

GP73 regulates Hepatic Steatosis by enhancing SCAP-SREBPs interaction

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Supplementary Information

Materials

Cell lines, cell culture and Transfection. HepG2 and 293T cell lines were purchased from the ATCC (Rockville, MD, USA). HL7702 cell line was purchased from the Chinese academy of science, Shanghai institute for cell resource center. HepG2 and 293T cells were grown in Dulbecco's modified Eagle's medium, and HL7702 cells were grown in RPMI 1640 medium. All media were supplemented with 10% fetal calf serum, together with penicillin, streptomycin, and L-glutamine. Lipofectamine 2000 reagent was used for transfection following the manufacturer's protocol (Invitrogen).

Reagents. tunicamycin (Tm), and other reagents were purchased from Sigma-Aldrich if not otherwise stated.

Supplementary Table S1: Table of antibodies

Antibody	Company	Catalog number	Dilution
GP73	Santa Cruz Biotechnology	sc-365817	1:500
SREBP1	Abcam	ab3259	1:500
SREBP2	ABclonal	A4123	1:1000
SCAP	Santa Cruz Biotechnology	sc-11355	1:500
α -Tubulin	Sigma	T6074	1:5000
GST	Proteintech	HRP-66001	1:2000
Flag	Sigma	F3165	1:5000
Myc	Sigma	M4439	1:5000

Supplementary Table S2: List of QRT-PCR primers used in this study

Name	Sequence (5'-3')
Mouse GP73	Forward: CGTCGCAGCATGAAGTCTC Reverse: CAGTAGTTGAAGCCTAGCACAAT
Human GP73	Forward: TGGCCTGCATCATCGTCTTG Reverse: CCCTGGAACTCGTTCTTCTTCA
Human SREBP1	Forward: ACAGTGACTIONCCCTGGCCTAT Reverse: GCATGGACGGGTACATCTTCAA
Human HMGR	Forward: GCTGGTAGGGAGTCAGAAGGA Reverse: TGGTGGTTTTCCGGGTCTTG
Human HMGCS1	Forward: GATGTGGGAATTGTTGCCCTT Reverse: ATTGTCTCTGTTCCAACCTCCAG
Human HMGCS2	Forward: GACTCCAGTGAAGCGCATTCT Reverse: CTGGGAAGTAGACCTCCAGG
Human ACC1	Forward: ATGTCTGGCTTGCACCTAGTA Reverse: CCCCAAAGCGAGTAACAAATTCT
Human FASN2	Forward: AAGGACCTGTCTAGGTTTGATGC Reverse: TGGCTTCATAGGTGACTTCCA
Human ACSS2	Forward: AAAGGAGCAACTACCAACATCTG Reverse: GCTGAACTGACACACTTGGAC

Human actin	Forward: AAGGAGCCCCACGAGAAAAAT Reverse: ACCGAACTTGCATTGATTCCAG
Mouse Edem	Forward: GGGGCATGTTCGTCTTCGG Reverse: CGGCAGTAGATGGGGTTGAG
Mouse HMGCR	Forward: TG TTCACCGGCAACAACAAGA Reverse: CCGCGTTATCGTCAGGATGA
Mouse actin	Forward: ATGACCCAAGCCGAGAAGG Reverse: CGGCCAAGTCTTAGAGTTGTTG

Supplementary Figure

(S1) SREBP-1 promoter activity of HL7702 cells after transfected with Flag-vector or Flag-GP73. The luciferase activity was measured 36 hrs post transfection and normalized based on transfection efficiency.

(S2) Quantification of Filipin intensities for cells in Fig. 1j using ImageJ. All values were normalized based on those of control images.

(S3,S4) QRT-PCR analysis of HMGSC1 in HepG2 (S3) or 293T (S4) cells transfected with Flag-vector or Flag-GP73 for the 24 hrs.

(S5,S6) QRT-PCR analysis of HMGSC2 in HepG2 (S5) or 293T (S6) cells transfected with Flag-vector or Flag-GP73 for the 24 hrs.

(S7,S8) QRT-PCR analysis of ACSS2 in HepG2 (S7) or 293T (S8) cells transfected with Flag-vector or Flag-GP73 for the 24 hrs.

(S9) Immunoblotting analysis of GP73 expression in Ctrl-1, Ctrl-2, GP73-1, and GP73-2 cells transfected with Flag-GP73 or Flag-vector. α -Tubulin was used as equal

loading control.

(S10) Signal quantification of confocal immunofluorescence images shown in Fig. 4b.

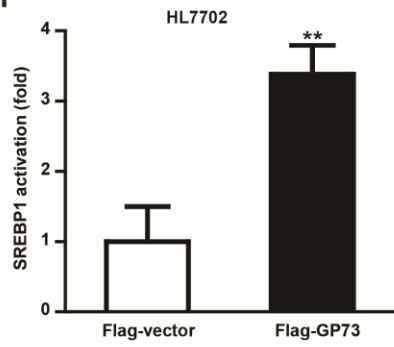
(S11) Representative confocal immunofluorescence images of GP73 localized in Golgi apparatus in Ctrl-1, Ctrl-2, GP73-1, and GP73-2 cells. SCAP, red; GM130, green. DAPI : blue Scale bar: 10 μ m.

