Electronic Supplementary Material

MicroRNA-132 provides neuroprotection for tauopathies via multiple signaling pathways

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Legends for Supplemental Figures

***Figure S1*. Validation of miRNA inhibitors, generation of partly aggregated Aβ, and schematics indicating the time course of neuroprotection experiments.** (**a**) Mouse primary neurons were transfected with LNA-containing oligonucleotide inhibitors for the indicated miRNAs, and their effects on validated direct mRNA targets [7] examined 48 hours later by qRT-PCR analysis**.** *P<0.05, **P<0.01 n=3, student t-test. **(b)** Aggregation of SEC-isolated Aβ (1– 42) was followed using a continuous thioflavin T (ThT)-binding assay and fluorescence values are expressed in relative fluorescence units and plotted versus time. The point at which sample was collected (*1⁄2t* max) and used for toxicity experiments is indicated with the blue oval. Freshly SEC-isolated, unaggregated monomer (i.e. equivalent to t=0) was used as a control. **(c)** Time course of neuroprotection experiments on mouse and human primary neurons exposed to Aβ or **(d)** excitotoxic glutamate. **(e)** Spearman rank for the correlation between miRNA levels in primary mouse cortical and hippocampal neurons at DIV-21 and their level of neuroprotection against Aβ toxicity exhibited in Figure 1a. The meta-analysis of miRNA expression was performed on the reported RNAseq and array-based datasets [57, 66].

***Figure S2*. MiR-132 directly targets Tau modifiers RBfox1, GSK3β, EP300, and Calpain 2. (a)** Quantification of three independent Western blot experiments shown in Fig. 4b indicates that miR-132 mimic reduces the levels of GSK3β, RBfox1, and EP300 proteins in primary mouse neurons. **(b)** Quantification of three independent Western blot experiments shown in Fig. 4f indicates that RBfox1 silencing reduces the levels of total Tau protein in primary neurons. **(c)** Quantification of three independent Western blot experiments shown in Fig.4h demonstrate that miR-132 reduces the levels of calpain 2 and cleaved caspase-3 and caspase-7. *P<0.05, **P<0.01 n=3, student t-test.

***Figure S3*. miR-132 reduces Gsk3β activity in mouse primary neurons.** GSK-3β was immunoprecipitated from mouse neurons transfected with miR-132 or control oligonucleotide, and its activity monitored using Tau-S396 as a substrate in an ELISA Assay (phosphoELISA™ Kit from

Invitrogen). miR-132 reduced GSK-3β-mediated phosphorylation of Tau, compared with the scrambled control oligonucleotide. *P<0.02.

***Figure S4*. Mapping of the RBfox1- Tau mRNA interaction in mouse primary neurons. (a)** Schematic presentation of the iCLIP experimental workflow. **(b)** RNA immunoprecipitated with the RBfox1-specific antibody was analyzed by qRT-PCR with primers amplifying various regions of Tau mRNA, including the coding region and 3'UTR. The data are presented as fold enrichment relative to IgG control; mean +/- SEM, n=3, *P<0.005. (**c**) Schematic view of the putative Rbfox1-binding GCAUG motifs in Tau mRNA. In mouse neurons, Rbfox1 binds to the Tau mRNA preferentially via the GCAUG site found in the coding region.

