Supplemental Figures and Tables

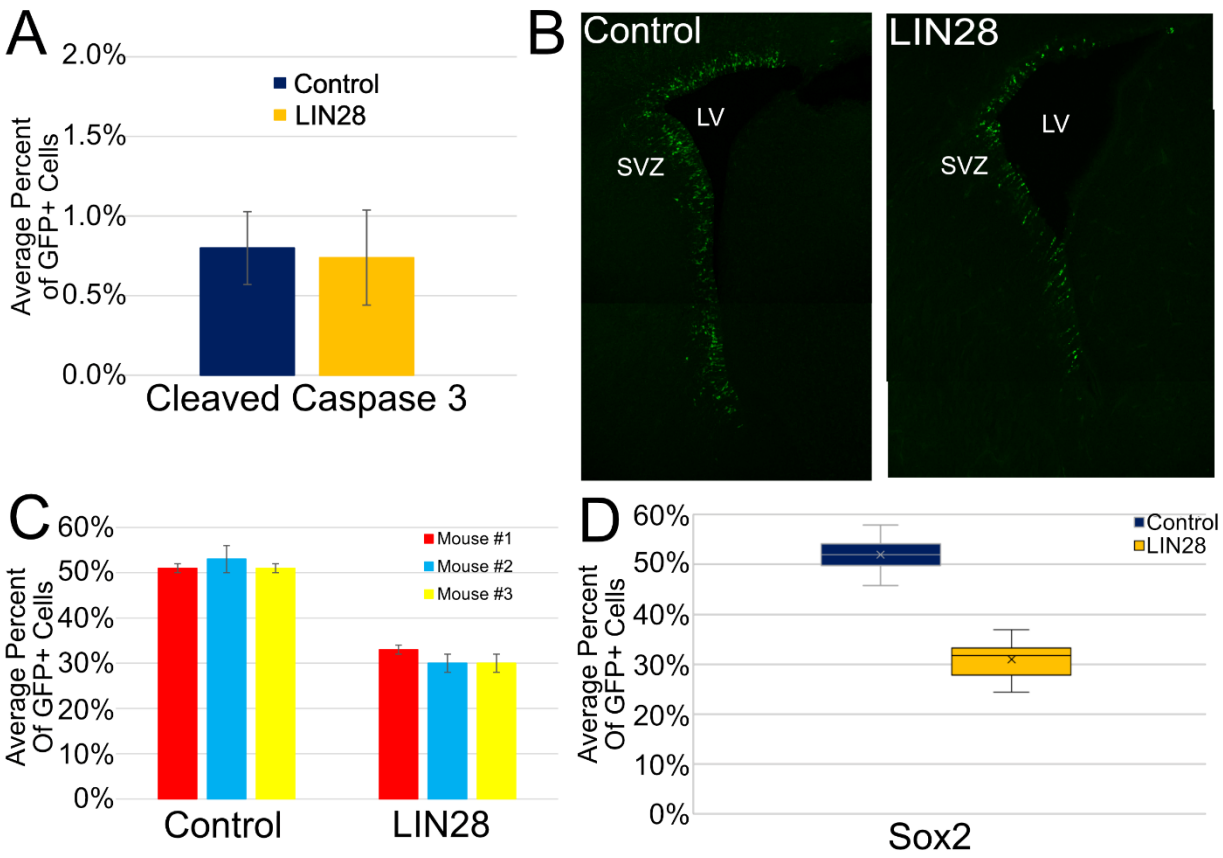

Figure S1 – Assessment of variability and effect of the LIN28::GFP plasmid.
A) Average percent of GFP+ cells positive for cleaved caspase 3, a marker for apoptosis, at 3 DPE. n = 7 slices.
B) Representative 10x micrographs depicting GFP+ electroporated cells in the dorsal and lateral SVZ regions in control (left) and LIN28 (right) at 1 DPE. LV, lateral ventricle; SVZ, subventricular zone; dorsal, up; lateral, left.
C) Bar graph depicting variability from mouse to mouse in both control and LIN28. Average percent of GFP+ cells positive for Sox2 from four slices per mouse, three mice.
D) Box and whisker plot showing variability between slices of different mice n = 12 slices. Average percent of GFP+ cells positive for Sox2.
In A, C, and D, data presented as mean ± S.E.M, Student's *t*-test. n.s., not significant.

