Supplementary Materials for

CYP1B1-AS1 regulates CYP1B1 to promote *Coxiella burnetii* pathogenesis by inhibiting ROS and host cell death.

Aryashree Arunima *et al*.

*Corresponding author: James E. Samuel

Email: jsamuel@tamu.edu

This PDF file includes:

Supplementary Text

Figs. S1 to S14

Tables S1 to S5

Data S1 to S9

References (1 to 2)

Other Supplementary Materials for this manuscript include the following:

Data S1 to S9

Supplementary Text

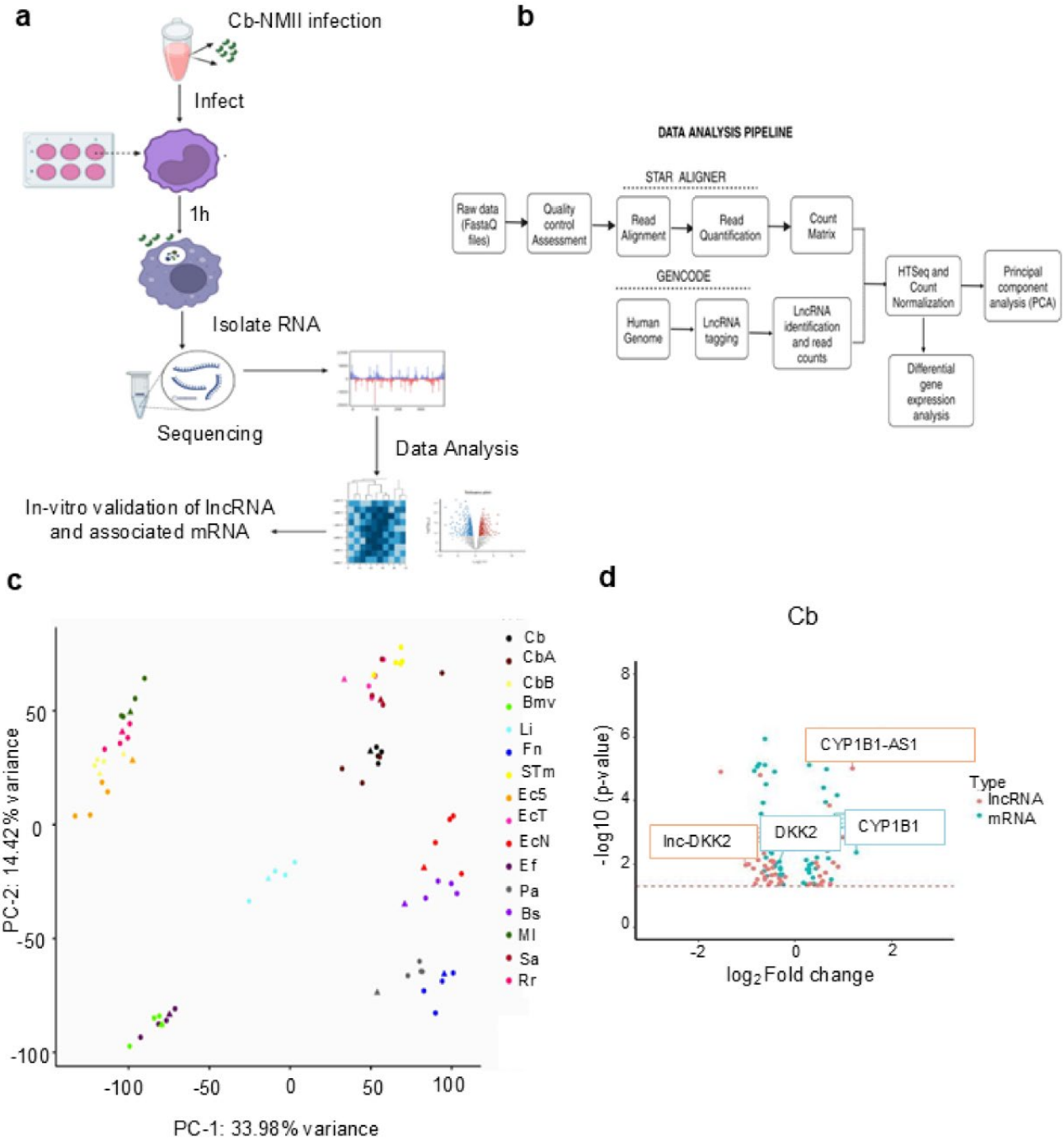

Fig S1. lnc-DKK2 and CYP1B1-AS1 as lncRNA signatures in *Coxiella burnetii* (Cb) infection. (a) Schematic workflow for transcriptome analysis of Cb and other pathogen infections. (b) Workflow for RNA-seq analysis, detailing the identification of differentially expressed (DE) lncRNAs and mRNAs using the STAR aligner, GENCODE, and ENSEMBL databases. (c) Principal component analysis (PCA) illustrating variance in RNA-seq data across different pathogens. (d) Scatter plot showing DE lncRNAs and mRNAs during Cb infection.

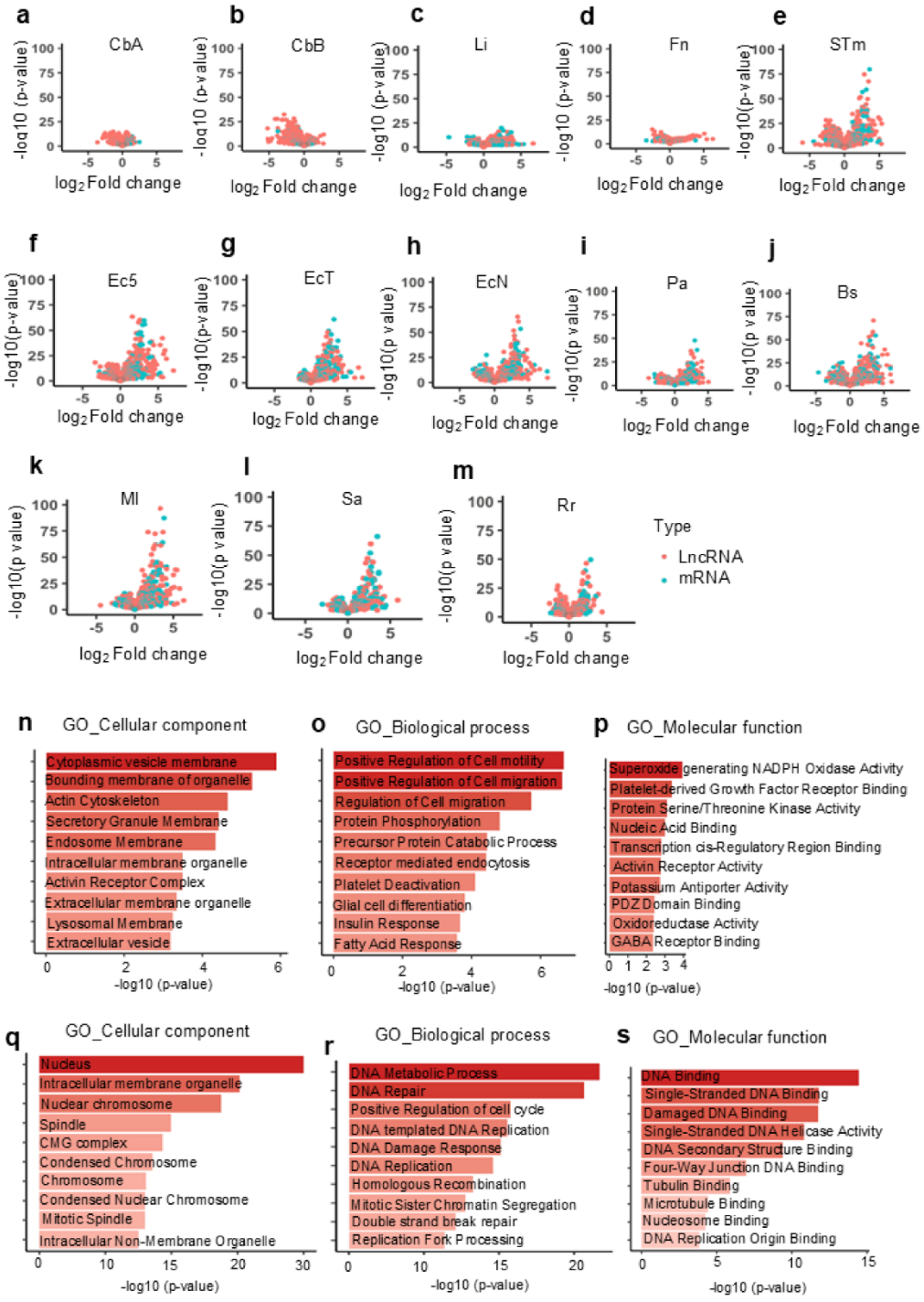

Fig S2. Global overview of DE host mRNA during Cb infection. Global overview of DE host mRNA-lncRNA during Cb infection. (a-m) Volcano plots of transcripts in various infection models: (a) *C. burnetii* Nine Mile Phase II *dotA*::Tn (CbA), (b) *C. burnetii* Nine Mile Phase II

dotB::Tn (CbB), (c) *Listeria innocua* (*L.innocua*; Li), (d) *Francisella novicida* U112 (*F. novicida*; Fn), (e) *Salmonella enterica* subsp. Typhimurium SL1344 (*S. Typhimurium*; STm), (f) *Escherichia coli* DH5α (*E. coli* DH5α; Ec5), (g) *Enterohemorrhagic Escherichia coli* O157:H7 (EHEC O157:H7; EcT), (h) *Enterohemorrhagic Escherichia coli* O157:H7Δ*stx* (EHEC Δ*stx;* EcN), (i) *Pseudomonas aeruginosa* PAO1 (*P. aeruginosa*; Pa), (j) *Bacillus subtilis* P31K6 (*B. subtilis*; Bs), (k) *Micrococcus luteus* (*M. luteus*; Ml), (l) *Staphylococcus aureus* JE2 (*S. aureus*; Sa), and (m) *Rhizobium radiobacter* (*R.radiobacter*; Rr). (n-p) Gene ontology (GO) analysis of upregulated DE mRNA-lncRNA pairs according to (n) Cellular Component, (o) Biological Process, and (p) Molecular Function. (q-s) GO analysis of downregulated DE mRNA-lncRNA pairs according to (q) Cellular Component, (r) Biological Process, and (s) Molecular Function. The color gradient indicates the proportion of transcripts corresponding to each annotation, with the top 10 significantly enriched terms shown ($P < 0.05$).

