METHODS

Clinical samples. Blood samples were collected from patients with RA and osteoarthritis (OA), and healthy volunteers under the premise of signing the informed and voluntary consent in Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou (China) and approval by the research ethics committee (GDREC 2015391H). All of the epidemiological investigations and classification from volunteers were carried out according to the criteria of American College of Rheumatology. The clinically relevant information and spread sheet are shown in table 1,

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

	RA (N=38)	**OA (N=17)**	**Healthy (N=29)**
Age (year(mean (range)))	53(30~73)	57(30~72)	45(24~74)
Sex (N(F/M))	24/14	14/3	22/7
Disease duration (year(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 factors; NA, not assessed.

RNA extraction, cDNA reverse transcription and real-time PCR. Total RNA of blood and cultured cells was isolated by using the FavorPrep^{TM} Blood/cultured Cell Total RNA mini kit (Favorgen Biotech) according to the manufacturer's protocol. Total RNA of synovial tissues were extracted with TRIzol (Invitrogen). UV spectrophotometry (NanoDrop Technologies) was used to measure the quality and concentration of RNA. 1 μg RNA was reverse transcribed with Maxima™ H Minus cDNA Synthesis Master Mix (Thermo). Quantification of gene expression was performed by ViiA 7 Real-Time PCR System (Applied Biosystems). All mRNA data

were normalized by the reference gene β-actin using ΔΔCT method for relative quantification. The primer sequences are seen in table 2.

Table 2. Primer sequences used for real-time PCR

Gena Name	Forward	Reverse
Human-Sirt1	5'-TTTGCCACCAAATCGTTACA-3'	5'-TCTGGCATGTCCCACTATCA-3'
Human-Sirt2	5'-TTCAAGAAACATCCGGAACC-3'	5'-GGAGTAGCCCCTTGTCCTTC-3'
Human-Sirt3	5'-CAGTCTGCCAAAGACCCTTC-3'	5'-AGAACACAATGTCGGGCTTC-3'
Human-Sirt4	5'-TGAAGATGAGCTTTGCGTTG-3'	5'-GCTGGCACAAATAACCCAAT-3'
Human-Sirt5	5'-TGTTCCGACCTTCAGAGGAG-3'	5'-CGCCGGTAGTGGTAGAACTC-3'
Human-Sirt6	5'-GTACGTCCGAGACACAGTCG-3'	5'-CTAGGATGGTGTCCCTCAGC-3'
Human-Sirt7	5'-CGCCAAATACTTGGTCGTCT-3'	5'-GCTTCTCCCTTTCTGAAGCA-3'
Human-IL-1β	5'-CCTGTCCTGCGTGTTGAAAGA-3'	5'-GGGAACTGGGCAGACTCAAA-3'
Human-IL-6	5'-AAATTCGGTACATCCTCGACGG-3'	5'-GGAAGGTTCAGGTTGTTTTCTGC-3'
Human-TNF-α	5'-AGAGGGAAGAGTTCCCCAGGGAC-3'	5'-TGAGTCGGTCACCCTTCTCCAG-3'
Rat-Sirt5	5'-AGCAAGATCTGCCTCACCAT-3'	5'-GGATTTCCAGCAGGTTCTTG-3'
Rat-IL-1β	5'-GCACAGTTCCCCAACTGGTA-3'	5'-AAGACACGGGTTCCATGGTG-3'
Rat-IL-6	5'-CCACCCACAACAGACCAGTA-3'	5'-GGAACTCCAGAAGACCAGAGC-3'
Rat-TNF-α	5'-CCAGGTTCTCTTCAAGGGACAA-3'	5'-CTCCTGGTATGAAATGGCAAATC -3'
Rat-MMP-3	5'-AAAGACAGGCACTTTTGGCG-3'	5'-ATCCACCTTTGTGCCAATGC-3'
Rat-Nrf-2	5'-AGCAGGCTGAGACTACCACT-3'	5'-TCCAGTGAGGGGATCGATGA-3'
Rat-MCP-1	5'-CAGTTAATGCCCCACTCACCT-3'	5'-ACAGCTTCTTTGGGACACCTG-3'
EGFP	5'-GGGGTACCATGAGTAAAGGAGAAGAACTTTTCACT-3'	5'-CGGGATCCTTATTTGTATAGTTC-3'ATCCATGCCA-3'

