Supporting Information

Successful genetic modification of porcine spermatogonial stem cells via an electrically responsive Au nanowire injector

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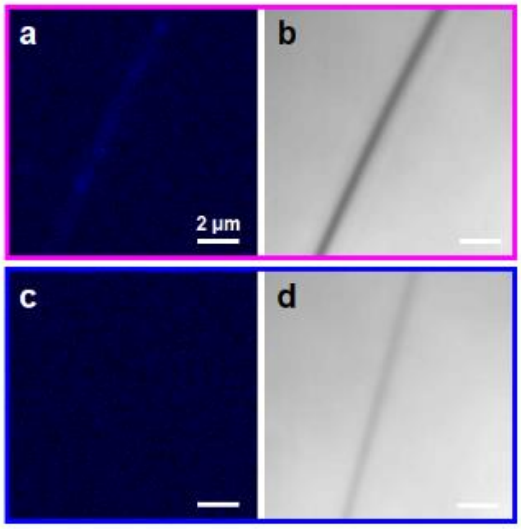

Fig. S1. (a) Fluorescence and (b) optical images of plasmid-attached E-R Au NWI after DAPI intercalation. Blue fluorescence of DAPI indicates that the plasmids were successfully loaded on the E-R Au NWI. (c) Fluorescence and (d) optical images of CA-modified E-R Au NWI (without plasmids) after DAPI intercalation. No fluorescence signals were observed. The scale bars indicates 2 μm.

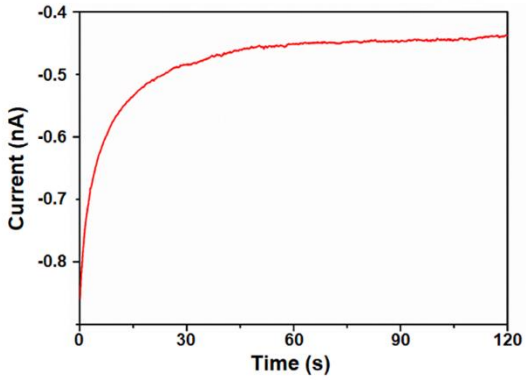

Fig. S2. Amperometric i-t curve during plasmid delivery into a pSSC via E-R Au NWI.

