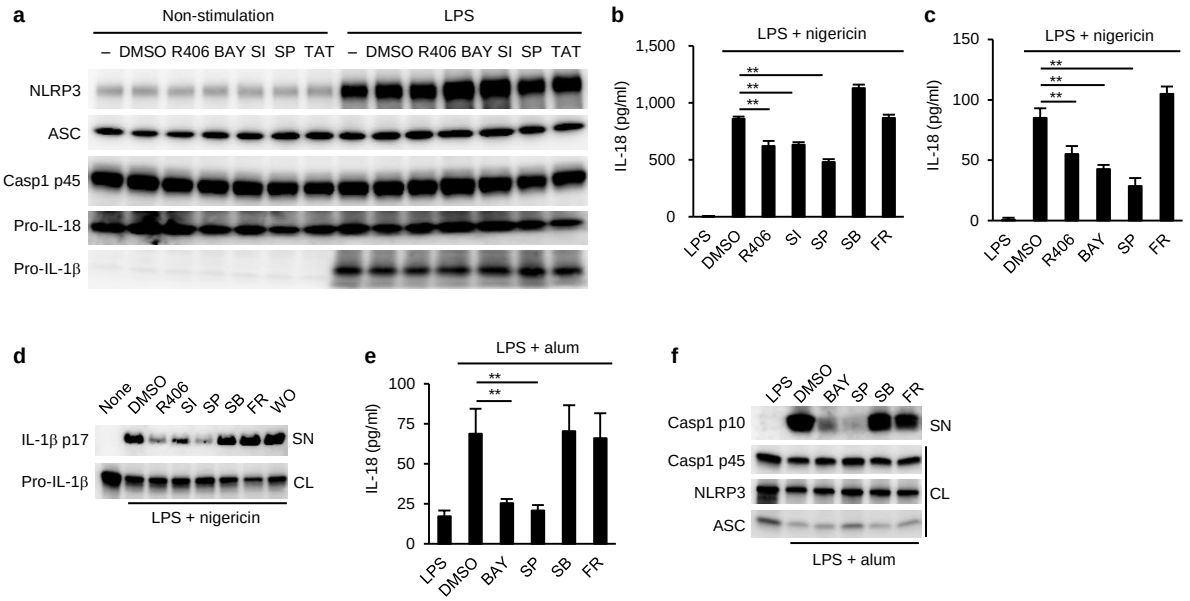
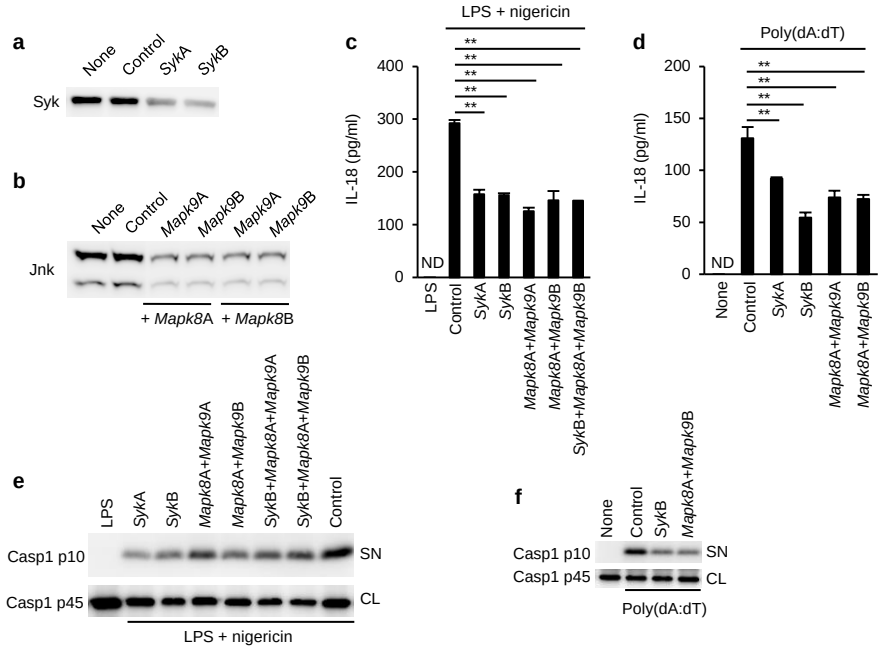
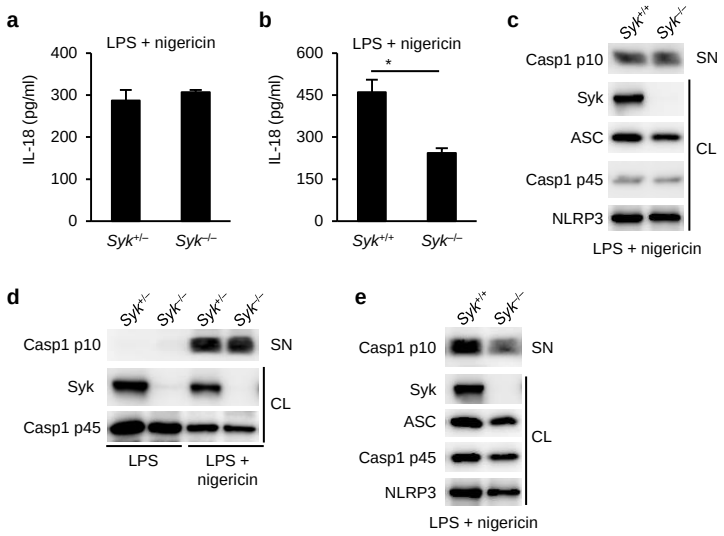


