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SUPPLEMENTARY INFORMATION

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N^{ε}-fatty acylation of multiple membrane-associated proteins by *Shigella* IcsB effector to modulate host function

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N^{ε}-fatty acylation of multiple membrane-associated proteins by *Shigella* IcsB effector to modulate host function

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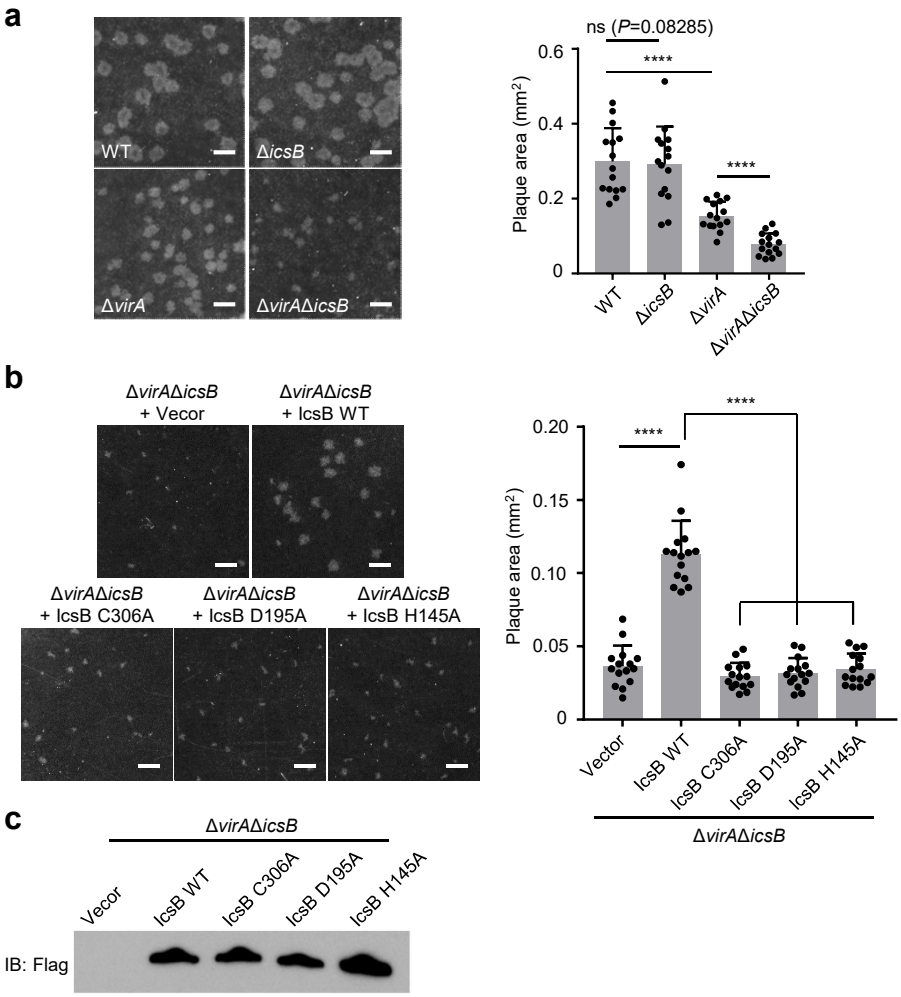
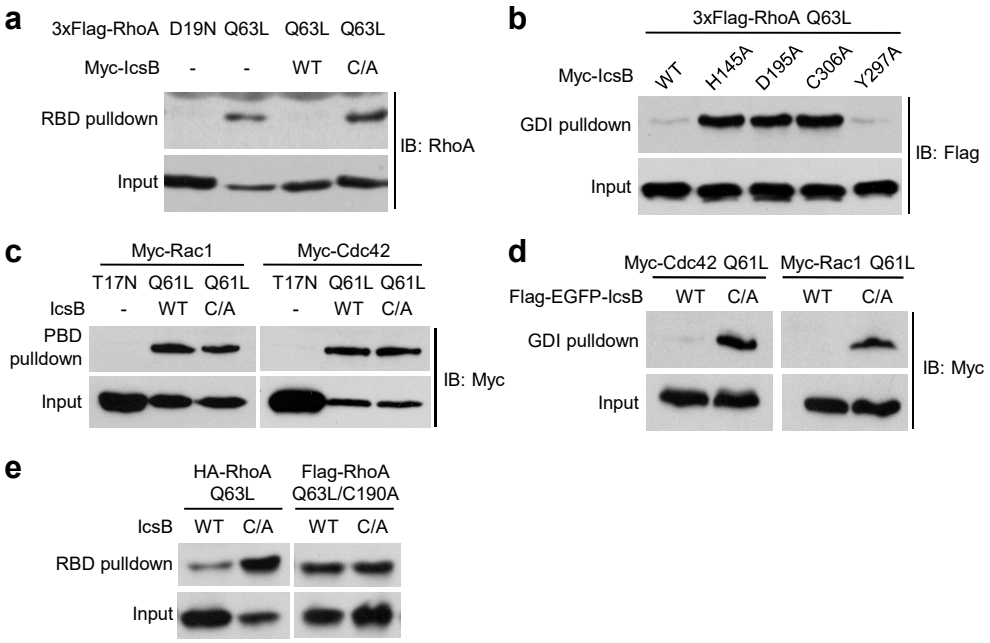
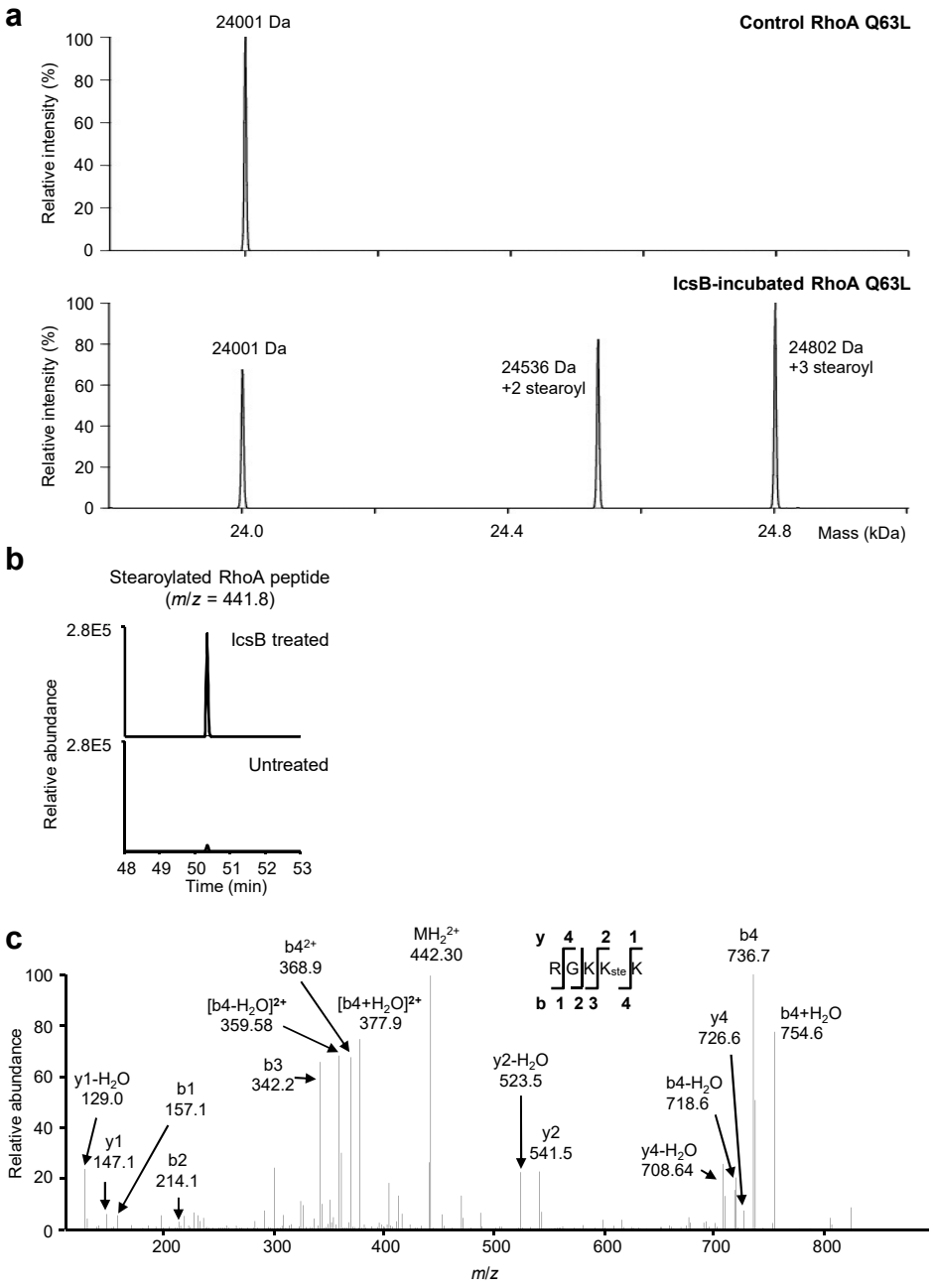