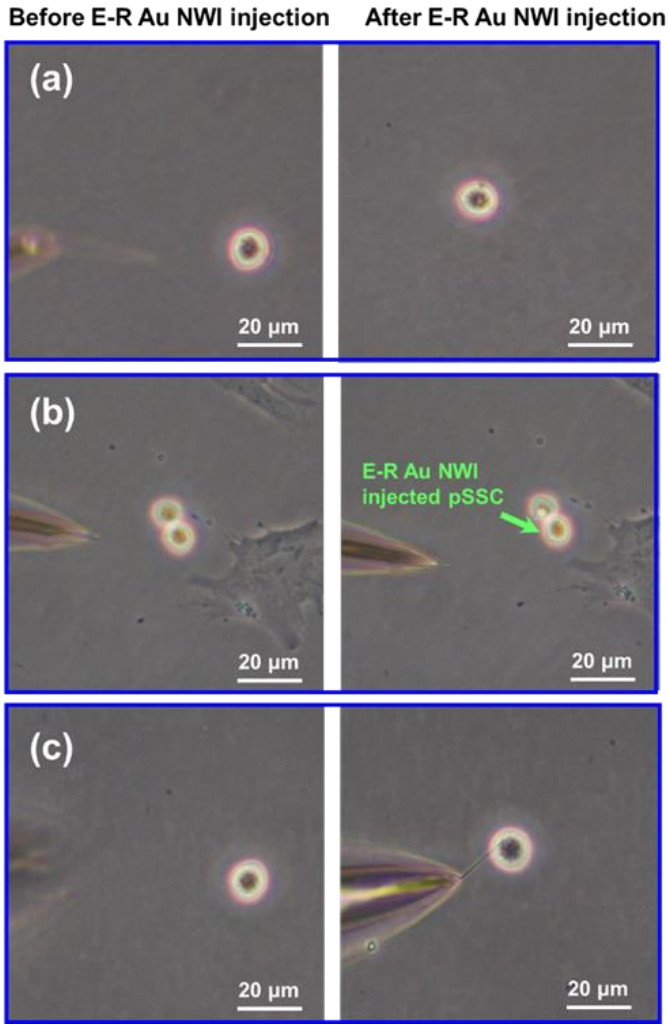

Fig. S3. Optical images of pSSCs before and after E-R Au NWI injection and DNA delivery. (a-c) Each image shows that E-R Au NWIs with diameter of less than 300 nm were used for introducing DNA into the nucleus of pSSCs and rarely induce cell damages on pSSCs. The scale bars indicate 20 μm.

