Supplementary Figures

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ASC has extracellular and prionoid activities that propagate inflammation

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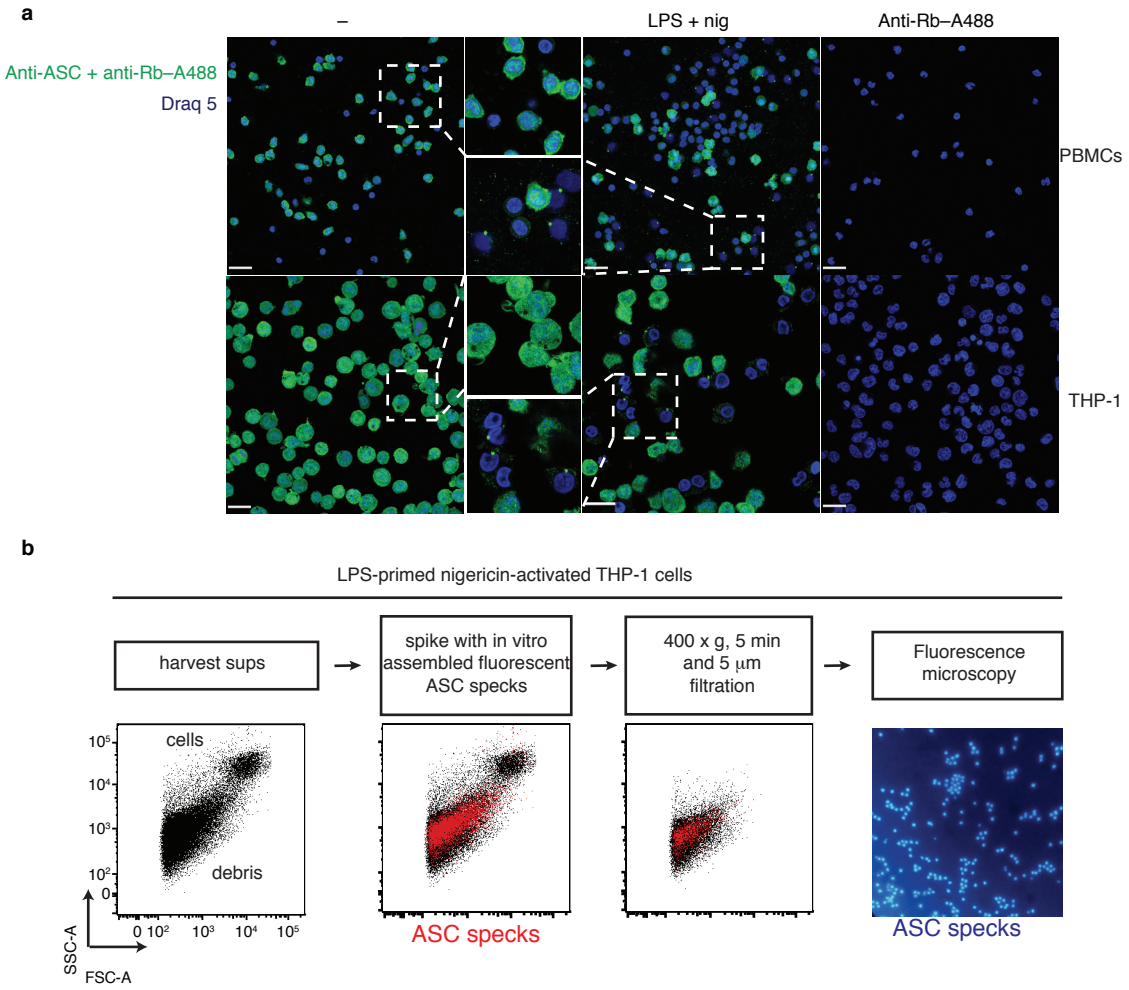
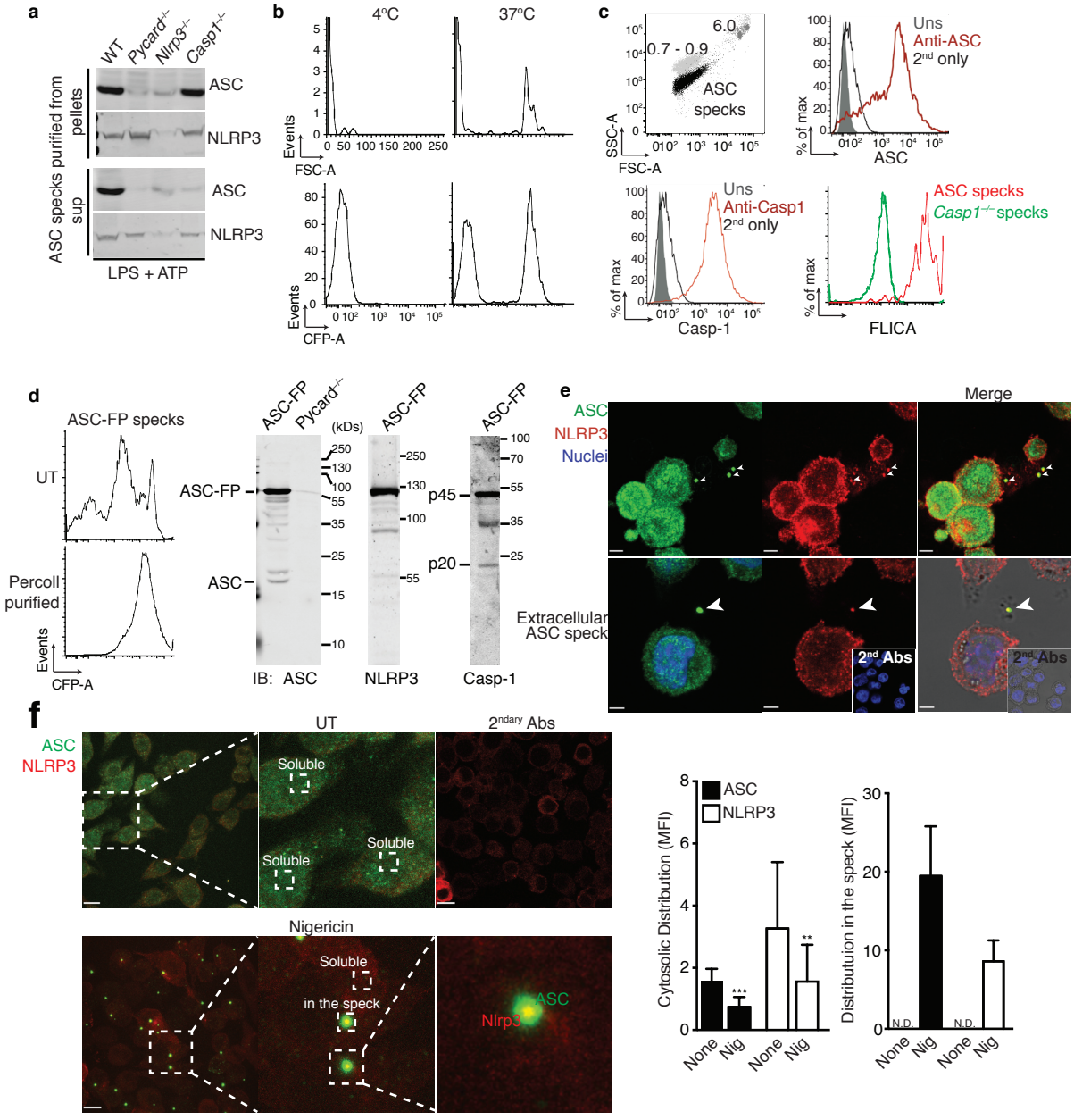
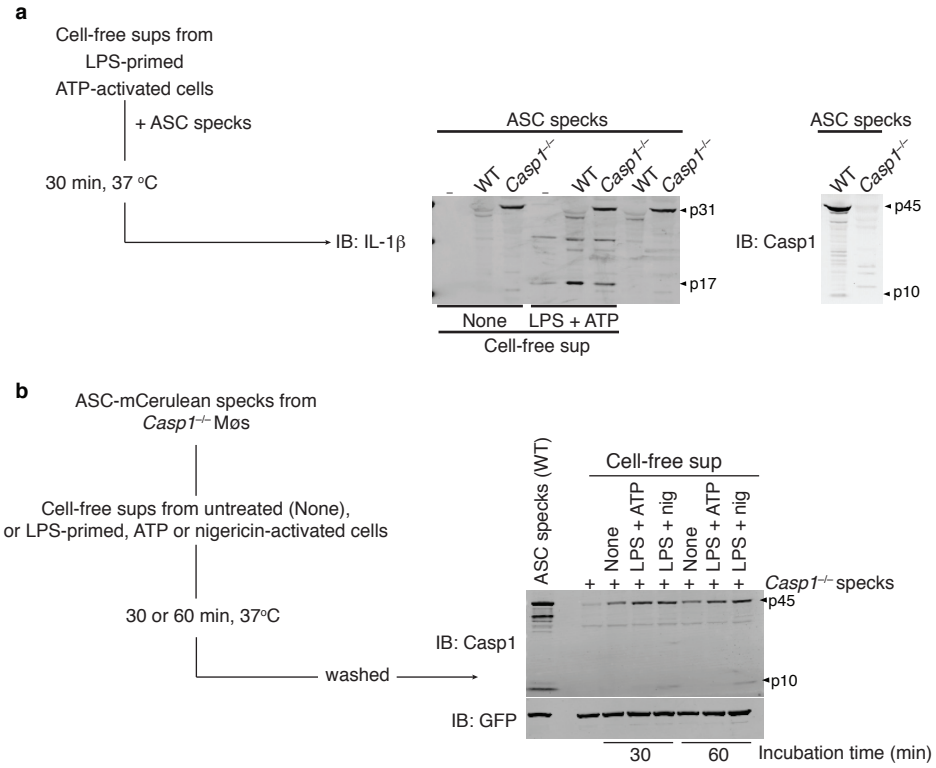


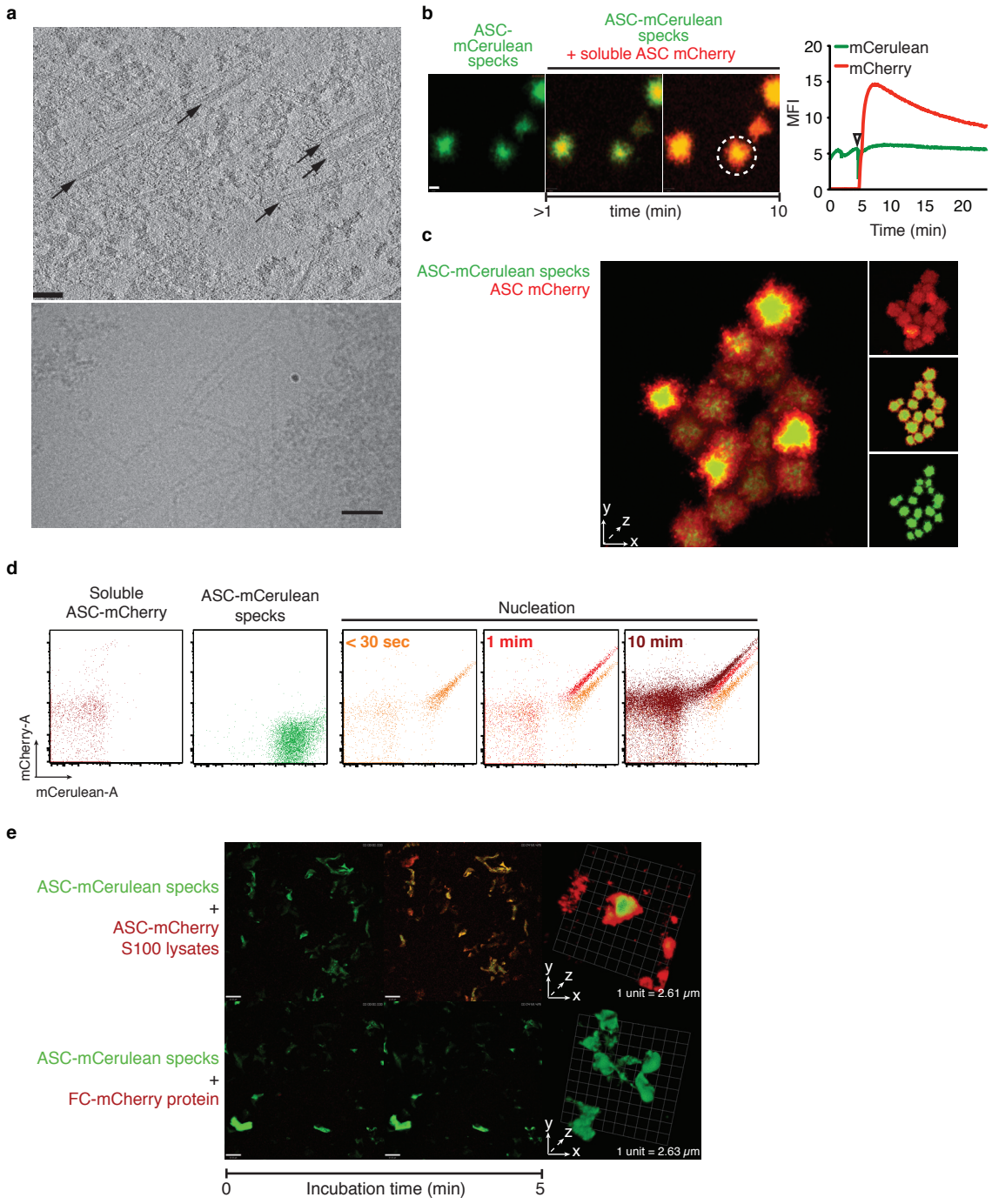
Supplementary Fig 1 ASC forms speck like clusters in different cells. (**a**) Confocal imaging of unstimulated (–) or LPS-primed (200 pg/ml), nigericin-activated (10 