SUPPLEMENTARY MATERIALS

Interleukin 18 function requires both interleukin 18 receptor and Na-Cl co-transporter

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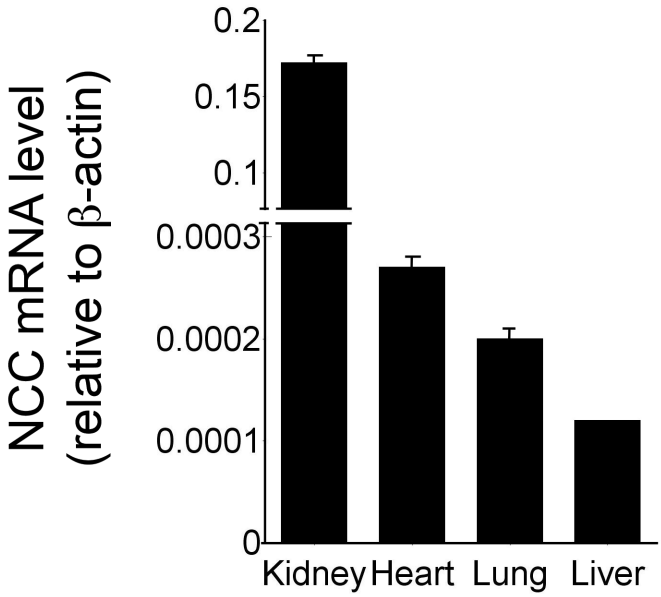
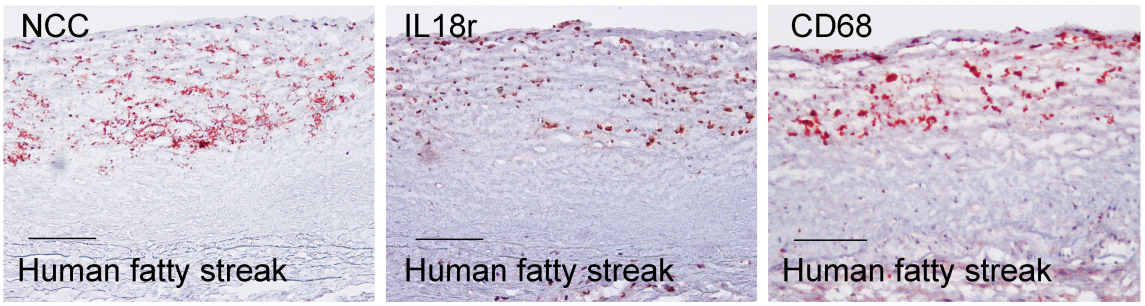
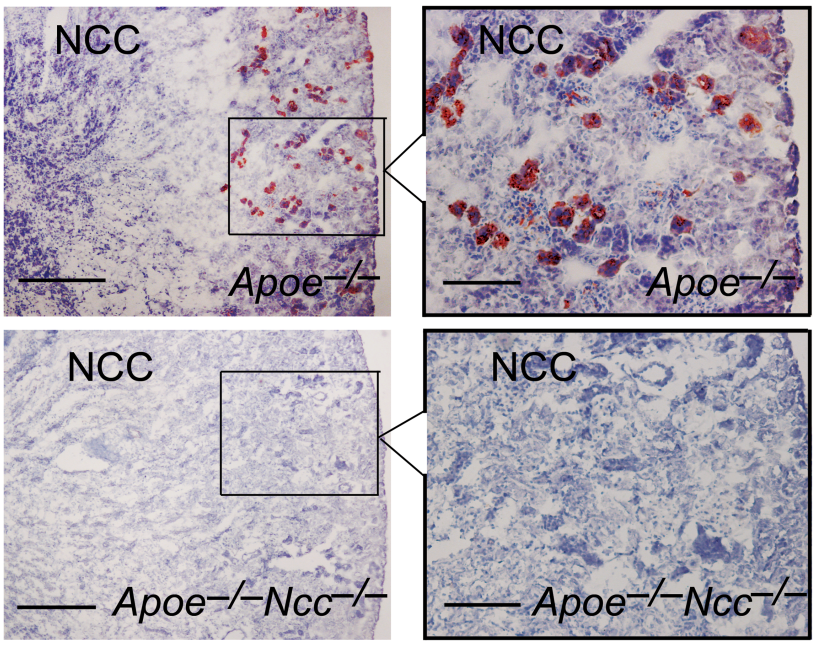


