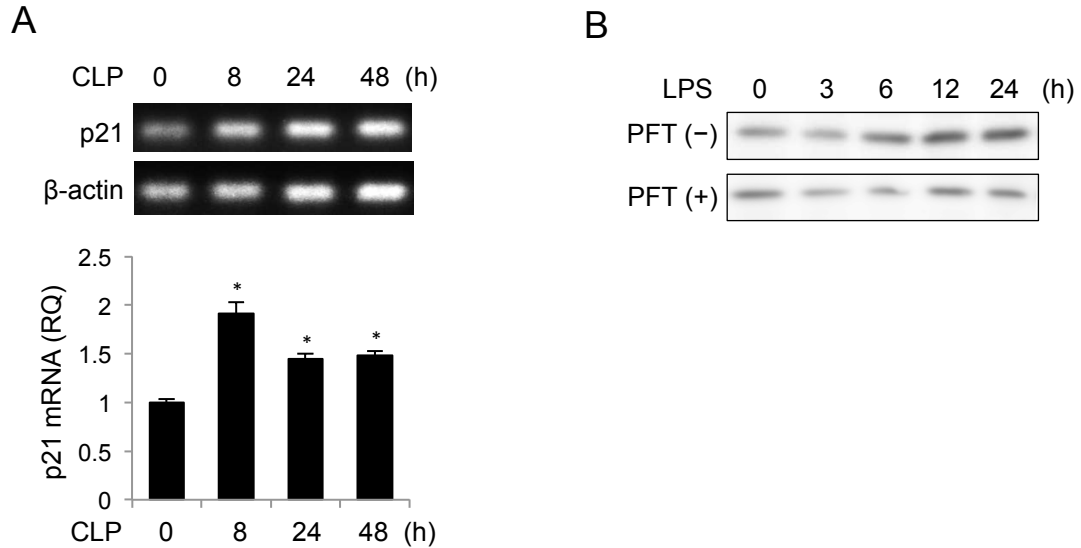


Supplementary Material

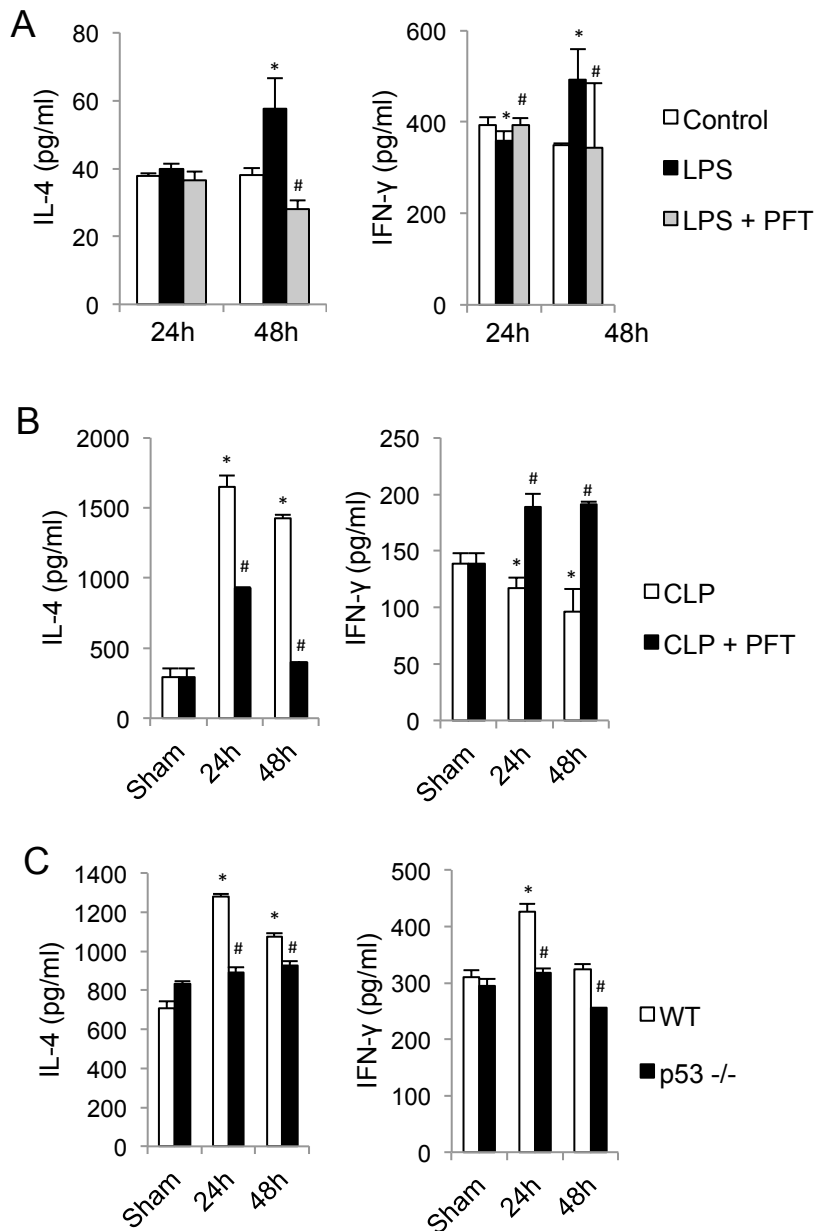
Novel Role of P53 in Septic Immunosuppression: Involvement in Loss and Dysfunction of CD4⁺ T Lymphocytes

