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SUPPLEMENTARY INFORMATION

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SERPINB1-mediated checkpoint of inflammatory caspase activation

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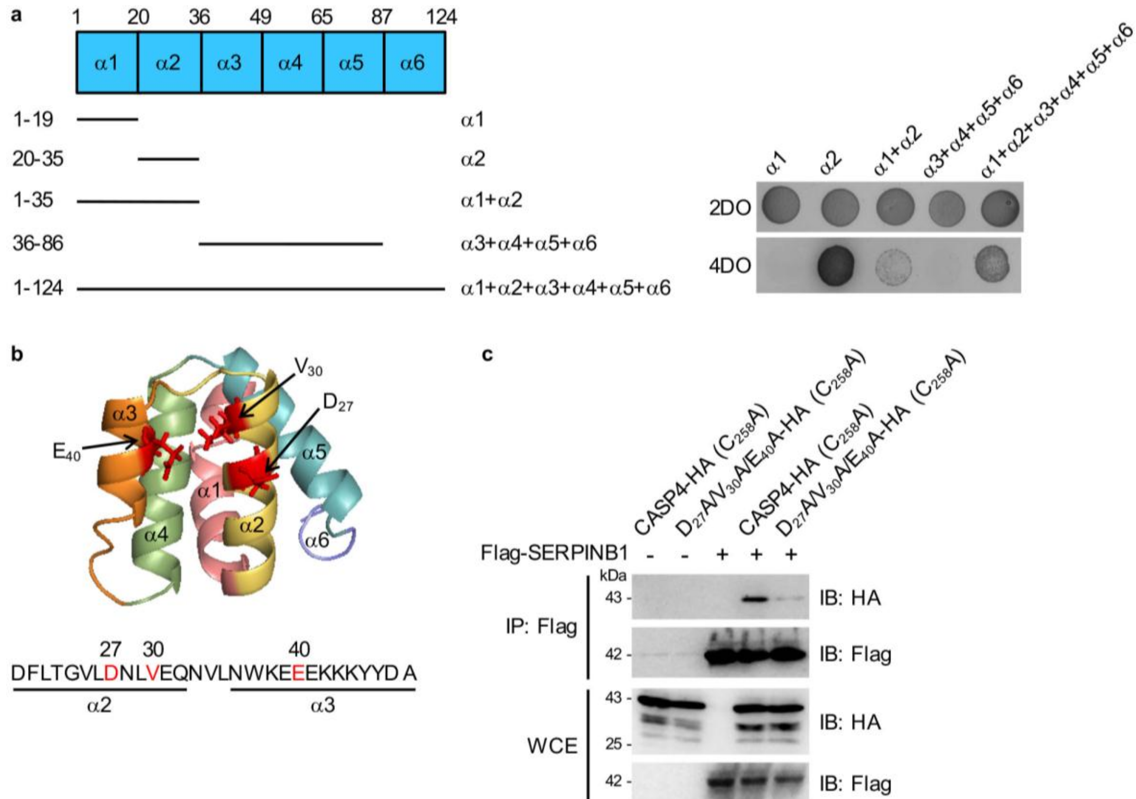
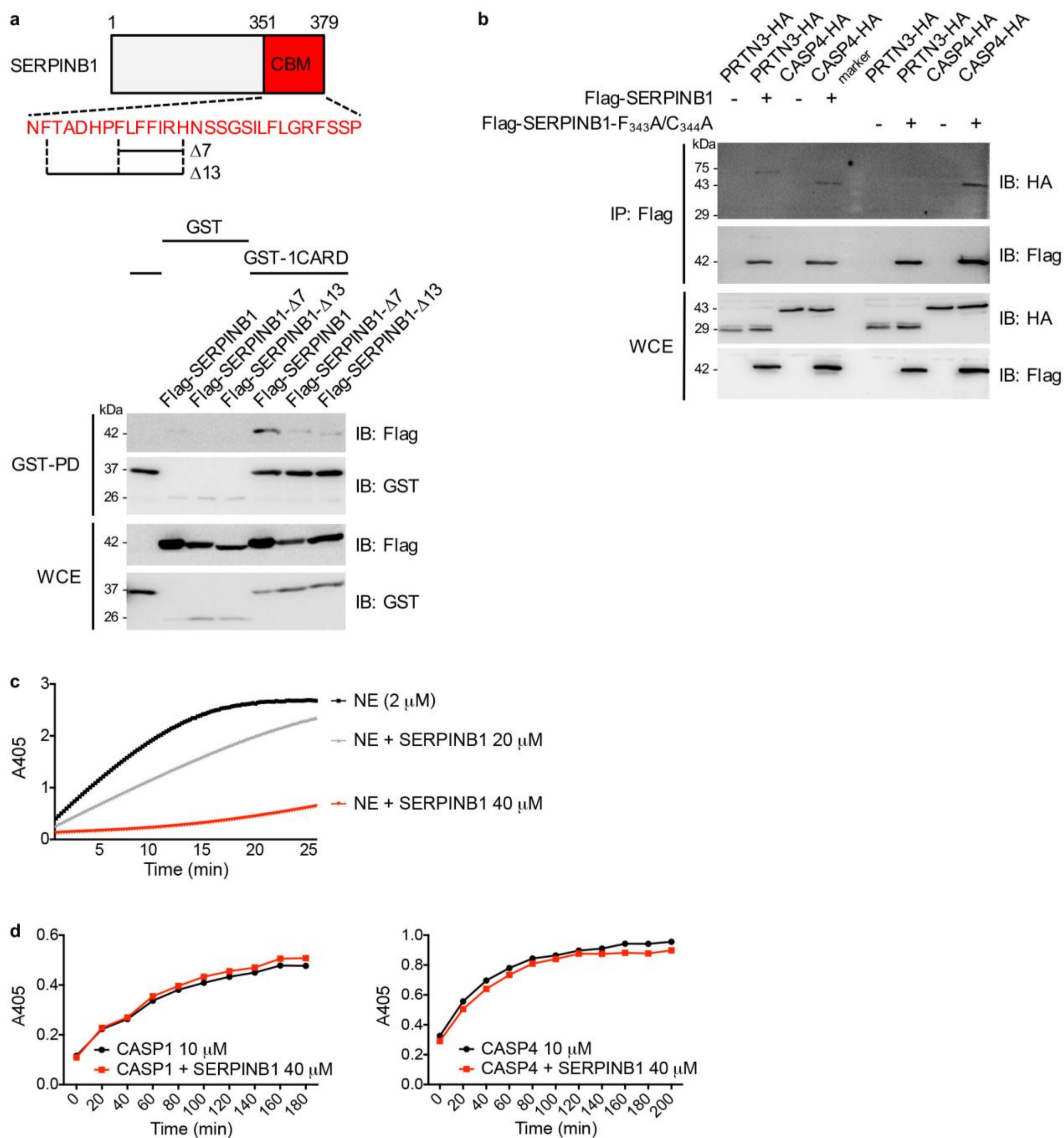


