Supplementary Information

Journal: Scientific Reports

Article title: Aβ42 oligomers disrupt calcium homeostasis via Piezo1 and induce neurotoxicity in differentiated SH-SY5Y cells

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Reference numbers in this file refer to the reference list of the main article.

Materials and methods

1. Cell experimental materials

SH-SY5Y proliferation and differentiation media were prepared by adapting published protocols [18–20] to local laboratory conditions. Proliferation medium (50 mL) contained 44 mL DMEM/F-12 (1:1; Gibco, 11320033), 5 mL heat-inactivated fetal bovine serum (hi-FBS; Gibco, 26010074), 0.5 mL 1× penicillin-streptomycin (VivaCell, C3420-0100), and 0.5 mL Mycoplasma Treatment Reagent 3 (B3; VivaCell, C3472-0100). Prepared medium was used within 2 weeks.

Differentiation medium I (50 mL) contained 47.4 mL DMEM/F-12 (1:1), 1.5 mL hi-FBS, 0.5 mL 1× penicillin-streptomycin, 0.5 mL B3 reagent, and 100 µL retinoic acid (10 µM; MCE, HY-14649R). Retinoic acid was added immediately before use, and prepared medium was used within 2 weeks.

Differentiation medium II (50 mL) contained 48.15 mL DMEM/F-12 (1:1), 0.5 mL hi-FBS, 0.5 mL 1× penicillin-streptomycin, 0.5 mL B3 reagent, 100 µL retinoic acid (10 µM), and 250 µL brain-derived neurotrophic factor (BDNF; 50 ng/mL; Apexbio, P1007). Retinoic acid and BDNF were added immediately before use.

Neuronal maturation medium (50 mL) contained 46.05 mL neuronal basal medium (Procell, PM151223), 1 mL B-27 supplement (Gibco, 17504044), 1 mL KCl (20 mM), 0.5 mL L-glutamine (2 mM; MCE, HY-N0390), 0.5 mL 1× penicillin-streptomycin, 0.5 mL B3 reagent, 100 µL dibutyryl cyclic AMP (2 mM; MCE, HY-B0764A), 250 µL BDNF (50 ng/mL), and 100 µL retinoic acid (10 µM). Retinoic acid and BDNF were added immediately before use, and prepared medium was used within 2 weeks.

2. Cell culture and differentiation

SH-SY5Y cells were purchased from Wuhan Procell Life Technology Co., Ltd. (catalogue no. CL-0208). Cells were seeded in T25 flasks with 5 mL proliferation medium, cultured at 37 °C to approximately 90% confluence, and passaged at a 1:3 ratio. Cells used for differentiation and subsequent experiments were maintained below passage 10.

SH-SY5Y cells were differentiated using a published protocol [17] with adjustments for local conditions.

Cells were cultured to 50–70% confluence, detached with trypsin, and seeded at approximately 2×10^4 cells/mL in poly-L-lysine-coated dishes. Cell density was determined with a hemocytometer. Cultures were maintained at 37 °C and sequentially exposed to proliferation medium, differentiation medium I, differentiation medium II, and neuronal maturation medium for 2–3 days per stage. Cells displaying stable, elongated neurite-like processes were used for experiments within the subsequent 3–5 days.

Differentiation was assessed by expression of the neuronal marker βIII-tubulin (Fig. S1a) and by morphology. βIII-tubulin expression increased after differentiation. Undifferentiated SH-SY5Y cells were rounded, grew in clusters, and showed few processes (Fig. S1b), whereas differentiated cells were dispersed and displayed elongated neurite-like processes (Fig. S1c,d).

3. Preparation of Aβ42 oligomers reagent

HFIP-treated Aβ42 monomer (MCE, HY-P1363B) was dissolved in anhydrous DMSO to 5 mM (22.57 mg/mL) and stored at −80 °C. The stock was diluted to 200 µM (0.9 mg/mL) with sterile double-distilled water and incubated at 4 °C for 24 h. Samples were centrifuged at 14,000 × g for 10 min at 4 °C, and the oligomer-containing supernatant was collected at an approximate concentration of 0.5 mg/mL. Aliquots were stored at −20 °C and used within 7 days.

4. Fluorescence experiment

Cells were seeded in 35-mm glass-bottom confocal dishes at 2×10^4 cells/mL and treated after differentiation. Cells were washed three times with 1–2 mL phosphate-buffered saline (PBS), fixed with 4% paraformaldehyde for 15 min at room temperature, and washed three times with PBS. For intracellular proteins or F-actin, cells were permeabilized with 0.1% Triton X-100 for 10 min and washed three times. Samples were stained according to the relevant reagent instructions, washed three times under light-protected conditions, and imaged by laser-scanning confocal microscopy [37–38].

5. Immunofluorescence test

Cells were seeded on 22-mm coverslips in 35-mm dishes at 2×10^4 cells/mL. After differentiation and treatment, cells were washed, fixed, and permeabilized as described above. Samples were blocked with 3% bovine serum albumin (BSA) for 30 min at room temperature, incubated overnight at 4 °C with primary antibody diluted in 3% BSA, and washed three times with PBS. Fluorescent secondary antibody was applied for 60 min at room temperature in the dark. Samples were washed three times, mounted under light-protected conditions, stored in the dark, and imaged by laser-scanning confocal microscopy within 24 h [37–38].

