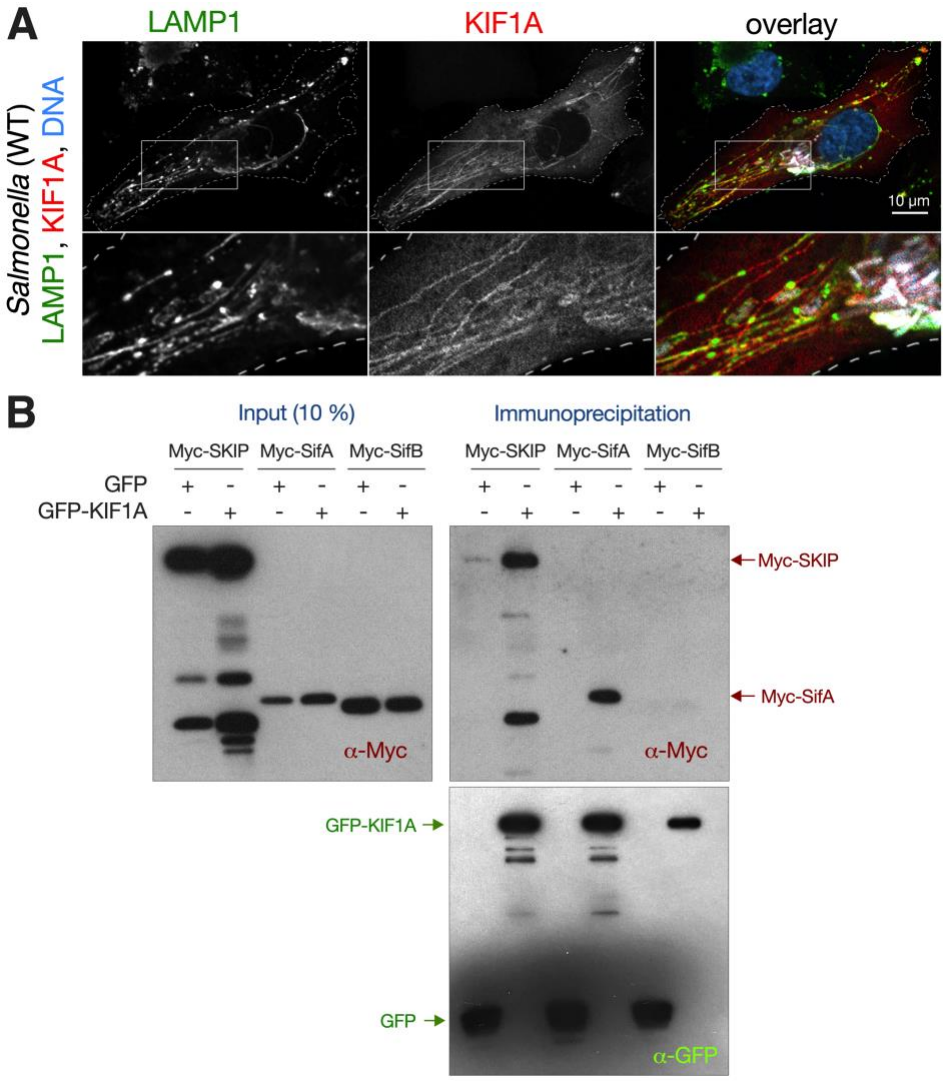

Fig. S1. KIF1A is present on *Salmonella* compartments and interacts with SifA and SKIP.

(**A**) HeLa cells were transfected with plasmids for the expression of GFP-KIF1A and further infected with a wild-type *Salmonella* strain expressing CFP. After 16 hours of infection, cells were fixed, immunostained and imaged for CFP (white), LAMP1 (green), KIF1A (red) and DNA (blue) using confocal microscopy. KIF1A is present on LAMP1 positive SCVs and SITs. The images in the lower row show the insets enlarged three times. Scale bar, 10 or 3,3 µm for the magnified insets. (**B**) HeLa cells were transfected with plasmids for the expression of GFP or GFP-KIF1A and various Myc-tagged proteins. Immunoprecipitations were performed with GFP-Trap beads. Input and immunoprecipitated proteins (IP) were analysed by Western blotting using anti-Myc and anti-GFP antibodies. KIF1A interacts specifically with SifA and SKIP but not with SifB.

