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Supplemental Information

Oral Gavage of Ginger Nanoparticle-Derived Lipid Vectors Carrying Dmt1 siRNA Blunts Iron Loading in Murine Hereditary Hemochromatosis

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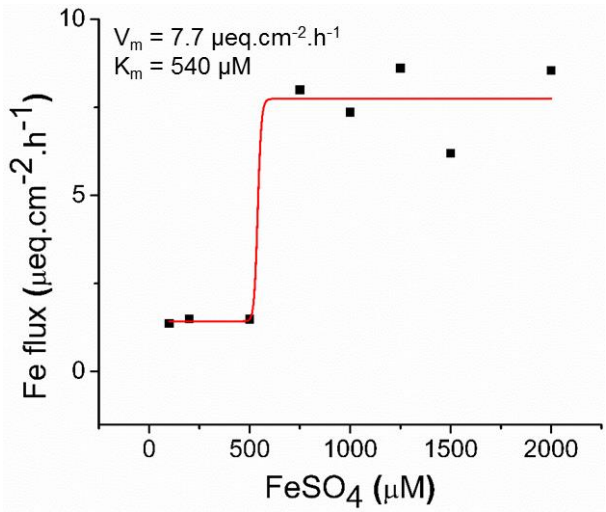

Figure S1. Transepithelial iron transport at different iron concentrations at pH 7.4 in WT mice. Ussing chambers were utilized to assess transepithelial Fe flux. Duodenal epithelial sheets from adult 129S6 male mice were used to determine the optimal $FeSO_4$ concentration. Data represent Fe flux at 100, 200, 500, 750, 1000, 1250, 1500 and 2000 µM $FeSO_4$ (n = 3).

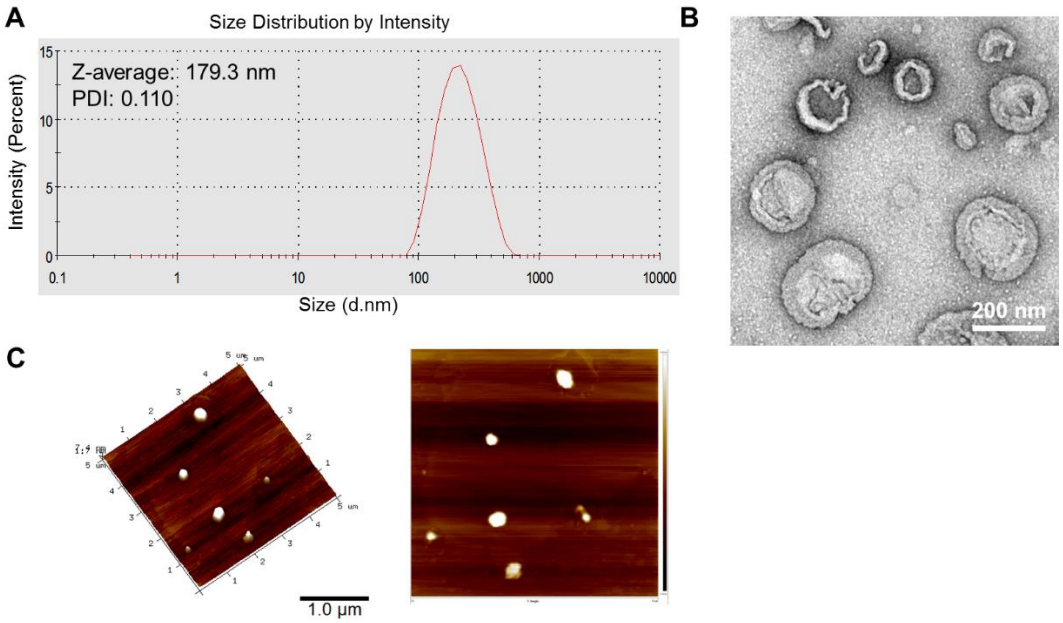

Figure S2. Characterization of ginger nanoparticle-derived lipid vector (GDLVs). GDLVs were measured by dynamic light scattering (DLS) (**A**), and visualized by transmission electron microscopy (TEM) (**B**) and atomic force microscopy (AFM) (**C**).

