SUPPLEMENTAL MATERIAL

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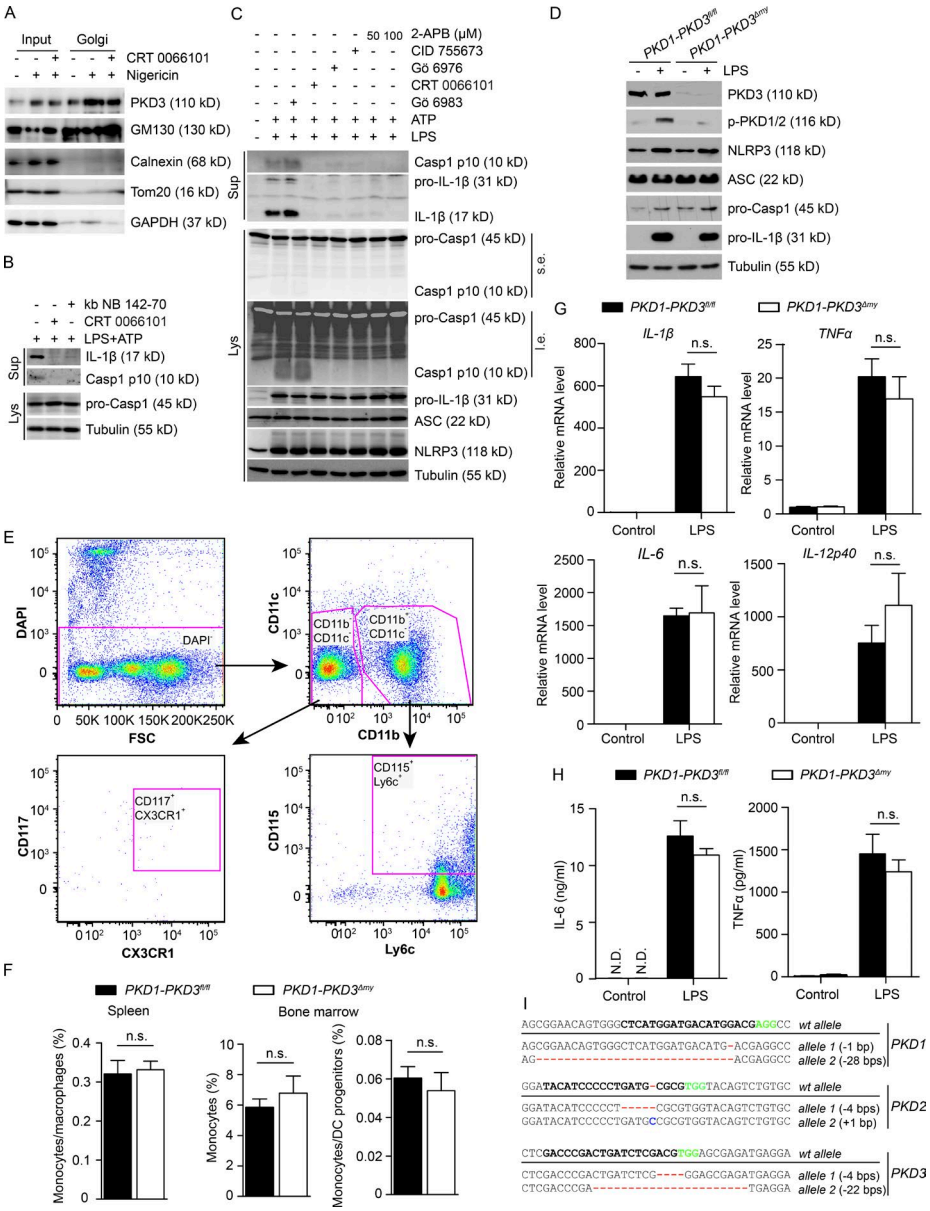

Figure S1. **PKD activity is required for the activation of the NLRP3 inflammasome, but not for lineage development of mouse myeloid cells, LPS priming, and secretion of other NF-κB–dependent cytokines.** (A) Immunoblotting of isolated Golgi fractions from THP-1 cells pretreated with DMSO or 10 μM CRT 0066101 (CRT) for 1 h, followed by treatment with 15 μM Nigericin in presence of DMSO or 10 μM CRT for 40 min (B) Immunoblotting of culture supernatants (Sup) and lysates (Lys) from LPS-primed BMDMs pretreated with DMSO, 10 μM CRT 0066101, or 20 μM kb NB 142–70 for 1 h, followed by ATP treatment in presence of indicated inhibitors for 40 min. (C) Immunoblotting of culture supernatants (Sup) and lysates (Lys) from LPS-primed peritoneal macrophages pretreated with DMSO, 5 μM Gö 6983, 10 μM CRT 0066101, 5 μM Gö 6976, 30 μM CID 755673, or 50 or 100 μM 2-APB for 1 h, followed by ATP treatment in the presence of DMSO or indicated inhibitors for 40 min. l.e.: long exposure; s.e.: short exposure. (D) Immunoblotting of lysates from BMDMs isolated from *LysM-Cre*–negative floxed *PKD1-PKD3* (*PKD1-PKD3*$^{fl/fl}$) control mice and *LysM-Cre*–positive myeloid-specific *PKD1-PKD3* double-KO (*PKD1-PKD3*$^{\Delta my}$) mice. Cells were treated with or without 1 μg/ml LPS for 4 h. (E) Gating parameters used for FACS analysis. (F) Comparison of the percentage of monocytes/macrophages in spleen and monocytes ($CD11b^+CD11c^-Ly6C^+CD115^+$), monocytes/DC