Western blot. Samples were lysed with Protein extraction buffer (1x Protease inhibitor cocktail from Roche; 150 mM NaCl; 50 mM Tris-HCl, pH 7.5; 100 mM DTT; 0.5 M EDTA; 100 mM PMSF) for 15 min. Soluble fractions were collected after centrifuged at 4 ℃, 12,000 rpm for 15 min. These protein concentration were determined by spectrophotometer at a wavelength of 595 um. 20 μg of protein sample was loaded by 12% SDS-PAGE then transferred onto PVDF membranes (Bio-Rad) and blocked with 5% BSA for 1 h. The membranes were probed for anti-SIRT5 antibody (1:1000; Abcam) and anti-β-actin antibody (1:2000; Santa Cruz Biotechnology) overnight at 4 ℃, after TBST washing, incubated with anti-rabbit and anti-mouse secondary antibodies (1:2000; Santa Cruz Biotechnology) for 2 h at room temperture. SuperSignal West Femto Maximum Sensitivity Substrate Kit (Thermo) was used to detect the bands under the Amersham Imager 600 (GE) Imaging System. Band densities quantification was managed by ImageJ software.

$CD14^+$ $CD16^-$ monocytes cell sorting. Peripheral blood mononuclear cells (PBMC) were isolated from healthy human whole blood using standard Ficoll density-gradient centrifugation kit (GE Healthcare). $CD14^+CD16^-$ monocytes were negative selected by Classical Monocyte isolation kit (Miltenyi Biotec) according to the manufacturer's protocol for stimulation and transfection experiments.

Cell stimulation. $CD14^+CD16^-$ monocytes and RASFs were cultured in 6-well plats (5×10^6 and 1×10^5 cell/well to ensure cell density reached 60-80%) in 2ml RPMI 1640 Medium with 10% Fetal Bovine Serum (FBS, Gibco) and Dulbecco's Modified Eagle Medium with 20% FBS, respectively. Cells then stimulated with 10-100ng/ml lipopolysaccharide (LPS from Escherichia coli J5 (Rc), LIST), 2 ng/ml IL-1β (R&D, Canada), 10 ng/ml TNF-α (Enzo Life Sciences, USA), 300 ng/ml Pam3CSK4 and 10 μg/ml Poly(I:C) HMW (both from InvivoGen), 100-200 μM suramin (Sigma) for 24h.

SIRT5 and shSIRT5 transfection. Selected $CD14^+CD16^-$ monocytes were transfected using Lipofectamine 3000 kit (Thermo Fisher Scientific). 2.5μg pcDNA3.1-SIRT5-Flag plasmid, empty pcDNA3.1(-) (Addgene) were used for overexpression. SIRT5 MISSION shRNA and shRNA Control Plasmid (Sigma) were

used for knockdown expression. After transfected for 48h at 37 ℃，cells subsequently stimulated with LPS or TNF-α for 24 h. Real-Time RCR was used to verify the transfection efficiency by SIRT5 specific primers.

Establishment of adjuvant-induced arthritis (AIA) in rats. SD Rats were divided into 7 groups (n=7~12) as following: (1) Healthy control (n=8), (2) AIA + Ad-EGFP (Vehicle control, n=8), (3) AIA+ Methotrexate (MTX, 0.76mg/ml) (n=7) , (4) AIA + Ad-SIRT5 (3×10^9 PFU for each joint) (n=8), (5) AIA + Ad-SIRT5 (1×10^{11} PFU) (n=12), (6) AIA + Ad-SIRT5 (1×10^{11} PFU) (n=8), equal volume of adenovirus was injected on day 0 and day 14, (7) AIA + Ad-SIRT5^{H158Y} (1×10^{11} PFU) (n=8). pAv/CMV/EGFP rSIRT5 vector (Ad-SIRT5), pAv/CMV/EGFP mtSIRT5 vector (Ad-SIRT5^{H158Y}) and control Adenovirus construction were packaged by Cyagen (USA). Mineral oil (Sigma) containing 5.0mg/ml *Mycobacterium tuberculosis* (*M. tuberculosis DES. H37 RA*, DIFCO) was grounded and rolled intensively until mixture turned into white colour. Rats received injection with adenovirus into knee-joints then subsequently injected 0.1 ml of mixture subcutaneously at the base of the tail on day 0. On day 28, the rats were sacrificed, and blood, organs and joint tissue were then harvested for biochemical assays, paw volume assessment and micro-CT analysis.

Paw Volume Assessment: Foot swelling determined by measuring paw volume with a plethysmograph (Ugo Basile, Italy or Kent Scientific Corp, Connecticut USA) every 3 day. Clinical scoring: The severity of arthritis can be objectively inspected on four paws and are scored on a scale of 0 ~ 4 for each paw according to the arthritis index in table 3.

