Electronic Supplementary Material (ESI) for Food & Function.

Figure S1

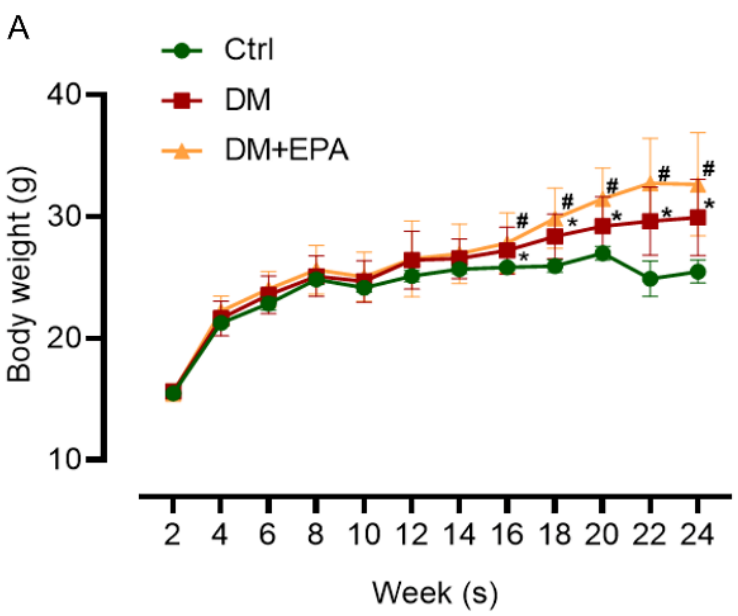


Figure S1. EPA increased body weight in the diabetic mice. (A) Body weight of mice. The data were summarized as means ± SD (n=6). **P*<0.05 vs. Ctrl; #*P*<0.05 vs. DM. Abbreviations: Ctrl, control; DM, diabetes mellitus; EPA, eicosapentaenoic acid.

Figure S2

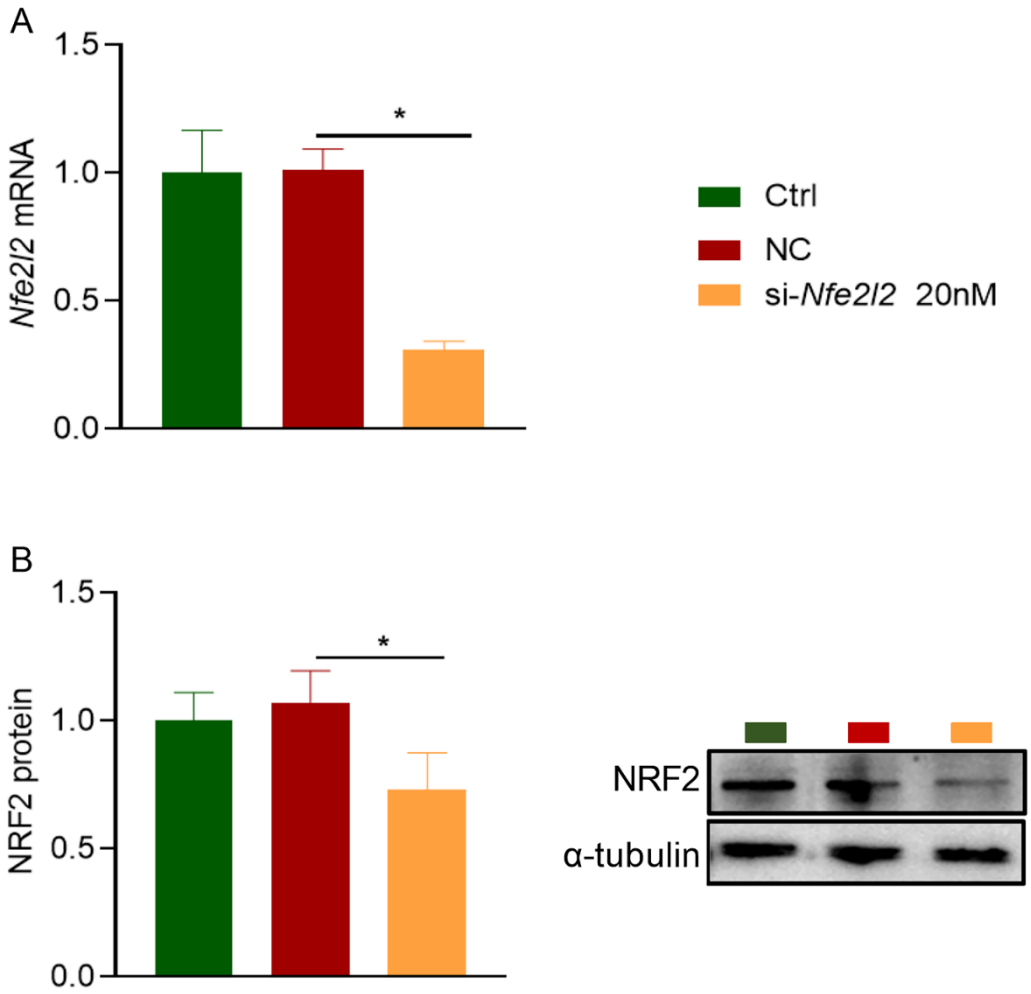


Figure S2. Validation of the efficacy of *Nfe2l2* small interfering RNA. (A) mRNA levels of *Nfe2l2*; (B) protein levels of NRF2. **P* < 0.05 vs. NC. The data were summarized as means ± SD (n=3). Abbreviations: NC, negative control; NRF2, NFE2 like bZIP transcription factor 2; si-*Nfe2l2*, *Nfe2l2*-siRNA.

Figure S3

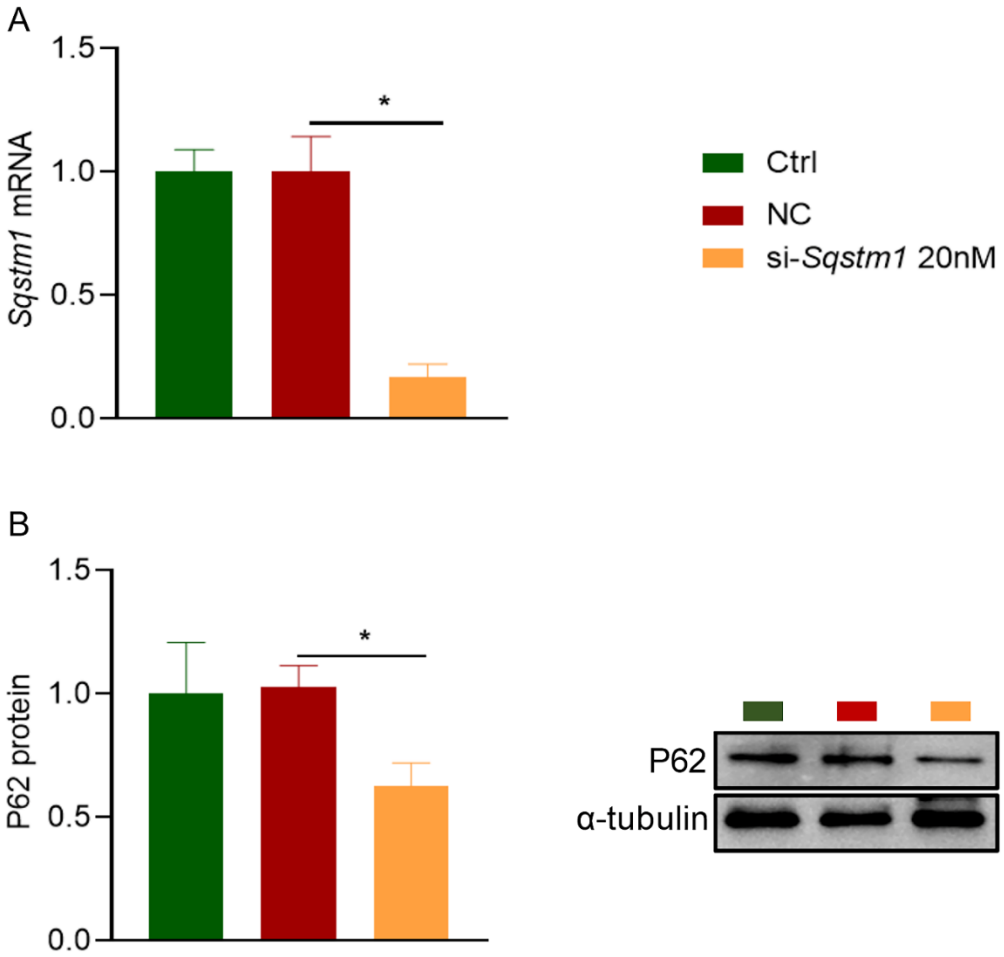


Figure S3. Validation of the efficacy of *Sqstm1* small interfering RNA. (A) mRNA levels of *Sqstm1*; (B) protein levels of P62; **P*<0.05 vs. NC. P62/*Sqstm1*, sequestosome 1. The data were summarized as means ± SD (n=3). Abbreviations: si-*Sqstm1*, *Sqstm1*-siRNA. Other abbreviations are the same as in Figure S2.

Figure S4

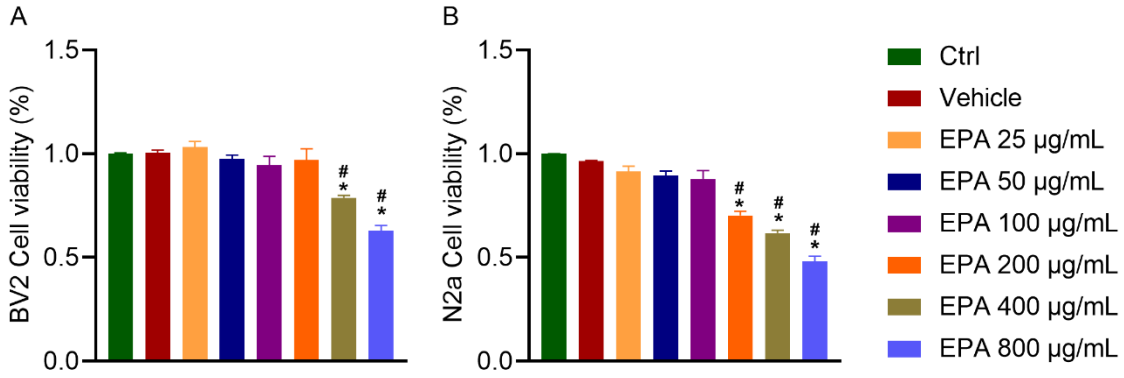


Figure S4. Cell viability test. (A) Viability of BV2 cells treated with EPA; (B) Viability of N2a cells treated with EPA; **P*<0.05 vs. Ctrl; $^{\#}$*P*<0.05 vs. Vehicle. The data were summarized as means ± SD (n=3). Abbreviations: Vehicle, dimethyl sulfoxide.