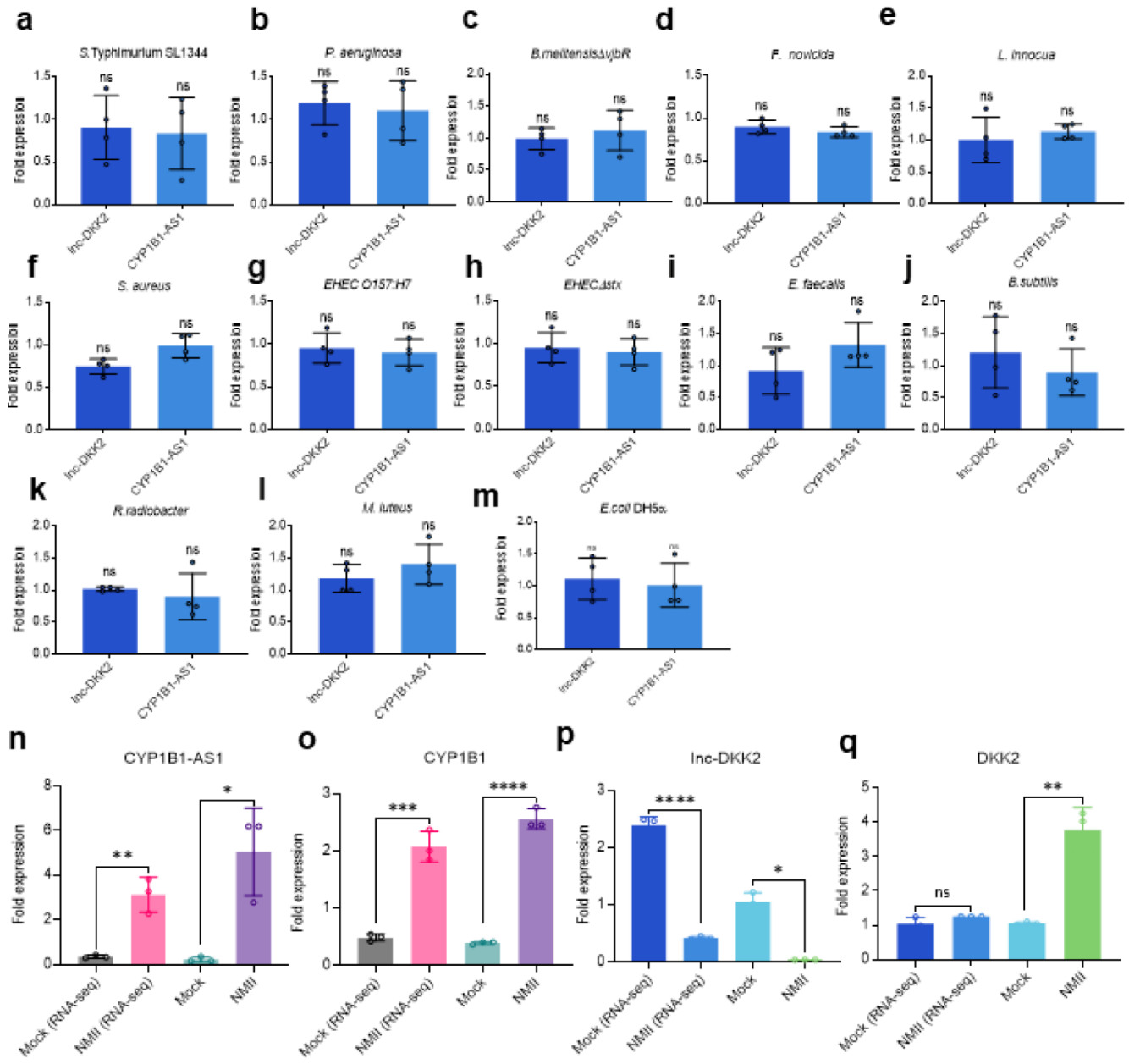

Fig S3. CYP1B1-AS1 and lnc-DKK2 as lncRNA signatures in Cb infection. (a-m) Quantitative real-time PCR (qPCR) analysis of CYP1B1-AS1 and lnc-DKK2 expression in various pathogens. (a) S. Typhimurium SL1344 (b) *P. aeruginosa* (c) *Brucella melitensis* (*B. melitensis* Δ*vjbR*) (d) *F. novicida* (e) *L. innocua* (f) *S.aureus* (g) EHEC 0157:H7 (h) EHEC Δ*stx* (i) *Enterococcus faecalis* (*E. faecalis*) (j) *B. subtilis* (k) *R. radiobacter* (l) *M. luteus* (m) *E. coli* DH5α. (n-q) Validation of host transcriptome data for (n) CYP1B1-AS1, (o) CYP1B1, (p) lnc-DKK2, and (q) DKK2 during

Cb infection. Gene expression was normalized to ACTB and compared to uninfected controls (Mock). Data are represented as mean ± SD, n=3. Statistical significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$; ****, $P < 0.0001$; ns, $P \geq 0.05$; Student's t-test (a-m), one-way ANOVA (n-q).

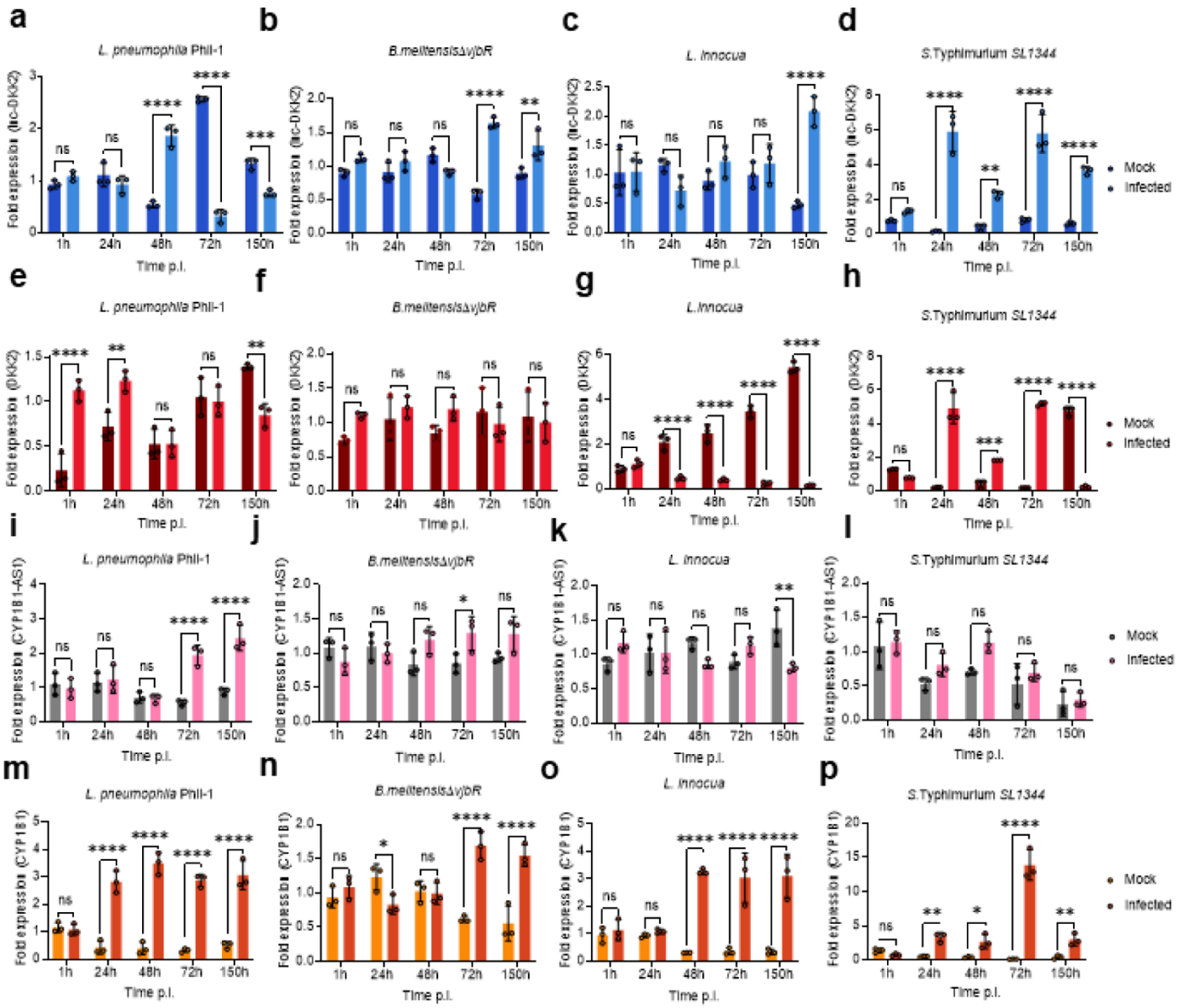

Fig S4. Temporal regulation of lnc-DKK2 and CYP1B1-AS1 in pathogenic infection models. Cells without siRNA treatment and infected with Cb were designated as NMII. RNA was harvested at the indicated time points for qPCR analysis, with gene expression normalized to ACTB and compared to uninfected controls (Mock). Expression levels of lnc-DKK2 in: (a) *Legionella pnuemophila* (*L. pnuemophila*) Phil-1, (b) *B. melitensis* Δ*vjbR*, (c) *L. innocua*, and (d) *S.* Typhimurium SL1344. Expression levels of DKK2 in: (e) *L. pnuemophila* Phil-1, (f) *B. melitensis*

Δ*vjbR*, (g) *L. innocua*, and (h) *S.* Typhimurium SL1344. Expression levels of CYP1B1-AS1 in: (i) *L. pnuemophila* Phil-1, (j) *B. melitensis* Δ*vjbR*, (k) *L. innocua*, and (l) *S.* Typhimurium SL1344. Expression levels of CYP1B1 in: (m) *L. pnuemophila* Phil-1, (n) *B. melitensis* Δ*vjbR*, (o) *L. innocua*, and (p) *S.* Typhimurium SL1344. Data are shown as mean ± SD, n=3. Statistical significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$; ****, $P < 0.0001$; ns, $P \geq 0.05$; one-way ANOVA.

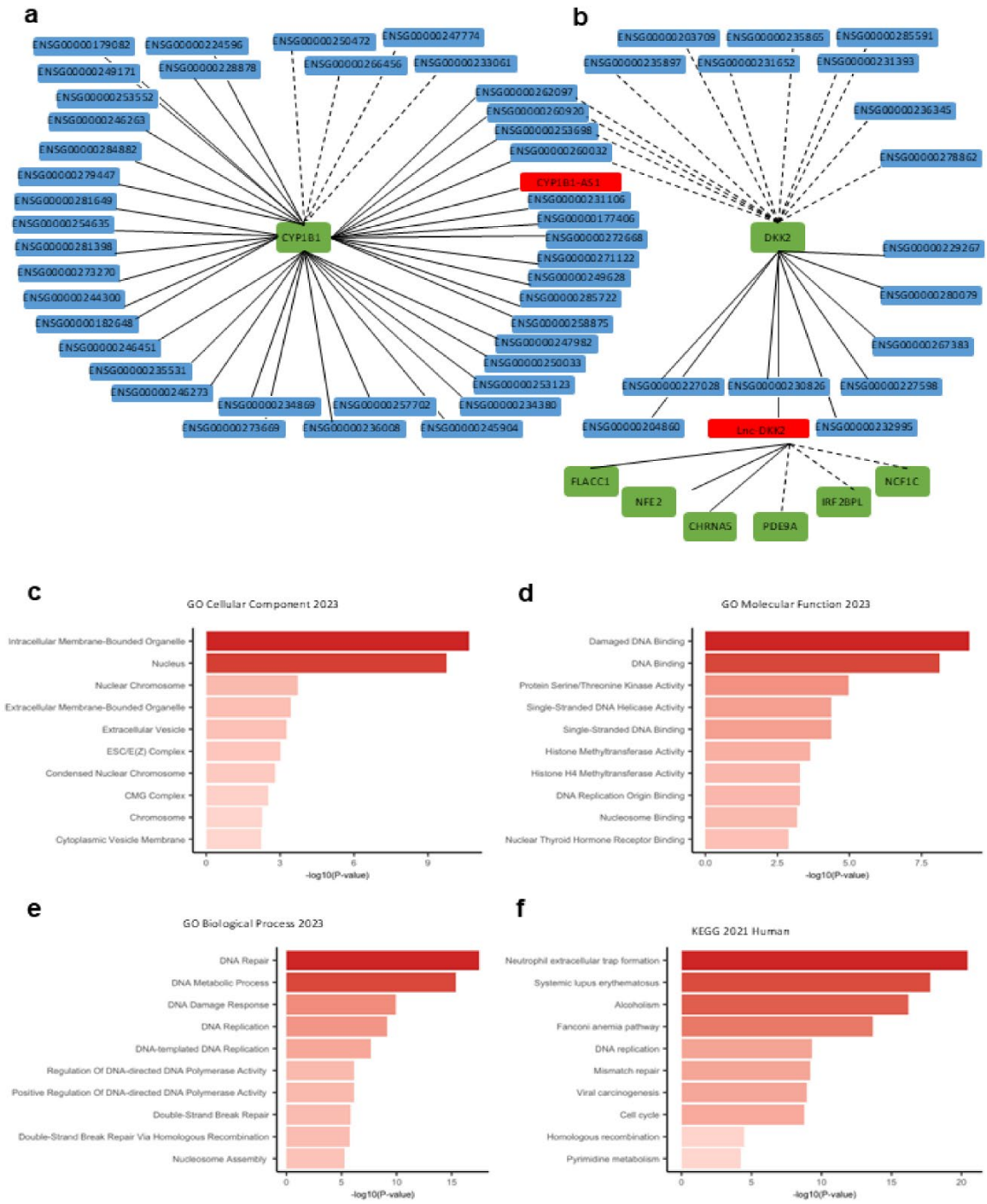

Fig S5. Coding non-coding expression network of CYP1B1-AS1 and lnc-DKK2. Partial map of coding-non-coding (CNC) co-expression network analysis of (a) CYP1B1 and CYP1B1-AS1; (b) lnc-DKK2 and DKK2 with other lncRNAs. Solid lines indicate positive correlations, dotted lines indicate negative correlations (Pearson correlation, -0.9 to 0.9). (c-e) Functional enrichment

analysis of correlated lncRNAs according to (c) GO cellular component, (d) GO molecular function, (e) GO biological process, and (e) KEGG pathways. The top 10 enriched terms ($P < 0.05$) are shown.

