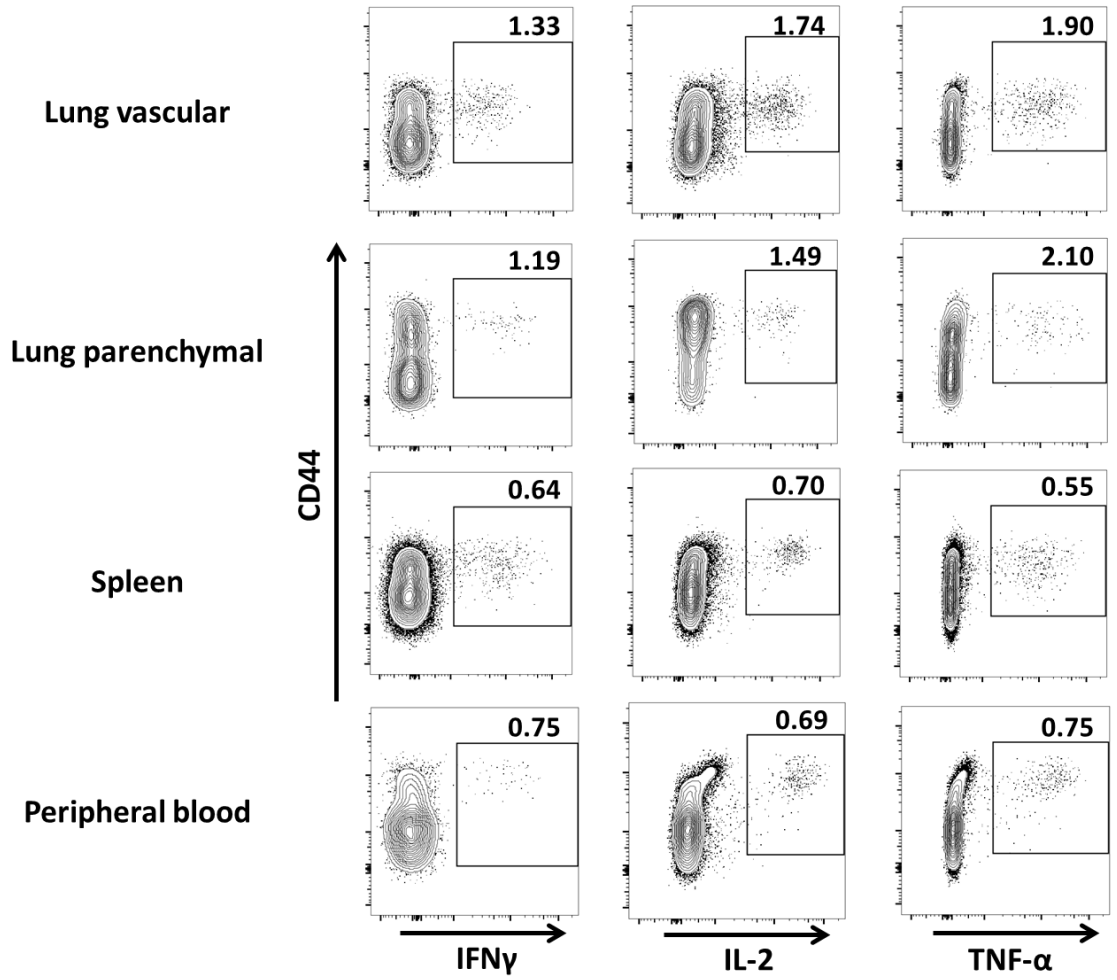
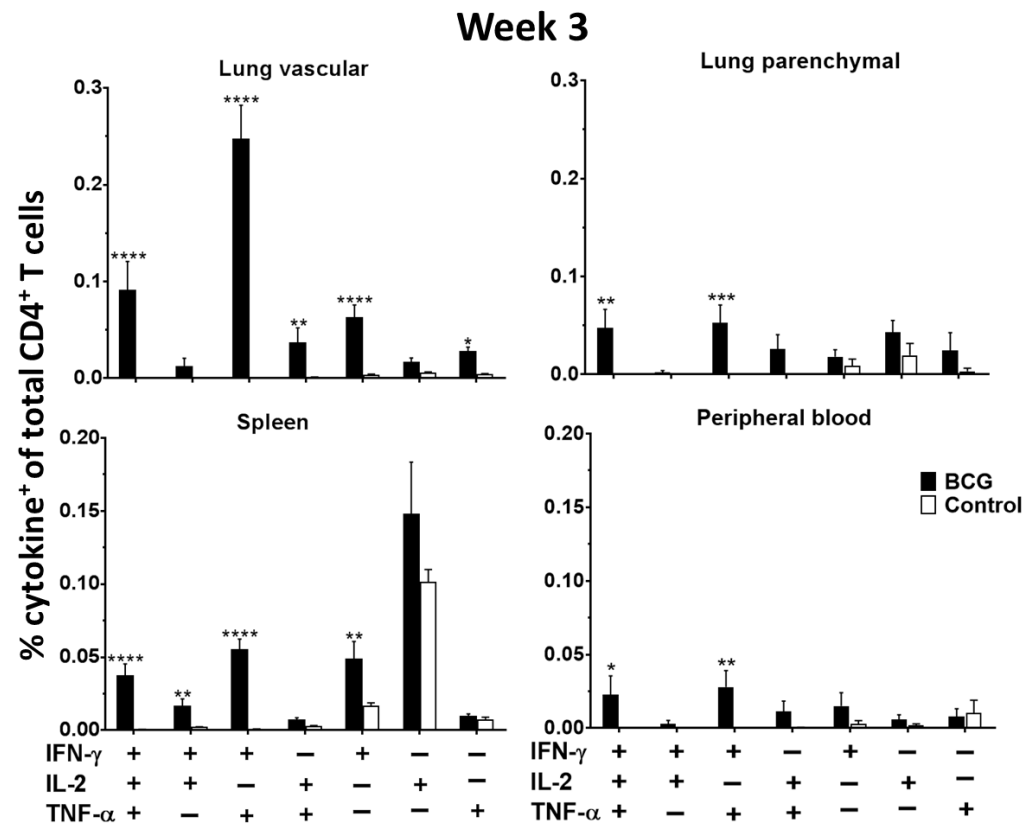