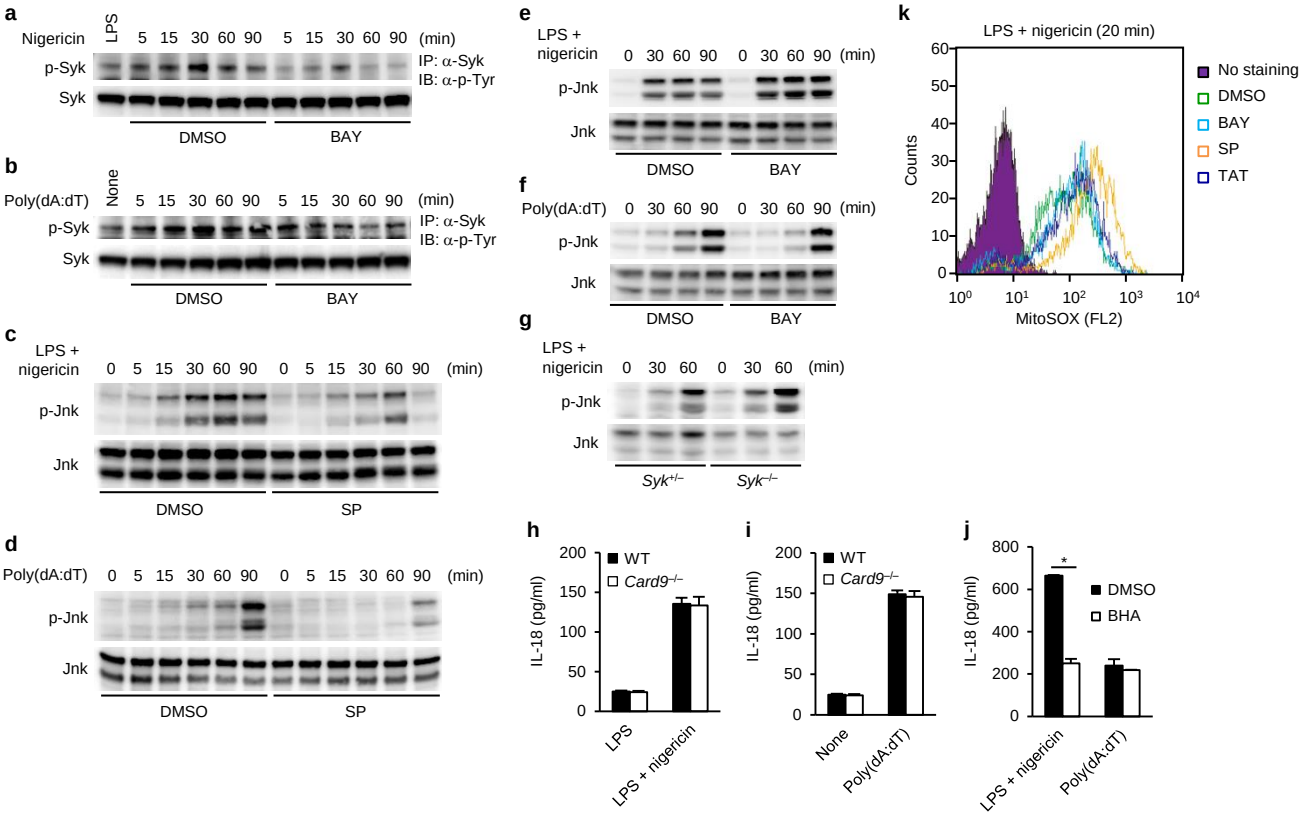
Supplementary Figure 1 Inhibition of Syk or JNK do not affect the expression of inflammasome molecules. (**a,d,f**) Immunoblotting of inflammasome molecules or (**b,c,e**) ELISA of IL-18 in peritoneal macrophages (**a,d–f**), BMDM (**b**), or U937 cells (**c**) primed with LPS for 4 h (**a**), followed by stimulation with nigericin for 90 min (**b–d**), or alum for 6 h (**e,f**). The indicated kinase inhibitors were added to the cultures 1 h before stimulation. CL, cell lysates; SN, supernatants. Data are shown as the means ± s.d. of triplicate samples of one experiment representative of three independent experiments. Data were analyzed by one-way ANOVA with Bonferroni multiple comparison test (**b,c,e**). * $P < 0.01$ and ** $P < 0.001$.