μM) human PBMCs (upper panel) or THP-1 monocytes (LPS 1 μg/ml, nigericin 10 μM, lower panel). Scale bars: 22 μm. (**b**) Flow charge and flow cytometry analysis and fluorescence imaging of ASC-mCerulean specks that were spiked into supernatants of LPS-primed (1 μg/ml), nigericin-activated (10 μM) THP-1 monocytes. Data is representative of two independent experiments.



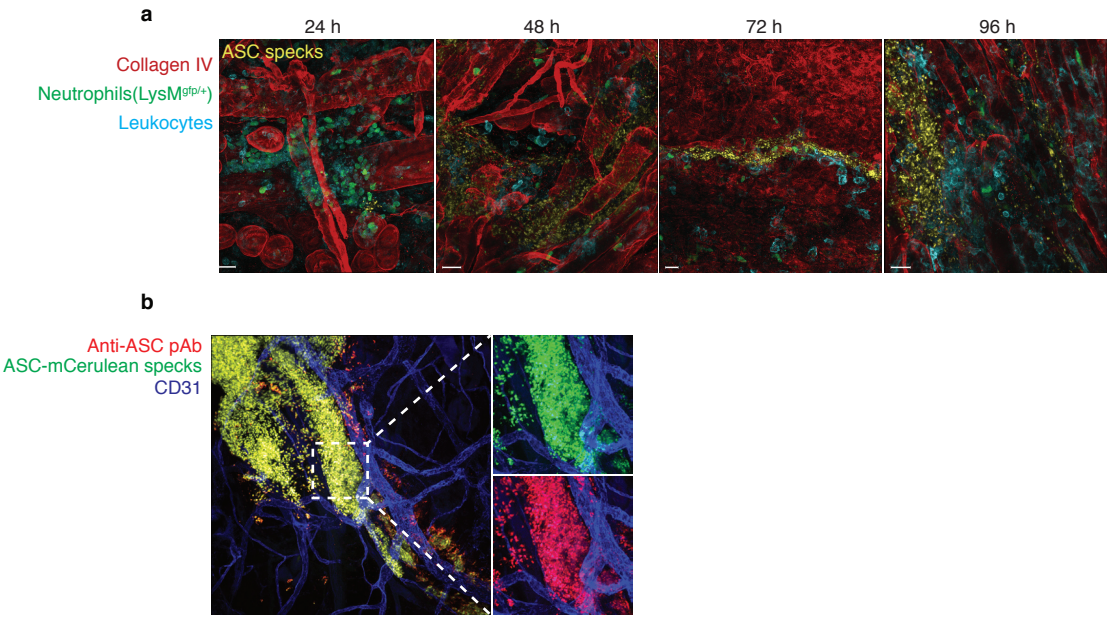
Supplementary Fig 2 (**a**) Immunoblot of ASC and NLRP3 in ASC specks purified from cell-free supernatants or from whole cell