Electronic supplementary information for

High content drug screening of primary cardiomyocytes based on microfluidics and real-time ultra-large-scale high-resolution imaging

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1. The simulation of concentration gradient generator

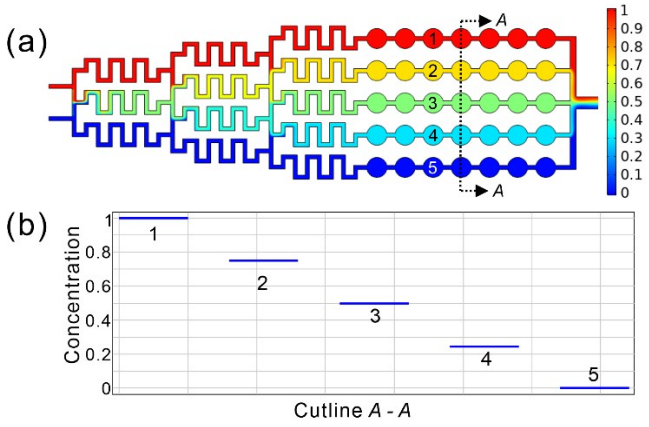

Fig. S1. The simulation results of concentration generator.

We use Comsol simulation software to simulate the effect of concentration gradient generator. The flow rate is set to 100 μm/s. Fig. S1a shows the simulation result for one unit of the microfluidic chip. It can be seen that the gradient generator can effectively generate five different concentrations. Fig. S1b is the concentration distribution curve of each unit along the cutline *A*-*A*. The concentration distribution in each unit is relatively uniform, which effectively guarantees the drug concentration

on the cardiomyocytes. Validation was performed using dyes.

2. Chip fabrication

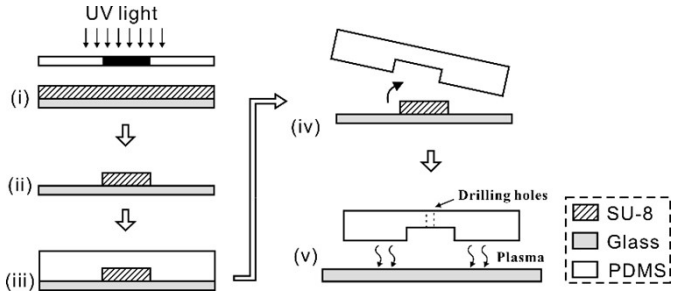

Fig. S2. The fabrication process of the microfluidic chip.

Fig. S2 illustrates the fabrication process of the microfluidic chips. (i) An SU-8 mold was firstly formed by photolithography; (ii) the mold was cured at 80 ◦C for 30 min; (iii) poured PDMS on and cured again at 80 ◦C for 2 h; (iv) peeled off PDMS channel; (v) drilled holes and bonded the PDMS on glass substrate by oxygen plasma.

3. Higher-resolution heatmaps of the cardiomyocytes' fluorescent intensity

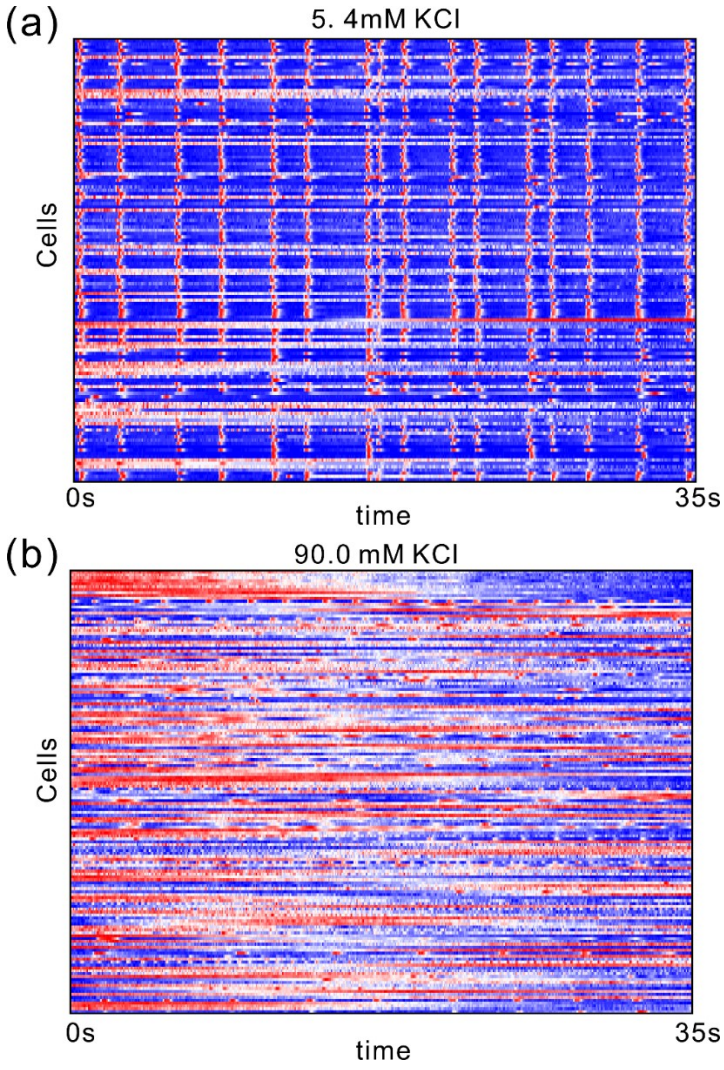

Fig. S3. The heatmaps of the cardiomyocytes' fluorescent intensity. (a) 1× Tyrode's solution (b) high K+ Tyrode's solution.

