Engineering the Hyperthermophilic Archaeon *Pyrococcus furiosus* to Overproduce its Cytoplasmic [NiFe]-Hydrogenase*

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Running title: *Homologous over-production of Pyrococcus furiosus hydrogenase*

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Supplemental Materials

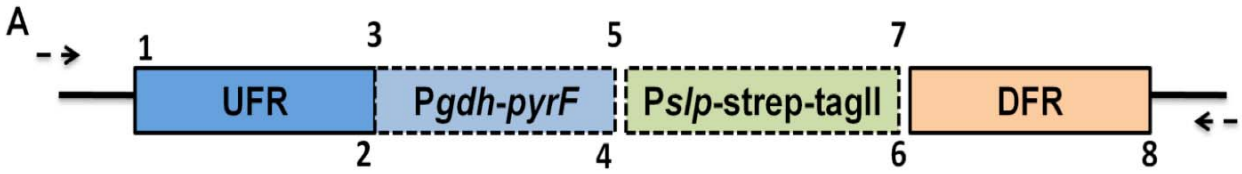
Supplemental Table S1: Primers used for overlapping PCR to construct the knock-in design and to screen by PCR to confirm the knock in. The primer number describes the position in the knock-in cassette shown in supplementary Figure S1.

Primer position	Sequence
Primers for knock in design	
1	tgagtatgaagctagggagaacgttgagcttaaggatata
2	gctcaagataaaatctttttttaaaccacctcccaatcagc
3	aaaaaagattttatcttgagctccattctttcacctc
4	gattgaaaatggagtgagctgagttaatga
5	agctcactccattttcaatcaaatagatattatcggcaaacac
6	caaattgtgggtgactccacacttttctccacctcccaataatc
7	tgttggagtcacccacaatttgaaaagagtgcaaggtatgttaagttaccc
8	cttctaagattgcctacaaaacttatattggctggacag
PCR Screening primer	
Forward primer	tatccacttgactggcgttgctcagctaagg
Reverse primer	ccagctgttgtccattgcatagagaaagacgc
qPCR primer	
PF0559 (Forward)	gctatgaagatgtggagaggttgg
PF0559 (Reverse)	cccgctgctgagttgcctc
PF975 (Forward)	gaagtcatgggagacgatggagtag
PF0975 (Reverse)	gcacttctaatttctgcccttaacttcttg
PF0894 (Forward)	gagaagtactatacatcggagacatg
PF0894 (Reverse)	gggagctttccgaatccgcc
PF0971 (Forward)	cgttgttgttgtgctagatcc
PF0971 (Reverse)	gatggcttcctctatgctctc
PF0983(Forward)	aatgaaggcagagggagaaaccca
PF0983(Reverse)	acttcatcggcctttccaagtcct

Supplemental Table S2: Purification of OE-SHI by combining conventional and affinity chromatography steps. Fractions from the DEAE-step were loaded onto a Streptactin affinity column and then a hydrophobic interaction (Phenyl Sepharose) column.

Step	Total Units (U)	Total Protein (mg)	Specific activity ($U.mg^{-1}$)	Yield (%)	Fold Purification
S100	3404	714	4.7	100	1
DEAE	2003	440	5.0	59	2
Streptactin	641	11	58	18	19
Phenyl Sepharose	90	0.33	272	3	84

Supplemental Figure S1: Design of overlapping PCR and screening PCR products. A) Schematic representation of the knock-in cassette. The abbreviations are: UFR, upstream flanking region; P_{gdh}-*pyrF*, marker driven by the promoter for the glutamate dehydrogenase gene; P_{slp}-*strep-tagII*, S-Layer protein promoter with codon-optimized strep-tag II sequence; DFR, downstream flanking region. Below the figure a whole sequence of the construct has been provided. B) Agarose gel showing corresponding PCR products. The samples are lane 1, UFR; lane 2, P*gdh-pyrF*; lane 3, P*slp-strep-tagII*; lane 4, DFR. C) Gel showing overlapping PCR product. D) PCR product obtained using the screening primer (Supplementary Table 1 and arrow marked in panel A) for COM1 strain and OE-SHI strain in lanes 1 and 2, respectively.



