Supporting Information

Au nanoclusters modulated macrophages polarization and synoviocytes apoptosis for enhanced rheumatoid arthritis treatment

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***In vitro* cell uptake test.** Au_{25}-ICG cluster nanoprobes were synthesized based on reported method.[1] RPMs and HFLS were incubated with indocyanine green (ICG)-conjugated gold nanoclusters for 4 h, then the cellular uptake of Au_{25} nanoclusters were assessed by CLSM (LMS-800, Carl Zeiss).

***In vitro* ROS assay.** The intracellular ROS generation was studied by DCFH-DA. Typically, HFLS was seeded in confocal plates at 1×10^5 cells/mL, and then incubated with Au_{25} nanoclusters (25 and 50 μg/mL) for 18 h. Subsequently, the cells were stimulated by another 6 h under TNF-α stimulation. After that the DCFH-DA was introduced incubated for 0.5 h to form green fluorescent substance (DCF). Finally, the intracellular green fluorescence was monitored by CLSM.

Cell migration. HFLS were seeded into 6 well plates to grow into a completely confluent monolayer. A linear wound was induced across the middle of each well with a gun head (200μL). HFLS were subsequently cultured in serum-free medium containing different concentrations of Au_{25} nanoclusters. The migrated distance in different groups were observed and imaged at 24 h.

Cell invasion assay. HFLS cells were plated in 24-well plates and 2×10^5 cells (100 μL) were added to upper chamber, and complete medium (600 μL) containing 20% FBS containing Au_{25} nanoclusters (25 and 50 μg/mL) was added to the lower chamber. HFLS were allowed to invade for 24 hours. The noninvaded cells on the upper surface of the membrane were removed by wiping with a cotton swab. Invaded cells were fixed in 4% paraformaldehyde, and stained with 0.5% crystal violet. The quantification of invading cells was assessed by counting three random fields (magnification 40x).

Cellular apoptosis assay and mitochondria membrane potential. HFLS were activated by TNF-α (40 ng/mL) for 6 h and seeded into 6-well plates (2×10^5 cells/well) or confocal dishes (3×10^5 cells/dish). Subsequently, 25μg/mL and 50μg/mL Au_{25} nanoclusters were added for 18 h incubation. Then, the apoptosis rate was determined by flow cytometry (NAVIOS, BECKMAN) according to an Annexin V-FITC apoptosis detection kit. The potential of the mitochondria membranes was tested by CLSM using JC-1 as a specific probe.

Immunofluorescence and TUNEL staining. After deparaffinization and rehydration, the sections of synovium were incubated with primary antibodies at 4 °C overnight, and then incubated with fluorescent-labelled secondary antibodies for 30 min. Another part was staining with TUNEL. After being counterstained with DAPI, digital fluorescence photographs were captured using a fluorescent microscope.

References

1. X. Jiang, B. Du and J. Zheng, *Nat. Nanotechnol.*, 2019, **14,** 874-882.

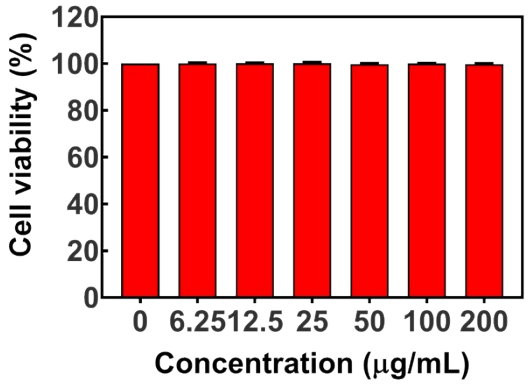

Fig. S1 Relative cell cytocompatibility analysis of RAW264.7 cell lines exposed with various concentrations of Au_{25} nanoclusters (n = 8, mean ± SEM).

