Supporting Information

Distribution of Particles in Human Stem Cell-Derived 3D Neuronal Cell Models: Effect of Particle Size, Charge, and Density

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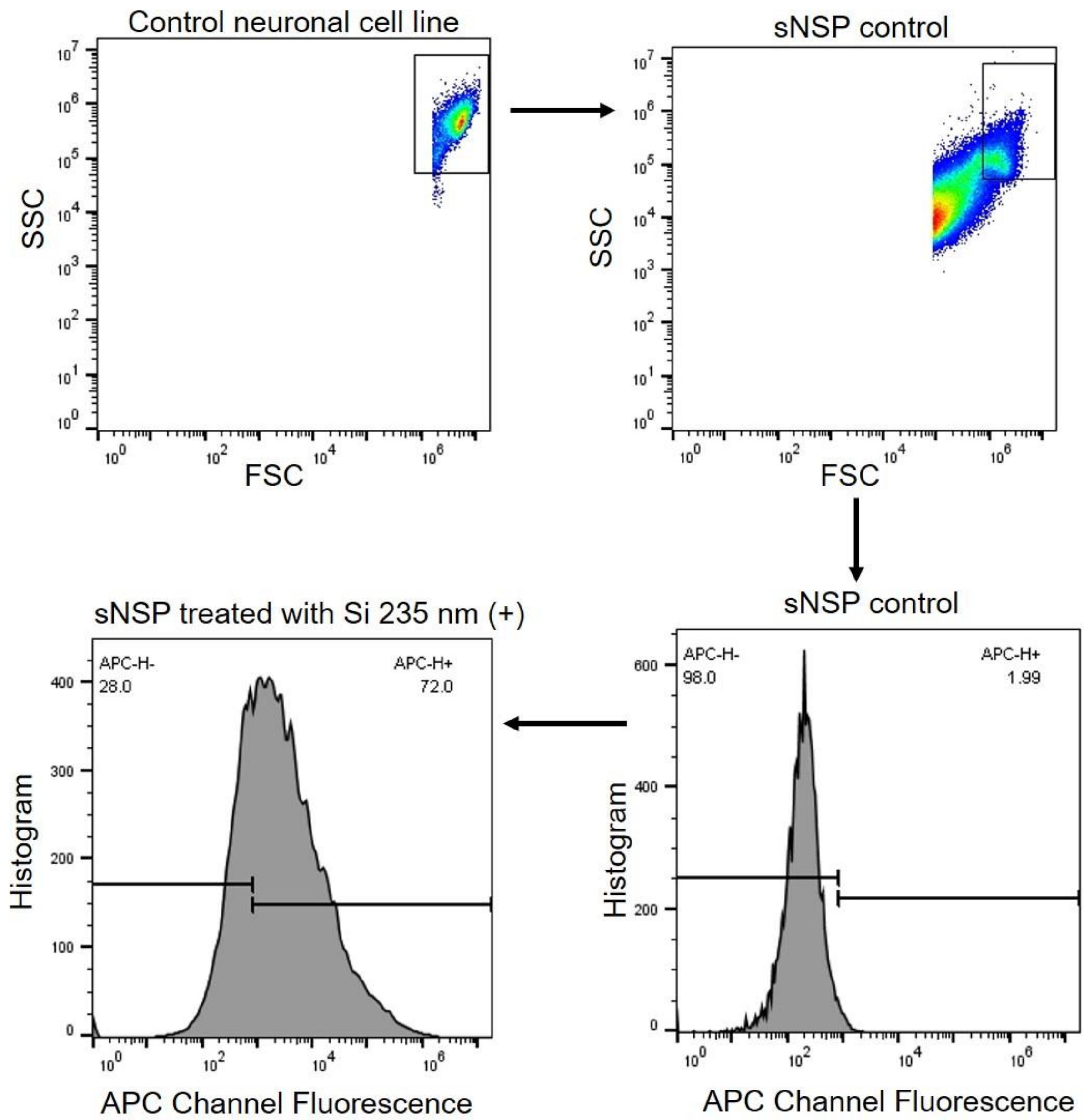

Figure S1. Gating strategy to assess association of particles with cells in the sNSP by flow cytometry. Single-cell population was determined by plotting FSC and SSC of untreated cells and used to determine fluorescence-positive (+) and fluorescence-negative (+) cells. This gate was applied to all samples treated with particles ($PLArg_{AF647}$-terminated Si 235 nm data are shown as an example).

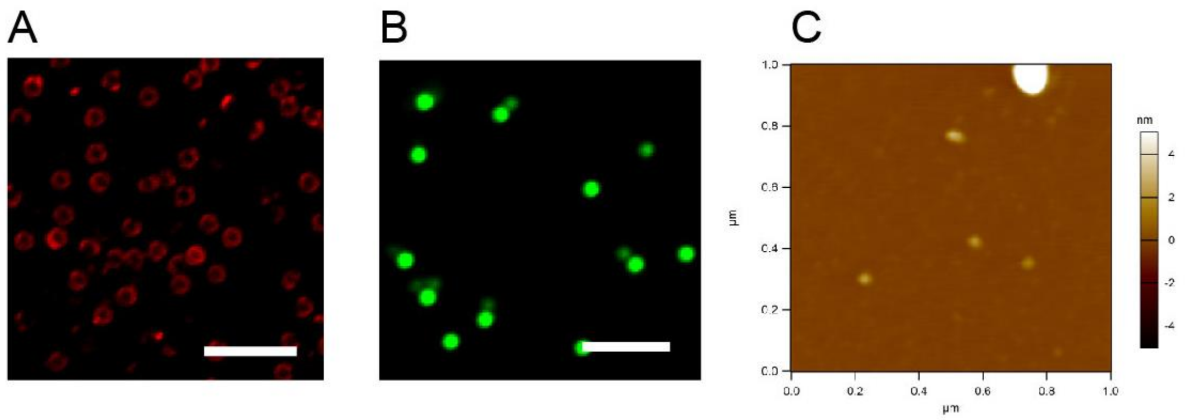

Figure S2. Particle imaging. Confocal microscopy images of (A) templated core–shell particles with silica cores (837 nm) and a layer of AF_{647}-labeled PLArg and (B) PEI-coated FITC-labeled (FluoroGreen) polystyrene particles (1000 nm). Scale bars are 5 µm. (C) AFM image of BG-EDA nanoparticles.

