

ThGM-CSF cells contribute to the protective immune response against *Listeria monocytogenes*

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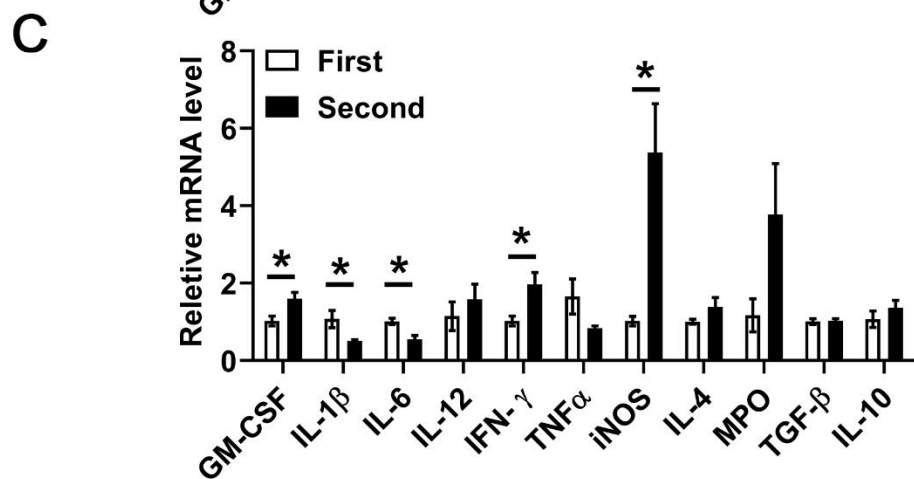
