# †Electronic supplementary information

For the paper: Surface coupling strength of gold nanoparticles affects cytotoxicity on neurons

## 1. Particle binding strength test

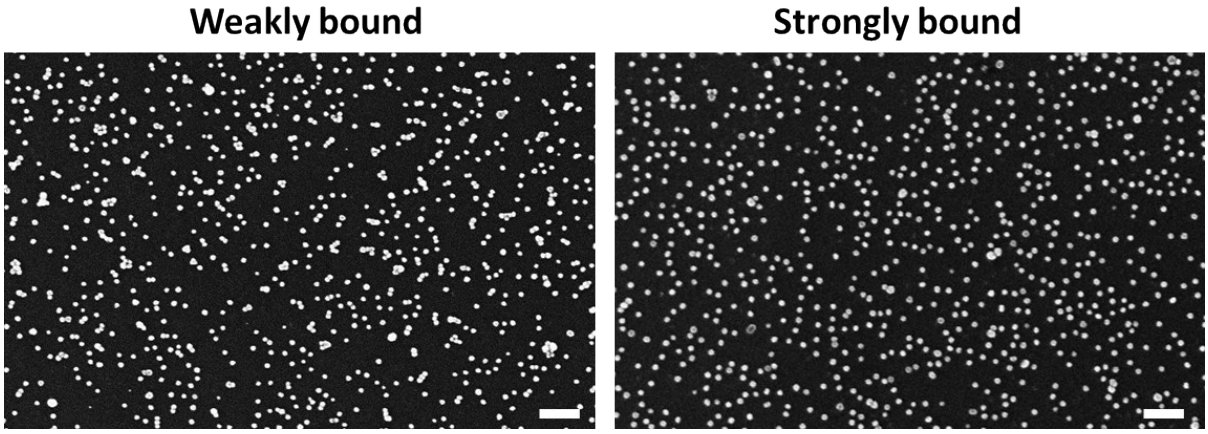


**Fig. S1** SEM images of 20 nm NPs after chemical modifications after applying an additional 1 min sonication treatment to illustrate the difference in particle binding strength. The left image shows weakly bound (WB) particles, which strongly aggregate during sonication (at least 8.6% particle agrregated), while the strongly bound (SB) particles remain separated (about 1.7% particles aggregated) after this treatment, right image. Noteworthy, during standard sample preparation for cell culture experiments, sonication was not used. Scale bar: 200 nm.

## 2. The cell survival analysis of neurons on WB and SB particles

Only the live cells (cell survival) are considered for the statistic analysis. We use the non-fouling molecules (PEG) as a backfill to prevent on-specific binding of the cells. Consequently, the dead cells did not stick as stably on the sample as the live cells, so they are easily removed during medium exchanged. If we normalize by number of dead cells, we artificially increase the cell viability. The adhesion of dead cells on the non-fouling surface is less defined than the adhesion of the vital neurons. Together with the fact, that we plated always the same number of cell on the surface, the number of live cells represents the most suitable characterization of the neuron viability for our system.

Furthermore, it is noteworthy that both particle density and particle size influence the number of adhesion molecules (AUT) on the surface. The density of surface tethered binding ligands is proportional to the surface coverage of AuNP. If we convert the particle density of 5 nm (density 150), 10 nm (density 199), and 20 nm (density 18) Au-NP as used in Fig. 5 of the main document to their surface coverages, then we obtain coverages of 0.29%, 1.56%, and 0.57% for 5 nm, 10 nm, and 20 nm particles respectively. Although the particle density of 20nm Au-NP is much smaller than of 5nm Au-NP, their capability to bind adhesion ligands is higher.

