Supporting Information

Table 1. RA, OA, and healthy volunteers' baseline data

	RA (N=39)	**OA (N=25)**	**Healthy (N=25)**
Age (years (mean (range)))	53 (30-73)	57 (30-72)	45 (24-74)
Sex (N (F/M))	24/15	19/6	20/5
Disease duration (years (mean (range))	5 (1~20)	8 (1~23)	NA
CRP (mg/L (mean (range)))	20.2 (1.4~93)	14.5 (3.13~39.5)	NA
ESR (mm/h (mean (range)))	49.5 (8~120)	22.3 (8~47)	NA
RF (>20 IU/mL)	31/38	NA	NA

CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; RF, rheumatoid factor; NA, not assessed.

Table 2 Rat arthritis index

Score	**Symptom**
0	No evidence of swelling and erythema of the joints, including the small joints of the forepaw and the phalangeal joint and the large joints such as the wrist and ankle.
1	Mild swelling and erythema of the ankle joint.
2	Mild swelling and erythema extending to the small joints.
3	Severe swelling and erythema of large joints.
4	Severe swelling and erythema encompassing the small and large joints.

Figure S1

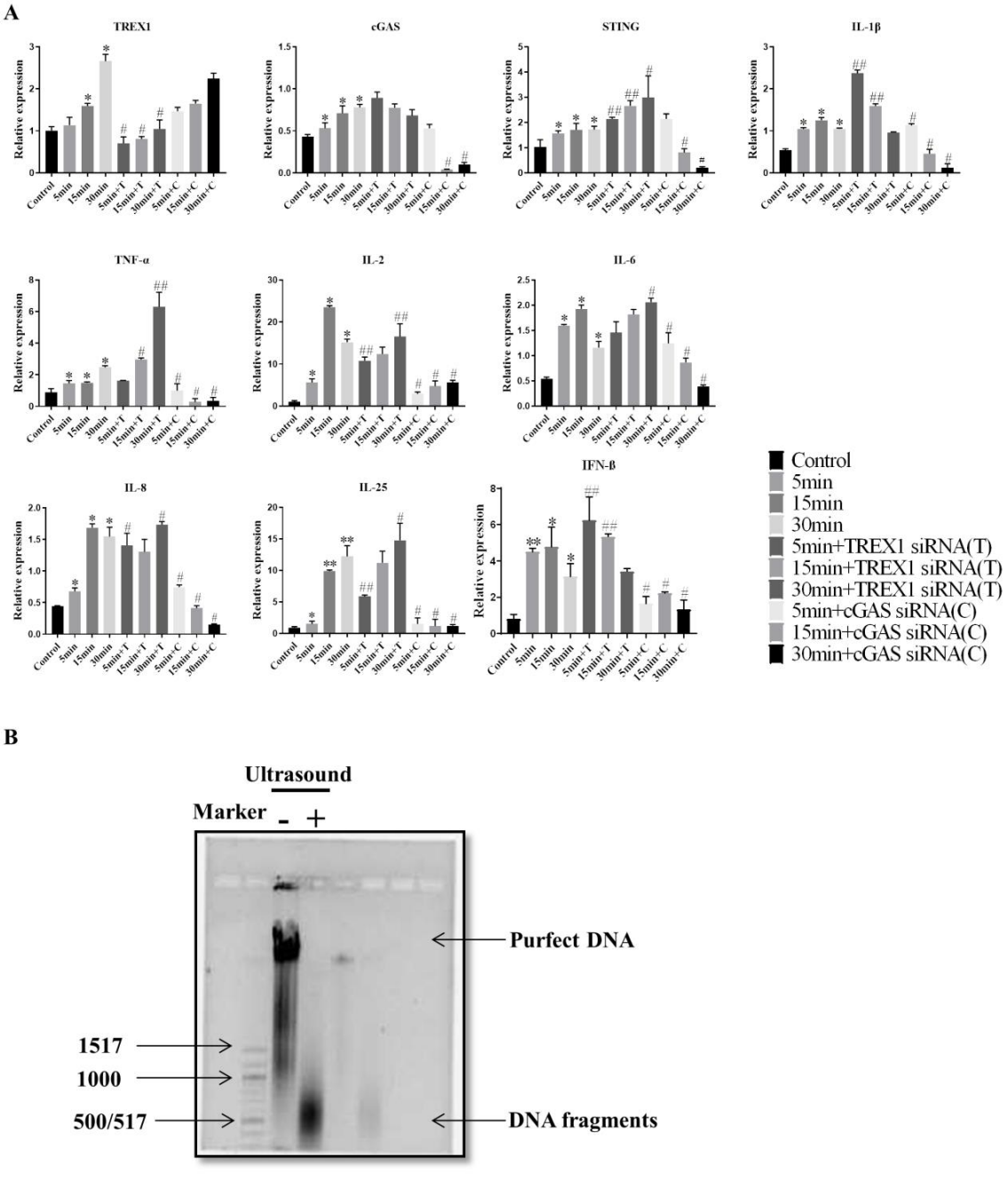


Figure S1. Role of TREX1 and cGAS in the production of proinflammatory mediators in RA-FLSs exposed to UV light. (**A**) RA-FLSs with siRNA-mediated silencing of TREX1 or cGAS were exposed to UV light for 0-30 min; total mRNA from the UV-

treated cells was then harvested, and the gene expression levels of proinflammatory mediators were determined by RT–PCR. The data shown are the means ± SEMs from three independent experiments. *P <0.05, **P<0.01, ***P<0.001 vs. control; #P <0.05 vs. UV treatment alone (T, TREX1 siRNA; C, cGAS siRNA). (**B**) Preparation of DNA fragments by ultrasonication. RA-FLSs were harvested for DNA extraction using a DNA extraction kit (Favorgen). DNA fragmentation was performed by ultrasonication to result in DNA fragments of 500 bp as determined by DNA gel electrophoresis.