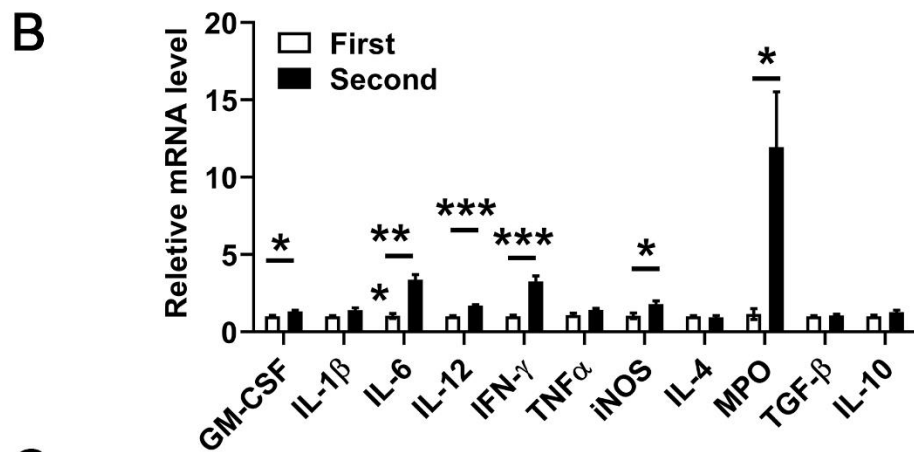
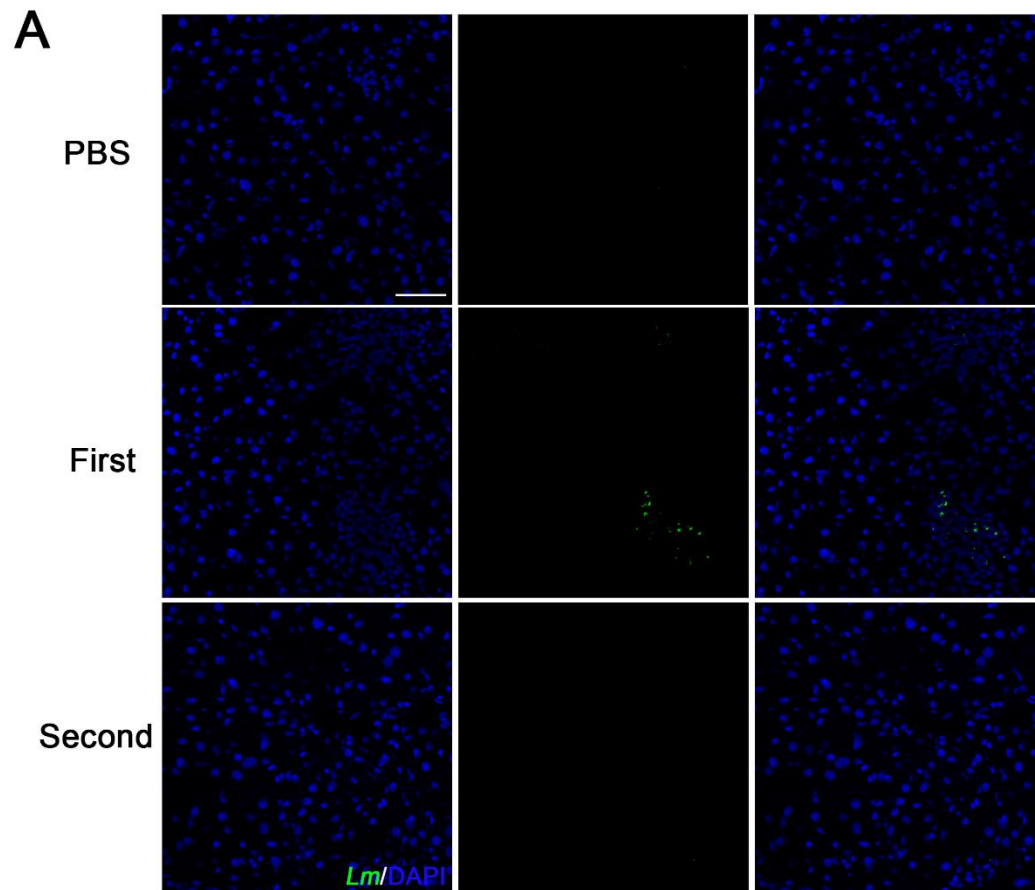
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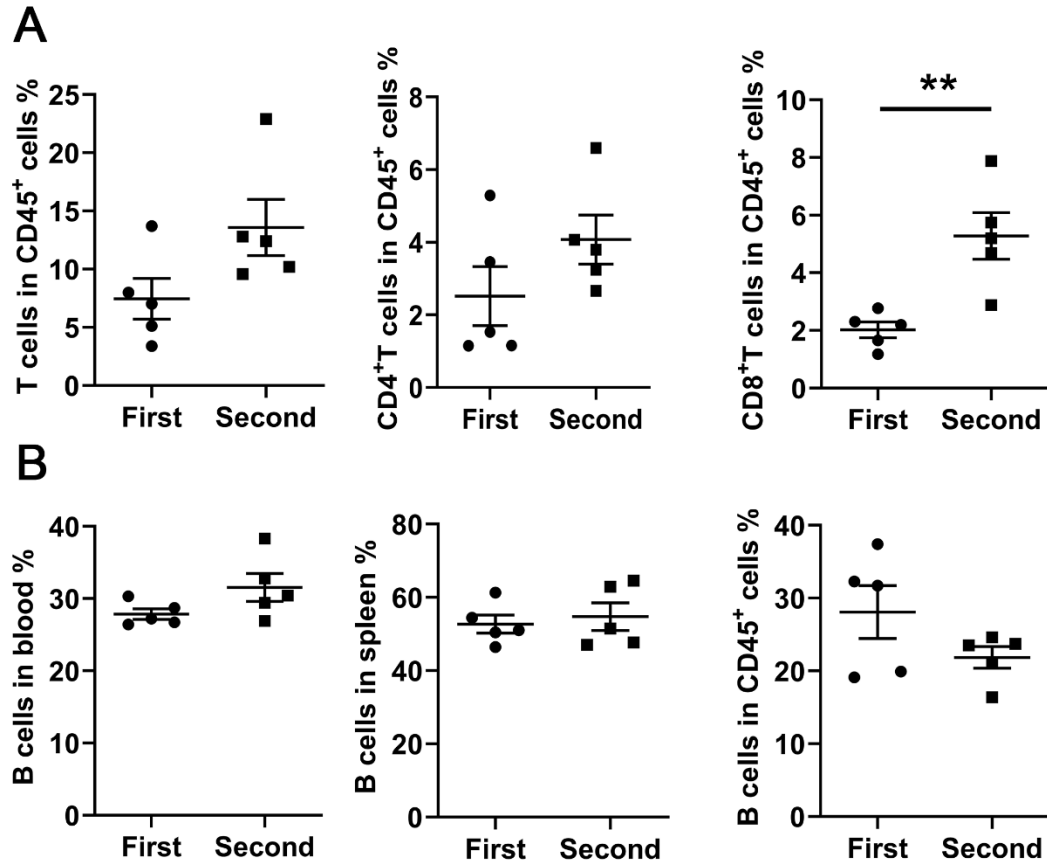
