

Supplementary Information

Low Carbohydrate High Fat Diet Curbs Glioblastoma by Shifting Lipids and Limiting Cancer Stem Cell Metabolic Flexibility

Shrita Sarkar,^{a§} Akash Ashokan,^{a,d§} Bapurao Surnar,^{a§} Bhabatosh Banik,^a Daniel G. Eichberg,^b Stefano Lepore,^d Lia Ficarò,^e Tatiana I. Slepak,^b Ricardo J. Komotar,^{b,d} Drew R. Jones,^e Daniel C. Bilbao,^{d,f} Eléonore Beurel,^{a,c} Michael E. Ivan,^{b,d} and Shanta Dhar^{a,d*}

^aBiochemistry and Molecular Biology, Miller School of Medicine, University of Miami, Miami, FL 33136.

^bDepartment of Neurological Surgery, Miller School of Medicine, University of Miami, Miami, FL, USA.

^cDepartment of Psychiatry and Behavioral Sciences, Miller School of Medicine, University of Miami, Miami, FL, USA.

^dSylvester Comprehensive Cancer Center, Miller School of Medicine, University of Miami, Miami, FL 33136.

^eDepartment of Biochemistry and Molecular Pharmacology, NYU Langone Medical Center, 435 E 30th St, New York, NY 10016, USA.

^fDepartment of Pathology and Laboratory Medicine, University of Miami School of Medicine, Miami, FL 33136

[§]*Equally contributed authors*

*Correspondence: shantadhar@med.miami.edu

Materials

All chemicals mentioned in the study were used without further purification unless stated otherwise. Phosphate buffered saline (1X PBS) (10010-023) for cell culture and Dulbecco's Modified Eagle Medium:Nutrient Mixture F-12 (DMEM/F-12) for N2 supplement (21331020) were purchased from Gibco. Epidermal growth factor (EGF) (315-09) and fibroblast growth factor (*b*FGF) (100-18B) were purchased from Peprotech. Human recombinant insulin (91077C-100MG), bovine serum albumin fraction V, fatty acid free (126575-10GM), apo-transferrin (T1147-500MG), progesterone (P8783-1G), putrescine (P5780-5G), sodium selenite (S9133-1MG), accutase (A6964-100ML), laminin (L2020), 2',7'-Dichlorofluorescein diacetate (DCFDA) (D6883), goat serum (G9023), D-(+)-glucose (G8270), oligomycin A (O4876-25MG), 2-deoxyglucose (2-DG) (D8375), carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP) (C2920), antimycin-A (A8674-25MG), rotenone (R8875-1G), etomoxir (E1905), cerulenin (C2389-5MG), perhexiline (SML0120), temozolomide (T2577-25MG), bis-2-(5-phenylacetamido-1,3,4-thiadiazol-2-yl)ethyl sulfide (BPTES) (SML0601), mitochondrial pyruvate carrier inhibitor (UK5099) (PZ0160), protease inhibitor cocktail (PIC) (P8340-1ML), antibody against HADHA (HPA015536-100UL), triton-X-100 (X100-500ML), n-propylgallate (P3130), sodium citrate dihydrate (W302600-1KG-K), glycine (G8898-1KG), sodium dodecyl sulfate (SDS) (L3771-100G), tween-20 (P1379-1L), and Trizma[®] base (T6066-1KG) were purchased from Sigma-Aldrich. Neurocult NS-A basal medium (5750) was purchased from StemCell Technology. Molecular biology grade water (SH30538.03) was purchased from HyClone. B-27[™] Supplement (50X) (17504044), MitoSOX[™] (M36008), CellLight[™] Mitochondria-GFP, BacMam 2.0 (C10600) CellLight[™] Mitochondria-RFP, BacMam 2.0 (C10505), L-Glutamine (25030081), penicillin/streptomycin (10378016), sodium pyruvate (11360070), radioimmunoprecipitation assay (RIPA) buffer (89900), bicinchoninic acid (BCA) assay kit (23225), NucBlue[™] fixed cell ReadyProbes[™] reagent (DAPI) (R37606), fetal bovine serum (FBS) (26140079) of a specific lot number (2531710RP), rat tail collagen

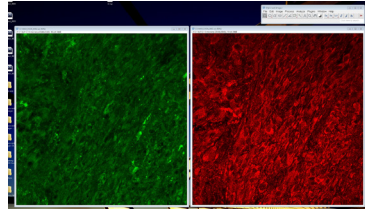
type I (A1048301), Countess™ cell counting chamber slides (C10228), 4% paraformaldehyde solution (J19943.K2), antibodies against CLOCK (PA1-520), and Prom1 (CD133) (MA1-219) were purchased from ThermoFisher Scientific. Primaria T25 (15705-068) and T75 (15705-070) tissue culture flasks were purchased from BD Primaria. Primary antibodies against Nestin (33475S), CD133 (64326S), vimentin (5741S), SOX2 (3579S), Ki-67 (9449), and Alexa Fluor® 555 secondary against rabbit (4413) were purchased from Cell Signaling Technology. Primary antibodies against FASN (ab128856), ACADM (ab110296), HKII (ab209847), GLUT1 (ab15309), nanog (ab109250), caspase 9 (ab202068), legumain (LGMN) (ab183028), cyclophilin (ab178397), β -actin (ab8226), HRP conjugated secondary against rabbit (ab205718), HRP conjugated secondary against mouse (ab205719), Alexa Fluor® 488 secondary against rabbit (ab150077), and Alexa Fluor® 488 secondary mouse (ab150113) were purchased from Abcam. The seahorse XF®96 FluxPak (102416-100) was purchased from Agilent. The Quick-DNA™ Fecal/Soil Microbe MiniPrep kit (D6010) was purchased from Zymo Research. The firefly luciferase lentifect™ purified lentiviral particles (LPP-HLUC-LV105-025-C) was purchased from GeneCopoeia. The astrocyte basal media and astrocytes SingleQuots™ kit (CC3186) and endothelial basal media (00190860) were purchased from Lonza. Cell strainers with pore size 100 μ m (10199-658) was purchased from VWR. Glycerol (A16205) was purchased from Alfa Aesar. The precast 4- 20% gradient gel (5671094), blotting grade blocker (1706404), Clarity™ western ECL substrate (1705061), precision plus protein dual color standard (1610374), and immune-blot PVDF membrane (1620177) were purchased from Bio-Rad. The male and female BALB/c athymic nude mice (Strain: 002019) were purchased from Jackson Laboratories. The standardized normal diet (2018) purified low fat diet (TD.08806), and purified low carbohydrate high fat diet (TD.06414) were purchased from Envigo.

Instruments

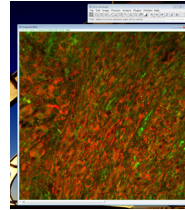
Water was purified by a Millipore Milli-Q Biocel water purification system (18.2 M Ω) containing a 0.22 μ m filter. Glycostress, mitostress, and fuel flex assays were carried out using a Seahorse XF®96 extra flux analyzer (Agilent Seahorse Biosciences, North Billerica, MA, USA). Confocal microscopy images

were obtained using an Olympus FluoView FV3000. IVIS images were obtained using the IVIS[®] Spectrum (In Vivo Imaging System) from PerkinElmer at the Cancer Modeling Shared Resource (CMSR) core facility of University of Miami Health System. The MRI images were obtained using a 9.4 Tesla BioSpec 94/20 USR, Bruker scanner. The ChemiDoc MP Imaging System from Bio-Rad was used to take the Western Blot images. Nanodrop was used to quantify the fecal DNA and RNA content and to check its purity. Open Field Test was performed using a Photobeam Activity System. The whole brain imaging was performed using the Olympus VS120 microscope at the core facility of University of Miami. The BioTek Synergy HT microplate reader was used for reading absorbance and fluorescence signals in 96-well cell plates. Cells were counted using the Invitrogen Countess[®] Automated Cell Counter. The stereotactic intracranial injection was performed using the digital stereotactic instrument setup from Stoelting. Decloaking chamber from Biocompare Medical was used for antigen retrieval step of immunofluorescence/immunocytochemistry. Keto-Mojo[®] GK+ blood glucose and β -ketone dual monitoring system was used to measure glucose and β -ketone levels in mice.

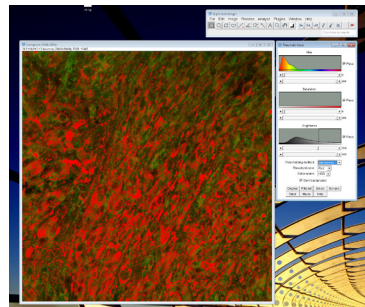
Step 1: Splitting red and green channels



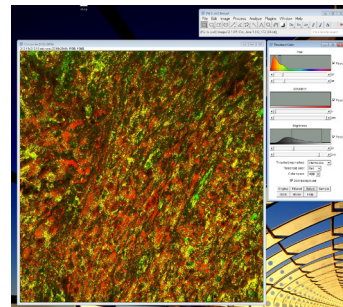
Step 2: Merging red and green channels



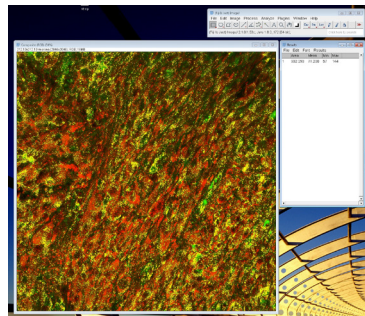
Step 3: Converting to RGB type and selecting color threshold



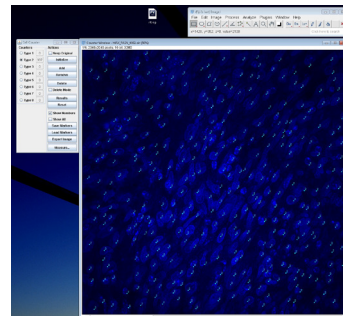
Step 4: Selecting yellow region from color threshold for red intensity in green channel



Step 5: Mean intensity measurement for red in green channel



Step 6: Manual counting of nuclei using cell counter plugin in ImageJ



Scheme 1: Quantification of the dual stained immunofluorescence images. Images showing the steps that were followed to quantify the dual stained immunofluorescence images for measuring red intensity in green channel. ImageJ software with Olympus viewer and cell counter plugins was used.

Table S1. Status of the clinical trials with diet supplementation in GBM.

Table S1. Current status of the clinical trials with diet supplement in GBM				
NCT Number	Clinical Trial Phase	Purpose	Intervention	Outcome
NCT03278249	NA	Active, not recruiting	Treatment with modified Atkins ketogenic diet in newly diagnosed malignant glioma.	Information related to the study was submitted to ClinicalTrials.gov but not available to public yet.
NCT01865162	Phase 1	Completed (Early termination due to slow recruiting and a smaller number of patients)	Safety and tolerability of ketogenic diet as an adjunct treatment for recurrent GBM.	Total 8 participants: 4 newly diagnosed and 4 recurrent patients. 4/4 in Group 1 and 1/4 in Group 2 completed 6 months under ketogenic diet with no AEs. In Group 2, 3/4 patients stopped diet due to GBM progression (2/3) and diet restrictiveness (1/3). Mean survival time from diet initiation: Group 1: 20 months Group 2: 12.8 months Mean survival time from diagnosis: Group 1: 21.8 months Group 2: 25.4 months Remarkable outcome: 1 patient from recurrent group 2 on bevacizumab experienced symptom reversal, tumor reduction, edema resolution.
NCT02302235	Phase 2	Completed	Evaluation of efficacy, safety, and tolerability of ketogenic diet as an adjunct to radiation therapy and chemotherapy in patients with GBM.	No results posted about the outcomes of 42 enrolled participants
NCT04691960	Phase 2	Recruiting	Tolerability of ketogenic diet adjunct with metformin.	No results posted
NCT03451799	Phase 1	Active, not recruiting	Primary outcome measures include safety evaluation. Secondary outcomes include feasibility of the diet, effect of diet on overall survival, progression time, quality of life, and cognitive functions in the patients with newly/recently diagnosed glioblastoma.	No results posted yet
NCT04730869	NA	Recruiting	Primary outcomes include study of daily blood glucose-to-ketone ration to investigate extent of ketosis in the patients.	No results posted yet
NCT00575146	Phase 1	Completed	Primary outcomes include investigation of tolerability of the ketogenic diet. Secondary outcomes include progression free survival, overall survival, frequency of seizures, measurement of ketosis, and quality of life.	Diet discontinuation: 3/20 patients, for poor tolerability. No serious AEs was observed. Urine ketosis achieved in 12/13 patients who were evaluable. Median progression free survival: 5 weeks Median survival from enrollment: 32 weeks. Median progression free survival for patients with bevacizumab treatment: 6/7 patients for 20.1 weeks.
NCT01754350	NA	Completed	Randomized clinical trial on clinically restricted ketogenic diet and intermittent fasting in combination with radiation therapy for recurrent GBM. Primary outcome includes progression free survival for 6 months. Secondary outcomes include progression free survival, overall survival, frequency of epileptic seizures, rate of ketosis, and quality of life.	Out of 20 patients, 17 patients developed ketosis at 6 days, glucose levels declined. No severe adverse effects. No significant changes in progression free survival.

Table S2. Detailed composition of normal diet (ND) and low carbohydrate high fat diet (LCHFD) used in the study.

Table S2. Composition of Normal Diet (ND) and Low Carbohydrate High Fat Diet (LCHFD) used in Our Study									
Normal Diet (ND)									
Macronutrients		Fatty Acids		Amino Acids		Vitamins		Minerals	
Crude Protein	18.6%	Palmitic Acid	0.7%	Aspartic Acid	1.4%	Vitamin A	15.0 IU/g	Calcium	1.0%
Fat (ether extract)	6.2%	Stearic Acid	0.2%	Glutamic Acid	3.4%	Vitamin D	1.5 IU/g	Phosphorus	0.7%
Carbohydrate (available)	44.2%	Oleic Acid	1.2%	Alanine	1.1%	Vitamin E	110 IU/g	Non-Phytate Phosph	0.4%
Crude Fiber	3.5%	Linolenic Acid	0.3%	Glycine	0.8%	Vitamin K3	50 mg/kg	Sodium	0.2%
Neutral Detergent Fiber	14.7%	Linoleic Acid	3.1%	Threonine	0.7%	Vitamin B1	17 mg/kg	Potassium	0.6%
Ash	5.3%	Total saturated fatty acids	0.9%	Proline	1.6%	Vitamin B2	15 mg/kg	Chloride	0.4%
Energy Density	3.1 kcal/g	Total monounsaturated fatty acids	1.3%	Serine	1.1%	Niacin	70 mg/kg	Magnesium	0.2%
Calories from Protein	24%	Total polyunsaturated fatty acids	3.4%	Leucine	1.8%	Vitamin B6	18 mg/kg	Zinc	70 mg/kg
Calories from Fat	18%			Isoleucine	0.8%	Pantothenic Acid	33 mg/kg	Manganese	100 mg/kg
Calories from Carbohydrate	58%			Valine	0.9%	Vitamin B12	0.08 mg/kg	Copper	15 mg/kg
				Phenylalanine	1.0%	Biotin	0.4 mg/kg	Iodine	6 mg/kg
				Tyrosine	0.6%	Folate	4 mg/kg	Iron	200 mg/kg
				Methionine	0.4%	Choline	1200 mg/kg	Selenium	0.23 mg/kg
				Cystine	0.3%				
				Lysine	0.9%				
				Histidine	0.4%				
				Arginine	1.0%				
				Tryptophan	0.2%				
Low Carbohydrate High Fat Diet (LCHFD)									
Macronutrients		Fatty Acids		Amino Acids		Vitamins		Minerals	
Protein	23.5%	Total Fat	342.7 g/kg	Aspartic Acid	15.9 g/kg	Vitamin A	8400 IU/kg	Calcium	7.9 g/kg
Carbohydrate	27.3%	Saturated Fat	119.2 g/kg	Glutamic Acid	8.2 g/kg	Vitamin D	2100 IU/kg	Phosphorus	4.8 g/kg
Fat	32.3%	Monounsaturated Fat	134.0 g/kg	Alanine	6.9 g/kg	Vitamin E	158 IU/kg	Sodium	1.4 g/kg
Fiber	6.6%	Polyunsaturated Fat	77.2 g/kg	Glycine	4.2 g/kg	Vitamin K	1.6 mg/kg	Potassium	4.9 g/kg
Energy Density	5.1 kcal/g	Butyric acid	0 g/kg	Threonine	10.1g/kg	Biotin	0.42 mg/kg	Chloride	2.2 g/kg
Calories from Protein	18.3%	Caproic acid	0 g/kg	Proline	23.9 g/kg	Choline	1377 mg/kg	Magnesium	0.711 g/kg
Calories from Carbohydrate	21.4%	Caprylic acid	0 g/kg	Serine	13.3 g/kg	Folic Acid	4.2 mg/kg	Zinc	56.7 mg/kg
Calories from Fat	60.3%	Capric acid	0 g/kg	Leucine	21.2 g/kg	Niacin	63 mg/kg	Manganese	14.5 mg/kg
		Lauric acid	0 g/kg	Isoleucine	13.3 g/kg	Pantothenate	30.8 mg/kg	Copper	8.5 mg/kg
		Myristic acid	3.1 g/kg	Valine	15.9 g/kg	Riboflavin	12.6 mg/kg	Iodine	0.28 mg/kg
Casein	265 g/kg	Palmitic acid	74.6 g/kg	Phenylalanine	11.7 g/kg	Thiamin	10.2 mg/kg	Iron	50.8 mg/kg
L-Cystine	4.0 g/kg	Palmitoleic acid	6.2 g/kg	Tyrosine	12.2 g/kg	Vitamin B6	12.1 mg/kg	Selenium	0.21 mg/kg
Maltodextrin	160 g/kg	Stearic acid	41.5 g/kg	Methionine	6.1 g/kg	Vitamin B12	0.05 mg/kg	Molybdenum	0.21 mg/kg
Sucrose	90 g/kg	Oleic acid	127.8 g/kg	Cystine	4.7 g/kg	Vitamin C	0 mg/kg	Chromium	1.37 mg/kg
Lard (Pig Fat)	310 g/kg	Linoleic acid	71.7 g/kg	Lysine	18.6 g/kg				
Soybean Oil	30 g/kg	Linolenic acid	5.5 g/kg	Histidine	6.6 g/kg				
Cellulose	65.5 g/kg	Saturated Fat (% of fat)	36.1%	Arginine	8.7 g/kg				
Mineral Mix (AIN-93G-MX)	48.0 g/kg	Monounsaturated Fat (% of fat)	40.6%	Tryptophan	2.7 g/kg				
Calcium Phosphate, dibasic	3.4 g/kg	Polyunsaturated Fat (% of fat)	23.4%						
Vitamin Mix (AIN-93-VX)	21 g/kg								
Choline Bitartrate	3.0 g/kg								
Cholesterol	347.5 g/kg								

Table S3. Detailed composition of low fat diet (LFD) used in a pilot study.

