Long Isoforms of NRF1 Negatively Regulate Adipogenesis via Suppression of PPARγ Expression

Peng Xue, Yongyong Hou, Zhuo Zuo, Zhendi Wang, Suping Ren, Jian Dong, Jingqi Fu, Huihui Wang, Melvin E. Andersen, Qiang Zhang, Yuanyuan Xu, Jingbo Pi

Table S1：Primer sequences for RT-qPCR

Gene	Forward (5'-3')	Reverse (5'-3')
Nrf1-All isoforms (Primer-A)	CAGCAAGTGAGATTCTGTACAATGC	TGACATTCTGATTGATGGGAGTGT
Nrf1-Long isoforms (Primer-L)	AACGATCGAACGGCTCAACT	GGAGCGAAAGCCACATTCAC
Nrf2	CGAGATATACGCAGGAGAGGTAAGA	GCTCGACAATGTTCTCCAGCTT
Cebpα	CGCAAGAGCCGAGATAAAGC	CGGTCATTGTCACTGGTCAACT
Cebpβ	AAGCTGAGCGACGAGTACAAGA	GTCAGCTCCAGCACCTTGTG
Cebpδ	GCCGTGCCCACCCTAGA	CGCTTGTGGTTGCTGTTGA
Pparγ1	GGGCTGAGGAGAAGTCACAC	TGGTTCACCGCTTCTTTCA
Pparγ2	TGCTGTTATGGGTGAAACTCTG	CTGTGTCAACCATGGTAATTTCTT
aP2	GCGTGGAATTCGATGAAATCA	CCCGCCATCTAGGGTTATGA
Lpl	CATGGATGGACGGTAACGGG	TTCTCTCTTGTACAGGGCGG
Pgc1α	CCGTAGGCCCAGGTACGA	TGCGGTATTCATCCCTCTTGA
Pgc1β	GGCCTTGTGTCAAGGTGGAT	GGTGCTTATGCAGTTCCGTACA
Adipsin	GCTATCCCAGAATGCCTCGTT	TTCCACTTCTTTGTCCTCGTATTG
Ho1	CCTCACTGGCAGGAAATCATC	CCTCGTGGAGACGCTTTACATA
Nqo1	TATCCTTCCGAGTCATCTCTAGCA	TCTGCAGCTTCCAGCTTCTTG
Glut4	CGCACTAGCTGAGCTGAAGGA	AGGAGCTGGAGCAAGGACATT
Gclc	TGGCCACTATCTGCCCAATT	GTCTGACACGTAGCCTCGGTAA
Gclm	ACATTGAAGCCCAGGATTGG	CCCCTGCTCTTCACGATGAC
18s	CGAACGTCTGCCCTATCAACTT	CCGGAATCGAACCCTGATT

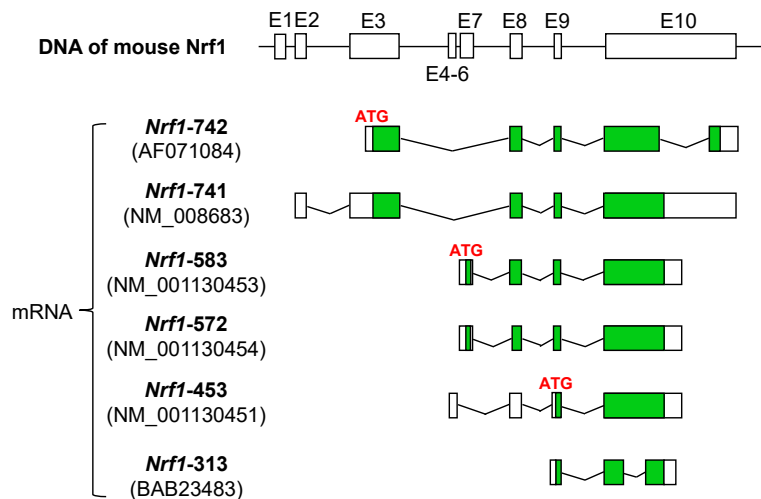**Table S2: Primers for cloning *of Pparγ2* promoter-Luciferase reporters**

Primer name	Sequences (5'- 3')	Positions of inserts
Pparγ2-Reverse	aaacggtaaAGATCTgctctgggtcaacaggagaa	
Pparγ2-F2601	tgtaacactGATATCgggcaaaagaagggcg	-2516 ~ +85 bp
Pparγ2-F1842	tgtaacactGATATCggcctacacatgccaagcaaa	-1757 ~ +85 bp
Pparγ2-F1411	tgtaacactGATATCcagtgatcccccatcatttgg	-1326 ~ +85 bp
Pparγ2-F934	tgtaacactGATATCcttgccaacctagcagggta	-849 ~ +85 bp
Pparγ2-F352	tgtaacactGATATCgtgggtcactggcgagacaat	-267 ~ +85 bp
Pparγ2-F258	tgtaacactGATATCcaactggctctcagtcagga	-173 ~ +85 bp
Pparγ2-F138	tgtaacactGATATCcgatgaccataagcctttttc	-53 ~ +85 bp

