Supporting Information

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SI Materials and Methods

Proteomic Analysis. Total protein concentrations were determined by the modified Bradford-HCl assay using γ-globulin as a standard and aligned among all samples. Separation of proteins by first-dimensional IEF electrophoresis was performed using IPG gels (Immobiline DryStrip, pH 3–11 NL; GE Healthcare). The strips were transferred onto SDS/PAGE gels. After the 2D PAGE, gels were stained with Sypro Ruby (Invitrogen). Differentially expressed spots were picked up manually and subjected to in-gel trypsin digestion. MS spectrometric analysis of the tryptic digests was performed using a 4700 MALDI-TOF/TOF mass spectrometer (Applied Biosystems). To identify proteins, sets of MS and MS/MS spectra were subjected to MS/MS Ion search (Matrix Science) against the Swiss Prot or the National Center for Biotechnology Information mammalian protein database.

Embryo Manipulation. Porcine nuclear transfer was performed as described previously (1). Porcine fibroblast cells from an adult male Meishan pig were cultured in DMEM with 10% FBS and used for donor cells within passage 8. Five picoliters of antibody solution or 10 pL of 500 ng/μL poly(A)-tailed RNA was injected into enucleated oocytes. After injection of antibody, embryos were kept for at least 1 h before fusion with donor cells, which allows the antibody to bind endogenous DJ-1. The total number of cleaved embryos was judged 48 h after nuclear transfer. Porcine ICSI followed the previous protocol (2) and the antibody was injected at least 1 h before sperm injection. Antibodies used are as follows: DJ-1 (sc-27004; Santa Cruz), rabbit IgG (sc-2027; Santa Cruz), and goat IgG (sc-2028; Santa Cruz).

Gene Expression Analyses. Incorporation of DJ-1 protein into extract-treated nuclei and distribution of DJ-1 protein in oocytes and embryos were examined by immunofluorescence analysis. The following antibody was used: DJ-1 (sc-27004). Anti-goat secondary antibody conjugated with Alexa488 (Invitrogen) was used. Nuclei were stained with propidium iodide. The amounts of proteins expressed in cells, oocytes, and embryos were measured by Western blotting. The following primary antibodies were used: DJ-1 (sc-27004), P53 (628201; Biolegend), P53(S20) [DR1023 (Calbiochem) or 55427 (ANASPEC)], Histone H2B (07-371; Millipore), β-Actin (clone AC15; Sigma), and Tubulin (T9026; Sigma). Anti-Tubulin, anti-Histone H2B, and anti–β-Actin antibodies were used for standardization. The blots were visualized using Immobilon Western HRP Substrate (Millipore). The amounts of transcripts expressed were measured by RT-PCR analysis. Total RNA from cells or from oocytes and embryos was extracted by a phenol-chloroform method or by a Picopure RNA extraction kit (Arcturus), respectively. Purified RNA was reverse transcribed by ReverTra Ace (TOYOBO) and PCR was performed by ExTaq (Takara) or KOD plus. Primers used are listed in Table S2. In both analyses, the band intensities were determined by a model 4.0 ATTO densitograph.

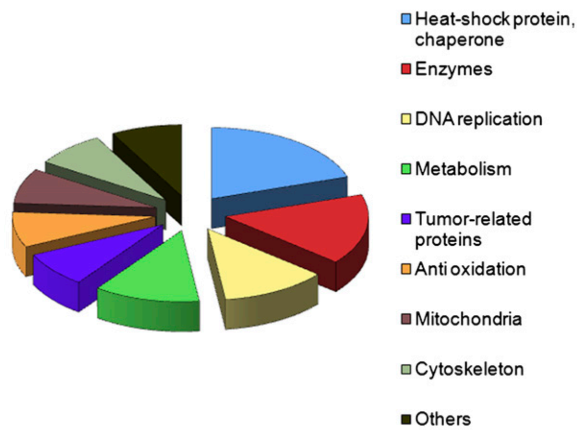
Microarray Analysis. Nuclear transfer embryos (23–26 embryos per each sample) were collected 28 h after nuclear transfer. Total RNA was purified by the use of a Picopure RNA extraction kit. Two rounds of amplification for isolated RNA were performed for microarray analysis (3). The final yield of biotinylated cRNA was hybridized to the Affymetrix GeneChip Porcine Genome Array. The output data were analyzed with GeneSpring software (Agilent).