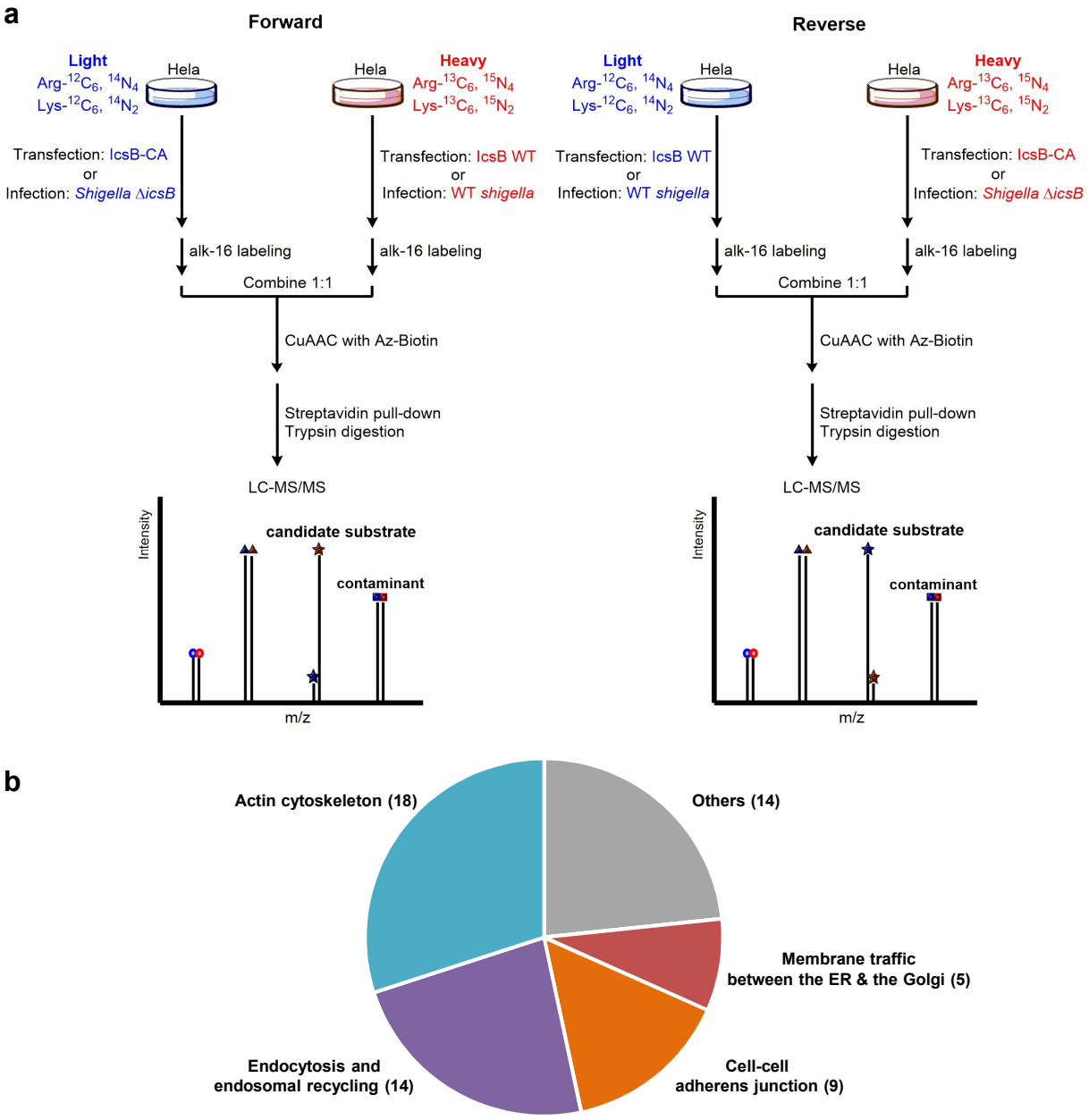
Supplementary Figure 1 | The putative catalytic motif of IcsB is necessary for intercellular spread of *S. flexneri*. **a**, **b**, HeLa cells were infected with *S. flexneri* WT, Δ*icsB*, Δ*virA*, Δ*virA*Δ*icsB* (**a**) or Δ*virA*Δ*icsB* complemented with WT, *icsB* or an indicated catalytically inactive mutant (**b**), and then subjected to plaque formation assay. The left panels (scale bar, 1 mm) show plaques formed by each strain and the graphs on the right indicates average area of plaques formed by each strain. To measure the plaque area, 15 plaques for each strain were examined and the data are shown as mean ± s.d. The two-tailed unpaired Student's *t*-test was performed in **a** and **b** (**** $P < 0.0001$; ns, not significant). **c**, The bacteria used in **b** were lysed and analyzed by anti-Flag immunoblotting (IB) to confirm the expression of IcsB. Data (**a**-**c**) are representative of three independent experiments.