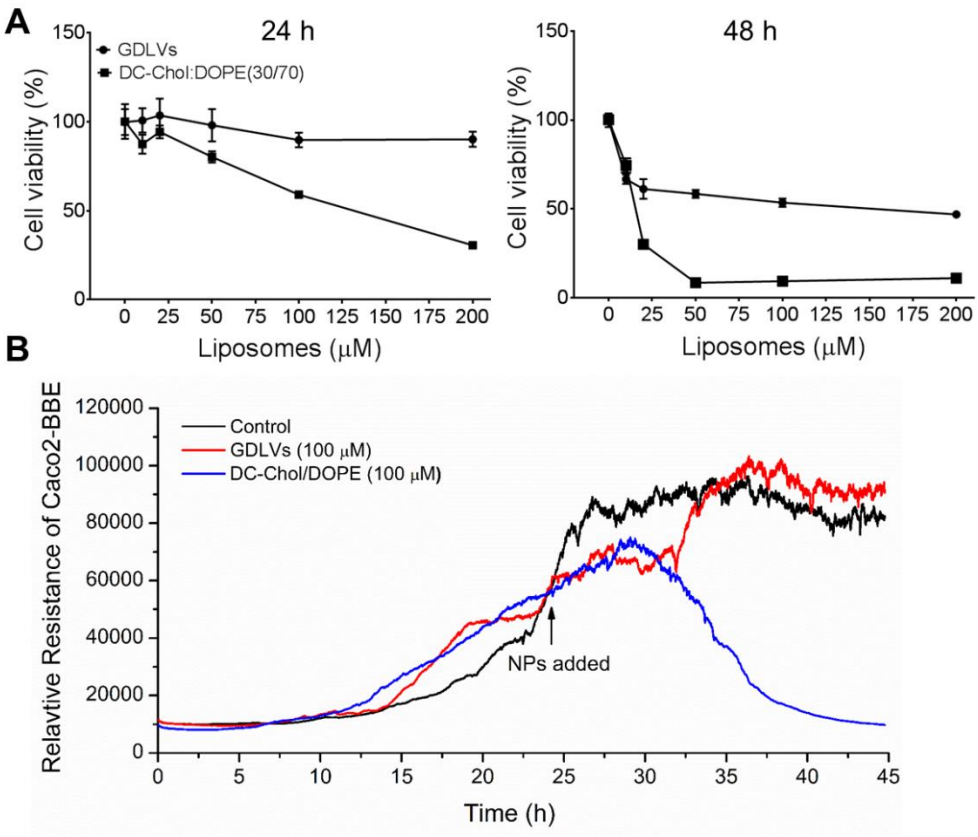

Figure S3. Biocompatibility of GDLVs. Colon-26 cell viability was measured by MTT assay after 24- or 48-hours exposure to GDLVs or commercial DC-Chol/DOPE liposomes (**A**). Electrical cell-substrate impedance sensing (ECIS) was used to monitor real-time barrier function (permeability) of Caco2-BBE cell monolayers (**B**). This image is representative of 4 identical experiments conducted.