progenitors ($CD11b^-CD11c^-CD117^+Cx3CR1^+CD115^+$) in bone marrow isolated from *PKD1-PKD3*$^{fl/fl}$ (n = 5) and *PKD1-PKD3*$^{\Delta my}$ (n = 6) mice. The values are expressed as means ± SEM. n.s., not significant (t-test). (G and H) Analysis of NF-κB–dependent cytokines in BMDMs isolated from *PKD1-PKD3*$^{fl/fl}$ and *PKD1-PKD3*$^{\Delta my}$ mice. Cells were stimulated with 1 μg/ml LPS for 4 h; the mRNA levels of *IL-1β*, *TNF*, *IL-6*, and *IL-12p40* relative to *Hprt* mRNA were analyzed by quantitative PCR (G), and the secretion of TNF and IL-6 was measured by ELISA (H). N.D., not detected. The values are expressed as means ± SEM; n.s.: not significant (t test). (I) Sequencing of *PKD1/2/3* alleles of the selected Raw-ASC clone after CRISPR/Cas9–mediated genome editing confirming gene disruptions. Sequences in bold indicate the targets of the gRNAs. "PAMs" are highlighted in green, the insertion is highlighted in blue, and "–" stands for one deleted nucleotide. Data shown in A–D, G, and H are representative of three independent experiments. The sequencing results shown in I were performed twice.

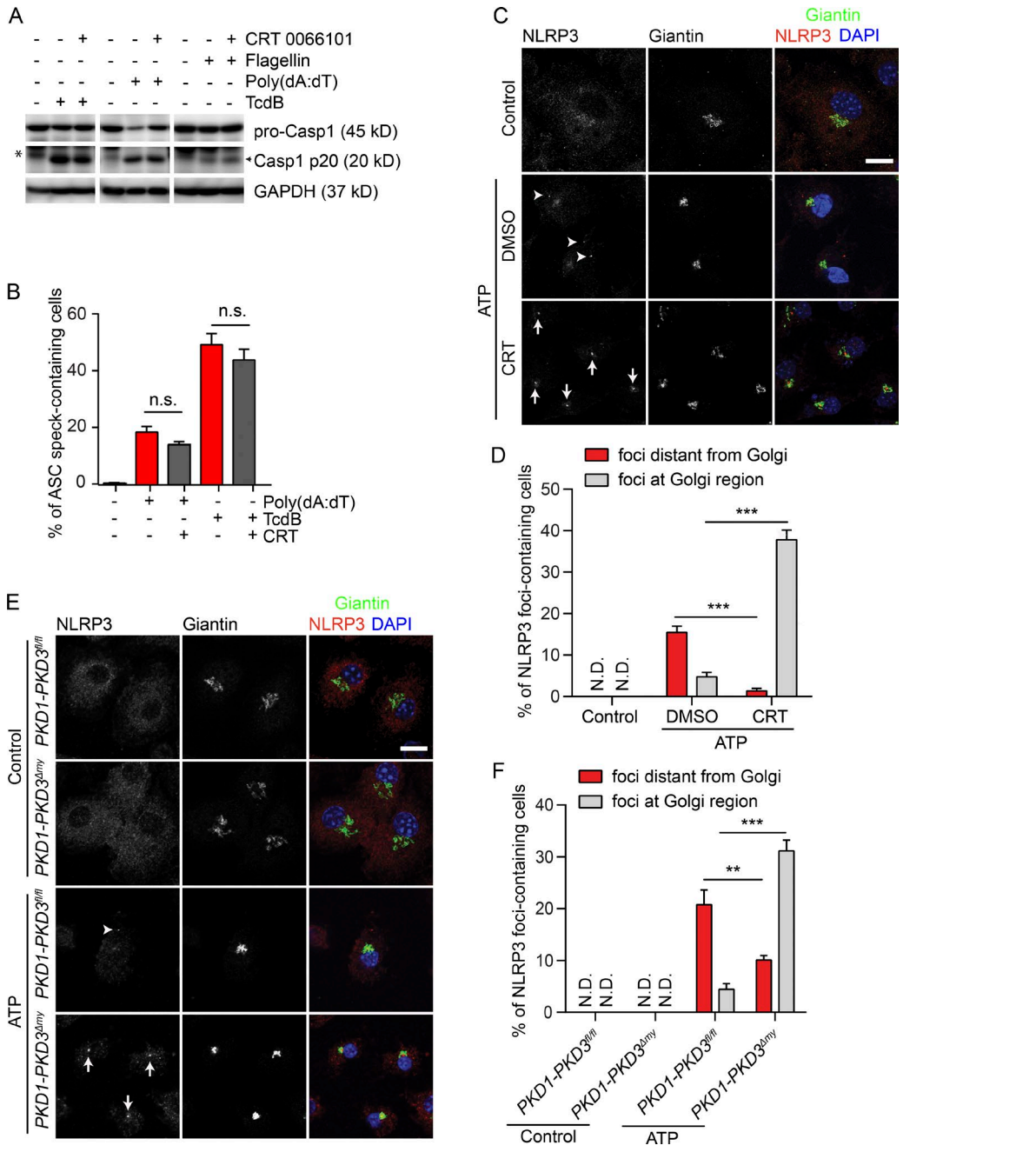

Figure S2. **Inhibition of PKD does not affect activation of other inflammasomes and deficiency of PKD sequesters NLRP3 to the Golgi region.** (A) Immunoblotting of lysates from LPS-primed BMDMs pretreated with DMSO or 10 µM CRT 0066101 for 1 h, followed by treatment with 0.1 nM of the PYRIN inflammasome activator recombinant TcdB for 2 h, 1 µg/ml of the AIM2 inflammasome activator