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Supplementary information

Rewiring of cortical glucose metabolism fuels human brain cancer growth

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Supplementary Information

Rewiring of cortical glucose metabolism fuels human brain cancer growth

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1. SUPPLEMENTARY METHODS

MALDI FT-ICR mass spectrometry imaging of clinical specimens

Clinical specimens, comprising cortex, enhancing, and non-enhancing regions, were sectioned at 10 µm thickness and then thaw-mounted onto indium-tin-oxide (ITO) slides. Serial sections were obtained for hematoxylin and eosin (H&E) staining. A high-resolution image of the entire H&E tissue samples was captured using image stitching technology (Zeiss Observer Z.1, Oberkochen, Germany) with a plan-apochromat lens (20×) equipped with an AxioCam MR3 camera. A matrix solution of 1,5-diaminonaphthalene hydrochloride was prepared at a concentration of 4.3 mg/mL in a mixture of 4.5 parts HPLC-grade water, 5 parts ethanol, and 0.5 parts 1 M HCl (v/v/v). A TM-sprayer (HTX imaging, Carrboro, NC) was used to apply a uniform layer of MALDI matrix, with the following four-pass spray method: a flow rate of 0.09 mL/min, spray nozzle velocity of 1200 mm/min, spray nozzle temperature of 75°C, nitrogen gas pressure at 10 psi, a track spacing of 2 mm, and Mass spectrometry imaging experiments were conducted in negative ion mode using a 15 Tesla SolariX XR FT-ICR MS (Bruker Daltonics, Billerica, MA). The instrument was calibrated with a tune mix solution (Agilent Technologies, Santa Clara, CA) via the electrospray source. Instrumental parameters included a pixel step size of 50 µm, covering the m/z range of 46–300. Each pixel was acquired with 200 shots at a laser power of 19% (arbitrary scale) and a frequency of 1000 Hz. Continuous accumulation of selected ions (CASI) mode was employed, with Q1 set to m/z 180 and an isolation window of 200.

The obtained datasets were preprocessed, visualized, and exported to imZML using SCiLS Lab 2023a Core (Bruker Daltonics, Billerica, MA). An in-house R (v4.1.1) pipeline was used to perform per-pixel natural abundance correction and produce fractional enrichment images. The pipeline uses rMSIproc v0.3.1[66] to import data and enviPat v2.7[67] to compute theoretical MS spectrum for each ^{13}C isotopologue. Natural abundance correction is performed by solving a system of linear equations, taking into account the theoretical isotopologue MS ratios and the experimental MS spectrum at each pixel[68].

iMFA at metabolic steady state

Several adaptations were made to a previously published *in vitro* INST-MFA method to make it applicable to *in vivo* data[69]. The model parameters comprised of reaction fluxes (expressed as vector $\boldsymbol{v}$), the pool sizes of mass-balanced metabolites (expressed as vector $\boldsymbol{c}$), the isotopologues of input metabolites (**R**), and the fraction of contribution of reactant isotopologues to the product isotopologues ($\boldsymbol{f}$) used only in pyrimidine model. The vector of model parameters, $\boldsymbol{x}$ is described in Supplementary Equation 1.

Metabolites inside the model boundary were mass-balanced and are called balanced metabolites. The metabolites outside the model boundary were not mass-balanced and are called input metabolites. Due to the complicated time-dependent nature of *in vivo* metabolite enrichments, this demarcation between input metabolites and balanced metabolites was important to establish the model. A set of linear mass balance equations were used to describe overall mass balance. The sum of the isotopologues of the input metabolites was constrained to 1. Supplementary Equation 2 describes the linear constraint equations. $\boldsymbol{S}$ is the stoichiometric matrix for a model with m balanced metabolites and n reactions. $\boldsymbol{L}$ is the linear constraints on the sum of input metabolite isotopologues, corresponding to p labeled input metabolites and r total isotopologues.

The time-dependent fractional isotopologue enrichment (MIDs) of balanced metabolites is described by a set of ordinary differential equations (ODEs, Supplementary Equation 3). The rate of change of the isotopologue *d* of metabolite *i* ($M_{i,d}$) is described by applying mass balance on the isotopologue.

An objective function was minimized to solve the model and estimate the optimal parameters (Supplementary Equation 4)[70]. The objective function (*obj*) was calculated as the sum of square of the differences between the measured values of isotope enrichments (M_{expt}) and the isotopic enrichments simulated by the model (M_{sim}) divided by the standard deviation of the experimental measurements (SD_{expt}). When the metabolite pool sizes were known, the difference between the known and simulated pool sizes were included in the objective function ($c_{expt} - c_{sim}$).

To estimate the fluxes and pool sizes, an initial parameter vector was selected randomly and the ODEswere solved to estimate the objective function. The objective function was minimized subject to linear constraints and parameter bounds. The optimization was performed in MATLAB R2021b with the Artelys Knitro toolbox (version 12.4)[71]. The solver *ode15s* was used to solve ODEs as Initial Value Problems (IVPs). All metabolites were unlabeled at time *t=0*. The optimization was performed for 100 randomly generated initial parameter vectors and the chi-square-goodness-of-fit test was used to select the optimal parameter vector at 95% confidence[72]. When the objective function was higher than the chi-square threshold, the parameter space with the lowest objective value was selected.

Dynamic iMFA

Previously described DMFA approaches were adapted to apply them to *in vivo* data[33,73]. The data for radiation-induced time-dependent change in metabolite pool sizes were incorporated into the model along with the MID-time profiles. The time-course flux profiles were parametrized by expressing them as B-splines (Supplementary Equation 5). B-spline is a parametric function that can be used to fit data without assuming a functional relationship between the input and output variables. It comprises of multiple polynomial segments joined together via “control points”. These control points control the shape of the b-spline curve and are hyperparameters in the model. Another hyperparameter is the b-spline order, which is equal to *d+1*, *d* being the degree of polynomials used to construct the b-spline[33].

The parameter vector comprised of the metabolite pool size at time *t=0* ($\boldsymbol{c_0}$), and the b-spline parameters expressed as vector ***cp*** and the isotopologues of input metabolites (**R**) (Supplementary Equation 6). The rate of change of metabolite pool size was expressed as a function of the stoichiometric matrix *S* and the time-dependent flux vector ***v(t)*** (Supplementary Equation 7). Mass balance on isotopologues follows Supplementary Equation 8.

The objective function from the steady state iMFA model was modified to include the terms for time-dependent metabolite pool size (Supplementary Equation 9). Only the relative time-dependent pool size could be measured experimentally. Hence, the relative change of the metabolite pool size at time *t* (c_t) relative to the pool size at time zero (c_0) was used in the objective function. The initial metabolite pool size values were the same as those used in the steady state model. The relative time-dependent concentration profiles are provided in Extended Data Fig. 6h.

To estimate the flux profiles, an initial parameter vector was randomly selected, and the ODEs were solved to minimize the objective function as done for the steady state model. The flux values at *t=0* were constrained to the 95% confidence intervals estimated from the steady state model. All metabolites were unlabeled at time *t=0*. The optimization was performed for 100 randomly

generated initial parameter vectors and the parameter vector with the lowest objective value was selected. To estimate the 95% confidence intervals, the parameter bounds were estimated as done for the steady state model. The determined parameter bounds were used to create the time-course flux profiles corresponding to the 95% confidence interval[74]. All the acceptable flux profiles were recorded and the minimum and maximum flux values at a certain time were reported as the 95% flux bound.

The b-spline hyperparameter selection was performed prior to parameter optimization. B-splines of orders 2,3, and 4 were tested and a spline of order 3 (quadratic b-spline) was selected after qualitative analysis of the results. A randomized approach was used to select the b-spline control points. All flux profiles were assumed to have the same control points. Control points in the range of [0.2,0.8] were tested at intervals of 0.05. Each time, one position was selected, and parameter optimization was performed for the same initial parameter vector. The control point with the lowest objective was recorded. To optimize the placement of a second control point, the same strategy was used. The second control point was placed at a minimum distance of 0.2 from the first control point. The procedure was repeated for 100 initial guesses. For both the GBM and normal cortex samples, the addition of a second control point did not reduce the minimum objective value for the 100 optimizations. Hence, one control point was used to simulate both conditions. Based on the model fit, 0.3 was selected as the control point for GBM because of a lower mean objective value and central position. 0.65 was selected for the normal cortex.

