Supplementary Table S1

Control Serine Residues with High Asp/Glu Replacement Percentages

Protein name	Species	Ser residue	# of homologs	# with a residue other than Ser at this site	Asp + Glu %
TUBA6	Human	292	208	207	100.0
EF2	Human	530	252	225	98.2
EF2	Human	677	316	215	98.1
Thyroid hormone receptor alpha (c-ErbA)	Chicken	381	75	52	96.2
Isoform 1 OF myosin-5A	Human	1118	85	75	96.0
Isoform 1 OF Krueppel-like factor 3	Human	275	72	22	95.5
14-3-3 protein beta/alpha	Cow	144	93	87	94.3
Multidrug resistance-associated protein 4	Human	906	77	69	94.2
Isoform 1 of cytoplasmic linker protein 2	Human	194	84	69	94.2
ISOFORM 1 OF MYOSIN-5A	Human	1712	85	69	94.2
Zinc finger protein 516	Human	273	155	152	94.1
EF2	Human	540	316	76	93.4
Phosphoribosyl pyrophosphate synthetase-associated protein 2	Human	189	134	128	93.0
TUBA6	Human	312	208	200	92.5
Isoform 1 of cytoplasmic linker protein 2	Human	551	84	78	92.3
Isoform 1 of myosin-5A	Human	347	85	77	92.2
Isoform 1 of myosin-5A	Human	1673	85	73	91.8
Isoform 1 of myosin-5A	Human	1127	85	72	91.7
Isoform 1 of myosin-5A	Human	1714	85	70	91.4
Isoform 1 of cytoplasmic linker protein 2	Human	854	84	79	91.1
Isoform 1 of cytoplasmic linker protein 2	Human	899	84	79	91.1
Tubulin beta-2C chain	Human	153	359	172	89.5
Tubulin beta-2C chain	Human	413	359	163	89.0
RAB31	Human	42	354	272	87.5
MAP kinase kinase 1	Rabbit	149	421	305	84.6
EF2	Human	773	316	248	79.4

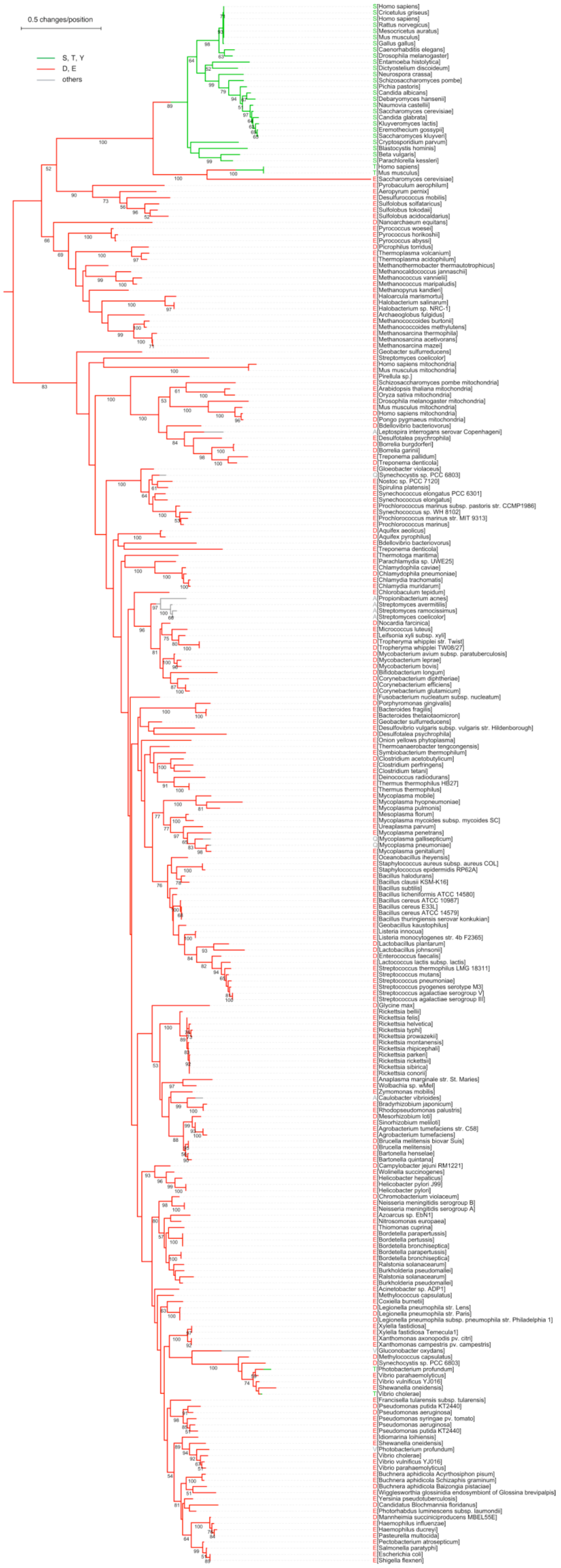

Table S2A. Detailed Phylogenetic Tree for eEF2
[related to Figure 4]

This tree adds the species names, the identities of the residues corresponding to S502, and the bootstrap values (when >50%) to the maximum-likelihood tree shown in Figure 4A.

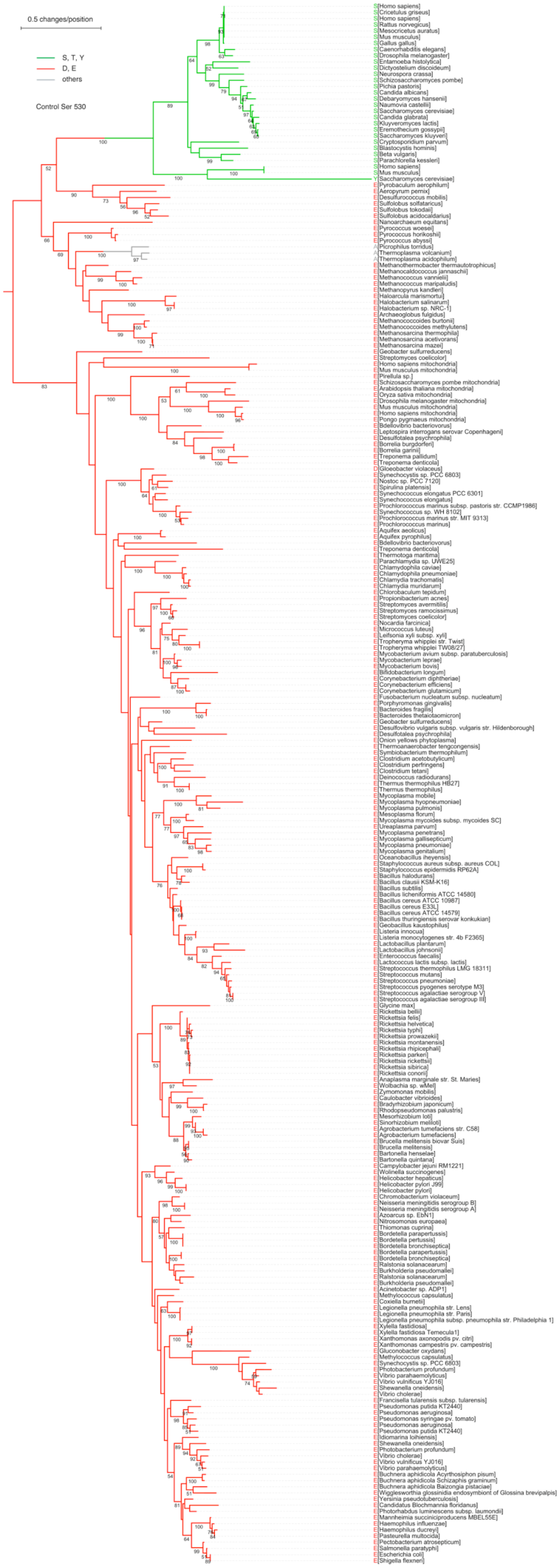

Table S2B. Detailed Phylogenetic Tree for Control Serine 530 in eEF2 [related to Figure 4]

