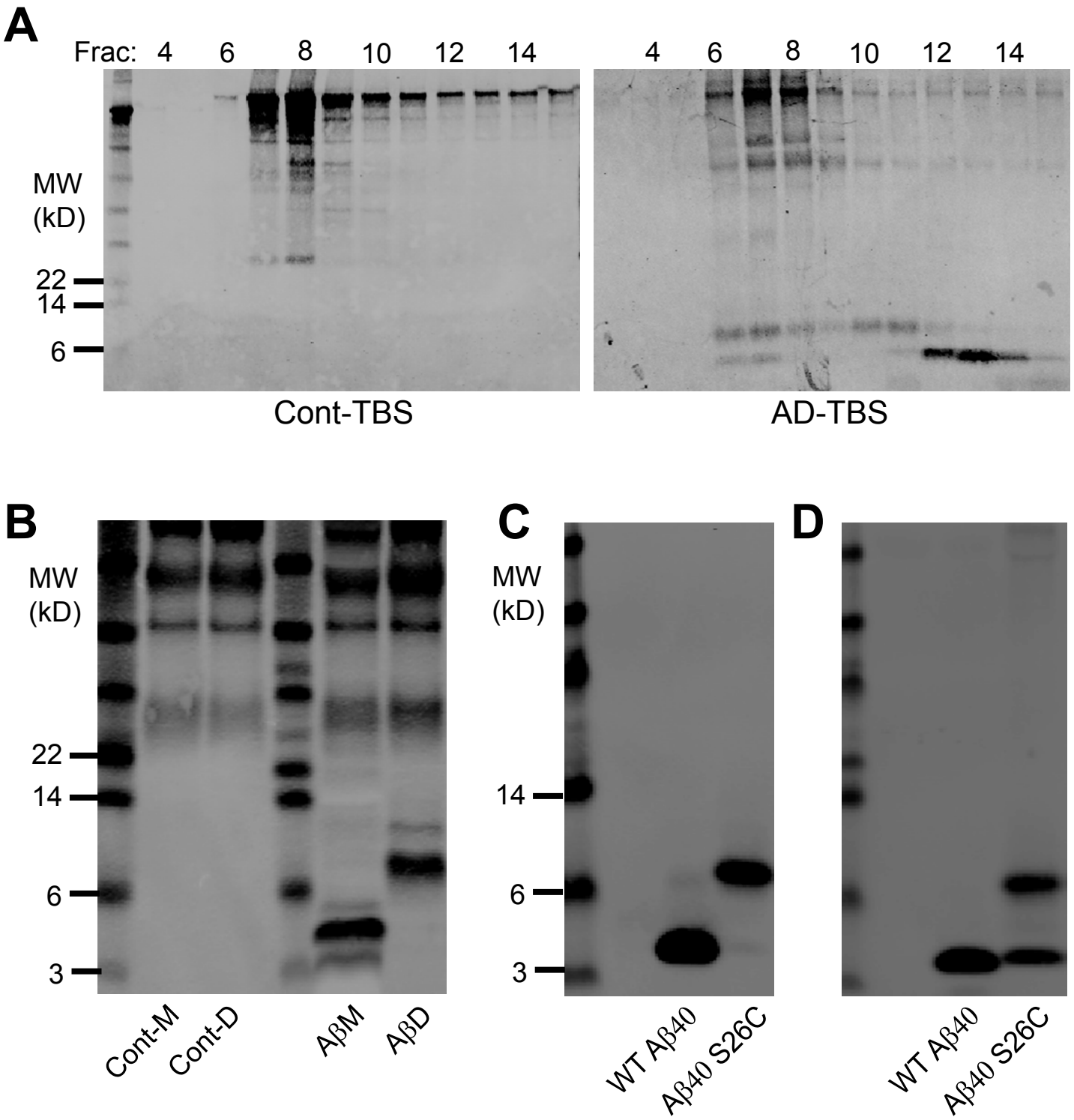

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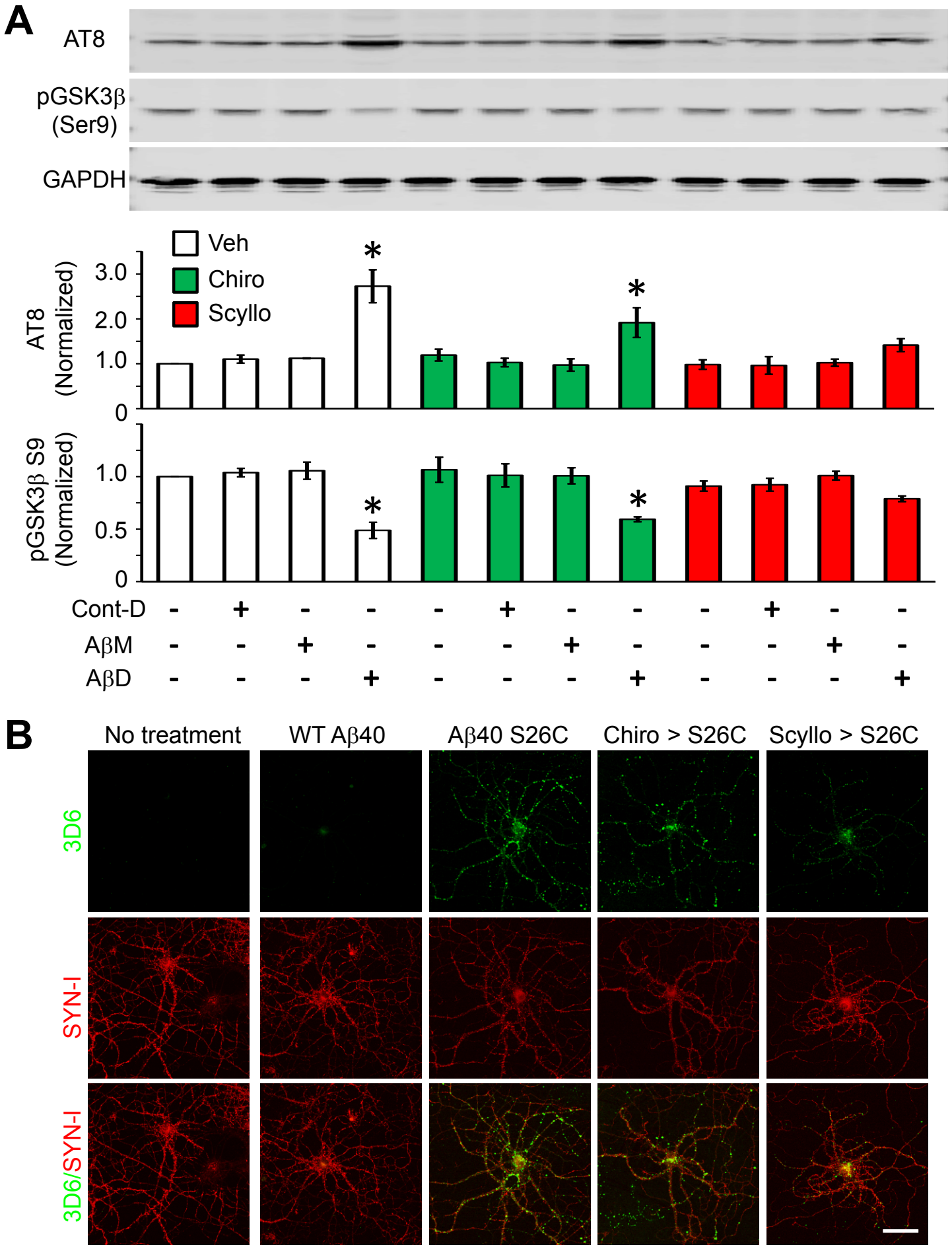