Table. 3 Rat arthritic index

Score	Symptom
0	There was no swelling and erythema evidence on the joint, including the small joints of the front foot and phalangeal joint, large joints including wrist and ankle.

1	There was mild swelling and erythema on ankle.
2	Mild swelling and erythema extended to small joint.
3	There was serious swelling and erythema on large joint.
4	Serious swelling and erythema encompassing on small and large joint.

Micro-CT analysis. At the end of the treatment period, the rats were humanly dispatched and the left hind paw was amputated and fixed in 4% PFA, then scanned using *in vivo* micro-CT scanner (SkyScan 1176, Bruker, Belgium). The following scanning parameters were used to obtain high-quality images of the joint of the rat: 35 μm resolution, 85 kV, 385 μA, 65 ms exposure time, 0.7° rotation step in 360°, and a 1 mm Al filter. The images were reconstructed using NRecon software (Bruker-micro CT, Belgium).

Micro-CT score was obtained from five disease related index of the micro-CT analysis for calcaneus including bone mineral density, bone volume fraction, cortical mineral density, trabecular number and total porosity. Micro-CT score was calculated using the formula as follows: (Acquired value – minimum value)/(maximum value – minimum value) or 1-(Acquired value – minimum value)/(maximum value– minimum value). The final micro-CT score is equally averaged from the above five aspect of bones (Micro-CT score: 0-0.2, mutilating; 0.4-0.6, moderate; 0.8-1, normal). The bone condition of rats is evaluated with radiological score according to their bone erosion severity with referenced to table 4.

Table. 4 Radiological Score

Radiological	**Score**	**Bone erosion severity**
0		Intact bony outlines
1		Slight bone erosion

2	Mild bone erosion
3	Definite bone erosion
4	Severe bone erosion
5	Mutilating, no bony outlines

Samples collection and haemotoxylin and eosin (H&E) staining. Experimental AIA ratswere sacrificed at day 28, the synovium, organs (brain, heart, kidney, liver, spleen and thymus) and joint samples were collected for weighting and H&E staining. Synovium samples were frozen in liquid nitrogen immediately and stored at -80 ℃ in optimum cutting temperature (OCT) formulation (Tissue-Tek) for cryo-sectioning preparation. Organs and joint tissue were fixed in 4% paraformaldehyde for 24 h, and subjected to dehydrate and embed in paraffin blocks at 60 ℃. 6 μm thick sections were dehydrated, deparaffinized, rehydrated according to the standard protocols. Synovium, joint and organs sections were double incubated with primary antobody anti-SIRT5 (1:500, Millipore), anti-CD68 (1:100, Abcam) and anti-vimentin (1:500, Abcam) antibody at 4 ℃ overnight. The fluorescence image was generated by secondary antibody (anti-rabbit FITC, anti-mouse Cy5, CST) in the dark at room temperature for 2 h. Then mounted with FluorSave™ Reagent (Millipore). Fluorescent images were taken from API Delta Vision Live-Cell Imaging System. Joints and organs section from AIA rat were subjected to H&E staining. Images were captured by light microscopy (Leica DM2500)

ELISA. Cytokine levels in rat plasma were determined using specific Quantikine enzyme-linked immune sorbent assay (ELISA) kit for CRP-1, IL-1β and IL-6 (both from R&D), MCP-1 and TNF-α (both from Thermo) according to the manufacturer's protocol. The absorption value was measured at 450 nm using SpectraMax Paradigm (Molecular Devices).

Hepatotoxicity analysis. Hepatotoxicity was detected with plasma of experimental

rats and measured by the department of Clinical Laboratory Diagnostic Center, Hospital of Macau University of Science and Technolgy.

Acetyl CoA and pyruvate analysis. For Acetyl CoA analysis, 50 mg **l**iver samples of rats were using 1 N perchloric acid/KOH to deproteinize. Add 100 ul 1 N perchloric acid each sample following sonicate thoroughly. Centrifuge at 10,000 × g then neutralize 50 ul supernatant with 5 ul 3 M $KHCO_3$ and vortex, put on ice for 5 min. Centrifuge then add 10 ul sample into duplicate wells. Add 50 ul reaction mix (Acetyl CoA Fluorometric Assay Kit, BioVision) for each well according to the protocol. Incubate for 10 min, 37 ℃. Measure fluorescence at Ex/Em = 535/587 nm with a plate reader. For pyruvate analysis, 25 mg liver was extracted with 100 ul Pyruvate Assay Buffer. Sonicate thoroughly and centrifuge at 10,000 × g, 10 min, 4 ℃ to remove insoluble material. Add 25 ul supernatant into 96 well with 25 ul Pyruvate Assay Buffer. Add 50 ul reaction mix (Pyruvate Fluorometric Assay Kit, BioVision) for each well according to the protocol. Incubation for 30 min, RT. Measure fluorescence at Ex/Em = 535/590 nm with a plate reader.

