SUPPLEMENTARY MATERIAL

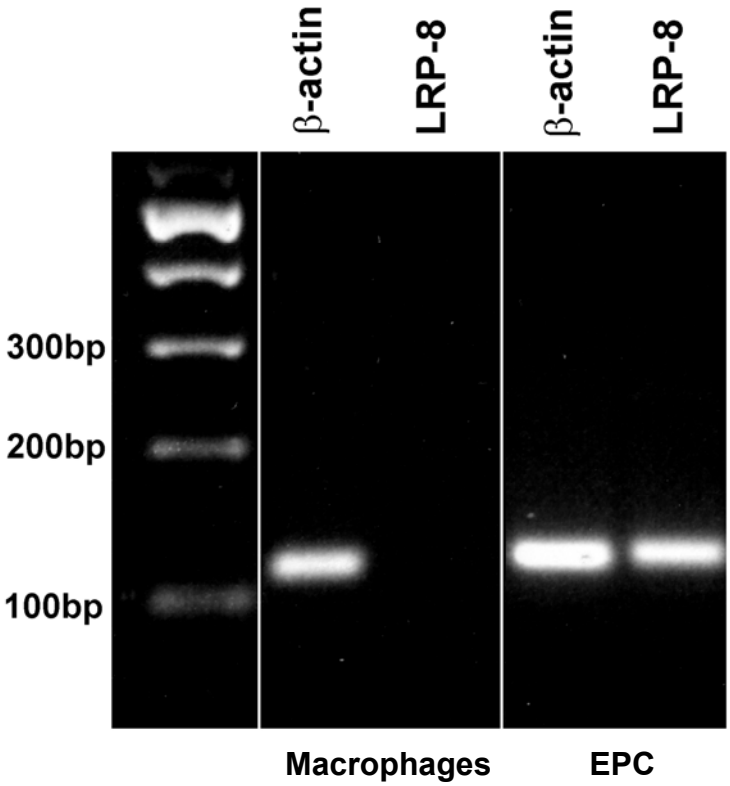

Figure S1. BM-derived murine macrophages do not express LRP8 (ApoER2). Total RNA was extracted from BM-derived murine macrophages or endothelial progenitor cells (EPC; as a control) using Absolutely RNA miniprep kit (Stratagene) according to the manufactures' instructions. One microgram of total RNA was used for cDNA synthesis using Superscript III and random hexamers (Invitrogen). RT-PCR was performed using AmpliTaq Polymerase and the following primers: LRP8: 5'-GTGATGATCAGAGGGACTGC-3' and 5'-AAGCCGATCTTGAGGTCAGT-3'; β-actin: 5'-AGTGTGACGTTGACATCCGT-3' and 5'-TGCTAGGAGCCAGAGCAGTA-3'. The PCR reaction was done in 25 µl solution containing 12.5 µl PCR Master Mix, 10 ng cDNA, and 400 nM of each primer, with the following settings: activation 95 °C for 2 min, 35 cycles of 94 °C for 30s, 57 °C for 30s, and 72 °C for 30s, followed by 72 °C for 10 min. The PCR products were analyzed on 2% agarose gel. Data shown are representative of two independent experiments.