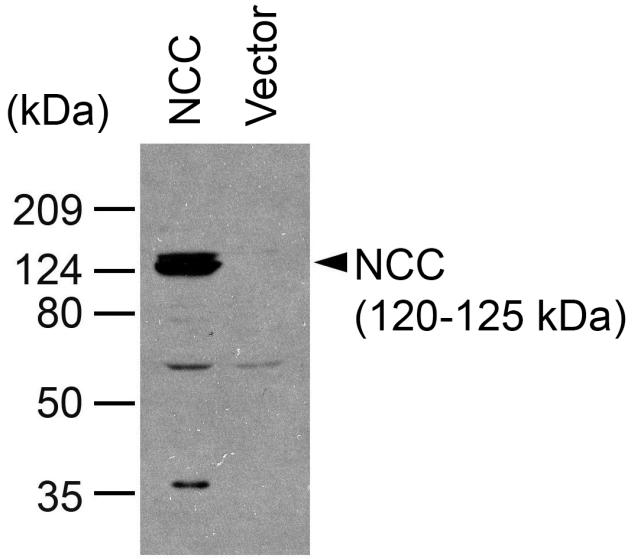
Supplementary Figure 1. RT-PCR to detect NCC mRNA levels in mouse kidney, heart, lung, and liver. Data are mean ± SEM from three to six independent experiments.



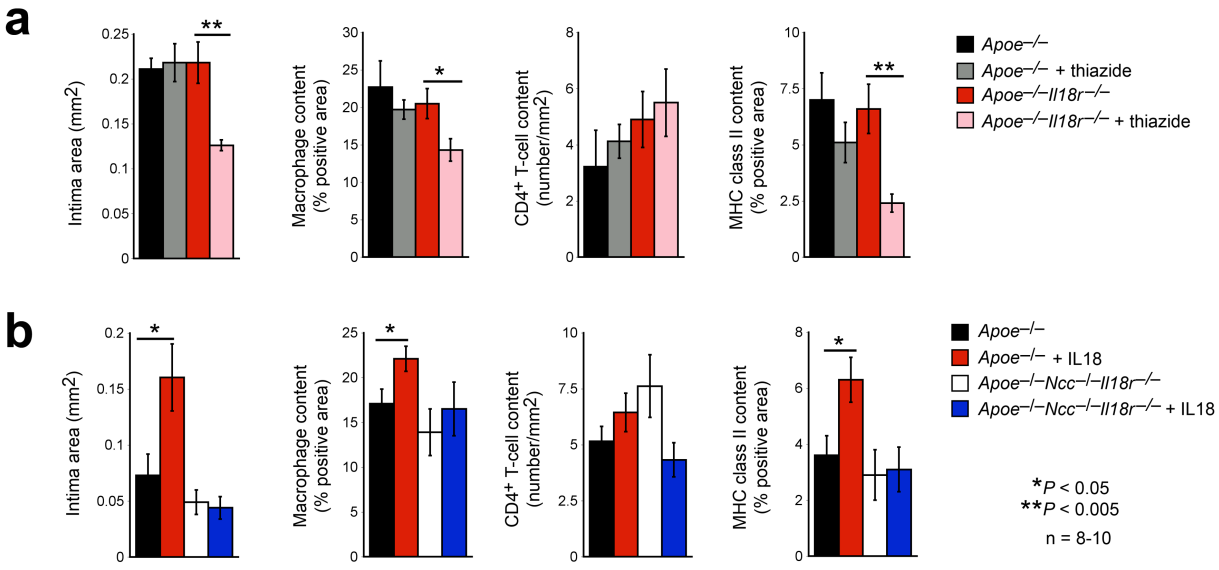
Supplementary Figure 2. Immunostaining of human carotid artery fatty streak frozen sections with antibodies against NCC (1:60, Millipore, Billerica, MA), IL18 receptor (1:100, R&D Systems, Minneapolis, MN), and CD68 (1:900, Dako, Carpinteria, CA). Bar: 200 μm.



