

Supplemental Information

Dexas1 is a homeostatic regulator of exercise-dependent proliferation and cell survival in the hippocampal neurogenic niche

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Supplemental Figures

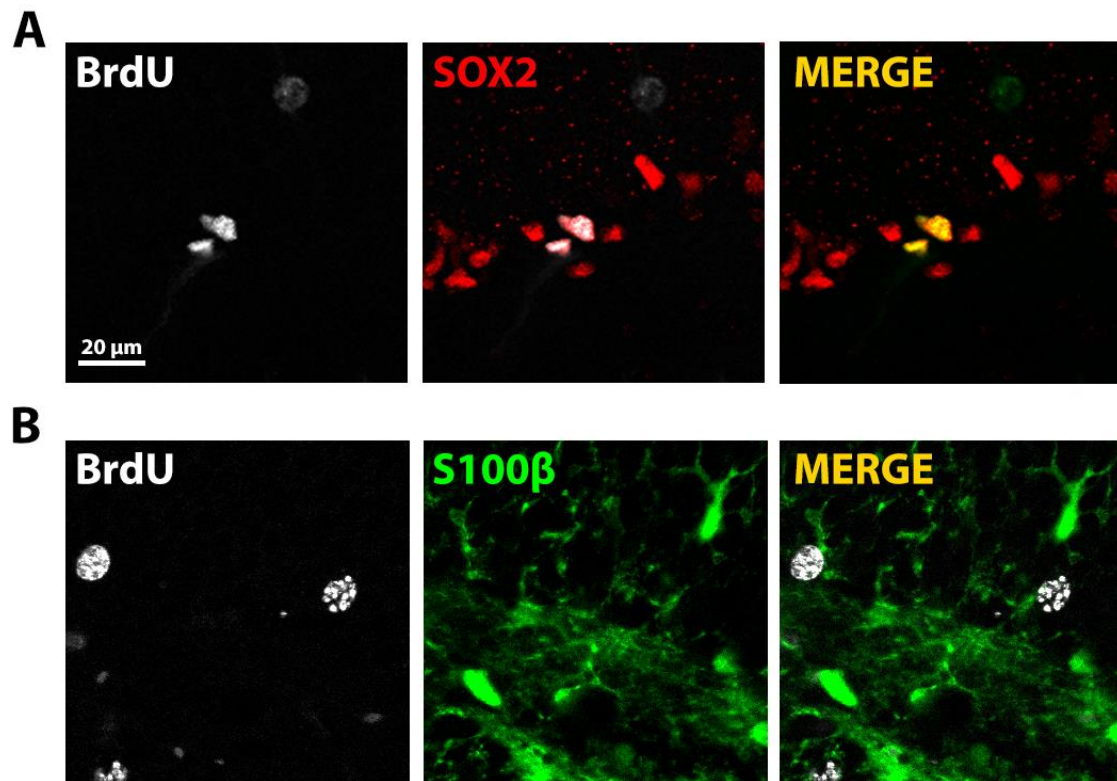


Figure S1. Co-localization of 28-days post-injection (DPI) label-retaining BrdU⁺ cells with SOX2 or S100 β within the Subgranular Zone (SGZ) and Dentate Gyrus (DG). (A) Representative photomicrograph of BrdU (white) and SOX2 (red) co-localization in the SGZ at 28 DPI. (B) Representative photomicrograph of BrdU (white) and S100B (green) expression in the DG at 28 DPI.