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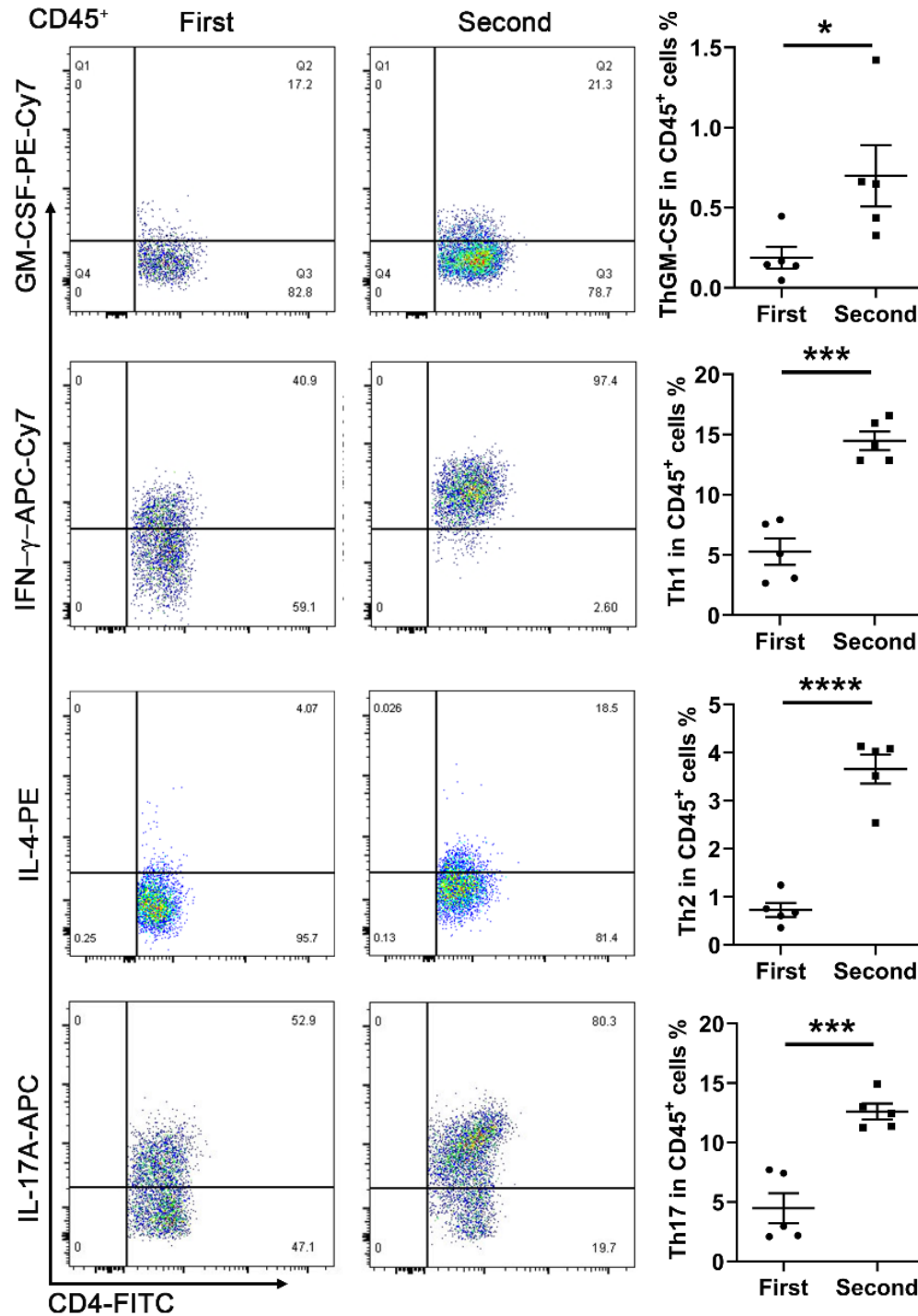
Supplementary Fig. 1 Multiple inflammatory factors were significantly increased during *L. monocytogenes* reinfection.

