Supplementary Experimental Procedures

Reagents
Hederagenin was obtained from extrasynthese.

Supplementary Figure Legends

Supl. Fig. 1
(A) Silencing of Nrf2 expression in HFL-1 cells. Immunoblot analysis of the Nrf2 in HFL-1 cells transfected with 75nm Nrf2 siRNA or scrambled siRNA for 48 h, [(i) low and (ii) high exposure]. (B) **Accumulation of Nrf2 upon proteasome inhibition.** Immunoblot analysis of Nrf2 in HFL-1 cells treated with 20 μM MG132 for 6h. GAPDH levels were used as loading control. (C) **Low exposure of the Nrf2 immunoblot from Fig. 1B.** Molecular mass markers are indicated on the left side of the Nrf2 blots. **(D) Quantification of the Nrf2 specific bands.** Graphs show the quantification of each specific band of the Nrf2 (1-5) of the immunoblots in (i) Fig. 1B, (ii) Fig 2B, (iv) and (v) Fig. 2C [cytosolic and nuclear fraction, respectively], (vi) Fig. 6E and (vii) Fig.7B. **(Diii, E) Low exposure** of (Diii) the Nrf2 immunoblot shown in Fig. 2C and (E) the β_2 immunoblot in the total extracts shown in Fig. 3Di. Statistical significance at $p < 0.05$ or $p < 0.01$ are denoted in graphs by a single (*) or double (**) asterisk, respectively.

Supl. Fig. 2
Silencing of Nrf2 expression in HFL-1 cells. Quantification of RNA expression levels of Nrf2 in HFL-1 cells transfected with 50 or 75nm Nrf2 siRNA (siNrf2) or scrambled siRNA (siCon) for 24 or 48 h. RNA expression levels in HFL-1 cells transfected with scrambled siRNA (siCon) were arbitrary set to 1. Statistical significance at $p < 0.01$ is denoted in graphs by double (**) asterisks.

