Supplemental Methods

Animals

Mice with ablation of TNF in T cells (T-TNF KO; $TNF^{flox/flox}$ ×$CD4\text{-}Cre^{+}$), in myeloid cells (M-TNF KO; $TNF^{flox/flox}$ ×Mlys-Cre/WT), in B cells (B-TNF KO; $TNF^{flox/flox}$ ×CD19-Cre/WT), TNF KO mice used were generated using gene targeting in 129Sv ES cells [1], cell type specific TNF KO mice were backcrossed at least 12 times to C57Bl/6N genetic background. Concomitant ablation of TNF in both T cells and myeloid cells (MT–TNF KO; $TNF^{flox/flox}$ ×Mlys-Cre/wt×$CD4\text{-}Cre^{+}$) was achieved by crossing M-TNF KO and T-TNF KO mice. To reduce the impact of the microbiota on the observed phenotype, $TNF^{flox/flox}$ mice (littermate controls from T-TNF KO and M-TNF KO breeding) were used as wild type (WT) controls for all experiments using cell-type specific TNF KO mice. tmTNF KI were generated and kindly provided by J. Sedgwick[2]. ROSA-STOP-EYFP×Mlys-Cre were generated by crossing of $Gt(ROSA)26Sor^{tm1(EYFP)Cos}$ mice (kindly provided by Dr. Anja Hauser, DRFZ, Berlin) with Mlys-Cre mice. Mice expressing only transmembrane TNF on myeloid cells (tm-M-TNF KI) were generated via crossing tmTNF KI mice with M-TNF KO mice, littermate controls were used as controls. C57Bl/6 mice were purchased from Charles River Laboratories. All animal procedures were carried out in accordance with German and Russian regulations for animal protection.

Induction of collagen-induced arthritis (CIA)

Chicken collagen type II (Sigma Co., C9301) was dissolved in 10 mM acetic acid (final concentration – 2mg/ml) overnight at 4 C with continuous stirring. Then, collagen solution was emulsified with complete Freund's adjuvant containing 8 mg/ml of heat-killed *M. Tuberculosis* H37RA. Mice were injected intradermally at the base of tail with a total of 100 mcl emulsion. Second injection of the same mixture was

performed at day 21 after the first injection. TNFR2 signalling was blocked via administration of anti-TNFR2 antibody (TR75-54.7; BioXcell; 100 mcg/mouse; i.p.; twice per week), IFNγ was blocked via administration of anti-IFNγ antibody (XMG1.2; DRFZ production; 100 mcg/mouse; i.p.; twice per week). Clinical assessment of CIA was performed as following: grade 0 - no swelling; grade 1 - swelling or focal redness of finger joints; grade 2 - mild swelling of wrist or ankle joints; grade 3 - severe swelling of the entire paw. The scores of four paws were summed up so that the maximal score per mouse is 12. Incidence of arthritis was calculated by dividing the number of mice showing swelling of any paws with number of total mice tested.

Induction of collagen antibody-induced arthritis (CAIA)

For induction of arthritis with arthritogenic antibodies, a cocktail containing five monoclonal antibodies against collagen type II and LPS from E. coli 0111:B4 as adjuvant were injected in accordance with the manufacturer's protocol (Chondrex, USA, Cat no: 53040). Clinical assessment for each paw was determined based on the following scale: 0 – no reddening and swelling (normal joints); grade 1 – minor, but definite redness or swelling confined to the ankle or wrist, or apparent redness and swelling limited to one digit; grade 2 – mild redness and swelling of the ankle or wrist, and/or apparent redness and swelling limited to individual digits, regardless of the number of affected digits; grade 3 – moderate redness and swelling of ankle or wrist/marked paw swelling; grade 4 - severe redness and swelling of the entire paw including digits. The total score for each mouse was calculated based on the sum of the scores for each of the four paws.

Histology

Knee joints were collected at the indicated time points, fixed overnight in 4% PFA at 4°C. Bone and joint tissue was decalcified in saturated EDTA for 10 days. Then the samples were embedded in paraffin and 3 µm sections were cut. Hematoxylin and eosin staining was done as described elsewhere. In CAIA experiments, knee joint sections were stained with Weigert's iron hematoxylin, 0.1% Safranin O and 0.04% fast green to assess general morphology and matrix proteoglycans. Immunofluorescent staining was performed using antibodies against podoplanin-PE (eBio8.1.1; ThermoFischer Scientific) or Thy1-Alexa488 (Clone: T24/31; DRFZ production) and nuclei were costained with DAPI. Images were acquired using a Karl Zeiss microscope (Axioplan) or Keyence Biorevo BZ-9000 (Keyence). For each image, the number of pixels fluorescent channel was quantified with ZEN and divided by a manually defined area (μm^2) only including tissue zones containing cells, as previously described[3]. Histopathological score was done in blind fashion. Each parameter was estimated from 0, which corresponds to the absence of inflammation/destruction to 3, which reflects strong inflammation/destruction by criteria described elsewhere [4].

