Supplemental Material

for the manuscript:

Deletion Strains Reveal Metabolic Roles for Key Elemental Sulfur Responsive Proteins in *Pyrococcus furiosus*

Stephanie L. Bridger[1], Sonya M. Clarkson[1], Karen Stirrett[2,3], Megan B. DeBarry[2], Gina L. Lipscomb[2], Gerrit J. Schut[1], Janet Westpheling[2], Robert A. Scott[1] and Michael W. W. Adams[1]*

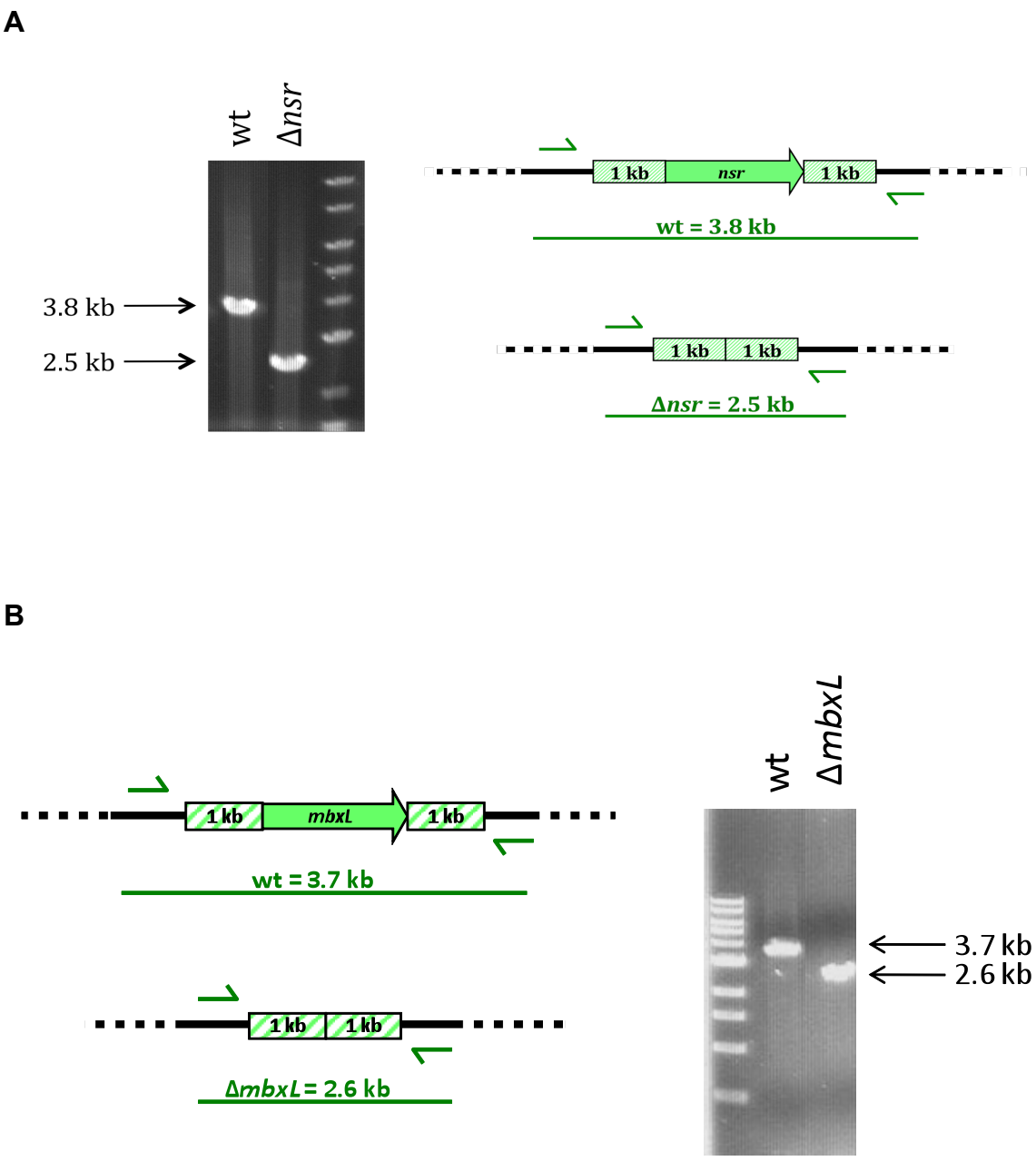

Figure S1. PCR confirmation of gene deletions *nsr* and *mbxL*. PCR confirmation of the *nsr* deletion (A, top) was obtained using primers KS023 and KS024, where the parent strain yielded a product of approximately 3.8 kb and the NSR1 strain a product of 2.5 kb. PCR confirmation of the *mbxL* deletion (B, bottom) was obtained using primers KS053MBXconF, where the parent strain yielded a product of approximately 3.7 kb and the ΔMbxL strain a product of 2.6 kb. PCR products obtained from the deletion strains were sequenced for further confirmation (data not shown).

