

Supplementary Materials

NMR Identification of the Binding Surfaces Involved in the *Salmonella* and *Shigella* Type III Secretion Tip-Translocon Protein-Protein Interactions

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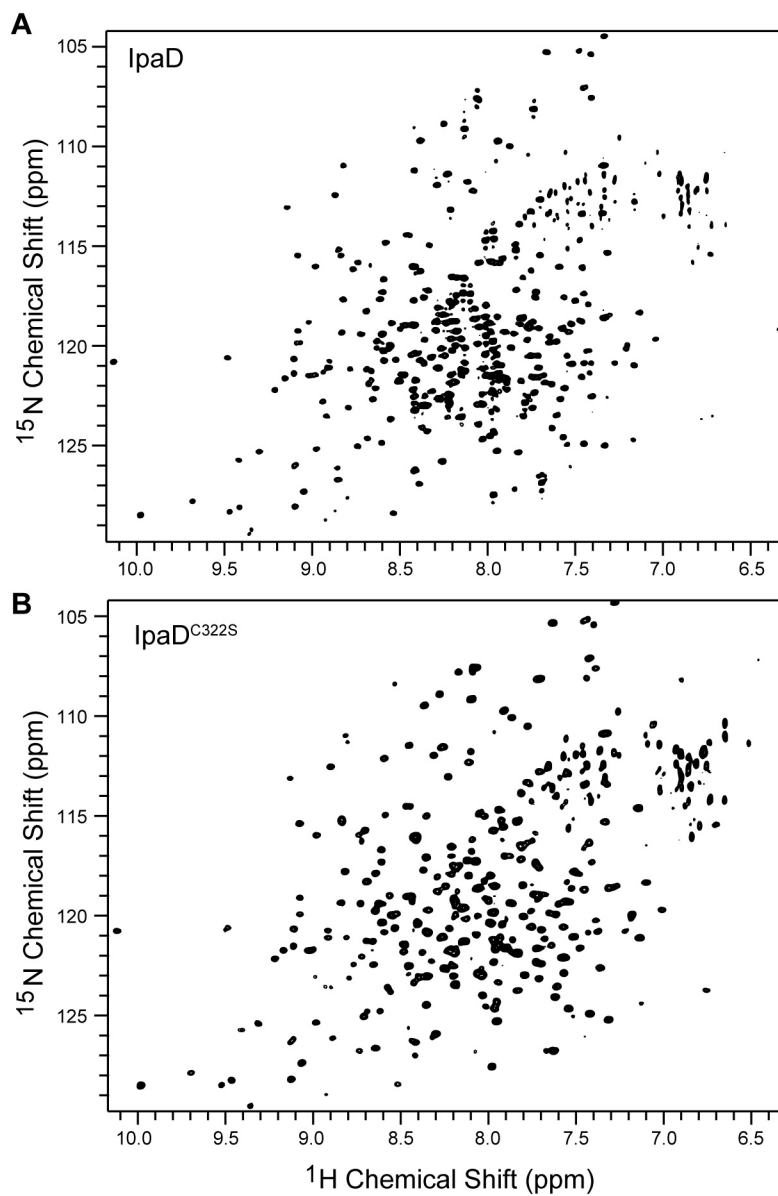


Figure S1. Comparison of ¹H-¹⁵N TROSY spectra of (A) IpaD and (B) IpaD^{C322S}.