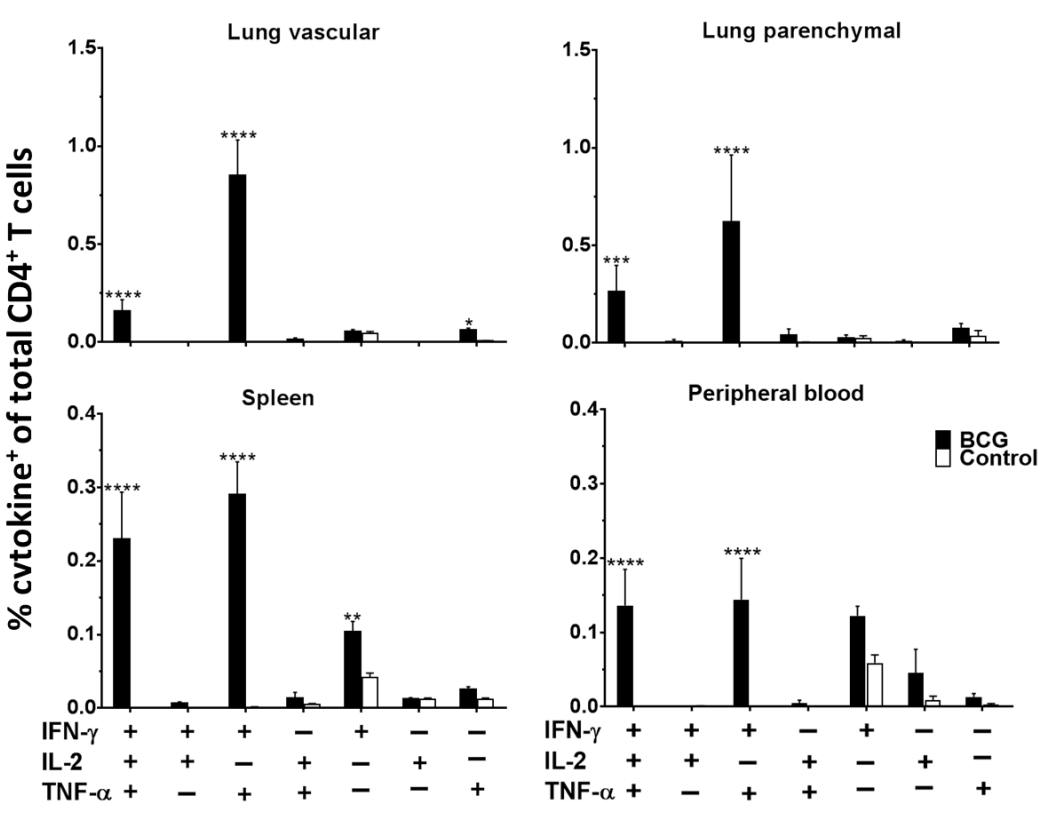