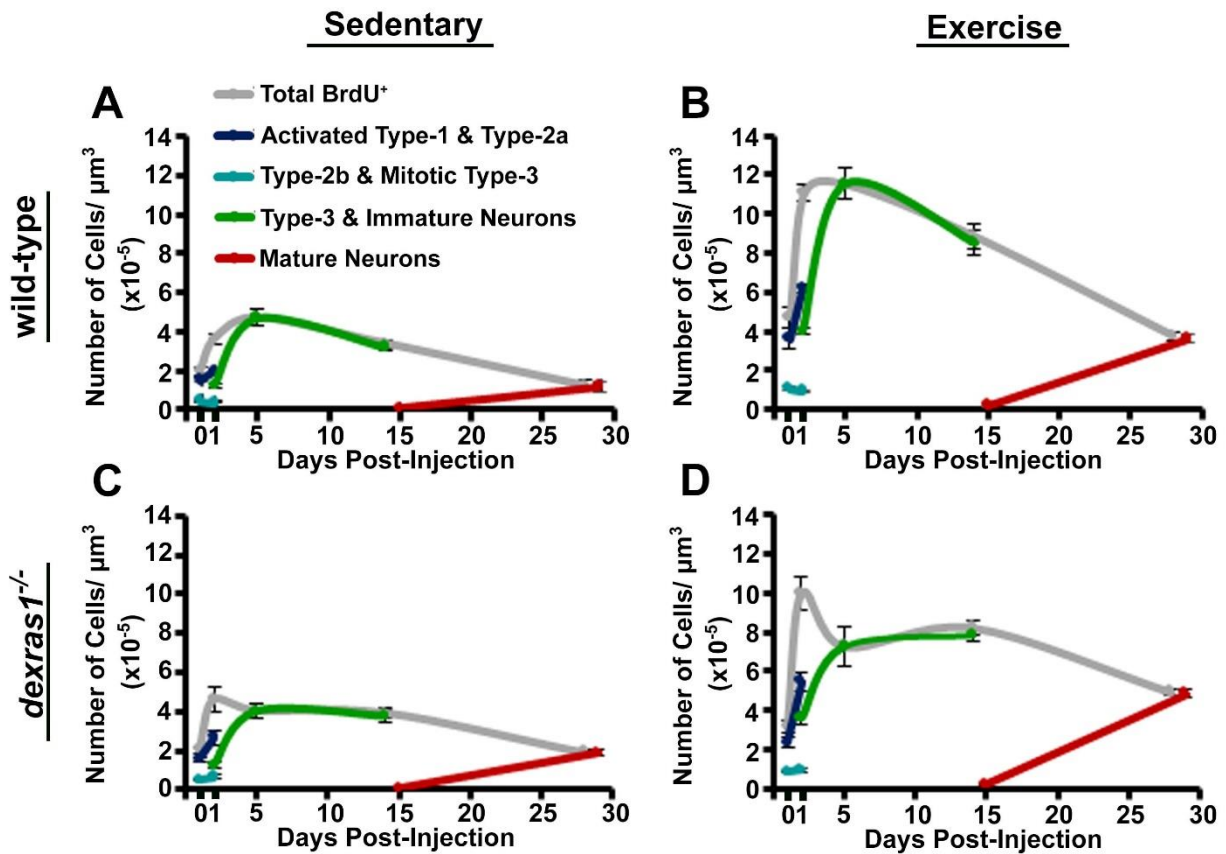


Figure S2. Fate of BrdU-labeled dividing cells in the SGZ of sedentary and exercised mice. (A-D) Quantification of the number of total BrdU⁺ cells (grey), activated type-1 & type-2a cells (blue), type-2b & mitotic type-3 cells (turquoise), post-mitotic type-3 & immature neurons (green), and mature neurons (red) per μm^3 of SGZ ($\times 10^{-5}$) from 1 hour to 28 days post-BrdU injection, in **(A)** sedentary wild-type mice, **(B)** exercised wild-type mice, **(C)** sedentary *dexas1*^{-/-} mice, and **(D)** exercised *dexas1*^{-/-} mice. All values represent mean \pm standard error.