Table S2C. Detailed Phylogenetic Tree for Control Serine 774 in eEF2 [related to Figure 4]

Table S2D. Detailed Phylogenetic Tree for DNA Topoisomerase II [related to Figure 4]

This tree adds the species names, the identities of the residues corresponding to T639, and the bootstrap values (when >50%) to the tree shown in Figure 4B.

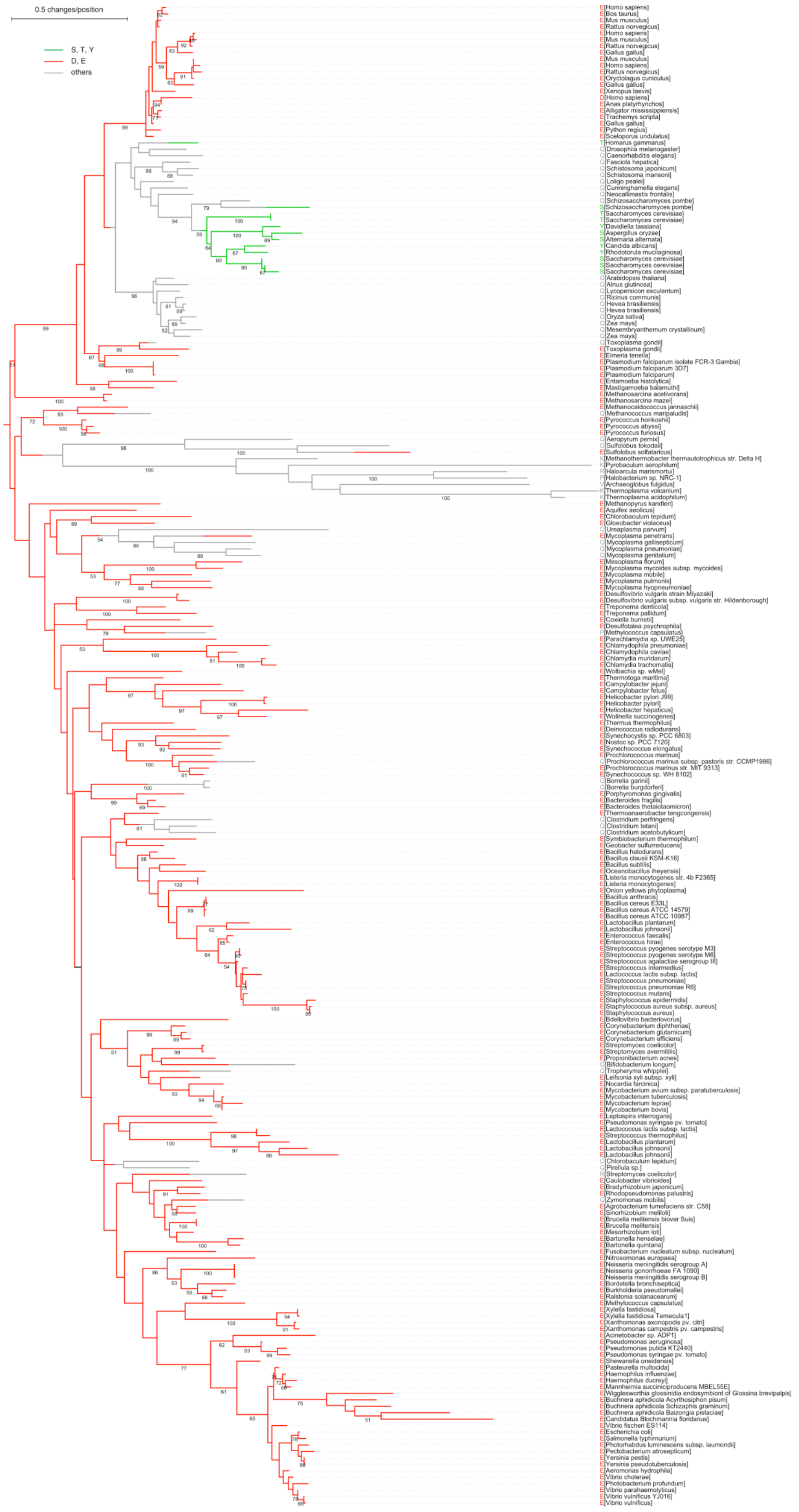

Table S3A. Detailed Phylogenetic Tree for Enolase [related to Figure 5]

This tree adds the species names, the identities of the residues corresponding to S10, and the bootstrap values (when >50%) to the tree shown in Figure 5A.
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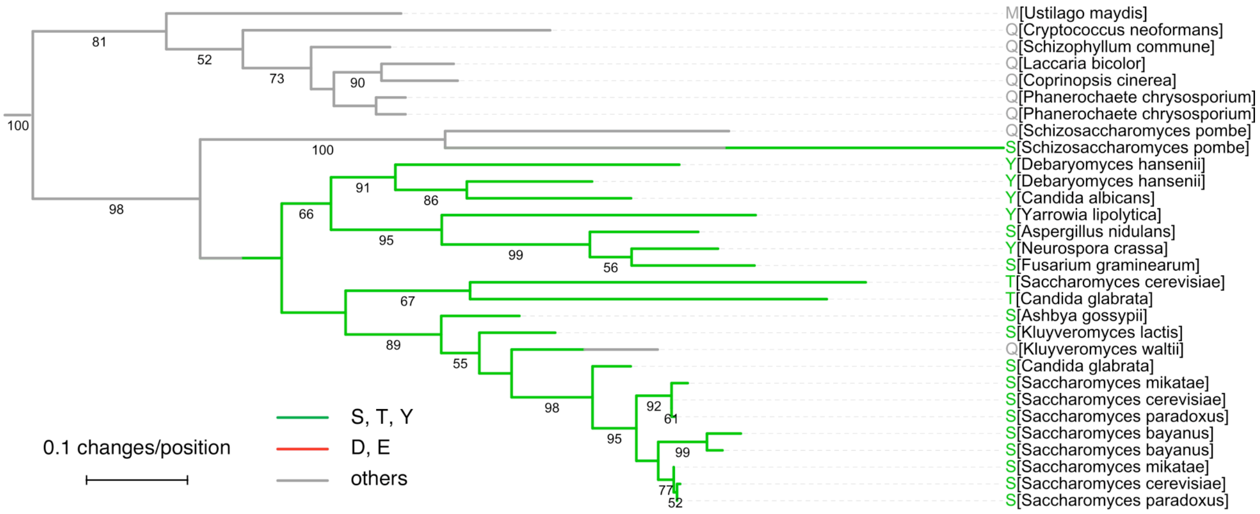

Table S3B. Detailed Phylogenetic Tree for the Fungal Enolases [related to Figure 5]

This tree adds the species names, the identities of the residues corresponding to S10, and the bootstrap values (when >50%) to the tree shown in Figure 5B.

