Supporting Information

Electro-triggered, Spatioselective, Quantitative Gene Delivery into a Single Cell Nucleus by Au Nanowire Nanoinjector

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Instruments. Au nanowire (NW) nanoinjector system consists of an optical microscope, three-dimensional piezoelectric stage (Sigma Koki) and an electrochemical workstation (CHI 660D). SEM images of Au NW nanoinjectors were taken on a Philips XL30S. Transmission electron microscope (TEM) and high-resolution TEM (HRTEM) images and selected area electron diffraction (SAED) patterns of a Au NW were taken on a JEOL JEM-2100F TEM operated at 200 kV. The fluorescence images of Au NWs and cells were obtained by using a laser scanning confocal microscope (LSM 510—Mets NLU, Carl Zeiss, Oberkochen, Germany) with excitation and emission wavelength filter (ex/em 494/530 nm for plasmid harboring cell, ex/em 528/595 nm for Cy3 DNA attached NW).

Subcellular insertion of a Au NW nanoinjector. Since Au NWs are 100 ~ 150 nm in diameter and mounted on a piezoelectric stage which controls the nanoinjectors with high spatial precision (< 200 nm), the Au NW nanoinjector could be inserted at subcellular level into a cell of which dimension is tens of micrometer.[1,2] Figure S1 shows the injection process of a Au NW nanoinjector into the cytoplasm (a-c) and nucleus (d-f) of SK-N-SH cells grown on a gelatin-coated glass slide. When the Au NW nanoinjector touched a nuclear membrane and penetrated into a nucleus, a small dent appeared in the nuclear membrane (Figure S1e), and then Au NW nanoinjector deeply penetrated into a nucleus. Since the Au NW was kept parallel to the substrate plane, it was laid on the focal plane, and was thus observed clearly and evenly during injection through an optical microscope. After ejecting the Au NW from a cell, the dent in nuclear membrane disappeared and the cell immediately recovered its original shape.