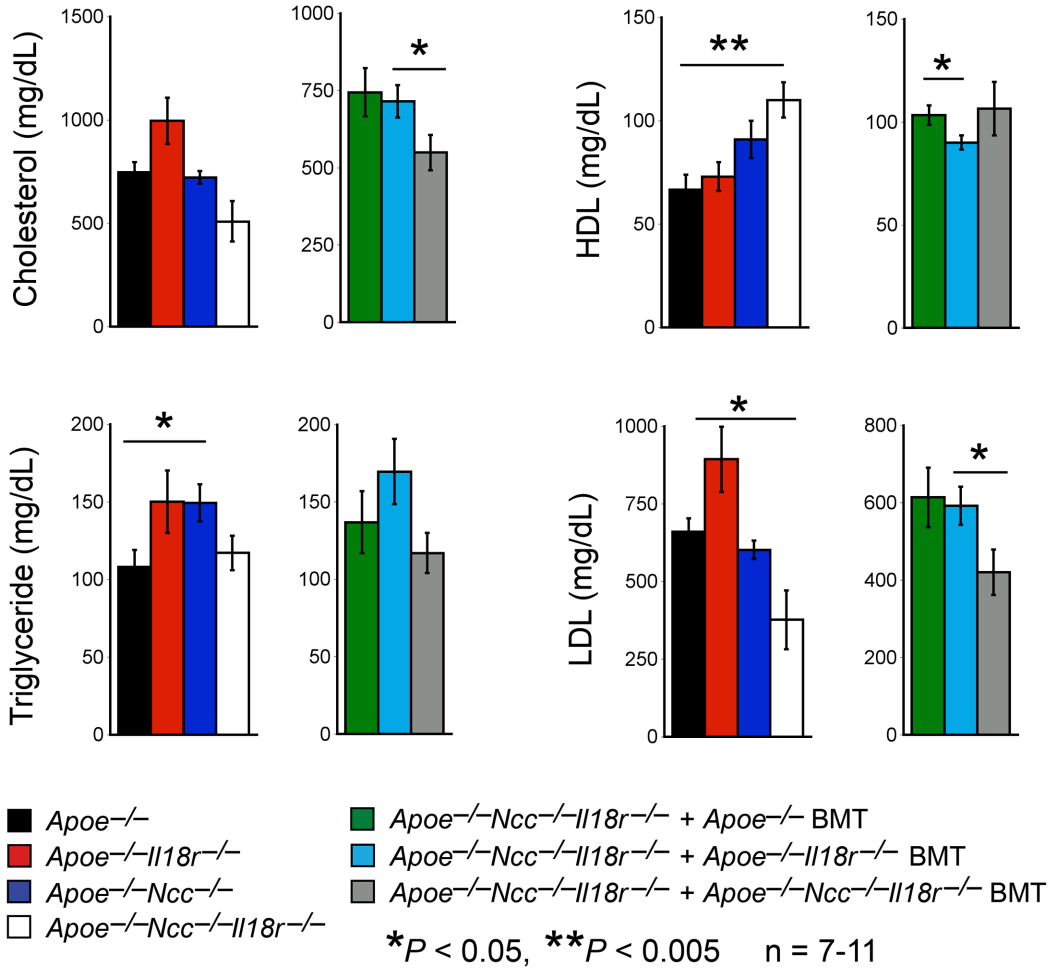
Supplementary Figure 3. Immunostaining of NCC on frozen kidney sections from *Apoe*$^{-/-}$ mice and *Apoe*$^{-/-}$*Ncc*$^{-/-}$ mice with rabbit anti-NCC polyclonal antibody (1:60, Millipore, Billerica, MA). Anti-NCC antibody from our prior study (*J Am Soc Nephrol.* 1998;9:1347) yielded the exact same immunostaining pattern (data not shown). Bar: 500 μm, Insert bar: 200 μm.



