

Supplementary data

Supplementary data include

- gating strategy of the beads for CBA (p. 70)
- gating strategy for analysis of leukocytes in mouse pinnae (p. 71–73)
- gating strategy for analysis of leukocytes in parotid LNs (p. 74)
- complete graphs with individual values from flow cytometry analysis (p. 75–92)

Supplement 1. Gating strategy of beads

Cytokine production from cultivated mouse pinnae and sera was evaluated by CBA. Gate for beads was set according to the size and granularity and the same gating was applied for serum as well as pinnae cytokines.

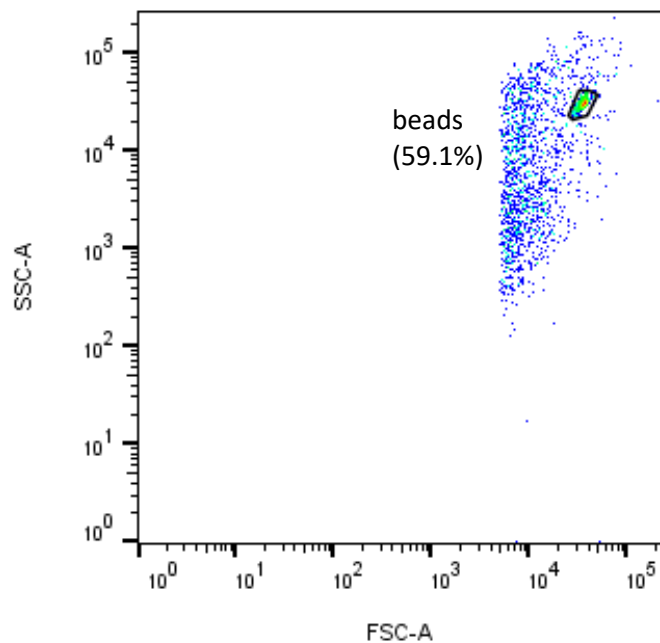


Figure S1. *Gating strategy of the beads for cytokine detection by CBA.* Beads formed compact population easily determined from debris by size (FSC-A) and granularity (SSC-A).

Supplement 2. Gating strategy of leukocytes

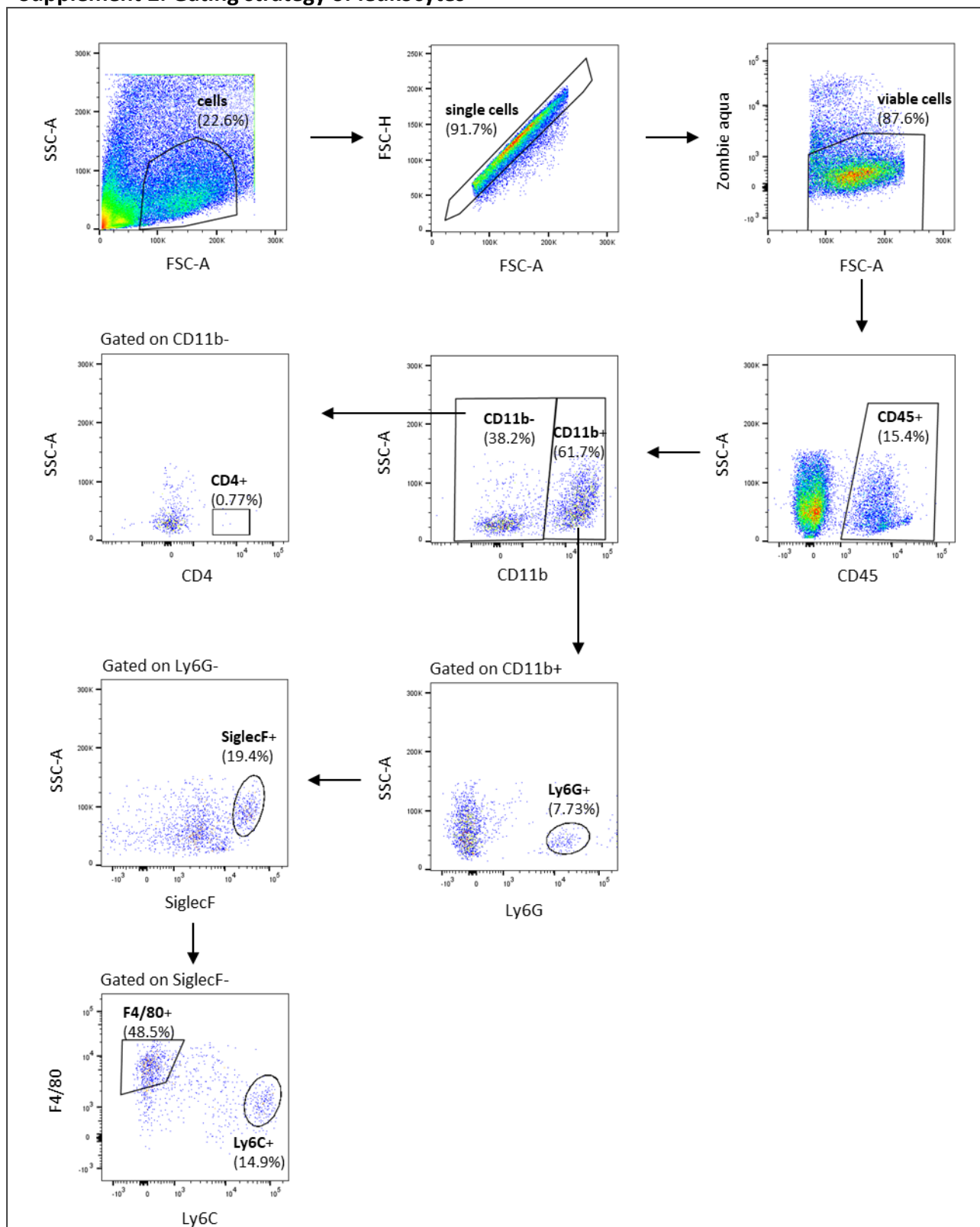


Figure S2. Representative dot plots of gating strategy for „general set“ of markers in dermis of mouse *pinnae*. Cellular debris, doublets and dead cells were excluded, and the remaining cells were then analyzed for specific leukocyte markers. Leukocytes (CD45+) were divided into myeloid (CD11b+) and lymphoid (CD11b-) cells. Lymphoid cells were further checked for CD4 expression. From myeloid cells, firstly population of neutrophils (Ly6G+) was separated and then eosinophils (SiglecF+). The remaining cells were analyzed for F4/80 (macrophages) and Ly6C (monocytes). Epidermis was gated in the same manner only borders of populations were slightly shifted.

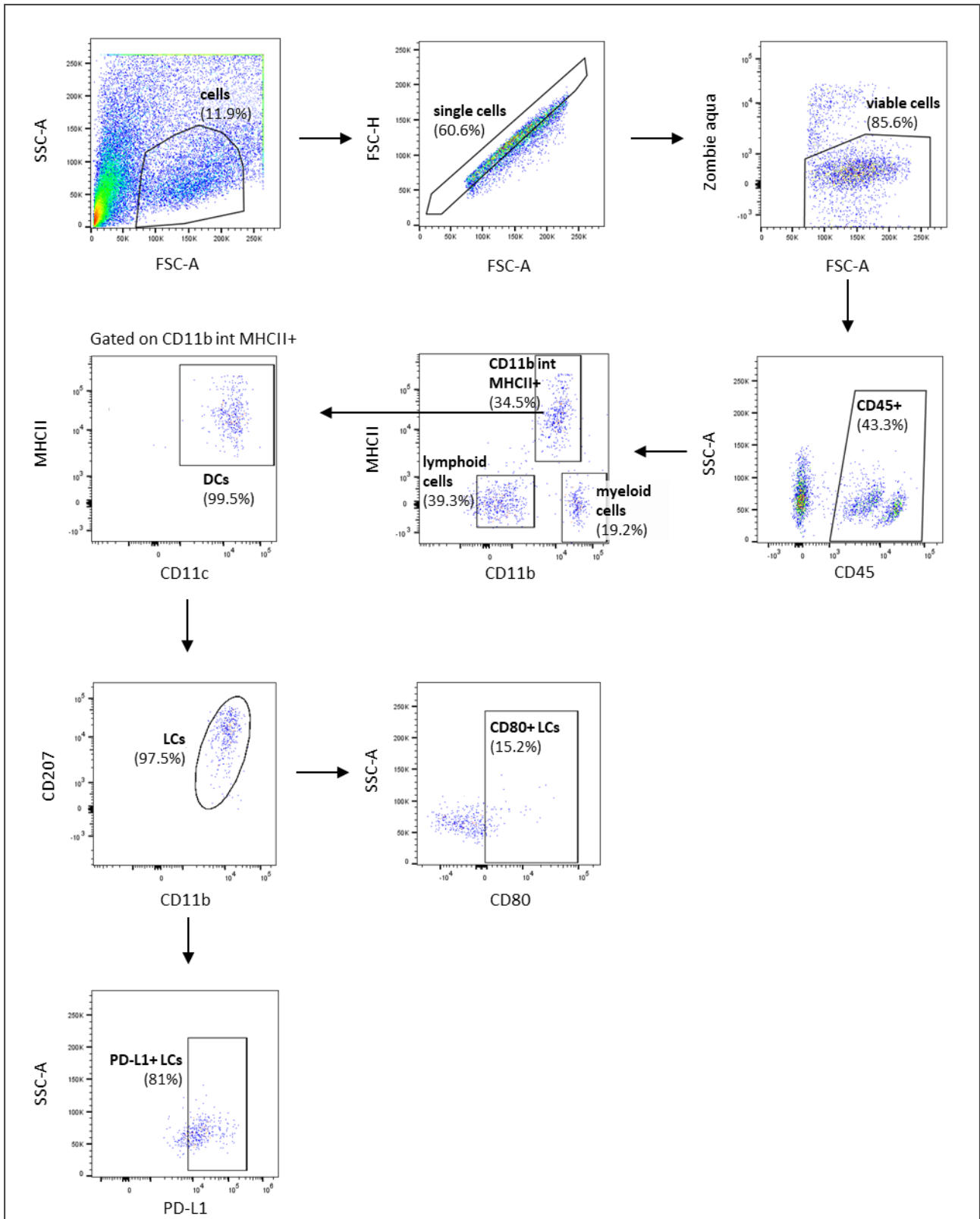


Figure S3. Representative dot plots of gating strategy for „Ag-presenting set“ of markers in epidermis of mouse pinnae. Cellular debris, doublets and dead cells were excluded, and the remaining cells were then analyzed for specific leukocyte markers. Leukocytes (CD45+) were divided by the expression of CD11b and MHCII. In epidermis, population of MHCII+ cells was CD11b intermediate. Later, these cells were shown to express CD11c and CD207, therefore, these cells were characterized as Langerhans cells (LCs). Gates for CD80 and PD-L1 were set according to the corresponding FMO controls.