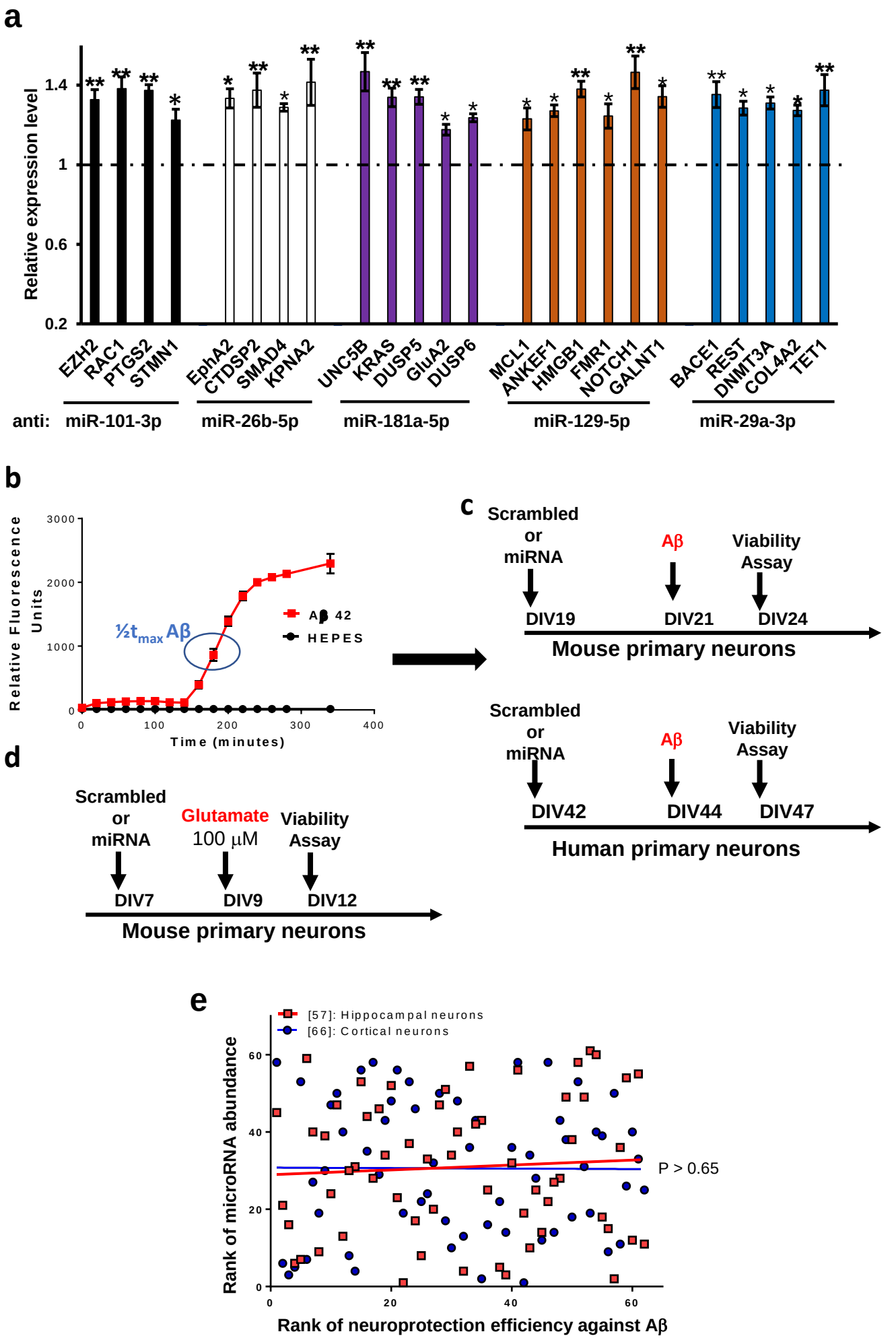
***Figure S5*. Schematic diagram of constructs used for the lentivirus production of LV-miR132 and the corresponding negative control (Empty vector).**

***Figure S6*. Representative images of PHF1-Tau staining in WT and PS19 brain sections, at 7.5 months.** Scale bar=100μm.

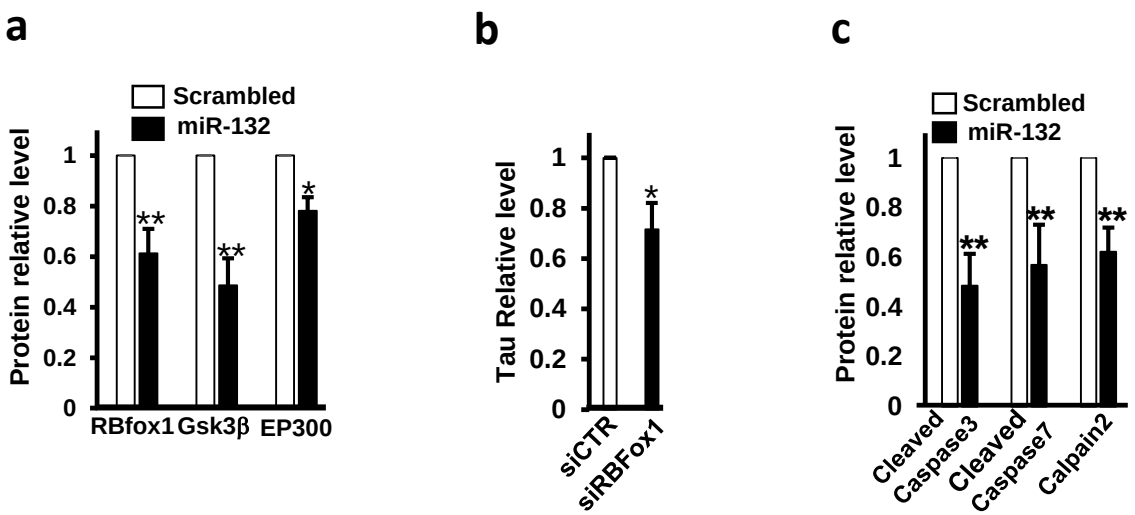
***Figure S7*. Stereotactic injections of the LV-miR132 to the CA1 reduces hippocampal atrophy in the PS19 mice. (a)** DAPI staining of PS19 mouse hippocampal CA1 region, injected with the LV-miR132 or control, **(b)** Image J quantification of the hippocampal area (mm2), in the brains injected with either LV-miR132 or control virus. For all quantifications, n = 14 mice, 15 sections per brain. All graphical data are shown as mean +/- SEM, Student's *t*-Tests, *P<0.01.