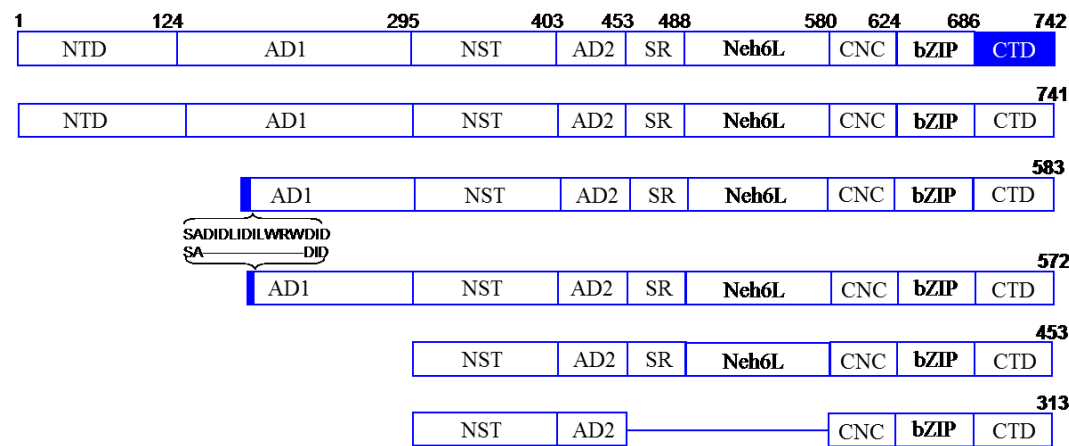Note: AGATCT, *BGL*II site; GATATC, *EcoR*V site; Red text, target sequences on *Pparg2* promoter.

