Myeloid sirtuin 6 deficiency accelerates experimental rheumatoid arthritis by enhancing macrophage activation and infiltration into synovium

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Contents

1. Supplementary Figures

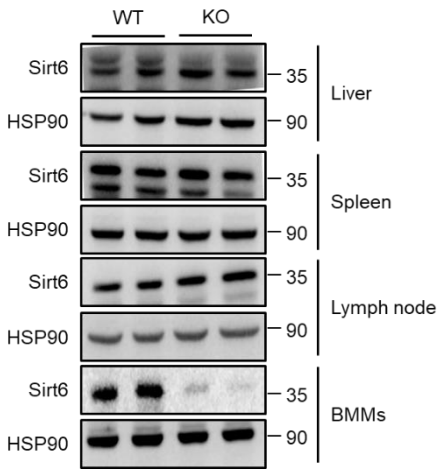

Figure S1. Generation of myeloid-specific Sirt6 KO (mS6KO) mice. Myeloid-specific deletion of Sirt6 was confirmed by Western blotting. BMMs, bone marrow macrophages.

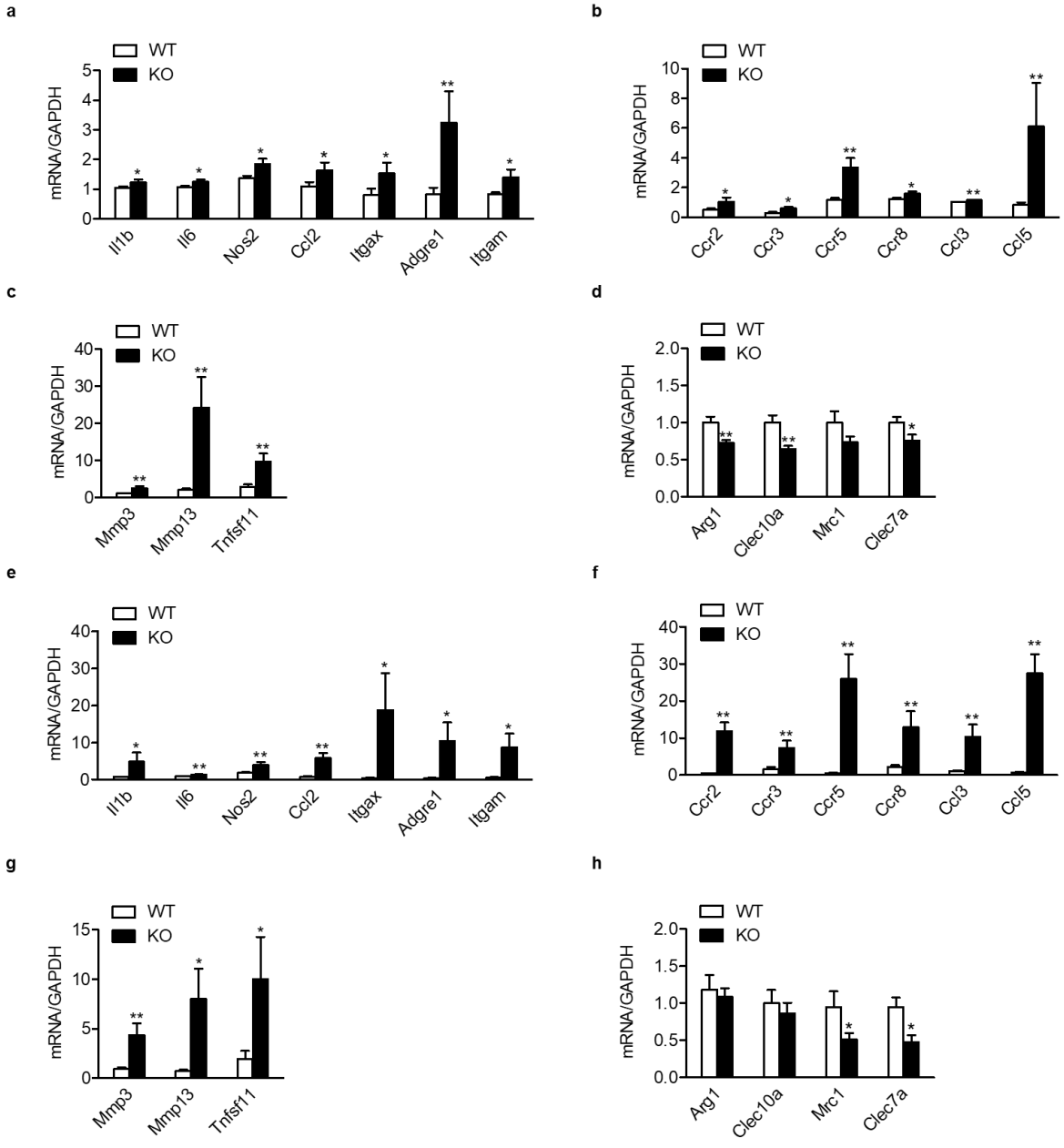

Figure S2. Effects of myeloid Sirt6 deficiency on the expression of M1/M2 macrophages- and inflammation-related genes in mS6KO mice with CIA (A-D) or K/BxN serum transfer arthritis (E-H). mRNA levels of (A, E) M1 macrophage-related genes (IL-1β, IL-6, iNOS, MCP-1, CD11c, F4/80, and