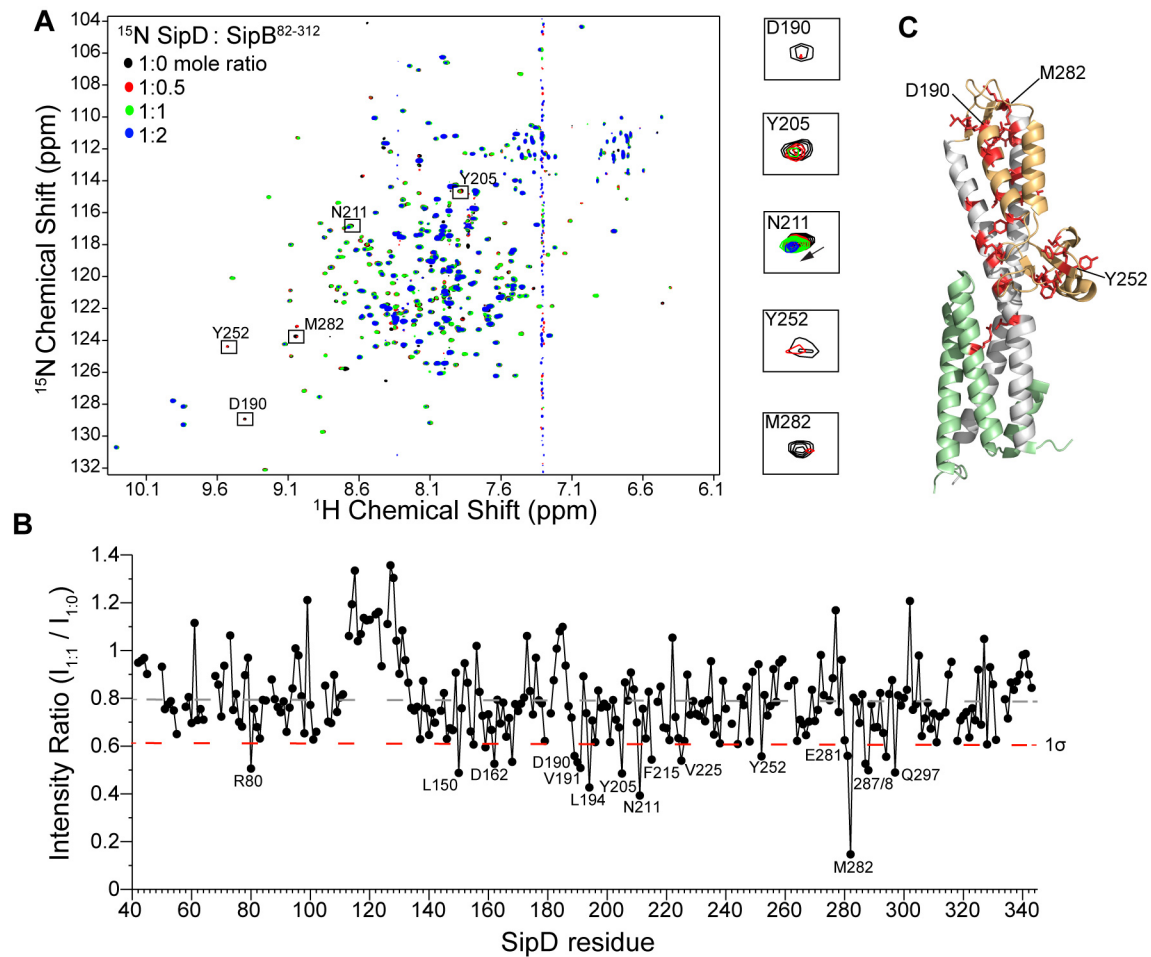


Figure S2. SipB⁸²⁻³¹² interacts with SipD^{C244S}. **(A)** Overlay of four ^1H - ^{15}N TROSY spectra of ^{15}N /ILV SipD^{C244S} with increasing concentrations of unlabeled SipB⁸²⁻³¹². **(B)** Plot of peak intensity ratio ($I_{1:1}/I_{1:0}$) at SipD^{C244S}:SipB⁸²⁻³¹² molar ratio of 1:1.1 **(C)** Residues with peak intensity ratio ($I_{1:1}/I_{1:0}$) lower than 1 standard deviation below average (1σ) are mapped onto the crystal structure of SipD^{C244S}.

