

Figure S1. Lactate promotes the synovial proliferation

(A) Quantification analysis in Figure 1H. **(B–C)** TUNEL assay to assess apoptotic cells treated with Nala (10 mM, 20 mM) (n = 6). **(D)** Quantification analysis in Figure 1L. **(E)** Quantification analysis in Figure 2C. **(F)** Quantification analysis in Figure 2J. **(G)** Quantification analysis in Figure 2L. **(H)** Quantification analysis in Figure 2M. Scale bar: 100 μm. ns = no significance, **p* < 0.05, ***p* < 0.01, ****p* < 0.001. Data are presented as mean ± SEM, and *p*-values are calculated using unpaired two-tailed t-test (A, E, G, H), or one-way ANOVA followed by Tukey’s post-hoc test (C, D, F).

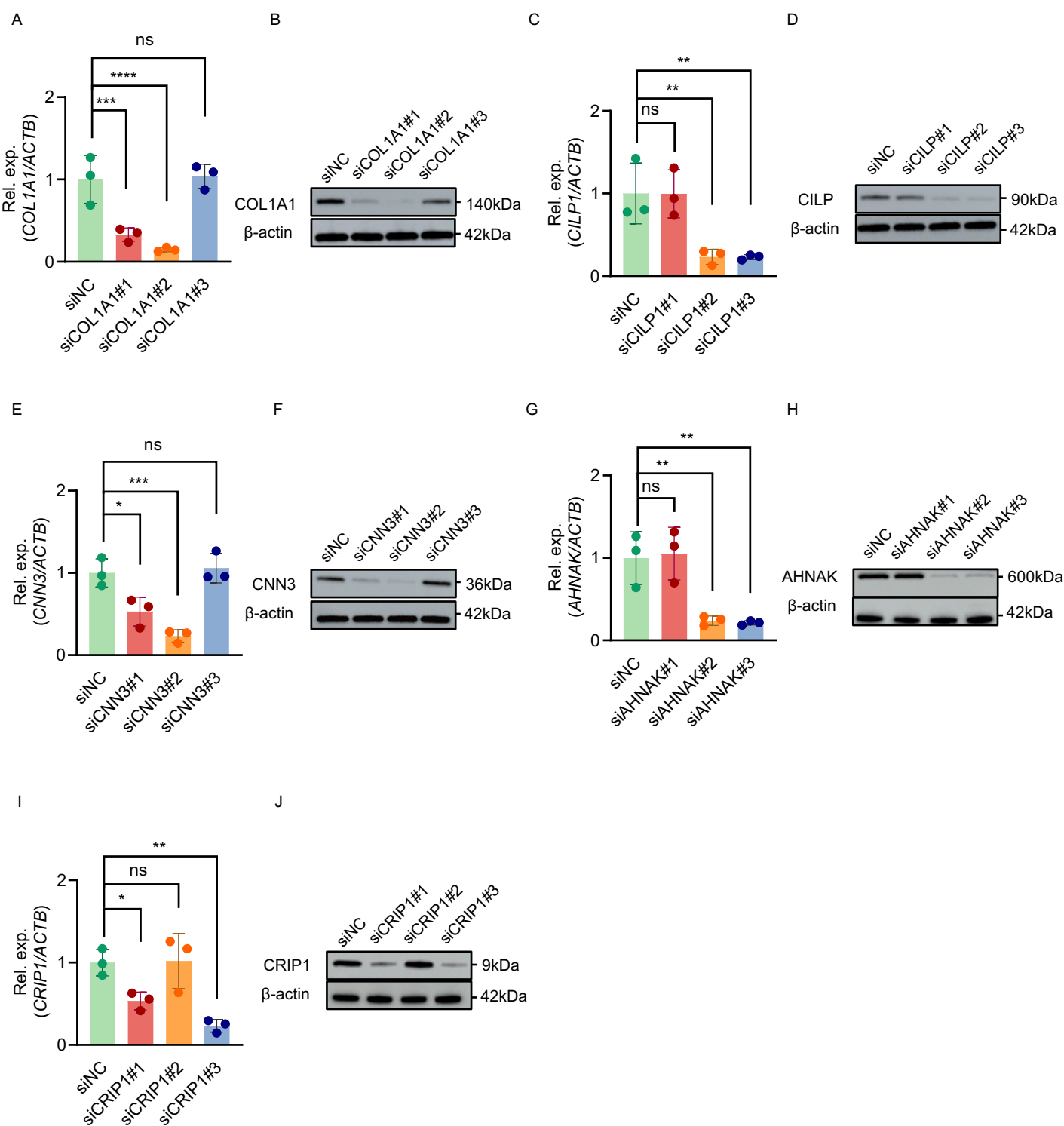


Figure S2. Efficiency validation of lactylation protein knockdown

qRT-PCR to quantify mRNA levels and western blotting to evaluate protein expression in RA-FLSs transfected with siCtrl or siRNA targeting *COL1A1*, *CILP*, *CNN3*, *AHNAK*, *CRIP1*. ns = no significance, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Data are presented as mean \pm SEM, and p -values are calculated using one-way ANOVA followed by Tukey's post-hoc test (A, C, E, G, I).