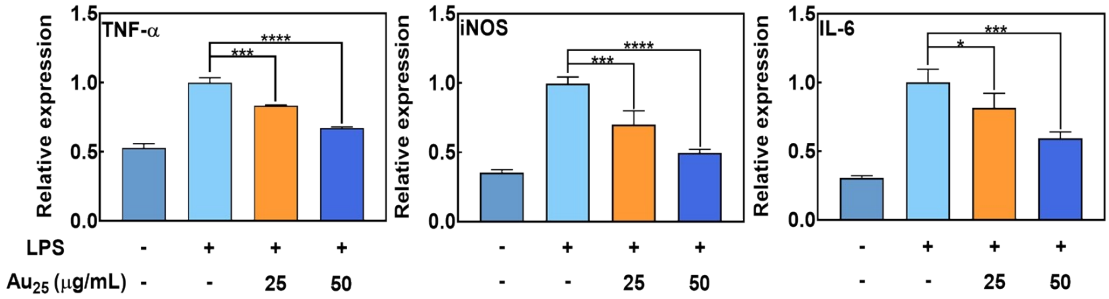

Fig. S2 mRNA levels of TNF-α, iNOS and IL-6 in RAW264.7 cells were evaluated by western blot analysis (n = 3, mean ± SEM). **p*<0.05, ****p*<0.001, *****p*<0.0001 *vs.* LPS-treated group, LPS: lipopolysaccharide.

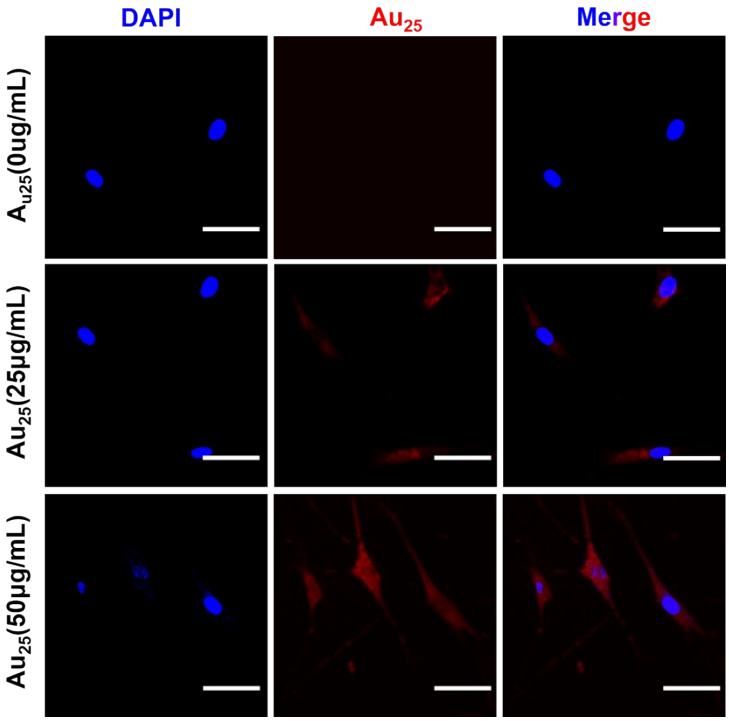

Fig. S3 CLSM images of HFLS after 4 h incubation with Au_{25} nanoclusters (scale bar: 50 μm).