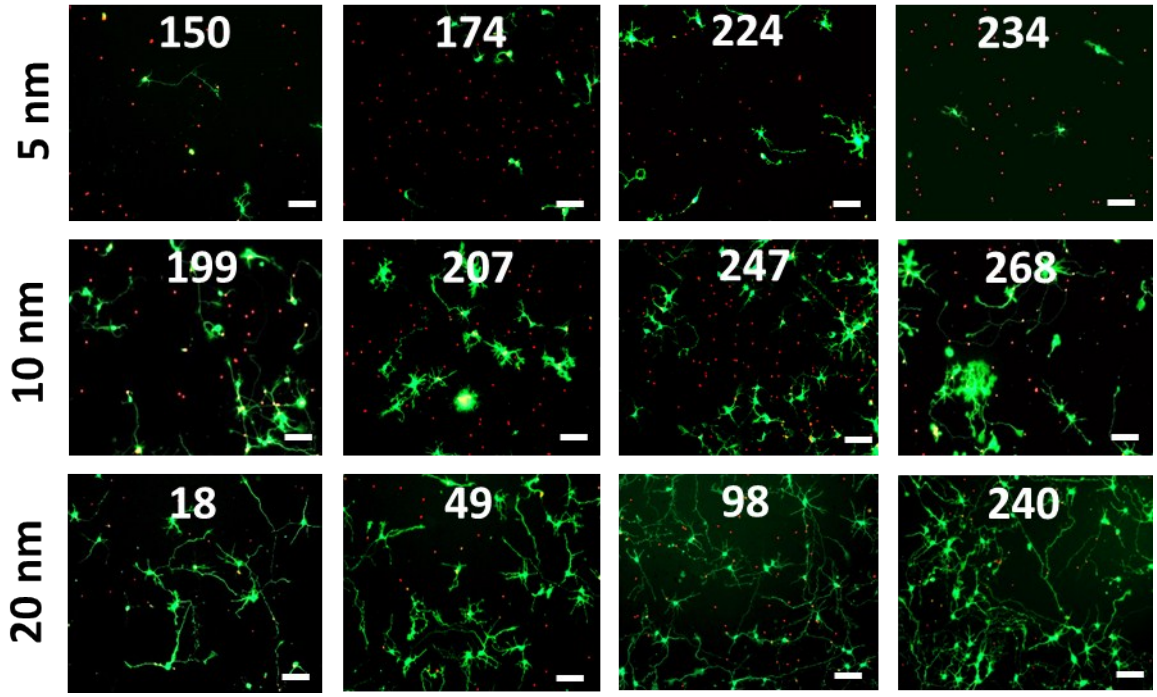


**Fig. S2** Live (green) and dead (red) fluorescence staining of DIV3 neurons on AUT-modified AuNPs of different sizes and densities with PEG backfill passivation. The particles are weakly bound to the substrate (WB particles). The particles densities (per $\mu m^2$) are shown in each image. Scale bar: 100 µm.