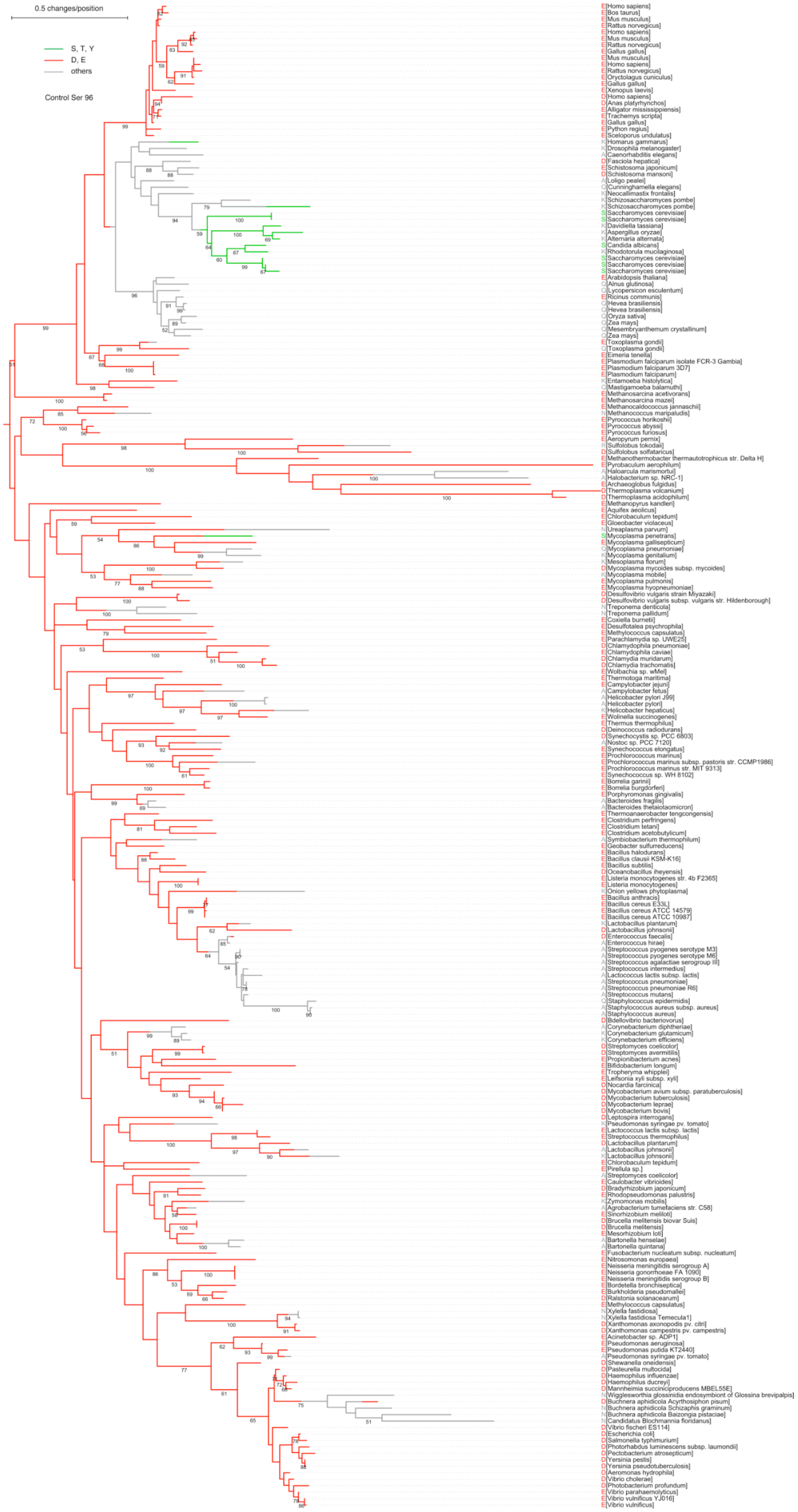

Table S3C. Detailed Phylogenetic Tree for Control Serine 96 in Enolase [related to Figure 5]

