1 **<u>Inventory of Supplemental Information</u>**

2 **SUPPLEMENTAL FIGURES**

3 Figure S1 is related to Figure 2.

4 Figure S2 is related to Figure 4.

5 Figure S3 is related to Figure 4.

6 Figure S4 is related to Figure 5.

7 Figure S5 is related to Figure 5.

8 Figure S6 is related to Figure 6.

9 **SUPPLEMENTAL TABLES**

10 Table S1 is related to Figure 3, and is provided as a separate Excel file.

11 Table S2 is related to Figure 3, and is provided as a separate Excel file.

12 Table S3 is related to Figure 6, and is provided as a separate Excel file.

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SUPPLEMENTAL FIGURES

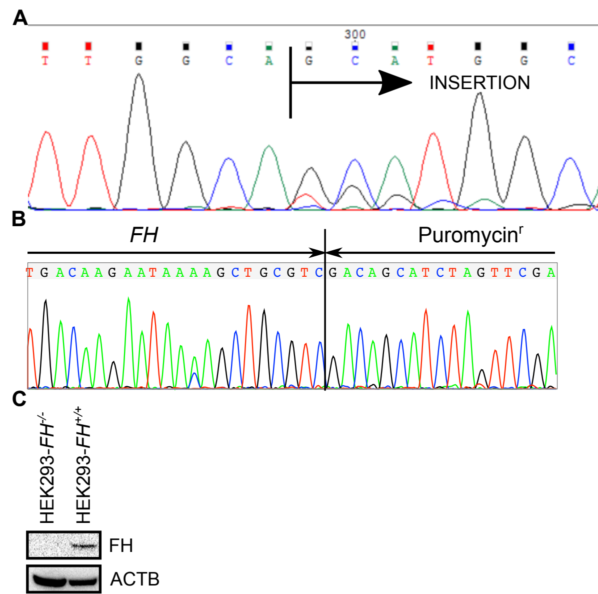

Figure S1, related to Figure 2. HEK293-*FH*$^{-/-}$ validation.

A-B. Exon 6 was isolated by PCR from HEK293-*FH*$^{-/-}$ gDNA. Two PCR products were identified. Genomic analysis of HEK293-*FH*$^{-/-}$ revealed an insertion (A) in one PCR product and a puromycin resistance cassette in the other PCR product (B).

C. Western blot analysis of HEK293-*FH*$^{-/-}$ show no FH protein compared to wildtype HEK293.

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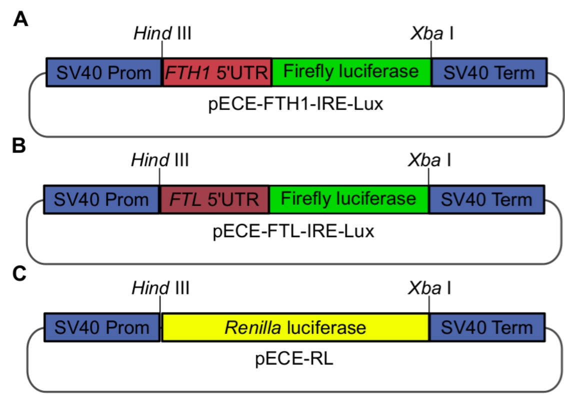

23 **Figure S2, related to Figure 4.** Luciferase construction vector maps.

24 A. Vector construction of pECE-FTH1-IRE-LUX. The 5' untranslated region (UTR) of FTH1 mRNA,
25 which contains an Iron Response Element under the repressional control of Iron Regulatory Proteins, was
26 placed in front of a firefly luciferase open reading frame, effectively placing the luciferase under Iron
27 Regulatory Protein control. SV40 is a constitutive promoter, removing any transcriptional regulation.

28 B. Vector construction of pECE-FTL-IRE-LUX. The 5' untranslated region (UTR) of FTL mRNA, which
29 contains an Iron Response Element under the repressional control of Iron Regulatory Proteins, was
30 placed in front of a firefly luciferase open reading frame, effectively placing the luciferase under Iron
31 Regulatory Protein control. SV40 is a constitutive promoter, removing any transcriptional regulation.

32 C. Vector construction of pECE-RL. *Renilla* luciferase open reading frame was placed directly behind the
33 constitutive SV40 promoter, reducing any transcriptional or translational control elements from its
34 production. pECE-RL was used as a transfection control.

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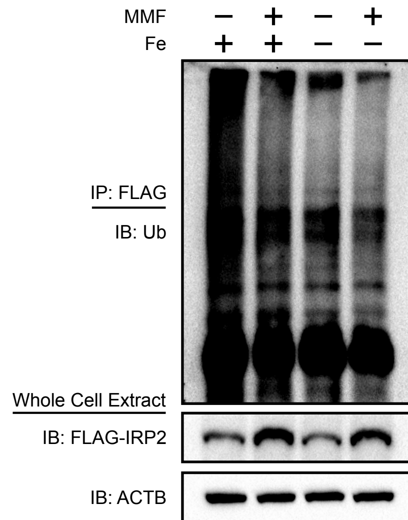

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37 **Figure S3, related to Figure 4.** IRP2 ubiquitylation is decreased by MMF.

38 Ubiquitylation of N-terminal flag-tagged IRP2. The day after transfection with flag-tagged IRP2 (N-Flag-IRP2
39 construct), HEK293 cells were treated with 80 μM monomethyl fumarate (MMF) (+) or vehicle control (-) for 2
40 days. MMF media was refreshed after 24 hours. After two days of MMF treatment, all cells were treated with 10
41 μM MG132; additionally, indicated cells were co-treated with 200 μM Fe (or vehicle) and/or 80 μM MMF (or
42 vehicle). 4 hours after treatment, cells were harvested for protein. Protein was normalized, and lysates were split
43 into whole cell extract for SDS-PAGE analysis or immunoprecipitation using anti-Flag conjugated beads.
44 Following overnight incubation, immunoprecipitated product was prepared for SDS-PAGE analysis. All samples
45 were resolved on SDS-PAGE and analyzed by Western blotting. IP=Immunoprecipitated product.

