Figure S3. Role of EGF receptor transactivation in IGF-1- and PACAP-associated phosphoproteomes in cortical neurons

(A) PACAP and IGF-1 transactivate the EGFR. Cortical neurons were treated for 10 min with either IGF-1 (2.5 ng/ml) or PACAP (1 nM) in the absence or presence of U73122 (10 μM) or Rp-cAMPS (10 μM). EGFR activation was measured by sequential immunoblotting with antibodies against phospho-Tyr1068 or phospho-Tyr845 EGFR and antibodies recognizing the EGFR independently of its phosphorylation state. (B) EGFR transactivation mediates the phosphorylation of Erk1,2 induced by PACAP. Cortical neurons were treated for 10 min with PACAP (1 nM) in the absence or presence of either U73122 (10 μM), Rp-cAMPS (10 μM) or AG1478 (250 nM) and Erk1,2 phosphorylation was analyzed by sequential immunoblotting with antibodies against phospho-Erk1,2 and total Erk1,2. Quantification of Erk1,2 phosphorylation level was performed by densitometric analysis using the NIH Image software. Data are means ± SEM of values obtained in three independent experiments. ** p<0.01, versus corresponding values measured in the absence of PACAP, §§ p<0.01, versus corresponding values measured in neurons treated with PACAP alone (ANOVA followed by Student Newman Keul’s test). (C) EGFR transactivation plays a minor role in PACAP-
associated phosphoproteome. Cortical neurons were exposed to PACAP (1 nM) + AG1478 (250 nM) for 10 min. Phosphoproteins were enriched by PMAC, separated onto 2-D gels and stained with silver. The arrowheads indicate the position of protein spots whose phosphorylation in response to PACAP treatment is altered in the presence of AG1478. Blots and gels representative of three experiments performed of different sets of cultured neurons are illustrated.