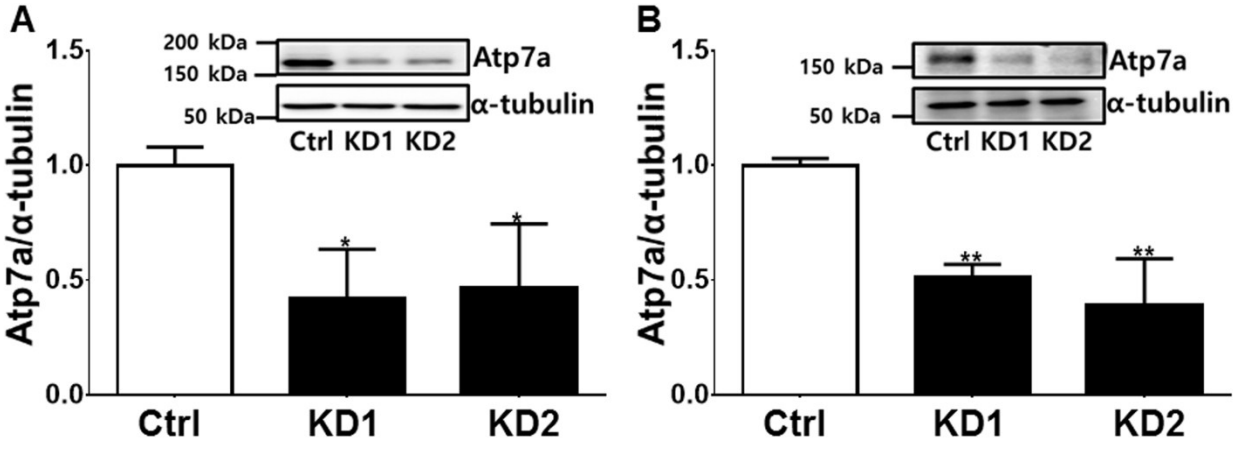
Supplemental Figure 1. Verification of Atp7a knockdown in IEC-6 and Caco-2 cells. IEC-6 and Caco-2 cells were transfected with negative-control (Ctrl) or Atp7a-targeting, shLentiviral plasmid vectors and two clonal populations, derived from a small number of individual cells, were selected for with puromycin (KD1 or KD2). Atp7a KD was verified at the protein level in all cell populations. In both panels, a representative western blot is shown as an inset; 2 other experiments produced comparable results for both proteins (not shown). Protein expression values are presented as means ± SDs. Data were analyzed by One-way ANOVA followed by Tukey's post hoc analysis. *p<0.05 and **p<0.01, as compared to control values. n = 3 independent experiments.

Supplemental Figure 2. Atp7a knockdown in IEC-6 cells alters expression of iron transport-related genes and proteins. Ctrl and Atp7a KD IEC-6 cells were grown for 8 days post-confluence, and then total cell lysates were prepared for western blot analysis. In all panels, a representative western blot is shown as an inset; 2 other experiments produced comparable results in each case (not shown). Protein expression values are means ± SDs. Data were analyzed by One-way ANOVA followed by Tukey's post hoc analysis. *p<0.05 and **p<0.01, as compared to control values. n = 3 independent experiments.

Supplemental Figure 3. Atp7a KD does not alter expression of metallothionein 1a. mRNA was purified from fully-differentiated Ctrl or Atp7a KD IEC-6 cells and SYBR-Green qRT-PCR was performed to quantify expression Mt1a. 200 μmol/L DFO was added to some wells for the last 24 hours to create an iron-deficient condition. No significant changes in expression were noted

between experimental groups. n = 3 independent experiments with 3 technical replicates per experiment.

Supplemental Figure 1.



