Cell Reports, Volume *27*

Supplemental Information

Early Seizure Activity Accelerates Depletion of Hippocampal Neural Stem Cells and Impairs Spatial Discrimination in an Alzheimer's Disease Model

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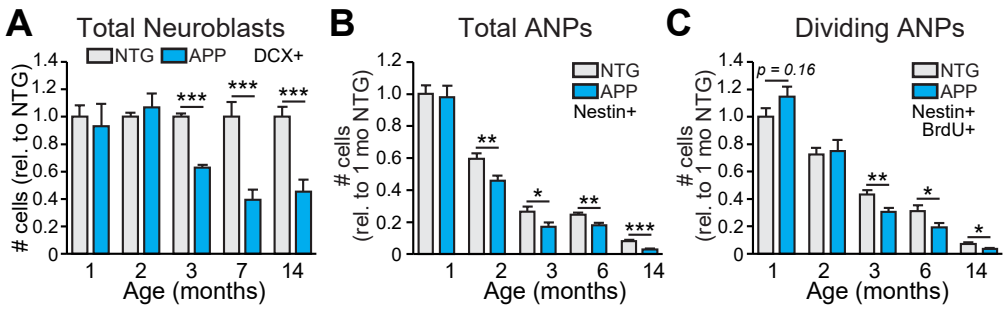

Figure S1. The effects of seizures on neuroblasts and amplifying neural progenitors (ANPs). Related to Figures 1 and 2.
(A) Optical density measurement of DCX at 1 month of age (n = 9-12 mice per genotype), and number of DCX+ neuroblasts at 2 (n = 6 mice per genotype), 3 (n = 8 mice per genotype), 7 (n = 9-10 mice per genotype), and 14 (n = 11-12 mice per genotype) months of age, normalized to NTG at each time point. Note that the 1 month time point is the same data as presented in Fig 1D.
(B) Numbers of Nestin+ ANPs were quantified in NTG and APP at 1 (n = 10-11 mice per genotype), 2 (n = 14 mice per genotype), 3 (n = 8 mice per genotype), 6 (n = 9-10 mice per genotype), and 14 (n = 11-12 mice per genotype) months of age. Cell counts are presented here as normalized to the average of 1-month-old NTG mice.
(C) Number of BrdU+ Nestin+ ANPs were quantified in NTG and APP mice at 1 (n = 9-10 mice per genotype), 2 (n = 8 mice per genotype), 3 (n = 8 mice per genotype), 6 (n = 8 mice per genotype), and 14 (n = 11-12 mice per genotype) months of age. Cell counts are presented here as normalized to the average of 1-month-old NTG mice.
*$p < 0.05$; **$p < 0.01$, ***$p < 0.001$, two-tailed unpaired Student's t-test comparing means between NTG and APP mice at each age (A-C).

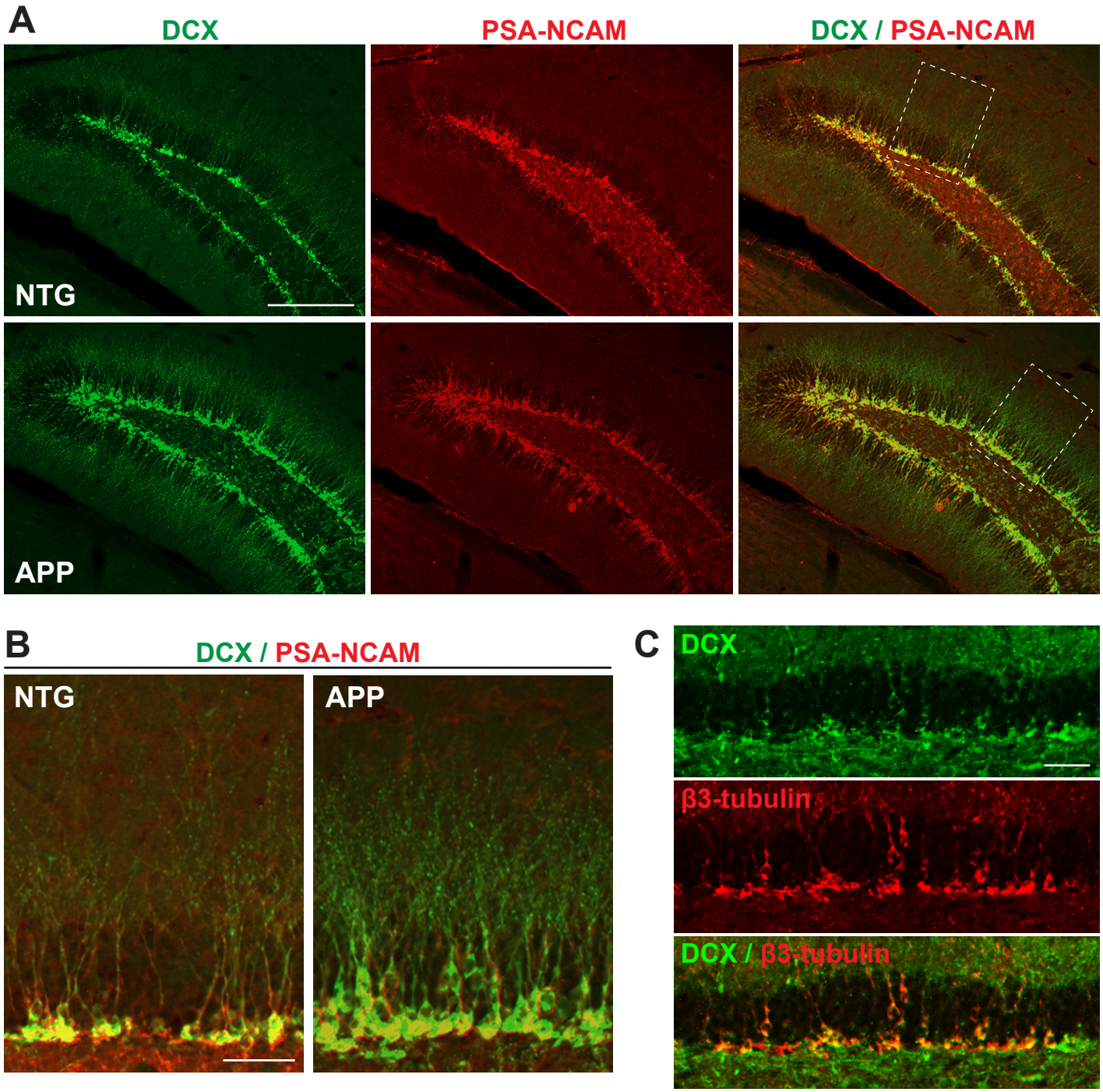

Figure S2. Doublecortin-expressing cells in the dentate gyrus of NTG and APP mice also express PSA-NCAM and β3-tubulin. Related to Figure 1.

Coronal sections from mice at 2 months of age were immunostained.

(A) Doublecortin (green, left panels) is expressed in cell bodies and in dendritic processes, as is PSA-NCAM (red, middle panels). Overlaid images (right panels) reveal coexpression of doublecortin and PSA-NCAM (yellow) in neurons in the subgranular zone of NTG mice (top panels) and APP mice (bottom panels). Scale bar, 200μm.