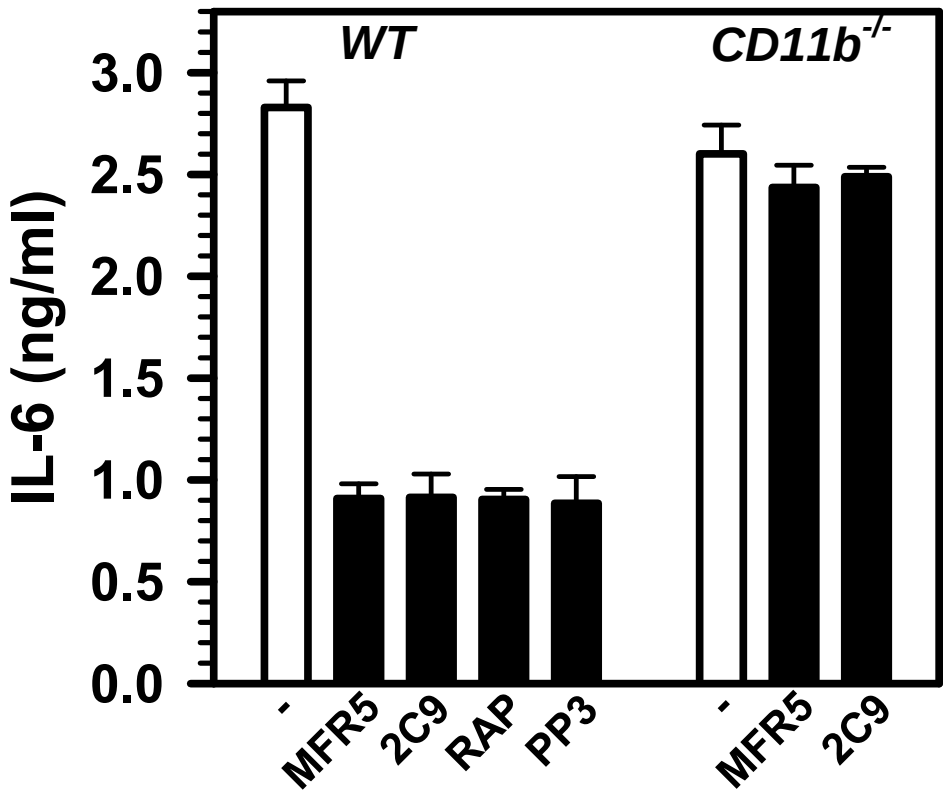

Figure S2. The IL-6 suppressive activity of APC is independent of integrin β1, integrin β3, ApoER2 or EGFR activity. LPS-stimulated *WT* and *CD11b*$^{-/-}$ BM-derived macrophages were treated with (filled bar) or without (open bar) 5 μg/ml APC in the presence of blocking antibodies/inhibitors for integrin β1 (MFR5; 20 μg/ml), integrin β3 (2C9.G2; 20 μg/ml), ApoER2 (RAP; 1 μM) or EGFR (PP3; 10 μM) at 37°C for 20 hours. IL-6 concentration in the conditioned media was determined by ELISA. Data shown were means ± S.D. of duplicate wells.

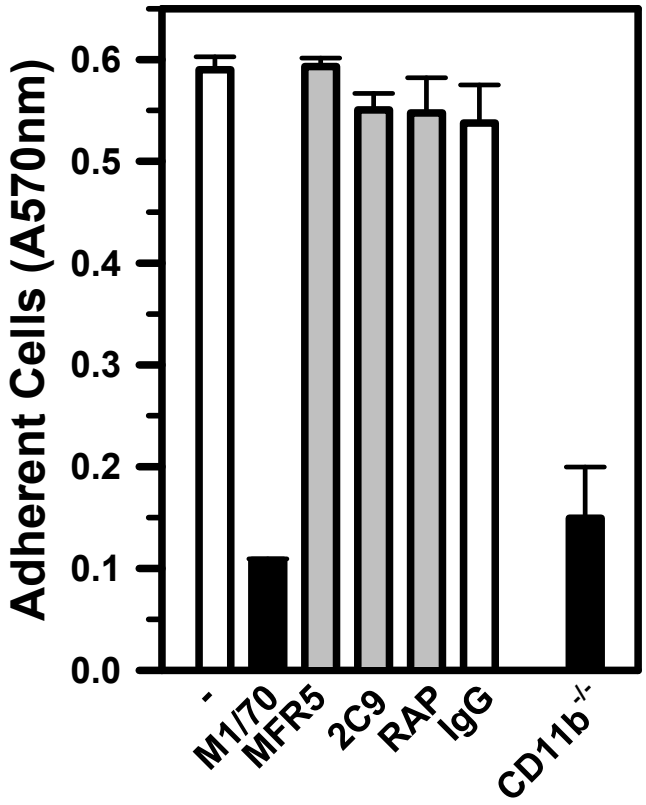

Figure S3. CD11b is critical to macrophage adhesion to GD-APC. *WT* BM-derived macrophages were allowed to adhere to hGD-APC-coated 24-well plates in the presence of function-blocking antibodies/inhibitors for CD11b/CD18 (M1/70; 20 μg/ml), integrin β1 (MFR5; 20 μg/ml), integrin β3 (2C9.G2; 20 μg/ml), ApoER2 (RAP; 1 μM) or control IgG. After washing, the number of adherent cells was quantified by absorbance at 570 nm of crystal violet-stained plates. The critical role of CD11b was further confirmed by the inability of *CD11b*$^{-/-}$ BM-derived macrophages to adhere to GD-APC. Data shown represent means ± SD of duplicate experiments.