Table S1. Composition of the experimental diets

Ingredient (g/kg)	Normal diet	HFD diet	HFD+EPA diet
Soybean	70	70	65
EPA	-	-	5
Lard	-	230	230
β-cornstarch	397.49	110.62	110.62
α-(dextrinized) cornstarch	132	36.87	36.87
Casein	200	250	250
Sucrose	100	200	200
AIN-93 mineral mixture	35	35	35
AIN-93 vitamin mixture	10	10	10
L-Cystine	3	3.75	3.75
Choline bitatrate	2.5	2.5	2.5
Cellulose	50	50	50
Tert-Butylhydroquinone	0.01	0.06	0.06
Cholesterol	-	1.2	1.2
Protein	203	253.75	253.75
Carbohydrates	629.49	347.49	347.49
Fat	70	300	300

Table S2. Antibodies used for Western Blot

Abbreviation	Full Name	Agent
BCL2	B-cell lymphoma-2	Proteintech
BAX	BCL2 Associated X Protein	Proteintech
cleaved Caspase3	Cleaved cysteine aspartate-specific protease3	Proteintech
Iba1	allograft inflammatory factor1	Cell Sinaling Technology
Cox2	cytochrome c oxidase subunit 2	Cell Sinaling Technology
IL-1β	interleukin 1β	Cell Signaling Technology
IL-6	interleukin 6	Proteintech
NOX4	NADPH oxidase 4	Proteintech
p-JNK	phospho-c-Jun N-terminal kinase	Cell Signaling Technology
JNK	c-Jun N-terminal kinase	Cell Signaling Technology
Neun	neuron specific nuclear protein	Cell Signaling Technology
p-ERK	phospho-extracellular regulated MAP kinase	Cell Signaling Technology
ERK	extracellular regulated MAP kinase	Cell Signaling Technology
p-P65	phosphor-RELA proto-oncogene NF-kB subunit	Affinity
P65	RELA proto-oncogene NF-kB subunit	Affinity
P62	sequestosome-1	Cell Signaling Technology
iNOS	inducible nitric oxide synthase	Cell Signaling Technology
NRF2	nuclear factor erythroid 2-related factor 2	Proteintech
HO1	heme oxygenase 1	Proteintech
NQO1	NAD(P)H quinone dehydrogenase1	Santa Cruz Biotechnology
KEAP1	Kelch-like ECH-associated protein 1	Santa Cruz Biotechnology
α-tubulin	Alpha Tubulin	Proteintech
Secondary Antibody	UltraPolymer Goat anti-Mouse IgG (H&L)-HRP	Proteintech
Secondary Antibody	UltraPolymer Goat anti-Rabbit IgG (H&L)-HRP	Proteintech
Secondary Antibody	Alexa Fluor® 488 Labeled Goat Anti-Rabbit IgG (H+L)	ZSGB-BIO
Secondary Antibody	Alexa Fluor® 594 Labeled Goat Anti-Mouse IgG (H+L)	ZSGB-BIO
Secondary Antibody	Alexa Fluor® 594 Labeled Goat Anti-Rabbit IgG (H+L)	ZSGB-BIO

Table S3. Sequences of primers and si-RNA

Gene name	Gene ID	Sequence
Il1α-forward	16175	TTTGACATGTATGCCTACTCGTCGG
Il1α-reverse	16175	CTGTGATGAGTTTTGGTGTTTCTGGC
Il1β-forward	16176	AAATGCCACCTTTTGACAGTGATG
Il1β-reverse	16176	GCAGCCCTTCATCTTTTGGG
Nos2-forward	18126	CAGCTGGGCTGTACAAACCTT
Nos2-reverse	18126	CATTGGAAGTGAAGCGTTTCG
36B4-forward	11837	GGCTGACTTGGTTGCTTTGG
36B4-reverse	11837	AGCAAAGGAAGAGTCGGAGG
Ccl2-forward	20296	TTAAAAACCTGGATCGGAACCAA
Ccl2-reverse	20296	GCATTAGCTTCAGATTTACGGGT
Il6-forward	16193	CCAGGTAGCTATGGTACTCCAGAA
Il6-reverse	16193	GCTACCAAACTGGATATAATCAGGA
Tnfα-forward	21926	TTGTCTTAATAACGCTGATTTGGT
Tnfα-reverse	21926	GGGAGCAGAGGTTCAGTGAT
Hmox1-forward	15368	AGGGTCAGGTGTCCAGAGAA
Hmox1-reverse	15368	CTTCCAGGGCCGTGTAGATA
Keap1-forward	50868	AAGGACCTTGTGGAAGACCA
Keap1-reverse	50868	CCCTGTCCACTGGAATTGAT
Nqo1-forward	18104	AGCGTTCGGTATTACGATCC
Nqo1-reverse	18104	AGTACAATCAGGGCTCTTCTCG
Nfe2l2-forward	18024	CATGATGGACTTGGAGTTGC
Nfe2l2-reverse	18024	CCTCCAAAGGATGTCAATCAA
Sqstm1-forward	18412	CCTTGCCCTACAGCTGAGTC
Sqstm1-reverse	18412	TGTTCCACATCAATGTCAACCT
si-Nfe2l2-forward	——	CGAGAAGUGUUUGACUUUATT
si-Nfe2l2-reverse	——	UAAAGUCAAACACUUCUCGTT
si-Sqstm1-forward	——	GGCACAGAAGACAAGAGUATT
si-Sqstm1-reverse	——	UACUCUUGUCUUCUGUGCCTT

negative control siRNA-forward	——	UUCUCCGAACGUGUCACGUTT
negative control siRNA-reverse	——	ACGUGACACGUUCGGAGAATT

Table S4. Original data table of blood glucose and blood insulin

Group	Blood glucose (mmol/L)						Blood insulin (μIU/ml)
	1st month	2nd month	3rd month	4th month	5th month	6th month	6th month
Ctrl1	5.6	8.5	12.1	6.6	7.1	4.7	3.17
Ctrl2	6.3	6.4	8.3	5.1	7.3	8.5	2.52
Ctrl3	5.2	6.7	10.3	7.3	6.7	6.7	3.58
Ctrl4	6.2	9.2	8	10.5	8.4	7.4	2.98
Ctrl5	5.2	8.9	8.3	7.3	7	7	3.25
Ctrl6	4.6	8.9	7.7	8.4	6.5	8	3.09
DM1	21	24	23.6	17.0	21.3	25.1	5.75
DM2	10.2	22.1	27.9	18.8	23.4	23.7	5.43
DM3	14.9	22.1	27.6	19.0	25.2	26.3	5.03
DM4	23.2	19.3	22.5	12.8	23.7	26.1	3.99
DM5	16.1	22.6	23.4	23.8	22.6	26.2	5.06
DM6	18.4	21	21.2	21.8	26.4	18.9	4.78
DM+EPA1	20.6	22.1	22.5	19.1	26.9	22.1	2.93
DM+EPA2	19.6	23.2	24.8	12.2	25.1	21.4	3.63
DM+EPA3	16.3	17.7	22.8	14.9	13.1	11.2	3.21
DM+EPA4	14.2	22.2	25.8	21.4	20	16.9	3.15
DM+EPA5	23.78	21.5	25.4	26.0	21.9	10.4	3.89
DM+EPA6	16.4	24.3	22.5	24.2	25.9	19.9	4.96

Table S5. Original data table of glucose tolerance test

Groups	0min	15min	30min	60min	90min	120min
Ctrl1	6.7	15.3	11.5	8.2	7.0	8.1
Ctrl2	6.4	22.0	17.0	15.3	8.4	6.8
Ctrl3	5.5	17.5	20.1	15.4	7.4	5.4
Ctrl4	5	14	14.6	11.3	6.4	5
Ctrl5	6.4	12.2	11.8	9.7	6.2	5.6
Ctrl6	7.9	14.3	9.7	6.7	7.2	6.5
DM1	7.8	14.2	20.4	15.2	14.5	8.4
DM2	24.4	34.3	43.5	38.9	35.3	32.5
DM3	24.3	29.0	42.9	32.8	34.0	27.2
DM4	24.4	34.9	46.0	39.7	37.6	31.0
DM5	25.6	37.3	47.0	34.0	36.2	33.1
DM6	25.4	36.5	40.3	37.4	33.9	31.3
DM+EPA1	12.1	24.0	29.9	24.5	23.6	20.3
DM+EPA2	5.4	21.8	26.1	14.9	18.9	14.8
DM+EPA3	10.3	23.1	38.0	32.8	25.0	24.8
DM+EPA4	6.1	16.1	28.3	12.0	5.7	6.9
DM+EPA5	15.5	36.2	19.8	26.6	27.3	22.7
DM+EPA6	22.8	34.6	40.1	36.6	26.8	27.0

Table S6. Original data table of insulin tolerance test

Groups	0min	15min	30min	60min	90min	120min
Ctrl1	5.2	3	2.9	2.8	2.8	2.6
Ctrl2	5.1	3.2	3.5	2.6	3.2	2.8
Ctrl3	5.7	3.4	3.4	3.1	3.3	5
Ctrl4	5.5	3.6	3.2	3.5	3.3	3.4
Ctrl5	7.2	3.9	4.2	2.3	2.9	3.8
Ctrl6	8.6	5.1	5.2	3.6	3.2	4.1
DM1	26.3	26	26.4	24.2	23.2	25.4
DM2	25	24.7	21.8	18.8	24	19
DM3	15.6	20.7	10	13.5	13.3	12.3
DM4	10.5	12	5.8	6.5	6.2	8
DM5	13	8.3	6.7	4.7	4.8	15.8
DM6	18.3	10	8.5	3.9	4.7	21.5
DM+EPA1	18	16.8	9.8	5.4	4.4	4.8
DM+EPA2	12.8	10.7	4.3	3.6	3.8	3.3
DM+EPA3	19.1	16.4	6.3	4.1	4.1	3.8
DM+EPA4	18.9	17.4	9.5	3.9	4	3.4
DM+EPA5	15.9	15.8	5.9	4.3	3.4	3.6
DM+EPA6	23.7	23.3	8.7	4.2	3.6	3.6

