Supporting Information

Immunocytochemistry

Primary cortical neurons grown on glass coverslips were fixed by 4% paraformaldehyde (PFA) in PBS for 15 min, washed with PBS 3 times, and subsequently permeabilized with 0.1% Triton X-100 (Sigma) in PBS. The cells were incubated with Rabbit anti-hPBEF (1:300, Bethyl Laboratories) and Mouse anti-MAP2 (1:300, Millipore) overnight at 4°C. The cells were then incubated with secondary antibodies conjugated with either FITC or Rhodamine (1:100, Chemicon) for 4 h at RT and counterstained with DAPI to label nuclei. Fluorescent images were taken by a Nikon FN1 epi-fluorescence microscopy (Nikon, NY, USA) equipped with a CoolSNAP-EZ CCD-camera (Photo-metrics, Tucson, AZ, USA).

Intracellular NAD^+ assay

The concentration of NAD^+ was measured in a similar way as previously described (Zhang et al. 2010) using NAD^+ assay kit according to the manufacturer's instructions (Bioassay Systems, Hayward, CA, USA). Cortical neurons cultured in a 6-well culture plate (~10^6 cells in each well) were washed with cold PBS for 2 times. Then the freshly harvested cortical neurons were placed into a 1.5 mL tube with a 100 μL NAD^+ extraction buffer and were homogenized. The extracts were heated at 60°C for 5 min, and 20 μL assay and 100 μL opposite extraction buffers were added to neutralize the extracts. The samples were briefly mixed, then spun down at 14,000 r.p.m. for 5 min. The supernatant was used for an NAD^+ enzymatic assay according to the manufacturer's instructions. The NAD^+ concentration was obtained from calibration curve using standard NAD^+ from the kit; NAD^+ content was expressed as a percentage of the value from the control group.

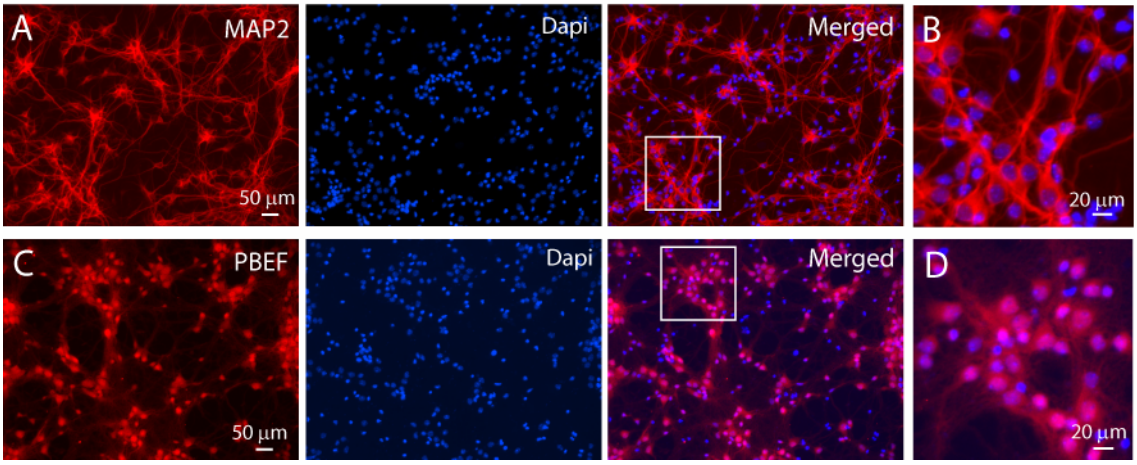
Intracellular ATP assay

The concentration of ATP was measured using an ATP assay kit (Bioassay Systems, CA, USA). Briefly, neurons cultured in a 6-well plate were washed with PBS and lysed by a lysis buffer. Each sample was placed into 1.5 mL tubes, sonicated and spun down at 14,000 r.p.m. for 5 min. The supernatant was used for ATP assay according to the manufacturer's instructions for a Fluoroskan Ascent FL luminometor (Thermo Scientific). The ATP concentration was obtained from a calibration curve using standard ATP from the kit; ATP content was expressed as a percentage of the value from control group.