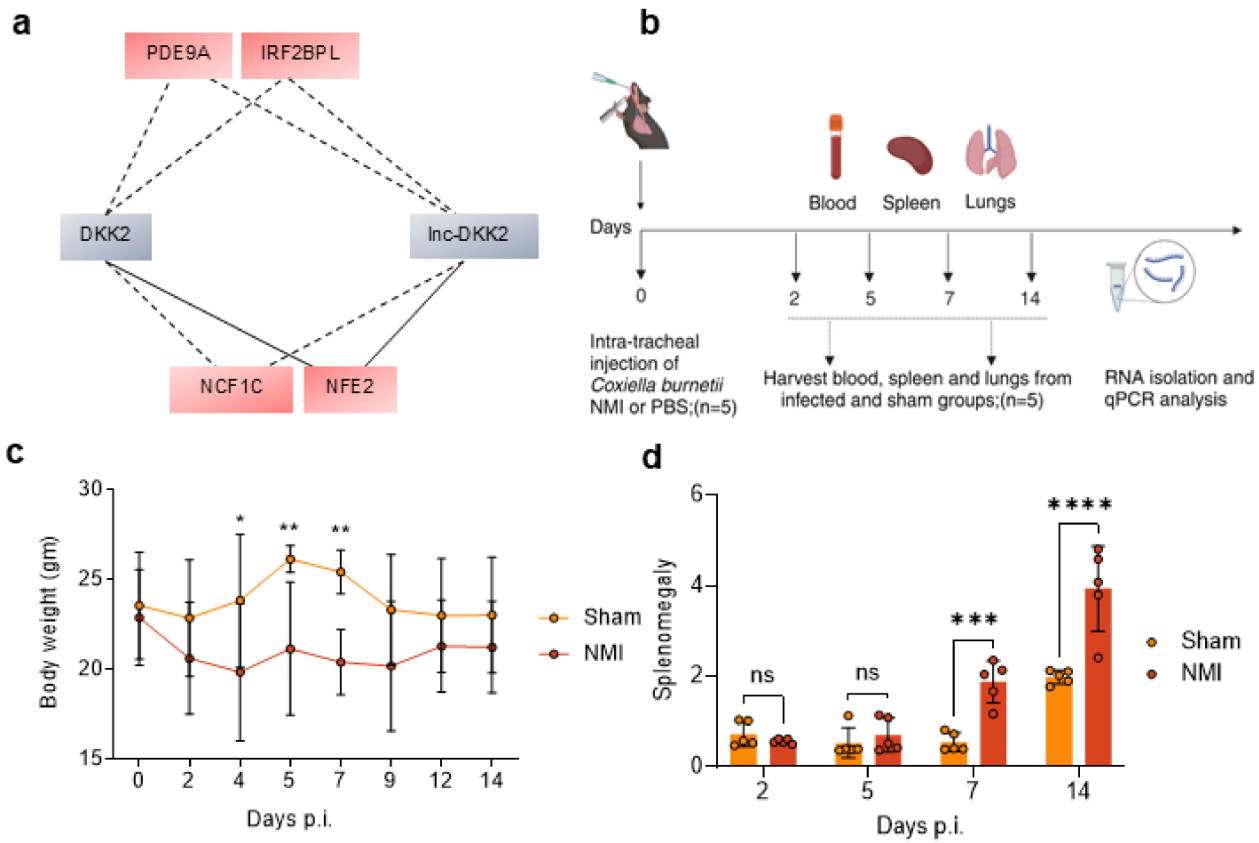

Fig S6. Intra-tracheal infection of C57BL/6 mice with Cb Nine Mile I (NMI) strain. (a) Partial map of coding-noncoding (CNC) co-expression network analysis of lnc-DKK2 and DKK2. The solid line between nodes represents a positive correlation, and dotted lines represents a negative correlation. Pearson correlation coefficient threshold: -0.9 to 0.9. (b) Infection timeline and workflow. C57BL/6 mice (n=5) were infected intra-tracheally with PBS (sham) or NMI at a dosage of 1×10^7 genome equivalents (GE)/ml. (c) Body weight change at 0 to 14 days p.i. (d) Spleen weight (% of body weight) comparing sham and NMI groups at 2, 5, 7, and 14 days p.i. Data are shown as mean ± SD, n=5. Statistical significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$; ****, $P < 0.0001$; ns, $P \geq 0.05$; one-way ANOVA.

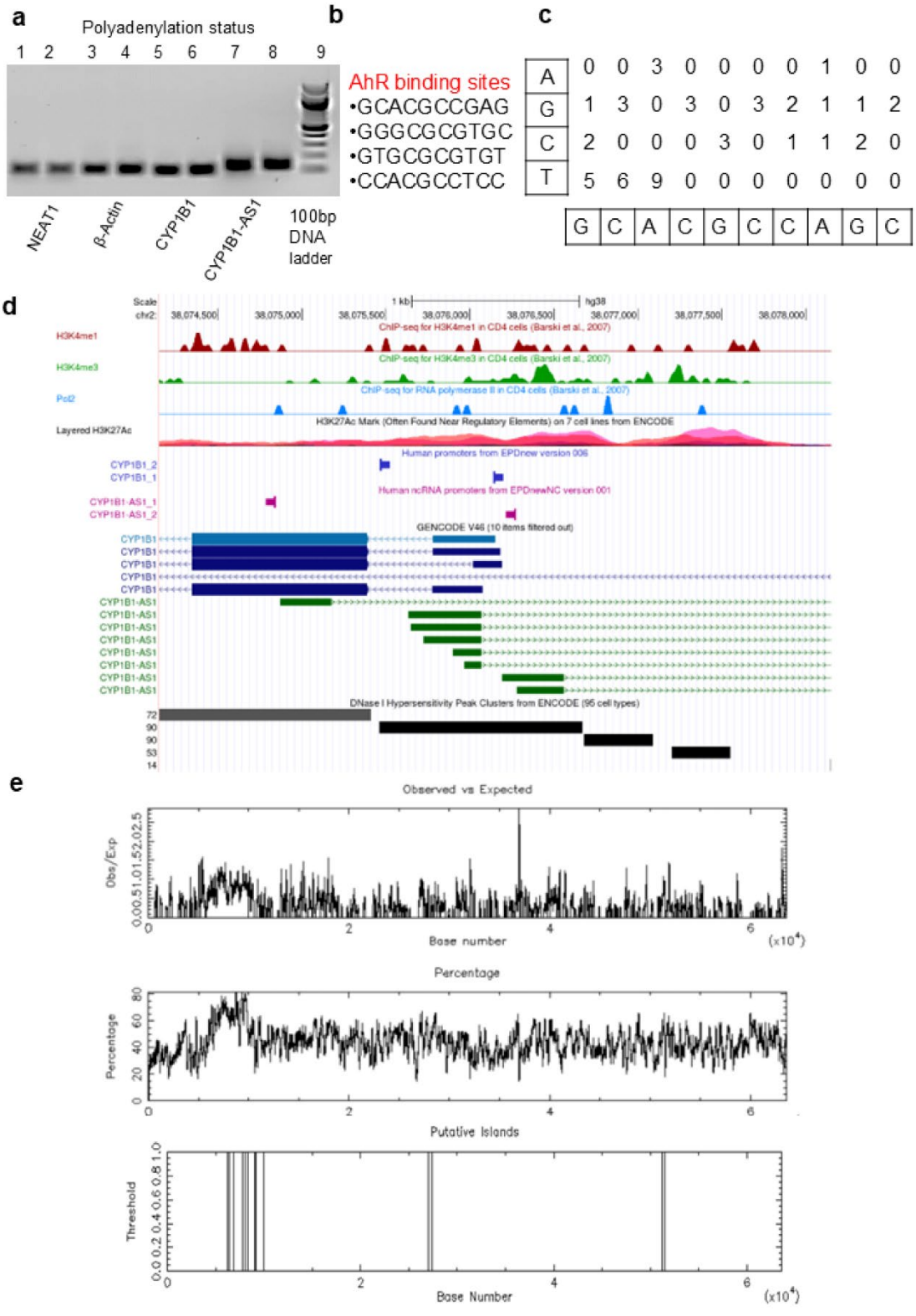

Fig S7. Regulatory regions prediction for CYP1B1-AS1 and CYP1B1. (a) Agarose gel electrophoresis image showing the poly-adenylation status of following samples (from left to

right); Lane 1&2: NEAT1; Lane 3&4: β-Actin; Lane 5&6: CYP1B1; Lane 7&8: CYP1B1-AS1; Lane 9: 100 bp DNA ladder (NEB; #N3231S). NEAT1 and β-Actin serves as poly-adenylated lncRNA and mRNA control, respectively. (b) Transcription factor binding site (TFBS) motif for AHR located upstream of the transcription start site (+1) of CYP1B1-AS1. (c) Position frequency matrix showing the expected occurrence of matches (random expectations) in a sequence of the same length as the query, based on the dissimilarity index. (d) TFBS regions predicted using UCSC and Expasy eukaryotic promoter databases ($P < 0.01$). ChIP-seq signals upstream of (+1) CYP1B1-AS1 indicate regions enriched for H3K27Ac, H3K4Me1, H3K4Me3, and DNase I hypersensitivity (HS) in human cell lines, suggesting active regulatory regions overlapping with AHR TFBS motifs. (e) Methylation prediction of the CYP1B1-AS1 regulatory region by EMBOSS CpGplot.