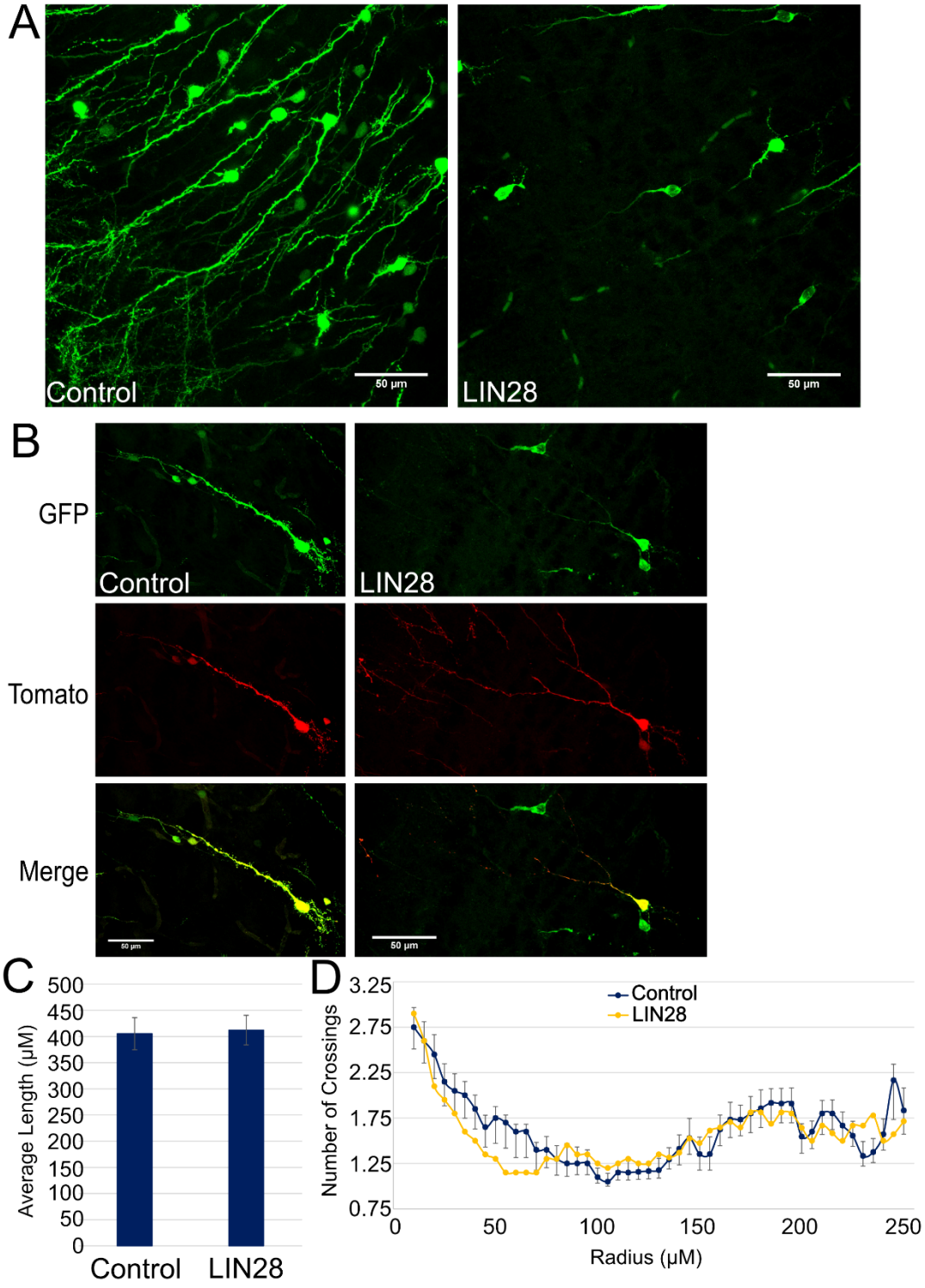

Figure S2 - LIN28 does not localize to the apical dendrites of granule neurons nor does it appear to affect dendritic morphology in the OB at 21 DPE.
A) Representative 40x micrographs depicting GFP+ (green) neurons in control (left) and LIN28 (right) at 21 DPE. Scale bars: 50µM.

