Reductions in the mitochondrial ABC transporter Abcb10 affect the transcriptional profile of heme biosynthesis genes

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Materials included:

5 supplemental figures and figure legends.

Supplemental Figure 1 Abcb10 mutants are expressed and localize to mitochondria

MEL cells stably expressing control shRNA or Abcb10-specific shRNA were transfected with empty vector, wild type Abcb10-GFP or mutant Abcb10-GFP and stable cells lines selected by growth in 1 mg/ml G418. Expression of wild type or mutant Abcb10-GFP was assessed by Western blotting with tubulin as a load control (bottom panel) (A) and localization was determined by immunofluorescence using Mfrn1-FLAG as a mitochondrial marker (B). Cells were processed as described in Materials and Methods.

Supplemental Figure 2 Abcb10-shRNA MEL cells show decreased steady-state mRNA for heme related transcripts.

qRT-PCR for *Pgbd, Urod, Ppox, Tfr1, Bcl-xl, Fth, Ftl* and *Sod2* was performed using the primers listed in Table 2.

Supplemental Figure 3 Abcb10-shRNA MEL cells show reduced transduction efficiency.

qRT-PCR for human *Abcb10* and mouse *β-actin* was performed on control shRNA and Abcb10-specific shRNA MEL cells transduced with lentivirus based expression of human Abcb10 as in Figure 5C.

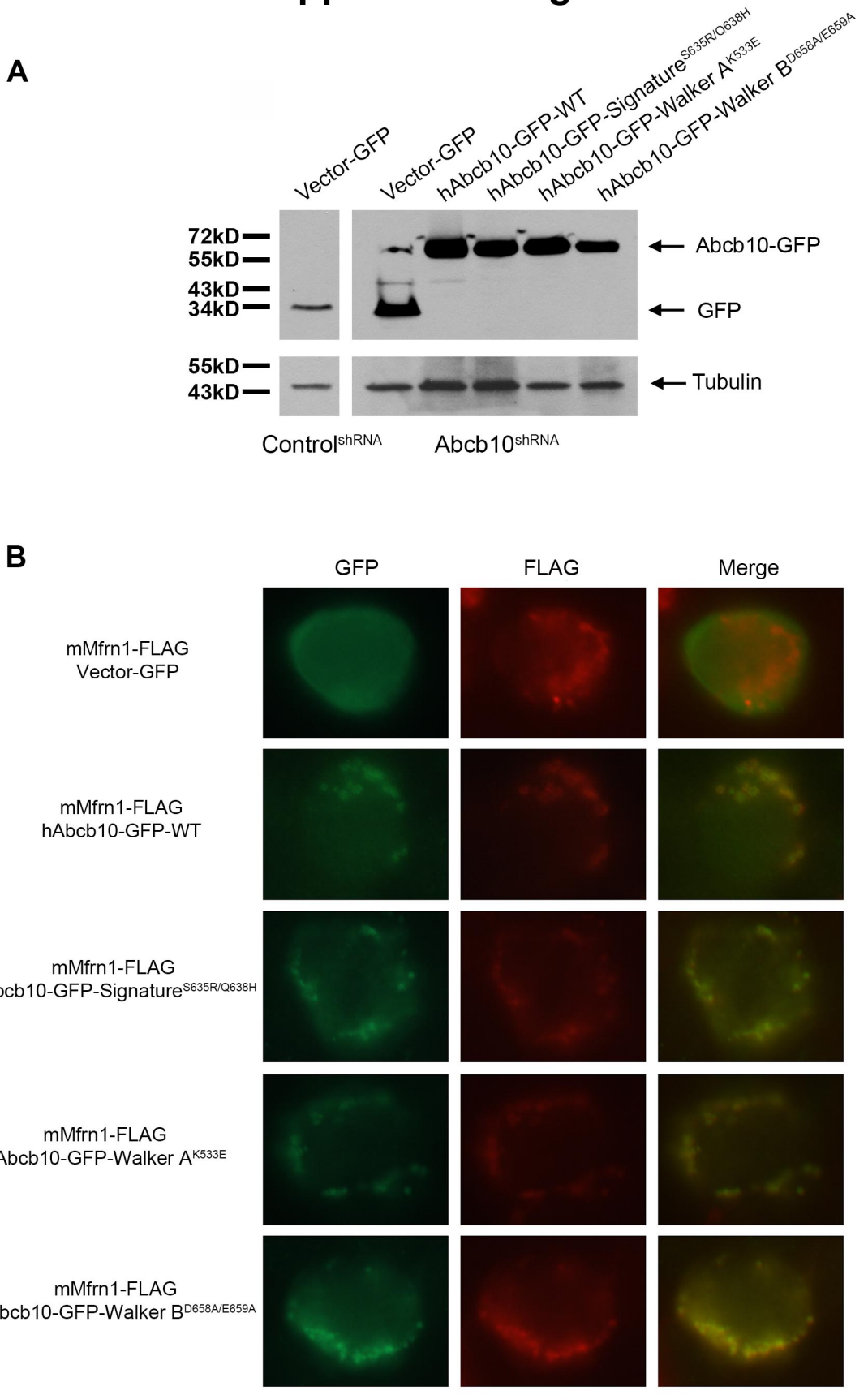
Supplemental Figure 4 Addition of SA did not alter *Alas2* or *β-Globin* mRNA levels in Abcb10-shRNA MEL cells.

