Supporting Information

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SI Materials and Methods

All animal procedures were approved by and performed according to the guidelines of the Peking University Animal Use and Care Committee and the Association for Assessment and Accreditation of Laboratory Animal Care.

Cell Culture and Differentiation of DA Neurons. Primitive neural stem cells (pNSCs) were induced from human embryonic stem cells (ESCs) as previously described (1). To induce them into dopamine (DA) neurons, pNSCs were firstly treated with 100 ng/mL sonic hedgehog (SHH) (C24II, R&D Systems) and 100 ng/mL fibroblast growth factor 8 (FGF8b) (R&D Systems) in differentiation medium containing DMEM/F12, 1×N2, 300 ng/mL cAMP (Sigma-Aldrich), and 0.2 mM vitamin C (Sigma-Aldrich) on a laminin and poly-L-ornithine (Sigma-Aldrich)-coated surface for 10 d. Then, the cells were incubated in differentiation medium with 10 ng/mL brain-derived neurotrophic factor (R&D Systems), 10 ng/mL glial cell-derived neurotrophic factor (R&D Systems), 10 ng/mL insulin-like growth factor-1 (R&D Systems), 1 ng/mL TGF-β3 (R&D Systems), and 0.5 mMdb-cAMP (Enzo Life Sciences) for another 14–21 d.

PD Model, Behavioral Testing, and Cell Transplantation. Adult male Sprague–Dawley rats (~200 g) were used to produce an animal model of Parkinson's disease (PD). Survival surgery was performed in a semisterile environment under general anesthesia using an established method (2). The surgical protocol for the PD animal model and in vivo recordings were similar to those described previously (3), with slight modifications. The PD model was created by unilateral injection of 4 μL 6-hydroxydopamine (6-OHDA) (3 μg/μL; Sigma-Aldrich) into the medial forebrain bundle [MFB; –2.5 mm anterior-posterior (AP), 2.0 mm medial-lateral (ML), 8.5 mm dorsal-ventral (DV)]. Half an hour before 6-OHDA injection, desipramine (25 mg/kg i.p.; Sigma-Aldrich) was given to protect noradrenergic neurons. Four weeks later, the rats were tested for apomorphine-induced asymmetric rotation (0.25 mg/kg s.c.; Sigma-Aldrich) for 30 min using a published protocol (4). The rats were placed in a quiet place for 30 min to habituate to the environment before apomorphine injection. An unbiased observer counted the number of rotations for 30 min after injection. Rats with >7 turns per minutes were used as PD model animals in the next part of the study.

Before transplantation, pNSC–DAns were digested with Accutase (Millipore) for 3–5 min at 37 °C, collected, resuspended in PBS at ~1×10^5 cells/μL, and placed on ice before use. The PD rats were randomly assigned to two groups, PD–PBS and PD–DA-like neuron (DAn). Rats in the PD–DAn group were transplanted with 4-μL pNSC–DAn suspension into two sites in the striatum on the lesioned side (site 1, 0.7 mm AP, 3.0 mm ML, 5.5 mm DV; site 2, 1.0 mm AP, 4.0 mm ML, 5.0 mm DV). The PD–PBS rats received PBS following the same procedure. The rats were immunosuppressed by daily injection of cyclosporine A (15 mg/kg, i.p.; Sigma-Aldrich) from 2 d before transplantation until the end of the experiment. Apomorphine-induced rotations were monitored every 4 wk after transplantation.

Microdialysis-Based HPLC for Evoked DA Release in Vivo. HPLC with an electrochemical detector (Waters) was used to measure the striatal level of DA and its metabolites in the collected samples, as described previously (5). Briefly, samples were eluted with a mobile phase containing (in mM) 70 KH_2PO_4, 0.11 EDTA, 0.72 SDS, and 20% (vol/vol) methanol. Solvent was delivered at 0.25 mL/min using an HPLC pump (Waters). HPLC detection was calibrated offline with standard curves for DA, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) (0.005, 0.01, 0.02, 0.05, 0.1, 0.3, and 1 μM; Fig. S1 *A* and *D*).

For HPLC identification of evoked DA release from cultured pNSC–DAns, differentiated pNSCs were washed four times with Krebs–Ringer solution containing (in mM) 120 NaCl, 3 KCl, 2 $CaCl_2$, 0.8 $MgSO_4$, 10 glucose, and 20 Hepes (pH 7.4). Then, these cells were incubated in the same solution for 5 min, and the solution was collected as the control. For DA release induced by high K^+, the cells were treated with depolarizing Krebs–Ringer containing (in mM) 83 NaCl, 50 KCl, 2 $CaCl_2$, 0.8 $MgSO_4$, 10 glucose, and 20 Hepes (pH 7.4) for 5 min, and then the solution was collected for HPLC analysis.