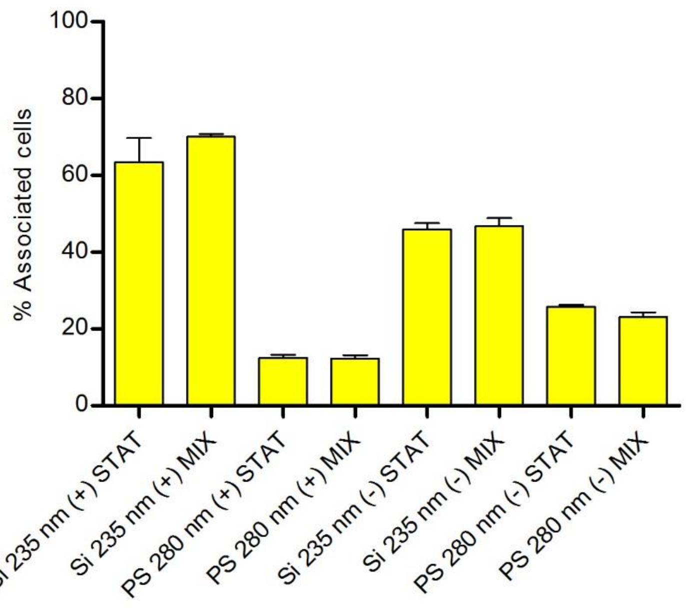

Figure S3. Comparison of static (STAT) and dynamic (MIX) cell culture conditions. Flow cytometry analysis of the association of particles with cells in the sNSP after incubation at 37 °C for 72 h under static or dynamic conditions and disassembly of the sNSP. The particles examined include PLArg-terminated (+) or PSS-terminated (−) Si particles (235 nm) and PEI-coated (+) or uncoated (−) PS particles (280 nm). Data are shown as the average mean ± standard error of the mean (n = 3).

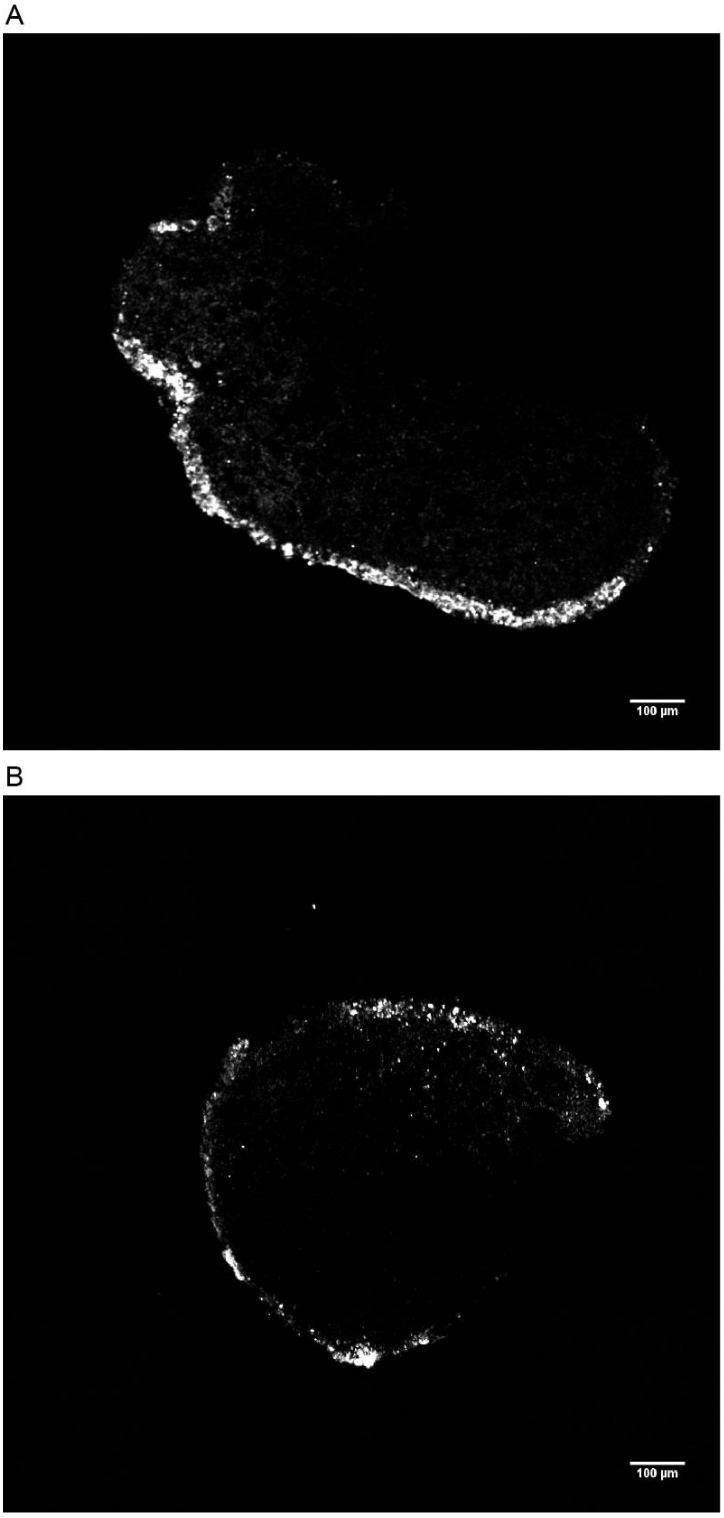

Figure S4. Particle distribution in sNSP. Confocal microscopy images showing a cross-section of sNSP incubated with (A) positively and (B) negatively charged Si (235 nm) particles coated with PLArg and PSS, respectively. Images show the fluorescence of AF_{647}-labeled particles in gray scale.

