Stem Cell Reports, Volume *9*

Supplemental Information

Accreditation of Biosafe Clinical-Grade Human Embryonic Stem Cells According to Chinese Regulations

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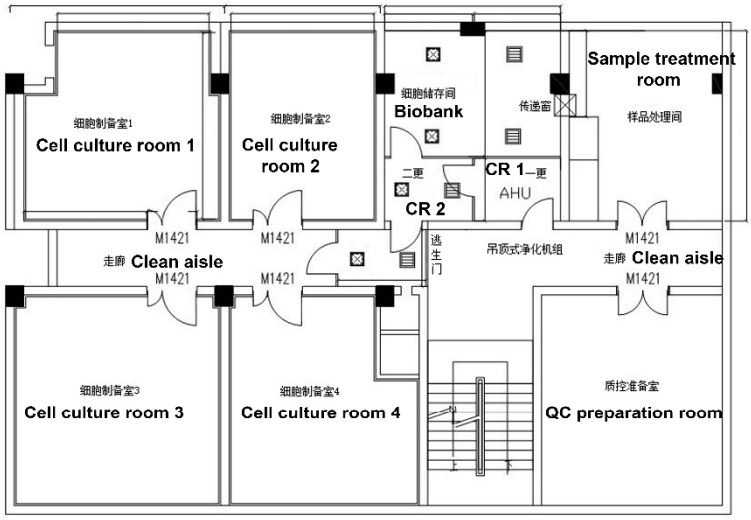
Supplementary information

A



B

C

Q-CTS-hESC-2--P47-4

Name	Q-CTS-hESC-2--P47-4
Sample Source	Q-Q-CTS-hESC--2017-03-15
Container	1/6 well
Incubator	Incubator 09
Generation	P47
Medium Type	E8
Passage Ratio	1:3
Operation Room	Clinical grade Room 2
Last Operating Time	2017.03.15, 17:00
Cell Type	Embryonic Stem Cell
Passage Operation	Passage Operation 806
Cells in the Same Generation	Cells in the Same Generation 171
Parent Cells	3
Own or Outside	Own
Last Operator	刘鑫

Figure S1 The GMP lab information and the digital system to trace the cell line. (**A**) The layout of GMP lab which mainly contains QC preparation room, sample treatment room, changing room, and four cell culture rooms. QC, quality control; CR, changing room. (**B**) The open interface of the software. (**C**) The display of one example cell line, Q-CTS-hESC-2.

