Supplementary Information

The PYRIN domain-only protein POP3 inhibits AIM2-like receptor inflammasomes and regulates responses to DNA virus infections

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a

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        10          20          30          40          50          60          70          80          90
ATGGAGAGTA AATATAAGGA GATACTCTTG CTAACCAGCC TGGATAACAT CACCGATGAG GAACTGGATA GGTTTAAGTG CTTTCTTCCA

       100         110         120         130         140         150         160         170         180
GATGAGTTTA ATATTGCCAC AGGCAAACTG CATACTCTAA ACAGCACGAG TAGCCAACTT GATTTAAAAC GCTGGCATGG TGTCTGCAGT

       190         200         210         220         230         240         250         260         270
GAGGAAGACC GTATTTTTCA GAAGCTGAAT TATATGCTTG TGGCAAAATG TCTTCGGGAA GAGCAGGAAA CAGGTATATG TGGGAGTCCC

       280         290         300         310         320         330         340
TCATCTGCCC GGTCCGTTTC TCAGTCAAGA CTTGGTCTTT CCTTTCATGG CATTTCTGGG AATGCATGTT GA
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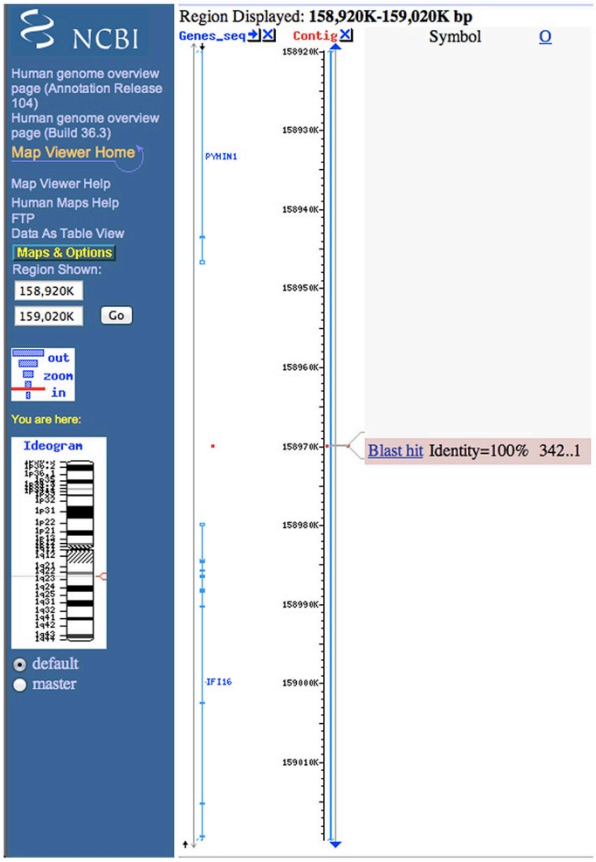
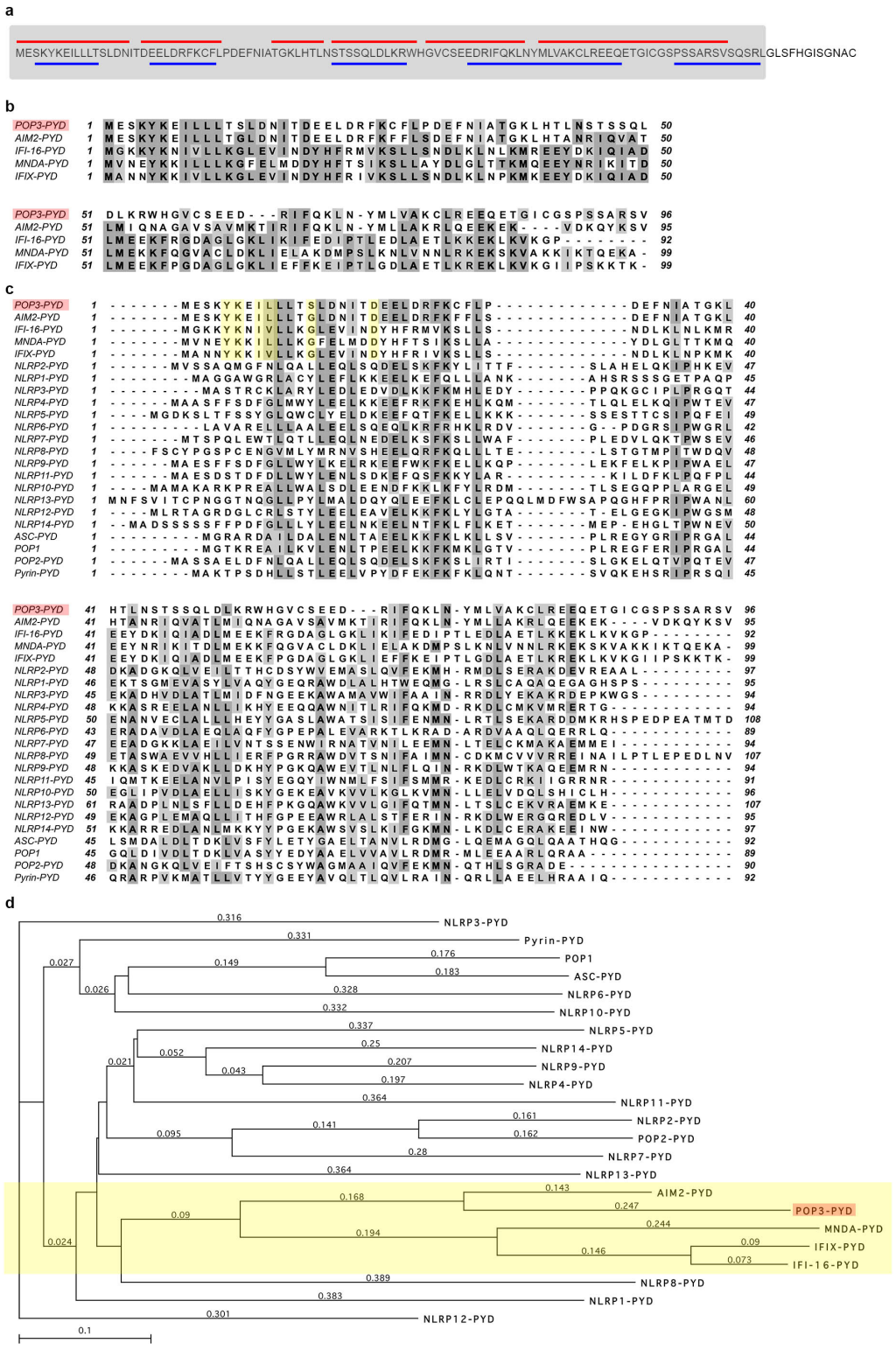
b

