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Supplemental information

CARD-only proteins regulate *in vivo* inflammasome responses and ameliorate gout

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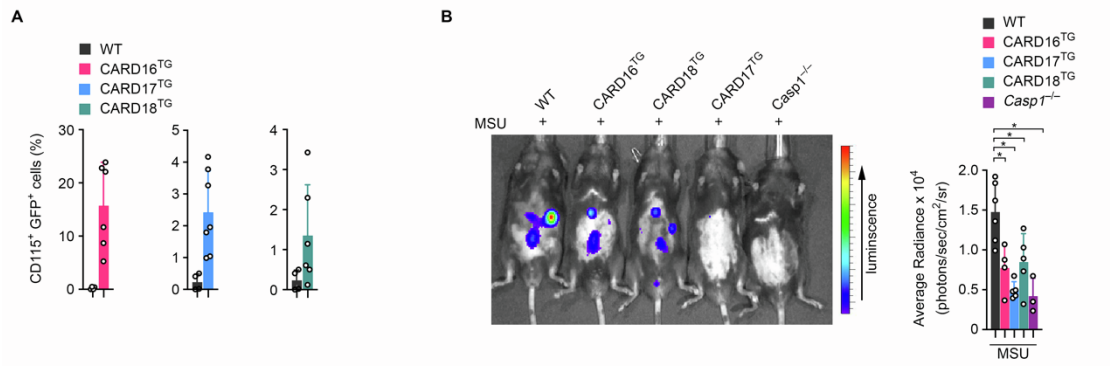
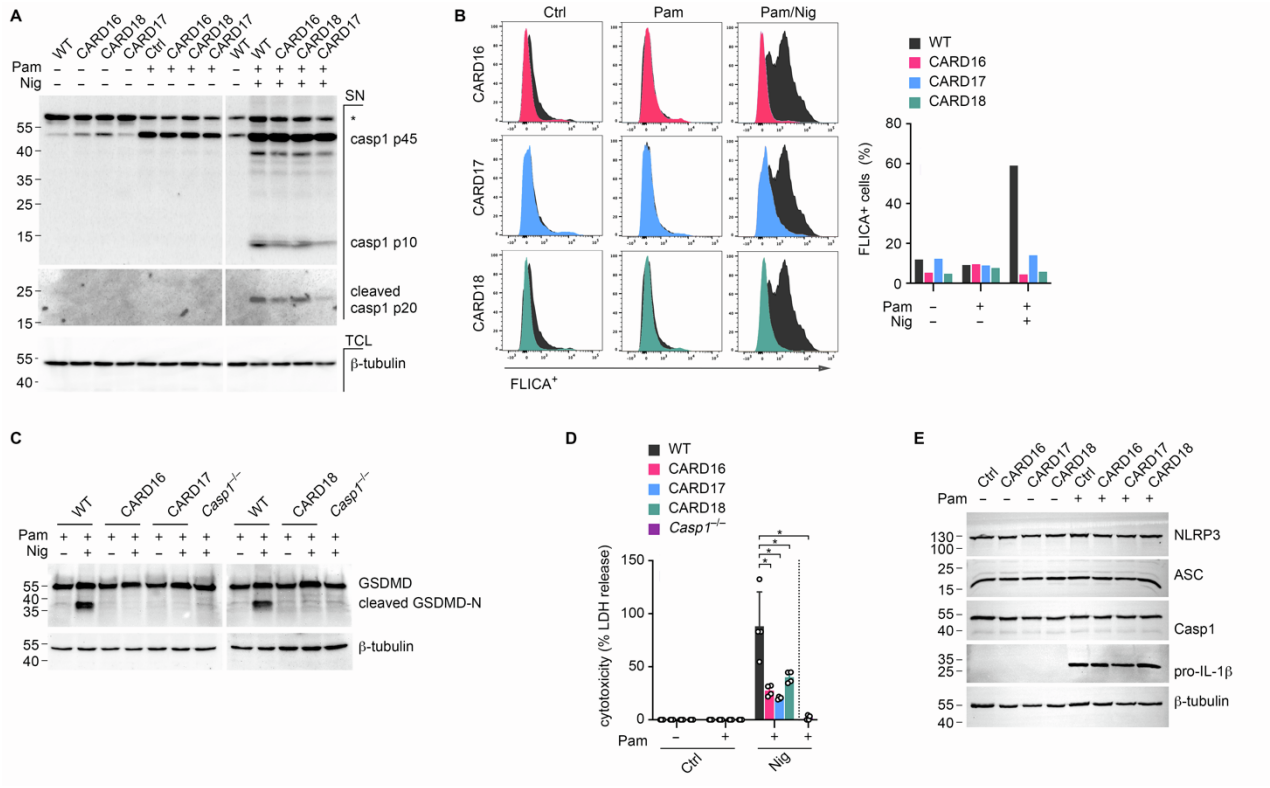


Supplemental Figure S1. COPs ameliorate MSU-induced peritonitis in mice related to Figure 1.

