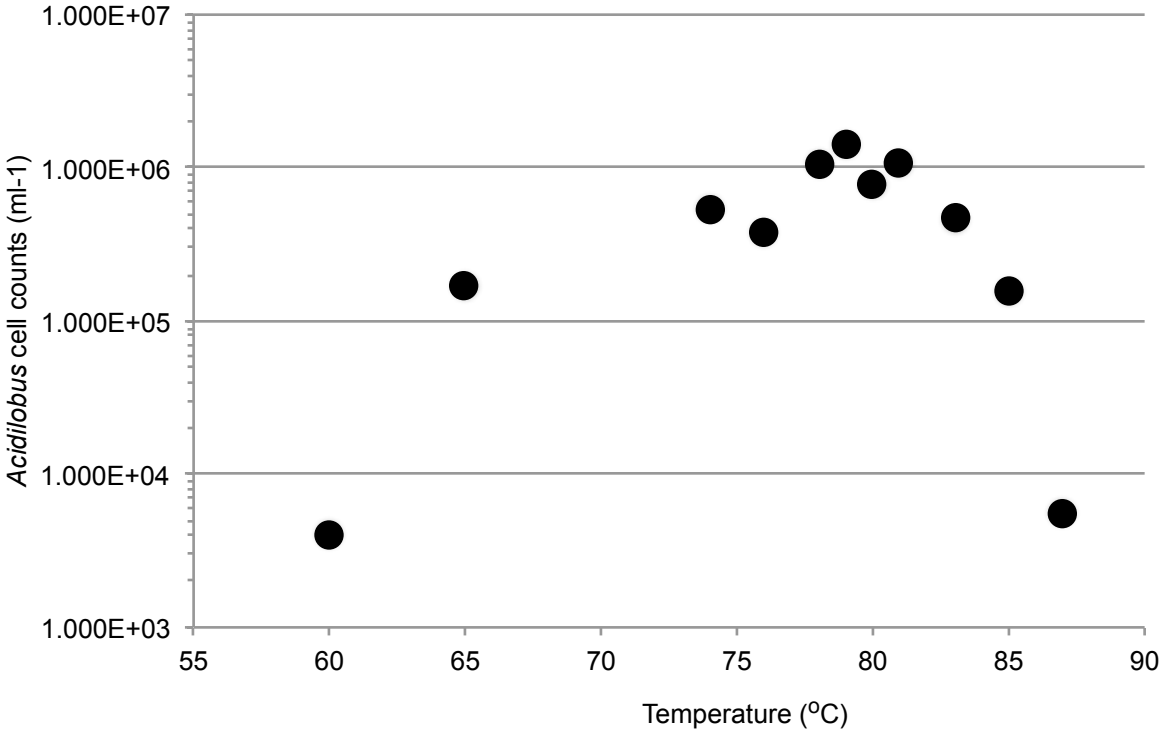
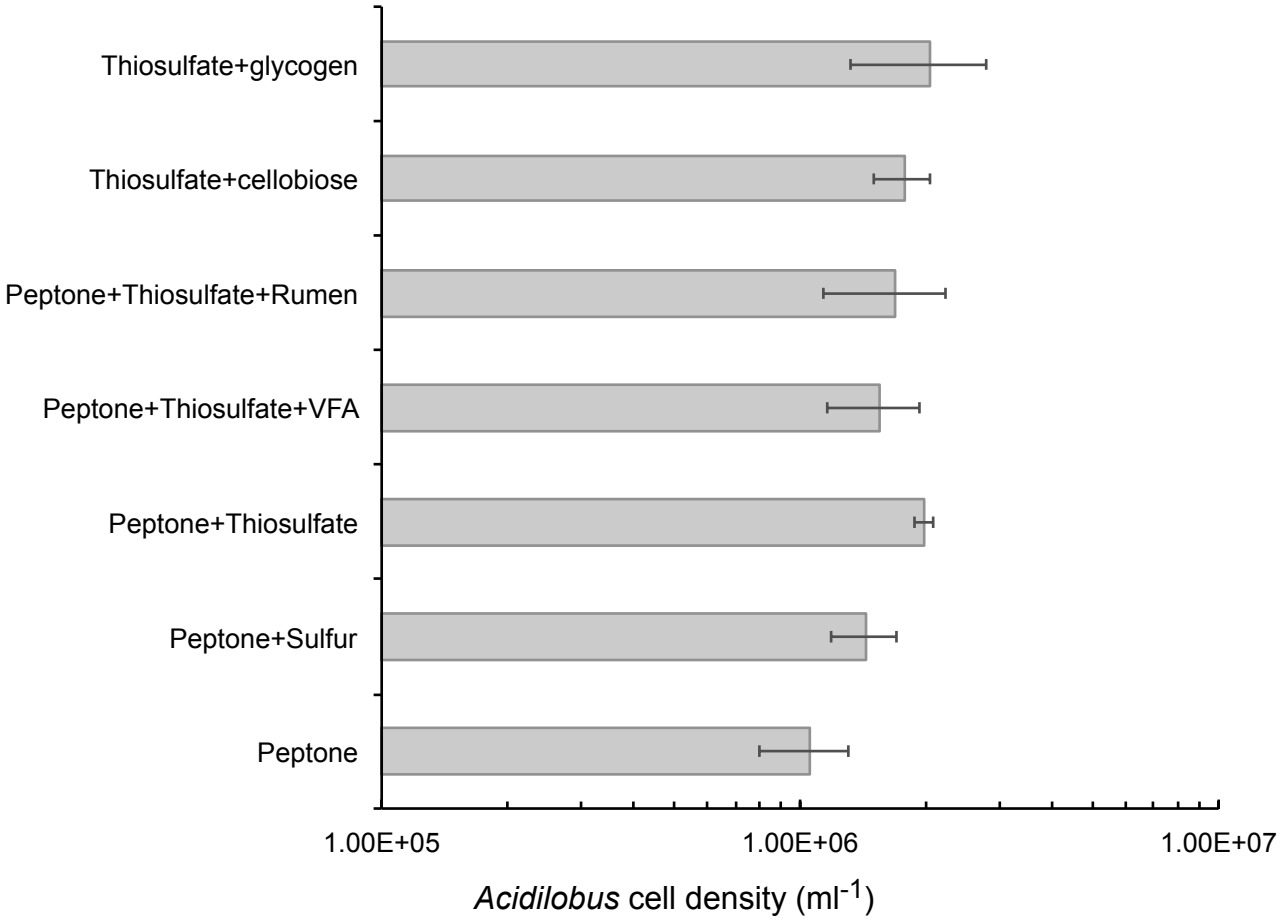
