# The p75NTR intracellular domain generated by neurotrophin-induced receptor cleavage potentiates Trk signaling

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## Summary

The p75 neurotrophin receptor (p75NTR) potentiates Trk signaling, but the underlying mechanisms remain uncertain. Here, we examine the relationship between p75NTR cleavage and Trk signaling. We found that, in PC12 cells, nerve growth factor (NGF) induces rapid and robust α-secretase- and γ-secretase-dependent cleavage of p75NTR, releasing the resulting intracellular domain into the cytosol. Brain-derived neurotrophic factor similarly induces p75NTR cleavage in primary cerebellar granule neurons. p75NTR cleavage occurs by means of Trk-dependent activation of MEK-Erk signaling and induction of α-secretase activity, and is independent of ligand binding to p75NTR. Neurons and PC12 cells lacking p75NTR display defects in neurotrophin-dependent Akt activation. Normal Akt activation is rescued using full-length p75NTR or the p75 intracellular domain, but not cleavage-resistant p75NTR. We then demonstrate that NGF-dependent growth arrest of PC12 cells requires p75NTR cleavage and generation of the intracellular domain. We conclude that generation of the soluble p75NTR intracellular domain by Trk-induced cleavage plays a fundamental role in Trk-dependent signaling events.

**Key words:** Neurotrophin, NGF, BDNF, Cerebellar granule neuron, PC12 cell, Cell cycle

## Introduction

The neurotrophins play crucial roles in the development and maintenance of the nervous system by binding to Trk receptors, cell-surface tyrosine kinases, and to the p75 neurotrophin receptor (p75NTR), a member of the tumor necrosis factor (TNF) receptor superfamily. Trk-dependent signaling cascades have been thoroughly characterized, and it is well established that Trk receptor activation facilitates neuronal survival, growth and synaptic activity (Patapoutian and Reichardt, 2001). p75NTR has several functions and participates in developmental and injury-induced apoptosis (Nykjaer et al., 2005), growth of developing neurons, neuronal survival, neuronal growth inhibition (Lee et al., 1992b; von Schack et al., 2001; Yamashita et al., 1999) and cell migration (Johnston et al., 2007; Snapyan et al., 2009). An emerging consensus is that p75NTR collaborates with a diverse group of cell-surface proteins to mediate discrete signaling events in a cell-specific and developmentally regulated manner (Barker, 2004).

It has long been known that p75NTR potentiates Trk signaling (Barker and Shooter, 1994; Hantzopoulos et al., 1994) and helps neurons respond to low doses of neurotrophins in vitro and in vivo (Davies et al., 1993; Ito et al., 2002; Ito et al., 2003; Lee et al., 1994; Yan et al., 1991; Lee et al., 1992b; von Schack et al., 2001; Yamashita et al., 1999). It is well established that p75NTR achieves this, at least in part, by enhancing neurotrophin binding to the TrkA receptor (for reviews, see Barker, 2007; Chao, 2003). However, the possibility that p75NTR signaling might synergize with signaling events initiated by the TrkA receptor has not been extensively explored (Wehrman et al., 2007). We have previously shown that overexpression of the p75NTR intracellular domain (p75ICD) induces activation of phosphoinositide 3-kinase (PI3K) and Akt in PC12 cells (Roux et al., 2001). In light of recent data showing that p75ICD is produced through a two-step process involving α- and γ-secretase activities (Kenchappa et al., 2006; Urra et al., 2007; Weskamp et al., 2004; Zampieri et al., 2005), we have considered the possibility that the p75ICD might facilitate Trk signaling.

In this report, we demonstrate that activation of Trk receptors results in cleavage of p75NTR through a α-secretase- and γ-secretase-dependent pathway in both PC12 cells and cerebellar granule neurons (CGNs). We demonstrate that the p75ICD plays an important role in facilitating neurotrophin-induced Akt phosphorylation and that generation of the p75ICD is required for nerve growth factor (NGF)-dependent growth arrest of PC12 cells. These studies reveal complex interplay between Trk activation, generation of the p75ICD and facilitation of Trk-induced signaling events.

## Results

### Cleavage of p75NTR in PC12 cells requires Trk activation, but not ligand binding to p75NTR

Recent studies revealed that p75NTR undergoes regulated intramembrane proteolysis in response to NGF, brain-derived neurotrophic factor (BDNF), pro-BDNF and myelin-associated glycoprotein (MAG). Direct ligand binding to p75NTR has been reported to activate p75NTR cleavage, but TrkA activation has also been suggested to initiate p75NTR processing (Kenchappa et al., 2006; Urra et al., 2007). To clarify the propensity of various ligands to induce p75NTR proteolysis, we directly compared NGF, BDNF and MAG for their ability to induce p75NTR cleavage in

PC12 cells. Previous studies have shown that p75ICD accumulation requires proteasome inhibition (Kanning et al., 2003) and we therefore included the proteosome inhibitor epoxomycin in these studies. Immunoblots using an antibody directed against the p75ICD revealed that 6 hours of NGF treatment (50 ng/ml) leads to the accumulation of a p75NTR cleavage product of 20 kDa, whereas cleavage products did not accumulate following BDNF exposure (50 ng/ml) (Fig. 1A). Treatment of PC12 cells with a MAG-Fc fusion protein (20 μg/ml) resulted in the production of a small amount of p75NTR cleavage product, but the amount produced was much lower than that induced by NGF. We then characterized the dose response and time course of NGF-induced cleavage of p75NTR. Fig. 1B shows that NGF at 2 ng/ml was sufficient to induce p75NTR cleavage and that maximal cleavage was observed using 10 ng/ml NGF. In addition, p75ICD could be detected by immunoblot only when proteasomal activity was inhibited using epoxomycin. We also found that accumulation of the p75ICD was rapid and observed detectable levels of p75ICD after 5 minutes of NGF treatment, with levels steadily increasing at later times (Fig. 1B, right).

To confirm the identity of the p75NTR cleavage products induced by NGF treatment, PC12 cells were exposed to NGF in the presence of the α-secretase inhibitor GM6001 and the γ-secretase inhibitors DAPT or compound XXI. The γ-secretase inhibitors blocked the NGF-dependent generation of the 20 kDa band and favored the accumulation of a 25 kDa product (supplementary material Fig. S1A). Production of the 20 and 25 kDa fragments was blocked by the α-secretase inhibitor GM6001 (supplementary material Fig. S1B). We conclude that NGF treatment of PC12 cells results in stepwise proteolysis of p75NTR, first through α-secretase activity, which generates the 25 kDa p75NTR C-terminal fragment (p75CTF), and subsequently through γ-secretase, which results in production of the 20 kDa p75ICD.

Because BDNF and NGF both bind p75NTR, but only NGF induces p75NTR cleavage, occupancy of p75NTR with ligand might not be sufficient to induce receptor cleavage. Of these two ligands, only NGF induces TrkA activation and we therefore asked whether TrkA activation was required to induce p75NTR cleavage, first by analyzing NGF-induced p75NTR cleavage in PC12nnr5 cells, a derivative PC12 cell line that does not express TrkA (Green et al., 1986). Fig. 1C shows that NGF was unable to induce p75NTR cleavage in this cell line. As a second approach, we assessed NGF-induced p75NTR cleavage in the absence or presence of the Trk kinase inhibitor K252A. Fig. 1D shows that cleavage of p75NTR is completely blocked in PC12 cells treated with K252A, indicating that TrkA activation is required for p75NTR cleavage induced by NGF. Proneurotrophins have been shown to induce p75NTR cleavage in sympathetic neurons (Kenchappa et al., 2006)

