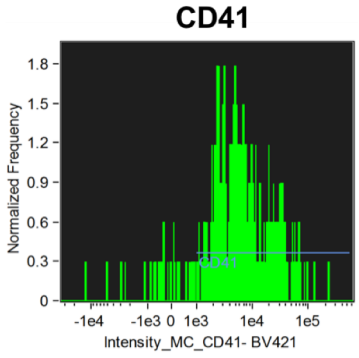
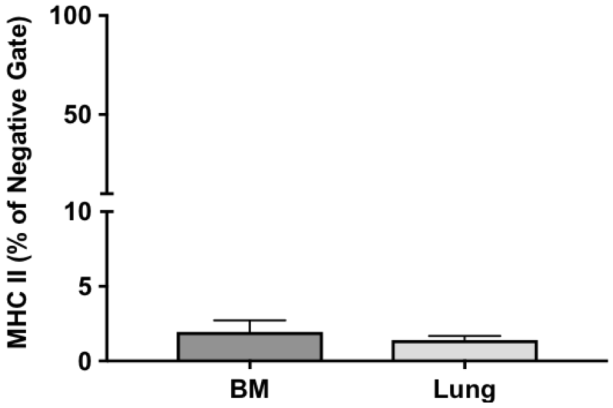
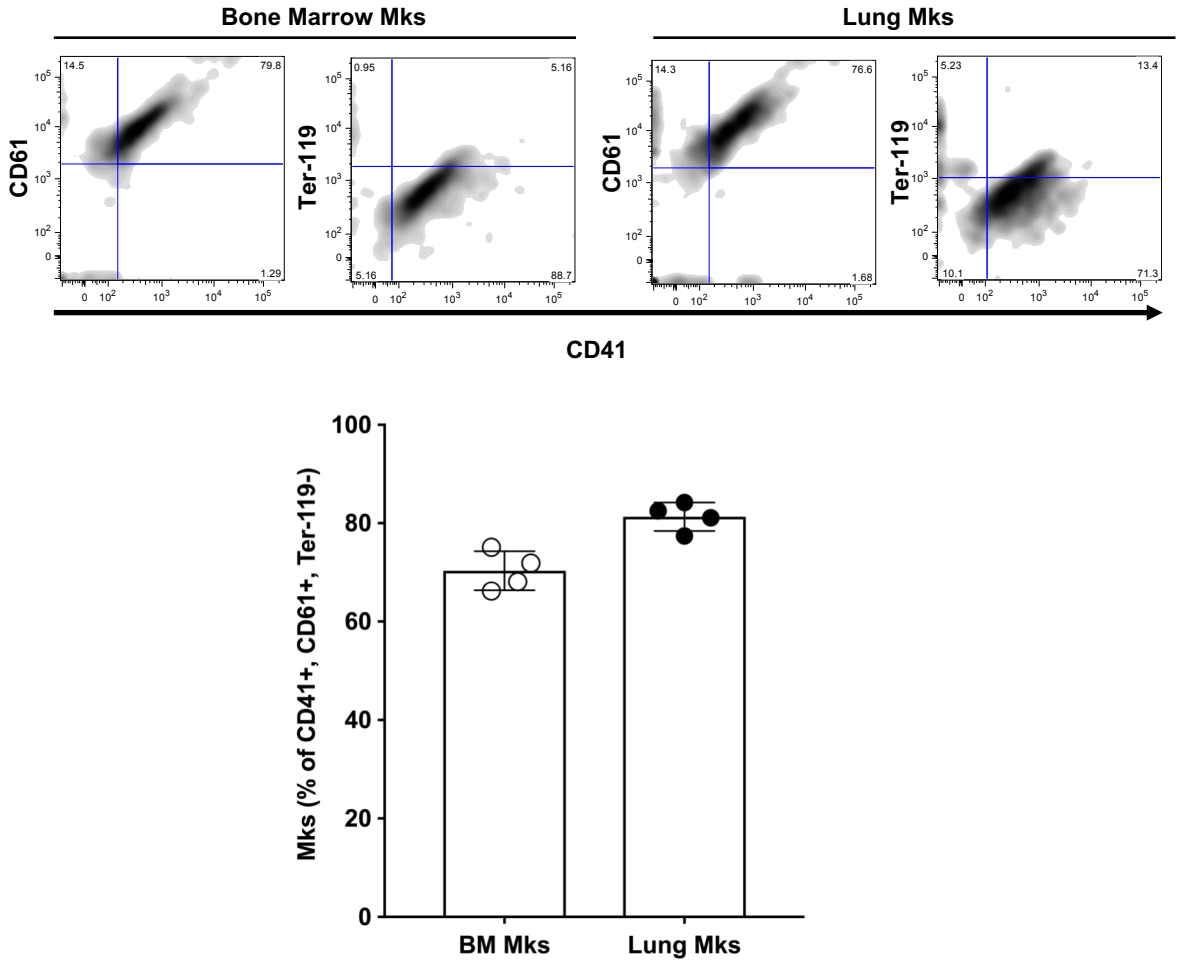


Intensity_MC_CD41- BV421

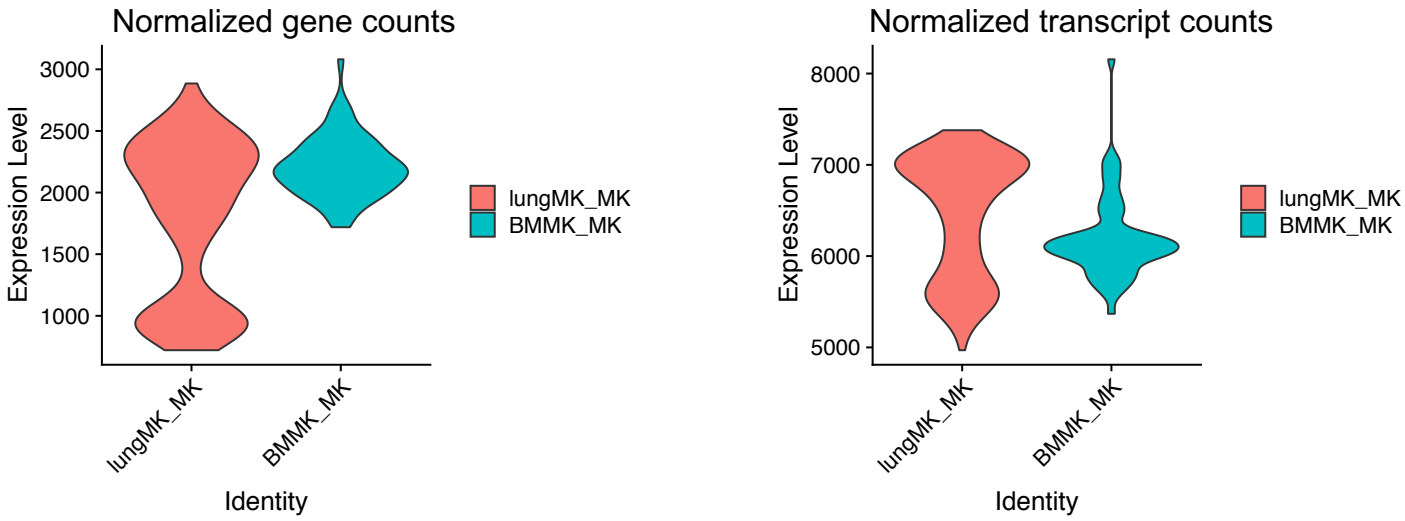
Population	Count	%Gated
Single Cells & Focus	337	100
CD41 & Single Cells & Focus	309	91.7



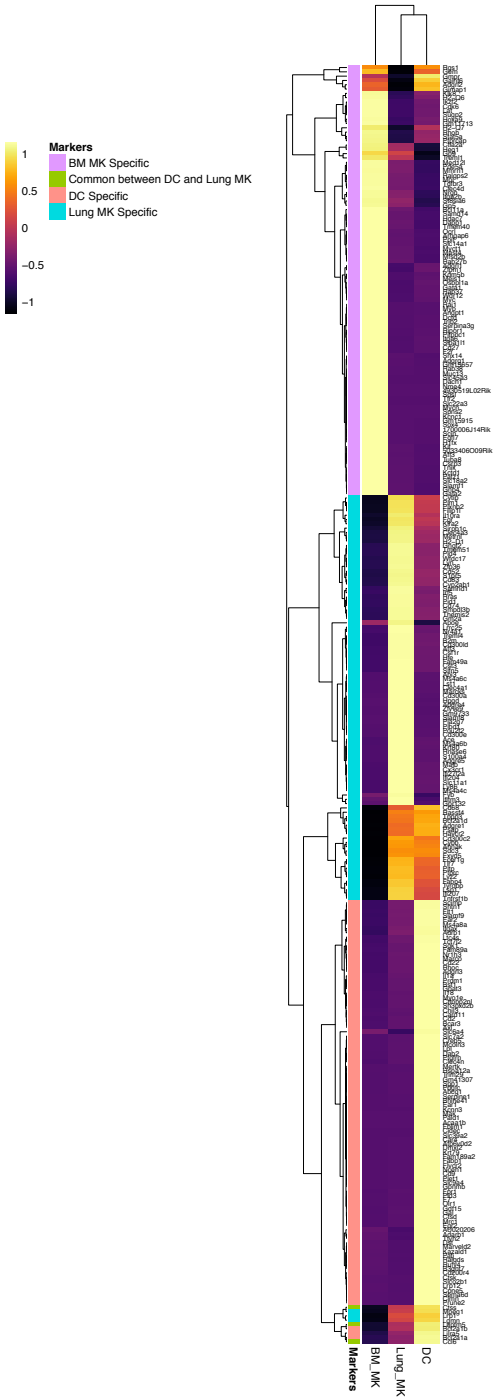
Supplementary S1. ImageStream confirmation of Mk isolation purity. Remaining non-Mk cells do not express MHC II.



