Supporting Information

Particle-mediated delivery of frataxin plasmid to a human sensory neuronal model of Friedreich's Ataxia

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EXPERIMENTAL

Materials

Silica particles (0.889 ± 0.003 μm, 5% w/v aqueous solution) were purchased from microParticles GmbH (Berlin, Germany). Poly(ethylenimine) (PEI) low molecular weight 50 wt% solution in water, poly-L-arginine hydrochloride, molecular weight >70000; poly(sodium 4-styrenesulfonate) (PSS) molecular weight ~70000, albumin from bovine serum (BSA), Dulbecco's phosphate buffered saline (DPBS), without calcium chloride or magnesium chloride, agarose, glucose, and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Sigma-Aldrich (St. Louis, MI, USA). Plasmid DNA (pEGFP, 3 kbp) was obtained from CSIRO (Australia) and pcDNA3-Luc (5.9 kbp) was a gift from Dr. Yunti Zhang. Sodium chloride (NaCl) was purchased from Chem-Supply (Gillman, Australia). Alexa Fluor *N*-hydroxysuccinimide (NHS) dyes (AF_{488} and AF_{555}), Alamar blue cell viability reagent, wheat germ agglutinin (WGA), Alexa Fluor 488 conjugate (WGA-AF_{488}), Hoechst 33342 (10 mg mL^{-1}

solution in water), lipofectamine, N2B27 medium, Dulbecco's modified Eagle's medium (DMEM)/F12 medium, insulin, transferrin, and selenium additives, N_2 supplement, retinol-free B27 supplement, penicillin-streptomycin, GlutaMAX, and PureLink RNA mini kit were purchased from Life Technologies (Scoresby, Australia). Dialysis tubing (molecular weight cutoff (MWCO) 3.5 kDa) and Nunc Lab-Tek II Chamber microscopy slides were purchased from Thermo Fisher Scientific (Scoresby, Australia). DNA electrophoresis sample loading dye was purchased from Bio-Rad (Gladesville, Australia). Brain-derived U-87 MG glioblastoma cell line (HTB-14) was purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). DMEM (with 4.5 g L^{-1} glucose and L-glutamine) and trypsin (10X) were purchased from Lonza (Basel, Switzerland). Fetal bovine serum was purchased from Bovogen Biologicals (Keilor East, Australia). Paraformaldehyde 4% aqueous solution (EM grade, 4% PFA) was purchased from Electron Microscopy Sciences. Vitronectin and Tesr-E8 basal medium were purchased from StemCell Technologies. Y-27632 was purchased from Selleckchem. Brain derived neurotrophic factor (BDNF), fibroblast growth factor-basic (FGF-basic), beta nerve growth factor (bNGF), and neutrotrophin-3 (NT-3) were purchased from PeproTech. CHIR99021 and SB431542 were purchased from Tocris Bioscience. Bone morphogenetic protein 2 (BMP2), goat anti-human TRKA (AF175), sheep anti-human/mouse/rat PV (AF5058), and goat anti-human SPP1 (AF1433) were purchased from R&D Systems. Mouse anti-human TRKB (NBP1-47898) was purchased from Novus Biologicals. Rabbit anti-human TRKC (701985) was purchased from Thermo Fisher Scientific. Mouse anti-human PRPH (H00005630-M02) was purchased from Abnova. Chicken anti-human/mouse/rat TUBB3 (ab41489) was purchased from Abcam. Secondary antibodies donkey Alexa Fluor 488 and donkey Alex Fluor 568 were purchased from Thermo Fisher Scientific, and donkey anti-chicken 488 was purchased from Jackson ImmunoResearch. Dako fluorescent mounting medium was purchased from Agilent Technologies. Donkey serum was purchased from Millipore (Millipore Corporation, Billerica, MA, USA) and Milli-Q water was obtained from a Millipore Milli-Q purification system.

Minimum information reporting in bio–nano experimental literature (MIRIBEL)

The studies conducted herein, including material characterization, biological characterization, and experimental details, conform to the MIRIBEL reporting standard

for bio–nano research,[25] and we include a companion checklist of these components herein.