Supplementary Figure 1

Caspase-4-CARD region responsible for SERPINB1 binding

a, Schematic diagram of caspase-4-CARD consisting of six α-helices (α1-α6). The full-length and truncated forms of caspase-4-CARD were co-transformed with the SERPINB1 carboxy-terminal region (aa 330-379) to yeast for the growth on two or four dropout (DO) plates. **b**, I-TASSER prediction of α-helical bundles of caspase-4-CARD. Model 1, among top five models generated by I-TASSER, with a C-score of 0.38, estimated TM-score of 0.76 ± 0.10, and estimated RMSD of 2.9 ± 2.1 Å**.** Three residues in α-helices 2 and 3 regions ($D_{27}/V_{30}/E_{40}$) marked with red color are predicted as a ligand binding site. Structure was prepared using PyMOL. **c**, The loss of SERPINB1-binding activity of caspase-4-CARD mutant. HA-tagged full-length enzymatically inactive caspase-4 containing wild-type CARD or mutant CARD ($D_{27}A/V_{30}A/E_{40}A$), and Flag-tagged SERPINB1 were transfected to 293T cells, and whole-cell extracts (WCEs) were subjected to co-immunoprecipitation (IP) with anti-Flag, followed by immunoblotting (IB) with anti-HA or anti-Flag antibody. Data in **a**,**c** are representative of two independent experiments.