(B) Inset of are indicated in overlaid images in A. Note that the increase in doublecortin- and PSA-NCAM-expressing neurons is evident at this age, consistent with findings in Figure 1 of the main paper. Scale bar, 50μm.

(C) Doublecortin-expressing cells (green, top panel) also express β3-tubulin, a marker of neuronal cells (red, middle panel; see overlay, bottom panel). Scale bar, 50μm.

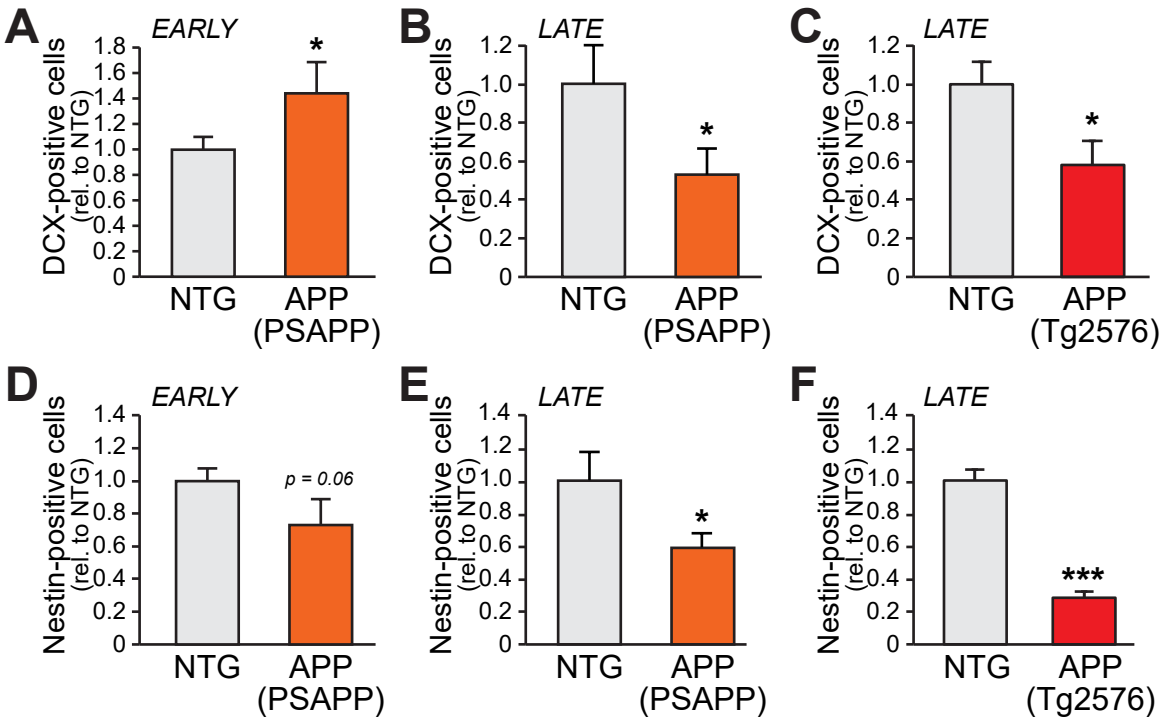

Figure S3. The PSAPP and TG2576 lines of transgenic APP mice exhibit alterations in immature neurons and neural stem cells similar to J20 APP mice. Related to Figures 1 and 2.

Brain sections from PSAPP mice at 5 months of age (n = 9-11 mice per genotype) and 12 months of age (n = 11-12 mice per genotype), and from Tg2576 mice at 10 months of age (n = 11 mice per genotype) were immunostained for doublecortin and nestin.

(A-B) Immunostaining of brain sections from PSAPP mice demonstrate increased number of DCX-expressing immature neurons at early disease stages (5 months of age, A) and decreased numbers at late disease stages (12 months of age, B) compared with age-matched controls.

(C) Tg2576 mice at late disease stages (10 months of age) also showed decreased DCX-expressing newborn neurons.

(D-E) PSAPP mice exhibit modest decreases in nestin-expressing neural stem cells at early disease stages (D) that further decrease at later disease stages (E).

(F) Tg2576 mice at late disease stages also show decreased Nestin-expressing neural stem cells compared with NTG controls.

For statistical analyses, one-tailed unpaired Student's t-tests were used since the hypothesis was that the direction of change in PSAPP and Tg2576 mice would mirror that observed in J20 mice in Figures 1 and 2, *$p < 0.01$, *** $p < 0.001$. Values indicate mean ± SEM.

