

## **Supplementary Materials**

### **Supplementary Method**

**Supplementary Figure S1.** qRT-PCR analysis in A549.

**Supplementary Figure S2.** Rescue of H<sub>2</sub>O<sub>2</sub> induced cell death in WT and MIB1 overexpression A549 cells.

**Supplementary Figure S3.** A549 cells with MIB1 overexpression is more sensitive to RSL3 treatment in mouse xenograft model.

**Supplementary Figure S4.** Illustration of hMIB1 and hNRF2 mutants used in this study.

**Supplementary Figure S5.** NRF2 ubiquitination level is reduced in MIB1 knockout MDA-MB-231 cells.

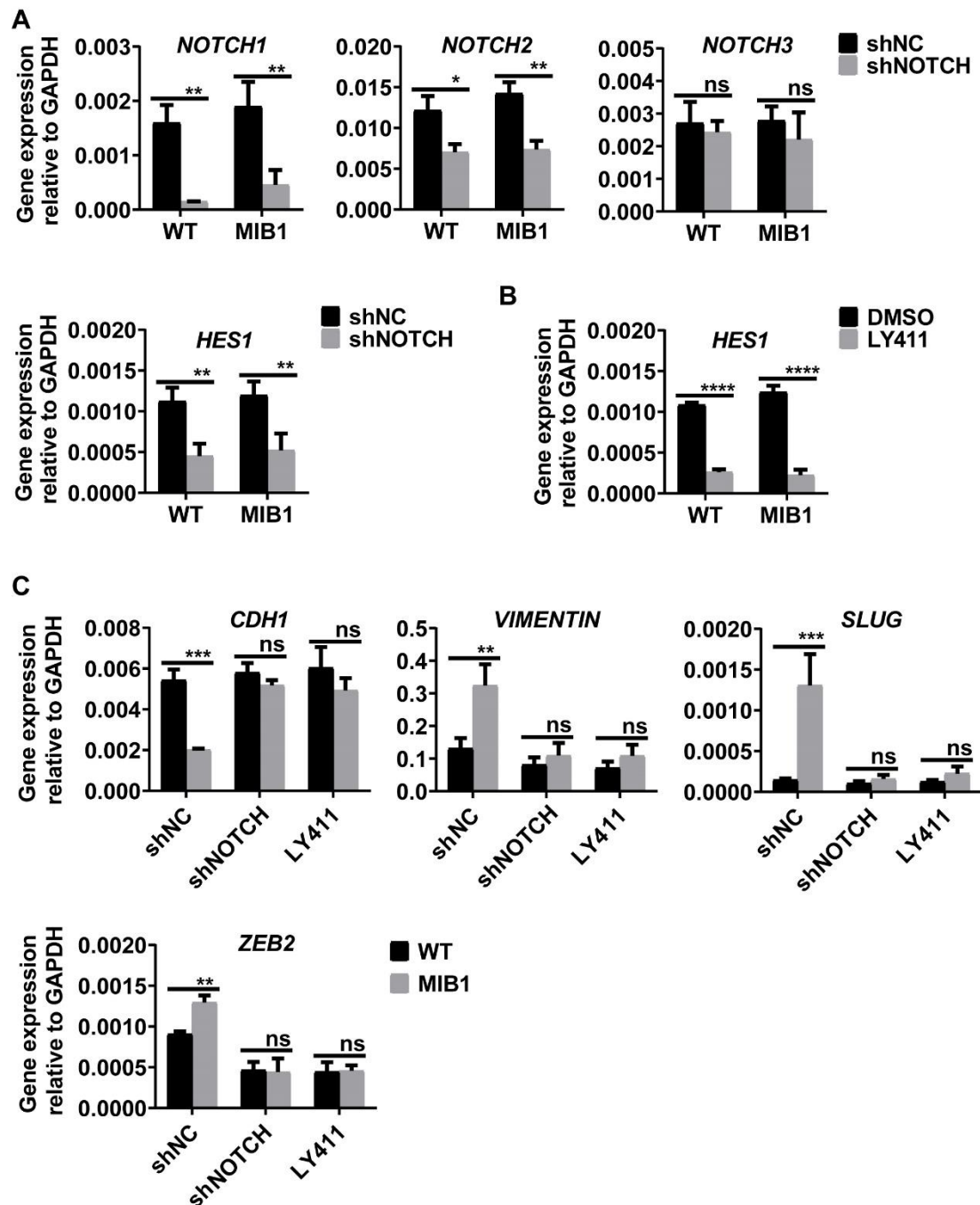
**Supplementary Table S1.** List of PCR primers used in this study;