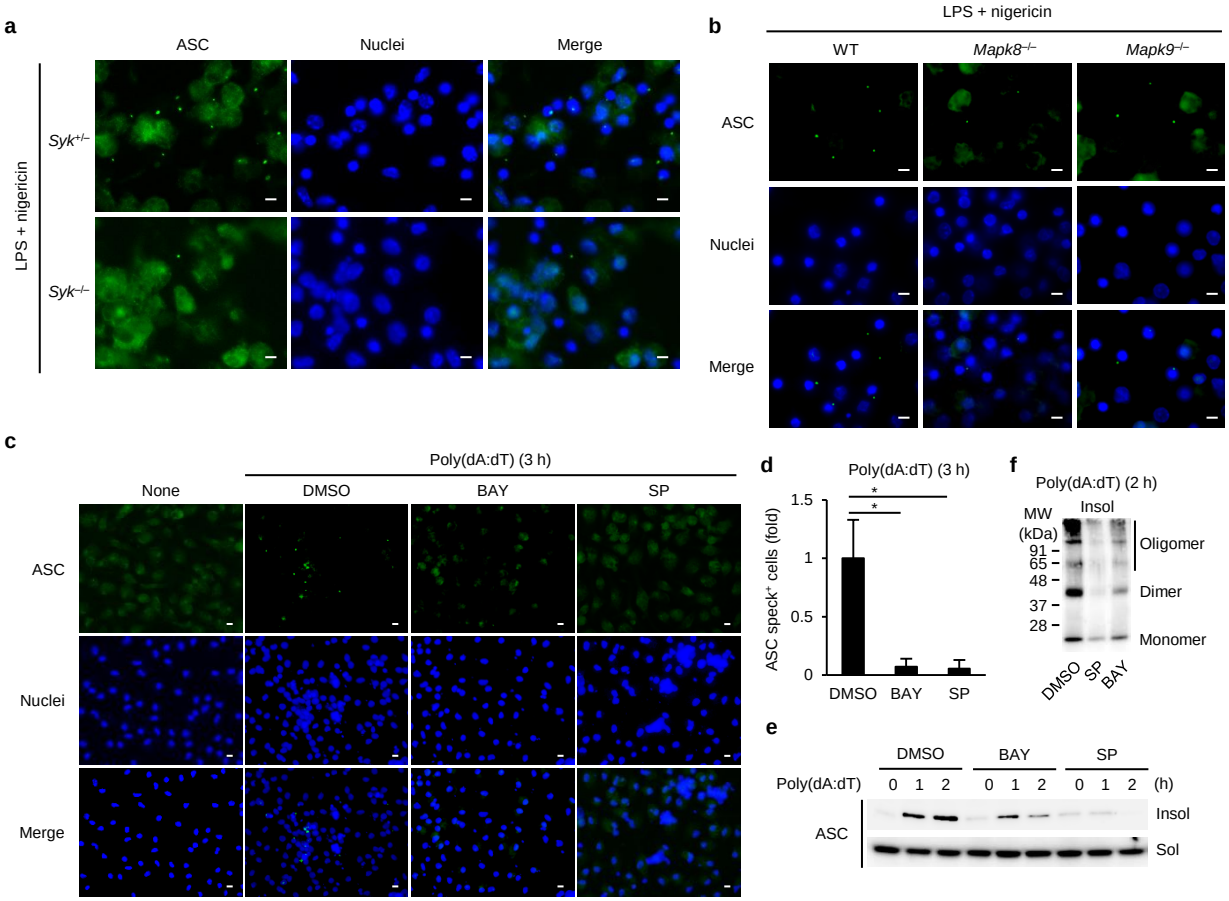
Supplementary Figure 2 Knockdown of *Syk* or *Mapk8,Mapk9* in primary macrophages. (**a,b,e,f**) Immunoblotting of caspase-1 or (**c,d**) ELISA of IL-18 in peritoneal macrophages unprimed (**a,b**), primed with LPS for 4 h, followed by stimulation with nigericin for 90 min (**c,e**), or unprimed macrophages stimulated with poly(dA:dT) for 3 h (**d,f**). Macrophages were transfected with siRNAs for 48 h. Control, negative control siRNA; CL, cell lysates; SN, supernatants; ND, not detected. Data are shown as the means ± s.d. of triplicate samples of one experiment representative of three independent experiments. Data were analyzed by one-way ANOVA with Bonferroni multiple comparison test (c,d). * $P < 0.001$.



