

Figure S1 Flow cytometry gating strategy used to define CD4⁺ T effector and central memory cells and the expression of transcription factors. Lymphocytes were gated based on FSC and SSC parameters and doublets were excluded (Single cells) in FSC-H versus FSC-A plots. Viable cells (Live cells) were defined as fixable viability dye (FVD) negative cells. CD4 T cells were defined as CD3⁺CD4⁺ and quadrants were set in contour plots, based on FMO stainings, to define effector memory (T_{EM}; CD44⁺CD62L⁻) and central memory (T_{CM}; CD44⁺CD62L⁺) cells. Gating of TEM cells expressing T-bet, RORγt and GATA-3 was defined based on FMO stainings. A similar analysis of the referred transcription factors was done gated in total CD44⁺ CD4⁺ T cells (T_{EM} plus T_{CM}). This is a representative example of lung cells isolated from a NcMP/CARB immunized mouse, 7 days i.p. post-infection with 1×10^7 *N. caninum* tachyzoites.

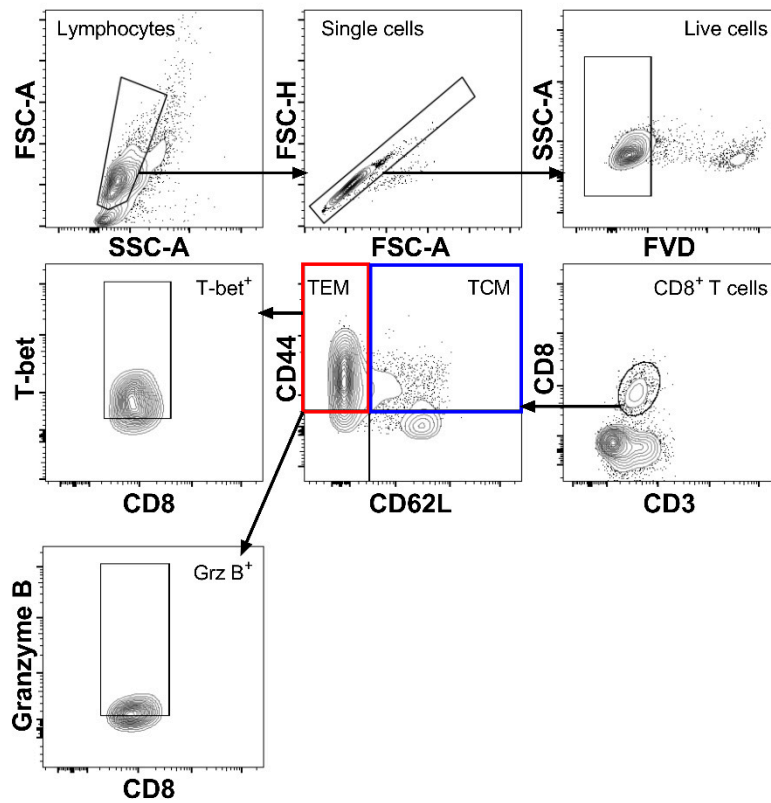


Figure S2 Flow cytometry gating strategy used to define CD8⁺ T effector and central memory cells and the expression of T-bet and Granzyme B. Lymphocytes were gated based on FSC and SSC parameters and doublets were excluded (Single cells) in FSC-H versus FSC-A plots. Viable cells (Live cells) were defined as fixable viability dye (FVD) negative cells. CD8⁺ T cells were defined as CD3⁺CD8⁺ and quadrants were set in contour plots, based on FMO stainings, to define effector memory (T_{EM}; CD44⁺CD62L⁻) and central memory (T_{CM}; CD44⁺CD62L⁺) cells. Gating of T_{EM} cells expressing T-bet (T-bet⁺) or granzyme B (Grz B⁺), was defined based on the respective FMO stainings. A similar analysis of T-bet- and granzyme B-expressing cells was done gated in total CD44⁺CD8⁺ T cells (T_{EM} plus T_{CM}). This is a representative example of lung cells isolated from a NcMP/CARB immunized mouse, 7 days i.p. post-infection with 1×10^7 *N. caninum* tachyzoites.