Supplementary S2. Isolated Mks from the lung and BM of naïve mice were cultured and stained for CD41+, CD61+, and Ter-119-.



Supplementary S3: BM Mks express relatively more genes but similar number of transcripts compared to Lung Mks.

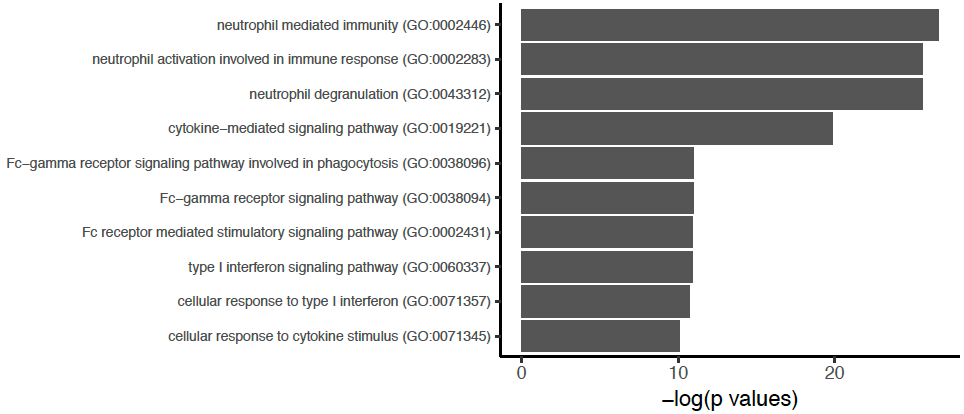


Supplementary S4. Lung and BM Mks are phenotypically and transcriptomically distinct.

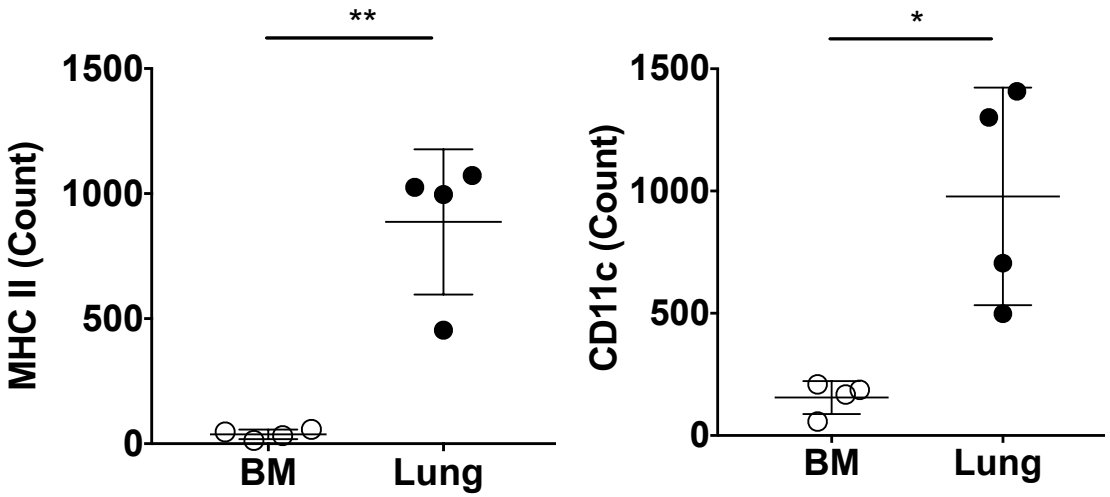
Heatmap showing cluster average expression of the top 100 markers for BM Mk, lung Mks, and monocyte lineage cells. Hierarchical clustering indicates a more similar profile between lung Mks and monocyte linage cells, compared to that of BM Mks.

BM Mks Enriched Pathways

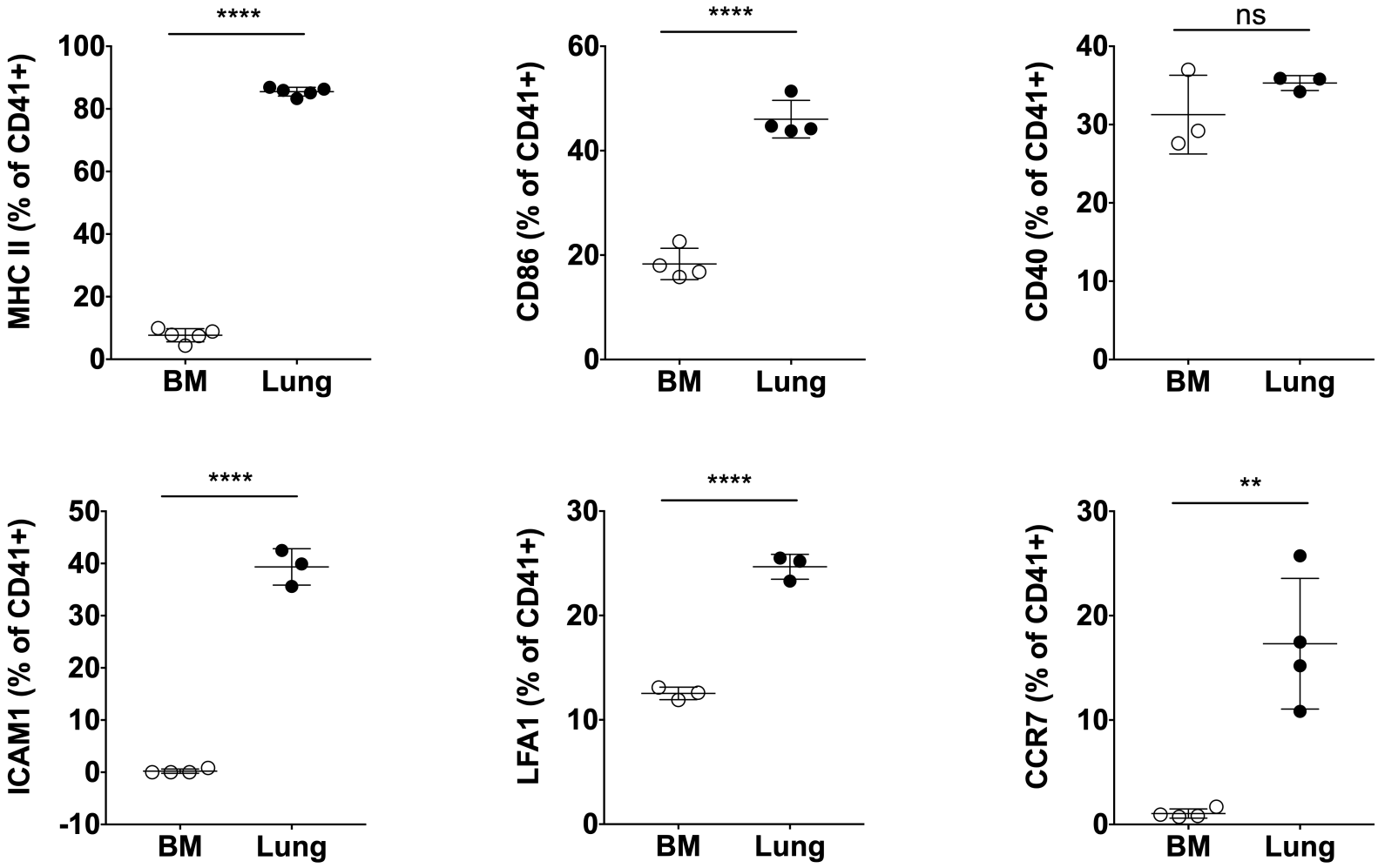
Lung Mks Enriched Pathways



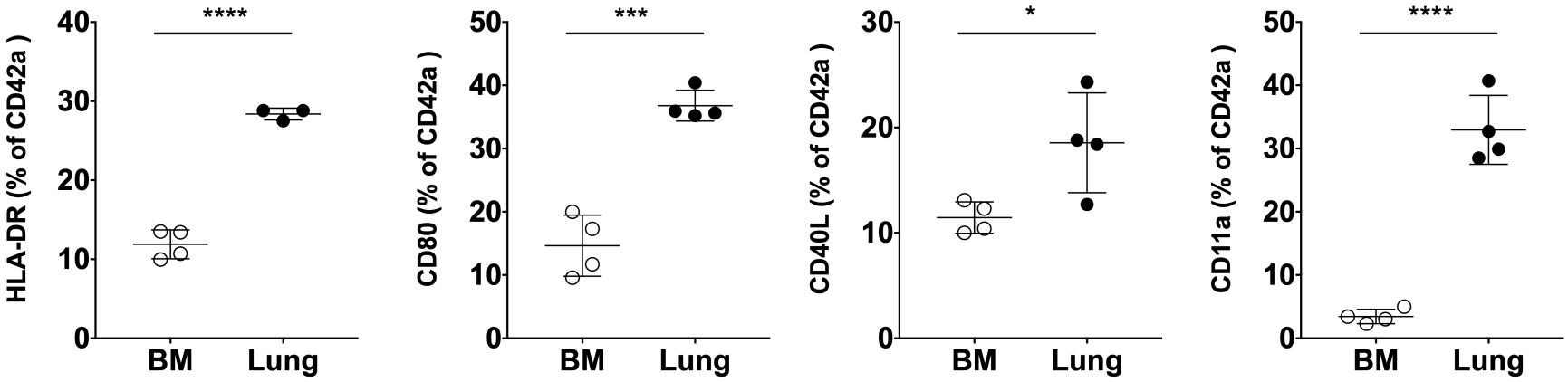
Supplementary S5: Pathway enriched for BM Mks or Lung Mks specific markers.



