SUPPLEMENTARY INFORMATION

The coenzyme B_{12} precursor 5,6-dimethylbenzimidazole is a flavin antagonist in *Salmonella*

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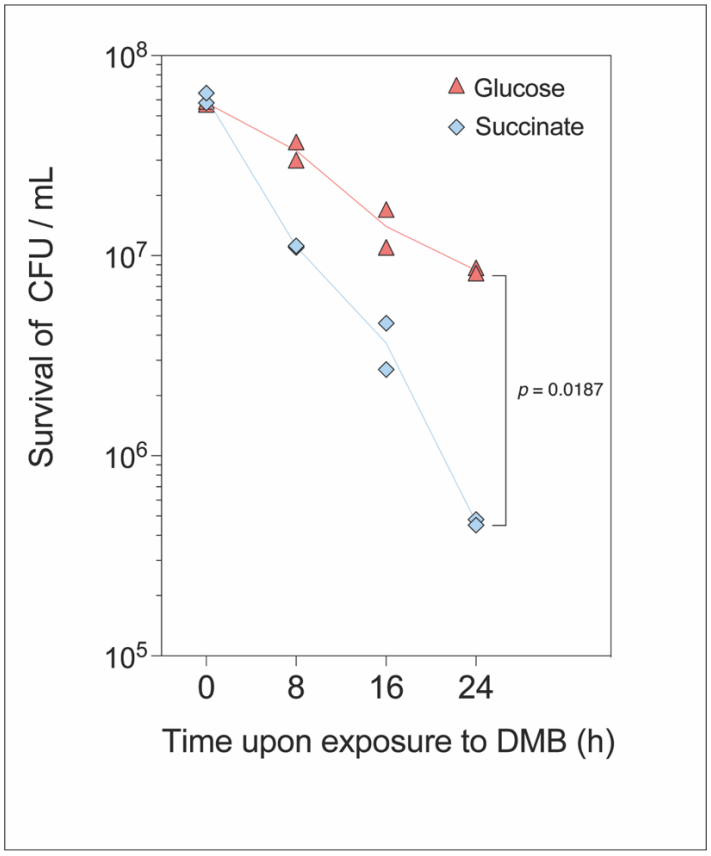


Figure S1: Cell survival upon exposure to DMB. NCE minimal medium supplemented with glucose or succinate as the main source of carbon/energy (As described under *Materials and Methods*) and DMB (4 mM) was inoculated (2% v/v) from an overnight culture of *S*. Typhimurium (JE9426) grown in LB. Cultures were grown at 37 °C with shaking (180rpm) and aliquots were taken at given time points. Appropriate dilutions were prepared in saline (0.9 % w/v NaCl), samples were plated in duplicate on LB agar and incubated overnight (~20 h) at 37 °C. Experiment was repeated two times with a representative graph shown. Student's t test was performed to compare the survival of cells grown in succinate to cells grown in glucose as the main source of carbon and energy.

