Supporting Information

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SI Materials and Methods

Histology. After 24 h of fixation in 10% formalin and embedding in paraffin, 4-μm-thick sections were stained with H&E (1). To score lung inflammation, slides were analyzed with respect to the following parameters: bronchitis, interstitial inflammation, edema, vasculitis, pleuritis, and thrombus formation. Each parameter was graded on a scale of 0 to 4 (0, absent; 1, mild; 2, moderate; 3, severe; 4, very severe). The total histopathological score was expressed as the sum of the scores for the different parameters, with a maximum score of 24. Areas with confluent inflammation were scored semiquantitatively and expressed as percentage of total lung area.

Granulocytes were stained with FITC-labeled anti-mouse Ly-6G mAb (Pharmingen) as described previously (2). Slides were photographed with a microscope equipped with a digital camera (Leica CTR500). Stained areas were analyzed with Image Pro Plus (Media Cybernetics) and expressed as percentage of the surface area. The average of 10 pictures at 200× magnification was used for analysis. To stain for C/EBPδ, lung tissue sections were boiled for 20 min in a 0.3% citrate buffer (pH 6.0), blocked with 10% normal goat serum, and incubated with a rabbit polyclonal antibody against C/EBPδ (2318; Cell Signaling Technologies) in a 1:1,000 dilution in PBS at 4 °C overnight. To stain for PAFR, lung tissue sections were blocked with Ultra V block (TA125-UB; Immunologic) and incubated with a rabbit polyclonal antibody against PAFR (sc-8744; Santa Cruz Biotechnology) in a 1:100 dilution in TBS-Tween20 at 4 °C overnight. For both C/EBPδ and PAFR, slides were incubated with Powervision poly-HRP anti rabbit IgG (DPVM-55HRP; Immunologic) for 30 min at room temperature and stained with 3-3′ diaminobenzidine dihydrochloride (BS04-999; Immunologic). Hematoxylin (1:10 in demineralized H_2O for PAFR staining) was applied as a counterstain.

Primers Used for Lightcycler Assays. The following promers were used for the LightCycler assays: for *pafr*, 5′-CTGGACCCTAGCAGAGTTGG-3′ and 5′-GCTACTGCGCATGCTGTAAA-3′; for *pIg-R*, 5′-CCTCTCCAGACACACAGCAA-3′ and 5′-CAGCTATGGTGCTGGACTGA-3′. The QuantiTect mouse *c/ebpδ* primer assay kit (QT00312809) was purchased from Qiagen.

Lung Permeability Assays. In brief, 100 μL of EB dye (5 mg/mL) was injected in the tail vein 90 min before the mouse was sacrificed. Lungs were flushed with 10 mL of PBS to wash out all remaining blood, after which the right lung was photographed using a Canon Powershot digital camera. Next the lungs were blotted dry, weighed, and homogenized in PBS (1 mL/100 μg tissue), after which two volumes of formamide were added. After 18 h of incubation at 60 °C, samples were centrifuged at 12,000 × *g* for 20 min, and optical densities of the supernatants were determined by spectrophotometry at 620 nm and 740 nm in 96-well plates using a BioTek Synergy HT Multi-Mode Microplate Reader. EB dye concentrations were calculated using the lung-specific correction factor as described previously (3).

Western Blot Analysis. After electrophoresis, proteins were transferred onto Immobilon-FL membranes (Millipore). The blots were blocked for 1 h at room temperature in Odyssey blocking buffer (LI-COR Biosciences)/PBS (1:1). Goat polyclonal α-C/EBPδ (M-17; Santa Cruz Biotechnology), α-PAFR (Cayman Chemical), and α-tubulin (B-5–1-2; Santa Cruz Biotechnology) were diluted 1:500 for C/EBPδ, 1:250 for PAFR, and 1:1,000 for α-tubulin in Odyssey blocking buffer/PBS with 0.2% Tween-20 (1:1). Membranes were incubated overnight, washed in PBS with 0.1% Tween-20, and incubated with IRDye 700-GAR (red) or IRDye 800-GAR (green) secondary antibodies diluted 1:5,000 in Odyssey blocking buffer/PBS with 0.2% Tween-20 and 0.02% SDS (1:1). After 1 h of incubation at room temperature, blots were washed in PBS with 0.1% Tween-20 and then scanned on a LI-COR Odyssey IR Imager.