Table S3: Primer sequences for ChIP PCR

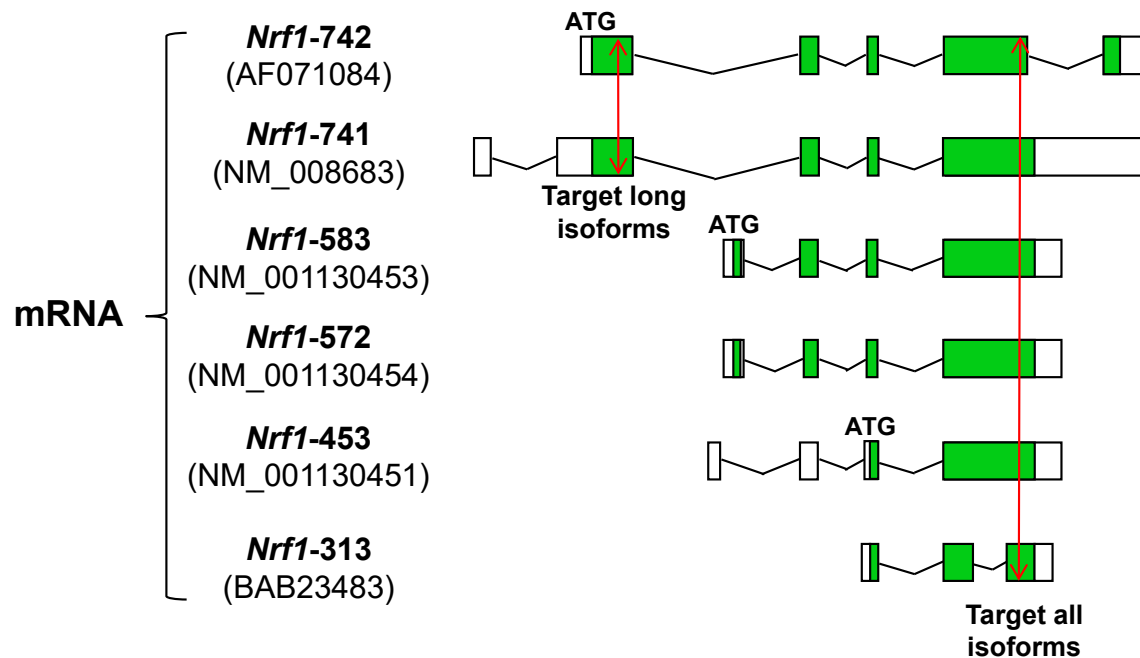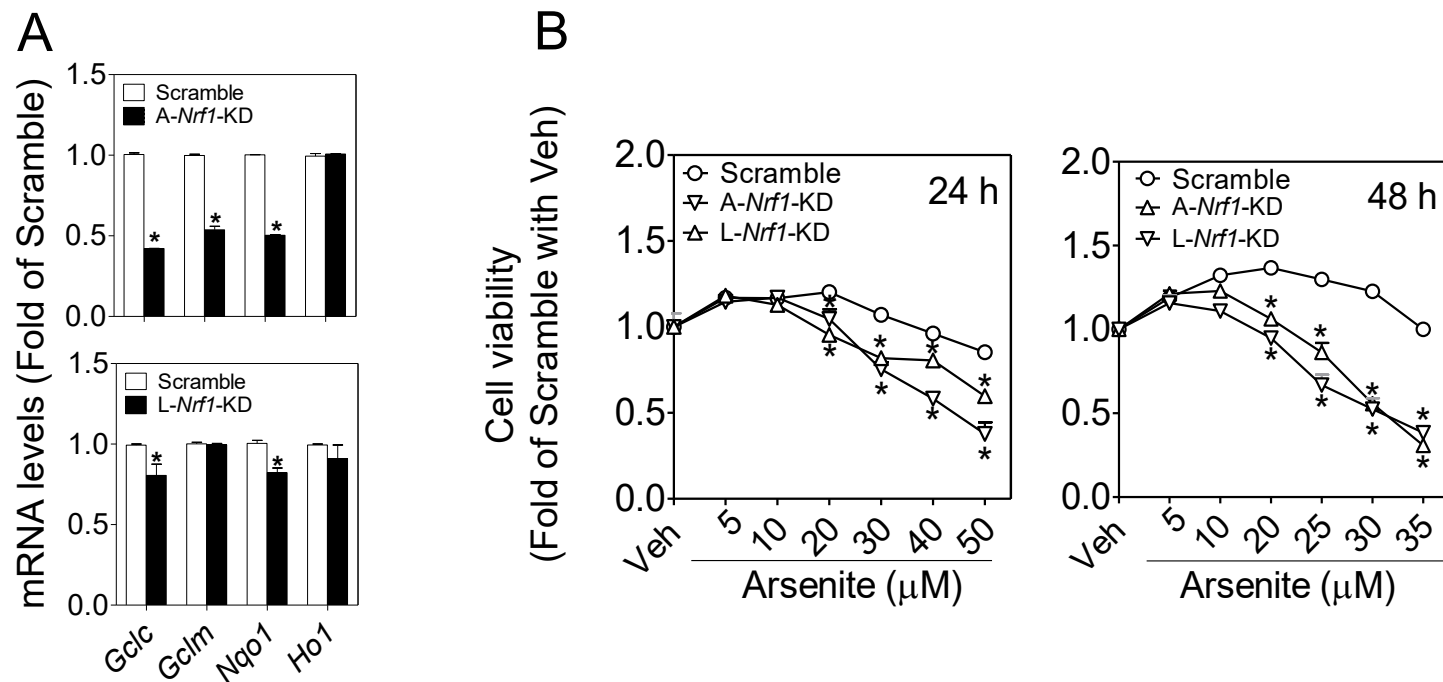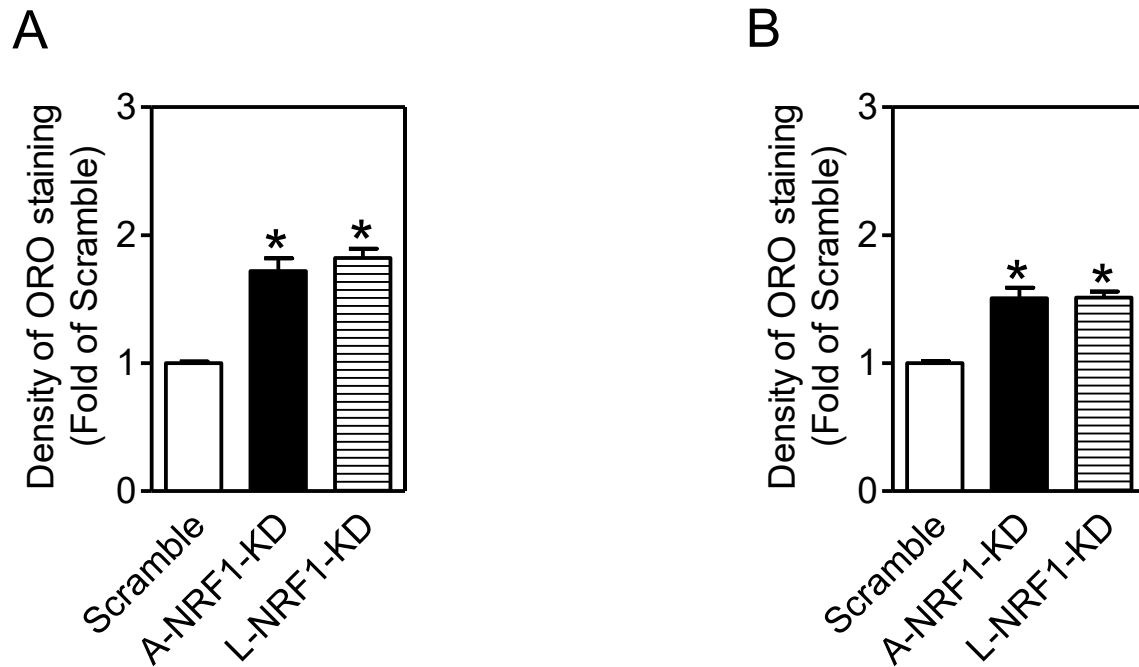| Primer name	Sequences (5'- 3')	Positions of *Ppary2* promoter
Primer1-F	GCTCTGTGCCAGGCTCTGTG	-2943 ~ -2500 bp
Primer1-R	CGCCCTTCTTTTGCCC	
Primer2-F	GGGCAAAAGAAGGGCG	-2516 ~ -2071 bp
Primer2-R	AAGTGGGGGCTGGTCTAGCA	
Primer3-F	TGCTAGACCAGCCCCCACTT	-2091 ~ -1664 bp
Primer3-R	TCCTGTCCCCAGATCCTGGTT	
Primer4-F	GGCCTACACATGCCAAGCAAA	-1757 ~ -1312 bp
Primer4-R	ATGGGGGATCACTGTGTTGGA	
Primer5-F	CAGTGATCCCCCATCATTTGG	-1326 ~ -830 bp
Primer5-R	ACCCTGCTAGGTTGGCAAGGA	
Primer6-F	CTTGCCAACCTAGCAGGGTA	-849 ~ -464 bp
Primer6-R	TGGTTGAAACTCCTAATCACACA	
Primer7-F	TGGCCAAATACGTTTATCTGGTG	-440 ~ -31 bp
Primer7-R	GGAAAAAGGCTTATGGTCATCGAG	
Primer8-F	GTGGGTCACTGGCGAGACAAT	-268 ~ +154 bp
Primer8-R	TGCAGCAACATCAGGAATGGA	

