

Supporting Information

for *Adv. Sci.*, DOI 10.1002/adv.202409154

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Leishmania Peptide via the Regulation of Fatty Acid Synthesis Metabolism

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Figure S1

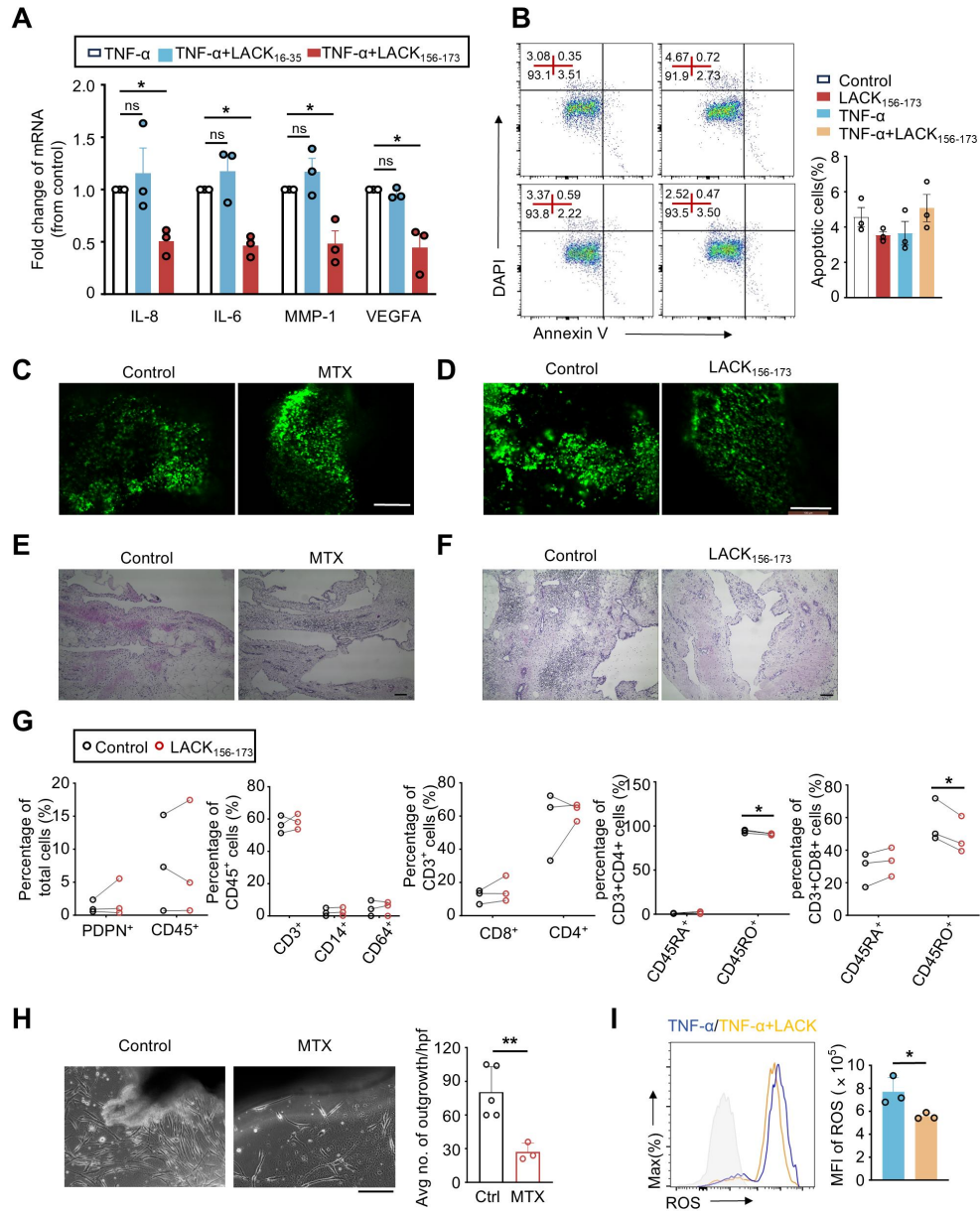


Figure S1. Synovial Explant and RA-FLS Response to LACK Peptides and MTX. (A) qRT-PCR analyses of IL-8, IL-6, MMP-1, VEGFA expression in PBS, LACK₁₆₋₃₅ or LACK₁₅₆₋₁₇₃ in primary human RA-FLS. **(B)** Flow cytometry detection of cell apoptosis percentage, n=3. **(C, D)** The activity of synovial explants cultured for 7 days was assessed by calcein staining. Scale bar =100µm. **(E, F)** HE staining of synovial explants. **(G)** Flow cytometry analysis showed changes in the proportion of subpopulations of synovial explant cells, n=3. **(H)** Microscope (left) and statistical analysis (right) of RA-FLS outgrowths from ST explants cultured with PBS or MTX (1µM) for 1 week. The scale bar represents 100 µm (n=3-5). **(I)** Flow cytometry showed the MFI of ROS, n=3. **p* < 0.05, ***p* < 0.01 versus vector control. The *p* values were determined by multiple unpaired t-tests (A), one-way ANOVA test (B), a paired t-test (G), and two-tailed unpaired t-test (H, I).

