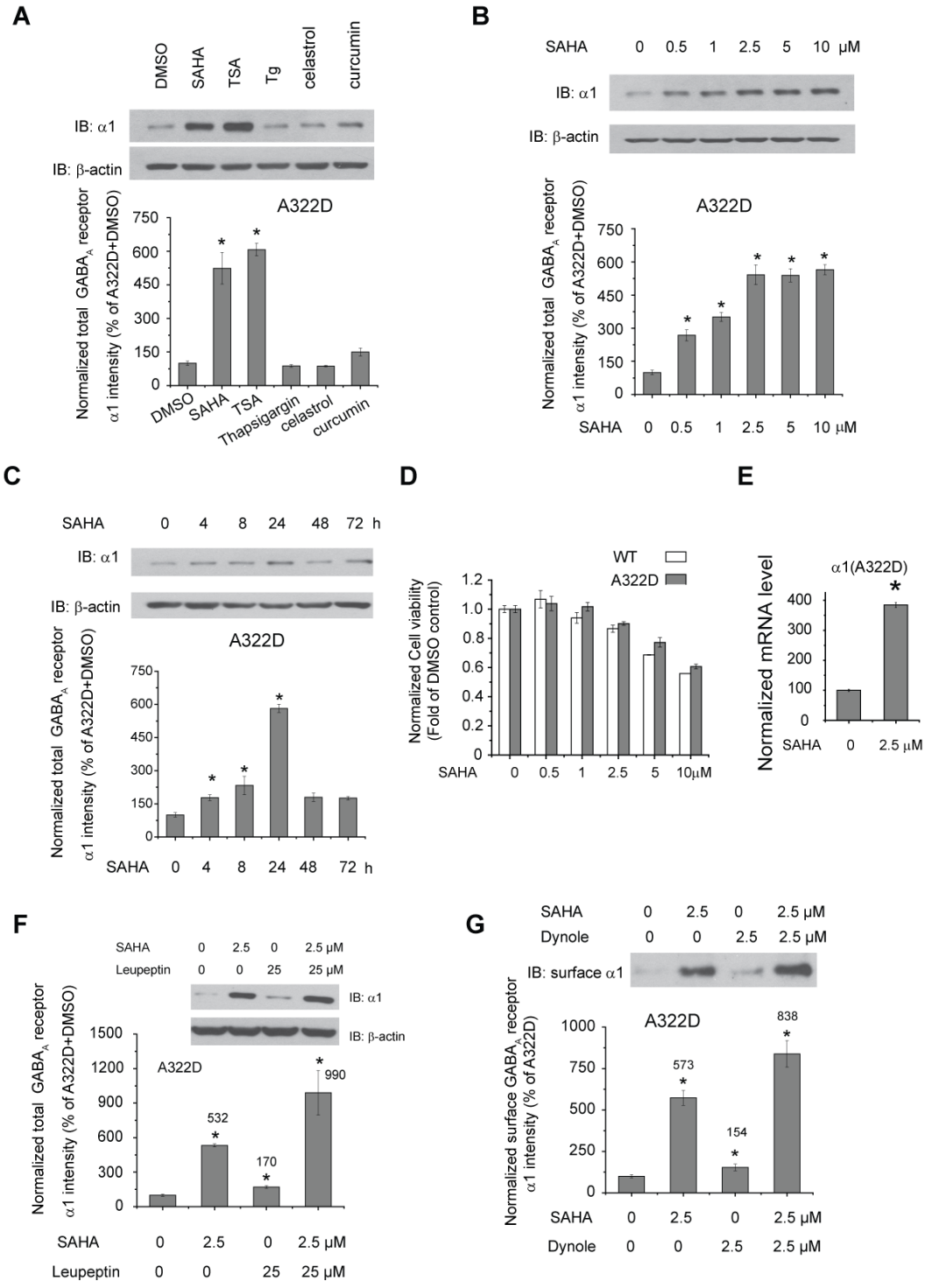

Figure S1, Related to Figure 1. SAHA's effect on the α1(A322D) subunit in HEK293 cells stably expressing epilepsy-associated α1(A322D)β2γ2 $GABA_A$ receptors.