Table S3. Composition of Low Fat Diet (LFD) used in Our Study									
Low Fat Diet (LFD)									
Macronutrients		Fatty Acids		Amino Acids		Vitamins		Minerals	
Protein	18.6%	Total Fat	42.1 g/kg	Aspartic Acid	12.6 g/kg	Vitamin A	6000 IU/kg	Calcium	5.6 g/kg
Carbohydrate	62.6%	Saturated Fat	10.4 g/kg	Glutamic Acid	38.2 g/kg	Vitamin D	1500 IU/kg	Phosphorus	3.5 g/kg
Fat	4.2%	Monounsaturated Fat	12.8 g/kg	Alanine	5.5 g/kg	Vitamin E	113 IU/kg	Sodium	1 g/kg
Fiber	3.7%	Polyunsaturated Fat	40.8 g/kg	Glycine	3.4 g/kg	Vitamin K	1.1 mg/kg	Potassium	3.6 g/kg
Energy Density	3.6 kcal/g	Butyric acid	0 g/kg	Threonine	8.0 g/kg	Biotin	0.30 mg/kg	Chloride	1.6 g/kg
Calories from Protein	20.5%	Caproic acid	0 g/kg	Proline	18.9 g/kg	Choline	1262.3 mg/kg	Magnesium	0.519 g/kg
Calories from Fat	10.5%	Caprylic acid	0 g/kg	Serine	10.5 g/kg	Folic Acid	3 mg/kg	Zinc	41.9 mg/kg
Calories from Carbohydrate	69.1%	Capric acid	0 g/kg	Leucine	16.8 g/kg	Niacin	45 mg/kg	Manganese	10.5 mg/kg
		Lauric acid	0 g/kg	Isoleucine	10.5 g/kg	Pantothenate	22 mg/kg	Copper	6.2 mg/kg
Casein	210 g/kg	Myristic acid	0.2 g/kg	Valine	12.6 g/kg	Riboflavin	9 mg/kg	Iodine	0.21 mg/kg
L-Cystine	3.0 g/kg	Palmitic acid	6.8 g/kg	Phenylalanine	9.2 g/kg	Thiamin	7.3 mg/kg	Iron	37.1 mg/kg
Maltodextrin	100 g/kg	Palmitoleic acid	0.4 g/kg	Tyrosine	9.7 g/kg	Vitamin B6	8.6 mg/kg	Selenium	0.15 mg/kg
Sucrose	90 g/kg	Stearic acid	3.4 g/kg	Methionine	4.8 g/kg	Vitamin B12	0.04 mg/kg	Molybdenum	0.15 mg/kg
Lard (Pig Fat)	20 g/kg	Oleic acid	12.2 g/kg	Cystine	3.5 g/kg	Vitamin C	0 mg/kg	Chromium	1 mg/kg
Soybean Oil	20 g/kg	Linoleic acid	14.2 g/kg	Lysine	14.7 g/kg				
Cellulose	37.15 g/kg	Linolenic acid	1.8 g/kg	Histidine	5.3 g/kg				
Mineral Mix (AIN-93G-MX)	35 g/kg	Saturated Fat (% of fat)	26.5%	Arginine	6.9 g/kg				
Calcium Phosphate, dibasic	2 g/kg	Monounsaturated Fat (% of fat)	32.7%	Tryptophan	2.1 g/kg				
Vitamin Mix (AIN-93-VX)	15 g/kg	Polyunsaturated Fat (% of fat)	40.8%						
Choline Bitartrate	2.75 g/kg								
Cholesterol	61 g/kg								
Corn Starch	465 g/kg								

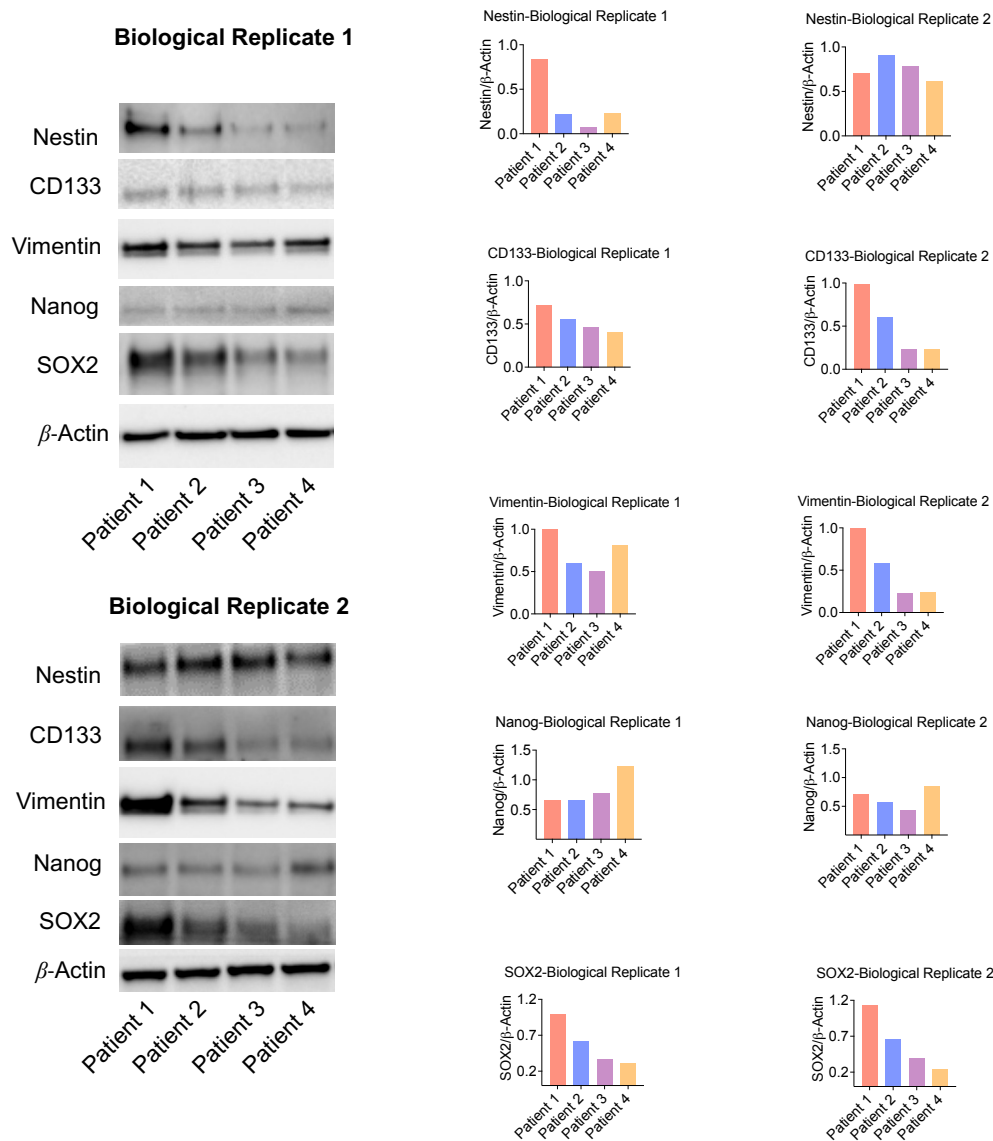


Figure S1. Expression of stemness marker proteins in patient derived GSCs. Western Blot quantification of stemness markers Nestin, CD133, Vimentin, Nanog, and SOX2 from two biological replicates in recurrent patients 1 and 2 compared to newly diagnosed patients 3 and 4. The quantification for each marker was performed with respect to the expression of β -Actin. All primary antibodies were used at a dilution of 1:1000 and the respective secondary antibodies were used at a dilution of 1:2000.

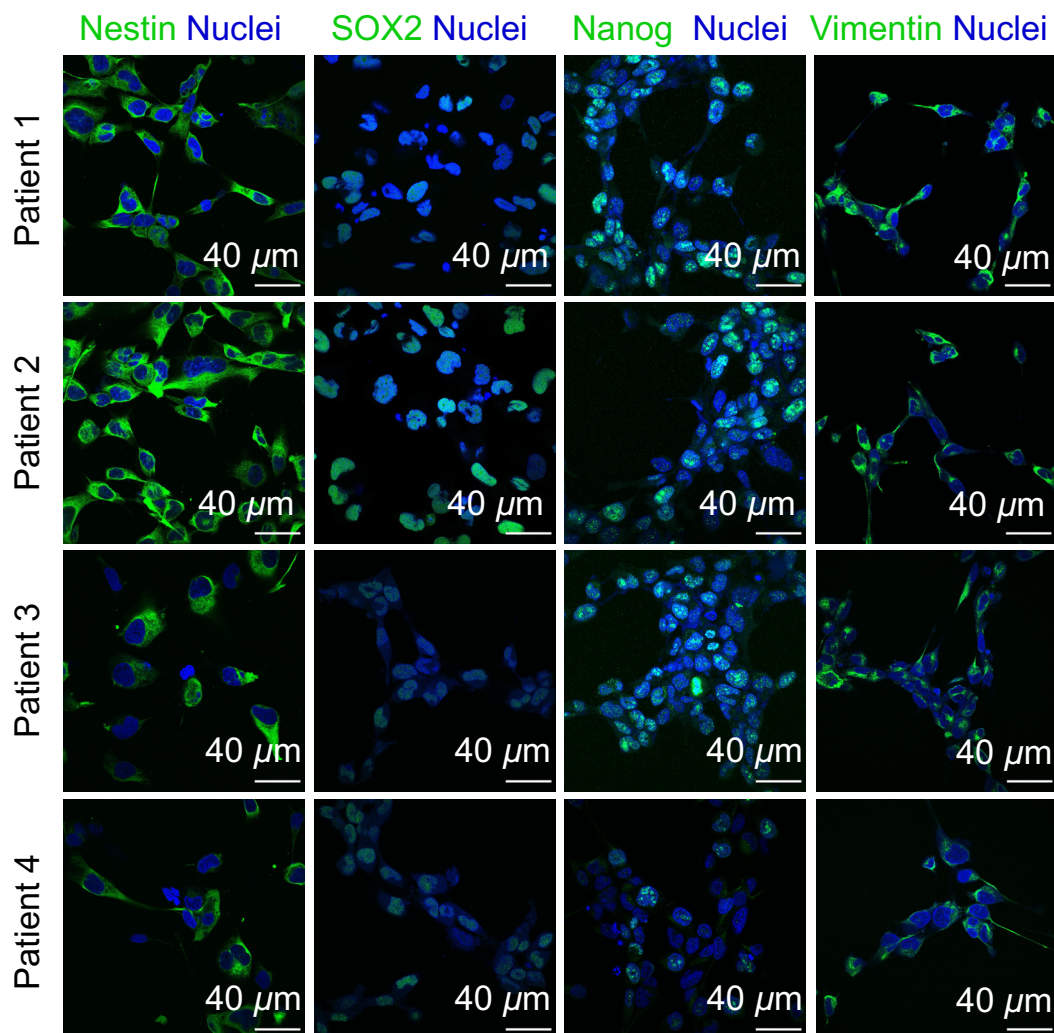


Figure S2. Immunofluorescence images showing the expression of stemness markers Nestin, SOX2, Nanog, and Vimentin in recurrent patients 1 and 2 compared to newly diagnosed patients 3 and 4. The images were taken at a scale of 40 μm . The primary antibodies for nestin, SOX2, nanog, and vimentin were used at a dilution of 1:1600, 1:400, 1:1000, and 1:200 respectively. The respective secondary antibody for each marker was used at a dilution of 1:200.

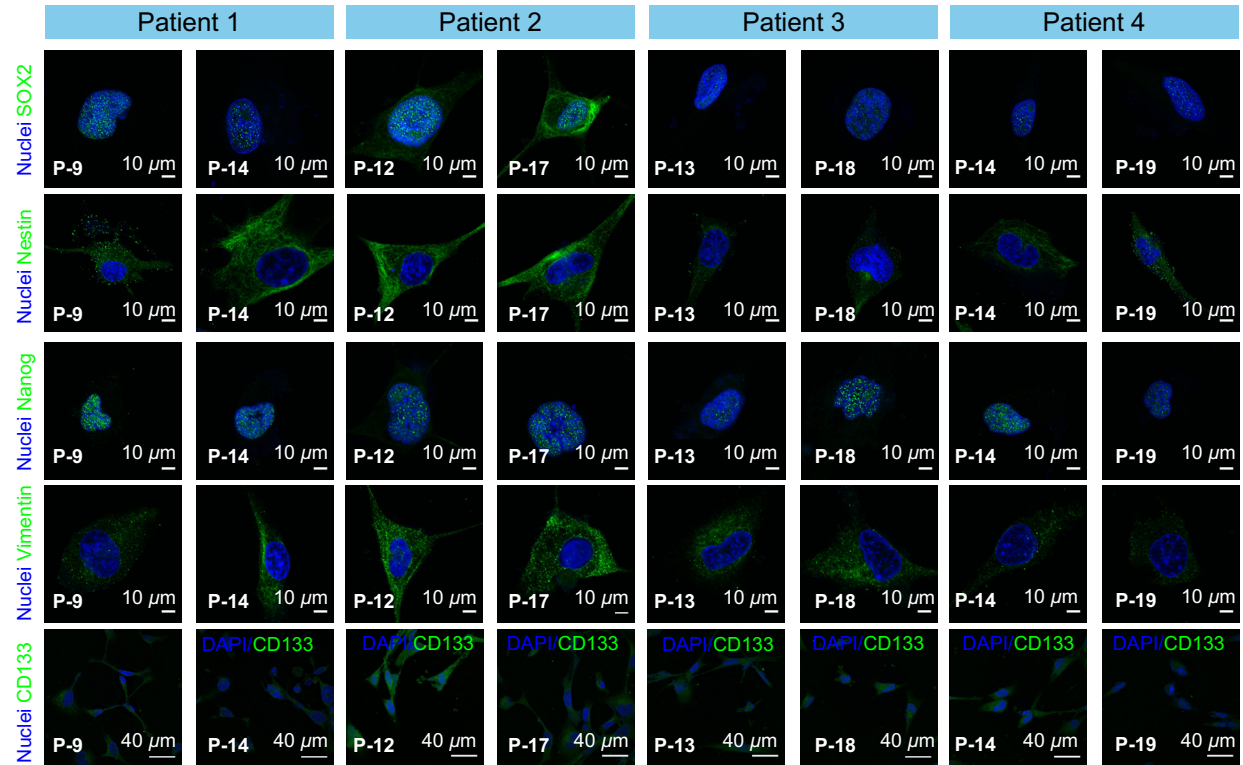


Figure S3. Immunofluorescence images showing the expression of the stemness markers SOX2, Nestin, Nanog, Vimentin and CD133 in the different patient derived GSCs from different passages of cell culture. The data suggested that the stemness marker profile remains unaltered at different passages if the growth conditions for the cells are maintained. Scale for images of SOX2, nestin, nanog, and vimentin is 10 μ m and for CD133 images the scale is 40 μ m. For patient 1 cells, passage 9 and 14 were analyzed. For patient 2 cells, passage 12 and 17 were analyzed. For patient 3 cells, passage 13 and 18 were analyzed. For patient 4 cells, passage 14 and 19 were analyzed.

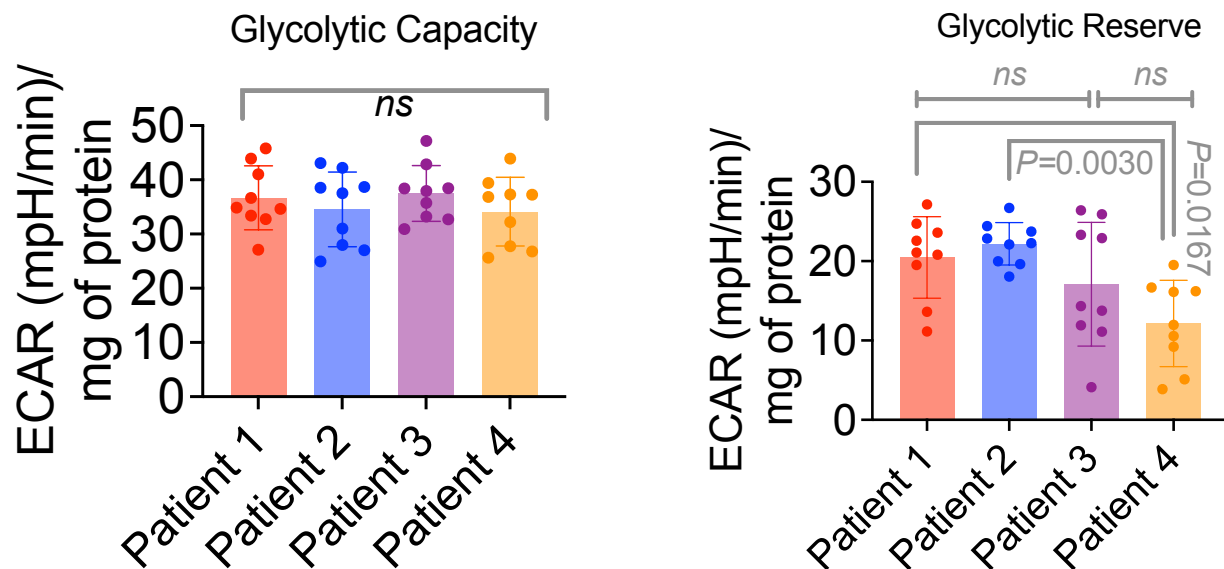


Figure S4. Glycolytic parameters in patient derived glioma stem cells. The seahorse glycostress assay was performed in the GSCs from recurrent patients 1 and 2 and newly diagnosed patients 3 and 4. The data revealed that the patient derived GSCs have low levels of glycolysis and there was a non-significant difference between their glycolytic capacity parameters and glycolytic reserve parameters. Glucose (10 mM), oligomycin (1 μ M), and 2-deoxyglucose (50 mM) were used as port injections. The data is represented from three biological replicates each with three technical replicates.