Metabolic model of purine pathways

The metabolic model of the purine synthesis pathway was curated on the basis of experimental data and current literature. The KEGG database was referenced for a list of reactions and the associated enzymes[75]. Data from the human proteome atlas was used to remove the reactions whose enzymes have low expression in GBM[76]. Consequently, purine model consists of 17 reactions associated with production and consumption of 6 nucleotides namely, IMP, inosine, GMP, GDP, guanosine, and AMP (Extended Data Fig. 6c, Supplementary Tables 3-4). Further, IMP was assumed to directly produce GMP and the intermediate XMP was excluded from the model because the enrichment data for XMP was not available. The input metabolites of our model include R5P, CO_2, Me-THF, hypoxanthine, guanine, adenosine, and glycine. All the ribose units (R5P, R1P, PRPP) were assumed to have the same enrichment. The rest of the metabolites are substrates in the purine pathway and outside the model boundary. The metabolites adenosine and hypoxanthine were assumed to be unlabeled based on our experimental data. The enrichment data for guanine was not available and it was assumed to be produced mainly from the degradation of old DNA and RNA units and hence was unlabeled in the model. The cellular carbon dioxide pool was also assumed to be unlabeled. Based on the stoichiometry balances, sink fluxes of inosine and guanosine are associated with degradation of these nucleosides to their corresponding nucleobases, hypoxanthine and guanine. Mass-balanced metabolites were assumed to be not accumulated in the treatment-naïve tissues and hence the same rate of production and consumption were expected by constraining the stoichiometric balances (Supplementary Equation 2). However, the enrichment of MIDs for mass-balanced metabolites were assumed to be accumulated over time based on our time course experiments (Supplementary Equation 3). Parameter values and their lower and upper confidence bounds associated with the lowest objective function were reported in Supplementary Tables 5 and 6. In the radiation treated tissues, we incorporated time-dependent changes of metabolite pool sizes (Supplementary Equations 7 and 8). Hence, for treatment-naïve and radiation treated conditions, iMFA at metabolic steady state and dynamic iMFA were performed, respectively.

The enrichment of methyl units cannot be measured experimentally, and the values were estimated from the enrichments of serine and glycine. To estimate the methyl unit enrichment, a pseudo-steady state assumption was applied. Serine, glycine, and the methyl enrichments were assumed to be in equilibrium at any given time. The corresponding equations for the metabolite isotopomers are represented in Supplementary Table 7. The variance-weighted sum-of-squared residuals was calculated between the experimental MID values of serine and glycine and the model estimated values. The optimization was performed from 10 initial guesses. To estimate the error in the estimated methyl enrichment, gaussian noise was added to the experimental values and the optimization was repeated 200 times.

Metabolic model of pyrimidine pathways

Pyrimidine model was implemented based on our available enrichment data for pyrimidines, the active reactions in KEGG database, and the enzymes that have high expression in GBM by checking the human protein atlas[75,76]. The pyrimidine model consists of six reactions associated with production and consumption of uridine and UMP (Supplementary Table 8 and Extended Data Fig. 6d). Flux bounds were selected based on our observation of optimization space and were relaxed to have no overlap with the flux confidence intervals (Supplementary Table 8). Parameter values and their lower and upper confidence bounds associated with the lowest objective function were reported in Supplementary Tables 9 and 10.

The input metabolites of our model include R5P, aspartate, CO2, and cytidine (Supplementary Table 11). We assumed that all ribose units including R5P, PRPP, and R1P share the same enrichment profiles. Mass-balanced metabolites were assumed to be not accumulated in the tissue and hence the same rate of production and consumption were expected for UMP and uridine by constraining the stoichiometric balances. However, the enrichment of MIDs for mass-balanced metabolites were assumed to be accumulated over time based on our time course experiments. Thus, for mass-balanced metabolites, mass isotopologue balances were written as shown in Supplementary Equation 10.

On the left side of Supplementary Equation 10, the rate of change of UMP and uridine isotopologues is calculated. c_{UMP} and $c_{URIDINE}$ denotes the concentration of UMP and uridine. v denotes fluxes described in Supplementary Table 8 and M_i represents isotopologues of input and mass-balanced metabolites with i heavy carbons. The first term shows that UMP gets labeling from the salvage flux ($v_{URIDINE \rightarrow UMP}$) through uridine MIDs ($M_{URIDINE,i}$ where i denotes the M+i enrichment). The second term shows UMP gets labeling from the de novo UMP synthesis flux ($v_{de\ novo}$) through R5P ($M_{R5P,j}$) and aspartate ($\widetilde{M}_{ASP,k}$) MIDs. In this model, we assumed that CO_2 is unlabeled. The produced UMP in de novo synthesis consists of nine carbons in which five carbons come from R5P to structure the ribose unit of UMP. The remaining four carbons in the uracil ring come from unlabeled CO_2 pool (2nd position), and aspartate (4th, 5th, and 6th positions). UMP de novo synthesis is a decarboxylation reaction and the carbon leaving the system as CO_2 might be labeled if the first carbon of aspartate is labeled. Therefore, we defined a new isotopologue distribution for aspartate ($\widetilde{M}_{ASP,k}$) that only contributes to the UMP labeling. Supplementary Table 12 shows aspartate isotopomers that correspond to each isotopologue of aspartate ($M_{ASP,M+i}$) that can enrich UMP in the same way. To do so, three isotopomer fractions were defined (f_1, f_2, f_3). For instance, suppose one labeled carbon in UMP comes from aspartate ($\widetilde{\boldsymbol{M}}_{\boldsymbol{ASP,M+1}}$), since the labeling of the first carbon of aspartate does not affect the labeling of UMP, the following isotopomers of aspartate can produce one labeled carbon in UMP: 0001, 0010, 0100, 1001, 1010, and 1100. The defined fractions f_i corresponded to $M_{ASP,i}$ helped us to

separate the isotopomers into isotopomers with labeled produced CO_2 and with unlabeled produced CO_2. For example, f_1 is the ratio of isotopomer 1000 to all isotopomers of M+1 (0001, 0010, 0100, 1000). To constrain the lower and upper bounds of fractions, we used the isotopomer distributions in mouse and human tumors reported in the literature[77]. Hence, we set the constraints to [0, 0.4], [0.2, 0.6], and [0, 1] for f_1, f_2, and f_3, respectively. To combine the probability of production of UMP M+i isotopologues ($M_{UMP,i}$) from aspartate and R5P, we multiplied M+j of R5P ($M_{R5P,j}$) to M+k of newly defined aspartate isotopologues ($\widetilde{M}_{ASP,k}$) where j+k=i. The third term in Supplementary Equation 10 represents the consumption of UMP in other reactions that are not included in this model ($v_{UMP \to UMP_{out}}$) and production of uridine from UMP. Uridine is synthesized from cytidine ($v_{CYTIDINE \to URIDINE}$) and UMP ($v_{UMP \to URIDINE}$) and is consumed in other reactions that are not included in this model ($v_{URIDINE \to URIDINE_{out}}$) and salvage pathway to synthesize UMP.

Metabolite pool sizes

Purine and pyrimidine pool sizes for mouse brain were obtained from literature (GDP[78], Inosine[79], AMP[78], Guanosine[79], GMP[80], IMP[81], Uridine[82], UMP[82]; Supplementary Table 13). Only the values reported with the measurement standard deviation were included in the objective function. When the measurement error was not available, the reported value was only used to set the bounds for the corresponding pool size parameter. Experimental data was used to estimate the pool sizes for GBM tissue. The average relative metabolite ion count between GBM and brain tissue was calculated and multiplied by the pool size in the brain. Standard error propagation techniques were used to estimate the standard deviation of the GBM pool sizes. GMP was not detected in the brain tissue but was detected in the GBM tissue. Hence, the bounds for GMP concentration were set to be higher than the pool size in the brain. The pool size data are reported in Supplementary Table 13.

Enrichment of input metabolites

The time-course isotopologue abundances of input metabolites are required to apply the metabolic model. The time-course enrichment of metabolites is complex in *in vivo* models and cannot be described by exact mathematical functions. A linear piecewise function was fit to the experimental time-point data to estimate the enrichment-time relationship of input metabolites. Supplementary Equation 11 describes the calculation of the slope of the isotopologue R_i between time points t_{m+1} and t_m. A linear function was selected because it requires the least assumptions and does not overfit the data. To account for the uncertainty in the experimental measurements, the input isotopologue abundances were allowed to vary within one standard deviation of the experimentally measured mean value.

Estimation of parameter confidence intervals

A similar approach to a previously described approach was used to determine the 95% confidence intervals for the estimated fluxes[72]. To estimate upper/lower confidence level of an estimated parameter, in each iteration, the estimated parameter is increased/decreased with a step size of 10% and other estimated parameters are kept unchanged. These parameters are set as initial guess of the optimization problem. The optimization problem is solved, and the iteration continues with an updated initial guess of the certain parameter until the increment of that parameter is small (0.1 for fluxes and concentrations and 0.01 for isotopomer fractions), or the parameter reaches its upper/lower bound. If optimization is infeasible or the change of objective value from its optimal value is higher than χ^2 of 95% confidence intervals, the step size is decreased in the following order: 10%, 5%, 2%, and 1%. When there is no reduction possible for the step size, the iterations

end. The updated value of the estimated parameter is recorded as the upper/lower confidence level of that parameter. The difference between GBM and cortex fluxes is statistically significant if there is no overlap between the flux confidence intervals in GBM and cortex.