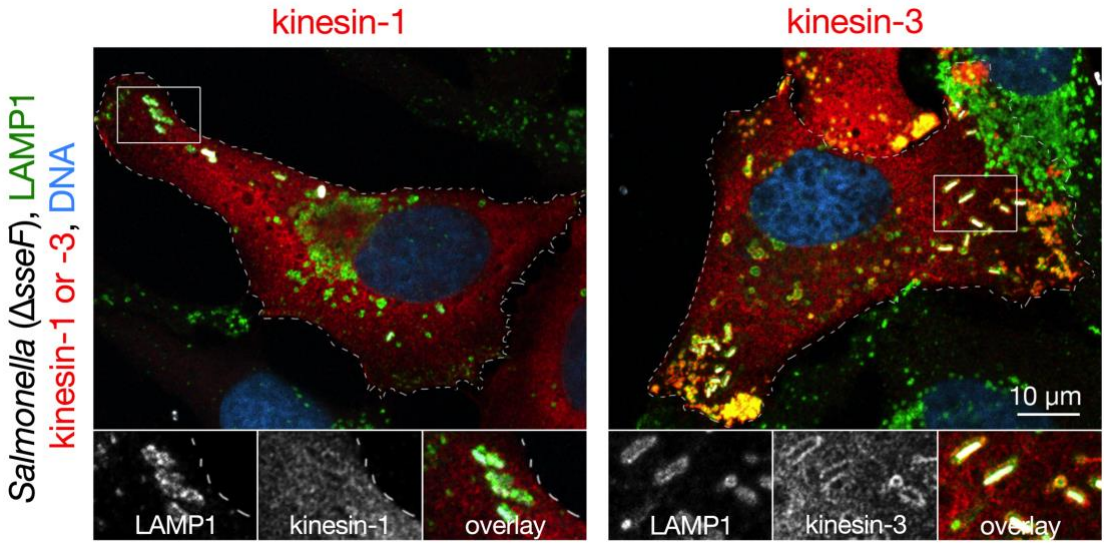

Fig. S2. Kinesin-3 is present on Δ*sseF* SCVs.

HeLa cells were transfected with plasmids for the expression of kinesin-1 (HA-KLC2 / HA-KIF5C) or kinesin-3 (FLAG-KIF1Bß) and further infected with a Δ*sseF Salmonella* mutants expressing GFP. After 16 hours of infection, cells were fixed, immunostained and imaged for GFP (withe), LAMP1 (green), kinesin-1/-3 (red) and DNA (light blue) using confocal microscopy. Unlike kinesin-3, kinesin-1 is not detected on Δ*sseF* SCVs. Scale bar, 10 or 5 µm for the magnified insets.

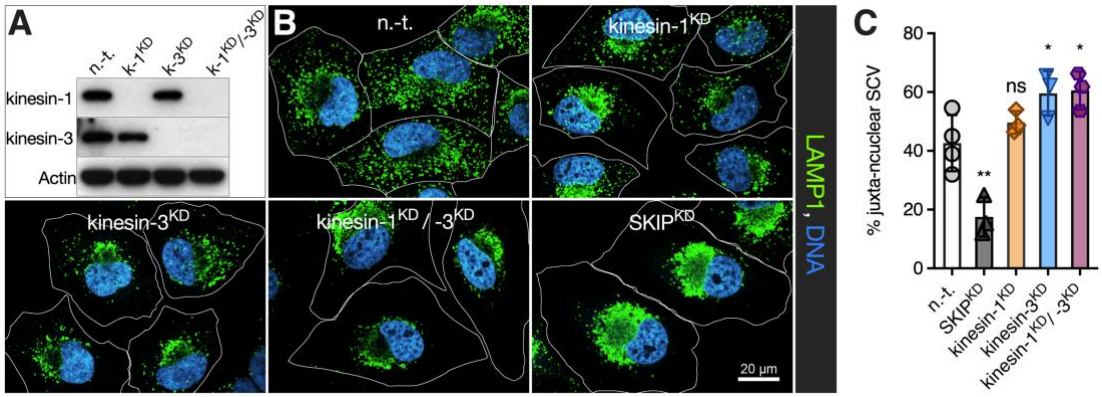

Fig. S3. Biochemical and microscopic analysis of the silencing of kinesin-1 and/or kinesin-3 in HeLa cells.

