Expanded View Figures

Figure EV1. Prdx4 does not impact priming of inflammasome components or associated factors (relates to Fig 3).

A qRT–PCR analysis of *Nlrp3*, *Il1b*, *Il18*, *ASC*, *caspase-1*, *Nlrp1*, *Trxnip*, and *Nos2* relative to *Gapdh* mRNA in Prdx4 WT and KO BMDMs, primed for 6 h with LPS or left untreated.

B Western blot analysis of NLRP3, pro-caspase-1, ASC, pro-IL-1β, Prdx4, and β-actin (loading control) in Prdx4 WT and KO BMDMs at 6 h after LPS stimulation.

C Western blot analysis of NLRP3, Prdx4, and β-actin (loading control) in Prdx4 WT and KO BMDMs at 6 h after LPS stimulation and CHX treatment for the time points indicated.

Data information: (A) Each dot represents a biological replicate; horizontal lines indicate mean. Vertical lines indicate SD (C). n.s. not significant (two-tailed *t*-test). Data are representative of one experiment with $n = 4$ mice per genotype with $n = 2$ technical replicates (A) or two (B, C) independent experiments with $n = 3$ mice per genotype.

Source data are available online for this figure.

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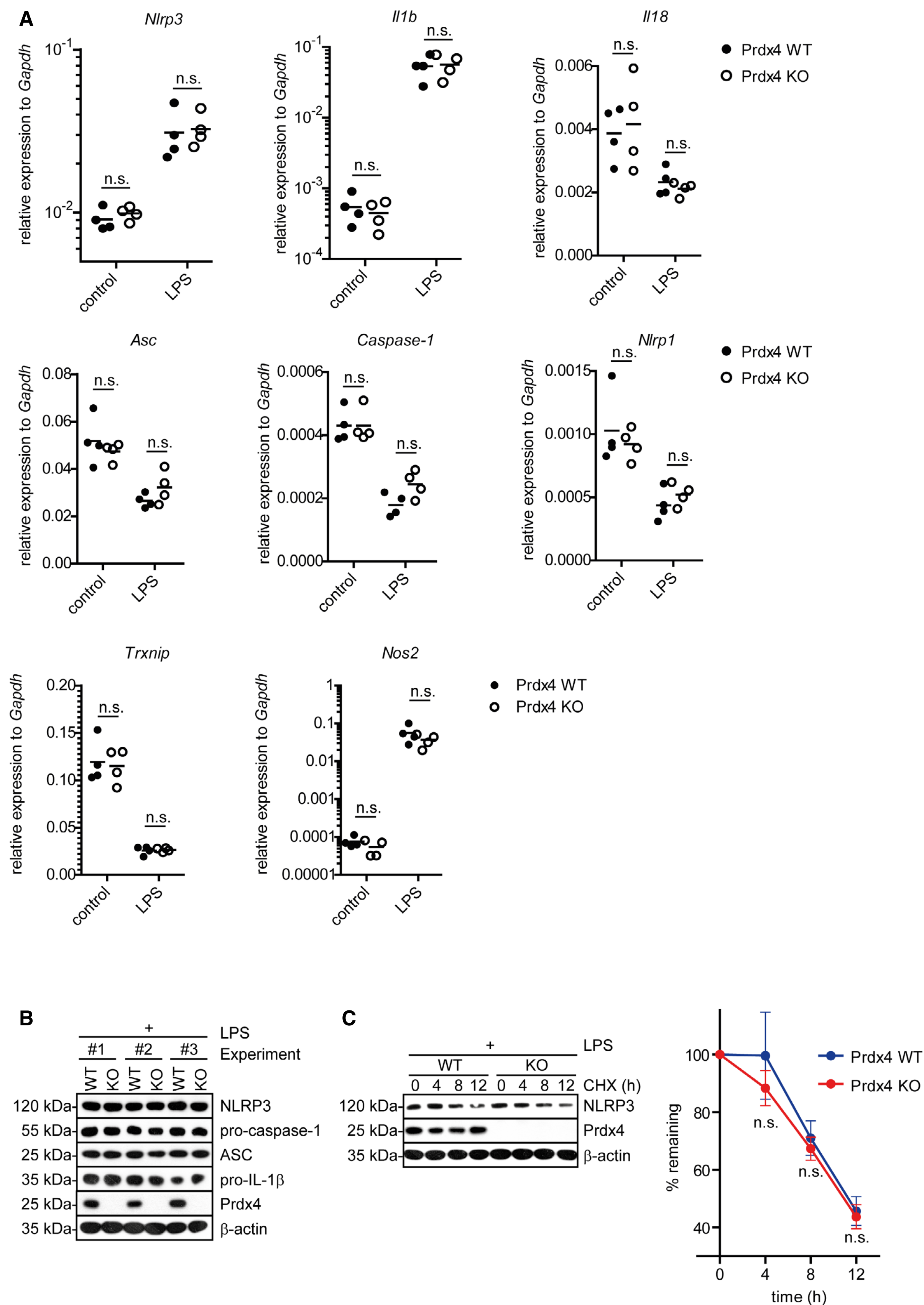
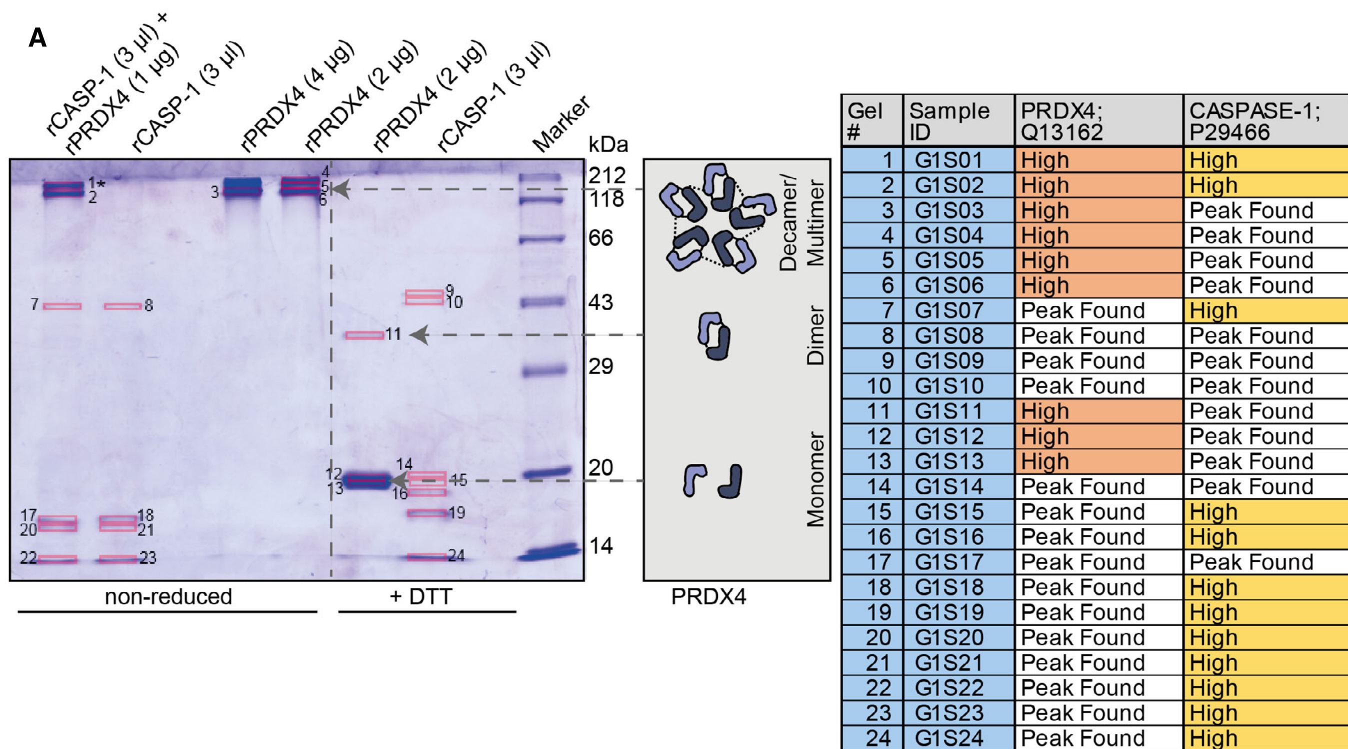
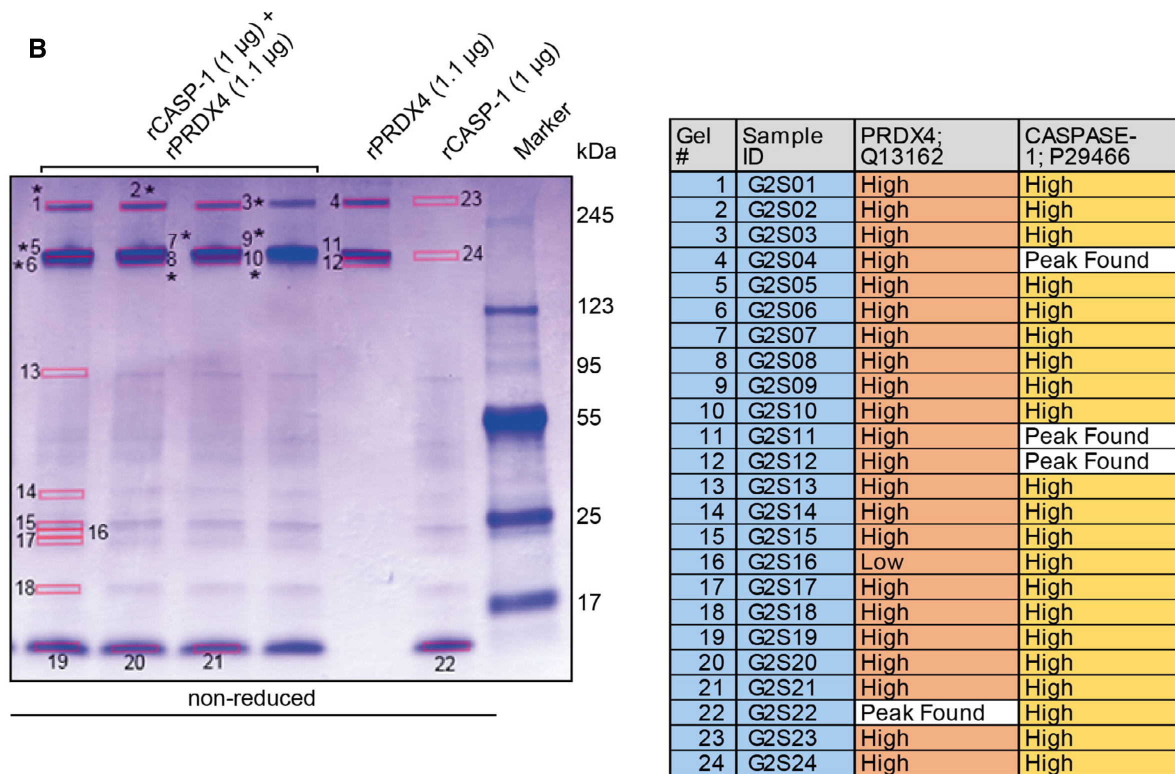