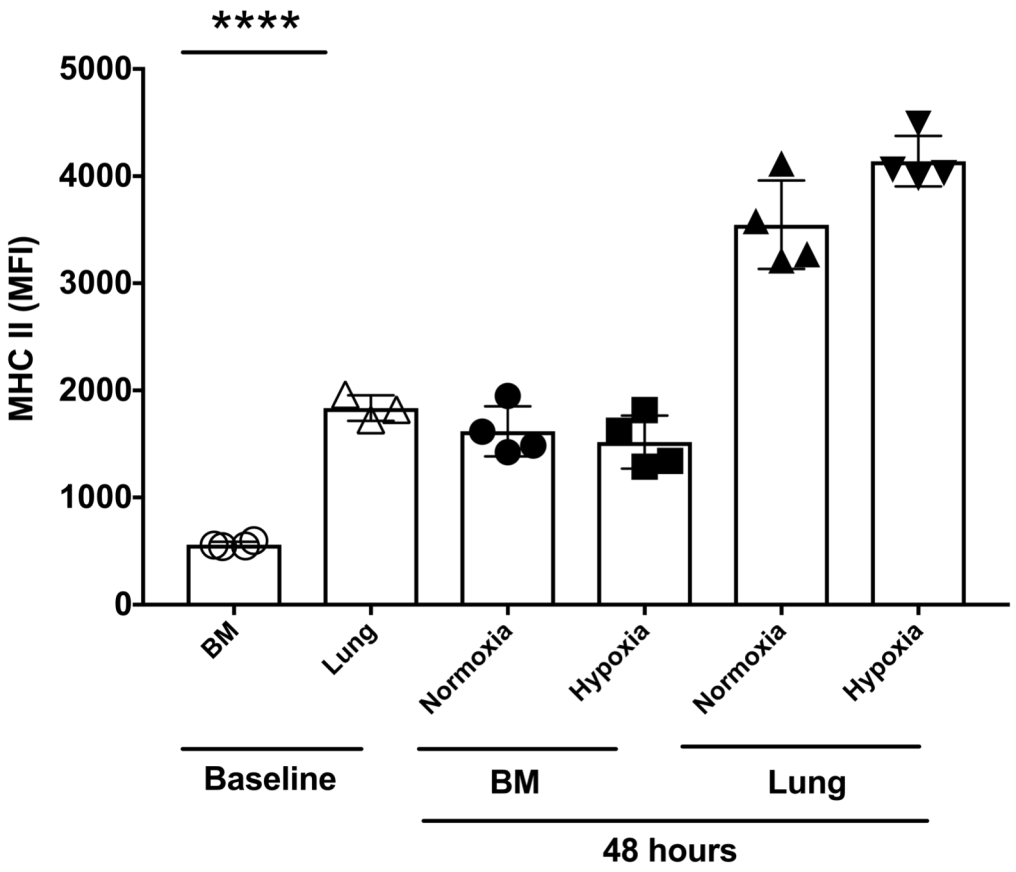
Supplementary S6. ImageStream data quantification of (*P<0.05).



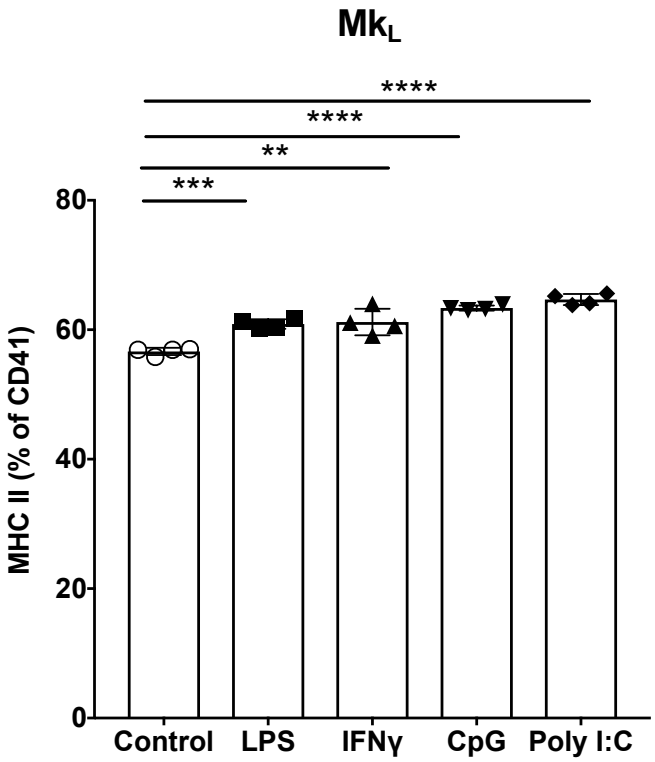
Supplementary S7. Mouse. Lung Mks expressed more MHC II and APC associated molecules compared to BM Mks (± SEM, *P<0.05).



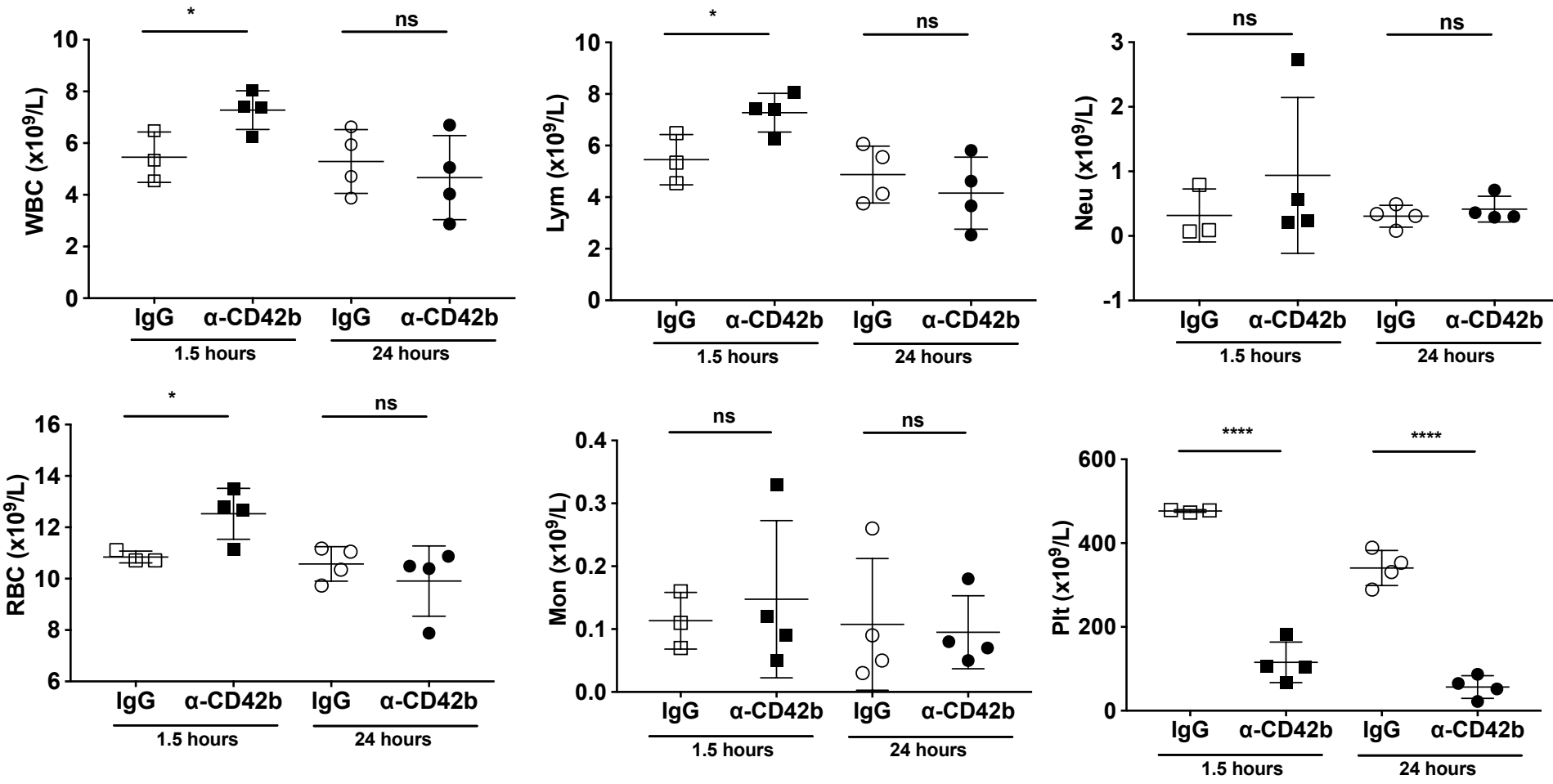
Supplementary S8. Primate. Lung Mks expressed more MHC II, and APC associated molecules compared to BM Mks (± SEM, *P<0.05).