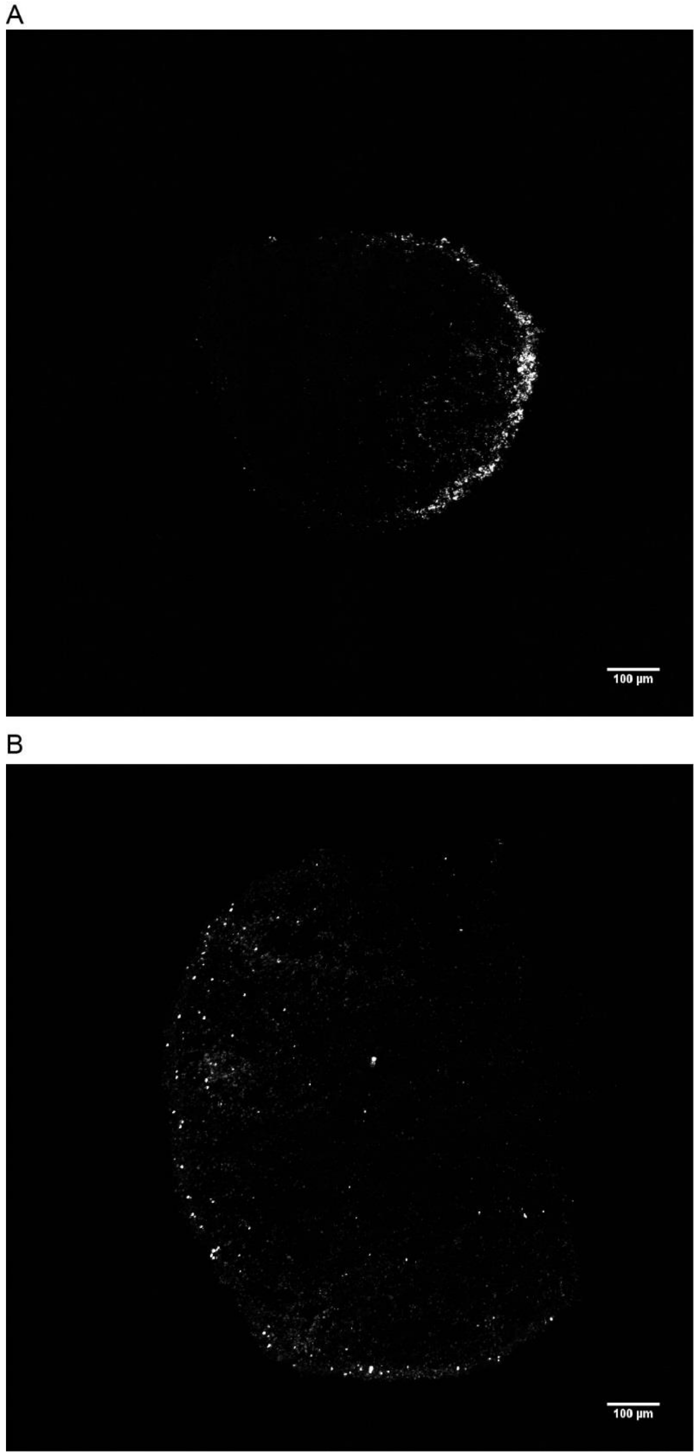

Figure S5. Particle distribution in sNSP. Confocal microscopy images showing a cross-section of sNSP incubated with (A) positively and (B) negatively charged Si (387 nm) particles coated with PLArg and PSS, respectively. Images show the fluorescence of AF_{647}-labeled particles in gray scale.