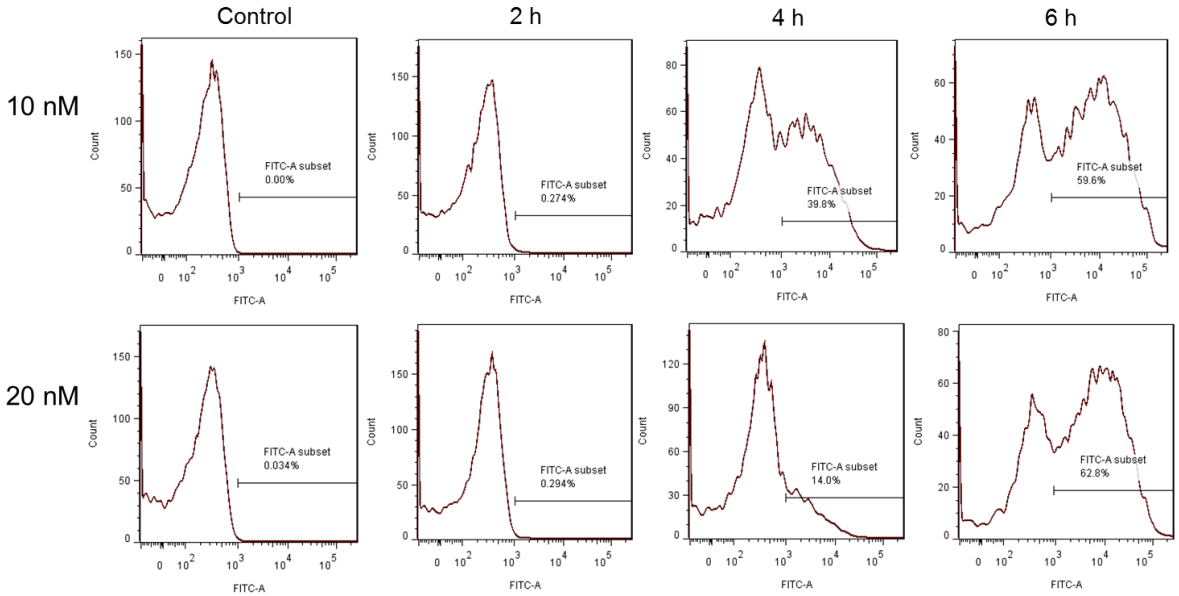

Figure S4. Cellular uptake of GDLVs/siRNA in Colon-26 cells measured by flow cytometry. Cellular uptake of GDLVs/siRNA in Colon-26 cells after 2, 4 or 6 hours. Colon-26 cells were treated with GDLVs-siRNA-FITC at 10 nM and 20 nM. The cell uptake efficiency was quantified by flow cytometry by gating for FITC fluorescence.

