SUPPLEMENTARY METHODS

Assessing the effect of zinc on the growth and viability of *S. aureus*

To assess whether zinc, at the concentrations used in our study, adversely affect the growth and viability of *S. aureus* Pig1, bacteria (~5 x10^5 CFU) were incubated with or without 100 µM zinc (Zn_2SO_4) in RPMI 1640 with 10% FBS. The assay was performed in triplicate in 96-well plates for 24 h at 37°C at which time ten-fold serial dilutions were plated on THA for enumeration of CFU.

Verification that the NAM used in our study is free of detectable endotoxin

To confirm the absence of endotoxin (pyrogen), we tested the two lots of NAM used in our study. The quantitative detection of bacterial endotoxin in aqueous solutions of NAM was determined by end-point chromogenic Limulus amebocyte lysate endochrome method (Endosafe; Charles River). Non-LAL reactive LAL reagent water was used as diluent for preparing reagents and test specimen. Two separate microplate assays were performed measuring high concentration range (0.15-1.2 EU/mL) and low concentration range (0.015-0.12 EU/mL). The linearity of the standard curve within the concentration range used to determine endotoxin levels was verified. At least 4 endotoxin standards, spanning the desired concentration range, and an endotoxin-free water blank were assayed in quadruplicate. The absolute value of the coefficient of correlation, r, was greater than or equal to 0.980. Replicate samples were run to establish proficiency and low coefficient of variation. The coefficient of variation, CV, which is equal to 100 times the s.d. of the group of values, divided by the mean, was less than the allowed 10%.

Reactive oxygen species assay

Murine neutrophils were plated in triplicate at 5x10^4 cells/well in 96-well plate in RPMI 1640, 10% FCS, 2 mM L-glutamine. Cells were treated with either NAM (1 mM) or PBS for designated times. Then cells were infected with *S. aureus* at the designated MOI(s). For reactive oxygen species (ROS) assays, Luminol detection reagent was added to the cells right before beginning to determine the relative light units (RLU) on a Veritas Microplate Lumiometer (Turner BioSystems).

Real-time reverse-transcriptase polymerase chain reaction

For the quantitative mRNA expression analysis of *Cebpe*, *Camp*, and *Ltf*, we isolated RNA from murine BMMC or BMDM by the use of the RNeasy Mini Kit (Qiagen). Subsequently, cDNAs were synthesized from high quality RNA samples with an oligo(dT) primer and random hexamers using Superscript III reverse transcriptase according to the manufacturer's recommendation (Invitrogen). Gene expression was quantified with real-time reverse-transcriptase polymerase chain reaction (RT-PCR; iCycler; Bio-Rad) using HotMaster Taq DNA Polymerase (Eppendorf) and SYBRGreen I

(Molecular Probes). Reactions were performed in triplicates using an iCycler iQ system (Bio-Rad). Sequences of the primer sets were used as followed: *Cebpe*: 5'- GGG CAA CCG AGG CAC CAG TC -3' (*forward*), 5'- CGC CTC TTG GCC TTG TCC CG -3' (*reverse*); *Ltf*: 5'- GAG CTG TGT TCC CGG TGC CC -3' (*forward*), 5'- CCG TGC TTC CTC TGG TAA AAG CCA -3' (reverse); *Camp*: 5'- ACT CCC AAG TCT GTG AGG TTC CGA -3' (*forward*), 5'- TGT CAA AAG AAT CAG CGG CCG GG -3' (*reverse*); *Actb*: 5'- GGA CTT CGA GCA AGA GAT GG -3' (*forward*), 5'- CCG CCA GAC AGC ACT GTG TT -3' (*reverse*). For each sample, the amount of the target genes and reference gene was determined using standard curves. mRNA levels were normalized against endogenous *Actb*. The results of real-time RT-PCR are presented as mean ± s.e.m. using either BMDM obtained from 3 mice or BMMC from 4 mice per experiment.

Transient transfection and luciferase assays

We designed a -230 *LTF* promoter reporter plasmid (LAC-LUC) including a C/EBP-binding site as previously described (15). For the reporter gene assay, $2x10^6$ U937 cells were transiently transfected either with 2 µg of the LAC-LUC luciferase reporter gene constructs or the empty-vector control (pGL3; Promega), as well as 0.2 µg of Renilla luciferase (pRL-SV40). Transfections were performed using the nucleofection technique with the Amaxa-Kit (Invitrogen) according to the manufacturer's instructions. After 16 h of transfection, the cells were treated with NAM (1 mM) for an additional 16 h. The lysates were harvested and luciferase activity measured by the Dual-Luciferase reporter assay system (Promega). For all transfection studies, luciferase activity was normalized using pRL-SV40 activity. Results represent the mean (± s.e.m.) of three independent experiments performed in triplicate.

T cell restimulation assay

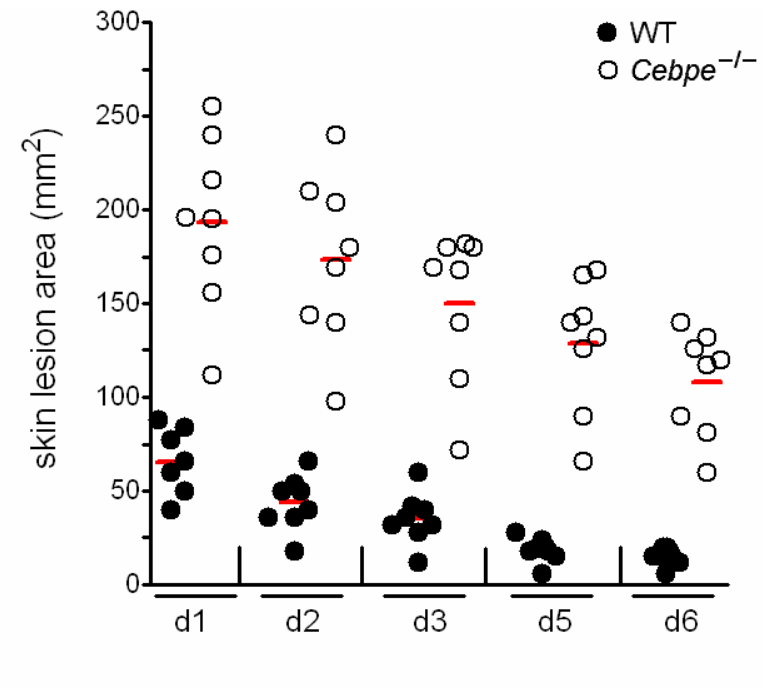
WT mice (n=3/group) were treated daily with NAM (250 mg/kg/day, i.p.) or with PBS (control), beginning 24 h prior to systemic (i.p.) infection with ~$1x10^7$ CFU *S. aureus*. In the two other groups mice were treated with NAM or PBS but not infected.

At 48 p.i. mouse spleen was disrupted by forcing the tissue through a 70 µm cell strainer using a 3 cc syringe plunger in RPMI 1640. Red blood cells were removed using ACK lysis buffer (Life Technologies). Total splenocytes were plated at $1x10^6$ cells/well in a 96-well round bottom plate in stimulation media [RPMI, 10% FCS, 2mM L-glutamine, 10mM HEPES, 10 µM nonessential amino acids, 1 mM sodium pyruvate, 100 U/mL penicillin, 100 µg/ml streptomycin, 1x β-mercaptoethanol (Gibco)]. Cells were untreated or were restimulated with 5 µg/mL anti-CD3 and 1 µg/mL anti-CD28 (BioLegend) for 48 h. Supernatants were assayed for cytokines by interleukin(-17 (Il17) or interferon-gamma (Ifng) ELISAs (BioLegend).

Genotyping of mice

First, mouse tail tips were digested in buffer containing 10 mM Tris-HCl (pH 8.0), 100 mM EDTA, 0.5% SDS and 0.1 mg/mL proteinase K (Sigma-Aldrich), overnight at 50^{o}C. Genomic DNA was then isolated by phenol/chloroform extraction followed by ethanol precipitation, and resuspended in 1 mL of Tris/EDTA buffer (pH 8). To determine the genotype of mice, 3 primers termed Neo1500 (5'- ATC GCC TTC TAT CGC CTT CTT GAC GAG -3'), mepsilon S (5'- GCT ACA ATC CCC TGC AGT ACC -3') and mepsilon AS (5'- TGC CTT CTT GCC CTT GTG -3') were utilized. To detect each allele, the following combinations of primers were used: mepsilon S and mepsilon AS for the WT allele, and mepsilon S and Neo1500 for the knockout allele of the *Cebpe* gene. Genomic PCR was performed using the FailSafe PCR buffer PreMix F (Epicentre Biotechnologies).

A



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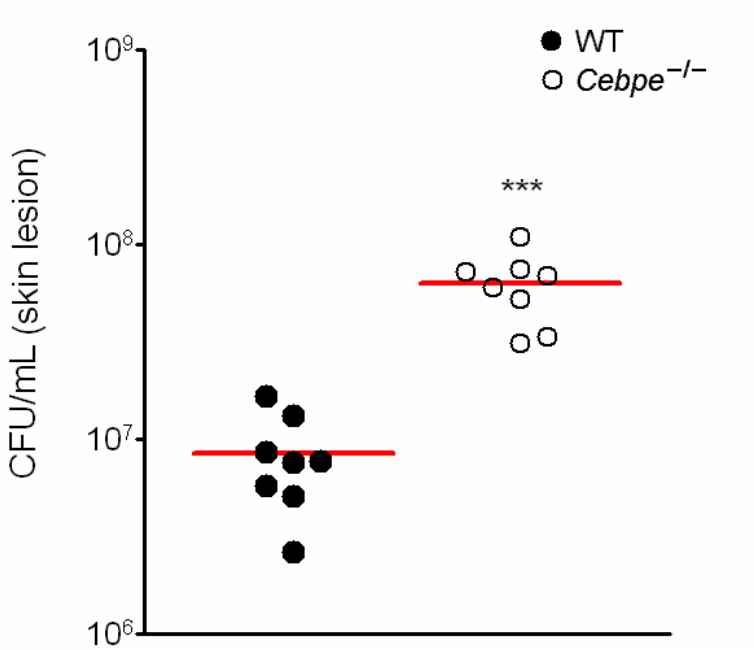
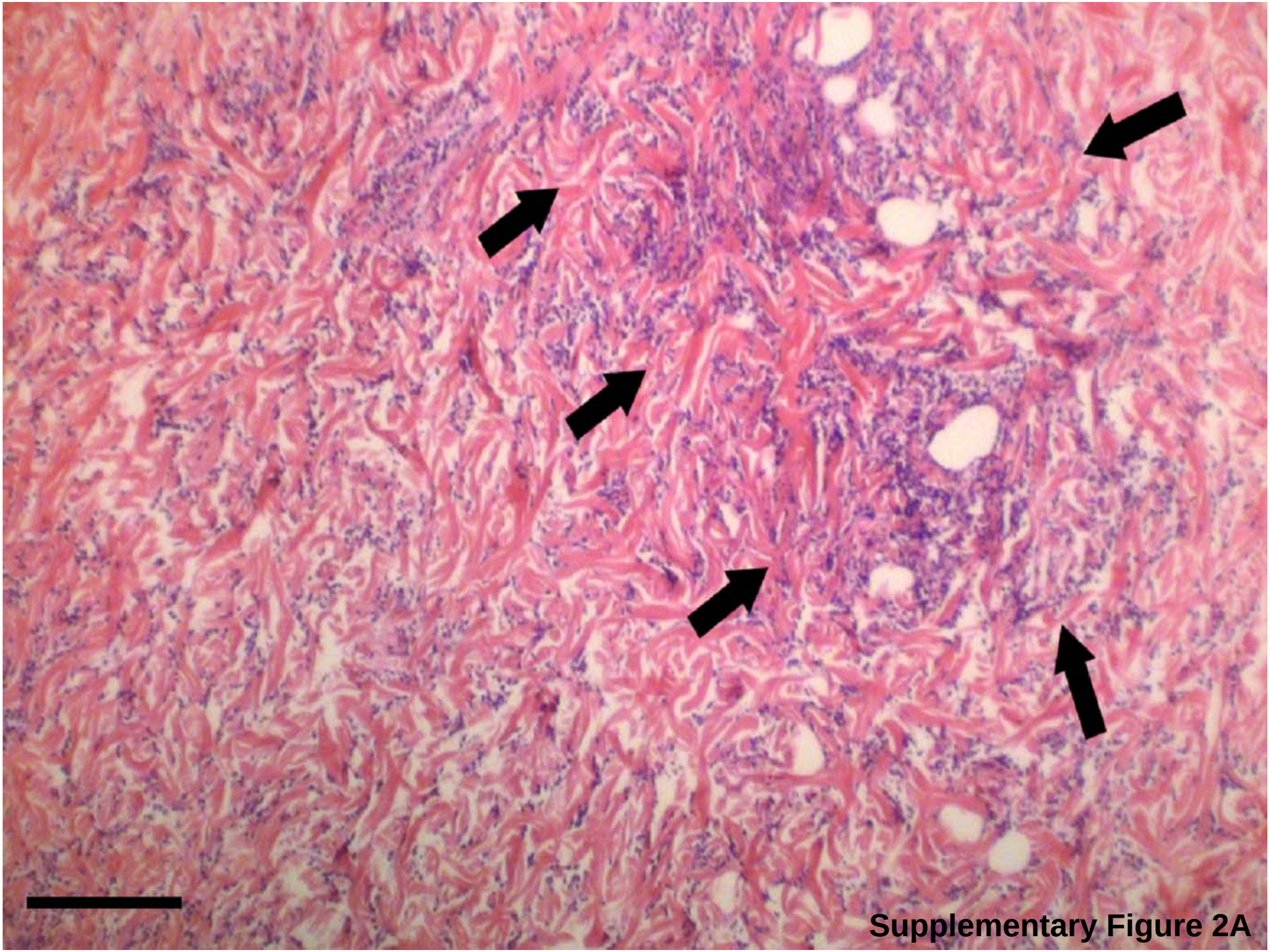
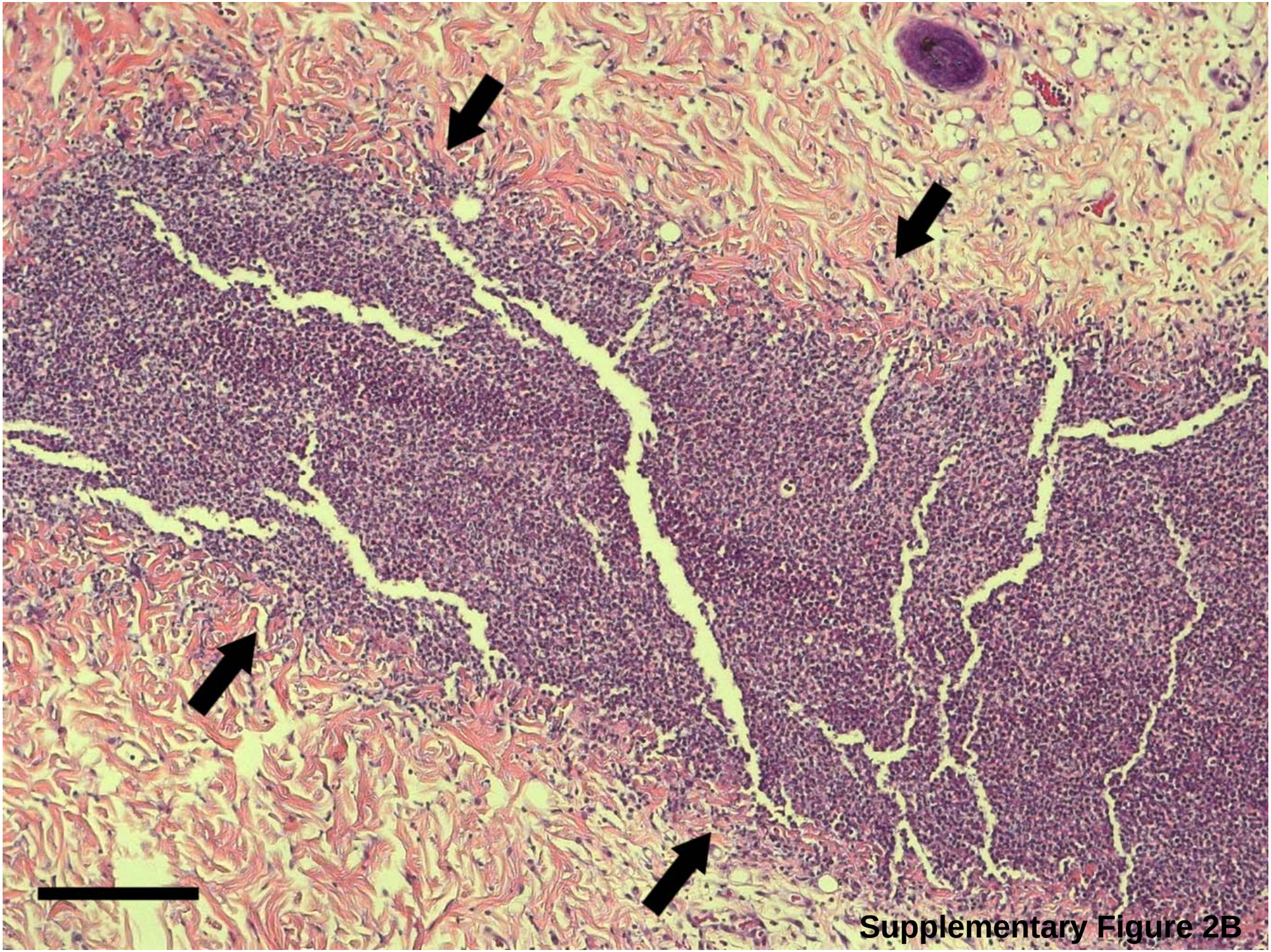
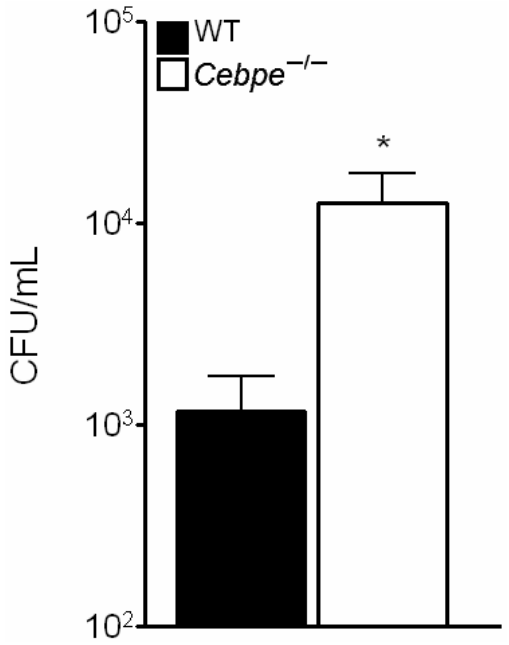