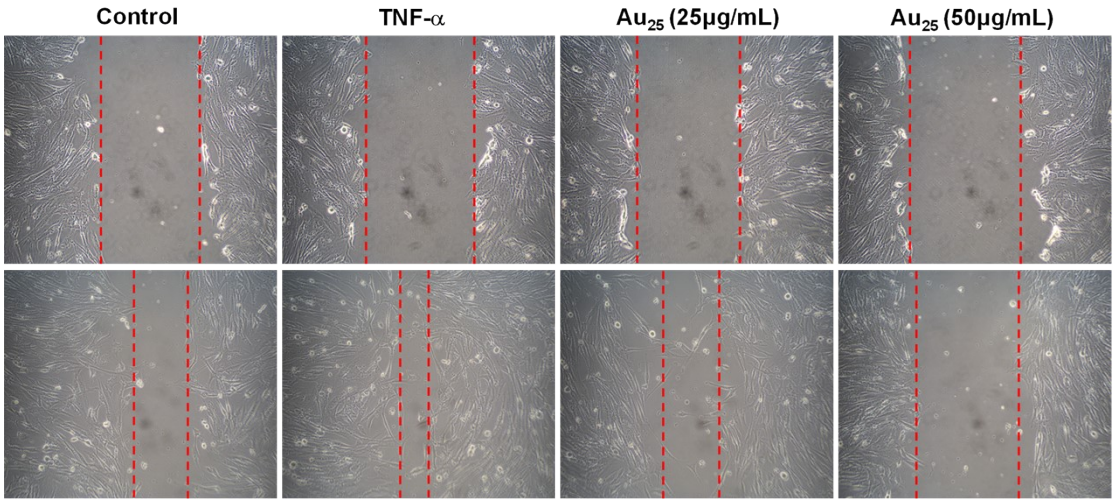

Fig. S4 Au_{25} nanoclusters inhibited HFLS migration. Representative photomicrographs of HFLS migration following inhibition with Au_{25} nanoclusters (25 and 50μg/mL) for 24 h (magnification×20).

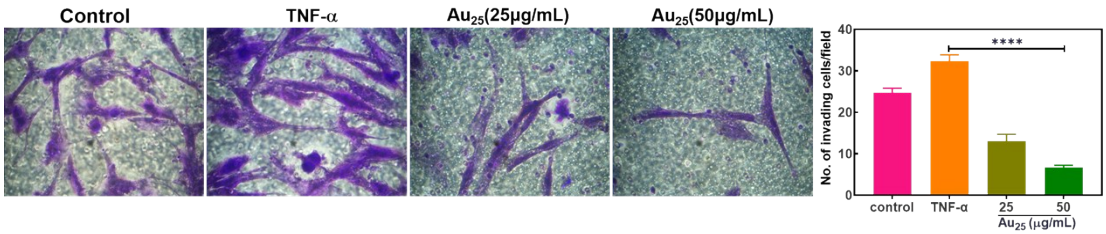

Fig. S5 Au_{25} nanoclusters inhibited HFLS invasion. Representative photomicrographs of HFLS invasion following inhibition with Au_{25} nanoclusters (25 and 50μg/mL) for 24 h (n=3, magnification×40).

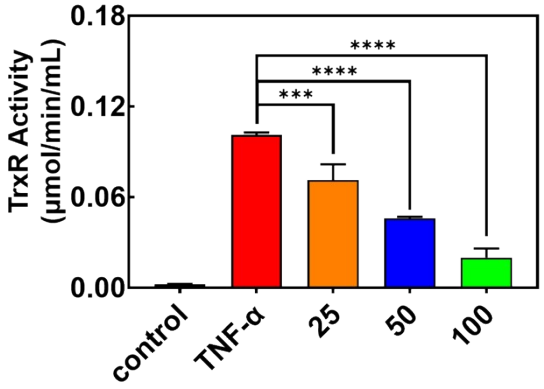

Fig. S6 Effect of different concentrations of Au_{25} nanoclusters (25,50,100 μg/mL) on TrxR activity in the cellular system was studied using thioredoxin reductase assay Kit.