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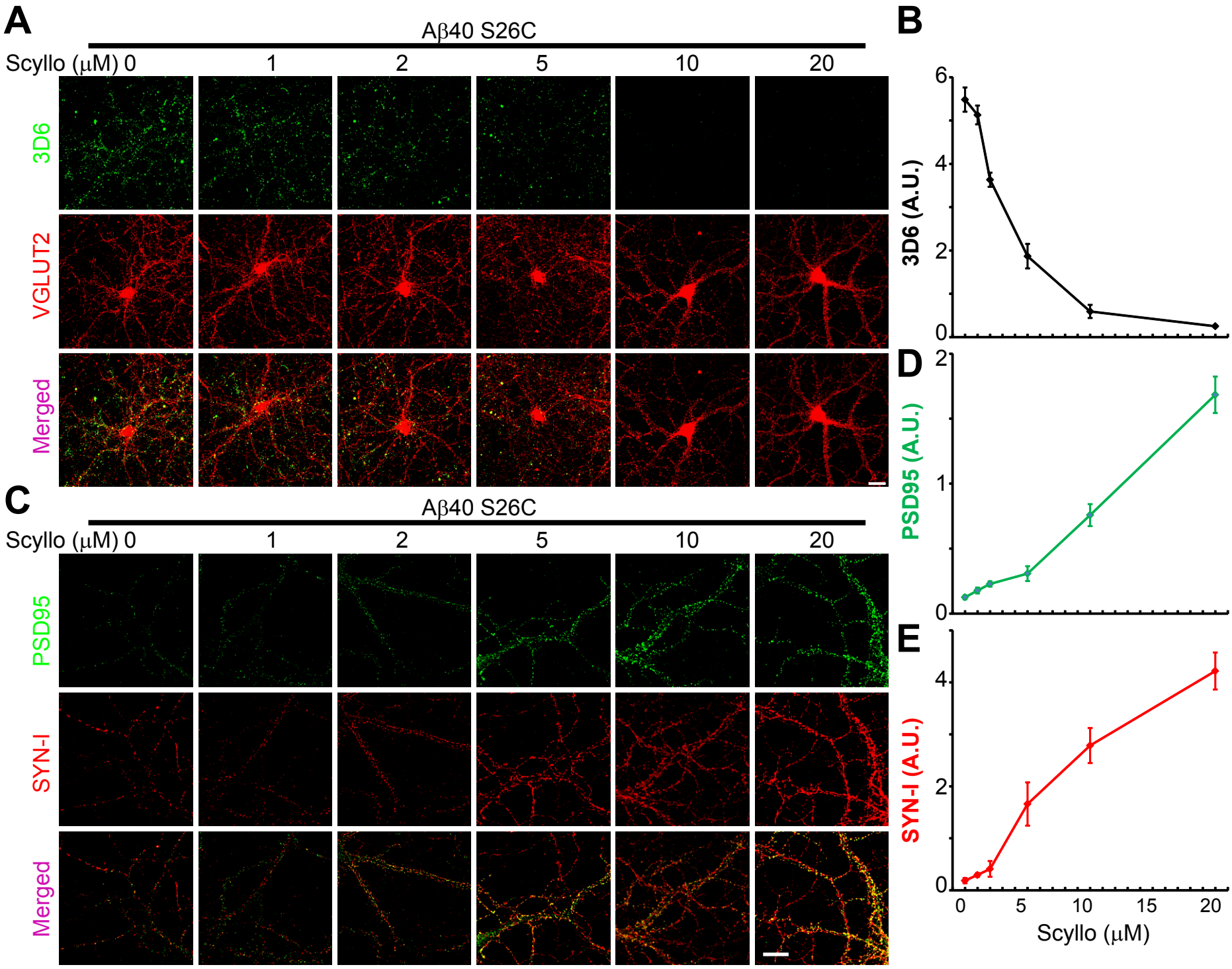

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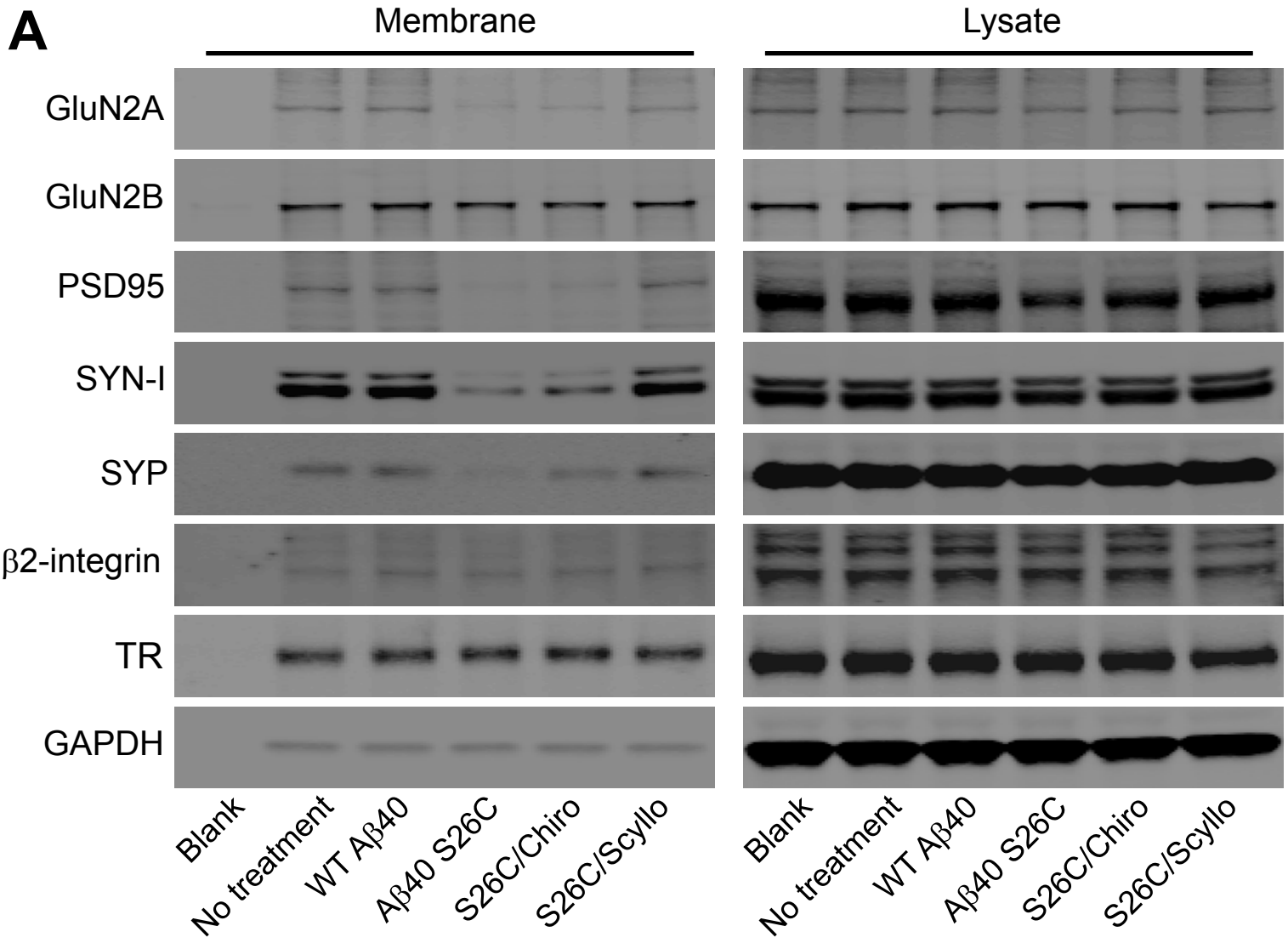