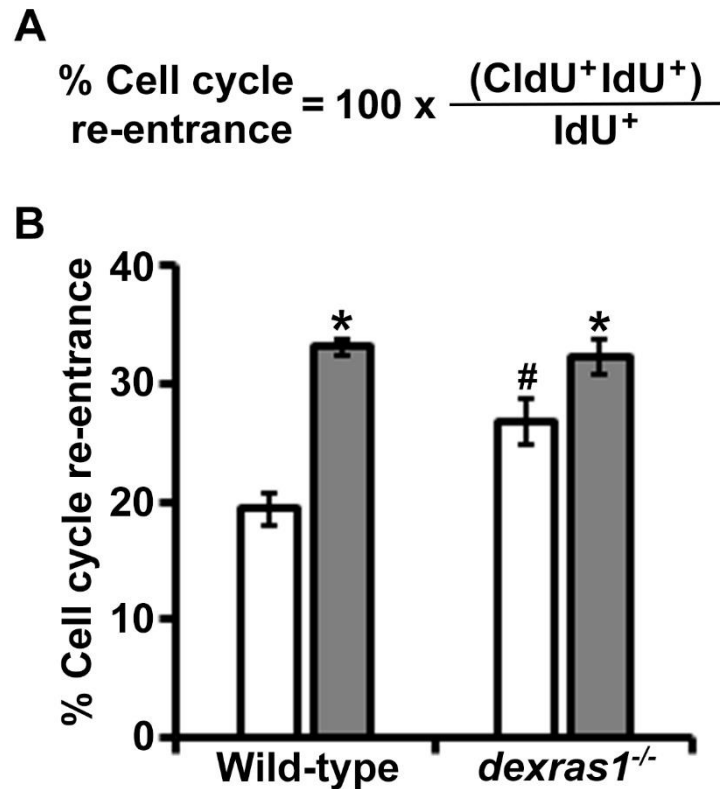


Figure S3. Exercise enhances cell cycle re-entrance of type-2 cells. Relates to Fig. 4. Wild-type and *dexas1*^{-/-} mice were injected with equimolar amounts of IdU and CldU, spaced 18-hrs apart, after either 5 days of sedentary or exercise conditions. **(A)** Formula used to calculate the estimated % cell cycle re-entrance of S-phase cells after 18-hrs. **(B)** Quantification of the % cell cycle entrance. Values represent mean ± standard error or estimated value. **p*<0.05 vs. sedentary control. #*p*<0.05 vs. wild-type control. *n*= 4-5 per group.