Figure 3

Neun (46-55kDa)

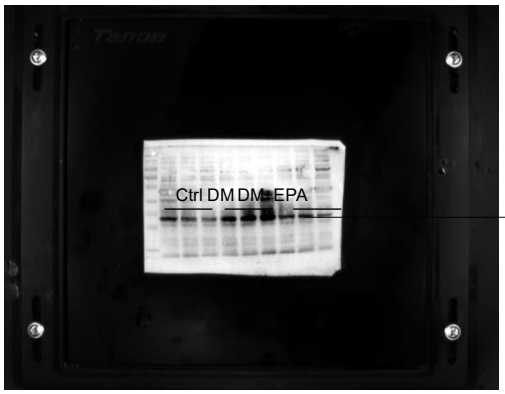
α-tubulin for Neun (55kDa)

Figure 4

BCL2 (26kDa)

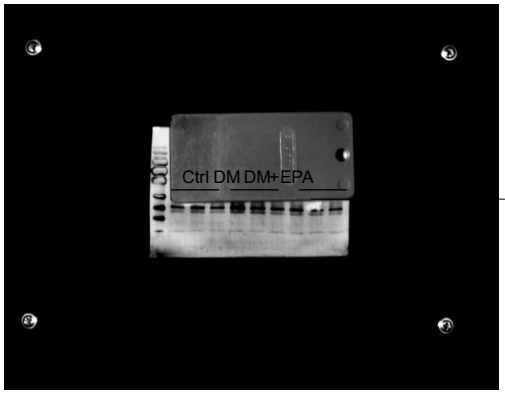
α-tubulin for BCL2 (55kDa)

Figure 4



BAX (20kDa)

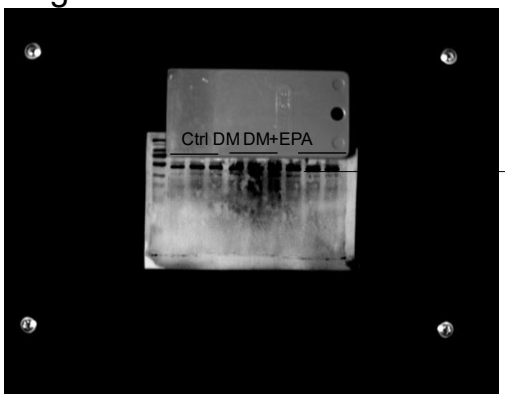
α-tubulin for BAX (55kDa)



cleaved Caspase3
(17-19kDa)

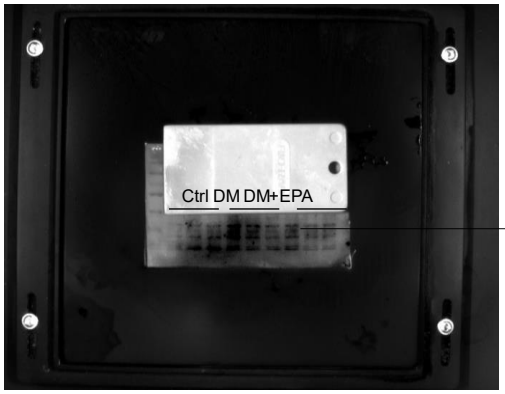
α-tubulin for cleaved
Caspase3 (55kDa)

Figure 4

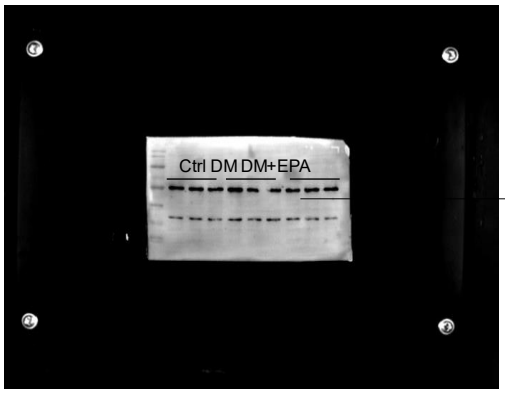


→ IL-1β (17-31kDa)

→ α-tubulin for IL-1β (55kDa)



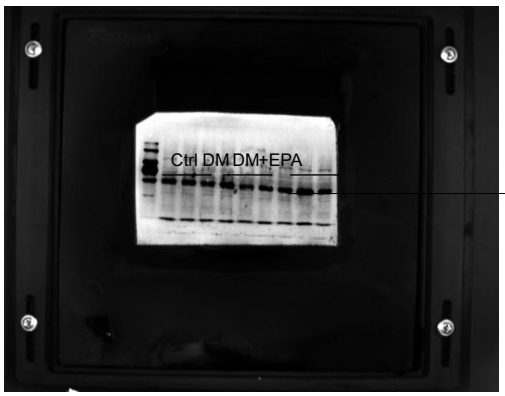
→ IL-6 (24kDa)



→ α-tubulin for IL6 (55kDa)

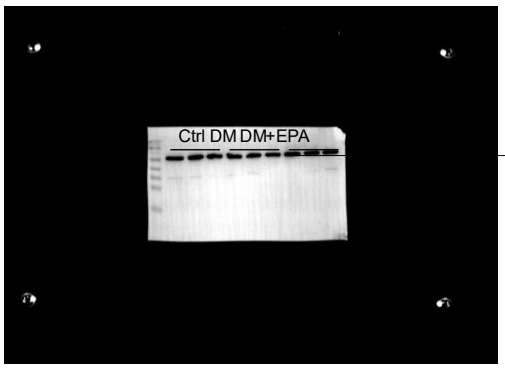
Figure 4

Iba1(17kDa)



α-tubulin for Iba1(55kDa)

Cox2 (74kDa)



α-tubulin for Cox2 (55kDa)

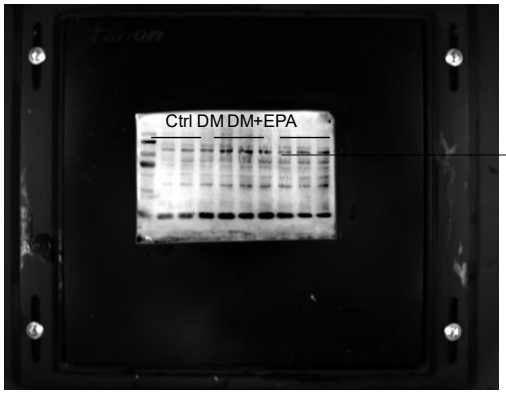
Figure 4

p-P65 (65kDa)

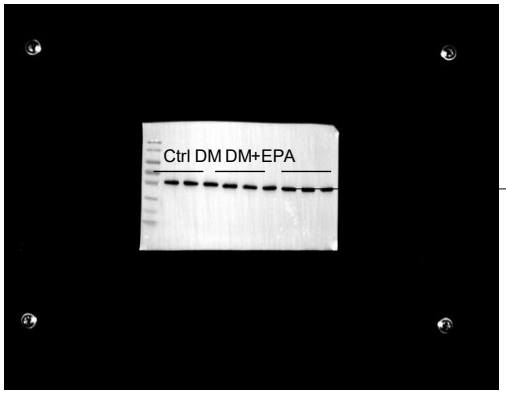
P65 (65kDa)

α-tubulin for p-P65/P65 (55kDa)

Figure 4

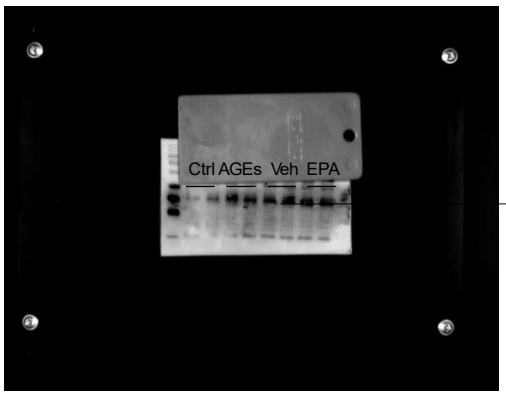


NOX4 (70kDa)

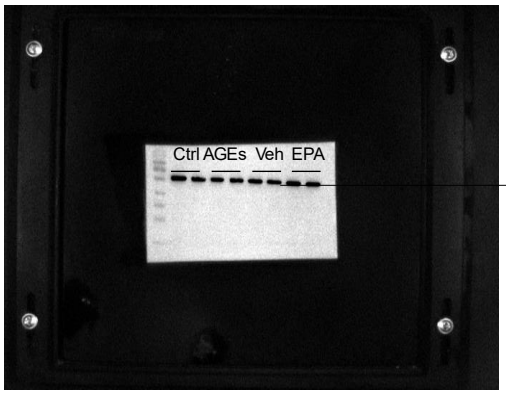


α-tubulin for NOX4 (55kDa)

Figure 5



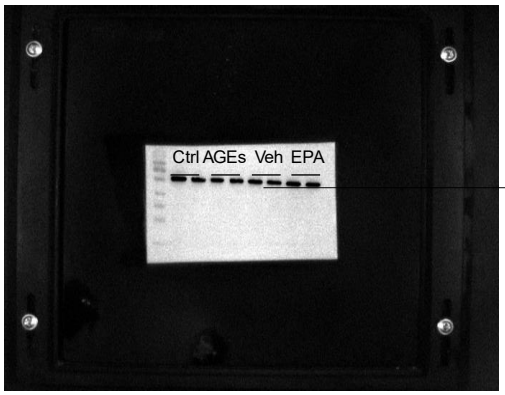
BCL2 (26kDa)



α-tubulin for BCL2 (55kDa)