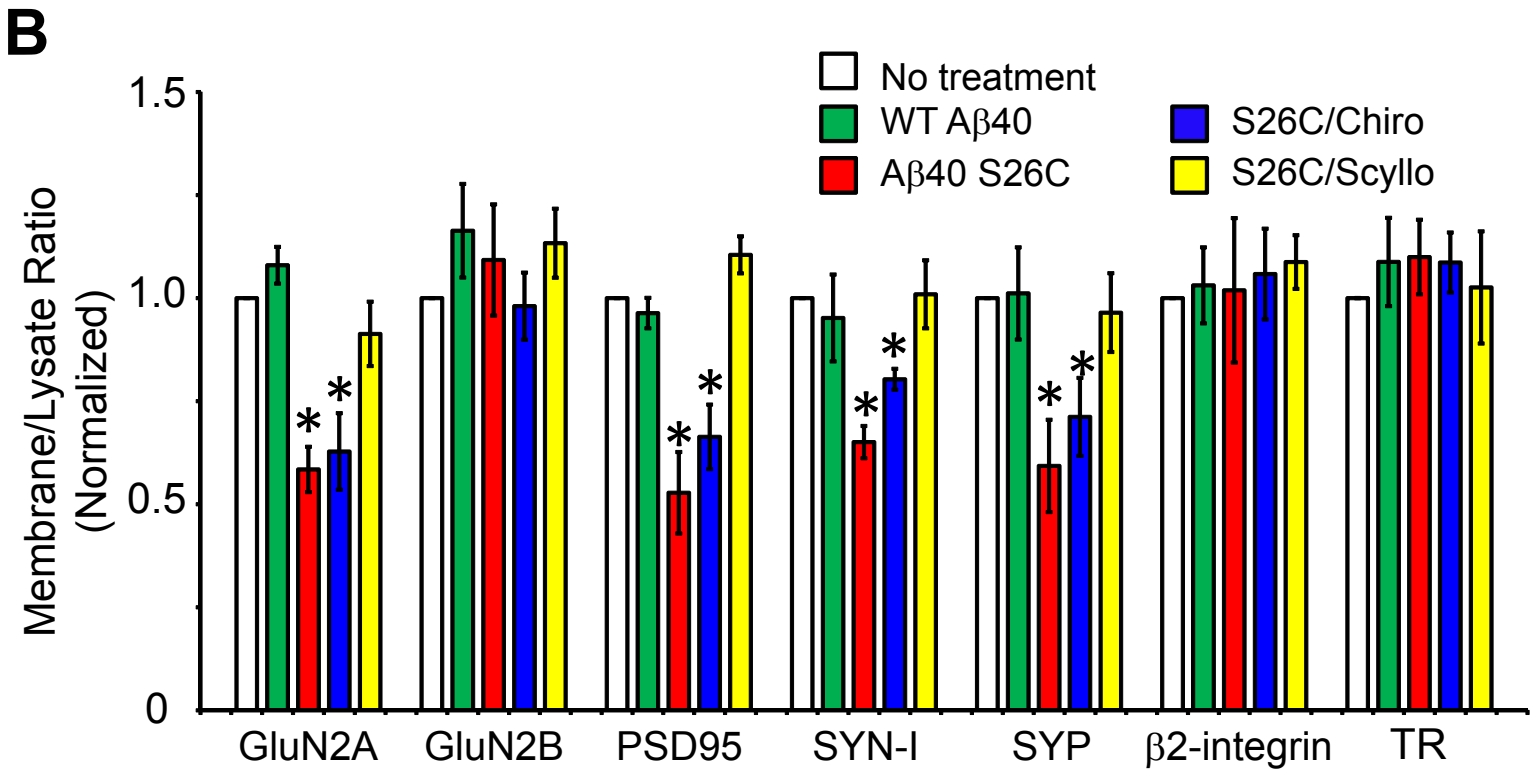

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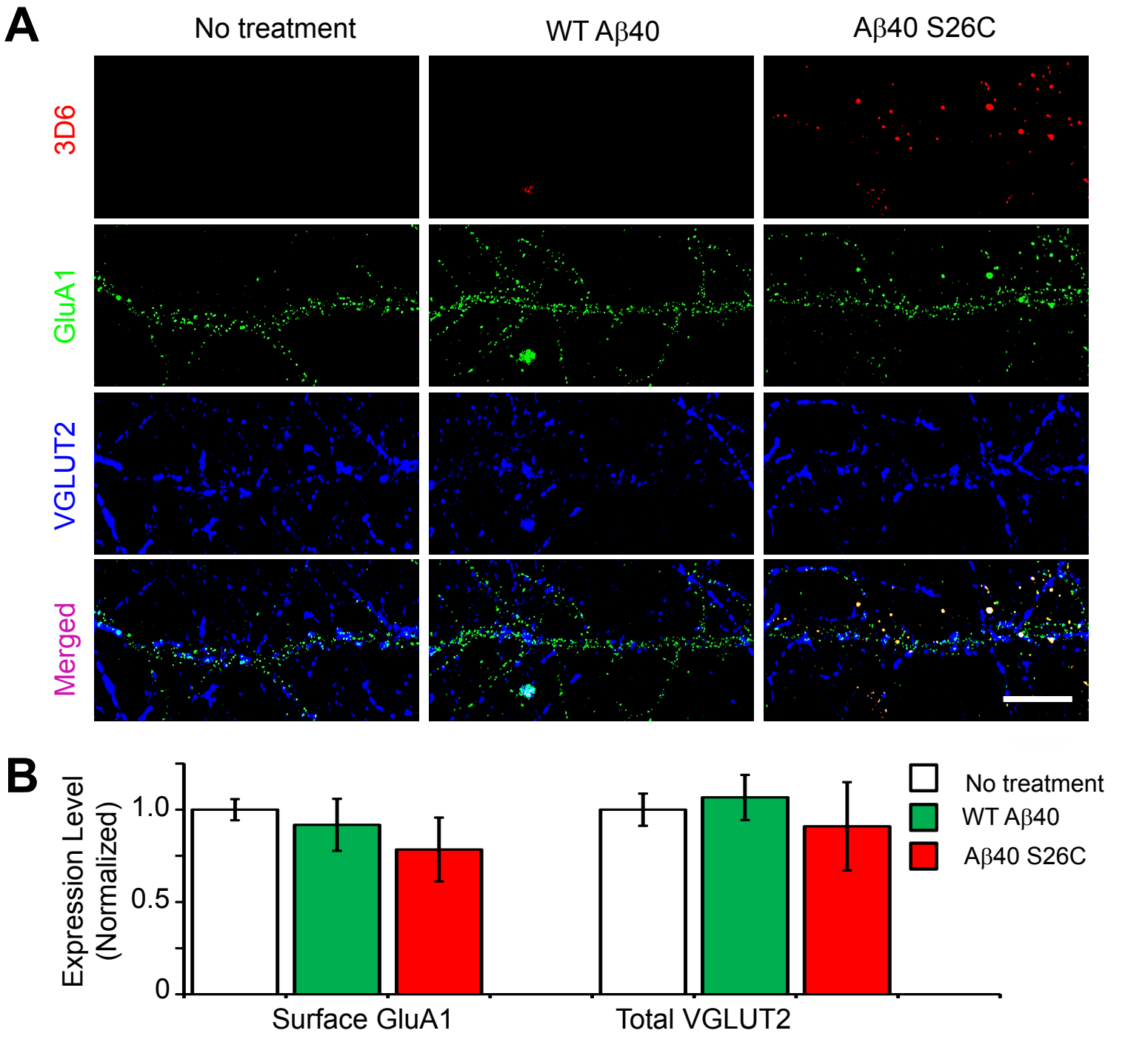

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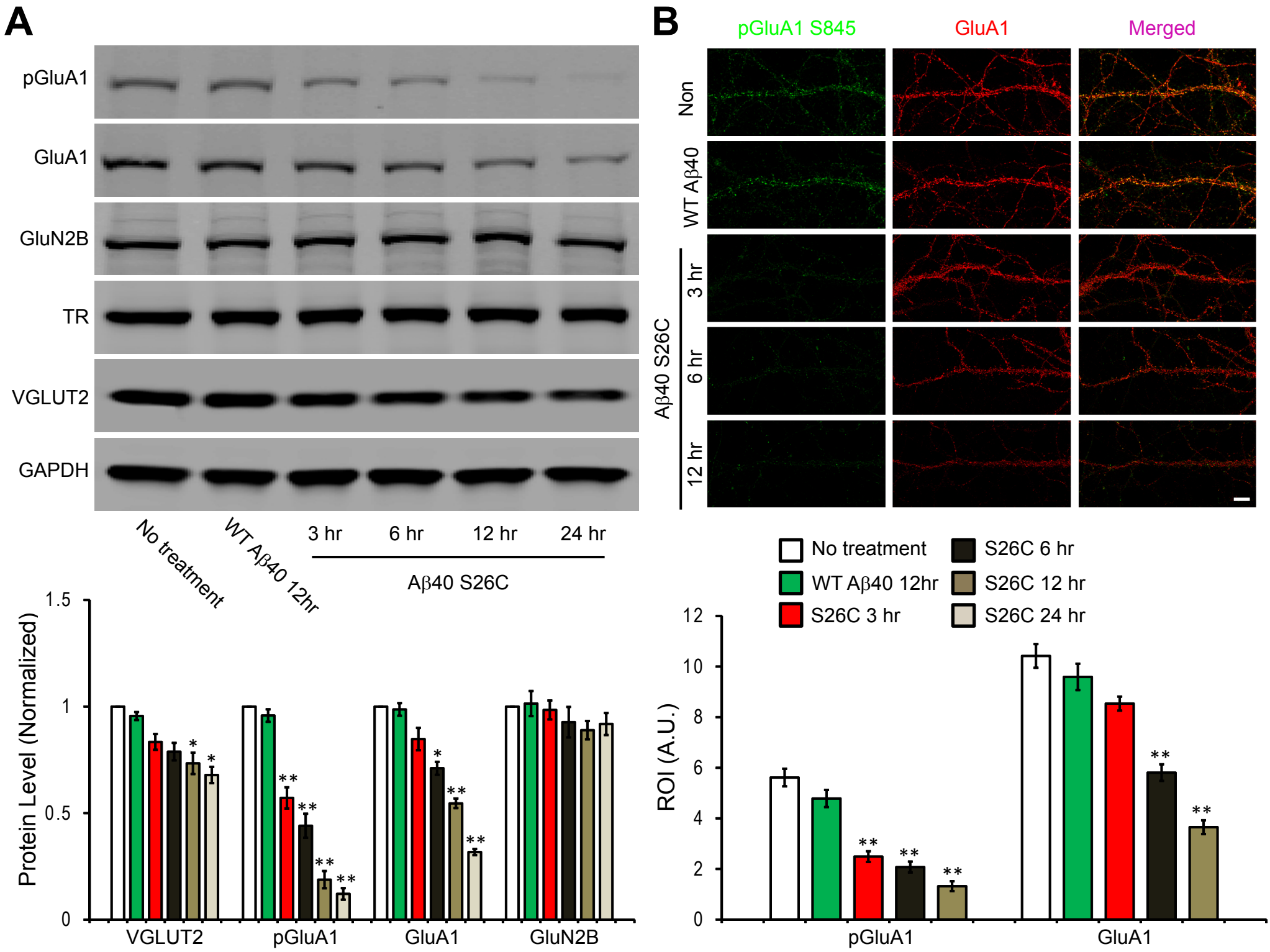

