

Electronic Supplementary Information

Dissecting binding of a β -barrel outer membrane protein by phage display

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PCR conditions for generating the ShuA loop deletion or alanine-substitution variants

One of the following DNA templates (~50 ng) was used to generate the ShuA loop deletion or alanine-substituted variants for phage display or protein expression: pEShuA¹⁸, PCR products containing overlapping regions of the ShuA gene with the mutation of interest, or phagemid DNA encoding a ShuA extracellular variant. Platinum DNA Polymerase (Invitrogen) was used according to the manufacturer's specifications with the oligonucleotides listed in Table SI 1 and the following PCR thermal cycling conditions: 1 cycle of 2 min at 98 °C, followed by 30 cycles of 1 min at 98 °C, 1 min at 60 °C, and 2 min at 68 °C, and finishing with 5 min incubation at 68 °C.

Table SI 1. Oligonucleotide sequences

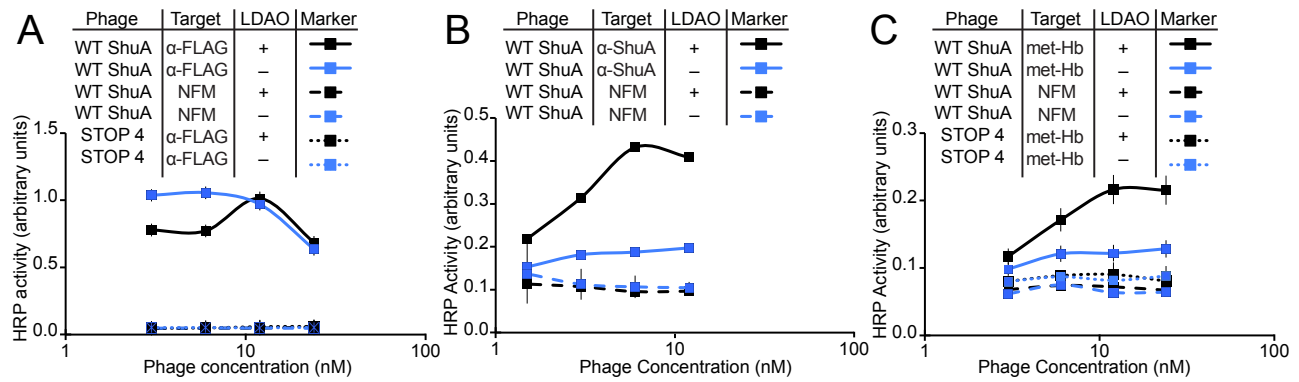
| Oligonucleotide Label | DNA Sequence (5' to 3') | Resulting ShuA variant |
|-----------------------|--|------------------------|
| OL_L1_Sb_F | TCG TGT CTT TGG TAC TGG CGG CAC GGG GGC CGC GAG CCT GGG ATT AGG CGC GAG CGC GTT | Ala-L1 |
| OL_L1_Sb_R | AAC GCG CTC GCG CCT AAT CCC AGG CTC GCG GCC CCC GTG CCG CCA GTA CCA AAG ACA CGA | Ala-L1 |
| OL_L2_Sb_F | TTG TGG CCT GGT CCA GTC GCG ATC GGG GTG ATG CGG CCG CGG CCG CTG CAG CAG CCG CGG CGA ATG ACG AGT CCA TTA ATA ACA TGC T | Ala-L2 |
| OL_L2_Sb_R | AGC ATG TTA TTA ATG GAC TCG TCA TTC GCC GCG GCT GCT GCA GCG GCC GCG GCC GCA TCA CCC CGA TCG CGA CTG GAC CAG GCC ACA A | Ala-L2 |
| OL_L3_Sb_F | TAC AAC AAC GAC GCG CGT GAA CCA AAA AAT GCG GCG GCC GCA GCG GCT GCG GCA GCC GCC AAC CCG ATG GTT GAT CGT TCA ACA ATT CAA | Ala-L3 |
| OL_L3_Sb_R | TTG AAT TGT TGA ACG ATC AAC CAT CGG GTT GGC GGC TGC CGC AGC CGC TGC GGC CGC CGC ATT TTT TGG TTC ACG CGC GTC GTT GTT GTA | Ala-L3 |
| OL_L4_Sb_F | GAA GTC CGT ATT AAT GCG CAA AAC GCA GCG GCA GCC GGC GAG TAT CGT GAA CAG ATA ACA | Ala-L4 |