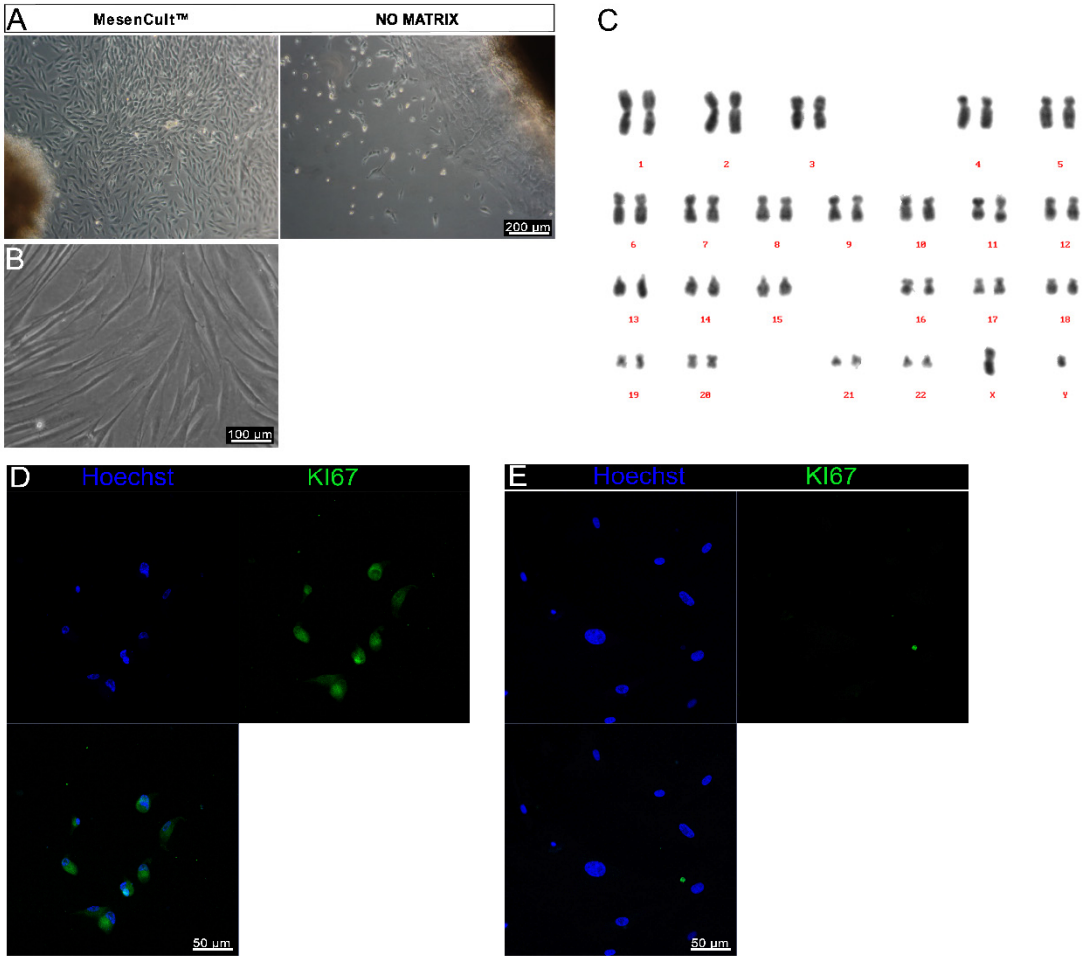


Figure S2 HFF derivation. (**A**) Bright field images of HFFs migrated out of the minced tissues after 7 days of attachment. The substrates in the right and left groups were no matrix and MesenCult™ separately. (**B**) Stable passaged HFFs in SCFM medium on MesenCult™ substrate. Scale bars, 200 μm. (**C**) Karyotype analysis of HFFs with normal 44 euchromosomes and one X chromosome, one Y chromosome. (**D**) Immunostaining of HFFs with KI67 (green) and Hoechst 33342 (blue) for nuclei. Scale bars, 50 μm. (**E**) Staining inactivated feeder cells with KI67 (green) and Hoechst 33342 (blue) for nuclei. Scale bars, 50 μm.

