SUPPLEMENTAL FIGURES

Figure S1

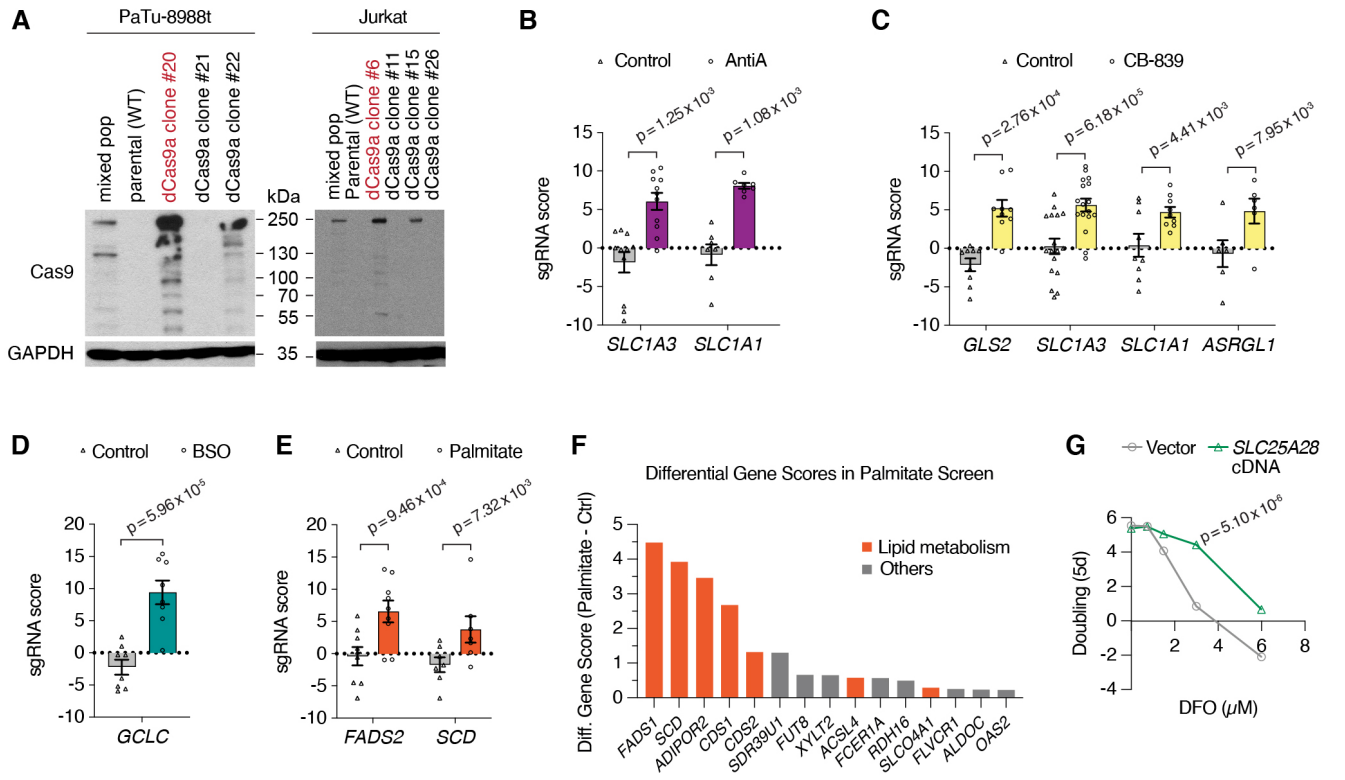

Figure S1. Positive selection CRISPRa screens for metabolic stressors identify resistance genes
Related to Figures 1 and 2

(A) Immunoblot analysis for Cas9 expression in PaTu-8988t and Jurkat single cell clones. Clones used for screens are highlighted in red.

(B-E) sgRNA scores of CRISPRa hits. Each data point represents a different sgRNA targeting the corresponding gene. Student's t-test, paired, two-tailed. 95% confidence interval. Hits from (B) Antimycin A, (C) CB-839, (D) BSO and (E) Palmitate were plotted.