| | | |
|-------------|--|---------|
| OL_L4_Sb_R | TGT TAT CTG TTC ACG ATA CTC GCC GGC TGC | Ala-L4 |
| OL_L5_Sb_F | CGC TGC GTT TTG CGC ATT AAT ACG GAC TTC TGA GTA TTA TCG TCA GGA ACA ACA TCC | Ala-L5 |
| OL_L5_Sb_R | GGG CGG CGC GGC GGC GGC CGC ACC GCA AGC AAA AAT CGA TTT TAG CTC GAG CTA AAA TCG ATT TTT GCT TGC GGT | Ala-L5 |
| OL_L6_Sb_F | GCG GCC GCC GCC GCG CCG CCC GGA TGT TGT TCC TGA CGA TAA TAC TCA | Ala-L6 |
| OL_L6_Sb_R | AGT TAT CGC GGT AGC AGT GAC GGT GCG AAA GAT GTT GAT GCC GAC AAA TGG TCA TCT | Ala-L6 |
| OL_L7A_Sb_F | AGA TGA CCA TTT GTC GGC ATC AAC ATC TTT CGC ACC GTC ACT GCT ACC GCG ATA ACT | Ala-L6 |
| OL_L7A_Sb_R | TGC CCA GGC ATT CCG CGC CCC GAC GGC GGC CGC AGC GGC AGC CGC GGC TGC GGC CGC CGC GGC GGG TCG CTT CTA TAC CAA CTA TTG GGT | Ala-L7A |
| OL_L7A_Sb_R | ACC CAA TAG TTG GTA TAG AAG CGA CCC GCC GCG GCG GCC GCA GCC GCG GCT GCC GCT GCG GCC GCC GTC GGG GCG CGG AAT GCC TGG GCA | Ala-L7A |
| OL_L7B_Sb_F | ACG ATT CTA AGC ACT TCT CGA TTG CGG CCG CCG CAG CCG CCG CGG CGG CGG CAG CCG CGG CCT TAC GTC CGG AAA CTA ACG AAA CTC AGG | Ala-L7B |
| OL_L7B_Sb_R | CCT GAG TTT CGT TAG TTT CCG GAC GTA AGG CCG CGG CTG CCG CCG CCG CGG CGG CTG CGG CGG CCG CAA TCG AGA AGT GCT TAG AAT CGT | Ala-L7B |
| OL_L8_Sb_F | AAG GAT TAC ATC TCC ACG ACC GTC GAT GCC GCG GCG GCG ACG ACT ATG TCG TAT AAC GT | Ala-L8 |
| OL_L8_Sb_R | ACG TTA TAC GAC ATA GTC GTC GCC GCC GCG GCA TCG ACG GTC GTG GAG ATG TAA TCC TT | Ala-L8 |
| OL_L9_Sb_F | TAA CCG TAC CCG CGG CAA AGA CAC CGA TGC CGC GGC AGC GGC GGC CAG CAT TAA CCC GGA TAC CGT TAC CA | Ala-L9 |
| OL_L9_Sb_R | TGG TAA CGG TAT CCG GGT TAA TGC TGG CCG CCG CTG CCG CGG CAT CGG TGT CTT TGC CGC GGG TAC GGT TA | Ala-L9 |
| OL_L10_Sb_F | TTC TCT GTT GGG TGG GTT GGT ACG TTT GCC GAT CGC GCA GCA GCG GCC GCC GCG GCT GCC GCG GCG GCA CCA GGC TAT GGC GTG AAT GAT | Ala-L10 |
| OL_L10_Sb_R | ATC ATT CAC GCC ATA GCC TGG TGC CGC | Ala-L10 |

