SUPPLEMENTARY DIGITAL CONTENT

Table S1. Oligonucleotides for real-time RT-PCR.

mRNA targets	Descriptions	Sense	Antisense
NOX-2	NOX-2 subunit of NADPH oxidase	ATGCAGGAAAGGAACAATGC	TTGCAATGGTCTTGAACTCG
p22phox	p22phox subunit of NADPH oxidase	GCGGTGTGGACAGAAGTACC	CTTGGGTTTAGGCTCAATGG
p47phox	p47phox subunit of NADPH oxidase	ATGACAGCCAGGTGAAGAAGC	CGATAGGTCTGAAGGCTGATGG
eNOS	Endothelial nitric oxide synthase	ATGGATGAGCCAACTCAAGG	TGTCGTGTAATCGGTCTTGC
C1qa	Complement C1q subcomponent subunit a	CGGGTCTCAAAGGAGAGAGA	TATTGCCTGGATTGCCTTTC
C1qb	Complement C1q subcomponent subunit b	CAGGGATAAAGGGGGAGAAA	TCTGTGTAGCCCCGTAGTCC
Tsp-1	Thrombospondin- 1	GCAGCACACACAGAAGCATT	CAATCAGCTCTCACCAGCAG
Mfge-8	Milk fat globule-EGF factor 8 protein	AAAGCTGTACCCTGTTTCGTG	GGAGGCTGACATCTGGT
IFNɣ	Interferon gamma	GCCCTCTCTGGCTGTTACTG	CCAAGAGGAGGCTCTTTCCT
IL-6	Interleukin-6	GATGGATGCTTCCAAACTGG	AGGAGAGCATTGGAAGTTGG
IL-10	Interleukin-10	GAATTCCCTGGGAGAGAAGC	GCTCCACTGCCTTGCTTTTA
IL-1b	Interleukin-1beta	GTCACTCATTGTGGCTGTGG	GCAGTGCAGCTGTCTAATGG
IL-17a	Interleukin-17a	CTTCACCTTGGACTCTGAGC	TGGCGGACAATAGAGGAAAC
IFN-α	Interferon alpha	GACTTTGGATTTCCCCTGGAG	AAGCCTTTGATGTGAAGAGGTTC
TGF-β	Transforming growth	AGGGCTACCATGCCAACTTC	CCACGTAGTAGACGATGGC

	factor beta		
VCAM-1	Vascular cell adhesion molecule-1	CTTCCAGAACCCTTCTCA	GGGACCATTCCAGTCACACTTC
TLR7	Toll-like receptor 7	GCCATCCAGCTTACATCTTCT	TTTGACCCAGGTAGAGTGTTTC
TLR9	Toll-like receptor 9	CTACAACAGCCAGCCCTTTA	GGACACACGGGTATGAATGT
GADPH	Glyceraldehyde-3-phosphate dehydrogenase	TGCACCACCAACTGCTTAGC	GGATGCAGGGATGATGTTCT
RPL13	Ribosomal protein L13	CCTGCTGCTCTCAAGGTTGTT	TGGTTGTCACTGCCTGGTACTT

Table S2. Maximal contractile response to U46619 in intact aortic rings in the presence of physiological salt solution (PSS), N(ω)-nitro-L-arginine methyl ester (L-NAME, 100μM) or apocynin (Apo, 10μM).

Experimental group	N	PSS (mN)	L-NAME (mN)	Apo (mN)
Control 4W	8	10.83±0.72	12.08±0.69	10.93±0.87
IMQ 4W	8	10.55±0.26	11.50±0.64	11.10±0.39
Control 8W	8	11.27±0.38	12.32±0.43	11.74±0.37
IMQ 8W	8	11.01±0.57	12.29±0.58	12.05±0.68

Values are expressed as Mean ± SEM. No differences were found among all experimental groups.

