**Supplementary materials and methods**

**Mice.** Wild-type C57BL/6 mice (WT) were purchased from The Jackson Laboratory (The Jackson Laboratory, Bar Harbor, ME). Breeding pairs of the Tlr9-/- mice on B6 background were provided by Dr. Daniela Verthely (FDA) with permission from Dr. Shizuo Akira (Osaka University, Japan). All the mice were bred and maintained under specific pathogen-free conditions and all experiments were performed in accordance with National Institutes of Health (NIH) guidelines under the National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS)-approved animal study protocol #A016-05-26.

***In vivo* treatment with TLR7 agonist Imiquimod.** Eight- to ten-week-old WT and Tlr9-/- mice were epicutaneously treated with fougera® IMIQUIMOD CREAM 5% (E Fougera & Co, Melville, NY) on both ears 3 times per week for indicated times. The cumulative dose was 50 mg/mice.

**Assessment of endothelium-dependent vasorelaxation.** Endothelium-dependent vasorelaxation was assessed by aortic ring myography, as previously described1. Briefly, thoracic aortic rings were harvested at euthanasia and stabilized in PSS buffer for 1 hour. A phenylephrine (PE) concentration corresponding to 80% maximum was added, and contraction was allowed to reach a stable plateau. To examine the endothelium-dependent relaxation, acetylcholine (Ach, 10-9 M to 10-4 M) was added cumulatively and a curve was generated. Ach-dependent relaxation were expressed as percentage of PE contraction.

**Cell isolation and flow cytometry.** Spleens were harvested and weighed, and single-cell suspensions were prepared. Total splenocyte numbers were counted. Single-cell suspensions then were stained with the following anti-mouse Abs: anti-B220 (RA3-6B2), anti-CD3 (17A2), anti-CD4 (GK1.5), anti-CD8 (53-6.7), anti-CD11b (M1/70), anti-CD11c (N418), anti-CD16/32 (93), anti-IL17A (TC11-18H10.1), anti-CD19 (6D5), anti-CD21 (7E9), anti-CD23 (B3B4), anti-CD34 (HM34), anti-CD40 (3/23), anti-CD44 (IM7), anti-CD45 (30-F11), anti-CD80 (16-10A1), anti-CD86 (GL1), anti-CD95 (Fas, SA367H8), anti-CD117 (ACK2), anti-CD138 (281-2), anti-F4/80 (BM8), anti-FoxP3 (FJK-16s), anti-GM-CSF (MP1-22E9), anti-IFN-γ (XMG1.2), anti-IgM (eB121-15F9), anti-IgD (11-26c.29), anti-IL10 (JES5-16E3), anti-IL-12 p40 (C15.6), anti-Ly-6C (HK1.4), anti-Ly6G (1A8), anti-MHC II (2G9, M5/114.15.2), anti-PDCA-1 (927), anti-Sca-1 (D7), and anti-TNFα (MP6-XT22). All of those antibodies were from Biolegend (San Diego, CA). Anti-IL12 p35 (SNKY35) and FITC conjugated mouse hematopoietic lineage antibody cocktail were from eBioscience™ (Thermo Fisher Scientific, Waltham, MA). Anti-CD11c (HL3), anti-CD3 (500A2). PE-conjugated anti-mouse TLR7 (A94B10) were from BD Pharmingen™ (BD Biosciences, San Jose, CA). FITC conjugated peanut agglutinin (PNA) was from Vector Laboratories (Burlingame, CA). FITC conjugated anti-mouse C3 was from Immunology Consultants Laboratory (Portland, OR). LIVE/DEAD Fixable Aqua Dead Cell Stain Kit (Thermo Fisher Scientific) was used in all flow experiments to exclude dead cells. Cells were acquired on a FACSCanto analyzer (BD Biosciences, San Jose, CA), and data were analyzed using FlowJo software (Tree Star, Ashland, OR).

**Intracellular Staining.** Cells were stimulated for 4 h with PMA/Ionomycin (50 ng/ml and 750 ng/ml, Sigma, St. Louis, MO) plus GolgiStop (BD Pharmingen), and analyzed for intracellular production of cytokines by staining with anti-cytokine Abs and subsequent flow cytometry, as previously described2.

**Bone marrow derived-DCs (BMDC) preparation**. Bone marrow cells were flushed from the femurs and tibiae of 8-wk-old WT and Tlr9-/- mice. These cells were cultured in RPMI 1640 medium containing 10% FBS and 40 ng/ml murine granulocyte-macrophage colony-stimulating factor (GM-CSF) for 7 days. Cell purity was confirmed by FACS analysis for CD11c (>95%).