(A) Total blood from WT and COP^{TG} mice was analyzed for CARD16, CARD17 and CARD18 expression by gating on GFP^{+} CD115^{+} blood monocytes by flow cytometry. Results are presented as percent GFP^{+} cells gated on CD115^{+} peripheral blood cells are shown (n=5-7).

(B) *In vivo* imaging of MPO activity correlating to MSU-induced neutrophil infiltration into the peritoneal cavity 7h after MSU crystal injection (3 mg) in WT, CARD16^{TG}, CARD17^{TG}, CARD18^{TG} and *Casp1*$^{-/-}$ mice (left) and average radiance (right) presented as photons/sec/cm^2/sr (n=4-5, mean ± s.d.). *p<0.05. The *in vivo* gout model has been performed twice.



Supplemental Figure S2. COPs inhibit caspase-1 activation and pyroptosis in mouse macrophages related to Figure 4.

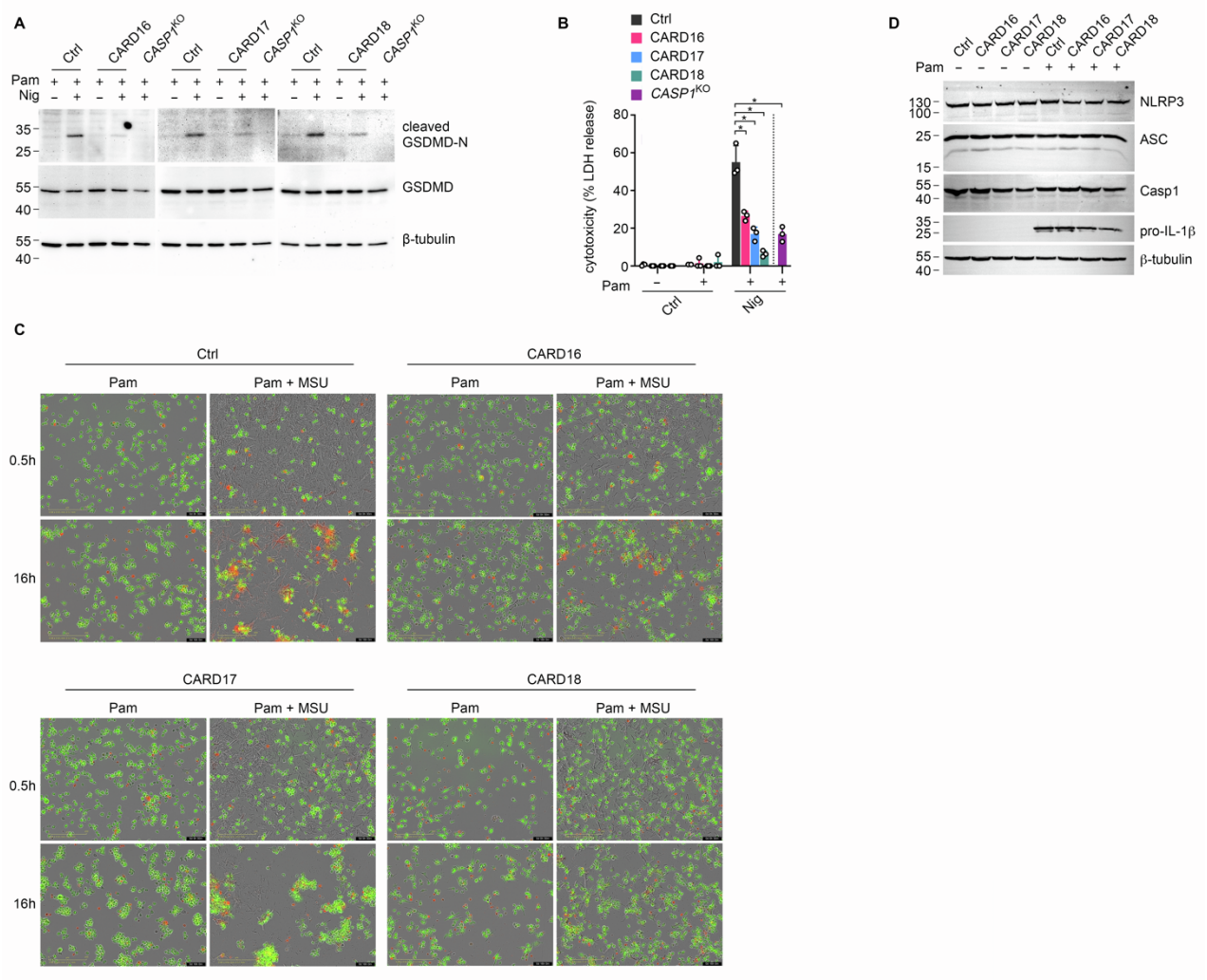
(A) Wild type (WT), CARD16^{TG}, CARD17^{TG} and CARD18^{TG} BMDM were primed with Pam3CSK4 (Pam) (1 µg mL^{-1}, 4h) and activated with nigericin (Nig) (15 µM, 45m) and culture supernatants (SN) and total cell lysates (TCL) were analyzed by SDS/PAGE and immunoblot.