Figure S2

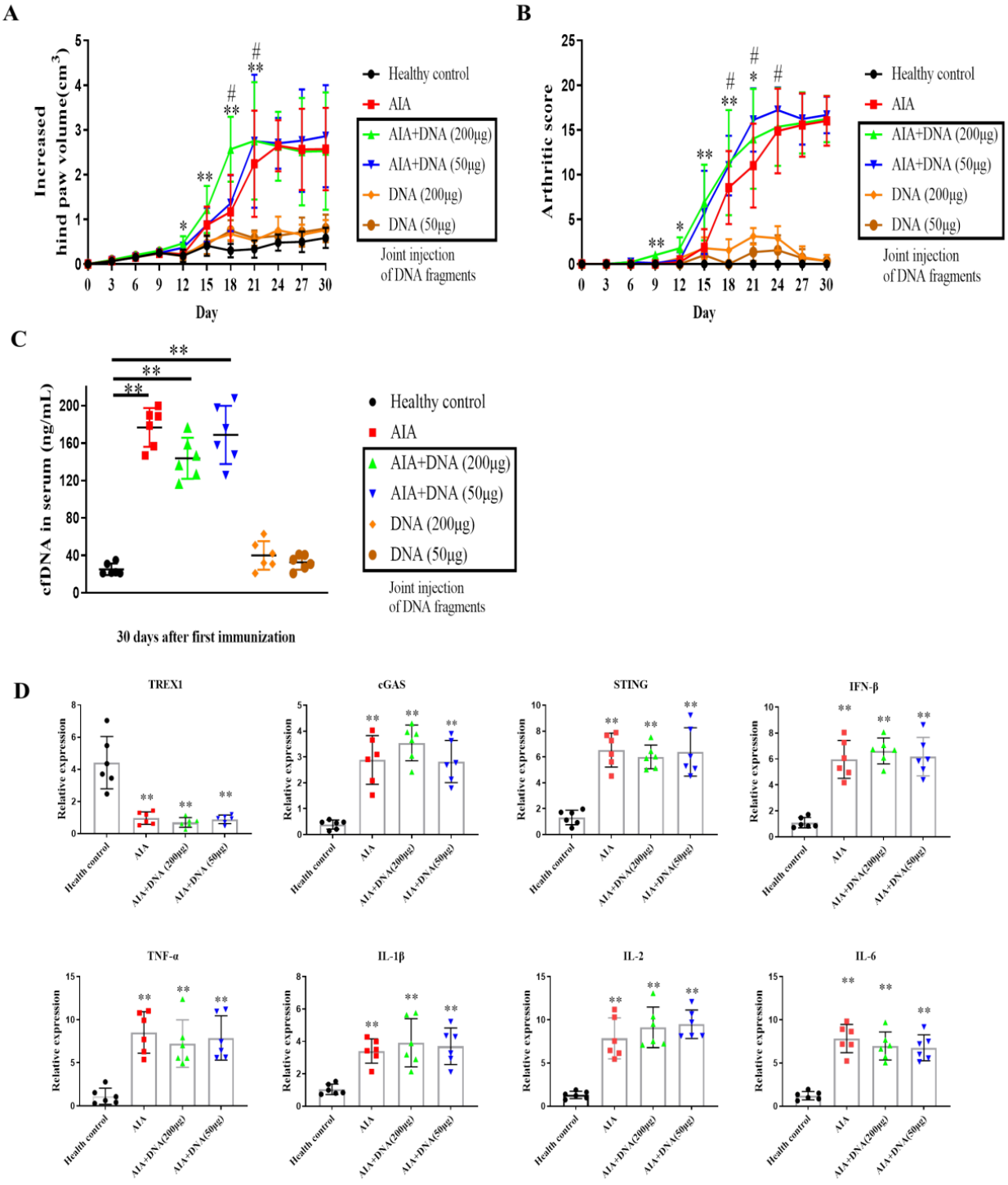

A
Increased hind paw volume(cm3)
Day
Healthy control
AIA
AIA+DNA (200μg)
AIA+DNA (50μg)
DNA (200μg)
DNA (50μg)
Joint injection of DNA fragments
B
Arthritic score
Day
C
cfDNA in serum (ng/mL)
30 days after first immunization
D
TREX1
cGAS
STING
IFN-β
TNF-α
IL-1β
IL-2
IL-6
Relative expression
Health control
AIA
AIA+DNA(200μg)
AIA+DNA(50μg)

Figure S2. The proinflammatory effect of joint injection with DNA fragments in AIA rat models. (**A & B**) Hind paw swelling and arthritis scores in DNA fragment-injected AIA rats. Rats in the three healthy control groups and the three AIA groups were injected with nucleic acid-free water or DNA fragments (200 μg or 50 μg), respectively, in the hind limb joint region and monitored for 30 days. Hind paw volumes (ml) and arthritis scores were determined every 3 days. The data are expressed as the mean values ± SEMs (n = 8). Images of hind paw swelling were acquired on Day 30. One-way ANOVA; *p<0.05, **p<0.01 for the AIA+DNA (200 μg) group compared with the AIA group; #p<0.05, ##p<0.01 for the DNA (200 μg) group vs. the healthy control group. (**C**) Detection of circulating free DNA (cfDNA) in the serum of DNA fragment-injected AIA rats. On Day 30, the serum was collected from rats in all treatment groups for extraction of cfDNA using a Dynabeads SILANE Viral NA Kit. The amount of cfDNA in each treatment group was quantified using Quant-iT^{TM} PicoGreen ® dsDNA Reagent and Kits. **p<0.01 compared with the healthy control group. (**D**) Gene expression analysis of TREX1 and cGAS signalling pathway components in AIA rats with or without injection of DNA fragments. PBMCs were harvested from rats in each treatment group for RNA preparation. RT–PCR was adopted to analyse the mRNA expression levels of proinflammatory cytokines. One-way ANOVA; *p<0.05, **p<0.01 compared with the healthy control group.