MFA of serine contribution in human tumors

The model comprised of three compartments which correspond to the cortex, enhancing tumor, and non-enhancing tumor (Extended Data Fig. 8o). Each compartment had its own 3PG pool which was used for *de novo* serine synthesis within the compartment. The compartments were linked by a common input of external serine from circulation. A source of unlabeled serine was included in the model since serine might be derived from protein breakdown. Autophagy has been shown to be a source of serine in *in vitro* models of glioma[83]. Further, *de novo* serine synthesis is rate limited by the levels of the PHGDH enzyme, and serine may not be in isotopic equilibrium with 3PG. An unlabeled serine source helped correct for this effect. The net serine input to the model from *de novo* synthesis, external serine uptake, and the unlabeled serine from recycle was assumed to be 1 unit. Forward and reverse SHMT fluxes and the fluxes of glycine to 5,10-methylene-THF were included in each compartment. Serine was assumed to be the only source of glycine and one-carbon unit. This is in accordance with previous findings which show that glycine is mainly derived from serine in the brain[84,85]. Further, serine has been described as a major source of glycine and one-carbon units in gliomas[42,83]. The enrichment of externally available serine was also assumed to be the same as the serine in circulation. Further, we assumed that tissues are homogenous and did not consider any cell-cell metabolic interactions in the serine-glycine pathway. These assumptions were important to reduce the model complexity and prevent the model from becoming highly underdetermined. Because of the various unknown factors, we only analyzed and reported the relative contributions of serine synthesis and uptake pathways that would yield labeled serine and did not analyze the absolute values. Sink fluxes were also included for serine, glycine, and formyl-THF to account for consumption not included in the model. The list of reactions and the associated carbon transitions are provided in Supplementary Table 14.

The model parameters x comprised of the fluxes v and the known IDV values D of 2PG/3PG, serine, and glycine in the three compartments. The MID of plasma serine was also included in the model parameters (Supplementary Equation 12). The fluxes in each compartment were mass balanced, which was described through the stoichiometric matrix $\boldsymbol{S}_{m\times n}$ corresponding to m metabolites and n reactions (Supplementary Equation 13). The external serine was not included in the mass balance. To make sure that the sum of all IDVs of a certain metabolite is always equal to 1, the sums of the IDVs of the external serine and the three tissue 3PG were constrained to 1. This added 4 equations to the model (one for external serine, 3 for 3PG in the three tissue compartments). These equations were represented by the matrix $\boldsymbol{L}_{4\times d}$ corresponding to d total IDV values in the model. The three equations constraining the new serine input to 1 were also included in the linear balance equations and are represented by the matrix $\boldsymbol{N}_{3\times n}$.

The IMM method was used to formulate the isotopic mass balance equations (Supplementary Equation 14)[86]. These equations were used to add non-linear constraints to the model. Additional linear constraints were applied to avoid the model converging to a trivial solution. The parameters were optimized to minimize the objective function, which was defined to minimize the differences between the experimentally measured MIDs and the MIDs simulated by the model (Supplementary Equation 15). The difference between the experimental and simulated values was normalized to the experimental standard deviation to account for experimental variation. An

L2 regularization term for the flux parameters was also included in the objective function because the model was underdetermined, i.e. we had more unknown parameters than the number of known values. The value of the regularization parameter was set to 0.1. To solve the model, local optimization was performed from 200 randomly selected initial points. Knitro toolbox (version 12.4) was used for the optimization in MATLAB R2021b. The solution with the lowest objective value was selected. To estimate the 95% confidence intervals using Monte Carlo simulations, gaussian noise was added to the experimental data and the optimization was repeated 1000 times. The distribution of the resulting solutions was used to determine the confidence intervals[72].

Normalization of *in vivo* enrichments to correct for inter-animal variability

The tissue MID data was saturated to the saturation ^{13}C mean enrichment of plasma glucose for each animal. Since some animals were sacrificed at time points before plasma enrichment reached saturation, a tracer kinetics model was used to estimate the saturation enrichment. The time-course plasma glucose ^{13}C enrichment profiles (f) fit to a two-compartment tracer kinetics model for each animal (Supplementary Equation 16)[87]. The rate of tracer infusion, $r = 0.012$ *mg/min/g*. The bolus infusion, $P = 0.4$ *mg/min/g*. Other parameters were the time after infusion (t), physiological glucose pool size (Q), the fractional contribution of two sources of labeled glucose (H_1, H_2), and the rate constant for glucose enrichment from the two sources (k_1, k_2). H_1, H_2, k_1, k_2, and Q were determined by fitting the experimental data at 4hr (which shows a steady state plateau) to Supplementary Equation 16, then Q were updated for each mouse by fitting the experimental data for all time points while the other kinetic parameters were assumed to be constant. Supplementary Equation 17 shows a property of H coefficients. Supplementary Equation 18 was used to calculate the saturation enrichment (SE) for each animal. To normalize tissue metabolite enrichment to plasma glucose enrichment, tissue metabolite enrichment was divided by SE for each mouse. The correction factor was calculated by dividing the saturation enrichment of a specific animal by the average saturation enrichment value across all animals. The correction factor for the 30-minute time point was set to 1 since the saturation enrichment could not be estimated accurately for these animals. To correct the data, labeled MID values were divided by the correction factor. M+0 was estimated by subtracting the corrected labeled MIDs from 100.

Generation and processing of RNA-seq

Total RNA was extracted from tumor samples obtained from three patients (1, 3, and 7; Extended Data Table 1) using standard protocols, and RNA sequencing was performed by Tempus (Chicago, IL) to generate raw sequencing data. For each sample, single-end sequencing generated 150 nucleobase reads for 39-85 million sequences. The quality of raw reads was checked using FastQC (version 0.12.1). Bam files were sorted by query name using samtools (version 1.13). Sequencing reads were aligned to human reference genome from Ensembl (Homo_sapiens.GRCh38.113.gtf.gz) using htseq-count (version 2.0.9) with the following command line options: --format=bam --order=name --type=exon --stranded=no.

Molecular subtype classification using bulk RNA-seq

We integrated our RNA-seq data with TCGA-GBM[5,88] RNA-seq data (accessed from GDC data portal https://portal.gdc.cancer.gov/ on Dec 13, 2024). Read counts were normalized to reads per kilobase per million mapped reads (RPKM) and transcript per million (TPM) using the Ensemble transcripts with the maximum transcript length. RPKM values were transformed to log2(1+RPKM) and Ensemble ids were converted to HGNC ids or gene names using biomaRt library (version

2.54.0) in R (4.2.2). Signature genes of molecular subtypes were defined previously in ref[63]. TPM values were imported to GenePattern[89] (https://www.genepattern.org/ accessed on Dec 16, 2024) single sample geneset enrichment analysis (ssGSEA version 5)[90] and sample normalization method was set to rank to calculate geneset enrichment of molecular subtypes. Hierarchical clustering grouped integrated RNA-seq samples based on the similarities of their enrichment scores. Since the molecular subtypes of TCGA-GBM were identified previously in ref[63], the samples from our patient cohort that clustered with the TCGA-GBM samples may have similar molecular subtypes as TCGA-GBM samples. To identify molecular subtypes of our patient cohort independent of TCGA-GBM, we imported log-normalized RPKM values to GlioVis portal[91] (https://gliovis.bioinfo.cnio.es/, version 0.20, accessed on Dec 17, 2024) and performed three available methods including support vector machine (SVM), k-nearest neighbor (KNN), and ssGSEA. A combined assessment of all methods was conducted to assign one subtype to each sample.

Molecular subtype classification using scRNA-seq

We used scRNA-seq of our patient cohort (from our unpublished manuscript, "Digital twins for In Vivo Metabolic Flux Estimations in Cancer Patients") to identify molecular subtypes of cells for each patient's tumor tissue. A list of signature genes of three molecular subtypes has been provided in[63], which includes 50 genes per molecular subtype. After integrating all patient samples and checking the quality control using Seurat (version 4.2.0), the UMI counts were used in AUCell (version 1.28.0) to calculate gene set enrichment scores based on the ranking of specified molecular subtype genes among all expressed genes in a cell[92]. Following AUCell guidelines, a threshold for each molecular subtype gene set was selected based on the global distribution of area under the curve of gene set rankings (by setting the selected thresholds to Global_k1). These thresholds were used to assign cells to molecular subtypes. This analysis was performed in R (version 4.4.2).

Transcriptional analysis of neurotransmitter signaling and serine metabolism genes in GBM and cortex

To understand the transcriptional regulation of serine related enzymes and neurotransmitters[93,94], we performed differential expression analysis on two available RNA-seq datasets using GEO2R[95] (https://www.ncbi.nlm.nih.gov/geo/geo2r/ accessed on Dec 27, 2024), an interactive web tool based on R (version 4.2.2): GSE59612 which includes normal cortex, enhancing and non-enhancing glioma samples[65] and GSE165595 which includes normal cortex and GBM samples[64]. Fold changes show the comparison of differential expressions in glioma samples over cortex samples with a false discovery rate less than 0.05 using Benjamini-Hochberg test.

Snapshot LC-MS metabolomics of tissues and plasma

LC-MS equipment and parameters for snapshot analysis of tissue metabolites are as described previously[96]. For targeted analysis, samples were run on an Agilent 1290 Infinity II LC-6470 Triple Quadrupole Tandem Mass Spectrometer system consisting of the 1290 Infinity II LC Flexible Pump (quaternary pump), the 1290 Infinity II Multisampler, the 1290 Infinity II Multicolumn Thermostat with 6-port valves, and the LC-6470 Triple Quadrupole Tandem Mass Spectrometer. Agilent Masshunter Workstation Software LC/MS Data Acquisition for 6400 Series Triple Quadrupole MS with Version B.08.02 was used for compound optimization, calibration, and data acquisition.