B) Representative 40x micrographs depicting GFP+ (green), tdTomato+ (red), and the overlap (yellow) of these two fluorescent markers in control (left) and LIN28 (right) neurons at 21 DPE. Scale bars: 50μM.
C) Bar graph depicting the average length of 21 DPE OB neurons. Analysis completed using the NeuronJ function of Fiji Image J. n = 20 neurons from 3 mice.
D) Scatter plot of Sholl analysis depicting the average number of crossings from the soma of 21 DPE neurons at 5 micron intervals. Analysis completed using the Sholl Analysis function of Fiji Image J. n = 20 neurons from 3 mice.
In C and D, data presented as mean ± S.E.M. C: Student's *t*-test. D: Two way ANOVA. n.s., not significant.

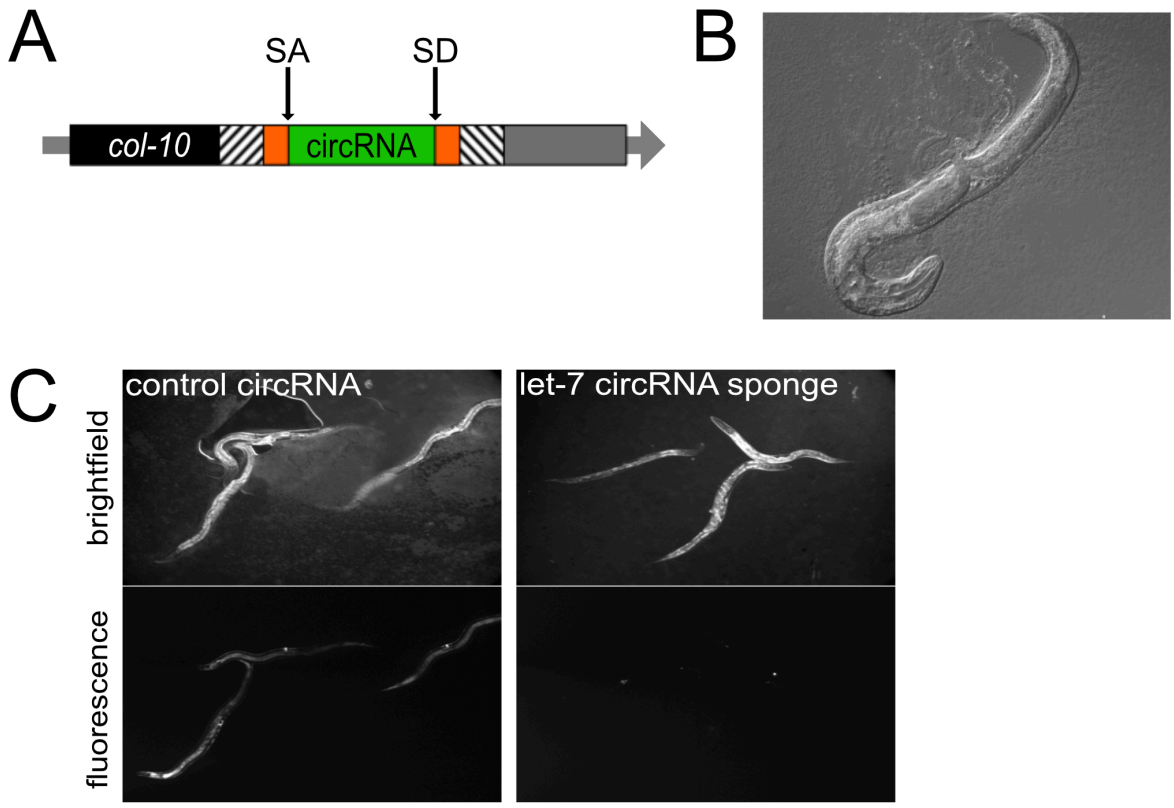

Figure S3 - A let-7 circRNA sponge effectively knocks down let-7 activity in *C. elegans*.
A) The circRNA-expressing constructs for *C. elegans*. The RNA is expressed from the *col-10* promoter, a constitutive epidermal promoter. Hatched regions indicate inverted repeat to form hairpin. SA, splice acceptor; SD, splice donor.
B) Micrograph of a let-7 circRNA sponge worm displaying adult bursting phenotype characteristic of a *let-7* mutant.
C) Adult *C. elegans* expressing control circRNA and let-7 circRNA sponge *col-19::GFP* adult-specific reporter in brightfield (top) and fluorescence (bottom). Sponge expressing animals express a co-injection marker (*ttx-3::GFP*) but not the adult-specific marker (*col-19::GFP*).