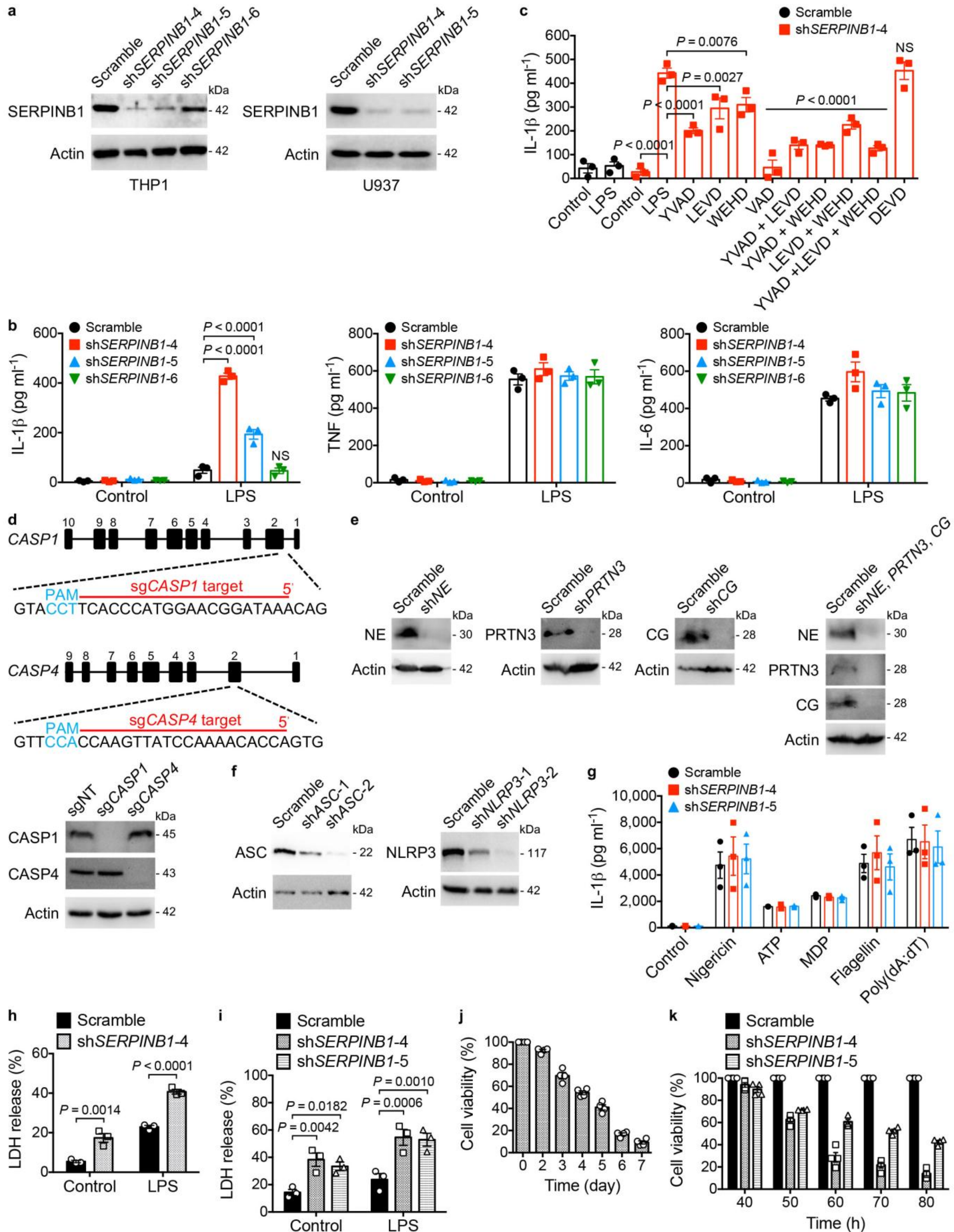


Supplementary Figure 2

The CARD-binding-motif of SERPINB1 targets caspase-1/-4

a, Schematic diagram of SERPINB1 deletion mutant constructs. GST-pulldown (GST-PD) assay of SERPINB1 deletion mutant (Δ7 and Δ13) binding to caspase-1-CARD. Flag-tagged SERPINB1 wild-type, Δ7, or Δ13 and GST-caspase-1-CARD were transfected to 293T cells, and WCEs were subjected to GST-pulldown, followed by immunoblotting (IB) using anti-Flag or anti-GST antibody. **b**, Co-immunoprecipitation (IP) assay of SERPINB1 wild-type or RCL mutant ($F_{343}A/C_{344}A$) binding to proteinase-3 (PRTN3) or caspase-4. Flag-SERPINB1 wild-type or $F_{343}A/C_{344}A$ and PRTN3-HA or caspase-4-HA were transfected to 293T cells, and WCEs were subjected to co-immunoprecipitation with anti-Flag, followed by immunoblotting using anti-HA or anti-Flag antibody. % indicates acrylamide percentage. **c**, Neutrophil elastase (NE) enzymatic assay; NE was incubated with SERPINB1 and the reactions were diluted into assay