CD11b), (B, F) chemokines (CCL3/5) and chemokine receptors (CCR2/3/5/8), (C, G) proteases (MMP3/13) and RANKL, (D, H) M2 macrophages-related genes in the ankle joints. Values are mean±SEM (n=10-12). *, $p<0.05$ and **, $p<0.01$ vs. WT.

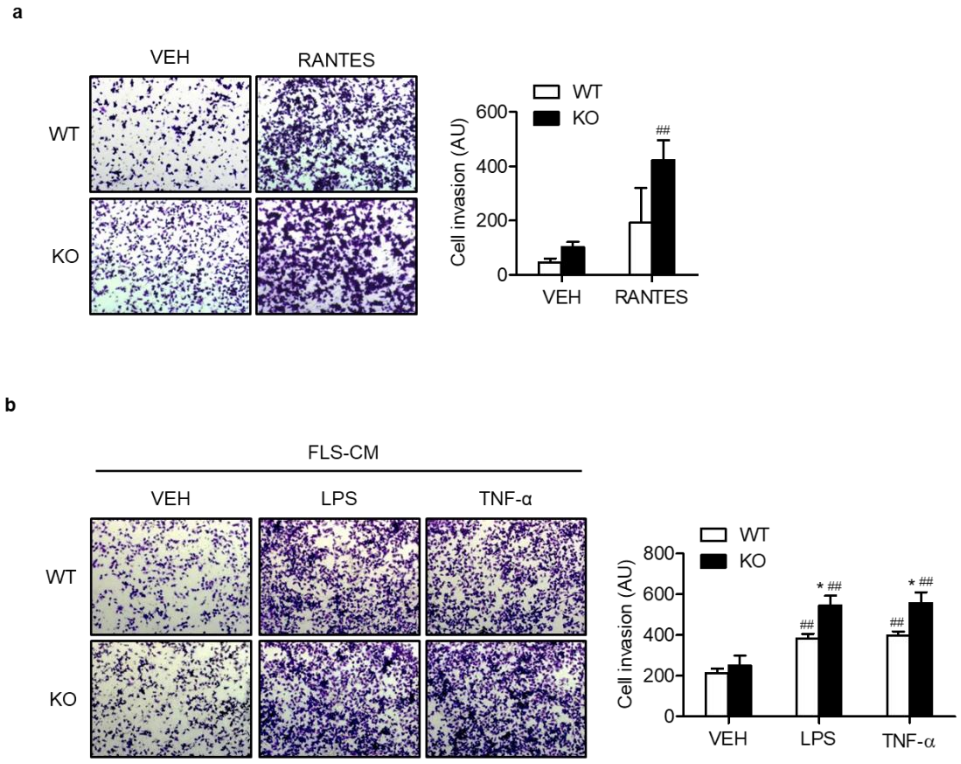

Figure S3. Regulation of macrophage invasion by Sirt6. (a, b) BMMs from WT or mS6KO mice were allowed to migrate through porous membranes for 24 h toward 20 ng/ml RANTES or fibroblast-like synoviocyte-conditioned medium (FLS-CM). Representative microphotographs (original magnification ×40). Mean number of cells in matrigel-coated trans-well chamber were counted (n=4). Values are mean±SEM. *, $p<0.05$ vs. WT; $^{\#\#}$, $p<0.01$ vs. VEH. VEH, Vehicle.