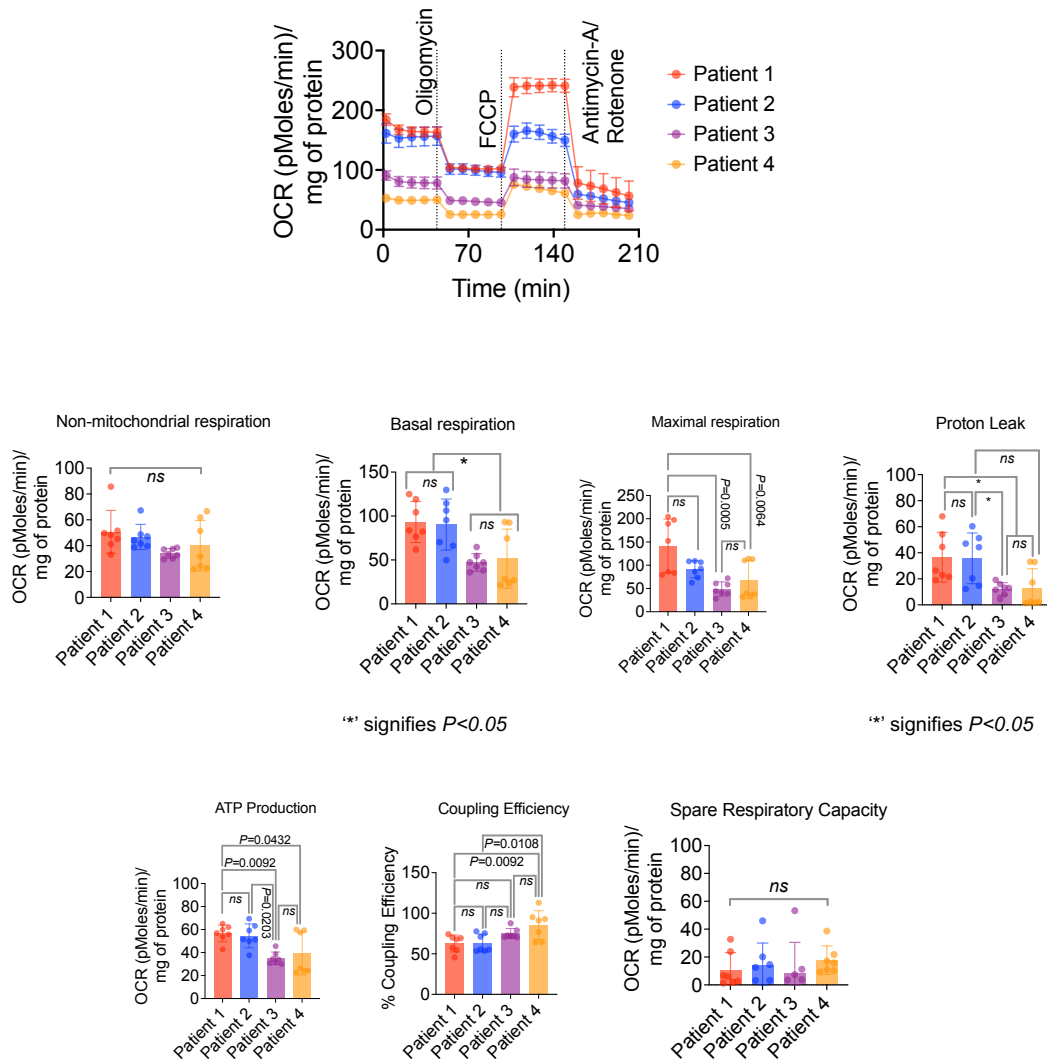


Figure S5. Oxidative phosphorylation analyses in patient derived glioma stem cells.

The seahorse mitostress assay was performed in the recurrent patients 1 and 2 GSCs and newly diagnosed patients 3 and 4. The data showed that the patient derived GSCs rely on mitochondrial respiration. Oligomycin ($1 \mu\text{M}$), FCCP ($1 \mu\text{M}$), and a combination of Antimycin-A and Rotenone ($1 \mu\text{M}$ each) were used as port injections. The above data is represented from two biological replicate studies, one with three technical replicates and another with four technical replicates.

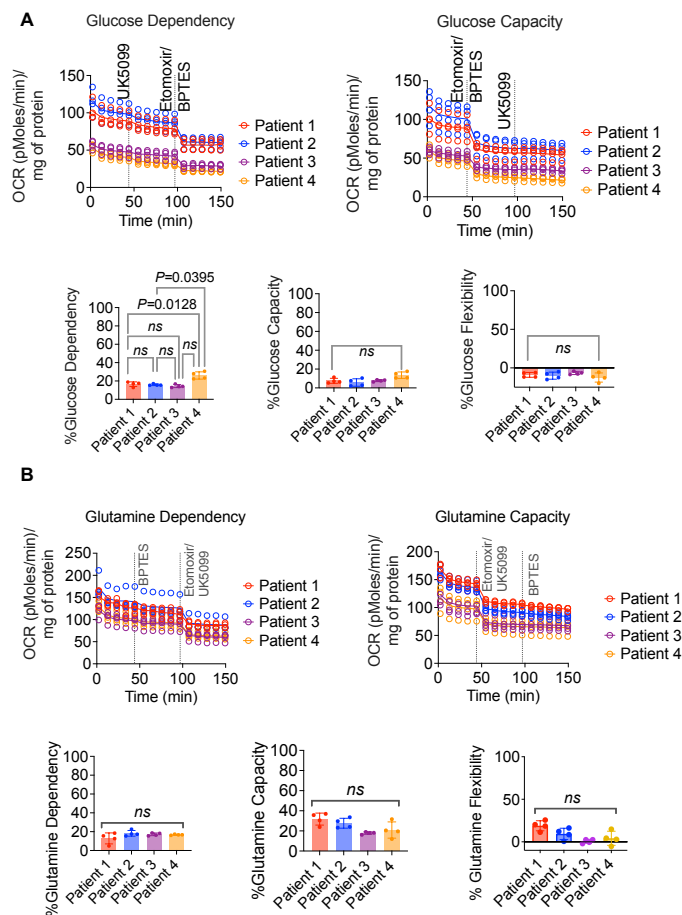


Figure S6. Glucose and glutamine utilization by recurrent and newly diagnosed patient derived GSCs. The results revealed that patient derived GSCs from varied background depend very less on the glucose or glutamine oxidation pathways for their energy requirements. The data also shows that there is no significant difference between the glucose utilization or glutamine utilization among the different patient derived GSCs. The compounds UK5099 (2 μ M) for glucose pathway inhibition, BPTES (3 μ M) for glutamine pathway inhibition, and etomoxir (4 μ M) for FAO inhibition were used as port injections for the respective pathways. The cells' reliance or dependency on a specific fuel pathway was measured by adding the respective metabolic inhibitor in the first port injection followed by the addition of a combination of the other two inhibitors. Further, the cells' ability to oxidize a specific fuel when the other fuel pathways are inhibited was measured by adding a combination of two inhibitors first followed by addition of the specific metabolic inhibitor and is named as fuel capacity.

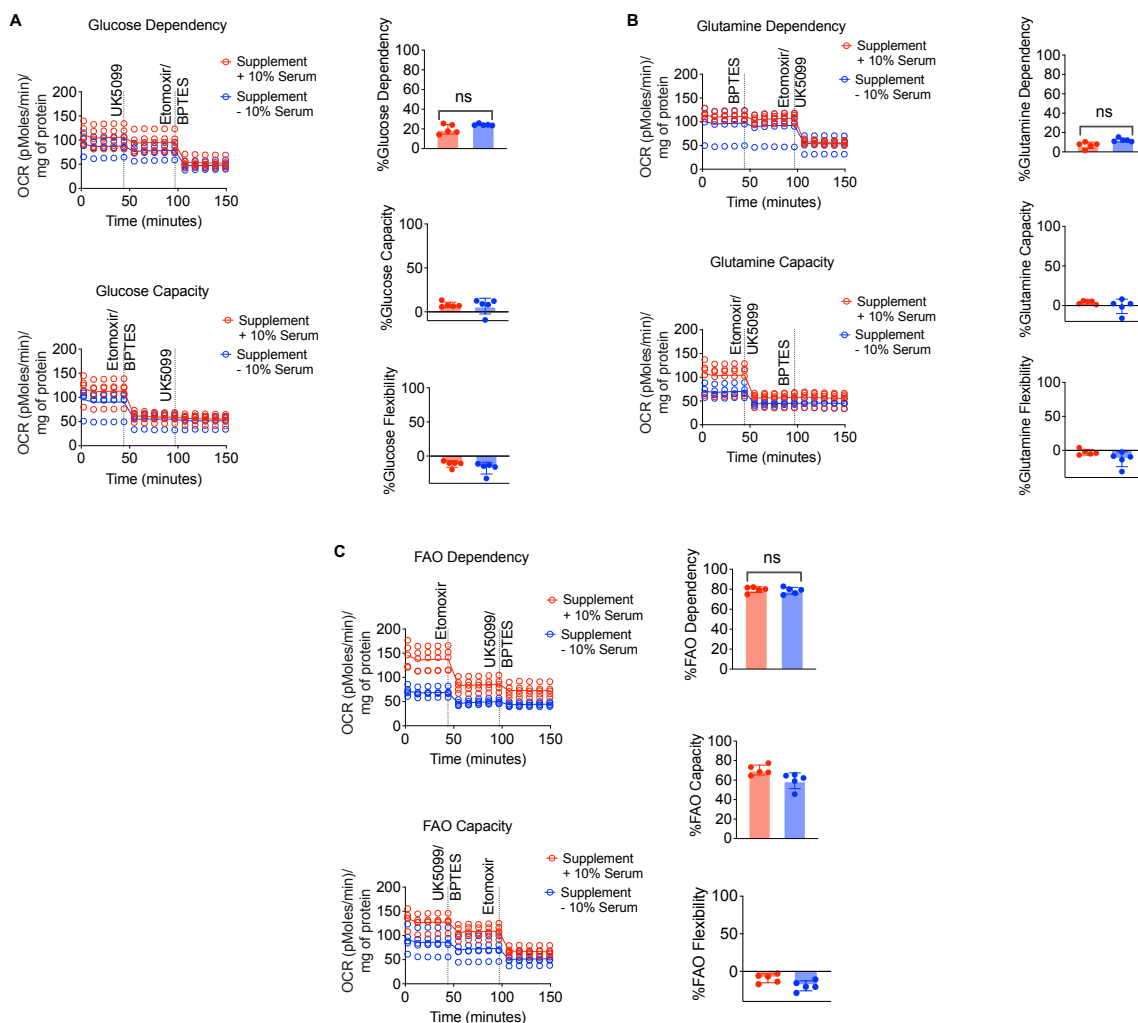


Figure S7. Effect of cell culture conditions on the metabolic profile of recurrent patient 1 derived GSC. Fuel flex analyses in recurrent patient 1 derived GSCs in presence and absence of serum. The cells were either grown in the presence of FBS or in their appropriate culture media in the absence of FBS prior to performing the metabolic utilization assay. The data probes that the altered metabolic preference of patient derived GSCs do not depend on the growth conditions but is a potential characteristic of the GSCs. The compounds UK5099 (2 μ M) for glucose pathway inhibition, BPTES (3 μ M) for glutamine pathway inhibition, and etomoxir (4 μ M) for FAO inhibition were used as port injections for respective pathways. All statistical significance was measured using the unpaired t test analysis.

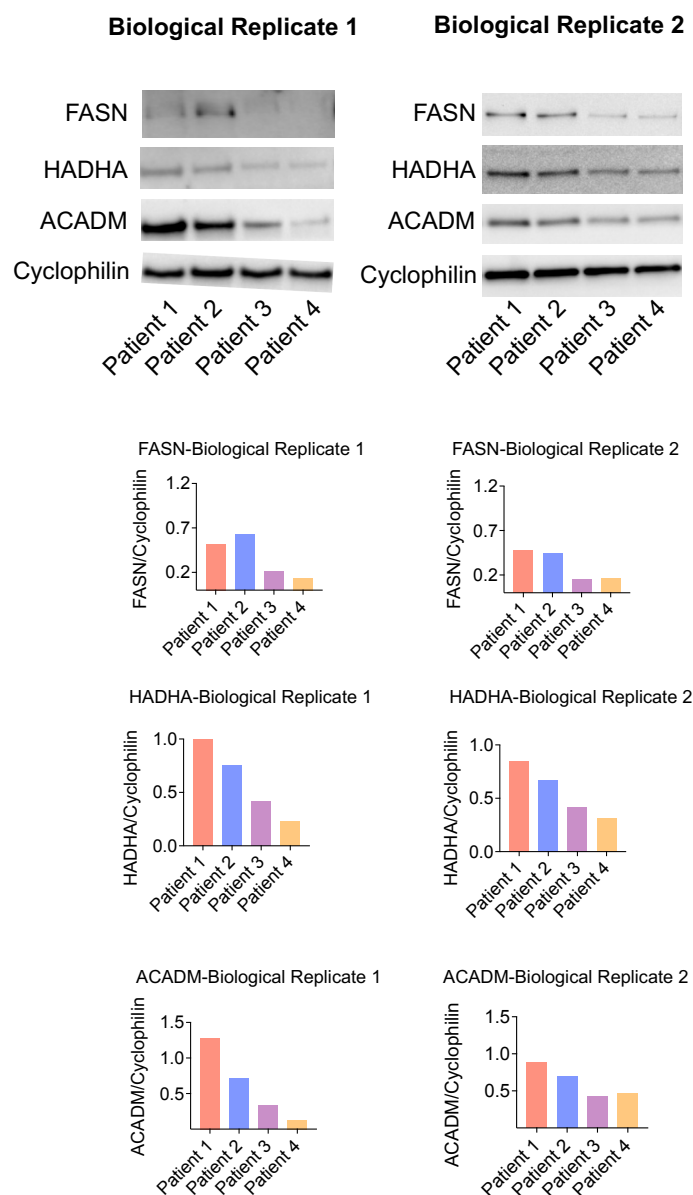


Figure S8. Expression of fatty acid metabolism related proteins in patient derived GSCs. Western blot quantification of FAO markers fatty acid synthase (FASN), hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase subunit A (HADHA), and medium-chain acyl-CoA dehydrogenase (ACADM) in recurrent patients 1 and 2 compared to newly diagnosed patients 3 and 4 from two biological replicate studies. The quantification for each marker was performed with respect to the expression of cyclophilin. All primary antibodies were used at a dilution of 1:1000 and the respective secondary antibodies were used at a dilution of 1:2000.

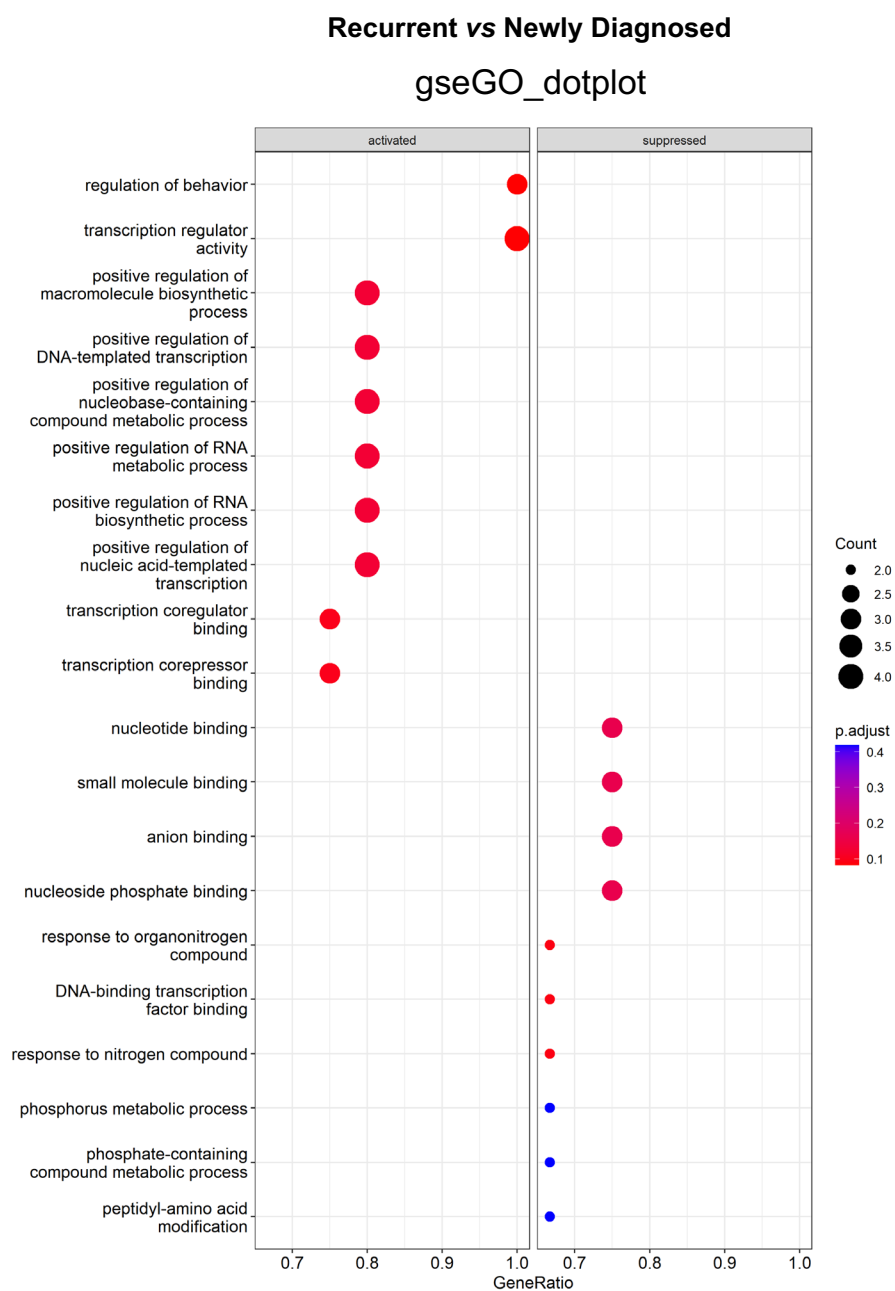


Figure S9. Gene sequence enrichment gene ontology (gseGO) dot plot revealing the activated and suppressed pathways between recurrent vs newly diagnosed patient derived GSCs based on the enriched gene sets.

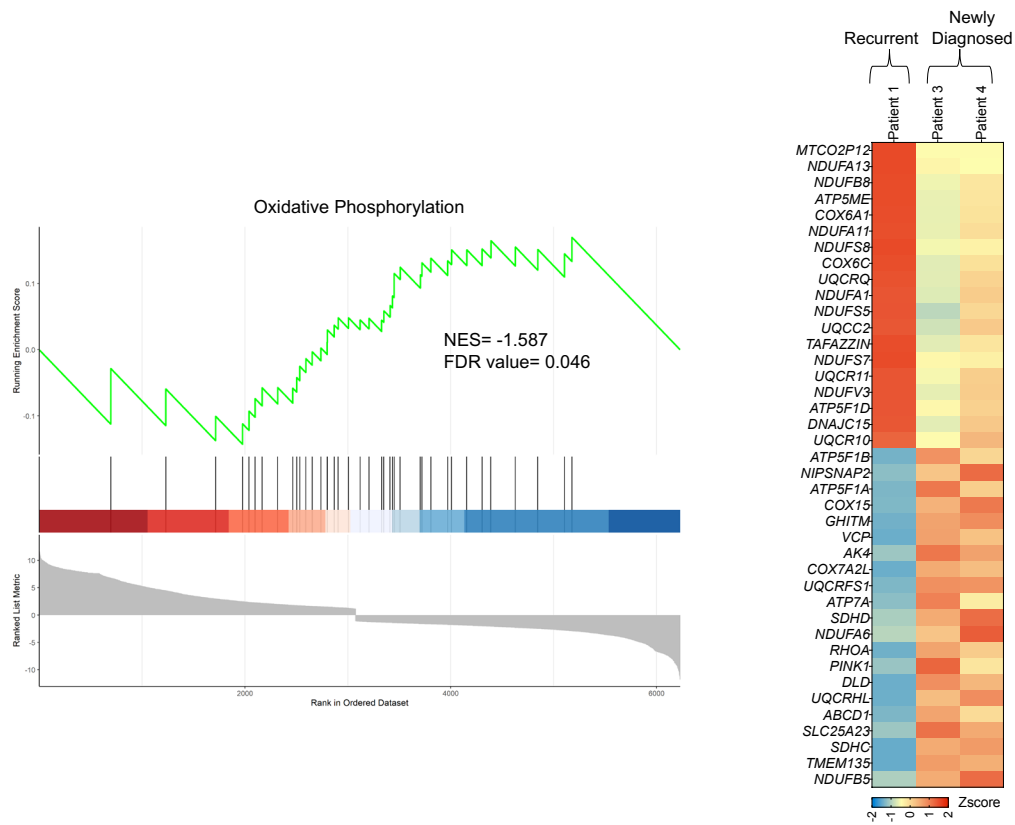


Figure S10. Heatmap and enrichment plot of enriched genes revealed through GSEA for oxidative phosphorylation when comparing recurrent patient 1 derived GSCs to the newly diagnosed patient derived GSCs.