(*A*) SAHA (2.5 μM, 24 h) and TSA (0.5 μM, 24 h) increase the total protein expression of the α1(A322D) subunit among the tested small molecules. IB: immunoblotting.

(*B*) Dose-response analysis of SAHA's effect (24 h) on total α1(A322D) subunit.

(*C*) Time-course study of SAHA's effect (2.5 μM) on total α1(A322D) subunit.

(*D*) SAHA treatment (2.5 μM, 24 h) does not substantially decrease cell viability in HEK293 cells stably expressing WT α1β2γ2 or α1(A322D)β2γ2 $GABA_A$ receptors according to MTT toxicity assay (n = 16).

(*E*) SAHA (2.5 μM, 24 h) increases the mRNA level of the α1(A322D) subunit according to quantitative RT-PCR analysis.

(*F*) Co-application of SAHA and leupeptin, a lysosomal protease inhibitor, produces slightly synergistic effect to increase total α1(A322D) subunit.

(*G*) Co-application of SAHA and dynole 34-2, a dynamin I inhibitor, produces additive effect to increase surface α1(A322D) subunit.

Each point is reported as mean ± SEM.

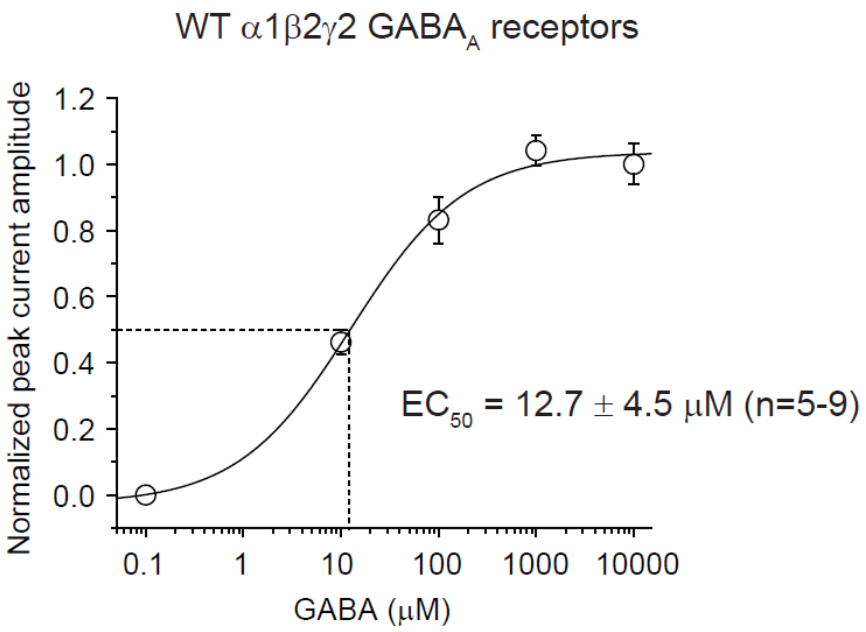

Figure S2, Related to Figure 2. Dose-response curve of GABA in HEK293 cells overexpressing WT α1β2γ2 GABA$_A$ receptors using whole-cell patch-clamping electrophysiology recording. Each point is reported as mean ± SEM.

