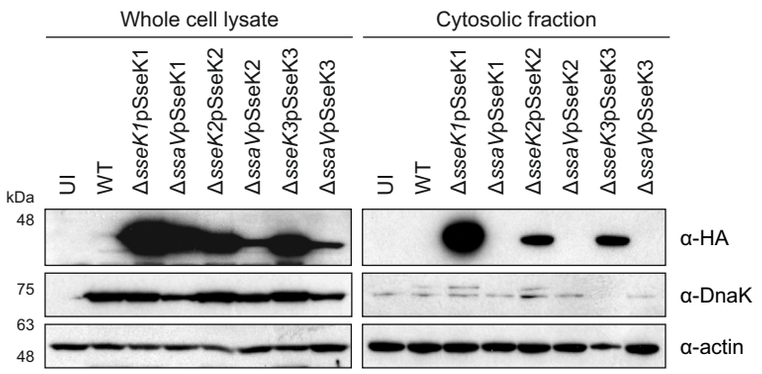
A



B

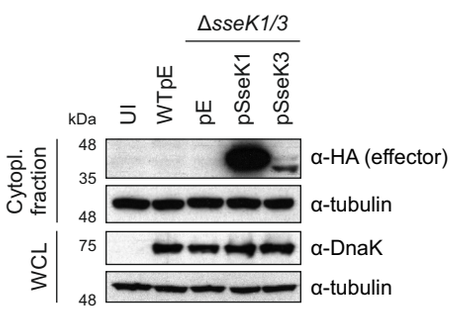


Fig S1. SPI-2 T3SS dependent translocation of SseK effectors into macrophages

(A-B) RAW264.7 macrophages were infected with the indicated *Salmonella* strains for 16 h. To compensate for the severe replication deficit Δ*ssaV* deletion mutant strains were used at 10x the MOI compared to WT *Salmonella*. Effector expression and translocation was analysed in whole cell lysates (WCL) and post-nuclear supernatants by SDS-PAGE and immuno-blotting of effectors (α-HA), *Salmonella* (α-DnaK), loading control (α-actin, α-tubulin). Data representative of two independent experiments. UI; uninfected.