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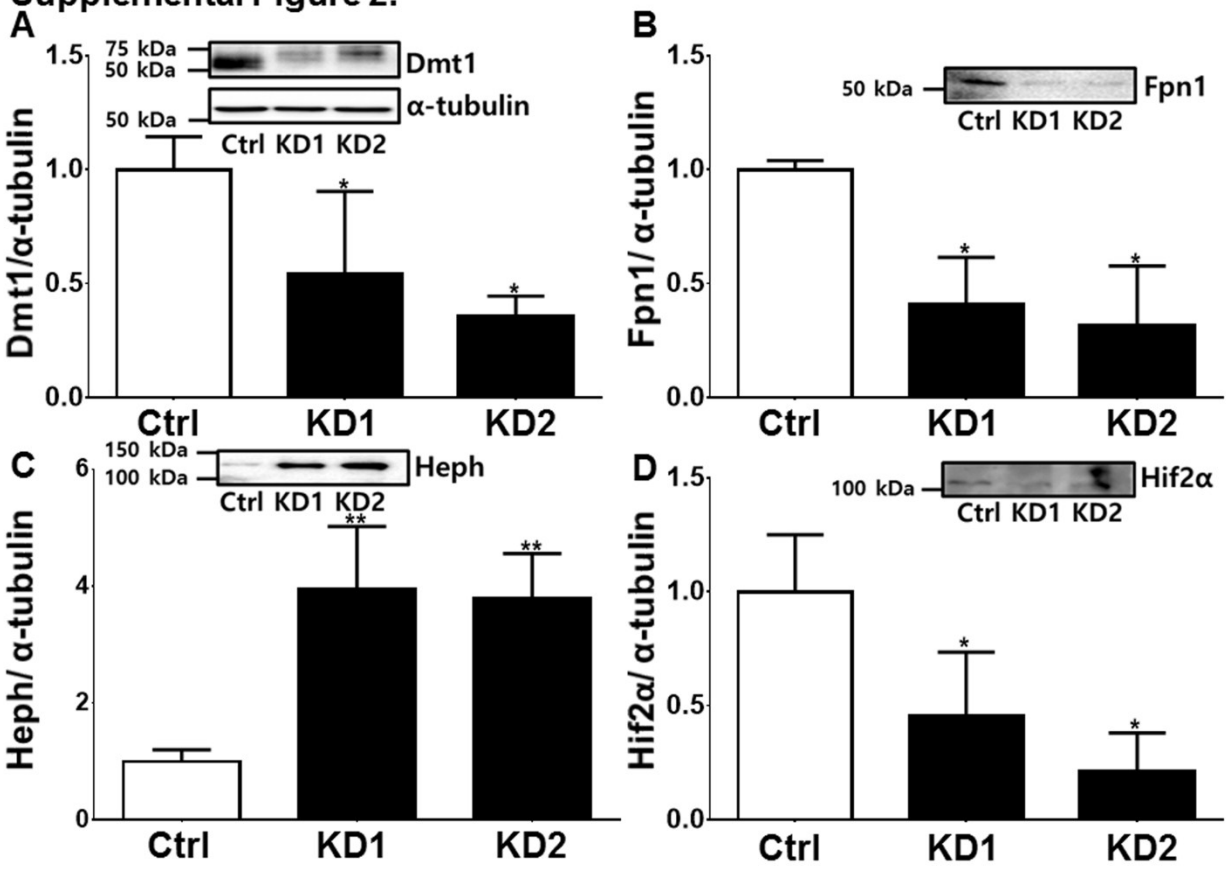
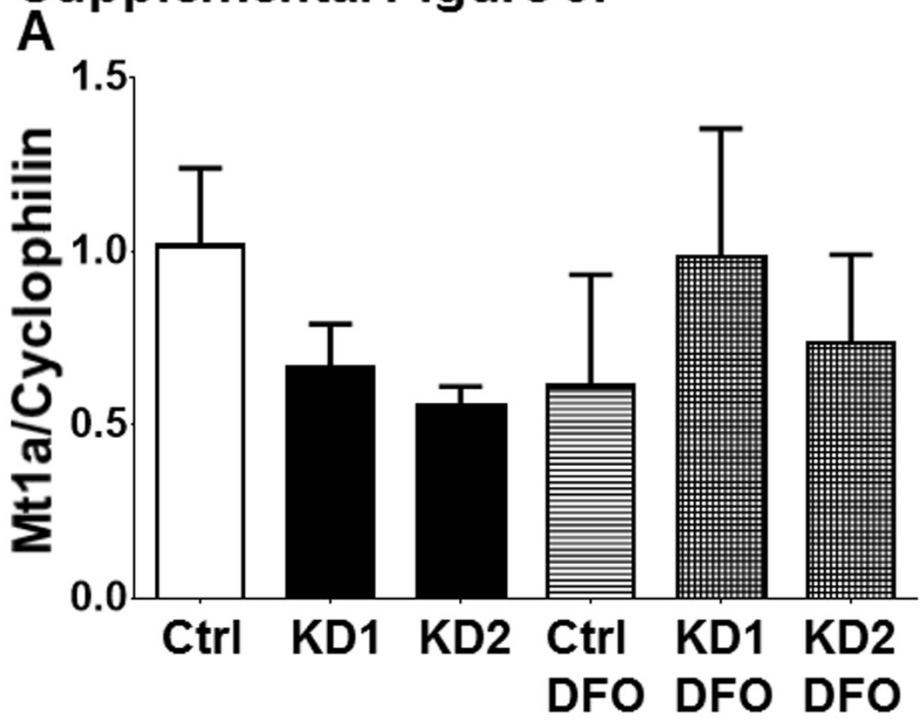
Supplemental Figure 2.