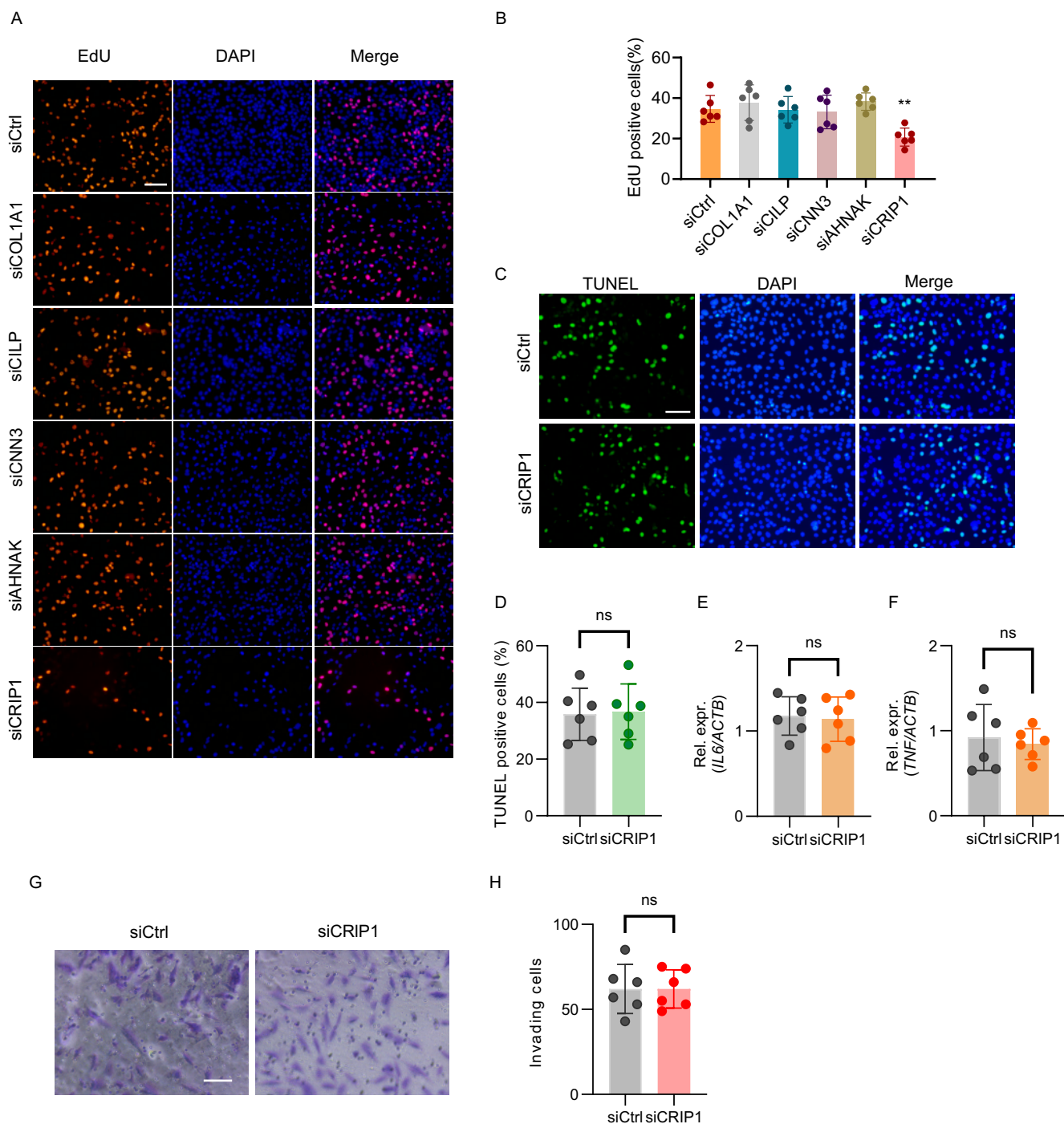


Figure S3. CRIP1 lactylation promotes the proliferation of RA-FLSs

(A–B) EdU incorporation assays and quantification of cell proliferation in RA-FLSs after knockdown of the candidate genes (*COL1A1*, *CILP*, *CNN3*, *AHNAK*, *CRIP1*). Scale bar: 100 μ m. **(C–D)** TUNEL assay to assess apoptotic cells in RA-FLSs after CRIP1 knockdown. Scale bar: 100 μ m. **(E–F)** qRT-PCR to quantify mRNA levels of *IL6* and *TNF*. **(G–H)** Transwell assays to measure cell invasion. ns = no significance, ** $p < 0.01$. Data are presented as mean \pm SEM, and p -values are calculated using one-way ANOVA followed by Tukey's post-hoc test (B), or unpaired two-tailed t-test (D, E, F, H).

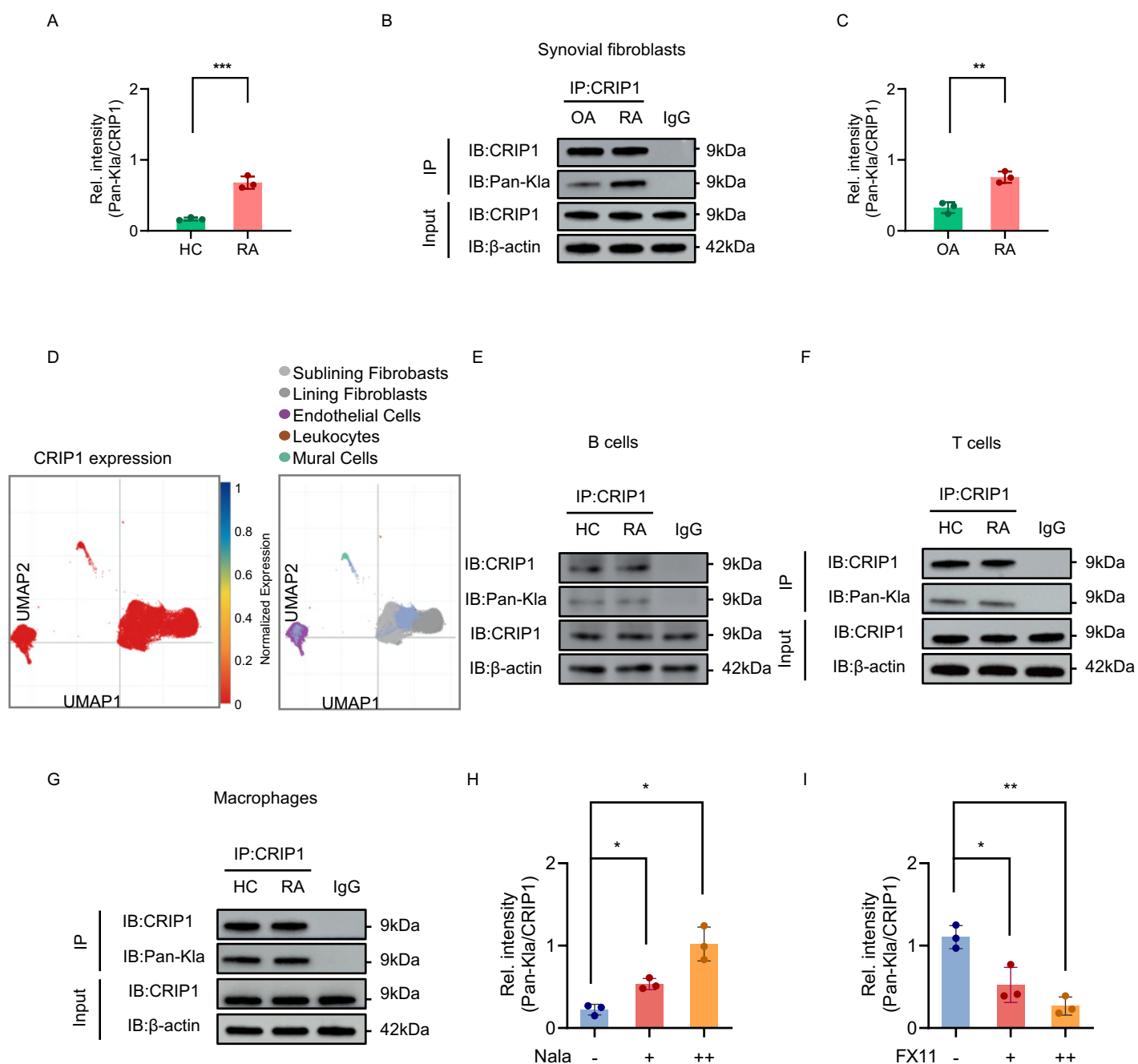


Figure S4. CRIP1 lactylation is elevated in RA-FLSs.