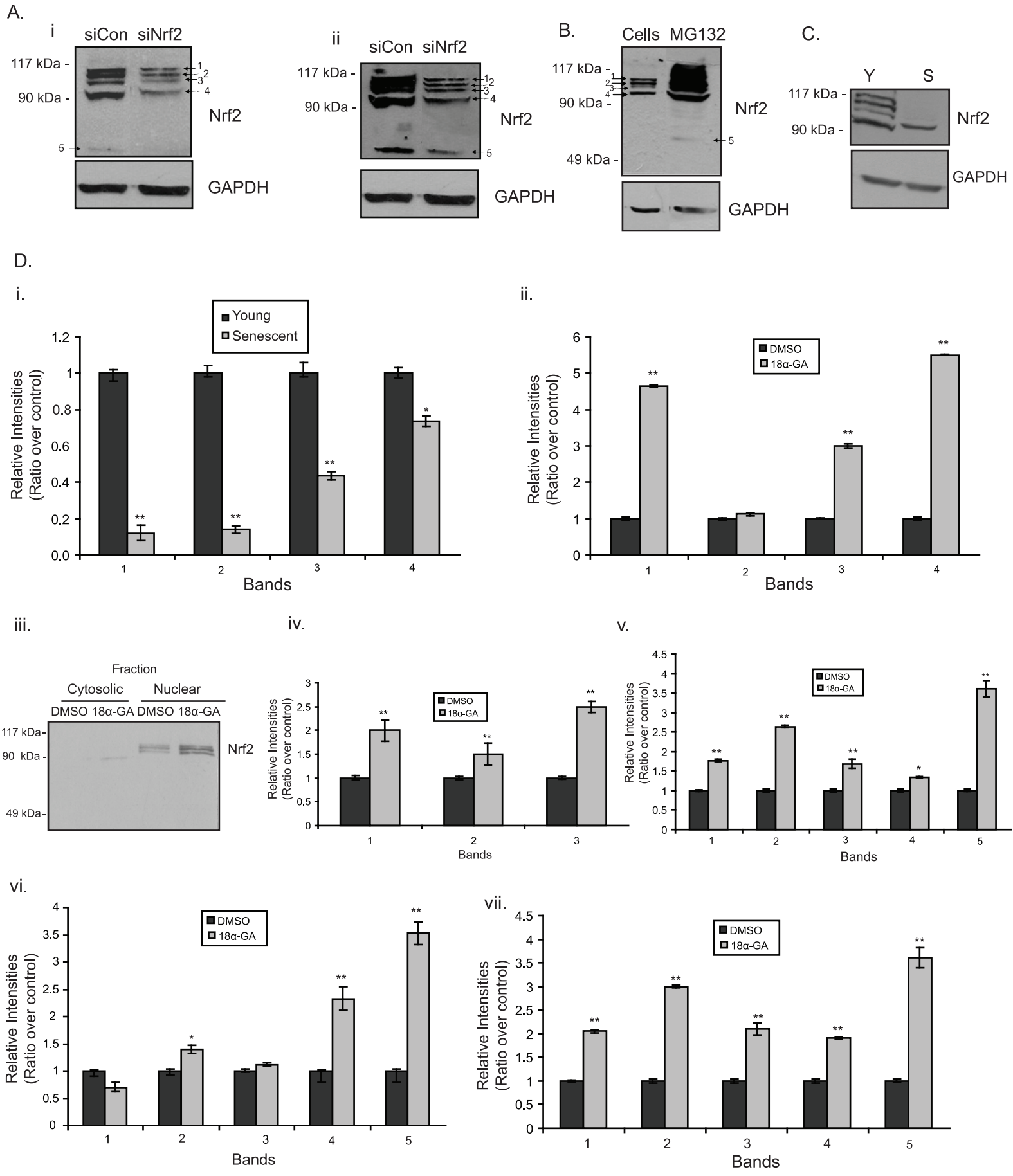
Supl. Fig. 3
Proteasome activation by 18α-GA and HED. (A) Manifold of CT-L proteasome activity in young HFL-1 cells treated with different concentrations of (i) 18α-GA and (ii) HED for 24 h. (B) Levels of CT-L, PGPH and T-L activities of HFL-1 cells treated with 2 μg/ml (i) 18α-GA, (ii) HED or DMSO for different time points. Use of proteasome inhibitor (MG132) in control reactions ensured the specificity of the enzymatic reaction. Activities in DMSO treated cells were arbitrary set to 1. Statistical significance at $p < 0.01$ is denoted in graphs by double (**) asterisks.

Supl. Fig. 4
Nrf-2 mediated proteasome activation by HED. (A) Immunoblot analysis and the relative quantification of proteasome subunits (β_5, β_1, β_2, α_4) in HFL-1 cells treated with 2 μg/ml HED or DMSO for 2 h. GAPDH levels were used as loading control. (Bi) Immunoblot analysis with the relative quantification of representative proteasome subunits (β_2, α_4) in total extracts and the relative elutions following proteasome immunoprecipitation in HFL-1 cells treated with 2 μg/ml HED or DMSO for 2 h. (Bii) Levels of CT-L proteasome activity in immunoprecipitated proteasomes of HFL-1 fibroblasts treated with 2 μg/ml HED. or DMSO for 2h. Activity or protein levels in DMSO treated cells were arbitrary set to 1. Blank samples represent the control immunoprecipitation samples. Immunoblot analysis of GAPDH (lower panel) in total extracts shows the equal starting protein quantity before immunoprecipitation. (C) Immunoblot analysis of Nrf2 and the relative quantifications of (i) all Nrf2 specific bands together and (ii) each specific band separately, in nuclear extracts of HFL-1 cells treated with 2 μg/ml HED or DMSO for 2 h. Lamin A/C was used as marker of nuclear fraction.

Molecular mass markers are indicated on the left side of the immunoprecipitation experiment and the Nrf2 blot. Statistical significance at $p < 0.05$ or $p < 0.01$ are denoted in graphs by a single (*) or double (**) asterisk, respectively.