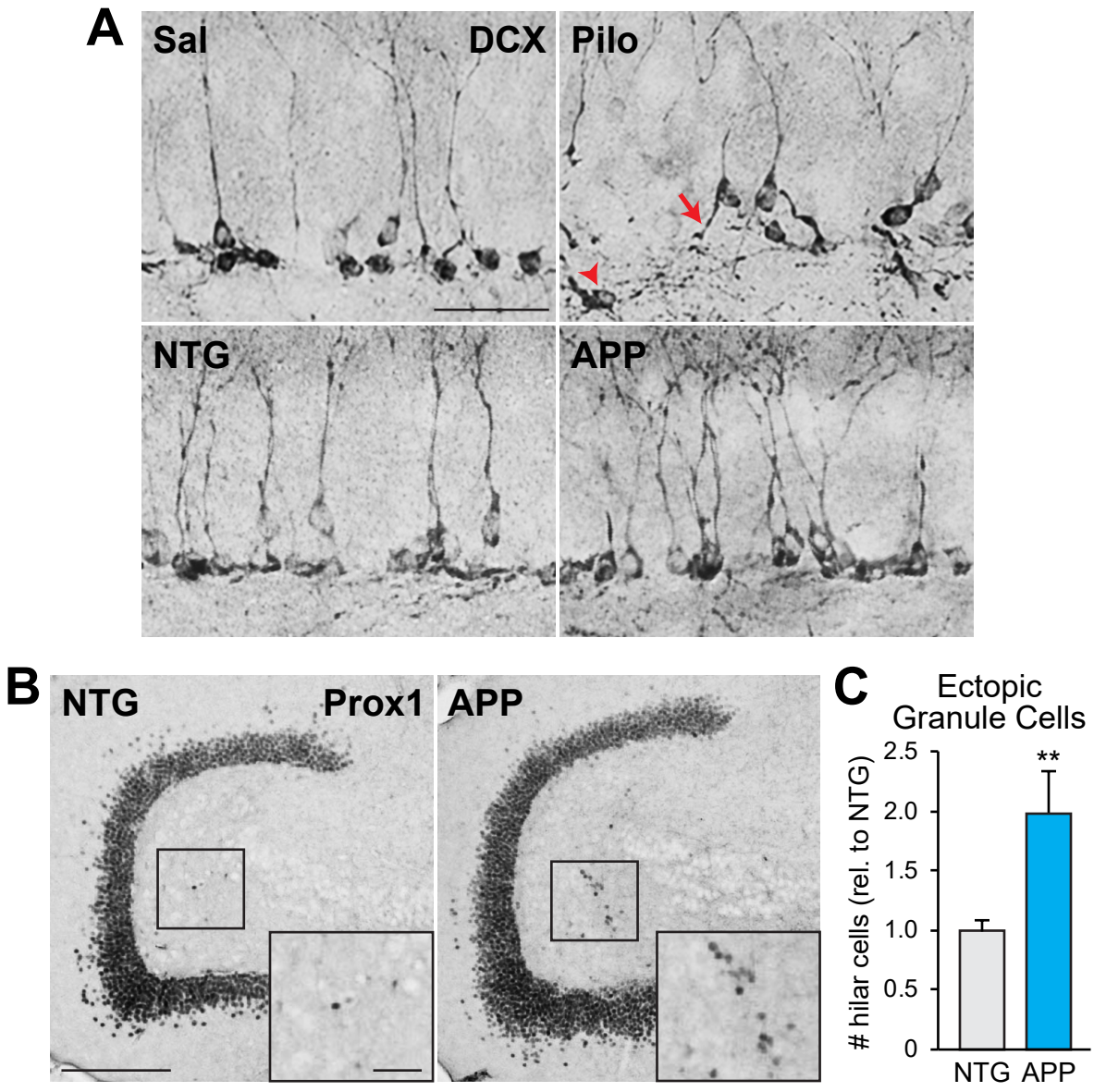

Figure S4. Newborn neurons in APP mice exhibit normal morphology, but show increased ectopic migration into the hilus compared to NTG mice. Related to Figure 1.

(A) Morphology of DCX+ cells is not obviously different between NTG and APP mice (3 months of age). In contrast, pilocarpine-treated wild-type mice (270-280 mg/kg, IP, 6 weeks post status epilepticus) showed altered neuronal polarity (arrow) and migration (arrowhead) compared to saline-treated mice. Scale bar, 50µm.

(B) APP mice show increased number of ectopic Prox1+ granule neurons in the hilus compared to NTG mice (6 months of age, n = 7-10 mice per genotype). Scale bar, 250µm, inset scale bar, 50µm.

**$p < 0.01$, two-tailed unpaired Student's t-test. Values indicate mean ± SEM.

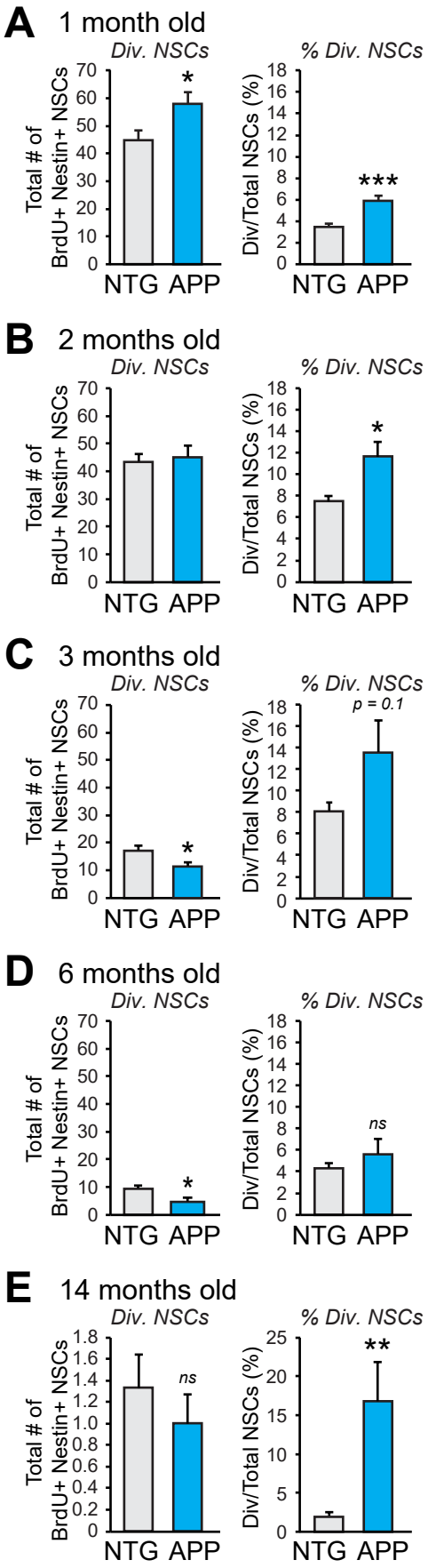

Figure S5. APP mice exhibit altered NSC division at different ages. Related to Figure 2.

(A-E) Neural stem cell (NSC) division is represented as total number of BrdU+ Nestin+ dividing NSCs (left), and as a percentage of dividing NSCs/total NSCs (right) in NTG and APP mice at 1 (A, n = 9-10 mice per genotype), 2 (B, n = 8 mice per genotype), 3 (C, n = 8 mice per genotype), 6 (D, n = 8 mice per genotype), and 14 (E, n = 11-12 mice per genotype) months of age.

*p < 0.05; **p < 0.01, ***p < 0.001, *ns*, not significant, two-tailed unpaired Student's t-test. Values indicate mean ± SEM.

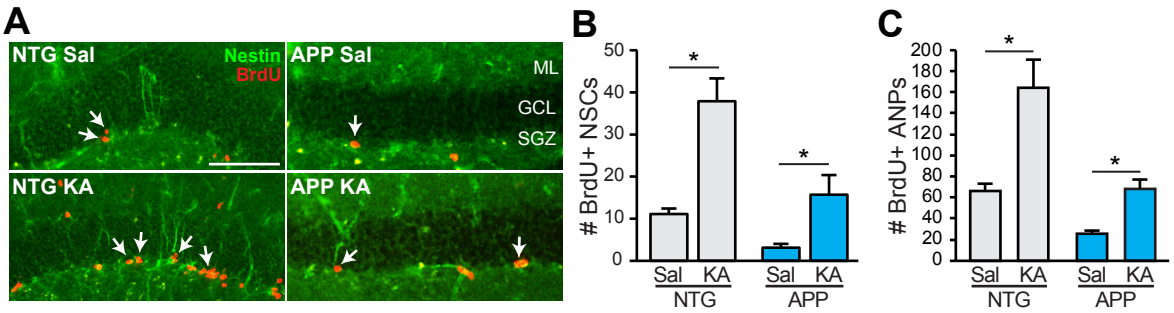

Figure S6. Kainic acid seizures induce NSC division in NTG and APP mice. Related to Figures 2 and 3.
(A) Representative images of Nestin/BrdU staining in 8-month-old NTG and APP mice that received intraperitoneal injection of saline or kainic acid (KA, 15 mg/kg). Scale bar, 100μm.
(B) Number of BrdU+ Nestin+ NSCs in the SGZ of NTG and APP mice injected with saline or kainic acid (n = 7-13 mice per genotype and treatment). Kruskal-Wallis test revealed significant differences between groups ($p < 0.0001$).
(C) Number of BrdU+ Nestin+ ANPs in the SGZ of NTG and APP mice injected with either saline or kainic acid (n = 7-13 mice per genotype and treatment). Kruskal-Wallis test revealed significant differences between groups ($p < 0.0001$).
*$p < 0.05$, Dunn post-hoc test. Values indicate mean ± SEM.