| | | |
|--------------|--|---------|
| | CGC GGC AGC CGC GGC GGC CGC TGC TGC GCG ATC GGC AAA CGT ACC AAC CCA CCC AAC AGA GAA | |
| OL_L11_Sb_F | ACT ACT TTG GTG TTG GGT AAC GCT GCC GCC GCG GCG GCC GCG GCG GCG GCA GCC GCC GCA GCG GCT GGT CGT AAC GGA AAA ATT TTC GTG | Ala-L11 |
| OL_L11_Sb_R | CAC GAA AAT TTT TCC GTT ACG ACC AGC CGC TGC GGC GGC TGC CGC CGC CGC GGC CGC CGC GGC GGC AGC GTT ACC CAA CAC CAA AGT AGT | Ala-L11 |
| OL_L1_Del_F | T CGT GTC TTT GGT ACT GGC GGC ACG GGG AGC CTG GGA TTA GGC GCG AGC GCG TTT | ΔL1 |
| OL_L1_Del_R | AAA CGC GCT CGC GCC TAA TCC CAG GCT CCC CGT GCC GCC AGT ACC AAA GAC ACG AA | ΔL1 |
| OL_L2_Del_F | TTG TGG CCT GGT CCA GTC GCG ATC GGG GTG ATA ATG ACG AGT CCA TTA ATA ACA TGC T | ΔL2 |
| OL_L2_Del_R | AGC ATG TTA TTA ATG GAC TCG TCA TTA TCA CCC CGA TCG CGA CTG GAC CAG GCC ACA A | ΔL2 |
| OL_L3_Del_F | TAC AAC AAC GAC GCG CGT GAA CCA AAA AAT AAC CCG ATG GTT GAT CGT TCA ACA ATT CAA | ΔL3 |
| OL_L3_Del_R | TTG AAT TGT TGA ACG ATC AAC CAT CGG GTT ATT TTT TGG TTC ACG CGC GTC GTT GTT GTA | ΔL3 |
| OL_L4_Del_F | TTG GTC GGA AGT CCG TAT TAA TGC GCA AAA CGG CGA GTA TCG TGA ACA GAT AAC A | ΔL4 |
| OL_L4_Del_R | TGT TAT CTG TTC ACG ATA CTC GCC GTT TTG CGC ATT AAT ACG GAC TTC CGA CCA A | ΔL4 |
| OL_L5_Del_F | TGA GTA TTA TCG TCA GGA ACA ACA TCC GGG CGG CCC GCA AGC AAA AAT C | ΔL5 |
| OL_L5_Del_R | GAG CTA AAA TCG ATT TTT GCT TGC GG G CCG CCC GGA TGT TGT TCC TGA CGA | ΔL5 |
| OL_L6_Del_F | GAC AGT TAT CGC GGT AGC AGT GAC GGT AAA GAT GTT GAT GCC GAC AAA TGG TCA TCT CGT | ΔL6 |
| OL_L6_Del_R | ACG AGA TGA CCA TTT GTC GGC ATC AAC ATC TTT ACC GTC ACT GCT ACC GCG ATA ACT GTC | ΔL6 |
| OL_L7A_Del_F | TAT TTG GCT CAT ATG CCC AGG CAT TCC GCG GTC GCT TCT ATA CCA ACT ATT GGG TGC CA | ΔL7A |
| OL_L7A_Del_R | TGG CAC CCA ATA GTT GGT ATA GAA GCG ACC GCG GAA TGC CTG GGC ATA TGA GCC AAA TA | ΔL7A |