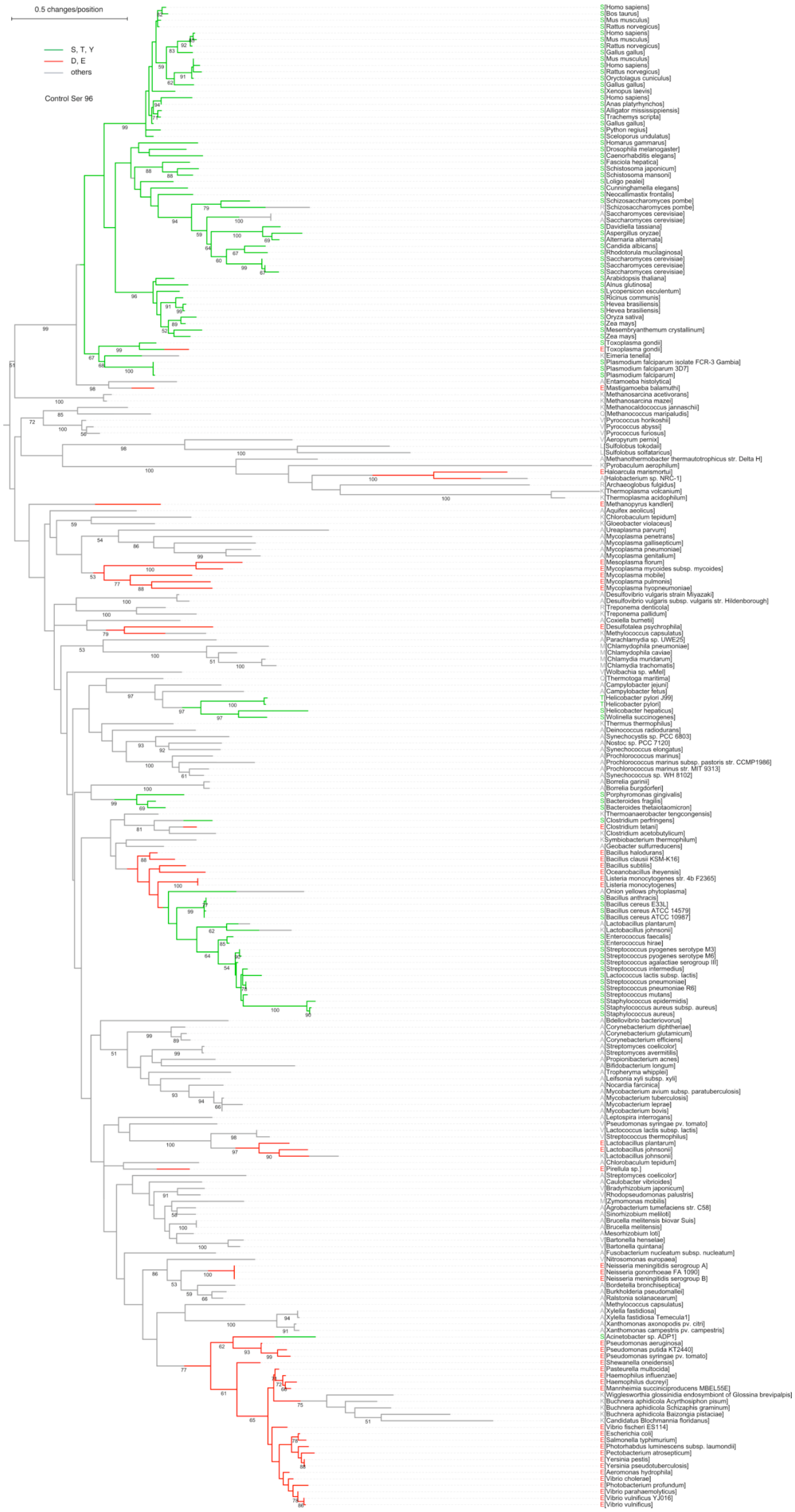

Table S3D. Detailed Phylogenetic Tree for Control Serine 159 in Enolase [related to Figure 5]

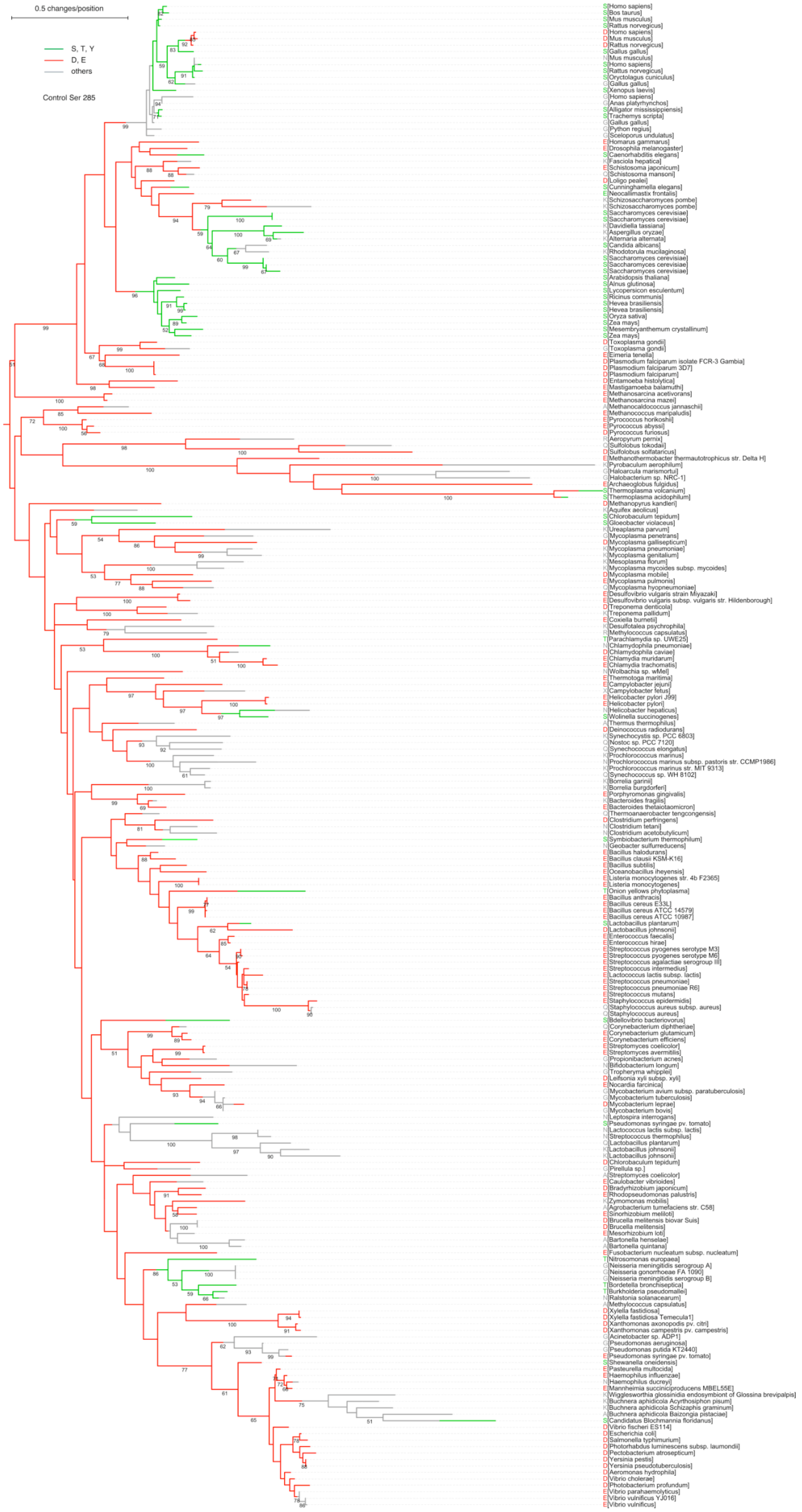

Table S3E. Detailed Phylogenetic Tree for Control Serine 285 in Enolase [related to Figure 5]