(F) Top 15 genes scoring as protective against palmitate toxicity in Jurkat CRISPRa screens. Differential gene scores (palmitate – untreated control) are plotted. Lipid metabolism-related genes are highlighted in orange.

(G) Fold change in the number (log_2) of control vector and *SLC25A28*-overexpressing cells after 5 days in the presence of indicated DFO concentrations

Figure S2

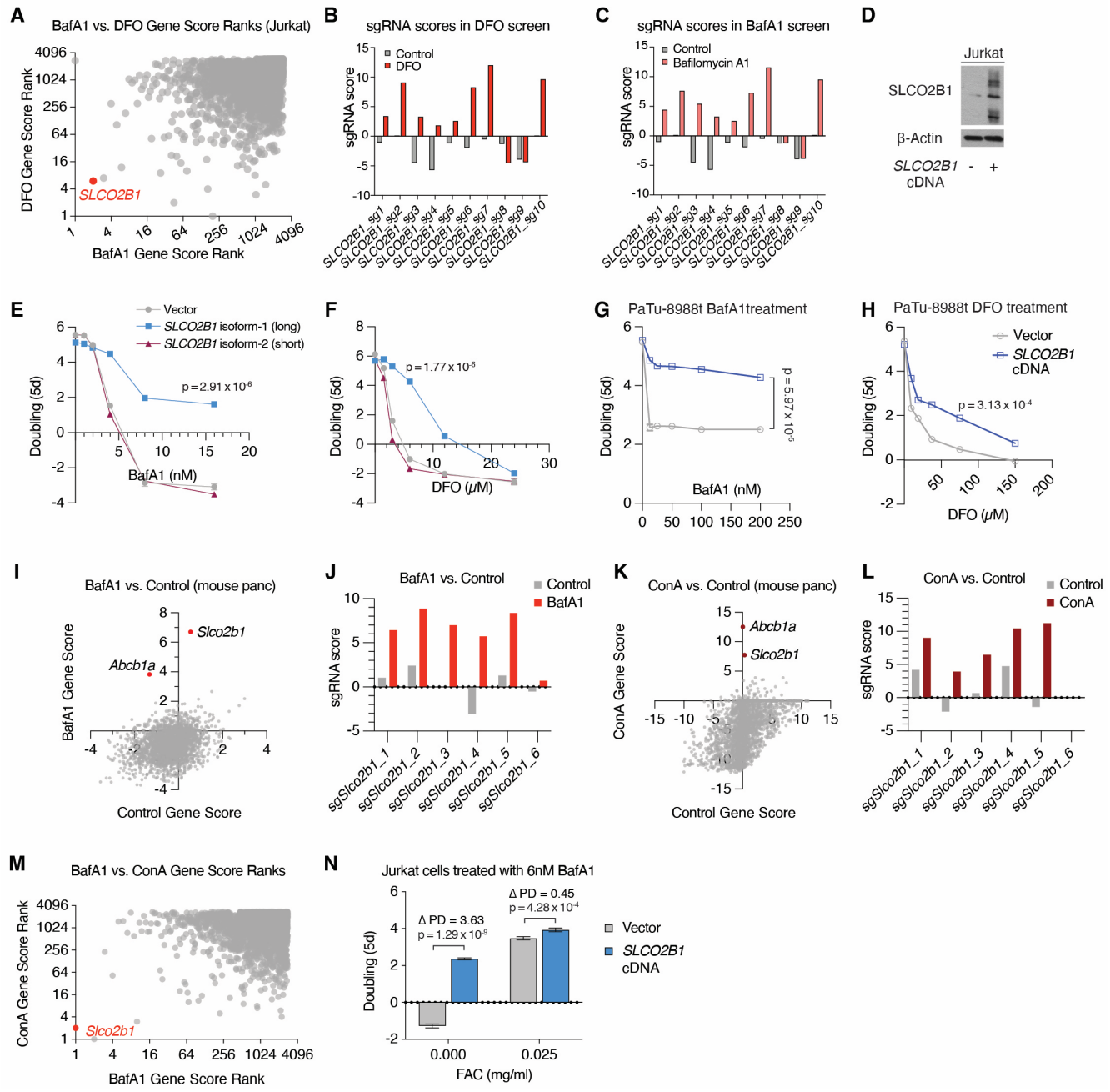

Figure S2. Positive selection CRISPRa screens reveal *SLCO2B1* as the common top hit providing resistance to iron restriction in human and mouse cells

Related to Figure 2

(A) Gene score ranks from BafA1 and DFO CRISPRa screens in Jurkat cells. *SLCO2B1* is highlighted as it has the lowest common rank, i.e. highest score.