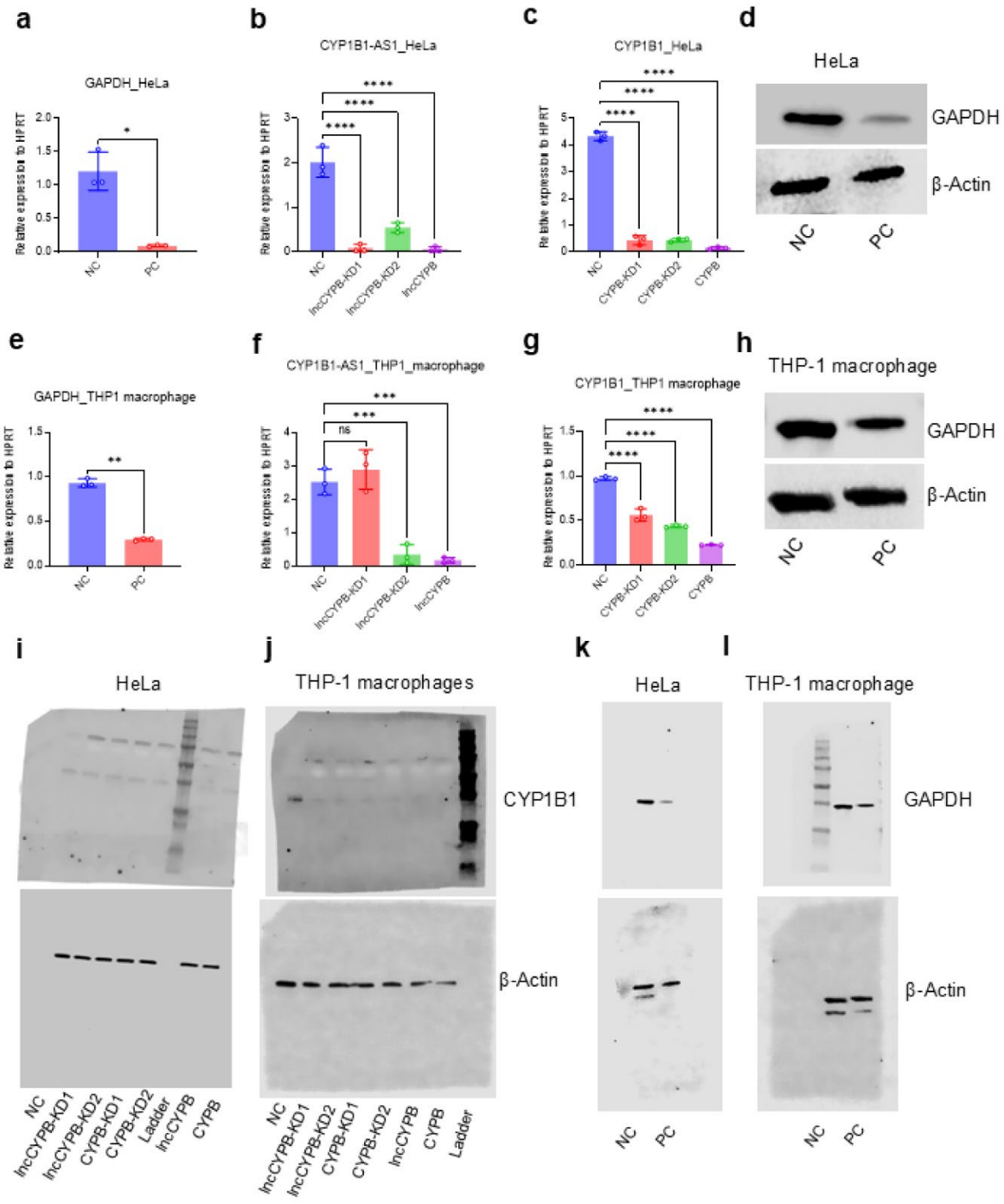

Fig S8. siRNA mediated knockdown efficiency in HeLa and THP-1 macrophages. Knockdown efficiency of different siRNA combinations targeting HeLa and THP-1 cells was assessed via qPCR. (a) Silencer™ Select GAPDH Positive Control siRNA was used to knock

down GAPDH in HeLa cells as a positive control (PC), while Silencer™ Select Negative Control No. 1 siRNA was used as a negative control (NC). (b-c) Knockdown efficiency of siRNAs targeting (b) CYP1B1-AS1 and (c) CYP1B1 was assessed via qPCR in HeLa cells. (d) Immunoblot analysis of GAPDH expression after knockdown in HeLa cells. (e) GAPDH knockdown efficiency in THP-1 macrophages using Silencer™ Select GAPDH Positive Control siRNA. (f-g) Knockdown efficiency of siRNAs targeting (f) CYP1B1-AS1 and (g) CYP1B1 was assessed via qPCR in THP-1 macrophages. (h) Immunoblot analysis of GAPDH expression in THP-1 macrophages after knockdown. Representative full immunoblots showing CYP1B1 expression after knockdown in (i) HeLa cells and (j) THP-1 macrophages, and GAPDH expression in (k) HeLa cells and (l) THP-1 macrophages. β-Actin serves as the loading control and Precision Plus Protein™ All Blue Prestained Protein Standards as protein ladder. For (a-c) and (e-g), (n=3), and error bars indicate mean ± SD. Statistical significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$; ****, $P < 0.0001$; ns, not significant, $P \geq 0.05$; one-way ANOVA.

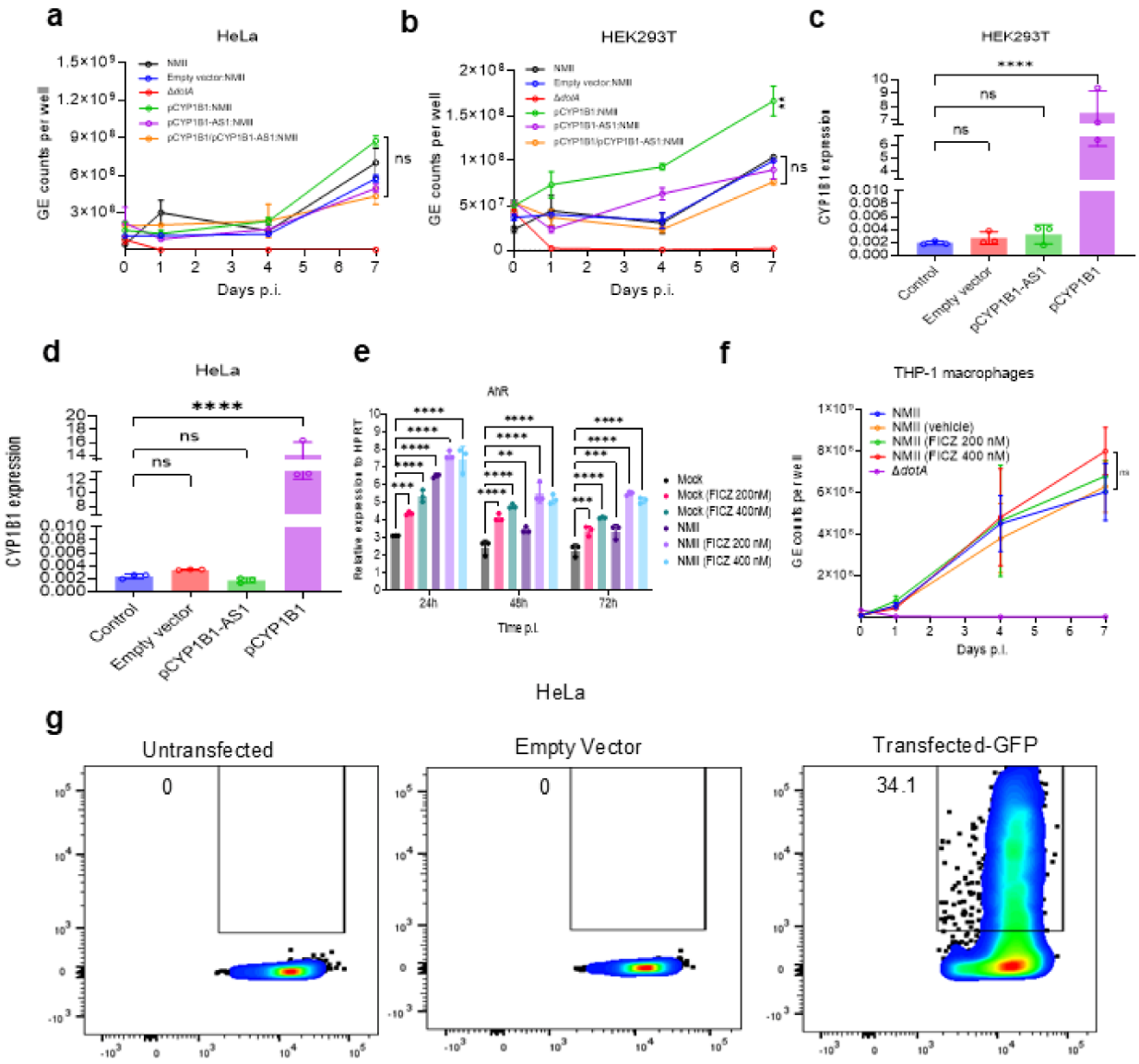

Fig S9. Over-expression study of CYP1B1-AS1 and CYP1B1. CYP1B1-AS1 and CYP1B1 were individually cloned into the pCDNA vector, generating constructs pCYP1B1-AS1 and pCYP1B1, respectively. The effect of over-expression on the growth curve of Cb was assessed by quantifying GE in host cells at indicated time points using qPCR. GE counts in overexpressing cell lines infected with Cb were designated as pCYP1B1-AS1: NMII, pCYP1B1: NMII and dual expression vectors as pCYP1B1-AS1/PCYP1B1: NMII. Growth curve analysis was performed in

(a) HeLa, and (b) HEK293T cells. Expression of CYP1B1 in cells over-expressing pCYP1B1-AS1 and pCYP1B1 was analyzed via qPCR in (c) HEK293T, and (d) HeLa cells. Gene expression was normalized to HPRT and compared to controls (mock and empty vector). (e) The impact of AHR overexpression on Cb replication was evaluated by treating THP-1 macrophages with FICZ (AHR agonist) at 200 nM and 400 nM concentrations, followed by infection at MOI of 50. Genome counts of Cb were obtained at indicated time points via qPCR. Cell lines infected with CbA (Δ*dotA*) served as the experimental control. "Vehicle" refers to cells treated with 0.1% DMSO. (f) qPCR analysis of AHR expression in cells treated with FICZ at the indicated concentrations and infected with Cb. RNA was harvested at specified time points for qPCR, with gene expression normalized to HPRT and compared to Mock and Mock treated with FICZ. (g) Transfection efficiency of pCDNA-eGFP, used as a representative construct in overexpression experiments, was assessed after 24 h in HeLa cells. For all data, n=3. Error bars indicate mean ± SD. Statistical significance was determined using one-way ANOVA for panels (a-e) and two-way ANOVA for panel (f): *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$; ****, $P < 0.0001$; ns, $P \geq 0.05$.

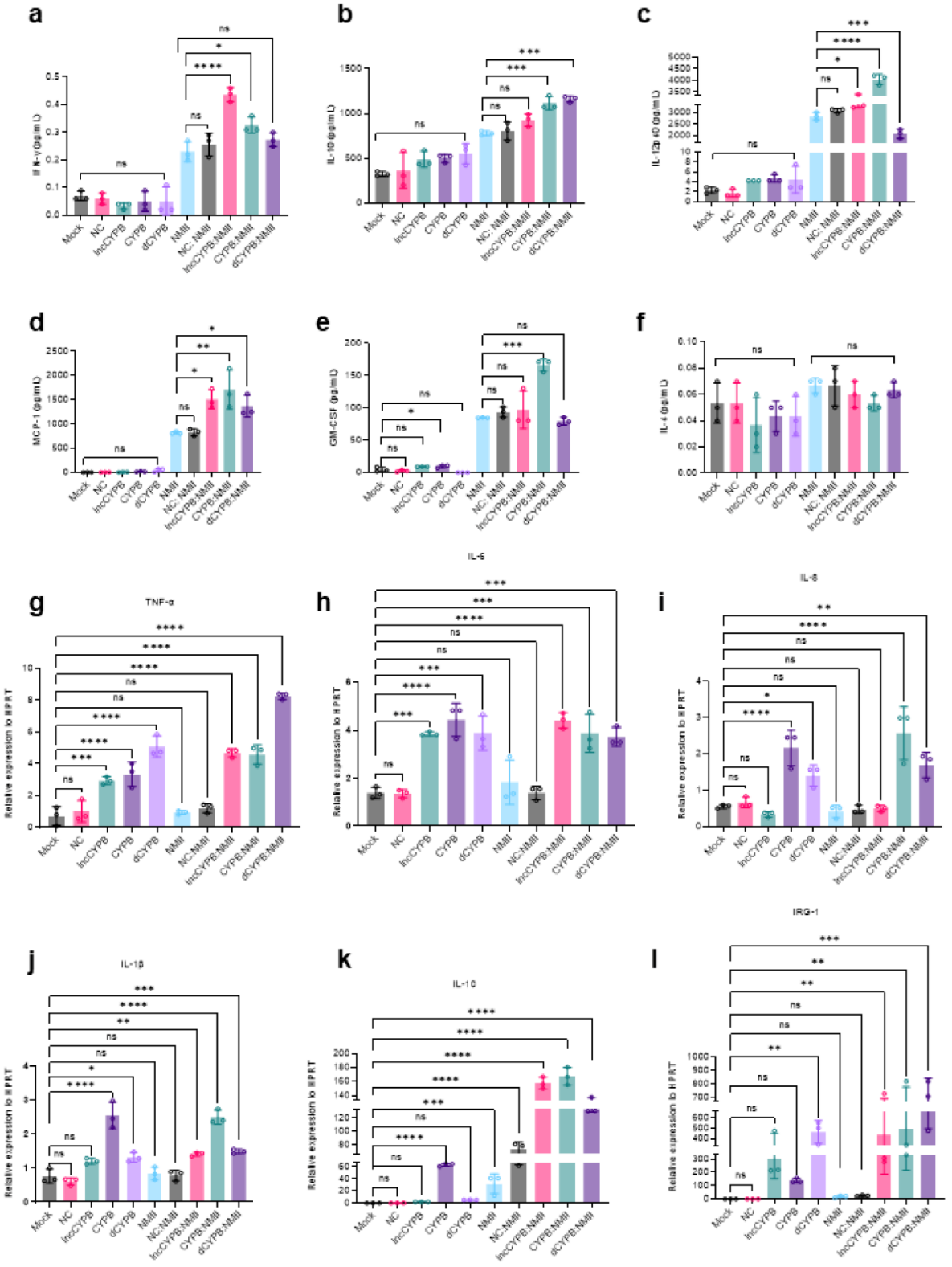
a
b
c
d
e
f
IL-6
g
TNF-α
h
i
IL-8
j
IL-1β
k
IL-10
l
IRG-1
Mock
NC
lncCYPB
CYPB
dCYPB
NMII
NC:NMII
lncCYPB:NMII
CYPB:NMII
dCYPB:NMII
Relative expression to HPRT

