

## Supplementary Information

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

Sonal Khare, Rojo A. Ratsimandresy, Lúcia de Almeida, Carla M. Cuda, Stephanie L. Rellick, Alexander V. Misharin, Melissa C. Wallin, Anu Gangopadhyay, Eleonora Forte, Eva Gottwein, Harris Perlman, John C. Reed, David R. Greaves, Andrea Dorfleutner & Christian Stehlik

<b>Supplementary Item &amp; Number</b>	<b>Title</b>
Supplementary Figure 1	<i>POP3</i> is a previously undescribed gene located between <i>IFI16</i> and <i>IFIX</i> .
Supplementary Figure 2	POP3 shows characteristic features of PYDs present in HIN-200 proteins.
Supplementary Figure 3	Silencing of POP3 specifically affects the AIM2 inflammasome.
Supplementary Figure 4	Gating strategy for immunophenotyping of peripheral blood and peritoneal lavage cells.
Supplementary Figure 5	Validation of POP3 function in mouse macrophages.
Supplementary Figure 6	Gating strategy for immunophenotyping of splenocytes.
Supplementary Figure 7	POP3 does not ameliorate MSU-induced peritonitis.
Supplementary References	

**a**

```

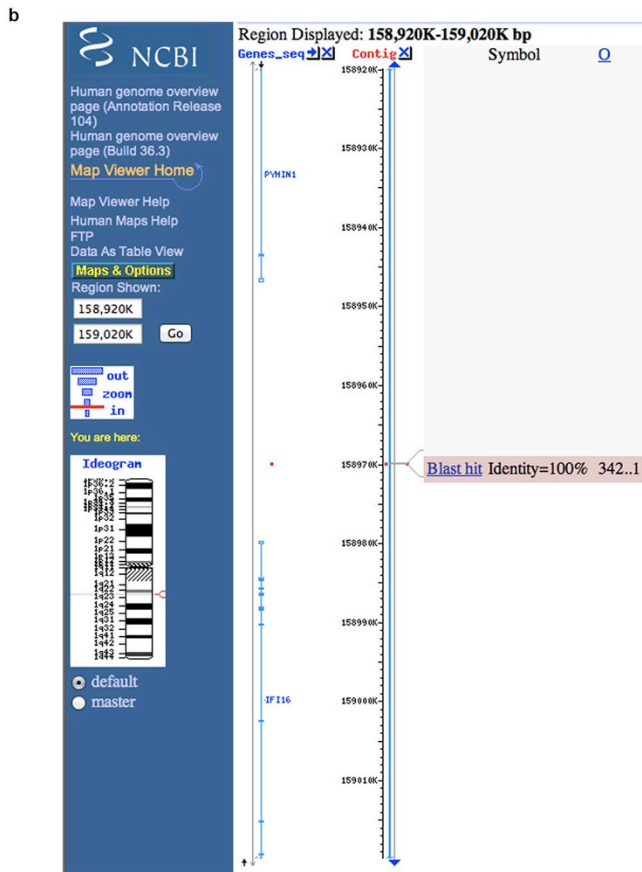
10      20      30      40      50      60      70      80      90
ATGGAGAGTA AATATAAGGA GATACTCTTG CTAACCAAGCC TGGATAACAT CACCGATGAG GAACTGGATA GGTTTAAGTG CTTTCTTCCA

100     110     120     130     140     150     160     170     180
GATGAGTTTA ATATTGCCAC AGGCAAACCTG CATACTCTAA ACAGCACGAG TAGCCAACCTT GATTTAAAC GCTGGCATGG TGTCTGCAGT

190     200     210     220     230     240     250     260     270
GAGGAAGACC GTATTTTCA GAAGCTGAAT TATATGCTTG TGGCAAATG TCTTCGGAA GAGCAGGAAA CAGGTATATG TGGGAGTCCC

280     290     300     310     320     330     340
TCATCTGCC GGTCCGTTTC TCAGTCAAGA CTTGGTCTTT CCTTTCATGG CATTCTGGG AATGCATGTT GA

```



**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.

**a**

```

MESKYKEILLTSLDNIIDEELDRFKCFLPDEFNIATGKLTHTLNSTSSQLDKRWHGVCSEEDRFQKLNLYMLVAKCLREEQETGICGSPSSARSVSQRLGLSFHGISGNAC