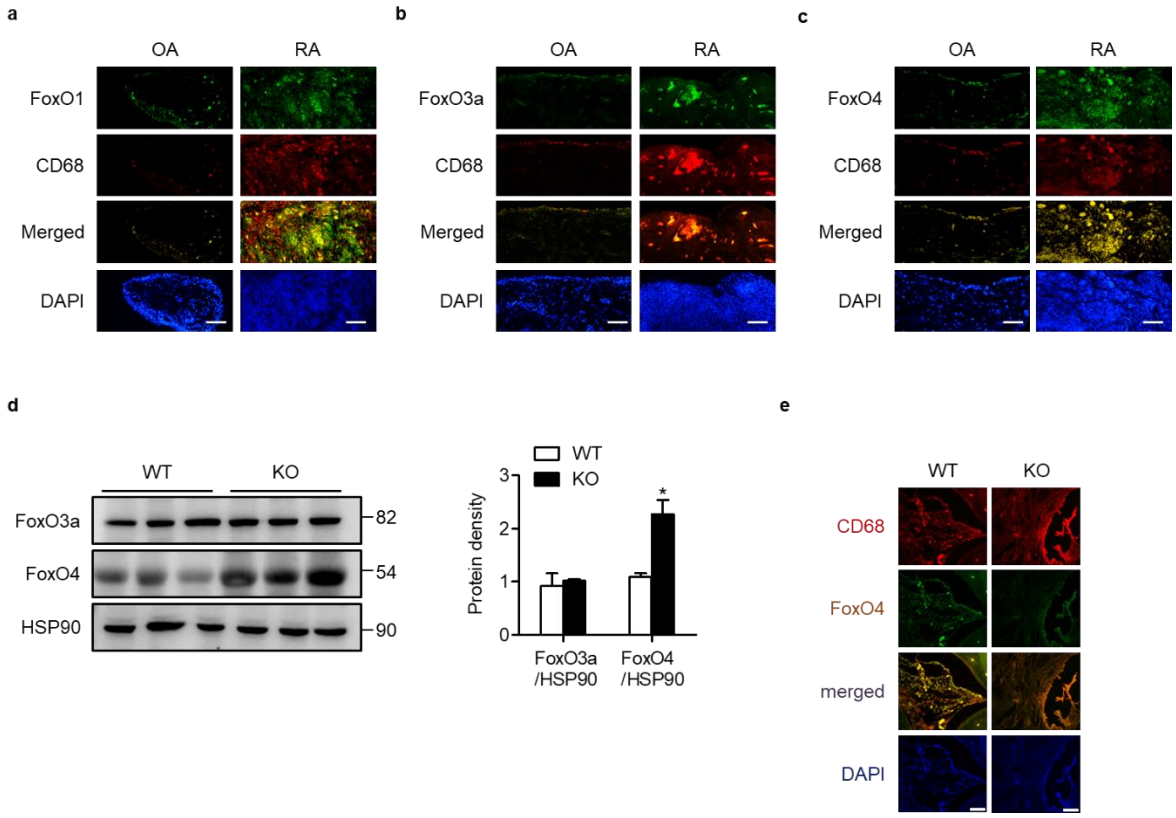

Figure S4. The expression of FoxO3a and FoxO4. **(a-c)** Staining of FoxO1, FoxO3a, and FoxO4 in the joint tissue from RA and OA patients (bar=100 μm). **(d)** Protein levels of FoxO3a and FoxO4 in the ankle joints of CIA mice (n=3). **(e)** Staining of FoxO4 and CD68 in the ankle joints of CIA mice (bar=100 μm). Values are mean±SEM. *, $p<0.05$ vs. WT.

2. Supplementary Tables

Table 1. Sequences and accession numbers for primers (forward, FOR; reverse, REV) used in real-time RT-PCR