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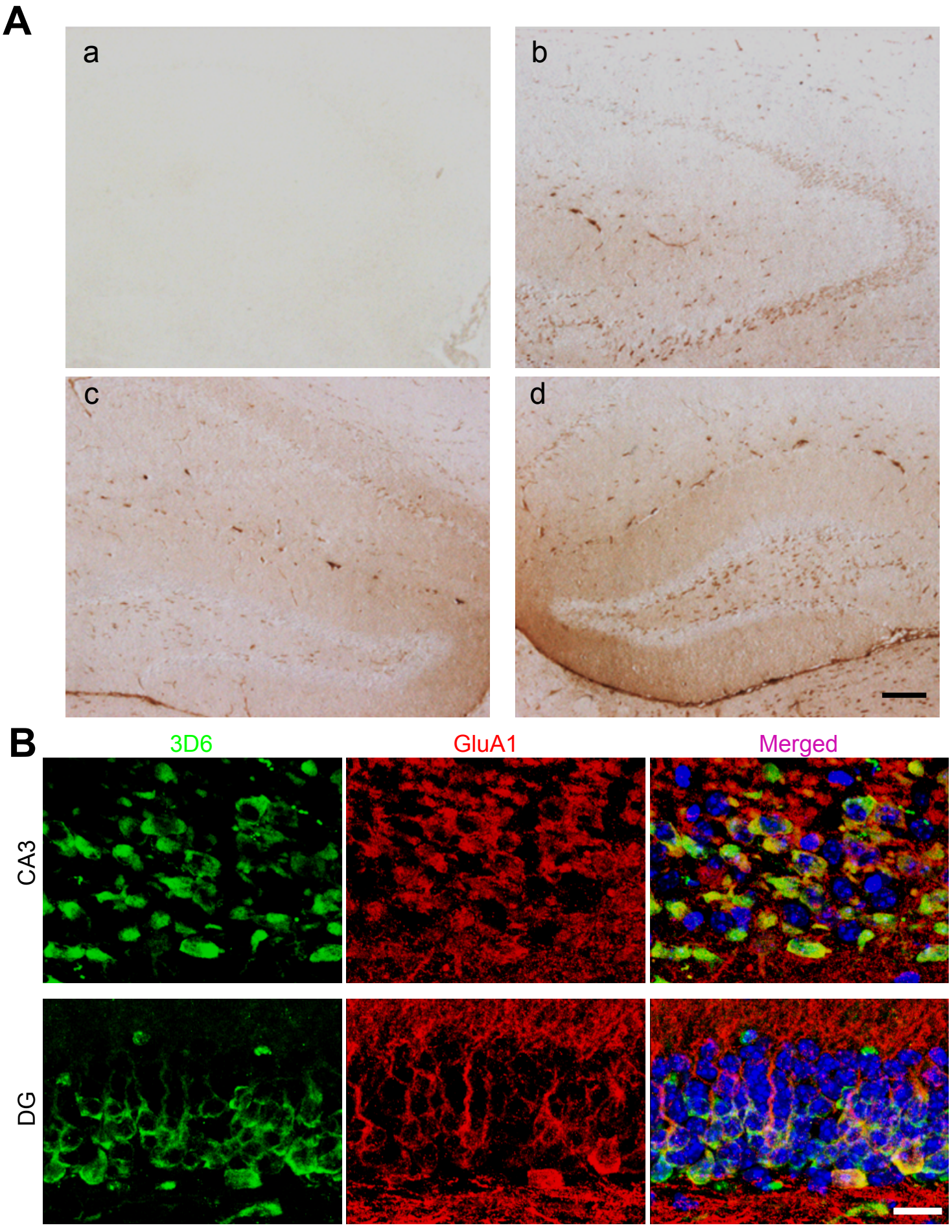

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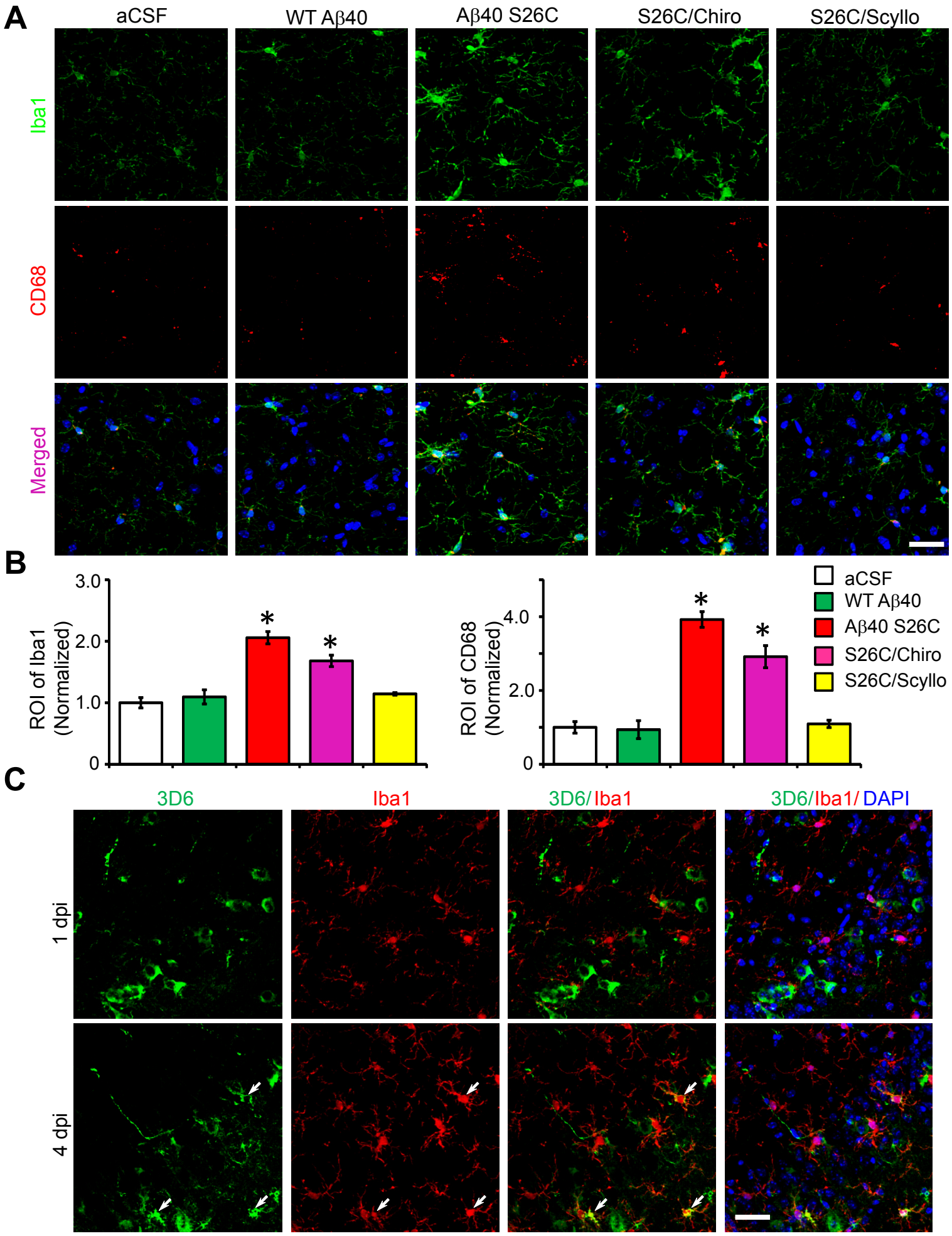