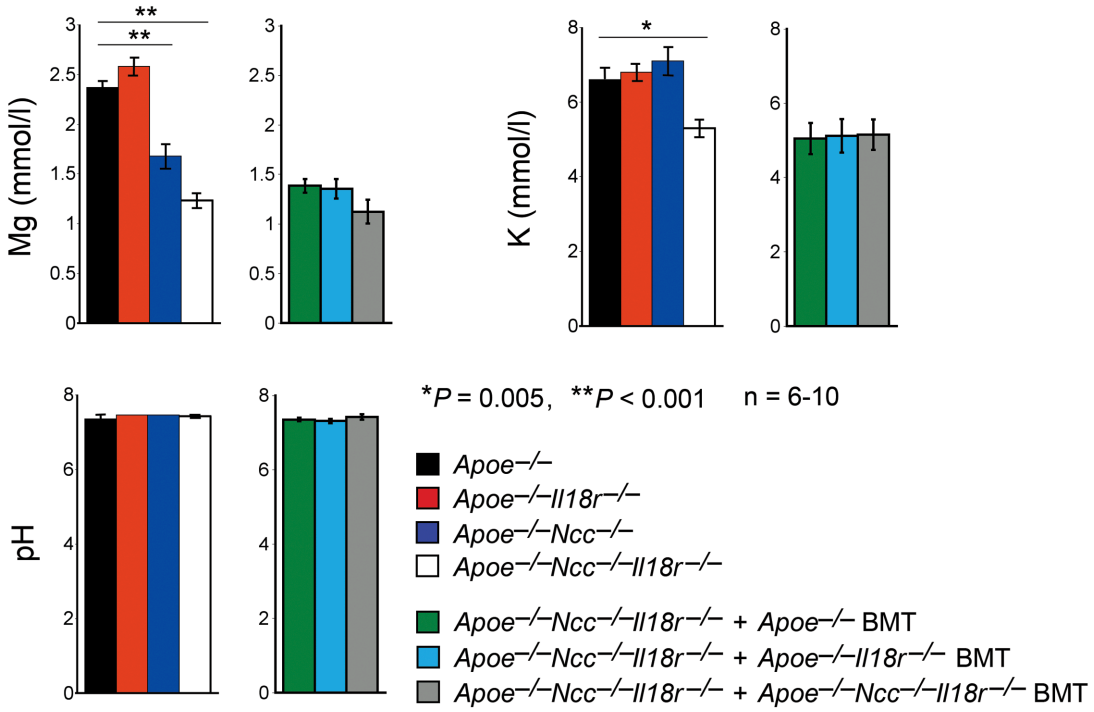
Supplementary Figure 4. Immunoblot analysis to detect NCC in NCC/pcDNA3.1- and pcDNA3.1-transfected COS-7 cells.



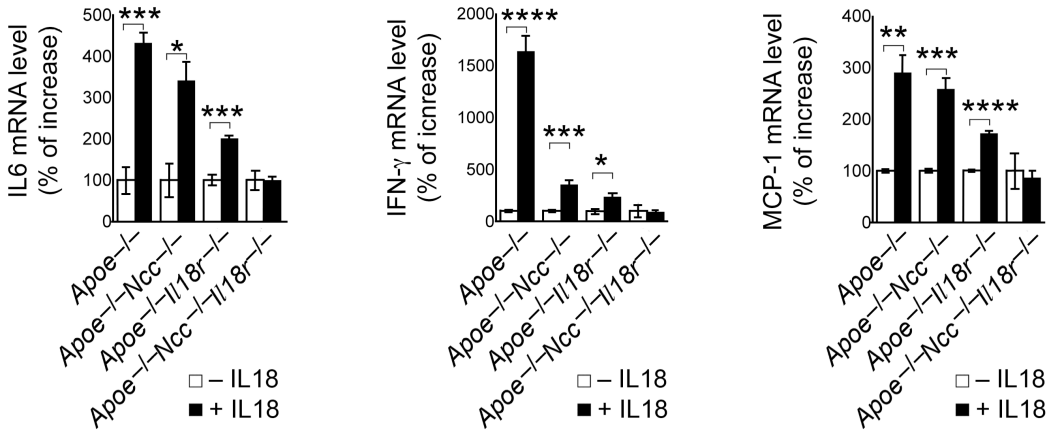
Supplementary Figure 5. Aortic root atherosclerotic lesion area, macrophage, $CD4^+$ T cell and MHC-II contents in mice treated with hydrochlorothiazide (3 months) (**a**) and mouse recombinant IL18 (4 weeks) (**b**) in different mice as indicated. Data are mean ± SEM, n=8-10 per experimental group.



