

Supplementary Materials for **Activation of a Bacterial Virulence Protein by the GTPase RhoA**

Matthias Christen, Lisette H. Coye, Jill S. Hontz, Doris L. LaRock, Richard A. Pfuetzner, Megha, Samuel I. Miller*

*To whom correspondence should be addressed. E-mail: millersi@u.washington.edu

Published 3 November 2009, *Sci. Signal.* **2**, ra71 (2009)
DOI: 10.1126/scisignal.2000430

This PDF file includes:

- Fig. S1. Assessment of the interaction of SseJ with various GTP γ S-loaded GTPases and their ability to stimulate its lipase activity.
- Fig. S2. Cotransfection of HeLa cells with plasmids encoding CA RhoA and SseJ results in the accumulation of cholesterol and neutral lipids in the enlarged endosomal compartment.
- Fig. S3. The expression of SseJ3x or RhoA alone does not induce the formation of BODIPY-labeled cholesterol esters.
- Table S1. Descriptions of the strains of *S. typhimurium* and the plasmids used in this study.
- References

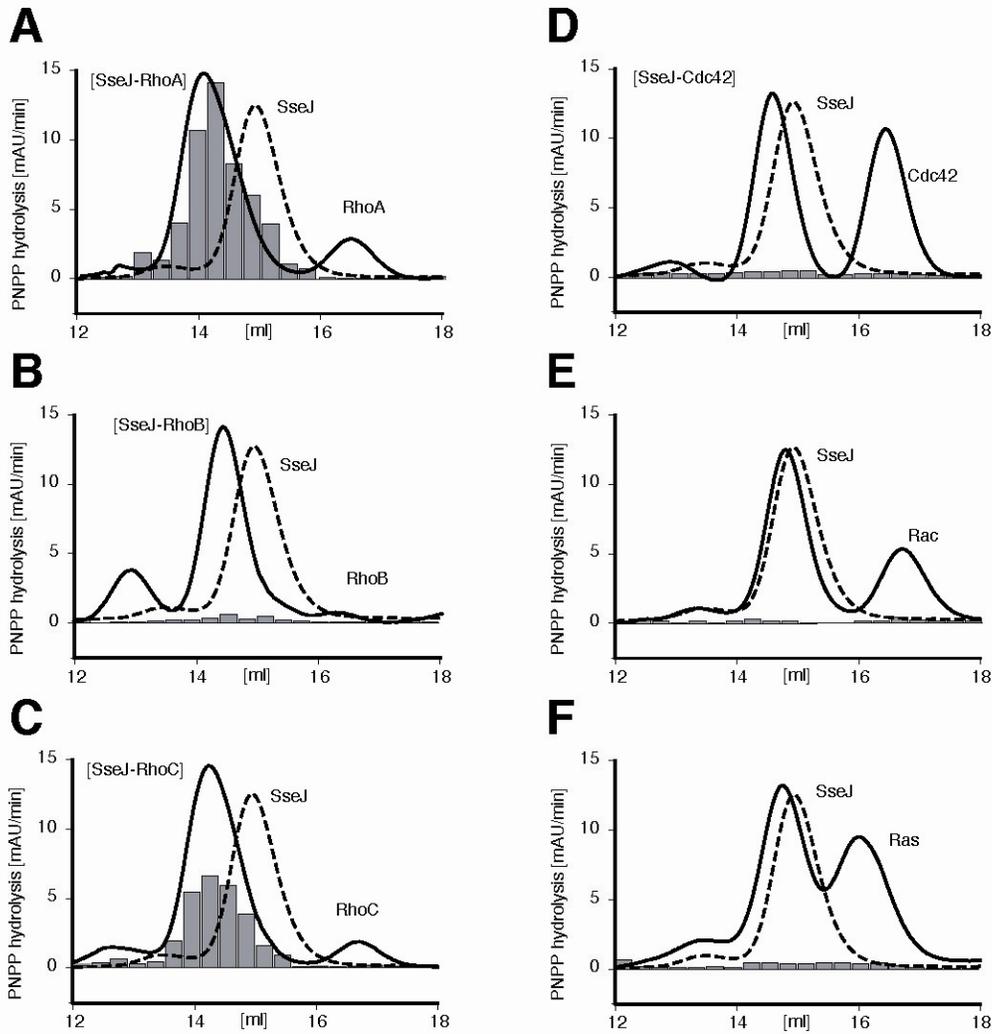


Fig. S1. Assessment of the interaction of SseJ with various GTP γ S-loaded GTPases and their ability to stimulate its lipase activity. (A to F) Gel filtration analyses of SseJ alone (dashed lines) or in the presence of GTP γ S-loaded GTPase (black lines) revealed the formation of an enzymatically active complex with RhoA-GTP γ S (A) and RhoC-GTP γ S (C), but not with RhoB-GTP γ S (B) Cdc42-GTP γ S (D), Rac-GTP γ S (E), or Ras-GTP γ S (F). The bar graph shows the lipase activity of eluted fractions from the gel filtration of SseJ and RhoA-GTP γ S. Each individual gel filtration shown is representative of three independent experiments.