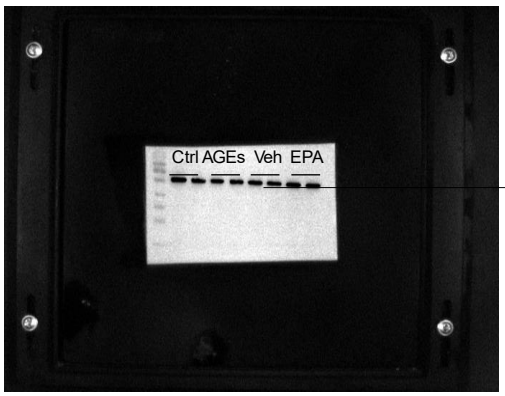
Figure 5

BAX (20kDa)



α-tubulin for BAX (55kDa)

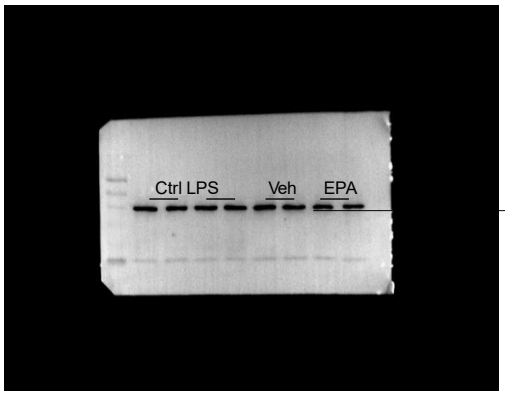
cleaved Caspase3
(17-19kDa)



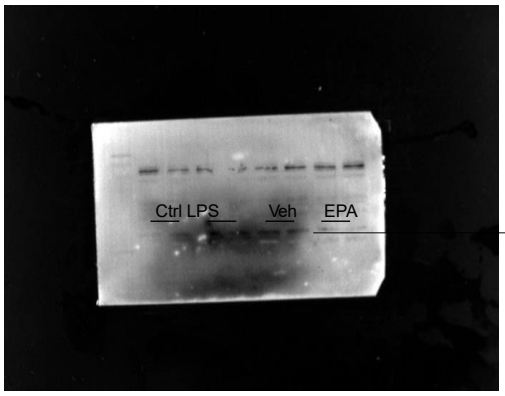
α-tubulin for cleaved
Caspase3 (55kDa)

Figure 6

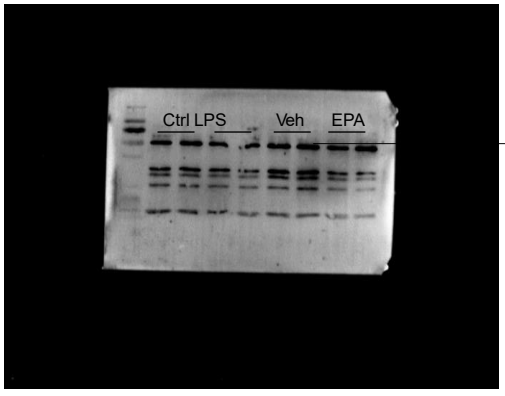
BCL2 (26kDa)



α-tubulin for BCL2 (55kDa)

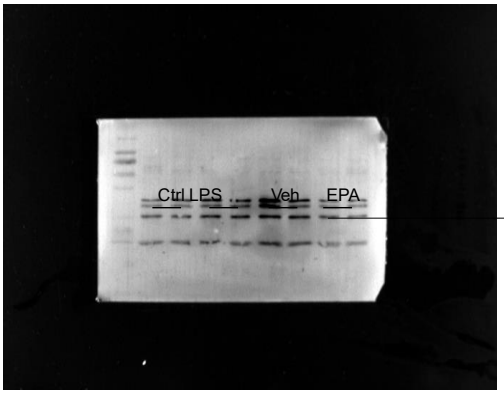


BAX (20kDa)

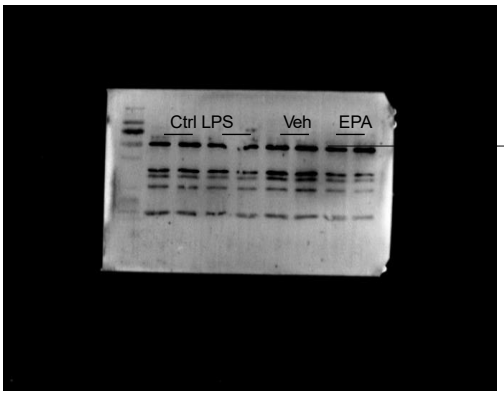


α-tubulin for BAX (55kDa)

Figure 6

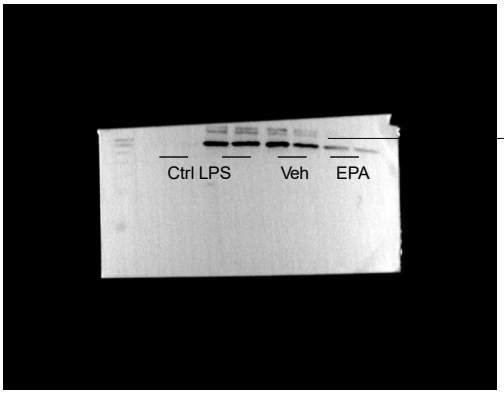


cleaved Caspase3 (17-19kDa)

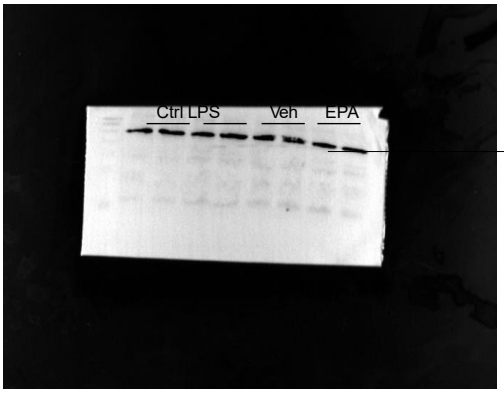


α-tubulin for cleaved Caspase3 (55kDa)

Figure 8

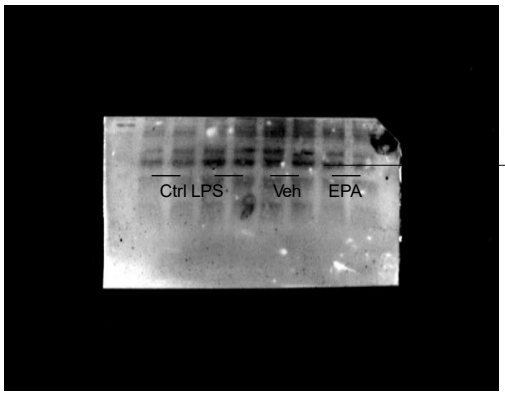


Cox2 (74kDa)

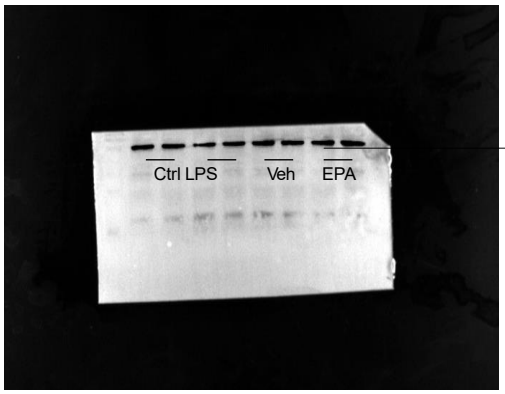


α-tubulin for Cox2 (55kDa)

Figure 8

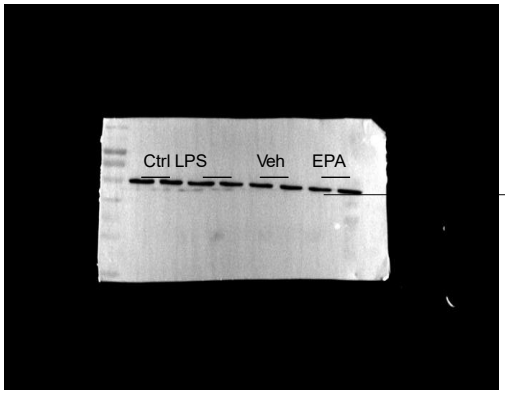


IL1β (17-31kDa)



α-tubulin for IL-1β (55kDa)

iNOS (130kDa)



α-tubulin for iNOS (55kDa)

Figure 8

p-JNK (46-54kDa)

JNK (46-54kDa)

α-tubulin for p-JNK/JNK (55kDa)

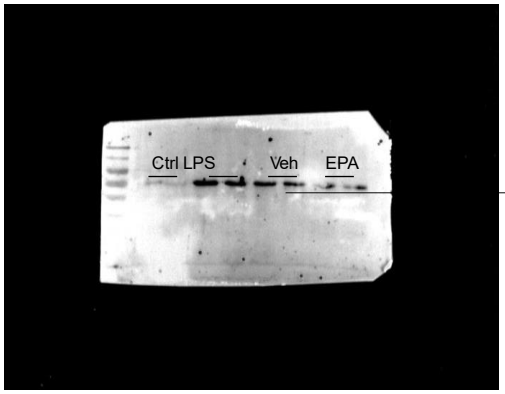
Figure 8

p-P65 (65kDa)

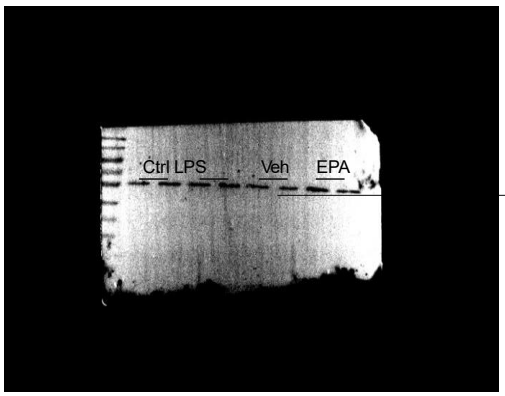
P65 (65kDa)

α-tubulin for p-P65/P65 (55kDa)