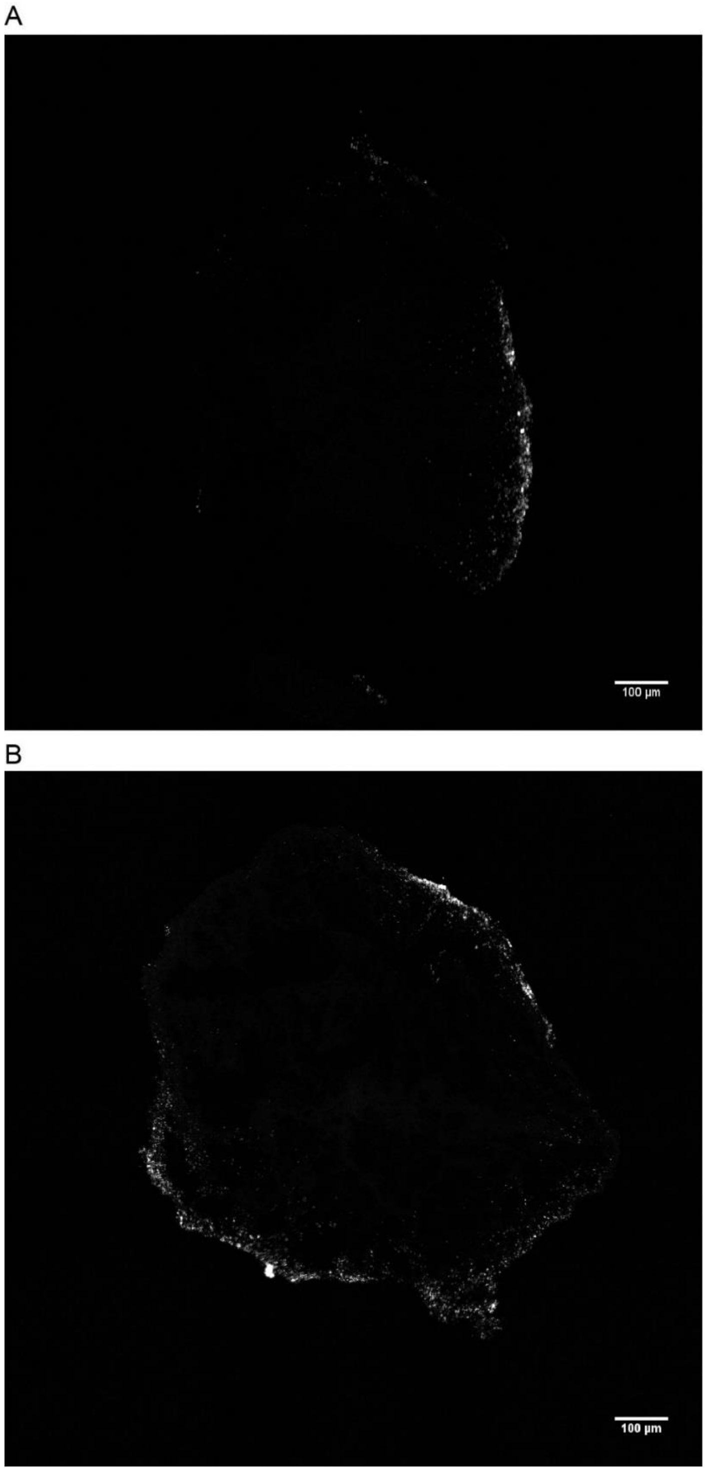

Figure S6. Particle distribution in sNSP. Confocal microscopy images showing a cross-section of sNSP incubated with (A) positively and (B) negatively Si (837 nm) particles coated with PLArg and PSS, respectively. Images show the fluorescence of AF_{647}-labeled particles in gray scale.

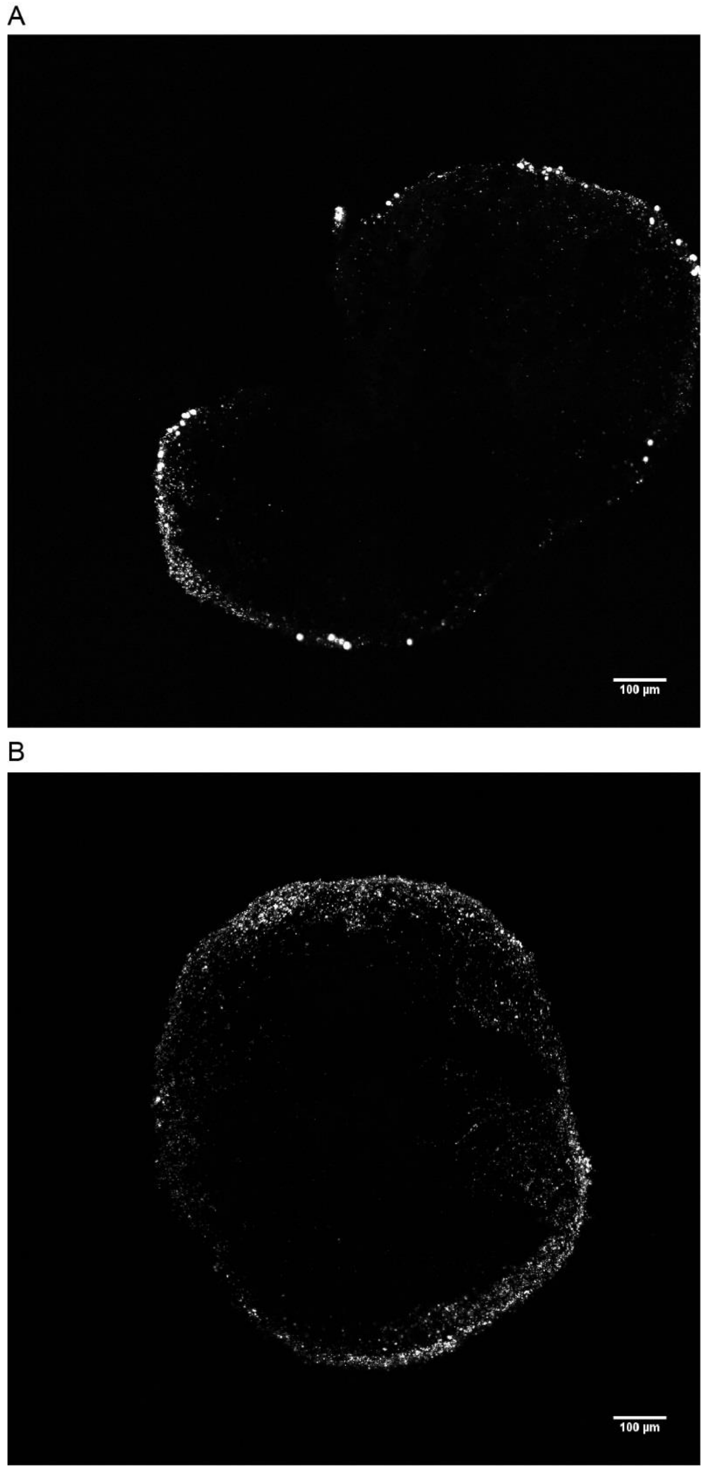

Figure S7. Particle distribution in sNSP. Confocal microscopy images showing a cross-section of NSPs incubated with (A) positively and (B) negatively PS (288 nm) particles coated with PEI and uncoated, respectively. Images show the fluorescence of FITC-labeled particles in gray scale.

