

Supplementary Tables

Supplementary Table 1. Key resources

Antibodies	Source	Identifier
Anti-NFE2L3	Abcepta (Antigen region: 666-694 aa)	Cat#AP19864B
Anti-NFE2L1	Cell Signaling Technology [®]	Cat#8052
Anti-NFE2L2	Cell Signaling Technology [®]	Cat#12721
Anti-SLC7A11	HuaBio	Cat#HA721868
Anti-TFRC	Zenbio	Cat#381603
Anti-GPX4	HuaBio	Cat#ET1706-45
Anti-HMGCR	Abcam	Cat#ab174830
Anti-HMGCR	Proteintech [®]	Cat#13533-1-AP
Anti-alpha Tubulin	Proteintech [®]	Cat#66031-1-Ig
Anti-rabbit IgG, HRP-linked	Cell Signaling Technology [®]	Cat#7074
Anti-mouse IgG, HRP-linked	Cell Signaling Technology [®]	Cat#7076
Chemical reagents	Source	Identifier
Cycloheximide	MCE [®]	Cat#HY-12320
Chloroquine	MCE [®]	Cat#HY-17589A
MG-132	Beyotime	Cat#S1748-5
Emodin	Sangon	Cat#A606281-0025
RSL3	MCE [®]	Cat#HY-100218A
FAC	MCE [®]	Cat#HY-B1645
Ferrostatin-1	MCE [®]	Cat#HY-100579
TRIzol [®]	Thermo Fisher	Cat#15596026
Commercial Assays	Source	Identifier
EndoFree Mini Plasmid Kit	TIANGEN	Cat# DP118-02
BCA Protein Quantification Kit	Vazyme	Cat#E112-01/02
HiScript [®] III RT Supermix for qPCR	Vazyme	Cat# R323-01
ChamQ Universal SYBR qPCR Master Mix	Vazyme	Cat# Q711-02

Dual Luciferase Reporter Assay Kit	Vazyme	Cat# DL101-01
miRNA 1st Strand cDNA Synthesis Kit (by tailing A)	Vazyme	Cat#MR201-01
miRNA 1st Strand cDNA Synthesis Kit (by stem-loop)	Vazyme	Cat#MR101-02
Cell Counting Kit-8	SparkJade®	Cat#CT0001-B
GSH and GSSG Assay Kit	Beyotime	Cat#S0053
Mito-Tracker Red CMXRos	Beyotime	Cat#C1049B
ROS Assay Kit	Beyotime	Cat#S0033S
BODIPY™ 581/591 C11	Thermo Fisher	Cat#D3861
Total Iron Content Colorimetric Assay Kit	APPLYGEN	Cat#E1042
One-step Mouse Genotyping Kit	Vazyme	Cat#PD101-01
SimpleChIP® Enzymatic Chromatin IP Kit (Magnetic Beads)	Cell Signaling Technology®	Cat#91820
Recombinant DNA	Source	Identifier
pCMV3-3Flag	Sino Biological	Cat#CF3vector
pCMV3-NFE2L3-3Flag	Sino Biological	Cat#HG21575-CF3
pGL3-WT (HMGR promoter)	Genscript	Cat#C9563EJ280-12
pGL3-Mut-1 (HMGR promoter)	Genscript	Cat#C9563EJ280-4
pGL3-Mut-2 (HMGR promoter)	Genscript	Cat#C9563EJ280-8
pGL3-Mut-3 (HMGR promoter)	Genscript	Cat#C9563EJ280-10
pmirGLO-WT (NFE2L3-3' UTR)	Tsingke	Cat#CD0105793-1
pmirGLO-Mut (NFE2L3-3' UTR)	Tsingke	Cat#CD0105793-2
Lentivirus and Oligonucleotides	Target Sequence (5' to 3')	Source
shNC	CCTAAGGTTAAGTCGCCCTCG	Cyagen (200307LVK504)
shNFE2L3-1	GCATGTAAAGTAGATATTATC	Cyagen (200328LVK501)

shNFE2L3-2	CGCGTAGACCTAGATCTTTAC	Cyagen (200328LVK502)
siHMGCRCR-NC	/	Tsingke
siHMGCRCR-1	GTTCCAGAATTTACGTCAA	Tsingke
siHMGCRCR-2	GCAGATACTTCTAAGGTTT	Tsingke
siHMGCRCR-3	CAGAATGTTGGTAGTTCAA	Tsingke
micrOFF inhibitor NC #22	/	Ribobio
miR-4465 inhibitors	/	Ribobio
micrON mimic NC #22	/	Ribobio
miR-4465 mimics	/	Ribobio