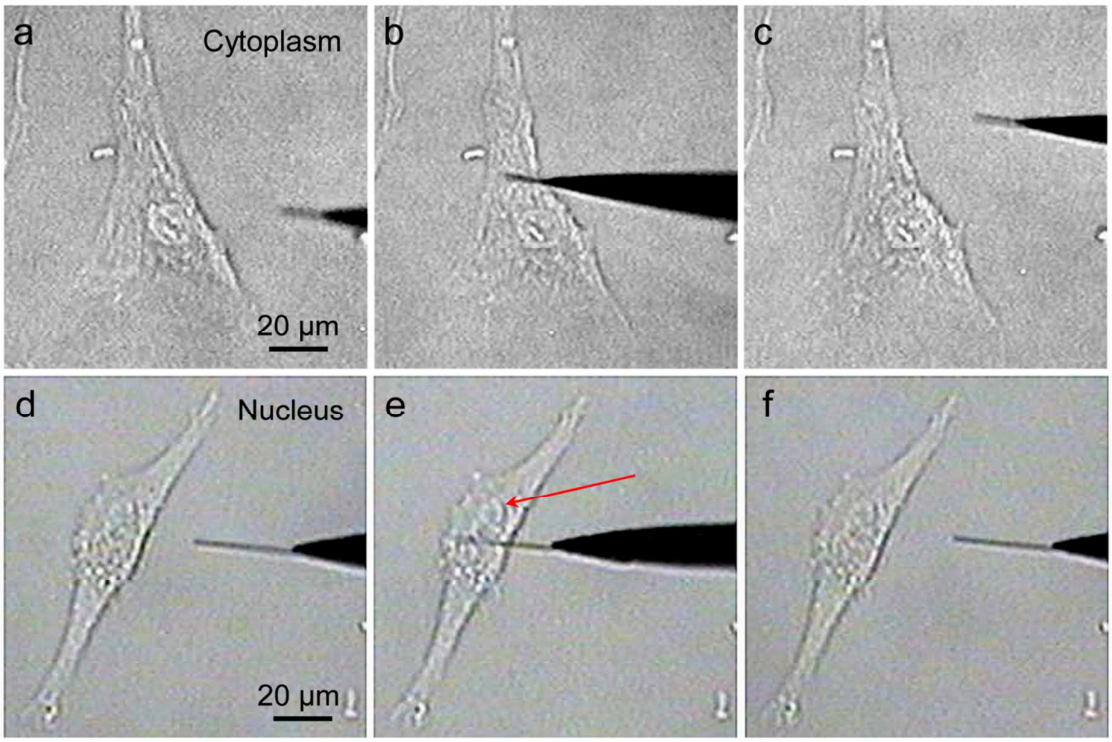

Figure S1. Optical images of SK-N-SH cells before (a,d), during (b,e), and after (c,f) insertion of Au NW nanoinjectors into the cytoplasm (a-c) or nucleus (d-f) of the cells. The red arrow in (e) denotes a nuclear membrane.

Fabrication of Au NW nanoinjectors. Au NWs were synthesized by the vapor transport method reported previously.[3] The Au slug was evaporated at 1100 °C in a horizontal furnace system. The Au vapor was carried to a c-cut sapphire substrate placed a few centimeters downstream for ~ 30 min by the flow of Ar gas at a rate of 100 sccm under the chamber pressure at ~ 10 Torr. Vertical Au NWs were grown on the substrate from the half-octahedral Au seed naturally formed on a c-cut sapphire substrate. To handle a single Au NW easily, a Au NW is attached to a conducting adhesive-coated macroscopic tungsten (W) tip mounted on a piezoelectric stage. As shown in Figure S2, the W tip was approached to a Au NW on a sapphire substrate and picked it up by softly touching. Whole part of W tip was insulated with nail varnish since the exposed W induces undesirable electrochemical reactions. The exposed Au NWs have diameters of 100 ~ 150 nm and lengths of tens of micrometers and are enclosed by the most stable {111} facets and has no twins or defects (Figure S3).

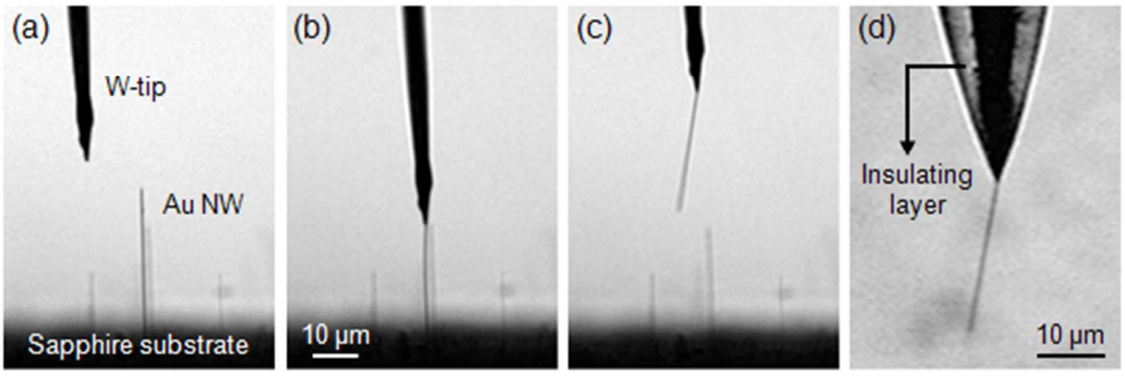

Figure S2. Optical images of the fabrication process of a Au NW nanoinjector.