Figure S2

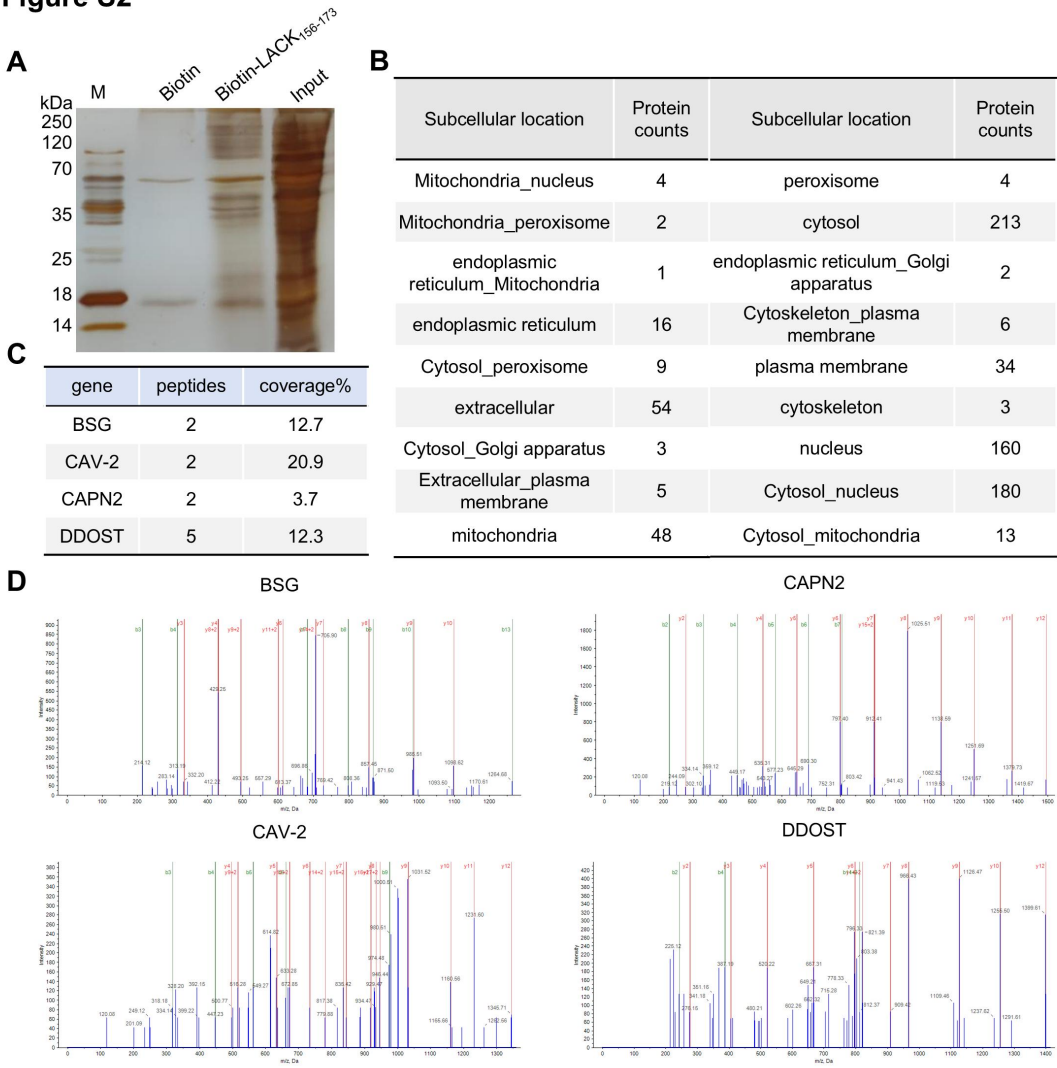


Figure S2. Proteins of RA-FLS that bind to LACK₁₅₆₋₁₇₃. (A) Silver staining of biotinylated LACK₁₅₆₋₁₇₃-associated proteins in peptide-protein pull-down assay. (B) Subcellular location of proteins binding to LACK₁₅₆₋₁₇₃ in RA-FLS, identified by mass spectrometry. (C) Proteomics analysis of LACK₁₅₆₋₁₇₃-interacted plasma membrane proteins with endocytic properties. (D) Secondary mass spectra of BSG, CAV2, CAPN2 and DDOST.

