

Anti-GBM serum effects on kidney function and glomerulosclerosis in mice

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Background & Aim

Antibody-induced glomerulonephritis (GN) is a condition caused by an inappropriate autoimmune response to renal antigens, such as the glomerular basement membrane (GBM), leading to progressive glomerulosclerosis and rapidly declining renal function for which there exist only few treatment options. Understanding the underlying mechanisms of GN is crucial for developing effective therapeutic strategies. In this study, we aimed to investigate the induction of antibody-induced GN by anti-GBM serum on kidney biomarkers, histology and transcriptome signatures.

Methods

Male C57BL/6J mice (n=12) were randomized into three groups (n=4 per group) and received either vehicle injection, 100, or 200 µl of anti-GBM serum. Renal endpoints included urine albumin-to-creatinine ratio (ACR), AI-assisted glomerulosclerosis scoring, histomorphometric analysis of fibrosis (Col3a1), and RNA sequencing (RNA-seq) analysis.

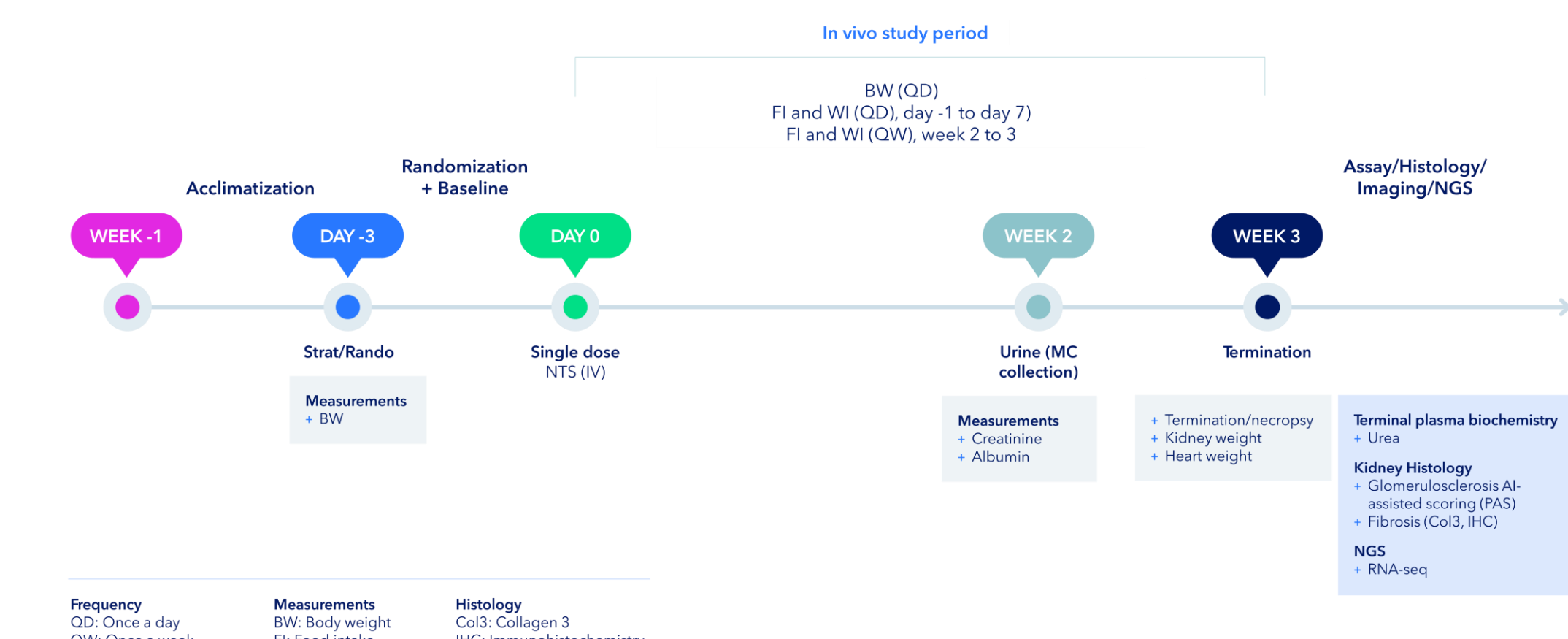
Conclusion

- + Anti-GBM serum induces fast onset of renal damage, glomerulosclerosis, and fibrosis in the mouse model of antibody-induced GN.
- + Anti-GBM also induces the up-regulation of genes involved in inflammatory and fibrosis.
- + The antibody-induced GN model in mice is highly applicable for probing test compounds with potential nephroprotective effects autoimmune GN.