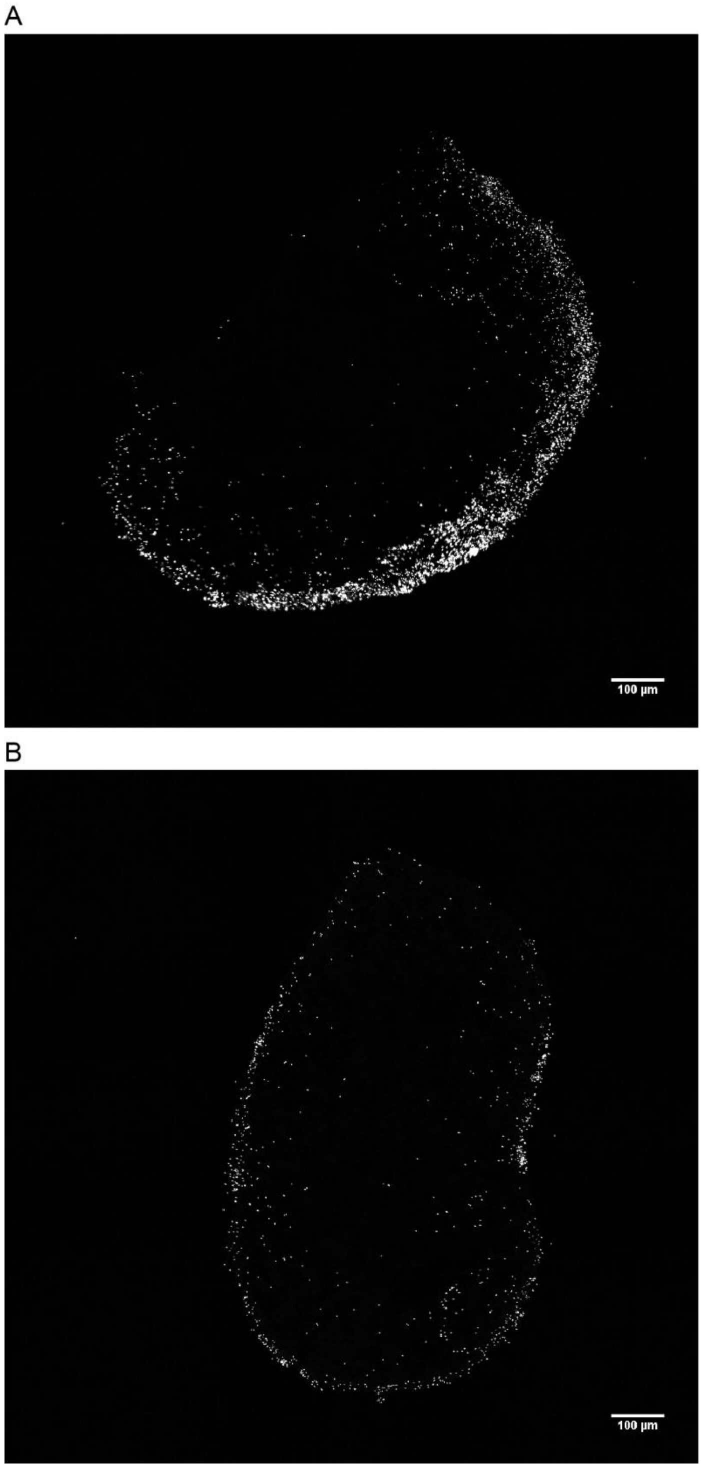

Figure S8. Particle distribution in sNSP. Confocal microscopy images showing a cross-section of sNSP incubated with (A) positively and (B) negatively PS (450 nm) particles coated with PEI and uncoated, respectively. Images show the fluorescence of FITC-labeled particles in grayscale.

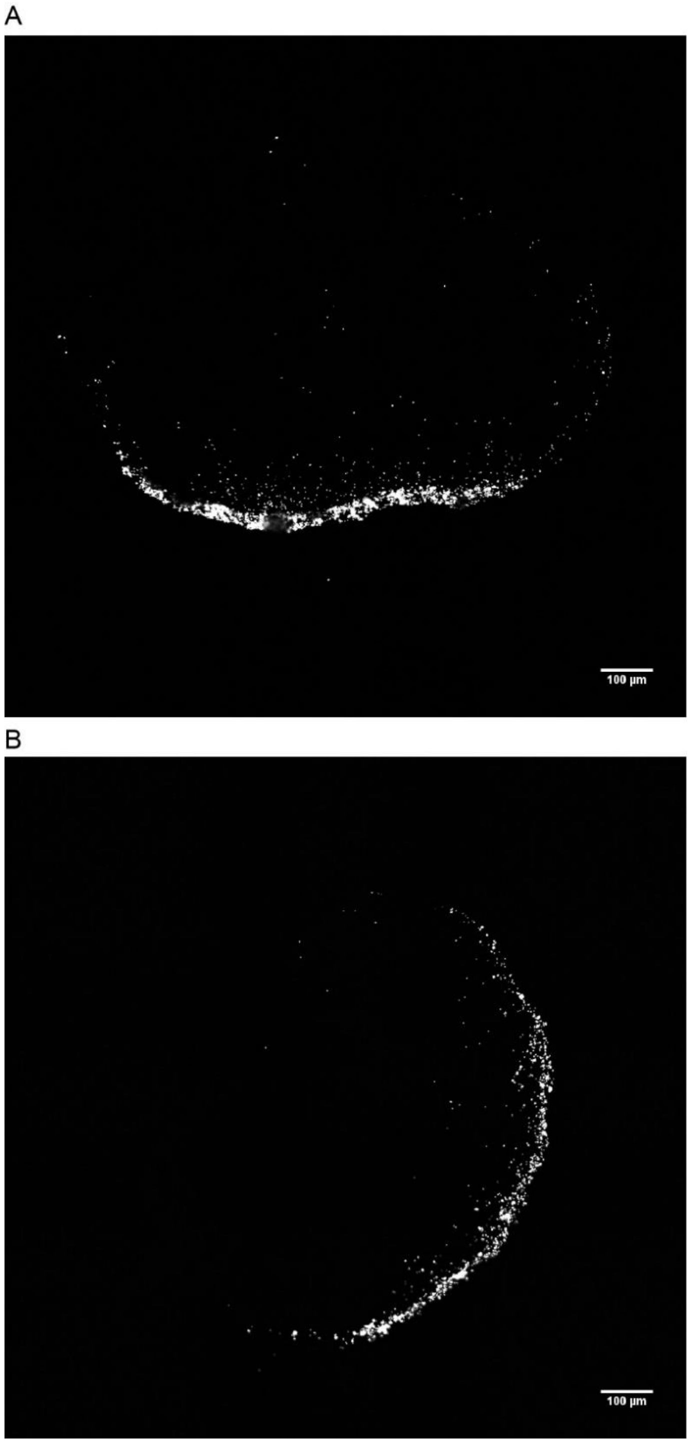

Figure S9. Particle distribution in sNSP. Confocal microscopy images showing a cross-section of sNSP incubated with (A) positively and (B) negatively PS (1000 nm) particles coated with PEI and uncoated, respectively. Images show the fluorescence of FITC-labeled particles in gray scale.