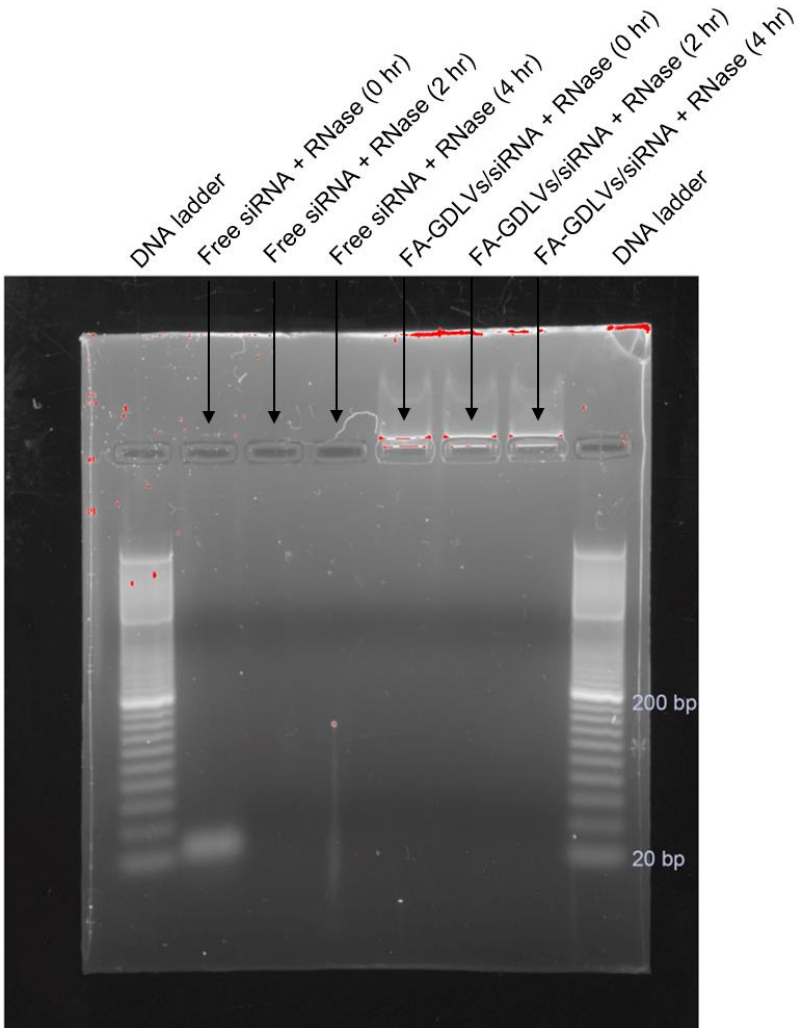

Figure S5. Stability of FA-GDLVs/siRNA. Free siRNA (10 μM) and FA-GDLVs/siRNA (10 μM) were treated with 10 μg/mL RNase (Sigma, cat#R-6513) for 0, 2, and 4hr. DNA ladder (Sigma, cat# p1598) was used as marker. 2% Agarose gel with 0.01%GelRed Nucleic Acid dye in TAE, running buffer: TAE; 110v, 40.0 min; loading volume: 10 μL (8 μL sample mixed with 2 μL RNA loading dye).

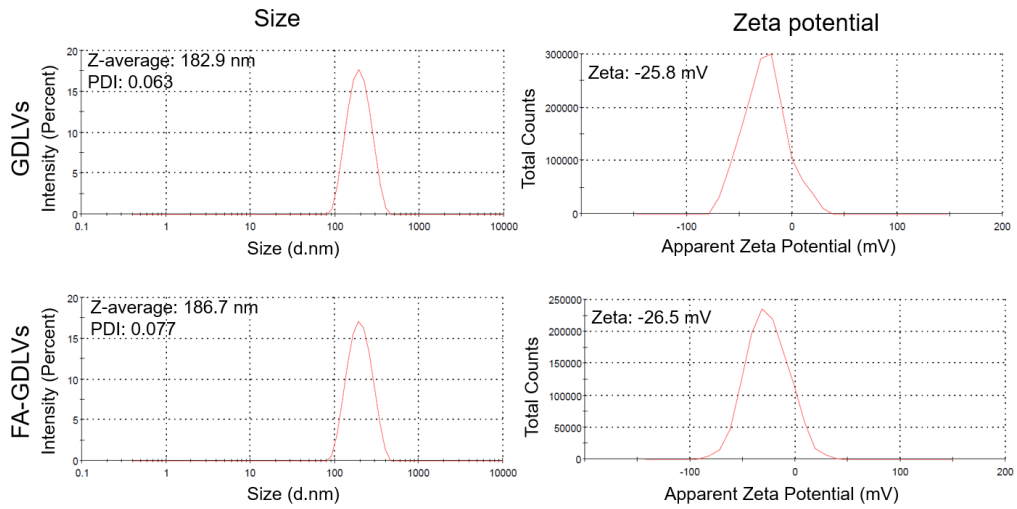

Figure S6. Characterization and comparison of GDLVs and FA-GDLVs. Size and zeta potentials of GDLV and FA-GDLVs were measured by dynamic light scattering (DLS).

