Golgi stress induces SIRT2 to counteract Shigella infection via defatty-acylation

Enzymes from pathogens often modulate host protein post-translational modifications (PTMs), facilitating survival and proliferation of pathogens. Shigella virulence factors IpaJ and IcsB induce proteolytic cleavage and lysine fatty acylation on host proteins, which cause Golgi stress and suppress innate immunity, respectively. However, it is unknown whether host enzymes could reverse such modifications introduced by pathogens’ virulence factors to suppress pathogenesis. Herein, we report that SIRT2, a potent lysine defatty-acylase, is upregulated by the transcription factor CREB3 under Golgi stress induced by Shigella infection. SIRT2 in turn removes the lysine fatty acylation introduced by Shigella virulence factor IcsB to enhance host innate immunity. SIRT2 knockout mice are more susceptible to Shigella infection than wildtype mice, demonstrating the importance of SIRT2 to counteract Shigella infection.

Results

Golgi stress upregulates SIRT2 via CREB3

The project initially started when we were interested in finding out how SIRT2 is regulated as we believe knowing its regulation is crucial for understanding its biological function. Interestingly, we found that...
Brefeldin A (BFA) drastically increased SIRT2 protein levels in multiple cell lines (Fig. 1A–B). BFA is known to induce Golgi stress. The ER stress inducers, Tunicamycin (Tm) and Thapsigargin (Tg), only slightly increased SIRT2 (Fig. 1C), which further confirmed the specificity of the stress response.

Quantitative real-time (RT)-PCR revealed that the SIRT2 transcript level increased more than 50-fold under BFA treatment when compared to the vehicle control group (Fig. 1D). Moreover, 24 h of Actinomycin D (ActD) or cycloheximide (CHX) treatment abolished the protein upregulation. This was consistent with the observation that the SIRT2 transcript increased primarily during 12–24 h of BFA treatment and suggested SIRT2 mRNA stability or protein stability was not the key attributes (Fig. 1E–F). We also examined several other sirtuins and HDACs under Golgi stress. SIRT2 mRNA and protein level increase was the most drastic (Fig. 1G–H). Taken together, the data suggest SIRT2 is upregulated by Golgi stress through transcriptional control.

Next, we tried to identify the transcription factor responsible for SIRT2 upregulation under Golgi stress. The Golgi apparatus is hypothesized to integrate and transduce stress stimuli to the nucleus. One of the mechanisms is through the CREB3 transcription factor. Thus,

![Fig. 1](https://example.com/fig1.png)

**Fig. 1** | Golgi stress transcriptionally upregulates SIRT2. A Immunoblots for SIRT2 in different cell lines treated with 5 μg/ml BFA for 24 h. HSP90 blots are shown as loading controls. Representative images from three independent experiments are shown. B Quantification of relative SIRT2 protein levels in different cell lines, normalized to HSP90. Statistical evaluation was done by a paired two-tailed Student’s t-test. n = 3 or 4 biological replicates. C In A549 cells, BFA treatment significantly increased SIRT2 protein level, while the ER stress inducers Tunicamycin (Tm) and Thapsigargin (Tg) only weakly induced SIRT2 expression. D RT-PCR analysis for SIRT2 and CREB3 mRNA levels in A549 cells treated with 5 μg/ml BFA for various incubation times. mRNA was normalized to 0 h. Statistical evaluation was done by an unpaired two-tailed Student’s t-test. n = 3 biological replicates. E ActD chase analysis showing SIRT2 upregulation is due to mRNA upregulation, which happens primarily during 12–24 h. A549 cells were treated with or without BFA at t = 0 h. During BFA treatment, ActD was added at t = 0, 12, 18, 23, 24 h. At t = 24 h, cells were collected and submit for western blot analysis. F CHX chase analysis showing SIRT2 upregulation is not due to protein stability. A549 cells were treated with or without BFA at t = 0 h. During BFA treatment, CHX was added at t = 0, 12, 24 h. At t = 24 h, cells were collected and submit for western blot analysis. G RT-PCR analysis for SIRT1-7 and HDAC11 mRNA level in A549 cells treated with 5 μg/ml BFA for 24 h. Fold change in mRNA was calculated by comparing samples with BFA treatment to control DMSO. n = 3 biological replicates. H Among all sirtuins and HDAC11 tested, SIRT2 protein level increase was the most drastic under Golgi stress. A549 cells were treated with BFA the indicated concentrations for 24 h before analyzed by western blot. α-tubulin blots were loading control. Data are represented as mean ± SEM. Statistical evaluation was done using unpaired two-tail Student’s t-test. ***p < 0.001.
we decided to test if CREB3 is the transcription factor controlling SIRT2 expression under Golgi stress.

We first confirmed that the CREB3 transcript was increased after BFA treatment (Fig. 1D). To test if CREB3 upregulates SIRT2 under Golgi stress, CREB3 KD cells were treated with or without BFA. The quantification of SIRT2 protein level, normalized to HSP90, is shown on the right. Statistical evaluation was done by two-way ANOVA.

Moreover, the SIRT2 promoter contains a strong CREB3 binding site (Fig. 2D), and is responsive to the expression of the active (cleaved) form of CREB3 or BFA treatment (Fig. 2E–F). Thus, the increased SIRT2 expression under Golgi stress is through CREB3.

While trying to understand the physiological relevance of BFA-induced Golgi stress and the possible role of SIRT2 upregulation, it came to our attention that an intracellular pathogen, Shigella flexneri, is known to induce Golgi fragmentation to inhibit host cell secretion13. Shigella utilizes a type III secretion system (TTSS) to inject multiple effectors into host cells for successful invasion. One of the effectors, IpaJ, induces Golgi stress by proteolytic cleavage of N-terminal glycine myristoylated proteins, such as ADP-ribosylation factors (ARFs)26.

