Supplementary file 1

MicroRNA-372-3p impairs fatty acid metabolism in hepatocellular carcinoma cells by targeting *CPT1A* and *ACSL4*

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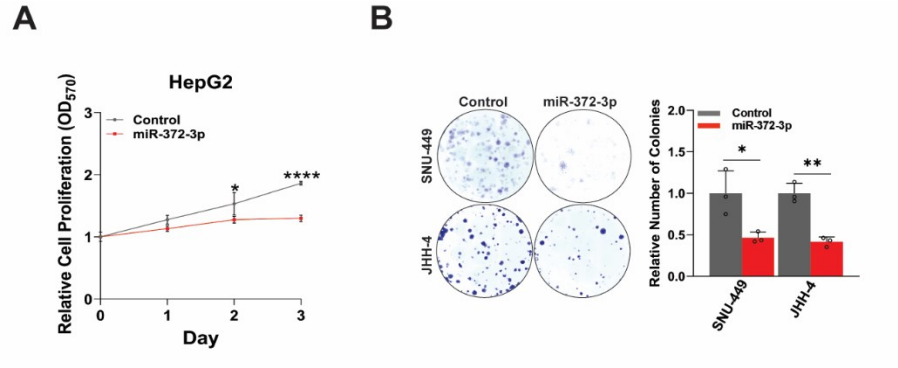

figure S1: Effects of miR-372-3p overexpression on HCC cell proliferation and colony formation. (A) MTT assay assessing the effect of miR-372-3p overexpression on HCC cell proliferation (n = 3). (B) Colony formation assay of control and 372-OE HCC cells, with representative images and quantification of colony numbers per well (n = 3). All experiments were quantified using Fiji software, normalized to the control group, and analyzed using Student's t-test. Data are presented as mean ± SD, with significance levels indicated as *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.

