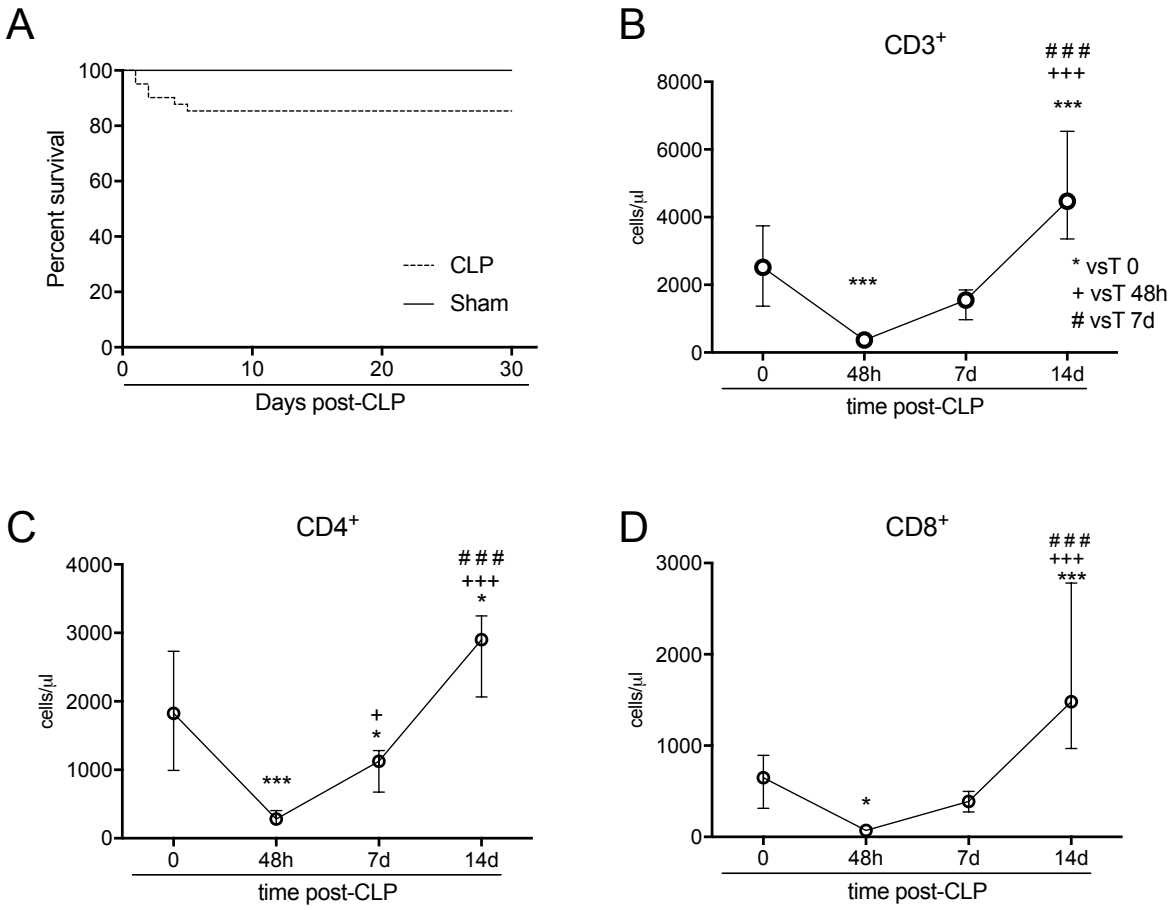

Figure S1. Dynamics of the recovery of circulating T cells after sepsis.

A. Kaplan-Meier survival curves of the mild sepsis model induced by double puncture with 25G needle and antibiotic and fluid treatment of BALB/c mice (n=16).