Fig S10. CYP1B1-AS1 and CYP1B1 regulate inflammation during Cb infection. Supernatants were collected 24 h p.i. from Mock, knockdowns of CYP1B1 (CYPB), CYP1B1-AS1 (lncCYPB), dual knockdowns of both genes (dCYPB), negative control (NC) cells, as well as their infected counterparts, for ELISA quantification of (a) IFN-γ, (b) IL-10, (c) IL-12p40, (d) MCP-1, (e) GM-CSF, (f) IL-4. Expression levels of (g) TNF-α, (h) IL-6, (i) IL-8, (j) IL-1β, (k) IL-10 and (l) IRG-1 were assessed using qPCR on RNA harvested from the infected and control cells at 6 h p.i. Gene expression was normalized to HPRT and compared to Mock. For all data, n=3, and error bars indicate mean ± SD. Statistical significance: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.0001$; ****, $P < 0.0001$; ns, not significant, $P \geq 0.05$; one-way ANOVA.

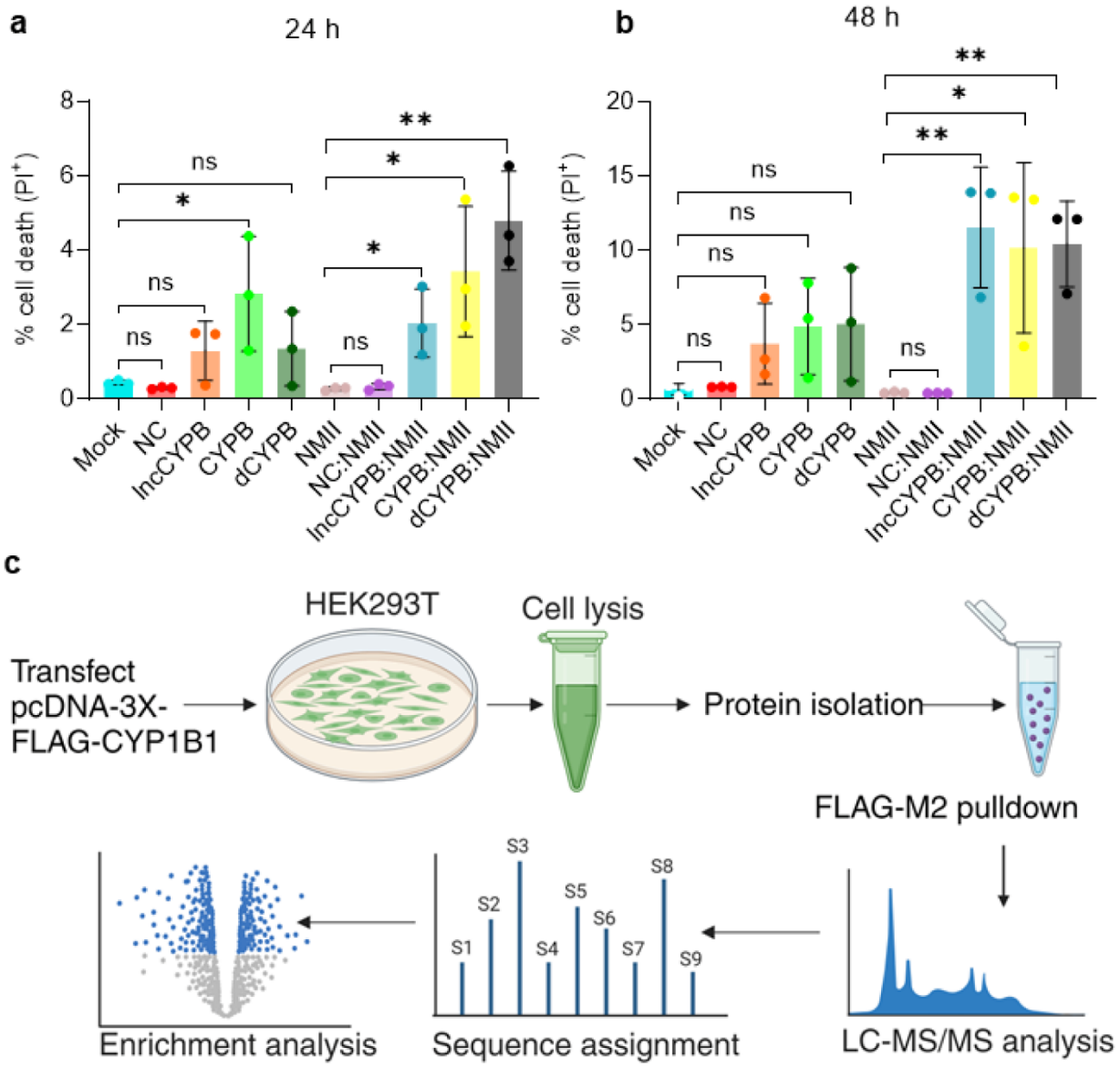

Fig S11. CYP1B1-AS1 and CYP1B1 regulate mitochondrial ROS and inhibit apoptosis. (a) ≈1×10^6 cells were stained with Propidium Iodide (PI) and FITC-Annexin-V at 24 h and 48 h p.i. according to manufacturer's instructions. Fluorescence was detected using flow cytometry to analyze the population of necrotic (PI^+; Q3) cells. Quantification of the necrotic population is shown as the percentage of cell death in lncCYPB, CYPB and dCYPB cells, as well as their Cb-infected counterparts at (a) 24 h p.i.; (b) 48 h p.i. The percentage of cell death was compared to

Mock and NMII as controls. Data represent three biological replicates from three independent experiments. Error bars indicate mean ± SD. Statistical significance: *, $P < 0.05$; **, $P < 0.01$; ns, not significant, $P \geq 0.05$; one-way ANOVA. (c) Schematic representation of the LC-MS/MS workflow for FLAG-CYP1B1 overexpression in HEK293T cells. 3XFLAG-CYP1B1 (pCDNA-CYP1B1) was transfected into HEK293T cells, and cells were harvested 48 h post-transfection. Protein was isolated, subjected to FLAG antibody-based immunoprecipitation, and the associated proteins were analyzed by LC-MS/MS.

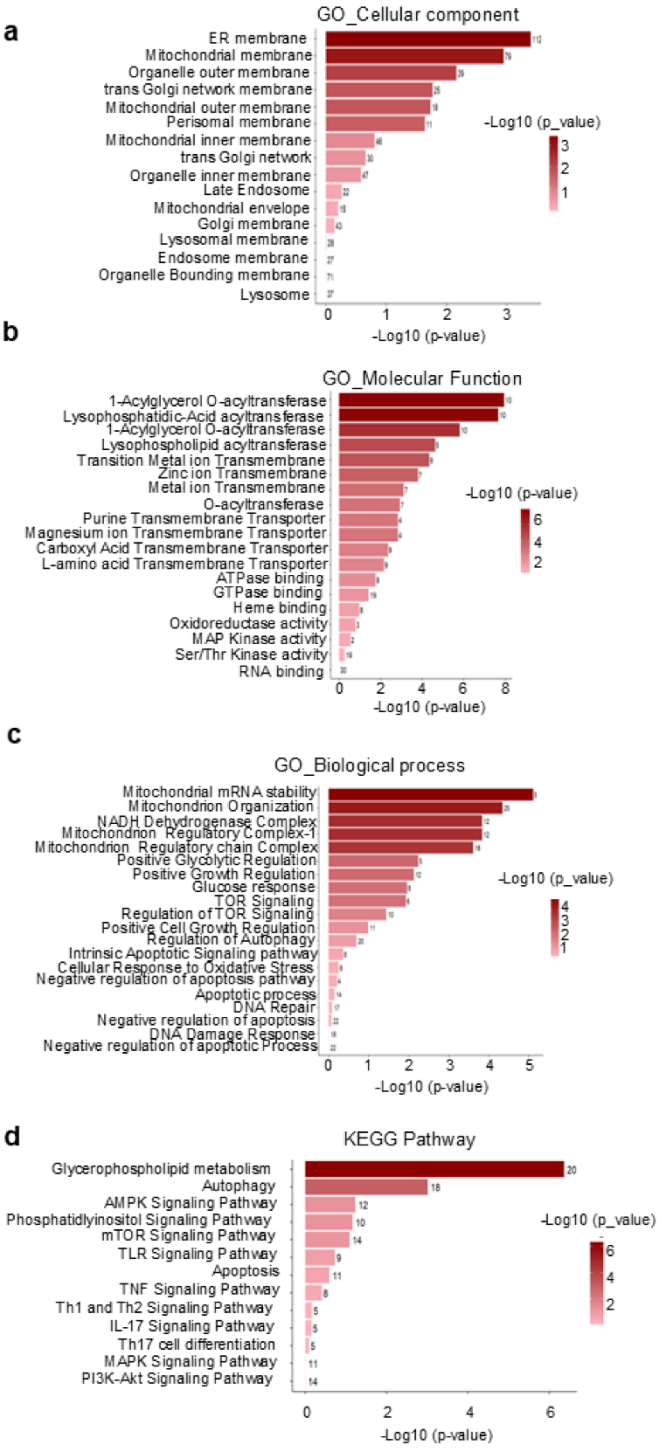

Fig S12. Functional enrichment analysis of 3XFLAG-CYP1B1 IP proteome. GO enrichment analysis was conducted on identified proteins based on (a) Cellular Component, (b) Molecular Function, and (c) Biological Process. (d) KEGG pathway analysis.

