

Expanded View Figures

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GlpG_Ec      MLMITSFANPRVAQAFVDYMATQGVI LTIQQHNQSDVWLADESQAERVRAELARFLENPA 60
GlpG_Ss      MLMITSFANPRVAQAFVDYMATQGVI LTIQQHNQSDVWLADESQAERVRAELARFLENPA 60
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GlpG_Ec      DPLYLAASWQAGHTGSGLHYRRYPFFAALRERAGPVTWVMMIACVVVFIAMQILGDQEV 120
GlpG_Ss      DPLYLAASWQAGHTGSGLHYRRYPFFAALRERAGPVTWVMMIACVVVFIAMQILGDQEV 120
*****
                ↓

GlpG_Ec      LWLAWPFDPTLKFEEFWRYPFTHALMHFSLMHILFNLLWVWYLGGAWEKRLGSGKLI 180
GlpG_Ss      LWLAWPFDPALKFEFWRYPFTHALMHFSLMHILFNLLWVWYLGGAWEKRLGSGKLI 180
*****
                ↓

GlpG_Ec      SALLSGYVQQKFGSPWFGGLSGVVYALMGYVWLRGERDPQSGIYLQRGLIIFALIWI 240
GlpG_Ss      SALLSGYVQQKFGSPWFGGLSGVVYALMGYVWLRGERDPQSGIYLQRGLIIFALIWI 240
*****

GlpG_Ec      WFDLFGMSMANGAHIAGLAVGLAMAFVDSL NARKRK 276
GlpG_Ss      WFDLFGMSMANGAHIAGLAVGLAMAFVDSL NARKRK 276
*****

AarA_Ps      MAEQQNPFSIKSKARFSLGAIATLTLVLLNIAVYFYQIVFASPLDSRESNLILFGANI 60
Rhom7_Ss     -----MSASSVKPLNVQLPAITLILFALCIGIFCYLAQWMSYEEVDQSALIHGANVA 53
                : * . *   : :   * : * * . * * : : *   : *   : : * * : * * :

AarA_Ps      QLSLTGDWWRYPISMM LHSNGTHLAFNCLALFVIGICERAYGKFKLLAIYIISGIGA 120
Rhom7_Ss     PLTLSGEPWRLSSIFLHSSVSHLLMNMFAFLVVGVAEQILGKWRLLITWLFSGVFGGL 113
                * : * : * *   * : * * * . : * * : * *   . * : * * : * *   : : * * : . *

AarA_Ps      FSAYWQYYEISNSDLWTDSTVYITICVGASGAIMCIAAASVIYLIKVVINKPNHPVI 180
Rhom7_Ss     ISACYALRE-----SEQIVISVGASGAILGIAGAAIATQFASG-----TGTYH 156
                : * * : *   :   * . * * * * * . * * : :   :   :

AarA_Ps      RQKYQIYNI IAMIATL IING-TQSGVDNAAHIGGAI I GALISTAYIIVPHKLRVAN-L 238
Rhom7_Ss     KNQRRVFPPLGMVALTLLYGARQTGIDNACHIGGLIAGGALGWL SARLSGQNRLVTE 216
                : : : : * : * * * * * *   * * : * * * . * * * * * . * .   : : * : . . *

AarA_Ps      TVIAASLLTMMIYLYSFTSNKHLEEREFIYQEYVTELDANQ----- 281
Rhom7_Ss     IVAGSLLLTGAIWLAQQQMDSESVLQVRQSLREAFYPQEIEQERRQKQQLAEERNAL 276
                * . : * * *   * : * . . : : * * : * : : . * : : : :

AarA_Ps      ----- 281
Rhom7_Ss     LSAPVSRQASGDLLAEIADIHMAISRDNMLYAAIENTNSIVVFDLGQKKILHTFTAP 336

AarA_Ps      ----- 281
Rhom7_Ss     IAKEKSVKHC GGCKDQGVRS LALSPDEKLIYATSF EANALSVIN VATGEIIQSI TTGA 396

AarA_Ps      ----- 281
Rhom7_Ss     DSLILSRDGT KAWVMNR TSNVPAIDLVTYQH VADIPLEKYDGTGTS GKP GAWVMALSP 456

AarA_Ps      ----- 281
Rhom7_Ss     ERTLLVPGAGRGNIVRINTITHQKEDFPAGDARGTISAMRFRPENGEVIFADSQGISRIS 516

AarA_Ps      ----- 281
Rhom7_Ss     VGDQQASIMTQWCSRSVYSVEGISPDGQYLALVSYGLQGYVILLNINAGQIIGVYPAS 576

AarA_Ps      ----- 281
Rhom7_Ss     NHLRFSADGRKIFVMAKNGLIQMDRTRS LDPQAIIRHPQYGNVACIPEP 625
    
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Figure EV1. Amino acid sequence alignments of *S. sonnei* GlpG (GlpG_Ss) with *E. coli* GlpG (GlpG_Ec), and *S. sonnei* Rhom7 (Rhom7_Ss) with *P. stuartii* AarA (AarA_Ps).

