SUPPLEMENTARY INFORMATION FOR

NLRP3 inflammasome activation in macrophage cell lines by prion protein fibrils as the source of IL-1β and neuronal toxicity

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Fig. S1

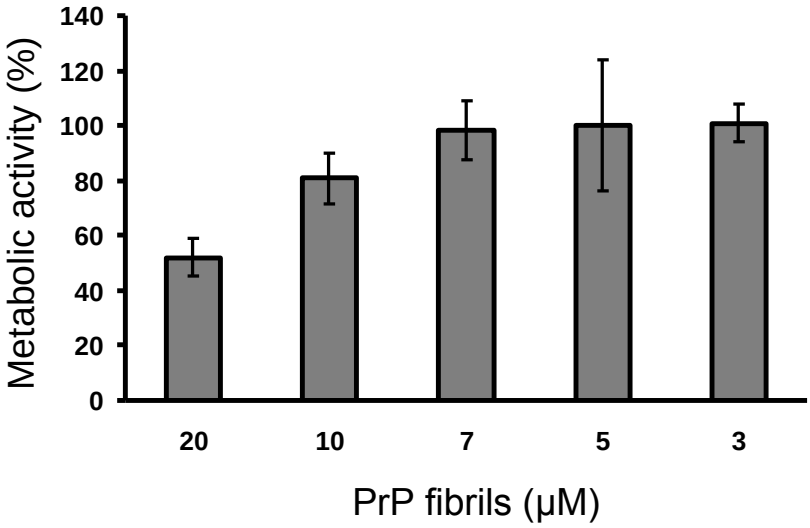

Fig. S1 PrP fibrils are toxic to macrophages at higher concentrations. After overnight