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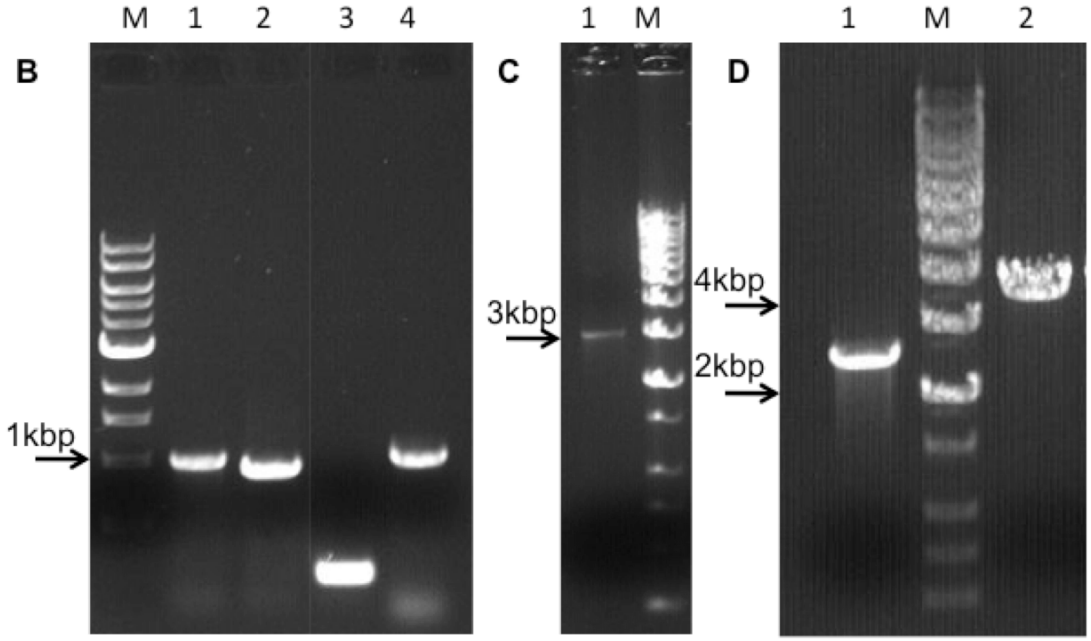
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M
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2
3
4
1kbp
C
1
M
3kbp
D
1
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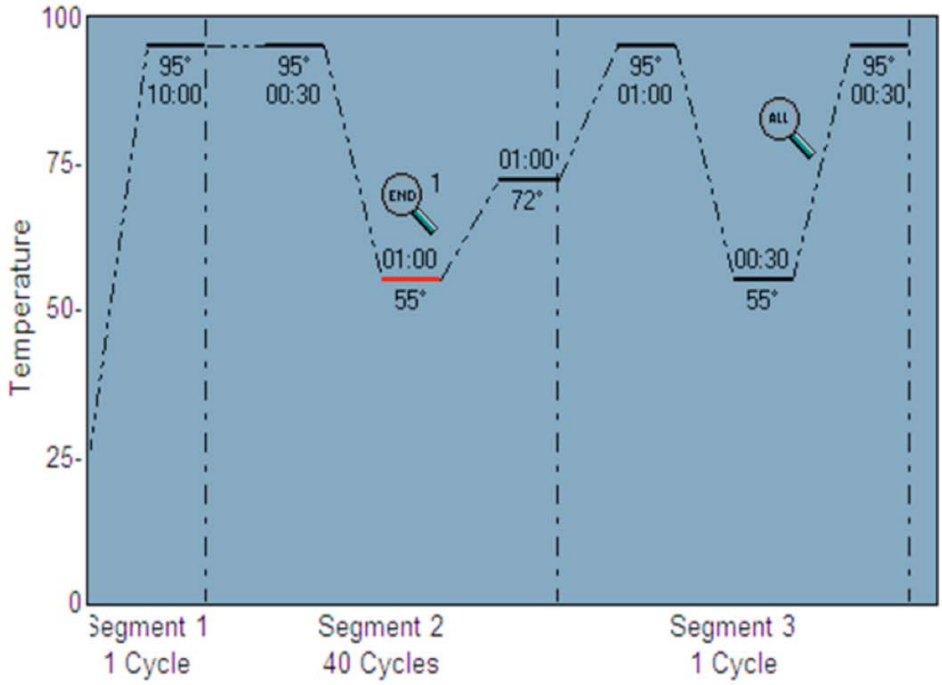
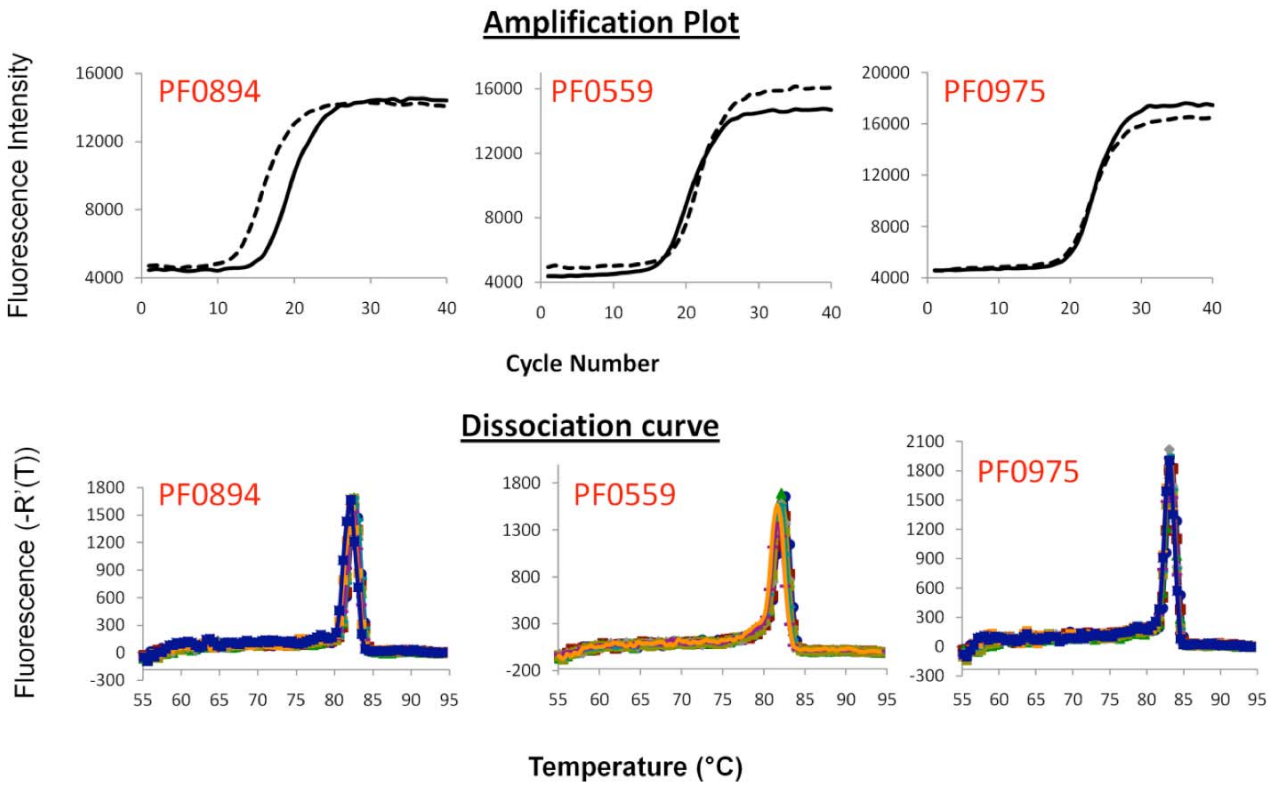
***Supplemental Figure S2*:** Details of the qPCR experiments including the program used, and the amplification plots and dissociation curves that were obtained. (A) The PCR program in which segment 1 was used to calculate the dissociation curve while segments 2 and 3 were used to calculate the amplification plot and critical threshold (C_t). The amplification plots and dissociation curves for the three experimental genes (PF0894, PF0559 and PF0975) and for the two housekeeping genes (PF0971 and PF0983) that were used as internal controls for Fig. 3 are shown in Figs. S2B and S2C, respectively.