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1 Study outline



Group	Animal	Gender	Number of animals	Treatment	Dosing Volume (µl)	Dosing concentration
1	Control	male	4	Vehicle	150	xx
2	Anti-GBM nephritis	male	4	NTS	100	xx
3	Anti-GBM nephritis	male	4	NTS	200	xx

2 Anti-GBM serum increases urine Albumin, ACR and KIM-1 to Creatinine

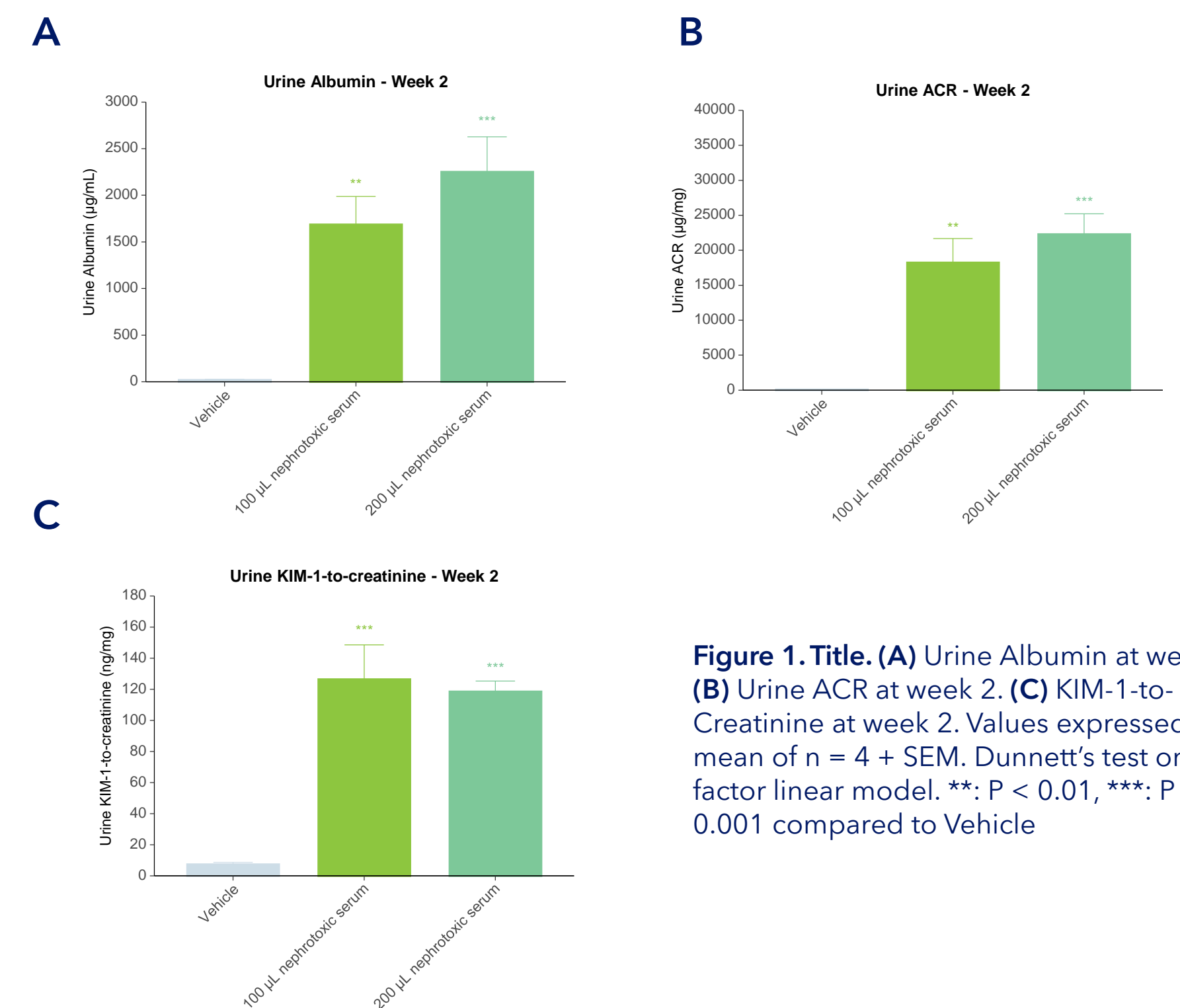


Figure 1. Title. (A) Urine Albumin at week 2. (B) Urine ACR at week 2. (C) KIM-1-to-Creatinine at week 2. Values expressed as mean of n = 4 + SEM. Dunnett's test one-factor linear model. **: P < 0.01, ***: P < 0.001 compared to Vehicle