```

**b**

```

POP3-PYD 1 MESKYKE ILLLTSLDNIIDEELDRFKCFLPDEFNIATGKLTHTLNSTSSQL 50
AIM2-PYD 1 MESKYKE ILLLTGLDNIIDEELDRFKFLLSDEFNIATGKLTHTNRIQVAT 50
IFI-16-PYD 1 MGKKYKNIVLLKGLLEVINDYHFRMVKSLNSDLKLNLMKREEYDKIQIAD 50
MND4-PYD 1 MVNEYKIVLLKGLFELMDDYHFTSIKSLLAYDLGLTTKMQEYNNRIKIID 50
IFI-X-PYD 1 MANNYKIVLLKGLLEVINDYHFRIVKSLNSDLKLNPKMKEEYDKIQIAD 50

POP3-PYD 51 DLKRWHGVCSEED--RIFQKLN-YMLVAKCLREEQETGICGSPSSARSV 96
AIM2-PYD 51 LMIQNA GAVSAVMKTRIFQKLN-YMLLAKRLQEEK--V D K Q Y K S V 95
IFI-16-PYD 51 LMEKFRGDAGLGLKLEIEFEIPTLEDLAETLKKKELKVKGP----- 92
MND4-PYD 51 LMEKFGQVACLDKLELA KDMPSLKNLVNLRKEKSKVAKKIKTQEK A 99
IFI-X-PYD 51 LMEKFKPGDAGLGLKLEIEFEIPTLGDLAETLKRREKLKVKGIIPSKKTK- 99