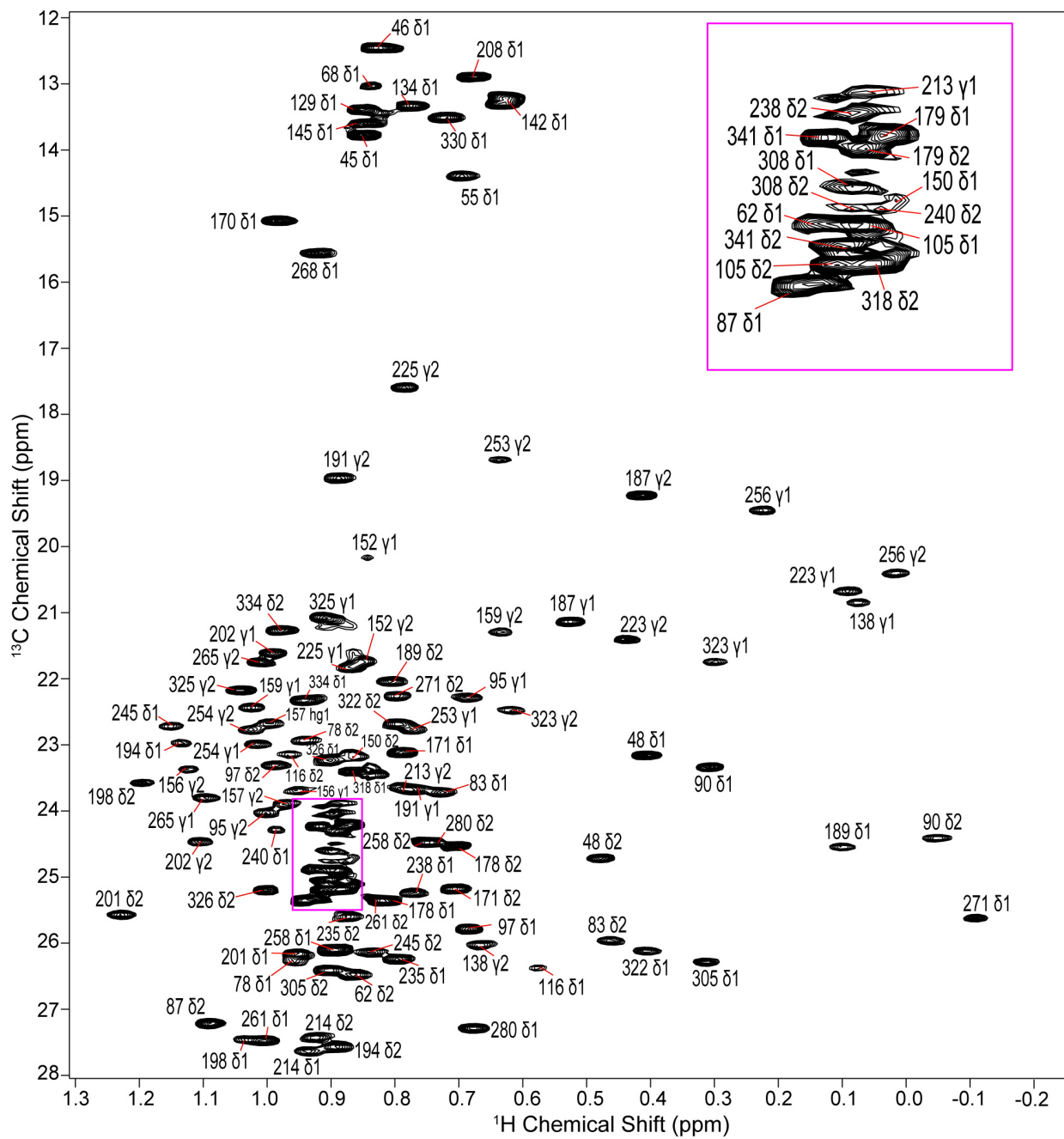


Figure S3. Complete stereospecific ILV assignments of SipD^{C244S}.

