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## Supplementary information

# Phase I/II trial of iPS-cell-derived dopaminergic cells for Parkinson's disease

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**Phase I/II trial of iPSC-derived dopaminergic cells for Parkinson's disease**

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## Supplementary Methods

1. Cell transplantation

Dopamine (DA) progenitors were bilaterally transplanted stereotactically into the putamen (Extended Data Table 4). The targets for the stereotactic procedures were determined using the iPlan neurosurgical navigation system (Brainlab AG, Munich, Germany) based on three-dimensional volume data from preoperative T1-weighted, T2-weighted, contrast-enhanced T1-weighted images and $^{18}$F-DOPA positron-emission tomography (PET) images. The targets were positioned to pass at least 9 mm through the dorsal and caudal regions of the putamen, where $^{18}$F-DOPA uptake was decreased. The mean coordinates of rostral, middle, and caudal target relative to a mid-commissural point were as follows, respectively: 25.6 mm lateral (s.d.=2.37), 3.9 mm superior (s.d.=2.22), and 4.5 mm anterior (s.d.=4.46); 26.4 mm lateral (s.d.=1.85), 4.4 mm superior (s.d.=2.05), and 1.4 mm anterior (s.d.=4.02); 26.9 mm lateral (s.d.=1.65), 4.6 mm superior (s.d.=2.49), and 1.8 mm posterior (s.d.=4.03). Three trajectories per hemisphere were designed to avoid sulci and blood vessels by referencing T2-weighted and contrast-enhanced T1-weighted images.

Surgical procedures were performed using a Leksell G frame stereotactic system (Elekta AB, Stockholm, Sweden) and an injection needle specifically designed for this study (TOP Corporation, Tokyo, Japan). All injections were made through a single burr hole placed in the frontal area. The location of the injection cannula tip was confirmed intraoperatively using cone-beam computed tomography (CT) with the Artis Zeego angiography system (Siemens Healthineers AG, Erlangen, Germany). Four to eight injections per trajectory were made at 1–2 mm intervals to transplant 2.1–5.5×$10^6$ cells per putamen.

2. Clinical behavior measures

The severity of dyskinesia was assessed using the Unified Dyskinesia Rating Scale (UDysRS[1]). Parkinsonian motor symptoms were evaluated with the MDS-UPDRS[2] parts I to IV, which include subscales for non-motor and motor aspects of daily living and motor function, with higher scores indicating more severe disease. The Hoehn and Yahr stage was also evaluated in a drug-on state with optimal dopaminergic medication. Additionally, motor symptom severity was assessed using the MDS-UPDRS part III and

the Hoehn and Yahr stage in a drug-off state, defined by the withdrawal of all anti-Parkinsonian medications for at least 12 hours. The bradykinesia subscale was examined by extracting items 3.1, 3.2, 3.4, 3.5, 3.8, and 3.14 from the MDS-UPDRS part III during the drug-off state[3]. Participants maintained a motor diary to record their predominant status in half-hour intervals using categories such as asleep, on-time, off-time, troublesome dyskinesia, and non-troublesome dyskinesia. This diary allowed for independent calculations of daily on-time and off-time, with and without troublesome or non-troublesome dyskinesia. After detailed instructions, participants completed the diaries for seven consecutive days. Additional patient-reported outcomes included the PDQ-39[4], the EQ-5D-5L quality of life questionnaire[5], and the WPAI questionnaire[6]. The LEDD was calculated using an updated formula published after the trial's initiation (Table 2)[7]. For completeness, we have retained the data calculated using an older formula in Extended Data Fig. 5g, h[8].

3. Imaging data acquisition

MRI scans were conducted using the 3-T MAGNETOM Skyla system equipped with a 32-channel head coil (Siemens Healthineers AG, Erlangen, Germany). Whole brain T1-weighted anatomical images were obtained using a magnetization-prepared rapid gradient echo sequence (MPRAGE) with the following parameters: repetition time, 1900 ms; echo time, 2.58 ms; flip angle, 9°; field of view, 230 × 230 mm; slices, 192; and voxel size, 0.9 × 0.9 × 0.9 mm. Additionally, 3 dimensional T2-weighted, fluid-attenuated inversion recovery (FLAIR), and T2*-weighted images were acquired in the same resolution covering the whole brain. Gadolinium-enhanced T1-weighted images were also obtained at enrollment for planning neurosurgical operations.