Figure S3

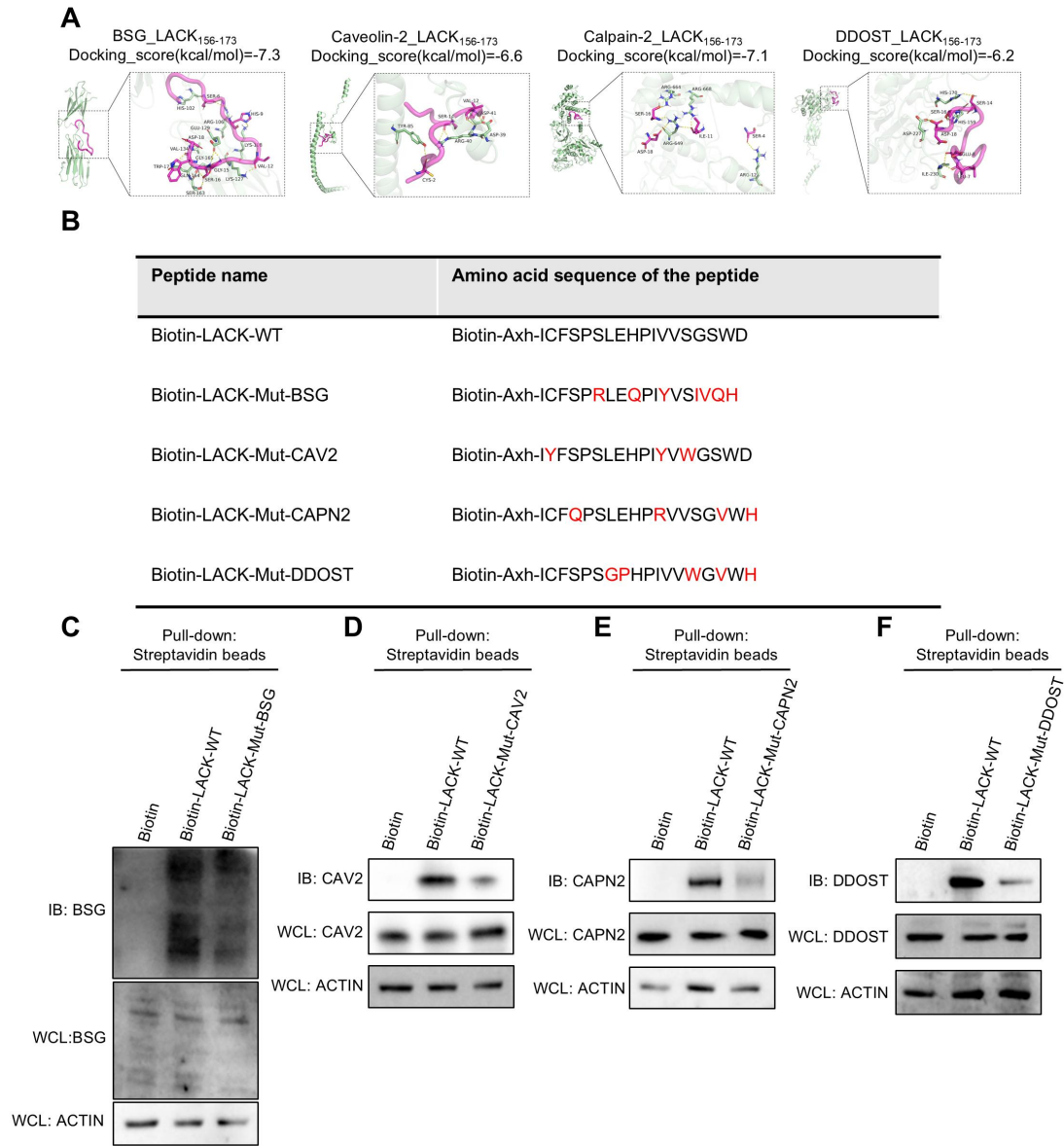


Figure S3. Mutagenesis of LACK₁₅₆₋₁₇₃ binding sites and their impact on protein interactions. (A) Docking models of LACK₁₅₆₋₁₇₃ interacting with BSG, Caveolin-2, Calpain-2, and DDOST. (B) Mutation sites of LACK₁₅₆₋₁₇₃. (C-F) Western blot analysis of pull-down assays assessing the interaction of mutated peptides with their respective proteins: LACK-Mut-BSG peptide with BSG protein (C), LACK-Mut-CAV2 peptide with CAV2 protein (D), LACK-Mut-CAPN2 peptide with CAPN2 protein (E), LACK-Mut-DDOST peptide with DDOST protein(F), or biotinylated WT LACK₁₅₆₋₁₇₃.