(B) WT, CARD16^{TG}, CARD17^{TG} and CARD18^{TG} BMDM were primed with Pam (1 µg mL^{-1}, 1h), activated with Nig (15 µM, 45m) and incubated with a FLICA substrate (10 µM). Active caspase-1 was determined by flow cytometry and FLICA^{+} cells from a representative experiment are presented. (n=3-4).

(C) WT, CARD16^{TG}, CARD17^{TG}, CARD18^{TG} and *Casp1*$^{-/-}$ BMDM were primed with Pam (1 µg mL^{-1}, 4h), activated with Nig (15 µM, 20m) and TCL were analyzed by SDS/PAGE and immunoblot.

(D) WT, CARD16^{TG}, CARD17^{TG}, CARD18^{TG} and *Casp1*$^{-/-}$ BMDM were primed with Pam (1 µg mL^{-1}, 4h), activated with Nig (15 µM, 20 min) and LDH release was quantified and presented as % cytotoxicity compared to maximum LDH release (n=3, mean ± s.d.). The dotted line indicates that *Casp1*$^{-/-}$ BMDM are only present in the primed and Nig activated treatment group as specificity control. *p<0.05.

(E) WT, CARD16^{TG}, CARD17^{TG}, CARD18^{TG} BMDM were left untreated or primed with Pam (1 µg mL^{-1}, 4h) and TCL were analyzed by SDS/PAGE and immunoblot.



