Epigenetic regulator UHRF1 suppressively orchestrates multiple pathogenesis in rheumatoid arthritis.

Yuuki Imai (✉ y-imai@m.ehime-u.ac.jp)

Ehime University <https://orcid.org/0000-0002-6119-0034>

Noritaka Saeki

Ehime University

Kazuki Inoue

Ehime University

Maky Ideta-Otsuka

Hoshi University

Kunihiko Watamori

Ehime University

Shinichi Mizuki

Matsuyama Red Cross Hospital

Katsuto Takenaka

Ehime University

Katsuhide Igarashi

Hoshi University

Hiromasa Miura

Ehime University

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Title

Epigenetic regulator UHRF1 suppressively orchestrates multiple pathogenesis in rheumatoid arthritis.

Noritaka Saeki^{1,2}, Kazuki Inoue¹, Maky Ideta-Otsuka⁴, Kunihiro Watamori⁵, Shinichi Mizuki⁶, Katsuto Takenaka⁷, Katsuhide Igarashi^{8, 9}, Hiromasa Miura⁵, Yuuki Imai^{1, 2, 3*}

¹Division of Laboratory Animal Research, Advanced Research Support Center, Ehime University, Ehime, Japan.

²Division of Integrative Pathophysiology, Proteo-Science Center, Ehime University, Ehime, Japan.

³Department of Pathophysiology, Ehime University Graduate School of Medicine, Ehime, Japan.

⁴Laboratory of Instrumental Analysis, School of Pharmacy and Pharmaceutical Sciences, Hoshi University, Shinagawa-ku, Tokyo, Japan

⁵Department of Bone and Joint Surgery, Ehime University Graduate School of Medicine, Ehime, Japan.

⁶The Center for Rheumatic Diseases, Matsuyama Red Cross Hospital, Ehime, Japan.

⁷Department of Hematology, Clinical Immunology and Infectious Diseases, Ehime University Graduate School of Medicine, Ehime, Japan.

⁸Laboratory of Biofunctional Science, School of Pharmacy and Pharmaceutical Sciences, Hoshi University, Shinagawa-ku, Tokyo, Japan

23 ⁹Institute for Advanced Life Sciences, Hoshi University, Shinagawa-ku, Tokyo, Japan

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25 The authors have no conflict of interest to declare.

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27 *Address all correspondence to:

28 Yuuki Imai MD, PhD

29 Professor,

30 Division of Integrative Pathophysiology, Proteo-Science Center, Graduate School of

31 Medicine, Ehime University.

32 Shitsukawa, Toon, Ehime, Japan 791-0295

33 Email: y-imai@m.ehime-u.ac.jp TEL: +81-89-960-5925, FAX: +81-89-960-5953

Abstract

Rheumatoid arthritis (RA) is characterized by chronic synovial inflammation with aberrant epigenetic alterations, eventually leading to joint destruction. However, epigenetic regulatory mechanisms underlying RA pathogenesis remain largely unknown. Here, we showed that Ubiquitin-like containing PHD and RING finger domains 1 (UHRF1)¹⁻³, a key molecule involved in maintenance of DNA methylation during cell division, is a central epigenetic regulator that orchestrates the suppression of expression of multiple factors that exacerbate RA. We found that murine arthritis tissue and human RA tissue, particularly synovial fibroblasts (SF), exhibit remarkable up-regulation of expression of *Uhrf1*. SF-specific *Uhrf1* conditional knockout mice showed more severe arthritic phenotypes and apoptosis-resistant SF. Integrative analysis of the transcriptome and methylome showed that expression of several cytokines including *Ccl20* was up-regulated in *Uhrf1*-deficient SF. In RA patients, disease activity scores, *CCL20* expression, Th17 accumulation and apoptosis resistance were negatively correlated with *UHRF1* expression in synovium. Finally, stabilization of UHRF1 by Ryuvudine administration diminished disease pathogenesis in arthritis model mice. Our results demonstrated that UHRF1 expressed in SF can contribute to suppression of multiple pathogenic events associated with RA such as Th17 recruitment, SF apoptosis and bone destruction, suggesting that targeting UHRF1 could represent a novel therapeutic strategy for RA.

Main

Rheumatoid arthritis (RA) is a systemic autoimmune disease, which has heterogeneous symptoms characterized by synovium hyperplasia and joint destruction. Despite the remarkable recent progress in RA therapeutics including DMARDs and biologics that can induce disease remission for a majority of RA patients, 17-23% of patients nonetheless fail to achieve remission after these treatments ^{4,5}. Therefore, precise clarification of the molecular mechanisms underlying RA pathogenesis is needed to identify novel therapeutic targets for RA. The etiology of RA is influenced by genetic and environmental factors ^{6,7}. In the past decade, genome wide association studies (GWAS) identified several genetic risk factors in RA patients ^{8,9}. However, the spectrum of RA pathogenesis cannot be explained solely based on genetics ¹⁰, particularly given the low concordance rate (12-15%) for RA among monozygotic twins ¹¹, which suggests that epigenetic alterations induced by environmental factors can also contribute to RA pathogenesis. Indeed, several studies reported that aberrant DNA methylation accounted for progression of various chronic inflammatory diseases ¹²⁻¹⁴. DNA methylation patterns clearly differ between osteoarthritis (OA) and RA ^{15,16}, between early and late phase of RA ¹⁷ and between treatment responders and non-responders ¹⁸. RA disease-discordant monozygotic twins also have differential variability in DNA methylation patterns ^{19,20}. Together, results from these cohort studies suggest an important role for epigenetic alterations that can affect RA heterogeneity and disease pathology.

Up-regulation of Uhrf1 expression in arthritis tissue

To identify a candidate epigenetic regulator in RA pathogenesis, we conducted a microarray analysis of gene expression using mRNA obtained from whole ankle tissue from collagen antibody-induced arthritis (CAIA) mice and two control mice (Ctrl^P and Ctrl^L) (Fig.1a). Principal component analysis (PCA) showed pronounced differences in the gene expression profiles between ankle tissues from CAIA mice and both control mice (Fig.1b). Subsequent microarray analysis revealed that 6,155 probes indicated differential expression (4,049 and 2,106 probes were up- and down-regulated, respectively) in CAIA ankle compared to Ctrl^L ankle (Fig.1c, Supplementary Table 1). KEGG pathway analysis showed enrichment of inflammatory- and rheumatoid arthritis-related genes among the up-regulated probes in CAIA (Extended Data Fig.1a, b, Supplementary Table 1). A subsequent gene set enrichment analysis (GSEA) to classify differentially counted probes in terms of epigenetic regulation (Fig.1d) showed that among the classified gene set, the *Uhrf1* probe count was the most elevated in CAIA ankle compared to both control ankles (Fig.1e). Up-regulation of *Uhrf1* mRNA was found not only in CAIA ankle but also in tissue from K/BxN serum transfer arthritis (STA) mice by RT-qPCR (Fig.1f, Extended Data Fig.2a). Analyses of public databases for gene expression (GSE89408) revealed that *UHRF1* mRNA was also significantly up-regulated in synovium from RA patients compared to healthy and osteoarthritis (OA) synovia (Fig.1g). UHRF1 is an essential player for DNA methylation homeostasis through its recognition of hemi-methylated DNA and recruitment of DNMT1 to maintain DNA methylation status during DNA replication¹⁻³. The physiological functions for Uhrf1

have been reported for several cell types including lymphocytes^{21,22}, chondrocytes²³ and vascular smooth muscle cells²⁴, but its functions in synovial cells are largely unknown. To assess Uhrf1 localization in synovial tissue, we performed immunohistochemical staining of tissue sections from arthritis mice. Uhrf1 expression frequently localized in cells positive for synovial fibroblast (SF)-markers (Podoplanin (Pdpn), Fap, Thy-1, Col6a1), whereas localization of Uhrf1 was limited in macrophage marker (F4/80, LysM)-positive cells and absent in CD3-positive T cells (Fig.1h, Extended Data Fig.2b). In contrast, Uhrf1-expressing cells were seen less frequently in healthy synovium (Extended Data Fig.2c). Moreover, substantial expression of *Uhrf1* mRNA was observed in primary cultured SF and synovial macrophages (SM) derived from both mouse models of arthritis (Extended Data Fig.2d, e). Taken together, these data suggest that Uhrf1 expression levels are dominantly increased in SF rather than SM during arthritis pathogenesis.

Synovial fibroblast-specific deletion of *Uhrf1* exacerbates arthritis pathogenesis

To understand physiological functions of Uhrf1 under arthritis conditions, we next established SF-specific *Uhrf1* conditional knockout mice (*Uhrf1*^{ΔCol6a1}). Under normal conditions, the body size of *Uhrf1*^{ΔCol6a1} mice was smaller than that for littermate control (*Uhrf1*^{fl/fl}) mice, although pathological hallmarks such as inflammation were not observed in several different tissues that were tested (Extended Data Fig.3a-c). Arthritis was induced in these mice using two different methods and development of hind paw swelling was monitored for 10 days. Measured swelling and clinical score for

hind paws were more severe in *Uhrf1*^{ΔCol6a1} mice than *Uhrf1*^{fl/fl} mice (Fig.2a, b).

Morphological analyses showed that hyperplasia of synovium as well as cartilage and bone destruction were also more severe in *Uhrf1*^{ΔCol6a1} mice than in *Uhrf1*^{fl/fl} mice (Fig.2c, d, Extended Data Fig.4a-e). Given the detectable presence of Uhrf1 expression in SM and a previous report that Tnf-α expression is regulated by Uhrf1 in macrophages²⁵, we also established macrophage-specific *Uhrf1* conditional knockout mice (*Uhrf1*^{ΔLysM}) (Fig. 1h, Extended Data Fig.2d, e). The *Uhrf1*^{ΔLysM} mice exhibited no notable phenotypes under either normal conditions or arthritis pathogenesis (Extended Data Fig.5a-e). Uhrf1 deficiency has also been reported to affect cell-cycle and/or apoptosis in certain cell types²⁶⁻²⁹. Histologically, we saw no difference in proliferative cell populations of Pdpn⁺ SF between *Uhrf1*^{ΔCol6a1} and *Uhrf1*^{fl/fl} mice (Extended Data Fig.6a), whereas the number of apoptotic Pdpn⁺ SF was significantly reduced in hyperplastic synovium of *Uhrf1*^{ΔCol6a1} mice compared to *Uhrf1*^{fl/fl} mice (Fig.2e). To confirm this observation, we isolated primary SF from arthritis tissue (Fig.2g) and carried out cell proliferation and apoptosis analyses *in vitro*. Although the BrdU incorporation rate was comparable between *Uhrf1*^{ΔCol6a1} and *Uhrf1*^{fl/fl} SF indicating a similar proliferation rate (Extended Data Fig.6b, c), primary SF derived from *Uhrf1*^{ΔCol6a1} mice were more resistant to apoptosis than those from *Uhrf1*^{fl/fl} mice (Fig.2f).

