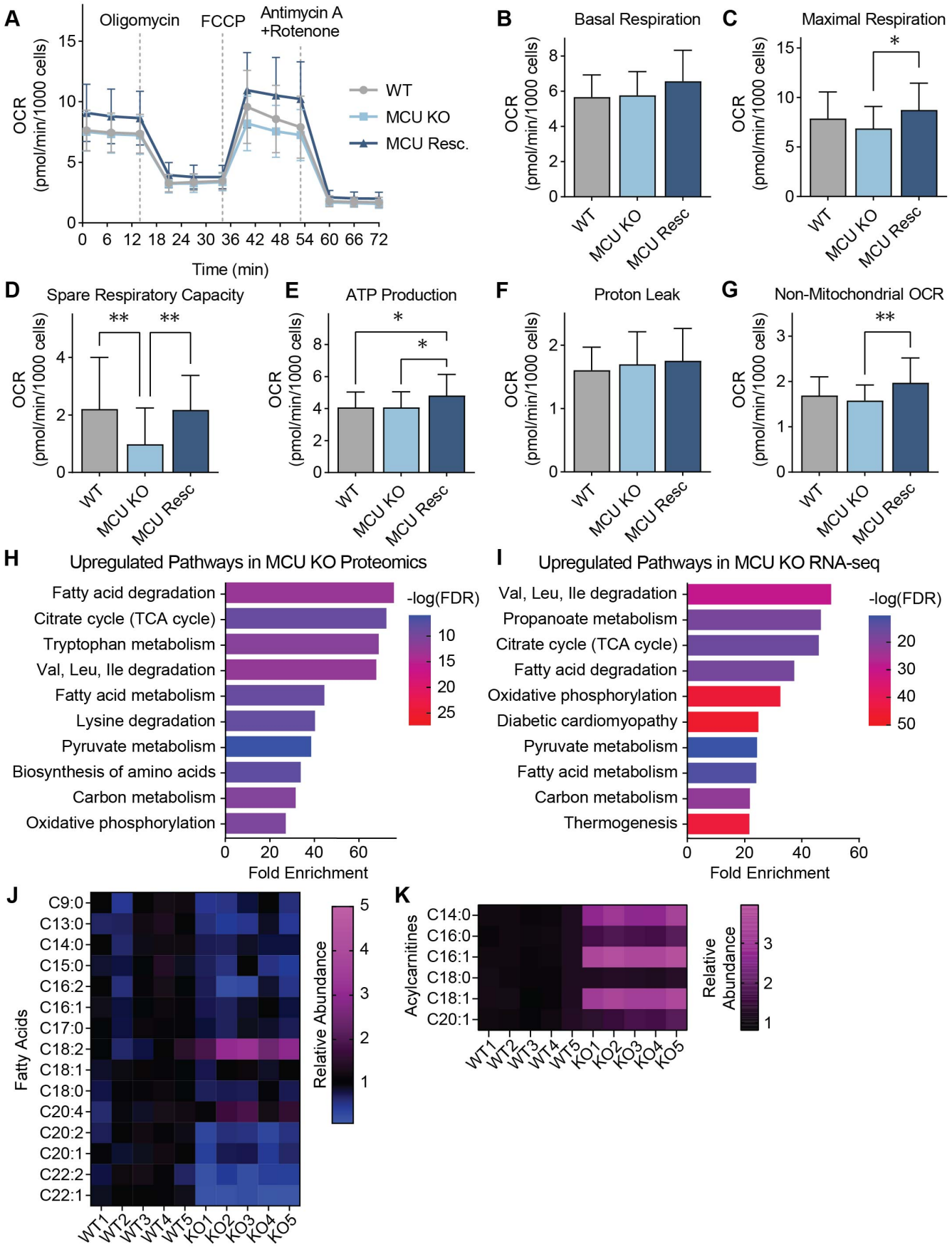
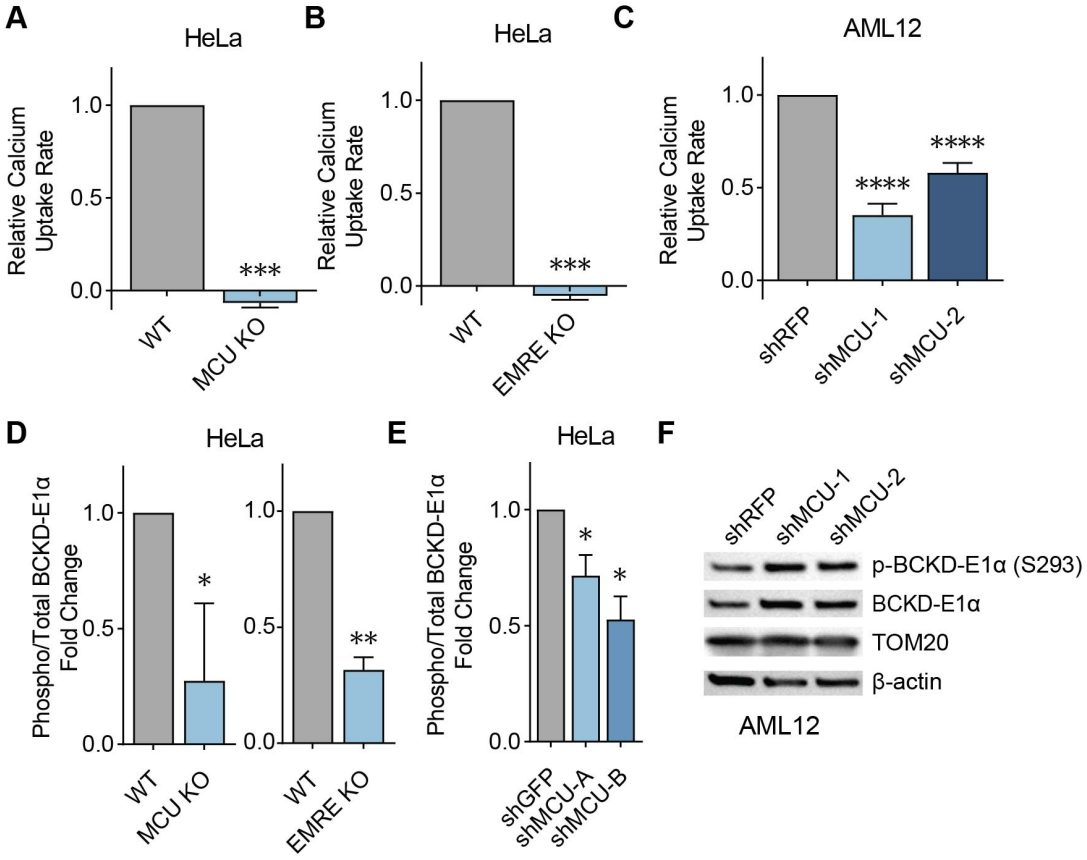
SUPPLEMENTAL FIGURE 1