Supplementary Figure 1

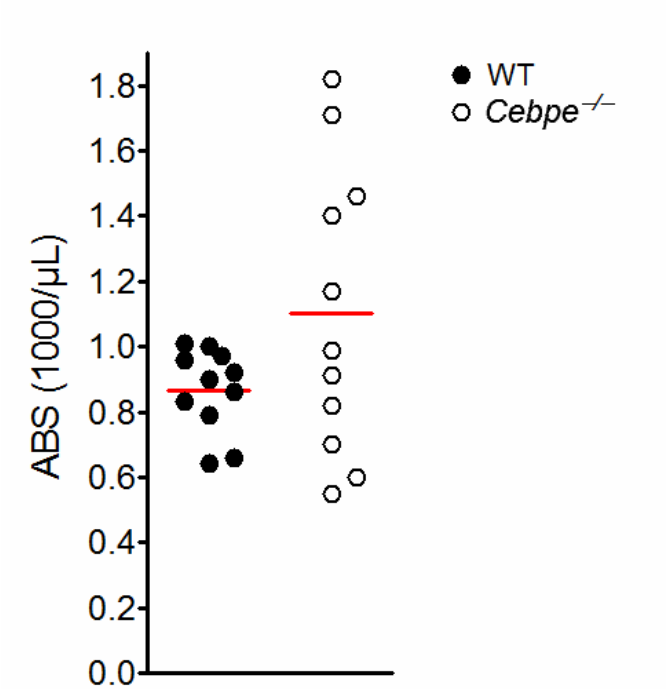


Supplementary Figure 2A


Supplementary Figure 2B

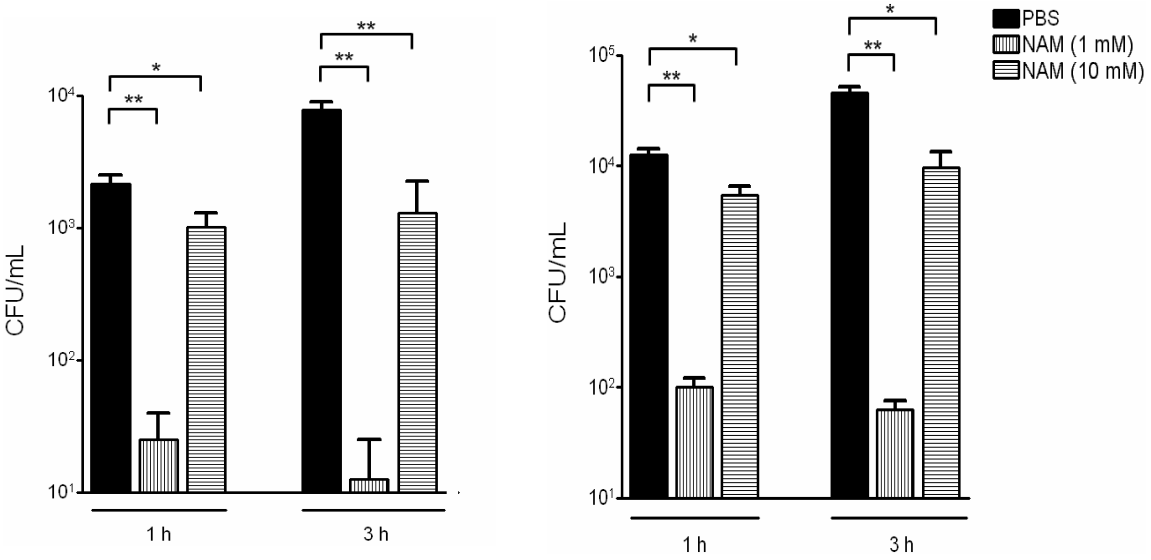


Supplementary Figure 3



Supplementary Figure 4

A



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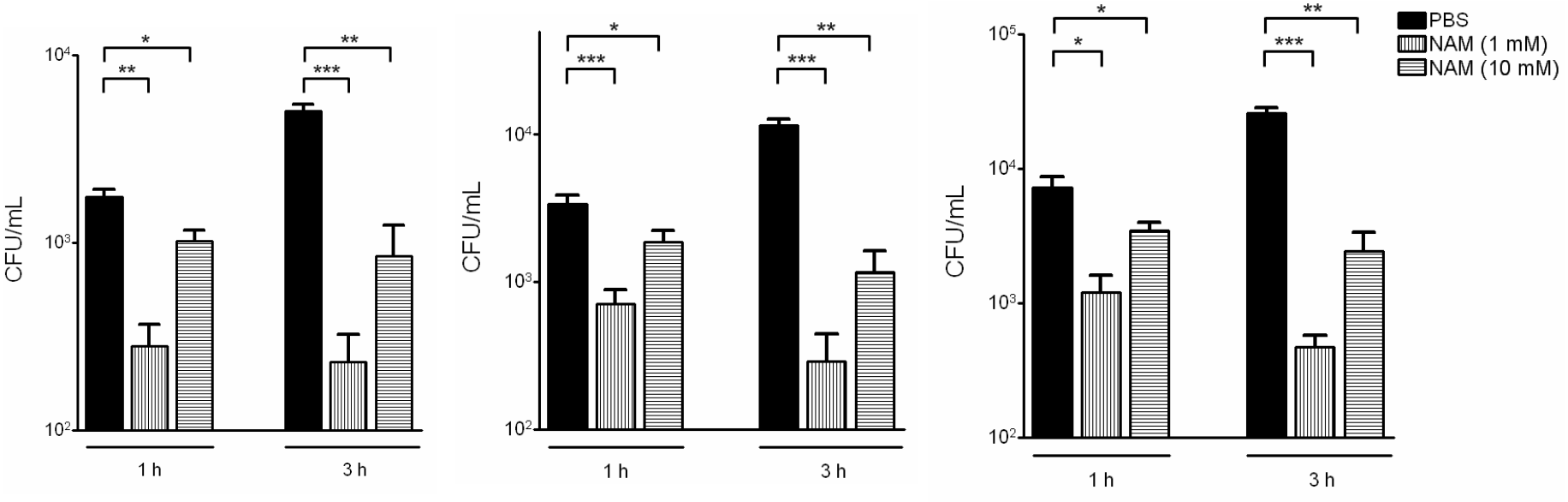
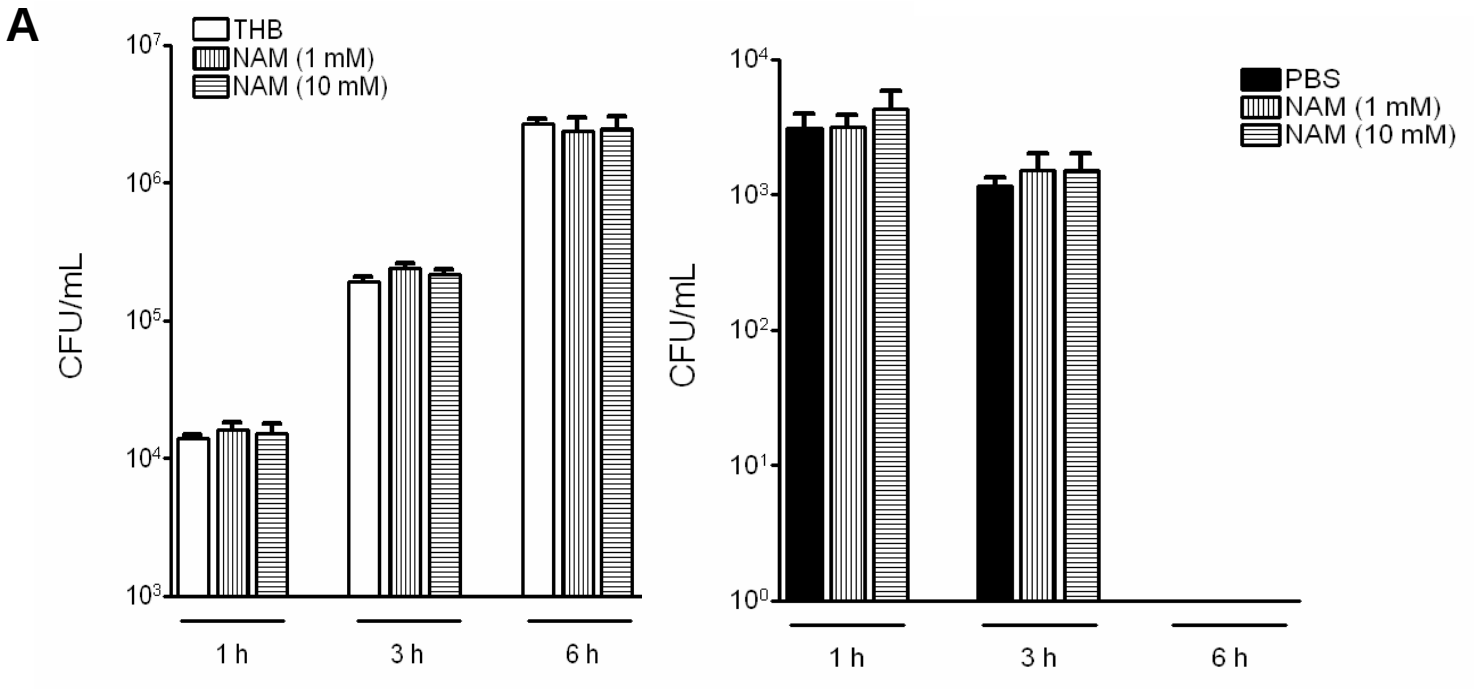
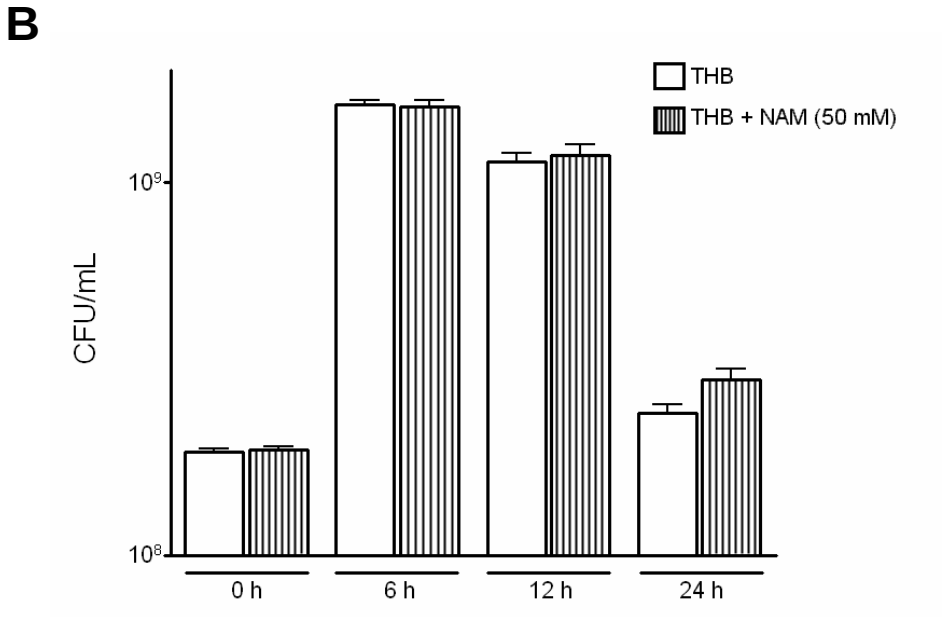
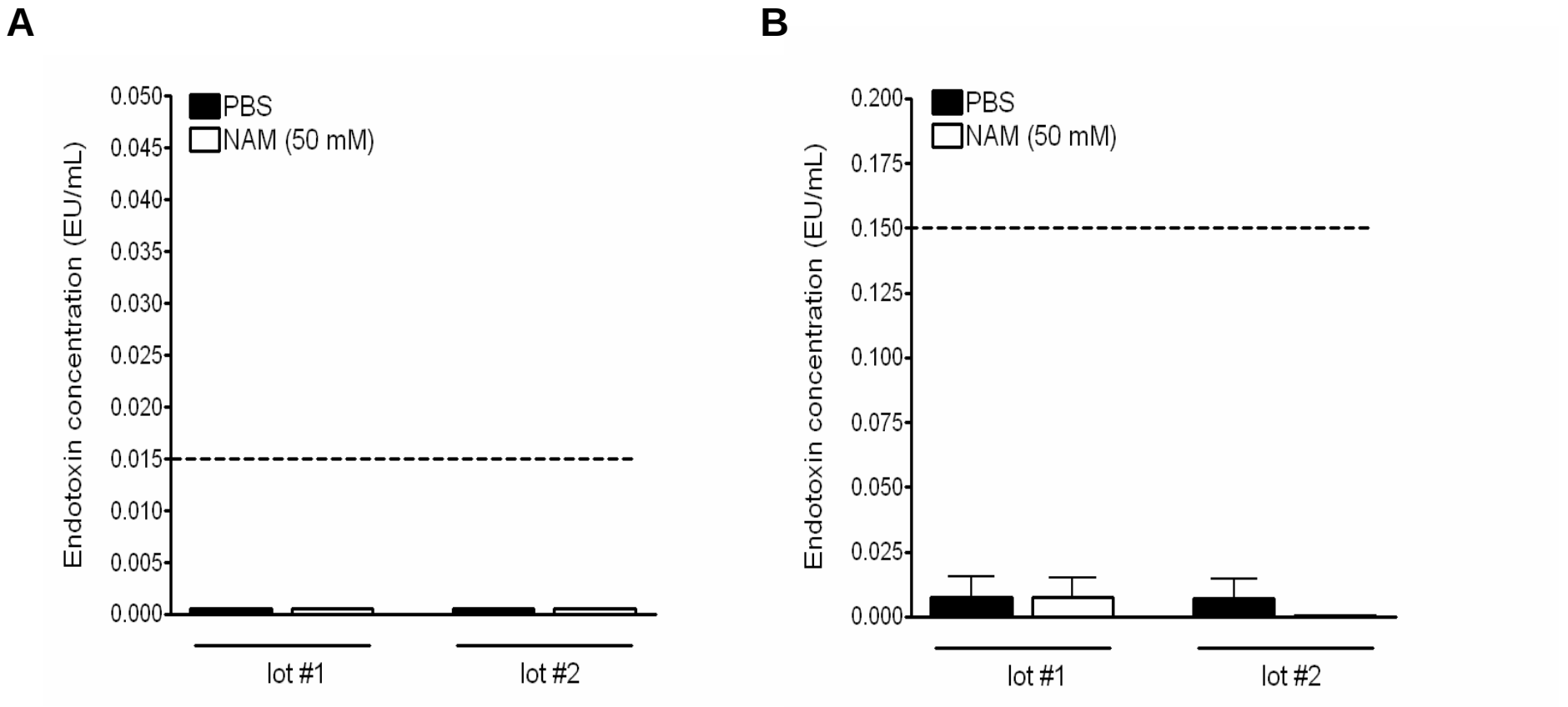


Supplementary Figure 5





Supplementary Figure 6



Supplementary Figure 7

A

Human Donor#1		Result		
	Ref. Range	t=0 h	t=24 h (PBS)	t=24 h (1 mM NAM)
Routine Blood Count				
WBC COUNT	*4-11 (1000/UL)*	9.2	8.9	8.9
Automated Differential				
POLYS	*%*	62	67	61
ABS POLYS	*1.8-8.0 (1000/UL)*	5.7	6	5.4
MONOS	*%*	6	5	7
ABS MONOS	*<0.8 (1000/UL)*	0.6	0.4	0.6

Human Donor#2		Result		
	Ref. Range	t=0 h	t=24 h (PBS)	t=24 h (1 mM NAM)
Routine Blood Count				
WBC COUNT	*4-11 (1000/UL)*	5.6	5.6	5.6
Automated Differential				
POLYS	*%*	59	66	51
ABS POLYS	*1.8-8.0 (1000/UL)*	3.3	3.7	2.9
MONOS	*%*	8	8	9
ABS MONOS	*<0.8 (1000/UL)*	0.4	0.4	0.5

Human Donor#3		Result		
	Ref. Range	t=0 h	t=24 h (PBS)	t=24 h (1 mM NAM)