**Supplementary Table S2.** List of antibodies used in this study.

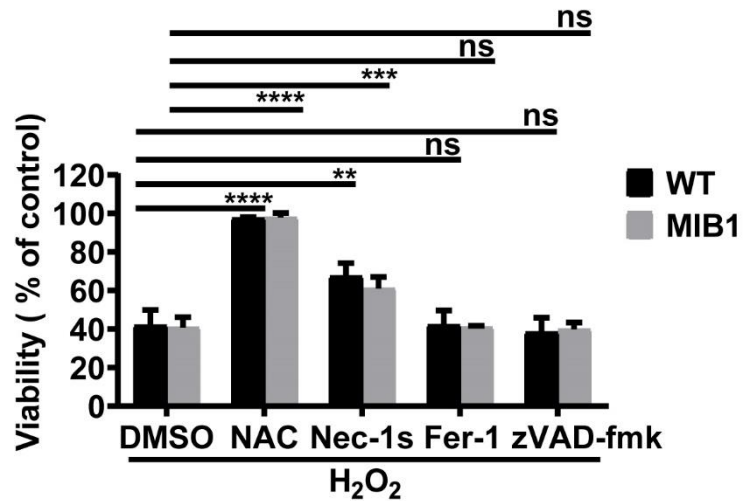
## **Supplementary Methods:**

### **Mouse xenograft model**

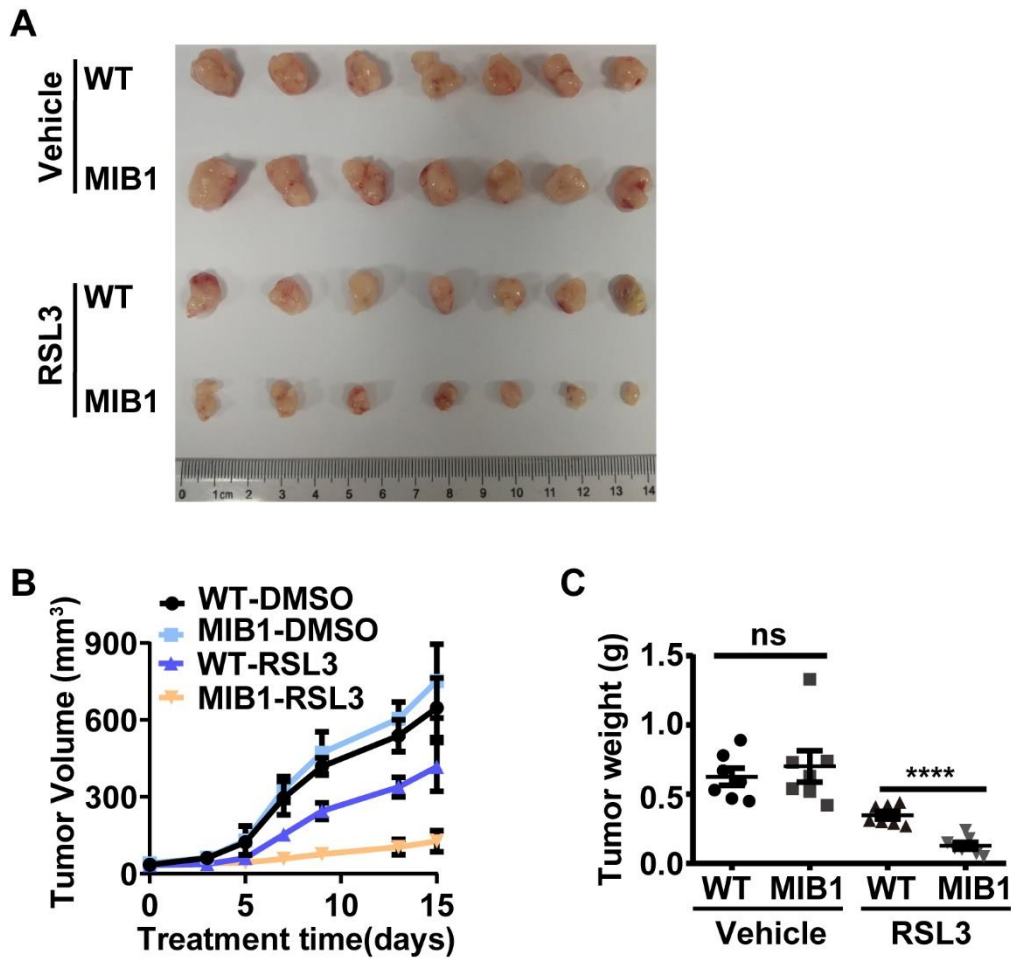
Five weeks old female B-NDG mice were purchased from Beijing BIOCYTOGEN Pharmaceutical Technology Co., LTD (BIOCYTOGEN, Beijing, China, catalog No. 110586). Mice at 7 weeks of age were subcutaneously injected with  $5 \times 10^6$  cells/200  $\mu$ l of PBS into the right flanks of mice. As soon as gross nodules from the tumor implants were detected, mice were randomly allocated into groups and then injected intratumorally with vehicle or 100 mg/kg (1S, 3R) - RSL3 twice a week for 2 weeks. Tumor volumes were measured by determining the lengths ( $L$ ) and widths ( $W$ ) of the tumors using a caliper. Tumor volume was calculated according to the following equation:  $V = (L * W * W) / 2$ . Mice were housed in the SPF-grade animal facility of the Guangzhou Institutes of Biomedicine and Health, Chinese Academy of Sciences. All animal experiments were approved by the Institutional Animal Care and Use Committee of Guangzhou Institutes of Biomedicine and Health (IACUC-GIBH).