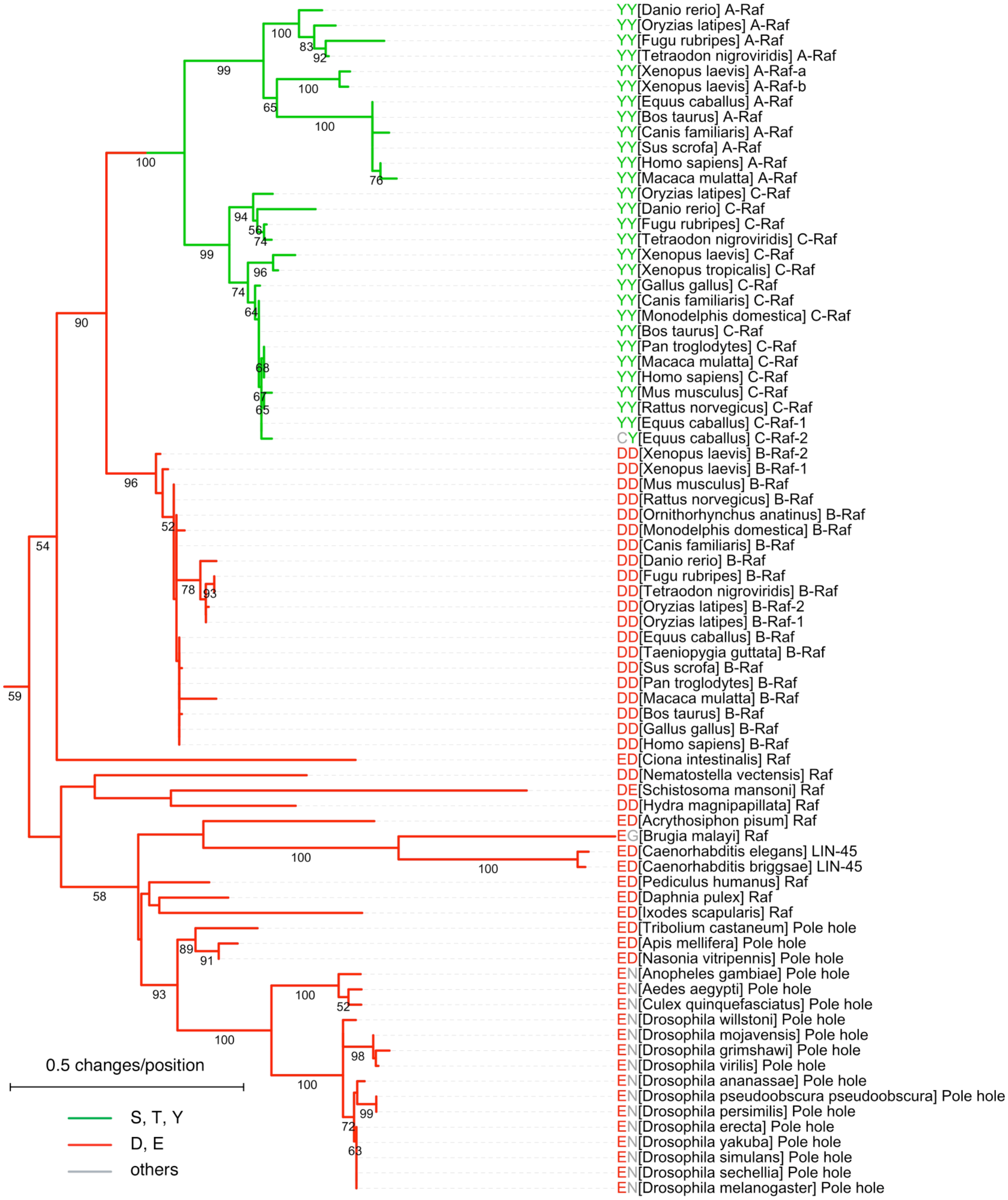

Table S4A. Detailed Phylogenetic Tree for Raf
[related to Figure 6]

This tree adds the species names, the identities of the residues corresponding to Y340 and Y341, and the bootstrap values (when >50%) to the tree shown in Figure 6A.

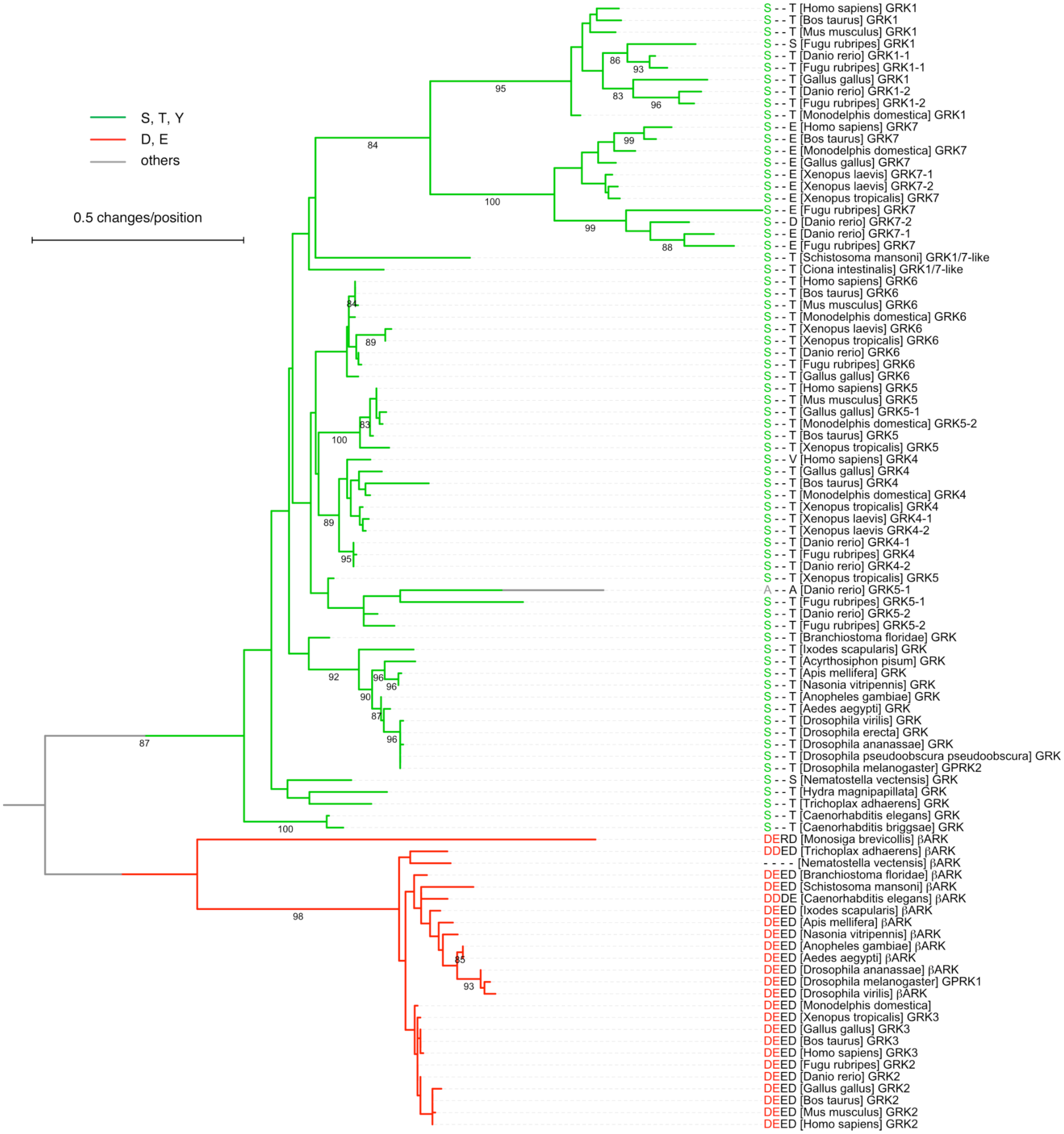

Table S4B. Detailed Phylogenetic Tree for S484 of GRK5
[related to Figure 6]
This tree adds the species names, the identities of the residues corresponding to S484 and T485, and the bootstrap values (when >50%) to the tree shown on the left in Figure 6C. Leaves, branches and internal nodes are colored according to the residue corresponding to S484.

