Fig. S1 Phylogenetic analysis of archaeal and eukaryotic EF-1A, and bacterial EF-Tu.
The maximum likelihood phylogenetic trees were inferred based on best-fitting empirical (LG+F+I+G4) and mixture (Q.pfam+C40+F+R6) models, respectively. Both the trees are rooted on the bacterial EF-Tu, and bootstrap values are shown for some nodes.

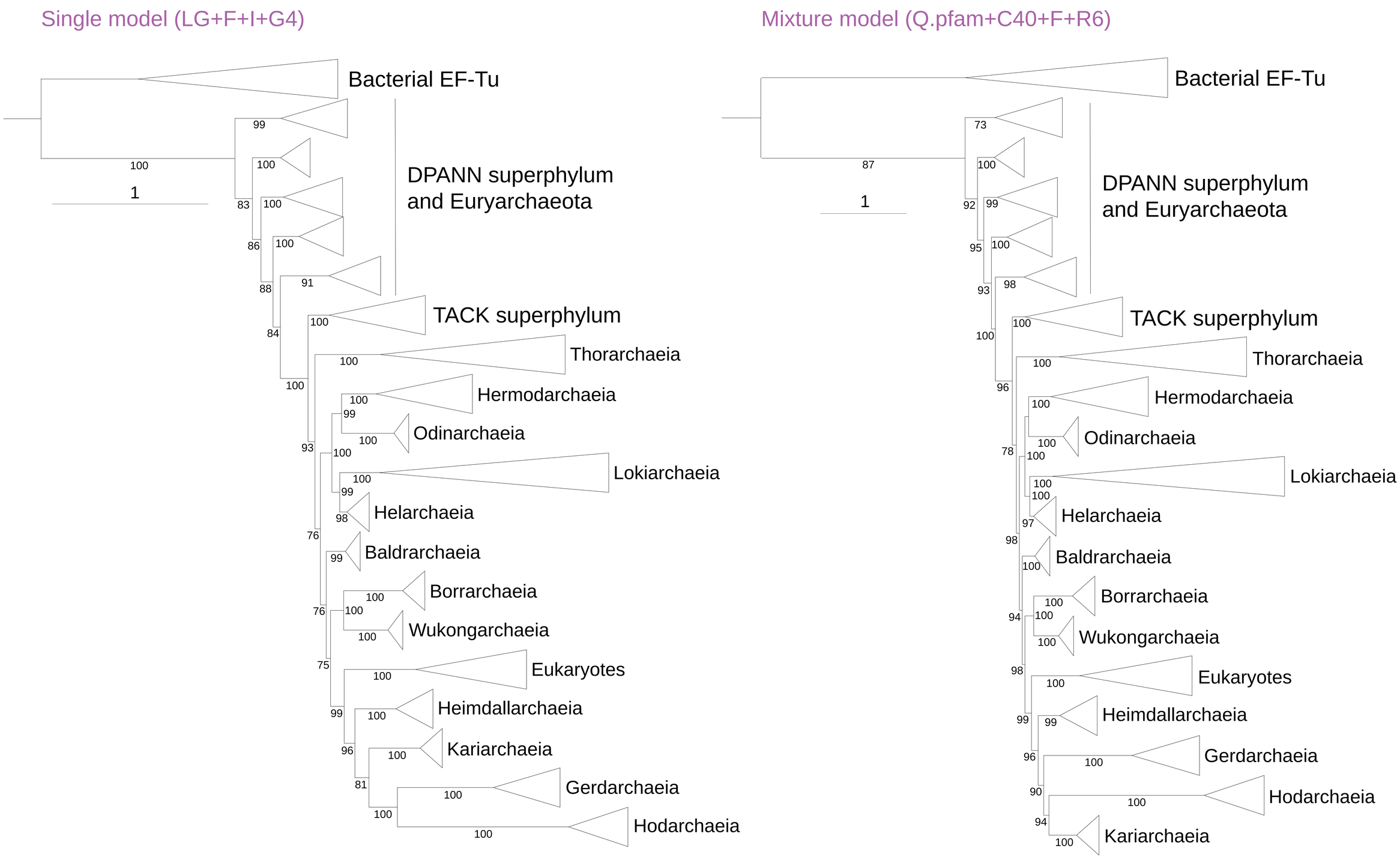

Fig. S2 Maximum likelihood phylogeny of archaeal and eukaryotic EF-1A, and bacterial EF-Tu.

The tree was reconstructed by maximum likelihood analysis using 195 amino acid sequences based on best-fitting model LG+F+I+G4 model, with option "-bb 1000", using IQ-tree. The tree is rooted on bacterial EF-Tu, and the bootstrap values were shown on nodes.