1. Knapp S, et al. (2006) Differential roles of CD14 and toll-like receptors 4 and 2 in murine *Acinetobacter* pneumonia. *Am J Respir Crit Care Med* 173:122–129.
2. van Zoelen MAD, et al. (2009) The receptor for advanced glycation end products impairs host defense in pneumococcal pneumonia. *J Immunol* 182:4349–4356.
3. Moitra J, Sammani S, Garcia JGN (2007) Re-evaluation of Evans blue dye as a marker of albumin clearance in murine models of acute lung injury. *Transl Res* 150:253–265.

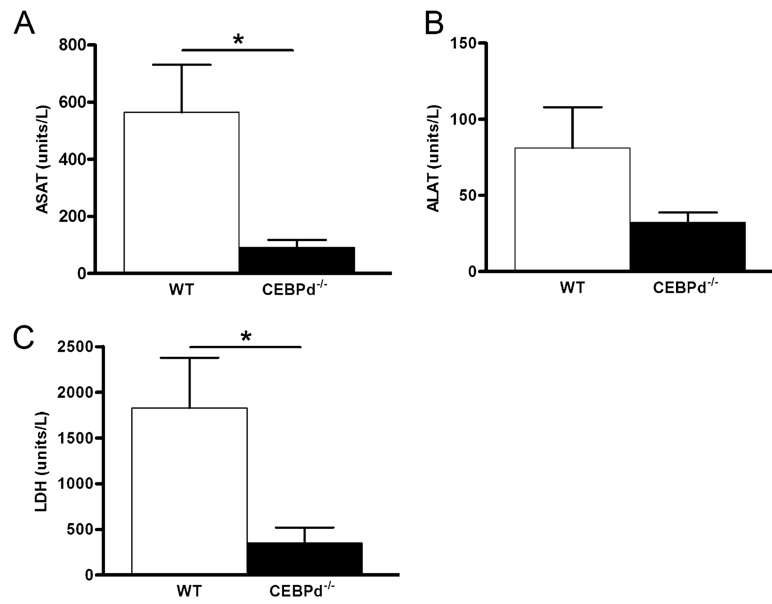

Fig. S1. WT mice succumb to systemic disease on *Streptococcus pneumoniae*-induced pneumonia. Levels of liver injury markers aspartate transaminase (ASAT) (*A*) and alanine transaminase (ALAT) (*B*) and general tissue injury marker lactate dehydrogenase LDH (*C*) in plasma of WT and C/EBPδ$^{-/-}$ mice at 48 h after intranasal inoculation of *S. pneumoniae*.