treatment of primed macrophages with different concentrations of PrP fibrils toxicity was assessed by XTT assay. Values were normalized to the fibril-untreated control. Error bars represent the SD of triplicate wells

Fig. S2

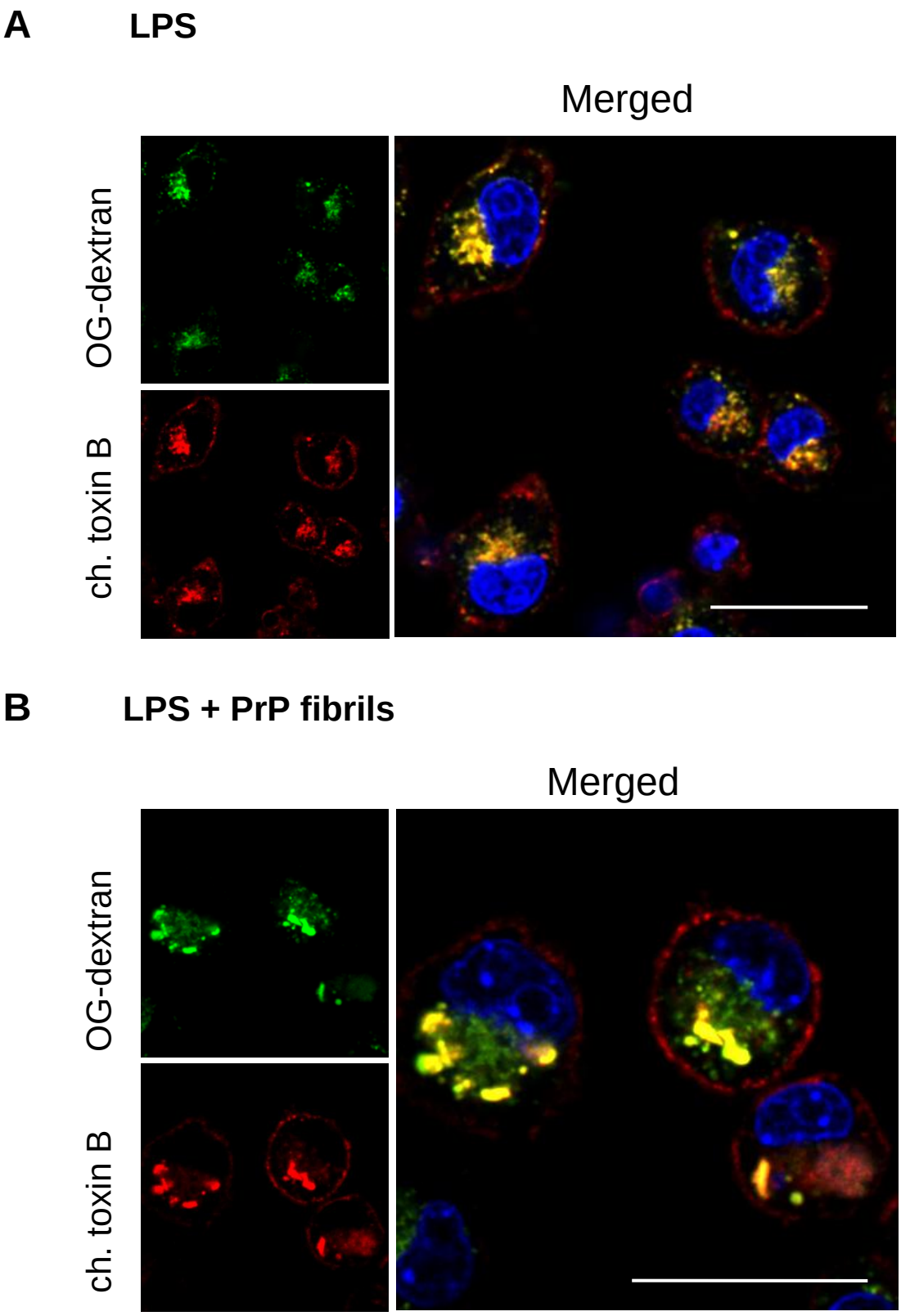
A
LPS
Merged
OG-dextran
ch. toxin B
B
LPS + PrP fibrils
Merged
OG-dextran
ch. toxin B