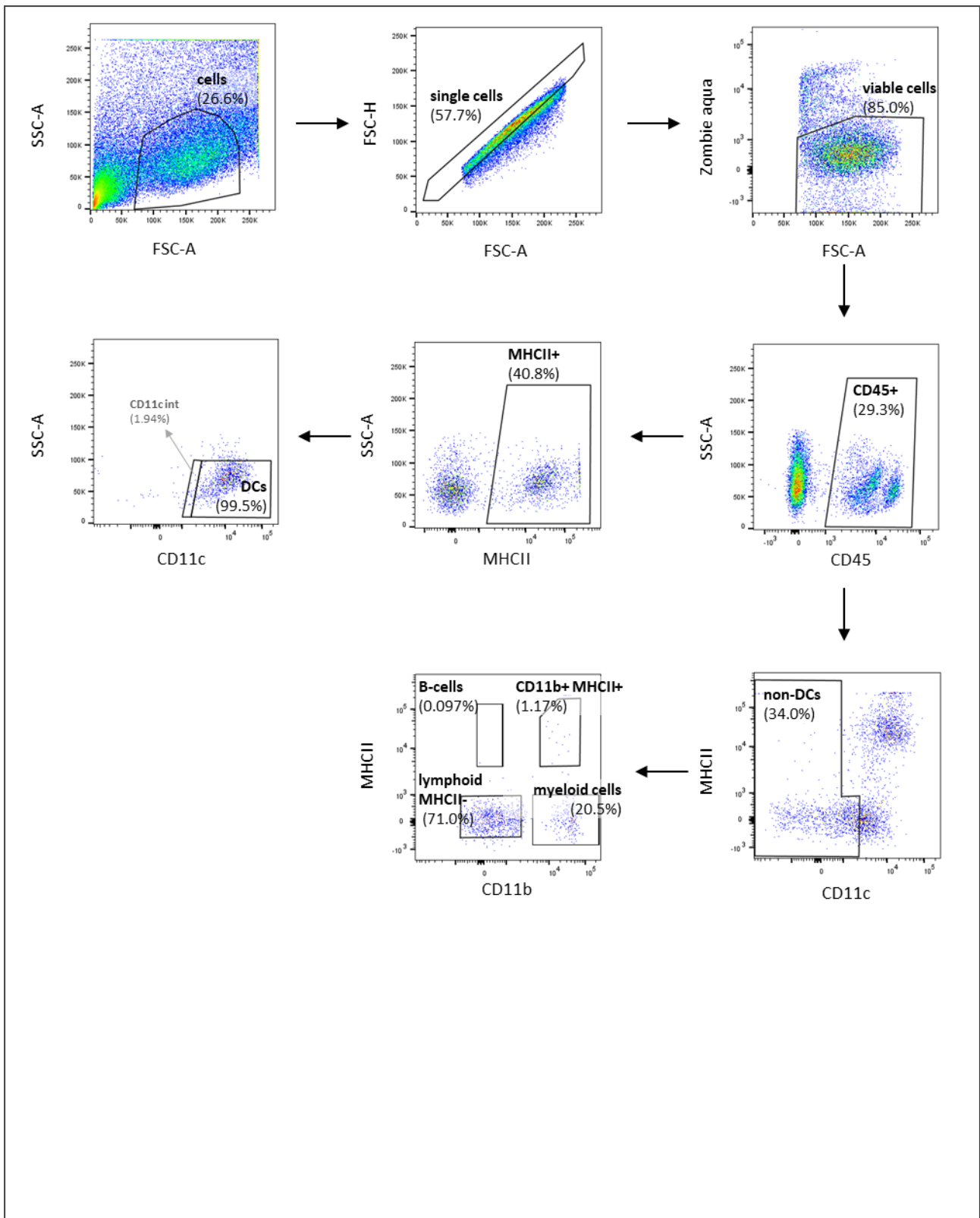


Figure S4. Representative dot plots of gating strategy for „Ag-presenting set“ of markers in dermis of mouse pinnae. Cellular debris, doublets and dead cells were „excluded, and the remaining cells were then analyzed for specific leukocyte markers. Leukocytes (CD45+) were analyzed for the expression of MHCII and afterwards for CD11c to characterize dendritic cells (DCs). Population of CD11c intermediate was appearing in the infected tissue. Cells not expressing CD11c with a possible expression of MHCII were characterized as non-DCs. From these cells, by the expression of CD11b and MHCII, B-cells (CD11b-MHCII+) and unspecified lymphoid cells (CD11b- MHCII-) were found. This gating allowed also visualization and possibly unspecific analysis of myeloid cells.

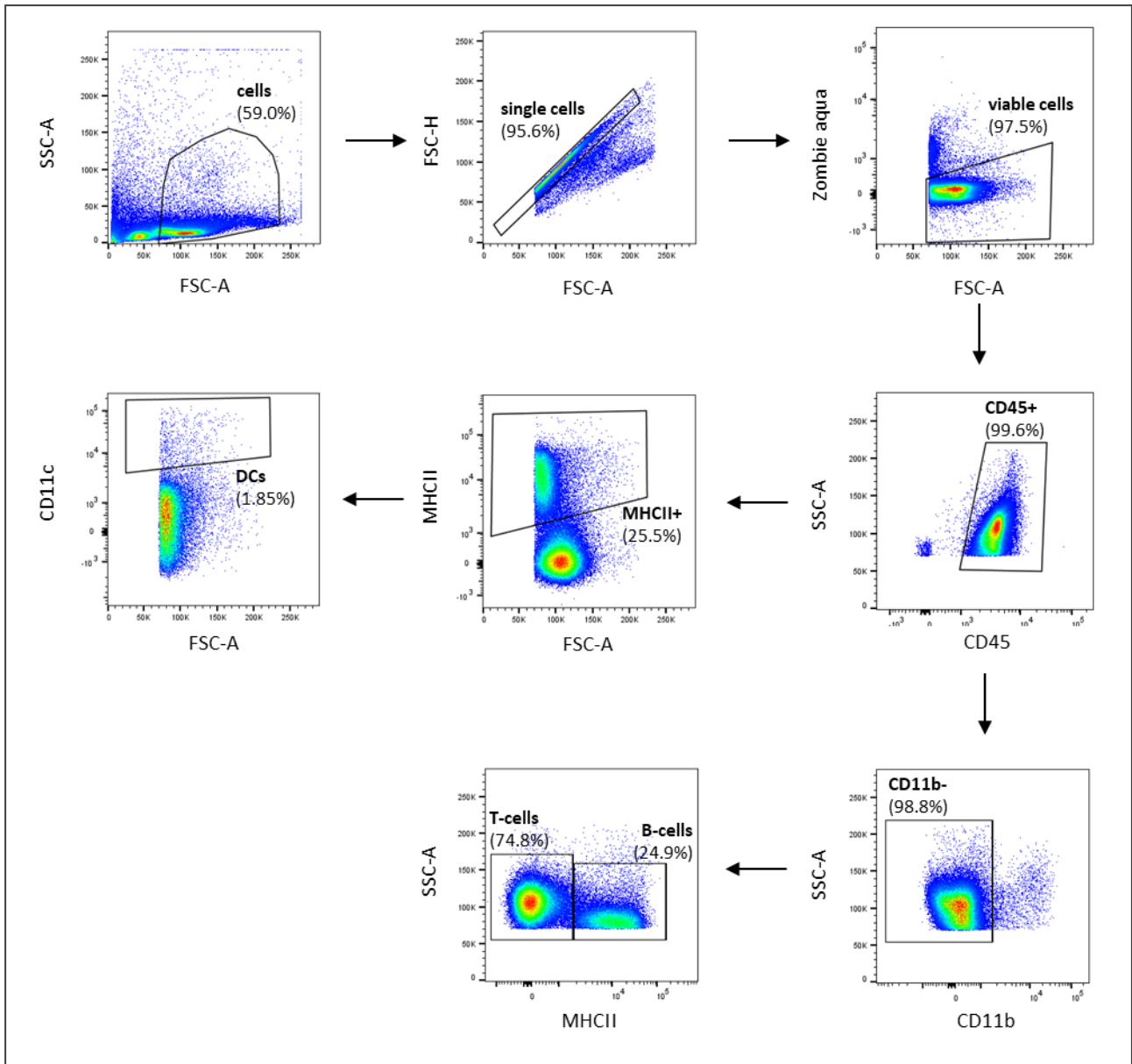


Figure S5. Representative dot plots of gating strategy for „Ag-presenting set“ of markers in parotid LNs. Cellular debris, doublets and dead cells were excluded, and the remaining cells were then analyzed for specific leukocyte markers. Leukocytes (CD45+) were analyzed for the expression of MHCII and afterwards for expression CD11c to characterize DCs. To examine lymphoid cells (CD11b-), leukocytes were separated according to the expression of CD11b. Lymphoid cells were divided into T-cells (CD11b- MHCII-) and B-cells (CD11b- MHCII+) based on the expression of MHCII.

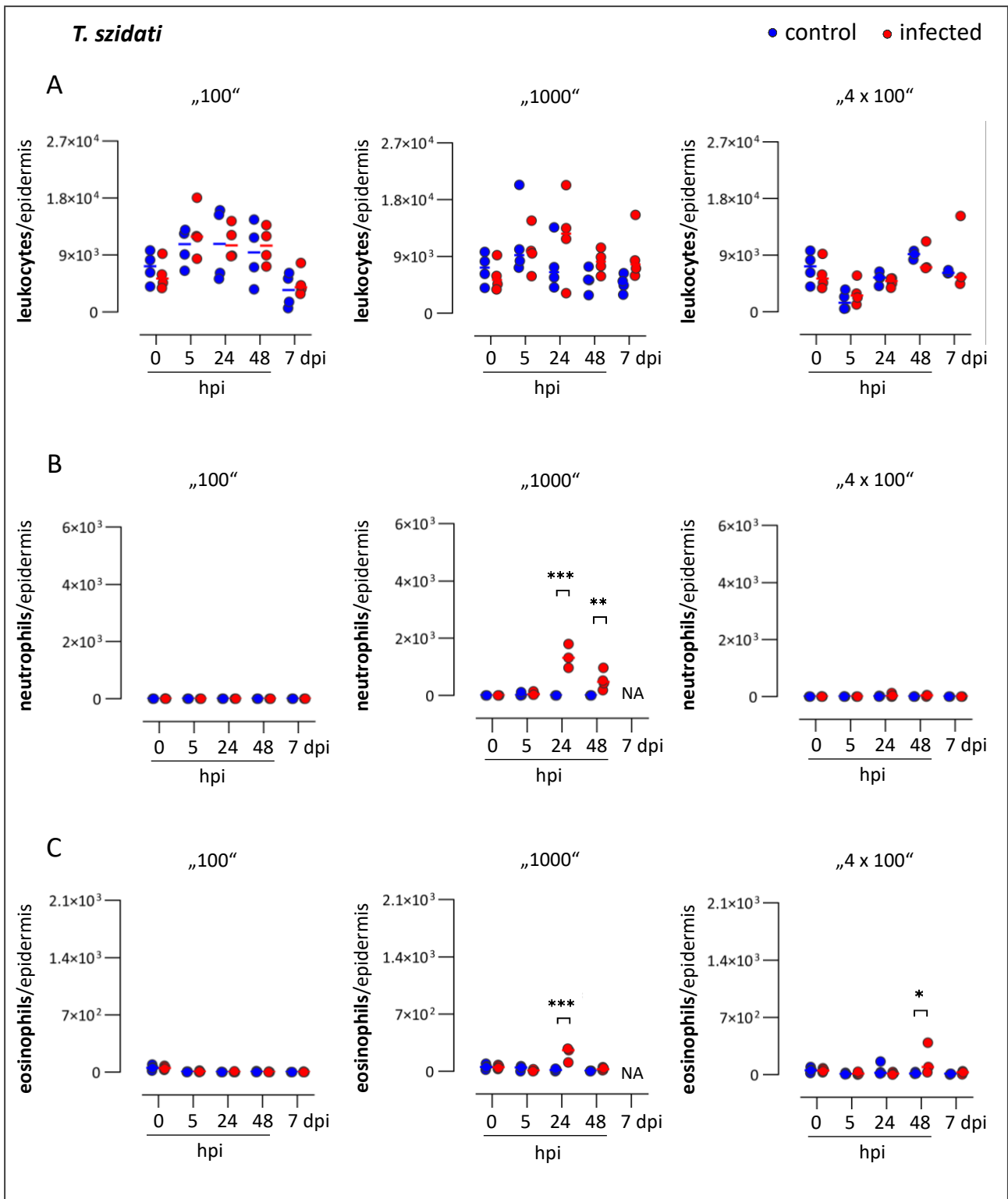


Figure S7. Dynamics of leukocyte populations in epidermis of mice infected by 100, 1000 or 4x100 cercariae of *T. szidati* expressed in absolute number of cells defined by flow cytometry. (A) -leukocytes (CD45+); (B) - neutrophils (CD45+ CD11b+ Ly6G+) and (C) - eosinophils (CD45+ CD11b+ SiglecF+). Significant differences between infected and contralateral control pinnae are shown. 2-way ANOVA; Sidak test; $n = 0-4$; hpi – hours post infection; dpi – days post infection; NA – not available

