**MATERIALS AND METHODS**

**hADSCs isolation**

5ml of sterile adipose tissue was provided by the Department of Plastic Surgery, Zhujiang Hospital, Southern Medical University, and the site was the inner left thigh fat. Type I collagenase digestion method was used to extract hADSCs, and the cell layer was resuspended in DMEM/F12 cell culture medium containing 10% FBS; inoculated into a 6cm culture dish and placed in an incubator for culture. 48 hours later, adipose stem cells(P2, P4, P8) were observed under the microscope(DMC4500, Leica). Adipogenic differentiation, osteogenic differentiation induced differentiation to identify the differentiation ability of hADSCs, These experiments were performed as described1.

**Neural induced differentiation**

We used this cell culture system (DMEM/F12+2% B27+20ng/ml EGF+20ng/ml bFGF+100u/ml P/S+2.5mmol/L NaHCO3) to induce neural differentiation of hADSCs with a pH of 6.5 to 7.0.

Flatten hADSCs on the coverslip for 24h, we washed with PBS twice, and fixed with 4% paraformaldehyde (about 2ml/well) for 30min, washing 3 times with PBS.We ruptured membrane with 0.25% Triton-X-100 (about 2ml/well) for 20min, washing 3 times with PBS.We blocked with 5% BSA for 30 min, and 150ul/well of primary antibody diluted in PBS containing 1% BSA (1:100 nestin, SOX9, abcam), incubating for 2 hour at room temperature, washing 3 times with PBS.We diluted with PBS containing 1% BSA Anti-fluorescence secondary antibody, 150ul/well, room temperature 2h, washing with 3 times. DAPI (1:2000, final concentration 1ug/ml), 37。C, 3-4min, washing twice with PBS. Neurosphere observed under fluorescence microscope or confocal microscope.

**Flow cytometry**

After the cells reaching 80-90%, we rinsed with PBS once, cell digestion, made cell suspension, centrifuge at 800 rpm for 5 min, discarded the supernatant. We washed with PBS twice, added 20ul primary antibody CD29-APC(BD), CD44-PE(BD), CD45- FITC(BD), and incubated on ice for 20 minutes in the dark. Finally we washed with an appropriate amount of PBS (200ul microplate, 1ml test tube), centrifuge (1000 rpm, 5 minutes, 4 degrees), aspirating the supernatant.We added an appropriate amount of PBS to suspend (500ul/ Tube) on the flow cytometer and analyzed the data.

**Open field test**

Open field activity test was conducted to assay spontaneous locomoter activity of mice with superMaze equipment(XmazeTM, XR-Xmaze). After all rats accustomed to test chamber(100cm×100cm×40 cm) in an hour before test, mice were placed on the center of the chamber followed allowance to freely explore the area for 10 minutes. After each animal was exposed to the box, the apparatus was cleaned with ethanol. Total running distance, average speed and resting time of the mice were recorded.

**Rotation behavior analysis**

Apomorphine hydrochloride(APO, Sigma-Aldrich, Taufkirchen, Germany) was dissolved in sterilized saline containing 0.02% ascorbic acid and was subcutaneously administered at 0.5 mg/kg body weight. Mice were injection subcutaneously with APO(0.5mg/kg), placed in square chamber (40cm square), and the number of contralateral turns in a 30-min period was recorded. The mice with more than 7 contralateral turns per minute were used as valid PD pathology animal models.

**Real-time PCR analysis**

Real-time PCR was carried out on the RocheLightCycler480 using SYBR green PCR master mix (both from Takara). GAPDH was used as an internal control. PCR analyses were conducted in triplicate for each sample.The reaction mix consisted of 6.35 ng cDNA, 0.5 μM forward and reverse primer mix,1X SYBR green PCR master mix.Reactions were run according to manufacturer protocols for at least 40 cycles.

**Western blot analysis**

The equivalent of 40 μg of protein was boiled for 10 min and resolved by SDS-PAGE(KeyGEN Bio TECH, China) using 12% Tris/Tricine gel (KeyGEN Bio TECH, China) and then transferred onto a PVDF membrane(Millipore Corporation, USA) and processed for immunolabeling the proteins of interest. The blots were blocked for 2h at RT with 5% nonfat milk (Bio-Red, USA) in Tris-buffered saline containing 0.1% Tween-20 (TBST). Membranes were incubated with the following antibodies: TH (1:1000, abcam-EP1532Y), TRADD(1:1000, Cell Signaling Techology-#3694), Cleaved-caspase-3(1:1000, Cell Signaling Techology- Asp175), Cleaved-caspase-8(1:1000, Cell Signaling Techology- Asp387) and FADD(1:1000, abcam-EPR4415) overnight at 4˚C. Then, the membranes were washed three times with TBST, incubated with secondary IgG-HRP antibodies(1:20000, Cell Signaling Techology#7074) for 2 h, and then washed again with TBST.β-actin antibody(1:4000, abcam-ab8227) was used as a loading control. Lastly, immunoreactive protein bands were visualized with Automatic chemiluminescence imaging analysis system(Tanon-5200, China), and band densities were quantified using imageJ software(National Institutes of Health).