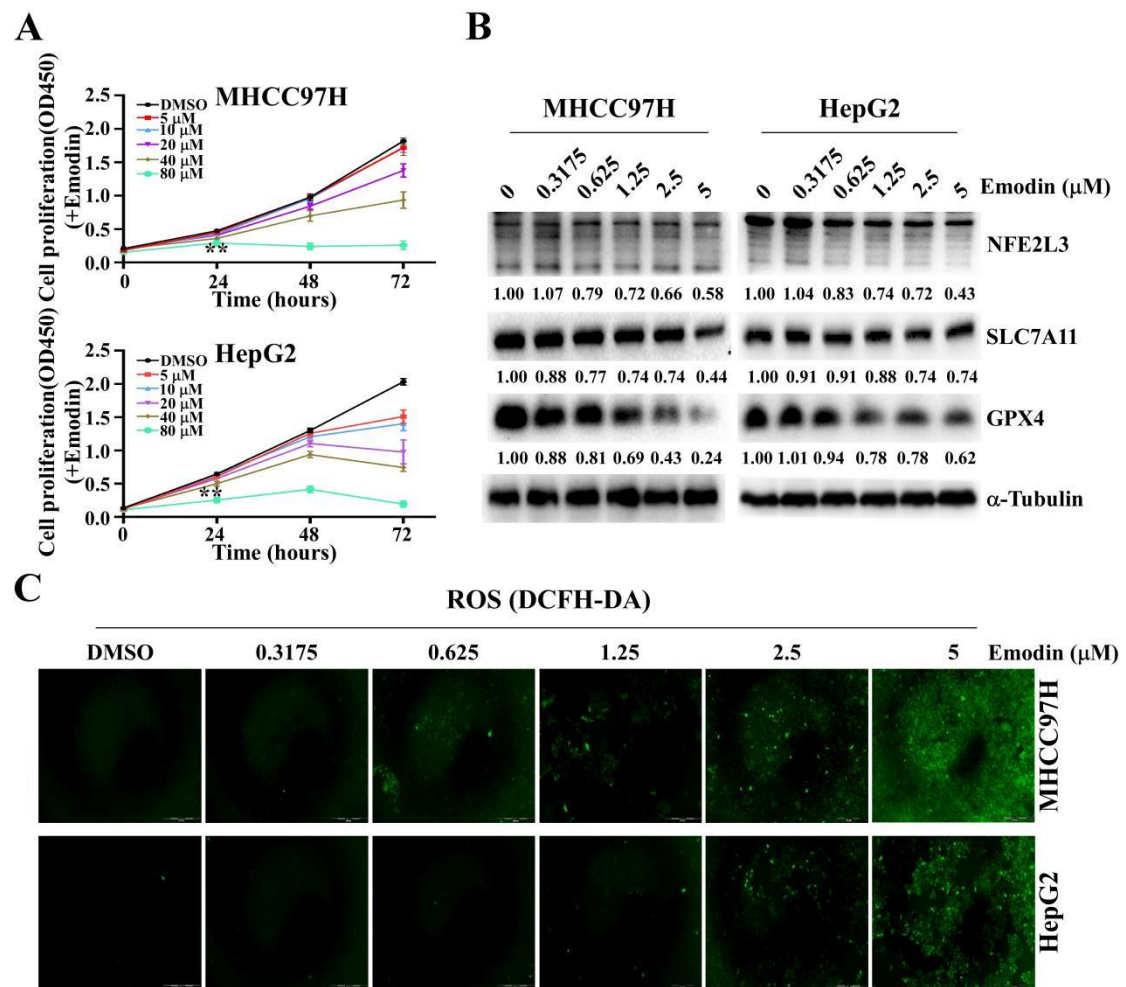
Supplementary Table 2. RT-qPCR and ChIP-qPCR primers

RT-qPCR primers	Source	Nucleotide sequences (5' to 3')
NFE2L1 F	Tsingke	GCTGGACACCATCCTGAATC
NFE2L1 R	Tsingke	CCTTCTGCTTCATCTGTGCG
NFE2L2 F	Tsingke	TTCCCGGTCACATCGAGAG
NFE2L2 R	Tsingke	TCCTGTTGCATACCGTCTAAATC
NFE2L3 F	Tsingke	CACAGATAGAACTTGAGCCGT
NFE2L3 R	Tsingke	GCGTTTACGACAGTTCTGCG
SLC7A11 F	Tsingke	GGTCCATTACCAGCTTTTGTACG
SLC7A11 R	Tsingke	AATGTAGCGTCCAAATGCCAG
TFRC F	Tsingke	GGCTACTTGGGCTATTGTAAAGG
TFRC R	Tsingke	CAGTTTCTCCGACAACTTTCTCT
GPX4 F	Tsingke	GAGGCAAGACCGAAGTAAACTAC
GPX4 R	Tsingke	CCGAACTGGTTACACGGGAA
HMGCRCR F	Tsingke	TGATTGACCTTTCCAGAGCAAG
HMGCRCR R	Tsingke	CTAAAATTGCCATTCCACGAGC
PSMA1 F	Tsingke	AAGGGCGCAATCAGAGCTT
PSMA1 R	Tsingke	CCAAACACTCCTGACGCATAAA
PSMA2 F	Tsingke	AGCTGCAAATGGTGTGGTATT