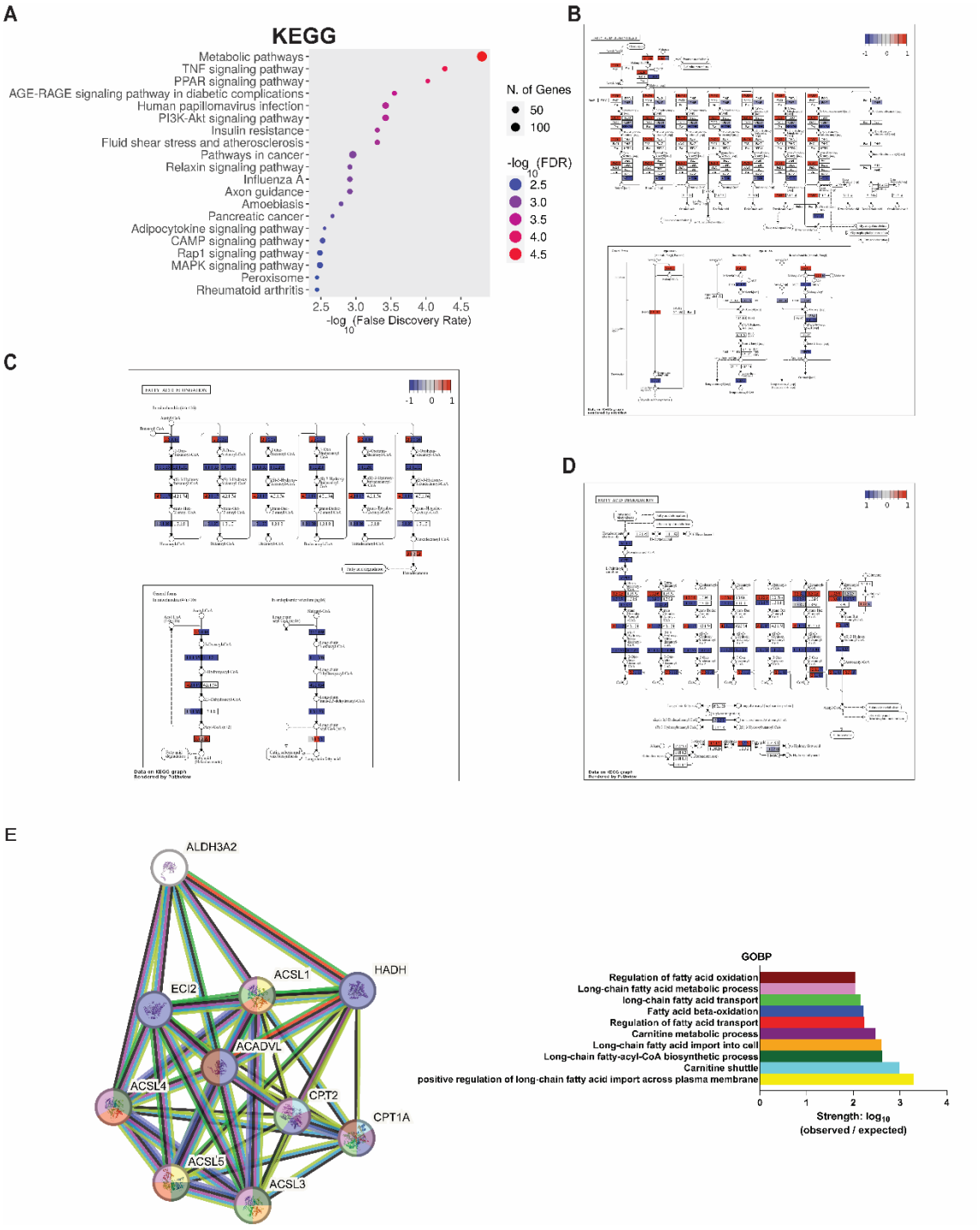

figure S2: Enrichment and network analysis of downregulated DEGs in fatty acid metabolism. (A) KEGG enrichment analysis of downregulated DEGs, with the Pathview analysis mapping DEGs onto KEGG pathways to illustrate expression patterns. Blue and red represent downregulated and upregulated genes, respectively. (B) Pathview analysis of downregulated DEGs in fatty acid

biosynthesis. (C) Pathview analysis of downregulated DEGs in fatty acid elongation. (D) Pathview analysis of downregulated DEGs in fatty acid degradation pathways. (E) STRING network analysis of ten core genes in fatty acid metabolism, analyzed using GOBP terms (left panel) and strength scores (FDR < 0.05, right panel).

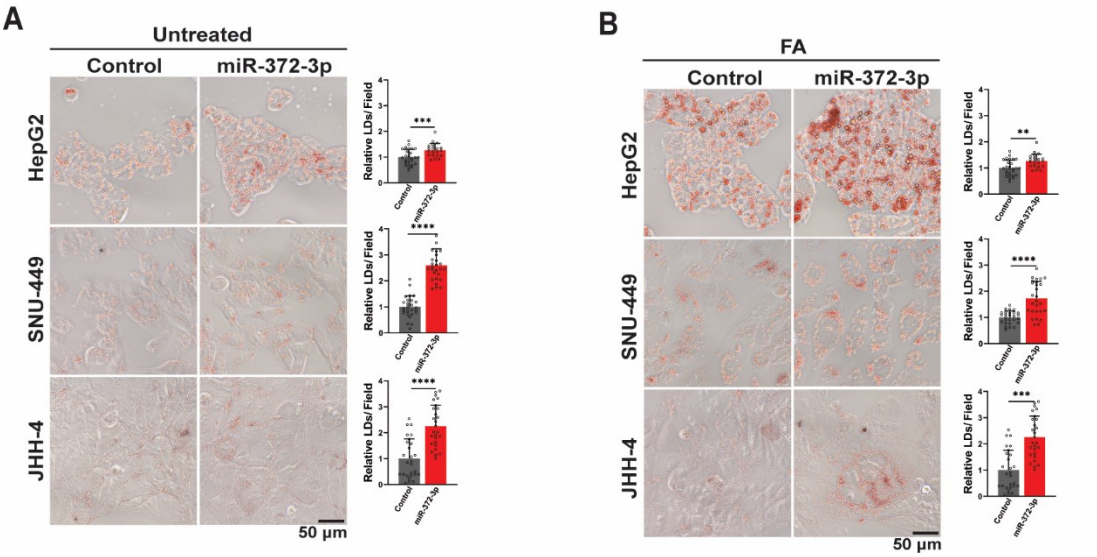

figure S3: Oil Red O (ORO) staining of control and 372-OE HCC cells under basal (A) and fatty acid (FA)-treated conditions (B), with representative images and quantification of lipid accumulation (n = 27). All experiments were quantified using quantified using Fiji, normalized to the control group, and analyzed using Student's t-test. Data are presented as mean ± SD, with significance levels indicated as *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0. 0001.