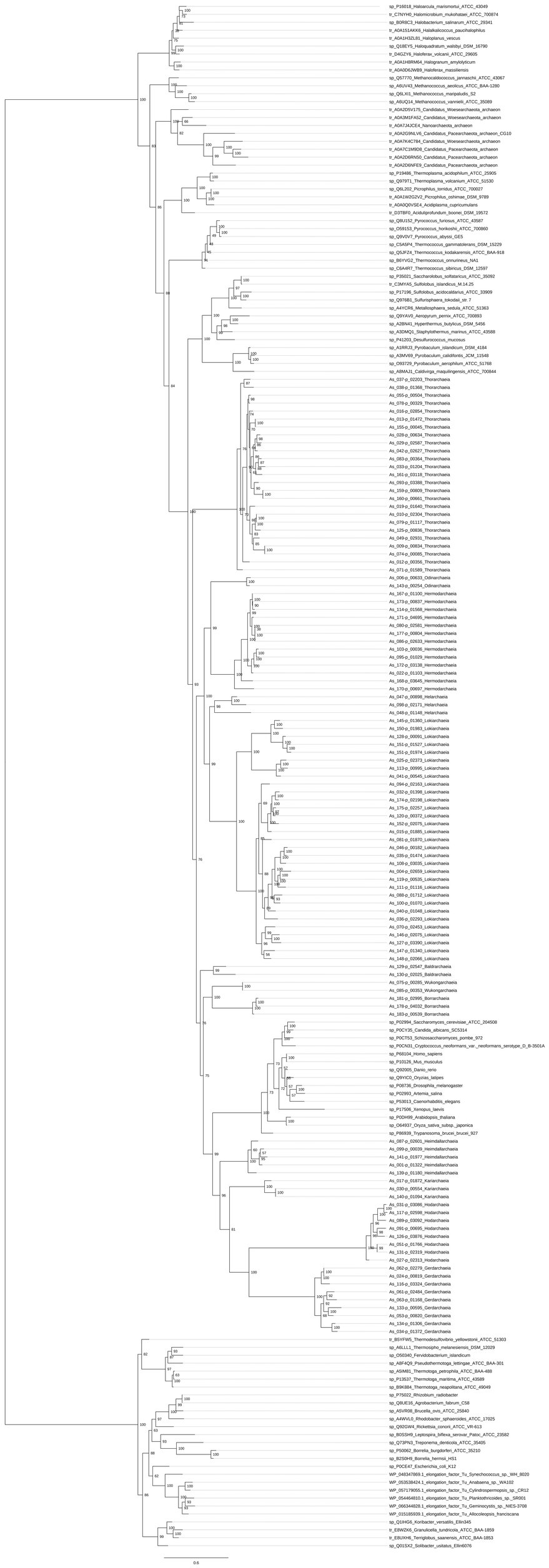

Fig. S3 Thermal stability range of the EF-1A/Tu and ancestral EF-1A.
The highest fluorescence intensity (a. u., arbitrary units) reflects that substantial protein unfolding occurred. Source data are provided as a Source Data file.