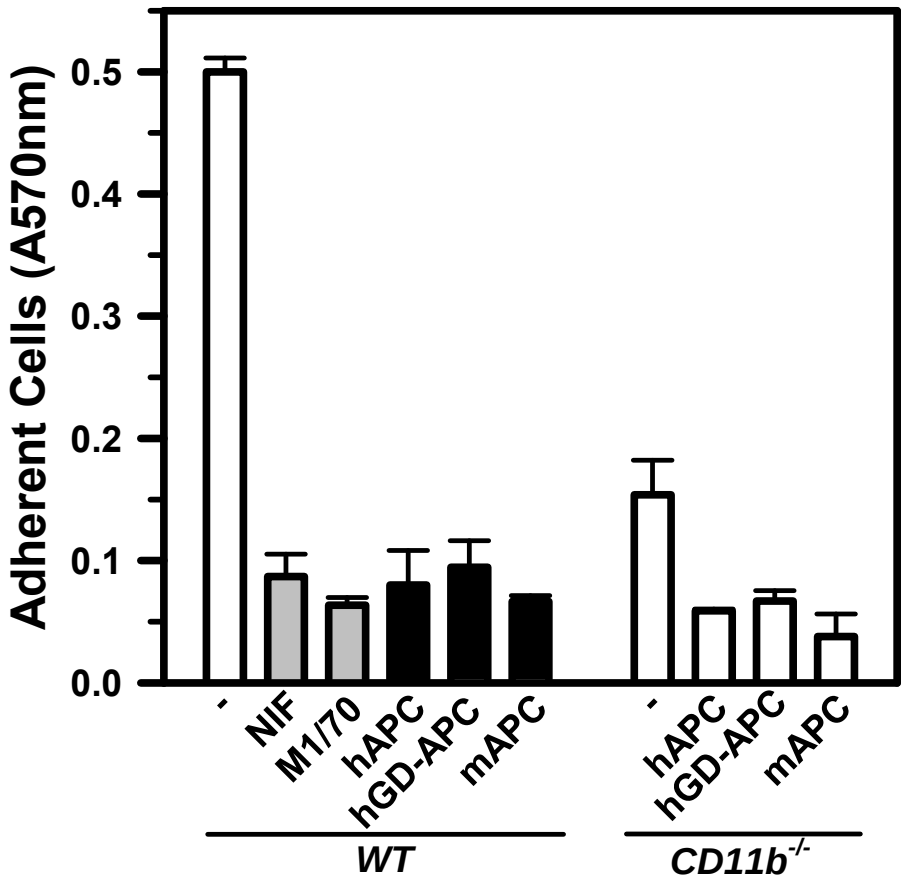

Figure S4. Macrophage adhesion to hAPC can be blocked by soluble hAPC and mAPC. WT and CD11b$^{-/-}$ BM-derived macrophages were allowed to adhere to hAPC-coated 24-well plates in the presence of PBS, 10 nM CD11b/CD18-specic antagonist (NIF) or 20 µg/ml blocking antibody (M1/70), 20 µg/ml hAPC, 20 µg/ml hGD-APC, or 20 µg/ml mAPC. After washing, the number of adherent cells was quantified by absorbance at 570 nm of crystal violet-stained plates. Data shown represent means ± SD of duplicate samples.