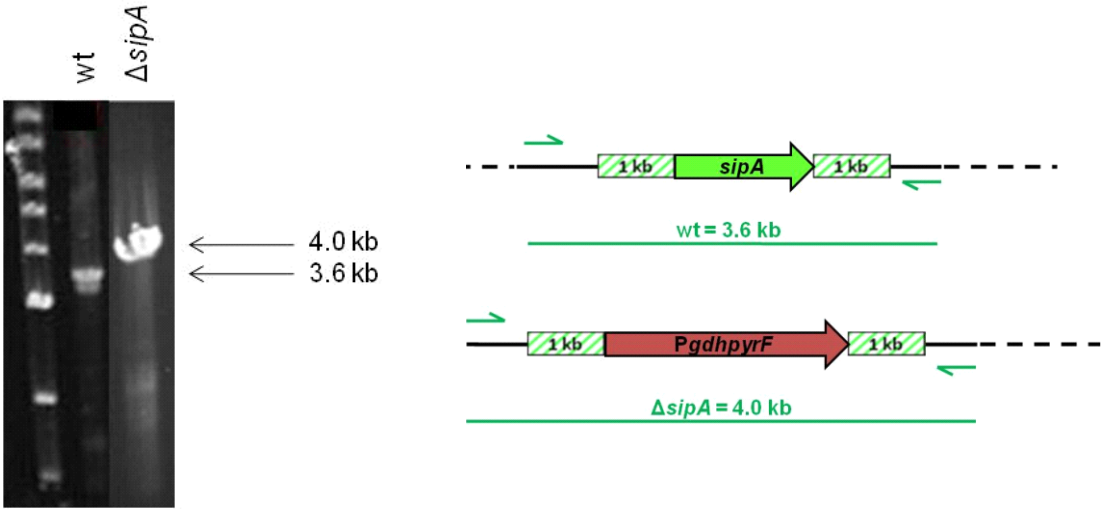

Figure S2. Confirmation of the *sipA* marker replacement deletion. Confirmation of the Δ*sipA* deletion was performed by PCR analysis using primers MD01 and MD014. The parent strain yielded a product of approximately 3.6 kb and the SIP1 strain a product of 4.0 kb. The latter was further confirmed by restriction enzyme digestion with both XbaI and EcoRI, giving products as expected (data not shown).

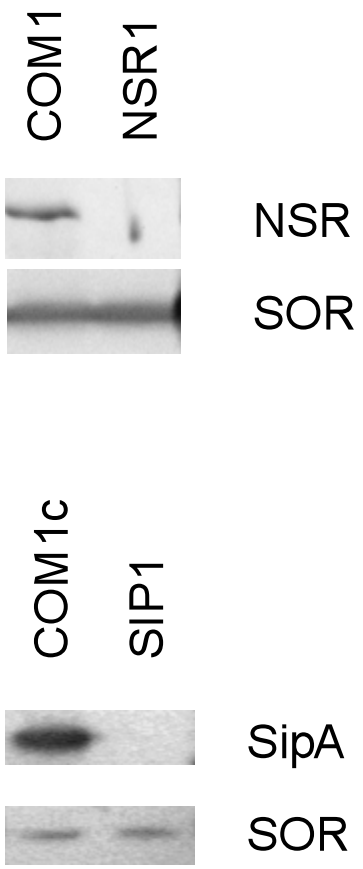

Figure S3. Western blot confirmation of NSR1 and SIP1 following S^0 addition. Polyclonal antibodies to NSR and SipA were generated against recombinant protein with an N-terminal His6 tag that was produced in *Escherichia coli* and purified in the presence of 6 M guanidinium chloride (Research Animal Resources Facility, Athens, GA). These and the polycolonal antibody to *P. furiosus* superoxide reductase ([SOR] a gift from Frank Jenney, Philadelphia College of Osteopathic Medicine, Suwanee, GA) were purified from serum using a protein A column (GenScript, Piscataway, NJ). Western blot analyses were performed on cell extracts prepared from cells harvested 30 min after S^0 addition (2g/L) with SOR used as a loading control.