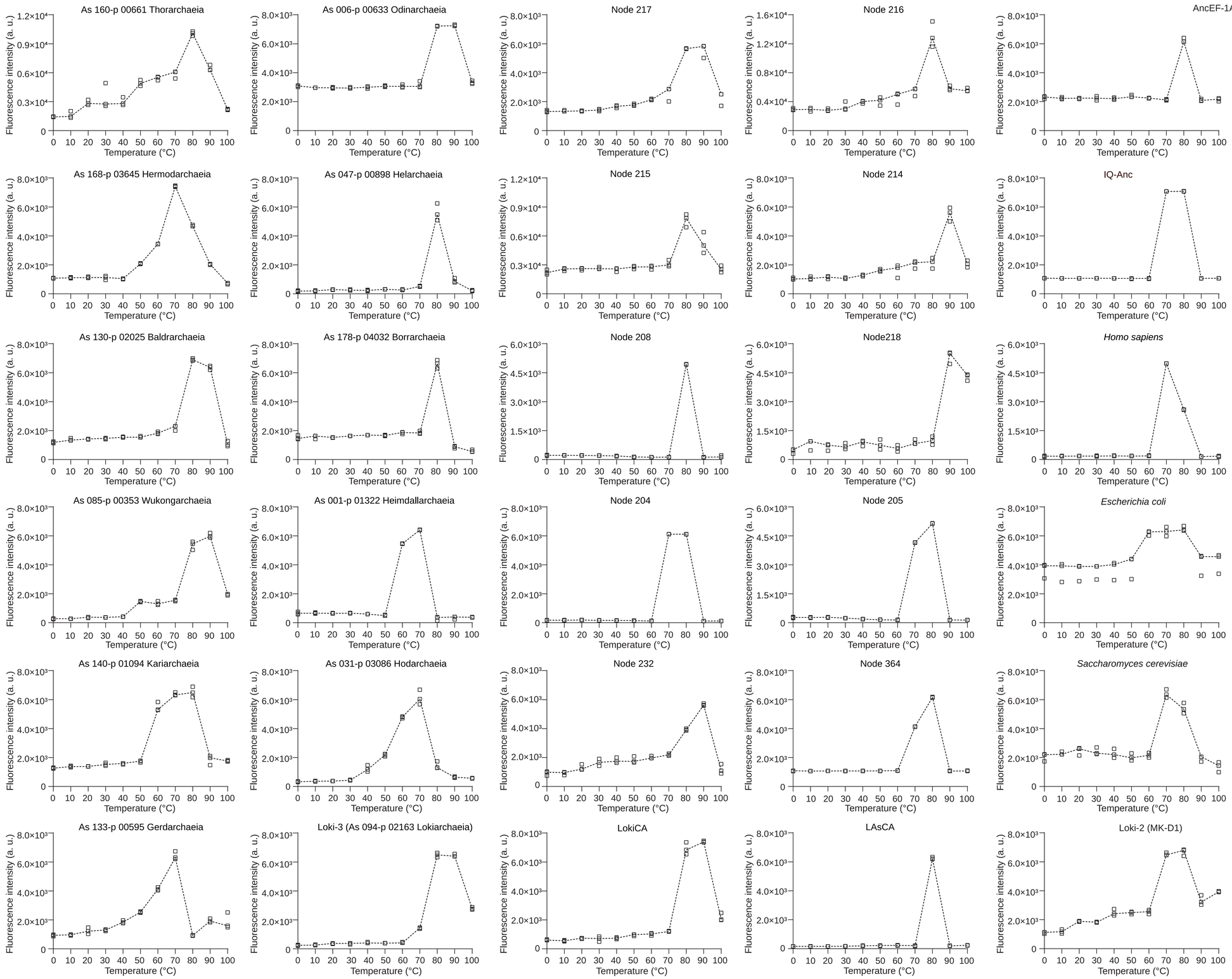

Fig. S4 Statistical analysis of the optimal GDP-binding temperature of the EF-1A/Tu and ancestral EF-1A. The testing EF-1A/Tu and ancestral EF-1A proteins were labeled with color according to their optimal temperature for GDP binding. Source data are provided as a Source Data file.