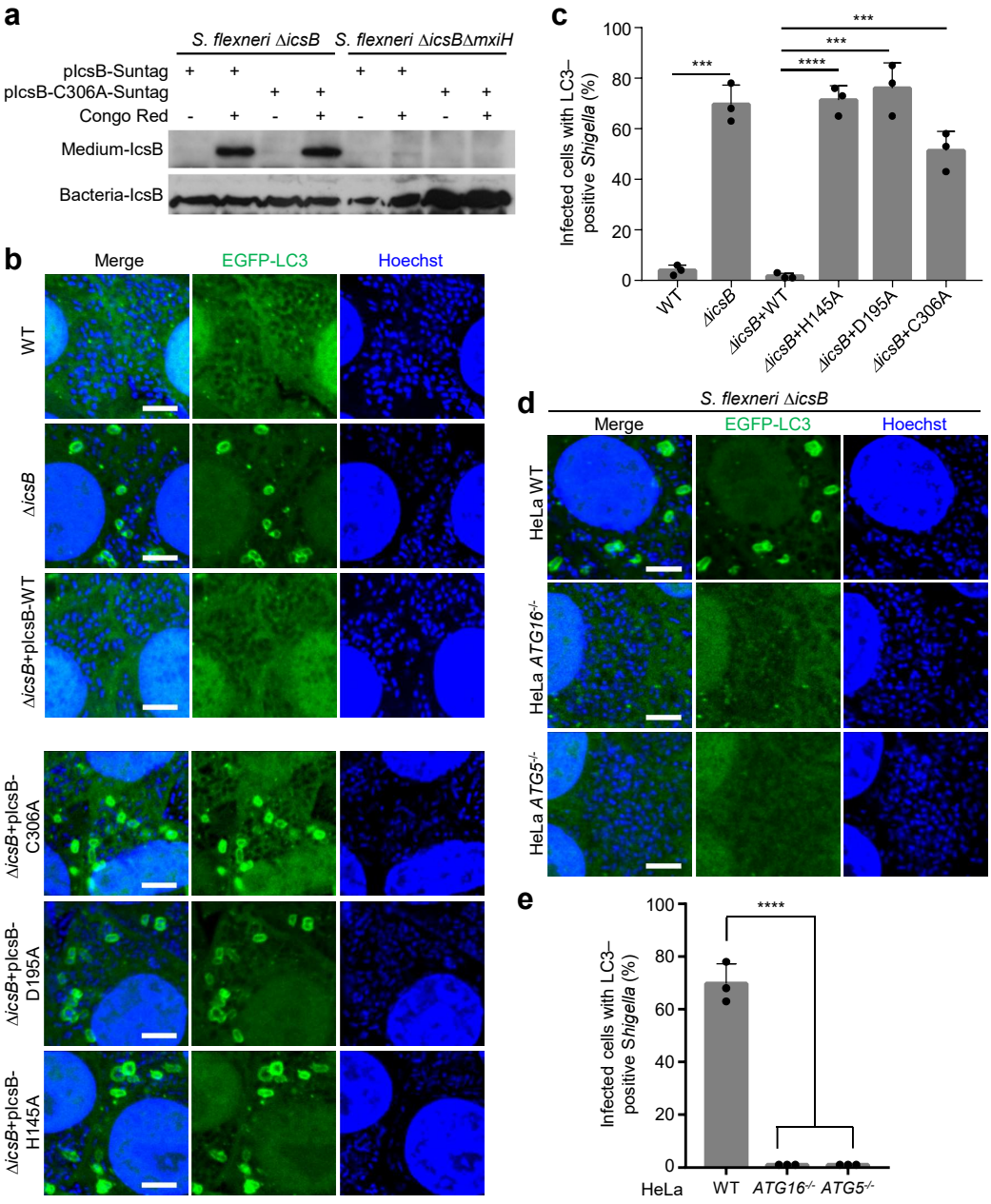
Supplementary Figure 2 | Ectopic expression of IcsB disrupts the binding of RhoGTPases to RhoGDI. a, Inactivation of RhoA by IcsB. 293T cells were co-transfected with Flag-RhoA and Myc-IcsB expression constructs as indicated. Cell lysates were subjected to GST-RBD pulldown assay. **b**, Disruption of RhoGDI binding to RhoA by IcsB. 293T cells were co-transfected with 3xFlag-RhoA Q63L and an indicated IcsB expression plasmid. Cell lysates were subjected to GST-RhoGDI pulldown assay. **c**, IcsB does not affect the activation state of Rac1/Cdc42. 293T cells were co-transfected with Myc-Rac1/Cdc42 and an indicated IcsB expression construct. Cell lysates were subjected to GST-PBD pulldown assay. **d**, Disruption of RhoGDI binding to Rac1/Cdc42 by IcsB. 293T cells were co-transfected with Myc-Rac1/Cdc42 and an indicated IcsB expression construct. Cell lysates were subjected to GST-RhoGDI pulldown assay. **e**, Effects of mutation of the prenylated cysteine in RhoA on its inactivation by IcsB. 293T cells were co-transfected with HA-RhoA Q63L (or Flag-RhoA Q63L/C190A) and an indicated IcsB expression construct. Cell lysates were subjected to GST-RBD pulldown assay. Shown in **a**-**e** are immunoblots of samples eluted from the GST beads (upper panels) and the input (lower panels). All data (**a**-**e**) are representative of three independent experiments.