***Figure S8*. Early supplementation of miR-132 prevents neuronal loss and Tau pathology in PS19 mice. (a)** Timeline of LV-miR132 injections to young PS19 mice and brain analysis. **(b)** Image J quantification of cells positive for cleaved caspase-3, NeuN, PHF-Tau and GFAP in CA1 and adjacent cortical layers. For all quantifications, n = 7 mice, 15 sections per brain. All graphical data are shown as mean +/- SEM, Student's *t*-Tests, *P<0.005.

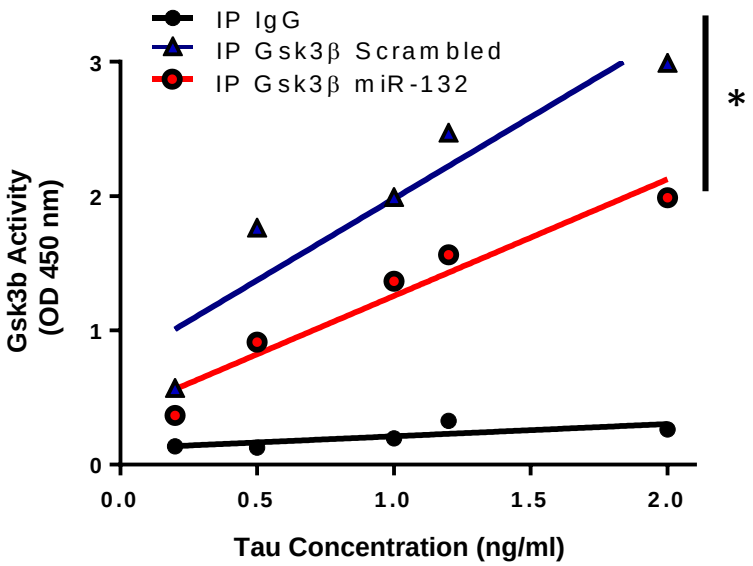