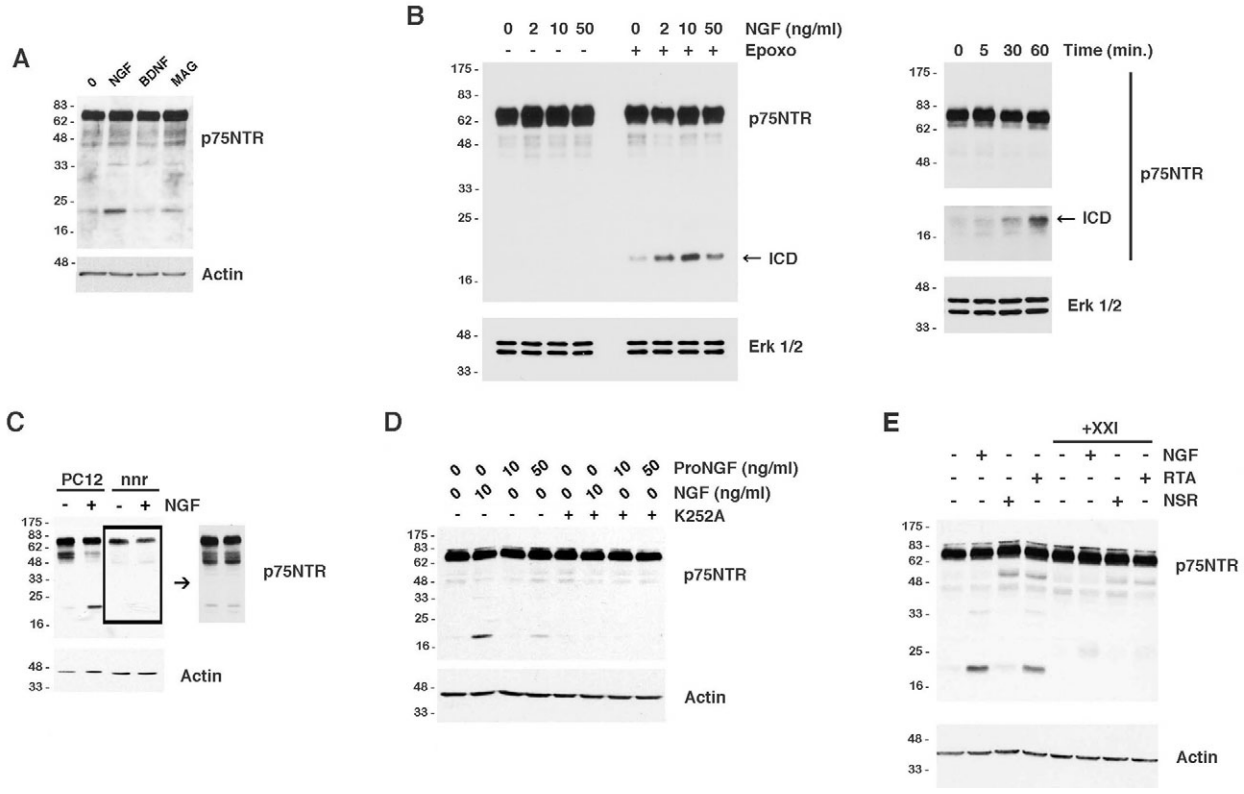


**Fig. 1. NGF-induced cleavage of p75NTR occurs independently of p75NTR binding, but requires Trk activation in PC12 cells.** (**A**) PC12 cells were pre-treated with epoxomycin (0.5 μM) for 1 hour and then treated with NGF (50 ng/ml), BDNF (50 ng/ml) or MAG-Fc (20 μg/ml) for 6 hours. (**B**) PC12 cells were pre-treated with epoxomycin (0.5 μM) for 1 hour and then treated with the indicated concentrations of NGF for 6 hours (left panel) or with 50 ng/ml NGF for the times shown (right panel). (**C**) PC12 cells and PC12nnr cells were pre-treated with epoxomycin (0.5 μM) for 1 hour and then treated with NGF (50 ng/ml) for 6 hours. (**D**) PC12 cells were pre-treated with epoxomycin (0.5 μM) for 1 hour in the absence or presence of the Trk-specific tyrosine kinase inhibitor K252A (250 nM) and then treated with NGF (10 ng/ml) or pro-NGF (at 10 or 50 ng/ml) for 6 hours. (**E**) PC12 cells were pre-treated with epoxomycin (0.5 μM) for 1 hour in the absence or presence of the γ-secretase inhibitor XXI, then treated with NGF (10 ng/ml), RTA IgG (100 μg/ml) or non-specific rabbit (NSR) IgG (100 μg/ml) for 6 hours. For A-E, lysates were analyzed by immunoblot, as indicated. Data are representative of three independent experiments, except for A and B for which data are representative of two independent experiments.

and we therefore tested whether proNGF induced p75NTR cleavage in PC12 cells. We previously showed that proNGF can be endocytosed and cleaved by furin to generate mature NGF, which then activates TrkA (Boutiler et al., 2008). Therefore, we compared NGF and pro-NGF for their ability to induce p75NTR cleavage in the absence or presence of the Trk kinase inhibitor K252A. Fig. 1D shows that proNGF does induce p75NTR cleavage in PC12 cells and that this is completely blocked by K252A. Therefore, it seems likely that proNGF-mediated p75NTR cleavage occurs only after proNGF is processed to mature NGF and thus becomes capable of activating TrkA in PC12 cells.

It is conceivable that occupancy of p75NTR by NGF might be required for a conformational change that allows cleavage to proceed. RTA is an antibody directed against the TrkA extracellular domain that has been previously shown to act as a potent TrkA agonist (Clary et al., 1994). To test whether ligand binding to p75NTR is required for its cleavage, we used RTA to activate the TrkA receptor on PC12 cells. Fig. 1E shows that RTA induced robust cleavage of p75NTR, whereas control IgG had no effect; we conclude that NGF-induced p75NTR cleavage requires TrkA activity, but does not require p75NTR occupancy.

### NGF-induced cleavage of p75NTR requires MEK-dependent activation of α-secretase

In our next set of experiments, we asked which of the canonical Trk signaling pathways is required for NGF-induced p75NTR cleavage. TrkA activation induces the PI3K-Akt cascade, the MEK-Erk pathway and the phospholipase Cγ (PLCγ) cascade. We found that inhibition of the PI3K and PLCγ pathways, using LY294002 and U73122, had no effect on p75NTR cleavage (data not shown). By contrast, U0126 and PD98059, chemically distinct MEK inhibitors, caused dose-dependent inhibition of p75NTR cleavage (Fig. 2A), indicating that TrkA-induced MEK activation is required for p75ICD accumulation. To determine whether MEK activation alone was sufficient to drive p75NTR cleavage, PC12 cells were infected either with recombinant adenovirus expressing a constitutively active form of MEK1 (MEK1*) or with recombinant adenovirus expressing GFP. Fig. 2B shows that MEK1* induces cleavage of p75NTR, whereas the control GFP virus did not.

To address whether MEK activates the initial α-secretase cleavage of p75NTR, we inhibited γ-secretase using compound XXI and then asked whether the NGF-dependent accumulation of the p75CTF is altered in the presence of U0126 or PD98059. Fig. 2C shows that p75CTF accumulation was almost completely blocked by MEK inhibition (compare lanes 6, 7 and 8), indicating that MEK activation of α-secretase is required for the NGF-dependent, α-secretase-mediated cleavage of p75NTR. Taken together, these data indicate that MEK activation induced by NGF-mediated TrkA signaling is sufficient to activate α-secretase-dependent cleavage of p75NTR and to promote production of a p75CTF fragment that is subsequently cleaved by γ-secretase to produce the soluble p75ICD.

### p75ICD generated by Trk activation accumulates in the cytosol

Previous studies have indicated that the p75ICD generated by cleavage accumulates in the cytoplasm and/or nucleus (Frade, 2005; Podlesniy et al., 2006). To determine the subcellular compartment in which the p75ICD accumulates after TrkA activation, we incubated PC12 cells with increasing amounts of NGF for 6 hours in the presence of epoxomycin (to inhibit proteasomal activity). We then produced purified nuclear and non-nuclear fractions, and assessed p75NTR content by immunoblot. NGF treatment clearly increased p75ICD in non-nuclear fractions, but the p75ICD was excluded from the nuclei, at all NGF concentrations tested (Fig. 3A). Immunoblots for Erk and histone deacetylase 1 (HDAC1) confirmed the purity of the non-nuclear and nuclear fractions. We also compared p75ICD accumulation after 6 hours and 24 hours of NGF treatment, in the presence and absence of epoxomycin. Results showed that p75ICD only accumulates in the presence of epoxomycin and only in the non-nuclear fraction (supplementary material Fig. S2). It is noteworthy that exposure to epoxomycin for 24 hours resulted in the accumulation of a high molecular weight form of HDAC1 in the cytosol that probably represents phosphorylated HDAC1 (Gu et al., 2005) and caused a significant reduction in the amount of full-length p75NTR. We also performed immunocytochemistry of PC12 cells that were incubated with or without NGF for 6 hours, in the presence of epoxomycin, using antibodies directed against the p75ICD or the p75NTR extracellular domain (p75ECD). Fig. 3B,C shows that NGF treatment resulted in the accumulation of

