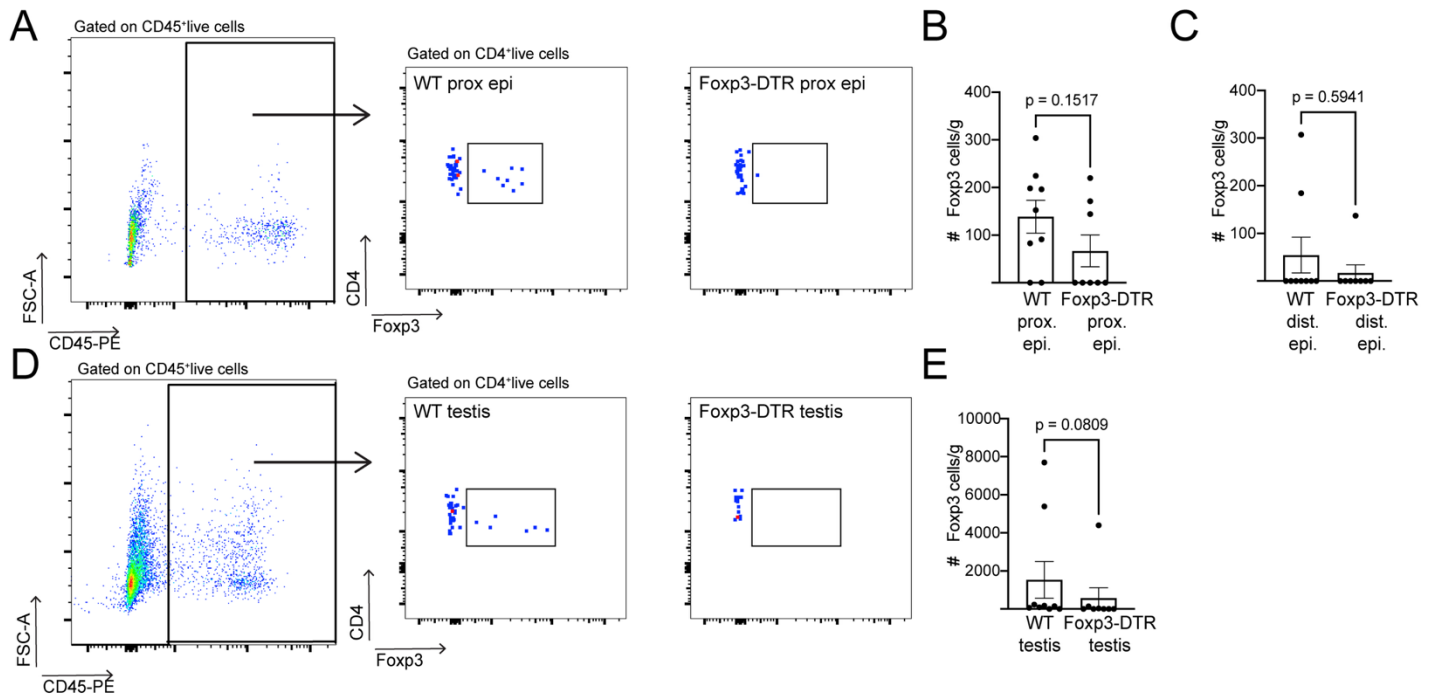


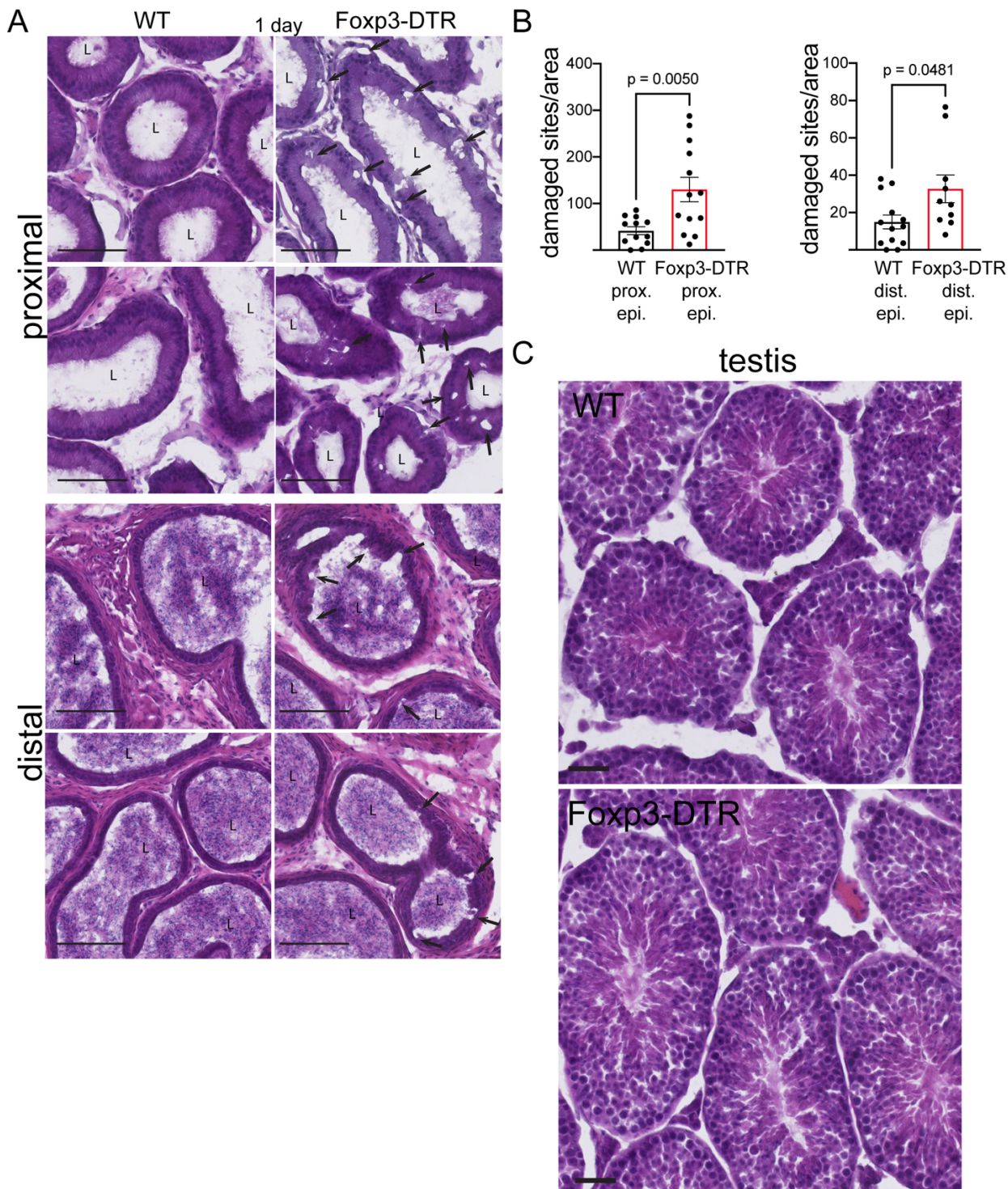
Chronic inflammation drives epididymal tertiary lymphoid structure formation and autoimmune fertility disorders in mice

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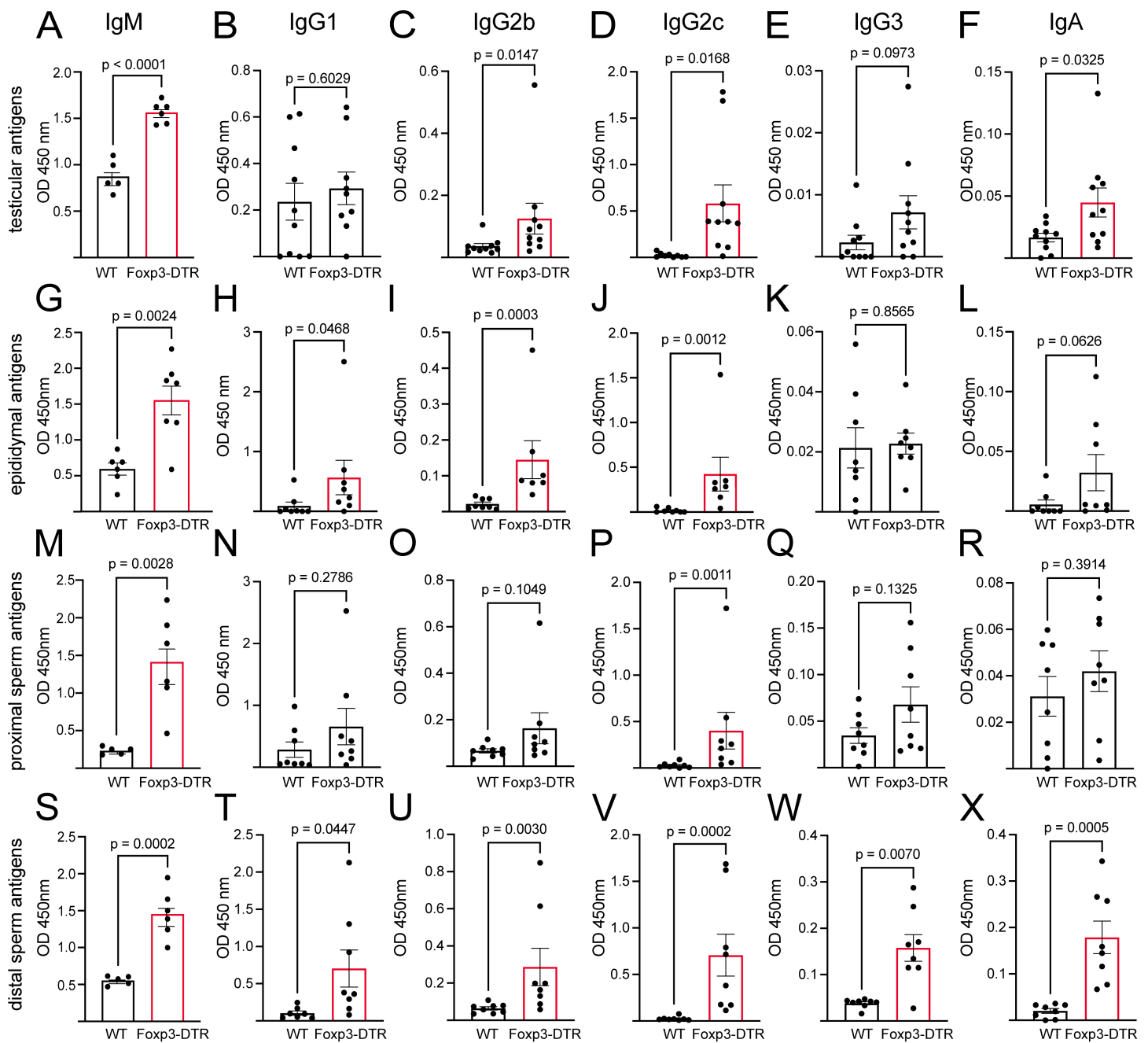
Supplementary Information:



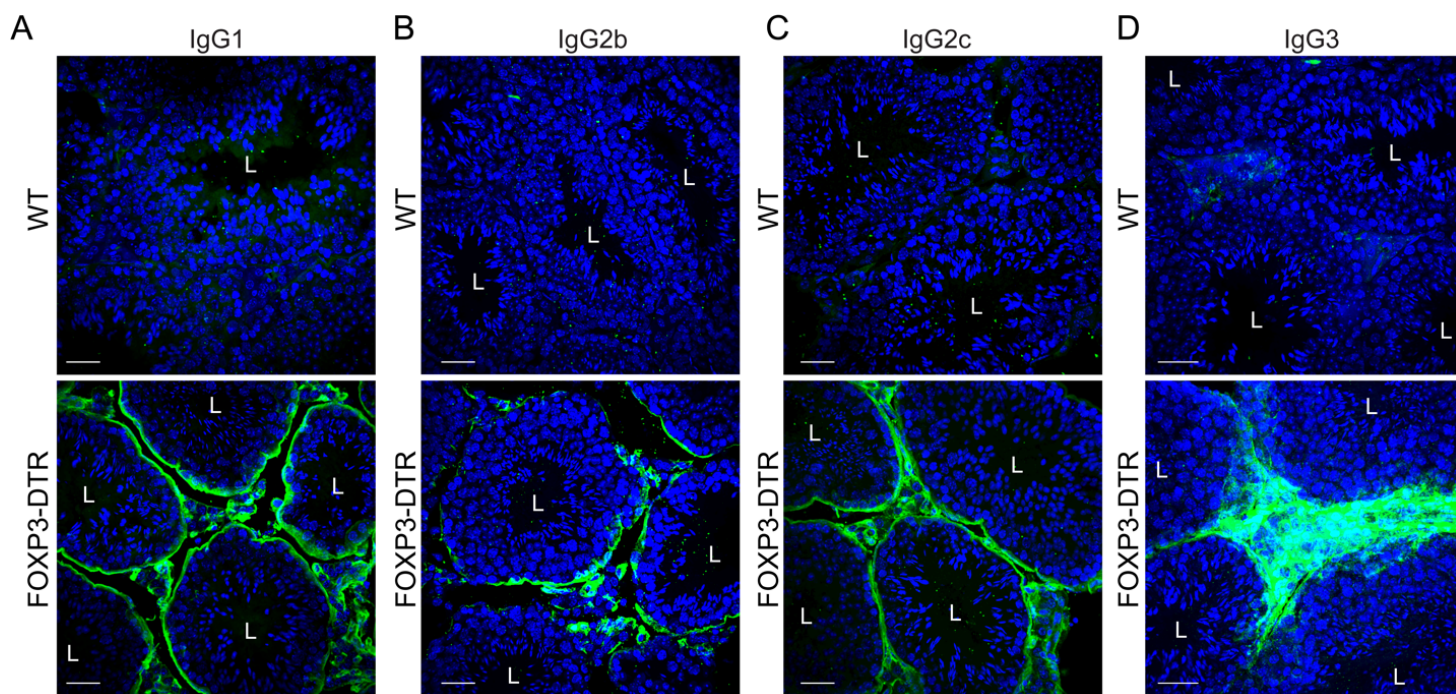
Supplementary Figure 1. Tissue-circulating Treg population in the epididymis and testis 1 day after DT treatment. **A)** Flow cytometry gating strategy and analysis of circulating Tregs (CD45-PE⁺Fxp3⁺CD4⁺CD45-BV711⁺) per gram of proximal **(B)** and distal epididymis **(C)**, and testis **(D-E)**. Each dot represents a pool of 2 organs from each mouse. n=9 (WT), n=8 (Fxp3-DTR). Data were analyzed using a two-sided Mann-Whitney test. Data are shown as means $\pm$ SEM. n= number of samples from different mice.