Figure S2: Appearance of enlarged lysosomes upon phagocytosis of PrP fibrils. Primed macrophages were incubated without fibrils (A) or with unlabeled fibrils (B) and with Oregon green dextran (green). Membrane structures were labeled by cholera toxin subunit B (red) and nuclei wit Hoechst (blue). Scale bar represents 25 μm. Representative of 2 independent experiments is shown

Fig. S3

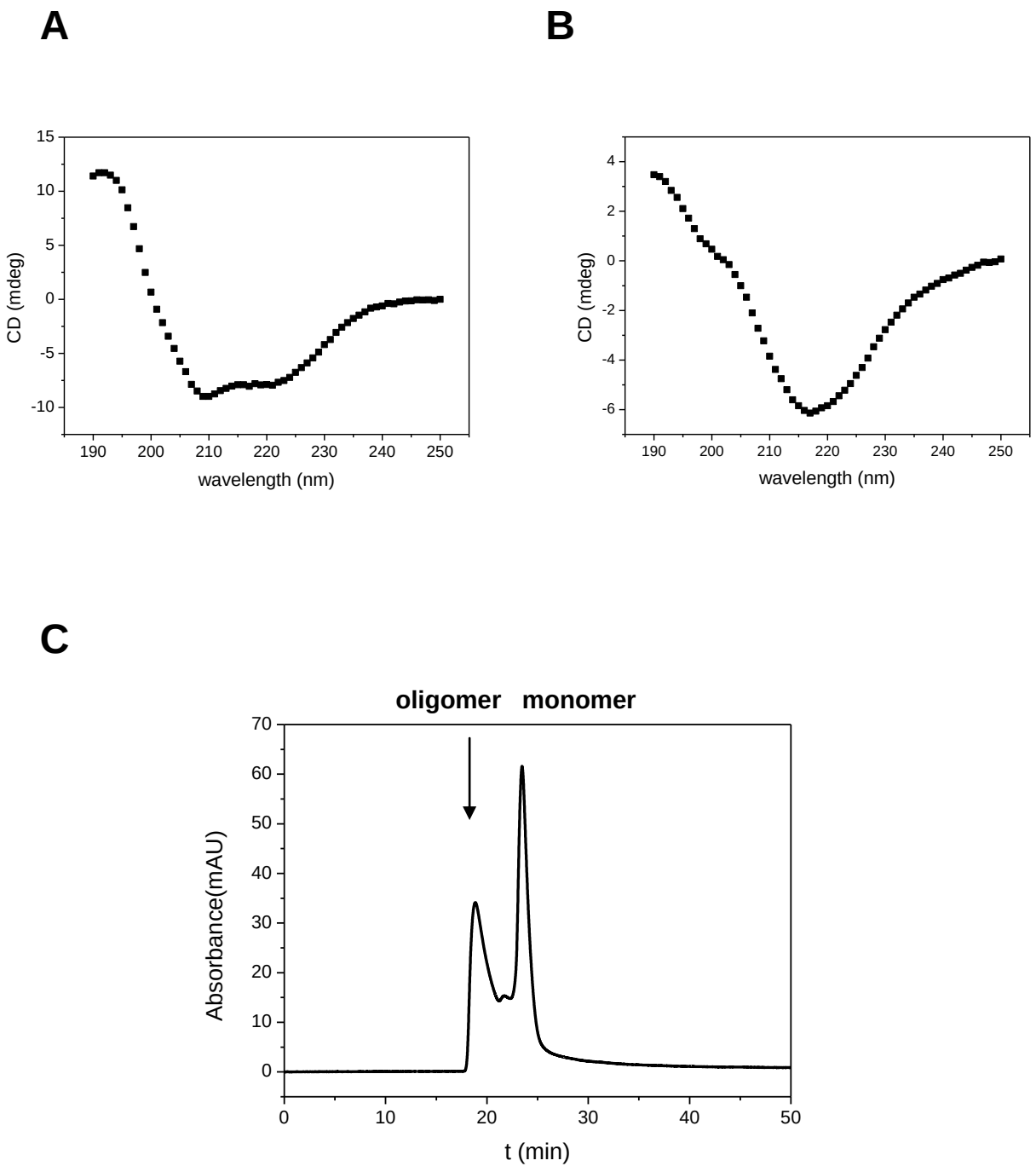
A
B
C
CD (mdeg)
wavelength (nm)
CD (mdeg)
wavelength (nm)
oligomer
monomer
Absorbance(mAU)
t (min)

Fig. S3 Characterization of different PrP forms. A) Far-UV CD spectrum of the monomeric α-form exhibits two minima at 208 nm and 220 nm, which is characteristic of an α-helical secondary structure, as expected for normal PrP. B) Far-UV CD spectrum of PrP fibrils has one minimum at 218 nm, which is characteristic of a β-secondary structure. C) The formation of oligomeric PrP was analyzed by size-exclusion chromatography. The relative yield of oligomeric PrP was determined from the area of the peak eluting at 18 min after loading