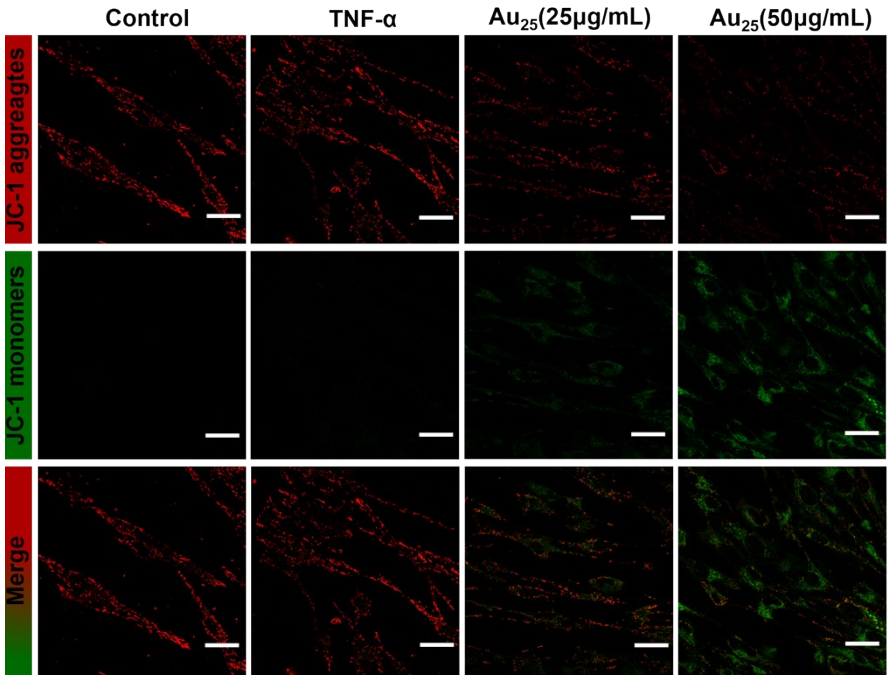

Fig. S7 The mitochondrial membrane potential was monitored by staining with the fluorescent dye JC-1 (scale bar: 50 μm).

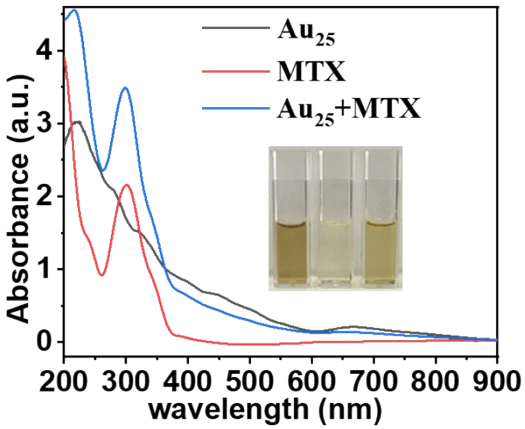

Fig. S8 UV–vis absorption of the Au_{25} (black lines), MTX (red lines) and Au_{25}+MTX (blue lines) at room temperature. Digital photos from left to right are Au_{25}, MTX and Au_{25}+MTX in deionized water.

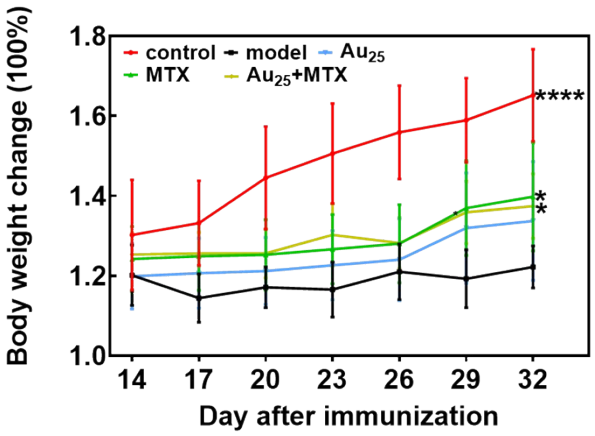

Fig. S9 Body weights of various treated rats as a function of time. Data are presented as mean ± S.D (n=7).

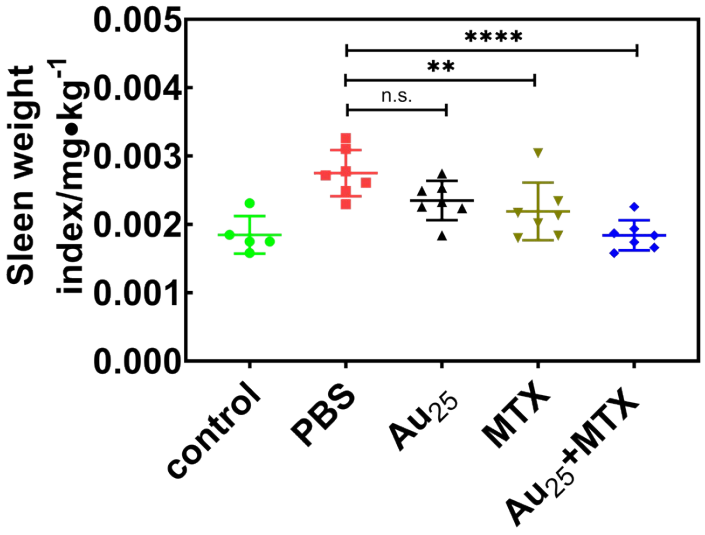

Fig. S10 Spleen index of various treated rats. Data are presented as mean ± S.D (n=7).