(A) Quantification of Figure 3G. (B–C) Co-IP of CRIP1 followed by immunoblotting for Pan-Kla to detect CRIP1 protein lactylation in FLSs from RA and OA patients (n = 3). (D) UMAP visualization of scRNA-seq data showing CRIP1 expression across distinct cell populations in synovial tissue. (E) Co-IP of CRIP1 followed by immunoblotting for Pan-Kla to detect CRIP1 protein lactylation in B cells from RA patients and healthy controls. (F) Co-IP of CRIP1 followed by immunoblotting for Pan-Kla to detect CRIP1 protein lactylation in T cells. (G) Co-IP of CRIP1 followed by immunoblotting for Pan-Kla to detect CRIP1 protein lactylation in macrophage. (H) Quantification analysis in Figure 3I. (I) Quantification analysis in Figure 3J. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Data are presented as mean \pm SEM, and p -values are calculated using unpaired two-tailed t-test (A, C) or one-way ANOVA followed by Tukey's post-hoc test (H, I).

Supplementary Figure 5

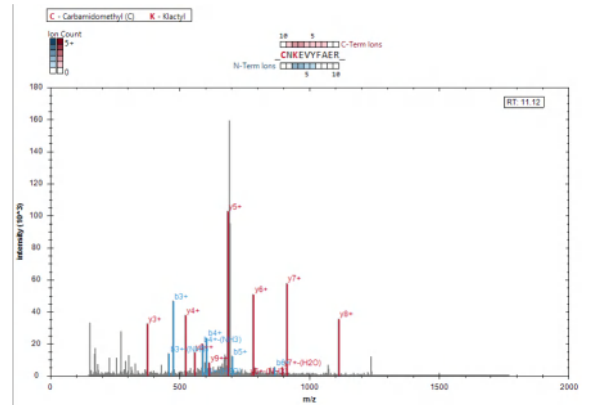
A

CRIP1 lactylated modification

CNKEVYFAER	(K9)
VTSLGKDWHRPCLK	(K22)
PCLKCEK	(K30)
TLTSGGHAHEHGKPYCNHPCYAAMFGPK	(K49)
GGAESHTFK	(K77)

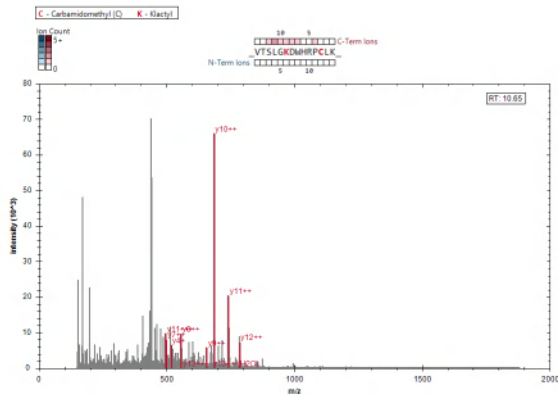
B

Lactylated modification (K9)



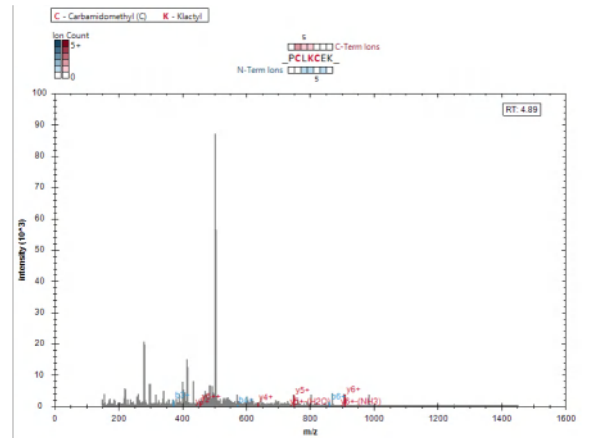
C

Lactylated modification (K22)



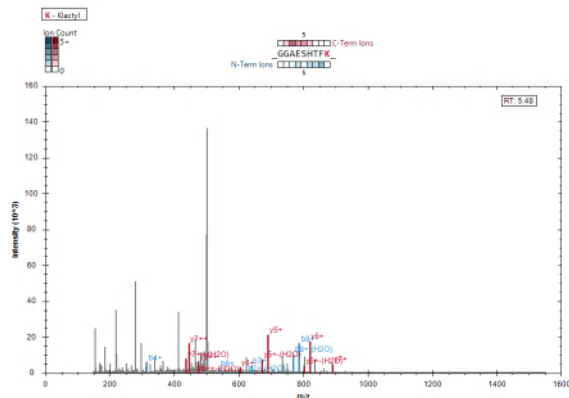
D

Lactylated modification (K30)

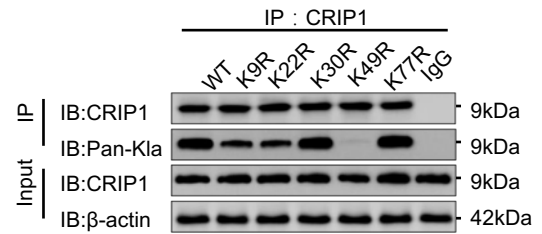


E

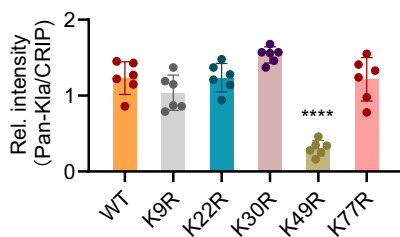
Lactylated modification (K77)



