SUPPLEMENTARY MATERIAL

Figure S1. LPS does not change expression of WGA and heparanase in glomeruli of Tnfr1−/− mice.

RNA isolation and real-time quantitative polymerase chain reaction (PCR). Total RNA extraction from kidneys and reverse transcriptase reactions were performed as described previously.[69] Real-time PCR was performed using the Applied Biosystems 7900 system and the SybrGreen intercalating dye method with HotStar DNA polymerase (Applied Biosystems, Foster City, CA). PCR was performed with a hot start at 95°C (10 min) followed by 40 cycles of alternating 95°C (15 s) / 59°C (60 s). For each sample, the number of cycles required to generate a given threshold signal (Ct) was recorded. The ratio of expression of the gene of interest relative to 18S expression was calculated for each sample and normalized to the ratio of the control group. Primers for 18S were designed using Primer3 and Blast. Primers for VEGF and VEGFR2 were from GETPrime (http://updepla1srv1.epfl.ch/getprime/). Synthesis was performed by Invitrogen Custom Primers (Camarillo, CA), with sequences as follows:

18S forward primer 5-GTT GGT GGA GCG ATT TGT CT-3,

18S reverse primer 5-GAA CGC CAC TTG TCC CTC TAT-3,

VEGF forward primer 5- GGT TTA AAT CCT GGA GCG T -3,

VEGF reverse primer 5- TTG TCA CAT CTG CAA GTA CG -3,

VEGFR2 forward primer 5- CTC TTT GCG CTA GGT ATC C -3,

VEGFR2 reverse primer 5- GAG TAA AGC CTA TCT CGC TG -3.

Supplementary material is linked to the online version of the paper at

http://www.nature.com/ki

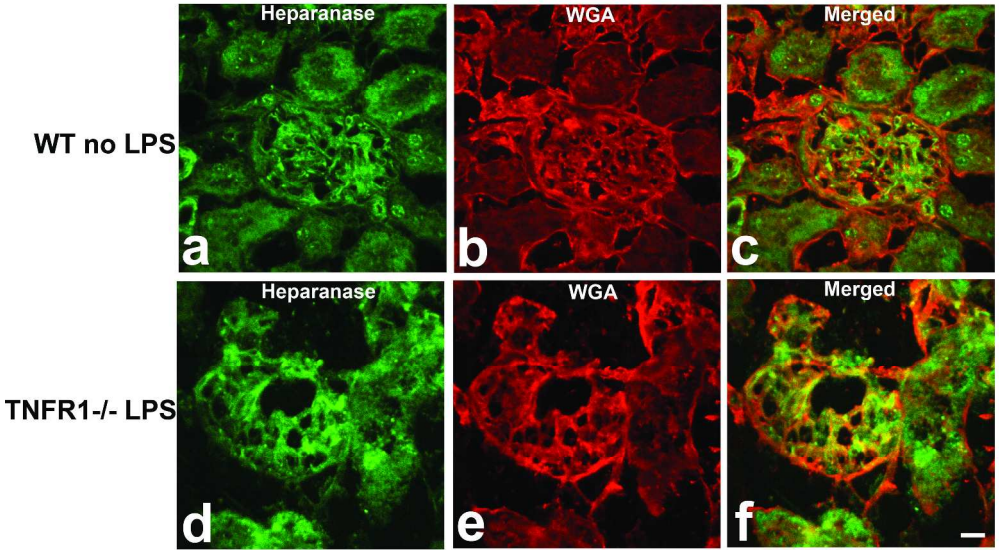

Figure S1. LPS fails to change expression of WGA and heparanse in glomeruli of *Tnfr1*$^{-/-}$ mice. Representative fluorescence photomicrographs of frozen kidney cortex sections from wild-type control mice (a-c) and *Tnfr1*$^{-/-}$ mice treated 24 h with 10 mg/kg LPS (d-e), incubated with antibodies against Heparanase-1 (green; a, d) and Alexa Fluor 594-WGA (red; b, e). Scale bar 20 μm.
314x173mm (300 x 300 DPI)