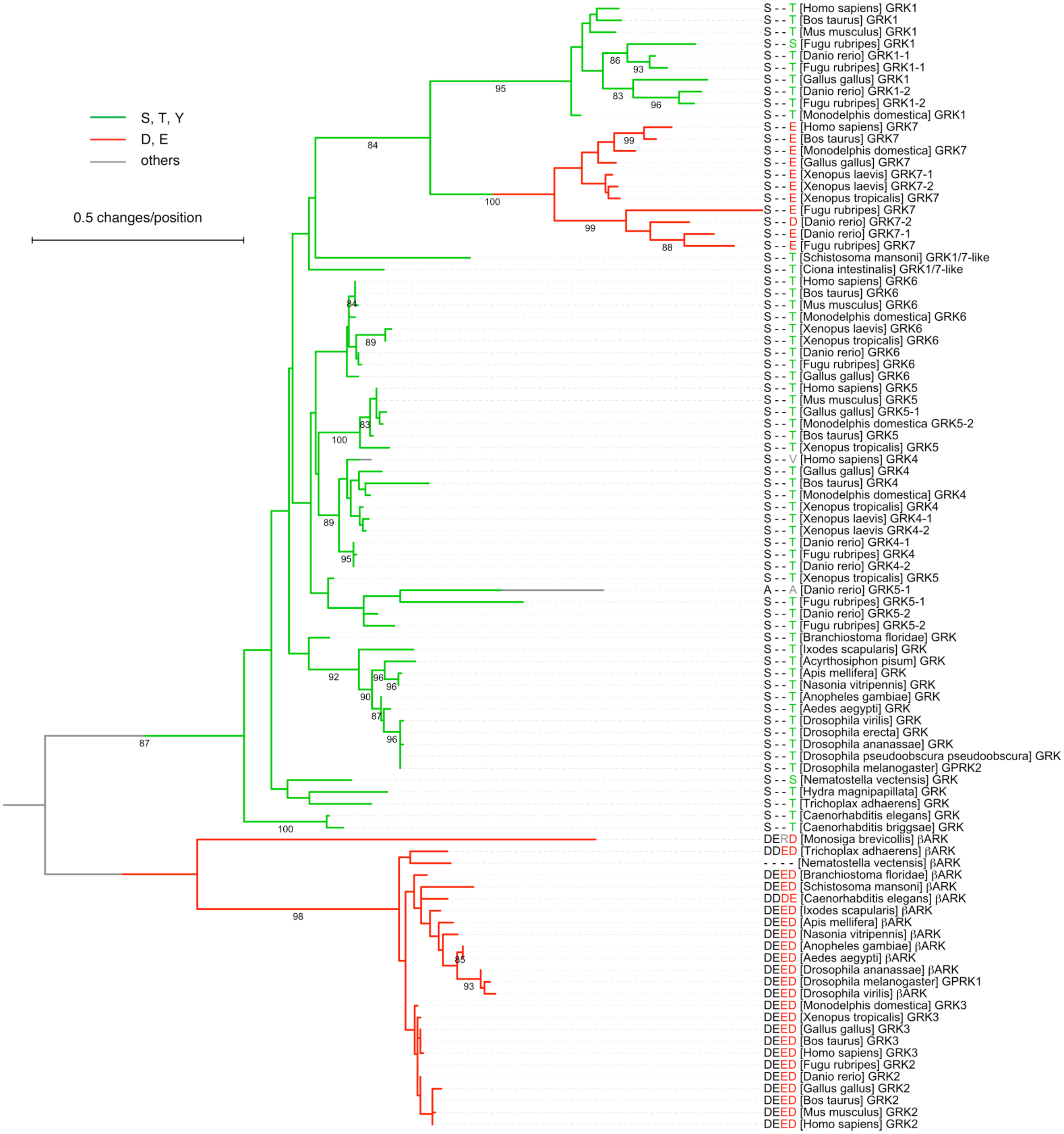

Table S4C. Detailed Phylogenetic Tree for T485 of GRK5 [related to Figure 6]

This tree adds the species names, the identities of the residues corresponding to S484 and T485, and the bootstrap values (when >50%) to the tree shown on the right in Figure 6C. Leaves, branches and internal nodes are colored according to the residue corresponding to T485.

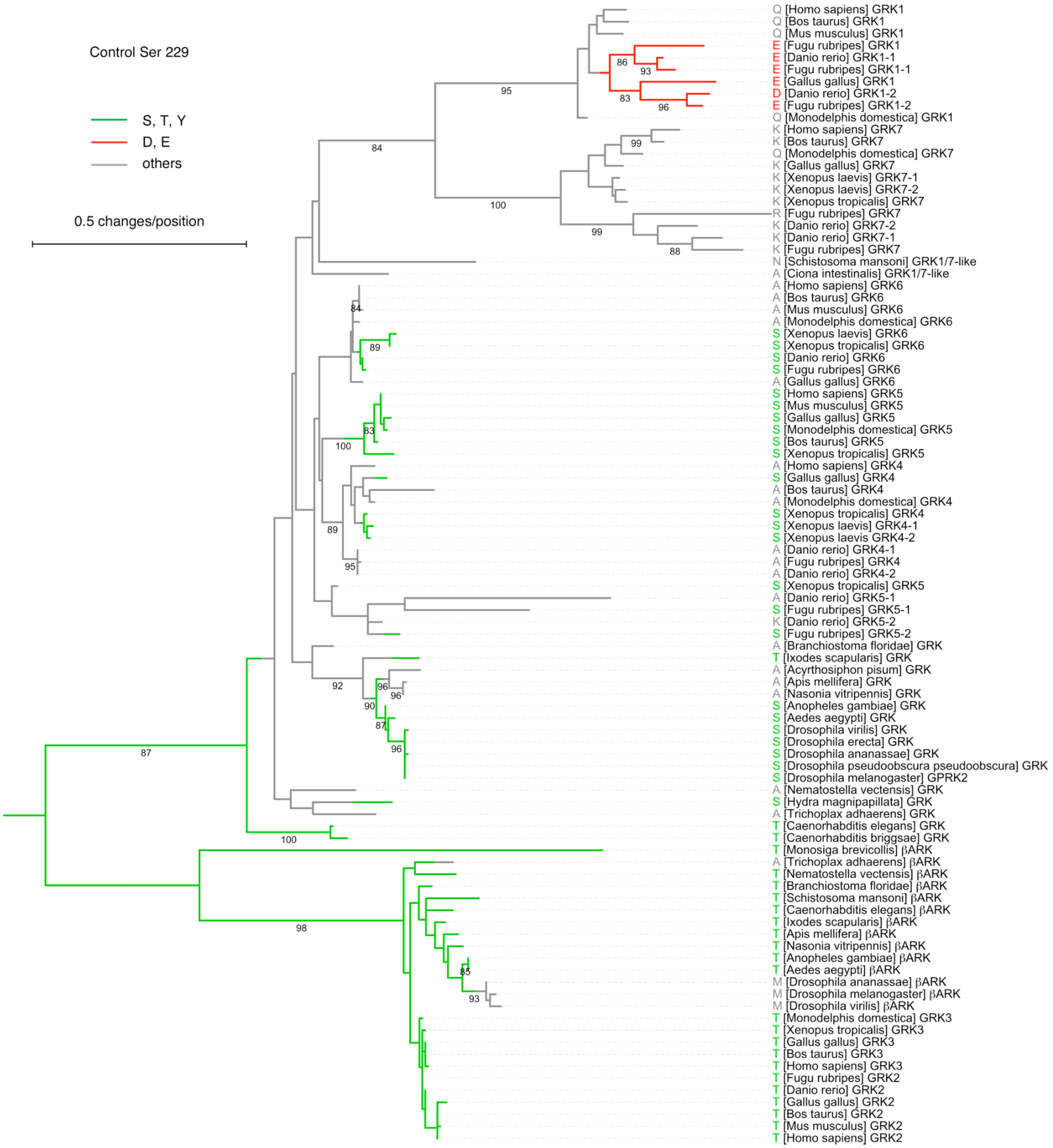

Table S4D. Detailed Phylogenetic Tree for Control Serine 229 in GRK5 [related to Figure 6]