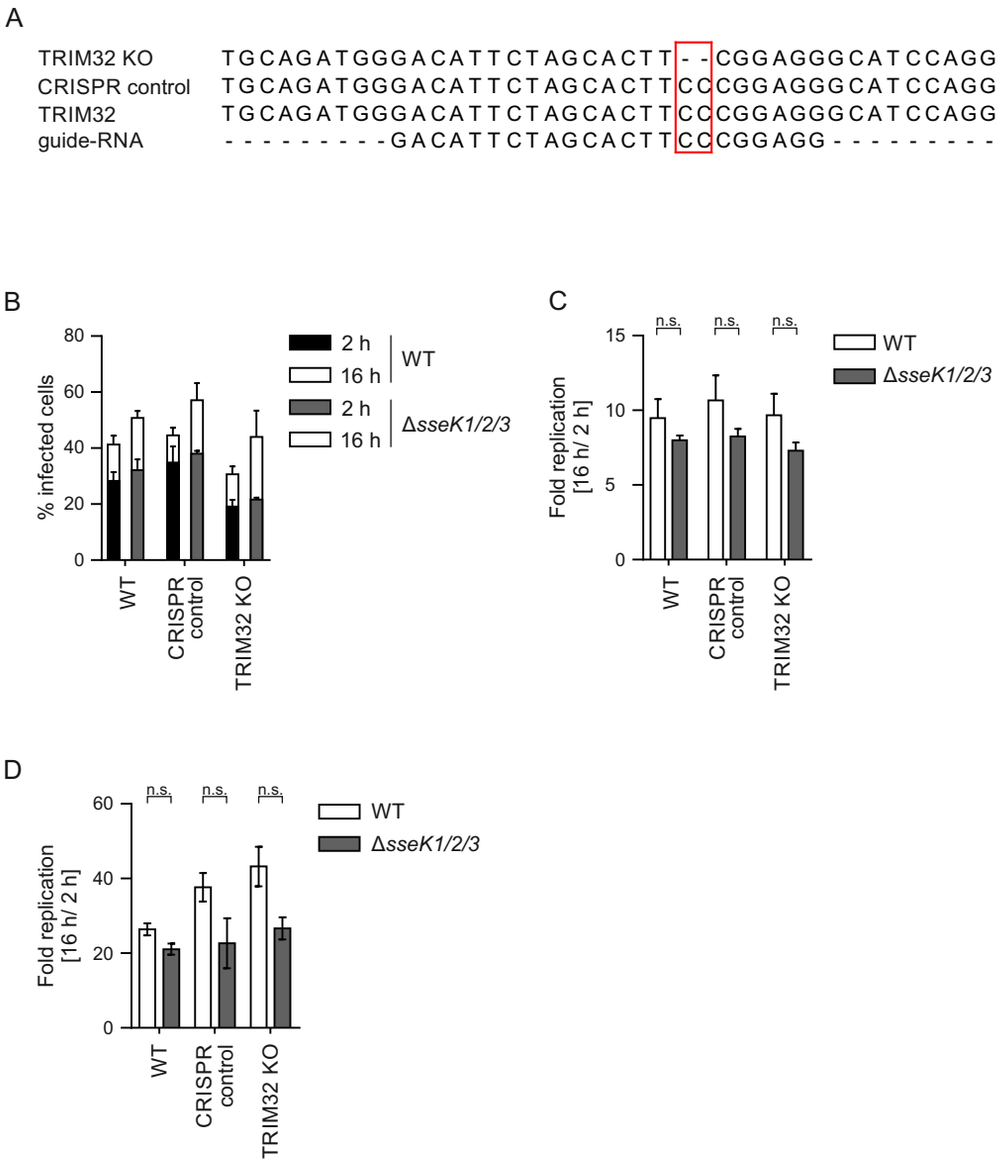


Fig S2. Analysis of the TRIM32 knockout RAW264.7 macrophages

(A) Sequence analysis of *Trim32* in WT and CRISPR generated TRIM32 knockout RAW264.7 macrophages. Alignment generated with Jalview. (B-C) TRIM32 knockout RAW264.7 macrophages were infected with the indicated GFP-expressing *Salmonella* strains for 2 h and 16 h. The infection rate (B) and fold geometric mean (C) were calculated after analysis by flow cytometry. The data represents the mean ± s.e.m. of three independent experiments. n.s. non significant. (D) TRIM32 deletion in macrophages does not affect *Salmonella* replication. Bacterial replication was analysed by gentamicin protection assay and calculated as fold colony-forming units (CFU) between 2 and 16 h.p.u. Values are the mean of three independent experiments ± s.e.m. Statistical analysis with Students *t*-test, differences not significant (n.s.).

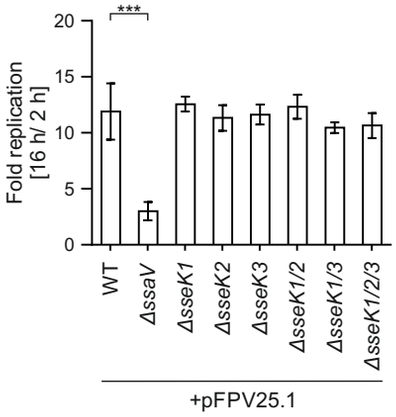


Fig S3. SseK deletion strains do not have a replication defect in macrophages

RAW264.7 macrophages were infected with the indicated GFP-expressing *Salmonella* strains for 2 h and 16 h. The GFP geometric mean was analysed by flow cytometry and the bacterial fold replication calculated. Data represents mean of three independent experiments ± s.e.m. *** $P < 0.001$.

