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Supplementary Materials for

Persistent repression of tau in the brain using engineered zinc finger protein transcription factors

Susanne Wegmann*, Sarah L. DeVos, Bryan Zeitler*, Kimberly Marlen, Rachel E. Bennett, Marta Perez-Rando, Danny MacKenzie, Qi Yu, Caitlin Commins, Riley N. Bannon, Bianca T. Corjuc, Alison Chase, Lisa Diez, Hoang-Oanh B. Nguyen, Sarah Hinkley, Lei Zhang, Alicia Goodwin, Annemarie Ledeboer, Stephen Lam, Irina Ankoudinova, Hung Tran, Nicholas Scarlott, Rainier Amora, Richard Surosky, Jeffrey C. Miller, Ashley B. Robbins, Edward J. Rebar, Fyodor D. Urnov, Michael C. Holmes, Amy M. Pooler, Brigit Riley, H. Steve Zhang, Bradley T. Hyman*

*Corresponding author. Email: bzeitler@sangamo.com (B.Z.); susanne.wegmann@dzne.de (S.W.); bhyman@mgh.harvard.edu (B.T.H.)

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/7/12/eabe1611/DC1)

Supplementary Materials

Supplemental Figures S1-S10

Supplemental Tables 1-6:

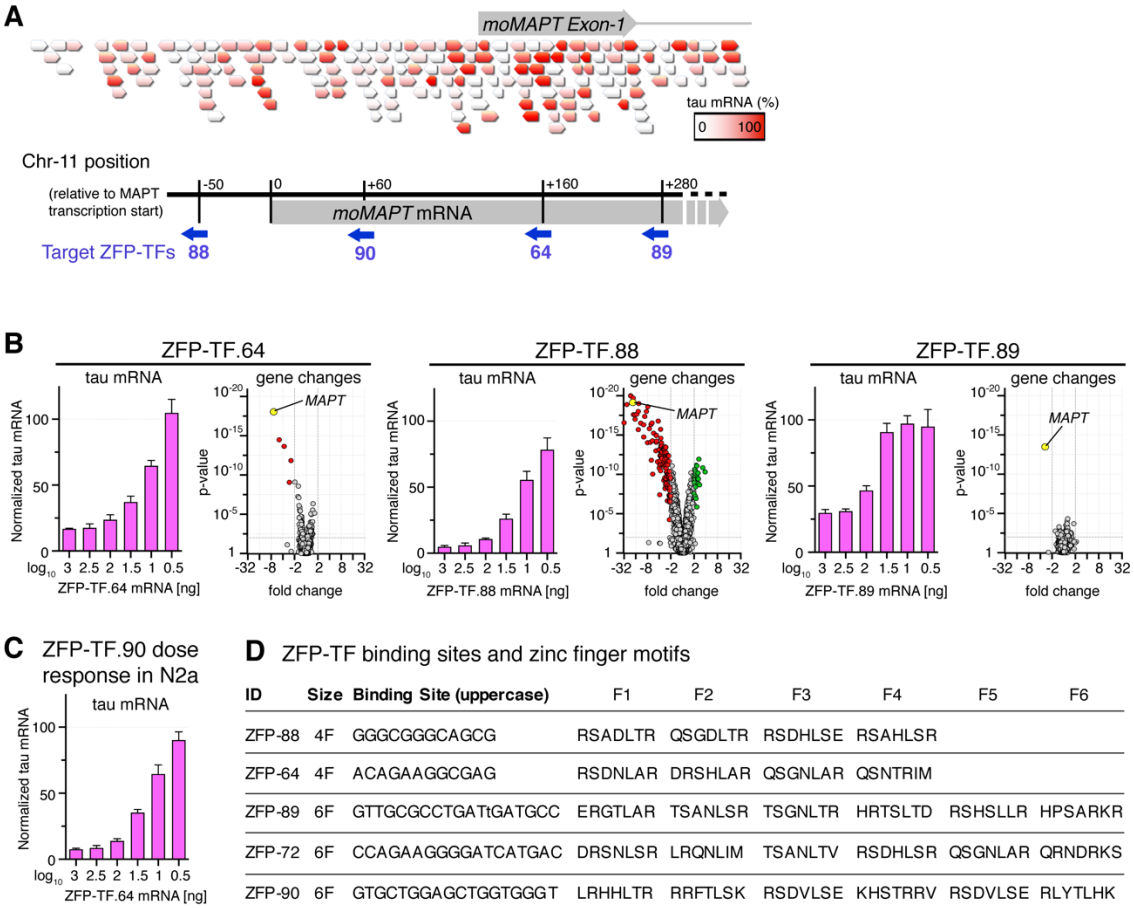
Supplemental Tables 1-4
contain the Affymetrix RNA array data from cells, primary neurons, and mice, and are provided as individual Supplemental Excel files (also available at: https://www.dropbox.com/sh/9txfpj24zfoqv1j/AADVLli3UJxxZrDzy_bTgeXFa?dl=0):

- **Supplemental Table 1:** RNA microarray data for ZFP-TF.89 and ZFP-TF.64, transfected in Neuro2A cells.
- **Supplemental Table 2:** RNA microarray data for ZFP-TF.88, transfected in Neuro2A cells.
- **Supplemental Table 3:** RNA microarray data for CMV.89 treated mouse cortical neurons.
- **Supplemental Table 4:** *In vivo* RNA array data from frontal cortex of AAV-PHP.B injected mice.

Supplemental Table 5: ZFP-TF amino acid sequences.

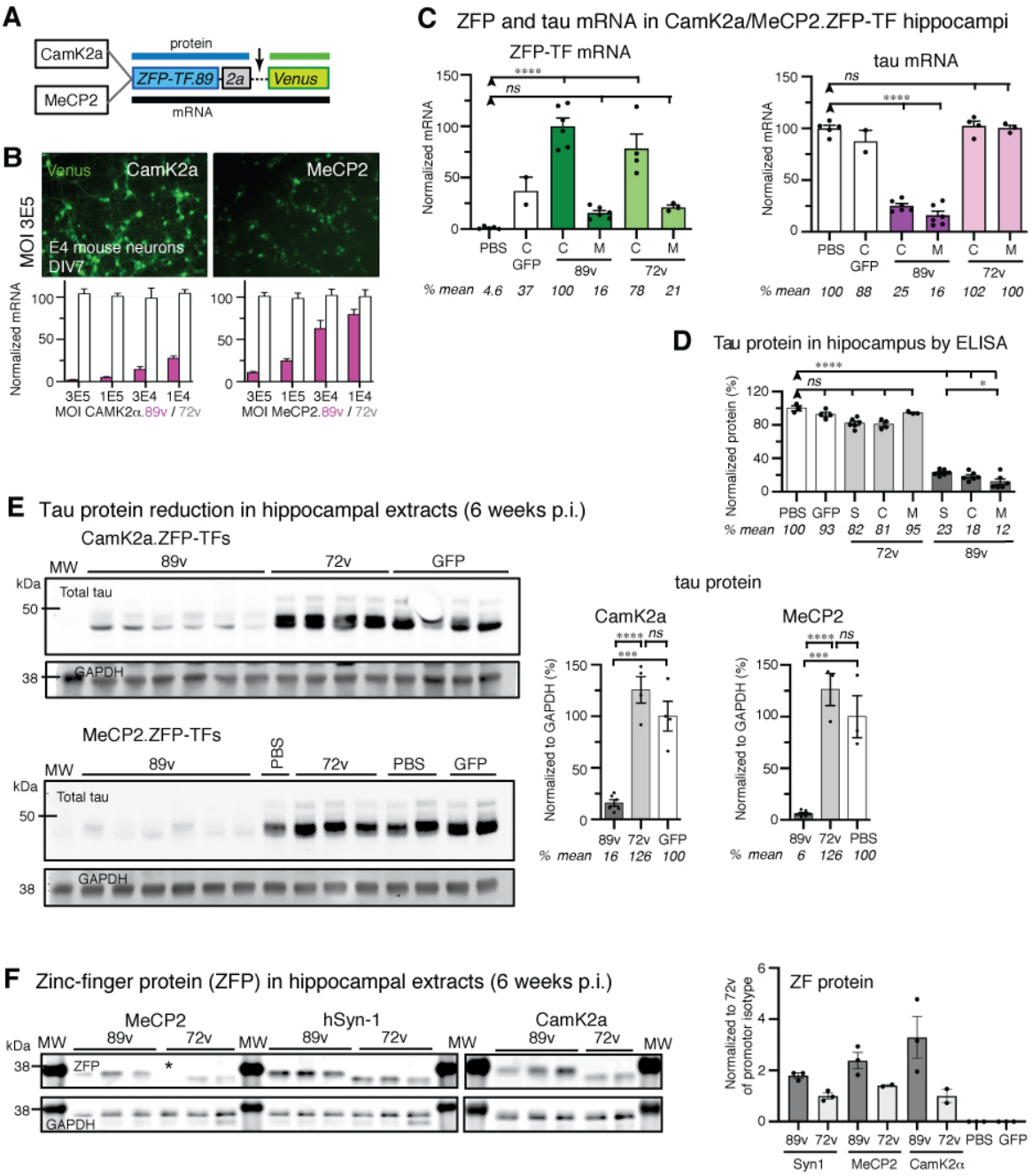
Supplemental Table 6: qPCR probes.