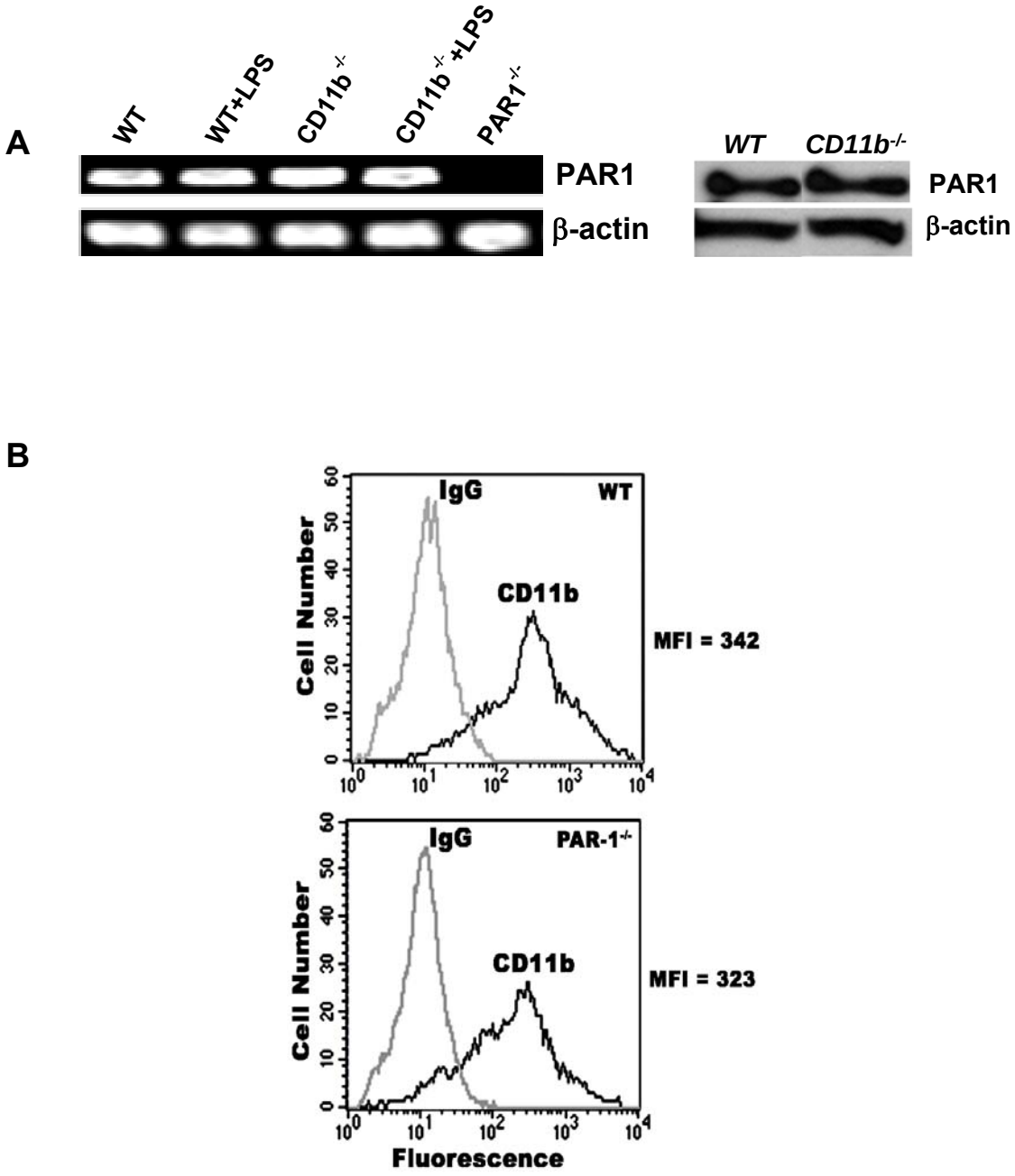

Figure S5. CD11b deficiency does not affect PAR-1 expression on BM-derived macrophages and vice versa. A) PAR-1 expression. Total RNA and cell lysates were prepared using *WT* and *CD11b*$^{-/-}$ BM-derived macrophages. RT-PCR was conducted using PAR-1 and β-actin-specific primers (see Table S1). Western blot was performed using rabbit anti-PAR-1 (H-111) and mouse anti-β-actin antibody, respectively. B) Surface CD11b expression. *WT* and *Par1*$^{-/-}$ BM-derived macrophages were stained with FITC-conjugated anti-CD11b mAb (M1/70). After washing, CD11b surface expression was analyzed by flow cytometry. Mean fluorescent intensity (MFI) was calculated using the FACScan program.