The single amino acid difference between the two versions of GlpG is highlighted by a purple arrow. The potential active site residues of Rhom7, Ser¹³³ and His¹⁸⁷, are highlighted by red arrows. Alignment performed by Clustal Omega.

Figure EV2. GlpG and Rhom7 do not influence the growth of *S. sonnei* under various conditions.

- A, B Wild-type *S. sonnei* or *S. sonnei* Δ glpG Δ rhom7 were grown in liquid LB in the presence of oxygen at 37°C with shaking at 180 rpm. Growth was assessed by measuring the optical density of cultures at 600 nm (OD₆₀₀) (A) and colony-forming units of bacteria (CFU) recovered from the same samples (B).
- C, D Bacteria were grown anaerobically in LB supplemented with 10 mM KNO₃ with shaking at 180 rpm. Growth was assessed by measuring the OD₆₀₀ as above (C) and CFU of bacteria recovered from the same cultures (D).
- E, F Comparisons of cellular respiration (NADH production) of *S. sonnei* Δ glpG Δ rhom7 (green) to wild-type *S. sonnei* (red) (E), or of *S. sonnei* Δ glpG Δ rhom7 + glpG (green) to wild-type *S. sonnei* (red) (F) in conditions provided by PM1-10 of the Biolog MicroArrays. Areas of overlap are coloured yellow, while differences are shown as patches of red or green. Wild-type *S. sonnei* seemed to respire better than *S. sonnei* Δ glpG Δ rhom7 + glpG in pH 9.5 + anthranilic acid (black box in E). However, this result was not reproducible.

Data information: In (A-D), data are presented as mean \pm SD from three independent experiments.