PET and CT scans were conducted using the Discovery IQ system (GE HealthCare, Waukesha, WI). A total of 79 slice brain images were acquired in list mode (event-by-event) with post-acquisition frame re-binning. An elastic headband restraint was used to minimize head movement during the scan. Brain images were reconstructed using an ordered subset expectation maximization (OSEM) algorithm with 12 subsets and 10 iterations, incorporating CT-based attenuation correction.

$^{18}$F-FLT (3–4 MBq/kg) was injected 60 minutes prior to a 15-minute brain scan, followed by a whole-body scan from the skull to the proximal femur at 3 minutes per frame. $^{18}$F-GE180 (3–4 MBq/kg) was intravenously administered over 60 seconds at the

beginning of the 90-minute dynamic data acquisition. The $^{18}$F-DOPA scan was conducted in a practically defined drug-off state after withdrawing all anti-Parkinsonian medications for at least 15 hours. Additionally, 150 mg of carbidopa was administered 1 hour before the scan to block peripheral dopa-decarboxylase activity and thereby prolong the availability of $^{18}$F-DOPA. $^{18}$F-DOPA (3–4 MBq/kg) was injected over 60 seconds at the beginning of the 90-minute dynamic data acquisition. Involuntary head movement was corrected post-acquisition with frame-by-frame realignment.

## 4. Imaging data analysis

MRI images were processed using Functional Magnetic Resonance Imaging of the Brain (FMRIB) software[9] as described for non-human primates to measure graft size[10]. The MRI-based measurement was consistent with those obtained by immunohistochemistry. Structural MRIs were up-sampled from 0.9 mm isotropic to 0.3 mm isotropic to reduce round-off error, and the brains were extracted. The T2-weighted image was co-registered using rigid-body translation to the T1-weighted image acquired one day after transplantation, where spherical volumes of interest (VOIs) with a radius of 3 mm were placed at the transplanted sites. The co-registered T2-weighted image was segmented into gray matter, white matter, cerebrospinal fluid, and other regions, including hyperintense graft tissue. The volume of the graft segments within the spherical VOIs was calculated and assumed to represent the graft volume. The co-registration was visually inspected, and the Statistical Parametric Mapping 12 (SPM12) tool was applied when necessary.

The distribution of $^{18}$F-FLT throughout the entire body was evaluated to confirm the typical physiological distribution pattern. Subsequently, brain images were classified as positive or negative based on a consensus between two independent readers, according to visual read classification.

The PET images of $^{18}$F-GE180 were assessed visually. Then, if the abnormal accumulation was suspected, the binding was estimated using the distribution volume ratio (DVR), calculated with the Logan reference tissue model[11]. Frontal cortical masks from Hammers adult atlases in Montreal Neurological Institute (MNI) space (www.brain-development.org)[12] were extracted and used as the pseudo reference region (PRR). These masks were transformed into individual T1-weighted images using FMRIB software[9] and then into integrated PET space using rigid-body transformation. The PRR was defined as the transformed frontal cortical masks in individual PET space, excluding voxels

suspected of being affected by inflammation. DVR was determined from dynamic 20 to 90-minute data using the PMOD Kinetic Modelling tool implemented in PMOD version 3.905 (PMOD Technologies LLC., Zurich, Switzerland)[13,14]. The k2ref of the reference tissue was set to 0.0271/min, following a previous study[11].

