Supplementary Material for

# Mineralocorticoid receptor antagonism in diabetes reduces albuminuria by preserving the glomerular endothelial glycocalyx

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## Supplementary Figures

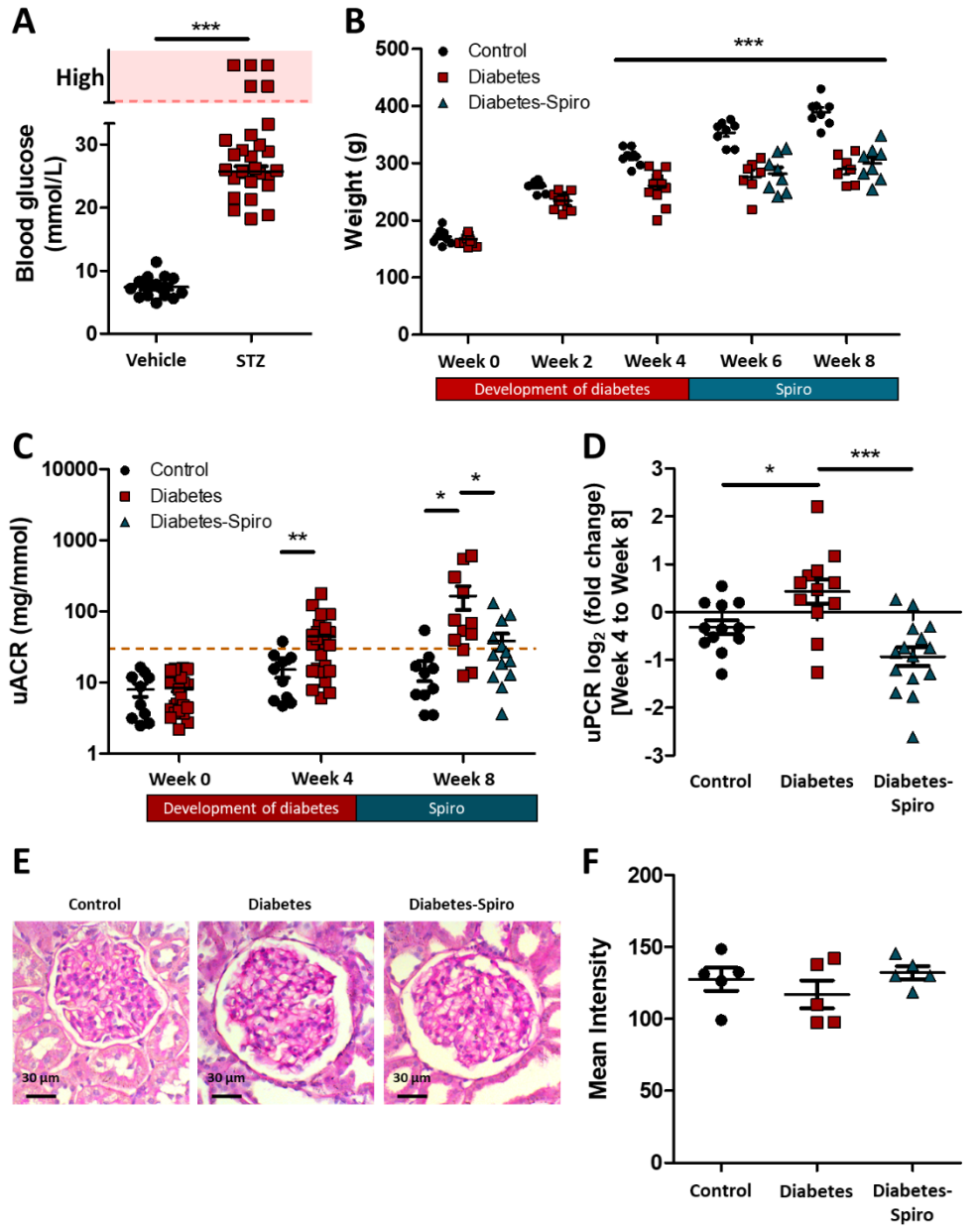
**Supplemental Figure 1.** Streptozotocin (STZ)-induced rats were hyperglycaemic, development of albuminuria and proteinuria in early diabetic nephropathy were limited by MR antagonism

**Supplemental Figure 2.** Transmission electron microscopy confirmed that MR antagonism prevented diabetes-induced glomerular endothelial glycocalyx damage.

**Supplemental Figure 3.** Early diabetic nephropathy was not associated with other endothelial, podocyte or glomerular parameter changes.