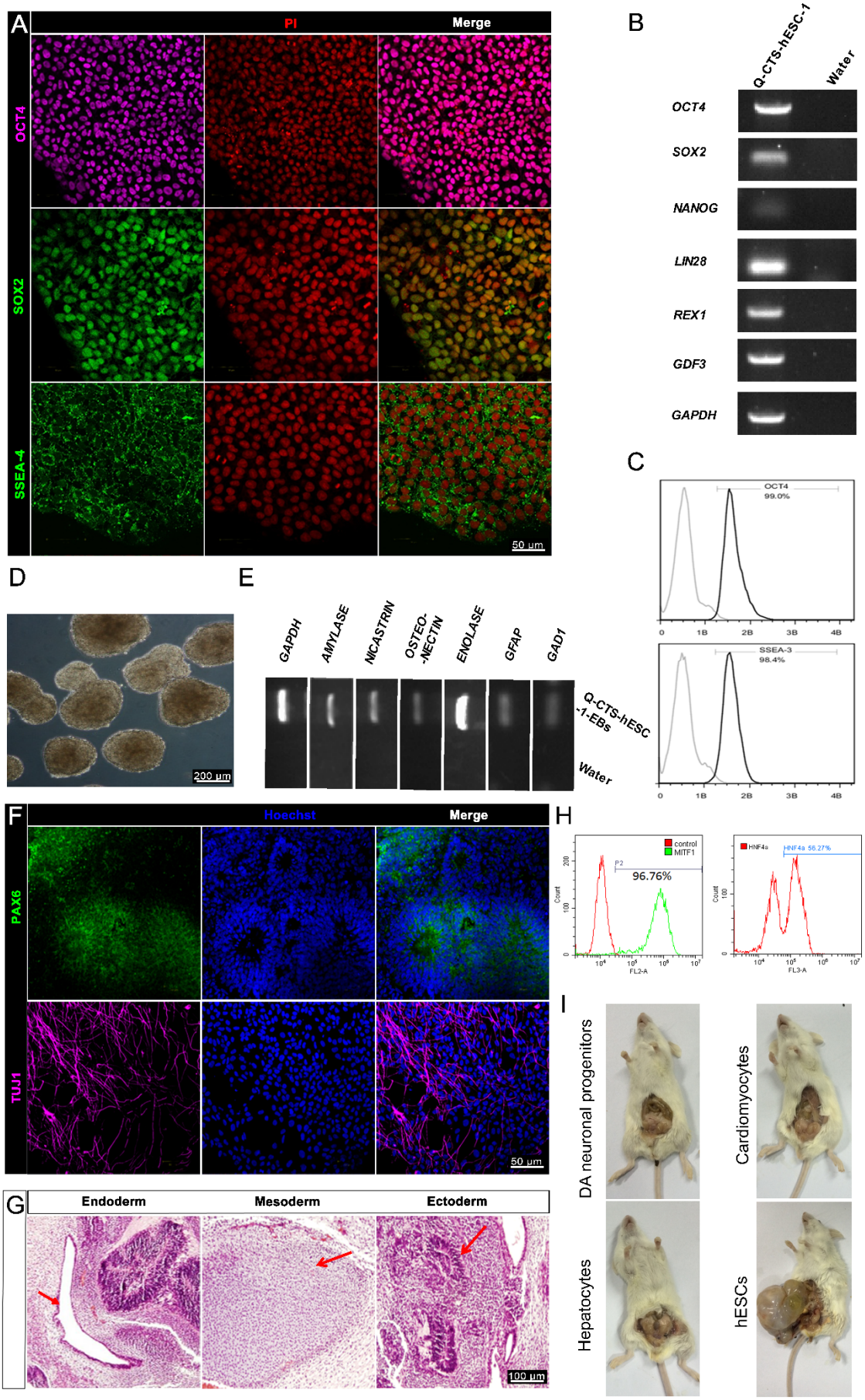


Figure S3 Pluripotent characterizations of Q-CTS-hESC-1 cells and flow cytometry, teratoma formation test for differentiated Q-CTS-hESC-2 cells. (**A**)

Immunofluorescence analysis of Q-CTS-hESC-1 cells. Positive nuclear transcription factors OCT4 (purple) and SOX2 (green) and expression of the ESCs surface antigen SSEA4 (green) were observed. Nuclei were stained by PI (red). Scale bars, 50 μm. (**B**) RT-PCR analysis of ESC specific genes confirmed their expression. (**C**) Quantitative flow cytometry analysis indicating robust expression of intracellular OCT4 and extracellular SSEA4 in Q-CTS-hESC-1 cells. (**D**) EB formation *in vitro*. Scale bars, 200 μm. (**E**) RT-PCR of EBs showing transcript for ectoderm (*GAD1, GFAP*) mesoderm (*ENOLASE, OSTEONECTIN*) and endoderm (*AMYLASE, NICASTRIN, ALSO NAMED NCSTN*) markers. (**F**) Neurons differentiated from Q-CTS-hESC-1 EBs. Top, neuroepithelial cells (stained with PAX6 (green)) formed rosettes. Bottom, neuronal lineages were detected TUJ1 (purple) positive. Nuclei were stained by Hoechst 33342 (blue). Scale bars, 50 μm. (**G**) Teratoma formation. All three germ layers tissues were presented on the teratoma dissection slices identified by staining with haematoxylin and eosin. As red arrows pointed, left, endoderm with glands; middle, mesoderm with cartilage tissues; right, ectoderm with nervous tissues. Scale bars, 100 μm. (**H**) Flow cytometry analysis of RPE cells (left) at P3 and hepatocytes (right) on day 9 for the differentiated cells from cell line Q-CTS-hESC-2. (**I**) Teratoma formation test for the differentiated cells DA neuronal progenitors, cardiomyocyte and hepatocytes specialized from hESCs for the cell line Q-CTS-hESC-2.

