Expanded View Figures

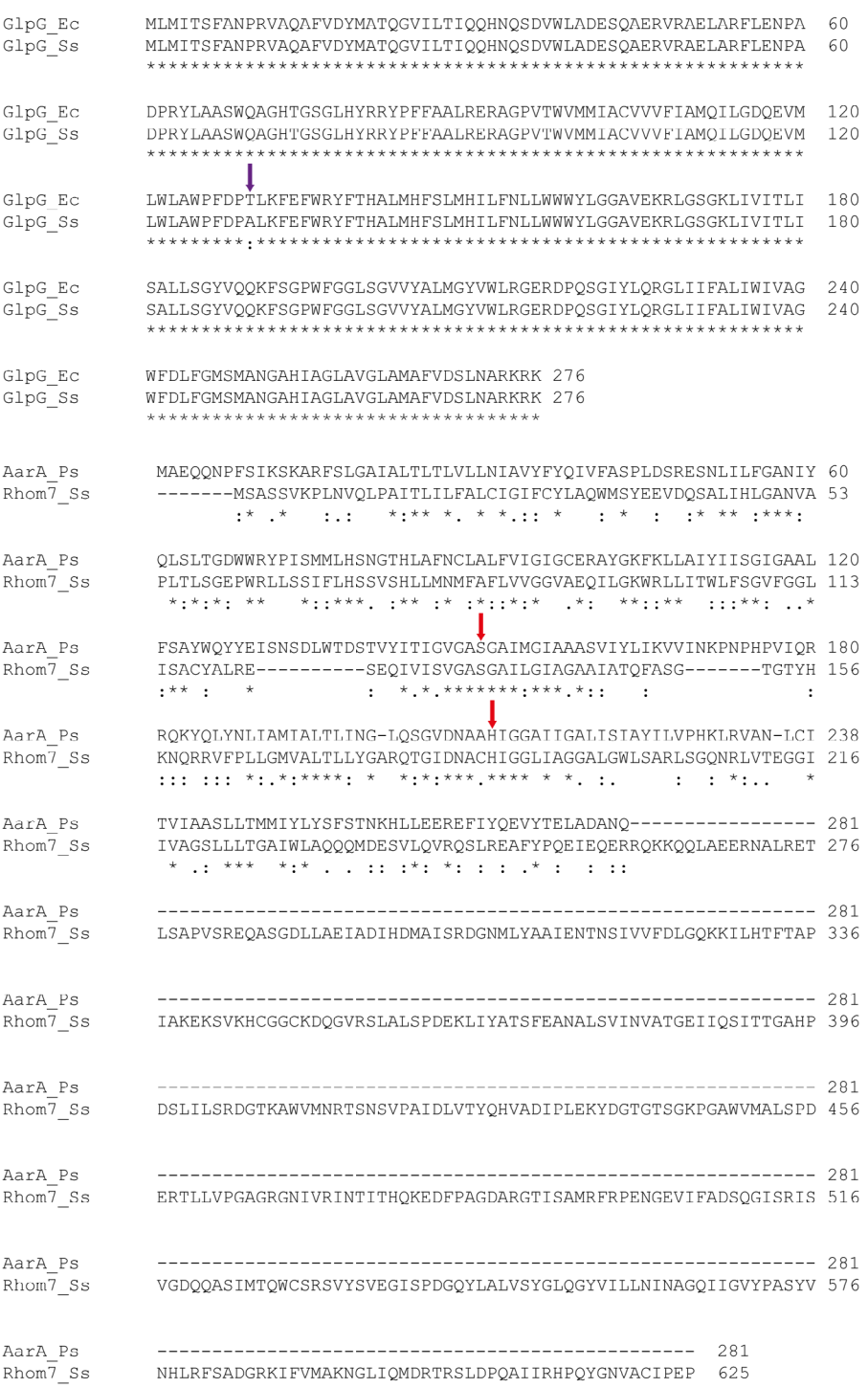

Figure EV1. Amino acid sequence alignments of *S. sonnei* GlpG (GlpG_Ss) with *E. coli* GlpG (GlpG_Ec), and *S. sonnei* Rhom7 (Rhom7_Ss) with *P. stuartii* AarA (AarA_Ps).

The single amino acid difference between the two versions of GlpG is highlighted by a purple arrow. The potential active site residues of Rhom7, Ser^{133} and His^{187}, 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 (OD_{600}) (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 KNO_3 with shaking at 180 rpm. Growth was assessed by measuring the OD_{600} 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 ± SD from three independent experiments.