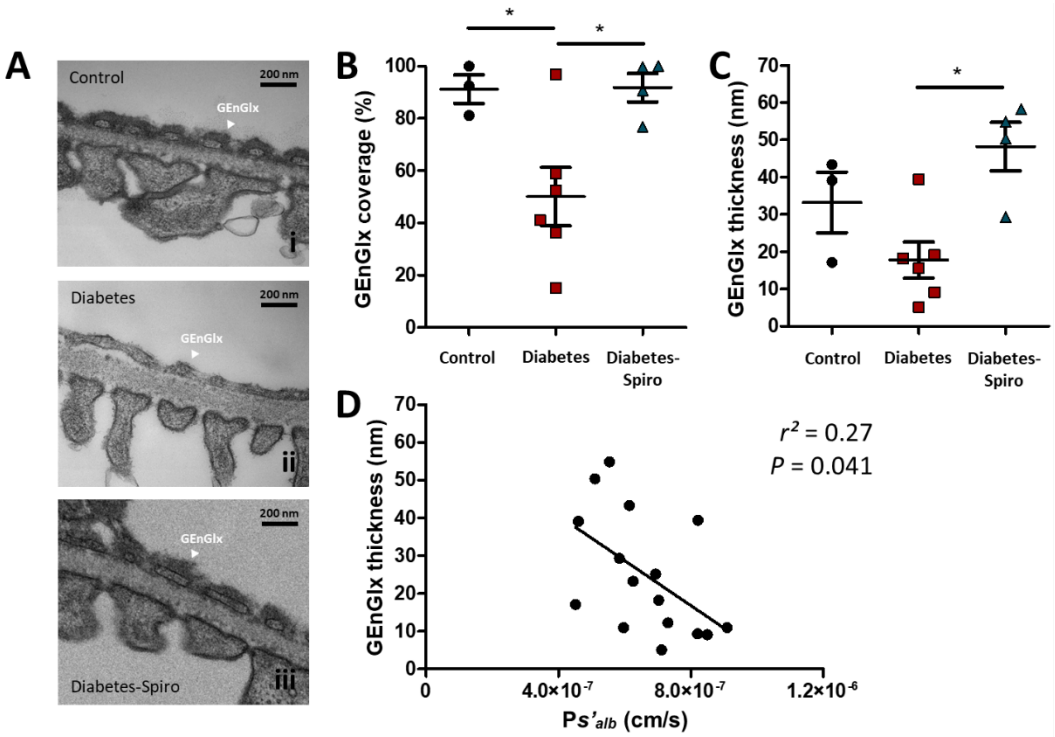
**Supplemental Figure 4.** The effect of infused hyaluronidase is focused on the endothelial glycocalyx, resulting in isolated GEnGlx degradation and no other detectable changes in the filtration barrier.

**Supplemental Figure 5.** Exposing human GEnC to diabetic conditions resulted in GEnGlx damage and *MMP* mRNA up-regulation that were ameliorated by MR antagonism.