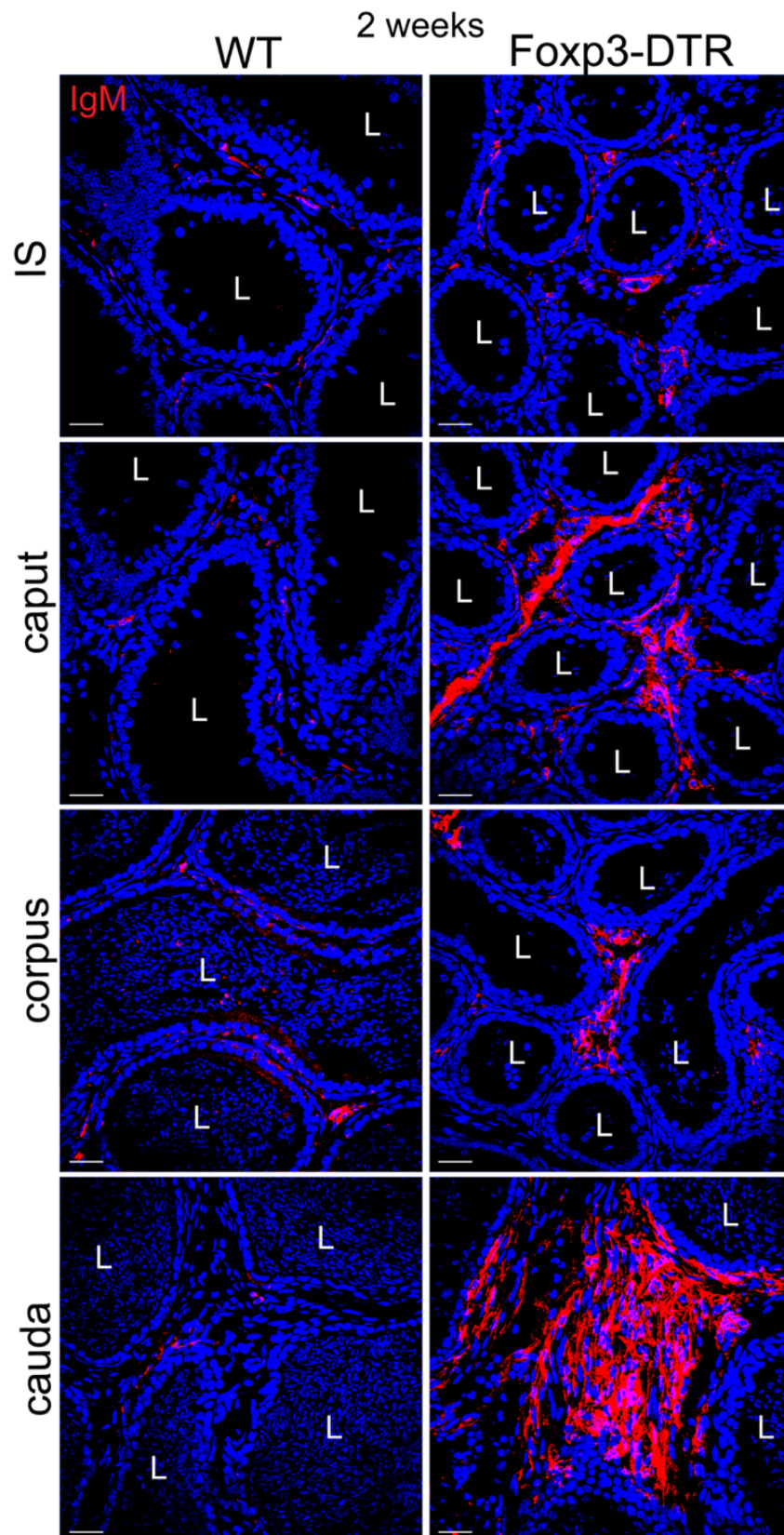
Supplementary Figure 2. Histological evaluation of epididymis and testis of WT and Foxp3-DTR mice 1 day after DT treatment. **A)** H&E staining of different epididymal regions: proximal (initial segments (IS) and caput) and distal (cauda) of DT-injected WT and Foxp3-DTR mice. $n=4$ epididymides from different mice. **B)** Quantification of the number of damaged sites normalized per tissue area (nm^2). Any morphological alteration in epididymal epithelia (arrows) was counted as a damaged site. Each area quantified is represented as a dot. Bars: 100 μm . L: Lumen. Data were analyzed using the two-sided Student's t-test (B, left panel) or the Mann-Whitney test (B, right panel). Data are shown as means $\pm$ SEM. $n=12$ (WT prox.), $n=13$ (Foxp3-DTR prox., WT dist.), $n=10$ (Foxp3-DTR dist.) from 4 from different mice. **C)** H&E staining in the testis of DT-injected WT and Foxp3-DTR mice. A complete process of spermatogenesis was observed in both groups. $n=4$ epididymides from different mice. Bars: 20 μm .



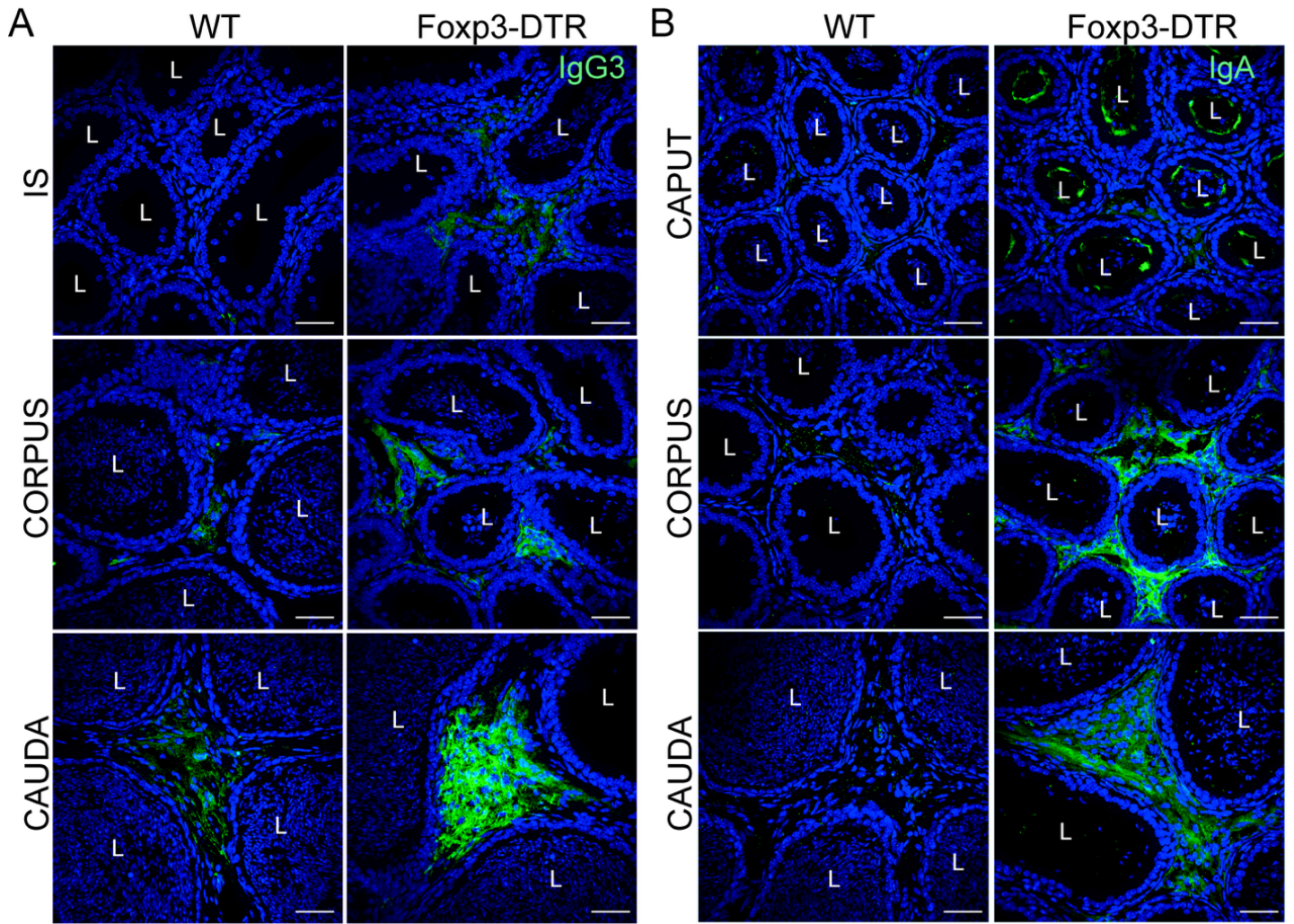
Supplementary Figure 3. Serum autoantibody isotypification of Foxp3-DTR mice 2 weeks after DT treatment by ELISA. Serum IgM, IgG1, IgG2b, IgG2c, IgG3, and IgA levels of DT-injected Foxp3-DTR and WT mice against testicular antigens (A-F), epididymal antigens (G-L), proximal sperm antigens (M-R), and distal sperm antigens (S-X). A: n=5 (WT: IgM), n=6 (Foxp3-DTR: IgM), n=10 (IgG2b, IgG3, IgA; WT: IgG1; Foxp3-DTR: IgG2c), n=9 (Foxp3-DTR: IgG1; WT: IgG2c). B: n=6 (WT: IgM), n=7 (Foxp3-DTR: IgM, IgG2b, IgG2c; WT: IgG3), n=8 (IgG1, IgG3, IgA; WT: IgG2b, IgG2c). C: n=5 (WT: IgM), n=6 (Foxp3-DTR: IgM), n=8 (IgG1, IgG2b, IgG2b, IgG3, IgA). D: n=5 (WT: IgM), n=6 (Foxp3-DTR: IgM), n=7 (WT: IgG1), n=8 (IgG2b, IgG2c, IgG3, IgA; Foxp3-DTR: IgG1). Data were analyzed using two-sided Student's t-test (A,D,E,G,K,M,Q-T,X) or Mann-Whitney test (B,C,F,H-J,L,N-P,U-W). Data are shown as means $\pm$ SEM. n= number of samples from different mice.



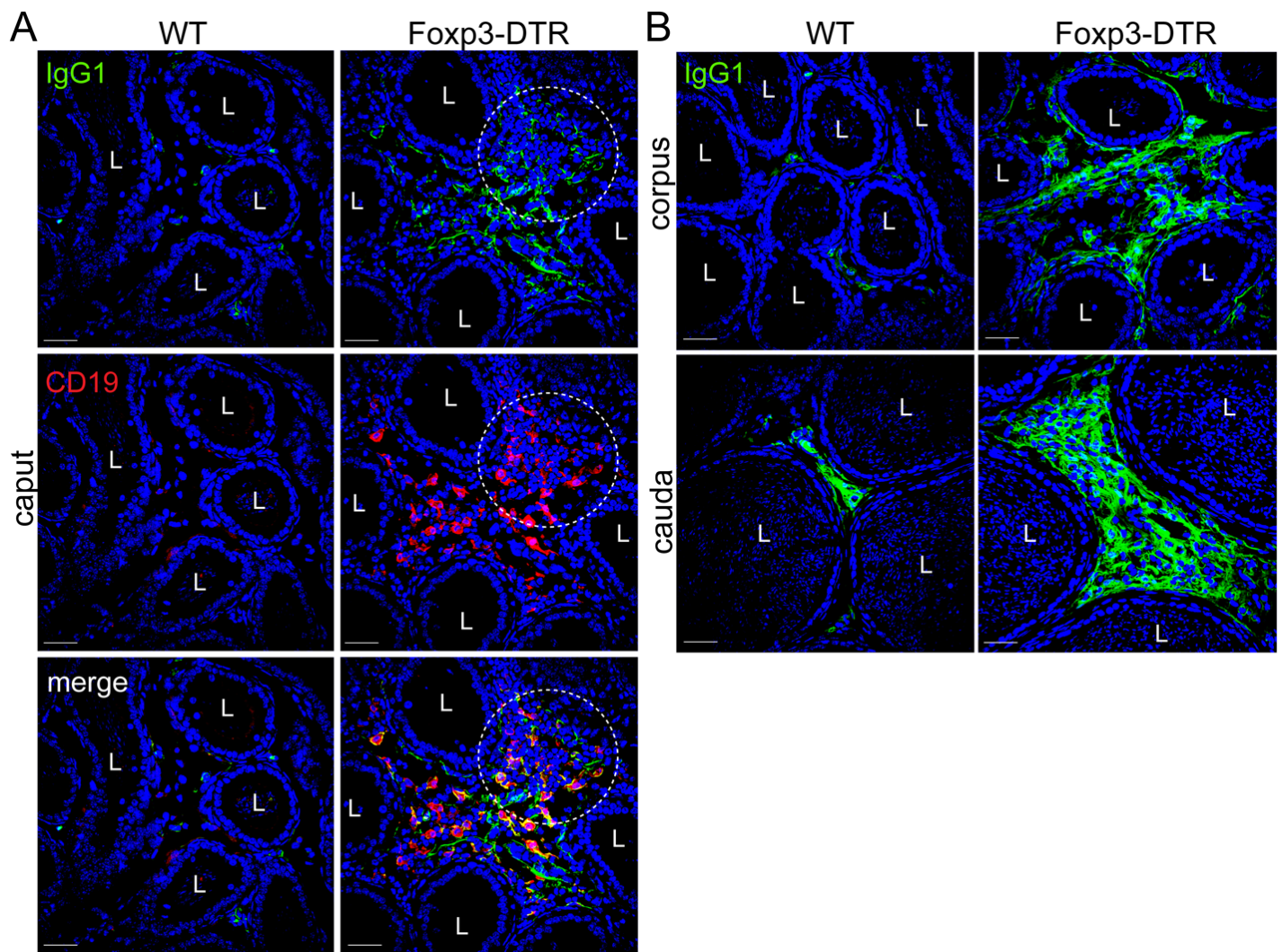
Supplementary Figure 4. Evaluation of IgG1, IgG2b, IgG2c, and IgG3 in the testis of Foxp3-DTR mice 2 weeks after Treg depletion by immunofluorescence. Confocal microscopy images showing immunolabeling of IgG1 (A), IgG2b (B), IgG2c (C), and IgG3 (D) (green) on testis of WT and Foxp3-DTR mice 2 weeks after DT treatment. n=3 testes from different mice. Nuclei are labeled with DAPI (blue). Bars: 20 μ m. L: Lumen.



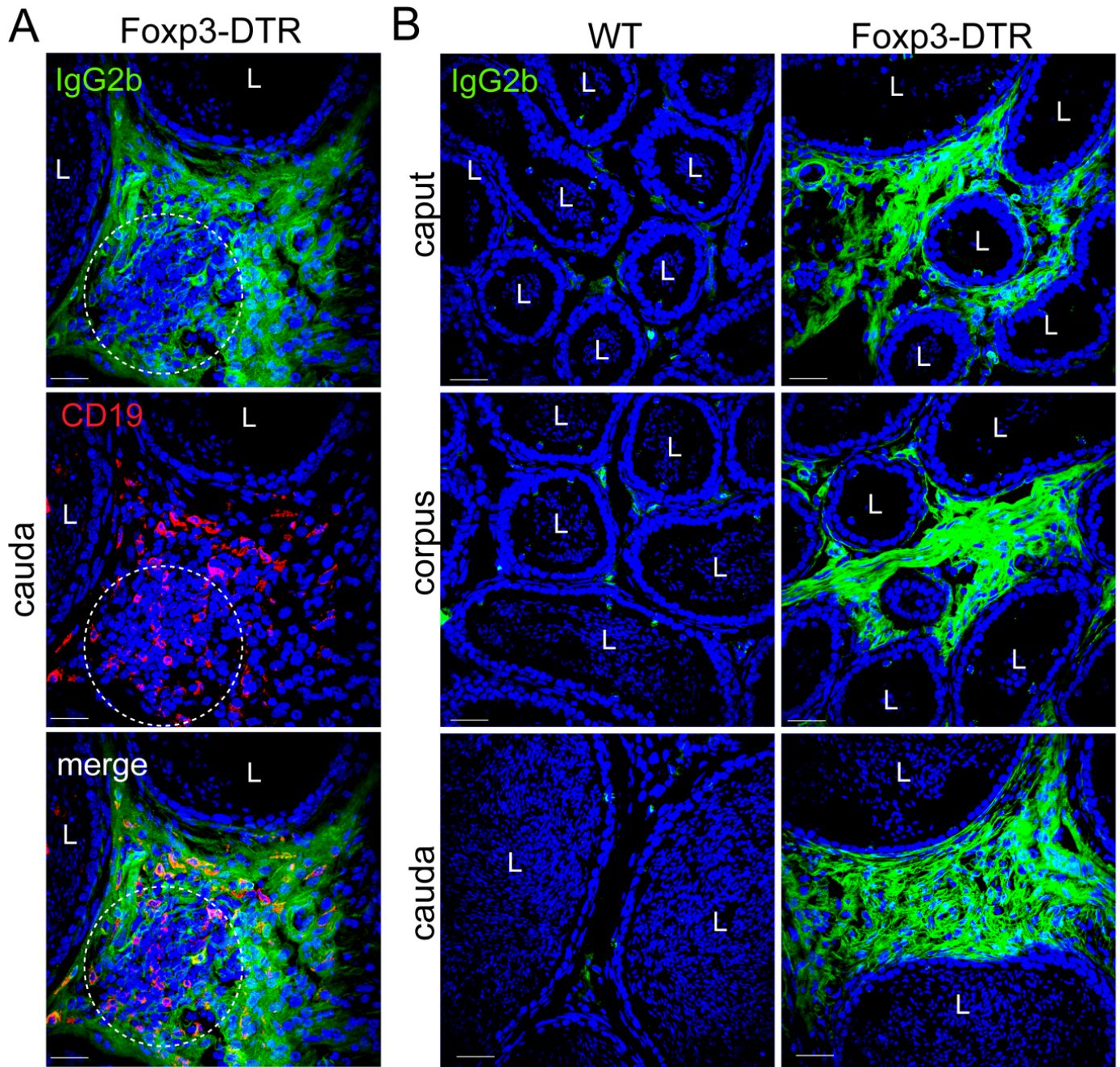
Supplementary Figure 5. Evaluation of IgM on all epididymal regions of Foxp3-DTR mice 2 weeks after Treg depletion by immunofluorescence. Confocal microscopy images showing immunolabeling of IgM (red) on all epididymal regions of WT and Foxp3-DTR mice 2 weeks after DT treatment. Proximal epididymis comprises initial segments (IS) and caput; Distal epididymis comprises corpus and cauda. n=3 epididymides from different mice. Nuclei are labeled with DAPI (blue). Bars: 20 μ m. L: Lumen.