A

B

Fig. S1. (A) Schematic diagram of mouse *Nrf1* genomic sequence (Gene ID: ENSMUSG00000038615) and depiction of different isoforms of *Nrf1* transcripts. Sequences are from the National Center for Biotechnology (www.ncbi.hlm.nih.gov) and Ensembl release 97 - July 2019 (www.ensemble.org). Green and white open boxes represent coding regions and untranslated regions, respectively. Solid black lines represent introns and 5'-flanking regions. The numbers in parentheses under each isoform are the accession numbers in GenBank. **(B)** Schematic protein structures of different isoforms of mouse NRF1. Abbreviations: NTD, N-terminal domain; AD1, acidic domain 1; NST, Asn/Ser/Thr-rich; AD2, acidic domain 2; SR, serine-repeat; Neh6L, Neh6-like; CNC, Cap 'n' collar; bZIP, basic-region zipper; CTD, C-terminal domain. Solid boxes represent unique sequences different from other isoforms. All the sequences are from the National Center for Biotechnology (www.ncbi.nlm.nih.gov). The ID numbers for mouse isoform-742, -741, -583, -572, -453 and -313 are AF071084, NM_008683, NM_001130453, NM_001130454, NM_001130451 and BAB23483, respectively.

Fig. S2. The target sites of shRNAs against mouse *Nrf1*. Sequences are from the National Center for Biotechnology (www.ncbi.hlm.nih.gov) and Ensemble Genome Browser (www.ensemble.org). Green and white open boxes represent coding regions and untranslated regions, respectively. Solid black lines represent introns. Solid red lines with arrowhead are the target sites of the shRNAs used. The numbers in parentheses under each isoform are the accession numbers in GenBank.
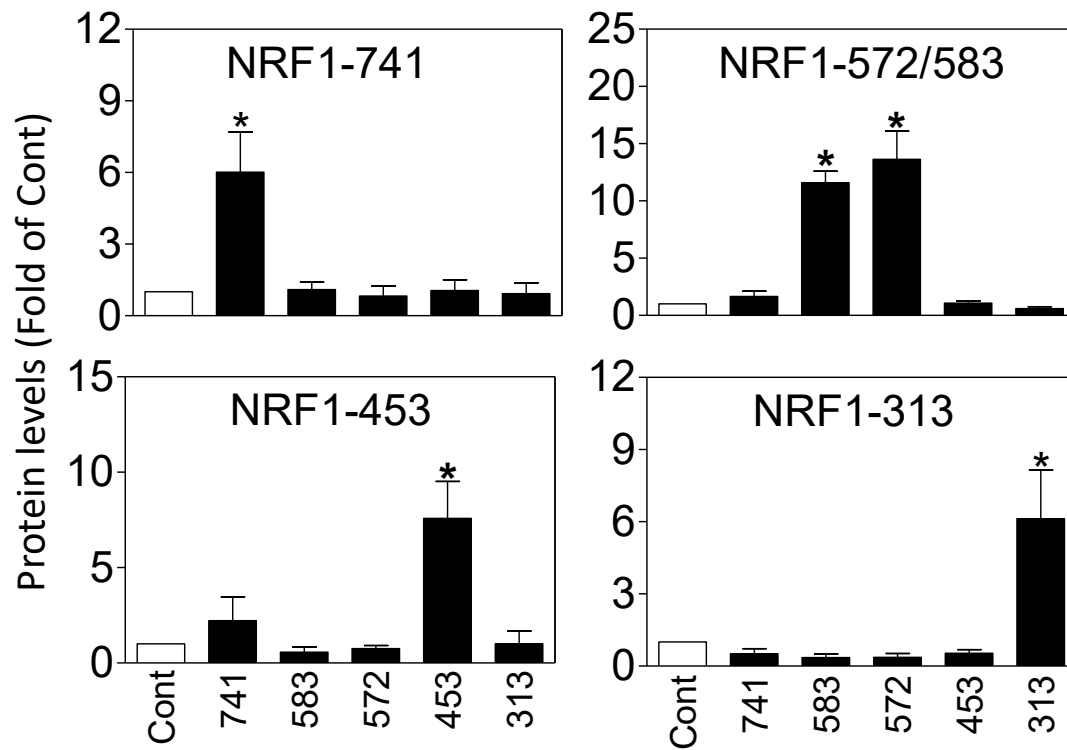
Fig. S3. Stable knockdown of *Nrf1* reduces the expression of antioxidant genes and increases sensitivity to arsenite-induced cytotoxicity in 3T3-L1 preadipocytes. (A) mRNA expression of ARE-dependent antioxidant genes. A-*Nrf1*-KD, knockdown of all isoforms of *Nrf1*; L-*Nrf1*-KD, knockdown of long isoforms of *Nrf1*. n = 3. *$p < 0.05$ vs. Scramble. (B) Cell viability following arsenite exposure for 24 and 48 hours. n = 3. *$p < 0.05$ vs. Scramble with the same treatment. Veh, Vehicle (medium).
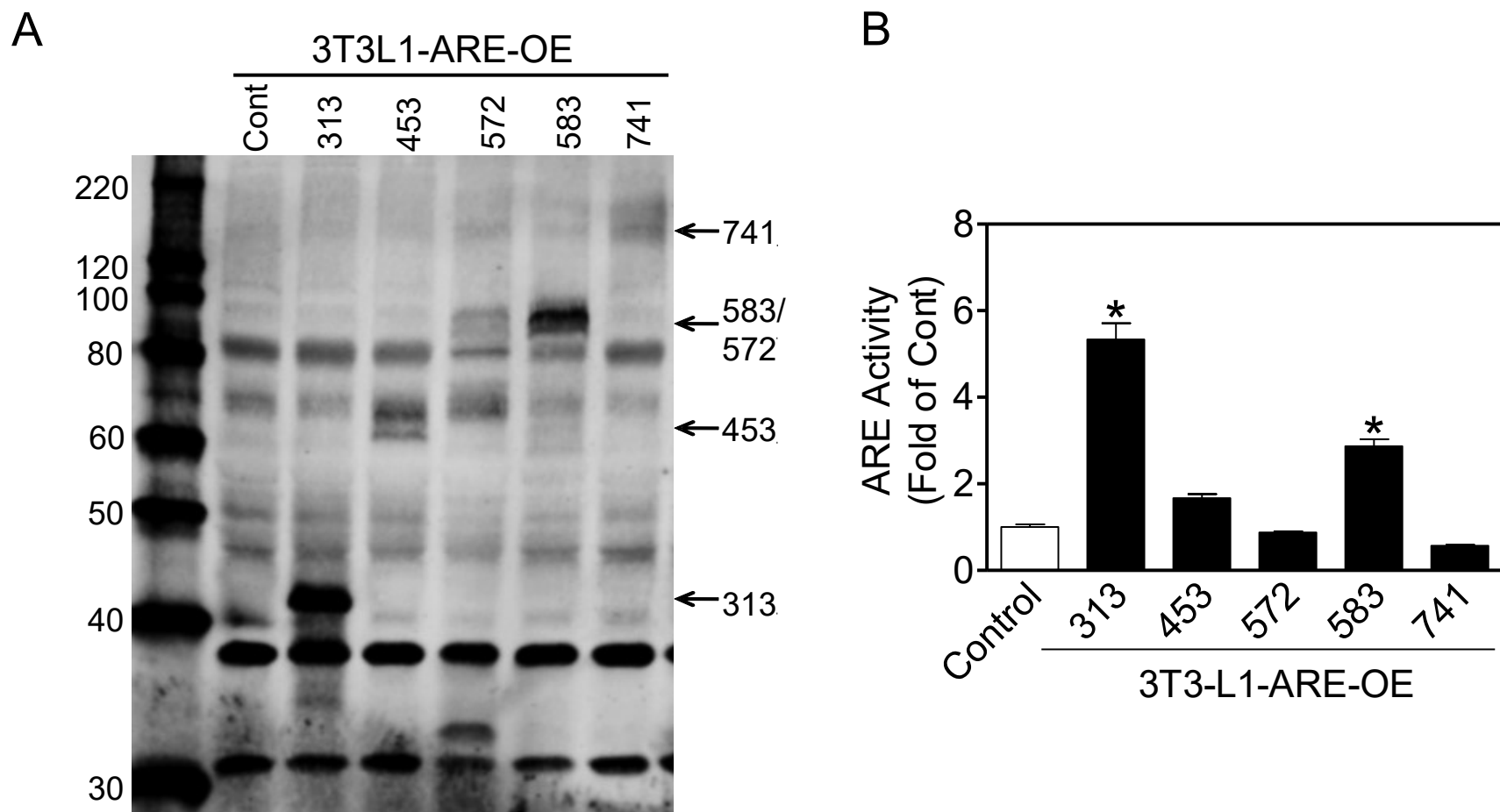
A

