<u>Supportive/ Supplementary material</u>

Supplementary methods

1. *Infection*

The HeLa cells were infected with *Shigella flexneri* 2457T (wild-type), *S. flexneri* BS103 (non-invasive, virulence plasmid-cured) [136] for one hour (multiplicity of infection was 500:1 to achieve a rate of infection of 25%, as measured by colony forming unit count), or left uninfected.

2. *Protein extraction, ubiquitin-specific active-site probe reaction and immunoprecipitation*

The cells were washed in PBS at 4°C, and lysed in the 0.1% NP-40 lysis buffer (0.1% NP-40, 150 mM NaCl, 20 mM $CaCl_2$, 50 mM Tris pH 7.4) containing PMSF (phenylmethylsulfonyl fluoride) to inhibit serine proteases. The protein concentration was measured and 4.5mg protein duplicate samples were incubated with 20ug HA-tagged bromoethyl ubiquitin probe (HA–Ub-Br2, Enzo) for 50 minutes at 37°C. Samples were then subjected to anti-HA immunoprecipitation to pull-down the active DUBs. For immunoprecipitation, protein lysates were first diluted in the NET buffer (50 mM Tris, 5mM EDTA, 150 mM NaCl, 0.5% NP-40, pH 7.4) to a protein concentration of 1 mg/ml, the HA Agarose beads (#A2095-1ML, Sigma Aldrich) were added and immunoprecipitation of duplicate samples was carried out at 4°C overnight. Samples were then washed four times in the NET buffer, centrifuged, and the immunoprecipitated proteins were eluted using 400ul 100 mM glycine pH 2.5 for 30 minutes at 4°C with gentle rotation.

3. *Proteomics*

The protein samples were precipitated using chloroform/methanol using a previously published method [137]. The supernatant was removed and the precipitated proteins were dried in a laminar hood. The proteins were then digested with trypsin and desalted via SepPak C18 columns (Waters), using exactly the same methods as published earlier [77, 138]. Peptides were then dried in a vacuum centrifuge and resuspended in 80 µL of 0.1% formic acid and 5% ACN. The LC-MS/MS analysis was accomplished by reverse phase (RP) liquid chromatography coupled directly in line with an ESI ion trap mass spectrometer (LCQ Deca XP Plus; ThermoElectron Corporation; San Jose, CA), similarly as published earlier [139], but using only an RP gradient.

Tandem mass spectra were extracted, charge state deconvoluted and deisotoped by Proteome Discoverer version 1.3. All MS/MS samples were analyzed using Sequest (Thermo Fisher Scientific, San Jose, CA, USA; version 1.3.0.339). Sequest was set up to search Human Uniprot fasta database (66987 entries) assuming the digestion

enzyme trypsin. Sequest was searched with a fragment ion mass tolerance of 1.00 Da and a parent ion tolerance of 2.0 Da. Oxidation of methionine and iodoacetamide derivative of cysteine were specified in Sequest as variable modifications. Scaffold (version Scaffold_3.4.9, Proteome Software Inc., Portland, OR) was used to validate MS/MS based peptide and protein identifications. Peptide identifications were accepted if they exceeded specific database search engine thresholds. Sequest identifications required at east deltaCn scores of greater than 0.10 and XCorr scores of greater than 1.5, 2.5, 3.0 and 3.5 for singly, doubly, triply and quadruply charged peptides. Protein identifications were accepted if they contained at least 2 identified peptides. Proteins that contained similar peptides and could not be differentiated based on MS/MS analysis alone were grouped to satisfy the principles of parsimony.