6. Western blot experiment

Cells were seeded in six-well plates and treated according to the experimental design. After treatment, cells were washed two or three times with PBS and lysed on ice for 10–15 min in 100 μL RIPA buffer containing 1% phosphatase inhibitor and 1% phenylmethylsulfonyl fluoride. Lysates were centrifuged at 12,000 × g for 15 min at 4 °C, and protein concentration in the supernatant was determined by BCA assay. Equal amounts of protein were separated by SDS-PAGE and transferred to polyvinylidene fluoride membranes. Membranes were blocked for 30 min at room temperature, incubated overnight at 4 °C with primary antibody, and washed three times for 10 min in Tris-buffered saline with Tween 20. HRP-conjugated secondary antibody was applied for 1 h at room temperature, followed by three washes. Signals were developed with enhanced chemiluminescence reagent (SQ201, Epizyme, Shanghai, China) according to the manufacturer's instructions, captured with a gel-imaging system, and quantified in ImageJ.

7. CCK8 experiment

Cells were seeded in 96-well plates at 1×10^4 cells per well and treated as specified. The medium was replaced with 100 μL fresh medium containing 10% CCK-8 reagent (Beyotime, C0037), and plates were incubated for 0.5–2 h. Absorbance at 450 nm was measured with a microplate reader. Blank wells containing medium and CCK-8 but no cells were used for background subtraction. Viability was normalized to the untreated control.

Cell viability (%) = (OD_{450} of treatment group / OD_{450} of control group) × 100%

For concentration-response experiments, cell viability was measured across a drug concentration gradient. Curves were fitted in GraphPad Prism, and half-maximal inhibitory concentrations (IC_{50}) were estimated (Fig. S1f,g). These values guided the concentrations used in subsequent experiments.

8. Kit-Based Assays

Mitochondrial membrane potential (JC-1), intracellular Ca^{2+} (Fluo-4 AM), ROS, and LDH release were measured with commercial kits from Beyotime Biotechnology: C2003S, S1060, S0034S, and C0016, respectively. Sample preparation, reaction conditions, signal acquisition, and data analysis followed the manufacturers' instructions.

Supplementary Data

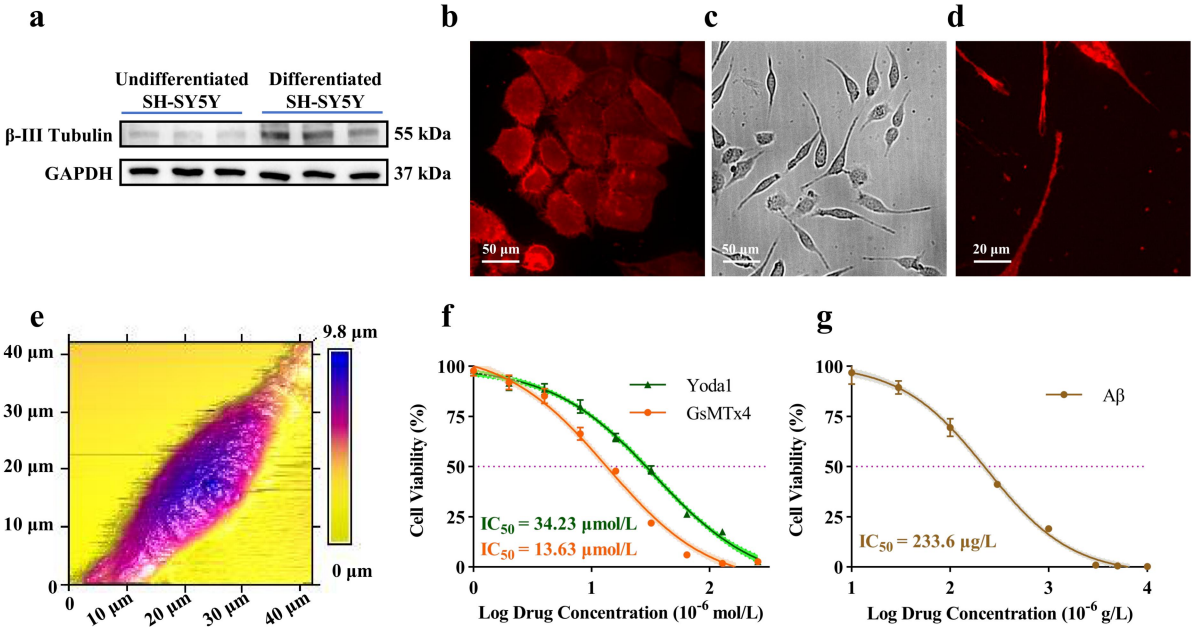


Fig. S1 Characterization of differentiated SH-SY5Y cells and treatment concentrations. (a) βIII-tubulin expression before and after differentiation by immunoblotting. (b) Undifferentiated cells grew in clusters and lacked neurite-like processes. (c) Differentiated cells were dispersed and displayed prominent neurite-like processes. (d) High-magnification image of neurite-like terminals after differentiation. (e) AFM height map of a differentiated SH-SY5Y cell. (f) IC_{50} estimates for Yoda1 and GsMTx4; shading indicates the 95% confidence interval. (g) IC_{50} estimate for Aβ42 oligomers; shading indicates the 95% confidence interval. In (a), the βIII-tubulin and GAPDH rows are cropped from their corresponding target-protein images of the same membrane; black borders and white space separate the displayed crops. Source images and membrane photographs are provided under "Source blots for Fig. S1a" below.

Western blot source images and membrane records

All western blot assays were performed on intact full-length PVDF membranes; no membrane trimming was performed before blocking and antibody incubation. ECL substrate was applied locally to target-band regions to conserve reagent.

