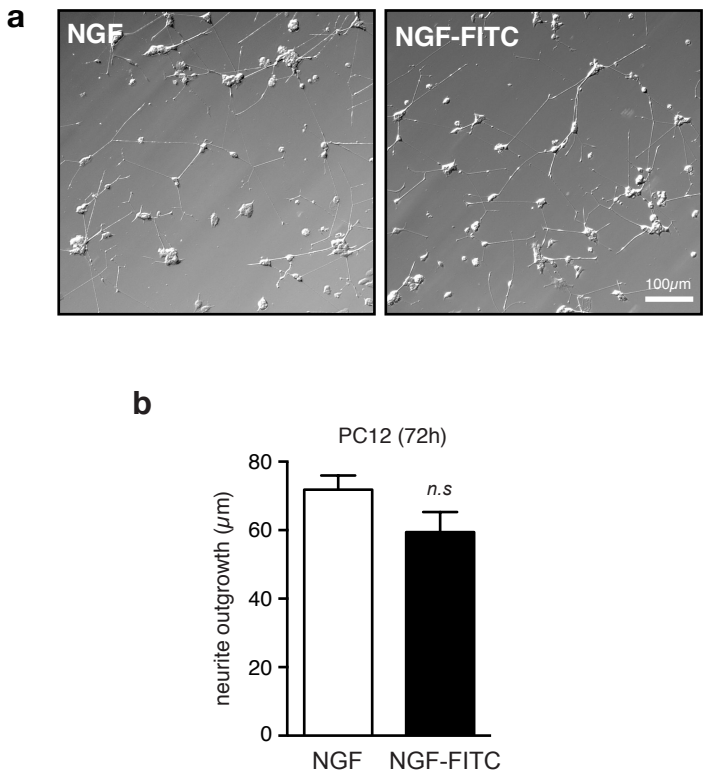
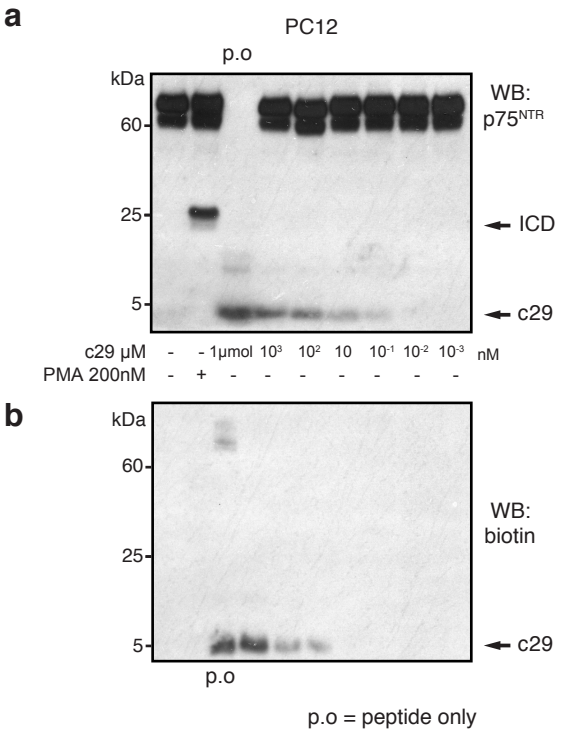
	Treatment	% cell population NGF- FITC	Statistics	Median population fluorescence	Statistics	Mean fluorescence per NGF-FITC positive cell	Statistics	Maximal fluorescence per cell	Statistics
Transfected HEK293	**$p75^{FL}$** n=6	67.9 ± 1.2 %	P = 0.20 *c.f.* $p75^{\Delta JUX}$	4821 ± 2121	P = 0.38 *c.f.* $p75^{\Delta JUX}$	7088 ± 2172	P = 0.48 *c.f.* $p75^{\Delta JUX}$	5214 ± 1493	P = 0.99 *c.f.* $p75^{\Delta JUX}$
	$p75^{\Delta JUX}$ n=3	79.2± 5.6 %	P = 0.11 *c.f.* $TrkA^{K538R}$	5210 ± 1680	P = 0.32 *c.f.* $TrkA^{K538R}$	8029± 2026	P = 0.43 *c.f.* $TrkA^{K538R}$	5210 ± 1681	P = 0.27 *c.f.* $TrkA^{K538R}$
	$TrkA^{K538R}$ n=9	73.0 ± 5.2 %	p=0.31 *c.f.* $p75^{FL}$	5027 ± 2047	P = 0.37 *c.f.* $p75^{FL}$	7861 ± 3104	P = 0.46 *c.f.* $p75^{FL}$	8482 ± 4612	P = 0.12 *c.f.* $p75^{FL}$
	$TrkA^{K538R}$ + $p75^{FL}$ *c.f* **$TrkA^{K538R}$** n=3	***112 ± 4.8%***	***P = 0.005***	119 ± 22.0%	P = 0.101	***111.7± 5.2%***	***P = 0.009***	***134.3 ± 9.6%***	***P = 0.002***
	$TrkA^{K538R}$+ $p75^{\Delta JUX}$ *c.f* **$TrkA^{K538R}$** n=3	103 ± 10%	P = 0.29	***63.8 ± 9.4%***	***P = 0.0013***	***92.8% ± 4.7%***	***P = 0.027***	***91.9 ± 1.7%***	***P = 0.0005***
	$TrkA^{K538R}$ + $p75^{FL}$ *c.f* **$TrkA^{K538R}$ + $p75^{\Delta JUX}$** n=3	109.4 ± 13.3%	P = 0.15	***191.4 ± 52.1%***	***P = 0.019***	***120.5 ± 3.8%***	***P = 0.0004***	***123.4 ± 8.8%***	***P = 0.005***
	$p75^{FL}$ + c29 *c.f.* **$p75^{FL}$** n=3	100.5 ± 14.2 %	P = 0.96	103.8 ± 11.2%	P = 0.29	105.2 ± 3.6%	P = 0.071	98.7 ± 4.3%	P = 0.32
	$TrkA^{K538R}$ + c29 *c.f* **$TrkA^{K538R}$** n=3	***112.2 ± 5.3 %***	***P = 0.008***	***140.5 ±21.3%***	***P = 0.015***	***125.0 ± 12.1%***	***P = 0.012***	***128.0 ± 11.7%***	***P = 0.007***
PC12	**PC12** n=3	67.3 ± 12.0 %		6313 ± 1383		7712 ± 1148		17308 ± 5494	
	PC12 + SC *c.f* **PC12** n=3	100.3 ± 5.1%	P = 0.20	97.0 ± 3.8%	P = 0.12	96.3 ± 7.4%	P = 0.22	100.4 ± 9.6%	P = 0.47
	PC12 + c29 *c.f.* **PC12 + SC** n=3	115.3 ± 13.4%	P = 0.06	***123.5 ± 8.4%***	***P = 0.004***	***121.4 ± 5.5%***	***P = 0.001***	***123.1 ± 5.2%***	***P = 0.0008***
	PC12 + c29 *c.f.* **PC12** n=3	115.7 ± 19.7%	P = 0.12	***127.4 ± 8.1%***	***P = 0.002***	***126.3 ± 4.8%***	***P = 0.0003***	***123.6 ± 17.3%***	***P = 0.038***