To test if SIRT2 is upregulated by pathogen-induced Golgi stress, we overexpressed IpaJ in HEK293T and A549 cells. Indeed, SIRT2 protein level was upregulated (Fig. 3A and Supplementary Fig. 2E). Infecting mouse bone-marrow-derived macrophage (BMDM) cells or mice with wildtype S. flexneri increased SIRT2 protein and mRNA levels while infection with the IpaJ deletion strain or IpaJ/VirA double deletion strain did not significantly change SIRT2 levels (Fig. 3B–C, Supplementary Fig. 2). Consistent with IpaJ upregulating SIRT2 on Chromatin 19 complement [39,389,400-39,391,600], reference: GRCh37.

E SIRT2-promoter driven firefly transcription in cells under Flag-tagged CREB3 N-terminal bZIP domain (CREB3-N) overexpression. The Renilla luciferase construct was used as an internal control. F Firefly luciferase transcription in cells treated with BFA. Cells were transfected with an empty pGL3-basic vector (ctrl) or pGL3-basic vector with 994 base pairs of SIRT2 5' UTR (Sirt2). G CREB3 chromatin IP followed by qPCR of the SIRT2 promoter using primers surrounding the putative CREB3 binding site. Results are from three separate experiments with three technical replicates each. Data are represented as mean ± SEM. Statistical evaluation was done using unpaired two-tail Student’s t-test. ***p < 0.001.
through CREB3, overexpression of IpaJ in CREB3 knockdown cells did not upregulate SIRT2 (Supplementary Fig. 2E). Infection of cells with enteropathogenic *E. coli*, as well as overexpression of EspG and VirA (Golgi stress-inducing bacterial toxins from enteropathogenic *E. coli* and *Shigella* respectively) also upregulated SIRT2 consistent with a conserved mechanism of SIRT2 regulation during bacteria-induced Golgi stress (Supplementary Fig. 2F–G). Overall, the data is consistent with the idea that *Shigella* infection upregulates SIRT2 via Golgi stress and suggest a potential role of SIRT2 in pathogen-host interaction.

**SIRT2 counteracts the action of IcsB**

To identify the role of SIRT2 during *Shigella* infection, we focused on the enzymatic activity of SIRT2. SIRT2 can hydrolyze long-chain fatty acyl groups on protein lysine residues. Interestingly, another effector from *Shigella*, IcsB, is able to fatty acylate host proteins. Therefore, we hypothesized that, upon *Shigella* infection, SIRT2 is upregulated to remove the PTM installed by IcsB as a host defense mechanism.

We first tested whether SIRT2 could reverse the fatty acylation added by IcsB in vitro. An alkyne-tagged long-chain fatty acid analog, Alk14, was used to metabolically label substrate proteins. Several known IcsB substrate proteins were tagged with a flag tag, co-overexpressed with IcsB and immunoprecipitated from HEK293T cell that were treated with Alk14. Next, we incubated the isolated proteins with purified recombinant SIRT2 with or without NAD+, and then conjugated the substrate proteins to BODIPY-azide using click chemistry to allow visualization of fatty acylation level by in-gel fluorescence. Hydroxylamine (NH$_2$OH) was used to remove cysteine modification to allow visualization of fatty acylation level by in-gel fluorescence. As expected, co-expression of SIRT2 with multiple IcsB substrate proteins significantly decreased their lysine fatty acylation levels, whereas co-expression of SIRT2-HY had much less effect (Fig. 4B–C, Supplementary Fig. 3B, 4A–B). Similar trends were observed when HEK293T cells were infected with *S. flexneri* instead of ectopically overexpressing IcsB (Supplementary Fig. 3A). Additionally, IcsB substrates showed higher lysine fatty acylation levels in SIRT2 knockdown cells upon IcsB overexpression or *Shigella* infection (Supplementary Fig. 3C–D). The effect of SIRT2 knockdown was less pronounced compared to SIRT2 overexpression. We reasoned that the overexpression of SIRT2 more closely mimics *Shigella* infection where SIRT2 is upregulated by Golgi stress. Global protein fatty acylation labeling also showed that *Shigella* infection increased the labeling of several bands and SIRT2 knockdown further increased the labeling (Supplementary Fig. 4C).

It is reported that the fatty acylation on Rho GTPase inhibits the protein-protein interaction between Rho GTPases and RhoGDI, which regulates the membrane cycling and signaling of Rho GTPases. We wanted to confirm these results and find out whether SIRT2 could rescue this effect. As shown in Fig. 5, lysine fatty acylation inhibits Rho GTPase and RhoGDI interaction, and this can be rescued by SIRT2 overexpression. Thus, SIRT2 works as a defatty-acylase to maintain Rho GTPase signaling during *Shigella* infection.

We tested other enzymes that can remove long-chain fatty acyl groups from protein lysine residues and found that HDAC1, but not SIRT6 or SIRT7, could also decrease IcsB substrate fatty acylation (Supplementary Fig. 3E), suggesting that HDAC1 could also be involved. However, HDAC1 is not regulated by Golgi stress (Fig. 1G), which is different from SIRT2. The strong activity of SIRT2 in comparison to SIRT6 and SIRT7 could be explained by the fact that SIRT2 has more efficient activity in vitro and it lacks substrate peptide sequence selectivity. Also, SIRT2 is the only sirtuin with a pre-dominant cytosolic localization, which allows the interaction with IcsB.
**Fig. 4 | SIRT2 is a potent lysine defatty-acylase that counteracts IcsB.**

A. In-gel fluorescence detection of lysine fatty acylation of Flag-tagged IcsB substrate proteins treated with 5 μM of SIRT2, with or without 1 mM of NAD⁺ in vitro. B. In-gel fluorescence detection of lysine fatty acylation of Flag-tagged IcsB substrates in HEK293T cells that were also transfected with Flag-tagged IcsB and SIRT2. Representative images from at least three independent experiments are shown. EV, empty vector. WT, SIRT2 WT. HY, SIRT2 HY. FL, fluorescence (indicative of lysine fatty acylation level on substrate proteins). Flag, anti-Flag immunoblot.

