Supplementary Information for

Electrochemical evaluation of sarcomeric α-actinin in embryoid bodies after gene silencing using an LSI-based amperometric sensor array.

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Bio-LSI chip device

The Bio-LSI chip consisted of 400 electrochemical sensors. The surface of the LSI was modified with Au and insulated with SU-8 3005 to prepare 50 µm diameter Au disk microelectrodes. The distance between the centers of the sensors was 250 µm. Potential stepping and data acquisition were conducted using a Bio-LSI control system.[1,2]

Calibration curve for HRP

A calibration curve for HRP (Wako Pure Chemical Industry LTD., Japan) was generated. HRP solution (0, 0.02, 0.1, 0.2 and 1 µg/ml) containing 1.0 mM $FcCH_2OH$, 5.0 mM H_2O_2 and 0.1 M KCl, was introduced onto the Bio-LSI chip. The detection of HRP is shown schematically in Fig. 1. Briefly, HRP catalyzed the oxidation of $FcCH_2OH$ to $FcCH_2OH^+$ using H_2O_2. The enzymatically generated $FcCH_2OH^+$ was reduced back to $FcCH_2OH$ at the electrode (0.05 V vs. Ag/AgCl). The responses at all sensor points were continuously monitored, and the currents were acquired 40 s after the potential step (Fig. S1).

Gene silencing with siRNA-WNT5a

In order to investigate the effect of WNT5a gene silencing, EBs (400 cells/drop, 5 days) were formed following siRNA-WNT5a transfection. WNT5a plays a key role in vascularization in small EBs. Silencing this gene leads to EBs differentiating into cardiomyocytes with elevated expression of WNT11 which is somehow suppressed in case of high Wnt5a expression. The sarcomeric α-actinin content of EBs was assessed and compared to control EBs. Electrochemical assessment was conducted on the basis of immunocytochemical staining with HRP-labeled secondary antibodies (Fig. S2). The current from the WNT5a-silenced EBs was significantly higher than that from EBs that were not gene silenced (Fig. S2). The results show that WNT5a gene silencing induced EBs to differentiate into cardiomyocytes. Silencing the gene led to a significantly higher cardiomyocyte differentiation, indicating the complex mechanism by which WNT5a directs the fate of cells.

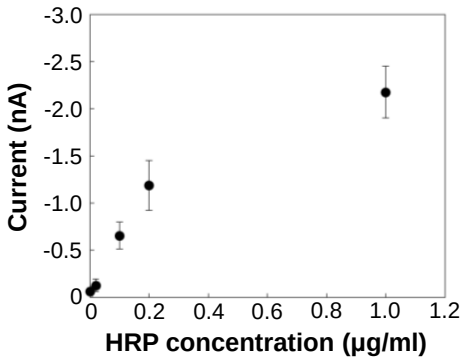

Fig. S1 Dependence of the electrochemical signals on the HRP concentration (0, 0.02, 0.1, 0.2 and 1 µg/ml). The signal was detected at the electrodes 40 s after the potential step to 0.05 V. Data points represent means ± SD of 400 independent sensors.

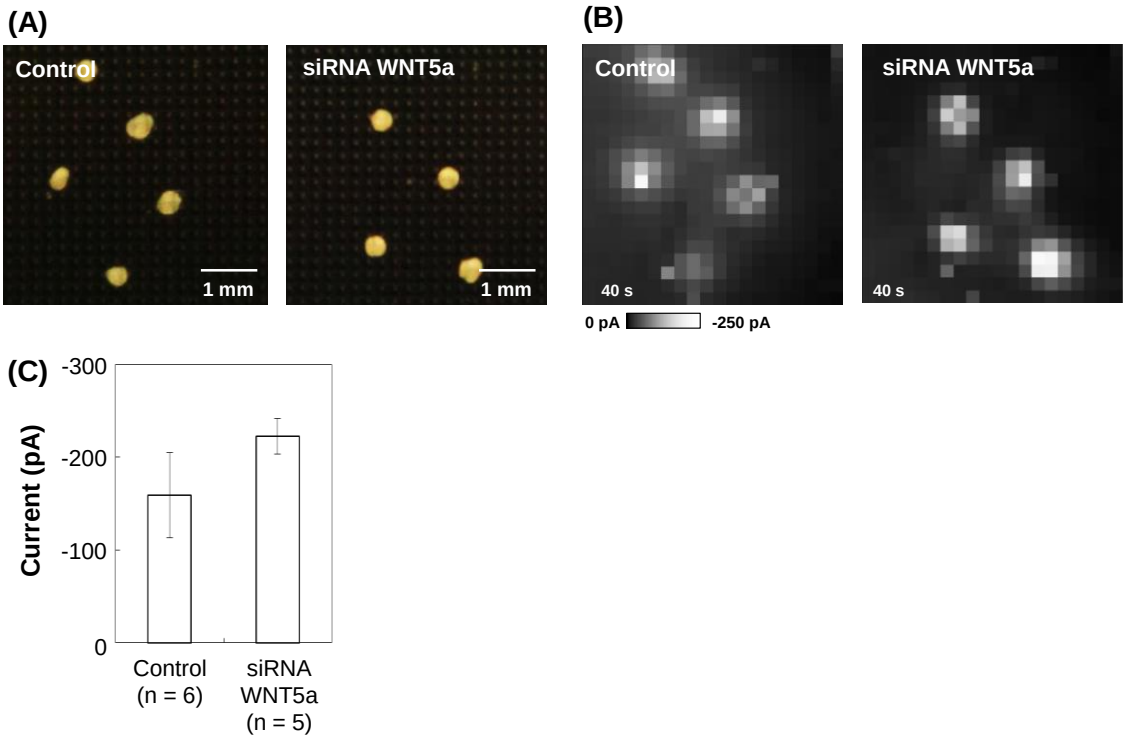

Fig. S2 Electrochemical analysis based on WNT5a gene silencing. Optical (A) and electrochemical (B) images were obtained through immunocytochemical staining of sarcomeric α-actinin in EBs using HRP-conjugated secondary antibody. (C) The currents from the EBs were plotted on the graph.

References

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2. M. Şen, K. Ino, K.Y. Inoue. T. Arai, T. Nishijo, A. Suda, R. Kunikata, H. Shiku and T. Matsue, *Biosens. Bioelectron.*, 2013, 18, 12-18.