| | | |
|----------------|---|---|
| OL_L7B_Del_F | TG TAT AAC GAT TCT AAG CAC TTC TCG ATT GAA ACT AAC GAA ACT CAG GAG TAC GGT TT | ΔL7B |
| OL_L7B_Del_R | AAA CCG TAC TCC TGA GTT TCG TTA GTT TCA ATC GAG AAG TGC TTA GAA TCG TTA TAC A | ΔL7B |
| OL_L8_Del_F | AAG GAT TAC ATC TCC ACG ACC GTC GAT GCG GCG GCG ACG ACT ATG TCG TAT AAC GT | ΔL8 |
| OL_L8_Del_R | ACG TTA TAC GAC ATA GTC GTC GCC GCC GCA TCG ACG GTC GTG GAG ATG TAA TCC TT | ΔL8 |
| OL_L9_Del_F | TAA CCG TAC CCG CGG CAA AGA CAC CGA TAG CAT TAA CCC GGA TAC CGT TAC CA | ΔL9 |
| OL_L9_Del_R | TGG TAA CGG TAT CCG GGT TAA TGC TAT CGG TGT CTT TGC CGC GGG TAC GGT TA | ΔL9 |
| OL_L10_Del_F | TTC TCT GTT GGG TGG GTT GGT ACG TTT GCC GAT CGC CCA GGC TAT GGC GTG AAT GAT T | ΔL10 |
| OL_L10_Del_R | AAT CAT TCA CGC CAT AGC CTG GGC GAT CGG CAA ACG TAC CAA CCC ACC CAA CAG AGA A | ΔL10 |
| OL_L11_Del_F | ATG ACC ACT ACT TTG GTG TTG GGT AAC GCT GGT CGT AAC GGA AAA ATT TTC GTG AGT | ΔL11 |
| OL_L11_Del_R | ACT CAC GAA AAT TTT TCC GTT ACG ACC AGC GTT ACC CAA CAC CAA AGT AGT GGT CAT | ΔL11 |
| pM1155a_NsiI_F | AGC TTC ATG CAT GCG ATT ACA AGG ATG ACG ACG AT | For sub-cloning into the phage display vector |
| pM1155a_NcoI_R | ATC CTC CAC CAC TAG TAC CAT GGT ACC AT | For sub-cloning into the phage display vector |
| pET22b_MscI_F | 5'-AGC CTG TGG CCA TGG CTA CTG AAA CCA TGA CCG TTA CGG CAA | For sub-cloning into the protein expression vector |
| pET22b_XhoI_R | 5'-TTG GCT CTC GAG CCA TTG ATA ACT CAC GAA AAT TTT TCC GTT ACG A | For sub-cloning into the protein expression vector |

*F and R designate forward and reverse primers, respectively.



SI Figure 1. Functional phage-displayed wild-type ShuA requires the detergent LDAO.

Phage-displayed wild-type ShuA or STOP4 (negative control phage) were incubated in the presence and absence of the detergent LDAO with immobilized **A**) anti-FLAG antibody (α -FLAG), **B**) anti-ShuA antibody (α -ShuA), or **C**) met-hemoglobin (met-Hb). Relative levels of the bound phage-displayed ShuA variants were quantified by anti-M13 antibody conjugated to HRP. Throughout this report, each data point represents the average of three replicates, and error bars indicate standard deviation around the mean.

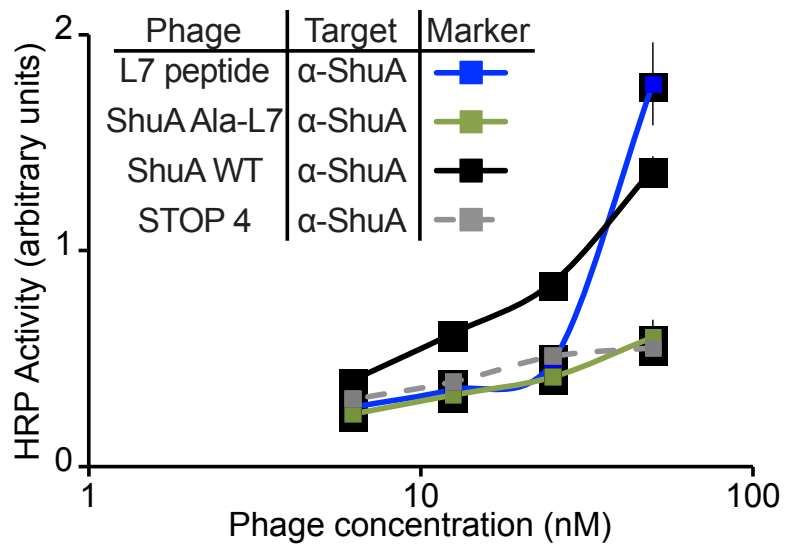


Figure SI 2. Phage-based ELISAs of a phage-displayed peptide, ShuA Loop 7. Phage-displayed ShuA L7 peptide, full-length ShuA alanine-substituted L7 variant (Ala-L7), wild-type ShuA (positive control phage), or STOP4 (negative control phage) were incubated with immobilized anti-ShuA antibody coated on microtiter plate wells. Relative levels of the bound phage-displayed ShuA variants and peptide were quantified by anti-M13 antibody conjugated to HRP. No binding was observed for phage-displayed ShuA Ala-L7 to the anti-ShuA antibody, but the phage-displayed peptide ShuA Loop 7 bound the immobilized target. Throughout this report, each data point represents the average of three replicates, and error bars indicate standard deviation around the mean.

