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Supplementary Materials for

Mycobacterial EST12 activates a RACK1–NLRP3–gasdermin D pyroptosis–IL-1β immune pathway

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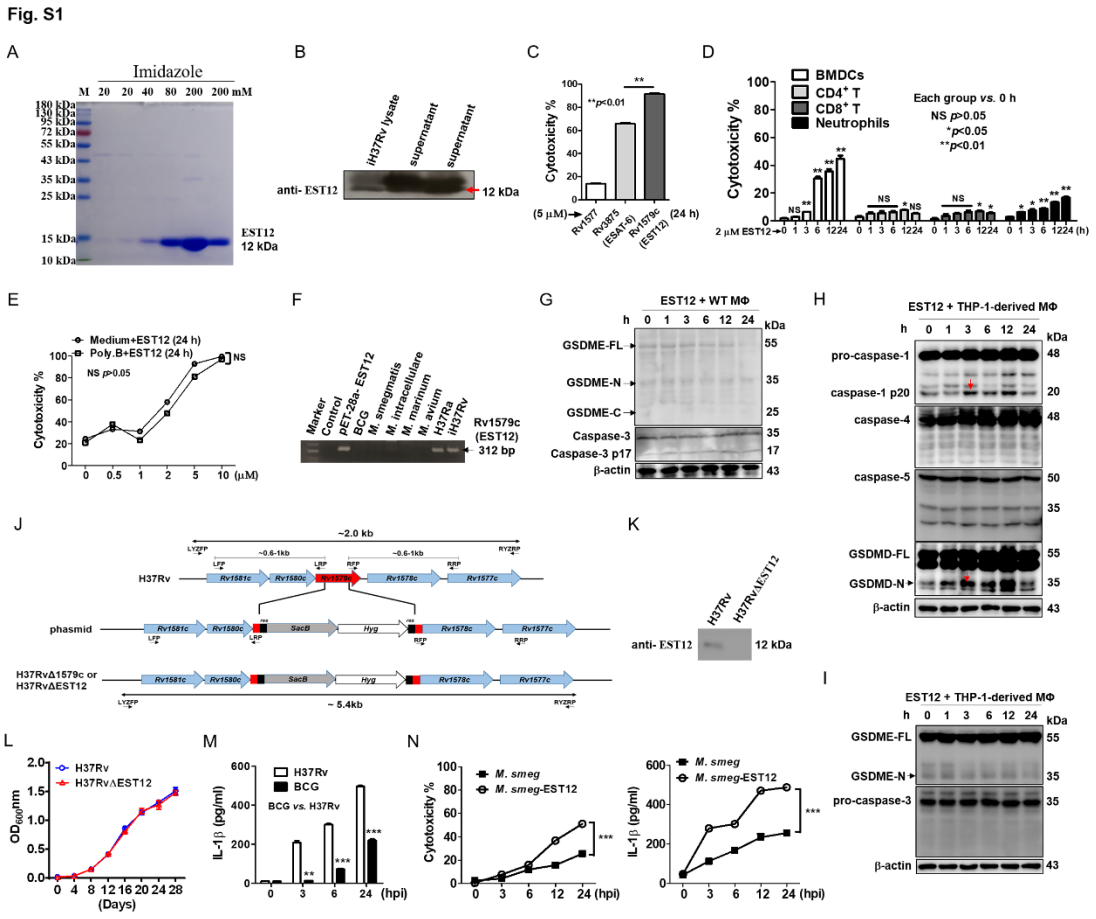


Fig. S1. Characterization and the cytotoxicity of EST12 protein. (**A**) Representative purified recombinant EST12 protein analyzed by SDS-PAGE. (**B**) Supernatants and whole bacterial lysates from H37Rv cultures were analyzed by WB. (**C-E**) Cytotoxicity assay by LDH. Peritoneal macrophages treated with Rv1577, ESAT-6 and EST12 (C). Mouse BMDCs, $CD4^+$ T, $CD8^+$ T cells and neutrophils treated with EST12. Unpaired *t*-test. (C, D). Peritoneal macrophages pretreated with Poly. B followed by EST12 treatment. Two-way ANOVA with Bonferroni's multiple comparison test (**E**). (**F**) Identification of EST12 gene in different mycobacterial strains by PCR. (**G-I**) Peritoneal macrophages (**G**) or THP-1-derived macrophages (**H, I**) treated with EST12 were detected for caspase-3/GSDME (**G, I**) and caspase-1/4/5/GSDMD (**H**) activation by WB. (**J-L**) Schematic of H37RvΔEST12 strain construction **(J)**. The

H37RvΔEST12 strain examined by WB **(K)**. The growth kinetics of the strains (**L**). (**M**) BMDMs infected with H37Rv and BCG were detected for IL-1β secretion by ELISA. Unpaired *t*-test. (**N**) BMDMs infected with *M. smeg* and *M. smeg*-EST12 were analyzed by LDH and ELISA. Two-way ANOVA with Bonferroni's multiple comparison test. The data are expressed as the mean ± SEM of n =3, NS, not significant ($p > 0.05$); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Fig. S2

