# Supporting Information

## Humphreys et al. 10.1073/pnas.1311680110

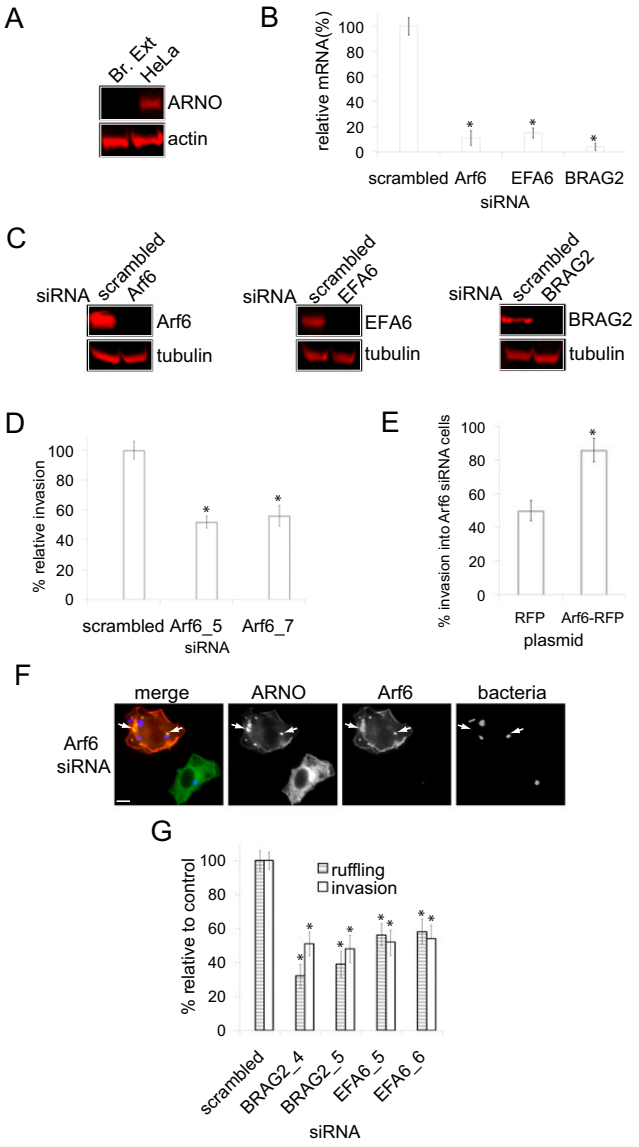


**Fig. S1.** Supplementary experiments. (*A*) Brain and HeLa cell extracts immunoblotted with antibodies to ARNO and actin as a loading control. (*B*) mRNA levels of ADP ribosylation factor 6 (Arf6), EFA6, or BRAG2 72 h after siRNA transfection. Values are relative to individual controls in each case. (*C*) Immunoblot of Arf6, BRAG2, or EFA6 after siRNA transfection with tubulin shown as control. (*D*) Influence of Arf6 depletion on *Salmonella* invasion. HeLa cells were transfected with scrambled or Arf6 siRNAs that each target distinct Arf6 mRNA sequences (Arf6 siRNA 5 or 7) 72 h before infection with *Salmonella* (10 min) encoding pM975 that expresses GFP inside pathogen-containing vacuoles. Error bars represent ± SEM. Asterisks indicate a significant difference vs. mock ($P < 0.05$, ANOVA; $n = 3$). (*E*) Restoration of invasion in Arf6-depeleted cells expressing Arf6-RFP. HeLa cells were transfected with Arf6 siRNAs and control RFP or Arf6-RFP before *Salmonella* infection as in *D*, and quantification of invasion in RFP-expressing cells was performed. Error bars represent ± SEM. Asterisks indicate a significant difference vs. mock ($P < 0.05$, *t* test; $n = 3$). (*F*) Restoration of ARNO-macropinosomes in Arf6-depeleted cells expressing Arf6-RFP. HeLa cells were transfected with Arf6 siRNA 72 h before coexpression of Arf6-RFP and CFP-tagged ARNO and with fluorescently labeled WT *Salmonella* (bacteria). Arrows indicate macropinosomes in cells expressing Arf6 and ARNO. (Scale bar: 8 μm.) (*G*) Influence of BRAG2 and EFA6 depletion on *Salmonella* invasion. HeLa cells were transfected with scrambled or BRAG2 and EFA6 siRNAs that each target distinct mRNA sequences (BRAG2 siRNAs 4 or 5, EFA6 siRNAs 5 or 6) 72 h before infection, and quantification of invasion was done as in *D*. Error bars represent ± SEM. Asterisks indicate a significant difference vs. mock ($P < 0.05$, ANOVA; $n = 3$).

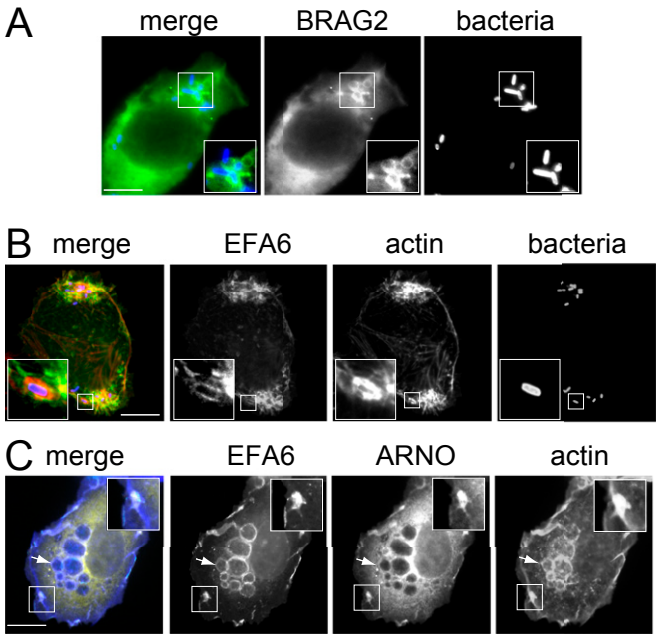


**Fig. S2.** Supplementary experiments. (*A*) Localization of BRAG2 during *Salmonella* invasion. HeLa cells expressing CFP-tagged BRAG2 were infected for 10 min with fluorescently labeled WT *Salmonella* (bacteria). (*Insets*) Magnified areas. (Scale bar: 8 μm.) (*B*) Localization of actin in HeLa cells expressing CFP-tagged EFA6 infected for 10 min with fluorescently labeled *Salmonella* (bacteria). Actin filaments were labeled by using Alexa Fluor 594–phalloidin. (*Insets*) Magnified areas. (Scale bar: 8 μm.) (*C*) Localization of CFP-tagged EFA6 and YFP-tagged ARNO in HeLa cells stained with Alexa Fluor 594–phalloidin to visualize actin. (*Insets*) Magnified areas. (Scale bar: 8 μm.)