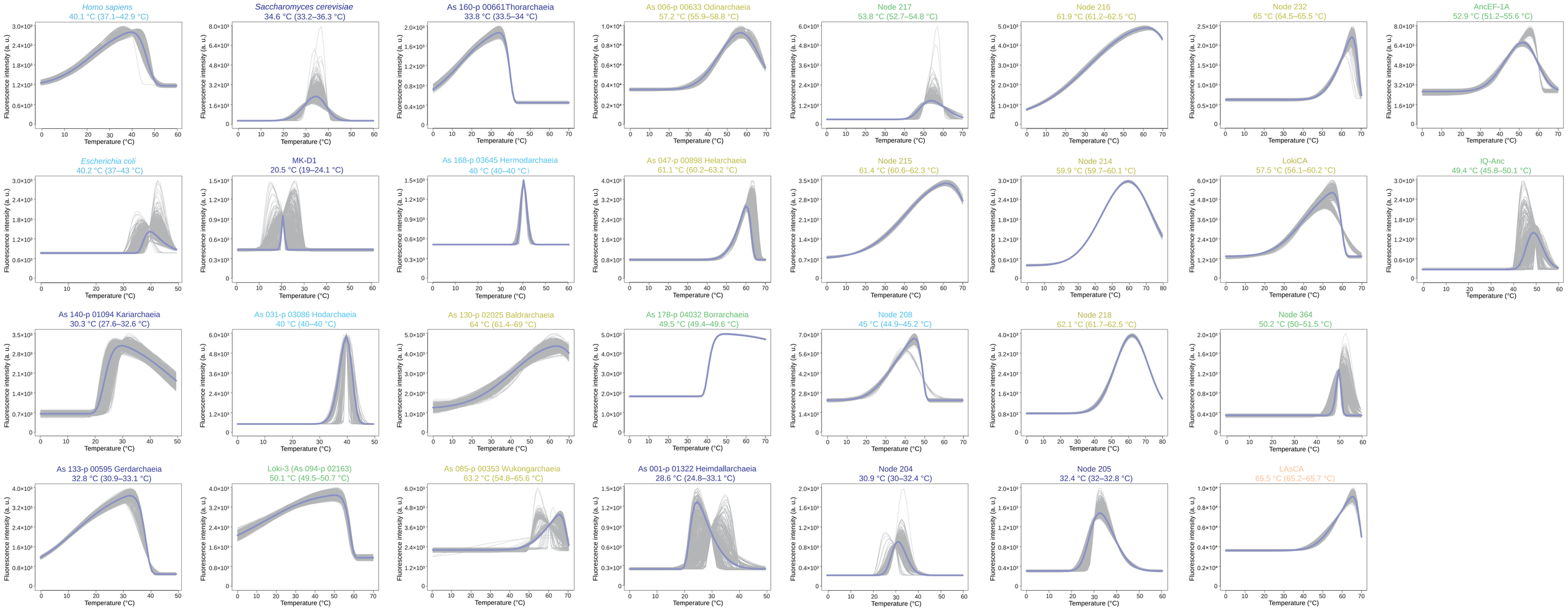

Fig. S5 Inferring evolutionary trajectory of optimal growth temperature of Asgard archaea based on the optimal GDP-binding temperature of EF-1A proteins. (a) Evolutionary of the Asgard and eukaryotic EF-1A proteins. The tree is a schematic representation according to maximum likelihood phylogeny of Asgard and eukaryotic EF-1A in Fig. S2. The testing EF-1A proteins were labeled with color according to their optimal temperature for GDP binding. Divergent evolution of Lokiarchaeia subgroup Loki-2 (blue) and Loki-3 (green) was highlighted. MK-D1 refers to Candidatus 'Prometheoarchaeum syntrophicum' strain MK-D1. (b) Evaluation of optimal GDP-binding temperature of the EF-1A/Tu and ancestral EF-1A proteins used in this study. Source data are provided as a Source Data file.