Quartz crystal microbalance with dissipation (QCM-D) monitoring

Layer-by-layer (LbL) film buildup of cationic poly-L-arginine (PLArg) and anionic PSS) and subsequent adsorption of DNA were studied using QCM-D. The gold crystal QCM surface was cleaned by exposure to Piranha solution (one part of 30% H_2O_2 in three parts of H_2SO_4) for 2 min, followed by rinsing in pure water and drying with nitrogen. *Caution: Piranha solution is highly corrosive. Extreme care should be taken when handling Piranha solution and only small quantities should be prepared.* PEI solution (2 mg mL^{-1}) was prepared in Milli-Q water with 1 M NaCl. PSS (1 mg ml^{-1}) and PLArg (1 mg ml^{-1}) solutions were prepared in 50 mM sodium acetate buffer pH 5.2, which was also used in washing steps after each layer. PEI was deposited on the electrode as the first (primer) layer. After 15 min of incubation, excess polymer was removed by rinsing for 5 min with water. Sequential deposition of PSS and PLArg (15 min adsorption followed by three washing cycles in buffer) was repeated until four PSS/PLArg bilayers or $(PSS/PLArg)_4$ were formed. To assess DNA binding to the PLArg-terminated film, DNA (10 µg mL^{-1} in 50 mM sodium acetate buffer pH 5.2) was introduced and adsorbed for 20 min, followed by washing with buffer. The mass of deposited nucleic acid was calculated using Sauerbrey's equation:[1]

$$\Delta F = -\frac{2F_0^2}{A(\mu_q \rho_q)^{1/2}} \Delta m$$

where F_0 is the resonance frequency of the crystal (9×10^6 Hz), μ_q is the shear modulus of the quartz (2.947×10^{13} g m^{-1} s^{-2}), ρ_q is the density of the quartz (2.648×10^6 g m^{-3}), and A is the piezoelectric area of the electrode.

Atomic force microscopy (AFM)

Silicon wafers were ultrasonically cleaned in ethanol for 2 h to remove any unwanted residues on the surface and dried under a nitrogen stream. PEI-$(PSS/PLArg)_2$ and PEI-$(PSS/PLArg)_2$-DNA films were prepared on silicon wafers using the adsorption and washing conditions as those described for QCM and dried under a gentle stream of nitrogen. AFM experiments were conducted on a JPK NanoWizard II BioAFM instrument. Typical scans were performed in tapping mode with MikroMasch silicon

cantilevers (NSC/CSC). The roughness of the air-dried films was analyzed using JPK SPM image processing software (version V.3.3.32).

Preparation of multilayered particles

Multilayered particles were prepared by LbL deposition of PEI-(PSS/PLArg)$_4$ on spherical silica particles. Silica particles (40 µL, 0.05 wt%) were washed by dispersing in 200 µL of Milli-Q water and mixed vigorously by vortexing, followed by centrifugation (500 *g*, 1.5 min). The supernatant was removed, and the dispersion and centrifugation cycles were repeated once with water then twice with buffer (50 mM sodium acetate buffer pH 5.2). Polyelectrolyte solutions were prepared as per the procedure described for QCM-D. To prime the LbL film buildup, PEI was adsorbed as the first layer. Specifically, 200 µL of PEI was added to 200 µL of the silica particle suspension in water. Adsorption was carried out for 15 min, followed by centrifugation (conditions as described above). The supernatant was removed, and the particles were re-dispersed in 500 µL of water, followed by centrifugation. The particles were washed in water three times. For the subsequent formation of PSS/PLArg bilayers, 500 µL of polyelectrolyte was added to 200 µL of particles in sodium acetate buffer. After 15 min adsorption, three washing cycles were performed using buffer as the dispersant. For the AF$_{488}$- or AF$_{555}$-labeled particles, the PLArg labeled with the dye was used as the seventh layer (see below for labeling method). To monitor the assembly process, a small volume of particle sample was removed after each deposition step for ζ-potential measurements. Particles were counted using an Apogee A50-Microflow cytometer.

