

mTOR inhibitor combined with NAC inhibits hepatic fibrosis

Table S1. Primer used for PCR

Target gene	Forward primer	Reverse primer	Product size (bp)
α -SMA	TGTGCTGGACTCTGGAGATG	GATCACCTGCCCATCAGG	292
Collagen type I	TTCCCTGGACCTAAGGGTACT	TTGAGCTCCAGCTTCGCC	113
MMP-2	CCCATACTTTACTCGGACCA	TGACCTTGACCAGAACACCA	420
MMP-9	AAATGTGGGTGTACACAGGC	TTCACCCGGTTGTGGAACT	309
MMP-13	CCCTCGAACACTCAAATGGT	GAGCTGCTTGCCAGGTTTC	312
TIMP-1	GGTTCCCTGGCATAATCTGA	GTCATCGAGACCCCAAGGTA	246
GAPDH	CCATCACCATCTTCCAGGAG	GCATGGACTGTGGTCATGAG	322

Abbreviation α -SMA: alpha-smooth muscle actin, GAPDH: Glyceraldehyde 3-phosphate dehydrogenase, MMP: matrix metalloproteinase, TIMP: tissue inhibitor metalloproteinase.

Table S2. List of antibodies indicating the dilution for each use

Antibody	Company, Cat NO.	Dilution
Phospho-Akt	Cell signaling, #9271	1:1000
Akt	Cell signaling, #9272	1:1000
Phospho-p70 S6 Kinase	Cell signaling, #9205	1:1000
p70S6 Kinase	Cell signaling, #9202	1:1000
Collagen alpha1	LifeSpan BioScience, #LS-C150353	1:1000
β -actin	Santa Cruz, #sc-47778	1:1000

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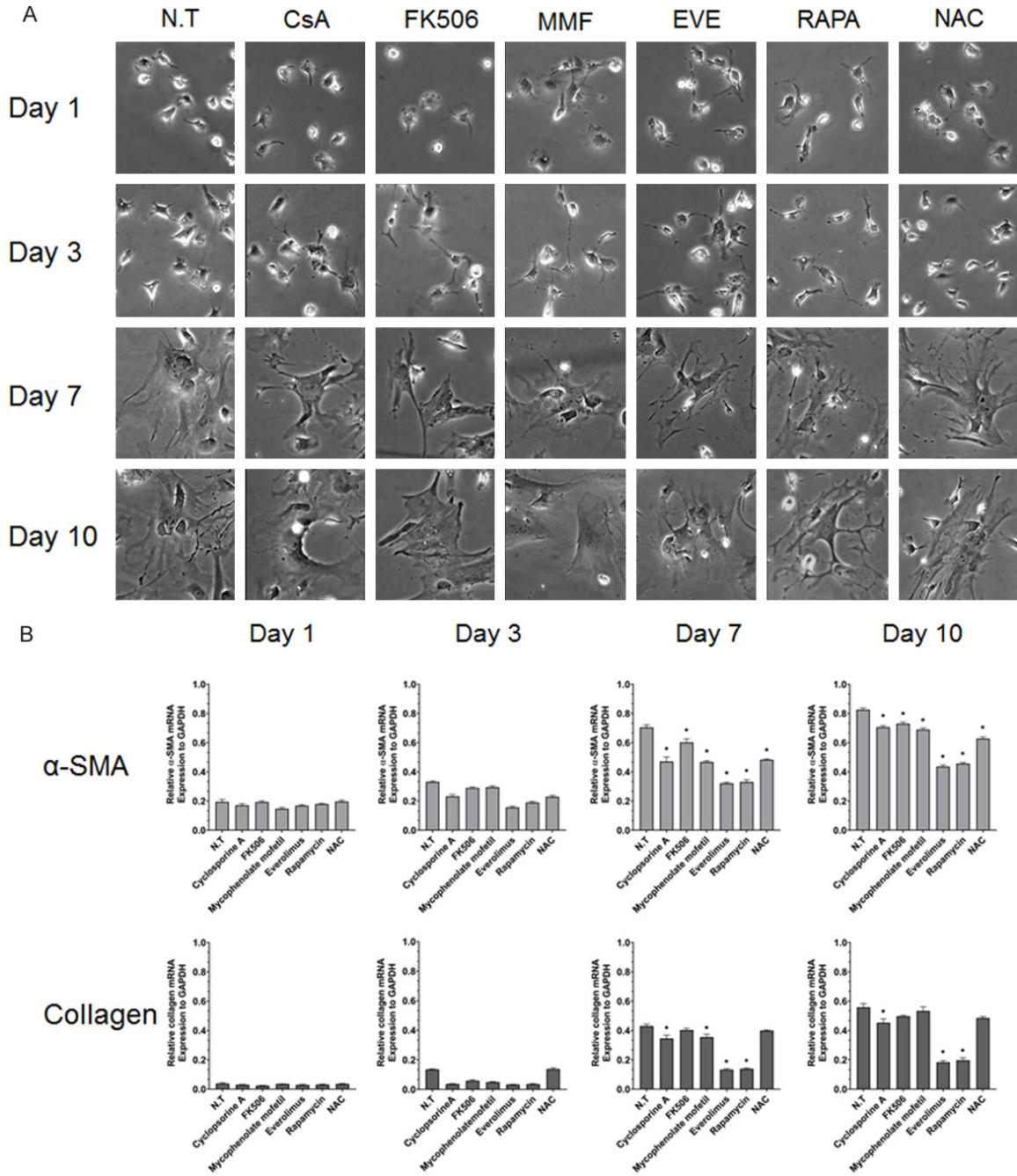