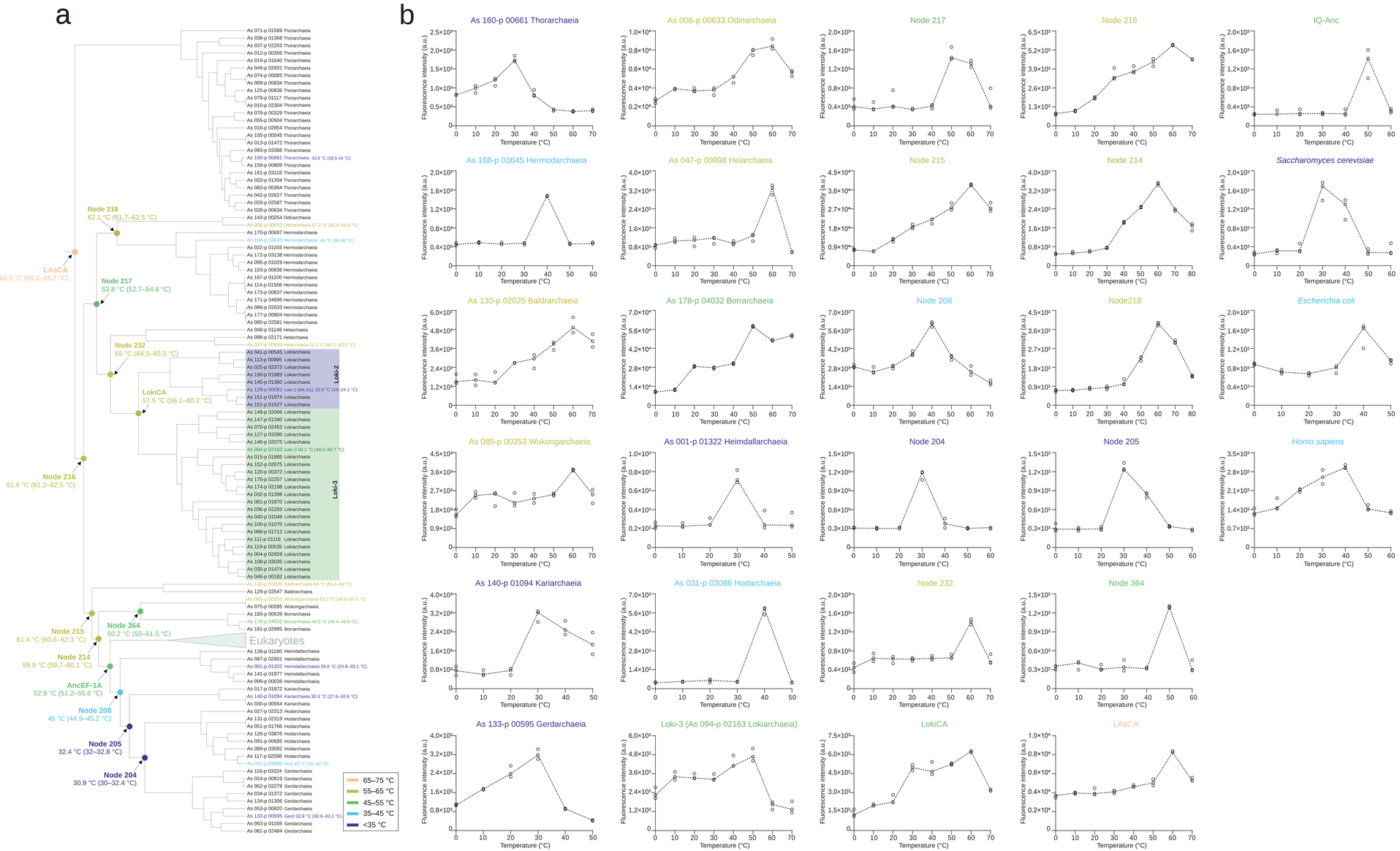

Fig. S6 Alignment of amino acid sequences of Asgard EF-1A. To trim the amino acid sequences of Asgard EF-1A, the N- and C-terminal residues (labeled in red) were removed. The average values of amino acid sequence similarity of the trimmed EF-1A within Asgardarchaeota phylum and Lokiarchaeia subgroups, and between the Lokiarchaeia subgroups were labeled in blue and red, respectively. The canonical EF-1A domain (PRK12317) was labeled.

Fig. S7 Correlation analysis between GDP-binding temperature and genomic-based inferred Asgard OGTs.
(a) Correlation analysis between GDP-binding temperature and our inferred Asgard OGTs. (b) Correlation analysis between GDP-binding temperature and the Asgard OGTs predicted by reference 4. Both panels a and b were subject to t-test, and the p value was less than 0.0001, which met the conditions for correlation analysis. Source data are provided as a Source Data file

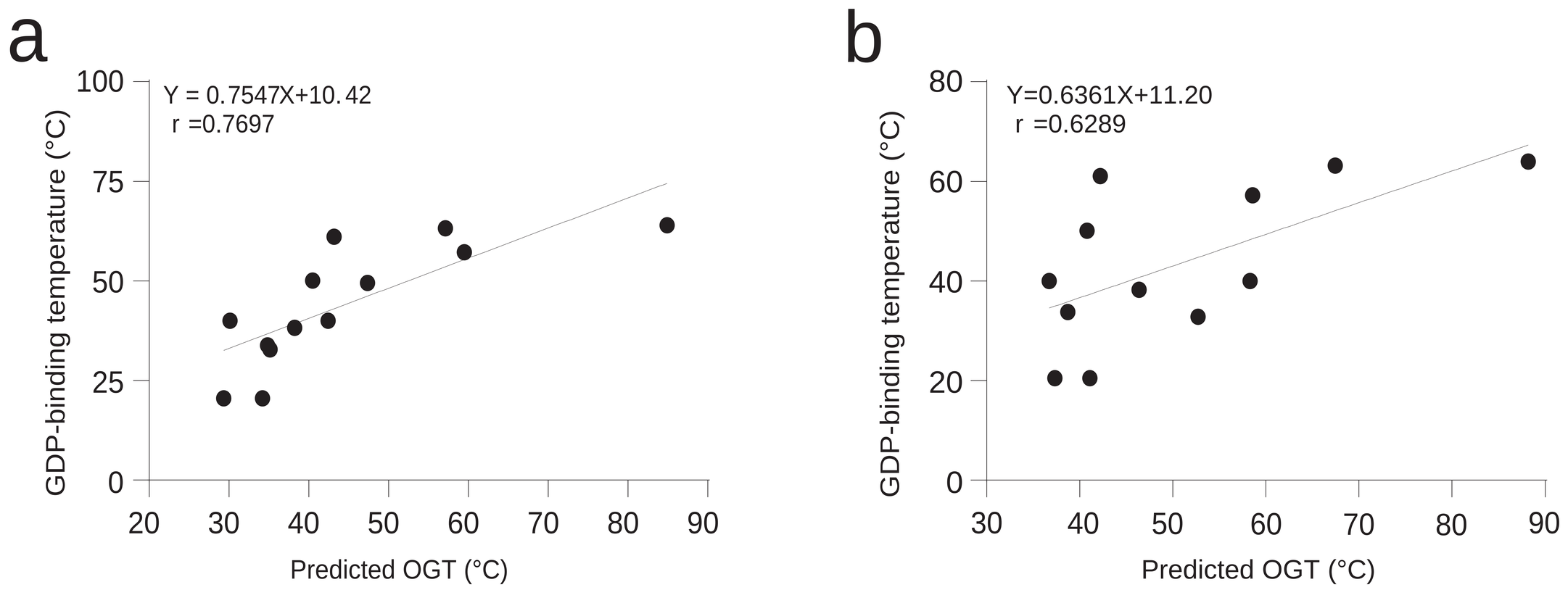

Fig. S8 Amino acid sequences of ancestral EF-1A proteins used here. The Posterior probabilities (PP) for amino acid states of the ancestral EF-1A are labeled.