Figure EV1.



Gel #	Sample ID	PRDX4; Q13162	CASPASE-1; P29466
1	G1S01	High	High
2	G1S02	High	High
3	G1S03	High	Peak Found
4	G1S04	High	Peak Found
5	G1S05	High	Peak Found
6	G1S06	High	Peak Found
7	G1S07	Peak Found	High
8	G1S08	Peak Found	Peak Found
9	G1S09	Peak Found	Peak Found
10	G1S10	Peak Found	Peak Found
11	G1S11	High	Peak Found
12	G1S12	High	Peak Found
13	G1S13	High	Peak Found
14	G1S14	Peak Found	Peak Found
15	G1S15	Peak Found	High
16	G1S16	Peak Found	High
17	G1S17	Peak Found	Peak Found
18	G1S18	Peak Found	High
19	G1S19	Peak Found	High
20	G1S20	Peak Found	High
21	G1S21	Peak Found	High
22	G1S22	Peak Found	High
23	G1S23	Peak Found	High
24	G1S24	Peak Found	High



Gel #	Sample ID	PRDX4; Q13162	CASPASE-1; P29466
1	G2S01	High	High
2	G2S02	High	High
3	G2S03	High	High
4	G2S04	High	Peak Found
5	G2S05	High	High
6	G2S06	High	High
7	G2S07	High	High
8	G2S08	High	High
9	G2S09	High	High
10	G2S10	High	High
11	G2S11	High	Peak Found
12	G2S12	High	Peak Found
13	G2S13	High	High
14	G2S14	High	High
15	G2S15	High	High
16	G2S16	Low	High
17	G2S17	High	High
18	G2S18	High	High
19	G2S19	High	High
20	G2S20	High	High
21	G2S21	High	High
22	G2S22	Peak Found	High
23	G2S23	High	High
24	G2S24	High	High

Figure EV2.

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Figure EV2. Analysis of caspase-1 HMW complex by mass spectrometry (relates to Fig 4).

A, B SDS–PAGE and mass spectrometry analyses of rPRDX4, rCASP-1, or co-incubated rPRDX4+rCASP-1. (A) rPRDX4, rCASP-1, or co-incubated rPRDX4+rCASP-1 as well as rPRDX4 and rCASP-1 treated with DTT were loaded onto the gel, and SDS–PAGE was performed on a 4% polyacrylamide stacking gel followed by a 10% gel for separation. The gray box depicts a schematic illustration of Prdx4 decamers/multimers, dimers and monomers. Excised spots (1–24), analyzed by in-gel digestion and LC-ESI MS, are indicated (red rectangles). Gel spots were digested with pepsin, and peptide extracts were analyzed by LC-ESI MS with an Orbitrap Velos mass spectrometer. Both HCD and ETD spectra were acquired. Caspase-1 was identified by three peptides together with Prdx4 in-gel spot 1 (asterisk). (B) SDS–PAGE was performed on a 4–20% gradient gel. Excised spots (1–24), analyzed by in-gel digestion and LC-ESI MS, are indicated (red rectangles). Gels spots were digested with pepsin, and peptide extracts were analyzed on two LC-ESI MS platforms. HCD spectra were acquired with a QExactive MS, and both HCD and ETD spectra were acquired using an Orbitrap Velos MS instrument. Spots where at least two caspase-1 peptides could be detected on Prdx4 gel spots are indicated by asterisk.

Source data are available online for this figure.

Figure EV3. Characterization of EV isolates, obtained from inflammasome-activated BMDMs (relates to Fig 6).

A Dynamic light scattering (DLS) measurements from EVs, isolated from the supernatant of unstimulated or LPS+ATP-treated BMDMs.

B Transmission electron microscopy (TEM) and high magnification of EVs, isolated from the supernatant of unstimulated or LPS+ATP-treated BMDMs. Scale bar indicates 100 nm.

C Reducing SDS–PAGE (left panel) or non-reducing SDS–PAGE (right panel) followed of EV and whole-cell lysates followed by Western blot analysis using antibodies to Prdx4, inflammasome components (NLRP3, pro-caspase-1, pro-IL-1β, and ASC), CD63 as a positive EV marker, Grp94, mitofilin, and cytochrome c as negative markers and β-actin as control.

Source data are available online for this figure.

