SUPPLEMENTAL DATA

Article Title: Cinnabarinic acid provides hepatoprotection against non-alcoholic fatty liver disease

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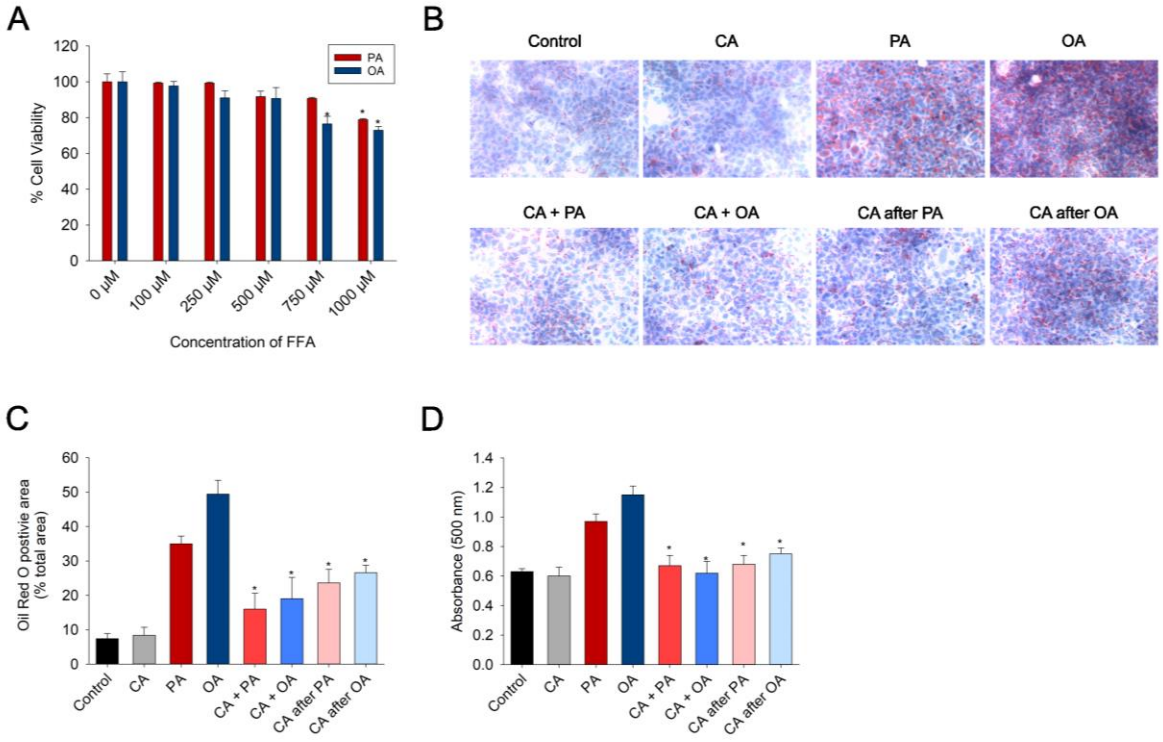
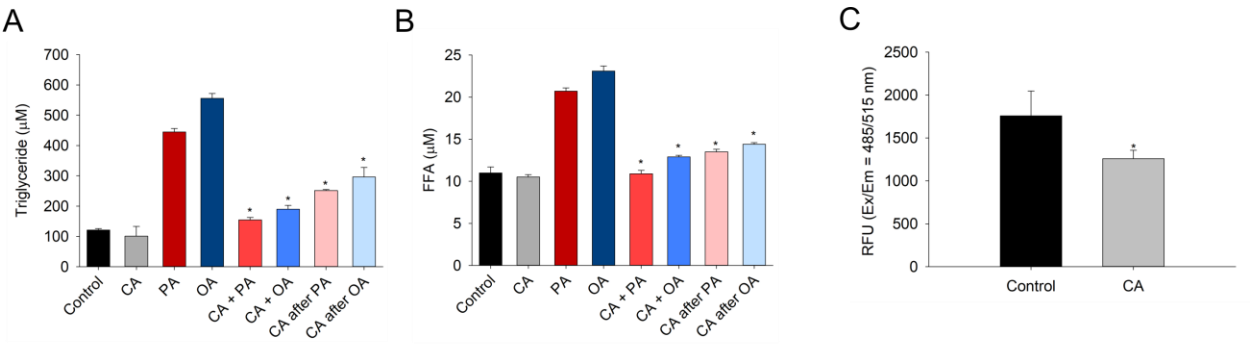
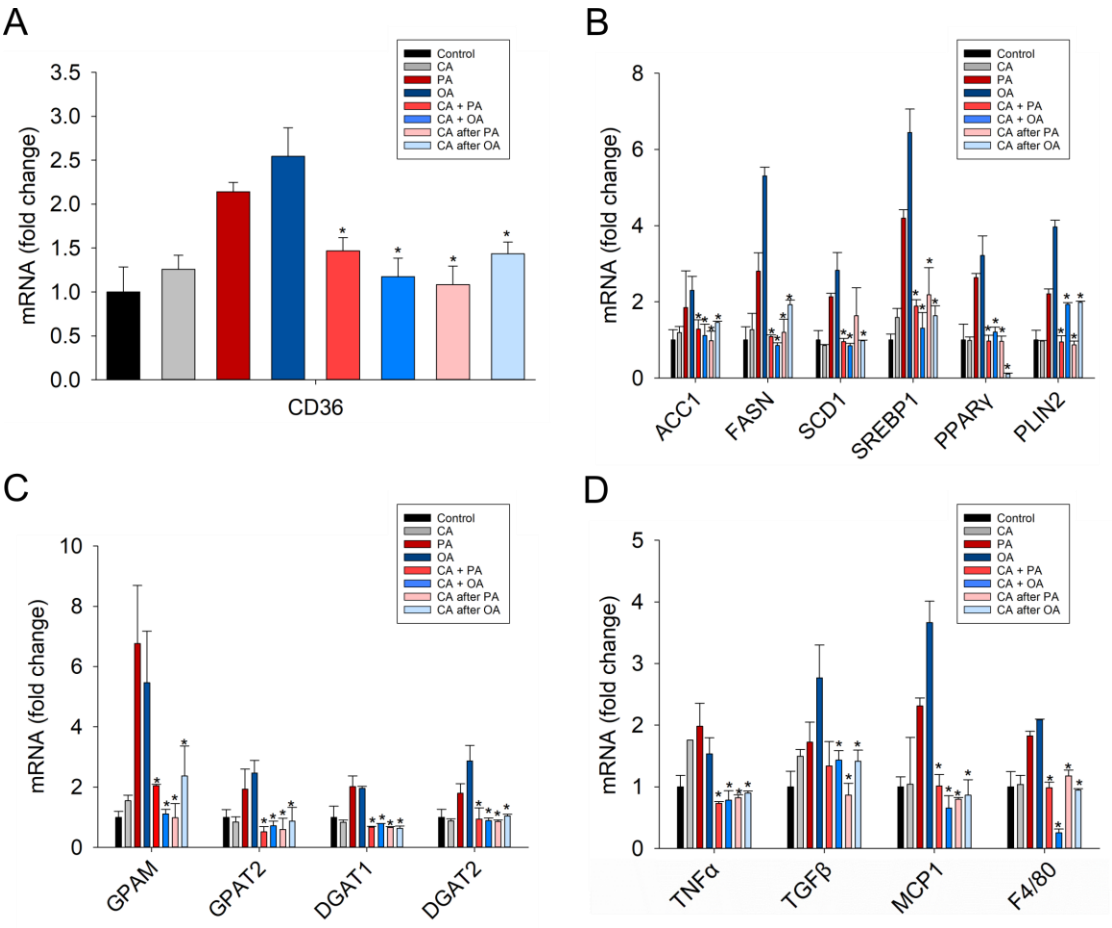