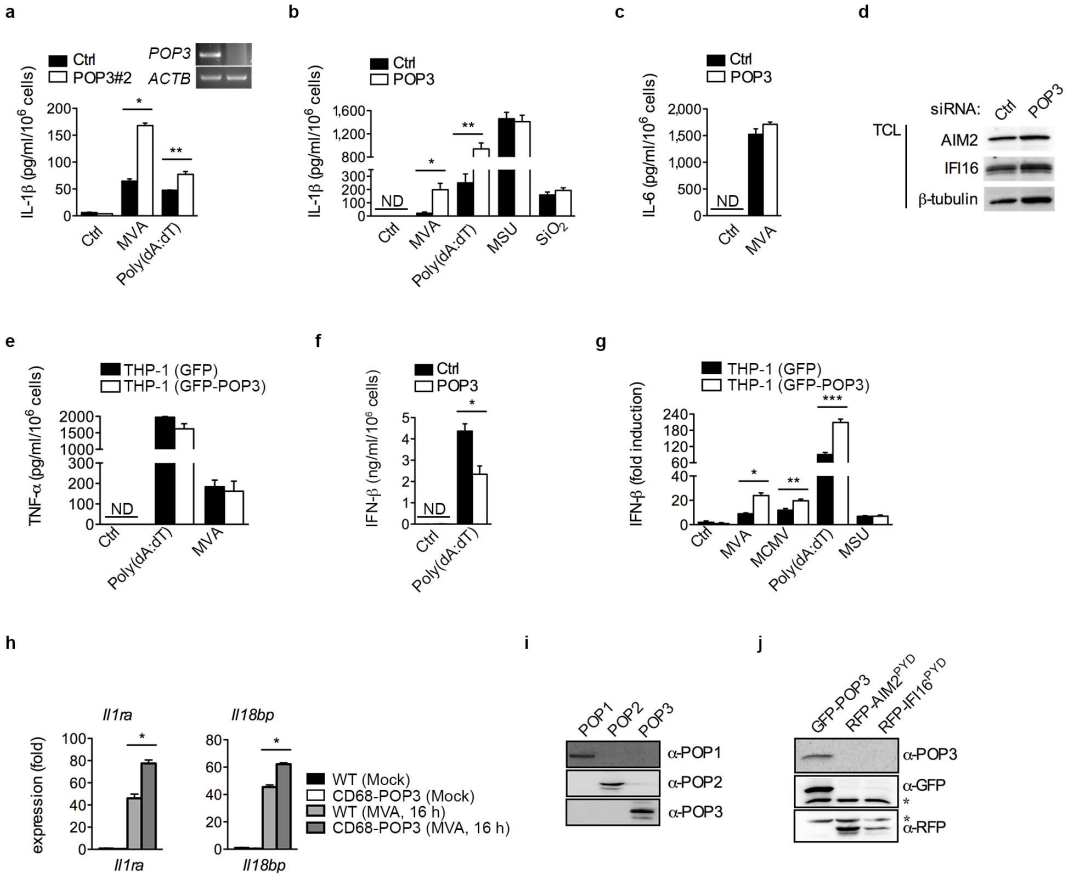


Supplementary Figure 1. *POP3* is a previously undescribed gene located between *IFI16* and *IFIX*. **(a)** cDNA showing the open reading frame of *POP3* (Genbank accession number: KF562078) **(b)** A nucleotide BLAST (blastn) analysis against the assembled human RefSeq genomes (http://blast.ncbi.nlm.nih.gov) detailing the genomic location of *POP3* within the HIN-200 cluster flanked by *IFI16* and *PYHIN1* on human chromosome 1q23.



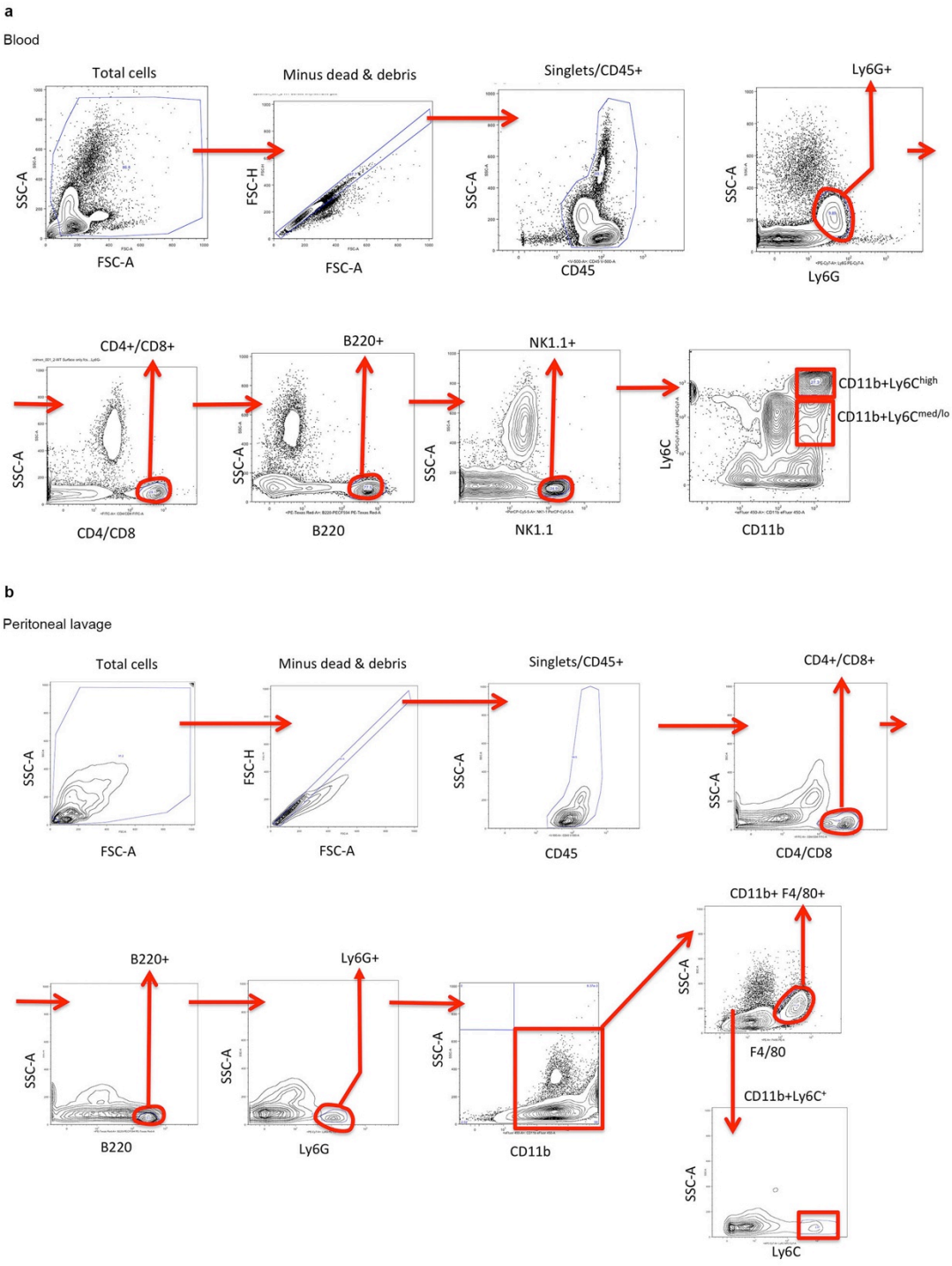
Supplementary Figure 2. POP3 shows characteristic features of PYDs present in HIN-200 proteins. **(a)** Amino acid sequence of POP3. The PYD is shaded grey.