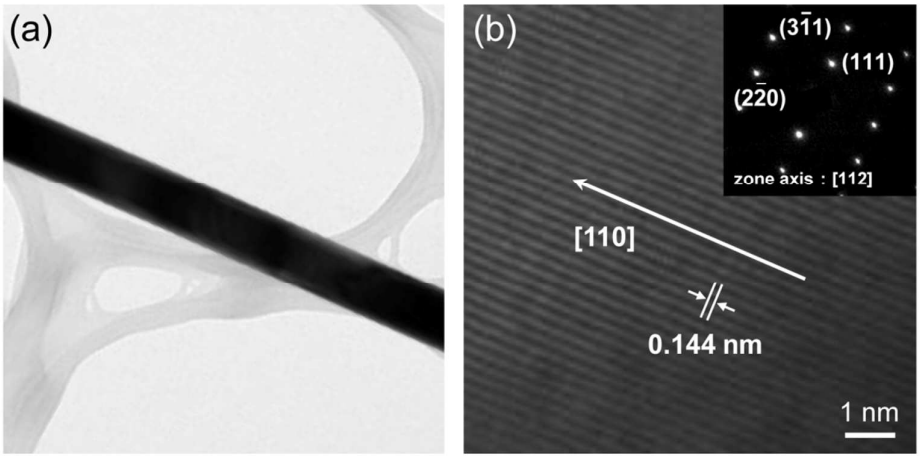

Figure S3. (a) TEM image, (b) HRTEM image and selected area electron diffraction (SAED) pattern of a Au NW, indicating that the Au NW is single-crystalline without twins or defects.

Electrochemical characteristics of Au NW nanoinjectors. Successful electric contact between a Au NW and a W tip was confirmed by electrochemical oxidation and reduction current of Au at 0.89 and 0.40 V, respectively, measured in a 50 mM sulfuric acid solution (Figure S4). These sharp peak shapes without splitting originate from well-defined single-crystalline Au (111) surfaces.[4]

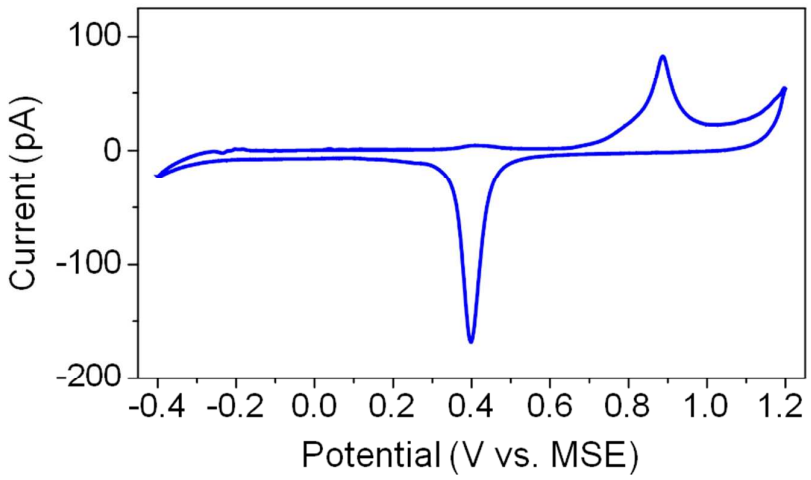

Figure S4. Cyclic voltammogram of a Au NW in a 50 mM sulfuric acid solution. Electrochemical currents for the oxidation and reduction of Au were observed at 0.89 V and 0.40 V (vs. mercury sulfate electrode (MSE)), respectively.

Mechanical characteristics of Au NW nanoinjectors. A Au NW nanoinjector was bent easily when pushed against the substrate and then recovered to its straight structure when retracted (Figure S5). The Au NW could be even bent into a U-shape (Figure S6). This indicates the Au NW nanoinjector is highly elastic and flexible, which originates from perfectly crystalline nature of a Au NW.[5]

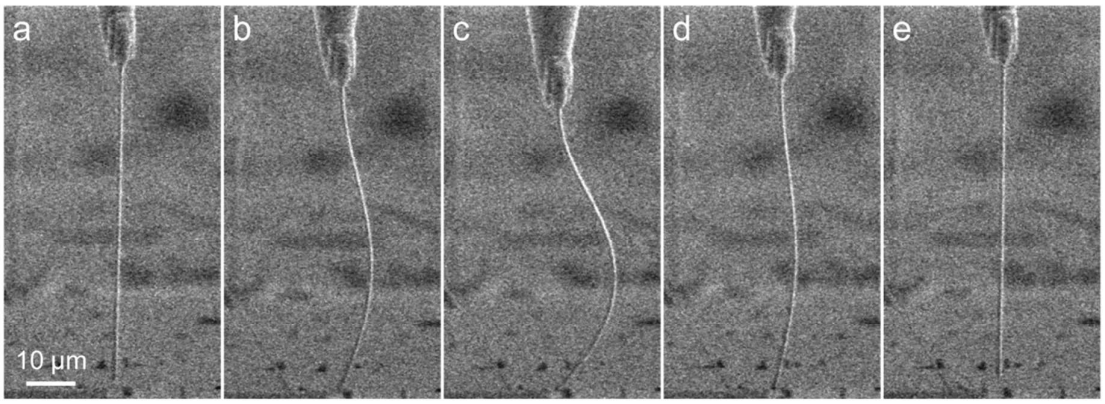

Figure S5. SEM images showing the flexible bending and recovery of a Au NW nanoinjector as it is pushed against a solid surface.

