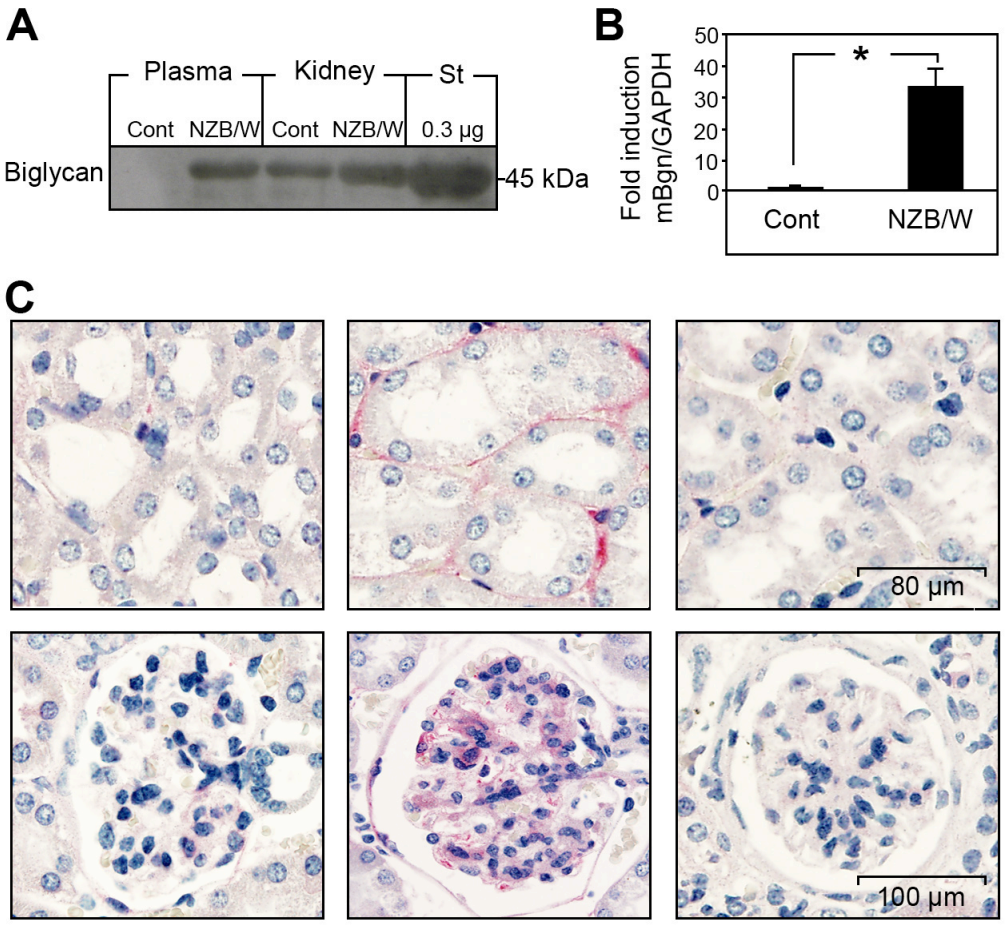
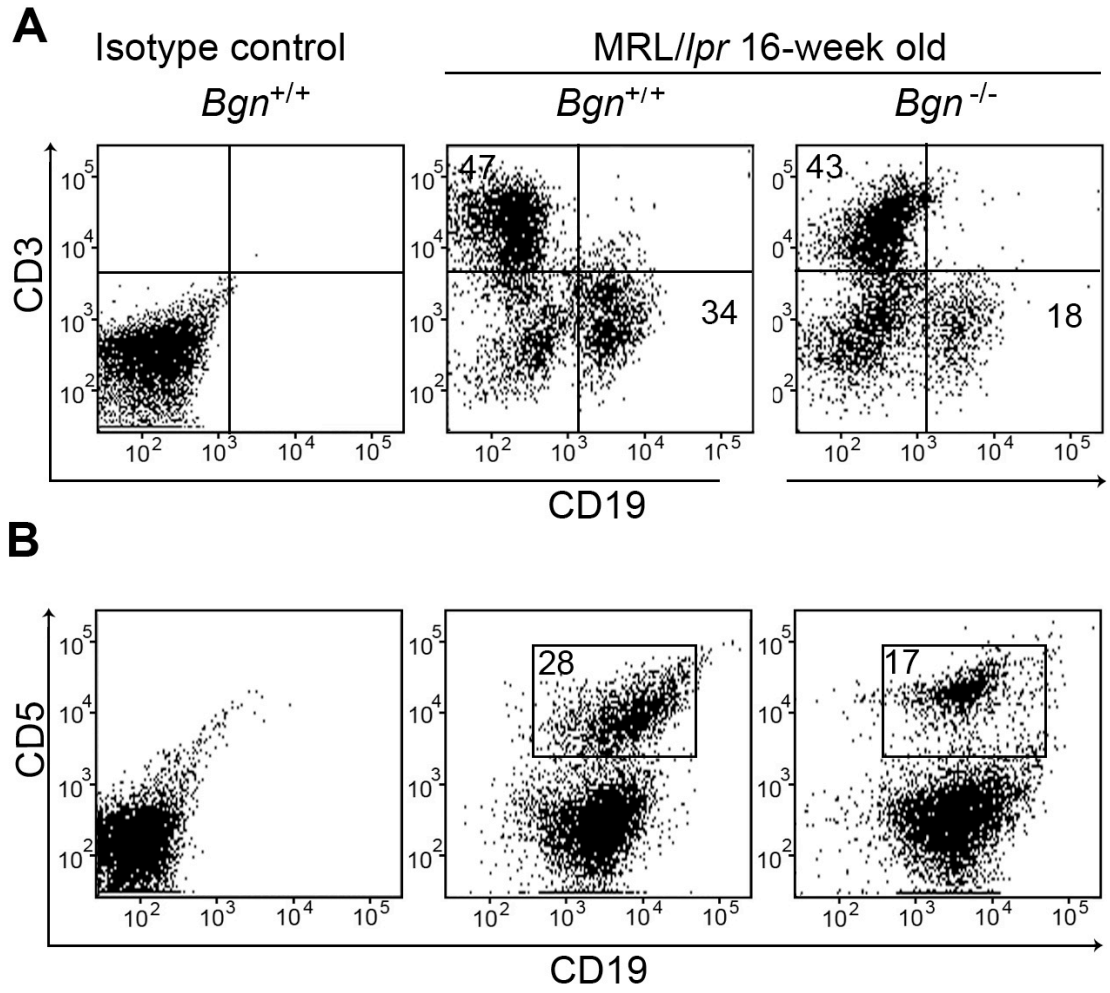
SUPPLEMENTAL FIGURE 1.