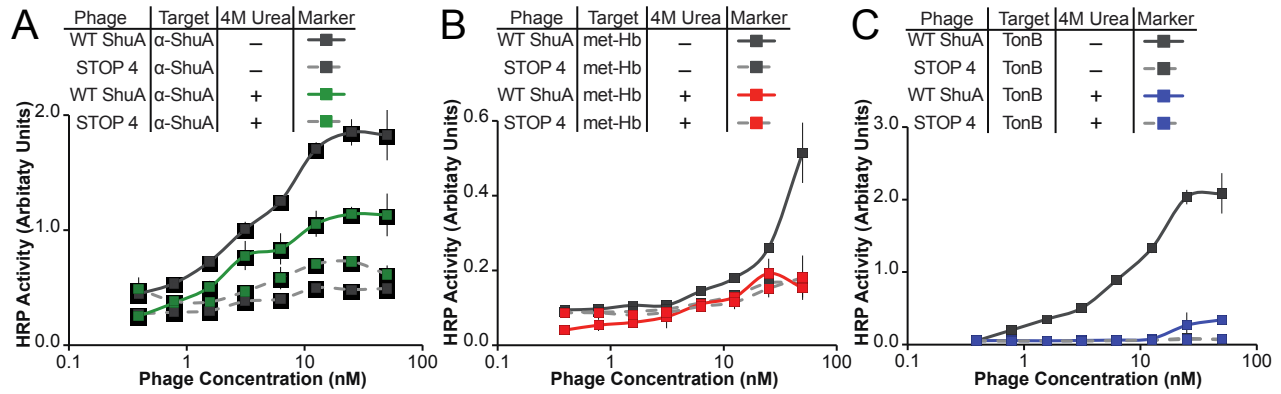


Figure SI 3. Phage-based ELISAs of phage-displayed wild-type ShuA treated with 4M urea.

Phage-displayed wild-type ShuA and STOP4 (negative control phage) were assayed for binding in the presence or absence of 4M urea. Following treatment with urea, the phage-displayed wild-type ShuA or STOP4 were purified from excess urea through a second PEG precipitation and incubated with the following immobilized targets: **A)** anti-ShuA antibody, **B)** met-Hemoglobin, or **C)** TonB. Relative levels of the bound phage-displayed ShuA were quantified by anti-M13 antibody conjugated to HRP. Phage-displayed wild-type ShuA treated with 4 M urea did not bind to the met-hemoglobin or TonB protein. However, binding is observed by urea-denatured, phage-displayed wild-type ShuA to the immobilized anti-ShuA antibody. Throughout this report, each data point represents the average of three replicates, and error bars indicate standard deviation around the mean.