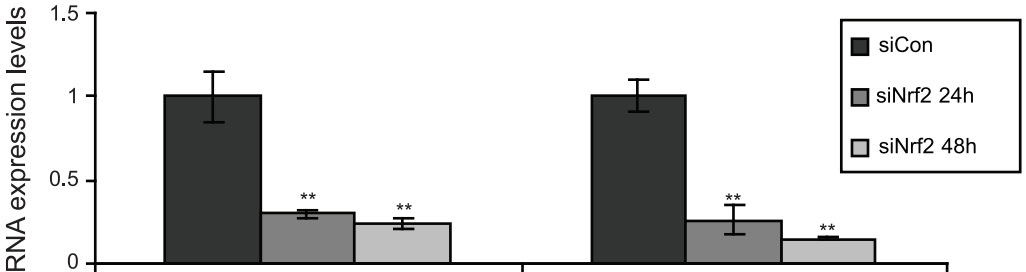
Supplementary Table 1. Primers used for Real-Time PCR analysis. h: human, m: mouse; R: reverse; F: forward.

Primers	Sequence
h GAPDH-R	5'-CATGGGTGGAATCATATTGGAA-3'
h GAPDH-F	5'-GAAGGTGAAGGTCGGAGT-3'
h Nrf2-R	5'-GACCGGGAATATCAGGAACA-3'
h Nrf2-F	5'-AAACCAGTGGATCTGCCAAC-3'
h Nqo1-R	5'- GGTGGATCACGCCTGTAAT-3'
h Nqo1-F	5'-AGTGCAGTGGTGTGATCTCG-3'
h β_1-R	5'-CAAACTGCACGGCCATGATA-3'
h β_1-F	5'-GAGGCATTCACTCCAGACTG-3'
h β_2-R	5'-ACAACCATCCCTTCAGTTGC-3'
h β_2-F	5'-TGCAAAGAGGGGATACAAGC-3'
h β_5-R	5'-CATCTCTGTAGGTGGCTTGGT-3'
h β_5-F	5'-AGGTTCTGGCTCTGTGTATGC-3'
h α_4-R	5'-ATGGAAAGGCCTACACATCG-3'
h α_4-F	5'-GGTGGTGTTCGAGGAAAGGA-3'
m GAPDH-R	5'-TGAAGTCGCAGGAGACAACCT-3'
m GAPDH-F	5'-TGAAGGGCATCTTGGGCTAC-3'
m β_1-R	5'-GATGGCAAAGGACTGTCTTACC-3'
m β_1-F	5'-AAGATCTGATGGCAGGAATCAT-3'
m β_5-R	5'-CATCTCTGTAGGTGGCTTGGT-3'
m β_5-F	5'-GGGCTCTGGCTCCGTGTATGC-3'
m α_3-R	5'-GTGCTTATCCCAGCCAATATAC-3'
m α_3-F	5'-GAGCCAATTCCCTGTGAGCAG-3'
m α_6-R	5'-TTCTTCAAGACCATCCAGGAAT-3'
m α_6-F	5'-CTGACCACAAAGAATGTTTCCA-3'
m HO-1-R	5'-CCAGAGTGTTCATTCGAG-3'
m HO-1-F	5'-CACGCATATACCCGCTACCT-3'



