Supplemental material

Singh et al., https://doi.org/10.1083/jcb.201802155

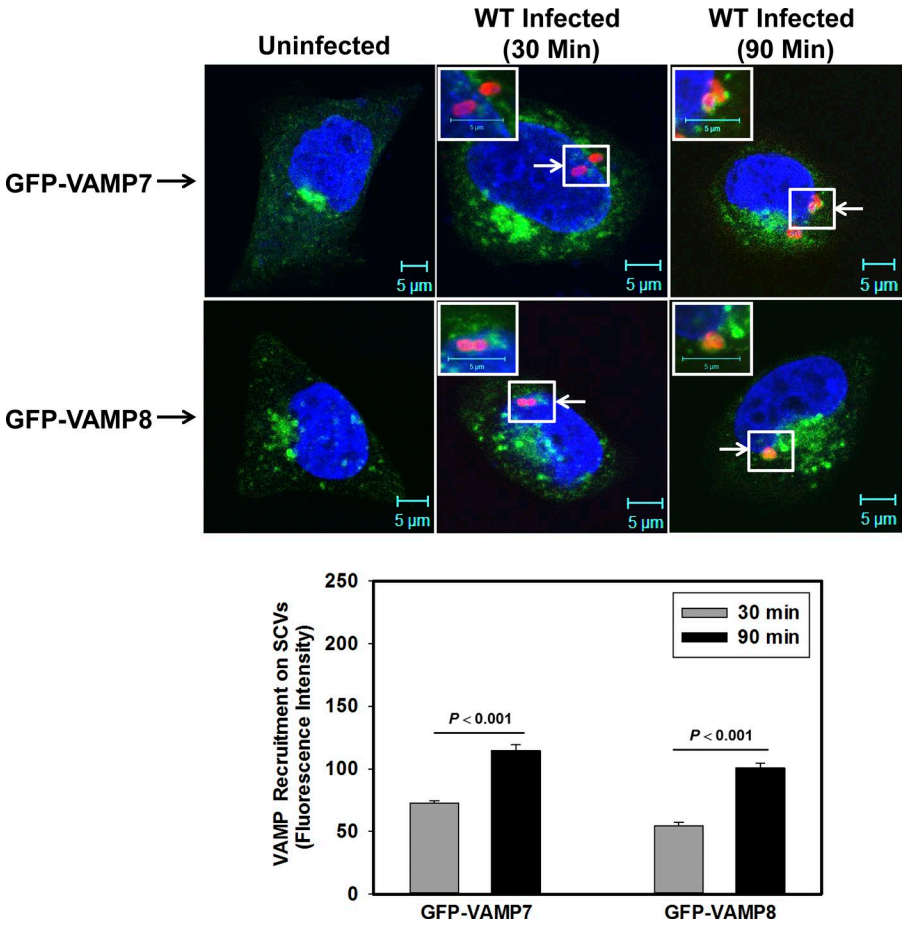

Figure S1. **Recruitment of VAMP7 and VAMP8 on SCV.** We analyzed the recruitment of VAMP7 and VAMP8 on SCVs (red) at indicated time points in HeLa cells overexpressing GFP-VAMP7 or GFP-VAMP8. Our results showed that SCVs significantly recruit both VAMP7 and VAMP8 after 90 min infection in HeLa cells. These results are consistent with previous findings that VAMP8 is recruited on SCV through SopB-generated PtdIns(3)P (Dai et al., 2007) and that SCVs also interact with VAMP7-positive vesicles to promote *Salmonella*-induced filament formation (Santos et al., 2015).

