Supplemental Materials

Molecular Biology of the Cell

Miller et al.

SUPPLEMENTAL FIGURE S1 High glucose induces activation of β1 integrins in glomerular endothelial cells

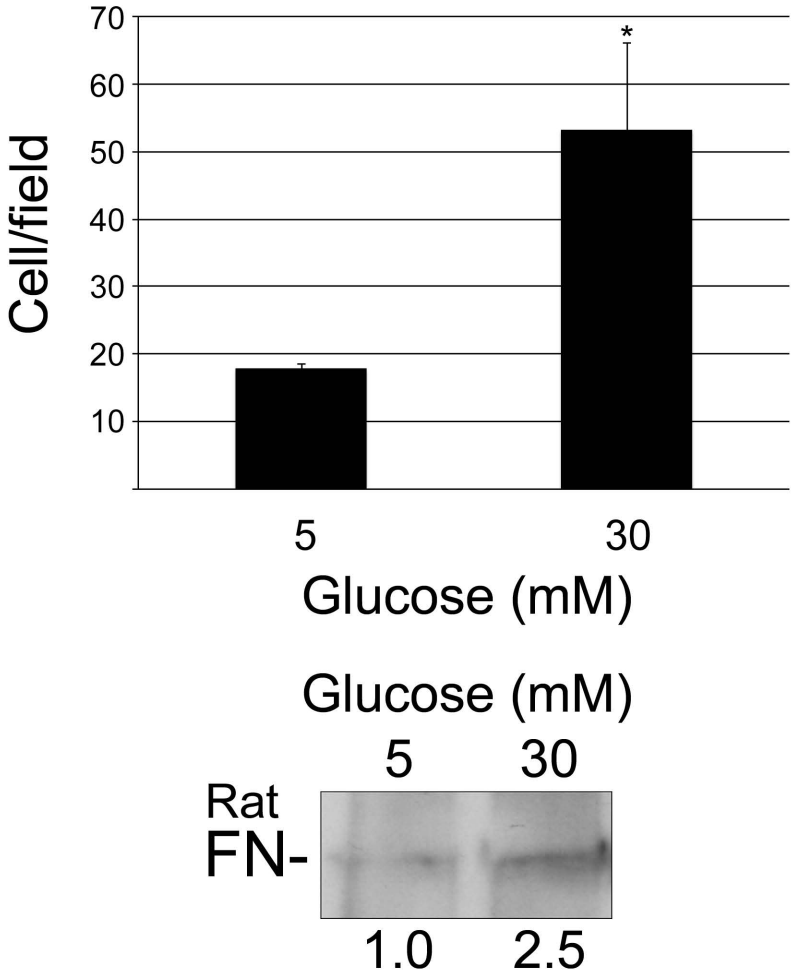
Glomerular endothelial cells were grown for 3 days in normal or high glucose. (A) Cells were then plated on surfaces coated with 9EG7 anti-β1 antibody and incubated for 30 min at 37°C before unattached cells were washed away. Attached cells were fixed and 3 wells per condition were counted. Bar graph shows the mean of two independent experiments ± 1 SD, *$p < 0.05$ compared to 5 mM glucose. (B) Cells were suspended in modified Tyrode's buffer with rat FN for 30 min at room temperature, pelleted through a sucrose cushion, lysed in SDS sample buffer, and immunoblotted with IC3 anti-rat FN monoclonal antibody. Relative densitometry values (below the lanes) are the mean of two experiments.

SUPPLEMENTAL FIGURE S2 Collagen IV and FN co-localize to the mesangium in glomeruli of diabetic mice

Kidney sections of streptozotocin (STZ)-treated mice (24 weeks) were stained sequentially with anti-collagen IV (red) and R457 anti-FN antiserum (green). Two representative fields are shown; scale bar = 50 μm.

Tissue staining—Mice of 129 Sv background, 8 weeks old, received a single intraperitoneal injection of streptozotocin (STZ) (100 mg/kg dissolved in citrate buffer, pH 4.5) to induce diabetes and were subsequently killed at 24 weeks post-STZ injection (Wang *et al.*, 1993). Frozen OCT-embedded mouse kidneys were cut into sections of 6 μm thickness on a cryostat and fixed in 4% paraformaldehyde and subsequently blocked with 5% goat serum in PBS before staining. For R457 and anti-collagen IV staining antibodies were diluted 1:100 in 5% goat serum in PBS. Secondary antibodies were diluted 1:600 in 5% goat serum in PBS. All images were captured using a Nikon Eclipse

Ti microscope equipped with a Hamamatsu C10600 ORCA-R2 digital camera.



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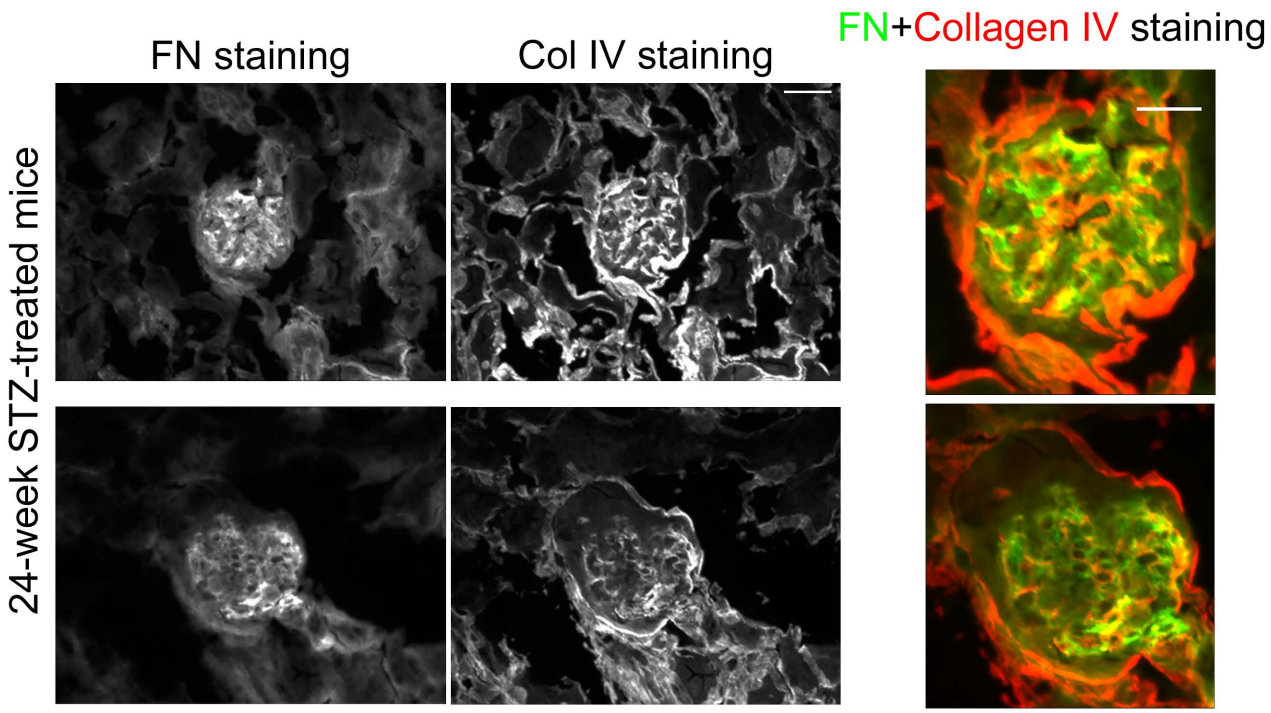

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