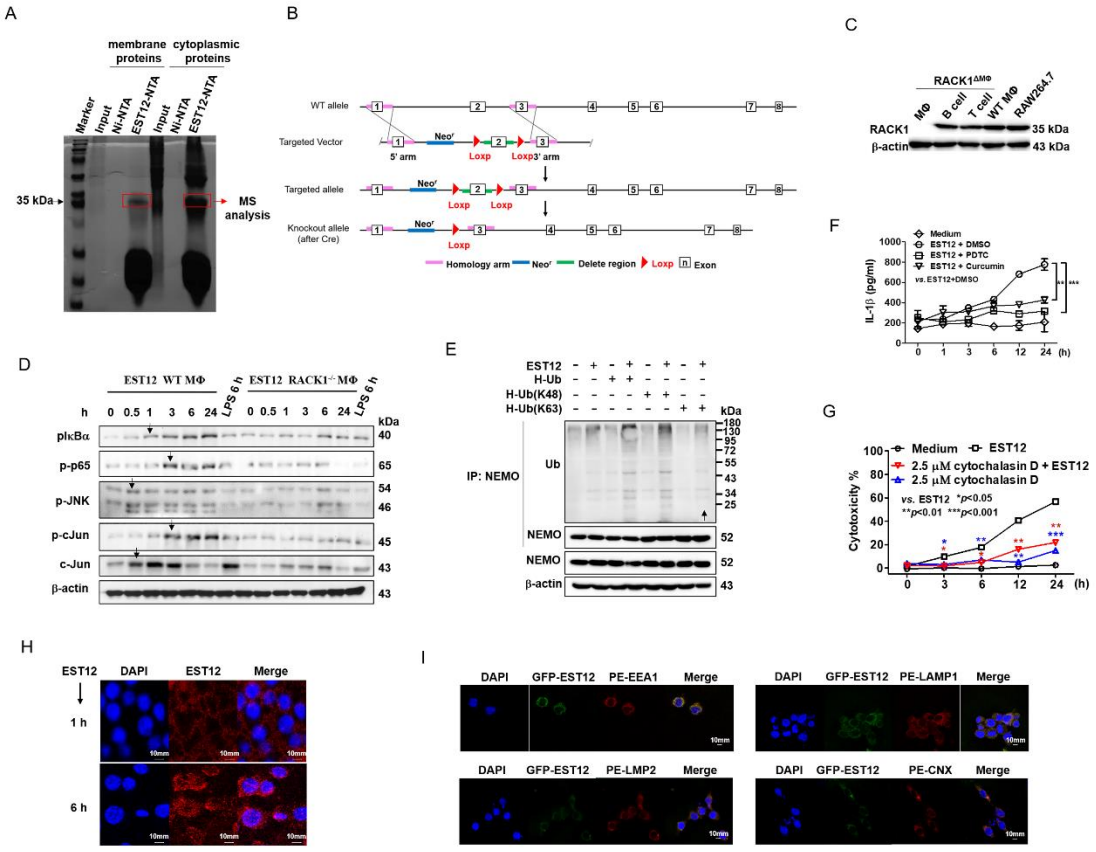


Fig. S2. EST12 induces the activation of NF-κB/Ap-1 pathways via RACK1. (**A**) An EST12-His pull-down experiment with RAW264.7 lysates analyzed by SDS-PAGE and coomassie brilliant blue staining. The specific bands were excised for MALDI-TOF/TOF MS analysis. (**B**) Schematic of the construction of mouse macrophage-specific deletion of RACK1. (**C**) WB analysis of RACK1 expression in the purified indicated cells. (**D**) WT and RACK1$^{-/-}$ peritoneal macrophages stimulated with EST12 were analyzed by WB (LPS as a control). (**E**) RAW264.7 cells transfected with the indicated plasmids and then stimulated with EST12 protein were subjected to IP with anti-NEMO and IB analysis. (**F**) Peritoneal macrophages pretreated with PDTC and curcumin, followed by EST12 stimulation, were evaluated for the secreted IL-1β by ELISA. Two-way ANOVA with Bonferroni's multiple comparison test. (**G**) Peritoneal

macrophages pretreated with cytochalasin D, followed by 2 μM EST12 treatment, were analyzed by LDH assay. Unpaired *t*-test. (**H**) RAW264.7 cells stimulated with EST12 were analyzed by confocal microscopy with anti-EST12 (red) and DAPI (blue). (**I**) RAW264.7 cells transfected with pEGFP-C1-EST12 were analyzed by confocal microscopy with anti-CNX/EEA1/LAMP1/LMP2 (red) and DAPI (blue) staining. The data are expressed as the mean ± SEM of n =3, * p <0.05, ** p <0.01, *** p <0.001.

