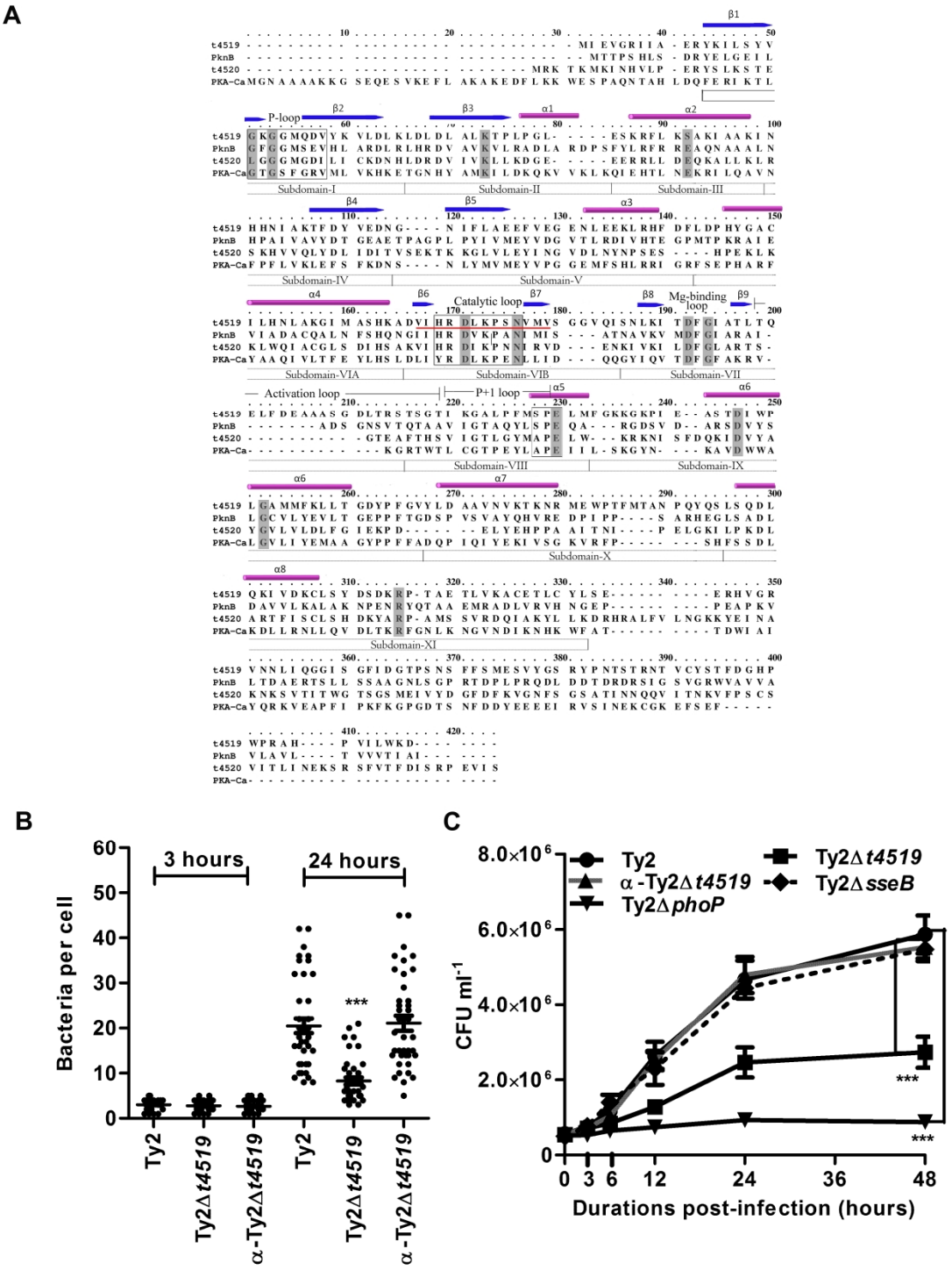
Supplementary information

Figure S1



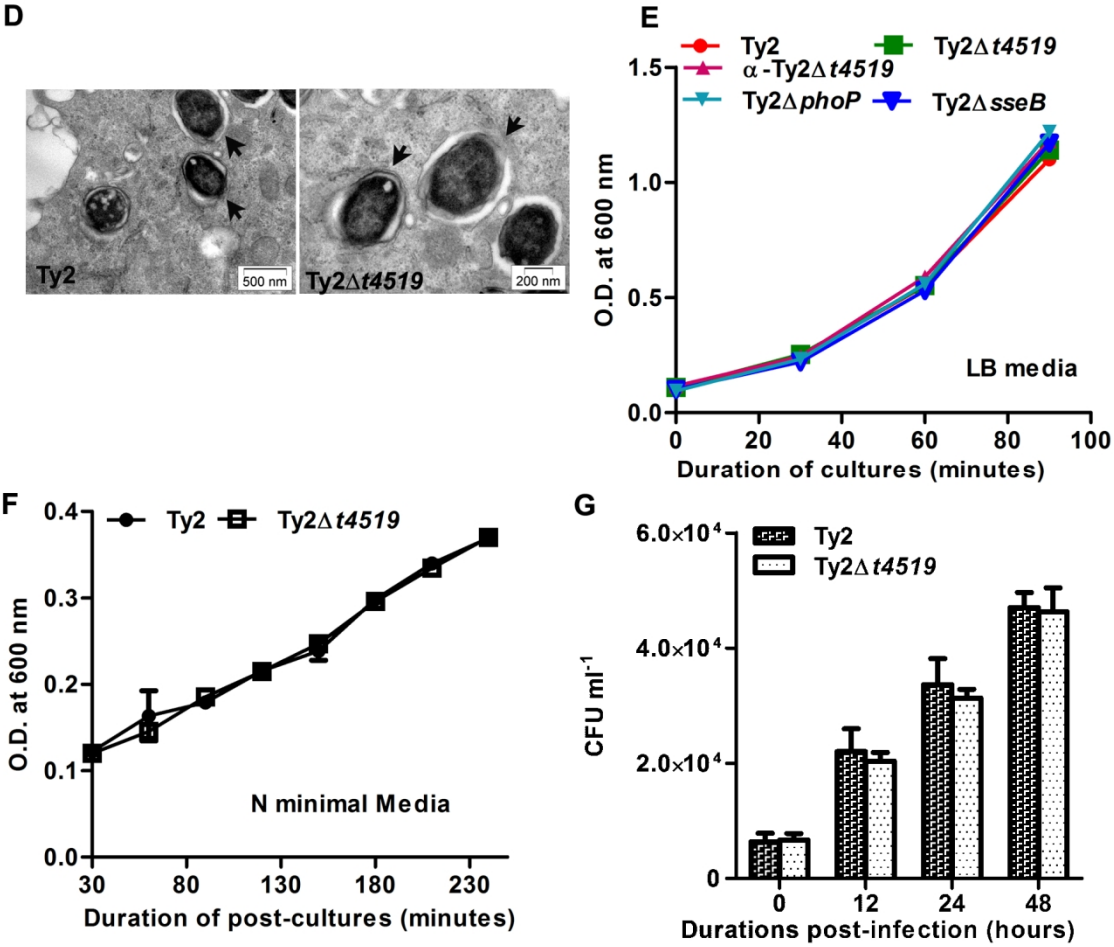

D
Ty2
500 nm
Ty2Δt4519
200 nm
E
Ty2
Ty2Δt4519
α-Ty2Δt4519
Ty2ΔphoP
Ty2ΔsseB
O.D. at 600 nm
LB media
Duration of cultures (minutes)
F
Ty2
Ty2Δt4519
O.D. at 600 nm
N minimal Media
Duration of post-cultures (minutes)
G
Ty2
Ty2Δt4519
CFU ml-1
Durations post-infection (hours)