Restimulation of cells *in vitro*

Spleen and lymph node cells were isolated at indicated time points. Single cell suspensions were made by using 70 µm cell strainer (BD Bioscience). For spleen, red blood cells were lysed with ACK lysis buffer. Paws with arthritis were dissected at the fur line, cut up into small pieces after removing skin and then digested with collagenase type IV (2 mg/ml) and DNAse1 (0,1 mg/ml) to make single cell suspension. Single cell suspensions were either used directly for the staining or were counted and resuspended at 8×10^6 cells/ml and restimulated overnight with chicken

type II collagen (100 mcg/ml). Brefeldin A (5 µg/ml; Sigma-Aldrich) was added for the last 4 hours of culture.

Flow cytometry

For flow cytometry, Fc receptors were blocked with antibody 2.4G2 (10 µg/ml), followed by staining with antibodies against various surface markers[5]. Antibodies for IL-17A PE (TC11-18H10), TNF-APC (MP6-XT22), TCRβ (H57-597) and secondary reagents streptavidin-PE, streptavidin-PerCP and streptavidin-Cy5 were from BD bioscience, anti-IFNγ-FITC (AN18.17.24), anti-CD4-biotin (GK1.5) and anti-CD4-Cy5 (GK1.5), anti-CD11c(N418), anti-CD19(1D3), anti-GL7(GL7) and anti-MHCII (M5.114.2) were purified from respective hybridomas and conjugated to fluorochromes in our facility. F4/80 (CI:A3-1), IL-12p40 (C17.8), CD11b (M1/70.15), Ly6C (HK1.4), Gr1 (RB6-8C5) were from ThermoFischer Scientific. Biotinylated Peanut agglutinin (PNA) was from Vector Labs. Foxp3 and intracellular cytokine staining were performed using FoxP3 staining kit from ThermoFischer Scientific. Cells were acquired using flow cytometers FACSCalibur/FACSCanto/LSRII (both from BD Biosciences). Data analysis was performed using FlowJo software (Tree Star, Inc).

Gene expression analysis

RNA from the indicated tissues and cells was isolated using TRI reagent according to manufacturer's instructions (Sigma-Aldrich). 1 µg of RNA was treated with RQ1 Dnase 1 (Promega Inc.) and cDNA was synthesized using Im-Prom II reverse transcriptase (Promega Inc.). Real-time PCR was performed using Brilliant II SYBR Green QPCR master mix (Agilent). PCR was performed using Stratagene Real-time PCR amplifier. All reactions were run using the following program: 95°C 10

min – 50 cycles of 95°C 30 sec, 60°C 30 sec and 72°C 30 sec. Following primers used analysis are depicted in Table S1.

Table S2. Primer sequences used for Real-time PCR

Gene	Forward primer	Reverse primer
TNF	gCCACCTTCgCAACAAgTCT	CCgggCACAgTCATAgCAC
MMP2	ggCCACggTATTCTggAAgC	gggCgTAACTTgAATCCgATCTA
IL-6	gTTCTCTgggAAATCgTggA	TgTACTCCAggTAgCTATgg
MMP9	ATCCCggCAATTCCTgTTCTC	ggggCTTTgTTTCCCTggg
TIMP1	ACCTgATCCgTCCACAAACAg	CTTggTTCCCTggCgTACTC
β-actin	CTCCTgAGCgCAAGTACTCTgTg	TAAAACgCAgCTCAgTAACAgTCC

ELISA

Anti-collagen antibody titers were measured as described elsewhere. Briefly, 96 well plates were coated with chicken collagen overnight at 4°C. Plates were blocked with 5% PBS/BSA for one hour, serial dilution of sera were applied and incubated overnight at 4°C. Antibodies were detected using goat anti-mouse IgG, IgG1, IgG2a (Southern Biotech) coupled with alkaline phosphatase, followed by pNPP substrate. Protein levels of TNF and IL-6 were measured using respective ELISA Ready-Set-Go! Kit (ThermoFischer Scientific) according to the manufacturer's instructions.

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