For HPLC identification of evoked DA release in vivo, rats were anesthetized with urethane (1.5 g/kg, i.p.; Sigma-Aldrich). A microdialysis probe (Φ200 μm, 4,000 μm sensor tip, CMA/MICRODIALYSIS AB) was inserted into the target striatum at the site of engraftment and perfused with artificial cerebrospinal fluid (aCSF) for recording (in mM) 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 1.3 $MgCl_2$, 1.3 NaH_2PO_4, 25 $NaHCO_3$, and 10 glucose (saturated with 95% O_2 and 5% CO_2) at a constant flow rate of 1 μL/min by a microinjection pump (CMA). Following an equilibration period of 3 h, dialysate samples were collected in the next 20 min as the control. Then the perfusion solution was switched to aCSF with 70 mM K^+ for depolarization, and the dialysate was collected for another 20 min for analysis.

Amperometric Recording in Vivo and in Striatal Slices. Electrochemical amperometric current (I_{amp}) was recorded in brain slices and in vivo using carbon fiber electrodes (CFEs), as previously described (3, 6). DA release was recorded as I_{amp} (oxidization amperometric current) by a CFE (Φ7 μm) with a 200-μm sensor tip held at 780 mV.

For amperometric recording of DA release in vivo, rats were anesthetized with urethane (1.5 g/kg, i.p.; Sigma-Aldrich). To stimulate the grafted pNSC–DAns for CFE recording, we designed a puffer-coupled CFE (pcCFE) consisting of a combined CFE and delivery tube (Φ 70 μm, ~100 μm from the CFE tip), fixed with glue (Fig. 6*B*). To quantify the evoked DA release from the intact side versus the damaged or cell-grafted side, we used the same pcCFE for both sides. DA overflow was recorded by the pcCFE placed in the striatum (in or near the grafted region: 1.0 mm AP, 4.0 mm ML, and 5.0 mm DV), after being evoked by a 5-s perfusion of 70 mM K^+ at 200 nl/s through the drug-delivery tube (Fig. 5*A*, *Inset*).

For amperometric recording of DA in striatal slices, rats were anesthetized with urethane and then transcardially perfused with ~50 mL ice-cold aCSF for slicing (in mM): 110 $C_5H_{14}ClNO$, 2.5 KCl, 0.5 $CaCl_2$, 7 $MgCl_2$, 1.3 NaH_2PO_4, 25 $NaHCO_3$, and 25 glucose (saturated with 95% O_2 and 5% CO_2). The brain was rapidly removed and cut into horizontal slices (300 μm thick) in the same aCSF. DA overflow was evoked by perfusion with 70 mM K^+ for 10 s and recorded by a CFE in recording aCSF at room temperature.

Amperometric signals were calibrated offline according to the standard curve of DA at 0.1, 1, 3 and 5 μM in aCSF. All amperometric recordings are expressed as both amperometric current (I_{amp}) and DA overflow concentration [DA].

Immunohistochemistry. Immunohistochemistry was performed as previously described (7) with slight modifications. Briefly, cul-

tured cells were fixed with 4% (wt/vol) paraformaldehyde (Sigma-Aldrich) in PBS. Rats were anesthetized and perfused with 0.9% saline and 4% (wt/vol) paraformaldehyde, and the brain was removed and postfixed overnight in 4% (wt/vol) paraformaldehyde. After dehydration with 10%, 20%, and 30% (wt/vol) sucrose, the brain was sectioned at 50 μm on a cryostat (Leica). The fixed cells and sections were washed three times for 5 min with PBS, and then permeabilized with 0.3% Triton X-100 in PBS containing 2% (wt/vol) BSA for 5 min and blocked with 2% (wt/vol) BSA in PBS for 1 h. The samples were then incubated with the primary antibodies rabbit anti-tyrosine hydroxylase (TH) (Millipore, 1:500), mouse anti-MAP2 (Sigma, 1:500), rabbit anti-Nurr1 (Santa Cruz, 1:500), rabbit anti-D2 receptor (Lifespan Bioscience, 1:500), rabbit anti–aldehyde dehydrogenase 1 (ALDH1A1) (Abcam, 1:200), or rabbit anti–vesicular monoamine transporter-2 (VMAT2) (Abcam, 1:200) at 4 °C overnight. After three washes with blocking solution, samples were incubated for 1 h with the secondary antibodies Alexa Fluor 594 goat anti-mouse IgG (H+L) (Invitrogen, A11032) or Alexa Fluor 594 goat anti-rabbit IgG (H+L) (Invitrogen, A11037) at room temperature. Nuclei were visualized by DAPI staining, and samples were observed using a Zeiss 710 inverted confocal microscope. Images were processed with Zeiss LSM Image Browser (version 3.0) and Adobe Photoshop (Adobe Systems Inc.).