Collectively, these data demonstrate that Uhrf1 expressed in SF, but not in SM, plays a role in suppressing arthritis pathogenesis through negative feedback mechanisms associated with various arthritis pathologies.

Uhrf1 regulates mRNA expression of multiple RA-exacerbating factors in synovial fibroblasts

Previous reports indicated that Uhrf1 could regulate gene expression genome-wide by regulating DNA methylation^{23,30}. To reveal Uhrf1-dependent changes in gene expression, we performed RNA-seq analysis using SF obtained from *Uhrf1^{fl/fl}* and *Uhrf1^{ΔCol6a1}* mice on day 4 in STA model (Fig.3a, b). PCA and hierarchical clustering analyses showed an apparently different gene expression profile between SF isolated from *Uhrf1^{fl/fl}* and *Uhrf1^{ΔCol6a1}* mice (Fig.3c, Extended Data Fig.7a). Subsequent expression analysis visualized with volcano plots indicated that there were more genes with up-regulated expression than those with down-regulated expression in SF from *Uhrf1^{ΔCol6a1}* mice versus those from *Uhrf1^{fl/fl}* mice (171 genes up-regulated and 89 genes down-regulated) (Fig.3d, Supplementary Table 2). KEGG pathway enrichment analyses revealed that the top two pathways for up-regulated genes were “Rheumatoid arthritis” and “Cytokine-cytokine receptor interaction” (Extended Data Fig.7b, d, Supplementary Table 2). Gene Ontology (GO) analyses had significant enrichment in the biological process termed “negative regulation of apoptotic process” among the up-regulated genes (Extended Data Fig.7c, e, Supplementary Table 2). Recent studies suggested that biological functions of Uhrf1 could be cell type-dependent²¹⁻²³. To test this hypothesis, we reanalyzed public RNA-seq databases (GSE92641, GSE85450) to compare changes in expression of genes that were affected by *Uhrf1* depletion in SF and other cell types. Differentially-expressed genes seen in the presence of *Uhrf1* depletion showed no marked overlap among all cell types considered (Fig.3e,

Extended Data Fig.7f, Supplementary Table 3), suggesting that there is indeed cellular specificity in regulation of gene expression by Uhrf1. We further analyzed DNA methylation status in SF by carrying out next-generation sequencing (MBD-seq) on methylated DNA enriched from the whole genome using methyl-CpG-binding domain protein 2 (MBD2) beads. To identify Uhrf1-mediated methylated DNA loci in SF, we performed peak calling using MACS14 with the locus of down-regulated methylation level as peaks. This analysis identified 18,649 Uhrf1-mediated peaks. Cis-regulatory element annotation system (CEAS) analysis showed that the distribution of methylated DNA peaks was altered in *Uhrf1*^{ΔCol6a1} SF against the genome background despite the presence of a nearly commensurate proportion of methylated DNA in the genome (Fig.3f, g). Moreover, the Uhrf1-mediated methylated DNA peak locus between SF and chondrocytes (GSE99335) largely did not overlap, supporting the cellular specificity of Uhrf1 function (Fig.3h). To identify genes targeted by Uhrf1 in SF, we investigated whether Uhrf1-mediated peaks localized at the region surrounding differentially expressed genes in *Uhrf1*^{ΔCol6a1} SF. Among the 171 genes showing Uhrf1-dependent up-regulation, 105 genes had peaks in the TSS (transcriptional start site) ± 50 kb (Fig.3i, k, Supplementary Table 2). These 105 genes were highly enriched in KEGG pathways termed “Cytokine-cytokine receptor interaction” and “Rheumatoid arthritis” and included 8 individual genes (Fig.3j, k Supplementary Table 2). GO biological process showed a tendency for these genes to be enriched in “negative regulation of apoptotic process”, although this enrichment was not statistically significant (Extended Data Fig.8a, Supplementary Table 2). We validated that *Uhrf1* deficiency altered

expression of these genes, including *Ccl20*, *Tnfsf11* (Fig.3l) and others (Extended Data Fig.8b, c) by RT-qPCR. In addition, among the 89 down-regulated genes, 39 had peaks in the gene body. Even though none of these genes was enriched in biological process, they may nonetheless contribute to RA pathogenesis (Extended Data Fig.8d, e, Supplementary Table 2). Notably, serum levels of the chemokine Ccl20 were significantly increased in *Uhrf1*^{ΔCol6a1} mice compared with *Uhrf1*^{fl/fl} mice in late-phase STA (Fig.3m). Ccl20 binds to its unique receptor, Ccr6, to recruit Th17 cells that have important roles in progression of autoimmune disease³¹⁻³³. Flow cytometry analysis showed significantly increased recruitment of Th17 cells in arthritis tissue from *Uhrf1*^{ΔCol6a1} mice in the late, but not early, phase of STA (Fig.3n). These data suggest that Uhrf1 orchestrates mRNA expression through control of DNA methylation status of loci for genes that encode multiple exacerbating factors, including Ccl20, that are derived from SF during arthritis pathogenesis.

UHRF1 suppresses several processes in RA pathogenesis

To translate our findings to human RA pathogenesis, we next examined the significance of UHRF1 in RA patients. We collected synovium specimens from patients with OA or RA. RT-qPCR analysis revealed that *UHRF1* mRNA expression levels were significantly elevated in RA synovium relative to those for OA, although the expression level was highly variable among the RA patients (Fig.4a). Meanwhile, mRNA expression levels of DNA methyltransferases (*DNMT1*, *DNMT3A* and *DNMT3B*) were similar between OA and RA samples, which is consistent with a previous report³⁴

(Extended Data Fig.9a), and suggests that RA-specific aberrant DNA methylation and/or RA heterogeneity of disease severity are dependent on UHRF1 expression level. Immunohistochemical staining detected UHRF1 expression in RA synovial fibroblasts (RASf), but not in samples from OA patients (Fig.4b). Several clinical parameters including swollen joint count 28 (SJC28), matrix metalloproteinase-3 (MMP3) and C-reactive protein (CRP) were negatively correlated with *UHRF1* mRNA expression levels in RA synovium, whereas there were no correlations with levels of *DNMT1*, *DNMT3A* and *DNMT3B* (Fig.4c, Extended Data Fig.9b-e). To test whether UHRF1 regulates expression of notable genes including cytokine- and RA-related pathways in both murine SF and RASf, *UHRF1* knockdown was performed in OASF and RASf. *UHRF1* mRNA suppression was significantly associated with up-regulation of *CCL20* mRNA expression in RASf but not OASF (Fig.4d, Extended Data Fig.9f). A negative correlation of mRNA expression was also found between *UHRF1* and *CCL20* in RA synovium tissue, supporting the regulation of *CCL20* expression by UHRF1 (Extended Data Fig.9g). Focusing on *CCL20*, we used flow cytometry to examine the proportion of Th17 cells in RA and OA synovia. Although we observed no large difference in the population of other leukocytes between OA synovium and RA synovium samples, the population of Th17 cells was elevated in RA (Fig.4e, Extended Data Fig.9h). To confirm that accumulation of Th17 cells is regulated by UHRF1 expression in human SF, we then investigated the correlation between the frequency of Th17 cells and *UHRF1* mRNA expression in SF in synovium samples from the same patients. We found that the Th17 frequency was indeed negatively correlated with

UHRF1 mRNA expression levels in human SF (Fig.4f). In addition, consecutive knockdown of *UHRF1* mRNA resulted in the resistance to FAS-induced apoptosis in RASF (Extended Data Fig.10a-c), similarly to that seen for murine SF lacking *Uhrf1* (Fig.2f). Collectively, these data demonstrate that UHRF1 suppressively affects arthritis pathogenesis by regulating Th17 recruitment and apoptosis of synovial fibroblasts both in murine models of arthritis and in human RA.

***Uhrf1* stabilization ameliorates arthritis pathogenesis**

The abovementioned results demonstrate that preservation of UHRF1 expression could attenuate RA pathogenesis. Although the precise molecular mechanisms underlying degradation of UHRF1 are largely unclear, a recent study reported that methylation of UHRF1 protein by SET8 (also called SETD8, PR-SET7 and KMT5A) promotes ubiquitination-dependent protein degradation of UHRF1³⁵. In addition, SET8 inhibitors (UNC0379, NSC663284, BVT948 and Ryuvidine) are reported to reduce methylation levels of other proteins^{36,37}. Thus, we initially assessed whether these inhibitors can stabilize UHRF1 protein levels using HEK293 cells. Cell cycle synchronization revealed that UHRF1 was the protein that showed the greatest degree of stabilization following Ryuvidine treatment in the G2/M phase, which is known as the UHRF1 degradation phase³⁵ (Fig.4g). We also administered Ryuvidine to STA model mice (Fig.4h). Immunofluorescent staining of tissue samples showed that sustainable *Uhrf1* expression was achieved by Ryuvidine treatment *in vivo* (Fig.4i). Ryuvidine treatment alleviated arthritis phenotypes in STA model mice compared to mice treated

with DMSO (Fig.4j, k) and also reduced histological pathology and Ccl20 serum levels (Fig.4l, m). These results collectively indicate that stabilization of Uhrf1 protein is a potential therapeutic strategy for RA.

Discussion

Epigenetic alterations have potential to be a mechanism that promotes RA heterogeneity and treatments that target proteins involved in epigenetic changes could be a novel therapeutic strategy for patients with RA, particularly those who do not respond to current treatments ^{38,39}. In this study, we identified UHRF1 as a central epigenetic regulator of DNA methylation that can suppressively modulate multiple exacerbating factors of RA (Extended Data Fig.10). Our integrative analyses of the transcriptome and methylome of synovial tissue from a mouse model of RA and patients with RA showed that CCL20 is a common UHRF1 target gene among cytokine- and RA-related genes. However, a role for other genes (*CSF3*, *TNFSF11*, *CCL5*, *TNFRSF9*, *IL2RB*, *IL12RB1* and *ACP5*) was not validated in RASF (Data not shown). These data indicate that regulation of the expression of specific gene(s) by UHRF1 is dependent on species and/or arthritis types. Intriguingly, expression levels of UHRF1, but not DNMTs, were diverse and negatively correlated with disease scores in RA patients, suggesting that UHRF1 could also serve as a biomarker for disease severity and/or one of the criteria for RA heterogeneity. Our finding that Ryuvudine treatment ameliorated arthritis provides support for the ability of UHRF1 stabilization to inhibit expression of multiple exacerbating factors in RA. These findings could

276 contribute to a basis for exploration of novel alternative therapeutic approaches,
277 especially for those patients who do not respond to existing treatments.