Supplemental Figure 1: *Overexpression of biglycan in plasma and kidneys from New Zealand Black/ White (NZB/W)-F1 hybrid mice*

A: Immunoblot for biglycan protein core after semi-purification from plasma (150 µl) and kidneys (150 µg) from 16-week-old female NZB/W-F1 mice vs. C57BL/6 control (Cont) mice. Standard (St) contained 0.3 µg of biglycan that was digested with chondroitinase ABC only. **B:** Enhanced biglycan mRNA expression in kidneys from 16-week-old NZB/W-F1 vs. C57BL/6 control mice quantitated by TaqMan analysis after normalization to GAPDH (given as means ± SD). The asterisks indicate statistical significance; n=4, $*P < 0.05$. **C:** Immunohistochemical analysis of biglycan expression patterns (APAAP, red) in the tubulointerstitium (upper panel) and glomeruli (lower panel) of renal tissue sections from 16-week-old NZB/W-F1 and control mice. Counterstaining was done with Meyer´s Hematoxylin. The negative control was performed in renal tissue sections from 16-week-old NZB/W-F1 mice by using an antigen-preadsorbed (mouse biglycan) antiserum. Scale bar = 80 µm for the upper panel and 100 µm for the lower panel.

SUPPLEMENTAL FIGURE 2.



Supplemental Figure 2: *Flow cytometric analysis of B- and T-lymphocytes in kidneys from 16-week old Bgn$^{+/+}$MRL/lpr and Bgn$^{-/-}$MRL/lpr mice*