Mice were intravenously injected with 2×10^3 CFU of *L. monocytogenes* followed by 2×10^4 CFU 7 days later. **(a)** Immunofluorescence staining to detect *L. monocytogenes* colonization in liver section at 48h after reinfection. Blue represents DAPI and green represents *L. monocytogenes*. 400 $\times$, Scale bar, 50 μ m. **(b, c)** qRT-PCR analysis of inflammatory cytokine expression in the spleen **(b)** and liver **(c)** of mice after 4 hpi during protective immunity (n=4). Data are the mean $\pm$ SEM, student's *t*-test, **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001.

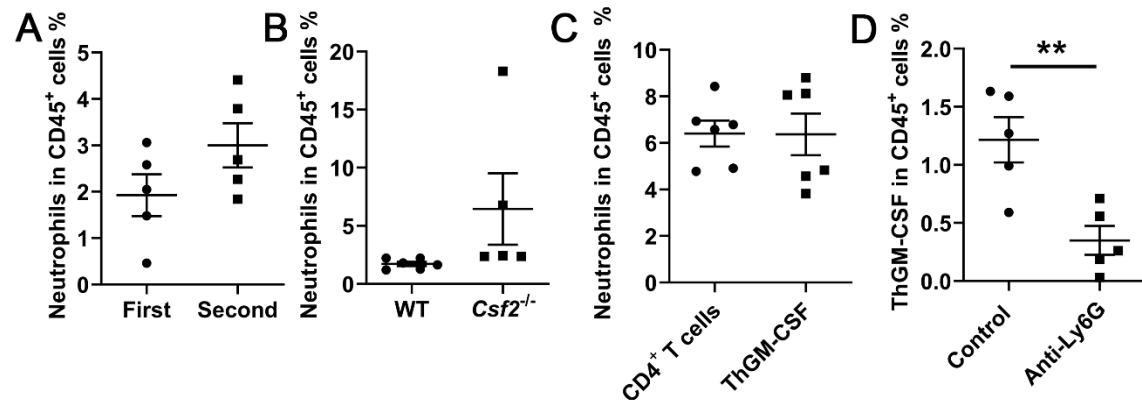


Supplementary Fig. 2 Increased T cell numbers in livers of reinfected mice.

Mice were intravenously injected with 2×10^3 CFU of *L. monocytogenes* followed by 2×10^4 CFU after 7 days. **(a)** Percentage of T cells among CD45⁺ cells in the liver detected by flow cytometry at 48 hpi (n=5). **(b)** Ratios of B cells in the blood, spleen, and CD45⁺ cells in the liver of infected mice detected by flow cytometry at 48 hpi (n=5). Data are the mean ± SEM, student's *t* test, **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001.

