Figure S1  IpaH9.8 specifically inhibits NF-κB pathway. (a) IpaH9.8 specifically targets NF-κB pathway. HeLa cells stably expressing IpaH9.8 or control vector were infected with Shigella harboring Afa expression plasmid (moi=10). After Shigella invasion, cell lysates were prepared at the indicated time points and subjected to immunoblotting analysis. (b) IpaH9.8 specifically inhibits NF-κB activity. Luciferase assays were performed after Shigella infection for 3 h of 293T cells transiently transfected with an NF-κB, Elk-1, or AP-1 reporter plasmid and empty vector or IpaH9.8 expressing plasmid. Results are presented as ‘fold increase’ relative to the activity of non-infected cells. Data are mean ± s.e.m. (n = 3).
Figure S2 IpaH9.8 acts at a level between RICK and IKKβ in the Nod1-RICK-NF-κB pathway. Luciferase assays of 293T cells transiently transfected with an NF-κB reporter plasmid and empty vector or FLAG-IpaH9.8 expressing plasmid, plus stimulation plasmids expressing Myc-Nod1, Myc-RICK, and Myc-IKKβ. Results are presented as ‘fold increase’ relative to the activity of non-stimulated cells. Data are mean ± s.e.m. (n = 4).
Figure S3 Binding domain of IpaH9.8 and NEMO or ABIN-1. (a) Deletion mutants of NEMO used in domain-mapping experiments. Numbers in parentheses indicate amino acids included in constructs (upper panel). GST-NEMO deletion mutants or GST were mixed with cell lysates expressing Myc6-IpaH9.8CA and the bound proteins were immunoblotted with the antibodies indicated (lower panel). (b) Deletion mutants of IpaH9.8 used in domain-mapping experiments (upper panel). GST-NEMO beads were mixed with cell lysates expressing several deletion mutants of Myc6-IpaH9.8CA and the bound proteins were immunoblotted with the antibodies indicated (lower panel). (c) Deletion mutants of ABIN-1 used in domain-mapping experiments (upper panel). GST-ABIN-1 deletion mutants or GST were mixed with cell lysates expressing Myc6-IpaH9.8CA and the bound proteins were immunoblotted with the antibodies indicated (lower panel). (d) Deletion mutants of IpaH9.8 used in domain-mapping experiments (upper panel). GST-ABIN-1 beads were mixed with cell lysates expressing several deletion mutants of Myc6-IpaH9.8CA and the bound proteins were immunoblotted with the antibodies indicated (lower panel). Interaction (+), absence of interaction (-).
Figure S4 IpaH9.8 promotes NEMO degradation. 293T cells were transfected with FLAG-NEMO with or without FLAG-IpaH9.8 or FLAG-IpaH9.8CA. Twenty-four hours after transfection, cells were treated with CHX (25 µg/ml) and cell extracts were prepared at the indicated time points. Nod1-dependent NF-κB activation was stimulated by the transfection with pcDNA-Nod1 and iE-DAP (10 ng/ml). Samples were subjected to immunoblotting. The remaining NEMO was quantified (lower graph).
Figure S5 NEMO K309 and K321 residues are ubiquitination sites mediated by IpaH9.8. (a) In vitro ubiquitination assay with GST-NEMO-WT or GST-NEMO-K309R/K321R and a mixture of E1, UbcH5b, ATP, ubiquitin, in the presence of GST-IpaH9.8 or GST-IpaH9.8CA. Samples were subjected to immunoblotting with anti-NEMO and -IpaH antibody. (b) IpaH9.8 equally binds to NEMO-WT and NEMO-K309R/K321R. Immunoprecipitation (IP) analysis of lysates of 293T cells expressing GFP-NEMO-WT or GFP-NEMO-K309R/K321R and Myc6-IpaH9.8CA. Proteins that were immunoprecipitated were immunoblotted with anti-Myc antibody.
Figure S6  IpaH9.8 modulates the host inflammatory response in a murine pneumonia infection model. (a) The number of viable bacteria in the lungs of mice infected with the S. flexneri WT strain, ∆ipaH9.8 mutant, ∆ipaH9.8/ipaH9.8 complementation strain, or ∆ipaH9.8/ipaH9.8CA complementation strain at 24 h and 48 h after inoculation. Data are means of values from 4 mice (± s.d.). *P<0.001 (b) Histological analysis of mouse lungs at 48 h after inoculation. Sections of infected lung were stained with hematoxylin and eosin. (c) Quantification of MPO in the lungs of mice 24 and 48 h after inoculation. Data are means of values from 4 mice (± s.d.). *P<0.01, ** P<0.05 (d) Measurement of cytokines in lung tissue infected with WT (closed bar), ∆ipaH9.8 (open bar), ∆ipaH9.8/ipaH9.8 (light gray bar), or ∆ipaH9.8/ipaH9.8CA (gray bar). Data are means of values from 4 mice (± s.d.). *P<0.01, ** P<0.05.
Figure S7 Full scans