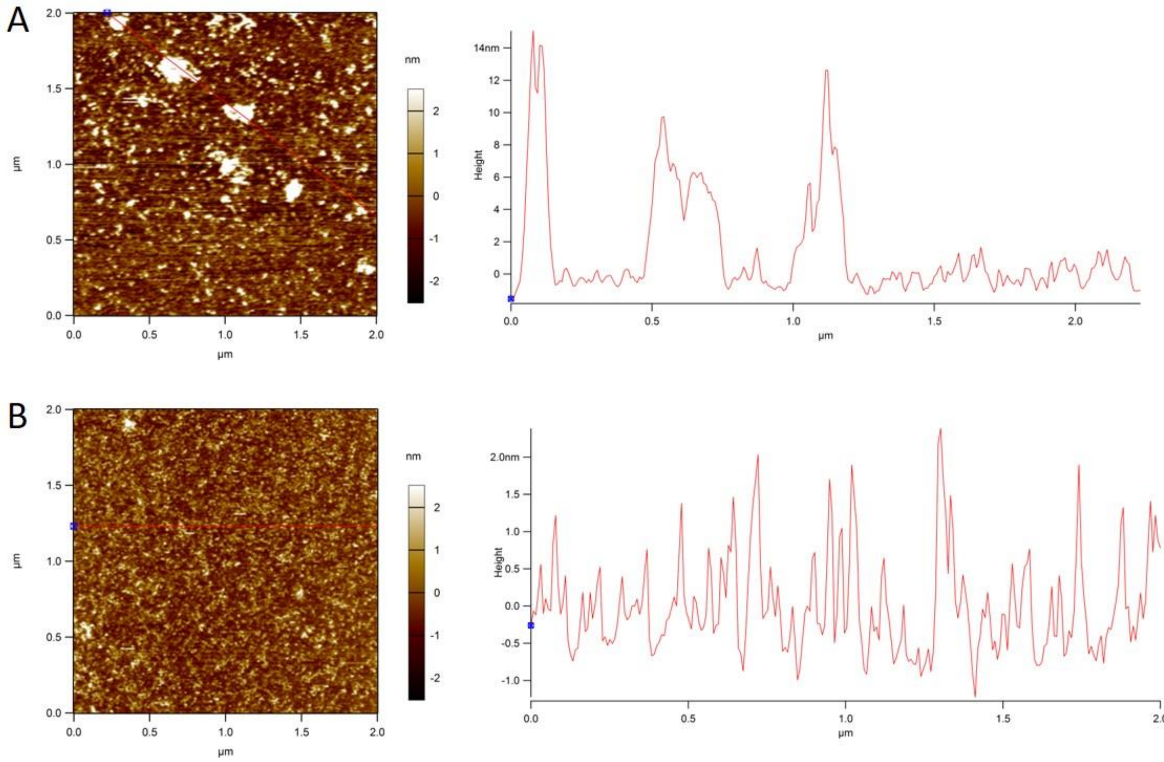

Figure S10. Aqueous AFM imaging of BG-EDA/DNA. Complexes were assembled in DPBS at a glycogen-to-DNA weight ratio of 20. AFM images and corresponding line profiles for (A) BG-EDA/DNA complexes and (B) mica substrate.

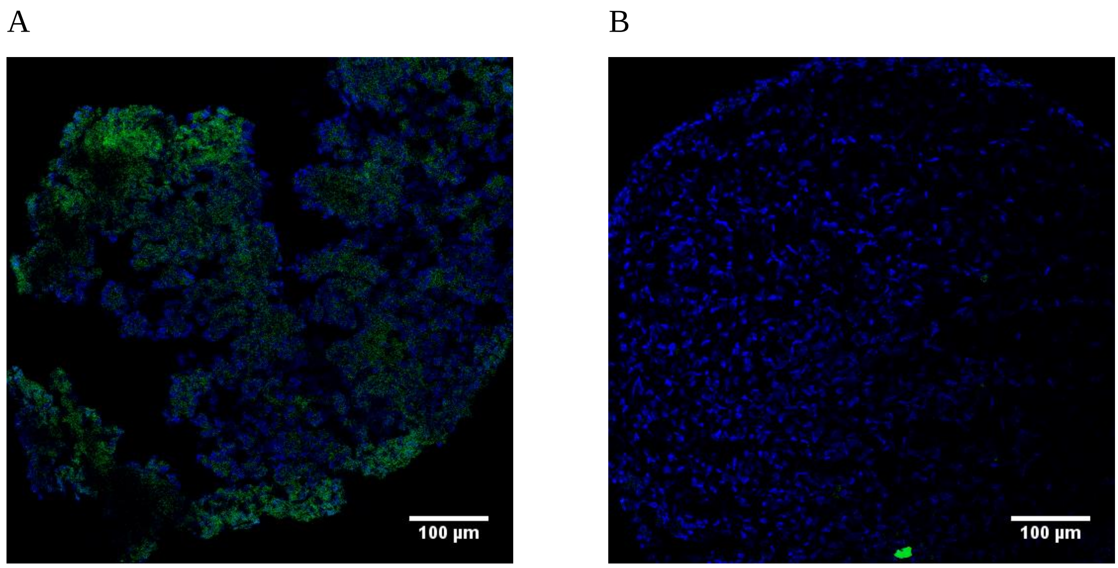

Figure S11. Transfection with BG-EDA/DNA complexes. Confocal microscopy images showing sections of (A) a sNSP transfected with BG-EDA complexed with GFP-expression plasmid and (B) untreated cells.