Supplementary Figure 6. Plasma levels of total cholesterol, HDL, triglyceride, and LDL from *Apoe*$^{-/-}$, *Apoe*$^{-/-}$*Il18r*$^{-/-}$, *Apoe*$^{-/-}$*Ncc*$^{-/-}$, *Apoe*$^{-/-}$*Ncc*$^{-/-}$*IL18r*$^{-/-}$ mice, and *Apoe*$^{-/-}$*Ncc*$^{-/-}$ *IL18r*$^{-/-}$ mice received BMT. Data are mean ± SEM, n=7-11 per experimental group.

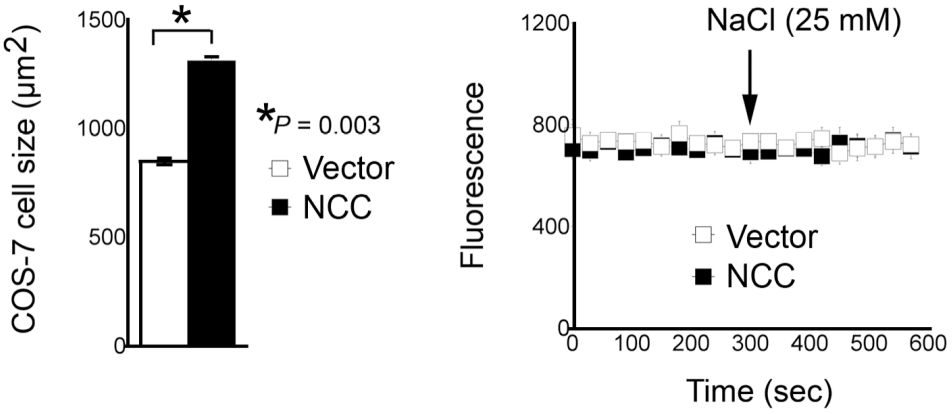


Supplementary Figure 7. Plasma levels of Mg^{2+}, K^{+}, and pH from *Apoe*$^{-/-}$, *Apoe*$^{-/-}$*Il18r*$^{-/-}$, *Apoe*$^{-/-}$*Ncc*$^{-/-}$, *Apoe*$^{-/-}$*Ncc*$^{-/-}$*IL18r*$^{-/-}$ mice, and *Apoe*$^{-/-}$*Ncc*$^{-/-}$*IL18r*$^{-/-}$ mice received BMT. Data are mean ± SEM, n=6-10 per experimental group. Samples with gross hemolysis were excluded.

