

Supplementary Materials

Koji Ishida, Kosuke Kaji, Shinya Sato, Hiroyuki Ogawa, Hirotetsu Takagi, Hiroaki Takaya, Hideto Kawaratani, Kei Moriya, Tadashi Namisaki, Takemi Akahane, Hitoshi Yoshiji

Department of Gastroenterology, Nara Medical University, Kashihara, Nara 634-8521, Japan

Address correspondence to:

Kosuke Kaji M.D., Ph.D.

Department of Gastroenterology, Nara Medical University

840 Shijo-cho, Kashihara, Nara 634-8521, Japan

E-mail: kajik@naramed-u.ac.jp

1. Supplementary figure legend

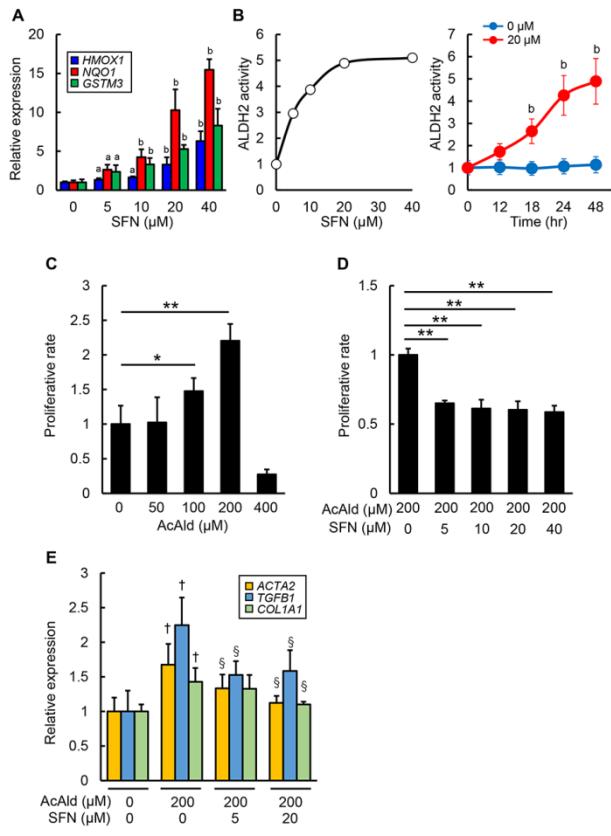
2. Supplementary Table. 1

Supplementary figure legend

Supplementary figure 1. Effects of sulforaphane on *in vitro* acetaldehyde-stimulated rat HSC activation.

(A) Relative mRNA expression levels of *HMOX1*, *NQO1* and *GSTM3* in HepG2 cells. The HepG2 cells were cultured with sulforaphane (SFN) (0, 5, 10, 20 and 40 μ M) for 24 h. (B) Dose- and Time-dependent effects of SFN on ALDH2 activity in HepG2 cells. The cells were cultured for 24 hr in dose-dependent assay and at 0 or 20 μ M of SFN in time-dependent assay.

(C) Cell proliferation of HSC-T6 cells stimulated by acetaldehyde (AcAld) (0, 50, 100, 200 and 400 μ M). (D) Cell proliferation of HSC-T6 cells co-incubated with 200 μ M of AcAld and treated with SFN (0, 5, 10, 20 and 40 μ M). (E) The effects of SFN on the mRNA expressions of *ACTA2*, *TGFB1*, *COL1A1* in the AcAld-stimulated HSC-T6 cells. The cells were cultured with AcAld (200 μ M) and SFN (0, 5 and 20 μ M) for 24 h. Quantitative values are relatively indicated as fold changes to the values of non-treatment groups. The mRNA expression levels were measured by qRT-PCR, and *GAPDH* was used as internal control for qRT-PCR. Data are mean \pm SD (n=8) (A-C). * p <0.05; ** p <0.01, indicating a significant difference between groups (A and B). † p <0.05; § p <0.05, indicating a significant difference compared with AcAld (0 μ M)/SFN (0 μ M) and AcAld (200 μ M)/SFN (0 μ M), respectively.