(B) *SLCO2B1* sgRNA scores in CRISPRa screens for DFO in Jurkat cells

(C) *SLCO2B1* sgRNA scores in CRISPRa screens for BafA1 in Jurkat cells

(D) Immunoblot analysis for SLCO2B1 expression in Jurkat cells expressing vector or *SLCO2B1* cDNA. β-actin was used as loading control.
(E) Fold change in the number (log_2) of vector, *SLCO2B1* long isoform and *SLCO2B1* short isoform expressing Jurkat cells after 5 days in the presence of indicated BafA1 concentrations.
(F) Fold change in the number (log_2) of vector, *SLCO2B1* long isoform and *SLCO2B1* short isoform expressing Jurkat cells after 5 days in the presence of indicated DFO concentrations.
(G) Fold change in the number (log_2) of vector, *SLCO2B1* long isoform and *SLCO2B1* short isoform expressing PaTu-8988t cells after 5 days in the presence of indicated BafA1 concentrations.
(H) Fold change in the number (log_2) of vector, *SLCO2B1* long isoform and *SLCO2B1* short isoform expressing PaTu-8988t cells after 5 days in the presence of indicated DFO concentrations.
(I) Gene scores of untreated vs. BafA1 (15 nM)-treated mouse AK196 pancreas-dCas9a cells. Gene score is the median log_2 fold change in the abundance of all sgRNAs targeting that gene during the screening period.
(J) *Slco2b1* sgRNA scores in positive selection CRISPRa screens for BafA1 treatment in mouse AK196 pancreas cells
(K) Gene scores of untreated vs. ConA (1 nM)-treated mouse AK196 pancreas-dCas9a cells.
(L) *Slco2b1* sgRNA scores in positive selection CRISPRa screens for ConA treatment in mouse AK196 pancreas cells
(M) Gene score ranks from BafA1 and ConA CRISPRa screens in mouse AK196 pancreas cells. Slco2b1 is highlighted as the lowest common rank, i.e., highest score.
(N) Fold change in the number (log_2) of control vector and *SLCO2B1*-overexpressing cells after 5 days in the presence of 6 nM BafA1 with or without 0.025 mg/ml FAC. ΔPD: Difference between the number of proliferation doublings.