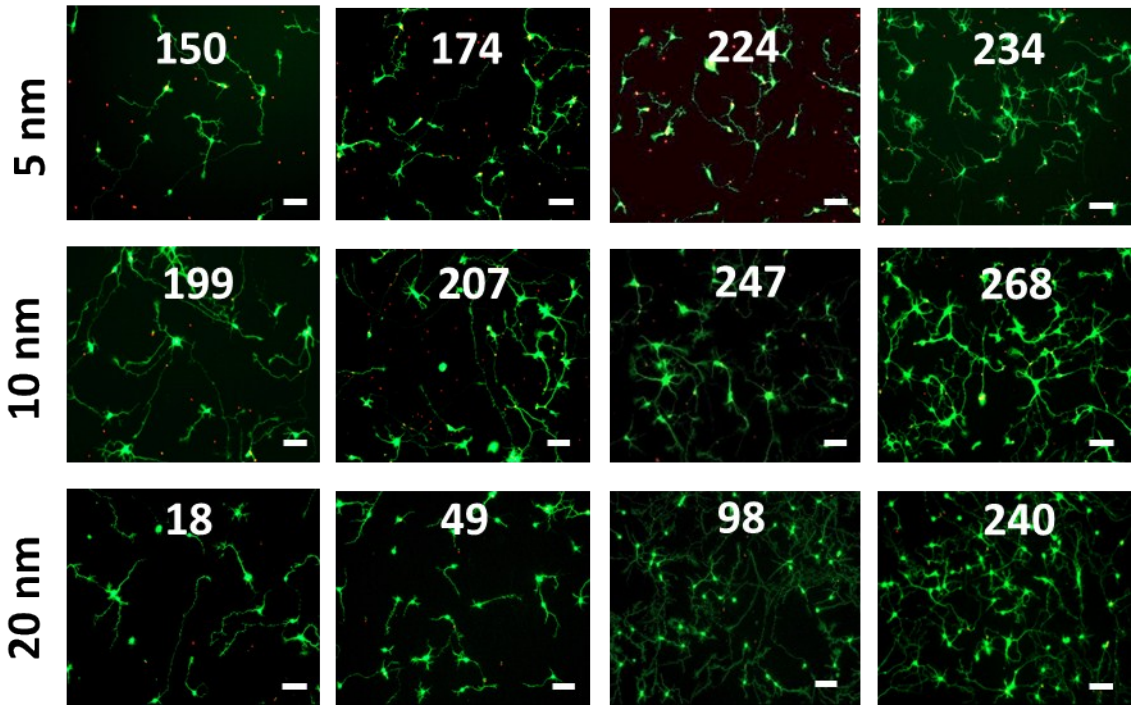


**Fig. S3.** Live (green) and dead (red) fluorescence images of the DIV3 neurons on AUT-modified AuNPs of different sizes and densities with PEG backfill passivation. The particles are strongly bound to the substrate (SB particles). The particles densities (per $\mu m^2$) are shown in each image. Scale bar: 100 µm

## 3. Cytotoxicity investigation

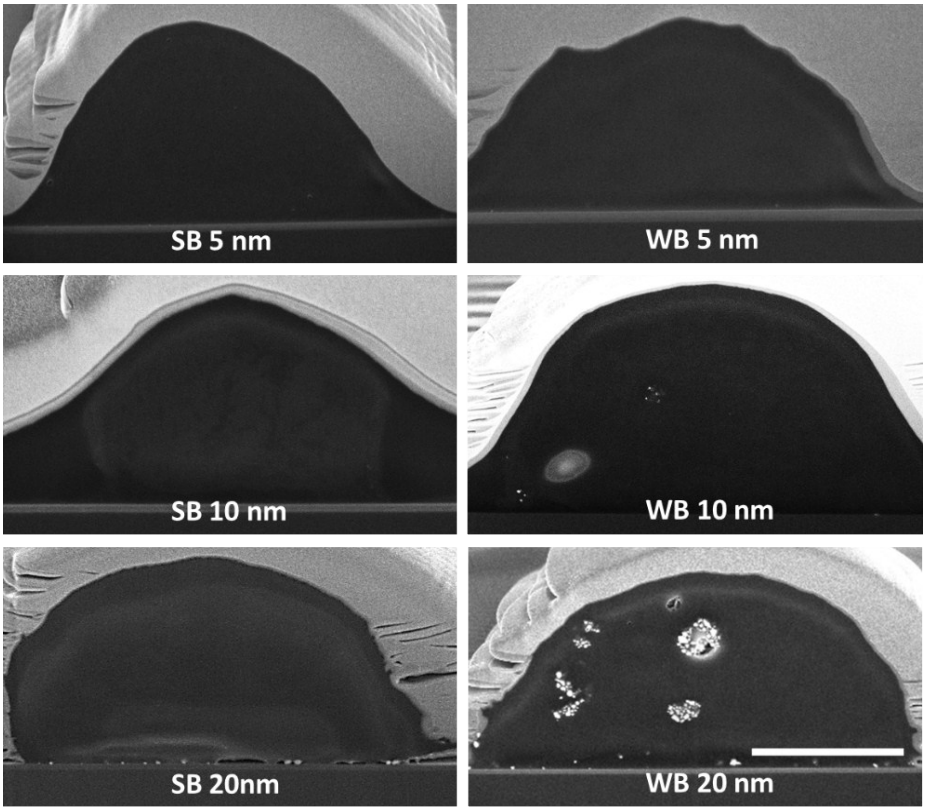


**Fig. S4** FIB cuts images of cells cultured on the 5 nm, 10 nm, and 20 nm particles for SB and WB. Scale bar: 2 µm.

| Size [nm] | Amount[(1)] [mg/l] | Density of as-prepared particles [$\mu l^{-1}$] | Particle density in culture medium [$ml^{-1}$] | Density of surface tethered AuNP in contact with cells[(2)] [$cm^{-2}$] |
|---|---|---|---|---|
| 5 | 5966 | $4.7 \times 10^{12}$ | $4.7 \times 10^{12}$ | $1.05 \times 10^{9}$ |
| 10 | 1536 | $1.5 \times 10^{11}$ | $5.1 \times 10^{11}$ | $2.28 \times 10^{9}$ |
| 20 | 152 | $1.9 \times 10^{9}$ | $2.5 \times 10^{10}$ | $3.6 \times 10^{9}$ |

**Table S1** The concentration of AUT-modified-AuNPs of 5 nm, 10 nm, and 20 nm sizes, the number of particles (per µL) is calculated from the amount of gold[(1)] measured from the atomic absorption spectroscopy (AAS) (See Method S1†). [(2)] The particles in contact with cells are calculated from the surface coverage of cells on SB NPs (see Fig. S7†).