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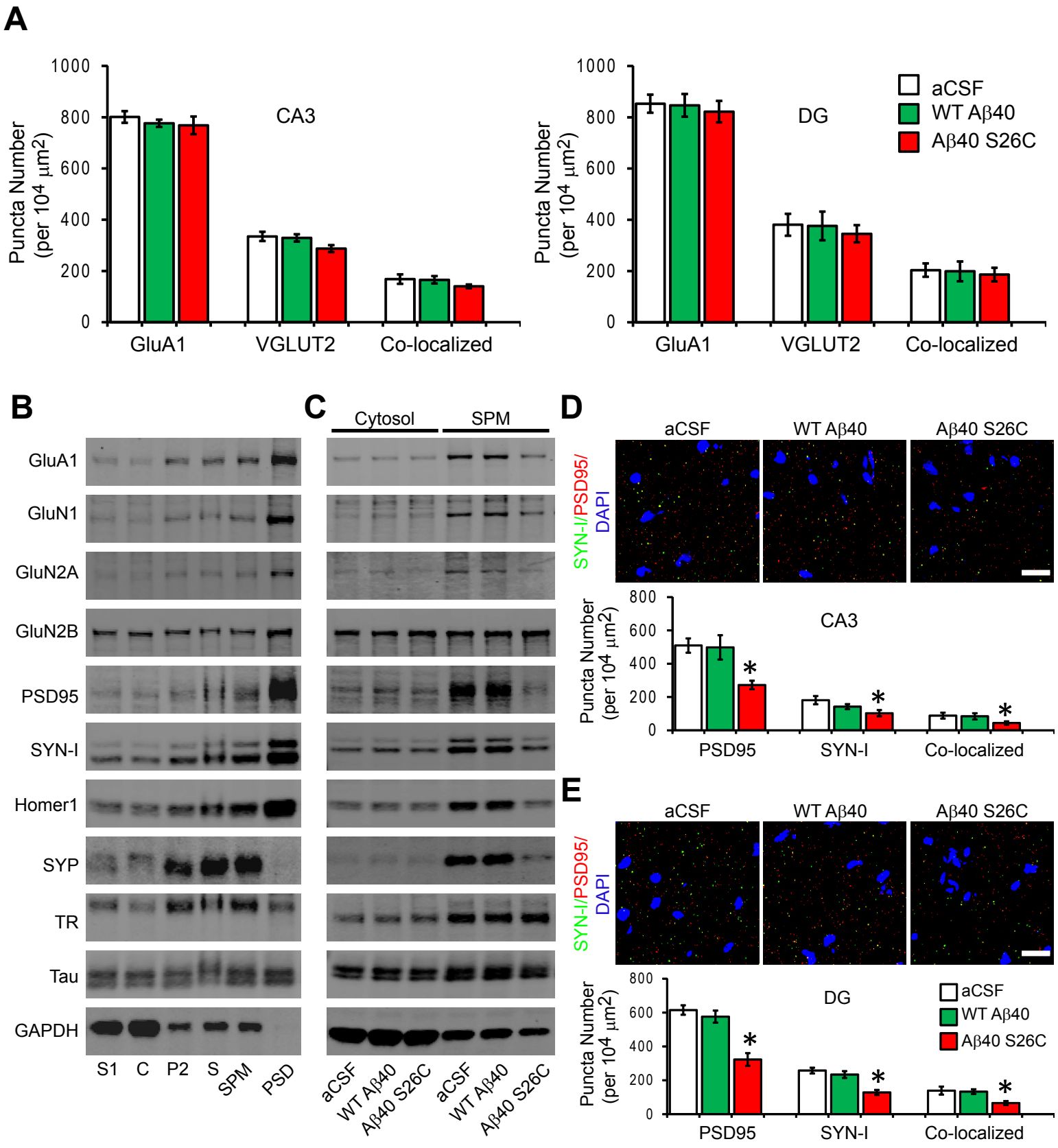

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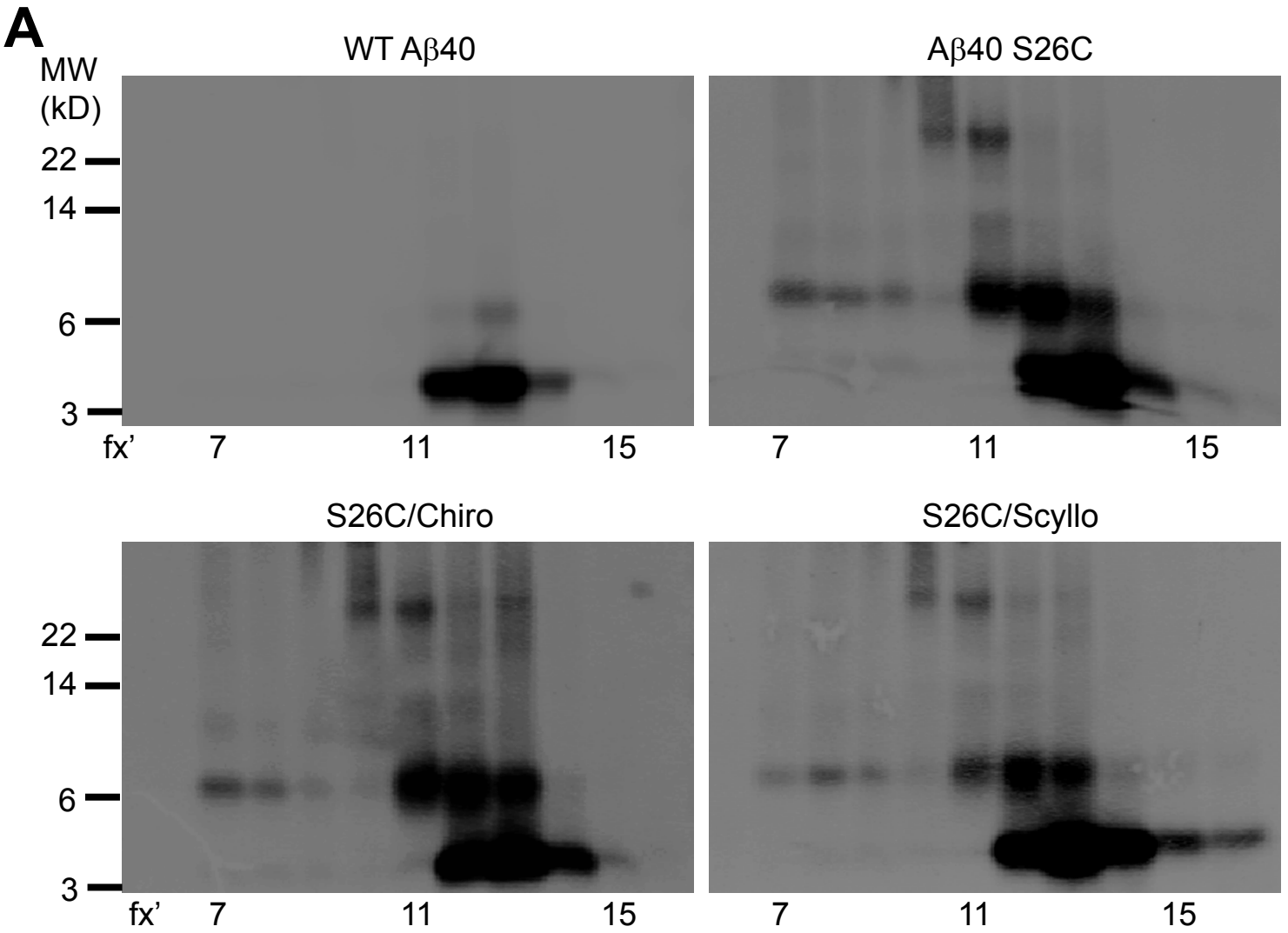

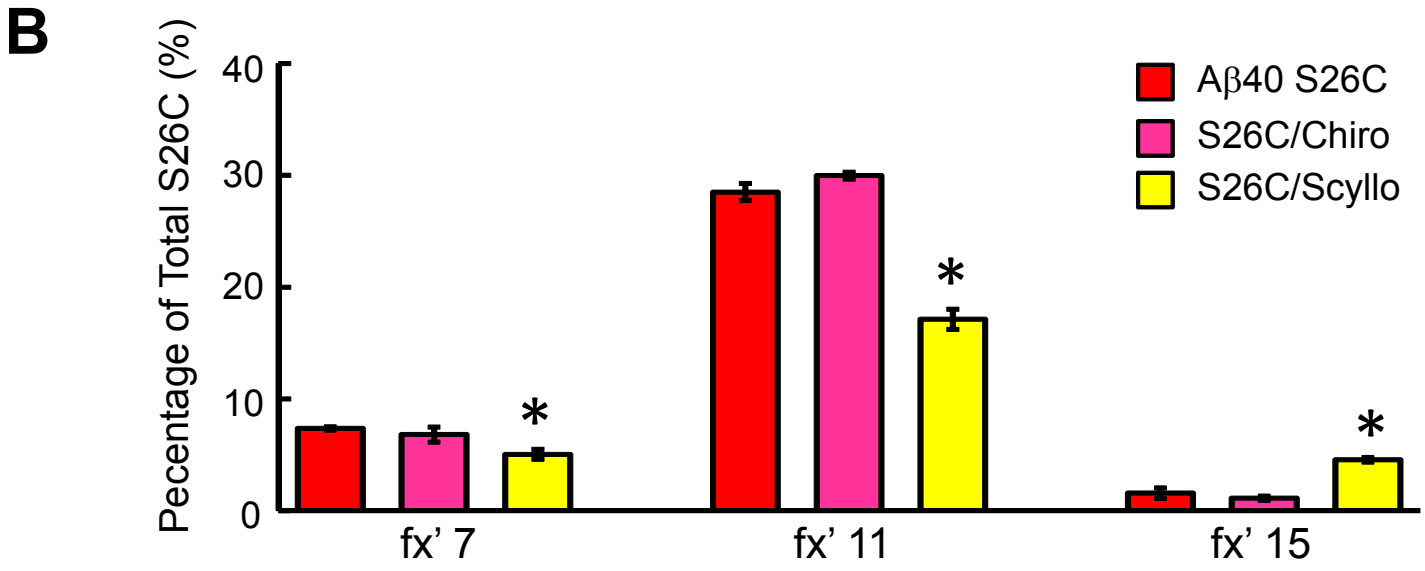

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Supplementary Figure Legends

Figure S1. Characterization of soluble Aβ species isolated by size-exclusion chromatography (SEC) from AD cerebral cortex or synthetic Aβ40 peptides. (**A**) Cont-TBS (left) and AD-TBS (right) were IP'ed with 3D6 (3 μg/ml), eluted with 4% LDS sample buffer, and subjected to SEC. IP-SEC of AD-TBS resolved Aβ dimers (frx 10-11) from monomers (frx 13-14) (WB: mAbs 6E10+2G3+21F12). (**B**) After 3d incubation on primary neurons, conditioned media (CM) were IP'ed with Aβ antiserum AW7, yielding monomers and dimers in the IP-SEC fractions of AD- but not Cont-TBS (WB: 6E10). (**C**, **D**) Blots (6E10) of CM containing synthetic WT Aβ40 or $(Aβ40\ S26C)_2$ dimer (5 ng for each) before (**C**) and after (**D**) 1 day treatment of hippocampal neurons (DIV18).