F



G



H

Homo sapiens
KTLTSGGHAHEHGKPYCNHPCYAAMFGPKGF
Mus musculus
KTLTSGGHAHEHGKPYCNHPCYSAMFGPKGF
Rattus norvegicus
KTLTSGGHAHEHGKPYCNHPCYSAMFGPKGF
Pan paniscus
KTLTSGGHAHEHGKPYCNHPCYAAMFGPKGF
Aphelocoma coerulescens
KTLTSGGHAHEHDGKPYCNHPCYAALFGPKGF

Figure S5. Identification of CRIP1 lactylation sites

(A) Identification of lysine lactylation sites on CRIP1 by LC-MS/MS. (B–E) MS/MS spectrum of lysine lactylation in CRIP1. (F–G) Co-IP of CRIP1 followed by immunoblotting for Pan-Kla in RA-FLSs expressing WT or its site-specific mutants CRIP1 (K9R, K22R, K49R, and K77R) (n = 6). (H) Protein sequence alignment analysis of the CRIP1 K49 site across multiple species. ****p < 0.0001. Data are presented as mean ± SEM, and p-values are calculated using one-way ANOVA followed by Tukey's post-hoc test (G).

Supplementary Figure 6

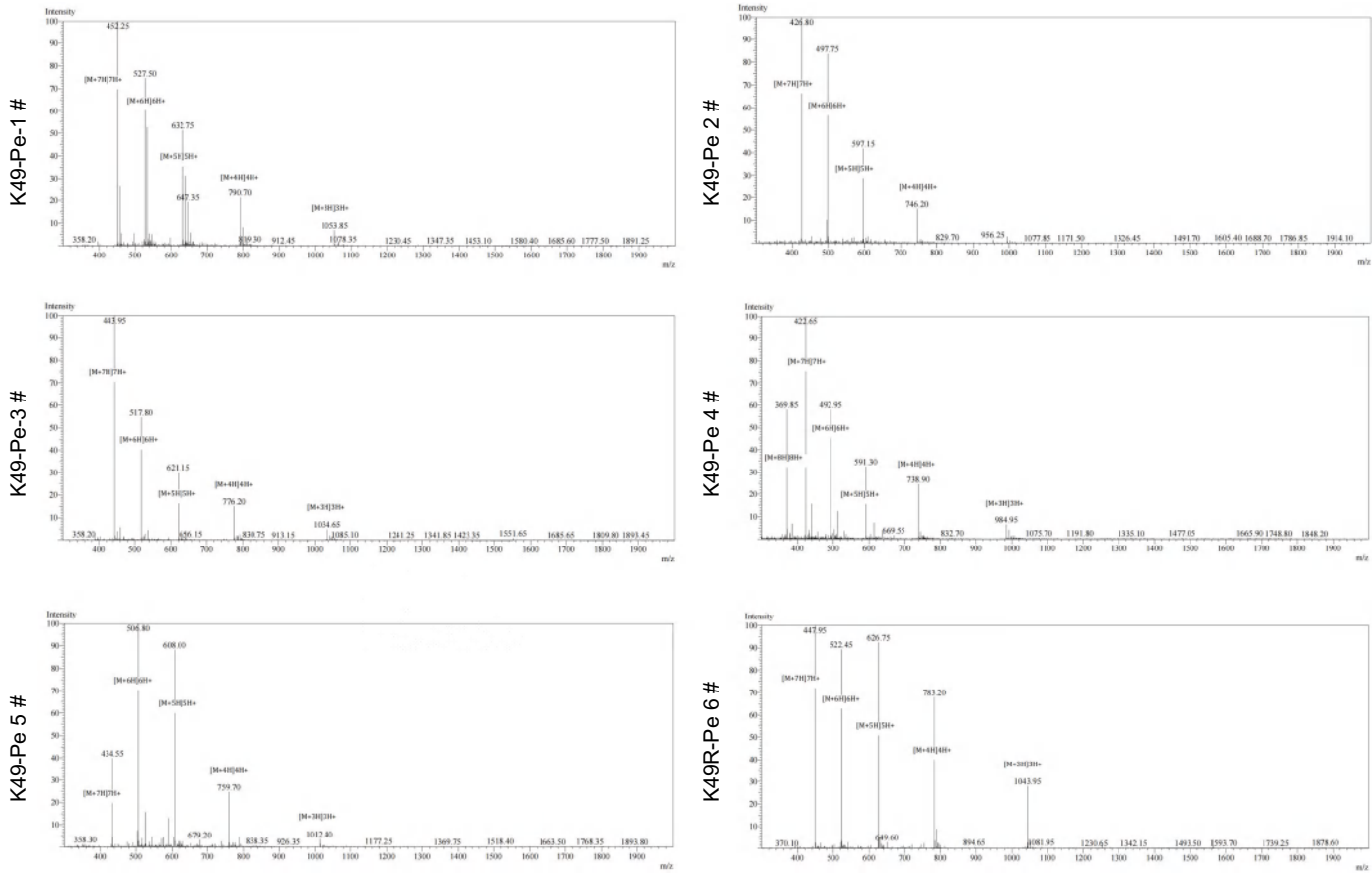


Figure S6. Validation of CRIP1 K49-region peptides

MS spectra of synthetic peptides (CRIP1 K49-Peptide 1–5 and K49R-Peptide 6).