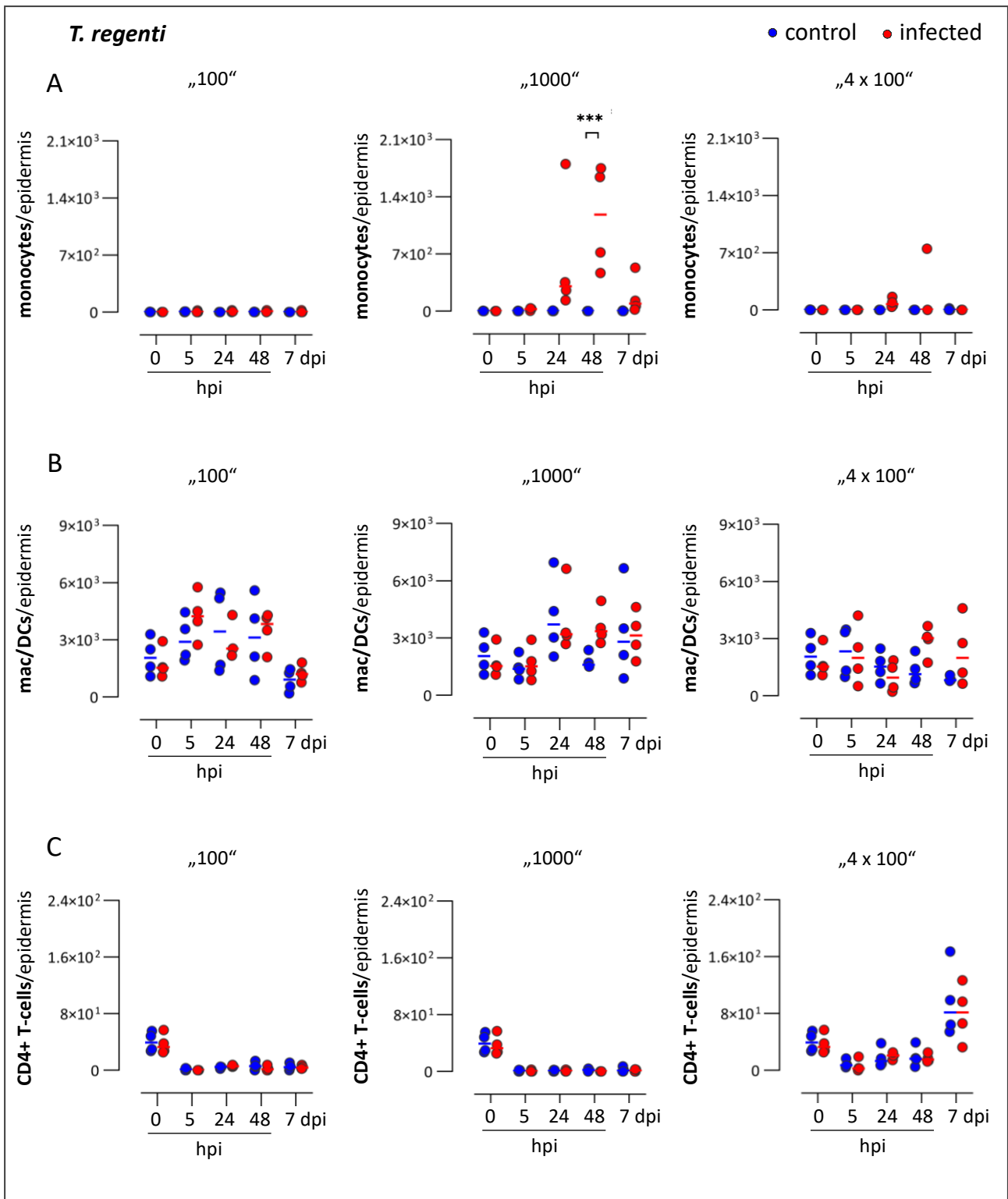


Figure S8. Dynamics of leukocyte populations in epidermis of mice infected by 100, 1000 or 4x100 cercariae of *T. regenti* expressed in absolute number of cells defined by flow cytometry. (A) - monocytes (CD45+ CD11b+ Ly6C+); (B) - macrophages/DCs (CD45+ CD11b+ F4/80+) and (C) - CD4+ T-cells (CD45+ CD11b- CD4+). Significant differences between infected and contralateral control pinnae are shown. 2-way ANOVA; Sidak test; $n = 3-4$; hpi – hours post infection; dpi – days post infection; mac – macrophages; DCs – dendritic cells

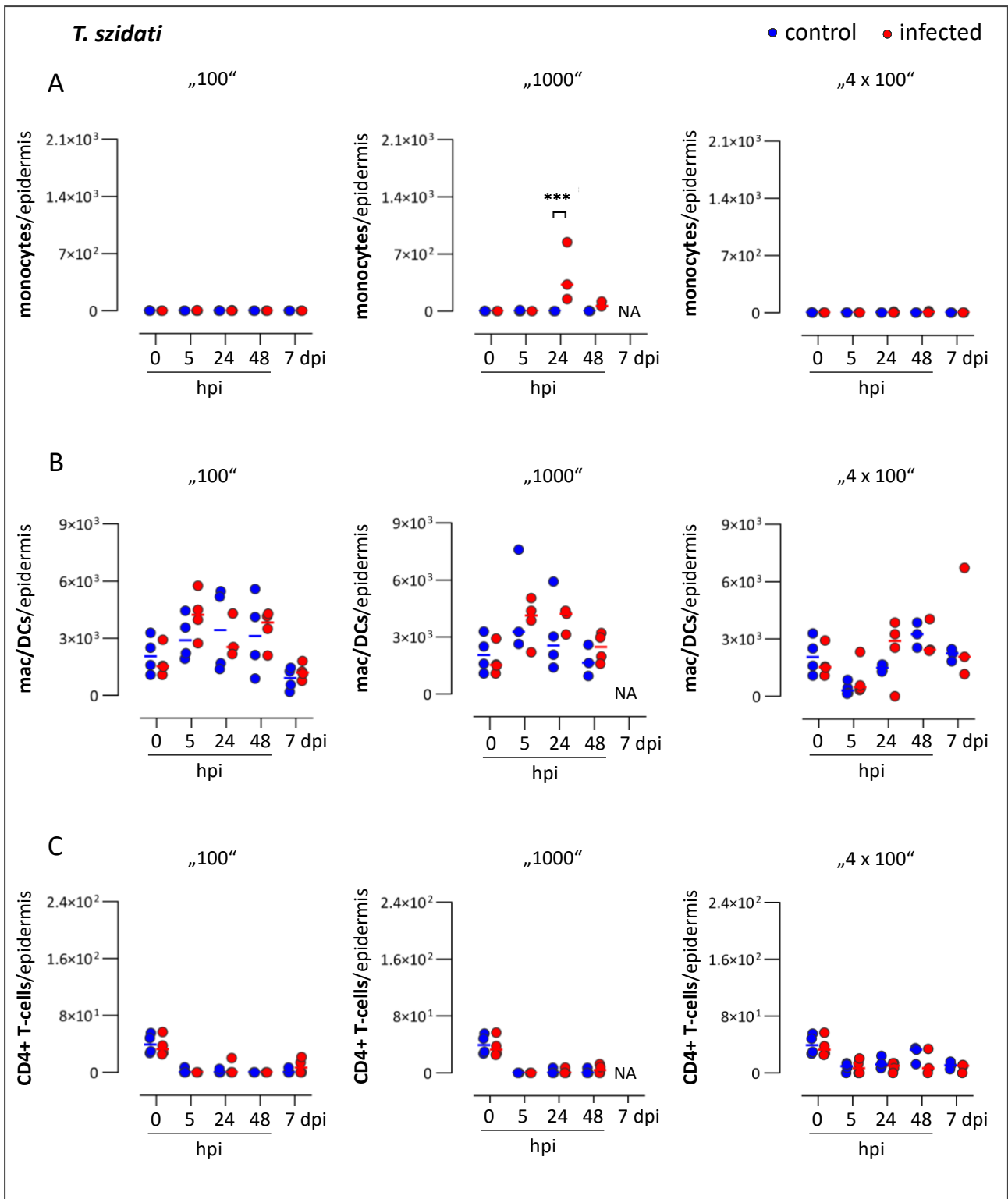


Figure S9. Dynamics of leukocyte populations in epidermis of mice infected by 100, 1000 or 4x100 cercariae of *T. szidati* expressed in absolute number of cells defined by flow cytometry. (A) - monocytes (CD45+ CD11b+ Ly6C+); (B) - macrophages/DCs (CD45+ CD11b+ F4/80+) and (C) - CD4+ T-cells (CD45+ CD11b- CD4+). Significant differences between infected and contralateral control pinnae are shown. 2-way ANOVA; Sidak test; $n = 0-4$; hpi – hours post infection; dpi – days post infection; mac – macrophages; DCs – dendritic cells; NA – not available