Fig. S3

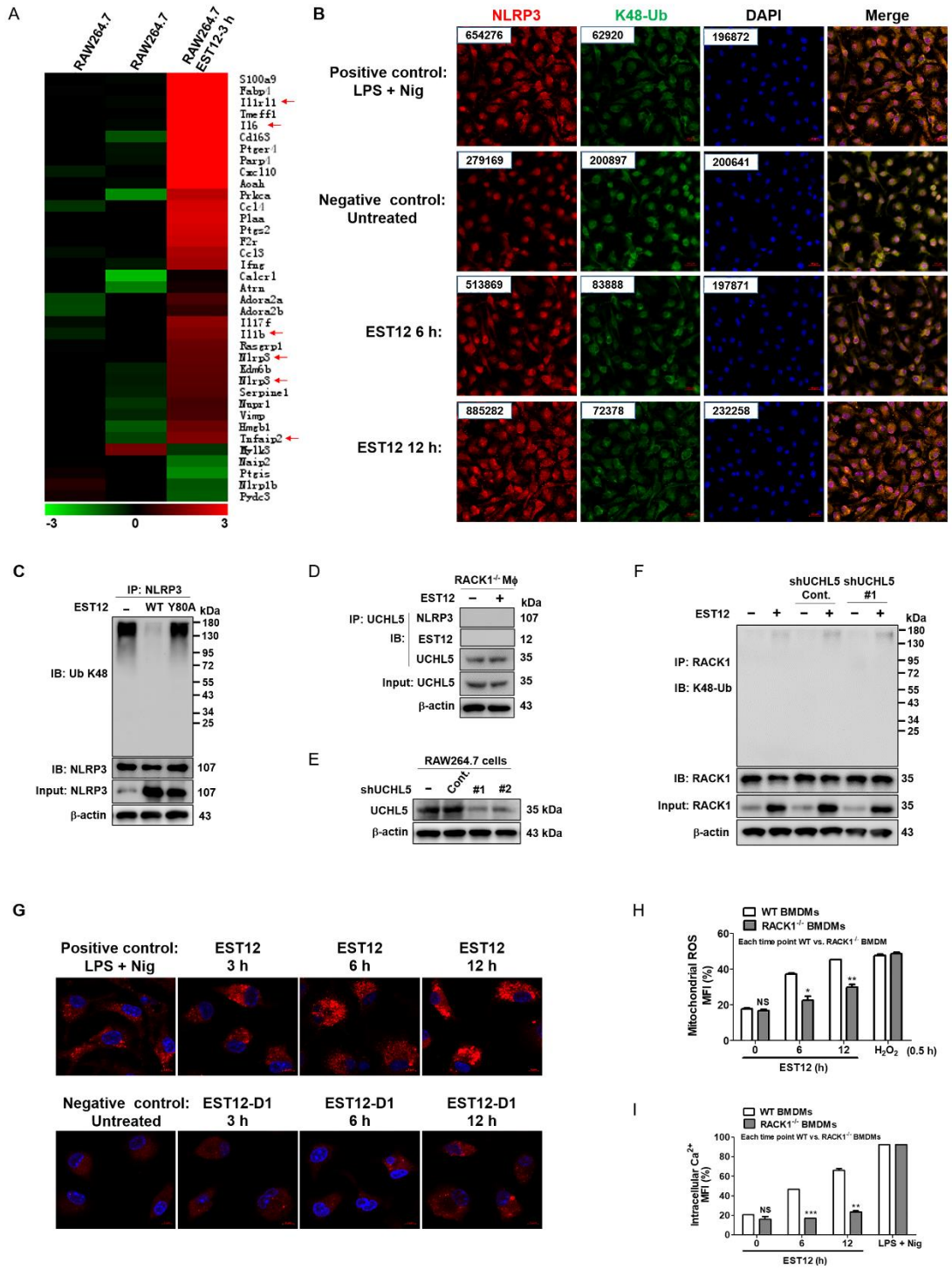


Fig. S3. EST12 induced NLRP3 K48-deubiquitination and inflammasome activation. (**A**) The microarray analysis. (**B**) BMDMs treated with EST12 or LPS+Nig were stained with anti-NLRP3 (green), anti-K48-Ub (red) and DAPI (blue) and analyzed by confocal microscopy. The integrated densities of NLRP3 and K48-Ub were

quantified as indicated numbers with Image J software. (**C, D, F**) BMDMs treated with EST12 or EST12-Y80A were immunoprecipitated with anti-NLRP3 (**C**), anti-RACK1 (**D**) and anti-UCHL5 (**F**) and IB analysis. (**E**) WB analysis of UCHL5 expression in RAW264.7 cells transfected with shRNAs-UCHL5. (**G**) Peritoneal macrophages treated with EST12 or EST12-D1 were analyzed by confocal microscopy with anti-ASC (red) and DAPI (blue). LPS + Nig as a positive control. (**H, I**) Statistical analysis of the MFI percentages of the mtROS (**H**) and intracellular Ca^{2+} (**I**). WT and RACK1$^{-/-}$ BMDMs treated with EST12 were analyzed by FCM. Treatments of H_2O_2 (**H**) or LPS+Nig (**I**) as positive controls. Unpaired *t*-test. The data are expressed as the mean ± SEM of n =3 cultures. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, NS, not significant ($p > 0.05$).

Fig. S4

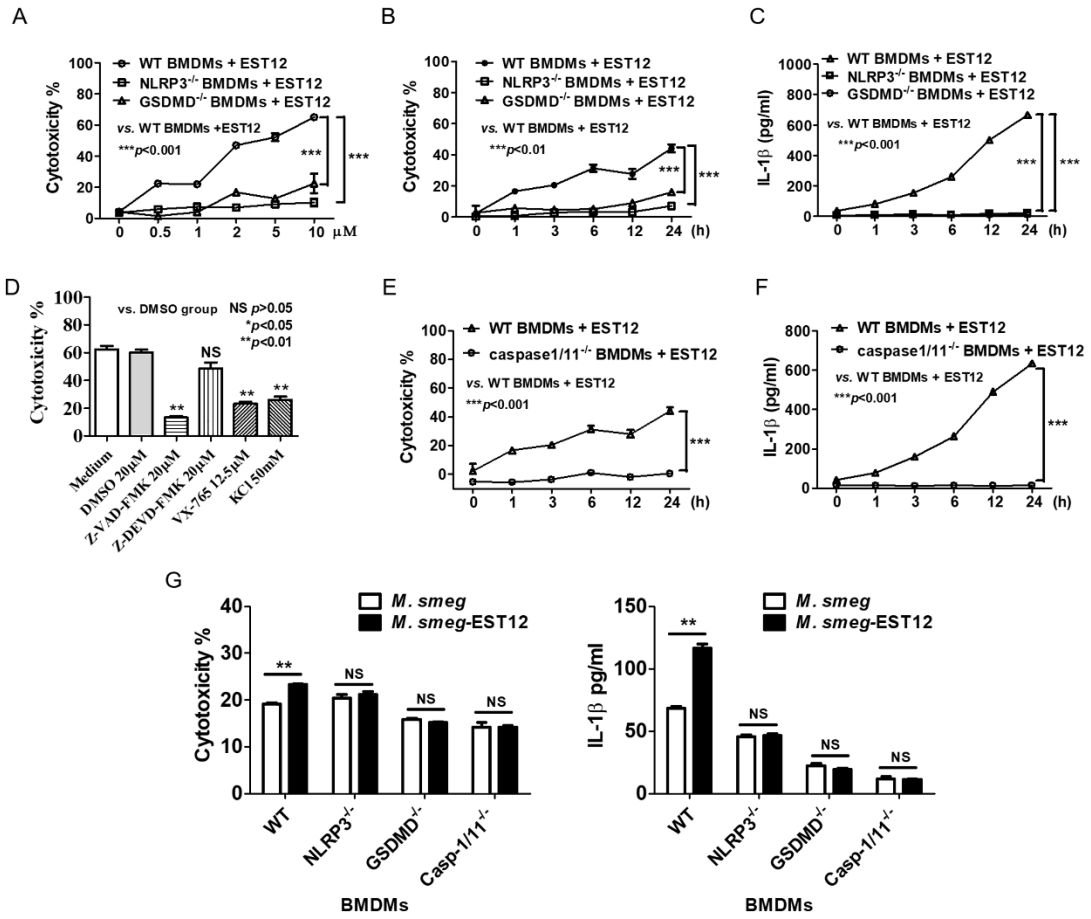


Fig. S4. Cytotoxicity effects of EST12 on WT/NLRP3$^{-/-}$/GSDMD$^{-/-}$/Caspase-1/11$^{-/-}$ BMDMs. (**A-C**) WT, NLRP3$^{-/-}$ and GSDMD$^{-/-}$ BMDMs treated with EST12 at the indicated doses (**A**) or for the indicated time periods (**B, C**) were analyzed for cytotoxicity by LDH assay (**A, B**) and IL-1β secretion by ELISA (**C**). Two-way ANOVA with Bonferroni's multiple comparison test. (**D**) Peritoneal macrophages pretreated with Z-VAD-FMK, Z-DEVD-FMK, VX-765, KCL and DMSO and then with EST12 stimulation for 24 h were evaluated by LDH assay. Unpaired *t*-test. (**E, F**) WT and caspase-1/11$^{-/-}$ BMDMs treated with EST12 were detected for LDH assay (**E**), secreted IL-1β by ELISA (**F**), two-way ANOVA with Bonferroni's multiple comparison test. (**G**) WT, NLRP3$^{-/-}$, GSDMD$^{-/-}$ and caspase-1/11$^{-/-}$ BMDMs infected with *M. smeg*

and *M. smeg*-EST12 were analyzed by LDH for cytotoxicity and by ELISA for secreted IL-1β. Unpaired *t*-test, *vs M. smeg* was employed for analysis. The data are expressed as the mean ± SEM of n =3. NS, not significant ($p > 0.05$); ** $p < 0.01$, *** $p < 0.001$.