Matusica et al. Supplementary Table 1

Matusica et al. Supp Figure 1



Matusica et al. Supp Figure 2



Supplementary Table 1. Analysis of NGF-FITC binding to transfected HEK cells and PC12 cells treated with c29

Summary of population-based flow cytometry results from steady-state ligand-binding assays of HEK293 cells transfected with various $p75^{NTR}$ and TrkA expression constructs or PC12 cells treated with c29 or scrambled (SC) peptides. The mean median and maximal fluorescence for cells incubated with NGF-FITC is reported as the average measurement of that parameter over independent experiments. The number of experiments per condition is indicated in the treatment column. Representative plots population fluorescence are presented in Fig 7. Where a measure is statistically significantly different from that of a control condition, the results are in bold text.

Supplementary Figure 1. Biological activity comparison between NGF and NGF-FITC.

(a) Phase contrast micrographs of PC12 cells cultured in the presence of 100ng/ml NGF or NGF-FITC for 72 hours, showing similar extent of cell differentiation. **(b)** Graph analysis of median neurite outgrowth of PC12 cells showing a minor but not significant loss of activity of NGF-FITC when compared to unlabeled NGF. (n=4 experiments; mean ± SEM; n.s. p =0.018; Student's t-test).

Supplementary Figure 2. Western blot analysis of c29 peptide internalization by PC12 cells.

Representative western blots showing the amount of c29 peptide internalized by PC12 cell cultures (100,000 cells) following 1 hour incubation with biotinylated c29 at concentrations ranging from 1μM to 1pM in 1ml of medium. Following washing cells were lysed in 200μl of lysis buffer. 20μg of lysate (~$1/10^{th}$ of the sample) was separated by SDS-gel electrophoresis and western blotted. $p75^{NTR}$ and c29 peptides were detected with **(a)** the $p75^{NTR}$ antibody 9992 raised against the intracellular domain of $p75^{NTR}$ or **(b)** vectastain ABC peroxidase substrate for avidin/biotin. Lane 1: control PC12 cell lysate, lane 2: lysate of PMA-treated PC12 cells illustrating maximal $p75^{NTR}$ cleavage, lane 3: 1μmol of peptide only (p.o) loaded directly onto the gel. Lanes 4-9: c29-treated PC12 cell lysates. The amount of biotinylated c29 peptide with the cells at concentrations of 1μM, 100nM and 10nM was in a similar range to the amount of $p75^{ICD}$ fragment generated by PMA cleavage of $p75^{NTR}$, and in excess of that produced under basal conditions. The concentrations of c29 at which TrkA-expressing cells bound more NGF-FITC was from 10nM -1μM (Fig 8c).