Fig. S4

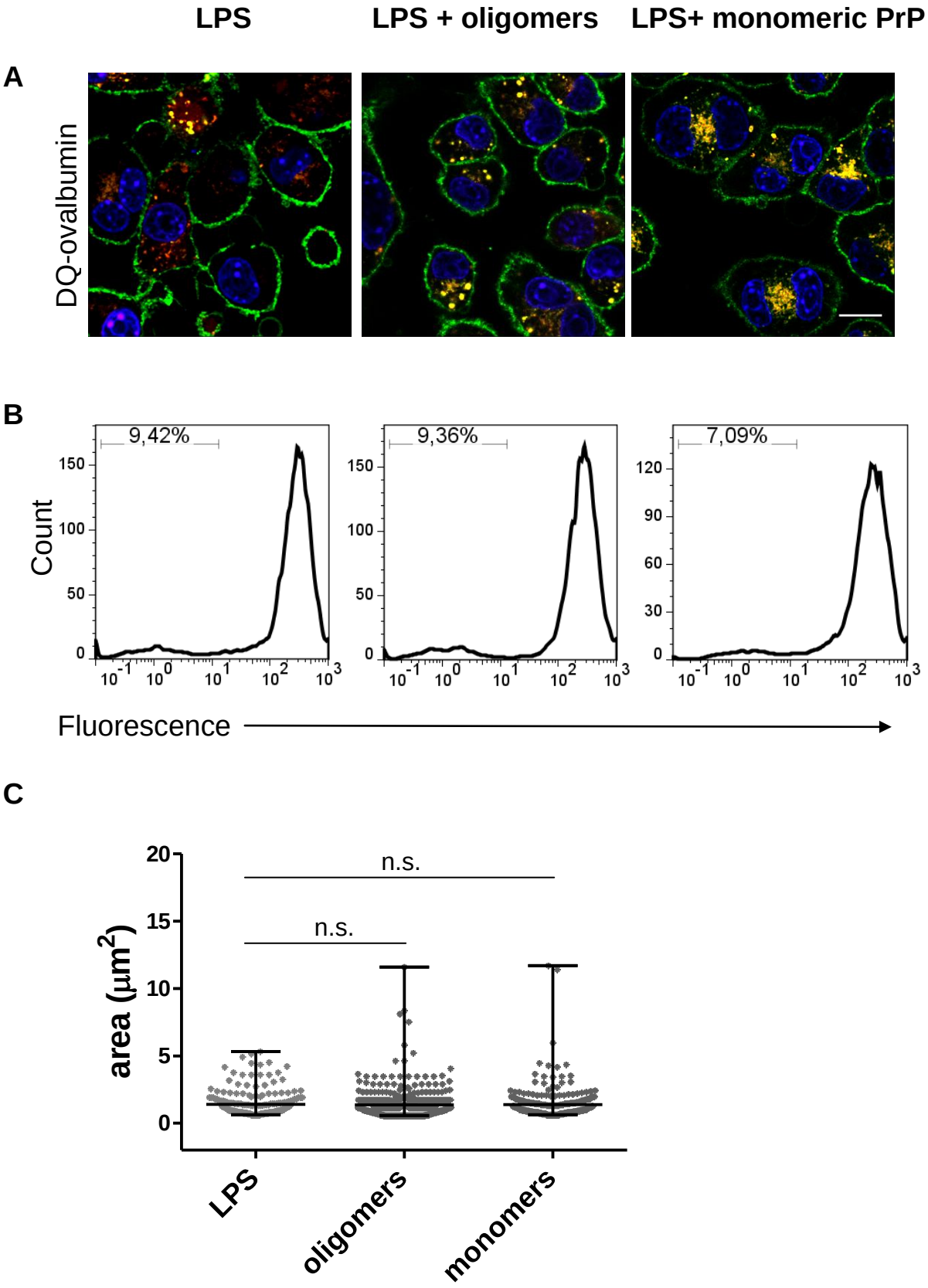
LPS
LPS + oligomers
LPS+ monomeric PrP
A
DQ-ovalbumin
B
9,42%
9,36%
7,09%
Count
Fluorescence
C
n.s.
n.s.
area (µm²)
LPS
oligomers
monomers

Figure S4: PrP oligomers and monomers do not cause lysosome swelling and destabilization. A) Macrophages were incubated with DQ-ovalbumin and with LPS (left) or oligomers (middle) or monomers (right) (green:cholera toxin subunit B, red: DQ-ovalbumin, blue: hoechst). Scale bar represents 10 μm. B) There was no loss of lysosome integrity upon treatment with PrP monomers or oligomers as followed by acridine orange stain. Response of live cells is shown. C) Areas of individual lysosomes from 50 cells are depicted. No significant increase in lysosome size was observed upon treatment with monomers or oligomers. Representative of 2 experiments is shown