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# Supplementary Materials for

## Novel reprogramming of neutrophils modulates inflammation resolution during atherosclerosis

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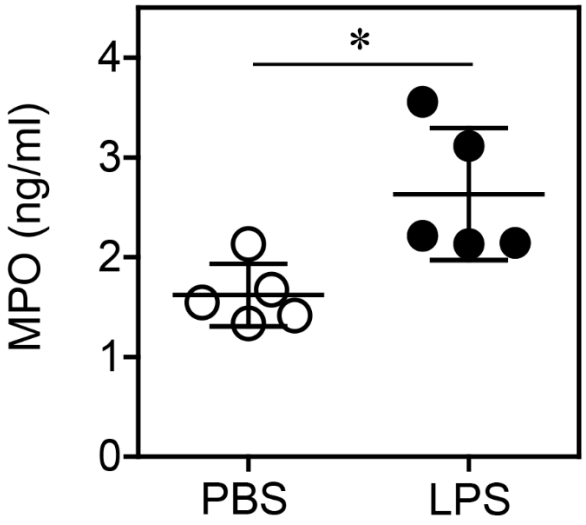


**Fig. S1. Subclinical endotoxin up-regulates MPO level in HFD-fed mice.** ApoE$^{-/-}$ mice were administrated with PBS or super-low-dose LPS together with HFD for 4 weeks. Circulating MPO level was determined by ELISA. Data are representative of two independent experiments, and error bars represent means ± s.e.m. *$P < 0.05$; Student's *t*-test (n = 5 for each group).

