Supplementary Figure 1. NGF and BDNF stimulate p75 receptor cleavage in RN22 cells.

RN22 cells were treated with 10 μM ZLLLH alone (Control) or in the presence of 100 ng/ml of NGF for indicated periods (A) or with 100 ng/ml of BDNF for 12 hrs (B). The cells were lysed and the lysates Western blotted using an antibody to the ICD of p75. The full length receptor (~ 75 kDa, FL), the carboxy-terminal fragment, which includes the transmembrane domain (~ 30 kDa, CTF), and the intracellular domain (~ 25 kDa, ICD) are indicated.
Supplementary Figure 2. The p75 chimeric receptor (p75-FasTM) co-immunoprecipitates with wild type p75 and blocks its cleavage by γ-secretase.
(A) 293 cells were transfected with wild type p75 receptor alone or co-transfected with p75-FasTM receptor. 48 hrs later the cells were treated with 10 µM ZLLLH alone or in the presence of 100 ng/ml of BDNF for 12 hr. Cell lysates were collected and approximately 500 µg of protein was analyzed by Western blotting using an antibody to the p75 ICD. The full length receptor (~ 75 kDa, FL), the carboxy-terminal fragment (~ 30 kDa, CTF), and the intracellular domain (~ 25 kDa, ICD) are indicated. Note that the p75 mutant receptor prevented the production of the ICD from the wild type receptor.

(B) 293 cells were transfected with GFP tagged p75 receptor alone or co-transfected with HA tagged wild type p75 or p75-FasTM chimera. Forty hours later the cells were lysed and immunoprecipitated with an HA antibody and Western blotted with anti-GFP or anti-HA (upper panels). Cell lysates were also subjected to GFP and HA Western blot analysis to confirm the expression of the proteins (lower panels).

(C) 293 cells were transfected with p75-FasTM, pcDNA3-NRIF and pFlag-TRAF6, and 48 hrs later treated with the proteasome inhibitor (10 µM ZLLLH) in the presence or absence of 1 µM PMA for 2 hrs. The cells were then lysed, immunoprecipitated with an NRIF antibody and Western blotted using anti-p75ICD (upper panel) or anti-NRIF (lower panel). Note that the full length (FL) and carboxy-terminal fragment (CTF) of the p75-FasTM receptor co-immunoprecipitated with NRIF, but not the ICD, since this chimeric receptor is resistant to γ-secretase-mediated cleavage.

(D) RN22 cells were transfected with wild type p75 or p75-FasTM or left untransfected. After 48 hrs, the plasma membrane was isolated from the cells and Western blotted using antibody to p75 or CYP-17 (a P450 enzyme found only in endoplasmic reticulum).
Supplementary method:

Plasma membrane preparation

RN22 cells were transfected with wild type p75 or p75-FasTM, and 48 hours later plasma membrane was isolated as described by Kanning et al (2003) and subjected to p75 and CYP-17 (a P450 enzyme found only in the endoplasmic reticulum, kindly provided by Dr. Michael Waterman, Vanderbilt University, Nashville, TN) Western blotting.