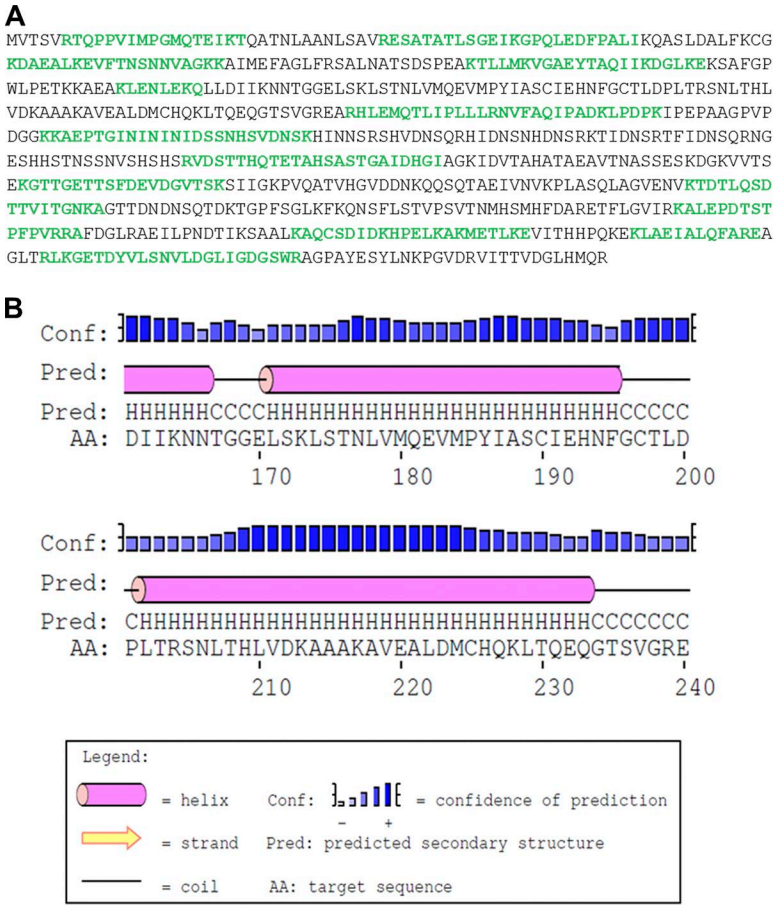

Figure S2. **Identification of Syn8 binding protein as SipA by mass spectrometry. (A)** To identify the Syn8 binding protein from *Salmonella*, GST-Syn8 was immobilized, and a pullout experiment was performed from SSPs as described in Materials and methods. The bound proteins were separated on a 12% SDS-PAGE and identified by mass spectrometry. The obtained peptides were mapped into the SipA sequence highlighted in green. **(B)** $SipA^{180–232}$ is a helical structure. To determine the secondary structure in SipA SM, the SipA protein sequence was analyzed by the PSIPRED Protein Sequence Analysis Workbench. The predicted output secondary structure shows that $SipA^{180–232}$ has helical structure.