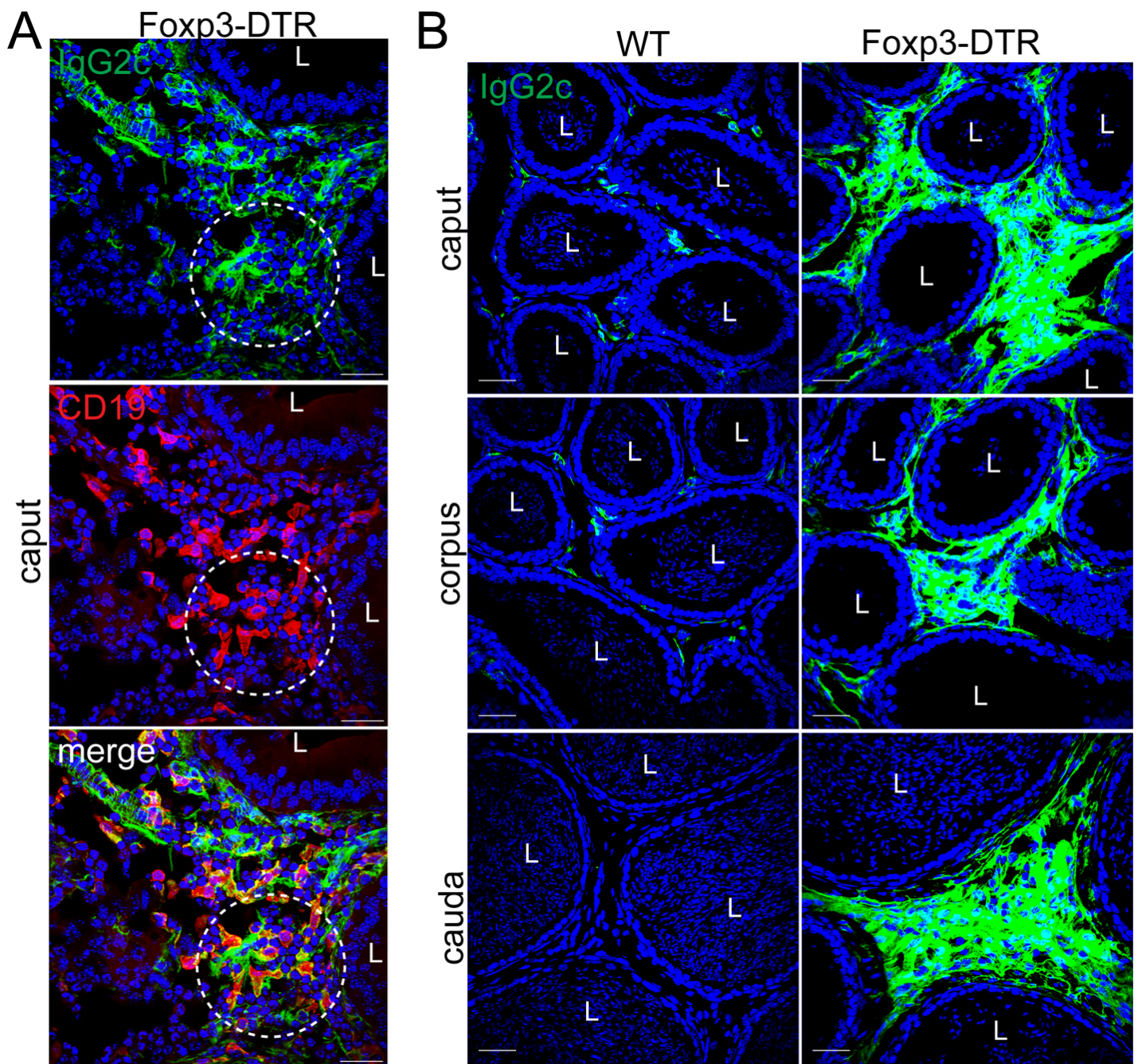
Supplementary Figure 6. Evaluation of IgA and IgG3 on all epididymal regions of Foxp3-DTR mice 2 weeks after Treg depletion by immunofluorescence. Confocal microscopy images showing immunolabeling of IgG3 (**A**) and IgA (**B**) (green) on all epididymal regions of WT and Foxp3-DTR mice 2 weeks after DT treatment. n=3 epididymides from different mice. Nuclei are labeled with DAPI (blue). Bars: 20 μm. L: Lumen.



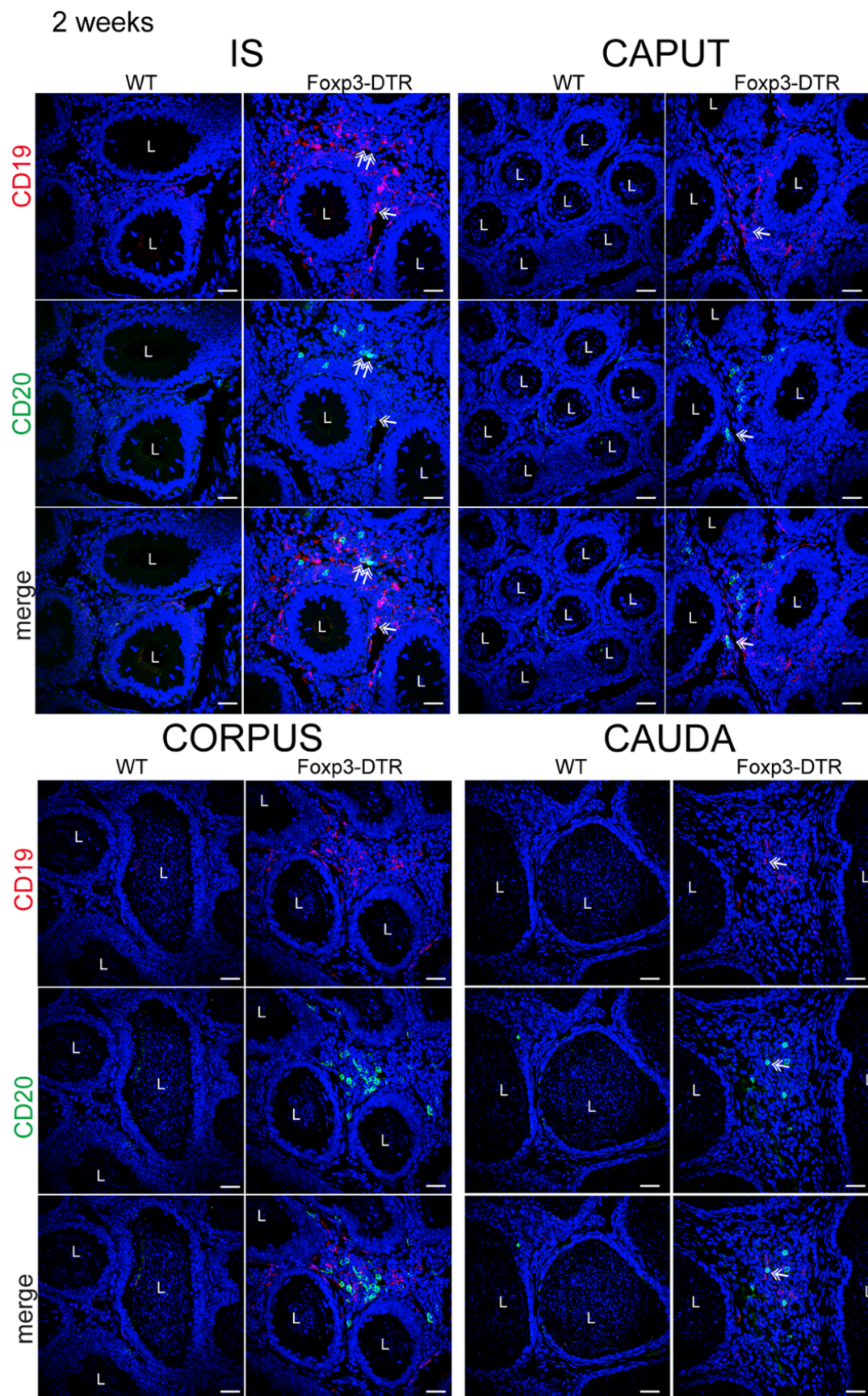
Supplementary Figure 7. Evaluation of IgG1 on all epididymal regions of Foxp3-DTR mice 2 weeks after Treg depletion. **A)** Confocal microscopy images showing immunolabeling of IgG1 (green) and CD19 (red) on the caput of WT and Foxp3-DTR mice 2 weeks after DT treatment. **B)** Confocal microscopy images showing immunolabeling of IgG1 (green) on corpus and cauda of WT and Foxp3-DTR mice 2 weeks after DT treatment. n=3 epididymides from different mice. Nuclei are labeled with DAPI (blue). Bars: 20 μ m. L: Lumen.



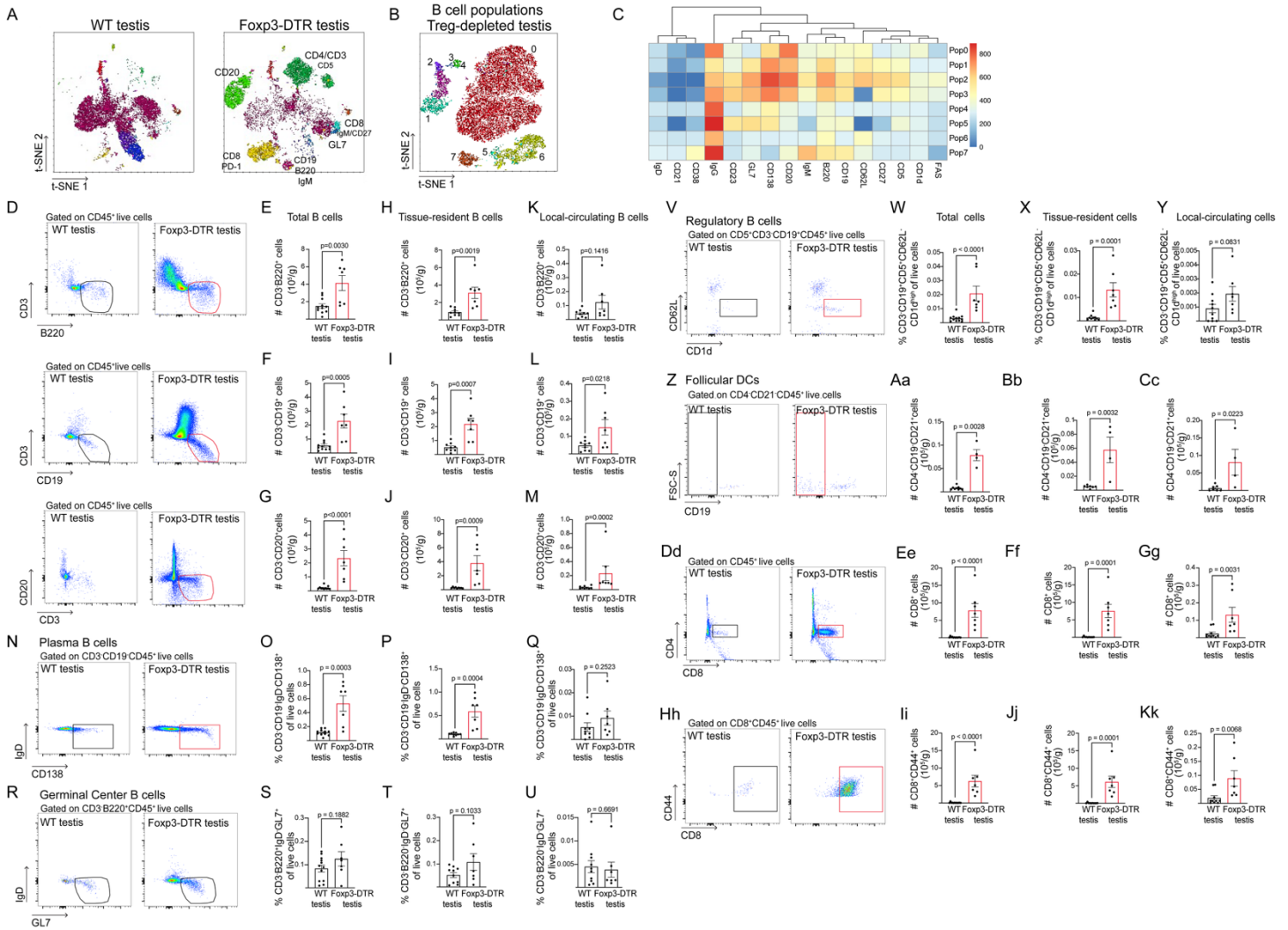
Supplementary Figure 8. Evaluation of IgG2b on all epididymal regions of Foxp3-DTR mice 2 weeks after Treg depletion. A) Confocal microscopy images showing IgG2b (green) immunolabeling and CD19 (red) on the cauda region of Foxp3-DTR mice 2 weeks after DT treatment. n=3 epididymides from different mice. **B)** Confocal microscopy images showing IgG2b (green) immunolabeling on caput, corpus, and cauda of WT and Foxp3-DTR mice 2 weeks after DT treatment. n=3 epididymides from different mice. Nuclei are labeled with DAPI (blue). Bars: 20 μ m. L: Lumen.



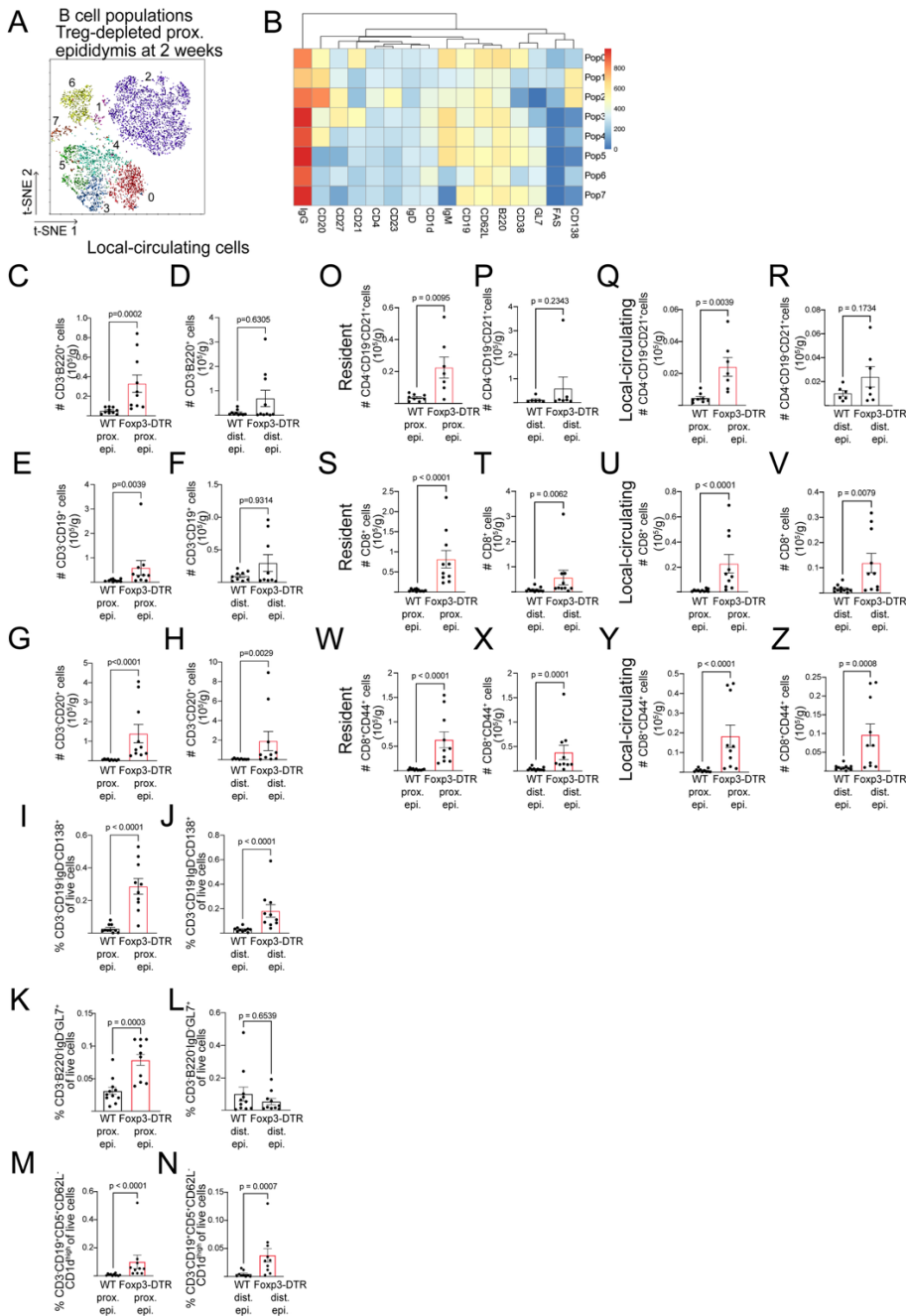
Supplementary Figure 9. Evaluation of IgG2c on all epididymal regions of Foxp3-DTR mice 2 weeks after Treg depletion. A) Confocal microscopy images showing IgG2c (green) immunolabeling and CD19 (red) on the caput region of Foxp3-DTR mice 2 weeks after DT treatment. n=3 epididymides from different mice. **B)** Confocal microscopy images showing IgG2c (green) immunolabeling on caput, corpus, and cauda of WT and Foxp3-DTR mice 2 weeks after DT treatment. n=3 epididymides from different mice. Nuclei are labeled with DAPI (blue). Bars: 20 μ m. L: Lumen.