Supplementary Table 1. List of primers used in q-PCR

Gene	Sense (5'-3')	Antisense (5'-3')
Mouse		
<i>Aldh2</i>	GAGCAGAGCCATGTCATGTG	TGTCACACATCCAGGCATCT
<i>Hmox1</i>	AACAAGCAGAACCCAGTCTATGC	AGGTAGCGGGTATATGCGTGGGCC
<i>Nqo1</i>	CAAGTTGGCCTCTCTGTGG	AAGCTGCGTCTAACTATATGT
<i>Gstm3</i>	CCCCAACTTGACCGAACG	GGTGTCATAACTGGTTCTCCA
<i>Nox1</i>	AAGCCATTGGATCACAAACCTCAC	ATCCATGGCCTGTTGGCTTC
<i>Nox2</i>	CCTTAGGCACTCAAGGCTGGTC	CTTGTCAGGGCAACAATT
<i>Nox4</i>	CCAGAATGAGGATCCCAGAA	ACCACCTGAAACATGCAACA
<i>Cd68</i>	TTCTGCTGTGAAATGCAAG	AGAGGGGCTGGTAGGTTGAT
<i>Lbp</i>	GGCTGCTGAATCTCTTCCAC	GAGCGGTGATTCCGATTAAA
<i>Tlr4</i>	GGCAGCAGGTGGAATTGTAT	AGGCCAGAGTTTGTCT
<i>Cd14</i>	GTCAGGAACTCTGGCTTGC	TGGCTTTACCCACTGAACC
<i>Tnfa</i>	ACGGCATGGATCTCAAAGAC	AGATAGCAAATCGGCTGACG
<i>Il1β</i>	GCCCATCCTCTGTGACTCAT	AGGCCACAGGTATTTGTCG
<i>Ccl2</i>	AGGTCCCTGTCATGCTTCTG	TCTGGACCCATTCTTCTTG
<i>Acta2</i>	CTGACAGAGGCACCACTGAA	CATCTCCAGAGTCCAGCACA

<i>Tgfb1</i>	TTGCTTCAGCTCCACAGAGA	TGGTTGTAGAGGGCAAGGAC
<i>Col1a1</i>	GAGCGGAGAGTACTGGATCG	GCTTCTTTCCTGGGTTTC
<i>Gapdh</i>	CTGCGACTTCAACAGCAACT	GAGTTGGATAGGCCTCTC
Human		
<i>HMOX1</i>	TCCGATGGGTCTTACACTC	TAAGGAAGCCAGCCAAGAGA
<i>NQO1</i>	TTACTATGGGATGGGGTCCA	TCTCCCATTTCAGGCAAC
<i>GSTM3</i>	CGCTCTTGCTTGCTTTT	TCTCCAAGTGTGCAATCTCG
<i>ACTA2</i>	GAGACCCTGTTCCAGCCATC	TACATAGTGGTGCCCCCTGA
<i>TGFB1</i>	GGGACTATCCACCTGCAAGA	CCTCCTGGCGTAGTAGTCG
<i>COL1A1</i>	CCAAATCTGTCTCCCCAGAA	TCAAAAACGAAGGGGAGATG
<i>BAMBI</i>	GGCAGCATCACAGTAGCATC	GATGCCACTCCAGCTACAT
<i>NOX1</i>	TTAACAGCACGCTGATCCTG	CTGGAGAGAATGGAGGCAAG
<i>NOX2</i>	TCACTCCTCCACCAAAACC	GGGATTGGGCATTCTTTAT
<i>NOX4</i>	CTTCCGTTGGTTGCAGATT	TGGGTCCACAACAGAAAACA
<i>GAPDH</i>	CCAAGGAGTAAGACCCCTGG	TGGTTGAGCACAGGGTACTT