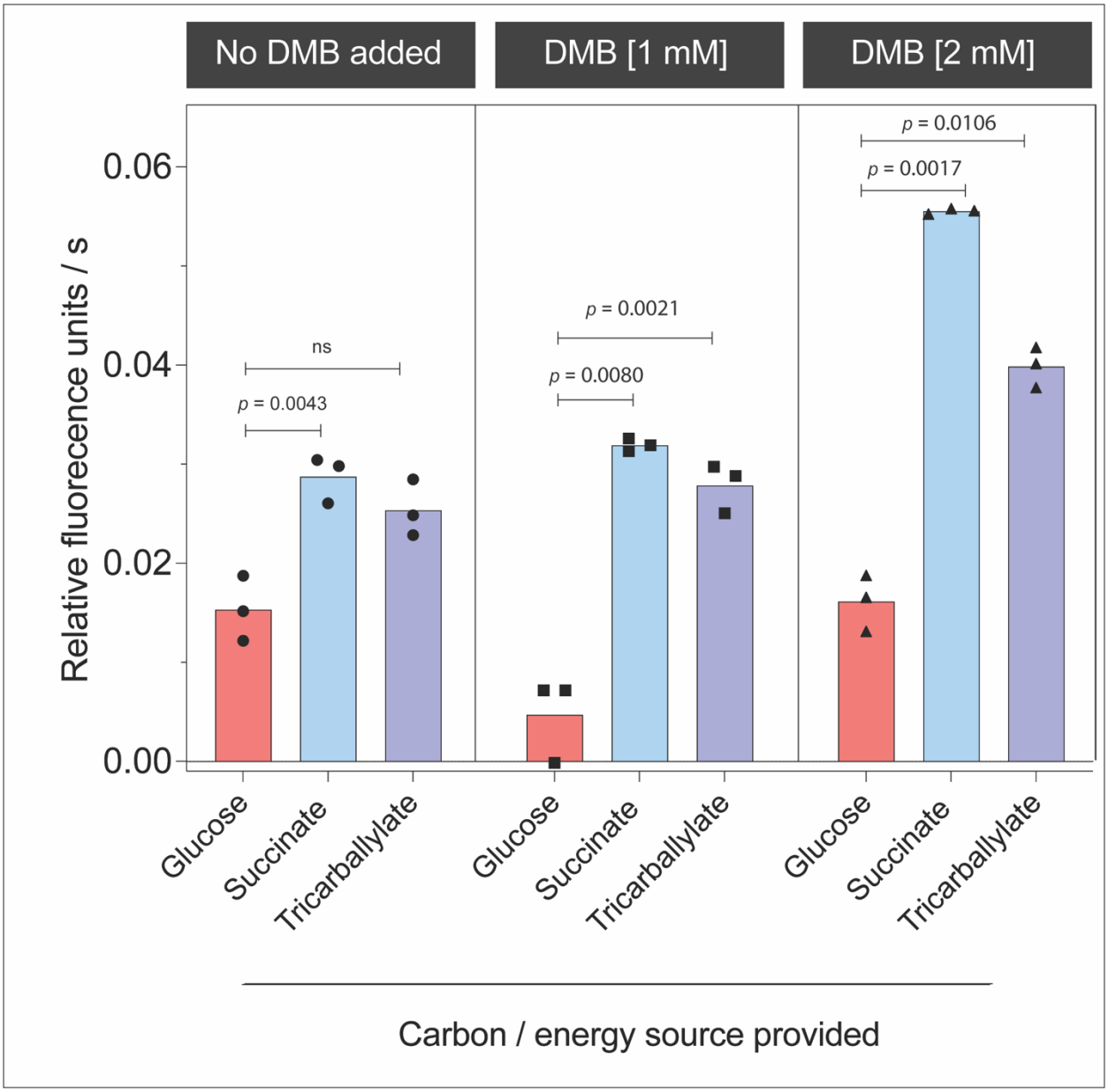


Figure S2: Dissipation of proton motive force by DMB is carbon source dependent. Cells were grown in NCE minimal medium supplemented with the indicated carbon and energy source as described under *Materials and Methods* and DMB was added to a final concentration as indicated; cultures were incubated for 15 min at 37 °C with shaking. EtBr was added to a final concentration of 40 mM, cultures were irradiated with 530 nm light, and fluorescence emitted at 600 nm was monitored over 15 min. The rate of EtBr accumulation is expressed as relative fluorescence units/s. Experiment was repeated two times with each condition containing three technical replicates, with a representative graph shown. A paired Student's t test was performed compared to the cells grown in glucose as the main source of carbon/ energy to determine statistical significance in each test condition (ns, not significant).