Routine Blood Count				
WBC COUNT	*4-11 (1000/UL)*	7.3	6.8	6.9
Automated Differential				
POLYS	*%*	70	72	76
ABS POLYS	*1.8-8.0 (1000/UL)*	5.1	4.9	5.2
MONOS	*%*	5	5	4
ABS MONOS	*<0.8 (1000/UL)*	0.4	0.3	0.2

Human Donor#4		Result		
	Ref. Range	t=0 h	t=24 h (PBS)	t=24 h (1 mM NAM)
Routine Blood Count				
WBC COUNT	*4-11 (1000/UL)*	7.6	6.9	6.7
Automated Differential				
POLYS	*%*	64	70	63
ABS POLYS	*1.8-8.0 (1000/UL)*	4.9	4.8	4.2
MONOS	*%*	8	1	2
ABS MONOS	*<0.8 (1000/UL)*	0.6	0.1	0.1

Human Donor#5		Result		
	Ref. Range	t=0 h	t=24 h (PBS)	t=24 h (1 mM NAM)
Routine Blood Count				
WBC COUNT	*4-11 (1000/UL)*	6.9	5.9	6.1
Automated Differential				
POLYS	*%*	67	60	70
ABS POLYS	*1.8-8.0 (1000/UL)*	4.6	3.5	4.3
MONOS	*%*	8	3	2
ABS MONOS	*<0.8 (1000/UL)*	0.5	0.2	0.1

B

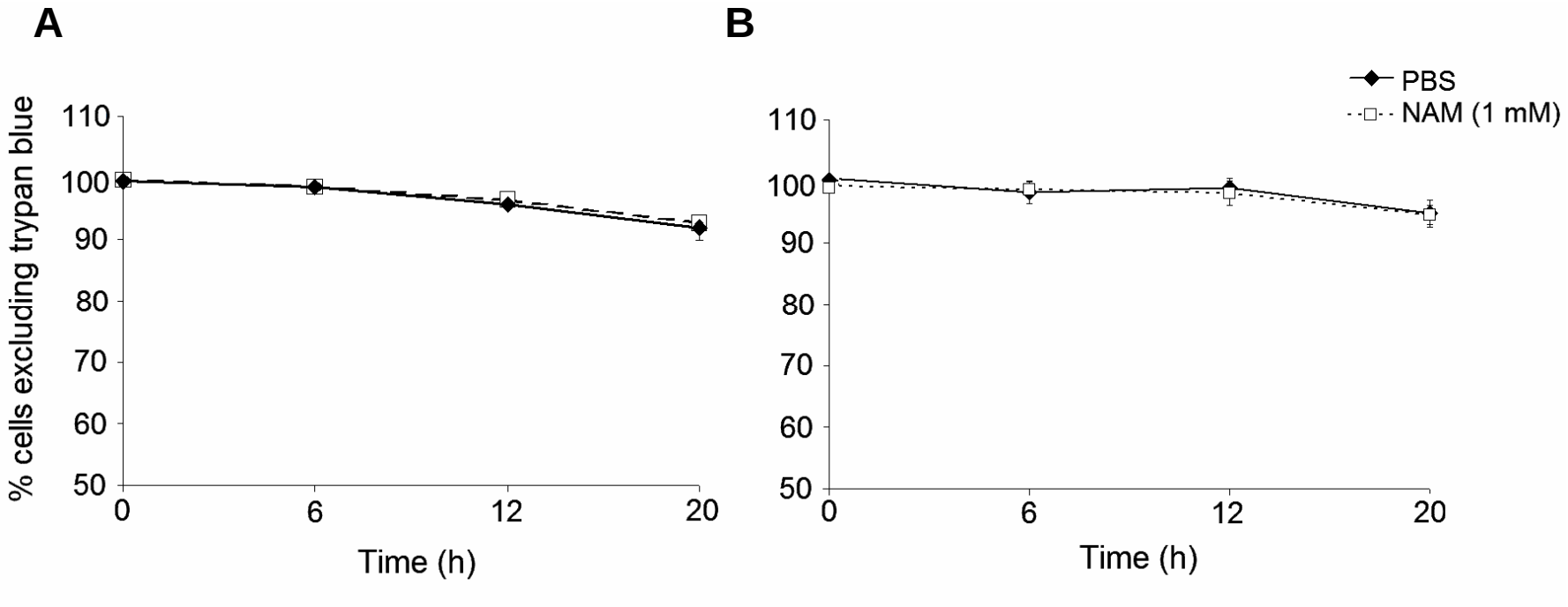
	P-value*		
	WBC COUNT	ABS POLYS	ABS MONOS
t=0h *vs.* t=24h PBS	0.04	0.63	0.06
t=0h *vs.* t=24h NAM	0.04	0.07	0.15
t=24h PBS *vs.* t=24h NAM	0.78	0.59	0.75

* Two-tailed, Paired Students *t* Test

	P-value*	
	POLYS %	MONOS %
t=0h *vs.* t=24h PBS	0.36	0.14
t=0h *vs.* t=24h NAM	0.94	0.24
t=24h PBS *vs.* t=24h NAM	0.56	0.54