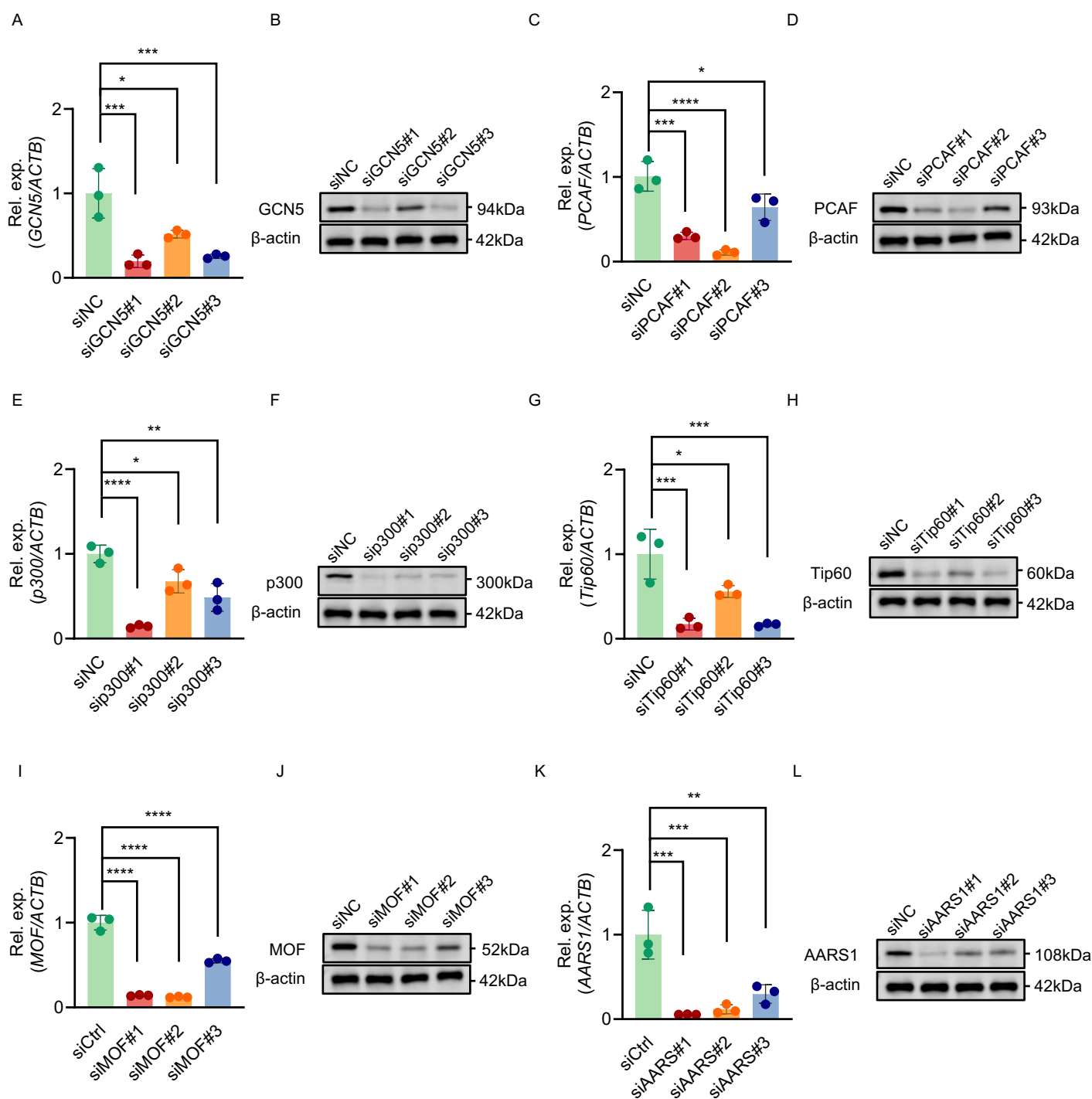


Figure S7. Efficiency validation of lactylation enzyme knockdown

qRT-PCR to quantify mRNA levels and western blotting to evaluate protein expression in RA-FLSs transfected with siCtrl or siRNA targeting *GCN5*, *PCAF*, *p300*, *KAT5*, *KAT8*, and *AARS1*. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Data are presented as mean \pm SEM, and p -values are calculated using one-way ANOVA followed by Tukey's post-hoc test (A, C, E, G, I, K).

