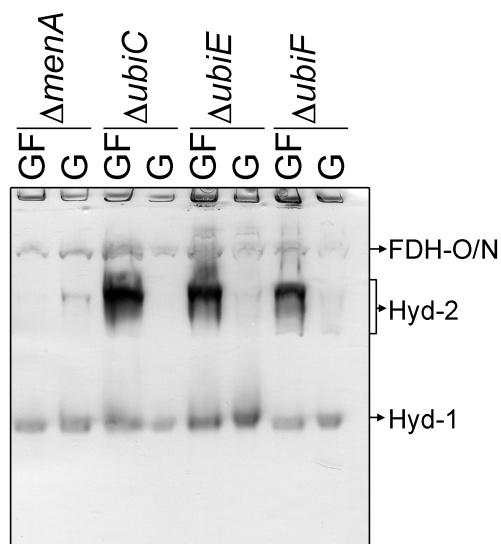


**Supplementary Material: Pinske et al.**

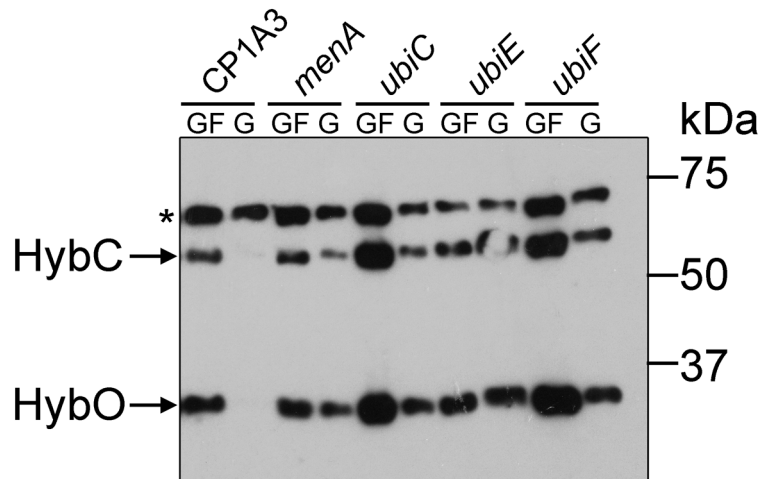
**Supplementary Figure S1:** Strains JW3901 ( $\Delta menA$ ), JW5713 ( $\Delta ubiC$ ), JW5581 ( $\Delta ubiE$ ) and JW0659 ( $\Delta ubiF$ ) were grown in GF medium or in glycerol only medium (G) and 25  $\mu$ g of protein were subjected to native-PAGE and subsequently stained for hydrogenase activity as described. The migration patterns of the hydrogen-oxidizing formate dehydrogenases N and O (FDH-O/N), Hyd-1 and Hyd-2 are shown on the right side.

Figure S1



**Supplementary Figure S2: Western blot analysis of Hyd-2 content in *hybA* and quinol mutants.** Strains CP1A3 ( $\Delta$ *hyaB hybA hycE*), JW3901 ( $\Delta$ *menA*), JW5713 ( $\Delta$ *ubiC*), JW5581 ( $\Delta$ *ubiE*) and JW0659 ( $\Delta$ *ubiF*) were grown in GF medium or in glycerol only medium (G) and 25  $\mu$ g of protein were subjected to SDS-PAGE and subsequently transferred onto nitrocellulose and challenged with anti-Hyd-2 antibodies as described in Materials and Methods. The migration of the large subunit HybC and the small subunit HybO is indicated as well as an unspecific cross-reacting species, which is marked with an asterisk.

Figure S2



**Supplementary Figure S3 (orig. Fig. 3): Hydrogenase 2 large subunit is membrane-associated in *frdA* mutants.** Strains MC4100, JW4115 ( $\Delta frdA$ ) and DHP-F2 ( $\Delta hypF$ ) were grown anaerobically in M9-minimal media containing either 0.4% (v/v) glycerol (G) or glycerol and 25 mM fumarate (GF) as indicated. Cells were disrupted and sub-cellular fractions prepared as described in the Materials and Methods. Aliquots of 50  $\mu$ g of protein from the membrane fraction (MF) or soluble fraction (SF) were separated on 10% (w/v polyacrylamide) SDS-PAGE, transferred onto nitrocellulose and challenged with antibodies raised against Hyd-2 (the two forms of HybC are indicated), the membrane bound TatC protein or the cytoplasmic FdhE protein. The star indicates an unspecific and unidentified cross-reacting band. The molecular mass standards are labelled on the right side of the image.

Figure S3