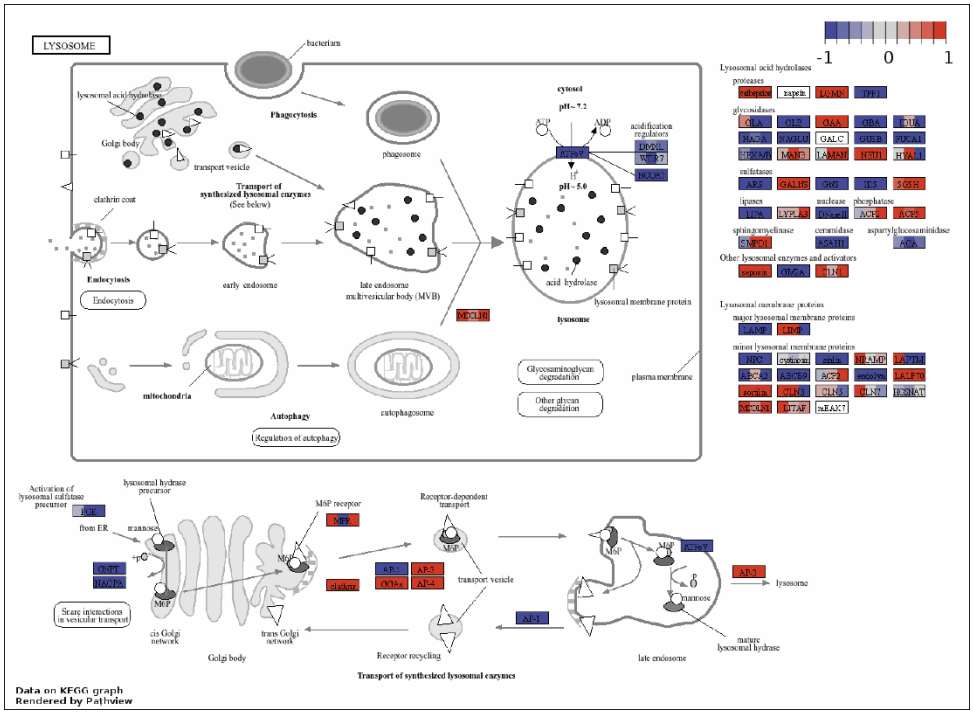

figure S4: Pathview analysis mapping DEGs onto the lysosome pathway in KEGG to illustrate expression patterns. Blue and red represent downregulated and upregulated genes, respectively.