Figure S1. The effect of various immunosuppressive drugs on the activation of hepatic stellate cells. A. Morphological observation of drug-treated HSCs. HSCs increased in size and exhibited a myofibroblast-like phenotype. Morphological observation conducted at 200 times magnification. B. HSCs were treated with immunosuppressants and antioxidants for 10 d and mRNA expression of hepatic fibrosis-related genes was analyzed by RT-PCR. The mRNA level of each gene was normalized to that of glyceraldehyde 3-phosphate dehydrogenase. Values are shown as mean \pm SEM. *P < 0.05 vs. non-treatment group.

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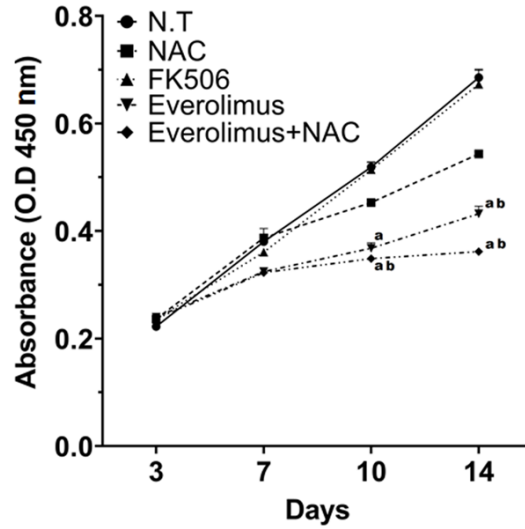


Figure S2. Treatment with everolimus and NAC inhibits the growth of HSCs. The effect of combination treatment on HSC viability was evaluated by a cell counting (CCK-8) assay. The cells were exposed to NAC, FK506, EVE, or EVE+NAC for 14 d. Data are represented as the mean \pm SEM of three independent experiments. ^aindicates $P < 0.05$ vs. non-treatment group, ^bindicates $P < 0.05$ for NAC treatment group vs. EVE+NAC treatment group at the same time point.

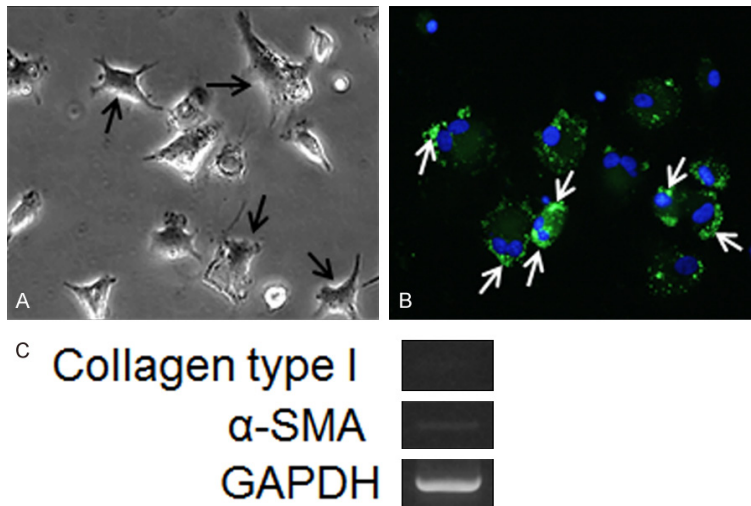


Figure S3. A. Phase-contrast image of rat HSCs in culture. Arrows (black) indicate cell bodies. Star-like morphology was observed. B. Quiescent HSCs exhibiting lipid droplets within the HSCs. HSCs incubated with BODIPY to stain the lipid vesicles green. Arrows (white) indicate lipid vesicles. C. In quiescent HSCs, fibrogenesis genes are downregulated. Morphological observation conducted at 200 times magnification.