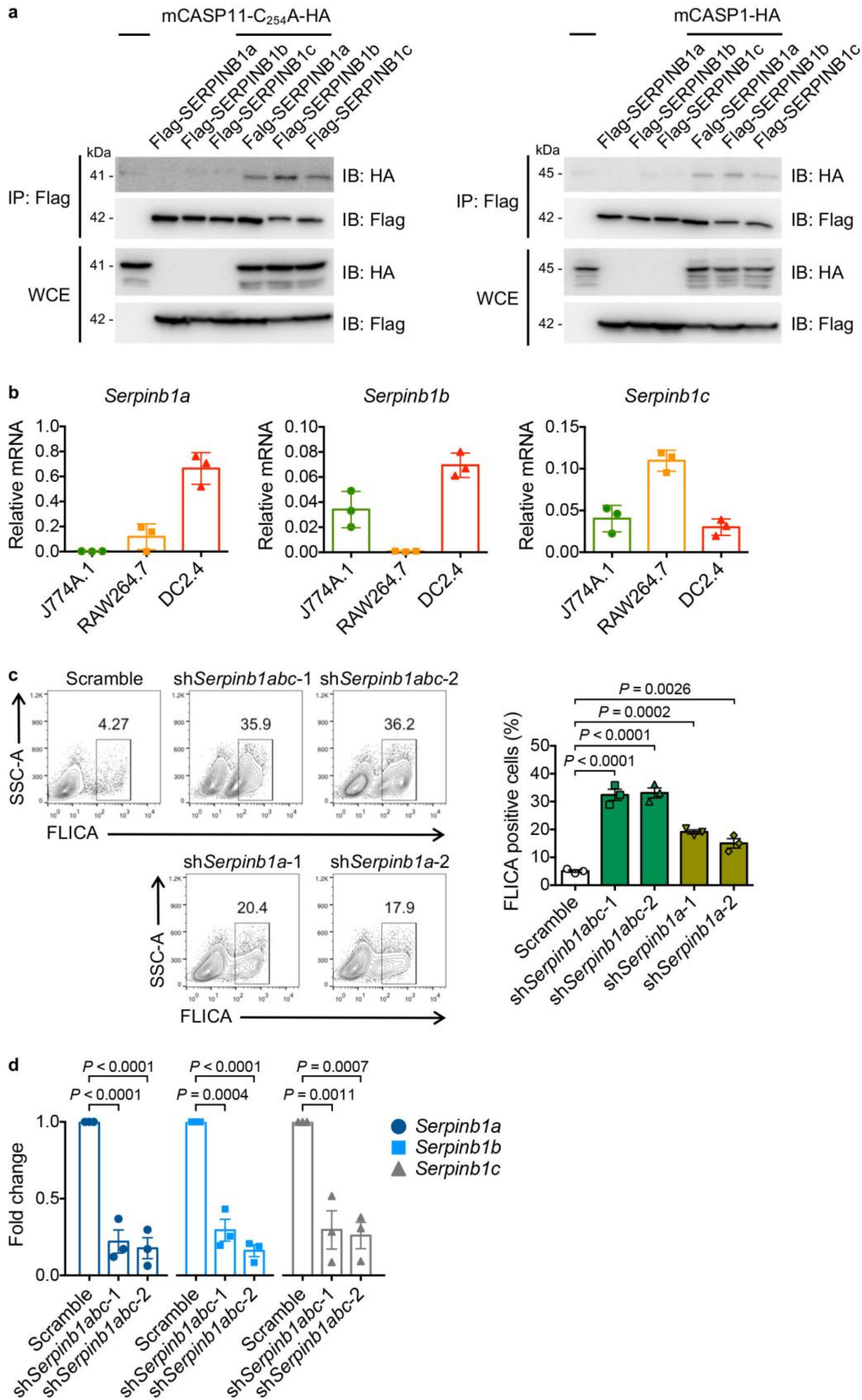
buffer containing chromogenic substrate (MeO-SucAAPV-pNA). Free p-nitroanilide (pNA) after cleavage from the substrate was measured at 400 nm. **d**, Caspase-1 or caspase-4 enzymatic assay; caspase-1 or caspase-4 was incubated with SERPINB1 and the reactions were diluted into assay buffer containing substrate (caspase-1; Ac-YVAD-pNA, caspase-4; Ac-LEVD-pNA). The reaction mixture was further incubated at 37 °C for hydrolysis of substrate and residual protease activity was quantified at the indicated time points. Data in **a**,**b**,**c**,**d** are representative of two independent experiments.