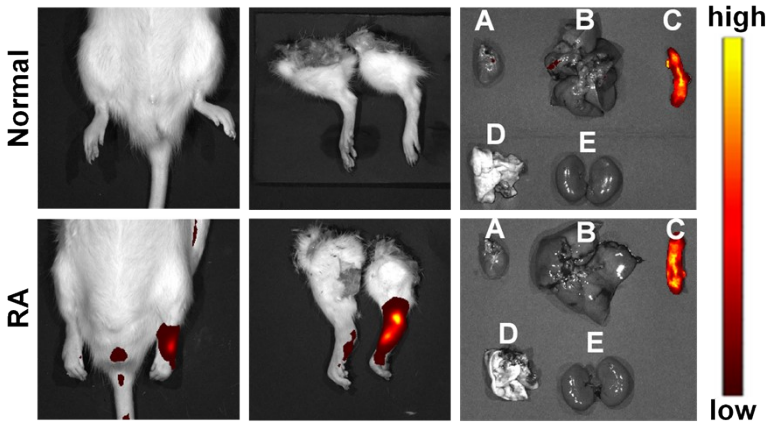

Fig. S11 Au_{25}-ICG fluorescence images showed the biodistribution in normal rat and AA rat with a unilateral inflamed joint (A, heart; B, liver; C, spleen; D, lung; E, kidney arthritic joint) at 8 h after tail vein injection.

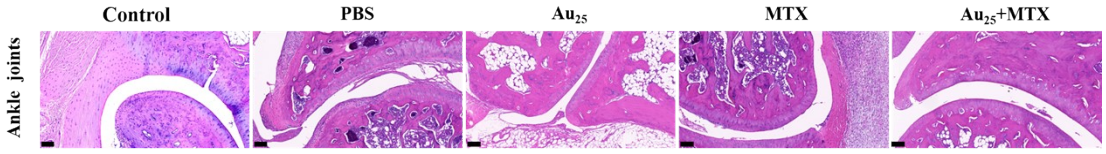

Fig. S12 Histopathology evaluation of ankle joints was identified using H&E (scale bars: 100 μm).

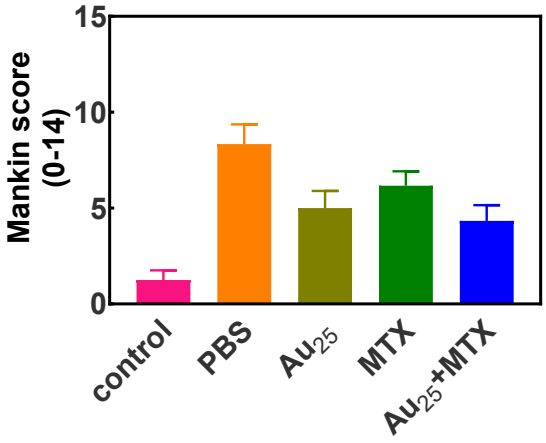

Fig. S13 Mankin score with different treatment groups.

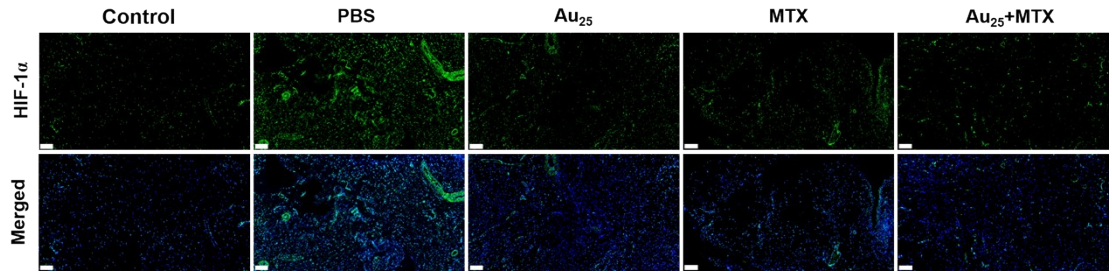

Fig. S14 Immunofluorescence analysis of HIF-1α in the synovium after treatment (scale bars: 100 μm).