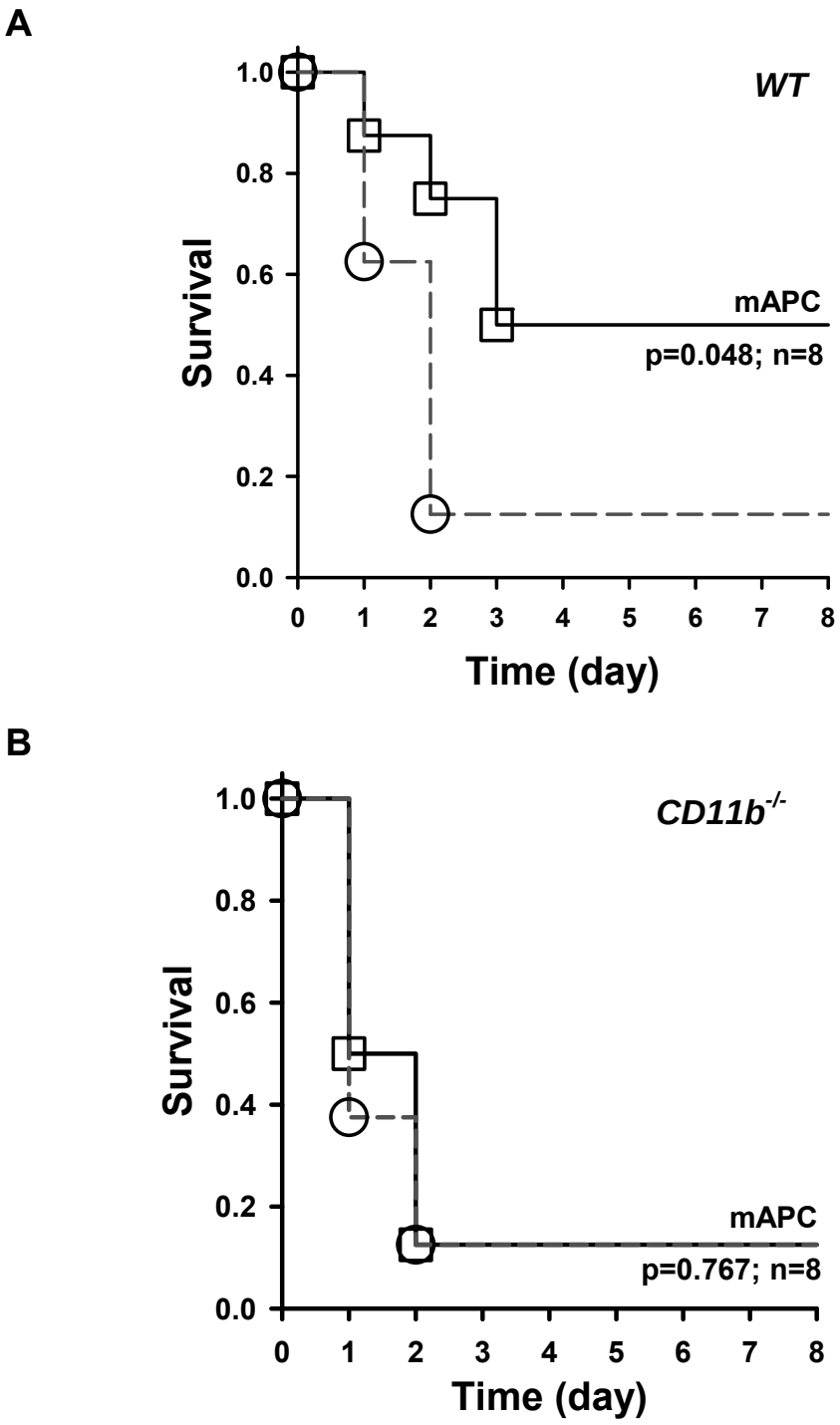

Figure S6. CD11b is critical to protection of endotoxemic mice by mAPC. *WT* (A) or *CD11b*$^{-/-}$ (B) mice were injected i.p. with a single dose (LD_{90}) of LPS, followed by two i.v. injections of 10 μg mAPC (□) or PBS (○) at 20 min and 8 hours post-LPS challenge. Survival was determined over a period of 12 days and shown in Kaplan–Meier plots. Log rank test between mAPC and PBS: p=0.048, n=8 for WT and p=0.767, n=8 for CD11b$^{-/-}$ mice.