a
Scramble
shSERPINB1-4
shSERPINB1-5
shSERPINB1-6
kDa
SERPINB1
Actin
THP1
U937
b
Scramble
shSERPINB1-4
shSERPINB1-5
shSERPINB1-6
IL-1β (pg ml-1)
TNF (pg ml-1)
IL-6 (pg ml-1)
P < 0.0001
P < 0.0001
NS
Control
LPS
c
IL-1β (pg ml-1)
P = 0.0076
P = 0.0027
P < 0.0001
P < 0.0001
P < 0.0001
NS
Control
LPS
YVAD
LEVD
WEHD
VAD
YVAD + LEVD
YVAD + WEHD
LEVD + WEHD
YVAD +LEVD + WEHD
DEVD
d
CASP1
PAM
sgCASP1 target
5'
GTACCTTCACCCATGGAACGGATAAACAG
CASP4
sgCASP4 target
GTTCCACCAAGTTATCCAAAACACCAGTG
sgNT
sgCASP1
sgCASP4
CASP1
CASP4
Actin
e
shNE
shPRTN3
shCG
shNE, PRTN3, CG
NE
PRTN3
CG
f
shASC-1
shASC-2
ASC
shNLRP3-1
shNLRP3-2
NLRP3
g
IL-1β (pg ml-1)
Control
Nigericin
ATP
MDP
Flagellin
Poly(dA:dT)
h
LDH release (%)
P = 0.0014
P < 0.0001
i
P = 0.0182
P = 0.0042
P = 0.0010
P = 0.0006
j
Cell viability (%)
Time (day)
k
Cell viability (%)
Time (h)

Supplementary Figure 3

SERPINB1 depletion-induced IL-1β secretion and cytotoxicity

a, Validation of silencing efficiency of three shRNAs targeting *SERPINB1* in THP1 and U937. Cells transiently transduced with scramble or sh*SERPINB1* lentivirus for 48 h and WCEs were immunoblotted with anti-SERPINB1 antibody. **b**, IL-1β, TNF and IL-6 cytokine secretion upon *SERPINB1* depletion. **c**, Effects of caspase inhibitors on IL-1β secretion upon *SERPINB1* depletion. **d**, Generation of *caspase-1* or *caspase-4* knockout cells. Location of sgRNA used for CRISPR/Cas9-mediated editing of the *caspase-1* and *caspase-4* locus. The sgRNA target and PAM sequences are shown in red and blue, respectively. **e**,**f**, Validation of silencing efficiency of shRNAs targeting neutrophil elastase (*NE*), proteinase-3 (*PRTN3*), and/or cathepsin G (*CG*), *ASC* or *NLRP3* in THP1. Whole-cell lysates (WCL) were immunoblotted with indicated antibodies. **g**, IL-1β release upon inflammasome stimulus in *SERPINB1*-depleted cells. **h**,**i**, Lactate dehydrogenase (LDH) release-based cytotoxicity of THP1 and U937 cells after *SERPINB1* depletion. Cytotoxicity was measured at 3 days post-transduction. **j**,**k**, ATP-based cell viability of THP1 and U937 cells upon *SERPINB1* depletion. Cells were transduced by scramble or sh*SERPINB1* lentivirus for 48 h. Cell viability was determined at the indicated time point after lentiviral transduction. Data in **a**,**d**,**e**,**f** are representative of two independent experiments. Data are presented as mean ± s.e.m. from n=3 independent experiments in **b**,**c**,**g**,**h**,**i** and from n=4 pooled from two independent experiments in **j**,**k**. *P* values were determined by two-way analysis of variance (ANOVA) with Bonferroni's comparison relative to scramble in **b**,**h**,**i**, and by one-way ANOVA with Dunnett's comparison relative to sh*SERPINB1*-4 LPS in **c**. NS, not significant.