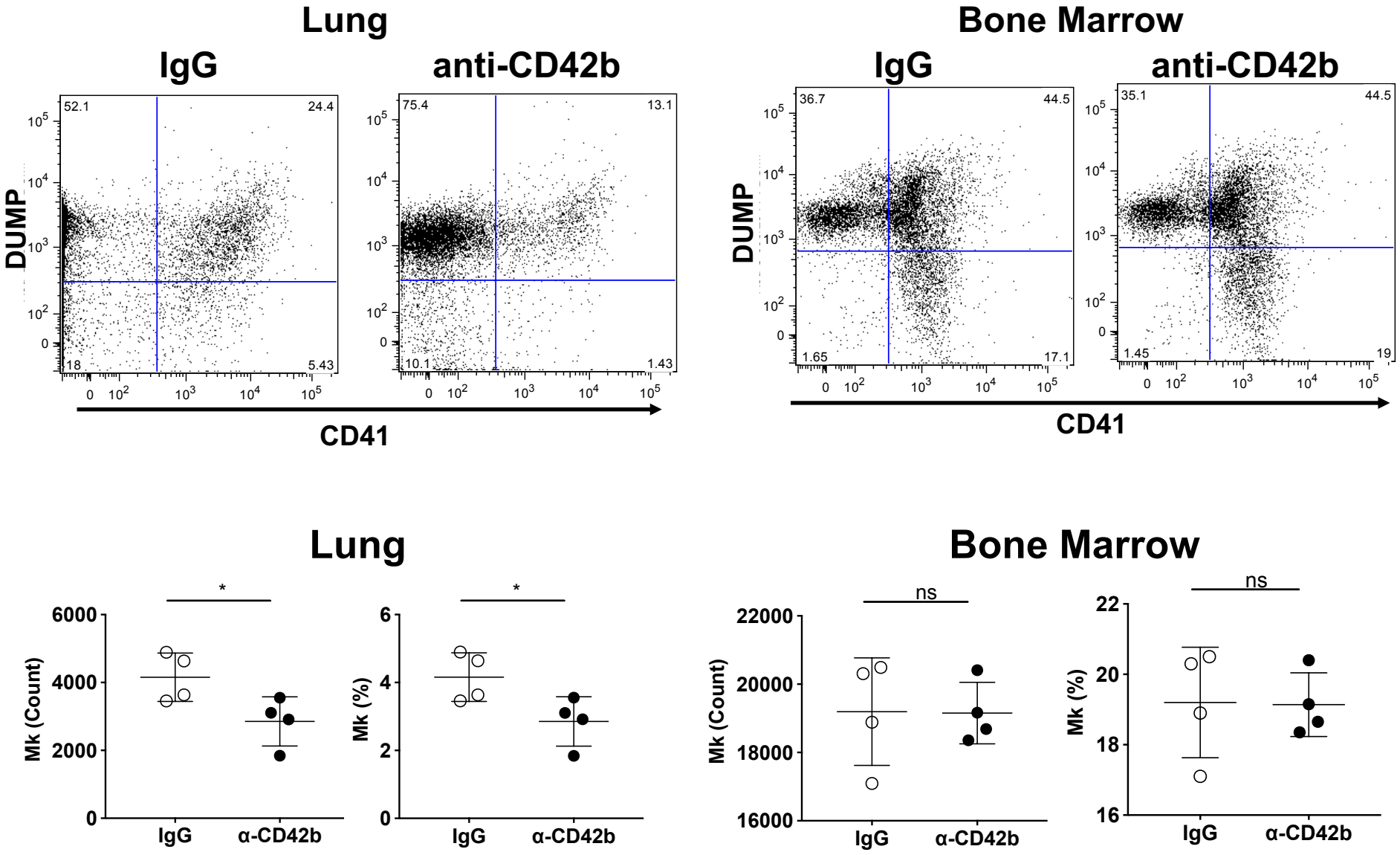
Supplementary S9. Mk MHC II expression is not O_2 responsive. BM and lung Mks were isolated and baseline MHC II expression determined. Mks were then cultured in normoxic or hypoxic (10% O_2) conditions for 48 hrs and MHC II expression determined. Hypoxia had no effect on BM Mk MHC II expression.



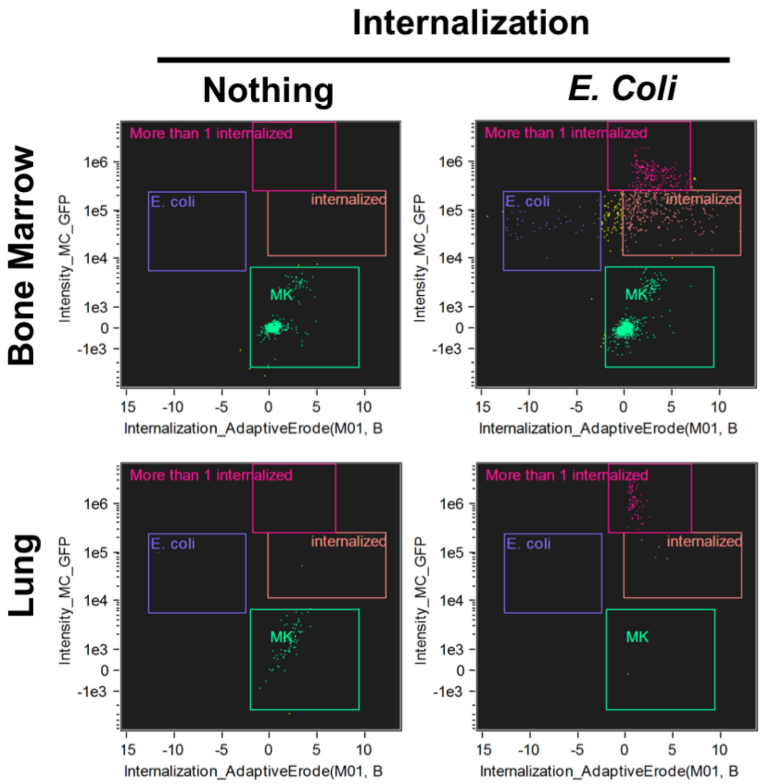
Supplementary S10. Lung Mks were incubated with immune stimuli for 48 hrs and MHC II expression determined (± SEM, *P<0.01 vs Control).



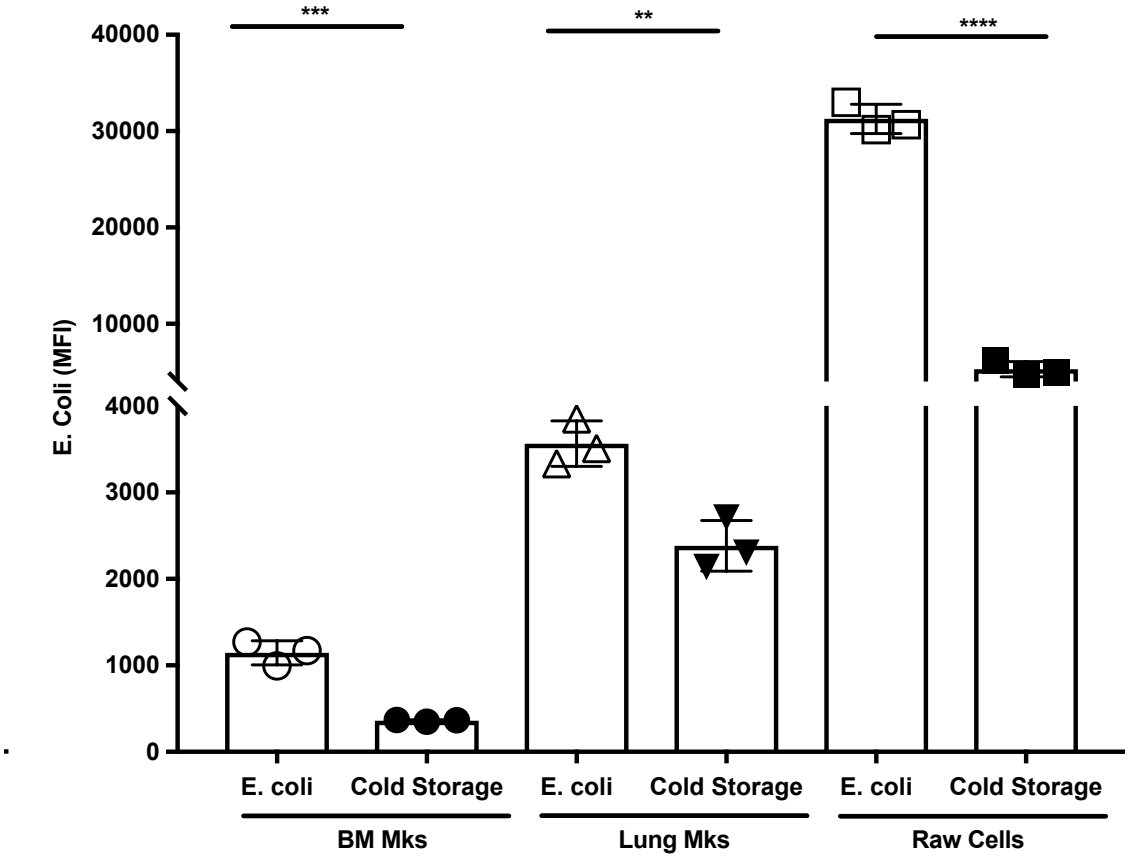
Supplementary S11. Anti-CD42b antibody depletes platelets. Mice were treated with 2 µg/g of platelet depleting antibody and 24 hrs later the complete blood count was taken to determine the platelet count.