Fig. S5

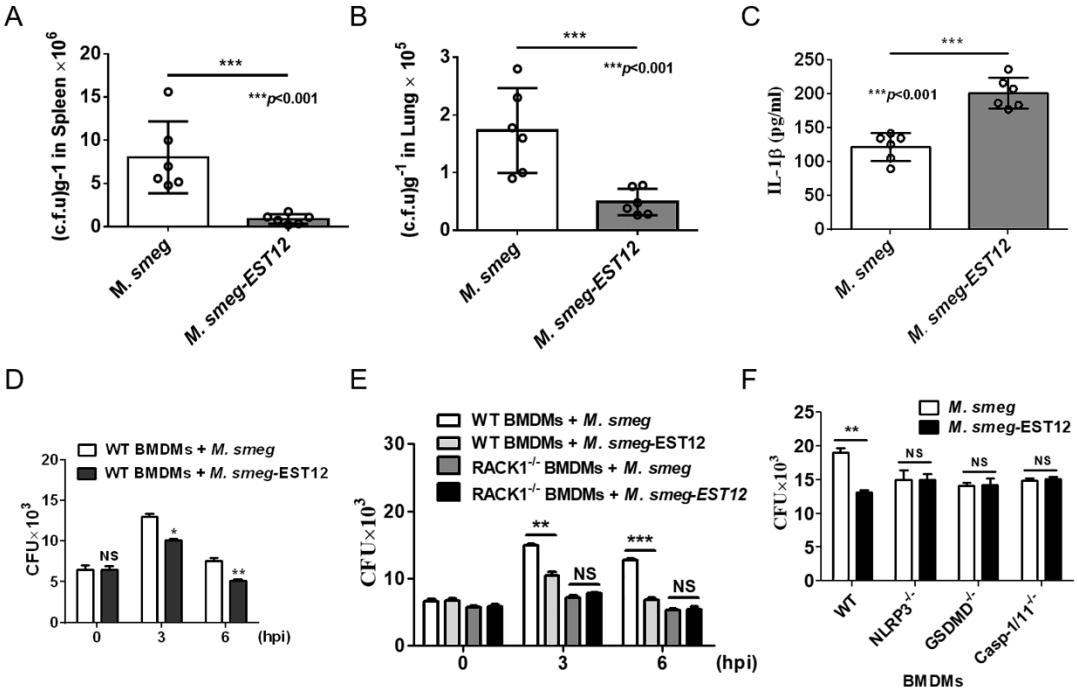


Fig. S5. *M. smeg*-EST12 increased IL-1β expression and bacterial clearance compared with *M. smeg*. (**A-C**) On day 0, WT mice (n=6 per group) were intravenously (*i.v.*) infected with WT-*M. smeg* or *M. smeg*-EST12. On Day 3, the mice were sacrificed for CFUs assay in spleens (A) and lungs (**B**). The serum IL-1β concentrations were analyzed by ELISA (**C**). (**D-F**) WT, RACK1$^{-/-}$, NLRP3$^{-/-}$, GSDMD$^{-/-}$ and caspase-1/11$^{-/-}$ BMDMs were infected with *M. smeg* and *M. smeg*-EST12 and CFUs were enumerated. Unpaired *t*-test. The data are expressed as the mean ± SEM. NS, not significant ($p > 0.05$); * $p < 0.05$, ** $p < 0.01$.

Fig. S6

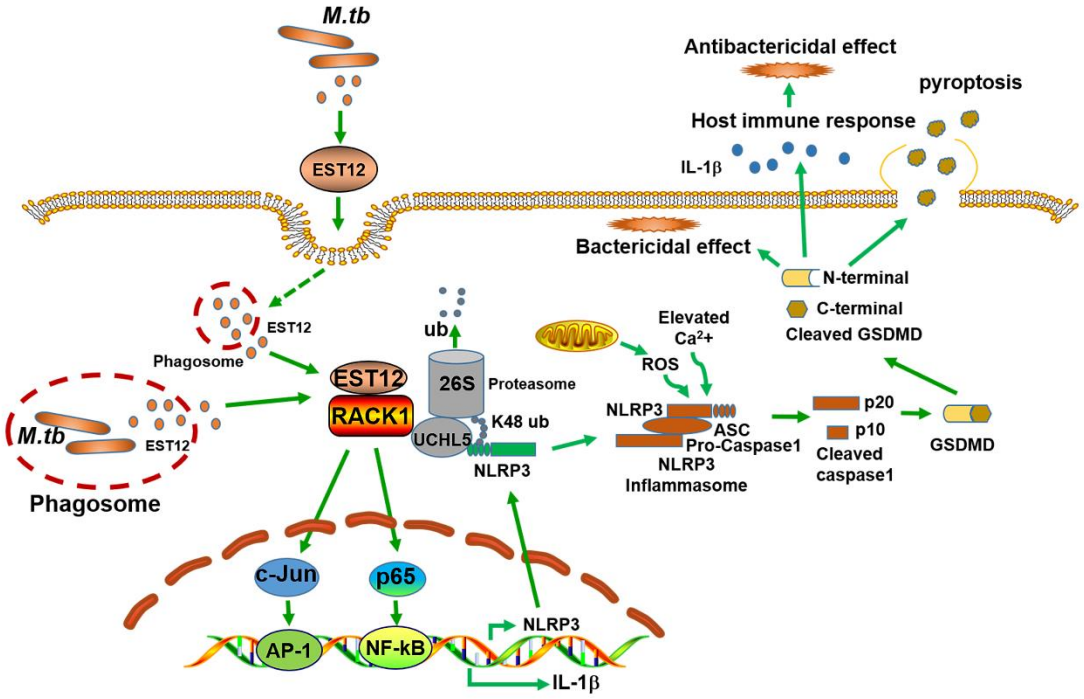


Fig. S6. A schematic mechanism model of *M.tb*-EST12-induced macrophage pyroptosis.