Figure S3

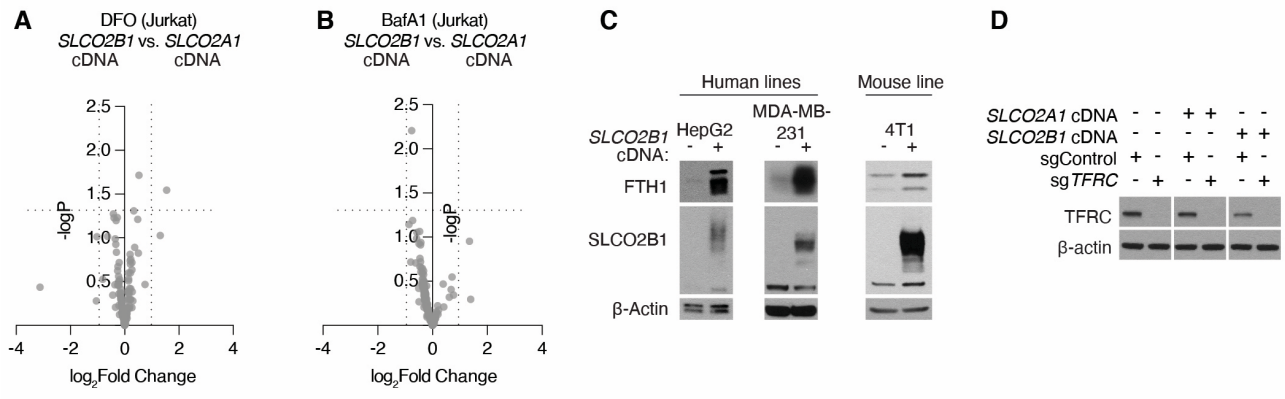

Figure S3. *SLCO2B1* overexpression leads to an increase in cellular iron availability
Related to Figures 2 and 3
(A-B) Volcano plots showing the $\log_2$ fold change in polar metabolite abundance between *SLCO2B1* and *SLCO2A1*-expressing Jurkat cells vs. -log P values. The dotted lines on x-axis indicate 2-fold difference; that on y-axis represents P = 0.05 (Student's t-test, two-tailed, 95% confidence interval). (A) DFO (3 μM)-treated and (B) BafA1 (3nM)-treated cells were assayed.
(C) Immunoblot analysis of FTH1 and SLCO2B1 in select human and mouse cell lines expressing vector or *SLCO2B1* cDNA. β-actin was used as loading control.
(D) Immunoblot analysis of TFRC in *TFRC* knockout Jurkat cells expressing vector, *SLCO2A1* or *SLCO2B1* cDNA. β-actin was included as loading control for immunoblots.

