Supplemental Information

Serine Racemase mediates subventricular zone neurogenesis via fatty acid metabolism

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A

SVZ

B

WT — Ki67 / DAPI — 20 µm

SR⁻ᐟ⁻ — Ki67 / DAPI — 20 µm

Ki67

*p<0.001

Integrated Density

WT   SR⁻ᐟ⁻

C

WT — GFAP/Hoechst

SR⁻ᐟ⁻ — GFAP/Hoechst

*p<0.001

No of GFAP⁺ cells per image

WT   SR⁻ᐟ⁻

D

WT — NEUN/Hoechst

SR⁻ᐟ⁻ — NEUN/Hoechst

*p<0.0001

No of NeuN⁺ cells per image

WT   SR⁻ᐟ⁻

Suppl Fig 1.

A

B

β-III Tubulin
*p<0.001

Neuroblasts/High powered field

WT   SR$^{-/-}$

C

Neuro D1
* p<0.001

Neuroblasts/High powered field

WT   SR$^{-/-}$

D

BrdU
*p=0.0084

Number of BrdU positive cells / field

WT   SR$^{-/-}$

E

SR$^{flox/flox}$ Nes-Cre$^+$

FASN

β-actin

FASN
Brain

Relative optical density/ (actin)

SR$^{flox/flox}$ Nestin-Cre+

F

SVZ

SR$^{flox/flox}$ Nes-Cre$^+$

FASN

β-actin

FASN
SVZ

Relative optical density/ (actin)

SR$^{flox/flox}$ Nestin-Cre+

Suppl Fig 2.

Suppl Fig 3.

A Phosphatidylcholine

Fold change (y-axis: 0 to 1.2)

SR^flox/flox vs Nestin-Cre+ (x-axis: 18 to 37)

B WT / NCAM DAPI — SR^-/- / NCAM DAPI

WT L-Ser / NCAM DAPI — SR^-/- L-Ser / NCAM DAPI

WT D-Ser / NCAM DAPI — SR^-/- D-Ser / NCAM DAPI

C Summed Intensity

Counts (y-axis: 0.0E+00 to 9.0E+08)

x-axis: Nes Cre_1-1, Nes Cre_1-2, Nes Cre_1-3, Nes Cre_2-1, Nes Cre_2-2, Nes Cre_2-3, SR Flox_1-1, SR Flox_1-2, SR Flox_1-3, SR Flox_2-1, SR Flox_2-2, SR Flox_2-3, QC-1, QC-2, QC-3, QC-4, QC-5, QC-6, QC-7, QC-8, QC-9, QC-10

D WT — BrdU GFAP — 20 µm

E SR Flox / mCD24 DAPI — Nes Cre / mCD24 DAPI — 20 µm

No of mCD24+ cells per mm^2 (y-axis: 0 to 15)

*p<0.001

SR^flox/flox   Nestin-Cre+

Suppl Fig 4.

A

B

C

Suppl Fig 5.

Suppl Fig 6.

Suppl Fig 7.

Supplemental Figures and Tables legend.

Suppl Fig 1. (**A**) Schematic of mouse SVZ (**B**) Ki67 staining in WT and SR$^{-/-}$ primary neurons derived from SVZ neural stem cells Scale Bar=20 μm. (**C**) GFAP (**D**) NeuN expression in differentiated WT and SR$^{-/-}$ neural stem cells in culture. DAPI (nuclear stain) is indicated in blue. Scale Bar= 100 μm. Data are representative of 3 independent experiments. Cells were counted in multiple high-powered fields. Error Bars are SD. *Related to Figure 1 and 2.*

Suppl Fig 2. (**A**) Phase contrast image of neuroblasts (purple arrows) on a bed of astrocytes (black arrows) in the neuroblast assay. Neuroblasts appear 3-4 days after induction of differentiation. Scale Bar=20 μm (**B**) Expression of β-III Tubulin, a marker for neuroblasts. *p<0.001 relative to WT control. Neuroblasts were counted in each of the different high powered fields in each genotype. (**C**) Expression of NeuroD1, a marker for neuroblasts. *p<0.001 relative to WT control. Data are representative of 3 independent experiments. Error Bars are SD. (**D**) BrdU (Bromo deoxy Uridine) incorporation in NSCs from WT and SR$^{-/-}$ mice SVZ after incubation for 18 h with 10 μM BrdU. BrdU positive cells were counted in the different high powered fields. *p=0.0084 relative to WT control. Error Bars refer to SD. (**E**) Expression of mouse FASN in whole brain lysates of SR $^{flox/flox}$ and nestin-cre+ mice. (**F**) Expression of mouse FASN in SVZ lysates of SR $^{flox/flox}$ and nestin-cre+ mice. All samples are run in duplicate and tissues pooled from 5-10

mice/experiment. Actin was a loading control. Plot shows densitometry of the blots. *Related to Figures 1 and 2.*