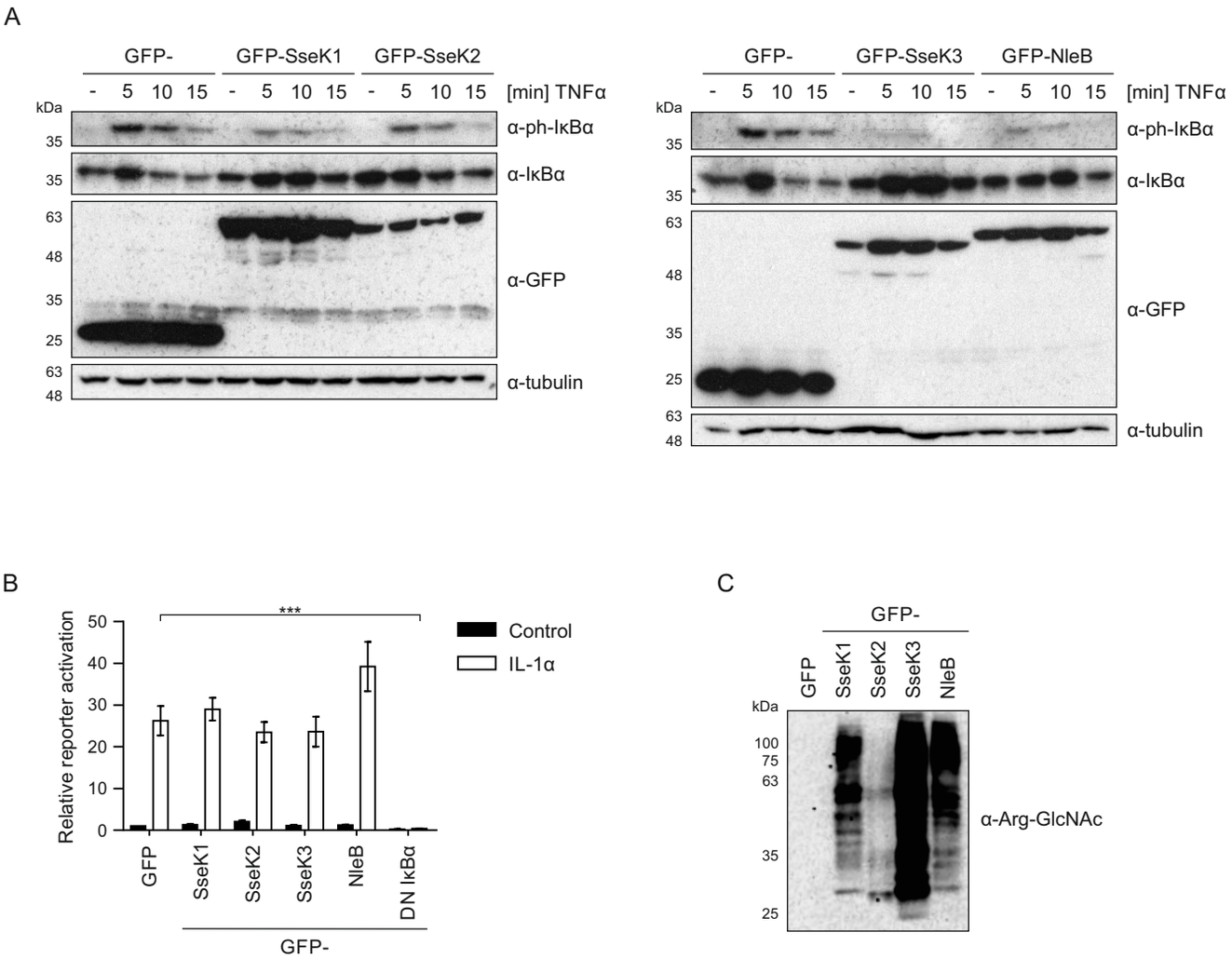
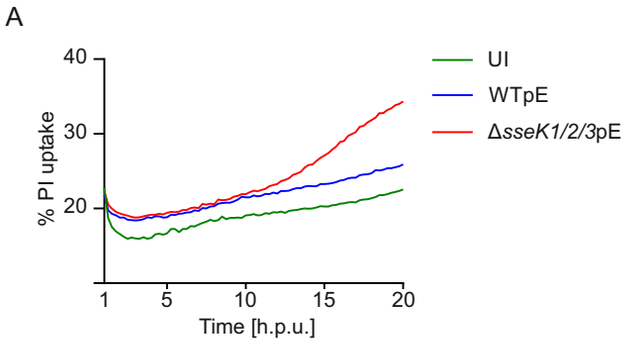
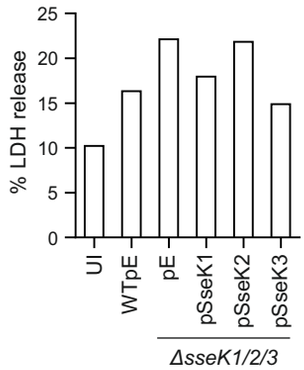
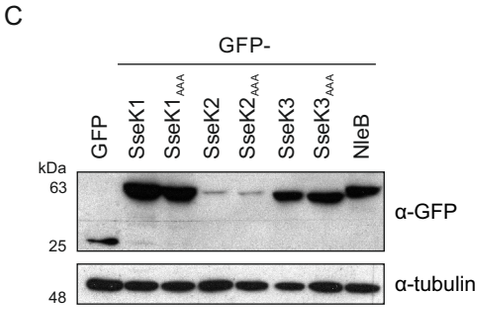