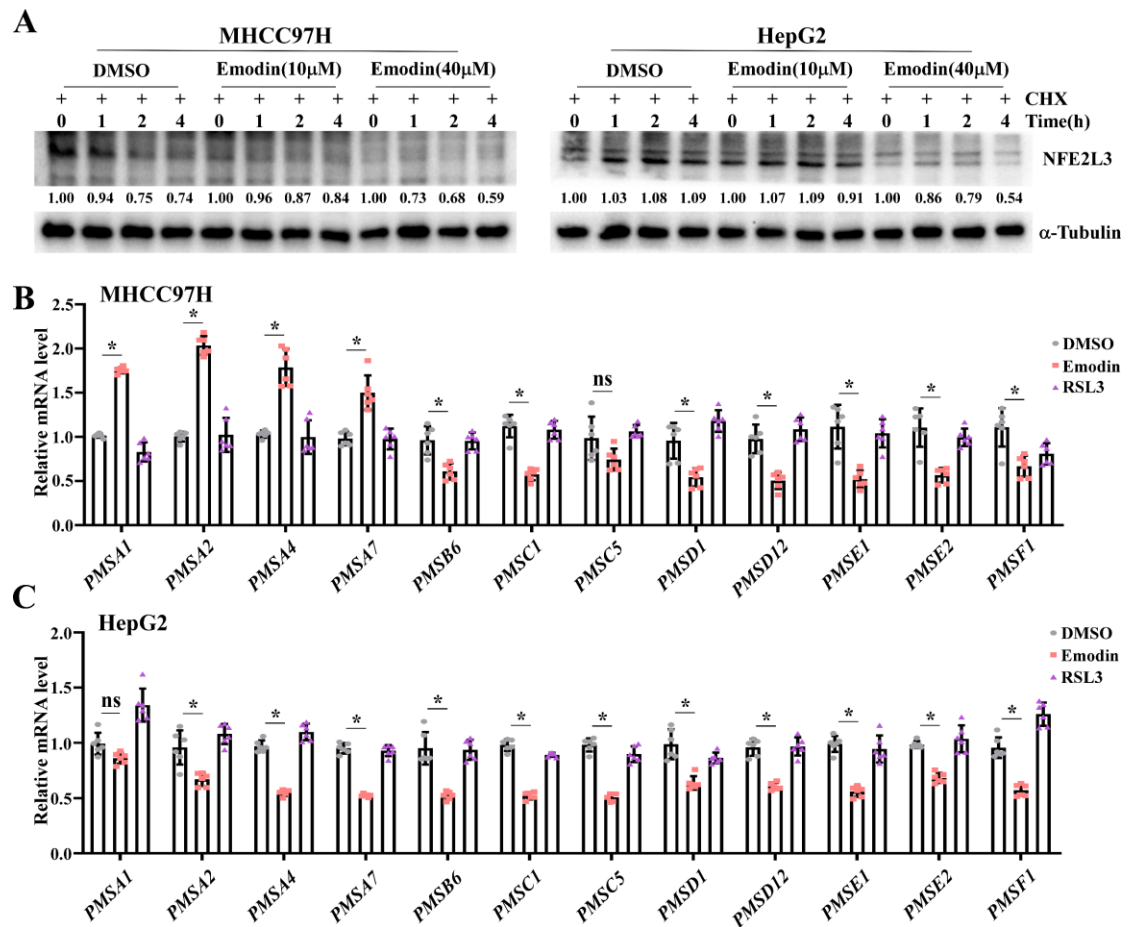
PSMA2 R	Tsingke	ACAAGCACTCTGTAATCGGGG
PSMA4 F	Tsingke	AGTGTGGCAGGCATAACTTCT
PSMA4 R	Tsingke	TCACAAGGTATTGGCTCCTGA
PSMA7 F	Tsingke	ACCGCCGATGCAAGGATAG
PSMA7 R	Tsingke	CTGCGTATAACGCTGCTTCAG
PSMB6 F	Tsingke	CGGGAATCATCATCGCAGG
PSMB6 R	Tsingke	GGA CTGCCTTACCATCATACCC
PSMC1 F	Tsingke	CTCAGAACACTACGTCAGCATT
PSMC1 R	Tsingke	CATGCACCTTGTGGTTGAGC
PSMC5 F	Tsingke	AGGCACAGAGGAACGAACTAA
PSMC5 R	Tsingke	AGGATGTACCTTGACCAACACTT
PSMD1 F	Tsingke	TCCGAGTCCGTAGACAAAATAGA
PSMD1 R	Tsingke	CCACACATTGTTTGGTGTAGTGA
PSMD12 F	Tsingke	ATGGTTACCGAAGGCAAGATTT
PSMD12 R	Tsingke	CTTTTCCATTGACCCGTAGGTT
PSME1 F	Tsingke	CCAGTGCCTGATCCAGTCAAG
PSME1 R	Tsingke	ACCACGATCTTTTCATTGCAGT
PSME2 F	Tsingke	TTTGGGGTAGCAATCCAGGAG
PSME2 R	Tsingke	CCAAGGCCCGGTAATCCAT
PSMF1 F	Tsingke	GTGGTGACACACGGTACTTC
PSMF1 R	Tsingke	ATACCGGAGGACATACAGGTC
miR-26a F	Tsingke	CGCGTTCAAGTAATCCAGGA
miR-26b F	Tsingke	GCGCGTTCAAGTAATTCAGG
miR-1297 F	Tsingke	CGCGCGCGTTCAAGTAATT
miR-4465 F	Tsingke	CGCTCAAGTAGTCTGACC
miR-4465 R	Tsingke	AGTGCAGGGTCCGAGGTATT
ACTB F	Tsingke	CATGTACGTTGCTATCCAGGC
ACTB R	Tsingke	CTCCTTAATGTCACGCACGAT
U6 F	Tsingke	GCTTCGGCAGCACATATACTAAAAT

U6 R	Tsingke	AACGCTTCACGAATTTGCGT
m-Nfe2l3 F	Tsingke	TGAGCCAAGCTATAAGCCATGA
m-Nfe2l3 R	Tsingke	AATGGTTCTTGTGCCTGTGAA
m-Hmgcr F	Tsingke	AGCTTGCCCGAATTGTATGTG
m-Hmgcr R	Tsingke	TCTGTTGTGAACCATGTGACTTC
m-Gpx4 F	Tsingke	GCCTGGATAAGTACAGGGGTT
m-Gpx4 R	Tsingke	CATGCAGATCGACTAGCTGAG
m-Actb F	Tsingke	GGCTGTATTCCCCTCCATCG
m-Actb R	Tsingke	CCAGTTGGTAACAATGCCATGT
ChIP-qPCR primers	Source	Nucleotide sequences (5' to 3')
HMGCR-1 F	Tsingke	GTCCTTGAGACTTCTAGCGGC
HMGCR-1 R	Tsingke	GCAGTGTTCTCTTAGCAGCC
HMGCR-2 F	Tsingke	ACCGGCCAGTTACAGGTTTG
HMGCR-2 R	Tsingke	AATCGGAGCGTGAGGGAAAA
HMGCR-3 F	Tsingke	AGCTGTGGGCGGTTGTTAAG
HMGCR-3 R	Tsingke	GGACGAGCCTTCGACCAAT
Reverse transcription primers (by stem-loop)	Source	Nucleotide sequences (5' to 3')
miR-4465	Tsingke	GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACTCCCCT

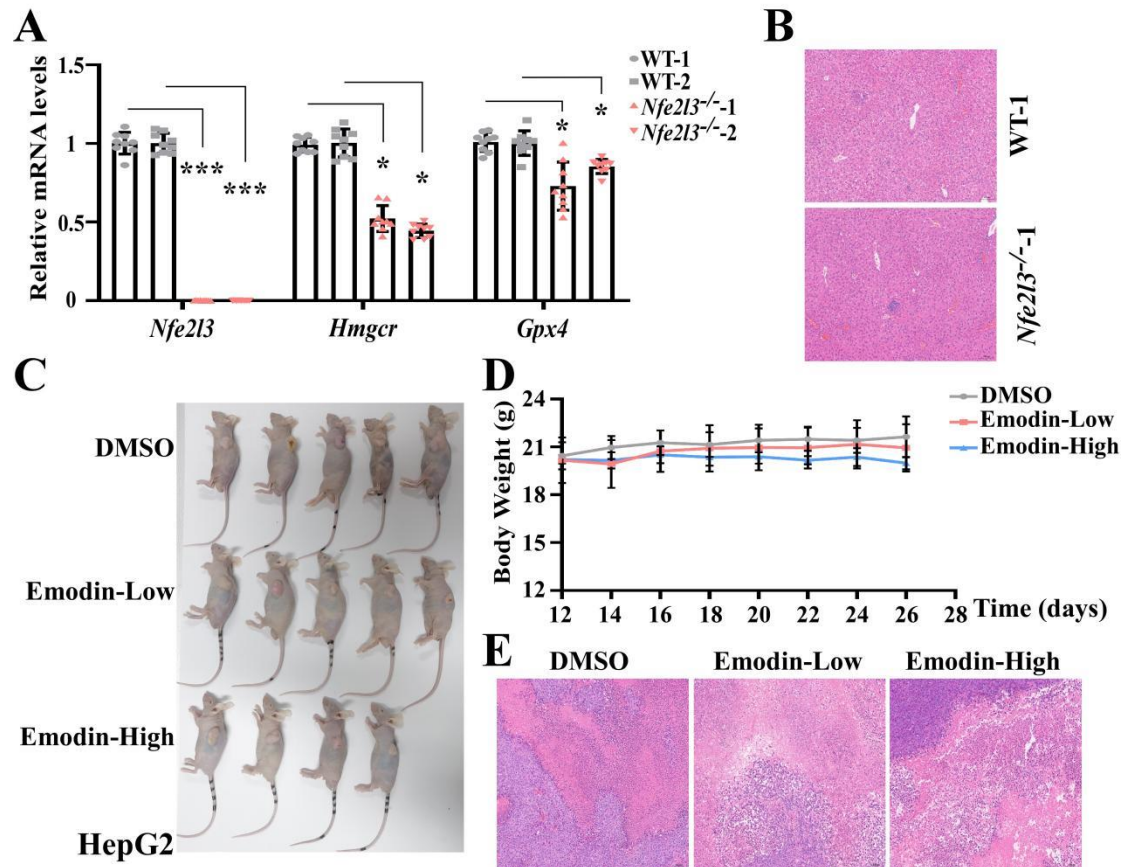
Supplementary Figures



Supplementary Fig. 1 (A) Cell viability was assessed using the CCK-8 assay after treatment with different concentrations of Emodin for 24, 48, 72 h. (B) NFE2L3, SLC7A11 and GPX4 were detected by western blot after treatment with a low concentration gradient of Emodin for 24 h in MHCC97H and HepG2. (C) MHCC97H and HepG2 cells were treated with a low concentration gradient of Emodin for 24 h and ROS were detected using the DCFH-DA probe. Scale bars, 200 μ m. Error bars represent means $\pm$ SD of at least three independent experimental replicates with ** $P < 0.01$.



Supplementary Fig. 2 (A) The stability of NFE2L3 in MHCC97H and HepG2 after treatment with DMSO, 10 μ M and 40 μ M Emodin followed by CHX. **(B-C)** The relative expression of proteasome genes in