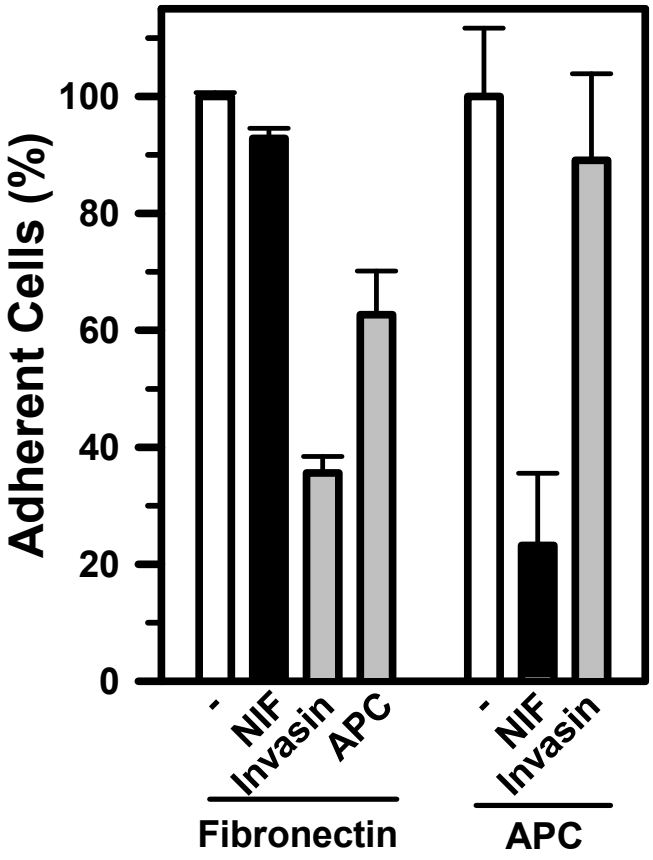

Figure S7. CD11b/CD18 but not the β_1 integrins is critical to macrophage adhesion to APC. BM-derived macrophages were allowed to adhere to fibronectin (5 µg/ml) or hAPC (20 µg/ml)-coated 24-well microtiter plate in the presence of 20 nM NIF, 10 µg/ml MBP-invasin or 5 ug/ml APC for 40 min at 37°C. After washing, adherent cells were determined by staining cells with 0.5% Crystol Violet and measuring absorption at 570nm. The number of adherent macrophages in the absence of inhibitors was assigned 100%. Data shown are means ± SD of duplicate experiments.

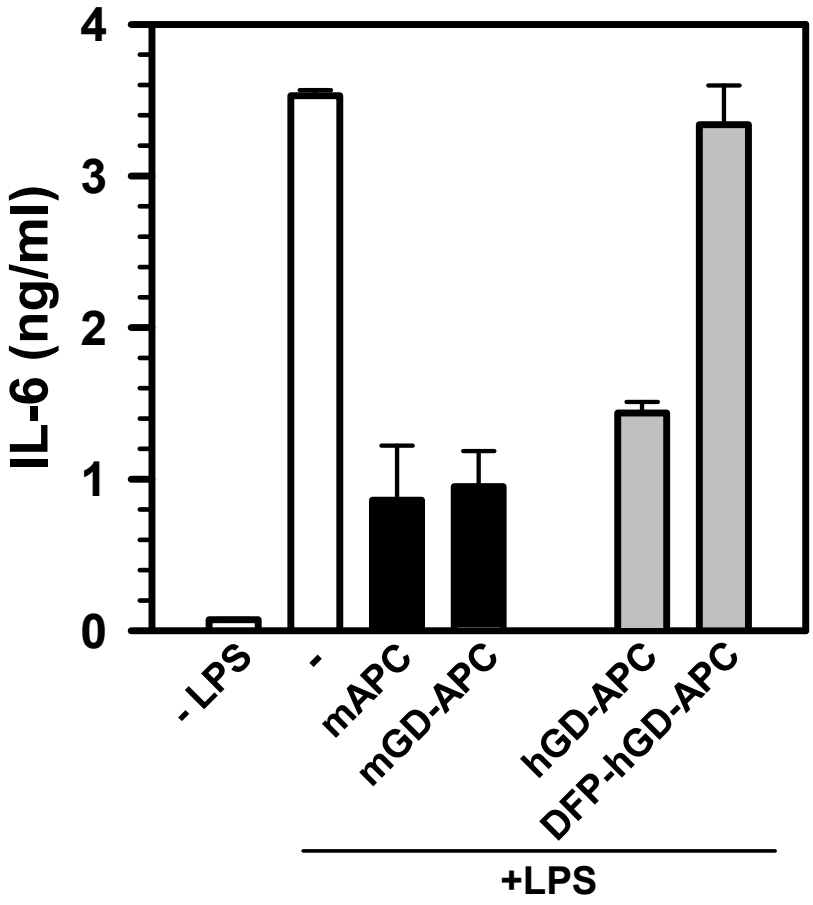

Figure S8. mGD-APC exhibits similar *in vitro* suppressive activity toward macrophages to mAPC. BM-derived macrophages were left untreated (-LPS) or stimulated with 50 ng/ml LPS in the presence of 0.09 μM mAPC, mGD-APC, hGD-APC, DFP-treated hGD-APC or vehicle control ("-"). Twenty hours later, IL-6 concentration in the conditioned media was determined by ELISA. Data shown were means ± S.D. of duplicated samples and are representative of two independent experiments.