Figure S4

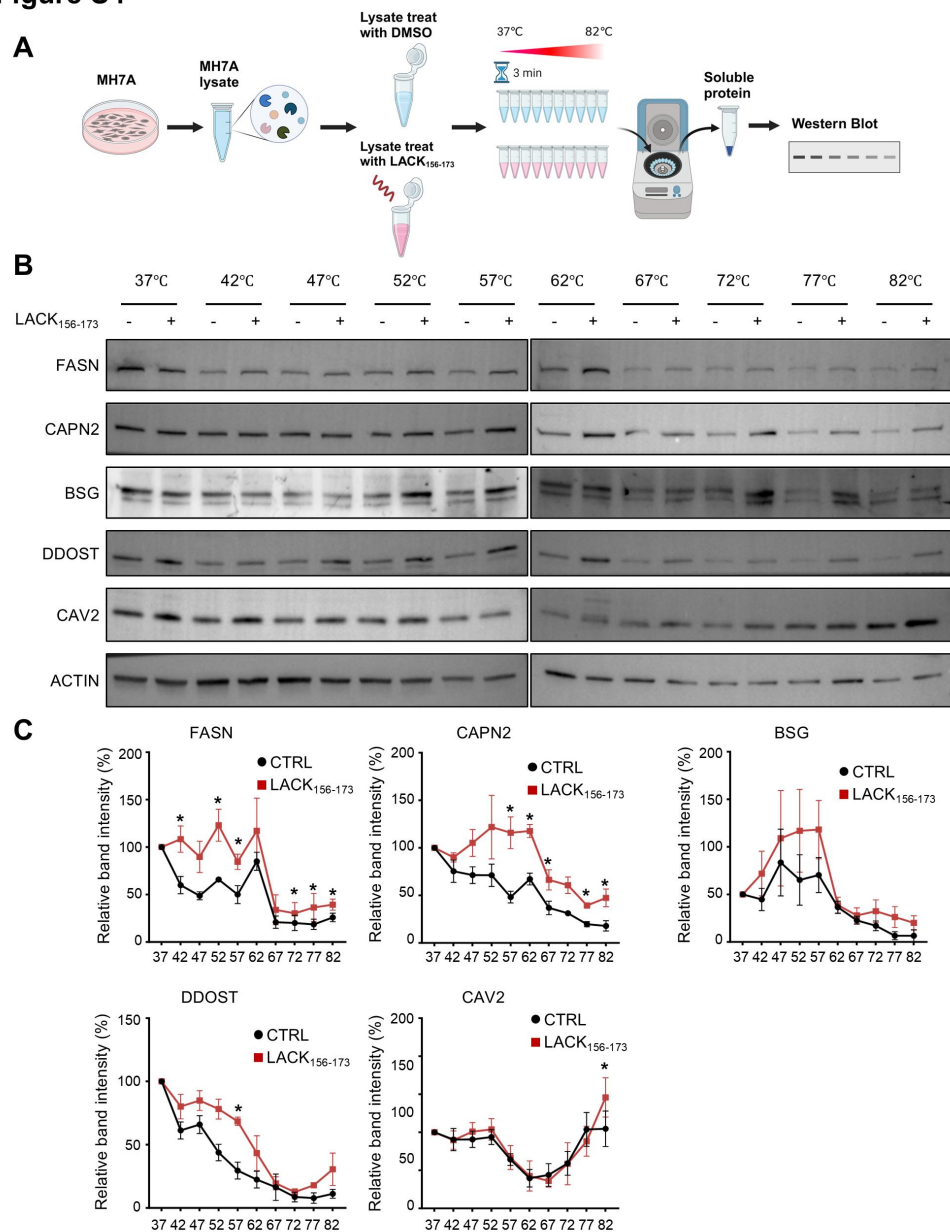


Figure S4. CETSA reveals the binding of LACK₁₅₆₋₁₇₃ to FASN, CAPN2, DDOST, and BSG. (A) Schematic of the CETSA-Western blot (CETSA-WB) experimental setup. Diagram is created in BioRender. Li, L. (2025) <https://BioRender.com/e43b074>. Agreement number: FT27VONHE6. (B) CETSA-WB assay demonstrating the thermal stabilization of FASN, CAPN2, BSG, DDOST, and CAV2 upon interaction with LACK₁₅₆₋₁₇₃ across a temperature range of 37°C to 82°C following 60 minutes of drug exposure. (C) Statistical analysis of the thermal stabilization data, showing significant binding of LACK₁₅₆₋₁₇₃ to the listed proteins. * $p < 0.05$ versus control. p -values were determined by multiple unpaired t-tests (C).