Generation of SIRT5 Knockout Rat (SIRT5$^{-/-}$). SIRT5$^{-/-}$ rat on a Sprague Dawley rats background were generated using CRISPR/Cas9 technology by Cyagen (USA). Rats were bred and maintained at International Institute for Traditional Chinese Medicine of Guangzhou University of Chinese Medicine. Animal care and experimental procedures were approved and conducted under the guide for the Laboratory Animal Research Committee Guidelines of Guangzhou University of Chinese Medicine. Rats were housed under standard laboratory chow within 12:12 h cycle of light in a quiet room (temperature 22 ℃ ± 1 ℃) during the experiment. The genotypes of SIRT5 transgenic rats were identified by PCR of tail genomic DNA using two pairs of primers as table 5. Rats were divided into 3 groups as following: (1) AIA + SIRT5$^{+/+}$ (n=14), (2) AIA + SIRT5$^{-/-}$ (n=10), (3) AIA + SIRT5$^{-/-}$ + Ethyl pyruvate (70mg/kg/2 days) (n=4). Rats injected 0.1 ml mixture of mineral oil containing 2.5mg/ml *M. tuberculosis* at the base of the tail on day 0. On day 28, all rats were sacrificed and collected blood, organs and joint tissue for biochemical

assays.

Table 5. Primer sequences used for genotyping.

Gene name	Primer
Step1-RatSirt5-F	5’-GGTCAGAAGGAGGCAACTTGTAGAAATC-3’
Step1-RatSirt5-R	5’-TGGTCTGGGACTCAAACACTTGGA-3’
Step2-RatSirt5-F	5’-GGTCAGAAGGAGGCAACTTGTAGAAATC-3’
Step2-RatSirt5-R	5’-TGGTCTGGGACTCAAACACTTGGA-3’
Step2-RatSirt5-Wt/He-R	5’-CGAGATGATGACTATGTGCTTTGCG-3’

Statistical analysis. Unpaired and paired t tests or one-way analysis of variance followed by Dunnett’s post-test were used, where appropriate, for statistical evaluation of the data by GraphPad Prism 7.0 software. Values are presented as mean (SEM). p value <0.05 were considered as significant.

Detailed Figure 1 Legends

Figure 1. The anti-inflammatory role of SIRT5 in RA. A) RNA expression of SIRT1-7 in PBMC samples from healthy controls (n = 5-20) and RA patients (n = 10-15). B) SIRT5 gene expression between healthy volunteers and patients with RA or osteoarthritis. Healthy volunteers (H, n=29), rheumatoid arthritis (RA, n=38), and osteoarthritis (OA, n=17). Unpaired t test, *P<0.05, **P<0.01, ***P<0.001. C) SIRT5 mRNA levels in PBMC and synovial tissues of AIA rats on day 30 after induction of AIA in SD rat (n=6). D) SIRT5 protein expression in rat synovial tissues on day 30 (n=4). E) Effect of MTX and SIRT5 inhibitor suramin on RASFs. The cells were treated with 20 or 40 μM MTX, 10 or 100 μM suramin. F) Effect of proinflammatory cytokines on RASFs. Different Toll-like receptor ligands [100 ng/ml LPS, 300 ng/ml Pam3, and 30 μg/ml poly (I: C)], 20 ng/mL TNF-α and 4 ng/mL IL-1β were incubated with RASFs for 24 h. The treated RASFs were then harvested for immunoblotting of SIRT5. G) Production of proinflammatory cytokines in human $CD14^{+}CD16^{-}$ monocytes with overexpression or H) knockdown of SIRT5 in the presence or absence of suramin. *P<0.05, **P<0.01, ***P<0.001 *vs* untransfected controls. I) The arthritic scores and J) hind paw volume of Ad-SIRT5 overexpression in AIA rats (n = 8-12). $^{\#}$P<0.05 *vs* healthy ctrl; *P<0.05, **P<0.01 *vs* Ad-EGFP vehicle ctrl. The data shown are the mean ± SEM. K) microCT radiographic images of Ad-SIRT5 overexpression in AIA rats. L) Histopathological sections (scale bar 100 μm) and M) Immunofluorescence images (scale bar 50 μm) of synovium tissue from Ad-SIRT5-treated AIA rat. Joints were harvested from AIA rats treated with Ad-EGFP