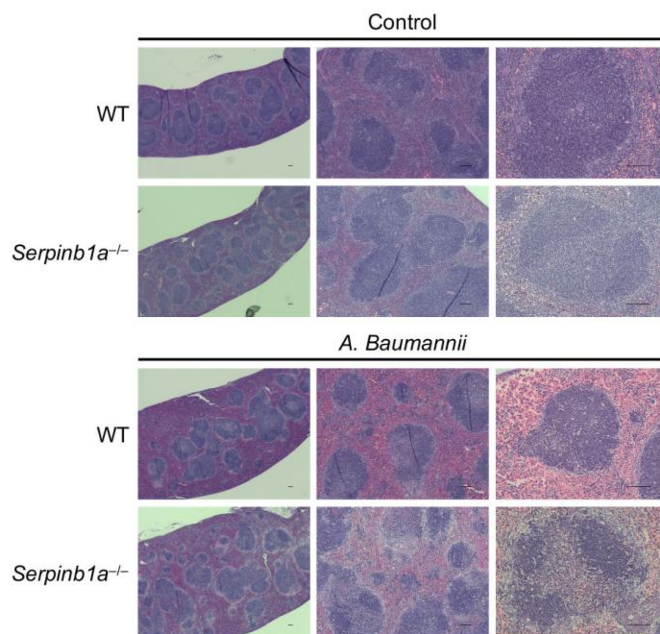


Supplementary Figure 4

Supplementary Figure 4

Murine SERPINB1 isoforms for murine caspase-1/-11 inhibition

a, Interaction between murine SERPINB1a,b,c and caspase-1/-11. HA-tagged caspase-1-C_{254}A or caspase-1 and Flag-tagged SERPINB1a,b,c were transfected to 293T cells, and WCEs were subjected to co-immunoprecipitation (IP) with anti-Flag, followed by immunoblotting (IB) using anti-HA or anti-Flag antibody. **b**, Relative mRNA quantification of murine *Serpinb1a,b,c* in myeloid cell lines. *Gapdh* was used as a normalization control. **c**, Detection of caspase-1 activation upon *Serpinb1a,b,c* depletion in DC 2.4 cells. **d**, Verification of *Serpinb1*-targeting shRNA silencing efficiency by quantitative PCR with reverse transcription (qRT-PCR) in BMDMs. mRNA expression was normalized to *18S* and fold change was calculated relative to scramble. Data in **a** are representative of two independent experiments. Data are presented as mean ± s.e.m. from n=3 independent experiments in **b**,**c**,**d**. *P* values were determined by one-way ANOVA with Dunnett's comparison relative to scramble in **c**,**d**.



Supplementary Figure 5

Supplementary Figure 5

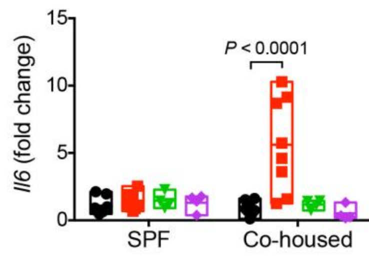
Spleen histopathology during *A. baumannii* infection

Hematoxylin-eosin-stained spleen sections from wild-type (WT) and *Serpinb1a*$^{-/-}$ mice at 6 hpi. Images are representative of two analyzed mice for each condition. Bar, 100 μm.

a

- WT
- *Serpinb1a*$^{-/-}$
- *Casp1*$^{-/-}$*Casp11*$^{-/-}$
- *Serpinb1a*$^{-/-}$*Casp1*$^{-/-}$*Casp11*$^{-/-}$

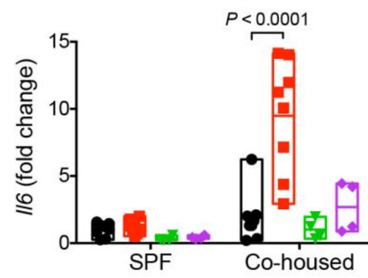
Blood



b

- WT
- *Serpinb1a*$^{-/-}$
- *Casp1*$^{-/-}$*Casp11*$^{-/-}$
- *Serpinb1a*$^{-/-}$*Casp1*$^{-/-}$*Casp11*$^{-/-}$

