# Central Role of CD169$^{+}$ Lymph Node Resident Macrophages in the Adjuvanticity of the QS-21 Component of AS01.

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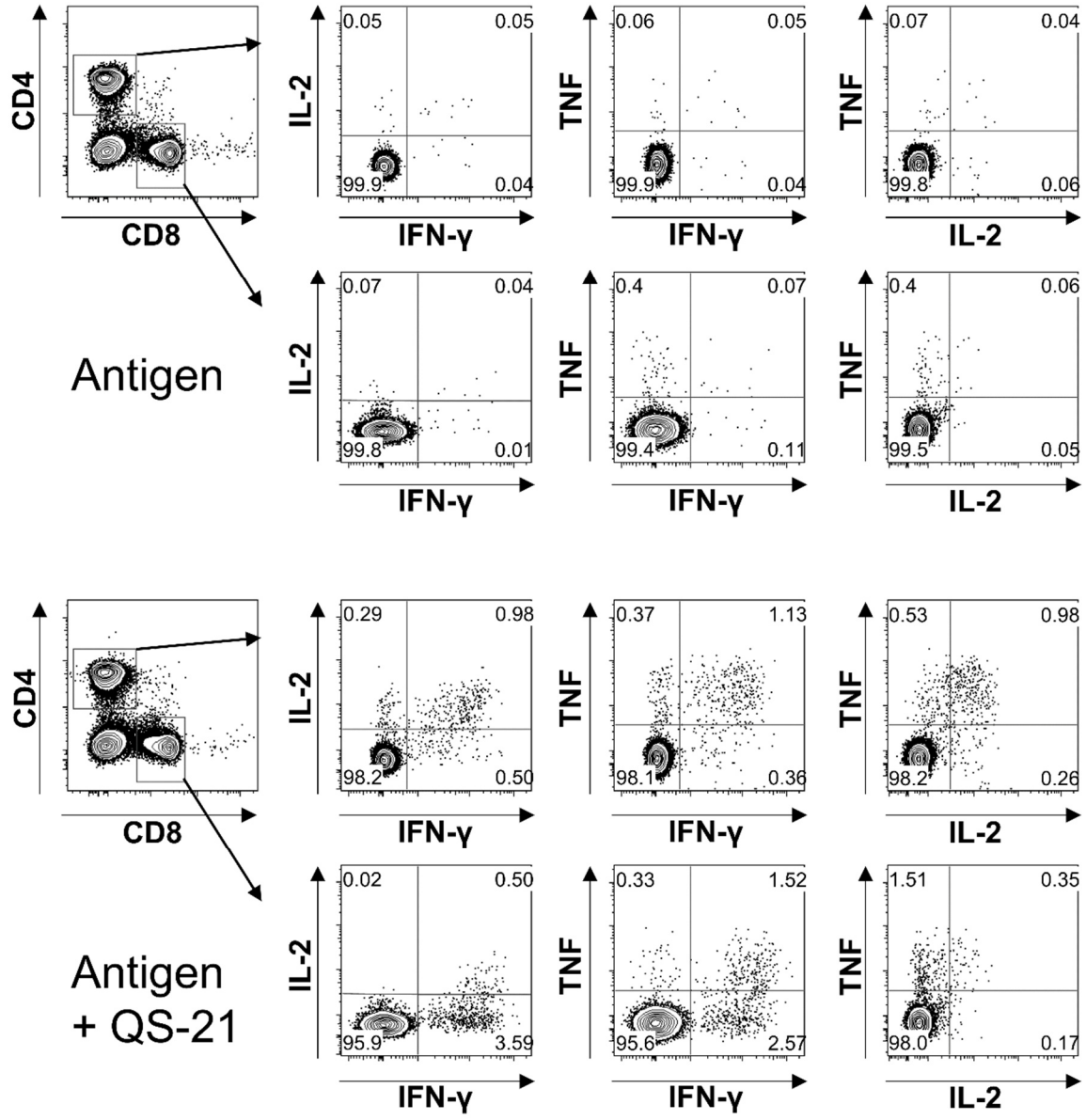
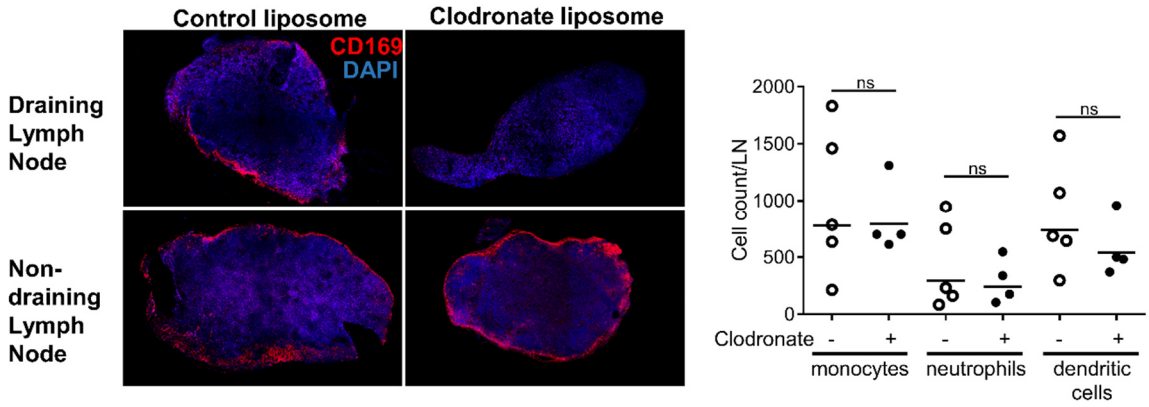
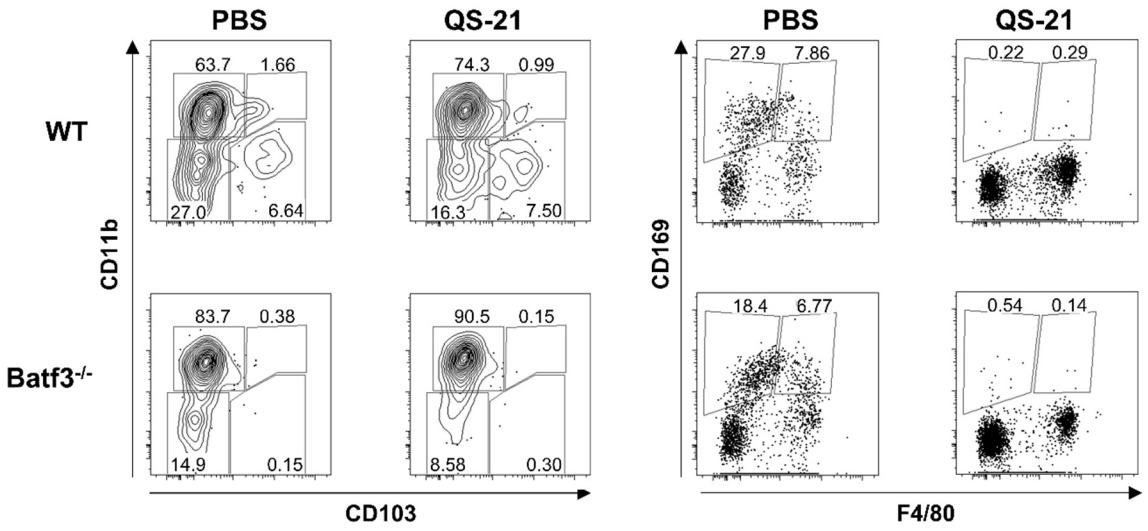
**Supplementary Figures**