Figure 8



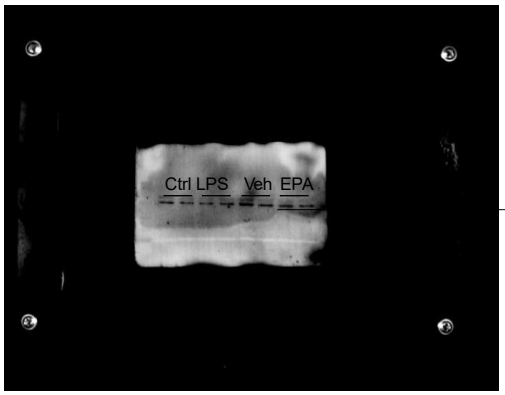
NOX4 (70kDa)



α-tubulin for NOX4 (55kDa)

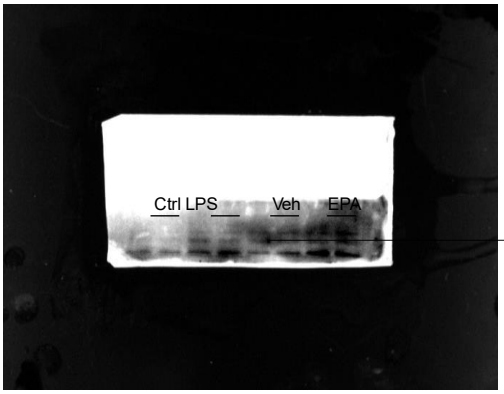
Figure 9

HO1 (28kDa)

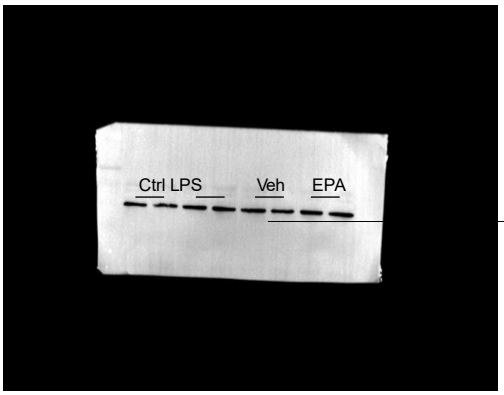


α-tubulin for HO1(55kDa)

Figure 9



NQO1(29kDa)

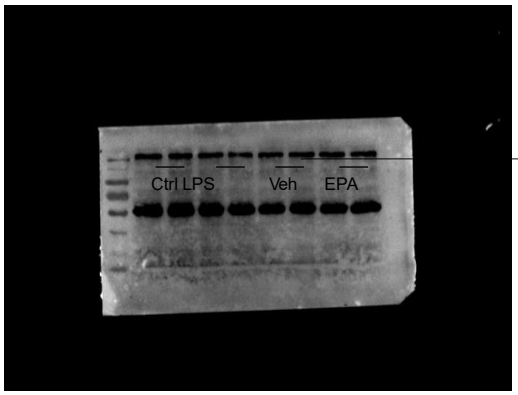


α-tubulin for NQO1(55kDa)

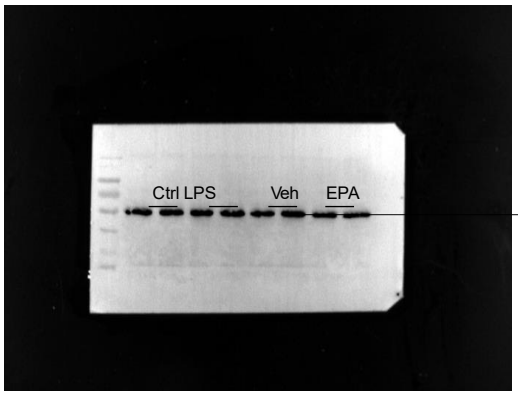
NRF2 (110kDa)

α-tubulin for NRF2 (55kDa)

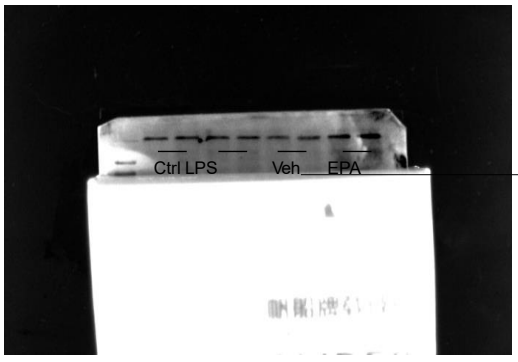
Figure 9



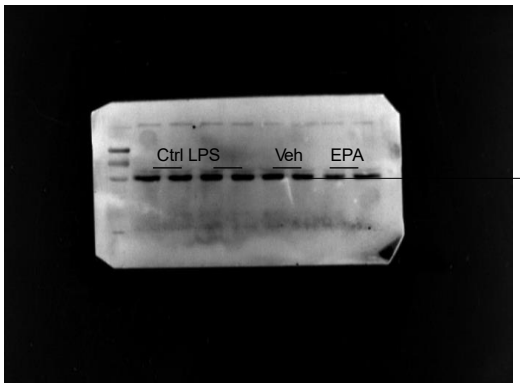
Cytosolic NRF2 (110kDa)



α-tubulin for Cytosolic NRF2 (55kDa)



Nuclear NRF2 (110kDa)



Lamin B for Nuclear NRF2 (66kDa)

Figure 11

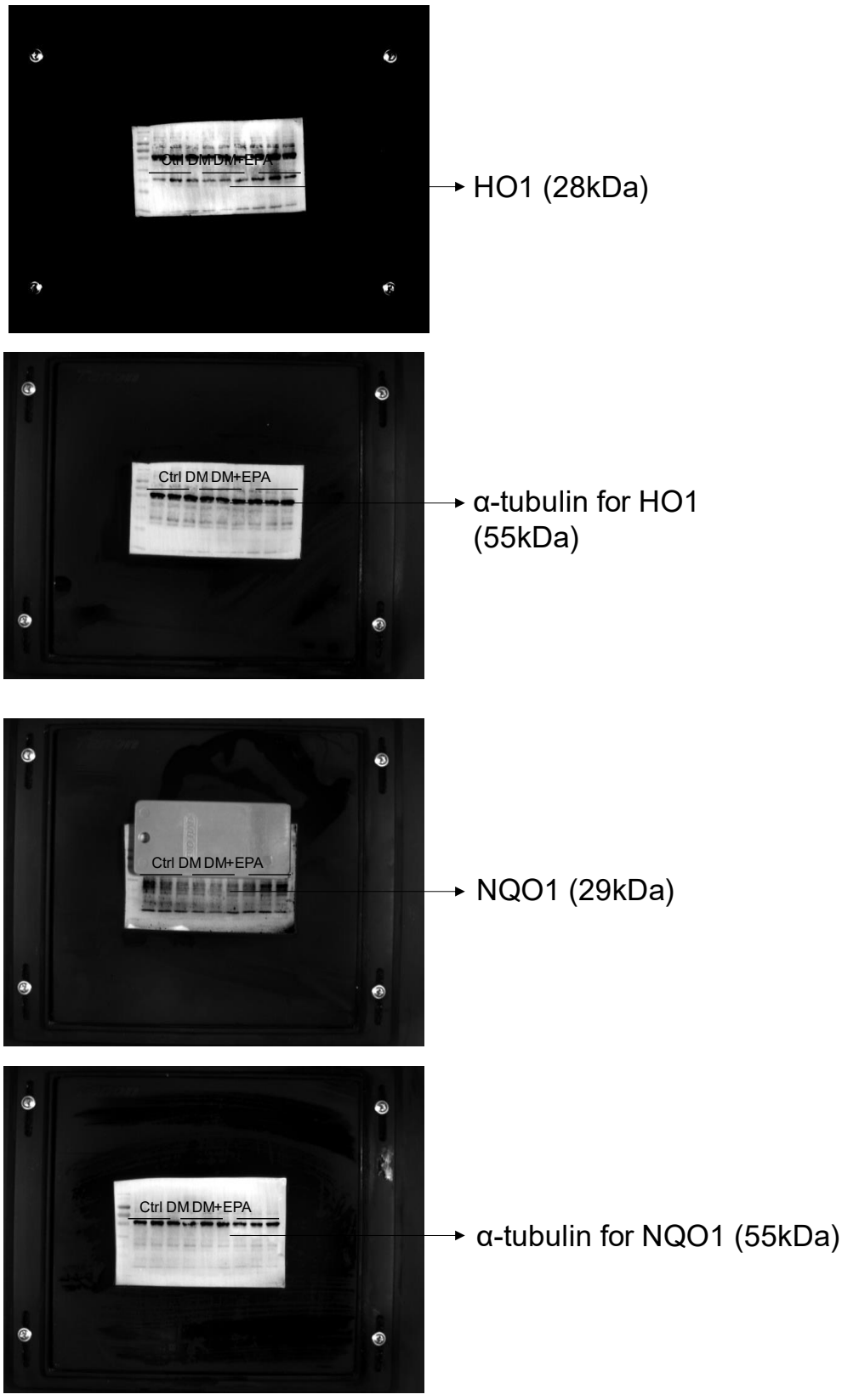
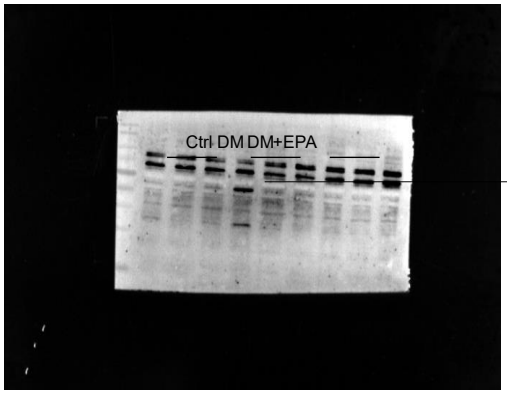
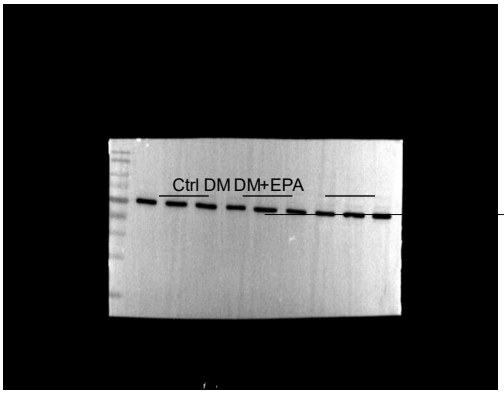