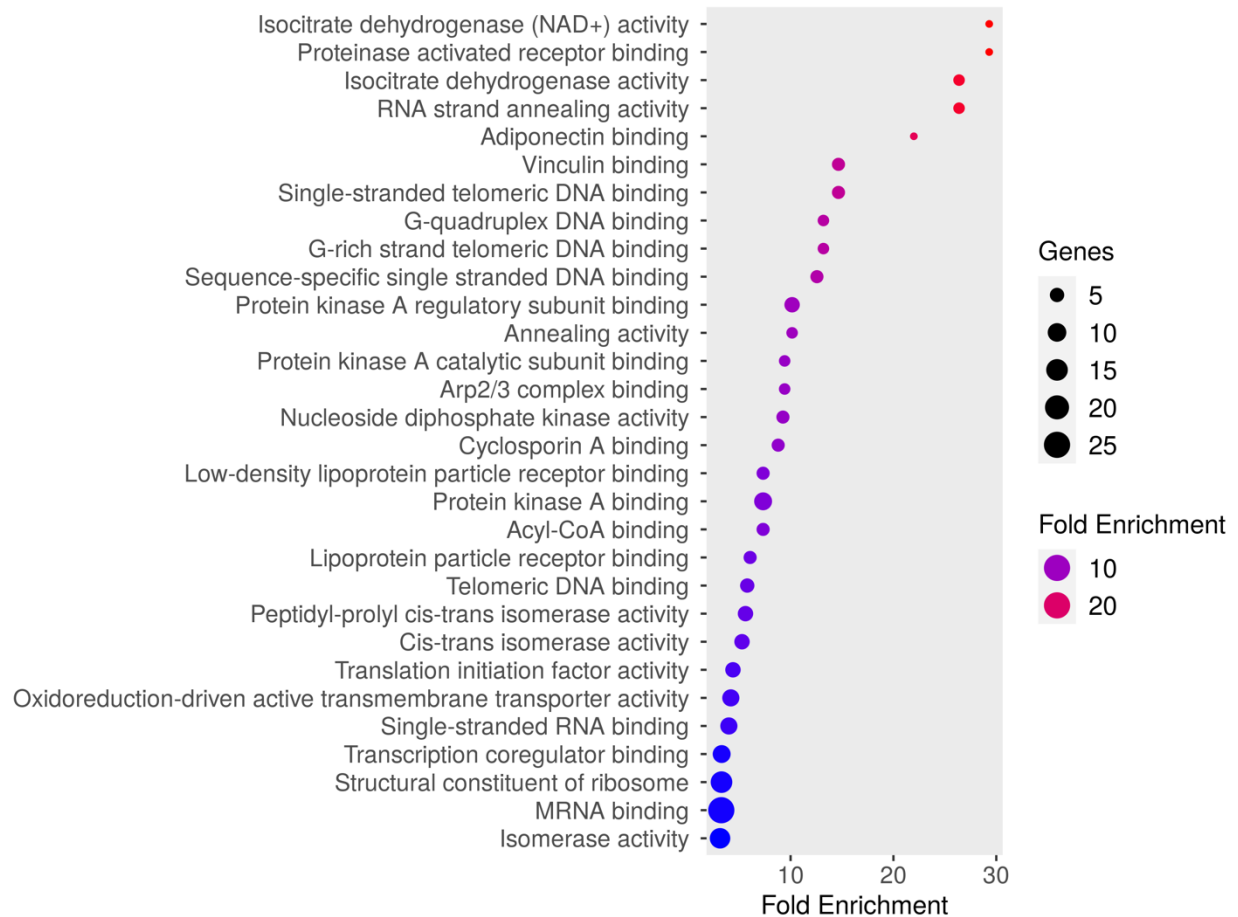


Figure S11. Top 30 pathways based on gene ontology terms for enriched molecular functions based on KEGG pathway analysis between patient derived GSCs and normal astrocytes.

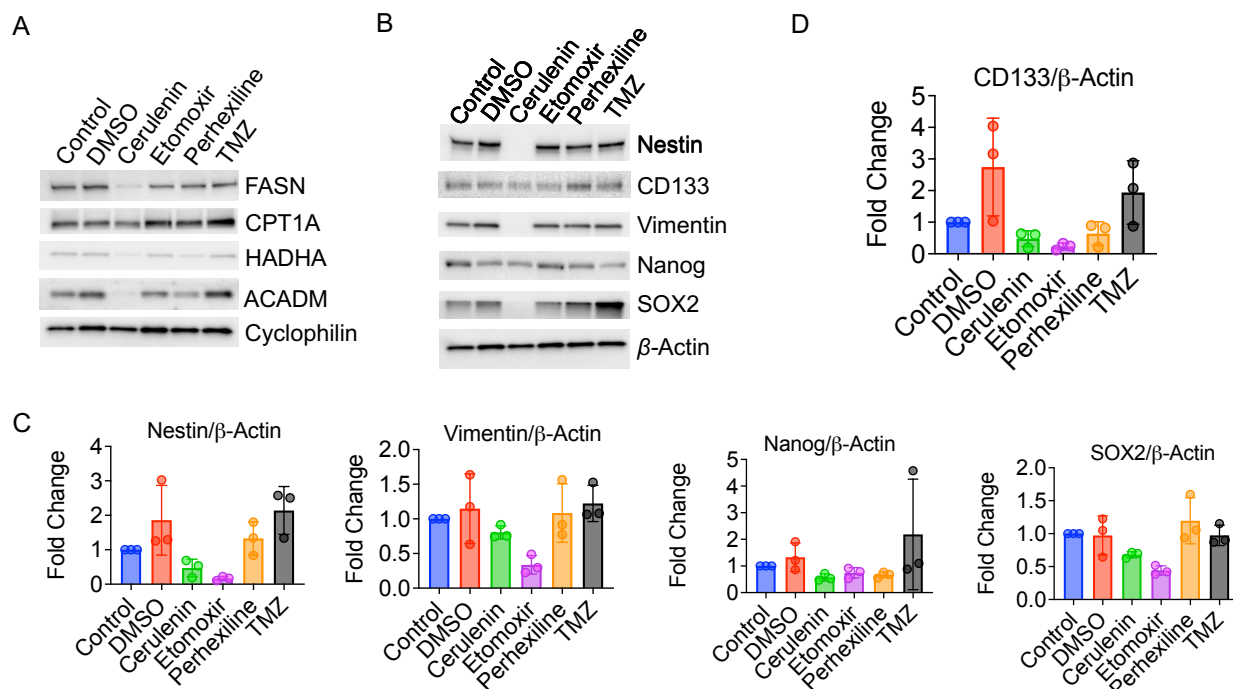


Figure S12. Pharmacologic inhibition of FAO with etomoxir, perhexiline, or FASN inhibitor cerulenin and stemness marker expression in patient-derived glioblastoma stem cells (GSC Patient 1). (A) Representative Western blot showing expression of FAO-associated proteins, FASN, CPT1A, ACADM, and loading control (cyclophilin) in GSC Patient 1 after 24 h treatment with vehicle (DMSO), etomoxir (50 μ M), perhexiline (10 μ M), cerulenin (25 μ M), or temozolomide (TMZ; 10 μ M). (B) Representative Western blot showing expression of stemness markers (nestin, vimentin, nanog, SOX2) and loading control (β -actin) under the same treatment conditions. (C) qRT-PCR quantification of NES, VIM, NANOG, and SOX2 mRNA levels normalized to β -actin (n = 3 independent experiments). (C) qRT-PCR quantification of cancer stemness CD133 mRNA levels normalized to β -actin (n = 3 independent experiments).

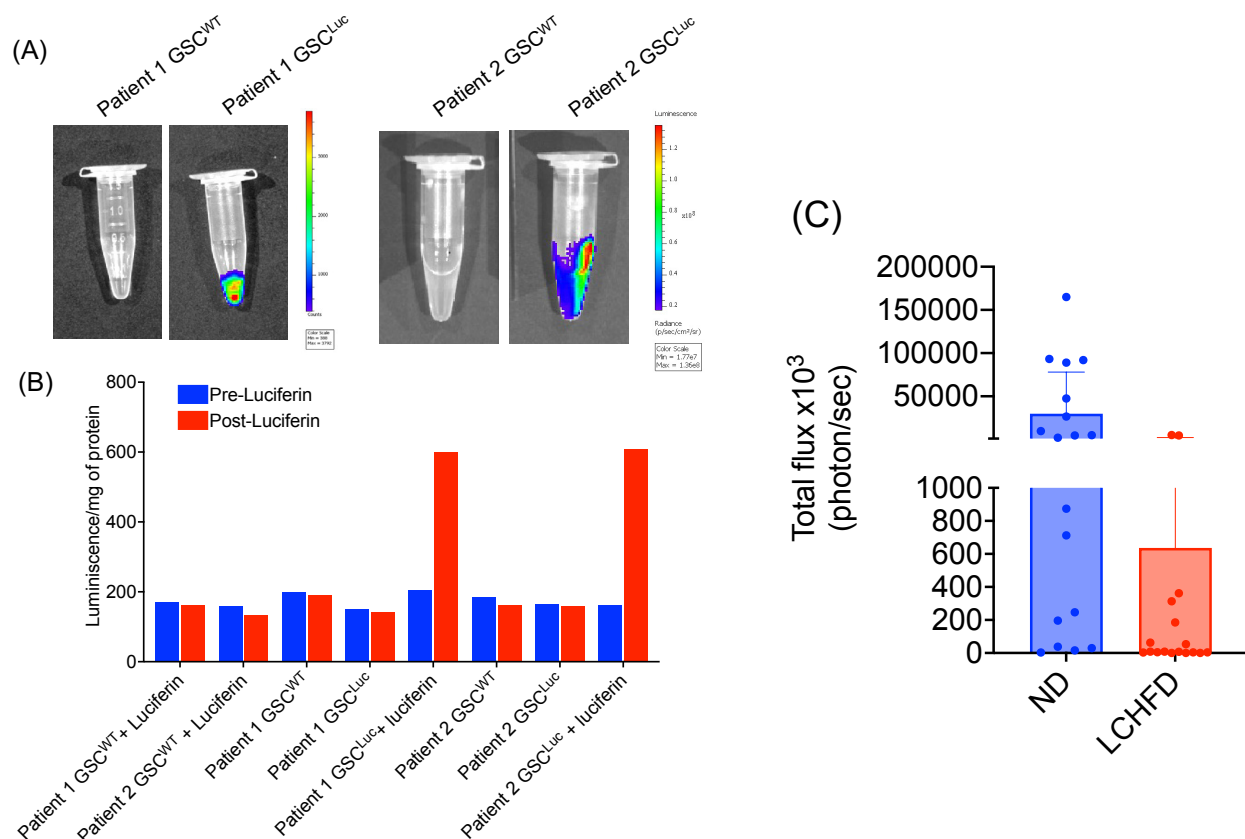


Figure S13. Characterization of Patient 1 GSC^{Luc} and Patient 2 GSC^{Luc} for efficiency of transfection and quantitative analyses of IVIS images from Figure 3 in the main manuscript. (A) IVIS images confirming the luciferase transfection in patient 1 and patient 2 GSCs. (B) Quantitative analysis supporting the successful transfection of luciferase in patient 1 GSCs as well as patient 2 GSCs. (C) Quantification of the background corrected total flux from the brain region of the patient 1 GSCs implanted animals through *In Vivo* Imaging System (IVIS) using LivingImage software.

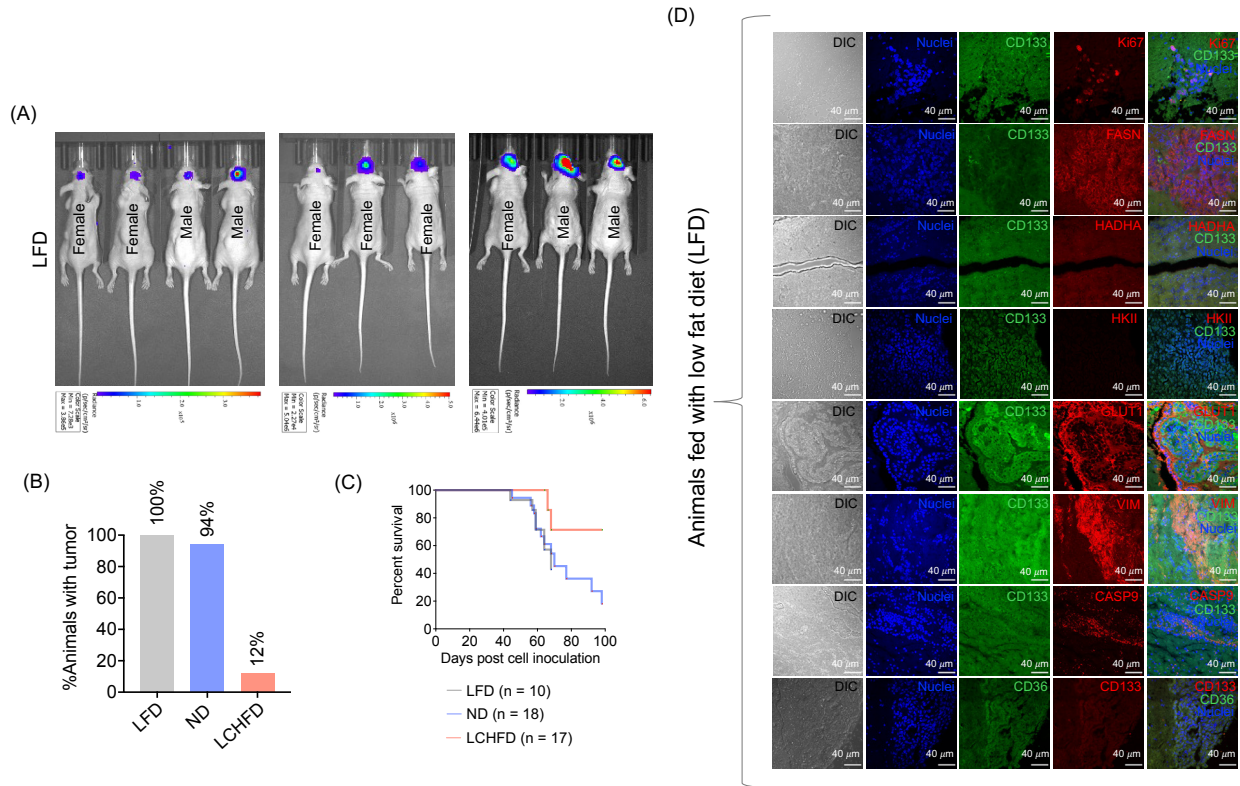


Figure S14. Effect of low fat diet (LFD) on orthotopic glioblastoma tumor progression. (A) IVIS images showing aggressive glioblastoma tumor formation in male and female BALB/c nude mice kept under LFD followed by the (B) percentage (%) of animals that formed tumor under LFD, ND, and LCHFD fed groups, showing aggressive tumor formation in all LFD animals. (C) Kaplan-Meier survival curve showing the survival percent of animals under the diet groups. (D) Immunofluorescence images of brain tumor regions analyzing the effects of low-fat diet (LFD) on the growth of patient 1 GSCs following the same experimental protocol as ND and LCHFD show no beneficial outcome on tumor growth and survival. Patient 1 GSC^{Luc} cells were used for the orthotopic implantation in the BALB/c nude mice.

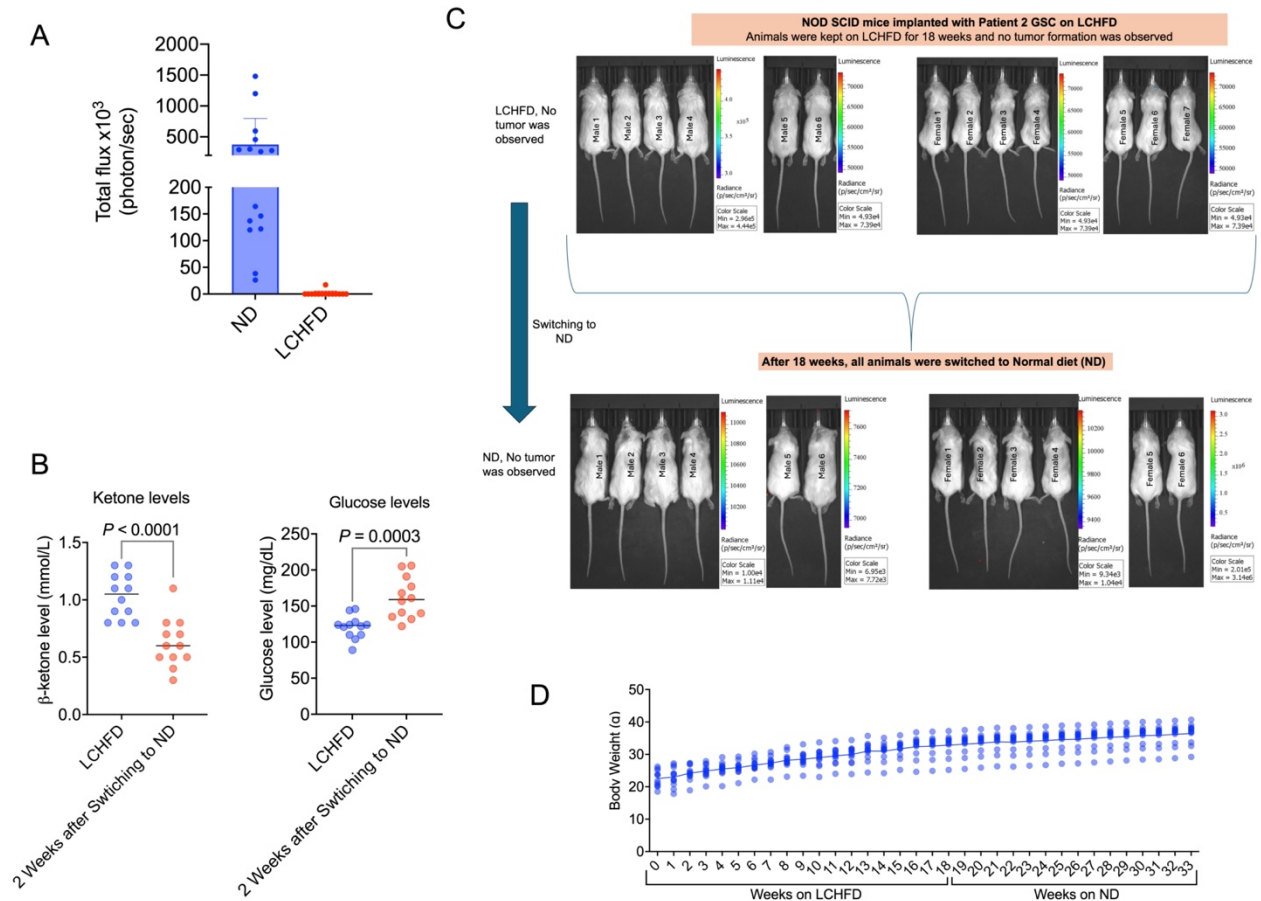


Figure S15. (A) Quantification of the background corrected total flux from the brain region of the patient 2 GSCs implanted animals through IVIS using LivingImage software. (B) Data showing the levels of β -ketone in mmol/L and glucose levels in mg/dL in LCHF and 2 weeks after switching back to ND. Statistical significance was determined using unpaired t test at 95% CI. (C) IVIS images of animals implanted with patient 2 GSCs while on LCHF. Following 18 weeks of LCHF, mice were switched to a ND and continued 15 weeks. Representative IVIS images depict tumor bioluminescence during the ND period. (D) Body weight of the animals during study duration.