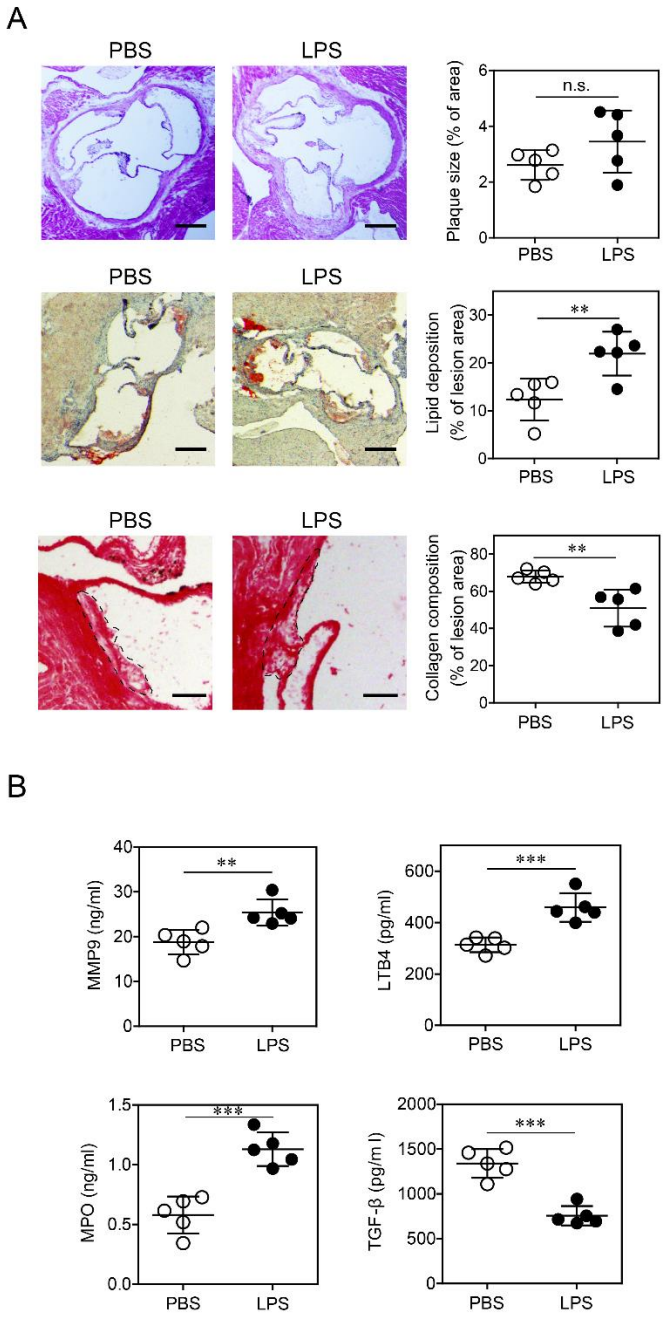


**Fig. S2. Subclinical endotoxemia exacerbates atherosclerotic pathogenesis in RD-fed mice.** ApoE$^{-/-}$ mice were administrated with PBS or super-low-dose LPS together with RD for 4 weeks**.** (**A**) Representative images of HE-stained atherosclerotic lesions and quantification of plaque size demonstrated as the percentage of lesion area within aortic root area (upper panels). Representative images of Oil Red O-stained atherosclerotic plaques and quantification of lipid deposition within lesion area (middle panels). Representative images of Picrosirius Red-stained atherosclerotic plaques and quantification of collagen content within lesion area (lower panels). Scale bar, 300 μm (upper and middle panels) and 100 μm (lower panels). (**B**) The levels of circulating MMP9, LTB4, MPO and TGF-β were