Plasmid Construction, RNA Synthesis, and Transfection. Site-directed mutagenesis was induced by different primer sets containing a point mutation (Table S2) to generate constructed plasmids containing pig DJ1(L166P) cDNA. The plasmids were used as templates for in vitro transcription. RNA synthesis and poly(A) tailing were performed with a MEGAscript T7 kit (Ambion). Antisense oligonucleotide and siRNA against porcine DJ-1 were designed and the sequences are listed in Table S2. *DJ-1* siRNA was transfected to fibroblast cells using Lipofectamine 2000 (Invitrogen) according to the vendor's instructions.

1. Miyamoto K, Hoshino Y, Minami N, Yamada M, Imai H (2007) Effects of synchronization of donor cell cycle on embryonic development and DNA synthesis in porcine nuclear transfer embryos. *J Reprod Dev* 53:237–246.
2. Binh NT, Van Thuan N, Miyake M (2009) Effects of liquid preservation of sperm on their ability to activate oocytes and initiate preimplantational development after intracytoplasmic sperm injection in the pig. *Theriogenology* 71:1440–1450.
3. Vassena R, et al. (2007) Tough beginnings: Alterations in the transcriptome of cloned embryos during the first two cell cycles. *Dev Biol* 304:75–89.

Fig. S1. Fibroblast cell nuclei were treated with oocyte extracts and nuclear proteins were separated by 2D PAGE. MII-EXT, MII oocyte extract-treated cells; GV-EXT, GV oocyte extract-treated cells; Fibro-EXT, fibroblast extract-treated cells; Non treatment, cells before extract treatment. pI, isoelectric point.

Fig. S2. Protein spots identified by MS/MS analysis. Protein spots marked by red circles were specifically and abundantly expressed in MII oocyte extract-treated cells. The number on the 2D PAGE gel corresponds to the spot number in Table S1. MII-EXT, MII oocyte extract-treated cells; pI, isoelectric point.



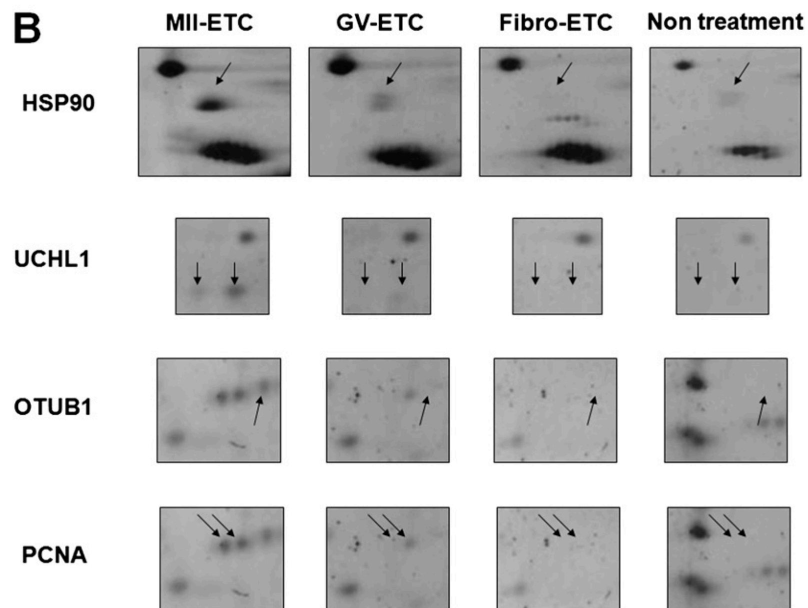


Fig. S3. Oocyte proteins that are specifically and abundantly accumulated in somatic nuclei after treatment with MII oocyte extracts. (*A*) Identified proteins are classified according to their cellular function. (*B*) Some examples of proteins that are highly expressed in MII oocyte extract-treated cells (MII-EXT). Protein spots are indicated by arrows. GV-EXT, GV oocyte extract-treated cells; Fibro-EXT, fibroblast extract-treated cells; Non treatment, cells before extract treatment.