Statistical Analysis. At least three independent experiments were performed for each type of assay. Comparisons were made with the two-tailed unpaired Student t test or one-way ANOVA as indicated. All tests were performed using the Statistical Package for the Social Sciences version 13.0, and significant differences were accepted at $P < 0.05$. All data are presented as mean ± SEM.

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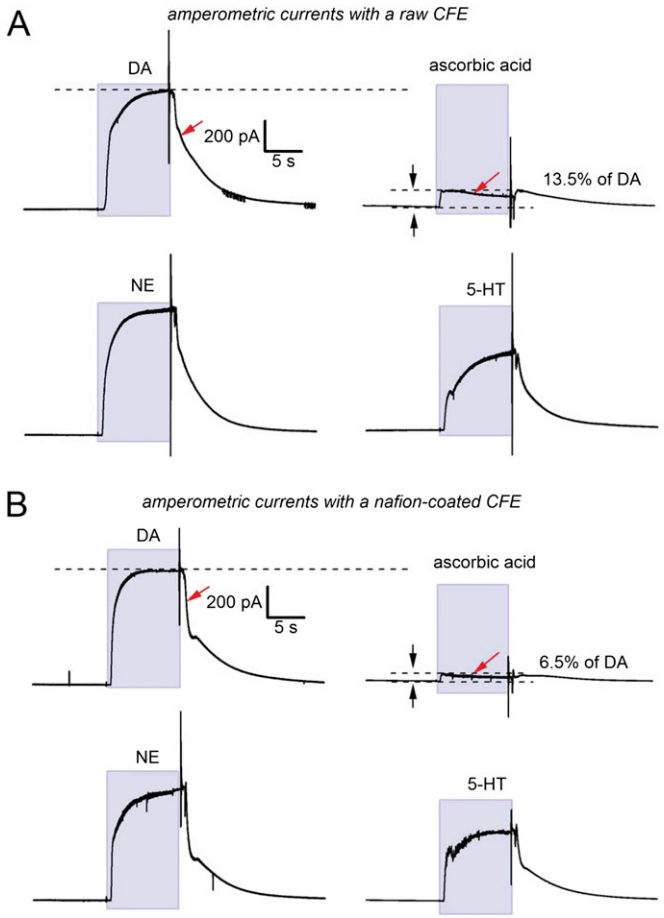

Fig. S1. Amperometry using a nafion-coated CFE (ncCFE) cannot distinguish DA from norepinephrine (NE) and serotonin (5-HT) in vitro; the ncCFE is insensitive to ascorbic acid. (*A*) With a raw (without nafion coating) CFE at 780 mV, typical amperometric current (I_{amp}) responses to 5 μM DA, ascorbic acid, NE, and 5-HT for 10 s. Similar results were obtained from three different electrodes. (*B*) With an ncCFE at 780 mV, responses to 5 μM DA, ascorbic acid, NE, and 5-HT for 10 s. Similar results were obtained from three different electrodes. ncCFEs were prepared according to Kehr (1999) (1) and ref. 2 with slight modifications. Raw CFE sensor tips were dipped into 5% (vol/vol) nafion solution and dried at 60 °C for 30 min. This treatment was repeated four times.