Supplementary tables

Table S1 Progress in clinical-grade hESC derivation

Year	Country	Feeder/ Substrate	Xeno -Free	Medium	Clinical Trials	Biosafety Test	Function *in vivo*	Ref
2006	United States	Mouse	No	containing KSR and Plasmanate	Yes	PASS	Yes	Klimanskaya et al., 2006; Schwartz et al., 2012)
2007	Australia and Singapore	Human	No	containing KSR	No	PASS	NA	Ludwig et al., 2006a
2010	Finland	Human	Yes	containing human serum albumin	No	NA	NA	Rajala et al., 2010
2011	United Kingdom	Human#	Yes	KSR/TeSR2	No		NA	Ilic et al., 2012
2012	Israel	Human	Yes	SCGM (commercially available)	No	PASS	NA	Tannenbaum et al., 2012
This study	China	Human	Yes	STEMedia (commercially available)	No	PASS	Yes	

Human ESCs were firstly cultured on HFF feeders and then transferred to StemAdhere substrate (commercially available)

Table S2 GMP reagents for feeder cell production and banking and hESC line derivation and banking

Reagent	Supplier	Catalog Number	Regulation
G-1™ medium	Vitrolife	10127	Manufactured under cGMP and FDA clearance
G-2™ medium	Vitrolife	10131	Manufactured under cGMP and FDA clearance
MesenCult™-XF Supplement (Mesencult™)	STEMCELL Technologies	05422	Manufactured under ISO 13485 medical device standards
CTS™ KnockOut™ DMEM (CTS-KO-DMEM)	Life Technologies	A12861-01	Manufactured under cGMP with DMF
CTS™ KnockOut™ SR Xeno Free medium (CTS-KOSR)	Life Technologies	12618-012	Manufactured under cGMP with DMF
NutriStem™ XF/FF Culture Medium (STEMedia)	Stemgent	01-0005	Manufactured under cGMP
Collagenase NB6	Serva	17458.04	Manufactured under cGMP and sterility tested according to EP
CTS™ TrypLE™ Select Enzyme (CTS-Tryple)	Life Technologies	A12859-01	Manufactured under cGMP with DMF
CTS™DPBS (CTS-DPBS)	Life Technologies	A1285801	Manufactured under cGMP with DMF
Xeno-free Penicillin/streptomycin	Lifeline Cell Technology	LS-1073	Manufactured under cGMP
FibroGRO™ Xeno-Free Human Fibroblast Expansion Medium (SCFM)	Millipore,	SCM037	Manufactured under cGMP with DMF
Essential 8™ Medium	Life Technologies	A1517001	Manufactured under cGMP
Vitronectin	Life	A14700	Manufactured under cGMP

Reagent	Supplier	Catalog Number	Regulation
	Technologies		
Non Essential Amino Acid (NEAA)	Life Technologies	11140050	Manufactured under cGMP
CTS™ GlutaMAX™-I Supplement (CTS-GlutaMAX)	Life Technologies	A12860-01	Manufactured under cGMP with DMF
β-mercaptoethanol	Life Technologies	21985023	Manufactured under cGMP
CTS™ KnockOut™ DMEM/F-12 (CTS-KO-DMEM/F12)	Life Technologies	A13708	Manufactured under cGMP with DMF
CTS™ N-2 Supplement	Life Technologies	A13707-01	Manufactured under cGMP with DMF
CTS™ CELLstart™ Substrate (CTS-CELLstart)	Life Technologies	A10142-01	Manufactured under cGMP with DMF
CTS™ Neurobasal® Medium (CTS-Neurobasal)	Life Technologies	A13712-01	Manufactured under cGMP with DMF
CTS™ B-27® Supplement (CTS-B27)	Life Technologies	A14867	Manufactured under cGMP with DMF
StemPro® Accutase® Cell Dissociation Reagent (Accutase)	Life Technologies	A11105-01	Manufactured under cGMP with DMF
Y-27632	Selleck	S1049	Manufactured under cGMP
CHIR99021	Stemgent	04-0004	Manufactured under cGMP
IWR-1	Calbiochem	681669	Manufactured under cGMP
Insulin	Life Technologies	12585-014	Manufactured under cGMP
RPMI1640	Life Technologies	31800-022	Manufactured under cGMP with Type II DMF
Dimethyl Sulfoxide	Sigma-Aldrish	D2348	BioPerformance Certified,