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Loki CA   : MAKDKPHLNLVIIGHIDHGKSTMVGHLLIKTGAVSDREARELEKLAQELDRESWKYAYVFDRLKEERQRGITIDLAFRKFETKNYYFTIIDAPGHADFVKNMITGASQADAAILVVSGKKGEFEAGIS-----------ANGQTREHAFLAKTLGVKQLVVAINKMD--TWNYSEDRYNEVKEEVSDLLKNV-- : 179
LAs CA    : --MSKPHLNLVIIGHVDHGKSTMVGRLLYETGAIDERTIRKFEEEAEKLGKESFKFAWILDRLKEERERGLTIDLAFRKFETKKYYFTIIDAPGHRDFIKNMITGASQADAAILVVSAKKGEFEAGIG-----------PGGQTREHAFLARTLGVNQLIVAVNKMDDSTVNYSQERYNEIKEEVSDFLKSI-- : 179
Node205   : MSKEKPHLNLVIIGHVDHGKSTMTGHLLYLCGAIDERTIEKYAKESEKMGKGSWKYAWVLDKLKEERERGLTIDIAFFKFETKKYYFTIIDAPGHRDFVKNMITGTSQADAAILVVSAEKGEFEAGIS-----------AEGQTREHALLAKTLGVNQLIVAVNKMDDPTVDWSQERYNEIKEEVSGRLKQF-- : 181
Node208   : MSKEKPHLNLVIIGHVDHGKSTMTGHLLYLCGAIDERTIEKFEKEAEKMGKGSFKYAWVLDKLKEERERGLTIDLAFFKFETKKYYFTIIDAPGHRDFVKNMITGTSQADAAILVVSAEKGEFEAGIS-----------AEGQTREHALLAKTLGVNQLIVAVNKMDDPTVDWSQERYNEIKEEVSGRLKQF-- : 181
Node214   : MSKEKPHLNLVIIGHVDHGKSTLMGHLLYLTGAIDERTIRKFEEEAEKLGKGSFKYAWVLDKLKEERERGLTIDLAFWKFETKKYYFTIIDAPGHRDFVKNMITGASQADAAILVVSAKKGEFEAGIG-----------PGGQTREHAFLAKTLGVNQLIVAVNKMDDPTVNWSQERYNEIKEEVSGLLKQI-- : 181
Node215   : MSKEKPHLNLVIIGHVDHGKSTLMGHLLYLTGAIDERTIRKFEEEAEKLGKGSFKYAWVLDKLKEERERGLTIDLAFWKFETKKYYFTIIDAPGHRDFVKNMITGASQADAAILVVSAKKGEFEAGIG-----------AGGQTREHAFLAKTLGVNQLIVAVNKMDDPTVNWSQERYNEIKEEVSGLLKQI-- : 181
Node216   : MSKEKPHLNLVIIGHVDHGKSTLMGHLLYLTGAIDERTIRKLEEEAEKLGKGSFKYAWVLDRLKEERERGLTIDLAFRKFETKKYYFTIIDAPGHRDFVKNMITGASQADAAILVVSAKKGEFEAGIG-----------AGGQTREHAFLARTLGVNQLIVAVNKMDDPTVNWSQERYNEIKEEVSDLLKQI-- : 181
Node364   : MSKEKPHLNLVIIGHVDHGKSTLMGHLLYLTGAIDERTIRKFEEEAEKLGKGSFKYAWVLDKLKEERERGLTIDLAFWKFETKKYYFTIIDAPGHRDFVKNMITGTSQADAAILVVSAKKGEFEAGIG-----------KGGQTVEHAVLAATLGVNQFIVAVNKMDDPTVNWSQDRYNEIKSNVDKLIKER-- : 181
Node217   : MSEKKPHLNLVIIGHVDHGKSTLMGHLLYLTGAISDREMRKLEKMAQKLDKGSFKYAFVLDRLKEERERGLTIDLAFRKFETKKYYFTIIDAPGHRDFVKNMITGASQADAAILVVSAKKGEFEAGIS-----------AGGQTREHAFLARTLGVNQLIVAVNKMDDPTVNWSQERYNEIKEEVSDLLKQV-- : 181
Node218   : MSEKKPHLNLVIIGHVDHGKSTLMGHLLYKTGAISDREMRKLEKLAQKLDKGSFKYAFVMDRLKEERERGLTIDLAFRKFETQKYYFTIIDAPGHRDFVKNMITGASQADAAILVVSARKGEFEAGIS-----------AGGQTREHAFLARTLGVNQLIVAVNKMDDSTVNWSQERYNEIKEEVSGLLEGK-- : 181
Node232   : MSEKKPHLNLVIIGHVDHGKSTMVGHLLILTGAISDREMRELEKMAQKLDKGSFKYAFVFDRLKEERERGLTIDLAFRKFETKKYYFTIIDAPGHRDFVKNMITGASQADAAILVVSAKKGEFEAGIS-----------AGGQTREHAFLARTLGVNQLIVAVNKMDDPTVNWSQERYNEIKEEVSDLLKQV-- : 181
Node204   : MSKEKPHLNLVIIGHVDHGKSTMTGHLLFLCGAIDERTIEKYAKESEKMGKGSWKYAWVLDKLKEERERGLTIDISFFKFETKKYVFTIIDAPGHRDFVKNMITGASQADAAILVVSAEKGEFEAGISAERGTPKGDWRIPGGQTREHALLAKTLGVNQLIVAVNKMD--TVDWSQERYNEIKEEVSALLKQFVE : 193