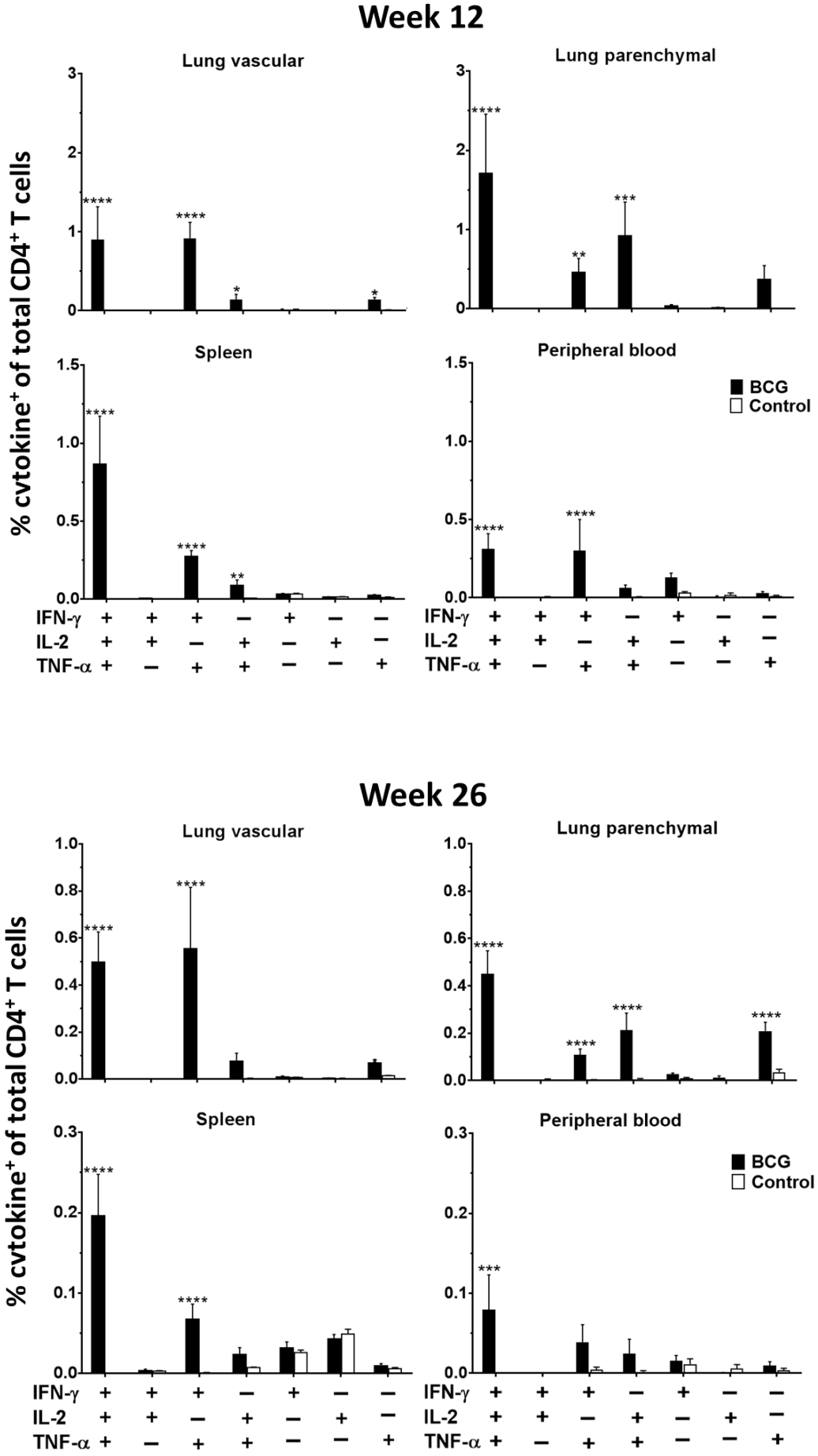
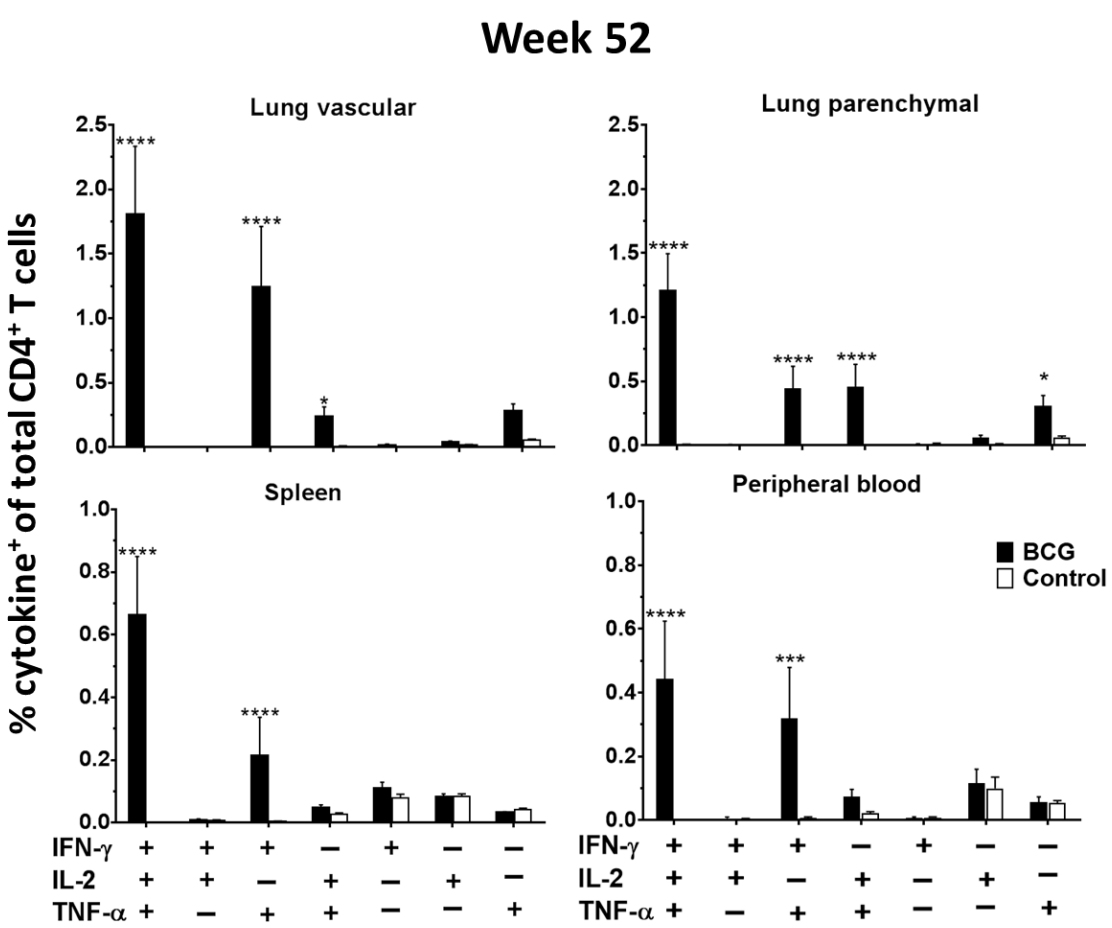