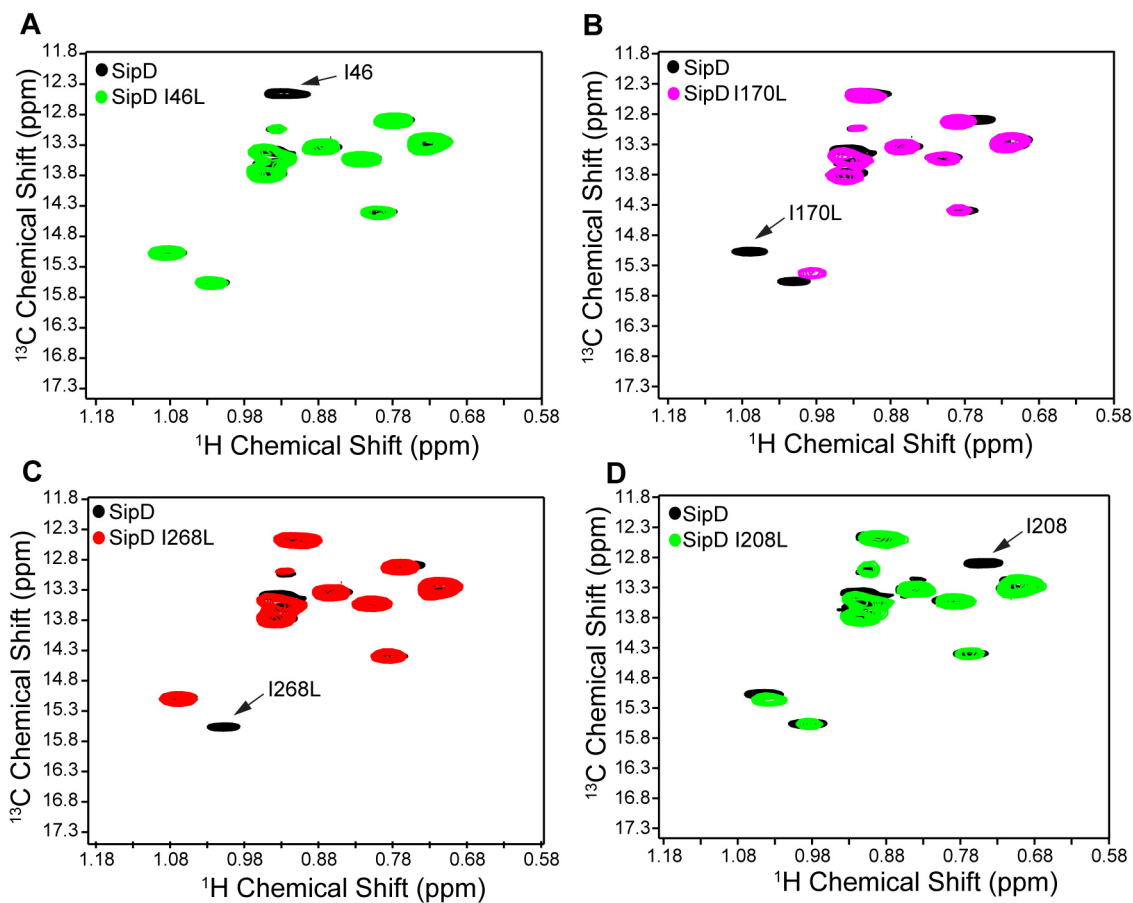


Figure S4. Ile to Leu mutations used in assigning the isoleucine $^{13}\text{C}\delta 1$ methyl peaks of SipD^{C244S}. Representative examples of ^1H - ^{13}C HSQC spectra of ^{15}N /ILV SipD^{C244S} with point mutations in (A) I46L (B) I170L (C) I268L (D) I208L overlaid with the ^1H - ^{13}C HSQC spectrum of wild type SipD^{C244S} used in assignment of isoleucine $^{13}\text{C}\delta 1$ peak.