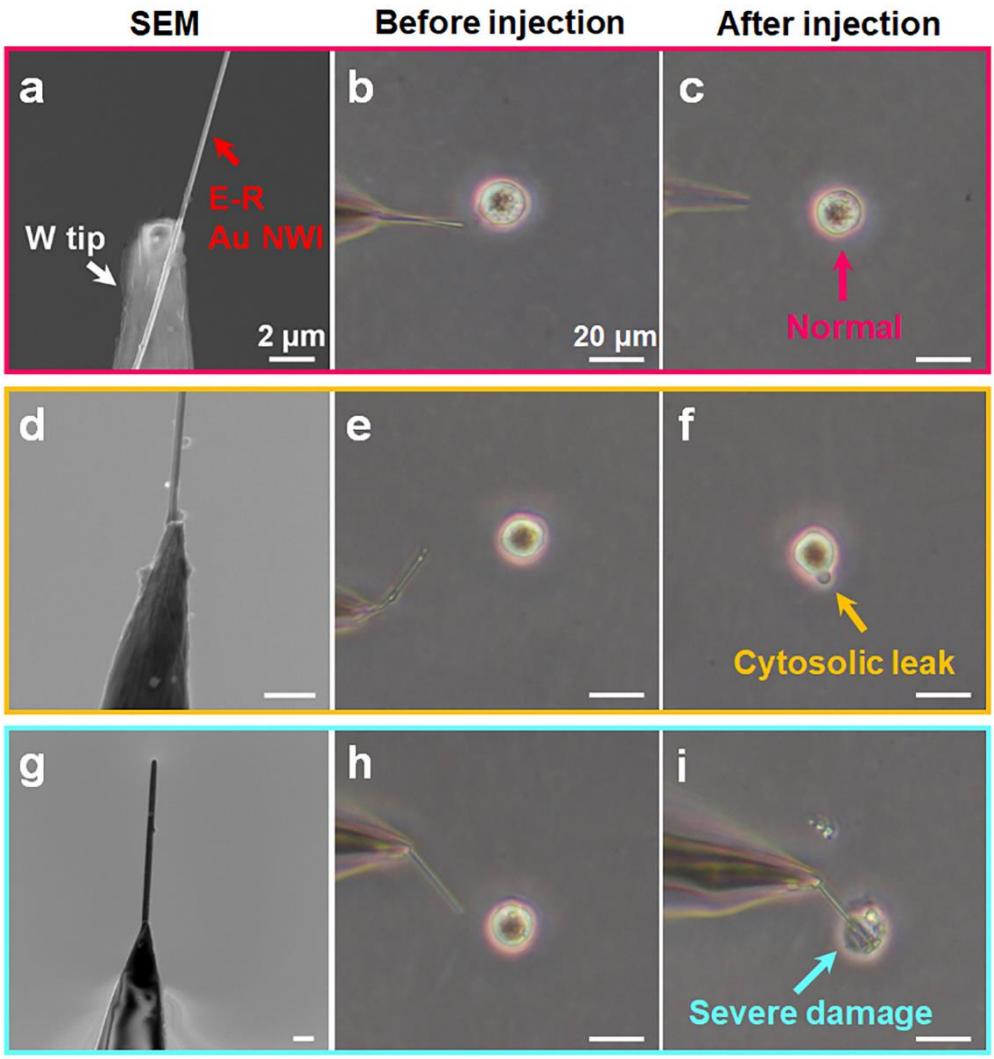

Fig. S4. (a) SEM image of E-R Au NWI (diameter of 278 nm) and optical images of a pSSC (b) before and (c) after the E-R Au NWI injection. The cell was viable, and no damage was observed after injection and the application of an electrical stimulus for 2 min. (d) SEM image of E-R Au NWI (diameter of 325 nm) and optical images of a pSSC (e) before and (f) after the E-R Au NWI injection. A cytosolic leak occurred in the cell after injection and the application of an electrical stimulus for 1 min. (g) SEM image of E-R Au NWI (diameter of 594 nm) and optical images of a pSSC (h) before and (i) after the E-R Au NWI injection. The thick E-R Au NWI resulted in severe damage to the cell after injection and the application of an electrical stimulus for 30 s. The scale bars in SEM images are 2 μm and the scale bars in all optical images are 20 μm.

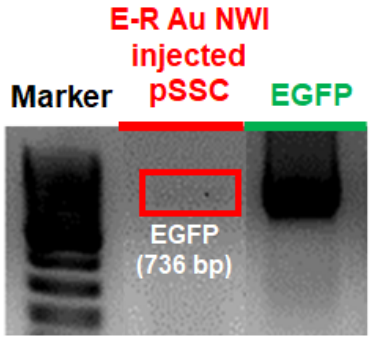

Fig. S5. PCR and gel electrophoresis analysis of pSSC after plasmid delivery using a rather thick (> 300 nm) E-R Au NWI. The EGFP fragment was observed even in the pSSC with a slight cytosol leak, suggesting that pSSCs can spontaneously recover from a slight cytosol leak.