A: Flow cytometric analysis of CD3-positive (T cells) and CD19-positive (B cells) mononuclear cells revealed a marked reduction of B lymphocytes in kidneys from 16-week old *Bgn*$^{-/-}$MRL/*lpr* vs. *Bgn*$^{+/+}$MRL/*lpr* mice. Mononuclear cells were purified by density gradient centrifugation, counted and 10^6 cells were immunostained with anti-CD3 (PerCP) and anti-CD19 (FITC) or the respective isotype controls, and analyzed by flow cytometry. **B:** Flow cytometric analysis of CD5- and CD19-positive B cells revealed a marked reduction of B1 lymphocytes (positive for both CD5 and CD19) in kidneys from 16-week old *Bgn*$^{-/-}$ MRL/*lpr* vs. *Bgn*$^{+/+}$MRL/*lpr* mice. Mononuclear cells were isolated as described above with subsequent selection of CD19 positive cells using MicroBeads and MACS Technology. Cells were then counted and 10^6 cells were immunostained with anti-CD5 (PE) or anti-CD19 (FITC) antibodies or the respective isotype controls, and analyzed by flow cytometry. For isolation of mononuclear cells 6 whole kidneys from each genotype were used **(A, B)**. The figures **(A, B)** show a representative flow cytometric analysis out of 5 independent experiments. Percentages given are the mean of the five experiments **(A, B)**.

Supplemental Table 1. Weights of the spleen and lymph nodes from 10-week old, biglycan-transgenic *Bgn*$^{+/+}$MRL and *Bgn*$^{+/+}$MRL/*lpr* mice and their respective controls

Weights	10-week old						
	MRL			MRL/*lpr*			
	Bgn$^{+/+}$	*Bgn*$^{+/+}$ pLIVE	*Bgn*$^{+/+}$ h*BGN*	*Bgn*$^{+/+}$	*Bgn*$^{+/+}$ pLIVE	*Bgn*$^{+/+}$h*BGN*	*Bgn*$^{-/-}$
Spleen (g)	0.10±0.04	0.10±0.03	0.11±0.04	0.15±0.03	0.15±.0.05	0.19±0.03	0.11±0.05
Spleen/BW ratio x1000	3.6±0.52	3.7±0.67	3.7±0.79	5.5±0.94*	5.7±0.56	6.5±0.87	3.7±0.86
Lymph nodes (g)	0.05±0.03	0.05±0.02	0.06±0.02	0.24±0.06*	0.26±0.09	0.41±0.08‡	0.07±0.03†
LN/BW ratio x1000	1.8±0.81	1.7±0.75	2.0±1.2	8.2±1.4*	8.7±1.5	14±2.3‡	2.6±1.0†

The weights of the spleen and lymph nodes (mediastinal, axillary, inguinal and mesenteric) were measured and the ratios of spleen/body weight and lymph node/body weight (LN/BW ratio) were calculated for each animal. pLIVE: mice transiently transfected for 7 days with the pLIVE vector. h*BGN*: mice transiently transfected for 7 days with human biglycan inserted into the pLIVE vector.

Values are given as the means ± SD for n=6 per group, asterisks indicating statistical significance; **P <0.05 Bgn*$^{+/+}$MRL*/lpr* versus *Bgn*$^{+/+}$MRL mice; *†P <0.05 Bgn*$^{-/-}$MRL*/lpr* versus age-matched *Bgn*$^{+/+}$MRL*/lpr* mice; *‡P <0.05* h*BGN* MRL/*lpr* versus age-matched *Bgn*$^{+/+}$MRL/*lpr* mice.

Supplemental Table 2. Weights of the spleen and lymph nodes from *Bgn*$^{+/+}$MRL, *Bgn*$^{+/+}$MRL/*lpr* and *Bgn*$^{-/-}$ MRL/*lpr* mice at 16 and 24 weeks of age

Weights	16-week old			24-week old		
	MRL	MRL/*lpr*		MRL	MRL/*lpr*	
	Bgn$^{+/+}$	*Bgn*$^{+/+}$	*Bgn*$^{-/-}$	*Bgn*$^{+/+}$	*Bgn*$^{+/+}$	*Bgn*$^{-/-}$
Spleen (g)	0.15±0.04	0.58±0.09*	0.26±0.04*†	0.18±0.04	0.75±0.09*	0.39±0.15*†
Spleen/BW ratio x1000	3.7±0.63	14±1.2*	5.9±0.98*†	4.2±0.23	17±2.0*	8.1±1.4*†
Lymph nodes (g)	0.07±0.03	0.96±0.33*	0.28±0.13*†	0.07±0.05	2.4±0.43*	0.75±0.21*†
LN/BW ratio x1000	1.8±0.72	23±1.2*	6.7±1.4*†	1.7±0.68	53±7.1*	16±4.4*†

The weights of the spleen and lymph nodes (mediastinal, axillary, inguinal and mesenteric) were measured and the ratios of spleen/body weight and lymph nodes/body weight (LN/BW) were calculated for each animal.

