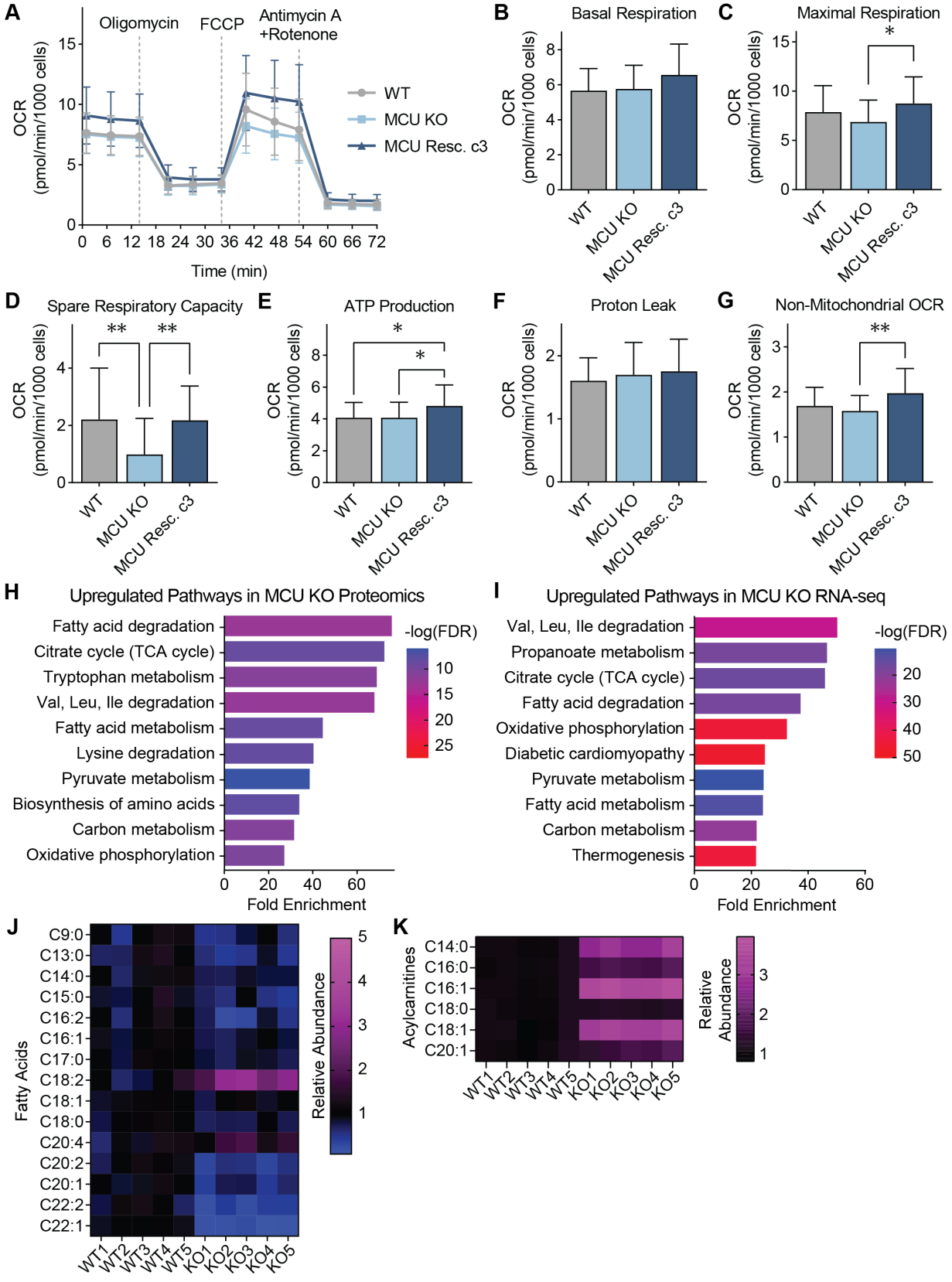