lysates of LPS-primed, ATP activated Wt, *Pycard*$^{-/-}$, *Casp1*$^{-/-}$ or *Nlrp3*$^{-/-}$ iMøs. (**b**) Flow cytometry analysis showing *in vitro* assembly of ASC-mCerulean specks from cytosolic lysates (S100) of untreated ASC-mCerulean expressing cells. (**c**) Flow cytometry analysis of *in vitro*-assembled ASC specks prepared as in **b**, that were left unstained (Uns) or stained with anti-ASC or anti-Caspase1 Abs followed by staining with A488-direclty conjugated secondary Abs. Samples were also stained with the caspase-1 inhibitor z-YVAD-FMK-FITC and compared to ASC specks generated from *Casp1*$^{-/-}$ iMøs. Beads with defined sizes (μm) were used as reference. (**d**) Flow cytometry analysis showing the purification with a 50% percoll cushion and immunoblot for ASC, NLRP3 and Caspase-1 in *in vitro*-assembled ASC-mCerulean specks prepared as in **b**. (**e**) Confocal imaging of LPS-primed and nigericin-activated WT THP-1 monocytes. Cells were fixed and stained with anti-ASC or anti-NLRP3 Abs coupled to fluorochrome-conjugated secondary Abs. Arrows show intra- and extracellular specks containing both endogenous ASC and NLRP3 proteins. Scale bar: 3.2 μm. (**f**) Confocal imaging of LPS-primed NLRP3-mCitrine expressing iMøs left untreated (UT) or activated with nigericin (10 μM). Cells were fixed and stained with anti-ASC Abs followed by staining with fluorescence-conjugated secondary Abs. The mean fluorescence intensity (MFI) of ASC or NLRP3 was compared in the cytosol (soluble) and on the specks. Data are from one representative out of two (**a**-**f**) independent experiments.