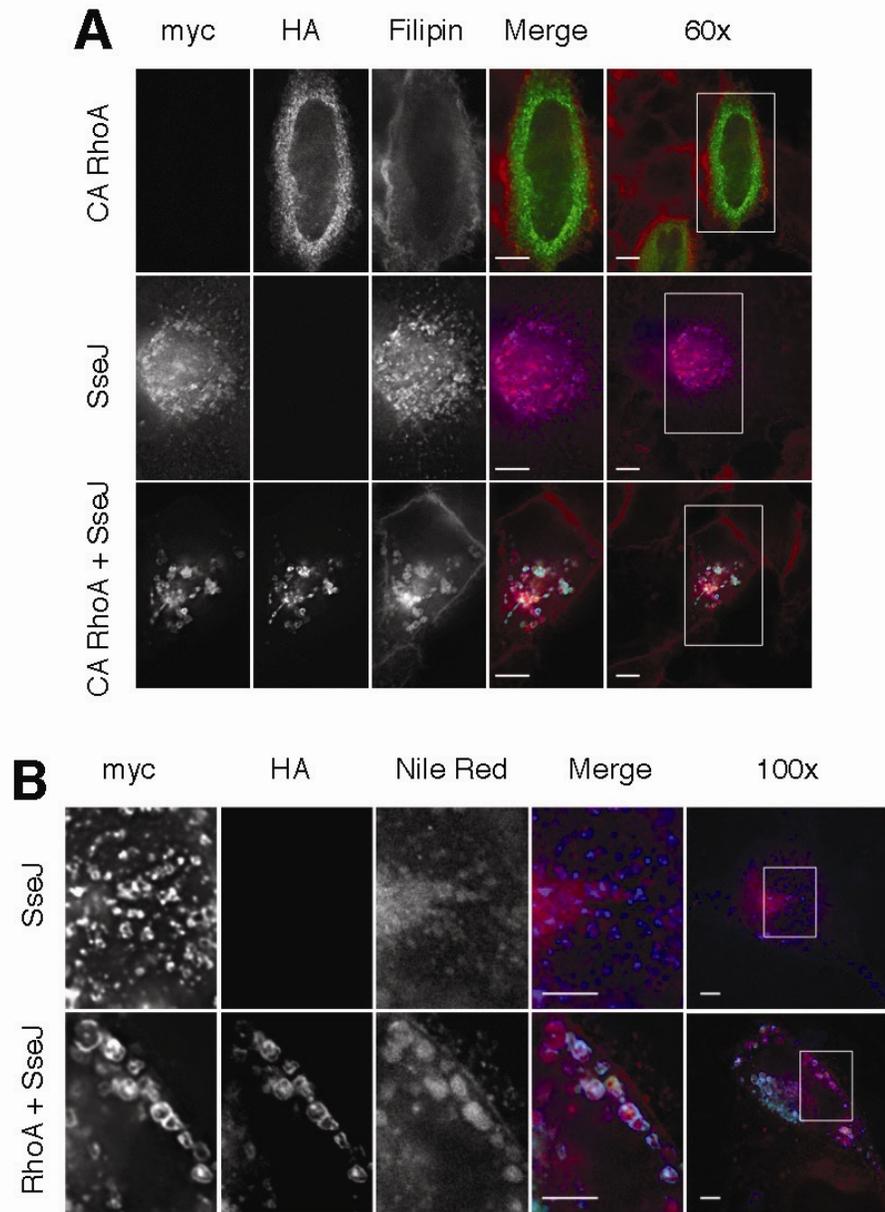


Fig. S2. Cotransfection of HeLa cells with plasmids encoding CA RhoA and SseJ results in the accumulation of cholesterol and neutral lipids in the enlarged endosomal compartment. **(A)** Ectopic expression of myc-SseJ and CA HA-RhoA in HeLa cells led to the formation of an enlarged endosomal compartment compared to that formed upon expression of myc-SseJ alone. The accumulation of cholesterol in the endosomal compartment was visualized by filipin staining. HeLa cells were incubated with antibodies against myc (blue), HA (green), and cholesterol (red). **(B)** Neutral lipids accumulated in the endosomal compartment as visualized by Nile Red staining. HeLa cells transfected with a plasmid encoding myc-SseJ or cotransfected with plasmids encoding CA HA-RhoA and myc-SseJ were incubated with antibodies against myc (blue), HA (green), and neutral lipids (red). Scale bars are 10 μm for (A) and 5 μm for (B). For both panels, the experiments were repeated four times.