Particle characterization

The LbL assembly on particles was monitored by measuring the change in ζ-potential after each layer deposition. For the ζ-potential measurements, 2 µL of sample (~8×10^6 particles µL^{-1}) was dispersed in 798 µL of water. To assess nucleic acid binding, DNA was complexed to the particles for 20 min in 30 µL of sodium acetate buffer pH 5.2 (2×10^6 particles per 0.5 µg of DNA), followed by the addition of 760 µL of Milli-Q water. All measurements were performed at 25 °C in folded capillary cells (DTS1070, Malvern Instruments) using a Zetasizer Nano-ZS instrument (Malvern Instruments).

For the scanning electron microscopy (SEM) analysis, silicon wafers were cleaned in a bath (100 mL of 80% H_2SO_4, 35 mL H_2O_2 and 20 mL Milli-Q water) at room temperature and dried with compressed nitrogen gas. Particle suspensions (2 µL of

stock suspended in 18 µL of 5 mM sodium acetate buffer) were deposited on the silicon wafer and air-dried. Samples were washed with Milli-Q water four times. Images were acquired using an FEI Quanta 200 field-emission scanning electron microscope at an operation voltage of 10 kV.

Labeling PLArg with Alexa Fluor-NHS dyes

For labeling, 10 mg of PLArg (equivalent to 0.057 mmol of arginine monomer) was dissolved in 5 mL of 50 mM sodium acetate buffer, pH 5.2. Either AF_{488} or AF_{555} (2.8×10^{-4} mmol) was added and the resulting solution was incubated for 2 h, protected from light, with mixing. Excess dye was removed by dialysis (MWCO 3.5 kDa) against Milli-Q water for 2 days (5 times water change). The product was freeze dried and stored at 4 °C until further use.

DNA–Particle complexation

Analysis of DNA–particle complexation was performed using agarose gel electrophoresis. Particle suspensions with varying numbers of particles per sample (1×10^5, 2×10^5, 2×10^6, 8×10^6, 3.6×10^7, and 7.2×10^7) were prepared in 50 mM sodium acetate buffer, pH 5.2. Model plasmid DNA (pEGFP, 3 kbp) was diluted in the same buffer and added to each particle suspension (100 ng of DNA per sample). After 20 min of incubation (room temperature, mixed by vortexing every 5 min), samples were centrifuged (600 *g*, 2 min) and 30 µL of the supernatant that contained unbound DNA was mixed with 6 µL of nucleic acid loading dye and loaded in a 1.6% agarose gel. Electrophoresis was carried out for 50 min at 110 V. Samples containing DNA only or particles only in buffer were also loaded as controls.

Cell culture

U87-MG human brain glioblastoma (ATCC HTB-14) cell line was cultured in DMEM supplemented with 10% fetal bovine serum. Cells were cultured at 37 °C, 5% CO_2 in a humidified atmosphere.

Particle–cell association

In 24-well plates, cells were seeded at 6×10^4 cells per well in 400 µL of medium and left overnight at 37 °C, 5% CO_2 in a humidified atmosphere. Particles labeled with

AF_{555} were added at varying particle-to-cell ratios of 10, 50, 200, and 500 for 24 h at 37 °C, 5% CO_2 in a humidified atmosphere. After incubation, the cells were detached by trypsinization (200 µL of 0.05% trypsin) and washed twice by centrifugation–resuspension cycles (300 *g*, 5 min). Cells were suspended in 200 µL of PBS containing 1% BSA and analyzed on a BD Accuri C6 flow cytometer. Data were acquired for at least 1×10^4 cells per sample.

Cellular uptake of fluorescently labeled particles by U87-MG cells

Cells were seeded in 8-well chamber slide at 6×10^4 cells per well in 400 µL of medium and left overnight at 37 °C, 5% CO_2 in a humidified atmosphere. The media was aspirated and replaced with 370 µL of fresh medium, to which 30 µL of AF_{555}-labeled particle suspension was added at a particle-to-cell ratio of 200. After incubation for 24 h (37 °C, 5% CO_2 in a humidified atmosphere), the cells were washed twice with DPBS and incubated for 5 min at room temperature with WGA solution at 10 µg mL^{-1} in DPBS, after which fixation was performed for 10 min at room temperature using 4% PFA. Nuclei were stained using Hoechst 33342 (1 µg mL^{-1}). Cells were imaged with a Nikon A1R confocal microscope equipped with a 60× oil objective.