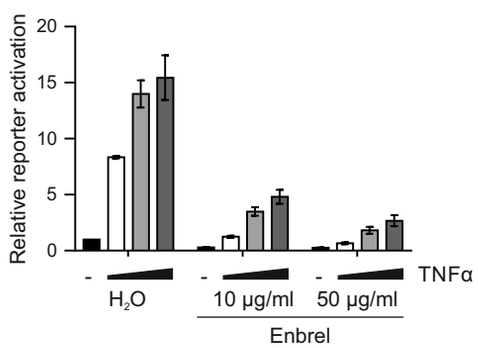
Fig S4. *Salmonella* SseK effectors inhibit TNFα mediated IκBα phosphorylation but do not inhibit IL-1α mediated NF-κB signalling

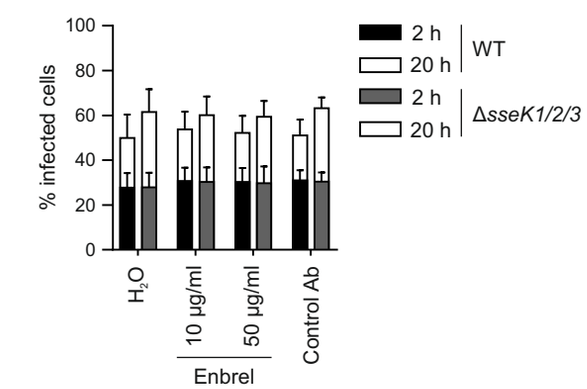
Inhibition of NF-κB signalling by GFP-SseK effectors following TNFα stimulation of transected 293ET cells. Cells were harvested after the indicated incubation with 50 ng/ml TNFα, lysed and analysed by SDS-PAGE and immuno-blotting to determine IκBα phosphorylation. Phospho IκBα (α-ph-IκBα), IκBα (α-IκBα), effectors (α-GFP), loading control (α-tubulin). (B) 293ET cells were co-transfected with an NF-κB -dependent luciferase reporter plasmid, pTK-Renilla luciferase plasmid and the indicated ptCMV-GFP-effector plasmids or dominant negative IκBα as a control. The NF-κB pathway was activated with 10 ng/ml IL-1α and luciferase activity measured in cell lysates after overnight stimulation. Results are presented as fold activation relative to unstimulated, GFP expressing control cells. These results were acquired at the same time as Fig. 3D and contain the same unstimulated data. Data shown are mean of five independent experiments ± s.e.m. *** $P < 0.001$. (C) SDS-PAGE and immuno-blotting of Arginine-GlcNAcylated proteins (α-Arg-GlcNAc) after expression of GFP-tagged effectors. This is a long exposure of Fig. 3C

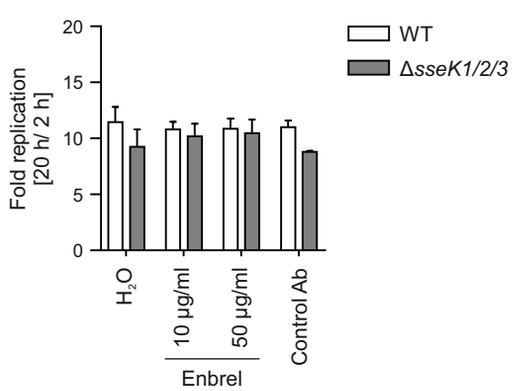

A
40
30
20
% PI uptake
1
5
10
15
20
Time [h.p.u.]
UI
WTpE
ΔsseK1/2/3pE

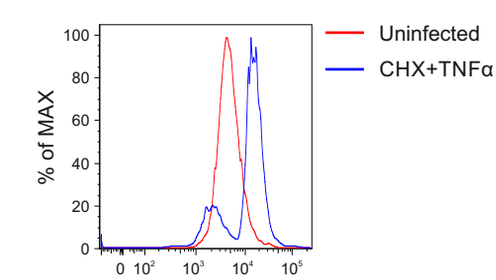

B
25
20
15
10
5
0
% LDH release
UI
WTpE
pE
pSseK1
pSseK2
pSseK3
ΔsseK1/2/3


C
GFP-
GFP
SseK1
SseK1AAA
SseK2
SseK2AAA
SseK3
SseK3AAA
NleB
kDa
63
25
48
α-GFP
α-tubulin