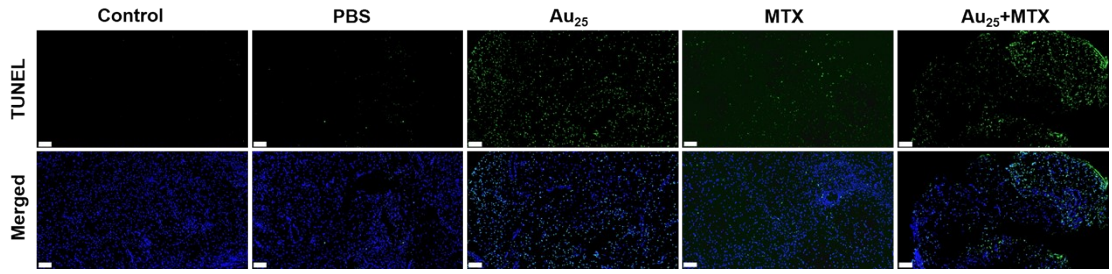

Fig. S15 TUNEL staining of synovium in different treatment groups on the 32th day (scale bars: 100 μm).

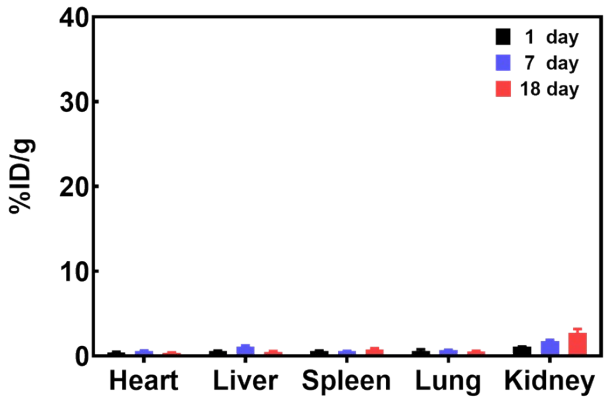

Fig. S16 Distribution of Au in main organs following multiple injection of Au_{25} nanoclusters at different timepoints.

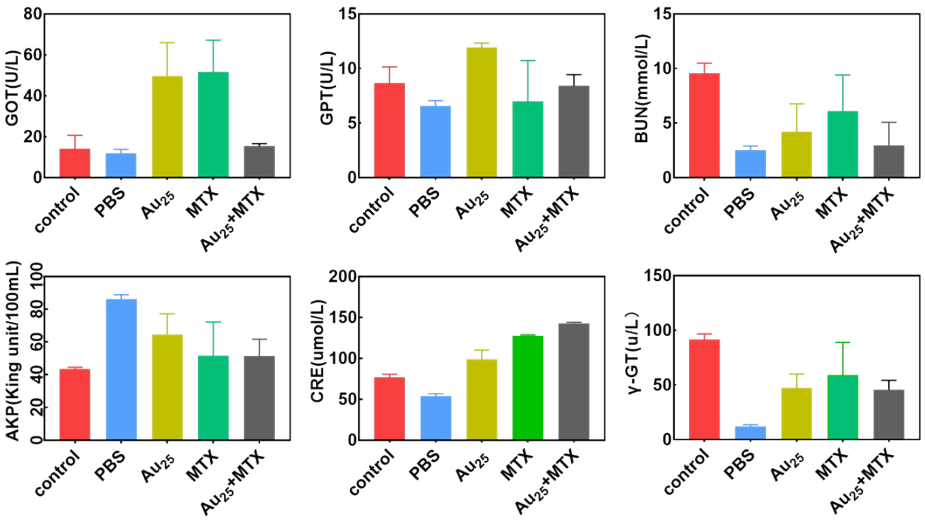

Fig. S17 Serum biochemical analysis results after 32 days treatments (n = 3). Data are presented as mean ± S.D. AST: aspartate transaminase; ALT: alanine aminotransferase; AKP: alkaline phosphatase; γ-GT: γ -glutamyl transpeptidase; CRE: creatinine; BUN: blood urea nitrogen.