Suppl Fig 3. Plots show fold changes in the expression (Y-axis) of lipids in the different classes that are significantly altered in the SVZ of adult SR $^{flox/flox}$ and nestin-cre+ mice following a global lipidomics screen by UPLC-MS/MS. Numbers on the X-axis in the different plots show the specific lipid molecules in the respective classes that are listed in Table S2. (**A**) Fatty Acid conjugates (**B**) Sphingomyelins (**C**) Phosphatidylcholine (**D**) Phosphatidic Acid (**E**) Ceramides (**F**) Sterols (**G**) Carnitines (**H**) Triacylglycerols (**I**) Diacylglycerols (**J**) Monoacylglycerols (**K**) Hexosyl Ceramides (**L**) Lysophosphatidic Acid. *Related to Figure 4.*

Suppl Fig 4. (**A**) Fold change in expression of phosphatidylcholine in the SVZ of SR $^{flox/flox}$ and nestin-cre+ mice from a global lipidomics screen by LC-MS/MS. Numbers on the x-axis indicate individual molecules within the group. The molecules are listed in Table S4. Y axis indicates fold change in expression relative to SR $^{flox/flox}$ control. (**B**) NCAM expression in WT and SR$^{-/-}$ NSCs grown under control, L and D-serine rescue conditions Scale Bar=20 μm (**C**) Summed intensity for all detected features in each LC-MS/MS sample injection in SR $^{flox/flox}$ and nestin-cre+ SVZ after merging positive and negative ionization modes. QC refers to quality control sample. (**D**) Representative enlarged section of the lateral ventricle of the SVZ of WT mice showing incorporation of BrdU (red) and GFAP (green). (**E**) Enlarged image of the lateral ventricle of SVZ in SR $^{flox/flox}$ and nestin-cre+ mice showing expression of mCD24 (red) in the different high powered fields.

Scale Bar=20 μm. Data are representative of 3 independent experiments with an N=4-8 mice per genotype/experiment. Error Bars are SD. DAPI is seen in blue. *Related to Figures 1, 2 and 4.*

Suppl Fig 5. (**A**) Expression of SPOT14 (green) in SR $^{flox/flox}$ and nestin-cre+ mice SVZ along the lateral ventricle per high powered field. Scale bar=50 μm. (top panel). Lower panel shows an enlarged section. (**B**) Expression of PSA-NCAM (pink) along the rostral migratory stream in SR $^{flox/flox}$ and nestin-cre+ mice. Panel shows an enlarged section. (**C**) Expression of GFAP (red) in the lateral ventricle (LV) of SVZ of adult SR $^{flox/flox}$ and nestin-cre+ mice per high powered field. Data represent 3 independent experiments with N=4-6 mice per genotype per experiment. Lower panel shows an enlarged section of the above figure. Scale bar=50 μm (top panel) and 20 μm (bottom panel). DAPI is indicated in blue. *Related to Figures 1 and 2.*

Suppl Fig 6. Densitometric plots of western blots of (**A**) DLX2 (**B**) OLIG2 from NSC lysates of age matched WT and SR $^{-/-}$ mice. (**C**) DLX2 (**D**) OLIG2 from SVZ tissue lysates of age matched SR $^{flox/flox}$ and nestin-cre+ SVZ. Samples were run in duplicate. In each lysate sample, SVZ tissue was pooled from N=5-10 mice/experiment. *Related to Figures 1 and 2*. (**E**) STRING analysis of SR with PGDH and PSPH in mouse database (**F**) human database. *Related to Figures 3 and 5.* (**G**) Simplified schematic of the different lipid classes that are under control of SR in the SVZ of adult mice based on a global lipidomics screen. SR alters lipid metabolism at the primary level by phosphorylation of ACC at Ser 79 and at the tertiary level by altering polymerization of ACC and its loss of catalytic

activity by SPOT14-MIG12 heterocomplex interaction, leading to decrease in malonyl CoA synthesis and decrease in *de novo* fatty acid synthesis by FASN. SVZ schematic was adapted from (Pircs et al., 2018). pACC (phospho Acetyl CoA Carboxylase), TCA (tricarboxylic acid). Ox phos (oxidative phosphorylation). *Related to Figures 4 and 5, Suppl figs 3 and 4.*

Suppl Fig 7. Plots show quantitative analysis of (**A**) NEUN (**B**) SOX2 (**C**) GFAP (**D**) NCAM positive staining in rescue experiments performed with SVZ NSCs from WT and SR$^{-/-}$ mice. Expression of (**E**) FASN (**F**) MCM2 (**G**) Prominin (**H**) NCAM (**I**) SOX2 (**J**) PGDH (**K**) PSPH in SVZ of age matched SR$^{flox/flox}$ and nestin-cre+ mice. Error Bars refer to SD. * indicates p value between the groups. (student's *t-test* and ANOVA). *Related to Figures 2, 3, 6, and 7.*

Suppl Tables Legend.