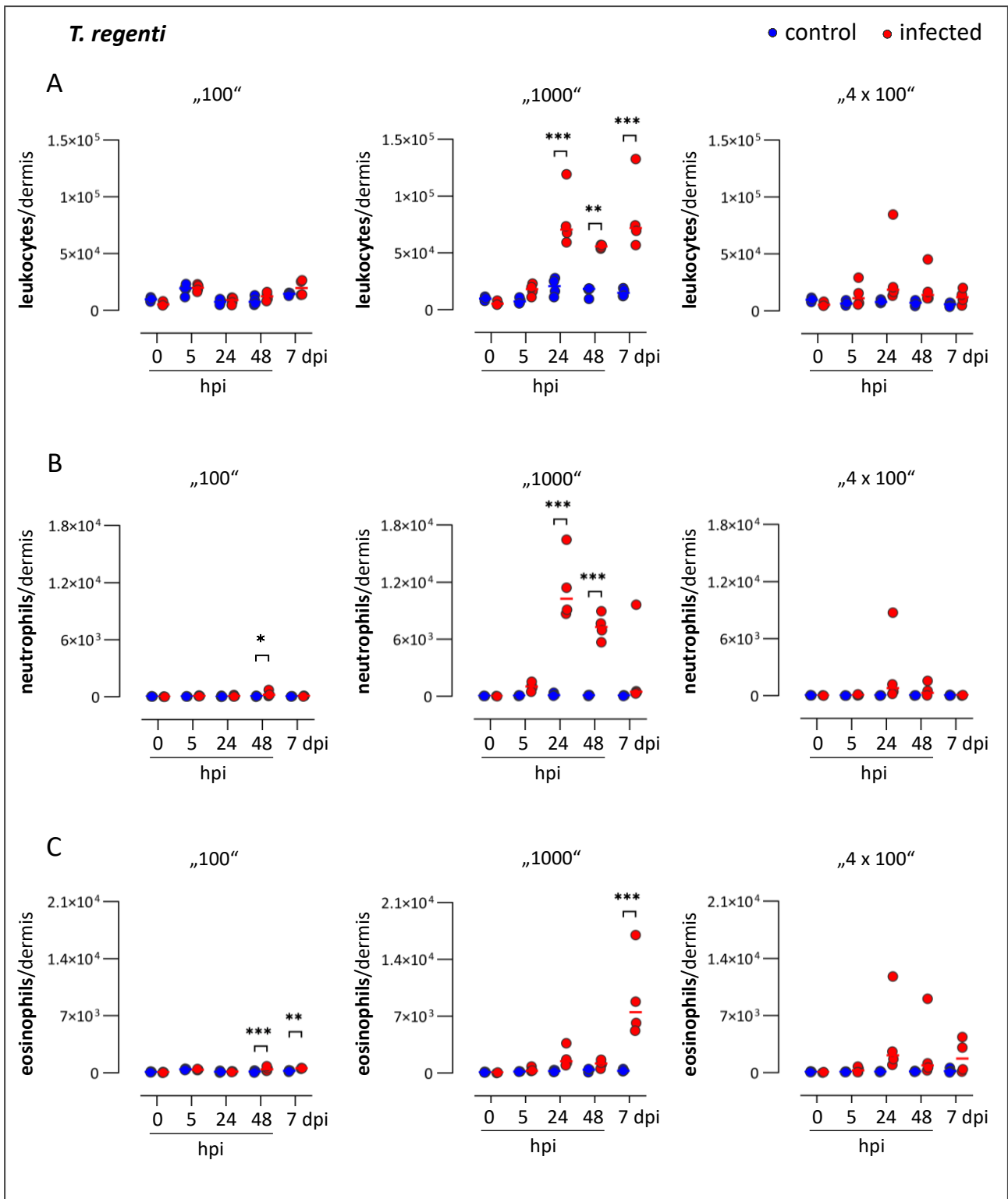


Figure S10. Dynamics of leukocyte populations in dermis of mice infected by 100, 1000 or 4x100 cercariae of *T. regenti* expressed in absolute number of cells defined by flow cytometry. (A) - leukocytes (CD45+); (B) - neutrophils (CD45+ CD11b+ Ly6G+) and (C) - eosinophils (CD45+ CD11b+ SiglecF+). Significant differences between infected and contralateral control pinnae are shown. 2-way ANOVA; Sidak test; $n = 3-4$; hpi – hours post infection; dpi – days post infection

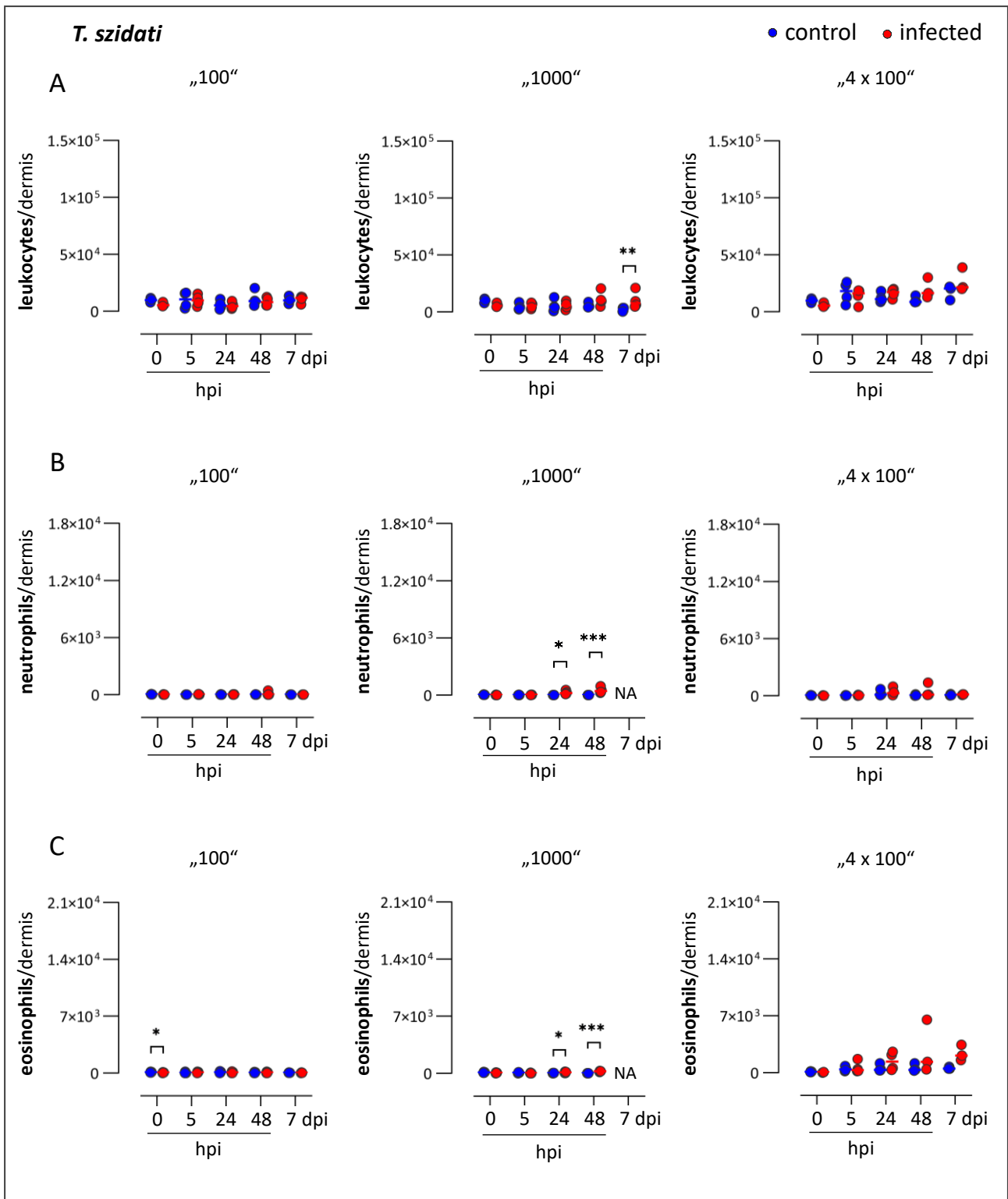


Figure S11. Dynamics of leukocyte populations in dermis of mice infected by 100, 1000 or 4x100 cercariae of *T. szidati* expressed in absolute number of cells defined by flow cytometry. (A) - leukocytes (CD45+); (B) - neutrophils (CD45+ CD11b+ Ly6G+) and (C) - eosinophils (CD45+ CD11b+ SiglecF+). Significant differences between infected and contralateral control pinnae are shown. 2-way ANOVA; Sidak test; $n = 0-4$; hpi – hours post infection; dpi – days post infection; NA – not available

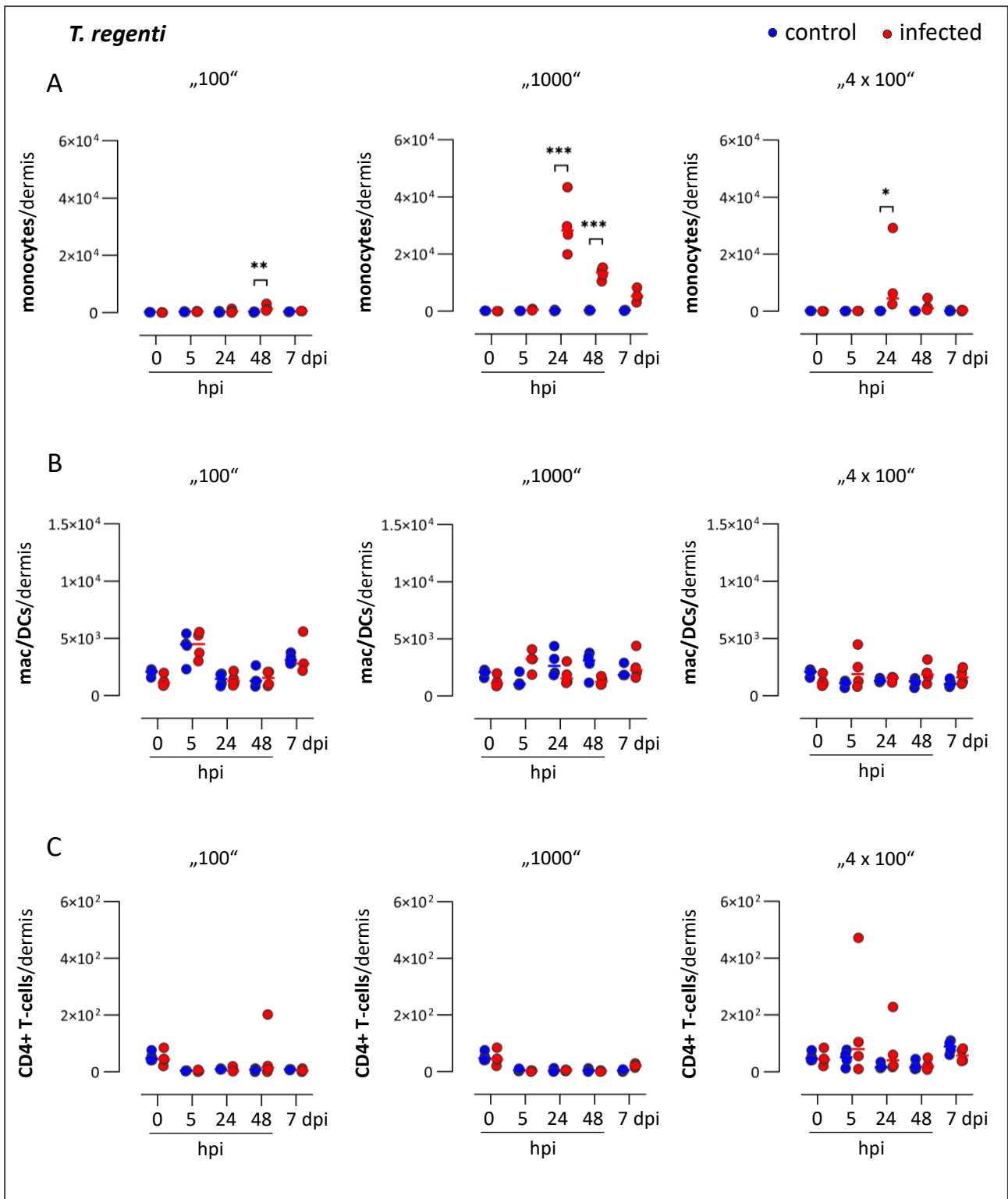


Figure S12. Dynamics of leukocyte populations in dermis of mice infected by 100, 1000 or 4x100 cercariae of *T. regenti* expressed in absolute number of cells defined by flow cytometry. (A) - monocytes (CD45+ CD11b+ Ly6C+); (B) - macrophages/DCs (CD45+ CD11b+ F4/80+) and (C) - CD4+ T-cells (CD45+ CD11b- CD4+). Significant differences between infected and contralateral control pinnae are shown. 2-way ANOVA; Sidak test; $n = 3-4$; hpi – hours post infection; dpi – days post infection; mac – macrophages; DCs – dendritic cells