(vehicle), MTX, and various doses of Ad-SIRT5 at day 28 and then subjected to histopathological examination of inflammatory symptoms, and immunofluorescence staining for SIRT5/vimentin expression. Sections were stained with hematoxylin and eosin (cartilage ×200, synovium ×200). Synovial hyperplasia, adhesion to articular cartilage, unclear boundaries, visible uneven articular cartilage, and even missing articular cartilage are indicated by a black arrow. Inflammatory cell infiltration was found in the proliferative synovial tissues marked by a red arrow. Neovascularization is shown by a blue arrow. Local bone trabeculae were missing in the bone cancellous, showing hyperplasia of multicellular giant cells, as illustrated by the yellow arrow. Connective tissue proliferation is labeled by a green arrow. N) Arthritic scores and O) the increased hind paw volume of $SIRT5^{+/+}$, $SIRT5^{-/-}$ and $SIRT5^{-/-}$ + EP AIA rats were measured for 28 days. $SIRT5^{-/-}$ SD rats generated by CRISPR/Cas9 technology were used to establish the AIA model, and the arthritic condition of rats was evaluated every 3 days to calculate the arthritic scores and hind paw volume. P) Characterization of succinylation distribution in rat tissues. Western blot analysis comparing lysine succinylation levels in rat organs derived from $SIRT5^{-/-}$ rats or $SIRT5^{+/+}$ rats with AIA. Equal amounts of tissues protein were loaded into each lane. Q) Hypersuccinylation of PKM2 in $SIRT5^{+/+}$ and $SIRT5^{-/-}$ SD rats. Total protein preparation from the synovium and liver of $SIRT5^{+/+}$ and $SIRT5^{-/-}$ SD rats was immunoprecipitated with an antibody against PKM2. R) Detection of acetyl CoA and pyruvate from livers of $SIRT5^{+/+}$, $SIRT5^{-/-}$ and $SIRT5^{-/-}$ +EP rats. **P<0.01, ***P<0.001, by One-way ANOVA. The data shown are the mean ± SEM.

Supplementary Figure Legends

Supplementary Fig. S1. Localization of SIRT5 in AIA rat synovial tissue sections, and the body and organ weights of AIA rats overexpressing SIRT5. A) Triple-staining of frozen sections from AIA rat synovial tissues with DAPI and antibodies against SIRT5, $CD68^+$ (monocytes/macrophages), or vimentin (synovial fibroblasts). SIRT5 appears as green, DAPI appears as blue, and $CD68^+$ and vimentin appear as red. Magnification, ×400, scale bar 25 μm. B) Body weight of Ad-SIRT5-treated AIA rats at different time points. C) Weight of the brain, heart, kidney and liver of Ad-SIRT5-treated AIA rats. D) Weight of the spleen and thymus from Ad-SIRT5-treated AIA rats. ##P<0.01, ###P<0.001 *vs* healthy ctrl; *P<0.05, **P<0.01 *vs* Ad-EGFP vehicle ctrl. The data shown are the mean ± SEM.

Supplementary Fig. S2. Micro-CT and radiological scores of Ad-SIRT5-treated AIA rats. A) BMD, Tb. N (mm^{-1}), TMD (g/cm^3), BV/TV, and total porosity percent of Ad-SIRT5-treated AIA rats. B) Micro-CT score and the disease severity evaluation standard for Ad-SIRT5-treated AIA rats. C) Radiological scores and bone erosion severity for Ad-SIRT5-treated AIA rats. $^{###}$P<0.001 *vs* healthy ctrl; *P<0.05, **P<0.01, ***P<0.001 *vs* Ad-EGFP vehicle ctrl by One-way ANOVA. The data shown are the mean ± SEM.

Supplementary Fig. S3. Proinflammatory cytokine detection in plasma serum of Ad-SIRT5-overexpressing AIA rats or SIRT5-deficient AIA rats. A) The ESR, CRP, and the proinflammatory cytokines MCP-1, TNF-α, IL-1β and IL-6 were

measured using ELISA in serum from the blood of Ad-SIRT5-treated AIA rats (n = 8). B) Body weight of $SIRT5^{+/+}$, $SIRT5^{-/-}$ and $SIRT5^{-/-}$ +EP AIA rats during progression of the disease; one-way ANOVA, ***P<0.001 *vs* $SIRT5^{+/+}$. C) Gene expression of TNF-α in PBMCs. D) IL-1β, IL-6 and MCP-1 in plasma serum were measured using ELISA from $SIRT5^{+/+}$, $SIRT5^{-/-}$ and $SIRT5^{-/-}$+EP SD rats. *P<0.05, **P<0.01 *vs* $SIRT5^{-/-}$.