The $^{18}$F-DOPA influx rate constants (Ki), which represent the rate of $^{18}$F-DOPA uptake and storage as $^{18}$F-dopamine, were calculated using the Patlak tissue model[15]. The reference region input function was obtained from circular regions with a 15 mm radius on each occipital cortex in four contiguous planes on the integrated PET images. Using individual T1-weighted images and the FMRIB software tool[16], the bilateral caudate, accumbens, and putamen were segmented. VOIs were created for the caudate nucleus by merging the caudate and accumbens structures and for the putamen, and these were rigid-body transformed to the $^{18}$F-DOPA images using T1-weighted images[17]. Ki was then calculated from the regression slope obtained from the time-activity curve of dynamic 30 to 90-minute data using the PMOD Kinetic Modelling tool implemented in PMOD version 3.905[15,18]. For display purposes, semiquantitative $^{18}$F-DOPA images were generated at 80–90 minutes post-injection by subtracting the occipital background signal and normalizing the result to the occipital activity.

## Supplementary Tables

Supplementary Table 1. Summary of quality control test of iPSC-derived DA progenitors

| **Patient** | | **PD01** | | **PD02** | **PD03** | **PD04** | **PD05** | **PD06** | **PD08** |
|---|---|---|---|---|---|---|---|---|---|
| **Test Items** | **Criteria** | **Results** | | | | | | | |
| Batch | | 180904 | 190507 | 190909 | 191002 | 20022 | 20048 | 21004 | 21047 |
| Morphology | Sphere shaped, no foreign body | Confirmed | Confirmed | Confirmed | Confirmed | Confirmed | Confirmed | Confirmed | Confirmed |
| Viability | 90% or more | 97.4% | 93.8% | 98.3% | 98.6% | 95.9% | 97.6% | 93.2% | 99.4% |
| Markers for DA progenitors | FOXA2 and TUJ1 co-positive cells (FCM): 80% or more | 90.7% | 97.7% | 90.9% | 98.5% | 98.70% | 99.20% | 96.60% | 98.50% |
| Markers for undifferentiated cells | OCT3/4 and TRA-2-49/6E co-positive cells (FCM): less than 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| Markers for early neurons | SOX1 and PAX6 co-positive cells (FCM): less than 0.1% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% | 0.0% |
| | POU5F1 and LIN28 (qPCR): less than 1% compared to undifferentiated cells | 0.36% and 0.40% | 0.00% and 0.10% | 0.09% and 0.06% | 0.10% and 0.00% | 0.02% and 0.13% | 0.10%and 0.21% | 0.52% and 0.64% | 0.05% and 0.08% |
| Sterility | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative |
| Mycoplasma | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative | Negative |
| Endotoxin | 0.5 EU/mL or less | < 0.1 | < 0.1 | < 0.1 | < 0.1 | < 0.2 | < 0.2 | < 0.2 | < 0.2 |
| **Reference test** | | | | | | | | | |
| Viable cells per sphere | | 6450 | 6160 | 11296 | 9895 | 21968 | 18118 | 10563 | 13415 |
| Neurite extension | | Positive | NA | NA | NA | Positive | Positive | Positive | Positive |
| DA release (pmol / DNA μg) (High potassium stimulation) | | NA | NA | NA | NA | 0.7 | 1.1 | 2.0 | 0.8 |

Supplementary Table 2. Adverse events during the trial

| MedDRA system organ class and preferred term | Low- dose subgroup (N = 3) | High- dose subgroup (N = 4) | Related to cell transplantation | Related to tacrolimus |
|---|---|---|---|---|
| Blood and lymphatic system disorders | | | | |
| Anemia | | 1 | Unlikely | Unlikely |
| Ear and labyrinth disorders | | | | |
| Tinnitus | 1 | | Unlikely | Unlikely |
| Vertigo | | 1 | Unlikely | Unlikely |
| Eye disorders | | | | |
| Vitreous floaters | | 1 | Unlikely | Unlikely |
| Gastrointestinal disorders | | | | |
| Abdominal discomfort | 1 | | Unlikely | Unlikely |
| Abdominal pain upper | 1 | | Unlikely | Unlikely |
| Dental caries | 1 | 1 | Unlikely | Unlikely |
| Diarrhea | | 1 | Unlikely | Unlikely |
| Nausea | 1 | 1 | Unlikely | Unlikely |
| Vomiting | 1 | | Unlikely | Unlikely |
| General disorders and administration site conditions | | | | |
| Application site pruritus | 2 | 2 | Unlikely | Unlikely |
| Chills | | 1 | Unlikely | Unlikely |
| Discomfort | | 1 | Unlikely | Unlikely |
| Pyrexia | 1 | | Unlikely | Unlikely |
| Therapeutic product effect decreased* | 1 | | Unlikely | Unlikely |
| Hepatobiliary disorders | | | | |
| Hepatic function abnormal | 1 | | Unlikely | Possible |
| Hyperbilirubinemia | | 1 | Unlikely | Unlikely |
| Infections and infestations | | | | |
| Conjunctivitis | | 1 | Unlikely | Unlikely |
| Cystitis | 1 | | Unlikely | Possible |
| Dermatophytosis of nail | 1 | | Unlikely | Possible |
| Nasopharyngitis | 1 | | Unlikely | Unlikely |
| Sinusitis | | 1 | Unlikely | Unlikely |
| Conjunctivitis bacterial | | 1 | Unlikely | Unlikely |
| Injury, poisoning, and procedural complications | | | | |
| Fall | | 2 | Unlikely | Unlikely |

