Supplementary methods

Plasmid construction

The pDsRed-mRXRα and pEGFP-mRXRα mammalian expression constructs encode wild-type mRXRα that have been tagged with the red fluorescent protein (RFP) and green fluorescent protein (GFP), respectively. The bacterial expression plasmid PETDuet-1-his-mRXRα and pET41a-mRXRα encode wild-type mRXRα that have been tagged with Histidine (his) and GST, respectively. The PETDuet-1-his-hNRF2 and pET41a-hNRF2 plasmids encode the his- and GST-tagged wild-type hNRF2, respectively. Plasmid pET41a-luc encoding GST-tagged luciferase was generated and used as negative control.

Plasmid pDsRed-mRXRα was made by PCR amplification from pSG5-mRXRα using the following pair of primers: 5'-CCGGAATTCACCATGGACACCAAACATTTC-3' and 5'-ACGCGTCGACTGGTGGCTTGATGTGGTGC-3'. The resulting fragment was cloned into expression vector pDsRed-C1 (Clonetech, China) between *Eco*RI and *Sal*I restriction sites. The cDNA for pEGFP-mRXRα was similarly created by PCR before being ,cloned into expression vector pEGFP-N1 between the *Eco*RI and *Sal*I sites. PETDuet-1-his-mRXRα was created by PCR amplification from pSG5-mRXRα using the following pair of primers: 5'-CCGGAATTCGATGGACACCAAACATTTCCTGCC-3' and 5'-ACGCGTCGACCTAGGTGGCTTGATGTGGTG-3', and cloned into PETDuet-1 between *Eco*RI and *Sal*I. pET41a-mRXR was created by PCR amplification from pSG5-mRXRα using the following pair of primers: 5'-CGGAATTCATGGACACCAAACATTTCCTG-3' and 5'-

ACGCGTCGACCTAGGTGGCTTGATGTGGTGC-3'. The resulting fragment was cloned into expression vector pET41a between *Eco*RI and *Sal*I. Using the same cloning strategy, the pET41a-mRXRα$^{1-229}$ encoding the GST-tagged N-terminal half of mRXRα, pET41a-mRXRα$^{230-467}$ encoding the GST-tagged C-terminal half of mRXRα, pET41a-mRXRα$^{1-139}$ encoding the GST-tagged AF-1 domain of mRXRα, and pET41a-mRXRα$^{140-205}$ encoding the GST-tagged DBD of mRXRα, were amplified by PCR and cloned into pET41a. The following pairs of primers were used: pET41a-mRXRα$^{1-229}$, 5'-CGGAATTCATGGACACCAAACATTTCCTG-3' and 5'-ACGCGTCGACCTAGCTGGTGGACTCCACCTCGTTC-3'; pET41a-mRXRα$^{230-467}$, 5'-CGGAATTC AGTGCCAACGAGGACATGCC-3' and 5'-ACGCGTCGACCTAGGTGGCTTGATGTGGTGC-3'; pET41a-mRXRα$^{1-139}$, 5'-CGGAATTCATGGACACCAAACATTTCCTG-3' and 5'-ACGCGTCGACCTA GATGTGCTTGGTGAAGGAGGC-3'; pET41a-mRXRα$^{140-205}$, 5'-CGGAATTCTGTGCTATCTGTGGGGACCG-3' and 5'-ACGCGTCGACCTA CATGCCCATGGCCAGGCACTTC-3'. PETDuet-1-his-hNRF2 was created by PCR amplification from pHyg-EF-hNRF2 using the following pair of primers: 5'-CGCGGATCCGACCATGGATTTGATTGACATAC-3' and 5'-ACGCGTCGACCTAGTTTTTCTTAACATCTG-3', and cloned into PETDuet-1 between *Bam*HI and *Sal*I. Plasmid pET41a-hNRF2 was made by PCR amplification from pHyg-EF-hNRF2 using the following pair of primers: 5'-GACGGATCCAGAATGGATTTGATTGAC-3' and 5'-ACGCGTCGACCTAGTTTTTCTTAACATCTG-3' and cloned into vector pET41a between *BamH*I and *Sal*I. Plasmid pET41a-hNRF2$^{17-338}$ expressing the N-terminus of NRF2, pET41a- hNRF2$^{339-605}$ expressing the C-terminus of NRF2, pET41a- hNRF2$^{17-110}$ expressing Neh2 domain of NRF2, pET41a- hNRF2$^{109-219}$ expressing Neh4 and

