Supporting Information

Costa et al. 10.1073/pnas.1003653107

SI Methods

Disruption of *hdrB1* or *hdrB2* in MM901, MM1263, or MM1264. Puromycin N-acetyl-transferase (*pac*) was inserted into the middle of *hdrB1* or *hdrB2* in MM1264 and MM1263 respectively. Regions for bases 100 to 400 and 400 to 700 in the ORF were PCR-amplified and ligated into pJK3 (1) into restriction sites ~400 bp upstream (ClaI-XhoI) and downstream (NotI-BamHI) of the plasmid encoded *pac* gene. The construct was linearized with SapI, transformed into MM901, MM1263, or MM1264 as appropriate, and disruptions were selected with medium containing puromycin. MM901 with *hdrB1* and *hdrB2* disruptions were designated MM1268 and MM1270, respectively. MM1263 and MM1264 with *hdrB2* or *hdrB1* disrupted were designated MM1271 and MM1269, respectively. Constructs were verified using Southern blots.

Testing the Viability of the His-Tagged Constructs. Strains with *pac* disruptions of *hdrB1* or *hdrB2* were grown to OD_{660} ~1.0 in McCas medium with 2.5 μg/mL puromycin and ~0.5 mL was transferred to 5 mL fresh McCas medium with a headspace of H_2/CO_2 (80:20) at 40 psi. MM1262 and MM1265 were grown to OD_{660} ~0.6 in formate medium and ~0.5 mL was transferred to 5 mL fresh formate medium with 0.2% casamino acids and a headspace of N_2/CO_2 (80:20) at 30 psi. Cultures were grown at 37 °C at 100 rpm agitation (Jeio Tech SK-600 shaker). Cell density (OD_{660}) was monitored.

1. Metcalf WW, Zhang JK, Apolinario E, Sowers KR, Wolfe RS (1997) A genetic system for Archaea of the genus *Methanosarcina*: Liposome-mediated transformation and construction of shuttle vectors. *Proc Natl Acad Sci USA* 94:2626–2631.

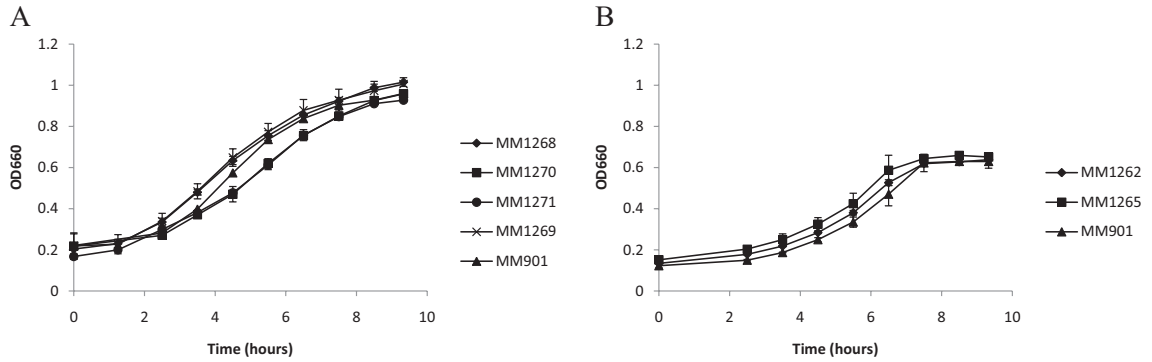

Fig. S1. Growth curves of strains with a His-tagged protein and the redundant copy inactivated compared with wild-type. Strains were constructed and growth curves were conducted as described in *SI Methods*. OD_{660}, optical density at 660 nm. Data are from duplicate cultures with error bars representing one SD. (*A*) Growth on H_2: MM901, wild type; MM1268, wild-type *hdrB2* with *hdrB1::pac*; MM1269, His-tagged HdrB2 with *hdrB1::pac*; MM1270, wild-type *hdrB1* with *hdrB2::pac*; MM1271, His-tagged HdrB1with *hdrB2::pac*. (*B*) Growth on formate: MM901, wild-type; MM1262, Δ*fdh2*; MM1265, His-tagged FdhA1 with Δ*fdh2*.

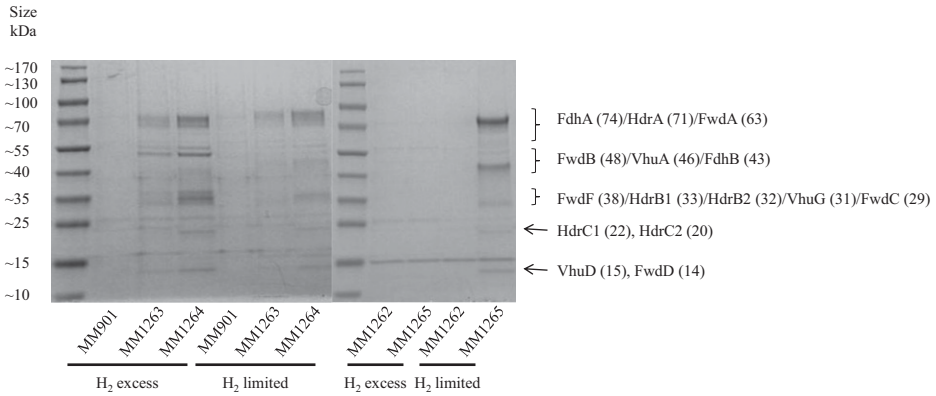

Fig. S2. SDS/PAGE for purifications of the complex from cultures grown with either excess or limiting H_2. Proteins in the size ranges of the purified bands are indicated with their predicted molecular weights in kDa in parentheses. MM901 and MM1262, controls; MM1265, FdhA1-6xHis; MM1263, HdrB1-6xHis; MM1264, HdrB2-6xHis.

Other Supporting Information Files

Table S1 (DOCX)
Table S2 (DOC)
Table S3 (DOC)