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Supplemental Information

Caspase Activation and Caspase-Mediated Cleavage of APP Is Associated with Amyloid β-Protein-Induced Synapse Loss in Alzheimer's Disease

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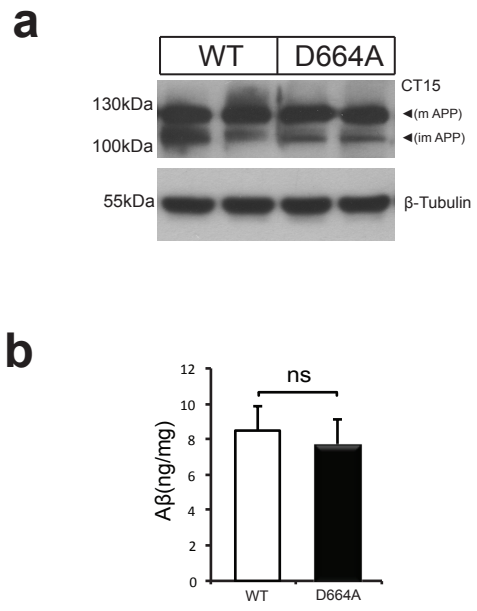
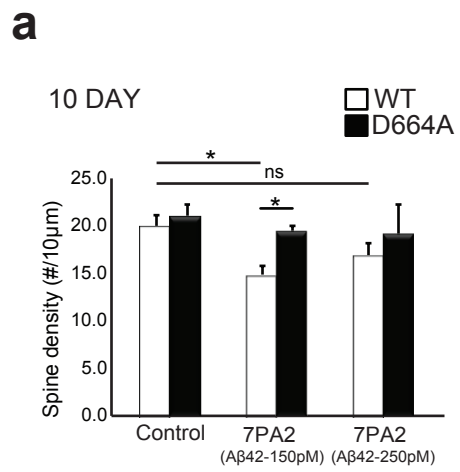
Figure S1



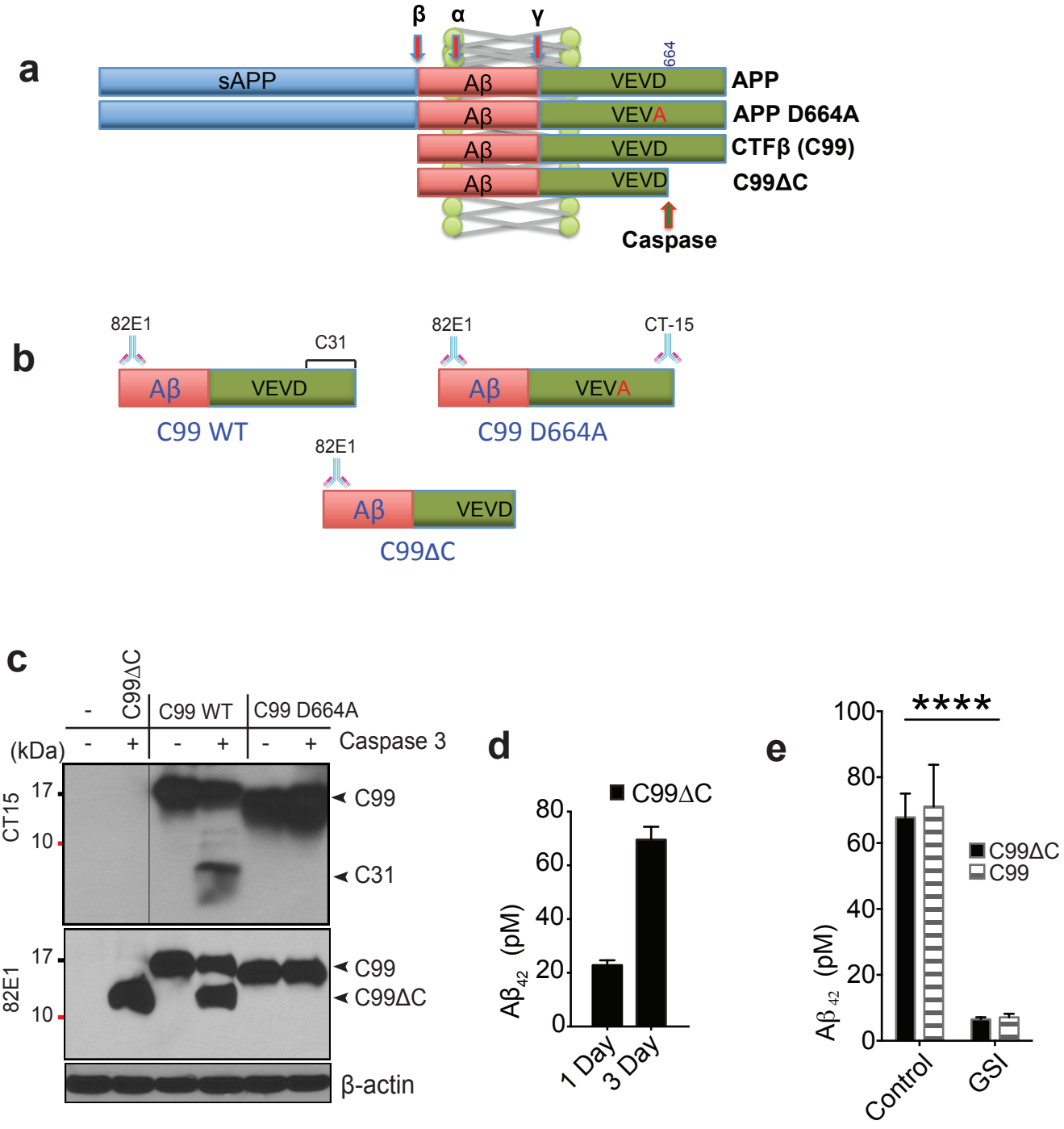
1. APP D664A KI mice show normal APP processing and synapse density. Western blotting of whole brain lysates showed comparable levels of (a) immature and mature full length APP species (CT-15). β-Tubulin was used as a loading control. (b) Levels of endogenous Aβ42 from WT and APP D664A KI mice measured by ELISA were comparable. (n=7 APP D664A KI mice and n=4 wild type control mice. NS: not-significant by Two-sided Student's t-test). Related to Figure 1.