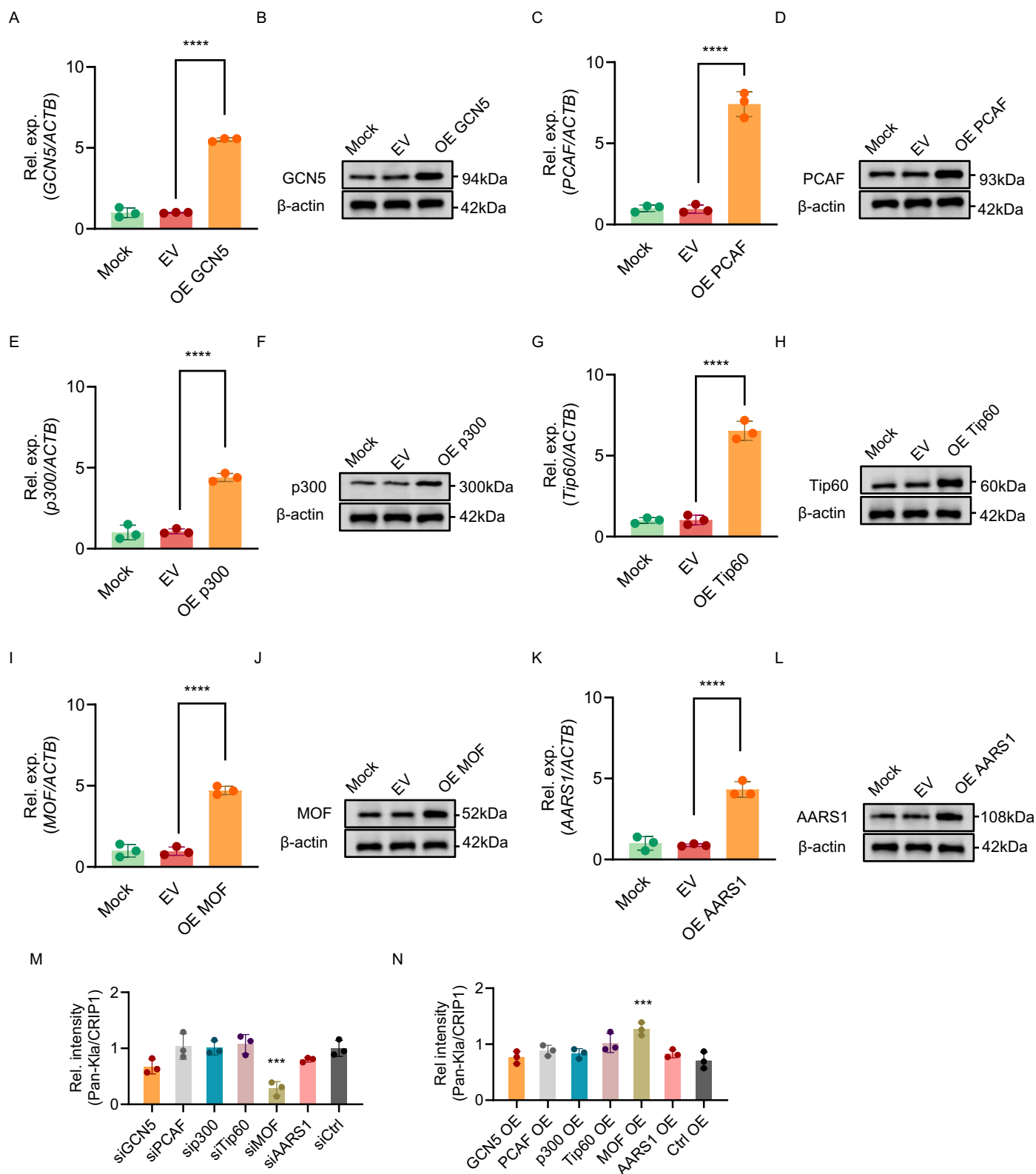


Figure S8. Overexpression of lactylation enzyme

(A–L) qRT-PCR to quantify mRNA levels, and western blotting to evaluate protein expression in RA-FLSs transfected with mock, empty vector, or overexpression constructs (*GCN5*, *PCAF*, *p300*, *Tip60*, *MOF*, and *AARS1*). (M–N) Quantification analysis of Figure 5A–B. *** $p < 0.001$, **** $p < 0.0001$. Data are presented as mean \pm SEM, and p -values are calculated using one-way ANOVA followed by Tukey's post-hoc test (A, C, E, G, I, K, M, N).

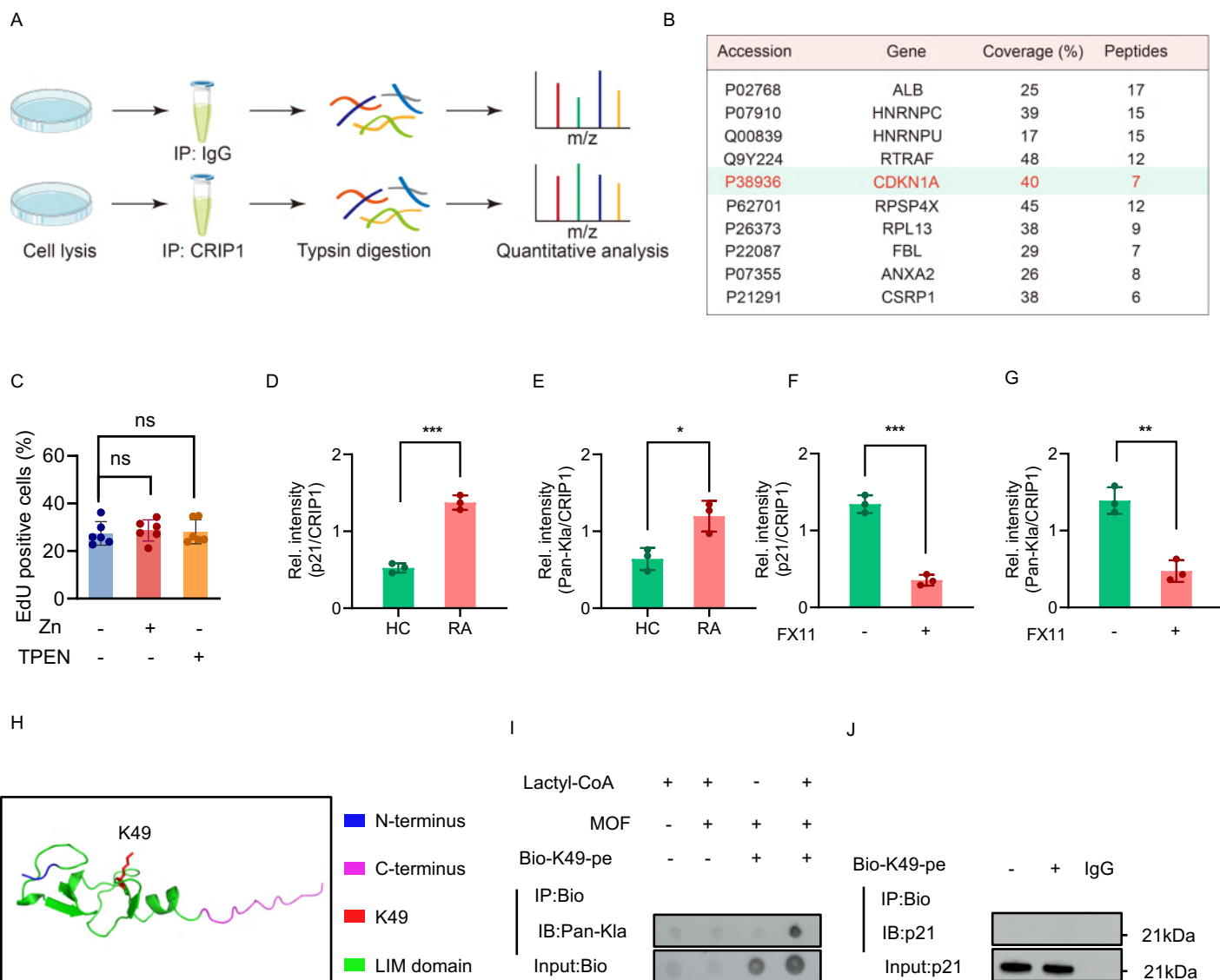


Figure S9. Analysis of CRIP1-interacting proteins

(A) Schematic diagram for the immunoprecipitation–mass spectrometry (IP–MS) analysis. CRIP1 or IgG control was immunoprecipitated from cell lysates, followed by trypsin digestion and LC-MS/MS-based quantitative proteomic analysis. **(B)** List of CRIP1-associated proteins identified by IP–MS analysis. **(C)** EdU to detect cell proliferation. RA-FLSs were treated with Zn (40 μ M) or TPEN (5 μ M). **(D–E)** Quantification analysis in Figure 6E. **(F–G)** Quantification analysis in Figure 6F. **(H)** Structural representation of CRIP1. **(I)** In vitro lactylation assay to assess the lactylation of Bio-tagged peptide. **(J)** Co-IP to assess the interaction between Bio-tagged peptide and p21. ns = no significance, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Data are presented as mean \pm SEM, and p -values are calculated using one-way ANOVA followed by Tukey's post-hoc test (C), or unpaired two-tailed t-test (D, E, F, G).