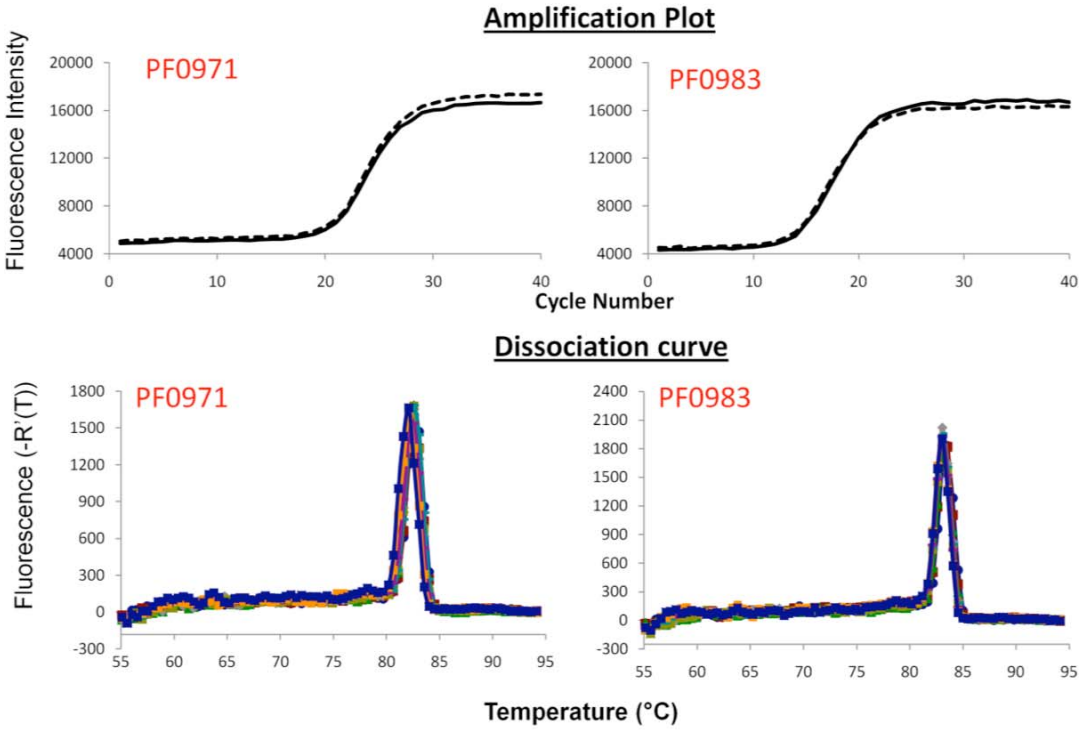
A : PCR program