278 **Methods**

279 **Antibodies**

280 The primary antibodies used in this study include: mouse monoclonal antibody against
281 human and mouse UHRF1 (Santa Cruz, USA, Cat. No. sc-373750); rat monoclonal
282 antibody against mouse Podoplanin (mPdpn; Wako, Japan, Cat. No. 015-24111);
283 mouse monoclonal antibody against human PODOPLANIN (hPDPN; BioLegend, USA,
284 Cat. No. 916606); rat monoclonal antibody against mouse Fap (mFap; R&D systems,
285 USA, Cat. No. MAB9727); rabbit polyclonal antibody against human FAP (hFAP; Bioss,
286 USA, Cat. No. bs-5758R); rabbit monoclonal antibody against human and mouse Thy-
287 1 (Cell Signaling Technology, USA, Cat. No. 13801); rabbit monoclonal antibody
288 against mouse F4/80 (Cell Signaling Technology, Cat. No. 70076); rabbit polyclonal
289 antibody against human and mouse CD3 (Sigma, USA, Cat. No. C7930); rabbit
290 polyclonal antibody against GPF (Cell Signaling Technology, Cat. No. 598); rabbit
291 polyclonal antibody against human and mouse cleaved caspase-3 (Cell Signaling
292 Technology, Cat. No. 9661); rabbit monoclonal antibody against human and mouse Ki-
293 67 (Abcam, UK, Cat. No. ab16667); and mouse monoclonal antibody against human
294 and mouse β -actin (MBL, Japan, Cat. No. M177-3). The secondary antibodies used
295 include: Alexa Fluor488-conjugated goat anti-rat IgG, Alexa Fluor488-conjugated goat
296 anti-rabbit IgG, Alexa Fluor568-conjugated goat anti-mouse IgG1, Alexa Fluor568-
297 conjugated goat anti-rat IgG and Alexa Fluor568-conjugated goat anti-rabbit IgG
298 (Molecular Probes, USA); and HRP-conjugated goat anti-mouse IgG (DAKO,
299 Denmark). Flow cytometry antibodies used include: FITC-conjugated rat antibody

against mouse CD45 (BioLegend, 30-F11); PE-conjugated rat antibody against mouse CD4 (BioLegend, GK1.5); Alexa Fluor647-conjugated rat antibody against mouse Ccr6 (BD Biosciences, USA, 140706); APC-conjugated mouse antibody against human CD45 (Miltenyi Biotec, Germany, 5B1); FITC-conjugated mouse antibody against human CD4 (BioLegend, OKT4); and PE-conjugated mouse antibody against human CCR6 (BioLegend, G034E3). Mouse monoclonal antibody against human FAS (MBL, CH-11, Cat. No. SY-001) was used to induce functional apoptosis.

Human synovial specimens

Human synovial specimens were obtained from patients with osteoarthritis (OA) or rheumatoid arthritis (RA) who underwent knee joint replacement surgery at the Ehime University Hospital and Matsuyama Red Cross Hospital. For histological analysis, synovial tissues were fixed with 4% PFA for 6-8 hours and then embedded in paraffin. To obtain human SF from OA and RA patients (OASF and RASF), synovial tissues were minced and treated with 1 mg/mL collagenase type IV (Sigma) in Dulbecco's Minimum Essential Medium GlutaMax (DMEM GlutaMax, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Sigma) and 1% antibiotic–antimycotic solution (anti-anti, Gibco) for 6-8 hours before filtration through a 40 µm cell strainer (Falcon). Filtered cells were seeded in culture dishes and the most adherent cells were considered to be OASF and RASF. Human SF were used within passage 5. Human SF were cultured in DMEM GlutaMax supplemented with 10% FBS and 1% anti-anti solution and cultured at 37 °C in a humidified atmosphere of 5% CO₂. All protocols to

collect synovial tissues were approved by the Institutional Review Board. All patients provided informed written consent to participate in the study.

Mice

Uhrf1 mutant knockout-first mice (B6Dnk;B6N-Uhrf1^{tm1a(EUCOMM)Wtsi/leg}) were obtained from The European Mouse Mutant Archive (EMMA). ACTB-Flpe mice, R26^{NZG} mice (FVB.Cg-*Gt(ROSA)26Sor*^{tm1(CAG-lacZ,-EGFP)Gih/J}) and *LysMCre* (B6.129P2-Lyz2^{tm1(cre)lfo/J}) mice were obtained from The Jackson Laboratory (USA). *Col6a1Cre* mice⁴⁰ (B6. Cg-Tg(Col6a1-cre) 1Gkl/Fimg) were kindly provided by Prof. George Kollias (Biomedical Sciences Research Centre, Greece). KRN mice⁴¹ were kindly provided by Prof. Christophe Benoist and Prof. Diane Mathis at Harvard Medical School. C57BL/6 (WT) and NOD/ShiJcl mice were obtained from CLEA Japan.

To generate Uhrf1 floxed mice (*Uhrf1*^{f/f}), knockout-first mice were crossed with ACTB-Flpe mice. *Uhrf1*^{f/f} mice were crossed with Cre mice to generate *Col6a1Cre; Uhrf1*^{f/f} (*Uhrf1*^{ΔCol6a1}) mice and *LysMCre; Uhrf1*^{f/f} (*Uhrf1*^{ΔLysM}) mice, respectively. To generate cell type-specific reporter mice, R26^{NZG} mice were crossed with *Col6a1Cre* mice and *LysMCre* mice, respectively. To generate K/BxN mice, KRN mice, which were backcrossed with C57BL/6 mice, were crossed with NOD/ShiJcl mice. All mice were housed in a specific-pathogen-free facility under climate-controlled conditions with a 12-hour light/dark cycle, and were provided with water and standard diet (MF, Oriental Yeast, Japan) *ad libitum*. Experiments involving animals were approved by the Animal

Experiment Committee of Ehime University and were performed in accordance with Ehime University Guidelines for Animal Experiments.

Arthritis model mice studies

At postnatal 7 weeks, female mice were subjected to collagen antibody-induced arthritis (CAIA) or K/BxN serum transfer arthritis (STA). CAIA induction was conducted as previously described⁴². Briefly, 5 mg anti-collagen 2 monoclonal antibody cocktail (Chondrex, USA, Redmond) was administered on day 0, followed by 50 µg LPS *i.p.* on day 3. For STA induction, 50 µL K/BxN serum was administered *i.p.* on days 0 and 3. STA mice were *i.p.* administered on days 1, 2, 4 and 5 a single dose of 0.8 µg/g Ryuvidine in DMSO and corn oil (16 µL/g body weight). We monitored the development of swelling by measuring hind paw thickness (ratio of average increased thickness of both hind paws) for 10 days and assigned clinical score: 0, no swelling; 1, mild swelling of the tarsals or ankle; 2, mild swelling of both the tarsals and ankle; 3, moderate swelling of tarsals and ankle; 4, severe swelling of tarsals and ankle (the sum score of both hind paws). For histological analysis, mice were anesthetized and then rapidly euthanized with reflux flow of PBS. Ankle tissues were obtained and fixed overnight with 4% PFA, followed by decalcification with 0.5 M EDTA for 2 weeks. The samples were embedded in paraffin after dehydration and 6-7 µm-thick paraffin sections were cut with a microtome (RM2255, Leica Biosystems, Germany). The sections were deparaffinized and used for safranin O-fast green-hematoxylin staining and tartrate resistant acid phosphatase (TRAP) staining (TRAP staining kit, Wako).

366

367 **Murine synovial cell studies**

368 Primary cultures of synovial fibroblasts (SF) and synovial macrophages (SM) were
369 obtained from swollen ankle tissues from CAIA and K/BxN STA mice, respectively, as
370 previously described ⁴². Briefly, mice on 4 and 10 days after arthritis induction were
371 removed blood by reflux flow of PBS under anesthesia, and then swollen ankles were
372 harvested by dislocation and treated with 1 mg/mL collagenase type IV (Sigma) in
373 DMEM GlutaMax supplemented with 10% FBS and 1% anti-anti for 1-2 hours with
374 shaking before filtration with a 40 µm cell strainer (Falcon). To obtain SF, filtered cells
375 were cultured for 1 hour on a culture dish pre-coated with collagen (Type I-C, Nitta
376 gelatin, Japan) in DMEM GlutaMax supplemented with 10% FBS and 1% anti-anti
377 solution. Non-adherent cells were then removed. To obtain SM, filtered bulk cells were
378 cultured for 1-2 weeks before fibroblastic cells were gently detached by trypsin
379 treatment. Cells remaining on the dish were used as SM. Primary SF and SM were
380 cultured in DMEM GlutaMax supplemented with 10% FBS and 1% anti-anti solution
381 before use in some experiments. The cells used for experiments were from passages
382 0-3. All cells were cultured at 37 °C in a humidified atmosphere of 5% CO₂.
383 Primary SF were seeded in 96-well plates at 1 x 10⁴ cells/well. To test the rate of cellular
384 proliferation, a BrdU assay was performed using a cell proliferation ELISA kit (Roche
385 Molecular Biochemicals, Germany). BrdU solution was added and cells were incubated
386 for additional 2 hours at 37 °C. After fixation of cells, BrdU incorporation was measured
387 according to the manufacturer's instructions. To assess apoptosis, cells were treated

with or without 25 ng/mL Tnf- α and 0.5 μ g/mL cycloheximide (CHX) for 8 hours. After fixation, the cells were stained with Alexa Fluor488-conjugated phalloidin (Thermo Fisher, USA) and DAPI for 30 min at room temperature. The number of nuclei per field was automatically counted using ImageJ. To calculate the percentage of living cells, the number of nuclei in the treated cells was divided by that for vehicle-treated cells. Immunocytochemical staining was performed as previously described⁴². Briefly, cells were fixed with 4% PFA for 5-10 min and then permeabilized with 0.5% Triton X-PBS for 5 min before blocking with 1%BSA-0.02% Triton X-PBS. Primary antibodies were added at 1:100 (anti-Uhrf1, mPdpn) and incubated for 1 hour at room temperature. After washing, secondary antibodies were incubated with 5 μ g/mL DAPI for 30 min at room temperature.