B. Quantification of the lysine fatty acylation levels in B. n = 4–6 biological replicates.

C. In-gel fluorescence detection of lysine fatty acylation of Flag-tagged Rac1 and Rac2 in HEK293T cells that were infected with equal number of WT or Ipaj deletion S. flexneri M90T cells. FL, fluorescence (lysine fatty acylation level). Flag, anti-Flag immunoblots.

D. Quantification of the lysine fatty acylation levels detected in D. n = 3 biological replicates. Data are represented as mean ± SEM. Statistical evaluation was done using one-way ANOVA. ***p < 0.001.
substrates. With the same multiplicity of infection (MOI), lysine fatty acylation levels in IcsB substrate proteins are moderately higher in cells infected with IpaJ deletion S. flexneri (Fig. 4D–E), consistent with the idea that SIRT2 is upregulated by IpaJ-induced Golgi stress. Taken together, this highlights the uniqueness of SIRT2 as the defatty-acylase and Golgi stress-mediated SIRT2 regulation.

**SIRT2 limits Shigella autophagosome escape**

Notably, the in-cell labeling results suggest that SIRT2 prefers certain substrates (Supplementary Fig. 3B). CHMP5, an ESCRTIII component, is one of the preferred substrates as it showed more than 9-fold decrease in fatty acylation level upon SIRT2 overexpression. CHMP5 K7R, a mutant that lacks the primary IcsB modification site, only had some very weak fatty acylation that could be removed by SIRT2, suggesting SIRT2 is able to remove fatty acylation from redundant sites (Supplementary Fig. 3F).

Host cells use autophagy to target intracellular bacteria and to restrict bacterial growth. IcsB is important for *Shigella* to escape host autophagy by modifying CHMP5. We hypothesized that SIRT2, working against IcsB, would promote the innate immunity and suppress *Shigella* escaping from autophagosomes. To test this hypothesis, SIRT2 knockout (SIRT2−/−) and wildtype (SIRT2+/+) mouse embryonic fibroblast (MEF) cells were infected with *S. flexneri*. Bacteria that were trapped in autophagosomes were visualized by staining LC3, an autophagosome-specific marker. Consistent with previous reports, more IcsB deletion (Δicsb) *Shigella* was trapped in autophagosomes than wildtype *Shigella* (Fig. 6). Excitingly, the percentage of SIRT2−/− MEF cells containing LC3 decorated wildtype *Shigella* was significantly less than those of SIRT2+/+ MEF cells (Fig. 6). This is further confirmed by LC3 turnover assay (Supplementary Fig. 5). The data supports that SIRT2 limits *Shigella* autophagosome escape.

**SIRT2 restricts Shigella infection in cells and in mice**

To further test if SIRT2 is able to restrict proliferation of *Shigella*, we performed a gentamycin killing assay to examine the intracellular *Shigella* growth. Equal number of SIRT2−/− and SIRT2+/+ MEF cells were infected with equal number of *S. flexneri* for 10 min before treated with gentamycin to kill extracellular bacteria. The intracellular bacteria number was measured by recoverable colony formation units (CFU) to give the MOI. This assay revealed that *Shigella* survived better without SIRT2, at both early and later time points (Fig. 7A). Similar trends were also observed in SIRT2−/− MEF cells that re-express empty vector or SIRT2, as well as in Caco2 and A549 control or SIRT2 knockdown cells (Fig. 7B–C, Supplementary Fig. 6A–B). CREB3 KD cells have increased bacterial load after infection which can be partially rescued by WT but not HY SIRT2, suggesting that regulation of SIRT2 is a key function for CREB3 during *Shigella* infection (Supplementary Fig. 6C–D). The protective effect of SIRT2 is impaired against *S. flexneri* IpaJ deletion strain, suggesting the relevance of Golgi stress-mediated SIRT2 regulation (Fig. 7D). Furthermore, SIRT2−/− MEF cells survived better under *Shigella* infection or BFA treatment conditions than SIRT2+/+ cells (Supplementary Fig. 6E–F).

We also examined the effect of SIRT2 in wildtype and SIRT2 knockout C57/B6J mice. Six to eight-week old mice were intranasally administrated with S. flexneri, closely monitored, and sacrificed after 1–3 days. *S. flexneri* present in the lung were quantified. Consistently, there were more *S. flexneri* in SIRT2−/− mice, which further confirmed our model that SIRT2 protects against *Shigella* infection (Fig. 7E, Supplementary Fig. 7). We observed drastically reduced physical activity in SIRT2−/− mice comparing with SIRT2+/+ mice after bacterial infection (attached video in supplementary information). Moreover, there were lower circulating cytokine (TNF-α and IL-17) levels in SIRT2−/− mice (Fig. 7F), suggesting that SIRT2 promotes the immune response against *S. flexneri* infection. Overall, the data demonstrate SIRT2 restricts *Shigella* infection both in cells and in vivo.