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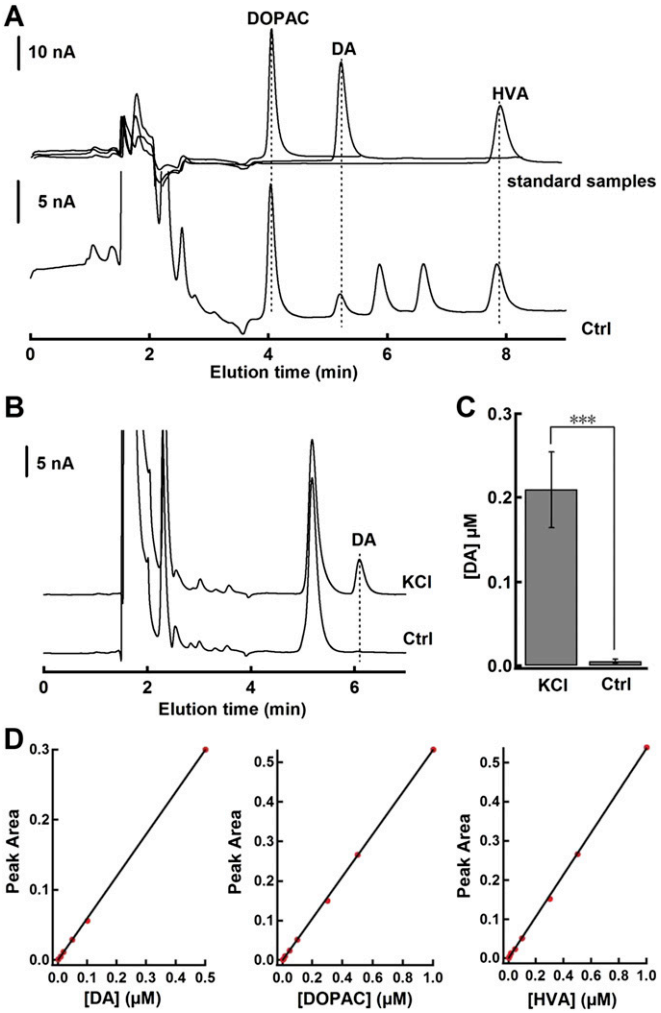

Fig. S2. Microdialysis-based HPLC recorded signals recorded from intact control striatum in response to stimulation with 70-mM K^+ in vivo. (*A*) Identification of DA, DOPAC, and HVA in striatal dialysate from an intact control rat, collected during 20 min of 70-mM K^+ stimulation. (*Upper* traces) HPLC chromatographs of standard samples of DOPAC (1 μM), DA (1 μM), and HVA (1 μM). (*Lower* traces) HPLC chromatograph of dialysate from intact control striatum (Ctrl). (*B*) The DA signal increased during 70-mM K^+ stimulation, indicating the stimulation dependence of DA release in the striatum. (*Upper* trace) HPLC chromatograph of dialysate from striatum in response to 20-min stimulation with 70-mM K^+ (KCl). (*Lower* trace) HPLC chromatograph of dialysate from striatum perfused for 20 min with aCSF as control (Ctrl). (*C*) Statistical analysis of DA signals recorded as in *B* (Student *t* test; $***P < 0.001$; $n = 6$ for KCl and Ctrl). (*D*) Calibration of [DA], [DOPAC], and [HVA] to the peak area of chromatogram traces.

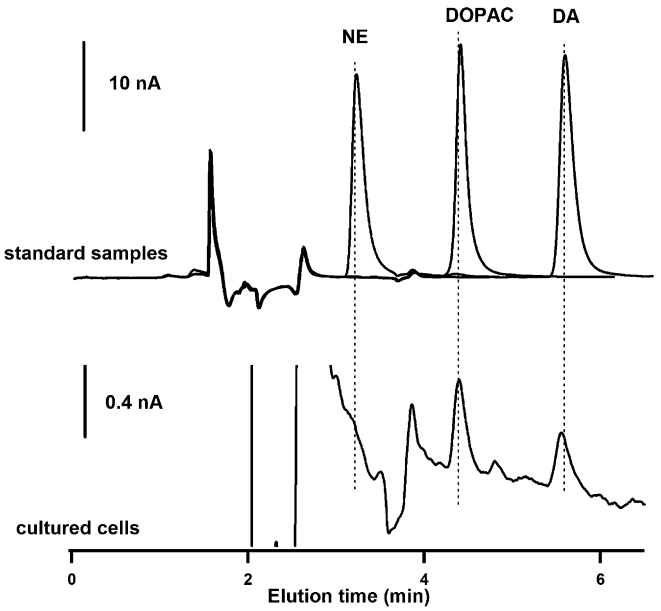

Fig. S3. DA and DOPAC, but not NE, were detected by HPLC in cultured pNSC–DAns. (*Upper*) Representative HPLC chromatogram of standard samples of NE (1 μM), DOPAC (1 μM), and DA (1 μM). (*Lower*) Representative HPLC chromatogram of sample from pNSCs (differentiated for 2 mo) in 50-mM K^+ medium.