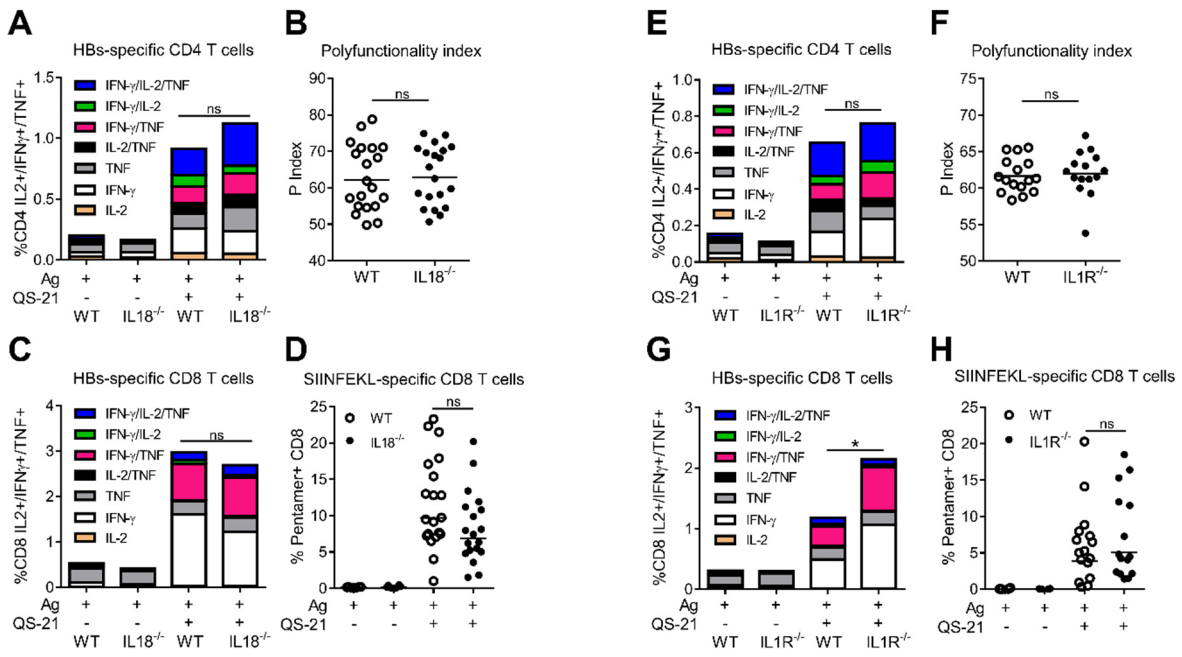

**Supplementary Figure 1.** Intracellular cytokine stain. Following antigenic peptide restimulation, T cells were gated on either CD4 or CD8-positive cells and IL-2, IFN-γ or TNF production was assessed. One mouse immunised with antigen alone and one mouse immunised with QS-21-adjuvanted antigen are shown.



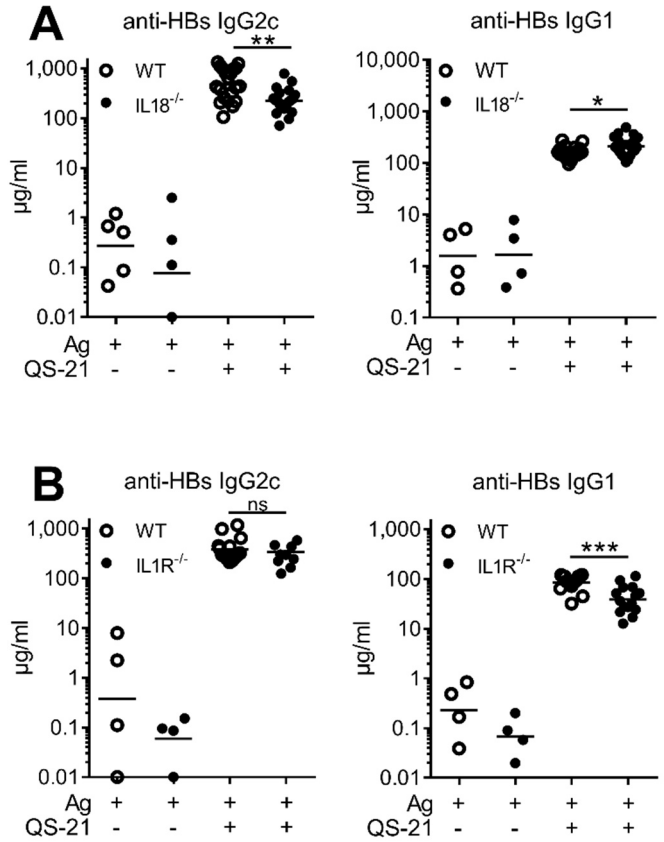

**Supplementary Figure 2.** Related to figure 4. A) 6 days after clodronate liposome injection, the draining (iliac) and non-draining (cervical) lymph nodes were recovered and stained with DAPI (nuclei - blue) and anti-CD169 (red) antibodies. B) DLNs were recovered at day 6 post clodronate liposome (CL) injection. Monocytes ($Lin^-CD11b^+$ $Ly6C^+$ $Ly6G^-$), neutrophils ($SSC^{hi}$ $Lin^-$ $CD11b^+$ $Ly6C^{int}$ $Ly6G^+$) and dendritic cells ($Lin^-CD11c^+$ $IA/IE^{hi}$) were quantified by flow cytometry (n=4-5).