Cells were transfected with a single siRNA for the knock-down of SKIP or a non-targeting siRNA pool (n.-t.) or siRNA pools for the knock-down of kinesin-1(KIF5B) and/or kinesin-3 (KIF1B). (**A**) Western blotting showing the expression of kinesin-1 and kinesin-3 in control cell lysates (n.-t.) or knock-down for one of both kinesins. Actin was used as a loading control. (**B**) After fixation and immunostaining for LAMP1, cells were imaged by confocal microscopy for LAMP1 (green) and DNA
(blue). Continuous lines delineate the cells. (**C**) The knocked-down cells were infected with wild-type *Salmonella* expressing GFP for 16 hours, fixed and stained for DNA. The percentages of juxta-nuclear bacteria were scored by epi-fluorescence microscopy. Bacteria were considered to be juxta-nuclear when they were located in the inner third of the cytoplasmic space between the nucleus and the plasma membrane. Data are means ± S.D. of at least three independent experiments. Ordinary one-way ANOVA and Dunnett's multiple comparisons test was used to compare the results in the knocked-down cells with those of the control cells. Not significant (ns), $P>0.05$; *, $P<0.05$; **, $P<0.01$.

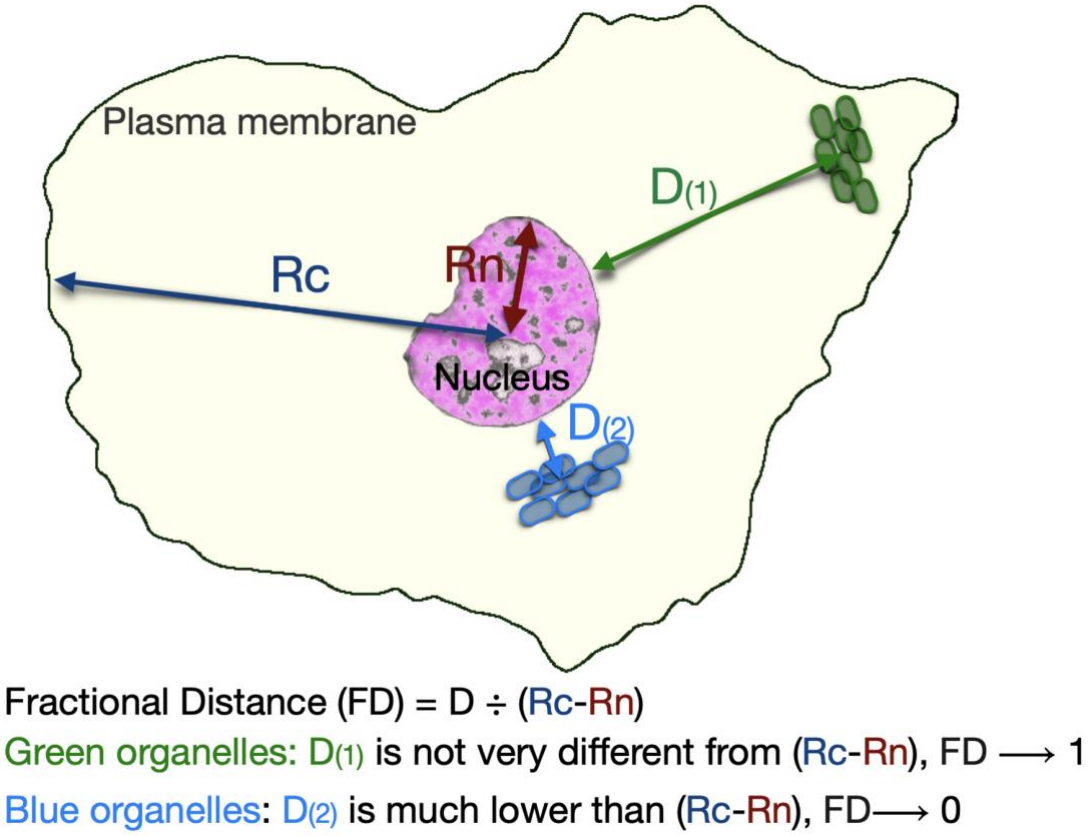

Fig. S4. Definition of fractional distance.