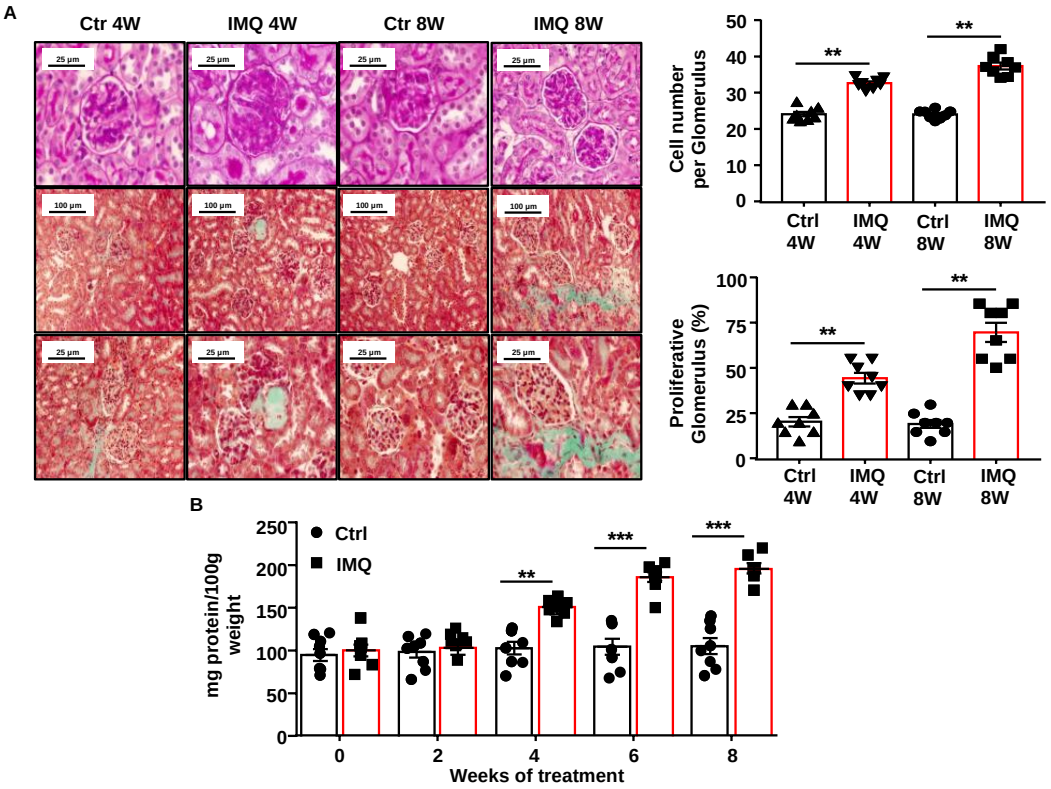

Figure S1. TLR7 activation by topical application of imiquimod promotes renal injury. (A) Kidney histopathology analysis. Kidney sections of control and IMQ-treated mice were stained with haematoxylin and eosin (H&E) and representative images are shown (top, original magnification: x40), and with periodic acid-Schiff and representative images are shown (intermediate and bottom, original magnifications: ×10 and x40, respectively). Beginning 4 weeks after topical imiquimod treatment, histopathologic assessment of the kidneys showed proliferative glomerular lesions, an enlarged hypercellular glomeruli, an increase in the mesangial matrix, and mild peritubular mononuclear cell infiltrates. Results are shown as mean ± SEM, obtained from 8-10 separate experiments. (B) Urinary albumin excretion was increased in imiquimod (IMQ)-treated mice compared with control mice after 4 weeks (W) of treatment. Experimental groups: Ctrl 4W (n=8), Ctrl 8W (n=8), IMQ 4W (n=8), IMQ 8W (n=8). Results were compared using 1-way ANOVA and Tukey *post hoc* test. Values are expressed as Mean ± SEM. **P<0.01, ***P<0.001 compared to their respective control group.