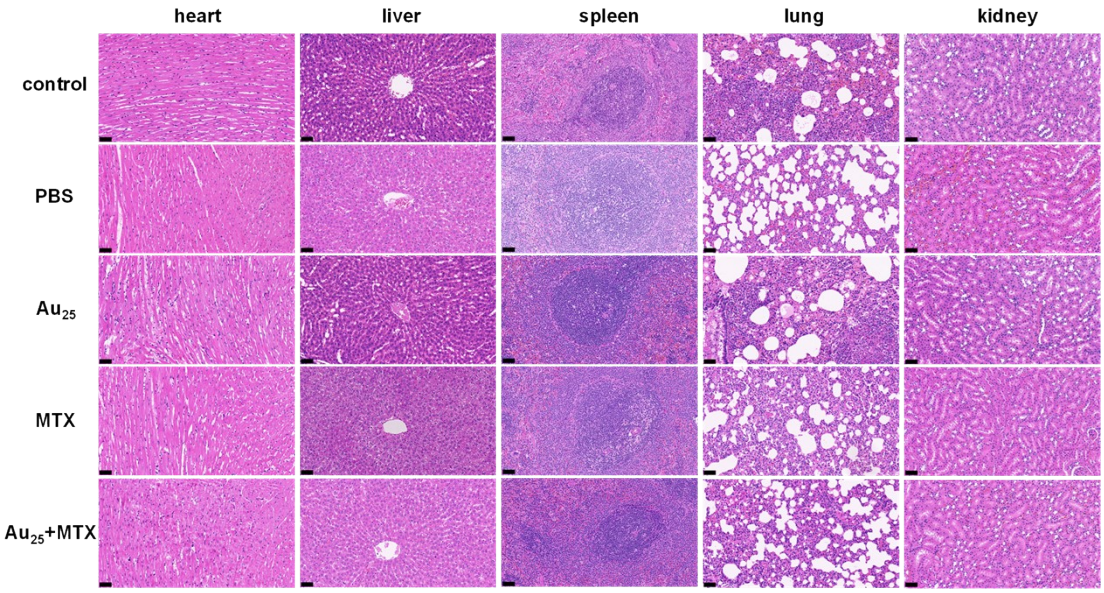

Fig. S18 H&E-stained images of major organ slices of rats from different groups after 32 days treatment (scale bars: 50 μm).

Table S1. Primers used in qRT-PCR.

Gene	Amplicon Size (bp)	Forward primer (5'→3')	Reverse primer (5'→3')
β-actin	120	AGTGTGACGTTGACATCCGT	TGCTAGGAGCCAGAGCAGTA
IL-6	88	CCCACCAAGAACGATAGTCAA	ATCAGTCCCAAGAAGGCAAC
TNF-a	133	GACAGTGACCTGGACTGTGG	TGAGACAGAGGCAACCTGAC
iNOS	94	GGAGCGAGTTGTGGATTGTC	CAGCCTCTTGTCTTTGACCC
CD206	110	AGTGGCTTTGGTTGAACGAC	CCAAAGGCCCGAAGATGAAG
IL-1β	98	GAAGAAGAGCCCATCCTCTG	TCATCTCGGAGCCTGTAGTG
Arg-1	134	CTCAAAGGGACAGCCACGAG	TAGGGATGTCAGCAAAGGGC

Table S2. global assessment.

content	score	symptoms
ears	0	no swelling and nodules
	1	one ear has swelling and nodules
	2	both ears have swelling and nodules
nose	0	no swelling in the tissue
	1	swelling in the tissue
tail	0	no swelling and nodules
	1	swelling and nodules
front paws	0	no swelling
	1	swelling in the left front foot claw
	1	swelling in the right front foot claws
hind paws	0	no swelling
	1	swelling in the left hind foot claws
	1	swelling in the right hind foot claws

Table S3. arthritis index score.

score	symptoms
0	no swelling
1	mild swelling in the ankle joint
2	mild swelling in the ankle to metatarsal joint or metacarpal joint
3	moderate swelling in the ankle to metatarsal joint or metacarpal joint
4	severe swelling of the entire paw