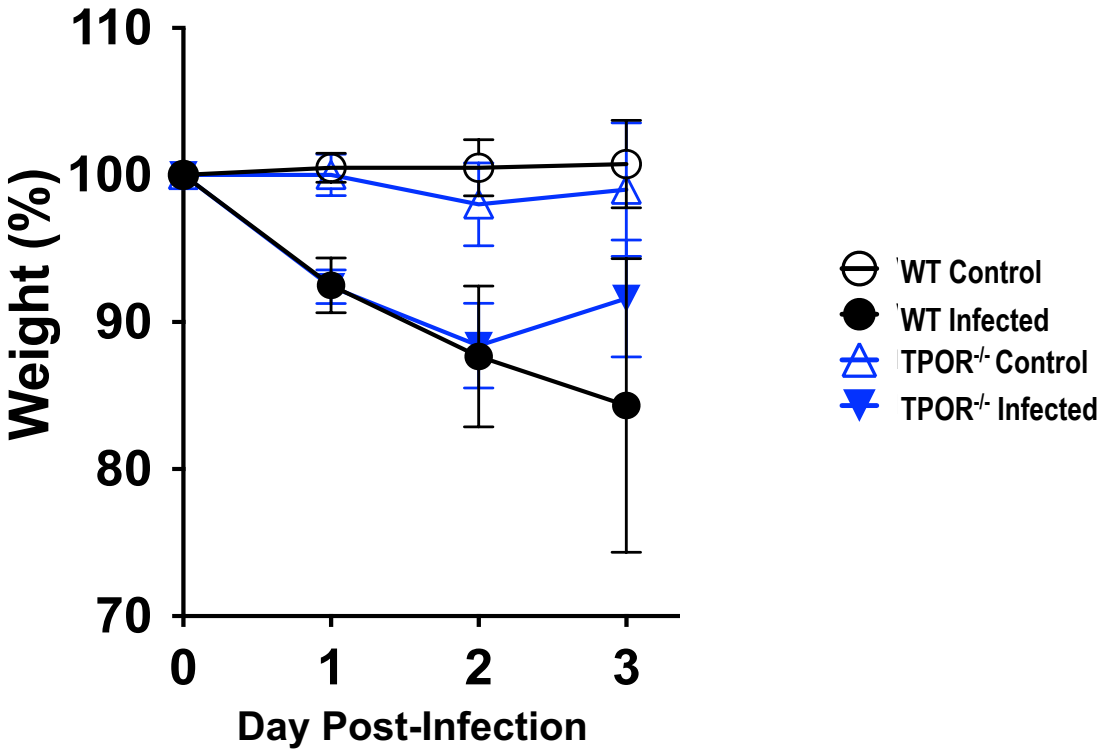
Supplementary S12. Anti-CD42b antibody depletes lung Mks. Mice were treated with 2 µg/g of platelet depleting antibody and 24 hrs later the number of lung and BM Mks quantified.



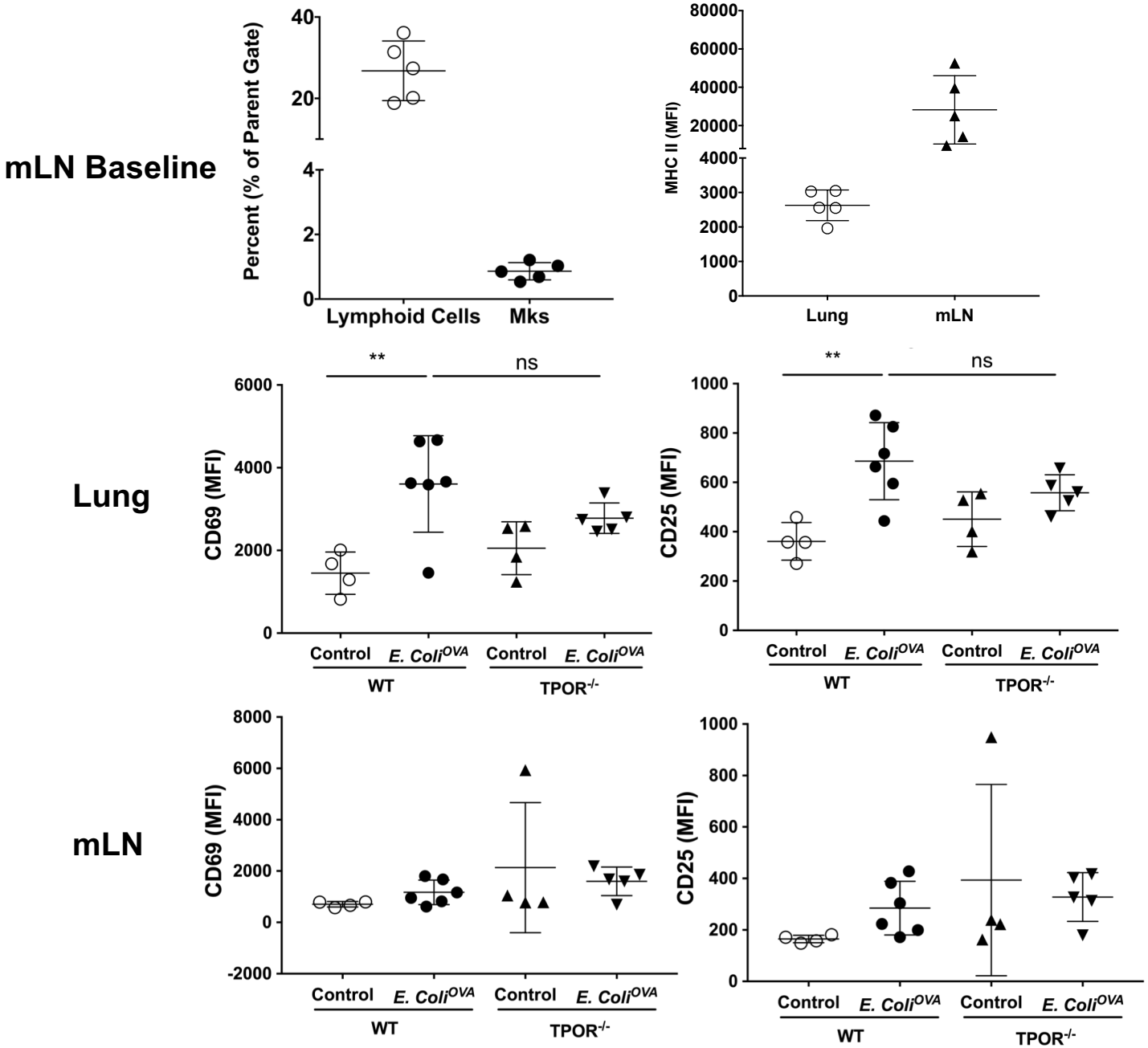
Supplementary S13. Lung Mks are more phagocytic than BM Mks as determined using Imagestream. BM and lung Mks were incubated with control buffer or GFP-*E Coli* and 30 mins.