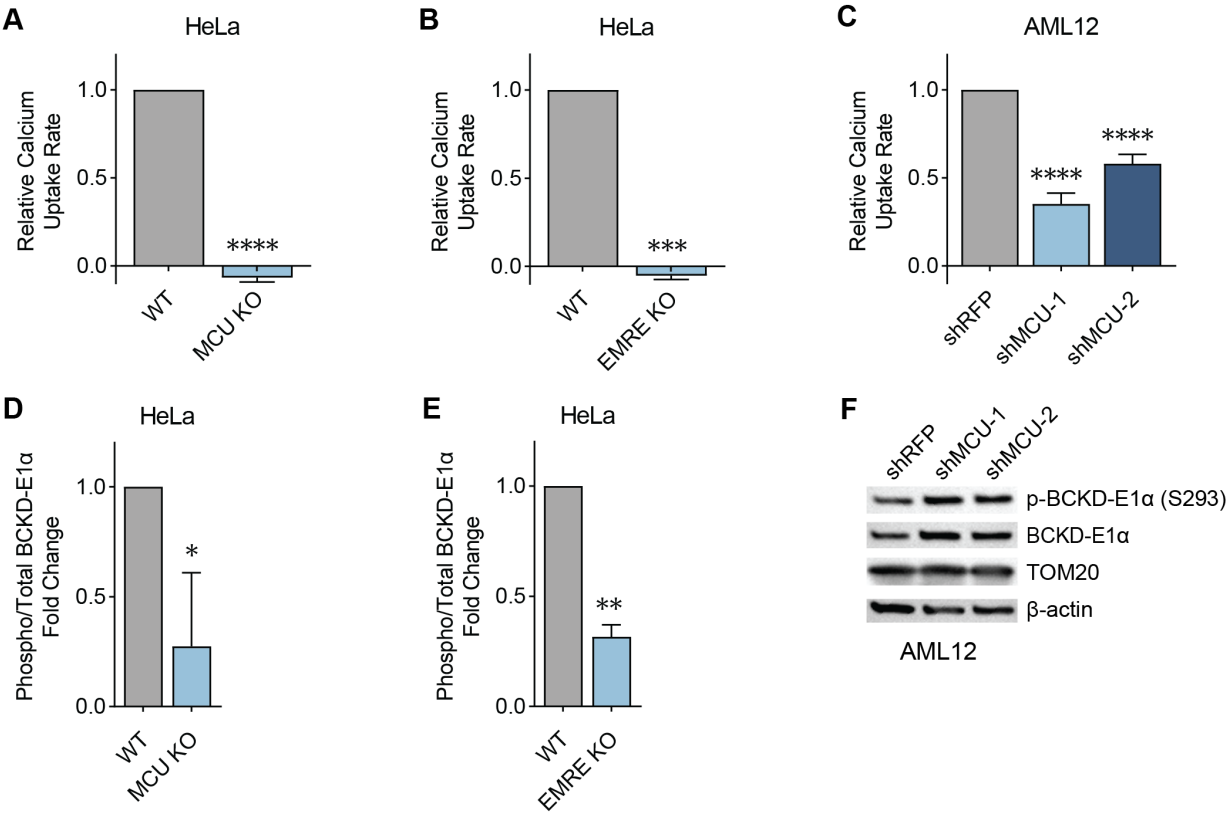
Supplemental Figure 1:



Supplemental Figure 1: (A-G) Seahorse extracellular flux analysis in WT, MCU KO, and MCU rescue HeLa cells; n=24-28; oxygen consumption rate at baseline and after indicated treatments are shown in (A);

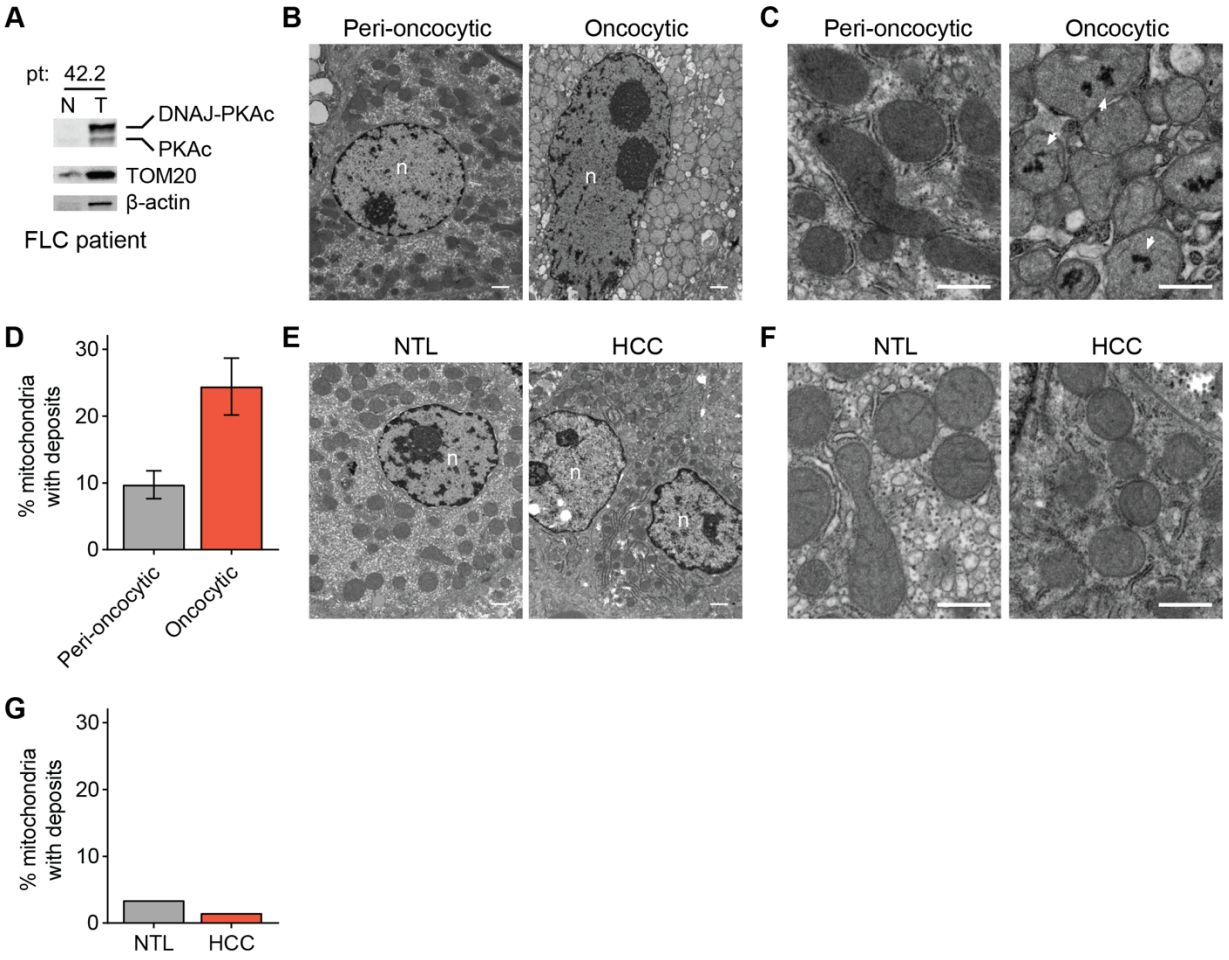
indicated mitochondrial parameters are shown in (B-G). Statistical significance was determined by the Tukey-Kramer test following one-way ANOVA; n=24-28. **(H-I)** Gene Set Enrichment Analysis of mitochondrial proteins (H) or RNAs coding for mitochondrial proteins (I) that show a statistically significant increase in MCU KO cells compared to WT cells. **(J, K)** Relative abundance of fatty acids (J) and acylcarnitines (K) in WT and MCU KO HeLa cells; loss of MCU decreases steady state levels of very long chain fatty acids, but increases acylcarnitines, suggesting activation of the mitochondrial FAO pathway. Error bars indicate standard deviation; * indicates a p-value < 0.05, ** indicates a p-value < 0.01.

