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SUPPLEMENTARY INFORMATION

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Free-standing kinked nanowire transistor probes for targeted intracellular recording in three dimensions

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Materials and Methods

Kinked nanowire synthesis. Kinked silicon nanowires with a 60° angle were synthesized by Au nanocluster catalyzed vapor-liquid-solid (VLS) method as previously described[1-4]. Specifically, gold nanoparticles with diameters 80, 100, or 150 nm (Ted Pella) were dispersed on Si substrates with 600 nm SiO_2 (Nova Electronic Materials, Flower Mound, TX). A heavily doped n-type (n^{++}) arm was first grown for 30 min using SiH_4 (1 sccm), PH_3 (4 sccm, 1000 ppm in H_2) and H_2 (60 sccm) at a total pressure of 40 torr and temperature of 460 °C. The growth was paused for 15 s by rapidly evacuating the chamber, followed by re-introducing reactant gases to generate the first 120° kink[2-4]. At this point the flux of PH_3 was reduced to 0.1 sccm (from 4 sccm) to decrease the doping level in the segment that serves as the active channel of the nanoFET. Growth of the lightly doped segment was continued for 30-60 s, and then a second 120° kink was introduced by another evacuation/reactant introduction cycle, where PH_3 flux was increased back to 4 sccm and growth was continued for 30 min to complete the other n^{++} arm of the probe. Under these growth conditions the two 120° kinks form a *cis*-geometry with high efficiency to yield an overall 60° probe tip[3,4].

Free-standing probe fabrication. Free-standing kinked nanowire probes were fabricated on a metal sacrificial layer that was deposited on a Si substrate with 600 nm SiO_2 surface layer (Nova Electronic Materials, Flower Mound, TX) using photolithography (PL) and electron-beam lithography (EBL). Key steps used in the fabrication were as follows. (1) PL and metal deposition (100 nm, Ni) were used to define an array of 5 mm x 5 mm sacrificial layer blocks, where each block is sufficiently large for fabrication of a free-standing probe end. (2) The 4.5 mm long bottom passivation layer and main body of the probes was fabricated by spin coating SU-8 2002 (2 μm, MicroChem) followed by PL, where one probe body was defined in each sacrificial layer block. (3) Contact pads and interconnects to the tip region were patterned by PL, metal evaporation and lift-off (Cr/Au, 5/200 nm) on top of the bottom SU-8 of the probe body. (4) 500 nm thick layer of SU-8 2000.5 (MicroChem) was deposited by spin coating, pre-baked at 65 °C for 2 min, and then kinked Si-nanowires dispersed in ethanol solution were deposited in the region near the end of each probe structure. (5) Dark-field optical microscopy was used to identify kinked Si-nanowires with desired orientation in each block near the tip region of the probe body, and then the chosen nanowires were immobilized on the surface by electron-beam

irradiation of the SU-8 in this region and post-baking (75 °C for 2 min) to yield a continuous SU8 passivation layer from the probe body defined by PL to the arms of the kinked nanowire. Subsequent development of the SU-8 removed all other deposited nanowires. (6) Metal contacts linking the arms of the kinked nanowires to the larger PL-defined probe interconnects were defined by EBL and metallization (Cr/Pd/Cr, 1.5/120/60 nm) on the SU-8. (7) Another 500 nm thick SU-8 layer was deposited and patterned by EBL to passivate the metal contacts to the kinked nanowire arms while leaving the kinked nanoprobe exposed. (8) A 50 μm thick layer of low stress SU-8 with SiO_2 nanoparticles (GLM 2060, Gersteltec) was patterned by PL as top passivation layer for the 4.5 mm long main body of the probe, and then post-baked at 57 °C for 3 h, followed by slow cooling to reduce internal stress, before development and final hard baking[5]. (9) Last, 300 and 500 nm thick layers of LOR 3A and S1805 (MicroChem) were deposited by spin-coating on the surface, and a small region covering the nanowire tip end was defined by PL. (10) The probe was released from the substrate by etching the Ni layer (40% $FeCl_3$:39% HCl:H_2O=1:1:20) in a small petri-dish, and then the solution was exchanged with deionized (DI) water. (11) The resulting probe body was manually removed from the solution with tweezers, and excess water was removed on absorbent material (Kimwipes, Kimberly-Clark). Then the probe was aligned and glued (Devcon 5-minute epoxy, ITW) to a printed circuit board connector (PCB_A16, NeuroNexus) on top of a 4 mm long, 15 μm thick silicon lever (raw A-style probe, NeuroNexus). The position of the probe was adjusted such that the nanowire probe extends beyond the Si support lever. (12) The metal pads on the probe and printed circuit board connector were electrically-connected using silver epoxy (E415G, Epoxy Technology), cured at 55 °C for 3-4 h, and subsequently passivated with silicone elastomer (Kwik-Sil adhesive, World Precision Instruments). Since 12 probe-end structures on a single substrate can be fabricated in ca. 6 days, it is possible to make 12 complete probes in ~7 days and satisfy research requirements.