3 Anti-GBM serum develops kidney fibrosis

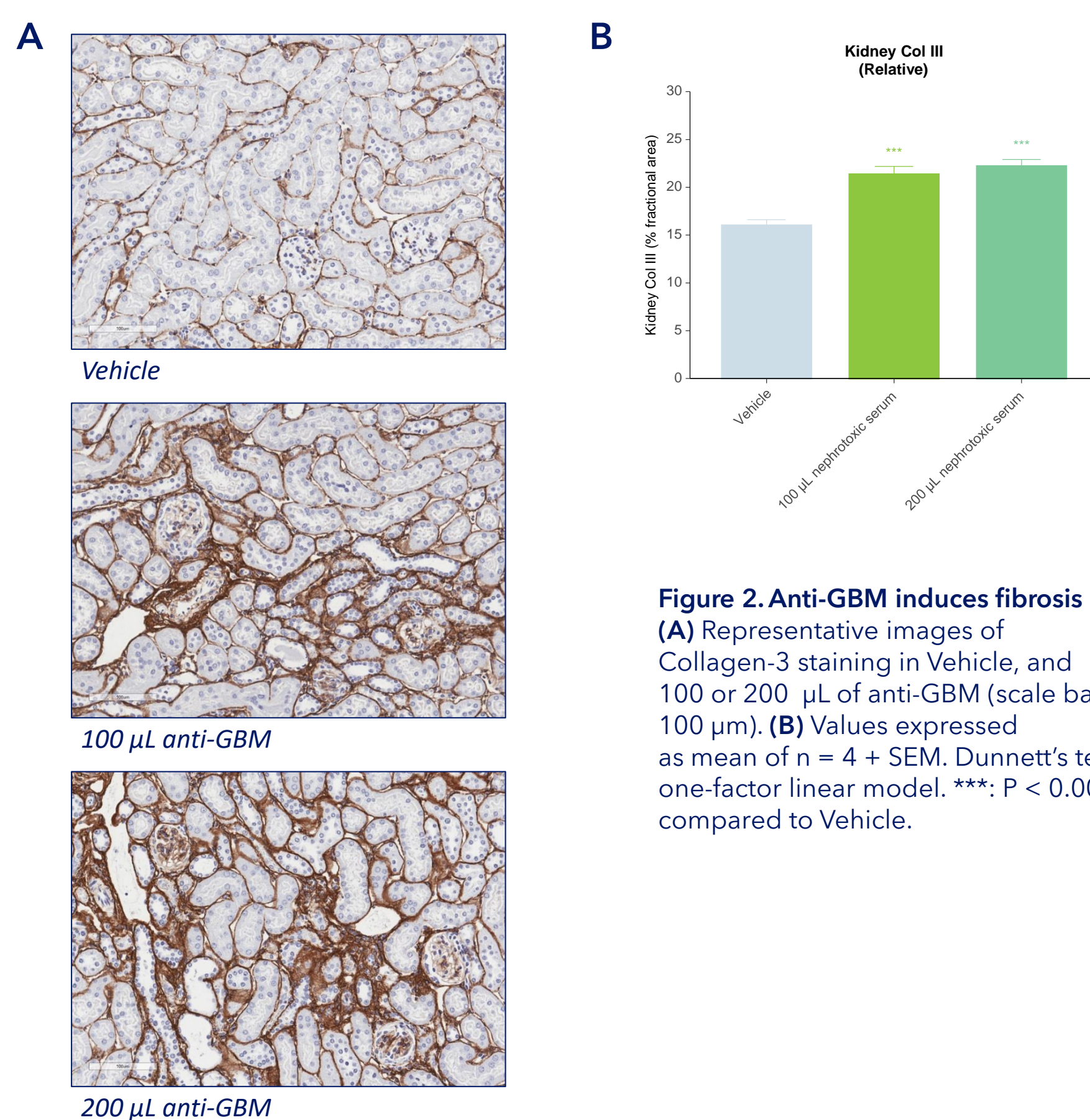


Figure 2. Anti-GBM induces fibrosis. (A) Representative images of Collagen-3 staining in Vehicle, and 100 or 200 µl of anti-GBM (scale bar, 100 µm). (B) Values expressed as mean of n = 4 + SEM. Dunnett's test one-factor linear model. ***: P < 0.001 compared to Vehicle.

