

Figure S1. Distribution of thiamine biosynthesis pathways, transporters and regulators in Archaea.

The phylogenetic species tree was constructed using the concatenated alignment of 78 universal bacterial proteins. The tree includes the analyzed representatives of three archaeal lineages possessing *thiR* orthologs.

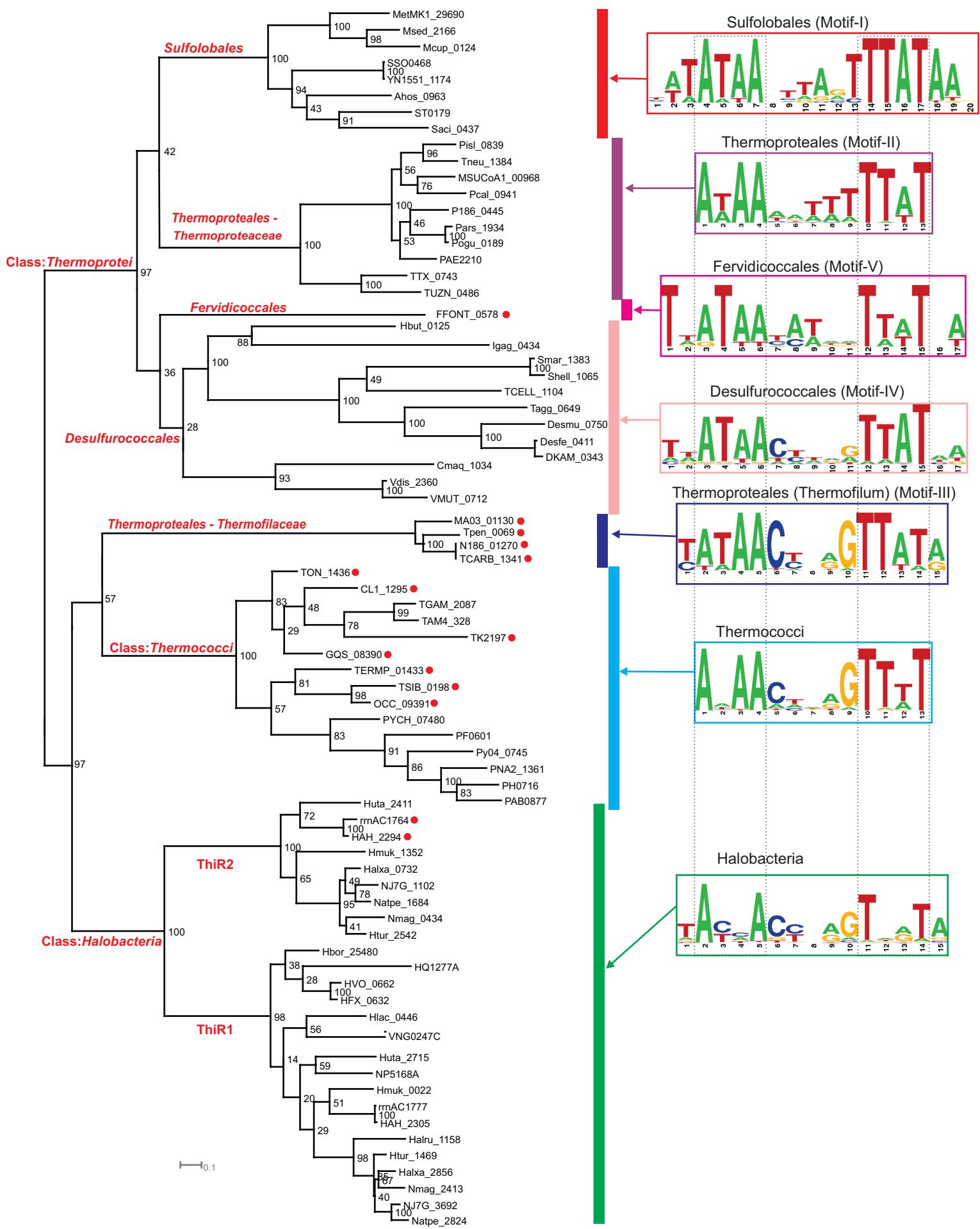


Figure S2. Phylogenetic tree of the ThiR proteins and their predicted lineage-specific DNA motifs.
Red dots show ThiRs that are predicted to be autoregulated, as the *thiR* genes belong to ThiR regulons.

DNA-binding domain, XRE family (COG2522)



Figure S4. Alignment of the thiamine regulators ThiR in Archaea. MK1_29690 denotes the characterized in this work ThiR protein from *Metallosphaera yellowstonensis*. Mk1. C-terminal domain in ThiR is aligned with thiamine phosphate synthase domain ThiN from *Pyrococcus furiosus*. (PDB ID 2PB9). Conserved residues in ThiR and ThiN proteins are marked with asterisk. Red asterisks indicate conserved residues that are presumably involved in thiamine phosphate binding, they were also bound phosphoric acid in 2PB9.

Table S3. ThiR-binding sites and their experimental validation in Metallosphaera yellowstonensis MK1.

First gene locus tag	ThiR regulated operon	Predicted ThiR-binding sites and synthetic DNA fragments used in fluorescence polarization DNA-binding assays ¹
MetMK1DRAFT_00023630	<i>thi4</i>	a ccccccggttggat <u>TATATAATCTAGTTTATAAAG</u> Gccact <u>a</u> cccccc
MetMK1DRAFT_00024350	<i>thiT</i>	a ccccccgttatgt <u>cTAAATAAGTAAGTTTATAAC</u> cgact <u>t</u> cccccc
MetMK1DRAFT_00019590	<i>tenA</i>	a ccccccataact <u>agATTATAAAATGATT</u> TATCccgcccacccccc
MetMK1DRAFT_00012900	<i>trxA(N.C.)</i> ²	a ggggaa <u>tataaacttttaaacggggg</u> g

¹ Predicted ThiR-binding sites are capitalized. DNA fragments from target gene upstream region are underlined.

Additional poly-cytosine oligonucleotides have been added on each side of DNA fragments to improve annealing of oligos.

For each DNA fragment, two complimentary single-stranded oligonucleotides were synthesized by IDT

at that one of these fragments (5'-3' shown here) was labeled by 6-carboxyfluorescein at 5' end (marked by red).

²NC, a negative control DNA fragments from promoter region of a gene that lack a ThiR-binding site.