Tumor implantation site

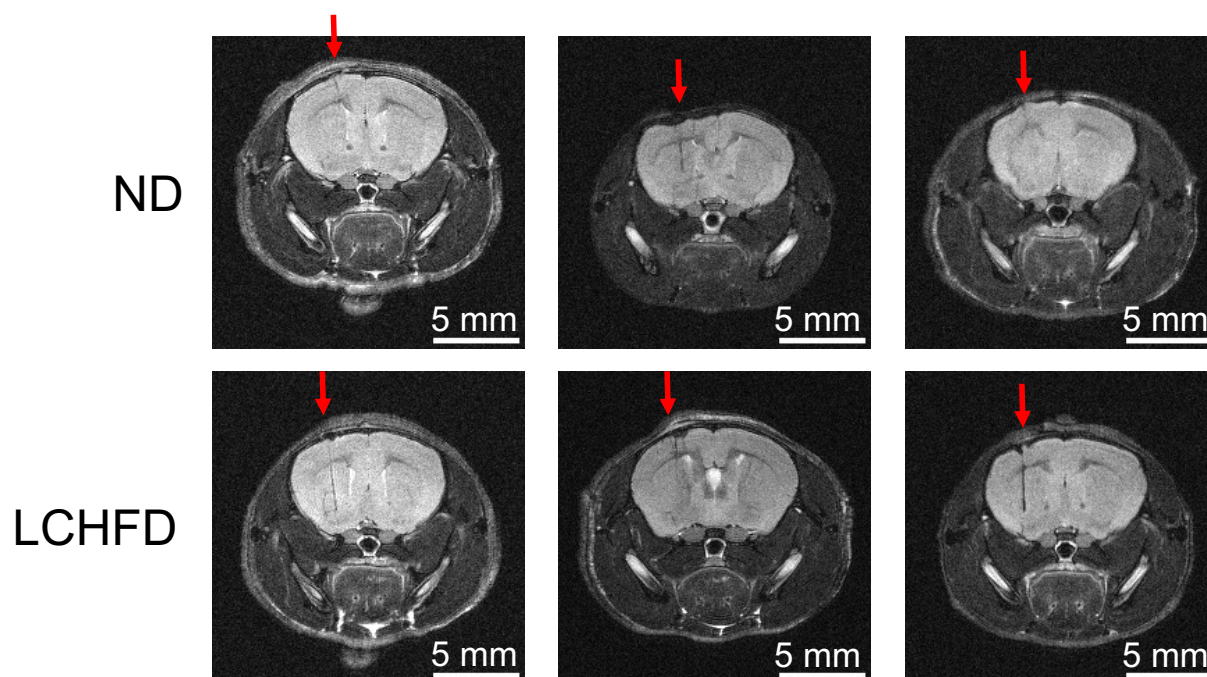


Figure S16. MRI images of tumor implantation site in ND and LCHF groups. Representative MRI Images visualizing the site of injection (marked with red arrow) in both Normal diet and Low carbohydrate high fat diet groups (Scale = 5 mm).

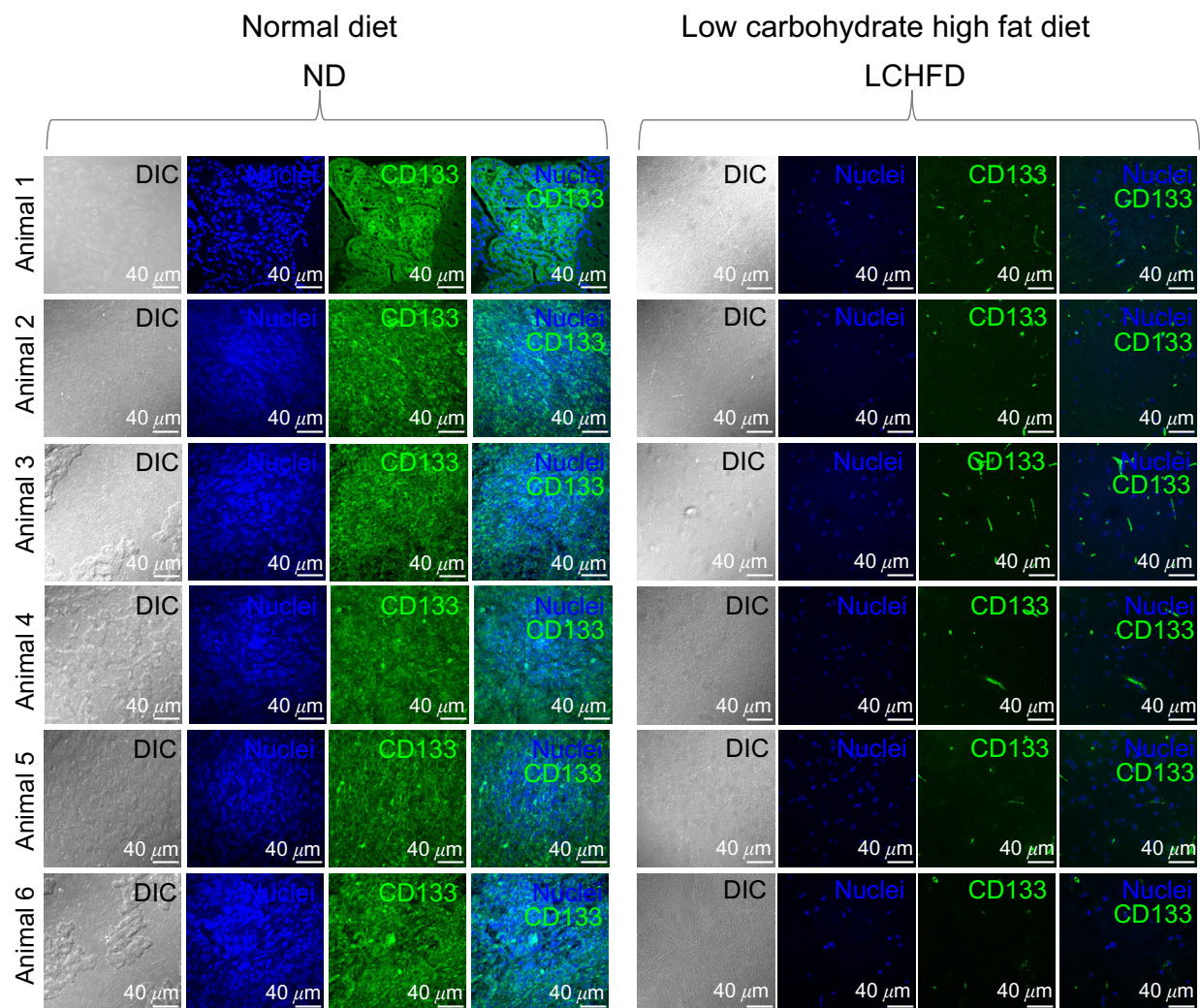


Figure S17. Immunofluorescence images used for the quantification of CD133 positive cells at the tumor inoculation site of ND and LCHFD fed mice.

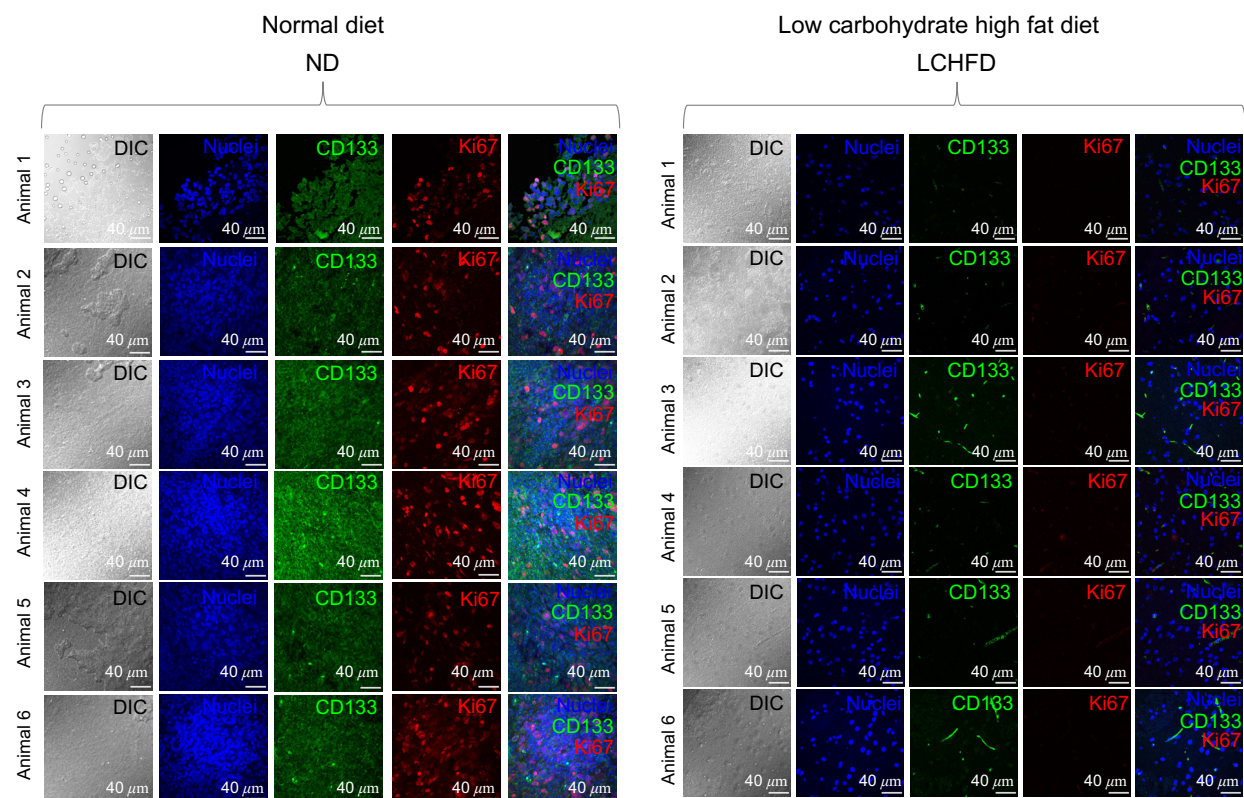


Figure S18. Immunofluorescence images used for the quantification of Ki67 expression in CD133 positive cells at the tumor inoculation site of ND and LCHFD fed mice.

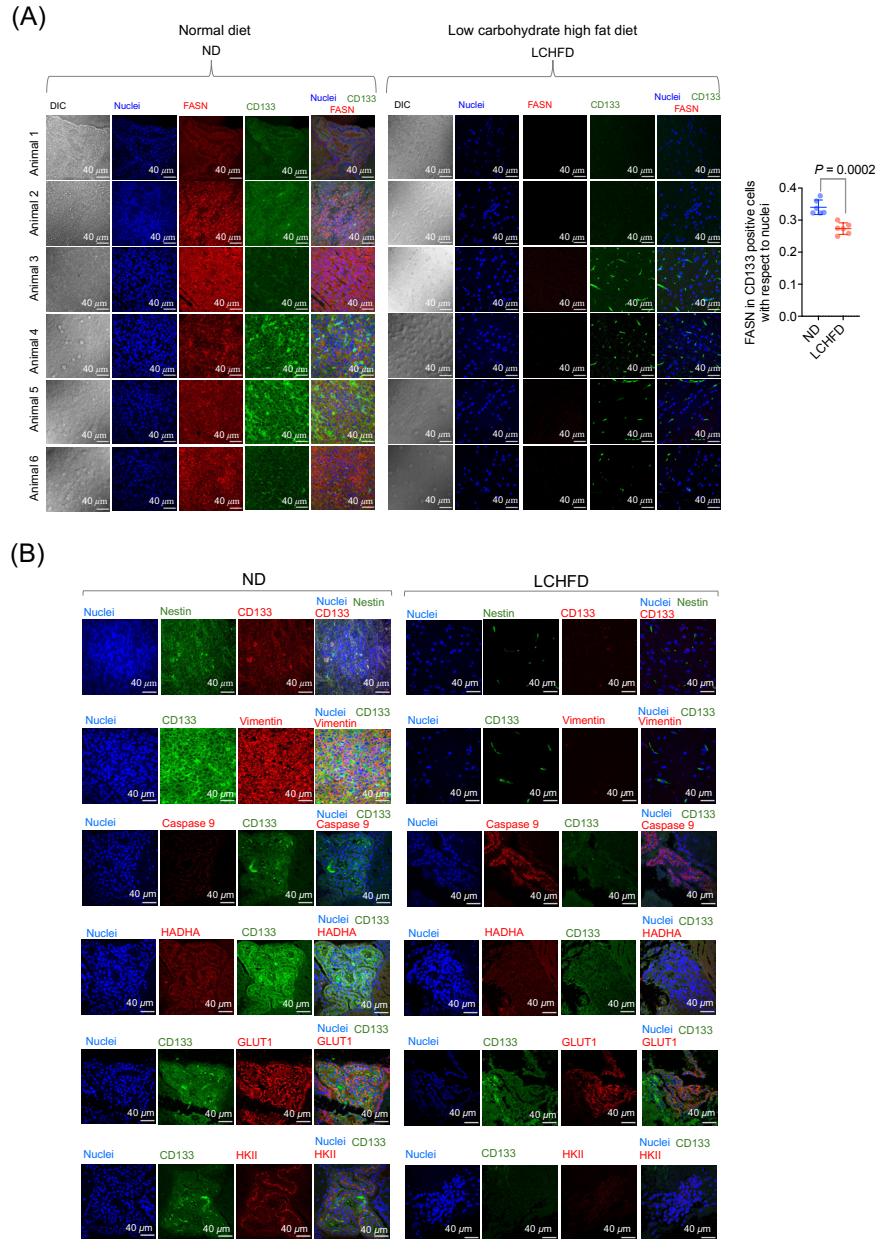


Figure S19. Tumor metabolic markers altered in the orthotopic brain by low carbohydrate high fat diet. (A) Immunofluorescence images used for the quantification of FASN expression in CD133 positive cells at the tumor inoculation site of ND and LCHFD fed mice. (B) Immunofluorescence images in coronal brain sections of mice from ND and LCHFD groups performed *ex vivo* revealing the reduction in Nestin, vimentin, HADHA, GLUT1, and HKII in CD133 positive cells in brain sections from LCHFD fed mice, whereas caspase 9 shows increase in LCHFD group.

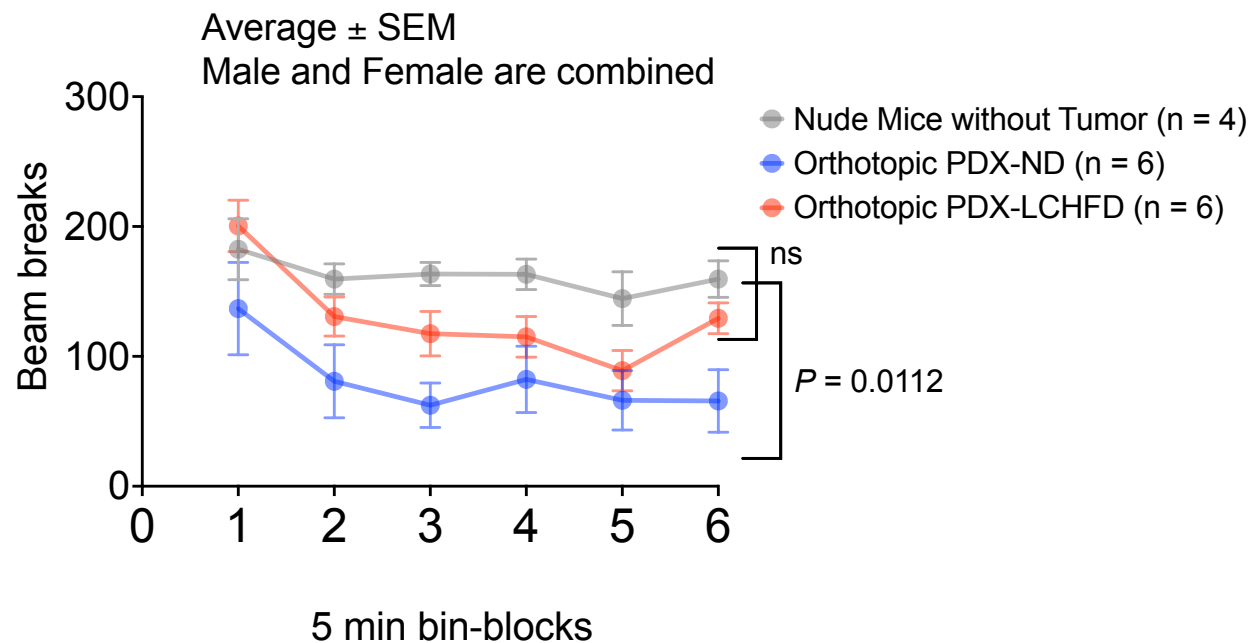


Figure S20. Average data showing the number of beam breaks by each animal from the respective groups for every 5-min bin-blocks, across a total interval of 30 min. Two-way ANOVA analyses with multiple comparison was conducted.