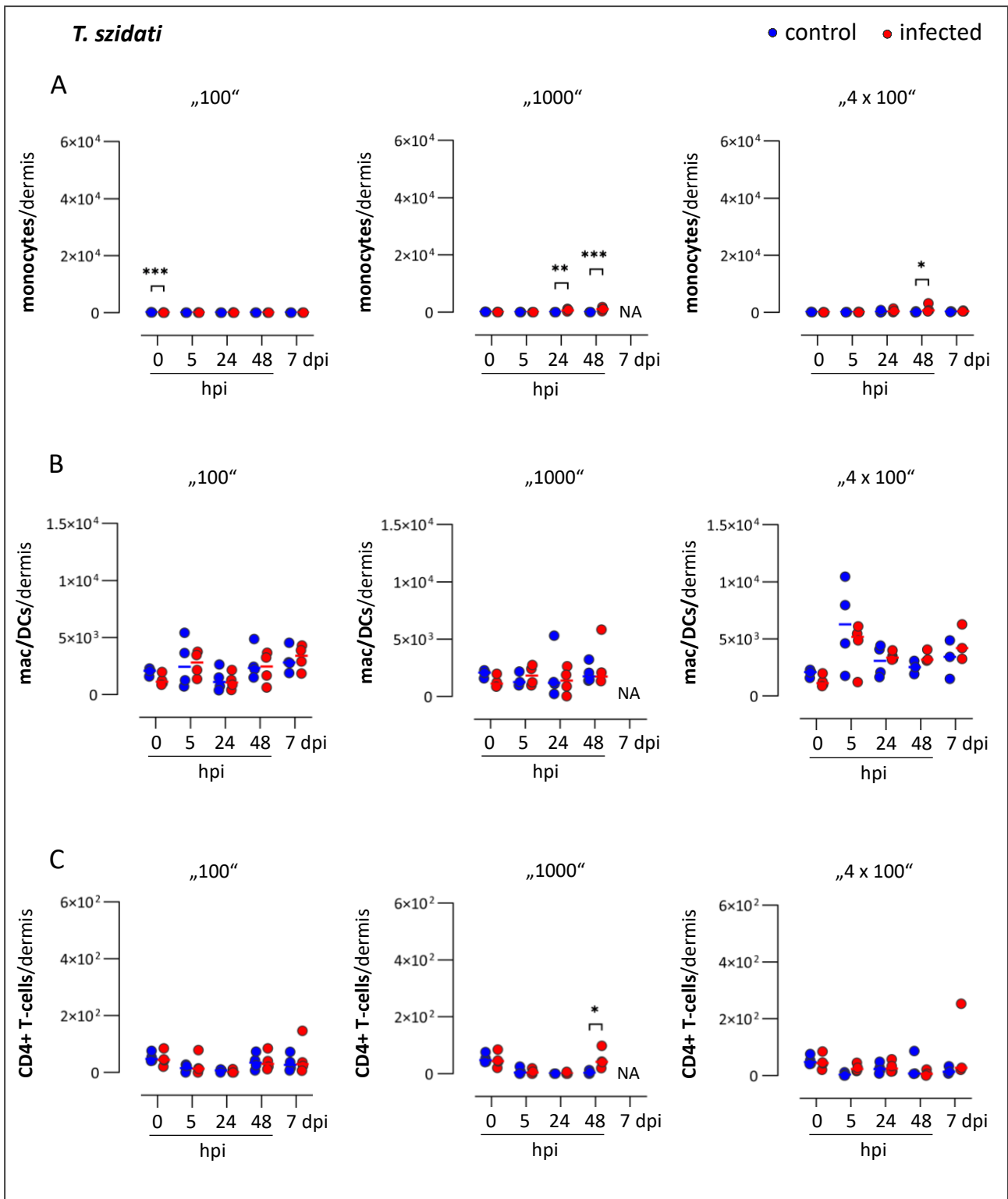


Figure S13. Dynamics of leukocyte populations in dermis of mice infected by 100, 1000 or 4x100 cercariae of *T. szidati* expressed in absolute number of cells defined by flow cytometry. (A) - monocytes (CD45+ CD11b+ Ly6C+); (B) - macrophages/DCs (CD45+ CD11b+ F4/80+) and (C) - CD4+ T-cells (CD45+ CD11b- CD4+). Significant differences between infected and contralateral control pinnae are shown. 2-way ANOVA; Sidak test; $n = 0-4$; hpi – hours post infection; dpi – days post infection; mac – macrophages; DCs – dendritic cells; NA – not available

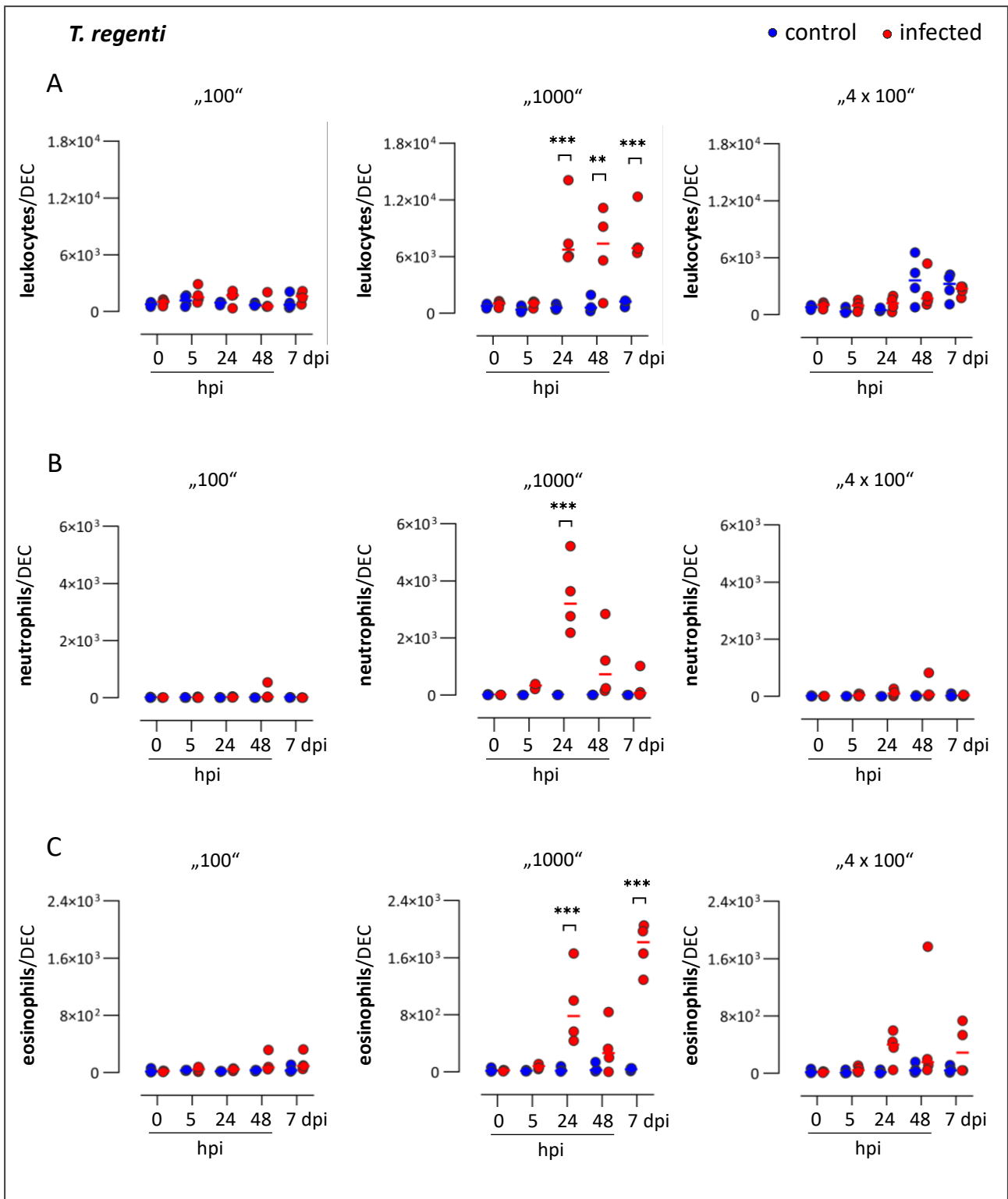


Figure S14. Dynamics of leukocyte populations in DEC of mice infected by 100, 1000 or 4x100 cercariae of *T. regenti* expressed in absolute number of cells defined by flow cytometry. (A) - leukocytes (CD45+); (B) - neutrophils (CD45+ CD11b+ Ly6G+) and (C) - eosinophils (CD45+ CD11b+ SiglecF+). Significant differences between infected and contralateral control pinnae are shown. 2-way ANOVA; Sidak test; $n = 3-4$; hpi – hours post infection; dpi – days post infection; DEC – dermal exudate cells

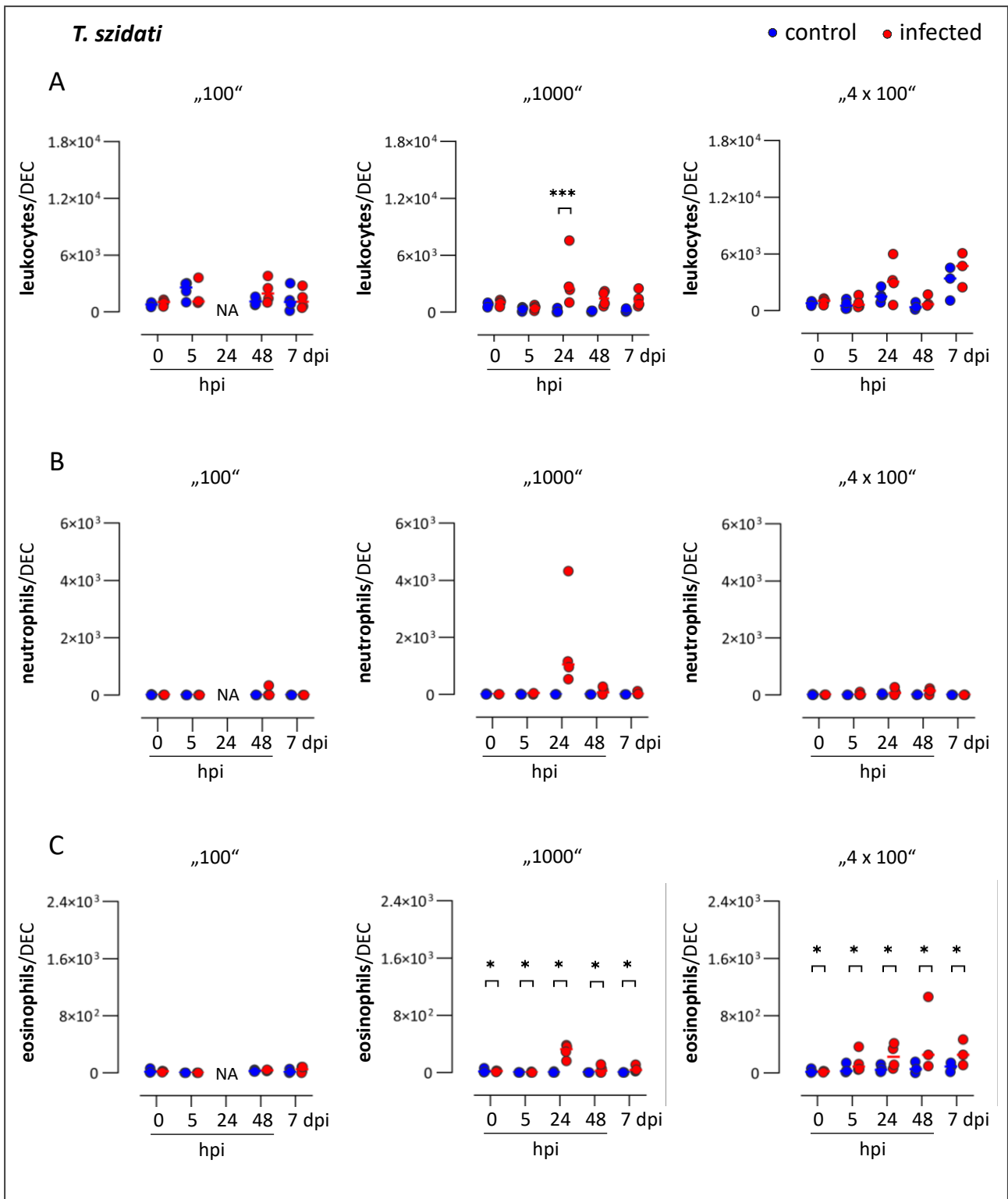


Figure S15. Dynamics of leukocyte populations in DEC of mice infected by 100, 1000 or 4x100 cercariae of *T. szidati* expressed in absolute number of cells defined by flow cytometry. (A) - leukocytes (CD45+); (B) - neutrophils (CD45+ CD11b+ Ly6G+) and (C) - eosinophils (CD45+ CD11b+ SiglecF+). Significant differences between infected and contralateral control pinnae are shown. 2-way ANOVA; Sidak test; $n = 0-4$; hpi – hours post infection; dpi – days post infection; DEC – dermal exudate cells; NA – not available