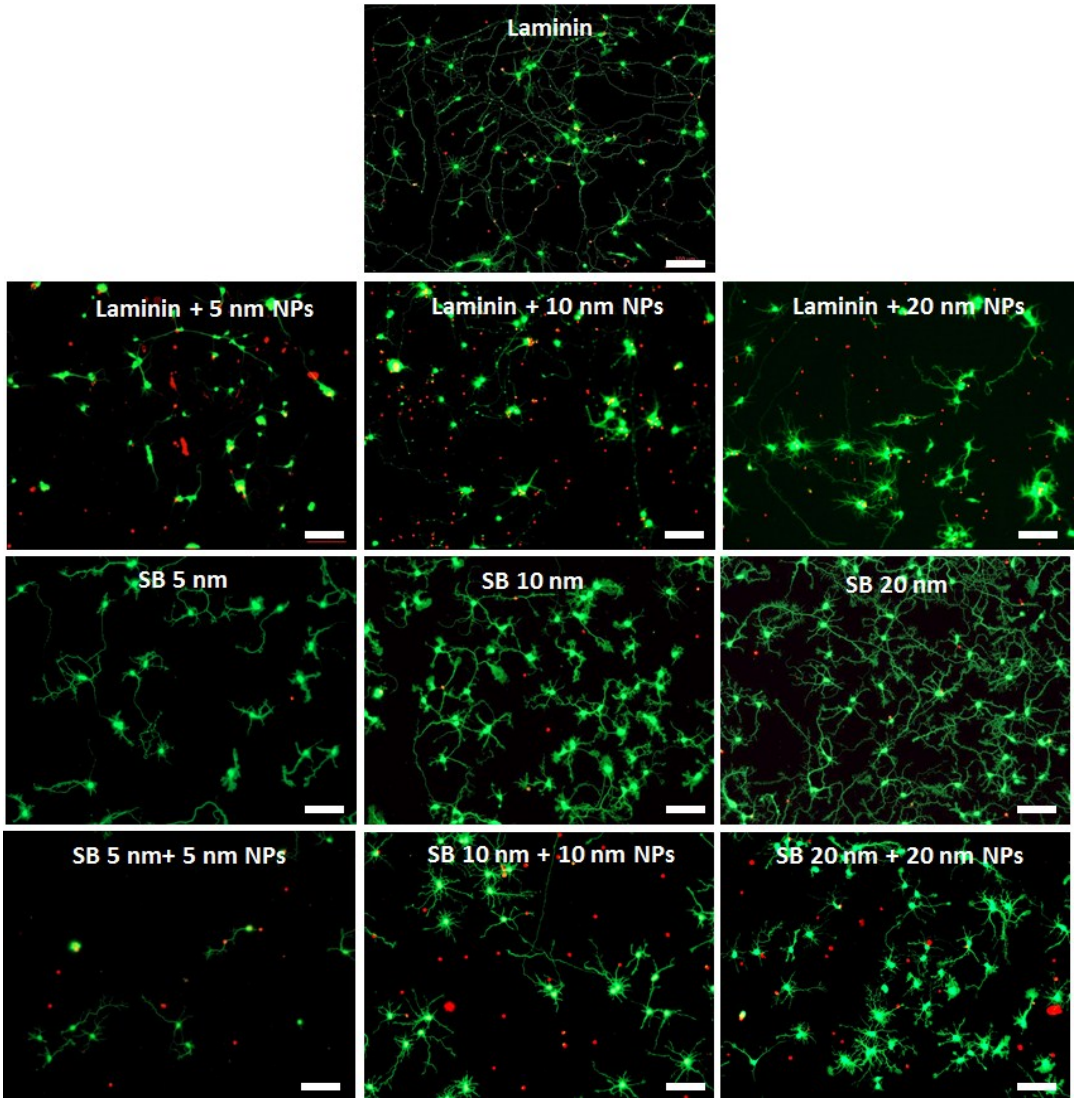


**Fig. S5** Live (green) and dead (red) fluorescence images of the DIV3 neurons on laminin coated $SiO_2$ (control), and SB NP samples (with and without added dispersed NPs). Laminin is used as coating for the reference sample to eliminate animal to animal variations and influences from varying culture conditions. It has been widely used as coating for in vitro studies of cortical cells. Scale bar: 100 µm.