Supplemental Table 7: RNAscope materials.



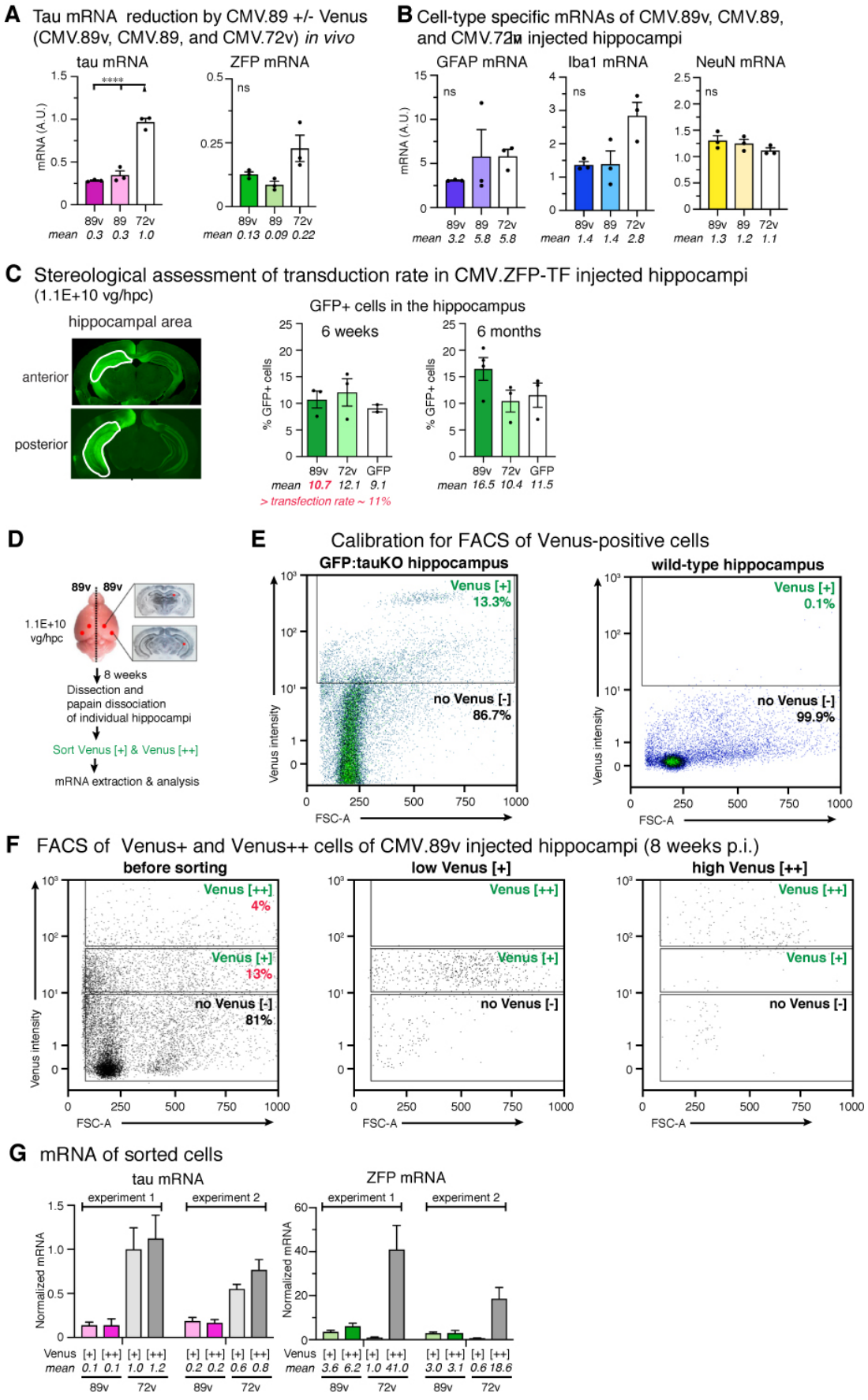
ID	Size	Binding Site (uppercase)	F1	F2	F3	F4	F5	F6
ZFP-88	4F	GGGCGGGCAGCG	RSADLTR	QSGDLTR	RSDHLSE	RSAHLSR		
ZFP-64	4F	ACAGAAGGCGAG	RSDNLAR	DRSHLAR	QSGNLAR	QSNTRIM		
ZFP-89	6F	GTTGCGCCTGATtGATGCC	ERGTLAR	TSANLSR	TSGNLTR	HRTSLTD	RSHSLLR	HPSARKR
ZFP-72	6F	CCAGAAGGGGATCATGAC	DRSNLSR	LRQNLIM	TSANLTV	RSDHLSR	QSGNLAR	QRNDRKS
ZFP-90	6F	GTGCTGGAGCTGGTGGGT	LRHHLTR	RRFTLSK	RSDVLSE	KHSTRRV	RSDVLSE	RLYTLHK

Supplemental Figure S1. Dose response and gene deregulation by mouse tau targeted ZFP-TFs. (A) Schematic of binding locations around the mouse MAPT (moMAPT) transcription start and tau mRNA repression activity of 185 moMAPT-targeted ZFPs tested in Neuro2a cells (top). The binding locations of candidate ZFP-TFs (88, 64, 89) around the mouse *MAPT* gene TSS in the mouse chromosome 11 are indicated in more detail (bottom). **(B)** Dose-response of tau mRNA repression to ZFP-TF mRNA expression (1E+3 to 1E+0.5 ng per 1E+5 cells) in N2a cells transduced by ZFP-TFs 64, 88, and 89 and transcriptomic changes (volcano plots of Affymetrix data collected from cells treated with 300 ng ZFP-TF mRNA for 24 hours). In these conditions, ZFP-TF.89 was least efficient with a maximum tau mRNA knock-down of ~75% at $10^{2.5}$-10^{3} ng ZFP-TF.89, but showed virtually no significant (>2-fold change, $p<0.01$) gene alterations additional to *MAPT*. RNA array data is available in Supplemental Tables 1 and 2. Bar graphs: Data presented as mean±SEM, n=3 mice per group. **(C)** Dose-response of tau mRNA repression to ZFP-TF mRNA expression (1E+3 to 1E+0.5 ng per 1E+5 cells) in N2a cells transduced by ZFP-TF.90. Data presented as mean±SEM, n=3 experimental and 5 technical replicates. **(D)** Zinc finger protein (F) amino acid sequences for zinc finger protein arrays of ZFP-TFs 88, 64, 89, 72, and 90.