Immunofluorescent staining

Immunofluorescent staining was performed as previously described^{23,42}. Briefly, deparaffinized 4-5 μ m-thick sections were boiled at 85-90 °C for 60 min with 0.05% citraconic acid solution (ImmunoSaver; Wako) to retrieve antigens. After blocking for 60 min (Blocking One Histo, Nakalai Tesque, Japan), the sections were incubated overnight at 4 °C with primary antibodies diluted in immune reaction enhancer solution (Can get signal, Toyobo, Japan) at 1:50 (anti-Uhrf1), 1:100 (anti-mPdpn, hPDPN, mFap, hFAP, Thy-1, F4/80, CD3, cleaved-caspase-3, Ki67) or 1:500 (anti-GFP). After washing with PBS, 5 μ g/mL secondary antibodies with DAPI were reacted for 60 min at room temperature.

410

411 **Flow cytometry**

412 To produce single cell suspensions, murine ankle tissue and human synovia samples
413 were digested with collagenase type IV. Bulk cells were stored at -80 °C before use.

414 Thawed cell suspensions were seeded in culture dishes and pre-incubated in medium
415 overnight at 37 °C. Non-adherent cells were used for flow cytometry analysis. Murine

416 cells were stained with anti-CD45, CD4, Ccr6 and 7-AAD (7-Amino-Actinomycin D;

417 Thermo Fisher). Human cells were stained with anti-CD45, CD4, CCR6 and 7-AAD.

418 Expression of cell surface markers were evaluated using FACS Aria (BD Biosciences)

419 and Gallios (Beckman Coulter, USA) instruments. Data were analyzed using FlowJo

420 software (Treestar Inc., USA).

421

422 **Cell cycle synchronization**

423 UNC0379, NSC663284, BVT948 and Ryuvudine were purchased from Cayman

424 Chemical (USA) as candidate chemical agents that stabilize UHRF1 protein. For

425 synchronization at the G1/S border, HEK293 cells were treated with 1 µg/mL

426 aphidicolin for 6 hours followed by treatment with 1 µg/mL aphidicolin with or without 1

427 µM of the indicated chemical agent for 16 hours. For synchronization in the G2/M

428 phase, cells were treated with 50 ng/mL nocodazole with or without 1 µM of the

429 indicated chemical agent for 16 hours.

430

431 **Real time RT-PCR**

Total RNA was extracted with Isogen (Nippon gene, Japan) and RNeasy spin column kits (Qiagen, USA). First-strand cDNA was synthesized from the total RNA using PrimeScript RT Master Mix (Takara Bio, Japan) and subjected to real time RT-PCR using TB Green Premix Ex Taq II (Takara Bio) with Thermal Cycler Dice (Takara Bio, Inc.) according to the manufacturer's instructions. Gene expression levels were normalized relative to those of the housekeeping gene *RPLP0* (*Rplp0*). Primer sequences for each gene are listed in Supplemental Table 4.

Western blotting

Cells were washed with PBS and dissolved in RIPA buffer with protease inhibitor cocktail (Nakalai Tesque). Whole cell extracts were separated by SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes, which were blocked with 3% BSA in TBS with 0.5% skim milk and 0.05% Triton X-100 (TBST). The membranes were then incubated with anti-Uhrf1 antibody and anti- β -actin antibody overnight at 4 °C. After washing with TBST, HRP-conjugated secondary antibody (1:5000) was bound for 1 hour at room temperature. Immunoreactive signals were detected with ECL prime (GE Healthcare, USA) and an ImageQuant LAS 4000 instrument (GE Healthcare).

siRNA experiments

siRNA specific for UHRF1 was purchased from Thermo Fisher Scientific. The sequence for the siRNA construct targeting UHRF1 gene is: UHRF1-1 (5'-

CUGCUUUGCUCCCAUCAAU-3'), UHRF1-2 (5'-GCCAUACCCUCUUCGACUA-3').
MISSION siRNA Universal Negative Control (Sigma) was used as a control siRNA. To
analyze gene expression in OASF and RASF, 5×10^4 cells were transfected with 2
pmol siRNA using an electroporation apparatus (Neon, Invitrogen, USA) as previously
described²³. Cells were used 48 hours after transfection. To analyze apoptosis
resistance, 1×10^4 cells/cm² cells were transfected with 3 pmol siRNA using
Lipofectamine RNAiMax (Thermo Fisher Scientific) according to the manufacturer's
instructions. On day 3 after the first transfection, siRNA was re-transfected and the
cells were then cultured for another 2 days. The transfected cells were re-seeded in 96-
well plates ($2.5\text{--}3.0 \times 10^3$ cells/well) and one day later were incubated with 0.5 µg/mL
anti-FAS antibody for 16 hours to induce functional apoptosis. After fixation, the cells
were stained with Alexa Fluor488-conjugated phalloidin (Thermo Fisher) and DAPI for
20 min at room temperature. The number of nuclei per field was automatically counted
using ImageJ. To calculate the percentage of living cells, the number of nuclei seen for
treated cells was divided by the number of nuclei seen for vehicle-control cells.

ELISA

Ccl20 protein concentration in mice serum was measured using Mouse Ccl20 ELISA
kit (R&D Systems). Absorbance at 450 nm was measured using a FluxStation3
(Molecular Devices, USA) according to the manufacturer's instructions.

Microarray analysis

Total RNA was extracted from whole ankle tissue using Isogen and an RNeasy Mini kit (Qiagen, USA). The total RNA was used to generate cRNA according to the GeneChip (Thermo Fisher) protocol. After reverse transcription by SuperScript II (Invitrogen) and conversion into double-stranded cDNA, a MinElute Reaction Cleanup Kit (Qiagen) was used for purification. The purified double-stranded cDNA was transcribed and labeled *in vitro* using a BioArray HighYield RNA Transcript Labeling Kit (Enzo Life Sciences, USA). The labeled cRNA was then purified using an RNeasy Mini kit (Qiagen). The purified cRNAs were hybridized to GeneChip Mouse Genome 430 2.0 arrays and washed and stained in a GeneChip Fluidics Station. The phycoerythrin-stained arrays were scanned to obtain digital image files, which were then analyzed using GeneChip Operating Software (Affymetrix, USA).

RNA-seq analysis

Murine SF were isolated from swollen ankle tissue on 4 days after K/BxN STA induction. After culturing for one day, high quality total RNA was obtained from the SF using RNeasy spin column kits and verified using an Agilent 2100 Bioanalyzer (Agilent Technologies, USA). RNA-seq analysis was performed as previously described^{23,42}. RNA sequence libraries were prepared using an Illumina TruSeq Stranded mRNA LT Sample Prep kit (Illumina, USA) according to the manufacturer's instructions. The libraries were subsequently validated for an average size of ~311-328 bp using a 2100 Bioanalyzer and an Agilent DNA1000 kit. Sequencing of paired-end reads (75 bp) was performed with a MiSeq Reagent kit V3 150 cycle on a MiSeq system (Illumina).

Sequence data were mapped on the mouse genome (mm10) using TopHat⁴³ and analyzed using Cufflinks⁴⁴.

MBD-seq analysis

MBD-seq was performed to analyze genome-wide methylated and/or non-methylated DNA regions as previously described²³. Briefly, methylated DNA was enriched by methyl-CpG-binding domain protein 2 (MBD2)-mediated precipitation and subjected to next-generation sequencing. Highly methylated DNA regions were identified by sequence reads mapped on the reference genome. Extracted DNA from murine SF was sonicated with a Covaris sonicator to obtain ~300 bp fragments. MBD2-mediated enrichment of methylated DNA was performed using the methylated DNA enrichment kit EpiXplore (Takara Bio) according to the manufacturer's instructions. The amount of enriched methylated DNA in 1 µg total DNA was measured using a Quantus Fluorometer (Promega, USA). Libraries for MBD-seq analysis were prepared using a QIAseq Ultralow Input Library Kit (Qiagen) according to the manufacturer's instructions and validated for an average size of ~300-700 bp using a TapeStation and the Agilent High Sensitivity D1000 ScreenTape kit. Each experiment was biologically replicated at least three times. Sequencing of paired-end reads (75 bp) was performed using the MiSeq Reagent kit V3 150 cycle on a MiSeq system (Illumina) and mapped on the mouse genome (mm10) using CLC Genomics Workbench (QIAGEN).

Analysis of sequencing data

Differentially expressed genes having expression levels that were significantly increased or decreased by more/less than twice/half that of the control, were extracted for further analyses. Hierarchical cluster analysis and principal-component analysis (PCA) were carried out using MeV ⁴⁵ and Gene Ontology analyses were performed DAVID Bioinformatics Resources 6.8 ⁴⁶ and Gene Set Enrichment Analysis (GSEA) ⁴⁷. For MBD-seq, peak calling was performed by MACS14 ⁴⁸ and integrative analyses were done using Cistrome Analysis Pipeline (<http://cistrome.org/ap/>) as previously described.

Statistical analysis

Two-tailed unpaired Student's *t*-test with Prism 8 was used to analyze differences between two groups. ANOVA followed by post-hoc Tukey's test with Prism 8 and SPSS (IBM) was applied to compare multiple groups. For all graphs, data are represented as the mean ± standard deviation. Statistical significance was accepted when $P < 0.05$.

Data availability

Data sets from the Microarray, RNA-seq and MBD-seq were deposited in the NCBI Gene Expression Omnibus under accession numbers GSE167190, GSE166746 and GSE166747 respectively.

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Author information

Affiliations

Division of Laboratory Animal Research, Advanced Research Support Center, Ehime University, Ehime, Japan.

Noritaka Saeki, Kazuki Inoue & Yuuki Imai

Division of Integrative Pathophysiology, Proteo-Science Center, Ehime University, Ehime, Japan.

Noritaka Saeki & Yuuki Imai

Department of Pathophysiology, Ehime University Graduate School of Medicine, Ehime, Japan.

563 Yuuki Imai
564
565 Laboratory of Biofunctional Science, School of Pharmacy and Pharmaceutical
566 Sciences, Hoshi University, Tokyo, Japan
567 Katsuhide Igarashi
568
569 Institute for Advanced Life Sciences, Hoshi University, Shinagawa-ku, Tokyo, Japan
570 Katsuhide Igarashi
571
572 Laboratory of Instrumental Analysis, School of Pharmacy and Pharmaceutical
573 Sciences, Hoshi University, Shinagawa-ku, Tokyo, Japan
574 Maky Ideta-Otsuka
575
576 Department of Bone and Joint Surgery, Ehime University Graduate School of Medicine,
577 Ehime, Japan.
578 Watamori Kunihiro & Hiromasa Miura
579
580 The Center for Rheumatic Diseases, Matsuyama Red Cross Hospital, Ehime, Japan.
581 Shinichi Mizuki
582
583 Department of Hematology, Clinical Immunology and Infectious Diseases, Ehime
584 University Graduate School of Medicine, Ehime, Japan.