Cell viability

Cells were plated on a 96-well plate at 1×10^4 cells per well in 100 µL of medium and left overnight at 37 °C, 5% CO_2 in a humidified atmosphere. Particle suspensions were prepared in 50 mM sodium acetate buffer pH 5.2, with varying numbers of particles per sample (1×10^5, 5×10^5, 2×10^6, and 5×10^6) at respective particle-to-cell ratios of 10, 50, 200, and 500. For samples containing model plasmid DNA (pcDNA3-Luc), particle suspensions were prepared in the same way and incubated with 0.5 µg of DNA for 20 min at room temperature in sodium acetate buffer prior to addition to U87-MG cells. Particles, with or without DNA, were added to cells and incubated for 24 h at 37 °C, 5% CO_2 in a humidified atmosphere. After incubation, the media was aspirated and replaced with 100 µL of fresh medium, to which 10 µL of Alamar blue reagent was added. After incubation for 4 h (37 °C, 5% CO_2 in a humidified atmosphere), cell fluorescence (excitation 560 nm/emission 590 nm) was measured on a Tecan Infinite M200 microplate reader.

Friedreich ataxia (FRDA) iPSC cell culture and differentiation to sensory neurons

The experiments were performed in accordance with the Guidelines of the National Health and Medical Research Council and approved by The University of Melbourne Human Ethics Committee (#1545394 and #0829937). Informed consents were obtained from human participants of this study. FRDA 'FA10' iPSC lines were maintained as bulk culture in feeder-free conditions on vitronectin-coated dish using Tesr-E8 basal medium. Differentiation of FRDA iPSC to sensory neurons was performed using previously published protocols.[27,28] Briefly, iPSCs were plated per laminin-coated organ culture dish in TeSR-E8 complete medium supplemented with 10 μM Y-27632 for 24 h. Then, TeSR-E8 complete medium was replaced with N2B27 medium containing 1:1 mix of neurobasal medium (NBM) and DMEM/F12 medium, 1% insulin/transferrin/selenium, 1% N_2 supplement, 1% retinol-free B27 supplement, 1% GlutaMAX, 1% penicillin-streptomycin, 0.3% glucose, and supplemented with CHIR99021 (3 μM) and SB431542 (10 μM). After 5 days, cells were harvested, spun at 200 *g* for 3 min and resuspended in NBM containing 1% insulin/transferrin/selenium, 1% N_2 supplement, 1% retinol-free B27 supplement, 1% penicillin-streptomycin, 1% GlutaMAX, and supplemented with 10 ng mL^{-1} of BMP2 and 20 ng mL^{-1} of bFGF. Cells were then cultured in ultralow attachment plates at 37 °C and 5% of CO_2 in a humidified incubator for 4–6 days to promote neurosphere (NSP) formation. The following day, the cells were cultured in NBM complete medium with 10 ng mL^{-1} BDNF, 10 ng mL^{-1} NGF, and 10 ng mL^{-1} NT-3 supplements to support differentiation to sensory neurons. After two weeks, NSPs were mechanically dissociated and plated onto poly-D-lysine and laminin-coated dishes for an additional 7 days in NBM supplemented with 10 ng mL^{-1} BDNF, 10 ng mL^{-1} NGF, and 10 ng mL^{-1} NT-3 to support maturation of neurons, cultured as monolayer.

Incubation of particles with FRDA iPSC-derived sensory neurons

Confocal microscopy. AF_{488}-labeled particles were used to examine particles uptake in sensory neurons. For samples without plasmid DNA, 30 μL of particle suspension (2×10^6 particles) in 50 mM sodium acetate buffer was prepared immediately prior to addition to cells. For samples containing particles with bound DNA, a particle suspension (2×10^6) was prepared in sodium acetate buffer and incubated with 0.5 μg of pcDNA3-Luc for 20 min at room temperature, prior to addition to human sensory

neurons cells. Particles were added to 3 weeks differentiated FRDA iPSC-derived sensory neurons for an incubation period of 24 h, after which cells were washed with DPBS, fixed with 4% PFA, and incubated with Cell Mask Deep Red Plasma membrane at 5 μg mL^{-1} in DPBS for 10 min at room temperature. Nuclei were stained using DAPI (1 μg mL^{-1}). Images were acquired on a Nikon A1R confocal microscope equipped with a 60× oil objective.