Supplementary Figure 1 Cytokine production by CD4$^+$ T cells in different tissue compartments. Following BCG immunisation, intravascular staining and ICS identified populations of antigen-specific (cytokine$^+$) CD4$^+$ T cells. Representative plots show IFN-γ, TNF-α and IL-2 production from CD4$^+$ T cells in the lung parenchyma, lung vasculature, spleen and peripheral blood of a mouse 5 weeks post-BCG vaccination.

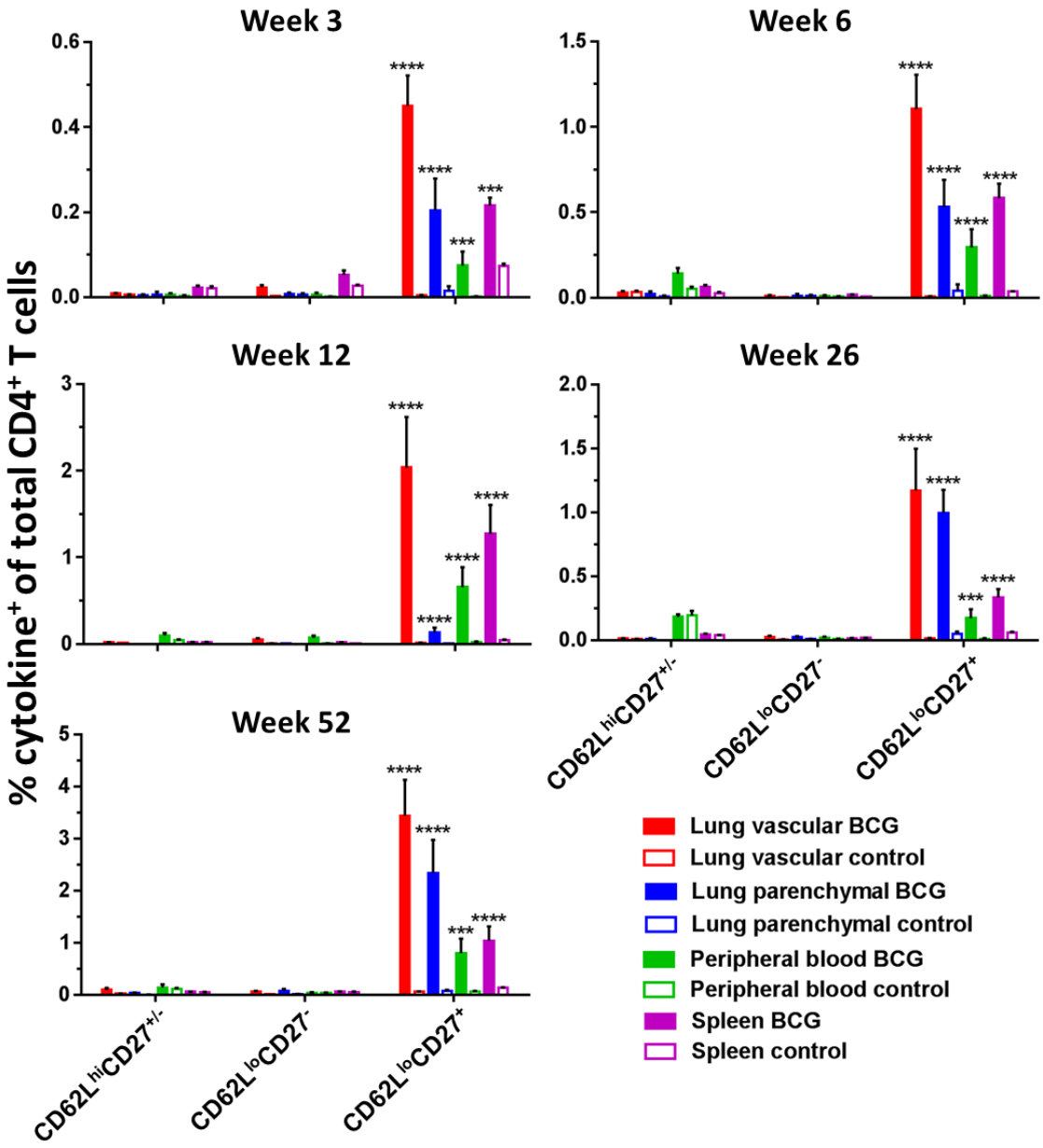

Week 3
Lung vascular
Lung parenchymal
Spleen
Peripheral blood
% cytokine+ of total CD4+ T cells
BCG
Control
IFN-γ
IL-2
TNF-α