Cardiomyocyte culture. Cardiac cells were isolated from intact ventricles of 1 to 4-day old neonatal Sprague/Dawley rats using 3–4 cycles (30 min each) of enzyme digestion using collagenase type II and pancreatin as previously described[6]. The cells were suspended in culture medium, composed of Medium-199 (Invitrogen, Grand Island, NY) supplemented with 0.6 mM $CuSO_4 \cdot 5H_2O$, 0.5 mM $ZnSO_4 \cdot 7H_2O$, 1.5 mM vitamin B12, 500 U ml^{-1} penicillin, 100 mg ml^{-1}

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streptomycin and 5 vol.% fetal bovine serum (FBS). The cardiac cells were seeded onto fibronectin/gelatin coated glass cover-slips at an initial cell density of 0.5-2×10^5 cm^{-2}. The culture was maintained at 37 °C with 5% CO_2 with the first full medium change in 24 hours, and following change of 50% medium every other day. Electrical measurements were conducted after 3-4 days of culture. All animal procedures conformed to US National Institutes of Health guidelines and were approved by Harvard University's Animal Care and Use Committee.

Patch-clamp recording. Patch pipettes were pulled from 1.5 mm diameter boron glass tubes (P-97 Flaming/Brown Micropipette Puller, Sutter Instruments). Pipettes were filled with intracellular medium (in mM, potassium gluconate 125, KCl 20, $CaCl_2$ 0.5, $MgCl_2$ 2, HEPES 10, EGTA 5, Na_2-ATP 2, pH 7.3), and the resistance was measured with 5 mV pulses to be ~7 MΩ. After a giga-seal was formed, the pipette was held at -70 mV before breaking the membrane to form a whole-cell patch. The data were recorded in current-clamp mode by setting I=0 with sampling rate of 20 KHz using Digi1440A and Clampex 10 software (MDS).

Intracellular recording. A free-standing nanowire probe was first exposed in ultraviolet light (430 nm, 120 s) to sensitize the photoresist protection layer before it was mounted on a micromanipulator (MP285, Sutter Instrument) for control during experiments. The probe tip was then immersed in DI water in a petri-dish, followed by developer solution (MF-CD-26, MicroChem), which dissolved the protection on the tip. The solution was changed to 1x phosphate buffer solution (PBS) for 1 h, and then incubated for 2 h with 1 mg/mL lipid vesicles of 1,2-dimyristoyl-sn-glycero-3-phospho- choline (DMPC, Avanti Polar Lipids Inc.) containing 1% 1-myristoyl- 2-{12-[(7-nitro-2−1,3-benzoxadiazol-4-yl) amino] dodecanoyl}-sn- glycero-3-phosphocholine (NBD-lipid, Avanti Polar Lipids Inc.) as fluorescent reporter. As described previously, this latter step yields lipid layers on the nanowire surface[3]. Last, the solution was changed to Tyrode solution (in mM, NaCl 138, KCl 4, $CaCl_2$ 2, $MgCl_2$ 1, Na_2HPO_4 0.33, HEPES 10, glucose 10, pH 7.4), and cultured cardiomyocyte cell samples were introduced at 35 °C. A fixed 0.1 V voltage was applied between the source and drain of the probe using a battery source. The resulting current was input to a current/voltage preamplifier (Model 1211, DL Instruments) at sensitivity of 10^{-6} A/V, low-pass filtered (0−3kHz, CyberAmp 380, Molecular Devices) and digitized at 20 kHz sampling rate (Axon Digi1440A, Molecular Devices). A Ag/AgCl reference

electrode was used to fix the extracellular solution potential at a constant value of 0 V in all recording experiments. With freshly made lipid solution (used within 1 hour), we have achieved >90% success rate of intracellular recording with lipid coated nanoFET probe (N=25).

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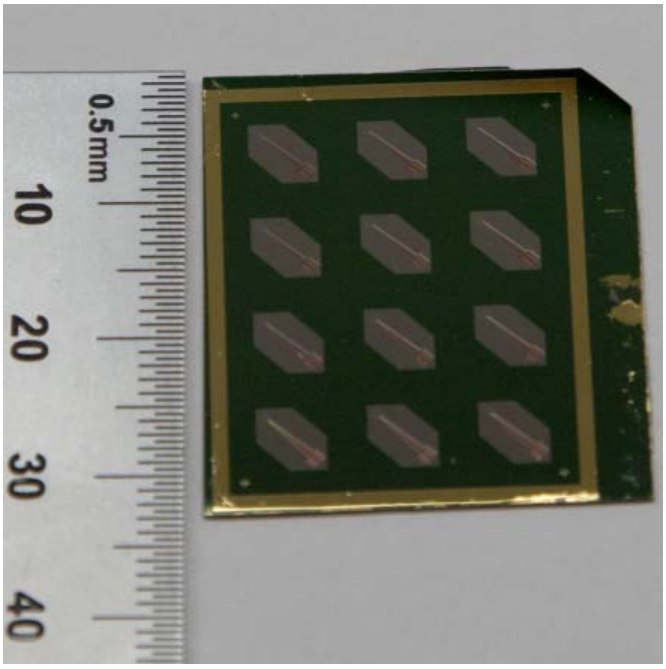