To validate if the effect of SIRT2 was through counteracting the effect of IcsB, we infected the same number of SIRT2−/− and SIRT2+/+ MEF cells with same number of wildtype and Δicsb *S. flexneri*. Consistently, wildtype *S. flexneri* proliferated much better in SIRT2−/− cells than in SIRT2+/+ cells (Fig. 8A). In contrast, Δicsb *S. flexneri* proliferated at similarly low levels in both SIRT2−/− and SIRT2+/+ cells, suggesting SIRT2 counteracts IcsB to limit *Shigella* infection (Fig. 8A). Interestingly, wildtype and Δicsb *S. flexneri* proliferated similarly in SIRT2−/− MEF cells, suggesting that SIRT2 is able to fully counteract IcsB (Fig. 8A). The same trend was also observed in vivo (Fig. 8B). In intranasally infected mice, we observed similar MOI for Δicsb *S. flexneri* in SIRT2−/− and SIRT2+/+ mouse lungs, but significantly higher MOI of wildtype *S. flexneri* in SIRT2−/− than in SIRT2+/+ mouse lungs (Fig. 8B). To further confirm the SIRT2/IcsB axis, we generated Δicsb *S. flexneri* rescued by either WT or catalytic dead C306A IcsB. When infected with WT IcsB *S. flexneri*, SIRT2−/− mice had much more bacterial colonization. SIRT2−/− and SIRT2+/+ mice showed no statistically significant difference in colonization by C306A S. flexneri (Fig. 8C). Similar, though less pronounced trends were observed in intraperitoneally infected mice (Supplementary Fig. 8), which is possibly due to complication from severe inflammation reactions. These results demonstrate the in vivo anti-bacterial role of SIRT2 as a defatty-acylase to counteract the action of IcsB.

**Discussion**

Emerging studies suggest that pathogens employ PTMs to modulate host protein functions to facilitate pathogenesis. Such PTMs are often highlighted with novel enzymatic reactions and high reaction
efficiency. One of the PTMs, protein fatty acylation, regulates the association of proteins with membranes, and is important for membrane trafficking, protein-protein interaction, and signal transduction. Fatty acylation on lysine residues, however, is less well studied. Multiple mammalian lysine defatty-acylases were identified, which strongly suggested the physiological relevance of this PTM. However, very little is known on mammalian lysine fatty acyltransferases, which has greatly limited the study of the function of this PTM. Our findings demonstrate that reversible lysine fatty acylation plays an important role in pathogen-host interaction, highlighting the importance of lysine fatty acylation in innate immunity.

SIRT2 was initially recognized as a lysine deacetylase, with its cellular function mostly attributed to its deacetylation activity. Many in vitro studies suggested that SIRT2 can also catalyze lysine defatty-acylation. However, the first physiological de-fatty acylation substrate, K-Ras4a, was only recently identified. Our findings here thus provide key insights that will help understand the physiological significance of SIRT2’s defatty-acylation activity and expands its substrate scope. While we believe the defatty-acylation activity of SIRT2 plays a predominate role in fighting Shigella infection, we do not rule out that the deacetylation activity could also be important.

As the hub of intracellular membrane trafficking, the Golgi apparatus possess a unique structure where the stacks are interconnected into a compact ribbon. The ribbon undergoes dynamical membrane fission, fusion, and cargo transport through cisternal maturation. Upon certain cellular events such as mitosis and stresses conditions, the ribbon can undergo distinct disassembly processes, which will lead to disruption of Golgi integrity and stress stimuli transduction to the nucleus. Our study revealed an important function of Golgi stress pathway in fighting Shigella infection. Through Golgi stress, cells sense Shigella infection and activate SIRT2 via the CREB3 transcription factor to enhance innate immunity. Among all of the known lysine defatty-acylases, SIRT2 is the most responsive to Golgi stress induced by pathogens. This highlights the unique role of SIRT2 in stress response.

Previously, SIRT2 was reported to be hijacked by other pathogens to work as deacetylase and to promote pathogen invasion.

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**Fig. 6 | SIRT2 suppresses Shigella autophagosome escape.** A Effect of SIRT2 knockout on S. flexneri autophagosome escape. Representative images of MEF cells infected with S. flexneri from three biological replicates are shown. Scale bar: 15 μm. White arrow: LC3-positive Shigella. B Quantitation of data shown in A. Percentage of MEF cells containing LC3-positive shigella was quantified. n = 3 biological replicates. Data is shown as mean ± SEM with >100 infected MEF cells counted for each experiment. Statistical evaluation was done using two-way ANOVA. Data are represented as mean ± SEM.
**Fig. 7** | SIRT2 restricts Shigella infection in cells and in mice model. **A** Effect of Sirt2 knockout on *S. flexneri* intracellular proliferation analyzed by the gentamycin killing assay. MEF Sirt2+/+ and Sirt2−/− cells were infected with *S. flexneri* for 10 min. Intracellular *S. flexneri* number and MEF cell number were counted at indicated time points to get MOI value. Data are presented as mean ± SEM with three biological replicates (two technical replicates). Statistical evaluation: unpaired two-tailed Student’s *t*-test.

**B** Effect of SIRT2 knockout on *S. flexneri* intracellular proliferation. MOI were determined after 10 min of infection and the indicated time of incubation in the presence of 50 μg/ml gentamicin in Sirt2−/− MEF with empty vector or SIRT2 knock-in (KI) and in A549 with control or SIRT2 knockdown (C). Data are presented as mean ± SEM (the SEM is very small and hard to see in the figure) with three biological replicates (two technical replicates). Statistical evaluation: unpaired two-tailed Student’s *t*-test.

**C** Effect of SIRT2 knockout on *S. flexneri* intracellular proliferation. MOI were determined after 10 min of infection in the presence of 50 μg/ml gentamicin in Sirt2+/+ MEF with empty vector or SIRT2 knock-in (KI) and in A549 with control or SIRT2 knockdown (C). Data are presented as mean ± SEM (the SEM is very small and hard to see in the figure) with three biological replicates (two technical replicates). Statistical evaluation: unpaired two-tailed Student’s *t*-test. **D** A549 control and SIRT2 KD cells were infected with wildtype and IpaJ deletion *S. flexneri* M90T cells for 10 min, then treated with gentamycin to kill extracellular bacteria. Intracellular *S. flexneri* number and mammalian cell number were counted to get MOI. Data are presented as mean ± SEM with nine biological replicates. Statistical evaluation: two-way ANOVA.