Week 6
Lung vascular
Lung parenchymal
Spleen
Peripheral blood
% cytokine+ of total CD4+ T cells
BCG
Control
IFN-γ
IL-2
TNF-α

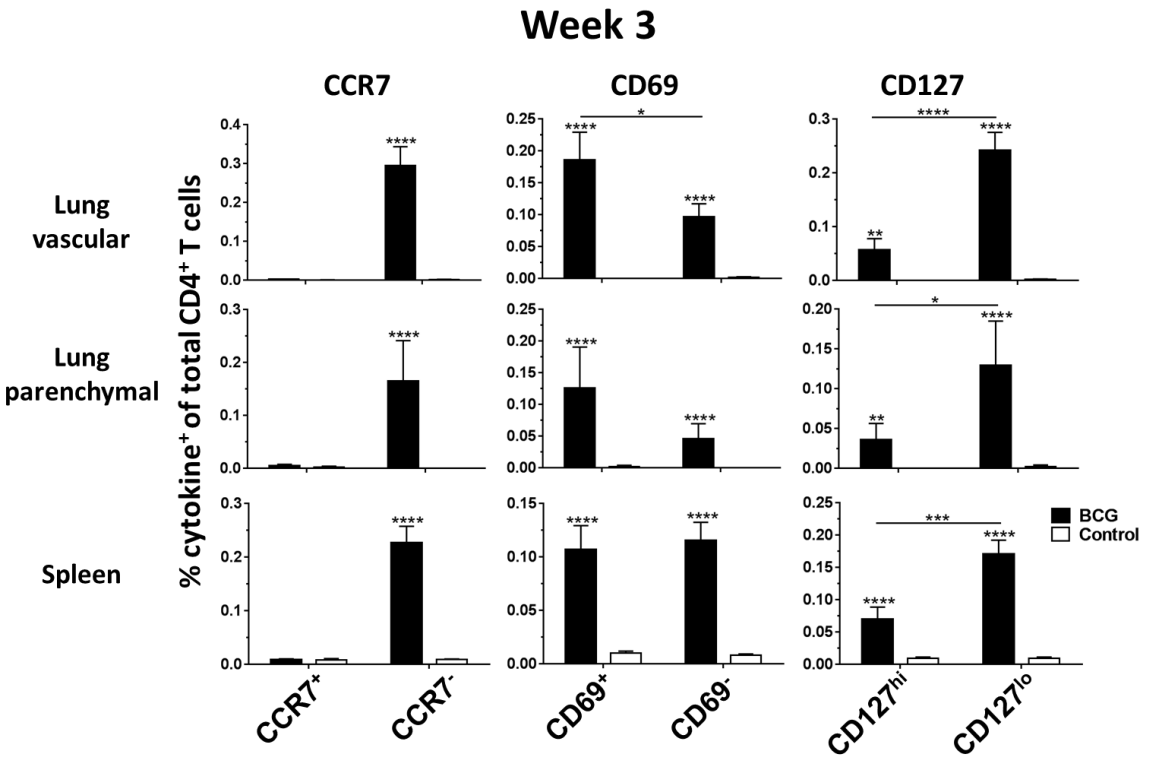

Week 12
Lung vascular
Lung parenchymal
Spleen
Peripheral blood
% cvtokine+ of total CD4+ T cells
BCG
Control
IFN-γ
IL-2
TNF-α
Week 26
Lung vascular
Lung parenchymal
Spleen
Peripheral blood
% cvtokine+ of total CD4+ T cells
BCG
Control
IFN-γ
IL-2
TNF-α