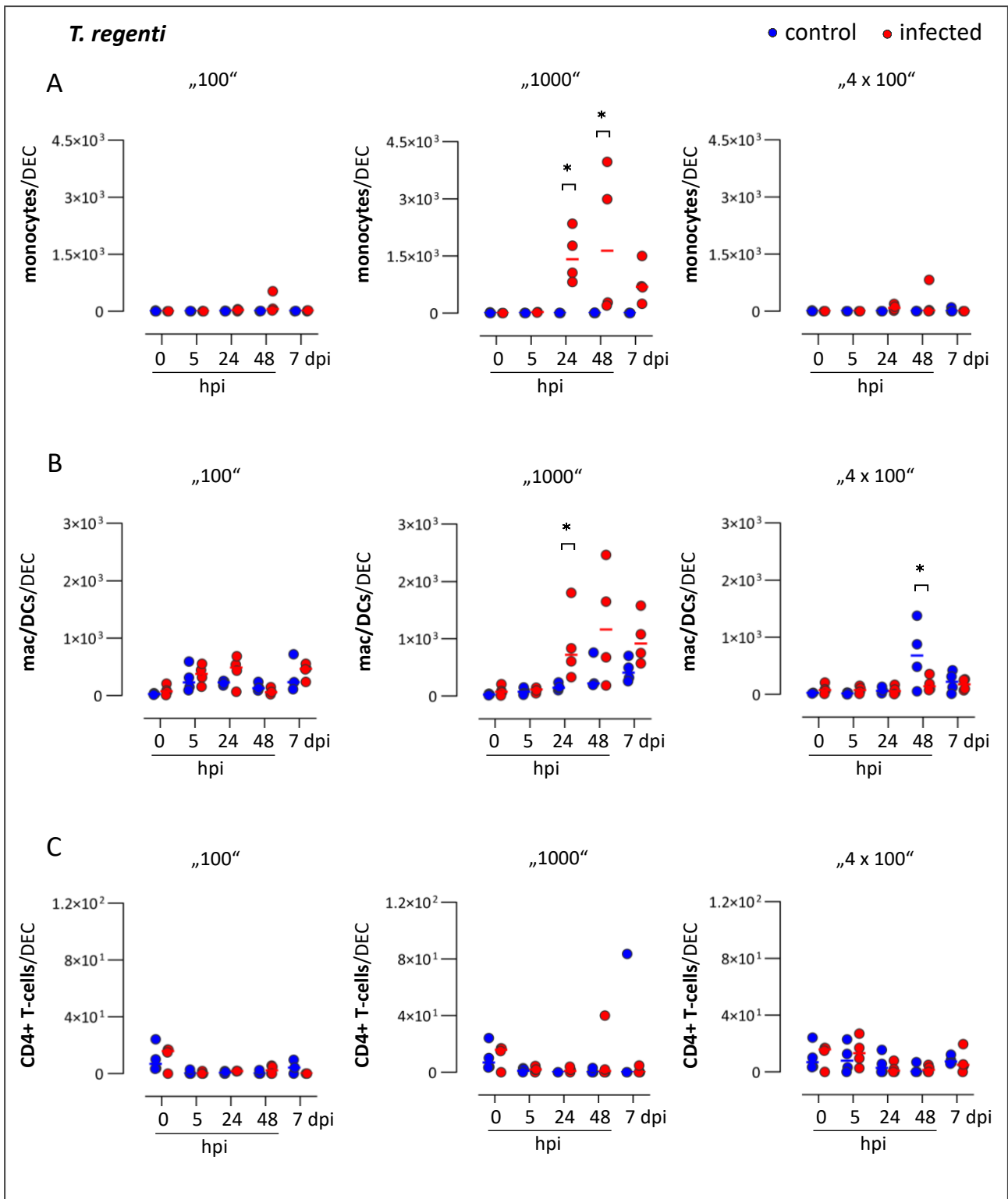


Figure S16. Dynamics of leukocyte populations in DEC of mice infected by 100, 1000 or 4x100 cercariae of *T. regenti* expressed in absolute number of cells defined by flow cytometry. (A) - monocytes (CD45+ CD11b+ Ly6C+); (B) - macrophages/DCs (CD45+ CD11b+ F4/80+) and (C) - CD4+ T-cells (CD45+ CD11b- CD4+). Significant differences between infected and contralateral control pinnae are shown. 2-way ANOVA; Sidak test; $n = 3-4$; hpi – hours post infection; dpi – days post infection; mac – macrophages, DCs – dendritic cells, DEC – dermal exudate cells

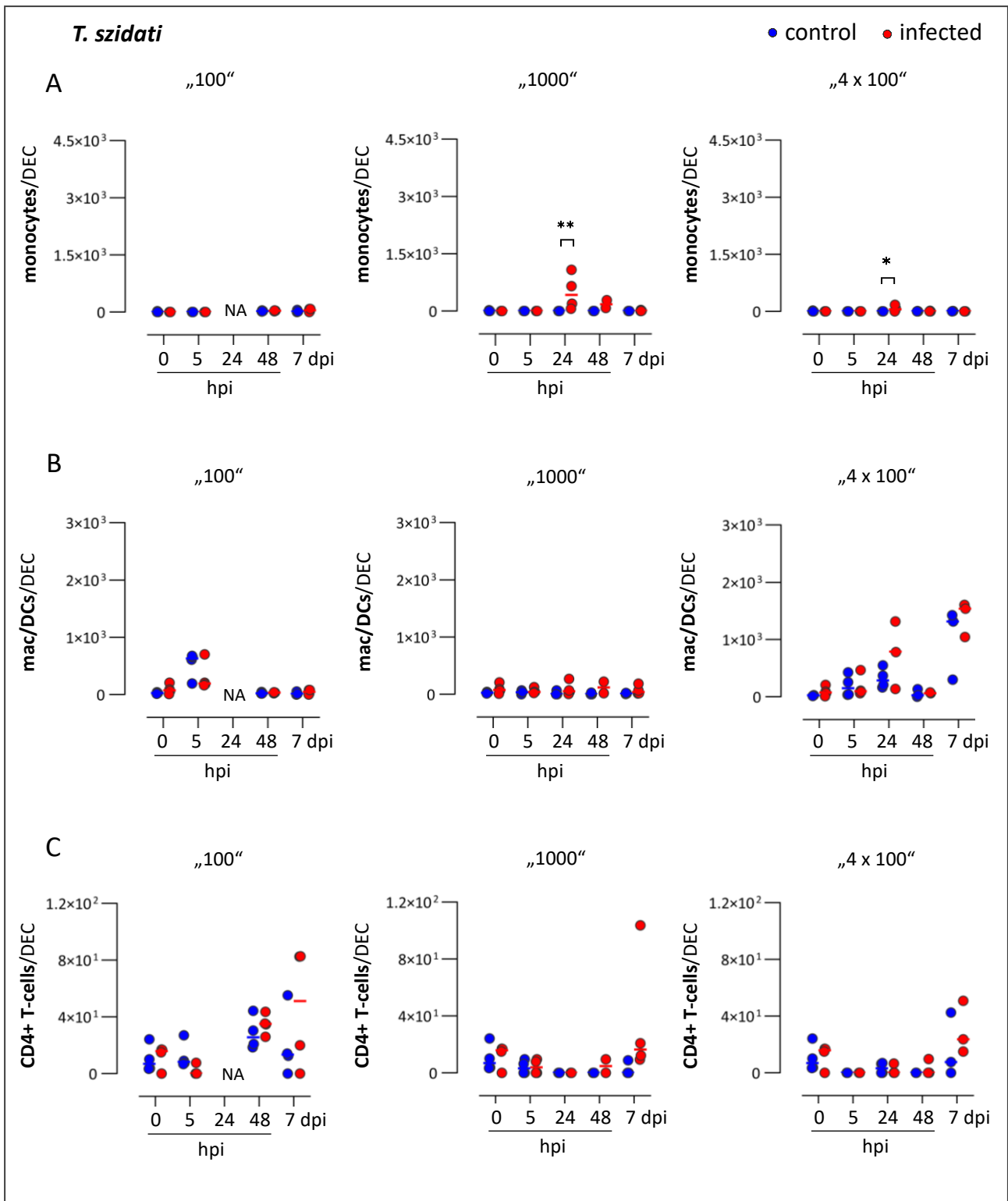


Figure S17. Dynamics of leukocyte populations in DEC of mice infected by 100, 1000 or 4x100 cercariae of *T. szidati* expressed in absolute number of cells defined by flow cytometry. (A) - monocytes (CD45+ CD11b+ Ly6C+); (B) - macrophages/DCs (CD45+ CD11b+ F4/80+) and (C) - CD4+ T-cells (CD45+ CD11b- CD4+). Significant differences between infected and contralateral control pinnae are shown. 2-way ANOVA; Sidak test; $n = 0-4$; hpi – hours post infection; dpi – days post infection; mac – macrophages, DCs – dendritic cells; DEC – dermal exudate cells; NA – not available