Supplemental Figure 1

(A-G) Seahorse extracellular flux analysis in WT, MCU KO, and MCU rescue HeLa cells. Oxygen consumption rates 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 mRNAs 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. All error bars indicate standard deviation; * indicates a p-value < 0.05 and ** indicates a p-value < 0.01.

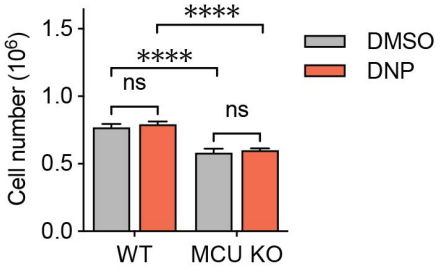
SUPPLEMENTAL FIGURE 2



Supplemental Figure 2

(A,B) Mitochondrial Ca2+ uptake rates in MCU KO (A) and EMRE KO (B) cells relative to WT controls are shown; n=3. **(C)** Mitochondrial Ca2+ uptake rates following MCU knockdown compared to control RFP knockdown in AML12 cells; n=8. **(D)** Quantification of immunoblots in Fig. 2E shown as the relative abundance of phosphorylated BCKD-E1α to total BCKD-E1α; n=3. **(E)** Quantification of immunoblots in Fig. 2F 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. All 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.

