

**Fig. S1. Quantitative proteome profiling.** (A) Histograms of log2-transformed SILAC ratios for all proteins quantified from RAW264.7 (black) and HeLa (grey). (B) Functional classification of the regulated RAW264.7 proteins (n=63) and HeLa proteins (n=61) based on UniProtKB annotation.



**Fig. S2.** Enriched GO terms and pathways. (A-C) Logarithmized corrected p-values (dash line, p < 0.01) are shown. Other results are shown in Fig. 2.



Fig. S3. Overview of RAW264.7 proteins (phosphorylation site) and cellular processes modulated by SPI2 effectors. Red or blue represent increased phosphorylation in wt *Salmonella* or in  $\Delta ssaR$  *Salmonella*, respectively.

In vitro kinase reaction



Fig. S4 LC-MS analysis of the tryptic digest of SteC and MBP after in vitro kinase assay (with or without ATP).

#### Phospho site: S9



Sequence: RVPFSLLR, S5-Phospho (79.96633 Da) Charge: +2, Monoisotopic m/z: 534.29156 Da (-0.17 mmu/-0.32 ppm) IonScore:26 Phospho site: S15 Sequence: GPSWDPFRDWYPHSR, S3-Phospho (79.96633 Da) Charge: +3, Monoisotopic m/z: 661.61578 Da (-0.22 mmu/-0.33 ppm), IonScore:30



Phospho site: S82 Sequence: QLSSGVSEIR, S3-Phospho (79.96633 Da) Charge: +2, Monoisotopic m/z: 578.27393 Da (-0.01 mmu/-0.02 ppm), IonScore:73

See Fig. 4F



Sequence: LPEEWSQWLGGSSWPGYVRPLPPAAIESPAVAAPAYSR, S13/S6/S12/ Y17

Phospho site: T174 /S176

Phospho site: S43/S49/S50/Y54

-Phospho (79.96633 Da)

Sequence: LATQSNEITIPVTFESR, T3/S5-Phospho (79.96633 Da) Charge: +2, Monoisotopic m/z: 993.48315 Da (+0.5 mmu/+0.5 ppm), IonScore:35 mà

#### Phospho site: \$199 Sequence: AQLGGPEAAKSDETAAK, S11-Phospho (79.96633 Da)

Charge: +3, Monoisotopic m/z: 575.26794 Da (-0.22 mmu/-0.38 ppm), IonScore:39



Fig. S5. MS/MS spectra of phosphopeptdes identified in human HSP27 from *in vitro* kinase assay.



Fig. S6. Representative images of the other conditions presented in Fig.5 panel B.