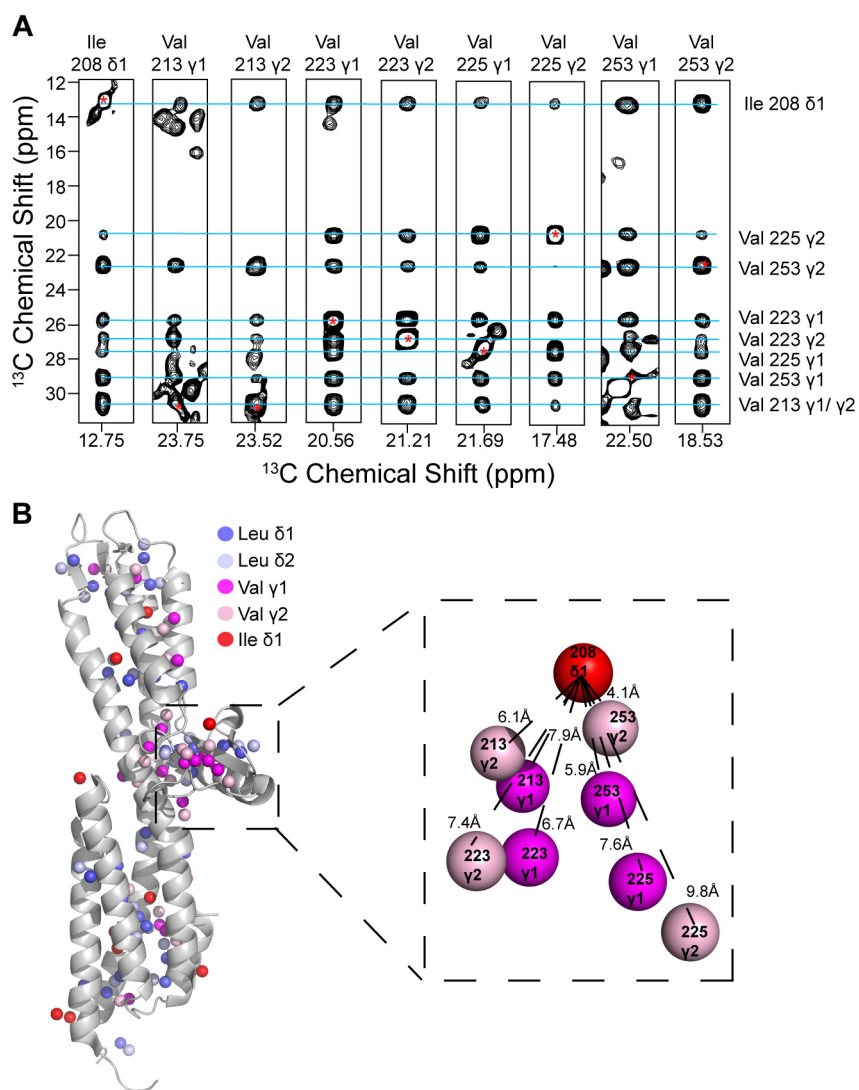


Figure S5. Representative strips from 3D ^1H - ^{13}C - ^{13}C HMQC-NOESY-HMQC used in assigning the ILV resonances of SipD^{C244S}. **(A)** Strips from a 3D ^1H - ^{13}C - ^{13}C HMQC-NOESY-HMQC dataset of perdeuterated ^{15}N /ILV-labeled SipD^{C244S}. Asterisks mark the cross peak for each residue; NOEs with other residues are shown in blue lines. **(B)** Example of distance information from the crystal structure of SipD^{C244S} used in assigning the ILV resonances of SipD^{C244S}, with ILV methyls shown in spheres (Ile, red; Leu, blue & light blue; Val, pink & light pink). An expanded region showing the ILV methyls close to I208 shown with distances that correlated with the NOE peak intensities.