The fractional distance (FD) was defined as the ratio of the organelle mean distance to nucleus border (D) to the difference between the mean radius of the cell (Rc) and the mean radius of the nucleus (Rn). The FD was calculated using an internally developed Macro based on the ImageJ Radial Profile plugin and applied to confocal images. The FD tends towards zero when the organelles are close to the nucleus (blue organelles) or towards one when they are close to the plasma membrane (green organelles).

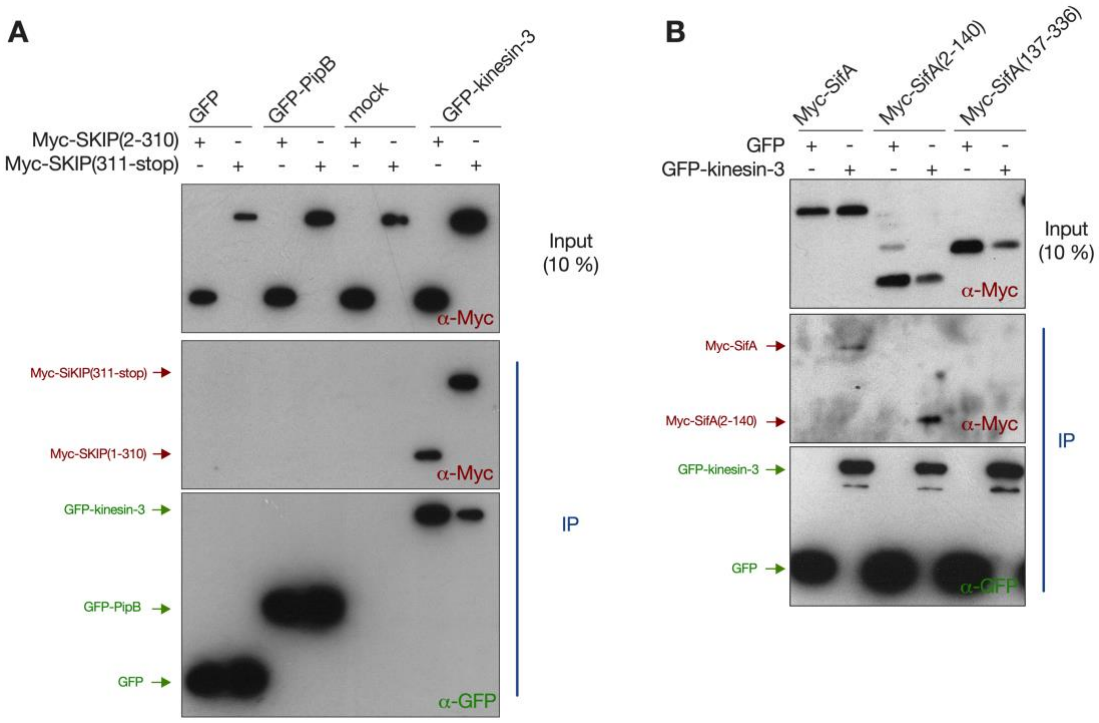

Fig. S5. Co-immunoprecipitation tests with kinesin-3.

Cos-7 cells were transfected with plasmids for the expression of various GFP- or Myc-tagged proteins. Immunoprecipitations were performed with GFP-Trap beads. Input and immunoprecipitated proteins (IP) were analysed by Western blotting using anti-Myc and anti-GFP antibodies. (**A**) Kinesin-3 interacts with the N- and C-terminal parts of SKIP. SKIP(2-310) and SKIP(311-stop) co-immunoprecipitate with GFP-kinesin-3 but not with GFP, GFP-PipB or beads only (mock). (**B**) Kinesin-3 interacts with the N-terminal domain of SifA. SifA and its N-terminal domain [SifA(1-140)] specifically co-immunoprecipitate with GFP-kinesin-3, while the C-terminal domain
[SifA(137-336)] does not.