**siRNA Transfection**

hADSCs (1.5×104cells) were plated and incubated in a 75-cm2 culture flask for 18–24 hours in order to reach 60%–70% confluence. Subsequently, the cells were washed and transfected with 4.8 μg of PTX3-specific or nonspecific control siRNA using transfection reagent in siRNA transfection medium as per the manufacturer’s protocol (RIBOBIO,Guangzhou). Following overnight incubation, the transfection medium was replaced with standard hADSCs growth culture medium, and the cells were cultured for an additional 48 hours. Real-time polymerase chain reaction (PCR) using PTX3 specific primers was used to validate the knockdown efficiency of siRNA after 3 days of transfection.

**Transplantation**

RhPTX3(R&D, USA), hADSCs in suspension (phosphate-buffered saline,PBS) were implanted at the striatumstereotaxic coordinates(AP, +0.9mm, ML, +2.2mm, DV, -2.8mm). A total of 1 X 105 cells were injected per point in a volume of 5uL with a 5uL Hamilton syringe (1.5 ul/min). RhPTX3( 4ul, 0.50 mg/ml) were injected.

**Immunohistochemical staining**

The animals were killed with Tribromoethanol (Sigma) and transcardially perfused with 4% paraformaldehyde (YONG JIN BIOTECH) in 0.1M PBS. The animals’ brains were extracted, post-fixed, paraffinized, cut at a thickness of 4µm( VTA+SNc and STR regions). A total number of six coronal sections per mouse were obtained. Sections were treated with SP kit ( ZSGB-BIO, SP-9001). The detailed procedure was carried out in accordance with the SP kit instructions. The sections were then incubated with the primary antibody anti-Tyrosine hydroxylase(TH, 1:500, abcam-EP1532Y). Using a bright-field microscope (model no. DM2500; Leica) and imageJ software(National Institutes of Health) measured the density of TH+ neurons of VTA+SNc and STR.

**Immunofluorescence**

The brains were removed, postfixed in 4% paraformaldehyde for 24 h at 4。C and then cryoprotected in 30% sucrose solution. Brain sections (6um &100um thickness) were prepared, washed twice in PBS, and incubated in 0.2% TritonX-100 for 60 min at RT. Samples were blocked with 0.5% bovine serum albumin (Sigma-Aldrich, A7906) for 60 min. After blocking, the sections were rinsed twice with modified PBS and incubated overnight at 4。C with primary antibody: Rabbit anti-TH (1:200, Millipore-AB152, USA). The sections were then rinsed with PBS and treated with a secondary antibody conjugated to a fluorescent dye. Secondary antibodies included Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488(Thermo Fisher Scientific, catalog # A-21206, RRID AB\_2535792) and then imaged by fluorescence microscope(Nikon,Ti2-E).

**Tunel assay**

DNA fragmentation in cells undergoing apoptosis was detected in the cryosections of brain tissue(6μm)(same as immunohistochemical staining for TH), using terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling(TUNEL), according to the manufacturer’s recommendations(Roche In Situ Cell Death Detection Kit,TMR). TUNEL-positive cells with apoptotic nuclear features were counted manually in five random microscopic fields in the perilesional areas, which were identified by TH expression, under 400X magnification by a blinded researcher.

**Fluoro-Jade C**

Fluoro-Jade staining was used to assess neuronal degenera-tion. Sections were rinsed in basic alcohol for 5 min followed by a 2 min rinse in 70% alcohol.The sections were then briefly rinsed in distilled water and incubated in 0.06% KMnO4 for 10 min, they were then briefly rinsed in distilled water to remove excess KMnO4 and incubated in 0.0001% Fluoro-Jade C( Biosensis, TR-100-FJT) stain in 0.1% acetic acid for 10 min. Following Fluoro-Jade C labeling, the sections were rinsed three times in distilled water, air dried for 10 min, cleared in xylene and covered with ProLong™ Gold Antifade Mountant with DAPI.