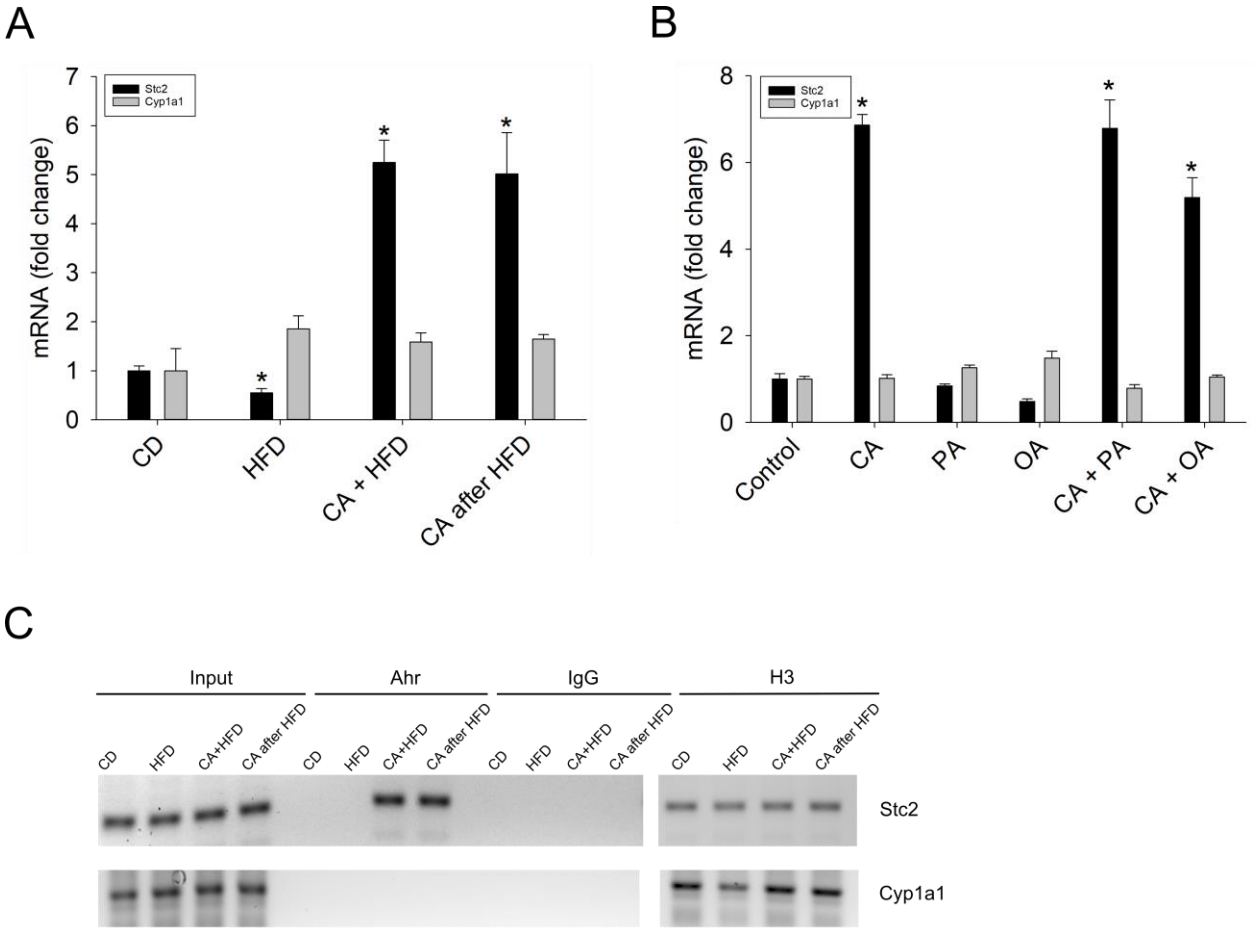
Supplemental Figure 1. Determination of (A) cell viability of AML12 cells treated with different concentrations of palmitic acid (PA) and oleic acid (OA) for 24 hrs. Cell viability was measured by a luminescent assay and expressed relative to BSA-treated control. Data are represented as mean ± SD (n=3). *p<0.05 compared to control group. (B) CA protects against palmitic acid (PA)/oleic acid (OA)-induced steatosis. Representative images of oil red O stained AML12 cells treated with 500µM BSA + DMSO (control), 30µM CA, 500µM PA, 500µM OA, 30µM CA+ 500µM PA/OA, 30µM CA after 500µM PA/OA. (C) Quantification of oil red O-stained images; area of stained lipid droplets was determined using ImageJ and normalized to the total area (3 images per treatment) (D) quantification of accumulated oil red O by colorimetry; absorbance measured at 500 nm. Data are represented as mean ± SD (n=3). *p<0.05 compared to PA/OA-only treatment group.