Fig. S4. Inhibition of *DJ-1* expression and specificity of anti–DJ-1 antibody. (*A*) siRNA against *DJ-1* was injected into GV oocytes and then in vitro matured to the MII stage. Although *DJ-1* transcripts were somewhat decreased, no significant degradation of DJ-1 proteins was observed during oocyte maturation. As a control, EGFP mRNA was injected at the same time. β-Actin (ACTB) was used as a loading control. (*B*) Oocytes injected with antisense oligonucleotide against pig *DJ-1* were in vitro matured to the MII oocytes and specific inhibition of *DJ-1* expression was examined. Although transcripts were efficiently down-regulated by the antisense oligonucleotide, no apparent degradation of DJ-1 proteins was detected (AS). As a control, sense oligonucleotide (S) and EGFP mRNA were injected. β-Actin was used as a loading control. (*C*) Goat polyclonal antibody against human DJ-1 specifically recognized porcine DJ-1 proteins in MII oocytes, observed by Western blotting.

Fig. S5. Timing of first cleavage was compared between NT embryos injected with DJ-1 antibody (DJ1-NT) and those with IgG (IgG-NT). The *y* axis represents ratios of cleaved embryos at different times (24 h, 27 h, and 30 h) to total cleaved embryos at 48 h.

Fig. S6. Molecular networks related to *P53* and *DJ-1* are depicted. Signaling pathways and relationship among these genes are shown. These pathways are based on published reports. Genes whose expression was disrupted after inhibition of DJ-1 function in nuclear transfer embryos in this report are marked with asterisk. Abnormal expression was detected by microarray analysis (more than twofold differences), RT-PCR, Western blotting analysis, or inhibitor treatment for P53. Large sets of genes and molecular pathways are perturbed after DJ-1 inhibition in NT embryos, including some critical genes involved in cell arrest or apoptosis.

Fig. S7. Fibroblast cells after treatment with specific siRNA against DJ-1. Many lipid droplets (some of them marked with arrows) accumulated 24 h after transfection with siRNA against pig DJ-1 (siRNA). As a control, cells were treated with lipofectamine 2000 without siRNA.

Table S1. Oocyte proteins that are incorporated into somatic nuclei during incubation in MII oocyte extracts