```

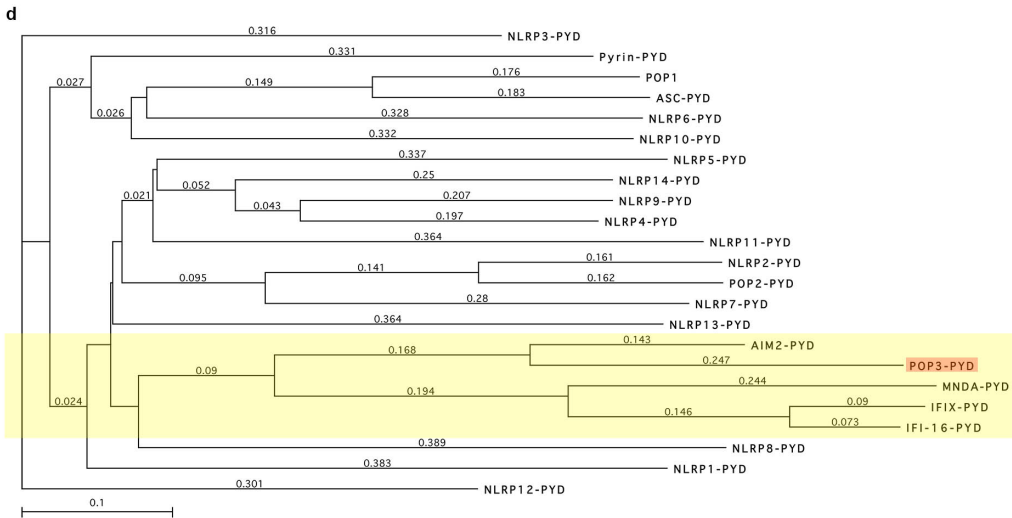
**c**

```

POP3-PYD 1 -----MESKYKEILLTSLDNIIDEELDRFKCFLP-----DEFNIATGKL 40
AIM2-PYD 1 -----MESKYKEILLTGLDNIIDEELDRFKFLLS-----DEFNIATGKL 40
IFI-16-PYD 1 -----MGKKYKNIVLLKGLLEVINDYHFRMVKSLNSDLKLNLMKREEYDKIQIAD 40
MND4-PYD 1 -----MVNEYKIVLLKGLFELMDDYHFTSIKSLLAYDLGLTTKMQEYNNRIKIID 40
IFI-X-PYD 1 -----MANNYKIVLLKGLLEVINDYHFRIVKSLNSDLKLNPKMKEEYDKIQIAD 40
NLRP2-PYD 1 -----MVSQAQMGFNLCALLLEQLSQDEEESKFKYLIITTF-----SLAHELQKIPHKEV 47
NLRP1-PYD 1 -----MAGGAWGRACYLEFLKKEELKEFQLLLANKN-----AHSRSSSGETPAQP 45
NLRP3-PYD 1 -----MASTRCKLARYLEDLEDVLLKFKMHLEDY-----PPQKGCIPLPGRQT 44
NLRP4-PYD 1 -----MAASFFSDFGLMWYLEELKKEEFKFKELKQM-----TLQLELKQIPWTEV 47
NLRP5-PYD 1 -----MGDKSLTFSSYGLQWCVLYELDKKEEFTFKELLKK-----SSESTTCSIQFEI 49
NLRP6-PYD 1 -----LAVARELLAALEELSQEQLKRRFRHKL RDV-----G-PDGRSIPWGR L 42
NLRP7-PYD 1 -----MTSPQLEWTLQTLLEQLNEDELKSFKSLWAF-----PLEDVLQKTPWSEV 46
NLRP8-PYD 1 -----FSCYPGSPCENGVMLYMRNVSHLEELQRKQLLLE-----LSTGTFPIITWDQV 48
NLRP9-PYD 1 -----MAESFSDFGLEWYKELRKEEFWKFKELLKP-----LEKFE LKPIPWAE L 47
NLRP10-PYD 1 -----MAESDSTDFDLWYLENLSDKEFGQFKKYLRM-----KILDFKLPQFPL 44
NLRP11-PYD 1 -----MAMAKARKPREALLWALS DLEENDFKKLLKFLYLRDM-----TLSEGQPLAR GEL 49
NLRP13-PYD 1 MNFSVITCPNGGTNQGLLPYLMALDQYQLEEFKLCLEPQQLMDFW SAPQGHPFRIPWANL 60
NLRP12-PYD 1 -----MLRTAGRDGLCRLLSTYLEELEAVELEKFKYLGTA-----TELGEKIPWGS M 48
NLRP14-PYD 1 -----MADSSSSFFPFDGFLLLYLEELNKEELNTFKLFLKET-----MEPEHGLTPWNEV 50
ASC-PYD 1 -----MGRARDAILDALENLTAELKFKKLLKLSV-----PLREGYRIPRGL 44
POP1 1 -----MGTKREAIKLVLENLTPLEKFKMKLGTV-----PLREGYRIPRGL 44
POP2-PYD 1 -----MASAELEDFLQALLLELSQDEESKFKSLIRTI-----SLKELQTVPQTEV 47
Pyrin-PYD 1 -----MAKTPSDHLLSTLEELVYDFEKFVKLQNT-----SVQKEHSRIIPRSQI 45

