

Supplementary Information

The hypertension drug Verapamil activates Nrf2 by promoting p62-dependent autophagic Keap1 degradation and prevents acetaminophen-induced cytotoxicity

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Supplementary Figure Legends

Supplementary Fig.1. Verapamil activates Nrf2 without causing cytotoxicity in MEF cells.

(A) Cell viability was estimated using a Cell titer-Glo assay kit. The live cell number was expressed as the absorbance at Luminescence in MEF cells. qRT-PCR analysis for mRNAs of GSTA1 (B), HO-1 (C), Nqo-1 (D). Data are presented as means \pm SD from three independent experiments. * $p < 0.05$ and N.S = not significant.

Supplementary Fig.2. Verapamil mediates Keap1 degradation via autophagic pathway in MEF cells.

(A) Lysates of MEF cells treated with Vera (100 μ M) for the indicated times were subjected to immunoblot analysis with antibodies to Keap1, LC3-II, and β -actin (loading control). Densitometric analysis of Keap1 immunoblots was obtained in (B) and LC3-II/ β -actin (D). qRT-PCR analysis for mRNAs of Keap1 (C), GSTA1 (E), HO-1(F), and Nqo-1 (G). Data are presented as means \pm SD from three independent experiments. * $p < 0.05$.

Supplementary Fig.3. Verapamil induces Nrf2 activation in Hepa1c1c7 cells. qRT-PCR

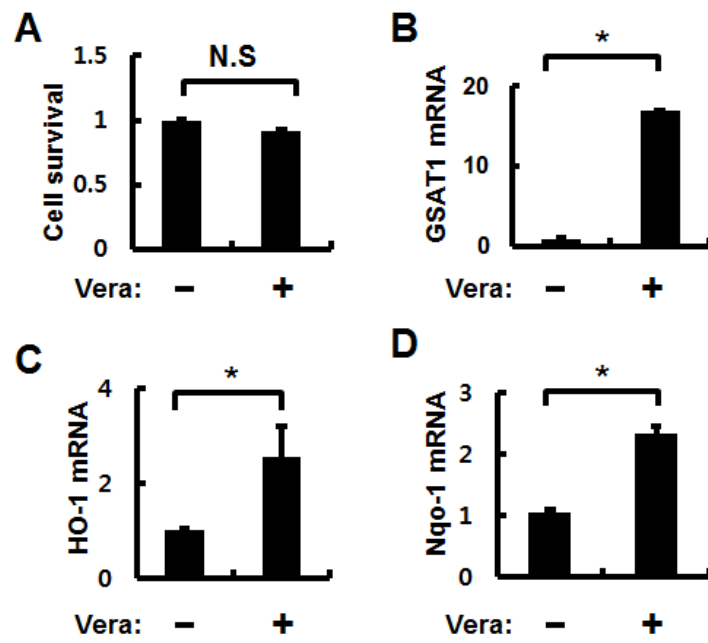
analysis for mRNAs of GSTA1 (A), HO-1 (B), and Nqo-1(C) in Hepa1c1c7 cells treated with Vera (100 μ M) for the indicated times. Data are presented as means \pm SD from three independent experiments. * $p < 0.05$.

Supplementary Fig.4. Verapamil increases autophagic flux in Hepa1c1c7 cell. (A) Lysates

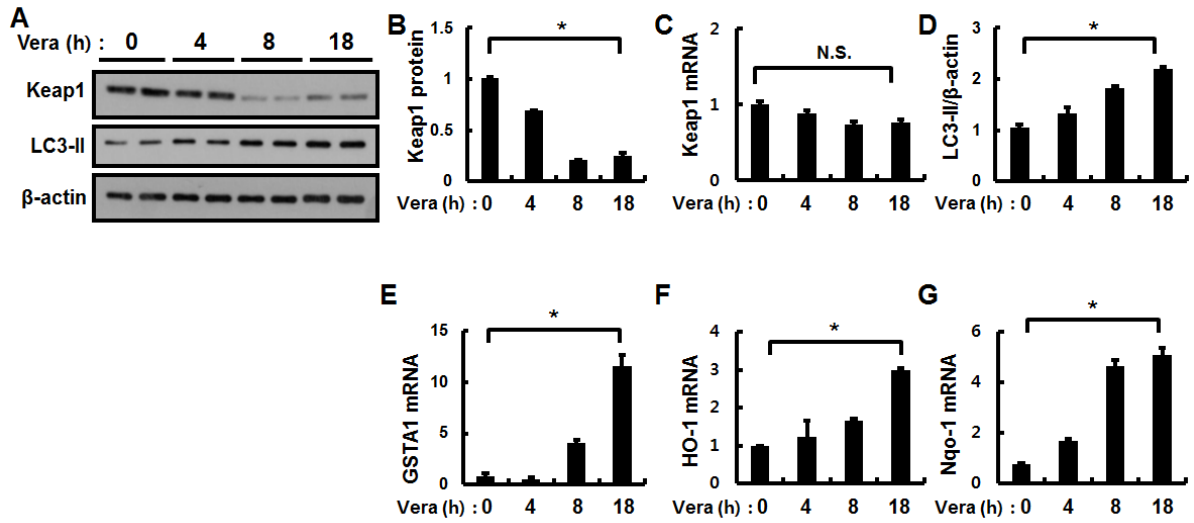
of Hepa1c1c7 cells treated with bafilomycin A1 (100 nM) and Vera (100 μ M) were subjected to immunoblot analysis with antibodies to LC3-II and β -actin (loading control). Densitometric analysis of Keap1 immunoblots was obtained in (B). Data are presented as means \pm SD from three independent experiments. * $p < 0.05$.