determined by ELISA. Data are representative of two independent experiments, and error bars represent means ± s.e.m. $^{**}P < 0.01$ and $^{***}P < 0.001$; Student's *t* test (n = 5 for each group).

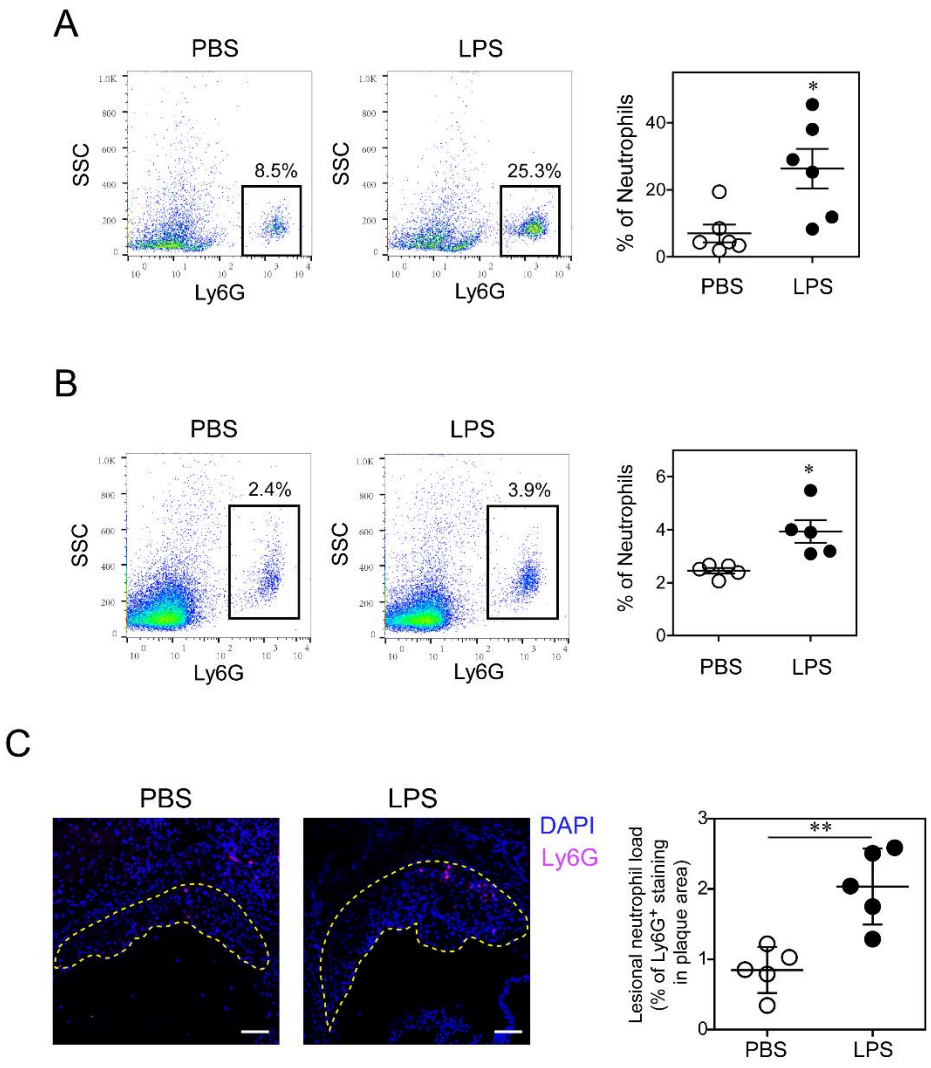


**Fig. S3. Subclinical endotoxin causes neutrophil expansion in atherosclerotic mice.** ApoE$^{-/-}$ mice were administrated with PBS or super-low-dose LPS together with HFD for 4 weeks. The frequency of Ly6G$^{+}$ neutrophils in the peripheral blood (**A**) and spleen (**B**) was analyzed with flow cytometry. (**C**) Representative confocal images and quantification of Ly6G$^{+}$ neutrophils in atherosclerotic plaques. Scale bar, 100 μm. Data are representative of two independent experiments, and error bars represent means ± s.e.m. *$P$ < 0.05 and **$P$ < 0.01; Student's t-test (n= 5 to 6 for each group).