The following pages reproduce the available source chemiluminescence images and matched bright-field photographs as complete image frames. No additional cropping, contrast adjustment, background removal or band editing was applied when assembling these pages. Bright-field photographs document the visible membrane boundaries; chemiluminescence images document the detected signal. The original source file identifiers are retained beneath each image pair.

For Figs. 2d, 4c and 5c, sample lanes 1–3 are the blank control group and sample lanes 4–6 are the Aβ42-treated group, with three biological replicates per group. For Fig. S1a, sample lanes 1–3 are undifferentiated cells and sample lanes 4–6 are differentiated cells, with three biological replicates per group. These lane numbers refer to sample lanes, excluding molecular-weight marker lanes.

Source image index

Source blots for Fig. 2b: two Co-IP image pairs.

Source blots for Fig. 2d: YAP, p-YAP, TAZ and p-TAZ image pairs; archived membrane and molecular-weight marker reference photographs.

Source blots for Fig. 4c: CaMKIIα, p-CaMKIIα and GAPDH image pairs.

Source blots for Fig. 5c: GSDMD/N-GSDMD/GAPDH, caspase-4 and caspase-1 image pairs; two membrane archive photographs.

Source blots for Fig. S1a: βIII-tubulin and GAPDH image pairs; a membrane archive photograph.

Image availability and interpretation

The GAPDH chemiluminescence file underlying Fig. 2d is unavailable because of a historical storage error. A photograph of the archived PVDF membrane is included as a physical record; it does not replace the missing chemiluminescence data. The lower edge of the CaMKIIα bright-field image for Fig. 4c is partially obscured. Caspase-1 in Fig. 5c was measured on a separate membrane without a loading control; Fig. 5d uses its unnormalized band gray values.

We have carefully rechecked all chemiluminescence raw images. All western blot signals were captured using highly sensitive ECL substrate (SQ201, Epizyme Biotechnology, Shanghai, China). Even under short exposure settings, the background remains low. None of the protein bands in our submitted images show signal saturation.

All provided chemiluminescence files are original raw images directly exported from the imaging system without any post-processing adjustment of brightness or contrast. No overexposed bands were observed during image acquisition. Therefore, no additional separate short-exposure raw images are available for this dataset.

Source blots for Fig. 2b

The Co-IP lane and protein assignments follow Fig. 2b. For both membranes, sample lanes 1–4 are, from left to right, input cell lysate, IgG control, anti-Aβ immunoprecipitation and anti-Piezo1 immunoprecipitation. The upper row in Fig. 2b shows Piezo1 (286 kDa), and the lower row shows Aβ (10 kDa). The corresponding full-frame source images are presented below in the same protein order; the original file identifiers are retained for traceability.

Piezo1 — upper protein row in Fig. 2b

Bright-field membrane photograph



Chemiluminescence image



Source files: Supp-Fig2b_Reciprocal-IP-Piezo1_brightfield and Supp-Fig2b_Reciprocal-IP-Piezo1_chemilum

Aβ — lower protein row in Fig. 2b

Bright-field membrane photograph



Chemiluminescence image



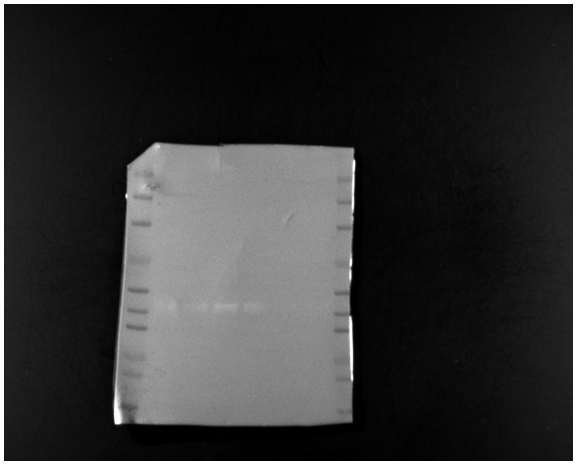
Source files: Supp-Fig2b_IP-Abeta42_brightfield and Supp-Fig2b_IP-Abeta42_chemilum

Source blots for Fig. 2d

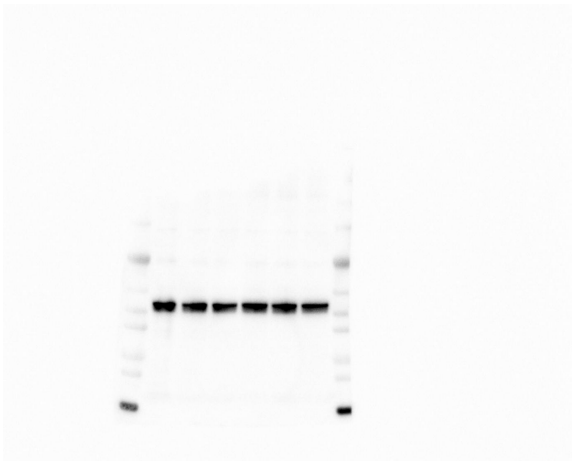
Sample lanes 1–3: blank control; sample lanes 4–6: Aβ42 treatment (three biological replicates per group). The samples were separated on the same SDS-PAGE gel and transferred onto a single PVDF membrane. Target-specific source captures are shown separately.

