

SUPPLEMENTAL MATERIAL

SUPPLEMENTAL FIGURE LEGENDS

Figure SI. 2-CIHA induces apoptosis of RAW 264.7 macrophages. A. Cells were treated with indicated concentrations of HA or 2-CIPA for 24h, and the apoptotic cells were determined under fluorescent microscopy after TUNNEL staining. B. Caspase 3 activity in cells treated with indicated concentrations of HA or 2-CIHA for 24h. N=3. *P<0.05 and **P<0.01 vs. Blank.

Figure SII. 2-CIHA induces mRNA expression of ER stress-associated proteins. THP-1 cells (A) or RAW 264.7 cells (B) were treated with 50 μ M of HA or 2-CIHA for 18h, and the mRNA levels of indicated proteins were determined by qRT-PCR. N=3. **P<0.01.

Figure SIII. 2-CIHA induces XBP1 mRNA splicing in RAW 264.7 cells. Cells were treated with 50 μ M of HA or 2-CIHA for 18h, and XBP1 mRNA splicing was determined by qRT-PCR.

Table SI. Primer sequences

Gene	Forward	Reverse	Genebank ID
Human ATF3 ¹	GCCATCCAGAACAAGCACCT	GGCTACCTCGGCTTTTGTGAT	NM_001674
Human CHOP	TTGACCCCTGCTTCTCTGGCTT	GCTCTGGGAGGTGCTTGTA	NM_001195056.1
Human GAPDH	CACTCCTCCACCTTTGACGCT	CCTGTTGCTGTAGCCAAATTCGT	NM_002046
Human GRP78	ATCACGCCGTCCTATGTCG	TTGGCGTCAAAGACCGTGT	NM_005347
Human XBP1 ¹	GGGAATGAAGTGAGGCCAGT	TGAAGAGTCAATACCGCCAGA	NM_005080
Mouse ATF3	CCTCCTGGGTCACTGGTATTTG	AGGGGTCTGTTGTTGACGGTAA	NM_007498.3
Mouse β -Actin	CCAGCCTTCCTTCTGGGTAT ^a	GAGGTCTTTACGGATGTCAACGT	NM_007393
	ACCTTCTACAATGAGCTGCG ^b	CTGGATGGCTACGTACATGG	
Mouse CHOP ²	GGCATCACCTCCTGTCTGTC	CTACCTCAGTCCCCTCTC	NM_007837
Mouse GRP78 ³	ACCTATTCCTGCGTCGGTGT	GCATCGAAGACCGTGTCTC	NM_001163434
Mouse XBP1 ²	GAACCAGGAGTTAAGAACACG	AGGCAACAGTGCAGAGTCC	NM_013842

^afor realtime PCR. ^bfor XBP1.

REFERENCES

1. Galluzzi L, De Santi M, Crinelli R, De Marco C, Zaffaroni N, Duranti A, Brandi G, Magnani M. Induction of endoplasmic reticulum stress response by the indole-3-carbinol cyclic tetrameric derivative ctet in human breast cancer cell lines.

PLoS One. 2012;7:e43249

2. Son SH, Goo YH, Chang BH, Paul A. Perilipin 2 (plin2)-deficiency does not increase cholesterol-induced toxicity in macrophages. *PLoS One*. 2012;7:e33063
3. Hosogai N, Fukuhara A, Oshima K, Miyata Y, Tanaka S, Segawa K, Furukawa S, Tochino Y, Komuro R, Matsuda M, Shimomura I. Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. *Diabetes*. 2007;56:901-911

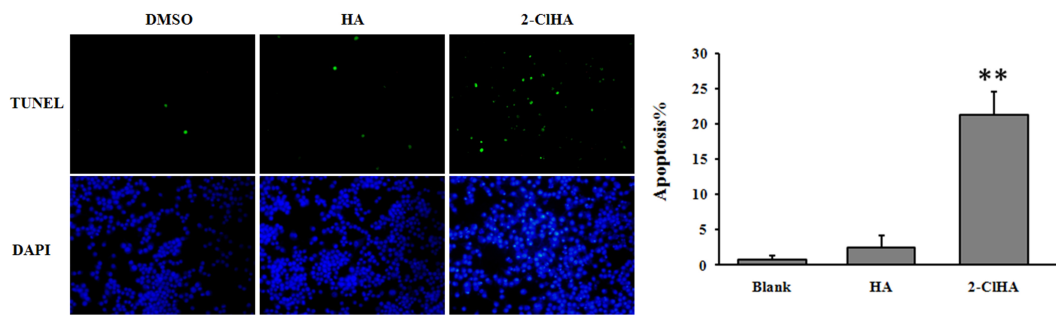
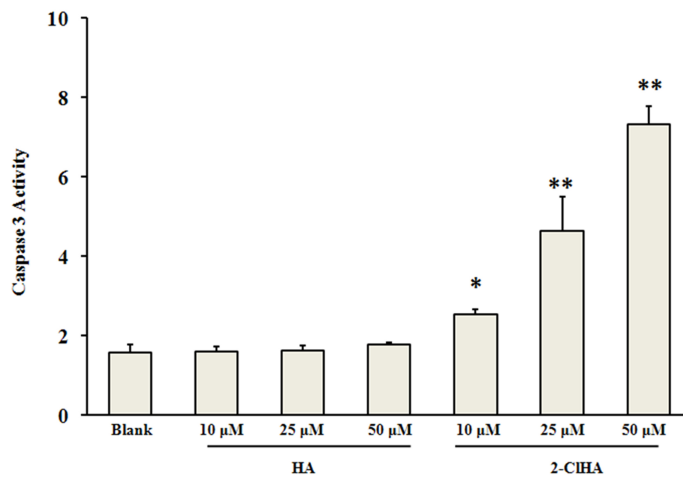
A**B**

