Supplementary Information

The Extended C-terminal α-Helix of the HypC Chaperone Restricts Recognition of Large Subunit Precursors by The Hyp-Scaffold Machinery During [NiFe]-Hydrogenase Maturation in *Escherichia coli*

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Table S1. List of primers and their sequence used in this study.

Name	Oligonucleotide primer sequence
hypC+HQLfor	5’ ATTGACGGCAACCAGCATCAACTCGCGAAAGTCGACG 3’
hypC+HQLrev	5’ GACGTCGACTTTCGCGAGTTGATGCTGGTTGCCGTCA 3’
hypC-GSfor	5’ GATTTAACGTTAGTCTGCGATGAAAACGGT 3’
hypC-GSrev	5’ ACCGTTTTCATCGCAGACTAACGTTAAATC 3’
hypCVstopfor	5’ CAAAACATGTTTGACTAAGAGCCGGATGTCGGC 3’
hypCVstoprev	5’ GCCGACATCCGGCTCTTAGTCAAACATGTTTTG 3’
hypCGstopfor	5’ GTTGAGCCGGATGTCTGAGCGCTGTTGTATGGC 3’
hypCGstoprev	5’ GCCATACAACAGCGCTCAGACATCCGGCTCAAC 3’
hypCDGfor	5’ ATCCGCACCATTGACGACAACCAGGCGAAAGTC 3’
hypCDGrev	5’ GACTTTCGCCTGGTTGTCGTCAATGGTGCGGAT 3’
hypCPADfor	5’ CAGCTGCGATGAAAACCCTGCGGACCGCGTGGGCCAGT GGGTAC 3’
hypCPADrev	5’ GTACCCACTGGCCCACGCGGTCCGCAGGGTTTTCATCG CAGCTG 3’

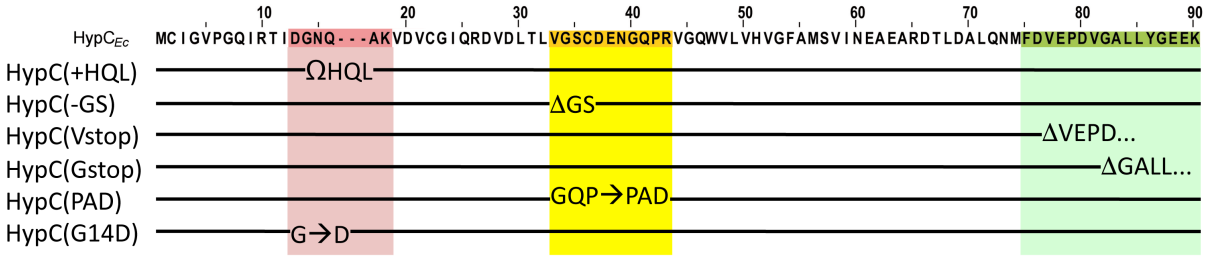

Figure S1. Schematic representation of the amino acid changes introduced into HypC. The HypC amino acid variants constructed are shown. The HypC polypeptides are shown schematically in linear representation below the amino acid sequence and the corresponding plasmids that encode them are shown on the left of the panel. The amino acid exchanges introduced are shown on top of the linear representation of the plasmids. The Δ signifies the deletion of the indicated amino acids while the Ω signifies the insertion of the indicated amino acids. The variable regions VR1, VR2, and VR3 are indicated by the colours red, yellow and green, respectively.

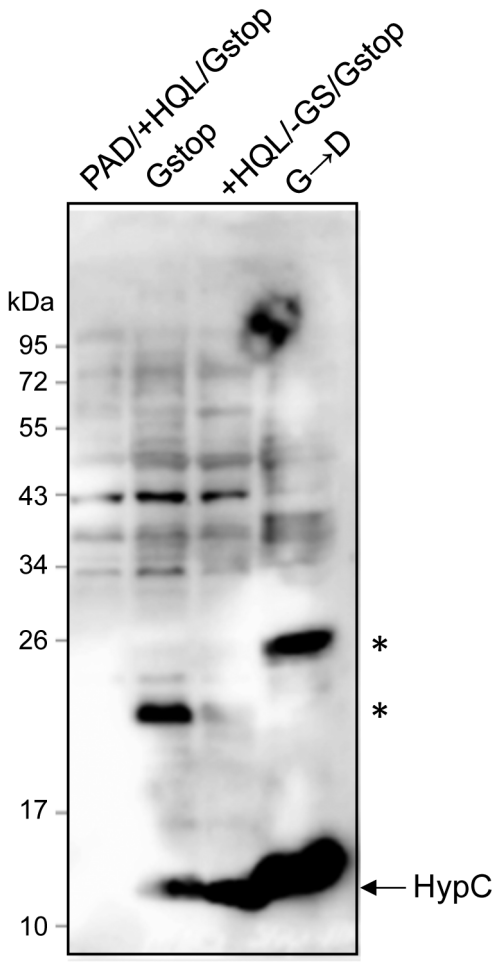

Figure S2. Immunoblot showing over-production of selected HypC variants. Samples of crude extracts (50 μg of protein) derived from strain SHH288 (Δ*hypC*Δ*hybG*) encoding the indicated HypC proteins grown anaerobically in M9 minimal medium containing glycerol and fumarate as carbon sources (0.5% w/v each) were separated in denaturing 12.5% (w/v) polyacrylamide gels and transferred to nitrocellulose membranes. The blot was challenged with antiserum raised against HypC (1:800 dilution). The migration position of HypC is indicated on the right of the panel and the molecular mass markers in kDa are shown on the left. Asterisks indicate possible dimeric forms of HypC.

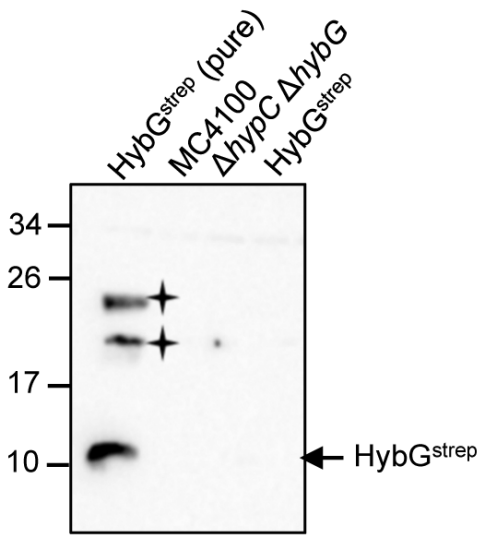

Figure S3. Immunoblot with anti-HybG antiserum. Samples of purified HybG^{Strep} (100 ng), and of crude extracts (50 μg of protein) derived from strains MC4100 (wild type), SHH288 (Δ*hypC*Δ*hybG*) and SHH288 transformed with plasmid phybGstrep encoding HybG^{Strep} were separated in 12.5% (w/v) polyacrylamide gels and transferred to nitrocellulose membranes. The blot was challenged with antiserum raised against HybG (1:1000 dilution). The migration position of HybG^{Strep} is indicated on the right of the panel and the molecular mass markers in kDa are shown on the left. The crosses to the right of the bands indicate a possible dimeric form of HybG^{Strep} and a further form that might represent a degradation product of the dimer.