Total YAP

Bright-field membrane photograph



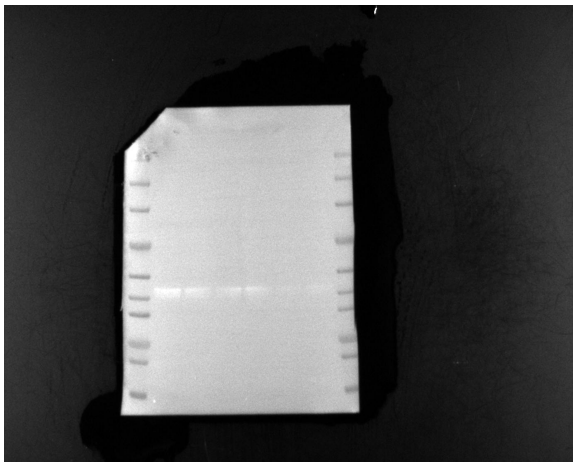
Chemiluminescence image



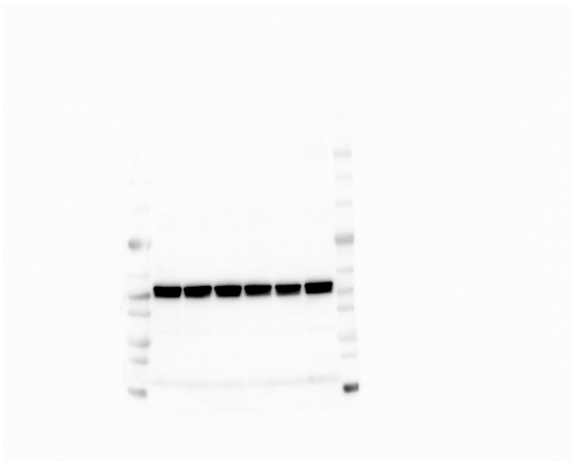
Source files: Supp-Fig2d_YAP_brightfield and Supp-Fig2d_YAP_chemilum

Phosphorylated YAP

Bright-field membrane photograph



Chemiluminescence image

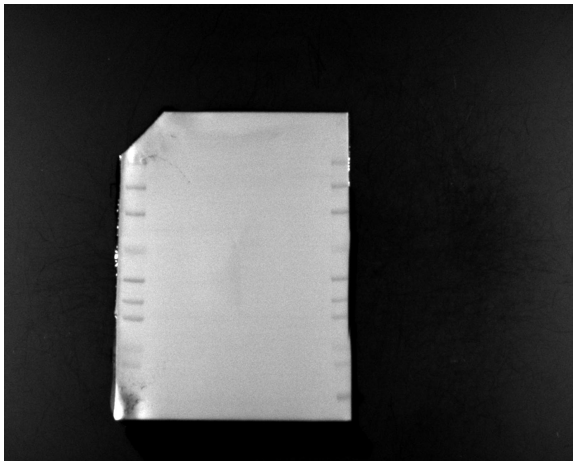


Source files: Supp-Fig2d_pYAP_brightfield and Supp-Fig2d_pYAP_chemilum

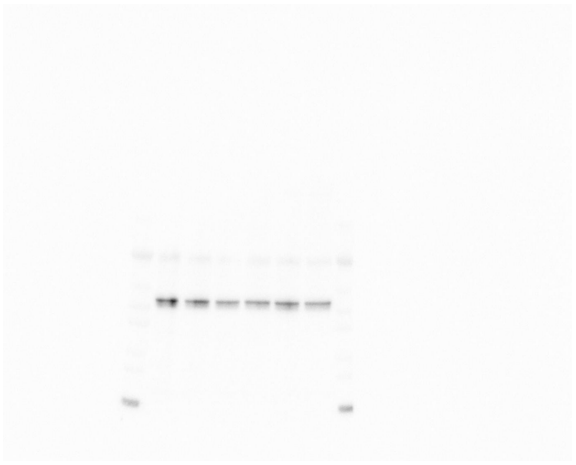
Source blots for Fig. 2d continued

Total TAZ

Bright-field membrane photograph



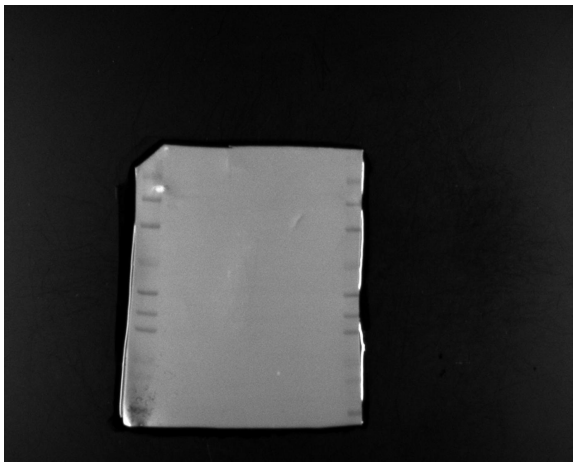
Chemiluminescence image



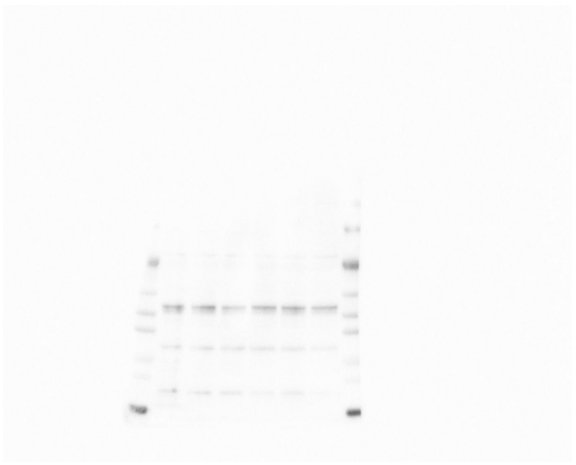
Source files: Supp-Fig2d_TAZ_brightfield and Supp-Fig2d_TAZ_chemilum

