN220Q230EY240GR250FLE260DL270MS280SEV290K..
T_thermophilus_1WWM LLE60EA70P...QAH80R90APL100VQAL110MAT120VE130LDW140LL150Q160AS170PS180AP190VHP...VR200AGY210IA220L230EE240MGR250LP

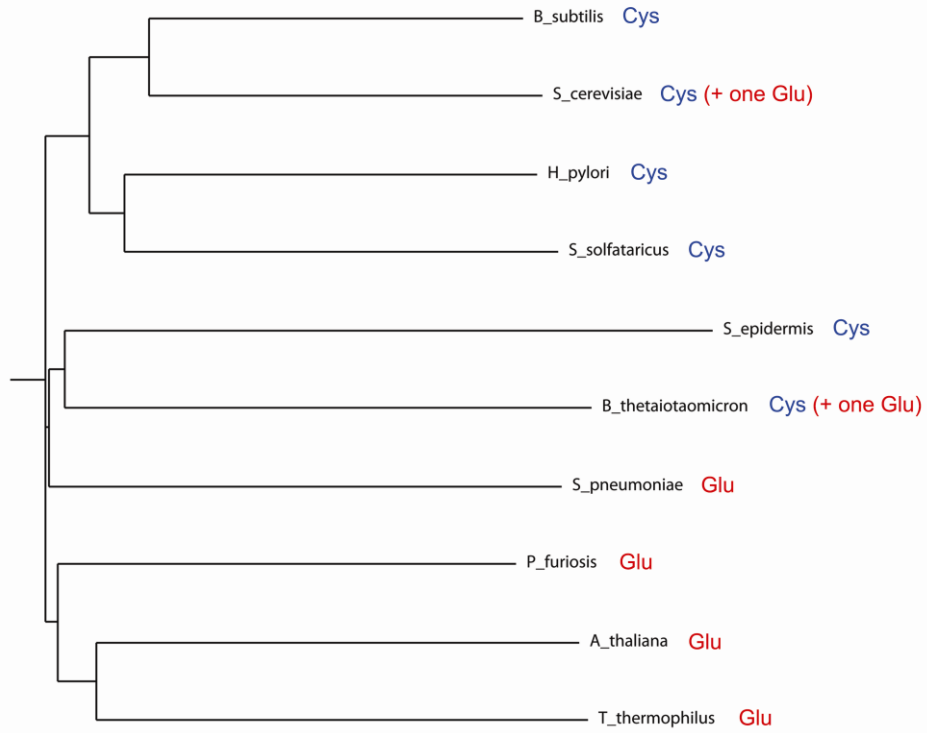
          120      130      140      150      160      170      180
P_furiosis_1RTW      FLEGF120TALYC130E140EKAY150Y160E170AWK180.WVREN190LK200ERS210PY220Q230EFIN240H250WSS260Q270E280F290GE300Y310V320KR330I340E350K360I370LN380SLA390E400KHGEFE410KER420
S_pneumoniae_1Z72    YAH.LLVMLV120IAEGLY130LDWG.SKDLALP140EVY150IH160SEWIN170LHRG180PF190AE200WV210QF220LV230DE240LN250RVG260KNRED270LTEL280EQ
A_thaliana_2F2G      YPVI120MTAFWA130IEAVY140Q150ESFA160H170CLED180GNK190TP200VEL210TGACH220RWG230ND240G250FK260Q270YCS280SV290KN300IA310ER320CLEN330ASGE340VLGE
T_thermophilus_1WWM YAYRV120VFFYF130LNGLFL140EAWA...HHVP150E160EG170PW180AE190LSQ200HWF210AP220E230Q240AVLYD250LE260V270LAR280GLWE290D...LDPE300V

          190      200      210
P_furiosis_1RTW      AREV190FK200EVSK210FEL220TFWD230IAYGGEG240NVL
S_pneumoniae_1Z72    QR..WNQ190AVALE200LAF210FD220IGYDV...
A_thaliana_2F2G      AEDV190LV200RVLE210LEV220AFW230MSRGGQ...
T_thermophilus_1WWM VR190TYLR200RILE210AEK220ATWS230LLL.....

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Figure S2.