Figure S2

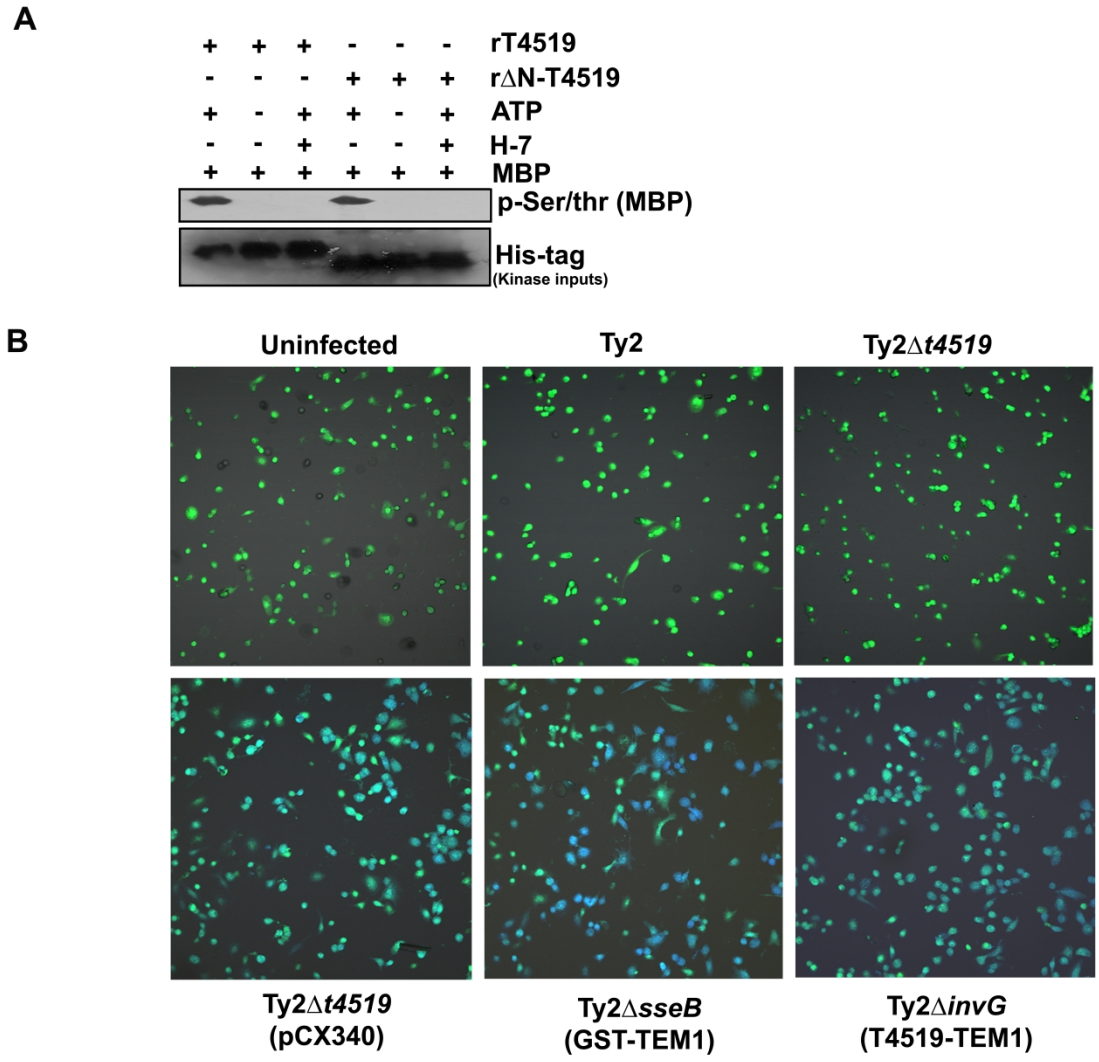

A
+ + + - - - rT4519
- - - + + + rΔN-T4519
+ - + + - + ATP
- - + - - + H-7
+ + + + + + MBP
p-Ser/thr (MBP)
His-tag
(Kinase inputs)
B
Uninfected
Ty2
Ty2Δt4519
Ty2Δt4519 (pCX340)
Ty2ΔsseB (GST-TEM1)
Ty2ΔinvG (T4519-TEM1)