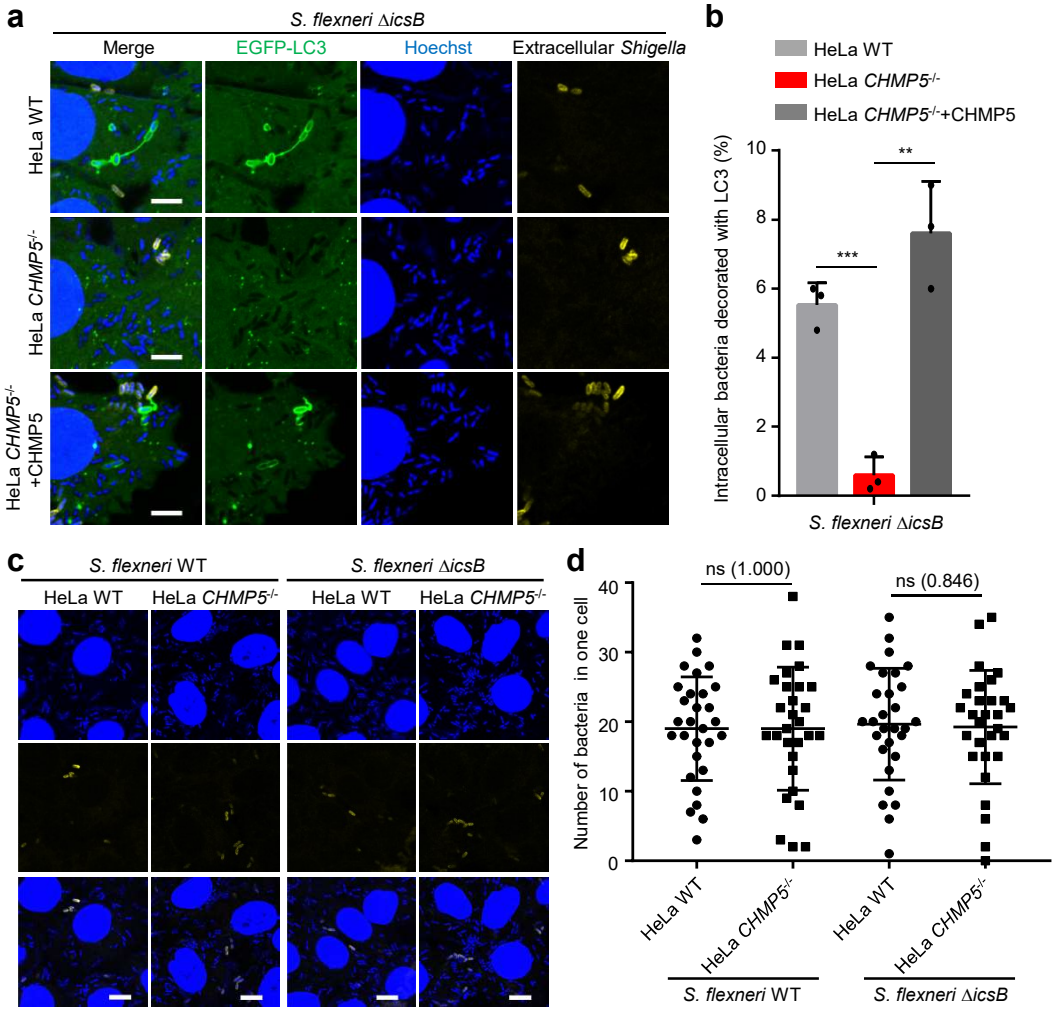
Supplementary Figure 3 | Mass spectrometry of *in vitro* modification of RhoA by IcsB. a, Total molecular mass measurement of *in vitro* IcsB-modified RhoA. Farnesylated RhoA Q63L was incubated with/without purified IcsB in the presence of stearoyl-CoA and IP_6. The total molecular mass was analyzed by ESI mass spectrometry. **b**, Extracted ion chromatograms of RhoA modified by IcsB *in vitro*. Shown are graphs for the stearoylated peptide RGKKK (*m*/*z* = 441.8). **c**, MS/MS mass spectrum of the stearoylated peptide RGKKK from *in vitro* IcsB-modified RhoA. All data (**a**-**c**) are representative of two independent experiments.



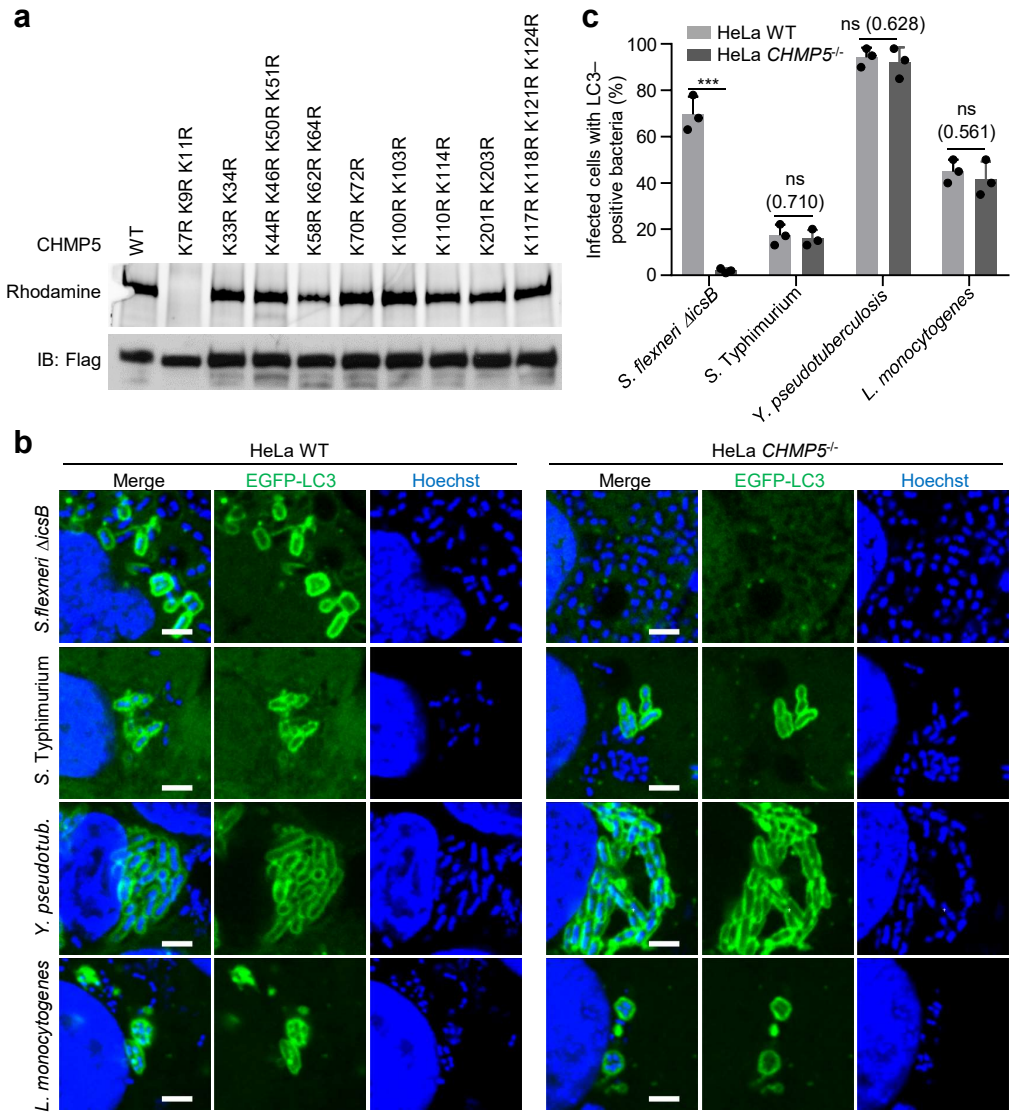
Supplementary Figure 4 | Proteomic profiling of IcsB-dependent fatty acylation modification of host proteins. a, Schematic diagram of click reaction-based quantitative proteomic profiling of host proteins modified under the IcsB-transfection or *Shigella*-infection condition. Experimental details are described in the Method session. **b**, Gene Ontology-based clustering analysis of the proteomic substrates of IcsB. The analysis was based on DAVID Bioinformatics Resources 6.7 (https://david.ncifcrf.gov) with modifications in consideration of the bacterial infection context used here.