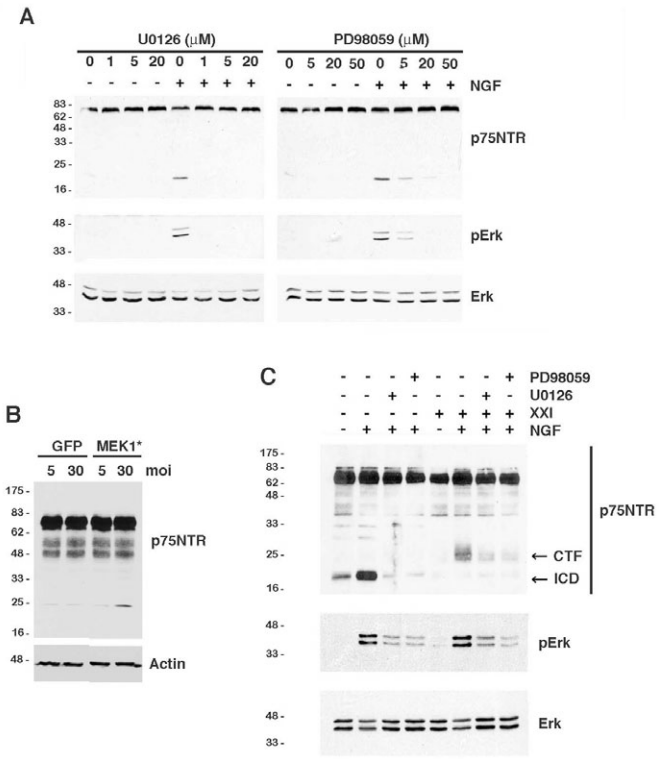


**Fig. 2. NGF-induced cleavage of p75NTR requires MEK-dependent activation of α-secretase.** (**A**) PC12 cells were pre-treated with epoxomycin (0.5 μM) for 1 hour in the absence or presence of the MEK inhibitors U0126 or PD98059, and then treated with 50 ng/ml NGF for 6 hours. (**B**) PC12 cells were infected with an adenovirus driving expression of GFP (control) or constitutively active MEK1 (MEK1*). 48 hours after infection, cells were treated with epoxomycin (0.5 μM) for 6 hours and then lysed. (**C**) PC12 cells were pre-treated with epoxomycin (0.5 μM) for 1 hour in the absence or presence of PD98059 (20 μM), U0126 (1 μM) and the γ-secretase inhibitor compound XXI (10 μM), and then treated with 50 ng/ml NGF for six additional hours. For A–C, lysates were analyzed by immunoblot, as indicated. Data are representative of three independent experiments, except for B for which they are representative of two independent experiments.

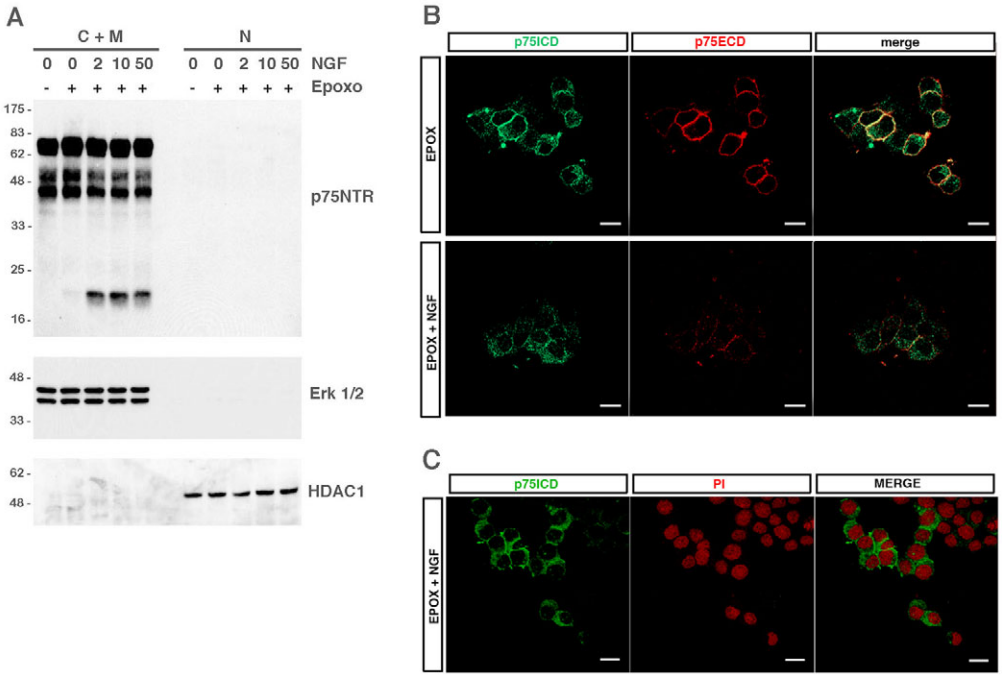


**Fig. 3. The p75ICD generated by NGF treatment accumulates in the cytosol.** (**A**) PC12 cells were pre-treated with epoxomycin (0.5 μM) for 1 hour and exposed to increasing concentrations of NGF (0-50 ng/ml) for 6 hours. Cells were then lysed and fractionated, and lysates were analyzed by immunoblot as indicated (C + M indicates cytosol and membrane, N indicates nuclei). (**B**) PC12 cells were pre-treated with epoxomycin (EPOX; 0.5 μM) for 1 hour, and then treated in the absence or presence of NGF (50 ng/ml) for 6 hours. Cells were next fixed, permeabilized, immunostained with an anti-p75ICD antibody and an anti-p75ECD antibody, and imaged by confocal microscopy. (**C**) PC12 cells were treated as in B, immunostained with an anti-p75ICD antibody, stained with PI and imaged by confocal microscopy. Scale bars: 10 μM. Data are representative of four independent experiments.

cytoplasmic, but not nuclear, p75ICD (Fig. 3C) and that this correlates with decreased cell-surface p75NTR (Fig. 3B). Although we cannot rule out the possibility that low amounts of p75ICD that are below our detection limit reach the nucleus, our data indicate that most of the p75ICD generated by TrkA activation in PC12 cells accumulates in the cytoplasmic compartment.

### A BDNF-TrkB signaling cascade facilitates p75NTR cleavage in cerebellar granule neurons

To determine whether neurotrophin-induced Trk activation also induces p75NTR cleavage in post-mitotic neurons, we examined CGNs. In our first experiments, we treated CGNs with increasing amounts of BDNF or NGF, in the presence of the proteasomal inhibitor epoxomycin, and examined cell lysates for the p75ICD using immunoblots. Fig. 4A shows that the p75ICD was present at low levels in untreated CGNs and that it accumulated in response to all concentrations of BDNF tested (10–300 ng/ml). By contrast, NGF exposure did not increase p75ICD levels at any concentration used (10–300 ng/ml). We then used compound XXI to determine whether BDNF-induced accumulation of the p75ICD required γ-secretase activity. We found that the compound inhibited p75ICD accumulation and caused a corresponding increase in p75CTF levels (Fig. 4B).

CGNs express p75NTR and TrkB, but do not express TrkA. To determine whether TrkB activation was required to induce p75NTR cleavage in post-mitotic neurons, we compared proNGF, proBDNF and BDNF for their ability to induce p75NTR cleavage, in the absence or presence of the Trk kinase inhibitor K252A. For this, we treated CGNs with each of these ligands in the presence of epoxomycin, and in the absence or presence of the Trk inhibitor K252A. Both BDNF and proBDNF caused p75NTR cleavage in CGNs, whereas proNGF had no effect (Fig. 4C). The accumulation of the p75ICD induced by BDNF and proBDNF was completely blocked by K252A, indicating that both ligands induce p75NTR cleavage through a TrkB-dependent pathway. We have recently shown that proBDNF is processed to produce BDNF isoforms that activate TrkB in CGNs (Boutilier et al., 2008). We therefore conclude that BDNF-dependent TrkB activation results in γ-secretase-dependent p75NTR cleavage, whereas occupancy of the p75NTR by either NGF or proNGF does not.

### p75ICD facilitates neurotrophin signaling in PC12 cells and CGNs

Because the p75ICD is generated by Trk activity in both PC12 cells and CGNs, we were interested in determining whether this fragment of the p75NTR might enhance signaling of Trk pathways. To begin to address this possibility, we asked whether blocking p75NTR cleavage using α-secretase or γ-secretase inhibitors altered NGF-induced Erk or Akt signaling in PC12 cells. Although proteasomal inhibition is required to biochemically detect the p75ICD, we presumed that this treatment results in the accumulation of non-physiological levels of the p75ICD; therefore, epoxomycin was excluded from these and subsequent signaling experiments. As expected, Erk and Akt became phosphorylated in PC12 cells when exposed to NGF concentrations ranging from 10-150 ng/ml but, interestingly, in the presence of compounds XXI or GM6001, NGF-induced Erk and Akt phosphorylation was attenuated. Fig. 5A shows that the robust phosphorylation of Akt induced by 10 ng/ml NGF is almost undetectable in the presence of compounds XXI or GM6001, and Erk phosphorylation is significantly reduced.