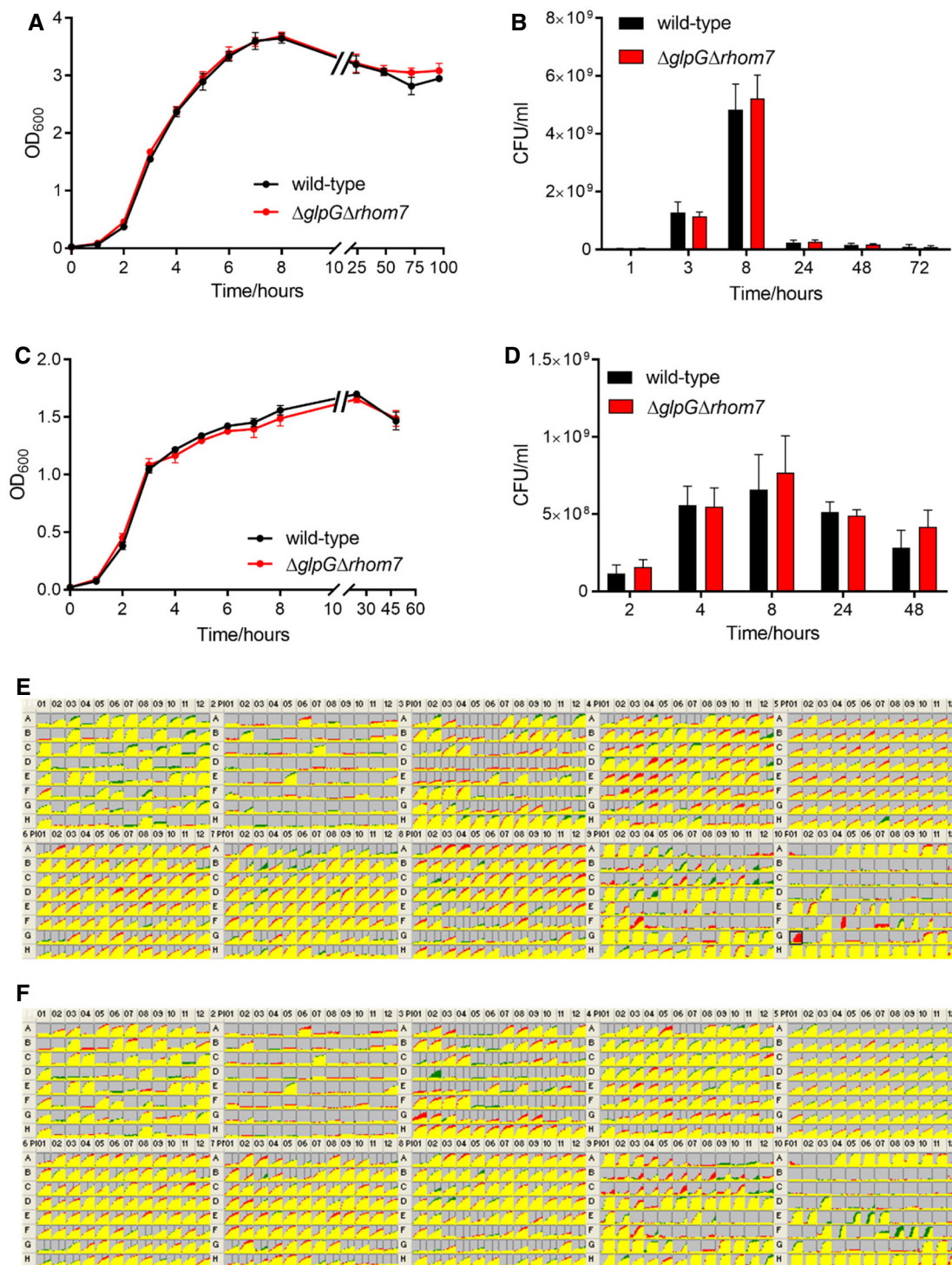


Figure EV2.

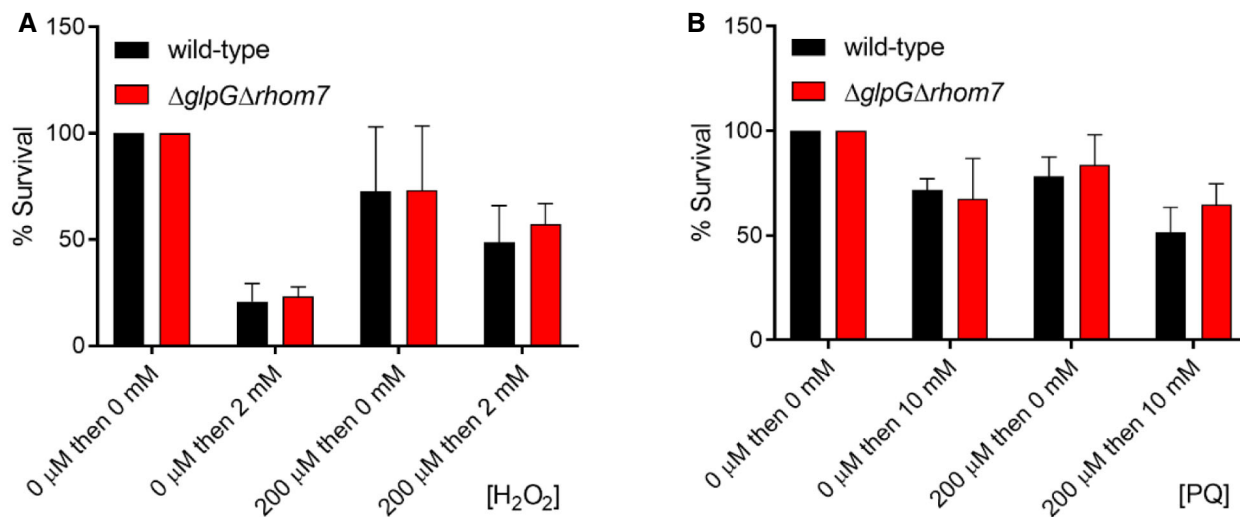


Figure EV3. GlpG and Rhom7 do not affect *S. sonnei* survival following oxidative stress.

A Resistance of wild-type *S. sonnei* or *S. sonnei* ΔglpGΔrhom7 to H₂O₂. *S. sonnei* was grown in LB to an OD₆₀₀ of ≈ 0.5 and then pre-incubated in 0 or 200 μM H₂O₂ for 20 min, before incubation in 0 or 2 mM H₂O₂ for 15 min. Bacterial survival was measured by plating samples to solid media. The percentage survival was calculated as the ratio of CFU recovered from H₂O₂-exposed bacteria compared to bacteria not exposed to H₂O₂.

B Resistance of wild-type *S. sonnei* or *S. sonnei* ΔglpGΔrhom7 to paraquat (PQ). *S. sonnei* was grown as above and then pre-incubated in 0 or 200 μM PQ for 15 min before incubation in 0 or 10 mM PQ for 45 min; samples were then plated to solid media to measure bacteria survival. The percentage survival was calculated as the ratio of CFU recovered from PQ-treated bacteria to those that were untreated.