Table S1. Table shows the 15 most important lipids identified in the lipidomics screen in the SVZ of age matched adult SR $^{flox/flox}$ and nestin-cre+ mice. Table shows unique identification number, lipid molecule and the class. *Related to Fig. 4F.*

Table S2. Table 2 identities all the lipid molecules from the lipidomics screen in the different lipid classes. Number in each table indicates the lipid molecule identified in the respective class (plotted in suppl fig. 3 on x axis), followed by the lipid ID and fold change in nestin-cre+ mice relative to SR $^{flox/flox}$ (on y-axis). *Related to Fig. 4E.*

Table S3. Table 3 shows the list of primer sequences used for gene expression studies using the SYBR Green method. *Related to Fig. 3B,3I and 5F-G*.

Table S4. Table shows all the lipids identified in the SVZ of SR $^{flox/flox}$ and nestin-cre+ lipidomics heatmap with m/z value, best matched lipid followed by their subclass. *Related to Fig. 4E*.

Table S5. Table shows the abbreviations of the different lipid subclasses identified in the UPLC-MS MS lipidomics screen. *Related to Figure 4 and Suppl figs 3 and 4.*

Supplemental Experimental Procedures:

Reagents: Purified L-serine and D-serine, were purchased from Sigma Chemical Corp. All salts for buffers and reagents were of research grade and high purity. Milli Q water was used to make buffers and solutions for experiments. HPLC grade solvents and water (Fisher Scientific) was used for estimation of D and L-Serine. Antibodies for rabbit Ki67 (Novus Bio; NB500-170SS), rabbit FASN (Bethyl Labs; A301-324A-T), mouse PSA-NCAM (DSHB Iowa; 5A5-c), mouse Spot14 (sc-137178; Santacruz Biotechnology), rabbit MCM2 (Bethyl Labs; IHC-00009-T), mouse BrdU (Sigma; B8434-25 μl), mouse CD24 (eBioscience; #14-0242-82), rabbit Prominin (Millipore; ZRB1013-25 μl) antibodies were purchased from the vendors mentioned in parenthesis next to the antibody. Antibodies for immunocytochemistry were rabbit NEUN (Cell Signaling Technology; D4G40), β III-Tubulin (RND Systems; #MAB1195-SP), mouse GFAP (Millipore; MAB360), rabbit OLIG2 (Novus Bio; 28667SS).

Generation of Nestin Cre SR mice: Nestin Cre (conditional deletion of SR) mice were generated by breeding SR flox mice (Basu et al., 2009) with nestin cre mice obtained from Jackson labs (number 003771) to generate nestin cre SR flox $^{+/-}$ or nestin cre (-) SR flox $^{+/-}$. The nestin cre (+) SR flox$^{+/-}$ mice were then bred with SR flox mice to obtain nestin cre SR flox/flox. From the litters obtained and after genotyping (Transnetyx Inc) nestin cre SR flox/flox mice were then bred with SR flox mice to obtain nestin cre SR flox/flox or SR flox/flox mice. The nestin cre SR flox/flox mice were used in experiments along with SR flox/flox mice as controls. All mice were age matched and genotyped before use in experiments (Giusti et al., 2014). _Note:_ Flox and SR Flox refer to SR $^{flox/flox}$ and Nestin Cre to nestin-cre+ (conditional deletion of SR) mice in this study.

BrdU (Bromo deoxy Uridine) labeling: Eight weeks old WT and age matched SR$^{-/-}$ mice (and SR $^{flox/flox}$, nestin-cre+ mice) were kept in a cage and administered 1 mg/ml BrdU (Bromo deoxy Uridine) in the water by means of a water bottle (_ad libitum_) contained within the cage for 14 days. The water was changed after 7 days with fresh solution of BrdU. On day 13 the mice were given a single i.p. injection of 200 μl BrdU (5 mg/ml) and the brains harvested after 18 h following euthanasia. The brains were perfused with 4 % PFA in PBS. The brains were sectioned after paraffin embedding. BrdU incorporation in the brain was detected using anti BrdU antibody staining by IHC with antigen retrieval at 60°C for 10 min. The imaging was performed using laser scanning confocal microscopy and a _z_ stack obtained. The number of BrdU positive cells were counted in each section and quantified (Doetsch et al., 1999).