The reduction in NGF-dependent signaling events induced by compounds XXI or GM6001 indicates that cleavage events mediated by α-secretase and γ-secretase might play an important role in neurotrophin signaling. These data must be interpreted with caution, however, because they do not shed light on the role of p75NTR cleavage in these events. To investigate a specific role for p75NTR cleavage in these pathways, we reduced endogenous p75NTR levels in PC12 cells using a lentivirus that encodes micro RNA (miRNA) targeting rat but not human p75NTR (Bertrand et al., 2008) and then assessed NGF-induced signaling. Fig. 5B shows that NGF-dependent Akt phosphorylation was strongly reduced in PC12 cells in which endogenous p75NTR levels were reduced. We

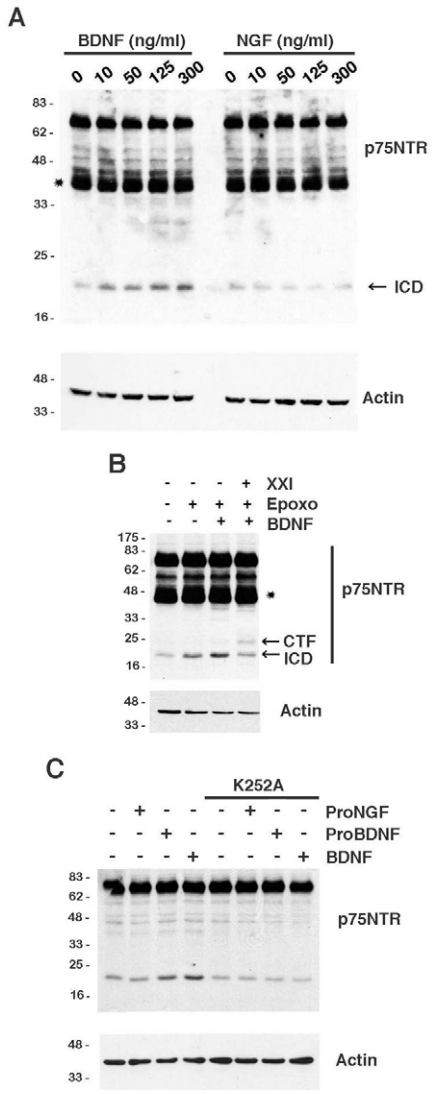


**Fig. 4. A BDNF-TrkB signaling cascade facilitates p75NTR cleavage in CGNs.** (**A**) Rat CGNs were pre-treated with epoxomycin (0.5 µM) for 1 hour, and then treated with BDNF or NGF at the concentrations indicated for 12 hours. (**B**) Rat CGNs were pre-treated with epoxomycin (0.5 µM) for 1 hour in the absence or presence of the γ-secretase inhibitor XXI (5 µM), and then treated with BDNF (50 ng/ml) for 6 hours. (**C**) Mouse CGNs were pre-treated with epoxomycin (0.5 µM) for 1 hour in the absence or presence of the inhibitor K252A (250 nM), and then treated with BDNF (50 ng/ml), pro-BDNF (50 ng/ml) or pro-NGF (50 ng/ml) for 12 hours. For A-C, lysates were analyzed by immunoblot, as indicated. The asterisk in A and B indicates a non-specific band that is detected in rat, but not mouse, CGNs using the anti-p75NTR antibody. Data are representative of three independent experiments, except for C for which they are representative of two independent experiments.

then asked whether we could rescue this signaling defect in the p75NTR-depleted cells by expressing p75NTR constructs derived from the human cDNA, which is not targeted by the rat-specific p75NTR miRNA. Interestingly, full-length p75NTR and the p75ICD restored the signaling defect, whereas p75NTR that was resistant to γ-secretase cleavage could not (Fig. 5B,C), consistent with the hypothesis that Trk-dependent generation of the p75ICD plays an important role in enhancing Trk signaling.

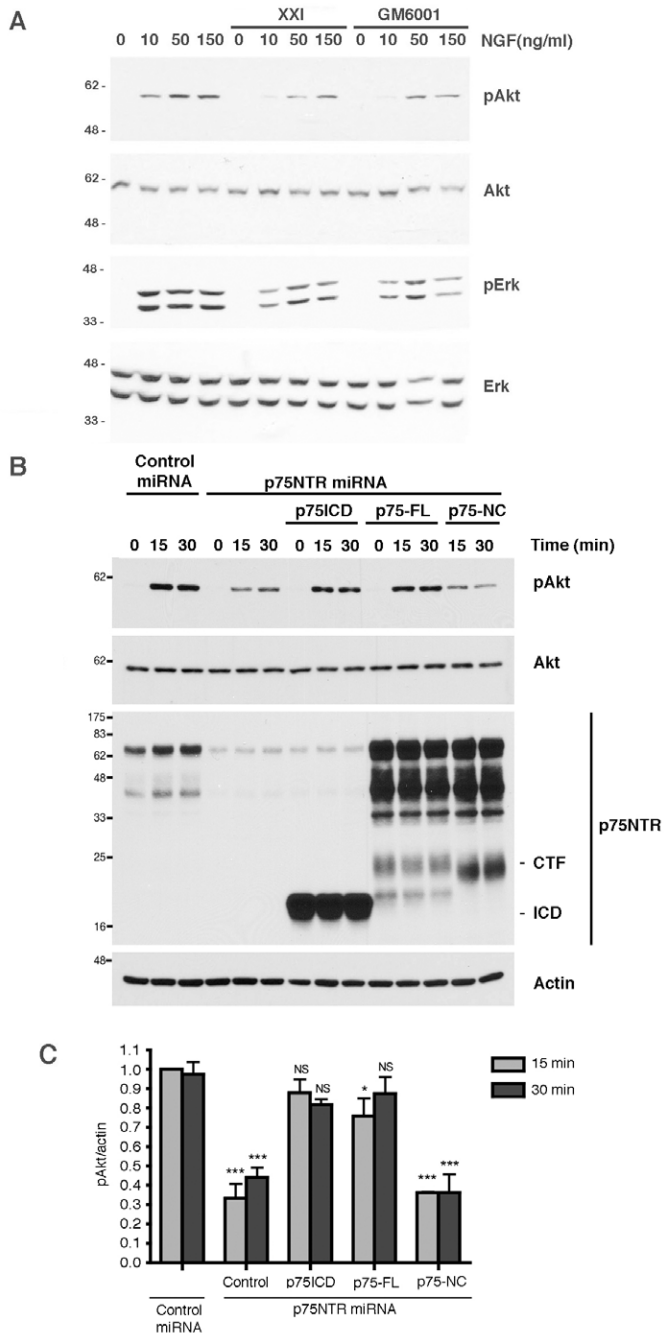


**Fig. 5. The p75ICD facilitates neurotrophin signaling in PC12 cells.** (**A**) PC12 cells were maintained for 1 hour in the absence or presence of the γ-secretase inhibitor compound XXI (5 µM) or the α-secretase inhibitor GM6001 (10 mM), and then cells were exposed to NGF (at 10, 50 or 150 ng/ml) for 3 minutes. Data are representative of three independent experiments. (**B**) PC12 cells were co-infected with a control lentivirus or with lentivirus encoding an miRNA targeting p75NTR, and with lentivirus overexpressing p75ICD, full-length wild-type p75NTR (p75FL) or a full-length non-cleavable form of p75NTR (p75NC). Cells were maintained in PC12 media for 6 days and then treated with NGF (10 ng/ml) for 0, 15 or 30 minutes, as indicated. (**C**) Quantification of three independent experiments from B was performed using ImageJ by measuring the relative pAkt/actin ratio in each condition, considering as 1 the ratio obtained for the second lane (control cells NGF treated for 15 minutes). Comparisons were made between control and indicated treatment groups using two-way ANOVA followed by Bonferroni post tests (*$P$<0.05, ***$P$<0.001). NS, not significant.