Figure S2



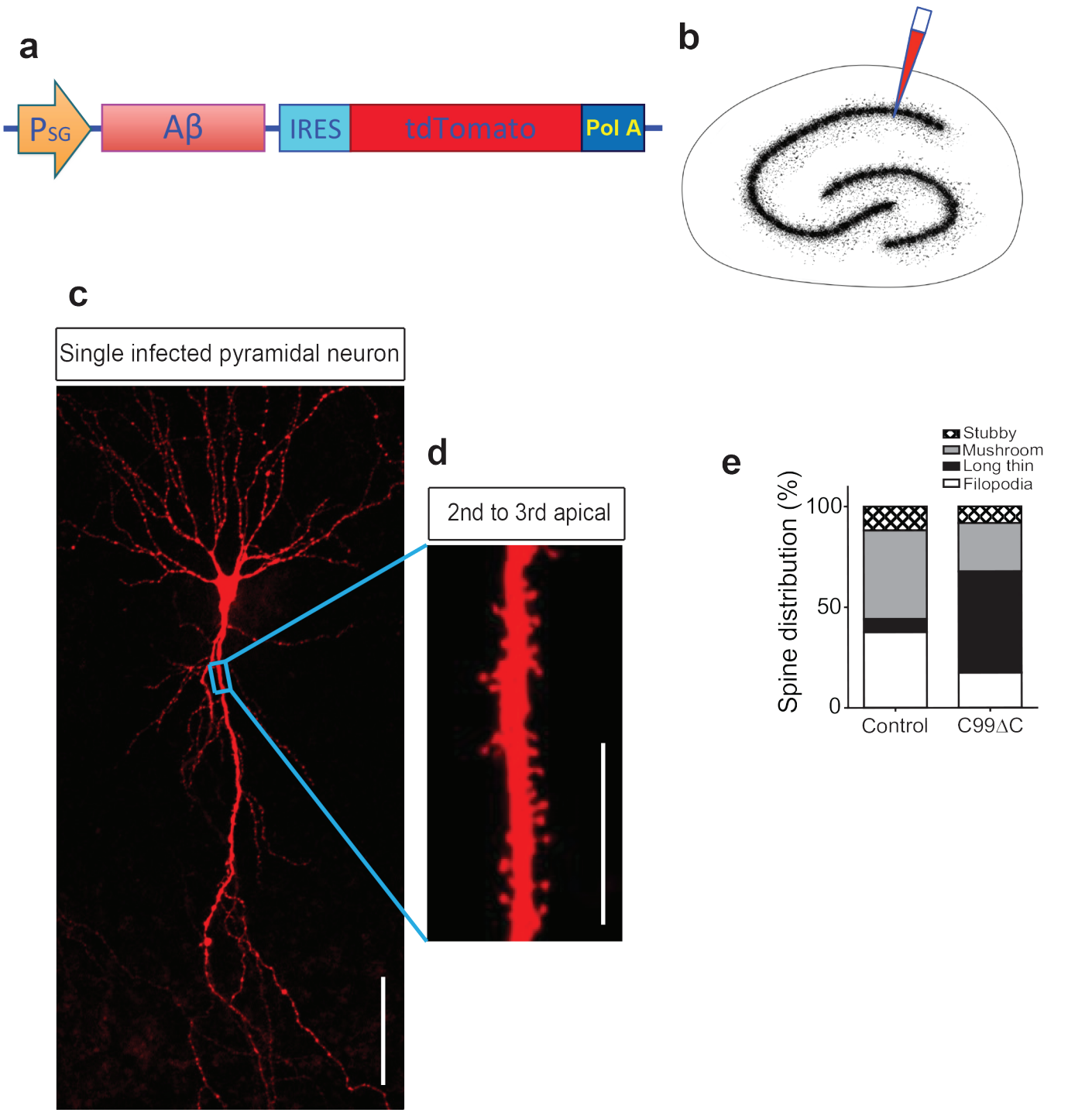
2. The APP D664A point mutation rescues Aβ-mediated synaptic loss in 10 days incubation of OTSC. (a) Quantification of dendritic spine density of hippocampal OTSCs from WT and APP D664A KI mice The OTSCs infected with Sindbis virus expressing GFP were incubated in 7PA2 media containing 150 and 250 pM $A\beta_{42}$ for 10 days in vitro. (n=10 neurons (CHO control), n=10 neurons (150 $A\beta_{42}$), n=10 neurons (250 $A\beta_{42}$) from 2 APP D664AKI mice and n=10 neurons (CHO control), n=10 neurons (150 $A\beta_{42}$), n=10 neurons (250 $A\beta_{42}$) from 2 wild type littermate. NS: not-significant, *P≤0.05 by Two-way ANOVA followed by Tukey's multiple comparisons test). Related to Figure 2.

Figure S3



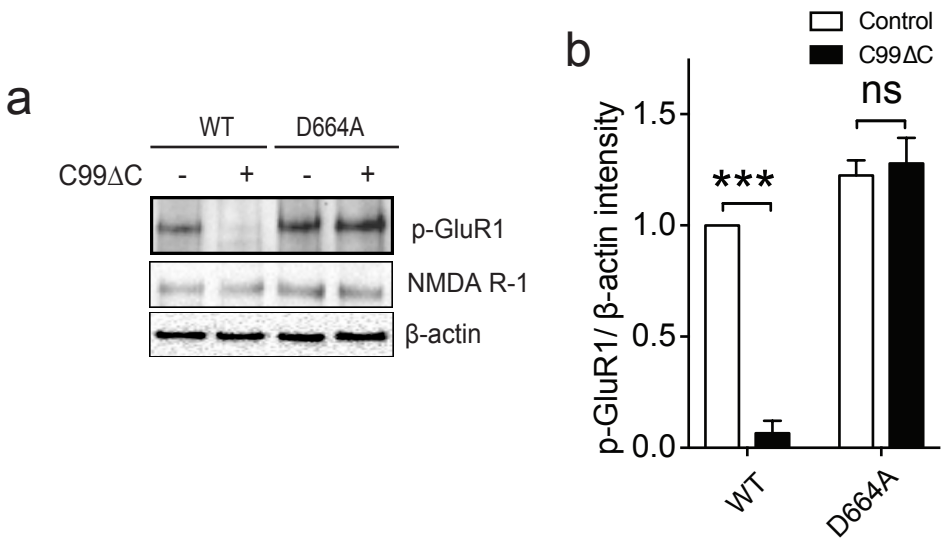
3. C99ΔC expresses Aβ, which is modulated by GSI. (a) Illustration of APP processing. Caspase cleaves C99 to C99ΔC and C31 fragment. (b) Vector construction of C99 WT, C99 D664A, C99ΔC for Sindbis virus preparation and in vitro caspase reaction and antibody binding epitopes. (c) Immunoblotting analysis of transient transfection of C99ΔC, C99 WT, and C99 D664A in 293T cells. Cell lysates were pre-incubated with or without purified caspase 3 in in vitro reactions. 82E1 antibody recognizes the N-terminus of Aβ and C99 fragments: C99ΔC, C99 WT and C99 D664A while CT15 recognizes the last 15 amino acids of the APP C-terminus present in C99 WT and C99 D664A but not in C99ΔC. Note that the mobility of the APP D664A CTF migrated slightly faster than wild type APP CTF due to the amino acid substitution. (d) Culture media of OTSCs densely infected C99ΔC Sindbis virus for 1day and 3 days were collected and analyzed for $A\beta_{42}$ levels by ELISA showed accumulation of Aβ in media over this time period (media collected from OTSCs of n=9 mice for each condition, triplicate experiments). (e) 10μM GSI treatment for 3 day in OTSCs effectively inhibited Aβ production from both C99 and C99ΔC constructs. (n=35 OTSC slices from 5 APP D664AKI mice and n=42 OTSC slices from 6 wild type littermate control mice. ****P≤0.0001 by Two-tailed Student's t-test). Related to Figure 3.

