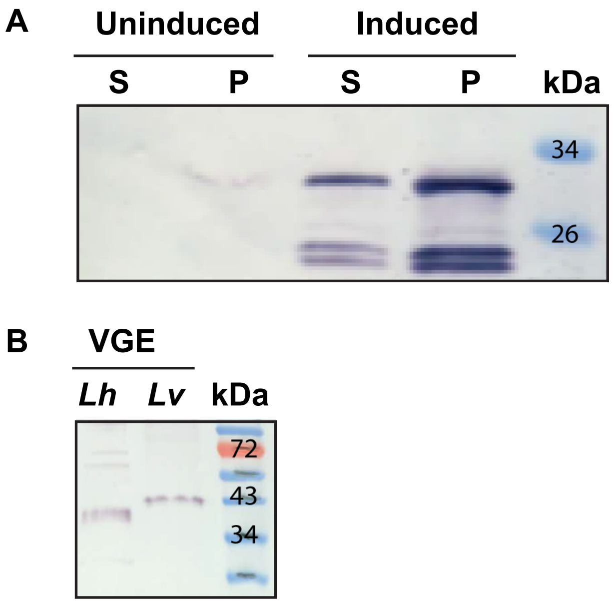
Figure S1



C

IpaD Helices & Residues (2J0O)	**Corresponding p40 Model Residues**	**Results of Comparison of IpaD's & p40's Helices**
#1: 40-66 (PV…LS)	4-25 (KP…MK)	Structurally aligned
#2: 72-102 (KT…RH)	40-45 (MI…LD) & 50-69 (IE…ET)	Structurally aligned to p40 helices #2 & #3 which are interrupted by a 4-residue turn
#3: 108-112 (KD…RE)	Absent	A short IpaD helical region that is absent in p40
#4: 131-175 (HR…LA)	76-103 (FT…FG)	Structurally aligned
#5: 192-206 (VN…KY)	154-171 (SN...IN)	Structurally aligned to set of discontinuous 3_{10}-helices in p40
#6: 220-230 (QE…EL)	Absent	Not applicable
#7: 250-263 (MT…NL)	189-191 (KKL)	Structurally aligned to a short 3_{10}-helix
#8: 273-321 (NA…SS)	Not modeled	Not modeled; a p40 TMH is predicted

Figure S1: Western analyses for p40 expression and comparison of the p40 3D model to IpaD crystal structure (Related to Main Figure 4 D – F)

(A) Anti-p40 antibody reacts at the expected molecular weight (top bands) for the His-tagged p40 central domain (CD, residues 26 – 240) from bacterial extracts, and **(B)** with venom extracts (VGE) from *L. heterotoma* (*Lh*) and *L. victoriae* (*Lv*) female wasps. In panel A, S = supernatant; P = pellet. Uninduced and induced refer to bacterial extracts prepared without or with IPTG induction for p40 CD expression. p40's identity was also confirmed with anti-His antibody (not shown). The p40-positive bands at higher molecular weights **(B)** suggest higher-order protein associations and/or post-translational modifications. **(C)** Comparison of the *Shigella flexneri* IpaD (2J0O) structure and *L. heterotoma* p40 model **(Figure 4 E)**: The residue numbers for the p40 model do not include the predicted signal peptide. The first and last model residues are 26 and 213 of the predicted full length protein, respectively. The p40 model lacks the short α-helix and β-hairpin at residues 208 – 251 in IpaD and the model's local quality drops in this region. 3_{10} helices are found in the p40 model, in addition to α-helices. 3_{10} and α-helices psi/phi angles are similar. These 3_{10} helices could render as α-helices, given slight conformational and energetic shifts in the model. Experimental methods are necessary to validate these p40 model predictions.