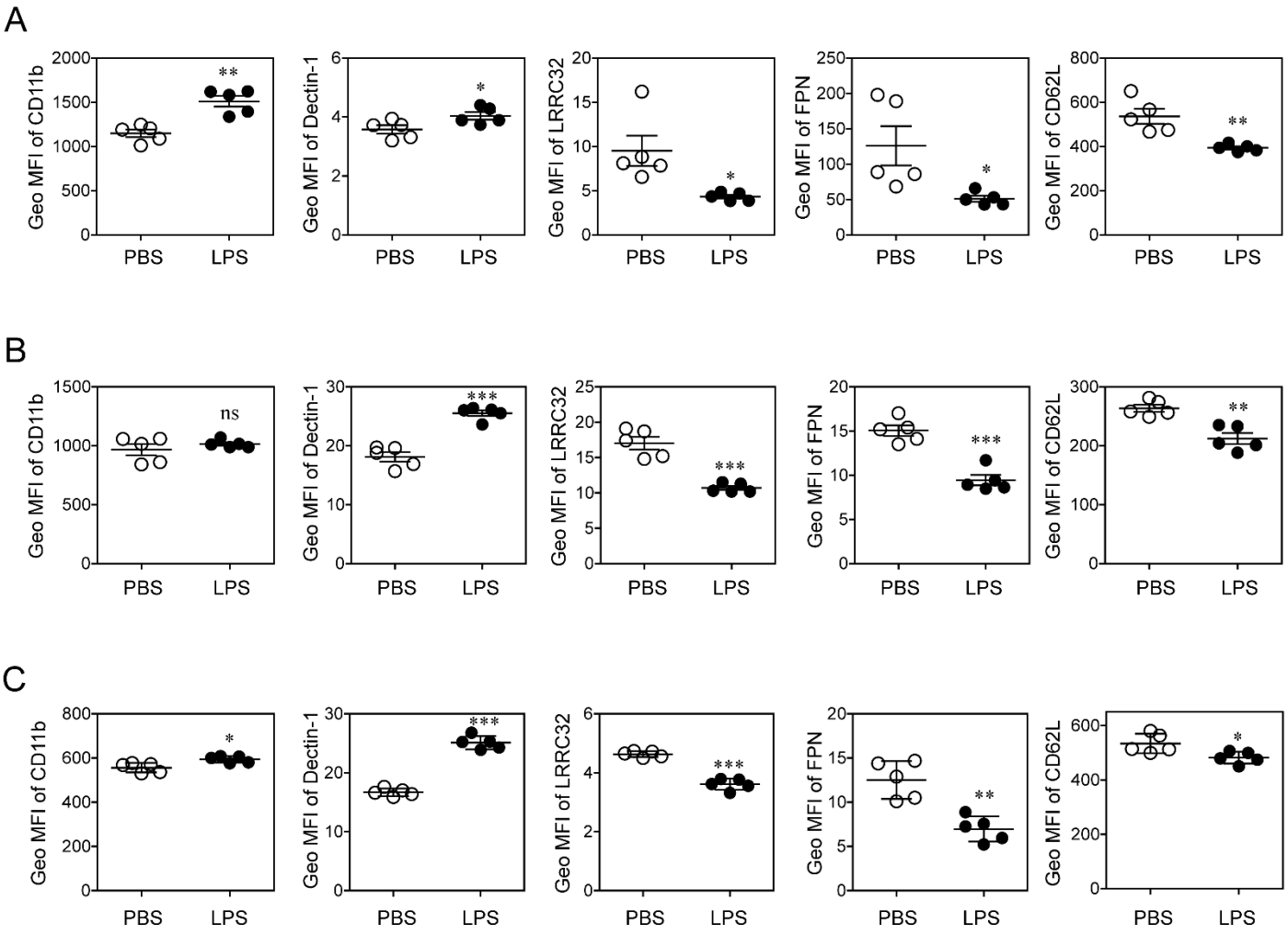


**Fig. S4. Subclinical endotoxin primes neutrophils into a proinflammatory state in atherosclerotic mice.** ApoE$^{-/-}$ mice were administrated with PBS or super-low-dose LPS together with HFD for 4 weeks. The surface phenotypes of Ly6G$^{+}$ neutrophils in the peripheral blood (**A**) spleen (**B**) and bone marrow (**C**) were analyzed by flow cytometry. The same data shown in Fig. 2 were re-analyzed with geometric mean fluorescence intensity (Geo MFI) as the parameter. Error bars represent means ± s.e.m. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001; Student's t-test (n= 5 for each group).

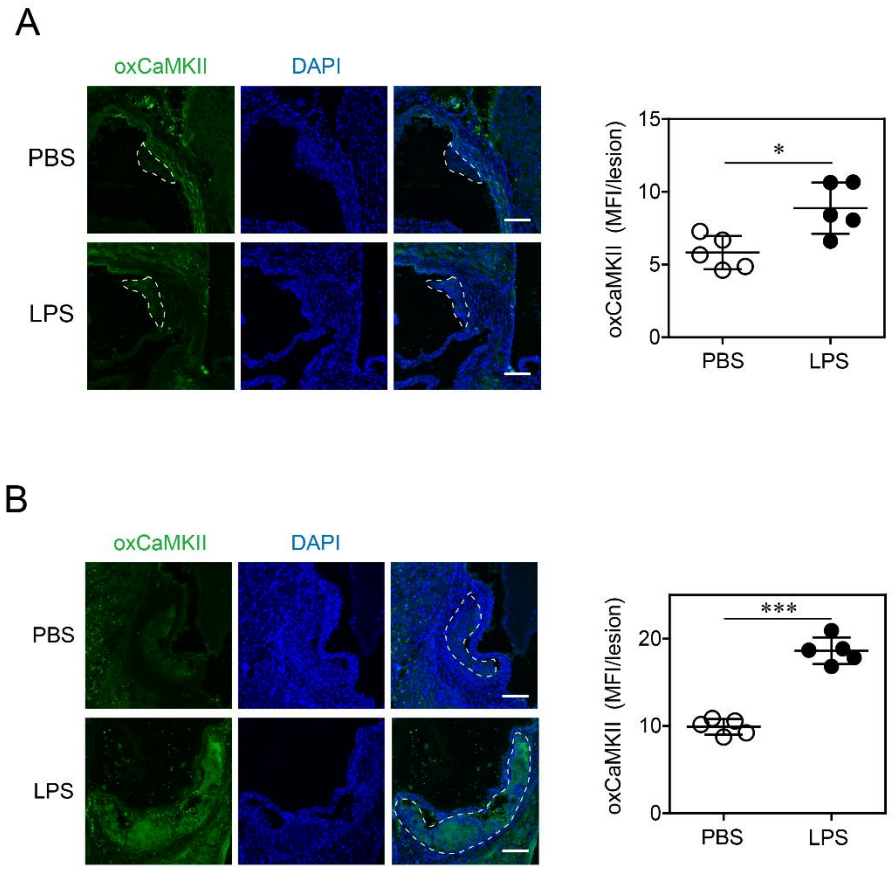


**Fig. S5. Subclinical endotoxin induces oxCAMKII elevation in vivo.** (**A**) ApoE$^{-/-}$ mice were administrated with PBS or super-low-dose LPS together with RD for 4 weeks. Representative confocal images and quantification of lesional oxCaMKII levels are displayed. (**B**) ApoE$^{-/-}$ mice were administrated with PBS or super-low-dose LPS together with HFD for 4 weeks. Representative confocal images and quantification of lesional oxCaMKII levels are displayed. Scale bar, 100 μm. Data are representative of two independent experiments, and error bars represent means ± s.e.m. **P* < 0.05 and ****P* < 0.001; Student's t-test (n = 5 for each group).