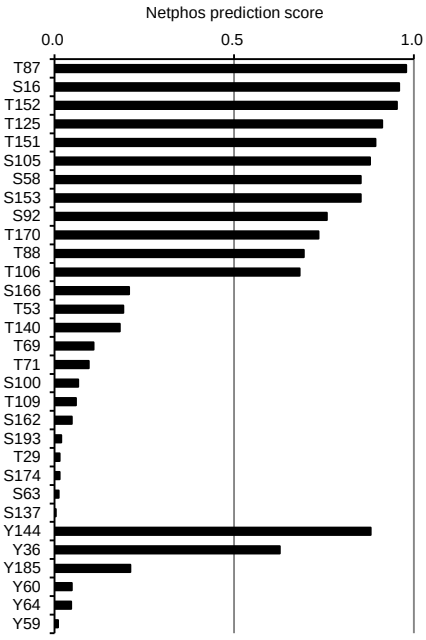
Supplementary Figure 3 Syk is not required for NLRP3 inflammasome activation in dendritic cells. (**a,b**) ELISA of IL-18 or (**c,d**) immunoblotting of inflammasome molecules in BMDCs (**a,c,d**) or BMDMs (**b,e**) primed with LPS for 4 h, followed by stimulation with nigericin for 90 min. BMDMs were prepared using L-cell conditioned medium. CL, cell lysates; SN, supernatants. Data are shown as the means ± s.d. of triplicate samples of one experiment representative of two independent experiments. Data were analyzed by two-tailed unpaired *t* test with Welch's correction (a,b). * $P < 0.001$.



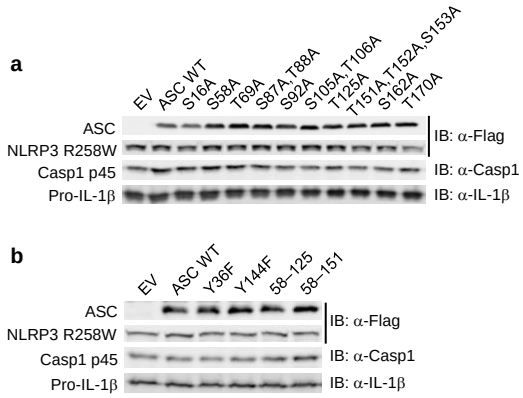
Supplementary Figure 4 Implication that Syk contributes to inflammasome activity through an unknown mechanism. (**a**–**g**) Immunoblotting of kinases, (**h**–**j**) ELISA of IL-18, or (**k**) FACS analysis of mitochondrial ROS in peritoneal macrophages primed with LPS for 4 h, followed by stimulation with nigericin for the indicated times (**a,c,e,g,h,j,k**), or unprimed macrophages stimulated with poly(dA:dT) for 3 h (**b,d,f,i,j**). The kinase inhibitors and BHA (25 μM) were added to the cultures 1 h before stimulation (**a**–**e,j,k**). The cells were incubated with nigericin for 20 min in the presence of MitoSOX (5 μM) and analyzed on a flow cytometer (**k**). Data are shown as the means ± s.d. of triplicate samples of one experiment. Data shown in **e,f,h**–**k** are representative of three independent experiments and those in **a**–**d,g** are representative of two independent experiments. Data were analyzed by two-tailed unpaired *t* test with Welch's correction (**h**–**j**). * $P < 0.001$.