| | | | | |
|---|---|---|---|---|
| Rib fracture | | 1 | Unlikely | Unlikely |
| Pneumocephalus | | 1 | Unlikely | Unlikely |
| Postoperative hypertension | | 1 | Unlikely | Unlikely |
| Wound | | 1 | Unlikely | Unlikely |
| Wound complication | 1 | 1 | Unlikely | Unlikely |
| Investigations | | | | |
| C-reactive protein (CRP) increased | 1 | | Unlikely | Unlikely |
| Gamma-glutamyltransferase (GGT) increased | 1 | | Unlikely | Possible |
| Weight decreased | | 1 | Unlikely | Unlikely |
| Urinary occult blood positive | | 2 | Unlikely | Unlikely |
| Magnetic resonance imaging head† | | 1 | Unlikely | Unlikely |
| Musculoskeletal and connective tissue disorders | | | | |
| Arthralgia | 1 | | Unlikely | Unlikely |
| Back pain | 1 | | Unlikely | Unlikely |
| Lumbar spinal stenosis | | 1 | Unlikely | Unlikely |
| Muscle tightness | 1 | | Possible | Unlikely |
| Nervous system disorders | | | | |
| Dyskinesia (moderate) | | 1 | Unlikely | Unlikely |
| Dystonia | | 1 | Unlikely | Unlikely |
| Dystonia | 1 | | Possible | Unlikely |
| Headache | 1 | | Unlikely | Unlikely |
| Hypoaesthesia | | 1 | Unlikely | Unlikely |
| Psychiatric disorders | | | | |
| Anxiety | | 1 | Unlikely | Unlikely |
| Claustrophobia | 2 | | Unlikely | Unlikely |
| Hallucination, olfactory | | 1 | Unlikely | Unlikely |
| Renal and urinary disorders | | | | |
| Hypertonic bladder | | 1 | Unlikely | Unlikely |
| Pollakiuria | | 1 | Unlikely | Unlikely |
| Renal impairment | 1 | | Unlikely | Unlikely |
| Renal impairment | 1 | 1 | Unlikely | Possible |
| Respiratory, thoracic, and mediastinal disorders | | | | |
| Rhinorrhea | 1 | | Unlikely | Unlikely |
| Skin and subcutaneous-tissue disorders | | | | |
| Alopecia | | 1 | Unlikely | Unlikely |

| | | | | |
|---|---|---|---|---|
| Eczema | 1 | | Unlikely | Unlikely |
| Vascular disorders | | | | |
| Orthostatic hypotension | | 1 | Unlikely | Unlikely |
| Peripheral coldness | | 1 | Unlikely | Unlikely |
| Product issues | | | | |
| Device breakage# | 1 | | Unlikely | Unlikely |

* A decline in the perceived efficacy of antiparkinsonian medication was documented.

† Hyperintense regions on brain T2-weighted and fluid-attenuated inversion recovery (FLAIR) images were reported.

