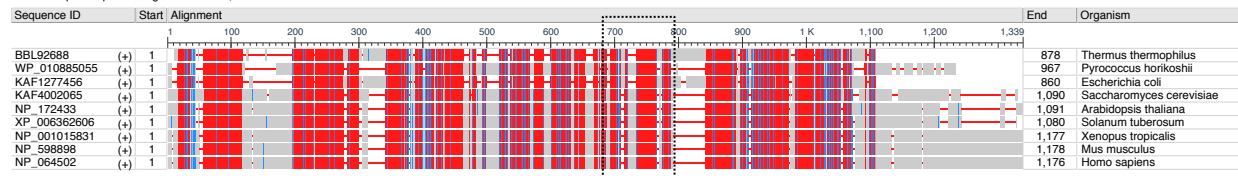


A

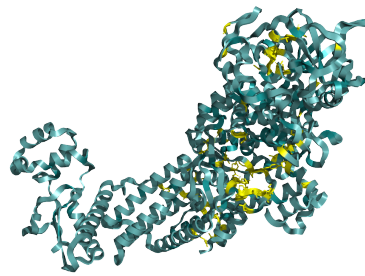
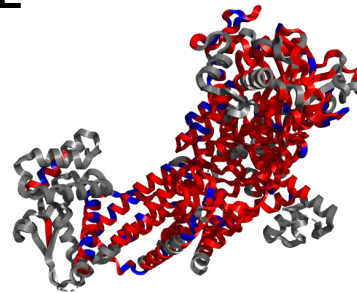
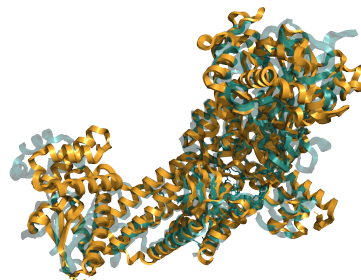
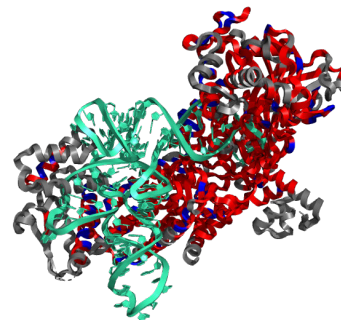
NCBI Multiple Sequence Alignment Viewer, Version 1.16.1

**B**

Thermus thermophilus 488 GPKAKRDTDMQ-----TFDSEWYLYRTPD[6]DPEKA-----SAMQPVQYIGGVHAVLHLLYSR 550
Pyrococcus horikoshii 546 N--KLRQKGLD---PEKLTPEFFOYIPLRF[6]LEKKTgIPARIHEMKKEFFYVYVPLNRCRSGKDLIPNHLTFPI 622
Escherichia coli 480 GMPALRETDTFD---TFMSEWYLYRTPCE[6]DSEKA-----NYWLPVDIYIGGIEHAIMHLLYFR 542
Saccharomyces cerevisiae 627 F-KDYDNEIGP1GISADQNTDEVFOYIIPRQDQ VKNFM-IPLPALQKLRFEFFYVYVPLDVISIGKDLIPNHLTFPI 701
Arabidopsis thaliana 614 HDGDMYKSGSL--IRPQQNDEWGVLYFC-DGP YPKSGIIPSAVLSEMKQEFYVYVPLDLRVSGKDLIPNHLTFPI 687
Solanum tuberosum 617 QGDMYGNDSH--VPRQLTDEINWFLFC-NGP FPKNS+ISSSLKEMKQEFYVYVPLDLRVSGKDLIPNHLSPCI 690
Xenopus tropicalis 612 QGKLGQGGASPIIRPQNTKEWVYVYVFKKAP FFKYT-IGREKLEKQEFYVYVPLDLRVSGKDLIPNHLSPFI 687
Mus musculus 613 QGGDLNQAESP1IRPQNTKEWVYVYVFKDAP FFKYT-IFREKLDLQKQEFYVYVPLDLRVSGKDLIPNHLSPYI 688
Homo sapiens 611 QGGNLHQAESPIIRPQNTKEWVYVYVFKKAP FFKYT-IAKREKLDLQKQEFYVYVPLDLRVSGKDLIPNHLSPYL 686