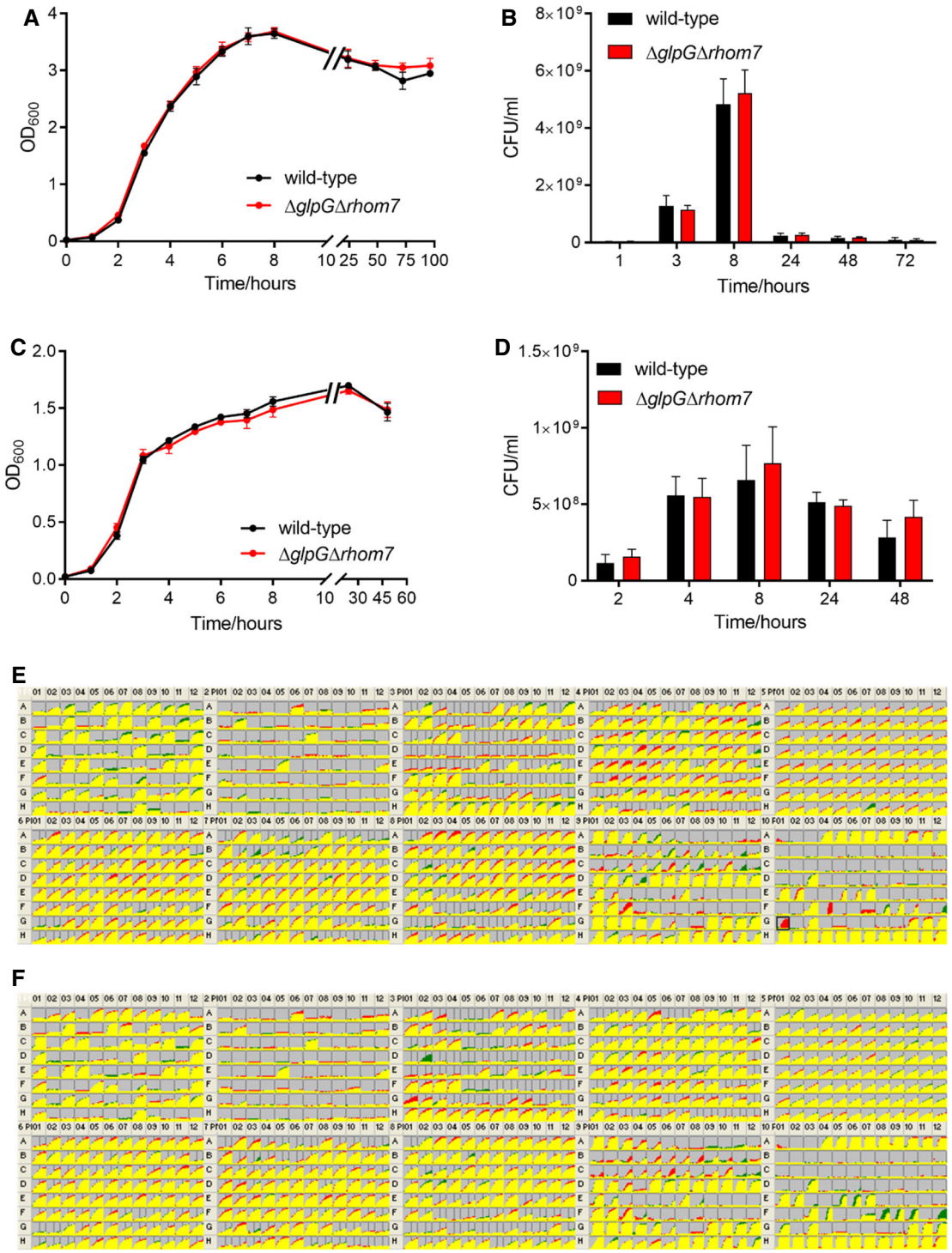

Figure EV2.

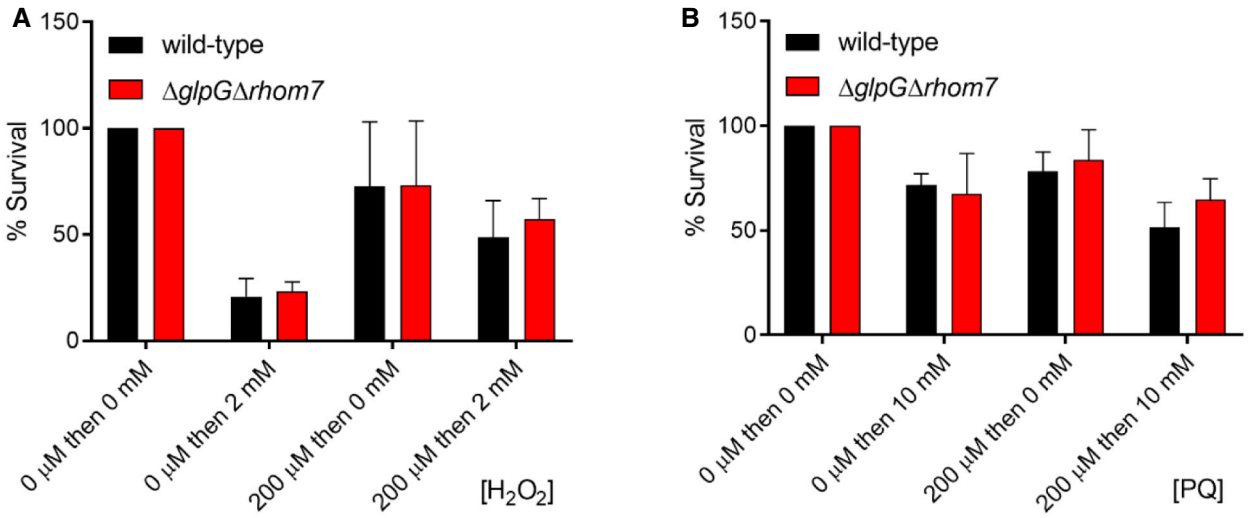

Figure EV3. GlpG and Rhom7 do not affect *S. sonnei* survival following oxidative stress.