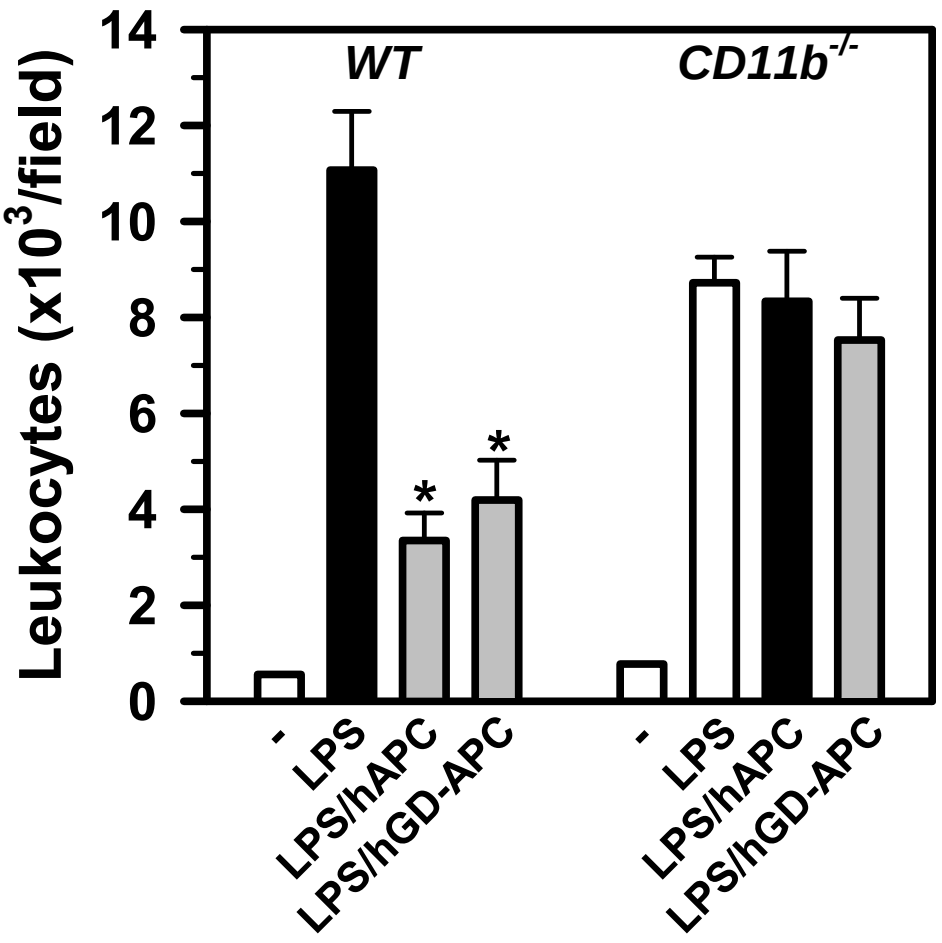

Figure S9. APC suppression of leukocyte infiltration in the lung in endotoxemic mice is dependent on CD11b. *WT* or *CD11b*$^{-/-}$ mice were injected i.p. with a single LD_{90} dose of LPS, followed by i.v. injections of 10 μg hAPC, 10 μg hGD-APC or PBS. Twenty hours later, mice were sacrificed, perfused with PBS, and then fixed in 1% PFA. Lungs were collected, embedded in paraffin, cut into 5 μm-thick tissue sections, and stained with H&E. The number of infiltrating leukocytes was determined using NIH ImageJ by counting 5 randomly selected view fields per section. Data shown were means ± S.E.M of three mice. *, $p<0.01$, two-tailed Student's t-test.