Micro dissection of adult mouse brain SVZ: The mice brain was dissected and removed following euthanasia and kept in a sterile petri dish containing sterile PBS. The petri dish containing the individual brain was placed under a dissecting microscope with light source at low magnification on the ventral surface and using fine forceps, the olfactory bulbs were removed by holding the cerebellum. The brain was then rotated on to the dorsal aspect and using a sterile blade a coronal cut was made at the level of the optic chiasm. To micro dissect the SVZ, the rostral portion of the brain was positioned to face the cut coronal section upwards. The microscope was focused to a higher magnification. The septum was discarded using fine curved forceps. The SVZ (thin tissue layer adjacent to the ventricle) was dissected by placing the tip of a fine curved forceps in the lateral corner of the ventricle adjacent to the corpus callosum and the other tip approximately 1 mm into the tissue immediately adjacent to the ventricle. The tissue was gently pressed down with the forceps and the triangular piece of tissue removed. The dissected SVZ was placed on a petri dish on ice. A total of 5-10 mice SVZ regions per genotype were pooled in each isolation (Walker and Kempermann, 2014).

Adherent monolayer stem cell culture: The neural stem cell adherent monolayer culture system is a valuable tool for determining the proliferative and differentiative potential of adult neural stem cells _in vitro_. The adherent monolayer cultures comprise mostly homogenous population of precursor cells and are useful for following the differentiation process in single cells. The SVZ was carefully dissected from WT, SR$^{-/-}$, SR $^{flox/flox}$ and nestin-cre+ SR mice brains as per the method in (Walker and Kempermann, 2014). The protocol for coating the 96 well and 24 well plate was followed exactly as mentioned. Differentiation of precursor cells in adherent monolayer cultures was followed as per Walker _et al_ protocol (Walker and Kempermann, 2014).

Neuroblast Assay: Neuroblasts (Type A cells) were visualized after plating the cells obtained from dissociation of adherent monolayer cultures (mentioned above) following accutase treatment. The dissociated monolayer cells were plated in a 15 μ 24 well plate at 2-3 X 10^5 cells/ml in complete NSC medium supplemented with 20 ng/ml EGF and 10 ng/ml b-FGF. The plated cells were incubated at 37°C incubator for 3-4 days to achieve 75-80 % confluence. Differentiation was induced by gradually replacing media with less growth factors (10 ng/ml b-FGF and 5 ng/ml EGF to no growth factors). The media was subsequently replaced with no growth factors. The differentiated cells (4-5 days post induction) were fixed with 4 % PFA in PBS and immunostained for PSA-NCAM (marker for neuroblasts), β-III tubulin (neuronal marker) and NeuroD1 (neuronal maturation). DAPI was used as a nuclear stain in the mounting media. The cells were visualized under a fluorescence microscope and positively stained cells were counted in each high-powered field and neuroblasts quantified (Azari and Reynolds, 2016; Azari et al., 2012).

Neural Stem Cell Immunocytochemistry: Staining of NSC's for expression of different markers was performed in a 15 μ 24 well plate (thin glass bottom; Ibidi; #82406). The cells were washed once with 0.5 ml PBS. The cells were fixed with 4 % PFA in PBS for 20 min at RT. After fixation, the cells were washed once in PBS. The cells were then blocked in 0.5 % triton X-100 in PBS for 60 min at RT. Following the blocking step, the cells were incubated with the different primary antibodies (1:500-1:1000 dilution) in blocking buffer for 60 min at RT. After incubation, the cells washed 3 times in PBS. The cells were incubated with anti-mouse or anti-rabbit Alexa fluor secondary antibodies (1:1000-1:2000 dilution) in blocking buffer for 30 min at RT in the dark. The cells were washed 3 times in PBS. A drop of the mounting medium (ProLong Diamond Antifade Mountant with DAPI; Invitrogen; #P36962) was added to each well and a circular coverslip placed gently in the well. The coverslip was tapped gently to remove any air bubbles. The plate was wrapped in foil and allowed to dry at 4°C in the dark. The cells were imaged using a fluorescence microscope.

Mouse FASN ELISA: Mouse FASN (fatty acid synthase) expression in mice brain and SVZ region was measured quantitatively using mouse FASN ELISA kit (CUSABIO cat# CSB-EL008435MO). A standard curve was generated from purified mouse FASN as per instructions. Lysates from whole brain and SVZ regions of age matched SR [flox/flox] and nestin-cre+ mice were homogenized in PBS containing protease inhibitors, sonicated and the harvested supernatant diluted (1:20 v/v) in PBS containing protease inhibitors. Protein concentration was estimated using BCA assay. Equal amounts of protein were added to each well of a 96 well plate in triplicate and the ELISA performed as per manufacturer's instructions. The absorbance at 450 nm was correlated to the amount of FASN (in pg/ml) in the sample based on a FASN standard curve.