Figure S4

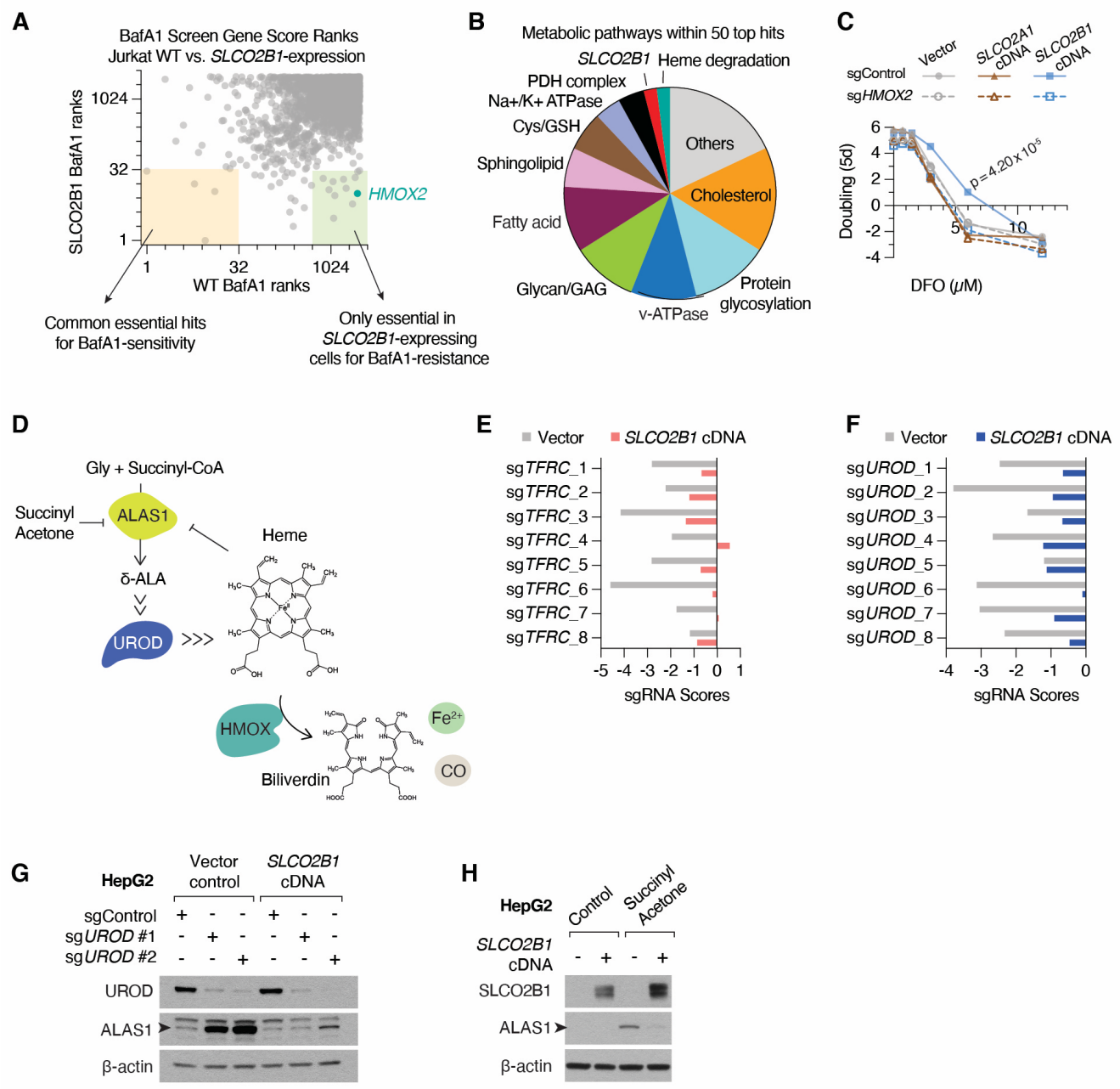

Figure S4. *SLCO2B1* expression mediates resistance to iron restriction through heme oxygenase and overcomes heme deficiency

Related to Figure 4

(A) Gene score ranks from BafA1 screens in parental (3 nM BafA1) and SLCO2B1-expressing (4nM BafA1) Jurkat cells. Common hits essential in BafA1 treatment, regardless of the genotype, are in yellow highlighted area (genes scoring within top 32 in both screens). Genes that are only essential in *SLCO2B1*-overexpressing cells for BafA1-resistance are in green highlighted area. *HMOX2*, shown in green circle, is one of the top hits specifically in *SLCO2B1*-overexpressing cells.

(B) Top 50 genes scoring as differentially essential upon BafA1 treatment in *SLCO2B1*-expressing cells were grouped in metabolic pathways in which they are functioning.
(C) Fold change in the number (log_2) of *HMOX2* knockout Jurkat cells expressing vector, *SLCO2A1* or *SLCO2B1* cDNA, during 5-day incubation with or without DFO at indicated concentrations. (Mean ± SEM, n= 3, Student's t-test, 95% confidence interval).
(D) Schematic depicting key reacting in heme biosynthesis and heme degradation pathways. Succinyl acetone is a potent inhibitor of heme biosynthesis, targeting ALAS1.
(E) *TFRC* sgRNA scores in CRISPR knockout screens of Jurkat cells expressing a control vector or *SLCO2B1* cDNA.
(F) *UROD* sgRNA scores in CRISPR knockout screens of Jurkat cells expressing a control vector or *SLCO2B1* cDNA.
(G) Immunoblot analysis for UROD and ALAS1 in UROD knockout HepG2 cells expressing a control vector or *SLCO2B1* cDNA. The position of ALAS1 band was shown with an arrow. β-actin was used as loading control.
(H) Immunoblot analysis of SLCO2B1 and ALAS1 in untreated control and 1 mM succinyl acetone-treated HepG2 cells expressing a control vector or *SLCO2B1* cDNA. β-actin was used as loading control.