poly(dA:dT) for 4 h, or 0.5 µg/ml of the NLRC4 inflammasome activator flagellin for 4 h in the presence of DMSO or 10 µM CRT. Arrows indicate the bands of Casp1 p20. Asterisk represents unspecific bands. (B) Quantification of ASC speck–containing BMDMs treated as in A. The values are expressed as means ± SEM. n.s., not significant (*t* test). (C) Confocal fluorescence imaging of LPS-primed BMDMs pretreated with DMSO or 10 µM CRT for 1 h, followed by treatment with 2.5 mM ATP for 20 min. Cells were coimmunostained with antibodies against giantin and NLRP3. Nuclei were stained with DAPI. Bar, 10 µm. The arrowhead indicates small cytoplasmic NLRP3 foci distant from the Golgi, whereas arrows indicate slightly bigger NLRP3 foci at the Golgi region. (D) Quantification of NLRP3 foci-containing BMDMs of experiments represented in C. N.D., not detected. The values are expressed as means ± SEM. ***, $P < 0.001$ (*t* test). (E) Confocal fluorescence imaging of LPS-primed BMDMs isolated from *PKD1-PKD3*$^{fl/fl}$ and *PKD1-PKD3*$^{\Delta my}$ mice. Cells were treated with 2.5 mM ATP for 20 min and coimmunostained with antibodies against giantin and NLRP3. Nuclei were stained with DAPI. Bar, 10 µm. Arrowhead indicates the NLRP3 small focus in cytoplasm; arrows indicate the NLRP3 foci at the Golgi region. (F) Quantification of NLRP3 foci-containing BMDMs of experiments represented in E. N.D., not detected. The values are expressed as means ± SEM. **, $P < 0.01$; ***, $P < 0.001$ (*t* test). Data shown are representative of at least three independent experiments.