Mouse Malonyl CoA ELISA: Estimation of mouse malonyl CoA in SVZ lysates was measured quantitatively using mouse malonyl CoA ELISA kit (CUSABIO cat# CSB-E12896m). A standard curve of purified malonyl CoA was generated as per manufacturer's instructions in the concentration range of 0-10 ng/ml. SVZ lysates from age matched SR [flox/flox] and nestin-cre+ mice were prepared in PBS containing protease inhibitors. The stock lysate was diluted 1:500 in PBS containing protease inhibitors and protein concentration estimated using BCA assay. Equal amounts of protein were added to each well of the 96 well ELISA plate in triplicate. The ELISA was performed as per the instructions in the manufacturer's catalog. Absorbance was read in a microplate reader at 450 nm and background correction of the wells was done at 540 nm. The amount of malonyl CoA (ng/ml) in the SVZ was estimated from the standard curve.

Quantitative lipidomic analysis: Quantitative lipidomic analysis on SVZ tissue from SR [flox/flox] and nestin- cre+ mice (N=5-8 mice pooled per set per genotype; N=2 sets per genotype) and run in triplicate was done at The Metabolomics Innovation Center (TMIC) University of Alberta Canada. The analysis was performed under the four different steps listed below.

Lipid Extraction. The extraction was performed strictly following the SOP established based on a modified Folch liquid-liquid extraction protocol. Each sample was extracted in duplicate. The mass of each sample aliquot (5.0 to 6.8 mg) was employed as a normalization factor for the extraction solvents and reagents. Briefly, each aliquot of SVZ brain tissue was vortexed and homogenized with 1.0 μL of

internal standard solution / mg of sample and 33.3 µL/mg of methanol, followed by extraction with 65.7 µL/mg of dichloromethane. A clean-up step was performed with 23.6 µL/mg of water. Samples were allowed to equilibrate at room temperature for 10 min and centrifuged at 16,000 g for 10 min at 4°C. An aliquot of the organic layer was evaporated to dryness with a nitrogen blowdown evaporator. The residue was immediately re-suspended in 7.0 µL/mg of NovaMT MixB, vortexed for 1 min, and diluted with 63.0 µL/mg of NovaMT MixA (see below). A pooled mixture of the extracts of both samples was prepared for quality control.

LC-MS Analysis. The LC-MS analyses were performed by strictly following the SOP in both positive and negative ionization with extraction duplicates. A total of 12 sample injections (2 samples with extraction duplicates and injection triplicates per genotype) and 10 quality control injections (a pooled mixture of the extracts of both samples) were performed in each ionization polarity. MS/MS spectra were acquired for all samples for identification. Parameters used for data acquisition are described below.

Instrument:	Thermo Vanquish UHPLC linked to Bruker Impact II QTOF Mass Spectrometer
Column:	Waters Acquity CSH C18 column, 1.7 µm.
MPA:	NovaMT MixA
MPB:	NovaMT MixB
Gradient:	NovaMT 26-min-gradient
Flow Rate:	250 µL/min.
Injection Volume:	5.0 µL for positive ionization and 10.0 µL for negative ionization
Column Oven Temperature:	42 °C
Mass Range:	m/z 150-1500
MS/MS Collision Energies:	10-80 eV

Data Processing. LC-MS data from 22 injections were independently processed in positive and negative ionization. Lipid features were extracted and aligned using NovaMT LipidScreener. The data acquired in positive and negative ionization from each sample extraction were combined, i.e. the detected features from all samples were merged into one feature-intensity table. Missing values were substituted by the average intensity of the sample group for features detected in at least 50% of injections within the group (Nestin Cre, SR Flox and QC); or by the global minimum for all sample and QC injections for features detected in less than 50% of injections within the group. Parameters used for data processing are below.

Minimum Intensity Cut-off:	3000 cts for negative ionization; 3000 cts for positive ionization
Minimum Peak Length:	6 spectra
Retention Time Tolerance	5 seconds
Mass Tolerance:	5 mDa
Feature Filtering:	Detection for ≥80% of injections in at least one sample group (Nes Cre, SRFlox and QC)

Lipid Identification. A three-tier identification approach based on MS/MS identification and MS match was employed for lipid identification. The parameters used for identification are described below.

Tier 1 (MS/MS identification):	MS/MS match score ≥ 500; precursor m/z error ≤ 5 mDa
Tier 2 (MS/MS identification):	MS/MS match score ≥ 100; precursor m/z error ≤ 5 mDa
Tier 3 (MS match):	Mass match with m/z error ≤ 5 mDa

After tier 3 identification, a six-tier filtering and scoring approach embedded in NovaMT LipidScreener was employed to restrict the number of matches and select the best identification option to determine the

lipid sub-classes for normalization. Data normalization was performed by using a set of 14 deuterated internal standards belonging to different lipid classes. The positively and putatively identified lipids were matched to one of the 14 internal standards according to lipid class similarity and expected retention time range for each class. Intensity ratios, i.e., intensity of each lipid divided by intensity of the matched internal standard, were calculated for normalization. Statistical analysis was performed with MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/). Non-informative features (i.e., internal standards) and features with low repeatability (RSD >20% for QC injections) were filtered out. The dataset was further normalized by auto-scaling and to the median intensity. Finally, the normalized and auto-scaled features were used for statistical analysis.