Quantitative polymerase chain reaction (Q-PCR) analyses. Cells were incubated with particles or particles with bound frataxin (FXN)-GFP expression plasmid (8.9 kbp) for 24 h (particle preparation as described above), after which cells were washed extensively with supplemented NBM and cultured for another 24 h or 13 days before harvesting. Q-PCR data are reported as fold change, which is defined as fold change of *FXN* expression levels relative to expression levels of housekeeping genes in the treated sample/fold change of *FXN* expression levels relative to expression levels of housekeeping genes in control samples.

Gene expression analyses

For each sample, total RNA was extracted using PureLink RNA mini kit according to the manufacturer's instructions and then processed to generate cDNA. Q-PCR analysis was performed on cDNA samples and experiments were conducted to obtain relative levels of each transcript normalized to the endogenous controls E74-like ETS Transcription Factor 1 (ELF1), glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and hydroxymethylbilane synthetase (HMBS) for each sample. The specific probes (Life Technologies) that were used in the present study were FXN (Hs00175940_m1) and Caspase 3 (CASP3; Hs00234387_m1). Probes used as controls were ELF1 (Hs00152844_m1), GAPDH (Hs02758991_g1), and HMBS (Hs00609297_m1).

Immunostaining analyses of differentiated FRDA iPSC

Cells were fixed with 4% PFA on ice for 10 min and permeabilized for 15 min at room temperature using 0.2% triton-X100 solution. Incubation with primary and secondary antibodies was performed in 10% normal donkey serum/DPBS blocking solution overnight at 4 °C and for 1 h at room temperature, respectively. The following antibody dilutions were used: goat anti-human TRKA (1:200); mouse anti-human TRKB

(1:200); rabbit anti-human TRKC (1:250); sheep anti-human/mouse/rat PV (1:500); goat anti-human SPP1 (1:500); mouse anti-human PRPH (1:200); and chicken anti-human/mouse/rat TUBB3 (1:2000). All Alexa Fluor secondary antibodies were used at 1:1000 dilution except for donkey anti-chicken secondary antibody, which was used at 1:200. Nuclei were stained using DAPI (1 μg mL^{-1}). Three washes with DPBS solution were performed at each step, allowing 3 min between each wash. Samples were mounted onto slides (Super Frost Plus Slide, Thermo Fisher Scientific) using Dako fluorescent mounting medium, imaged immediately or stored at 4 °C. For long storage, samples were kept at −80 °C.

Statistical analyses

Statistical analyses were performed using GraphPad Prism 5 software. Q-PCR data are presented as the mean ± standard error of the mean, with $n = 3$ biological replicate experiments and $n > 3$ technical replicate samples for each replicate experiment.