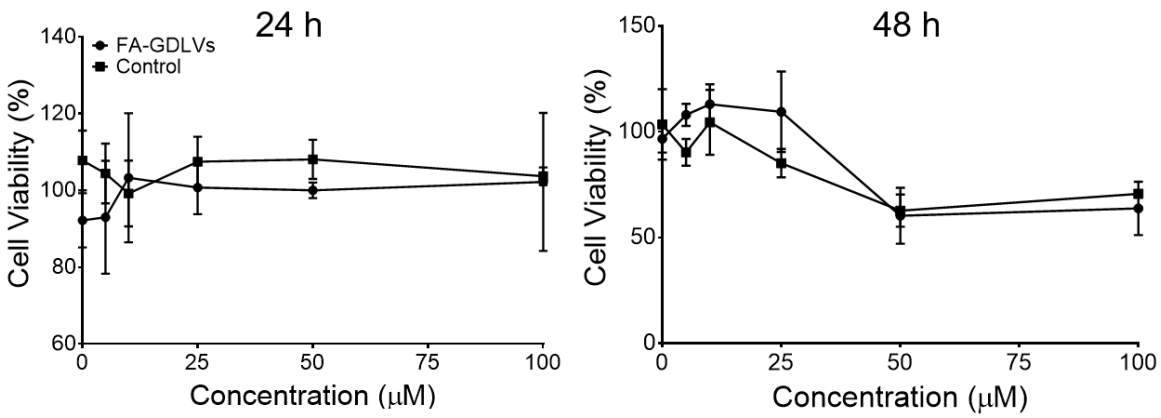

Figure S7. Biocompatibility of FA-GDLVs. Colon-26 cell viability was measured by MTT assay after 24- or 48-hours exposure to FA-GDLVs or control (PBS). FA-GDLVs did not show growth inhibition compared to the control groups. There is thus no IC_{50} value for FA-GDLVs up to 100 µM (n=4).

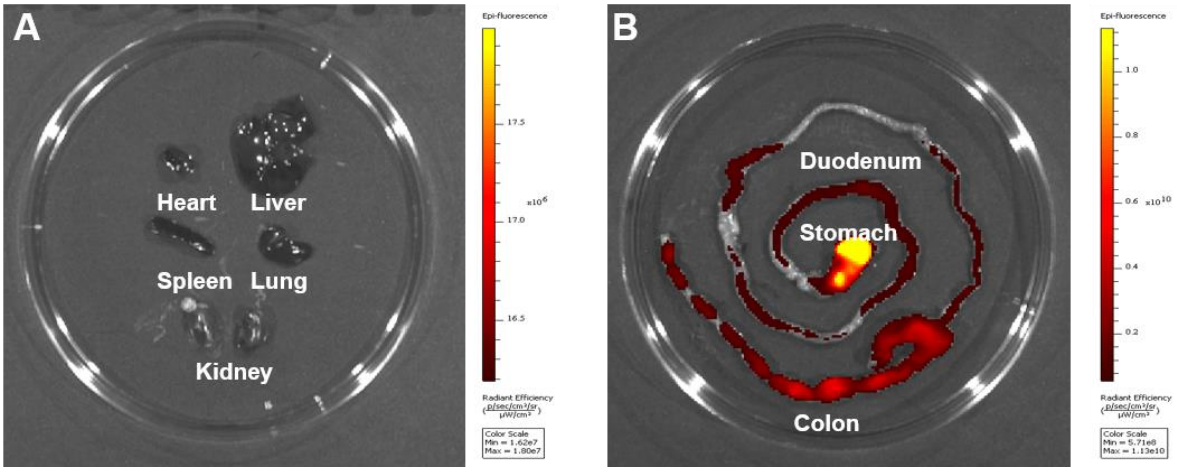

Figure S8. Biodistribution of FA-GDLVs. Mice (n=5) were fasted for 4 hours before gavage and images were captured by an IVIS series preclinical *in vivo* imaging system (Perkin Elmer; MA, USA) 4h post-gavage. Concentration of FA-GDNVs was 1 mg/mL; concentration of labeling dye (DiR) was 10uM; gavage volume was 0.1 mL. A. An image of organs (heart, liver, spleen, lung and kidney) (**A**). An image of the whole gut (**B**). Representative images are shown.

