FIG S1 Sequence alignment of HbpA proteins from *C. diphtheriae* strains 1737, 07-18, and 09-15. Regions in bold and boxed at the N- and C-terminus show the signal sequence and transmembrane regions, respectively. Amino acids in red are the putative Hb-binding site. The underlined sequence is a region of low sequence similarity and charged residues. Stars indicate identical conserved residues; dots indicate similar amino acids.

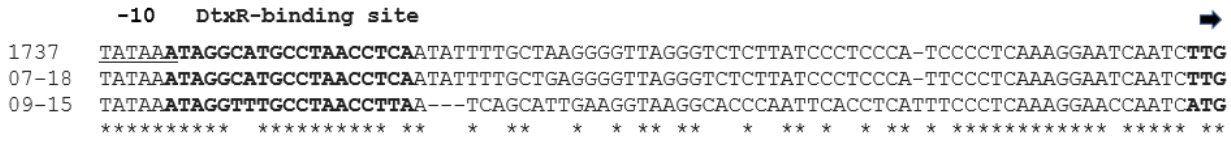

FIG S2 DNA sequence alignment of the upstream region of *hbpA* in *C. diphtheriae* strains 1737, 07-18, and 09-15. The *hbpA* promoter -10 sequence is underlined, bold sequence shows the DtxR binding site, and the start codon is in bold with the arrow above showing direction of transcription. Stars indicate conserved nucleotides.

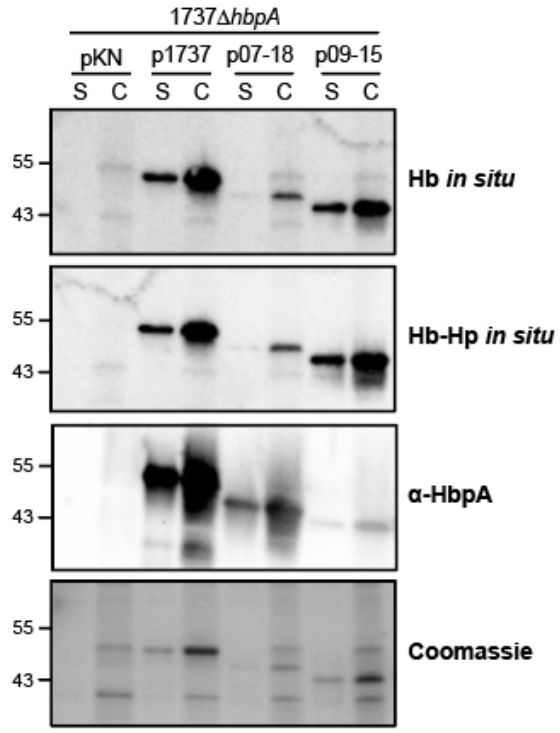

FIG S3 Austrian HbpA proteins expressed from plasmid pKN2.6Z in strain 1737Δ*hbpA* showed similar hemoprotein binding and cellular localization to that observed in their native strains. Supernatant (S) and cellular (C) protein fractions of each strain grown in low iron mPGT (0.25 μM $FeCl_3$) medium were separated by SDS-PAGE and then stained with Coomassie blue (bottom panel), probed with α-HbpA antibody (third panel) or assessed for binding to Hb or Hb-Hp as indicated using an *in situ* method. pKN indicates vector only control. A representative experiment is shown.

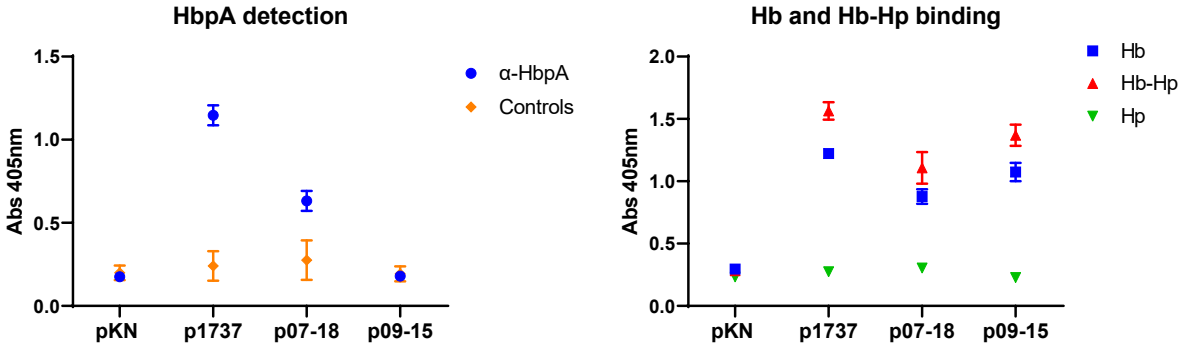

FIG S4 Austrian HbpA proteins expressed from plasmid pKN2.6Z in strain 1737Δ*hbpA* showed similar surface exposure and hemoprotein binding to that observed in their native strains. Bacteria were grown in low iron mPGT (0.25 μM $FeCl_3$) medium and the ELISA experiments were performed as described in Fig. 4A and B. Results show the mean and standard deviation of at least three experiments.