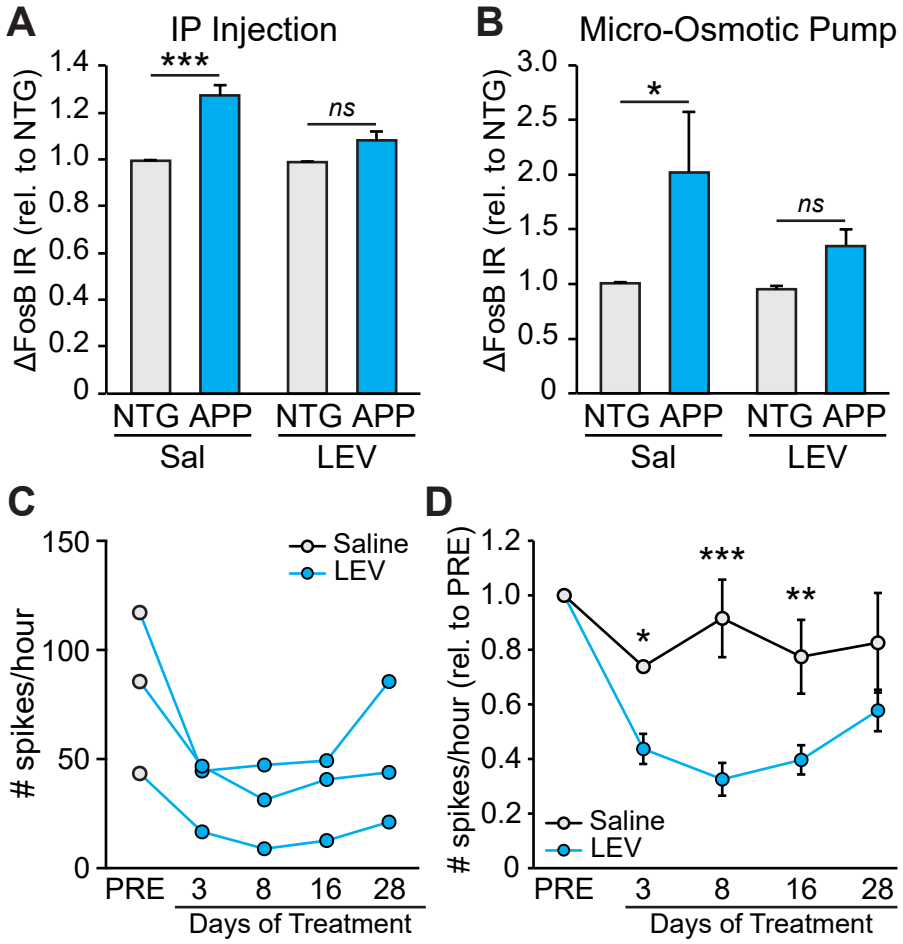

Figure S7. Treatment of APP mice with the antiepileptic drug levetiracetam normalizes ΔFosB, a seizure-induced transcription factor, and epileptic spikes. Related to Figures 4 and 5.

(A) Mice in Figure 6 were injected with levetiracetam (LEV, 75 mg/kg, IP), or an equivalent volume of saline, 3 times a day for 2 weeks (n = 9-11 mice per genotype/treatment), and then sacrificed and brains were processed for immunostaining. Δ FosB immunoreactivity (IR) is increased in saline-treated APP mice compared with saline-treated NTG mice, but is normalized in LEV-treated APP mice compared to LEV-treated NTG mice. Two-way ANOVA revealed a significant effect of LEV treatment ($p < 0.01$), genotype ($p < 0.0001$), and an interaction between treatment and genotype ($p < 0.01$).

(B) Mice in Figure 7 were implanted with Alzet micro-osmotic pumps designed to release either saline or 75 mg/kg/day of LEV for 28 days (n = 6-8 mice per genotype/treatment). LEV delivered via micro-osmotic pumps similarly reduced ΔFosB IR in APP mice. Two-way ANOVA revealed a significant effect of genotype ($p < 0.05$).

*$p < 0.05$, ****$p < 0.0001$, *ns*, not significant, Tukey post-hoc tests (A-B).

(C-D) Mice received implantation of chronic EEG electrodes, allowed to recover, and baseline EEG recordings were performed. Mice were then implanted with Alzet micro-osmotic pumps designed to release 75 mg/kg/day of LEV for 28 days.

(C) Number of spikes exhibited by individual mice during baseline recordings, and then at 3, 8, 16, and 28 days of LEV treatment.

(D) Data in panel C was normalized to baseline spike frequency (blue circles), and plotted with data from mice that received saline-filled micro-osmotic pumps as controls (gray circles). Two-way repeated measures ANOVA revealed a significant effect of LEV treatment ($p < 0.05$), and an interaction between treatment and time ($p < 0.01$).

Holm-Sidak post-hoc tests indicated significant differences between saline and LEV groups at 3, 8, and 16 days of treatment.

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, *ns*, not significant. Values indicate mean ± SEM.

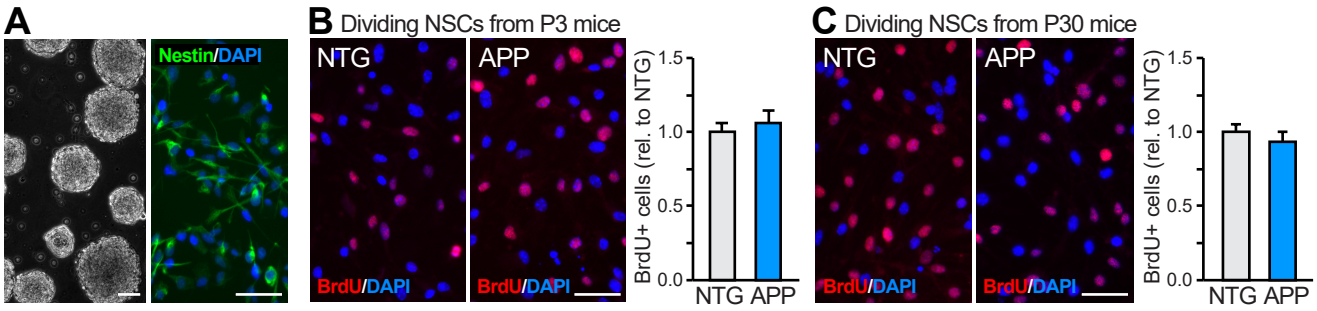

Figure S8. NSCs division is similar in dissociated neurospheres from NTG and APP mice. Related to Figure 4.
(A) Representative images of neurospheres grown in vitro from hippocampal NSCs (left) that can be dissociated, plated, and immunostained for nestin and DAPI to confirm their identity (right).
(B) Example images of BrdU+ NSCs generated from dissociated neurospheres originating from mice at postnatal day 3 (P3, left), with quantification (right; n = 3 mice per genotype).
(C) Example images of BrdU+ dividing NSCs generated from dissociated neurospheres originating from mice at postnatal day 30 (P30, left), with quantification (right; n = 4 mice per genotype).
Scale bars: (A) left, 100µm; right, 50µm. (B, C) 50µm. Two-tailed unpaired Student's t test. Values indicate mean ± SEM.