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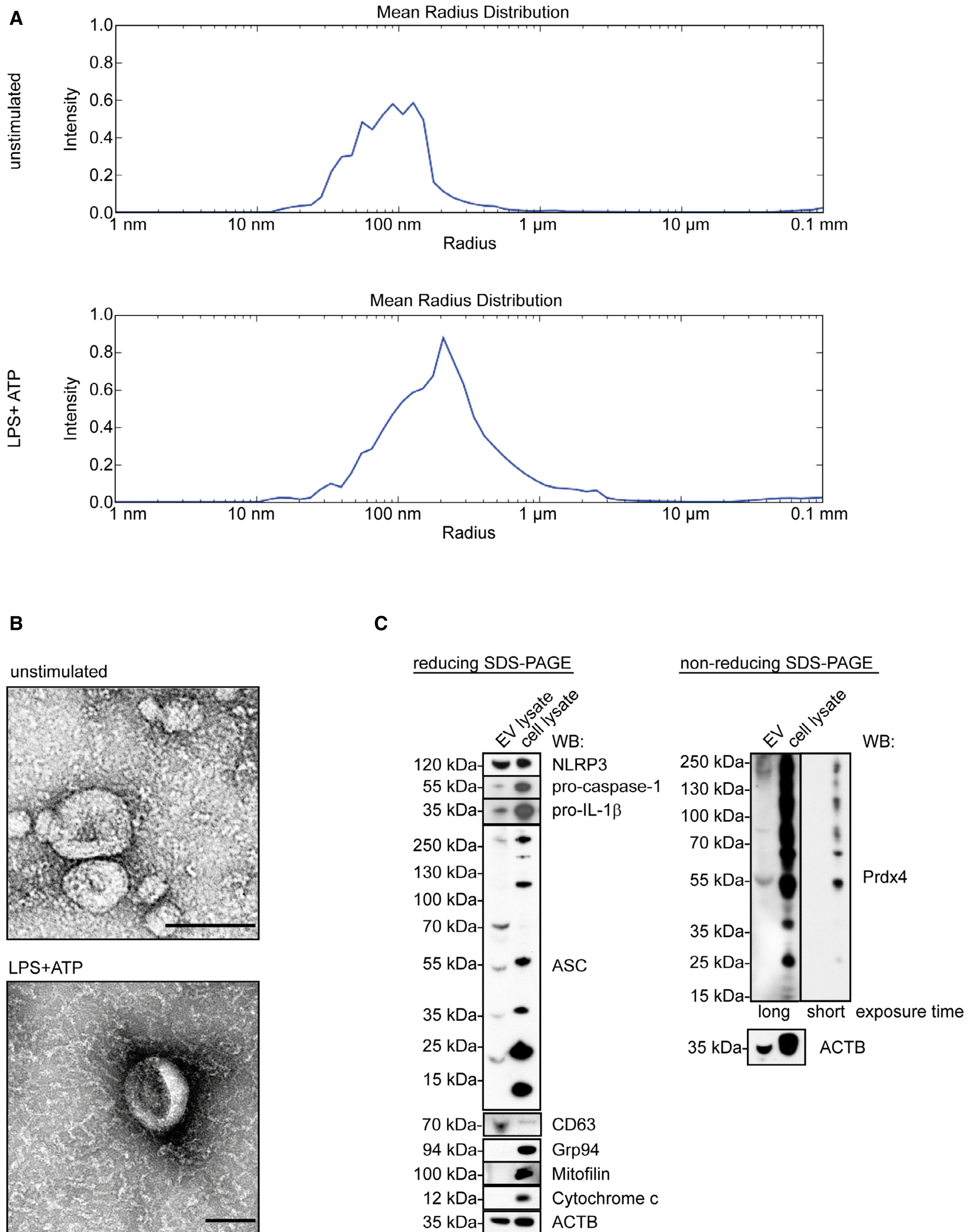

Figure EV3.