Figure S3

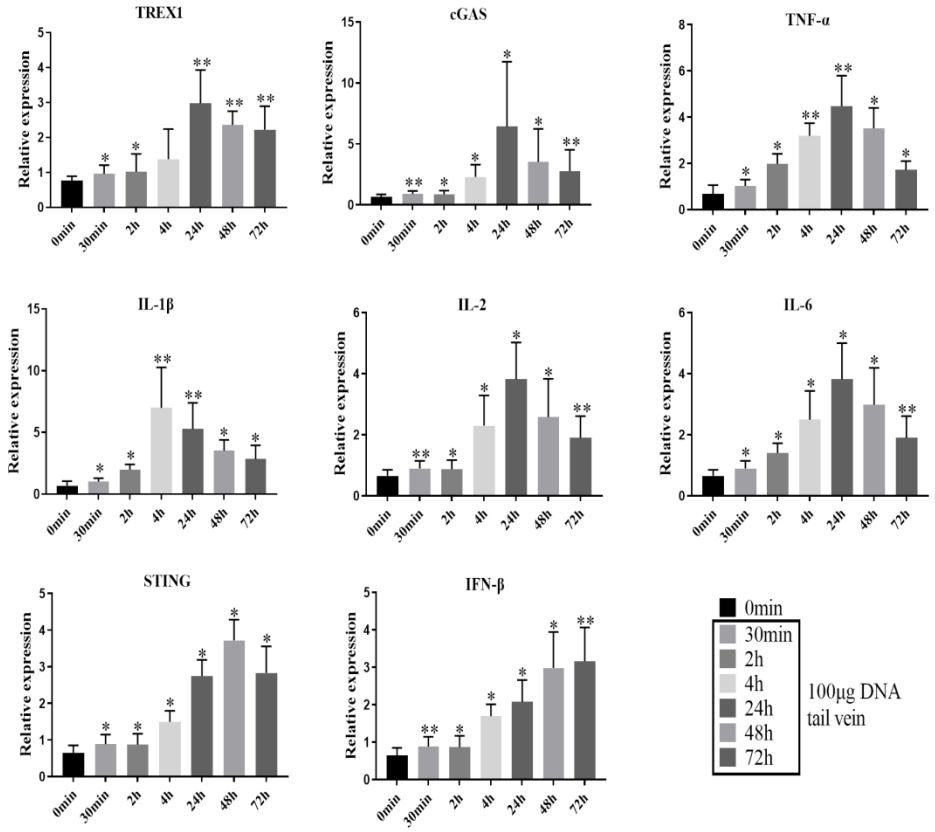
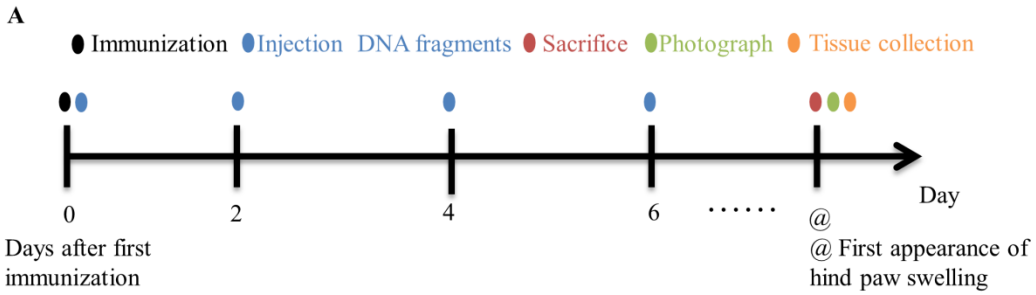


Figure S3. The proinflammatory effect of DNA fragments in healthy control SD rats. Healthy SD rats were injected via the tail vein with 100 μg of DNA fragments (sonicated DNA from dissected rat muscle tissue). At 0 to 72 h after DNA fragment injection, PBMCs were collected from the DNA fragment-injected SD rats (n = 8) for RNA extraction. The gene expression of TREX1, cGAS, TNF-α, IL-1β, IL-2, IL-6, STING, and IFN-β was then validated by RT–PCR. One-way ANOVA; *$p<0.05$, **$p<0.01$ compared with the untreated control group.

Figure S4-1


A
Immunization
Injection
DNA fragments
Sacrifice
Photograph
Tissue collection
0
2
4
6
.....
@
Day
Days after first immunization
@ First appearance of hind paw swelling

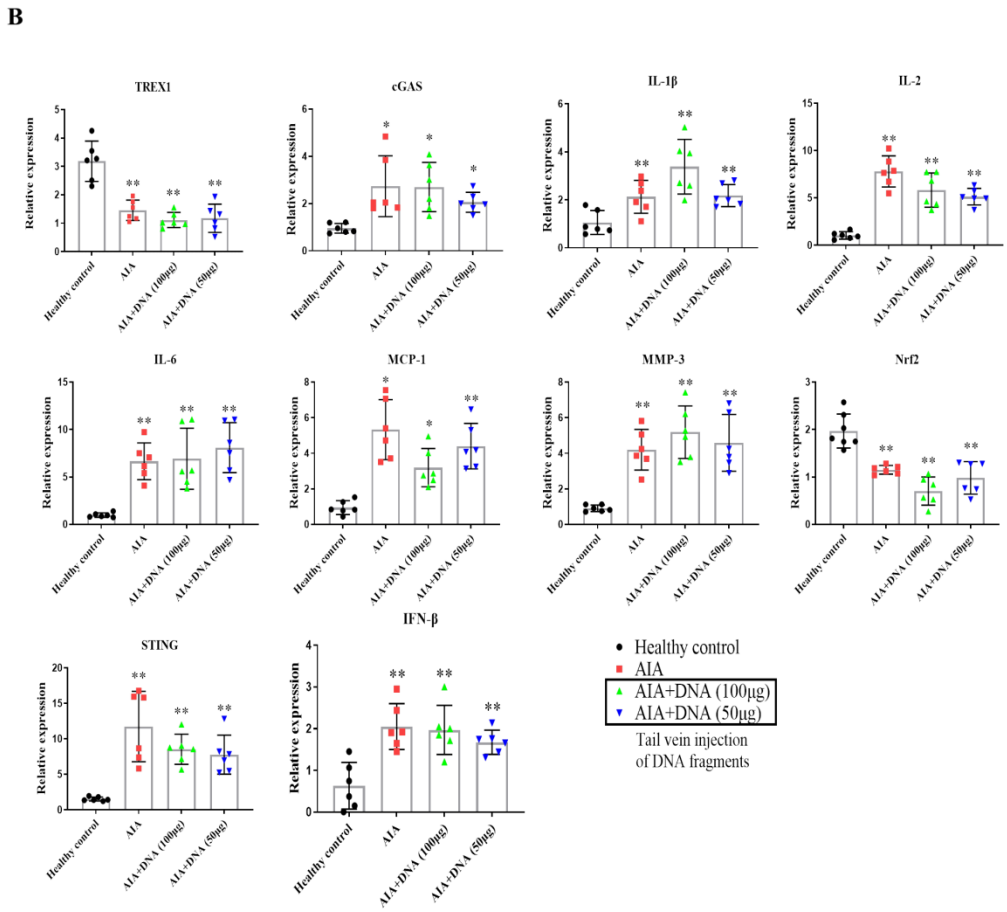
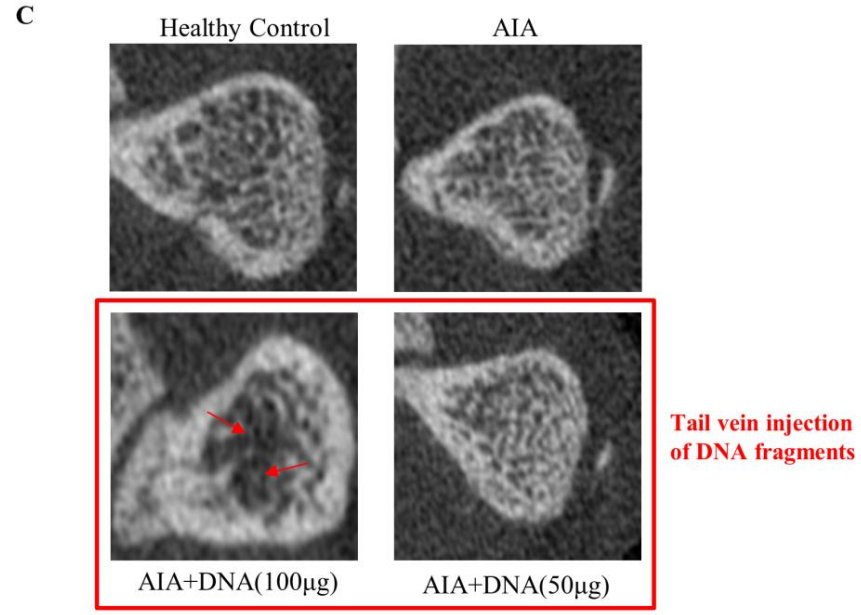