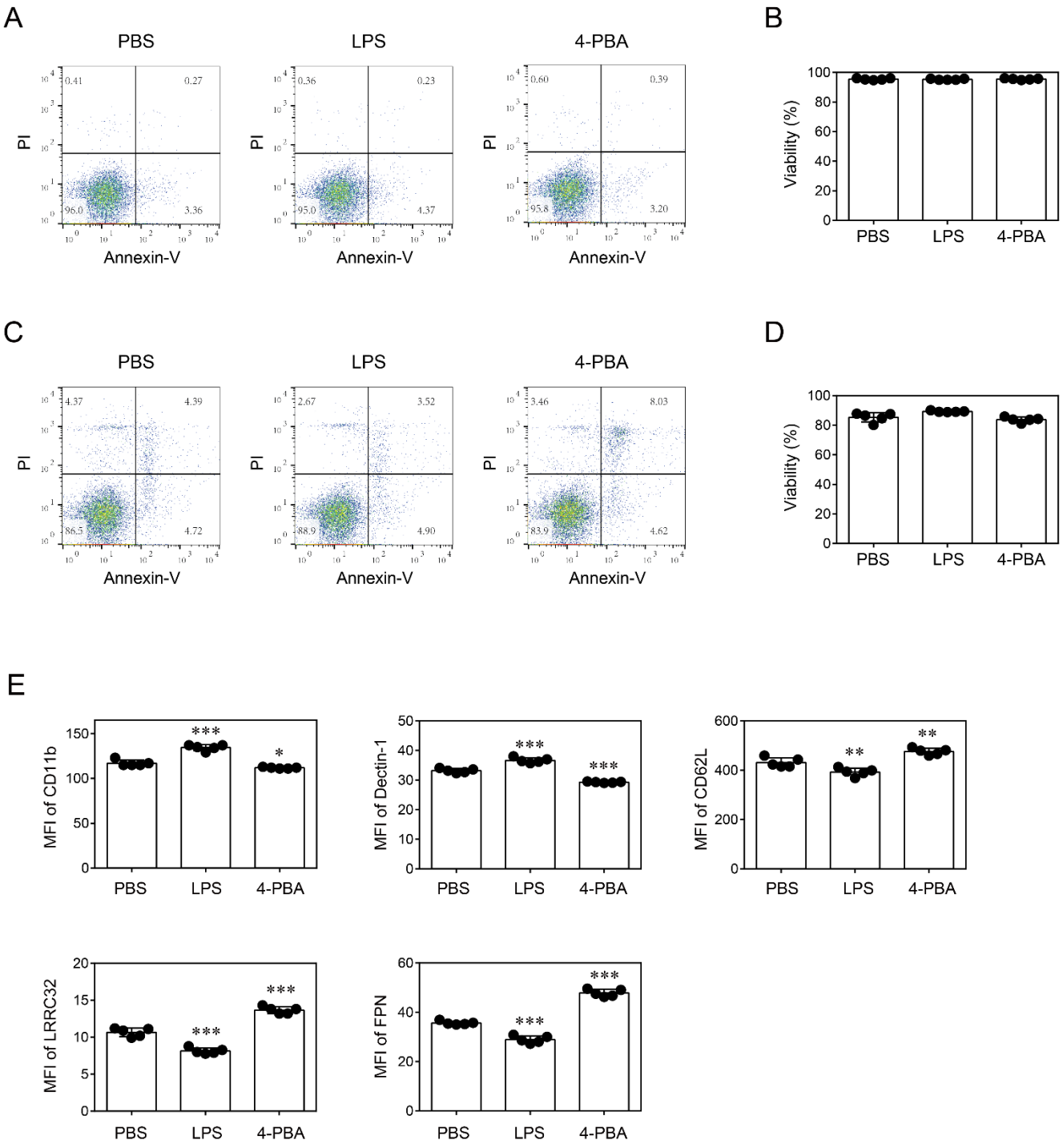


**Fig. S6. Neutrophils maintain viability after in vitro polarization.** Bone marrow neutrophils were purified and treated with PBS, super-low-dose LPS (100 pg/ml) or 4-PBA (1 mM) for 24 h (**A**, **B**) and 48 h (**C**, **D**). The cells were stained with Annexin-V and PI, and the viability (percentage of Annexin-V$^-$/PI$^-$ population) was determined by flow cytometry. (**E**) Surface phenotype of neutrophils was analyzed by flow cytometry after polarization for 24 h. Data are representative of three independent experiments, and error bars represent means ± s.e.m. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001 as compared with PBS group; one-way ANOVA. (n = 5 for each group).

