## **1** Supporting Information

## 2 Figure S1. SDS-PAGE of purified wild-type RnfG and variant RnfG-T166A from

3 *Methanosarcina acetivorans*. Lane 1, molecular mass standards; lane 2, purified RnfG (15 μg);

4 lane 3, purified RnfG-T166A (15  $\mu$ g). Lanes 1-3 were stained with Coomassie brilliant blue

5 R250. Lanes 4 and 5 correspond to lanes 2 and 3 exposed to UV illumination prior to protein

6 staining.

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Figure S2. Sequence alignment of RnfG from Methanosarcina acetivorans with NqrC from 8 *Vibrio cholerae*. Only the C-terminal domains are aligned showing potential FMN-binding 9 motifs (**bold and underlined**) previously proposed for NqrC (). The threonine 166 residue of M. 10 acetivorans RnfG is shown in **bold italic**. Shaded residues are potential FMN-binding sites for 11 RnfG. Aligned with NCBI blasp. RnfG, MA0661, gene id 1472553; NqrC, VC2293, gene id 12 13 4808844. 14 15 NqrC GLWSMMYAFVAVETDGNTVSGLTYYEQGETPGLGGEVENPAWRAQWVGKKLFDENHKPAI 203 16 G M+ + +D +T++G + ETPGLG + P ++ Q+V + D + Rnfg GAOGMIQLLAGISSDFSTITGFOVMKHSETPGLGALITTPEFOGOFVDLPVADTS---- 151 17 18 19 NgrC KIVKGGAPQGSEHGVDGLSGATLTSNGV 231 20 + K G VD +SGAT++S V 21 RnfG -LTKNGGQ-----VDAISGATISSQAV 172 22 Figure S3. SDS-PAGE of RnfB from Methanosarcina acetivorans. Lane 1, molecular 23 24 weight standards; Lane 2, purified RnfB stained with Coomassie blue R250; Lane 3, Western blot of purified RnfB using anti-His antibody coupled to alkaline phosphatase. The lanes were 25

loaded with  $15 \mu g$  of protein.



28	Figure S4. Sequence comparisons of the RnfB subunit from <i>Methanosarcina acetivorans</i> .
29	Panel A, alignment with RnfB from Rhodobacter capsulatus. The predicted N-terminal
30	transmembrane helix using the TMHMM and HMMTOP algorithms [54] is highlighted in grey
31	and conserved cysteines are highlighted in black. Panel B, alignment with partial sequences of
32	CdhE and AcsC subunits from the CO dehydrogenase/acetyl-CoA synthase complex of
33	Methanosarcina thermophila (M.t.), Moorella thermoacetica (M.th.), and RnfB from
34	Rhodobacter capsulatus (R.c.).
35 36 37 38 39 40 41 42 43 44 45 46 47 48	<pre>(A) M.a. MSSVLINSIAVLAGLGFAVGVMLVIASKVFKIDSNPLIDDVASLLPGANGGGGFAGAACAEAIVEQGAPVNSG 75 R.cMIAAAASMSALGLGLGYLLGAAARKFHVETPPIVEEIAKILPGTNGGAGGFPGONGLAEAMAEGNAPVTAG 71 * * ** ** * * * * * * * * * * * * * *</pre>
49 50 51 52	M.a. ECPAGAIRVTEFLAEIDQEKCTACGACVAICPQKAIELR 264 R.c. VCPTEAIVSRVKPKTLKTWYWDKPQPGLVAASAETAA 187 **
53 54 55 56 57 58	(B) M.a. 43 SLLPGANOGGCGFAGCAACAEAIVEQGAPVNSCPVGGFEV 82 R.c. 39 KILPGTNOGACGFPGCNGLAEAMAEGNAPVTACTPGGRDV 78 M.t. 11 KYLPQTNOGECGEPTCMAFASKLIDRSGKTSDCPPLVKEK 50 M.th. 9 KQLPKKNOGECGTPTCLAFAMNLASGKASLDSCPYVSDAA 49

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## 59 Figure S5. Membrane topology of RnfA, RnfD and RnfE predicted with the

60 **HMMTOP algorithm**. The SGAT and SGTF motifs are highlighted. The topology was