Transient transfection of neuronal cell cultures

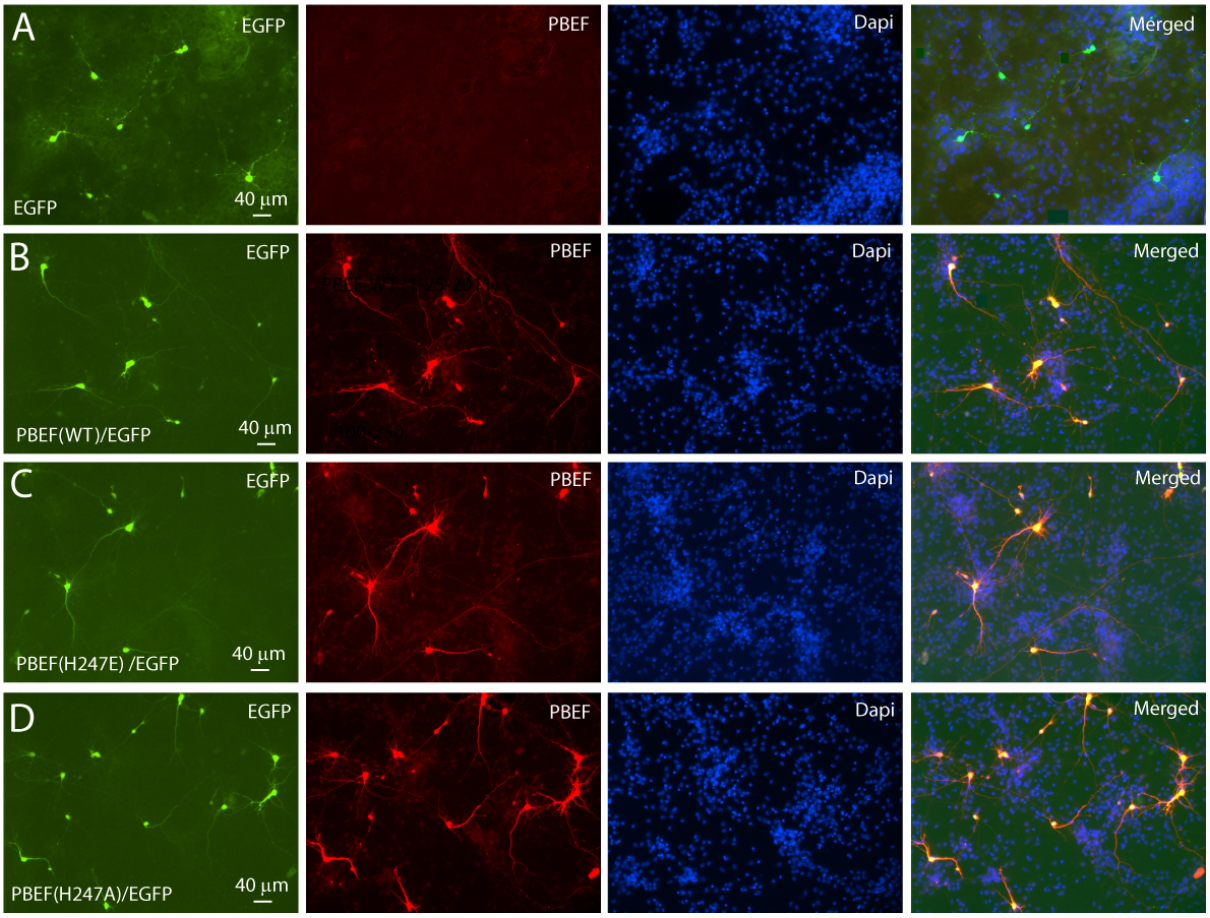
Neurons cultured on glass coverslips were transfected with DNA plasmids with a CAGGS promoter (pCAGGS) encoding EGFP, wild type (WT) and mutant hPBEF (i.e., H247A and H247E) cDNAs using Lipofectamine^{TM} 2000 (Cat#11668-019, Invitrogen), according to the manufacturer's specifications. Neurons expressing WT or mutant hPBEFs were identified with cotransfection of EGFP using a fluorescent microscope. For one well in a 24-well plate, 0.8 μg of WT, or mutant hPBEF DNA plasmid, and 0.8 μg of EGFP DNA plasmid were mixed with 50 μL antibiotics-free neurobasal medium in the first tube; and 2 μL Lipofectamine^{TM} 2000 was mixed with 50 μL antibiotics-free neurobasal medium in a second tube. Then the diluted DNA and Lipofectamine^{TM} 2000 were mixed gently and incubated for 20 min at room temperature (RT). The DNA-Lipofectamine^{TM} 2000 complexes were gently added into culture well containing 500 μL antibiotics-free neurobasal medium. After the neurons were cultured at 37 °C for 6 h, the medium was replaced with normal culture medium. To determine the efficiency of cotransfection, neurons were immunostained with antibody against hPBEF 2 days after transfection.