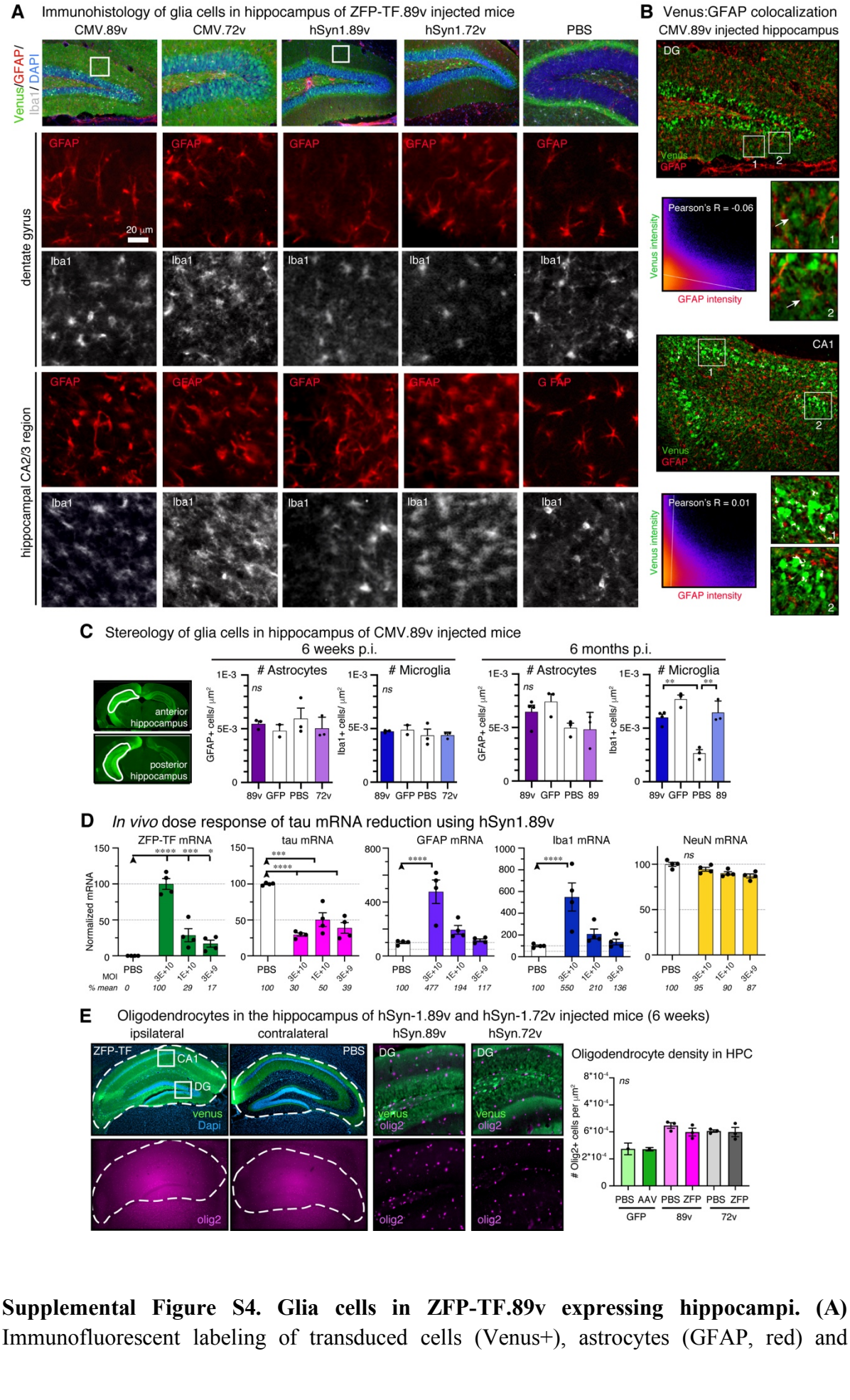
Supplemental Figure S2. Tau repression using ZFP-TF.89v under CamK2α and MeCP2 promotors. (A) AAV9 ZFP-TF.2a.Venus constructs with the neuronal CamK2α and MeCP2 promotors for neuron-specific ZFP-TF expression. **(B)** Expression of AAV ZFP-TF.89v under CamK2α and MeCP2 promotors in primary cortical mouse neurons (DIV 7; 4 days p.i.) leads to a dose dependent repression of tau mRNA (pink bars). ZFP-TF.72v control constructs did not show tau repression (white bars). Data presented as mean±SD. n=3 independent experiments, data normalized to tau mRNA in ZFP-FT.72v expressing neurons at highest dose (MOI=3E5). **(C)** *In vivo* mRNA levels of ZFP-TF (green) and tau (pink) in CamK2α.89v (C)- and MeCP2.89v (M)-injected hippocampi after 6 weeks. Even at low ZFP-TF transcript levels, MeCP2.89v leads to a >80% reduction in tau mRNA. Control CamK2α.72v- or MeCP2.72v-injected hippocampi did not reduce tau mRNA compared to non-injected (NI) and PBS-injected mice. Data are presented as mean±SEM. One-way ANOVA, Sidak's test

for multiple comparisons, reference for significance indicated by arrowhead, n=3 mice per group. **(D)** Tau protein reduction in hippocampal extracts of CamK2α.89v (C), MeCP2.89v (M), and hSyn1.89v (S) injected animals determined by ELISA of lysates 6 weeks p.i.. All three neuronal promotors reduced tau protein by reduced total tau protein by ~80-90%. ZFP-TF.72v expressing, non-injected, and PBS-injected hippocampi had similar tau protein levels. Data are presented as mean±SEM. One-way ANOVA with Sidak post-test, n=3 mice per group. **(E)** Tau protein reduction in hippocampal extracts of CamK2α.89v (C) and MeCP2.89v (M) injected animals determined by western blot confirm the results of tau protein reduction by ELISA in d). Data are presented as mean±SEM. One-way ANOVA with Sidak post-test, n=3 mice per group. **(F)** Western blot analysis of ZFP-TF protein expression in hippocampal lysates under the three neuronal promotors. Notably, among the different neuronal promotors, MeCP2.89v showed the strongest repression of tau mRNA and protein *in vivo* although the ZFP-TF mRNA levels were low and ZFP protein levels were similar compared to CamK2α.89v and hSyn1.89v. Data are presented as mean±SEM. One-way ANOVA with Sidak post-test, n=3 mice per group.



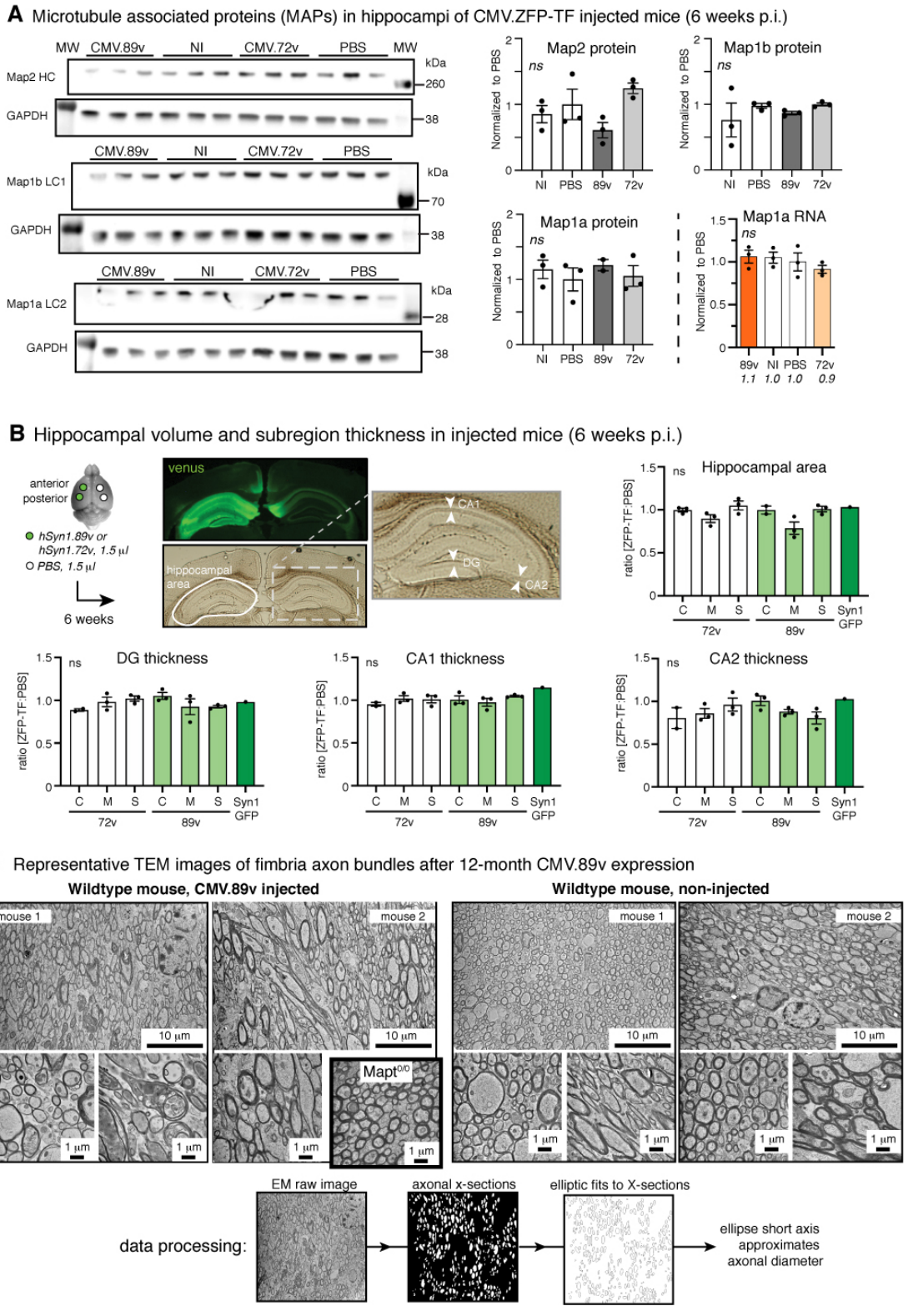
Supplemental Figure S3. Parameters of tau repression and cell transduction by CMV.89v. (A) Tau mRNA reduction by ZFP-TFs +/- Venus (CMV.89, .89v, .72v) in the

