Online supplement

Macrophage-inducible C-type lectin Mincle expressing dendritic cells contribute to control splenic *Mycobacterium bovis* BCG infection in mice

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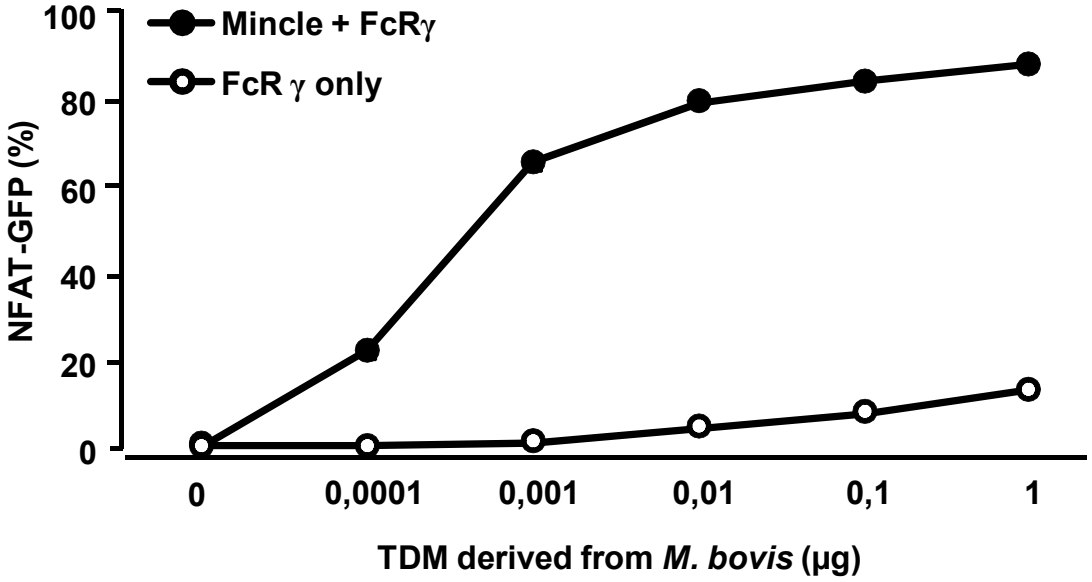

Figure S1: *M. bovis* derived TDM is a ligand for Mincle. NFAT-GFP reporter cells expressing FcRγ only (FcRγ), or Mincle + FcRγ were stimulated with the indicated amounts of plate-coated TDM derived from *M. bovis* for 18 h. Subsequently, cells were washed off the plates and induction of NFAT-GFP (1) was analyzed by flow cytometry. Data are shown as mean values of triplicate determinations, and the experiment was repeated two times with similar results.

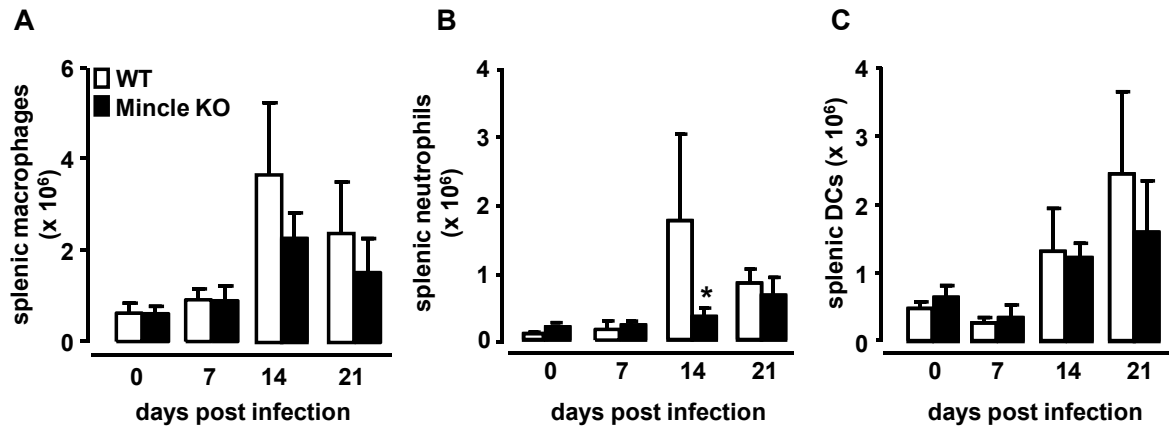

Figure S2: Quantification of splenic macrophages, neutrophils, and DC after intravenous *M. bovis* BCG infection of WT and Mincle KO mice. WT and Mincle KO mice were intravenously infected with *M. bovis* BCG ($8x10^5$ CFU/mouse) and at indicated time points, numbers of splenic phagocytes were determined. Values are shown as mean ± SD of n = 3-5 mice per time point and treatment group. * $p < 0.05$ (Mann-Whitney U test).