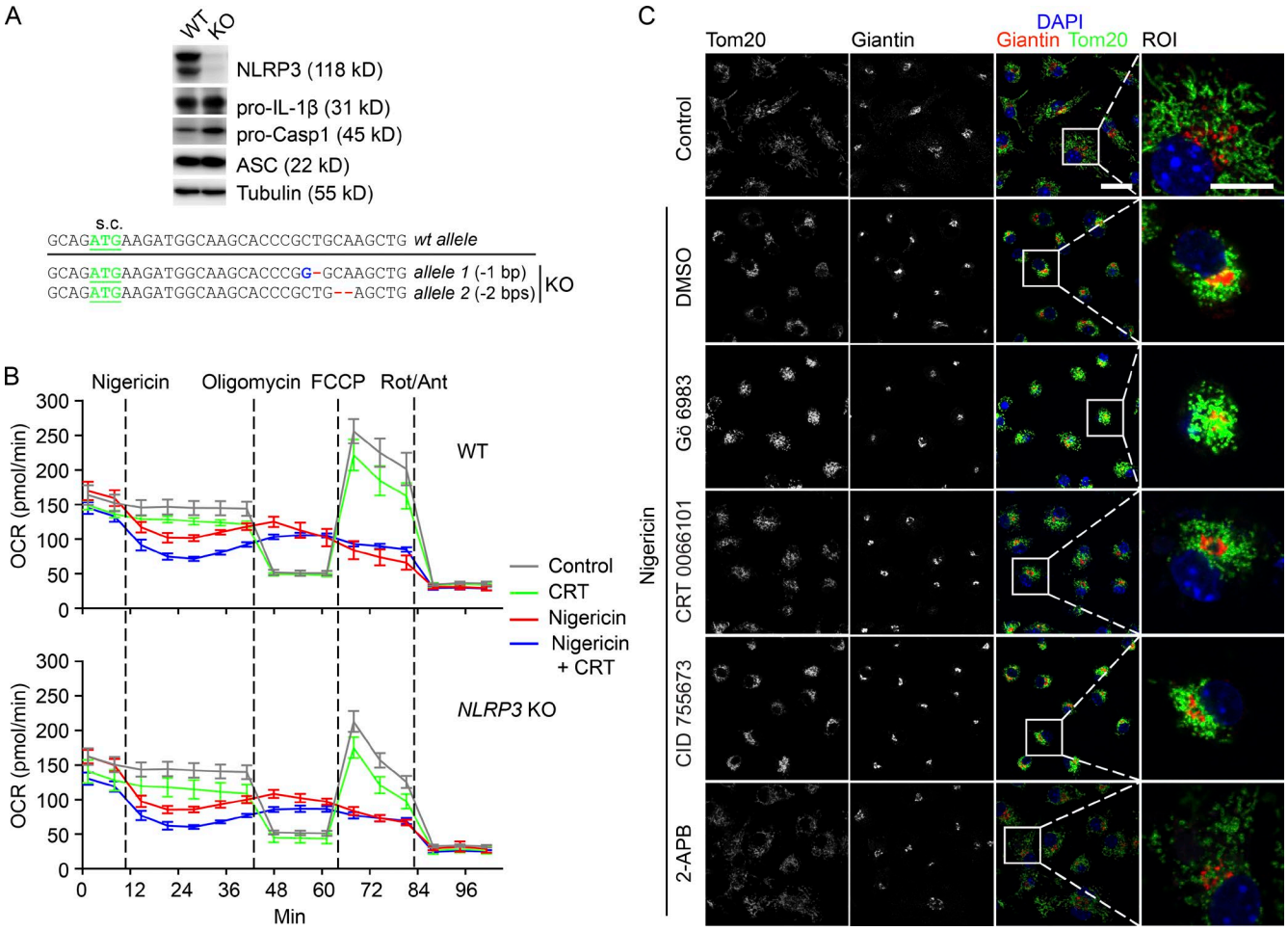

Figure S3. **Inhibition of PKD does not affect the mitochondrial dysfunction and mitochondrial clustering around the Golgi upon NLRP3 inflammasome activation.** (A) Validation of a *NLRP3*-KO THP-1 cell line generated by CRISPR/Cas9–mediated genome editing system by immunoblotting and sequencing. s.c., start codon (ATG in green). The mutation is highlighted in blue. Dash indicates deleted nucleotides. Data shown are representative of at least three independent experiments. (B) Measurement of oxygen consumption rate (OCR) of WT and *NLRP3* KO THP-1 cells. PMA-differentiated cells pretreated with DMSO or 10 µM CRT 0066101 were treated with 15 µM nigericin, 2.0 µM oligomycin, 1.0 µM FCCP, and 0.5 µM rotenone/antimycin (Rot/Ant) as indicated. For each group, values were obtained from eight wells and are expressed as means ± SEM. (C) Confocal fluorescence imaging of LPS-primed BMDMs pretreated with DMSO, 5 µM Gö 6983, 10 µM CRT 0066101, 30 µM CID 755673, or 50 µM 2-APB for 1 h, followed by ATP