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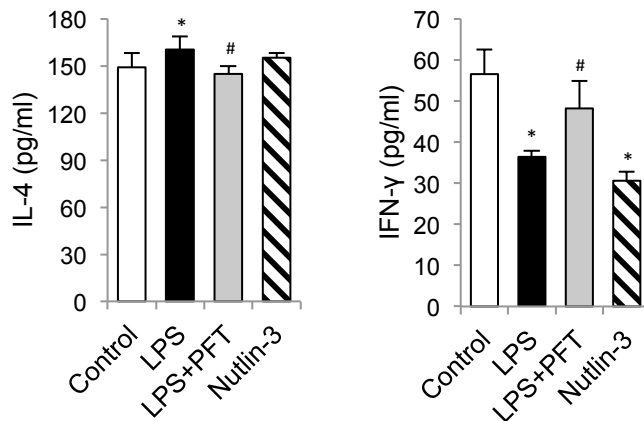
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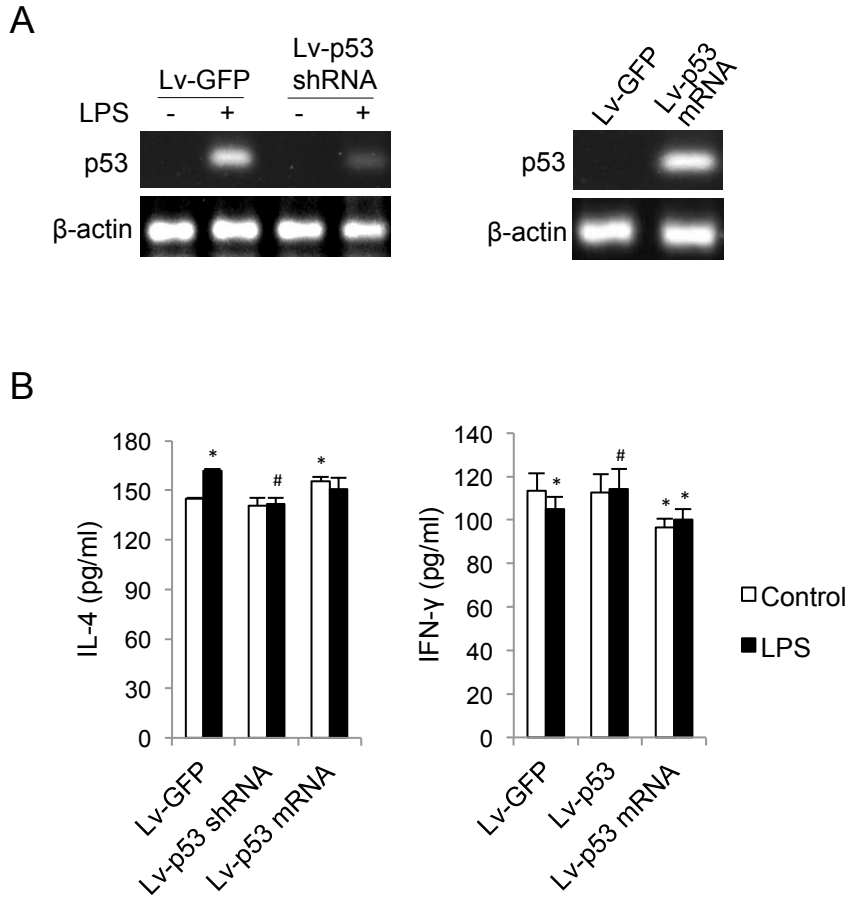
Supplementary Fig. 1. p21 expression in splenic CD4⁺ T Cells after CLP. (A) C57BL/6 mice (n=20) were subjected to CLP, and mice were sacrificed at 8, 24, and 48 hours, respectively. Splenic CD4⁺ T cells were isolated and subjected to mRNA analysis of p21 by PT-PCR (top panel) and qPCR (bottom panel). β -actin was used as loading control. The values of sham group (0) were taken as 1, and were means \pm SD. of at least three independent experiments. (B) C57BL/6 mice (n=20 for each group) were subjected to CLP with or without a 30min prior intraperitoneal injection of p53 inhibitor Pifithrin- α (PFT, 2mg/kg), after indicated time mice were sacrificed. Splenic CD4⁺ T cells were isolated and subjected to analysis of p21 protein level by Western blot. β -actin was used as loading control. The value *Significant difference when compared with 0 group ($P < 0.05$).