**E** Recoverable CFU in lung homogenates from 6 to 8-week old Sirt2+/+ and Sirt2−/− C57BL/6 J mice intranasally infected with 1 million *S. flexneri* M90T wildtype strain. Statistical evaluation: unpaired two-tailed Student’s *t*-test. Data are presented as mean ± SEM with three mice per group. LOD, limit of detection.

**F** Cytokine profile in Sirt2+/+ and Sirt2−/− mice lung homogenates after intranasal infection with 1 million *S. flexneri* for 2 days. *n* = 18 (TNFα) or 14 (IL–17A) biological replicates. Cytokine levels in PBS-challenged mice fell below the detection limit for the experimental setup so were not included in the graph. Data are represented as mean ± SEM. Statistical evaluation: unpaired two-tailed Student’s *t*-test. ***p < 0.001.

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**Article**

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Here our study discovered an anti-bacterial role of SIRT2 by counteracting the fatty acyltransferase IcsB from *S. flexneri*. Many other pathogen fatty acyltransferases have been identified or predicted. For example, MARTX from *Vibrio cholerae* shares sequence similarities and substrate proteins with IcsB. In addition, heat labile toxin (LTA1) that shares sequence similarities with cholera toxin, also disrupts Golgi apparatus in a pattern that is reminiscent of the actions of BFA. This suggests that the mechanism of SIRT2’s anti-bacterial role is potentially broader. One limitation of our study is that we used a mouse lung infection model for *Shigella*. While this model is useful for deciphering local immune protection, it does not mimic bacillary dysentery, the usual disease caused by *Shigella* infection in humans.

In addition to lysine fatty acylation, pathogenic bacteria use many other PTMs to help infect host cells. Here we reveal lysine fatty acylation as a pathogen-induced PTM that can be efficiently removed by host enzymes. This suggests that other pathogen-induced PTMs may also be reversed by host enzymes and studying them may provide insights into mechanisms of innate immune responses.

**Methods**

**Reagents, antibodies and plasmids**

Tunicamycin, Thapsigargin, cycloheximide were purchased from Sigma-Aldrich (St. Louis, MO); Actinomycin D and gentamicin were from Thermo Fisher. Brefeldin A was from Cell Signaling Technology (Danvers, MA). NAD+ was purchased from VWR (Radnor, PA). Alk14 and BODIPY-azide were synthesized as previously described.

The following antibodies were purchased from Cell Signaling Technology (Danvers, MA): SIRT2 (D4050, #12650, used at 1:1000), PARP (#9542, used at 1:1000), LC3 (#3868, used at 1:1000), and anti-rabbit IgG HRP-linked Antibody (#7074, used at 1:2000). The following antibodies were from Santa Cruz: β-actin (C-4, sc-47778, used at 1:2000), GRP78 (H-129, sc-13968, used at 1:500). Anti-Flag M2 antibody conjugated with horseradish peroxidase (A8592, used at 1:3000) and anti-flag M2 affinity gel (A220) were purchased from Sigma. Anti-CREB3 (ab180119, used at 1:2000) was from Abcam (Cambridge, MA). Cy3-conjugated goat anti-rabbit IgG (H + L) was purchased from Thermo Fisher Scientific (A10520, used at 1:1000).

IcsB gene was synthesized by Integrated DNA Technologies (Coralville, IA) after codon optimization from *Shigella flexneri*. The gene fragment was later cloned into pCMV4a between EcoRI and XhoI using primers 5'- AGT CAG GAA TTC ACC ATG GAT TAC AAA GAT CAC G -3' and 5'- AGT CAG CTC GAG CTA AAT ATT TGA ATG GGA GTT GAT GA -3' (the restriction sites are underlined).

*IpaJ* was synthesis by Integrated DNA Technologies and was cloned into pEGFP-C1-FLAG-GFP-Ubl4A-C (Addgene #86923) between BglII and SalI with N-terminal Flag-tag followed by EGFP using primers 5'- AGT CAG GAA TTC ACC ATG TCG GAA CAA CGG AAG AAG TTA ATG TTC ATG CAG CAG ACA AAT ATG GGT GGA GAT TTG GGA AAT ATT ATG TTC CTG CAA CAA GTG AGT AAT ATG GGA GAT TTG GA -3' and 5'- AGT CAG CTC GAG CTA AAT ATT TGA ATG GGA GTT GAT GA -3' (the restriction sites are underlined).

*Ipaf* was synthesis by Integrated DNA Technologies and was cloned into pEGFP-C1-FLAG-GFP-UbI4A-C (Addgene #86923) between BglII and Sall with N-terminal Flag-tag followed by EGFP using primers 5'- AGT CAG AGA TCT ATG TCG GAA CAA CGG AAG AAG TTA ATG TTC ATG CAG CAC ACA AAT ATG GGT GGA GAT TTG GA -3' and 5'- AGT CAG GTC GAC TTA ATG TTA ATG ATA ATG GGT GTT GA -3' (the restriction sites are underlined).

For lentivirus generation, *Ipaf* was cloned into pCDH-SIRT2-Flag (Addgene # 102624) between EcoRI and XhoI.

The following plasmids were purchased from Addgene:

- *Ipaf* gene was synthesized by Integrated DNA Technologies (Coralville, IA) after codon optimization from *Shigella flexneri. 5a* plasmid pWR100 193718 – 195202 with N-terminal Triple Flag-tag. The gene fragment was later cloned into pCMV4a between EcoRI and XhoI using primers 5'- AGT CAG GAA TTC ACC ATG GAT TAC AAA GAT CAC G -3' and 5'- AGT CAG CTC GAG CTA AAT ATT TGA ATG GGA GTT GAT GA -3' (the restriction sites are underlined).