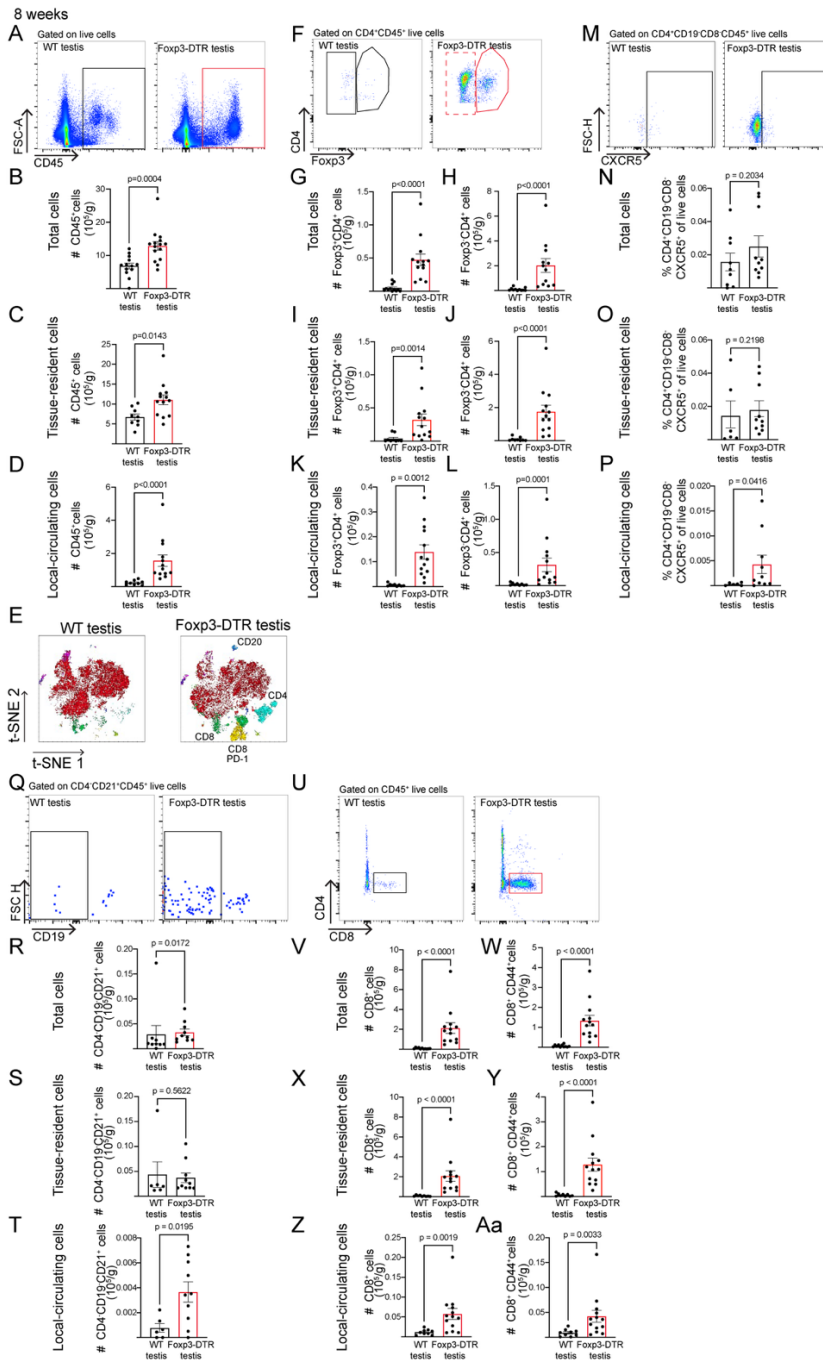
Supplementary Figure 10. Localization and characterization of CD20⁺ and CD19⁺ B cells in the epididymis 2 weeks after Treg depletion. Immunolabeling of CD19 (red) and CD20 (green) B cells in the IS, caput, corpus, and cauda regions of DT-injected WT and Fxp3-DTR mice. Double-headed arrows indicate double-positive cells. n=3 epididymides from different mice. Nuclei are labeled with DAPI (blue). Bars: 20 μm. L: Lumen.



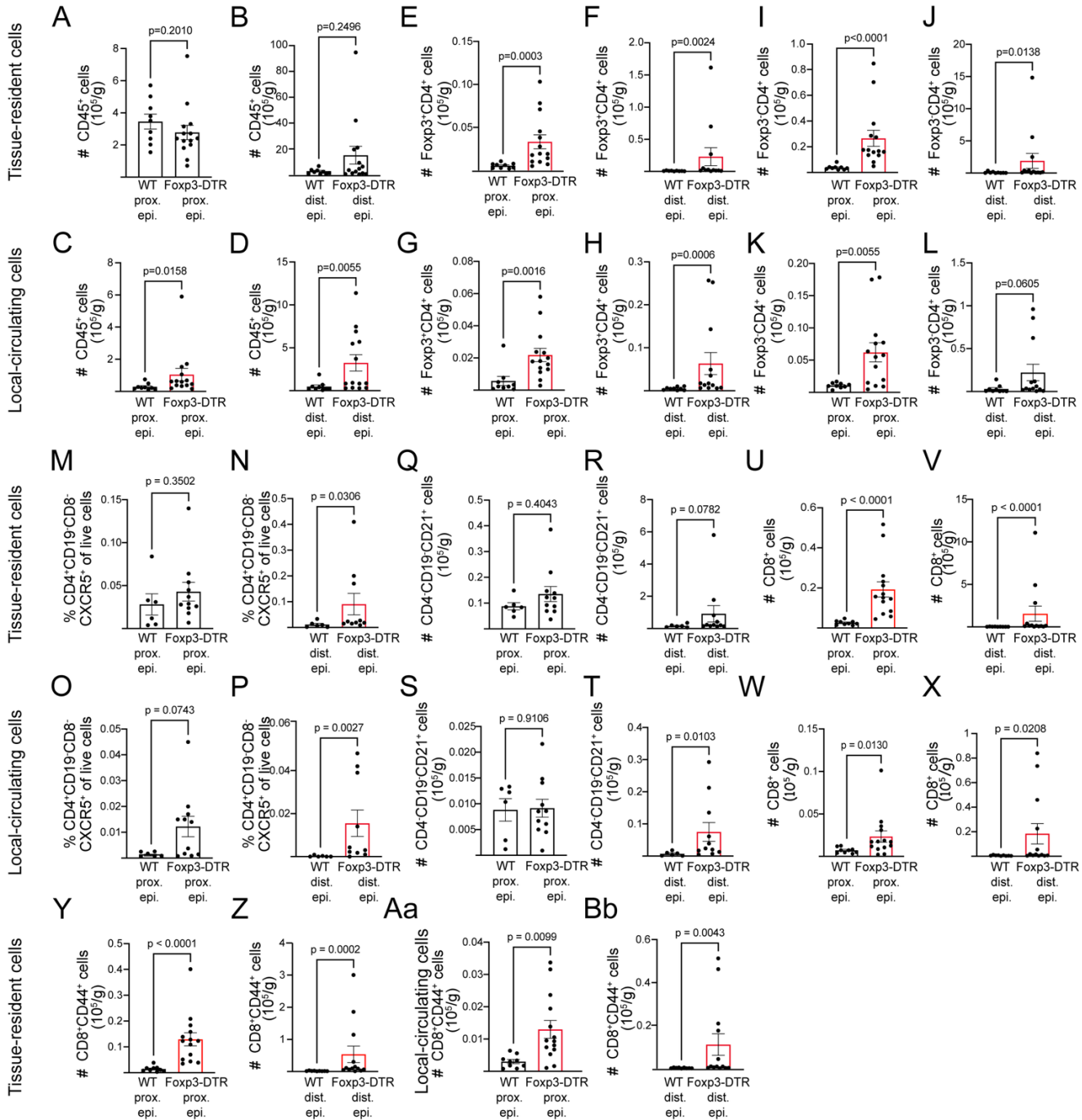
Supplementary Figure 11. B cell populations analysis in the testis of Foxp3-DTR mice 2 weeks after Treg depletion by flow cytometry. t-SNE and FlowSOM pan-populations analysis of testis from WT and Foxp3-DTR mice. The most relevant populations that relatively increased in the Foxp3-DTR samples are highlighted: **(A)** light green: CD20; dark green: CD3/CD4/CD5; yellow: CD8/PD-1; cyan: CD8; blue: IgM/CD27; light brown: GL7; magenta: CD19/B220/IgM) and of the B cell populations of testis from Foxp3-DTR mice **(B)** 0 red:CD20/CD138; 1 cyan:CD20/CD138/B220/GL7; 2 violet:CD20/CD138/GL7/CD23/B220/CD62L/CD27; 3 blue:CD20/CD138/GL7/B220/CD23; 4 green:CD138/IgG; 5 turquoise:CD138/GL7/CD23/CD5; 6 yellow:B220/CD62L; 7 orange:CD19/B220/IgM/CD38) and its relative marker expression heatmap **(C)**. Flow cytometry gating strategy and absolute count (#) of total **(D-G)**; n=11 (WT), n=7 (Foxp3-DTR)), resident **(H-J)**; n=9 (WT), n=7 (Foxp3-DTR)), and local-circulating **(K-M)**; n=9 (WT), n=7 (Foxp3-DTR)) B cells (CD3⁺B220⁺, CD3⁺CD19⁺, and CD3⁺CD20⁺) per gram of testis. Flow cytometry gating strategy and relative abundance of total **(N-O)**; n=11 (WT), n=7 (Foxp3-DTR)), resident **(P)**; n=9 (WT), n=7 (Foxp3-DTR)), and local-circulating **(Q)**; n=9 (WT); n=7 (Foxp3-DTR)) Plasma B cells (CD3⁺CD19⁺IgD⁻CD138⁺) in the testes. Flow cytometry gating strategy and relative abundance of total **(R-S)**; n=12 (WT), n=7 (Foxp3-DTR)), resident **(T)**; n=10 (WT); n=7 (Foxp3-DTR)), and local-circulating **(U)**; n=9 (WT), n=7 (Foxp3-DTR)) GC B cells (CD3⁺B220⁺IgD⁻GL7⁺) in the testes. Flow cytometry gating strategy and relative abundance of total **(V-W)**; n=12 (WT), n=7 (Foxp3-DTR)), resident **(X)**; n=10 (WT), n=7 (Foxp3-DTR)), and local-circulating **(Y)**; n=10 (WT); n=7 (Foxp3-DTR)) Bregs (CD3⁺CD19⁺CD5⁺CD62L⁻CD1d^{hi}) in the testes. Flow cytometry gating strategy and absolute count (#) of total **(Z-Aa)**; n=9 (WT), n=4 (Foxp3-DTR)), resident **(Bb)**; n=7 (WT); n=4 (Foxp3-DTR)), and local-circulating **(Cc)**; n=7 (WT), n=4 (Foxp3-DTR)) follicular DC (CD4⁺CD19⁺CD21⁺) in the testes. Flow cytometry gating strategy and absolute count (#) of total **(Dd-Ee)**; n=12 (WT), n=7 (Foxp3-DTR)), resident **(Ff)**; n=10 (WT); n=7 (Foxp3-DTR)), and local-circulating **(Gg)**; n=10 (WT), n=7 (Foxp3-DTR)) CD8⁺ T cells per gram of testis. Flow cytometry gating strategy and absolute count (#) of total **(Hh-Ii)**; n=12 (WT), n=7 (Foxp3-DTR)), resident **(Jj)**; n=10 (WT); n=7 (Foxp3-DTR)), and local-circulating **(Kk)**; n=10 (WT), n=7 (Foxp3-DTR)) activated CD8⁺ T cells (CD4⁺CD8⁺CD44⁺) per gram of testis. Data were analyzed using two-sided Student's t-test (E-F,H-J, L,O,P,S,T,Y,Bb,Cc) or the Mann-Whitney test (G,K,M,Q,U,W,X,Aa,Ee,Gg,Ii-Kk). Data are shown as means ± SEM. Each dot represents a pool of 2 organs from each mouse. n= number of samples from different mice.