4 Inflammatory and fibrotic signalling intensifies following the anti-GBM dosage

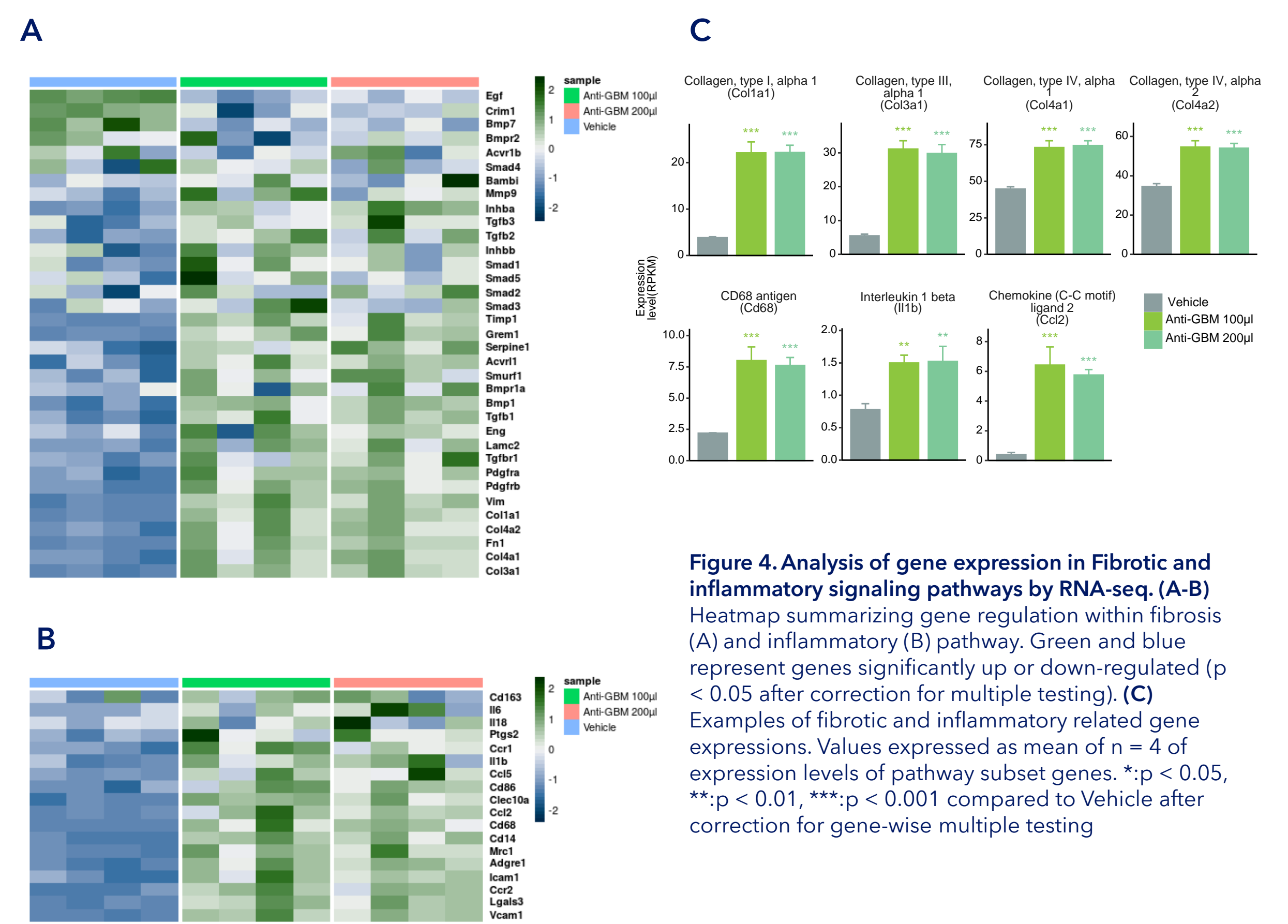


Figure 4. Analysis of gene expression in Fibrotic and inflammatory signaling pathways by RNA-seq. (A-B) Heatmap summarizing gene regulation within fibrosis (A) and inflammatory (B) pathway. Green and blue represent genes significantly up or down-regulated (p < 0.05 after correction for multiple testing). (C) Examples of fibrotic and inflammatory related gene expressions. Values expressed as mean of n = 4 of expression levels of pathway subset genes. *: p < 0.05, **: p < 0.01, ***: p < 0.001 compared to Vehicle after correction for gene-wise multiple testing