qRT-PCR for *Mfrn1, Abcb10*, *β-Globin* and *Alas2* was performed using the primers listed in Table 2.

Supplemental Figure 5 Addition of hemin does not increase *Mfrn1*, *β-Globin*, *Alas2 or Fech* transcripts but does increase *HO-1* transcripts in shRNA Abcb10 MEL cells.

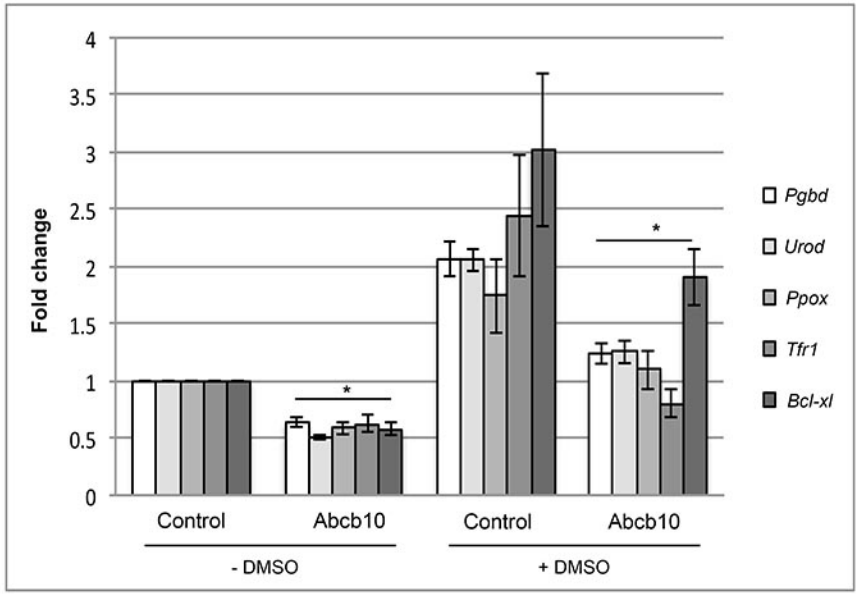
qPCR for *Mfrn1, β-Globin*, *Alas2, Fech* and *Heme Oxygenase 1 (HO-1)* was performed on undifferentiated and differentiated control shRNA and Abcb10-specific shRNA MEL cells grown for three days with or without 75 μM hemin.