MHCC97H and HepG2 treated with Emodin and RSL3 for 24 h. Error bars represent means $\pm$ SD of at least three independent experimental replicates with * $P < 0.05$. ns: not significant difference.



Supplementary Fig. 3 (A) The expression of *Nfe2l3*, *Hmgcr* and *Gpx4* mRNA in liver tissue from *Nfe2l3*^{-/-} mice were detected via RT-qPCR. (B) HE staining results of liver tissue from *Nfe2l3*^{-/-} mice. (C-E) Images, body weight, and HE staining of tissue sections from subcutaneously transplanted HCC tumor tissue in nude mice treated with DMSO, low and high concentrations of Emodin. Error bars represent means $\pm$ SD of at least three independent experimental replicates with * $P < 0.05$, *** $P < 0.001$.

Supplementary Fig. 4

Fig2A

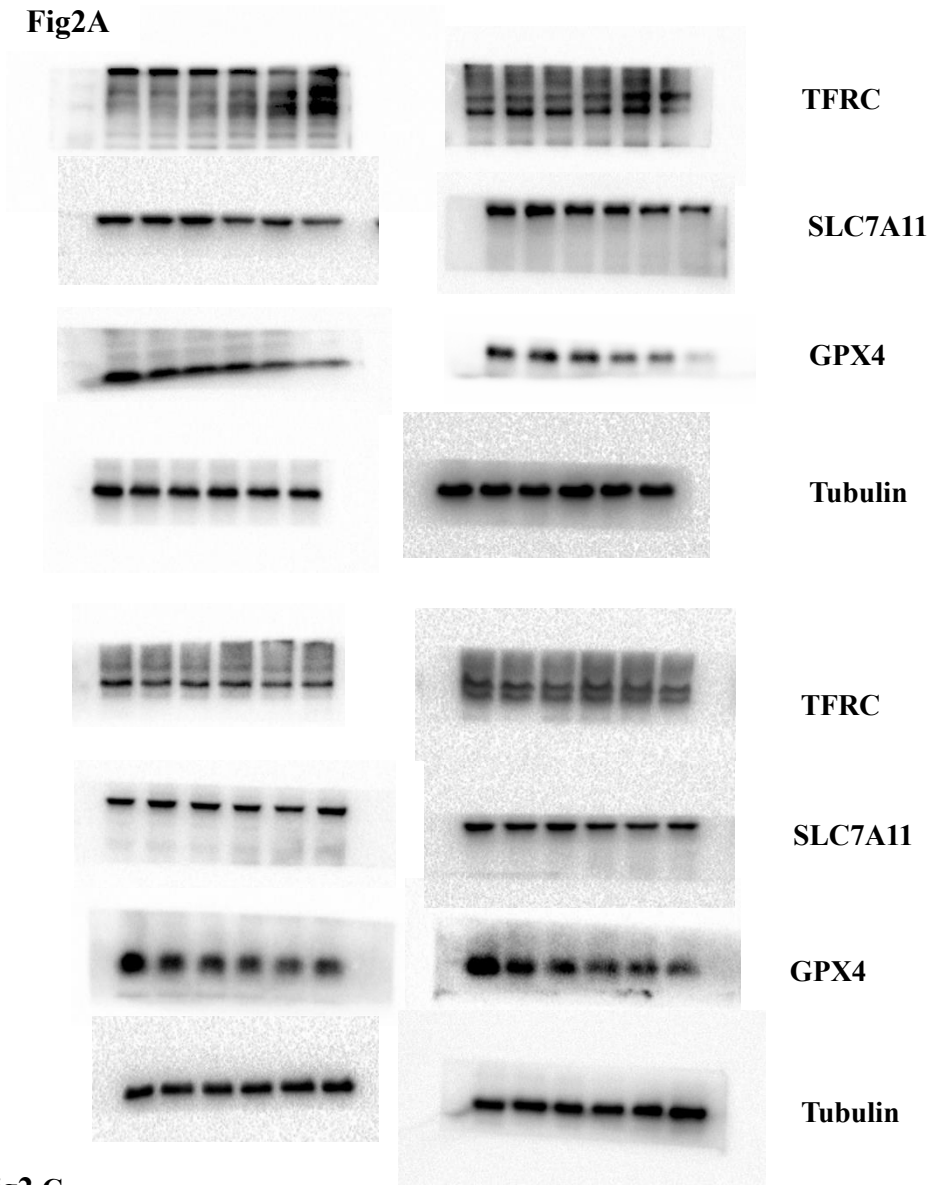
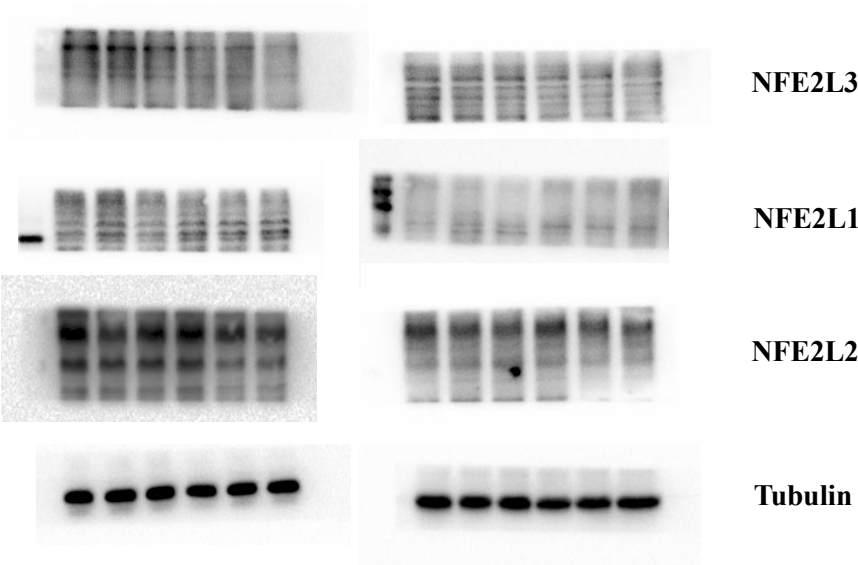