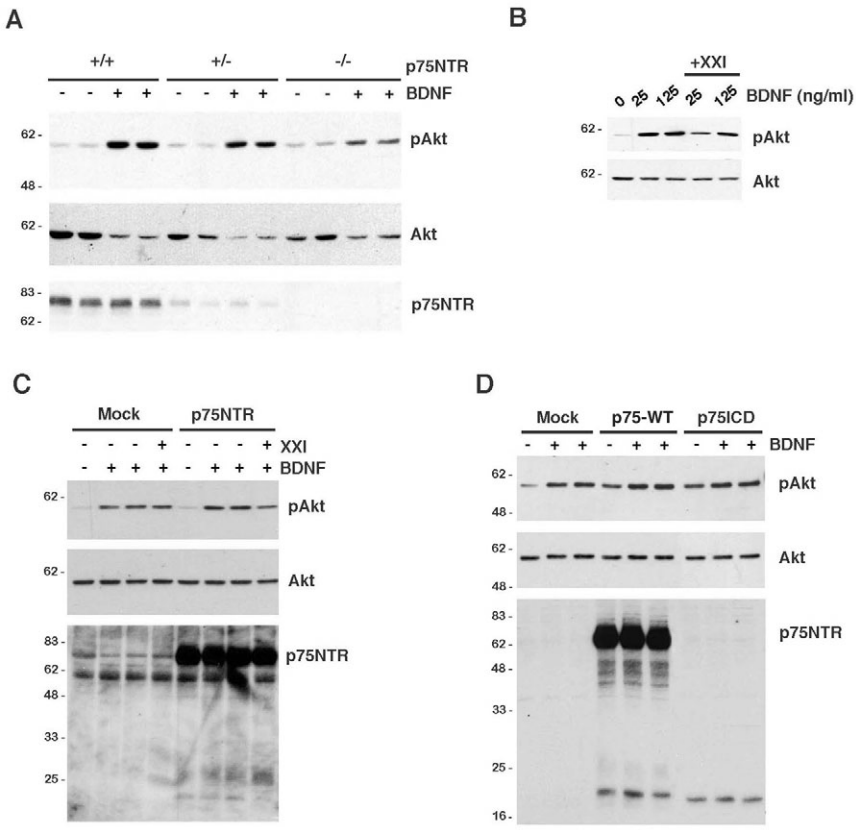


**Fig. 6. The p75ICD facilitates Akt signaling in CGNs.** (**A**) CGNs prepared from *p75ntr*+/+, *p75ntr*–/+ or *p75ntr*–/– littermates were treated with BDNF (5 ng/ml) for 5 minutes. (**B**) Rat CGNs were maintained in the absence or presence of the γ-secretase inhibitor XXI (20 μM) for 1 hour and then cells were exposed to BDNF (5 ng/ml) for 3 minutes. (**C**) CGNs prepared from *p75ntr*–/– pups were transfected with a plasmid encoding p75NTR and, the next day, treated with the γ-secretase inhibitor XXI (10 μM) for 6 hours, followed by 5 ng/ml BDNF for 5 minutes. (**D**) CGNs prepared from *p75ntr*–/– pups were transfected with plasmids encoding full-length p75NTR (p75WT) or p75ICD plasmid and, the next day, treated with BDNF (5 ng/ml) for 5 minutes. For A-D, lysates were analyzed by immunoblot, as indicated. Data are representative of two independent experiments, except for B for which data are representative of three independent experiments.

To extend these results to primary neurons, we asked whether TrkB signaling in primary CGNs was potentiated by p75ICD generation. As a first step, we compared BDNF-induced Akt phosphorylation in CGNs derived from *p75ntr*+/+, *p75ntr*+/–, *p75ntr*–/– littermates. We found that the robust BDNF-dependent phosphorylation of Akt observed in CGNs derived from *p75ntr*+/+ animals was moderately reduced in *p75ntr*+/– neurons and strongly reduced in neurons derived from *p75ntr*–/– mice (Fig. 6A). BDNF-induced Erk phosphorylation showed a similar, albeit less dramatic, variance with p75NTR genotype (data not shown). We then asked whether γ-secretase cleavage facilitates BDNF signaling events and found that compound XXI reduced BDNF-dependent phosphorylation of Akt, but had no effect on BDNF-induced TrkB phosphorylation (Fig. 6B; supplementary material Fig. S3). We conclude that γ-secretase-dependent cleavage events are required for optimal BDNF signaling in CGNs and that p75NTR plays an important role in regulating neurotrophin-induced Akt activation in these cells.

To determine whether p75NTR cleavage is required for BDNF signaling in CGNs, expression plasmids encoding full-length p75NTR were electroporated into *p75ntr*–/– CGNs. Twenty-four 24 hours later, cells were treated with BDNF in the absence or presence of the γ-secretase inhibitor compound XXI. Fig. 6C shows that the Akt phosphorylation that was conferred by the full-length receptor was lost if cells were co-treated with compound. This suggests that generation of the p75ICD is required for normal BDNF-dependent Akt activation in CGNs. To directly test this possibility, expression plasmids encoding full-length p75NTR or the p75ICD were electroporated into *p75ntr*–/– CGNs. Twenty-four hours later, CGNs were treated with BDNF and analyzed for Akt activation. Interestingly, both basal and, to a lesser extent, BDNF-induced activation of Akt were enhanced in the presence of p75NTR and the p75ICD (Fig. 6D). We conclude that γ-secretase-induced cleavage of p75NTR and generation of the p75ICD are required for facilitation of Akt phosphorylation in primary CGNs.

## p75NTR cleavage promotes NGF-induced growth arrest of PC12 cells

To assess whether the effects of the p75ICD on Trk signaling have functional consequences, we turned to NGF-induced growth arrest of PC12 cells. It is well established that TrkA activation is required for NGF-induced differentiation and cell-cycle exit (Green et al., 1986; Gryz and Meakin, 2003; Loeb and Greene, 1993; Rende et al., 2000). Interestingly, recent studies have suggested that p75NTR contributes to NGF-dependent growth arrest of PC12 cells (Ito et al., 2002; Ito et al., 2003). To confirm this, we reduced endogenous p75NTR levels using lentiviral-driven miRNA and showed that growth arrest induced by NGF is reduced in PC12 cells lacking this receptor. We then asked whether expression of p75NTR constructs resistant to the miRNA knockdown were capable of rescuing NGF-induced growth arrest. Fig. 7 shows that expression of full-length p75NTR or the p75ICD rescued the growth-arrest phenotype, but full-length p75NTR that was cleavage resistant did not. We and others have shown that massive overexpression of full-length p75NTR or the p75ICD induces caspase cleavage and cell death (Bhakar et al., 2003; Kenchappa et al., 2006; Salehi et al., 2006), but supplementary material Fig. S4 shows that the modest overexpression of p75NTR or p75ICD used in this paradigm has no effect on caspase activation. Therefore, we conclude that cleavage of p75NTR and generation of the p75ICD contributes to NGF-induced growth arrest in PC12 cells.

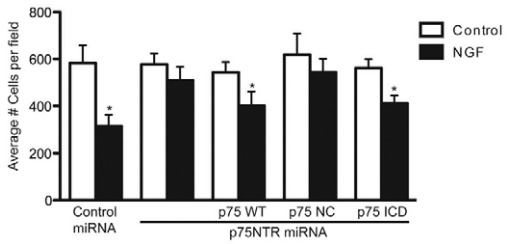


**Fig. 7. p75NTR cleavage promotes NGF-induced cell-cycle exit.** PC12 cells were infected with a control lentivirus or with lentivirus encoding an miRNA targeting p75NTR, and then co-infected with lentivirus overexpressing full-length wild-type p75NTR (p75WT), a full-length non-cleavable form of p75NTR (p75NC) or p75ICD. Cells were treated with 10 ng/ml NGF 1 day after seeding and, 6 days later, cells were fixed and cell number was determined on a MetaExpress apparatus. Significant differences between non-treated and NGF-treated cells were determined using two-way ANOVA followed by Bonferroni post tests (*$P$<0.001).

## Discussion

The p75NTR is involved in a wide array of functions, in part because p75NTR participates in several distinct cell-surface signaling platforms. A complex comprising p75NTR and sortilin mediates apoptotic responses to proneurotrophins, whereas a p75NTR, LINGO-1 and Nogo receptor (NgR1) complex functions as a receptor for myelin-based growth inhibitors (Bandtlow and Dechant, 2004; Barker, 2004; Nykjaer et al., 2005). Intriguingly, regulated intramembrane proteolysis of p75NTR might be a key step in the activation of each of these p75NTR signaling pathways (Domeniconi et al., 2005; Kenchappa et al., 2006). p75NTR also forms complexes with members of the Trk receptor family; this association enhances Trk activation and signaling under physiological conditions (Barker, 2007). However, the mechanisms that allow p75NTR to facilitate Trk signaling remain uncertain. In this study, we show that Trk activation causes robust p75NTR cleavage in cell lines and in primary neurons, and that proteolytic processing of p75NTR and generation of the p75ICD is required to enhance Trk signaling events.

Previous studies have shown that occupancy of p75NTR by NGF or BDNF induced regulated intracellular proteolysis of the receptor, and that MAG binding to NgR1 resulted in p75NTR cleavage (Domeniconi et al., 2005; Kenchappa et al., 2006; Urra et al., 2007). We directly compared NGF, BDNF and MAG for their capacity to induce p75NTR processing in PC12 cells, and found that NGF treatment caused rapid and obvious cleavage of p75NTR. MAG had a much more modest effect and BDNF did not induce any p75NTR cleavage. We used several lines of evidence to show that the robust p75NTR cleavage that was induced by NGF relied on Trk receptor activation. In this regard, our results are entirely consistent with earlier findings from the Bronfman group (Urra et al., 2007).