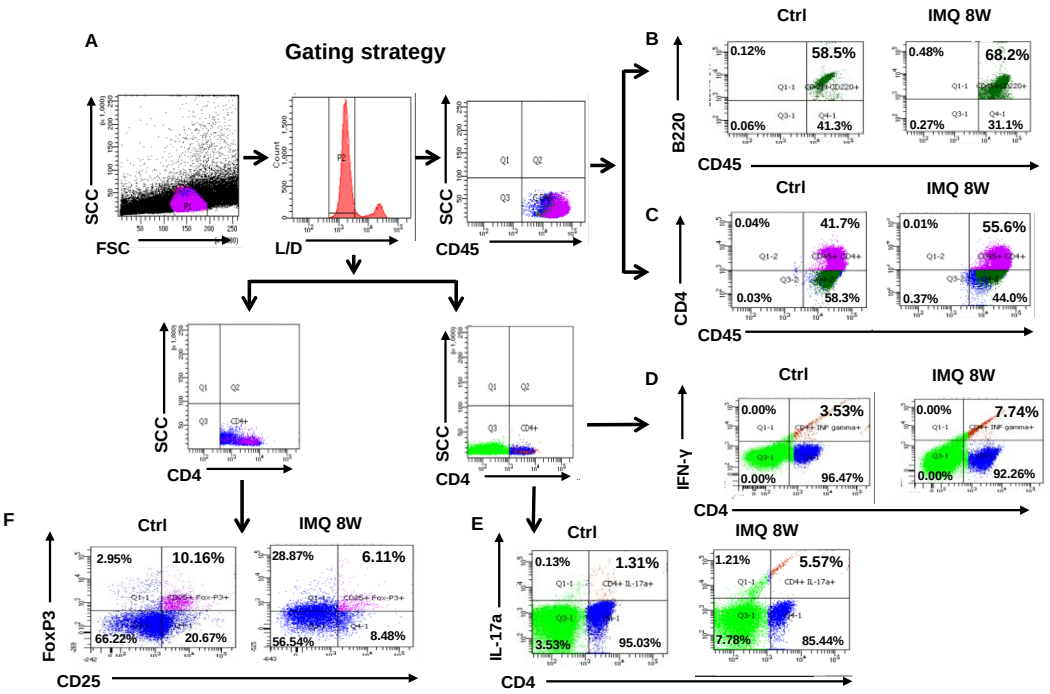

Figure S2. Gating strategy and representative flow cytometry of spleens from all experimental groups. (A) Live singlet cells were gated for total leukocytes (CD45+), (B) total B cells (B220+), (C) total T cells (CD4+), (D) Th1 cells (CD4+ IFNγ+), (E) Th17 cells (CD4+ IL17a+) and (F) Treg cells (CD25+ FoxP3+). Percentages of mean ± SEM are shown. Experimental groups: Ctrl 8W (n=8), IMQ 8W (n=8).

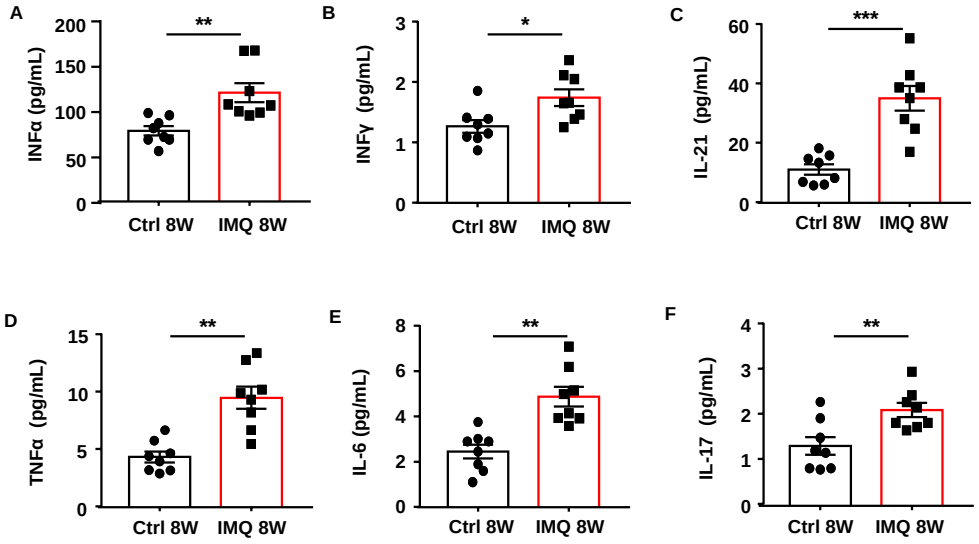

Figure S3. TLR7 activation by topical application of imiquimod raises plasma levels of proinflammatory cytokines. Plasma levels of IFN-α (A), IFN-γ (B), IL-21 (C), TNF-α (D), IL-6 (E), and IL-17 (F) were measured by ELISA in control (Ctrl) and imiquimod (IMQ)-treated mice after 8 weeks (W) of treatment. Experimental groups: Ctrl 8W (n=8) and IMQ 8W (n=8). Results were compared by 1-way ANOVA and Tukey *post hoc* test. Values are expressed as Mean ± SEM. *P<0.05, **P<0.01, ***P<0.001 compared to the control group.