Spot no.*	Protein name	Accession ID	Mascot PS†	Classification
1–3	Peroxiredoxin-2: *Sus scrofa* (pig)	PRDX2_PIG	420,366,344	Antioxidation
4	Heat-shock protein HSP 90α: *Bos taurus* (bovine)	HSA90A_BOVIN	376	Chaperone
5, 6	Proliferating cell nuclear antigen: *B. taurus* (bovine)	PCNA_BOVIN	214,278	DNA replication
7	Ubiquitin thioesterase OTUB1: *Homo sapiens* (human)	OTUB1_HUMAN	102	Ubiquitin enzyme
8, 9	Protein DJ-1: *B. taurus* (bovine)	PARK_BOVIN	33,144	Chaperone, oncogene
10	Heat shock protein β1: *B. taurus* (bovine)	HSPB1_BOVIN	72	Chaperone
11	14-3-3 protein ζ/δ: *B. taurus* (bovine)	1433Z_BOVIN	94	Oncogene
12, 13	Ubiquitin carboxyl-terminal hydrolase isozyme L1: *S. scrofa* (pig)	UCHL1_PIG	224,76	Ubiquitin enzyme
14	Heat-shock cognate 71-kDa protein: *Equus caballus* (horse)	HSP7C_HORSE	117	Chaperone
	Protein-arginine deiminase type-6: *H. sapiens* (human)	PADI6_HUMAN	63	Arginine deiminase
15	Medium-chain specific acyl-CoA dehydrogenase, mitochondrial precursor: *S. scrofa* (pig)	ACADM_PIG	107	Mitochondria
16	Peroxiredoxin-4: *B. taurus* (bovine)	PRDX4_BOVIN	47	Antioxidation
17	Thioredoxin-dependent peroxide reductase, mitochondrial precursor: *B. taurus* (bovine)	PRDX3_BOVIN	121	Mitochondria
18	Polymerase δ-interacting protein 2: *H. sapiens* (human)	PDIP2_HUMAN	87	DNA replication
19	Alcohol dehydrogenase [NADP+]: *S. scrofa* (pig)	AK1A1_PIG	112	Metabolism
20	DNA replication complex GINS protein PSF1: *B. taurus* (bovine)	PSF1_BOVIN	67	DNA replication
21	Uncharacterized protein C6orf118: *H. sapiens* (human)		75	Others
22	α-Enolase: *H. sapiens* (human)	ENOA_HUMAN	74	Glycolytic enzyme
23	Actin, aortic smooth muscle: *B. taurus* (bovine)	ACTA_BOVIN	37	Cytoskeleton
24	Lamin-A/C: *S. scrofa* (pig)	LMNA_PIG	230	Nuclear membrane
25	PREDICTED: similar to Nitrilase family, member 2 (*Pan troglodytes*)	gi|114588194	105	Putative tumor suppressor
26	Vimentin: *H. sapiens* (human)	VIME_HUMAN	229	Cytoskeleton
27	DnaJ homolog subfamily B member 11 precursor: *Canis familiaris* (dog)	DJB11_CANFA	213	Chaperone
28–30	Galactokinase: *B. taurus* (bovine)	GALK1_BOVIN	102,130,177	Metabolism
31	Spermine synthase: *B. taurus* (bovine)	SPSY_BOVIN	300	Metabolism

*See Fig. S2.
†Mascot protein score (Mascot PS) was calculated by MASCOT MS/MS Ion search.

Table S2. Primer, siRNA, and oligonucleotide sequences

Gene	Forward primer (5′–3′)	Reverse primer (5′–3′)
Gene expression analysis (pig genes)		
ACTB	GCCCATCTACGAGGGGTACG	CACGTCGCACTTCATGATCG
DJ-1	CGAGGTTCAGTTAAGTCTTCAGA	CGGGAGGGTTCCGGGCAGCCCGCTCG
MDM2	AATGAATCCTCCCCTTCCAC	TTGGGATAGGAAGGCTTGTG
P53	CCTCACCATCATCACACTGG	GGCTTCTTCTTTTGCACTGG
Cloning of pig DJ-1		
Pig DJ-1 cloning 1	CGGAATTCCGAGGTTCAGTTAAGTCTTCAGA	CGGGATCCCGGGAGGGTTCCGGGCAGCCCGCTCG
Pig DJ-1 cloning 2	CGGGGTACCCCGAGGTTCAGTTAAGTCTTCAGA	CCCATCGATGGGAGGGTTCCGGGCAGCCCGCTCG
Pig DJ-1 L166P	CTTCGAGTTTGCTCCGGCCATTGTTGAGG	CTCAACAATGGCCGGAGCAAACTCGAA
siRNA against pig DJ-1		
Sense	GAAGCAAAGUUACGACGCAUU	
Antisense	UUCUUCGUUUCAAUGCUGCGU	
Oligonucleotide against pig DJ-1		
DJ-1 sense	CAGAAAATACAACATAAGAATGGCT	
DJ-1 antisense	AGCCATTCTTATGTTGTATTTTCTG	