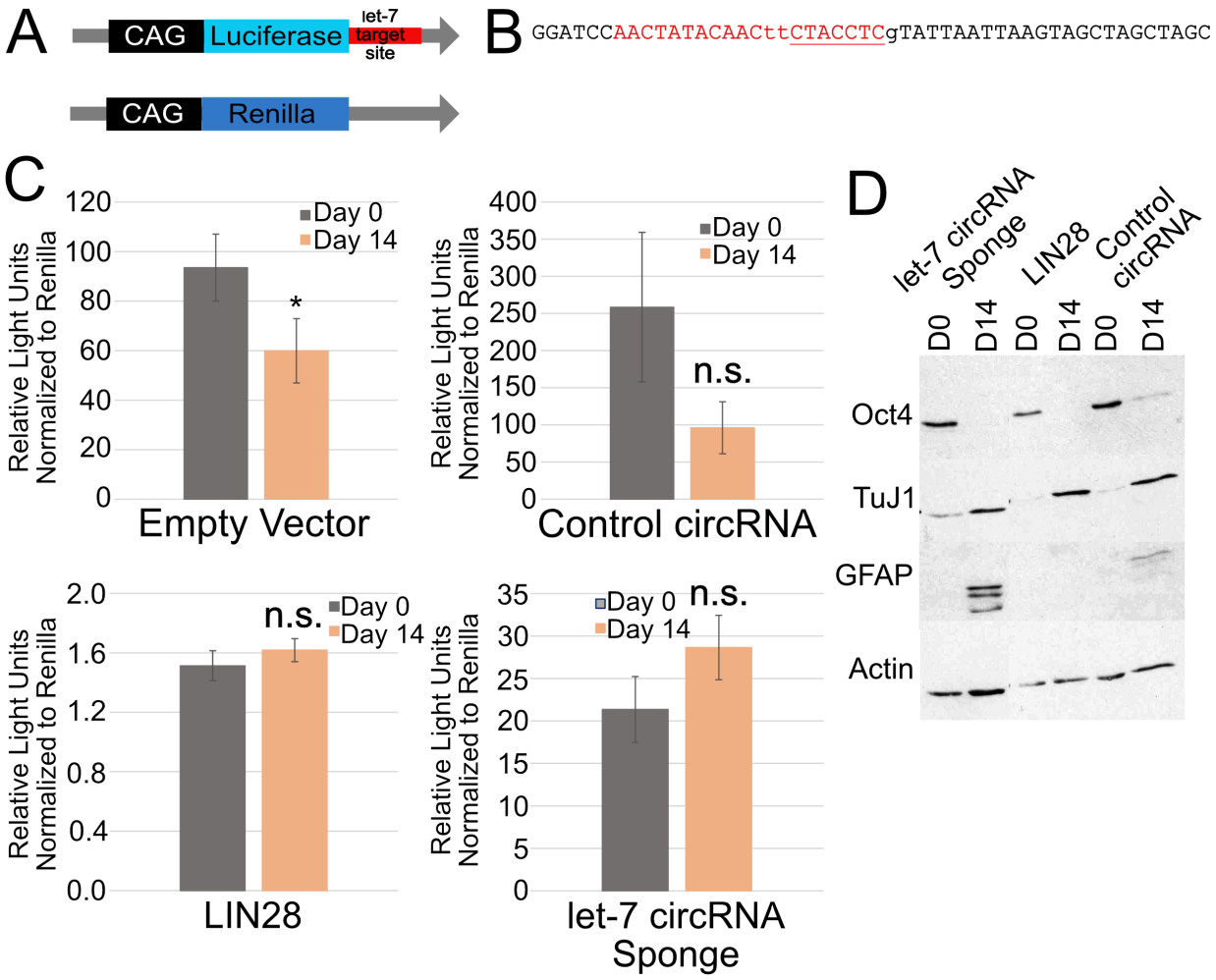

Figure S4 - The let-7 circRNA sponge blocks let-7 activity in P19 cells.
A) Diagram illustrates the luciferase reporter plasmid construct (top) and the renilla plasmid construct (bottom). Both open reading frames are expressed under the CAG promoter. The luciferase reporter contains a let-7A target site in the 3'UTR.
B) Let-7 target sight design. Red, microRNA target sequence; the seed sequence is underlined.
C) Bar graphs depicting the luciferase assay. Luciferase levels were normalized to its own internal renilla expression. n = 3-4 differentiations per cell line.
D) Representative western blot showing the results of gliogenesis in the various P19 cell lines. D0, day 0; D14, day 14. Oct4, pluripotency marker; TuJ1, neuron marker. Actin was used as the loading control. n = 3-4 differentiations per cell line.
In C, data presented as mean ± S.E.M. *, $p \leq 0.05$ versus control, paired *t*-test. n.s., not significant

Table S1. The the sequence of the let-7 circRNA construct with target sites aligned.

CTTATACAGACCTACCTC
ACTATACAGATCTACCTC
ACTATACAAATCTACCTC