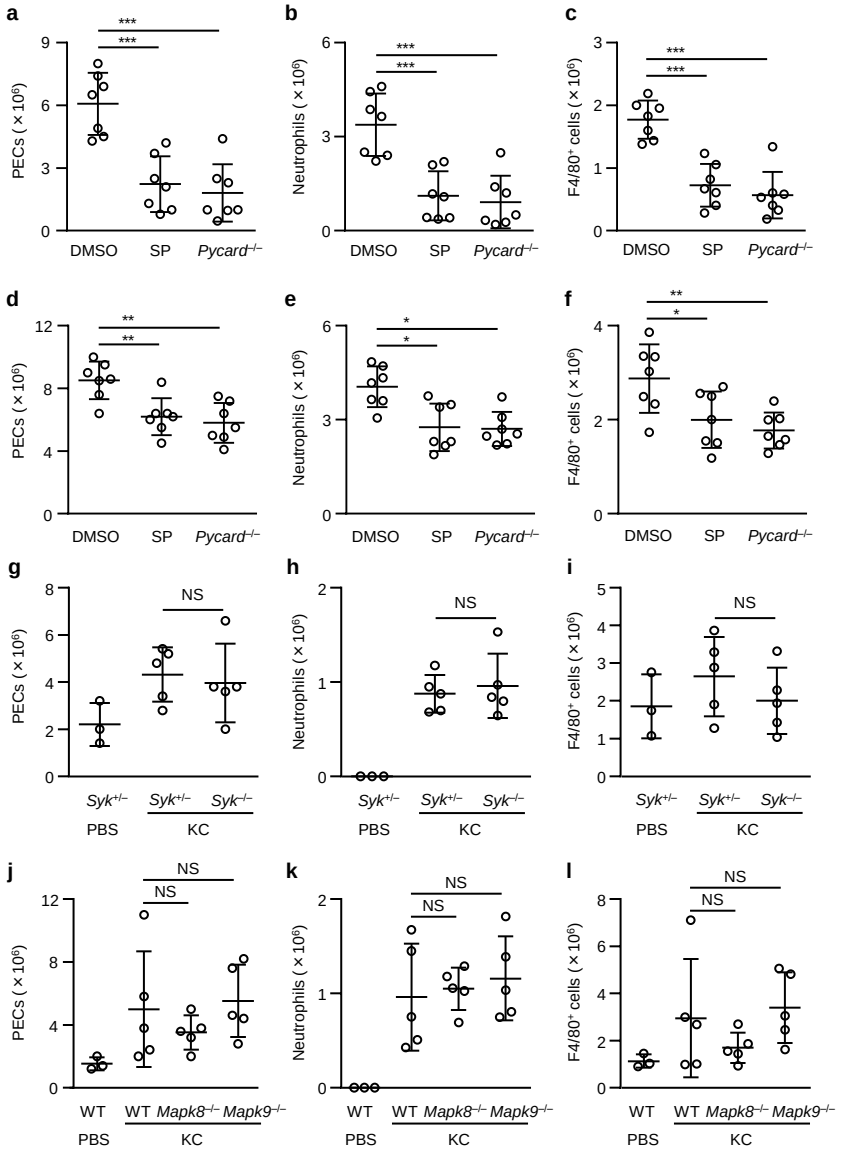
Supplementary Figure 5 Requirement of Syk and JNK for ASC speck formation induced by poly(dA:dT). (**a–d**) ASC staining or (**e,f**) immunoblotting of ASC in peritoneal macrophages primed with LPS for 4 h, followed by stimulation with nigericin for 90 min (**a,b**), or unprimed macrophages stimulated with poly(dA:dT) for the indicated times (**c–f**). The kinase inhibitors were added to the cultures 1 h before stimulation (**c–f**). ASC is shown in green, nuclei in blue (**a–c**). The number of ASC speck-positive cell was counted and normalized to that of the solvent control (**d**). The Triton-insoluble fraction was treated with DSS before immunoblotting (**f**). Data are shown as the means ± s.d. of triplicate samples of one experiment. Data shown in **c–f** are representative of three independent experiments and those in **a,b** are representative of two independent experiments. Data were analyzed by Kruskal-Wallis test with Dunn's multiple comparison test (**d**). Scale bar, 10 mm. * $P < 0.05$.