5 AI-assisted Glomerulosclerosis scoring increases after anti-GBM induction

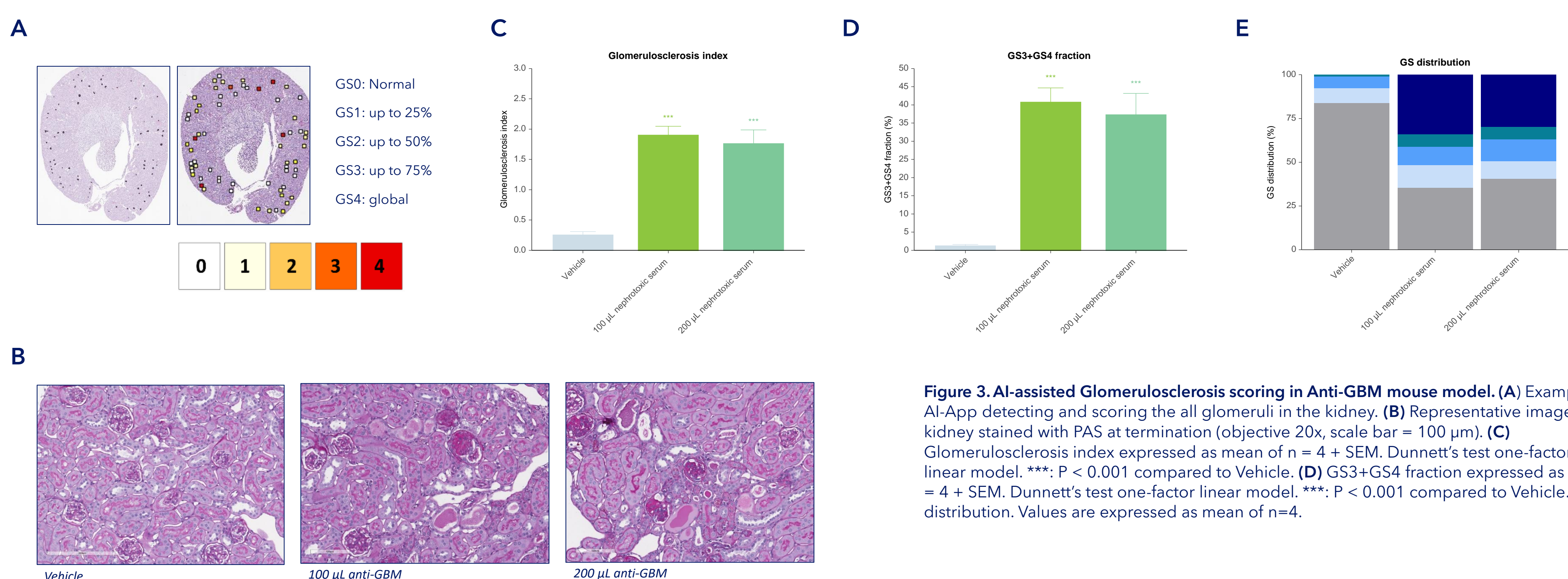


Figure 3. AI-assisted Glomerulosclerosis scoring in Anti-GBM mouse model. (A) Example of the AI-App detecting and scoring the all glomeruli in the kidney. (B) Representative images of kidney stained with PAS at termination (objective 20x, scale bar = 100 µm). (C) Glomerulosclerosis index expressed as mean of n = 4 + SEM. Dunnett's test one-factor linear model. ***: P < 0.001 compared to Vehicle. (D) GS3+GS4 fraction expressed as mean of n = 4 + SEM. Dunnett's test one-factor linear model. ***: P < 0.001 compared to Vehicle. (E) GS % distribution. Values are expressed as mean of n=4.