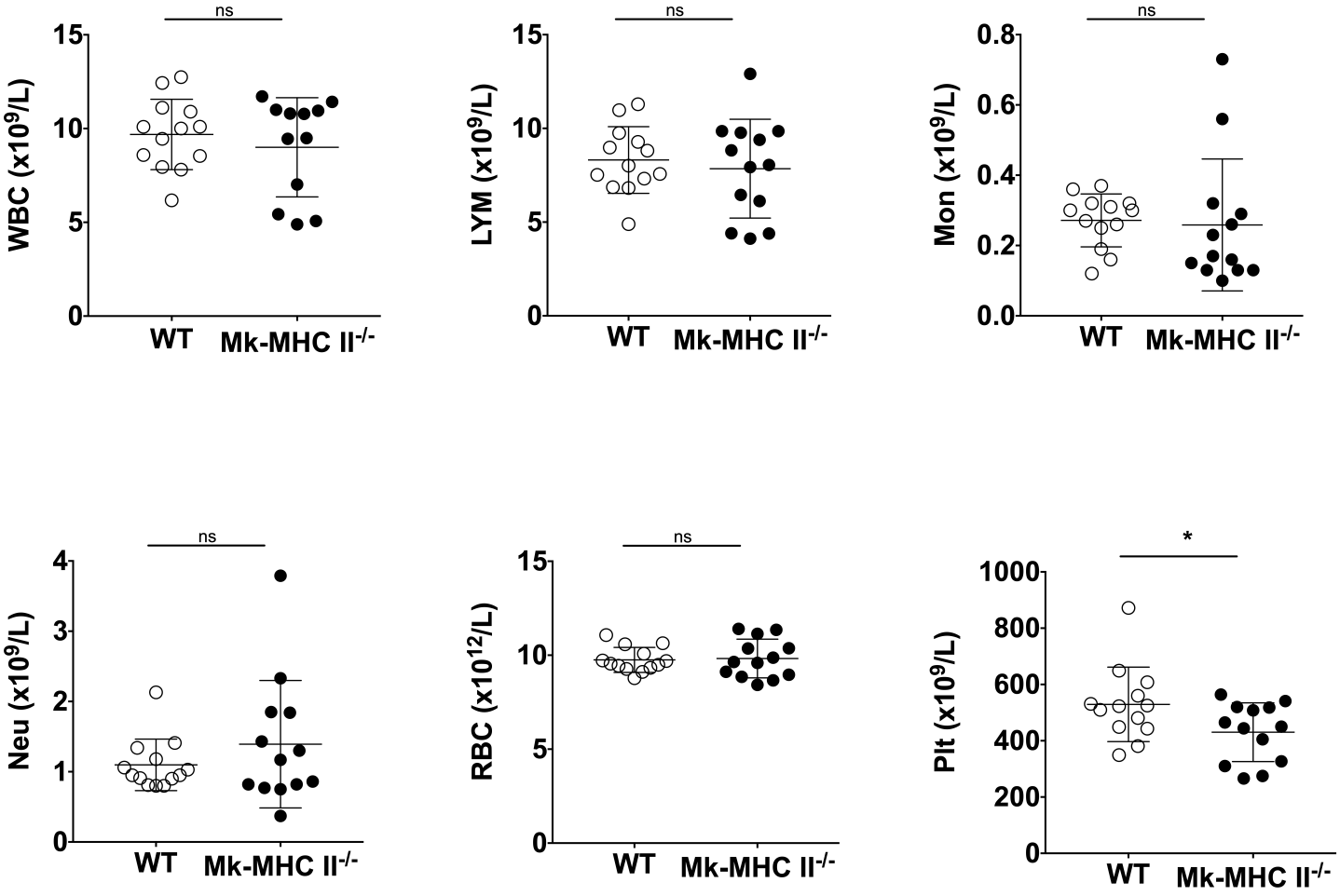
Supplementary S14. pHrodoGreen bacteria were incubated with isolated Mks or RAW cells for 2 hours in 37˚C or in ice.



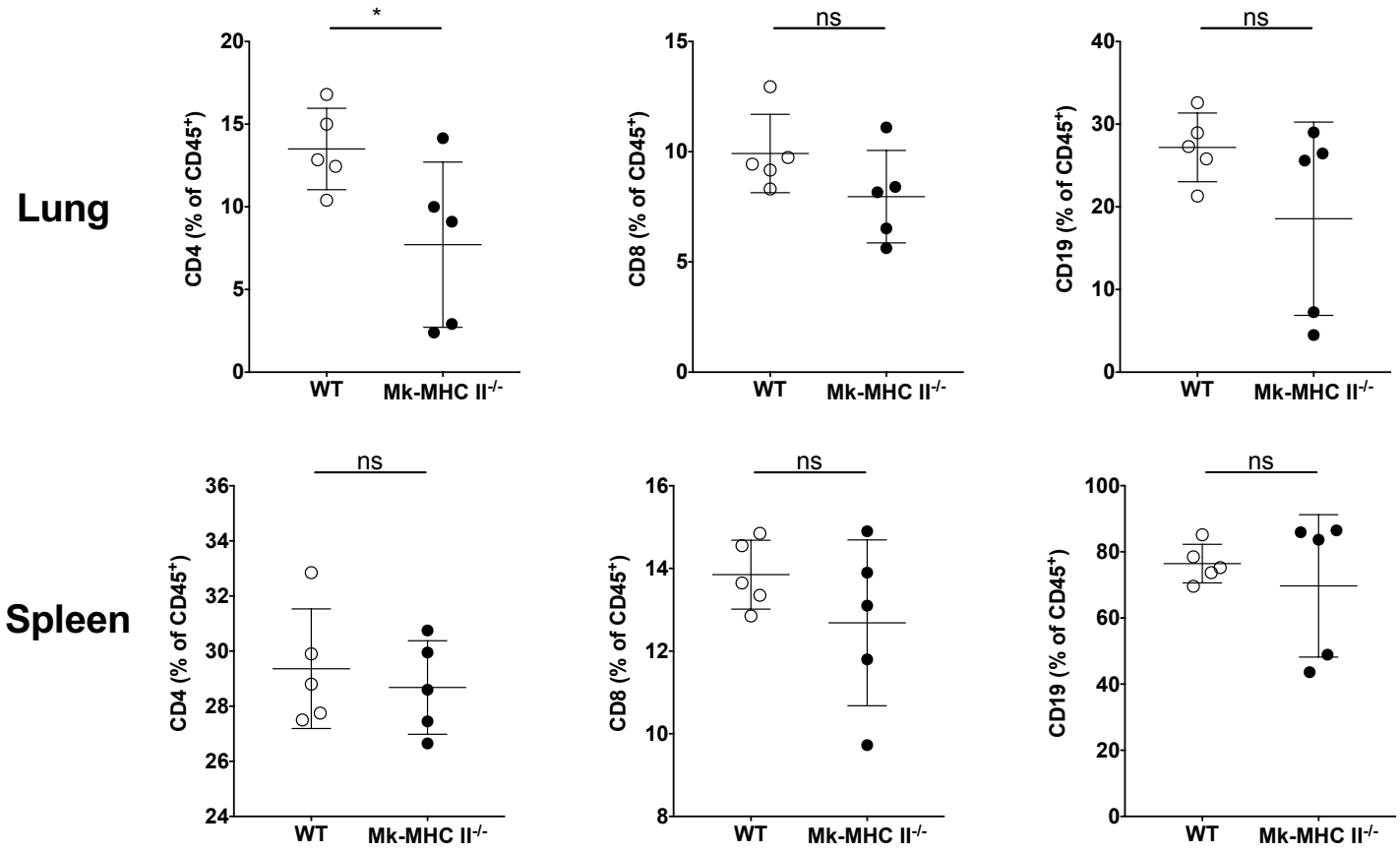
Supplementary S15. WT and TPOR$^{-/-}$ mice post-infection weight loss (± SEM, *P<0.05).



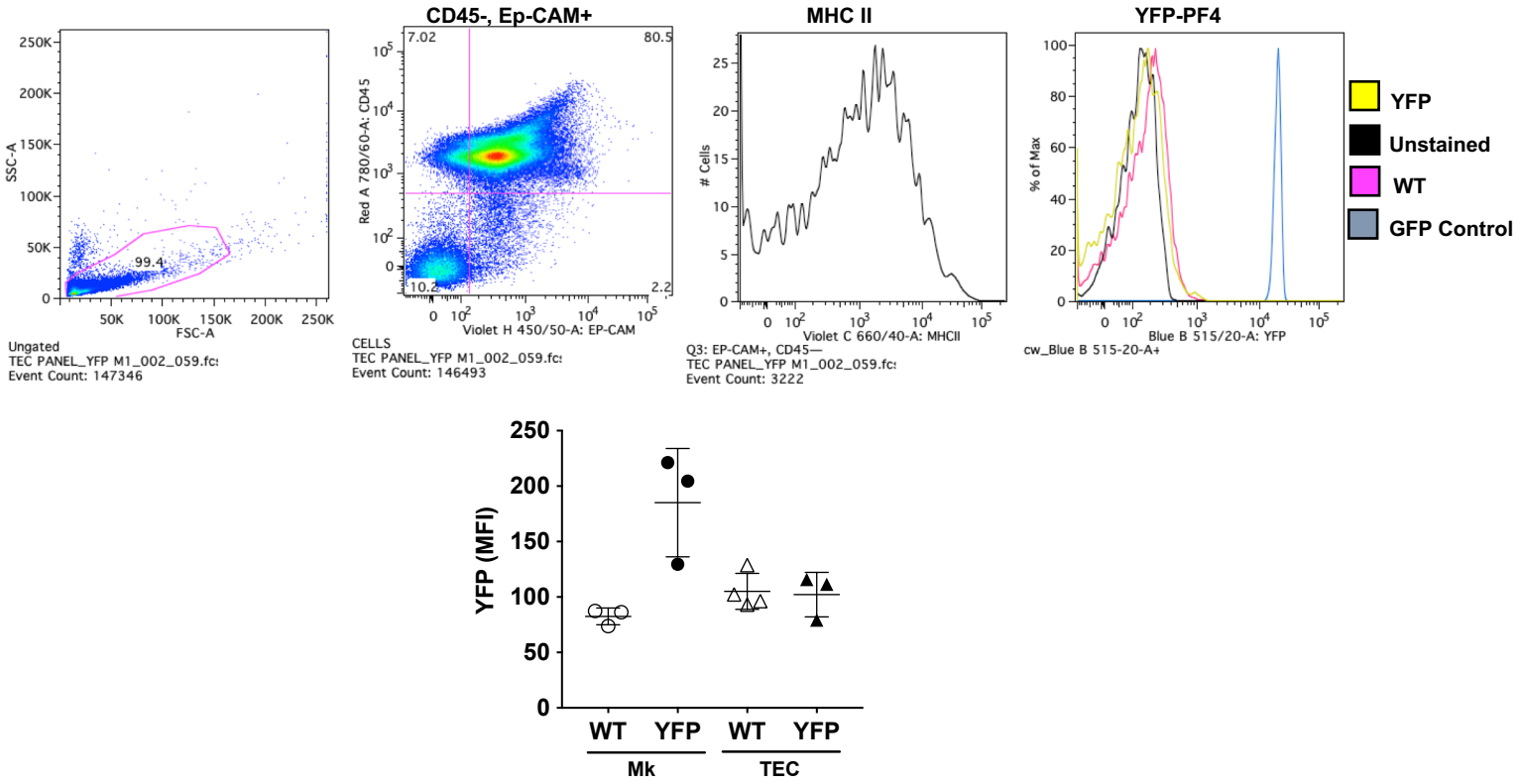
Supplementary S16. Mediastinal lymph nodes (mLN) contain Mks that highly express MHC II. Mice lacking Mks did not mount an antigen specific T cell response (± SEM, *P<0.05).