Phosphorylated TAZ

Bright-field membrane photograph



Chemiluminescence image

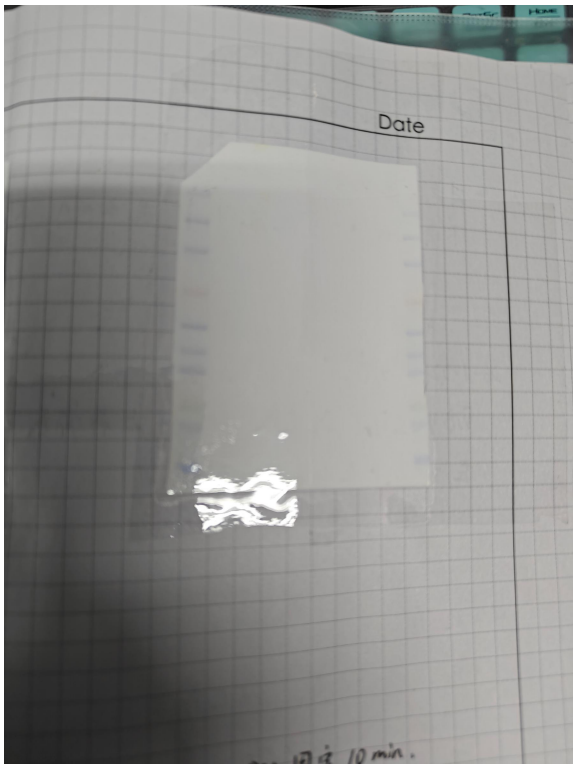


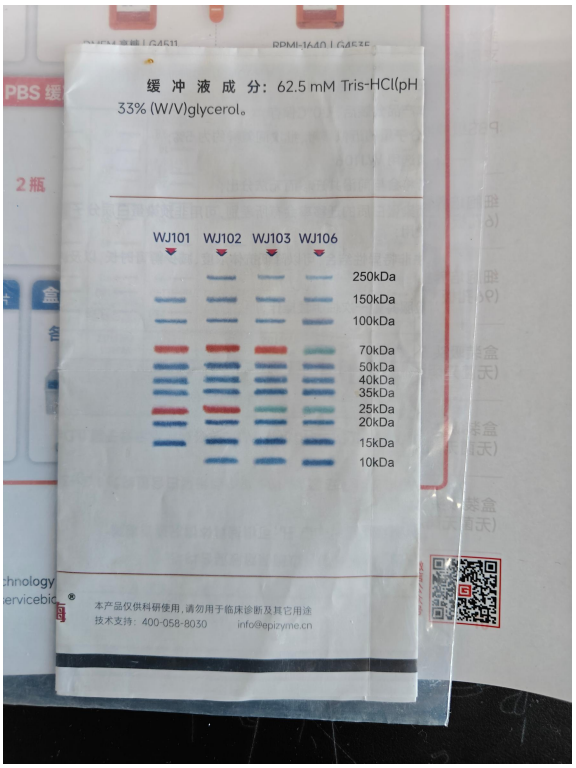
Source files: Supp-Fig2d_pTAZ_brightfield and Supp-Fig2d_pTAZ_chemilum

The GAPDH source chemiluminescence file is unavailable because of a historical storage error. The following page contains the archived-membrane photograph and the supplied marker reference.

Membrane record and marker reference for Fig. 2d

The notebook photograph documents the archived membrane. The marker-product photograph is a molecular-weight reference, not an additional blot or exposure.





Left: Supp-Fig2d_notebook_archive.jpg. Right: Supp-western blot marker kDa.jpg.

Source blots for Fig. 4c

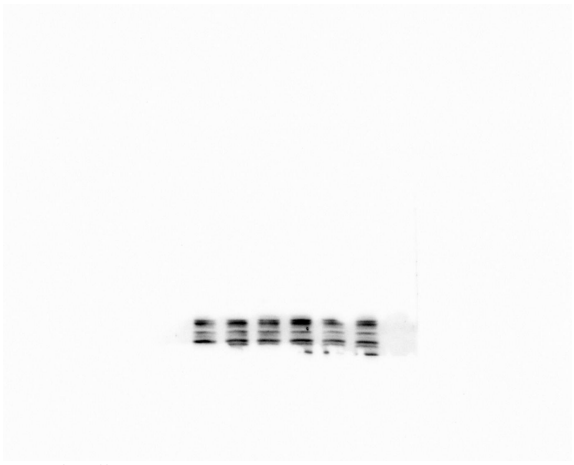
Sample lanes 1–3: blank control; sample lanes 4–6: Aβ42 treatment (three biological replicates per group). The samples were separated on the same SDS-PAGE gel and transferred onto a single PVDF membrane. ECL substrate was applied locally to target-band regions. The lower edge of the CaMKIIα bright-field image is partially obscured by the imaging background.