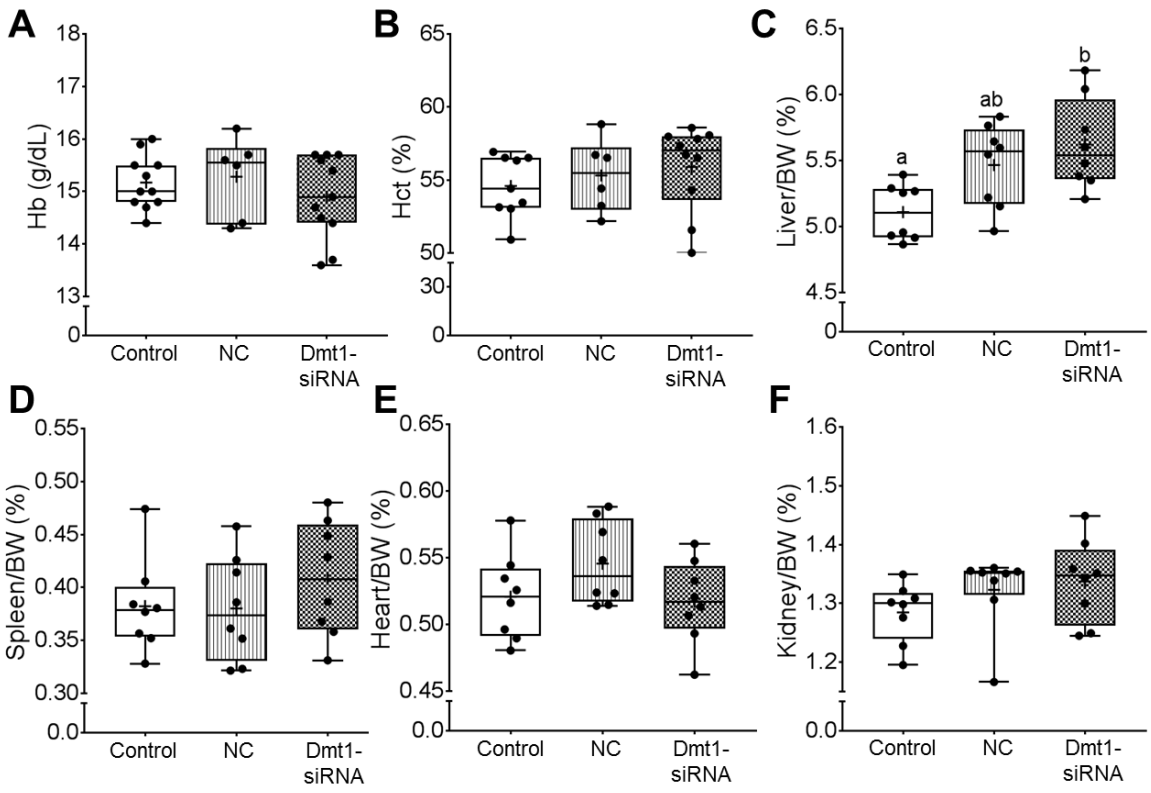

Figure S9. Hb concentrations and tissue weights of Hepc$^{-/-}$ mice treated with FA-GDLVs loaded with Dmt1 siRNA. Female Hepc$^{-/-}$ mice (19 days old) were orally gavaged daily for 16 days with saline (control), negative control-siRNA (NC) or Dmt1-siRNA FA-GDLVs. Mice were fed a low-iron diet on days 9-16. Shown are Hb levels (A), Hct levels (B), and relative liver (**C**), spleen (**D**), heart (**E**) and kidney weights (**F**). Data were analyzed by one-way ANOVA and are presented a box plots for n = 6-11 mice per group. Groups labeled with different letters are statistically different from one another (C).