Solvents used for LC-MS of metabolite extracts from tissues are as follows: Solvent A: 97% water, 3% methanol, 15 mM acetic acid, 10 mM tributylamine at pH of 5; Solvent C: 15 mM acetic acid and 10 mM tributylamine in methanol; Washing Solvent D: acetonitrile; LC system seal washing solvent: 90% water, 10% isopropanol; needle wash solvent: 75% methanol, 25% water. LC-MS reagents are as follows: GC-grade Tributylamine 99% (Acros Organics), LC-MS grade acetic acid, Optima (Fisher Chemical), InfinityLab deactivator additive and ESI-L low concentration tuning mix (Agilent Technologies), LC-MS grade solvents of water, and acetonitrile, methanol (Millipore), isopropanol (Fisher Chemical).

An Agilent ZORBAX RRHD Extend-C18, 2.1 × 150 mm and a 1.8 μm and ZORBAX Extend Fast Guards for UHPLC were used in the separation. The LC gradient profile is: at 0.25 mL/min, 0–2.5 min, 100% A; 7.5 min, 80% A and 20% C; 13 min, 55% A and 45% C; 20 min, 1% A and 99% C; 24 min, 1% A and 99% C; 24.05 min, 1% A and 99% D; 27 min, 1% A and 99% D; at 0.8 mL/min, 27.5–31.35 min, 1% A and 99% D; at 0.6 mL/min, 31.50 min, 1% A and 99% D; at 0.4 mL/min, 32.25–39.9 min, 100% A; and at 0.25 mL/min, 40 min, 100% A. Column temperature is kept at 35°C, samples are at 4°C, and injection volume is 2 μL.

The mass spectrometer is calibrated with the Agilent ESI-L low concentration tuning mix. Source parameters are as follows: gas temperature 150°C, gas flow 10 L/min, nebulizer 45 psi, sheath gas temperature 325°C, sheath gas flow 12 L/min, capillary –2000 V, and delta EMV –200 V. Dynamic multiple reaction monitoring (dMRM) scan type is used with 0.07 min peak width, and acquisition time is 24 min. dMRM transitions and other parameters for each compound are listed in Supplementary Table 15. Delta retention time of ±1 min, fragmentor of 40 eV, and cell accelerator of 5 eV are incorporated in the method.

^{13}C and ^{15}N metabolomics of tissues and plasma

Isotope labeling was determined using an Agilent system consisting of an Infinity Lab II UPLC coupled with either a 6545 or 6230 QTOF mass spectrometer (Agilent Technologies, Santa Clara, CA) operated by the University of Michigan Metabolomics Core using a JetStream ESI source in negative mode. The following source parameters were used: Gas Temp: 250 °C, Gas Flow: 13 L/min, Nebulizer: 35 psi, Sheath Gas Temp: 325 °C, Sheath Gas Flow: 12 L/min, Capillary: 3500 V, Nozzle Voltage: 1500 V.

The UPLC was equipped with a 10-port valve configured to allow the column to be either eluted to the mass spectrometer or back-flushed to waste. The chromatographic separation was performed on an Agilent ZORBAX RRHD Extend 80Å C18, 2.1 × 150 mm, 1.8 μm column with an Agilent ZORBAX SB-C8, 2.1 mm × 30 mm, 3.5 μm guard column. The column temperature was 35 °C. Mobile phase A consisted of 97:3 water/ methanol and mobile phase B was 100% methanol; both A and B contained tributylamine and glacial acetic acid at concentrations of 10mM and 15mM, respectively. The column was backflushed with mobile phase C (100% acetonitrile, no additives) between injections for column cleaning.

The LC gradient was as follows: 0-2.5 min, 0% B; 2.5-7.5 min, linear ramp to 20% B min, 7.5-13 min, linear ramp to 45% B; 13-21 min linear ramp to 99% B and held at 99% B until 25 min. At 25 minutes, the 10-port valve was switched to reverse flow (backflush) through the column, and the solvent composition changed to 95% C and held there for 3 min. From 28 to 28.5 min, the flow rate was ramped to 0.8 mL/min, held until 32.5 min, then reduced to 0.6mL/min. From 32.5 to 33.25 the solvent was ramped from 99% to 0% C while flow was simultaneously ramped down from 0.6-0.4mL/min and held until 39.9 min., at which point flow rate was returned to starting conditions at 0.25mL/min. The 10-port valve was returned to restore forward flow through the column at 40 min. An isocratic pump was used to introduce reference mass solution through the

reference nebulizer for dynamic mass correction. Total run time was 40 min. The injection volume was 5 µL.

Metabolite abundance in tissues

In analyses comparing metabolite abundance in human and mouse glioma to cortex, LC-MS values (total AUC of labeled and unlabeled species acquired via either Snapshot metabolomics or ^{13}C metabolomics methods described above) obtained for compounds in tissues from infused and non-infused mice and patients were preprocessed and used to generate relative abundance values with corrections for false discovery rate with MetaboAnalyst (www.metaboanalyst.ca, version 6.0), and results were used to generate volcano plots. In analyses comparing metabolite abundance in orthotopic GBM and cortex from mice on control diets to those from mice on -SG diets in unlabeled mice, values were preprocessed as above followed by principal component analysis and hierarchical clustering with MetaboAnalyst (versions 5.0 and 6.0). Corresponding heatmaps were generated using Morpheus Matrix Visualization and analysis tool (https://software.broadinstitute.org/morpheus).

Stable isotope tracing in patient-derived gliomaspheres

Patient-derived 374gs gliomaspheres were seeded as single cells into ultra-low adhesion 6-well plates at a density of 100,000 cells per mL in complete media containing 17.5 mM [U^{13}C]-glucose (99%, Cambridge Isotope Laboratories) and either 0 mM or 0.25 mM serine and glycine. Isotope label-containing media was replenished every 24 hours to avoid nutrient exhaustion, and metabolite extraction was performed as described[97] after a total incubation time of 96 h. Cell suspensions were collected and vacuum-filtered onto a 0.45 µm nylon filter (EMD Millipore) on a glass filter (EMD Millipore). Filters were rapidly washed with cold 150 mM ammonium acetate. Filters were then flipped cell-side down in extraction buffer (80% methanol at -80°C) and allowed to stand for 25 minutes at -80°C before being flipped back (cell-containing side facing up). Spheres were pipetted several times to promote detachment from the filter. Extracts were collected into microtubes and centrifuged at 10,000×g for 5 min at 4°C. Supernatants were collected into HPLC vials (Thermo Scientific), evaporated and stored at -80°C until processing for LC-MS.

Metabolite fractions were dried and resuspended in 100 µl 50% CAN, and 5 µl was loaded onto a Luna 3µm NH2 100A 150 × 2.0 mm column (Phenomenex). The chromatographic separation was performed on a Vanquish Flex (Thermo Scientific) with mobile phases A (5 mM ammonium acetate buffer at pH 9.9) and B (acetonitrile) and a flow rate of 200 µL/min. A linear gradient from 15% A to 95% A over 18 minutes was followed by 9 min isocratic flow at 95% A and re-equilibration to 15% A. Metabolites were detected with a Thermo Scientific Q Exactive mass spectrometer run with polarity switching (+3.5 kV/− 3.5 kV) in full scan mode with an m/z range of 70-975. Maven (version 8.1.27.11) was used to quantify metabolites by area under the curve (TopArea) using expected retention time and accurate mass measurements (< 5 ppm). ^{13}C labeling measurements from LC-MS were corrected for natural ^{13}C isotope abundance and ^{12}C impurity present in the ^{13}C-glucose tracers using AccuCor[98,99] version 0.3.1. Data analysis was performed using in-house scripts in the R programming language. Peak intensities were normalized to cell number or protein concentration.

Animal growth, complete blood counts, and blood chemistry

C57BL/6J mice (Jackson Laboratories) were placed on either control diets or serine/glycine-restricted diets at 8 weeks of age and remained on respective diets indefinitely. Animal weights and behavior were monitored regularly (approximately every 3 to 14 days). After approximately 9 months, whole blood was drawn from the submandibular vein into K^{2+} EDTA anticoagulant tubes (for complete blood counts) and serum separator tubes (for serum chemistries). Complete blood counts were performed with a Heska Element HT5 (Heska Corporation, Loveland, CO) automated veterinary hematology analyzer. For chemistries, whole blood was allowed to clot and separated into serum by centrifugation. Serum chemistries were determined with a Liasys 330 (AMS Alliance, Guidonia, Italy) automated wet chemistry analyzer. Hematological assays were performed within the ULAM In Vivo Animal Core Pathology Laboratory at the University of Michigan. Quality control was performed daily using manufacturer-provided reagents, and the laboratory is a participant in an external independent quarterly quality assurance program (Veterinary Laboratory Association Quality Assurance Program).