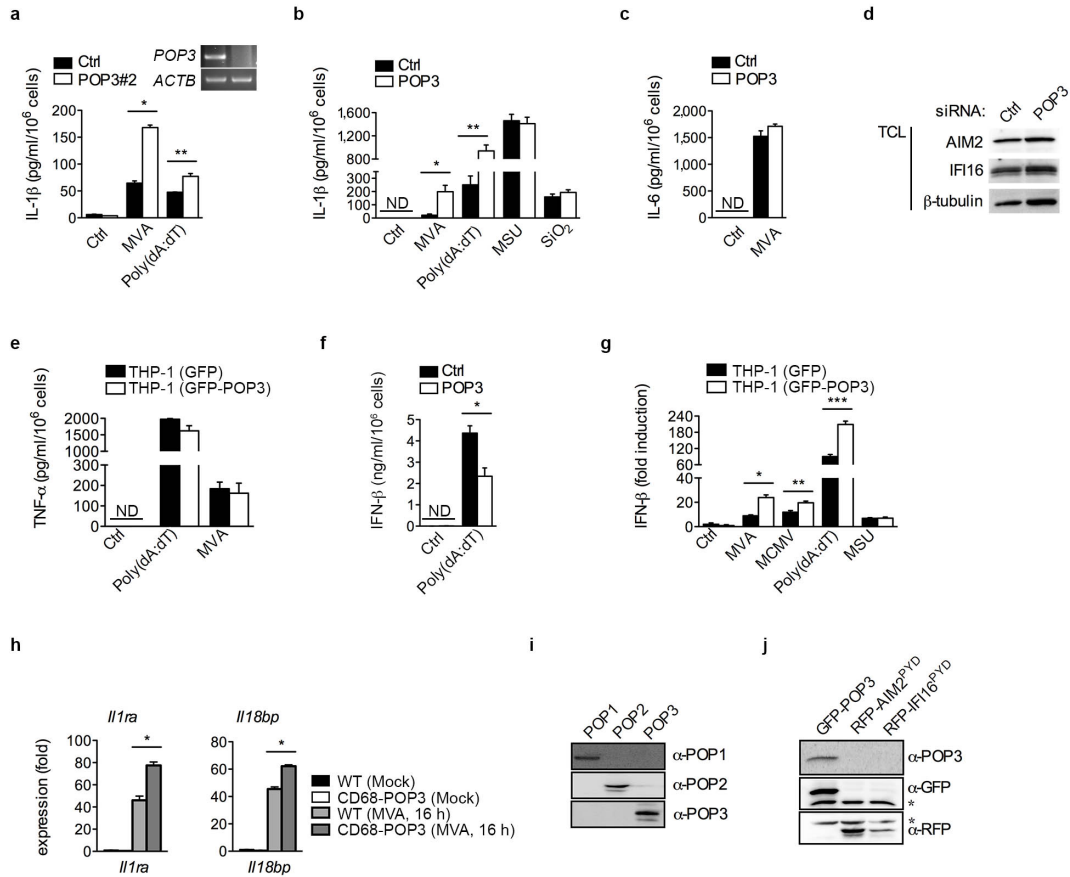
POP3-PYD 41 HTLNSTSSQLDKRWHGVCSEED--RIFQKLN-YMLVAKCLREEQETGICGSPSSARSV 96
AIM2-PYD 41 HTANRIQVATLMIQNA GAVSAVMKTRIFQKLN-YMLLAKRLQEEK--V D K Q Y K S V 95
IFI-16-PYD 41 EYDKIQIADLMEKFRGDAGLGLKLEIEFEIPTLEDLAETLKKKELKVKGP----- 92
MND4-PYD 41 EYNRKIVTDLMEKFRQVACLDKLELA KDMPSLKNLVNLRKEKSKVAKKIKTQEK A 99
IFI-X-PYD 41 EYDKIQIADLMEKFRGDAGLGLKLEIEFEIPTLGDLAETLKRREKLKVKGIIPSKKTK- 99
NLRP2-PYD 41 DKADGKQLVEI LTHCDYSYVWEMASLQVFEKMH-RMDLSERAKDEVREAA L----- 97
NLRP3-PYD 41 EKTSGMEVASYLVAQYGEQRWDLALHTWEQMG-LRSICAQAGEGHSPS----- 95
NLRP4-PYD 41 EKADHVDLALMIDFNGEKAWAMAVWIFAAIN-RRDLYEAKKRDEPKWGS----- 94
NLRP5-PYD 41 KKASREELANLLIKHYEEQAWNITLRI FQKMD-RKDLCKMVMRERTG----- 94
NLRP6-PYD 50 ENANVECLA LLLH EY YGASLAWATSISIFENMN-LRTLSEKARDDMKRHSPEDPEATMTD 108
NLRP7-PYD 43 ERADAVDLAQLAQFYGP EAL E VARKTLKRA D-ARDVAAQLQERRLQ----- 89
NLRP8-PYD 47 EADGKLAETILVNTSSENWIRNATVNI L EEMN-LTELCMKAKENMMLQ----- 94
NLRP9-PYD 49 ETASWAEVYHLLIERFPGRRAWDVTSNIFAIMN-CKDMCVVVRREINAILPTLEPEDLNV 107
NLRP10-PYD 48 KKASKEDVAKLLDKHYPGKQWVETLNLFLQIN-RKDLWTKAQEEMRN----- 94
NLRP11-PYD 45 IQMTKEELANVLPISYEQYIWNMLF SIFSMMR-KEDLCRKIIGRRNR----- 91
NLRP12-PYD 50 EGLIPVDLAE LLLSKYGEKEAVKVV LKGLKVMN-LLELV DQLSHICLH----- 96
NLRP13-PYD 61 RAADPLNLSFLLEHFFPKGQAWKVV L G I FQTMN-LTSLCEKVR AEMKE----- 107
NLRP14-PYD 49 EKAGPLEMAQLLITFHGPEEAWRLALSTFERIN-RKDLWERGQREDLV----- 95
NLRP14-PYD 45 KKARR EDLANLMKYYYPGEKAWSVSLKIFGKMN-LKDLCEKAKKEEINW----- 92
ASC-PYD 45 L SMDALD L TDKLVASYYEDYAAELVAVLRDMG-LQEMAGLQQAATHQG----- 92
POP1 45 GQDIDVLDLTKLVASYYEDYAAELVAVLRDMG-LQEMAGLQQAATHQG----- 89
POP2-PYD 48 DKANGKQLVEI FTSHSCSYWAGMAAIQVFEKMN-QTHLSGRADE----- 90
Pyrin-PYD 46 QRARPVKMATLLVTYGEEYAVQLTLQVLR AIN-QRLLA EELHRAA I Q----- 92