Figure S2. Pre-treatment with scyllo-inositol of hippocampal neurons inhibits soluble Aβ oligomer-induced tau phosphorylation and binding of oligomer to the cell surface. (**A**) Representative blots showing phosphorylation of endogenous rat Tau (rTau) at AT8 epitope (Ser202/205) and GSK3β at Ser9 epitope in cultures of hippocampal neurons (DIV19). Neurons were treated with vehicle, chiro- or scyllo-inositol for 1 day, followed by replacing media with those containing human Cont-D, AβM or AβD SEC fractions. GAPDH, loading control. Histograms: mean levels +/- s.e.m. (n = 4 independent experiments) of phosphorylation of rat tau at AT8 epitope and GSK3β at Ser9, normalized to the respective levels in untreated sister cultures. * significantly different from untreated neurons ($p < 0.05$ by one-way ANOVA test). (**B**) Confocal images of membrane-bound Aβ detected by 3D6 (green) and endogenous synapsin I (SYN-I, red) on primary hippocampal neurons (DIV21) after 1 d pre-treatment with chiro- or

scyllo-inositol (20 μM) followed by medium exchange and exposure to synthetic wt Aβ40 or $(Aβ40\ S26C)_2$ oligomers for 3 h. Scale bar, 50 μm.

Figure S3. Dose response of scyllo-inositol in inhibiting Aβ oligomer binding to the neuronal surface and preventing oligomer-induced synaptic loss. (**A**) Confocal images of membrane-bound Aβ oligomers detected by mAb 3D6 (green) and endogenous VGLUT2 (red) in hippocampal neurons (DIV21) after 3 hr treatment with $(Aβ40\ S26C)_2$ oligomers (250 nM) and increasing final concentrations of scyllo-inositol in the CM. Scale bars, 20 μm. (**B**) Intensity of 3D6 surface staining (A.U.) as a function of increasing scyllo-inositol in the CM. (**C**) Confocal icc of endogenous PSD95 (green) and synapsin I (SYN-I, red) in hippocampal neurons (DIV18) after 1 d treatment with $(Aβ40\ S26C)_2$ oligomers (250 nM) plus increasing concentrations of scyllo-inositol. Scale bars, 20 μm. (**D, E**) Intensity of PSD95 and SYN-I on surface membranes (A.U.) as a function of increasing scyllo-inositol in the CM. Fifteen cells per condition were analyzed. Error bars, s.e.m.

Figure S4. Scyllo-inositol inhibits Aβ oligomer-induced internalization of synaptic proteins in hippocampal neurons. (**A**) Representative blots showing the biotinylated synaptic membrane proteins in hippocampal neurons (DIV18) under different conditions of treatment. Western blotting of the same proteins in cell lysates served as a control. (**B**) Bars: mean level of membrane protein/lysate ratio normalized to values in parallel cultures without treatment. *significantly different from neurons without treatment ($p < 0.01$ by one-way ANOVA test). Four independent experiments; error bars, s.e.m.

Figure S5. Short-term treatment with Aβ oligomers does not induce glutamate receptor internalization in primary hippocampal neurons. (**A**) Confocal images of surface-labeled Aβ (3D6, red), surface-labeled GluA1 (green) and total VGLUT2 (blue) of hippocampal neurons (DIV18) after 3 hr treatment with synthetic wt Aβ40 or $(Aβ40\ S26C)_2$ oligomers. Scale bar, 10 μm. (**B**) Histograms: mean intensity of surface GluA1 and total VGLUT2 after indicated treatments. Error bars, s.e.m. (**C, D**) Confocal images of surface-labeled Aβ (3D6, red), surface-labeled GluA1 (green) and total VGLUT2 (blue) in hippocampal neurons (DIV18) after 3 hr (**C**) or 12 hr (**D**) treatment with $(Aβ40\ S26C)_2$ oligomers. Arrows: co-localized puncta with 3D6, GluA1 and VGLUT2. Scale bar, 2 μm.

Figure S6. Short-term treatment with Aβ oligomers decreases the phosphorylation of glutamate receptor GluA1 at serine 845 in primary hippocampal neurons. (**A**) Representative blots showing certain synaptic membrane proteins in hippocampal neurons (DIV18) under different conditions of treatment. GAPDH is a control. Histograms: average level of synaptic or membrane protein in different conditions normalized to GAPDH. Data are from 4 independent experiments. (**B**) Confocal images of pGluA1 S845 (green) and total GluA1 (red) in hippocampal neurons (DIV18) after treatment with synthetic wt Aβ40 or $(Aβ40\ S26C)_2$ oligomers for different times. Scale bar, 10 μm. Histograms: mean intensity of pGluA1 and total GluA1 after indicated treatments. Error bars, s.e.m. Asterisks indicate data significantly different from neurons without treatment (*, $p < 0.05$; **, $p < 0.01$ by one-way ANOVA test).

Figure S7. Soluble Aβ oligomers bind to neuronal surfaces *in vivo*. (**A**) Immunohistochemistry of membrane-bound Aβ detected by 3D6 in mouse hippocampus 1 day

after icv microinjection of synthetic wt Aβ40 (**a**) or $(Aβ40\ S26C)_2$ oligomers (**b-d**). **b**,**d**: ipsilateral side; **c**, contralateral side. Scale bar, 50 μm. (**B**) Confocal images of membrane-bound Aβ detected by 3D6 (green) in the granule cell layer of CA3 and DG of hippocampus 1 day after icv injection of $(Aβ40\ S26C)_2$ oligomers, showing co-localization with GluA1 (red). DAPI was shown in blue. Scale bar, 10 μm.

Figure S8. Scyllo-inositol inhibits soluble Aβ oligomer-induced activation of microglia *in vivo*. (**A**) Confocal images showing the pattern of microglia labeled by Iba1 (green) and CD68 (red), and DAPI (blue) in CA3 of hippocampus 1 day after icv injection of synthetic wt Aβ40 or $(Aβ40\ S26C)_2$ oligomers in the presence or absence of chiro- or scyllo-inositol (20 μM). Scale bar, 20 μm. (**B**) Histograms: mean intensity of Iba1 and CD68 under indicated conditions. * significantly different from mice injected with aCSF ($p < 0.05$ by one-way ANOVA test). Fifteen slices per condition were analyzed. Error bars, s.e.m. (**C**) Confocal images showing the pattern of $(Aβ40\ S26C)_2$ oligomers labeled by 3D6 (green), microglia labeled by Iba1 (red), and DAPI (blue) in CA3 of hippocampus 1 day or 4 days after icv injection of synthetic $(Aβ40\ S26C)_2$ oligomers. Arrows indicate microglia bound with Aβ40 oligomers. Scale bar, 20 μm.

Figure S9. Longer-term, but not short-term injection of soluble Aβ oligomers induces synaptic loss *in vivo*. (A) Histograms represent mean density of pre-synaptic puncta labeled by VGLUT2, post-synaptic puncta labeled by GluA1 and co-localized synaptic puncta in the molecular layer of CA3 or DG of hippocampus 1 day after icv injection of wt Aβ40 or $(Aβ40\ S26C)_2$ oligomers. Fifteen slices per condition were analyzed. Error bars, s.e.m. (**B**) Representative blots of synaptic and other membrane proteins in different centrifugal fractions

isolated from mouse hippocampus (see Supplementary Methods). S1: supernatant 1; C: cytosol; P2: pellet 2; S: synaptosome; SPM: synaptic plasma membranes; PSD: post-synaptic density. (**C**) Representative blots of synaptic and other membrane proteins in total cytosol or synaptic plasma membrane (SPM) fractions of mouse hippocampus 4 d after icv injection of aCSF, wt Aβ40 or $(Aβ40\ S26C)_2$ oligomers. GAPDH: loading control. (**D**, **E**) Confocal images of pre-synaptic puncta labeled by Synapsin I (green), post-synaptic puncta labeled by PSD95 (red), and DAPI (blue) in the molecular layer of CA3 (**D**) or DG (**E**) of hippocampus 4 d after icv injection of aCSF, wt Aβ40 or $(Aβ40\ S26C)_2$ oligomers. Scale bar, 20 μm. Histograms: mean density of SYN-I, PSD95 and co-localized synaptic puncta under indicated conditions. * significantly different from mice injected with aCSF ($p< 0.05$ by one-way ANOVA test). Fifteen slices per condition were analyzed. Error bars, s.e.m.