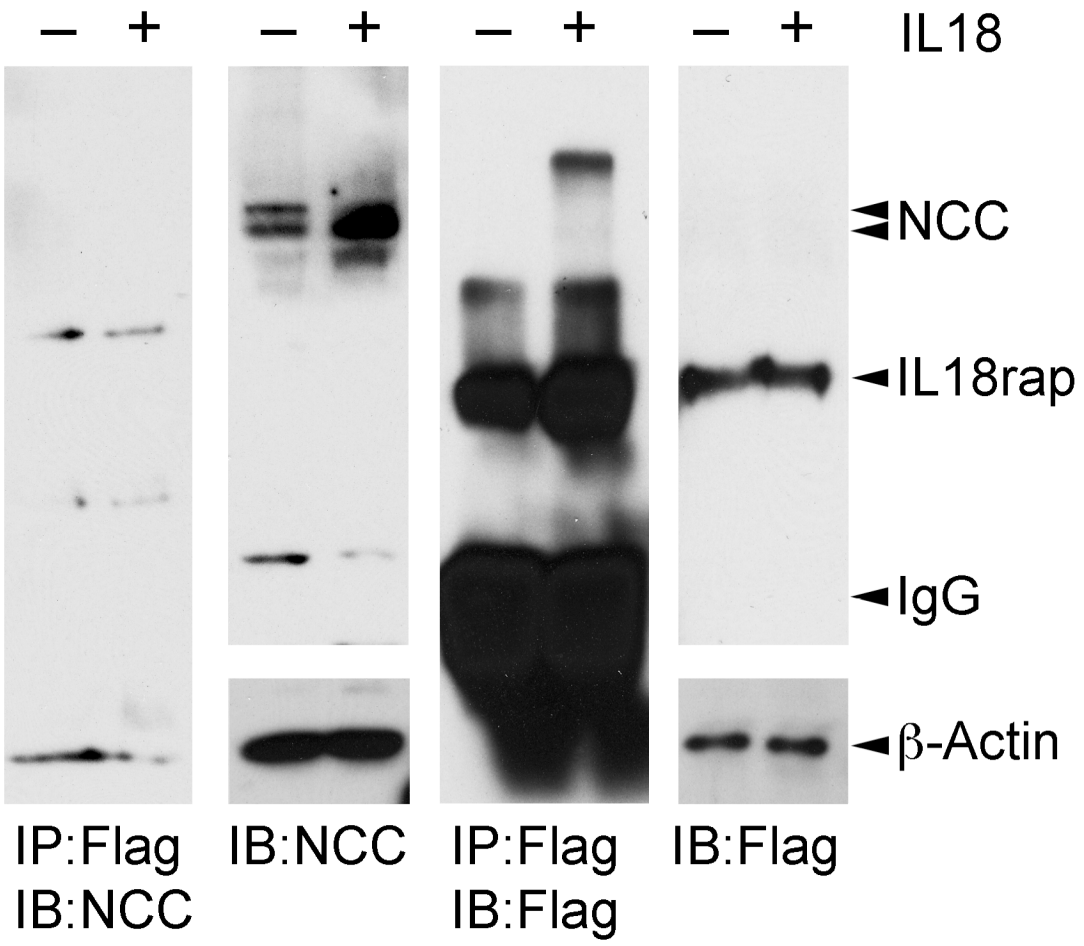


*P < 0.05, **P < 0.01, ***P < 0.005, ****P < 0.001

Supplementary Figure 8. RT-PCR determined IL6, INF-γ, and MCP-1 mRNA levels in IL18-treated and untreated macrophages from different mice, as indicated. Data are mean ± SEM from three to six independent experiments.



Supplementary Figure 9. Cell volume and intracellular Cl^- concentrations in COS-7 cells transfected with NCC/pcDNA3.1 or vector alone. Data are mean ± SEM from three to six independent experiments.



Supplementary Figure 10. NCC and IL18rap expression and co-immunoprecipitation from COS-7 cells. COS-7 cells were transfected with both NCC/pcDNA3.1 and Flag-IL18rap/pcDNA3.1. After 48 hours, cells were treated with or without IL18 for 15 minutes and then lysed in RIPA buffer and immunoprecipitated with anti-Flag antibody, followed by immunoblot analysis with either rabbit anti-mouse NCC polyclonal antibody (panel 1) or anti-Flag antibody (panel 3). The same cell lysates were used for immunoblot analysis to detect NCC (panel 2) and Flag-IL18rap (panel 4) expressions. Actin blots in panels 2 and 4 were used to ensure equal protein loading. IP: immunoprecipitation; IB: immunoblot.