a
S. flexneri ΔicsB
S. flexneri ΔicsBΔmxiH
pIcsB-Suntag
pIcsB-C306A-Suntag
Congo Red
Medium-IcsB
Bacteria-IcsB
b
Merge
EGFP-LC3
Hoechst
WT
ΔicsB
ΔicsB+pIcsB-WT
ΔicsB+pIcsB-C306A
ΔicsB+pIcsB-D195A
ΔicsB+pIcsB-H145A
c
Infected cells with LC3–positive Shigella (%)
WT
ΔicsB
ΔicsB+WT
ΔicsB+H145A
ΔicsB+D195A
ΔicsB+C306A
d
S. flexneri ΔicsB
Merge
EGFP-LC3
Hoechst
HeLa WT
HeLa ATG16-/-
HeLa ATG5-/-
e
Infected cells with LC3–positive Shigella (%)
HeLa
WT
ATG16-/-
ATG5-/-

Supplementary Figure 5 | The fatty acyltransferase activity of IcsB mediates *Shigella* escape from autophagy and analyses of host autophagic response to *S. flexneri* infection. a, *S. flexneri* Δ*icsB* or Δ*icsB*Δ*mxiH* harboring pME6032-IcsB WT/C306A-24xSuntag-Flag and pBAD24-IpgA were cultured in 2xYT broth containing 1 mM IPTG and 0.02% L-(+)-arabinose, and treated with or without 30 μg/mL Congo Red (to activate the T3SS). The supernatants of bacteria culture containing were subjected to anti-Flag immunoprecipitation followed by anti-Flag immunoblotting analyses. The pellets were also lysed and analyzed by anti-Flag immunoblotting analyses. **b**, **c**, Effects of IcsB catalytic residues mutation on *Shigella* escape from autophagy. HeLa cells stably expressing EGFP-LC3 were infected with *S. flexneri* WT, Δ*icsB* or Δ*icsB* complemented with WT IcsB or a catalytically inactive mutant. **b**, Fluorescence images taken at 2 h post-infection (scale bar, 3 μm). **c**, Percentages of infected cells containing LC3-positive *Shigella*. At least 200 infected cells were examined for each experiment and data are presented as mean ± s.d. from three replicates. **d**, **e**, Analyses of host autophagic response to *S. flexneri* infection. WT or indicated knockout HeLa cells stably expressing EGFP-LC3 were infected with *S. flexneri* Δ*icsB*. **d**, Fluorescence images taken at 2 h post-infection are (scale bar, 3 μm). **e**, Percentages of infected cells containing LC3-positive *Shigella*. At least 200 infected cells were examined for each experiment and data are presented as mean ± s.d. from three replicates. Two-tailed unpaired Student's *t*-test was performed (**c**, **e**) (** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$). All data (**a**-**e**) are representative of three independent experiments.



Supplementary Figure 6 | CHMP5 is required for *S. flexneri*-induced autophagy but not *S. flexneri* invasion into host cells. a, **b**, Effects of *CHMP5* deficiency on bacterial autophagosome formation in response to *S. flexneri* infection. Indicated HeLa cells expressing EGFP-LC3 were infected with *S. flexneri* Δ*icsB* (MOI=8). Extracellular bacteria were stained by the antibody against *Shigella* LPS. **a**, Representative fluorescence images (scale bar, 7 μm). **b**, Percentages of LC3-positive intracellular bacteria at 2 h post-infection. At least 100 intracellular bacteria were examined for each experiment and the data are presented as mean ± s.d. from three replicates. **c**, **d**, Effects of *CHMP5* deficiency on *S. flexneri* invasion. Indicated HeLa cells were infected with WT or Δ*icsB* mutant of *S. flexneri* (MOI=8) for 2 h. Total *S. flexneri* are indicated by the Hoechst 33342 signal and extracellular *S. flexneri* were stained by the antibody against *Shigella* LPS. **c**, Representative fluorescence images (scale bar, 10 μm). **d**, The number of intracellular *S. flexneri* in one cell was counted and the statistics is shown (mean ± s.d., n=30). Two-tailed unpaired Student's *t*-test was performed (** $P < 0.01$; *** $P < 0.001$; ns, not significant). Data (**a**-**d**) are representative of at least two independent experiments.



Supplementary Figure 7 | CHMP5 is specifically involved in host autophagy targeting intracellular *S. flexneri*. **a**, Lysine mutation analyses of CHMP5 in its modification by IcsB. 293T cells were co-transfected with IcsB and Flag-CHMP5 (WT or an indicated K to R mutant). Transfected cells were metabolized with Alk-16 for and then subjected to in-gel fluorescence assay. **b**, **c**, Effects of *CHMP5* deficiency on autophagosome formation in response to other bacterial infections. WT or *CHMP5*$^{-/-}$ HeLa cells stably expressing EGFP-LC3 were infected with *S. flexneri* Δ*icsB*, *S.* Typhimurium SL1344, *Y. pseudotuberculosis* IP2666 (ΔHEMOJ(T)) or *L. monocytogenes* EGD strain. **b**, Fluorescence images taken at 2 h post-infection (scale bar, 1.5 μm). **c**, Percentages of infected cells containing LC3-positive *Shigella*. At least 200 infected cells were examined for each experiment and data are presented as mean ± s.d. from three replicates. Two-tailed unpaired Student's *t*-test was performed (*** $P < 0.001$; ns, not significant). All data (**a**-**c**) are representative of three independent experiments.