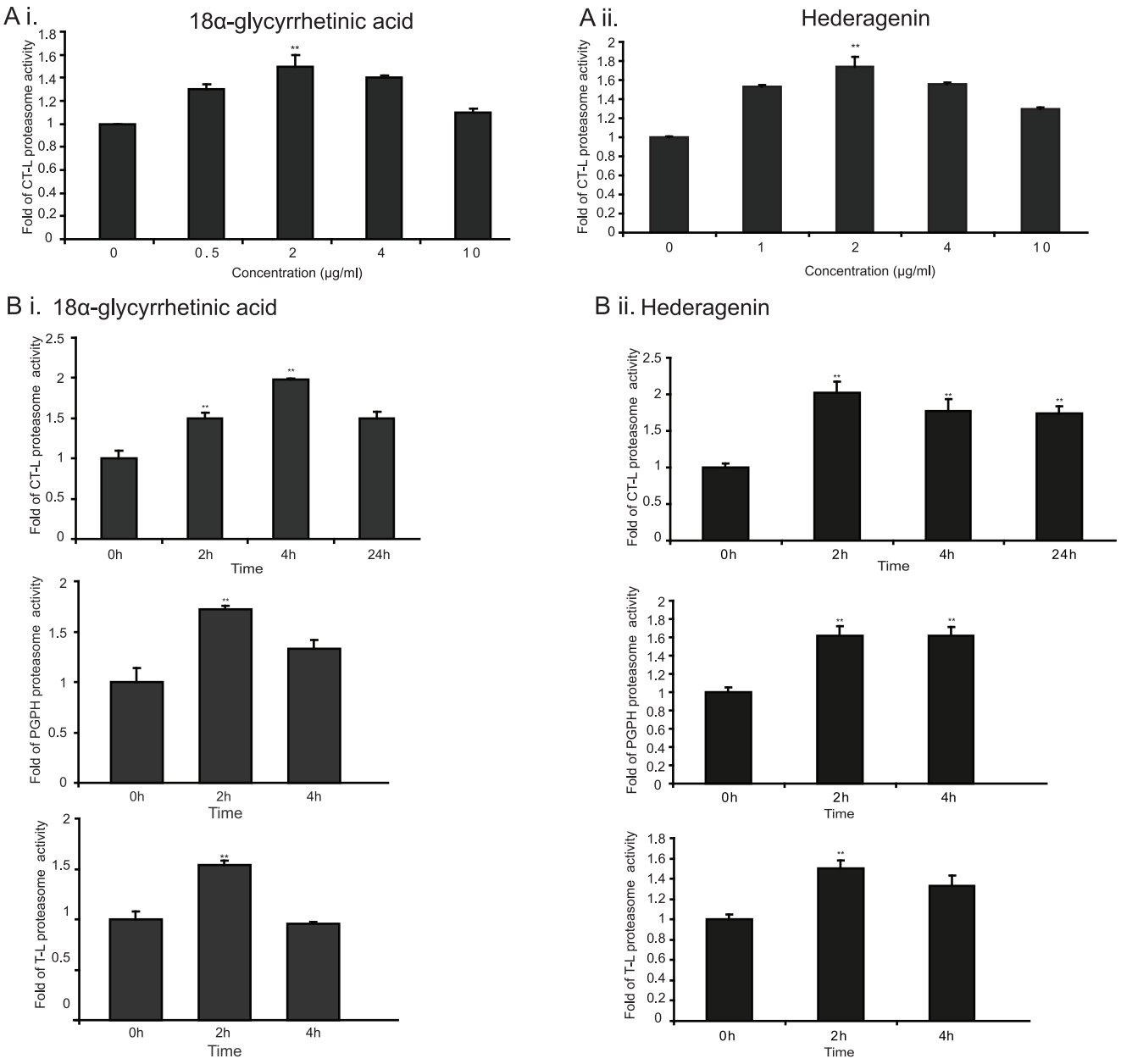
S. Figure 1

Nrf2-proteasome axis in senescence

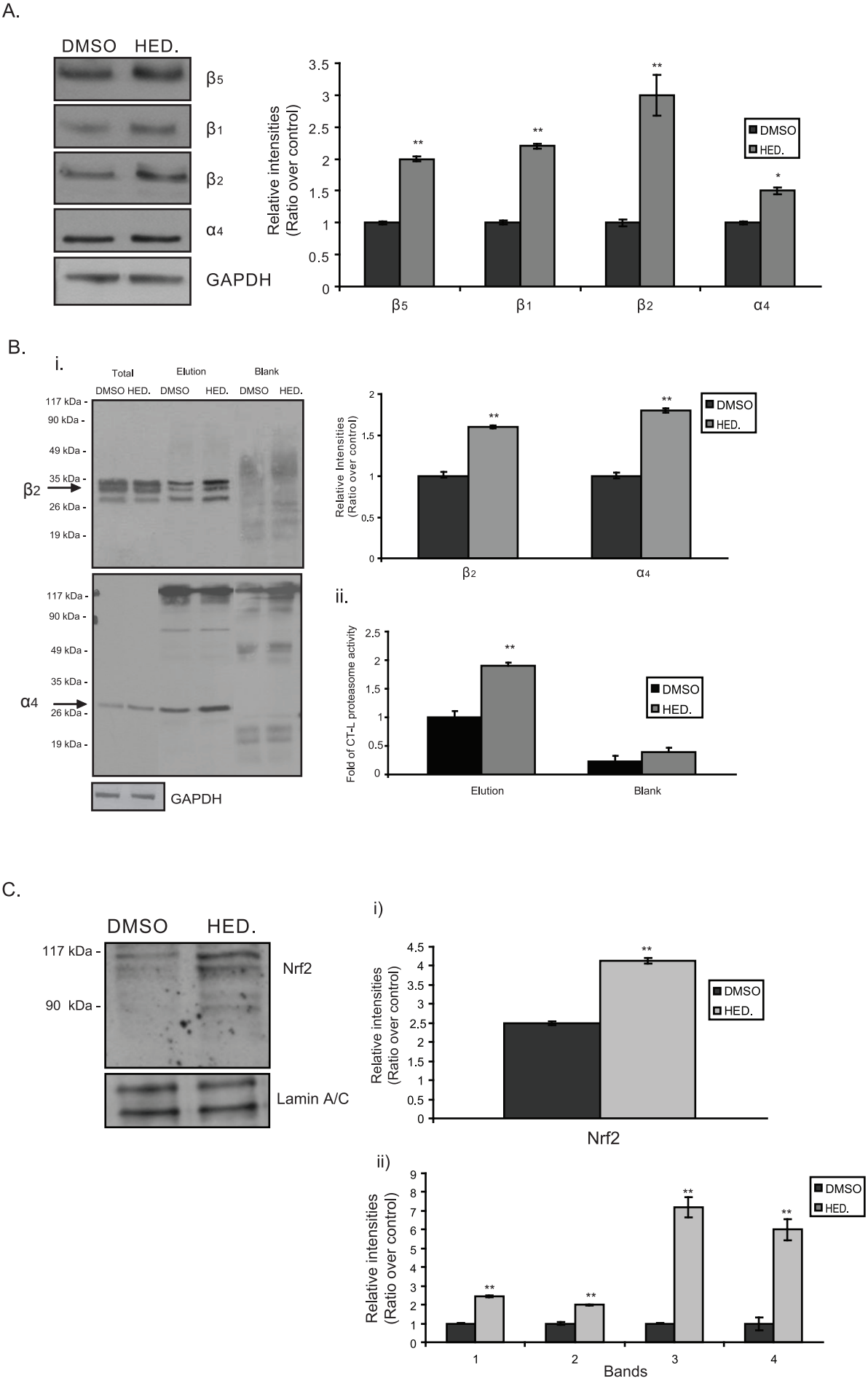


	50 nm	75 nm
siCon	1	1
siNrf2 24h	0.3	0.26
siNrf2 48h	0.24	0.15

S. Figure 2


A i.
18α-glycyrrhetinic acid
Fold of CT-L proteasome activity
Concentration (μg/ml)
A ii.
Hederagenin
Fold of CT-L proteasome activity
Concentration (μg/ml)
B i. 18α-glycyrrhetinic acid
Fold of CT-L proteasome activity
Time
Fold of PGPH proteasome activity
Time
Fold of T-L proteasome activity
Time
B ii. Hederagenin
Fold of CT-L proteasome activity
Time
Fold of PGPH proteasome activity
Time
Fold of T-L proteasome activity
Time

S. Figure 3


A.
DMSO HED.
β5
β1
β2
α4
GAPDH
Relative intensities (Ratio over control)
DMSO
HED.
β5
β1
β2
α4
B.
i.
Total
Elution
Blank
DMSO HED. DMSO HED. DMSO HED.
117 kDa
90 kDa
49 kDa
35 kDa
26 kDa
19 kDa
β2
α4
GAPDH
Relative Intensities (Ratio over control)
ii.
Fold of CT-L proteasome activity
Elution
Blank
C.
DMSO HED.
117 kDa
90 kDa
Nrf2
Lamin A/C
i)
Relative intensities (Ratio over control)
Nrf2
ii)
Relative intensities (Ratio over control)
Bands

S. Figure 4