551 FTFKFLHDLgmvVVEEFPQLFTQGNVlaTD[53]AVNSKSKNGVWVGPVKEGADJARITLFAAPPENNVWTE 679
 623 FNIVAIFRE----EHPKGLIANGVGLT--[2]QMSKSKGNVLANIDAIENGADVVALYINLAHSDDFDRKH 692
 543 FFKLMBDAgmvNSDEPAKQLCCQGNVLA-DA[44]SKMSKSKNGIDPQVVERGADTVLRFNMFASPADMTLEWQE 661
 702 YHVALFPK---KF--NPKIRANGHML-NN SKMSKSTGNMFLRQVVEFGADAARAFADAGDVEDANFSE 771
 688 YHVALMAN---NM--NPKIRANGHML-NS SKMSKSTGNMFLRQVVEFGADTFLADAGDVEDANFVE 757
 691 YHVTAMPK---HH--NPKIRANGHML-NS SKMSKSTGNMFLRQVVEFGADTFLADAGDVEDANFVE 760
 688 YHVTAMPK---DSEKMPVAVRANGHLL-NS SKMSKSTGNMFLRQVVEFGADTFLADAGDVEDANFVE 759
 689 YHVTAMPK---QSDKMPVAVRANGHLL-NS SKMSKSTGNMFLRQVVEFGADTFLADAGDVEDANFVE 760
 687 YHVTAMPK---QSDKMPVAVRANGHLL-NS SKMSKSTGNMFLRQVVEFGADTFLADAGDVEDANFVE 758

680 GVGA----KRFKRIYRVA[6]ETS9VQAALGKORELYGKLTAKVTDLEALR-FYNTAIALMFLNALYE 757
 693 EVG----KLRKQIERFYLISQ[1]AEY+VKGNVELKIDRNMALRLNAIKETTNALERF-TRTAVQAFYSIMDLR 765
 662 GVGA----NRFKRWKLYE[4]GDVAALNVQALYDQKALRDLVHTIAKVTDDIGRQLFYNTAIALMFLNALYK 738
 772 NANAAILRFLKEMWAE-FTK ESN-LRTGELTFDPIAEHEMNALEKTEQYALTN-YKNALYGLFDPQAARD 845
 758 TANAAILRFLKEMWAEVLDV ESN-LRTGEPSTYADKVFEMHNALEKTERAYDCL-FKREALEKGFVDPQAARD 832
 761 TANAAILRFLKEMWAEVLSA EFS-LRNGPSTYADRVFANINIAVTAENYSETH-FKREALEKGFVDPQAARD 835
 760 MADAGILRELYTWVVKEMLAN FDS-LRSGTSHYNDRVFASINAGIKVTKQNTKEM-FKREALEKGFVDPQAARD 834
 761 MADAGILRELYTWVVKEMLAS CSS-LRSGFADSNDRVFASEMAGIKVTKQNTKEM-FKREALEKGFVDPQAARD 835
 759 MADAGILRELYTWVVKEMVAN WDS-LRSGASTYNDRVFASEMAGIKVTKQNTKEM-FKREALEKGFVDPQAARD 833

C**E****D****F**

Supplemental Figure S5. LeuRS protein alignments and conservation analysis. (A,B) LeuRS protein alignments, performed similarly to the ValRS alignments (Fig. S2A-B), from nine species indicated in the figure with a zoom-in of the sequence alignment corresponding to the boxed region in panel A. (C) The structural model of *P. horikoshii* LeuRS (teal) with conserved residue positions by identity across all species highlighted yellow. (D) Protein structure alignment of LeuRS from *P. horikoshii* (teal) with a model of LeuRS from *S. tuberosum* generated using Phyre2 (Kelley et al. 2015) (orange). (E,F) The Phyre2 *S. tuberosum* structural model colored to match the conservation alignment from panel A in the absence (E) and presence (F) of tRNA^{val} (teal).