References

1. G. Sauerbrey, *Z. Phys.*, 1959, **155**, 206.

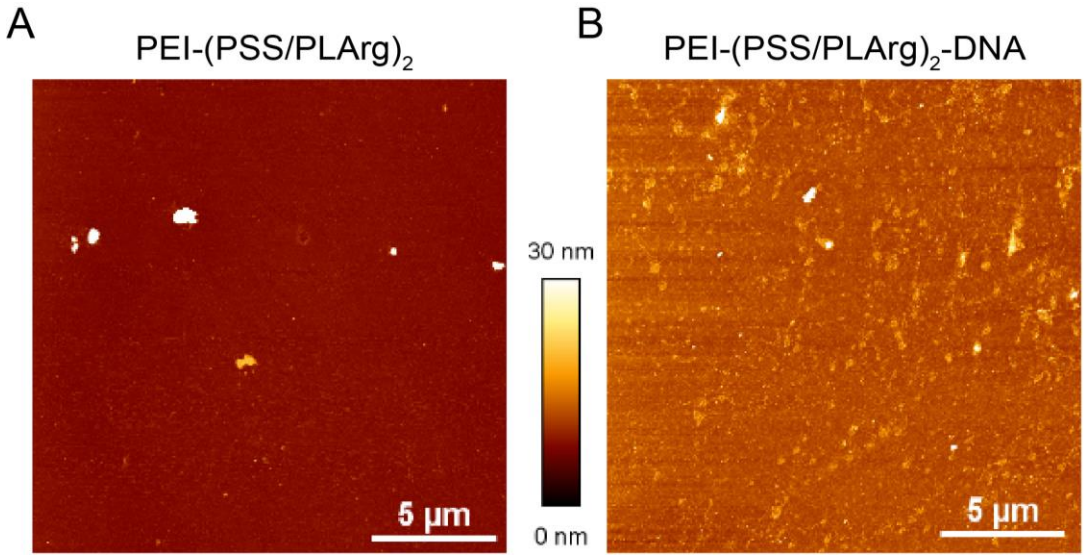

Figure S1. AFM analysis of plasmid DNA adsorption on PLArg-terminated LbL films. (A) PEI-(PSS/PLArg)$_2$ multilayer film; root mean square (RMS) = 0.6 nm. (B) PEI-(PSS/PLArg)$_2$-DNA multilayer film; RMS = 1.4 nm.

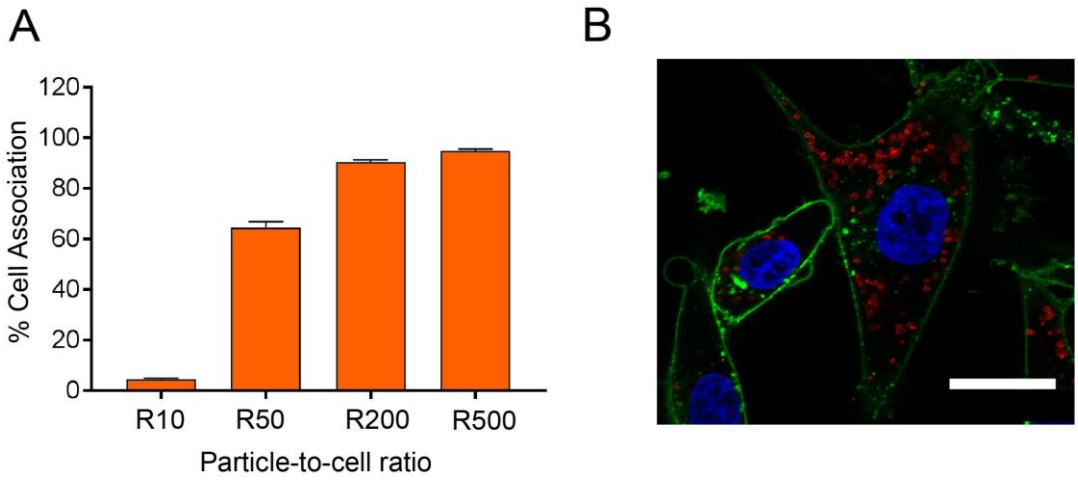

Figure S2. Characterization of DNA binding. (A) Association of particles with U87-MG cells after incubation for 24 h with AF$_{555}$-labeled core–shell particles (without DNA) at particle-to-cell ratios of 10:1 (R10), 50:1 (R50), 200:1 (R200), and 500:1 (R500). The *y*-axis corresponds to the percentage of AF$_{555}$-positive cells. (B) Confocal microscopy image showing internalization of AF$_{555}$-labeled PLArg-terminated LbL particles (red) in U87-MG cells. Cell membranes were stained with WGA-AF$_{488}$ (green). Nuclei were stained with Hoechst 33342 (blue). Scale bar 20 µm.