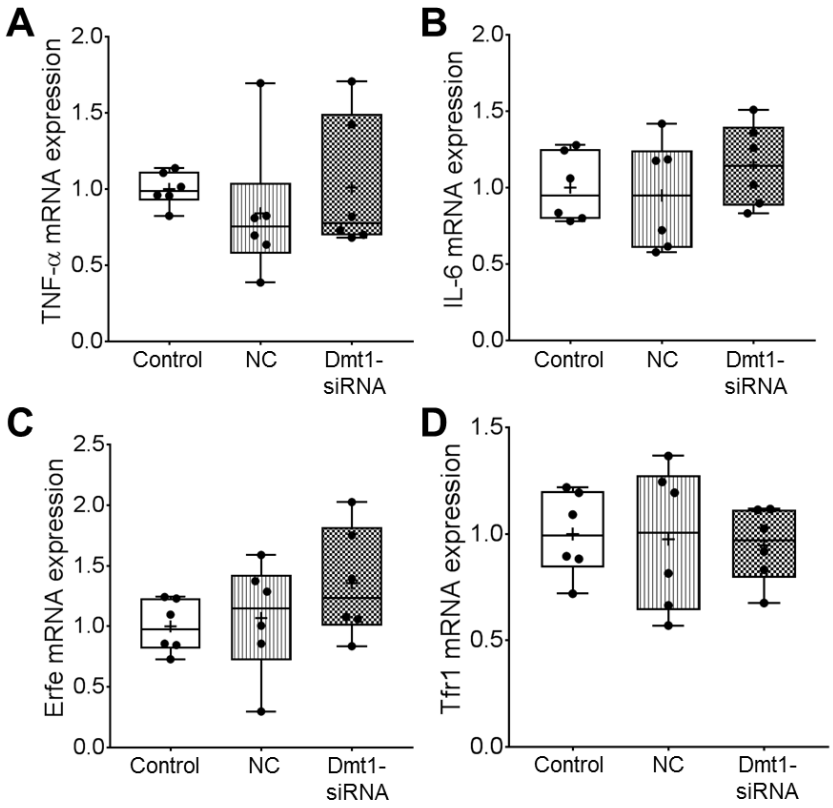

Figure S10. Hepatic TNF-α and IL-6, and bone marrow Erfe and Tfr1, mRNA expression in Hepc$^{-/-}$ mice was not affected by administration of FA-GDLVs loaded with Dmt1 siRNA. Female Hepc$^{-/-}$ mice (19 days old) were orally gavaged daily for 16 days with saline (control), negative control-siRNA (NC) or Dmt1-siRNA FA-GDLVs. Mice were fed a low-iron diet on days 9-16. Shown are TNF-α (**A**) and IL-6 (**B**) mRNA expression in liver, and Erfe (**C**) and Tfr1 (**D**) mRNA expression in bone marrow, which were normalized to expression of CypA. Data were analyzed by one-way ANOV A and are presented a box plots for n = 6 mice per group.

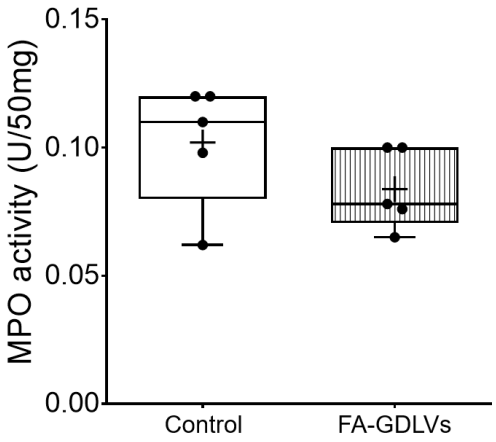

Figure S11. MPO activity in duodenum of FA-GDLVs-treated mice. Mice were gavaged with saline (control) or FA-GDLVs, and then 4 hours later, mice were euthanized and duodenal scrapes were collected for the assay (n=5/group).