The predicted α-helices are marked with blue lines (bottom), while the corresponding α-helices of AIM2, as determined by crystal structure[1], are marked with a red line (top). **(b)** ClustalW alignment of the amino acid sequences corresponding to the PYDs of POP3 and human HIN-200 members. **(c)** ClustalW alignment of all human PYDs. The characteristic amino acid motifs found in HIN-200 members, which are also present in POP3, are highlighted in yellow. **(d)** Phylogenetic tree cluster analysis of sequences used in b. The HIN-200 cluster, which includes POP3 is highlighted in yellow.

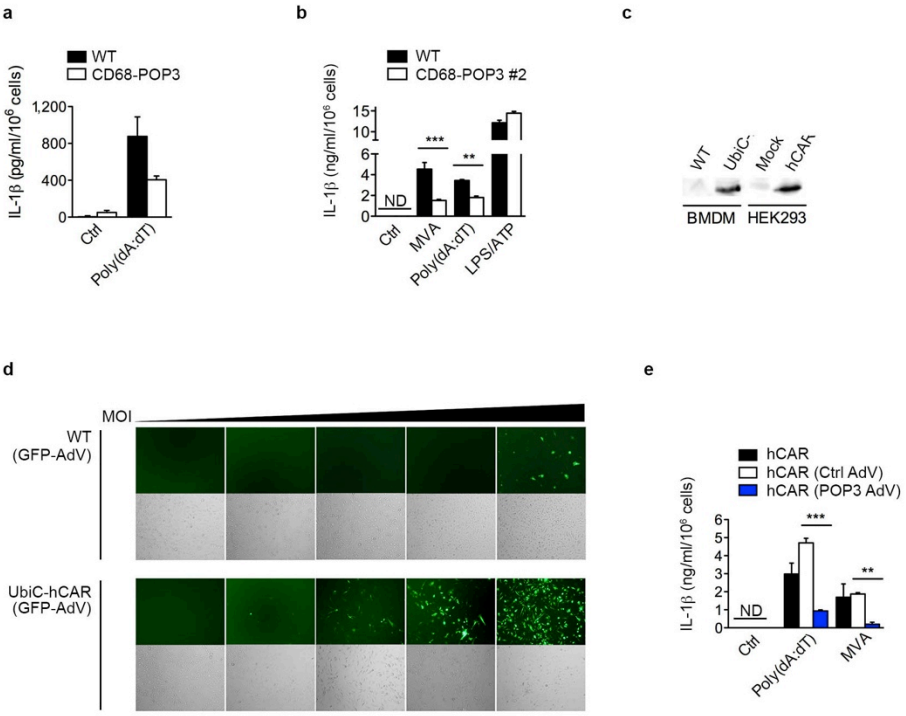


Supplementary Figure 3. Silencing of POP3 specifically affects the AIM2 inflammasome. **(a, c, d)** hMΦ were transfected with either control or **a,** POP3#2 or **c, d,** POP3 siRNAs and infected with MVA or transfected with poly(dA:dT) as indicated for 16 h and analyzed for **a,** mature IL-1β and **c,** IL-6 by ELISA (n = 3 ± s.e.m.) and **d,** TCL from Figure 3f, were analyzed in parallel for expression of AIM2 and IFI16 by immunoblot. **(b, f)** THP-1 cells were transfected with siRNAs as above, and infected with MVA, transfected with poly(dA:dT) or treated with MSU or SiO_2, as indicated for 16 h and analyzed for **b,** IL-1β secretion and **f,** IFN-β by ELISA (n = 3 ± s.e.m.). (**e, g)** THP-1 (GFP) and THP-1 (GFP-POP3) cells were analyzed for secretion of **e,** TNFα and **g,** IFN-β in response to MVA and MCMV infection, transfection of poly(dA:dT) and treatment with MSU as indicated by ELISA (n = 3 ± s.e.m.). **(h)** WT and POP3 transgenic BMDM were infected

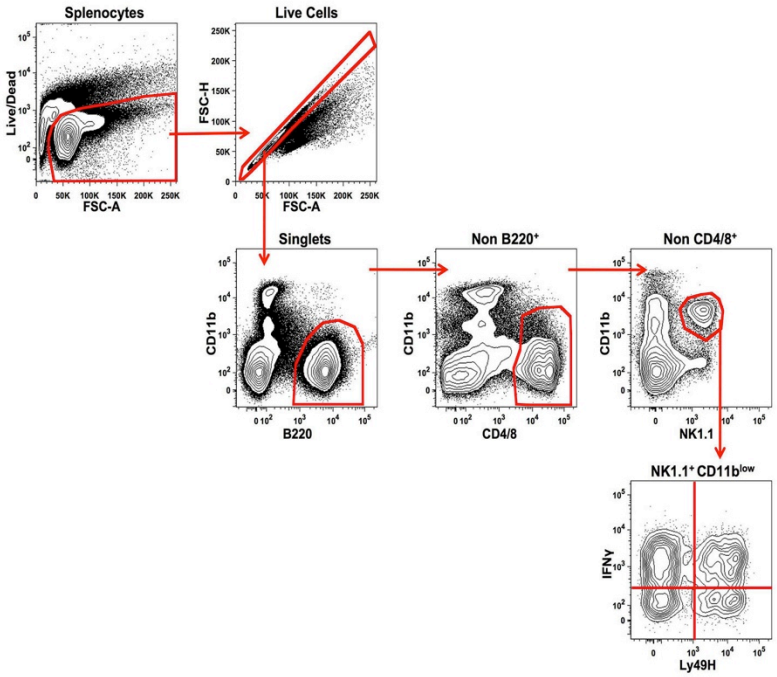
with MVA and analysed for mRNA expression of *IL1ra* and *Il18bp* (n=3 ± s.e.m.). **(i)** The POP3 antibody does not cross-react with other POP family members. HEK293 cells were transfected with Myc-tagged POP1, POP2 and POP3 and immunoprobed with our custom POP1, POP2 and POP3-specific antibodies. **(j)** The POP3 antibody does not cross-react with the related PYDs of AIM2 and IFI16. HEK293 cells were transfected with GFP or RFP-tagged POP3, AIM2-PYD and IFI16-PYD and immunoprobed with our POP3 antibody and with GFP and RFP antibodies as control. * denotes a cross-reactive protein. Data are representative of 3 experiments (a), 2 experiments (b-d, f) and 1 experiment (e, g-i). (a) **P*<0.0001, ***P*=0.0049; (b) **P*=0.0199, ***P*=0.0495; (f) **P*<0.0001; (g) **P*=0.0031, ***P*=0.0161, ****P*=0.0013; (h) **P*=0.0029 (*Il1ra*); **P*=0.0009 (*Il18bp*).



