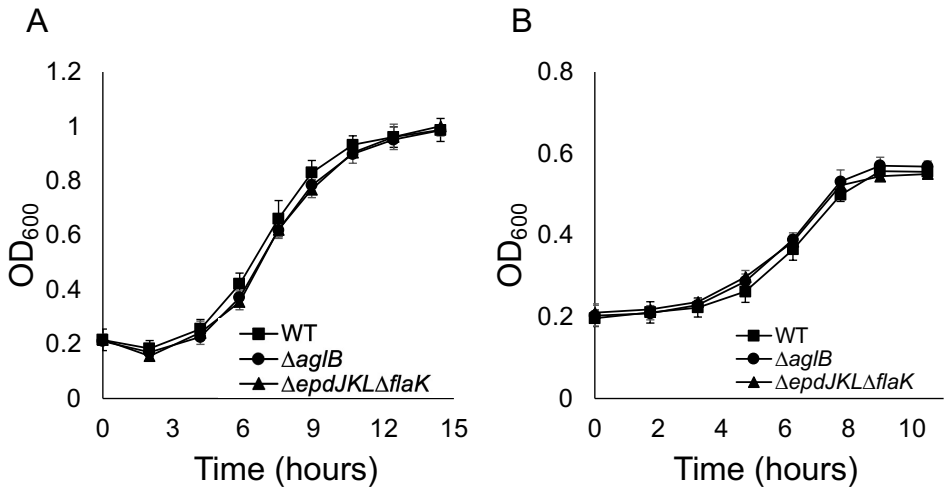

Figure S1. Growth of strain JJ wild type (WT), the Δ*aglB* strain, and the Δ*epdJKL* Δ*flaK* strain. Cultures were grown in triplicate in medium with H_2 (A) or formate (B) as the sole electron donor for growth. Tubes were grown on a shaking platform with agitation at 250 rpm to prevent surface-attached biomass from accumulating. Data are presented as means and standard deviations of triplicate cultures.