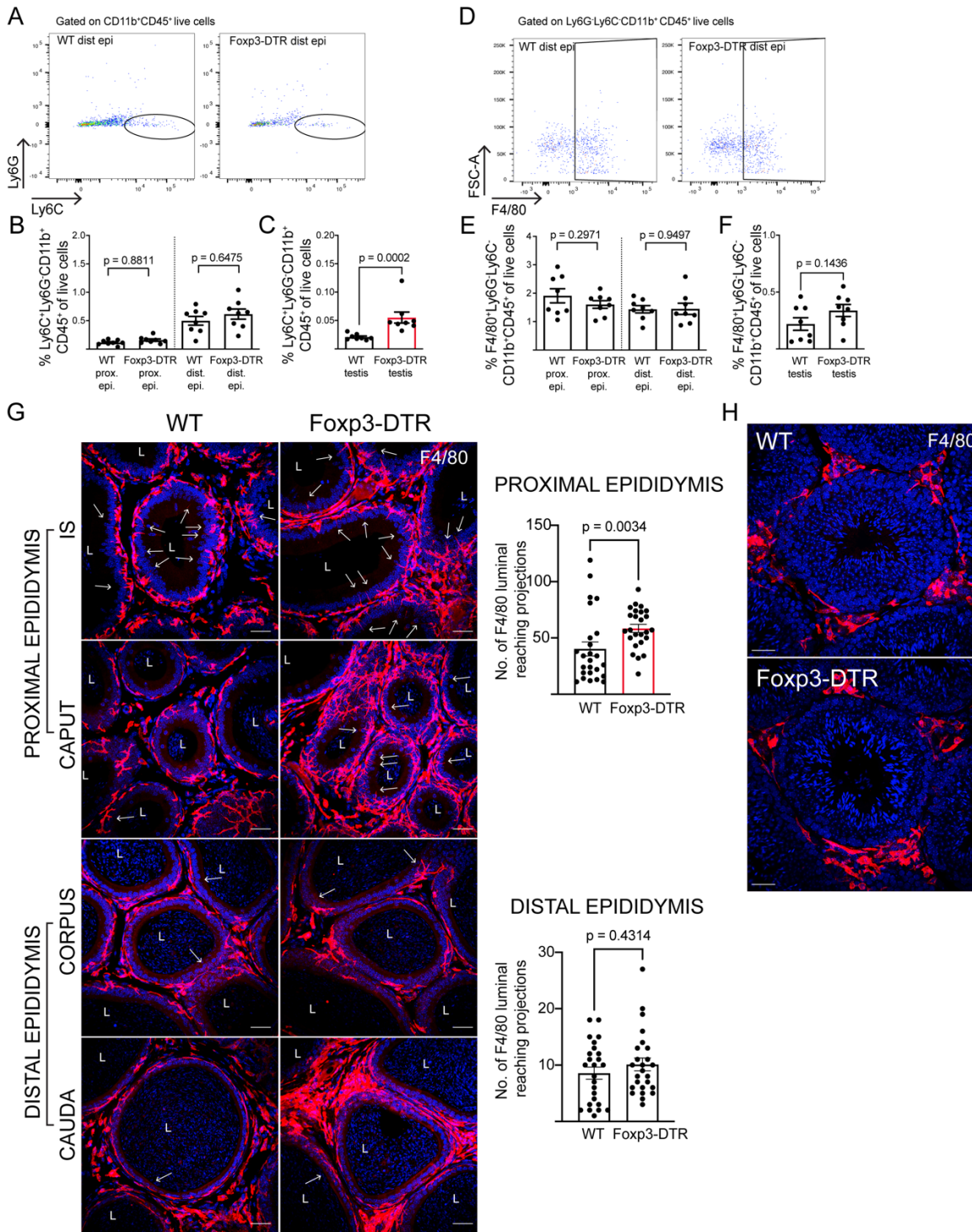
Supplementary Figure 12. Characterization of CD20⁺ and CD19⁺ B cells in the epididymis 2 weeks after Treg depletion. **A-B**) t-SNE and FlowSOM analysis of the B cell population of proximal epididymis from Foxp3-DTR mice. The most relevant populations that relatively increased in the Foxp3-DTR samples are highlighted: **(A)** 0 red:B220/CD38/CD62L/IgM/CD21/CD20; 1 magenta:CD20/CD138; 2 violet:CD20/CD27/CD23/CD62L/CD138; 3 blue:B220/CD62L/IgM/CD21/CD27; 4 cyan:CD20/IgM/CD19/B220; 5 green:B220/GL7/IgM/CD19/CD62L/CD38; 6 yellow:B220/CD62L/CD1d; 7 brown:CD19/CD62L/B220/CD38; and its relative marker expression heatmap **(B)**. n=3. Absolute count (#) of local-circulating B cells (CD3⁺B220⁺ **(C-D)**, CD3⁺CD19⁺ **(E-F)**, and CD3⁺CD20⁺ **(G-H)**) cells per gram of proximal and distal epididymis by flow cytometry. n=10. Relative abundance of local-circulating Plasma B cells (CD3⁺CD19⁺IgD⁺CD138⁺) in the proximal **(I)** and distal **(J)** epididymides. n=10. Relative abundance of local-circulating GC B cells (CD3⁺B220⁺IgD⁺GL7⁺) in the proximal **(K)** and distal **(L)** epididymides. n=11 (WT), n=10 (Foxp3-DTR). Relative abundance of local-circulatory Bregs (CD3⁺CD19⁺CD5⁺CD62L⁺CD1d^{hi}) in the proximal **(M)** and distal **(N)** epididymides. n=11 (WT prox.), n=10 (Foxp3-DTR prox. and dist.), n=9 (WT dist.). Absolute count (#) of tissue-resident and local-circulating follicular DC (CD4⁺CD19⁺CD21⁺ cells) in the proximal **(O,Q)** and distal **(P,R)** epididymides. n=8 (WT prox. resident/circulating), n=7 (Foxp3-DTR), n=6 (WT dist. resident/circulating). Absolute count (#) of tissue-resident and local-circulating CD8⁺ (CD4⁺CD8⁺) **(S-V)** and CD8⁺CD44⁺ T cells **(W-Z)** per gram of proximal and distal epididymis. n=11 (WT), n=10 (Foxp3-DTR). Data were analyzed using two-sided Student's t-test (O,Q,R) or Mann-Whitney test (C-H,I-N,P, S-Z). Data are shown as means ± SEM. Each dot represents a pool of 2 organs from each mouse. n= number of samples from different mice.



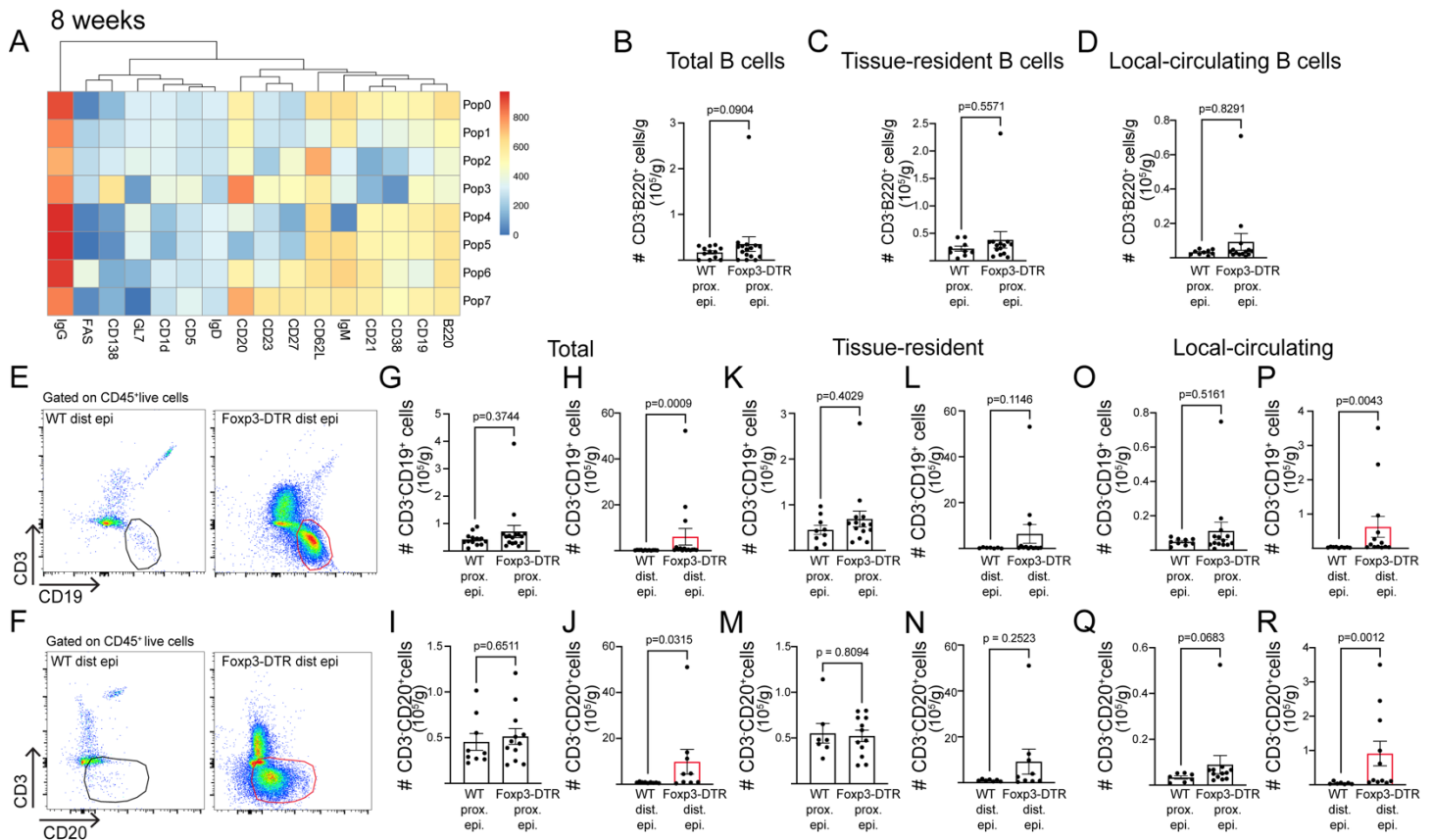
Supplementary Figure 13. T cell and DC infiltration in the testis of DT-injected Foxp3-DTR mice 8 weeks after Treg ablation by flow cytometry. **A**) Flow cytometry gating strategy and absolute count (#) of total (**B**), resident (**C**), and local-circulating (**D**) CD45⁺ cells per gram of testis. n=13(WT total); n=15 (Foxp3-DTR total), n=9 (WT resident/circulating), 13 (Foxp3-DTR resident/circulating). **E**) t-SNE and FlowSOM pan-populations analysis of DT-treated WT and Foxp3-DTR testes. The most relevant populations that relatively increased in the Foxp3-DTR samples are highlighted: Blue:CD20; cyan:CD4; green:CD8; yellow:CD8/PD-1. **F**) Flow cytometry gating strategy and absolute count (#) of total (**G,H**), tissue-resident (**I-J**), and local-circulatory (**K,L**) Tregs and CD4⁺ T cells per gram of testis. n=12 (WT total Tregs, Foxp3-DTR total TCD4⁺), n=13 (Foxp3-DTR total/resident/circulating Tregs and Foxp3-DTR resident/circulating TCD4⁺), n=11 (WT total TCD4⁺), n=9 (WT resident/circulating Tregs and TCD4⁺). **M**) Flow cytometry gating strategy and relative abundance of total (**N**), tissue-resident (**O**), and local-circulatory (**P**) Tfh in the testes. n=9 (WT total), n=10 (Foxp3-DTR total/resident/circulating), n=6 (WT resident/circulating). **Q**) Flow cytometry gating strategy and absolute count (#) of total (**R**), tissue-resident (**S**), and local-circulatory (**T**) CD21⁺ follicular DCs cells per gram of testis. n=9 (WT total), n=10 (Foxp3-DTR total/resident/circulating), n=6 (WT resident/circulating). **U**) Flow cytometry gating strategy and absolute count (#) of total (**V-W**), tissue-resident (**X-Y**), and local-circulatory (**Z-Aa**) CD8 and activated CD8⁺ T lymphocytes. n=11 (WT total), n=13 (Foxp3-DTR total/resident/circulating), n=9 (WT resident/circulating). Data were analyzed using two-sided Student's t-test (C,K,T), or Mann-Whitney test (B,D,G-J,L,N-P,R,S,V-Aa). Data are shown as means ± SEM. Each dot represents a pool of 2 organs from each mouse. n= number of samples from different mice.



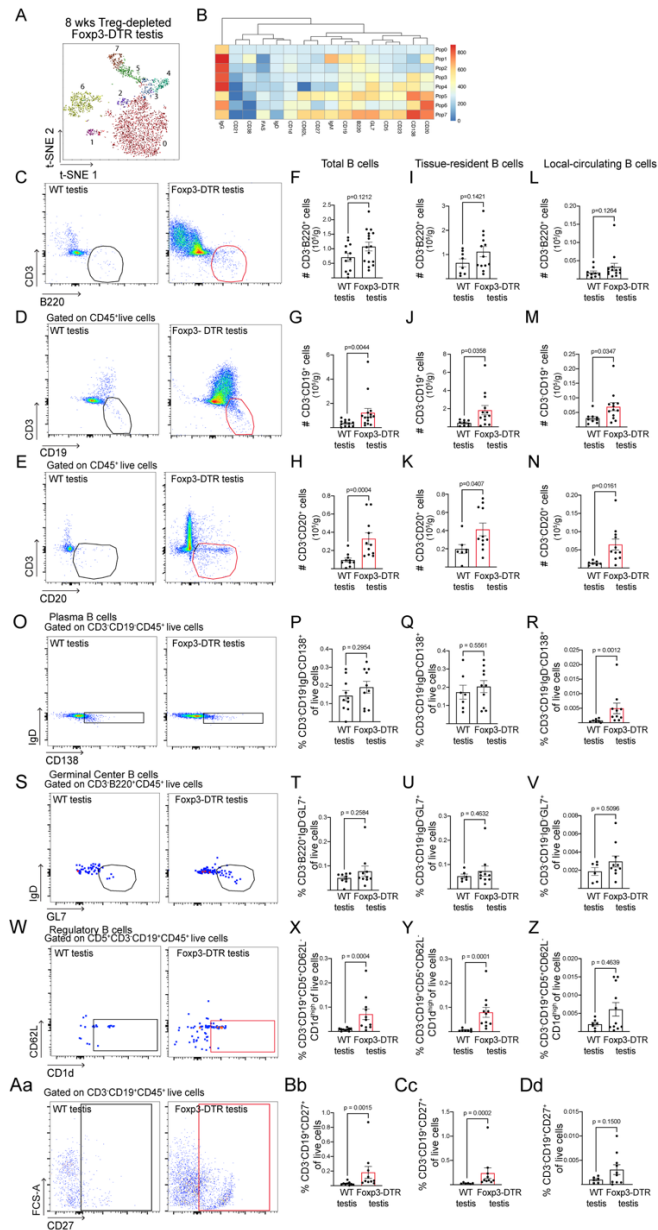
Supplementary Figure 14. T and DC infiltration in the epididymis 8 weeks after Treg depletion by flow cytometry. Absolute count (#) of tissue-resident (A,B) and local-circulatory (C,D) CD45⁺ cells per gram of proximal and distal epididymis. n=9 (WT), n=14 (Foxp3-DTR). Absolute count (#) of tissue-resident (E,F) and local-circulatory (G,H) Tregs per gram of proximal and distal epididymides. n=9 (WT), n=14 (Foxp3-DTR). Absolute count (#) of tissue-resident (I,J) and local-circulatory (K,L) CD4 T lymphocytes per gram of proximal and distal epididymis. n=9 (WT), n=14 (Foxp3-DTR). Relative abundance of tissue-resident (M,N) and local-circulatory (O,P) T follicular helper cells (Tfh, CD4⁺CD8⁻CD19⁺CXCR5⁺ of live cells) in the proximal and distal epididymides. n=6 (WT), n=11 (Foxp3-DTR prox. resident and Foxp3-DTR circulating), n=10 (Foxp3-DTR dist. resident). Absolute count (#) of tissue-resident (Q,R) and local-circulatory (S,T) follicular DCs per gram of proximal and distal epididymis. n=6 (WT), n=11 (Foxp3-DTR). Absolute count (#) of tissue-resident (U,V) and local-circulatory (W,X) CD8⁺ and activated CD8⁺ (Y-Bb) T lymphocytes per gram of proximal and distal epididymis. n=9 (WT), n=14 (Foxp3-DTR prox. resident and Foxp3-DTR circulating), n=13 (Foxp3-DTR dist. resident). Data were analyzed using two-sided Student's t-test (S, Aa) or Mann-Whitney test (A-R, T-Z, Bb). Data are shown as means ± SEM. Each dot represents a pool of 2 organs from each mouse. n= number of samples from different mice.