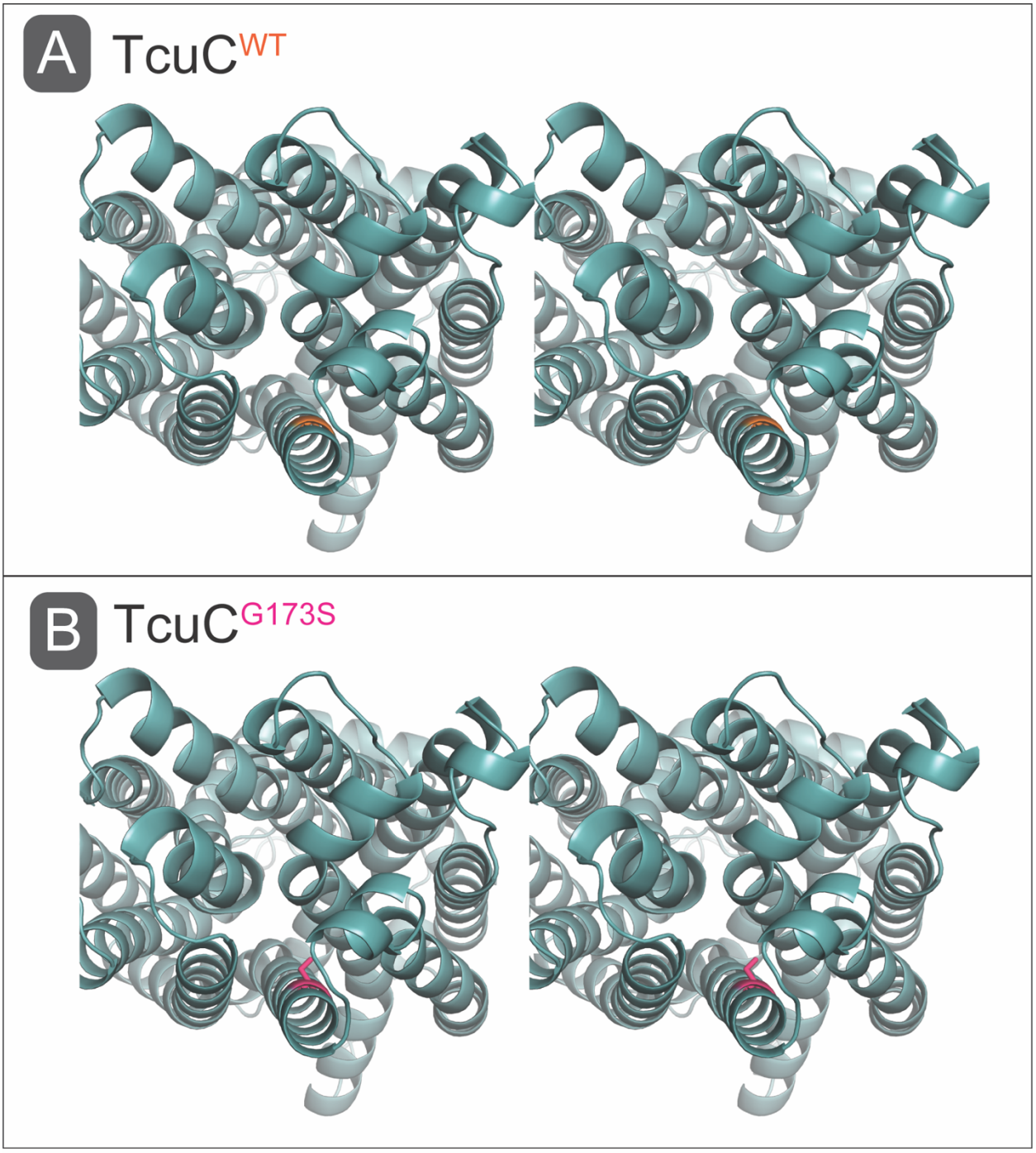


Figure S3: Mutation in *tcuC58*$^{+}$ (JE26839, JE26841) likely affected an amino acid residue located within the transmembrane channel of the tricarballylate transporter,TcuC. Stereoview of predicted ribbon structures (AlphaFold) [1, 2] showing native TcuC^{WT} (A) and TcuC^{G173S} (B). Glycine residue (G173) is shown in orange in the wildtype structure whereas the mutated serine residue is shown in pink in TcuC^{G173S}. Mutation simulation and figure was prepared using PyMOL.