Fig2 C



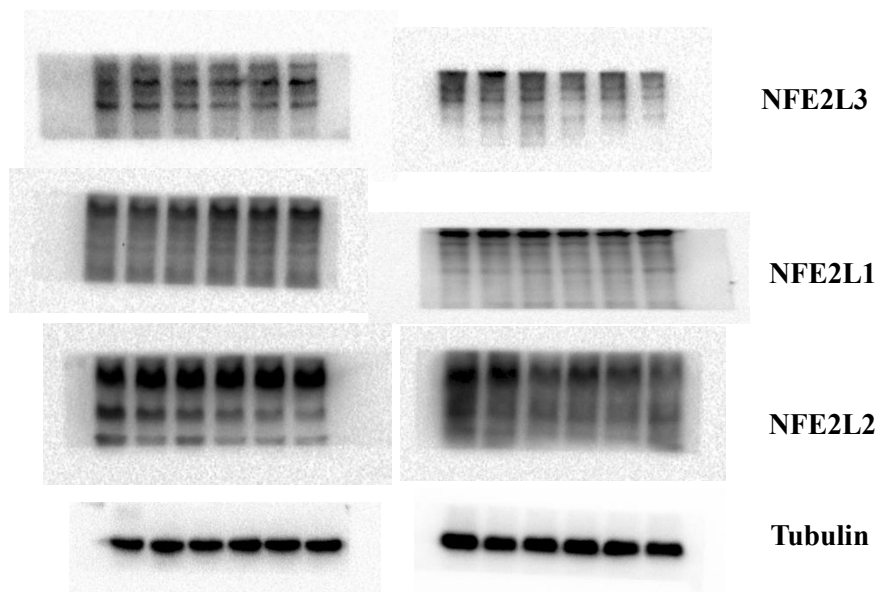


Fig2 E

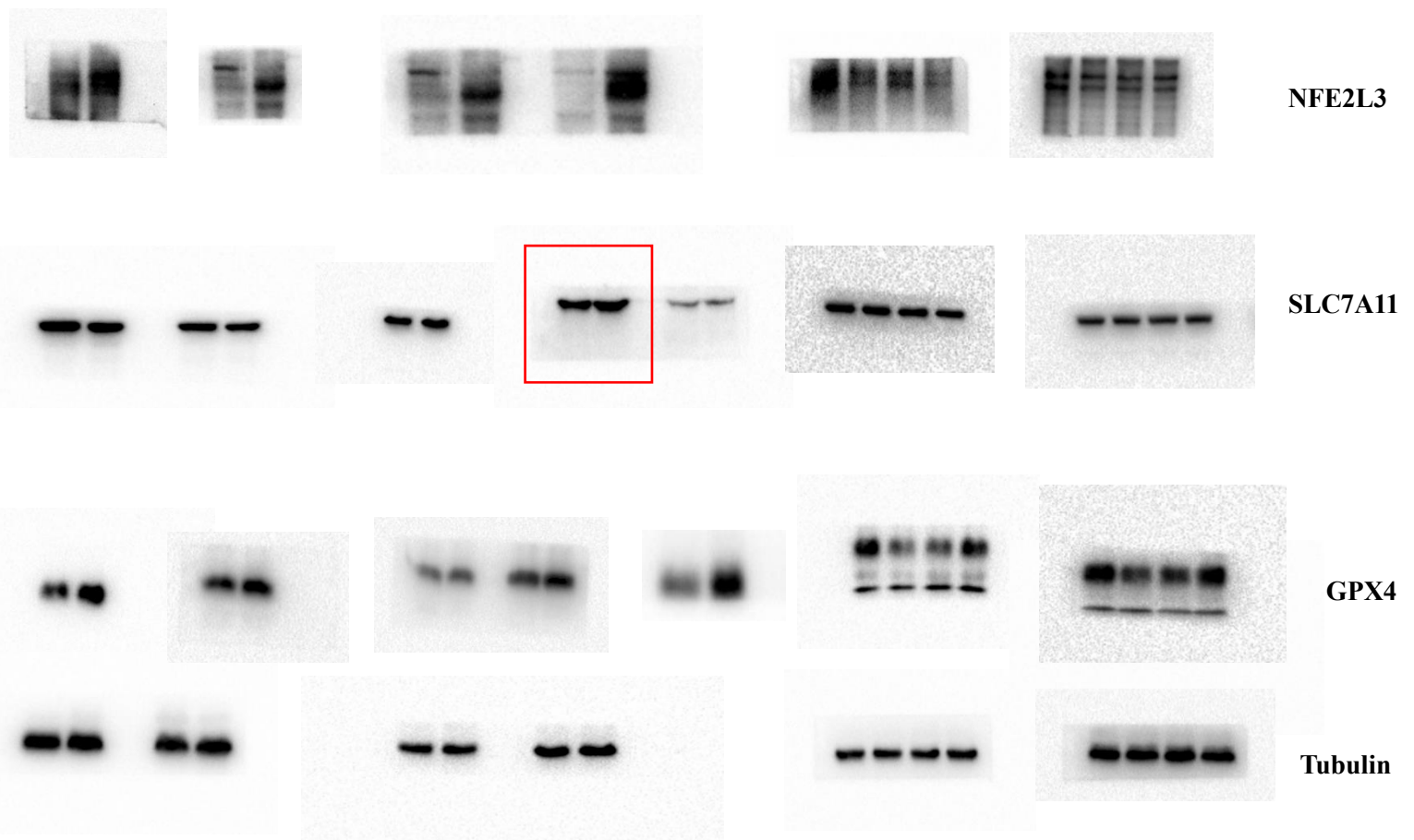


Fig4 C

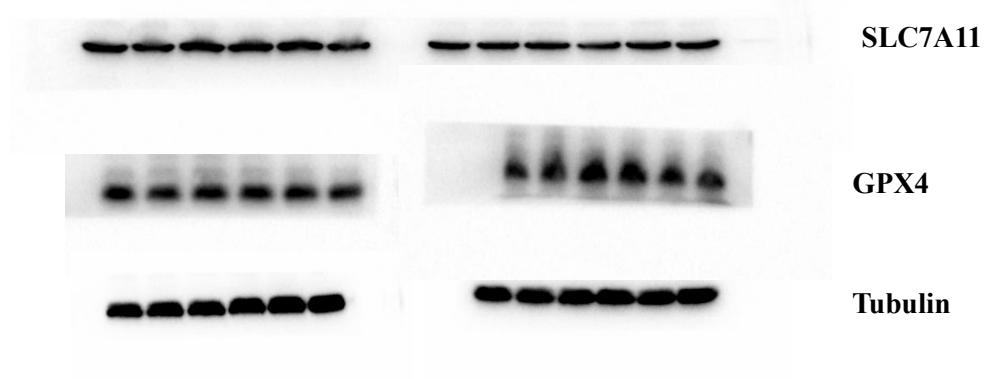


Fig4 D

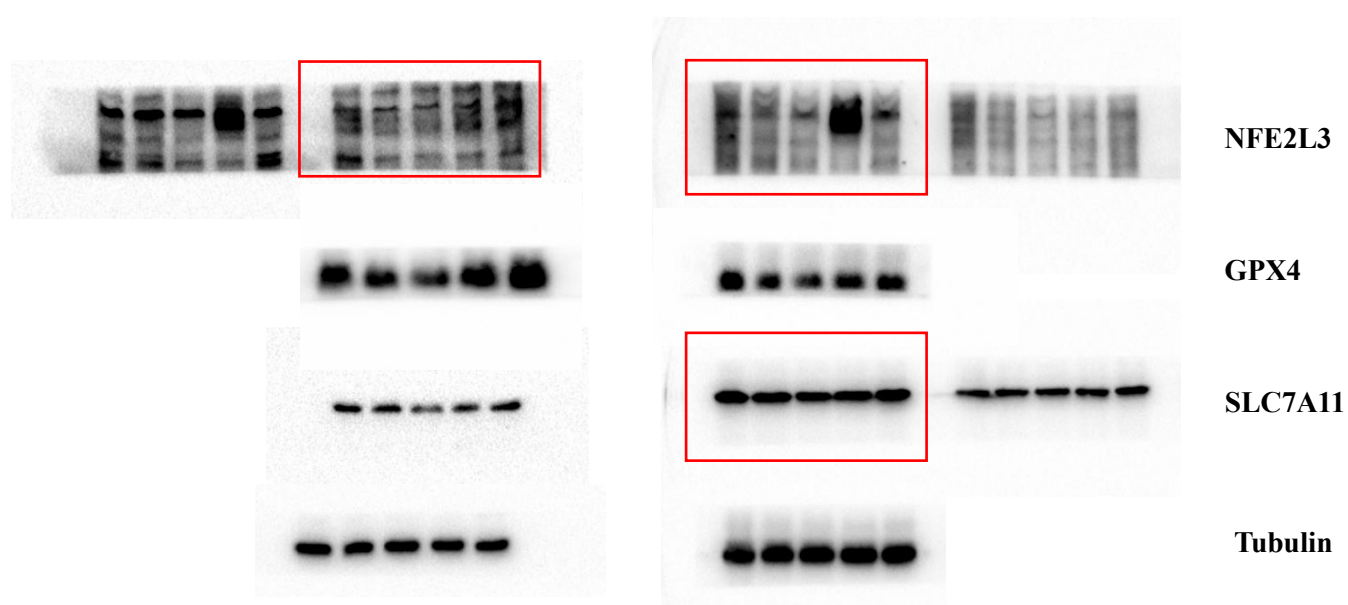