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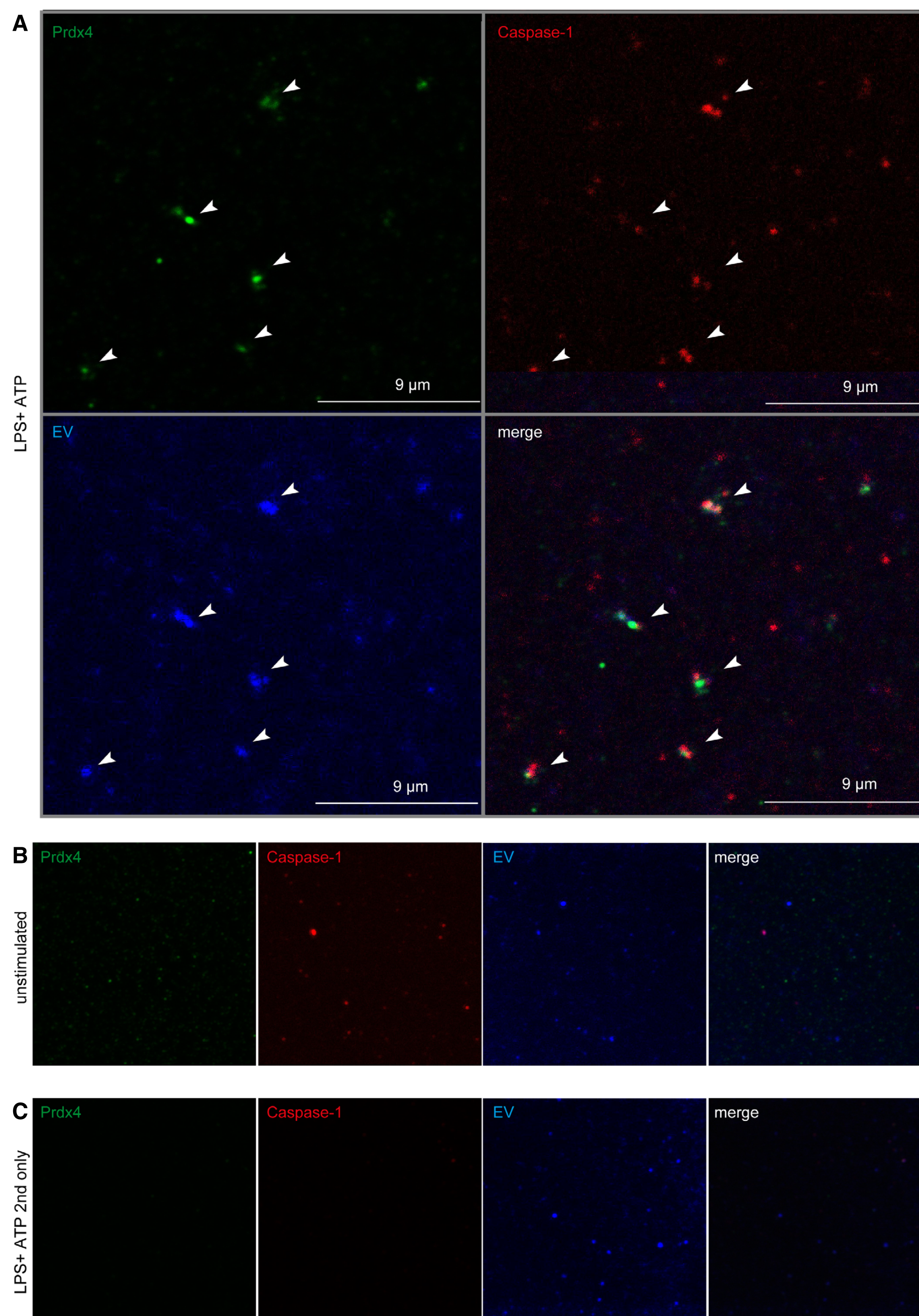

Figure EV4. Prdx4 and caspase-1 co-localize in EVs (relates to Fig 6).

A–C Fluorescence microscopy of EVs isolated from the supernatant of LPS+ATP-treated (A) or unstimulated (B) BMDMs. EV membrane was stained with CellVue Burgundy, EVs were then fixed and stained with antibodies to Prdx4 (green) or caspase-1 (red) (A, B) or second antibodies only as control (C).

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