Loki CA   : --GYPVE----KIPFVPVSALTGDNLTEKSTTLPWYDGPTLIEALDQFELPEKPTDKPLRLPIQDVYKIKGAGVVPVGRVETGVLKVGDKVIIMPTGY-----FGEVRSIEMHHEEMPQAEPGDNIGFNIRGITMKDVHRGDVVGHA-DNPPTVVTPNGNFTGQIIVIW----------H-PTAIAQGYTPVVHA : 351
LAs CA    : --GYKVD----KIPFIPVSAWTGDNLTEKSPNMPWYDGPTLLEALDTFEVPPKPIDKPLRIPIQDVYSITGVGTVPVGRVETGVLKVGDKVIFMPPGV-----TGEVKSIEMHHEQMPQAEPGDNIGFNVRGIEKKDIKRGDVAGHV-DNPPTVAE---EFTGQIIVIY----------H-PTAIAVGYTPVIHA : 348
Node205   : --GYDLD----KVPFVPVSGMTGDNLTDKSVTLPWYDGPTLLEALDTFEVPPKPTDKPLRLPIQDVYSITGIGTVPVGRVETGVLKPGDKVIFAPSGG-----KAEVKSIEMHHEQLPQAEPGDNIGFNVKGIAKKDIRRGDVVGHPGDNPPKVAK---TFTATVIVIK----------G-PTAIAAGYTPVIHC : 351
Node208   : --GYDLD----KVPFVPVSGMTGDNLTEKSVTVPWYDGPTLLEALDTFEVPPKPIDKPLRLPIQDVYSITGIGTVPVGRVETGVLKPGDKVIFAPSGY-----KAEVKSIEMHHEQLPQAEPGDNIGFNVKGISKKDIRRGDVVGHPGDNPPTVAK---EFTATVIVIK----------G-PTAIAAGYTPVIHC : 351
Node214   : --GYDLD----KVPFVPVSAWTGDNLTEKSVTVPWYDGPTLLEALDTFEVPPKPIDKPLRIPIQDVYSITGVGTVPVGRVETGVLKVGDKVIFMPSGL-----TGEVKSIEMHHEQLQQAEPGDNIGFNVKGISKKDIRRGDVAGHPGDNPPTVAK---EFTGQIIVIY----------H-PTAIAAGYTPVIHA : 351
Node215   : --GYDLD----KVPFVPVSAWTGDNLTEKSVTVPWYDGPTLLEALDTFEVPPKPIDKPLRIPIQDVYSITGVGTVPVGRVETGVLKVGDKVIFMPPGL-----TGEVKSIEMHHEQLQQAEPGDNIGFNVKGISKKDIRRGDVAGHP-DNPPTVAK---EFTGQIIVIY----------H-PTAIAAGYTPVIHA : 350
Node216   : --GYDVD----KVPFIPVSAWTGDNLTEKSVTVPWYDGPTLLEALDTFEVPPKPIDKPLRIPIQDVYSITGVGTVPVGRVETGVLKVGDKVIFMPPGL-----TGEVKSIEMHHEQLQQAEPGDNIGFNVRGISKKDIKRGDVAGHV-DNPPTVAE---EFTGQIIVIY----------H-PTAIAAGYTPVIHA : 350
Node364   : --ELDKK----KVPYVPVSAWTGDNLTEKSVTVPWYDGPTLLEALDTFEVPPKPIDKPLRIPIQDVYSITGVGTVPVGRVETGVLKVGDKVIFMPSGG-----KGEVKSIEMHHEQLQKAEPGDNIGFNVKGISKKDIRRGDVAGHP-DNPPTVAK---EFTGQLFVIY----------HPPTAIAAGYTPVIHA : 351
Node217   : --GFPVD----KVPFIPVSAWTGDNLTEKSTTMPWYDGPTLLEALDTFELPPKPIDKPLRIPIQDVYSITGVGTVPVGRVETGVLKVGDKVIFMPPGV-----TGEVKSIEMHHEQLQQAEPGDNIGFNVRGISKKDIKRGDVAGHV-DNPPTVVE---EFTGQIIVIY----------H-PTAIAAGYTPVIHA : 350
Node218   : --GYDVD----KIPFIPVSAWTGDNLTEPSVTVPWYKGPTLLEALDTFELPPKPVDKPLRIPIQDVYTITGVGTVPVGRVETGVLKVGDKVIFMPPGV-----TGEVKSIEMHHERLEQAEPGDNIGFNVRGIGKGDIKRGDVAGHV-DNPPTVVE---EFTGQIIVIH----------H-PTAIAAGYTPVIHA : 350
Node232   : --GFPVD----KVPFIPVSAWTGDNLTEKSVTVPWYDGPTLLEALDTFELPPKPVDKPLRIPIQDVYSITGVGTVPVGRVETGVLKVGDKVIFMPPGL-----TGEVRSIEMHHEQLQQAEPGDNIGFNIRGISKKDIKRGDVAGHV-DNPPTVVE---EFTGQIIVIY----------H-PTAIAPGYTPVIHA : 350
Node204   : GSGYRLDGKGLGVKFVPTSGMTGDNLTDKSDKMPWYDGPSLLEALDTFEVPEKPTDKPLRLPIQDVYSITGIGTVPVGRVESGVLKPGDKVIFAPSKYGIKPLTAEVKSIEMHHEQLPQAEPGDNIGFNVKGIAKKDILRGDVVGHPGDNPPKVAD---TFTATVIVIKGLGKPGKKANLPPTGIAAGYTPVIHC : 385