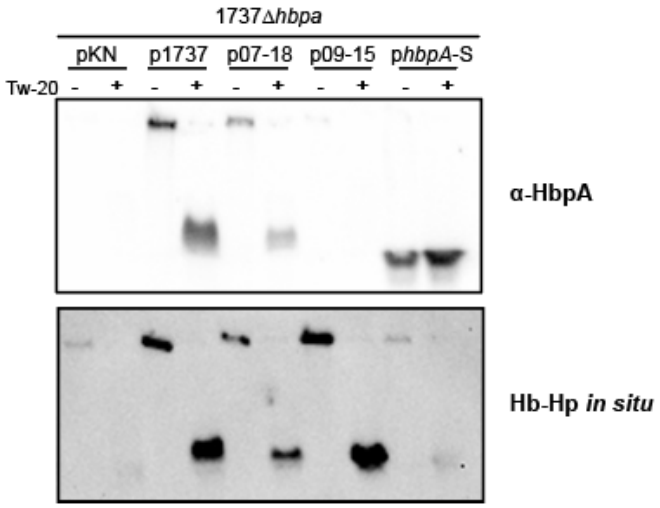

FIG S5 The Austrian HbpA proteins expressed in 1737Δ*hbpA* form large aggregates. Native gels were used to compare HbpA proteins present in supernatant fractions from *C. diphtheriae* strain 1737Δ*hpbA* that carried cloned *hbpA* genes from the strains indicated, expressed from plasmid pKN2.6Z (pKN indicates vector only control). Protein levels were normalized based on OD_{600} of the culture. Proteins in the supernatant fraction were separated by native PAGE, and then probed with α-HbpA antibody (upper panel) or assessed for Hb-Hp binding *in situ* (lower panel). The presence (+) or absence (-) of 0.1% Tween-20 is indicated. Plasmid p*hbpA*-S expresses the HbpA-S protein, which runs as a monomer and was used as a control (23). A representative experiment is shown.

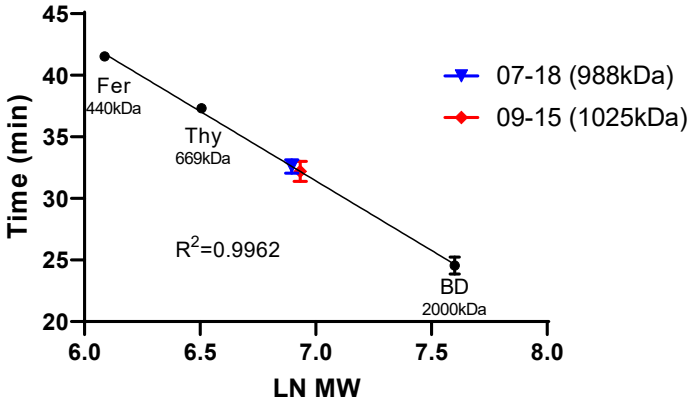

FIG S6 Analysis of SEC molecular weight standards for HbpA aggregate size calculation. High molecular weight SEC standards were assessed using a Superose 6 Increase 3.2/300 column. Retention time for blue dextran (BD), thyroglobulin (Thy), and ferritin (Fer) was plotted against the natural log of their molecular weight. A line of best fit (R^2 = 0.9962) was generated using GraphPad Prism 9.1.2 and was used to calculate an estimated size for HbpA protein from *C. diphtheriae* Austrian strains 07-18 and 09-15 (shown in parenthesis). Retention times are average and standard deviation of three replicates.

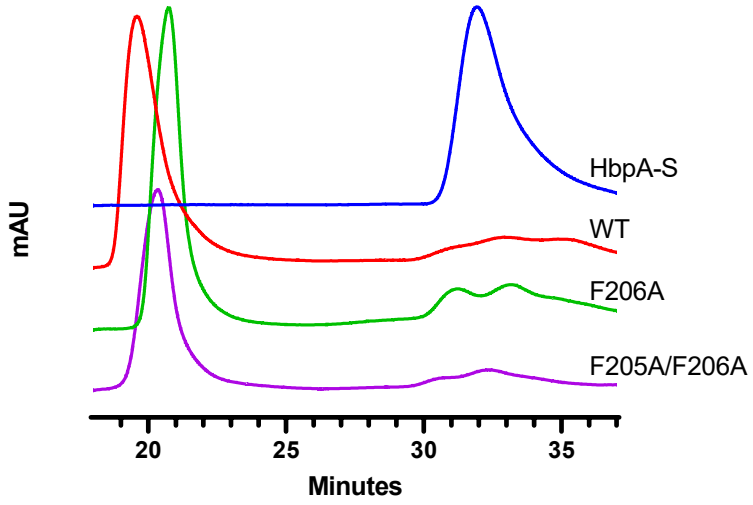

FIG S7 SEC was used to assess protein aggregation for purified recombinant HbpA proteins containing amino acid substitutions in the Hb-binding site. A lower elution time (minutes) indicates a larger molecular weight species. Samples were run on a Superdex 200 increase 3.2/300 column to identify presence of aggregates; each trace shows a representative sample from at least three replicates.