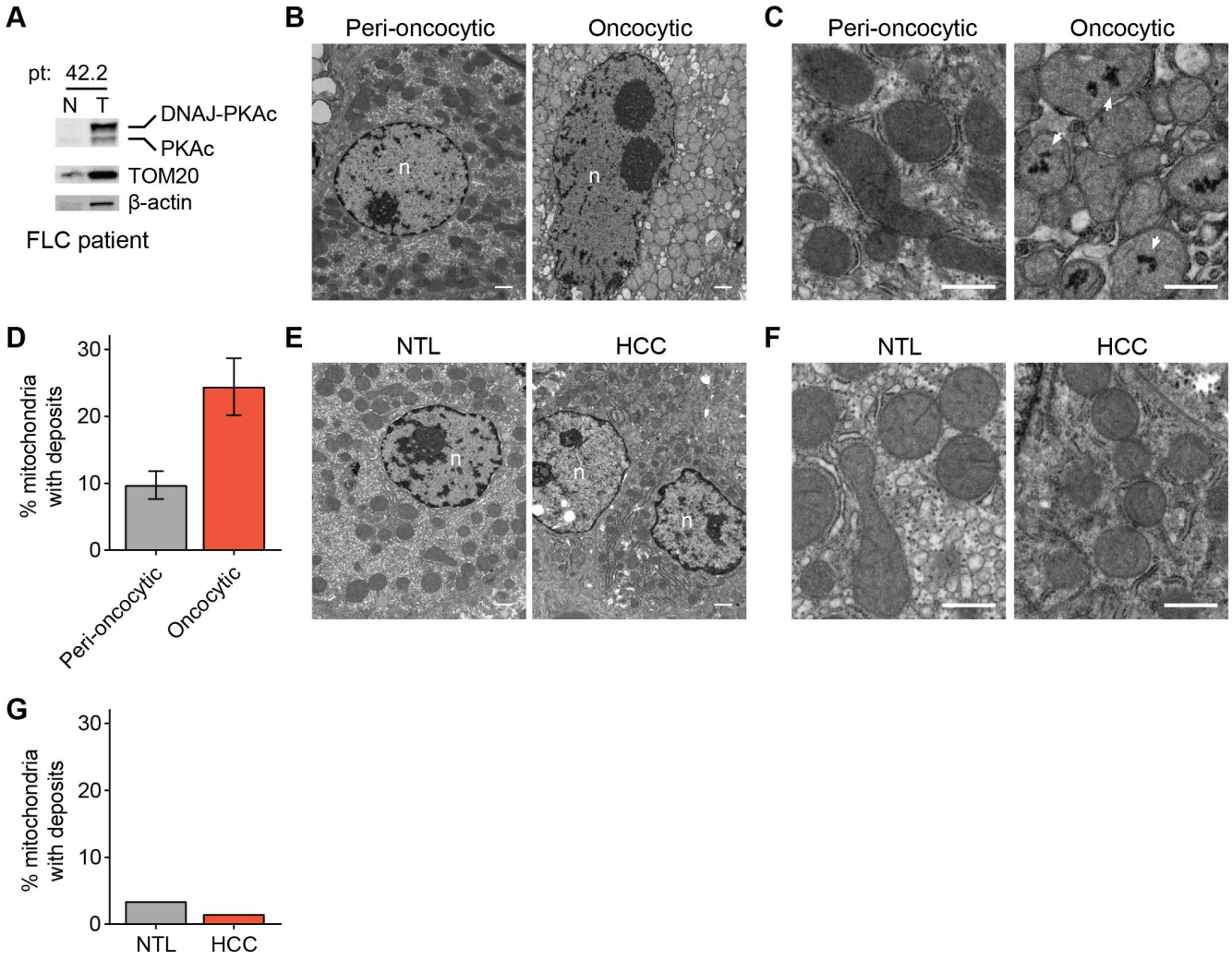
SUPPLEMENTAL FIGURE 3



Supplemental Figure 3

Cell numbers of WT and MCU KO cells with and without 2 M μDNP. Cells were counted three days after plating and treatment; n=3. Statistical significance was determined by Dunnett's multiple comparisons test following one-way ANOVA