B. Changes in the absolute counts of circulating $CD3^+$ T-cells.

C. Changes in the $CD4^+$ T-cells counts.

D. Changes in the $CD8^+$ T-cells counts. (n=13-16 per time-point). Comparison to Time0: *p<0.05, ***p<0.001; comparison to T48h: +p<0.05, +++p<0.001; comparison to T7d: ###p<0.001 using ANOVA with Tukey post-hoc test. Mean ±SD values are shown.

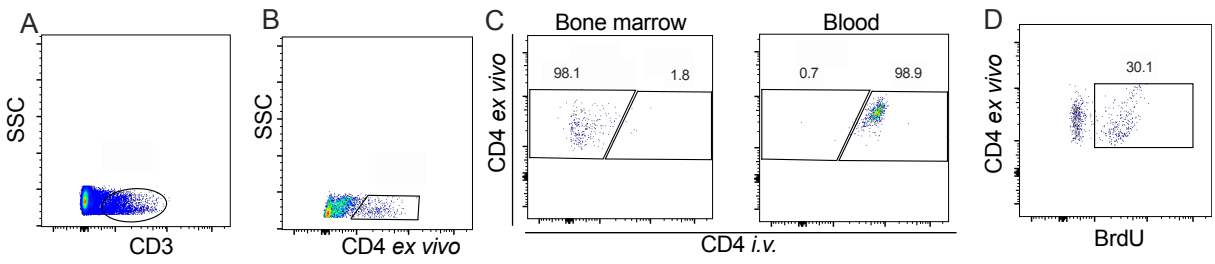

Figure S2. Bone marrow proliferating CD4⁺ T-cells are tissue-resident. Septic mice were injected on day 6 with a bolus of BrdU and on day 7 after CLP were administered *i.v.* with anti-CD4-APC antibody (CD4 *i.v.*) and were sacrificed 3 minutes later. Bone marrow was flushed out from the femurs and stained with another clone and fluorochrome anti-CD4-eFluor450 antibody (*ex vivo*), then the cells were stained for BrdU. As an internal control of appropriate *in vivo* labeling, blood sample was concomitantly analyzed.
A Representative cytograms from 5 experiments are presented.
A. CD3⁺ T cells are gated first,
B. The *ex vivo* stained CD4⁺ T-cells population (all CD4⁺ T-cells) is gated and analyzed in
C. as all CD4⁺ T cells (*ex vivo* stained) vs. *i.v.* stained CD4⁺ T-cells. D. CD4 *i.v.*-negative cells from the bone marrow are shown.

