

Data S1. Detailed procedure of primary culture of rat atrial cardiomyocytes.

### **Materials and methods**

*Primary culture of rat atrial cardiomyocytes.* Scissors, forceps and other materials were sterilized. Procedures were performed in the cell culture hood to ensure sterility. Then, two 6-cm cell culture dishes were placed on ice and filled with 10 ml PBS. Rats were briefly dipped in 75% ethanol solution for sterilization, then sacrificed by cervical dislocation. The chest was opened along the sternum to allow access to the chest cavity and heart. Whole hearts were excised from the body with scissors and transferred immediately into the sterile cell culture dish containing cold PBS (without  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ). The blood was gently squeezed out from the heart with forceps to prevent clot formation inside the lumen of the heart. Atria were isolated from the ventricles and residual lung tissues and washed in the PBS solution with gentle agitation, then transferred into another 6-cm cell culture dish filled with 10 ml PBS. Atria were transferred to a 6-cm cell culture dish containing 10 ml 0.08% trypsin. Tissue was cut into small pieces and incubated with gentle agitation at room temperature for 15 min. The trypsin was aspirated with a 3 ml serological pipette and the remaining myocardium was transferred to a 15-ml conical tube containing 7 ml pre-warmed 0.1% type II collagenase solution.

The myocardium was incubated at 37°C for 10 min with intermittent agitation and then the supernatant containing cardiomyocytes was transferred into a new conical tube which contained an equal amount of DMEM (Invitrogen; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (HyClone; Cytiva) to stop the collagenase digestion. Next, 7 ml collagenase solution was added into the tube containing the remaining undigested heart tissue. Heart tissue was gently triturated using a glass rod and agitated gently for 10 min at 37°C. After the undigested tissue sank, the supernatant containing cardiomyocytes was transferred to a new conical tube which contained an equal amount of cell culture medium to stop the collagenase digestion. A sterile strainer (80  $\mu\text{m}$  nylon mesh) was pre-wetted with cell culture medium, then placed in a new 50 ml conical tube and cells were passed through the cell strainer. The conical tube containing cardiomyocytes was centrifuged for 5 min at 350 x g at room temperature.

The supernatant was aspirated and the cell pellet was re-suspended with 3 ml cell culture medium. Cells were plated into a T25 cell culture flask and incubated for 1 h in a cell culture incubator to allow the differential attachment of non-myocardial cells. Then, non-adherent cells (enriched in cardiomyocytes) were transferred into a new T25 cell culture flask and incubated for an additional 1 h in a cell culture incubator with 5% CO<sub>2</sub> at 37°C. Non-adherent cells (enriched in cardiomyocytes) were then transferred into a new 15-ml conical tube and cells were counted using a hemocytometer. Cells were plated onto a 6-well plate with coverslips at a density of 10<sup>6</sup> cells/ml, then 5-BrdU was added at a final concentration of 0.1 mmol/l. The medium was replenished after 24 h and thereafter every 48 h. Beating myocardial cells were observed with an inverted microscope (DFC295; Leica Microsystems GmbH) and video graphs of beating cells were recorded with a digital camera (Coolpix5400; Nikon Corporation). Cells were cultured for ≤2 weeks for further study.

Video S1. Video graph of beating cardiomyocytes; magnification, x200.