B
TREX1
cGAS
IL-1β
IL-2
IL-6
MCP-1
MMP-3
Nrf2
STING
IFN-β
Relative expression
Healthy control
AIA
AIA+DNA (100μg)
AIA+DNA (50μg)
Healthy control
AIA
AIA+DNA (100μg)
AIA+DNA (50μg)
Tail vein injection of DNA fragments

Figure S4-2


C
Healthy Control
AIA
AIA+DNA(100μg)
AIA+DNA(50μg)
Tail vein injection
of DNA fragments

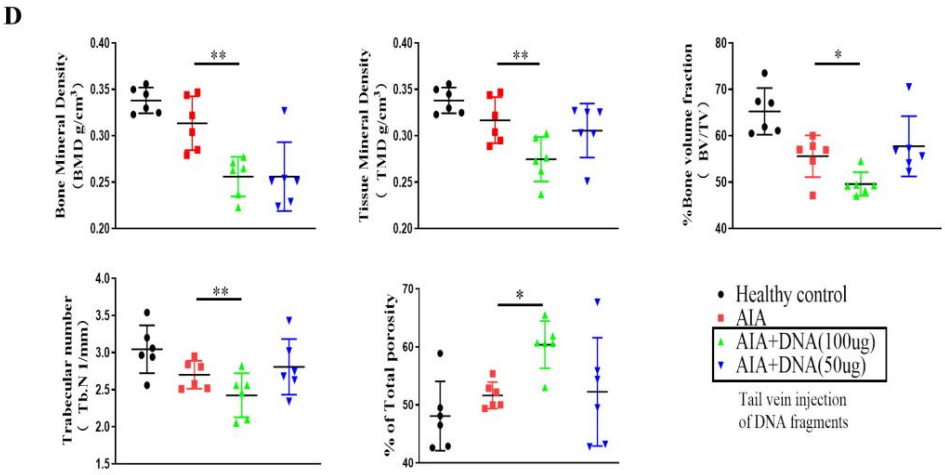

D
Bone Mineral Density (BMD g/cm³)
Tissue Mineral Density (TMD g/cm³)
%Bone volume fraction (BV/TV)
Trabecular number (Tb.N 1/mm)
% of Total porosity
Healthy control
AIA
AIA+DNA(100ug)
AIA+DNA(50ug)
Tail vein injection
of DNA fragments

Figure S4-1 and S4-2. Correlation of arthritis symptoms and the release of proinflammatory cytokines in AIA rats injected with DNA fragments. (**A**) Experimental scheme of the AIA rat models with tail vein DNA injection. SD rats were injected with complete adjuvant for arthritis induction (on Day 0), and the resulting AIA rats were then injected with 50 μg or 100 μg of DNA fragments (sonicated DNA from rat-dissected muscle tissue) via the tail vein beginning on Day 0 at intervals of 2 days until the day (@) when hind paw swelling first became visible. Hind paw swelling, the arthritis score and blood samples were monitored and collected regularly as indicated in the schedule. (**B**) Gene expression analysis of TREX1-cGAS signalling pathway components in AIA rats with tail vein injection of DNA fragments. On Day 12 (@), the AIA rats with hind paw swelling were sacrificed, and PBMCs were collected for RNA extraction and gene expression analysis. The gene expression of cytokines (IL-1β, IL-2, IL-6, MCP-1, MMP-3, Nrf2, STING, IFN-β) was analysed by RT–PCR (n = 6-8). One-way ANOVA; *$p<0.05$, **$p<0.01$ compared with healthy controls. (**C**) Representative radiographic Micro-CT images indicating bone destruction in the calcaneus region in the DNA fragment-injected AIA rats. The red arrows indicate the area of destruction in the calcaneus region of the hind limb joint. (**D**) Bone destruction in AIA rats with tail vein injection of DNA fragments. Micro-CT analysis of hind limb joints from DNA fragment-injected AIA rats was conducted to assess the extent of bone damage. Micro-CT scores were calculated from five disease-related indices: bone mineral density (BMD), trabecular number (mm-1) (Tb. N), cortical bone tissue mineral density (g/cm3) (TMD), bone volume fraction (BV/TV), and total porosity (as a percentage of total area). *$p<0.05$, **$p<0.01$ compared with AIA rats by one-way ANOVA.