hippocampus 6 weeks after AAV injection. Both 89v and 89 show similar tau repression activity *in vivo*. Data presented as average per animal, mean±SEM. One-way ANOVA compared to group indicated by black arrow head in significance labels. Sidak's test for multiple comparison, n=3 mice per group and 3 tissue slices per hippocampus. **(B)** Cell-type specific markers (GFAP, Iba1, and NeuN) of ZFP-TFs +/- Venus (CMV.89, .89v, .72v) injected hippocampi show no difference in GFAP, Iba1 and NeuN mRNA levels between the groups. Data presented as average per animal, mean±SEM. One-way ANOVA compared to group indicated by black arrow head in significance labels. Sidak's test for multiple comparison, n=3 mice per group and 3 tissue slices per hippocampus. **(C)** Assessment of CMV.89v and CMV.72v transduction efficiency by stereological counting of Venus+ cells in the hippocampal formation. Data presented as mean±SEM. n=3 mice per group, 3-4 sections per animal. One-way ANOVA, Tukey's test for multiple comparison. **(D)** Injection scheme for FACS experiments: AAV CMV.89v was injected into both hemispheres (2x 1.5 µl =1.1E+10 vg/hippocampus). After 8 weeks, the hippocampi were extracted and dissociated using papain. After FACS by Venus fluorescence (GFP channel), the RNA was extracted and analyzed. **(E)** The calibration and gating for FACS of Venus-positive cells was setup in the GFP channel: a dissociated hippocampus from a tau knock-out mouse expressing GFP in place of murine *MAPT* (TauKO (*72*), $Mapt^{tm1(EGFP)Klt}$, Jackson Laboratories; left scatter plot) was used as Venus+ control, and the hippocampus of a wildtype mouse (C57/B6; right scatter plot) was used to control for background fluorescence. Scatter plots show Venus intensity (y-axis) versus forward scatter (FSC-A, x-axis). **(F)** FACS of Venus++, Venus+ and Venus- cells from hippocampi of CMV.89v injected C57/B6 mice shows that ~17% (4% Venus++ and 13% Venus+) of hippocampal cells were transduced by AAV CMV.89v. **(G)** Tau and ZFP mRNA in Venus++ and Venus+ cells from CMV.89v and CMV.72v hippocampi from two different experiments, each analyzing 2 hippocampi per group. Even in CMV.89v cells with low ZFP-TF expression (Venus+) tau mRNA was reduced by 80-90% (compared to Venus+ in CMV.72v cells in experiment 1). Data presented as mean±SEM. N=2 hippocampi per group per experiment.



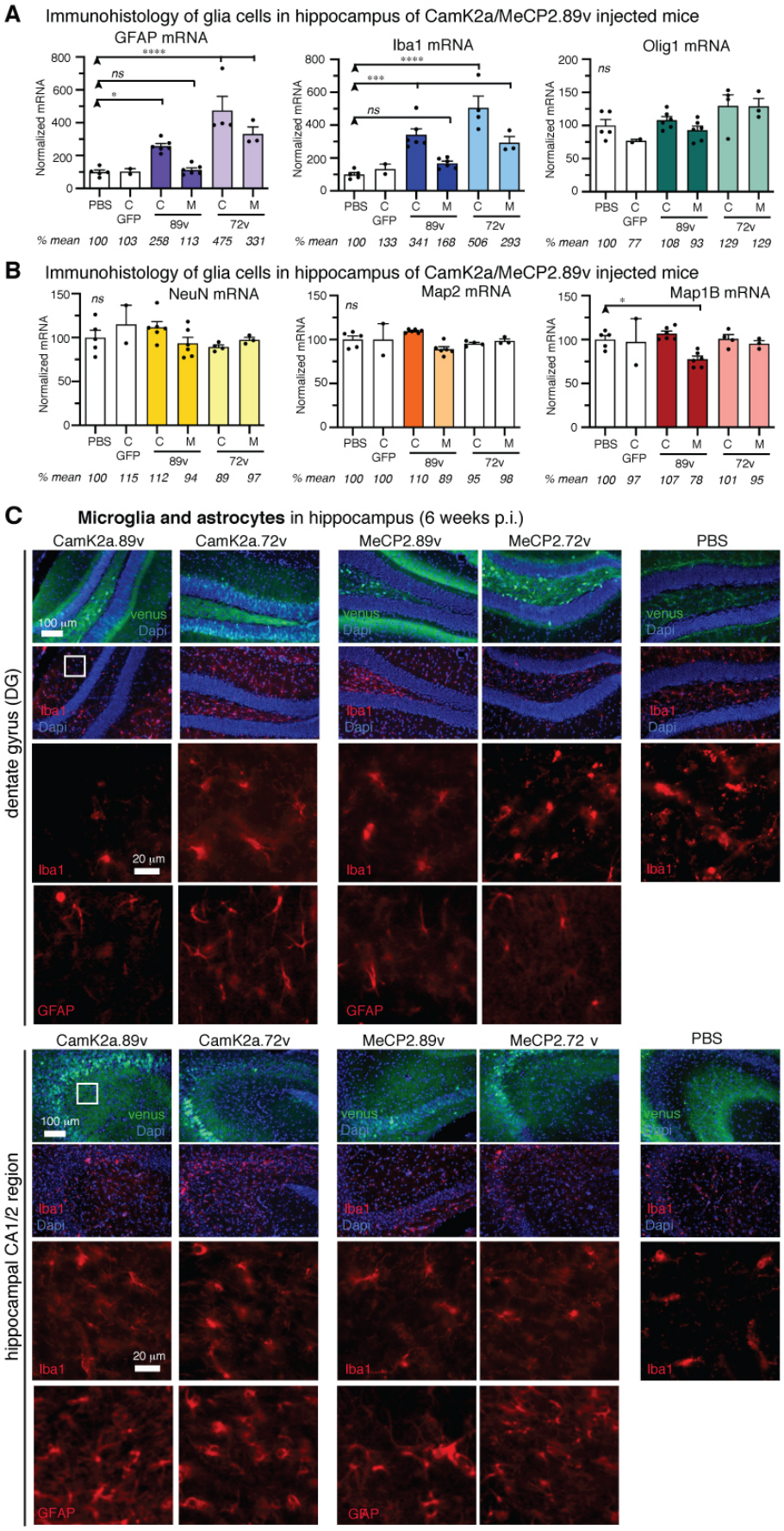
Supplemental Figure S4. Glia cells in ZFP-TF.89v expressing hippocampi. (A) Immunofluorescent labeling of transduced cells (Venus+), astrocytes (GFAP, red) and

microglia (Iba1, white) in the hippocampus (coronal brain sections) of animals injected with CMV.ZFP-TFs (CMV.89v, CMV.72v), hSyn1.ZFP-TFs (hSyn1.89v, hSyn1.72v), or with PBS. Higher-magnification images of glia cells in the dentate gyrus (top) and the hippocampal area CA2/CA3 (bottom) are shown as well. Arrow heads indicate AAV CMV.ZFP-TF transduced astrocytes expressing Venus. **(B)** No colocalization (Pearson's R-value of ~0.0) is detected for Venus and GFAP in images from a CMV.89v injected hippocampi, indicating no expression of ZFP-TFs in GFAP+ astrocytes. However, some GFAP- cells (reminiscent of tufted astrocytes; white arrows) show Venus expression. Minor colocalization occurs at the somatic periphery of some Venus+ neurons (white areas in insets). **(C)** Stereological counting of astrocytes (GFAP) and microglia (Iba1) in the hippocampus of CMV.89v and CMV.72v injected mice shows an increase in the number of microglia but not astrocytes wit time. Data presented as mean±SEM. n=3 mice per group, 3-4 sections per animal. One-way ANOVA, Tukey's test for multiple comparison. **(D)** Dose response of ZFP, tau, and cell-type specific mRNA (glia cells: GFAP and Iba1; neurons: NeuN) after bilateral dual hippocampal injections of different amounts (3 µl per hemisphere with 3E+10, 1E+10, or 3E+9 vg) of hSyn1.89v. All doses led to efficient (>50%) tau reduction after 4 weeks. Only the highest dose of hSyn1.89v resulted in an elevation of astroglial GFAP and microglial Iba1 mRNA. NeuN mRNA levels remained unchanged. Data presented as mean±SEM. One-way ANOVA compared to group indicated by black arrowhead in significance labels. Sidak's test for multiple comparison, n=4 mice per group, 1 hippocampus per mouse. **(E)** Immunohistological assessment of the number of oligodendrocytes (Olig2) in the hippocampal formation of hSyn1.89v and hSyn1.72v expressing mice reveals no changes in the hippocampal density of these glial cells compared to PBS or GFP injected animals. No colocalization of Olig2 with Venus expressing cell bodies was observed for any of the neuronal promotors. Data presented as mean±SEM. n=3 mice per group, 3-4 sections per animal. One-way ANOVA, Tukey's test for multiple comparison.