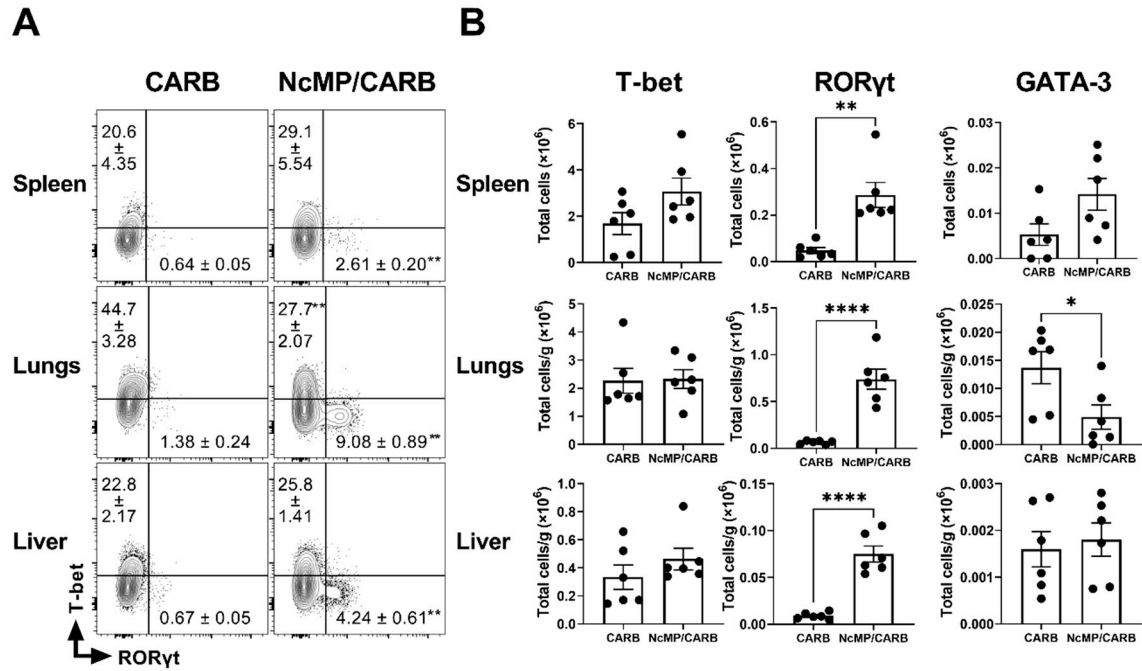


Figure S3: Expression of transcription factors T-bet and RORγt in CD4⁺ T_{EM} cells of infected mice. (A) Representative contour-plot analysis of T-bet and RORγt expression in CD4⁺ T cells T-bet in the indicated organs of sham-immunized (CARB) or immunized (NcMP/CARB) mice, as indicated, 7 days after i.p. challenged with 1×10⁷ *N. caninum* tachyzoites. Numbers within contour plots correspond to mean percentage values ± SEM of cells in the respective analysis regions. (B) Numbers of CD4⁺ T_{EM} cells expressing T-bet, RORγt and GATA-3, in the indicated organs of infected mice of NcMP/CARB and CARB mouse groups. Results are representative of two independent experiments that yielded concordant results (n=6 group; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001, Mann-Whitney).

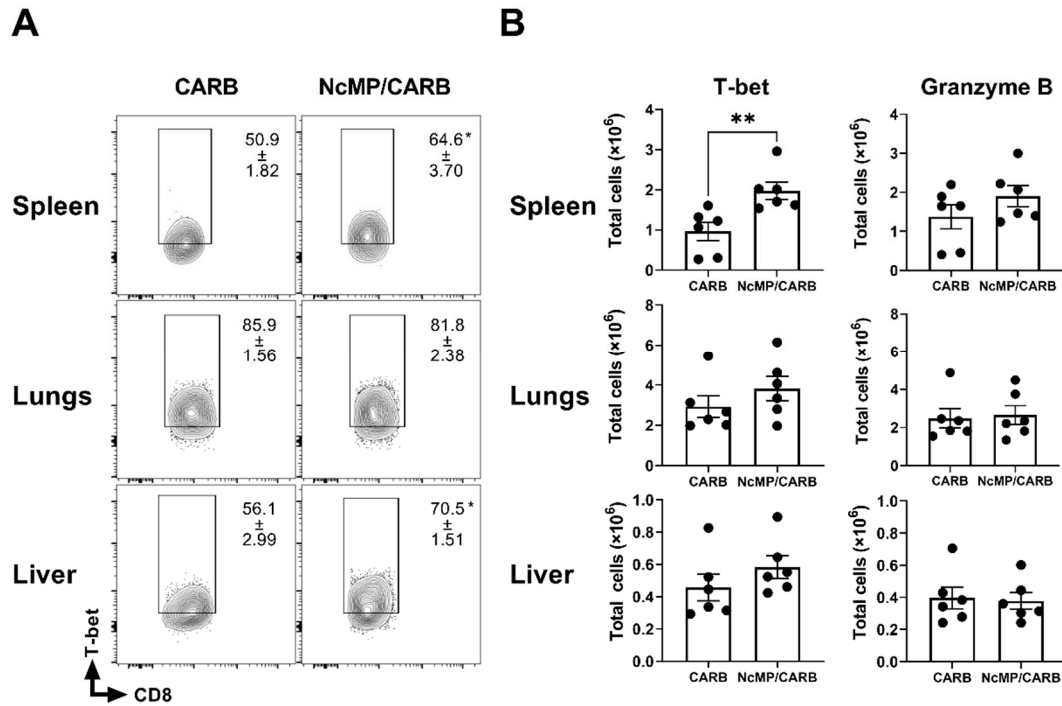


Figure S4 Expression of transcription factor T-bet and of granzyme B in CD8⁺ T_{EM} cells of infected mice. **(A)** Representative contour-plot analysis of gated CD8⁺ T_{EM} cells expressing T-bet in the indicated organs of sham-immunized (CARB) or immunized (NcMp/CARB) mice, as indicated, 7 days after i.p. challenged with 1×10⁷ *N. caninum* tachyzoites. Numbers within contour plots correspond to mean percentage values ± SEM of T-bet expressing cells within the CD8⁺ T cell population. **(B)** Numbers of CD8⁺T_{EM} T-bet⁺ or granzyme B⁺ cells in the indicated organs of immunized and sham-immunized mice. Results are representative of two independent experiments that yielded concordant results (n=6 group; **P*< 0.05; ***P*< 0.01, Mann-Whitney).