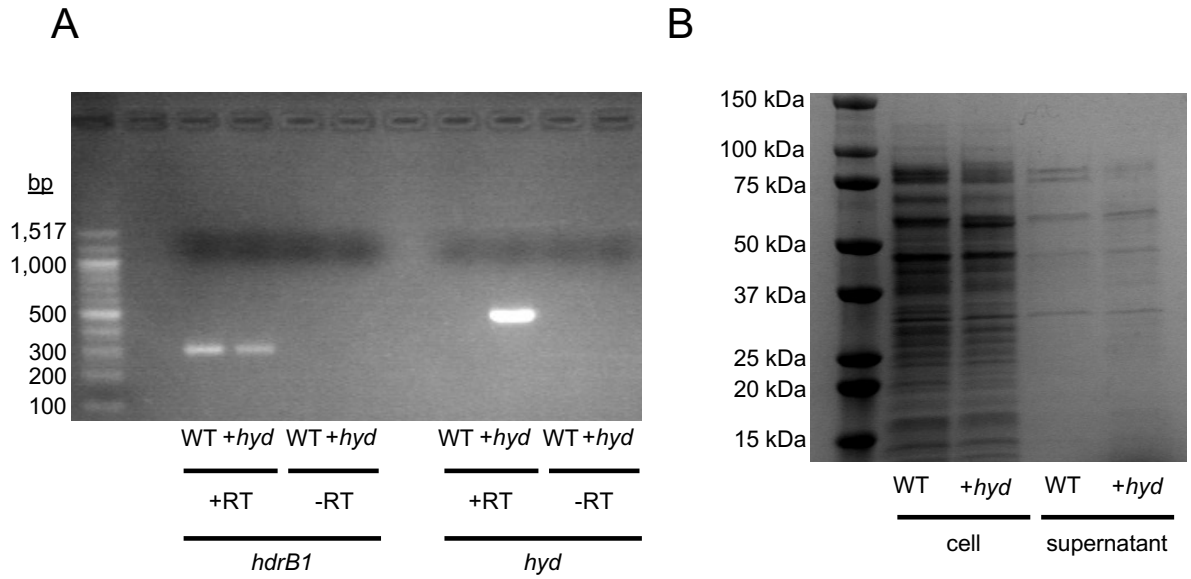

Figure S2. Expression of the hydrogenase from the pLW40-iron plasmid. (A) Reverse transcription-PCR (RT-PCR) demonstrating the expression of the genes encoding heterodisulfide reductase subunit B1 (a genomic locus present in the WT background) or the plasmid borne hydrogenase. RNA was extracted from 1.5 ml of stationary phase culture with an RNA Clean & Concentrator^{TM}-25 kit (Zymo Research) with an on column DNase treatment following manufacturer's instructions. Purified RNA was converted to cDNA using an iScript^{TM} cDNA Synthesis Kit (Bio-Rad) following manufacturer's instructions. cDNA of interest was amplified with GoTaq® Green Master Mix (Promega) using primers HdrB1-RT-F (5'-TTGGAATACAATTGCACCCATTTGAACAG) and HdrB1-RT-R (5'-CAAAATAAAGTACTTCCGTTAAGTG) to amplify a 300 bp fragment of hdrB1 or primers hyd-RT-F (5'- GAATTGAAGGCGATGGAAAA) and hyd-RT-R (5'-CATGATGCAGGATGAACAGG) to amplify a 494 bp fragment of the plasmid borne hydrogenase (hyd). Controls that were not subjected to cDNA synthesis (-RT) were included to verify that bands are not a product of incomplete DNase treatment. (B) Coomassie Blue stained SDS-PAGE gel of proteins from WT strain JJ and a strain carrying the pLW40-iron plasmid. For cell associated proteins, ~1ml of culture material was concentrated, lysed in ddH2O, and loaded onto the gel. For supernatants, 5ml of culture was pelleted by centrifugation, the supernatant was concentrated ~50x in a 10 kDA molecular weight cut-off spin column, and the resulting material was loaded onto the gel. We did not identify bands corresponding to the predicted size of the large and small subunits (~52 and ~31 kDa, respectively) in either fraction; however, activity observed in experiments (Figs. 6 and S3) strongly suggest the presence of functional enzyme.

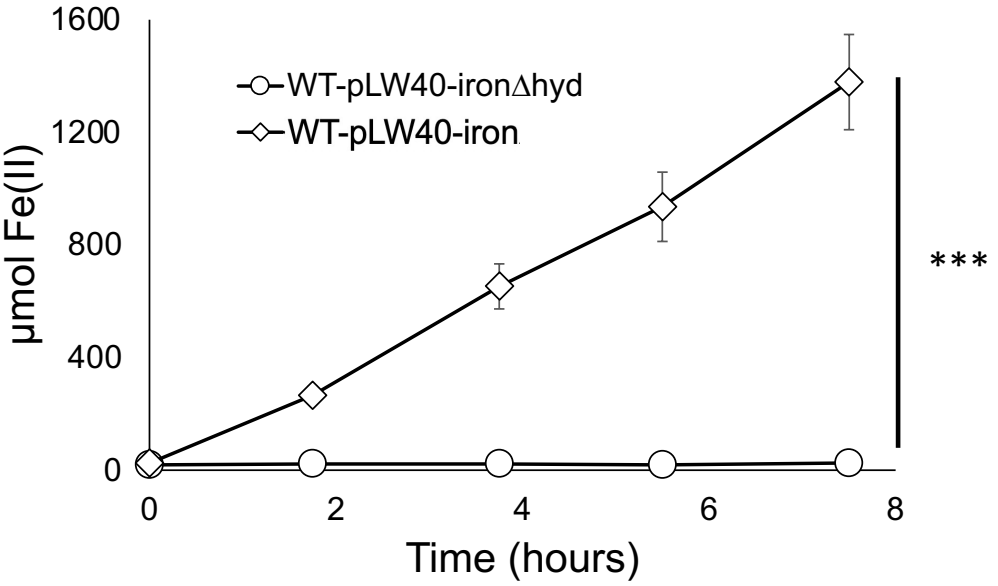

Figure S3. Plasmids carrying genes encoding the putative Tat secretion system without the predicted iron oxidizing hydrogenase are not sufficient for iron oxidation, suggesting that genomically encoded hydrogenases and formate dehydrogenases are not involved in iron oxidation. To generate a plasmid carrying genes encoding Tat but lacking genes encoding the hydrogenase, the genomic island depicted in figure 6A was PCR amplified with primers HydIsland-Fg (5'-TCTCTTCTTCTTCAGGGAGCTCGAGAGAGTCAGAACCGATGGTTAAAC) and hyd-us-Rg (5'-TTCAATTCTAGTTACCGGTTTTATTTCAACTGCCATTTAG) to amplify a fragment upstream of the hydrogenase genes and hyd-ds-fg (5'-AATAAAACCGGTAACTAGAATTGAAAACTACTTTTAGGTGATGCAAGC) and HydIsland-Rg (5'-ATTAACTAGTAATTCTTATCTCGAGAAAGTTGGATTAAATTAAGATCAAC) to amplify a fragment downstream of the hydrogenase genes. These fragments were placed into XhoI cut pLW40neo via Gibson assembly as described in the Materials and Methods. Fe^0 oxidation activity was measured as described in the Materials and Methods. Data are means and standard deviations of triplicate experiments. ***, $P < 0.001$.