Analysis of Gene expression: RNA was isolated from SVZ of WT and SR$^{-/-}$, SR $^{flox/flox}$ and nestin-cre+ mice using RNA isolation kit (Ambion). Gene expression studies related to glucose homeostasis and pancreas development were performed using the SYBR green method. cDNA synthesis was performed using High Capacity cDNA synthesis kit (Applied Biosystems). 100 ng of synthesized cDNA was used in each well of a 96 well 0.1 ml MicroAmp PCR plate (Applied Biosystems) along with SYBR Green master mix, nuclease free water and gene specific primers (1 μM final concentration) in a total volume of 10 μl. 18S rRNA was used as a housekeeping gene and non-template control was added in each plate as a control. Samples were run in triplicate. The PCR was run on an ABI Step One Plus instrument. The raw data obtained was analyzed on Step One data analysis software and mean C_T values obtained. ΔC_T and $\Delta\Delta C_T$ were calculated and fold change in gene expression obtained from $2^{exp-\Delta\Delta CT}$. The primer sequences spanning exon-exon boundaries for qPCR using SYBR Green are listed in Table S3.

Immunohistochemistry: Immunohistochemical experiments were performed on WT, SR$^{-/-}$, SR flox and nestin cre mice brain coronal and saggital sections. Briefly, the mice were euthanized by CO_2 narcosis and perfused with 4% paraformaldehyde (PFA) in PBS by cardiac puncture technique. The brain was removed and incubated at 4°C for 48 h in 4% PFA in PBS with gentle shaking. The tissue was paraffin embedded at the Johns Hopkins Oncology Core Services and sectioned at 4 μ thickness. The sections were de paraffinized using Histoclear (5 min X 2) followed by sequential incubations in absolute alcohol (5 min X 2), 95% alcohol (5 min X 2) and PBS (10 min X 2). The sections were permeabilized in 0.5% Triton X-100 in PBS for 10 min. Antigen retrieval was performed in 10 mM citrate buffer + 0.05% Tween at 60°C for 10 minutes. The sections were cooled on ice for 15 min. The sections were blocked with 1% BSA in PBS for 30 min. The sections were incubated with primary antibody (1:500 dilution in blocking buffer) a 4°C overnight. The slides were washed in PBS (10 min X 2) followed by incubation with secondary antibody (1:1000 dilution in blocking buffer) at RT for 30 min and protected from light. The sections were washed in PBS (10 min X 2). The slides were then cover slipped with mounting medium containing DAPI and edges sealed with transparent nail polish. The slides were allowed to dry at 4°C and observed using confocal microscopy.

Confocal Microscopy: Fluorescently stained mice brains SVZ sections from age matched WT, SR$^{-/-}$, SR $^{flox/flox}$ and nestin-cre+ mice were imaged on LSM 780 and LSM 810 confocal microscope (Zeiss) at 10X and 20X magnification at the microscopy core facility at Johns Hopkins. Images were acquired at different high powered fields in each section. All settings and parameters were kept constant during image acquisition. Scale bars were added to each image using Zen black software.

Rescue in monolayer adherent cultures: Rescue experiments in adherent monolayer cultures were performed by isolating NSC's from the SVZ of age matched adult WT, SR$^{-/-}$, SR $^{flox/flox}$ and nestin-cre+ mice. Following isolation based on protocol mentioned above, the cells were plated in 500 μl of growth factor media in coated 24 well plates. After 24 h, the media was replaced completely in each well with fresh media containing either an equimolar mixture of L and D-serine (100 μM) or individually with L-serine (0.6 mM) and D-serine (0.1 mM). Control cells did not receive any L or D-serine. The media with L and D-serine were replaced every 2-3 days (50 % volume in each well). Both L and D serine were made fresh on the day of addition. The cells were grown in culture for 7-10 days following which differentiation was induced by gradual removal of growth factors as mentioned in (Walker and Kempermann, 2014). All media added to rescue group of cells contained freshly prepared L and D-serine. The cells were harvested 3-4 days after induction of differentiation at which time neuroblasts were present. For

immunocytochemistry, the cells were fixed, permeabilized and stained in the 24 well plate. The cells were covered with a coverslip in the well following staining with a drop of antifade mounting medium and stored in the dark at 4°C.