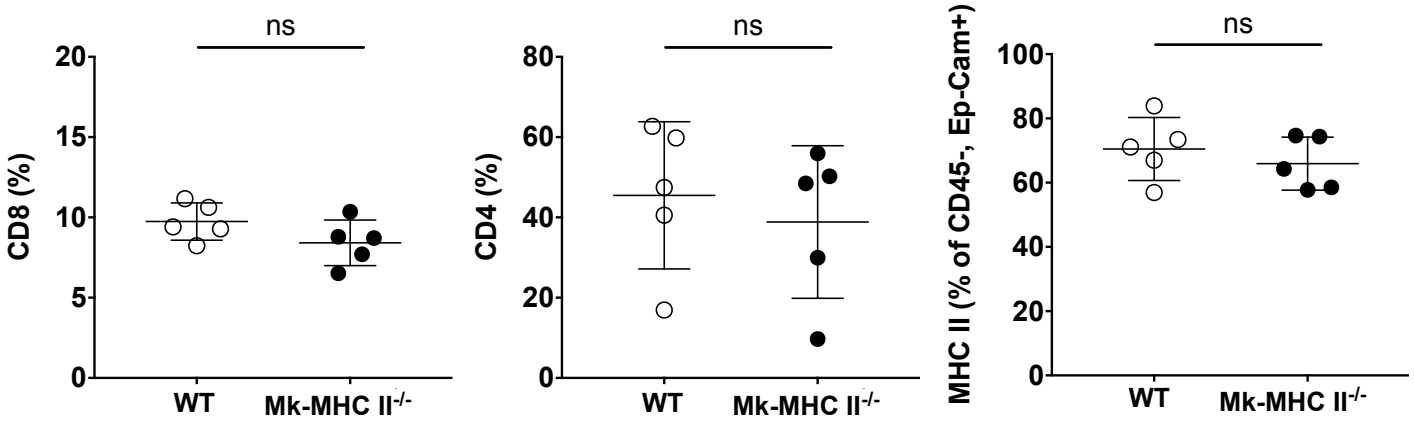
Supplementary S17. Mk-MHC $II^{-/-}$ phenotype. Peripheral blood complete blood count of WT and Mk-MHC $II^{-/-}$ mice (± SEM, *P<0.05).



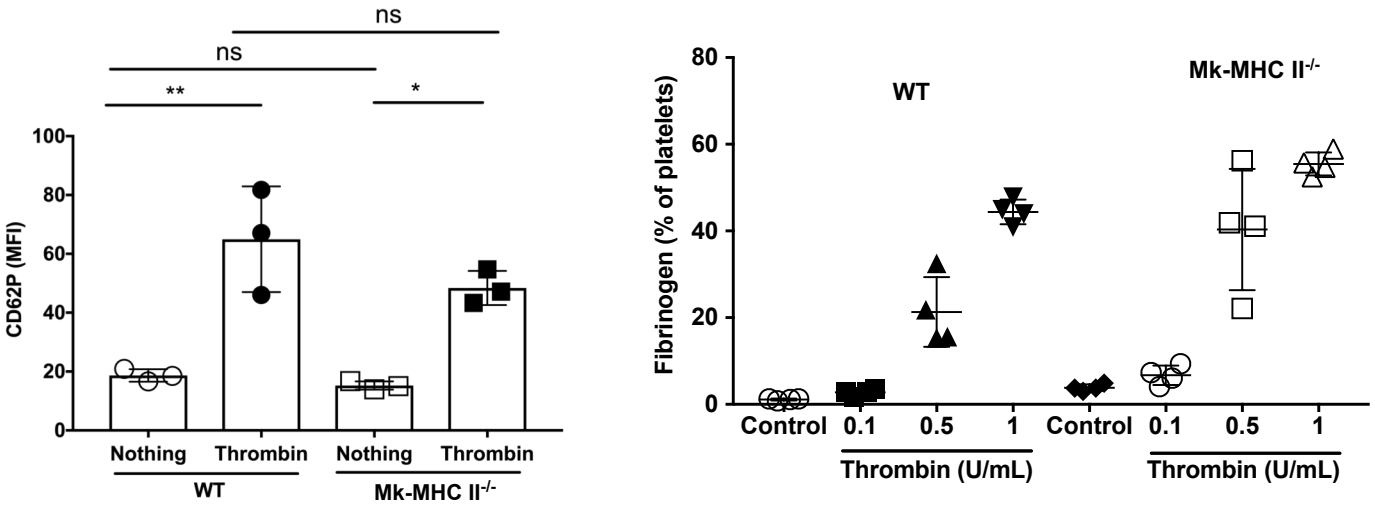
Supplementary S18. T and B cells in WT and Mk-MHC II$^{-/-}$ mice.



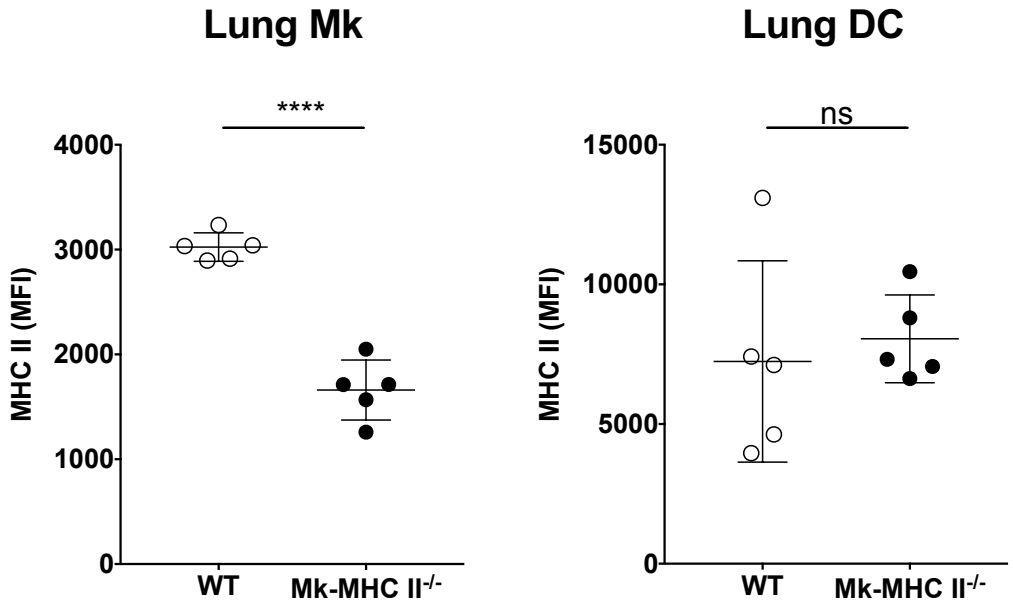
Supplementary S19. Thymic epithelial cells (TEC) do not express PF4. The thymus from PF4^{cre}-eYFP$^{flx/flx}$ mice were isolated and cell fluorescence determined by flow cytometry. Lung Mks, but not TEC were YFP positive.



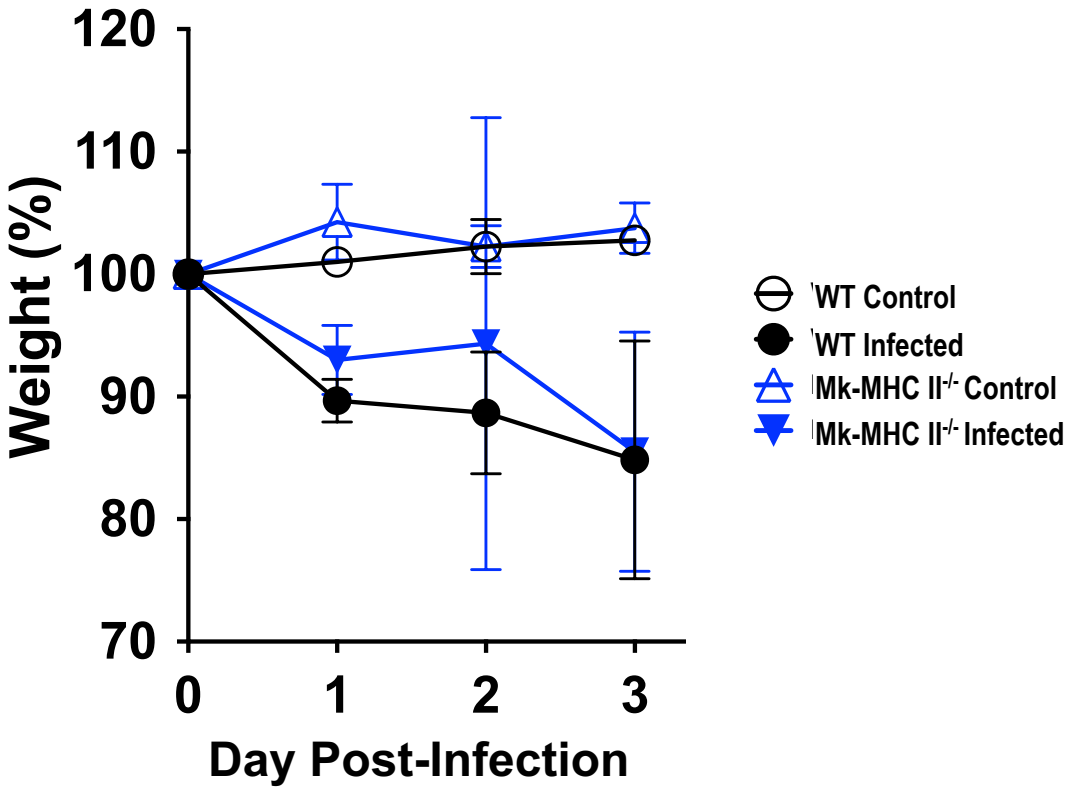
Supplementary S20. WT and Mk-MHC II$^{-/-}$ have similar numbers of thymic T cells and TEC MHC II expression.