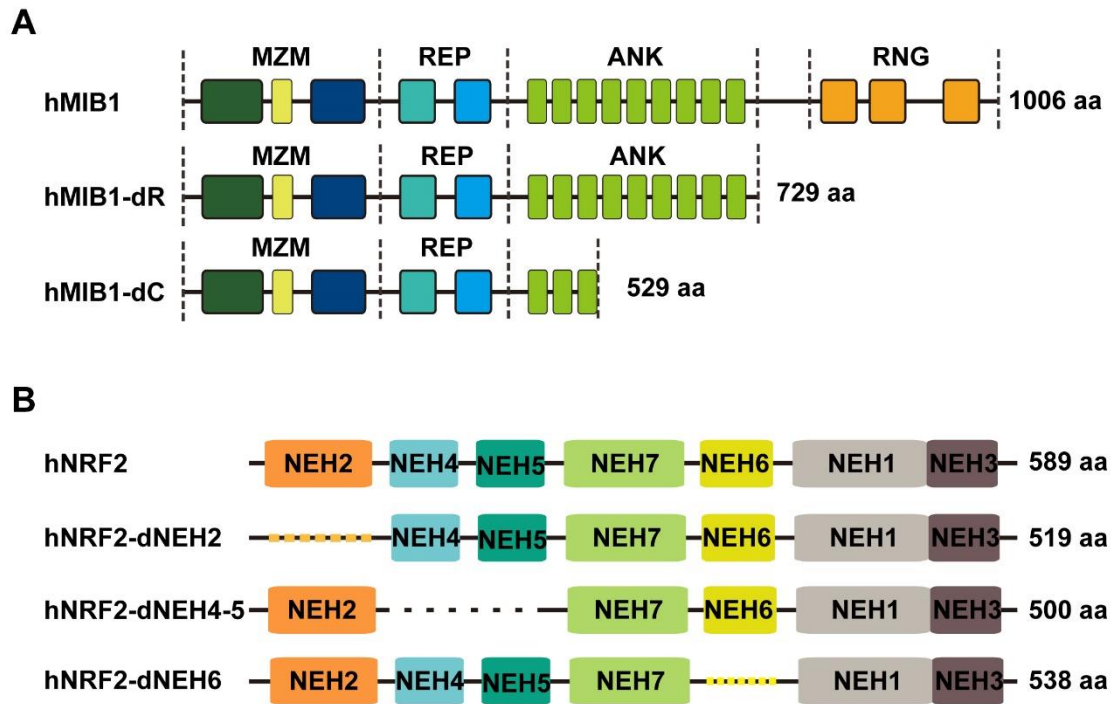
**Supplementary Figure S1.** qRT-PCR analysis in A549. **A**, qRT-PCR analysis for the expression levels of the indicated genes in shNOTCH1/2/3 infected stable A549 cell lines. *HES1* is a NOTCH target gene and it is inhibited by shNOTCH treatment. **B**, Notch pathway inhibitor LY411575 (LY411) treatment inhibits *HES1* expression. **C**, The effects of Notch inhibition (by shNOTCH or LY411575) on the expression of EMT related genes. Data represent mean  $\pm$  SD from three biological repeats and *p*-value determined by unpaired t test. ns: no significance; \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ ; \*\*\*\*:  $p < 0.0001$ .



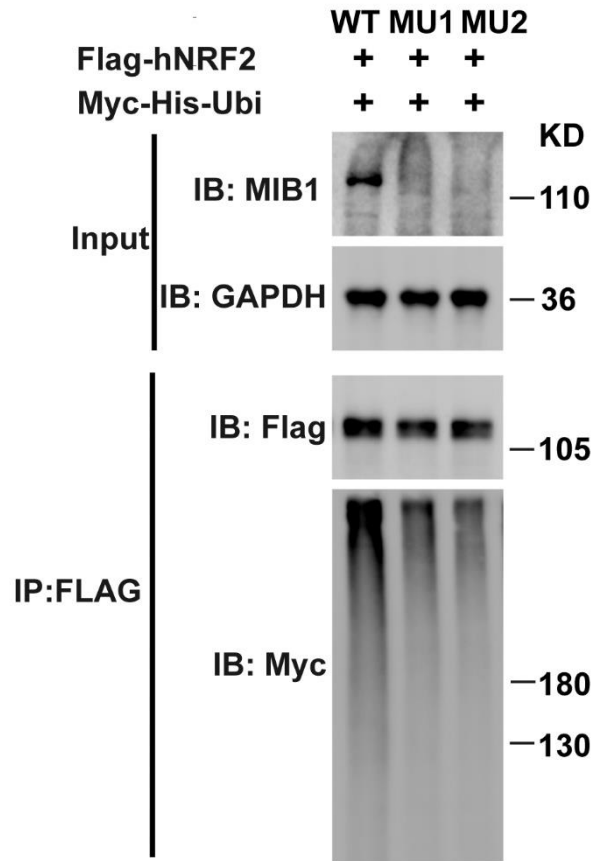
**Supplementary Figure S2.** Rescue of H<sub>2</sub>O<sub>2</sub> induced cell death in WT and MIB1 overexpression A549 cells. Cells were treated with H<sub>2</sub>O<sub>2</sub> together with the indicated inhibitors. NAC and Nec-1s partially rescue H<sub>2</sub>O<sub>2</sub> induced cell death. Data represent mean  $\pm$  SD from three independent repeats and *p*-value is determined by ordinary one-way ANOVA with Dunnet's multiple comparisons test. ns: no significance; \*\*: *p*<0.01; \*\*\*: *p*<0.001; \*\*\*\*: *p*<0.0001.