```



**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  $\alpha$ -helices are marked with blue lines (bottom), while the corresponding  $\alpha$ -helices of AIM2, as determined by crystal structure<sup>1</sup>, 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)** hM $\Phi$  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-1 $\beta$  and **c**, IL-6 by ELISA ( $n = 3 \pm$  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<sub>2</sub>, as indicated for 16 h and analyzed for **b**, IL-1 $\beta$  secretion and **f**, IFN- $\beta$  by ELISA ( $n = 3 \pm$  s.e.m.). **(e, g)** THP-1 (GFP) and THP-1 (GFP-POP3) cells were analyzed for secretion of **e**, TNF $\alpha$  and **g**, IFN- $\beta$  in response to MVA and MCMV infection, transfection of poly(dA:dT) and treatment with MSU as indicated by ELISA ( $n = 3 \pm$  s.e.m.). **(h)** WT and POP3 transgenic BMDM were infected

with MVA and analysed for mRNA expression of *IL1ra* and *Il18bp* ( $n=3 \pm$  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

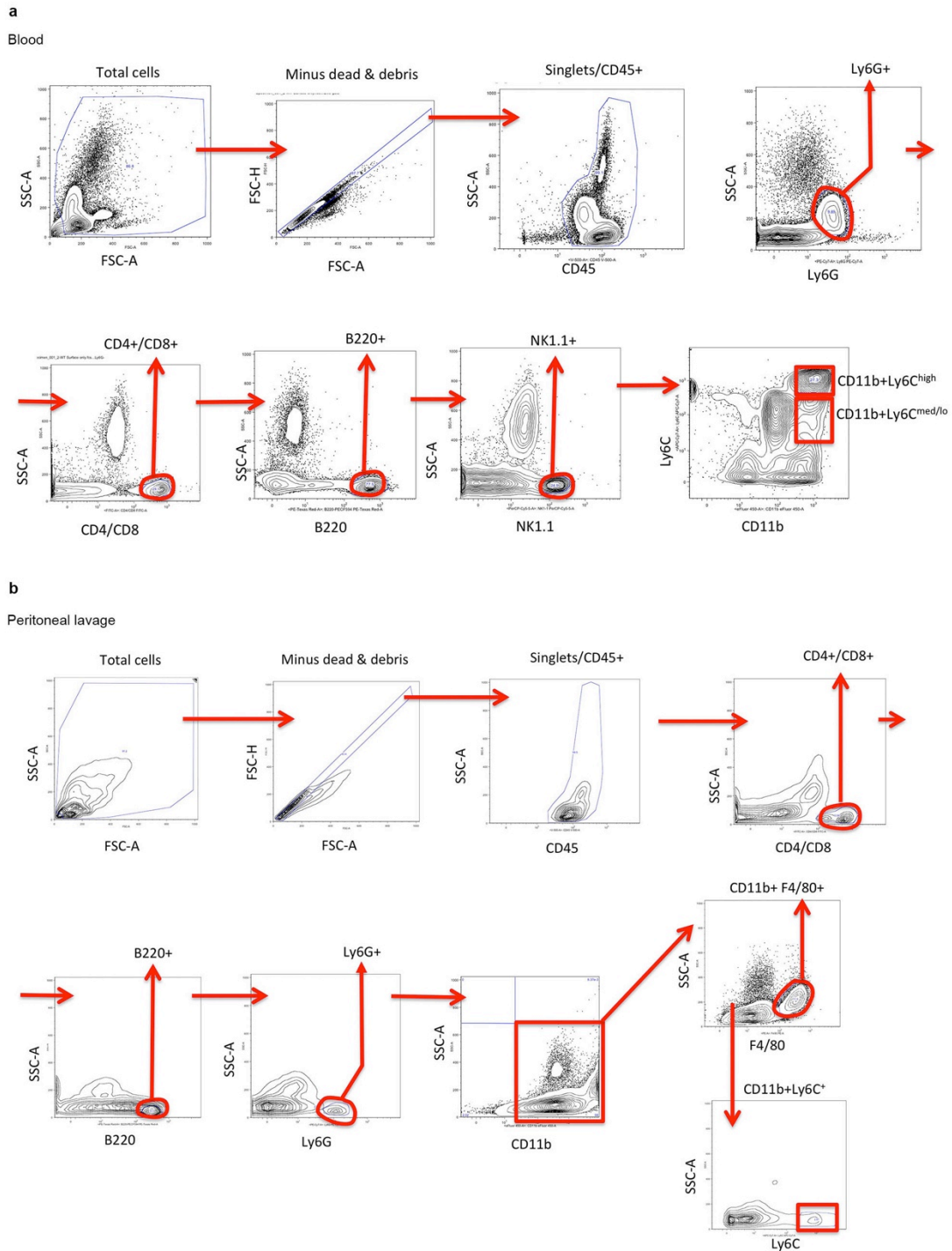
and IFI16-PYD and immunoprobed with our POP3 antibody and with GFP and