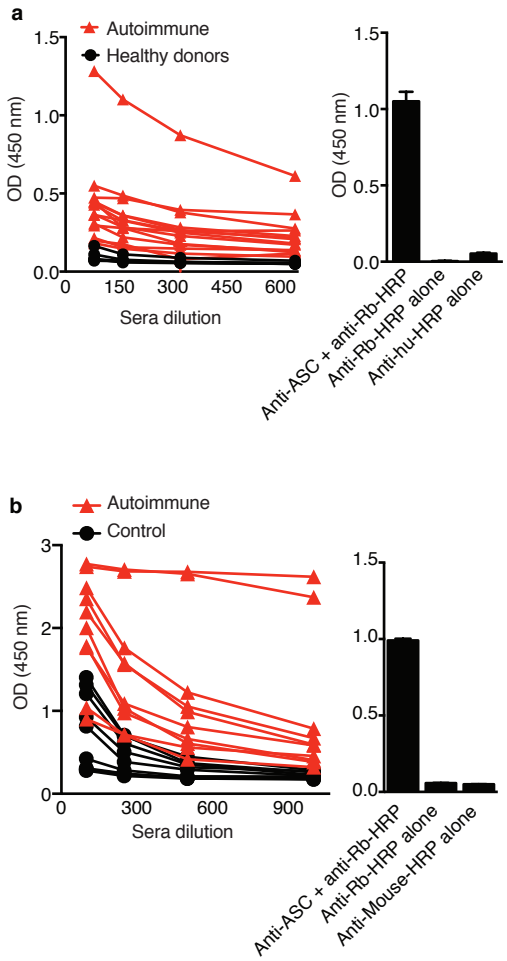
Supplementary Fig 3 ASC specks recruit pro-caspase-1 from cell-free supernatants. (**a**) Immunoblot for IL-1β and Caspase-1 in cell-free supernatants from untreated or LPS-primed (500 ng/ml, 3h), ATP activated (2.5 mM) iMøs before and after addition of fluorescent recombinant ASC-mCerulean specks generated from WT or *Casp1*$^{-/-}$ iMøs. (**b**) Immunoblot for Caspase-1 and GFP in in recombinant ASC-mCerulean specks from WT or *Casp1*$^{-/-}$ iMøs that were incubated with cell-free supernatants of unstimulated (None), or LPS-primed and ATP, or nigericin-activated iMøs. Data are from one representative of two independent experiments.



Supplementary Fig 4 ASC has prionoid features (**a**) Cryo-EM of in vitro-assembled ASC-mCerulean specks. Scale bars: 100 nm and 200 nm. (**b**) Time-lapse confocal imaging of the *in vitro* nucleation of soluble ASC-mCherry (red) by ASC-mCerulean specks (green). Scale bar: 4.4 μm. Graph shows mean fluorescence intensity over time of ASC-mCerulean specks after addition of soluble ASC-mCherry. (**c**) Rendered 3D confocal image of z-stack sections of ASC-mCerulean specks after incubation with soluble ASC-mCherry. (**d**) Flow cytometry of pre-assembled ASC-mCerulan specks incubated with soluble ASC-mCherry for the indicated time. (**e**) Confocal imaging of the nucleation of soluble ASC-mCherry, or FC-mCherry protein by ASC-mCerulean specks. Images were recorded every 30 seconds at 63X magnification. Data are from one representative of two (**a**,**d**,**e**) or three (**b**,**c**) independent experiments.



Supplementary Fig 5 ASC specks are resistant to proteases *in vivo* (**a**) *Ex vivo* confocal imaging of whole mount staining of the ears of LysM$^{gfp/+}$ transgenic reporter mice at 24, 48, 72 or 96 hours post injection of 1 μg of ASC-mCerulean specks in 10 μl of PBS. (**b**) *Ex vivo* confocal imaging of subcapsular sinus macrophages of popliteal draining LNs from LysM$^{gfp/+}$ mice injected with 2.5 μg of ASC-mCerulean specks and re-injected 4 hours later via the same route with PE-labeled anti-ASC Abs. Scale bars: 20 μm. Data are from a representative out of two independent experiments.



Supplementary Fig 6 Autoimmune sera contains anti-ASC speck Abs (**a**) ELISA of anti-ASC specks Abs in sera from autoimmune patients (n = 14), or healthy donors (n = 7). (**b**) ELISA of anti-ASC specks Abs in sera from autoimmune (n = 10), or control (n = 10) mice. ELISA plates were coated with 50 μg/ml of ASC specks or PBS. Anti-ASC specks Abs in sera were detected with specific anti-human or anti-mouse HRP conjugated secondary Abs. The absorbance (OD) at 450 nm was normalized against the OD obtained in wells coated with PBS and assayed with anti-human or mouse-HRP Abs. The reactivity of secondary Abs against ASC specks without human or mouse sera is shown.