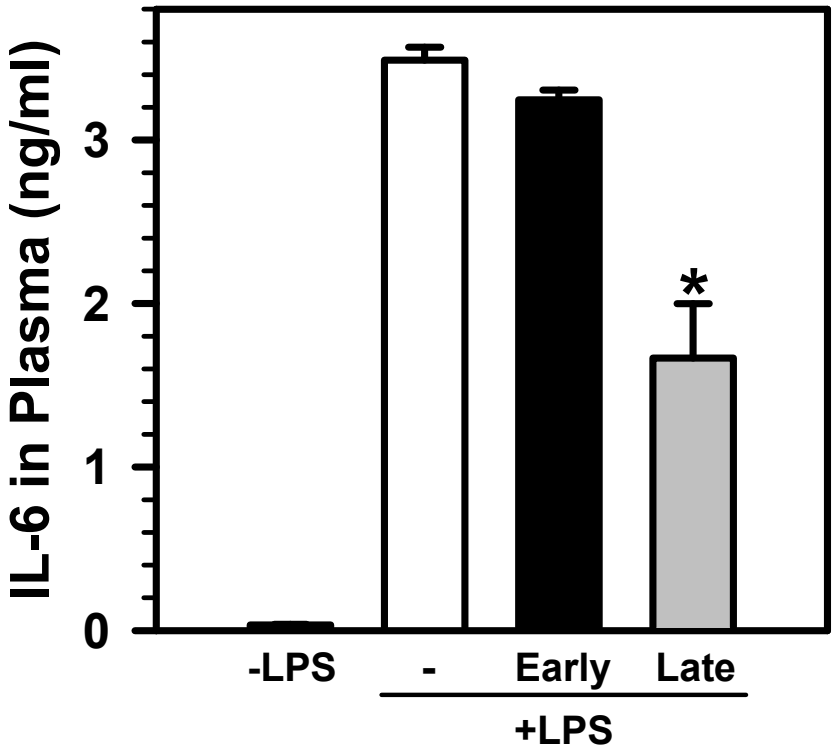

Figure S10. The two-dose late intervention regimen is superior to the early intervention regimen in GD-APC suppression of IL-6 production *in vivo*. In the early intervention regimen (black bar), WT mice were injected i.v. with 10 μg hGD-APC, followed by i.p. injection of a single LD_{90} dose of LPS. In the two-dose late intervention regimen (gray bar), WT mice were injected i.p. with a single LD_{90} dose of LPS, followed by two intravenous injections of 10 μg hGD-APC at 20 min and 8 hours post-LPS challenge. Twenty hours later, mice were sacrificed and IL-6 concentrations in the blood were determined by ELISA. Data shown were means ± S.D. *, $P<0.001$, LPS vs. LPS+hGD-APC; n=3-4.

Table s1. Primers used for qRT-PCR

β-Actin	NM_007393	Forward (5’ to 3’)	AGTGTGACGTTGACATCCGT
		Reverse (5’ to 3’)	TGCTAGGAGCCAGAGCAGTA
ApoER2	NM_ 001080926	Forward (5’ to 3’)	GTGATGATCAGAGGGACTGC
		Reverse (5’ to 3’)	AAGCCGATCTTGAGGTCAGT
TNF-α	NM_013693	Forward (5’ to 3’)	ATGAGAAGTTCCCAAATGGC
		Reverse (5’ to 3’)	CTCCACTTGGTGGTTTGCTA
IL-6	NM_031168	Forward (5’ to 3’)	CTCTGGGAAATCGTGGAAAT
		Reverse (5’ to 3’)	CCAGTTTGGTAGCATCCATC
IL-12A	NM_008351	Forward (5’ to 3’)	GTCTTAGCCAGTCCCGAAAC
		Reverse (5’ to 3’)	CAGGTCTTCAATGTGCTGGT
NOS2A	NM_010927	Forward (5’ to 3’)	GGAGCATCCCAAGTACGAGT
		Reverse (5’ to 3’)	CCCATGTACCAACCATTGAA
STAT3	NM_011486	Forward (5’ to 3’)	GAGAAGCAGCAGATGTTGGA
		Reverse (5’ to 3’)	CATGTCTCCTTGGCTCTTGA
NFκB1	NM_008689	Forward (5’ to 3’)	GGAGAAGGCTGGAGAAGATG
		Reverse (5’ to 3’)	GCTCATACGGTTTCCCATTT
TRAIL	NM_009425	Forward (5’ to 3’)	TGCAGGTTAAGAGGCAACTG
		Reverse (5’ to 3’)	GTGAGCTGCCACTTTCTGAG
Wnt5A	NM_009524	Forward (5’ to 3’)	CAAATAGGCAGCCGAGAGAC
		Reverse (5’ to 3’)	CAGCCACAGGTAGACAGCTC
BCL-3	NM_033601	Forward (5’ to 3’)	TGCTGAACCTGCCTACTCAC
		Reverse (5’ to 3’)	GTTATTCTGGACCACAGCGA