Supplementary Fig.5. Verapamil phosphorylates p62 at Ser351 in Hepa1c1c7 cells. (A) Lysates of Hepa1c1c7 cells treated with Vera (100 μ M) for the indicated times were subjected to immunoblot analysis with antibodies to p62 (p-S351) and β -actin (loading control).

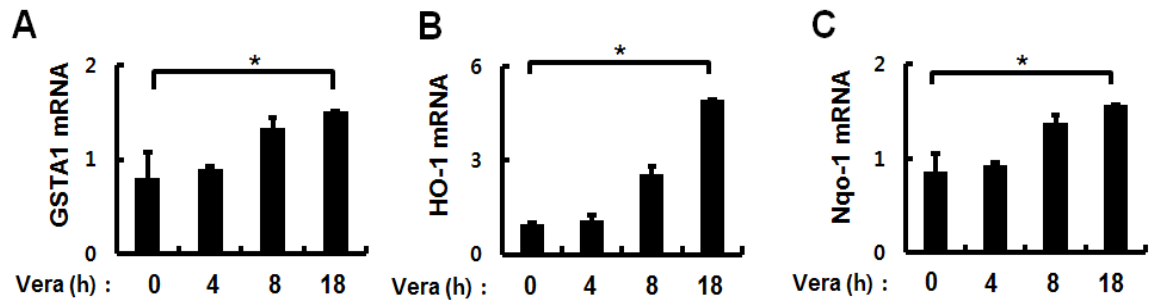
Supplementary Fig.6. Verapamil prevents APAP-induced apoptotic cell death in Hepa1c1c7 cells. Hepa1c1c7 cells pretreated with Vera and incubated in the absence or presence of APAP. Total RNA isolated from the cells was subjected to qRT-PCR analysis for mRNAs of HO-1. Data are presented as the means \pm SD from three independent experiments. * $P < 0.05$.



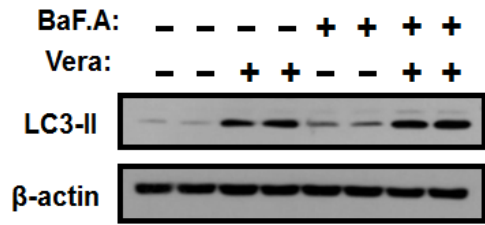
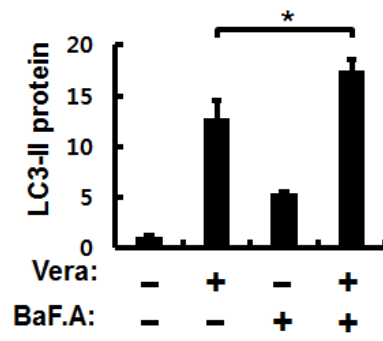
Supplementary Fig.1.

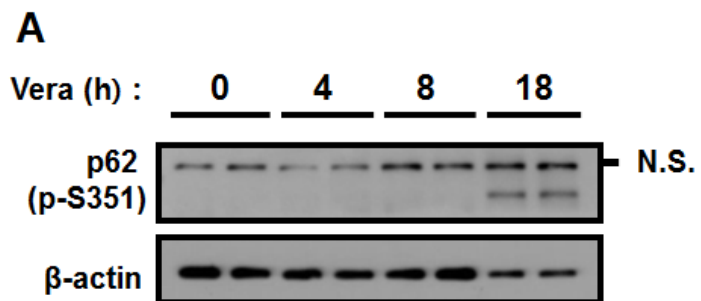


Supplementary Fig.2.

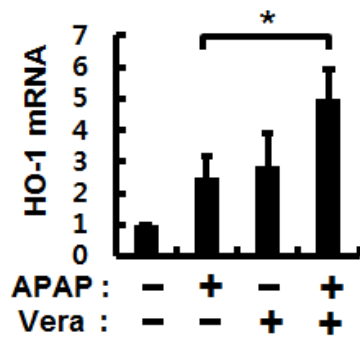


Supplementary Fig.3.

A**B****Supplementary Fig.4**



Supplementary Fig.5



Supplementary Fig.6