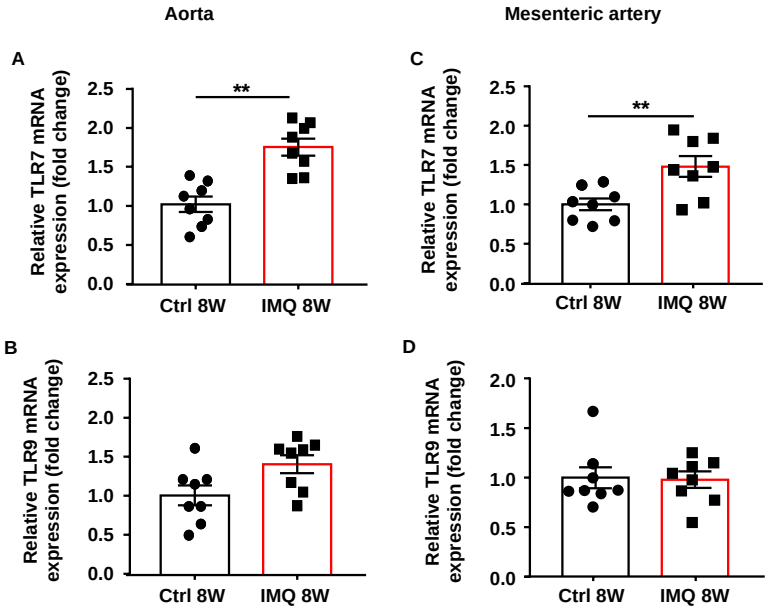

Figure S4. Increases in vascular TLR7 mRNA expression were found after TLR7 activation by topical application of imiquimod (IMQ). In contrast, no significant changes were found in TLR9 mRNA expression after the application of IMQ. Aortic and mesenteric arteries TLR7 mRNA (A, C) and TLR9 mRNA (B, D) levels measured by reverse transcriptase-polymerase chain reaction were assessed in control (Ctrl) and imiquimod (IMQ)-treated mice after 8 weeks (W) of treatment. Experimental groups: Ctrl 8W (n=8), IMQ 8W (n=8). Results were compared using 1-way ANOVA and Tukey *post hoc* test. Values are expressed as Mean ± SEM. **P<0.01 compared to the control group.