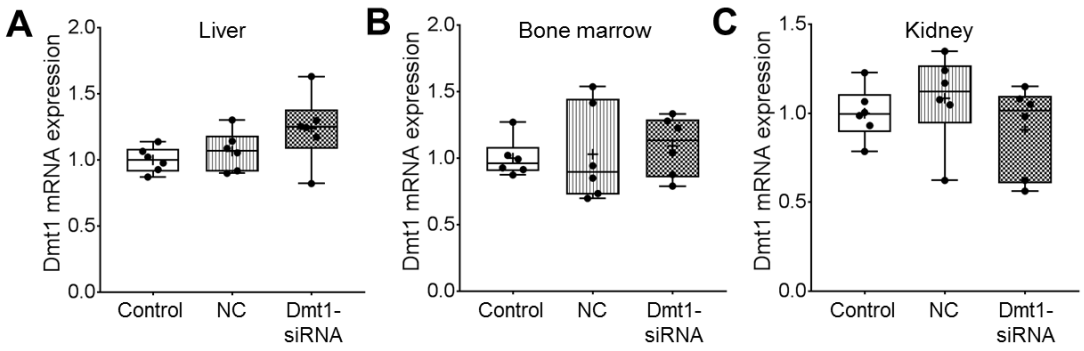

Figure S12. Dmt1 mRNA expression in liver, bone marrow and kidney of Hepc$^{-/-}$ mice was not affected by administration of FA-GDLVs loaded with Dmt1 siRNA. Female Hepc$^{-/-}$ mice (19 days old) were orally gavaged daily for 16 days with saline (control), negative control-siRNA (NC) or Dmt1-siRNA FA-GDLVs. Mice were fed a low-iron diet on days 9-16. Dmt1 mRNA expression in liver (**A**), bone marrow (**B**) and kidney (**C**) was normalized to expression of CypA. Data were analyzed by one-way ANOVA (with no significant changes noted) and are presented as box plots for n=6 mice/group.

Constituent, mM	pH 5.0	pH 6.5	pH 7.4
Na^+	119.6	140.4	144.5
K^+	5.2	5.2	5.2
Mg^{2+}	1.2	1.2	1.2
Ca^{2+}	1.2	1.2	1.2
Acetate	50	—	—
Cl^-	74	115	115
Citrate	25	25	25
$H_2PO_4^-$	—	7.6	3.5
HPO_4^{2-}	—	1.4	5.5

Table S1. Composition of experimental buffers. Different buffer systems were chosen based on their pKa that was best suited or closer to the pH of the buffer solution used for the studies. Henderson-Hasselbalch equation was used to calculate the ratio of acid and its corresponding conjugate base. The buffers were then titrated using 1N HCl to confirm the buffering capacity and arrive at the titration curve. The stability of the buffer with time was determined by measuring the pH at 25^{o}C at regular interval for 24 hours. The final osmolarity of the solution was 296mOsms.

Transcript	Forward (5’ to 3’)	Reverse (5’ to 3’)
Dmt1	GTGATCCTGACCCGGTCTATCG	TGAGGATGGGTATGAGAGCAAAGG
Epo	ATGAAGACTTGCAGCGTGGA	AGGCCCAGAGGAATCAGTAG
Erfe	ACTCACCAAGCAGCCAAGAA	TTCTCCAGCCCCATCACAGT
TNF-α	CACAAGATGCTGGGACAGTGA	TCCTTGATGGTGGTGCATGA
IL-6	CTGCAAGAGACTTCCATCCAGTT	AGGGAAGGCCGTGGTTGT
Tfr1	AACTTACCCATGACGTTGATTGAACC	ACAGCCACTGTAGACTTAGACCCATATC
CypA	CTTACGACAAGCAGCCCTTCATG	AGCTGTTTTTAACTCACTGCTGTTGTA

Table S2. Primer Sequences