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Supplemental information

A TLR4-independent critical role for CD14 in intracellular LPS sensing

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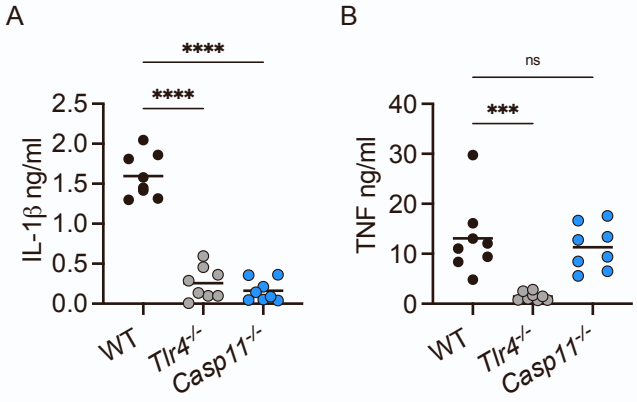

Figure S1. Noncanonical inflammasome activation by LPS *in vivo* is caspase-11 and CD14-dependent.

(A and B) Plasma IL-1β (A) and TNF (B) in IFN-γ (1 μg; 3 h)-primed mice 6 h post-LPS (20 mg/kg) injection. Each circle represents a mouse, and horizontal lines represent mean. Data from two experiments are shown. ***p < 0.001; ****p < 0.0001; ns: non-significant, one-way ANOVA/ Dunnett's test. Related to Figure 1.