Figure S1. Parallel multi-probe fabrication. A 3 x 4 array of kinked nanowire probes fabricated in a single batch; the image was recorded prior to release and attachment of free-standing probe to the printed circuit board connector. A larger number of probes could be batch fabricated using larger substrates, where the size limitation in this demonstration was determined by the EBL instrument (JEOL JSM-7000F) sample chamber.

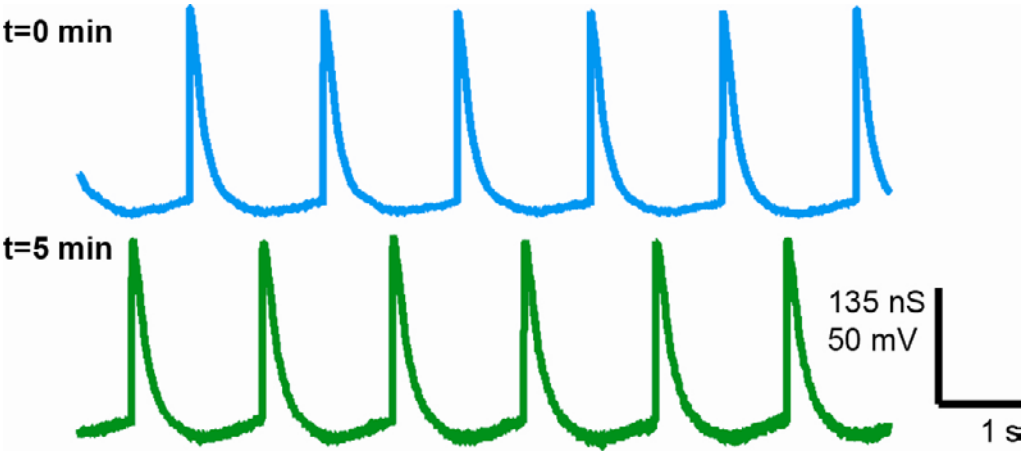

Figure S2. Stability of nanoFET intracellular recording. Initial trace (cyan; time, t = 0 min) and a subsequent trace (green) after 5 min of continuous measurement from the same beating cardiomyocyte cell. The t = 0 min trace corresponds to ca. 1min following internalization of the nanoFET into the cell. The amplitude (mV)/frequency (Hz) of the action potentials in the t = 0 and 5 min traces are 85/0.6 and 83/0.6, respectively. The results demonstrate highly stable action potential recording using the nanoFET probes, and the absence of apparent leakage on the 5 min time scale, even for beating cells. In addition, these results are consistent with the average stable recording time, 5.4 min (N = 10), and are substantially longer than the average stable recording time we could achieve with the patch-clamp on the same culture of cells and manipulator setup, 2.4 min (N = 11).

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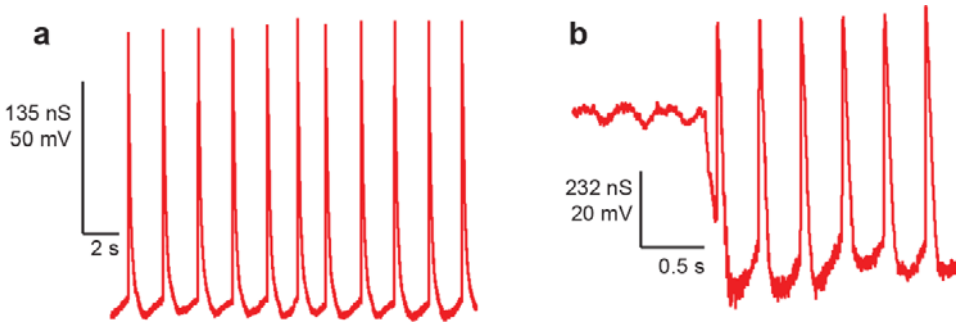

Figure S3. Additional examples of full amplitude action potentials and resting potential recorded by nanoFET probes. a. Stable action potential signals from beating cardiomyocyte with amplitude of 90 mV. **b.** Baseline shift corresponding to a resting potential of -47 mV, with action potential amplitude of 70 mV. The data shown in **a** and **b** were recorded in separate experiments using distinct nanoFET probes and cells (both DIV3). Other experimental details are the same as described in the Methods.

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