Figure S1. Apigenin shows binding with NGF in ultrafiltration assay.

The direct binding of NGF with luteolin analogs was tested using an ultrafiltration-based approach. The un-filtrated supernatant was analyzed by HPLC-MS/MS to quantify apigenin, fisetin, and isorhamnetin in the presence or absence of NGF, as in Fig. 1B. Apigenin, fisetin, and isorhamnetin were identified at around 7.1 min, 5.2 min, and 7.8 min of migration, respectively. The chemical structures of the three compounds were shown.

Figure S2. Luteolin/NGF-induced differentiation is not mediated by p75NTR.

(A) Cultured PC12 cells were treated with luteolin for 48 hours, and the expression level of p75NTR (~75 kDa) was quantified by western blotting. α-Tubulin (~52 kDa) was used as loading control. The representative gels were shown. **(B)** Cultured PC12 cells were pre-incubated with p75NTR inhibitor TAT-Pep5 (20 μg/mL) for 3 hours, before the NGF/luteolin cotreatment. After 48 hours, the proportion of differentiated cells were analyzed for each well, according to at least five randomly selected views, as in Fig. 3B. Values are expressed as percentage of total number of cell, in mean ± SEM, $n = 3$.

Figure S3. Luteolin enhances the affinity of NGF to PC12 cells.

PC12 cells were treated with 5 ng/mL DyLight 650-biotinylated NGF for 30 min in the absence or presence of 5 μM luteolin. Digital images were taken to show the binding of NGF on the cells. One representative picture result was shown. Scale bar = 5 μm. Values are expressed as the fold of change (× Basal) against the control group without luteolin in the culture, in mean ± SEM, $n = 4$. (*) $p < 0.05$.