Figure S5

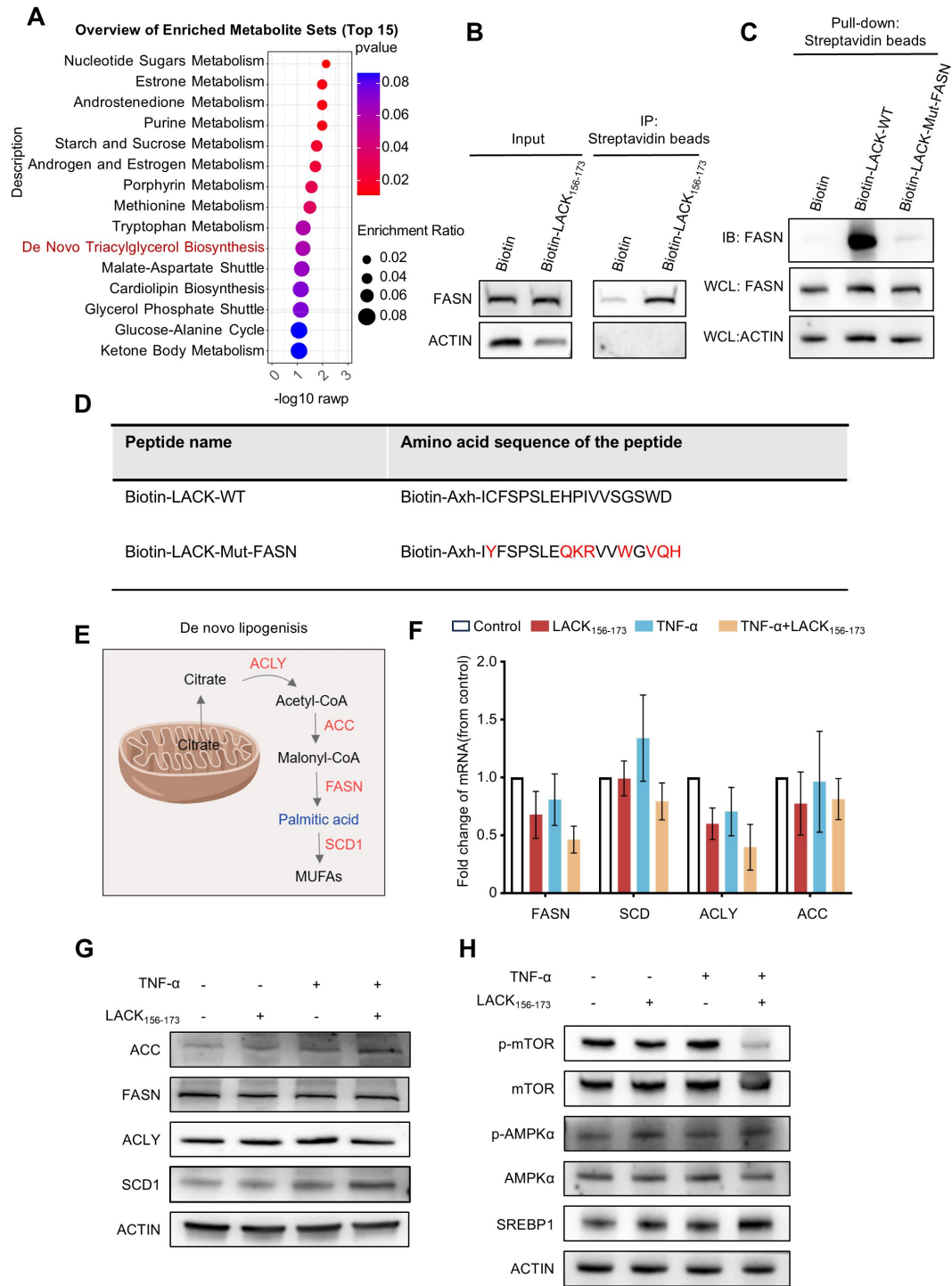


Figure S5. Impact of LACK₁₅₆₋₁₇₃ on fatty acid synthesis and key metabolic pathways in RA-FLSs. (A) Pathway analysis of significantly altered metabolites ($p < 0.05$, FDR < 0.05) in MH7A cells treated with LACK₁₅₆₋₁₇₃, following TNF- α stimulation ($n=4$). (B) Co-immunoprecipitation (CO-IP) analysis detecting the interaction between LACK₁₅₆₋₁₇₃ and FASN in MH7A cells. (C) Immunoblot analysis showing the interaction between FASN and biotinylated LACK₁₅₆₋₁₇₃ or -LACK-Mut-FASN peptides. (D) Schematic representation of the mutation site for LACK₁₅₆₋₁₇₃. (E) Overview of the de novo fatty acid synthesis pathway, highlighting key enzymes: ACLY, ACC, FASN, SCD1. (F) qRT-PCR analysis of mRNA expression changes in FASN, SCD1, ACLY, and ACC. (G) Western blot analysis assessing protein expression changes in FASN, SCD1, ACLY, and ACC. (H) Western blot analysis of protein expression of mTOR, AMPK α , SREBP1, and the phosphorylation of mTOR and AMPK α in RA-FLSs. The p values were determined by one-way ANOVA test (F).