Fig5 F

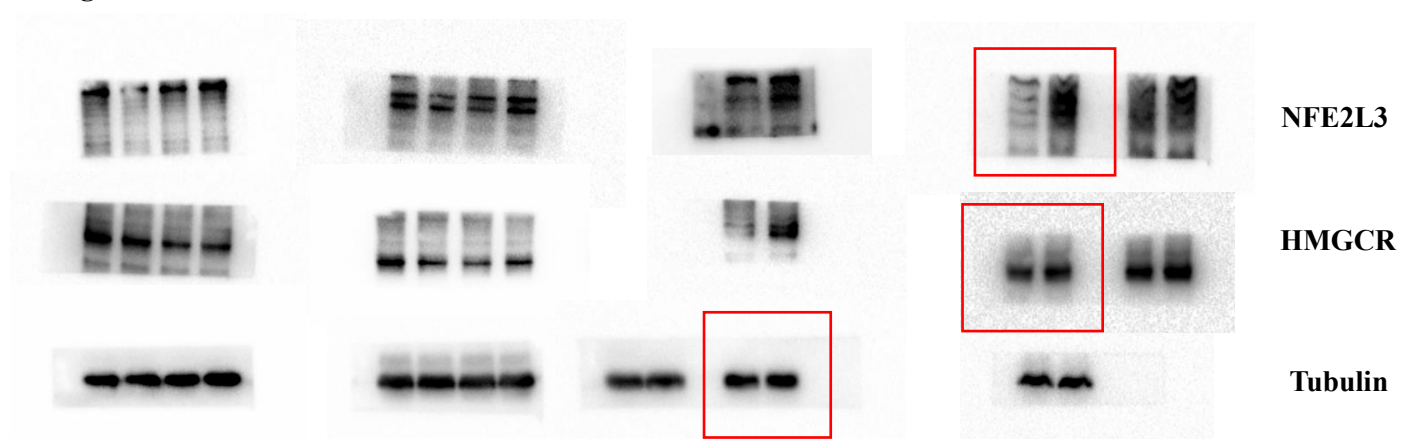


Fig5 H

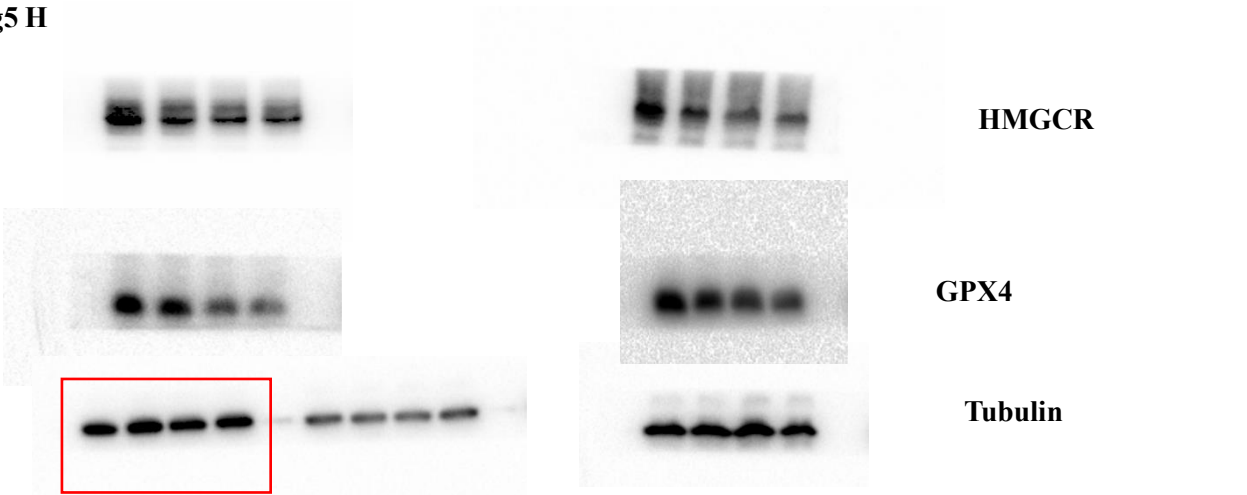


Fig6 A

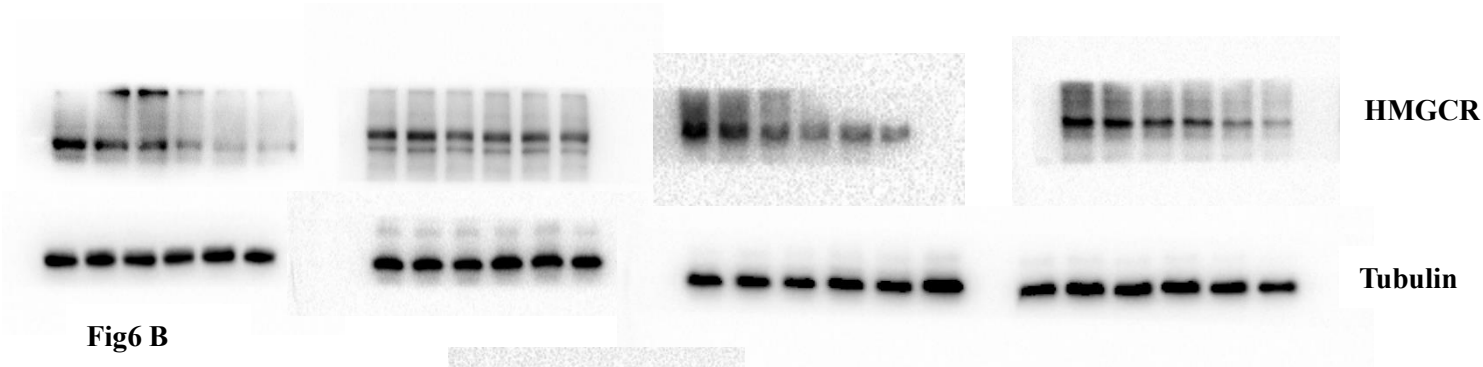


Fig6 B