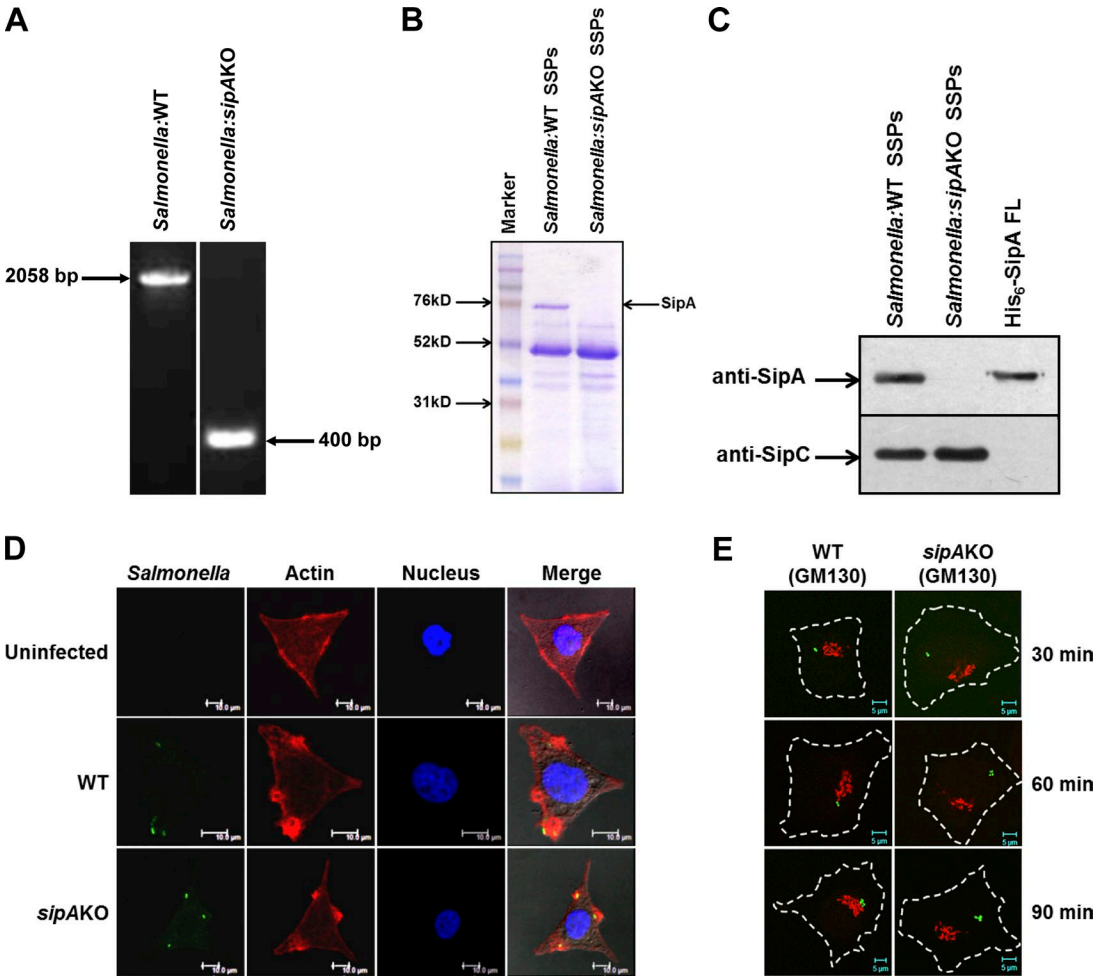

Figure S3. **Generation and characterization of *sipA*KO *Salmonella*. (A)** To validate the generation of *Salmonella:sipA*KO, PCR was performed by *sipA* gene-specific end-to-end primers using genomic DNA prepared from *Salmonella:*WT and *Salmonella:sipA*KO. Primers amplified a 400-bp fragment from *Salmonella:sipA*KO compared with 2,058-bp fragment from *Salmonella:*WT, demonstrating the deletion of *sipA*. **(B)** SSPs of *Salmonella:*WT and *Salmonella:sipA*KO resolved in SDS-PAGE and stained with Coomassie showing the absence of SipA protein in the *Salmonella:sipA*KO strain. **(C)** Western blot analysis of SSPs of *Salmonella:*WT and *Salmonella:sipA*KO using anti-SipA antibody showing the absence of SipA in *Salmonella:sipA*KO. His_6-SipAFL and anti-SipC antibody were used as controls. To characterize the *Salmonella*:*sipA*KO strain, we determined the actin rearrangement activity and its ability to move near Golgi. **(D)** Cells infected with indicated strains of GFP-*Salmonella* were stained with phalloidin (red) to label actin in HeLa cells. Confocal image showing reduced actin bundling in the *Salmonella*:*sipA*KO strain. **(E)** HeLa cells infected with GFP-*Salmonella:*WT or GFP-*Salmonella*:*sipA*KO were stained with GM130 to label the Golgi in host cells at indicated times. Confocal image showing *Salmonella:sipA*KO unable to move near the Golgi.