CaMKIIα

Bright-field membrane photograph



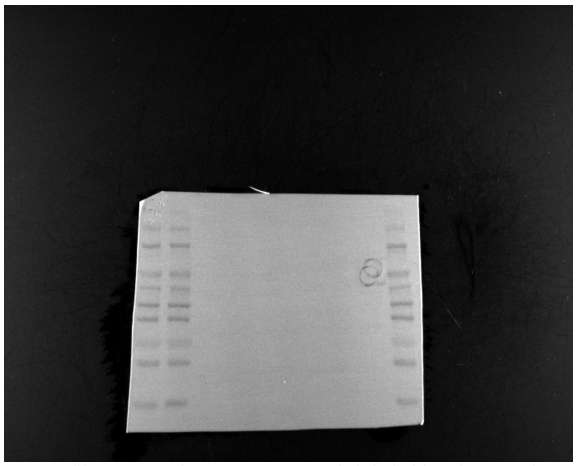
Chemiluminescence image



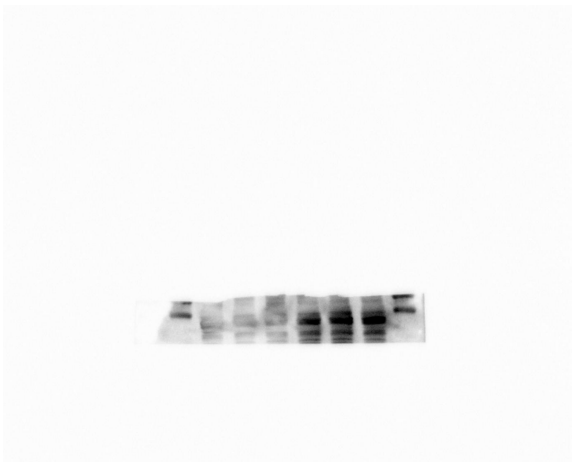
Source files: Supp-Fig4c_CaMKIIa_brightfield and Supp-Fig4c_CaMKIIa_chemilum

Phosphorylated CaMKIIα

Bright-field membrane photograph



Chemiluminescence image



Source files: Supp-Fig4c_p-CaMKIIa_brightfield and Supp-Fig4c_p-CaMKIIa_chemilum

Source blots for Fig. 4c continued

GAPDH

Bright-field membrane photograph



Chemiluminescence image

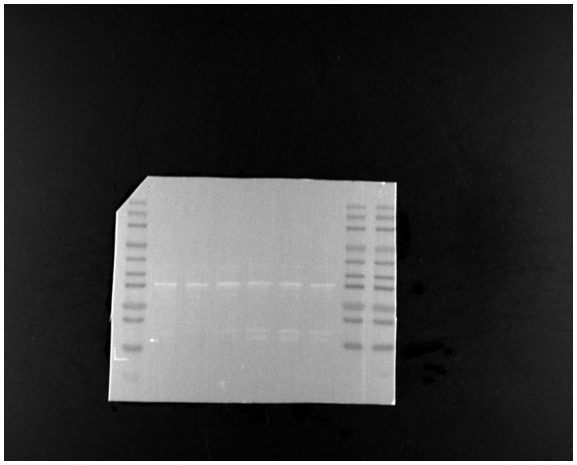
Source files: Supp-Fig4c_GAPDH_brightfield and Supp-Fig4c_GAPDH_chemilum

Source blots for Fig. 5c

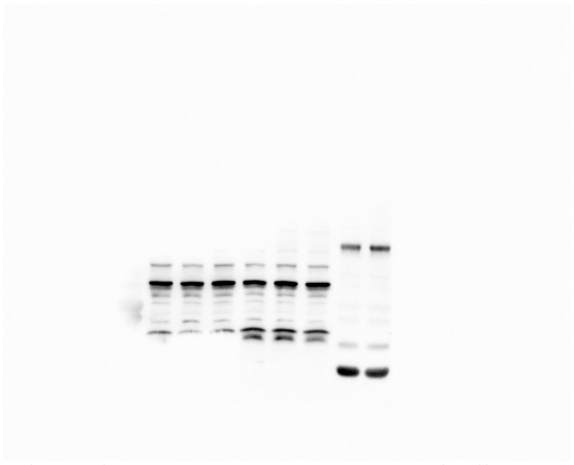
Two separate PVDF membranes were used. On each membrane, sample lanes 1–3 are the blank control and sample lanes 4–6 are the Aβ42-treated group (three biological replicates per group). The caspase-4 pathway membrane provided GSDMD, N-GSDMD, caspase-4 and GAPDH signals. Caspase-1 was detected on the separate membrane shown on the following page.

Caspase-4 pathway membrane — GSDMD, N-GSDMD and GAPDH

Bright-field membrane photograph



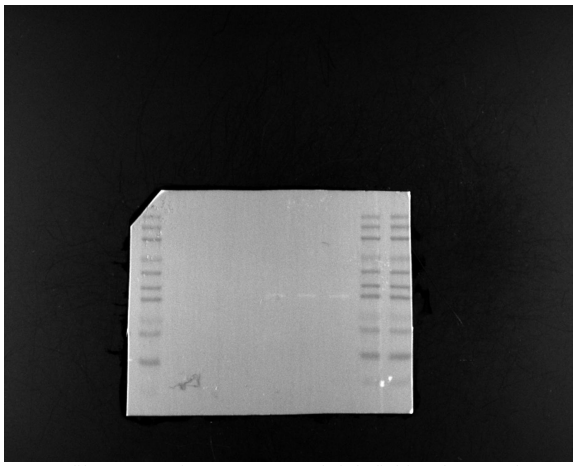
Chemiluminescence image



Source files: Supp-Fig5c_GSDMD_N-GSDMD_GAPDH_brightfield and Supp-Fig5c_GSDMD_N-GSDMD_GAPDH_chemilum