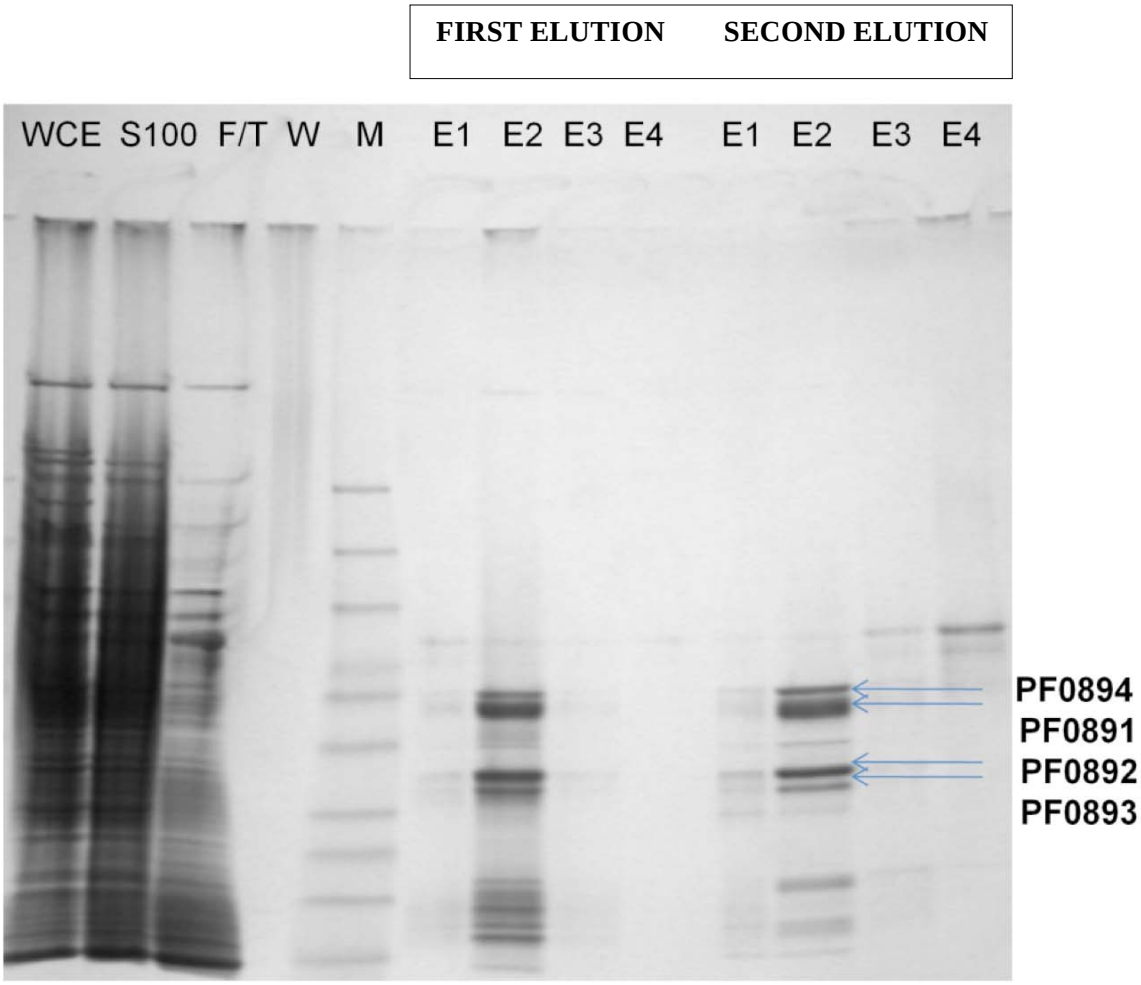
B : qPCR results for the three experimental genes