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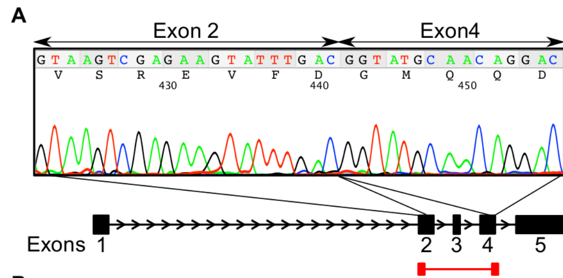

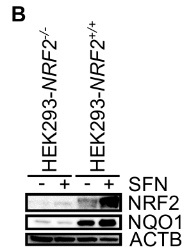

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48 **Figure S4, related to Figure 5.** Validation of HEK293-*NRF2*$^{-/-}$.

49 A. *NRF2* was isolated by PCR from HEK293-*NRF2*$^{-/-}$ gDNA. Genomic analysis of HEK293-*FH*$^{-/-}$ revealed

50 a large deletion of DNA between exons 2 and 4.

51 B. Western blot analysis of HEK293-*NRF2*$^{-/-}$ revealed an absence of NRF2 protein and the canonical NRF2

52 target gene NQO1 compared to wildtype HEK293. Additionally, 4 µM of the strong NRF2 activator

53 sulforaphane (SFN), was able to induce NRF2 and NQO1 in wildtype cells, but not in the *NRF2*$^{-/-}$ cells.

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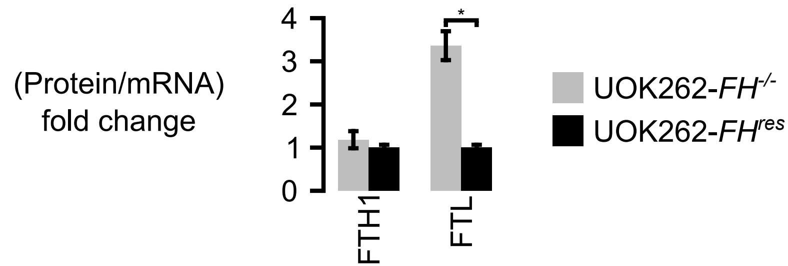

56 **Figure S5, related to Figure 5.** Translation of FTL and FTH1 in UOK262-*FH*$^{-/-}$ and UOK262-*FH*^{res}.

57 Densitometry quantitation of FTL and FTH1 protein was conducted on UOK262-*FH*$^{-/-}$ and UOK262-*FH*^{res}

58 immunoblots, using ACTB as a loading/normalization control. Relative fold changes in protein level were divided

59 by relative fold changes in mRNA levels within each cell type. * represents $p<0.05$ by student's t-test calculated

60 from 3 independent sets of experiments.

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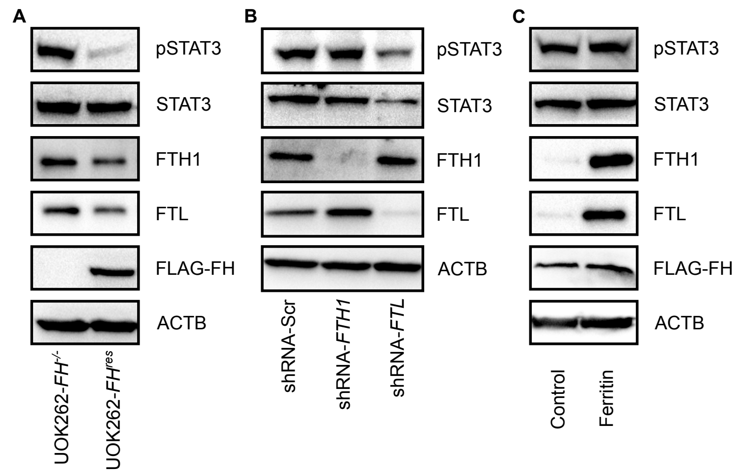

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63 **Figure S6, related to Figure 6.** STAT3 signaling is altered in UOK262-*FH*$^{-/-}$ versus UOK262-*FH*^{res}, but ferritin

64 overexpression or knockdown cannot alter STAT3 signaling.

65 A. Western blot of UOK262-*FH*$^{-/-}$ and UOK262-*FH*^{res} show a change in STAT3 phosphorylation level.

66 B. Western blot of UOK262 cells transduced with shRNAs targeting *FTH1* show no change in STAT3

67 signaling relative to shRNA-Scr (a nontargeting shRNA control). shRNA targeting *FTL* show decreases in

68 both total STAT3 and pSTAT3.

69 C. Western blot of UOK262-*FH*^{res} cells transduced with pLKO-CMV empty vector (Control) or pLKO-CMV-

70 FTL and pLKO-CMV-FTH1 (Ferritin) show no change in STAT3 signaling with ferritin overexpression.

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72 **SUPPLEMENTAL TABLES**

73 **Table S1, related to Figure 3**. Proteomics analysis of IRP2. Table S1 is provided as a separate Excel file.

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75 **Table S2, related to Figure 3**. Proteomics analysis of IRP2-A523R. Table S2 is provided as a separate Excel file.

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77 **Table S3, related to Figure 6**. Differentially expressed genes between HLRCC kidney tumors and normal kidney

78 tissues. Table S3 is provided as a separate Excel file.