Katsuto Takenaka

Contributions

NS, KI and YI planned the study and designed the experiments. NS, KI, MO and KI generated the microarrays, and NS, MO and KI performed MBD-seq and also assisted with data interpretation. NS performed all other experiments with support and advice from TK and the other authors. NS, MH, MS, KW, YI collected human specimens. NS and YI wrote the manuscript with input from the other authors.

Corresponding authors

Correspondence to Yuuki Imai.

Ethics declarations

Competing interests

The authors declare no competing interests.

Figure legends

Fig. 1 : Up-regulation of the epigenetic regulator Uhrf1 in arthritis tissue.

a. Protocol for analysis of collagen antibody-induced arthritis (CAIA) model. PBS (Ctrl^P) or LPS (Ctrl^L) was administered as a control. **b.** PCA analysis using microarray data obtained from ankle tissue. **c.** Heatmap of differentially expressed gene probes in ankle tissue ($\text{Log}_2\text{FC} > 1$, $P < 0.01$). Log_{10} transformed read counts are scaled from 1.0 to

3.0. **d.** Expression of genes related to epigenetic regulation classified by Gene Set Enrichment Analysis (GSEA). Log10 transformed read counts are scaled to minimum to maximum values **e.** Relative probe counts detected in CAIA ankle compared to Ctrl^P or Ctrl^L ankles. **f.** RT-1PCR of *Uhrf1* mRNA expression in CAIA (n=4) and STA (n=3) ankles; Mean±SD is shown. ** indicates $P<0.01$ by ANOVA followed by Tukey's test and unpaired *t*-test. **g.** *UHRF1* mRNA expression in synovium biopsies from healthy, osteoarthritis (OA) and rheumatoid arthritis (RA) patients by RNA-seq. Data are registered in the Gene expression omnibus (GSE89408). Mean±SD is shown. ** indicates $P<0.01$ by ANOVA followed by Tukey's test. **h.** Immunofluorescent staining for Uhrf1 (Red), Pdpn, Fap, Thy-1, CD45, F4/80 and CD3 (Green) in WT STA ankle tissue. Scale bar represents 50 µm.

Fig. 2: Specific *Uhrf1* depletion in synovial fibroblasts exacerbates arthritis pathogenesis.

a, b. Development of **(a)** hind paw thickness and **(b)** Clinical score in *Uhrf1^{fl/fl}* and *Uhrf1^{ΔCol6a1}* mice after CAIA (n=4-5) and STA (n=12-15) induction. **c.** Representative images of safranin O, fast green and eosin staining of ankle tissue on day 10 after arthritis induction. Scale bar represents 500 µm. **d.** Left, high-magnification images of synovium. Scale bar represents 50 µm. Right, quantification of synovium thickness in normal (n=3-5), CAIA (n=4-5) and STA (n=9-11) from *Uhrf1^{fl/fl}* and *Uhrf1^{ΔCol6a1}* mice. **e.** Left, immunofluorescent staining for Pdpn (Green), cleaved caspase-3 (Cl-Casp3; Red) and DAPI (Blue) in synovium from *Uhrf1^{fl/fl}* (n=5) and *Uhrf1^{ΔCol6a1}* (n=5) mice. Scale bar

represents 50 μm . Right, quantification of $\text{Cl-Casp3}^+ \text{Pdpn}^+$ cells (arrow) among Pdpn^+ cells in the synovium region. **f.** Left, phalloidin (Green) and DAPI (Blue) staining of *Uhrf1^{fl/fl}* SF (n=3) and *Uhrf1 ΔCol6a1* SF (n=3) derived from STA mice after treatment with 0.5 $\mu\text{g/mL}$ cycloheximide (CHX) and 20 ng/mL $\text{Tnf-}\alpha$ for 8 hours. Scale bar represents 200 μm . Right, quantification of cell numbers after treatment relative to those for vehicle treatment. Mean $\pm$ SD are shown. * and ** indicate $P < 0.05$ and $P < 0.01$ versus *Uhrf1^{fl/fl}*, respectively, by unpaired *t*-test.

Fig. 3: Uhrf1 suppresses expression of multiple genes involved in RA via modulation of DNA methylation.

a. Top, representative phase contrast images of *Uhrf1^{fl/fl}* SF and *Uhrf1 ΔCol6a1* SF derived from STA mice on day 4 after arthritis induction. Bottom, immunostaining for Uhrf1 (Red), Pdpn (Green) and DAPI (Blue). Scale bar represents 50 μm . **b.** RT-qPCR measurement of *Uhrf1* mRNA expression levels in *Uhrf1^{fl/fl}* SF (n=3) and *Uhrf1 ΔCol6a1* SF (n=3) derived from STA mice on day 4 after arthritis induction. **c.** PCA analysis of RNA-seq data. **d.** Volcano plot showing log₂-fold change (Log₂FC) and statistical significance (*P* value) of differences between *Uhrf1 ΔCol6a1* SF and *Uhrf1^{fl/fl}* SF. **e.** Venn diagram comparing up-regulated genes (Log₂FC > 1, $P < 0.05$) following *Uhrf1*-depletion in SF, chondrocytes and hematopoietic stem cells (HSC) by RNA-seq analysis of data from this study and data in public databases (GSE92641, GSE85450). **f.** Quantification of methylated DNA after enrichment from genome DNA using MBD-beads. **g.** Distribution of Uhrf1-mediated methylated DNA annotated using given intervals and

scores with genome features by CEAS (cis-regulatory element annotation system). **h.** Venn diagram to compare Uhrf1-mediated methylated DNA loci between SF and chondrocytes using MBD-seq data from this study and from a public database (GSE99335). **i.** Venn diagram for 171 genes having up-regulated expression in *Uhrf1 Δ Col6a1* SF and 105 genes having Uhrf1-mediated methylated DNA peaks within the transcriptional start site (TSS) region ($\pm$ 50 kb). **j.** KEGG pathway analysis of 105 up-regulated with peaks assigned using DAVID Bioinformatics Resources. Significantly enriched pathways are illustrated by gene counts and *P* values. **k.** Representative Uhrf1-mediated methylated DNA peaks visualized by igv (integrative genome viewer). **l.** RT-qPCR measurement of *Ccl20* and *Tnfrsf11* mRNA expression in *Uhrf1^{fl/fl}* SF and *Uhrf1 Δ Col6a1* SF (n=3). **m.** Quantification of Ccl20 serum levels in *Uhrf1^{fl/fl}* and *Uhrf1 Δ Col6a1* on day 0 (n=10) and day 10 (n=16) after STA induction. **n.** Left, flow cytometry analysis of the population of Th17 cells (CD45⁺, CD4⁺, Ccr6⁺) in *Uhrf1^{fl/fl}* and *Uhrf1 Δ Col6a1* derived from STA mice on day 4 (n=6-8) and day 10 (n=9-10). Right, quantification of CD45⁺ CD4⁺ Ccr6⁺ cells among CD45⁺ cells. Mean $\pm$ SD is shown. *N.S.*: not significant versus *Uhrf1^{fl/fl}*. * and ** indicate *P*<0.05 and *P*<0.01 versus *Uhrf1^{fl/fl}*, respectively, by unpaired *t*-test.

Fig. 4: Uhrf1 stabilization attenuates arthritis pathogenesis.

a. Expression levels of *UHRF1* mRNA in synovium obtained from OA (n=32) and RA (n=26) patients. **b.** Immunofluorescent staining for UHRF1 (Red), PDPN (Green) and DAPI (Blue) in OA and RA synovium. Scale bar represents 30 μ m. **c.** Spearman's

673 correlation between *UHRF1* mRNA expression in RA synovium (n=19-20) and swollen
674 joint count in 28 joints (SJC28) as well as levels of MMP3 and C-reactive protein
675 (CRP). **d.** mRNA Expression levels of *UHRF1* and *CCL20* in RASF transfected with
676 *UHRF1* siRNA (n=4-5). **e.** Left, flow cytometry to measure proportion of Th17 cells
677 (CD45⁺, CD4⁺, CCR6⁺) in OA (n=14) and RA (n=21) synovium tissue. Right,
678 quantification of CD45⁺ CD4⁺ CCR6⁺ cells among 7-AAD⁻ cells. **f.** Spearman's
679 correlation between proportion of Th17 cells and *UHRF1* mRNA expression level in
680 OASF (n=10) and RASF (n=12) obtained from synovium of the same patients. **g.**
681 Western blot analysis of *UHRF1* expression in HEK293 cells. The cell cycle was
682 synchronized with aphidicolin (G1/S phase) or nocodazole (G2/M phase) before the
683 cells were treated with UNC0379 (U), NSC663284 (N), BVT948 (B) or Ryuvidine (R). **h.**
684 Protocol to assess efficacy of Ryuvidine in STA. **i.** Left, immunofluorescent staining for
685 Uhrf1 (Red), Pdpn (Green) and DAPI (Blue) in STA with or without Ryuvidine
686 treatment. Scale bar represents 50 μ m. Right, quantification of Uhrf1⁺ Pdpn⁺ (arrow)
687 per population of Pdpn⁺ cells. **j, k.** Development of **(j)** hind paw thickness and **(k)**
688 Clinical score in STA with or without Ryuvidine injection. DMSO; n=8-12, Ryuvidine;
689 n=10-12. **l.** Left, representative safranin O, fast green and eosin staining in STA. Scale
690 bar represents 200 μ m. Right, quantification of synovium thickness in STA after
691 Ryuvidine treatment. **m.** Quantification of *Ccl20* serum levels in STA after Ryuvidine
692 treatment. Mean $\pm$ SD is shown. * and ** indicate $P < 0.05$ and $P < 0.01$ versus OA,
693 siControl or DMSO, respectively, by unpaired *t*-test and ANOVA followed by Tukey's
694 test.

Extended data figure and tables

Extended data Fig. 1: Number of rheumatoid arthritis-related genes having differential expression is increased in CAIA ankle tissue.

a, b. KEGG pathway analysis of **(a)** up- and **(b)** down-regulated gene probes by microarray analysis. The top 10 terms that showed significant enrichment are illustrated with gene counts and *P* values.