Fig. 1a anti-Flag

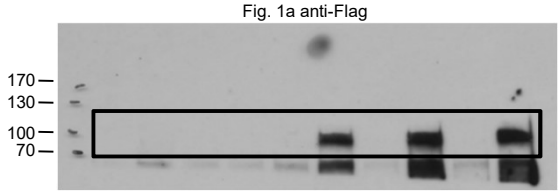


Fig. 1b anti-Flag

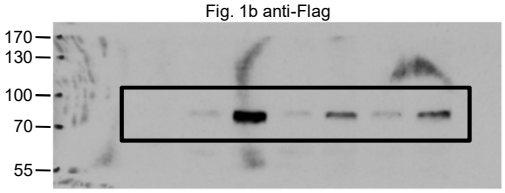


Fig. 1a anti-GAPDH

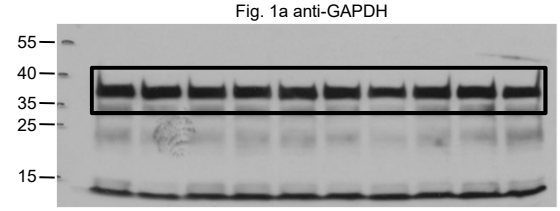


Fig. 1b anti-Tubulin

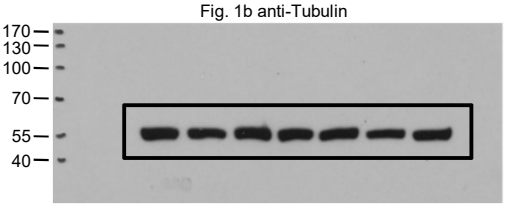
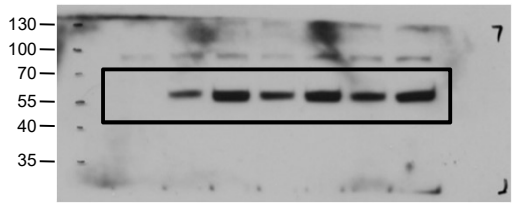


Fig. 1d anti-Flag



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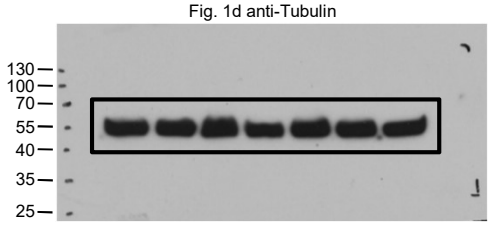


Fig. 2a anti-RhoA

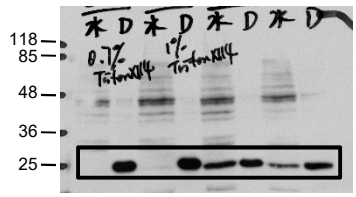


Fig. 2b anti-Myc (Middle)

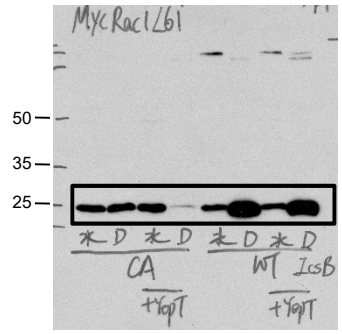


Fig. 2b anti-Myc (Lower)

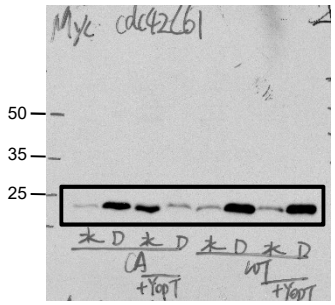


Fig. 2b anti-Flag (Upper)

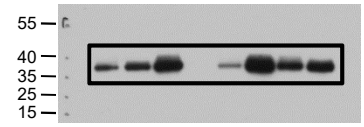


Fig. 2d anti-EGFP (Middle)

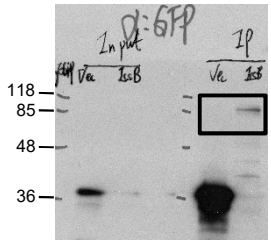


Fig. 2d anti-HA

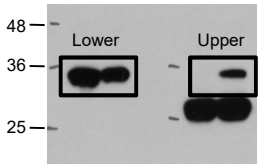


Fig. 2e anti-RhoA

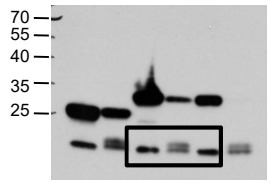
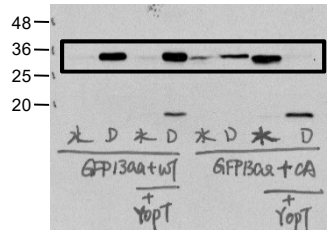


Fig. 2f anti-EGFP



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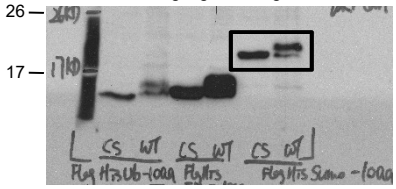


Fig. 2h anti-Flag (Upper)

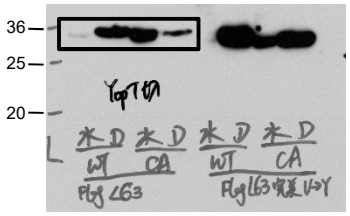


Fig. 2h anti-Flag (Middle)

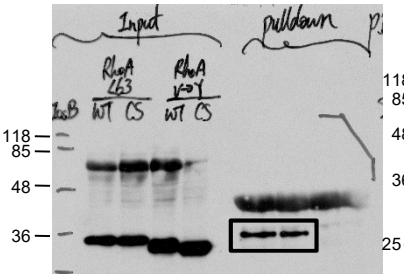


Fig. 2h anti-Flag (Lower)

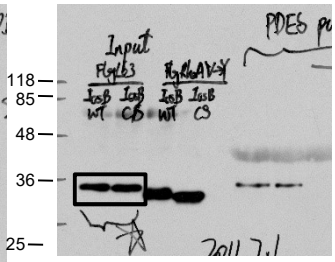


Fig. 3e ^{3}H autoradioactive exposure

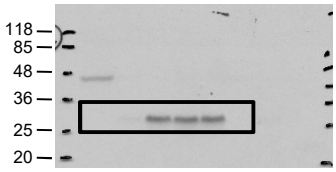


Fig. 3f anti-Flag (Left)