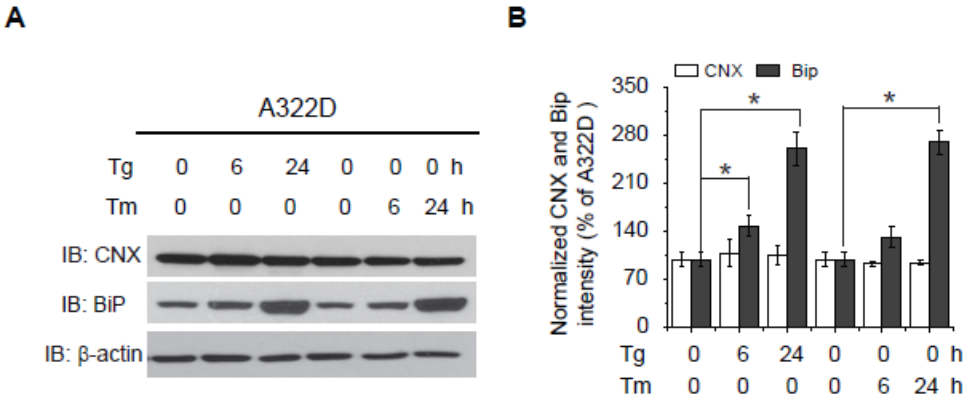

Figure S3, Related to Figure 3. Thapsigargin (Tg, 0.5 μM) and tunicamycin (Tm, 10 μg/ mL), potent UPR inducers, increase the protein level of BiP, but not calnexin (CNX) in HEK293 cells expressing α1(A322D)β2γ2 $GABA_A$ receptors. Each point is reported as mean ± SEM.

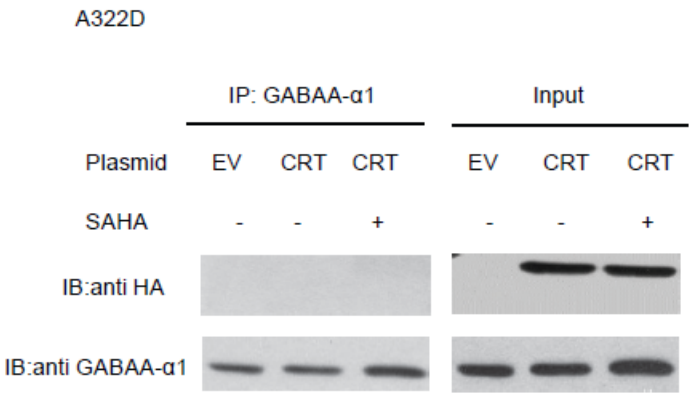

Figure S4, Related to Figure 5. An interaction was not detected between the α1(A322D) subunit and endogenous calreticulin or overexpressed HA-tagged calreticulin with or without SAHA treatment in HEK293 cells expressing α1(A322D)β2γ2 $GABA_A$ receptors. EV: empty vector; CRT: calreticulin.

Table S1, Related to Figure 3. List of primers for genes analyzed.

Name	Accession number	Forward (5'>3')	Reverse (5'>3')
GRP78	NM_005347	GCCTGTATTTCTAGACCTGCC	TTCATCTTGCCAGCCAGTTG
GABRA1	NM_000806	GTCACCAGTTTCGGACCCG	AACCGGAGGACTGTCATAGGT
XBP1	NM_005080	TTACGAGAGAAAACTCATGGC	GGGTCCAAGTTGTCCAGAATGC
GAPDH	NM_002046	GTCGGAGTCAACGGATT	AAGCTTCCCGTTCTCAG

EXTENDED EXPERIMENTAL PROCEDURES

Reagents

SAHA and celastrol were obtained from Cayman Chemical; TSA and leupeptin from Sigma-Aldrich; dynole 34-2 from Tocris Bioscience; and curcumin, thapsigargin and castanospermine from Enzo Life Science. The pCMV6 plasmids containing human $GABA_A$ receptor α1, β2, and γ2 subunits and pCMV6 Entry Vector plasmid (pCMV6-EV) were obtained from Origene. The human $GABA_A$ receptor α1 subunit missense mutation A322D or N38Q/N138Q was constructed using QuickChange II site-directed mutagenesis Kit (Agilent Genomics), and the cDNA sequences were confirmed by DNA sequencing. The pCR(calreticulin)-HA plasmid and pcDNA3.1-BiP plasmid were provided by Professor Tohru Mizushima (Kumamoto University). The Ap^r-M8-CNX plasmid was provided by Professor Michael Brenner (Harvard Medical School). The mouse monoclonal anti-α1 (clone BD24), anti-β2/3 (clone 62-3G1), and rabbit polyclonal anti-γ2 antibodies were obtained from Millipore. The rabbit polyclonal anti-α1 antibody came from R&D systems; the mouse monoclonal anti-β-actin antibody from Sigma; and the rabbit polyclonal anti-calnexin and anti-Hsp70, mouse monoclonal anti-calreticulin and anti-Hsp90, and rat polyclonal anti-Grp94 antibodies from Enzo Life Sciences. The rabbit polyclonal anti-Derlin1 and anti-HA tag antibodies were obtained from Abgent. The rabbit monoclonal anti-BAG2, monoclonal anti-BiP, and polyclonal anti-HDAC7 antibodies were obtained from Epitomics. The rabbit polyclonal anti-ubiquitin antibody was obtained from Cell Signaling.