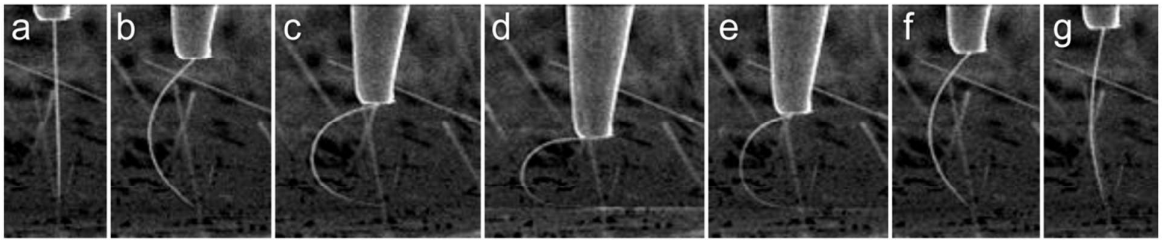

Figure S6. SEM images showing U-shape bending (a-d) and complete recovery (e-g) of a Au NW as the Au NW is pushed against a solid surface.

Preparation of a DNA-attached Au NW nanoinjector. Linear DNAs for fluorescence observation were prepared by amplifying 23S ribosomal DNA from *Neisseria gonorrhoea* (ATCC 10150). PCR was performed in 50 μl reactions containing 1× *Taq* buffer, 0.2 mM dGTP, 0.2 mM dATP, 0.2 mM dTTP, 0.125 mM dCTP, 0.05 mM dCTP-Cy3 (EBIOGEN Inc., Korea), 2 units of *Taq* polymerase (Takara Shuzo Co., Japan), 5 pM Ngo13Fw forward primer (5'-HS-$(CH_2)_6$-GCGAAGTAGAATAACGACGCATC-3'), and 5 pM MS38R reverse primer (5'-CCCGACAAGGAATTTCGCTACCTTA-3'). PCR condition was as follows; 35 cycles each consisting of first denaturation at 94°C for 4 min and second denaturation at 94°C for 30 s, annealing at 52°C for 30 s and extension at 72°C for 1 min 30 s and final extension at 72°C for 5 min in that order. To immobilize linear DNAs on a Au NW, as-prepared Au NW nanoinjector was incubated in 1 M KH_2PO_4 (pH 6.7) solution containing 100 nM thiolated linear DNAs at room temperature for 9 h and excess DNAs were washed with 0.2% (w/v) sodium dodecyl sulfate (SDS) for 5 min.

For amplification of thiolated linear DNA coding enhanced green fluorescent protein (EGFP) including CMV promoter and SV40 polyadenylation signals at 5' and 3' region of the gene, thiol-modified primer set was used.: Forward primer, 5'-HS-$(CH_2)_6$-TTACCGGATAAGGCGCAGCG-3'; Reverse primer, 5'-CGCCCTTTGACGTTGGAGTC-3'. This linear DNA fragment was attached onto NW nanoinjector in the same way mentioned above.

pEGFP-N1s, EGFP-coding plasmids, were prepared with DNA-spin^{TM} plasmid DNA extraction kit (iNtRON co., Korea) according to the manufacturer's instructions. For the attachment of pEGFP-N1 plasmids to a Au NW, first the Au NW was functionalized with cysteamine ($HSCH_2CH_2NH_2$) via Au-S bond, of which -NH_2 group is positively charged as -NH_3^+ in neural solution and therefore able to attract negatively charged plasmid.[6] A Au NW nanoinjector was incubated in a 20 mM cysteamine aqueous solution for 30 min.[7] The

cysteamine-coated Au NW nanoinejctor was then incubated in a solution of 100 nM pEGFP-N1 in 1 M KH_2PO_4 (pH 6.7) at room temperature for 9 h and excess DNA was washed with 0.2% (w/v) SDS for 5 min. This method can attach plasmid on a Au NW without any chemical modification as well as it lowers the potential to release DNA since electrostatic attraction is rather weaker than a chemical bond.[8]

Cell cultivation. SK-N-SH cell was cultivated in DMEM supplemented with penicillin (100 units/mL), streptomycin (0.1 mg/mL), and 10% FBS and maintained in a 5% CO_2, water-saturated atmosphere at 37°C. The glass slide for cell fixing was treated with 0.01% (w/v) gelatin for 30 min and then was incubated with cells in culture media.