BDNF has been shown to induce p75NTR cleavage in primary sympathetic neurons. In PC12 cells, however, BDNF does not activate p75NTR processing (Kenchappa et al., 2006) (and this paper). We found that a bivalent TrkA-specific antibody that functions as a TrkA agonist was an effective trigger of p75NTR cleavage in PC12 cells. Therefore, although p75NTR cleavage might be activated by ligand binding in some circumstances, p75NTR occupancy is not a prerequisite for receptor cleavage mediated by TrkA receptor activation. Taken together with data showing that BDNF treatment of CGNs caused TrkB- and γ-secretase-dependent p75NTR cleavage, we conclude that Trk-dependent p75ICD generation is a physiological event that occurs in primary neurons.

Two earlier studies reported that p75ICD can accumulate in the nucleus (Frade, 2005; Podlesniy et al., 2006), but nuclear accumulation of the p75ICD after NGF treatment of PC12 cells was not observed in our studies. Our results are more consistent with the non-nuclear location of the p75ICD reported by Kenchappa and colleagues (Kenchappa et al., 2006). Thus, the ability of the p75ICD to accumulate in different subcellular compartments might vary with cell type.

We have found that MEK signaling is necessary and sufficient for Trk-induced p75NTR cleavage. A role for mitogen-activated protein kinase (MAPK) signaling in receptor processing is not limited to p75NTR, as cleavage of other type I transmembrane proteins requires activation of the MAPK pathway (Fan and Derynck, 1999; Gechtman et al., 1999). The precise protease(s) that mediates α-secretase-dependent p75NTR cleavage in vivo is not certain, but p75NTR cleavage induced by PMA is blocked in cells lacking ADAM17, a member of the disintegrin and metalloprotease family (Weskamp et al., 2004). Interestingly, Erk phosphorylates ADAM17 at threonine 735 and phosphorylation at this site reportedly enhances cell-surface tumor necrosis factor-α converting enzyme (TACE) activity (Diaz-Rodriguez et al., 2002; Fan and Derynck, 1999; Soond et al., 2005). Determining precisely how TrkA-induced MEK activation causes p75NTR cleavage will be an important topic for future studies.

Why is the p75ICD generated as a function of Trk activation? Because it is well established that p75NTR can act as a positive physiological modulator of Trk action (Barker and Shooter, 1994; Hantzopoulos et al., 1994; Hempstead et al., 1991; Lee et al., 1994), we considered the possibility that generation of the p75ICD induced by Trk activation feeds back to facilitate Trk-dependent signaling cascades. We found that depletion of p75NTR from PC12 cells or from CGNs dramatically reduced neurotrophin-induced Akt phosphorylation and showed that full-length p75NTR or the p75ICD, but not a cleavage-resistant p75NTR, rescues the neurotrophin signaling defects in both PC12 cells and CGNs. It is noteworthy that the main effect of p75ICD overexpression in CGNs is to increase basal Akt phosphorylation, whereas its main effect in PC12 cells is to facilitate NGF-induced Akt phosphorylation. We believe this reflects differences in the expression levels obtained in each system (lentivirus for PC12 cells; electroporation for CGNs), because we have previously shown that the p75ICD does increase basal Akt phosphorylation in PC12nnr cells if very highly overexpressed (using adenovirus) (Roux et al., 2001). It is also important to note that p75NTR dimerization is crucial for signaling (Vilar et al., 2009) and that the cleavage-resistant p75NTR used in our studies might not precisely mimic the signaling properties of the full-length receptor. Nonetheless, experiments with chemical secretase inhibitors and the p75NTR constructs used in this study are uniformly consistent with the hypothesis that p75NTR cleavage plays an important role in modulating Trk signaling.

Our data suggest that Trk-mediated MEK activation induces α-secretase activity and p75NTR cleavage is then initiated. Once released from its transmembrane tether, p75ICD accumulates in the cytosol, where it positively modulates Akt signaling. We previously showed that overexpression of the p75ICD inhibits cytoplasmic protein tyrosine phosphatase (PTPase) activity and

increases Akt phosphorylation (Roux et al., 2001). Therefore, our working hypothesis is that the p75ICD functions to inhibit PTEN activity, thereby indirectly enhancing Akt phosphorylation. Determining precisely how the p75ICD acts to regulate PTEN is the subject of ongoing studies.

Although it is well established that NGF-induced PC12 growth arrest is dependent on Trk activation, we have found that PC12 growth arrest induced by NGF also requires p75NTR cleavage and generation of the p75ICD. A defect in NGF-induced growth arrest was observed in PC12 cells lacking endogenous p75NTR expression, consistent with previous findings (Ito et al., 2003). We showed that this could be rescued by expression of full-length p75NTR, but not of a non-cleavable form of p75NTR. Importantly, direct expression of the p75ICD also rescued NGF-induced growth arrest in cells depleted in endogenous p75NTR. Taken together, our data show that NGF-dependent activation of TrkA induces cleavage of p75NTR and that newly generated p75ICD then feeds back to enhance Trk signaling and facilitate Trk-dependent growth arrest. We speculate that this mechanism might explain why neuroblastoma cells require p75NTR for NGF-induced growth arrest and why primary neuronal precursor cells require p75NTR for BDNF-induced differentiation (Lachyankar et al., 2003; Hosomi et al., 2003).

In closing, our findings reveal a novel and intimate bi-directional signaling pathway between Trk receptors and p75NTR. Neurotrophin-induced Trk activation functions as the initial trigger for p75NTR cleavage, and the p75 intracellular domain that is generated functions to enhance Trk signaling pathways and facilitate neuronal differentiation.

## Materials and Methods

### Animals

Sprague Dawley rats and C57BL/6 mice were purchased from Charles River Laboratory (Wilmington, MA). Mice bearing the p75NTRExonIII mutation (Lee et al., 1992a) were originally obtained from the Jackson Laboratory (Bar Harbor, ME) and maintained on a C57BL/6 background.

### Reagents and plasmids

Antibodies directed against Akt, phospho-Akt, Erk and phospho-Erk (pErk) were obtained from Cell Signaling Technology (Danvers, MA). The antibody against actin was obtained from MP Biomedicals (Irvine, CA). The polyclonal and monoclonal antibodies against p75NTR, and the polyclonal antibodies against TrkA (RTA and 203) and TrkB (RTB) have been previously described (Clary et al., 1994; Hempstead et al., 1992; Huang et al., 1999; Majdan et al., 1997). Horseradish-peroxidase-conjugated secondary antibodies were purchased from Jackson ImmunoResearch (West Grove, PA); NGF, BDNF and proBDNF were from Alomone labs (Jerusalem, Israel); and proNGF was from Scil Proteins (Saale, Germany). Cell-culture reagents were from BioWhittaker (Walkersville, MD). All other reagents were from Sigma, Gibco or Calbiochem. MAG fused to immunoglobulin heavy chain (MAG-Fc) was purified on protein A-sepharose beads from media conditioned by HEK293T cells transfected with a plasmid encoding MAG-Fc.

Wild-type human p75NTR and rat cleavage-resistant p75NTR plasmids were generous gifts from Moses V. Chao (New York University, NY). Human p75NTR and p75ICD (amino acids 275-427 plus a methionine at the N terminus) were cloned into pDONR221. Overlap PCR was used to generate full-length human p75NTR that was cleavage resistant. For this, we employed the strategy of Zampieri et al. and replaced the p75NTR transmembrane domain with the corresponding region in murine Fas (Zampieri et al., 2005). After sequence fidelity was confirmed by sequencing, open reading frame constructs were subcloned into pLenti-OE-IRES-RFP and used to generate lentiviral particles.

### Cell culture, transfections and infections

The rat pheochromocytoma PC12 line and the PC12nnr5 cells, a derivative PC12 cell line that does not express TrkA (Green et al., 1986), were maintained in 10% CO2 at 37°C in DMEM supplemented with 6% bovine calf serum, 6% horse serum, 2 mM L-glutamine and 100 mg/ml penicillin/streptomycin. HEK293T cells were maintained in 5% CO2 at 37°C in DMEM supplemented with 10% bovine calf serum, 2 mM L-glutamine and 100 mg/ml penicillin/streptomycin. CGNs were prepared from 8- to 10-day-old (P8-10) rats or 6- to 8-day-old (P6-8) mice, as previously described (Boutilier et al., 2008). PC12 cells were transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) and CGNs were transfected with the Rat Neuron Nucleofector kit (Amaxa, Gaithersburg, MD), both using the manufacturer's protocol. p75NTR and TACE knockdowns were achieved by infecting PC12 cells with lentivirus encoding miRNA with sequences directed against the respective rat mRNAs. miRNA targeting sequences were designed using the Invitrogen miR prediction algorithm and cloned following the manufacturer's instructions into a variant of the pcDNA6.2/GW-EmGFP-miR vector (Invitrogen) in which the EmGFP has been replaced with mRFP. The mRFP-miRNA expression cassette was then amplified by PCR and subcloned into the pRRLsinPPTeGFP vector, such that the eGFP was replaced. VSV-G pseudotyped viral particles were produced in HEK293T cells. Particles were purified by ultracentrifugation, resuspended in DMEM and the amount of active viral particles was determined by titration in HEK293T cells. For miRNA-mediated knockdown, PC12 cells were transduced at a multiplicity of infection (MOI) of 6 and analyzed 6 days post-transduction.

