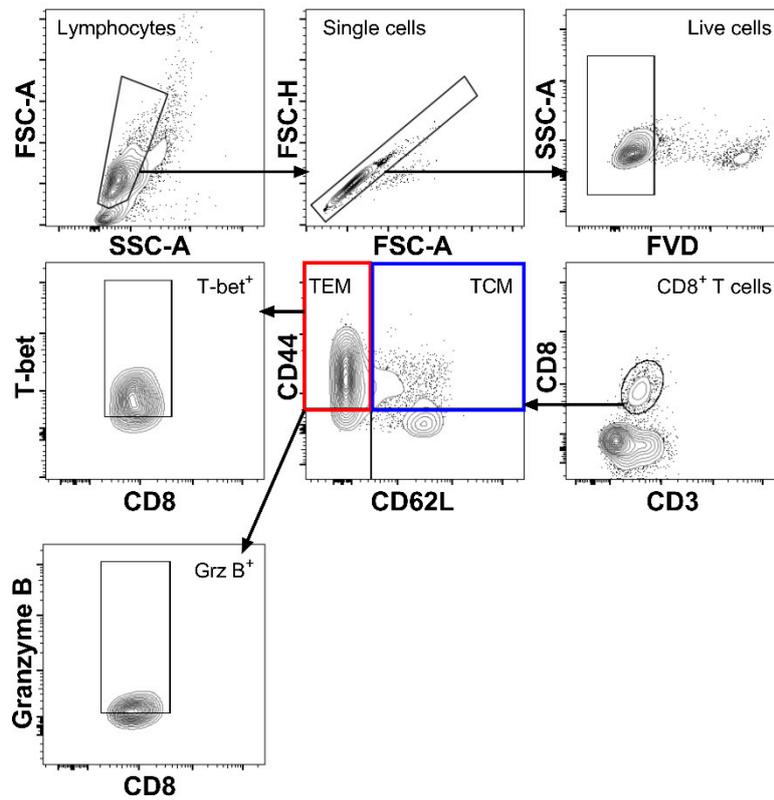
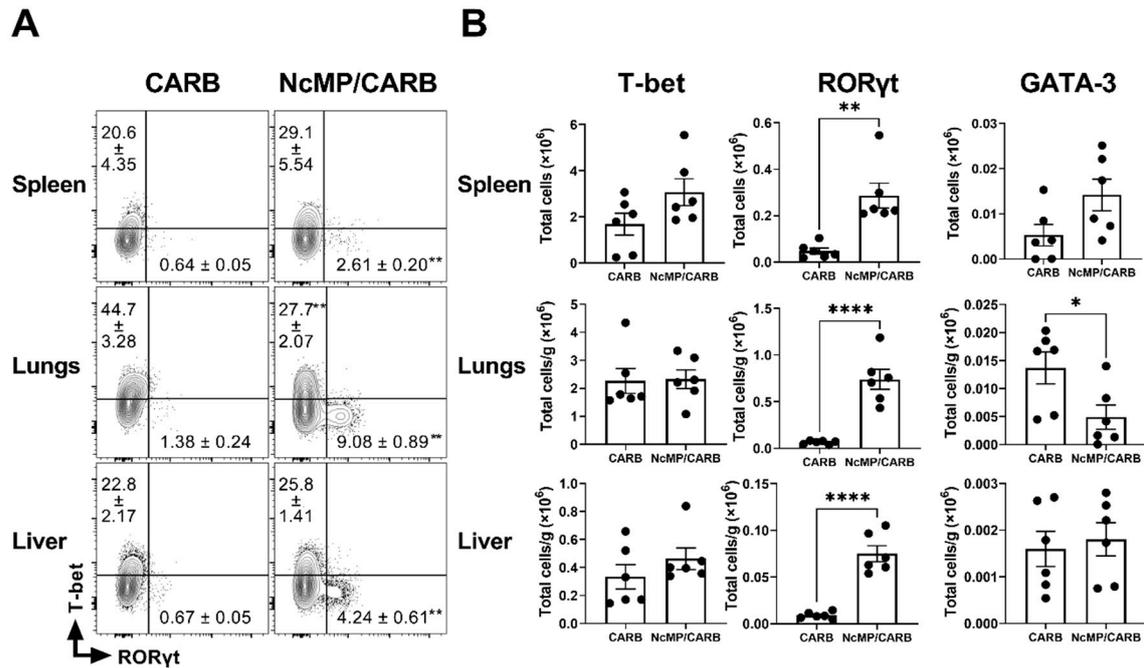