4. The Ca^{2+} ion signal rhythm of cardiomyocytes treated with 10 small molecules.

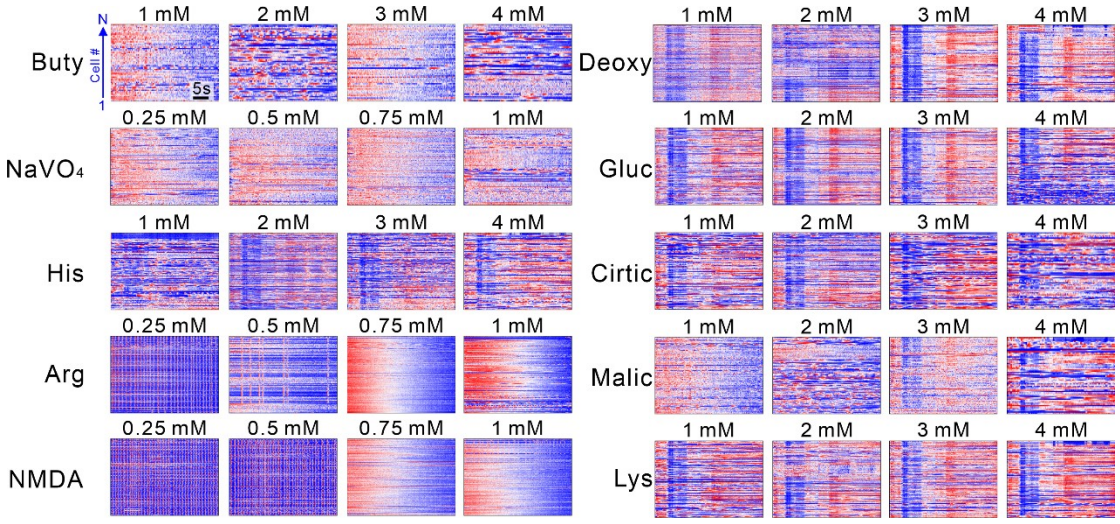

Fig. S4 The Ca2+ ion signal rhythm of 500 cardiomyocytes was unified and homogenized to generate heatmaps for 10 small molecules. Note the control for each concentration resembles exactly the pattern of 5.4 mM KCl in Fig. 3d.

NMDA and Arg have similar effects on cardiomyocyte rhythm, indicating that the NMDA receptor pathway is likely to be regulated by phosphorylation[1-3]. However, clinical trials have proved that low concentration of Arg in blood often predicts the risk of cardiac insufficiency and heart failure[4, 5]. In our experiment, 0.25 mM Arg was not found adverse effect on Ca^{2+} ion signal rhythm. Both Arg and $NaVO_4$ are alkaline phosphatase inhibitors, but $NaVO_4$ has a greater impact on cardiomyocytes. At high concentrations, the stationary phase for $NaVO_4$ is significantly prolonged. Deoxy has amphiphilicity and can change the permeability of cell membranes[6]. The heatmaps for Deoxy showed that the Ca^{2+} ion signal of cardiomyocytes was more sensitive to the change of membrane permeability. As the concentration increased, the membrane permeability became higher, which enhanced the pump-in throughput of Ca^{2+} ions while decreased the pump-out throughput of Na^{+} and K^{+} ions. Correspondingly, the fluorescent intensity of the Ca^{2+} signal increased and the stationary phase was prolonged.

The effect of His on the Ca^{2+} ion signal of cardiomyocytes mainly lied in prolongation of the stationary phase, while Butyrate significantly shortened the stationary phase and accelerated the beating rhythm. Citric, Malic, VC and Lys are often considered to have cardioprotective effects due to their antioxidant function[7]. The results for VC were relatively definite, and the Ca^{2+} ion signal rhythm decreased linearly with concentration,

which indicates that VC may have a direct influence on the biological regulation mechanism of Ca^{2+} ion equilibrium in cardiomyocytes. The three drugs, Citric Acid, Malic Acid and L-Lysine, at any concentration, affect the synchronization of cardiomyocytes. The reason may be that the drug molecules have a lot of charges, which interfere with the equilibrium of extracellular ion concentration.

5. The definition of the three basic signal characteristics (FWHM, Δt and AUC)

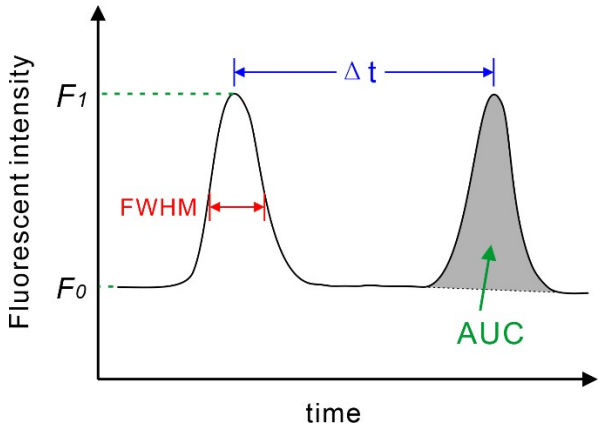

Fig. S4. The definition of the three basic signal characteristics.

Fig. S5 shows the definition of the three basic signal characteristics (FWHM, Δt and AUC).

Reference

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