Table S1. LC-MS/MS analysis.

No.	Protein Name	Protein ID (UniProt)	Numbers of unique peptides	Species	Protein Score[#]
1	RACK1	P68040	7	MOUSE	13.56
2	Ldha	G5E8N5	6	MOUSE	12.86
3	Eno1	P17182	6	MOUSE	10.82
4	Eef1a1	P10126	4	MOUSE	8.9
5	Rps3a2	D3Z6C3	3	MOUSE	6.44
6	TOM34	Q9CYG7	3	MOUSE	6.33

[#] A higher score indicates a more confident match.

Table S2. Diffraction data collection and refinement statistics.

Space group	P 1 21 1
Data Collection	
Cell dimensions	
a, *b*, *c* (Å)	26.539 60.14 28.121
α, β, γ (º)	90 107.847 90
Resolution (Å)	15.7-1.9 (1.97-1.90)[a]
R_{merge} (%)	14.3(21.3)
I/s (I)	5.71(2.37)
Completeness (%)	92(85)
Redundancy	1.9(1.8)
Refinement	
Resolution (Å)	15.7-1.9(1.97-1.90)[a]
No. reflections	6582 (634)
R_{work}/R_{free}(%)	20.47/24.33(28.67/31.28)
No. of atoms	
Protein	74
Water	37
R.m.s deviations	
Bond lengths (Å)	0.003
Bond angles (º)	0.56
Ramachandran plot	
favored region (%)	100
allowed region (%)	0
outlier region (%)	0

[a]Data for outer shell shown in parentheses

Table S3. Reagent sources.

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Cryopyrin (6F12) Antibody	Santa Cruz Biotechnology	Cat# sc-134306 Lot# RRID:AB_2152550
Human/Mouse NLRP3/NALP3 Antibody	R＆D systems	Cat # MAB7578
Caspase-1 P10 (M-20) Antibody	Santa Cruz Biotechnology	Cat# sc-514 Lot# RRID:AB_2068895
Caspase-1 P20 antibody	AdipoGen	Cat# AG-20B-0042-C100, RRID:AB_2755041
UCHL5 antibody	Abcam	Cat# ab133508, RRID:AB_2814821
Anti-ubiquitin (linkage-specific K48)	Abcam	Cat# ab140601, RRID:AB_2783797
GSDME antibody	Abcam	Cat# ab215191, RRID:AB_2737000
Caspase 3/P17/P19 Antibody	Proteintech	Cat# 19677-1-AP, RRID:AB_10733244
Caspase-4 antibody	ABclonal	Cat# A6495, RRID:AB_2767094
Caspase-5 antibody	ABclonal	Cat# A16733, RRID:AB_2768720
Goat anti-ASC polyclonal antibody	Santa Cruz Biotechnology	Cat# sc-22514-R Lot# RRID:AB_2174874
GAPDH (G-9) antibody	Santa Cruz Biotechnology	Cat# sc-365062 Lot# RRID:AB_10847862
Phospho-I B (Ser32) (14D4) rabbit mAb antibody	Cell Signaling Technology	Cat# 2859 Lot# RRID:AB_561111
Rabbit anti-NF-KappaB P65, phospho (Ser536) mAb	Cell Signaling Technology	Cat# 3033 Lot# RRID:AB_331284
Phospho-JNK (Thr183/Tyr185) (G9) mouse mAb	Cell Signaling Technology	Cat# 9255 Lot# RRID:AB_2307321
Phospho-C-Jun (Ser73) (D47G9) XP rabbit mAb	Cell Signaling Technology	Cat# 3270 Lot# RRID:AB_2129575
C-Jun (60A8) rabbit mAb	Cell Signaling Technology	Cat# 9165 Lot# RRID:AB_2130165
Anti-Rabbit IgG (H+L), F(Ab')2 fragment (Alexa Fluor® 594 conjugate) antibody	Cell Signaling Technology	Cat# 8889 Lot# RRID:AB_2716249
Anti-mouse IgG (H+L), F(Ab')2 fragment (Alexa Fluor® 488 Conjugate)	Cell Signaling Technology	Cat# 4408, RRID:AB_10694704
IKKgamma (F-10) antibody	Santa Cruz Biotechnology	Cat# sc-166398 Lot# RRID:AB_2011719

Rabbit polyclonal antibody anti-GSDMD	Ding et al., 2016	The rabbit GSDMD polyclonal antibody was a gift from Feng Shao.
Ubiquitin antibody	Proteintech	Cat# 10201-2-AP Lot# RRID:AB_671515
Goat Anti-Mouse IgG (H+L), HRP conjugate antibody	Proteintech	Cat# SA00001-1 Lot# RRID:AB_2722565
Goat Anti-Rabbit IgG (H+L), HRP conjugate antibody	Proteintech	Cat# SA00001-2 Lot# RRID:AB_2722564
Actin antibody	Proteintech	Cat# 60008-1-Ig Lot# RRID:AB_2289225
Rabbit anti-RACK1 polyclonal antibody	Abcam	Cat# ab62735 Lot# RRID:AB_956255
EST12 rabbit polyclonal antibody	This paper	N/A
Bacterial strains		
M.tb H37Rv	ATCC	ATCC 93009
M.tb H37RvΔEST12	This paper	N/A
BCG	ATCC	ATCC 35734
BCG-EST12	This paper	N/A
M. smeg	ATCC	ATCC 19420
M. smeg-EST12	This paper	N/A
M. smeg-EST12-D1	This paper	N/A
M. smeg-EST12-D2	This paper	N/A
M. smeg-EST12-E55A	This paper	N/A
M. smeg-EST12-F76A	This paper	N/A
M. smeg-EST12-Y80A	This paper	N/A
M. intracellulare	ATCC	ATCC 13950
M. avium	ATCC	ATCC 25291
M. marinum	ATCC	ATCC 927
Escherichia coli (*E. coli)* DH5α	ATCC	ATCC 25922
E.coli BL-21	ATCC	ATCC BAA-1025
Chemicals, Peptides, and Recombinant Proteins		
Middlebrook 7H9 broth	BD Difco™	Cat# 271310
Middlebrook 7H10 agar	BD Difco™	Cat# 262710
BD BBL™ Middlebrook OADC Enrichment	BD Difco™	Cat# 212351
Fluid Thioglycollate Medium	BD Difco™	Cat# 225650
propidium iodide (PI)	Biolegend	Cat# 421301
DAPI	Sigma	Cat# D9542
Fetal bovine serum (FBS)	Gibco	Cat# 10099141
GM-CSF	Peprotech	Cat# 315-03-20
M-CSF	Peprotech	Cat# 315-02-50