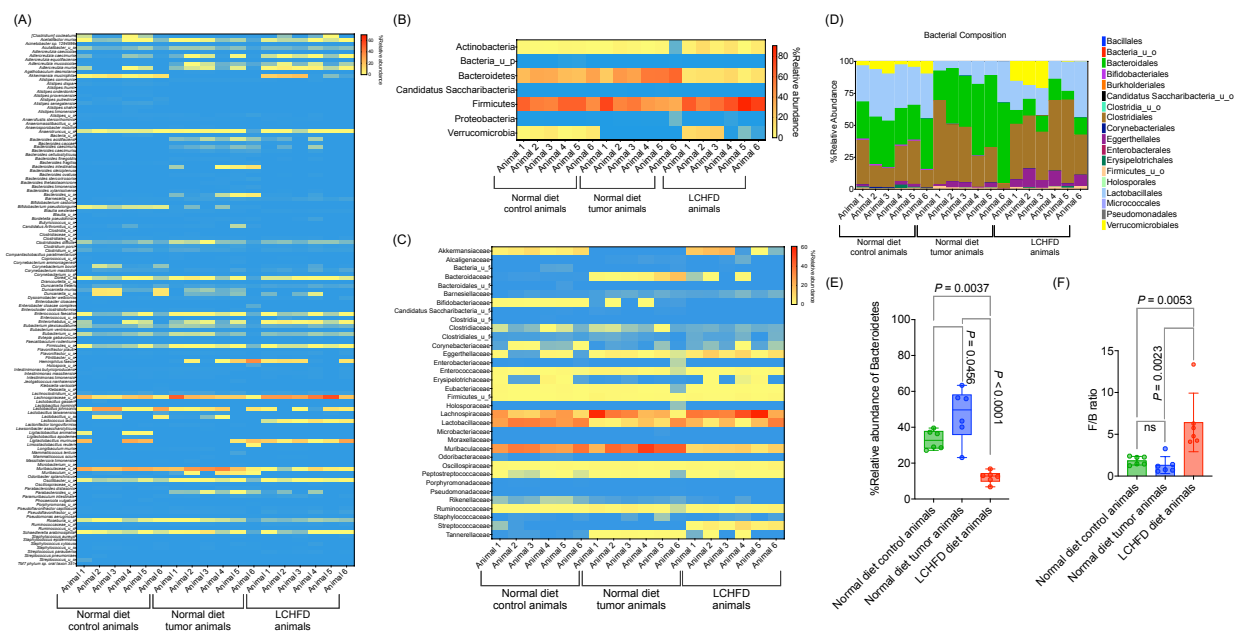


Figure S21. Dietary intervention improves the gut-microbiome structure. (A) Heatmap showing the relative abundance of the different microbial species found in the gut microbiome of the animals in the ND (n = 6) and LCHFD (n = 6) groups. Relative abundance of the various microbial (B) phylum and (C) family in the tumor bearing animal under ND and animals without tumor under the LCHFD group. (D) Relative abundance showing the differential bacterial composition in animals from ND and LCHFD groups. (E) Data showing the ratio between abundance of Firmicutes and Bacteroidetes (F/B ratio) at the top and %relative abundance of Bacteroidetes in ND and LCHFD groups. The statistical significance was measured using unpaired t test at 95% confidence interval and the data is represented as mean $\pm$ S.D.

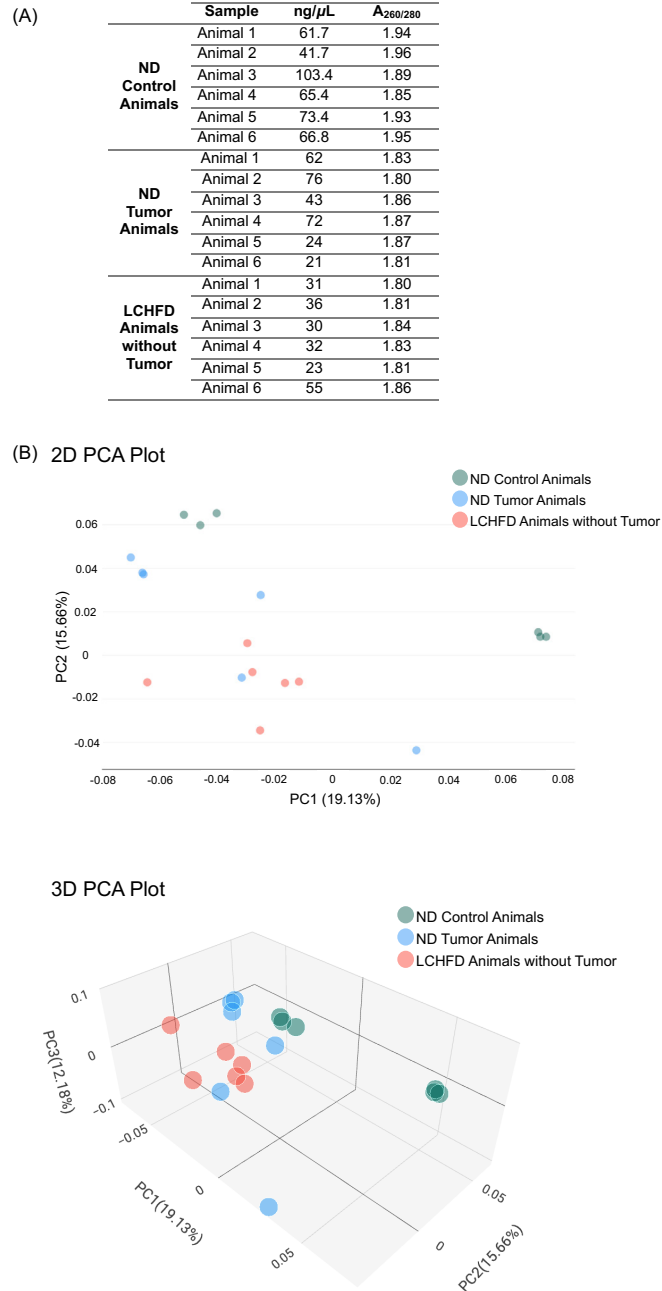
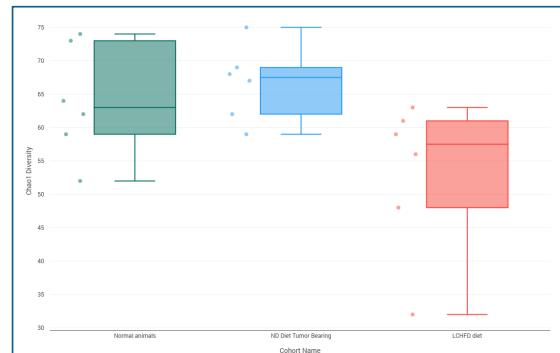
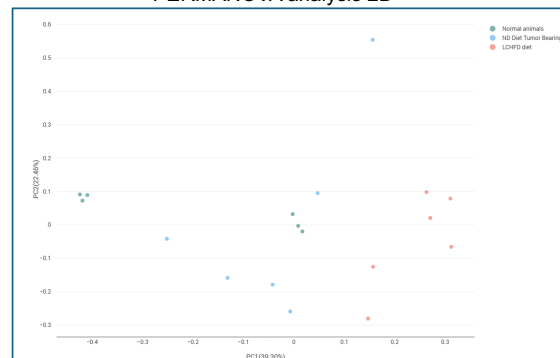


Figure S22. (A) Purity of isolated DNA from the fecal samples of animal under the varying diet forms. The table shows fecal DNA concentration and purity of the DNA used for microbiome analyses. (B) 2-dimensional and 3-dimensional principal component analysis (PCA) graphs.

**A. Alpha diversity: Chao1
Wilcoxon Rank Sum Test**



**B. Beta diversity: Bray-Curtis
PERMANOVA analysis 2D**



**C. Beta diversity: Bray-Curtis
PERMANOVA analysis 3D**

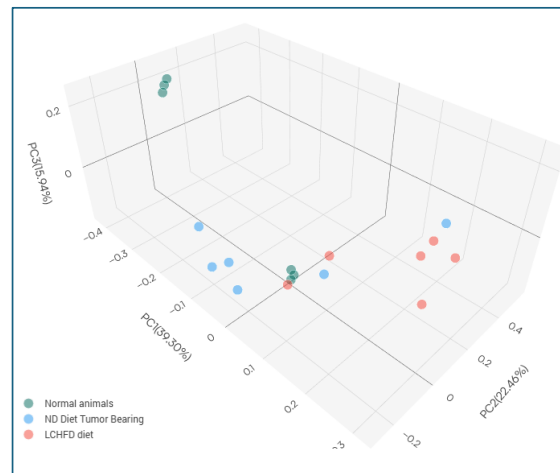


Figure S23. Differences in α -diversity (Chao1 richness) between Normal, ND diet tumor bearing and LCHFD animals were assessed using the Wilcoxon rank-sum test. B-diversity based on Bray-Curtis dissimilarity was compared using PERMANOVA between three groups and represented in 2D and 3D analysis.

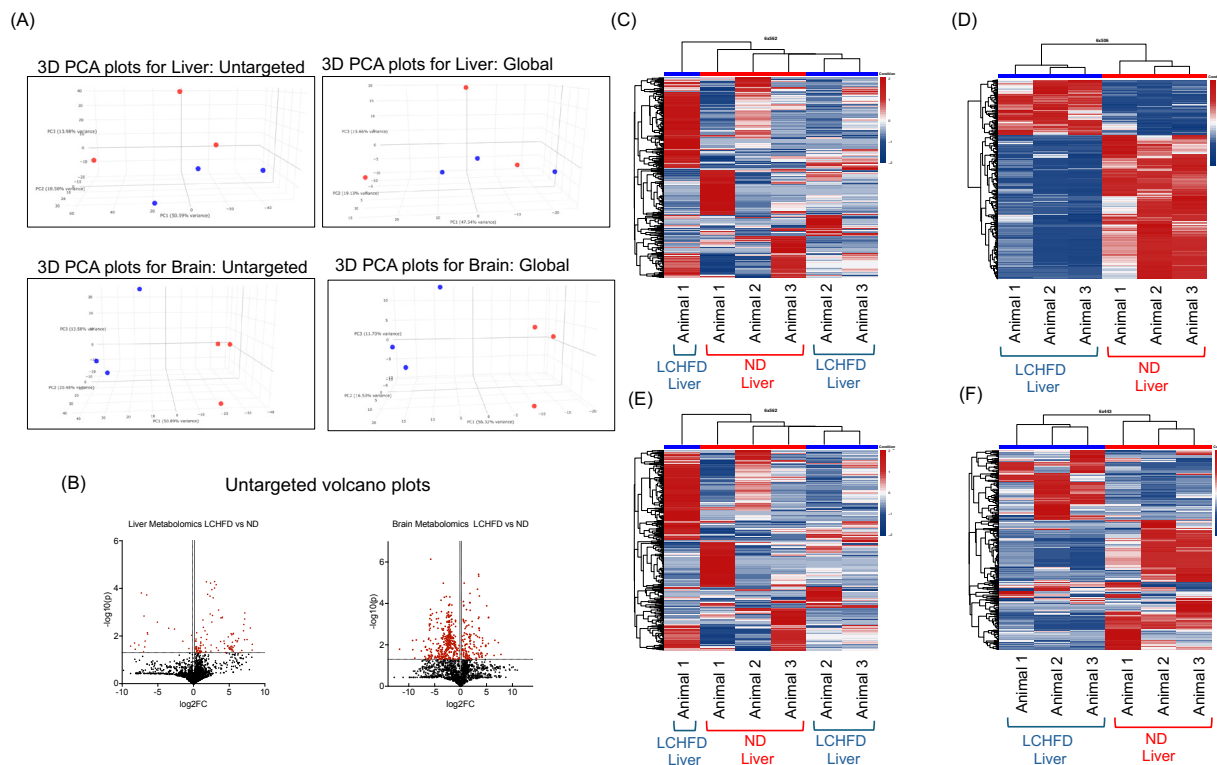


Figure S24. Metabolomic mapping of liver and brain samples from different diet fed animals which had tumor implantation. (A) The 3D-Principal components analysis of samples from liver (on the top panel) and brain (on the bottom panel) through untargeted and global metabolomics between HFD and ND. liver samples are on the bottom panel. Samples were analyzed with a 3 component PCA model, and the resulting score plots were generated. (B) Volcano plot representing lipids present in liver samples (on the left panel), and brain samples (on the right panel) using the metabolites for HFD vs ND in untargeted metabolomics. Samples were clustered with the complete method and Euclidian distance function. Unsupervised clustering analysis of significant metabolites ($P < 0.05$) from liver samples from HFD vs ND in untargeted (C) and global (D) metabolomics. Unsupervised clustering analysis of significant metabolites ($P < 0.05$) from brain samples from HFD vs ND in untargeted (E) and global (F) metabolomics.

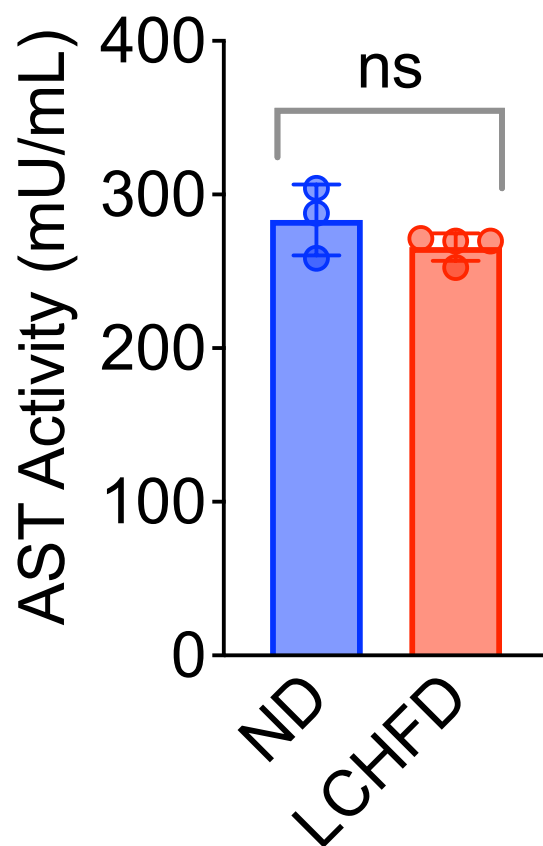


Figure S25. Data showing that distinguished metabolic features in LCHFD fed animals do not induce hepatotoxicity in the animals. The statistical significance was performed by unpaired t test between two groups. Each point represents an animal, and the data is represented as mean $\pm$ S.D.

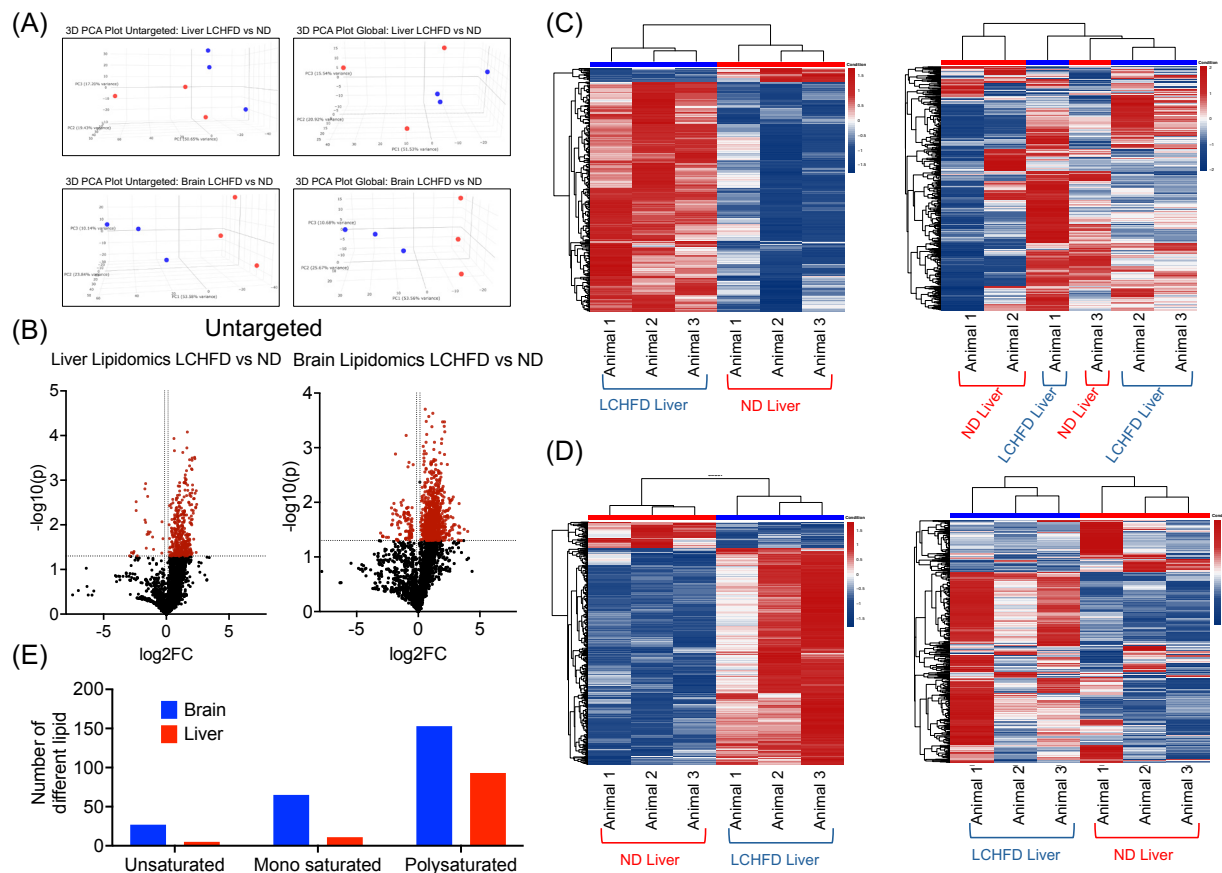


Figure S26. Dietary intervention regulated lipid distribution between peripheral organ and brain. (A) The 3D-Principal components analysis of samples from liver (on the top panel) and brain (on the bottom panel) through untargeted and global lipidomic analysis between HFD and ND. Samples were analyzed with a 3 component PCA model, and the resulting score plots were generated. (B) Volcano plot representing lipids present in liver samples (on the left panel), and brain samples (on the right panel) using the lipids for HFD vs ND in untargeted lipidomics. (C) Unsupervised clustering analysis of significant lipids ($p < 0.05$) from liver samples from HFD vs ND in untargeted and global lipidomics. (D) Unsupervised clustering analysis of significant lipids ($p < 0.05$) from brain samples from HFD vs ND in untargeted and global lipidomics.