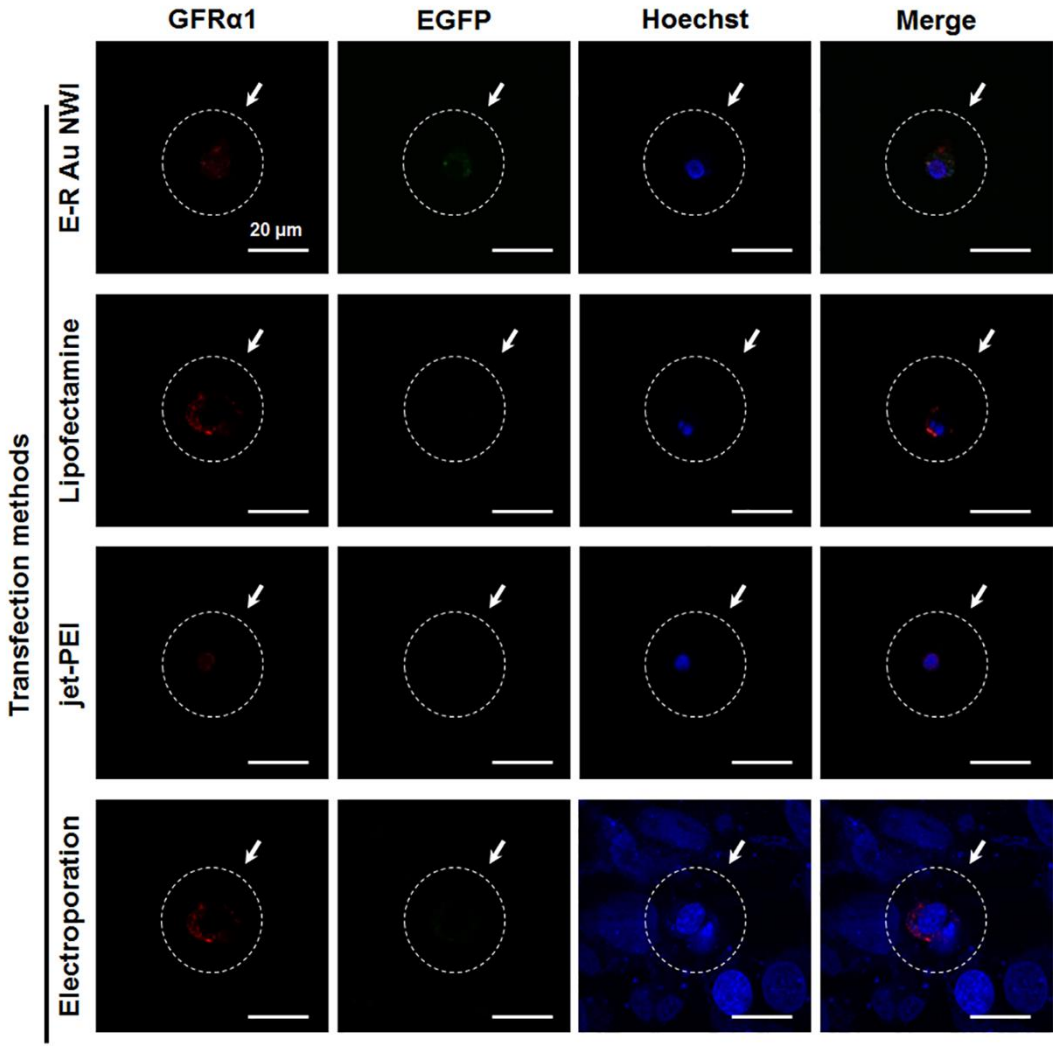

Fig. S6. Confocal microscope images of pSSCs transfected through Lpofectamine, jetPEI, electroporation and E-R Au NWI-based gene delivery system. Blue fluorescence (arrow) from DNA staining with Hoechst33342 was observed in the nucleus of pSSCs (dotted circle) and green fluorescence derived from EGFP was observed in the cytoplasm of pSSCs (dotted circle). The scale bars indicate 10 μm.

