Mitochondrial dysfunction reactivates α-fetoprotein expression that drives copper-dependent immunosuppression in mitochondrial disease models

Kimberly A. Jett¹, Zakery N. Baker¹, Amzad Hossain¹, Aren Boulet¹, Paul A. Cobine², Sagnika Ghosh³, Philip Ng⁴, Orhan Yilmaz¹, Kris Barreto⁵, John DeCoteau⁵, Karen Mochoruk⁵, George N. Ioannou⁶,⁷,⁸, Christopher Savard⁶,⁷,⁸, Sai Yuan⁹, Osama H.M.H. Abdalla¹⁰,¹¹, Christopher Lowden¹⁰,¹¹, Byung-Eun Kim⁹, Hai-Ying Mary Cheng¹⁰,¹¹, Brendan J. Battersby¹², Vishal M. Gohil³ and Scot C. Leary¹,¹,¹³,¹⁴,*

Supplemental Figures
Figure S1. A) The Sco1<sup>hep</sup> thymus exhibits progressive thinning of the cortex from P27 to P47, with disruption of the cortico-medullary boundary, accumulation of tingible body macrophages (denoted with a *) and increased vascularity (denoted with a >). Scale bar, 100µm. B) LacZ staining in the liver (4X), spleen (4X), heart (4X) and lung (2X) upon intracardiac (IC) or intraperitoneal (IP) administration of vehicle or helper-dependent adenovirus. C&D) Restoration of Sco1 expression in the Sco1<sup>hep</sup> liver normalizes C) metal ion levels (t-test, n=3, Cu and Fe, p<0.01; Zn, p<0.05) and D) CTR1 abundance. Control refers to wild-type littermates.
**Figure S2.** Change in body weight (g) over time in A) $Coa5^{hep}$ (*Control*, n=23-64; $Coa5^{hep}$, n=6-31) and B) $Cox10^{hep}$ (*Control*, n=11-31; $Cox10^{hep}$, n=5-16) mice. C) $Coa5^{hep}$ livers have a severe COX (t-test, n=5) and copper deficiency relative to livers from *Control* littermates. D) Plasma copper, iron and zinc levels in $Coa5^{hep}$ (t-test, n=4) and $Cox10^{hep}$ (t-test, n=8) plasma relative to age-matched, littermate *Controls.*
**Figure S3.** A) Body and organ weight are unaffected in *Control* mice injected with *Sco1*<sub>hep</sub> plasma relative to those injected with *Control* plasma (t-test, n=6-7). B) Metal content (t-test, n=3) and C) OXPHOS subunit abundance are unaltered in livers from mice fed a high fat (HF) diet compared to those fed normal chow. *Control* and *Sco1*<sub>hep</sub> liver extracts were included for comparative purposes and tubulin served as an internal loading control. D) Lower magnification showing a greater number of PBMCs, with black boxes depicting the region of interest shown in Figure 3B.
Figure S4. A) Individual box plots of significantly up- or downregulated plasma proteins in hep compared to Control mice. Red and blue circles denote data from the Sco1 and Cox10 models, respectively (open circles, Control animals; closed circles, hep animals). HF denotes plasma from mice fed a high fat diet. B) Afp mRNA levels are significantly higher in the Sco1hep liver (ANOVA; p < 0.02) but not the heart, when compared to Control tissues from age-matched littermates (n=4, all tissues and genotypes). Transcript levels were normalized to Gapdh mRNA abundance. C) PBMC viability is similarly reduced upon treatment with Sco1hep plasma or recombinant AFP (rAFP, 1µg).
**Figure S5.**

**A)** *Afp* mRNA levels are significantly higher in the *Ctrl* heart (ANOVA; *p* < 0.005) but not the liver, when compared to *Control* tissues from age-matched littermates (heart, *n*=6; liver, *n*=10 for both genotypes). Transcript levels were normalized to *Gapdh* mRNA abundance. **B)** The *Ctrl* heart has elevated levels of AFP and the ISR marker phospho-eIF2α. Equal amounts of *Control* and hep liver extracts from the *Sco1* and *Cox10* lines were included in these analyses for comparative purposes. N.B. original data for actin, total and phospho-eIF2α livers from both hep models are shown in Figure 2E. **C)** The viability of PBMCs isolated from *Control* mice is reduced when co-cultured with AFP produced by baculovirus (bac AFP, the rAFP used in Figure S4C of this study) but not with AFP isolated from *E. coli*. *Control* and *Sco1* hep plasma were included in these analyses as negative and positive controls, respectively.
Figure S6. Representative flow plots of peripheral PBMCs show that $Sco1^{hep}$ mice show have a higher percentage of cells positive for the cell surface expression of A) the apoptotic marker Annexin V and B) the activation marker CD44.