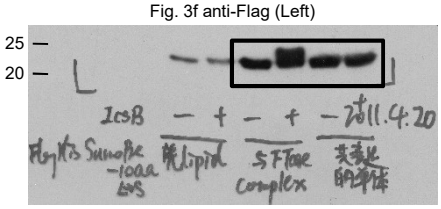


Fig. 3e Coommassie staining

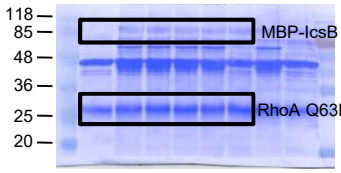


Fig. 3f anti-Flag (Right)

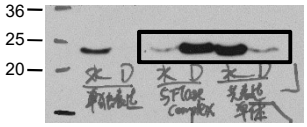


Fig. 4c In-gel fluorescence (Left)

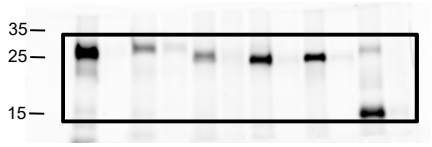
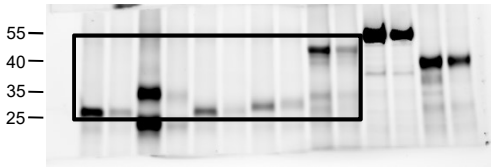


Fig. 4c In-gel fluorescence (Right)



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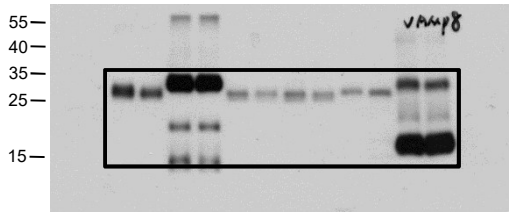

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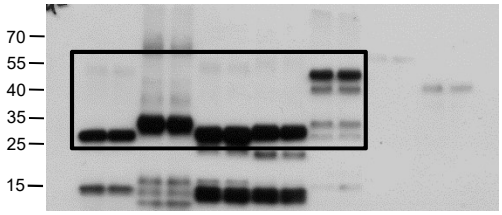


Fig. 4d In-gel fluorescence (Left)

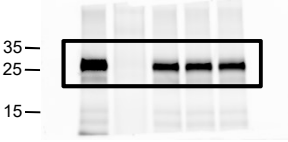


Fig. 4d In-gel fluorescence (Middle)

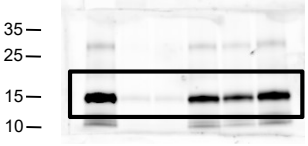


Fig. 4d In-gel fluorescence (Right)

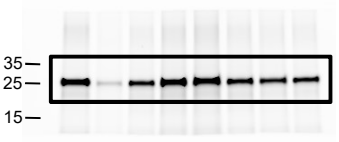


Fig. 4d anti-Flag (Left)

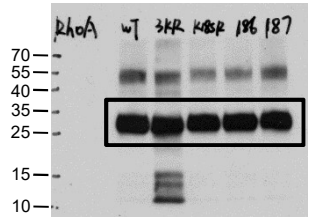


Fig. 4d anti-Flag (Middle)

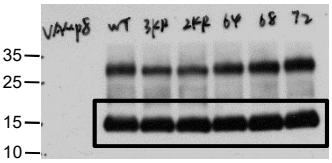


Fig. 4d anti-Flag (Right)

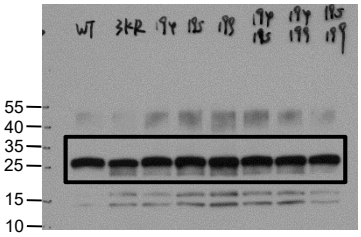


Fig. 5b anti-Flag

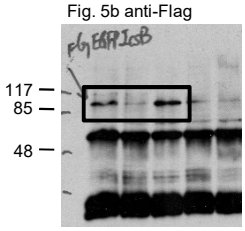


Fig. 5b anti-Cadherin

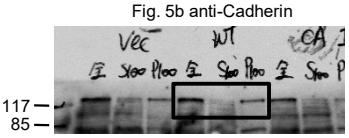


Fig. 5b anti-Erk2

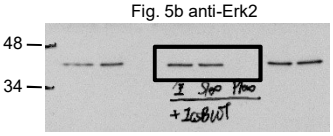


Fig. 5e In-gel fluorescence (Left)

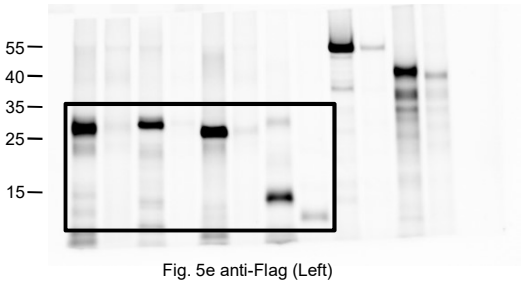


Fig. 5e In-gel fluorescence (Right)

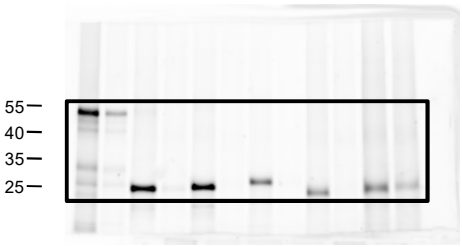


Fig. 5e anti-Flag (Left)

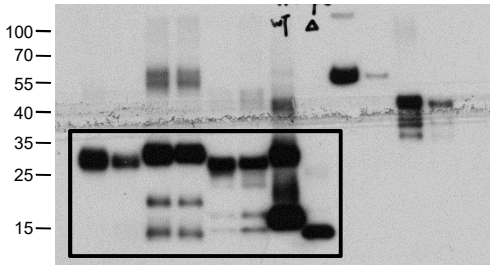


Fig. 5e anti-Flag (Middle)

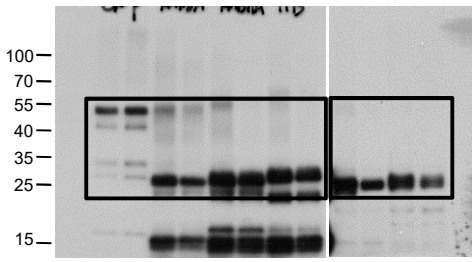


Fig. 5e anti-Flag (Right)