Reagent	Supplier	Catalog Number	Regulation
(DMSO)			Hybridoma, USP
IMDM	Life Technologies	12440053	Manufactured under cGMP and ISO 13485 standard
Oncostatin M (OSM)	R&D	295-OM/CF	Manufactured under cGMP
HGF	R&D	294-HG/CF	Manufactured under cGMP
Dexamethasone (Dex)	Sigma-Aldrish	D4902	Manufactured under cGMP
MesenCult™-ACF Freezing Medium (ACF)	STEMCELL Technologies	05490	Manufactured under cGMP and ISO 13485 standard
STEM-CELLBANKER® GMP (CELLBANKER)	Zenoaq	STEM-CELLBANKER	Manufactured under cGMP with FDA clearance

Table S3 Primer sequences for RT-PCR

Gene name	Forward primers	Reverse primers	Product size (bp)
OCT4	GACAGGGGGAGGGGAGGAG CTAGG	CTTCCCTCCAACCAGTTGCC CCAAAC	144
SOX2	GGGAAATGGGAGGGGTGCA AAAGAGG	TTGCGTGAGTGTGGATGGGA TTGGTG	161
NANOG	CAGCCCCGATTCTTCCACCA GTCCC	CGGAAGATTCCCAGTCGGGT TCACC	380
LIN28	GCAGAAGATCACTCCGTTCC A	CGCACATTGAACCACTTACA GT	191
REX1	CAGATCCTAAACAGCTCGCA GAAT	GCGTACGCAAATTAAAGTCC AGA	306
GDF3	CTTATGCTACGTAAAGGAGC TGGG	GTGCCAACCCAGGTCCCGG AAGTT	631
AMYLASE	AATGATGCTACTCAGGTCAG AGATT GTC	TGTCCTCGTTGATTGTCATG GTTATCC	461
NICASTRIN	CGAGGATGGTCTACGATATG GAGAAGG	TCAGCCAGAACAACGCCAG AGAT	307
ENOLASE	GCTCCGTGACCGAGTCTCTT	TAGCCAACAGGTGACCGAA GG	301
OSTEONECTIN	CCAGGTGGAAGTAGGAGAA TT	CTCAGTCAGAAGGTTGTTGT C	427
GAD1	GGAACTAGCGAGAACGAGG AAG	AGGAGGTTGCGGACGAAGA T	235
GFAP	TGAGTCGCTGGAGGAGGAG AT	GTCGTTGGCTTCGTGCTTGG	283
GAPDH	AGGCATCCTCACCCTGAAGT A	CACACGCAGCTCATTGTAGA	103

Supplementary methods

Ethical approval

Clinically discarded oocytes that were not suitable for *in vitro* fertilization (IVF) were used in this study for parthenogenetic hESC derivation. Early-stage embryos (for fertilized hESC derivation) used in this study were obtained from clinically discarded embryos that were not suitable for transplantation. Both oocytes and early embryos had been cryopreserved for at least five years. Foreskin tissues (for feeder cell derivation) used in this study were obtained from children who were undergoing foreskin resection surgery. The study was approved by the "Animal and Medical Ethics Committee of the Institute of Zoology, Chinese Academy of Sciences" (Ethical No. IOZ15033 and IOZ15038). Written consent was obtained from the couples or the donor's parents. After donation, a thorough assessment of the donors' medical history and infectious diseases was conducted. Only donors without any pathogenic microorganism infection or genetic disease were selected. All information was secured to protect the privacy and confidentiality of the donors. No financial benefits were involved in the donation process. The guidelines legislated and posted by the Ministry of Health of the People's Republic of China were closely followed for all tissue and cell handling procedures.

