Media constituents:

- Fibroblast media: DMEM (Lonza) supplemented with 10% (v/v) FBS and 50 U/mL Penicillin/ Streptomycin.
- pre-iNPC media: DMEM/F-12 GlutaMAX™ (ThermoFisher) supplemented with 1% (v/v) B-27, 1% (v/v) N-2, 20ng/ml FGFb (PeproTech). 20ng/ml EGF (PeproTech) and 5ng/ml Heparin
- iNPC media: DMEM/F-12 GlutaMAX™ (ThermoFisher) supplemented with 1% (v/v) B-27, 1% (v/v) N-2, and 40ng/ml FGFb (PeproTech).
- iNeuron media: DMEM/F-12 GlutaMAX™ supplemented with 2% (v/v) B-27, 1% (v/v) N-2.

Fibroblast culture: culture fibroblasts in 10 mL fibroblast media in T75 culture flasks at 37°C, 5% CO_2. Change the culture media every 2 days and split when ~80% confluent. For splitting, remove media and wash cells with 1x sterile PBS. Add an appropriate volume of 1x trypsin to cover adherent cells and incubate for ~2mins at 37°C or until the cells dislodge. Centrifuge trypsinised cells at 550xg for 4 minutes at RT and re-suspend the cell pellet in fresh fibroblast media. Seed ~$1x10^6$ cells into a fresh T75 culture flask in fibroblast media and incubate at 37°C, 5% CO_2.

Transduction of fibroblasts: coat desired number of wells of a 6-well culture plate in fibronectin (Merck) (5ug/mL in PBS) for 5-30mins prior to seeding. Remove excess fibronectin and immediately seed ~200,000 cells per well. Incubate for 24 hours at 37°C, 5% CO_2. Transduce fibroblasts using retroviral vectors (MOI 5-10) expressing Kruppel-like factor 4 (*Klf4*), POU transcription factor *Oct-3/4 (Oct3/4)*, SRY-related HMG-Box Gene 2 (*Sox2*), and c-*Myc.* Change the culture media 16 hours post-transduction to fibroblast media. After 24 hours, change to pre-iNPC media containing epidermal growth factor (EGF, 20 ng/mL; PeproTech), heparin (5 ng/mL; PeproTech) and (FGF, 20 ng/mL; PeproTech). Perform media changes every 2 days; remove EGF and heparin 10 days post-transduction and increase concentration of FGF to 40ng/ml.

iNPC culture: coat 10cm petri dishes in fibronectin (5 µg/mL in PBS) for 5-30mins prior to seeding; remove excess fibronectin immediately before seeding. Culture iNPCs in 10mL iNPC media in 10 cm petri-dishes at 37 °C, 5% CO_2. Perform media changes every two days and split when ~90% confluent. For splitting, remove media and incubate cells in an appropriate volume of accutase at 37 °C, 5% CO_2 until they dislodge. Collect cells and centrifuge at 200xg for 4 mins at RT. Re-suspend the cells in fresh iNPC media and re-plate at a suitable density to encourage contact-mediated recovery.

Differentiation of iNPCs into iNeurons: coat desired number of wells of a 6-well culture plate in fibronectin (5 µg/mL in PBS) for 5-30mins prior to seeding. Remove excess fibronectin and immediately seed iNPCs into 6-well culture plates at a density of ~200,000 cells per well in 2 mL iNPC media. Change to iNeuron media 24 hours after seeding and maintain for 48 hours. At day

3, change the media to iNeuron media + γ-secretase inhibitor IX (DAPT, 2.5 μM; Tocris) and maintain for 48 hours. At day 5, change the media to iNeuron media + smoothened agonist (SAG, 0.5 μM) + all-trans retinoic acid (RA, 1 μM) + forskolin (2.5 μM) for 6 days with a media change every 2 days. At day 11, culture the cells in iNeuron media + SAG (0.5 μM; Millipore) + all-trans retinoic acid (1 μM; Sigma) + forskolin (2.5 μM; Sigma) + growth factors (all from PeproTech): brain-derived neurotrophic factor (BDNF, 20 ng/mL), ciliary neurotrophic factor (CNTF, 20 ng/mL) and glial cell-line derived neurotrophic factor (GDNF, 10 ng/mL) for 4 days. Collect fully differentiated iNeurons at day 16.