IL-4	Peprotech	Cat# 214-14-20
PMA	Sigma	Cat# P1585
Curcumin	Sigma	Cat# 78246
PDTC	Sigma	Cat# P8765
Z-VAD-FMK	Targetmol	Cat# T6013
Z-DEVD-FMK	Targetmol	Cat# T6005
VX-765	Targetmol	Cat# T6090
Cytochalasin D	Invitrogen	Cat# PHZ1063
Ni NTA agarose	Qigen	Cat# 30310
LPS	Sigma	Cat# L2630
Nigericin	Millipore	Cat# 481990
ploymyxin B	Sigma	Cat# P1004
Gentamicin	Sigma	Cat# E003632
Protein A/G magnetic beads	MCE	Cat HY-K0202
Pierce™ High Capacity Endotoxin Removal Resin	Thermo Fisher Scientific	Cat# 88270
MitoSOX Red Mitochondrial Superoxide Indicator	Thermo Fisher Scientific	Cat# M36008
Fluo 4-AM	Beyotime	Cat# S1060
IPTG	Sigma	Cat# I6758
NEOFECT™ DNA transfection reagent	Neofect Biotech	Cat# TF201201
jetPEI®-Macrophage	Polyplus Transfection	Cat#103-05N
Immobilon WesternBright ECL HRP substrate	Advansta	Cat#K-12045-D10
PMSF	Roche	Cat#10837091001
TRIzol Reagent	Invitrogen	Cat# 15596026
ReverTra Ace® qPCR RT Kit	Toyobo Life Science	Cat# FSQ-101
SYBR® Green Realtime PCR Master Mix	Toyobo Life Science	Cat# QPK-201
Critical Commercial Assays		
EasySepTM mouse $CD4^+$ T Cell Isolation Kit	Stemcell Technologies	Cat#19852
EasySep™ Mouse Naïve CD8+ T Cell Isolation Kit	Stemcell Technologies	Cat#19858
CD19 Microbeads mouse	Miltenyi Biotec	Cat#: 130-052-201
CytoTox 96 Non-Radioactive Cytotoxicity Assay	Promega	Cat# G1780
Mouse IL-1β ELISA kit	Dakewe Biotech	Cat# 1210122
Experimental models: Cell lines		
RAW264.7	The China Center for	Cat# GDC0143

	Type Culture Collection (CCTCC)	
THP1	The China Center for Type Culture Collection (CCTCC)	Cat# GDC0100
Experimental models: organisms/strains		
Mouse: C57BL/6	Animal Laboratory Center of Wuhan University	http://shydw.whu.edu.cn/
Mouse: B6.129S6-Nlrp3$^{tm1Bhk/J}$	The Jackson Laboratory	Stock No: 021302
Mouse: B6.129P2-Lyz2$^{tm1(cre)Ifo}$/J	The Jackson Laboratory	Stock No: 004781
Mouse: Gnb2l1$^{Fl/Fl}$	Dr. Jiyan Zhang	N/A
Mouse: GSDMD$^{-/-}$	Dr. Feng Shao	N/A
Mouse: caspase-1/11$^{-/-}$	Dr. Feng Shao	N/A
Oligonucleotides		
Primers for PCR, see Table S2	This paper	N/A
Primers for cloning, see Table S3	This paper	N/A
Recombinant plasmids		
pET-28a-Rv1579c	This paper	N/A
pET-22b-Rv1579c	This paper	N/A
pMV261-Rv1579c	This paper	N/A
pMV261-Rv1579c-D1	This paper	N/A
pMV261-Rv1579c-D2	This paper	N/A
pMV261-Rv1579c-E55A	This paper	N/A
pMV261-Rv1579c-F76A	This paper	N/A
pMV261-Rv1579c-Y80A	This paper	N/A
pEGFP-C1-Rv1579c	This paper	N/A
pAsRED2-C1-RACK1	This paper	N/A
pSilencer 1.0-U6-sh-UCHL5	This paper	N/A
pET-28a- Rv1579c -D1	This paper	N/A
pET-28a- Rv1579c -D2	This paper	N/A
pET-28a- Rv1579c -Y80A	This paper	N/A
pET-28a- Rv1579c -F76A	This paper	N/A
pET-28a- Rv1579c -E55A	This paper	N/A
wild-type ubiquitin (H-Ub)	Drs. Hongbing Shu and Yanyi Wang	N/A
ubiquitin mutant H-Ub (K48)	Drs. Hongbing Shu and Yanyi Wang	N/A
ubiquitin mutant H-Ub (K63)	Drs. Hongbing Shu	N/A

	and Yanyi Wang		
Software and Algorithms			
GraphPad Prism	GraphPad Software	https://www.graphpad.com.	RRID: SCR_002798

Table S4. Primer sequences for molecular cloning.

Primer names	Primer sequences	Restriction Endonuclease
pET-28a-Rv1579c	Forward: TAGGATCCATGACCCCGATCAACC Reverse: TTAAGCTTCGATGGCGACCCCG	*Bam*HI *Hin*dIII
pET-22b-Rv1579c	Forward: TAGGATCCATGACCCCGATCAACC Reverse: TTAAGCTTCGATGGCGACCCCG	*Bam*HI *Hin*dIII
pMV261-Rv1579c	Forward: TAGGATCCATGACCCCGATCAACC Reverse: TTAAGCTTCGATGGCGACCCCG	*Bam*HI *Hin*dIII
pEGFP-C1-Rv1579c	Forward:ATCTCGAGAATGACCCCGATCAACCGG Reverse: ATGAATTCATCGATGGCGACCCCGCCC	*Xho*I *Eco*RI
pAsRED2-C1-RACK1	Forward: GCAAGCTTCTATGACCGAGCAGATGAC Reverse: AGCTGCAGAGCGGGTACCAATAGTTA	*Hin*dIII *Pst*I
pSilencer 1.0-U6-sh-UCHL5 1#	Oligo1: GGAGTGGTGTCTCATGGAAAGTTCA Oligo2: AGCTTGAACTTTCCATGAGACACCACTCCGGCC Oligo3: AGCTTCTTTCCATGAGACACCACTCCTTTTT Oligo4: AATTAAAAAGGAGTGGTGTCTCATGGAAAGA	Oligo1+Oligo 2 Oligo3+Oligo 4
pSilencer 1.0-U6-sh-UCHL5 2#	Oligo1: GGGTCTTCACCGAGCTCATTATTCA Oligo2: AGCTTGAATAATGAGCTCGGTGAAGACCCGGCC Oligo3: AGCTTTAATGAGCTCGGTGAAGACCCTTTTT Oligo4: AATTAAAAAGGGTCTTCACCGAGCTCATTAA	Oligo1+Oligo 2 Oligo3+Oligo 4
pET-28a-EST12-D1	Forward: TTGGATCCGTGACCCCGATCAA Reverse: TTAAGCTTGGCAAGCCAGTCAC	*Bam*HI *Hin*dIII
pET-28a-EST12-D2	Forward: TTGGATCCATGTGCTCACCCGA Reverse: TTAAGCTTCGATGGCGACCCCG	*Bam*HI *Hin*dIII
pET-28a-EST12-Y80A	Forward CTTGCCTTCCACGCGTCGGCTCCCGGCAACGACCCGCTGC	