ACTATGCAGCCCTACCTC
TCTATATAAACCTACCTC
AATATGTAAACCTACCTC
ACTATGTAAACCTACCTC
TCTATACAGATCTACCTC
ACTGTACAAATCTACCTC
ACTATGCAACCCTACCTC
ATTATACAAACCTACCTC
ACTGTACAACTCTACCTC
ACTATACACACCTACCTC
ACTATACAAGTCTACCTC
ACTGTACAAATCTACCTC
ACTATATAAATCTACCTC
ACTATACAAATCTACCTC
ACTGTATAACTCTACCTC
ATTATGCAGACCTACCTC
TCTGTACAGATCTACCTC
AATATGCAAACCTACCTC
ACTATACAAACCTACCTC
ATTGTACAAATCTACCTC
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ACTATGCAAACCTACCTC
TTTATGCAGGCCTACCTC
ACTGTACAAGTCTACCTC
ACTATACACACCTACCTC
ACTATATAGACCTACCTC
ACTATACAAATCTACCTC
ACTATGCACACCTACCTC
ATTGTACAGATCTACCTC
ACTATACAGCTCTACCTC
ACTATACAAACCTACCTC
ACTGTACAAATCTACCTC
ATTATACAACCCTACCTC

Table S2. The let-7 sponge phenocopies a *let-7(0)* phenotype.

Genotype	Young Adults with Adult Lateral Alae	Death by Bursting
Wildtype	100% (n=22)	0% (n=30)
let-7(0)	0% (n=30)	100% (n=20)
let-7 circRNA sponge	0% (n=30)	100%* (n=20)
Control circRNA	100% (n=10)	0%* (n=20)

*Transgenic animals carrying the circRNA construct and a GFP co-transformation marker were first selected as bright GFP+ L4 stage larvae then monitored for bursting.