Figure S5

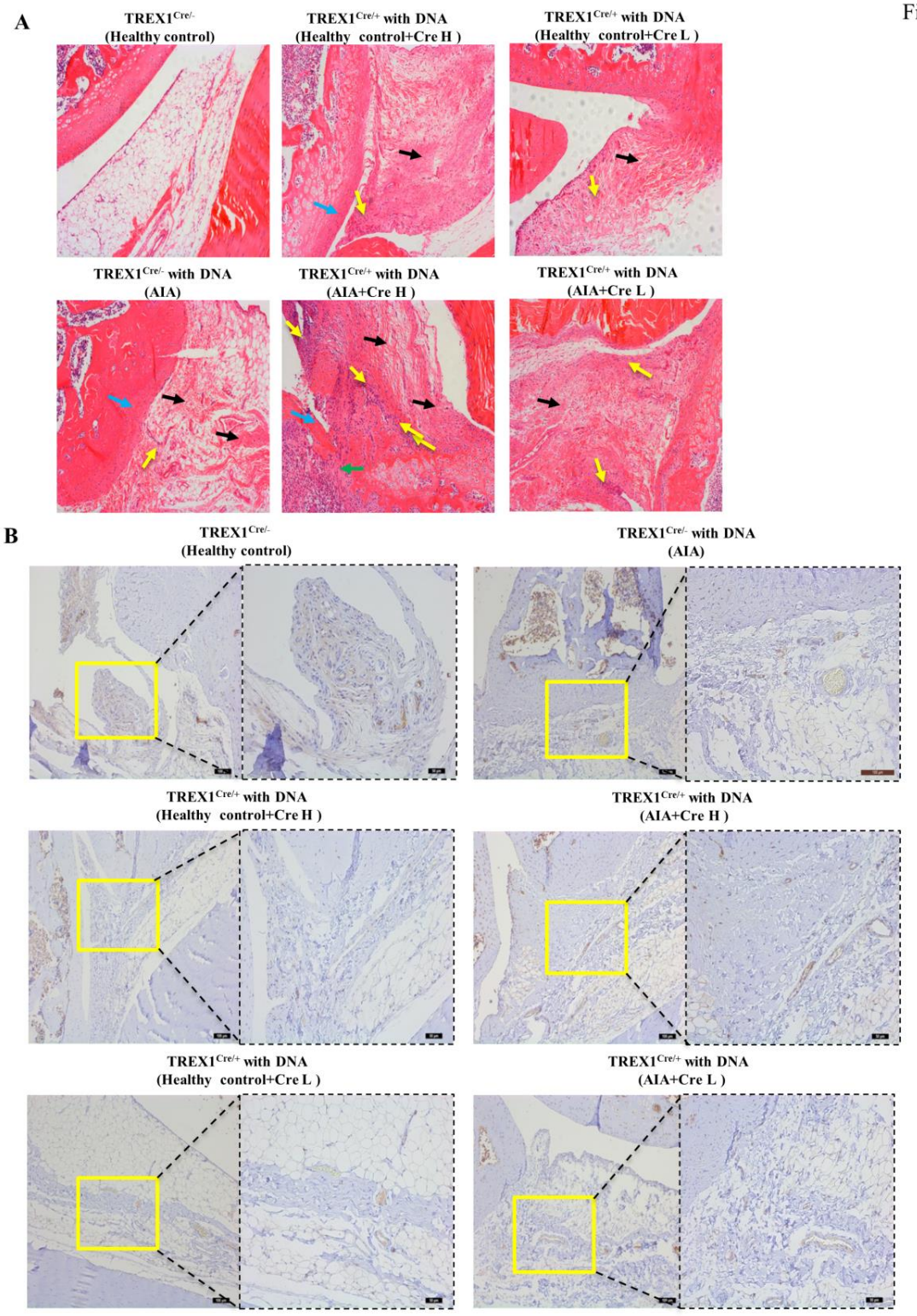

A
TREX1Cre/- (Healthy control)
TREX1Cre/+ with DNA (Healthy control+Cre H)
TREX1Cre/+ with DNA (Healthy control+Cre L)
TREX1Cre/- with DNA (AIA)
TREX1Cre/+ with DNA (AIA+Cre H)
TREX1Cre/+ with DNA (AIA+Cre L)
B
TREX1Cre/- (Healthy control)
TREX1Cre/- with DNA (AIA)
TREX1Cre/+ with DNA (Healthy control+Cre H)
TREX1Cre/+ with DNA (AIA+Cre H)
TREX1Cre/+ with DNA (Healthy control+Cre L)
TREX1Cre/+ with DNA (AIA+Cre L)

Figure S5. Histopathological analysis of knee joints in AIA model TREX1 conditional knockout ($TREX1^{Cre}$) rats injected with DNA fragments. (**A**) Representative histological sections of knee joints stained with H&E (n = 6-8). The yellow arrows indicate synovial cell proliferation with infiltration of neutrophils, lymphocytes, plasma cells, and other inflammatory cells; the black arrows indicate interstitial fibrotic cell proliferation accompanied by neovascularization; the blue arrow indicates cartilage thickening; and the green arrows indicate regions where the villus-like synovium stretched into the joint cavity, adhered and invaded into the cartilage, and destroyed the articular cartilage surface. (**B**) Immunohistochemical staining of TREX1 in the knee synovial tissue of AIA model $TREX1^{Cre}$ rats. The yellow boxes indicate the knee synovial region.