Extended data Fig. 2: Uhrf1 is highly expressed in arthritis-derived SF.

a. Protocol for analysis of K/BxN serum transfer arthritis (STA) model mice. **b, c.** Immunofluorescent staining for Uhrf1 (Red), GFP (Green) and DAPI (Blue) in **(b)** *Col6a1Cre; Rosa26-EGFP* and *LysMCre; Rosa26-EGFP* after STA induction and **(c)** Uhrf1 (Red), Fap (Green), F4/80 (Green) and DAPI (Blue) in *WT* normal ankle tissue. Arrows indicate Uhrf1⁺ GFP⁺ and Uhrf1⁺ Fap⁺ cells. Scale bar represents 50 μ m. **d, e.** RT-qPCR measurement of mRNA expression of SF markers (*Cdh11*, *Col6a1*, *Tnfsf11*), macrophage markers (*Cd68*, *Emr1*, *Tnfrsf11a*) and *Uhrf1* in primary cultures of SF and SM derived from **(c)** CAIA ankle and **(d)** STA ankle. Mean $\pm$ SD is shown. *N.S.*; not significant. ** indicates *P* < 0.01 by unpaired *t*-test.

Extended data Fig. 3: SF-specific *Uhrf1* depletion has no pathological effect under healthy conditions.

a. Macroscopic images of SF-specific *Uhrf1* knockout mice (*Uhrf1*^{ΔCol6a1}) and littermate control mice (*Uhrf1*^{fl/fl}). b. Quantification of body weight of 7-week-old *Uhrf1*^{fl/fl} and *Uhrf1*^{ΔCol6a1} mice (n=23). Mean±SD is shown. ** indicates *P*<0.01 by unpaired *t*-test. c. Hematoxylin-eosin (H.E.) and Masson's trichrome (M.T.) staining of intestine, liver, lung and kidney tissue from *Uhrf1*^{fl/fl} and *Uhrf1*^{ΔCol6a1} mice. Scale bar represents 100 μm.

Extended data Fig. 4: Morphological comparison of arthritic ankles from *Uhrf1*^{fl/fl} and *Uhrf1*^{ΔCol6a1} mice

a. Left, immunofluorescent staining for *Uhrf1* (Red), *Pdpn* (Green) and DAPI (Blue) in hyperplastic synovia from *Uhrf1*^{fl/fl} (n=5) and *Uhrf1*^{ΔCol6a1} mice (n=5). Scale bar represents 50 μm. Right, quantification of *Uhrf1*⁺ *Pdpn*⁺ (arrow) among *Pdpn*⁺ cells in the synovium region. b. Left, safranin O, fast green and eosin staining of ankle tissue on day 10 after arthritis induction. Scale bar represents 50 μm. Right, relative cartilage area (safranin O positive region) per fields in normal (n=3-4), CAIA (n=4-5) and STA (n=7-8) *Uhrf1*^{fl/fl} and *Uhrf1*^{ΔCol6a1} mice. c. TRAP staining of ankle tissue on day 10 after arthritis induction. Scale bar represents 50 μm. d. Left, binarized image of TRAP staining. Scale bar represents 50 μm. Right, relative TRAP-positive area in the entire ankle area (as in Panel c) in normal (n=3-4), CAIA (n=4-5) and STA (n=7) *Uhrf1*^{fl/fl} and *Uhrf1*^{ΔCol6a1} mice. e. Representative μCT image of arthritic ankles from *Uhrf1*^{fl/fl} and *Uhrf1*^{ΔCol6a1} mice. Arrowhead indicates bone damage. Scale bar represents 1 mm. Mean±SD is shown. * and ** indicate *P*<0.05 and *P*<0.01 versus *Uhrf1*^{fl/fl}, respectively, by unpaired *t*-test.

Extended data Fig. 5: Macrophage-specific *Uhrf1* depletion does not result in an arthritic phenotype.

a. *Uhrf1* mRNA expression in bone marrow-derived macrophages (BMDM) from *Uhrf1^{fl/fl}* and *Uhrf1^{ΔLysM}* mice. **b, c.** Development of **(b)** hind paw thickness and **(c)** Clinical score in *Uhrf1^{fl/fl}* and *Uhrf1^{ΔLysM}* mice after STA induction (n=8-9). **d, e.** Representative **(d)** safranin O, fast green and eosin staining and **(e)** TRAP staining in ankle tissue on day 10 after STA induction. Scale bar represents 1 mm. Mean±SD is shown. * indicates $P<0.05$ by unpaired *t*-test. *N.S.*; not significant versus *Uhrf1^{fl/fl}*.

Extended data Fig. 6: *Uhrf1* depletion does not affect cell proliferation in SF.

a. Left, immunofluorescent staining for Pdpn (Green), Ki67 (Red) and DAPI (Blue) in synovium from *Uhrf1^{fl/fl}* (n=5) and *Uhrf1^{ΔCol6a1}* (n=5) mice after STA induction. Scale bar represents 50 μm. Right, quantification of Ki67⁺ Pdpn⁺ cells (arrow) among Pdpn⁺ cells in the synovium region. **b.** Left, quantification of relative *Uhrf1* protein levels in primary *Uhrf1^{fl/fl}* SF (n=6) and *Uhrf1^{ΔCol6a1}* SF (n=5) derived from STA ankles. Right, representative western blot analysis. **c.** BrdU ELISA assay in SF from *Uhrf1^{fl/fl}* (n=4) and *Uhrf1^{ΔCol6a1}* (n=4) mice. Mean±SD is shown. *N.S.*; not significant versus *Uhrf1^{fl/fl}*. ** indicates $P<0.01$ versus *Uhrf1^{fl/fl}*, respectively, by unpaired *t*-test.

Extended data Fig. 7: GO and pathway analysis of differentially expressed genes in *Uhrf1^{ΔCol6a1}* SF.

a. Heatmap of differentially expressed genes in *Uhrf1^{fl/fl}* SF and *Uhrf1^{ΔCol6a1}* SF derived from STA mice on day 4 after induction. Log10 transformed read counts subtracted from the mean are scaled to -0.1 to 0.1. **b-e.** Left, (**b, d**) KEGG pathway analysis and (**c, e**) GO analysis among (**b, c**) up-regulated and (**d, e**) down-regulated genes using DAVID Bioinformatics Resources. Significantly enriched terms are illustrated by gene counts and *P* values. Right, heatmap of genes included in the (**b**) KEGG pathways “Rheumatoid arthritis” and “Cytokine-cytokine receptor interaction” and (**c**) GO biological process “Negative regulation of apoptotic process”. Log10-transformed read counts subtracted from the mean are scaled to -0.3 to 0.3. **f.** Venn diagram to compare genes exhibiting down-regulation following *Uhrf1*-depletion in SF, chondrocytes and hematopoietic stem cells (HSC) based on RNA-seq data from this study and public databases (GSE92641, GSE85450).

Extended data Fig. 8: GO analysis of genes that are directly regulated by Uhrf1 in SF.

a. GO biological processes among 105 up-regulated genes with peaks assigned using DAVID Bioinformatics Resources. Enriched pathways are illustrated by gene counts and *P* values. **b, c.** Representative mRNA expression of genes included in the (**b**) KEGG pathways “Rheumatoid arthritis” and “Cytokine-cytokine receptor interaction” and (**c**) GO biological process “Negative regulation of apoptotic process” in SF from *Uhrf1^{fl/fl}* and *Uhrf1^{ΔCol6a1}* mice (n=3) as measured by RT-qPCR. Mean ± SD is shown. * and ** indicate *P* < 0.05 and *P* < 0.01 versus *Uhrf1^{fl/fl}*, respectively, by unpaired *t*-test. **d.**

Venn diagram for 89 down-regulated genes in *Uhrf1*^{ΔCol6a1} SF and 39 genes having Uhrf1-mediated methylated DNA peaks within the gene body. **e.** KEGG pathway analysis of 39 down-regulated genes with peaks assigned using DAVID Bioinformatics Resources. Significantly enriched pathways are illustrated by gene counts and *P* values.

Extended data Fig. 9: UHRF1 expression level is negatively correlated with RA severity.

a. *DNMTs* mRNA expression in OA (n=32) and RA (n=26) synovium measured by RT-qPCR. Mean±SD is shown. *N.S.*; not significant versus OA by unpaired *t*-test. **b.** Spearman's correlation between *UHRF1* mRNA expression in RA synovium and TJC28, SDAI (n=19-20). **c-e.** Spearman's correlation between (**c**) *DNMT1*, (**d**) *DNMT3A* and (**e**) *DNMT3B* mRNA expression in RA synovium and TJC28, SJC28, SDAI, MMP3 and CRP (n=19-20). **f.** *UHRF1* and *CCL20* mRNA expression levels in OASF treated with UHRF1 siRNA (n=5). Mean±SD is shown. *N.S.*; not significant versus siControl. * indicate *P*<0.05 versus siControl by ANOVA followed by Tukey's test. **g.** Spearman's correlation between *CCL20* and *UHRF1* mRNA expression in OA (n= 28) and RA synovium (n=19). * indicate *P*<0.05. **h.** Left, flow cytometry analysis to analyze proportion of Th17 cells (CD45⁺, CD4⁺, CCR6⁺) in OA (n=14) and RA (n=21) synovium tissue. Right, quantification of total CD45⁺ cells, CD45⁺ CD4⁻ CCR6⁻ cells, CD45⁺ CD4⁻ CCR6⁺ cells, CD45⁺ CD4⁺ CCR6⁻ cells and CD45⁺ CD4⁺ CCR6⁺ cells among 7-AAD⁻ cells. Mean±SD is shown.

Extended data Fig. 10: Consecutive *UHRF1* knockdown induces resistance to apoptosis in RASF.

a. Schematic protocol of consecutive *UHRF1* knockdown and experimental apoptosis induction in RASF. **b.** RT-qPCR of *UHRF1* mRNA expression in RASF transfected twice with *UHRF1* siRNA (n=8). **c.** Left, phalloidin (Green) and DAPI (Blue) staining of RASF transfected twice with *UHRF1* siRNA (n=9) after treatment with 0.5 µg/mL anti-FAS antibody for 16 hours. Scale bar represents 200 µm. Right, quantification of cell numbers after apoptosis induction relative to that for vehicle treatment. Mean ± SD is shown. * and ** indicate $P < 0.05$ and $P < 0.01$ by ANOVA followed by Tukey's test.

Extended data Fig. 11: *UHRF1* negatively modulates gene expression of multiple RA-exacerbating factors in RA synovium.

Among genes involved in maintaining DNA methylation, *UHRF1* mRNA expression level is elevated as a whole and levels are more diverse in RA synovium than in OA synovium. Insufficient *UHRF1* expression levels that arise in response to as yet unknown effects could accelerate progression of RA pathogenesis. In contrast, increased levels of *UHRF1* can reduce mRNA expression levels of genes that encode multiple RA-exacerbating factors such as RA-related, cytokine-related and anti-apoptosis-related genes by altering DNA methylation. These results suggest that *UHRF1* stabilization could be a new strategy for RA therapeutic treatment.