Quantification of released target DNAs into cell. DNAs for quantification study were prepared by PCR process using 1× *Taq* buffer, 0.2 mM dNTP, 2 units of *Taq* polymerase, 5 pM Ngo13 forward primer, and 5 pM MS38R reverse primer. To obtain linear DNAs injected in cells after Au NW nanoinjector-based delivery, whole DNAs from cells were extracted with TRIZOL®-LS reagent according to the manufacturer's instructions. The quantification of intracellular target DNAs was performed by qPCR with Ngo13Fw forward primer and Ngo 3R reverse primer (5'-TTACCTACCCGTTGACTAAGTAAGC-3'). The qPCR conditions were as follows; 40 cycles of 95°C for 20 s, 52°C for 15 s, and 72°C for 20 s. The specificity of the qPCR amplification was checked with a melting curve analysis from 72°C to 95°C following the final cycle of the PCR. The absolute quantification of target DNAs was calculated as standard curve obtained by applying dilution series (1.5, 7.5, and 15 ng). The measurement of qPCR was performed by three times.

Cell viability test. Trypan blue is used as an indicator for the cell viability since it only penetrates into dead cells and stains them blue. Trypan blue stain solution (0.4%) was purchased from Life Technologies Co. To investigate whether the multiple DNA deliveries into a cell using Au NW nanoinjectors affect the cell viability, three Au NW nanoinjectors were inserted into the same cell and electric pulse (-0.8 V vs. SCE for 2 min) was applied after each insertion. Thereafter, trypan blue solution was added into the medium (0.03% in medium), and the cell was monitored for 7 hours. Neither trypan blue inclusion nor changes in cell morphology were observed during this time period (Figure S7).

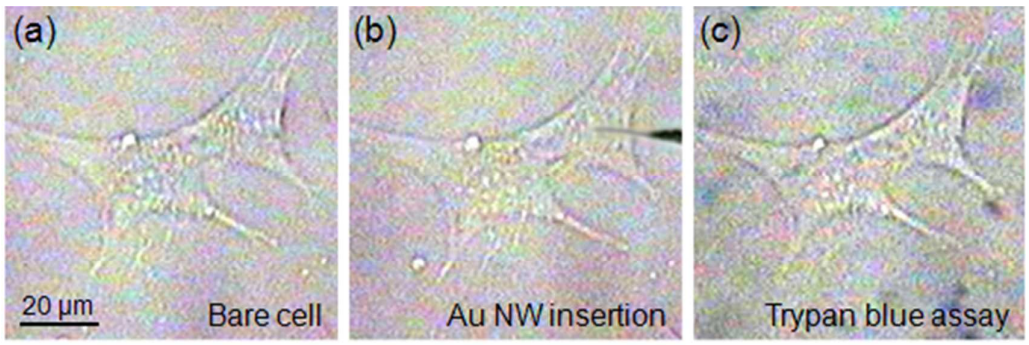

Figure S7. Optical images of the SK-N-SH cell before (a) and during (b) the electrically triggered gene delivery by a Au NW nanoinjector when we applied an electric pulse of −0.8 V for 2 min. (c) Trypan blue assay result of the cell after three rounds of the delivery. The interval of delivery was 5 min. No intranuclear trypan blue staining clearly verifies that the cell is alive after three rounds of delivery.

Delivery of plasmid DNAs into cells. To examine the successful expression of DNAs, we released pEGFP-N1 plasmid DNA into nucleus and cytoplasm by Au NW nanoinjector. For the successful expression of extracellular DNAs, DNAs should pass both a cell membrane and a nuclear membrane without any damage. The expression of DNAs in medium and cytoplasm is much more difficult than that of DNA released directly into nucleus, because DNAs must survive from both highly acidic endosome and/or cytoplasmic nuclease. As expected, distinct fluorescence signals were observed only from the cells of which the plasmid was directly injected into nucleus (Fig. 5d-f and Figure S8a-b), while no fluorescence was observed in the cells of which DNAs were released into cytoplasm (Figure S8c-h).

