Supplementary information

MATERIALS and METHODS

Reagents and animals

TPEN (cat. T3952) and CQ (cat. T0876) were bought from TOPSCIENCE (Shanghai, China). DMSO (cat. A503039) was purchased from Sangon Biotech (Shanghai, China). Anti-blzf1 (cat. Ab155510), also known as anti-Golgin45, was bought from ABCAM (Shanghai, China). Anti-GRASP55 (cat. sc-136395) and anti-Aβ (cat. sc-28365) were bought from Santa Cruz (Shanghai, China). Anti-NeuN (GB11138) and DAPI (G1012) was bought from Servicebio (Wuhan, China).

KM mouse were obtained from our university. All mouse were 2.5 m.o.a., 25-30g of weight and raised in animal care facility under controlled humidity and temperature, with ad libitum access to water and food. All experimental operations were in compliance with the Guide for Care and Use of laboratory animals published by the National Institute of Health, United States (NIH Publication No.85-23, revised 1985).

Intraperitoneal injection of TPEN and CQ

TPEN was dissolved in DMSO, and then diluted with 0.9% saline to final concentration of 5mM (5% DMSO). CQ was prepared by dissolving in DMSO and then diluted by castor oil to a final concentration of 4mg/mL. TPEN (10mg/kg) and CQ (50mg/kg, 100mg/kg) were performed to mouse daily by i.p. injection for 30 days.

NOR tests

This test mainly has three steps which are habituation, training and retention sessions. At first, mice were put into the arena for 10 minutes to reduce anxiety caused by strange environment. Then, training trial was carried out. Two identical objects were fixed in the back of the box. Mice were put into the box for 10 minutes and the time that mice explored each object should be recorded. A retention session was performed 24 hours later. During retention session, one of the object should be replaced by an object with different shape and color. The time spent on each object should be recorded again, new one as (a) and old one as (b). Climbing on the object was not regard as exploring. The discrimination index (DI) was calculated as (a)/((a)+(b)).

Electron Microscopy

Hippocampus from brain of mouse were dissected and cut into small pieces (thick of one dimension <1 mm). Sample was fixed in 2.5% pre-cold glutaraldehyde for 2-4 h. 0.1 M PB buffer (pH7.4) was used to wash samples for 3 times with 15 min each. Then, samples were postfixed with 1% OsO4 in 0.1 M PB buffer at room temperature for 2 h. 0.1 M PB buffer (pH7.4) was used to wash samples for 3 times with 15 min each again. The samples were further dehydrated with ethanol and embedded with Epon at 37℃ overnight, then 60℃ for 48 h. The samples were ultrasectioned to 60 nm, stained with 2% uranyl, and contrasted with lead citrate.

Paraffin Section and Immunofluorescence

The newly dissected total cerebrum was quickly transferred to ice-cold 4% paraformaldehyde (PFA) solution. The fixed tissues were cut into portions and imbedded with paraffin after dehydrate. Tissues should be cut into 5μm slices. Paraffin sections were deparaffinized in xylene twice with 5 min each. After successively hydrating with 100% ,95% ethanol, tissues were rinsed in distilled water and PBS-Tween 20. Blocking step was performed with 3% BSA for 30 minutes at room temperature. Sections were incubated with primary antibody overnight at 4℃. After rinsing in PBS-Tween 20, sections were incubated with secondary antibody for 30 minutes at room temperature. Then, PBS-Tween 20 was used

to rinse sections and DAPI was used to counterstain. Sections were washed with PBS-Tween 20 again and dehydrated through 95% ethanol for 2 min, 100% ethanol for 2×3 min. Finally, coverslip were sealed with anti-fade mouting medium. Nikon Eclipse Ti-SR was used to acquire all images.

ITC Procedure

All the proteins for ITC experiments were expressed and purified individually. The Golgin45 C-terminal peptide with GST tag was obtained by GST beads. GRASP55 (2–208) with a His6 tag and its mutants were purified using affinity beads (nickel-nitrilotriacetic acid agarose). All samples were collected at each step and analyzed by SDS-PAGE. The measurements were conducted using an ITC-200 microcalorimeter (MicroCal) at 23 °C. All the samples were dialyzed into 20 mM Tris, 100 mM NaCl, 5mM EDTA/5mM $ZnSO_4$ buffer prior to the ITC experiments.

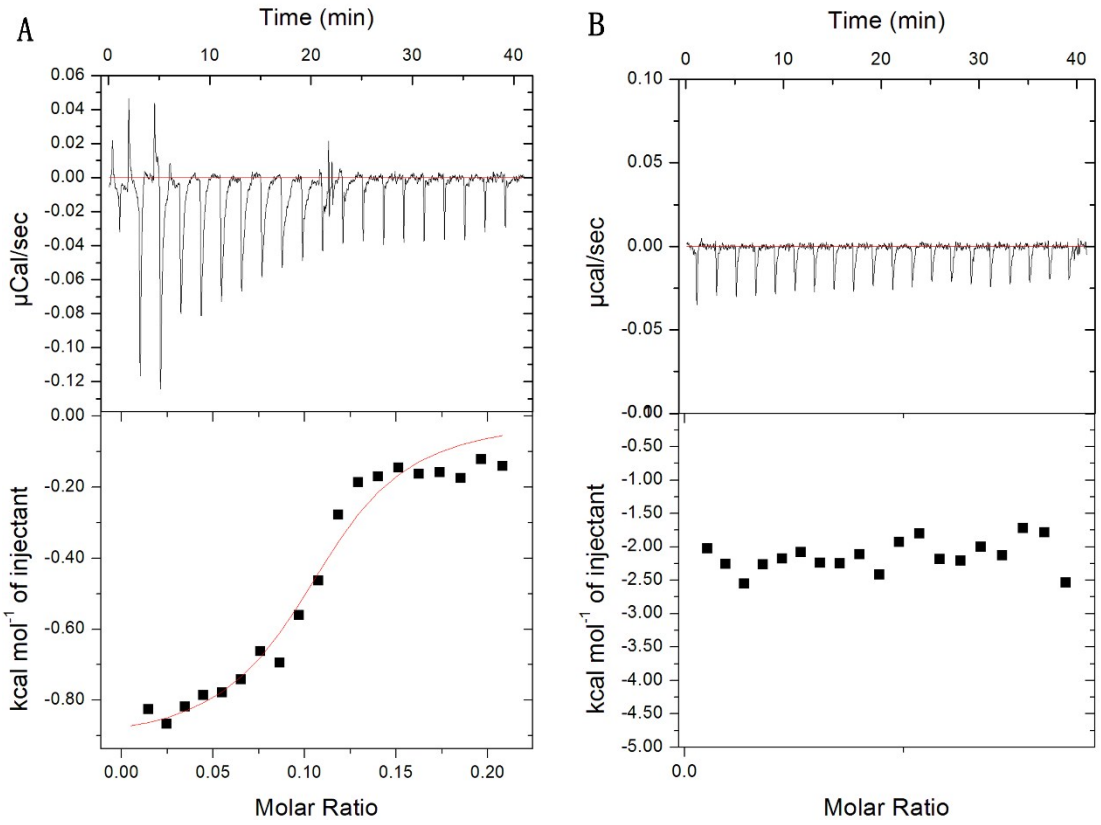

Fig.S1. ITC experiments of GRASP55-Golgin45 C-terminal interaction. GRASP55 titrate Golgin45 C-terminal peptides with 5mM $ZnSO_4$ (A) and with 5mM EDTA (B).