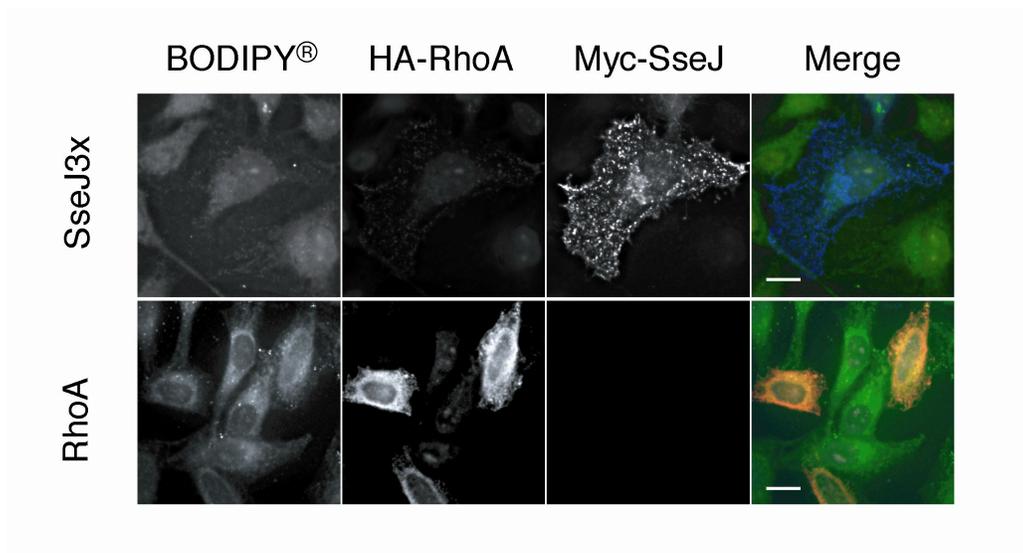


Fig. S3. The expression of SseJ3x or RhoA alone does not induce the formation of BODIPY-labeled cholesterol esters. HeLa cells were transfected with plasmids encoding myc-SseJ3x or CA HA-RhoA for 24 hours and were then incubated for 2h with 3.5 μ M PEDAl followed by incubation with antibodies against myc (blue) and HA (red). Scale bars are 10 μ m.

Table S1. Descriptions of the strains of *S. typhimurium* and the plasmids used in this study.

<i>Strain or Plasmid</i>	<i>Description</i>	<i>Source or Reference</i>
<i>S. typhimurium</i>		
CS401	14028s <i>phoN2 zxx::6251 Tn10d-Cm Str^r</i>	ATCC
JAF43	CS401 Δ <i>sseJ</i> Camr Str ^r	(1)
CS806	JAF43 pJAF111	(1)
CS807	JAF43 pKF35	this work
Plasmids		
pWSK29	Expression vector, Amp ^r	
pJAF111	SseJ-HA in pWSK29	(1)
pKF35	SseJ-S151A-HA in pWSK29	this work
myc-RhoA	pCDNA3.1RhoA 2xmyc (N terminus) Amp ^r	cDNA Resouce center
myc-RhoACA	pCDNA3.1RhoAG14V 2xmyc (N terminus) Amp ^r	cDNA Resouce center
HA-RhoACA	pCDNA3.1RhoAG14V 3xHA (N terminus) Amp ^r	cDNA Resouce center
myc-SseJ	pCMV-myc-3x-SseJ Amp ^r	(2)
his-RhoB	pNIC28	Declan A Doyle

References

1. J. A. Freeman, M. E. Ohl, S. I. Miller, The *Salmonella enterica* serovar typhimurium translocated effectors SseJ and SifB are targeted to the *Salmonella*-containing vacuole. *Infect. Immun.* **71**, 418–427 (2003).
2. M. B. Ohlson, Z. Huang, N. M. Alto, M.-P. Blanc, J. E. Dixon, J. Chai, S. I. Miller, Structure and function of *Salmonella* SifA indicate that its interactions with SKIP, SseJ, and RhoA family GTPases induce endosomal tabulation. *Cell Host Microbe* **4**, 434–446 (2009).