Intracranial tumor bioluminescence

To monitor intracranial tumor establishment and growth in mice, we employed bioluminescence imaging, which leverages the expression of luciferase in intracranial tumors. For each measurement, mice intracranially implanted with luciferase-expressing tumors were intraperitoneally injected with 150-225 mg/kg D-luciferin. Mice were then placed under anesthesia (2% isoflurane inhalation) and imaged 10 minutes after injection using an IVIS™ Spectrum imaging system (PerkinElmer) managed by the University of Michigan Center for Molecular Imaging.

Histological staining of intracranial tissues and quantification

In experiments comparing tumor burden and proliferation index in orthotopic mouse models, tissue harvests from mice on control diets and -SG diets were performed once control mice neared humane endpoints. Mice were deeply anesthetized, and blood was collected by cardiac puncture into EDTA-coated vials for plasma preparation. Mice were then decapitated, and brains were fixed and embedded for histopathologic analysis, which was performed as previously[7] by H&E staining and Ki-67 staining. The primary antibody for Ki-67 (BD Biosciences 550609) was used at a 1:1000 dilution. Immunohistochemical staining was performed using the Vectastain Elite ABC Kit (PK-6102), which included the secondary antibody (1:200). To determine Ki-67 positive cells in tumors, DAB staining was quantified using QuPath (https://qupath.github.io, version 0.5.1). Tumor regions in each field were identified based on H&E staining. Stain vectors were estimated, and positive cells were detected in resulting images using Optical Density sum parameters. Three intensity thresholds were applied to detect all DAB-positive cells, categorized by the intensity of the staining. The percentage of Ki-67 positive cells was defined as the sum of cells detected across all three intensity thresholds, divided by the total number of cells in the tumor region.

2. SUPPLEMENTARY TABLES

Supplementary Table 1

Molecular subtype classification of patient tumors by bulk RNA-seq and scRNA-seq. Three of the four methods used for RNA-seq-based classification (Extended Data Fig. 1) were matched for each patient's tissue.

Patient ID	Molecular subtype classification based on	
	bulk RNA-seq	**scRNA-seq**
P1	Proneural	Enhancing: Mesenchymal, Non-enhancing: Mesenchymal
P2	N/A	Enhancing: Mesenchymal, Non-enhancing: N/A
P3	Proneural	Enhancing: N/A, Non-enhancing: N/A
P4	N/A	Enhancing: Proneural, Non-enhancing: Proneural
P5	N/A	Enhancing: N/A, Non-enhancing: N/A
P6	N/A	Enhancing: Mesenchymal, Non-enhancing: Mesenchymal
P7	Classical	Enhancing: Mesenchymal, Non-enhancing: Classical
P8	N/A	Non-enhancing: Mesenchymal

Supplementary Table 2

Molecular subtypes of orthotopic models. Molecular subtypes of orthotopic patient-derived models used in this study were approximated previously by expression of transcriptional markers (HF2303) and methylation profiling (GBM12 and GBM38)[16-19].

Mouse model	Molecular subtype
GBM38	Proneural
GBM12	Classical
HF2303	Mesenchymal

Supplementary Table 3

List of purine model reactions and associated flux bounds. An irreversible reaction type refers to reactions that proceed only in one direction. Reaction type sink refers to the reactions that consume the metabolite and are not included in the model. Flux bounds were set according to the experimental data. AMP: adenosine monophosphate; Me-THF: 5-methytetrahydrofolate; CO2: carbon dioxide; GLY: glycine; IMP: inosine monophosphate; GDP: guanosine diphosphate; GMP: guanosine monophosphate; R5P: ribose-5-phosphate.

Reaction No.	Reaction	Reaction Type	Flux Lower Bound (pmol/mg/hr)	Flux Upper Bound (pmol/mg/hr)
1	R5P + GLY + 2Me-THF + CO_2 == IMP	Irreversible	0.01	100
3	IMP == INOSINE	Irreversible	0.01	1000
2	R5P + HYPOXANTHINE == IMP	Irreversible	0.01	1000
4	R5P + HYPOXANTHINE == INOSINE	Reversible (forward)	0.01	1000
5	INOSINE == 0 (INOSINE == R5P + HYPOXANTHINE)	Reversible (backward)	0.01	1000
6	IMP == GMP	Irreversible	0.01	1000
7	R5P + GUANINE == GMP	Irreversible	0.01	1000
8	GMP == GUANOSINE	Irreversible	0.01	1000
9	R5P + GUANINE == GUANOSINE	Reversible (forward)	0.01	1000
10	GUANOSINE == 0 (GUANOSINE == R5P + GUANINE)	Reversible (backward)	0.01	1000
11	GMP == GDP	Irreversible	0.01	1000
12	GDP == 0	Sink	0.01	1000
13	IMP == AMP	Irreversible	0.01	1000
14	ADENOSINE == AMP	Irreversible	0.01	1000
15	AMP == 0	Sink	0.01	1000
16	ADENOSINE == INOSINE	Irreversible	0.01	1000
17	AMP == IMP	Reversible (backward)	0.01	1000

Supplementary Table 4

List of metabolites included in purine model. Input metabolites are not mass balanced. Some input metabolites had zero to low isotopic enrichment and were considered unlabeled.

Metabolite No.	Metabolite	Metabolite Type	No. of Carbon Atoms
1	Ribose-5-Phosphate (R5P)	Input	5
2	Glycine (GLY)	Input	2
3	Carbon Dioxide (CO_2)	Input (unlabeled)	1
4	5-Methyltetrahydrofolate (Me-THF)	Input	1
5	Inosine Monophosphate (IMP)	Mass-Balanced	10
6	Inosine	Balanced	10
7	Hypoxanthine	Input (unlabeled)	5
8	Guanine	Input (unlabeled)	5
9	Guanosine Monophosphate (GMP)	Mass-Balanced	10
10	Guanosine Diphosphate (GDP)	Mass-Balanced	10
11	Guanosine	Mass-Balanced	10
12	Adenosine Monophosphate (AMP)	Mass-Balanced	10
13	Adenosine	Input (unlabeled)	10

Supplementary Table 5

Estimated purine fluxes for GBM tissue using iMFA at metabolic steady state. Flux bounds in the table represent 95% confidence intervals and were used as lower and upper bounds of fluxes at t=0 in dynamic iMFA.

Reaction No.	Reaction	Flux (pmol/mg/hr)	Lower Bound (pmol/mg/hr)	Upper Bound (pmol/mg/hr)
1	R5P + GLY + 2C-THF + CO_2 == IMP	11.75	8.91	12.11
2	IMP == INOSINE	68.22	35.58	94.69
3	R5P + HYPOXANTHINE == IMP	328.92	218.18	345.37
4	R5P + HYPOXANTHINE == INOSINE	208.02	98.60	269.60
5	INOSINE == 0	323.29	262.87	537.60
6	IMP == GMP	149.59	127.90	181.68
7	R5P + GUANINE == GMP	171.00	84.60	219.42
8	GMP == GUANOSINE	247.40	182.03	249.87
9	R5P + GUANINE == GUANOSINE	622.18	453.57	1000
10	GUANOSINE == 0	869.57	627.58	1000
11	GMP == GDP	73.19	65.45	128.42
12	GDP == 0	73.19	65.45	128.42
13	IMP == AMP	149.31	120.15	161.67
14	ADENOSINE == AMP	91.81	89.98	144.72
15	AMP == 0	214.67	165.19	272.10
16	ADENOSINE == INOSINE	47.05	33.20	79.62
17	AMP == IMP	26.45	11.32	33.10

Supplementary Table 6

Estimated purine fluxes for cortex tissue using iMFA at metabolic steady state. Flux bounds in the table represent 95% confidence intervals and were used as lower and upper bounds of fluxes at t=0 in dynamic iMFA.

Reaction No.	Reaction	Flux (pmol/mg/hr)	Lower Bound (pmol/mg/hr)	Upper Bound (pmol/mg/hr)
1	R5P + GLY + 2C-THF + CO_2 == IMP	3.63	1.74	7.70
2	IMP == INOSINE	22.93	7.48	38.19
3	R5P + HYPOXANTHINE == IMP	102.95	80.90	1000
4	R5P + HYPOXANTHINE == INOSINE	42.08	17.05	141.34
5	INOSINE == 0	95.65	61.32	364.06
6	IMP == GMP	54.37	0.99	89.38
7	R5P + GUANINE == GMP	56.67	41.31	913.42
8	GMP == GUANOSINE	35.43	13.31	164.02
9	R5P + GUANINE == GUANOSINE	80.39	57.06	191.45
10	GUANOSINE == 0	115.81	83.11	252.35
11	GMP == GDP	75.61	51.54	815.41
12	GDP == 0	75.61	51.54	815.41
13	IMP == AMP	97.78	69.86	750.28
14	ADENOSINE == AMP	20.48	1.98	41.01
15	AMP == 0	49.75	6.53	391.27
16	ADENOSINE == INOSINE	30.63	0.95	55.19
17	AMP == IMP	68.51	2.91	127.56

Supplementary Table 7

Equations to estimate methyl unit enrichment. The number in subscript corresponds to the presence (1) or absence (0) of 13C carbon at the position. SER: serine; GLY: glycine; ME: 5, 10-Methylene tetrahydrofolate.