Ctrl DM DM+EPA
HO1 (28kDa)
Ctrl DM DM+EPA
α-tubulin for HO1 (55kDa)
Ctrl DM DM+EPA
NQO1 (29kDa)
Ctrl DM DM+EPA
α-tubulin for NQO1 (55kDa)

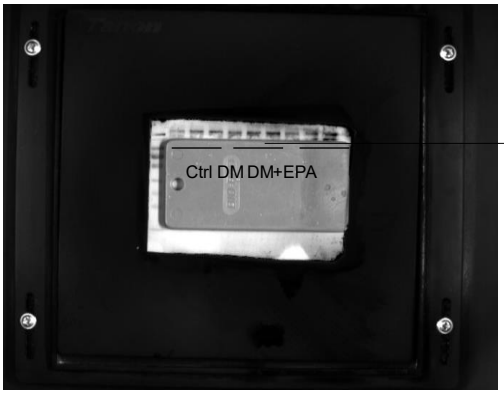
Figure 11



KEAP1 (55-70kDa)



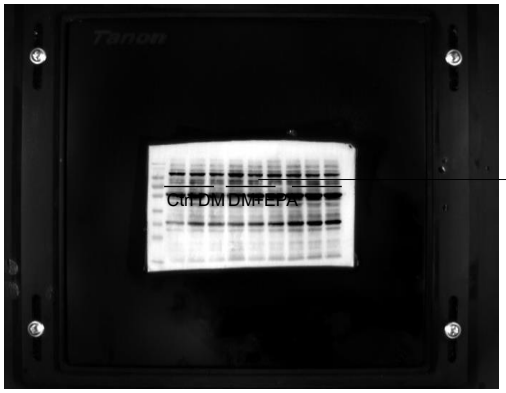
α-tubulin for KEAP1 (55kDa)



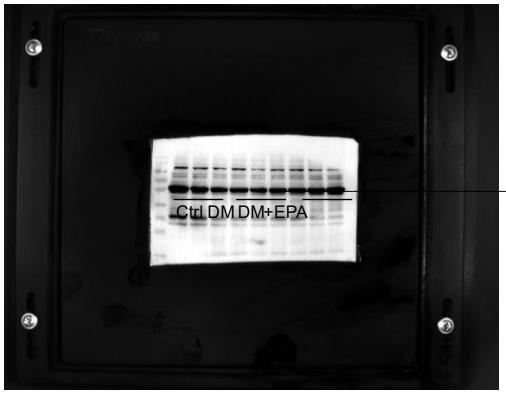
NRF2 (110kDa)

α-tubulin for NRF2 (55kDa)

Figure 11



Cytosolic NRF2
(110kDa)



α-tubulin for Cytosolic NRF2
(55kDa)

Nuclear NRF2
(110kDa)

Lamin B for Nuclear NRF2
(66kDa)

Figure 12

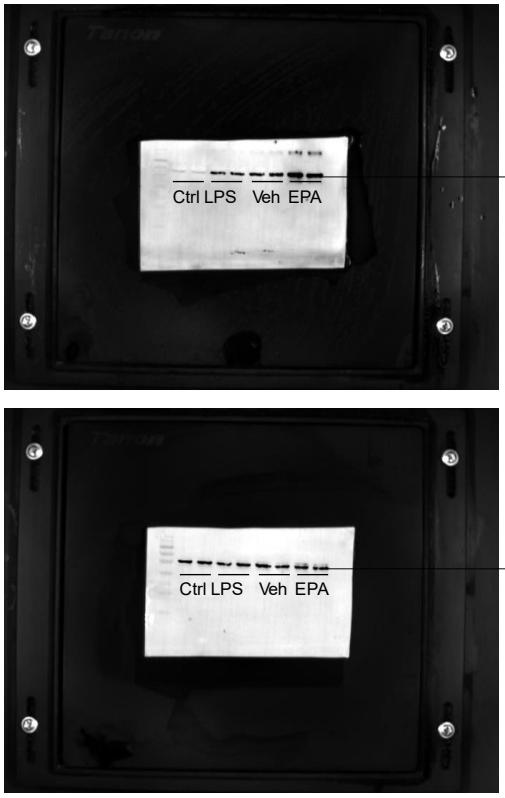


Figure 13

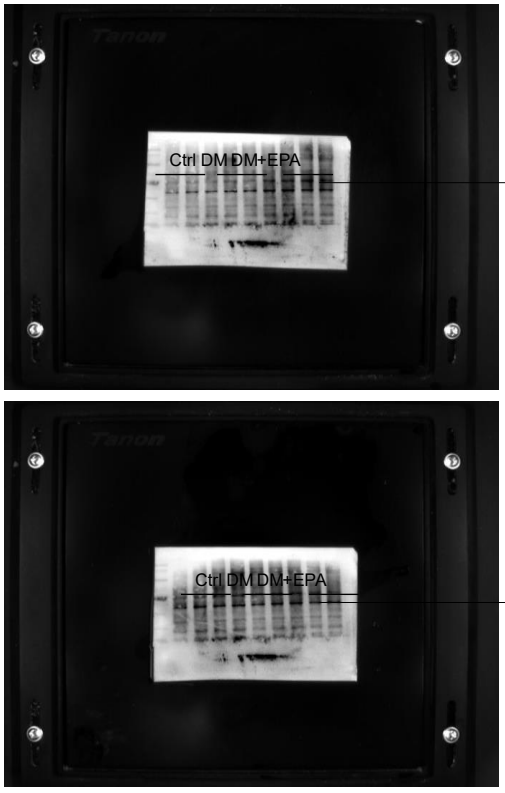
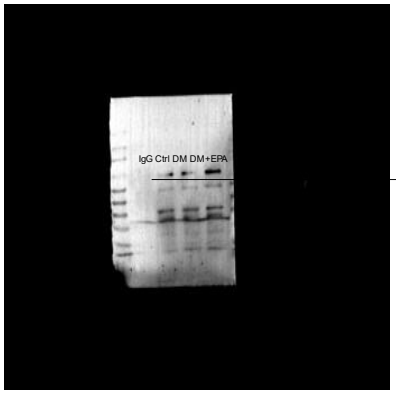
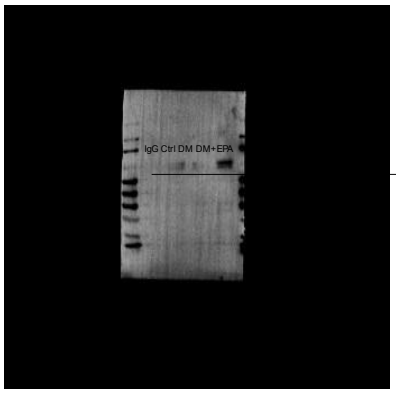


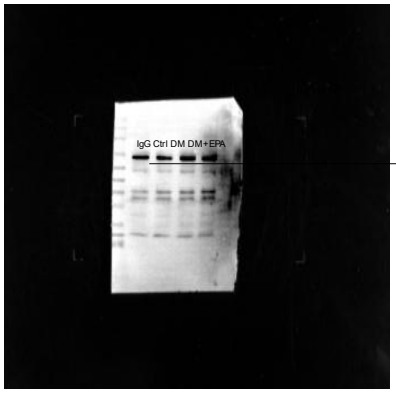
Figure 13



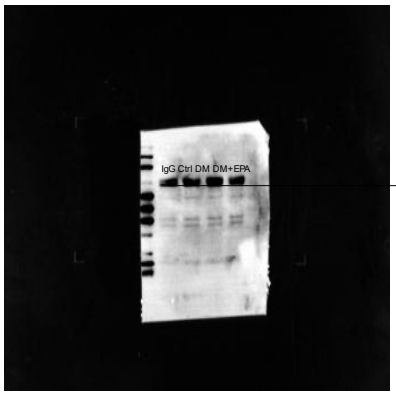
IP-P62 (62kDa)



IP-KEAP1 (55kDa)



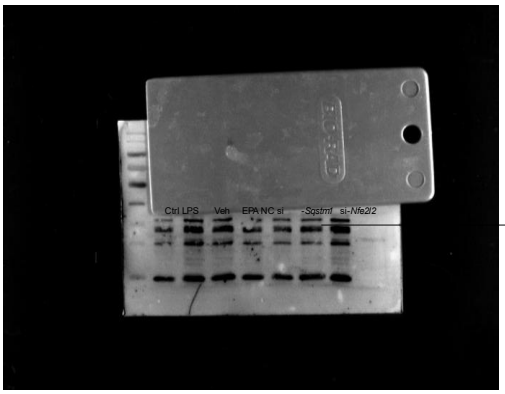
Input-p62
(62kDa)



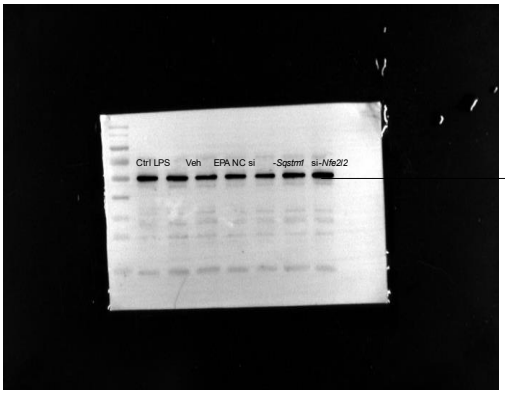
Input-KEAP1
(55kDa)

α-tubulin for
input-P62/KEAP1
(55kDa)