Supplementary Figure 6 Prediction of phosphorylation sites in mouse ASC. Possible phosphorylation sites in the amino acid sequence of mouse ASC were predicted by using the online program NetPhos 2.0. The threshold is 0.5.



Supplementary Figure 7 Reconstitution of inflammasome system in HEK293 cells. (**a, b**) Immunoblotting of inflammasome molecules in reconstituted HEK293 cells transfected as described in Fig. 5a,b.



Supplementary Figure 8 Involvement of ASC and JNK in inflammatory responses to MSU and Alum *in vivo*. (**a–f**) Infiltration of inflammatory cells in the peritoneal cavity induced by intraperitoneal injection of MSU (**a–c**) or alum (**d–f**) at 6 h after injection. Two hours before and 30 min later administration of the irritants, the mice were intraperitoneally treated with JNK inhibitor. (**g–l**) Infiltration of inflammatory cells in the peritoneal cavity induced by intraperitoneal injection of KC or PBS at 1.5 h after injection. Absolute numbers of PECs (**a,d,g,j**), Gr-1$^+$ F4/80$^-$ neutrophils (**b,e,h,k**), and F4/80$^+$ monocytes and macrophages (**c,f,i,l**) in the peritoneum were then determined. Data are shown as dots, and the bars indicate the means ± s.d. (n = 7 for **a–f**; n = 5 for **g–l**; n = 3 for PBS control in **g–l**). Data were analyzed by one-way ANOVA with Bonferroni (**a–d,f**) or Tukey-Kramer (**g–l**) multiple comparison test, or Kruskal-Wallis test with Dunn's multiple comparison test (**e**). NS, no significant difference. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

Supplementary Table 1 List of kinase inhibitors

Inhibitor	Abbreviation	Target	Final conc.
R406	R406	Syk	1 μM
BAY 61-3606	BAY	Syk	10 μM
Syk inhibitor I	SI	Syk	1 μM
PP2	PP2	Src	5 μM
SP600125	SP	JNK	40 μM
TAT-TI-JIP$_{153-163}$	TAT	JNK	40 μM
SB203580	SB	p38	10 μM
FR180204	FR	Erk	10 μM
Wortmannin	WO	PI3K	10 nM

Supplementary Table 2 Prediction of kinase-specific phosphorylation sites in ASC from different species

Tested kinase	Target protein	Code	Position	Peptide	Score
Syk family	Mouse ASC	Y	144	SVLTEGQ**Y**QAVRAET	1.092
JNK family	Mouse ASC	T	29	KFKMKLL**T**VQLREGY	1.312
JNK family	Mouse ASC	T	87	ELAEQLQ**T**TKEESGA	1.625
JNK family	Mouse ASC	T	88	LAEQLQT**T**KEESGAV	1.479
JNK family	Mouse ASC	S	100	GAVAAAA**S**VPAQSTA	1.333
JNK family	Mouse ASC	S	105	AASVPAQ**S**TARTGHF	1.688
JNK family	Mouse ASC	S	153	AVRAETT**S**QDKMRKL	1.458
JNK family	Mouse ASC	S	193	LVMDLEQ**S*********	1.438
Syk family	Human ASC	Y	146	KVLTDEQ**Y**QAVRAEP	1.723
JNK family	Human ASC	Y	187	ALRESQS**Y**LVEDLERY	1.308
JNK family	Human ASC	S	106	GIQAPPQ**S**AAKPGLHA	2.500
JNK family	Human ASC	T	154	QAVRAEP**T**NPSKMRK	1.333
JNK family	Human ASC	T	166	MRKLFSF**T**PAWNWTC	2.146
JNK family	Human ASC	S	195	LVEDLER**S*********	1.542
Syk family	Zebrafish ASC	Y	152	KVITNED**Y**CTIRNKE	1.892
JNK family	Zebrafish ASC	S	40	QEPRV**T**KSAIEKLKD	1.354
JNK family	Zebrafish ASC	T	160	CTIRNKE**T**PQKKMRE	5.104
JNK family	Zebrafish ASC	T	170	KKMRELL**T**GPITCAG	1.521