Supplementary Fig. 2. IL-4 and IFN- γ secretion by splenic CD4⁺ T cells from WT or p53^{-/-} CLP mice. (A) Splenic CD4⁺ T cells were isolated from C57BL/6 mice (n=20) and treated with LPS (1 μ g/ml) in the presence or absence of PFT (20 μ M). After indicated time points, cell supernatants were collected and subjected to ELISA analysis of IL-4 and IFN- γ . The original values were means \pm SD. of at least three independent experiments. *Significant difference when compared with respective control ($P < 0.05$). #Significant difference when compared with respective LPS group ($P < 0.05$). (B) C57BL/6 mice (n=20 for each group) were subjected to CLP with or without a 30 min prior interperitoneal injection of PFT (2 μ g/ml). After indicated time points, mice were sacrificed. (C) WT (n=20) and p53^{-/-} mice (n=20) were subjected to CLP, and mice were sacrificed at indicated time points. Splenic CD4⁺ T cells were isolated and cultured for further 3 days in the presence of growth stimuli. Afterwards cell supernatants were used for ELISA analysis of IL-4 and IFN- γ . The original values were means \pm SD. of at least three independent experiments. *Significant difference when compared with respective sham group ($P < 0.05$). #Significant difference when compared with respective CLP group or WT ($P < 0.05$).



Supplementary Fig. 3. IL-4 and IFN- γ secretion by cultured Jurkat cells. Jurkat cells were treated with LPS in the presence or absence of PFT (20 μ M), or treated with Nutlin-3 (10 μ M) for 24 hours. Afterwards cell supernatants were used for ELISA analysis of IL-4 and IFN- γ . The original values were means \pm SD. of at least three independent experiments. *Significant difference when compared with control ($P < 0.05$). #Significant difference when compared with LPS group ($P < 0.05$).



Supplementary Fig. 4. Effect of p53 interference on IL-4 and IFN- γ secretion in Jurkat cells. (A) Jurkat cells were transfected with p53 shRNA or mRNA expressing lentivirus (Lv-p53 shRNA or Lv-p53 mRNA). GFP expressing lentivirus (Lv-GFP) was used as a cell control. After stable expression, Lv-GFP and Lv-shRNA transfected cells were treated with LPS (1 μ g/ml) for 24 hours and subjected to mRNA analysis of p53 by PCR (left panel). Lv-p53 mRNA expressing cells were subjected to PCR analysis of p53 directly (right panel). β -actin was used as loading control. (B) Transfected cells were exposed to LPS for 24 hours and subjected to ELISA analysis of IL-4 and IFN- γ . The original values were means \pm SD. of at least three independent experiments. *Significant difference when compared with Lv-GFP control ($P < 0.05$). #Significant difference when compared with Lv-GFP LPS group ($P < 0.05$).