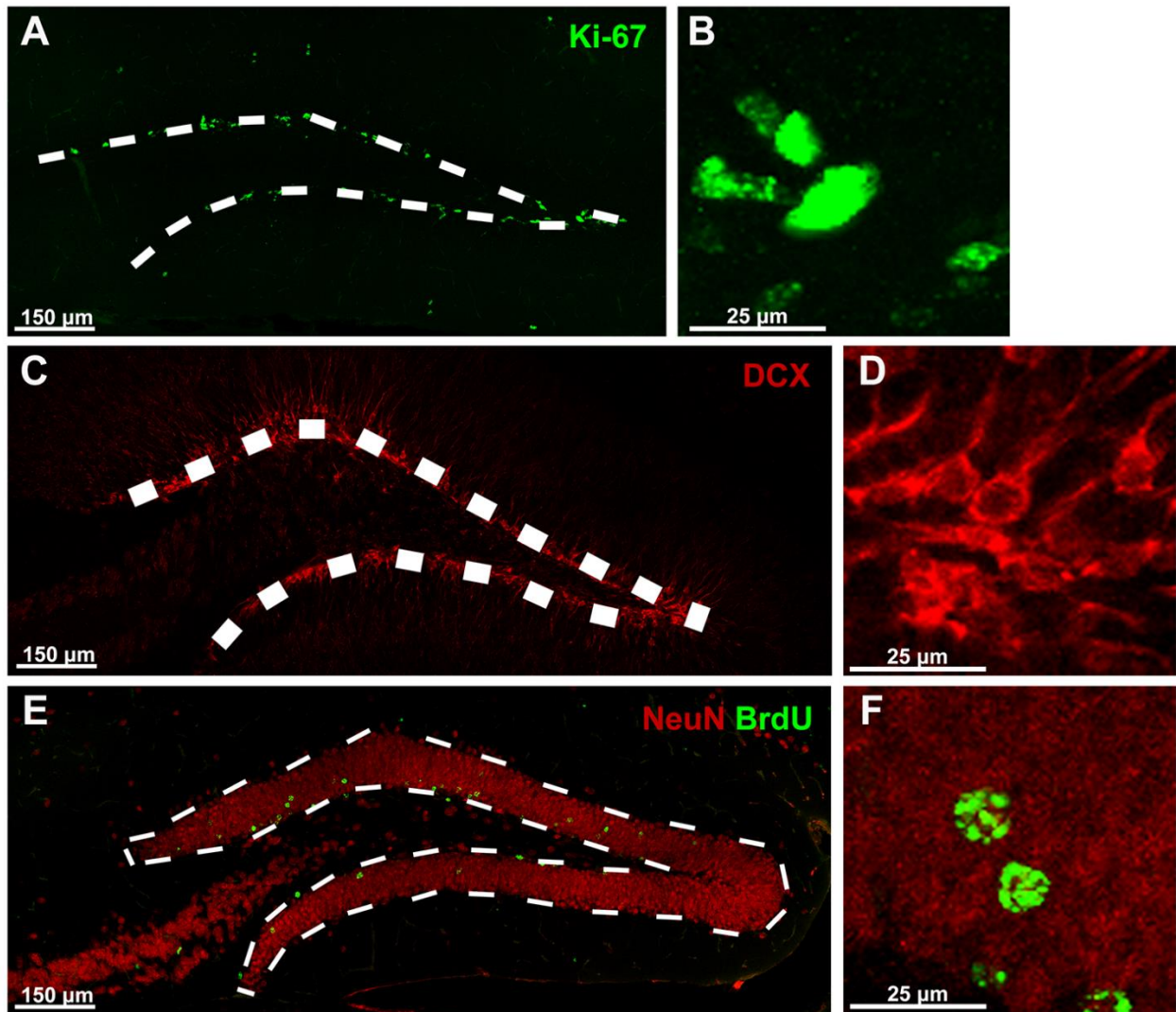


Figure S4. Delimited hippocampal area used for density measurements (A, C, E) Representative photomicrographs of **(A)** Ki-67, **(C)** DCX, and **(E)** NeuN/ BrdU. White hashed lines represent the area used for density measurements. **(B, D, F)** Zoomed-in images of **(B)** Ki-67, **(D)** DCX, and **(F)** NeuN/ BrdU. Images are represented as a **(A-B, E-F)** collapsed z-stack image (30- μm) or **(C-D)** a single z-stack image (5- μm) acquired at 40x magnification.