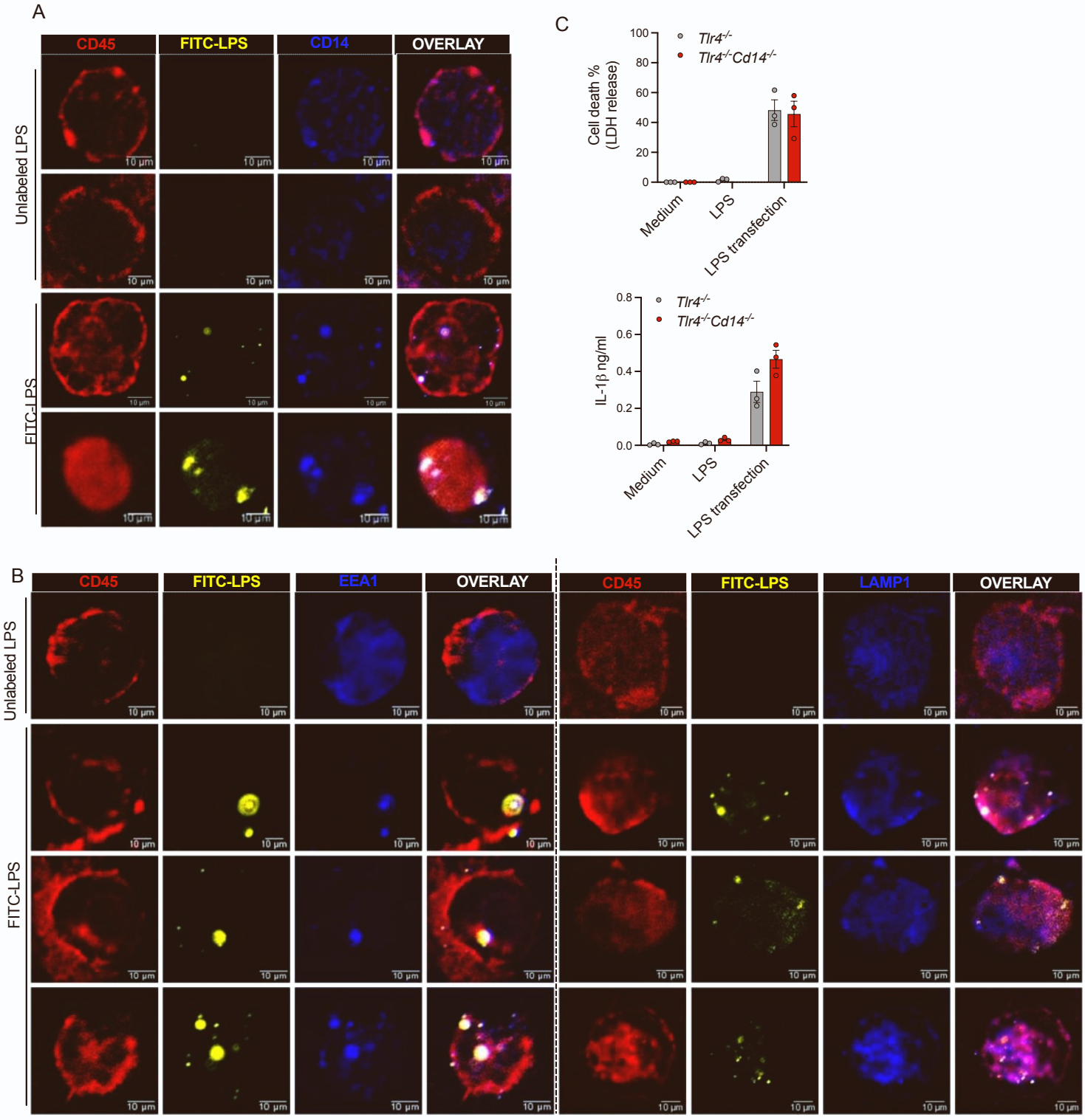

Figure S2. CD14 is dispensable for LPS-transfection induced caspase-11 activation.
(A and B) Confocal images of CD11b+ myeloid cells from unlabeled LPS- or FITC-LPS-injected *Tlr4*$^{-/-}$ mice stained as indicated. Scale bar: 10 µM. **(C)** Cell death (LDH release) and IL-1β secretion by indicated BMDMs primed with 10 ng/ml IFN-γ and 0.5 µg/ml Pam3CSK4 for 3 h and stimulated for 16 h with 1 µg/ml LPS or transfected with 1 µg/ml LPS using lipofectamine 2000. Images representative of four experiments (A and B) and data from three experiments are shown (C). Data are presented as the mean ± s.e.m (C). Related to Figure 4.

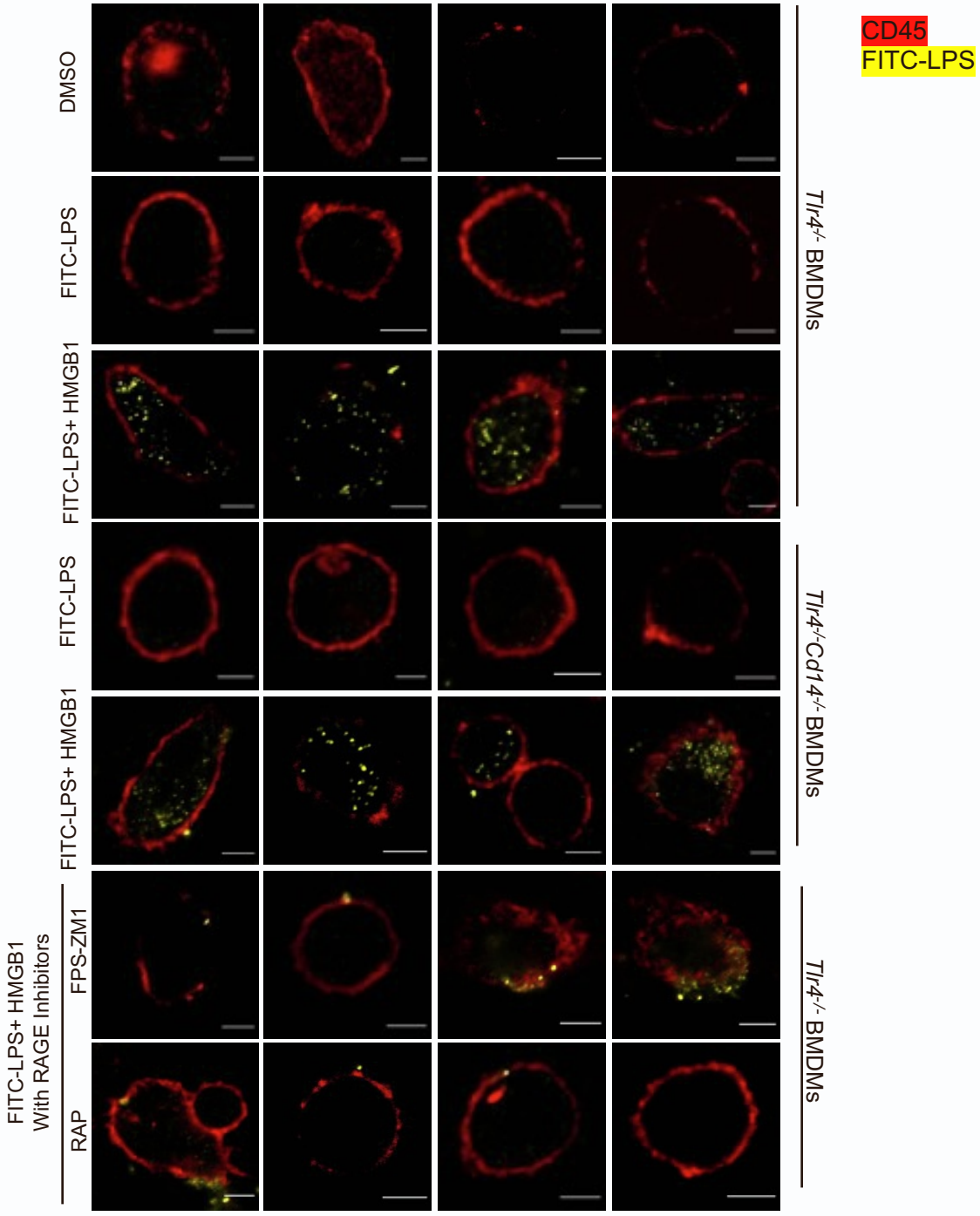

Figure S3. CD14 is dispensable for HMGB1-mediated LPS internalization in BMDMs.
Confocal images of indicated BMDMs pretreated with DMSO or RAGE inhibitors (1 μM FPS-ZM1 or 10 μM RAP), stimulated with FITC-LPS (100 ng/ml) or FITC-LPS (100 ng/ml) complexed with HMGB1 (400 ng/ml), and stained with anti-CD45 (red) and anti-FITC (yellow) antibodies. Scale bar: 5 μM. Images representative of three experiments are shown. Related to Figure 4.