S. No.	Equation
1	$SER_{000} = GLY_{00}ME_0$
2	$SER_{001} = GLY_{00}ME_1$
3	$SER_{100+010} = GLY_{10+01}ME_0$
4	$SER_{101+011} = GLY_{10+01}ME_1$
5	$SER_{110} = GLY_{11}ME_0$
6	$SER_{111} = GLY_{11}ME_1$
7	$ME_0 + ME_1 = 1$

Supplementary Table 8

List of pyrimidine model reactions and associated flux bounds. Reaction type irreversible refers to reactions that proceed only in one direction and a reversible reaction can proceed in forward and backward directions. Reaction type sink refers to the reactions that consume the metabolite and are not included in the model. UMP: uridine monophosphate; CO_2: carbon dioxide; R5P: ribose-5-phosphate; ASP: aspartate.

Reaction No.	Reaction	Reaction Type	Flux Lower Bound (pmol/mg/hr)	Flux Upper Bound (pmol/mg/hr)
1	R5P + ASP + CO_2 == UMP + CO2	Irreversible	0.01	50
2	URIDINE == UMP	Reversible (forward)	0.01	200
3	UMP == URIDINE	Reversible (backward)	0.01	100
4	CYTIDINE == URIDINE	Irreversible	0.01	400
5	UMP == 0	Sink	0.01	200
6	URIDINE == 0	Sink	0.01	400

Supplementary Table 9

Estimated pyrimidine fluxes for GBM tissue. Flux bounds represent 95% confidence intervals.

Reaction No.	Reaction	Flux (pmol/mg/hr)	Lower Bound (pmol/mg/hr)	Upper Bound (pmol/mg/hr)
1	R5P + ASP + CO_2 == UMP + CO2	8.73	8.73	8.90
2	URIDINE == UMP	57.93	55.03	57.93
3	UMP == URIDINE	32.15	30.24	32.47
4	CYTIDINE == URIDINE	29.12	22.41	29.71
5	UMP == 0	34.50	25.32	41.46
6	URIDINE == 0	3.35	2.71	3.87

Supplementary Table 10

Estimated pyrimidine fluxes for normal cortex tissue. Flux bounds represent 95% confidence intervals.

Reaction No.	Reaction	Flux (pmol/mg/hr)	Lower Bound (pmol/mg/hr)	Upper Bound (pmol/mg/hr)
1	R5P + ASP + CO_2 == UMP + CO2	1.28	1.28	1.28
2	URIDINE == UMP	33.91	30.52	33.91
3	UMP == URIDINE	30.38	30.38	30.38
4	CYTIDINE == URIDINE	22.89	22.21	22.89
5	UMP == 0	4.81	4.57	5.15
6	URIDINE == 0	19.36	17.47	19.36

Supplementary Table 11

List of metabolites included in pyrimidine model. Input metabolites are not mass balanced. Some input metabolites had zero to low isotopic enrichment and were considered unlabeled.

Metabolite No.	Metabolite	Metabolite Type	No. of Carbon Atoms
1	Ribose-5-Phosphate (R5P)	Input	5
2	Aspartate (ASP)	Input	4
3	Carbon Dioxide (CO_2)	Input (unlabeled)	1
4	Cytidine	Input (unlabeled in cortex and labeled in GBM)	9
5	Uridine	Mass-Balanced	9
6	Uridine Monophosphate (UMP)	Mass-Balanced	9

Supplementary Table 12

Contribution of aspartate labeling in UMP labeling.

Newly defined aspartate isotopologues contributed to UMP labeling	Aspartate isotopomers that produce unlabeled CO_2	Aspartate isotopomers that produce labeled CO_2
$\widetilde{\boldsymbol{M}}_{\boldsymbol{ASP},\boldsymbol{M+0}} = \boldsymbol{M}_{\boldsymbol{ASP},\boldsymbol{M+0}} + \boldsymbol{f}_1\,\boldsymbol{M}_{\boldsymbol{ASP},\boldsymbol{M+1}}$	0000	f_1: 1000
$\widetilde{\boldsymbol{M}}_{\boldsymbol{ASP},\boldsymbol{M+1}} = (\boldsymbol{1}-\boldsymbol{f}_1)\,\boldsymbol{M}_{\boldsymbol{ASP},\boldsymbol{M+1}} + \boldsymbol{f}_2\,\boldsymbol{M}_{\boldsymbol{ASP},\boldsymbol{M+2}}$	$(1-f_1)$: $0001, 0010, 0100$	f_2: $1001, 1010, 1100$
$\widetilde{\boldsymbol{M}}_{\boldsymbol{ASP},\boldsymbol{M+2}} = (\boldsymbol{1}-\boldsymbol{f}_2)\,\boldsymbol{M}_{\boldsymbol{ASP},\boldsymbol{M+2}} + \boldsymbol{f}_3\,\boldsymbol{M}_{\boldsymbol{ASP},\boldsymbol{M+3}}$	$(1-f_2)$: $0011, 0101, 0110$	f_3: $1011, 1101, 1110$
$\widetilde{\boldsymbol{M}}_{\boldsymbol{ASP},\boldsymbol{M+3}} = (\boldsymbol{1}-\boldsymbol{f}_3)\,\boldsymbol{M}_{\boldsymbol{ASP},\boldsymbol{M+3}} + \boldsymbol{M}_{\boldsymbol{ASP},\boldsymbol{M+4}}$	$(1-f_3)$: 0111	1111

Supplementary Table 13

Purine and pyrimidine pool sizes used in metabolic models.

Metabolite	Pool Size in Mouse Brain (Mean ± SD, pmol/mg tissue)	Pool Size in GBM (Mean ± SD, pmol/mg tissue)	Additional Bounds on Pool Size (pmol/mg tissue)
GDP	182 .1± 8.2	59.0 ± 22.1	
Inosine	126.7 ± 18.3	94.1 ± 26.8	
AMP	172 ± 6.0	95.4 ± 48.9	
Guanosine	245.8 ± 31.6	70.5 ± 22.0	
GMP	157 ± 40		GBM: [172,304]
IMP	~200		Brain: [125,300] GBM: [30,70]
Uridine	9.52 ± 1.21	21.85 ± 12.80	
UMP	3.16 ± 1.26	2.31 ± 1.10	

Supplementary Table 14

List of reactions in serine MFA model. SER: serine, GLY: glycine, 3PG: 3-phosphoglycerate, Me-THF: 5,10-methylene-THF

S. No.	Reaction	Carbon Transition
1	SER_{plasma} == SER_{cortex}	abc == abc
2	$3PG_{cortex}$ == SER_{cortex}	abc == abc
3	$SER_{unlabeled}$ == SER_{cortex}	abc == abc
4	SER_{cortex} == GLY_{cortex} + $Me\text{-}THF_{cortex}$	abc == ab + c
5	GLY_{cortex} + $Me\text{-}THF_{cortex}$ == SER_{cortex}	ab + c == abc
6	GLY_{cortex} == $Me\text{-}THF_{cortex}$ + CO_2	ab == b + a
7	SER_{cortex} == 0	
8	GLY_{cortex} == 0	
9	$Me\text{-}THF_{cortex}$ == 0	
10	SER_{plasma} == $SER_{enhancing}$	abc == abc
11	$3PG_{enhancing}$ == $SER_{enhancing}$	abc == abc
12	$SER_{unlabeled}$ == $SER_{enhancing}$	abc == abc
13	$SER_{enhancing}$ == $GLY_{enhancing}$ + $Me\text{-}THF_{enhancing}$	abc == ab + c
14	$GLY_{enhancing}$ + $Me\text{-}THF_{enhancing}$== $SER_{enhancing}$	ab + c == abc
15	$GLY_{enhancing}$ == $Me\text{-}THF_{enhancing}$ + CO_2	ab == b + a
16	$SER_{enhancing}$== 0	
17	$GLY_{enhancing}$ == 0	
18	$Me\text{-}THF_{enhancing}$ == 0	
19	SER_{plasma} == $SER_{nonenhancing}$	abc == abc
20	$3PG_{nonenhancing}$ == $SER_{nonenhancing}$	abc == abc
21	$SER_{unlabeled}$ == $SER_{nonenhancing}$	abc == abc
22	$SER_{nonenhancing}$ == $GLY_{nonenhancing}$ + $Me\text{-}THF_{nonenhancing}$	abc == ab + c
23	$GLY_{nonenhancing}$ + $Me\text{-}THF_{nonenhancing}$== $SER_{nonenhancing}$	ab + c == abc
24	$GLY_{nonenhancing}$ == $Me\text{-}THF_{nonenhancing}$ + CO_2	ab == b + a
25	$SER_{nonenhancing}$== 0	
26	$GLY_{nonenhancing}$ == 0	
27	$Me\text{-}THF_{nonenhancing}$ == 0	

Supplementary Table 15

Transitions and retention times of compounds in tissue LC-MS experiments.