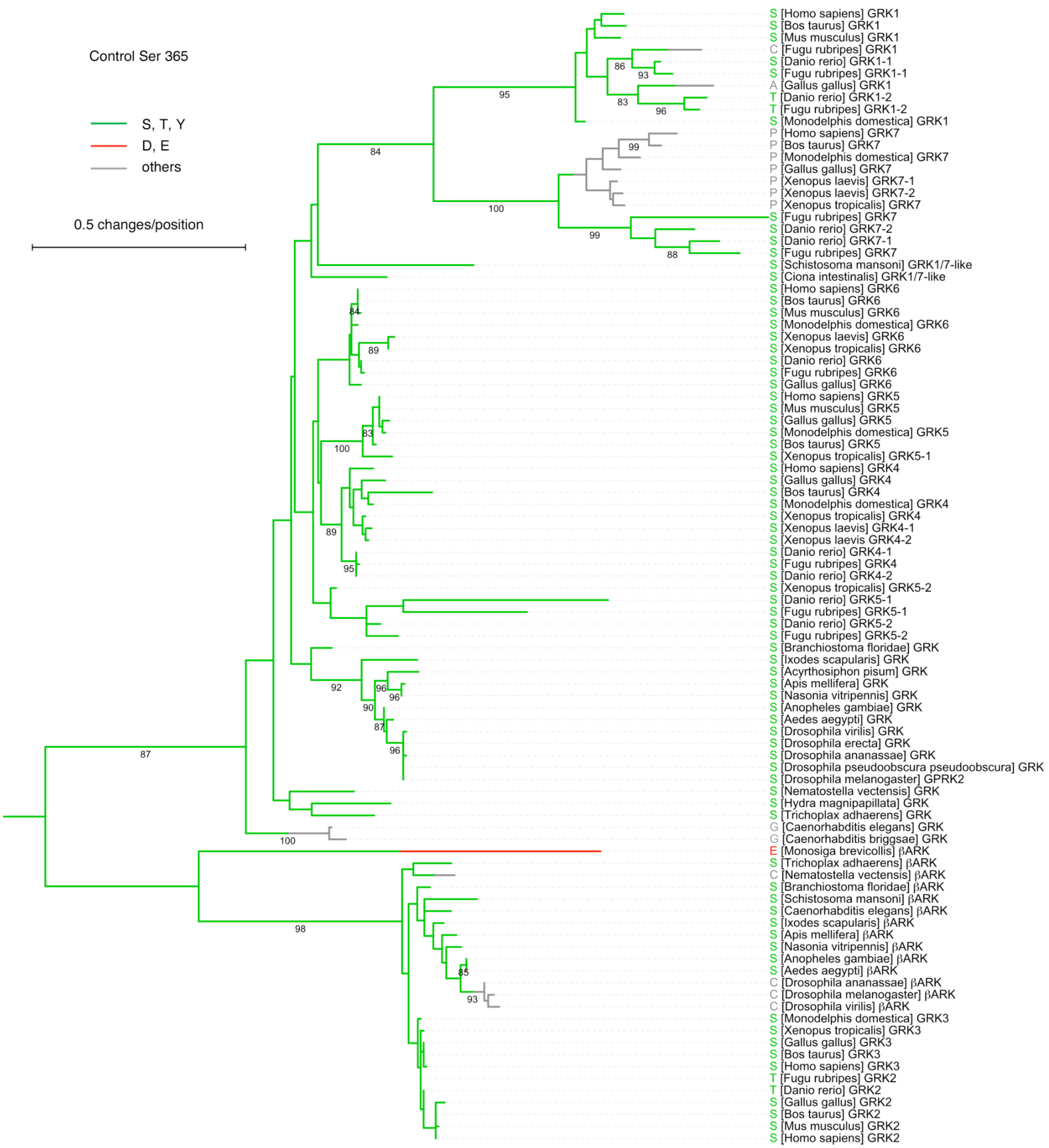

Table S4E. Detailed Phylogenetic Tree for Control Serine 365 in GRK5 [related to Figure 6]