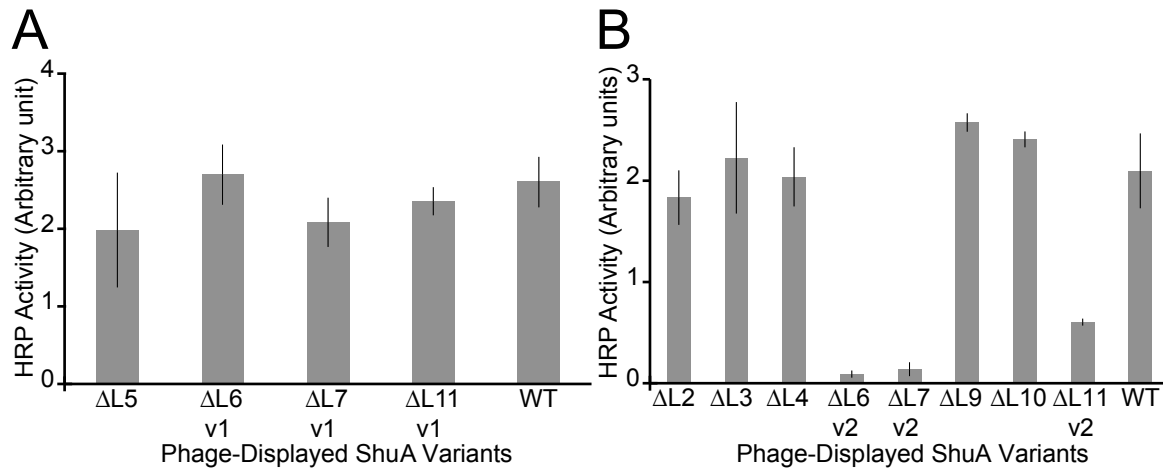


Figure SI 4. Phage-based ELISAs of ShuA extracellular loop deletion variants. To evaluate display levels, 50 nM of phage-displayed ShuA extracellular loop deletion variants, $\Delta L2$ through $\Delta L11$, or wild-type ShuA (positive control) were assayed for binding to immobilized anti-FLAG antibody, which can recognize a FLAG epitope fused to the N-terminus of the ShuA variants. **A)** Phage-displayed ShuA $\Delta L5$, $\Delta L6v1$, $\Delta L7v1$, and $\Delta L11v1$ demonstrated display levels similar to wild-type ShuA (WT) **B)** No or low display levels were observed for ShuA $\Delta L6v2$, $\Delta L7v2$, and $\Delta L11v2$ when one or two native amino acid residue(s) were removed as compared to wild-type ShuA (WT) and its loop deletion variants, ShuA $\Delta L2$, $\Delta L3$, $\Delta L4$, $\Delta L9$, and $\Delta L10$. Thus, this report focuses on the loop deletion variants that demonstrated similar display levels.