Name	Transition	Retention time (min)
2-Aminoadipate	160.1 -> 142.0	4.2
2-Deoxyadenosine	310.1 -> 250.0	6.5
2-Deoxyribose	193.1 -> 59.2	1.4
2-Deoxyribose 5-phosphate	213.0 -> 79.1	9.5
2-Deoxyuridine	227.1 -> 42.2	2.7
2-Hydroxyglutarate	147.0 -> 85.1	13.3
2-Isopropylmalic acid	175.1 -> 113.0	14.7
2-Ketobutyrate	101.0 -> 57.2	12.4
3-Hydroxykynurenine	223.1 -> 162.0	2.3
3-Methylglutarate	145.1 -> 101.1	14.0
3PG/2PG	185.0 -> 97.0	14.8
4-Hydroxybenzoic acid	137.0 -> 93.0	11.9
4-Methyl-2-oxovaleric acid	129.1 -> 85.1	13.7
4-Pyridoxic acid	182.0 -> 138.0	15.2
5-Hydroxyindoleacetate	190.1 -> 144.0	13.0
Aconitate	173.0 -> 85.1	15.1
Adenine	134.0 -> 107.0	2.8
Adenosine	326.1 -> 134.0	6.5
Adenylosuccinate	462.0 -> 134.1	16.3
Adipate	145.1 -> 101.1	14.5
ADP	426.0 -> 159.0	15.3
AICAR	257.1 -> 42.2	2.2
Allantoin	157.0 -> 97.0	1.5
alpha-Ketoglutarate	145.0 -> 101.0	14.0
AMP	346.0 -> 79.0	11.8
Arginine	173.1 -> 131.0	1.0
Argininosuccinate	289.0 -> 131.1	4.7
Asparagine	131.0 -> 113.0	1.4
Aspartate	132.0 -> 88.0	4.2
ATP	506.0 -> 159.0	17.2
Beta-hydroxybutyrate	103.0 -> 59.1	7.8
cAMP	328.0 -> 79.0	13.4
Carbamoylaspartate	175.0 -> 132.0	13.1
Carbamoylglutamic acid	189.1 -> 146.0	13.2
Carnitine	220.1 -> 146.0	1.3
CDP	402.0 -> 158.9	14.2
Citrate/Isocitrate	191.0 -> 111.0	15.0
Citrulline	174.1 -> 131.0	1.3
CMP	322.0 -> 97.0	9.8
CoA	766.1 -> 686.2	17.8
Creatine	130.1 -> 88.1	1.3
Creatinine	112.0 -> 68.1	1.4
CTP	482.0 -> 158.8	17.0
Cystathionine	221.1 -> 134.0	1.4
Cysteine	120.0 -> 33.2	1.3
Cytidine	242.1 -> 109.0	1.5
dADP	410.0 -> 79.1	16.0
dAMP	330.0 -> 134.1	12.4

dCDP	386.0 -> 79.0	15.0
dCMP	306.0 -> 79.0	10.4
dTDP	401.0 -> 97.0	15.4
dTTP	481.0 -> 158.8	17.3
FAD	784.1 -> 437.0	17.0
Fructose 1,6-biphosphate	338.9 -> 97.0	14.7
Fructose 6-phosphate (Hexose	259.0 -> 79.0	7.2
gamma-Aminobutyric acid	102.0 -> 84.1	1.3
gamma-Glu-Cys	249.1 -> 171.0	8.1
GAP/DHAP	169.0 -> 79.0	10.4
GDP	442.0 -> 158.9	15.2
GlcNAc	220.0 -> 160.0	1.4
Gluconic acid	195.1 -> 129.0	5.6
Glucosamine 6-phosphate	258.0 -> 79.0	1.6
Glucose (hexose)	179.1 -> 89.0	1.3
Glutamate	146.0 -> 102.0	3.6
Glutamine	145.1 -> 127.0	1.4
Glutathione (oxidized)	611.1 -> 306.1	13.3
Glutathione (reduced)	306.1 -> 128.1	8.4
Glyceric acid	105.0 -> 75.1	6.0
GTP	522.3 -> 158.9	17.0
Guanine	150.0 -> 66.0	1.9
Guanosine	282.1 -> 150.0	4.1
Histidine	154.1 -> 93.1	1.2
Homocitrate	205.0 -> 125.0	15.4
Hydroxyproline	130.1 -> 128.0	2.1
Hypoxanthine	135.0 -> 92.0	2.1
IDP	427.0 -> 135.0	14.1
IMP	347.0 -> 79.0	10.7
Inosine	267.1 -> 135.0	3.3
Itaconate	129.0 -> 85.1	13.7
ITP	507.0 -> 409.0	17.2
Ketovalerate	115.0 -> 71.2	14.2
Kynurenine	207.1 -> 190.0	4.4
Lactate	89.0 -> 43.3	6.7
Leucine/Isoleucine	130.0 -> 45.0	2.1
Malate	133.0 -> 115.0	13.4
Malonic acid	103.0 -> 59.2	12.3
Maltose	341.1 -> 179.0	1.4
Methionine	148.0 -> 47.2	1.7
Mevalonic acid	147.1 -> 128.9	9.7
MTA	356.1 -> 296.0	11.7
myo-Inositol	239.1 -> 179.0	1.3
N-acetylaspartate	174.0 -> 88.1	13.7
N-acetylaspartylglutamate	303.1 -> 285.0	16.2
N-Acetylglutamate	188.1 -> 128.0	14.0
N-Acetylneuraminic acid	308.1 -> 87.0	6.0
NAD	662.1 -> 540.0	9.5
NADH	664.1 -> 79.0	15.3
NADP	742.1 -> 620.0	14.6
Orotic acid	155.0 -> 42.0	9.1
Pantothenate	218.0 -> 88.0	11.8

Phenylalanine	164.1 -> 147.0	5.0
Phosphoenolpyruvate	167.0 -> 79.0	15.3
Phosphorylethanolamine	140.0 -> 79.0	1.7
Phosphoserine	184.0 -> 97.0	10.1
Proline	114.0 -> 68.1	1.3
Pyridoxal hydrochloride	166.1 -> 138.0	1.9
Pyridoxal phosphate	246.0 -> 79.0	14.7
Pyruvic acid	87.0 -> 43.2	9.3
Riboflavin	375.1 -> 255.0	12.6
Ribose 5-phosphate	229.0 -> 79.0	9.4
S-Adenosylhomocysteine	383.1 -> 248.0	5.3
Sedoheptulose 7-phosphate	289.0 -> 79.0	8.2
Serine	104.0 -> 74.1	1.3
Sphinganine	300.3 -> 199.0	8.8
Succinate	117.0 -> 73.1	12.5
Taurine	124.0 -> 80.0	1.3
Taurocholic acid	514.0 -> 514.0	20.6
Thiamine	264.1 -> 234.0	1.2
Threonine	118.0 -> 74.1	1.3
Thymine	125.0 -> 42.2	2.6
Tryptophan	203.1 -> 116.0	7.7
Tyrosine	180.0 -> 119.0	2.2
UDP	403.0 -> 79.0	14.8
UDP-GlcNAc	606.1 -> 385.0	14.0
UDP-Glucose	565.0 -> 322.9	14.0
UMP	323.0 -> 79.1	10.4
Uracil	111.0 -> 42.2	1.7
Uric acid	167.0 -> 124.0	3.8
Uridine	243.1 -> 200.0	2.3
UTP	483.0 -> 385.0	17.0
Xanthine	151.0 -> 108.0	2.3
Xanthosine	283.1 -> 151.0	8.2
Xylulose 5-phosphate	229.0 -> 79.0	8.3

3. SUPPLEMENTARY EQUATIONS

Supplementary Equation 1

$$\boldsymbol{x} = [\boldsymbol{v}, \boldsymbol{c}, \boldsymbol{f}, \boldsymbol{R}]$$

Where ***x*** represents iMFA at steady state model parameters including reaction fluxes (***v***), pool sizes of mass-balanced metabolites (***c***), fractional contributions of reactant isotopologues to the product isotopologues (***f***), and isotopologues of input metabolites (***R***).

Supplementary Equation 2

$$\begin{bmatrix} S_{m\times n} & 0_{m\times m} & 0_{m\times q} & 0_{m\times r} \\ 0_{p\times n} & 0_{p\times m} & 0_{p\times q} & L_{p\times r} \end{bmatrix} x = \begin{bmatrix} 0_{m\times 1} \\ 1_{p\times 1} \end{bmatrix}$$

Where *S* is the stoichiometric matrix for a model with *m* balanced metabolites and *n* reactions. *L* is the linear constraints on the sum of input metabolite isotopologues, corresponding to *p* labeled input metabolites and *r* total isotopologues.

Supplementary Equation 3

$$\frac{dM_{i,d}}{dt} = \frac{1}{c_i}.\left(\sum_{\substack{j=1 \\ S_{ij}>0}}^{n} S_{ij}.v_j \left(\prod_{\substack{k \\ \sum q=d \\ S_{kj}<0}} M_{k,q} \right) + \sum_{\substack{j=1 \\ S_{ij}<0}}^{n} S_{ij}.v_j.M_{i,d} \right)$$

Where the rate of change of the isotopologue *d* of metabolite *i* ($M_{i,d}$) is described by applying mass balance on the isotopologue. *c* represents pool size of metabolite *i*. $S_{i,j}$ represents stoichiometric coefficients of metabolite *i* in reactions *j* in which metabolite *i* is a product and v_j is the flux of reaction *j*. $M_{k,q}$ represents combinations of isotopologues *q* of reactants *k* that produce isotopologue *d* of metabolite *i*.