Supplemental Figure 2. Quantification of (A) triglyceride, (B) free fatty acid and (C) free fatty acid uptake in AML12 cells treated with 500μM BSA + DMSO (control), 30μM CA, 500μM PA, 500μM OA, 30μM CA+ 500μM PA/OA, 30μM CA after 500μM PA/OA. Triglyceride content was measured using luminescence assay, whereas free fatty acid content and free fatty acid uptake was determined fluorometrically. Data are represented as mean + SD (n=3). *$p<0.05$ compared to PA/OA-only treatment group.



Supplemental Figure 3. Expression of mRNAs encoding genes involved in (A) free fatty acid transport, (B) fatty acid synthesis, (C) triglyceride synthesis, and (D) inflammation. AML12 cells were treated with 500μM BSA+ DMSO (control), 30μM CA, 500μM PA, 500μM OA, 30μM CA+ 500μM PA/OA , 30μM CA after 500μM PA/OA. mRNA message was analyzed by qRT-PCR and normalized to 18S rRNA. Results are expressed as fold of the value found in control treatment arbitrarily set at 1. For statistical analysis, a mixed-effects multivariate ANOVA (MANOVA) model was used. After an overall significant F test from MANOVA model, the post hoc multiple-comparison tests were performed for the pre-specified comparisons adjusted by Tukey procedure. Data are represented as mean ± SD (n=3). *p<0.05 compared to PA/OA-only treatment group.



Supplemental Figure 4. CA treatment activates AhR signaling by upregulating Stc2 expression. mRNA expression of Stc2 and Cyp1a1 measured by qRT-PCR in (A) *in vivo* and (B) *in vitro* models of NAFLD, normalized to 18S rRNA. Data are represented as mean ± SD. *p<0.05 compared to control diet(CD)/control group. (B) ChIP analysis of AhR binding to Stc2 and Cyp1a1 promoters in mice liver tissue. IgG and histone H3 antibodies were used as negative and positive controls respectively. The XRE clusters in the Stc2 and Cyp1a1 promoters (Patil et al., 2022) were PCR-amplified and PCR products separated and visualized on 5% polyacrylamide gels.