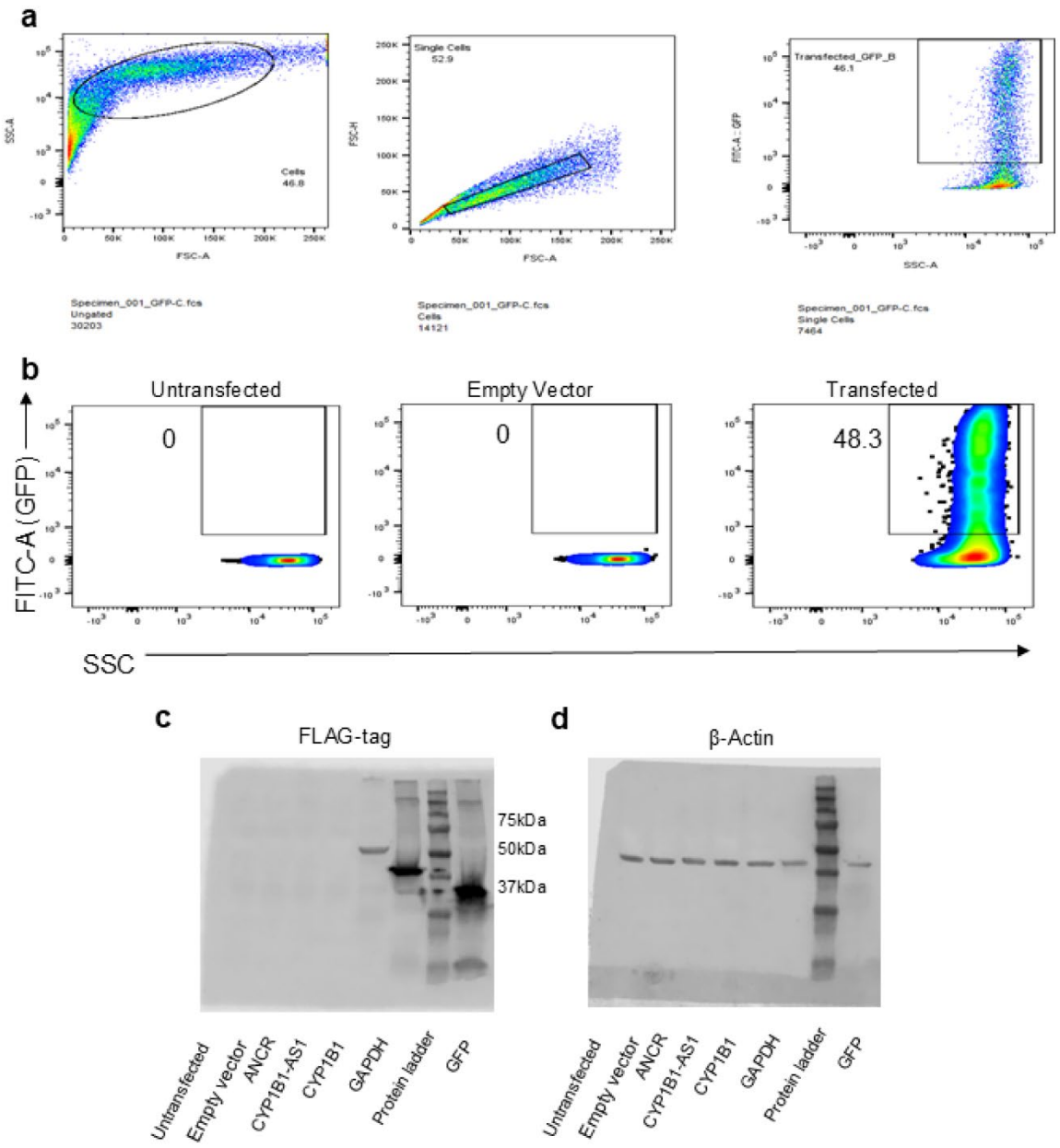

Fig S13. Flow cytometry and immunoblot analysis for coding potential assessment of CYP1B1-AS1. (a) Flow cytometry gating strategy used to assess transfection efficiency of pCDNA constructs for coding potential analysis. (b) Transfection efficiency of pCDNA-eGFP in

HEK293T cells 24 h post-transfection for coding potential assessment. (c) Representative full blot showing FLAG tag signal to assess the coding potential of CYP1B1-AS1 in transfected cells. (d) Loading control used in transfected cells for the coding potential assay. The protein ladder used was Precision Plus Protein™ All Blue Prestained Protein Standards.

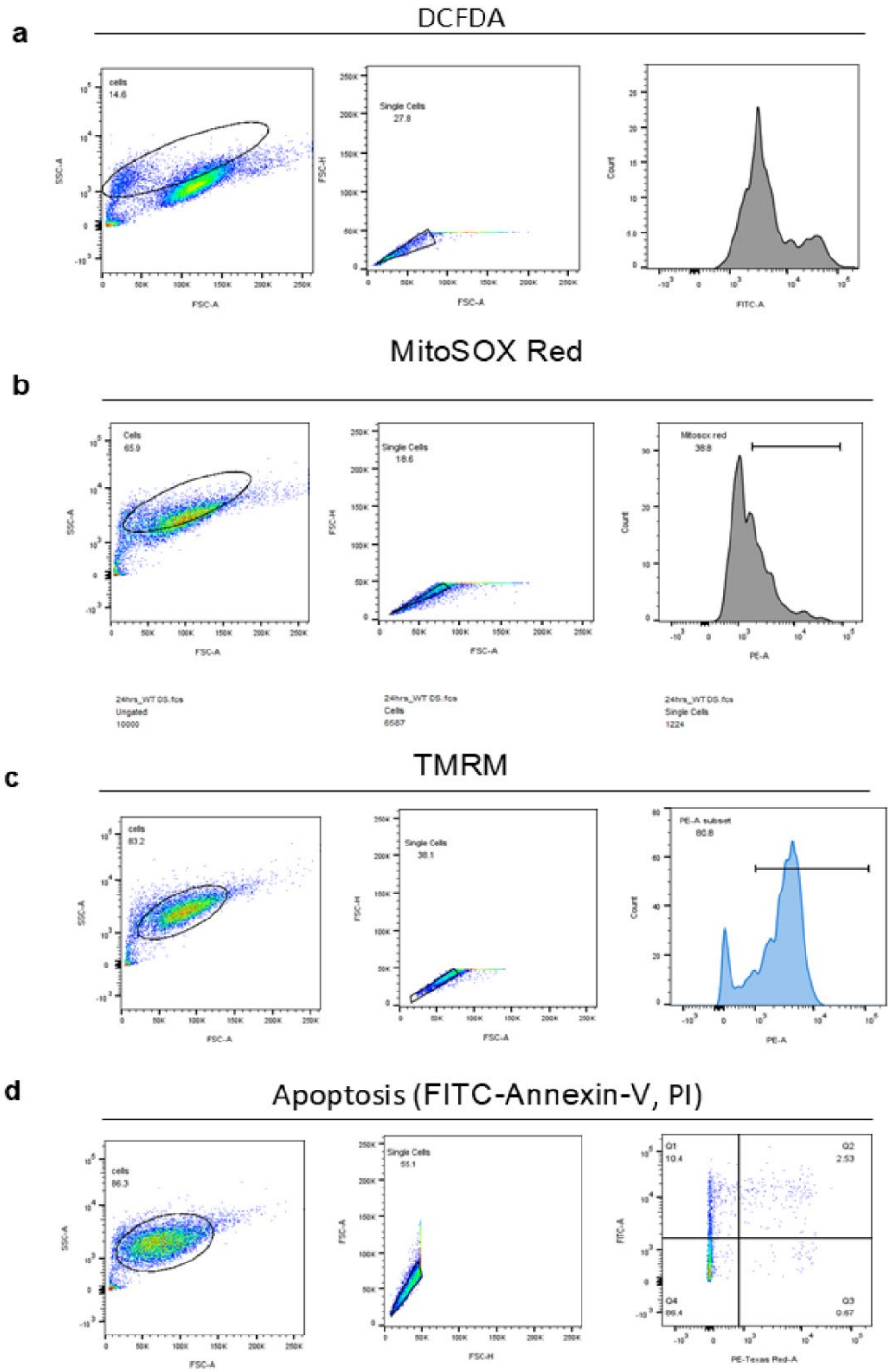

Fig S14. Flow cytometry gating strategies for (a) Total reactive oxygen species (ROS) assay using DCFDA dye, (b) Mitochondrial ROS assessment using MitoSOX™ Red dye, (c)

Mitochondrial membrane potential assessment using MitoProbe™ TMRM dye, and (d) Apoptosis measurement using FITC-Annexin-V and PI.

Table S1. Bacterial strains and cell lines used in the study

Bacterial Strain	**Strain code name**	**Alternate name**	**Source**	**Gram-negative/ Gram-positive**	**Pathogen/ Non-Pathogen**
Coxiella burnetii Nine Mile Phase II	Cb	NMII	James Samuel Laboratory, TAMHSC	Gram-negative	Pathogen
Coxiella burnetii Nine Mile Phase II *dotA*::Tn	CbA	Δ*dotA*	James Samuel Laboratory, TAMHSC	Gram-negative	Pathogen; attenuated
Coxiella burnetii Nine Mile Phase II *dotB*::Tn	CbB	Δ*dotB*	James Samuel Laboratory, TAMHSC	Gram-negative	Pathogen; attenuated
Escherichia coli DH5α	Ec5	DH5α	MCLAB	Gram-negative	Non-Pathogen
Enterohemorrhagic Escherichia coli O157:H7	EcT	EHEC	ATCC43895	Gram-negative	Pathogen
Enterohemorrhagic Escherichia coli O157:H7 Δ*stx* (nontoxicogenic)	EcN	EHECΔ*stx*	ATCC700728	Gram-negative	Pathogen; attenuated
Bacillus subtilis P31K6	Bs	*B.subtilis*	ATCC6051	Gram-positive	Non-Pathogen
Francisella novicida U112	Fn	*F.novicida*	BEI Resources, #NR-13	Gram-negative	Pathogen
Pseudomonas aeruginosa PAO1	Pa	*P. aeruginosa*	ATCC47085	Gram-negative	Pathogen
Staphylococcus aureus JE2	Sa	*S. aureus*	BEI Resources, #NR-46543	Gram-positive	Pathogen
Salmonella enterica subsp. Typhimurium SL1344	STm	*S.* Typhimurium	ATCC53648	Gram-negative	Pathogen
Rhizobium radiobacter	Rr	*R. radiobacter*	James Samuel	Gram-negative	Opportunistic pathogen

			Laboratory, TAMHSC		
Micrococcus luteus	Ml	*M.luteus*	James Samuel Laboratory, TAMHSC	Gram-positive	Opportunistic pathogen
Listeria innocua	Li	*L.innocua*	ATCC33090	Gram-positive	Non-Pathogen
Enterococcus faecalis	Ef	*E.faecalis*	ATCC19433	Gram-positive	Opportunistic pathogen
Brucella melitensis Δ*vjbR*	Bmv	*B.melitensis*	Paul deFigueiredo laboratory, TAMHSC	Gram-negative	Pathogen; attenuated
Legionella pneumophila Philadelphia (Phil)-1	Lp	*L.pneumophila*	Jeffrey Cirillo laboratory, TAMHSC	Gram-negative	Pathogen
Mammalian cell Lines	**Source**	**Identifier**			
THP-1	ATCC	TIB-202			
HEK293T	ATCC	CRL-3216			
HeLa	ATCC	CCL-2			

Table S2. Plasmids used in the study

Plasmid name	Backbone	Genotype Information	Resistance	Source	Identifier
Coding potential and FLAG-IP constructs					
pcDNA3.1/Zeo(+)		Untagged, CMV and T7 promoter, Mammalian expression vector, *f1 ori*, high copy	Amp^r, Zeo^r	Invitrogen™	V86020
pcDNA-FLAG	pcDNA3.1/ Zeo (+)	N-term 3XFLAG tag downstream of CMV and T7 promoter cloned at NheI/EcoRI sites, Mammalian expression vector, *f1 ori*	Amp^r, Zeo^r	This study	N/A
pcDNA-ANCR	pcDNA-FLAG	ANCR cloned downstream of N-term 3XFLAG tag sequence, Mammalian expression vector, *f1 ori*	Amp^r, Zeo^r	This study	N/A
pcDNA-eGFP	pcDNA-FLAG	eGFP cloned downstream of N-term 3XFLAG tag sequence, Mammalian expression vector, *f1 ori*	Amp^r, Zeo^r	This study	N/A
pcDNA-GAPDH	pcDNA-FLAG	GAPDH cloned downstream of N-term 3XFLAG tag sequence, Mammalian expression vector, *f1 ori*	Amp^r, Zeo^r	This study	N/A
pcDNA-CYP1B1	pcDNA-FLAG	CYP1B1 cloned downstream of N-term 3XFLAG tag sequence, Mammalian expression vector, *f1 ori*	Amp^r, Zeo^r	This study	N/A
pcDNA-CYP1B1-AS1	pcDNA-FLAG	CYP1B1-AS1 cloned downstream of N-term 3XFLAG tag sequence, Mammalian expression vector, *f1 ori*	Amp^r, Zeo^r	This study	N/A
pCDNA-cFLAG	pcDNA3.1/ Zeo (+)	C-term 3XFLAG tag downstream of CMV and T7 promoter cloned at NheI/EcoRI sites, Mammalian expression vector, *f1 ori*	Amp^r, Zeo^r	This study	N/A
pCDNA-CBU0937	pCDNA-cFLAG	CBU_0937 cloned upnstream of C-term 3XFLAG tag sequence,	Amp^r, Zeo^r	This study	N/A