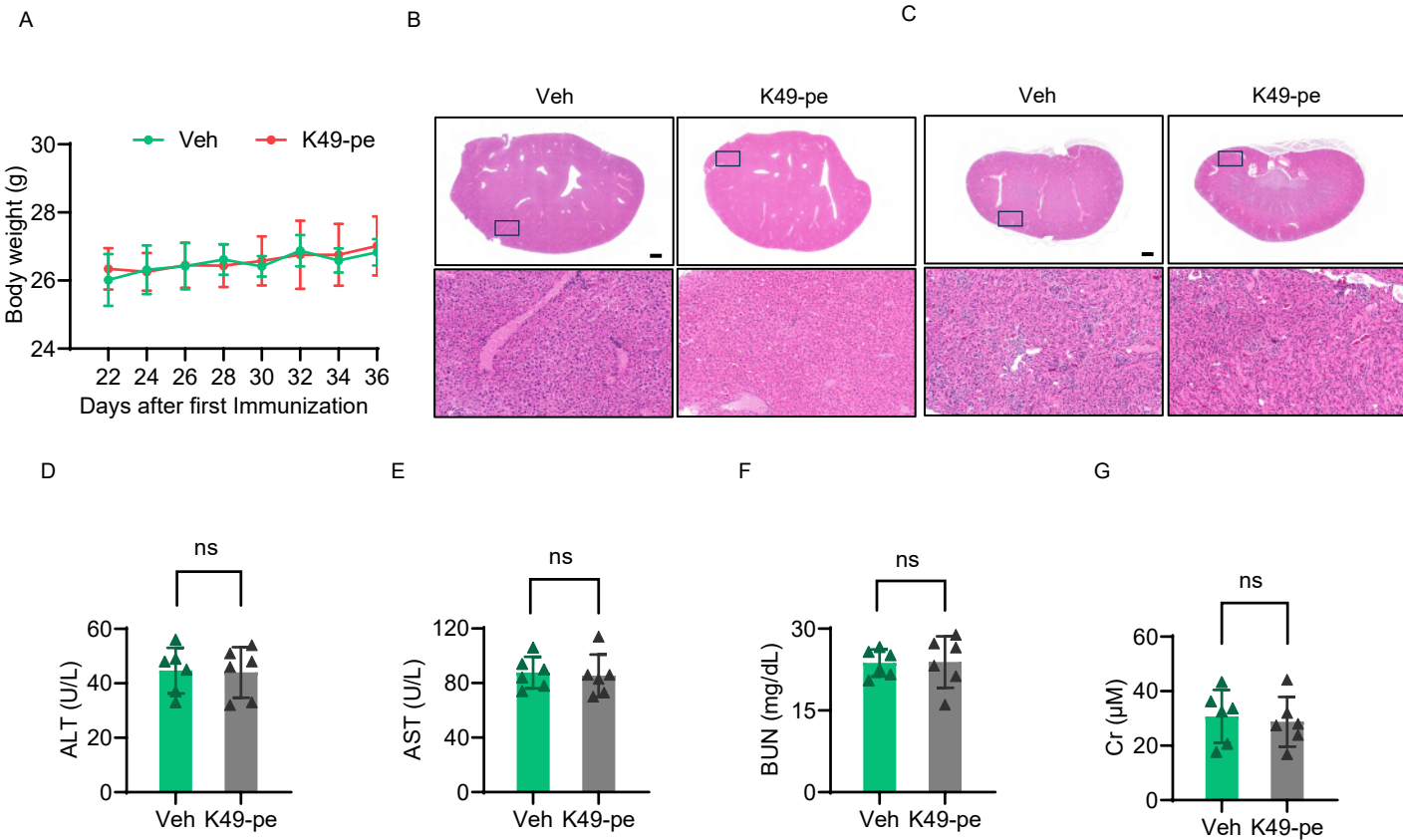


Figure S10. Evaluation of the hepatic and renal toxicity of K49-peptide

(A) Body weight in DBA/1 mice treated with either vehicle or K49-peptide. **(B–C)** Histological analysis of liver and kidney tissues. Scale bar, 500 μ m. **(D–G)** Serum ALT, AST, BUN, and Cr levels. ns = no significance. Data are presented as mean \pm SEM, and *p*-values are calculated using two-way ANOVA with Bonferroni’s post hoc test (A), or unpaired two-tailed t-test (D–G). ALT, alanine transaminase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Cr, creatinine.

SUPPLEMENTARY TABLE

Supplementary Table 1. Baseline data for patients

	HC (n=32)	RA (n=38)	p value
Female: Male	20:12	30:10	-
Age (years)	40.16 (19-72)	47.11 (22-75)	0.0546
CRP (IU/ml)	2.65 (0.5-9.1)	30.26 (4-120.5)	<0.0001
ESR (mm/h)	11.72 (5-34)	39.11 (10-96)	<0.0001
Disease duration (years)	-	7.48 (1.1-15.3)	-
DAS28-ESR	-	5.02 (1.9-8.4)	-
Anti-CCP antibody (U/ml)	-	153.44 (19.6-410)	-
RF positive	-	63.2%	-
ANA-positive	-	52.6%	-

Statistical significance was determined using unpaired two-tailed Student's t test. ESR, Erythrocyte sedimentation rate; CRP, C-reactive protein; DAS 28: Disease activity score 28; Anti-CCP antibody: Anti-cyclic citrullinated peptide Antibody; RF: Rheumatoid factor. ANA, Anti-nuclear antibodies.

Supplementary Table 2. Sequence of human oligonucleotides

COL1A1-siRNA #1	sense	CAUUGGUAAUGUUGGUGCUTT
	antisense	AGCACCAACAUAUACCAAUGTT
COL1A1-siRNA #2	sense	CCUCAAGAUGUGCCACUCUTT
	antisense	AGAGUGGCACAUCUUGAGGTT
COL1A1-siRNA #3	sense	UCACCCACCGACCAAGAAATT
	antisense	UUUCUUGGUCGGUGGGUGATT
CILP- siRNA #1	sense	GGCUUCUUGUCCAACCCUATT
	antisense	UAGGGUUGGACAAGAAGCCTT
CILP- siRNA #2	sense	GAACUUCAUCAUGACGAATT
	antisense	UUCGUCAUUGAUGAAGUUCTT
CILP- siRNA #3	sense	GCAUCCUGAAGAUCACAAATT
	antisense	UUUGUGAUCUUCAGGAUGCTT
CNN3- siRNA #1	sense	GCAUCUUUAUGAUCCCAAATT
	antisense	UUUGGGAUCAUAAAGAUGCTT
CNN3- siRNA #2	sense	GGAACAAAUGGUUCGGAAATT
	antisense	UUUCCGAACCAUUUGUUCCTT
CNN3- siRNA #3	sense	GUACCAGAAGAGACAUCUATT
	antisense	UAGAUGUCUCUUCUGGUACTT