Western blot: Whole brain and SVZ lysates were run on a 1 mm 4-12% Bis-Tris gel (Novex Life Technologies; Thermo Fisher) with MES SDS running buffer (Invitrogen; #NP0002-02) initially at 75 V and then at 120 V. The samples were then transferred to a pre-wet immobilon PVDF transfer membrane (Merck Millipore Ltd; Pore Size 0.45 μm) and sandwiched between wet filter paper and cassette holder. The entire apparatus was placed in a wet transfer apparatus (Bio-Rad) and run at 90 V for 90 min on ice. After transfer, the membrane was removed and incubated in blocking buffer containing Tris buffered saline + Tween 20 (TBS-T) containing 5% BSA for 30 minutes at room temperature (RT). The membrane was incubated on a shaker with the respective primary antibody at 1:1000 dilution in blocking buffer overnight at 4°C. After overnight incubation, the membrane was rinsed 3 times with TBS-T and washed in TBS-T at RT for 15 minutes per wash. The membrane was washed a total of 4 times. After washing, the membrane was incubated with HRP conjugated secondary antibody (IgG; mouse or rabbit) (GE Healthcare UK) at 1:5000 dilution in blocking buffer and incubated at RT with shaking for 1 h. After incubation with secondary antibody, the membrane was washed 4 times with TBS-T. The duration of each wash was 15 min. After the last wash, the membrane was incubated with Enhanced Chemiluminescent (ECL) reagent (Thermo Scientific; Super Signal West Pico Plus Peroxide solution and Luminol enhancer solution in a 1:1 (v/v) for 5 min in the dark. The excess ECL reagent was removed using a kimwipe and the membrane was placed in between a plastic sheet and in a cassette holder and developed on an 8" X 10" Ultra Cruz autoradiography film (Santa Cruz Biotechnology) in a developer.

Time spent sniffing novel odor. Mouse olfactory behavior was tested in age matched WT and SR$^{-/-}$ mice (N=20-25 per group) were collected and brought to the behavior suite and the mice placed in clean cages with new bedding. The mice were placed in an adjacent room before testing and acclimatized in their new cage for 5 minutes. The new cage had bedding 2-2.5 inches deep. The mice were prepared as mentioned above for this test. Using two disposable pipettes two drops of vanilla extract were placed on one side of the cage and two drops of water were placed on the opposite side of the cage. This eliminates the confounding principle of investigating a novel "object" versus a novel odor. The mice are timed for 2 minutes and recorded for the amount of time it spends sniffing the vanilla extract within the allotted 2 minutes. The data are recorded if the mouse is within 1.5 inches of the placed vanilla extract. The mice are then returned to their original cages. The data were compared between the groups.

Blood Glucose Estimation. Blood glucose level was monitored by tail bleeding immediately before and at indicated times after injection using Contour glucometer (Bayer Co, Japan) and Contour blood glucose test strips (Cat#7097C; Ascensia Diabetes care Inc, NJ). Blood glucose measurements were obtained from tail veins at indicated time points post injection. A small drop of blood from the tail was placed on a new glucose strip each time, inserted into the glucometer and value recorded.

Insulin ELISA: Quantitative estimation of insulin from brain homogenates and SVZ (following microdissection) of age matched WT, SR$^{-/-}$, SR $^{flox/flox}$ and nestin-cre+ mice was performed using the Ultra Sensitive Mouse Insulin ELISA Kit (Cat# 90080; Crystal Chem). The protocol was followed exactly as mentioned for the low range assay. Standard curve for insulin was developed prior to the actual experiment (low range). Each individual sample was done in quadruplicate. The samples were pooled from N=4-10 mice per genotype and analyzed. The absorbance was read at 450 nm in a 96 well plate reader and also at 630 nm and data plotted after correction at 630 nm.

Protein-protein Interaction Network Analysis. The search tool for retrieval of interacting genes (STRING) (https://string-db.org) database was applied to predict functional interactions of proteins. To seek potential interactions between differentially expressed genes, the STRING tool was employed with k means clustering. Edges between clusters are shown in dashed lines. Interactions within a cluster is shown in solid lines. Text mining, databases, and co-expression as well as species limited to *Homo sapiens* and *Mus musculus* were applied to construct the protein-protein interaction networks (Szklarczyk D et al).

Statistics: Statistical tests were computed using KaleidaGraph (Synergy Software, Reading PA). Data are represented as mean ± SD. Unpaired student's t test was used for pairwise comparison with a fixed control condition. For multiple pairwise comparisons with different control and treatment conditions, one-way ANOVA analysis followed by Tukey's post hoc test was used. Values with $p < 0.05$ were considered significant.