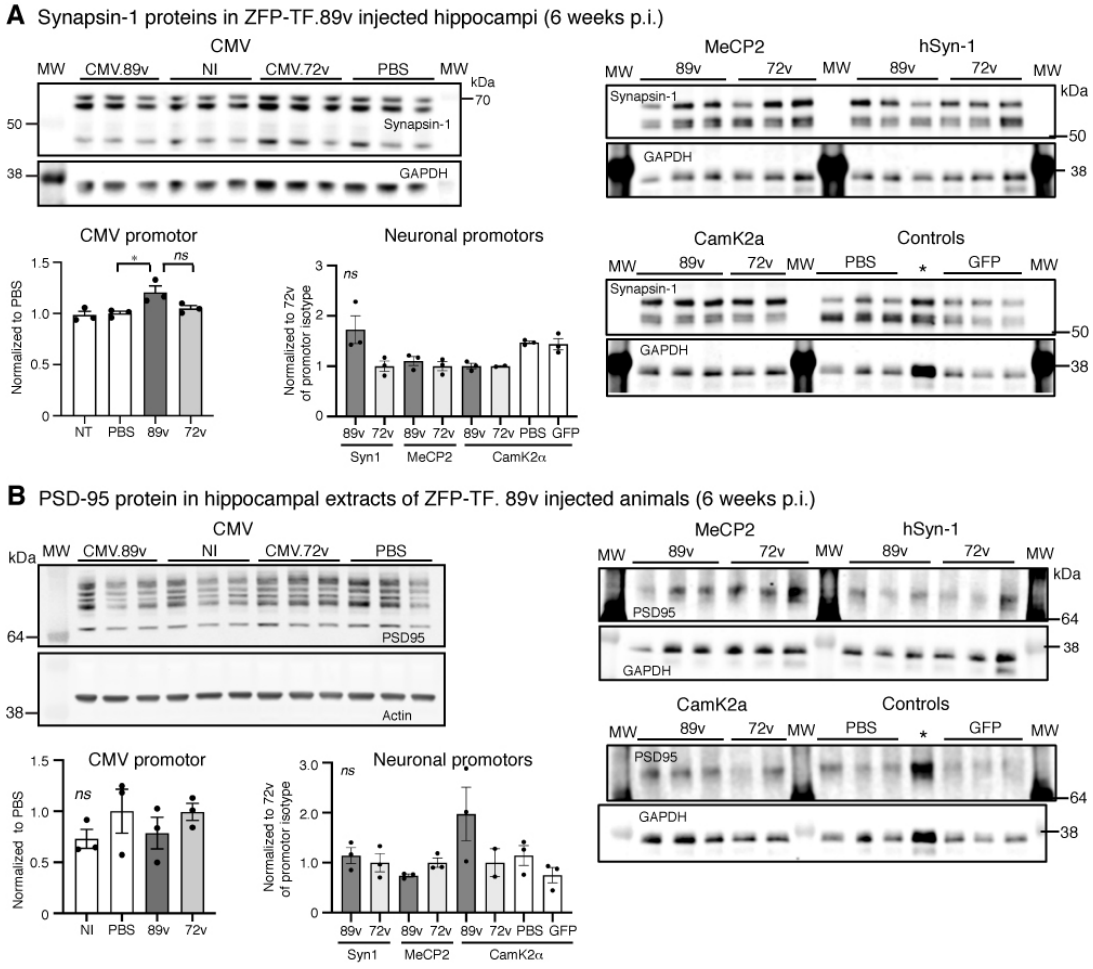
Supplemental Figure S5. No overt neuronal changes upon tau repression by ZFP-TF.89v. (A) Western blots and quantification of Map2, Map1a, and Map1b in hippocampal lysates 6 weeks after AAV injection. No significant difference is detected in CMV.89v compared to CMV.72v and PBS or GFP injected mice. Map1a mRNA was neither elevated, for mRNA of Map1a and Map2 see Figure 3c. Data presented as mean±SEM. One-way ANOVA compared to PBS-injected hippocampi. Tukey's test for multiple comparison, n=3 mice per group. **(B)** Coronal brain sections of mice unilaterally expressing ZFP-TF.89v, ZFP-

TF.72, or GFP for 6 weeks were evaluated for changes in hippocampal volume (area covered by hippocampal formation in individual brain sections) and sub-region (DG, CA1, CA2) layer thickness. Hippocampal area and layer thicknesses were normalized to the PBS-injected control hemisphere in the same brain section. Data are presented as mean±SEM. One-way ANOVA with Tukey's correction for multiple comparison. n=3 mice per group, n=3 brain sections per animal. For sub-region thickness, n=5 linear distance measurements across neuronal layer per sub-region. **(C)** Representative TEM micrographs of axon cross-sections in the fornix (white outline area in brain section image) of long-term (12 month) CMV.89v injected mice (top panel; overview and two close-ups from two mice), a genetic tau knock-out mouse ($Mapt^{0/0}$; inset in CMV.89v mouse-1 panel), and non-injected wildtype mice (bottom panel). Analysis of axonal cross-sections revealed no difference in axon diameter between CMV.89v and non-injected wildtype mice, neither across animals (violin plot; data shown as median (green) and interquartile range (pink)), nor across groups (bar graph; data shown as mean±SEM, two-tailed Student's t-test, n=3 CMV.89v and n=2 non-injected mice). The myelination (dark layer around axons) appears variable across individual axons in each animal.

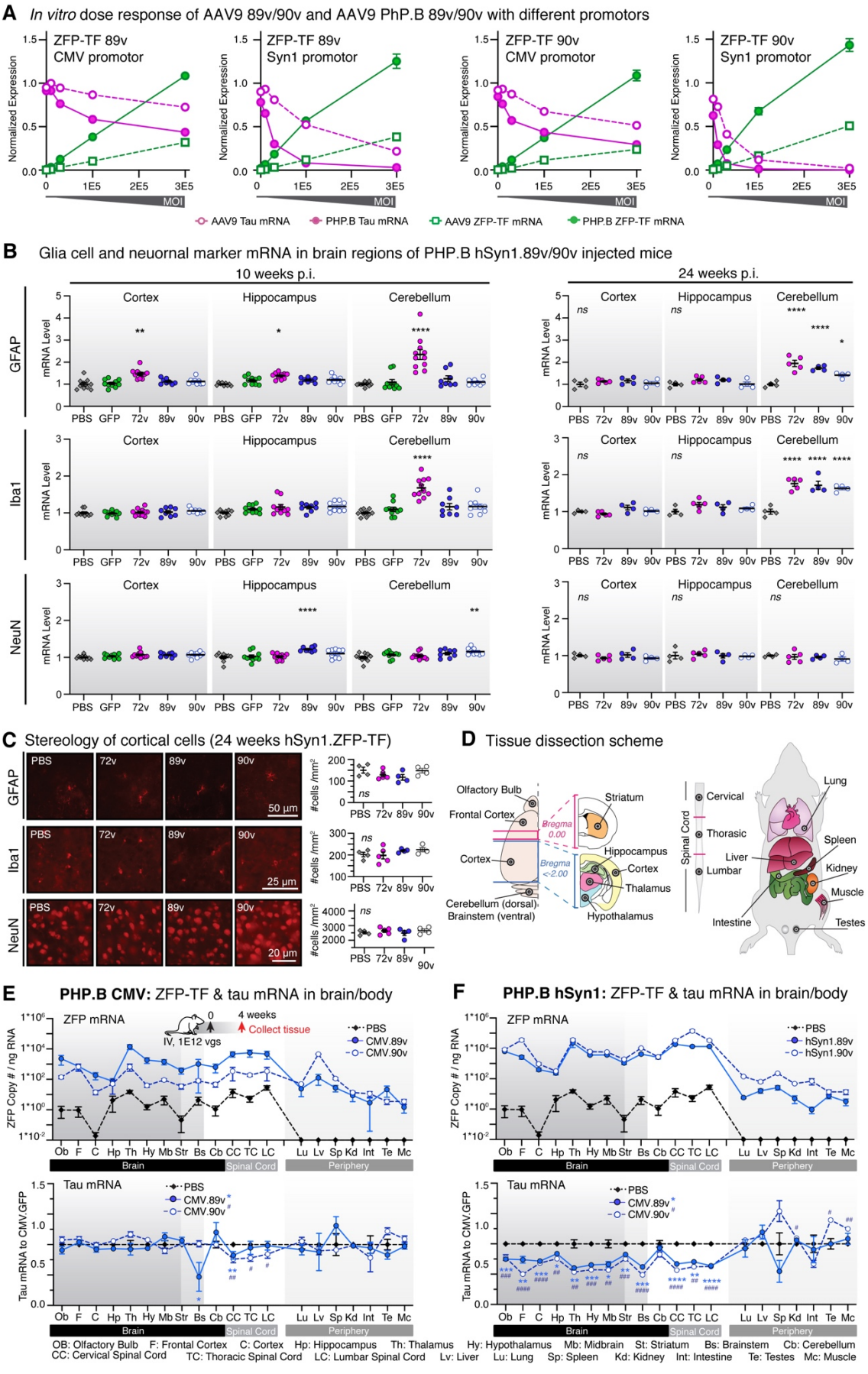


Supplemental Figure S6. Cell-type specific RNA in CamK2α.89v and MeCP2.89v injected hippocampi. (A) Astrocyte (GFAP) and microglia (Iba1) mRNA levels were

