Supplementary Information

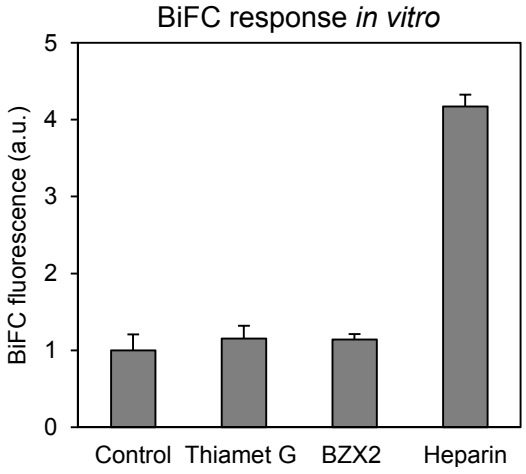

Figure S1. OGA/OGT (*O*-GlcNAcase/*O*-GlcNAc transferase) inhibitor do not directly regulate purified tau aggregation *in vitro*. Tau-BiFC cell lysates (0.4 mg/mL) was incubated with Thiamet G or BZX2 (0.1 mg/mL). After 48 h, BiFC fluorescence intensity was quantified by microplate reader (λex = 430 nm; λem = 525 nm). $p > 0.05$.

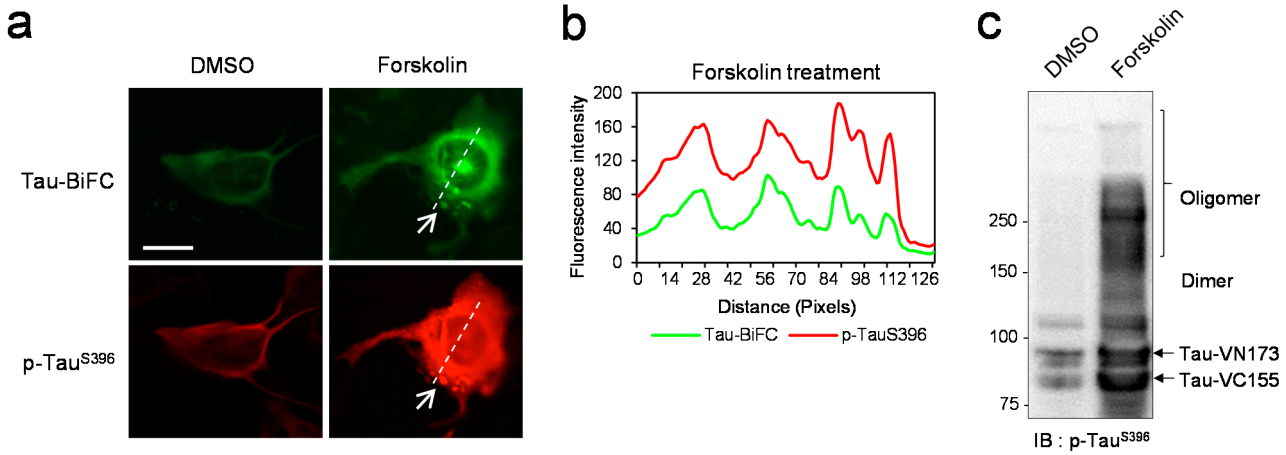

Figure S2. Intracellular tau oligomerization and aggregation were showed by native gel analysis and immune-fluorescence microscopy. (**a**) Confocal microscopy images of Tau-BiFC clearly showed the formation of tau aggregates (arrows) induced by forskolin (90 μM). Most of the tau aggregates were co-localized with tau stained with anti-phospho-Ser396 antibody. Scale bar = 50 μm; (**b**) Correlation graph between tau phosphorylation and tau aggregation. Fluorescence intensities of tau-BiFC cell according to the distance (Pixels, dotted line in (**a**)) were quantified by ImageJ software (Version 1.48, National Institutes of Health, Bethesda, MD, USA); and (**c**) Native gel analysis visualized the formation of tau oligomers induced by forskolin. Under basal condition, only two bands of tau-BiFC fragments are visible at 76 and 85 kDa. Upon the treatment of forskolin, ladders of tau dimers and oligomers appeared in the range of 150–300 kDa.

1. Supplementary Methods

1.1. Quantification of Tau-BiFC Response in Vitro

To quantify tau-BiFC response by Thiamet G and BZX2 *in vitro*, tau-BiFC cell lysates were prepared by using CelLytic M (Sigma, St. Louis, MO, USA) containing protease and phosphatase inhibitor cocktail (Sigma). 0.4 mg/mL of tau-BiFC cell lysates were incubated with Thiamet G (0.01 mg/mL) or BZX2 (0.01 mg/mL) or Heparin (0.01 mg/mL). After 48 h, 40 µL tau-BiFC cell lysates were transferred to a black 384-well plate and the BiFC fluorescence signal was measured at excitation wavelength of 430 nm in Flexstation2 spectrophotometer (Molecular Devices, Sunnyvale, CA, USA).

1.2. Immunofluorescence Analysis

To detect tau phosphorylation in tau-BiFC cells, the cells were fixed in 3.7% formaldehyde (Sigma) after 48 h of forskolin treatment. Then, the fixed cells were incubated in PBS containing 0.1% triton-X for permeabilization followed by PBS wash. The tau-BiFC cells underwent blocking step with 4% BSA for 1 h, and incubated with primary phospho-tau Ser396 antibody (1:500, Abcam, Cambridge, MA, USA) overnight at 4 °C. Next day, tau-BiFC cells were stained with Alexa Fluor 633-conjugated secondary antibodies (1:1000, Abcam). Images were obtained by confocal microscopy.