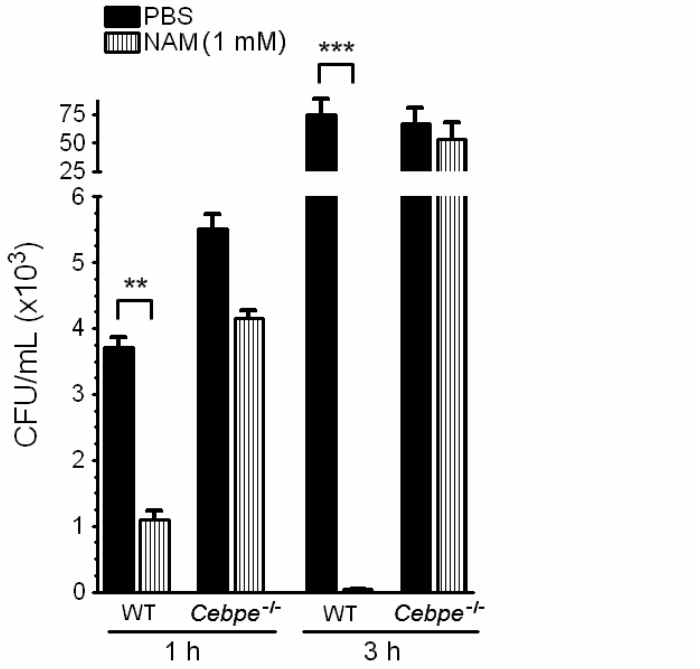
* Two-tailed, Paired Students *t* Test

Supplementary Figure 8



Supplementary Figure 9

A



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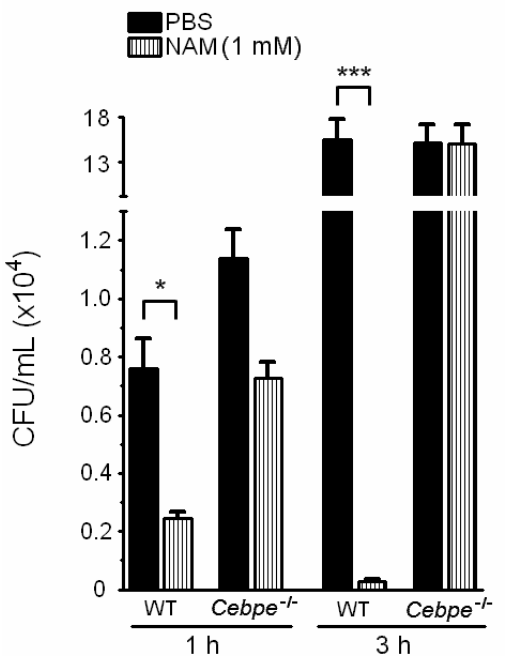
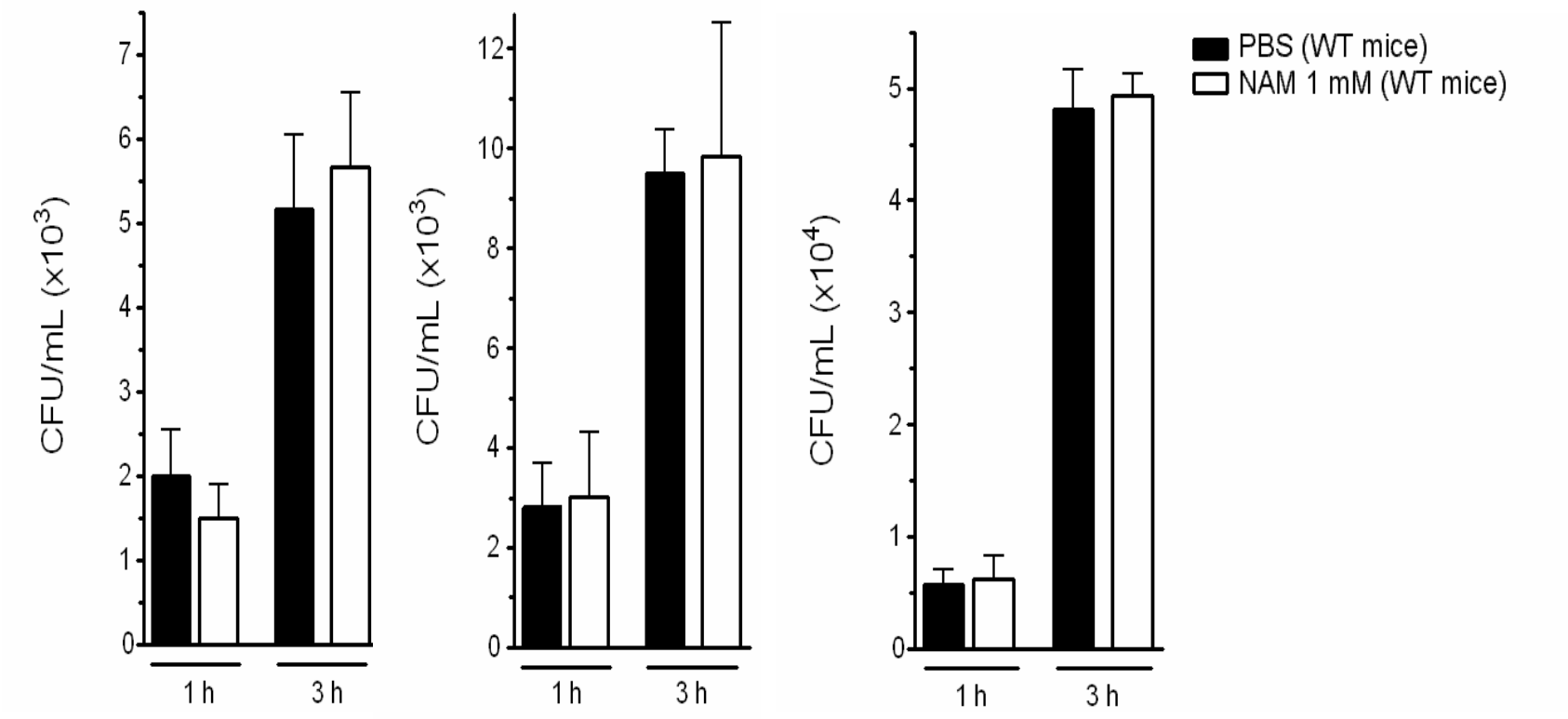
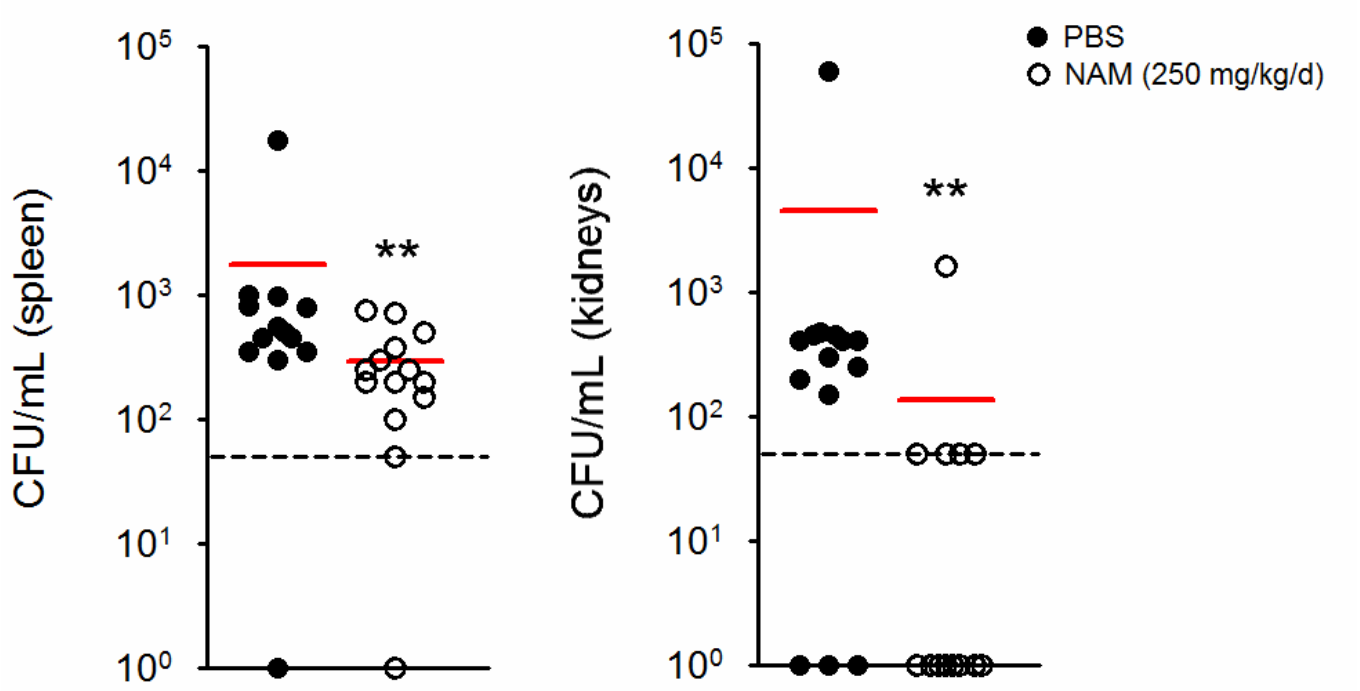