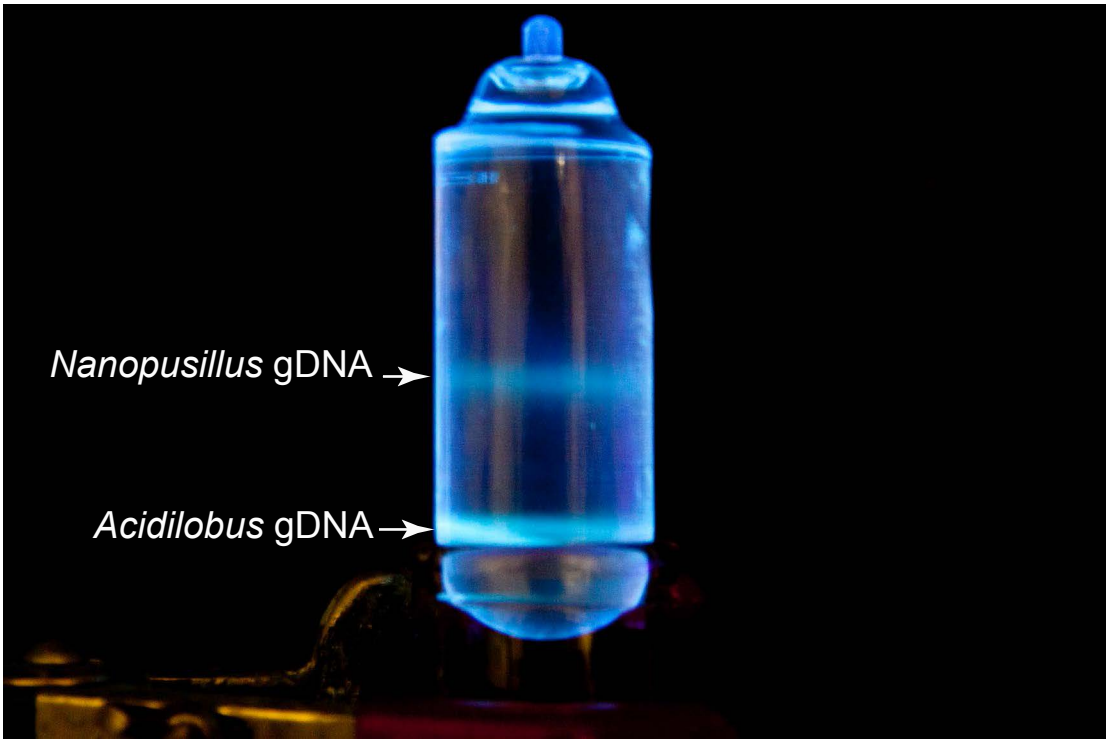