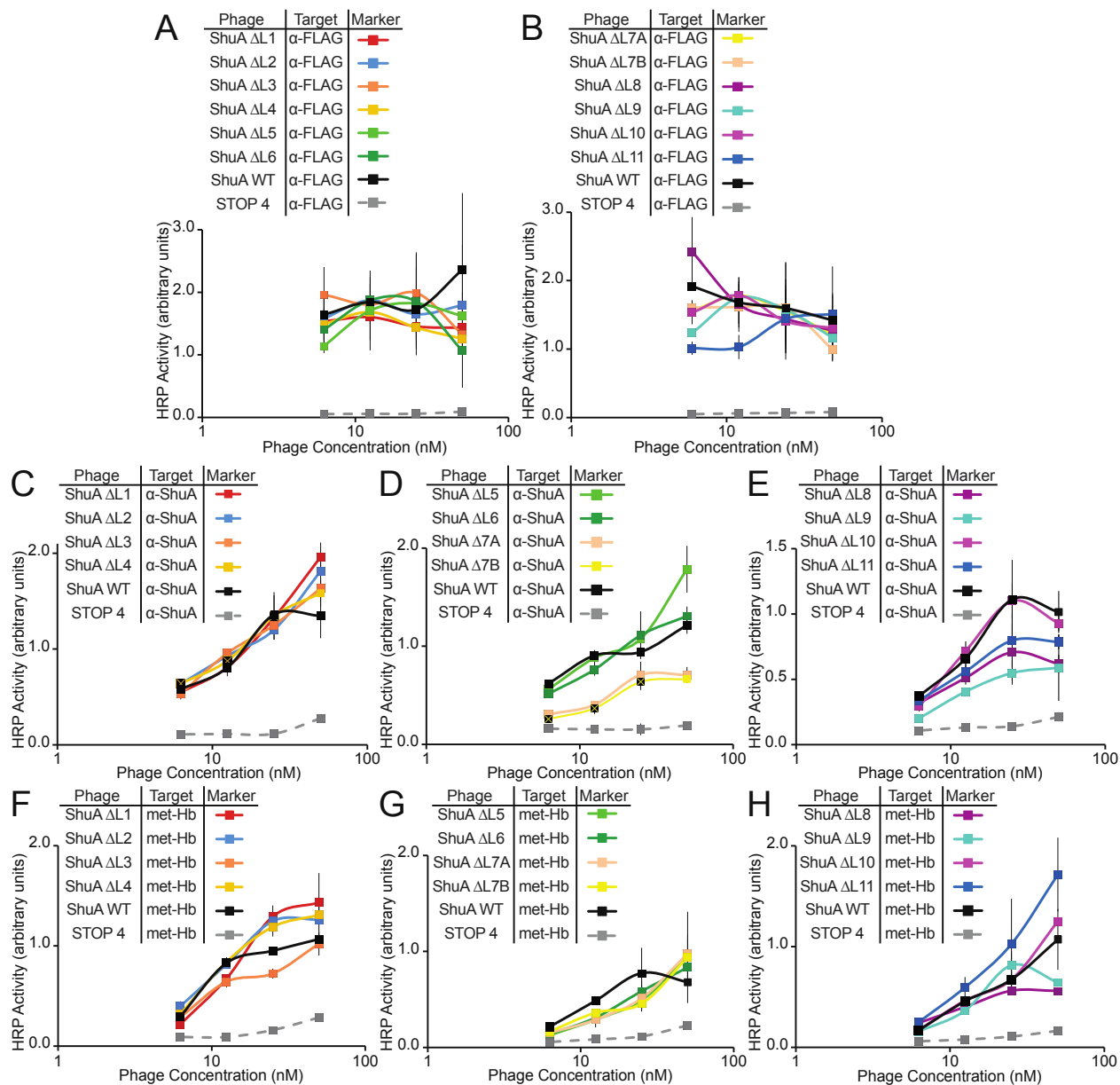


Figure SI 5. Functional ShuA extracellular loop deletion variants displayed on the phage surface. Phage-displayed ShuA loop deletion variants, Δ L1 through Δ L11, wild-type ShuA (positive control phage) or STOP4 (negative control phage) were incubated with immobilized **A** and **B**) anti-FLAG antibody (α -FLAG), **C**, **D**, and **E**) anti-ShuA antibody (α -ShuA), or **F**, **G**, **H**) methemoglobin (metHb). Relative levels of the bound phage-displayed ShuA variants were quantified by anti-M13 antibody conjugated to HRP. Throughout this report, each data point represents the average of three replicates, and error bars indicate standard deviation around the mean.

Figure SI 6. SDS-PAGE analysis of TonB.

Eluted fractions of a 92 residue C-terminal fragment of TonB (residue 142 – 239) from *S. dysenteriae* were purified by size exclusion chromatography, and visually examined in a 12% Tris-glycine SDS-PAGE gel stained by Coomassie blue. **Lane L.** PageRuler Plus pre-stained protein ladder (ThermoFisher Scientific). **Lanes 1 – 9.** Samples (15 μ L) of the 2 mL fractions were collected through size exclusion chromatography. This purified TonB protein was coated on microtiter plates for TonB binding ELISAs with phage-displayed and detergent-solubilized ShuA variants, using conditions described above.

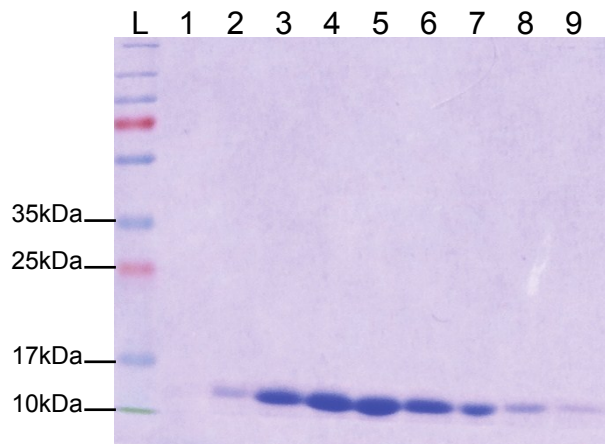


Figure SI 7. SDS-PAGE analysis of met-hemoglobin. A

sample of met-hemoglobin purified from human red blood cells was visually examined in a 12% Tris-glycine SDS-PAGE gel stained by Coomassie blue. **Lane L.** PageRuler Plus pre-stained protein ladder **Lane 2.** Sample (5 μ L) of 1 mg/mL met-hemoglobin. This protein sample was coated on microtiter plates for hemoglobin binding ELISAs with phage-displayed and detergent-solubilized ShuA variants, using conditions described above.