Supplemental Figure 2:



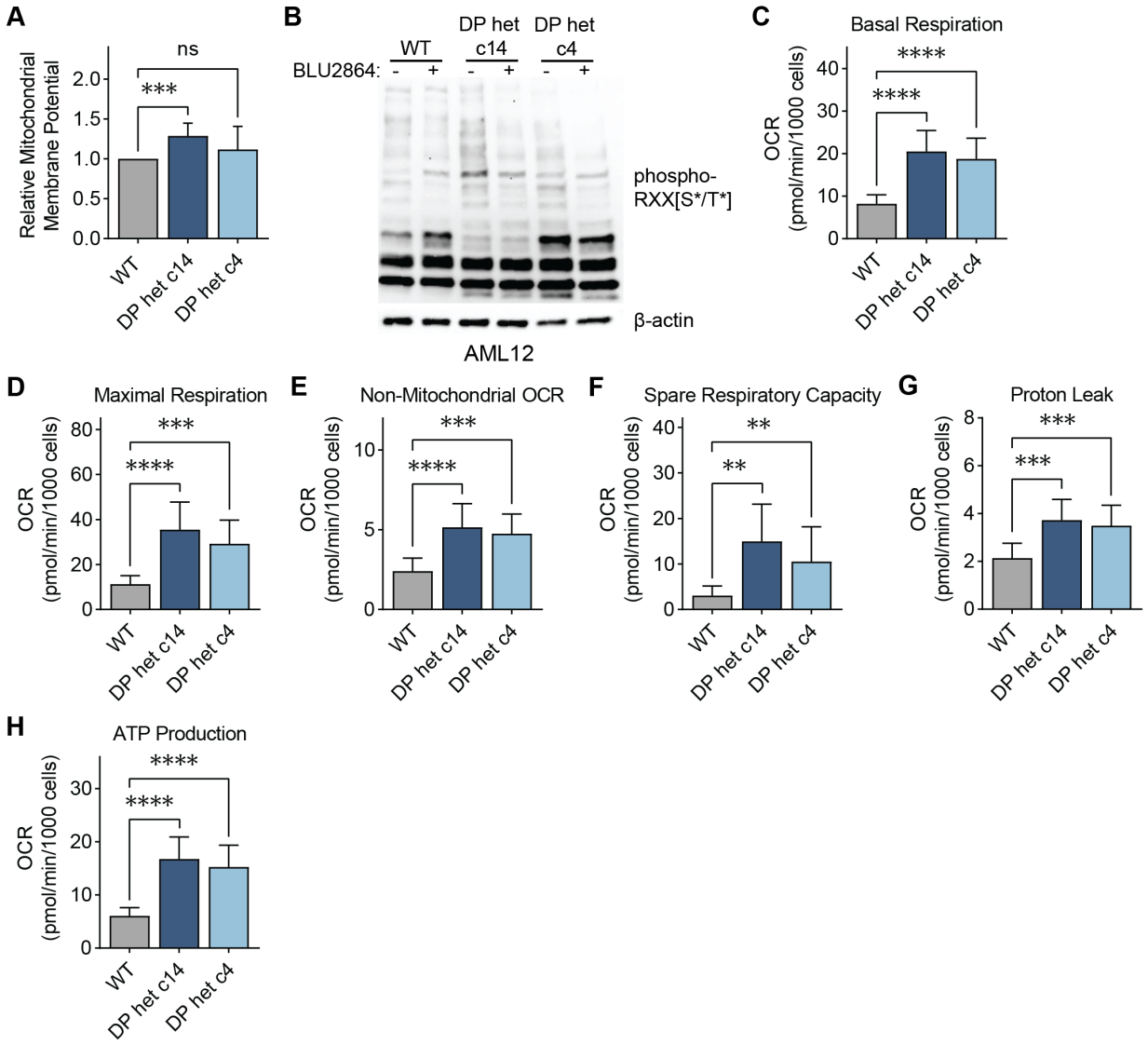
Supplemental Figure 2: (A-B) Mitochondrial Ca^{2+} uptake rates in MCU KO (A), EMRE KO (B) cells relative to WT controls are shown; n=3. **(C)** Mitochondrial Ca^{2+} uptake rates following MCU knockdown compared to control RFP knockdown in AML12 cells; n=8. **(D, E)** Quantification of immunoblots in Figure 2G (D) and Fig 2H (E) shown as the relative abundance of phosphorylated BCKD-E1α to total BCKD-E1α; n=3. **(F)** Immunoblots of phosphorylated and total BCKD-E1α in AML12 cells with or without MCU knockdown. Error bars indicate standard deviation. * indicates a p-value < 0.05, ** indicates a p-value < 0.01, *** indicates a p-value < 0.001, and **** indicates a p-value < 0.0001.