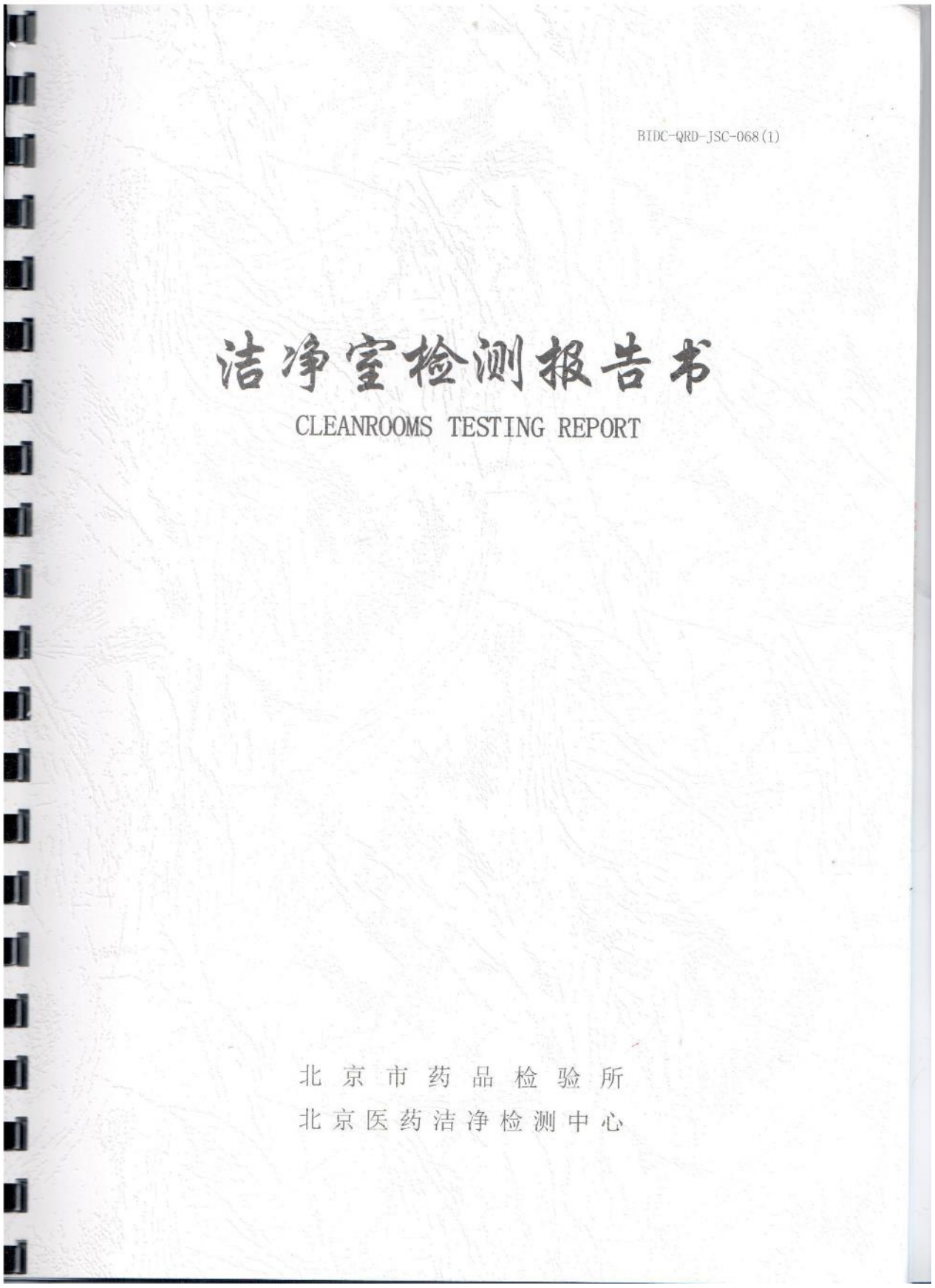
Cell transplantation and immunohistochemistry