Data information: In (A) and (B), data are presented as mean ± SD from three independent experiments.

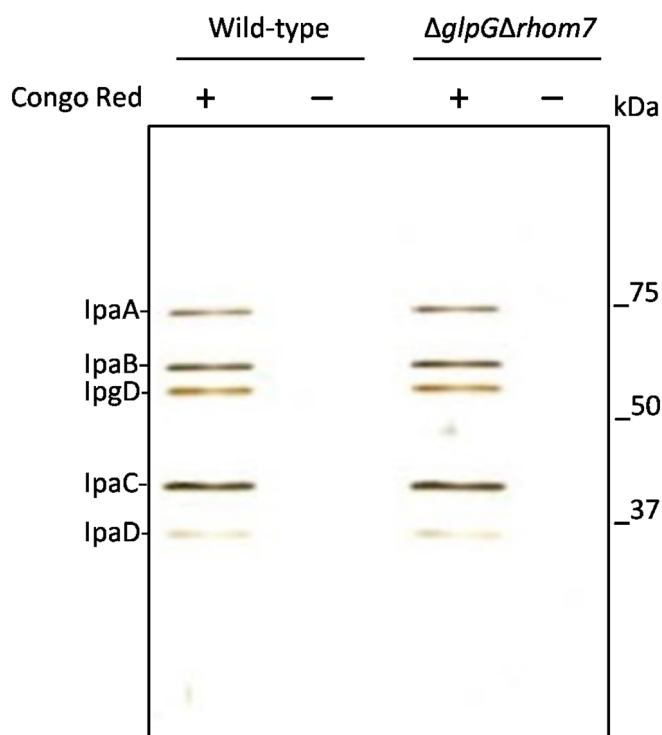


Figure EV4. T3SS-mediated secretion of Ipas by *S. sonnei* is rhomboid-independent.

T3SS-mediated secretion of effectors by wild-type *S. sonnei* and *S. sonnei* ΔglpGΔrhom7. Bacteria were grown to exponential phase (OD₆₀₀ ≈ 0.5), and Congo red (final concentration, 200 μg/ml) was added to bacteria to induce secretion. Supernatants were analysed by SDS-PAGE and silver staining. Sizes of a molecular weight marker are shown in kDa.

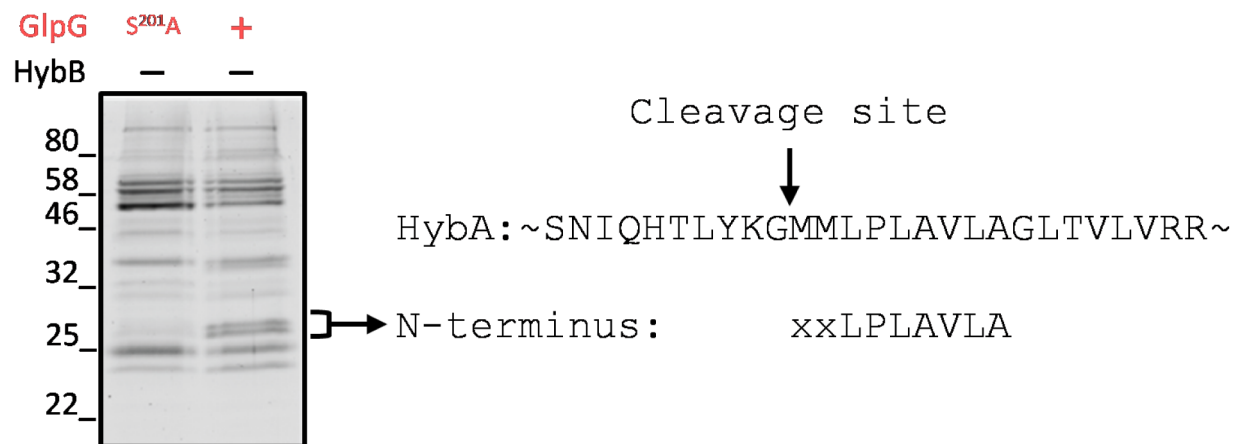


Figure EV5. Identification of HybA cleavage site by GlpG.

N-terminal sequencing of cleaved chromosomally encoded sfCherry-3xFLAG-tagged HybA by chromosomally encoded GlpG in *S. sonnei* in the absence of HybB. Bacteria were grown anaerobically in LB supplemented with 0.5% fumarate, 0.5% glycerol to $OD_{600} = 0.5$ before lysis. HybA was purified by anti-FLAG affinity chromatography prior to analysis by SDS-PAGE. Two bands only present in the GlpG (+) sample with molecular weight consistent with cleaved HybA were subject to N-terminal sequencing and gave the same result.