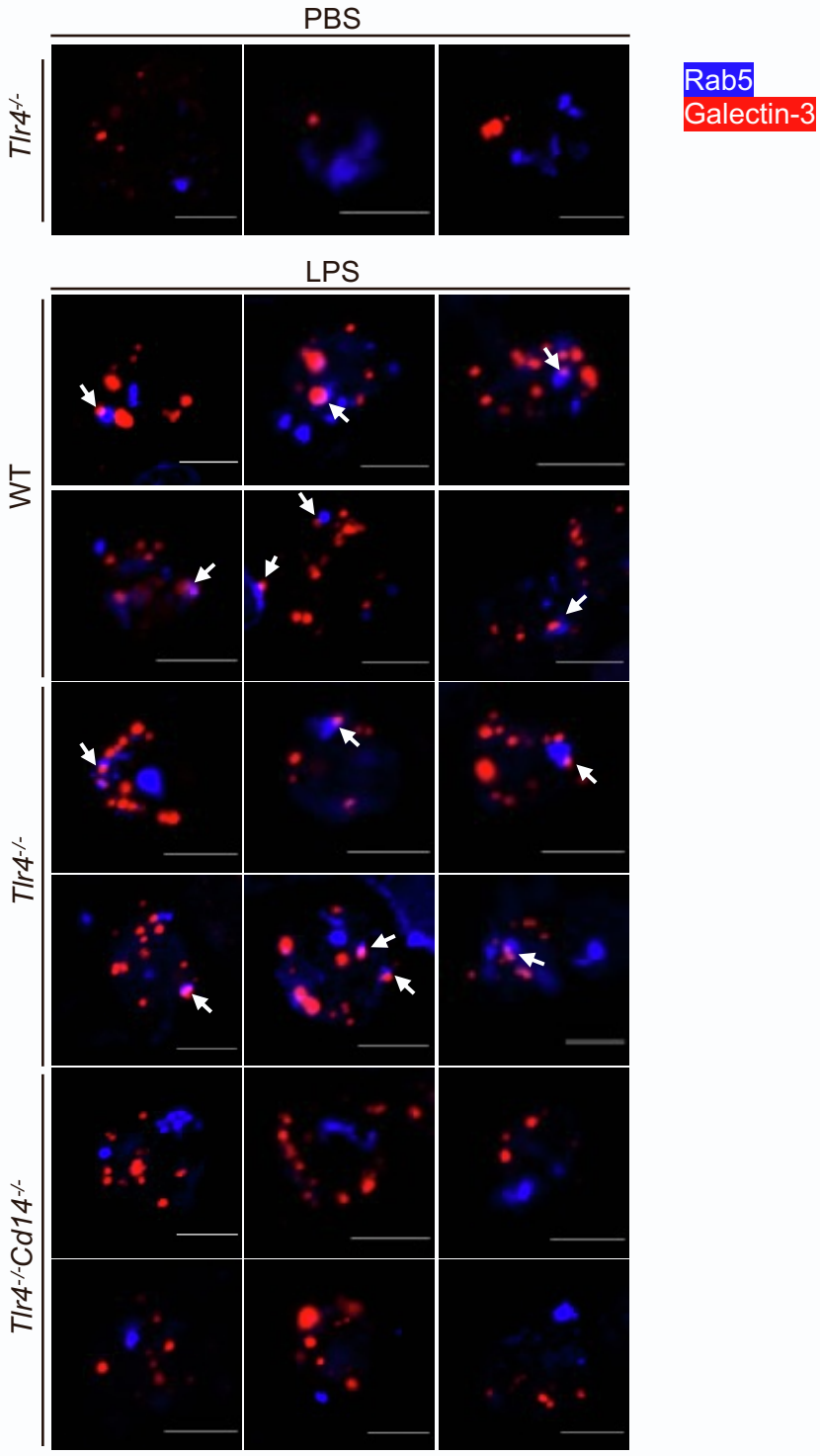

Figure S4. Endosomal membrane disruption upon LPS internalization is CD14-dependent.
Confocal images of CD11b+ myeloid cells from PBS- or LPS (25 mg/kg)-injected WT, *Tlr4*$^{-/-}$ and *Tlr4*$^{-/-}$ *Cd14*$^{-/-}$ mice stained for Rab5 (blue) and galectin-3 (red). Scale bar: 5 μM. White arrows indicate the colocalization of Rab5^{+} compartments and galectin-3. Images representative of three experiments are shown. Related to Figure 4.