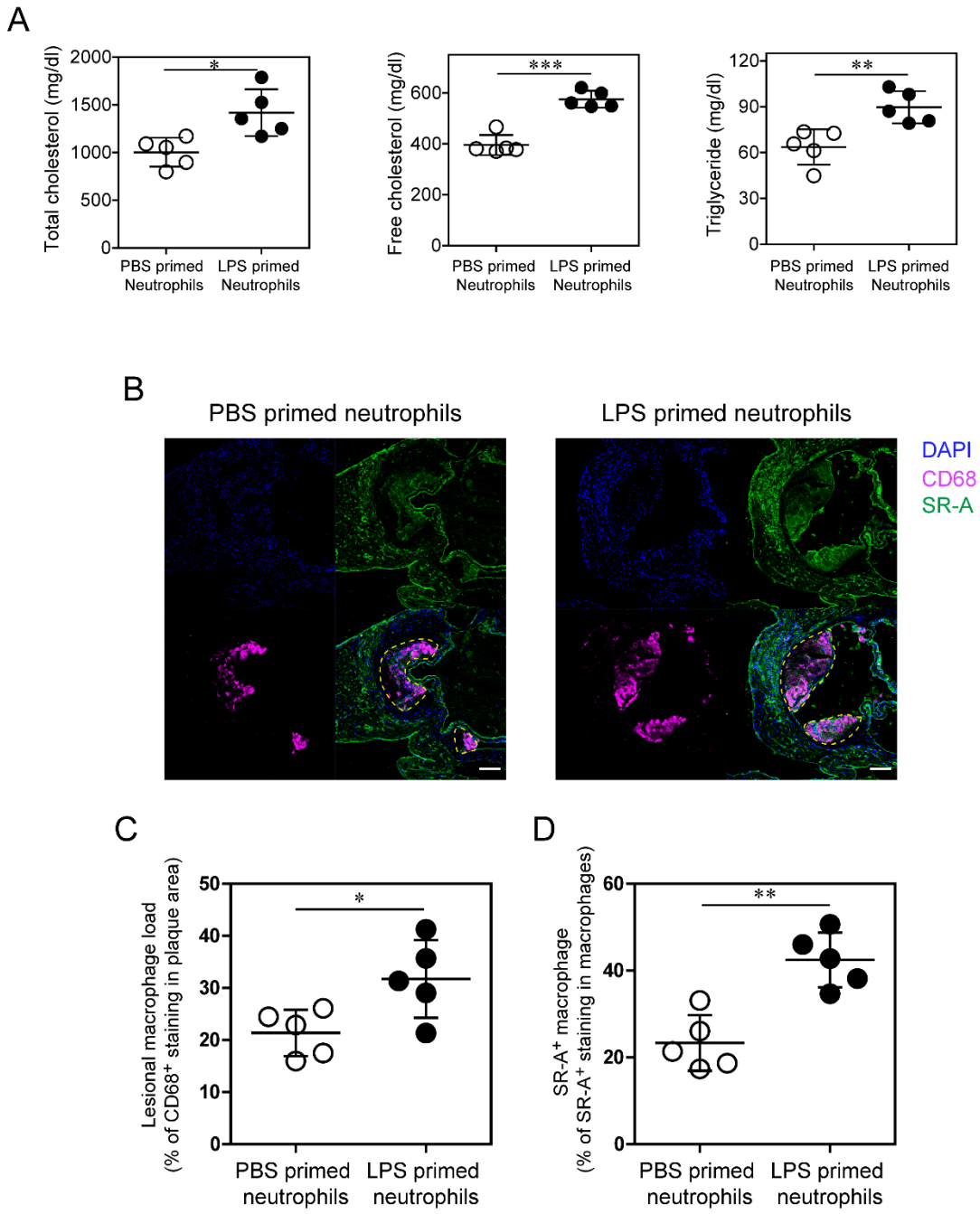


**Fig. S7. Transfusion of superlow-dose LPS–polarized neutrophils elevates plasma lipid levels and modulates lesional macrophages.** Neutrophils purified from ApoE$^{-/-}$ mice were treated with PBS or super-low-dose LPS (100 pg/ml) for 24 h. PBS- or LPS-treated neutrophils ($2 \times 10^6$ cells per mouse) were then adoptively transferred by intravenous injection to HFD-fed ApoE$^{-/-}$ mice once a week for 4 weeks. (**A**) Plasma samples were collected 1 week after the last neutrophil transfer, and the levels of total cholesterol, free cholesterol and triglycerides were determined. (**B**) Representative confocal images of CD68 and SR-A staining in atherosclerotic plaques. Scale bar, 100 μm. (**C**) Quantification of CD68$^+$ macrophage load in plaques. (**D**) Quantification of the frequency of SR-A$^+$ staining within macrophage population. Data are representative of two independent experiments, and error bars represent means ± s.e.m. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001; Student's *t*-test (n= 5 for each group).