		Mammalian expression vector, *f1 ori*			
pcDNA-cGFP	pCDNA-cFLAG	eGFP cloned upnstream of C-term 3XFLAG tag sequence, Mammalian expression vector, *f1 ori*	Amp^r, Zeo^r	This study	N/A
Promoter Dual Luciferase constructs					
pGL4Luc-Rluc	pGL4.10	Mammalian Expression bdirectional reporter vector, Luciferase, Renilla luciferase in Asp718/NheI sites, *ori*, high copy	Amp^r	Addgene	64034
pF-Luc	pGL4Luc-Rluc	Promoter region of ACTB (P_{ACTB}),750 bp upstream of transcriptional start site (+1) of ACTB cloned between BgIII and HindIII	Amp^r	This study	N/A
pR-Luc	pGL4Luc-Rluc	Promoter region of ACTB (P_{ACTB}) cloned between HindIII and BgIII	Amp^r	This study	N/A
pCYP1B1-FL-RL	pGL4Luc-Rluc	Promoter region of CYP1B1-AS1 ($P_{CYP1B1\text{-}AS1}$), 600bp upstream of (+1) site of CYP1B1-AS1 was cloned at NheI/HindIII site	Amp^r	This study	N/A
Overexpression constructs					
pCYP1B1-AS1	pcDNA3.1/ Zeo (+)	CYP1B1-AS1 cloned downstream of CMV and T7 promoter, Mammalian expression vector, *f1 ori*	Amp^r, Zeo^r	This study	N/A
pCYP1B1	pcDNA3.1/ Zeo (+)	CYP1B1 cloned downstream of CMV and T7 promoter, Mammalian expression vector, *f1 ori*	Amp^r, Zeo^r	This study	N/A

Amp^r- Ampicillin resistance; Zeo^r-Zeocin resistance;

Table S3. siRNA used in the study

Knockdown name	siRNA ID	Identifier	Manufacturer/Source	Target gene	Species
CYPB-KD1	s3805	4392421	Silencer™ Select Pre-Designed siRNA, ThermoFisher Scientific	CYP1B1	*Homo sapiens*
CYPB-KD2	s3806	4392421	Silencer™ Select Pre-Designed siRNA, ThermoFisher Scientific	CYP1B1	*Homo sapiens*
lncCYPB-KD1	n507309	4392421	Silencer™ Select Pre-Designed siRNA, ThermoFisher Scientific	CYP1B1-AS1	*Homo sapiens*
lncCYPB-KD2	n507310	4392421	Silencer™ Select Pre-Designed siRNA, ThermoFisher Scientific	CYP1B1-AS1	*Homo sapiens*
Negative control (NC)		4390844	Silencer™ Select Negative Control No. 1 siRNA, ThermoFisher Scientific	non-targeting control	*Homo sapiens*
GAPDH-KD (Positive control, PC)		4390850	Silencer™ Select GAPDH Positive Control siRNA, ThermoFisher Scientific	GAPDH	*Homo sapiens*
CYPB			s3805 and s3806 pairs used at indicated concentrations to achieve atleast 80% knockdown	CYP1B1	*Homo sapiens*
lncCYPB			n507309 and n507310 pairs used at indicated concentrations to achieve atleast 80% knockdown	CYP1B1-AS1	*Homo sapiens*
dCYPB			Knockdown of both CYP1B1 and CYP1B1-AS1 using s3805, s3806, n507309 and n507310	CYP1B1; CYP1B1-AS1	*Homo sapiens*

Table S4. Primers used in the study

Gene name	Sequence (5' to 3')	Species	Reference
qPCR primers			
com1-FWD	CGCGTTGTCTTCAAAGAACT	*Coxiella burnetii*	(1)
com1-REV	GCGTCGTGGAAAGCATAATA	*Coxiella burnetii*	(1)
com1 Taqman Probe	CGGCCAATCGCAATACGCTG	*Coxiella burnetii*	(1)
CYP1B1-AS1-RTFWD	AGACTCTGCTCTACCAGGGG	*Homo sapiens*	This study
CYP1B1-AS1-RTREV	TGGTCACCTTGGAATGGCTC	*Homo sapiens*	This study
CYP1B1-RTFWD	CCTCCTCTTCACCAGGTATCC	*Homo sapiens*	This study
CYP1B1-RTREV	CCAGGACATAGGGCAGGTTG	*Homo sapiens*	This study
lnc-DKK2-RTFWD	ACGAGAACAAACACACAACATAC	*Homo sapiens*	This study
lnc-DKK2-RTREV	GCTTTCTCTTGTGGGCATTTAG	*Homo sapiens*	This study
DKK2-RTFWD	GGACTCCTGAAGTAGACAGAGTA	*Homo sapiens*	This study
DKK2-RTREV	CTGCAGAGGTTTGGGAAGAA	*Homo sapiens*	This study
AHR-RTFWD	GCCGGTGCAGAAAACAGTAAA	*Homo sapiens*	This study
AHR-RTREV	AGCCAAACGGTCCAACTCTG	*Homo sapiens*	This study
HPRT-RTFWD	CCTGGCGTCGTGATTAGTGAT	*Homo sapiens*	This study
HPRT-RTREV	AGACGTTCAGTCCTGTCCATAA	*Homo sapiens*	This study
ACTB-RTFWD	GGATCAGCAAGCAGGAGTATG	*Homo sapiens*	This study
ACTB-RTREV	AGAAAGGGTGTAACGCAACTAA	*Homo sapiens*	This study
NEAT1-RTFWD	CTTCCTCCCTTTAACTTATCCATTC	*Homo sapiens*	This study
NEAT1-RTREV	CTCTTCCTCCACCATTACCAACAATAC	*Homo sapiens*	This study
TUG1-RTFWD	TAGCAGTTCCCCAATCCTTG	*Homo sapiens*	This study
TUG1-RTREV	CACAAATTCCCATCATTCCC	*Homo sapiens*	This study
CYP1B1-AS1-mRTFWD	ACTGCTGGCTCTGTTTCATTA	*Mus Musculus*	This study
CYP1B1-AS1-mRTREV	CTCTCTTCTCCTCATGCTTTCTC	*Mus Musculus*	This study
CYP1B1-mRTFWD	CTAGCCAGCAGTGTGATGATATT	*Mus Musculus*	This study
CYP1B1-mRTREV	CGAGGATGGAGATGAAGAGAAAC	*Mus Musculus*	This study
ACTB-mRTFWD	GAGGTATCCTGACCCTGAAGTA	*Mus Musculus*	This study
ACTB-mRTREV	CACACGCAGCTCATTGTAGA	*Mus Musculus*	This study
Cytokine primers for qPCR			
IL1B-RTFWD	AGCCATGGCAGAAGTACCTG	*Homo sapiens*	This study
IL1B-RTREV	CCTGGAAGGAGCACTTCATCT	*Homo sapiens*	This study
TNFA-RTFWD	AGCCCATGTTGTAGCAAACC	*Homo sapiens*	This study

TNFA-RTREV	ATGAGGTACAGGCCCTCTGA	*Homo sapiens*	This study
IL8-RTFWD	CACTGCGCCAACACAGAAAT	*Homo sapiens*	This study
IL8-RTREV	TCTCAGCCCTCTTCAAAAACTTC	*Homo sapiens*	This study
IL10-RTFWD	CGAGATGCCTTCAGCAGAGT	*Homo sapiens*	This study
IL10-RTREV	GGCAACCCAGGTAACCCTTA	*Homo sapiens*	This study
IL-6-RTFWD	CTTCGGTCCAGTTGCCTTCT	*Homo sapiens*	This study
IL-6-RTREV	GGGCGGCTACATCTTTGGAA	*Homo sapiens*	This study
IL-10-RTFWD	TCAAGGCGCATGTGAACTCC	*Homo sapiens*	This study
IL-10-RTREV	GATGTCAAACTCACTCATGGCT	*Homo sapiens*	This study
IRG1-RTFWD	TGTTTCACATAGCCAGCCAAT	*Homo sapiens*	This study
IRG1-RTREV	CCGTTCACAAAAGCAGCATA	*Homo sapiens*	This study
Cloning primers for promoter luciferase assay			
promCYP1B1-AS1-FWD	CTAGCTAGCACGGAACTCGGGGTCGTC	*Homo sapiens*	This study
promCYP1B1-AS1-REV	CCCAAGCTTAGCGGGTGGCGTGGTAG	*Homo sapiens*	This study
PromACTB-Rluc-FWD	CCCAAGCTTGCGGCCTCCAGATGGTCT	*Homo sapiens*	This study
PromACTB-Rluc-REV	GAAGATCTGGTGGCGCGTCGCGCC	*Homo sapiens*	This study
PromACTB-Fluc-FWD	GAAGATCTGCGGCCTCCAGATGGTCT	*Homo sapiens*	This study
PromACTB-Fluc-REV	CCCAAGCTTGGTGGCGCGTCGCGCC	*Homo sapiens*	This study
Restriction cloning primers			
N-termFLAG-AA-Fw	CTAGCTAGCATGGACTACAAGGACCACG	*Escherichia coli*	This study
N-termFLAG-AA-Rw	CCGGAATTCCGCTGCCTTGTCGTCGT	*Escherichia coli*	This study
Gibson Cloning primers for coding potential assessment constructs and FLAG-IP			
pCDNA3.1-FLAG-DANCR FWD	CGTCTTAAAAAAAAAAAAAAGCGCCCTGTAGCGGCGCATT	*Escherichia coli*	This study
pCDNA3.1-FLAG-DANCR REV	CTCCAGCTGACAAAGAGGCCCGCTGCCTTGTCGTCGTCGT	*Escherichia coli*	This study
DANCR-204 FWD	ACGACGACGACAAGGCAGCGGGCCTCTTTGTCAGCTGGAG	*Homo sapiens*	This study
DANCR-204 REV	AATGCGCCGCTACAGGGCGCTTTTTTTTTTTTTTAAGACGGTGTC	*Homo sapiens*	This study

pCDNA3.1-FLAG-GAPDH FWD	ACATGGCCTCCAAGGAGTAAAGCTGGGGCTCTAGGGGGTA	*Escherichia coli*	This study
pCDNA3.1-FLAG-GAPDH REV	CCGACCTTCACCTTCCCCATCGCTGCCTTGTCGTCGTCGT	*Escherichia coli*	This study
GAPDH FWD	ACGACGACGACAAGGCAGCGATGGGGAAGGTGAAGGTCGG	*Homo sapiens*	This study
GAPDH REV	TACCCCCTAGAGCCCCAGCTTTACTCCTTGGAGGCCATGTG	*Homo sapiens*	This study
pCDNA3.1-FLAG-CYP1B1 FWD	CCAAGGAAACTTGCCAATAAGCGCCCTGTAGCGGCGCATT	*Escherichia coli*	This study
pCDNA3.1-FLAG-CYP1B1 REV	GGGCTGAGGCTGGTGCCCATCGCTGCCTTGTCGTCGTCGT	*Escherichia coli*	This study
CYP1B1 FWD	ACGACGACGACAAGGCAGCGATGGGCACCAGCCTCAGCCC	*Homo sapiens*	This study
CYP1B1 REV	AATGCGCCGCTACAGGGCGCTTATTGGCAAGTTTCCTTGGCTTGTAAA	*Homo sapiens*	This study
pCDNA3.1-FLAGCYP1B1-AS1-FWD	AAGTGCAGTTTAATAAAAAAGCGCCCTGTAGCGGCGCATT	*Escherichia coli*	This study
pCDNA3.1-FLAGCYP1B1-AS1-REV	TGTGTTAGCAACCATAGTTGCGCTGCCTTGTCGTCGTCGT	*Escherichia coli*	This study
CYP1B1-AS1-FWD	ACGACGACGACAAGGCAGCGCAACTATGGTTGCTAACACA	*Homo sapiens*	This study
CYP1B1-AS1-REV	AATGCGCCGCTACAGGGCGCTTTTTTATTAAACTGCACTT	*Homo sapiens*	This study
pCDNA3.1-FLAG-eGFP FWD	TGGACGAGCTGTACAAGTAACACGCGCCCTGTAGCGGCGC	*Escherichia coli*	This study
pCDNA3.1-FLAG-eGFP REV	TCCTCGCCCTTGCTCACCATCGCTGCCTTGTCGTCGTCGTCC	*Escherichia coli*	This study
eGFP FWD	ACGACGACGACAAGGCAGCGATGGTGAGCAAGGGCGAGGA	*Escherichia coli*	This study
eGFP REV	GCGCCGCTACAGGGCGCGTGTTACTTGTACAGCTCGTCCATGCC	*Escherichia coli*	This study
pcdna3.1 (+)-C-term 3XFLAG-GFP FWD	GCATGGACGAGCTGTACAAGATGGACTACAAGGACCACGA	*Escherichia coli*	This study
pcdna3.1 (+)-C-term 3XFLAG-GFP REV	TCCTCGCCCTTGCTCACCATGGGTCTCCCTATAGTGAGTCG	*Escherichia coli*	This study
cGFP FWD	GACTCACTATAGGGAGACCCATGGTGAGCAAGGGCGAGGA	*Escherichia coli*	This study