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Fig. 1 : Up-regulation of the epigenetic regulator Uhrf1 in arthritis tissue.

a. Protocol for analysis of collagen antibody-induced arthritis (CAIA) model. PBS (Ctrl^P) or LPS (Ctrl^L) was administered as a control. **b.** PCA analysis using microarray data obtained from ankle tissue. **c.** Heatmap of differentially expressed gene probes in ankle tissue (Log₂FC > 1.1, $P < 0.01$). Log₁₀ transformed read counts are scaled from 1.0 to 3.0. **d.** Expression of genes related to epigenetic regulation classified by Gene Set Enrichment Analysis (GSEA). Log₁₀ transformed read counts are scaled to minimum to maximum values. **e.** Relative probe counts detected in CAIA ankle compared to Ctrl^P or Ctrl^L ankles. **f.** RT-PCR of *Uhrf1* mRNA expression in CAIA (n=4) and STA (n=3) ankles. Mean $\pm$ SD is shown. ** indicates $P < 0.01$ by ANOVA followed by Tukey's test and unpaired *t*-test. **g.** *UHRF1* mRNA expression in synovium biopsies from healthy, osteoarthritis (OA) and rheumatoid arthritis (RA) patients by RNA-seq. Data are registered in the Gene expression omnibus (GSE89408). Mean $\pm$ SD is shown. ** indicates $P < 0.01$ by ANOVA followed by Tukey's test. **h.** Immunofluorescent staining for Uhrf1 (Red), Pdpn, Fap, Thy-1, CD45, F4/80 and CD3 (Green) in WT STA ankle tissue. Scale bar represents 50 μ m.

Fig. 2 : Specific *Uhrf1* depletion in synovial fibroblasts exacerbates arthritis pathogenesis.

a, b. Development of (a) hind paw thickness and (b) Clinical score in *Uhrf1^{fl/fl}* and *Uhrf1^{ΔCol6a1}* mice after CAIA (n=4-5) and STA (n=12-15) induction. **c.** Representative images of safranin O, fast green and eosin staining of ankle tissue on day 10 after arthritis induction. Scale bar represents 500 μm. **d.** Left, high-magnification images of synovium. Scale bar represents 50 μm. Right, quantification of synovium thickness in normal (n=3-5), CAIA (n=4-5) and STA (n=9-11) from *Uhrf1^{fl/fl}* and *Uhrf1^{ΔCol6a1}* mice. **e.** Left, immunofluorescent staining for Pdnpn (Green), cleaved caspase-3 (Cl-Casp3; Red) and DAPI (Blue) in synovium from *Uhrf1^{fl/fl}* (n=5) and *Uhrf1^{ΔCol6a1}* (n=5) mice. Scale bar represents 50 μm. Right, quantification of Cl-Casp3⁺ Pdnpn⁺ cells (arrow) among Pdnpn⁺ cells in the synovium region. **f.** Left, phalloidin (Green) and DAPI (Blue) staining of *Uhrf1^{fl/fl}* SF (n=3) and *Uhrf1^{ΔCol6a1}* SF (n=3) derived from STA mice after treatment with 0.5 μg/mL cycloheximide (CHX) and 20 ng/mL Tnf-α for 8 hours. Scale bar represents 200 μm. Right, quantification of cell numbers after treatment relative to those for vehicle treatment. Mean±SD are shown. * and ** indicate $P<0.05$ and $P<0.01$ versus *Uhrf1^{fl/fl}*, respectively, by unpaired *t*-test.

Fig. 3 : Uhrf1 suppresses expression of multiple genes involved in RA via modulation of DNA methylation.

a. Top, representative phase contrast images of *Uhrf1^{fl/fl}* SF and *Uhrf1^{ΔCol6a1}* SF derived from STA mice on day 4 after arthritis induction. Bottom, immunostaining for Uhrf1 (Red), Pdpn (Green) and DAPI (Blue). Scale bar represents 50 μm. **b.** RT-qPCR measurement of *Uhrf1* mRNA expression levels in *Uhrf1^{fl/fl}* SF (n=3) and *Uhrf1^{ΔCol6a1}* SF (n=3) derived from STA mice on day 4 after arthritis induction. **c.** PCA analysis of RNA-seq data. **d.** Volcano plot showing log2-fold change (Log2FC) and statistical significance (*P* value) of differences between *Uhrf1^{ΔCol6a1}* SF and *Uhrf1^{fl/fl}* SF. **e.** Venn diagram comparing up-regulated genes (Log2FC>1, *P*<0.05) following *Uhrf1*-depletion in SF, chondrocytes and hematopoietic stem cells (HSC) by RNA-seq analysis of data from this study and data in public databases (GSE92641, GSE85450). **f.** Quantification of methylated DNA after enrichment from genome DNA using MBD-beads. **g.** Distribution of Uhrf1-mediated methylated DNA annotated using given intervals and scores with genome features by CEAS (cis-regulatory element annotation system). **h.** Venn diagram to compare Uhrf1-mediated methylated DNA loci between SF and chondrocytes using MBD-seq data from this study and from a public database (GSE99335). **i.** Venn diagram for 171 genes having up-regulated expression in *Uhrf1^{ΔCol6a1}* SF and 105 genes having Uhrf1-mediated methylated DNA peaks within the transcriptional start site (TSS) region (± 50 kb). **j.** KEGG pathway analysis of 105 up-regulated with peaks assigned using DAVID Bioinformatics Resources. Significantly enriched pathways are illustrated by gene counts and *P* values. **k.** Representative Uhrf1-mediated methylated DNA peaks visualized by igv (integrative genome viewer). **l.** RT-qPCR measurement of *Ccl20* and *Tnfsf11* mRNA expression in *Uhrf1^{fl/fl}* SF and *Uhrf1^{ΔCol6a1}* SF (n=3). **m.** Quantification of Ccl20 serum levels in *Uhrf1^{fl/fl}* and *Uhrf1^{ΔCol6a1}* on day 0 (n=10) and day 10 (n=16) after STA induction. **n.** Left, flow cytometry analysis of the population of Th17 cells (CD45⁺, CD4⁺, Ccr6⁺) in *Uhrf1^{fl/fl}* and *Uhrf1^{ΔCol6a1}* derived from STA mice on day 4 (n=6-8) and day 10 (n=9-10). Right, quantification of CD45⁺ CD4⁺ Ccr6⁺ cells among CD45⁺ cells. Mean±SD is shown. N.S.: not significant versus *Uhrf1^{fl/fl}*. * and ** indicate *P*<0.05 and *P*<0.01 versus *Uhrf1^{fl/fl}*, respectively, by unpaired *t*-test.

Fig. 4 : Uhrf1 stabilization attenuates arthritis pathogenesis.

a. Expression levels of *UHRF1* mRNA in synovium obtained from OA ($n=32$) and RA ($n=26$) patients. **b.** Immunofluorescent staining for *UHRF1* (Red), *PDPN* (Green) and DAPI (Blue) in OA and RA synovium. Scale bar represents 30 μm . **c.** Spearman's correlation between *UHRF1* mRNA expression in RA synovium ($n=19-20$) and swollen joint count in 28 joints (SJC28) as well as levels of MMP3 and C-reactive protein (CRP). **d.** mRNA Expression levels of *UHRF1* and *CCL20* in RASF transfected with *UHRF1* siRNA ($n=4-5$). **e.** Left, flow cytometry to measure proportion of Th17 cells (CD45^+ , CD4^+ , CCR6^+) in OA ($n=14$) and RA ($n=21$) synovium tissue. Right, quantification of CD45^+ CD4^+ CCR6^+ cells among 7-AAD $^-$ cells. **f.** Spearman's correlation between proportion of Th17 cells and *UHRF1* mRNA expression level in OASF ($n=10$) and RASF ($n=12$) obtained from synovium of the same patients. **g.** Western blot analysis of *UHRF1* expression in HEK293 cells. The cell cycle was synchronized with aphidicolin (G1/S phase) or nocodazole (G2/M phase) before the cells were treated with UNC0379 (U), NSC663284 (N), BVT948 (B) or Ryuvidine (R). **h.** Protocol to assess efficacy of Ryuvidine in STA. **i.** Left, immunofluorescent staining for *Uhrf1* (Red), *Pdpn* (Green) and DAPI (Blue) in STA with or without Ryuvidine treatment. Scale bar represents 50 μm . Right, quantification of *Uhrf1* $^+$ *Pdpn* $^+$ (arrow) per population of *Pdpn* $^+$ cells. **j, k.** Development of (j) hind paw thickness and (k) Clinical score in STA with or without Ryuvidine injection. DMSO; $n=8-12$, Ryuvidine; $n=10-12$. **l.** Left, representative safranin O, fast green and eosin staining in STA. Scale bar represents 200 μm . Right, quantification of synovium thickness in STA after Ryuvidine treatment. **m.** Quantification of *Ccl20* serum levels in STA after Ryuvidine treatment. Mean $\pm$ SD is shown. * and ** indicate $P < 0.05$ and $P < 0.01$ versus OA, siControl or DMSO, respectively, by unpaired *t*-test and ANOVA followed by Tukey's test.

a

b

Extended data Fig. 1: Number of rheumatoid arthritis-related genes having differential expression is increased in CAIA ankle tissue.

a, b. KEGG pathway analysis of (a) up- and (b) down-regulated gene probes by microarray analysis. The top 10 terms that showed significant enrichment are illustrated with gene counts and *P* values.

Extended data Fig. 2: Uhfr1 is highly expressed in arthritis-derived SF.

a. Protocol for analysis of K/BxN serum transfer arthritis (STA) model mice. **b, c.** Immunofluorescent staining for Uhfr1 (Red), GFP (Green) and DAPI (Blue) in **(b)** *Col6a1Cre; Rosa26-EGFP* and *LysMCre; Rosa26-EGFP* after STA induction and **(c)** Uhfr1 (Red), Fap (Green), F4/80 (Green) and DAPI (Blue) in WT normal ankle tissue. Arrows indicate Uhfr1⁺ GFP⁺ and Uhfr1⁺ Fap⁺ cells. Scale bar represents 50 μ m. **d, e.** RT-qPCR measurement of mRNA expression of SF markers (*Cdh11*, *Col6a1*, *Tnfsf11*), macrophage markers (*Cd68*, *Emr1*, *Tnfrsf11a*) and *Uhfr1* in primary cultures of SF and SM derived from **(c)** CAIA ankle and **(d)** STA ankle. Mean $\pm$ SD is shown. N.S.; not significant. ** indicates $P < 0.01$ by unpaired t -test.