# Dislodgement of dental restoration was reported.

Supplementary Table 3. Inclusion criteria

1) Parkinson's disease (PD) in accordance with the Movement Disorder Society (MDS) Clinical Diagnostic Criteria (2015)
2) Poor response to existing drug treatments
3) ≥ 50 years and < 70 years of age at the time of informed consent
4) PD duration for at least 5 years
5) Suffering from ON and OFF
6) Hoehn and Yahr stage 3 or worse at OFF time
7) Hoehn and Yahr stage 3 or better at ON time
8) 30% or more levodopa response from anti-Parkinsonian medication OFF state
9) Decrease pattern characteristic to PD in the basal ganglia region on $^{123}$I-ioflupane scan
10) Organ functions as follows
   i) Neutrophil count ≥ 2,000/μL
   ii) Platelet count ≥ $5.0 \times 10^4$/μL
   iii) Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) ≤ 3.0 × upper limit of normal at the study site
   iv) Total bilirubin ≤ 1.5 × upper limit of normal at the study site
   v) Estimated Glomerular Filtration Rate (eGFR) ≥ 60 mL/min/1.73 $m^2$
   vi) eGFR (mL/min/1.73 $m^2$) = $194 \times Cr^{-1.094} \times age^{-0.287}$ (× 0.739 for females)

Supplementary Table 4. Exclusion criteria

| |
|---|
| 1) Symptomatic organic lesions as detected by head magnetic resonance imaging (MRI) |
| 2) Abnormal immune function |
| 3) Demented or deemed at high risk of dementia |
| 4) Bleeding tendency or abnormal coagulation function |
| 5) Infection or history of infection with hepatitis B virus, Human Immunodeficiency Virus (HIV), Human T-cell Leukemia Virus type 1 (HTLV-1) |
| 6) Active infection with hepatitis C or syphilis |
| 7) Contraindication to immunosuppressive drug (tacrolimus) and concomitant drugs (e.g., levodopa, carbidopa, MRI contrast) |
| 8) Concurrently treated for malignant neoplasm, epilepsy, mental diseases, and other serious diseases (e.g., cerebrovascular disorder, heart disease, chronic respiratory disease, inadequately controlled hypertension, diabetes mellitus) |
| 9) History of malignant neoplasm, epilepsy, cerebral hemorrhage, mental diseases, and neurosurgery |
| 10) Pregnant or lactating |
| 11) Judged as inappropriate by the investigators |

Supplementary Table 5. Endpoints

1) Primary Endpoints
  1-1) Incidence and severity of adverse events
  1-2) Graft overgrowth at 24 months (> 3 $cm^3$ by MRI)

2) Secondary Safety Endpoints
  2-1) Uptake of fluorine-18-fluorothymidine ($^{18}$F-FLT) indicating cell proliferation
  2-2) Uptake of fluorine-18-flutriciclamide ($^{18}$F-GE180) indicating inflammation or immune response
  2-3) Change in Dyskinesia score
  2-4) Change in graft sizes

3) Secondary Efficacy Endpoints
  3-1) MDS Unified Parkinson's Disease Rating Scale (MDS-UPDRS) part III at OFF time (no anti-Parkinsonian medication more than 12 hours)
  3-2) MDS-UPDRS part III at ON time
  3-3) Daily reported ON and OFF duration
  3-4) Levodopa equivalent daily dose (LEDD)
  3-5) MDS-UPDRS part II
  3-6) MDS-UPDRS part I
  3-7) MDS-UPDRS part I+ II+ III (OFF)
  3-8) MDS-UPDRS part I+ II+ III (ON)
  3-9) Hoehn and Yahr severity
  3-10) Bradykinesia subscale
  3-11) Uptake of fluorine-18-L-dihydroxyphenylalanine ($^{18}$F-FDOPA) evaluating dopaminergic neuronal function
  3-12) Uptake of $^{123}$I-ioflupane assessing the concentration of dopamine transporters
  3-13) 39-item Parkinson's Disease Questionnaire (PDQ-39)
  3-14) EuroQol 5-dimension 5-level (EQ-5D-5L)
  3-15) Work Productivity and Activity Impairment (WPAI) questionnaire: General Health V2.2
  3-16) Nursing care category