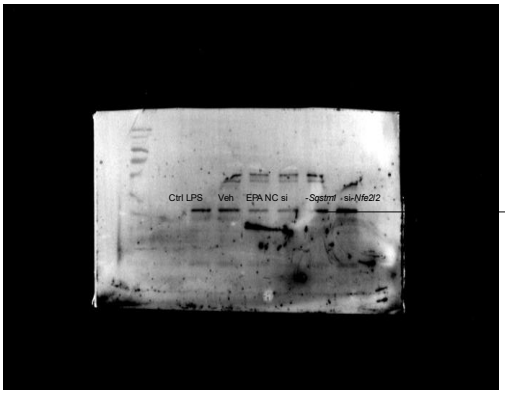
Figure 14



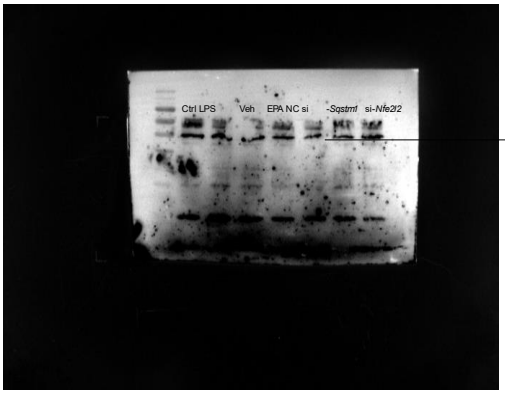
IL-1β (31kDa)



α-tubulin for IL-1β (55kDa)

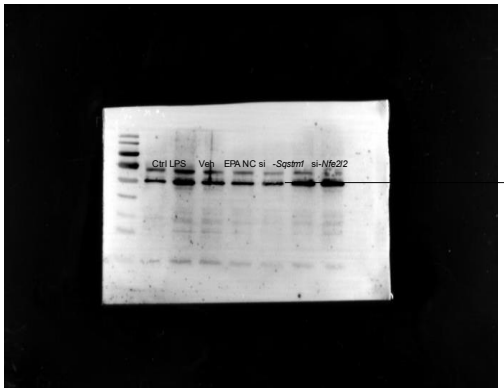


IL-6 (24kDa)



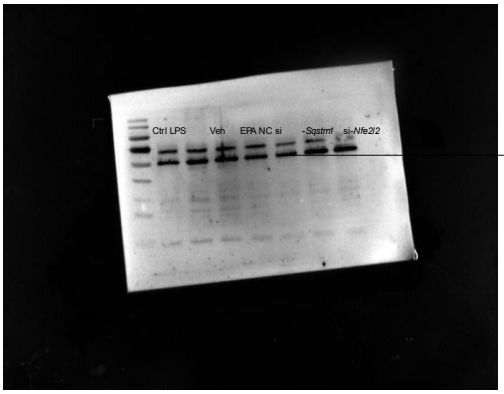
α-tubulin for IL-6 (55kDa)

Figure 14



p-P65 (65kDa)

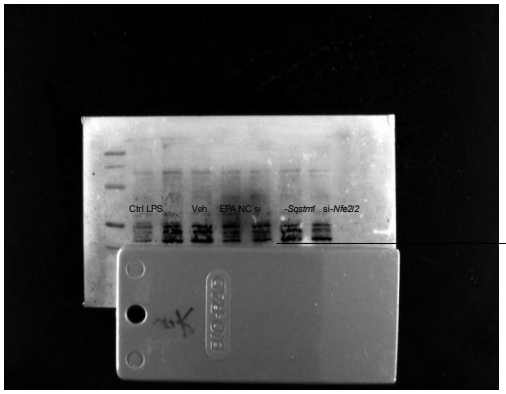
P65 (65kDa)



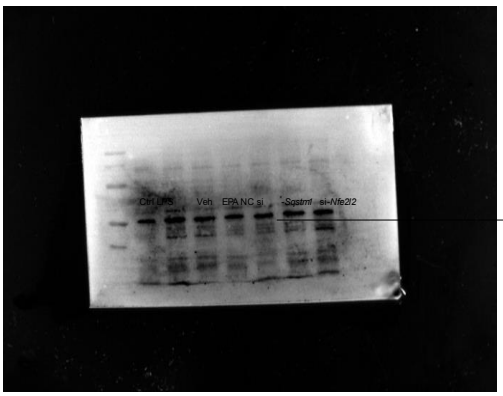
α-tubulin for p-P65/P65 (55kDa)

Figure 14

p-JNK (46-54 kDa)

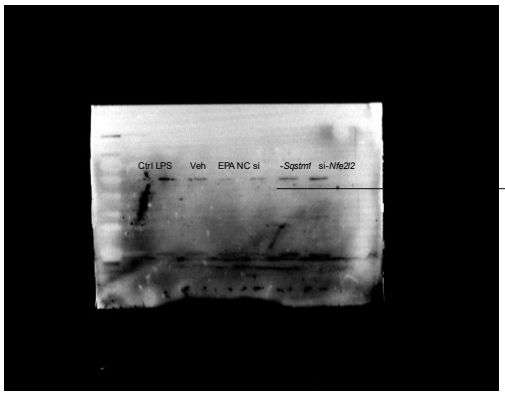


JNK (46-54 kDa)

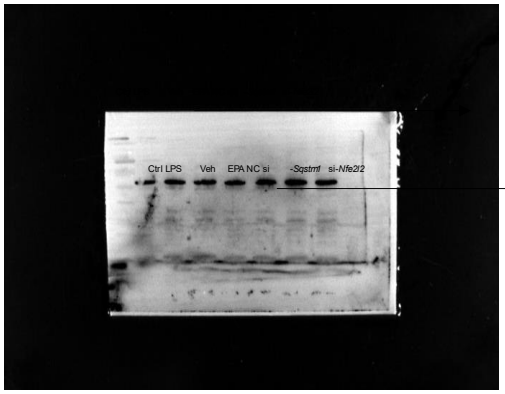


α-tubulin for p-JNK/JNK (55kDa)

Figure 14

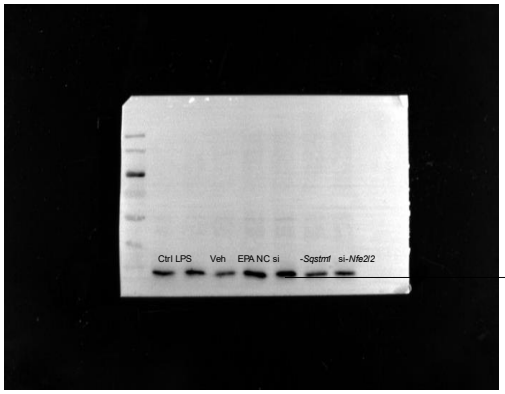


NOX4 (70kDa)

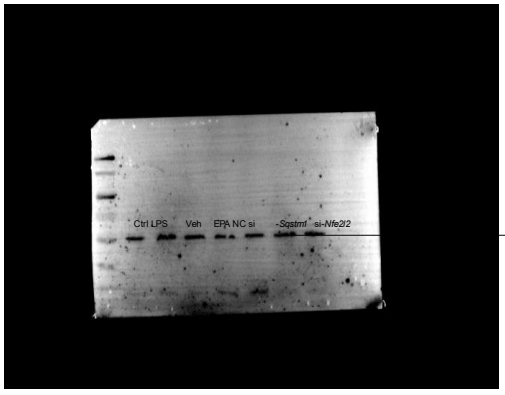


α-tubulin for NOX4 (55kDa)

Figure 15

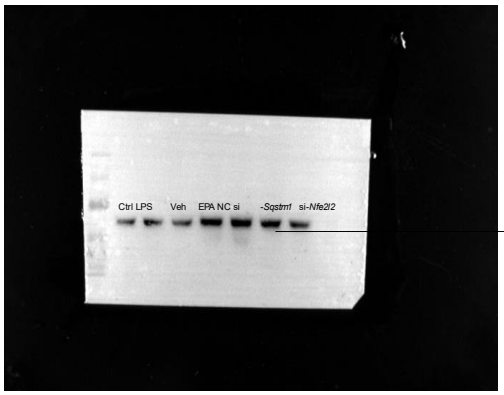


HO1 (28kDa)



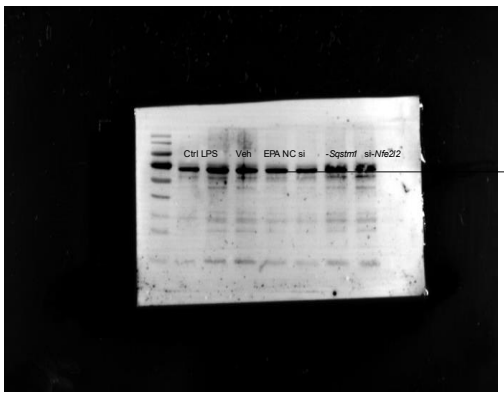
α-tubulin for HO1 (55kDa)

Figure 15

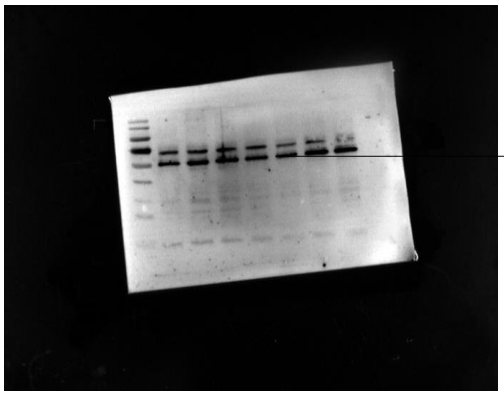


NQO1 (29kDa)

α-tubulin for NQO1 (55kDa)

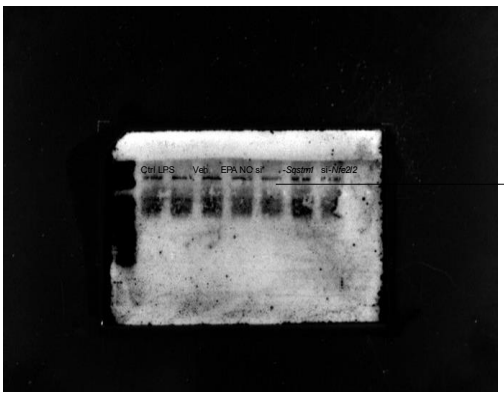


KEAP1 (55-60 kDa)

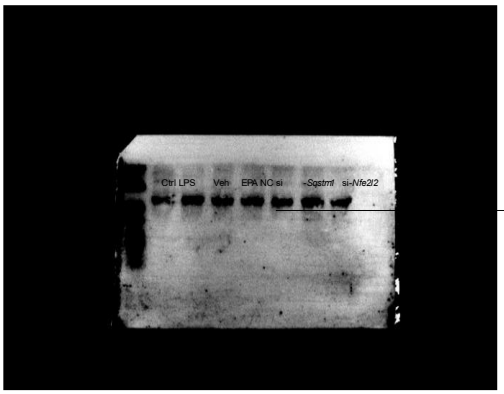


α-tubulin for KEAP1(55kDa)