Lung

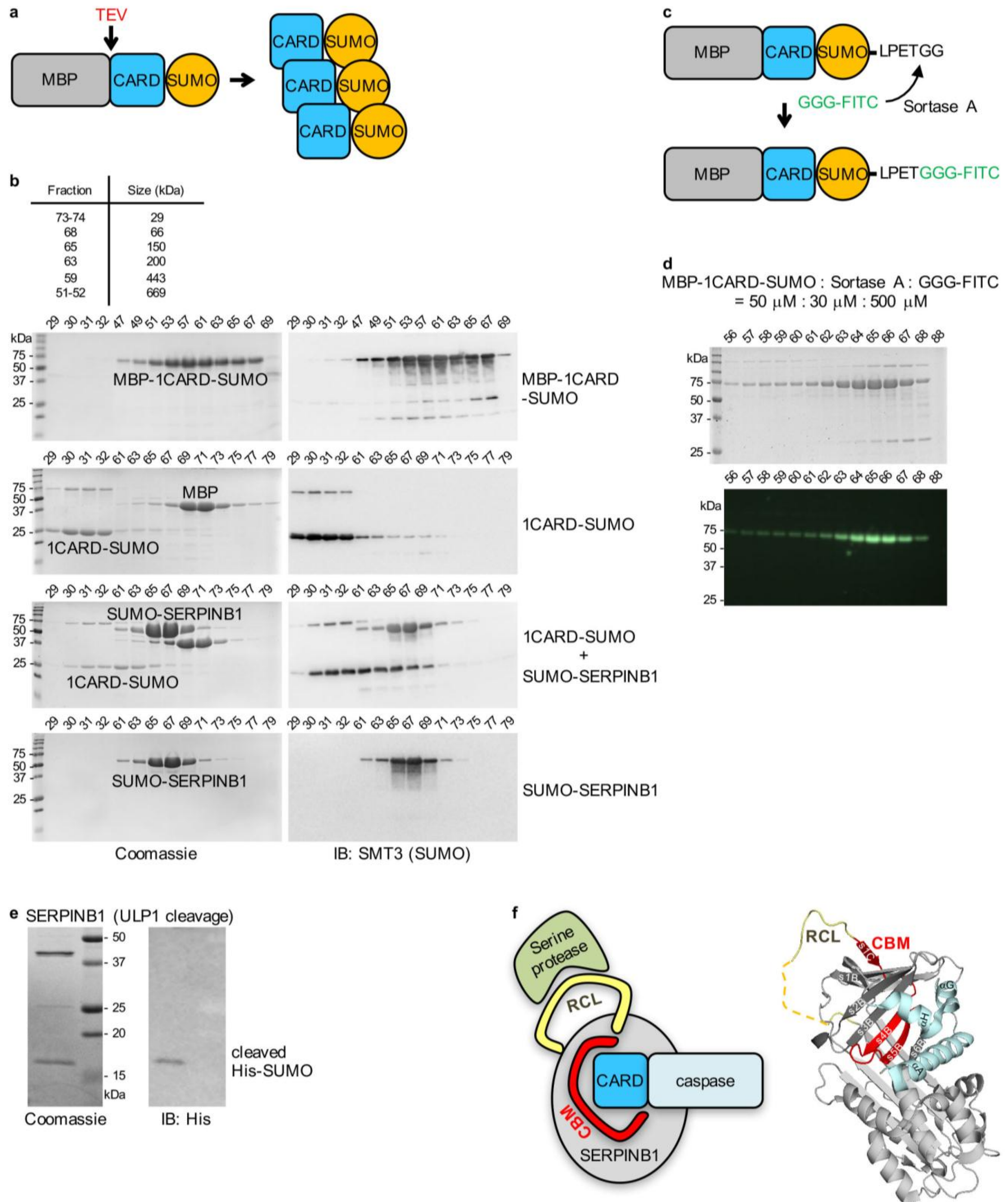


Supplementary Figure 6

Supplementary Figure 6

Gene expression of *Serpinb1a*$^{-/-}$ mice upon co-housing

a,**b**, Peripheral leukocytes and lung *Il6* mRNAs of wild-type (WT), *Serpinb1a*$^{-/-}$, *Casp1*$^{-/-}$*Casp11*$^{-/-}$ and *Serpinb1a*$^{-/-}$*Casp1*$^{-/-}$*Casp11*$^{-/-}$ mice either housed in SPF facility or co-housed with pet-store mice (n=8 for wild-type or *Serpinb1a*$^{-/-}$, n=4 for *Casp1*$^{-/-}$*Casp11*$^{-/-}$ or *Serpinb1a*$^{-/-}$*Casp1*$^{-/-}$*Casp11*$^{-/-}$). mRNA expression was normalized to *18S* and fold change was calculated relative to the average of SPF-housed wild-type mice. Data are presented as floating bars (min to max) with line at mean. *P* values were determined by two-way ANOVA with Bonferroni's comparison relative to co-housed wild-type mice.



Supplementary Figure 7

Protein purification and Sortase A-mediated FITC labeling

a, Schematic diagram of a sandwich-tagged caspase-1-CARD protein oligomerization assay. **b**, SDS-PAGE of size-exclusion chromatography fractions was either stained with Coomassie Blue (left) or immunoblotted with anti-SMT3 antibody (right). **c**, Schematic diagram of Sortase A-mediated fluorescein isothiocyanate- (FITC)-conjugation of the sandwich-tagged caspase-1-CARD protein. **d**, Sortase A-mediated FITC labeling. MBP-1CARD-SUMO, Sortase A and GGG-FITC peptide were incubated at the indicated ratio, followed by size-exclusion chromatography. The indicated FITC labeled fractions were visualized (bottom). The corresponding SDS-PAGE Coomassie Blue gel is shown (top). **e**, The SUMO cleavage from His-SUMO-SERPINB1 by ULP1 protease to exclude the potential SUMO-SUMO interaction. After ULP1 protease treatment, protein was either stained with Coomassie Blue (left) or immunoblotted with anti-His antibody (right). **f**, Illustration of SERPINB1 function as a protease inhibitor with dual specificity. SERPINB1 binds to neutrophil serine protease through the RCL domain and interacts with inflammatory caspase through the CBM region. Human native SERPINB1 structure; PDB 4GA7. The CBM of SERPINB1 consists of β-strands (s1C, s4B and s5B) depicted in red color. The surrounding α-helices (αH, αG and αA) are colored in light cyan and β-strands (s3B, s2B, s1B and s6B) are shown in dark gray. The RCL of SERPINB1 is shown in yellow dashed lines indicate missing residues of the RCL in the structure. Structure was prepared using PyMOL. Data in **b**,**d**,**e** are representative of two independent experiments.