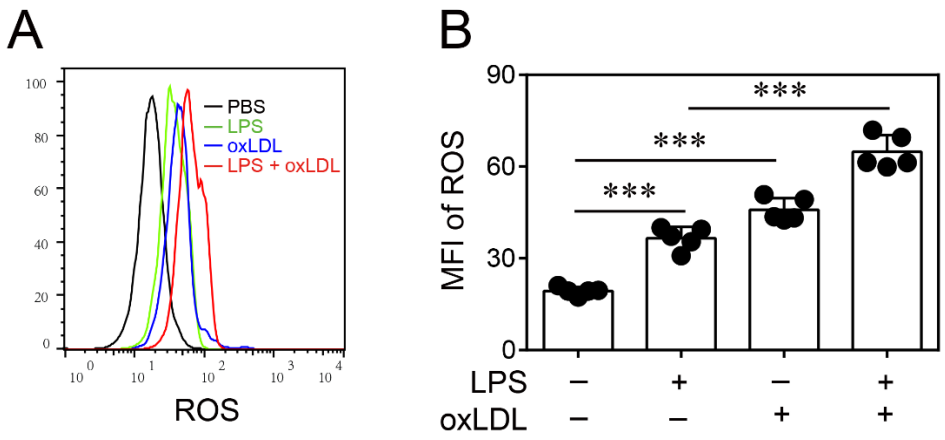


**Fig. S8. Superlow-dose LPS and oxLDL treatment elevates ROS accumulation in neutrophils.** Neutrophils were purified from the bone marrow of wild-type C57 BL/6 mice and treated with PBS, super-low dose LPS (100 pg/ml) and/or oxLDL (10 μg/ml) for 2 d. (**A**) Representative histogram of ROS level determined by Cell ROX labeling. (**B**) Quantification of ROS level in neutrophils. Data are representative of three independent experiments, and error bars represent means ± s.e.m. ***$P < 0.001$; ANOVA (n= 5 for each group).

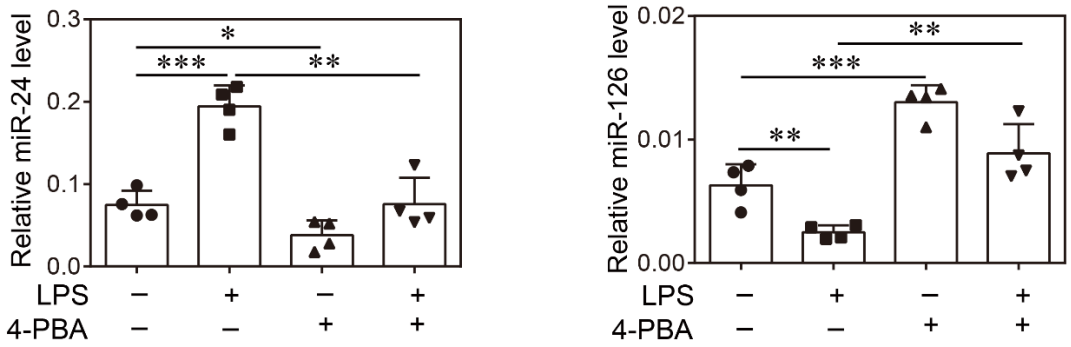


**Fig. S9. 4-PBA reverses superlow-dose LPS–induced differential regulation of miR-24 and miR-126 in neutrophils.** Neutrophils were purified from the bone marrow of wild-type C57 BL/6 mice and treated with PBS, super-low dose LPS (100 pg/ml) and/or 4-PBA (1 mM) for 2 d. The levels of miR-24 and miR-126 were determined by real-time RT-PCR**.** Data are representative of three independent experiments, and error bars represent means ± s.e.m. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001; one-way ANOVA (n= 4 for each group).