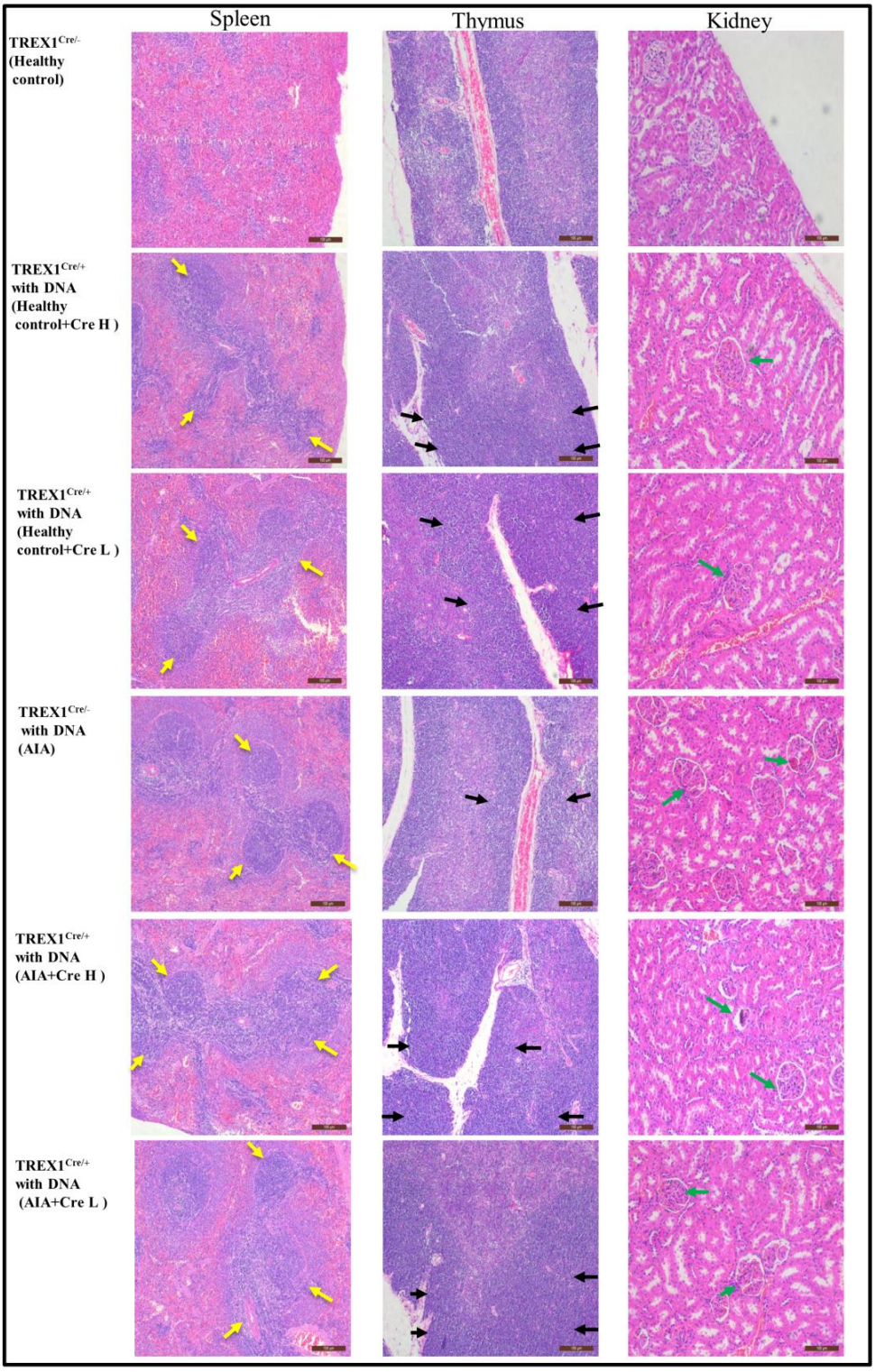


Figure S6. Histopathological staining of the spleen, thymus and kidney in AIA model TREX1^{Cre} rats injected with DNA fragments. Representative histological sections of the spleen, thymus and kidney were prepared and stained with H&E (n = 6-8). The yellow arrows indicate white pulp enlargement and fusion and lymphocyte proliferation; the black arrows indicate the clear boundary between the thymic cortex and medulla, cortical thickening and the increase in T lymphocytes; and the green arrows indicate glomerular oedema, the indistinct glomerular structure, and fibrosis.