Figure S5

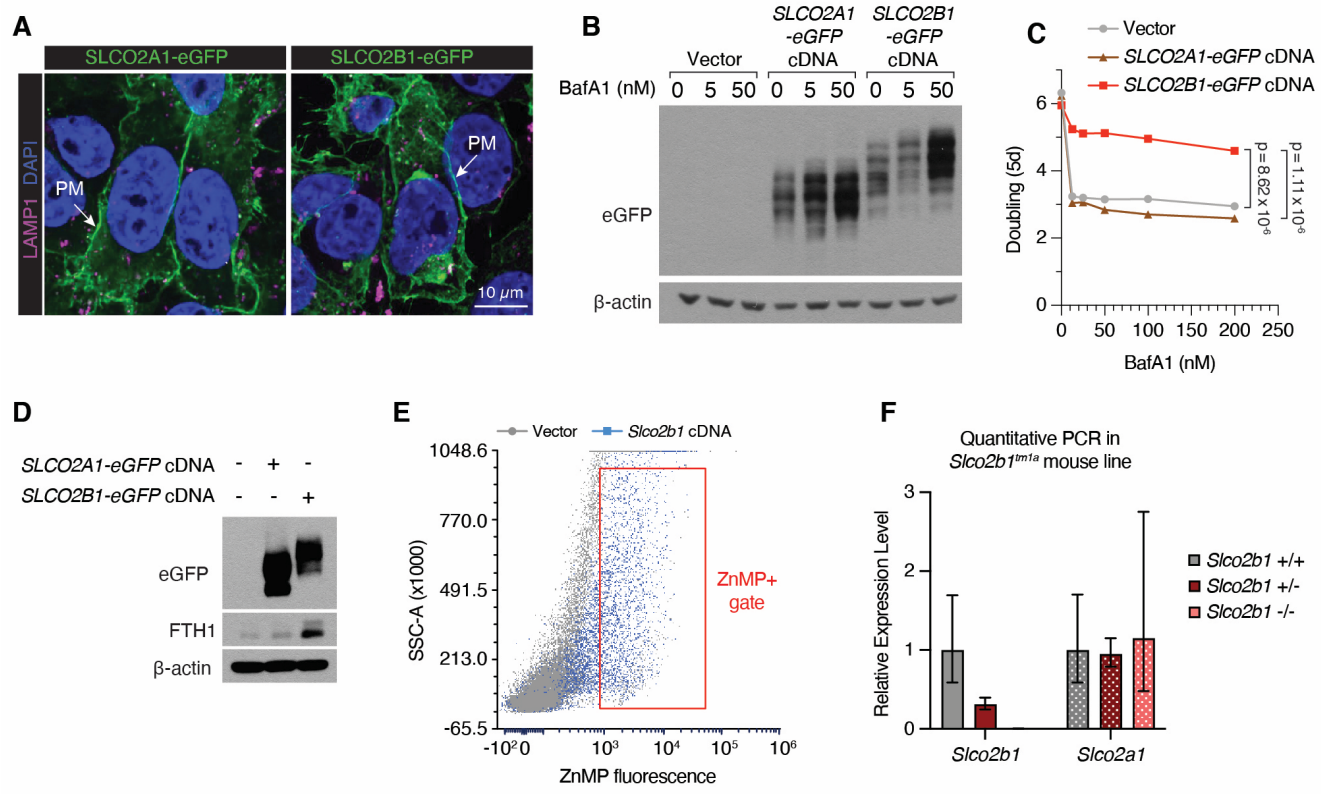

Figure S5. SLCO2B1 localizes to plasma membrane

Related to Figures 5 and 6

(A) Confocal microscopy images of PaTu-8988t cells expressing SLCO2A1-eGFP or SLCO2B1-eGFP. Cell were immunostained for eGFP and LAMP1 (lysosomal marker). DAPI was used as nuclear counterstain. PM: plasma membrane.

(B) Immunoblot analysis for eGFP in PaTu-8988t cells expressing a control vector, SLCO2A1-eGFP or SLCO2B1-eGFP, either untreated or treated with indicated concentration of BafA1. β-actin was used as loading control.

(C) Fold change in the number (log_2) of PaTu-8988t cells expressing a control vector, SLCO2A1-eGFP or SLCO2B1-eGFP cDNA, during 5-day incubation with or without BafA1 at indicated concentrations. (Mean ± SEM, n= 3, Student's t-test, 95% confidence interval).

(D) Immunoblot analysis for eGFP and FTH1 in PaTu-8988t cells expressing a control vector, SLCO2A1-eGFP or SLCO2B1-eGFP cDNA. β-actin was used as loading control.

(E) Flow cytometry analysis of ZnMP uptake in mouse HY15549 pancreas cells expressing a control vector or *Slco2b1* cDNA, treated with1 μM ZnMP for 15 min. ZnMP+ gate shown here was applied in dose-dependent ZnMP uptake assays

(F) Relative expression levels of *Slco2b1* and *Slco2a1* genes in wild-type (+/+), heterozygous (+/-) and homozygous mutant (+/-) mice from *Slco2b1*^{tm1a} mouse line. Expression levels were normalized to β-actin. Specific loss of *Sloc2b1* expression in homozygous mutants was observed; *Slco2a1* expression was assayed as a control. (Mean ± SEM, n= 3, Student's t-test, 95% confidence interval).