Gene	Sequences for primers	Accession No.
Foxo1	FOR: GAGTGGATGGTGAAGAGCGT	NM_019739
	REV: TGCTGTGAAGGGACAGATTG	
Il1b	FOR: GGTCAAAGGTTTGGAAGCAG	NM_008361
	REV: TGTGAAATGCCACCTTTTGA	
Il6	FOR: CCACGGCCTTCCCTACTTC	NM_031168
	REV: TTGGGAGTGGTATCCTCTGTGA	
Nos2	FOR: TTCTGTGCTGTCCCAGTGAG	NM_010927
	REV: TGAAGAAAACCCCTTGTGCT	
Ccl2	FOR: ATTGGGATCATCTTGCTGGT	NM_011333
	REV: CCTGCTGTTCACAGTTGCC	
Itgax	FOR: CACTCAGTGACTGCCCAAAA	NM_021334
	REV: CCTCAAGACAGGACATCGCT	
Adgre1	FOR: TTTCCTCGCCTGCTTCTTC	NM_010130
	REV: CCCCGTCTCTGTATTCAACC	
Itgam	FOR: AAGGATTCAGCAAGCCAGAA	NM_008401
	REV: TAGCAGGAAAGATGGGATGG	
Ccr2	FOR: AGCACATGTGGTGAATCCAA	NM_009915
	REV:TGCCATCATAAAGGAGCCA	
Ccr3	FOR:CATAGGGTGTGGTCTCAAAGC	NM_009914
	REV:AAAGGACTTAGCAAAATTCACCA	
Ccr5	FOR: GCAGGGTGCTGACATACCAT	NM_009917
	REV:ATCCGTTCCCCCTACAAGAG	
Ccr8	FOR: GCGGTGAAGAAATCAGGGTA	NM_007720
	REV: CTCAGAAGAAAGGCTCGCTC	
Ccl3	FOR: GTGGAATCTTCCGGCTGTAG	NM_011337
	REV: ACCATGACACTCTGCAACCA	
Ccl5	FOR: CCACTTCTTCTCTGGGTTGG	NM_013653
	REV: GTGCCCACGTCAAGGAGTAT	
Mmp3	FOR: CCCCTGATGTCCTCGTGGTA	NM_010809
	REV: GCACATTGGTGATGTCTCAGGTT	
Mmp13	FOR: GGTCCTTGGAGTGATCCAGA	NM_008607
	REV: TGATGAAACCTGGACAAGCA	
Tnfsf11	FOR: CCCACAATGTGTTGCAGTTC	NM_011613
	REV: TCCTGAGACTCCATGAAAACG	
Gapdh	FOR: CGTCCCGTAGACAAAATGGT	NM_008084
	REV: TTGATGGCAACAATCTCCAC	
Arg-1	FOR: CAGAAGAATGGAAGAGTCAG	NM_007482
	REV: CAGATATGCAGGGAGTCACC	
Clec10a	FOR: ATGATGTCTGCCAGAGAACC	NM-010796
	REV: ATCACAGATTTCAGCAACCTT	
Mrc1	FOR: CTCGTGGATCTCCGTGACAC	NM_008625
	REV: GCAAATGGAGCCGTCTGTGC	
Clec-7a	FOR: AGGTTTTTCTCAGTCTTGCCTTC	NM_020008
	REV: GGGAGCAGTGTCTCTTACTTC	

Table S2. **Characteristics of the study patients**

Characteristics	OA (n=23)	RA (mild) (n=7)	RA (severe) (n=13)
Age (years)	55.4 ± 1.2	48 ± 7.2	57.9 ± 3.9
Sex	F = 22, M = 1	F = 6, M = 1	F = 12, M = 1
Disease duration (months)	35.8 ± 8.1	28.3 ± 13	40.7 ± 9.2
ESR (mm/h)	25.8± 2.9	47.9± 8.5	73± 5.7
CRP (mg/l)	0.6 ± 0.1	7.8 ± 3.2	25.2 ± 5.2
Tender joint	N/A	2.1 ± 0.5	9.5 ± 1.1
Swollen joint	N/A	1.3± 0.5	8.2± 0.9
Pain VAS (mm)	N/A	25.7± 4.3	64.6± 5.5
DAS28-ESR	N/A	4.1± 0.3	6.4± 0.2
DAS28-CRP	N/A	3.1 ± 0.2	5.4 ± 0.2
RF (%)	0	100	100
Anti-CCP (%)	0	100	100
Use of DMASRDs (%)	N/A	0	0

Data are mean±SEM. Abbreviations: RA, rheumatoid arthritis; OA, osteoarthritis; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; VAS, visual analogue scale; DAS28, disease activity score; RF, rheumatoid factor; Anti-CCP, Anti-cyclic citrullinated peptide; DMARD, disease modifying anti-rheumatic drug; N/A, not applicable.