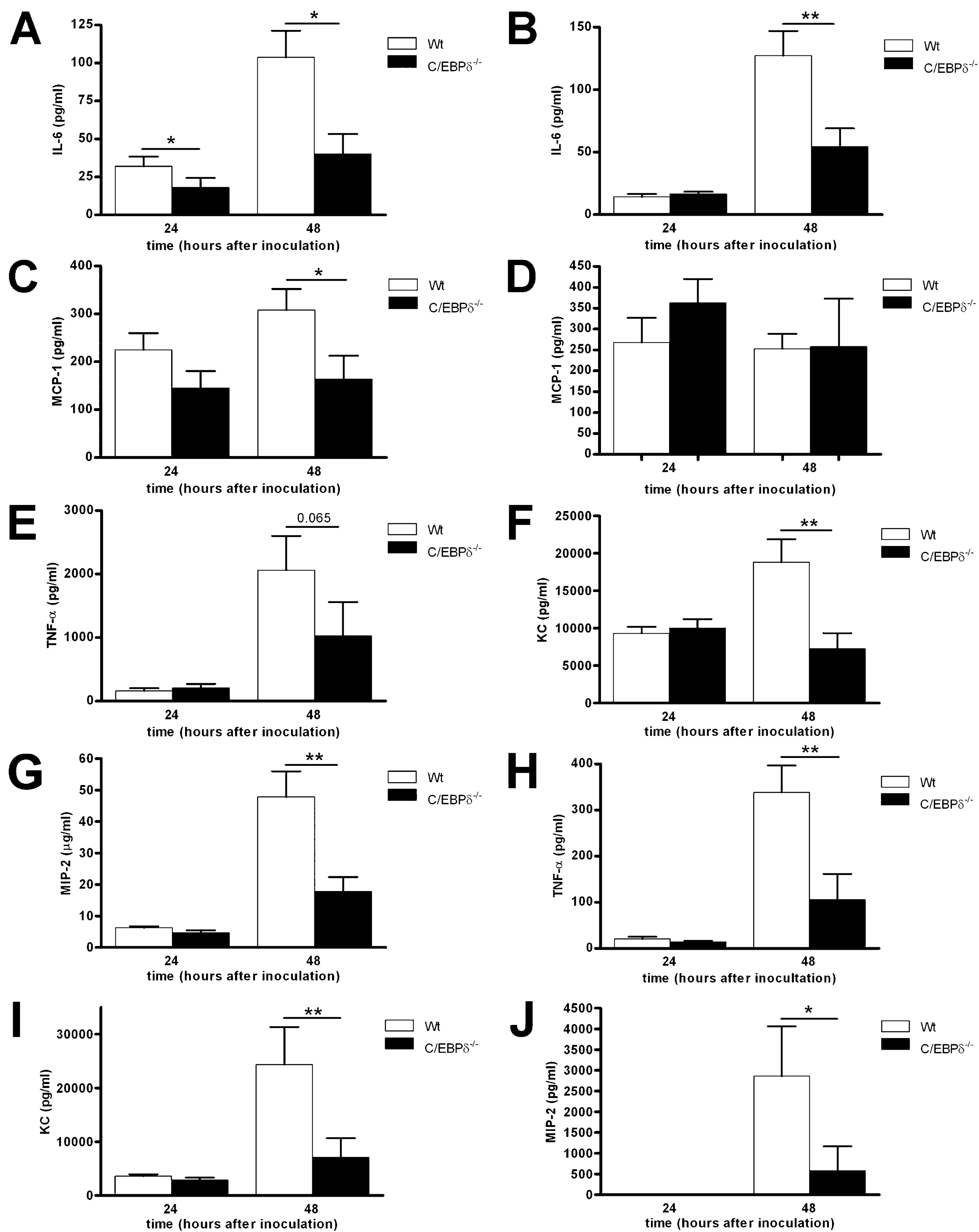

Fig. S2. C/EBPδ deficiency limits *S. pneumoniae*-induced inflammation. IL-6 and MCP-1 levels in liver (*A* and *C*) and kidney (*B* and *D*) and TNF-α, KC, and MIP-2 levels in lung (*E*–*G*) and plasma (*H*–*J*) of WT and C/EBPδ$^{-/-}$ mice after inoculation with *S. pneumoniae*. Data are mean ± SEM (n = 7–8). *$P < 0.05$; **$P < 0.01$. P values of 0.1–0.05 are indicated above the bars.