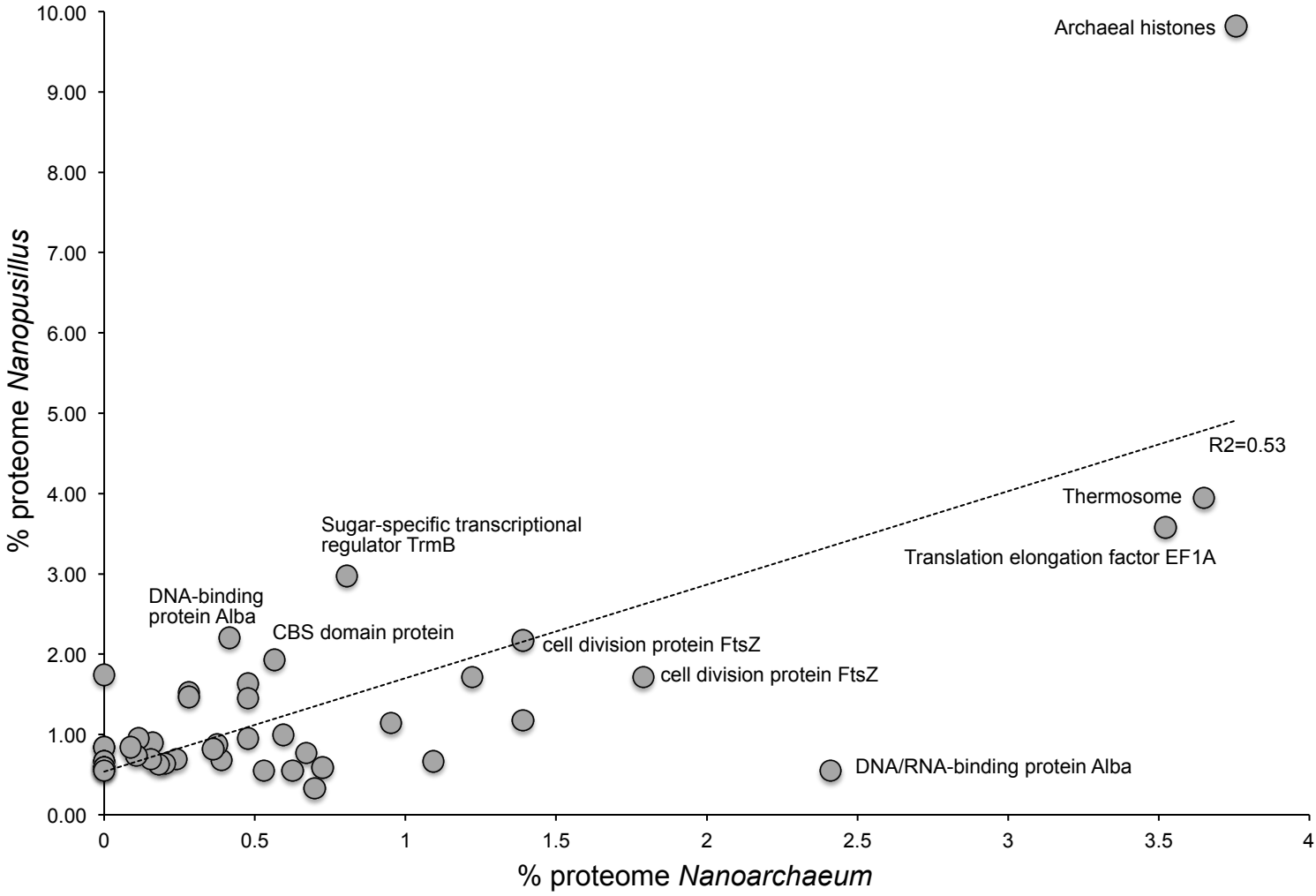
Supplementary Figure 1. Effect of temperature on *Acidilobus* sp.7A growth in co-culture with *N. acidilobi,* based on cell density measured after 3 days.