RFP antibodies as control. \* denotes a cross-reactive protein. Data are

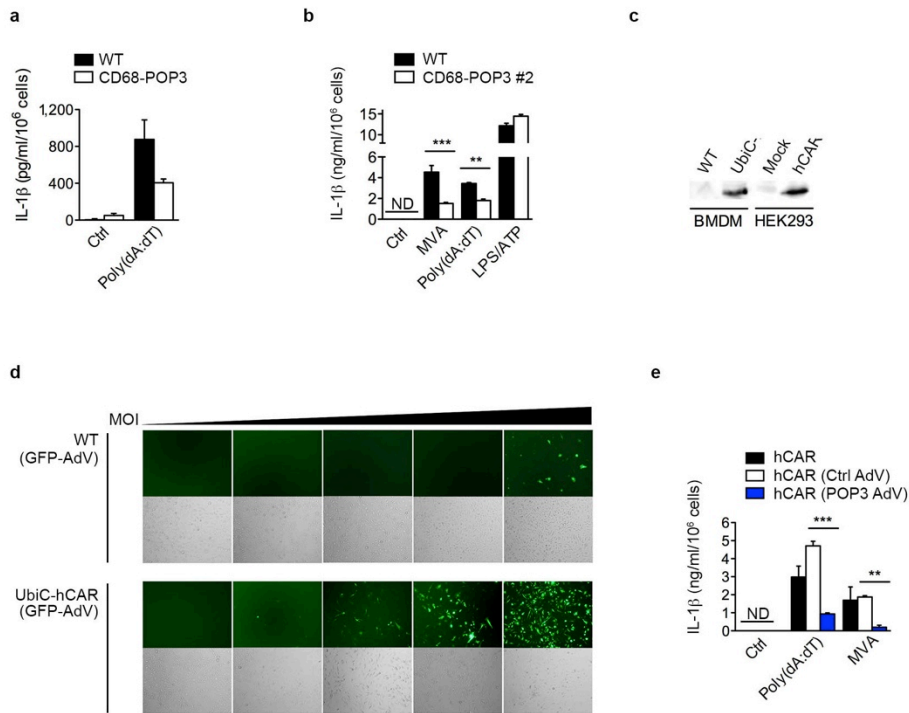
representative of 3 experiments (a), 2 experiments (b-d, f) and 1 experiment (e,

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*).