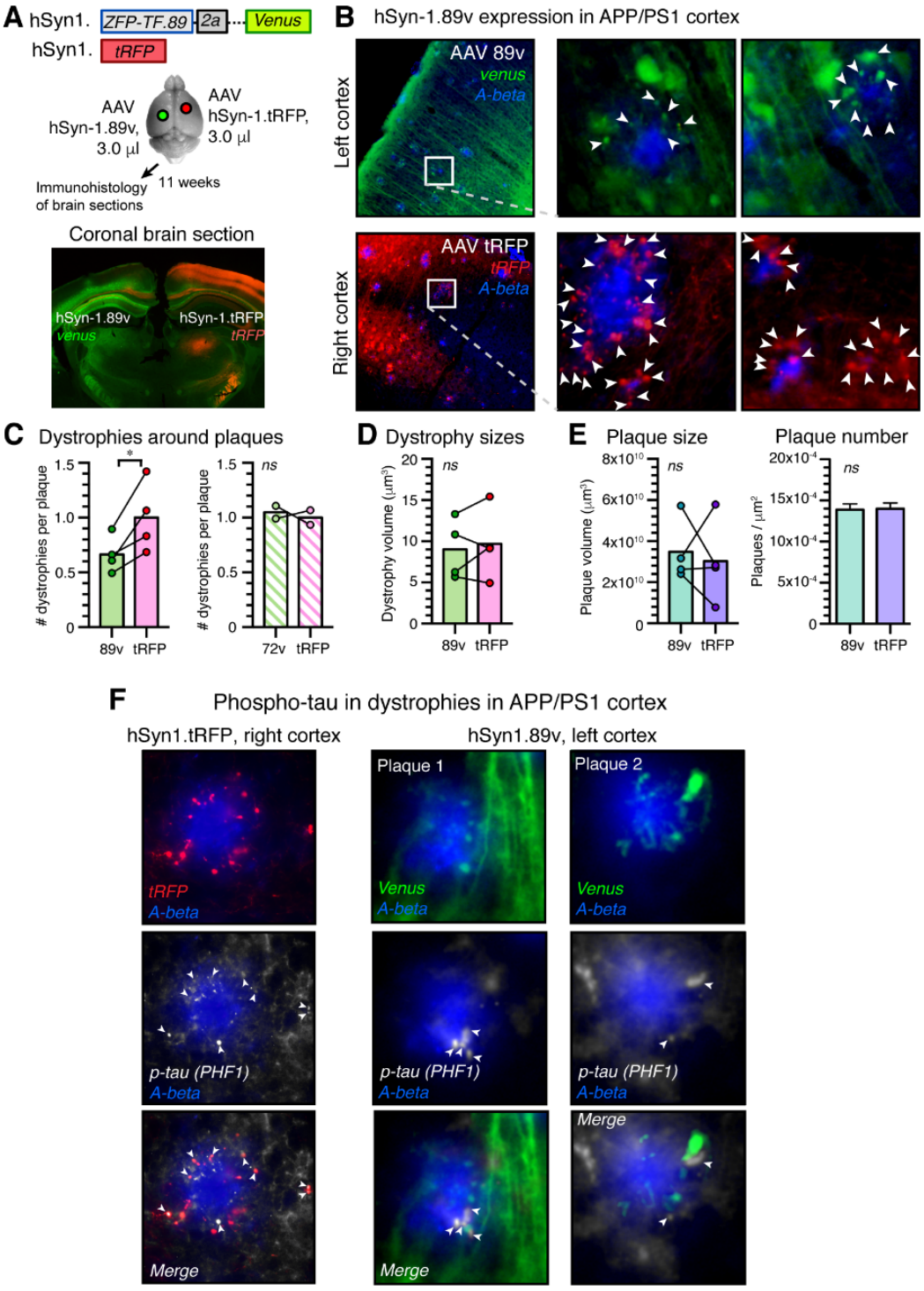
elevated in ZFP-TF-treated hippocampi. 72v led to higher GFAP and Iba1 levels than 89v, and CaMK2α (C) > MeCP2 (M). The increase in glial marker mRNA levels in 89v-treated hippocampi did not correlate with tau reduction. For example, the MeCP2 (M) construct strongly repressed tau, but resulted in a small increase in glial mRNA levels. No changes in the oligodendrocyte marker Olig1 were observed. Data are presented as mean ± SEM. One-way ANOVA compared to group indicated by black arrowhead in significance labels. Sidak's test for multiple comparisons, n=6 hippocampi per group. **(B)** Neuronal mRNA markers were largely unchanged upon 72v and 89v expression driven by CamK2α and MeCP2 promotors. Small but significant changes were detected for Map1B after MeCP2.89v treatment. Data are presented as mean ± SEM. One-way ANOVA compared to group indicated by black arrowhead in significance labels. Sidak's test for multiple comparisons, n=6 hippocampi per group. **(C)** Immunofluorescent labeling of transduced cells (Venus+), astrocytes (GFAP) and microglia (Iba1) in the hippocampus (coronal brain sections) of animals injected with CamK2α.89v, CamK2α.72v, MeCP2.89v, or MeCP2.72v, or with PBS. Higher-magnification images of glia cells in the dentate gyrus (top) and the hippocampal area CA1/CA2 (bottom) are shown as well.



Supplemental Figure S7. Synaptic proteins in hippocampal lysates from ZFP-TF.89v expressing mice. (A) Western blots and quantification of pre-synaptic marker protein synapsin-1 in hippocampal lysates of mice expressing ZFP-TF.89v under CMV, hSyn1, CamK2α, and MeCP2 promotors. No significant changes in synapsin-1 were detected, except a mild (~20%) increase in CMV.89v compared to control (CMV.72v, PBS, and non-injected (NI)) hippocampi. Data presented as mean±SEM, one-way ANOVA, Tukey's test for multiple comparison, n=3 mice per group. **(B)** Western blots and quantification of post-synaptic marker protein PSD95 in hippocampal lysates of mice expressing ZFP-TF.89v under CMV, hSyn1, CamK2α, and MeCP2 promotors. No significant changes in PSD95 were detected. Data presented as mean±SEM, one-way ANOVA, Tukey's test for multiple comparison, n=3 mice per group.