Movie S1. Pyroptosis effect of EST12 protein on murine peritoneal macrophage. Representative video of PI uptake by WT mouse peritoneal macrophages after treatment with EST12. WT mouse peritoneal macrophages were treated with 2 μM EST12 for 4 h, and then stained with 5 ng/ml PI to monitor cell membrane integrity. Imaging was carried out using Zeiss LSM 880 with a 63×/1.49 NA oil objective. The total time was 100 min, and images were acquired every 2.5 min.

Movie S2. Effect of EST12 protein on the RACK1$^{-/-}$ peritoneal macrophage. Representative video of PI uptake by RACK1$^{-/-}$ mouse peritoneal macrophages after treatment with EST12. RACK1$^{-/-}$ mouse peritoneal macrophages were treated with 2 μM EST12 for 4 h, and then stained with 5 ng/ml PI to monitor cell membrane integrity. Imaging was carried out using Zeiss LSM 880 with a 63×/1.49 NA oil objective. The total time was 100 min, and images were acquired every 2.5 min.

Data file S1. Full wwPDB X-ray structure validation report.

WORLDWIDE PDB
PROTEIN DATA BANK

Preliminary Full wwPDB X-ray Structure Validation Report (i)

Nov 12, 2019 04:05 AM EST

This is a Preliminary Full wwPDB X-ray Structure Validation Report.

This report is produced by the standalone wwPDB validation server.
The structure in question has not been deposited to the wwPDB.
This report should not be submitted to journals.

We welcome your comments at *validation@mail.wwpdb.org*
A user guide is available at
https://www.wwpdb.org/validation/2017/XrayValidationReportHelp
with specific help available everywhere you see the (1) symbol.

The following versions of software and data (see references (1)) were used in the production of this report:

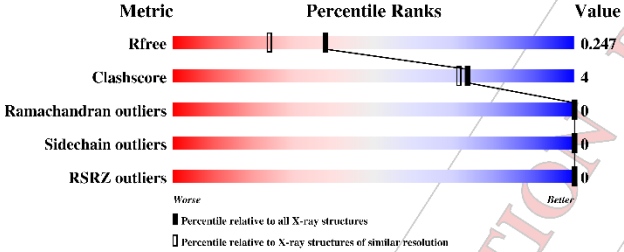
MolProbity	:	4.02b-467
Xtriage (Phenix)	:	1.13
EDS	:	2.6.1
Percentile statistics	:	20171227.v01 (using entries in the PDB archive December 27th 2017)
Refmac	:	5.8.0158
CCP4	:	7.0.044 (Gargrove)
Ideal geometry (proteins)	:	Engh & Huber (2001)
Ideal geometry (DNA, RNA)	:	Parkinson et al. (1996)
Validation Pipeline (wwPDB-VP)	:	2.6.1

1 Overall quality at a glance (i)

The following experimental techniques were used to determine the structure:
X-RAY DIFFRACTION

The reported resolution of this entry is 1.90 Å.

Percentile scores (ranging between 0-100) for global validation metrics of the entry are shown in the following graphic. The table shows the number of entries on which the scores are based.



Metric	Whole archive (#Entries)	Similar resolution (#Entries, resolution range(Å))
R_{free}	111664	5502 (1.90-1.90)
Clashscore	122126	6115 (1.90-1.90)
Ramachandran outliers	120053	6048 (1.90-1.90)
Sidechain outliers	120020	6048 (1.90-1.90)
RSRZ outliers	108989	5379 (1.90-1.90)

The table below summarises the geometric issues observed across the polymeric chains and their fit to the electron density. The red, orange, yellow and green segments on the lower bar indicate the fraction of residues that contain outliers for >=3, 2, 1 and 0 types of geometric quality criteria respectively. A grey segment represents the fraction of residues that are not modelled. The numeric value for each fraction is indicated below the corresponding segment, with a dot representing fractions <=5% The upper red bar (where present) indicates the fraction of residues that have poor fit to the electron density. The numeric value is given above the bar.

Mol	Chain	Length	Quality of chain
1	A	74	93% 7%

2 Entry composition (i)

There are 2 unique types of molecules in this entry. The entry contains 608 atoms, of which 0 are hydrogens and 0 are deuteriums.

In the tables below, the ZeroOcc column contains the number of atoms modelled with zero occupancy, the AltConf column contains the number of residues with at least one atom in alternate conformation and the Trace column contains the number of residues modelled with at most 2 atoms.

- Molecule 1 is a protein.

Mol	Chain	Residues	Atoms					ZeroOcc	AltConf	Trace
			Total	C	N	O	S			
1	A	74	571	354	100	114	3	0	1	0

- Molecule 2 is water.

Mol	Chain	Residues	Atoms		ZeroOcc	AltConf
			Total	O		
2	S	37	37	37	0	0

3 Residue-property plots (i)

These plots are drawn for all protein, RNA and DNA chains in the entry. The first graphic for a chain summarises the proportions of the various outlier classes displayed in the second graphic. The second graphic shows the sequence view annotated by issues in geometry and electron density. Residues are color-coded according to the number of geometric quality criteria for which they contain at least one outlier: green = 0, yellow = 1, orange = 2 and red = 3 or more. A red dot above a residue indicates a poor fit to the electron density (RSRZ > 2). Stretches of 2 or more consecutive residues without any outlier are shown as a green connector. Residues present in the sample, but not in the model, are shown in grey.