Figure S6

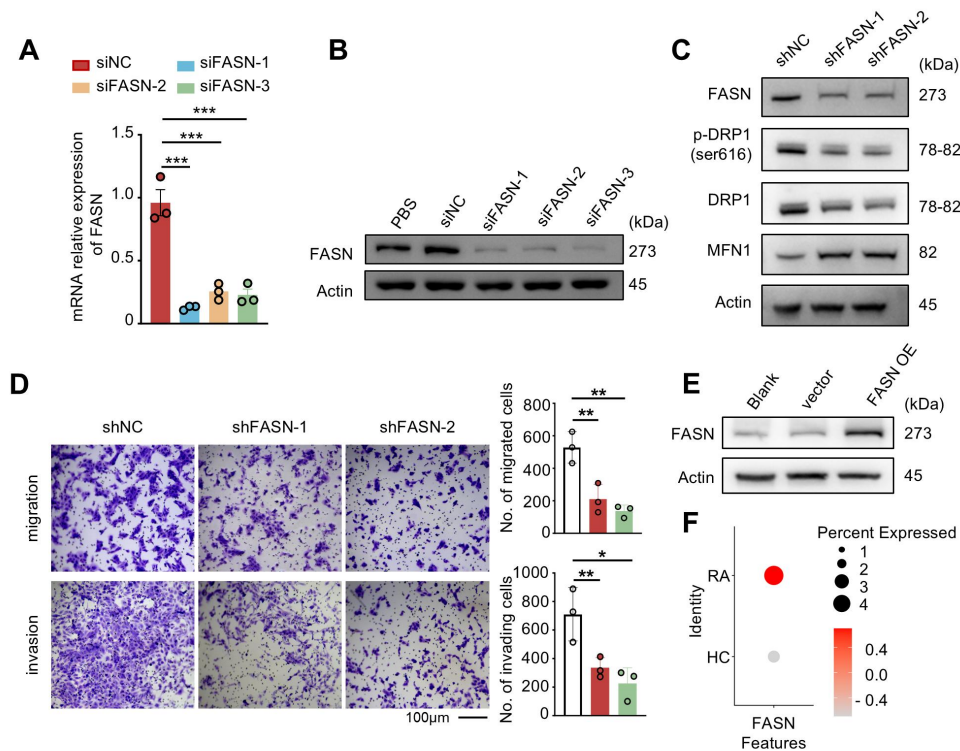


Figure S6. FASN expression and its impact on migration, invasion in MH7A cells. (A) RT-qPCR analysis of FASN expression in MH7A cells transfected with small interfering RNA (siRNA). **(B)** Western blot analysis of FASN protein levels in MH7A cells transfected with siRNA. **(C)** Western blot analysis of FASN protein expression in MH7A cells transfected with shFASN. **(D)** Transwell assay assessing the migration and invasion of MH7A cells transfected with shFASN. **(E)** Western blot analysis of FASN expression in MH7A cells overexpressing FASN. **(F)** Bubble chart shows the expression level of FASN in RA and healthy control (HC) FLS. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ versus vector control. The p -values were determined by one-way ANOVA (A, D).

Figure S7