B

Fig. S4. The quantification of Oil-red O staining in Fig. 3A. (A) DMI treatment for 5 days. **(B)** DMIRI treatment for 5 days. The images were analyzed by Image J software and expressed as mean $\pm$ SD. n = 3. *p < 0.05 vs. Scramble.
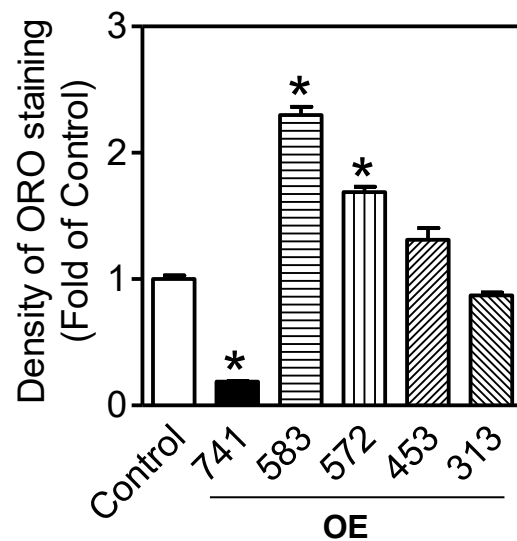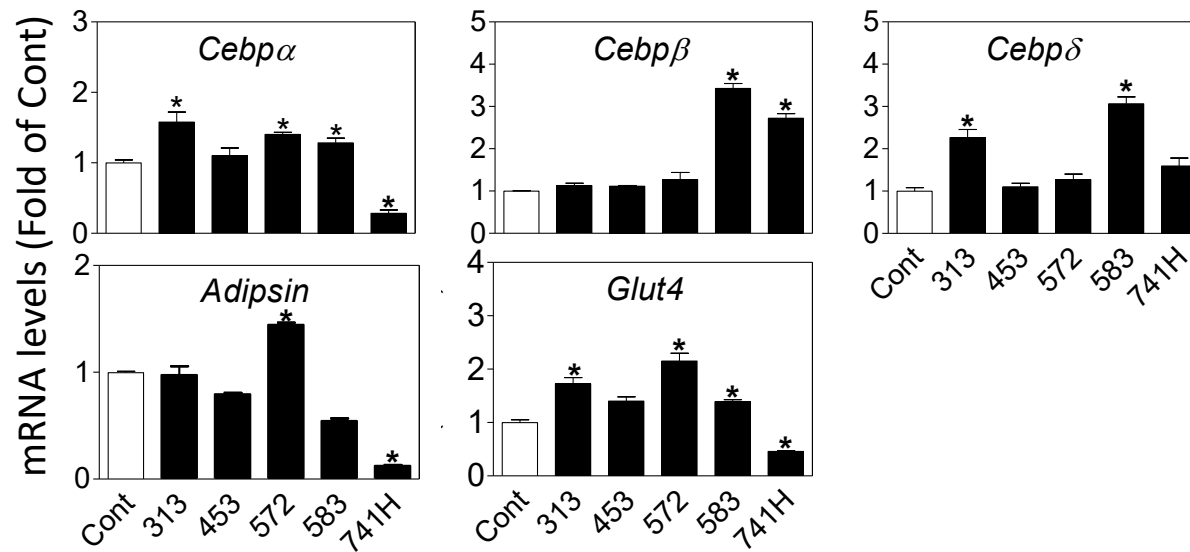
Fig. S5. Quantification of Western blot in Fig. 5B. The data was normalized with β-ACTIN and presented as the fold of Cont. Cont, Control; NRF1-313, -453, -572/583 and -741 refer to the cells overexpressed indicated isoforms of NRF1, respectively. n = 3. * $p < 0.05$ vs. Cont.

Fig. S6. Effect of overexpression of various isoforms of NRF1 on ARE activity in 3T3-L1 cells under basal condition. (A) Representative image of NRF1 immunoblots in 3T3-L1 cells which were overexpressed various isoforms of NRF1 and cignal Lenti ARE reporter. 3T3L1-ARE-OE cells, 3T3-L1-ARE cells generated in our previous study [31]) were overexpressed with various isoforms of NRF1. Cont (Control), 313, 453, 572, 583 and 741 represent the 3T3-L1-ARE cells that were transduced with lentivirus expressing an empty vector and various isoforms of NRF1, respectively. (B) ARE activity in 3T3L1-ARE-OE cells under normal culture condition. $n = 3$; * $p < 0.05$ vs. Control.
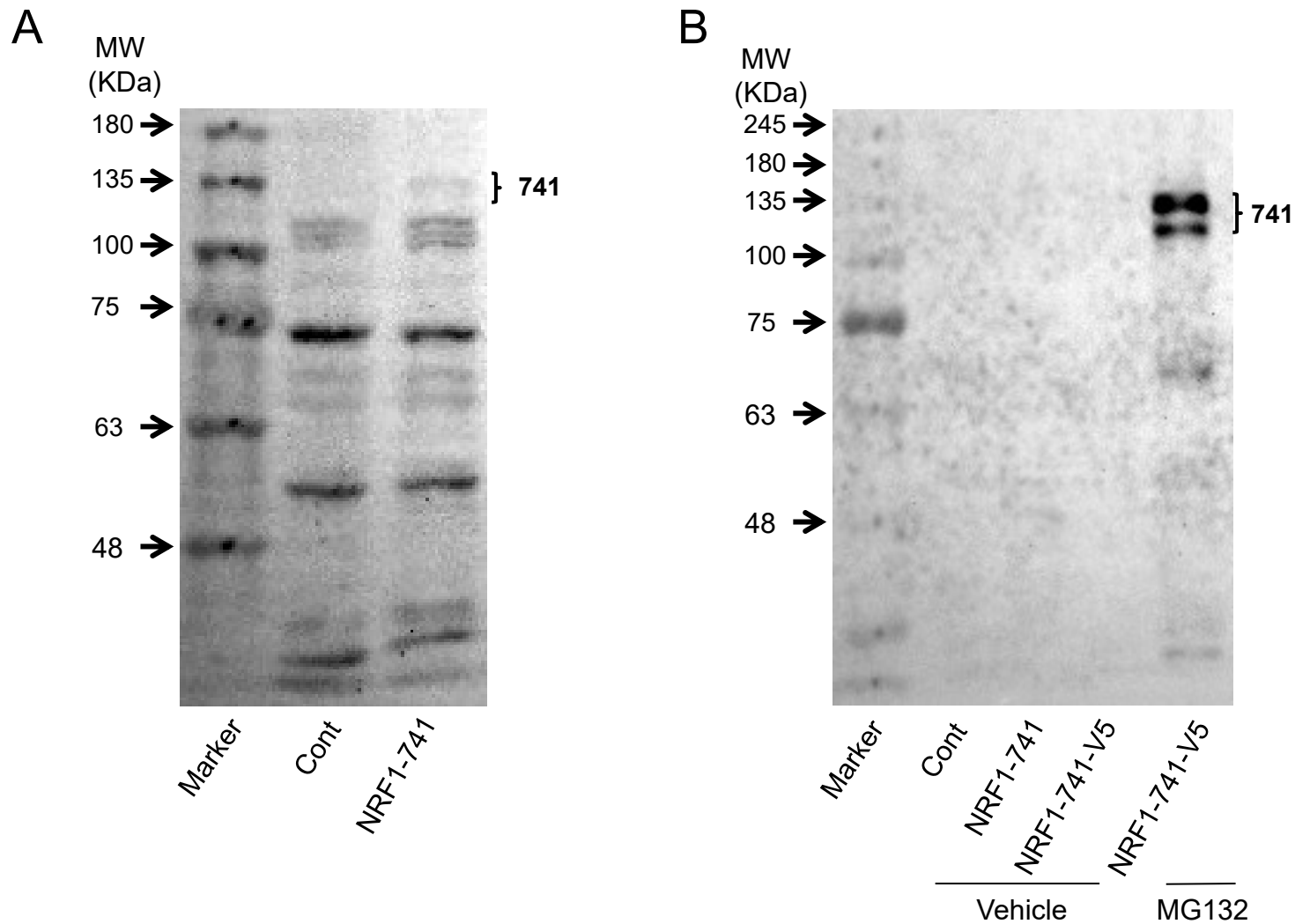
Fig. S7. The quantification of Oil-red O staining in Fig. 5C. Representative images of Oil-red O staining in Control, NRF1-741, -583, -572, -453, and -313 cells induced by hormonal cocktail of DMI were analyzed by Image J software and expressed as mean $\pm$ SD. n = 3. * $p < 0.05$ vs. Control.