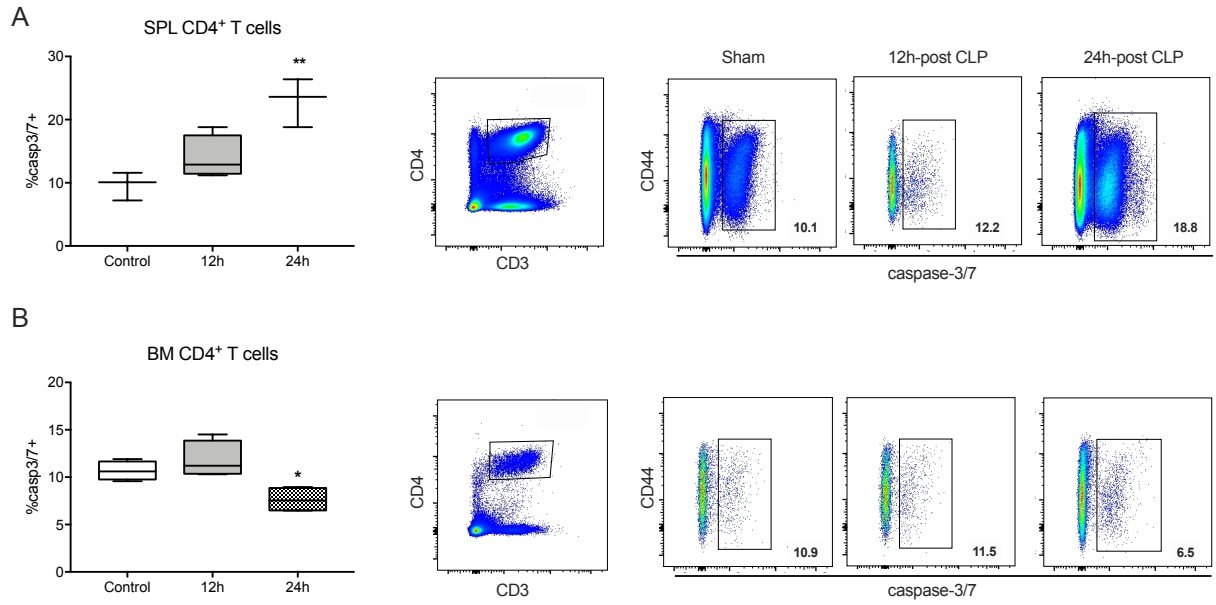

Figure S3. Sepsis induces apoptosis of CD4⁺ T-cells in the spleen, but not in the bone marrow. Cells were collected from the spleens and femurs of control and septic mice at indicated times after CLP and stained with Vybrant FAM caspase-3/-7 reagent.
A.The results and gating strategy of the analysis of caspase-3/-7 activity in the splenic CD4⁺ T-cells (n=5 per group)
B.The results and gating strategy of the analysis of caspase-3/-7 activity in bone marrow CD4⁺ T-cells (n=5 per group). Box and whiskers graphs present p25-p75 (box), mean and p10-p90 (whiskers). *p<0.05, **p<0.01 using Anova with Tukey post-hoc test.