Supplemental Tables

Table S1. Label Retaining Assay Cell Counts

| Total BrdU (number of cells per μm^3) ($\times 10^{-5}$) | | | | | | |
|--|------------------|-----------------|-----------------|-----------------|------------------|-----------------|
| Genotype | Condition | 1 HPI | 1 DPI | 5 DPI | 14 DPI | 28 DPI |
| wild-type | Sedentary | 2.0 \pm 0.2 | 3.6 \pm 0.3 | 5.3 \pm 0.5 | 3.4 \pm 0.2 | 1.3 \pm 0.3 |
| wild-type | Exercise | 4.7 \pm 0.6* | 11.1 \pm 0.4* | 12.2 \pm 0.8* | 8.9 \pm 0.6* | 2.3 \pm 0.2 |
| <i>dexas1</i> ^{-/-} | Sedentary | 2.0 \pm 0.1 | 4.6 \pm 0.6 | 4.4 \pm 0.4 | 3.9 \pm 0.2 | 1.9 \pm 0.1 |
| <i>dexas1</i> ^{-/-} | Exercise | 3.1 \pm 0.3*# | 10.0 \pm 0.8* | 7.8 \pm 0.1*# | 8.2 \pm 0.4* | 4.9 \pm 0.2*# |
| Total Type 2a (BrdU⁺ Ki-67⁺ DCX⁻) (number of cells per μm^3) ($\times 10^{-5}$) | | | | | | |
| Genotype | Condition | 1 HPI | 1 DPI | 5 DPI | 14 DPI | 28 DPI |
| wild-type | Sedentary | 1.5 \pm 0.1 | 2.0 \pm 0.2 | ND | ND | ND |
| wild-type | Exercise | 3.6 \pm 0.5* | 6.1 \pm 0.2* | ND | ND | ND |
| <i>dexas1</i> ^{-/-} | Sedentary | 1.5 \pm 0.9 | 2.7 \pm 0.4 | ND | ND | ND |
| <i>dexas1</i> ^{-/-} | Exercise | 2.3 \pm 0.3*# | 5.5 \pm 0.5* | ND | ND | ND |
| Total Type 2b (BrdU⁺ Ki-67⁺ DCX⁺) (number of cells per μm^3) ($\times 10^{-6}$) | | | | | | |
| Genotype | Condition | 1 HPI | 1 DPI | 5 DPI | 14 DPI | 28 DPI |
| wild-type | Sedentary | 4.4 \pm 0.8 | 4.0 \pm 0.2 | ND | ND | ND |
| wild-type | Exercise | 10.8 \pm 1.2 | 9.3 \pm 0.6* | ND | ND | ND |
| <i>dexas1</i> ^{-/-} | Sedentary | 4.8 \pm 0.6 | 6.8 \pm 1.1# | ND | ND | ND |
| <i>dexas1</i> ^{-/-} | Exercise | 8.4 \pm 0.7 | 9.1 \pm 0.8* | ND | ND | ND |
| Total Type 3 (BrdU⁺ Ki-67⁻ DCX⁺) (number of cells per μm^3) ($\times 10^{-5}$) | | | | | | |
| Genotype | Condition | 1 HPI | 1 DPI | 5 DPI | 14 DPI | 28 DPI |
| wild-type | Sedentary | ND | 1.2 \pm 0.1 | 4.7 \pm 0.4 | 3.2 \pm 0.2 | ND |
| wild-type | Exercise | ND | 4.0 \pm 0.2* | 11.5 \pm 0.8* | 8.5 \pm 0.6* | ND |
| <i>dexas1</i> ^{-/-} | Sedentary | ND | 1.2 \pm 0.2 | 4.0 \pm 0.4 | 3.8 \pm 0.3 | ND |
| <i>dexas1</i> ^{-/-} | Exercise | ND | 3.6 \pm 0.3* | 7.3 \pm 1.0*# | 7.8 \pm 0.4* | ND |
| Newborn Neurons (BrdU⁺ NeuN⁺) (number of cells per μm^3) ($\times 10^{-5}$) | | | | | | |
| Genotype | Condition | 1 HPI | 1 DPI | 5 DPI | 14 DPI | 28 DPI |
| wild-type | Sedentary | ND | ND | ND | 0.10 \pm 0.01 | 1.2 \pm 0.3 |
| wild-type | Exercise | ND | ND | ND | 0.18 \pm 0.02* | 3.6 \pm 0.2* |
| <i>dexas1</i> ^{-/-} | Sedentary | ND | ND | ND | 0.07 \pm 0.02 | 1.9 \pm 0.1 |
| <i>dexas1</i> ^{-/-} | Exercise | ND | ND | ND | 0.20 \pm 0.03* | 4.8 \pm 0.2*# |

ND : Not detected

* : Significant at $p \leq 0.05$ within the same genotype.

: Significant at $p \leq 0.05$ between genotypes of the same condition.