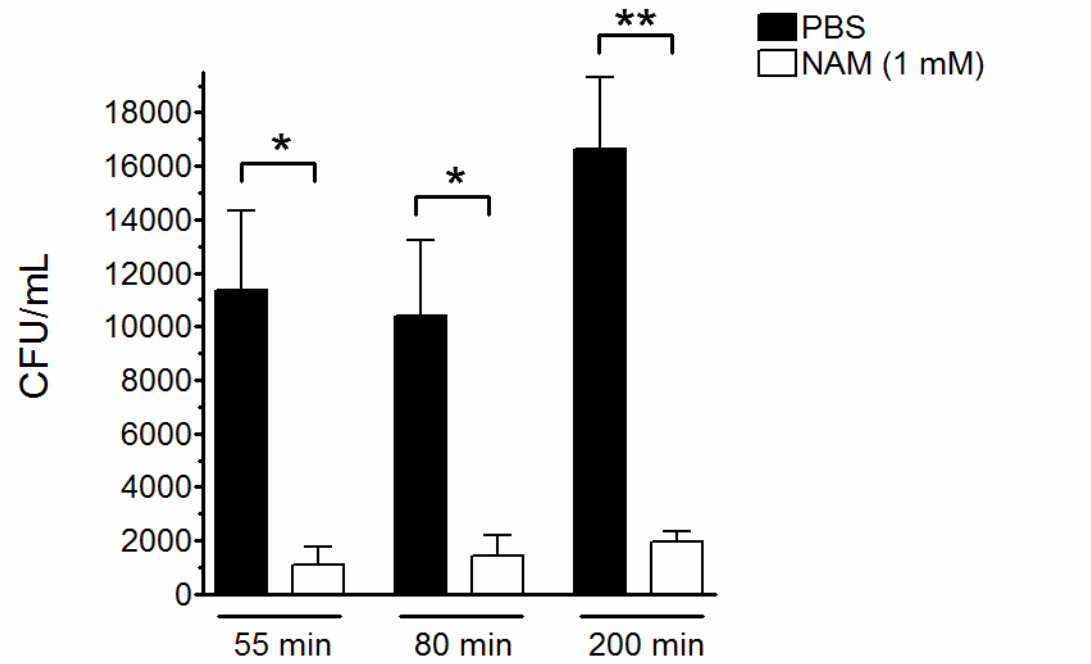
Supplementary Figure 10



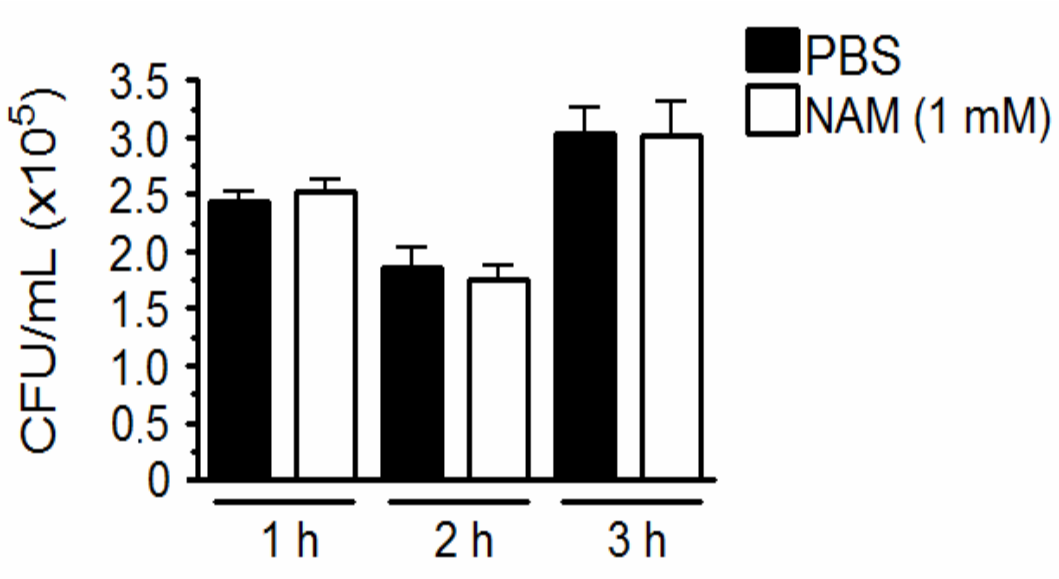
Supplementary Figure 11



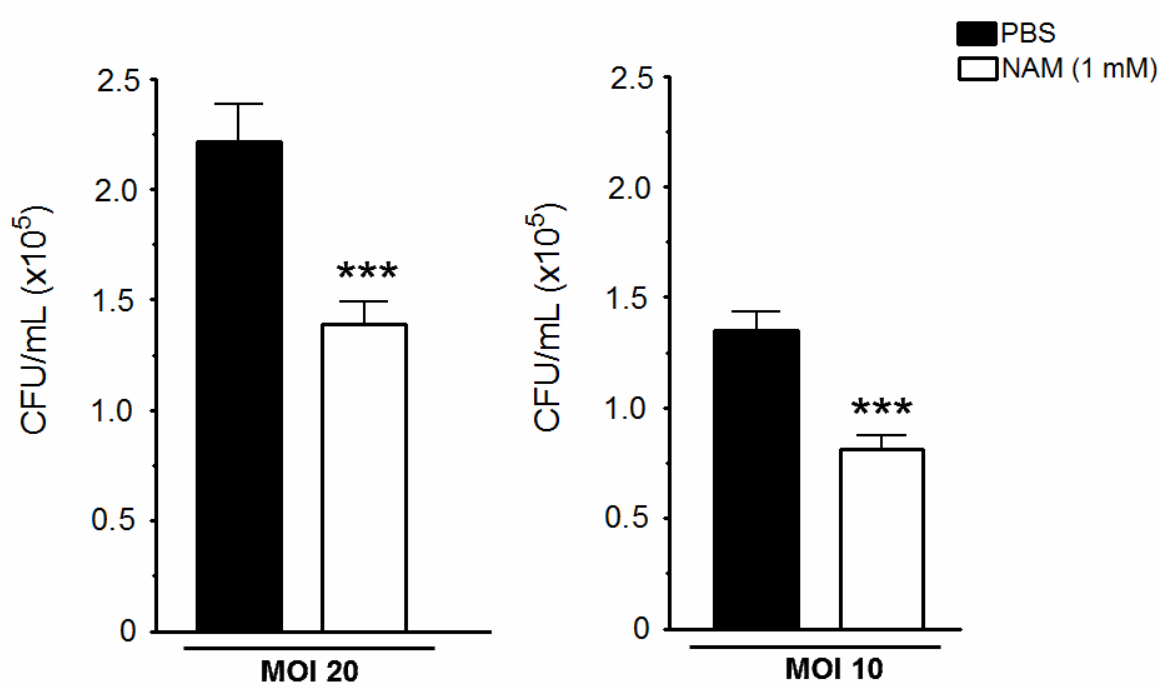
Supplementary Figure 12



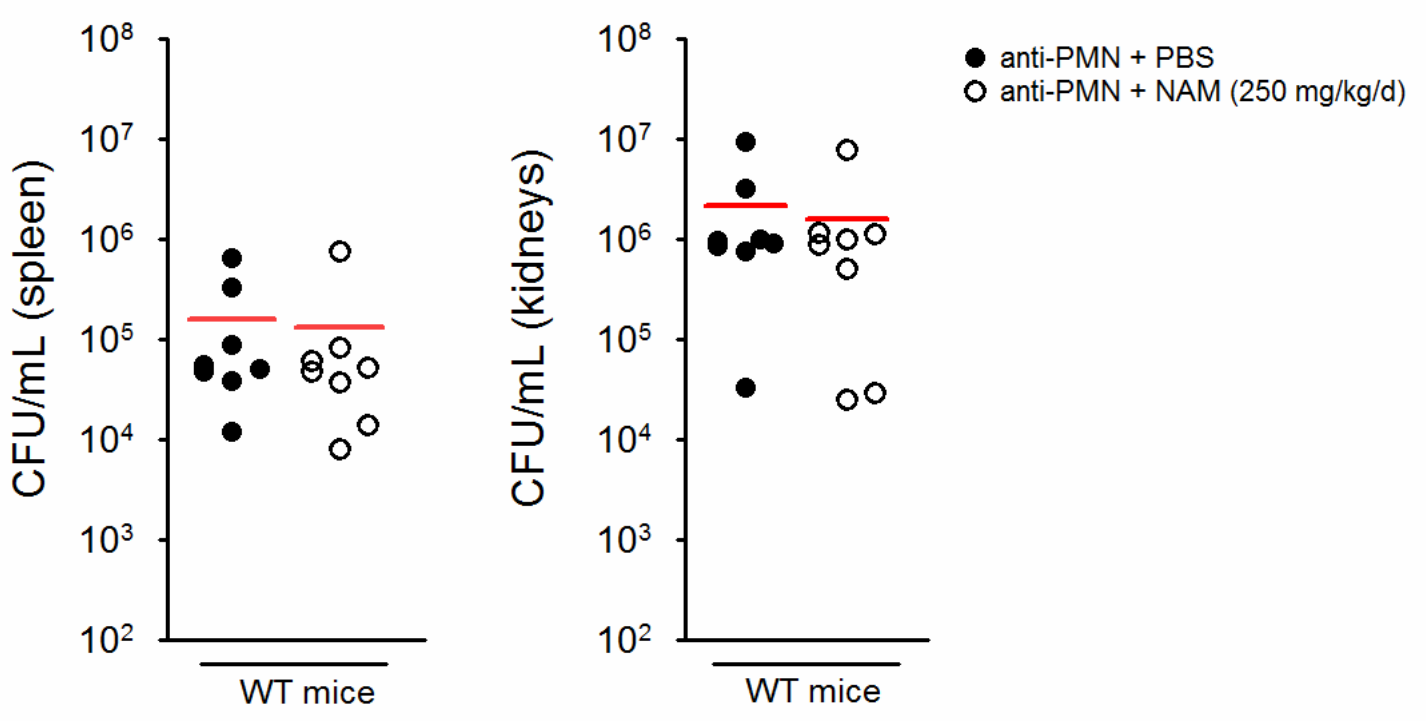
Supplementary Figure 13



Supplementary Figure 14



Supplementary Figure 15



Supplementary Figure 16

PBS for 24h (n=12)

Parameter (Units)	Normal Range	Results													
Leukocytes:		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	Mean	S.D.
WBC (K/uL)	*1.8-10.7*	8.66	4.60	4.34	4.68	3.50	7.42	4.50	2.46	3.22	2.30	2.26	2.08	4.17	2.07
NE (K/uL)	*0.1-2.4*	1.70	1.03	1.15	1.17	0.73	2.07	0.47	0.65	0.98	0.79	0.76	0.67	1.01	0.46
LY (K/uL)	*0.9-9.3*	6.34	3.19	2.87	3.17	2.58	4.62	3.72	1.59	2.00	1.21	1.28	1.25	2.82	1.55
MO (K/uL)	*0.0-0.4*	0.50	0.25	0.22	0.19	0.16	0.38	0.25	0.16	0.21	0.23	0.11	0.12	0.23	0.11
NE (%)	*6.6-38.9*	19.65	22.49	26.56	25.01	20.73	27.85	10.35	26.49	30.51	34.35	33.57	32.17	25.81	6.84
LY (%)	*55.8-91.6*	73.20	69.23	66.15	67.78	73.81	62.30	82.71	64.57	62.07	52.61	56.81	59.91	65.94	8.19
MO (%)	*0.0-7.5*	5.79	5.44	5.18	4.11	4.45	5.06	5.51	6.70	6.47	10.00	4.68	5.90	5.77	1.53

NAM (250 mg/kg/d) for 24h (n=10)

Parameter (Units)	Normal Range	Results												
Leukocytes:		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	Mean	S.D.	*P* value
WBC (K/uL)	*1.8-10.7*	3.30	2.18	2.12	2.94	3.80	5.16	2.78	5.38	4.24	4.44	3.63	1.16	**0.46**
NE (K/uL)	*0.1-2.4*	0.54	0.31	1.61	1.20	1.65	0.95	0.65	1.00	0.90	0.83	0.86	0.38	**0.41**
LY (K/uL)	*0.9-9.3*	2.49	1.72	1.38	1.50	1.96	3.97	1.89	3.84	3.13	3.11	2.50	0.96	**0.56**
MO (K/uL)	*0.0-0.4*	0.25	0.12	0.09	0.19	0.12	0.19	0.18	0.28	0.17	0.36	0.20	0.08	**0.38**
NE (%)	*6.6-38.9*	16.22	14.42	28.88	40.66	43.33	18.47	23.40	18.67	21.34	18.63	24.4	10.12	**0.71**
LY (%)	*55.8-91.6*	75.37	78.97	65.33	51.05	51.65	76.92	67.88	71.36	73.82	70.08	68.24	9.80	**0.56**
MO (%)	*0.0-7.5*	7.52	5.55	4.22	6.35	3.11	3.77	6.47	5.28	4.03	8.11	5.44	1.67	**0.64**

Supplementary Figure 17A

PBS for 48h (n=5)

Parameter (Units)	Normal Range	Results						
Leukocytes:		*1*	*2*	*3*	*4*	*5*	mean	S.D.
WBC (K/uL)	*1.8-10.7*	3.46	1.88	1.86	2.30	2.22	2.34	0.65
NE (K/uL)	*0.1-2.4*	0.60	0.60	0.40	0.53	0.41	0.51	0.10
LY (K/uL)	*0.9-9.3*	2.55	1.13	1.26	1.51	1.57	1.60	0.56
MO (K/uL)	*0.0-0.4*	0.23	0.12	0.18	0.21	0.17	0.18	0.04
NE (%)	*6.6-38.9*	17.29	32.16	21.51	23.11	18.40	22.49	5.89
LY (%)	*55.8-91.6*	73.59	60.24	67.59	65.57	70.92	67.58	5.13
MO (%)	*0.0-7.5*	6.52	6.62	9.75	8.97	7.47	7.87	1.44

NAM (250 mg/kg/d) for 48h (n=5)

Parameter (Units)	Normal Range	Results							
Leukocytes:		*1*	*2*	*3*	*4*	*5*	mean	S.D.	*P*-value
WBC (K/uL)	*1.8-10.7*	6.34	1.86	3.30	4.64	2.70	3.77	1.76	**0.15**
NE (K/uL)	*0.1-2.4*	1.41	0.29	0.59	1.01	0.58	0.78	0.44	**0.25**
LY (K/uL)	*0.9-9.3*	4.37	1.41	2.41	3.16	1.81	2.63	1.18	**0.13**
MO (K/uL)	*0.0-0.4*	0.34	0.14	0.12	0.26	0.20	0.21	0.09	**0.53**
NE (%)	*6.6-38.9*	22.22	15.43	17.76	21.75	21.48	19.73	2.99	**0.39**
LY (%)	*55.8-91.6*	68.85	75.58	73.06	68.03	67.04	70.51	3.65	**0.33**
MO (%)	*0.0-7.5*	5.37	7.27	3.72	5.50	7.40	5.85	1.53	**0.06**

Supplementary Figure 17B

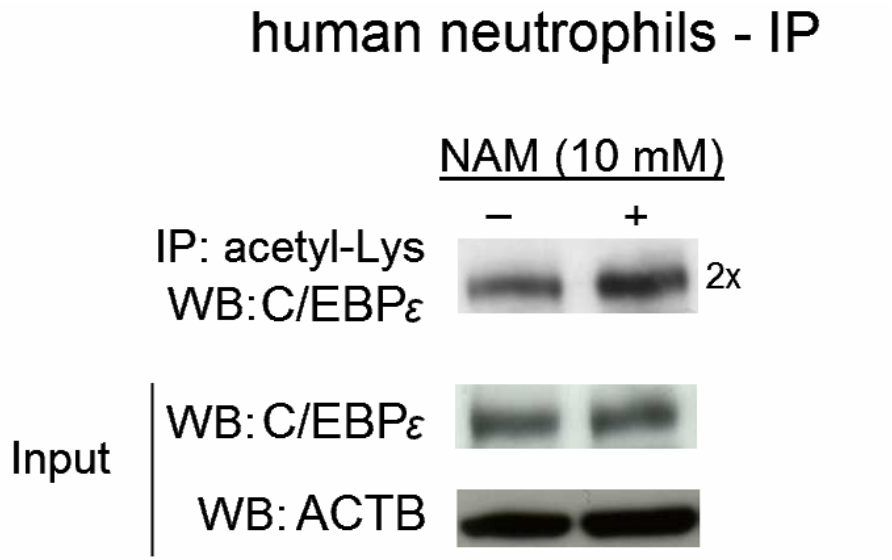
PBS for 72h (n=8)

Parameter (Units)	Normal Range	Results									
Leukocytes:		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	Mean	S.D.
WBC (K/uL)	*1.8-10.7*	4.40	7.18	6.60	3.02	6.28	5.32	6.34	5.80	5.62	1.35
NE (K/uL)	*0.1-2.4*	0.47	1.43	1.68	1.16	2.47	0.99	1.39	2.31	1.49	0.66
LY (K/uL)	*0.9-9.3*	3.52	5.29	4.56	1.71	3.53	3.92	4.47	3.19	3.77	1.08
MO (K/uL)	*0.0-0.4*	0.39	0.25	0.31	0.10	0.17	0.36	0.35	0.24	0.27	0.10
NE (%)	*6.6-38.9*	10.63	19.93	25.49	38.30	39.28	18.64	21.98	39.89	26.77	11.08
LY (%)	*55.8-91.6*	79.96	73.64	69.13	56.75	56.22	73.72	70.48	54.93	66.85	9.57
MO (%)	*0.0-7.5*	8.85	3.53	4.77	3.26	2.71	6.7	5.49	4.15	4.93	2.04

NAM (250 mg/kg/d) for 72h (n=8)

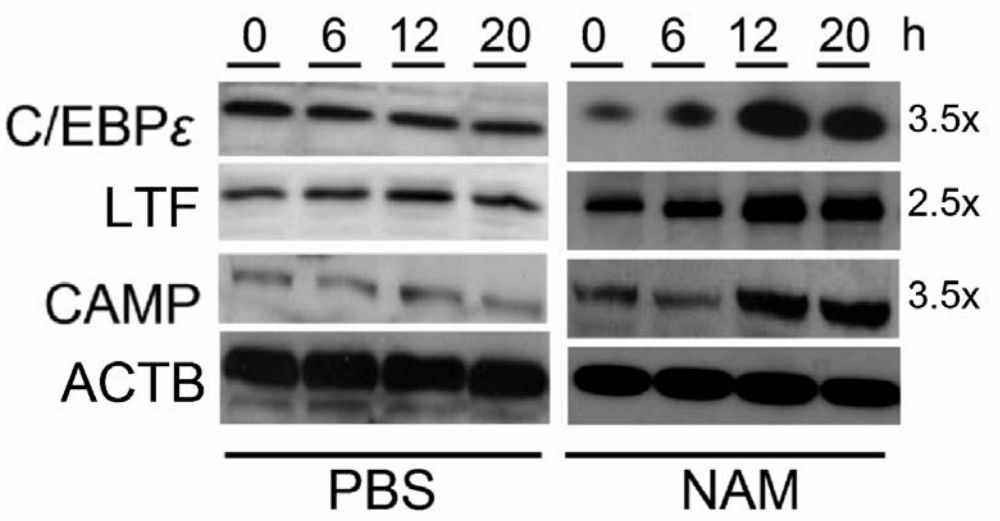
Parameter (Units)	Normal Range	Results										
Leukocytes:		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	Mean	S.D.	*P*-value
WBC (K/uL)	*1.8-10.7*	2.20	4.08	5.66	2.24	3.62	6.22	3.06	5.48	4.07	1.57	**0.05**
NE (K/uL)	*0.1-2.4*	0.51	0.86	1.05	0.62	1.22	2.28	0.97	1.84	1.17	0.61	**0.33**
LY (K/uL)	*0.9-9.3*	1.60	2.88	4.34	1.35	2.10	3.75	1.92	3.47	2.68	1.10	**0.06**
MO (K/uL)	*0.0-0.4*	0.06	0.24	0.26	0.22	0.22	0.14	0.12	0.11	0.17	0.07	**0.04**
NE (%)	*6.6-38.9*	23.25	20.96	18.47	27.89	33.59	36.70	31.63	33.52	28.25	6.69	**0.75**
LY (%)	*55.8-91.6*	72.55	70.58	76.68	60.42	57.97	60.36	62.64	63.30	65.56	6.79	**0.76**
MO (%)	*0.0-7.5*	2.89	5.82	4.53	9.61	6.08	2.30	3.77	2.02	4.63	2.52	**0.79**