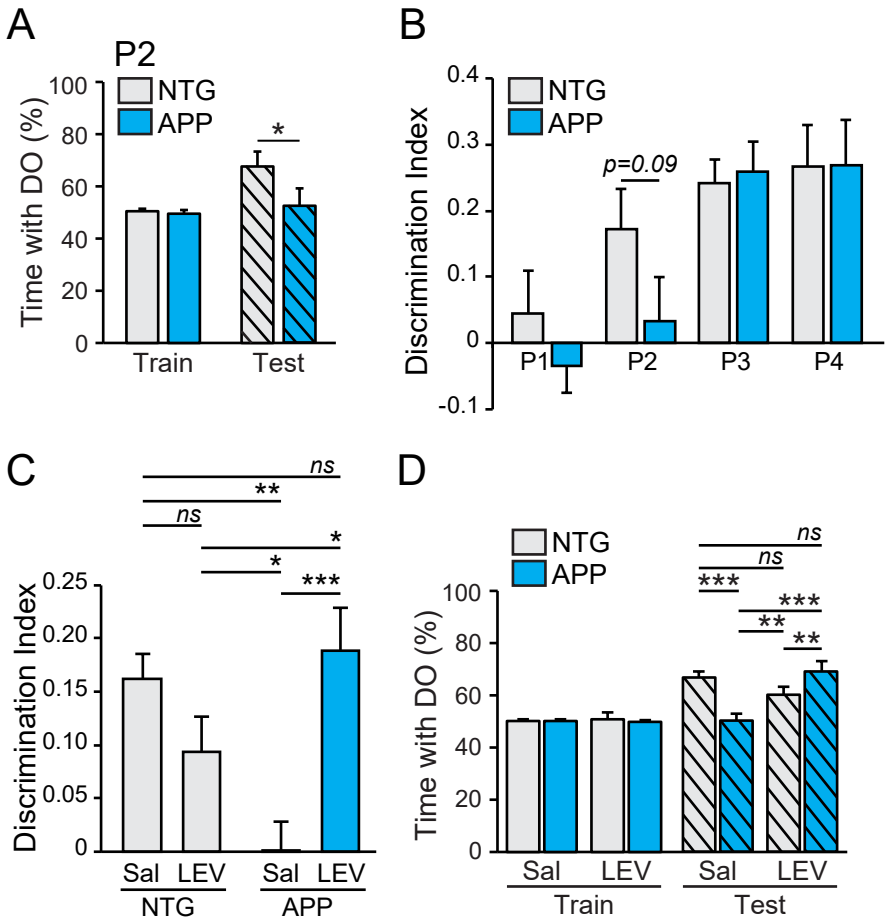

Figure S9. Spatial discrimination index of NTG and APP mice. Related to Figure 5.
(A-B) Additional analyses of data presented in Figure 5B.
(A) Comparison of spatial discrimination performance at position 2 in untreated NTG and APP mice. Two-way ANOVA revealed a significant effect of test phase ($p < 0.05$). *$p < 0.05$, Holm-Sidak post-hoc test. For simplicity, post-hoc comparisons for only the "Test" phase are indicated.
(B) Discrimination index was calculated as the difference between the percent of time spent with displaced object during the testing and training phases of the spatial discrimination task in untreated NTG and APP mice. Two-way ANOVA revealed a significant effect of object position ($p < 0.0001$). $p = 0.09$, Fisher's LSD post-hoc test.
(C-D) Additional analyses of data presented in Figure 5C.
(C) Discrimination index at position 2 in saline- or LEV-treated NTG and APP mice. Two-way ANOVA revealed a significant effect of LEV treatment ($p < 0.05$) and an interaction between genotype and treatment ($p < 0.001$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ns, not significant, Newman-Keuls post-hoc test.
(D) Comparison of spatial discrimination performance at position 2 in saline- or LEV-treated NTG and APP mice. Two-way ANOVA revealed a significant effect of LEV treatment ($p < 0.001$), test phase ($p < 0.0001$), and an interaction between treatment and test phase ($p < 0.001$). **$p < 0.01$, ***$p < 0.001$, *ns*, not significant, Holm-Sidak post-hoc test. For simplicity, post-hoc comparisons for only the "Test" phase are indicated. Values indicate mean ± SEM.

Table S1. Raw values for normalized data in Figures 1-S8. Related to Figures 1-S8 and STAR Methods

Figure	Parameter	Groups	Avg ± SEM	Additional cohort	Unit
1g	DCX+ staining	NTG (1 mo) APP (1 mo)	148.39 ± 12.24 137.93 ± 24.23		Sum of % threshold area covered by DCX expression in the granule cell layer in every 10th section through the rostral-caudal extent of hippocampus.
	DCX+ immature neurons	NTG (2 mo) APP (2 mo) NTG (3 mo) APP (3 mo) NTG (7 mo) APP (7 mo) NTG (14 mo) APP (14 mo)	665.83 ± 45.79 903.50 ± 95.33 515.63 ± 17.18 394.63 ± 39.91 167.40 ± 14.57 54.67 ± 15.06 50.58 ± 3.19 19.36 ± 7.33		Cell numbers
2c	Nestin+ NSCs	NTG (1 mo) APP (1 mo) NTG (2 mo) APP (2 mo) NTG (3 mo) APP (3 mo) NTG (6 mo) APP (6 mo) NTG (14 mo) APP (14 mo)	977.36 ± 38.86 910.67 ± 52.63 373.00 ± 28.36 144.83 ± 23.20 217.50 ± 9.65 124.63 ± 28.97 172.40 ± 13.72 85.22 ± 15.12 72.83 ± 3.91 9.45 ± 4.39	 592.50 ± 49.18 411.88 ±49.26	Cell numbers
2f	Nestin+ BrdU+ NSCs	NTG (1 mo) APP (1 mo) NTG (2 mo) APP (2 mo) NTG (3 mo) APP (3 mo) NTG (6 mo) APP (6 mo) NTG (14 mo) APP (14 mo)	45.11 ± 3.32 58.10 ± 4.30 43.63 ± 2.76 45.25 ± 4.17 17.38 ± 1.59 11.50 ± 1.55 9.57 ± 1.13 4.88 ± 1.33 1.33 ± 0.31 1.00 ± 0.27		Cell numbers
2i	Nestin+ Ki67+ NSCs	NTG APP	50.25 ± 4.26 57.13 ± 7.21		Cell numbers
3b, c	BrdU+ EdU+ Nestin+ NSCs	NTG (3 wk) APP (3 wk) NTG (2 mo) APP (2 mo) NTG (6 mo) APP (6 mo) NTG (12 mo) APP (12 mo)	39.83 ± 5.21 76.67 ± 13.70 18.25 ± 2.10 13.33 ± 2.82 3.43 ± 0.84 1.25 ± 0.49 3.33 ± 0.67 0.40 ± 0.24		Cell numbers
4a	Ki67+ Nestin+ NSCs/ Nestin+ NSCs	NTG sal APP sal NTG lev APP lev	18.53 ± 2.15 30.68 ± 5.97 17.96 ± 2.04 26.04 ± 3.91	7.75 ± 1.25 18.22 ± 4.09 5.06 ± 1.25 7.83 ± 3.10	% Cell numbers
4b	Nestin+ NSCs	NTG sal APP sal NTG lev APP lev	484.50 ± 60.04 225.75 ± 45.75 494.60 ± 55.44 365.00 ± 57.04	186.2 ± 17.93 134 ± 18.87 173.33 ± 7.80 184.80 ± 25.70	Cell numbers
4c	DCX+ staining	NTG sal APP sal NTG lev APP lev	198.73 ± 7.23 259.61 ± 25.71 157.16 ± 22.38 132.59 ± 46.75	110.03 ± 8.28 181.82 ± 32.31 83.13 ± 4.31 116.29 ± 24.65	Sum of % threshold area covered by DCX expression in the granule cell layer in every 10th section through the rostral-caudal extent of hippocampus.
S1a	DCX+ staining	NTG (1 mo) APP (1 mo)	148.39 ± 12.24 137.93 ± 24.23		Sum of % threshold area covered by DCX expression in the granule