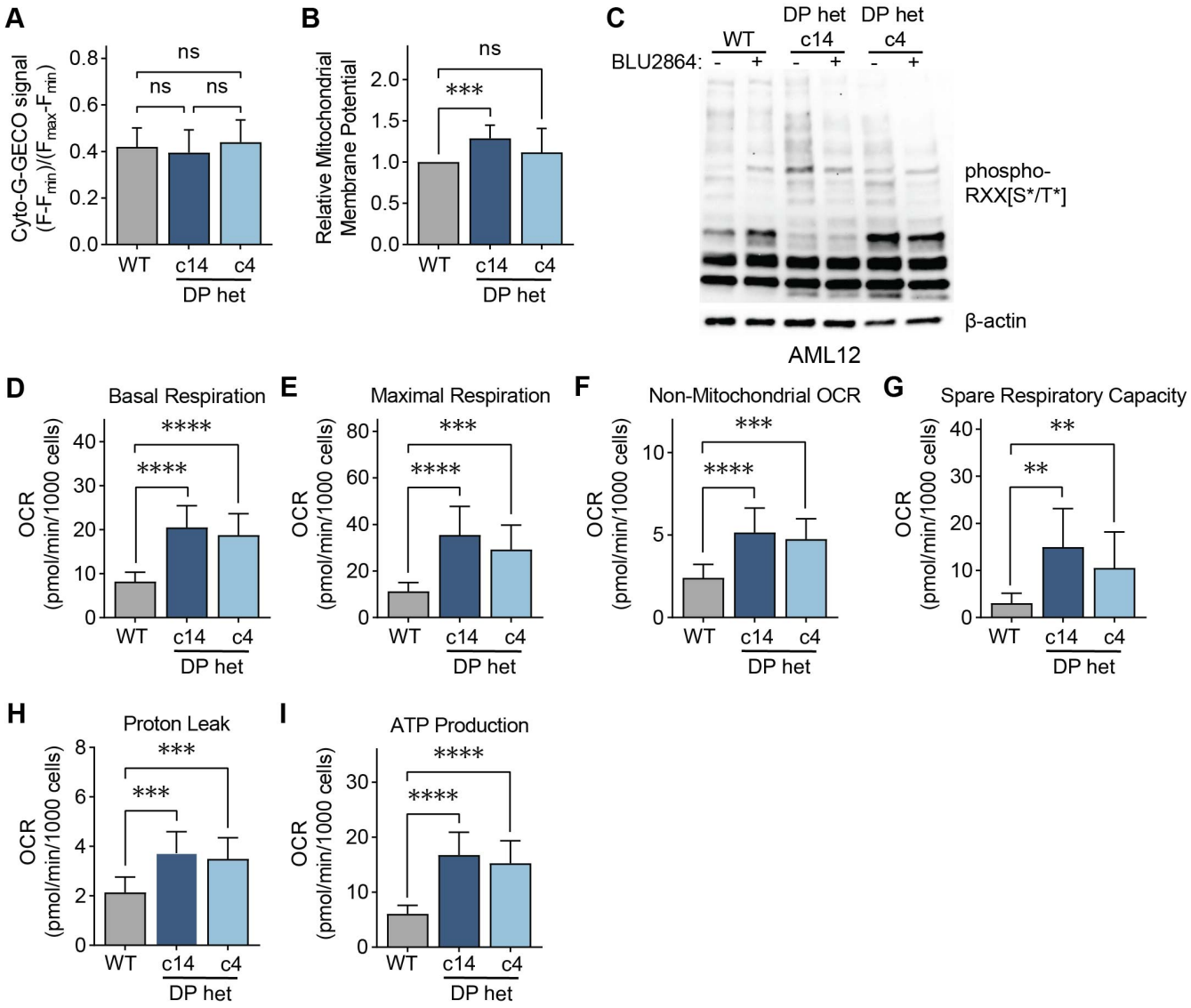
SUPPLEMENTAL FIGURE 4



Supplemental Figure 4

(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 (peri-oncocytic) cells from the tumor periphery 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 Ca2+ 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 (HCC) sections from HCC patient 7; scale bar = 1 μm; nuclei are labeled n. **(F)** Electron micrographs of samples shown in (E) 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.

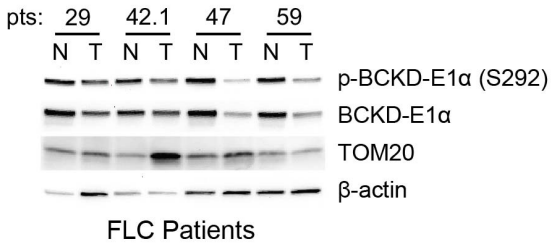
SUPPLEMENTAL FIGURE 5



Supplemental Figure 5

(A) Baseline cyto-G-GECO fluorescence normalized to minimum and maximum signals in AML12 WT, c14, and c4 cells; statistical significance was determined by one-way ANOVA; n=11-12. **(B)** Resting mitochondrial membrane potential was measured by the difference in TMRM fluorescence before and after CCCP addition, normalized to WT AML12 cells. **(C)** Immunoblot of AML12 lysates with a phospho-PKA substrate motif antibody after 5 μM BLU2864 or DMSO treatment for 4 days. **(D-I)** Indicated mitochondrial parameters of AML12 cells from Seahorse extracellular flux analysis shown in Fig. 5I; statistical significance was 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 6



Supplemental Figure 6

Immunoblots of phosphorylated and total BCKD-E1α in non-tumor (N) and tumor (T) lysates from FLC patients.

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.