Loki CA   : HTAQVACKFVELSKKLDPKTGQVIEDNPQFLKKNDAAIVKLQPIKKLCLEKYKDFPELGRFAVRDMGRTVAVGVVKDIETG : 432  mean posterior probability (PP) =0.90
LAs CA    : HTAQVACRFTELISKLDPRTGQVVEENPDFIKQGDAAIVKLKPIRPMVIEKYSDFPQLGRFAVRDMGRTVAVGVVIDVKPK : 429  mean PP =0.92
Node205   : GTAQVACRFAEIKEKIDRRTGQVKEANPDFLKNGDRATVVMRPVRPMVVEKFSDFPQLGRFAVRDMGQTVAVGIVKDVQPK : 432  mean PP =0.88
Node208   : GTAQVACRFAELKSKIDRRTGQVKEANPDFLKNGDAATVVMVPTRPMVVEKYSDFPQLGRFAVRDMGQTVAVGIVKDVTPK : 432  mean PP =0.91
Node214   : HTAQVACRFAELKSKIDRRTGQVVEENPDFLKSGDAAIVKLVPTRPMVIEKYSDFPQLGRFAVRDMGRTVAVGIVKDVTPK : 432  mean PP =0.93
Node215   : HTAQVACRFAELISKIDRRTGQVVEENPDFLKQGDAAIVKFVPTRPMVIEKYSDFPQLGRFAVRDMGRTVAVGIVKDVTPK : 431  mean PP =0.94
Node216   : HTAQVACRFEELISKIDPRTGQVVEENPDFLKQGDAAIVKFKPIRPMVIEKYSDFPQLGRFAVRDMGRTVAVGVVKDVTPK : 431  mean PP =0.94
Node364   : HTAQVACTFAELQSKIDRRTGQVVEENPDYLKSGDGAIVKLVPTRPMVIEKYSDFPQLGRFAVRDMGRTVAVGIVKDVTPK : 432  mean PP =0.92
Node217   : HTAQVACKFEELISKIDPRTGQVIQEKPDFLKQGDAAIVKFKPIRPLVIEKYSDFPQLGRFAVRDMGRTVAVGVVKEVTPK : 431  mean PP =0.92
Node218   : HTAQVACKFTELISKIDPRTGQVIQEKPDFIKQGDAAIVKFKPIRPLVIEKYSDFPQLGRFAVRDMGRTVAVGVVKEVTPK : 431  mean PP =0.91
Node232   : HTAQVACKFEELIQKIDPRTGQVIQEKPDFLKQGDAAIVKFKPIRPLVIEKYSDFPQLGRFAVRDMGRTVAVGVVKEVKPK : 431  mean PP =0.92
Node204   : GTAQVACRFAIIKEKIDRRTGQVKEANPDFLKQGDRATVVMRPVRPMVVEKFSDFPQLGRFAVRDMGQTVAVGIVKDVQPK : 466  mean PP =0.81
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