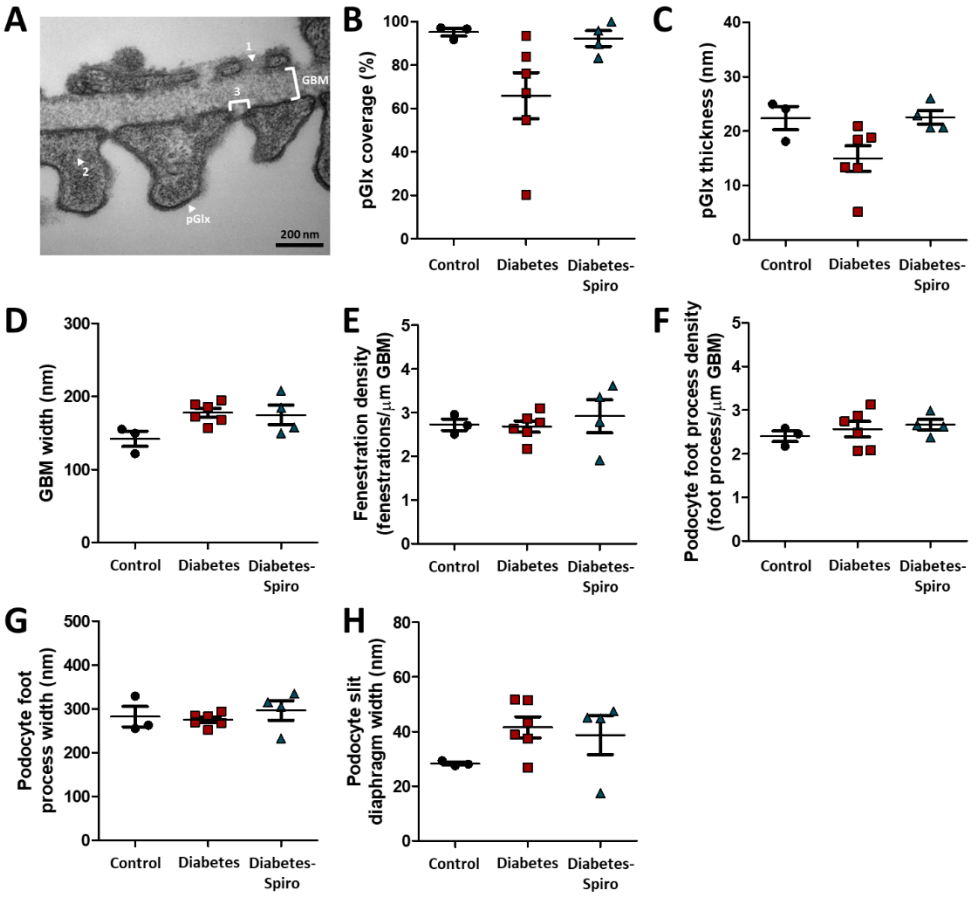



**Supplemental Figure 1. Streptozotocin (STZ)-induced rats were hyperglycaemic, development of albuminuria and proteinuria in early diabetic nephropathy were limited by MR antagonism.** (**A**) Rats were hyperglycaemic at week 3 post-STZ (vehicle, *n*=14; STZ, *n*=27). Unpaired t test was used for statistical analysis. (**B**) Body weight was not significant between groups when compared at week 0 but from week 4 post-STZ both vehicle and spironolactone (spiro) treated diabetic rats were significantly lower than control rats (control, *n*=8; diabetes, *n*=7; diabetes-spiro, *n*=8). Repeated measures two-way ANNOVA was used for statistical analysis followed by Bonferroni's multiple comparisons. (**C**) Albuminuria levels stabilise in diabetic rats treated with spiro but progressively worsen in rats given vehicle. Urinary albumin:creatinine ratio

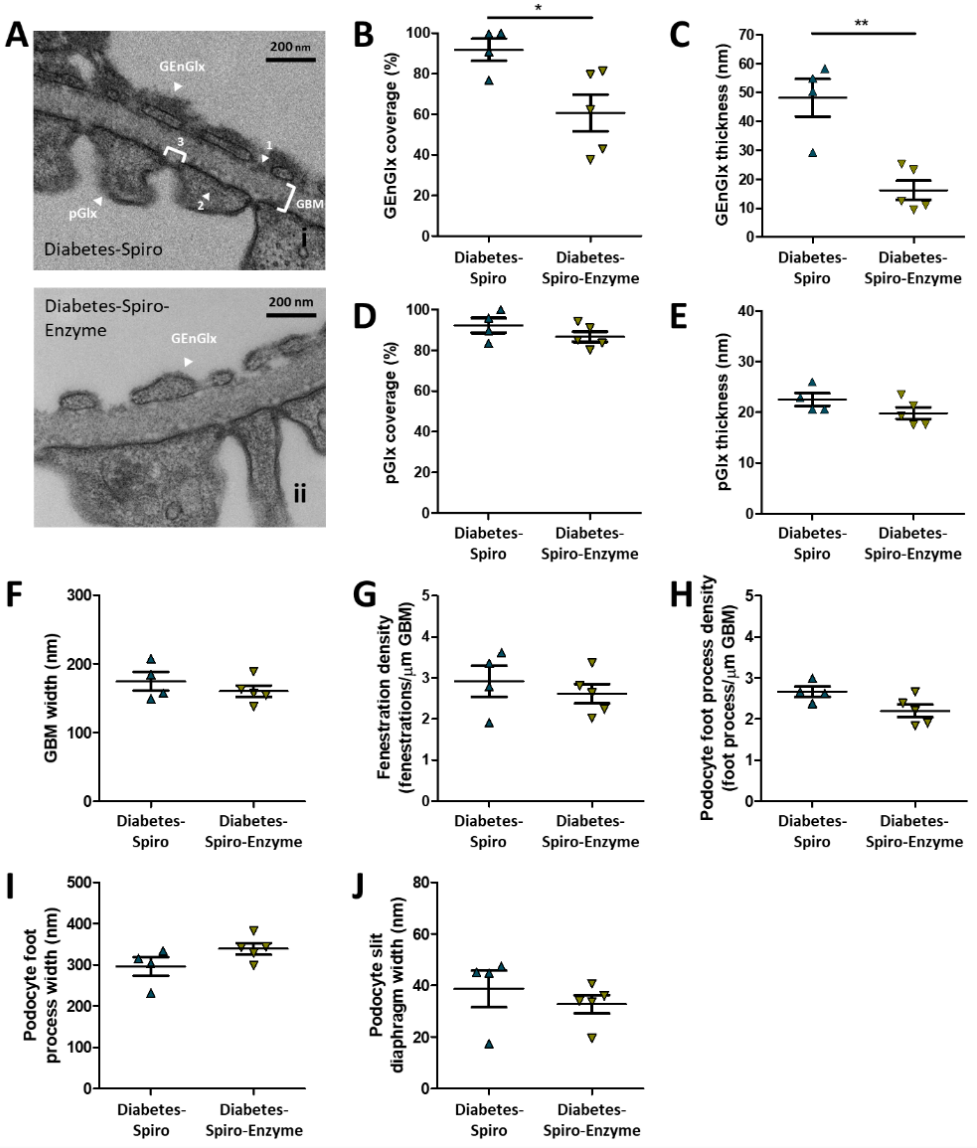
(uACR) was determined at week 0, week 4, and week 8 (control, *n*=10; diabetes, *n*=12; diabetes-spiro, *n*=14). A base-10 log scale is used for the Y axis. Repeated measures two-way ANNOVA (Week 0 to Week 4) and one-way ANOVA (Week 8) was used for statistical analysis followed by Bonferroni's and Tukey's multiple comparisons, respectively. (**D**) Urinary protein:creatinine ratio (uPCR) was determined at week 4 and week 8 showing treatment with spiro reduced the fold change in uPCR from initiation of treatment (control, *n*=12; diabetes, *n*=12; diabetes-spiro, *n*=15). Data was log transformed and presented as $\log_2$ (fold change). (**E**) Representative images of glomeruli stained with periodic acid–Schiff (PAS) are shown for control, diabetes, and diabetes-spiro samples. Bars = 30 μm. (**F**) Quantification of PAS staining at week 8 post-STZ show no significant differences. One-way ANOVA was used for statistical analysis. Each dot, triangle, or square on the graphs represent a rat. Data are expressed as mean ± SEM. * $P<0.05$; ** $P<0.01$; *** $P<0.001$.