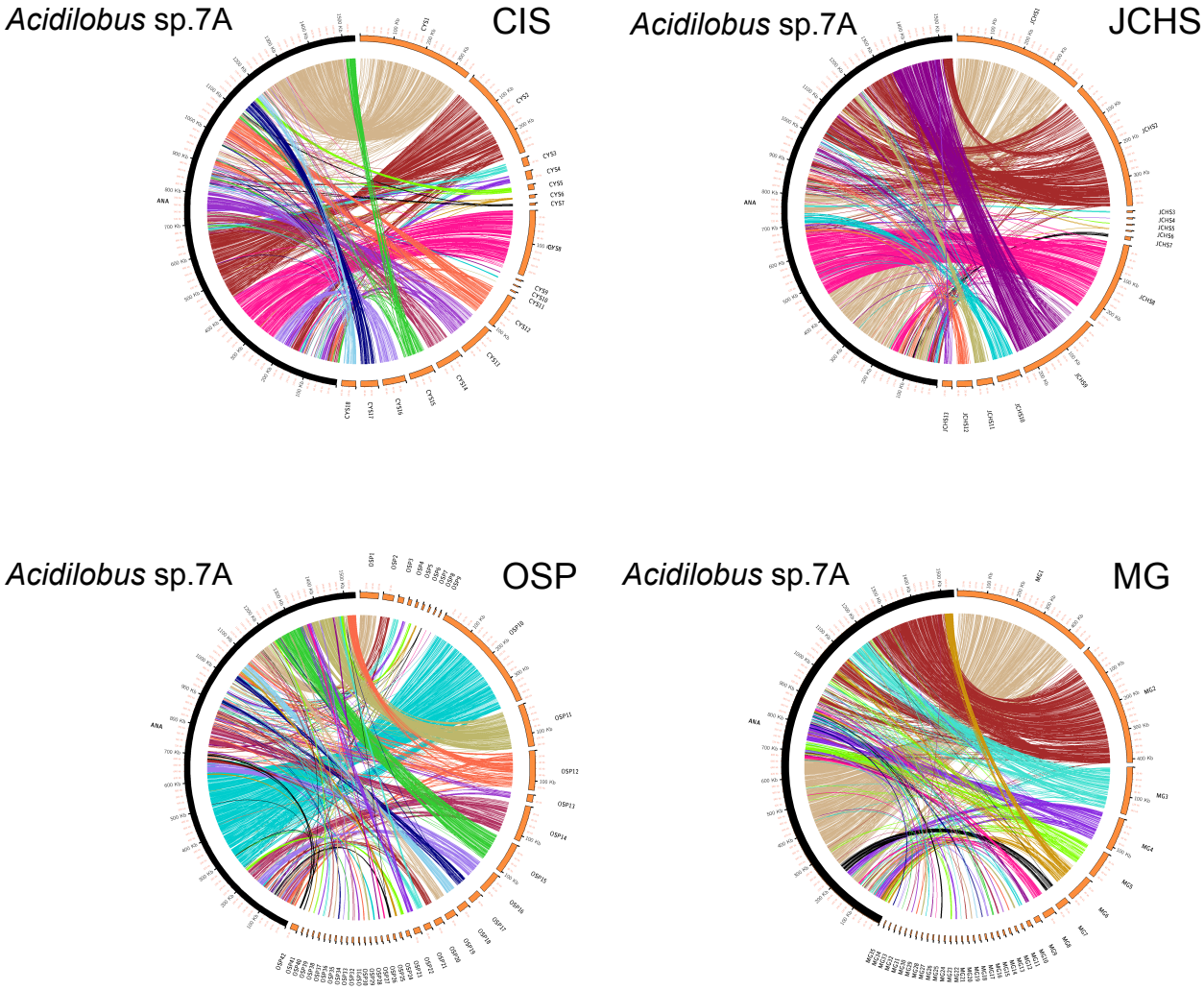
Supplementary Figure 2. Effect of variations in media composition on *Acidilobus* growth in co-culture with *N. acidilobi*, based on cell density measured after 3 days. Scale bars correspond to standard deviation based on 3 replicate cultures.



