# Supplementary figures

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1. Clonogenic assays of Detroit562,Fadu, SCC9, SCC25 cells that had been exposed to different concentrations of RSL3 for 24 h.
2. c. Cell viability (b) and LDH release assays(c) after exposure to 5 μM RSL3. The cells were co-treated with liproxstatin-1 (Lipro-1, 1 μM), The error bars represent standard deviation from three replicates. \* P < 0.01 relative to the control or the differently treated groups.

Text

Description automatically generated with low confidence Fig.S2

1. b. Validated efficiency of shRNAs targeting ALKBH5 as indicated by both qPCR (a) and Western blot analysis (b) of Detroit 562 and FaDu HPSCC cells.
2. The global m6A levels in mRNA of control and ALKBH5-knockdown HPSCC cells were measured by the EpiQuik™ m6A RNA Methylation Quantification Kit, n = 3,nonparametric Mann–Whitney test.
3. Validated efficiency of GV141-ALKBH5-WT plasmid transfection as indicated by Western blot analysis.

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1. Principal component analysis (PCA) showing that two repeats (shCON: control1 and control2, shALKBH5: shK5\_1 and shK5\_2) of each sample clustered together.
2. Heat map of the 295 most significantly altered proteins based on their intensities and hierarchical clustering analysis; 175 genes were upregulated genes, and 120 genes were downregulated genes as identified by RNA-seq (two replicates).
3. Gene Ontology (GO) (G) and Kyoto Encyclopedia of Genes and Genomes (KEGG) (H) analyses of 175 significantly enriched upregulated genes and 120 significantly enriched downregulated genes as identified by RNA-seq.
4. GSEA plots showing the pathways of the overlapping genes identified by m6A-seq and RNA-seq.

Graphical user interface

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1. qPCR analysis and Western blotting assays were performed to analyze the relative NFE2L2/NRF2 levels after knockdown of ALKBH5 in Detroit 562 and FaDu cells, n = 3, nonparametric Mann–Whitney test
2. qPCR analysis and Western blot assays were performed to analyze the relative NFE2L2/NRF2 levels in Detroit 562 cells after transfection with GV141, GV141-ALKBH5-mut (H204A mutant of ALKBH5) and GV141-ALKBH5-WT, n = 3, nonparametric Mann–Whitney test.
3. The wild-type or mutant (m6A motif mutated) sequence of NFE2L2/NRF2–3’UTR was inserted into a GV306 vector between Firefly and Renilla elements
4. Validated efficiency of siRNAs targeting IGF2BP1/2/3 as indicated by both qPCR and Western blot analysis of Detroit 562 cells.
5. qPCR analysis was performed to analyze the relative NFE2L2/NRF2 levels after silencing IGF2BP1/2/3 in Detroit 562 and FaDu cells, n = 3, nonparametric Mann–Whitney test.
6. The NFE2L2/NRF2 mRNA half-life (t 1/2) was detected by real-time PCR in Detroit 562 and FaDu cells transfected with siCON and siIGF2BP1/2/3.
7. NFE2L2/NRF2 correlated with IGF2BP2 expression in TCGA HNSCC Dataset.

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Fig.S5

1. Validated efficiency of siRNAs silencing NFE2L2/NRF2 as indicated by both qPCR and Western blot analysis of Detroit 562 cells.
2. Validated efficiency of transfection NFE2L2/NRF2 WT plasmid as indicated by Western blot analysis of FaDu cells.
3. Western blotting assays were performed to analyze NFE2L2/NRF2,GPX4,FTH1 levels after transfection with NRF2-WT plasmid in FaDu control and ALKBH5-overexpressed cells.
4. e. Cell viability assay (d), LDH release assay (e) and colony formation assay were performed with WT and ALKBH5-overexpressed FaDu cells transfected with a control vector (GV306) or GV306 NFE2L2/NRF2-WT and subsequently treated with 5μm RSL3.
5. NFE2L2/NRF2 correlated with GPX4 expression in TCGA HNSCC Dataset
6. NFE2L2/NRF2 functional association data including the protein and genetic interactions, pathways, co-expression, co-localization is summarized by GeneMANIA (<http://genemania.org>).

# Supplementary data

## Supplementary 1

## Supplementary Materials and methods

## Supplementary 2

Mutation sequences

## Supplementary 3

Primers and siRNA