**Supplemental Figure 2. Transmission electron microscopy confirmed that MR antagonism prevented diabetes-induced glomerular endothelial glycocalyx damage.** Rats were perfusion-fixed for transmission electron microscopy (TEM) with cacodylate buffer containing glutaraldehyde and Alcian blue. (**A**) Representative electron micrographs of the glomerular capillary wall are shown for control, diabetes, and diabetes-spironolactone (spiro) samples. Labels indicate glomerular endothelial glycocalyx (GEnGlx). Bars = 200 nm. Quantification at week 8 post-STZ of (**B**) GEnGlx coverage, and (**C**) GEnGlx thickness (control, *n*=3 rats (9 glomeruli); diabetes, *n*=6(13); diabetes-spiro, *n*=4(8)). In B and C, one-way ANOVA was used for statistical analysis followed by Tukey's multiple comparisons. (**D**) The rate of glomerular albumin leakage ($Ps'_{alb}$) is weakly associated with GEnGlx thickness measured by TEM (*n*=16). Each dot, triangle, and square on the graph represents a rat. Data are expressed as mean ± SEM. * *P*<0.05.

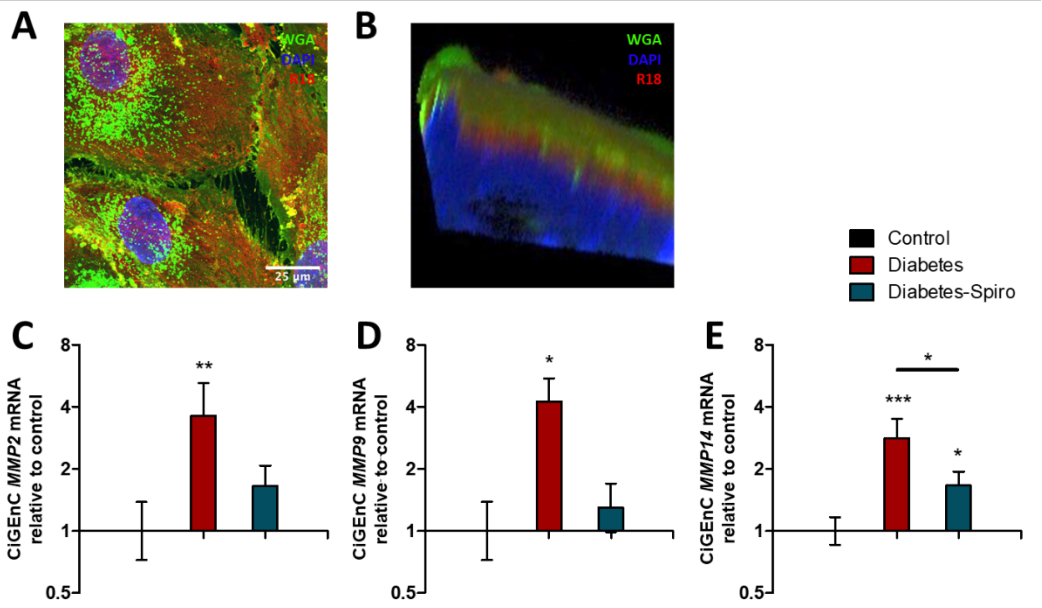



**Supplemental Figure 3. Early diabetic nephropathy was not associated with other endothelial, podocyte or glomerular parameter changes.** Rats were perfusion-fixed for transmission electron microscopy with cacodylate buffer containing glutaraldehyde and Alcian blue. (**A**) Representative electron micrographs of the glomerular capillary wall with labels indicating podocyte glycocalyx (pGlx), glomerular basement membrane (GBM), (1) fenestration, (2) podocyte foot process, and (3) slit diaphragm. Bar = 200 nm. Quantification at week 8 post-STZ of (**B**) pGlx coverage, (**C**) pGlx thickness, (**D**) GBM width, (**E**) fenestration density, (**F**) podocyte foot process density, (**G**) podocyte foot process width, and (**H**) slit diaphragm width (control, *n*=3 rats (9 glomeruli); diabetes, *n*=6(13); diabetes-spiro, *n*=4(8)). In B-H, one-way ANOVA was used for statistical analysis. Each dot, triangle, or square on the graph represents a rat. Data are expressed as mean ± SEM.




**Supplemental Figure 4. The effect of infused hyaluronidase is focused on the endothelial glycocalyx, resulting in isolated GEnGlx degradation and no other detectable changes in the filtration barrier.** Rats were perfusion-fixed for transmission electron microscopy with cacodylate buffer containing glutaraldehyde and Alcian blue. (**A**) Representative electron micrographs of the glomerular capillary wall are shown for diabetes-spiro, and diabetes-spiro-enzyme samples. Labels indicate glomerular endothelial glycocalyx (GEnGlx), podocyte glycocalyx (pGlx), glomerular basement membrane (GBM), (1) fenestration, (2) podocyte foot process, and (3) slit diaphragm. Bars = 200 nm. TEM quantification at week 8 post-STZ of (**B**) GEnGlx coverage, and (**C**) GEnGlx thickness (diabetes-spironolactone (spiro), *n*=4 rats (8 glomeruli); diabetes-spiro-enzyme, *n*=5(14)) confirmed enzyme degradation of GEnGlx. Quantification at week 8 post-STZ of (**D**) pGlx coverage, (**E**) pGlx thickness, (**F**) GBM width, (**G**) fenestration density, (**H**) podocyte foot

process density, (**I**) podocyte foot process width, and (**J**) slit diaphragm width. In B-J, unpaired t test was used for statistical analysis Each triangle on the graph represents a rat. Data are expressed as mean ± SEM. * $P<0.05$; ** $P<0.01$.




**Supplemental Figure 5. Exposing human GEnC to diabetic conditions resulted in GEnGlx damage and *MMP* mRNA up-regulation that were ameliorated by MR antagonism.** Human conditionally immortalized glomerular endothelial cells (CiGEnC) maintained in the presence of glucose, insulin, TNF-α and IL-6 to mimic a diabetic environment. Representative images of CiGEnC control samples stained with WGA lectin showing (**A**) 3D projection of Z-stack images and (B) 3D volume rendering with a sliced view showing endothelial glycocalyx stained with WGA lectin. DAPI, nuclear label; R18, membrane label. Bar = 25 µm. Gene expression in CiGEnC was determined using TaqMan RT-qPCR for (**C**) *MMP2*, (**D**) *MMP9*, and (**E**) *MMP14*. The $2^{-\Delta\Delta CT}$ method of quantification was used to calculate the fold change, normalized to *GAPDH* (*n*=6 per group). In C-E, one-way ANOVA was used for statistical analysis followed by Tukey's multiple comparisons. Data are expressed as mean ± SEM. * *P*<0.05; ** *P*<0.01; *** *P*<0.001.