Supplemental Figure 3:



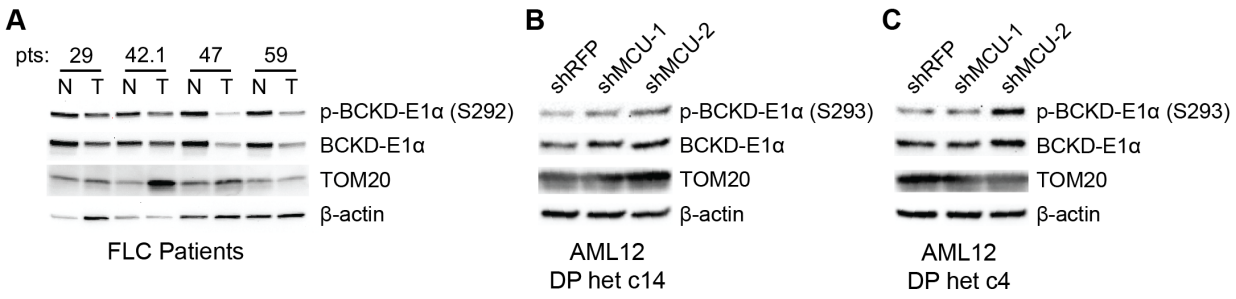
Supplemental Figure 3: (A) Immunoblot of tumor (T) and non-tumor liver (N) samples from FLC patient 42.2 showing fusion protein expression. **(B)** Electron micrographs at 10,000x magnification of oncocytic liver cells and proximal cells from the tumor border (peri-oncocytic) of FLC Patient 42.2; normal tumor sample was not dissected in this surgery; scale bar = 1 μm; nuclei are labeled n. **(C)** Electron micrographs of samples shown in (B) at 25,000x magnification; white arrowheads mark representative Ca^{2+} deposits in the oncocytic cells; scale bar = 600 nm. **(D)** Percentage of mitochondria with Ca^{2+} deposits in EM samples shown in (C); the mean is reported from manual counting of >500 mitochondria per sample by two independent, blinded analysts; error bars indicate standard deviation. **(E)** Electron micrographs at 10,000x magnification of non-tumor (NTL) and tumor sections from HCC patient 7; scale bar = 1 μm; nuclei are labeled n. **(F)** Electron micrographs of samples shown in (F) at 25,000x magnification; scale bar = 600 nm. **(G)** Percentage of mitochondria with Ca^{2+} deposits in EM samples shown in (F); >100 mitochondria per sample were quantified by an independent, blinded analyst

Supplemental Figure 4:



Supplemental Figure 4: (A) Resting mitochondrial membrane potential measured by the difference in TMRM fluorescence before and after CCCP addition, normalized to WT AML12 cells. **(B)** Immunoblot of AML12 lysates with a PKA substrate motif antibody after 5 μM BLU2864 or DMSO treatment for 4 days. **(C-G)** Indicated mitochondrial parameters of AML12 cells from Seahorse extracellular flux analysis in Figure 4L; statistical significance determined by the Dunnett test following Welch's one-way ANOVA; n=10-16. All error bars indicate standard deviation; ns indicates non-significant, ** indicates a p-value < 0.01, *** indicates a p-value < 0.001, and **** indicates a p-value < 0.0001

Supplemental Figure 5:



Supplemental Figure 5: (A) Immunoblots of phosphorylated and total BCKD-E1α in non-tumor (N) and tumor (T) lysates from FLC patients. **(B, C)** Immunoblots of phosphorylated and total BCKD-E1α in c14 (B) and c4 (C) after MCU knockdown.

Supplemental Table 1. Patient Information

Patient ID	Diagnosis	Age	Sex
7	Hepatocellular carcinoma; history of HCV	70	Female
9	Fibrolamellar carcinoma	27	Male
17	Fibrolamellar carcinoma	14	Female
29	Fibrolamellar carcinoma	20	Male
42.1*	Fibrolamellar carcinoma	26	Male
42.2*	Fibrolamellar carcinoma	27	Male
47	Fibrolamellar carcinoma	26	Male
58	Fibrolamellar carcinoma	18	Female
59	Fibrolamellar carcinoma	18	Female

*Patient 42.1 and 42.2 refer to the same individual; the latter resection was performed following tumor recurrence.