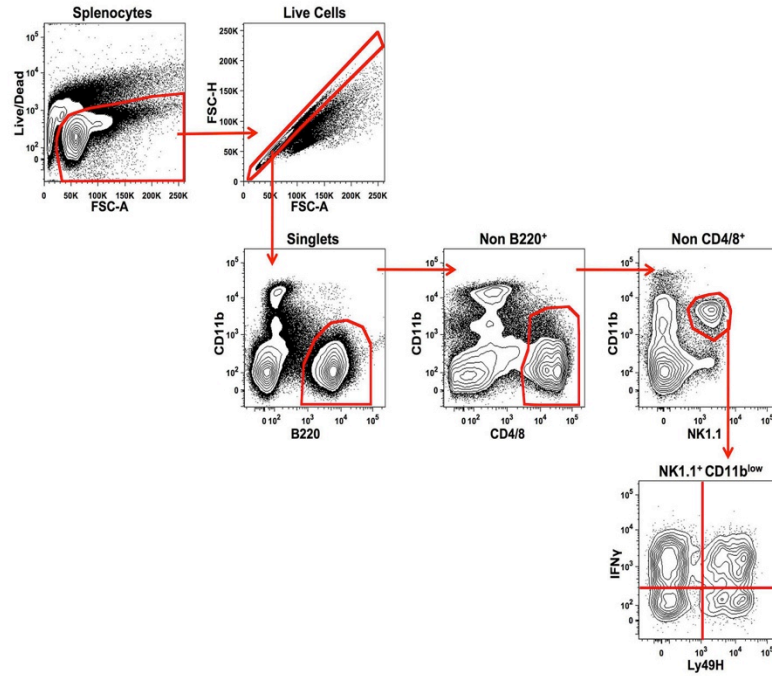
**Supplementary Figure 4.** Gating strategy for immunophenotyping of peripheral blood and peritoneal lavage cells. **(a)** Peripheral blood cells and **(b)** peritoneal lavage cells obtained 6 h after MCMV infection were gated according to established cell surface markers, as indicated.



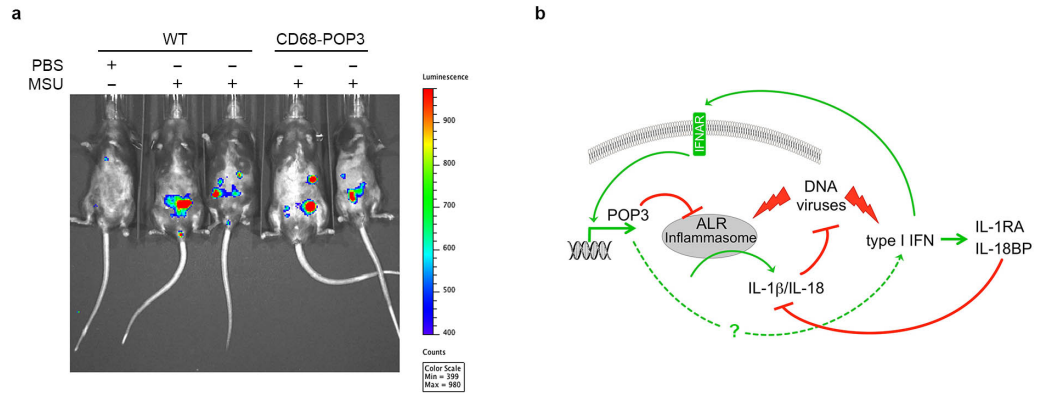
**Supplementary Figure 5.** Validation of POP3 function in mouse macrophages.

**(a)** Thioglycollate-elicited PM were isolated by peritoneal lavage, transfected with poly(dA:dT) for 16 h and analyzed for mature IL-1 $\beta$  by ELISA ( $n = 3 \pm$  s.e.m.). **(b)** BMDM isolated from a 2<sup>nd</sup> 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-1 $\beta$  by ELISA ( $n = 3 \pm$  s.e.m.). **(c)** BMDM of UbiC-hCAR TG mice were immunoprobed for expression of hCAR <sup>$\Delta$ cyt</sup> using HEK293 cells transiently transfected with hCAR <sup>$\Delta$ cyt</sup> 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-1 $\beta$  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).