Figure S3

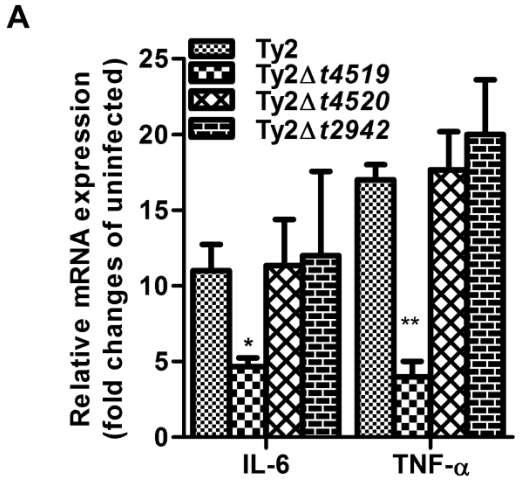


Figure S4

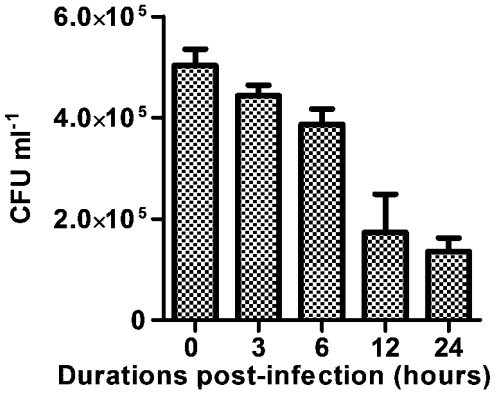


Table S1: List of plasmids and bacterial strains used in this study.

Vector/Bacterial Strain	**Genotype**	**Reference**
pBluescriptSK+		Stratagene
pCVD442		Addgene (11074)
pCVD442-Ty2Δ*t4519*		This study
pCVD442-Δ*t4520*		This study
pCVD442-Δ*t4521*		This study
pCVD442-Δ*phoP*		This study
pCVD442-Δ*sseB*		This study
pET28a+		Novogen
pET28a+-*t4519*		This study
pET28a+ΔN-*t4519*		This study
pET28a+Δpkc-*t4519*		This study
pET28a+ΔC-*t4519*		This study
pET28a+T4520		This study
pET28a+T2544		This study

pET28a+T2942		(1)
pQE-60		D. Chakraborty, India
pQE60-*t4519*		This study
pCX340		Eric Oswald, France
pCX340-*t4519*		In this study
pCX312		Eric Oswald, France
Salmonella enterica subsp. enterica serovar Typhi Ty2 (Ty2)		ATCC
Salmonella serovar Typhimurim LT214028s (LT2)		ATCC
E. coli DH5α		T Ramamurthy, India
DH5α-λ pir		T Ramamurthy, India
SM10λ-pir		T Ramamurthy, India
BL21(DE3)		Novogen
Ty2Δ*t4519*	*t4519*(-)	This study
Ty2Δ*t4520*	*t4520*(-)	This study

Ty2Δ*t4521*	*t4521*(-)	This study
Ty2Δ*phoP*	*phoP*(-)	This study
Ty2Δ*sseB*	*sseB*(-)	This study
Ty2Δ*invG*	*invG*(-)	This study
Ty2Δ*t2942*	*T2942*(-)	(1)
Ty2Δ*t4519*-pQE60-*t4519*	*t4519* compelemnt (+)	This study
Ty2Δ*t4519*-pCX340-*t4519*	*t4519-βlactamase* fusion (+)	This study
Ty2Δ*sseB*-pCX340-*t4519*	*sseB*(-) and *t4519-βlactamase* fusion (+)	This study
Ty2Δ*invG*-pCX340-*t4519*	*invG*(-) and *t4519-βlactamase* fusion (+)	This study
Ty2Δ*t4519*-pCX340		This study
LT2-*t4519*	*t4519* compelemnt (+)	This study

Table S2: List of primers was used in this study.