Supplemental Table 1. Primer sequences for quantitative RT-PCR

Species	Gene	Forward Primer	Reverse primer
Human	ACAA1	GCGGTTCTCAAGGACGTGAAT	GTCTCCGGGATGTCACTCAGA
Mouse	ACAA1	CCAACATTGCTGGTGGCATC	CCCATCCCAGACAGGGACAT
Human	ACADL	TGCAATAGCAATGACAGAGCC	CGCAACTACAATCACAACATCAC
Mouse	ACADL	TGCACACATACAGACGGTGC	CATGGAAGCAGAACCGGAGT
Human	ACC1	GCAGGTCACACGTCTCTTTAT	CCAGCCTGTCATCCTCAATATC
Mouse	ACC1	TAACAGAATCGACACTGGCTGGCT	ATGCTGTTCCTCAGGCTCACATCT
Human	ACOX1	TGCTGATGAAGTATGCCCAGGTGA	TCCCACAAGGAAGGACCTGACAAA
Mouse	ACOX1	TCATGTGGTTTAAAAACTCTGTGC	GCAGGAACATGCCCAAGTGA
Human	CD36	AAACGGCTGCAGGTCAACCTATTG	TCATCACCAATGGTCCCAGTCTCA
Mouse	CD36	TCATGCCAGTCGGAGACATGCTTA	AACTGTCTGTACACAGTGGTGCCT
Human	CPT1A	GCAAAGGCGACATCAATCCGAACA	ACCAAAGGCTACGAATGGGAAGGA
Mouse	CPT1A	GTCCCTCCAGCTGGCTTATC	CATGCGGCCAGTGGTGTCTA
Human	CPT2	GCTGCCTATTCCCAAACTTG	CATGCAGTTCTTTTCCAATCCC
Mouse	CPT2	TCGTACCCACCATGCACTAC	CTTCTGTCTTCCTGAACTGGCT
Human	DGAT1	CCTACCGCGATCTCTACTACTT	GGGTGAAGAACAGCATCTCAA
Mouse	DGAT1	AACCTGGCCACAATCATCTGCTTC	ATGATGCCAGAGCAAACACGGAAC
Human	DGAT2	ATTGCTGGCTCATCGCTGT	GGGAAAGTAGTCTCGAAAGTAGC
Mouse	DGAT2	TTCTGCACAGACTGCTGGCTGATA	TCACCAGCTGGATGGGAAAGTAGT
Human	F4/80	CAGACCAAGGAGTGGAATGTAG	GCCTTCTGGATTGGGATGAA
Mouse	F4/80	TCAAATGGATCCAGAAGGCTCCCA	TGCACTGCTTGGCATTGCTGTATC
Human	FASN	TACGACTACGGCCCTCATTT	CCATGAAGCTCACCCAGTTATC
Mouse	FASN	GGTGTGGTGGGTTTGGTGAATTGT	TTGCTGAGGTTGGACAGCAGGATA
Human	GPAM	CTAGCAAGTCCTGTGCCATTA	CGACCAATGTGGAGAGATCAA
Mouse	GPAM	ATGAAACGCACACAAGGCAC	CCCTTATGGACGTCTCGCTC
Human	GPAT2	TGTGGTCGTCAGGCTTTGG	GGTCCGTTATGCTTCTGTGGA
Mouse	GPAT2	GCACATACCCACCAGTTTTGA	AGGATACGCTGTACCTCTTTCT
Human	MCP1	TCGCTCAGCCAGATGCAATCAATG	CACAGCTTCTTTGGGACACTTGCT
Mouse	MCP1	TCACCTGCTGCTACTCATTCACCA	AGCACAGACCTCTCTCTTGAGCTT
Human	MOGAT1	AGGCCATGAAGGTAGAGTTTG	CCCGAGCAGCAGGTATTT
Human	PLIN2	TTGCAGTTGCCAATACCTATGC	CCAGTCACAGTAGTCGTCACA
Mouse	PLIN2	CAGCTCTCCTGTTAGGCGT	CGGAGGACACAAGGTCGTAG
Human	PPARγ	TACTGTCGGTTTCAGAAATGCC	GTCAGCGGACTCTGGATTCAG
Mouse	PPARγ	AGGGCGATCTTGACAGGAAAGACA	AAATTCGGATGGCCACCTCTTTGC
Human	RNA18S	GGACAGGATTGACAGATTGAT	AGTCTCGTTCGTTATCGGAAT
Mouse	RNA18S	CTCAACACGGGAAACCTCAC	CGCTCCACCAACTAAGAACG
Human	SCD1	AACTGGTGATGTTCCAGAGGAGGT	CGCAAGAAAGTGGCAACGAACACA
Mouse	SCD1	CAGGTTTCCAAGCGCAGTTC	ACTGGAGATCTCTTGGAGCA
Human	SREBP1C	GGAGCCATGGATTGCACTTT	TCCCAGCATAGGGTGGGTCAAATA

Mouse	SREBP1C	GGAGCCATGGATTGCACATT	GGCCCGGGAAGTCACTGT
Human	TGFβ	GGAAATTGAGGGCTTTCGCC	CCGGTAGTGAACCCGTTGAT
Mouse	TGFβ	TAAAGAGGTCACCCGCGTGCTAAT	ACTGCTTCCCGAATGTCTGACGTA
Human	TNFα	GCCCATGTTGTAGCAAACCCTCAA	GTTATCTCTCAGCTCCACGCCATT
Mouse	TNFα	TAGCCCACGTCGTAGCAAAC	ACAAGGTACAACCCATCGGC

Supplemental Table 2. Primer sequences for ChIP

Species	**Gene**	**Forward Primer**	**Reverse primer**
Mouse	CYP1A1	CTATCTCTTAAACCCCACCCCAA	CTAAGTATGGTGGAGGAAAGGGTG
Mouse	STC2	CTCAGTCCATTCGGCCATTGCCC	AGGAAGCGGAGCGCCTCCGC