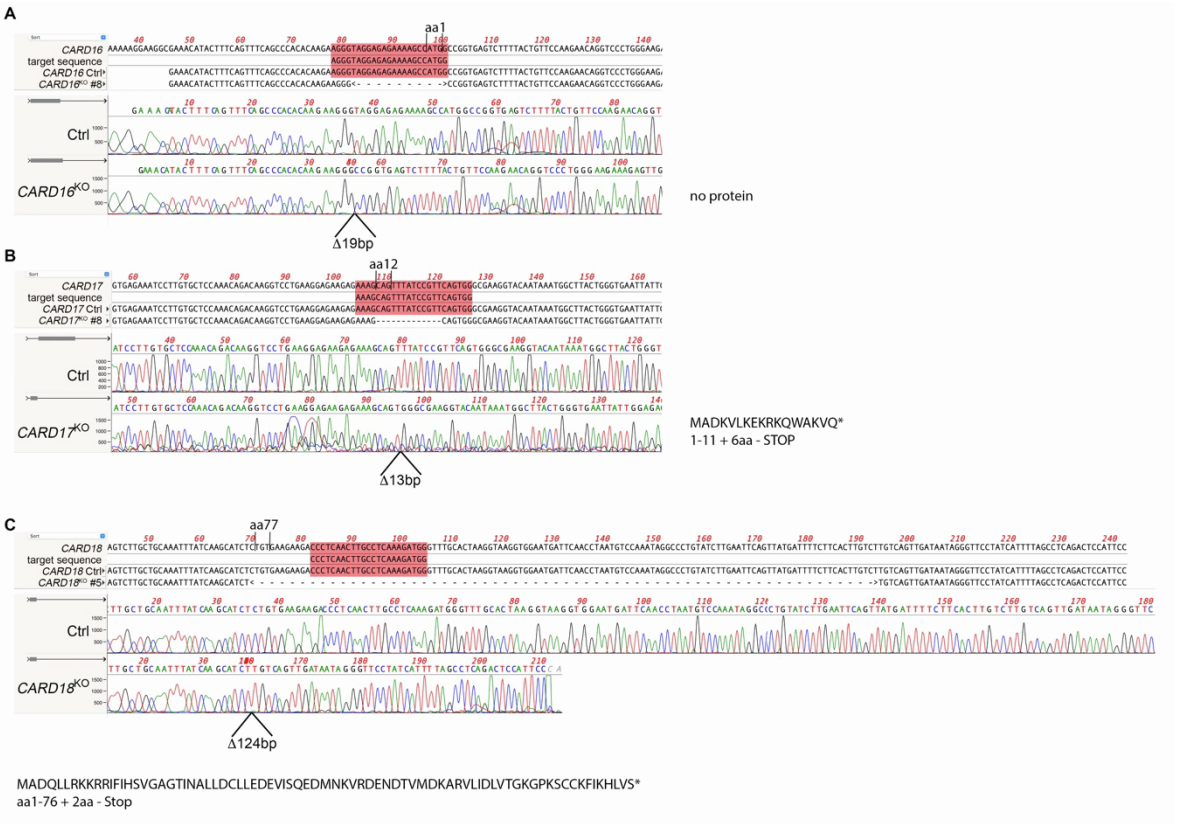
Supplemental Figure S3. COPs inhibit caspase-1 activation and pyroptosis in human macrophages related to Figure 4.

(A) Control (Ctrl), CARD16, CARD17 and CARD18 expressing as well as *CASP1*^{KO} THP-1 cells were primed with Pam3CSK4 (Pam) (1 µg mL^{-1}, 4h), activated with nigericin (Nig) (15 µM, 20m) and TCL were analyzed by SDS/PAGE and immunoblot.

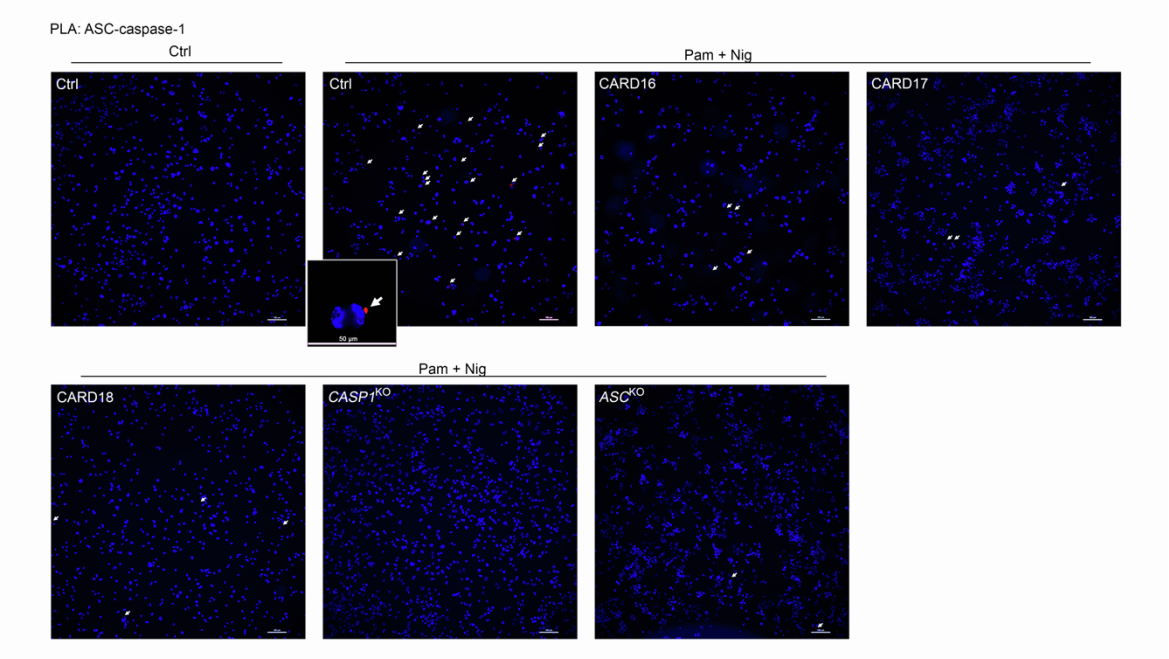
(B) Ctrl, CARD16, CARD17 and CARD18 expressing as well as *CASP1*^{KO} THP-1 cells were primed with Pam (1 µg mL^{-1}, 4h), activated with Nig (15 µM, 20 min) and LDH release was quantified and presented as % cytotoxicity compared to maximum LDH release (n=3, mean ± s.d.). The dotted line indicates that *CASP1*^{KO} THP-1 cells are only present in the primed and Nig activated treatment group as specificity control. *p<0.05.