Supplemental Figure 3.



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Supplemental Table 1. Sequences of qRT-PCR primers

Gene	§F/R	Primer sequence
Atp7a	F	5'- TGAACAGTCATCACCTTCATCGTC -3'
	R	5'- GCGATCAAGCCACACAGTTCA -3'
Cyclophilin	F	5'- CTTGCTGCAATGGTCAACC -3'
	R	5'- TGCTGTCTTTGGAACTTTGTCTGC -3'
Ctr1	F	5' - AGAAGTCCAGACCTGGTTAGGGATC - 3'
	R	5' - TGTGGTTCATCCTCAGGTCC - 3'
Dcytb	F	5'- CGTGTTTGATTATCACAATGTCCG -3'
	R	5'- CACCGTGGCAATCACTGTTCC -3'
Dmt1	F	5'- GCATCTTGGTCCTTCTCGTCTGC -3'
	R	5'- AACACACTGGCTCTGATGGCTCC -3'
Fpn1	F	5'- TCGTAGCAGGAGAAAACAGGAGC -3'
	R	5'- GGAACCGAATGTCATAATCTHGC -3'
Heph	F	5'- ACACTCTACAGCTTCAGGGCATGA -3'
	R	5'- CTGTCAGGGCAATAATCCCATTCT -3'
Mt1a	F	5'- CTTCTTGTCGCTTACACCGTTG-3'
	R	5'- CAGCAGCACTGTTCGTCACTTC-3'
Tfr1	F	5'- ATTGCGGACTGAGGAGGTGC -3'
	R	5'- CCATCATTCTCAGTTGTACAAGGGAG -3'
*hnDcytb	F	5'- CCTCTTTGGAACAGTGATTGCC -3'
	R	5'- GAAGAAGGCTACAGACTTACAGGACA -3'
hnHeph	F	5'- TTCCACGGACAGACACTGAG -3'
	R	5'- TCAATATGGCAGCACCAGCA -3'
hnFpn1	F	5'- TGCAGTGTCTGTGTTTCTGGTGG -3'
	R	5'- ATGTAACTGCACTCACCTTTAAGTCTGG -3'

§F: forward/R: reverse

*hn: heteronuclear