Cell Culture and Transfection

HEK293 cells and SH-SY5Y cells came from ATCC and were maintained in Dulbecco's Modified Eagle Medium (DMEM) (Hyclone) with 10% heat-inactivated fetal bovine serum (Sigma-Aldrich) and 1% Pen-Strep (Hyclone) at 37°C in 5% CO_2. Monolayers were passaged

upon reaching confluency with TrypLE Express (Invitrogen). HEK293 cells were grown in 6-well plates or 10-cm dishes and allowed to reach ~70% confluency before transient transfection using Fugene 6 (Roche) or Lipofectamine 2000 (Invitrogen) according to the manufacturer's instruction. Stable cell lines expressing α1β2γ2 and α1(A322D)β2γ2 receptors were generated using the G-418 selection method. Briefly, cells were transfected with α1:β2:γ2 (1:1:1) and α1(A322D):β2:γ2 (1:1:1) plasmids, and then maintained in DMEM supplemented with 0.6 mg/mL G418 (Enzo Life Sciences) for 15 days. G-418 resistant cells were selected for follow-up experiments.

Western Blot Analysis

Cells were harvested with TrypLE Express and then lysed with lysis buffer (50 mM Tris, pH 7.5, 150 mM NaCl, and 1% Triton X-100) supplemented with Roche complete protease inhibitor cocktail. Lysates were cleared by centrifugation (15,000 × *g*, 10 min, 4 °C). Protein concentration was determined by MicroBCA assay (Pierce). Endoglycosidase H (endo H) or Peptide-N-Glycosidase F (PNGase F) (New England Biolabs) enzyme digestion was performed according to published procedure (Gallagher, et al., 2005). Aliquots of cell lysates were separated in a 10% SDS-PAGE gel, and Western blot analysis was performed using the appropriate antibodies. Band intensity was quantified using Image J software from the NIH.

MTT cell toxicity assay

Cell viability was measured by quantitative colorimetric assay with MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) (from Amresco) and showed the level of mitochondrial activity in living cells described in published literatures (Denizot and Lang, 1986; Mosmann, 1983). HEK293 cells were plated in a 96-well plate, incubated at 37°C overnight, and then treated with SAHA for 24 h. To each well, 500 μg/mL MTT (final concentration) was added,

and then the plate was incubated at 37 °C for 3 h. MTT was removed, and the crystals were dissolved by DMSO. The absorbance at 595 nm was measured using a microplate reader (Molecular Device). Cell viability was expressed as the ratio of the signal obtained from SAHA-treated samples over DMSO control samples, and data were presented as mean ± SEM.