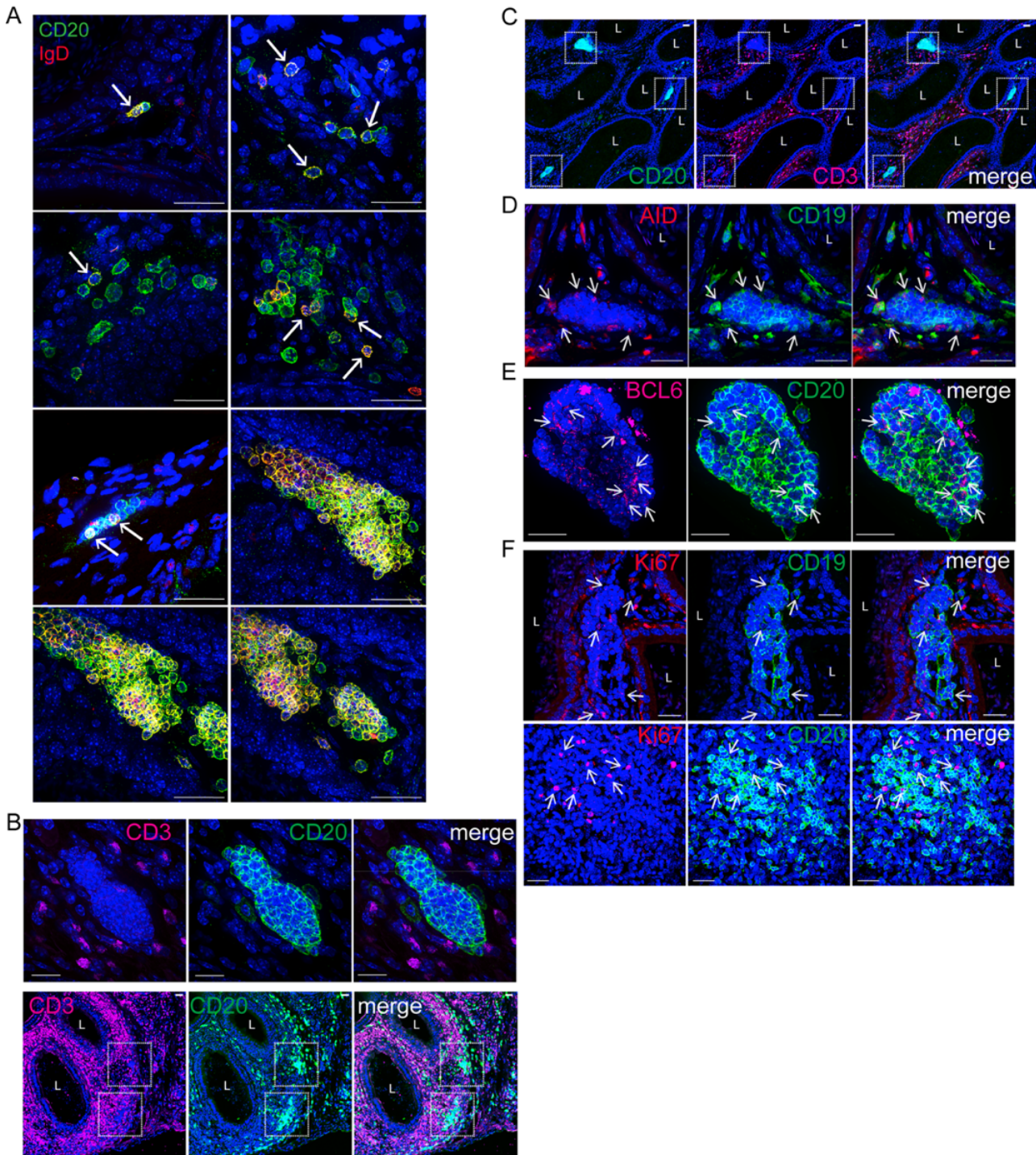
Supplementary Figure 15. Myeloid cell analysis 8 weeks after Treg depletion. **A)** Flow cytometry gating strategy and relative abundance of monocyte (CD45⁺CD11b⁺Ly6G⁺Ly6C⁺ of live cells) in the proximal and distal epididymides (**B**) and testis (**C**). n=8. Each dot represents a pool of 2 organs from each mouse. n= number of samples from different mice. **D)** Flow cytometry gating strategy and relative abundance of macrophages (CD45⁺CD11b⁺Ly6G⁺Ly6C⁺F4/80⁺) in the proximal and distal epididymides (**E**) and testis (**F**). n=8. Each dot represents a pool of 2 organs from each mouse. n= number of samples from different mice. **G)** Imaging and quantification of the number of F4/80 luminal-reaching projections (red, arrows) in the proximal (IS and caput) and distal (corpus and cauda) epididymis of DT-injected WT and Foxp3-DTR mice per area of tissue (110,540 μm^2). Each image quantification is represented as a dot. n=25 images from 4 epididymis from different mice. **H)** Immunolabeling of F4/80⁺ cells (red) on testis from WT and Foxp3-DTR mice. n=4 testes from different mice. Nuclei are labeled with DAPI (blue). Bars: 20 μm . L: Lumen. Data were analyzed using two-sided Student's t-test (B,E,F) or Mann-Whitney test (C,G). Data are shown as means $\pm$ SEM.



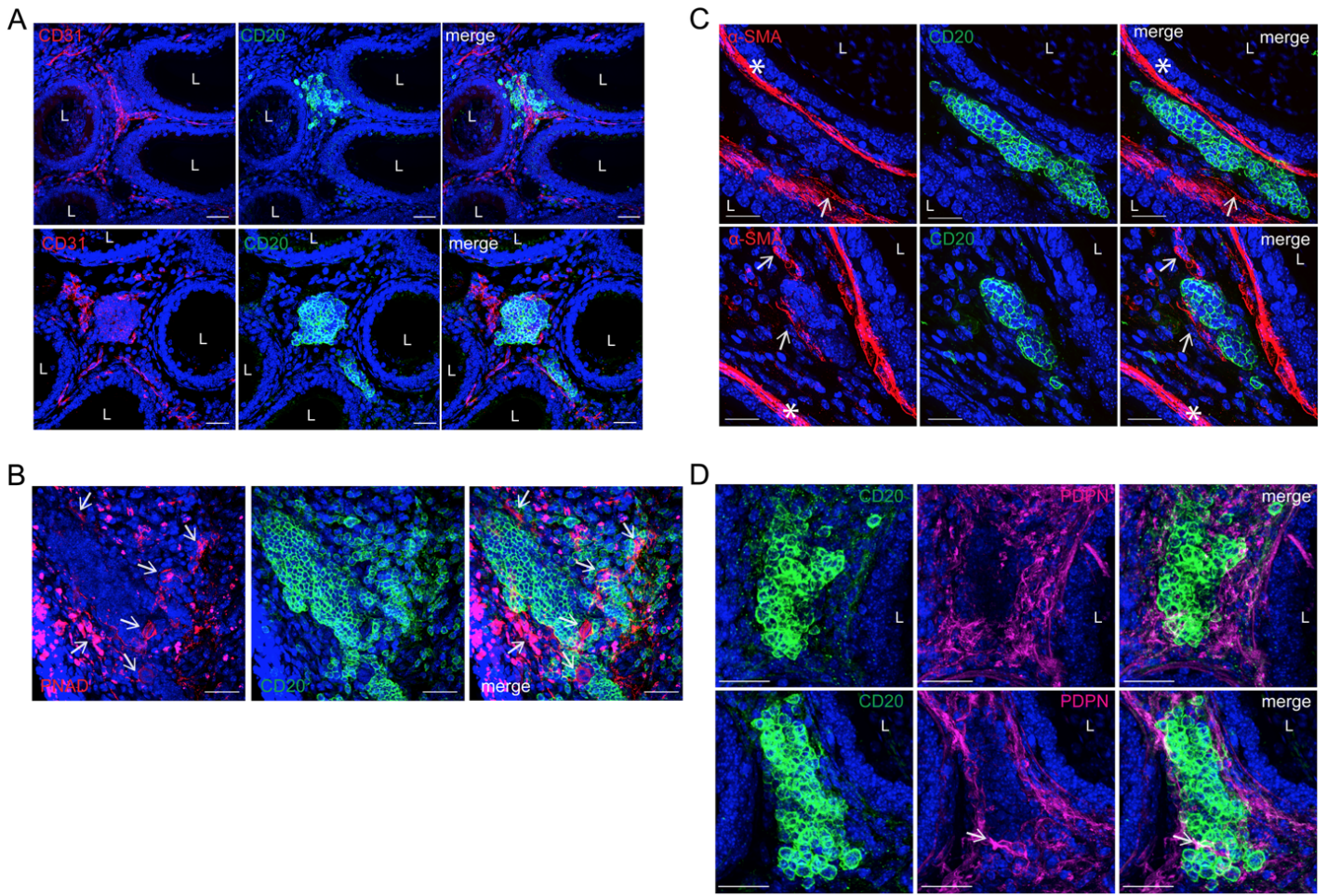
Supplementary Figure 16. B cell analysis in the epididymis of Foxp3-DTR mice 8 weeks after Treg depletion by flow cytometry. **A)** Relative marker expression heatmap of t-SNE and FlowSOM analysis of 8-week Treg-depleted distal epididymis. $n=3$. Absolute count (#) of total (**B**), tissue-resident (**C**), and local-circulating (**D**) B220⁺ B cells per gram of proximal epididymis. $n=12$ (WT total), $n=16$ (Foxp3-DTR total), $n=8$ (WT resident/circulating), $n=13$ (Foxp3-DTR resident/circulating). **E-F)** Flow cytometry gating strategy and absolute count (#) of total (**G-J**), tissue-resident (**K-N**), and local-circulating (**O-R**) B cells (CD3⁺CD19⁺ and CD3⁺CD20⁺) per gram of proximal and distal epididymis. **E-J:** $n=13$ (WT prox. CD19), $n=16$ (Foxp3-DTR prox. CD19), $n=12$ (WT dist. CD19, Foxp3-DTR prox. CD20), $n=15$ (Foxp3-DTR dist. CD19), $n=9$ (WT prox., WT and Foxp3-DTR dist. CD20). **K-N:** $n=9$ (WT prox. CD19, Foxp3-DTR dist. CD20), $n=14$ (Foxp3-DTR prox. CD19), $n=7$ (WT dist. CD19, WT prox. and dist. CD20), $n=13$ (Foxp3-DTR dist. CD19), $n=12$ (Foxp3-DTR prox. CD20). **O-R:** $n=9$ (WT prox. and dist. CD19), $n=14$ (Foxp3-DTR prox. CD19), $n=13$ (Foxp3-DTR dist. CD19), $n=7$ (WT prox. and dist. CD20), $n=12$ (Foxp3-DTR prox. CD20), $n=11$ (Foxp3-DTR dist. CD20). Data were analyzed using two-sided Student's t-test (**M**), or Mann-Whitney test (**B-D, G-L, N-R**). Data are shown as means $\pm$ SEM. Each dot represents a pool of 2 organs from each mouse. n = number of samples from different mice.



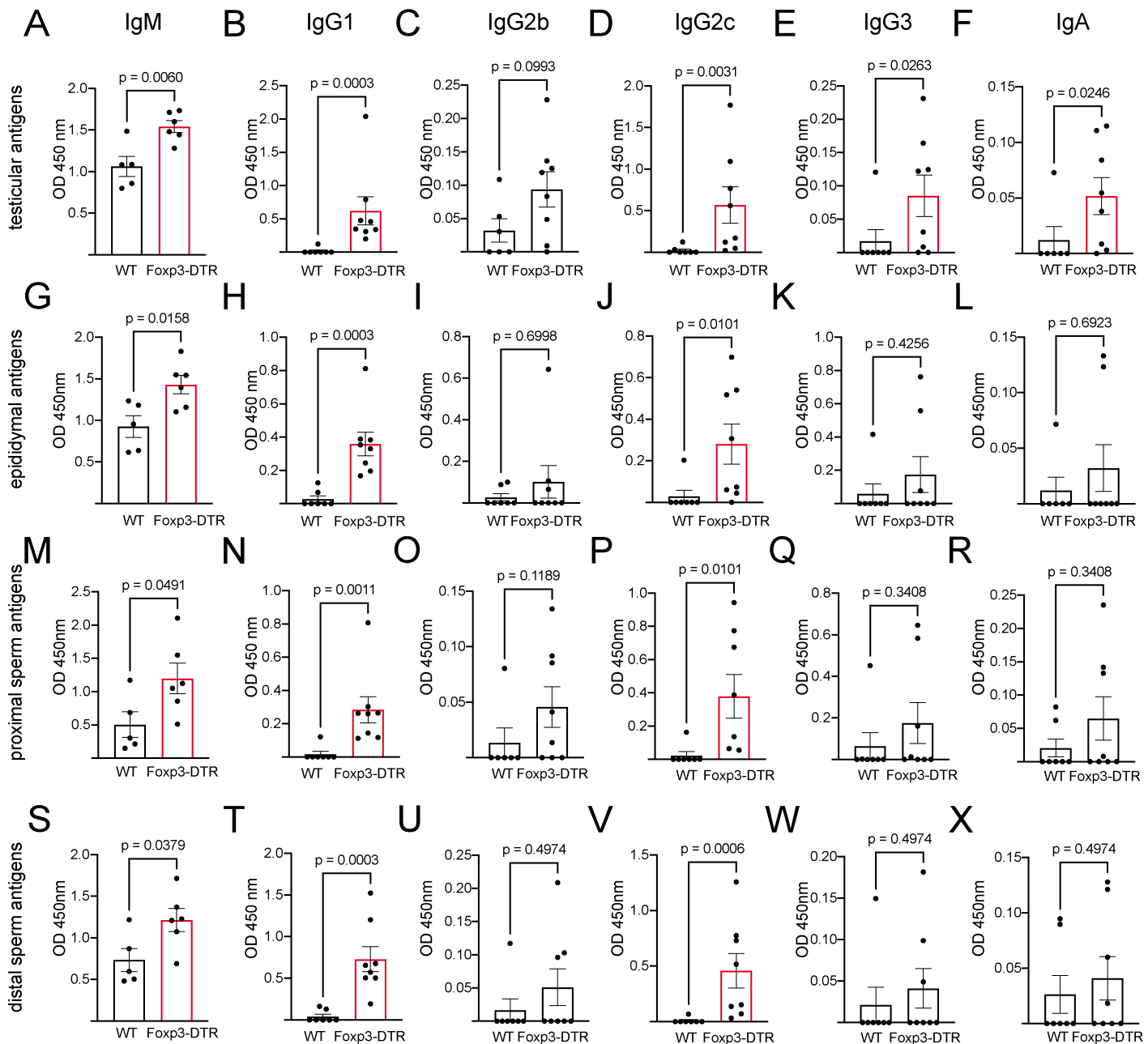
Supplementary Figure 17. B cell analysis in the testis of Foxp3-DTR mice 8 weeks after Treg depletion by flow cytometry. A-B) t-SNE and FlowSOM B cell populations analysis of testes from Foxp3-DTR mice. The most relevant populations that relatively increased in the Foxp3-DTR samples are highlighted: (A). 0 red:B220/IgG; 1 magenta:CD20/B220/CD19/IgM; 2 purple:B220/CD19/GL7; 3 blue:CD20/CD138/GL7; 4 cyan:CD19/GL7/CD23/CD138; 5 green:CD20/CD138/B220/GL7/CD19/IgM/CD27/CD62L; 6 yellow:CD20/CD19; 7 brown:CD20/CD138/CD23/CD5/GL7/B220/CD19/CD27/CD62L) and relative marker expression heatmap (B). n=3. **C-E)** Flow cytometry gating strategy and absolute count (#) of total (**F-H**), tissue-resident (**I-K**), and local-circulating(**L-N**) B220⁺, CD19⁺, and CD20⁺ B cells per gram of testis from DT-treated WT and Foxp3-DTR mice. C,F,I,L: n=12 (WT total), n=16 (Foxp3-DTR total), n=8 (WT resident), n=13 (Foxp3-DTR resident/circulating), n=9 (WT circulating). D,G,J,M: n=12 (WT total), n=15 (Foxp3-DTR total), n=9 (WT resident/circulating), n=13 (Foxp3-DTR resident/circulating). E,H,K,N: n=10 (WT total), n=11 (Foxp3-DTR total/resident/circulating), n=7 (WT resident/circulating). **O)** Flow cytometry gating strategy and relative abundance of total (**P**), tissue-resident (**Q**), and local-circulating (**R**) plasma B cells in the testes. n=10 (WT and Foxp3-DTR total, Foxp3-DTR resident/circulating), n=7 (WT resident/circulating). **S)** Flow cytometry gating strategy and relative abundance of total (**T**), tissue-resident (**U**), and local-circulating (**V**) GC B cells in the testes. n=9 (WT total), n=10 (Foxp3-DTR total/resident/circulating), n=6 (WT resident/circulating). **W)** Flow cytometry gating strategy and relative abundance of total (**X**), tissue-resident (**Y**), and local-circulating (**Z**) Bregs in the testes. n=10 (WT total), n=11 (Foxp3-DTR total/resident/circulating), n=7 (WT resident/circulating). **Aa)** Flow cytometry gating strategy and relative abundance of total (**Bb**),tissue-resident (**Cc**), and local-circulating (**Dd**) memory B cells in the testes. n=9 (WT total), n=10 (Foxp3-DTR total/resident/circulating), n=6 (WT resident/circulating). Data were analyzed using two-sided Student's t-test (I,J,K,M,N,P,Q,T,U,Dd), or Mann-Whitney test (F-H,L,R,V,X-Z,Bb,Cc). Data are shown as means ± SEM. Each dot represents a pool of 2 organs from each mouse. n= number of samples from different mice.