Supplementary Figure 1



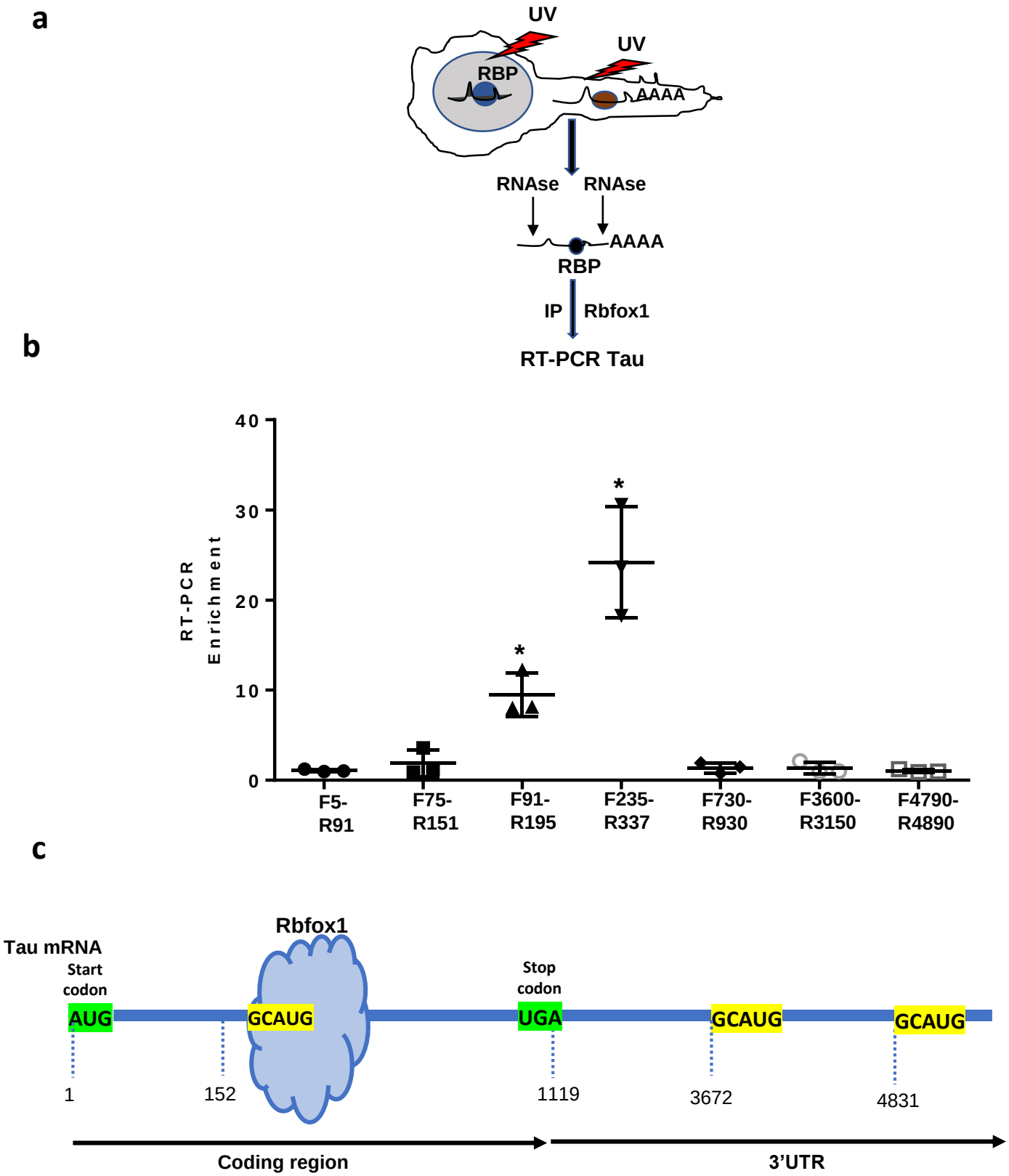
Supplementary Figure 2



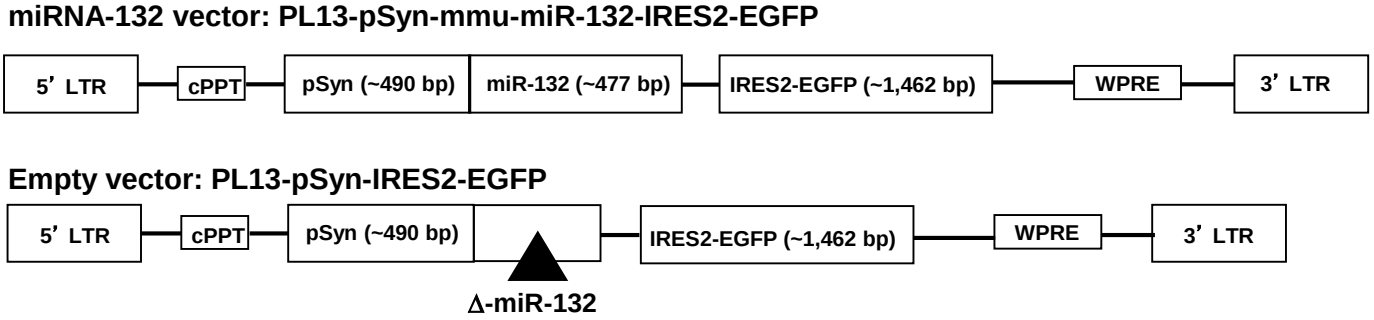
Supplementary Figure 3



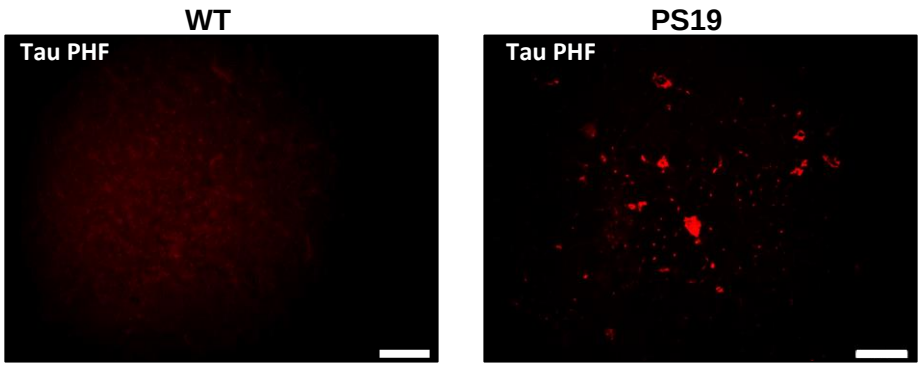
Supplementary Figure 4



Supplementary Figure 5

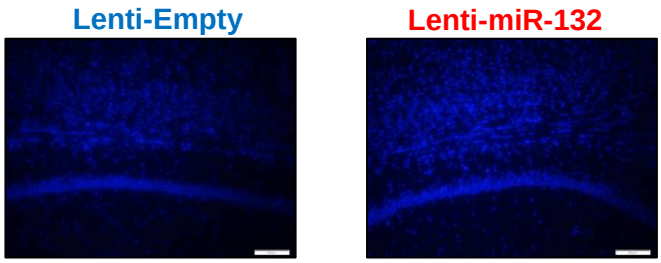


Supplementary Figure 6



Supplementary Figure 7

a



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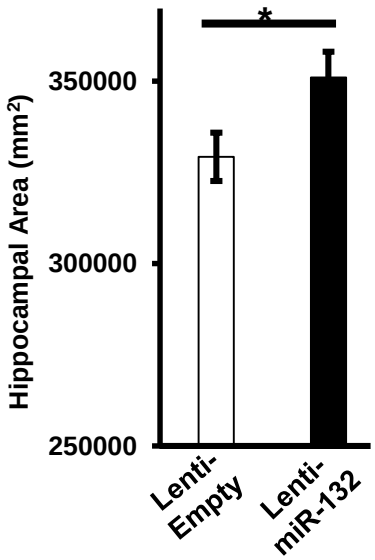