**Endothelial progenitor cell (EPC) differentiation assays**. EPCs were isolated from bone marrow, and their capacity to differentiate into endothelial cells (ECs) was quantified by fluorescence microscopy, as previously described3 4. Briefly, femurs and tibias were flushed with a buffer solution (15 mM EDTA in Hanks’ balanced salt solution) and bone marrow cells were separated on Histopaque 1083. After collecting the cells from the interface, red blood cells were lysed by ACK lysis buffer. Cells were then plated in triplicate onto 24-well fibronectin-coated plates (3.5×106/well) in MCDM 131 media supplemented with EGM-2 MV BulletKit (Lonza, Walkersville, MD) and 5% fetal bovine serum. Cells were cultured for 1 week with a media change every 3 days. On day 7, cells were incubated with DiI-labeled acetylated low-density lipoprotein (Biomedical Technologies, Stoughton, MA) and Fluorescein labeled Griffonia Simplicifolia Lectin I (GS-I; Vector, Burlingame, CA) for 4 hours. Images from 5 random fields per well were obtained using a ZOE Fluorescent Cell Imager (BIO-RAD, Hercules, CA).

**Kidney harvesting and urinalysis.** Mice were anesthetized, and kidneys were perfused with cold PBS via left-sided cardiac puncture. Kidney was frozen in Tissue-Tek® O.C.T. Compound and frozen at -80°C until sectioning at 10-micron thickness for immunofluorescence staining. Immune complex deposition was assessed by immunofluorescence staining of IgG and C3 on frozen kidney sections as previously described5. Briefly, kidney sections were stained with Alexa Fluor 555 conjugated Donkey anti-Mouse IgG (H+L) antibody (Invitrogen, Carlsbad, CA) and FITC conjugated goat anti-mouse C3 (Immunology Consultants Laboratory, Portland, OR). For both IgG and C3, glomerular staining was graded in blinded fashion by intensity on a 0 to 3+ scale for at least 10 glomeruli per mouse; an average score was then calculated. Urine albumin and creatinine were quantified using a mouse Albuwell ELISA kit and Creatinine Companion Kit (Exocell, Philadelphia, PA), and albumin/creatinine ratios were calculated.

**Quantification of serum autoantibodies and cytokines.** Serum total IgG and anti-dsDNA were determined by ELISA according to the manufacturer’s instructions (Thermo Fisher Scientific, Waltham, MA and Alpha Diagnostics, Owings Mills, MD, respectively). Anti-histone and anti-RNP/Sm autoantibodies were quantified as previously described6. Briefly, Nunc MaxiSorp ELISA plates were precoated with histones (20 mg/ml, Sigma, St. Louis, MO) and ribonucleoprotein-Smith Ag complex (RNP/Sm; 20 mg/ml; ImmunoVision, Springdale, AR) in PBS at 4°C overnight. Plates were blocked with 3% FCS for 1 h at 37°C, washed, and incubated with 1:100 dilutions of mouse sera for 1 h at 37°C. Plates were washed, and specific Abs were detected with a 1/1000 dilution of alkaline phosphatase-conjugated goat anti-mouse IgG (103605; Southern Biotech, Birmingham, AL) for 1 h at 37°C and developed with a phosphatase substrate for 30 min at 37°C. Anti-nuclear Abs (ANAs) were detected by immunofluorescence on HEp-2 slides (MBL International, Woburn, MA).

**Quantification of gene expression in spleen and kidney.** Approximately 20 mg of spleens and one kidney/mouse were excised and homogenized with TRIzol reagent (Invitrogen, Carlsbad, CA) for RNA extraction using the Qiagen RNeasy kit. The cDNA was synthesized using BIO-RAD iScript reverse transcription supermix according to manufacturer instructions. Quantitative real-time PCR was performed on BIO-RAD CFX96 Real-Time System thermocycler (BIO-RAD, Hercules, CA using specific TaqMan primers and probes (Thermo Fisher Scientific, Waltham, MA). *Gapdh* was used as the house-keeping gene to normalize the expression of the target genes, and the WT imiquimod-treated mice Ct was used in the delta delta Ct calculations for the determination of the fold gene expression. The primers (Assay ID) were as follows: *Gapdh*, Mm99999915\_g1; *IL-1b*, Mm00434228\_m1; Tnf, Mm00443258\_m1; Il12a, Mm00434169\_m1; Mx-, Mm00487796\_m1; Isg15, Mm01705338\_s1; Tlr7, Mm04933178\_g1; Ifng, Mm01168134\_m1; Cxcl10, Mm00445235\_m1; Ciita, Mm00482914\_m1; ISG15, Mm01705338\_s1; and IFIT1, Mm00515153\_m1 (Thermo Fisher Scientific, Waltham, MA).