Key Resources Table

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit anti Ki67 antibody	Novus Bio	NB500-170SS
Rabbit anti FASN antibody	Bethyl Labs	A301-324A-T
Mouse anti PSA-NCAM antibody	DSHB Iowa	5A5-c
Mouse anti Spot14 antibody	Santacruz Biotechnology	sc-137178
Rabbit anti MCM2 antibody	Bethyl Labs	IHC-00009-T
Mouse anti BrdU antibody	Sigma	B8434-25
Mouse anti CD24 antibody	eBioscience	14-0242-82
Rabbit anti Serine Racemase antibody D5V9Z	Cell Signaling Technology	#29798
Rabbit anti Prominin antibody	Millipore	ZRB1013-25
Rabbit anti NeuN antibody	Cell Signaling Technology	D4G40
Mouse anti β-III tubulin antibody	R&D Systems	MAB1195-SP
Mouse anti GFAP antibody	Millipore	MAB360
Rabbit anti Olig2 antibody	Novus Bio	28667SS
Rabbit anti Akt antibody	Cell Signaling Technology	9272S
Rabbit anti phospho-Akt (S473) antibody	Cell Signaling Technology	4060S
Rabbit β-actin antibody	ABClonal	NO:AC026
Goat anti mouse Alexa 547 secondary antibody	Invitrogen	R37121
Goat anti rabbit Alexa 488 secondary antibody	Abcam	ab150077
Bacterial and virus strains		

N/A		
Biological samples		
N/A		
Chemicals, peptides, and recombinant proteins		
L-serine	Sigma	S4500
D-serine	Sigma	S4250
Ascensia Contour Blood Glucose meter	Bayer Healthcare LLC	7151B
Contour Blood Glucose test strips	Ascensia Diabetes Care	7097C
Critical commercial assays		
Mouse FASN ELISA Kit	CUSABIO	CSB-EL008435MO
Mouse Malonyl CoA ELISA Kit	CUSABIO	CSB-E12896m
Mouse Insulin ELISA Kit	Crystal Chem	#90080
Deposited data		
N/A		
Experimental models: Cell lines		
N/A		
Experimental models: Organisms/strains		
SR$^{-/-}$ mouse (Serine Racemase knock out mouse)	Basu et al; Molecular Psychiatry 2009	Joe Coyle, Harvard Medical School
WT mouse C57 BL/6	Jackson Labs	#000664
SR $^{flox/flox}$ mice		Joe Coyle, Harvard Medical School

Nestin Cre mice (background)	Jackson Labs	003771
Nestin-Cre+ mice	This paper	
Oligonucleotides		
Spot14, Citrate Lyase, Cpt1a, Mig12, Acc primers	Knobloch et al; Nature 2013; Table 3, this paper.	
Mouse PSPH qPCR primer pair	Sino Biological	MP201727
Mouse PHGDH qPCR primer pair	Sino Biological	MP201627
HACoA primer	Table 3, this paper	
ECH1 primer	Table 3, this paper	
Recombinant DNA		
N/A		
Software and algorithms		
Zen Black	Zeiss	https://www.zeiss.com/microscopy/us/products/microscope-software/zen.html
Image J	NIH	https://imagej.nih.gov/ij
KaleidaGraph v4.1	Synergy Software	https://www.synergy.com

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Supplemental References

Basu, A.C., Tsai, G.E., Ma, C.L., Ehmsen, J.T., Mustafa, A.K., Han, L., Jiang, Z.I., Benneyworth, M.A., Froimowitz, M.P., Lange, N.*, et al.* (2009). Targeted disruption of serine racemase affects glutamatergic neurotransmission and behavior. Mol Psychiatry *14*, 719-727.

Giusti, S.A., Vercelli, C.A., Vogl, A.M., Kolarz, A.W., Pino, N.S., Deussing, J.M., and Refojo, D. (2014). Behavioral phenotyping of Nestin-Cre mice: implications for genetic mouse models of psychiatric disorders. J Psychiatr Res *55*, 87-95.

Doetsch, F., Caille, I., Lim, D.A., Garcia-Verdugo, J.M., and Alvarez-Buylla, A. (1999). Subventricular zone astrocytes are neural stem cells in the adult mammalian brain. Cell *97*, 703-716.

Walker, T.L., and Kempermann, G. (2014). One mouse, two cultures: isolation and culture of adult neural stem cells from the two neurogenic zones of individual mice. J Vis Exp, e51225.

Azari, H., and Reynolds, B.A. (2016). In Vitro Models for Neurogenesis. Cold Spring Harb Perspect Biol *8*.

Azari, H., Sharififar, S., Fortin, J.M., and Reynolds, B.A. (2012). The neuroblast assay: an assay for the generation and enrichment of neuronal progenitor cells from differentiating neural stem cell progeny using flow cytometry. J Vis Exp.

Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, et al. (2015). STRING v10: protein-protein interaction networks, integrated over the tree of life. Nucleic Acids Res, 43:D447–52.

Pircs, K., Petri, R., Jakobsson, J. (2018). Crosstalk between MicroRNAs and Autophagy in Adult Neurogenesis: Implications for Neurodegenerative Disorders. Brain Plast 3(2),195-203.