A



B

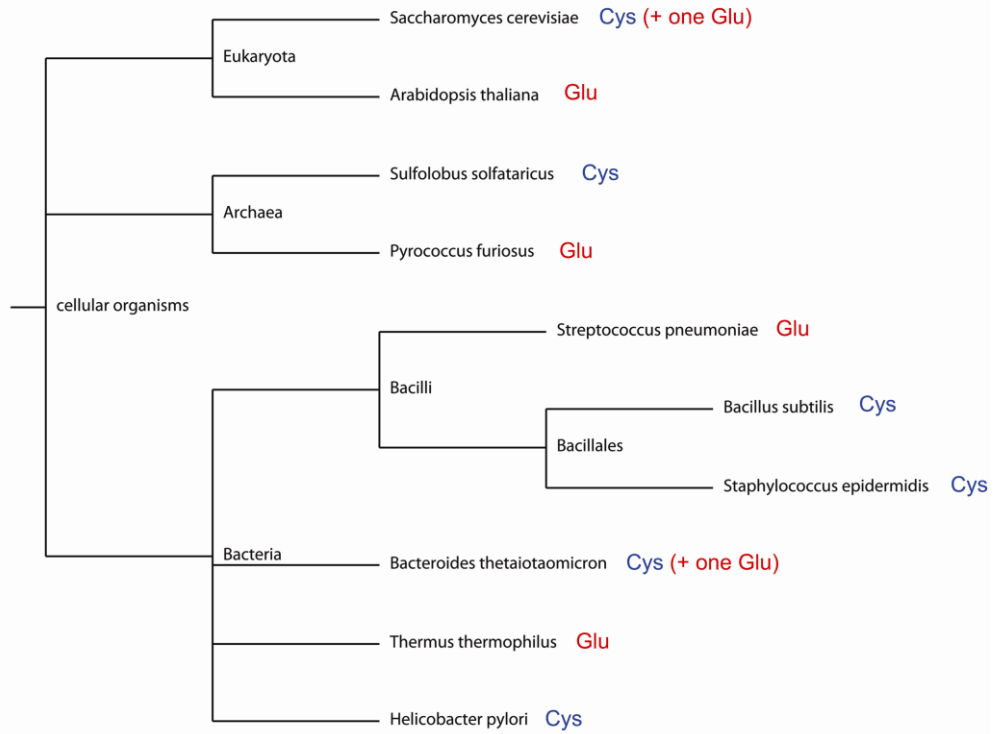


Figure S3.

Sc_THI20 1 10 20 30 40
 Sc_THI21 MTYSTVSNINTPPPYLTLACNEKLPTVSIAGTDPSSGAGVEADVKTIT
 Sc_THI22 MVIILLGLCTLGFPRTAFCPSSIMTNSSTVSNINTPPPYLTLACNEKLPTVMSIAGSDSSGAGVEADVKTIT

Sc_THI20 50 60 70 80 90 100 110
 Sc_THI21 AHRCYAMTCITALNAQTPVKVYSINNTPEKVVFOITLESNLRDMKCNVIKTGMLTAAIEVLHEKLLQLGE
 Sc_THI22 AHRCYAMTCITALNAQTPVKVYSINNTPEKVVSOILDANLQDMKCDVIKTGMLTAAIEVLHEKLLQLGE

Sc_THI20 120 130 140 150 160 170 180
 Sc_THI21 NRPKLVVDPVLVATSGSSLAGKDIVSLITEKVAFFADILTPNIPFCYKLLGGERKVNGLQDIFQIAKDIA
 Sc_THI22 NRPKLVVDPVLVATSGSSLAGKDIASLITEKIAPFADILTPNIPFCFKLLGEDREISKLRDIFEVAKDIA

Sc_THI20 190 200 210 220 230 240 250
 Sc_THI21 KITKCSNILVKGGHIPWNEDEKYEKITDVLFLGAEQRFITFKGNFVNTTHTHGTGCTLASAIASNLARGYS
 Sc_THI22 RITKCSNILVKGGHIPCDDGKEKHITDVLVFLGAEQRFITFKGNFVNTTHTHGTGCTLASAIASNLARGYS

Sc_THI20 260 270 280 290 300 310 320
 Sc_THI21 LPQSVYGGIEYVQNAVAIGCDVTKETVKDNGPINHVYAVEIPELEKMLSDECFTASDAVHKKPKVKSAAADKI
 Sc_THI22 LQSVYGGIEYVQNAVAIGCDVTKKAVR.VGPIHVYAVEIPELEKMLDDECFTASDAVFKKPIEGSIDKI

Sc_THI20 330 340 350 360 370 380 390
 Sc_THI21 PGGNFFYELINHPKVKPHWDSYINHEFVKKVADGTLERKKQFFIEQDYLYLVDYARVHCIAAGSKAPCLE
 Sc_THI22 PGGNFFYELINHPKVKPHWDSYVNHDFVKKVADGSLERKKQFFIEQDYLYLVNYARVSCIAAGSKSPCLE

Sc_THI20 400 410 420 430 440 450 460
 Sc_THI21 DMEKEELVIVGGVRETEMGCHERRLRKEVFGVKDPPDYFOKIKRGPALRAYSRVFNQDVSRRGNWQELVVALNPC
 Sc_THI22 DLEKELVIVADCARNELEHERRLRKEVFGVKDPPDYFOKIQRGPALRAYCRYLIQDISRRGNWQELVVALNPC

Sc_THI20 470 480 490 500 510 520 530
 Sc_THI21 LMGYGEALTKMKGKVTAPEGSVYHEWCETYSASSWYREAMDGEKLLNHILETYPPEQLDTLVTIYAEVCE
 Sc_THI22 LMGYVHALTKMKDEVTAPEGSVYREWCBETYSASSWCHAMLEGEKLLNHILETYPPEQLDTLVTIYAEVCE

Sc_THI20 540 550
 Sc_THI21 LETNFWTAAEYE
 Sc_THI22 LETNFWTAAEYE

Figure S4.