Quantitative RT-PCR

The relative expression levels of target genes were analyzed using quantitative RT-PCR according to published procedure (Mu, et al., 2008). Briefly, the cells were incubated with drugs at 37 °C for the indicated amount of time before total RNA was extracted from the cells using RNeasy Mini Kit (Qiagen #74104). cDNA was synthesized from 500 ng of total RNA using QuantiTect Reverse Transcription Kit (Qiagen #205311). Quantitative PCR reactions (45 cycles of 15 s at 94°C, 30 s at 57°C, and 30 s at 72°C) were performed using cDNA, QuantiTect SYBR Green PCR Kit (Qiagen #204143) and corresponding primers in the StepOnePlus system (Applied Biosystems) and analyzed using StepOne v2.2 software (Applied Biosystems). The forward and reverse primers for *GABRA1*, *HSPA5* (Gene name of BiP) and *GAPDH* (housekeeping gene control) are listed in Table S1. Threshold cycle (C_T) was extracted from the PCR amplification plot, and the ΔC_T value was defined as: ΔC_T = C_T (target gene) - C_T (housekeeping gene). The relative mRNA expression level of target genes of drug-treated cells was normalized to that of untreated cells: Relative mRNA expression level = 2 exp [- (ΔC_T (treated cells) - ΔC_T (untreated cells))]. Each data point was evaluated in triplicate and measured three times.

Confocal Immunofluorescence

The labeling of cell surface and total α1 subunits and confocal immunofluorescence microscopy analysis was performed according to published procedure (Eshaq, et al., 2010). To label cell

surface α1 subunits, HEK293 cells on coverslips were incubated on ice in 100 μL of HEPES buffer (HEPES 25 mM, NaCl 140 mM, KCl 5.4 mM, $CaCl_2$ 1.8 mM, glucose 15 mM, pH=7.4) containing mouse anti-α1 antibody (clone BD24, 1:100) for 1 h, and then incubated on ice with 1 mL of buffer containing an Alexa 546-conjugated goat anti-mouse antibody (1:200). To label total α1 subunits, HEK293 cells were then fixed with 4% paraformaldehyde, permeabilized with saponin (0.2%), blocked with goat serum for 0.5 h at room temperature, labeled with mouse anti-α1 antibody (clone BD24, 1:100) for 1 h, and then incubated at room temperature with 1 ml of buffer containing an Alexa 488-conjugated goat anti-mouse antibody (1:200) for 1 h. The coverslips were then mounted and sealed. For confocal immunofluorescence microscopy, an Olympus IX-81 Fluoview FV1000 confocal laser scanning system was used. A 60X objective collected images using FV10-ASW software.

Biotinylation of Cell Surface Proteins

HEK293 cells or SH-SY5Y cells stably overexpressing α1β2γ2 or α1(A322D)β2γ2 receptors were plated in 10-cm dishes for surface biotinylation experiments according to published procedure (Lachance-Touchette, et al., 2011). Intact cells were washed twice with ice-cold PBS and incubated with the membrane-impermeable biotinylation reagent Sulfo-NHS SS-Biotin (0.5 mg／mL; Pierce) in PBS containing 0.1 mM $CaCl_2$ and 1 mM $MgCl_2$ (PBS+CM) for 30 min at 4 °C to label surface membrane proteins. To quench the reaction, cells were incubated with 10 mM glycine in ice-cold PBS+CM twice for 5 min at 4 °C. Sulfhydryl groups were blocked by incubating the cells with 5 nM N-ethylmaleimide (NEM) in PBS for 15 min at room temperature. Cells were solubilized for 1 h at 4 °C in lysis buffer (Triton X-100, 1%; Tris–HCl, 50 mM; NaCl, 150 mM; and EDTA, 5 mM; pH 7.5) supplemented with Roche complete protease inhibitor cocktail and 5 mM NEM. The lysates were cleared by centrifugation (16,000 × g, 10 min at 4 °C)

to pellet cellular debris. The supernatant contained the biotinylated surface proteins. The concentration of the supernatant was measured using microBCA assay (Pierce). Biotinylated surface proteins were affinity-purified from the above supernatant by incubating for 1 h at 4 °C with 100 µL of immobilized neutravidin-conjugated agarose bead slurry (Pierce). The samples were then subjected to centrifugation (16,000 ×g, 10 min, at 4 °C). The beads were washed six times with buffer (Triton X-100, 0.5%; Tris–HCl, 50 mM; NaCl, 150 mM; and EDTA, 5 mM; pH 7.5). Surface proteins were eluted from beads by boiling for 5 min with 200 µL of LSB / Urea buffer (2x Laemmli sample buffer (LSB) with 100 mM DTT and 6 M urea; pH 6.8) for SDS-PAGE and Western blotting analysis.