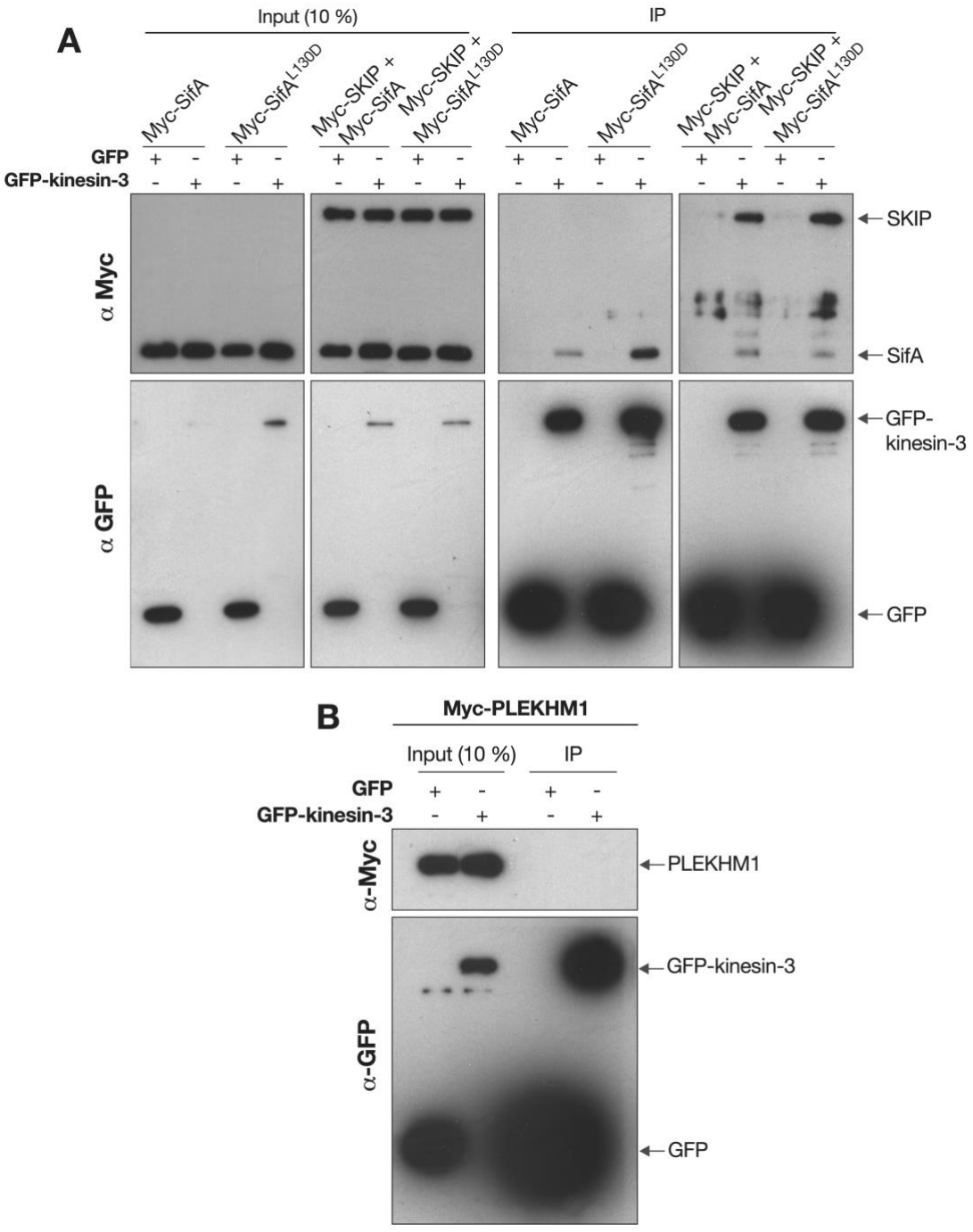

Fig. S6. Co-immunoprecipitation tests with kinesin-3.

Cos-7 cells were transfected with plasmids for the expression of GFP or GFP-kinesin-3 and various Myc-tagged proteins. Immunoprecipitations were performed with GFP-Trap beads. Input and immunoprecipitated proteins (IP) were analysed by Western blotting using anti-Myc and anti-GFP antibodies. (**A**) SifA and SKIP do not compete for kinesin-3 binding. SifA or SifA^{L130D} specifically co-immunoprecipitate with GFP-kinesin-3 independently of the presence of SKIP. (**B**) Kinesin-3 does not bind PLEKHM1.