Figure S1. 2-CIHA induces apoptosis of RAW 264.7 macrophages. A. Cells were treated with indicated concentrations of HA or 2-CIPA for 24h, and the apoptotic cells were determined under fluorescent microscopy after TUNNEL staining. B. Caspase 3 activity in cells treated with indicated concentrations of HA or 2-CIHA for 24h. N=3. *P<0.05 and **P<0.01 vs. Blank.

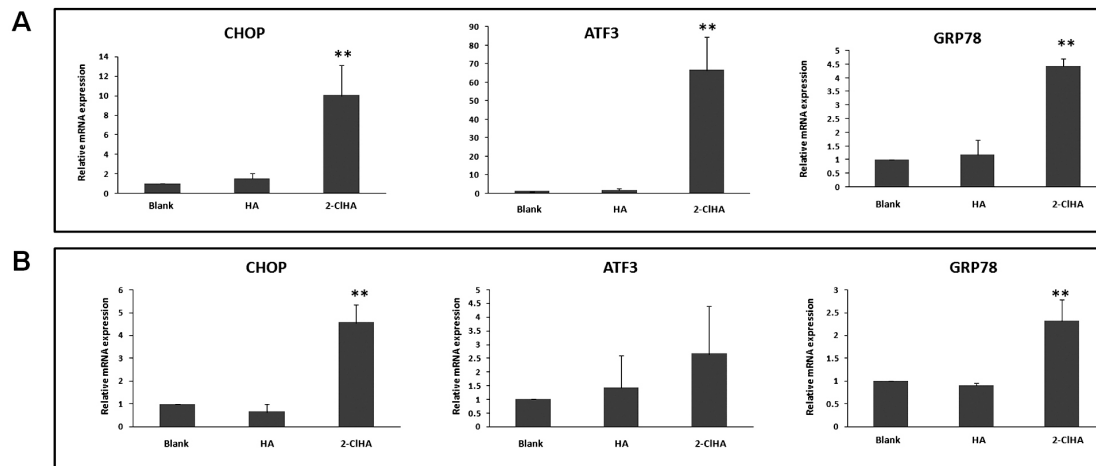


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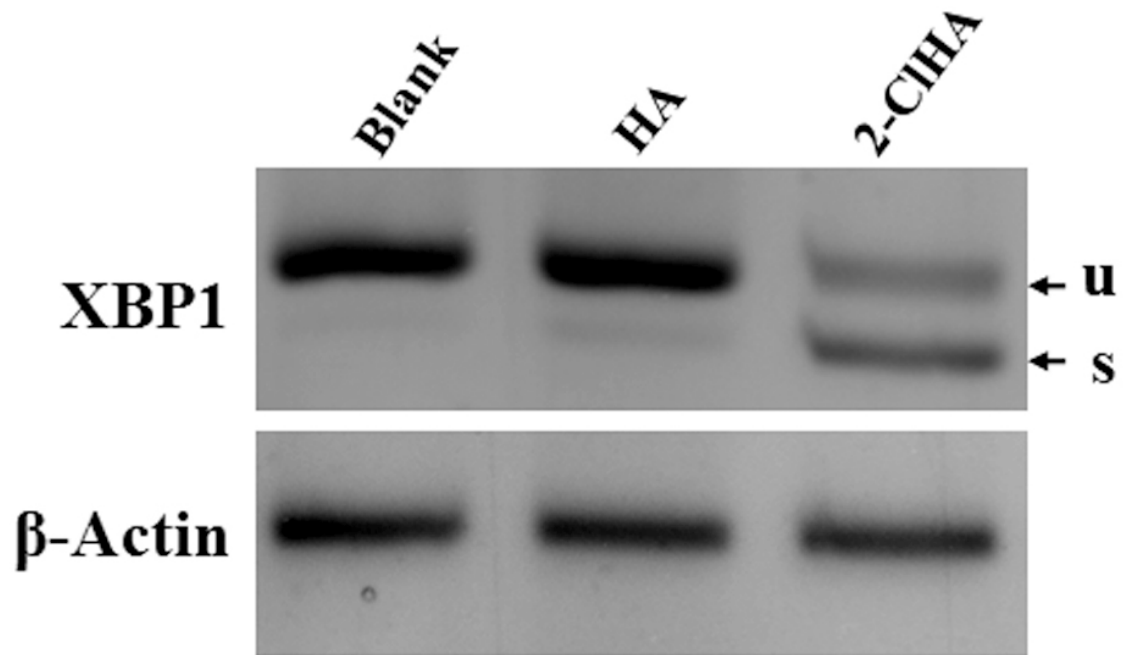


Figure SIII. 2-CIHA induces XBP1 mRNA splicing in RAW 264.7 cells. Cells were treated with 50 μ M of HA or 2-CIHA for 18h, and XBP1 mRNA splicing was determined by qRT-PCR.