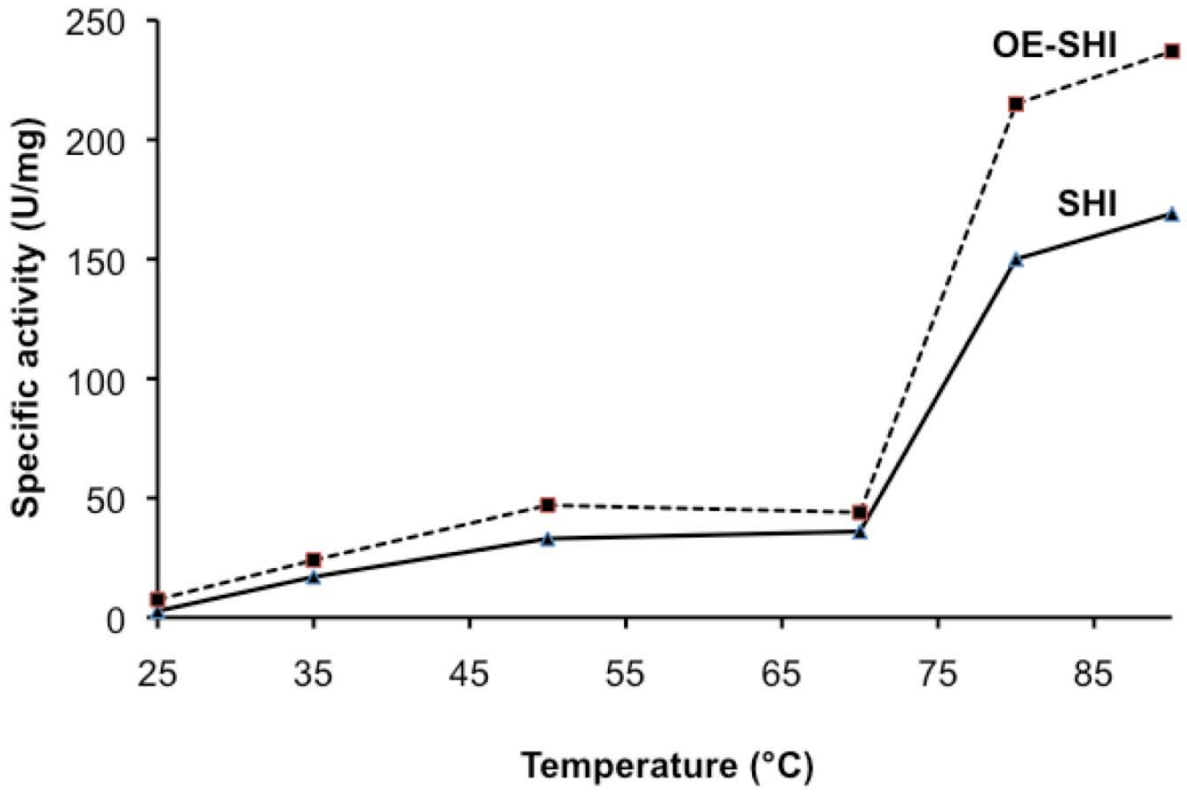
C: qPCR results for the two control housekeeping genes



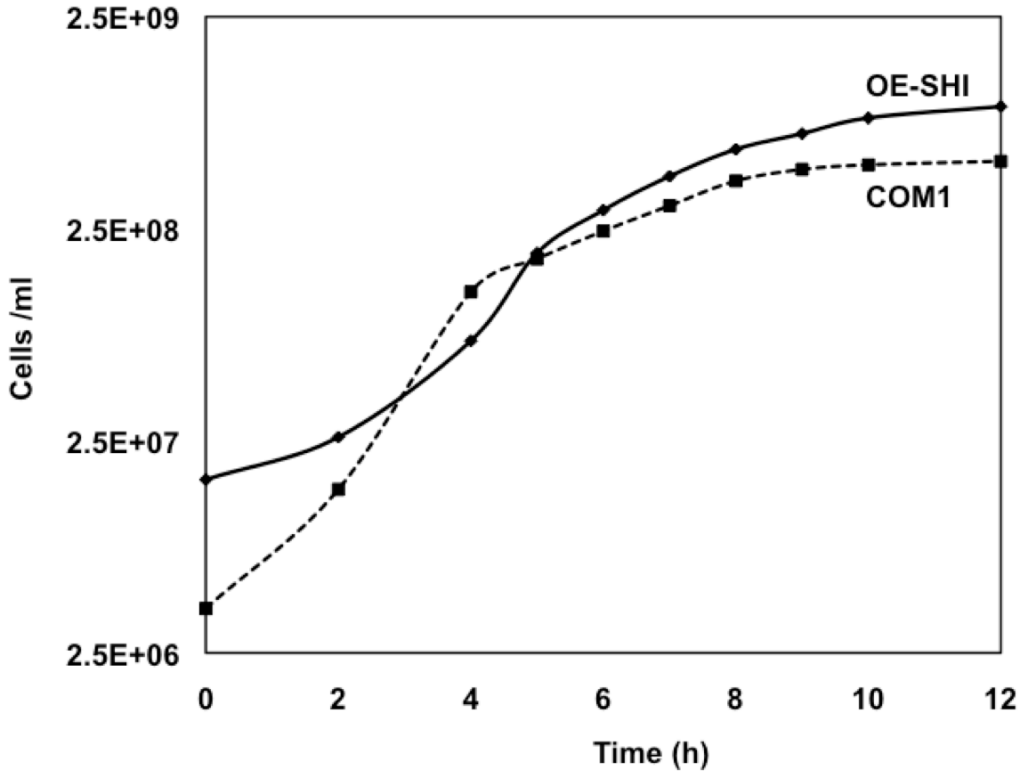
Supplemental Figure S3: SDS-PAGE of the fractions from the one step affinity purification of affinity tagged OE-SHI. WCE: whole cell extract, S100: cytoplasmic extract, F/T: flow thorough, W: column wash, M: protein molecular weight marker, E1-E4: show the different elution fractions for two separate strep-tactin elution steps as mentioned in material and method section.