Figure S7

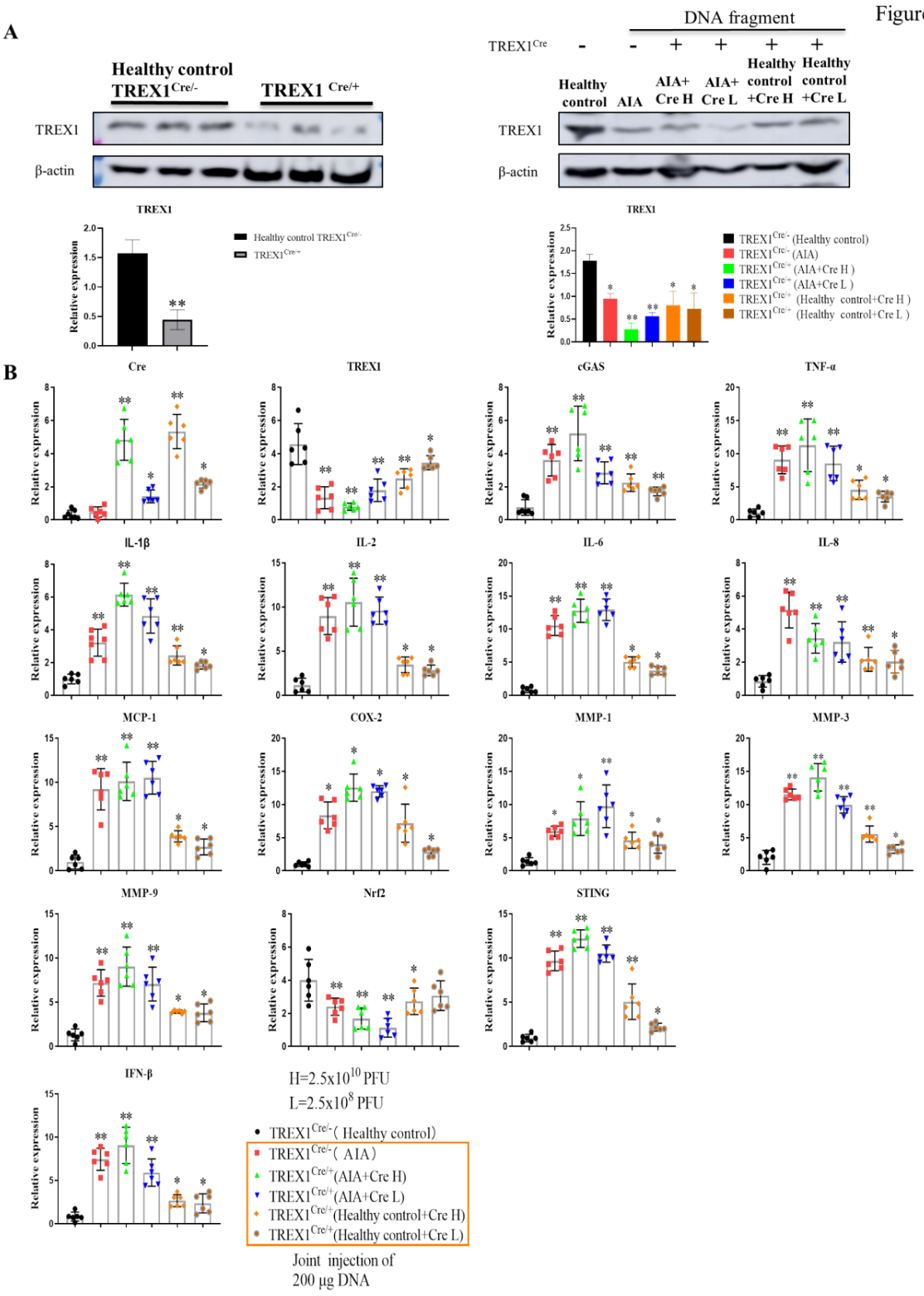

A
Healthy control
TREX1Cre/-
TREX1 Cre/+
TREX1
β-actin
TREX1
Relative expression
Healthy control TREX1Cre/-
TREX1Cre/+
DNA fragment
TREX1Cre
Healthy control
AIA
AIA+ Cre H
AIA+ Cre L
Healthy control +Cre H
Healthy control +Cre L
TREX1Cre/- (Healthy control)
TREX1Cre/- (AIA)
TREX1Cre/+ (AIA+Cre H)
TREX1Cre/+ (AIA+Cre L)
TREX1Cre/+ (Healthy control+Cre H)
TREX1Cre/+ (Healthy control+Cre L)
B
Cre
TREX1
cGAS
TNF-α
IL-1β
IL-2
IL-6
IL-8
MCP-1
COX-2
MMP-1
MMP-3
MMP-9
Nrf2
STING
IFN-β
H=2.5x10^10 PFU
L=2.5x10^8 PFU
TREX1Cre/-(Healthy control)
TREX1Cre/-(AIA)
TREX1Cre/+(AIA+Cre H)
TREX1Cre/+(AIA+Cre L)
TREX1Cre/+(Healthy control+Cre H)
TREX1Cre/+(Healthy control+Cre L)
Joint injection of
200 μg DNA

Figure S7. Gene expression analysis of TREX1, cGAS signalling mediators, proinflammatory cytokines and metalloproteinases in PBMCs or synovium from DNA fragment-injected AIA model TREX1^{Cre} rats. (**A**) TREX1 protein expression in AIA model TREX1^{Cre} rats. Synovial samples were harvested from all treatment groups for protein extraction and Western blotting using antibodies against TREX1 and β-actin (as the loading control). The bar charts show the quantitation of TREX1 protein expression. (**B**) mRNA expression analysis of TREX1, cGAS signalling mediators, proinflammatory cytokines and metalloproteinases. PBMC samples were collected from all treatment groups for RNA extraction and RT–PCR analysis of gene expression using specific primer sets (n = 6-8). One-way ANOVA; *$p<0.05$, **$p<0.01$ compared with healthy controls.

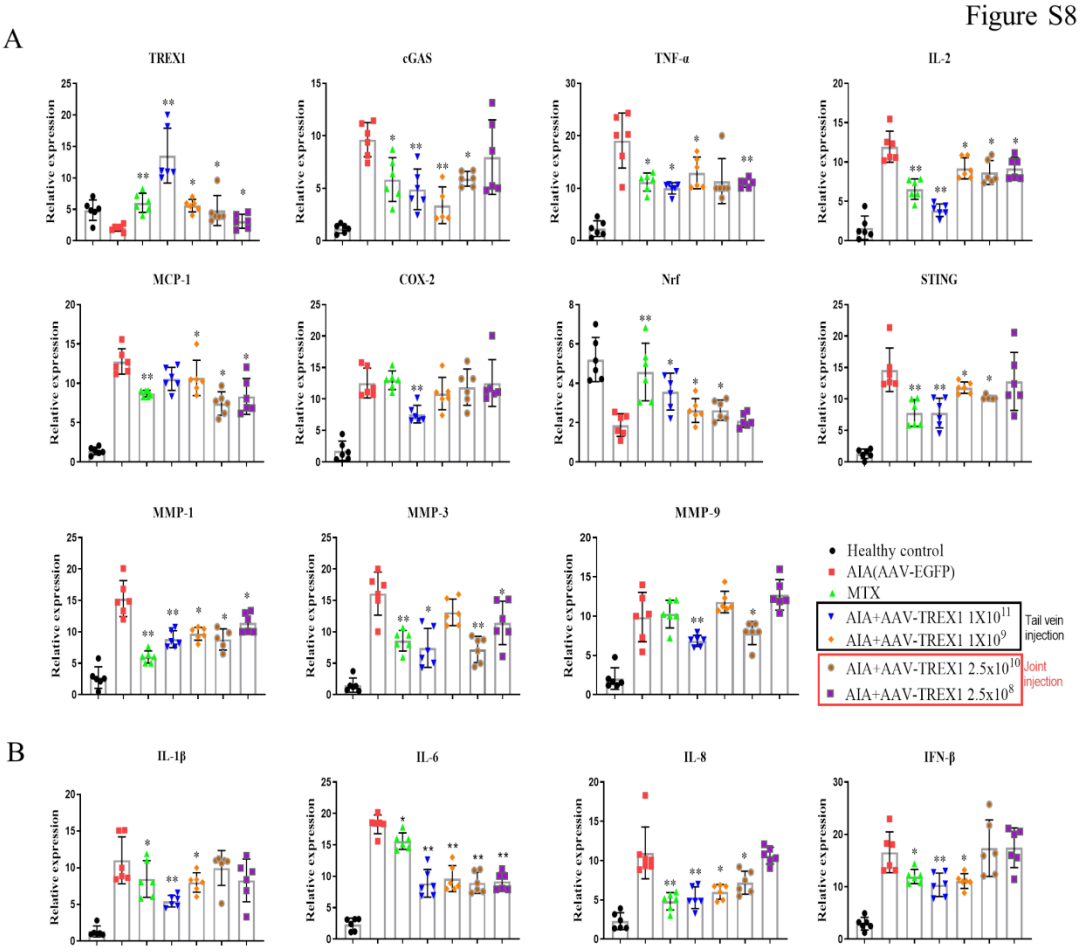


Figure S8. Gene expression analysis of TREX1, cGAS signalling mediators, proinflammatory cytokines and metalloproteinases in PBMCs from AAV-TREX1-injected AIA rats. (**A**) mRNA expression analysis of TREX1, cGAS signalling mediators, proinflammatory cytokines and metalloproteinases. PBMC samples were collected from all treatment groups for RNA extraction and RT–PCR analysis of gene expression using specific primer sets (n = 6). One-way ANOVA; *p<0.05, **p<0.01 compared with AIA rats. (**B**) Gene expression analysis of SASP factors in PBMCs from AAV-TREX1-injected AIA rats.