D
20
15
10
5
0
Relative reporter activation
-
-
-
TNFα
H2O
10 μg/ml
50 μg/ml
Enbrel


E
100
80
60
40
20
0
% infected cells
2 h
20 h
WT
2 h
20 h
ΔsseK1/2/3
H2O
10 μg/ml
50 μg/ml
Control Ab
Enbrel


F
20
15
10
5
0
Fold replication
[20 h/ 2 h]
WT
ΔsseK1/2/3
H2O
10 μg/ml
50 μg/ml
Control Ab
Enbrel


G
100
80
60
40
20
0
% of MAX
0
10²
10³
10⁴
10⁵
Uninfected
CHX+TNFα

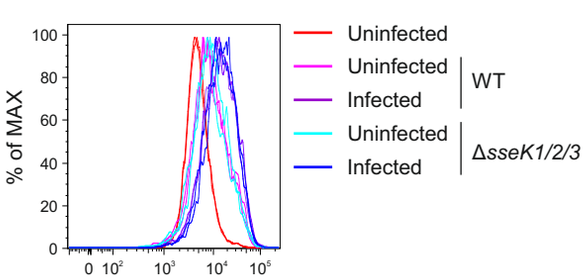

100
80
60
40
20
0
% of MAX
0
10²
10³
10⁴
10⁵
Uninfected
Uninfected
Infected
WT
Uninfected
Infected
ΔsseK1/2/3

Fig S5. SseK effectors inhibit TNFα driven cell death

(A) Representative experiment of Fig. 8A. RAW264.7 macrophages were infected with wild-type or mutant *Salmonella* strains and cell death assayed by propidium iodide (PI) uptake over time. (B) Representative experiment of Fig. 8B. LDH release from RAW264.7 macrophages infected for 20 h with the indicated *Salmonella* strains. Cell death was calculated relative to max. (C) Cell lysates from Fig. 8C were analysed by SDS-PAGE and immuno-blotting to determine effector protein expression levels during transfection LDH experiments. Effectors (α-GFP), loading control (α-tubulin). (D) The TNFα inhibitor Enbrel (Etanercept) inhibits TNFα driven NF-kB pathway activation in RAW264.7 macrophages. Cells were pre-incubated with Enbrel for 30 min and then stimulated with TNFα (10, 50, 100 ng/ml) for 16 h. Data is normalised to untreated water control cells and is the mean of three independent experiments ± s.e.m. (E-F) RAW264.7 macrophages were pre-treated with the indicated drugs for 30 min and then infected in the presence of the inhibitor for 2 and 20 h with the indicated GFP-expressing strains. The control antibody (Ab) was used at 50 μg/ml. The infection rate (E) and fold geometric mean (F) was calculated after analysis by flow cytometry. Data represents the mean of three independent experiments and differences are not significant. (G) Representative histograms of Fig. 8F. Caspase-3/7 activity was analysed using the SR-DEVD-FMK FLICA probe at 20 h.p.u. in infected RAW264.7 macrophages. Treatment with 50 μg/ml cyclohexamide and 50 ng/ml TNFα for 20 h was used as a positive control.

Table S1

***S*. Typhimurium 12023 strains**		
Name	**Description**	**Source or Reference**
wild-type	12023 *S.* Typhimurium wild-type	NTCC
Δ*ssaV*	Δ*ssaV*::km	(1)
Δ*sseL*	Δ*sseL*::km	(2)
Δ*sseK1*	Δ*sseK1*::km	(3)
Δ*sseK2*	Δ*sseK2*::km	(3)
Δ*sseK3*	Δ*sseK3*::km	(3)
Δ*sseK1/2*	Δ*sseK1*/Δ*sseK2*::km	(3)
Δ*sseK1/3*	Δ*sseK1*/Δ*sseK3*::km	(3)
Δ*sseK1/2/3*	Δ*sseK1*/Δ*sseK2*/Δ*sseK3*::km	(3)