					cell layer in every 10th section through the rostral-caudal extent of hippocampus.
	DCX+ neuroblasts	NTG (2 mo) APP (2 mo) NTG (3 mo) APP (3 mo) NTG (7 mo) APP (7 mo) NTG (14 mo) APP (14 mo)	992.50 ± 28.05 1058 ± 102.75 640.38 ± 14.36 402.25 ± 11.91 506.90 ± 53.50 199.56 ± 37.43 56.58 ± 4.00 25.55 ± 4.99		Cell numbers
S1b	Nestin+ ANPs	NTG (1 mo) APP (1 mo) NTG (2 mo) APP (2 mo) NTG (3 mo) APP (3 mo) NTG (6 mo) APP (6 mo) NTG (14 mo) APP (14 mo)	853.36 ± 43.08 836.44 ± 57.67 567.00 ± 38.86 427.17 ± 21.51 225.75 ± 27.21 145.5 ± 22.09 208.40 ± 12.05 152.89 ± 12.17 68.67 ± 6.12 23.64 ± 4.68	 463.63 ± 34.93 361.75 ± 44.07	Cell numbers
S1c	Nestin+ BrdU+ ANPs	NTG (1 mo) APP (1 mo) NTG (2 mo) APP (2 mo) NTG (3 mo) APP (3 mo) NTG (6 mo) APP (6 mo) NTG (14 mo) APP (14 mo)	137.67 ± 8.42 157.5 ± 10.29 99.50 ± 6.58 103 ± 11.31 59.50 ± 4.19 42.00 ± 4.09 42.71 ± 5.69 26.25 ± 4.58 9.58 ± 1.78 4.64 ± 1.08		Cell numbers
S3a	DCX+ immature neurons	NTG PSAPP	163.91 ± 15.82 241.89 ± 40.51		Cell numbers
S3b	DCX+ immature neurons	NTG PSAPP	32.45 ± 6.56 16.33 ± 4.13		Cell numbers
S3c	DCX+ immature neurons	NTG Tg2576	53.09 ± 6.14 30.80 ± 6.82		Cell numbers
S3d	Nestin+ NSCs	NTG PSAPP	159.09 ± 11.83 116.22 ± 25.10		Cell numbers
S3e	Nestin+ NSCs	NTG PSAPP	71 ± 12.06 41.75 ± 6.10		Cell numbers
S3f	Nestin+ NSCs	NTG Tg2576	103.50 ± 6.95 29.78 ± 3.79		Cell numbers
S4c	Prox1+ hilar granule cells	NTG APP	83.50 ± 7.22 165.86 ± 29.57		Cell numbers
S7a	ΔFosB IR	NTG sal APP sal NTG LEV APP LEV	1.109 ± 0.007 1.426 ± 0.088 1.102 ± 0.004 1.240 ± 0.059	1.075 ± 0.004 1.366 ± 0.066 1.072 ± 0.003 1.102 ± 0.020	Arbitrary units (intensity)
S7b	ΔFosB IR	NTG sal APP sal NTG LEV APP LEV	1.682 ± 0.022 4.881 ± 1.448 1.434 ± 0.045 2.860 ± 0.549	1.047 ± 0.003 1.186 ± 0.107 1.051 ± 0.005 1.187 ± 0.061	Arbitrary units (intensity)
S8b	BrdU+ cells	NTG APP	41.60 ± 2.45 44.12 ± 3.66		Cell numbers
S8c	BrdU+ cells	NTG APP	52.94 ± 2.84 49.34 ± 3.66		Cell numbers

Table S2. Statistical values for comparisons in Figures 1-S9. Related to Figures 1-S9 and STAR Methods.