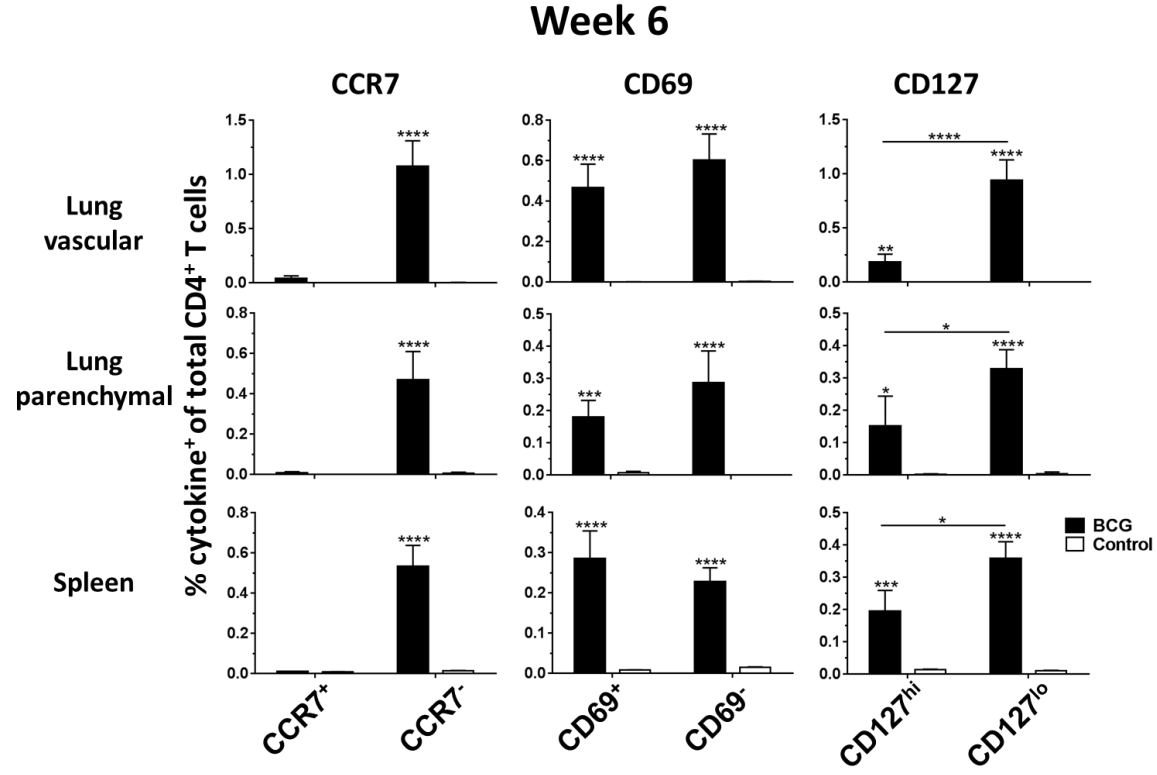


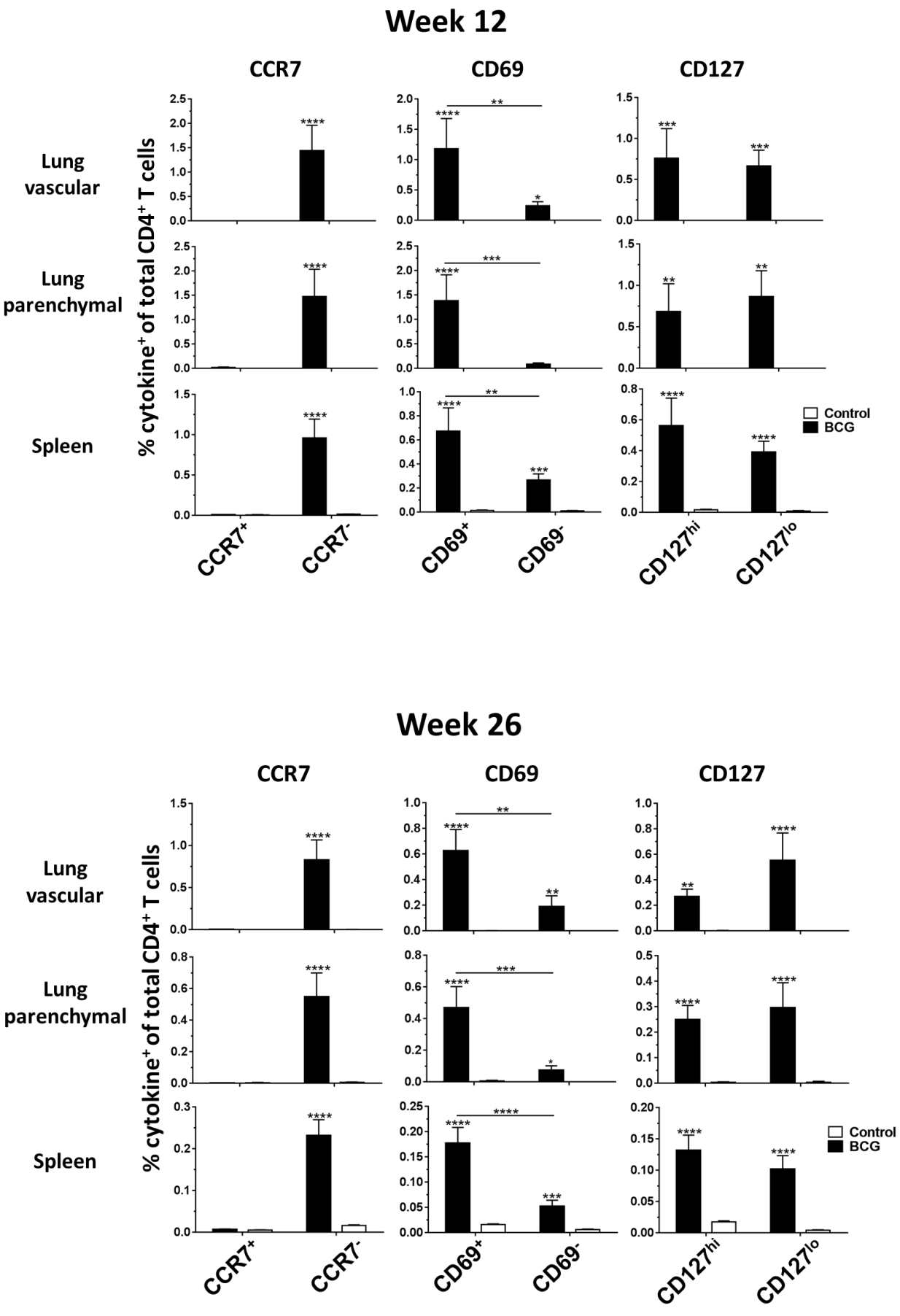
Supplementary Figure 2 Production of IFN-γ, IL-2 and TNF-α by CD4⁺ T cells post-BCG vaccination. Following BCG immunisation, intravascular staining and ICS identified populations of antigen-specific (cytokine⁺) CD4⁺ T cells in the lungs, spleen and peripheral blood producing IFN-γ, IL-2 or TNF-α alone or in combination. Graphs show frequencies of antigen-specific CD4⁺ T cells in all compartments as a % of the total CD4⁺ T cells in the same compartment at each time point. For all graphs, bars represent mean ± SEM (*n*=6). Two-way ANOVA with Sidak's post-test, comparing BCG and control, * *P*<0.05, ** *P*<0.01, *** *P*<0.001, **** *P*<0.0001.