Supplementary Figure 17C



Supplementary Figure 18

A



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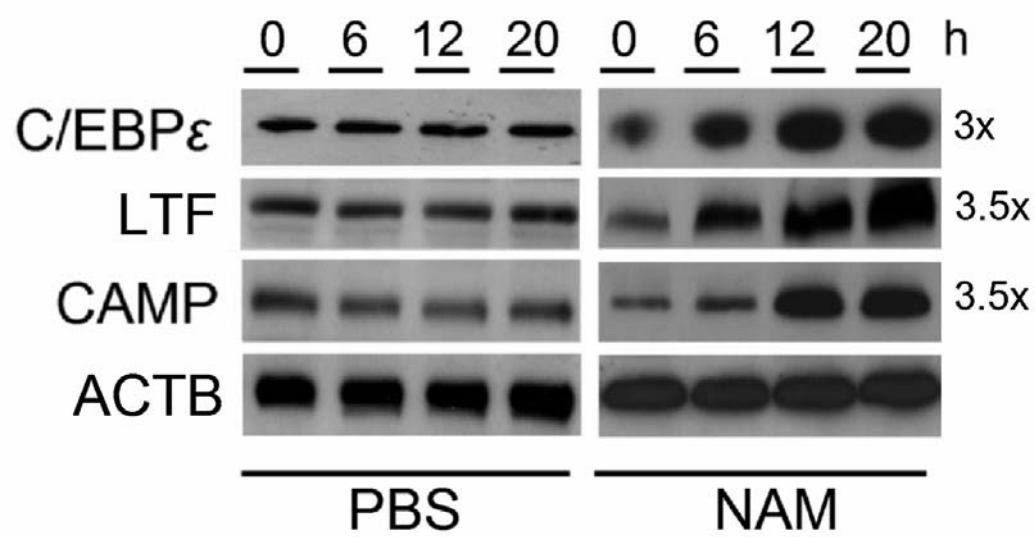
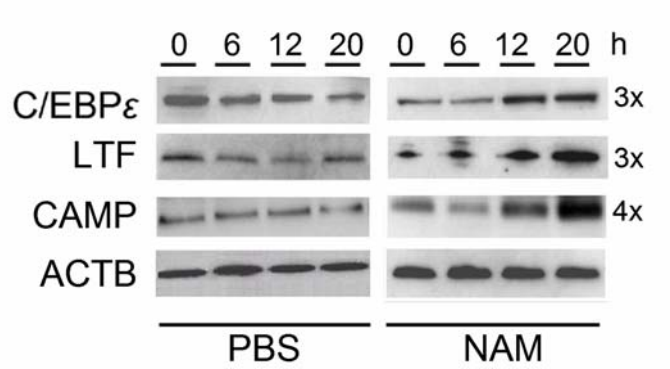


Supplementary Figure 19

A



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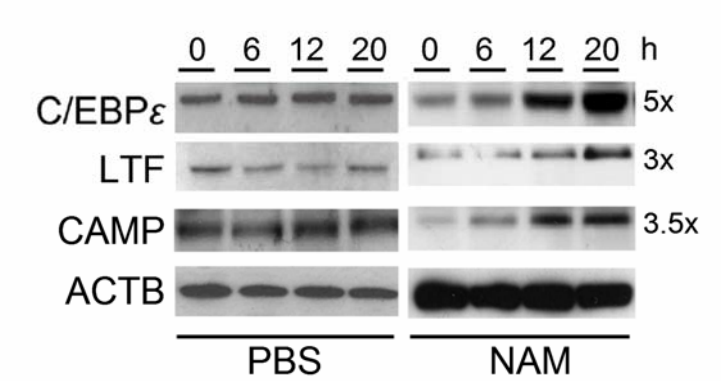

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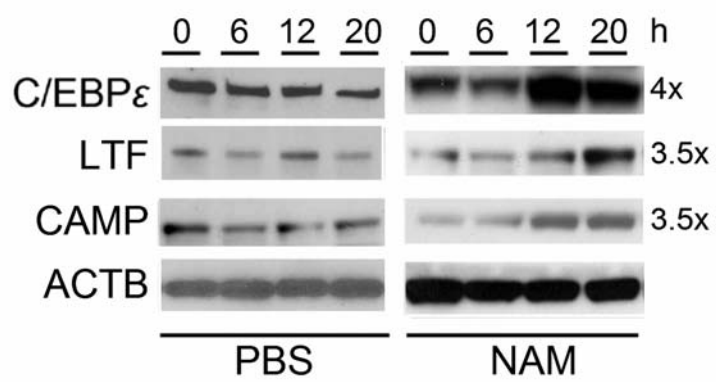
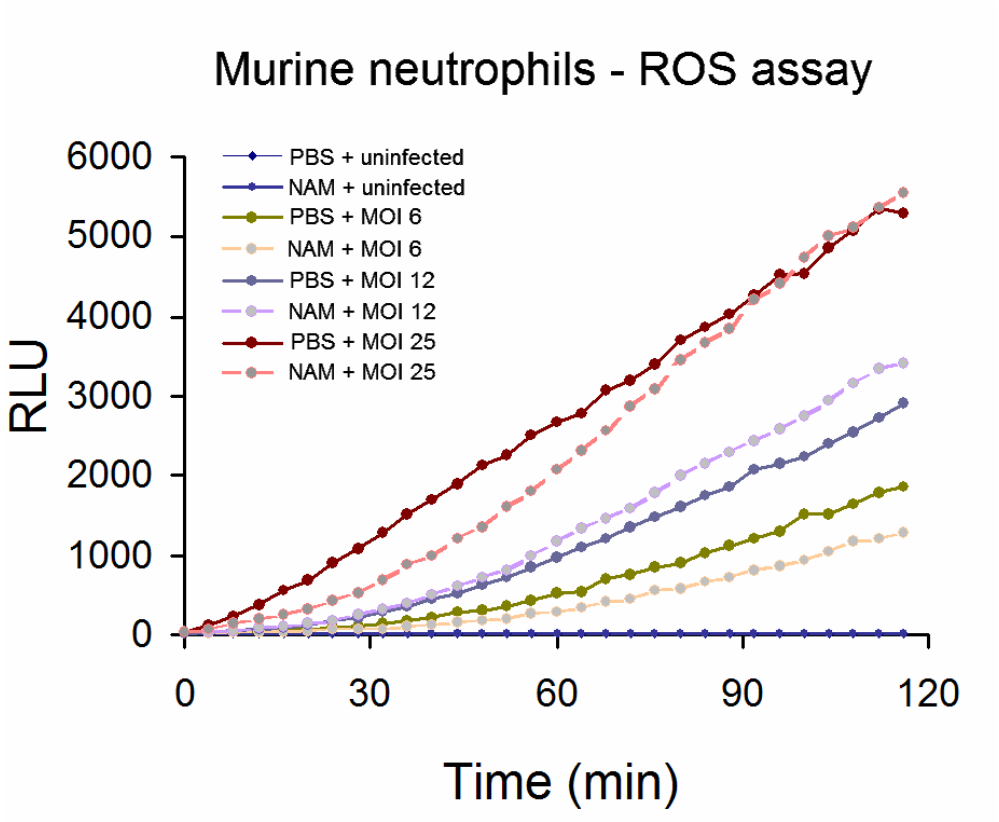


Supplementary Figure 20

A



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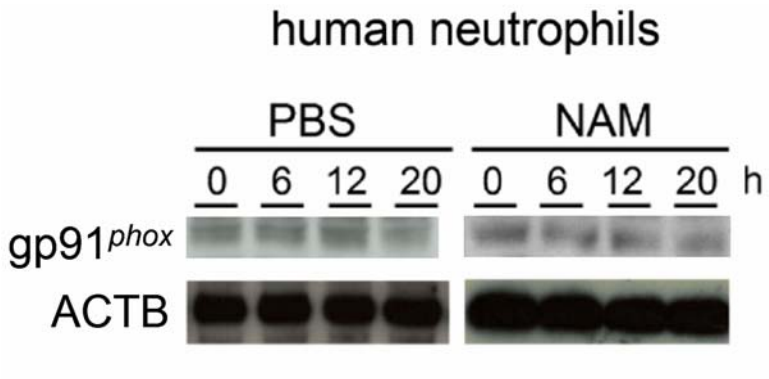
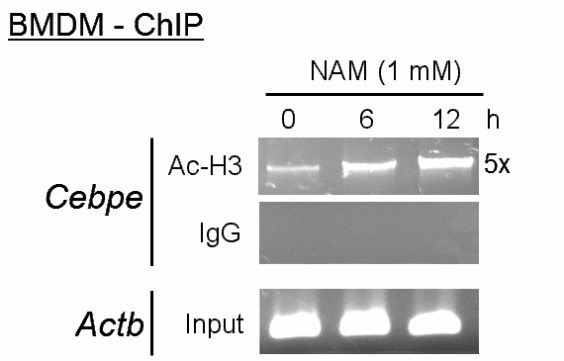


Supplementary Figure 21

A



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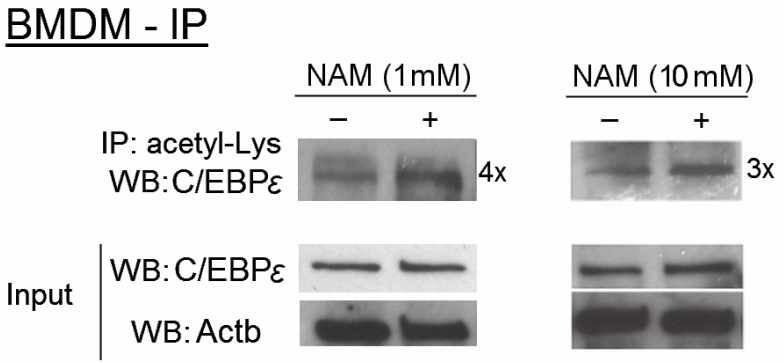
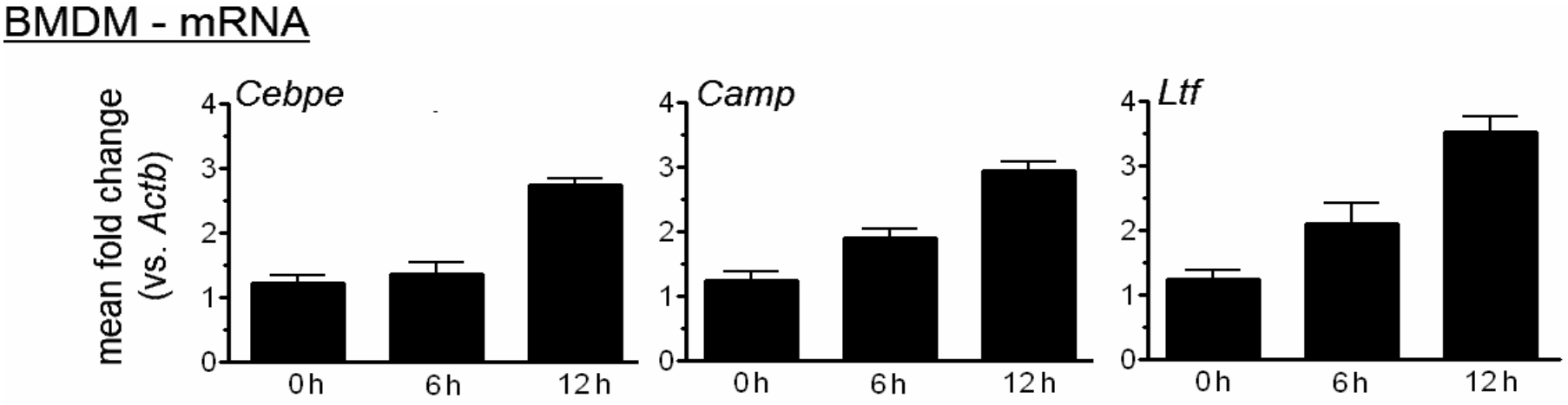