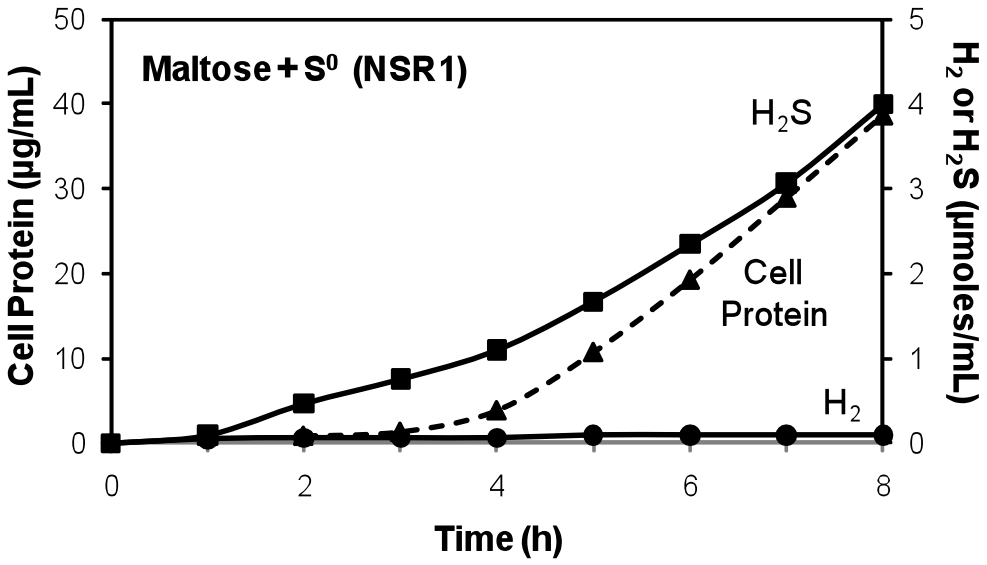

Figure S4. H_2S is produced during growth of NSR1 in the presence of S^0. 100 ml bottle cultures stirred at 98°C with 5 g/L maltose, 0.5 g/L yeast extract, 20 µM uracil, and 2 g/L S^0. Samples of headspace gas and media were transferred to secondary assay vials at each point for subsequent hydrogen gas and sulfide analyses, and cell growth was monitored by assaying total cell protein. Cell growth (closed triangles/dotted line), hydrogen gas (closed circles), and hydrogen sulfide (closed squares).

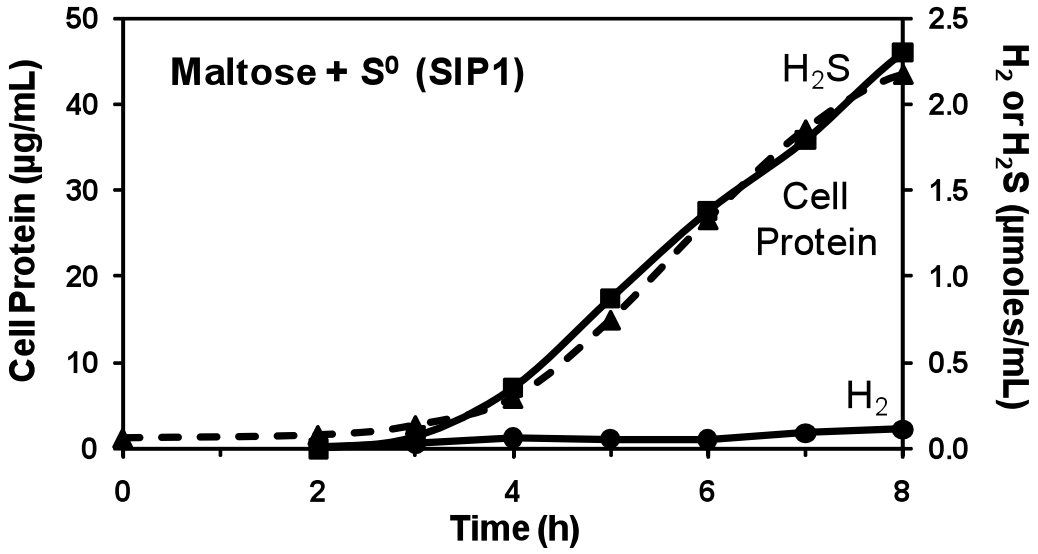

Figure S5. H_2S production follows growth of SIP1 in the presence of S^0. 100 ml bottle cultures stirred at 98°C with 5 g/L maltose, 0.5 g/L yeast extract, and 2 g/L S^0. Samples of headspace gas and media were transferred to secondary assay vials at each point for subsequent hydrogen gas and sulfide analyses, and cell growth was monitored by assaying total cell protein. Cell growth (closed triangles/dotted line), hydrogen gas (closed circles), and hydrogen sulfide (closed squares).