- pEGFP-C1-FLAG-GFP-Ubl4A-C (Addgene #86923) between BglII and SalI with N-terminal Flag-tag followed by EGFP using primers 5'- AGT CAG GAA TTC ACC ATG TCG GAA CAA CGG AAG AAG TTA ATG TTC ATG CAG CAG ACA AAT ATG GGT GGA GAT TTG GGA AAT ATT ATG TTC CTG CAA CAA GTG AGT AAT ATG GGA GAT TTG GA -3' and 5'- AGT CAG CTC GAG CTA AAT ATT TGA ATG GGA GTT GAT GA -3' (the restriction sites are underlined).

- P2A-pCMV4a-Flag-Flag (Addgene #102624) between EcoRI and XhoI.

- pCDH-SIRT2-Flag (Addgene # 102624) between EcoRI and XhoI.

- pCDH-CMV-MCS-EF1a-Puro (Addgene #102624) between EcoRI and XhoI.

- pCDH-CMV-MCS-EF1a-GFP (Addgene #102624) between EcoRI and XhoI.

- pCDH-CMV-MCS-EF1a-Luc (Addgene #102624) between EcoRI and XhoI.
and XhoI with N-terminal Flag-tag using primers 5′- AGT CAG GAA TTC ATG TCG GAA CAA CAG AAG A-3′ and 5′- AGT CAG CTC GAG TTA CAA AGC CTC ATT AGT TAT AAC TAT GGA G-3′. pcDH-SIRT2-Flag was previously cloned28.

Human CHMP3 was amplified from HEK293T cDNA library and cloned into pCMV4a between EcoRI and XhoI with C-terminal Flag-tag using primers 5′- AGT CAG GAA TTC ATC ACC ATG ACG CCA CTC TTC TGG G-3′ and 5′- AGT CAG CTC GAG TTA CAA AGC CTC ATT AGT TAT AAC TAT GGA G-3′. pcDH-SIRT2-Flag was previously cloned28.

Human RHOD was amplified from HEK293T cDNA library and cloned into pGEX4T3 between BamHI and EcoRI using primers 5′- AGT CAG GAA TTC ACC ATG AAC CGA CTC TTC GGG TTA CTA GCC CGG GCT CGA GAT CTG CGA TCT AAG TAC CAC AGT TCT TGC GAG TCT CTA GTC TTC CCA CAA GAG TCG GTT CAT GGT GAA GAC TTA CTC G-3′ (the mutation sites are underlined).

Cell culture, transfection and transduction
Human MCF7, Hela, MDA-MB-231, A2780, and HeyA8 were grown in DMEM media (Invitrogen) supplemented with non-essential amino acids and 15% (v/v) heat-inactivated FBS. A549 cells were cultured in DMEM media (Invitrogen) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS; Invitrogen, Carlsbad, CA). MEF cells were grown in DMEM supplemented with non-essential amino acids and 15% (v/v) heat-inactivated FBS. AS49 cells were cultured in RPMI-1640 (Invitrogen) with 10% (v/v) heat-inactivated FBS. HEK293T were cultured in DMEM supplemented with 10% (v/v) heat-inactivated calf serum (Sigma).

For transient overexpression in HEK293T cells, mammalian expression vectors were transfected using PEI MAX 40 K (Polyplus-Transfection). Empty vector was transfected as negative control. To transduce AS49 for overexpression or knockdown, lentiviral infection was performed as previously described31. The pkLO.1-puro lentiviral shRNAs constructs for Luciferase and human CREB3 were purchased from Sigma-Aldrich. Luciferase shRNA (SHC007), CREB3 shRNA1 (TRCN0000020342), CREB3 shRNA2 (TRCN0000020343) were used. SirT2 WT and KO MEF, SirT2 KO MEF with stable SirT2 re-expression and SirT2 stable knockdown AS49 and HEK293T cells were generated as previously described32.

For ActD and CHX chase experiments, AS49 cells were treated with or without BFA at t = 0 h. During BFA treatment, ActD was added at t = 0, 12, 18, 23, 24 h; or CHX was added at t = 0, 12, 24 h. at t = 24 h, cells were collected and submit for western blot analysis.

For IpaJ transfection of control and ShCREB3 AS49 cells, cells were kept at less than or equal to 50% confluence for at least 3 passages to ensure maintenance of CREB3 knockdown and equal level of SirT2F (SirT2 levels may be regulated by confluence). In 6-well plates, control cells were transfected with 2 μg IpaJ-flag and ShCREB3 cells transfected with 0.7 μg IpaJ-flag to ensure equal expression levels of IpaJ. Cells were collected for immunoblot analysis 48 h after transfection.

Bacterial strains and infection
Shigella flexneri 5a M90T is a kind gift from Prof. Neal M. Alto from UT Southwestern. ΔIcsB M90T was generated using the lambda red recombineering system30. Briefly, M90T containing pkD46 was transformed with KanR cassette from pkD4. Kanamycin resistant colonies were selected and cured for pkD46 to generate IcsB-Δkan. The resistance was later removed with pcP20, resulting in a traceless gene deletion. The KanR cassette was amplified using primers 5′- ACA TCC CCACAA TCA CCA AGT AAT GAG GAC TGA ATA ATG TAT GTA GGC TGG AGC TGC TCT-3′ and 5′- AAA GTC TAT CAT ATC GTG TGC GAC ACA TTT CTA TGG CCT TAT GGG AAT TAG CCA TGA TGG TCC-3′ (the external overlap with IcsB was underlined).