Caspase-4 pathway membrane — caspase-4

Bright-field membrane photograph



Chemiluminescence image

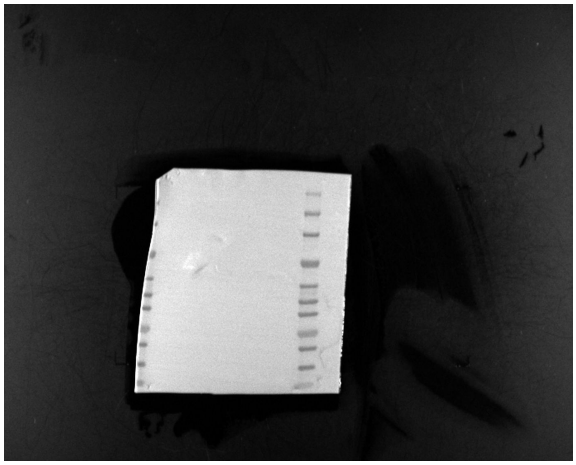
Source files: Supp-Fig5c_caspase-4_brightfield and Supp-Fig5c_caspase-4_chemilum

Source blots for Fig. 5c continued

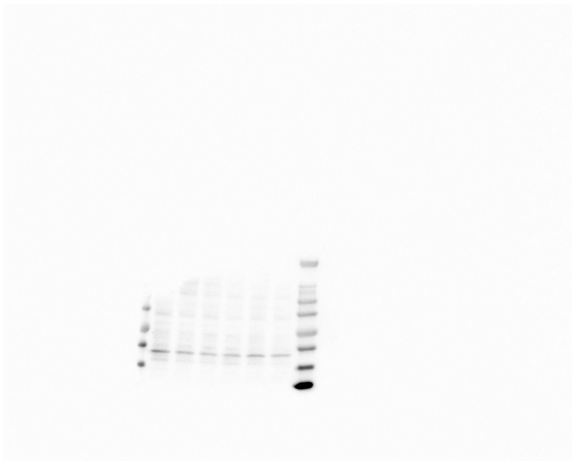
The caspase-1 membrane was used for negative verification without an internal loading control. The caspase-1 analysis in Fig. 5d was performed directly on raw band gray values, without loading-control normalization. The GAPDH shown in Fig. 5c belongs to the other membrane and is not a caspase-1 loading control.

Separate membrane — caspase-1

Bright-field membrane photograph



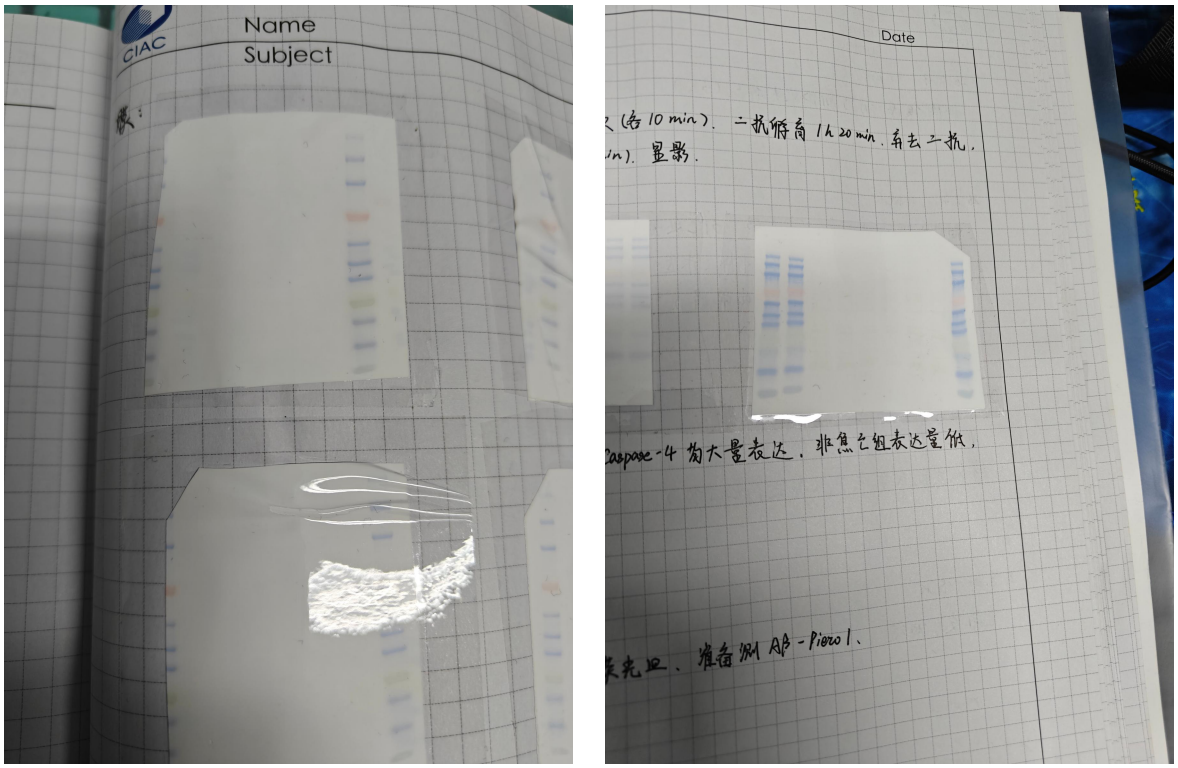
Chemiluminescence image



Source files: Supp-Fig5c_caspase-1_brightfield and Supp-Fig5c_caspase-1_chemilum

Archived membrane records for Fig. 5c

Laboratory notebook photographs of the physical membrane archives. Original photograph identifiers are retained.



