Supplemental figure 1

(A)

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  1 MNRAFSRKKD KTWMHTPEAL SKHFIPYNAK FLGSTEVEQP KGTEVVRDAV 50
 51 RKLKFARHIK KSEGQKIPKV ELQISIYGVK ILEPKTKEVQ HNCQLHRISF 100
101 CADDKTDKRI FTFICKDSES NKHLCYVFDS EKCAEEITLT IGQAFDLAYR 150
151 KFLESGGKDV ETRKQIAG                                  168
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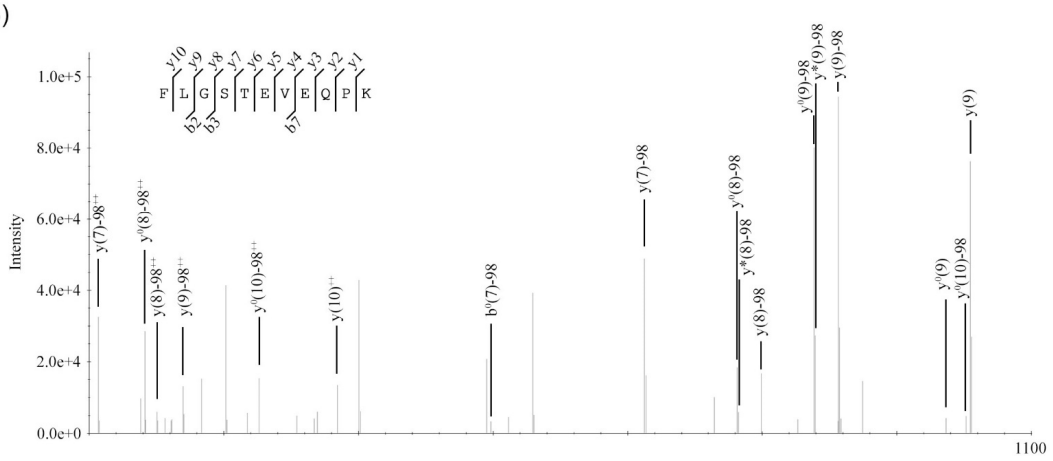
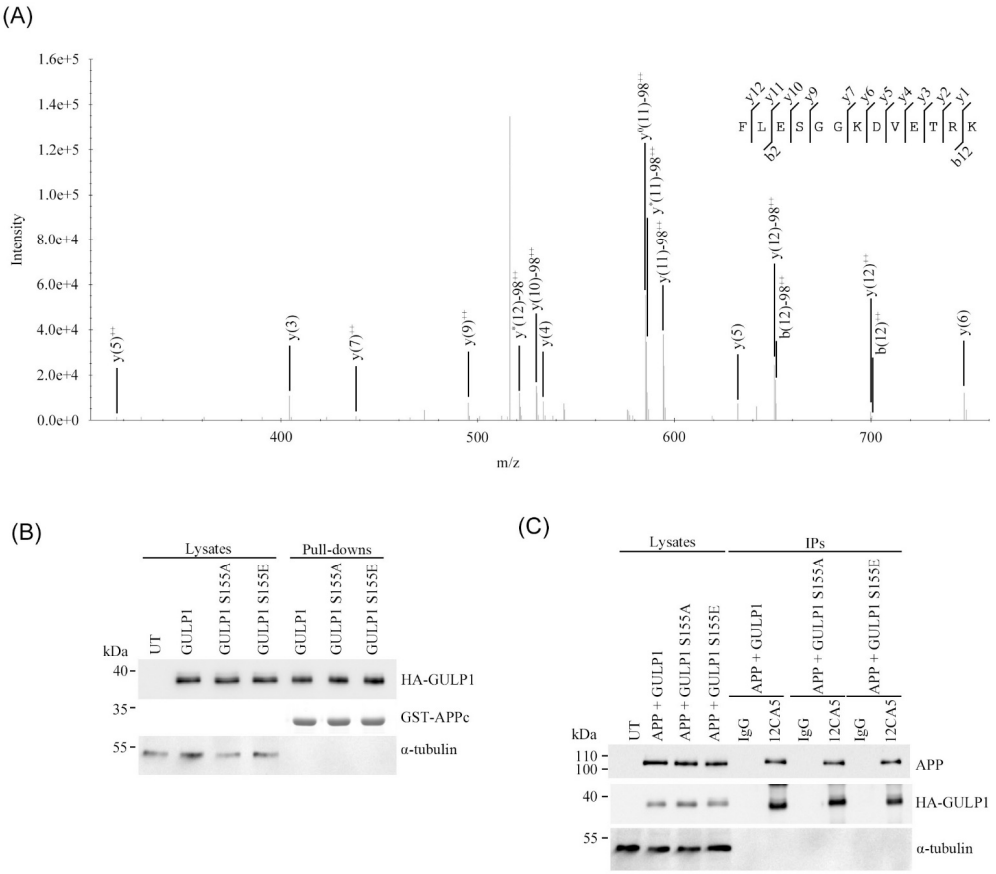
(B)



Supplemental figure 1. Identification of PKCζ target residues in GULP1 PTB by mass spectrometry. (A) Amino acid sequence of human GULP1 PTB domain (residues 1-168). Residues sequenced by mass spectrometry are bold. Residues T35 and S155 are boxed (B) Tandem MS/MS spectrum for GULP1 phosphopeptide FLGSTEVEQPK (residues 31-41). The m/z of the fragment ions is plotted against intensity. The m/z range has been zoomed to highlight the fragment ions which unambiguously define the phosphorylation site within the peptide. Nomenclature of fragment ions is as described (Roepstorff and Fohlman 1984). The presence of y(7) ions with neutral loss of phosphoric acid (97.9769 Da) assigns the phosphorylation to the specific threonine residue corresponding to GULP1 T35.

Supplemental figure 2



Supplemental figure 2. Phosphorylation of GULP1 S155 does not alter GULP1-APP interaction. (A) Tandem MS/MS spectra of GULP1 phosphopeptide FLESGGKDVETRK (residues 152-164). All the y ions past y(10) showed a peak with neutral loss of phosphoric acid (97.9769 Da), identifying GULP1 S155 as the phosphorylation site. Nomenclature of fragment ions is as described (Roepstorff and Fohlman 1984). (B) Bacterially expressed GST-APPc was used as bait for GST pull-down assay from GULP1, GULP1 S155A or GULP1 S155E-transfected cell lysate. GULP1 were detected by a rat anti-GULP1. Bottom panel shows Coomassie Blue staining of GST-APPc bait used. (C) Co-immunoprecipitation was performed from cells transfected with APP+HA-GULP1, APP+HA-GULP1 S155A or APP+HA-GULP1 S155E using a mouse anti-HA antibody 12CA5. APP and GULP1 in the immunoprecipitates (IPs) were detected by a rabbit anti-APP and a rat anti-GULP1.

Roepstorff, P. and J. Fohlman (1984). "Proposal for a common nomenclature for sequence ions in mass spectra of peptides." Biomed Mass Spectrom 11(11): 601.