Figure S4



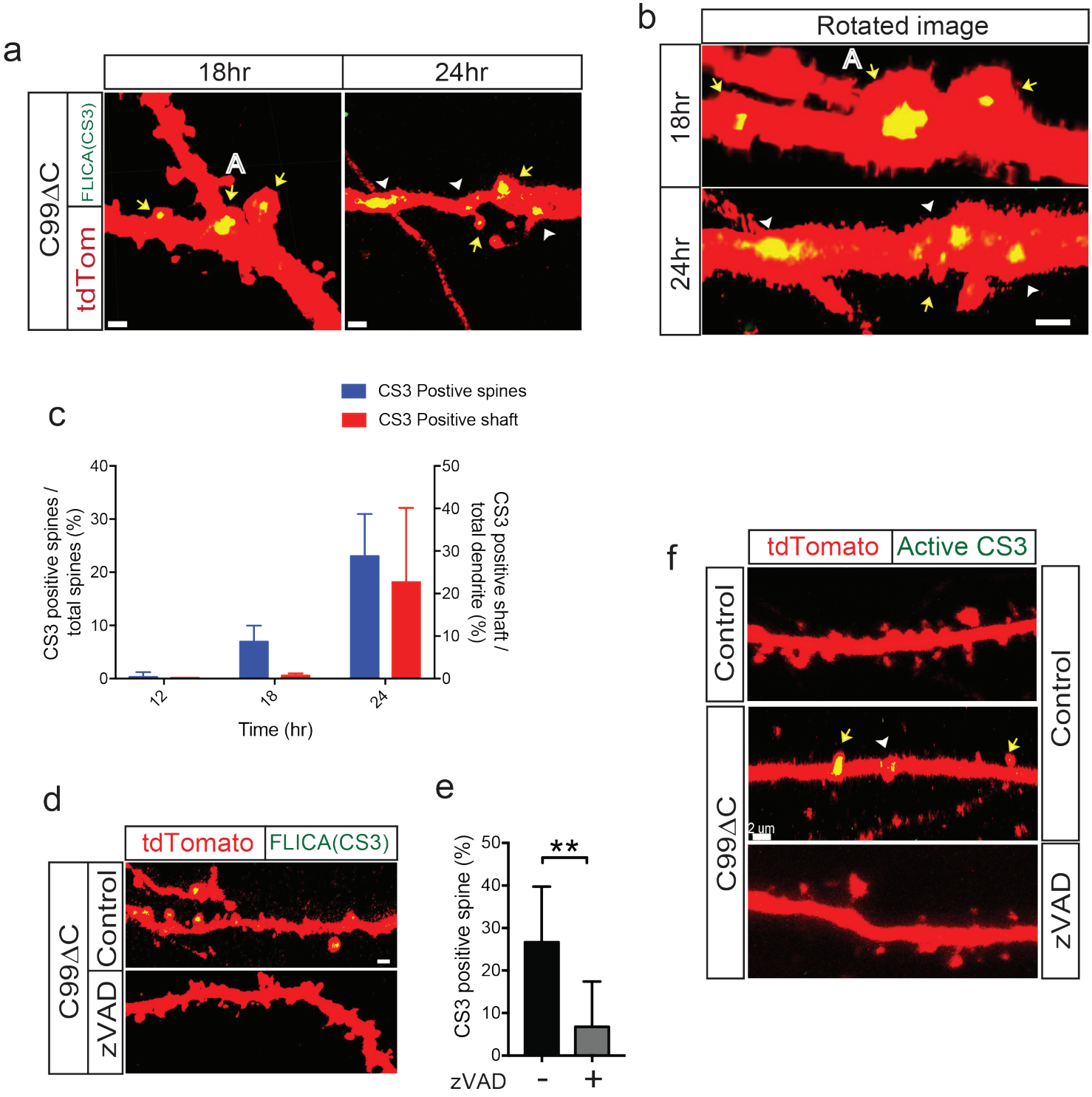
4. The morphology of hippocampal pyramidal neuron infected C99ΔC expressing Sindbis virus coexpressing tdTomato fluorescent protein. (a) Construction of C99ΔC coexpressing tdTomato protein in Sindbis virus. (b) Illustration of location of virus injection into the CA1 hippocampal region in OTSCs. (c) Representative confocal microscopy image of pyramidal neuron infected virus in CA1 region of hippocampus. Scale bar = 100μm. (d) Magnified apical dendrite between 2nd and 3rd branches that was selected for spine density measurements. Scale bar = 20μm. (e) Distribution of spine types in dendritic segments 24 hours after infection with Sindbis virus expressing C99ΔC or control tdTomato virus. Results were tabulated from a total of 430 spines imaged from 4 neurons infected with control tdTomato virus and 215 spines from 6 neurons infected with C99ΔC virus from a total of 5 wild type mice. Related to Figure 3.