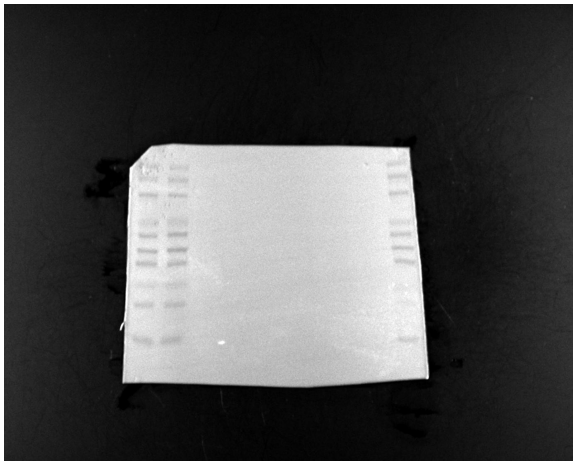
Left: Supp-Fig5c_notebook_archive_1.jpg. Right: Supp-Fig5c_notebook_archive_2.jpg.

Source blots for Fig. S1a

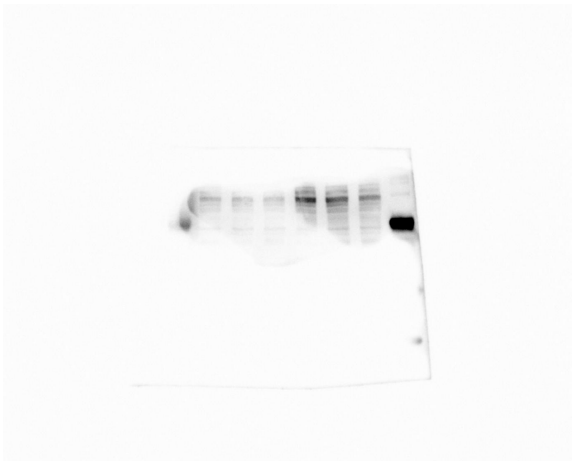
Sample lanes 1–3: undifferentiated SH-SY5Y cells; sample lanes 4–6: differentiated SH-SY5Y cells (three biological replicates per group). Samples were separated on the same SDS-PAGE gel and transferred onto a single PVDF membrane.

βIII-tubulin

Bright-field membrane photograph



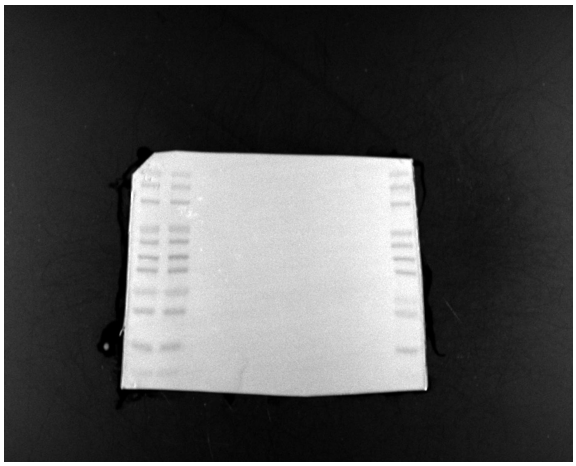
Chemiluminescence image



Source files: Supp-FigS1_βIII-tubulin_brightfield and Supp-FigS1_βIII-tubulin_chemilum

GAPDH

Bright-field membrane photograph



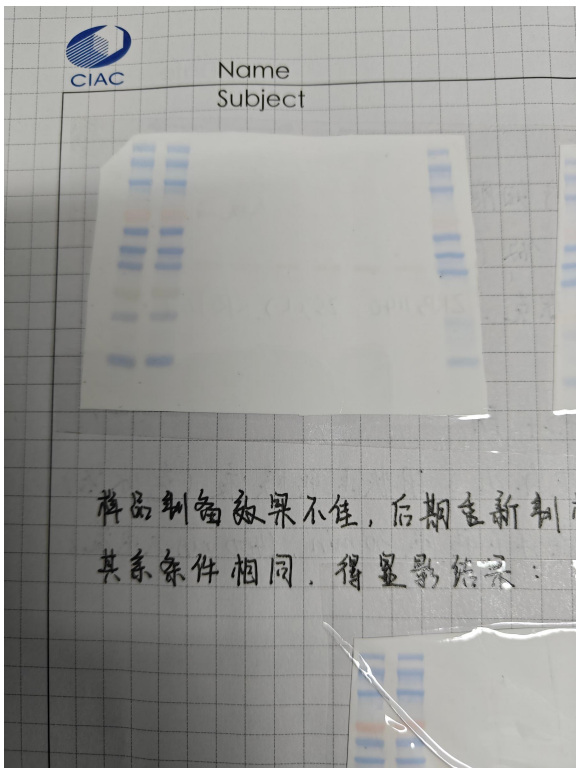
Chemiluminescence image



Source files: Supp-FigS1_GAPDH_brightfield and Supp-FigS1_GAPDH_chemilum

Archived membrane record for Fig. S1a

Laboratory notebook photograph



Source file: Supp-FigS1_notebook_archive