Supplementary Table 3 List of primers

Primer No.	Primer used for	Direction	Sequence
1	mNLRP3 cloning	Fw	CCTGCGGCCGCAACGAGTGTCCGTTGCAAG
2	mNLRP3 cloning	Rv	CCTGGTACCCTACCAGGAAATCTCGAAGACTA
3	mSyk cloning	Fw	AGCTTGCGGCCGCGGGAAGTGCTGTGGACAGCGCC
4	mSyk cloning	Rv	CTAGAGTCGACTTAGTTAACCACGTCGTAGTAG
5	mJNK1 cloning	Fw	CAACTATCGATGAGCAGAAGCAAACGTGACAAC
6	mJNK1 cloning	Rv	CGCACGGATCCTCATTGCTGCACCTGTGCTAAAGG
7	mJNK2 cloning	Fw	CAACTATCGATGAGTGACAGTAAAAGCGATGG
8	mJNK2 cloning	Rv	CGCACGGATCCTCACCGGCAGCCTTCCAGGGGTCC
9	NLRP3-R258W mutant	Fw	CCACTGCTGGGAGGTGAGCCTCAG
10	NLRP3-R258W mutant	Rv	TCACCTCCCAGCAGTGGATAAAGAA
11	ASC-S16A mutant	Fw	AACTTGGCCGGGGATGAACTCAAAAAG
12	ASC-S16A mutant	Rv	ATCCCCGGCCAAGTTTTCAAGAGC
13	ASC-S58A mutant	Fw	CTTGTCGCCTACTATCTGGAGTCGTATG
14	ASC-S58A mutant	Rv	ATAGTAGGCGACAAGTTTGTCAGTGAG
15	ASC-T69A mutant	Fw	GAGCTCGCCATGACTGTGCTTAGAG
16	ASC-T69A mutant	Rv	AGTCATGGCGAGCTCCAAGCCATAC
17	ASC-S87A/T88A mutant	Fw	CTGCAAGCCGCTAAAGAAGAGTCTGGA
18	ASC-S87A/T88A mutant	Rv	CTTCTTTAGCGGCTTGCAGCTGCTCAGC

Primer No.	Primer used for	Direction	Sequence
19	ASC-S92A mutant	Fw	GAAGAGGCCGGAGCTGTGGCAGCTG
20	ASC-S92A mutant	Rv	CAGCTCCGGCCTCTTCTTTAGTCGT
21	ASC-S105A/T106A mutant	Fw	CTCAGGCCGCAGCCAGAACAGGACAC
22	ASC-S105A/T106A mutant	Rv	CTGGCTGCGGCCTGAGCAGGGACACTG
23	ASC-T125A mutant	Fw	AGGGTCGCAGAAGTGGACGGAGTGCTG
24	ASC-T125A mutant	Rv	CACTTCTGCGACCCTGGCAATGAGTGC
25	ASC-T151A/T152A/S153A mutant	Fw	AGAGGCCGCCGCCCAAGACAAGATGAGGAAG
26	ASC-T151A/T152A/S153A mutant	Rv	CTTGGGCGGCGGCCTCTGCACGAACTGCCTG
27	ASC-S162A mutant	Fw	AACTTGGCCGGGGATGAACTCAAAAAG
28	ASC-S162A mutant	Rv	ATCCCCGGCCAAGTTTTCAAGAGC
29	ASC-T170A mutant	Fw	AACCTGGCCTGCAAGGACTCCCTC
30	ASC-T170A mutant	Rv	CTTGCAGGCCAGGTTCCAGGATGG
31	ASC-Y36F mutant	Fw	GAAGGCTTTGGGCGCATCCCACGC
32	ASC-Y36F mutant	Rv	GCGCCCAAAGCCTTCTCGCAGTTG
33	ASC-Y144F mutant	Fw	GGACAGTTCCAGGCAGTTCGTGCA
34	ASC-Y144F mutant	Rv	TGCCTGGAACTGTCCTTCAGTCAG
35	Deletion of FLAG-tag	Fw	CTACCATGGCGGCCGCGAATTCATCG
36	Deletion of FLAG-tag	Rv	GCGGCCGCCATGGTAGATCAATTCTGA