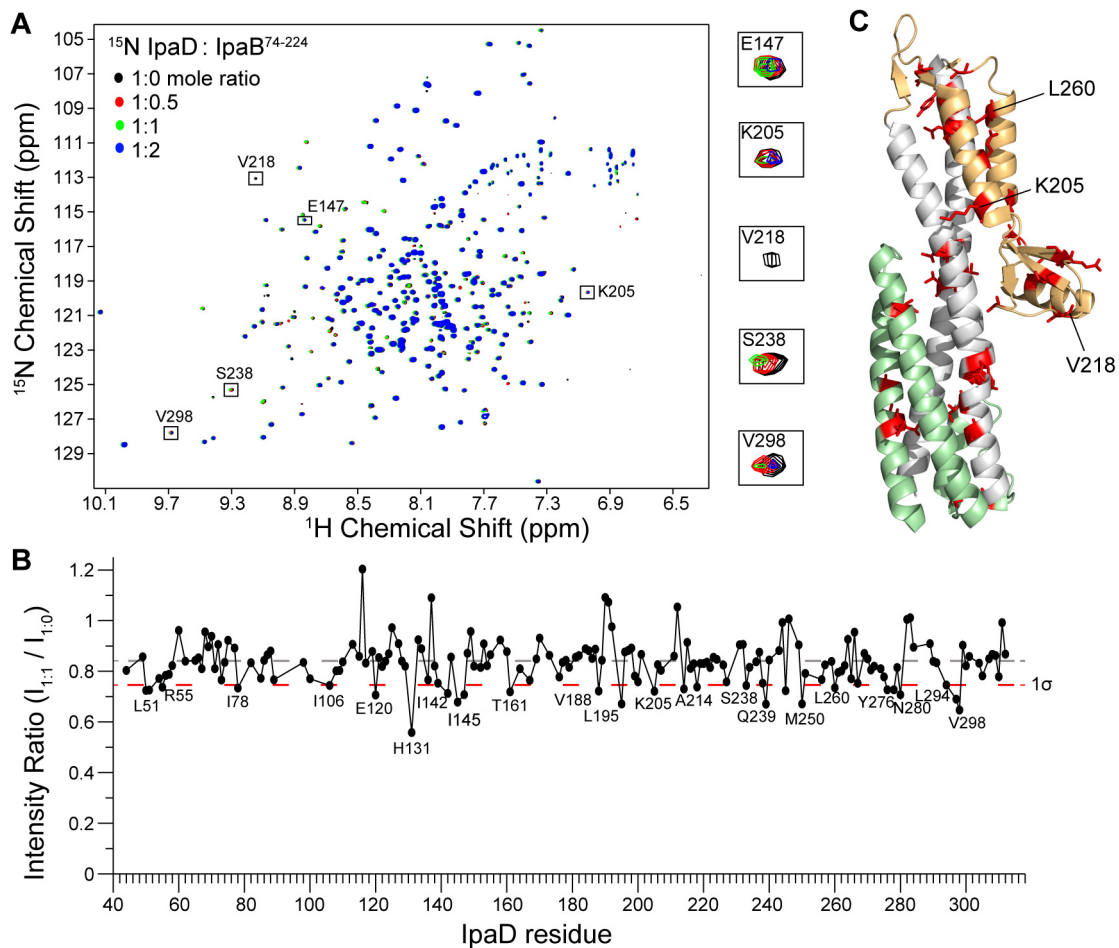


Figure S6. IpaB⁷⁴⁻²²⁴ interacts with IpaD. **(A)** Overlay of four ^1H - ^{15}N TROSY spectra of ^{15}N /ILV IpaD with increasing concentrations of unlabeled IpaB⁷⁴⁻²²⁴. **(B)** Plot of peak intensity ratio ($I_{1:1}/I_{1:0}$) at IpaD:IpaB⁷⁴⁻²²⁴ molar ratio of 1:1.1. **(C)** Residues with peak intensity ratio ($I_{1:1}/I_{1:0}$) lower than 1 standard deviation below average (1σ) are mapped onto the crystal structure of IpaD.