Supplementary S21. Platelets from Mk-MHC II$^{-/-}$ mice had normal activation. Washed platelets from WT and Mk-MHC II$^{-/-}$ mice were stimulated with thrombin (1 U/mL). CD62P expression and fibrinogen binding were determined by flow cytometry. Platelets from WT and Mk-MHC II$^{-/-}$ mice had similar platelet activation.

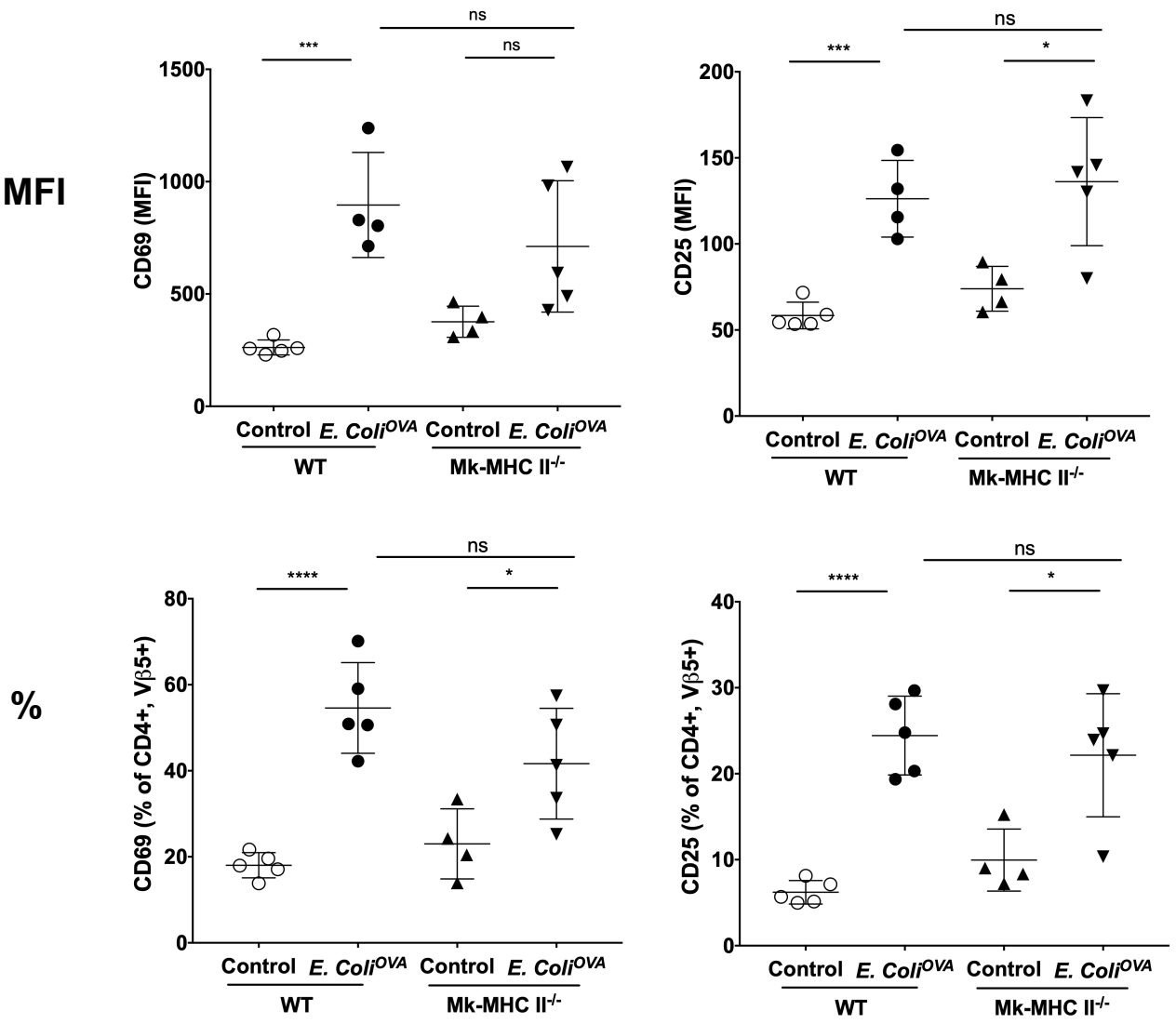


Supplementary S22. MHC II expression on lung Mks and DCs from WT and Mk-MHC $II^{-/-}$ mice. Mks, but not DCs had decreased MHC II expression.

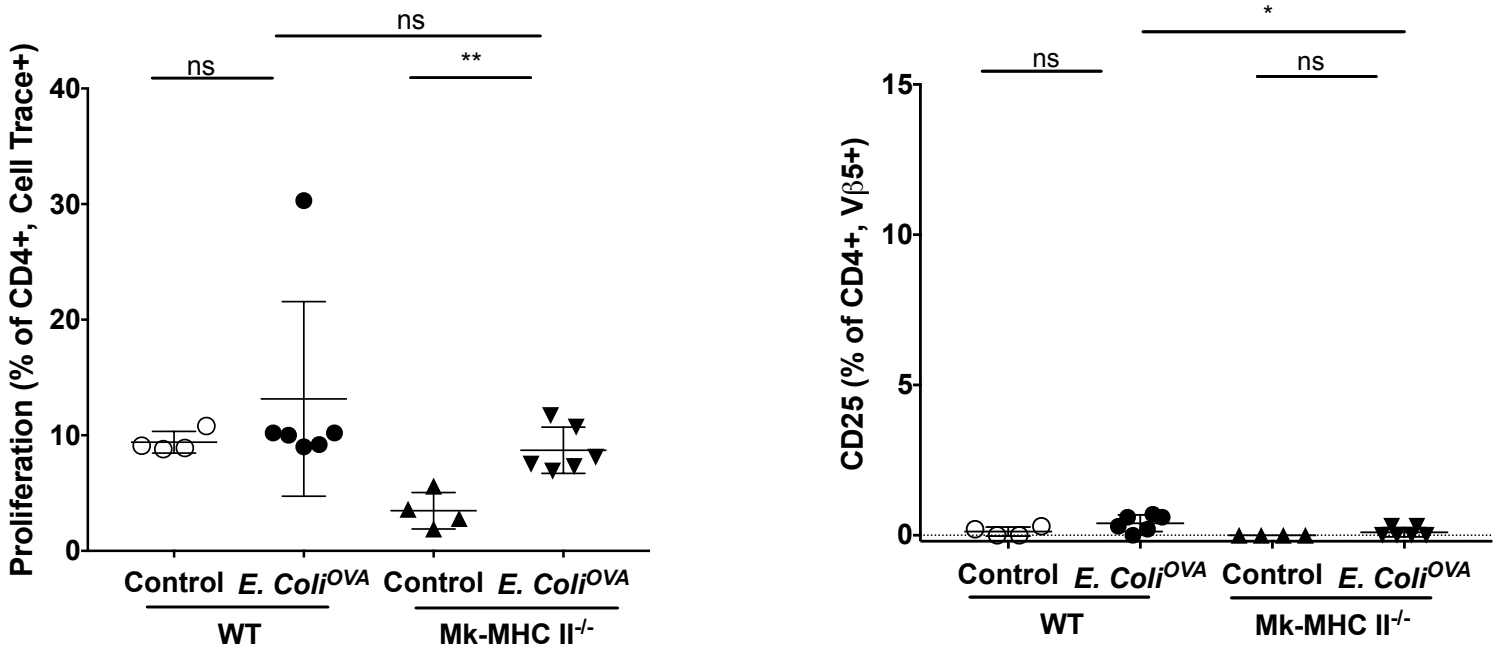


Supplementary S23. WT and Mk-MHC $II^{-/-}$ mice had similar post-infection weight loss (± SEM, *P<0.05).

mLN



Supplementary S24. By D3 Post infection there is no statistically significant difference in T cell activation the mLN between WT and Mk-MHC II$^{-/-}$ mice during *E. coli* infection (± SEM, *P<0.05).



•**Supplementary S25.** The Vβ5- CD4 T cells are not activated show that the Vβ5+ activation is antigen specific (± SEM, *P<0.05).