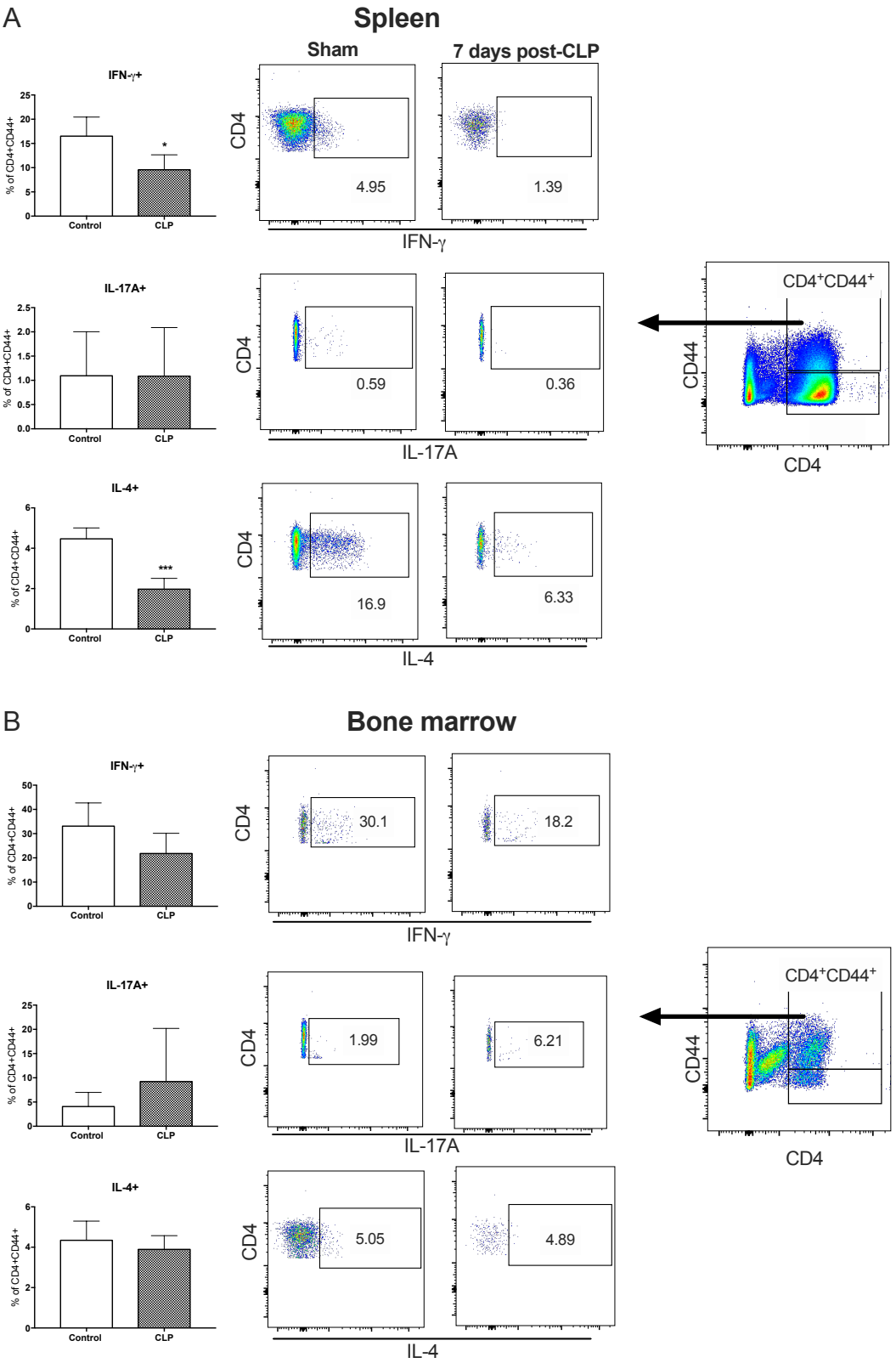

Figure S4. Phenotype of the memory CD4⁺ T-cells in the spleen and bone marrow during sepsis. Isolated cells were stimulated with PMA, ionomycin and brefeldin A for 5 hours and stained intracellularly.

