Anti-EGF antibody
SH-SY5Y Immunoprecipitation test

A. SH-SY5Y cells were collected into SDS, cell lysates sonicated for 30. Mass-normalized (BCA) aliquots were resolved on 5–20% gradient SDS-PAGE and electrotransferred onto a nitrocelulose membrane. The membrane was incubated with the anti-EGF primary antibody (1:3000 diluted, overnight incubation), followed by incubation with anti-rabbit HRP-linked secondary antibody (1:5000, 2h), and submitted to ECL-based detection.

B. For the immunoprecipitation, cells were lysed in non-denaturating buffer (10mM Tris-Hcl, 7.5; 0.5% NP-40; 150 mM NaCl; 0.5 mM EDTA; protease and phosphatase inhibitors), and samples were IPed using 5 µL of EGF antibody (‘αEGF’) + 25 µL ProteinG Dynabeads, or with rabbit IgGs (‘rbIgGs’,same dilution as the EGF antibody) + 25 µL ProteinG Dynabeads. The following steps were performed as in A. ‘*’ high pro-EGF (described to be around 140 kDa); ‘**’low pro-EGF (cleaved; around 70kDa, also reported in body fluids).