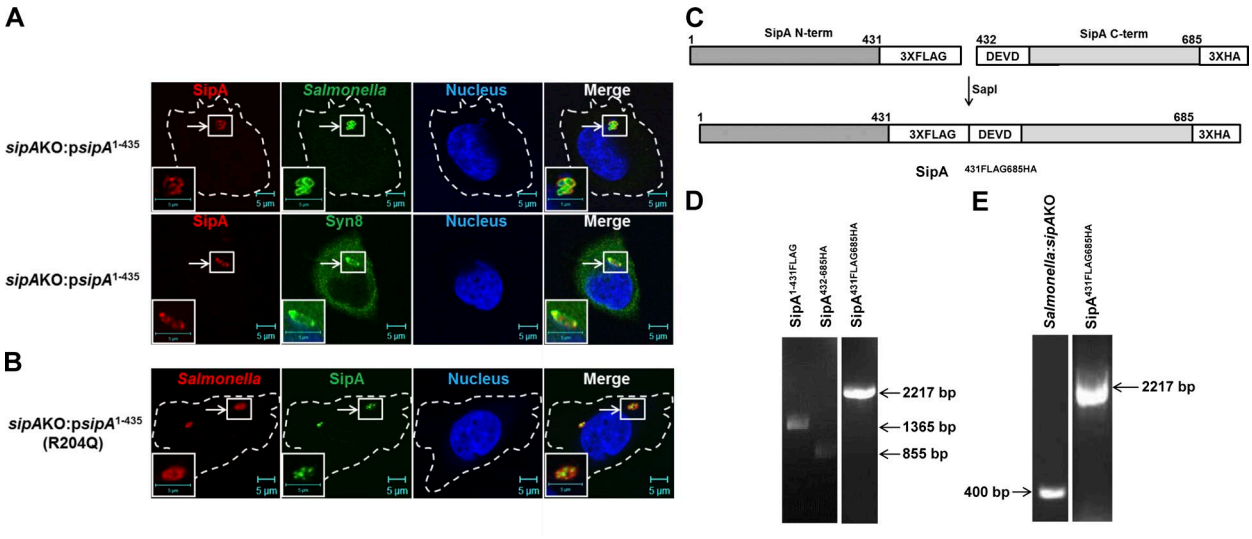

Figure S4. **Localization of SipA on SCVs containing *sipA*KO:SipA$^{1–435}$ *Salmonella* and generation of SipA$^{431FLAG-685HA}$ construct. (A)** HeLa cells were infected with *sipA*KO:SipA$^{1–435}$ *Salmonella* strain, and localization of SipA and Syn8 on SCV 90 min p.i. was determined using anti-FLAG (red) and anti-Syn8 (green) antibodies, respectively. *Salmonella* were stained with anti-*Salmonella* antibody (green). **(B)** Similarly, HeLa cells were infected with *sipA*KO:SipA$^{1–435: R204Q}$ (red), and localization of SipA on SCVs was determined using anti-FLAG (green). Our results showed that *sipA*KO:SipA$^{1–435}$ recruits Syn8 on SCVs. Arrows indicate colocalization of the respective proteins (A, bottom) or on SCVs (A [top] and B). **(C)** Schematic diagram showing the assembly of SipA N-terminal and C-terminal fragments in SipA$^{431FLAG-685HA}$ construct via a type IIS restriction enzyme, *Sap*I. **(D)** PCR analysis was performed by SipA N-terminal, C-terminal, and FL specific primers using plasmid DNA prepared from the pRE112-sipA$^{431FLAG-685HA}$ clone. **(E)** PCR analysis showing amplification of *sipA* from gene-specific end-to-end primers using genomic DNA prepared from *Salmonella:sipAKO* and *Salmonella*:*sipA*$^{431FLAG-685HA}$.

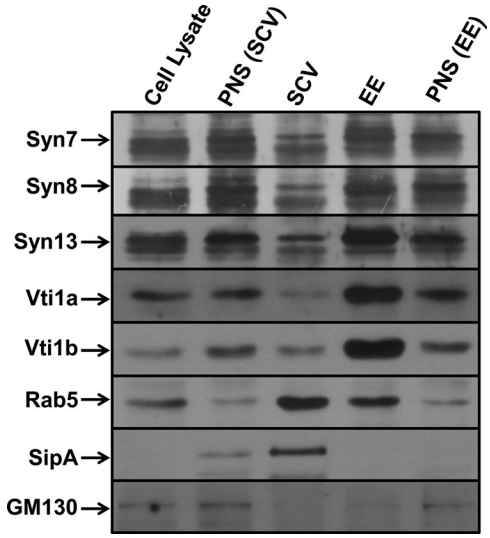

Figure S5. **Determination of the presence of different proteins on SCVs and endosomes.** Proteins (25 µg) from purified SCVs (90 min p.i.) and EEs were separated by SDS-PAGE followed by Western blot analysis using specific antibody to determine the presence of indicated protein. As anti-SipA antibody was less sensitive, SipA was detected by anti-FLAG antibody on purified SCVs containing *Salmonella:*sipA$^{431FLAG-685HA}$. Respective PNS was used as control.

References

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