Whole-Cell Patch Clamp Electrophysiology Recording

Whole-cell currents were recorded 48 h post transfection using HEK293 cells using published procedure (Eshaq, et al., 2010). The glass electrodes were pulled from thin-walled borosilicaste capillary glass (Kimble-Chase) and fire-polished on a DMZ Universal puller (Zeitz Instruments), having a tip resistance of 3–5 MΩ. The internal solution contains 153 mM KCl, 1 mM $MgCl_2$, 5 mM EGTA, 10 mM HEPES, and 2 mM MgATP (pH 7.3). The external solution contains 142 mM NaCl, 8 mM KCl, 6 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM glucose, and 10 mM HEPES (pH 7.4). Coverslips containing HEK293 cells were placed in a RC-25 recording chamber (Warner Instruments) on the stage of an Olympus IX-71 inverted fluorescence microscope and superfused with external solution. Fast GABA application was accomplished with a pressure-controlled perfusion system (Warner Instruments) positioned within 50 µm of the cell utilizing a Quartz MicroManifold with 100-µm inner diameter inlet tubes (ALA Scientific). The whole cell GABA-induced currents were recorded at a holding potential of -20 mV in voltage clamp mode using an

Axopatch 200B amplifier (Molecular Devices). The signals were filtered at 2 kHz and detected at 10 kHz using pClamp10 acquisition software.

ER Fraction Enrichment

Cells were harvested with TrypLE Express and then lysed with lysis buffer without detergent (50 mM Tris, pH 7.5, 150 mM NaCl) supplemented with Roche complete protease inhibitor cocktail. Lysates were centrifuged at 800 ×*g* for 10 min at 4 °C to pellet nuclei and cell debris. The supernatant was further centrifuged at 10,000 ×*g* for 15 min at 4 °C to pellet mitochondria. The supernatant was then ultracentrifuged at 100,000 ×*g* for 65 min at 4 °C to pellet the ER. The ER pellet was then resuspended with lysis buffer with detergent (50 mM Tris, pH 7.5, 150 mM NaCl, and 1% Triton X-100) supplemented with Roche complete protease inhibitor cocktail for immunoprecipitation.

Immunoprecipitation

Cell lysates (500 µg) were pre-cleared with 30 µL of protein A/G plus-agarose beads (Santa Cruz) and 1.0 µg of normal mouse IgG for 1 hour at 4°C to remove nonspecific binding proteins. The pre-cleared cell lysates were incubated with 2.0 µg of mouse anti-α1 antibody (clone BD24) for 1 hour at 4°C, and then with 30 µL of protein A/G plus agarose beads overnight at 4°C. The beads were collected by centrifugation at 8000 ×g for 30 s, and washed three times with lysis buffer. The α1 subunit complex was eluted by incubation with 30 µL of SDS loading buffer in the presence of DTT. The immunopurified eluents were separated in 8% SDS-PAGE gel, and Western blot analysis was performed.

siRNA Transfection

HEK293 cells were seeded at approximately 2.5 x 10^5 cells per well in 6-well plates for the siRNA treatment. Cells were allowed to reach ~70% confluency before transfection. The

following small interfering RNA (siRNA) duplexes were obtained from Dharmacon: calnexin (J-003636-07-0005), BiP (J-008198-06-0005), HDAC7 (J-009330-10-0005), and Non-Targeting (NT) siRNA (D-001810-01-20) was used as a negative control. Cells were transfected with corresponding 50 nM siRNA using HiPerfect Transfection Reagent (Qiagen) according to the manufacturer's transfection protocol prior to protein analysis. Forty-eight hours post-transfection, cells were harvested, lysed, and subjected to SDS-PAGE and Western blot analysis.

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