Supplementary Figure 22

A



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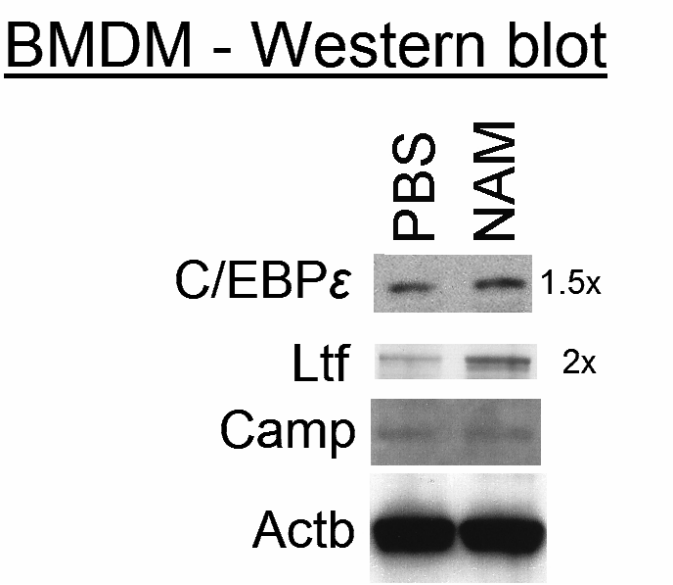
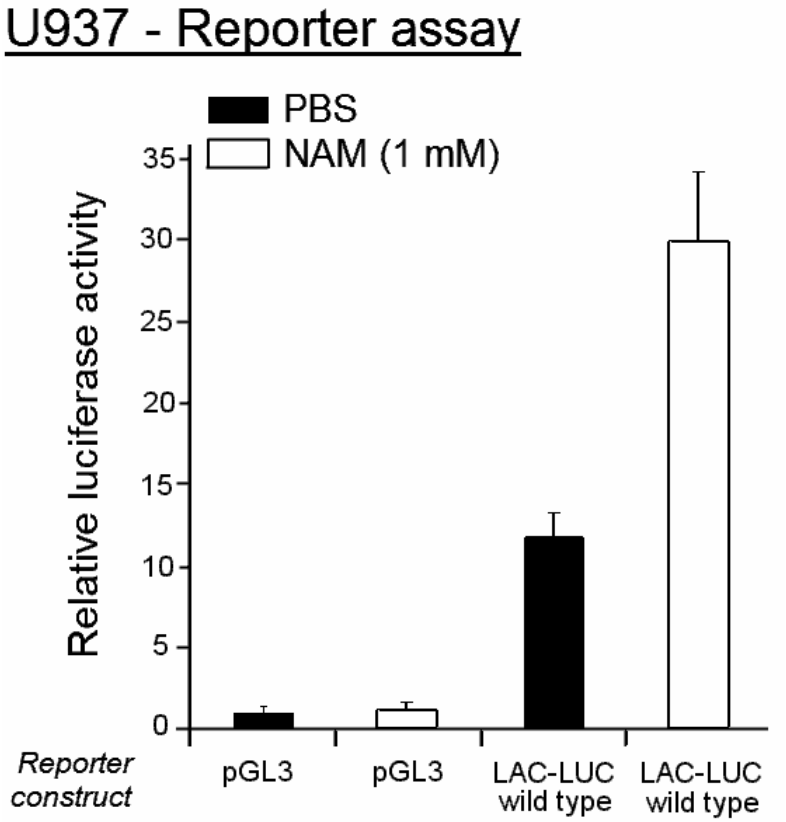
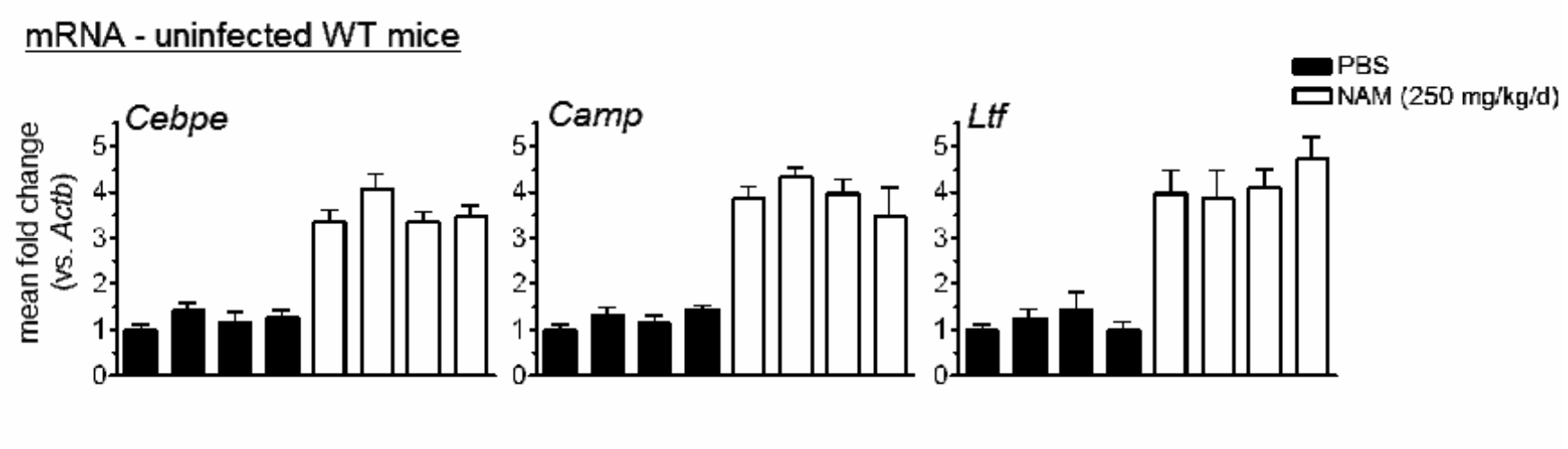


Supplementary Figure 23



Supplementary Figure 24

A



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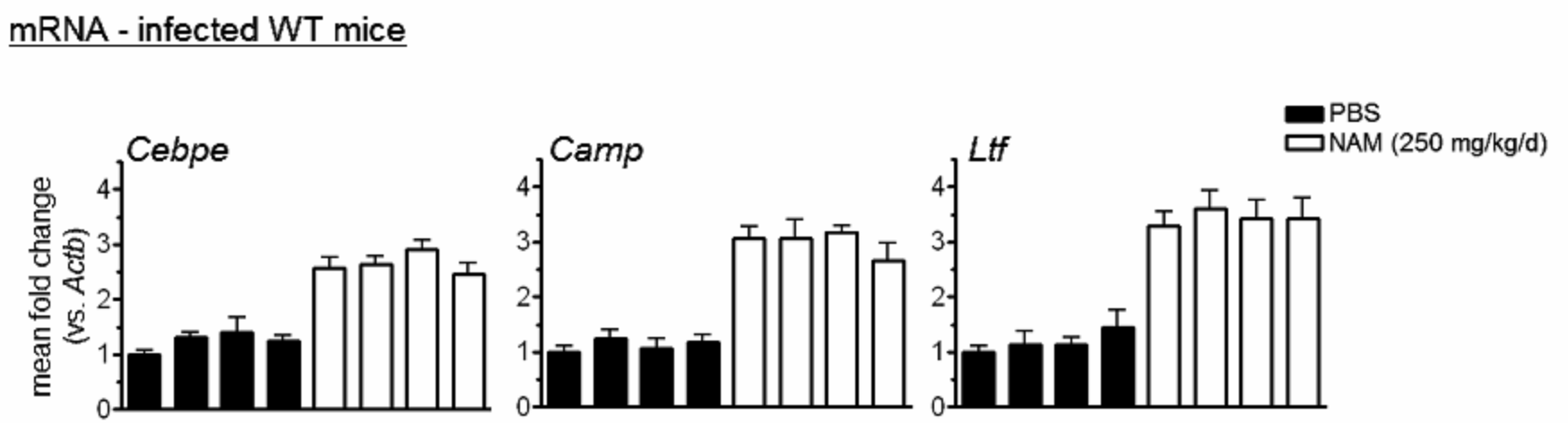
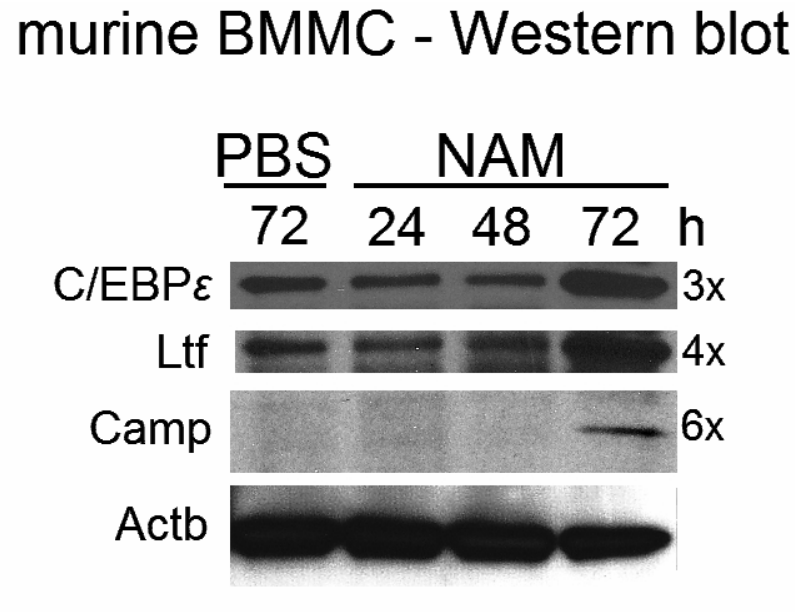
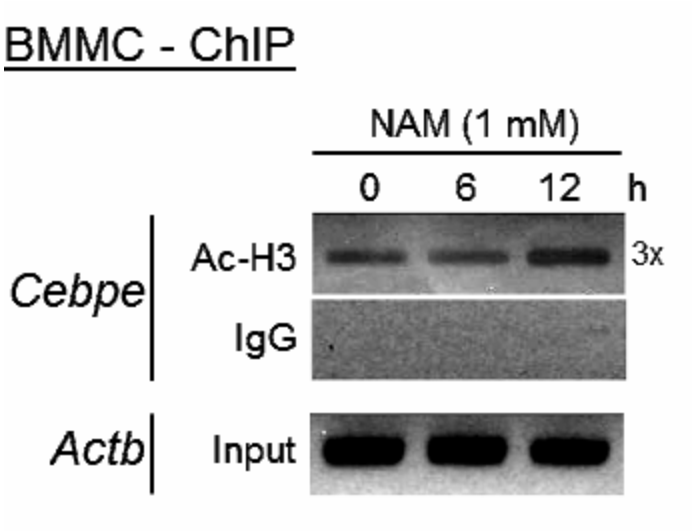


Supplementary Figure 25



Supplementary Figure 26

A



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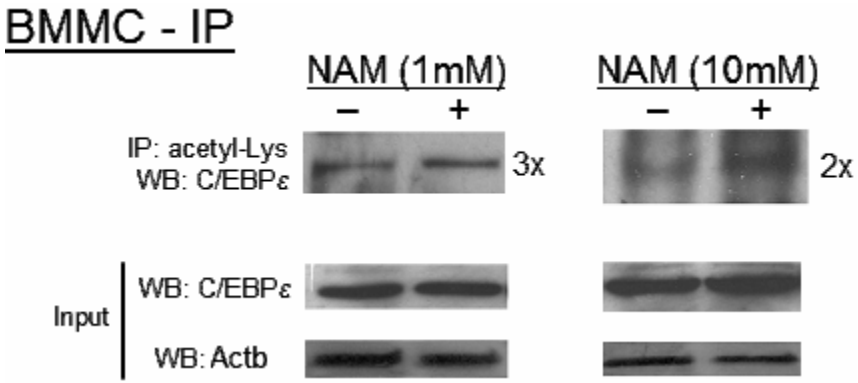
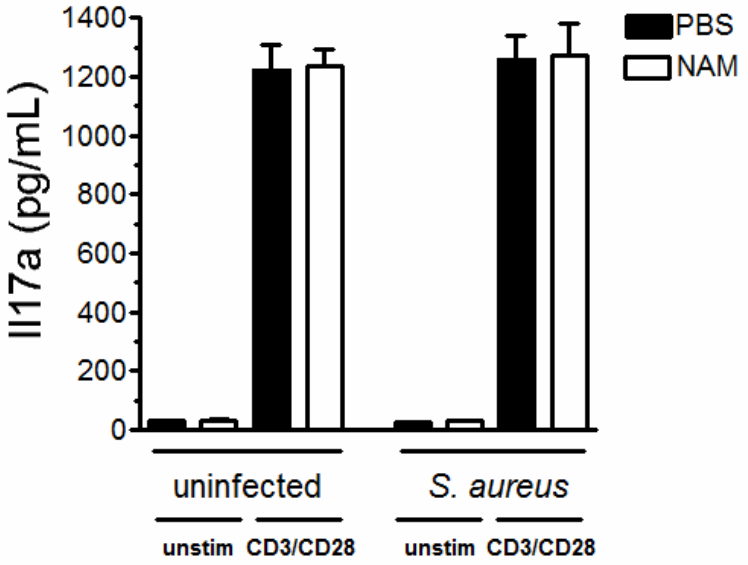


Supplementary Figure 27

A



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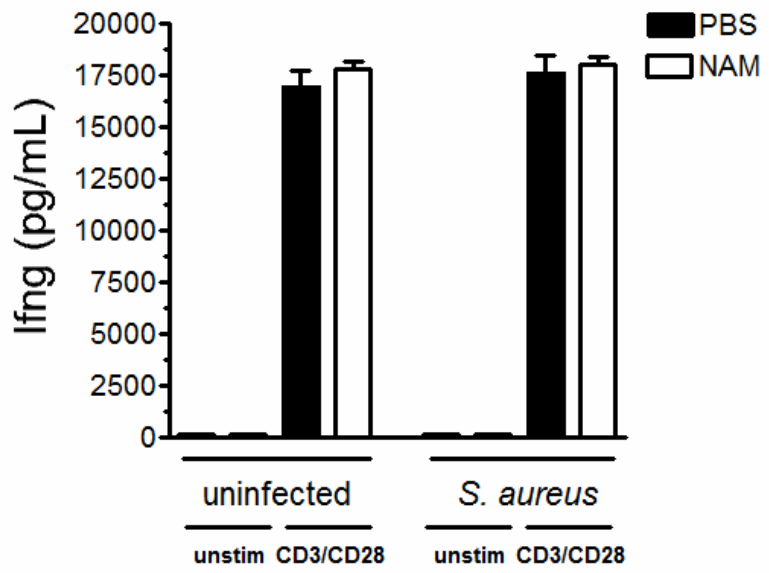

Supplementary Figure 28

Supplementary Figure 1. *Cebpe*$^{-/-}$ mice are highly susceptible to *S. aureus* subcutaneous challenge. (A) Graph shows the significantly larger area of skin lesions from *Cebpe*$^{-/-}$ compared to WT mice infected s.c. with ~1x10^8 CFU *S. aureus* ($P < 0.001$; red bar represents mean). **(B)** Higher CFU were recovered from these skin lesions of *Cebpe*$^{-/-}$ mice on day 6 p.i.; red bar indicates mean. *** $P < 0.001$.

Supplementary Figure 2. *Cebpe*$^{-/-}$ mice have increased infiltration of phagocytic cells. WT and C/EBP$\varepsilon^{-/-}$ mice (n=8/genotype) were injected s.c. with *S. aureus.* Representative H&E staining of skin lesions from **(A)** WT and **(B)** *Cebpe*$^{-/-}$ mice after 24 h of infection. Arrows indicate the area of infection with predominantly neutrophil invasion. Scale bar represents 200 µm.

Supplementary Figure 3. Blood derived from *Cebpe*$^{-/-}$ mice is defective in clearance of *S. aureus.* Peripheral blood drawn from WT or *Cebpe*$^{-/-}$ mice (n=5/group) was pooled and inoculated in triplicate with ~5x10^3 CFU/mL *S. aureus* for 1 h, at which time the surviving CFU were quantified and compared between the two groups (* $P < 0.05$). Data are means ± s.e.m.

Supplementary Figure 4. No difference in the population of circulating neutrophils in the blood of naive WT or *Cebpe*$^{-/-}$ mice. CBC with automated differential was performed on whole blood taken from naive mice (n=11/group) to determine the population of neutrophils.