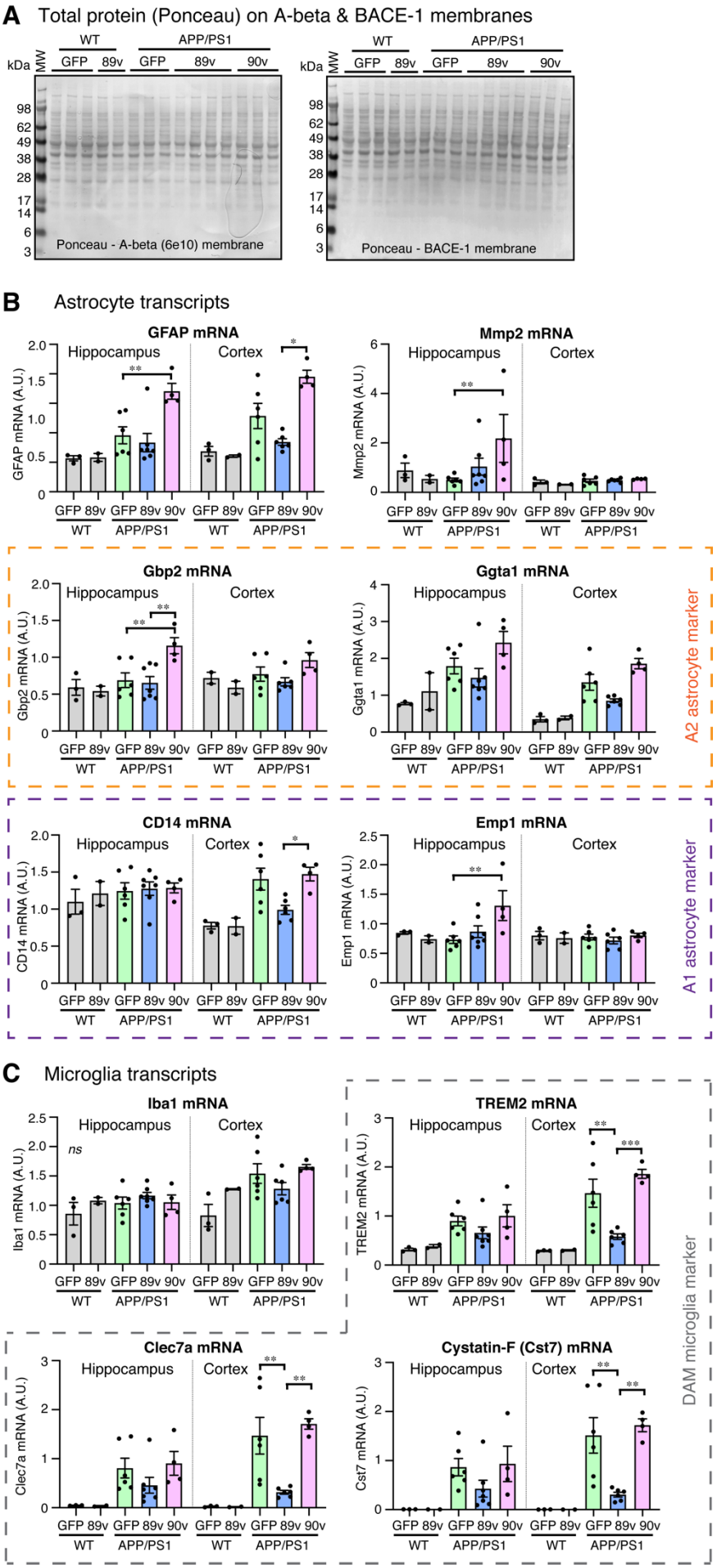

A
In vitro dose response of AAV9 89v/90v and AAV9 PhP.B 89v/90v with different promotors
ZFP-TF 89v CMV promotor
ZFP-TF 89v Syn1 promotor
ZFP-TF 90v CMV promotor
ZFP-TF 90v Syn1 promotor
Normalized Expression
MOI
AAV9 Tau mRNA
PHP.B Tau mRNA
AAV9 ZFP-TF mRNA
PHP.B ZFP-TF mRNA
B
Glia cell and neuornal marker mRNA in brain regions of PHP.B hSyn1.89v/90v injected mice
10 weeks p.i.
24 weeks p.i.
GFAP
Iba1
NeuN
Cortex
Hippocampus
Cerebellum
mRNA Level
PBS GFP 72v 89v 90v
C
Stereology of cortical cells (24 weeks hSyn1.ZFP-TF)
#cells /mm²
50 µm
25 µm
20 µm
D
Tissue dissection scheme
Olfactory Bulb
Frontal Cortex
Striatum
Bregma 0.00
Hippocampus
Cortex
Bregma <-2.00
Thalamus
Hypothalamus
Cerebellum (dorsal)
Brainstem (ventral)
Spinal Cord
Cervical
Thorasic
Lumbar
Lung
Spleen
Liver
Kidney
Muscle
Intestine
Testes
E
PHP.B CMV: ZFP-TF & tau mRNA in brain/body
ZFP mRNA
IV, 1E12 vgs
4 weeks
Collect tissue
PBS
CMV.89v
CMV.90v
ZFP Copy # / ng RNA
Tau mRNA
Tau mRNA to CMV.GFP
Ob F C Hp Th Hy Mb Str Bs Cb CC TC LC Lu Lv Sp Kd Int Te Mc
Brain
Spinal Cord
Periphery
F
PHP.B hSyn1: ZFP-TF & tau mRNA in brain/body
hSyn1.89v
hSyn1.90v
OB: Olfactory Bulb F: Frontal Cortex C: Cortex Hp: Hippocampus Th: Thalamus Hy: Hypothalamus Mb: Midbrain St: Striatum Bs: Brainstem Cb: Cerebellum
CC: Cervical Spinal Cord TC: Thoracic Spinal Cord LC: Lumbar Spinal Cord Lv: Liver Lu: Lung Sp: Spleen Kd: Kidney Int: Intestine Te: Testes Mc: Muscle

Supplemental Figure S8. *In vitro* and *in vivo* activity and side effects of AAV PHP.B 89v. **(A)** Normalized tau (pink) and ZFP (green) mRNA expression by ZFP-TF.89v or ZFP-TF.90v across multiple viral doses in primary neurons. For both ZFP-TFs and both promotor types, the CMV and hSyn1, AAV9-PHP.B serotypes (closed circles) showed superior dose response of tau repression compared to conventional AAV9 (open circles). Data presented as mean±SEM, n=6 experiments. **(B)** Glia cell (GFAP, Iba1) and neuronal (NeuN) mRNA levels upon tau reduction by PHP.B hSyn1.89v and hSyn1.90v at 10 weeks and 24 weeks after retroorbital IV injection of 2.5E+12 vg/mouse. Some elevation in GFAP and in Iba1 was detected in cortex, hippocampus and cerebellum of controls injected with hSyn1.72v, but not in hSyn1.89v- or hSyn1.90v-injected mice after 10 weeks. After 24 weeks, GFAP and Iba1 were increased in the cerebellum of all AAV-PHP.B ZFP-TF injected mice. Interestingly, hSyn1.89v injected animals showed increased neuronal NeuN transcripts in the hippocampus after 10 weeks, which was normalized again after 24 weeks. Data presented as mean±SEM, two-way ANOVA, Tukey's test for multiple comparison, n=5-12 mice per group. **(C)** Stereological assessment of astrocytes (GFAP), microglia (Iba1) and neurons (NeuN) in the cortex showed no increased number of these cell types after 24 weeks of hSyn1.89v or hSyn1.90v expression. Data presented as mean±SEM, two-way ANOVA, n=5 mice per group, 3-5 brain sections per mouse. **(D)** Brain, spinal cord, and body tissue sampled for whole-body analysis of ZFP-TF expression and tau reduction. **(E)** ZFP-TF and tau mRNA levels across multiple brain regions and body parts at 4 weeks after retro-orbital IV injection of 1E12 vgs/mouse of CMV.89v, CMV.90v, or PBS. Data for tau mRNA are normalized to mice treated with PBS and significance is indicated for CMV.89v (*) and CMV.90v (#). Data presented as mean±SEM, n=6-8 mice per group, student's t-test for each ZFP-TFs versus PBS in each tissue. **(F)** ZFP-TF and tau mRNA levels across multiple brain regions and body parts at 4 weeks after retro-orbital IV injection of 1E12 vg/mouse of hSyn1.89v, hSyn1.90v, or PBS. Data for tau mRNA are normalized to mice treated with PBS and significance is indicated for hSyn1.89v (*) and hSyn1.90v (#). Data presented as mean±SEM, n=6-8 mice per group, student's t-test for each ZFP-TFs versus PBS in each tissue.



Supplemental Figure S9. Reduced neuritic dystrophies around plaques in APP/PS1 mice with cortical injection of AAV9 hSyn1.89v. (A) Injection scheme and experimental design of AAV9 hSyn1.89v and control AAV9 hSyn1.tRFP injections into the somatosensory cortex of 4.5-month-old APP/PS1 mice. Representative coronal brain section showing 89v (Venus; green) and turboRFP (tRFP; red) expression in the cortex after 11 weeks. **(B)** Representative images of the somatosensory cortex of APP/PS1 mice with A-beta plaques (blue) injected with hSyn1.89v (green) and hSyn1.tRFP (red) into opposite hemispheres. The inset and additional zoom-in images (white star) show a "corona" of blob-like neuritic dystrophies (dendritic and axonal swellings; white arrowheads) around cortical plaques. Dystrophies are filled with cytosolic proteins, including AAV-encoded Venus and tRFP. **(C)** Quantification

of dystrophies in the somatosensory cortex of APP/PS1 mice injected with either hSyn1.89v and hSyn1.tRFP, or with hSyn1.72v and hSyn1.tRFP. Dystrophies filled with Venus in the left cortex and dystrophies filled with tRFP in the right cortex were counted in the immediate vicinity of amyloid plaques and normalized to the number of amyloid plaques; data are shown normalized to the number of dystrophies per plaque in the AAV9 hSyn1.tRFP-treated hemisphere for each mouse. The presence of hSyn1.89v in cortical neurons reduced the number of dystrophies per plaque when compared to control tRFP. Data are presented as mean per group, with single points showing the average per mouse. Student t-test, n=4 for hSyn1.89v and n=2 for hSyn1.72v mice, 4-10 cortical brain sections per animal, 5-10 plaques per sections. **(D)** Dystrophy size (volume extrapolated from circular outlines of individual dystrophies) in hSyn1.89v- and hSyn1.tRFP-expressing neurites. Data represent mean per group, and single points show the average per mouse. Student's t-test, n=4 mice, 4-10 cortical brain sections per animal, 130-260 dystrophies per animal. **(E)** Quantification of cortical amyloid plaque sizes (volume extrapolated from elliptic outlines of individual plaques) revealed no differences between hSyn1.89v- and hSyn1.tRFP-treated hemispheres. Data are presented as mean per group, and single points show the average per mouse. Student's t-test, n=4 mice, 3 cortical brain sections per animal, 50-200 plaques per animal. **(F)** In immunofluorescently labeled brain sections of injected APP/PS1 mice. Neurites filled with tRFP (red; in right cortex) represent a subset of dystrophies (white arrowheads) that are positive for phosphorylated tau (PHF1 antibody recognizing tau pS396/pS404). In neurites filled with 89v (green; in left cortex; bottom two pane rows), tau is absent. As a result, no phospho-tau can be detected in 89v dystrophies.