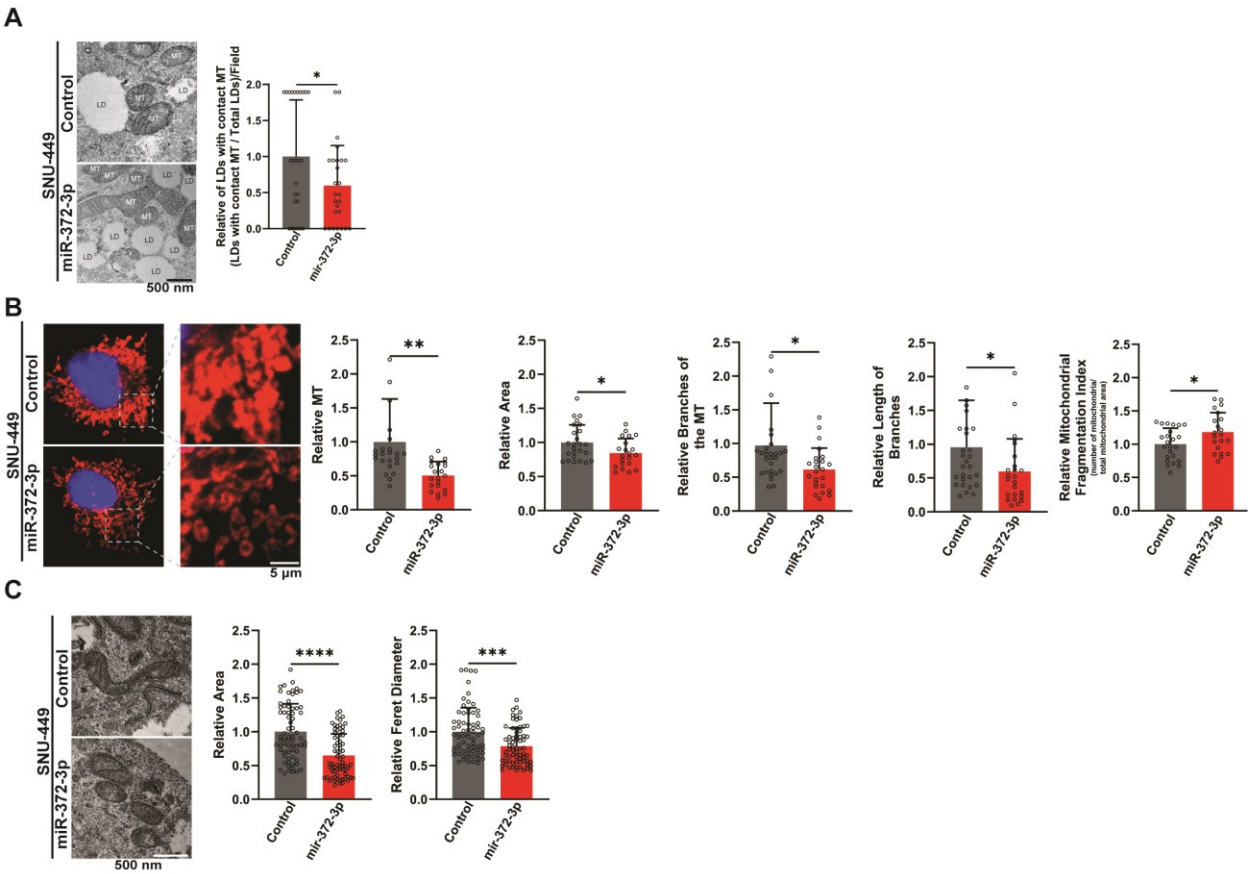

figure S5: Structural and morphological analysis of interaction between lipid droplets (LDs) and mitochondria (MT) and mitochondrial dynamics. (A) Analysis of LD-MT contact by transmission electron microscopy (TEM) micrographs showing LDs in contact with MT (left panel), with quantification of relative LD-MT contact per field (right panel, n = 26). (B) Confocal microscopy analysis of mitochondrial morphology maximum intensity projection (MIP) images illustrating mitochondrial fission in control and 372-OE HCC cells (left panel), with quantification of relative mitochondrial number and morphology (right panel, n = 21-29). (C) TEM analysis of mitochondrial structure with representative TEM micrographs showing mitochondrial structure (left panel), with quantification of relative mitochondrial areas and Feret diameter (right panel, n = 65-70). All experiments were quantified using quantified using EN 2.3 Lite and Fiji software, normalized to the control group, and analyzed using Student's t-test. Data are presented as mean ± SD, with significance levels indicated as *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

Table S1: Primer sequences used in this study.

Primer	Sequence 5→3′
Cloning primers	
F_EcoRI_miR-372-3p	GCTGAATTCACTTGCGATCGCCGCCTTG
R_BamHI_miR-372-3p	CGAGGATCCAGCCGCCCTCTGAACCTTC
Top_WT_3'UTR_*CPT1A*	CGCGGCCGCGGCTCATGCTACAGCGTCGTGAAAC
Bottom_WT_3'UTR *CPT1A*	TCGAGTTTCACGACGCTGTAGCATGAGCCGCGGCCGCGAGCT
Top_MUT_3'UTR *CPT1A*	CGCGGCCGCAGTATTCAAGAAGCAACCAGCACCC
Bottom_MUT_3'UTR_*CPT1A*	TCGAGGGTGCTGGTTGCTTCTTGAATACTGCGGCCGCGAGCT
Top_WT_3'UTR_*ACSL4*	CGCGGCCGCAGCAAAAGTGCTGCAGGGCTTTAC
Bottom_WT_3'UTR_*ACSL4*	TCGAGTAAAGCCCTGCAGCACTTTTGCTGCGGCCGCGAGCT
Top_MUT_3'UTR_*ACSL4*	CGCGGCCGCAGCAAAAGTGCGCGTCGTGAAAC
Bottom_MUT_3'UTR_*ACSL4*	TCGAGTTTCACGACGCGCACTTTTGCTGCGGCCGCGAGCT
Top_WT_3'UTR_*CPT2*	CGCGGCCGCAGCTGGGTGTGGTGGCATGC
Bottom_WT_3'UTR_*CPT2*	TCGAGCATGCCACCACACCCAGCTGCGGCCGCGAGCT
Top_MUT_3'UTR_*CPT2*	CGCGGCCGCAGCTGGGTGTGCGTCGTGAAAC
Bottom_MUT_3'UTR_*CPT2*	TCGAGTTTCACGACGCACACCCAGCTGCGGCCGCGAGCT
Top_mir-372-3p_Silencer	GATCCACGCTCAAATGTCGCAGCACTTTCCACACCAAAGTGCTGCGACATTTGAG CGTTTTTTTGGAAA
Buttom_miR-372-3p_Silencer	AGCTTTTCCAAAAAAACGCTCAAATGTCGCAGCACTTTGGTGTGGAAAGTGCTGC GACATTTGAGCGTG
qRT-PCR primers	
F_U6	CTCGCTTCGGCAGCACA
F_miR-372-3p	AGTGCTGCGACATTTGAGCG
R_universal_miR	CTCGCTTCGGCAGCACA
F_CPT1A	GGTGTCTAAATATCTCGCTGTGG
R_CPT1A	GGACACGTACTCTGGGTTATTC
F_ ACSL4	CCAAAGAACACCATTGCCATC
R_ ACSL4	AGCCTCAGATTCATTTAGCCC
F_ CPT2	TTGAGTGCTCCAAGTACCATG
R_ CPT2	GCAAACAAGTGTCGGTCAAAG
F_ ECI2	GACAGGGCAACATTTCATACAC
R_ ECI2	CCTCTCCCGCTGTTAACTTC
F_RPL19	GCTCTTTCCTTTCGCTGCT
R_RPL19	CATTGGTCTCATTGGGGTCT