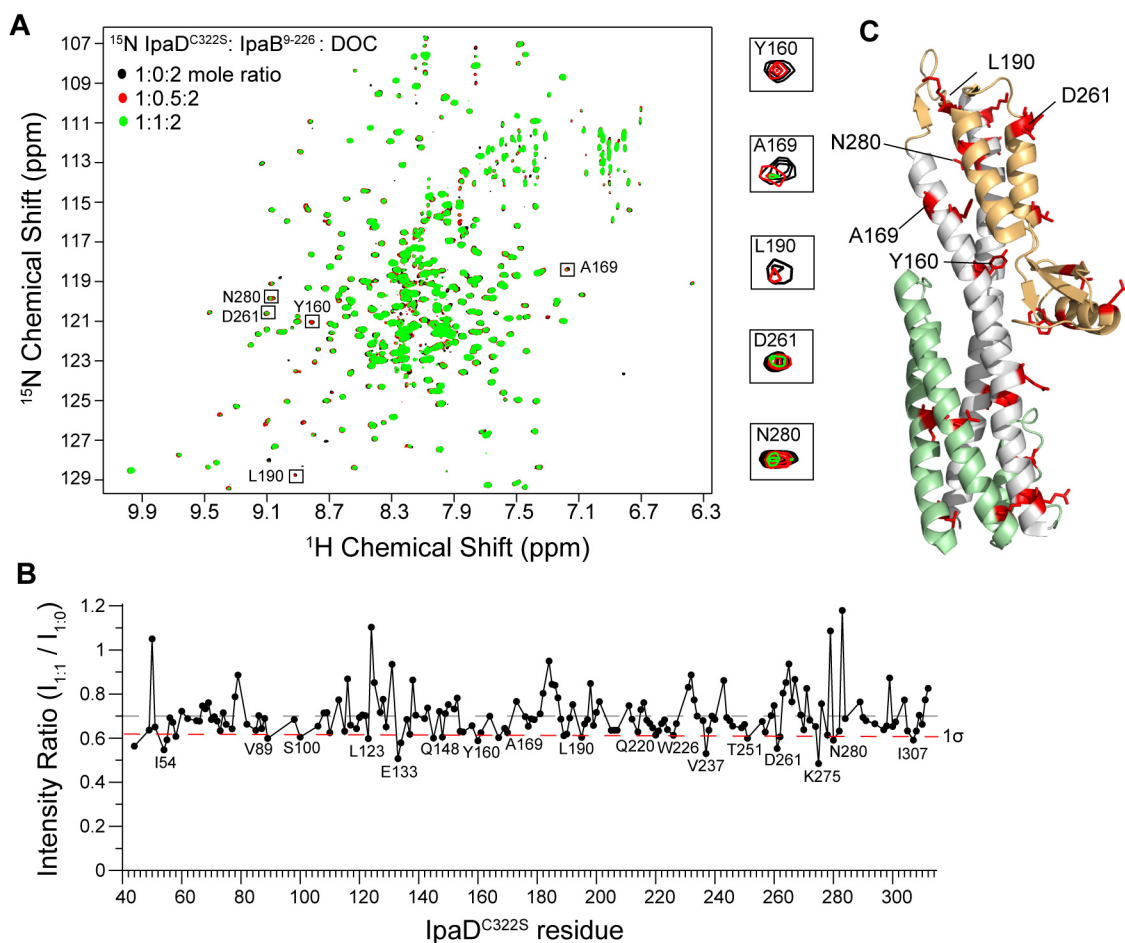


Figure S7. Deoxycholate (DOC) did not alter the surfaces of IpaD involved in binding IpaB. **(A)** Overlay of three ^1H - ^{15}N TROSY spectra of ^{15}N /ILV IpaD^{C322S} in the presence of 0.4 mM DOC with increasing concentrations of unlabeled IpaB⁹⁻²²⁶. **(B)** Plot of peak intensity ratio ($I_{1,1}/I_{1,0}$) at IpaD^{C322S}:IpaB⁹⁻²²⁶ molar ratio of 1:1.1. **(C)** Residues with peak intensity ratio ($I_{1,1}/I_{1,0}$) lower than 1 standard deviation below average (1σ) are mapped onto the crystal structure of IpaD. The presence **(C)** or absence of DOC (Fig. 3C) resulted in the same surfaces of IpaD perturbed upon titration with IpaB.

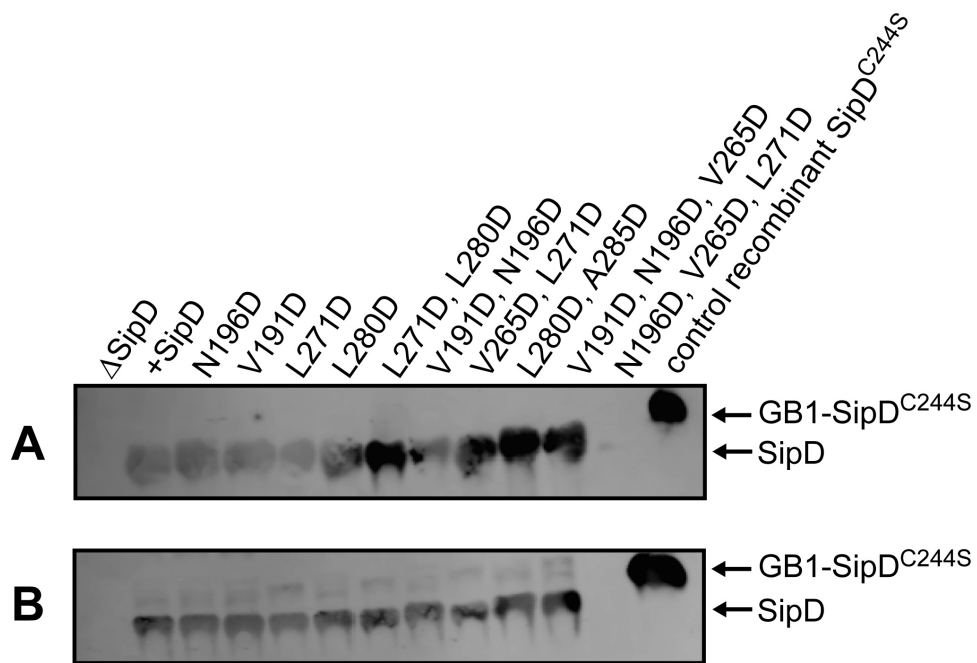


Figure S8. Immunoblotting using SipD antibodies of (A) cultured supernatants and (B) whole-cell lysates of *Salmonella typhimurium* strains containing *sipD* gene knockout (Δ SipD), exogenous *sipD* introduced by a plasmid (+SipD), and exogeneous SipD with site directed mutants. Recombinant GB1-SipD^{C244S} was used as a positive control.