Extended data Fig. 3: SF-specific *Uhrf1* depletion has no pathological effect under healthy conditions.
a. Macroscopic images of SF-specific *Uhrf1* knockout mice (*Uhrf1^{ΔCol6a1}*) and littermate control mice (*Uhrf1^{fl/fl}*).
b. Quantification of body weight of 7-week-old *Uhrf1^{fl/fl}* and *Uhrf1^{ΔCol6a1}* mice (n=23). Mean±SD is shown.
 ** indicates $P < 0.01$ by unpaired *t*-test. **c.** Hematoxylin-eosin (H.E.) and Masson's trichrome (M.T.) staining of intestine, liver, lung and kidney tissue from *Uhrf1^{fl/fl}* and *Uhrf1^{ΔCol6a1}* mice. Scale bar represents 100 μm .

Extended data Fig. 4: Morphological comparison of arthritic ankles from *Uhrf1^{fl/fl}* and *Uhrf1^{ΔCol6a1}* mice.

a. Left, immunofluorescent staining for *Uhrf1* (Red), *Pdpn* (Green) and DAPI (Blue) in hyperplastic synovia from *Uhrf1^{fl/fl}* (n=5) and *Uhrf1^{ΔCol6a1}* mice (n=5). Scale bar represents 50 μ m. Right, quantification of *Uhrf1*⁺ *Pdpn*⁺ (arrow) among *Pdpn*⁺ cells in the synovium region. **b.** Left, safranin O, fast green and eosin staining of ankle tissue on day 10 after arthritis induction. Scale bar represents 50 μ m. Right, relative cartilage area (safranin O positive region) per fields in normal (n=3-4), CAIA (n=4-5) and STA (n=7-8) *Uhrf1^{fl/fl}* and *Uhrf1^{ΔCol6a1}* mice. **c.** TRAP staining of ankle tissue on day 10 after arthritis induction. Scale bar represents 50 μ m. **d.** Left, binarized image of TRAP staining. Scale bar represents 50 μ m. Right, relative TRAP-positive area in the entire ankle area (as in Panel c) in normal (n=3-4), CAIA (n=4-5) and STA (n=7) *Uhrf1^{fl/fl}* and *Uhrf1^{ΔCol6a1}* mice. **e.** Representative μ CT image of arthritic ankles from *Uhrf1^{fl/fl}* and *Uhrf1^{ΔCol6a1}* mice. Arrowhead indicates bone damage. Scale bar represents 1 mm. Mean $\pm$ SD is shown. * and ** indicate $P < 0.05$ and $P < 0.01$ versus *Uhrf1^{fl/fl}*, respectively, by unpaired *t*-test.

Extended data Fig. 5: Macrophage-specific *Uhrf1* depletion does not result in an arthritic phenotype.
a. *Uhrf1* mRNA expression in bone marrow-derived macrophages (BMDM) from *Uhrf1^{fl/fl}* and *Uhrf1^{ΔLysM}* mice.
b, c. Development of (**b**) hind paw thickness and (**c**) Clinical score in *Uhrf1^{fl/fl}* and *Uhrf1^{ΔLysM}* mice after STA induction (n=8-9). **d, e.** Representative (**d**) safranin O, fast green and eosin staining and (**e**) TRAP staining in ankle tissue on day 10 after STA induction. Scale bar represents 1 mm. Mean±SD is shown. * indicates $P<0.05$ by unpaired *t*-test. N.S.; not significant versus *Uhrf1^{fl/fl}*.

Extended data Fig. 6: *Uhrf1* depletion does not affect cell proliferation in SF.

a. Left, immunofluorescent staining for Pdpn (Green), Ki67 (Red) and DAPI (Blue) in synovium from *Uhrf1*^{fl/fl} (n=5) and *Uhrf1*^{ΔCol6a1} (n=5) mice after STA induction. Scale bar represents 50 μm. Right, quantification of Ki67⁺ Pdpn⁺ cells (arrow) among Pdpn⁺ cells in the synovium region. **b.** Left, quantification of relative *Uhrf1* protein levels in primary *Uhrf1*^{fl/fl} SF (n=6) and *Uhrf1*^{ΔCol6a1} SF (n=5) derived from STA ankles. Right, representative western blot analysis. **c.** BrdU ELISA assay in SF from *Uhrf1*^{fl/fl} (n=4) and *Uhrf1*^{ΔCol6a1} (n=4) mice. Mean±SD is shown. N.S.; not significant versus *Uhrf1*^{fl/fl}. ** indicates $P < 0.01$ versus *Uhrf1*^{fl/fl}, respectively, by unpaired *t*-test.

Extended data Fig. 7: GO and pathway analysis of differentially expressed genes in *Uhrf1*^{ΔCol6a1} SF.

a. Heatmap of differentially expressed genes in *Uhrf1*^{fl/fl} SF and *Uhrf1*^{ΔCol6a1} SF derived from STA mice on day 4 after induction. Log10 transformed read counts subtracted from the mean are scaled to -0.1 to 0.1. **b-e.** Left, (**b**, **d**) KEGG pathway analysis and (**c**, **e**) GO analysis among (**b**, **c**) up-regulated and (**d**, **e**) down-regulated genes using DAVID Bioinformatics Resources. Significantly enriched terms are illustrated by gene counts and *P* values. Right, heatmap of genes included in the (**b**) KEGG pathways “Rheumatoid arthritis” and “Cytokine-cytokine receptor interaction” and (**c**) GO biological process “Negative regulation of apoptotic process”. Log10-transformed read counts subtracted from the mean are scaled to -0.3 to 0.3. **f.** Venn diagram to compare genes exhibiting down-regulation following *Uhrf1*-depletion in SF, chondrocytes and hematopoietic stem cells (HSC) based on RNA-seq data from this study and public databases (GSE92641, GSE85450).

Extended data Fig. 8: GO analysis of genes that are directly regulated by Uhrf1 in SF.

a. GO biological processes among 105 up-regulated genes with peaks assigned using DAVID Bioinformatics Resources. Enriched pathways are illustrated by gene counts and *P* values. **b, c.** Representative mRNA expression of genes included in the **(b)** KEGG pathways “Rheumatoid arthritis” and “Cytokine-cytokine receptor interaction” and **(c)** GO biological process “Negative regulation of apoptotic process” in SF from *Uhrf1^{fl/fl}* and *Uhrf1^{ΔCol6a1}* mice (*n*=3) as measured by RT-qPCR. Mean±SD is shown. * and ** indicate *P*<0.05 and *P*<0.01 versus *Uhrf1^{fl/fl}*, respectively, by unpaired *t*-test. **d.** Venn diagram for 89 down-regulated genes in *Uhrf1^{ΔCol6a1}* SF and 39 genes having Uhrf1-mediated methylated DNA peaks within the gene body. **e.** KEGG pathway analysis of 39 down-regulated genes with peaks assigned using DAVID Bioinformatics Resources. Significantly enriched pathways are illustrated by gene counts and *P* values.

Extended data Fig. 9: UHRF1 expression level is negatively correlated with RA severity.

a. *DNMTs* mRNA expression in OA (n=32) and RA (n=26) synovium measured by RT-qPCR. Mean±SD is shown. *N.S.*; not significant versus OA by unpaired *t*-test. **b.** Spearman' s correlation between *UHRF1* mRNA expression in RA synovium and TJC28, SDAI (n=19-20). **c-e.** Spearman' s correlation between (**c**) *DNMT1*, (**d**) *DNMT3A* and (**e**) *DNMT3B* mRNA expression in RA synovium and TJC28, SJC28, SDAI, MMP3 and CRP (n=19-20). **f.** *UHRF1* and *CCL20* mRNA expression levels in OASF treated with *UHRF1* siRNA (n=5). Mean±SD is shown. *N.S.*; not significant versus siControl. * indicate *P*<0.05 versus siControl by ANOVA followed by Tukey' s test. **g.** Spearman' s correlation between *CCL20* and *UHRF1* mRNA expression in OA (n= 28) and RA synovium (n=19). * indicate *P*<0.05. **h.** Left, flow cytometry analysis to analyze proportion of Th17 cells (CD45⁺, CD4⁺, CCR6⁺) in OA (n=14) and RA (n=21) synovium tissue. Right, quantification of total CD45⁺ cells, CD45⁺ CD4⁻ CCR6⁻ cells, CD45⁺ CD4⁻ CCR6⁺ cells, CD45⁺ CD4⁺ CCR6⁻ cells and CD45⁺ CD4⁺ CCR6⁺ cells among 7-AAD⁻ cells. Mean±SD is shown.

Extended data Fig. 10: Consecutive *UHRF1* knockdown induces resistance to apoptosis in RASF.
a. Schematic protocol of consecutive *UHRF1* knockdown and experimental apoptosis induction in RASF. **b.** RT-qPCR of *UHRF1* mRNA expression in RASF transfected twice with *UHRF1* siRNA (n=8). **c.** Left, phalloidin (Green) and DAPI (Blue) staining of RASF transfected twice with *UHRF1* siRNA (n=9) after treatment with 0.5 μ g/mL anti-FAS antibody for 16 hours. Scale bar represents 200 μ m. Right, quantification of cell numbers after apoptosis induction relative to that for vehicle treatment. Mean $\pm$ SD is shown. * and ** indicate $P<0.05$ and $P<0.01$ by ANOVA followed by Tukey's test.

Extended data Fig. 11: UHRF1 negatively modulates gene expression of multiple RA-exacerbating factors in RA synovium.

Among genes involved in maintaining DNA methylation, *UHRF1* mRNA expression level is elevated as a whole and levels are more diverse in RA synovium than in OA synovium. Insufficient UHRF1 expression levels that arise in response to as yet unknown effects could accelerate progression of RA pathogenesis. In contrast, increased levels of UHRF1 can reduce mRNA expression levels of genes that encode multiple RA-exacerbating factors such as RA-related, cytokine-related and anti-apoptosis-related genes by altering DNA methylation. These results suggest that UHRF1 stabilization could be a new strategy for RA therapeutic treatment.

Figures

Figure 1

Up-regulation of the epigenetic regulator Uhrf1 in arthritis tissue. Please see .pdf for full caption.

Figure 2

Specific Uhf1 depletion in synovial fibroblasts exacerbates arthritis pathogenesis. Please see .pdf for full caption.

Figure 3

Uhrf1 suppresses expression of multiple genes involved in RA via modulation of DNA methylation. Please see .pdf for full caption.

Figure 4

Uhrf1 stabilization attenuates arthritis pathogenesis. Please see .pdf for full caption.

Supplementary Files

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- [SupplementaryTable1.xlsx](#)

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