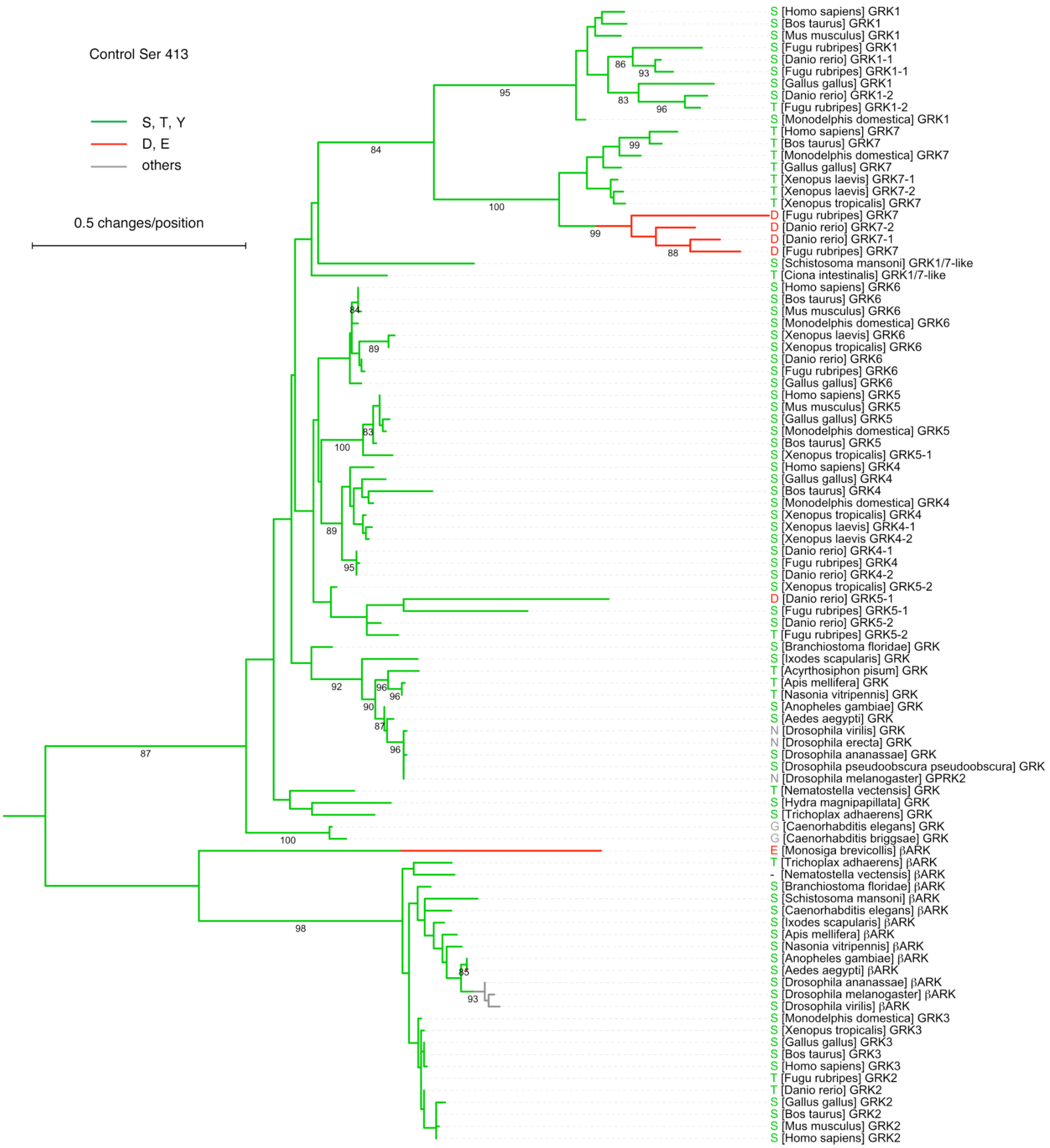

Table S4F. Detailed Phylogenetic Tree for Control Serine 413 in GRK5

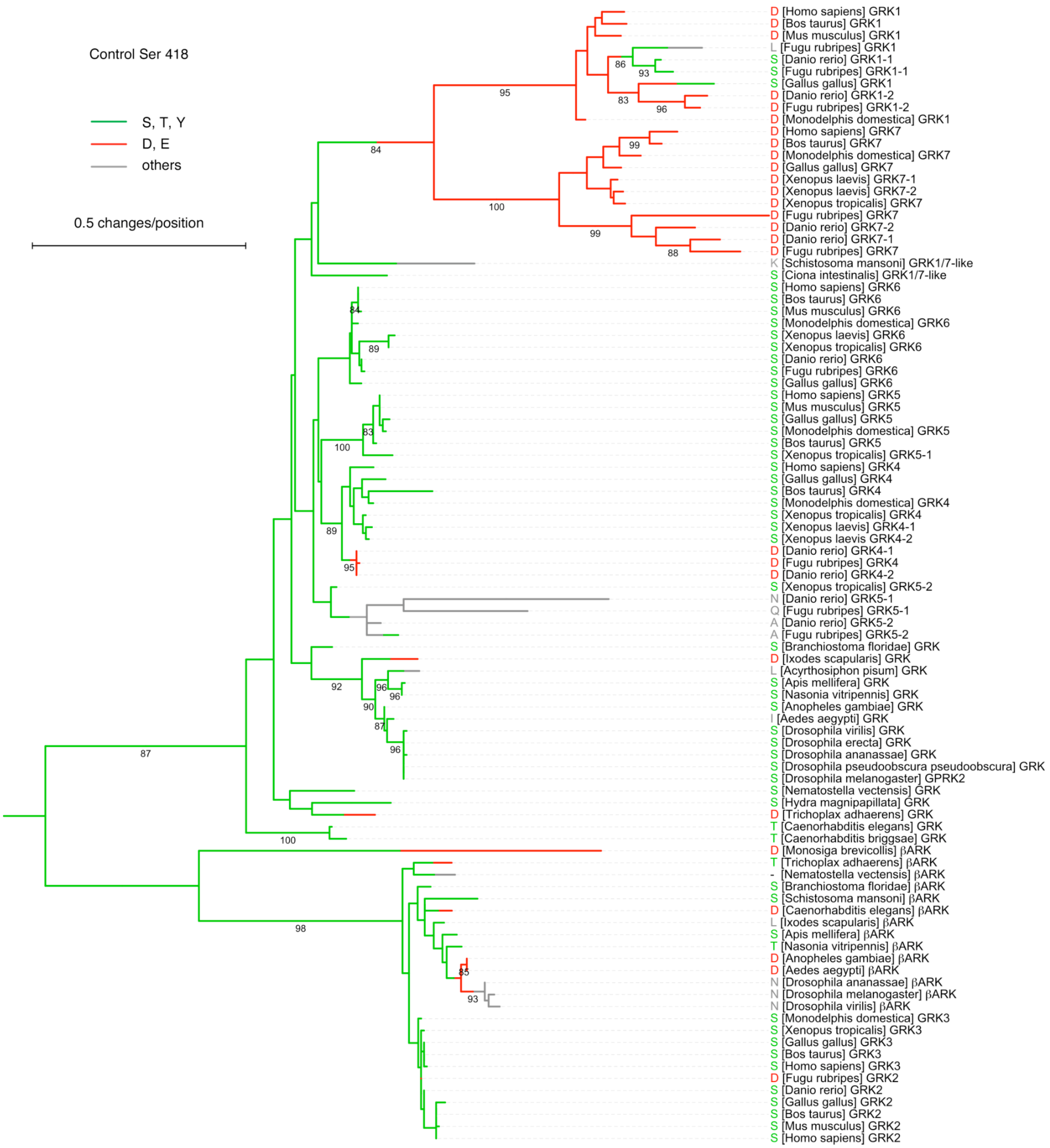

Table S4G. Detailed Phylogenetic Tree for Control Serine 418 in GRK5

Table S4H. Detailed Phylogenetic Tree for the CMGC Family of Protein Kinases

Because a full species tree for the CMGC family of kinases would be too large to display, we have restricted the tree shown here to CMGC kinases from four species: *Homo sapiens, Saccharomyces cerevisiae, Arabidopsis thaliana,* and *Monosiga brevicollis*. The tree is labeled with the species names, the identities of the residues corresponding to the TEY motif in ERK2, and the bootstrap values (when >50%). Some of the internal nodes are labeled with the inferred sequence of the hypothetical ancestral proteins to trace the evolution of the primordial TDY motif to the sequences present in the present-day ERK2 and CDK1 proteins.

Note that in GSK3 and some of its close relatives, the tyrosine phosphosite is hypothesized to be autophosphorylated co-translationally and then to act as a structural phosphate, resistant to dephosphorylation (Cole et al., 2004). Thus, it is possible that a primordial pY that acted as a permanent negative charge (persisting today in GSK3) evolved into both the Glu-containing kinases like CDK1, which have a different permanent negative charge in this position, and the kinases like ERK2, where the tyrosine phosphate turns over and acts as a conditional negative charge.

Supplemental Reference

Cole, A., Frame, S., and Cohen, P. (2004). Further evidence that the tyrosine phosphorylation of glycogen synthase kinase-3 (GSK3) in mammalian cells is an autophosphorylation event. Biochem J *377*, 249-255.