Supplementary Figure 3. Separation of *Acidilobus* and *Nanopusillus* gDNA based on equilibrium density ultracentrifugation in bisbenzimide-CsCl gradient



Supplementary Figure 4. Comparison of the top 50 relative most abundant proteins in *Nanopusillus acidilobi* versus *Nanoarchaeum equitans* (data from Giannone et al, 2011). The proteins indicated as 0% on the *Nanoarchaeum* axis are not encoded in the genome.



Supplementary Figure 5. Circos-based genome alignments between *Acidilobus* sp.7A and *Acidilobus*-like populations from Yellowstone (Jay et al 2014)

	N. equitans	Nst1	N. acidilobi	Note
Size	491kb	593kb (7 contigs)	606 kb	
G+C %	31.6%	24.3%	24.3%	
Split Proteins				
Reverse gyrase	NEQ 318-434	Nst 337-402	Nps 2030-2705	Same site
Glu-tRNA^{Gln} amidotransferase	NEQ 245-396	Nst 197-449	Nps 2280-3415	Same site
Predicted RNA-binding protein	NEQ 438-506	Nst 176-251	Nps 30-3140	Same site
Archaeosine tRNA-guanine transglycosylase	NEQ 124-305	Nst 096-232	Nps 1135-3235	Same site
RNA polymerase subunit B	NEQ 156-173	Nst 632-633	Nps 545-550	Same site
Large helicase-related protein	NEQ 003-409	Nst 172-239	Nps 745-3195	Different site
DNA polymerase I	NEQ 068-528	Nst 417	Nps 2105	Not split in *Nst1-Nac*
Topoisomerase I	NEQ 045-324	Nst 174	Nps 45	Not split in *Nst1-Nac*
P-loop ATPase-acetyltransferase fusion protein	NEQ 096-495	Nst 401	Nps 2025	Not split in *Nst1-Nac*
Alanyl-tRNA synthetase	NEQ 211-547	Nst 054	Nps 290	Not split in *Nst1-Nac*
Diphthamide synthase sub. DPH2	-	Nst 222-440	Nps 3285-2235	Absent in *N.eq.*
Uncharacterized conserved protein (arCOG04253)	-	Nst 474-480	Nps 2410	Absent in *N.eq.*
tRNAs				
cis-spliced tRNAs	Ile, Met, Trp, Tyr	Ile, Tyr	Ile, Tyr	
trans-joined tRNAs	iMet, His, Lys, Gln, Glu (2)	none	none	
RNase P	Absent	Present	Present	
Gluconeogenesis-Glycolysis	Absent	Present	Present	
Polyamine biosynthesis	Absent	Present	Present	
ATP synthase	Incomplete	Absent	Absent	
Glutamate dehydrogenase	Present	Absent	Absent	

Supplementary Table 1. Comparison of genomic features (split genes and metabolic features) among sequenced Nanoarchaeota.

Supplementary References

Giannone, R. J. et al. Life on the edge: functional genomic response of Ignicoccus hospitalis to the presence of Nanoarchaeum equitans. ISME J 9, 101-114 (2015).

Jay, Z. J. et al. Predominant Acidilobus-Like Populations from Geothermal Environments in Yellowstone National Park Exhibit Similar Metabolic Potential in Different Hypoxic Microbial Communities. Appl. Env. Microbiol. 80, 294-305 (2014).