Cell-based studies were performed at least three independent times with comparable results. Data represent mean \pm SEM. Student's t test was used for statistical analysis:

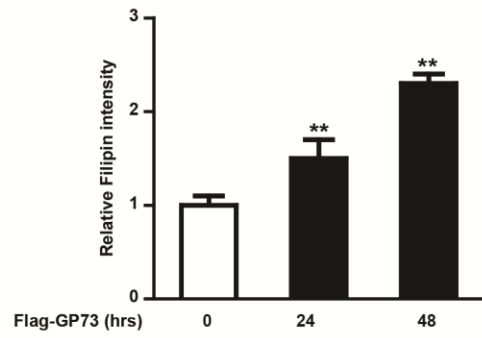
**p < 0.01.

Supplementary Figure

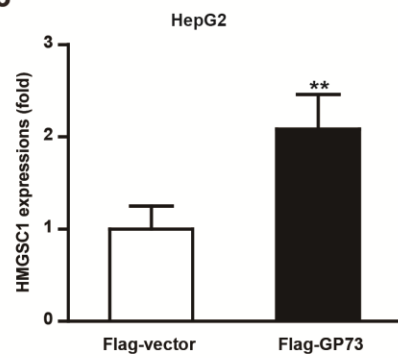
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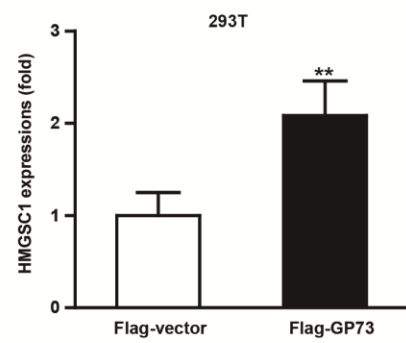
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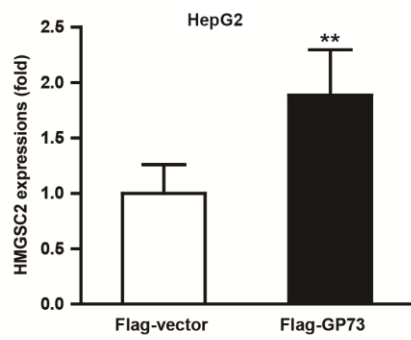
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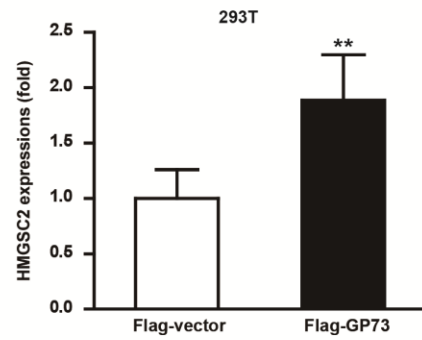
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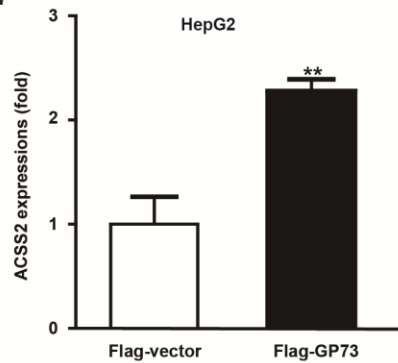
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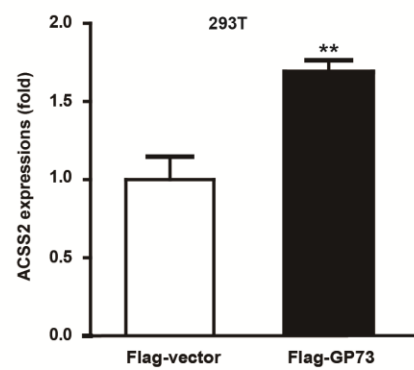
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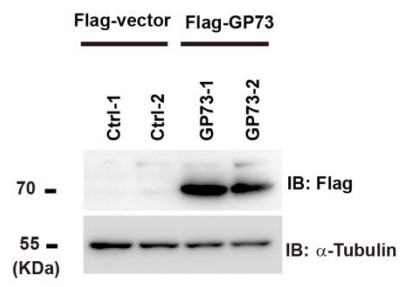
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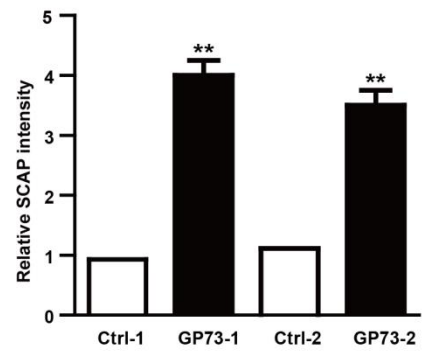
S8



S9



S10



S11