**Supplementary Figure S3.** A549 cells with MIB1 overexpression is more sensitive to RSL3 treatment in mouse xenograft model. Mice were injected subcutaneously with WT or MIB1 overexpression A549 cells then treated with RSL3. Animals were sacrificed at 15 days after RSL3 treatment and tumors analyzed. **A**, Morphology of dissected tumors at day 15. **B**, Growth curve of tumors at the indicated time points. **C**, Weight of tumors dissected at day 15. Each group contains 7 mice and data represent the average volume (B) or weight (C) of 7 tumors. *p*-value in (C) was determined by unpaired t test. ns: no significance; \*\*\*\*: *p*<0.0001.



**Supplementary Figure S4.** Illustration of hMIB1 and hNRF2 mutants used in this study. **A**, Structures of WT hMIB1 and hMIB1 mutants used in this study. In hMIB1-dR mutant, the c-terminal RNG domains are deleted. In hMIB1-dC mutant, the RNG domain plus six ANK repeats are removed. Both mutants are expected to be catalytically inactive. **B**, Three hNRF2 mutants with either the NEH2, NEH4-5 or NEH6 domain deleted are used in this study. The NEH2 domain is the site of KEAP1-CUL3-RBX1 complex mediated ubiquitination in NRF2.



**Supplementary Figure S5.** NRF2 ubiquitination level is reduced in MIB1 knockout MDA-MB-231 cells.

**Supplementary Table S1.** List of PCR primers used in this study.

<b>Gene</b>	<b>Primer 1</b>	<b>Primer 2</b>
full-length hMIB1	5'-GCCACCATGAGTAACTCCCG GAATAA-3'	5'-ATACAAAAGAATCCTTCGTT CA-3'
hMIB1-dR	5'-GCCACCATGAGTAACTCCCG GAATAA-3'	5'-GGCAGCATCCACCTTCCCCA CATCTT-3'
hMIB1-dC	5'-GCCACCATGAGTAACTCCCG GAATAA-3'	5'-TCGGCGCTTGTTCGAGCAT TC-3'
full-length hNRF2	5'-GATGACAAGCTCGAGTGTA CAGCCACCATGGATTTGATTGA CATACTTTGG-3'	5'-AGGGAGAGGGGCTCAGAAT TCCTAGTTTTTCTTAACATCTG GCTTCTTAC-3'
hNRF2-dNEH2	5'-GATGACAAGCTCGAGTGTA CAGCCACCATGTCTGCCAACTA CTCCCAGGTT-3'	5'-AGGGAGAGGGGCTCAGAAT TCCTAGTTTTTCTTAACATCTG GCTTCTTAC-3'
hNRF2-dNEH4-5	5'-AATATTGAAAATGACAAGCT GGTGAGACTACCATGGTTCC-3'	5'-GTCATTTCAATATTTGATTT GGAATGTGGGCAACCTGGGA GTAGTTG-3'
hNRF2-dNEH6	5'-AGTGTCAAACAGAATGGTC CTAAAACACCAGTACATTCTTC TGGGG-3'	5'-ATTCTGTTTGACACTGCTTT CAGGGTGGTTTTGGTTGAAAG CTTTGCAAAG-3'
NRF2 (qPCR)	5'-CACATCCAGTCAGAAACCA GTGG-3'	5'-GGAATGTCTGCGCCAAAAG CTG-3'
HMOX1 (qPCR)	5'-CCAGGCAGAGAATGCTGAG TTC-3'	5'-AAGACTGGGCTCTCCTTGTT GC-3'
CDH1 (qPCR)	5'-TGCCCAGAAAATGAAAAAG G-3'	5'-GTGTATGTGGCAATGCGTTC-3'
VIM (qPCR)	5'-GACGCCATCAACACCGAGTT -3'	5'-CTTTGTTCGTTGGTTAGCTGG T-3'
SNAIL1 (qPCR)	5'-ACTGCAACAAGGAATACCTC AG-3'	5'-GCACTGGTACTTCTTGACAT CTG-3'
SLUG (qPCR)	5'-CATGCCTGTCATACCACA AC-3'	5'-GGTGTTCAGATGGAGGAGGG -3'
ZEB2 (qPCR)	5'-CAAGAGGCGCAAACAAGCC -3'	5'-GGTTGGCAATACCGTCATCC -3'
KLF8 (qPCR)	5'-CCCAAGTGGAACCGATTGA CC-3'	5'-GACGTGGACACCACAAG GG-3'
NOTCH1 (qPCR)	5'-GCGACAACGCCTACCTCTG -3'	5'-AAGCCATTGATGCCGTCC -3'
NOTCH2 (qPCR)	5'-TCAGCCGGGATACTATG AG-3'	5'-CTGGCAGTGTCTGGAAT GT-3'
NOTCH3 (qPCR)	5'-CTCATCCGAAACCGCTCTAC AG-3'	5'-AGCAAGCGCACGATGTC CT-3'
HES1	5'-AACACTGATTTTGGATGCTC	5'-CCTCGGTATTAACGCCCTC-3'