AHNAK- siRNA #1	sense	CCACCAUCUACUUUGACAATT
	antisense	UUGUCAAAAGUAGAUGGUGGTT
AHNAK- siRNA #2	sense	UGCAGGAGGUGACGCAGAATT
	antisense	UUCUGCGUCACCUCCUGCATT
AHNAK- siRNA #3	sense	GGAUGUAGAUGUUCCTCAAATT
	antisense	UUUGGGAACAUCUACAUCCTT
CRIP1- siRNA #1	sense	CGCUGAGCACGAAGGCAAATT
	antisense	UUUGCCUUCGUGCUCAGCGTT
CRIP1- siRNA #2	sense	AGUGCAACAAGGAGGUGUATT
	antisense	UACACCUCCUUGUUGCACUTT
CRIP1- siRNA #3	sense	CUGCCUGAAGUGCGAGAAATT
	antisense	UUUCUCGCACUUCAGGCAGTT
GCN5-siRNA #1	sense	GGCUACCUACAAGGUCAAUUATT
	antisense	UAAUUGACCUUGUAGGUAGCCTT
GCN5-siRNA #2	sense	AGACACCAAGCAGGUCUAUUUTT
	antisense	AAAUAGACCUUGCUUGGUGUCUTT
GCN5-siRNA #3	sense	CCACCUGAAGGAGUAUCACAUTT
	antisense	AUGUGAUACUCCUUCAGGUGGTT
PCAF-siRNA #1	sense	CCUAAACCGCAUCAACUAUUGTT
	antisense	CAAUAGUUGAUGCGGUUUAGGTT

PCAF-siRNA #2	sense	GCAGAUACCAAACAAGUUUAUTT
	antisense	AUAAACUUGUUUGGUAUCUGCTT
PCAF-siRNA #3	sense	CGAACUCUAAUCCUCACUCAUTT
	antisense	AUGAGUGAGGAUUAGAGUUCGTT
P300-siRNA #1	sense	CCCGGUGAACUCUCCUAUAAUTT
	antisense	AUUAUAGGAGAGUUCACCGGGTT
P300-siRNA #2	sense	ACCUCGUGAUGCCACUUAUUATT
	antisense	UAAUAAGUGGCAUCACGAGGUTT
P300-siRNA #3	sense	CCAGCCUCAAAACUACAAUAAATT
	antisense	UUUAUUGUAGUUUGAGGCUGGTT
Tip60-siRNA #1	sense	CAAGUGUCUUCAGCGUCAUUUTT
	antisense	AAAUGACGCUGAAGACACUUGTT
Tip60-siRNA #2	sense	UCGAAUUGUUUGGGCACUGAUTT
	antisense	AUCAGUGCCCAAACAAUUCGATT
Tip60-siRNA #3	sense	GACUUCAACAAACGUCUGGAUTT
	antisense	AUCCAGACGUUUGUUGAAGUCTT
MOF-siRNA #1	sense	ACAUCGGGAACUACGAAAUUGTT
	antisense	CAAUUUCGUAGUCCCGAUGUTT
MOF-siRNA #2	sense	AUGGCAAAGACCAUAAGAUUUTT
	antisense	AAAUCUUAUGGUCUUUGCCAUTT
MOF-siRNA #3	sense	GCAAGAUCACUCGCAACCAAATT
	antisense	UUUGGUUGCGAGUGAUCUUGCTT

AARS1-siRNA #1	sense	GUCAACCAGGACGACCCUAAUTT
	antisense	AUUAGGGUCGUCCUGGUUGACTT
AARS1-siRNA #2	sense	ACAUGGUGAAGGACAUCAUUATT
	antisense	UAAUGAUGUCCUUCACCAUGUTT
AARS1-siRNA #3	sense	CCCAGGCAACAUGAAGGAUAATT
	antisense	UUAUCCUUCAUGUUGCCUGGGTT

Supplementary Table 3. Sequence of Primers

<i>ACTB</i>	Forward	CGCGAGAAGATGACCCAGAT
	Reverse	TCACCGGAGTCCATCACGAT
<i>COL1A1</i>	Forward	GAGGGCCAAGACGAAGACATC
	Reverse	CAGATCACGTCATCGCACAAAC
<i>CILP</i>	Forward	GCCCTGGTGAGTGGACAAC
	Reverse	GTCAGTGGTCCGAGCCTCTA
<i>CNN3</i>	Forward	GAAGAAGGTCAACGAGTCCTCA
	Reverse	AGTCTGAACCTGGGTCATGTT
<i>AHNAK</i>	Forward	TACCCTTCCTAAGGCTGACATT
	Reverse	TTGGACCCTTGAGTTTTGCAT
<i>CRIP1</i>	Forward	CCTGCCTGAAGTGCGAGAAAT
	Reverse	CCTTTAGGCCCAAACATGGC
<i>PCAF</i>	Forward	GAATCGCCGTGAAGAAAGCG
	Reverse	TACAAGACTCCTCGGCCTTG
<i>P300</i>	Forward	ACCAGGAATGACTTCTAGTTTGA
	Reverse	GGGTTTGCCGGGGTACAATA
<i>Tip60</i>	Forward	AACAAACGTCTGGATGAATGGG
	Reverse	AGGAAGTCCGTTCTTAGTGGG
<i>MOF</i>	Forward	GCTGGACGAGTGGGTAGAC
	Reverse	TTTGGTTGCGAGTGATCTTGC
<i>AARS1</i>	Forward	CCGATGTCCAGAAACGAGTGT

	Reverse	ATGACAAGAGGCTGGTTGGG
<i>IL6</i>	Forward	ACTCACCTCTTCAGAACGAATTG
	Reverse	CCATCTTTGGAAGGTTTCAGGTTG
<i>TNF</i>	Forward	CCTCTCTCTAATCAGCCCTCTG
	Reverse	GAGGACCTGGGAGTAGATGAG
<i>IL1B</i>	Forward	ATGATGGCTTATTACAGTGGCAA
	Reverse	GTCGGAGATTCGTAGCTGGA
<i>MMP1</i>	Forward	AAAATTACACGCCAGATTTGCC
	Reverse	GGTGTGACATTACTCCAGAGTTG
<i>MMP9</i>	Forward	AGACCTGGGCAGATTCCAAC
	Reverse	CGGCAAGTCTTCCGAGTAGT