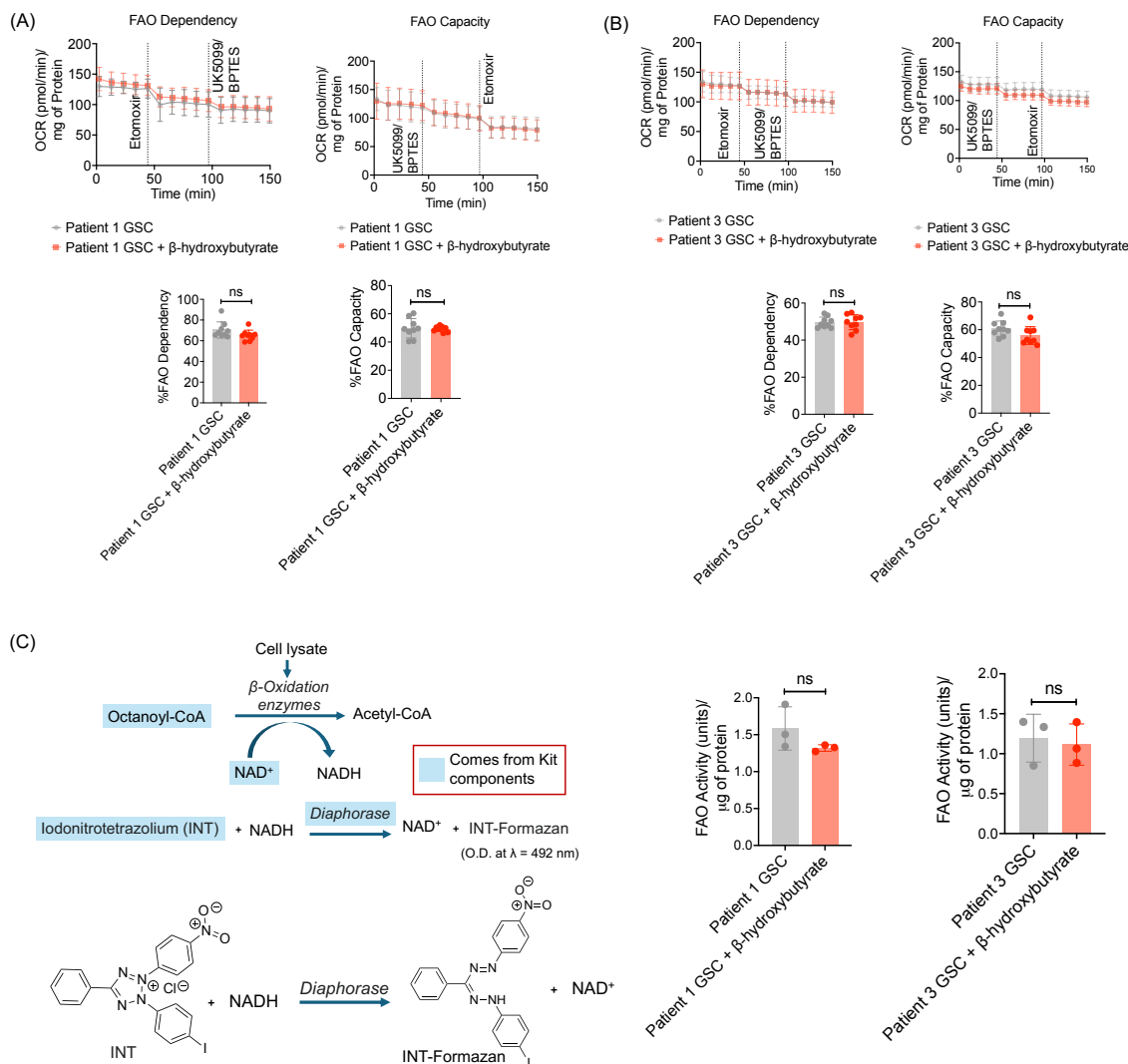


Figure S27. Effect of ketone bodies such as β -hydroxybutyrate on patient GSCs.

(A) Fuel flex analyses for fatty acid substrate utilization by recurrent patient 1 derived GSCs after treating with 5 mM β -hydroxybutyrate for 24 h. (B) Fuel flex analyses for fatty acid substrate utilization in newly diagnosed patient 3 derived GSCs after treating with 5 mM beta hydroxybutyrate for 24 h. The compounds UK5099 (2 μM) for glucose pathway inhibition, BPTES (3 μM) for glutamine pathway inhibition, and etomoxir (4 μM) for FAO inhibition were used as port injections for respective pathways. (C) Schematic representation of enzyme reactions occurring in fatty acid oxidation assay using assay kit and FAO activity in patient 1 GSC and patient 3 GSCs upon treating with 5 mM β -hydroxybutyrate for 24 h. All statistical significance was measured using the unpaired t test analyses.

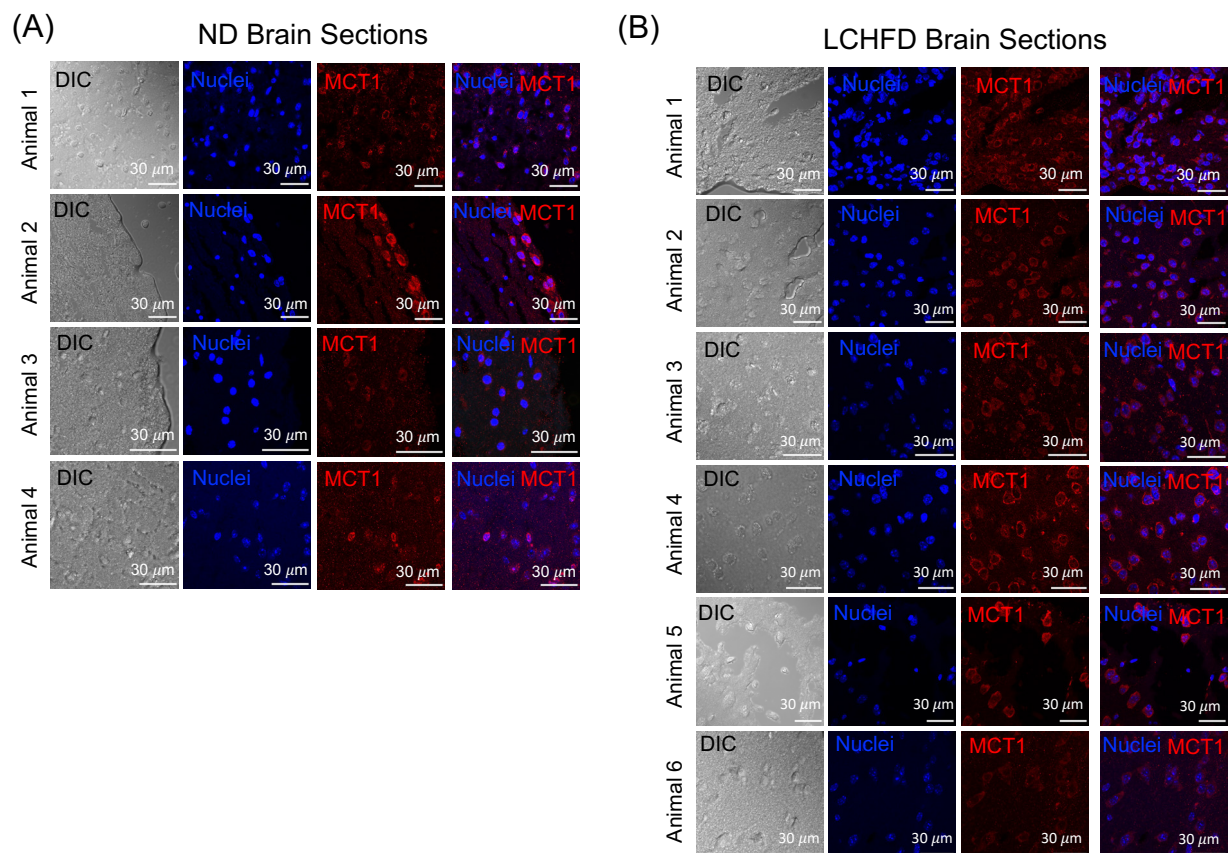


Figure S28. Immunofluorescence images showing the expression of monocarboxylate transporter 1 (MCT1) in the coronal brain sections of (A) ND and (B) LCHFD fed mice. All images were taken at Ex/Em: 359/461 nm for blue channel, and Ex/Em: 553/568 nm for red channel. All scans were kept constant at 2 μs /pixel.

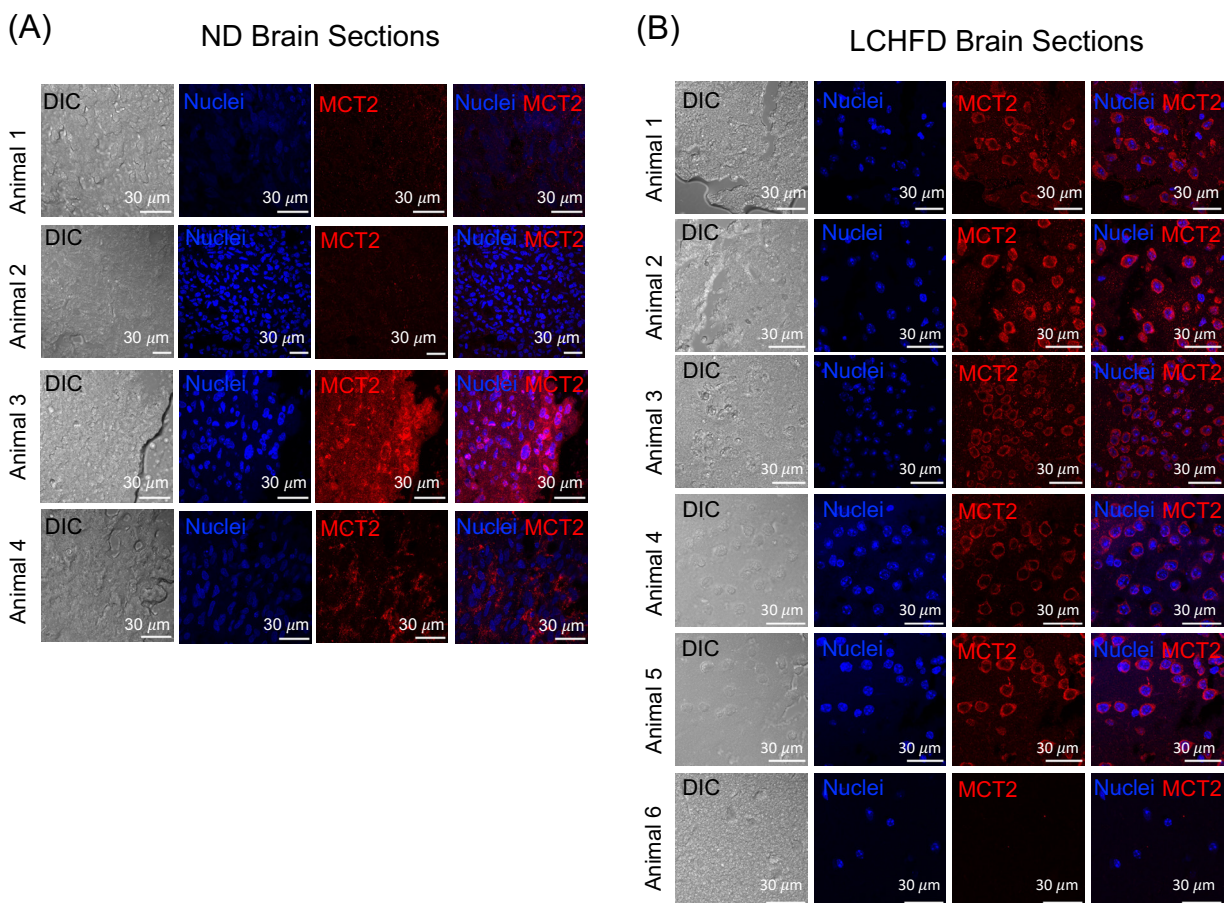
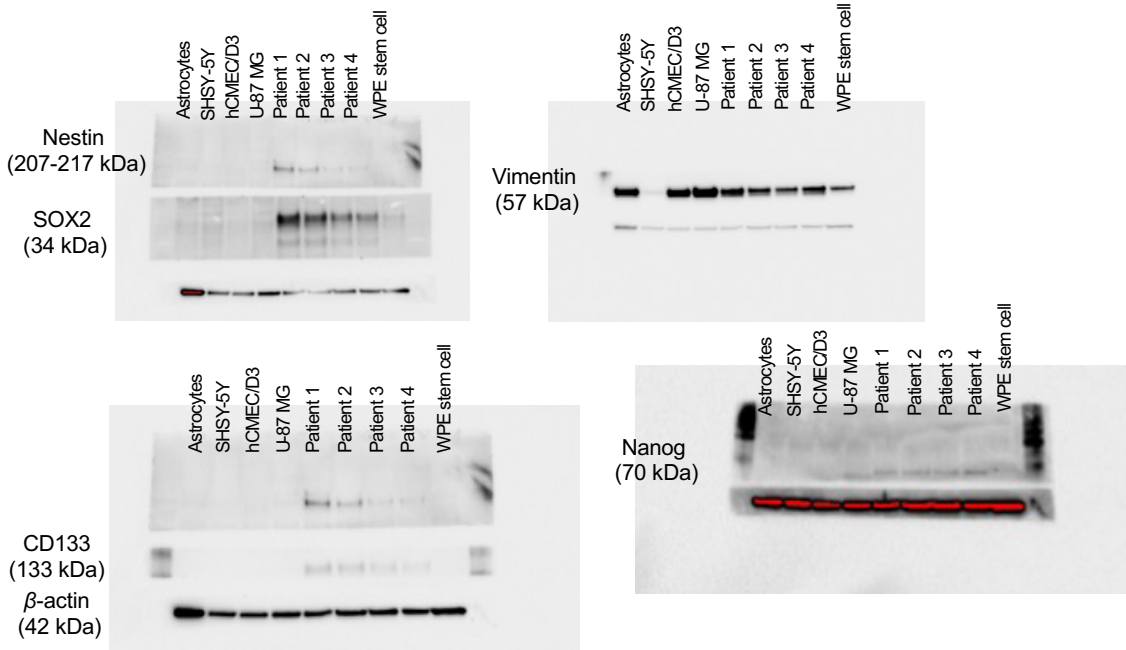
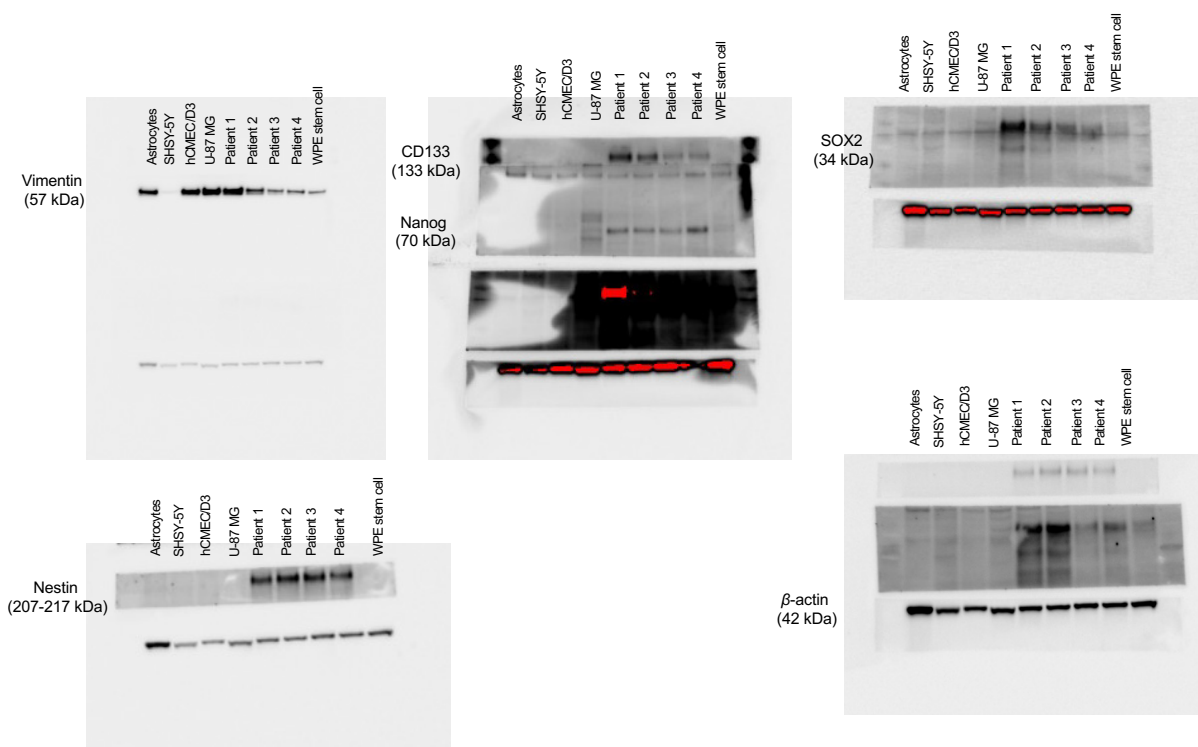


Figure S29. Immunofluorescence images showing the expression of monocarboxylate transporter 2 (MCT2) in the coronal brain sections of (A) ND and (B) LCHFD fed mice. All images were taken at Ex/Em: 359/461 nm for blue channel, and Ex/Em: 553/568 nm for red channel. All scans were kept constant at 2 μ s/pixel.

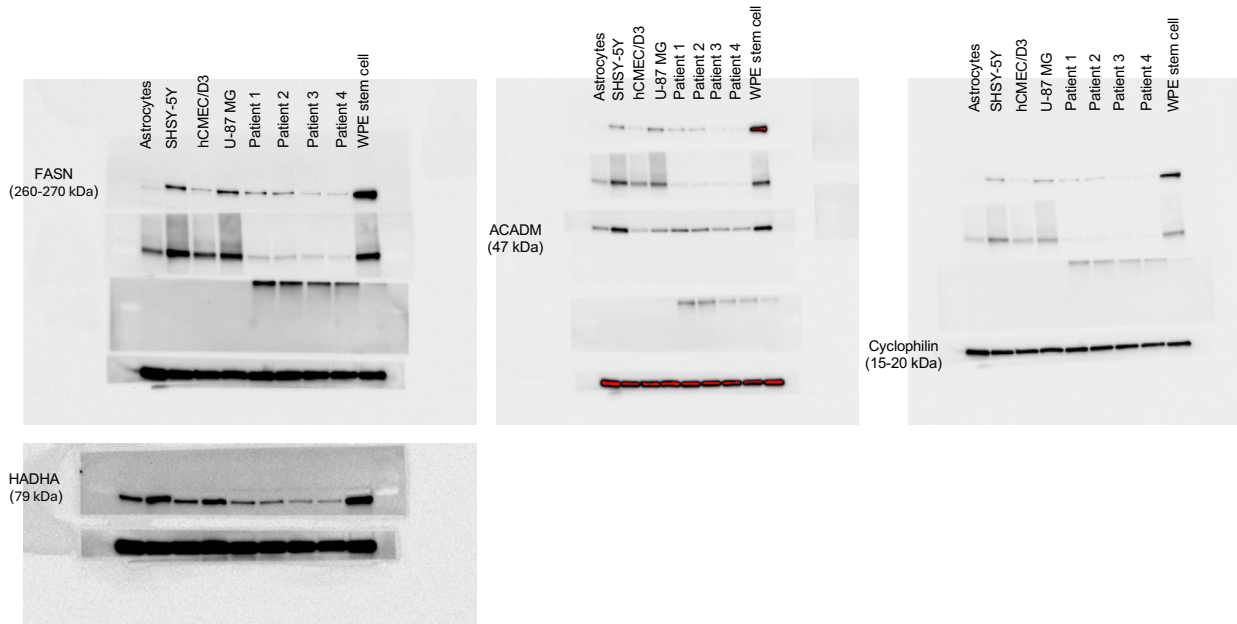
Uncropped blots For Supplementary Figure S1