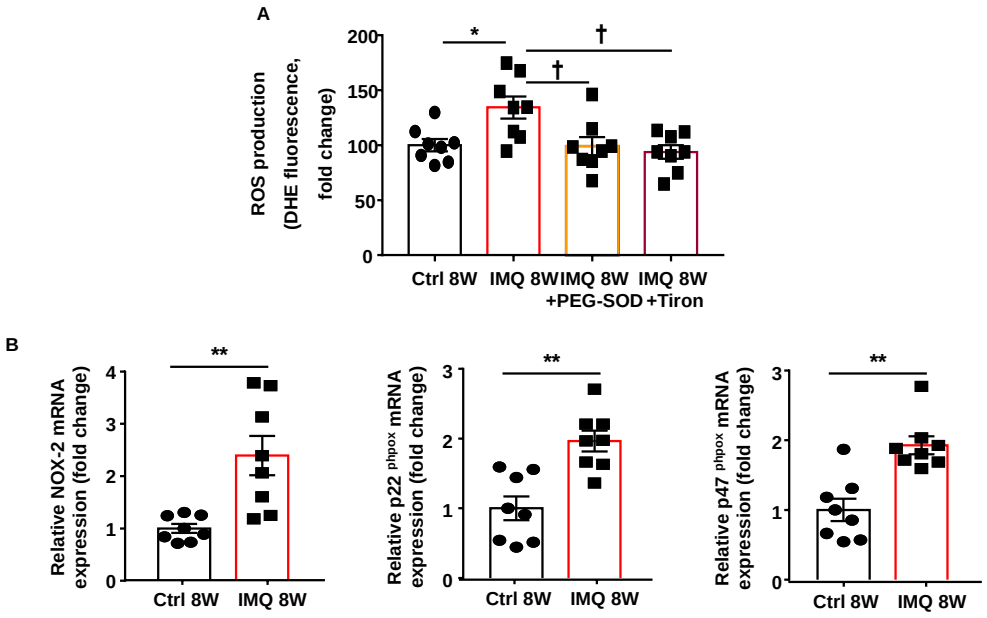

Figure S5. TLR7 activation promotes a significant increase in NADPH oxidase activity in mesenteric arteries from imiquimod (IMQ)-treated mice. (A) NADPH-stimulated ROS production, measured by dihydroethidium (DHE) fluorescence in a microplate reader, in homogenates of mesenteric arteries from control (Ctrl) or IMQ-treated mice incubated with polyethylene glycol-conjugated superoxide dismutase (PEG-SOD) (250U/ml) or Tiron (10 µM). (B) mRNA expression of NADPH oxidase subunits NOX-2, p22phox and p47phox in mesenteric arteries homogenates from control (Ctrl) and IMQ-treated mice IMQ). Data are represented as a ratio of arbitrary units of mRNA ($2^{-\Delta\Delta Ct}$). Experimental groups: Ctrl 8W (n=8), IMQ 8W (n=8). Results were compared using 1-way ANOVA and Tukey *post hoc* test. Values are expressed as Mean ± SEM. *P<0.05 and **P<0.01 compared to the control group; † P<0.05 compared to the IMQ-treated group.

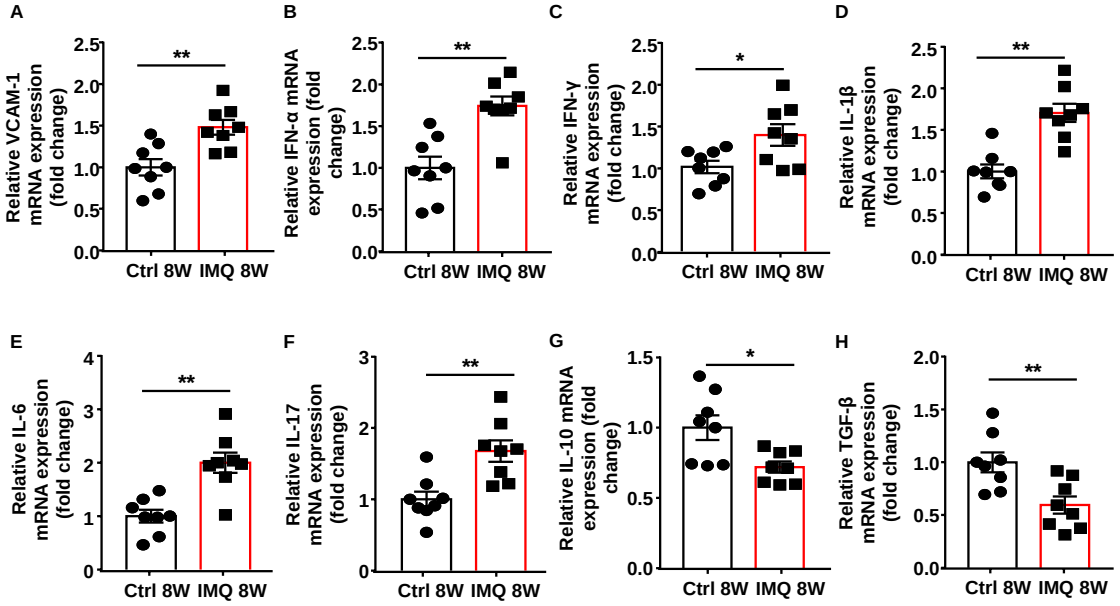

Figure S6. TLR7 activation promotes a higher gene expression of vascular adhesion molecules and proinflammatory cytokines in mesenteric arteries from imiquimod (IMQ)-treated mice. (A) mRNA expression of vascular cell adhesion molecule-1 (VCAM-1). mRNA expression of the proinflammatory cytokines IFN-γ (B), IFN-α (C), IL1β (D) IL-6 (E), and IL-17 (F), and the anti-inflammatory cytokine IL-10 (G) and TGF-β (H) in mesenteric arteries homogenates from control (Ctrl) and imiquimod IMQ-treated mice after 8 weeks (8 W) of treatment. Data are calculated using the $2^{-\Delta\Delta Ct}$ method. Experimental groups: Ctrl 8W (n=8), IMQ 8W (n=8). Results were compared using 1-way ANOVA and Tukey *post hoc* test. Values are expressed as Mean ± SEM. *P<0.05, **P<0.01 compared to the control group.