Figure S9

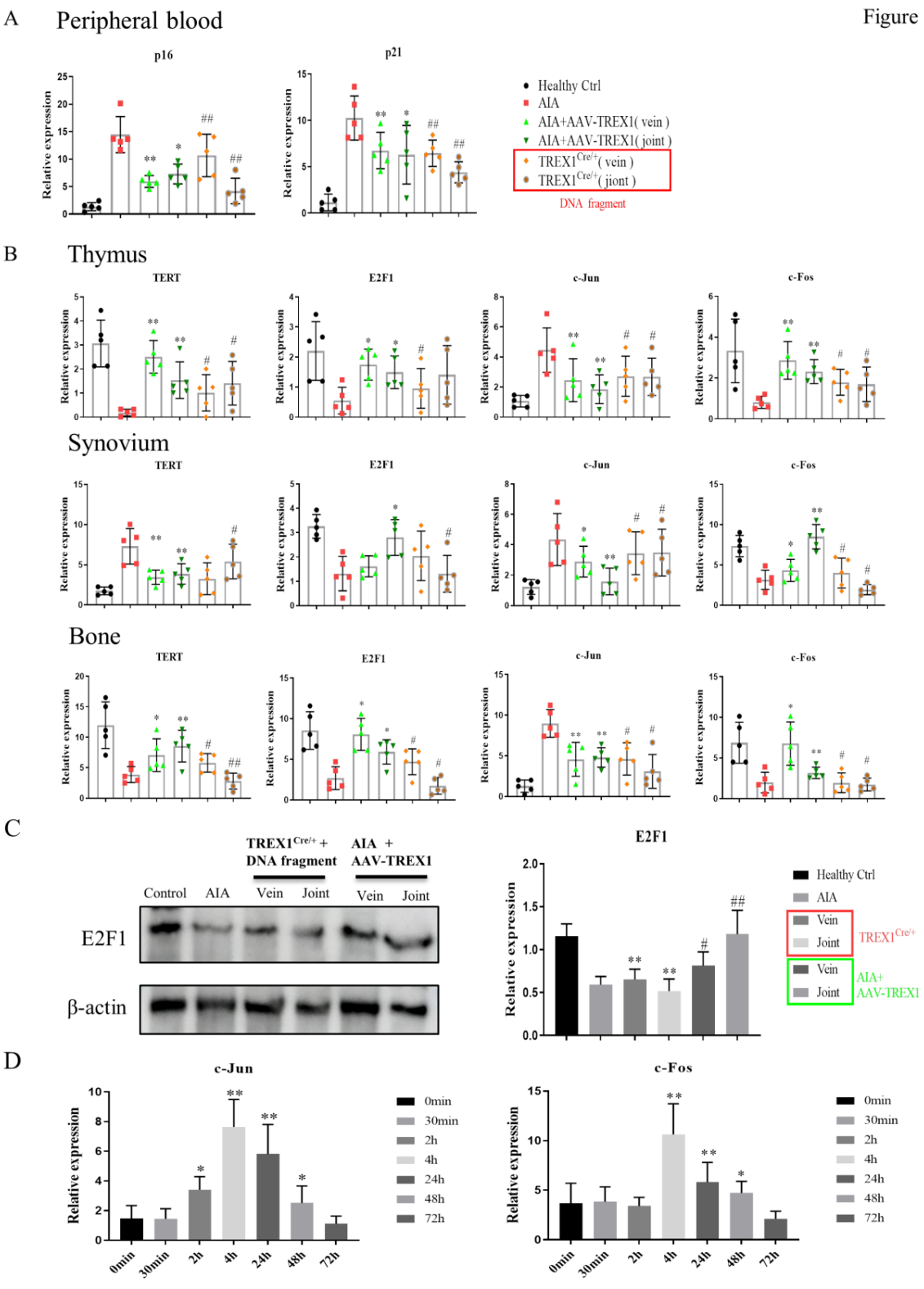

A
Peripheral blood
p16
p21
Relative expression
Healthy Ctrl
AIA
AIA+AAV-TREX1(vein)
AIA+AAV-TREX1(joint)
TREX1Cre/+(vein)
TREX1Cre/+(jiont)
DNA fragment
B
Thymus
TERT
E2F1
c-Jun
c-Fos
Synovium
Bone
C
TREX1Cre/+ + DNA fragment
AIA + AAV-TREX1
Control
AIA
Vein
Joint
E2F1
β-actin
Healthy Ctrl
AIA
TREX1Cre/+
AIA+ AAV-TREX1
D
c-Jun
c-Fos
0min
30min
2h
4h
24h
48h
72h

Figure S9. Analysis of the transcription factors and tissue-specific regulators of TREX1 in AAV-TREX1-injected AIA rats and $TREX1^{Cre}$ rats. (**A, B**) mRNA expression analysis of p16, p21, TERT, E2F1, c-Jun and c-Fos. Peripheral blood, thymus, synovium and bone samples were collected from healthy control, AIA model, AIA rats with tail vein or joint injection of AAV-TREX1, and $TREX1^{Cre}$ rats with tail vein or joint injection of AAV-Cre for RNA extraction and RT–PCR analysis of gene expression using specific primer sets (n = 6). One-way ANOVA; *p<0.05, **p<0.01 compared with the AIA model group; #p<0.05, ##p<0.01 compared with the healthy control group. (**C**) Expression of the E2F1 transcription factor in AAV-Cre-injected $TREX1^{Cre}$ rats and AAV-TREX1-injected AIA rats. Synovial tissues were harvested from these treatment groups for protein extraction and Western blot analysis using antibodies against E2F1 and β-actin (as the loading control). The bar charts show the quantitation of E2F1 expression relative to actin expression using ImageJ software. *P <0.05, **P<0.01 compared with healthy control rats; #P <0.05, ## P<0.01 compared with AIA rats. (**D**) The time-dependent expression of c-Jun and c-Fos in DNA fragment-injected healthy control SD rats. Healthy control SD rats were injected with 100 μg of DNA fragments (sonicated DNA from rat dissected muscle tissue) via the tail vein. Peripheral blood was then collected from the animals (n = 8) at 0 min, 30 min, 2 h, 4 h, 24 h, 48 h and 72 h for RNA extraction. The gene expression of c-Jun and c-Fos was then validated by RT–PCR. One-way ANOVA; *p<0.05, **p<0.01 compared with untreated control animals.