Figure	Parameter	Groups	Test used	Values	P value
1d	Spikes per hour	NTG, APP (1 mo) NTG, APP (2 mo) NTG, APP (4-6 mo)	Student t-test, 2-tailed	$t_6 = 2.514$ $t_4 = 4.209$ $t_6 = 3.879$	P = 0.0456 P = 0.0136 P = 0.0082
1g	DCX+ staining	NTG, APP (1 mo)	Student t-test, 2-tailed	$t_{19} = 0.4153$	P = 0.6826
	DCX+ immature neurons	NTG, APP (2 mo) NTG, APP (3 mo) NTG, APP (7 mo) NTG, APP (14 mo)	Student t-test, 2-tailed	$t_{10} = 2.247$ $t_{14} = 2.785$ $t_{17} = 5.374$ $t_{21} = 4.024$	P = 0.0484 P = 0.0146 P < 0.0001 P = 0.0006
2c	Nestin+ NSCs	NTG, APP (1 mo) NTG, APP (2 mo) NTG, APP (3 mo) NTG, APP (7 mo) NTG, APP (14 mo)	Student t-test, 2-tailed	$t_{18} = 1.011$ $t_{26} = 5.002$ $t_{14} = 3.041$ $t_{17} = 3.399$ $t_{21} = 10.81$	P = 0.3254 P < 0.0001 P = 0.0088 P = 0.0034 P < 0.0001
2f	Nestin+ BrdU+ NSCs	NTG, APP (1 mo) NTG, APP (2 mo) NTG, APP (3 mo) NTG, APP (7 mo) NTG, APP (14 mo)	Student t-test, 2-tailed	$t_{17} = 2.353$ $t_{14} = 0.3248$ $t_{14} = 2.647$ $t_{13} = 2.649$ $t_{21} = 0.8050$	P = 0.0309 P = 0.7501 P = 0.0191 P = 0.0201 P = 0.4299
	BrdU+ Nestin+ NSCs/ Nestin+ NSCs	NTG, APP (2 mo, inset)	Student t-test, 2-tailed	$t_{14} = 2.925$	P = 0.0111
2i	Nestin+ Ki67+ NSCs	NTG, APP	Student t-test, 2-tailed	$t_{14} = 0.8213$	P = 0.4253
	Ki67+ Nestin+ NSCs/ Nestin+ NSCs	NTG, APP	Student t-test, 2-tailed	$t_{14} = 2.141$	P = 0.0504
3b, c	BrdU+ EdU+ Nestin+ NSCs	NTG, APP (3 wk) NTG, APP (2 mo) NTG, APP (6 mo) NTG, APP (12 mo)	Student t-test, 2-tailed	$t_{10} = 2.514$ $t_{12} = 1.429$ $t_{13} = 2.309$ $t_{12} = 3.162$	P = 0.0307 P = 0.1784 P = 0.0380 P = 0.0082
4a	Ki67+ Nestin+ NSCs/ Nestin+ NSCs	NTG, APP (saline) NTG, APP (LEV)	2-way ANOVA	Genotype, $F_{1,35} = 8.785$ Treatment, $F_{1,35} = 6.929$ Interaction, $F_{1,35} = 3.095$	P = 0.0054 P = 0.0125 P = 0.0873
		NTG sal v NTG LEV NTG sal v APP sal NTG sal v APP LEV NTG LEV v APP sal NTG LEV v APP LEV APP sal v APP LEV	Holm-Sidak post-hoc	$t_{35} = 0.6278$ $t_{35} = 3.397$ $t_{35} = 0.2205$ $t_{35} = 4.242$ $t_{35} = 0.8379$ $t_{35} = 3.055$	P = 0.7923 P = 0.0085 P = 0.8267 P = 0.0009 P = 0.7923 P = 0.0170
4b	Nestin+ NSCs	NTG, APP (saline) NTG, APP (LEV)	2-way ANOVA	Genotype, $F_{1,35} = 7.003$ Treatment, $F_{1,35} = 2.925$ Interaction, $F_{1,35} = 4.273$	P = 0.0121 P = 0.0961 P = 0.0462
		NTG sal v NTG LEV NTG sal v APP sal NTG sal v APP LEV NTG LEV v APP sal NTG LEV v APP LEV APP sal v APP LEV	Holm-Sidak post-hoc	$t_{35} = 0.2567$ $t_{35} = 3.390$ $t_{35} = 0.6227$ $t_{35} = 3.303$ $t_{35} = 0.4029$ $t_{35} = 2.628$	P = 0.9036 P = 0.0104 P = 0.9011 P = 0.0110 P = 0.9036 P = 0.0497
4c	DCX+ staining	NTG, APP (saline) NTG, APP (LEV)	2-way ANOVA	Genotype, $F_{1,35} = 2.658$ Treatment, $F_{1,35} = 19.77$ Interaction, $F_{1,35} = 6.738$	P = 0.1120 P < 0.0001 P = 0.0137
		NTG sal v NTG LEV NTG sal v APP sal NTG sal v APP LEV NTG LEV v APP sal NTG LEV v APP LEV APP sal v APP LEV	Holm-Sidak post-hoc	$t_{35} = 1.331$ $t_{35} = 3.039$ $t_{35} = 1.873$ $t_{35} = 4.606$ $t_{35} = 0.6716$ $t_{35} = 4.899$	P = 0.3470 P = 0.0177 P = 0.1942 P = 0.0003 P = 0.5063 P = 0.0001
5b	Time spent with DO	NTG (P1, P2, P3, P4)	2-way RM ANOVA	Position, $F_{3,26} = 0.7074$ Test phase, $F_{1,26} = 38.39$ Interaction, $F_{3,26} = 2.992$	P=0.5563 P<0.0001 P=0.0492
		NTG P1 train v test	Holm-Sidak post-hoc	$t_{26} = 0.7706$	P = 0.4479

		NTG P2 train v test NTG P3 train v test NTG P4 train v test		t_{26} = 3.049 t_{26} = 3.990 t_{26} = 4.436	P = 0.0104 P = 0.0014 P = 0.0006
		APP (P1, P2, P3, P4)	2-way RM ANOVA	Position, $F_{3,23}$ = 7.271 Test phase, $F_{1,23}$ = 21.95 Interaction, $F_{3,23}$ = 7.783	P = 0.0013 P = 0.0001 P = 0.0009
		APP P1 train v test APP P2 train v test APP P3 train v test APP P4 train v test	Holm-Sidak post-hoc	t_{23} = 0.6623 t_{23} = 00.5999 t_{23} = 04.730 t_{23} = 04.533	P = 0.7641 P = 0.7641 P = 0.0004 P = 0.0004
5c	Time spent with DO	NTG, APP (saline) NTG, APP (LEV)	2-way RM ANOVA	Treatment, $F_{3,26}$ = 8.161 Test phase, $F_{1,26}$ = 59.59 Interaction, $F_{3,26}$ = 8.261	P = 0.0005 P < 0.0001 P = 0.0005
		NTG sal train v test NTG LEV train v test APP sal train v test APP LEV train v test	Holm-Sidak post-hoc	t_{26} = 5.593 t_{26} = 3.250 t_{26} = 0.02989 t_{26} = 7.192	P < 0.0001 P = 0.0064 P = 0.9764 P < 0.0001
S1a	DCX+ staining	NTG, APP (1 mo)	Student t-test, 2-tailed	t_{19} = 0.4153	P = 0.6826
	DCX+ neuroblasts	NTG, APP (2 mo) NTG, APP (3 mo) NTG, APP (7 mo) NTG, APP (14 mo)	Student t-test, 2-tailed	t_{10} = 0.6150 t_{14} = 12.76 t_{17} = 4.606 t_{21} = 4.892	P = 0.5523 P < 0.0001 P = 0.0003 P < 0.0001
S1b	Nestin+ ANPs	NTG, APP (1 mo) NTG, APP (2 mo) NTG, APP (3 mo) NTG, APP (7 mo) NTG, APP (14 mo)	Student t-test, 2-tailed	t_{18} = 0.2284 t_{26} = 3.104 t_{14} = 2.290 t_{17} = 3.233 t_{21} = 5.764	P = 0.8219 P = 0.0046 P = 0.0381 P = 0.0049 P < 0.0001
S1c	Nestin+ BrdU+ ANPs	NTG, APP (1 mo) NTG, APP (2 mo) NTG, APP (3 mo) NTG, APP (7 mo) NTG, APP (14 mo)	Student t-test, 2-tailed	t_{17} = 1.471 t_{14} = 0.2674 t_{14} = 2.987 t_{13} = 2.278 t_{21} = 2.321	P = 0.1596 P = 0.7931 P = 0.0098 P = 0.0403 P = 0.0304
S3a	DCX+ immature neurons	NTG, PSAPP	Student t-test, 1-tailed	t_{18} = 2.022	P = 0.0292
S3b	DCX+ immature neurons	NTG, PSAPP	Student t-test, 1-tailed	t_{20} = 1.921	P = 0.0346
S3c	DCX+ immature neurons	NTG, Tg2576	Student t-test, 1-tailed	t_{19} = 2.382	P = 0.0139
S3d	Nestin+ NSCs	NTG, PSAPP	Student t-test, 1-tailed	t_{18} = 1.642	P = 0.0590
S3e	Nestin+ NSCs	NTG, PSAPP	Student t-test, 1-tailed	t_{21} = 2.221	P = 0.0187
S3f	Nestin+ NSCs	NTG, Tg2576	Student t-test, 1-tailed	t_{18} = 8.863	P < 0.0001
S4c	Prox1+ hilar granule cells	NTG, APP	Student t-test, 2-tailed	t_{15} = 3.181	P = 0.0062
S5a	Nestin+ BrdU+ NSCs	NTG, APP	Student t-test, 2-tailed	t_{17} = 2.353	P = 0.0309
	BrdU+ Nestin+ NSCs/ Nestin+ NSCs	NTG, APP	Student t-test, 2-tailed	t_{17} = 4.250	P = 0.0005
S5b	Nestin+ BrdU+ NSCs	NTG, APP	Student t-test, 2-tailed	t_{14} = 0.3248	P = 0.7501
	BrdU+ Nestin+ NSCs/ Nestin+ NSCs	NTG, APP	Student t-test, 2-tailed	t_{14} = 2.925	P = 0.0111
S5c	Nestin+ BrdU+ NSCs	NTG, APP	Student t-test, 2-tailed	t_{14} = 2.647	P = 0.0191
	BrdU+ Nestin+ NSCs/ Nestin+ NSCs	NTG, APP	Student t-test, 2-tailed	t_{14} = 1.766	P = 0.0991
S5d	Nestin+ BrdU+ NSCs	NTG, APP	Student t-test, 2-tailed	t_{13} = 2.649	P = 0.0201
	BrdU+ Nestin+ NSCs/ Nestin+ NSCs	NTG, APP	Student t-test, 2-tailed	t_{13} = 0.8650	P = 0.4027
S5e	Nestin+ BrdU+ NSCs	NTG, APP	Student t-test, 2-tailed	t_{21} = 0.8050	P = 0.4299
	BrdU+ Nestin+ NSCs/ Nestin+ NSCs	NTG, APP	Student t-test, 2-tailed	t_{21} = 3.049	P = 0.0061
S6b	BrdU+ Nestin+ NSCs/ Nestin+ NSCs	NTG, APP (saline) NTG, APP (KA)	Kruskal-Wallis test	KW statistic = 26.70	P < 0.0001