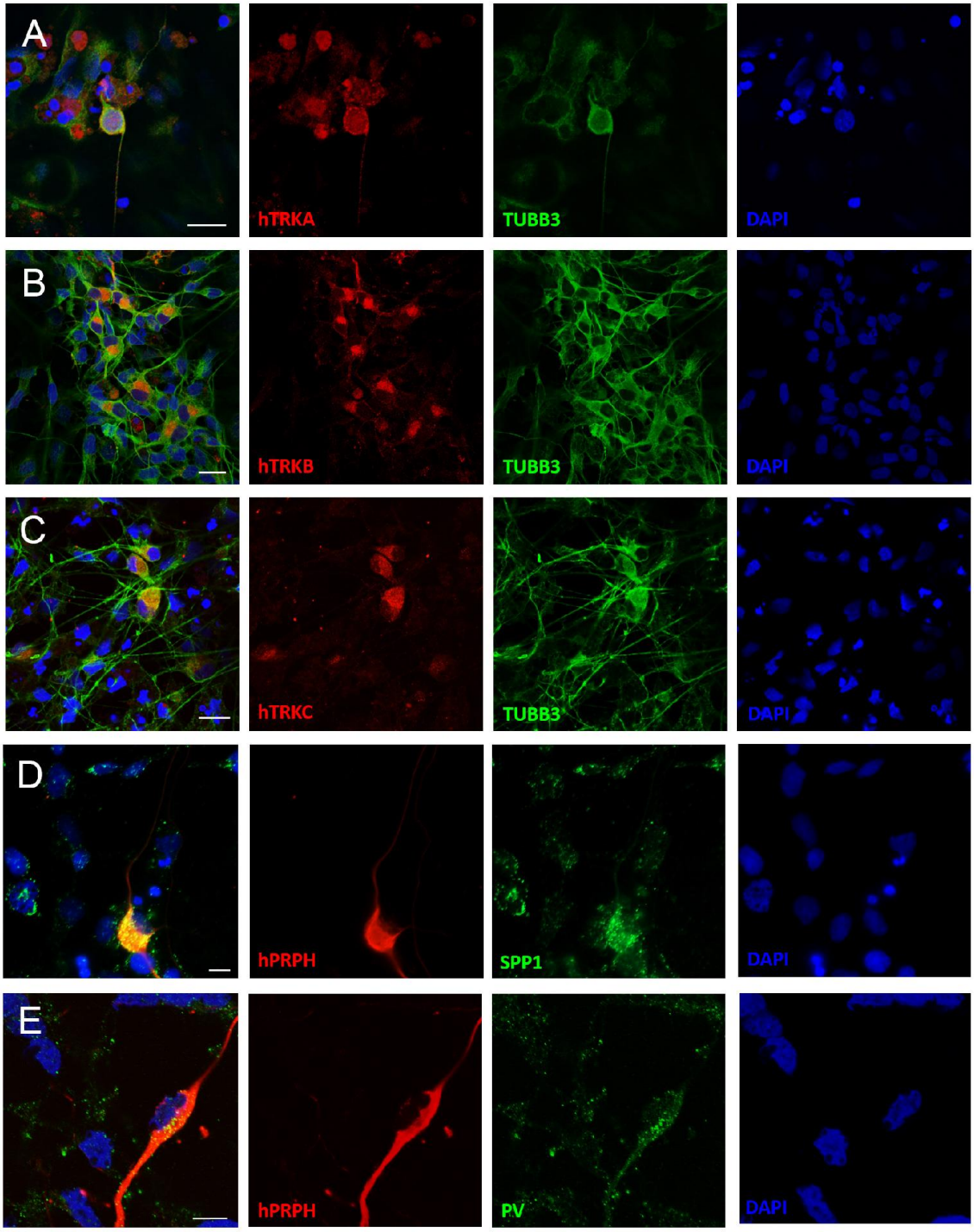

Figure S3. Characterization of FRDA-derived sensory neurons. Neurons show expression of sensory neuronal markers (A) TRKA (red) and β III tubulin (green), (B) TRKB (red) and β III tubulin (green), (C) TRKC (red) and β III tubulin (green), (D) peripherin (PRPH, red) and osteopontin (SSP1, green), and (E) peripherin (red) and parvalbumin (PV, green). DAPI-stained nuclei are shown in blue. Scale bar (A–D) 20 μm and (E) 10 μm.

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		x
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?	x	
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**?		N/A
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): N/A		

***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?	x	
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?		x
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?	x	
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).		N/A
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		N/A
Explanation for **No** (if needed): Flow cytometry data analysed as % Cell association based on a shift of single cell population, not MFI. Main experiments are performed on differentiated FRDA iPSC (not routinely tested for mycoplasma).		

*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**?		N/A
3.5 Is the methodology used to **equalise dosage** provided?		N/A
3.6 Is the **delivered dose** to tissues and/or organs (in vivo) reported, as % injected dose per gram of tissue (%ID g^{-1})?		N/A
3.7 Is **mass of each organ/tissue measured** and **mass of material** reported?		N/A
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?		N/A
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.