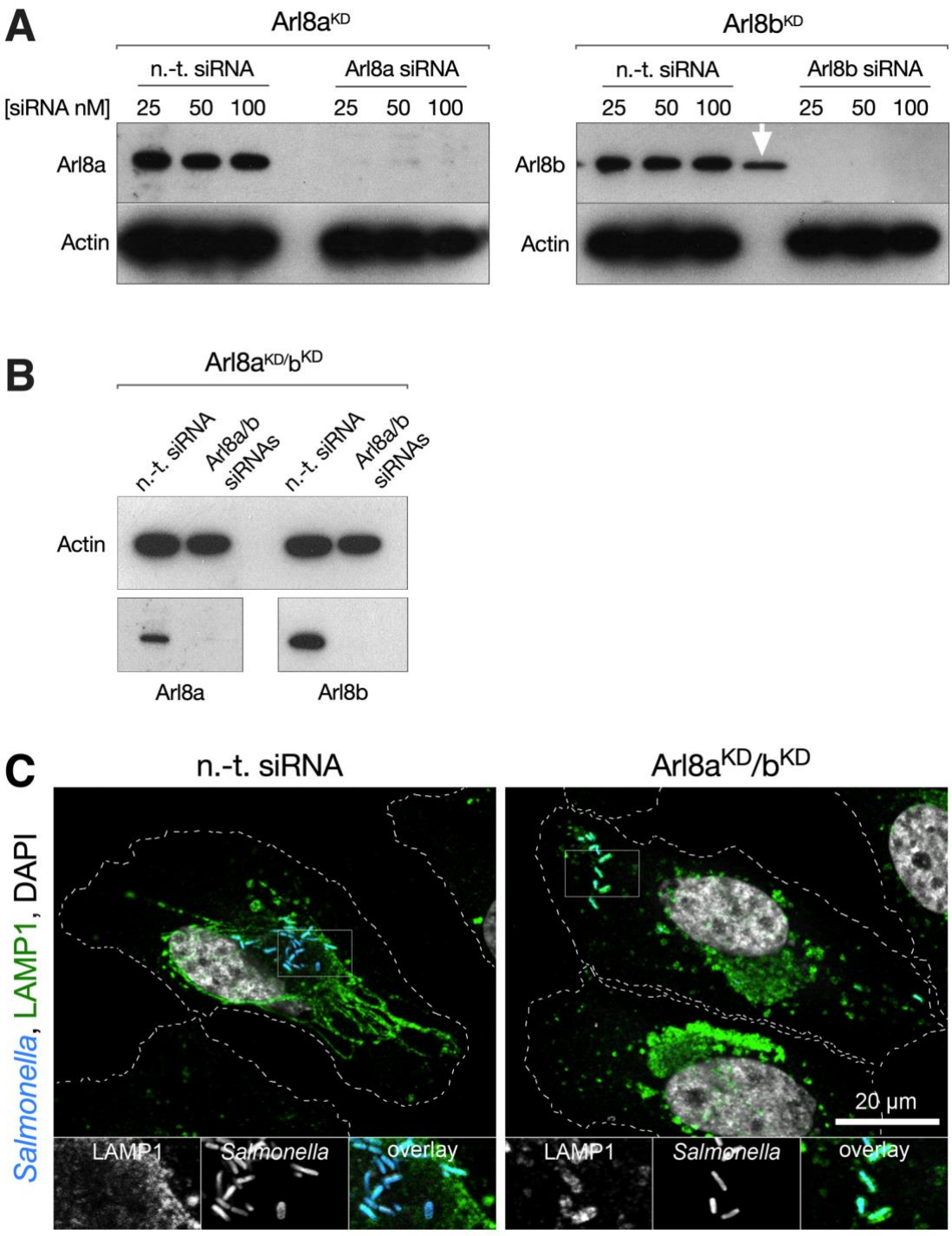

Fig. S7. Biochemical analysis and consequences of Arl8a and/or Arl8b silencing in *Salmonella*-infected cells.

HeLa cells were transfected with a non-targeting siRNA pool (n.-t.) or siRNA pools for the knock-down of Arl8a and/or Arl8b. (**A**) Western blotting showing the expression of Arl8a or Arl8b in control (n.-t.) or knock-down cell lysates for the corresponding GTPase. The white arrow indicates a non-specific band in the Protein Ladder line. (**B**) Western blotting showing expression of Arl8a and Arl8b in control (n.-t.) or

knockdown cell lysates for both GTPases. (**A** and **B**) Actin was used as a loading control. (**C**) Control (n.-t.) and Arl8a^{KD}/b^{KD} cells were infected with wild-type *Salmonella* for 16 hours, fixed and immunostained. Confocal images [(*Salmonella* (blue), LAMP1 (green) and DNA (white)] illustrate the disappearance of SITs and the more peripheral positioning of wild-type SCVs in cells silenced for Arl8a/b. Scale bar, 20 or 10 µm for the magnified insets.