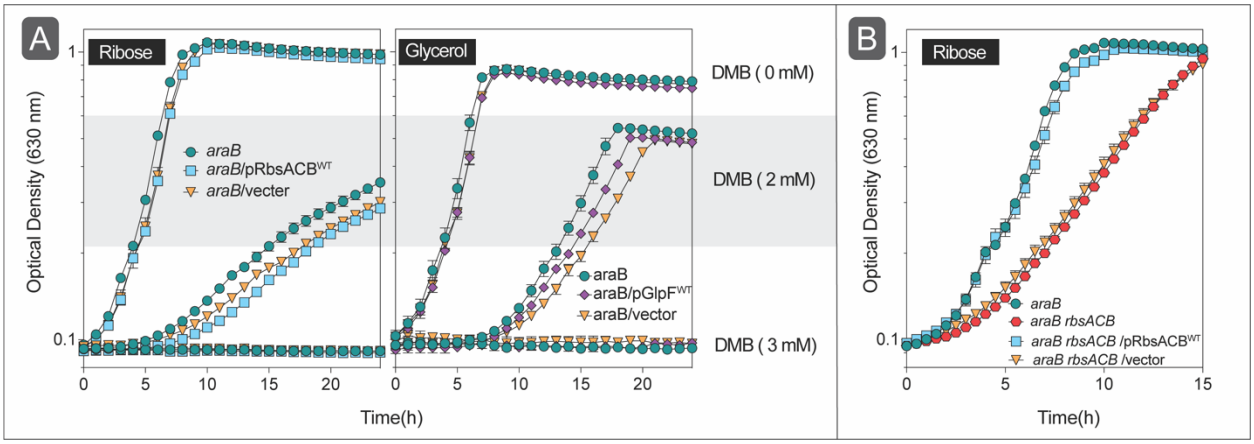


Figure S4: Overexpression of *rbsABC* or *glpF* is not sufficient to gain DMB resistance in *S*. Typhimurium. A. ABC type ribose transporter genes *rbsACB* were overexpressed in trans in the cells grown in ribose (left panel) and glycerol uptake facilitator *glpF* in cells grown in glycerol (right panel). Cultures were grown in NCE minimal medium supplemented with or without providing DMB as indicated. When used as the carbon/energy source ribose or glycerol was added to a final concentration of 22 or 20 mM respectively. **B.** Expression of *rbsACB* from pRbsACB^{WT} (pRBS1) is sufficient to rescue the growth rate of a *rbsACB* mutant strain when cells were grown in ribose as the sole source of carbon and energy. Cells were grown in NCE minimal medium supplemented with ribose (22 mM). L-(+)-Arabinose was added at a 0.5 mM final concentration to the medium for induction. Error bars represent standard deviation of three technical replicates and each experiment was completed with three biological replicates, with a representative graph shown.