A
Total protein (Ponceau) on A-beta & BACE-1 membranes
WT
APP/PS1
GFP
89v
90v
kDa
MW
98
62
49
38
28
17
14
6
3
Ponceau - A-beta (6e10) membrane
Ponceau - BACE-1 membrane
B
Astrocyte transcripts
GFAP mRNA
Mmp2 mRNA
Gbp2 mRNA
Ggta1 mRNA
CD14 mRNA
Emp1 mRNA
Hippocampus
Cortex
GFAP mRNA (A.U.)
Mmp2 mRNA (A.U.)
Gbp2 mRNA (A.U.)
Ggta1 mRNA (A.U.)
CD14 mRNA (A.U.)
Emp1 mRNA (A.U.)
GFP 89v
GFP 89v 90v
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A1 astrocyte marker
C
Microglia transcripts
Iba1 mRNA
TREM2 mRNA
Clec7a mRNA
Cystatin-F (Cst7) mRNA
Iba1 mRNA (A.U.)
TREM2 mRNA (A.U.)
Clec7a mRNA (A.U.)
Cst7 mRNA (A.U.)
ns
DAM microglia marker

Supplemental Figure S10. Reduced neuritic dystrophies around plaques in APP/PS1 mice with cortical injection of AAV9 hSyn1.89v. (A) Ponceau staining of total protein on western blot membranes immunoprobed for A-beta (6e10 antibody) and BACE-1 in Figure 5D. **(B)** Transcript levels of astrocyte genes. Increased GFAP and MMP2 levels are detected in APP/PS1 mice injected with AAV-PHP.B 90v compared to GFP and 89v expressing mice. Signature genes of neurotoxic astrocyte state 'A1': Epithelial Membrane Protein 1 (Emp1) is not affected and CD14 is reduced in cortex of AAV-PHP.B 89v injected APP/PS1 mice. Signature genes of neuroprotective astrocyte state 'A2': Guanylate Binding Protein 1 (Gbp1) is elevated in AAV-PHP.B 90v injected APP/PS1 mice, whereas as elevation of Alpha-1,3-galactosyltransferase (Ggta1) does not reach significance in the same mice. **(C)** Transcript levels of microglia genes. No significant changes in the housekeeping gene Iba1. Disease activated microglia (DAM) marker genes, TREM2, Cst7, and Clec7a are generally upregulated in APP/PS1 mice, and significantly reduced in APP/PS1 injected with AAV-PHP.B 89v. Data in (B) and (C) are presented as mean±SEM, n=4-6 mice per group, one-way ANOVA, Sidak's test for multiple comparison.

Supplemental Table 5. ZFP-TF amino acid sequences.

ZFP-TF ID	AA sequence (**helix**, intramodule linker, intermodule linker)
ZFP-88	MAPKKKRKVGVPAAMAERPFQCRICMRNFS**RSADLTR**HIRTHTGEKPFACDICGRK FA**QSGDLTR**HTKIHTGSQKPFQCRICMRNFS**RSDHLSE**HIRTHTGEKPFACDICGR KFA**RSAHLSR**HTKIHLRQKDAARGSGGDAKSLTAWSRTLVTFKDVFVDFTREEWKL LDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPD SETAFEIKSSVRS
ZFP-64	MAPKKKRKVGVPAAMAERPFQCRICMRNFS**RSDNLAR**HIRTHTGEKPFACDICGRK FA**DRSHLAR**HTKIHTGSQKPFQCRICMRNFS**QSGNLAR**HIRTHTGEKPFACDICGR KFA**QSNTRIM**HTKIHLRQKDAARGSGGDAKSLTAWSRTLVTFKDVFVDFTREEWKL LDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPD SETAFEIKSSVRS
ZFP-89	MAPKKKRKVGVPAAMAERPFQCRICMRNFS**ERGTLAR**HIRTHTGEKPFACDICGRK FA**TSANLSR**HTKIHTHPRAPIPKPFQCRICMRNFS**TSGNLTR**HIRTHTGEKPFACD ICGRKFA**HRTSLTD**HTKIHTGSQKPFQCRICMRNFS**RSHSLLR**HIRTHTGEKPFAC DICGRKFA**HPSARKR**HTKIHLRQKDAARGSGGDAKSLTAWSRTLVTFKDVFVDFTR EEWKLLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQ ETHPDSETAFEIKSSVRS
ZFP-90	MAPKKKRKVGVPAAMAERPFQCRICMRNFS**LRHHLTR**HIRTHTGEKPFACDICGRK FA**RRFTLSK**HTKIHTGSQKPFQCRICMRNFS**RSDVLSE**HIRTHTGEKPFACDICGR KFA**KHSTRRV**HTKIHTGSQKPFQCRICMRNFS**RSDVLSE**HIRTHTGEKPFACDICG RKFA**RLYTLHK**HTKIHLRQKDAARGSGGDAKSLTAWSRTLVTFKDVFVDFTREEWK LLDTAQQIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHP DSETAFEIKSSVRS
ZFP-72	MAPKKKRKVGVPAAMAERPFQCRICMRNFS**DRSNLSR**HIRTHTGEKPFACDICGRK FA**LRQNLIM**HTKIHTGSQKPFQCRICMRNFS**TSANLTV**HIRTHTGEKPFACDICGR KFA**RSDHLSR**HTKIHTGSQKPFQCRICMRNFS**QSGNLAR**HIRTHTGEKPFACDICG RKFA**QRNDRKS**HTKIHLRGSGGDAKSLTAWSRTLVTFKDVFVDFTREEWKLLDTAQ QIVYRNVMLENYKNLVSLGYQLTKPDVILRLEKGEEPWLVEREIHQETHPDSETAF EIKSSVRS

Supplemental Table 6. qPCR probes.

Commercially available qPCR probes					
Gene	**Exons**	**IDT Assay ID**	**Label**	**Multiplex with**	**Master mix**
Mapt (tau)	1 - 2	Mm.PT.58.42179817	FAM	ATP5B / EIF4A2	QuantiFast
Atp5b	7 - 8	Mm.PT.53a.17279462	Cy5	MAPT / EIF4A2	QuantiFast
Eif4a2	7 - 8	Mm.PT.53a.9498195.g	HEX	MAPT / ATP5B	QuantiFast
*Gapdh	3 - 4	Custom (Primer:Probe 2:1)	FAM		SsoFast
*ZFP ("MCS")	1 - 1	Custom(Primer:Probe 2:1)	FAM		SsoFast
Aif1 (Iba1)	5 - 7	Mm.PT.58.7014816	HEX	GFAP	QuantiFast
Gfap	4 - 5	Mm.PT.58.10570926	FAM	AIF1	QuantiFast
Olig2	1 - 2	Mm.PT.58.42319010	FAM		SsoFast
Map1a	7 - 8	Mm.PT.58.43650614.g	FAM		SsoFast
Map1b	2 - 4	Hs.PT.58.15363622	HEX	MAP2	SsoFast
Map2	6 - 7	Hs.PT.58.40791337	FAM	MAP1B	SsoFast
Rbfox3 (Neun)	8 - 10	Mm.PT.58.11738514	FAM	LGI1	SsoFast
Cst7	1 - 2	Mm.PT.58.9316716	FAM	Clec7a	SsoFast
Clec7a	5 - 6	Mm.PT.58.31649377	HEX	Cst7	SsoFast
Trem2	1 - 2	Mm.PT.58.7992121	FAM	App	SsoFast
Gbp2	10 - 11	Mm.PT.58.13279677	HEX	Ggta1	SsoFast
Ggta1	4 - 6	Mm.PT.58.10423746	FAM	Gbp2	SsoFast
Cd14	1 - 2	Mm.PT.58.8871572.g	HEX	Emp1	SsoFast
Emp1	1 - 2	Mm.PT.58.41401052	FAM	Cd14	SsoFast
App	2 - 3	Mm.PT.58.32315349	FAM	Trem2	SsoFast
Psen1	3 - 4	Mm.PT.58.32806614	HEX	Bace1	SsoFast
Bace1	1 - 3	Mm.PT.58.17407402	FAM	Psen1	SsoFast
Mme	12 - 13	Mm.PT.58.9990556	HEX	Ece1	SsoFast
Ece1	2 - 4	Mm.PT.58.28861053	FAM	Mme	SsoFast
Mmp2	11 - 12	Mm.PT.58.33561647	HEX	Mmp9	SsoFast
Mmp9	8 - 9	Mm.PT.58.10100097	FAM	Mmp2	SsoFast

***Custom qPCR Probes**		
GAPDH	Probe	/56FAM/CCC ATC ACC /ZEN/ATC TTC CAG GAG CGA GA/3IABkFQ/
	Primer 1	AGA TGA TGA CCC TTT TGG CTC
	Primer 2	CCC TTC ATT GAC CTC AAC TAC AT
ZFP ("MCS")	Probe	/56-FAM/AGC ACG TTG /ZEN/CCC AGG AGG TCA C/3IABkFQ/
	Primer 1	GGA ACG GTG CAT TGG AAC G
	Primer 2	GTT CGA ATC CCA ATT CTT TGC C

Supplemental Table 7. RNAscope materials.

Product name	Article #	Manufacturer
RNAscope Hydrogen Peroxide solution	ACD #322335	RNAscope
RNAscope Target retrieval solution	ACD #322000	RNAscope
RNAscope Protease III	ACD #322337	RNAscope
HybEZ II hybridization system	ACD #PN 321710	RNAscope
RNAscope probe-MmMAPT-no-X-Hs	ACD #417481	RNAscope
RNAscope Multiplex FL v2 AMP1	ACD #323101	RNAscope
RNAscope Multiplex FL v2 AMP2	ACD #323102	RNAscope
RNAscope Multiplex FL v2 AMP3	ACD #323103	RNAscope
RNAscope Multiplex FL v2 HRP-C1	ACD #323104	RNAscope
TSA Plus Cy5	#NEL744E001KT	Akoya Biosciences
RNAscope Multiplex FL v2 HRP blocker	ACD #323107	RNAscope