A. The results and gating strategy for splenocytes (n=5 per group)

B. The results and gating strategy for bone marrow cells (n=5 per group). *p<0.05, ***p<0.001 using Student's *t*-test.

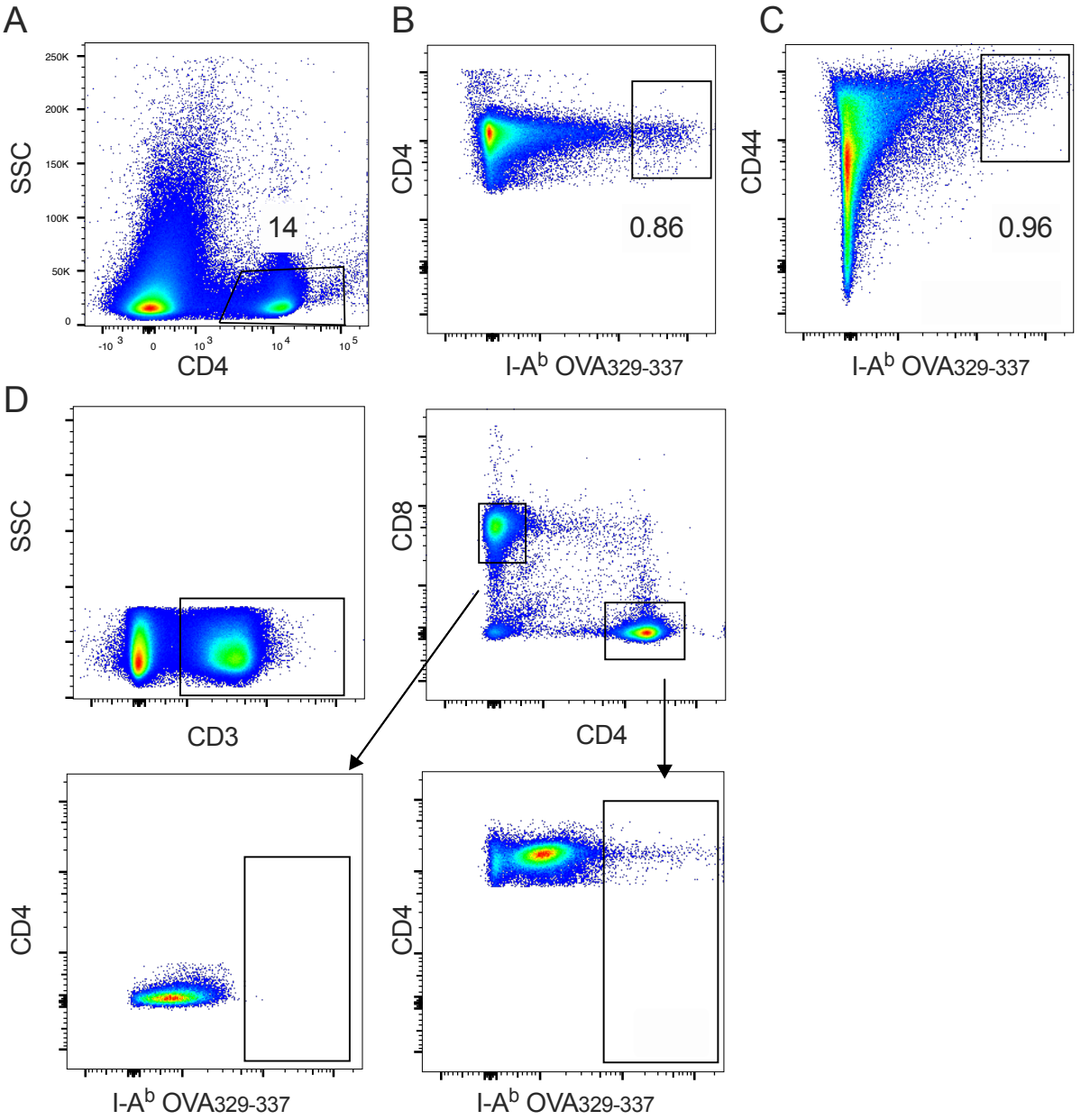

Figure S5. Tetramer-based analysis of transferred OT-II T-cells. Additional gating strategy to the Figure 4 is shown to confirm that 30 days after immunization the B. I-A^b tetramer-positive CD4$^+$ T-cells C. are also CD44$^+$ after immunization with ovalbumin D. tetramer-enriched gating strategy with internal control from CD8$^+$ T-cells showing no I-A^b-specific staining.