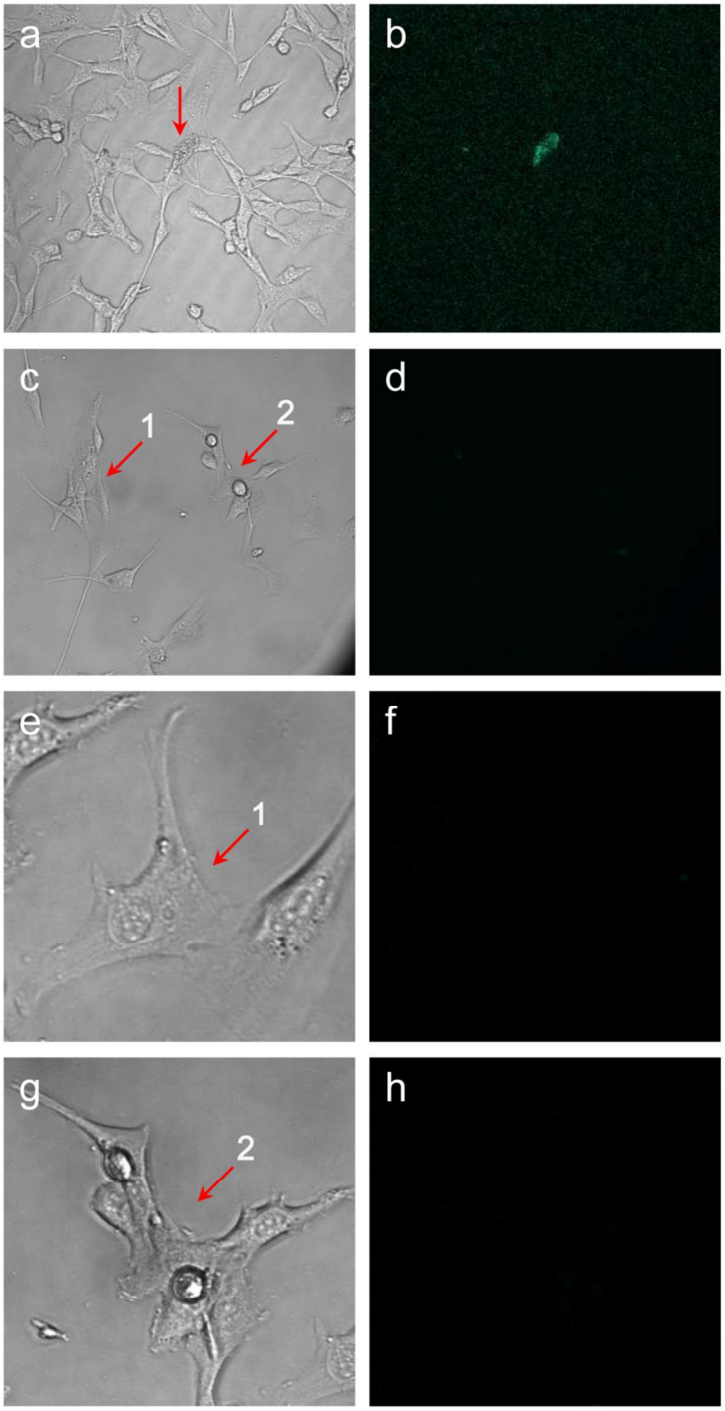

Figure S8. The images of the SK-N-SH cell after delivery of pEGFP-N1 plasmid DNAs. The cell was grown in a culture medium for 48 h after DNA delivery for GFP expression.

(a) Optical and (b) fluorescence images of the cell of which DNAs were delivered into nucleus. (c) Optical and (d) fluorescence images of the cell of which DNAs were delivered

into cytoplasm. (e) and (g) are enlarged optical images of cell 1 and 2 in (c), respectively. (f) and (h) are the fluorescence images of (e) and (g), respectively.

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