Neh5 domains, pET41a-hNRF2$^{109-338}$ expressing Neh4, Neh5 and the region coding 209 to 338 amino acid of NRF2, and pET41a-hNRF2$^{209-316}$ expressing the region 209 to 316 amino acid of NRF2, were made by PCR amplification from pHyg-EF-hNRF2 using the following pair of primers, respectively: pET41a-hNRF2$^{17-338}$, 5'-GACGGATCCAGAATGGATTTGATTGAC-3' and 5'-GACGAATTCTTATTATTCTGCTGTGCTTTC-3'; pET41a-hNRF2$^{339-605}$, 5'-GACGGATCCTTCAATGATTCTGAC-3' and 5'-GACGAATTCTTATTAGTTTTTCTTAACATC-3'; pET41a-hNRF2$^{17-110}$, 5'-GACGGATCCAGAATGGATTTGATTGAC-3' and 5'-CTGGAATTCCTATTATTTGGGAATGTGGGCAAC-3'; pET41a-hNRF2$^{109-219}$, 5'-CAGGGATCCCCCAAATCAGATGCTTTG-3' and 5'-CTGGAATTCCTATTATTTGGCTTCTGGACTTG-3'; pET41a-hNRF2$^{109-338}$, 5'-CAGGGATCCCCCAAATCAGATGCTTTG-3' and 5'-GACGAATTCTTATTATTCTGCTGTGCTTTC-3'; pET41a-hNRF2$^{209-316}$, 5'-CAGGGATCCGAGACTACCATGGTTCCAAG-3' and 5'-GACGAATTCTTATTATTCTGCTGTGCTTTC-3'. The resulting fragments were cloned into pCDNA3.1/V5 (Invitrogen) between *BamH*I and *Eco*RI, followed by sub-cloning into plasmid pET41a between *BamH*I and *Eco*RI. Plasmid pET41a-luc encoding GST-tagged luciferase was made by PCR amplification from pGL3-Control (Promega, China) using the following pair of primers: 5'-CGCGAATTCATGGAAGACGCCAAAAACAT-3' and 5'-ATAGTCGACAATTACACGGCGATCTTTCC-3'. The resulting fragments were cloned into vector pET41a between *Eco*RI and *Sal*I. The expression vectors for GFP-tagged NRF2 mutants were created by PCR amplification from pHyg-EF-hNRF2. The resulting fragments were cloned into expression vector pCDNA3.1B/V5/his between

*BamH*I and *Eco*RI, followed by sub-cloning into pEGFP-C1 between *Kpn*I and *Apa*I. The sequences for the primers used are as follows: pEGFP-hNRF2$^{17-316}$, 5'-GACGGATCCAGAATGGATTTGATTGAC-3' and 5'-GACGAATTCTTATTATTCTGCTGTGCTTTC-3'; pEGFP-hNRF2$^{17-110}$, 5'-GACGGATCCAGAATGGATTTGATTGAC-3' and 5'-CTGGAATTCCTATTATTTGGGAATGTGGGCAAC-3'; pEGFP-hNRF2$^{109-219}$, 5'-CAGGGATCCCCCAAATCAGATGCTTTG-3' and 5'-CTGGAATTCCTATTATTTGGCTTCTGGACTTG-3'; pEGFP-hNRF2$^{109-316}$, 5'-CAGGGATCCCCCAAATCAGATGCTTTG-3' and 5'-GACGAATTCTTATTATTCTGCTGTGCTTTC-3'; pEGFP-hNRF2$^{339-605}$, 5'-GACGGATCCTTCAATGATTCTGAC-3' and 5'-GACGAATTCTTATTAGTTTTTCTTAACATC-3'. pEGFP-mNrf2$^{201-329}$ coding the region amino acid 201-329 of mNrf2, was cloned by PCR amplification from pcDNA3.1/V5-mNrf2. The resulting fragments were cloned into pEGFP-C1 between *Xho*I and *Eco*RI. pcDNA3.1b-hNRF2$^{\Delta DIDLID}$ coding mutant NRF2 with DIDLID domain deletion was cloned by PCR amplification from pHyg-EF-hNRF2 using the following pair of primers: 5'- TTGGGTACCAGTCGAGAAGTATTTGACTTCA -3' and 5'- CGCTCTAGACTAGTTTTTCTTAACATCTGGC -3' between *Xho*I and *Xba*I. pcDNA3.1b-hNRF2$^{\Delta DIDLID+\Delta 209-316}$ coding mutant NRF2 with deletions of DIDLID and the region amino acid 201-329 was made from pcDNA3.1b-hNRF2$^{\Delta DIDLID}$ using Site-directed Mutagenesis Kit (Stratagene, China) with the following pair of primers: 5'-TTGAAAATGACAAGCTGGTTATTGATGTTTCTGATCTATC -3' and 5'-GATAGATCAGAAACATCAATAACCAGCTTGTCATTTTCAA -3'. pcDNA3.1b-hNRF2$^{\Delta DIDLID+\Delta 209-316}$ was subsequently used as PCR template for cloning pEGFP-