Table S1. Plasmids used in this study

Name	Designation	Application	Reference
V202	pEGFP-C1	Expression of EGFP in eukaryotic cells	Clontech
C259	pCMV-Myc-SifA	Expression of Myc-SifA in eukaryotic cells	(Boucrot et al., 2005)
C1059	pCMV-Myc-SifA^{L130D}	Expression of Myc- SifA^{L130D} in eukaryotic cells	(Zhao et al., 2015)
C497	pCMV-Myc-SifA (2-140)	Expression of Myc-SifA(2-140) in eukaryotic cells	(Diacovich et al., 2009)
C1057	pCMV-Myc-SifA (137-336)	LR recombination of C1074 with pCMV-Myc^{GW}. Expression of Myc-SifA(137-336) in eukaryotic cells	This study
C1074	pDONR-SifA(137-336)	Gateway entry plasmid: BP recombination of the PCR product (oligos O-691/O-693 using *S.* Typhimurium 12023 genomic DNA as template) with pDONR™ / Zeo	This study
C252	pSK-KIAA0842	Full length human SKIP in pBlueScript II SK(+)	Obtained from the Kazuza DNA Research Institute
C254	pCMV-HA-SKIP	Expression of HA-SKIP in eukaryotic cells	(Dumont et al., 2010)
C253	pCMV-Myc-SKIP	Expression of Myc-SKIP in eukaryotic cells	(Boucrot et al., 2005)
C901	pDONR-SKIP	Gateway entry plasmid: BP recombination of the PCR product (oligos O-680/O-681 using C252 as template) with pDONR™ / Zeo	This study
C904	pEGFP-SKIP	LR recombination of C901 with pEGFP-C1^{GW}. Expression of EGFP-SKIP in eukaryotic cells	This study
C469	pCMV-Myc-SKIP(2-310)	Expression of Myc-SKIP(2-310) in eukaryotic cells	(Dumont et al., 2010)
C1275	pDONR-SKIP(311-stop)	Gateway entry plasmid: BP recombination of the PCR product (oligos O-780/O-681 using C253 as template) with pDONR™ / Zeo	This study
C1278	pCMV-Myc-SKIP(311-stop)	LR recombination of C1275 with pCMV-Myc^{GW}. Expression of Myc-SKIP(311-stop) in eukaryotic cells	This study
C417	pSK-KIAA0356	Full length human PLEKHM1 in pBlueScript II SK(+)	From the Kazuza DNA Research Institute
C1227	pDONR-PLEKHM1	Gateway entry plasmid: BP recombination of the PCR product (oligos O-869/O-870 using C417 as template) with pDONR™ / Zeo	This study
C1233	pMyc-PLEKHM1	LR recombination of C1227 with pCMV-Myc^{GW}. Expression of Myc-PLEKHM in eukaryotic cells	This study
C443	pCMV-Myc-PipB2	Expression of Myc-PipB2 in eukaryotic cells	(Henry et al., 2006)
C330	pMyc-SifB	Expression of Myc-SifB in eukaryotic cells	(Deiwick et al., 2006)
C1188	pGFP-KIF1A	Expression of GFP- KIF1A in eukaryotic cells	(Guardia et al., 2016)
C1189	pFLAG-KIF1Bß	Expression of FLAG- KIF1Bß in eukaryotic cells	(Schlisio et al., 2008)
C1225	pDONR-KIF1Bß	Gateway entry plasmid: BP recombination of the PCR product (oligos O-871/O-837 using C1189 as template) with pDONR™ / Zeo	This study
C1232	pEGFP-KIF1Bß	LR recombination of C1225 with pEGFP-C1^{GW}. Expression of EGFP-kinesin-3 in eukaryotic cells	This study
C1288	pDONR-KIF1Bß(363-stop)	Gateway entry plasmid: BP recombination of the PCR product (oligos O-904/O-837 using C1189 as template) with pDONR™ / Zeo	This study
C1292	pCMV-Myc-KIF1Bß(363-stop)	LR recombination of C1288 with pCMV-Myc^{GW}. Expression of Myc-ML-kinesin-3 in eukaryotic cells	This study
C1193	pDONR-KIF1Bß(1324-end)	Gateway entry plasmid: BP recombination of the PCR product (oligos O-835/O-837 using C1189 as template) with pDONR™ / Zeo	This study
C1197	pGST-KIF1Bß(1324-end)	LR recombination of C1193 with pDEST-15. Expression of GST- KIF1Bß(1324-end) in E. coli	This study
C1198	P[His]$_6$-KIF1Bß(1324-end)	LR recombination of C1193 with pDEST-17. Expression of [His]$_6$- KIF1Bß(1324-end) in E. coli	This study
C1190	pCB6-HA-KHC	Expression of HA-KHC (rat KIF5C) in eukaryotic cells	(Sanger et al., 2017)
C1192	pCB6-HA-KLC2	Expression of HA-KLC (mouse KLC2) in eukaryotic cells	(Sanger et al., 2017)
C974	pGG2-CFP	pFPV25 derivative for *Salmonella* expression of CFP under control of the rpsM promoter	(Moest et al., 2018)