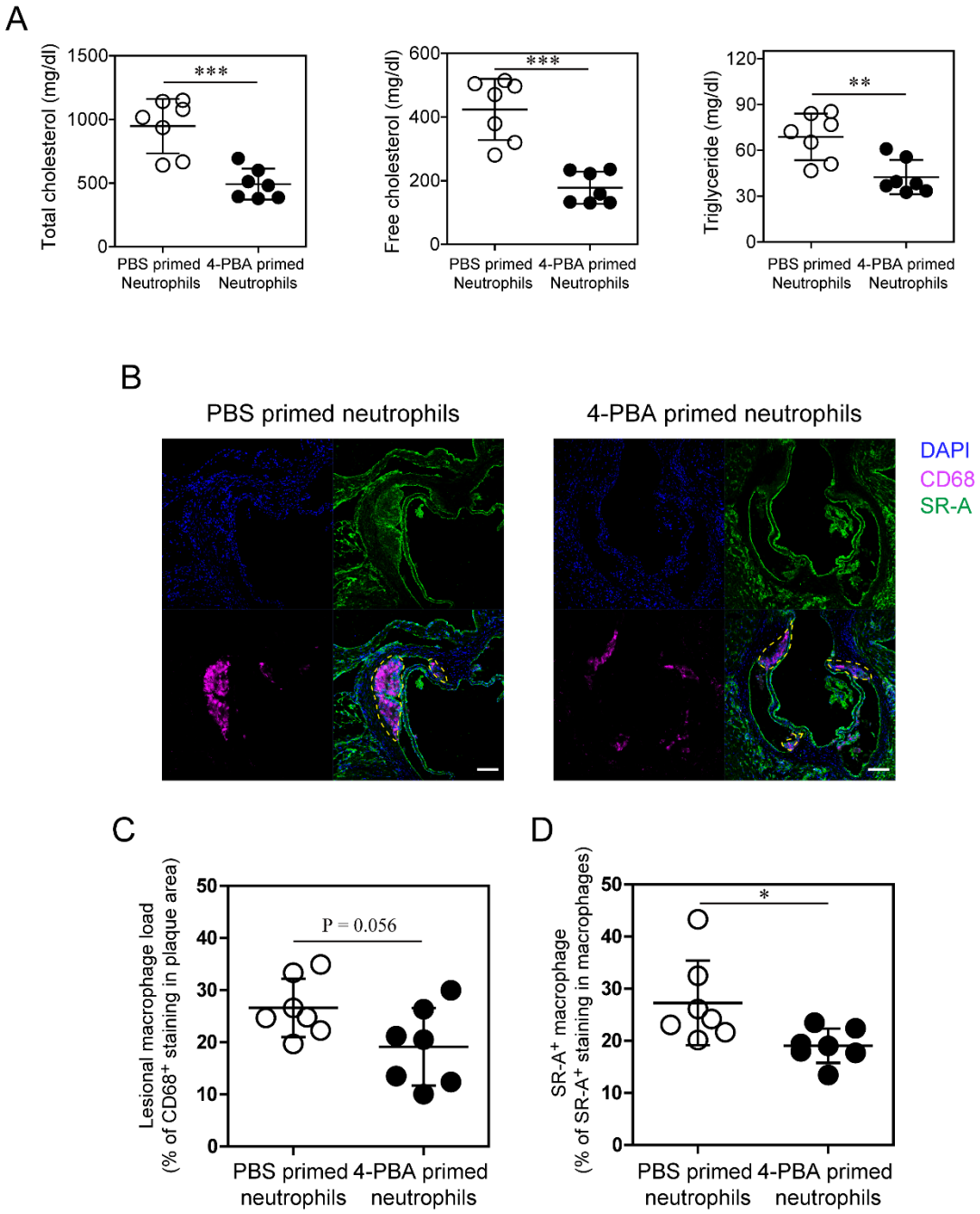


**Fig. S10. Transfusion of 4-PBA–polarized neutrophils down-regulates plasma lipid levels and reduces lesional macrophage activation.** Neutrophils purified from ApoE$^{-/-}$ mice were treated with PBS or 4-PBA (1 mM) for 24 h. PBS- or 4-PBA-treated neutrophils ($2 \times 10^6$ cells per mouse) were then adoptively transferred by intravenous injection to HFD-fed ApoE$^{-/-}$ mice once a week for 4 weeks. (**A**) Plasma samples were collected 1 week after the last neutrophil transfer, and the levels of total cholesterol, free cholesterol and triglycerides were determined. (**B**) Representative confocal images of CD68 and SR-A staining in atherosclerotic plaques. Scale bar, 100 µm. (**C**) Quantification of CD68$^+$ macrophage load in plaques. (**D**) Quantification of the frequency of SR-A$^+$ staining within macrophage population. Data are representative of two independent experiments, and error bars represent means ± s.e.m. **P* < 0.05, ***P* < 0.01 and ****P* < 0.001; Student's *t*-test (n= 7 for each group).