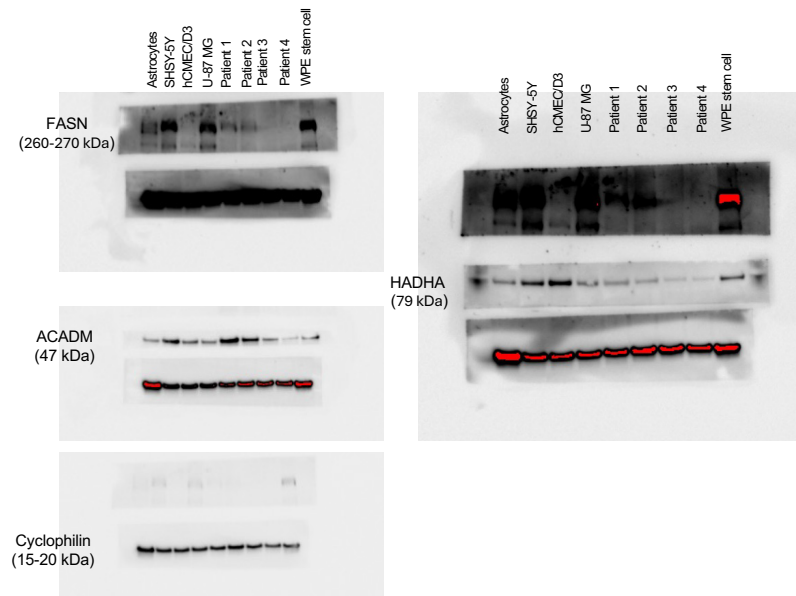
Uncropped blots Figure 2A of Main Manuscript



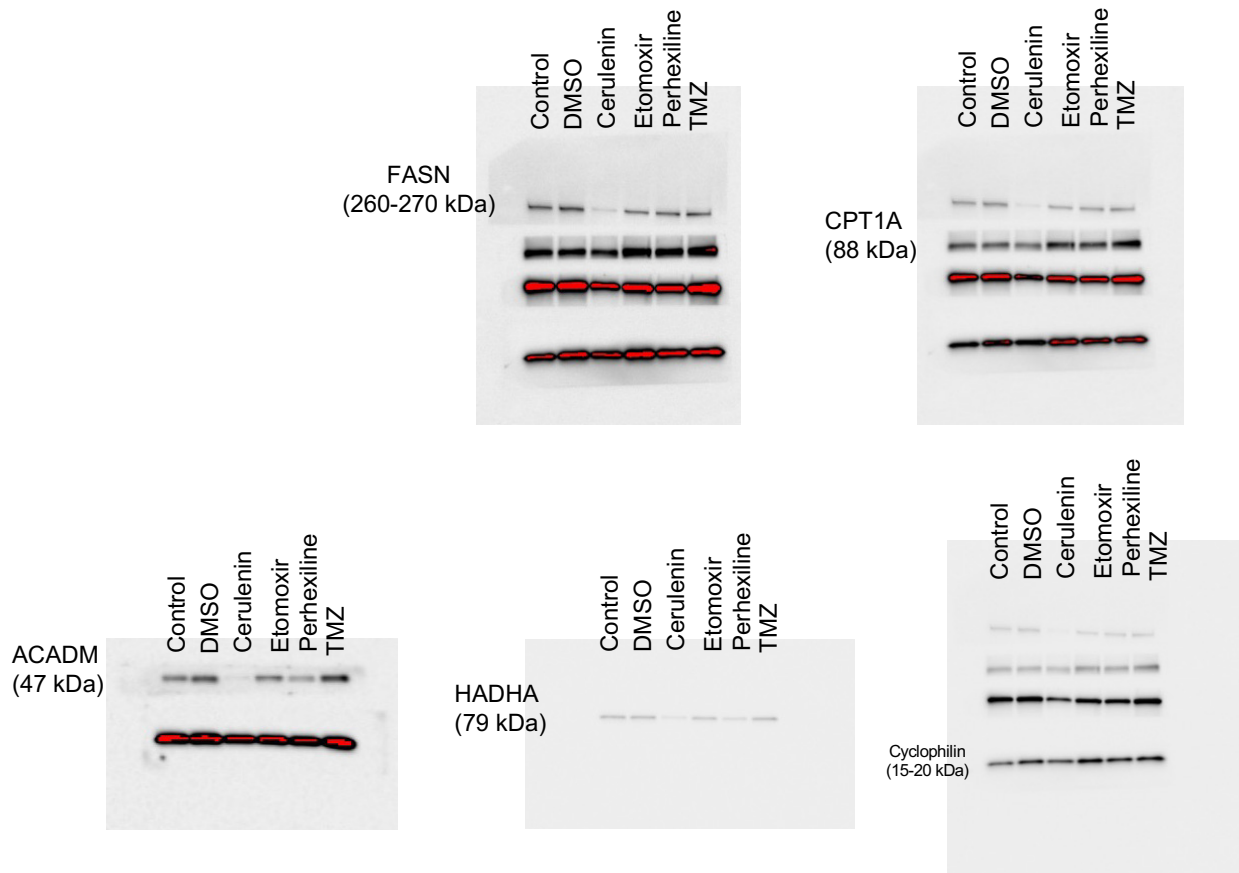
Uncropped blots of Fig. 2F of main manuscript



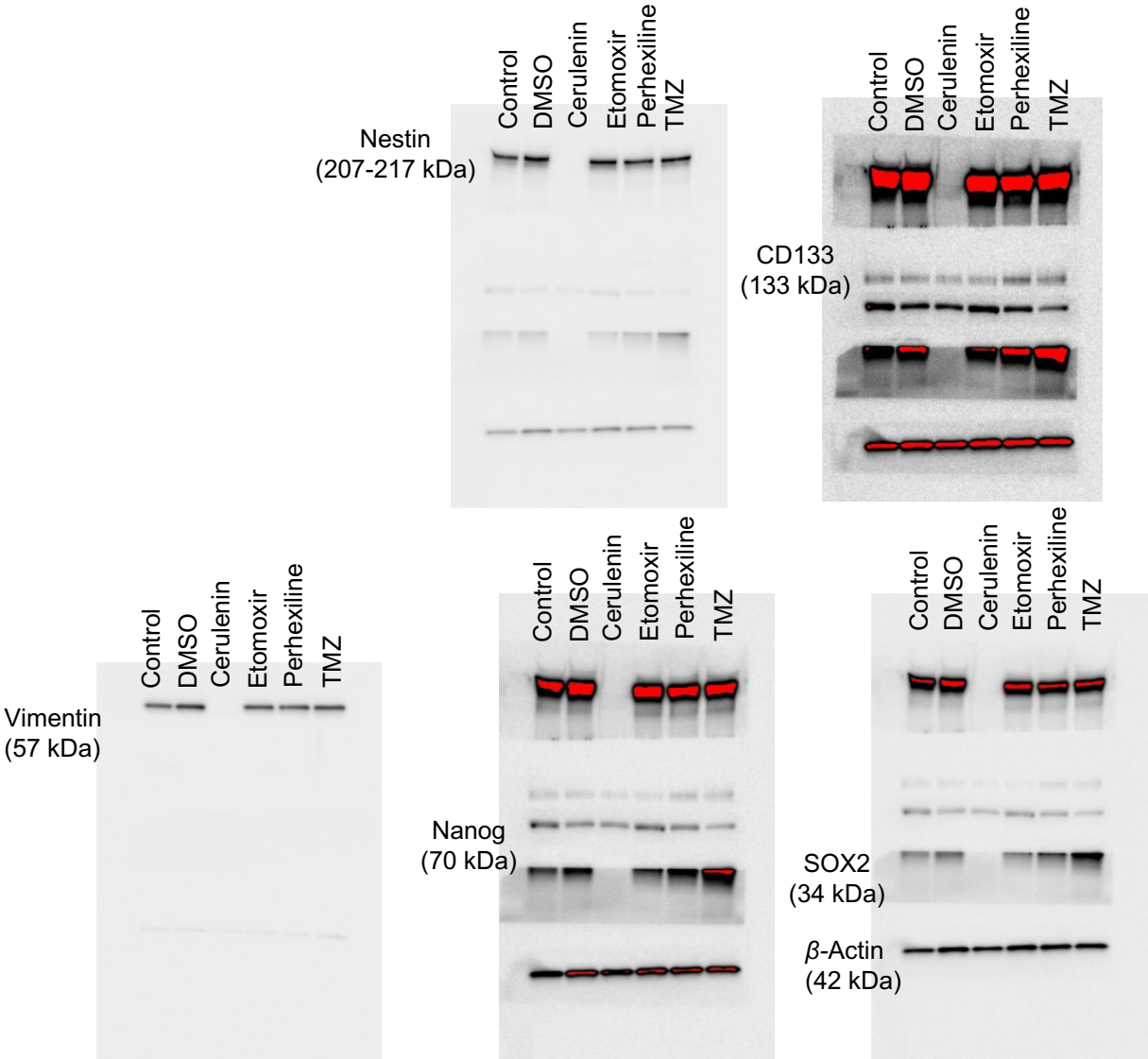
Uncropped blots for Supplementary Figure S8



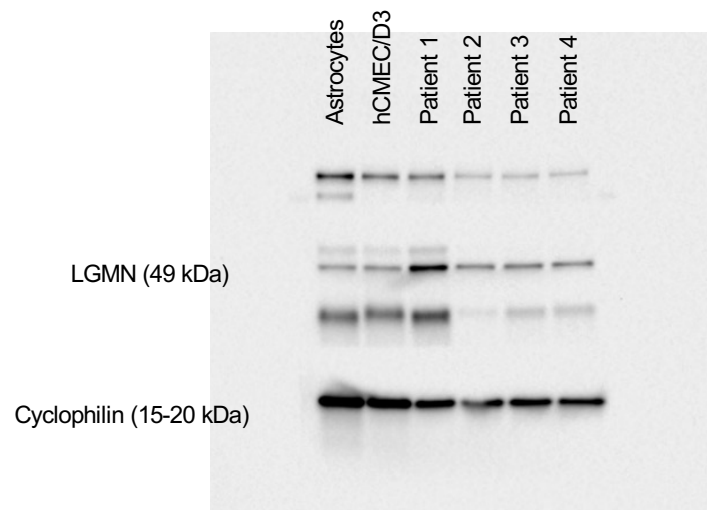
Uncropped blots for Figure S12A



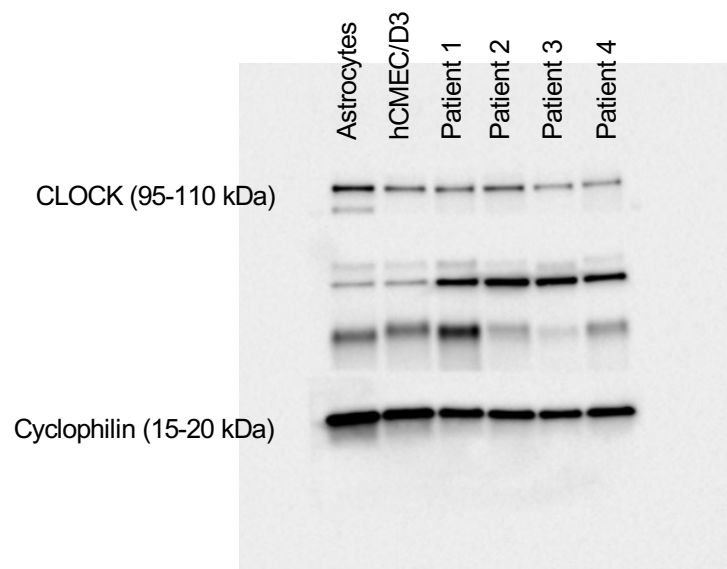
Uncropped blots for Figure S12B



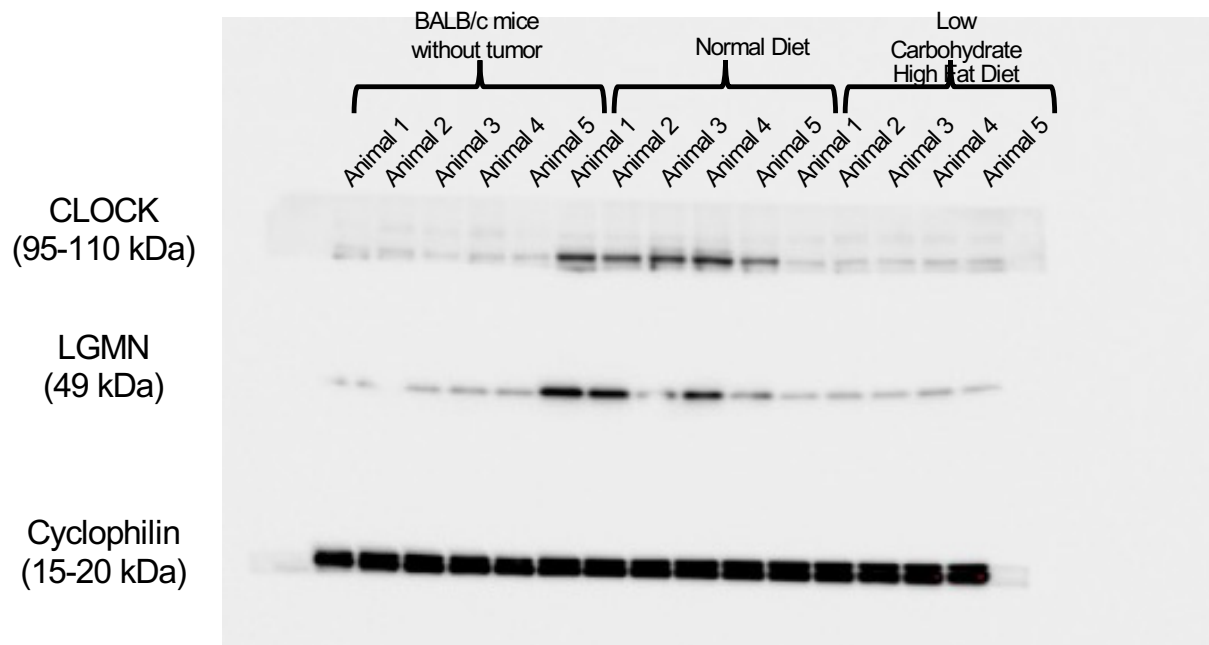
Uncropped blots of top panel of Main manuscript Figure 9C



Uncropped blots of bottom panel of Main manuscript Figure 9C



Uncropped blots of main manuscript Fig. 9D.



References

1. P. Sm, Glioma stem cell lines expanded in adherent culture have tumor-specific phenotypes and are suitable for chemical and genetic screens. *Cell Stem Cell* 4, 568-580 (2009).
2. A. Ashokan *et al.*, Simultaneous targeting of peripheral and brain tumors with a therapeutic nanoparticle to disrupt metabolic adaptability at both sites. *Proc. Natl. Acad. Sci. U.S.A.* 121, 1-12 (2024).
3. B. Ewing, L. Hillier, M. C. Wendl, P. Green, Base-calling of automated sequencer traces usingPhred. I. Accuracy assessment. *Genome Res.* 8, 175-185 (1998).
4. D. Kim, B. Langmead, S. L. Salzberg, HISAT: a fast spliced aligner with low memory requirements. *Nat. Methods.* 12, 357-360 (2015).
5. A. McKenna *et al.*, The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res.* 20, 1297-1303 (2010).
6. M. Pertea *et al.*, StringTie enables improved reconstruction of a transcriptome from RNA-seq reads. *Nat. Biotechnol.* 33, 290-295 (2015).
7. C. Trapnell *et al.*, Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. *Nat. Biotechnol.* 28, 511-515 (2010).
8. L. Florea, L. Song, S. L. Salzberg, Thousands of exon skipping events differentiate among splicing patterns in sixteen human tissues. *F1000Res.* 2, 1-24 (2013).

9. H. Jiang, W. H. Wong, Statistical inferences for isoform expression in RNA-Seq. *Bioinformatics* 25, 1026-1032 (2009).
10. S. R. Eddy, Profile hidden Markov models. *Bioinformatics (Oxford, England)* 14, 755-763 (1998).
11. C. Xie *et al.*, KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases. *Nucleic Acids Res.* 39, W316-W322 (2011).
12. M. Kanehisa, S. Goto, S. Kawashima, Y. Okuno, M. Hattori, The KEGG resource for deciphering the genome. *Nucleic Acids Res.* 32, D277-D280 (2004).
13. R. D. Finn *et al.*, Pfam: the protein families database. *Nucleic Acids Res.* 42, D222-D230 (2014).
14. E. V. Koonin *et al.*, A comprehensive evolutionary classification of proteins encoded in complete eukaryotic genomes. *Genome Biol.* 5, 1-28 (2004).
15. R. L. Tatusov, M. Y. Galperin, D. A. Natale, E. V. Koonin, The COG database: a tool for genome-scale analysis of protein functions and evolution. *Nucleic Acids Res.* 28, 33-36 (2000).
16. M. Ashburner *et al.*, Gene ontology: tool for the unification of biology. *Nat. Genet.* 25, 25-29 (2000).
17. R. Apweiler *et al.*, UniProt: the universal protein knowledgebase. *Nucleic Acids Res.* 32, D115-D119 (2004).
18. Y. Deng *et al.*, Integrated nr database in protein annotation system and its localization. *Comput. Eng.* 32, 71-74 (2006).
19. S. F. Altschul *et al.*, Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res.* 25, 3389-3402 (1997).

20. S. Djebali *et al.*, Landscape of transcription in human cells. *Nature* 489, 101-108 (2012).
21. S. Andrews (2010) FastQC: a quality control tool for high throughput sequence data. (Babraham Bioinformatics, Babraham Institute, Cambridge, United Kingdom).
22. S. Chen, Y. Zhou, Y. Chen, J. Gu, fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* 34, i884-i890 (2018).
23. M. Kawakami, K. Kawakami, R. K. Puri, Intratumor administration of interleukin 13 receptor-targeted cytotoxin induces apoptotic cell death in human malignant glioma tumor xenografts. *Mol. Cancer Ther.* 1, 999-1007 (2002).
24. G. H. Putri, S. Anders, P. T. Pyl, J. E. Pimanda, F. Zanini, Analysing high-throughput sequencing data in Python with HTSeq 2.0. *Bioinformatics* 38, 2943-2945 (2022).
25. S. R. Eddy, "A new generation of homology search tools based on probabilistic inference" in *Genome Informatics 2009: Genome Informatics Series Vol. 23*. (World Scientific, 2009), https://doi.org/10.1142/9781848165632_0019, pp. 205-211.
26. C.-H. Kang, J.-S. So, Antibiotic and heavy metal resistance in *Shewanella putrefaciens* strains isolated from shellfishes collected from West Sea, Korea. *Mar. Pollut. Bull.* 112, 111-116 (2016).
27. J. Huerta-Cepas *et al.*, Fast genome-wide functional annotation through orthology assignment by eggNOG-mapper. *Mol. Biol. Evol.* 34, 2115-2122 (2017).
28. T. Wu *et al.*, clusterProfiler 4.0: A universal enrichment tool for interpreting omics data. *Innov.* 2, 100141-100151 (2021).

29. T. Aramaki *et al.*, KofamKOALA: KEGG Ortholog assignment based on profile HMM and adaptive score threshold. *Bioinformatics* 36, 2251-2252 (2020).
30. S. Darmanis *et al.*, A survey of human brain transcriptome diversity at the single cell level. *Proceedings of the National Academy of Sciences* 112, 7285-7290 (2015).
31. D. Torre, A. Lachmann, A. Ma'ayan, BioJupies: Automated Generation of Interactive Notebooks for RNA-Seq Data Analysis in the Cloud. *Cell Systems* 7, 556-561.e553 (2018).
32. S. X. Ge, E. W. Son, R. Yao, iDEP: an integrated web application for differential expression and pathway analysis of RNA-Seq data. *BMC Bioinformatics* 19, 534 (2018).