### Cell treatment and harvesting

PC12 cells were pretreated for 1 hour in DMEM containing 0.1% BSA in the absence or presence of the indicated inhibitor(s) prior to addition of neurotrophin at the concentrations and for the times indicated. CGNs were pretreated for 1 hour in serum-deprived Sato media in the absence or presence of the indicated inhibitor(s) prior to addition of neurotrophin at the concentration and for the times indicated. Cells were lysed either directly in Laemmli sample buffer or in RIPA buffer (150 mM NaCl, 1% Igepal, 0.5% SDS, 0.5% deoxycholate, 10 mM Tris pH 8) complemented with a protease inhibitor cocktail (Complete Mini Protease Inhibitor Tablets, Roche Molecular Biochemicals, Basel, Switzerland) and sodium orthovanadate (0.5 mM). Fractions were prepared with a Nuclear/Non-nuclear Cytosol Fractionation kit (BioVision, Mountain View, CA) using the manufacturer's protocol.

### Immunoblotting, immunoprecipitation and immunocytochemistry

For immunoblotting, samples were boiled for 5 minutes, separated by SDS-PAGE and transferred onto nitrocellulose membranes. Membranes were blocked in TBST (10 mM Tris pH 8.0, 150 mM NaCl, 2% Tween 20) supplemented with 5% (w/v) dried skim milk powder or with 2% (w/v) BSA (for immunoblotting with phospho-antibodies). The incubation with primary antibodies was performed in blocking solution and the incubation with secondary antibodies was performed in TBST supplemented with 5% (w/v) dried skim milk. Membranes were extensively washed in TBST after each incubation and immunoreactive bands were detected using enhanced chemiluminescence. For immunoprecipitations, the TrkB-specific RTB antibody was incubated with protein A-sepharose beads overnight at 4°C. The cell lysates were then added to the antibody–protein A-sepharose complex and incubated for 3 hours at 4°C. Immunocomplexes were washed extensively with RIPA lysis buffer and eluted by boiling in Laemmli sample buffer. For immunocytochemistry, cells were grown on coverslips coated with poly-L-lysine (PLL). After treatment, cells were fixed for 30 minutes at room temperature in 2% paraformaldehyde and then incubated for 1 hour at room temperature in solution A (Tris-buffered saline at pH 7.5, 10 mM $MgCl_2$, 10 mM $CaCl_2$) containing 2% donkey serum and 0.2% Triton X-100. Primary antibody (polyclonal anti-p75ICD antibody at 1:500, monoclonal anti-p75ECD at 1 μg/ml) was incubated overnight at 4°C. Cells were washed three times with solution A before incubating with secondary antibody (donkey anti-rabbit IgG antibody coupled to Cy3) for 1 hour at room temperature. Cells were washed three times, treated for 30 minutes at room temperature with RNase A at 1 mg/ml, and nuclei were stained with propidium iodide (PI) at 1 μg/ml for 15 minutes. Coverslips were mounted in an anti-fading mounting media (Dako) and kept at 4°C until imaging was performed on a LSM510 confocal microscope (Carl Zeiss MicroImaging).

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## References

**Bandtlow, C. and Dechant, G.** (2004). From cell death to neuronal regeneration, effects of the p75 neurotrophin receptor depend on interactions with partner subunits. *Sci. STKE* **2004**, pe24.