Supplementary Table 6. Primer sets for single-cell RT-qPCR

| Gene | Forward primer | Reverse primer |
|---|---|---|
| AADC | AGCCCCTACTTCTTCGCCTA | GAGCCAGTCCATCATCACAG |
| ACTB | CATGTACGTTGCTATCCAGGC | CTCCTTAATGTCACGCACGAT |
| ALCAM | CCGTGTCATGCACAATATCTGC | CTTTAGTCCTTCAACCTCCTGC |
| ALDH1A1 | ATGCTTCCGAGAGGGGGCGA | CCCAACCTGCACAGTAGCGCA |
| AQP1 | CTGGGCATCGAGATCATCGG | ATCCCACAGCCAGTGTAGTCA |
| BARHL1 | GACTAAATGGAAGCGACAGACG | TGCGGGTAGAAATAAGGCGAC |
| BLBP | GTCTGTTGTTAGCCTGGATGGAGAC | AGTGGCGAACAGCAACCACATCA |
| BMP4 | ATGATTCCTGGTAACCGAATGC | CCCCGTCTCAGGTATCAAACT |
| BMPR1 | TGAAATCAGACTCCGACCAGA | TGGCAAAGCAATGTCCATTAGTT |
| BMPR2 | GACAGGAGACCGTAAACAAGG | CCATATCGACCTCGGCCAATC |
| BRN3a | AGCAAGCAGCCTCACTTTGC | CTTGAAAGGATGGCTCTTGC |
| CALB1 | GGCTCACGTATTACCCACAGA | GAAGCCACTGTGGTCAGTATCA |
| CD29 | CCTACTTCTGCACGATGTGATG | CCTTTGCTACGGTTGGTTACATT |
| CORIN | CACAGCCAGGGTCTGGTGGAATGCAG | GAGAGCTACCACCACATGAATCAAGG |
| DAT | TCATCGCCACATCCTCCA | ACCAGCTCACGGTCCTTCTC |
| DLK1 | CTTTCGGCCACAGCACCTAT | TGTCATCCTCGCAGAATCCAT |
| EMX1 | ACCGGGACCCTCTCCATTT | GCTTCTGCCGTTTGTACTTTGT |
| EMX2 | CGGCACTCAGCTACGCTAAC | CAAGTCCGGGTTGGAGTAGAC |
| EN1 | AGCGCAGGGCACCAAATAC | GGACGATCCGAATAACGTGTG |
| EN2 | GGTCTACTGTACGCGCTACTCG | CTTTGTTCGGGTTCTTCTTCTTTG |
| FN1 | AAAAAGACAGACGAGCTTCCCCAACT | GGGTGACGAAAGGGGTCTTTTGA |
| FOXA2 | TTCAGGCCCGGCTAACTCT | AGTCTCGACCCCCACTTGCT |
| FOXG1 | CCGCACCCGTCAATGACTT | CCGTCGTAAAACTTGGCAAAG |
| GAPDH | GGTCGGAGTCAACGGATTTG | TCAGCCTTGACGGTGCCATG |
| GATA6 | CTCAGTTCCTACGCTTCGCAT | GTCGAGGTCAGTGAACAGCA |
| GBX2 | GGTAACTTCGACAAGGCGGAGG | GGTCGTCTTCCACCTTTGACTCG |
| GDF7 | TGATGTCGCTTTACCGGAGC | CTGCCGATTCGTCTTGGGT |
| GFAP | GTTCTCTCGGAGTATCTGG | GATATCCCACCTCATAAAAACC |
| GLI1 | GGTCCCATCAGGGAGGAAAG | TCGTCCAAGCTGGAGAGGTC |
| GSC | GAGGAGAAAGTGGAGGTCTGGTT | CTCTGATGAGGACCGCTTCTG |
| HES1 | TCAACACGACACCGGATAAAC | GCCGCGAGCTATCTTTCTTCA |
| HES5 | GCACATTTGCCTTTTGTGAA | CACACTCAGGAGCCTTTTGG |