(qPCR)	TG-3'	
GAPDH (qPCR)	5'-AGGGCTGCTTTTAACTCTGG T-3'	5'-CCCCACTTGATTTTGGAGGG A-3'

**Supplementary Table S2.** List of antibodies used in this study.

<b>Name of Antibody</b>	<b>Company (Cat. No)</b>	<b>Dilution Factor</b>
Rabbit anti MIB1 antibody	Abcam, ab124929. RRID: AB_11127834	WB: 1:1000 IHC:1:200
Rabbit anti NRF2 antibody	Abcam, ab62352. RRID: AB_944418	WB: 1:2000
Rabbit anti E-Cadherin antibody	Cell Signaling Technology, 3195S. RRID: AB_2291471	WB: 1:1000
Rabbit anti N-Cadherin antibody	Cell Signaling Technology, 13116S. RRID: AB_2687616	WB: 1:1000 IF: 1:200
Rabbit anti HMOX1 antibody	GeneTex, GTX101147. RRID: AB_1950502	WB: 1:1000
Rabbit anti GPX4 antibody	Abcam, ab125066. RRID: AB_10973901	WB: 1:5000
Rabbit anti SLC7A11 antibody	Abcam, ab37185. RRID: AB_778944	WB: 1:1000
Mouse anti TFRC antibody	Invitrogen, 13-6800. RRID: AB_2533029	WB: 1:1000
Rabbit anti FTH1 antibody	Cell Signaling Technology, 3998S. RRID: AB_1903974	WB: 1:1000
Mouse anti ACSL4 antibody	Santa Cruz, sc-271800. RRID: AB_10715092	WB: 1:1000
GAPDH	GeneTex, GTX627408. RRID: AB_11174761	WB: 1:5000
Mouse anti FLAG antibody	Sigma, F1804. RRID: AB_262044	WB: 1:5000
Rabbit anti HA antibody	Sigma, H6908. RRID: AB_260070	WB: 1:1000
Rabbit anti MYC antibody	Proteintech, 16286-1-AP. RRID: AB_11182162	WB: 1:5000
Rabbit anti VIMENTIN antibody	Abcam, ab92547 RRID: AB_10562134	IHC: 1:500
Mouse anti SNAIL1 antibody	Cell Signaling Technology, 3895S. RRID:	WB: 1:1000

WB: western blot; IF: immunofluorescence staining; IHC: Immunohistochemistry.