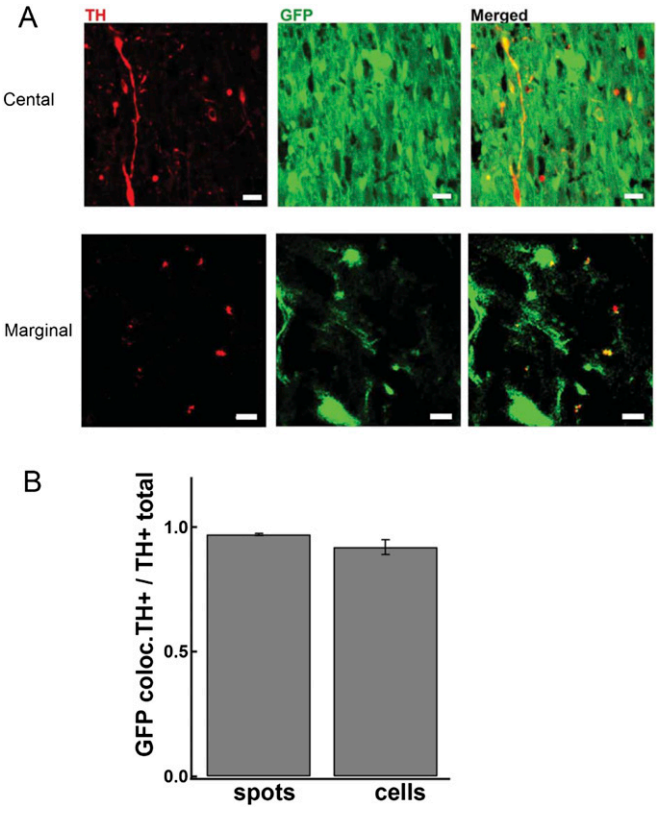

Fig. S4. TH-positive staining of cell bodies and processes of grafted pNSC–DAns after 16 wk of transplantation. (*A*) Representative micrographs showing the TH staining of pNSC–DAn-transplanted striatum. (*Upper* panels) TH-positive cells and spots are colocalized with GFP in the center of the graft. (*Lower* panels) TH-positive spots are colocalized with GFP in the margin of the graft. [Scale bar, (*Upper* panels) 20 μm and 5 μm (*Lower* panels).] (*B*) Statistics showing that >90% of the TH-positive cells and spots were also GFP-positive, as well in the grafted striatum.

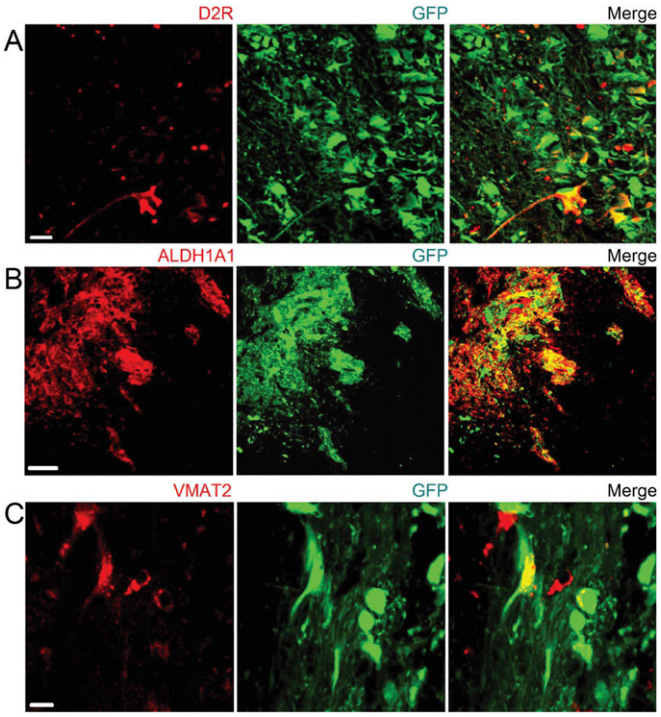

Fig. S5. Long-term survival of mature dopaminergic neurons in the grafted region 16 wk after transplantation. (*A*–*C*) Immunostaining showing the expression of D2 receptor, ALDH1A1, and VMAT2 in GFP-labeled pNSC–DAns in striatum. [Scale bar, (*A*) 20 μm, (*B*) 50 μm, and (*C*) 10 μm.]