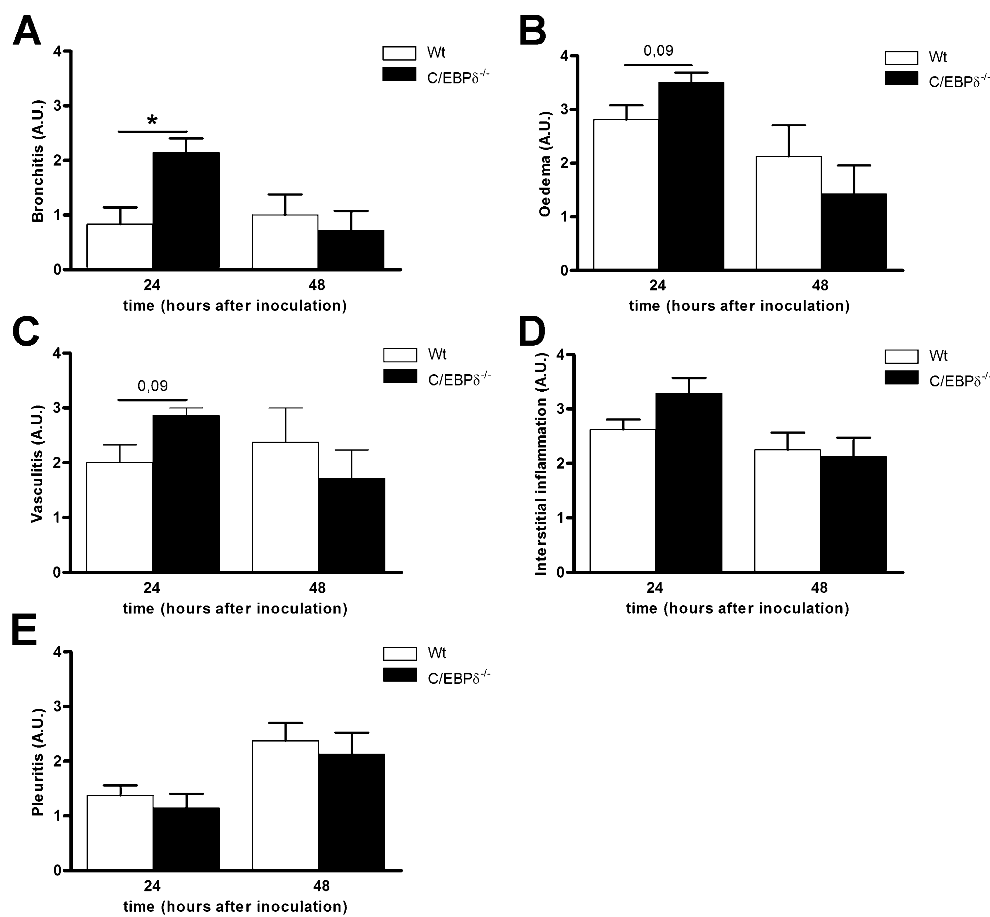

Fig. S3. C/EBPδ deficiency has no major impact on lung histology. Overview of the histological parameters scored to provide the total histological score as indicated in Fig. 2*H*. Graphical representation of bronchitis (*A*), edema (*B*), endothelialitis (*C*), interstitial inflammation (*D*), and pleuritis (*E*) in WT and C/EBPδ$^{-/-}$ mice. Data are mean ± SEM (n = 7–8). $^{*}P < 0.05$. P values of 0.1–0.05 are indicated above the bars.

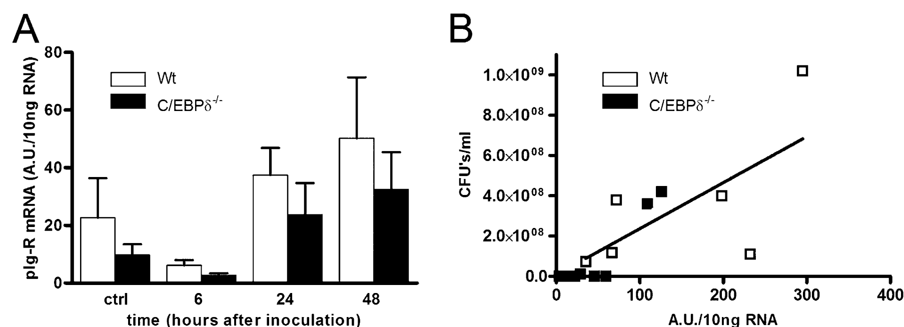

Fig. S4. *pIg-R* mRNA levels do not differ between WT and C/EBPδ$^{-/-}$ mice during *S. pneumoniae*-induced pneumonia. (A) *pIg-R* mRNA levels in lungs of WT and C/EBPδ$^{-/-}$ mice at different time points after intranasal inoculation with *S. pneumoniae*. Data are mean ± SEM (n = 7–8). (*B*) Correlation between *pafr* mRNA levels and bacterial outgrowth in blood of WT and C/EBPδ$^{-/-}$ mice at 48 h after intranasal inoculation with *S. pneumoniae*.

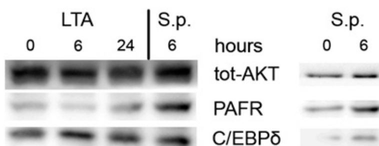

Fig. S5. C/EBPδ and PAFR are up-regulated on stimulation with lipoteichoic acid or *S. pneumoniae* in Calu3 cells. Representative Western blots of C/EBPδ (32 kDa), PAFR (48 kDa), and total-Akt (70 kDa) levels in Calu3 lung epithelial cells stimulated with 10 ng/mL of lipoteichoic acid or 1×10^8 CFU of growth-arrested *S. pneumoniae* (S.p.) for the times indicated. The results of two independent experiments are shown.