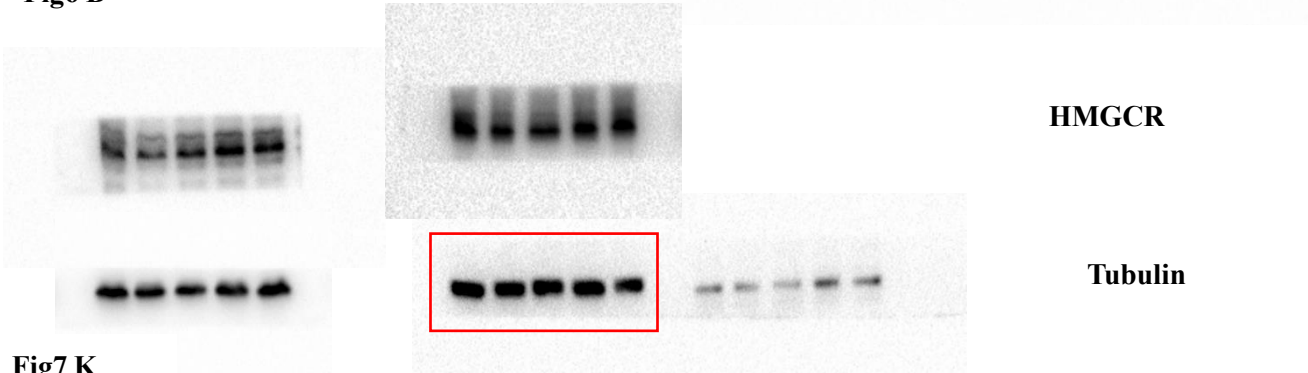


Fig7 K

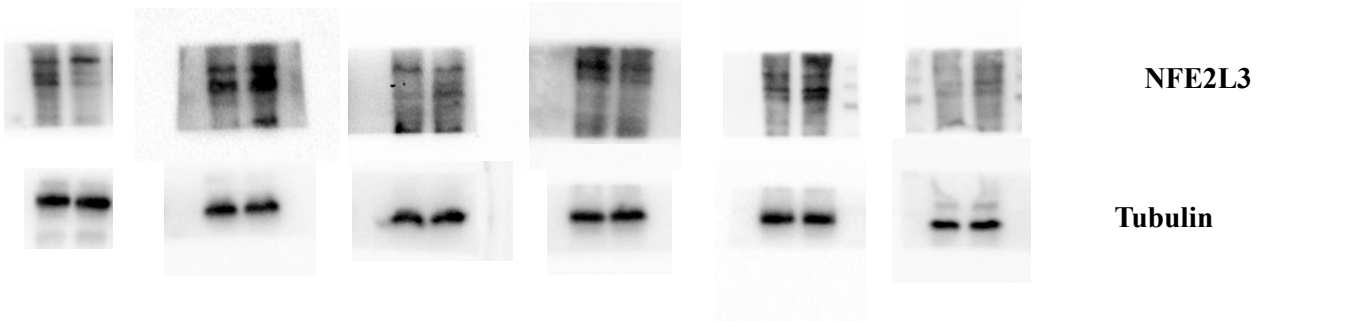
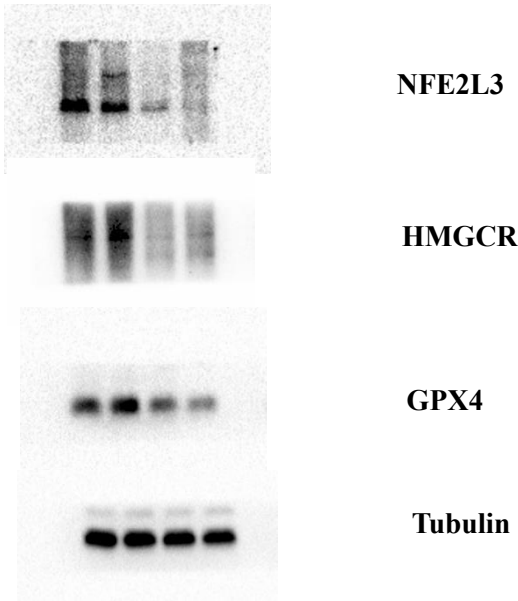
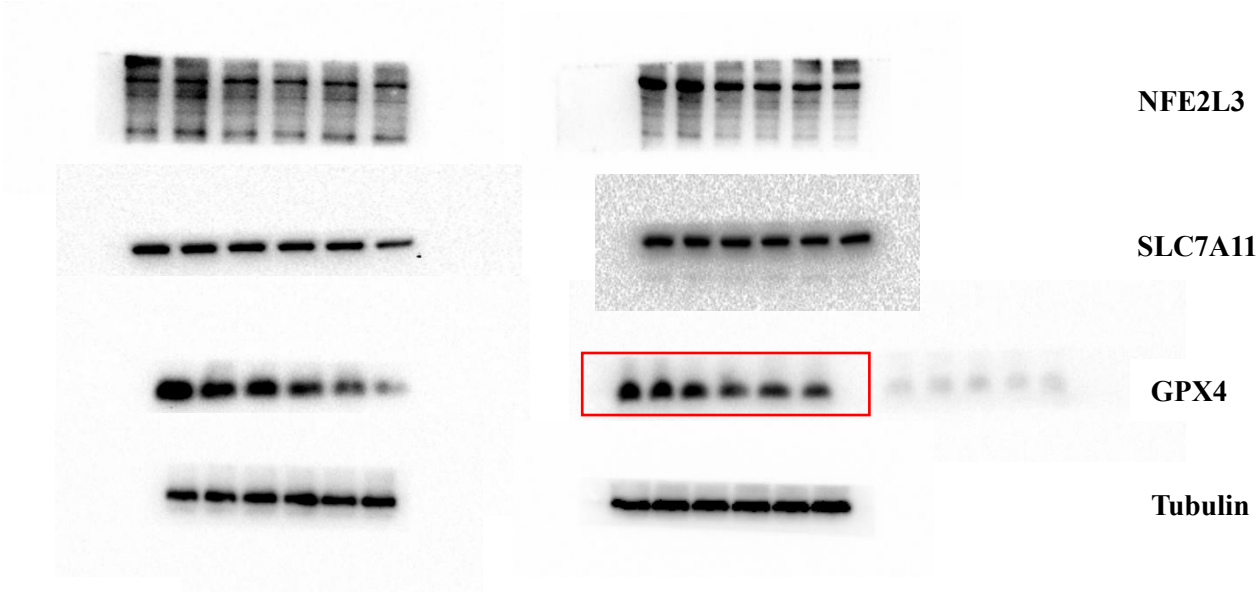


Fig8 A



S1 B



S2 A