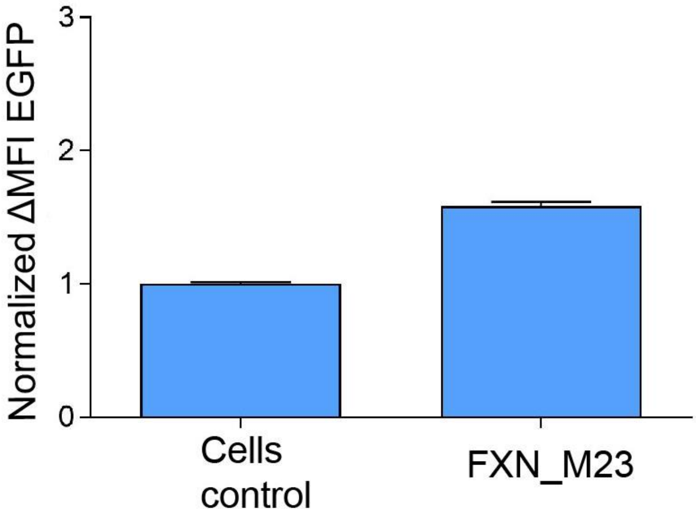

Figure S12. Glycogen-mediated plasmid delivery 4 days post-transfection. Flow cytometry results showing the MFI after transfection with frataxin-expressing plasmid complexed with BG-EDA. MFI was normalized against "Cells control". Data are shown as the average mean ± standard error of the mean (*n* = 2). FXN-M23 is pPB-ef1a-FXN-IRES-eEGFP-neo.

Minimum Information Reporting in Bio–Nano Experimental Literature

The MIRIBEL guidelines were introduced here: https://doi.org/10.1038/s41565-018-0246-4

The development of these guidelines was led by the ARC Centre of Excellence in Convergent Bio-Nano Science and Technology: https://www.cbns.org.au/. Any updates or revisions to this document will be made available here: http://doi.org/10.17605/OSF.IO/SMVTF.

The MIRIBEL guidelines were developed to facilitate reporting and dissemination of research in bio–nano science. Their development was inspired by various similar efforts:

- MIAME (microarray experiments): *Nat. Genet.* **29** (2001), 365; http://doi.org/10.1038/ng1201-365
- MIRIAM (biochemical models): *Nat. Biotechnol.* **23** (2005) 1509; http://doi.org/10.1038/nbt1156
- MIBBI (biology/biomedicine): *Nat. Biotechnol.* **26** (2008) 889; http://doi.org/10.1038/nbt.1411
- MIGS (genome sequencing): *Nat. Biotechnol.* **26** (2008) 541; http://doi.org/10.1038/nbt1360
- MIQE (quantitative PCR): *Clin. Chem.* **55** (2009) 611; http://doi.org/10.1373/clinchem.2008.112797
- ARRIVE (animal research): *PLOS Biol.* **8** (2010) e1000412; http://doi.org/10.1371/journal.pbio.1000412
- *Nature*'s reporting standards:
    - Life science: https://www.nature.com/authors/policies/reporting.pdf; e.g., *Nat. Nanotechnol.* **9** (2014) 949; http://doi.org/10.1038/nnano.2014.287
    - Solar cells: https://www.nature.com/authors/policies/solarchecklist.pdf; e.g., *Nat. Photonics* **9** (2015) 703; http://doi.org/10.1038/nphoton.2015.233
    - Lasers: https://www.nature.com/authors/policies/laserchecklist.pdf; e.g., *Nat. Photonics* **11** (2017) 139; http://doi.org/10.1038/nphoton.2017.28
- The "TOP guidelines": e.g., *Science* **352** (2016) 1147; http://doi.org/10.1126/science.aag2359

Similar to many of the efforts listed above, the parameters included in this checklist are **not** intended to be definitive requirements; instead they are intended as 'points to be considered', with authors themselves deciding which parameters are—and which are not—appropriate for their specific study.

This document is intended to be a living document, which we propose is revisited and amended annually by interested members of the community, who are encouraged to contact the authors of this document. Parts of this document were developed at the annual International Nanomedicine Conference in Sydney, Australia: http://www.oznanomed.org/, which will continue to act as a venue for their review and development, and interested members of the community are encouraged to attend.

After filling out the following pages, this checklist document can be attached as a "Supporting Information" document during submission of a manuscript to inform Editors and Reviewers (and eventually readers) that all points of MIRIBEL have been considered.

Supplementary Table 1. Material characterization*

Question	**Yes**	**No**
1.1 Are "**best reporting practices**" **available** for the nanomaterial used? For examples, see *Chem. Mater.* **28** (2016) 3535; http://doi.org/10.1021/acs.chemmater.6b01854 and *Chem. Mater.* **29** (2017) 1; http://doi.org/10.1021/acs.chemmater.6b05235	not applicable	
1.2 If they are available, **are they used**? If not available, ignore this question and proceed to the next one.		
1.3 Are extensive and clear instructions reported detailing all steps of **synthesis** and the resulting **composition** of the nanomaterial? For examples, see *Chem. Mater.* **26** (2014) 1765; http://doi.org/10.1021/cm500632c, and *Chem. Mater.* **26** (2014) 2211; http://doi.org/10.1021/cm5010449. Extensive use of photos, images, and videos are strongly encouraged. For example, see *Chem. Mater.* **28** (2016) 8441; http://doi.org/10.1021/acs.chemmater.6b04639	x	
1.4 Is the **size** (or **dimensions**, if non-spherical) and **shape of** the nanomaterial reported?	x	
1.5 Is the **size dispersity** or **aggregation** of the nanomaterial reported?	x	
1.6 Is the **zeta potential** of the nanomaterial reported?	x	
1.7 Is the **density (mass/volume)** of the nanomaterial reported?	x	
1.8 Is the amount of any **drug loaded** reported? 'Drug' here broadly refers to functional cargos (e.g., proteins, small molecules, nucleic acids).	x	
1.9 Is the **targeting performance** of the nanomaterial reported, including **amount** of ligand bound to the nanomaterial if the material has been functionalised through addition of targeting ligands?	not applicable	
1.10 Is the **label signal** per nanomaterial/particle reported? For example, fluorescence signal per particle for fluorescently labelled nanomaterials.		x
1.11 If a material property not listed here is varied, has it been **quantified**?	not applicable	
1.12 Were characterizations performed in a **fluid mimicking biological conditions**?	x	
1.13 Are details of how these parameters were **measured/estimated** provided?	x	
Explanation for **No** (if needed): 1.10 The label signal per particle was not quantified because the flow cytometry data was analyzed as %Cell Association based on a shift in the fluorescence of the treated cells relative to the control and not by using mean fluorescence intensity (MFI).		