C1-hNRF2$^{\Delta DIDLID+\Delta 209-316}$ and pET-41a-hNRF2$^{\Delta DIDLID+\Delta 209-316}$ coding mutant hNRF2 with the double deletions, between *Xho*I and *Bam*HI. For pEGFP-C1-hNRF2$^{\Delta DIDLID+\Delta 209-316}$, the following pair of primers were used: 5'-GAGCTCGAGGAAGTCGAGAAGTATTTGACTTCA -3' and 5'-GACGGATCCCTAGTTTTTCTTAACATCTGGC -3'. For pET-41a-hNRF2$^{\Delta DIDLID+\Delta 209-316}$ the following pair of primers were used: 5'-CGGGGATCCAGTCGAGAAGTATTTGACTTCA -3' and 5'-CGCCTCGAGCTAGTTTTTCTTAACATCTGGC -3'.

GST pull-down assay and immnuprecipitation

Mutant forms of NRF2 or mRXRα fused to GST were expressed in *Escherichia coli* DH5α cells and purified with glutathione-Sepharose beads (Pharmacia, China). The purified proteins were visualized by staining with Coomassie blue. The GST pull-down assay was carried out as described previously (1). Approximately 2 μg of purified and GST-bound mutant proteins and his-tagged fusion proteins were used for the pull-down assay. The beads were washed five times before collection by centrifugation. The bound proteins were analysed by SDS-PAGE, followed by immunoblotting with antibody against GST or the indicated protein. The input in each experiment represented 10% of the total amount used unless otherwise specified. The immune complexes were analysed by immunoblotting with an antibody directed against NRF2 or RXRα.

The region of NRF2 that interacts with RXRα was determined by transfecting HEK293 cells with various pEGFPC1 plasmids encoding different NRF2 deletion mutants. Twenty-four hr post transfection, aliquots (each of approximately 500 μg protein) of whole cell lysate were mixed with 5 μg bacteria expressed his-RXRα. The

mixture was subjected to immnuprecipitation with antibody against RXRα as described previously (19). The antigen-antibody complex was immunoprecipitated following incubation overnight at 4^{o}C with protein G-agarose. The precipitates were analysed by SDS-PAGE, and immunoblotting with an antibody directed against GFP or RXRα.

Development of stable cell lines

MCF7 cells were stably transfected with the plasmid pEGFP-mRXRα. After selection in the culture medium containing 0.8 mg/ml G418, one clone named as MCF7-RXRα, which maintained stable over-expression of GFP-mRXRα after multiple passages, was used for this study. A549 cells were also stably transfected with the pEGFP-mRXRα plasmid. After selection in culture medium containing 0.8 mg/ml G418, a cell line named A549-mRXRα was generated after combination of more than 50 clones. Similarly, a cell line named A549-EGFP was generated after A549 cells were stably transfected with the empty pEGFP vector, and used at negative control.

References

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