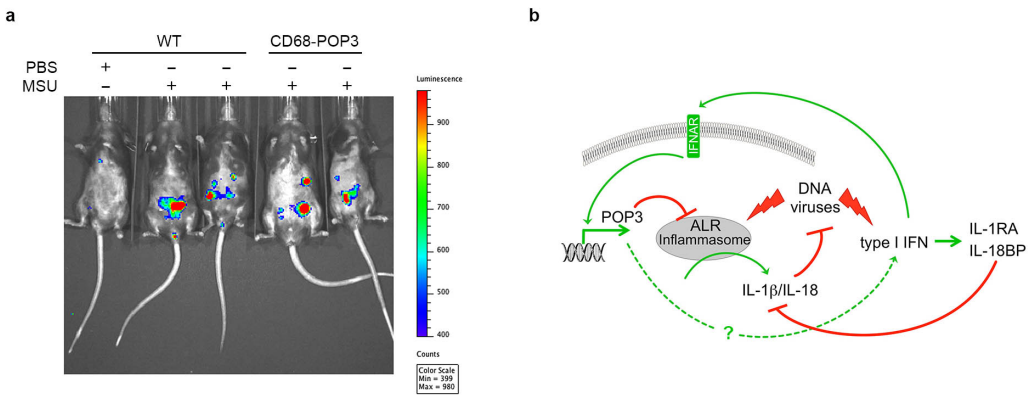
Supplementary Figure 4. Gating strategy for immunophenotyping of peripheral blood and peritoneal lavage cells. **(a)** Peripheral blood cells and **(b)** peritoneal lavage cells obtained 6 h after MCMV infection were gated according to established cell surface markers, as indicated.



Supplementary Figure 5. Validation of POP3 function in mouse macrophages. **(a)** Thioglycollate-elicited PM were isolated by peritoneal lavage, transfected with poly(dA:dT) for 16 h and analyzed for mature IL-1β by ELISA (n = 3 ± s.e.m.). **(b)** BMDM isolated from a 2nd line of CD68-POP3 TG mice were infected with MVA, treated with LPS/ATP or transfected with poly(dA:dT) for 16 h and analyzed for mature IL-1β by ELISA (n = 3 ± s.e.m.). **(c)** BMDM of UbiC-hCAR TG mice were immunoprobed for expression of $hCAR^{\Delta cyt}$ using HEK293 cells transiently transfected with $hCAR^{\Delta cyt}$ as a control. **(d)** WT (top panel) and UbiC-hCAR TG (bottom panel) BMDM were infected with increasing MOI of a GFP-expressing AdV and analyzed by fluorescence and phase contrast microscopy. **(e)** UbiC-hCAR TG BMDM were infected with low MOI of AdV expressing GFP or GFP-POP3 and transfected 48 h later with poly(dA:dT) or infected with MVA for 16 h and analyzed for secreted IL-1β by ELISA. Data are representative of two (a-c, e) and one (d) experiments. (b) ***P=0.0097, **P=0.0038; (e) ***P=0.0001, **P=0.0058.



Supplementary Figure 6. Gating strategy for immunophenotyping of splenocytes. Splenocytes obtained 36 h after MCMV infection were gated according to established cell surface markers, as indicated.



Supplementary Figure 7. POP3 does not ameliorate MSU-induced peritonitis. **(a)** WT and CD68-POP3 TG mice were i.p. injected with PBS or MSU crystals (10 mg/mouse) and mice were imaged for MPO activity *in vivo* 5 h later (n=3-7), showing representative examples. **(b)** Model of the type I IFN-induced regulatory loop of cytosolic DNA-induced inflammasome response that involves POP3. (a) Data are representative of one experiment.

References

1. Jin, T., Perry, A., Smith, P., Jiang, J. and Xiao, T.S. Structure of the Absent in Melanoma 2 (AIM2) Pyrin Domain Provides Insights into the Mechanisms of AIM2 Autoinhibition and Inflammasome Assembly. *J. Biol. Chem.* **288,** 13225-35 (2013).