Primer Name	**5'<-----Sequence----->3'**
t4519mut 5armFP	GGTAAAGGTGGGATGCAAGA
t4519mut 5armRP	AACATCTGCTTTATGAGATGCC
t4519mut 3armFP	GCTACGCTAACACAAGAAC
t4519mut 3armRP	TCACATGCCTTAACAAGCG
t4519 Full FP	CATATGATCATGATTGAAGTCGGAAGAATCATTGCA
t4519 Full RP	CTCGAGTCAATCTTTCCAAAGAATTACAGGGTG
t4519 RTFP	GTGCCAAAATCGCTGCAAA
t4519 RTRP	AACATCTGCTTTATGAGATGCC
16sRNA RTFP	TGGGTTAAGTCCCGCAACG
16sRNA RTRP	TGAGGTCCGCTTGCTCTCG
t4519 N-ter(del)FP	GAATTCCATATGGTGCCAAAATCGCTGCAAA
t4519 N-ter(del)RP	CTCGAGTCAATCTTTCCAAAGAATTACAGGGTG
t4519 C-ter(del)FP	CATATGATCATGATTGAAGTCGGAAGAATCATTGCA
t4519 c-ter(del)RP	CTCGAGTCACATGCCTTAACAAGCG
phoPmut 5armFP	GAATTCGTCGTGCTGGTGCTTTCTCT
phoPmut 5armRP	CGCATCATCGTCATCTTCAC
phoPmut 3armFP	GATATCGGTAGCGGCGTGTTGTTAAG
phoPmut 5armRP	TCTAGACGGCGTATTGTTCCGTAATC
sseBmut 5armFP	GAATTCCAGGAAACATCTTATGGGG

sseBmut 5armRP	GATATCTTCAGCGTTCTTCTGGAC
sseBmut 3armFP	GATATCGCTGGATAAAGGTGGCCTAC
sseBmut 3armRP	TCTAGAGAATACGTTTTCTGCGCTATC
invGmut 5armFP	5’ ATGGCGCTACAGCTAAAGGAG 3’
invGmut 5armRP	5’ CCCACGAAGGTATTGTTCAGAC 3’
invGmut 3armFP	5’ CTGGTTAAAGCGCTGGATGT 3’
invGmut 3armRP	5’ CCACATTACGTTCCCCAATC 3’
t4520mut 5armFP	AGCACTGAACTCGGTGGTGGC
t4520mut 5armRP	TTCGGGGTGACTTTCTGACGG
t4520mut 3armRP	CATATGGCGATAACCAATATACCTGAA
t4520mut 3armFP	CTCGAGCGGTCTAGATATATCAAAGGTG
t4521mut 5armFP	TGGTTAGCTCGTTCTTCG
t4521mut 5armRP	TGTTGAACCGCCATGTCC
t4521mut 3armFP	CATATGGCTCAATATGTTCAATGGTGC
t4521mut 3armRP	CTCGAGGCTTGTAATGGATGGATCTTG
T4520 FP full length	CATATGATGAGGAAAACAAAAATGAAG
T4520 RP full length	CTCGAGTGATATTACCTCCGGTCTAG
T2544 FP full length	CATATGGAAGGGATCTATATCACCGGGA
T2544 RP full length	CTCGAGTTAAAAGGCGTAAGTAATGCCG
T2942 FP full length	CCATGGACCGGAGAGAAGAAGATG
T2942 RP full length	AGATCTGAACCGGTAACCCACGCCAAGAT
hGAPDH RTFP	GAGAACGGGAAGCTTGTCATC

hGAPDH RTRP	CATGACGAACATGGGGGCATC
hTNF-α FP	GTGACAAGCCTGTAGCCCATGTTG
hTNF-α RP	CTTGATGGCAGAGAGGAGGTTGAC
hIL-6 FP	GACCAGTGATGATTTTCACCAGGC
hIL-6 RP	GCACTGGCAGAAAACAACCTGAAC