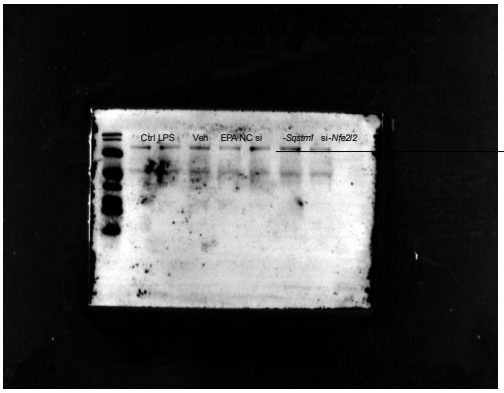
Figure 15



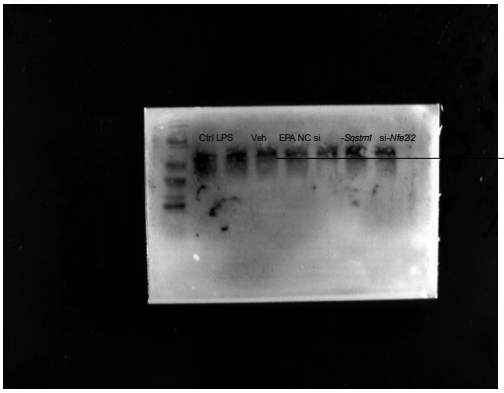
NRF2 (110kDa)



α-tubulin for NRF2 (55kDa)

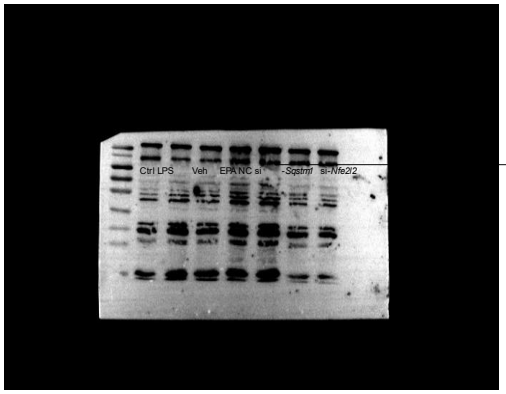


Cytosolic NRF2 (110kDa)

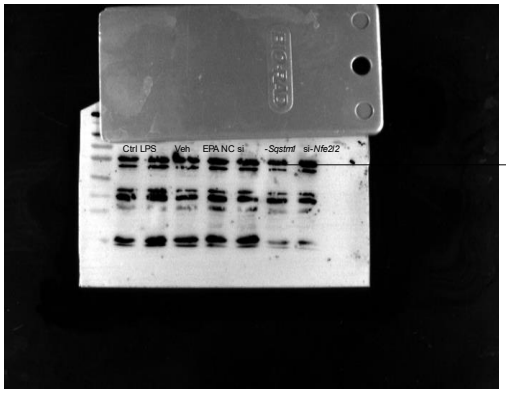


α-tubulin for Cytosolic NRF2 (55kDa)

Figure 15

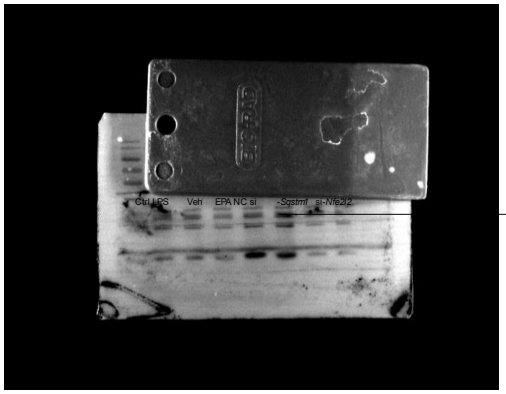


Nuclear NRF2 (110kDa)

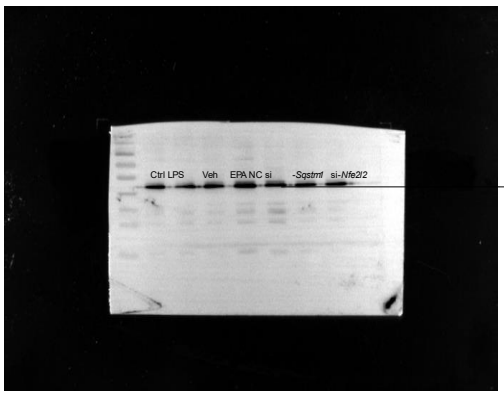


Lamin B for Nuclear NRF2 (66kDa)

Figure 16

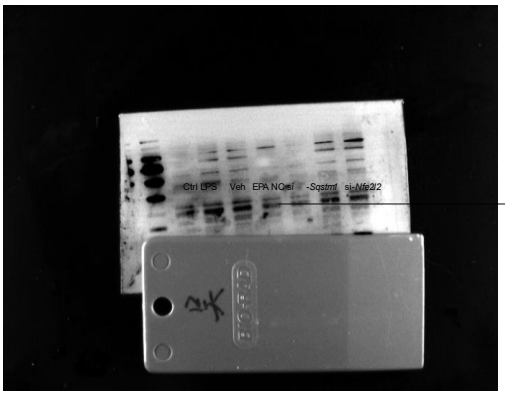


BCL2 (26kDa)

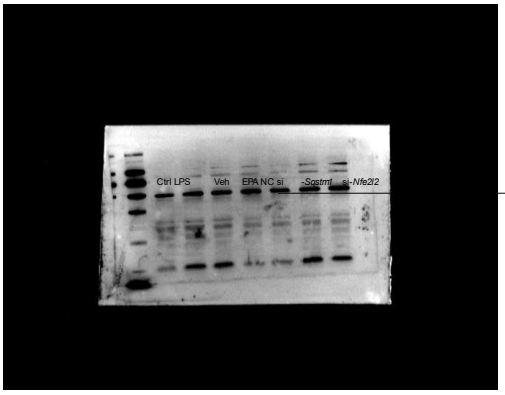


α-tubulin for BCL2 (55kDa)

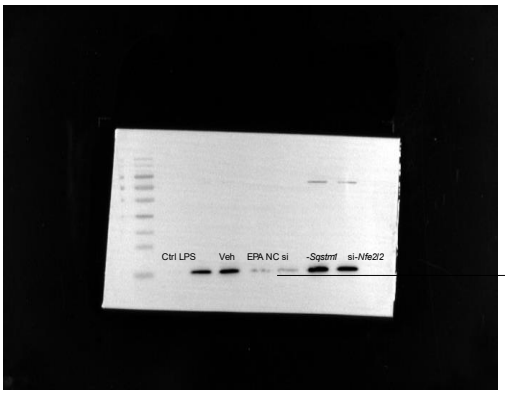
Figure 16



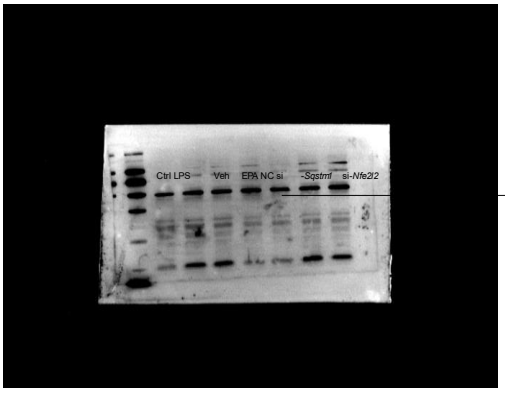
BAX (20kDa)



α-tubulin for BAX (55kDa)

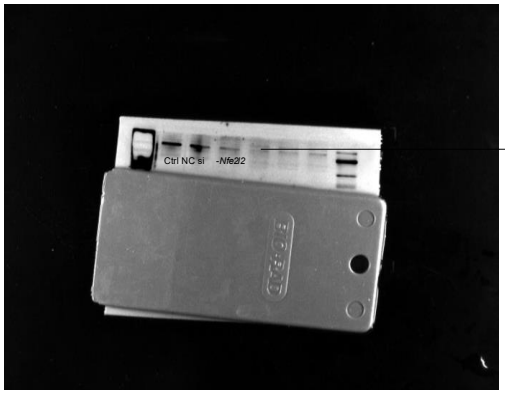


cleaved Caspase3
(17-19 kDa)



α-tubulin for cleaved
Caspase3 (55kDa)

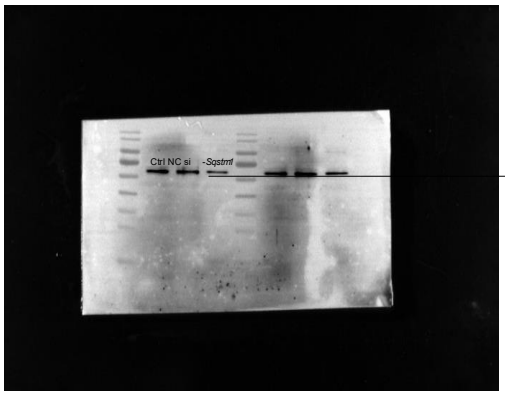
Figure S2



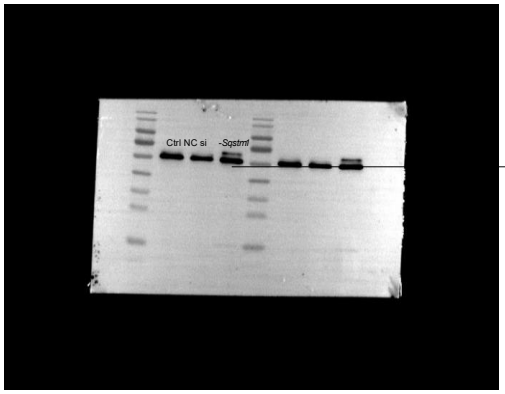
NRF2 (110kDa)

α-tubulin for NRF2 (55kDa)

Figure S3



P62 (62kDa)



α-tubulin for P62 (55kDa)