Table S2. Fold changes in BrdU⁺ cells relative to genotype, condition and time

| Fold change in BrdU within the same harvest time and genotype | | | | | | |
|--|------------------|--------------|--------------|--------------|---------------|---------------|
| ((Exercise – Sedentary) / Sedentary) | | | | | | |
| Genotype | Condition | 1 HPI | 1 DPI | 5 DPI | 14 DPI | 28 DPI |
| wild-type | Sedentary | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| wild-type | Exercise | 1.38 | 2.06 | 1.32 | 1.62 | 1.99 |
| <i>dexas1^{-/-}</i> | Sedentary | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| <i>dexas1^{-/-}</i> | Exercise | 0.56 | 1.18 | 0.79 | 1.10 | 1.58 |
| Fold Change in BrdU relative to 1 HPI within the same genotype and condition | | | | | | |
| ((DPI – 1 HPI)/1 HPI) | | | | | | |
| Genotype | Condition | 1 HPI | 1 DPI | 5 DPI | 14 DPI | 28 DPI |
| wild-type | Sedentary | 0.00 | 0.83 | 1.66 | 0.72 | -0.37 |
| wild-type | Exercise | 0.00 | 1.35 | 1.60 | 0.89 | -0.20 |
| <i>dexas1^{-/-}</i> | Sedentary | 0.00 | 1.26 | 1.15 | 0.93 | -0.06 |
| <i>dexas1^{-/-}</i> | Exercise | 0.00 | 2.17 | 1.47 | 1.61 | 0.56 |
| Fold Change in BrdU relative between harvest times within the same genotype and condition | | | | | | |
| ((DPI – previous DPI)/ previous DPI) | | | | | | |
| Genotype | Condition | 1 HPI | 1 DPI | 5 DPI | 14 DPI | 28 DPI |
| wild-type | Sedentary | 0.00 | 0.83 | 0.46 | -0.36 | -0.63 |
| wild-type | Exercise | 0.00 | 1.35 | 0.10 | -0.27 | -0.58 |
| <i>dexas1^{-/-}</i> | Sedentary | 0.00 | 1.26 | -0.05 | -0.10 | -0.51 |
| <i>dexas1^{-/-}</i> | Exercise | 0.00 | 2.17 | -0.22 | 0.06 | -0.40 |

Table S3. Antibody concentrations for immunofluorescence, immunohistochemistry and western blotting.

| Antibody | Dilution | Cat. No. | Company |
|---|---|-----------------|---------------------------|
| rat anti-BrdU/ CldU | 1:4,000 (BrdU IHC) 1:1,000 (BrdU/ CldU IF) | OBT0030G | Bio-Rad Laboratories |
| mouse monoclonal anti-BrdU | 1:2,000 | 347580 | BD Biosciences |
| mouse monoclonal anti-IdU | 1:4,000 | 32D8.D9 | Novus Bio |
| rat anti-Ki-67 | 1:1,000 | SolA15 | eBioscience |
| goat polyclonal anti-Doublecortin | 1:100 | C-18 | Santa Cruz Biotechnology |
| mouse monoclonal anti-NeuN | 1:1,000 | A60 | EMD Millipore |
| goat polyclonal anti-Sox2 | 1:1,000 | Y-17x | Santa Cruz Biotechnology |
| mouse anti-GFAP | 1:1,000 | A21282 | Life Technologies |
| rabbit anti-S100B | 1:500 | ab52642 | Abcam |
| rabbit anti-p-CREB (Ser133) | 1:100 | 9198S | Cell Signaling Technology |
| rabbit anti-NR2A | 1:10,000 | ab133265 | Abcam |
| rabbit anti-NR2B | 1:10,000 | 4207S | Cell Signaling Technology |
| rabbit anti-Phospho-p44/42 MAPK (T202/Y204) | 1:5,000 | 9101S | Cell Signaling Technology |
| rabbit anti-p44/42 MAPK | 1:1,000 | 9102S | Cell Signaling Technology |
| rabbit anti-actin | 1:10,000 | | Sigma-Aldrich |
| anti-rat IgG or anti-rabbit IgG | 1:300 | | Vector Laboratories |
| Alexa-Fluor secondary antibodies | 1:1,000 | | Life Technologies |
| goat anti-Rabbit IgG H&L (HRP) | 1:100,000 | | Life Technologies |

Table S4. qPCR Primers

| Gene | Forward | Reverse |
|---------------|-----------------------------|-----------------------------|
| <i>bdnf</i> | 5'-AGCATGAAATCTCCCAGCCT-3' | 5'-CGGTCCCCAAGGTTCTAGAC-3' |
| <i>trkb</i> | 5'-CGCCCTGTGAGCTGAACTCTG-3' | 5'-CTGCTTCTCAGCTGCCTGACC-3' |
| <i>vegf-a</i> | 5'-CCAGGAGGACCTTGTGTGAT-3' | 5'-GGGAAGGGAAGATGAGGAAG-3' |
| <i>dexas1</i> | 5'-CCACACAACCTGAGGACCTT-3' | 5'-TTCACAGCAGGTGACTGTCC-3' |
| <i>gapdh</i> | 5'-TCCATGACAACCTTTGGCATT-3' | 5'-GTTGCTGTTGAAGTCGCAGG-3' |