References

1. **Chowdhury R, Mandal RS, Ta A, Das S. 2014**. An AIL family protein promotes Type Three Secretion System-1 independent invasion and pathogenesis of Salmonella enterica serovar Typhi. Cellular microbiology. doi: 10.1111/cmi.12379.

Figure legends

Fig. S1. T4519, a putative Ser/Thr protein kinase of *S.* Typhi, is required for macrophage survival and replication. (A) Primary sequence alignment between the catalytic subunit of PKA (PKA-Ca), *M. tuberculosis* PknB (residues 1 to 350), T4519 and T4520 using Clustal X. The conserved motifs are shown in boxes and the invariant residues are marked in grey. Subdomains are denoted by roman numerals. PROSITE Database Ser/Thr kinase active site signature sequence is underlined red. Corresponding secondary structural information is also provided. (B) Visual counts of intracellular bacteria as in Fig 1C. Dots represent bacterial counts per cell in random fields. Statistical significance was calculated by Nonparametric test. (C) RAW 264.7 macrophages were infected with Ty2 or the mutant. Intracellular CFU counts at the indicated time points from the cells subjected to gentamicin protection assay were plotted. (D) THP-1 cells

were infected with Ty2 or Ty2Δ*t4519* strain and processed for Transmission Electron Microscopy. Images are representative of two independent experiments. Arrowheads indicate intact bacteria within phagososmes. Micron bars are shown at the lower right corners. (E and F) *In vitro* growth kinetics of *S.* Typhi strains cultured in Luria betani broth or N Minimal Medium. Optical density of the cultures at 600 nm (OD_{600}) was measured at indicated times. Data represent two independent experiments. (G) Gentamicin protection assay was done as above with HT-29 cells infected with Ty2 or Ty2Δ*t4519* and intracellular CFU counts after overnight culture in Luria agar plate were plotted. Error bars, mean ± SD of three independent experiments. ***$p<0.001$ (Student's t test).

Fig. S2. T4519 is a secreted Serine/Threonine protein kinase. (A) *In vitro* kinase assays. Full-length rT4519 protein or rΔN-T4519 (2 µg) was incubated with the substrate (MBP, 5 µg) for 1 hour. Kinase activities were monitored by immunoblots for phosphorylation of MBP. The reaction was performed in the absence or presence of ATP (10 mM) and H7-dihydrochloride (1 µM). One of two independent experiments is shown. (B) PMA-differentiated THP-1 cells were infected with the Ty2 or mutant strains as indicated in the figure and subjected to gentamicin protection assay. Some of the mutant strains expressed TEM1 fusion proteins. Cells were cultured as in fig. 1 for 6 hour followed by loading with CCF2-AM (1 µM) and analyzed by confocal microscopy after excitation at 410 nm wavelength. Emission was captured at 510 nm (green) and 450 nm (blue). One of the two independent experiments is shown.

Fig. S3. T4519 regulates cytokine/chemokine responses. RT-qPCR shows GAPDH-normalized expression of cytokines and chemokines at 12 hours post-infection of Ty2 or mutant strains. Error bars, mean ± SD of three independent experiments. *$p<0.05$; **$p<0.01$; compared with Ty2 infection (Student's t test).

Fig. S4. Human monocyte-derived macrophages were infected with the Ty2 strain at a MOI of 10 and live intracellular bacterial counts were analyzed in a gentamicin protection assay as in Fig. 1 at the indicated time points.