Supplemental Fig 1



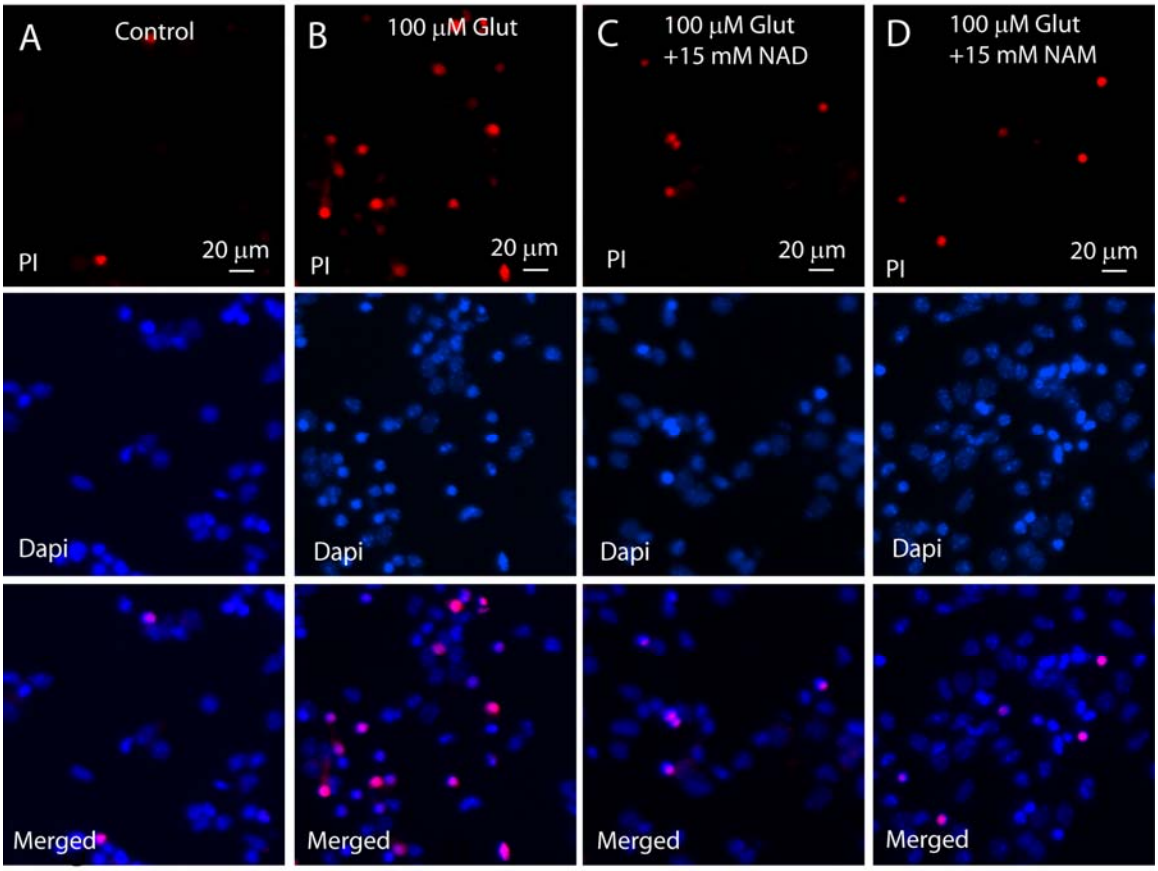
Supplemental Fig 1. Expression of MAP2 and PBEF in cultured neurons. Immunostaining for MAP2 (A) and PBEF (B) in cortical cultured neurons. Right panels are the high resolution images of the boxed regions in merged images.

Supplemental Fig 2



Supplemental Fig 2. Co-transfection of neuronal cultures (A) Epi-fluorescence images of PBEF and EGFP signals in neurons transfected by EGFP alone. Notice there is no EGFP+ cell that expresses enhanced PBEF. (B-D) Epi-fluorescence images of PBEF and EGFP signals in neurons cotransfected by WT hPBEF (B), H247E (C) and H247A (D) mutants with EGFP. Notice the enhanced PBEF expression levels of EGFP+ cells as compared with cells without transfection. Our data from 4-8 immunostaining experiments indicate 100% EGFP+ cells express enhanced WT and mutant hPBEF, therefore, EGFP can be considered to be a marker for PBEF overexpressing neurons after transfection. During imaging, we adjusted exposure time so that PBEF signals in non-transfected neurons are weak.

Supplemental Fig 3



Supplemental Fig 3. Representative images of PI staining to determine neuronal death induced by glutamate excitotoxicity. A-D) Images of PI and Dapi staining of neurons under control condition (A), after glutamate stimulation in the absence (B) and the presence of 15 mM NAD^+ (C) and 15 mM NAM (D).