Supplementary Equation 4

$$obj = \sum \left(\frac{M_{expt} - M_{sim}}{SD_{expt}} \right)^2 + \sum \left(\frac{c_{expt} - c_{sim}}{SD_{expt}} \right)^2$$

Where *obj* represents the objective function of iMFA at steady state. *M* and *c* denote isotopic enrichments and pool sizes for experimental measurements (*expt*) with their standard deviations (*SD*) and model simulated values (*sim*).

Supplementary Equation 5

$$\boldsymbol{v}(t) = \boldsymbol{CP} * \boldsymbol{N}(t)$$

Where *v(t)* represents time-variant fluxes. *CP* and *N(t)* denote the vector of b-spline parameters, and a time-dependent vector, respectively.

Supplementary Equation 6

$$\boldsymbol{x} = [\boldsymbol{cp}, \boldsymbol{c_0}, \boldsymbol{R}]$$

Where *x* represents the parameters of the dynamic iMFA model including parameters of b-spline (*cp*), metabolite pool sizes at time t=0 (c_0), and isotopologues of input metabolites (*R*).

Supplementary Equation 7

$$\frac{d\boldsymbol{c}}{dt} = \boldsymbol{S} * \boldsymbol{v}(t)$$

Where the rate of change of metabolite pool sizes (*c*) is defined as a function of stoichiometric matrix *S* and the time-dependent flux vector *v(t)*.

Supplementary Equation 8

$$M_{i,d}\frac{dc_i}{dt} + c_i\frac{dM_{i,d}}{dt} = \sum_{\substack{j=1 \\ S_{ij}>0}}^{n} S_{ij}.v_j \left(\prod_{\substack{k \\ \sum q=d \\ S_{kj}<0}} M_{k,q} \right) + \sum_{\substack{j=1 \\ S_{ij}<0}}^{n} S_{ij}.v_j.M_{i,d}$$

Where the rate of change of the isotopologue *d* of metabolite *i* ($M_{i,d}$) and time-dependent pool size of metabolite *i* (c_i) is described by applying mass balance on the isotopologue. $S_{i,j}$ represents stoichiometric coefficients of metabolite *i* in reactions *j* in which metabolite *i* is a product and v_j is the flux of reaction *j*. $M_{k,q}$ represents combinations of isotopologues *q* of reactants *k* that produce isotopologue *d* of metabolite *i*.

Supplementary Equation 9

$$obj = \sum\left(\frac{M_{expt} - M_{sim}}{SD_{expt}}\right)^2 + \sum\left(\frac{c_{0,expt} - c_{0,sim}}{SD_{expt}}\right)^2 + \sum\left(\frac{\left(\frac{c_t}{c_0}\right)_{expt} - \left(\frac{c_t}{c_0}\right)_{sim}}{SD_{expt}}\right)^2$$

Where *obj* represents the objective function of dynamic iMFA. *M* and *c* denote isotopic enrichments and pool sizes for experimental measurements (*expt*) with their standard deviations (*SD*) and model simulated values (*sim*). c_t represents metabolite pool size at time *t* and c_0 is the metabolite pool size at time *0*.

Supplementary Equation 10

$$\frac{dM_{UMP,i}}{dt} = \frac{1}{c_{UMP}}\left(v_{URIDINE\rightarrow UMP}.M_{URIDINE,i} + v_{de\ novo}.M_{R5P,j}.\widetilde{M}_{ASP,k} - \left(v_{UMP\rightarrow URIDINE} + v_{UMP\rightarrow UMP_{out}}\right).M_{UMP,i}\right);\ j+k=i$$

$$\frac{dM_{URIDINE,i}}{dt} = \frac{1}{c_{URIDINE}}\left(v_{CYTIDINE\rightarrow URIDINE}.M_{CYTIDINE,i} + v_{UMP\rightarrow URIDINE}.M_{UMP,i} - \left(v_{URIDINE\rightarrow UMP} + v_{URIDINE\rightarrow URIDINE_{out}}\right).M_{URIDINE,i}\right)$$

Where *M*, *c*, and *v* denote isotopologues, pool sizes, and fluxes, respectively. $\widetilde{M}_{ASP,k}$ represents newly defined aspartate isotopologue distribution based on fractional contribution (*f*) of aspartate labeling in uridine monophosphate (UMP) labeling defined in Supplementary Table 12.

Supplementary Equation 11

$$\frac{dR_i(t)}{dt} = \begin{cases} \dfrac{R_i(t_2) - R(t_1)}{t_2 - t_1} & t_1 \le t < t_2 \\ \cdots & \\ \cdots & \\ \dfrac{R_i(t_{m+1}) - R_i(t_m)}{t_{m+1} - t_m} & t_m \le t < t_{m+1} \end{cases}$$

Where the rate of change of isotopologues (*i*) of input metabolites (*R*) are approximated by difference quotient formula between time points t_{m+1} and t_m.

Supplementary Equation 12

$$\boldsymbol{x} = [\boldsymbol{v}, \boldsymbol{D}]$$

Where *x* represents MFA model parameters including fluxes (*v*) and isotopomer distribution values (*D*).

Supplementary Equation 13

$$\begin{bmatrix} \boldsymbol{S}_{m\times n} & \boldsymbol{0}_{m\times d} \\ \boldsymbol{N}_{3\times n} & \boldsymbol{0}_{3\times d} \\ \boldsymbol{0}_{4\times n} & \boldsymbol{L}_{4\times d} \end{bmatrix} \boldsymbol{x} = \begin{bmatrix} \boldsymbol{0}_{n\times 1} \\ \boldsymbol{1}_{7\times 1} \end{bmatrix}$$

Where matrix $\boldsymbol{S}_{m\times n}$ is stoichiometric matrix of *m* metabolites in *n* reactions. $\boldsymbol{L}_{4\times d}$ represents linear constraints on the sum of IDVs of input metabolites (external serine and 3PG of each tissue sample). $\boldsymbol{N}_{3\times n}$ represents linear constraints on serine input fluxes (serine synthesis, external serine uptake, and unlabeled serine from recycling) in each compartment to 1.

Supplementary Equation 14

$$\sum_{\substack{j=1 \\ S_{ij}>0}}^{n} S_{ij}.v_j \left(\prod_{\substack{k \\ \sum q=d \\ S_{kj}<0}} M_{k,q} \right) + \sum_{\substack{j=1 \\ S_{ij}<0}}^{n} S_{ij}.v_j.M_{i,d} = 0$$

Where the rate of change of the isotopologue *d* of metabolite *i* ($M_{i,d}$) is assumed 0 at steady state. $S_{i,j}$ represents stoichiometric coefficients of metabolite *i* in reactions *j* in which metabolite *i* is a product and v_j is the flux of reaction *j*. $M_{k,q}$ represents combinations of isotopologues *q* of reactants *k* that produce isotopologue *d* of metabolite *i*.

Supplementary Equation 15

$$obj = \sum \left(\frac{\sum y_{sim} - M_{expt}}{SD_{expt}} \right)^2 + 0.1 * ||[v, y]||_2$$

Where *obj* represents the objective function of serine MFA model. M_{expt} denotes experimental isotopic enrichments with their standard deviations (SD_{expt}) and model simulated IDVs (y_{sim}). *v* represents fluxes. The second term represents L2 regularization.

Supplementary Equation 16

$$f(k_1, k_2, H_1, Q) = \left(P - \frac{r}{k_1}\right)\frac{H_1}{Q}e^{-k_1 t} + \left(P - \frac{r}{k_2}\right)\frac{H_2}{Q}e^{-k_2 t} + \frac{r}{Q}\left(\frac{H_1}{k_1} + \frac{H_2}{k_2}\right)$$

Where *f* represents the time-course plasma glucose ^{13}C enrichment profiles. *r* denotes the rate of tracer infusion (*mg/min/g*). *P* denotes the bolus infusion (*mg/min/g*). Other parameters are the time after infusion (*t*), physiological glucose pool size (*Q*), the fractional contribution of two sources of labeled glucose (H_1, H_2), and the rate constant for glucose enrichment from the two sources (k_1, k_2).

Supplementary Equation 17

$$H_1 + H_2 = 1$$

Where H_1 and H_2 are the fractional contribution of two sources of labeled glucose.

Supplementary Equation 18

$$SE = \frac{r}{Q}\left(\frac{H_1}{k_1} + \frac{H_2}{k_2}\right)$$

Where *SE* represents saturation enrichment or the plateau of Supplementary Equation 16. *r* denotes the rate of tracer infusion (*mg/min/g*). Other parameters are the physiological glucose pool size (*Q*), the fractional contribution of two sources of labeled glucose (H_1, H_2), and the rate constant for glucose enrichment from the two sources (k_1, k_2).