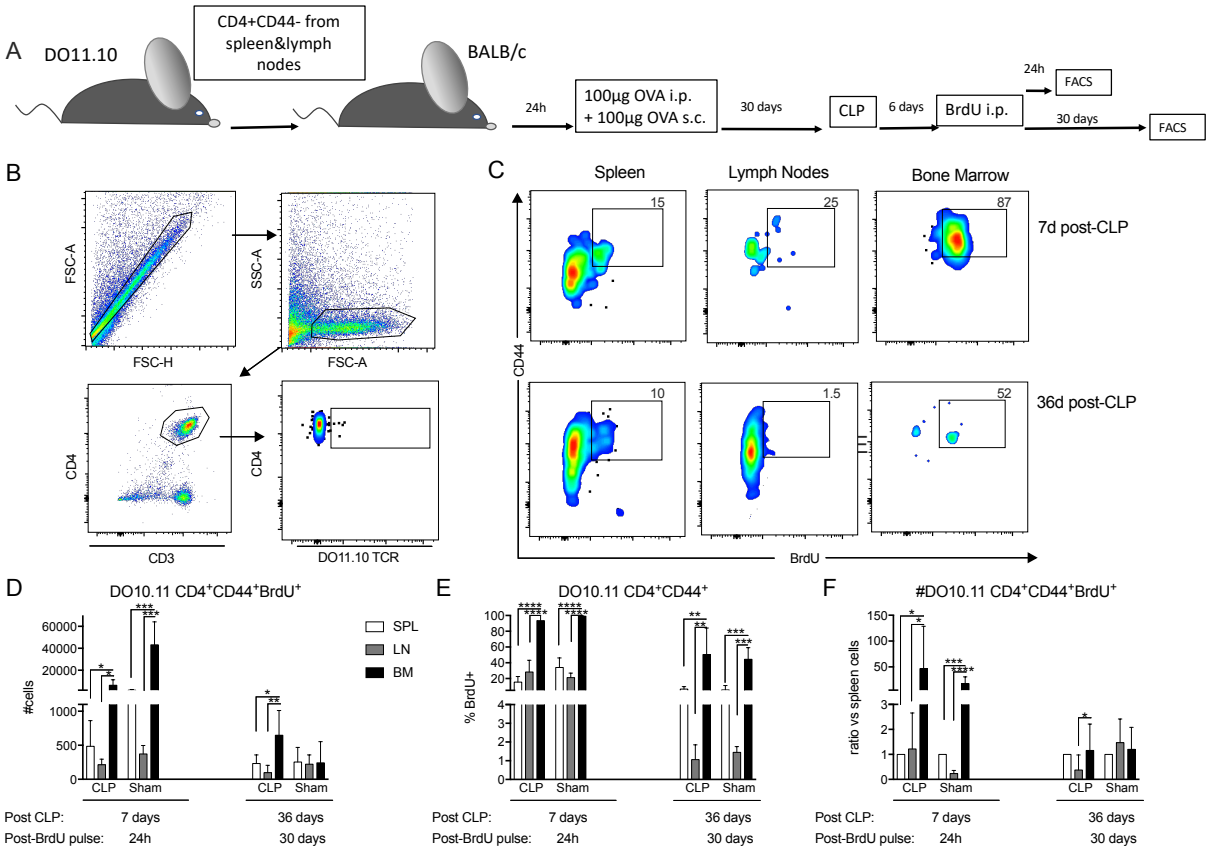

Figure S6. Bone marrow supports proliferation of DO11.10 specific antigen-experienced memory CD4⁺ T-cells in sepsis.

A. Experimental design. $CD4^+CD44^-$ naïve T-cells were isolated from young DO11.10 mice and transferred to BALB/c recipients. Then, mice were immunized with 100 µg of ovalbumin *i.p.* and 100 µg *s.c*. Thirty days later mice were subjected to CLP and received bolus of BrdU on day 6^{th} after surgery. Cells were analyzed 24 hours or 30 days later by flow cytometry.

B. Plots showing flow cytometry gating strategy of the transplanted DO10.11 TCR-Tg $CD4^+$ T-cells after nanobeads enrichment.

C. Representative flow cytometry plots showing analysis of $BrdU^+$ cells that are $CD4^+$TCR $DO10.11^+CD44^+$ from mice that had CLP surgery 7 days (upper row) and 36 days (lower row) before.

D. Total number of the TCR $DO10.11^+CD4^+BrdU^+$ cells in lymph nodes, spleen and bone marrow of mice post-CLP and sham mice at different time after BrdU injection.

E. Percentage of $BrdU^+$ cells among the TCR $DO10.11^+CD4^+$ cells in lymph nodes, spleen and bone marrow of mice post-CLP and sham mice at different time after BrdU injection.

F. Normalized ratios of the number of TCR $DO10.11^+CD4^+BrdU^+$ cells in lymph nodes and bone marrow versus the number of TCR $DO10.11^+CD4^+BrdU^+$ in the spleen. The results are from mice post-CLP and sham mice at different time after BrdU injection. Data from two independent experiments (n=6-8 in each group). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 using Anova with Tukey post-hoc test.