Supplementary Table 1. NGF treatment of TrkA and p75<sup>NTR</sup> expressing cells did not affect the release of the cytosolic enzyme LDH (lactate dehydrogenase) into the conditioned media.

TrkA and p75<sup>NTR</sup> expressing SK-N-BE cell lines were treated with increasing doses of NGF for 48 h. LDH release in the conditioned media was analysed using the enzymatic assay from Sigma, as described by the manufacturer.

<table>
<thead>
<tr>
<th>NGF (ng/ml)</th>
<th>0</th>
<th>50</th>
<th>100</th>
<th>200</th>
<th>LDH (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TrkA</td>
<td>29.5±1.3</td>
<td>30.3±1.7</td>
<td>29.5±2.0</td>
<td>28.5±1.7</td>
<td></td>
</tr>
<tr>
<td>p75&lt;sup&gt;NTR&lt;/sup&gt;</td>
<td>28.9±1.8</td>
<td>29.7±0.9</td>
<td>30.5±1.6</td>
<td>27.9±1.1</td>
<td></td>
</tr>
</tbody>
</table>
Supplementary Figure 1  p75<sup>NTR</sup> activates β and α cleavage of APP

p75<sup>NTR</sup> and TrkA stably transfected SK-N-BE cells were treated with increasing concentrations of NGF for 48 h. Western blot analysis was performed as described in the Materials and Methods section by using a polyclonal antibody against the C-terminus of APP (Chemicon International). NGF treatment increased β and α cleavage of APP in p75<sup>NTR</sup> but not in TrkA expressing cells. No additional modification of APP processing was observed. Please note that prior to NGF treatment, the baseline levels of α- and β-APP-CTF were already higher in cells expressing p75<sup>NTR</sup> than in those expressing TrkA alone. NGF treatment of TrkA cells produced a slight decrease in both β- and α-cleavage of APP. (B) shows a longer exposure of the same blot.

β- and α-APP-CTF were identified as shown previously ([S1--S3]) and were confirmed by using: (i) non-transfected CHO cells; (ii) CHO cells stably transfected with APP; (iii) CHO cells stably transfected with both APP and BACE1; (iv) H4 cells stably transfected with APP; and (v) H4 cells stably transfected with APP<sub>C105</sub>. CHO<sub>APP+BACE</sub> cells were a generous gift from Dr Rudy E. Tanzi, and CHO<sub>APP</sub>, H4<sub>APP</sub>, and H4<sub>APP-C105</sub> were from Dr Dora M. Kovacs.
Supplementary Figure 2  Western blot analysis of hippocampus of normally aged mice

Hippocampus from 5 and 30 month-old animals was analysed as described in the Materials and methods section. The expression levels of p75NTR, TrkA, BACE1, and β-APP-CTF were analyzed by Western blot with the following antibodies: p75NTR, sc-8317 (Santa Cruz); TrkA, sc-118 (Santa Cruz); BACE1, ab2077 (Abcam); APP-CTF, AB5352 (Chemicon). The primary antibody was followed by an HRP-conjugated mAb and detected by chemiluminescence as described [S1,S2].

References


Figure 1

Panel A shows a Western blot analysis of NGF-treated samples with 0 and 200 ng/ml concentrations. The kDa markers are indicated on the left, and bands labeled as APP, β-APP-CTF, and α-APP-CTF are visible.

Panel B displays a similar analysis with p75NTR and TrkA proteins. Bands for β-APP-CTF and α-APP-CTF are indicated.
Figure 2