**Barker, P. A.** (2004). p75NTR is positively promiscuous: novel partners and new insights. *Neuron* **42**, 529-533.

**Barker, P. A.** (2007). High affinity not in the vicinity? *Neuron* **53**, 1-4.
**Barker, P. A. and Shooter, E. M.** (1994). Disruption of NGF binding to the low affinity neurotrophin receptor p75LNTR reduces NGF binding to TrkA on PC12 cells. *Neuron* **13**, 203-215.
**Bertrand, M. J., Kenchappa, R. S., Andrieu, D., Leclercq-Smekens, M., Nguyen, H. N., Carter, B. D., Muscatelli, F., Barker, P. A. and De Backer, O.** (2008). NRAGE, a p75NTR adaptor protein, is required for developmental apoptosis in vivo. *Cell Death Differ.* **15**, 1921-1929.
**Bhakar, A. L., Howell, J. L., Paul, C. E., Salehi, A. H., Becker, E. B., Said, F., Bonni, A. and Barker, P. A.** (2003). Apoptosis induced by p75NTR overexpression requires Jun kinase-dependent phosphorylation of Bad. *J. Neurosci.* **23**, 11373-11381.
**Boutilier, J., Ceni, C., Pagdala, P. C., Forgie, A., Neet, K. E. and Barker, P. A.** (2008). Proneurotrophins require endocytosis and intracellular proteolysis to induce TrkA activation. *J. Biol. Chem.* **283**, 12709-12716.
**Chao, M. V.** (2003). Neurotrophins and their receptors: a convergence point for many signalling pathways. *Nat. Rev. Neurosci.* **4**, 299-309.
**Clary, D. O., Weskamp, G., Austin, L. R. and Reichardt, L. F.** (1994). TrkA cross-linking mimics neuronal responses to nerve growth factor. *Mol. Biol. Cell* **5**, 549-563.
**Davies, A. M., Lee, K. F. and Jaenisch, R.** (1993). p75-deficient trigeminal sensory neurons have an altered response to NGF but not to other neurotrophins. *Neuron* **11**, 565-574.
**Diaz-Rodriguez, E., Montero, J. C., Esparis-Ogando, A., Yuste, L. and Pandiella, A.** (2002). Extracellular signal-regulated kinase phosphorylates tumor necrosis factor alpha-converting enzyme at threonine 735, a potential role in regulated shedding. *Mol. Biol. Cell* **13**, 2031-2044.
**Domeniconi, M., Zampieri, N., Spencer, T., Hilaire, M., Mellado, W., Chao, M. V. and Filbin, M. T.** (2005). MAG induces regulated intramembrane proteolysis of the p75 neurotrophin receptor to inhibit neurite outgrowth. *Neuron* **46**, 849-855.
**Fan, H. and Derynck, R.** (1999). Ectodomain shedding of TGF-alpha and other transmembrane proteins is induced by receptor tyrosine kinase activation and MAP kinase signaling cascades. *EMBO J.* **18**, 6962-6972.
**Frade, J. M.** (2005). Nuclear translocation of the p75 neurotrophin receptor cytoplasmic domain in response to neurotrophin binding. *J. Neurosci.* **25**, 1407-1411.
**Gechtman, Z., Alonso, J. L., Raab, G., Ingber, D. E. and Klagsbrun, M.** (1999). The shedding of membrane-anchored heparin-binding epidermal-like growth factor is regulated by the Raf/mitogen-activated protein kinase cascade and by cell adhesion and spreading. *J. Biol. Chem.* **274**, 28828-28835.
**Green, S. H., Rydel, R. E., Connolly, J. L. and Greene, L. A.** (1986). PC12 cell mutants that possess low- but not high-affinity nerve growth factor receptors neither respond to nor internalize nerve growth factor. *J. Cell Biol.* **102**, 830-843.
**Gryz, E. A. and Meakin, S. O.** (2003). Acidic substitution of the activation loop tyrosines in TrkA supports nerve growth factor-dependent, but not nerve growth factor-independent, differentiation and cell cycle arrest in the human neuroblastoma cell line, SY5Y. *Oncogene* **22**, 8774-8785.
**Gu, H., Liang, Y., Mandel, G. and Roizman, B.** (2005). Components of the REST/CoREST/histone deacetylase repressor complex are disrupted, modified, and translocated in HSV-1-infected cells. *Proc. Natl. Acad. Sci. USA* **102**, 7571-7576.
**Hantzopoulos, P. A., Suri, C., Glass, D. J., Goldfarb, M. P. and Yancopoulos, G. D.** (1994). The low affinity NGF receptor, p75, can collaborate with each of the Trks to potentiate functional responses to the neurotrophins. *Neuron* **13**, 187-201.
**Hempstead, B. L., Martin-Zanca, D., Kaplan, D. R., Parada, L. F. and Chao, M. V.** (1991). High-affinity NGF binding requires coexpression of the trk proto-oncogene and the low-affinity NGF receptor. *Nature* **350**, 678-683.
**Hempstead, B. L., Rabin, S. J., Kaplan, L., Reid, S., Parada, L. F. and Kaplan, D. R.** (1992). Overexpression of the trk tyrosine kinase rapidly accelerates nerve growth factor-induced differentiation. *Neuron* **9**, 883-896.
**Hosomi, S., Yamashita, T., Aoki, M. and Tohyama, M.** (2003). The p75 receptor is required for BDNF-induced differentiation of neural precursor cells. *Biochem. Biophys. Res. Commun.* **301**, 1011-1015.
**Huang, E. J., Wilkinson, G. A., Farinas, I., Backus, C., Zang, K., Wong, S. L. and Reichardt, L. F.** (1999). Expression of Trk receptors in the developing mouse trigeminal ganglion: in vivo evidence for NT-3 activation of TrkA and TrkB in addition to TrkC. *Development* **126**, 2191-2203.
**Ito, H., Nomoto, H. and Furukawa, S.** (2002). Role of low-affinity p75 receptor in nerve growth factor-inducible growth arrest of PC12 cells. *J. Neurosci. Res.* **69**, 653-661.
**Ito, H., Nomoto, H. and Furukawa, S.** (2003). Growth arrest of PC12 cells by nerve growth factor is dependent on the phosphatidylinositol 3-kinase/Akt pathway via p75 neurotrophin receptor. *J. Neurosci. Res.* **72**, 211-217.
**Johnston, A. L., Lun, X., Rahn, J. J., Liacini, A., Wang, L., Hamilton, M. G., Parney, I. F., Hempstead, B. L., Robbins, S. M., Forsyth, P. A. et al.** (2007). The p75 neurotrophin receptor is a central regulator of glioma invasion. *PLoS Biol.* **5**, e212.
**Kanning, K. C., Hudson, M., Amieux, P. S., Wiley, J. C., Bothwell, M. and Schecterson, L. C.** (2003). Proteolytic processing of the p75 neurotrophin receptor and two homologs generates C-terminal fragments with signaling capability. *J. Neurosci.* **23**, 5425-5436.
**Kenchappa, R. S., Zampieri, N., Chao, M. V., Barker, P. A., Teng, H. K., Hempstead, B. L. and Carter, B. D.** (2006). Ligand-dependent cleavage of the P75 neurotrophin receptor is necessary for NRIF nuclear translocation and apoptosis in sympathetic neurons. *Neuron* **50**, 219-232.
**Lachyankar, M. B., Condon, P. J., Daou, M. C., De, A. K., Levine, J. B., Obermeier, A. and Ross, A. H.** (2003). Novel functional interactions between Trk kinase and p75 neurotrophin receptor in neuroblastoma cells. *J. Neurosci. Res* **71**, 157-172.
**Lee, K.-F., Li, E., Huber, J., Landis, S. C., Sharpe, A. H., Chao, M. V. and Jaenisch, R.** (1992a). Targeted mutation of the gene encoding the low affinity NGF receptor leads to deficits in the peripheral sensory nervous system. *Cell* **69**, 737-749.
**Lee, K. F., Li, E., Huber, L. J., Landis, S. C., Sharpe, A. H., Chao, M. V. and Jaenisch, R.** (1992b). Targeted mutation of the gene encoding the low affinity NGF receptor p75 leads to deficits in the peripheral sensory nervous system. *Cell* **69**, 737-749.
**Lee, K. F., Davies, A. M. and Jaenisch, R.** (1994). p75-deficient embryonic dorsal root sensory and neonatal sympathetic neurons display a decreased sensitivity to NGF. *Development* **120**, 1027-1033.
**Loeb, D. M. and Greene, L. A.** (1993). Transfection with trk restores "slow" NGF binding, efficient NGF uptake, and multiple NGF responses to NGF-nonresponsive PC12 cell mutants. *J. Neurosci.* **13**, 2919-2929.
**Majdan, M., Lachance, C., Gloster, A., Aloyz, R., Zeindler, C., Bamji, S., Bhakar, A., Belliveau, D., Fawcett, J., Miller, F. D. et al.** (1997). Transgenic mice expressing the intracellular domain of the p75 neurotrophin receptor undergo neuronal apoptosis. *J. Neurosci.* **17**, 6988-6998.
**Nykjaer, A., Willnow, T. E. and Petersen, C. M.** (2005). p75NTR-live or let die. *Curr. Opin. Neurobiol.* **15**, 49-57.
**Patapoutian, A. and Reichardt, L. F.** (2001). Trk receptors: mediators of neurotrophin action. *Curr. Opin. Neurobiol.* **11**, 272-280.
**Podlesniy, P., Kichev, A., Pedraza, C., Saurat, J., Encinas, M., Perez, B., Ferrer, I. and Espinet, C.** (2006). Pro-NGF from Alzheimer's disease and normal human brain displays distinctive abilities to induce processing and nuclear translocation of intracellular domain of p75NTR and apoptosis. *Am. J. Pathol.* **169**, 119-131.
**Rende, M., Brizi, E., Conner, J., Treves, S., Censier, K., Provenzano, C., Taglialatela, G., Sanna, P. P. and Donato, R.** (2000). Nerve growth factor (NGF) influences differentiation and proliferation of myogenic cells in vitro via TrKA. *Int. J. Dev. Neurosci.* **18**, 869-885.
**Roux, P. P., Bhakar, A. L., Kennedy, T. E. and Barker, P. A.** (2001). The p75 neurotrophin receptor activates Akt (protein kinase B) through a phosphatidylinositol 3-kinase-dependent pathway. *J. Biol. Chem.* **276**, 23097-23104.
**Salehi, A. H., Morris, S. J., Ho, W. C., Dickson, K. M., Doucet, G., Milutinovic, S., Durkin, J., Gillard, J. W. and Barker, P. A.** (2006). AEG3482 is an antiapoptotic compound that inhibits Jun kinase activity and cell death through induced expression of heat shock protein 70. *Chem. Biol.* **13**, 213-223.
**Snapyan, M., Lemasson, M., Brill, M. S., Blais, M., Massouh, M., Ninkovic, J., Gravel, C., Berthod, F., Gotz, M., Barker, P. A. et al.** (2009). Vasculature guides migrating neuronal precursors in the adult mammalian forebrain via brain-derived neurotrophic factor signaling. *J. Neurosci.* **29**, 4172-4188.
**Soond, S. M., Everson, B., Riches, D. W. and Murphy, G.** (2005). ERK-mediated phosphorylation of Thr735 in TNFalpha-converting enzyme and its potential role in TACE protein trafficking. *J. Cell Sci.* **118**, 2371-2380.
**Urra, S., Escudero, C. A., Ramos, P., Lisbona, F., Allende, E., Covarrubias, P., Parraguez, J. I., Zampieri, N., Chao, M. V., Annaert, W. et al.** (2007). TrkA receptor activation by nerve growth factor induces shedding of the p75 neurotrophin receptor followed by endosomal gamma-secretase-mediated release of the p75 intracellular domain. *J. Biol. Chem.* **282**, 7606-7615.
**Vilar, M., Charalampopoulos, I., Kenchappa, R. S., Simi, A., Karaca, E., Reversi, A., Choi, S., Bothwell, M., Mingarro, I., Friedman, W. J. et al.** (2009). Activation of the p75 neurotrophin receptor through conformational rearrangement of disulphide-linked receptor dimers. *Neuron* **62**, 72-83.
**von Schack, D., Casademunt, E., Schweigreiter, R., Meyer, M., Bibel, M. and Dechant, G.** (2001). Complete ablation of the neurotrophin receptor p75NTR causes defects both in the nervous and the vascular system. *Nat. Neurosci.* **4**, 977-978.
**Wehrman, T., He, X., Raab, B., Dukipatti, A., Blau, H. and Garcia, K. C.** (2007). Structural and mechanistic insights into nerve growth factor interactions with the TrkA and p75 receptors. *Neuron* **53**, 25-38.
**Weskamp, G., Schlondorff, J., Lum, L., Becherer, J. D., Kim, T. W., Saftig, P., Hartmann, D., Murphy, G. and Blobel, C. P.** (2004). Evidence for a critical role of the tumor necrosis factor alpha convertase (TACE) in ectodomain shedding of the p75 neurotrophin receptor (p75NTR). *J. Biol. Chem.* **279**, 4241-4249.
**Yamashita, T., Tucker, K. L. and Barde, Y. A.** (1999). Neurotrophin binding to the p75 receptor modulates Rho activity and axonal outgrowth. *Neuron* **24**, 585-593.
**Yan, H., Schlessinger, J. and Chao, M. V.** (1991). Chimeric NGF-EGF receptors define domains responsible for neuronal differentiation. *Science* **252**, 561-563.
**Zampieri, N., Xu, C. F., Neubert, T. A. and Chao, M. V.** (2005). Cleavage of p75 neurotrophin receptor by alpha-secretase and gamma-secretase requires specific receptor domains. *J. Biol. Chem.* **280**, 14563-14571.