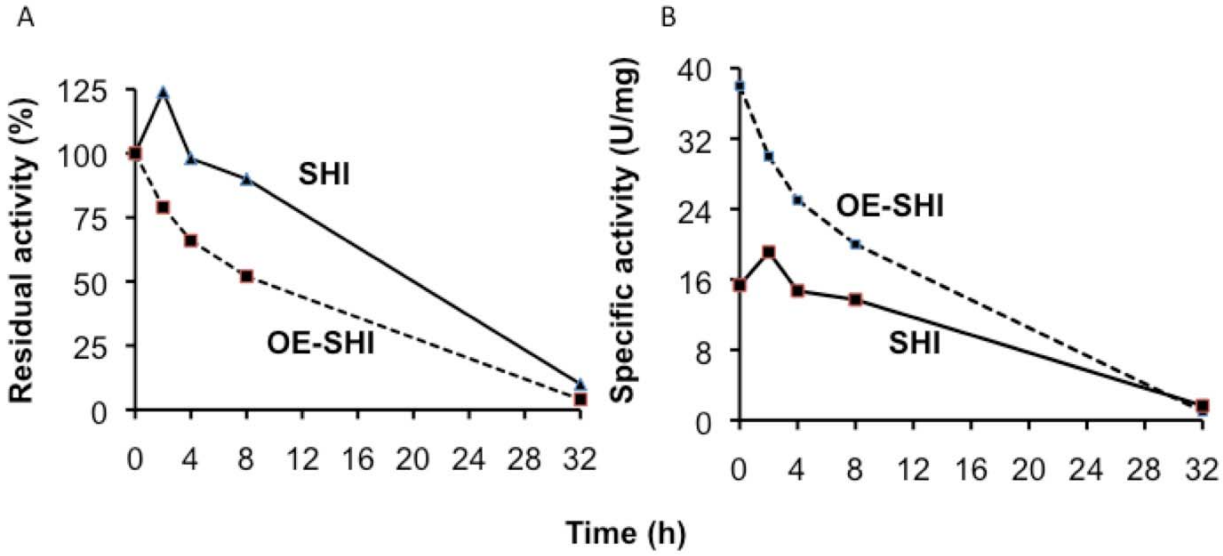
Supplemental Figure S4: MV-linked H_2 evolution specific activity comparison between pure OE-SHI and native SHI at different temperatures with both protein samples having the same protein concentration of 0.1 mg/ml.



Supplemental Figure S5: Growth of the parental COM1 and OE-SHI strains. Cells were grown in a 20-liter fermenter using maltose as the carbon source.



Supplemental Figure S6: H_2 oxidation specific activity of pure OE-SHI and SHI were compared at different times after incubation at 90°C with both proteins at the same concentration (0.1 mg/ml). The activity was measured by the H_2-dependent reduction of NADP (0.4 mM) in 100 mM EPPS buffer, pH 8.4, at 80°C using 1 atmosphere of H_2. The data have been plotted in terms of residual activity (%) vs time (A) and specific activity vs time (B).



Supplemental Figure S7: A: Structural stability of OE-SHI and SHI as measured at 25°C by fluorescence spectroscopy of the tryptophan fluorescence emission spectrum at different time points after incubation of the proteins (0.1 mg/ml in 100 mM EPPS, pH 8.4) at 90°C. Both proteins showed emission maxima of 355 nm. B: Relative fluorescence intensity of SHI and OE-SHI during 16 hours at 90°C.