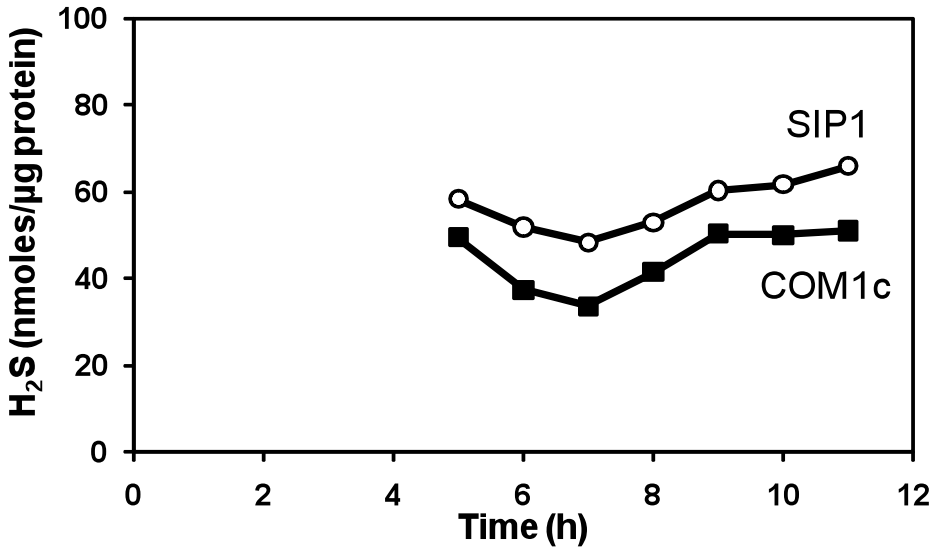

Figure S6. Amount of sulfide generated per unit protein in COM1c and SIP1. Cultures were grown in 100 ml bottles stirred at 98°C with 5 g/L maltose, 0.5 g/L yeast extract and 2 g/L S^0. Total cell protein and sulfide production were determined at each time point. COM1c (closed squares), SIP1 (open circles).

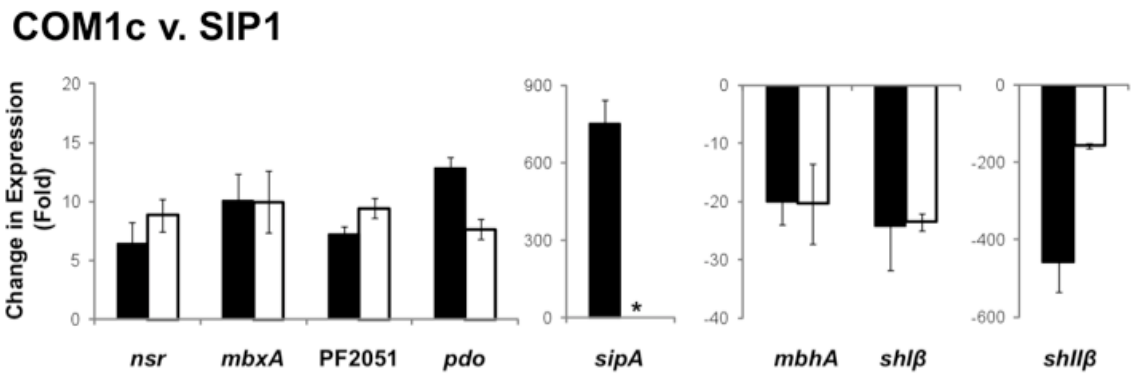

Figure S7. Quantitative RT-PCR of Select S^0 Response Genes in SIP1. Total RNA was prepared from COM1c and SIP1 cells harvested before and 30 min after S^0 addition (2 g/L). For gene clusters of interest, the first gene in the operon was selected for analysis. Constitutively expressed gene PF0971 (*por* gamma subunit) was used as a control. Ratio of change in gene expression within 30 min of S^0 addition in SIP1 (open bars) compared to the control strain COM1c (closed bars). Asterisk (*) indicates QPCR confirmation of deleted gene product.