Supplementary Information

NGF-NPs conjugation ratio

In order to estimate the conjugation ratio between NGF and the NPs, the mass of a single particle was calculated based on particles average diameter (23.0 ± 2.1 nm), sphere volume and iron oxide density (5.242 g/cm^3), as 3.34×10^{-17} g. The mass of a single NGF protein is 26.8 kDa which is equivalent to 4.45×10^{-20} g. As described in the experimental details section, the amount of NGF conjugated to NPs is 89 ± 3.1 µg/mg NPs. Therefore, by dividing the amount of NGF by the mass of a single particle and a single NGF protein, the number of NGF molecules attached to a single NP is estimated as 70 (67 ± 18).

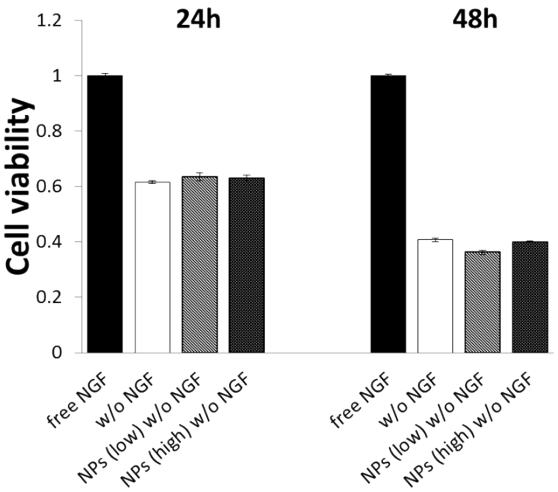

Fig. S1 XTT viability assay of PC12 cells treated in the presence (50ng/ml) of NGF ('free NGF') compared to PC12 cells treated in the absence of NGF ('w/o NGF') and to cells treated in the absence of NGF with non-conjugated NPs at two concentrations - low and high concentrations (0.6µg/ml and 20µg/ml, respectively). Absorbance at 450nm (630nm background). Measurements were normalized to control ('free NGF') (n=3).

The experiment depicted the necessity of NGF for PC12 cell survival. Lack of NGF, with or without NPs treatment, resulted in viability levels of 0.6 after 24 hours and of 0.4 after 48h. It can be seen that at both time points the addition of NPs (low or high concentrations) has no effect on the viability. These PC12 cells that were seeded on collagen-coated plates cease to proliferate and begin the differentiation process. Therefore, NGF is essential for their survival. An additional viability assay that was performed for PC12 cells in suspension that were incubated with non-conjugated NPs (at high concentrations) has shown a viability level of above 0.8 (data not shown).

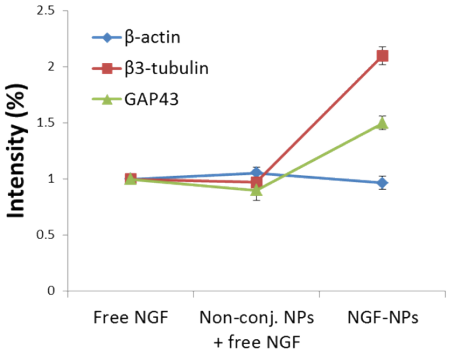

Fig. S2 RNA was extracted from PC12 cells treated with free NGF, non-conjugated NPs with free NGF and NGF-NPs. Intensity of gel bands of electrophoresed PCR products of differentiation related genes (β3-tubulin and GAP43) vs. a control gene (β-actin) (presented in Figure 6). Intensities are normalized to free NGF. Data analysis was performed using ImageJ software (US National Institutes of Health, Bethesda, MD, USA).

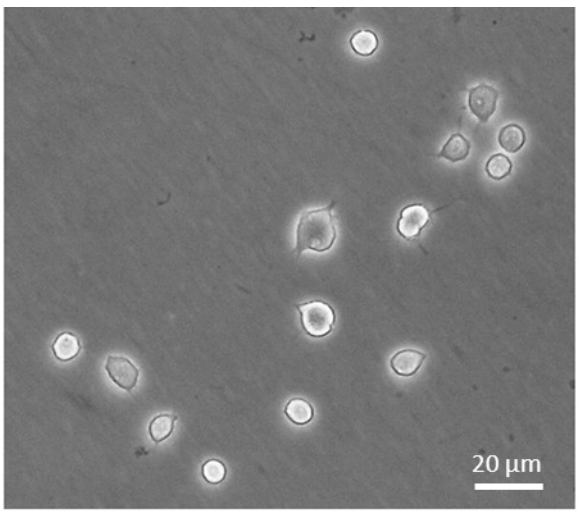

Fig. S3 PC12 cells 48h after treatment with NGF-NPs following TrkA inhibition. No neurite outgrowth is observed.