- Molecule 1:

Chain A:

4 Data and refinement statistics (i)

Property	Value	Source
Space group	P 1 21 1	Depositor
Cell constants a, b, c, α, β, γ	26.54Å 60.14Å 28.12Å 90.00° 107.85° 90.00°	Depositor
Resolution (Å)	19.34 – 1.90 30.07 – 1.90	Depositor EDS
% Data completeness (in resolution range)	92.3 (19.34-1.90) 93.5 (30.07-1.90)	Depositor EDS
R_{merge}	(Not available)	Depositor
R_{sym}	(Not available)	Depositor
$< I/\sigma(I) >$ [1]	1.35 (at 1.91Å)	Xtriage
Refinement program	phenix.refine 1.10.1_2155, PHENIX 1.10.1_2155	Depositor
R, R_{free}	0.205 , 0.244 0.206 , 0.247	Depositor DCC
R_{free} test set	657 reflections (10.05%)	wwPDB-VP
Wilson B-factor (Å^2)	31.7	Xtriage
Anisotropy	0.518	Xtriage
Bulk solvent k_{sol}(e/Å^3), B_{sol}(Å^2)	0.37 , 51.4	EDS
L-test for twinning[2]	$< \|L\| > = 0.50$, $< L^2 > = 0.33$	Xtriage
Estimated twinning fraction	No twinning to report.	Xtriage
F_o,F_c correlation	0.94	EDS
Total number of atoms	608	wwPDB-VP
Average B, all atoms (Å^2)	39.0	wwPDB-VP

Xtriage's analysis on translational NCS is as follows: *The largest off-origin peak in the Patterson function is 9.92% of the height of the origin peak. No significant pseudotranslation is detected.*

[1]Intensities estimated from amplitudes.

[2]Theoretical values of $< |L| >$, $< L^2 >$ for acentric reflections are 0.5, 0.333 respectively for untwinned datasets, and 0.375, 0.2 for perfectly twinned datasets.

5 Model quality

5.1 Standard geometry

The Z score for a bond length (or angle) is the number of standard deviations the observed value is removed from the expected value. A bond length (or angle) with $|Z| > 5$ is considered an outlier worth inspection. RMSZ is the root-mean-square of all Z scores of the bond lengths (or angles).

Mol	Chain	Bond lengths		Bond angles	
		RMSZ	$\#\|Z\| > 5$	RMSZ	$\#\|Z\| > 5$
1	A	0.32	0/584	0.52	0/793

There are no bond length outliers.

There are no bond angle outliers.

There are no chirality outliers.

There are no planarity outliers.

5.2 Too-close contacts

In the following table, the Non-H and H(model) columns list the number of non-hydrogen atoms and hydrogen atoms in the chain respectively. The H(added) column lists the number of hydrogen atoms added and optimized by MolProbity. The Clashes column lists the number of clashes within the asymmetric unit, whereas Symm-Clashes lists symmetry related clashes.

Mol	Chain	Non-H	H(model)	H(added)	Clashes	Symm-Clashes
1	A	571	0	544	4	0
2	S	37	0	0	3	0
All	All	608	0	544	4	0

The all-atom clashscore is defined as the number of clashes found per 1000 atoms (including hydrogen atoms). The all-atom clashscore for this structure is 4.

All (4) close contacts within the same asymmetric unit are listed below, sorted by their clash magnitude.

Atom-1	Atom-2	Interatomic distance (Å)	Clash overlap (Å)
1:A:22:GLN:NE2	2:S:28:HOH:O	2.31	0.63
1:A:10:ASN:ND2	2:S:33:HOH:O	2.28	0.60
1:A:68:HIS:ND1	2:S:29:HOH:O	2.32	0.59
1:A:51[B]:ARG:NH1	1:A:60:GLU:HG3	2.37	0.40

There are no symmetry-related clashes.

5.3 Torsion angles (i)

5.3.1 Protein backbone (i)

In the following table, the Percentiles column shows the percent Ramachandran outliers of the chain as a percentile score with respect to all X-ray entries followed by that with respect to entries of similar resolution.

The Analysed column shows the number of residues for which the backbone conformation was analysed, and the total number of residues.

Mol	Chain	Analysed	Favoured	Allowed	Outliers	Percentiles	
1	A	73/74 (99%)	73 (100%)	0	0	100	100

There are no Ramachandran outliers to report.

5.3.2 Protein sidechains (i)

In the following table, the Percentiles column shows the percent sidechain outliers of the chain as a percentile score with respect to all X-ray entries followed by that with respect to entries of similar resolution.

The Analysed column shows the number of residues for which the sidechain conformation was analysed, and the total number of residues.

Mol	Chain	Analysed	Rotameric	Outliers	Percentiles	
1	A	60/59 (102%)	60 (100%)	0	100	100

There are no protein residues with a non-rotameric sidechain to report.

Some sidechains can be flipped to improve hydrogen bonding and reduce clashes. There are no such sidechains identified.

5.3.3 RNA (i)

There are no RNA molecules in this entry.

5.4 Non-standard residues in protein, DNA, RNA chains (i)

There are no non-standard protein/DNA/RNA residues in this entry.

5.5 Carbohydrates (i)

There are no carbohydrates in this entry.

5.6 Ligand geometry (i)

There are no ligands in this entry.

5.7 Other polymers (i)

There are no such residues in this entry.

5.8 Polymer linkage issues (i)

There are no chain breaks in this entry.

6 Fit of model and data

6.1 Protein, DNA and RNA chains

In the following table, the column labelled '#RSRZ> 2' contains the number (and percentage) of RSRZ outliers, followed by percent RSRZ outliers for the chain as percentile scores relative to all X-ray entries and entries of similar resolution. The OWAB column contains the minimum, median, 95^{th} percentile and maximum values of the occupancy-weighted average B-factor per residue. The column labelled 'Q< 0.9' lists the number of (and percentage) of residues with an average occupancy less than 0.9.

Mol	Chain	Analysed	<RSRZ>	#RSRZ>2	OWAB(Å^2)	Q<0.9
1	A	74/74 (100%)	-0.24	0 100 100	27, 37, 51, 57	0

There are no RSRZ outliers to report.

6.2 Non-standard residues in protein, DNA, RNA chains

There are no non-standard protein/DNA/RNA residues in this entry.

6.3 Carbohydrates

There are no carbohydrates in this entry.

6.4 Ligands

There are no ligands in this entry.

6.5 Other polymers

There are no such residues in this entry.