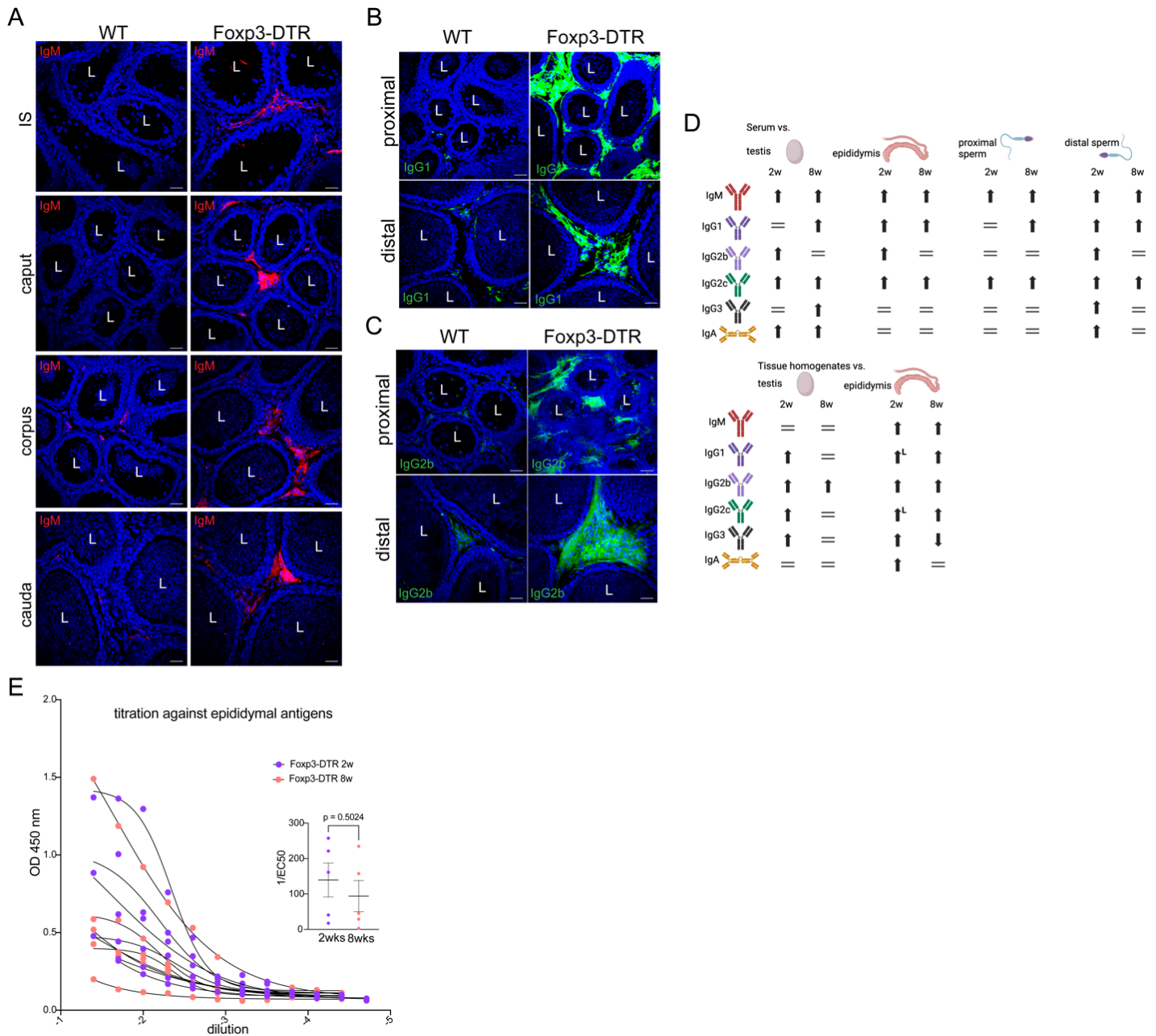
Supplementary Figure 18. TLS characterization in the distal epididymides of 8-week DT-injected Foxp3-DTR mice. **A)** Immunolabeling of IgD (red) and CD20 (green) of different TLS development stages in the distal region of DT-injected Foxp3-DTR mice. Arrows indicate double-positive cells. Please note that the last three pictures are different views of the same TLS shown in Fig. 7B. See also Suppl. Movies 2-5. n=5. **B)** Immunolabeling of CD3 (magenta) and CD20 (green) in the epididymal distal region of DT-injected Foxp3-DTR mice. See also Suppl. Movies 6-7. n=5. Dashed squares indicate TLSs surrounded by T-cells. **C)** Immunolabeling of CD3 (magenta) and CD20 (green) in the epididymal distal region of DT-injected Foxp3-DTR mice, low magnification. Dashed squares indicate TLSs surrounded by T cells. **D)** Immunolabeling of AID (red) and CD19 (green) in the epididymal distal region of DT-injected Foxp3-DTR mice. Arrows indicate double-positive cells. n=4. **E)** Immunolabeling of CD20 (green) and BCL6 (magenta) in the DT-injected Foxp3-DTR distal epididymis. Arrows indicate double-positive cells. n=3. **F)** Immunolabeling of Ki-67 (red) and CD19 (green) or CD20 (green) in the epididymal distal region of DT-injected Foxp3-DTR mice. Arrows indicate double-positive cells. n=5. Nuclei are labeled with DAPI (blue). Bars: 20µm. L:Lumen. n= number of epididymides from different mice.



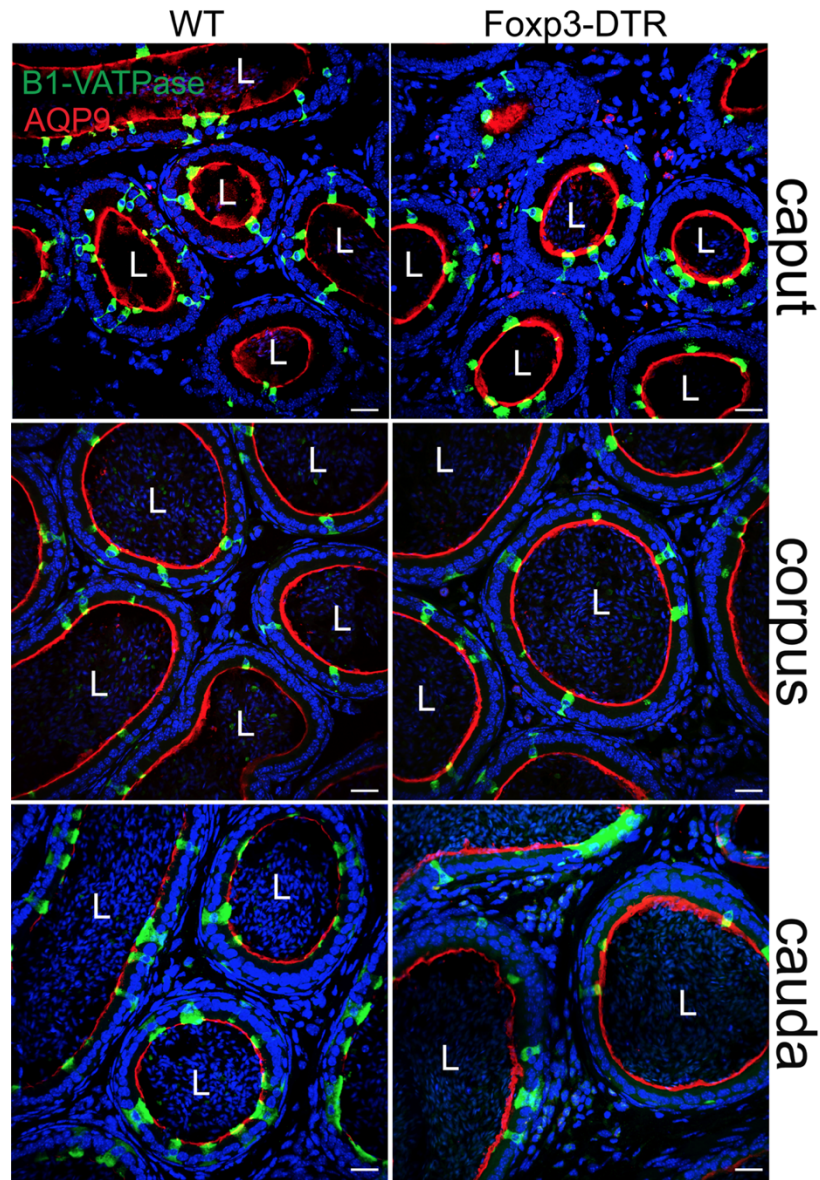
Supplementary Figure 19. TLS characterization in the distal epididymides of 8-week DT-injected Foxp3-DTR mice. **A)** Immunolabeling of CD31 (red) and CD20 (green) in the epididymal distal region of DT-injected Foxp3-DTR mice. See also Suppl. Movie 8. n=5. **B)** Immunolabeling of PNAD (red, arrows) and CD20 (green) in the distal region of DT-injected Foxp3-DTR mice. n=5. **C)** Immunolabeling of α -SMA (red) and CD20 (green) in the distal region of DT-injected Foxp3-DTR mice. Arrows indicate fibroblasts, and asterisks indicate muscle cells. n=5. **D)** Immunolabeling of PDPN (magenta, arrow) and CD20 (green) in the distal region of DT-injected Foxp3-DTR mice. See also Suppl. Movies 9-10. n=5. Nuclei are labeled with DAPI (blue). Bars: 20 μ m. L: Lumen. n= number of epididymides from different mice.



Supplementary Figure 21. Serum autoantibody isotypification of Fxp3-DTR mice 8 weeks after DT treatment by ELISA. Autoantibody isotypification (IgM, IgG1, IgG2b, IgG2c, IgG3, and IgA) of DT-injected Fxp3-DTR and WT mouse serum against testicular (A-F), epididymal (G-L), proximal sperm (M-R), and distal sperm (S-X) antigens. n=5-8. A-F; G-L; S-X: n=5 (WT IgM); n=6 (Fxp3-DTR IgM); n=7 (WT IgG1, IgG2b, IgG2c, IgG3, IgA); n=8 (Fxp3-DTR IgG1, IgG2b, IgG2c, IgG3, IgA). M-R: n=5 (WT IgM); n=6 (Fxp3-DTR IgM); n=7 (WT IgG1, IgG2c, IgG3, IgA); n=6 (WT IgG2b); n=8 (Fxp3-DTR IgG1, IgG2b, IgG2c, IgG3, IgA). Data were analyzed using two-sided Student's t-test (A,C,G,M,S) or Mann-Whitney test (B,D-F,H-L,N-R,T-X). Data are shown as means $\pm$ SEM. n= number of samples from different mice.

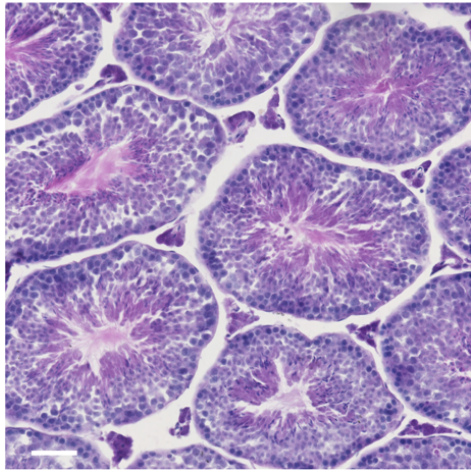


Supplementary Figure 22. Autoantibody deposition in the epididymis 8 weeks after Treg depletion. A) Confocal microscopy images showing immunolabeling of IgM (red) on all epididymal regions of WT and Foxp3-DTR mice 8 weeks after DT treatment. n=3 epididymis from different mice. **B)** Confocal microscopy images showing IgG1 (green) immunolabeling on proximal and distal epididymis of WT and Foxp3-DTR mice 8 weeks after DT treatment. n=4 epididymis from different mice. **C)** Confocal microscopy images showing IgG2b (green) immunolabeling on proximal and distal epididymis of WT and Foxp3-DTR mice 8 weeks after DT treatment. n=3 epididymis from different mice. Nuclei are labeled with DAPI (blue). Bars: 20 μ m. L: Lumen. **D)** Summary of serum and tissue autoantibody profiles against epididymal, testicular, and proximal, and distal sperm antigens 2 and 8 weeks after DT treatment. L indicates the detection of an isotype in the epididymal lumen. The figure was created with BioRender.com. **E)** Foxp3-DTR serum total IgG titers (calculated as 1/EC50) against epididymal antigens 2 and 8 weeks after DT treatment. n=5 number of samples from different mice. Data were analyzed using two-sided Student's t-test (E). Data are shown as means $\pm$ SEM.

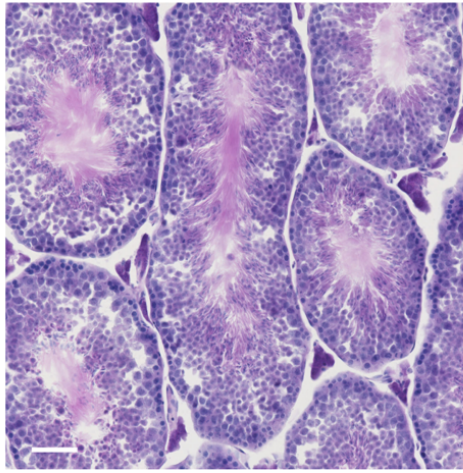


Supplementary Figure 23. Epithelial integrity of the epididymal epithelia 8 weeks after Treg depletion. Confocal microscopy images showing B1 V-ATPase⁺ cells (a marker of clear cells (CCs); green) and AQP9⁺ cells (a marker of principal cells; red) in all epididymal regions of WT and Foxp3-DTR mice 8 weeks after DT injection. n=3 epididymis from different mice. Nuclei are labeled with DAPI (blue). Bars: 20 μ m. L: Lumen.

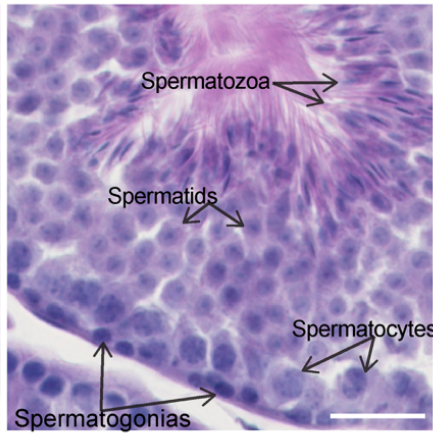
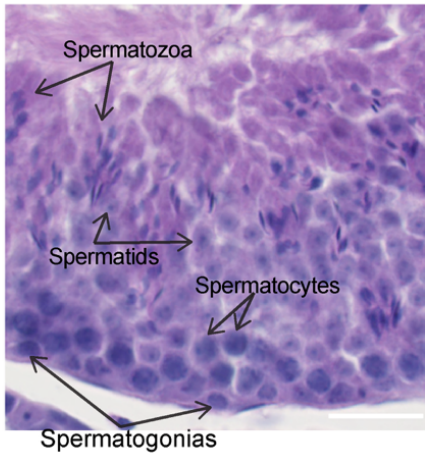
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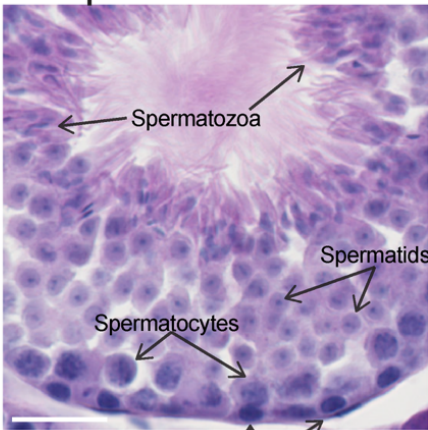
Foxp3-DTR