A Resistance of wild-type *S. sonnei* or *S.sonnei* Δ*glpG*Δ*rhom7* to H_2O_2. *S. sonnei* was grown in LB to an OD_{600} of ≈ 0.5 and then pre-incubated in 0 or 200 μM H_2O_2 for 20 min, before incubation in 0 or 2 mM H_2O_2 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 H_2O_2-exposed bacteria compared to bacteria not exposed to H_2O_2.

B Resistance of wild-type *S. sonnei* or *S. sonnei* Δ*glpG*Δ*rhom7* 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.

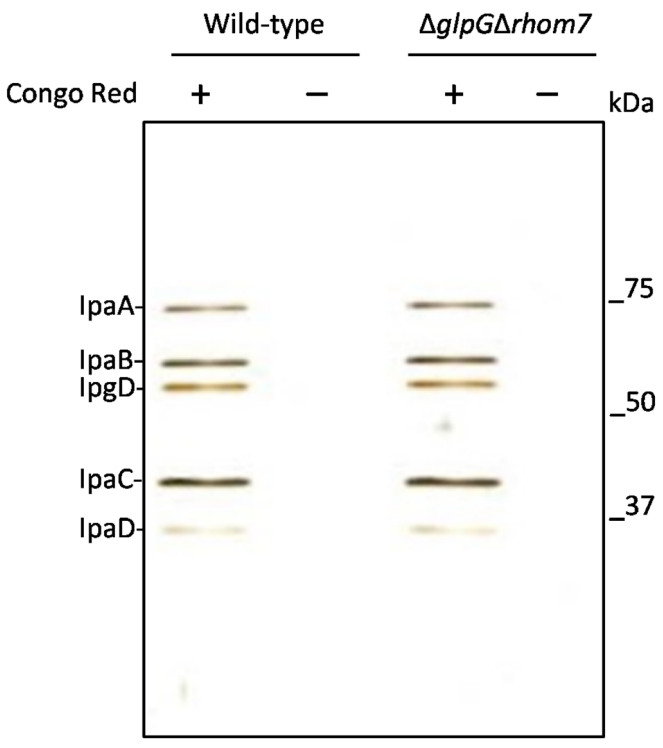

Figure EV4. T3SS-mediated secretion of Ipas by *S. sonnei* is rhomboid-independent.

T3SS-mediated secretion of effectors by wild-type *S. sonnei* and *S. sonnei* Δ*glpG*Δ*rhom7*. Bacteria were grown to exponential phase (OD_{600} ≈ 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.

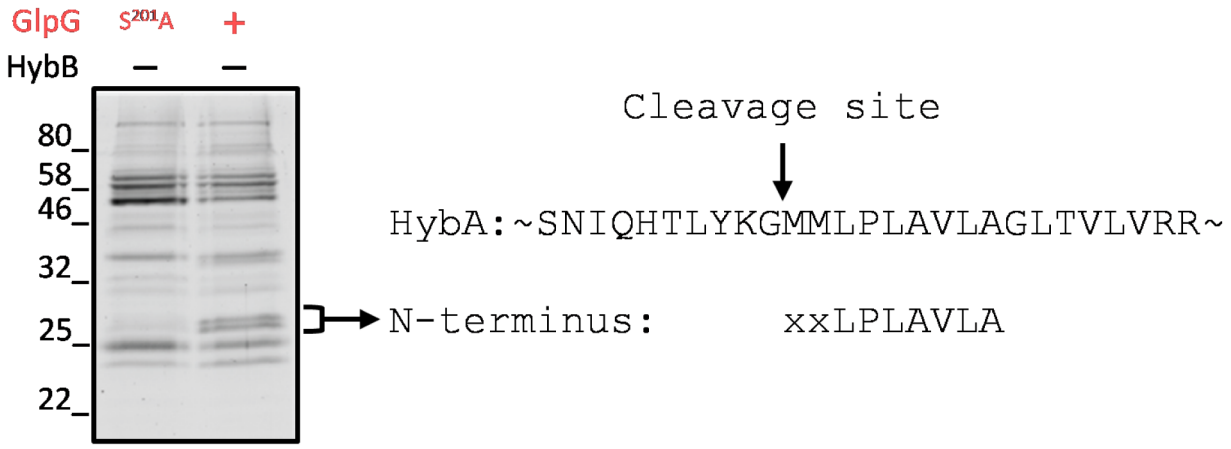

Figure EV5. Identification of HybA cleavage site by GlpG.
N-terminal sequencing of cleaved chromosomally encoded sfCherry-3xFLAG-tagged HybA by chromosomally encoded GlpG in *S. sonnei* in the absence of HybB. Bacteria were grown anaerobically in LB supplemented with 0.5% fumarate, 0.5% glycerol to OD_{600} = 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.