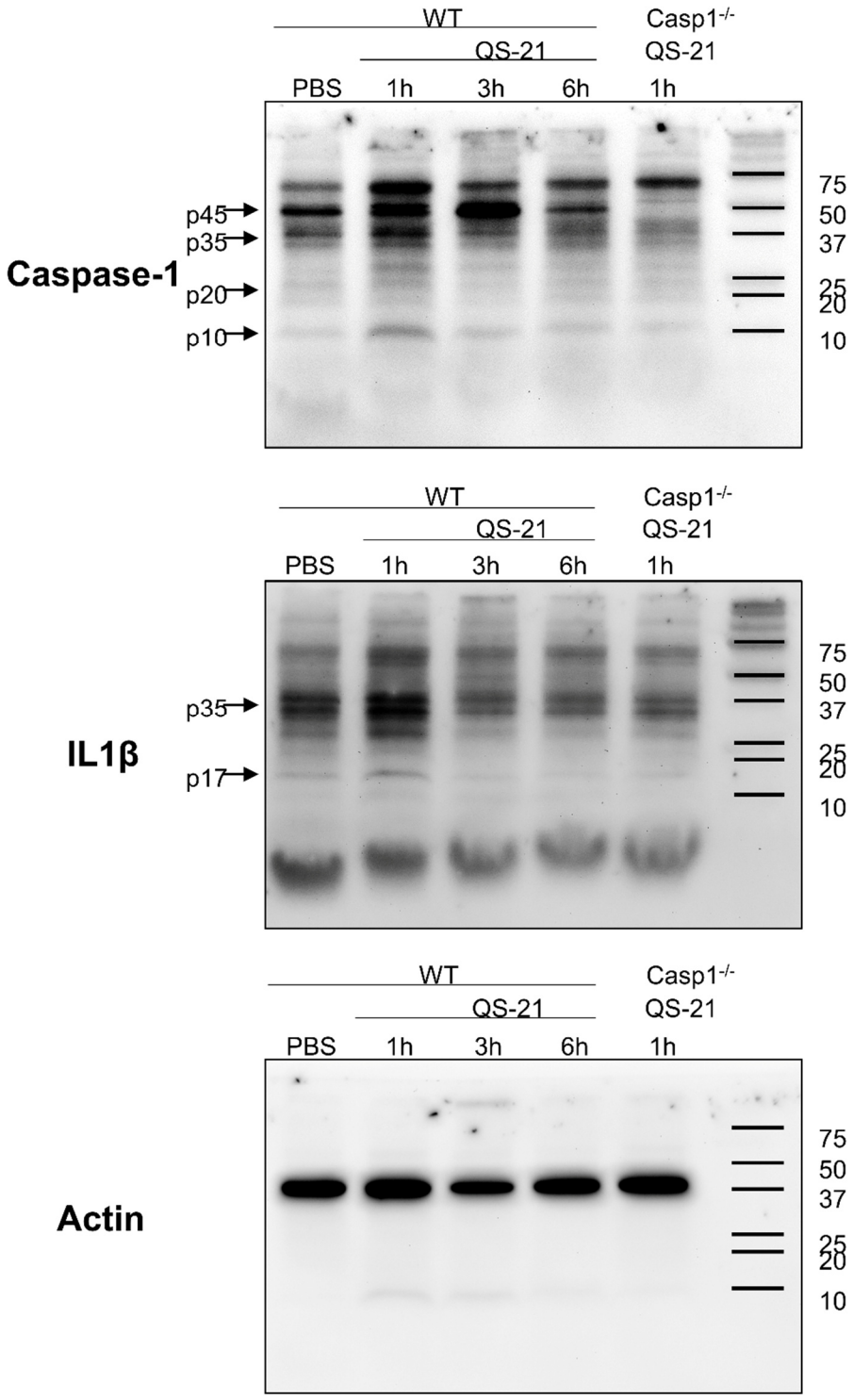

**Supplementary Figure 3.** Related to figure 5. 24h after PBS or QS-21 injection, dendritic cell and CD169+ cell subset frequencies in WT and Batf3$^{-/-}$ mice were analysed by flow cytometry.




**Supplementary Figure 4.** *Antigen-specific T cell responses are normal in IL-18 and IL-1R KO mice.* A-B) WT and IL-18 KO mice were immunised as in figure 1A. At day 21, cytokine production (IL-2, IFN-γ, TNF) of HBs-specific CD4 (A) and CD8 (C) T cells were evaluated by intracellular staining. The data is represented as median of 6 (Ag) or 20 (QS-21) mice. CD4 T cell polyfunctionality index was calculated as described (B) and the frequency of OVA-specific circulating CD8 T-cells (D) was assessed by pentamer staining (Ag: n=6, QS-21: n=20). WT and IL-1 receptor KO mice were immunised as in 1A. At day 21, cytokine production (IL-2, IFN-γ, TNF) of HBs-specific CD4 (E) and CD8 (G) T cells were evaluated by intracellular staining. The data is represented as the median value of 4 (Ag) or 16 (QS-21) mice. The CD4 T cell polyfunctionality index was calculated as described (F) and the frequency of OVA-specific circulating CD8 T-cells (H) were assessed by pentamer staining (Ag: n=6, QS-21: n=16)




**Supplementary Figure 5.** *Antibody production partially depends on IL-18 and IL-1R-mediated signalling events.* WT and IL-18 KO (A) or IL1-R KO (B) mice were immunised as in 1A. Anti-HBs IgG1 and IgG2c in the serum at day 21 were measured by ELISA (Ag: n=4-5, QS-21: n=20). Each point represents a single mouse and the horizontal bar represents the geometric mean. Statistical significance was determined by a non-parametric Mann Whitney t-test. The data represents a pool of 2 independent experiments.




**Supplementary Figure 6.** Uncropped immunoblots for Figure 6B