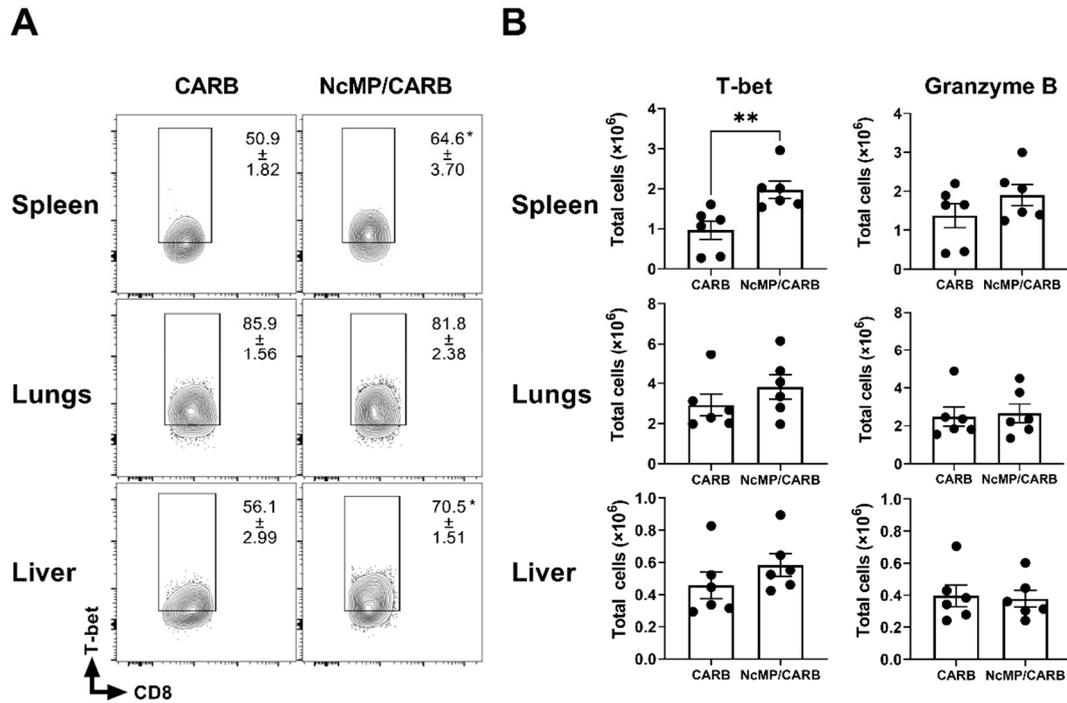
**Figure S1** Flow cytometry gating strategy used to define CD4<sup>+</sup> T effector and central memory cells and the expression of transcription factors. Lymphocytes were gated based on FSC and SSC parameters and doublets were excluded (Single cells) in FSC-H versus FSC-A plots. Viable cells (Live cells) were defined as fixable viability dye (FVD) negative cells. CD4 T cells were defined as CD3<sup>+</sup>CD4<sup>+</sup> and quadrants were set in contour plots, based on FMO stainings, to define effector memory (TEM; CD44<sup>+</sup>CD62L<sup>-</sup>) and central memory (TCM; CD44<sup>+</sup>CD62L<sup>+</sup>) 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<sup>+</sup> CD4<sup>+</sup> T cells (TEM plus TCM). This is a representative example of lung cells isolated from a NcMP/CARB immunized mouse, 7 days i.p. post-infection with  $1 \times 10^7$  *N. caninum* tachyzoites.



**Figure S2** Flow cytometry gating strategy used to define CD8<sup>+</sup> 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<sup>+</sup> T cells were defined as CD3<sup>+</sup>CD8<sup>+</sup> and quadrants were set in contour plots, based on FMO stainings, to define effector memory (T<sub>EM</sub>; CD44<sup>+</sup>CD62L<sup>-</sup>) and central memory (T<sub>CM</sub>; CD44<sup>+</sup>CD62L<sup>+</sup>) cells. Gating of T<sub>EM</sub> cells expressing T-bet (T-bet<sup>+</sup>) or granzyme B (Grz B<sup>+</sup>), 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<sup>+</sup>CD8<sup>+</sup> T cells (T<sub>EM</sub> plus T<sub>CM</sub>). This is a representative example of lung cells isolated from a NcMP/CARB immunized mouse, 7 days i.p. post-infection with 1 × 10<sup>7</sup> *N. caninum* tachyzoites.



**Figure S3:** Expression of transcription factors T-bet and ROR $\gamma$ t in CD4<sup>+</sup> T<sub>EM</sub> cells of infected mice. (A) Representative contour-plot analysis of T-bet and ROR $\gamma$ t expression in CD4<sup>+</sup> 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 1×10<sup>7</sup> *N. caninum* tachyzoites. Numbers within contour plots correspond to mean percentage values ± SEM of cells in the respective analysis regions. (B) Numbers of CD4<sup>+</sup> T<sub>EM</sub> cells expressing T-bet, ROR $\gamma$ t 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<sup>+</sup> T<sub>EM</sub> cells of infected mice. **(A)** Representative contour-plot analysis of gated CD8<sup>+</sup> T<sub>EM</sub> 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 1×10<sup>7</sup> *N. caninum* tachyzoites. Numbers within contour plots correspond to mean percentage values ± SEM of T-bet expressing cells within the CD8<sup>+</sup> T cell population. **(B)** Numbers of CD8<sup>+</sup>T<sub>EM</sub> T-bet<sup>+</sup> or granzyme B<sup>+</sup> 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).