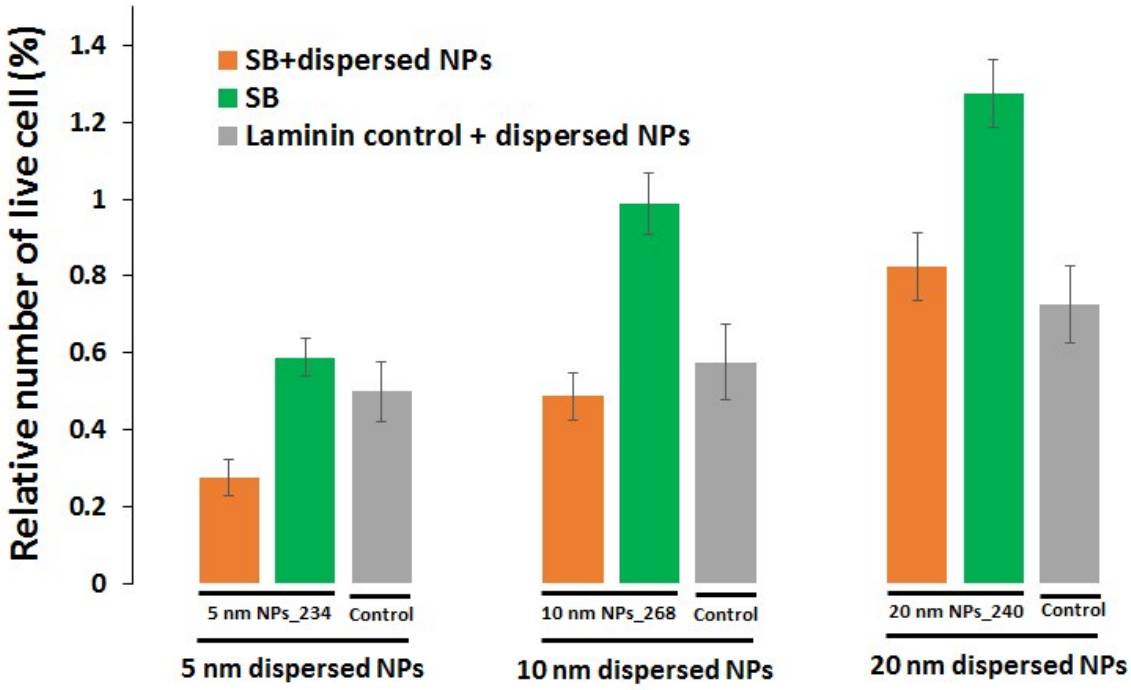


**Fig. S6** The statistical analysis of the relative live cell number on SB particles (with and without adding dispersed AuNP) and laminin coated $SiO_2$ samples (with added the nanoparticles). The relative number of live cells are calculated by dividing the live cell number of each sample by the live cell number of laminin coated $SiO_2$ sample (particle-free). The density of each particle size is given per $\mu m^2$ for 5 nm, 10 nm, and 20 nm as 234, 268, and 240 respectively. (The cell cultures were repeated at least three times, N=6; and variation shown by standard error, SE)

**Method S1:** Preparation and characterization of AUT-functionalized AuNPs: Briefly, glass beads (1-1.3 mm, Carl Roth GmbH + Co. KG) were silanized with APTES according to a procedure reported by Nath. 2.5 g of silanized glass beads were washed once with 1 mL of water, then 2 mL cit-AuNP (5 nm, 10 nm, and 20 nm) were added. The glass beads were incubated overnight, the liquid phase was removed and the beads were rinsed 3 times with water. Successful immobilization of the AuNP was evident by a color change of the glass beads from colorless (before immobilization) to red (after immobilization). Upon addition of 1mL water, 50 µL AUT (2 mM solution in ethanol and 50 µL HCl (0.1 M) and subsequent sonication ligand exchange occurred and the formed AUT-AuNPs were released from the solid support. The AuNP dispersion was removed from the glass beads and allowed to stand for two hours to ensure complete formation of an AUT coat on the particles surface. The AUT-AuNPs were further purified three times by centrifugation and redispersion in acidified water. After the last centrifugation step, the supernatant was removed and the concentrated AUT-AuNP pellet was used for cell tests.