Supplementary Figure 8

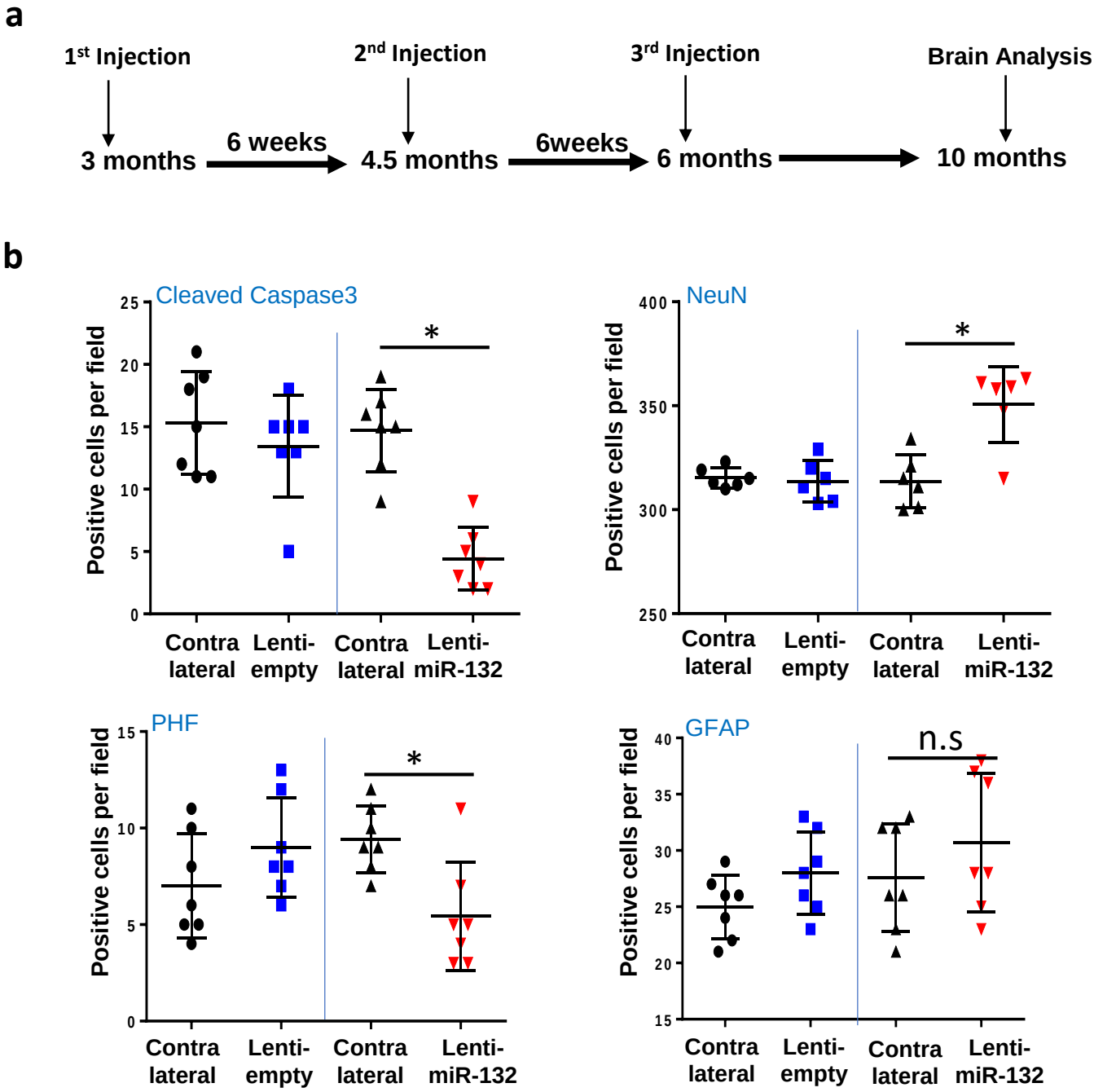

Table S1: List of primers used for real-time PCR experiments

Gene	Accession No.	Primer sequence 5' to 3'	
ACTB	NM_001101.3	F	CACCTTCTACAATGAGCTGCGTGTG
		R	ATAGCACAGCCTGGATAGCAACGTAC
18S rRNA	NR_003286.2	F	ACCACATCCAAGGAAGGCAG
		R	CCGCTCCCAAGATCCAACTA
PABP2	NM_004643.3	F	CAGTTGGCGTGAAGAGAGGA
		R	AGTACACGAGAAGGAGCACC
FOXO3a	NM_001455.3	F	GGCAAAGCAGACCCTCAAAC
		R	TGAGAGCAGATTTGGCAAAGG
Calpain 2	NM_009794.3	F	GAGGTCCTCAACCGCTTCAA
		R	AGCTTGCCACTCCCATCTTC
GSK3β	NM_001347232.1	F	AGCCTTCAGCTTTTGGTAGCAT
		R	CTGCTCCTGGTGAGTCCTTT
EP300	NM_177821.6	F	CTATGGGCTATGGACCTCGC
		R	TGAGCTTGTTGAGGCAGAGTAG
Rbfox1	NM_001359724.1	F	TCTTATGGCGTGCCCATGAT
		R	ACCGGAAAGGGATGTTGGAC
Tau F5-R91	NM_001038609.2	R	ACCAGTATGGCTGACCCT
		F	TCGCCAGGAGTTTGACACAA
Tau F75-R151	NM_001038609.2	R	TCTTGGTCTTGGAGCAGAGTG
		F	GAACCAGTATGGCTGACCCTC
Tau F91-R195	NM_001038609.2	R	ATGCCTGCTTCTTCGGCTTT
		F	CCGAGGTGTGGCGATCTTC
Tau F235-R337	NM_001038609.2	R	TGTGACTCAAGCTCGTGTGG
		F	CACCCGGGACGTGTTTGATA
Tau F730-R930	NM_001038609.2	R	TCTGCAGGCGGCTCTTACTA
		F	CATCACGTGGTGGGCTAGAA
Tau F3600-R3150	NM_001038609.2	R	TCTTCGCCCTGTTACGTTGT
		F	GCTGATTTGTGTCCCTCCCC
Tau F4790-R4890	NM_001038609.2	R	TGAGGGTGGAGGTGGTAATCA
		F	TCTTCGCCCTGTTACGTTGT
Bim	NM_207680	R	GGCGGACAATGTAACGTAACA
		F	CGGAGACGAGTTTAACGCTTA