Supplementary Figure 5. NAM enhances clearance of *S. aureus* from the blood of healthy human volunteers. (A) Bacterial counts (means ± s.e.m.) recovered from the blood of 4 human volunteers after the inoculation with either 4x10^3 CFU/mL (*Left*) or 1.3x10^4 CFU/mL (*Right*) *S. aureus*. Significantly less CFU were recovered from NAM- versus PBS-treated blood after 1 h and 3 h of infection. * $P < 0.05$; ** $P < 0.01$. **(B)** Similar findings were observed using peripheral blood from an additional 5 volunteers inoculated with 2.5x10^3 CFU/mL (*Left*), 5.3x10^3 CFU/mL (*Middle*), and 1.3x10^4 CFU/mL (*Right*) *S. aureus*. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Note that under similar experimental settings, NAM- and PBS-treated blood from an additional 3 human volunteers was inoculated with *S. aureus* and yielded consistent results (data not shown).

Supplementary Figure 6. NAM does not have direct anti-staphylococcal activity. (A) NAM (1 mM and 10 mM, in either THB or PBS) or THB/PBS (control) was inoculated in triplicate with *S. aureus* (~1x10^4 CFU/mL in THB or PBS) for 1 h, 3 h, and 6 h. No differences were observed between NAM-treated and control-treated samples at any time point. **(B)** *S. aureus* (~1x10^8 CFU/mL in THB) was incubated with or without 50 mM NAM (50-fold higher concentration than the standard concentration

used throughout our study). No difference in CFU was observed at any of the time points analyzed. Data are means ± s.e.m. This assay was performed on three independent occasions.

Supplementary Figure 7. NAM used in the study is endotoxin (pyrogen) free. Two separate lots (lot #1, lot #2) of NAM, used throughout our study, were tested to confirm the absence of endotoxin. The quantitative detection of bacterial endotoxin in aqueous solutions of NAM (50 mM) was determined by end-point chromogenic Limulus amebocyte lysate endochrome method (Refer to Supplementary Methods). Two separate microplate assays were performed in quadruplicate measuring **(A)** low concentration range (0.015-0.12 EU/mL) and **(B)** high concentration range (0.15-1.2 EU/mL). Dashed line indicates the limit of detection. Data are means ± s.e.m.

Supplementary Figure 8. Ex vivo NAM treatment of human blood does not alter monocyte and neutrophil counts. Blood from five healthy human volunteers was treated ex vivo with PBS or NAM (1 mM) for 24 h. Complete blood count (CBC) with automated differential was performed on the blood at 0 h and 24 h.

Supplementary Figure 9. Viability of human neutrophils is not affected by ex vivo treatment with NAM. (A) Neutrophils were isolated from the blood of three healthy human volunteers, and then treated ex vivo with PBS or NAM (1 mM) for the indicated times. Trypan blue analysis revealed no differences between the viability of PBS and NAM-treated neutrophils. **(B)** Blood from 3 healthy human volunteers was treated ex vivo with PBS or NAM (1 mM) for the indicated times, at which point neutrophils were isolated. Trypan blue analysis revealed no differences between the viability of neutrophils isolated from PBS and NAM-treated blood.

Supplementary Figure 10. NAM shows C/EBP*ε*-dependent clearance of *S. aureus* from murine blood. Peripheral blood from WT and ***Cebpe*$^{-/-}$** mice (n=6/group) was pooled and treated with either NAM (1 mM) or PBS. After 24 h, triplicate blood samples were inoculated with **(A)** 2.3x10^3 CFU/mL or **(B)** 5.2x10^3 CFU/mL *S. aureus* for 1 h and 3 h. Significantly less CFU were recovered from NAM- versus PBS-treated blood of WT mice after 1 h and 3 h of infection (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$). In contrast, CFU recovered from the NAM-treated blood of *Cebpe*$^{-/-}$ mice were not statistically different to CFU from PBS-treated blood at each time point ($P > 0.05$). Data are means ± s.e.m.

Supplementary Figure 11. Pretreatment of mouse blood with NAM for 4 h is not sufficient to promote clearance of *S. aureus*. Peripheral blood from WT mice (n=3/group) was pooled, and treated with either NAM (1 mM) or PBS. After 4 h of pretreatment with NAM, triplicate blood samples

were inoculated with *S. aureus* at $4x10^3$ CFU/mL (*Left)*, $6.3x10^3$ CFU/mL (*Middle*), or $1.38x10^4$ CFU/mL (*Right*) for 1 h and 3 h, at which time surviving CFU were quantitated. Similar CFU were recovered from NAM-treated blood and PBS-treated blood at each time point (all $P > 0.05$). Data are means ± s.e.m. The assay was repeated and yielded similar results (data not shown).

Supplementary Figure 12. Effect of NAM on in vivo clearance of *S. aureus* strain COL (MRSA) by WT mice. WT mice (n=14/group) were treated daily with NAM (250 mg/kg/day, i.p.) or with PBS (control), beginning 24 h prior to systemic (i.p.) infection with ~$1.5x10^7$ CFU *S. aureus* strain COL (MRSA). CFU count in spleen and kidneys of WT mice at 48 h p.i. Dashed line indicates the limit of detection. Red bar indicates mean. ** $P < 0.01$.

Supplementary Figure 13. Effect of NAM on clearance of *S. aureus* by human neutrophils. Neutrophils were isolated from the blood of three healthy human volunteers and subsequently treated ex vivo with PBS or NAM (1 mM) for 20 h before infecting them with $6x10^7$ CFU/mL pre-opsonized *S. aureus*. Gentamicin was added after 20 min of infection to kill all extracellular bacteria. CFU/mL reflects the number of surviving intracellular bacteria recovered at the indicated times. Data are means ± s.e.m.; * $P < 0.05$; ** $P < 0.01$.

Supplementary Figure 14. Effect of NAM on clearance of *S. aureus* by human peripheral mononuclear cells. Monocytes isolated from the blood of three healthy human volunteers were treated ex vivo with PBS or NAM (1 mM) for 20 h and then infected with pre-opsonized *S. aureus* for the indicated times. CFU/mL reflects the number of bacteria recovered at the indicated times. Data are means ± s.e.m.

Supplementary Figure 15. Effect of NAM on clearance of *S. aureus* by BMDM. BMDM were isolated from WT mice (n=3), treated with PBS or NAM (1 mM) for 20 h, and then infected with *S. aureus* at the indicated MOI. Gentamicin was added after 30 min of infection to kill all extracellular bacteria. CFU/mL reflects the number of surviving intracellular bacteria recovered at 24 h p.i. Data are means ± s.e.m.; *** $P < 0.001$.

Supplementary Figure 16. Effect of NAM on in vivo clearance of a lower inoculum of *S. aureus* by WT mice depleted of neutrophils. WT mice were treated daily with NAM (250 mg/kg/day, i.p.) or with PBS (control), beginning 24 h prior to systemic (i.p.) infection with ~$9x10^5$ CFU *S. aureus*. Neutrophil depletion was performed in parallel. CFU count in spleen and kidneys of WT mice at 48 h p.i. Dashed line indicates the limit of detection. Red bar indicates mean. Comparing PBS and NAM-treated WT mice depleted of neutrophils, $P > 0.05$ for both spleen and kidneys.

Supplementary Figure 17. NAM treatment does not induce granulocytosis in WT mice. WT mice (see table for numbers of mice used) were treated in vivo with NAM (250 mg/kg/d, i.p.) or PBS (control) for **(A)** 24 h, **(B)** 48 h, or **(C)** 72 h, at which time CBC analysis with automated differential was performed on the mouse blood.

Supplementary Figure 18. NAM increases acetylation of C/EBP*ε* in in human neutrophils. Neutrophils isolated from the peripheral blood of three healthy human volunteers were treated with 10 mM NAM for 6 h. Lysates were subjected to immunoprecipitation (*IP*) with an antibody against pan-acetylated lysine residues (*acetyl-Lys*), followed by Western blot (*WB*) with an antibody against C/EBP*ε*. Acetylation of C/EBP*ε* increased 2-fold in the 10 mM NAM-treated samples.

Supplementary Figure 19. NAM increases levels of C/EBP*ε*, LTF, and CAMP in human neutrophils. (A-B) Neutrophils were isolated from the blood of healthy human volunteers and then treated ex vivo with PBS or NAM (1 mM) for the indicated times. As indicated by Western blot, levels of C/EBP*ε*, LTF and CAMP were increased in the lysates of NAM-treated versus PBS-treated neutrophils. Fold-changes indicated: 20 h versus 0 h. *[Note that C/EBPε in **(B)** is shown in Figure 3A. **(B)** illustrates the remainder of the protein expression data].*

Supplementary Figure 20. Increased levels of C/EBP*ε*, LTF, and CAMP in human neutrophils isolated from NAM-treated blood. Blood from three healthy human volunteers (**A-C,** respectively) was treated ex vivo with PBS or NAM (1 mM) for the indicated times, at which point neutrophils were isolated. As indicated by Western blot, levels of C/EBP*ε*, LTF and CAMP were increased in the lysates of neutrophils isolated from NAM-treated versus PBS-treated human blood. Fold-changes indicated: 20 h versus 0 h.

Supplementary Figure 21. NAM does not enhance ROS production by *S. aureus*-infected neutrophils. (A) Neutrophils isolated from blood of WT mice (pooled from n=9 mice) were plated in 96-well plates ($5x10^4$ cells/well), treated with PBS or NAM (1 mM) for 18 h, and then infected with *S. aureus* at MOI 20, 12, or 6 (bacteria:macrophage) in triplicate in the presence of ROS detection reagent (Luminol). Luminescence was measured at 30, 60, 90, and 120 min. **(B)** Levels of gp91phox (NOX2) in human neutrophils treated with NAM. Neutrophils were isolated from the blood of three healthy human volunteers and then treated ex vivo with PBS or NAM (1 mM) for the indicated times. As indicated by the representative Western blot, levels of gp91phox (NOX2; large subunit of flavocytochrome b) were unchanged in the lysates of NAM-treated versus PBS-treated neutrophils.

Supplementary Figure 22. NAM increases acetylation of histone H3 at the *Cebpe* promoter region and of C/EBP*ε* in BMDM. (A) Effect of NAM on histone acetylation in BMDM. WT BMDM were treated with 1 mM NAM for 6 h and 12 h followed by ChIP using an antibody against acetylated histone H3 (*Ac-H3*) or *IgG* (negative control). The samples were analyzed by PCR using primers specific for the *Cebpe* promoter region. The input chromatin was included as a positive control using primers for the *Actb* gene. After 12 h of treatment with NAM, histone acetylation increased 5-fold compared to untreated BMDM. **(B)** Effect of NAM on acetylation of C/EBP*ε* protein in BMDM. Lysates from BMDM treated with 1 mM NAM (*Left*) or 10 mM NAM (*Right*) for 6 h were subjected to immunoprecipitation (*IP*) with an antibody against pan-acetylated lysine residues (*acetyl-Lys*), followed by Western blot (*WB*) with an antibody against C/EBP*ε*. Acetylation of C/EBP*ε* increased 4-fold in the 1 mM NAM-treated samples.

Supplementary Figure 23. NAM increases mRNA and protein level of C/EBP*ε* and downstream antimicrobials in BMDM. (A) mRNA expression of *Cebpe* and the downstream target genes *Camp* and *Ltf* after treatment of BMDM with 1 mM NAM over time. **(B)** Levels of C/EBP*ε*, Ltf, and Camp in BMDM from WT mice. Western blot shows an increase in expression of C/EBP*ε* and Ltf after addition of 1 mM NAM to WT BMDM for 12 h compared to control (PBS).

Supplementary Figure 24. Effect of NAM on *LTF* reporter gene activity. A reporter assay was performed using U937 pro-monocytic cells transiently transfected with either a reporter construct containing a gDNA fragment of the *LTF*-promoter (*LAC-LUC*), or control vehicle (*pGL3*). U937 cells were treated with 1 mM NAM or PBS for 16 h. Data are representative of three independent experiments; data are means ± s.e.m.

Supplementary Figure 25. NAM increases mRNA expression of *Cebpe* and downstream antimicrobials in BMMC. (A) mRNA expression of *Cebpe, Camp,* and *Ltf* in BMMC isolated from NAM-treated WT mice. Non-infected WT mice (n=4) received either NAM (250 mg/kg/day, i.p.) or PBS (control). After 72 h, BMMC were extracted and real-time RT-PCR expression analysis was performed. **(B)** mRNA levels of *Cebpe, Camp,* and *Ltf* in BMMC at 48 h p.i. Representative data (means ± s.e.m.) of 4 out of the 9 mice per treatment group are shown in **A** and **B**.

Supplementary Figure 26. Increased levels of C/EBP*ε*, Ltf, and Camp in BMMC from NAM-treated WT mice. BMMC were isolated from WT mice (n=3/group/timepoint) after mice had been treated in vivo with either NAM (250 mg/kg/d) or PBS (control) for the indicated times. Indicated fold-changes were determined by densitometry.

Supplementary Figure 27. NAM increases acetylation of histone H3 at the *Cebpe* promoter region and of C/EBP*ε* in BMMC. (A) BMMC harvested from WT mice were treated with 1 mM NAM for 6 h and 12 h, followed by ChIP analysis using an antibody against acetylated histone H3 (*Ac-H3*) or *IgG* (negative control). The samples were analyzed by RT-PCR using primers specific for the *Cebpe* promoter region, and the *input* chromatin was included as a positive control using primers for the *Actb* gene. By 12 h, histone acetylation increased 3-fold compared to untreated BMMC. Data are representative of two independent experiments performed in triplicate (n=3 mice). **(B)** Blots display IP results from BMMC treated with 1 mM (*Left*) and 10 mM (*Right)* NAM. Acetylation of *C/EBPε* increased 3-fold in the 1 mM NAM treated samples, and 2-fold in the 10 mM treated samples. Data are representative of two independent experiments performed in triplicate (n=3 mice).

Supplementary Figure 28. No differences in Il17 or Ifng following T-cell stimulation. Splenocytes taken from WT mice administered NAM or PBS daily for three days with or without *S. aureus* infection, do not secrete different levels of **(A)** Il17a or **(B)** Ifng following in vitro restimulations.