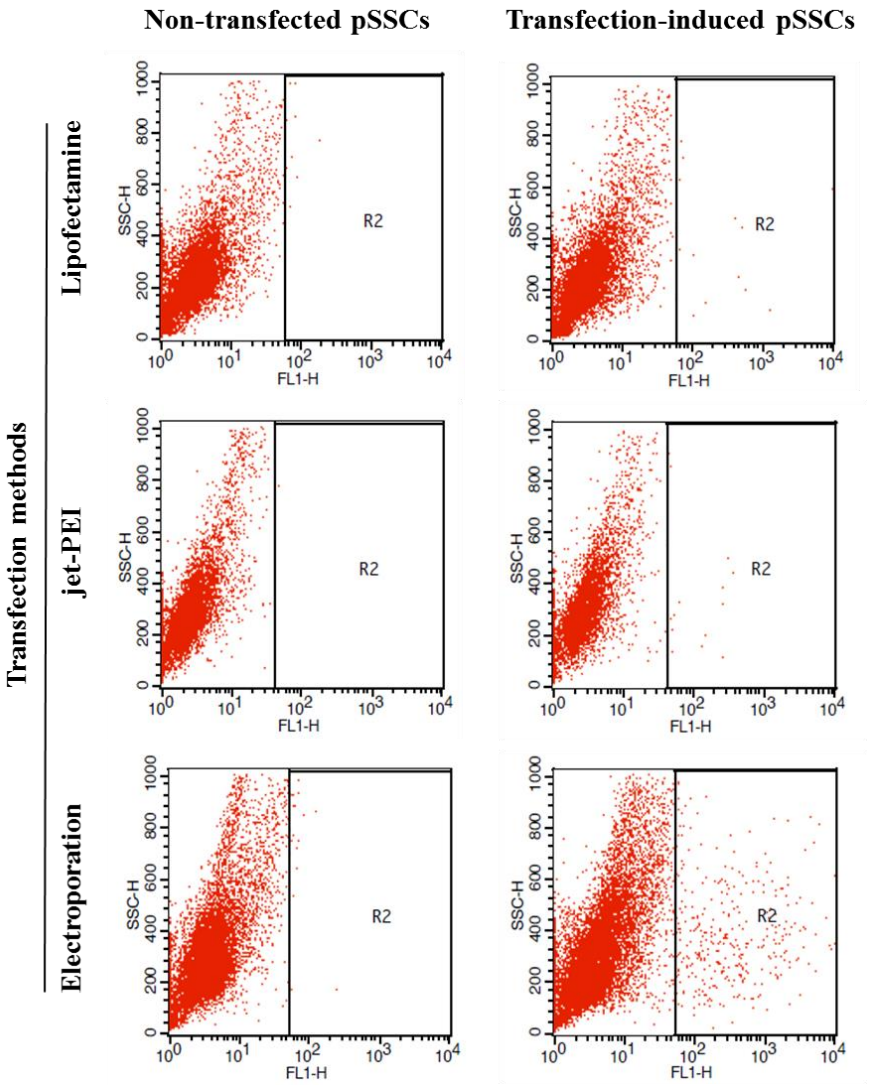

Fig. S7. Scatter plot of pSSCs transfected through Lipofectamine-, jetPEI-, and electroporation-based gene delivery systems. A day after transfection, transfection efficiency were measured by flow cytometry. Region 2 (R2) represents the pSSC population expressing EGFP.

Table S1. List of oligonucleotide primers and PCR cycling conditions.

Genes	GenBank number	Primer sequence		Size (bp)	Temp[a] (°C)	Application
		Sense (5′ >3′)	Antisense (5′ >3′)			
EGFP	U55762.1	AGCAAGGGCGAGGAGCTGTT	TGTACAGCTCGTCCATGCCG	736	58	mRNA detection
		GCGACGTAAACGGCCACAAG	GGGGTAGCGGCTGAAGCACT	167	60	Identification of target genes integrated into gDNA
hBMP2	M22489.1	TAGCGTTGCTGCTTCCCCAG	GCTGGGGGTGGGTCTCTGTT	185	60	Identification of target genes integrated into gDNA
GAPDH	NM_001206359.1	GATGGTGATGGCCTTTCCATTG	AGGGCTGCTTTTAACTCTGGCAA	180	60	mRNA detection and identification of target genes integrated into gDNA

[a]Temp =Temperature

Table S2. Effects of E-R Au NWI diameters on morphological normality and transfection efficiency of pSSCs after transfection.

Diameters (D, nm) of E-R Au NWI	No. of pSSCs transfected by E-R Au NWI	No. (%)[a] of pSSCs without abnormal morphological change after transfection	No. (%)[a] of successfully transfected pSSCs
D < 300	25	21 (84.0)[b]	14 (56.0)[b]
300 ≤ D < 500	22	1 (4.5)[c]	3 (13.6)[c]
D ≥ 500	20	0 (0)[c]	1 (0.5)[c]

Model effect of treatments in each parameter, which is indicated as the *p* value, was < 0.0001 and <0.0001 in the percentage of pSSCs without abnormal morphological change after transfection and the percentage of successfully transfected pSSCs.

[a]Percentage of number of pSSCs transfected by E-R Au NWI.

[bc]Different subscripts within a column are significantly different, $p < 0.0005$.

Table S3. Information on cellular morphology and transfection success after transfection of pSSCs using E-R Au NWI with different diameters.