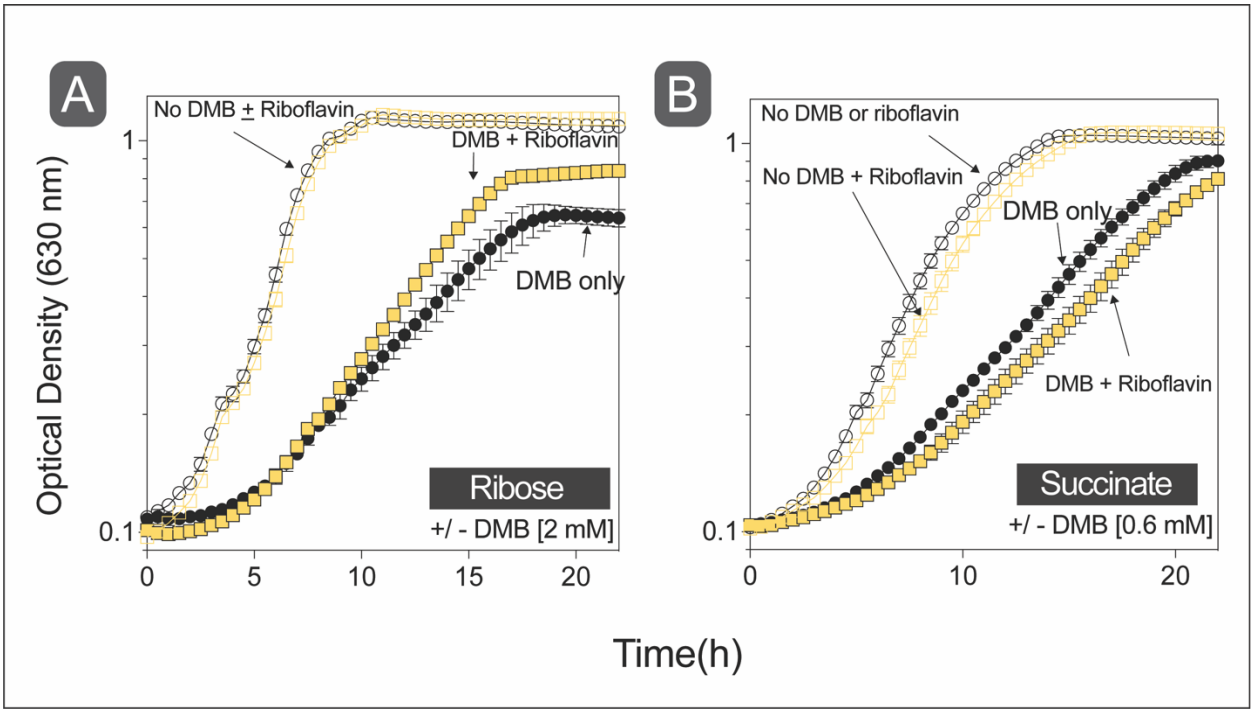


Figure S5: Cells grown in ribose responds to exogenous riboflavin under DMB stress, but not when grown in succinate. Cells were grown in NCE minimal medium supplemented with ribose (**A**) or succinate (**B**) as the main source of carbon and energy as described under *Materials and Methods* with (squares) or without (circles) riboflavin (0.75 mM). Cultures with added DMB are shown in filled shapes. Error bars represent standard deviation of three technical replicates and each experiment was completed with three biological replicates, with a representative graph shown.

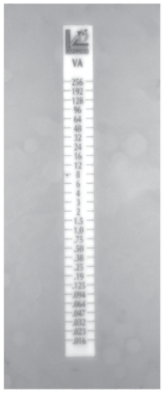
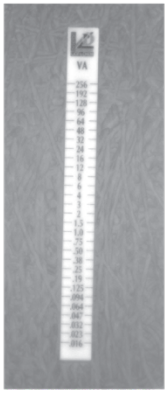
	No additive	DMB [1 mM]	DMB [2 mM]	EDTA [0.25 mM]
				
MIC (μg/ mL)	> 256	> 256	> 256	12

Figure S6: Exposure to sub-lethal levels of DMB does not cause an increase in susceptibility to vancomycin. An overnight culture (~20 h) of *S.* Typhimurium (JE9426) grown in LB was diluted to a ~ 1 x 10^8 CFU/ mL in saline (0.9 % w/v NaCl) and aliquots (150 mL) were spread onto Muller Hinton agar plates supplemented with DMB or EDTA as indicated. Plates were dried at RT for 10 min, sterilized E-strips were placed, and plates were incubated at 37 °C for ~18 -20 h. Experiment was replicated three times, with representative pictures shown. EDTA was used as a positive control for an outer membrane disorganizing agent [3]

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2. Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, Tunyasuvunakool K, Bates R, Zidek A, Potapenko A, Bridgland A, Meyer C, Kohl SAA, Ballard AJ, Cowie A, Romera-Paredes B, Nikolov S, Jain R, Adler J, Back T, Petersen S, Reiman D, Clancy E, Zielinski M, Steinegger M, Pacholska M, Berghammer T, Bodenstein S, Silver D, Vinyals O, et al. (**2021**). Highly accurate protein structure prediction with AlphaFold. **Nature** 596: 583-589. doi: 10.1038/s41586-021-03819-2

3. Vaara M (**1992**). Agents that increase the permeability of the outer membrane. **Microbiol Rev** 56: 395-411. doi: 10.1128/mr.56.3.395-411.1992