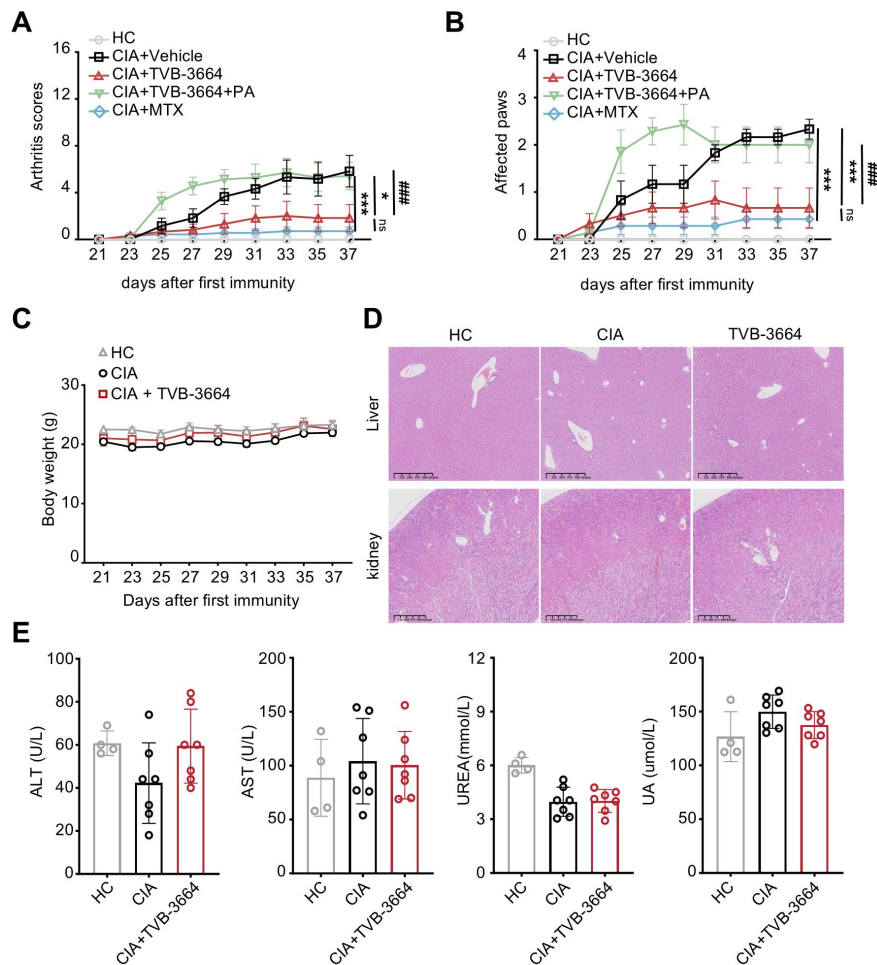


Figure S7. Evaluation of Clinical and Histological Effects of MTX and TVB-3664 Treatment in CIA Mice. (A-B) Statistical analyses of clinical scores and affected paws in CIA mice treated with TVB-3664 with or without PA, or treated with MTX (1mg/kg/week, oral gavage). ### $p < 0.001$, represents TVB-3664 versus TVB-3664+PA. (C) The statistical analyses of body weight in untreated and TVB-3664-treated mice. (D) Histological examination of the liver and kidney tissues after H&E staining in CIA treated with TVB-3664. Scale bar represents 625 μ m. (E) Detection of ALT, AST, UREA, and UA in serum from HC, CIA and TVB-3664 treated CIA mice. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ versus vector control. The p values were determined by two-way ANOVA test (A-C), one-way ANOVA test (E).