Table S2

Plasmids		
Name	**Description**	**Source or Reference**
pFPV25.1	*rpsM::gfpmut3a* promoter fusion in pFPV	(4)
pWSK29-SseL-2HA	pWSK29 containing C-terminal 2HA-tagged SseL with 300 bp endogenous promoter ($Carb^R$)	(5)
pE	pWSK29 empty control plasmid ($Carb^R$)	(6)
pSseK1	pWSK29 containing C-terminal 2HA-tagged SseK1 with 200 bp endogenous promoter ($Carb^R$)	This study
pSseK2	pWSK29 containing C-terminal 2HA-tagged SseK2 with 200bp endogenous promoter ($Carb^R$)	This study
pSseK3	pWSK29 containing C-terminal 2HA-tagged SseK3 with 1000 bp endogenous promoter ($Carb^R$)	This study
m3psinrevκB-*luc*	*luc* gene under control of NF-κB consensus promoter	F. Randow
m6pPAC-RLuc	Constitutively active Renilla luciferase for macrophage transduction	This study
pEGFP-N1	GFP control plasmid	Clontech
ptCMV-GFP-SseK1	SseK1 with N-terminal GFP-tag	This study

ptCMV-GFP-SseK1$_{AAA}$	SseK1 mutant DAD$_{223-225}$ mutated to AAA with N-terminal GFP-tag	This study
ptCMV-GFP-SseK2	SseK2 with N-terminal GFP-tag	This study
ptCMV-GFP-SseK2$_{AAA}$	SseK2 mutant DAD$_{239-241}$ mutated to AAA with N-terminal GFP-tag	This study
ptCMV-GFP-SseK3	SseK3 with N-terminal GFP-tag	This study
ptCMV-GFP-SseK3$_{AAA}$	SseK3 mutant DAD$_{226-228}$ mutated to AAA with N-terminal GFP-tag	This study
ptCMV-GFP-NleB	NleB with N-terminal GFP-tag	This study
ptCMV-GFP-DN IκBα	Dominant negative human IκBα (S32A/ S36A) with N-terminal GFP-tag	This study
pRLTK	Constitutively active Renilla luciferase	F. Randow
p4κB:Luc	*luc* gene under control of NF-κB consensus promoter	F. Randow
pEAKMMP-AU1-TLR4	Human TLR4 for NF-κB pathway activation	F. Randow
m4pGFP-SseK1	SseK1 with N-terminal GFP-tag	This study
m4pGFP-SseK3	SseK3 with N-terminal GFP-tag	This study
m4pGFP-NleB	NleB with N-terminal GFP-tag	This study
m4pGFP-FADD	Murine FADD with N-terminal GFP-tag	This study
m4pGFP-TRADD	Murine TRADD with N-terminal GFP-tag	This study
m6pPAC-FLAG-GFP	GFP control plasmid	This study
m6pPAC-FLAG-FADD	Murine FADD with N-terminal FLAG-tag	This study
m6pPAC-FLAG-FADD$_{R117A}$	Murine FADD with Arginine 117 mutated to Alanine with N-terminal FLAG-tag	This study
m6pPAC-FLAG-TRADD	Murine TRADD with N-terminal FLAG-tag	This study
m6pPAC-FLAG-TRADD$_{R233A}$	Murine TRADD with Arginine 233 mutated to Alanine with N-terminal FLAG-tag	This study
m6pPAC-FLAG-TRIM32	Murine TRIM32 with N-terminal FLAG-tag	This study