(C) Ctrl, CARD16, CARD17 and CARD18 expressing THP-1 cells were primed with Pam (1 µg mL^{-1}) and incubated with Cytotox Red (250 nM) for 3h followed by activation with MSU crystals (100 µg mL^{-1}). THP-1 cells were imaged every 30 min for 16h (IncuCyte). Representative images at 30 min and 16h are presented, scale bars indicate 200 µm and the 16h video file is available as Supplemental video 1 (WT), Supplemental video 2 (CARD16), Supplemental video 3 (CARD17), Supplemental video 4 (CARD18).

(D) Ctrl, CARD16, CARD17 and CARD18 expressing THP-1 cells were left untreated or primed with Pam (1 µg mL^{-1}, 4h) and TCL were analyzed by SDS/PAGE and immunoblot.



Supplemental Figure S4. COP gene knock-out in human THP-1 cells related to Figure 6. **(A-C)** THP-1 cells were stably transfected with CRISPR/Cas9 control (Ctrl) plasmid or with plasmids containing CRISPR/Cas9 and gRNAs targeting **(A)** CARD16, **(B)** CARD17, and **(C)** CARD18. The gRNA sequence is boxed in red. The targeting region was PCR amplified, sequence analyzed and the deletion, the resulting frame shift and premature stop in the amino acid sequence indicated.



Supplemental Figure S5. COPs disrupt inflammasome assembly related to Figure 7D.
PMA-differentiated Control (Ctrl), CARD16, CARD17, CARD18 expressing and *CASP1*^{KO} and *ASC*^{KO} THP-1 cells were left untreated, primed with Pam3CSK4 (Pam) (1 µg mL^{-1}, 3h), activated with nigericin (Nig) (10 µM, 15m) as indicated and subjected to proximity ligation assay (PLA) between caspase-1 and ASC, co-stained with DAPI (blue) and analyzed by fluorescence microscopy, showing representative results. The red PLA signal is marked by arrowheads and a magnified image is shown for nigericin-activated Ctrl cells (scale bar=50 µm).

Oligonucleotides	SOURCE	IDENTIFIER
GFP genotyping Fwd: cctacggcgtgcagtgcttcagc	Integrated DNA Technologies	N/A
GFP genotyping Rev: cggcgagctgcacgctgcgtcctc	Integrated DNA Technologies	N/A
CARD16 gRNA: agggtaggagagaaaagccatgg	Integrated DNA Technologies	N/A
CARD17 gRNA: aaagcagtttatccgttcagtgg	Integrated DNA Technologies	N/A
CARD18 gRNA: cctcaacttgcctcaaagatgg	Integrated DNA Technologies	N/A
Ctrl gRNA: acggaggctaagcgtcgcaa	Integrated DNA Technologies	N/A
CARD16 genotyping: Fwd: tgggtgtttcccaatatgtgta	Integrated DNA Technologies	N/A
CARD16 genotyping: Rev: tgccttttctttctaaagcctg	Integrated DNA Technologies	N/A
CARD17 genotyping: Fwd: aatgaggttgccttctggtg	Integrated DNA Technologies	N/A
CARD17 genotyping: Rev: tcgggccttatccataactg	Integrated DNA Technologies	N/A
CARD18 genotyping: Fwd: ggctcgagtcttgattgacc	Integrated DNA Technologies	N/A
CARD18 genotyping: Rev: ggaatggagtctgaggctaaaa	Integrated DNA Technologies	N/A

Supplemental Table 1. Oligonucleotide sequences. related to Key resource table.