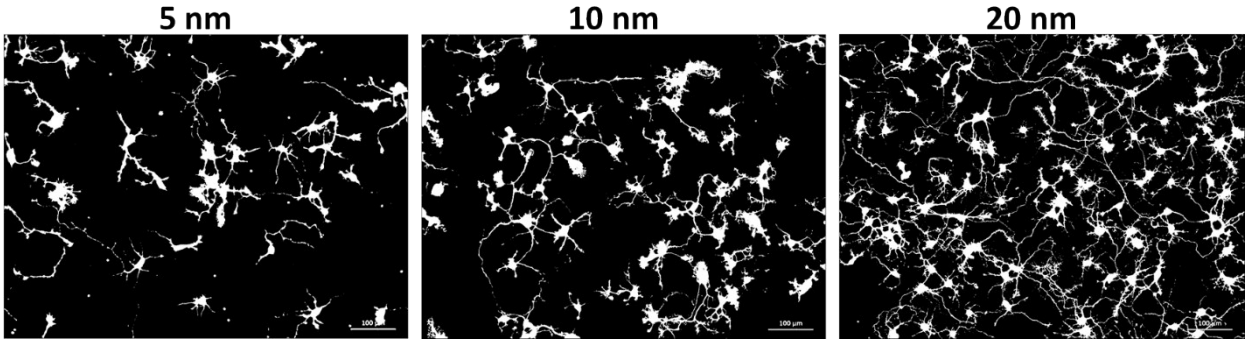


**Fig. S7** The average coverage of cells cultured on the WB particles in one sample is statistically estimated as 4.5 %, 8.7 %, and 14.6 % for 5 nm, 10 nm, and 20 nm particles respectively. The cell coverage is studied with the cells cultured on the highest densities of nanoparticles for each particle size. The only number of WB NPs underline the surface coverage of cells are considered to interact with the cells and they are called accessible NPs. These accessible NPs are $1.05 \times 10^9$, $2.28 \times 10^9$, $3.6 \times 10^9$ particles for 5 nm, 10 nm, and 20 nm NPs respectively.

| Size [nm] | $\lambda_{max}$ (UV-vis) [nm] | z-average (DLS) [nm] |
|---|---|---|
| 5 | 532 | 85 ± 2 |
| 10 | 532 | 129 ± 5 |
| 20 | 530 | 90 ± 4 |

**Table S2:** Characterization of AUT-AuNP.

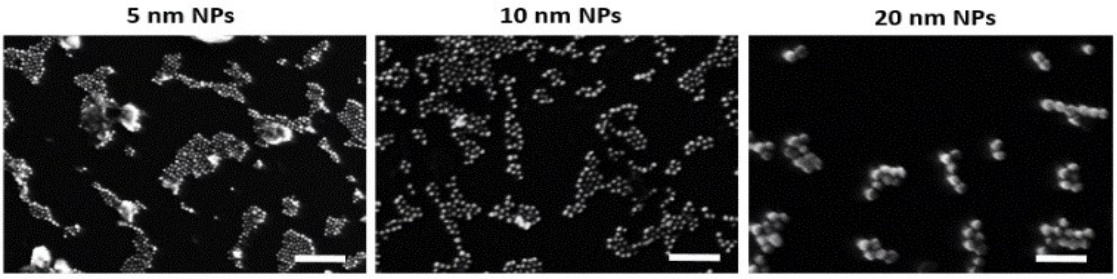


**Fig. S8** SEM images of colloidal 5 nm, 10 nm, and 20 nm AUT-functionalized NPs deposited on a silicon wafer (1 µl droplet for each particle size). The solvent of the colloidal solution was entirely evaporated to allow SEM investigation which leads to particle aggregation. Scale bar: 100 nm.