Fig. S8. Overexpression of L-NRF1-741 suppressed the expression of adipogenic genes in 3T3-L1 cells. The mRNA expression of adipogenic markers in control and various *Nrf1*-OE cells under normal culture condition. Cont (Control), 313, 453, 572, 583, and 741 refer to the cells overexpressing indicated isoforms of NRF1. * $p < 0.05$ vs. Control. n = 3-5.

```
AATGATGGGACAACACATTGTAATAAACATTAGCTGTCTTAAACAACCCACACCGTGTAGCTATTTTGCTTCACTTGTGTGTTTT
ATGTGTTATGTGGTTCAAATATGCCACCAGATATGTAAGCAACATTTATTGAACCTGCTCTGTGCCAGGCTCTGTGTTTATTTAA
ACTATGACTTTTGTTTATTGATTGGTTTTAAAGTCCATAAGAGTACACACATTTTATTCATTAGTACCCTAGCAACAAGCTTAAG
GCTTGCCATCTCTTATATTGTCAATAAATATTTGTTGGCCGGGCAGTGGTGGTGCACACCTTTAATCCCAGCACTTGGGAGGCAG
AGGCAGGTGGATTTCTGAGTTCGAGGCCAGCCTGGTCTACAGAGTGAGTTCCAGGACAACCAGGGCTACACAGAGAAACCCTGTC
TCAAAAAACCAAATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATAGATATT
TGTTGAACAAATGAATGAAATAGAATGATCAAGAGGCTTTTGCATCAAGTAAAGCATAGGGCAAAGAAGGGCGTTAAAACACAA
TCCTAGGAGCAGATGCTACCAGATTCATGTACTGCTGAATTAACTGCTAAAATAATTTGTGTGATTGAATAACAAGCACACAATA
GAAGTAGAGGTTCAGAAAGCCTAGTTAACCAGTAGATACACAAGTTAAATGTTCCTTTTGAAAGGAAGGGAAGGAGGAAGAGAAT
GAGGGAAAAGGAAGAAATAGGTAGAGTCCAAAATTCCATCCCTTGGAAGAAGGTTGGGAAGACTATATCTATGTGGAGCCCAACC
CAGATAATAACAGAGACTAAAACAAAACCTGAGAACCAGGTGAAATTGCTGACCTTAGAAATCAAATGTAGAAGATCCACTCTGA
GTCAAATGAAATGAGTGAGCTACATTGAGAGAATGAATAGACATGGGAAAGAAGGTATTGCTAGACCAGCCCCCACTTTCACCAT
AGTCATTGGTATTGAAAACGGGATAATATAGGTACTCATTAATATCCCCTTAAGGAAGAAGCTCCTCTTCCTCTATCCCGATGGT
TCCTGAGCACTGGAGACAAGTTCCAATTCCAGCCTCGCCTAATCTTTTTAATTTAGAAGACACAGGTATTATCAAGGGGCCTCCA
TTTCTCATCTTTTTACACTCCTTCTAAAATAGTATCACTCTGCCAAATGTTAGCATTTCAAATACCCATGCAAAATCTTAAGGAG
GTTCTAATAAATGTTTCCCTTTTGAGGGTGGGATACTGAGAATCAAACCCAGGGCCTACACATGCCAAGCAAAAGCTCTACCACA
AAGCTACACCTCTCCCCCTCAACTATGTTTAAAAGGTTAAACCAGGATCTGGGGACAGGAATAAGGGAACCAAGTGATATATTGA
ATTTCATAGGAAGTGAAGCTGAAGTTTTACATTCTAGACACAGATGCCAGGGATAGCACACAGGAGTATCTATAGATAGAATAGG
ACTCCAGCTTTTGTTCTATTCTGTTAAAATTTGTTTCTGTTTGTTTCTGGTTTTGACTTTAATGATTAATTTAGTGATTGGTTTA
GTGATTGCTTTTGACCTGTAAGTTCCACCAAAAAATTTGGACTCCATTACATCCTTAAGTAGTGACGTTTCTAAGCATTGAGAAG
AAAAGAGAAATTATTTTATTAAGAATTCAAGTTAAATAGAGATAAAACTAATCCAACACAGTGATCCCCCATCATTTGGACTACT
GAAAAACATACTTTAACTTGAATTACTTTAGTAATGTTTGGGGGGGAGTCATTAAATGATGTAATTGCCAACTTCTCGATTCACT
GACTGATATTTAGACCTAGTGAGCATTTTCCCATTGAGCTATTGCTTCATTATTGACAGAGGAACTCTTGCTAGTCCCCTTGAAG
AATGAATAGTTACTCAACATAGATTAACTAGATATATGGTTATTCCATTTGGGGGTATCAGGATAAATATAATTCAGGGAATTAT
TGCCATCTGATACACTGCCCTGTGTAAGGGCTCTTTTAAAGTCCACAAGTCACTGAATTATATTAGGTCACTTATGTGACAAGGG
CTGCTCCCACGTTAGCAGTTTGGCACAGCTAGGTTTTTTTTTCTGCCAAAAAGGGCAAAGGCCTTAAGCAAGAAGCCAGAGTTTTC
CTGATTACAAAACTGACCACAATTCCTTGCCAACCTAGCAGGGTAATTTTTTTTTCTTCTGGTAATACATTATTCTCAGGGAGGG
CTCCAATTTTTTCATTGTGACATAGCACTTATCACTTAAACATCAACCATTGGAATACTACTGCAGTTGCTATTGATAGATAAAC
AAATTTAAAACACAAACTTTGATATATAAAATTTAAATTTTACTAGCCTTTATTCTGTCAACTATTCCTTTTTATAGAATTTGGA
TAGCAGTAACATTTTGGACCTTTTATAGACTTGTTGAATAAATCACCTTAGAAATGTATTTTGTTACCTGTGTGGGTAACAAAAT
CTAAAATAAGAATGTGTATATGTTTGAGTACAAGAATATTCTTCAGATGTGTGATTAGGAGTTTCAACCAAAGATAAATACTTAA
GAAAAACTTTGGCCAAATACGTTTATCTGGTGTTTCATAACTTAGAGATTAAGGTTTTCTATTTTAAAAGCCACTGGTGTGTATT
TTACTGCAATTTTAAAAAGCAATCAATATTGAACAATCTCTGCTCTGGTAATTCCAACTACTGTACAGTTCACGCCCCTCACAGA
ACAGTGAATGTGTGGGTCACTGGCGAGACAATGTAGCAACGTTTTCCTTGTAATGTACCAAGTCTTGCCAAAGCAGCAGACAGCA
TTATGACACACCATTTTGTCACAACTGGCTCTCAGTCAGGACAGTGCCAGCCAATTCAGGCCTGATTCTTTCTGTGTTTATTCCC
ACCTCTCCCAAATATTTGAAAACTGGTGTCTTGACTTATTAGCATATTCATAAGCTCGATGACCATAAGCCTTTTTCCTTTAACC
                         +1
AACCAATCTTTTGCAAGACATAGACAAAACACCAGTGTGAATTACAGCAAATCTCTGTTTTATGCTGTTATGGGTGAAACTCTGG
GAGATTCTCCTGTTGACCCAGAGCATGGTGCCTTCGCTGATGCACTGCCTATGAGCACTTCACAAGGTAAAGT
```

Fig. S9. Identification of ARE sites on the promoter region of mouse *Pparγ2* gene. The ARE sites were defined according to the consensus sequences proposed by Chorley BN, et al. (Nucleic Acids Research, 2012, 40, No.15. DOI:10.1093/nar/gks409.) . Yellow highlights, ARE sites; +1, transcriptional start site.

Fig. S10. Overexpression of L-NRF1-741 in 3T3-L1 cells. (A) NRF1 protein levels detected by antibody (#12936-1-AP, Proteintech Group, Inc) against NRF1 in Control and NRF1-741 cells. Cont, Control; NRF-741 refers to the cells overexpressing isoform of 741 aa of NRF1. **(B)** Western blot analysis of NRF1 protein with antibody against V5 epitope tag in Control, NRF1-741, NRF1-741-V5 cells with or without 10 μM MG132 treatment. NRF1-741-V5 refers to the cells overexpressing V5-tagged NRF1-741 isoform.