treatment for 40 min in the presence of DMSO or indicated inhibitors. Cells were coimmunostained with anti–Tom20 and anti-giantin antibodies. Nuclei were stained with DAPI. Regions of interest (ROIs) are indicated by boxes. Bars: 20 µm; (ROI) 10 µm. Data shown are representative of three independent experiments.

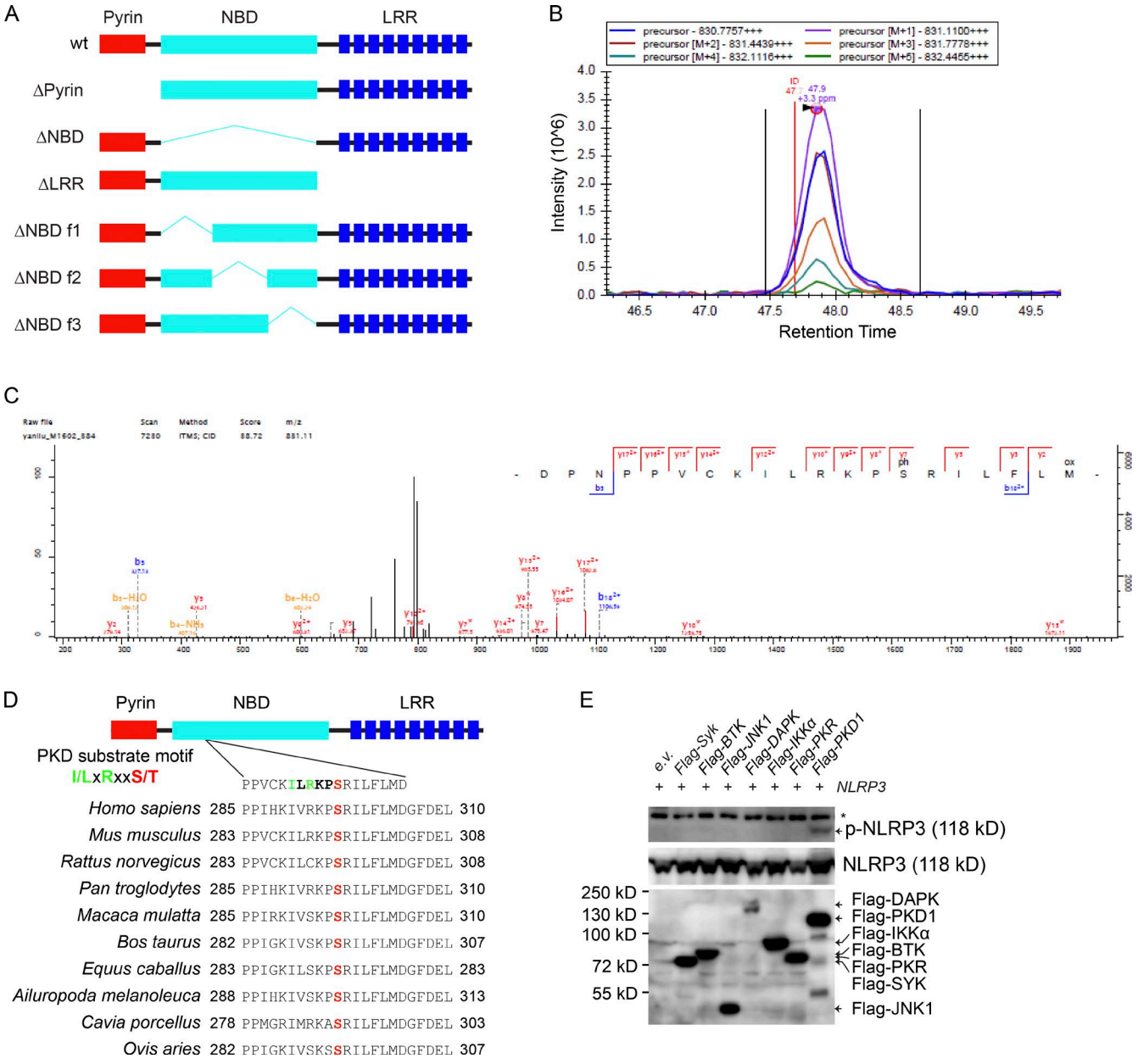

Figure S4. **PKD phosphorylates NLRP3 at Ser293.** (A) Truncation mutants of NLRP3 used for identification of the phosphorylation site. (B, C) Identification of the target phosphosite by shotgun mass spectrometry and unbiased database search. (B) The MS1 signal of the precursor ion target (charge 3+) and its isotope envelope (precursor M+1 to M+5). The isotope dot product (idotp) score reported by Skyline is 0.98. The red vertical line denoting the time points where MS2 scan was triggered. (C) The MS2 spectrum triggered was identified as the targeted phosphopeptide through an unbiased search by 1% FDR at the peptide and protein levels. The spectrum was exported from MaxQuant. (D) Alignment