## 4. Immunostaining for actin and vinculin of neurons on WB and SB particles

Immunofluorescence staining was performed as the similarly procedure described.[11] In brief, DIV3 neurons were rinsed 2 times with pre-warmed PBS at $37^0$ C, and fixed with 4% paraformaldehyde for 7 min in $4^0$ C. The samples were then rinsed three times with PBS, and the cells were permeabilized in a blocking buffer (1% bovine serum abumin + 2 % goat serum in PBS) with 0.1% Triton X for 15 min at RT. The samples were rinsed three times with PBS and stored in the blocking buffer for 1 hour at RT. The samples were incubated with a first antibody (Monoclonal anti-vinculin, Sigma-Aldrich) with a 1:200 concentration ratio in the blocking buffer solution for 1,5 hours. After rinsing three times with PBS, the samples were stored with a second antibody (Alexa Fluor 488 anti-mouse, Life technologies) with 1:500 ratio, Alexa Fluor 633 Phalloidin (ThermoFisher Scientific) with 1:40 ratio, and DAPI (Life Technologies) with 1:500 ratio together in the blocking buffer for 1,5 hours in dark condition at RT. Finally the samples were rinsed three times with PBS and imaged by the fluorescence microscopy.

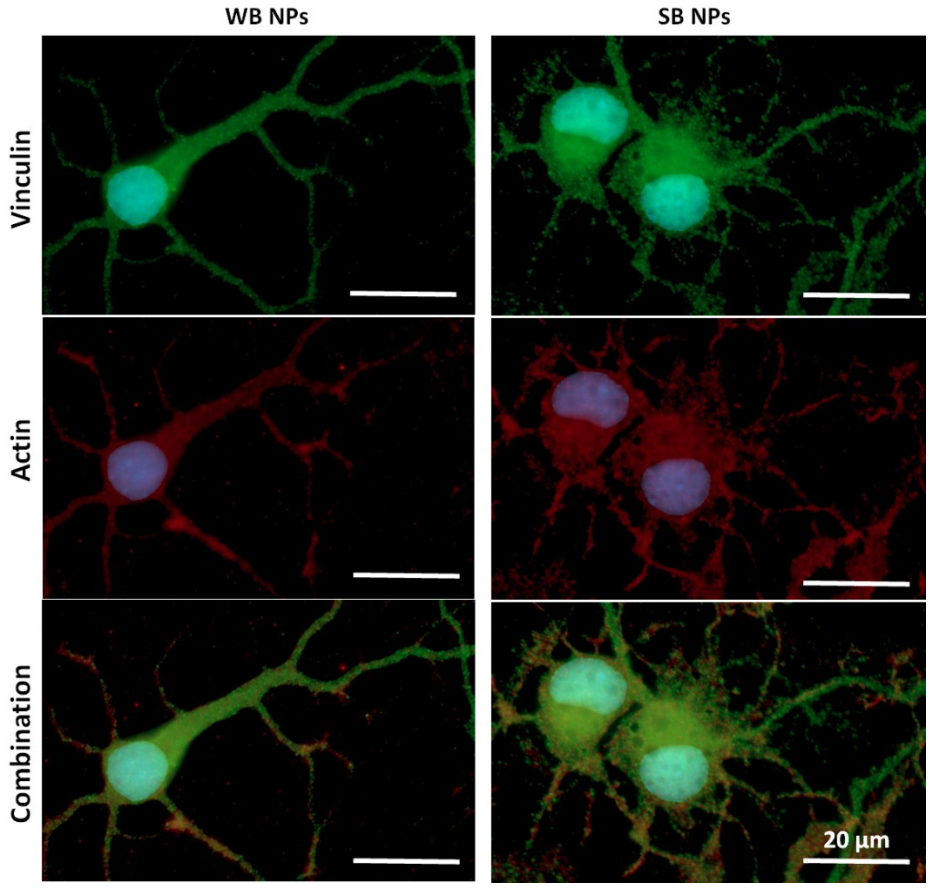


**Fig. S9** Fluorescent micrographs indicating the distributions of actin (red), vinculin (green), and nuclei (blue) in the neuron on weakly bound (WB) and strongly bond (SB) particles. Here, neuron cells were cultured for 3 days on 20 nm NPs of 240 particle density (per $\mu m^2$) for both binding strengths.