Twenty-four hours before transplantation, the DA neuronal progenitors were stained with Molday ION™ (Biopal) according to the manufacturer's instructions. Then, the cells were digested into single cells using TrypLE and resuspended in cell culture medium supplemented with 0.05% DNase at a density of 6.25×10^5 cells/mL. The methods for anesthetizing and fixing the rat PD models were similar to those described in the modeling section. The cell transplantation position was AP = +1.0 mm, ML = +2.5 mm with respect to the bregma and DV = -4.5/-5.0 mm with respect to the dura. Then, 5 μL of cell suspension was injected into each animal at a rate of 0.8 μL per minute. After injection, the needle remained in situ for a further 5 minutes to prevent diffusion. The

control animals were injected with 5 μL of cell culture medium only. Three months after cell transplantation, the rats were deeply anesthetized with pentobarbital (25 mg/kg, i.v.). The bodies were transcardially perfused with heparinized normal saline followed by 4% (w/v) PFA. The brain tissue was removed from the skulls and sliced in the coronal plane with a calibrated Lucite brain slice apparatus. Immediately after primary fixation, the brain tissues were post-fixed in 4% PFA for 1-3 days and then rinsed and soaked successively in 10% (w/v), 20% (w/v), and 30% (w/v) sucrose solutions to “sink”. The brains were cut coronally into 40 μm serial sections on a frozen sledge microtome and stored free-floating in cryoprotectant medium (30% (w/v) sucrose, 30% (w/v) ethylene glycol in PBS) at -20°C.

After washing with cold PBS, the coronal brain tissue sections were permeated and blocked with 1% (w/v) BSA and 0.3% (v/v) Triton. Primary antibodies diluted in 2% BSA were added to the sections and incubated for one hour at room temperature. The primary antibodies used were anti-tyrosine hydroxylase (TH, Santa Cruz, sc-14007, 1:200), anti-Nuclei (HNA, Millipore, clone 235-1, 1:200), and anti-GFAP (Millipore, clone EP672Y, 1:250). After sufficient washing in dilution media, appropriate secondary antibodies (FITC, Jackson ImmunoResearch, 1:200) were added and incubated for 1 hour at room temperature. Finally, the nuclei were stained with Hoechst 33342 (10 μg/mL) for 10 minutes at room temperature.

Supplementary information Doc1



BTDC-QRD-JSC-068(1)

洁净室检测报告书

CLEANROOMS TESTING REPORT

北京市药品检验所

北京医药洁净检测中心

CMA
150100140007
资质有效期至:2021.08.09
ilac-MRA
CNAS
检 测
CNAS L1669

洁净室检测报告书

报 告 编 号： JE1600403

申 检 单 位： 中国科学院动物研究所北京干细胞库

检 测 区 域： 北京干细胞库细胞间

检测区域地址： 北京市海淀区北四环西路25-2北京干细胞库

检 测 单 位： 北京市药品检验所
（北京医药洁净检测中心）

报 告 日 期： 2016.6.15

结论： 本区域按《医药工业洁净厂房设计规范》（GB50457—2008）检测，结果符合十万级、万级规定。

授权签字人：

Supplementary information Doc2

2012000599S

中国食品药品检定研究院

检验报告

报告编号：SH201402035

检品名称：O-CTS-hESC-2人胚胎干细胞

供样单位：中国科学院动物研究所

检定目的：合同检验

检验依据：约定方法

中国食品药品检定研究院检验报告

报告编号:SH201402035　　第1页，共6页

检品名称	Q-CTS-hESC-2人胚胎干细胞	检品编号	SH0417201301875
供样单位	中国科学院动物研究所	菌号/代次	编号：P40、代次：P40代
检品来源	中国科学院动物研究所北京干细胞库	检品状态	液体
检验目的	合同检验	检品数量	50个
检验项目	部分检验	收样日期	2013年11月6日
检验依据	约定方法		

检验项目	标准规定	检验结果
[细胞鉴别试验]		
细胞形态检查	报告结果	细胞呈二维克隆生长，克隆边缘清晰、表面光滑，克隆内细胞与细胞之间的连接紧密，看不清细胞界限
种属鉴别（同工酶法）	人源细胞B型	符合规定
细胞株鉴别（人源STR图谱分析）	报告结果	Amelogenin: X; vWA: 17,18; D21S11: 30.2,32.2; D18S51: 13,14; PentaE: 10,12; D5S818: 9,10; D13S317: 8,11; D7S820: 12; D16S539: 9,11; FGA: 20,22; D3S1358: 16,18; TH01: 6,9; D8S1179: 13,15; TPOX: 8; CSF1PO: 9,13; PentaD: 13,15。

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(The full part of the documents could be asked from the authors.)