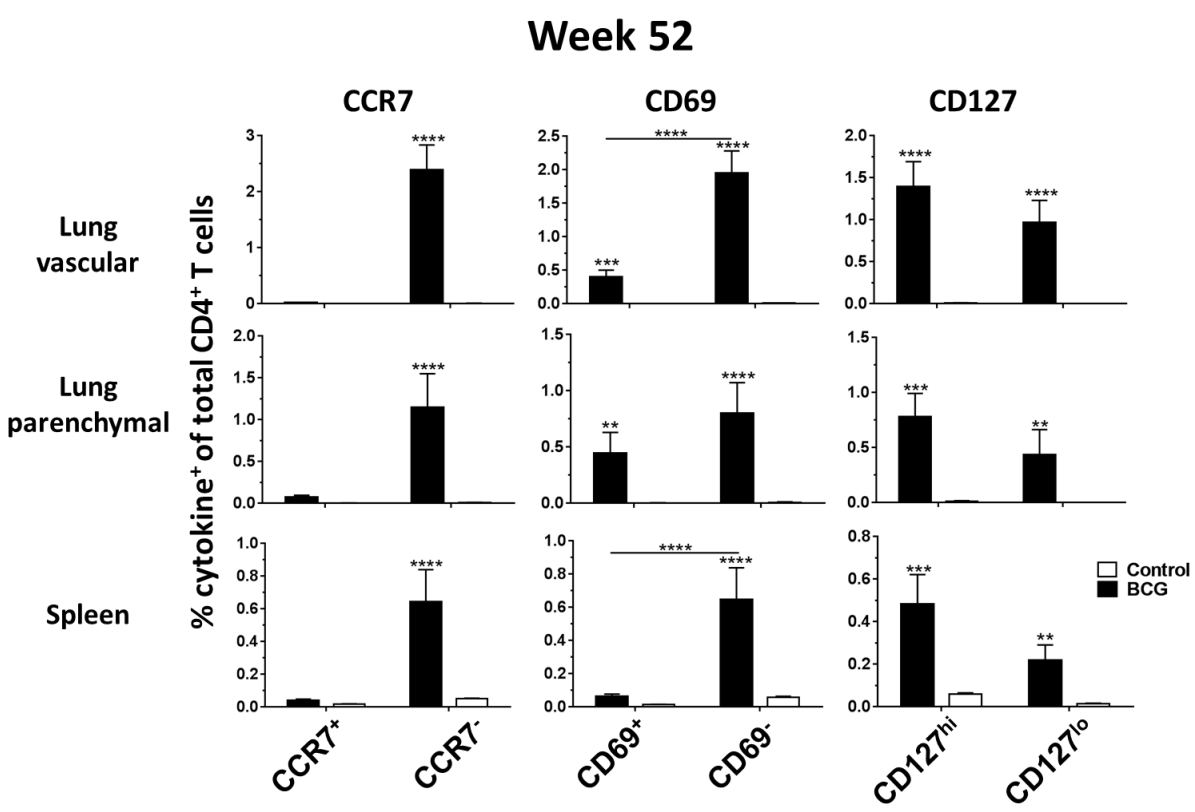


Supplementary Figure 3 BCG-induced antigen-specific CD4⁺ T cells display an effector phenotype at all time points measured post-BCG vaccination. Following BCG immunisation, intravascular staining and ICS identified populations of antigen-specific (cytokine⁺) CD4⁺ T cells. Graphs show frequency of antigen-specific CD4⁺ CD44^{hi} T cells in all compartments displaying combinations of CD62L and CD27 cell surface markers as a % of total CD4⁺ T cells in that compartment. Bars represent mean ± SEM (*n*=6). Two-way ANOVA with Sidak's post-test, comparing BCG and control, *** *P*<0.001, **** *P*<0.0001.