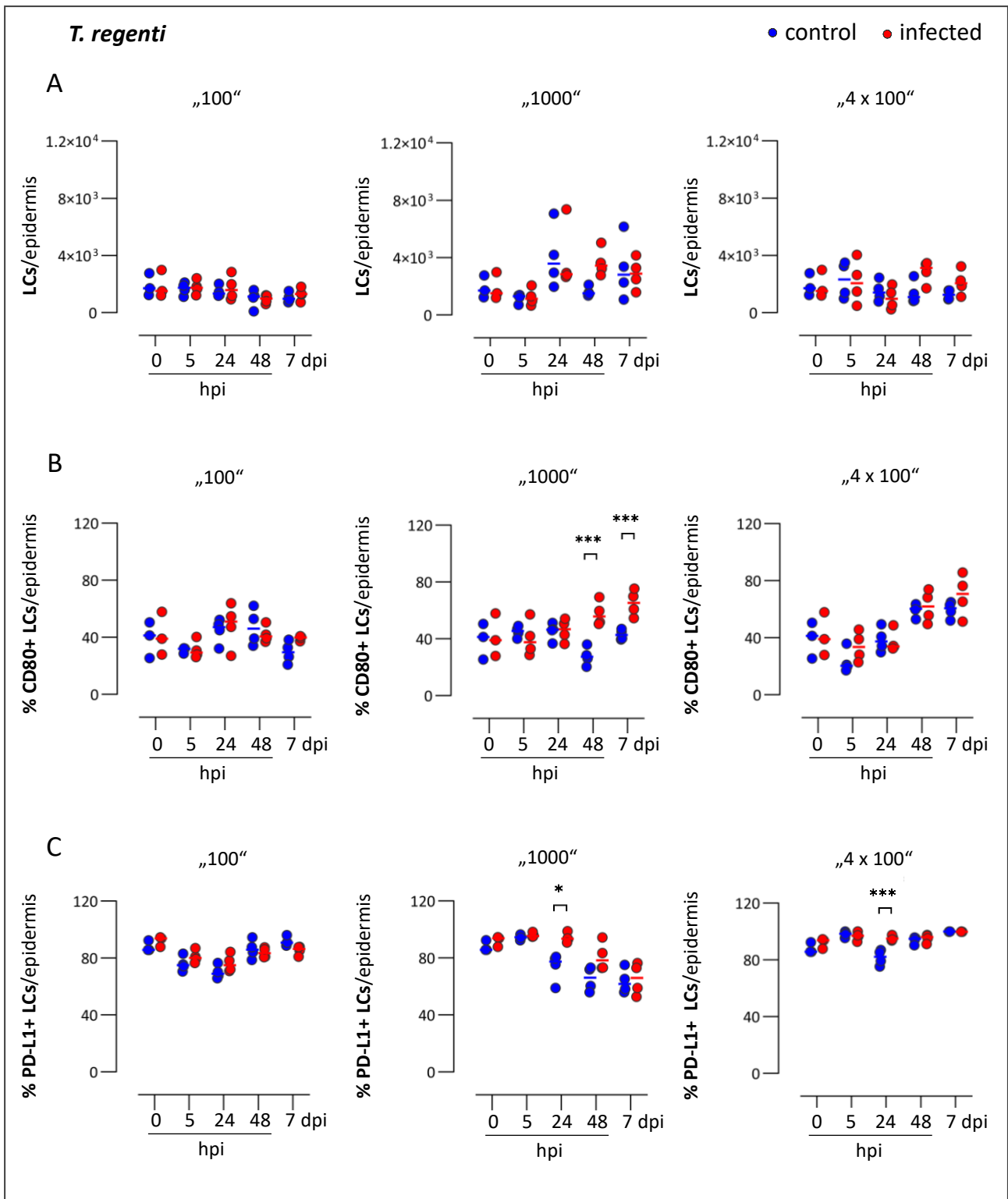


Figure S18. Dynamics of LCs and their activation status in the epidermis of mice infected by 100, 1000 or 4x100 cercariae of *T. regenti* were detected by flow cytometry. (A) - LCs (CD11c+ MHCII+ C207+); (B) – proportion of CD80+ LCs; (C) - proportion of PD-L1+ LCs. Significant differences between infected and contralateral control pinnae are shown. 2-way ANOVA; Sidak test; $n = 3-4$; hpi – hours post infection; dpi – days post infection; LCs – Langerhans cells; PD-L1 – programmed death ligand 1

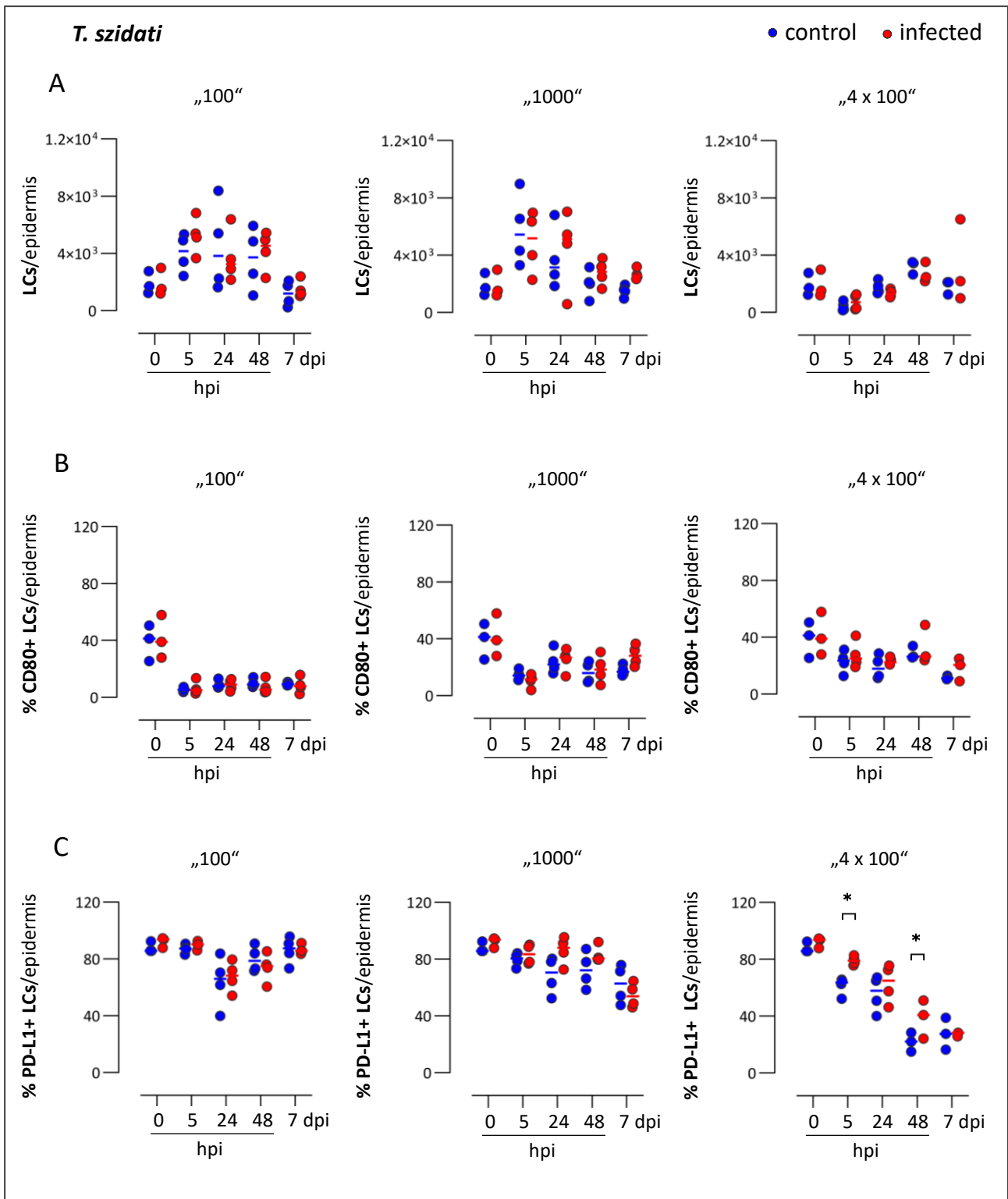


Figure S19. Dynamics of LCs and their activation status in the epidermis of mice infected by 100, 1000 or 4x100 cercariae of *T. szidati* were detected by flow cytometry. (A) - LCs (CD207+ CD11c+ MHCII+); (B) – proportion of CD80+ LCs; (C) - proportion of PD-L1+ LCs. Significant differences between infected and contralateral control pinnae are shown. 2-way ANOVA; Sidak test; $n = 3-4$; hpi – hours post infection; dpi – days post infection; LCs – Langerhans cells; PD-L1 – programmed death ligand 1

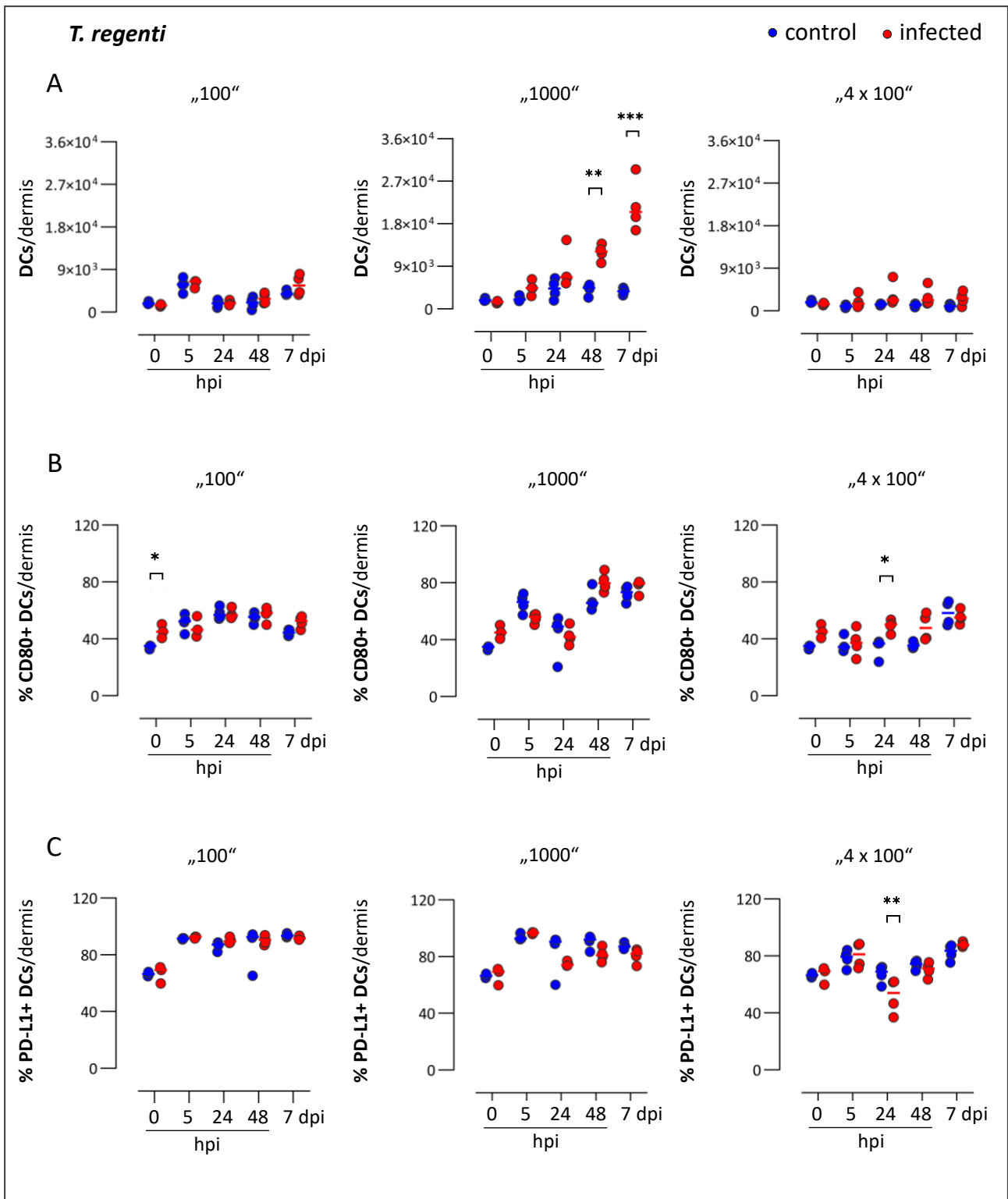


Figure S20. Dynamics of DCs and their activation status in the dermis of mice infected by 100, 1000 or 4x100 cercariae of *T. regenti* were detected by flow cytometry. (A) - DCs (CD11c+ MHCII+); (B) – proportion of CD80+ DCs; (C) - proportion of PD-L1+ DCs. Significant differences between infected and contralateral control pinnae are shown. 2-way ANOVA; Sidak test; $n = 3-4$; hpi – hours post infection; dpi – days post infection; DCs – dendritic cells; PD-L1 – programmed death ligand 1