Supplemental Table 1 Demographic and clinical characteristics of RA patients

Item	Patients(n=26)
Age (y)	
Range	28-79
Mean $\pm$ SD	59.29 $\pm$ 12.80
Female, No.(%)	24 (92%)
Disease duration (y)	
Median (IQR)	10 (2-15)
Tender joint count, Median(IQR)	3 (0-7)
Swollen joint count,Median (IQR)	2 (1-6)
Patient Global Assessment (0–100) , mean $\pm$ SD	49.37 $\pm$ 27.93
Physician Global Assessment (0–100), mean $\pm$ SD	40.93 $\pm$ 23.12
DAS28-ESR, mean $\pm$ SD	4.47 $\pm$ 1.83
C-Reactive protein (mg/L), Median (IQR)	30.70 (5.30-44.19)
IgM-rheumatoid factor-positive, No. (%)	16 (76.19%)
Anti-cyclic citrullinated peptide-positive, No. (%)	14(66.67%)
Erythrocyte sedimentation Rate, mm/hour	55.59 $\pm$ 37.73

DAS28-ESR = Disease Activity Score-28 for Rheumatoid Arthritis with Erythrocyte sedimentation Rate; IQR = interquartile range; SD = standard deviation. Categorical data are expressed as the number (%); continuous data distributed normally are expressed as the mean $\pm$ SD; continuous data distributed non-normally are expressed as the median (IQR).

Supplemental Table 2 Sequence of primers and oligonucleotides

Primers		
Human β -actin	FORWARD	CTCCATCCTGGCCTCGCTGT
	REVERSE	GCTGTCACCTTCACCGTTCC
Human IL-1 β	FORWARD	TTTGAAGCTGATGGCCCTAAACA
	REVERSE	TGTAGTGGTGGTCGGAGATTCGT
Human IL-6	FORWARD	GCTGCAGGACATGACAACTCATC
	REVERSE	TCTGAGGTGCCCATGCTACATTT
Human IL-8	FORWARD	TGCTAAAGAACTTAGATGTCAGTGCAT
	REVERSE	TGGTCCACTCTCAATCACTCTCA
Human MMP-1	FORWARD	GCTAACAAATACTGGAGGTATGATG
	REVERSE	GTCATGTGCTATCATTTTGGGA
Human VEGFA	FORWARD	CAAGACAAGAAAATCCCTGTGG
	REVERSE	GCTTGTCACATCTGCAAGTACG
Human SCD	FORWARD	GCACATCAACTTCACCACATTCTTC
	REVERSE	CAGCCACTCTTGAGTTTCCATCTC
Human ACC	FORWARD	TCTCCTCCAACCTCAACCACTATG
	REVERSE	ATCCGCCCATCCGCTGAC
Human ACLY	FORWARD	AGACCTATGACTATGCCAAGACTATCC
	REVERSE	GCGATGCTGCCTCCAATGATG
Human FASN	FORWARD	ACAGCGGGGAATGGGTACT
	REVERSE	GACTGGTACAACGAGCGGAT
Oligonucleotides		
siFASN_001	Human	GCATCAATGTCCTGCTGAA
siFASN_002	Human	GCGTTGACCTGGTCTTGAA
siFASN_003	Human	GCATGGCTATCTTCCTGAA
shFASN-1	Human	GCTGCTAGATGTAGGTGTTAG
shFASN-2	Human	CGAGAGCACCTTTGATGACAT
siBSG	Human	TGGGCCTGGTACAAGATCA
siCAV2	Human	CAGTGCAGACAATATGGAA
siCAPN2_	Human	GAGGCCATCACGTTTCAGA
siDDOST	Human	GCTGGAGTTTGTCCGCATT