Figure S5



5. D664A KI protects C99ΔC-infection mediated reduction of GluR1 phosphorylation in OTSCs of wild type mice. (a) Western blot and (b) quantification of p-GluR1 (Ser845) from densely infected OTSCs from APP WT and D664A KI mice. C99ΔC decreased p-GluR1 levels but unchanged in APP D664A mice. NMDAR-1 levels were not altered. β-actin antibody blotted for loading control. (n=21 slices from 3 APP D664AKI mice and n=21 slices from 3 wild type littermate. NS: not-significant, ***P≤0.001 by Two-way ANOVA followed by Tukey's multiple comparisons test). Related to Figure 6.

Figure S6



6. C99ΔC-induced local caspase activation in dendritic spines and dendritic shafts. (a) Representative confocal images of OTSCs infected with C99ΔC Sindbis virus for 18hr and 24hr after staining with FLICA caspase 3 reporter. Yellow arrows indicate sites of local caspase activation in dendritic spines. White arrowheads show the positive signal in dendritic shaft. Scale bar = 1μm. (b) Rotated Imaris-reconstructed 3D image of dendrite from figure 6a. Yellow arrow indicates caspase activation localized inside of dendritic spine. White arrowhead indicates caspase activation localized inside of dendritic shaft. ("A"), caspase signal inside of dendritic spine, is rotated spine of Fig. 6a (18hr). Scale bar = 2μm. (c) Quantification of FLICA caspase reporter positive dendritic spines and shafts to total dendritic spine and shaft showed that the caspase signal was more apparent in dendritic spines at 18 hours as compared to dendritic shafts. n=6 neurons [12hr C99ΔC], n=11 neurons [18hr C99ΔC], n=8 neurons [24hr C99ΔC] from a total of 12 mice. (d) Representative image and (e) quantification of FLICA caspase 3 activity after zVAD treatment to inhibit caspase activation in WT OTSC. (n=5 neurons [C99ΔC control] and n=4 neurons [C99ΔC + zVAD] from 4 wild type littermate controls. **P≤0.01 by Two-tailed Student's t-test). The value represents the mean and the upper error bars represent the SEM. Scale bar = 2μm. (f) Immunohistochemistry of C99ΔC-induced caspase 3 activation in WT OTSC activated caspase 3 specific antibody. Yellow arrows indicate positive signal in spines while white arrowhead shows evidence of patchy activation in dendritic shaft. Treatment with zVAD (10μM) inhibited the C99ΔC-induced local caspase activation in dendrites. Related to Figure 7.