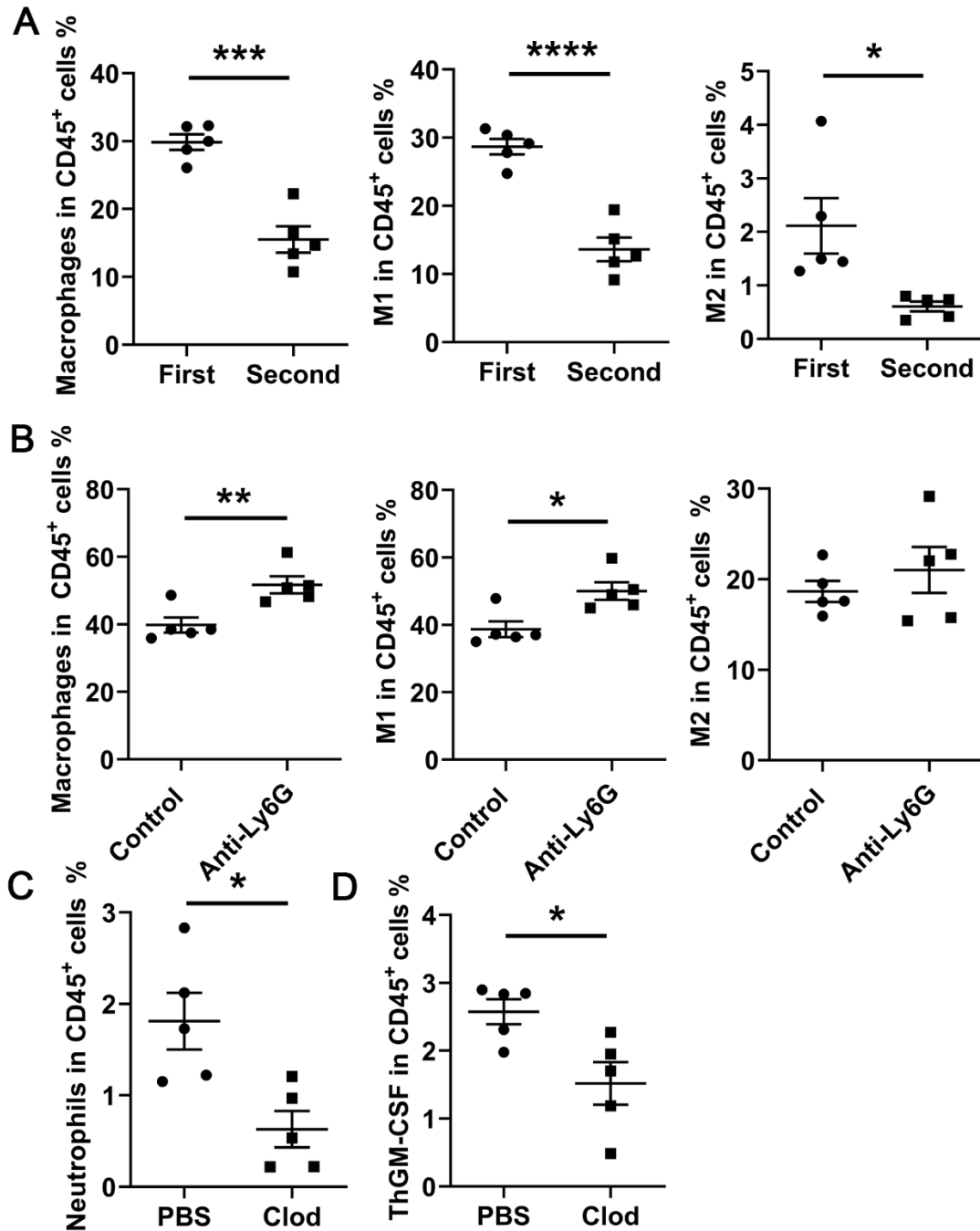


Supplementary Fig. 3 Subsets of CD4⁺ T cells in *L. monocytogenes* reinfection in the liver. Mice were injected intravenously with 2×10^3 CFU of *L. monocytogenes* followed by 2×10^4 CFU after 7 days. The ratios of ThGM-CSF, Th1, Th2, and Th17 cells in CD45⁺ cells in the liver were detected by flow cytometry at 48 hpi (n=5). Data are the mean $\pm$ SEM, student's *t* test, **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001.



Supplementary Fig. 4 ThGM-CSF cells did not affect neutrophil infiltration in the liver.

(a) Mice were intravenously injected with 2×10^3 CFU of *L. monocytogenes* followed by 2×10^4 CFU after 7 days. The ratios of neutrophils were detected in CD45⁺ cells of mice livers at 48 hpi (n=5). (b) The percentages of neutrophils in CD45⁺ cells of the liver of WT and Csf2^{-/-} mice at 48 h after reinfection (n=5-6). (c) *In vitro* cultured ThGM-CSF cells were intravenously injected into mice 1 h before infection. The ratios of neutrophils in CD45⁺ cells of the liver at 48 hpi are shown (n=6). (d) Detection of ThGM-CSF cells ratios in CD45⁺ cells of the liver of anti-Ly6G treated and reinfected mice by flow cytometry at 48 hpi (n=5). Data are the mean $\pm$ SEM, student's *t*-test, **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001.



Supplementary Fig. 5 Macrophages participate in protective immunity

(a) Macrophage ratios in CD45⁺ cells of the liver of reinfected mice were detected by flow cytometry at 48 hpi (n=5). (b) Percentages of macrophages in CD45⁺ cells in the liver of anti-Ly6G treated and reinfected mice at 48 hpi (n=5). (c, d) Neutrophil and ThGM-CSF cells ratios in CD45⁺ cells of the liver in clodronate liposomes treated and reinfected mice were detected by flow cytometry at 48 hpi (n=5). Data are the mean $\pm$ SEM, student's *t*-test, **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001.