Figure S10. Scyllo-inositol shifts Aβ oligomers to smaller species. (**A**) Representative Western blots (mAb 6E10) of SEC fractions of conditioned media (CM) of hippocampal neurons (DIV18) treated for 1 day with wt Aβ40 or $(Aβ40\ S26C)_2$ oligomers +/- chiro- or scyllo-inositol (20 μM). (**B**) Bars: mean percentages of the total $(Aβ40\ S26C)_2$ signals in the CM present in SEC fractions 7, 11 or 15 after the indicated treatments. *, significantly different from CM containing $(Aβ40\ S26C)_2$ alone ($p < 0.01$, one-way ANOVA test). Data are from 3 independent experiments; error bars, s.e.m.

Supplementary Materials and methods

Human brain sample preparation. Frozen human cerebral cortices were provided by C. Lemere (BWH/HMS) or M. Frosch (MGH/HMS) under IRB-approved human studies protocols and by M. Farrell (Beaumont Hospital, Dublin) in accord with local Ethics Committee guidelines and ERC/IRB approval. Each subject's clinical and neuropathological diagnoses are provided in Supplementary Table 1. Samples of temporal or frontal cortex containing white and grey matter were weighed. Freshly prepared, ice cold Tris-buffered saline (TBS) consisting of 20 mM Tris-HCl, 150 mM NaCl, pH 7.4, was added to the frozen cortex at 4:1 (TBS volume:brain wet wt) and homogenized with 25 strokes at a setting of 10 on a mechanical Dounce homogenizer. The homogenate was spun at 175,000 g in a TLA100.2 rotor on a Beckman TL 100. The supernate (called TBS extract) was aliquoted and stored at -80°C.

Immunoprecipitation/Western blot (IP/WB) analysis of Aβ. We used an IP/WB protocol described previously (Jin et al., 2011; Shankar et al., 2008; Walsh et al., 2002) to detect Aβ in TBS brain extracts or culture media. These were IP'ed with either Aβ antiserum AW7 (1:50) and Protein A sepharose (PAS; Sigma) or Aβ monoclonal antibody (mAb) 3D6 (3 μg/ml, gift of Elan, plc) and Protein G agarose (PGA; Roche) plus PAS. After bead washing, the immunoprecipitates were eluted with 10 μL 4 % LDS sample buffer, heated at 65 °C for 5 min and centrifuged at 14,000 rpm for 5 min. The supernatant was electrophoresed on a 26-well 4-12 % bis-Tris gel using MES running buffer (Invitrogen). Proteins were transferred to 0.2 μm nitrocellulose and Western blotted (WB) for Aβ with 1 μg/ml each of 6E10 (Covance) + 2G3 + 21F12 (mAb's from Elan, plc) using the LiCor Odyssey Infrared Imaging System.

Immunoprecipitation (IP)-size exclusion chromatography (SEC).

TBS extracts of AD or control cortex were IP'ed with 3D6 (3 μg/ml) + 15 μL PAS and 15 μL PGA. After bead washing, the precipitates were eluted with 10 μL 4 % LDS sample buffer, heated at 65 °C for 5 min and centrifuged at 14,000 rpm for 5 min. The supernate was transferred to 500 μL TBS. IPed samples or culture medium (500 μL) was injected onto a Superdex 75 (10/30HR) column (Amersham Biosciences, Piscataway, NJ) and eluted at a flow rate of 0.8 ml/min into 1 ml SEC fractions using 50 mM ammonium acetate, pH 8.5. 750 μL were removed and stored at -80ºC. The remaining 250 μL were lyophilized, reconstituted in 15 μL of 2X LDS sample buffer, heated at 65 ºC for 5 min and used for WB analysis. Soluble Aβ monomer-rich or dimer-rich SEC fractions from AD brain TBS extracts (AD-TBS) and the corresponding fractions from control brain extracts (Cont-TBS) were pooled separately and lyophilized prior to addition to the culture medium of primary cultured hippocampal neurons.

The concentration of soluble Aβ oligomers in the medium was measured by oligomer-specific, enzyme-linked immunoassays (o-ELISAs), as described previously (Yang et al., 2013). In brief, an Aβ aggregate-selective monoclonal (NAB61) was used for capture, and a monoclonal to the free N-terminus (3D6B) was used for detection.

Production & characterization of cross-linked synthetic dimers. Aβ40 S26C was synthesized by the Biopolymer Laboratory at UCLA Medical School and the correct sequence and purity confirmed by amino acid analysis, reverse-phase HPLC and mass spectrometry(Shankar et al., 2008). Disulfide-bonded Aβ dimers were generated by atmospheric oxidation of a 20 μM solution of Aβ40 S26C in 20 mM ammonium bicarbonate, pH 8.0, for 4

days at RT. To facilitate disassembly of aggregates formed during oxidation, the peptide solution was lyophilized and subsequently incubated in 5 M GuHCl, Tris-HCl, pH 8.0, for 4 hr. Disulfide crossed-linked Aβ dimers were then separated from unreacted monomer and higher aggregates by size exclusion chromatography. Briefly, two Superdex 75 10/30 HR columns were linked in series and eluted with 50 mM ammonium acetate, pH 8.5, at a flow rate of 0.5 ml/min. Fractions (0.5 ml) were collected, and an aliquot of each was electrophoresed on 16% tris-tricine polyacrylamide gels and detected by silver staining. SEC fractions found to contain exclusively dimeric Aβ were pooled and the peptide content determined by comparison to known standards. Samples were stored at -80 ºC until used.

Hippocampal neuronal cultures and calcium phosphate transfection: Primary hippocampal cultures were generated from E18 Sprague-Dawley rat embryos (Jin et al., 2011). The hippocampus was dissected in Hank's Balanced Salt Solution buffered with HEPES and dissociated with 0.125 % trypsin (Invitrogen) for 15 min at 37 °C followed by trituration. Dissociated cells were plated at a density of 1.5 x 10^5 cells/cm^2 in 6-well plates pre-coated with poly-D lysine (100 μg/ml), or 1 x 10^4 cells/cm^2 on 24-well plates with coverslips coated with poly-D lysine (100 μg/ml). After 4 days culturing in Neurobasal medium with B-27 supplement (Invitrogen) and glutamax, cytosine arabinofuranoside was added to reduce glial proliferation. Half the medium was exchanged every 4 days.

Calcium phosphate transfection was performed with CalPhos™ Mammalian Transfection Kit (Clonetech). In brief, 100 μl of Solution A (3 μg pCMV-EGFP, 12.4 μl 2M CaCl2, 84.6 μl H2O) was dropped into 100 μl Solution B (2x HBS), while gently vortexing Solution B. After

incubation of the mixture at RT for 20 min, 50 μl of transfection solution was added to each well of the 24-well plate, which was gently moved back and forth to distribute transfection solution evenly. After incubation of plates at 37 °C for 6 h in a CO_2 incubator, the transfection solution/medium was removed from culture plates, and cells were washed with fresh media. The cells underwent treatments and immunostaining 4 days after transfection.

Intracerebroventricular (ICV) injection: Intracerebroventricular (ICV) injection was performed as described previously (Cirrito et al., 2003). Wild type mice (C57/Bl6, 5 mos old, female) were anesthetized using 1.5–2.5 % isoflurane. The head was shaved, and the skin was transected along the midline to expose the skull from several millimeters anterior and posterior to bregma and lambda. The animal was then placed in a small-animal stereotaxic device equipped with dual manipulator arms and an anesthetic mask (David Kopf Instruments, Tujunga, CA). To ensure the skull was level for each animal, measurements and adjustments were made so that bregma and lambda were at equal heights (with 0.1 mm tolerance), as well as 2 points equidistant from midline. Bore holes (0.75 mm) were made above the left cerebroventricle (coordinates: bregma -0.4 mm, 1.0 mm lateral; -2.5 mm relative to dura mater). Synthetic Aβ40 S26C (5 ng, 2.5 μl vol.) was injected at 0.5 μl/min via a Hamilton syringe into the ventricle of anesthetized mice. Mice were sacrificed 1 to 7 days post-injection for analysis.