Supplementary Table 1: List of shRNAs

Organism	Name	Sense primer (5'–3')
Human	sh*SERPINB1*-4	GGCGTTGAGTGAGAACAATCC
Human	sh*SERPINB1*-5	GCTACATCGAGGACCTTAAGT
Human	sh*SERPINB1*-6	GCATCGCAACTTTCTGCATGT
Human	sh*NE*	GATCGACTCTATCATCCAACG
Human	sh*PRTN3*	GAACAAACTGAACGACGTTCT
Human	sh*CG*	GCCACCCTCAATATAATCAGC
Human	sh*ASC*-1	GATGCGGAAGCTCTTCAGTTT
Human	sh*ASC*-2	GCTCTTCAGTTTCACACCAGC
Human	sh*NLRP3*-1	CAGGTTTGACTATCTGTTCTA
Human	sh*NLRP3*-2	GAAGTGAAAGCCAAAGCTAAA
Mouse	sh*Serpinb1a*,*b*,*c*-1	GACCTGAAGTGCAAGGTGCTG
Mouse	sh*Serpinb1a*,*b*,*c*-2	GAACTTAGCATGGTCATTCTG
Mouse	sh*Serpinb1a*-1	GGACAGTATGACCAAACTTGT
Mouse	sh*Serpinb1a*-2	GCTATACCCTCAACTCTAACC

shRNA sequences were designed by Invitrogen Block-iT RNAi and cloned into pLKO.1-TRC vector.

Supplementary Table 2: List of sgRNAs

Gene	sequence
Human *CASP1*	T TTATCCGTTCCATGGGTGA
Human *CASP4*	T GGTGTTTTGGATAACTTGG

sgRNA sequences were cloned into pL-CRISPR.EFS.PAC vector.

Supplementary Table 3: List of qRT-PCR primers

Gene	Sense primer (5'-3')	Antisense primer (5'-3')
Mouse *18S*	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
Mouse *Gapdh*	AAGGTCATCCCAGAGCTGAA	CTGCTTCACCACCTTCTTGA
Mouse *Serpinb1a*	ATACCCTCAACTCTAACCTGG	CATACAGAATGTAGCAATGCC
Mouse *Serpinb1b*	ACTCAACTCTAACCTGTGCTG	TCTCGCAGAGCACCTGAATAA
Mouse *Serpinb1c*	CATCCTCAACTCTAACCTGGG	CAGCATCCAACAACAGTGCCT
Mouse *Il6*	TCCAGTTGCCTTCTTGGGAC	GTACTCCAGAAGACCAGAGG
Mouse *Cox2*	CCAGCACTTCACCCATCAGTT	ACCCAGGTCCTCGCTTATGA

Primers were designed by NCBI/Primer-BLAST.

Supplementary Table 4: Genes in volcano plot in Fig. 6e

Gene	log_2 (fold change)	Linear fold change	*P* values	Gene sets
Ccl3	2.11	4.32	0.000000674	Chemokine signaling, TLR signaling
Cdh1	3.27	9.68	0.00000109	Cell migration and adhesion, ECM remodeling, Lymphocyte activation
Hgf	2.15	4.45	0.00000396	Cytokine signaling, Growth factor signaling
Ms4a2	3.9	14.9	0.00000448	Fc receptor signaling, Lymphocyte activation
Chil3	3.03	8.14	0.0000184	Pathogen response
Chil4	3.08	8.47	0.0000197	Pathogen response
Fcer1a	3.44	10.8	0.000025	Fc receptor signaling, Lymphocyte activation
Gata2	2.01	4.03	0.0000277	Differentiation and maintenance of myeloid cells
Clec5a	1.32	2.49	0.0000329	Lymphocyte activation
Mmp8	2.75	6.73	0.0000542	ECM remodeling
Cpa3	3	8.02	0.0000818	Metabolism
Il1r2	2.67	6.36	0.000127	Cytokine signaling, Growth factor signaling
Il1rl1	3.1	8.55	0.000202	Cytokine signaling
Cx3cr1	-1.74	0.3	0.000105	Chemokine signaling
Hpgd	-1.48	0.359	0.000805	Metabolism
Il5ra	-1.01	0.498	0.000845	Angiogenesis, Cytokine signaling, Fc receptor signaling, Growth factor signaling, Lymphocyte activation
Pdfgb	-1.51	0.351	0.00173	Antigen presentation, ECM remodeling, Metabolism, TLR signaling
Cd36	-1.99	0.252	0.00375	Antigen presentation, ECM remodeling, Metabolism, TLR signaling

P values were determined by two-tailed unpaired *t*-test.