Table S2. Oligonucleotides used in this study

O-680	PLEKHM2GWFw	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAGCCGGGGGAGGTGAAGGACC
O-681	PLEKHM2GWRev	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTATCAGCACCAGGGGTCTCGGGAGGC
O-691	WZ05F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATTTTAAAATCGCATCCACAAATGACGGCC
O-693	WZ07-R336	GGGGACCACTTTGTACAAGAAAGCTGGGTCCTATAAAAAACAACATAAACAGCCGCTTTGTTG
O-780	SKIP 311-X_GW_fw	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGAGGTCATCAGGGTCACCAAGAAG
O-835	KIF1B(1324-) FW	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGGTAGTCCAGGTATGCAGAGAAGGAG
O-837	KIF1B(-STOP)Rev	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAGTATTTCGACTGGCTCGGGCATC-3'
O-869	GW FW PLEKHM1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCTTTCAGTGGTGGAGAATGGACTG
O-870	GW Rev PLEKHM1	GGGGACCACTTTGTACAAGAAAGCTGGGTCTTAAACGGCGAAAATGTTCTGTTCC
O-871	GW FW KIF1Bß	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGGATCGGGAGCCTCAGTGAAG-3'
O-885	KIF1B(-1561)Rev	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTCCTAAACAAGATTGAGAAATTCGTTTTTTCC-3'
O-904	KIF1Bß(363-)FW	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCGTTCGTGAATTAAAGGAGGAGG-3'

Table S3. Bacterial strains

Name	Description	Reference
12023	Wild-type *S.* Typhimurium (*Salmonella enterica* subsp. *enterica* serovar Typhimurium), strain 12023	Laboratory stock
AAG020	12023 *pipB2-2HAchr*::FRT	This study
HH109	12023 *ssaV*::*aphT*	(Deiwick et al., 1998)
126	12023 *sseJ-2HAchr*::FRT	Provided by D.W. Holden
WZ040G	12023 *sifA-2HA(L130D)chr*::FRT	(Zhao and Méresse, 2015)
AAG022sc4	12023 *pipB2-2HAchr*::FRT, *ΔsifA*::FRT	(Schroeder et al., 2010)
TM29	12023 *pipB2-2HAchr*::FRT, *ΔsseF*::km	This study
AAG023	12023 *pipB2-2HAchr*::FRT, *ΔsopD2*::Cm	This study
YZ012	12023 Δ*sspH2*::FRT	Provided by D.W. Holden
127	12023 Δ*sseJ*::km	(Henry et al., 2006)
AAG04	12023 *SseJ-2HAchr*::FRT, Δ*pipB2*::km	This study
TH146	12023 Δ*sifA*::FRT, ΔpipB2::km	(Henry et al., 2006)
PH011	12023 Δ*sifA*::FRT, p*sifA-2HA*	(Boucrot et al., 2005)

FRT: 128 bp scar remaining after excision of the antibiotic resistance FRT cassette (Datsenko and Wanner, 2000).

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