of NLRP3 orthologues of different species. The serine residue phosphorylated by PKD is highlighted in red. (E) Immunoblotting of lysates from cells coexpressing NLRP3 with FLAG-tagged Syk, BTK, JNK1, DAPK, IKKα, PKR, or PKD1. Asterisk represents an unspecific band. Data shown in E are representative of three independent experiments; data shown in B and C are from one experiment.

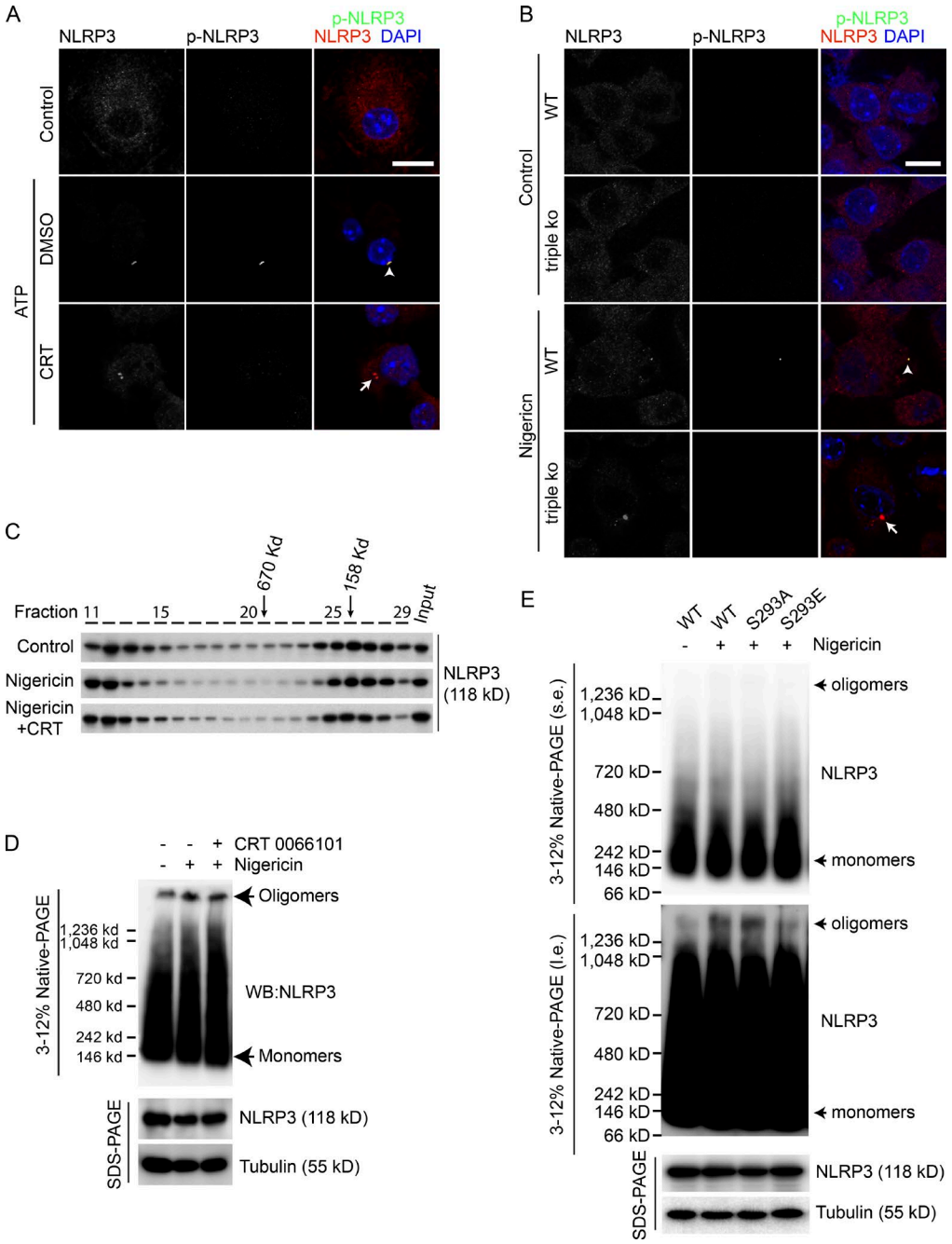

Figure S5. **PKD phosphorylates NLRP3 to activate the NLRP3 inflammasome.** (A) Confocal fluorescence imaging of LPS-primed BMDMs pretreated with DMSO or 10 µM CRT 0066101 for 1 h, followed by 5 mM ATP treatment in the presence of DMSO or 10 µM CRT 0066101 for 20 min. Cells were co-immunostained with anti-NLRP3 and anti–p-NLRP3 (Ser293) antibodies. Nuclei were stained with DAPI. Bar, 10 µm. Arrowhead indicates colocalization of NLRP3 and p-NLRP3; arrow indicates NLRP3 foci without p-NLRP3 signal. (B) Confocal fluorescence imaging of LPS-primed WT and *PKD1/2/3* triple-KO (triple ko) Raw-ASC cells treated with 10 µM nigericin for 1 h. Cells were coimmunostained with anti-NLRP3 and anti–p-NLRP3 (Ser293) antibodies. Nuclei were stained with DAPI. Bar, 10 µm. Arrowhead indicates colocalization of NLRP3 and p-NLRP3; arrow indicates NLRP3 focus without p-NLRP3 signal. (C) Immunoblotting of collected fractions (from fraction 11 to fraction 29) of gel filtration using an anti-NLRP3 antibody. Differentiated THP-1 cells were pretreated with DMSO or 10 µM CRT 0066101 for 1 h, followed by treatment with 15 µM nigericin in the presence of DMSO or 10 µM CRT for 40 min. (D) Immunoblotting of native PAGE and SDS-PAGE of lysates isolated from THP-1 cells pretreated with DMSO or 10 µM CRT for 1 h, followed by treatment with 15 µM nigericin in presence of DMSO or 10 µM CRT for 40 min. (E) Immunoblotting of native PAGE and SDS-PAGE of lysates from *NLRP3*-KO THP-1 cells reconstituted with NLRP3 WT, S293A mutant, or S293E mutant. PMA-differentiated cells were treated with or without 15 µM nigericin for 40 min. Data shown are representative of three independent experiments.