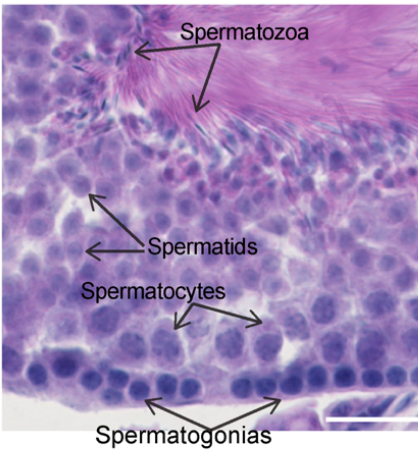
B WT



Foxp3-DTR

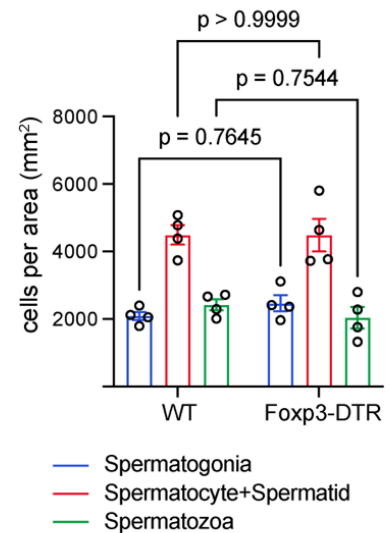


Spermatogonias

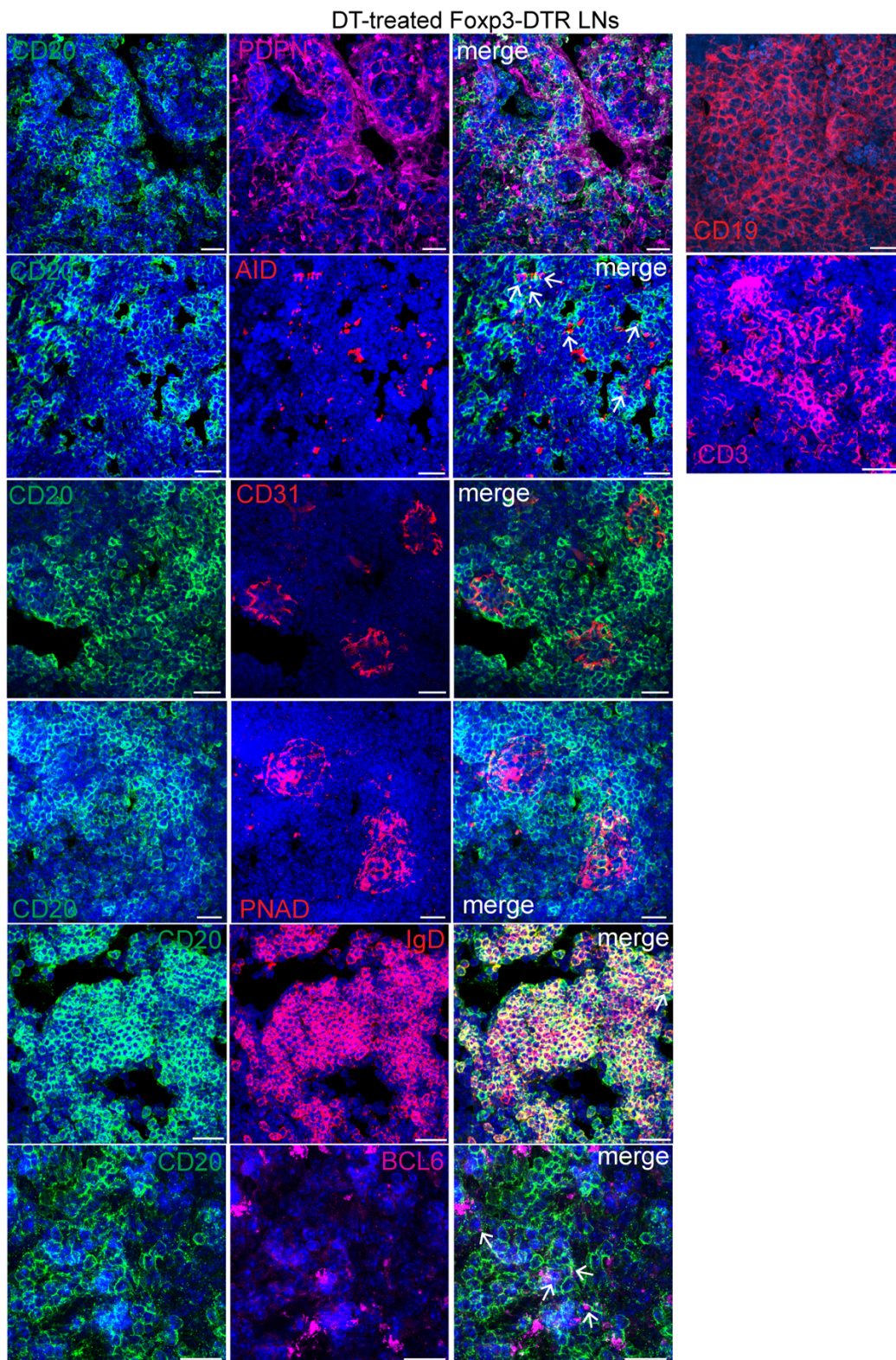


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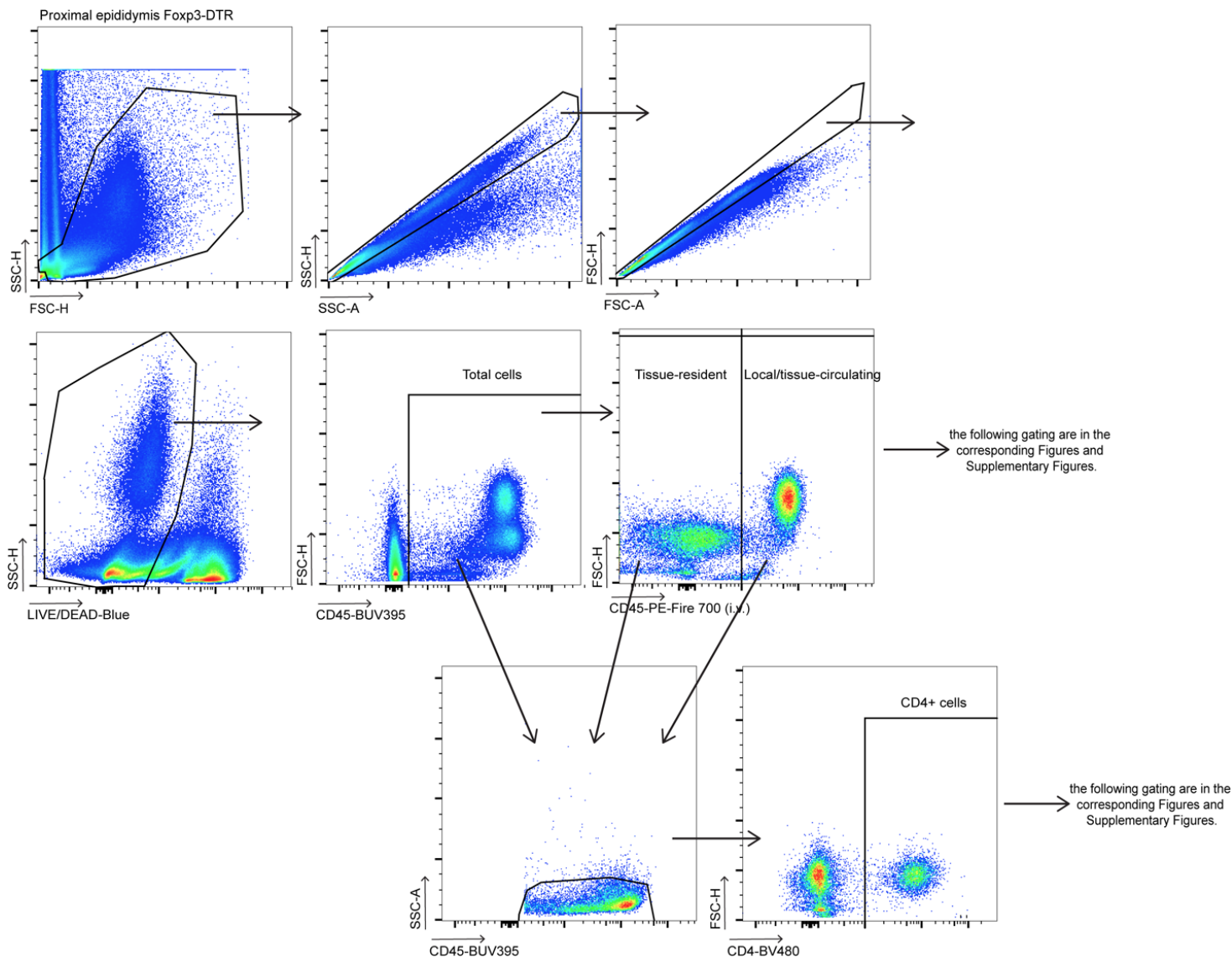
C



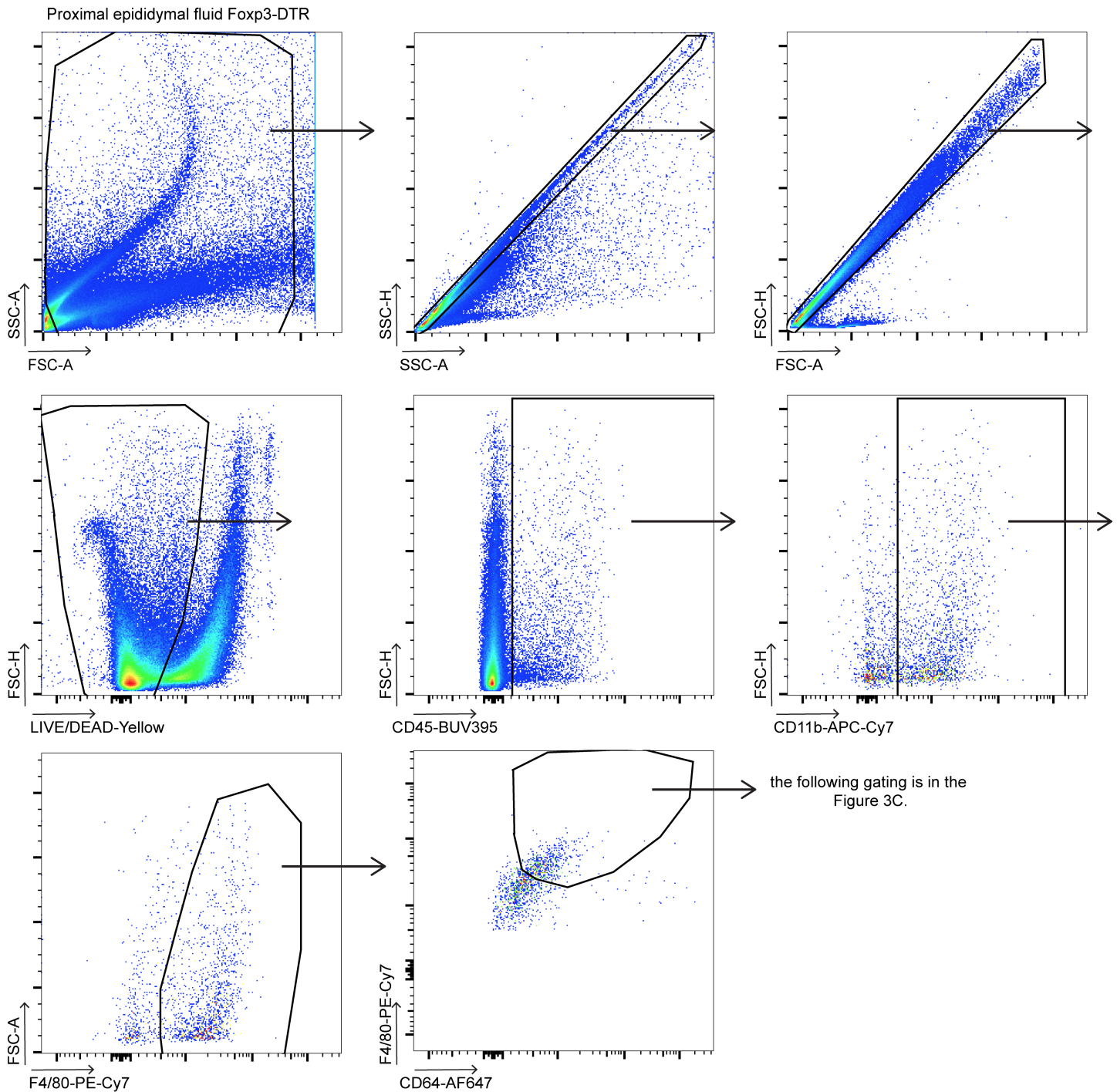
Supplementary Figure 24. Spermatogenesis 8 weeks after Treg depletion. **A)** H&E staining of WT and Foxp3-DTR testis 8 weeks after DT treatment. Bars: 25 μ m. **B)** A complete process of spermatogenesis was observed in both groups (n=4 testes from different mice). Bars: 20 μ m. **C)** Quantification of the various sperm cell developmental stages involved in mouse spermatogenesis was performed. Each dot represents the mean of four images quantified per testis per mouse. Data were analyzed using 2-way ANOVA. Data are shown as means $\pm$ SEM. n= number of samples from different mice.



Supplementary Figure 25. Confocal microscopy images of the positive controls using lymph nodes (LNs). Immunolabeling of CD20 (green), PDPN (magenta), AID (red), CD31 (red), PNAD (red), IgD (red), BCL6 (magenta), CD19 (red), and CD3 (magenta) in the lymph nodes (LNs) of DT-injected Foxp3-DTR mice. Bars: 20 μ m.



Supplementary Figure 26. Flow cytometry gating strategy used to study total, tissue-resident, and local/tissue-circulating immune cells in the epididymis. SSC-H: side scatter height, FSC-H: forward scatter height, SSC-A: side scatter area, FSC-A: forward scatter area, Viability dye: LIVE/DEAD™ Fixable Blue Dead Cell, CD45: pan immune cell marker, CD4: lymphocyte T helper marker.



Supplementary Figure 27. Flow cytometry gating strategy used to study myeloid immune cells in the epididymis. SSC-H: side scatter height, FSC-H: forward scatter height, SSC-A: side scatter area, FSC-A: forward scatter area, Viability dye: LIVE/DEAD™ Fixable Yellow Dead Cell, CD45: pan immune cell marker, CD11b: pan myeloid cell marker, F4/80: macrophage marker, CD64: macrophage marker.

	Normozoospermic (n=26)	Pathologic (n=12)	p-value
Volume (mL)	3.20 ± 1.28 [1.5 - 7]	3.33 ± 1.32 [1.5 - 5]	0.8524
Concentration (x10⁶ sperm/mL)	141.85 ± 145.62 [17.06 – 683.73]	112.45 ± 105.23 [25.42 – 313.32]	0.5781
Progressive motility (%)	47.20 ± 13.15 [33.07 – 85.74]	17.85 ± 6.43 [6.85 – 27.03]	< 0,0001
Morphology (Normal Forms %)	7.82 ± 3.38 [4 - 18]	9.04 ± 3.33 [4 - 14]	0.3544
Round Cells (%)	1.79 ± 1.94 [0.07 – 6.37]	4.29 ± 7.35 [0.46 – 26.55]	0.1889

Supplementary Table 1. Descriptive parameters of the semen samples included in the study were divided into normozoospermic (n=26) and pathologic (n=12) groups. Results are indicated as mean ± standard deviation (SD), and [minimum and maximum] values. Student's t-test (two-sided) was used to compare the groups. n= number of samples from different patients.

	no-RC (n=22)	RC (n=14)	p-value
Volume (mL)	3.17 ± 1.22 [1.5 - 7]	3.57 ± 1.33 [1.5 - 5]	0.3572
Concentration (x10 ⁶ sperm/mL)	129.49 ± 151.58 [17.06 – 683.73]	111.88 ± 91.22 [30.34 – 313.32]	0.6983
Progressive motility (%)	39.75 ± 19.23 [6.85 – 85.74]	33.92 ± 16.90 [7.64 – 61.11]	0.3595
Morphology (Normal Forms %)	8.86 ± 3.52 [4 - 18]	7.61 ± 3.11 [4 - 14]	0.2825
Round Cells (%)	0.60 ± 0.25 [0.07 – 0.96]	5.18 ± 6.48 [1 – 26.55]	0.0085

Supplementary Table 2. Descriptive parameters of the semen samples included in the study were divided into no-RC (n=22) and RC (n=14) groups. Results are indicated as mean ± standard deviation (SD), and [minimum and maximum] values. Student's t-test (two-sided) was used to compare the groups. n= number of samples from different patients.