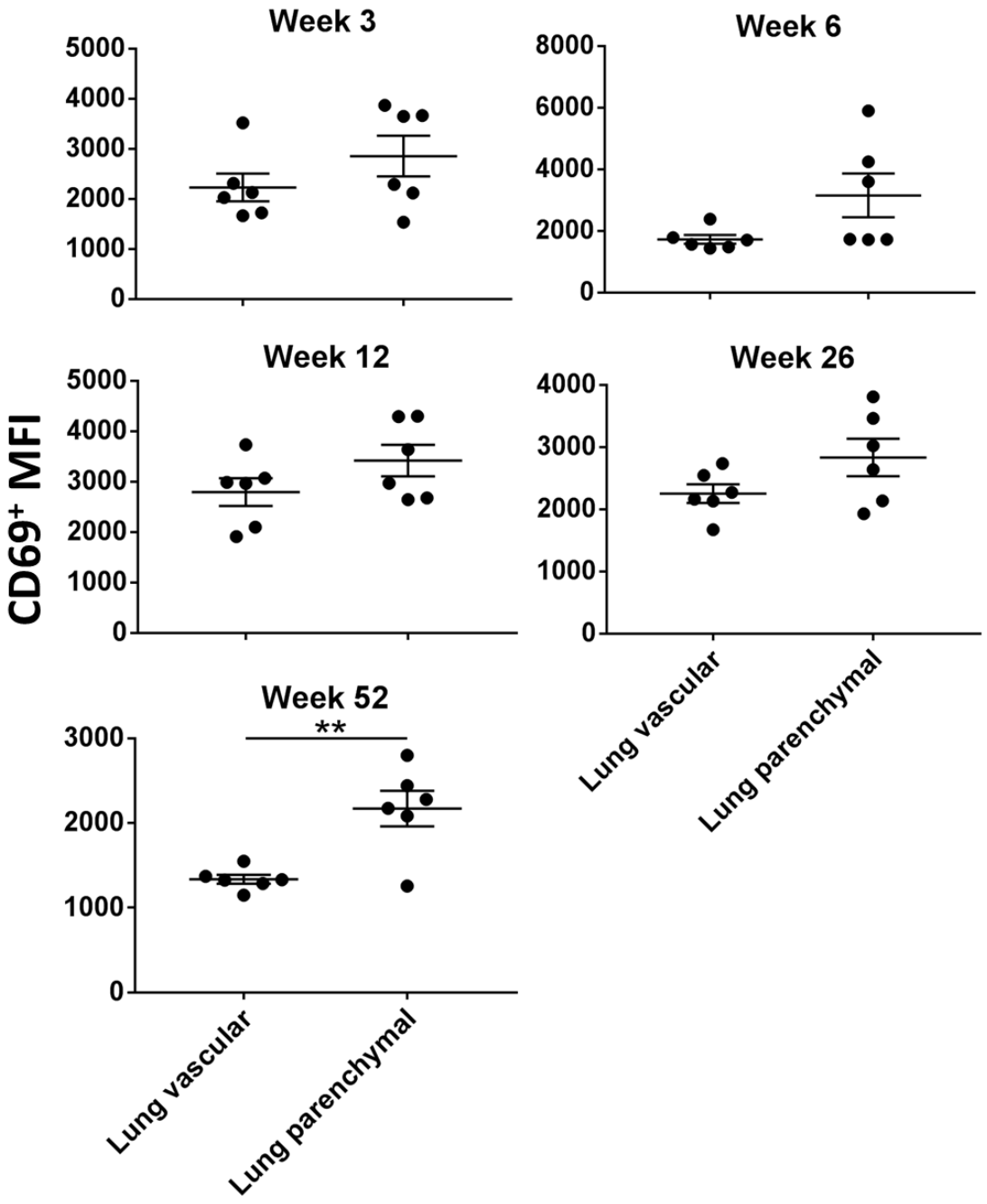

Week 3
CCR7
CD69
CD127
Lung vascular
Lung parenchymal
Spleen
% cytokine+ of total CD4+ T cells
BCG
Control
CCR7+
CCR7-
CD69+
CD69-
CD127hi
CD127lo


Week 6
CCR7
CD69
CD127
Lung vascular
Lung parenchymal
Spleen
% cytokine+ of total CD4+ T cells
BCG
Control
CCR7+
CCR7-
CD69+
CD69-
CD127hi
CD127lo


Week 12
CCR7
CD69
CD127
Lung vascular
Lung parenchymal
Spleen
% cytokine+ of total CD4+ T cells
Control
BCG
CCR7+
CCR7-
CD69+
CD69-
CD127hi
CD127lo
Week 26
CCR7
CD69
CD127
Lung vascular
Lung parenchymal
Spleen
% cytokine+ of total CD4+ T cells
Control
BCG
CCR7+
CCR7-
CD69+
CD69-
CD127hi
CD127lo



Supplementary Figure 4 BCG-induced antigen-specific CD4$^+$ T cells are CCR7$^-$ but heterogeneous for CD69 and CD127 at all time points measured post-BCG vaccination. Following BCG immunisation, intravascular staining and ICS identified populations of antigen-specific (cytokine$^+$) CD4$^+$ T cells. Graphs show frequencies of antigen-specific CD4$^+$ CD62L^{lo} T cells in all compartments displaying CCR7, CD69 and CD127 cell surface markers as a % of total CD4$^+$ T cells in that compartment. For all graphs, bars represent mean ± SEM (*n*=6). Two-way ANOVA with Sidak's post-test, comparing BCG with control and BCG with BCG, * *P*<0.05, ** *P*<0.01, *** *P*<0.001, **** *P*<0.0001.



Supplementary Figure 5 Expression of CD69 on BCG-induced antigen-specific CD4$^+$ T cells in the lung at all time points measured post-BCG vaccination. Following BCG immunisation, intravascular staining and ICS identified populations of antigen-specific (cytokine$^+$) CD4$^+$ T cells. Graphs show MFI of cytokine$^+$ CD69$^+$ populations in the lung parenchyma and lung vasculature. For all graphs, lines represent mean ± SEM (*n*=6). Unpaired two-tailed t-tests, ** P<0.01.