| | | |
|---|---|---|
| HOXA2 | GGATGAAGGAGAAGAAGGCGG | CTGCCATCGGCGATTTCCAGG |
| HOXB4 | GCGCAAAGTTCACGTGAGCAC | GGAACCAGATCTTGATCTGGCG |
| KCNJ6 | GCTCGAAGCTCCTACATCACC | CTCTTTGGCACTAAGGGATGG |
| KLF4 | CAGCTTCACCTATCCGATCCG | CTCCCTGCCATAGAGGAGG |
| LMX1A | GATCCCTTCCGACAGGGTCTC | GGTTTCCCACTCTGGACTGC |
| LMX1B | TGTGCAAGGGTGACTACGAGAA | TTCATGTCCCCATCTTCATCCT |
| LRP4 | CAGGAGGTGGTAGTGGATAC | GACGATCAAGGTTCTCCCAG |
| LRTM1 | ATTGCCACTTGCTCGGTCTT | TCCTTTCCCTTCCAGGTGTCT |
| MAP2 | GGATCAACGGAGAGCTGAC | TCAGGACTGCTACAGCCTCA |
| MEIS1 | GGGCATGGATGGAGTAGGC | GGGTACTGATGCGAGTGCAG |
| MKI67 | AAGCCCTCCAGCTCCTAGTC | GCAGGTTGCCACTCTTTCTC |
| MSX1 | AGTTCTCCAGCTCGCTCAGC | GGAACCATATCTTCACCTGCGT |
| NANOG | GGCTCTGTTTTGCTATATCCCCTAA | CATTACGATGCAGCAAATACGAGA |
| NCAM | GGCATTTACAAGTGTGTGGTTAC | TTGGCGCATTCTTGAACATGA |
| NESTIN | AACTCCCGGCTGCAAACAC | GGACTGGGAGCAAAGATCCA |
| NKX2.1 | AACCAAGCGCATCCAATCTCAAGG | TGTGCCCAGAGTGAAGTTTGGTCT |
| NKX6.1 | ATCTTCTGGCCCGGAGTG | TCTTCCCGTCTTTGTCCAAC |
| NR4A2 | CGAAACCGAAGAGCCCACAGGA | GGTCATAGCCGGGTTGGAGTCG |
| OLIG3 | AGCCGTCTCAACTCGGTCT | CATGGCTAGGTTCAGGTCGTG |
| OTX1 | GCGTCGTCGCTGAGTACAC | ACATGGGATAAGAGGCTGCTG |
| OTX2(primer2) | CAAAGTGAGACCTGCCAAAAAGA | TGGACAAGGGATCTGACAGTG |
| PAX2 | GACCAAAGTTCAGCAGCCTTTC | CAGGATCCCATTGATGGAGTAG |
| PAX6_Ogura | CTGGCTAGCGAAAAGCAACAG | CCCGTTCAACATCCTTAGTTTATCA |
| PAX7 | ACCCCTGCCTAACCACATC | GCGGCAAAGAATCTTGGAGAC |
| PAX8 | ATCCGGCCTGGAGTGATAGG | TGGCGTTTGTAGTCCCCAATC |
| PITX3 | GGGCCAGGAGCACAGCGACTCA | GCTGCCGCCGCTGCTTCTTTTT |
| POU5F1 | AGACCATCTGCCGCTTTGAG | GCAAGGGCCGCAGCTT |
| PTCH1 | ACTTCAAGGGGTACGAGTATGT | TGCGACACTCTGATGAACCAC |
| SHH | GATGTCTGCTGCTAGTCCTCGTC | TTTGGGGTGCCTCCTCTTC |
| SIX6 | ACCTGTGGACAAGTACCGAGT | TTGCTGGGGTTAGGGTATGGA |
| SMO | TCGAATCGCTACCCTGCTG | CAAGCCTCATGGTGCCATCT |
| SNCA | GTGTGACAGCAGTAGCCCAGAAG CAGTG | CCTTCTTCATTCTTGCCCAACTGGTCC |
| SOX1 | GCGGAGCTCGTCGCATT | GCGGTAACAACTACAAAAAACTTGTAA |
| SOX17 | CGCTTTCATGGTGTGGGCTAAGGACG | TAGTTGGGGTGGTCCTGCATGTGCTG |
| SOX2 | GCCGAGTGGAAACTTTTGTCG | GGCAGCGTGTACTTATCCTTCT |