Trials	Diameters (D, nm) of E-R Au NWI	Cellular morphology after transfection	Successes (O) or failures (X) for pSSC transfection
1	D < 300	Normal*	X
2	D < 300	Normal*	X
3	D < 300	Normal*	O
4	D < 300	Normal*	O
5	D < 300	Normal*	O
6	D < 300	Normal*	O
7	D < 300	Normal*	O
8	D < 300	Normal*	O
9	D < 300	Normal*	O
10	D < 300	Normal*	O
11	D < 300	Normal*	X
12	D < 300	Normal*	O
13	D < 300	Normal*	O
14	D < 300	Normal*	X
15	D < 300	Normal*	X
16	D < 300	Normal*	X
17	D < 300	Normal*	O
18	D < 300	Normal*	O
19	D < 300	Normal*	O
20	D < 300	Normal*	X
21	D < 300	Cytosolic leak‡	O
22	D < 300	Cytosolic leak‡	X
23	D < 300	Normal*	X
24	D < 300	Cytosolic leak‡	X
25	D < 300	Cytosolic leak‡	X
26	300 ≤ D < 500	Cytosolic leak‡	O
27	300 ≤ D < 500	Cytosolic leak‡	O
28	300 ≤ D < 500	Cytosolic leak‡	X
29	300 ≤ D < 500	Normal*	X
30	300 ≤ D < 500	Cytosolic leak‡	X
31	300 ≤ D < 500	Cytosolic leak‡	X
32	300 ≤ D < 500	Cytosolic leak‡	X
33	300 ≤ D < 500	Cytosolic leak‡	X
34	300 ≤ D < 500	Cytosolic leak‡	O
35	300 ≤ D < 500	Cytosolic leak‡	X
36	300 ≤ D < 500	Severe damaged§	X
37	300 ≤ D < 500	Severe damaged§	X
38	300 ≤ D < 500	Cytosolic leak‡	X
39	300 ≤ D < 500	Severe damaged§	X
40	300 ≤ D < 500	Cytosolic leak‡	X
41	300 ≤ D < 500	Cytosolic leak‡	X
42	300 ≤ D < 500	Severe damaged§	X
43	300 ≤ D < 500	Cytosolic leak‡	X
44	300 ≤ D < 500	Severe damaged§	X
45	300 ≤ D < 500	Cytosolic leak‡	X
46	300 ≤ D < 500	Cytosolic leak‡	X
47	300 ≤ D < 500	Cytosolic leak‡	X
48	D ≥ 500	Severe damaged§	X
49	D ≥ 500	Severe damaged§	X
50	D ≥ 500	Severe damaged§	X
51	D ≥ 500	Severe damaged§	X
52	D ≥ 500	Severe damaged§	O

53	D ≥ 500	Severe damaged[§]	X
54	D ≥ 500	Severe damaged[§]	X
55	D ≥ 500	Severe damaged[§]	X
56	D ≥ 500	Severe damaged[§]	X
57	D ≥ 500	Severe damaged[§]	X
58	D ≥ 500	Severe damaged[§]	X
59	D ≥ 500	Severe damaged[§]	X
60	D ≥ 500	Severe damaged[§]	X
61	D ≥ 500	Severe damaged[§]	X
62	D ≥ 500	Severe damaged[§]	X
63	D ≥ 500	Severe damaged[§]	X
64	D ≥ 500	Severe damaged[§]	X
65	D ≥ 500	Cytosolic leak[‡]	X
66	D ≥ 500	Severe damaged[§]	X
67	D ≥ 500	Cytosolic leak[‡]	X

*Success or failure for transfection of pSSCs was determined by detection of EGFP gene expression in pSSCs after 1 day from E-R Au NWI based gene delivery.
[‡]'Cytosolic leak' means pSSCs with extruded cytosol following physical stimulus derived from E-R Au NWI based gene delivery.
[§]'Severe damaged' means pSSCs with abnormal morphologies such as shrinking or bursting following physical stimulus derived from E-R Au NWI based gene delivery.
[*]'Normal' means pSSCs without 'cytosolic leak' and 'severe damaged'.
Cell morphology was observed under inverted microscope.