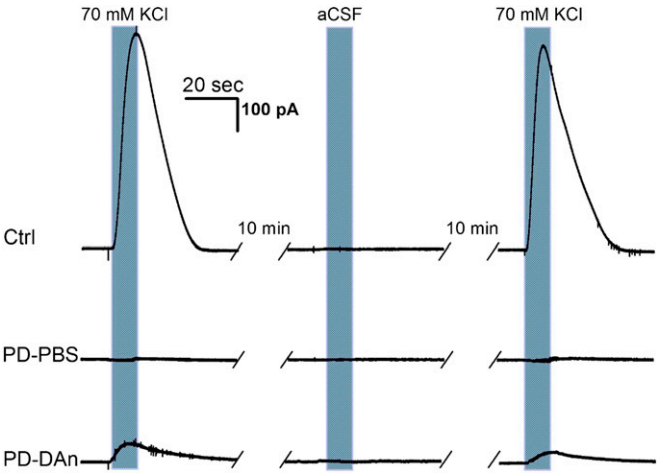

Fig. S6. Repeatable DA release induced by 70-mM K^+ in intact control, PD–PBS, and PD–DAn striatal slices. Representative CFE recordings of DA overflow in response to 10-s stimulation with 70-mM K^+, aCSF, and 70-mM K^+, with 10-min intervals between recordings, in intact control (Ctrl), PD–PBS, and PD–DAn rat striatal slices.

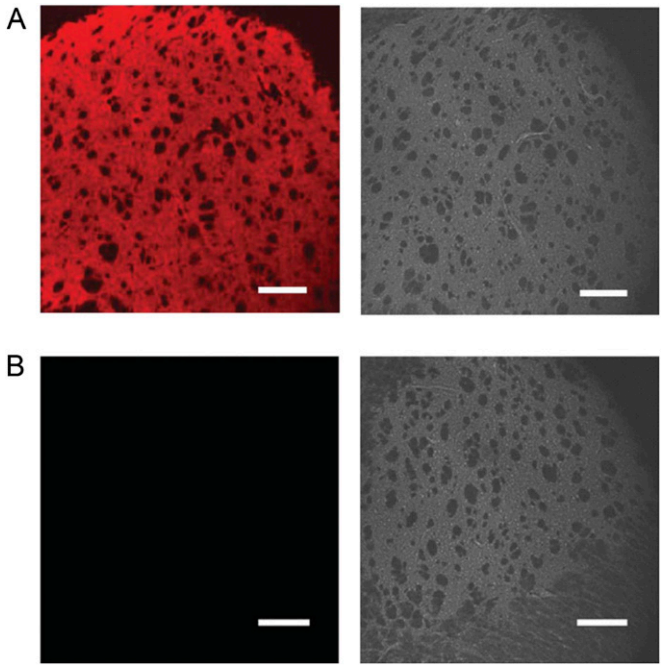

Fig. S7. TH-staining is absent from the 6-OHDA–lesioned striatum. (*A*) TH immunostaining (*Left*) with the corresponding DIC image (*Right*) of an intact striatal section. (*B*) TH immunostaining (*Left*) with the corresponding DIC image (*Right*) of a striatal section on the 6-OHDA–lesioned side. (Scale bar, 50 μm.)

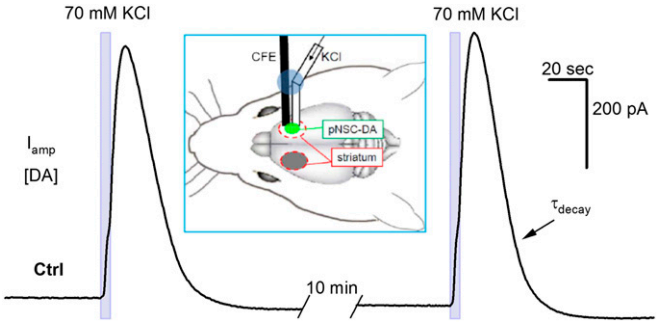

Fig. S8. High K^+ depolarization-induced DA overflows are reproducible in the striatum in vivo. Representative striatal I_{amp} signals in response to 5-s stimulation by local application of 70-mM K^+ in rat striatum in vivo. (*Inset*) The I_{amp} signal-recording system in vivo. A pcCFE with puffer tube was inserted into the grafted striatum (green) ($n = 6$).