Gentamycin killing assay
S. flexneri infection was done as previously described31. Briefly, -1 x 10⁶ mammalian cells were seeded into 6-well dishes. S. flexneri M90T was inoculated overnight at 30°C in Luria-Bertani (LB) broth (Fisher, Hampton NH) with shaking. Before infection, M90T culture was back diluted 1:100 in fresh LB broth and incubated at 37°C with shaking until the OD₆₀₀ reached 0.4. The bacteria were further incubated with 0.03% Congo Red in PBS for 15 min before adding 20 μl bacterial suspension to mammalian cells for an MOI of ~8. The infection was facilitated by centrifugation at 1000g at room temperature for 10 min. After 10 min, the mammalian cells were washed with PBS five times and incubated in fresh media containing 50 μg/ml gentamycin. At the indicated time points (30 min to 5 h), mammalian cells were washed with PBS three times, collected and counted under a light microscope. The cells were then lysed with 0.5% Triton X-100 in PBS and the cell lysates were plated on LB agar and colonies forming units (CFU) were counted after overnight incubation at 37°C.

Infection of mammalian cells with enteropathogenic E. coli
Enteropathogenic E. coli (EPEC) strain JPN15 (serotype O127:H6) was purchased from Bei Resources (NR-50517). EPEC were cultured overnight in LB media at 37°C. The following day, the EPEC culture was diluted 1:200 in LB media and grown to OD₆₀₀ = 0.4. EPEC was added to a 70% confluence well of AS49 cells grown in a 6-well plate at an MOI of 0.5 for 18 h before collection.

Western blot analysis
The proteins of interest were detected using enzyme-linked fluoro-escence (ECL Plus; Pierce Biotechnology Inc.) and visualized using the Typhoon 9400 Variable Mode Imager (GE Healthcare, Piscataway, NJ). Quantification of the western blots was done using ImageJ software.

RT-PCR analysis of mRNA levels
Total mRNA was extracted using the RNeasy Mini Kit (Qiagen, CA, USA) according to the manufacturer's instructions and then reverse transcribed to cDNA using SuperScript Vilo cDNA Synthesis Kit (Thermo Fisher). Real-time PCR were performed on QuantStudio™ 7 Flex Real-Time PCR System using SYBR™ Green PCR Master Mix (Applied Biosystems) and the primers shown in Table S1.

Luciferase assay for SirT2 promoter activity
The SirT2 promoter corresponding to the region –1 to –994 nucleotides upstream of the Sir2 transcription start site was cloned into the pGL3-basic vector (Promega) to create pGL3-994. Firefly/renilla luciferase ratio was determined by co-transfecting AS49 cells with pGL3-994 and pGL4.73 (Renilla luciferase) for 24 h. Cells were either co-transfected with N-terminal flag tagged CREB3 (1-220) or treated with 5 μg/mL BFA for an additional 24 h. SirT2 promoter was cloned into pGL3-basic using Gibson cloning using the primers 5′- GTG CTA GCC GGC GCT TGA ATG CAG TCT CTA AAC AGA ATG CCT CGG CCG CCT TCT TTA GTG TTT TGG GTC CGT CTA TGG CCG GCG TG-3′. CREB3 (1-220) was cloned into pCMV5 between EcoRI and XhoI with primers 5′- AGT CAG GAA TTC ATC ACC ATG ACG CCA CTC TTC TGG G-3′ and 5′- AGA ATG GCG CCG GGC CTT TCT TGG GAG TCT CTA GTC TTC CCA CAA GAG TCG GTT CAT GGT GAA GAC TTA CTC G-3′ (the mutation sites are underlined).
Chromatin IP
Approximately 1.5 x 10^7 A549 cells in a 15 cm dish were treated with 5 μg/mL BFA or DMSO for 24 h. Cells were crosslinked with 1% formaldehyde for 10 min and processed according to ChIP-IT® Express Enzymatic Magnetic Chromatin Immunoprecipitation Kit (Active Motif, 33009) manufacturer recommendations using an α-CREB3 antibody (Proteintech, 11275-1-AP). Chromatin inputs and eluted ChIP samples were analyzed via qPCR using the primers 5'-AGA CTC TAG ACC CCT GGT GG -3' and 5'-TTG GAG GGG AGA AGA ACA -3' which flank the putative CREB3 binding site and result in a 472 bp amplification product. qPCR was carried out on a QuantStudio 7 Flex (Applied Biosystems) using SYBR green (Abclonal, Piscataway, NJ) with setting of Green fluorescence. Quantity One (Bio-Rad, Hercules, CA) was used for the addition of TBTA (1 mM), CuSO4 (1 mM) and eluted proteins with Alk14 labeling was re-suspended in 20 μL of 1 mM solution in DMF.

Detection of fatty acylation on protein of interest using Alk14
HEK293T cells were transfected with IcsB and the gene of interest for 24 h, and then treated with Alk14 for 6 h before harvest. Alternatively, HEK293T cells transfected with gene of interest overnight were infected with M90T in the presence of Alk14 for 3 h. The cell pellets were processed as previously described.

Defatty-acylation assay by sirtuins in vitro
HEK293T cells were transfected with IcsB and substrate genes for 24 h and treated with Alk14 for 6 h before harvest. The immunoprecipitated substrate proteins with Alk14 labeling were pulled down using anti-Flag affinity gel. The affinity gel containing the pulldown proteins was suspended in 25 μL of assay buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 2 mM MgCl2, 1 mM DTT) with 5 μM of SIRT2, with or without 1 mM NAD+. The defatty-acylation reaction was allowed to proceed for 30 min at 37 °C and quenched by washing the affinity gel with IP wash buffer (25 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.2% Nonidet P-40).