		NTG saline v KA APP saline v KA	Dunn post-hoc	Z = 2.795 Z = 2.391	P = 0.0104 P = 0.0336
S6c	BrdU+ Nestin+ ANPs/ Nestin+ ANPs	NTG, APP (saline) NTG, APP (KA)	Kruskal-Wallis test	KW statistic = 27.86	P < 0.0001
		NTG saline v KA APP saline v KA	Dunn post-hoc	Z = 2.651 Z = 2.375	P = 0.0161 P = 0.0351
S7a	ΔFosB IR	NTG, APP (saline) NTG, APP (LEV)	2-way ANOVA	Genotype, $F_{1,35}$ = 35.61 Treatment, $F_{1,35}$ = 10.49 Interaction, $F_{1,35}$ = 9.472	P < 0.0001 P = 0.0026 P = 0.0040
		NTG sal v APP sal NTG LEV v APP LEV	Tukey post-hoc		P < 0.0001 P = 0.2037
S7b	ΔFosB IR	NTG, APP (saline) NTG, APP (LEV)	2-way ANOVA	Genotype, $F_{1,26}$ = 9.096 Treatment, $F_{1,26}$ = 2.391 Interaction, $F_{1,26}$ = 1.729	P = 0.0057 P = 0.1341 P = 0.2001
		NTG sal v APP sal NTG LEV v APP LEV	Tukey post-hoc		P = 0.0316 P = 0.6005
S7d	Spike per hour	Sal (pre, 3d, 8d, 16d) LEV (pre, 3d, 8d, 16d)	2-way ANOVA	Time, $F_{3,12}$ = 18.51 Treatment, $F_{1,4}$ = 18.58 Interaction, $F_{3,12}$ = 6.774	P < 0.0001 P = 0.0125 P = 0.0063
		Sal (pre) v LEV (pre) Sal (3d) v LEV (3d) Sal (8d) v LEV (8d) Sal (16d) v LEV (16d)	Holm-Sidak post-hoc	t_{16} = 0.000 t_{16} = 2.744 t_{16} = 5.377 t_{16} = 3.456	P > 0.9999 P = 0.0286 P = 0.0002 P = 0.0097
S8b	BrdU+ cells	NTG, APP	Student t-test, 2-tailed	t_4 = 0.5571	P = 0.6072
S8c	BrdU+ cells	NTG, APP	Student t-test, 2-tailed	t_6 = 0.7937	P = 0.4576
S9a	Discrimination index	NTG (P1, P2, P3, P4) APP (P1, P2, P3, P4)	2-way ANOVA	Genotype, $F_{1,49}$ = 1.496 Position, $F_{3,49}$ = 9.605 Interaction, $F_{3,49}$ = 0.8249	P = 0.2272 P < 0.0001 P = 0.4865
		NTG v APP (P1) NTG v APP (P2) NTG v APP (P3) NTG v APP (P4)	Fisher's LSD post-hoc	t_{49} = 1.008 t_{49} = 1.757 t_{49} = 0.2310 t_{49} = 0.01285	P = 0.3184 P = 0.0851 P = 0.8183 P = 0.9898
S9b	Time spent with DO	NTG, APP	2-way ANOVA	Genotype, $F_{1,13}$ = 3.357 Test phase, $F_{1,13}$ = 5.206 Interaction, $F_{1,13}$ = 2.399	P = 0.0899 P = 0.0400 P = 0.1454
		NTG v APP (train) NTG v APP (test)	Holm-Sidak post-hoc	t_{26} = 0.1697 t_{26} = 2.388	P = 0.8665 P = 0.0484
S9c	Discrimination index	NTG, APP (saline) NTG, APP (LEV)	2-way ANOVA	Genotype, $F_{1,26}$ = 0.6029 Treatment, $F_{1,26}$ = 5.350 Interaction, $F_{1,26}$ = 20.83	P = 0.4445 P = 0.0289 P = 0.0001
		NTG sal v NTG LEV NTG sal v APP sal NTG sal v APP LEV NTG LEV v APP sal NTG LEV v APP LEV APP sal v APP LEV	Newman-Keuls post-hoc		ns P < 0.01 ns P < 0.05 P < 0.05 P < 0.001
S9d	Time spent with DO	NTG, APP (saline) NTG, APP (LEV)	2-way RM ANOVA	Treatment, $F_{3,26}$ = 8.161 Test phase, $F_{1,26}$ = 59.59 Interaction, $F_{3,26}$ = 8.261	P = 0.0005 P < 0.0001 P = 0.0005
		NTG sal v NTG LEV (train) NTG sal v APP sal (train) NTG sal v APP LEV (train) NTG LEV v APP sal (train) NTG LEV v APP LEV (train) APP sal v APP LEV (train) NTG sal v NTG LEV (test) NTG sal v APP sal (test) NTG sal v APP LEV (test) NTG LEV v APP sal (test) NTG LEV v APP LEV (test) APP sal v APP LEV (test)	Newman-Keuls post-hoc		P > 0.9999 P > 0.9999 P > 0.9999 P > 0.9999 P > 0.9999 P > 0.9999 P = 0.0600 P < 0.0001 P = 0.1300 P = 0.0082 P = 00017 P < 0.0001