cGFP REV	TCGTGGTCCTTGTAGTCCATCTTGTACAGCTCGTCCATGCC	*Escherichia coli*	This study
pcdna3.1 (+)-C-term 3XFLAG FWD	CACAGTTCGATCTTTATTTTATGGACTACAAGGACCACGA	*Escherichia coli*	This study
pcdna3.1 (+)-C-term 3XFLAG REV	ACAACCCTGTCACAAGTCATGGGTCTCCCTATAGTGAGTCG	*Escherichia coli*	This study
CBU0937 FWD	GACTCACTATAGGGAGACCCATGACTTGTGACAGGGTTGT	*Coxiella burnetii*	This study
CBU0937 REV	TCGTGGTCCTTGTAGTCCATAAAATAAAGATCGAACTGTGC	*Coxiella burnetii*	This study
Gibson Cloning primers for overexpression construct			
pCDNA3.1+CYP1B1 FWD	GGAGACCCAAGCTGGCTAGCATGGGCACCAGCCTCAGCCC	*Homo sapiens*	This study
pCDNA3.1+CYP1B1 REV	GGGCTGAGGCTGGTGCCCATGCTAGCCAGCTTGGGTCTCCC	*Homo sapiens*	This study
pCDNA3.1+CYP1B1-AS1 FWD	GGAGACCCAAGCTGGCTAGCCAACTATGGTTGCTAACACAAGAATC	*Homo sapiens*	This study
pCDNA3.1+CYP1B1-AS1 REV	TGTGTTAGCAACCATAGTTGGCTAGCCAGCTTGGGTCTCC	*Homo sapiens*	This study

FWD- Forward primer; REV- Reverse primer

Table S5. Reagents used in the study

Primary Antibodies	**Source**	**Identifier**
Anti-*C. burnetii* guinea pig sera	(2)	James Samuel Laboratory, TAMHSC
Anti-LAMP1 mouse monoclonal antibody [H4A3]	abcam	ab25630
Anti-CYP1B1 Rabbit antibody [EPR14972]	abcam	ab185954
Anti-DYKDDDDK (FLAG) Tag mouse Monoclonal Antibody (FG4R)	Invitrogen™	MA1-91878
Anti-beta Actin Rabbit antibody	abcam	ab8227
Anti-GAPDH mouse monoclonal antibody [6C5]	abcam	ab8245
Secondary Antibodies	**Source**	**Identifier**
Goat anti-Guinea Pig IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647	Invitrogen™	#A-21450
Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 488	Invitrogen™	#A-11001
IRDye® 680RD Goat anti-Mouse IgG Secondary Antibody	LI-COR Biosciences	926-68070
IRDye® 680RD Goat anti-Rabbit IgG Secondary Antibody	LI-COR Biosciences	926-68071
IRDye® 800CW Goat anti-Rabbit IgG Secondary Antibody	LI-COR Biosciences	926-32211
Hoechst 33342	Thermo Scientific™	62249
Cell culture medium	**Source**	**Identifier**
Cytiva HyClone™ RPMI 1640 Media	Cytiva HyClone™	SH30255.01
DMEM - high glucose	Sigma-Aldrich	D6429
Gibco™ Sodium Pyruvate (100 mM)	Thermo Scientific™	11360070
Gibco™ Glucose Solution	Thermo Scientific™	A2494001
Avantor® Seradigm, Fetal Bovine Serum (FBS), US origin	Avantor, VWR	97068-085
PMA - Phorbol myristate acetate	InvivoGen	tlrl-pma
Bacterial growth medium	**Source**	**Identifier**
Brain Heart Infusion Broth	Millipore-Sigma	53286
Tryptic Soy Broth - Dehydrated Culture Media	Millipore-Sigma	22092
Luria-Bertini Medium	MP Biomedicals	0910053-CF
2X ACCM-2 Powder	Sunrise Science Products	4700-300
Legionella Enrichment Broth Base	Millipore-Sigma	94423
Legionella Growth Supplement	Millipore-Sigma	42981
BD BBL™ Prepared Plated Media: BCYE Agar (for *Legionella*)	BD	BD 221808
Cell Culture Plates	**Source**	**Identifier**
6-well plate, clear, tissue culture-treated	Greiner Bio-One	657160
24-well plate, clear, tissue culture-treated	Greiner Bio-One	662160

Corning™ Costar™ 96-Well, Cell Culture-Treated, Flat-Bottom Microplate	Corning™	3599
Other Reagents	**Source**	**Identifier**
TRIzol™ Reagent	Invitrogen™	15596026
Direct-zol RNA Miniprep Plus kits	Zymo Research	R2072
iScript™ Select cDNA Synthesis Kit	Biorad	1708897
DNase I, RNase-free (1 U/μL)	Invitrogen™	EN0521
SsoAdvanced™ Universal SYBR® Green Supermix	Biorad	1725271
PrimeSTAR® HS DNA Polymerase with GC Buffer	Takara Bio	R044B
M-PER™ Mammalian Protein Extraction Reagent	Thermo Scientific™	78501
Halt™ Protease Inhibitor Cocktail, EDTA-Free (100X)	Thermo Scientific™	78437
4x Laemmli Sample Buffer	Biorad	1610747
2x Laemmli Sample Buffer	Biorad	1610737
Precision Plus Protein™ All Blue Prestained Protein Standards	Biorad	1610373
Bicinchoninic acid assay (BCA)	Thermo Scientific™	#23225
Renilla Luciferase Assay System	Promega	E2810
Luciferase Assay System	Promega	E1500
FICZ (6-Formylindolo[3,2-b] carbazole)	Sigma-Aldrich	SML1489
Lipofectamine™ RNAiMAX Transfection Reagent	Invitrogen™	13778150
Lipofectamine™ 3000 Transfection Reagent	Invitrogen™	L3000015
Lipofectamine™ LTX Reagent with PLUS™ Reagent	Invitrogen™	15338100
Phosphate Buffered Saline (PBS)	Cytiva HyClone™	SH30256.01
Gentamicin solution	Sigma-Aldrich	G1397
Actinomycin D	Gibco™	11805017
Gibson Assembly® Master Mix	New England Biolabs (NEB)	E2611L
HindIII-HF®	New England Biolabs (NEB)	R3104S
NheI-HF®	New England Biolabs (NEB)	R3131S
T4 DNA Ligase	New England Biolabs (NEB)	M0202S
GenElute™ Bacterial Genomic DNA Kits	Sigma-Aldrich	NA2120
TaqMan™ Fast Advanced Master Mix	Applied Biosystems	4444557
High Pure PCR Template Preparation Kit	Roche	11796828001
Round Cover Slip German Glass	Electron Microscopy Sciences	50-949-439

Paraformaldehyde 4% in 0.1M Phosphate BufferSaline, pH 7.4	Electron Microscopy Sciences	15735-85
Ultrafree® Centrifugal Filter, 0.5 mL Sample Volume	Millipore	UFC30VV
MitoTracker™ Green FM Dye	Invitrogen™	M46750
MitoSOX™ Mitochondrial Superoxide Indicators	Invitrogen™	M36005
DCFDA / H2DCFDA - Cellular ROS Assay Kit	abcam	ab113851
MitoProbe™ TMRM Assay Kit	Invitrogen™	M20036
FITC Annexin V Apoptosis Detection Kit with PI (Annexin A5 Apoptosis Detection Kit)	Biolegend	640914
Cell Staining Buffer	Biolegend	420201
ANTI-FLAG® M2 Affinity Gel	Sigma-Aldrich	A2220
UltraPure™ 1 M Tris-HCI Buffer, pH 7.5	Invitrogen™	15567027
NaCl (5 M), RNase-free	Invitrogen™	AM9760G
EDTA (0.5 M), pH 8.0, RNase-free	Invitrogen™	AM9260G
NP-40 Surfact-Amps™ Detergent Solution	Thermo Scientific™	85124
Halt™ Protease and Phosphatase Inhibitor Cocktail	Thermo Scientific™	78442
12% Mini-PROTEAN® TGX™ Precast Protein Gels, 10-well, 50 µl	Biorad	4561044
Disposable Probe	Omni™ International	30750H
Immobilon®-FL PVDF Membrane	Millipore-Sigma	IPFL00010
ProLong™ Diamond Antifade Mountant	Invitrogen™	P36965
Software	**Source**	**Identifier**
Image J	NIH	N/A
FlowJo v. 10	BD biosciences	N/A
Fluoview FV3000 Laser Scanning Confocal Microscope	Olympus LS	N/A
GraphPad Prism v. 10	Graph Pad, San Diego, USA	N/A
Gen 3.5	BioTek	N/A
DESeq2 R package	GitHub	N/A
UpSetR	GitHub	N/A
Cytoscape	https://cytoscape.org/	N/A
R package (R 3.6.0)	www.r-project.org	N/A
Equipment	**Source**	**Identifier**
MaxQ™ HP Incubated and Refrigerated Console Shakers	Thermo Scientific™	SHKE435HP
New Brunswick™ Galaxy® 170 R High-Capacity CO_2 Incubators	Eppendorf	N/A
Nuaire Model NU-4850 US Autoflow Water Jacket Humidified CO2 Incubator	Nuaire	N/A
Nanodrop™ One	Invitrogen™	A38189

BioTek Cytation 5 Cell Imaging Multimode Reader	Agilent	N/A
CFX Opus 384 Real-Time PCR System	Biorad	12011452
QuantStudio™ 6 Flex Real-Time PCR System	Applied Biosystems	4485691
Omni Tissue Homogenizer (TH)	Omni^{TM} International	TH220
ChemiDoc Imaging System	Biorad	12003153

Data S1. (separate file)

Total Differentially Expressed (DE) lncRNAs Identified Across Different Infections.

Data S2. (separate file)

Differentially Expressed lncRNAs and Their Associated mRNA Pairs Across Different Infections.

Data S3. (separate file)

Alignment Properties and Raw Counts of Transcripts Identified in RNA-seq Analysis.

Data S4. (separate file)

Host Transcriptome Changes in Response to *C. burnetii* NMII Infection and a List of Differentially Expressed lncRNAs and Associated mRNAs.

Data S5. (separate file)

Coding-Noncoding Network Analysis of CYP1B1-AS1, CYP1B1, lnc-DKK2, and DKK2.

Data S6. (separate file)

Mass Spectrometry Results of 3XFLAG-CYP1B1 Immunoprecipitation (IP) Analysis and Peptide Information.

Data S7. (separate file)

Functional enrichment analysis of 3XFLAG-CYP1B1 IP proteins

Data S8. (separate file)

Protein-Protein Interaction Analysis in 3XFLAGCYP1B1 IP results

Data S9. (separate file)

Mass Spectrometry Results of CBU0937-3XFLAG IP analysis and Peptide Information.

References

1. A. E. Gregory *et al.*, Soluble antigens derived from Coxiella burnetii elicit protective immunity in three animal models without inducing hypersensitivity. *Cell Rep Med* **2**, 100461 (2021).

2. E. D. R. Case *et al.*, Primary Murine Macrophages as a Tool for Virulence Factor Discovery in Coxiella burnetii. *Microbiol Spectr* **10**, e0248421 (2022).