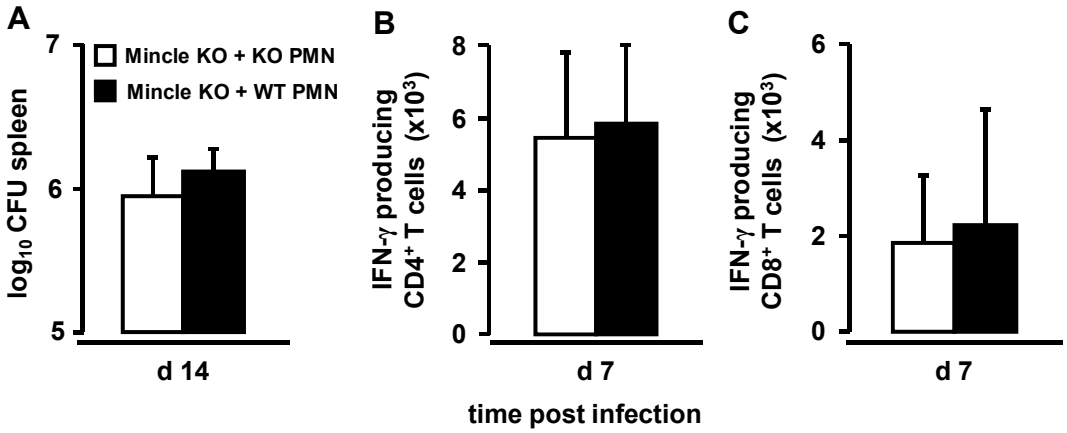

Figure S3: Effect of adoptive transfer of bone marrow neutrophils on splenic antimycobacterial immunity in *M. bovis* BCG infected Mincle KO mice. Mincle KO mice were intravenously infected with *M. bovis* BCG ($8x10^5$ CFU/mouse) and on day 0, 3, and 5 of infection, mice received bone marrow-derived WT or Mincle KO neutrophils ($5x10^6$ cells/mouse i.v.). **(A)** On day 14 post infection, mycobacterial loads were determined in the spleens of Mincle KO mice transfused with either Mincle KO neutrophils (white bars), or WT neutrophils (black bars). **(B, C)** On day 7 post infection, splenic IFN-γ producing $CD4^+$ T cells and $CD8^+$ T cells were determined, as indicated. Values are shown as mean ± SD of n = 3-6 mice per time point and treatment group. The data are representative of at least two independently performed experiments.

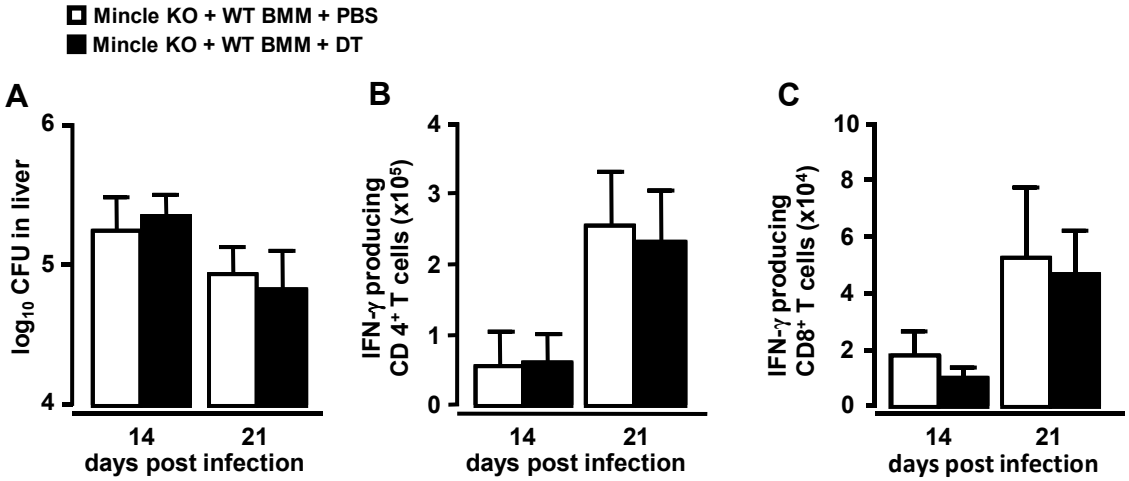

Figure S4: Effect of adoptive transfer of WT bone marrow-derived monocytes on hepatic antimycobacterial immunity in Mincle KO mice. Mincle KO mice were infected intravenously with *M. bovis* BCG ($8x10^5$ CFU/mouse) followed by adoptive transfer of bone marrow-derived monocytes ($5x10^6$ cells/mouse) derived from transgenic $zDC^{+/DTR}$ donor mice. Subsequently, mice were either treated i.p. with PBS or diphtheria toxin, and on day 14 and 21 post infection, hepatic mycobacterial CFU (**A**), or numbers of IFN-γ producing $CD4^+$ T cells (**B**) or $CD8^+$ T cells (**C**) were determined. The data are shown as mean ± SD of n = 3-6 mice per time point and treatment group. The experiment was repeated two times with similar results.

References

1. **Miyake Y, Toyonaga K, Mori D, Kakuta S, Hoshino Y, Oyamada A, Yamada H, Ono K, Suyama M, Iwakura Y, Yoshikai Y, Yamasaki S.** 2013. C-type lectin MCL is an FcRγ-coupled receptor that mediates the adjuvanticity of mycobacterial cord factor. Immunity **38:**1050-1062.