*Ideally, material characterization should be performed in the same biological environment as that in which the study will be conducted. For example, for cell culture studies with nanoparticles, characterization steps would ideally be performed on nanoparticles dispersed in cell culture media. If this is not possible, then characteristics of the dispersant used (e.g., pH, ionic strength) should mimic as much as possible the biological environment being studied.

Supplementary Table 2. Biological characterization*

Question	Yes	No
2.1 Are **cell seeding details**, including **number of cells plated, confluency at start of experiment,** and **time between seeding and experiment** reported?	not applicable	
2.2 If a standardised cell line is used, are the **designation and source** provided?	x	
2.3 Is the **passage number** (total number of times a cell culture has been subcultured) known and reported?	not applicable	
2.4 Is the last instance of **verification of cell line** reported? If no verification has been performed, is the time passed and passage number since acquisition from trusted source (e.g., ATCC or ECACC) reported? For information, see *Science* **347** (2015) 938; http://doi.org/10.1126/science.347.6225.938	x	
2.5 Are the results from **mycoplasma testing** of cell cultures reported?	x	
2.6 Is the **background signal of cells/tissue** reported? (E.g., the fluorescence signal of cells without particles in the case of a flow cytometry experiment.)	x	
2.7 Are **toxicity studies** provided to demonstrate that the material has the expected toxicity, and that the experimental protocol followed does not?	not applicable	
2.8 Are details of media preparation (**type of media, serum,** any **added antibiotics**) provided?	x	
2.9 Is a **justification of the biological model** used provided? For examples for cancer models, see *Cancer Res.* **75** (2015) 4016; http://doi.org/10.1158/0008-5472.CAN-15-1558, and *Mol. Ther.* **20** (2012) 882; http://doi.org/10.1038/mt.2012.73, and *ACS Nano* **11** (2017) 9594; http://doi.org/10.1021/acsnano.7b04855	x	
2.10 Is characterization of the **biological fluid** (*ex vivo*/*in vitro*) reported? For example, when investigating protein adsorption onto nanoparticles dispersed in blood serum, pertinent aspects of the blood serum should be characterised (e.g., protein concentrations and differences between donors used in study).	not applicable	
2.11 For **animal experiments**, are the ARRIVE guidelines followed? For details, see *PLOS Biol.* **8** (2010) e1000412; http://doi.org/10.1371/journal.pbio.1000412	not applicable	
Explanation for **No** (if needed):		

*For *in vitro* experiments (e.g., cell culture), *ex vivo* experiments (e.g., in blood samples), and *in vivo* experiments (e.g., animal models). The questions above that are appropriate depend on the type of experiment conducted.

Supplementary Table 3. Experimental details*

Question	Yes	No
3.1 For cell culture experiments: are **cell culture dimensions** including **type of well**, **volume of added media**, reported? Are cell types (i.e.; adherent vs suspension) and **orientation** (if non-standard) reported?	x	
3.2 Is the **dose of material administered** reported? This is typically provided in nanomaterial mass, volume, number, or surface area added. Is sufficient information reported so that regardless of which one is provided, the other dosage metrics can be calculated (i.e. using the dimensions and density of the nanomaterial)?	x	
3.3 For each type of imaging performed, are details of how **imaging** was performed provided, including details of **shielding, non-uniform image processing**, and any **contrast agents** added?	x	
3.4 Are details of how the dose was administered provided, including **method of administration, injection location, rate of administration,** and details of **multiple injections**?	not applicable	
3.5 Is the methodology used to **equalise dosage** provided?	not applicable	
3.6 Is the **delivered dose** to tissues and/or organs (in vivo) reported, as % injected dose per gram of tissue (%ID g^{-1})?	not applicable	
3.7 Is **mass of each organ/tissue measured** and **mass of material** reported?	not applicable	
3.8 Are the **signals of cells/tissues with nanomaterials** reported? For instance, for fluorescently labelled nanoparticles, the total number of particles per cell or the fluorescence intensity of particles + cells, at each assessed timepoint.		x
3.9 Are **data analysis details**, including **code used** for analysis provided?		x
3.10 Is the **raw data** or **distribution of values** underlying the reported results provided? For examples, see *R. Soc. Open Sci.* **3** (2016) 150547; http://doi.org/10.1098/rsos.150547, https://opennessinitiative.org/making-your-data-public/, http://journals.plos.org/plosone/s/data-availability, and https://www.nature.com/sdata/policies/repositories		x
Explanation for **No** (if needed): Flow cytometry data presented as %Cell Association based on the shift of single cell population; single time point.		

* The use of protocol repositories (e.g., *Protocol Exchange* http://www.nature.com/protocolexchange/) and published standard methods and protocols (e.g., *Chem. Mater.* **29** (2017) 1; http://doi.org/10.1021/acs.chemmater.6b05235, and *Chem. Mater.* **29** (2017) 475; http://doi.org/10.1021/acs.chemmater.6b05481) are encouraged.