For click chemistry and in gel fluorescence, the immunopurified proteins with Alk14 labeling was re-suspended in 20 μL IP washing buffer. Rh-N3 (3 μL of 1 mM solution in DMF, final concentration 200 μM) was added to the above suspension, followed by the addition of TBT (1 μL 10 mM solution in DMF, final concentration 500 μM), CuSO4 (1 μL of 40 mM solution in H2O, final concentration 2 mM), and TCEP (1 μL of 40 mM solution in H2O, final concentration 2 mM). The click chemistry was allowed to proceed at room temperature for 30 min. The reaction mixture was mixed with 10 μL of 6 × protein loading buffer and heated at 95 °C for 10 min. After centrifugation at 16,000 g for 2 min at room temperature, 15 μL of the supernatant was treated with hydroxylamine (pH7.4, 1 μL of 5 M solution in H2O, final concentration 300 mM) or equivalent volume of water (negative control) at 95 °C for 7 min. The samples were resolved by SDS-PAGE. Rhodamine fluorescence signal was recorded by Typhoon 9400 Variable Mode Imager (GE Healthcare Life Sciences, Piscataway, NJ) with setting of Green (532 nm)/580BP300 PMT 500 V (normal sensitivity), and analyzed by Fiji software. Quantity One (Bio-Rad, Hercules, CA) was used for quantification of the fluorescence intensity.

SIRT2 was expressed and purified from E. coli. Human SIRT2 (aa38-356) was cloned and inserted into pET28a vector for the expression of N-terminal His6-SUMO fusion protein. The SIRT2 protein was expressed in E. coli BL21 and purified using Ni affinity column purification. The desired fractions were pooled, concentrated and buffer exchanged, and the His6-SUMO tag was removed by overnight incubation at 4 °C with UPL1, followed by Ni-affinity column purification to remove any undigested SIRT2. The tag-free SIRT2 was further purified on a Superdex 75 column (Bio-Rad, Hercules, CA). The protein was eluted with 20 mM Tris-HCl, pH 8.0, 500 mM NaCl. After concentration, the target protein was frozen and stored at −80 °C.

GDI pulldown
GST-RhoGDI were purified from E. coli strain BL21(DE3) cells using glutathione resin. For the pulldown assay, 500 μg lysates from transfected cells were incubated with 60 μg GST-RhoGDI pre-coupled to glutathione resin. After three washes in IP wash buffer, the bound proteins were eluted and analyzed by immunoblotting.

Immunofluorescence
MEF cells were infected with M90T at MOI of 1001 in glass bottom dishes (MatTek, Ashland MA). After 3 h, cells were washed with PBS three times and fixed with pre-chilled methanol for 10 min. Then the cells were permeabilized with 0.25% Triton X-100 for 10 min, blocked with 1% (w/v) BSA/0.1% Tween-20 in PBS for 1 h and incubated with LC3 antibody overnight. The cells were washed with 0.1% Tween-20 in PBS three times and incubated with Cy3-conjugated goat anti-rabbit IgG (H + L) secondary antibody for 1 h. The cells were washed with 0.1% Tween-20 in PBS three times and mounted with Fluromount-G (SouthernBiotec, Homewood AL) containing DAPI before imaged on Cytation 5 (BioTek, Winooski VT). The number of MEF cells that contained Shigella and the number of MEF cells that contained LC3-positive Shigella were counted manually.

Intraperitoneal shigellosis mice model study
The S. flexneri intraperitoneal infection mouse study was done as previously described. Briefly, 6-week old wildtype and SIRT2 knockout C57/B6j mice (Jackson Lab, Sirt2^floxed/floxed) were intraperitoneally administrated with 150 million Shigella per 20 g of mouse body weight. After 17 h, mice were sacrificed, and tissue were harvested. The peritoneal wash was also collected. Tissues were washed in PBS with gentamicin (50 μg/mL) to remove superficial bacteria, rinsed twice in antibiotic-free PBS, and then mechanically homogenized in PBS with 2.5% Triton X-100 (200 mg of tissue per ml of PBS). The tissue extracts were diluted and plated onto Hektoen Enteric Agar plate. Tissue extracts and peritoneal wash from mice without Shigella infection were used as blank control. Colonies were counted after 18 h of culture at 37 °C. All animal experiments were approved by Cornell University’s Institutional Animal Care and Use Committee.

Intranasal shigellosis mice model study
The S. flexneri intranasal infection mouse study was done as previously described. Briefly, 6–8-week-old wildtype and SIRT2 knockout C57/B6j mice were intranasally administrated with 1 million Shigella. After 3 days (or at indicated time points), mice were sacrificed, and lung tissue were harvested. The broncho-alveolar lavage (BAL) fluid was also collected. Tissues were mechanically homogenized in PBS with 2.5% Triton X-100. The tissue extracts were diluted and plated onto Hektoen Enteric Agar plate. Tissue extracts and BAL fluid from mice without Shigella infection were used as blank control. Colonies were counted after 18 h of culture at 37 °C. All animal experiments were approved by Cornell University’s Institutional Animal Care and Use Committee.

Statistics and reproducibility
Statistical analysis and generation of graphs was done with GraphPad Prism 9. p-values are shown on relevant graphs to indicates the significance of experimental results as calculated by the methods indicated in the figure legends. ***p-values < 0.001, below the limit of presentation by the statistics software. Shown blots and gel images are representative of at least three biological replicates.

Reporting summary
Further information on research design is available in the Nature Research Reporting Summary linked to this article.
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Author contributions
M.W. designed and performed experiments except those noted below. Y.Z. and G.P.K. performed a variety of experiments required for the revision. J.C. made the initial discovery that SIRT2 is able to remove RID mediated lysine fatty acylation. G.P.K., X.L., M.Z., and Y.Z. performed the mice study. T.Y. and D.H. contributed to studies involving BMDM. N.A.S. cloned multiple mammalian expression vectors encoding SIRT2 substrates. M.Y. synthesized Alk14. I.R.P. purified SIRT2 recombinant protein. H.L. directed the studies. M.W. and H.L. wrote the manuscript with input from all authors. All authors reviewed and approved the manuscripts.

Competing interests
HL is a founder and consultant of Sedec Therapeutics. Other authors have no competing interests.

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