

Figure S1C

Figure S1D



**Figure S1: Mass spectrometry reveals that Cysteine<sup>58</sup> undergoes oxidation under aerobic conditions.** TpxD-cysteines in the reduced state were blocked with iodoacetic acid (IAA). Samples were resolved by SDS-PAGE and stained with Coomassie blue. A band corresponding to ~18 kDa was cut from the gel, reduced, and then incubated with iodoacetamide (IAM), to label the cysteines that were oxidized during the aerobic growth. The oxidation state of the cysteines was identified by mass spectrometry. (A and B) Relative abundance of the oxidized (Ox) and reduced (Red) states of the peptide containing Cys<sup>58</sup> (VLSVVPSIDTGIC<sup>58</sup>STQTR), in D39 grown under aerobic conditions without (A) and with (B) DTT treatment prior to IAA alkylation. (C and D) Relative abundance of the reduced state (Red) of the peptide containing Cys<sup>92</sup>,(WC<sup>92</sup>GAEGLDNAIMLSDYFDHSFGR) in D39 grown under aerobic conditions without (D) DTT treatment prior to IAA alkylation.

## Figure S2



Fig. S2: TpxD improves pneumococcal survival upon challenge with  $H_2O_2$ . Bacteria were grown under aerobic conditions to  $OD_{620}=0.3$  and challenged with the indicated  $H_2O_2$  concentrations for 15 min. Survival percentage was calculated by dividing the CFU of cultures after exposure to  $H_2O_2$  by the CFU of the control culture without  $H_2O_2$ . Results are the mean of two independent experiments.