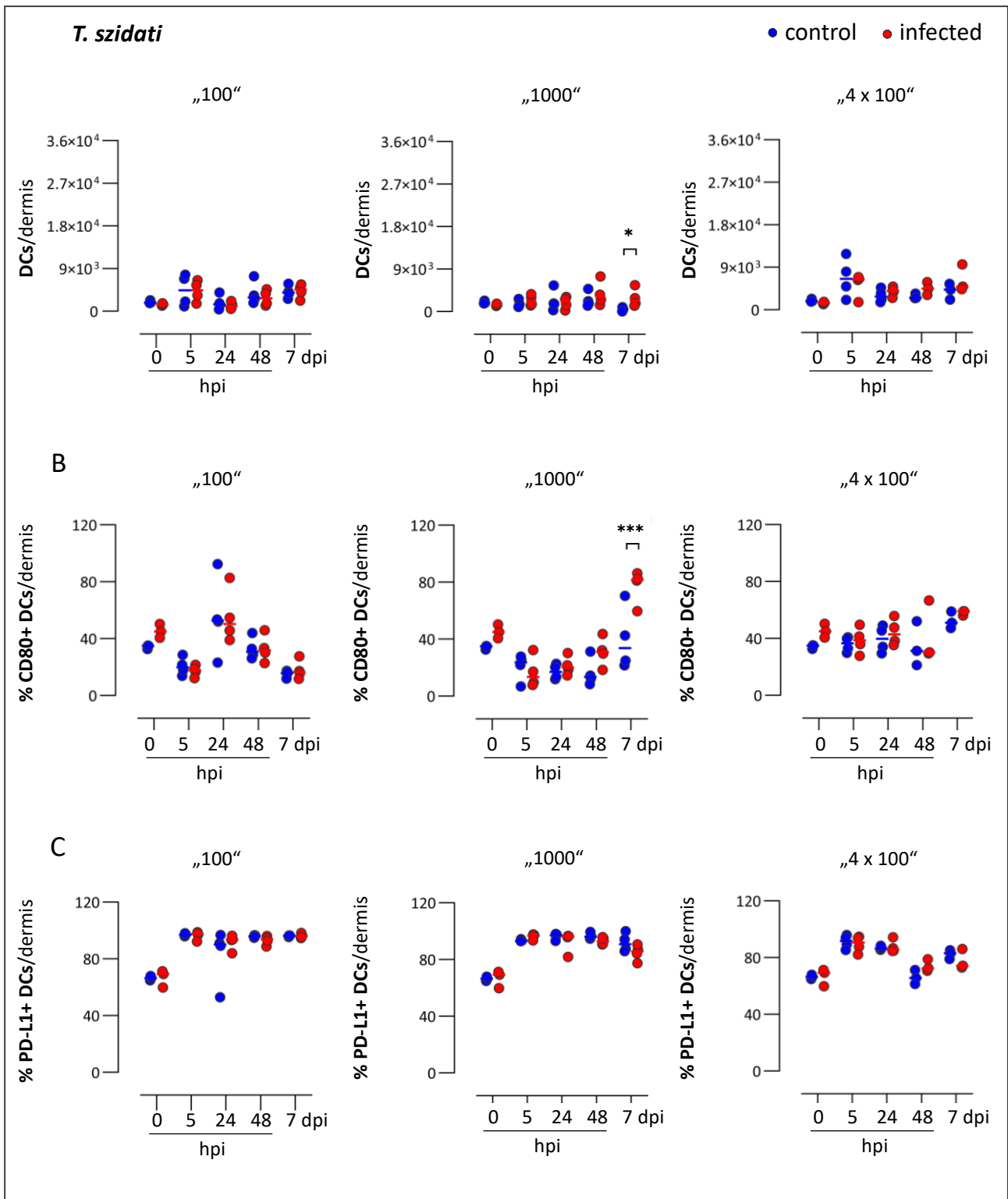


Figure S21. Dynamics of DCs and their activation status in the dermis of mice infected by 100, 1000 or 4x100 cercariae of *T. szidati* were detected by flow cytometry. (A) - DCs (CD45+ CD11c+ MHCII+); (B) - proportion of CD80+ DCs; (C) - proportion of PD-L1+ DCs. Significant differences between infected and contralateral control pinnae are shown. 2-way ANOVA; Sidak test; $n = 3-4$; hpi - hours post infection; dpi - days post infection; DCs - dendritic cells; PD-L1 - programmed death ligand 1

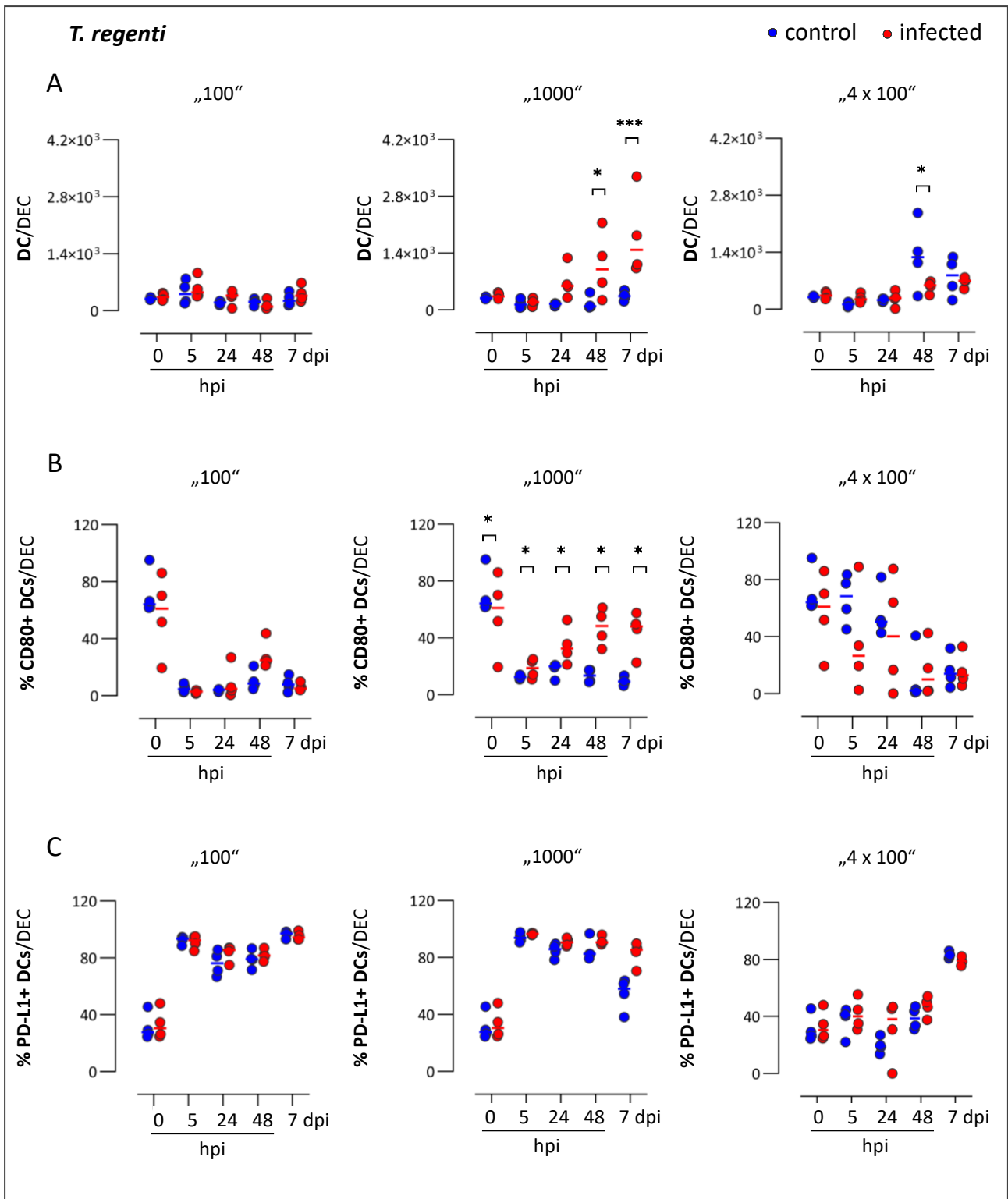


Figure S22. Dynamics of DCs and their activation status in DEC of mice infected by 100, 1000 or 4x100 cercariae of *T. regenti* were detected by flow cytometry. (A) - DCs (CD11c+ MHCII+); (B) – proportion of CD80+ DCs; (C) - proportion of PD-L1+ DCs. Significant differences between infected and contralateral control pinnae are shown. 2-way ANOVA; Sidak test; $n = 3-4$; hpi – hours post infection; dpi – days post infection; DCs – dendritic cells; PD-L1 – programmed death ligand 1; DEC – dermal exudate cells

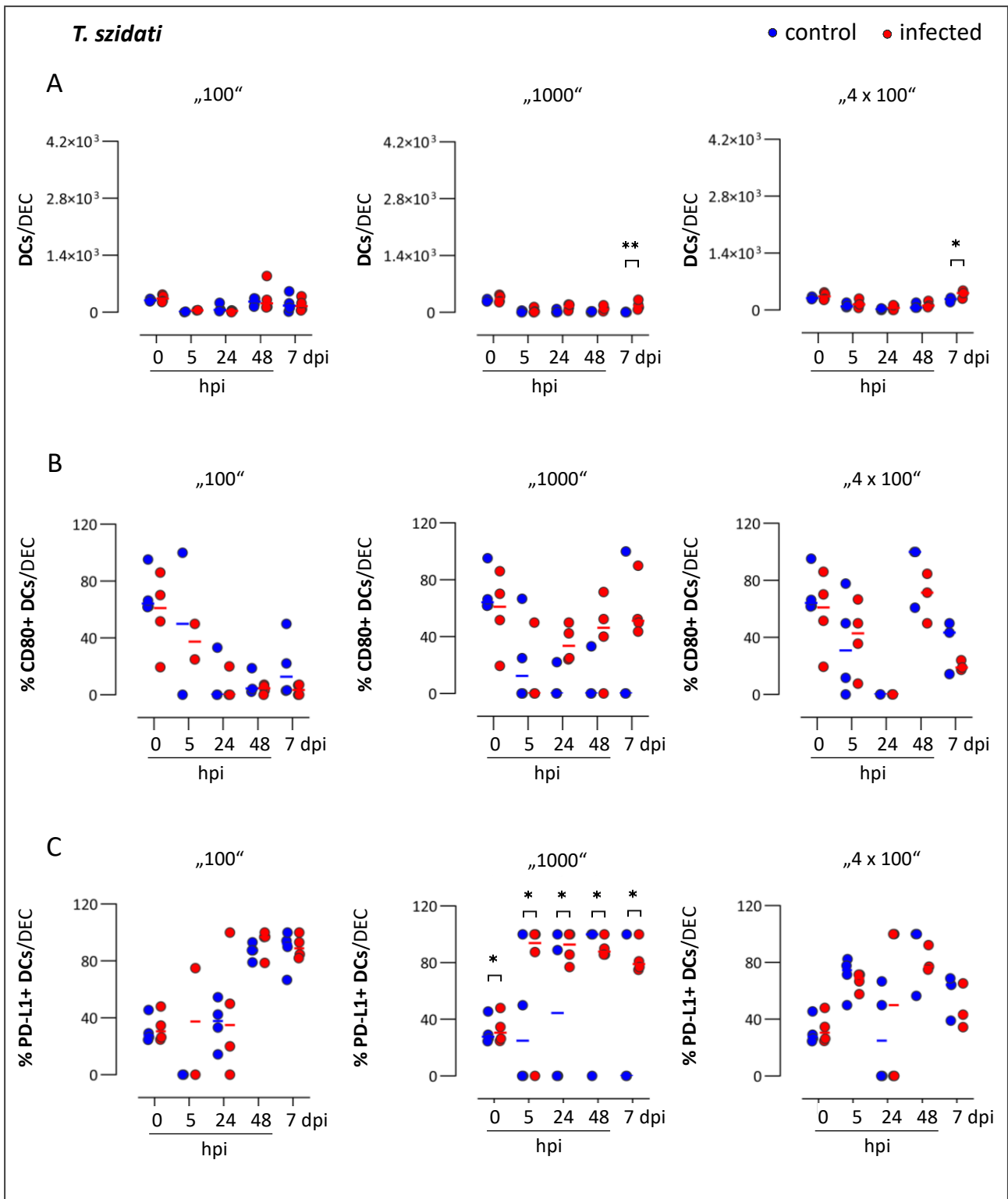


Figure S23. Dynamics of DCs and their activation status in the DEC of mice infected by 100, 1000 or 4x100 cercariae of *T. szidati* were detected by flow cytometry. (A) - DCs (CD11c+ MHCII+); (B) – proportion of CD80+ DCs; (C) - proportion of PD-L1+ DCs. Significant differences between infected and contralateral control pinnae are shown. 2-way ANOVA; Sidak test; $n = 3-4$; hpi – hours post infection; dpi – days post infection; DCs – dendritic cells; PD-L1 – programmed death ligand 1; DEC – dermal exudate cells