**Lactate dehydrogenase(LDH) assay**

LDH activity in cell culture medium was determined by a commercial kit according to the Nanjing Jiancheng Bioengineering Institute’s instructions. Briefly, the cell culture medium was collected and treated with LDH-assay. The absorbance was measured with a microplate reader(BioTek, ELX808, USA) at 450 nm.The cell death ratio was calculated by the following formula according to the manufacturer’s instructions.

**Figure S1.Characterization of Human adipose-derived mesenchymal stem cells (hADSCs).** A.P2, P4, and P8 were cytoplasmic and highly refractive. No significant changes in cell morphology, still showed a shuttle type, like the growth of colonies; B.hADSCs of P2, P4 and P8 all entered the logarithmic growth phase on the 3rd day after inoculation, peaked at 7th day; C.The hADSCs were induced to differentiate into adipocytes (left panel), osteoblasts (middle panel), or neurospheres; D,F,H.The flow sorting results showed that most hADSCs were expressed CD29 and CD44,which meet the chara -cteristics of mesenchymal stem cells, do not express CD45, which is characteristic of hematopoietic stem cells.E,G.Identification of neurospheres, immunofluorescence staining Nestin, SOX9, positive neurosphere expression.(scale bars = 100um)

**Fig.S2. Brain slice activity.** The LDH activity in the culture medium were analysed to assess cell viability, the LDH results showed that after day 5, LDH activity tended to be stable.(mice number in each group is equal or above 3, \*p<0.05,\*\*p<0.001, \*\*\*p<0.0001, versus 1day group).

**Fig.S3. 6-OHDA brain slice model construction.** The brain slices were exposed to 6-OHDA concentration gradients of 0, 100nM, 200nM, 400nM, 600nM, and 1200nM for 1h. The results showed that when the 6-OHDA concentration was 600nM, most of the dopaminergic neurons were damaged(mice number in each group is equal or above 3, scale bars = 100um ).

**Fig.S4. rhPTX3 treatment of 6-OHDA-induced mice behavioral improvement.** A.Different concentrations (0, 500ng, 1000ng, 2000ng, 3000ng) of rhPT3 were used to treat 6-OHDA-induced mice. At 5 weeks, open field experiment, total distance moved, velocity and resting times in 10 min; Side-biased rotational behavior in the apomorphine-induced rotation test was observed from 5 weeks.(mice number in each group is equal or above 5, \*p<0.05 versus 0ng group)

**Fig.S5.** **rhPTX3 treated the TH+ positive density improvement of6-OHDA-induced mice .** A.Different concentrations (0, 500ng, 1000ng, 2000ng) of rhPT3 were used to inject into 6-OHDA-induced mice. Immunofluorescent staining for TH in the VTA+SNc and STR ( mice number in each group is equal or above 5, scale bars = 100um).

**Fig.S6.rhPTX3 treated the TH+ positive density improvement of 6-OHDA-induced brain slices.** A.Different concentrations (0, 100ng, 500ng, 1000ng, 5000ng) of rhPT3 were used to treat 6-OHDA-induced brain slice for 4 day with serum-free medium. In the VTA+SNc and STR, rhTPX3(2000ng) incresed TH expression(mice number in each group is equal or above 5)

**Video S1-5:** The results of APO-induced contralateral rotations of vehicle group (Video S1)、6-OHDA group (Video S2)、hADSCs group (Video S3)、si-PTX3 group (Video S4) and rhPTX3 group(Video S5) .

Apomorphine caused a significant contralateral turning in the animals that were lesioned by 6-OHDA. The total net number of rotations in the hADSCs group、si-PTX3 group and rhPTX3 group was below 7 rotations/min-the accepted criteria for successful PD models, while the total net number of rotations in the 6-OHDA NS group was above the level of 7 rotations/min.

**Video S6-15:** Immunofluorescence 3D video of brain slices in VTA + SNc (vehicle group-Video S6、6-OHDA group-Video S7、hADSCs group-Video S8、si-PTX3 group-Video S9 and rhPTX3 group-Video S10) and STR regions(vehicle group-Video S11、6-OHDA group-Video S12、hADSCs group-Video S13、si-PTX3 group-Video S14 and rhPTX3 group-Video S15).

**Table S1:** RNA-seq differential gene expression table.

**Table S2:** Image J estimate the gray value of differential protein in human cytokine array.

**Table S3:** Evaluate the expression of cytokines in different groups through label-free quantitative proteomics.

**Table S4:** Protein identification and quantitative analysis parameters.

**Table-DNA bank**：DAN sequence and PTX3 small interference sequence