Fig. 5f In-gel fluorescence RhoA

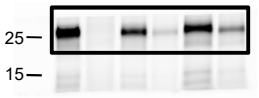


Fig. 5f In-gel fluorescence Rab13

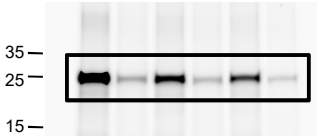


Fig. 5f In-gel fluorescence VAMP8

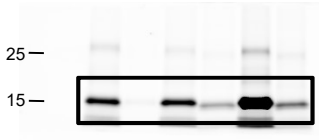


Fig. 5f anti-Flag RhoA

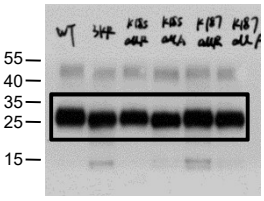


Fig. 5f anti-Flag Rab13

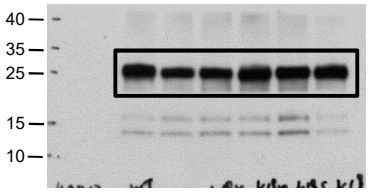


Fig. 5f anti-Flag VAMP8

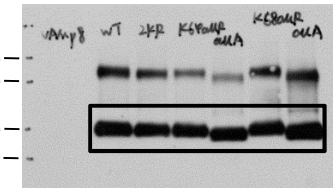


Fig. 6c In-gel fluorescence

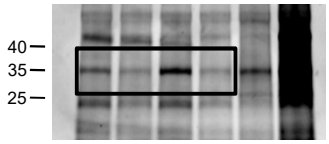


Fig. 6d In-gel fluorescence

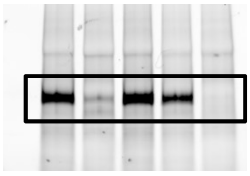


Fig. 6c anti-Flag

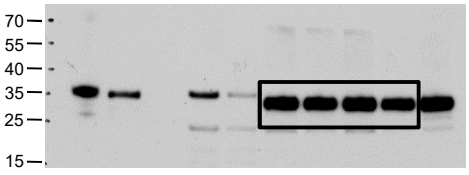
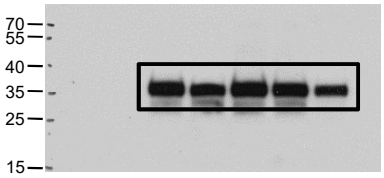
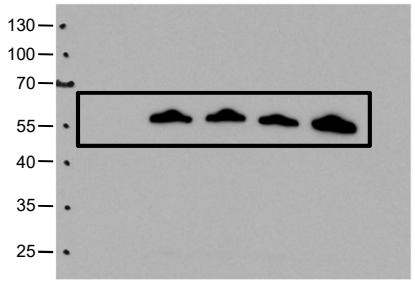


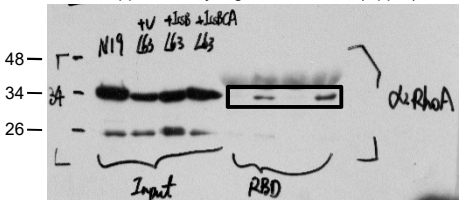
Fig. 6d anti-Flag



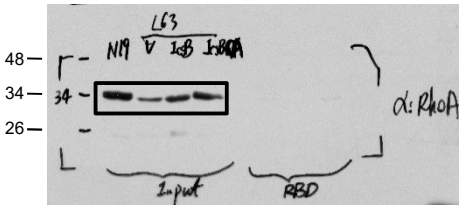
Supplementary Fig. 1c anti-Flag



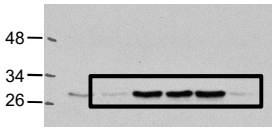
Supplementary Fig. 2a anti-RhoA (Upper)



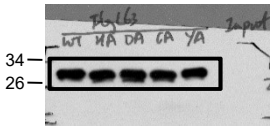
Supplementary Fig. 2a anti-RhoA (Lower)



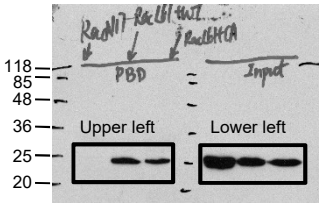
Supplementary Fig. 2b anti-Flag (Upper)



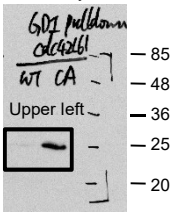
Supplementary Fig. 2b anti-Flag (Lower)



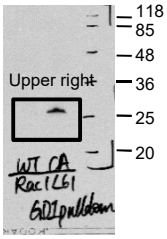
Supplementary Fig. 2c anti-Myc



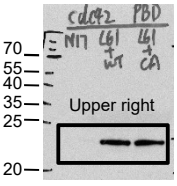
Supplementary Fig. 2d anti-Myc



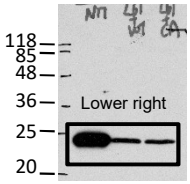
Supplementary Fig. 2d anti-Myc



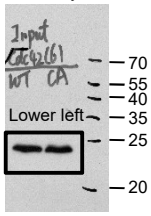
Supplementary Fig. 2c anti-Myc



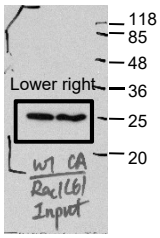
Supplementary Fig. 2c anti-Myc



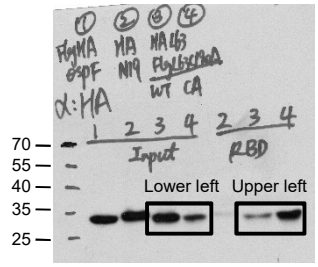
Supplementary Fig. 2d anti-Myc



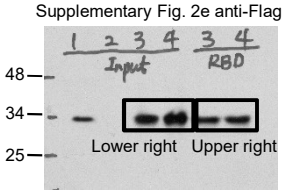
Supplementary Fig. 2d anti-Myc



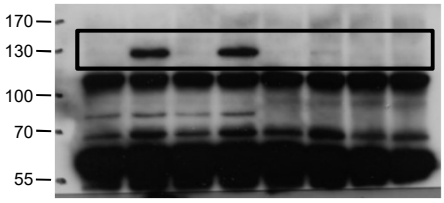
Supplementary Fig. 2e anti-HA



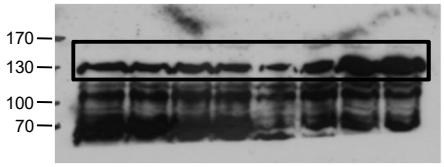
Supplementary Fig. 2e anti-Flag



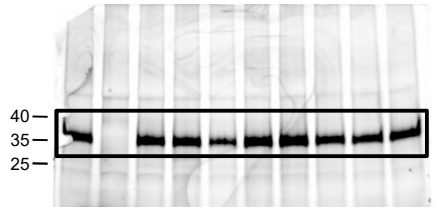
Supplementary Fig. 5a anti-Flag (Upper)



Supplementary Fig. 5a anti-Flag (Lower)



Supplementary Fig. 7a In-gel fluorescence



Supplementary Fig. 7a anti-Flag