Preparation of synaptic plasma membrane and PSD fractions: Synaptoplasma membrane and PSD were prepared as described previously (Linhoff et al., 2009) with minor modifications. Two hippocampi were dissected from each mouse under different treatment conditions and collected in 1 mL ice-cold Buffer A (0.32 M sucrose, 10 mM Tris, pH 7.4, 1 mM Na_3VO_4, 5

mM NaF, 1 mM EDTA, and 1 mM EGTA, plus protease inhibitors). Tissues were then homogenized in a glass grinding vessel using a rotating Teflon pestle (2,000 rpm) with at least 20 passes to create a Dounce homogenate. The homogenate was centrifuged at 1,000 × g for 10 min to remove nuclei and incompletely homogenized material (P1). The resulting supernatant (S1) was spun at 10,000 × g for 15 min to obtain a P2. The supernatant (S2) was defined as the cytosolic fraction. The P2 was subsequently resuspended in 1 mL Buffer B (6 mM Tris, pH 8.1, 0.32 M sucrose, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, plus protease inhibitors) using a motorized pestle mixing/grinding rod (Kontes) directly in the microfuge tube with 30 pulses. A sucrose gradient was prepared with (bottom to top) 1.2 M, 1.0 M, 0.8 M sucrose, 6 mM Tris, pH 8.1, using 3 mL of each. The P2 fraction was layered over the sucrose gradient and centrifuged for two hours at 82,500 x g. Material at the interface between the 1.0/1.2 M layers was collected (synaptosome fraction). Five volumes of hypotonic 6 mM Tris, pH 8.1, buffer was added to the collected fraction and allowed to sit on ice for ~10 min with occasional swirling. The sample was centrifuged for 20 min at 25,000 x g. The pellet was resuspended in 1 mL of Buffer B (synaptic plasma membrane fraction). The sample was then centrifuged for 30 min at 150,000 x g, and the pellet was resuspended in 1 mL of Buffer C (6 mM Tris, pH 8.1, 0.5% Triton X-100, 75 mM KCl, 1 mM EDTA, 1 mM EGTA, 1 mM DTT, plus protease inhibitors). The resuspended sample was homogenized with a Dounce homogenizer and allowed to sit on ice for 15 minutes. The sample was centrifuged for 20 minutes at 32,000 x g. The pellet was resuspended in 1 mL Buffer D (6 mM Tris, pH 8.1, 2% SDS, 1 mM DTT, plus protease inhibitors). The suspension was placed at RT for 10 min to allow solubilization and then placed back on ice (post-synaptic density (PSD) fraction). Protein concentrations were determined using the bicinchoninic acid (BCA) assay.

Surface Biotinylation: Different cultures in 6-well plates were rinsed 3 times with D-PBS (Invitrogen), biotinylated with Sulfo-NHS-SS-biotin (1 mg/ml, Thermo) at RT for 30 min, quenched with 50 mM Tris buffer (pH. 8.0), rinsed with D-PBS an additional 3 times, and lysed in 0.2 ml lysis buffer (0.1% SDS, 1% NP-40, 50 mM HEPES, pH 7.4, 2 mM EDTA, 100 mM NaCl, 5 mM Na3VO4, 40 μM p-nitrophenyl phosphate, plus protease inhibitors). The lysates were centrifuged at 12,000 rpm for 25 min. The supernatant was collected, and the concentration of protein was determined by BCA. Three-fourth of the lysate was pulled down with 40 μl strepavidin-agrose beads (Sigma) at 4 °C overnight, followed by washing with lysis buffer for three times, and elution with 40 μl 2X SDS sample buffer (Invitrogen).

Western blotting: Various cultures in 6-well plates were lysed in 0.2 ml lysis buffer (0.1% SDS, 1% NP-40, 50 mM HEPES, pH 7.4, 2 mM EDTA, 100 mM NaCl, 5 mM Na_3VO_4, 40 μM p-nitrophenyl phosphate, plus protease inhibitors). The lysates were spun at 12,000 rpm for 25 min. Supernatants were collected and denatured. Protein concentrations were by BCA. Twenty μg of total protein or 20 μl of the respective elution was loaded in each lane, separated by 4-12 % SDS-PAGE and blotted onto nitrocellulose membrane. The blot was blocked for 1 hr at RT, followed by incubation overnight at 4 °C with primary antibodies. Membranes were rinsed and incubated for 1 h with fluorescence-conjugated goat anti-rabbit or mouse IgG (1:5,000; Invitorgen). Blots were scanned using a Licor Odyssey system.

Immunocytochemistry, surface labeling, and immunohistochemistry: Hippocampal neurons cultured for 18 days were treated under different conditions as specified. After treatment, cells

were fixed in 4 % paraformaldehyde (PFA) + 4 % sucrose for 15 min, followed by permeabilization with cold methanol for 3 min. Neurons were rinsed 3 times with PBS. After blocking in blocking buffer (5 % BSA and 0.02 % sodium azide in PBS), neurons were incubated with primary antibodies at 4 ^{o}C overnight. After rinsing with PBS 3 times, cells were incubated with fluorescence-conjugated IgG (Invitrogen, 1:1,000) at 4 ^{o}C overnight. After rinsing 3 times with PBS, coverslips were placed with mounting medium (Southern Biotech). For labeling of surface-bound proteins, immunocytochemistry was performed under the non-permeabilization condition.

For surface labeling, cultured neurons were washed with PBS, followed by preincubation with primary antibodies against GluA1 or Aβ at 37 °C for 5 min. After washing with ice-cold PBS, neurons were fixed with 4 % PFA + 4 % sucrose for 15 min, then blocked in blocking buffer for 1 hr, followed by fluorescence-conjugated IgG incubation at RT for 1 hr. For immunolabeling of excitatory synaptic markers, cultures were then permeabilized with cold methanol for 3 min. Neurons were blocked in blocking buffer for 1 hr, followed by incubation with primary antibodies for VGLUT2 at 4 ^{o}C overnight. Neurons were washed with PBS and incubated with fluorescence-conjugated IgG (Invitrogen, 1:1,000) at RT for 1 hr, washed with PBS and mounted with mounting medium.

For immunohistochemistry, mice were sacrificed by CO2 inhalation, perfused once with 0.01M PBS and once with 4% paraformaldehyde (PFA) and their brains quickly removed. Brains were immersed in 4% PFA for 24 hr and in 30% sucrose for 24 hr and frozen in liquid nitrogen and stored at -80 ^{o}C. Brains were sliced into sections of 40 μm on a cryoslicer and sections were immediately transferred into PBS and stored at 4 ^{o}C. For staining, brain sections were washed

thoroughly in PBS and blocked in blocking buffer for 1 h at RT. Brain sections were incubated with primary antibodies overnight at 4^{o}C. The next day, sections were washed with PBS 3 times and incubated with fluorescence-conjugated IgG for 1 hr at RT, or with biotinylated secondary antibody for 1 h at RT and developed using Vector ELITE ABC kits (Vector Laboratories) and 3,3-Diaminobenzidine (DAB: Sigma). Sections were subsequently washed with PBS and mounted on glass slides.

Reagents and antibodies: Scyllo-inositol and chiro-inositol were by Transition Therapeutics (Toronto, Ontario, Canada). The following mouse mAbs were used: 3D6, 2G3, 21F12 (Elan), 6E10 (Covance), Transferrin receptor (Invitrogen), GluA1 (Millipore), PSD95 (Millipore), Synaptophysin (Millipore), β-tubulin (Sigma) and GAPDH (Millipore). The following polyclonal antibodies were used: AT8 (rabbit, Thermo), pGSK3b S9 (rabbit, Millipore), GluN1 (rabbit, Millipore), GluN2A (rabbit, Millipore), GluN2B (rabbit, Invitrogen), GluA1 (rabbit, Calbiochem), GluA1 S845 (rabbit, Millipore), b2-integrin (rabbit, Cell signaling), Synapsin I (rabbit, Millipore), Homer1 (rabbit, Millipore), Tau (rabbit, DAKO), VGLUT2 (guinea pig, Millipore). The following secondary antibodies were used: anti-mouse AlexaFluor 488, 546, 790; anti-rabbit AlexaFluor 488, 546, 633, 680; anti-guinea pig AlexaFluor546, 633 (Invitrogen); goat anti-mouse biotinylated secondary antibody (Vector Laboratories).

Image acquisition and analysis: Neurons or brain sections under different conditions were photographically captured in a random manner. Confocal microscopy was performed on a Zeiss LSM710 confocal microscope using a 20x air objective (NA: 0.75) or 63x oil objective (NA: 1.4), averaging of 2 frames, and set to 1024 x 1024 pixel resolution. Images were captured in a

Z-stack manner (5-10 stacks, interval 0.5-1.0 μm) and maximum pixel intensity projections were created. Within the same experiment, the settings for laser power, master gain and digital offset were used in the same manner, and the brightest pixels were still under saturation. Digital images were taken and the fluorescence intensity was quantified using the Zen Black software (Zeiss). For puncta analysis, the average synaptic puncta density was calculated using ImageJ 1.45 (NIH) as described previously (Ippolito and Eroglu, 2010). For the same experiment, threshold parameters for image analysis were identical. In each experiment, at least 15 cells or brain sections per condition were analyzed and the mean and SEM were calculated. Data shown represent the average of the mean values from at least 3 independent experiments.

Statistical Analysis

Data are expressed as mean ± SEM. Significance was assessed with one-way analysis of variance (ANOVA) followed by Bonfferoni post-hoc test using Prism 5.0 GraphPad Software (San Diego, CA). A value of $p < 0.05$ was considered significant.

Animal Use Approval

All animal protocols were approved by the Harvard Medical Area Standing Committee on Animals, and studies were performed in accordance with all state and federal regulations. The Harvard Medical School animal management program is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International (AAALAC), and meets all National Institutes of Health standards as demonstrated by an approved Assurance of Compliance (A3431-01) filed at the Office of Laboratory Animal Welfare (OLAW).

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Table S1. Clinical and histopathological information on brain samples used for analysis

Case #	Npath Dx	Age	Gender	Tissue
1	Control	89	F	Frontal cortex
2	Control	85	M	Temporal cortex
3	AD	69	F	Frontal cortex
4	AD	75	F	Temporal cortex