Supplemental Figure 1

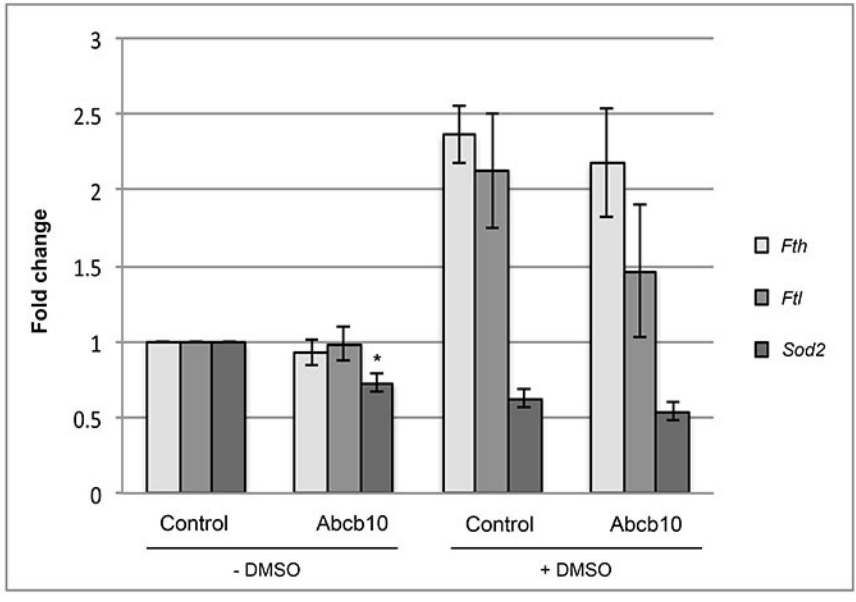


Supplemental Figure 2

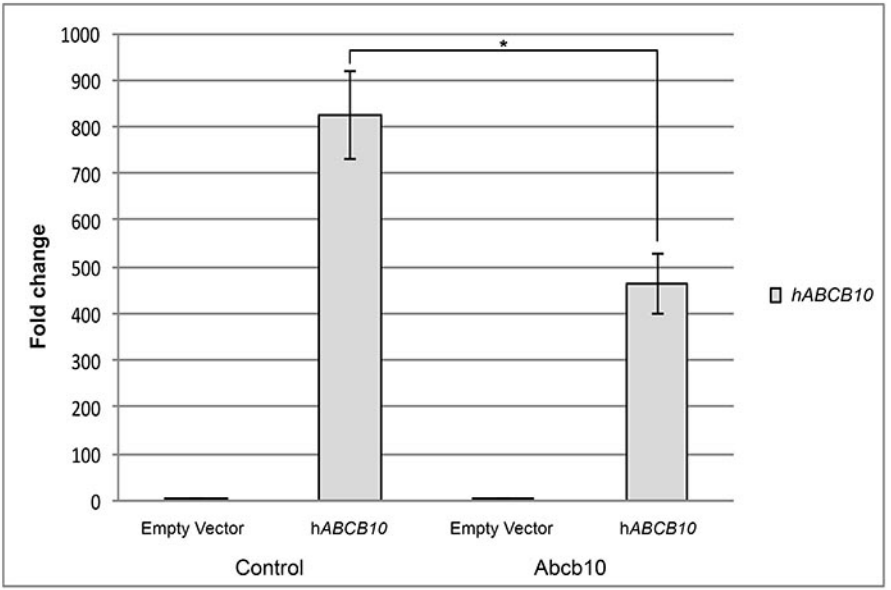
A



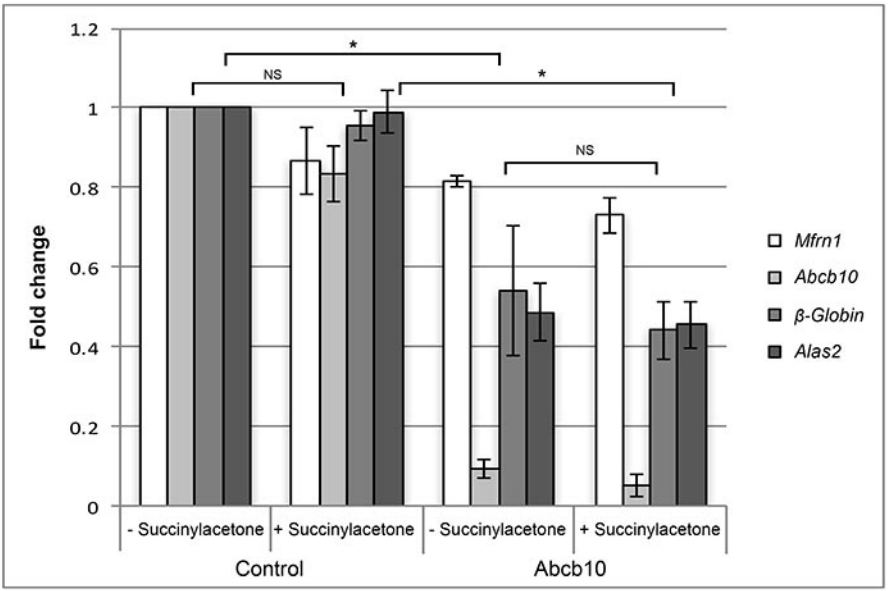
B



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5