Table S3

Cloning primers			
Use		**Species**	**5’ to 3’ nucleotide sequence**
200 bp-SseK1	FW	*S.* Typhimurium 12023	CGCGGGGAATTCAAATATGATGCCATTTCTGG
200 bp-SseK1	REV	*S.* Typhimurium 12023	CGCGGGGGATCCCTGCACATGCCTCGCCCATG
200 bp-SseK2	FW	*S.* Typhimurium 12023	CGCGGGGAATTCAATGGGCGCTTAGGTTTAGAG
200 bp-SseK2	REV	*S.* Typhimurium 12023	CGCGGGGGATCCCCTCCAAGAACTGGCAGTTA
1000 bp-SseK3	FW	*S.* Typhimurium 12023	CGCGGGGAATTCCACAGCAATTAATCTTCTGCCCG
1000 bp-SseK3	REV	*S.* Typhimurium 12023	CGCGGGGGATCCTCTCCAGGAGCTGATAGTCAAAC
SseK1	FW	*S.* Typhimurium 12023	CGCGGGACATGTCA ATGATCCCACCATTAAATAG
SseK1	REV	*S.* Typhimurium 12023	CGCGGGGCGGCCGCCTACTGCACATGCCTCGCCCATG
$SseK1_{AAA}$	FW	*S.* Typhimurium 12023	ATAGTGGGTGTATATATCTTGCTGCTGCTATGATTATCACGGAAAAACT
$SseK1_{AAA}$	REV	*S.* Typhimurium 12023	AGTTTTTCCGTGATAATCATAGCAGCAGCAAGATATATACACCCACTAT
SseK2	FW	*S.* Typhimurium 12023	CGCGGGACATGTCAATGGCACGTTTTAATGCCGC
SseK2	REV	*S.* Typhimurium 12023	CGCGGGGCGGCCGCTTACCTCCAAGAACTGGCAG
$SseK2_{AAA}$	FW	*S.* Typhimurium 12023	GCGGTGGGTGCATATATCTTGCTGCTGCTATGTTACTTACTGATAAACT
$SseK2_{AAA}$	REV	*S.* Typhimurium 12023	AGTTTATCAGTAAGTAACATAGCAGCAGCAAGATATATGCACCCACCGC
SseK3	FW	*S.* Typhimurium 12023	CGCGGGCCATGGCAATGTTTTCTCGAGTCAGAGGTTTTC
SseK3	REV	*S.* Typhimurium 12023	CGCGGG GCGGCCGCTTATCTCCAGGAGCTGATAGTC
$SseK3_{AAA}$	FW	*S.* Typhimurium 12023	GGTGGCTGCATATATCTTGCTGCTGCTATGTTACTTACAGGTAAAC
$SseK3_{AAA}$	REV	*S.* Typhimurium 12023	GTTTACCTGTAAGTAACATAGCAGCAGCAAGATATATGCAGCCACC
NleB	FW	*E.coli* O157:H7 Sakai	CGCGGGACATGTCCATGTTATCTTCATTAAATGTC
NleB	REV	*E.coli* O157:H7 Sakai	CGCGGGGCGGCCGCTTACCATGAACTGCTGG

FADD	FW	*Mus musculus*	CGCGGGACATGTCCATGGACCCATTCCTGGTGCTG
FADD	REV	*Mus musculus*	CGCGGGGCGGCCGCTCAGGGTGTTTCTGAGGAAGAC
$FADD_{R117A}$	FW	*Mus musculus*	CTGGAAAAGACTGGCCGCCGAGCTGAAGGTGTC
$FADD_{R117A}$	REV	*Mus musculus*	GACACCTTCAGCTCGGCGGCCAGTCTTTTCCAG
TRADD	FW	*Mus musculus*	CGCGGGACATGTCCATGGCAGCCGGTCAGAATGG
TRADD	REV	*Mus musculus*	CGCGGGCGTCTCCGGCCGTTAGGCCAGGCCGCCATCCG
$TRADD_{R233A}$	FW	*Mus musculus*	CTCAAGTGGCGCAGGGTGGGGGCCTCGCTGCAGCGTAACTGTCG
$TRADD_{R233A}$	REV	*Mus musculus*	CGACAGTTACGCTGCAGCGAGGCCCCCACCCTGCGCCACTTGAG
Trim32	FW	*Mus musculus*	CGCGGGACATGTCCATGGCTGCGGCTGCAGCAGCTTC
Trim32	REV	*Mus musculus*	CGCGGGGCGGCCGCTTAAGGGGTGGAATATCTTCTCAG
DN IκBα	FW	*Homo sapiens*	CGCGGGACATGTCCATGTTCCAGGCGGCCGAGCGCCCCCAGGAGTGGGC
DN IκBα	REV	*Homo sapiens*	CGCGGGGCGGCCGCTCATAACGTCAGACGCTGGCCTCCAAACACACAGT
RLuc	FW		CGCGGGCCATGGCAATGACTTCGAAAGTTTATGA
RLuc	REV		CGCGGGGCGGCCGCTTATTGTTCATTTTTGAGAA

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