| | | |
|---|---|---|
| SPRY1 | GCAGTGGCAGTTCGTTAGTTG | CAGTAGGCTGAATCTCTCTCTCA |
| SST | ACCCAACCAGACGGAGAATGA | GCCGGGTTTGAGTTAGCAGA |
| T | ATGGAGGAACCCGGAGACA | TGAGGATTTGCAGGTGGACA |
| TBR1 | GCCTTTCTCCTTCTATCATGCTC | GTCAGTGGTCGAGATAATGGGA |
| TH | GCAGTTCTCGCAGGACATTG | CGGCACCATAGGCCTTCA |
| TPH2 | TCAGCTACTTGGCAGCTCAAC | CTTGCCACTTTCGGTAGCAG |
| TTR | TGGGAGCCATTTGCCTCTG | AGCCGTGGTGGAATAGGAGTA |
| TUBB3 | TGATGAGCATGGCATCGAC | GGCCTGAAGAGATGTCCAAA |
| VIM | AGTCCACTGAGTACCGGAGAC | CATTTCACGCATCTGGCGTTC |
| WNT1 | GAACTGTCCCACTGCTCCAG | GCGGAGGTGATAGCGAAGA |
| WNT5A | TTCGCCCAGGTTGTAATTGA | GCAGAGAGGCTGTGCTCCTA |
| ZBTB16 | CCACCCCTACGAGTGTGAGT | CTCAAAGGGCTTCTCACCTG |

Supplementary Table 7. Summary of surgical procedure

| Pt | Hemi-sphere | Cells /sphere | Cell # ($x10^4$) | Vol (μL) | Deposits (Cau-Mid-Ros) | Interval (mm) | Trajectory length in the putamen caudal | middle | rostral |
|---|---|---|---|---|---|---|---|---|---|
| PD01 | Lt | 6,450 | 210.3 | 15.0 | 12 (4-4-4) | 2 | | | |
| | Rt | 6,160 | 246.4 | 18.0 | 12 (4-4-4) | 2 | | | |
| PD02 | Lt | 11,296 | 257.5 | 15.9 | 12 (4-4-4) | 2 | 9.19 | 11.7 | 10.98 |
| | Rt | | 246.3 | 15.4 | 12 (4-4-4) | 2 | 8.95 | 11.46 | 10.98 |
| PD03 | Lt | 9,895 | 239.5 | 15.4 | 12 (4-4-4) | 2 | 9.08 | 10.13 | 10.01 |
| | Rt | | 246.4 | 17.1 | 12 (4-4-4) | 2 | 9.78 | 10.13 | 8.73 |
| PD04 | Lt | 21,968 | 527.2 | 21.2 | 22 (7-8-7) | 1 | 11.73 | 11.51 | 11.36 |
| | Rt | | 536.0 | 19.0 | 22 (7-8-7) | 1 | 13 | 12.55 | 11.66 |
| PD05 | Lt | 18,118 | 541.7 | 21.0 | 22 (8-6-8) | 1 or 2 | 14.97 | 15.92 | 14.15 |
| | Rt | | 539.9 | 20.8 | 20 (7-6-7) | 1 or 2 | 16.74 | 16.27 | 15.68 |
| PD06 | Lt | 10,563 | 540.8 | 23.8 | 22 (8-8-6) | 1 | 14.14 | 13.47 | 14.06 |
| | Rt | | 533.4 | 20.9 | 21 (7-7-7) | 1 | 13.47 | 16.4 | 16.74 |
| PD08 | Lt | 13,415 | 554.0 | 21.1 | 20 (7-7-6) | 1 or 2 | 15.77 | 15.48 | 14.59 |
| | Rt | | 543.3 | 21.3 | 21 (7-7-7) | 1 or 2 | 16.4 | 16.95 | 15.84 |

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