Values are given as the means ± SD for n=6 per group; **P <0.05* versus age-matched *Bgn*$^{+/+}$MRL mice; *†P <0.05* *Bgn*$^{-/-}$MRL*/lpr* versus age-matched *Bgn*$^{+/+}$MRL*/lpr* mice.

Supplemental Table 3. Plasma levels of selected chemokines and cytokines in *C57BL/6, TLR2*$^{-/-}$, *TLR4*$^{-/-}$ and *TLR2*$^{-/-}$*/TLR4-M* mice overexpressing biglycan and their respective controls

Genotype		MCP-1 (pg/ml)	RANTES (pg/ml)	MIP-1α (pg/ml)	TNFα (pg/ml)	IL-1β (pg/ml)	CXCL13 (pg/ml)
C57BL/6	Cont	21±4.6	94±12	2.1±0.33	2.8±1.2	6.2±1.5	124±8.0
	pLIVE	21±5.2	92±11	2.1±0.29	2.6±0.54	6.0±1.4	120±10
	h*BGN*	46±5.6*	133±14*	6.8±1.1*	6.7±1.2*	11±1.58*	170±16*
TLR2$^{-/-}$	Cont	23±3.0	89±6.7	1.8±0.44	2.8±0.35	6.5±1.2	125±10
	pLIVE	22±2.5	91±6.7	2.1±0.47	3.0±0.43	6.3±1.0	123±10
	h*BGN*	34±5.3*†‡	123±25*	4.9±0.90*†	3.8±0.59*†	8.7±2.1*†‡	153±14*‡
TLR4$^{-/-}$	Cont	22±5.4	94±11	2.3±0.56	3.0±0.30	6.4±0.77	123±6.7
	pLIVE	23±5.1	93±9.5	2.0±0.67	2.8±0.30	6.1±1.2	122±7.8
	h*BGN*	28±5.3*†	113±17*†	3.3±1.4*†	3.1±0.79†	7.1±0.55*†	136±12*†
TLR2$^{-/-}$*/TLR4-M*	Cont	21±3.3	91±7.1	2.1±0.55	2.5±0.49	6.2±1.4	120±13
	pLIVE	19±2.7	91±10	2.3±0.58	2.7±0.49	5.9±1.5	122±8.7
	h*BGN*	23±7.6†	92±8.7†	2.1±0.30†	2.7±0.24†	6.6±1.1†	121±13†

pLIVE: mice transiently transfected for 7 days with the pLIVE vector. h*BGN*: mice transiently transfected for 7 days with human biglycan inserted into the pLIVE vector. Values are given as means ± SD for n=6 per group; **P <0.05* h*BGN* versus control (Cont) mice of the same genotype; *†P <0.05* h*BGN TLR2*$^{-/-}$, h*BGN TLR4*$^{-/-}$ or h*BGN TLR2*$^{-/-}$*/TLR4-M* versus h*BGN C57BL/6* mice. *‡P <0.05* h*BGN TLR2*$^{-/-}$ versus h*BGN TLR4*$^{-/-}$ mice.

Supplemental Table 4. Characteristics of healthy controls and patients with diabetic nephropathy and acute renal allograft rejection

Characteristics	Healthy Controls (n=8)	Diabetic Nephropathy (n=7)	Acute Rejection (n=6)
Age (years)	52±8	63±8	44±19
Sex (F/M)	4/4	2/5	2/4
Known duration of diabetes (years)§	-	13.5 (8.9-18.3)	-
Glycated hemoglobin (%)	-	7.9±1.5	-
Serum creatinine (mg/dl)	0.9±01	1.3±0.4	2.1±0.8
eGFR (ml/min/1.73m^2)§	>60	52 (39-67)	37 (24-53)
Albuminuria (mg/g Crea)§	-	725 (666-789)	119 (86-158)
Blood pressure (mmHg):			
Systolic	133±15	136±18	153±23
Diastolic	82±7	83±9	98±16

Values are given as means ± SD.

§Values are geometric means, with 95% confidence intervals in parentheses

eGFR: Glomerular filtration rate calculated according to the Modification of Diet in Renal Disease (MDRD) formula

Creatinine (Crea)