**Immunofluorescence staining of the spleen and kidney.** Frozen spleen and kidney sections were fixed with 4% PFA for 10 min, followed by permeabilization in 0.2% Triton X-100 for 8min. The sections were blocked by 1% BSA and 1% donkey/rat serum in PBS for 30 min. The kidney sections were stained with hamster-anti-mouse CD11c (clone HL3, BD Pharmingen™, San Jose, CA) and rat-anti-mouse F4/80 (clone BM8, Biolegend, San Diego, CA) or hamster-anti-mouse CD3 (clone 500A2, BD Pharmingen™, San Jose, CA) and rat-anti-mouse IFN-γ (XMG1.2, Biolegend, San Diego, CA), and counterstained with DAPI, before being mounted in ProLong Gold (Life Technologies, Washington, DC) and examined by Leica SP5 confocal microscopy (Wetzlar, Germany).

**Statistical analysis** Statistical analysis was performed using Mann-Whitney U test in GraphPad Prism (GraphPad Software, San Diego, CA). Multiple comparisons were analyzed by one-way ANOVA. For endothelium-dependent vasorelaxation, curves were first analyzed using an asymmetric (5-parameter) logistic equation, and the significance of each data point was determined by two-way analysis of variance. A *p* value less than 0.05 was considered significant.

**Figure legends**

**Supplementary Figure 1. Untreated WT mice and untreated Tlr9-/- mice do not differ in spleen size and immune responses.** (A) Spleen weight, spleen weight/body weight, and total number of splenocytes of 14-week old untreated WT and Tlr9-/- mice (n=4-7/group). (B) Representative images of frozen kidney sections quantifying immune complex deposition, C3: green; IgG: red. Bar, 100 µm. (n=6/group). (C) Representative flow cytometry plot of myeloid subsets and TNF-α and IL12-p35 production by splenic CD11b+ myeloid cells of 14-week old untreated WT and Tlr9-/- mice (n=8-12/group). (D) Representative flow cytometry plot of splenic CD4+ T cell subsets of 14-week untreated WT and Tlr9-/- mice (n=8-12/group). (E) Representative flow cytometry plot of splenic B cell subsets of 14-week untreated WT and Tlr9-/- mice (n=8-12/group).

**Supplementary Figure 2. TLR9-deficient mice display accelerated death rates and weight loss after long-term imiquimod treatment.** (A) Death rates in imiquimod treated-WT mice and imiquimod treated-Tlr9-/- mice after 4-5 weeks of imiquimod administration. (B). Final body weight in age matched untreated and imiquimod treated-WT mice, and age matched untreated and imiquimod treated-Tlr9-/- mice after 5 weeks of treatment.

**Supplementary Figure 3. TLR9 deficiency did not affect endothelial progenitor cells (EPCs) in differentiation to mature ECs in imiquimod-induced autoimmunity.** WT and Tlr9-/- mice were treated with imiquimod for 3 weeks, and EPCs from bone marrow cells were isolated and in vitro differentiated into mature endothelial cells (ECs). Upper panel, representative images of mature ECs staining with DiI-labeled acetylated low-density lipoprotein (Red) and Fluorescein labeled Griffonia Simplicifolia Lectin I (Green); lower panel, 9X magnification of upper panel. Bar, 100 µm. Data are representative of 5 mice in each group.

**Supplementary Figure 4. Gating strategy for Identification of plasmacytoid DCs (pDCs) from spleen.** Total splenocytes were first gated on CD45+live cells, followed by gating on CD19-CD11b- cells, then gating on CD11c+PDCA-1+ cells; pDCs were further gated in B220+CD3- cells. PDCs were B220+PDCA-1+CD11c+ cells. Percentage of pDCs was calculated in total live CD45+ cells.

**Supplemental Figure 5. TLR9 deficiency accelerates imiquimod-driven hematopoiesis.** (A) Gating strategy for Identification of bone marrow (BM) progenitor populations. The BM cells were first gated by live and lineage- cells, the myeloid progenitor cells (MP) were defined by c-kit+Sca-1- and LSK cells were defined by c-kit+Sca-1+. MPs were further stained with CD16/32 versus CD34 to differentiate between granulocyte myeloid progenitors (GMPs) and common myeloid progenitors (CMPs). (B) WT and TLR9-/- mice were treated with imiquimod for 1 week. Bone marrow (BM) cells were isolated and analyzed MPs and LSKs. (C) MPs were further analyzed for GMPs and CMPs. (D) WT and TLR9-/- mice were treated with imiquimod for 3 weeks. WT and TLR9-/- mice were treated with imiquimod for 3 weeks. Bone marrow (BM) cells were isolated and analyzed MPs and LSKs. MPs were further analyzed for GMPs and CMPs. Bone marrow derived DCs (BMDCs) from WT and Tlr9-/- mice were stimulated with media (UN), imiquimod (1 µg/ml), IFN-γ (40 ng/ml), and imiquimod (1 µg/ml) + IFN-γ (40 ng/ml) for 4h (n=4). Gene expression of IL-12 p35 (E) and TLR7 (F) was determined. \* *p*<0.05, \*\* *p*<0.01, \*\*\* *p*<0.001.

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