SUPPLEMENTARY MATERIALS AND METHODS

Isolation and characterization of EVs from human MSCs
Human mesenchymal stem cells (hMSCs) [American Type Culture Collection (ATCC) PCS-500-012, ATCC, Manassas, VA, USA] were cultured in DMEM containing 10% FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin at 37°C in an incubator with normoxic (95% air, 5% CO₂) and hypoxic (5% air, 95% CO₂) conditions. Cells at 80% to 90% confluence were treated with apoptogenic reagents for 24 h. Fifteen milliliters of each hMSC culture was pelleted at 300×g for 10 min to remove dead cells. Next, supernatants from both cell cultures were obtained by centrifugation at 750×g for 5 min and then at 1500×g for 15 min to remove large debris and apoptotic bodies, respectively. Extracellular vesicles (EVs) from supernatants were pelleted and washed three times (45 min at 14000×g, room temperature) (Fig. 1A). The size and morphology of EVs were confirmed using transmission electron microscopy. Nanoparticle tracking analysis (NTA) (Malvern, Grovewood road, UK) was conducted to reveal size and zeta potential using minor modification of manufacturer’s instruction. Samples were processed in duplicate and diluted with PBS over a range of concentration to obtain between 100 and 1000 particles per image before the analysis with the NTA system. The samples were mixed before introducing into the chamber, and the camera level was set to obtain an image that had sufficient contrast to clearly identify particles while minimizing background noise with video recording (capture duration: 60 s). Afterwards, the capture videos (three videos per sample) were processed and analyzed. The delivery of EVs to cardiomyocytes was evaluated using PKH26-stained EVs, according to the manufacturer’s instructions. Isolated EVs (up to 200 µg protein) were added to wells containing 2 mL of PKH26 (10⁻⁶ M in diluent buffer), and after 60 s the staining reaction was stopped by adding 7 mL of PBS.

Electrophysiologic evaluation
Optical mapping and ventricular tachycardia induction studies were performed in five rats from each group. For optical mapping, the rats (250–300 g) were anesthetized with ketamine (80 mg/kg) and xylazine (4 mg/kg), injected with heparin (200 U/kg, IV), and then the heart was excised and perfused with a Langendorff apparatus with physiological Tyrode's solution, containing (in mmol/L) 122 NaCl, 25 NaHCO₃, 4.81 KCl, 2 CaCl₂, 2.75 MgSO₄, and 5 glucose (pH 7.4), and gassed with 95% O₂ and 5% CO₂ at 37.0±0.2°C. The hearts were placed in a chamber and perfused with blebbistatin (3–5 µM) for 10 min to reduce motion artifacts. The hearts were stained with rhod-2 AM (Invitrogen, Grand Island, NY, USA) for calcium and RH237 (Invitrogen) for voltage imaging. Fluorescence images were collected using dual cameras (MiCAM Ultima, BrainVision, Tokyo, Japan) at 1024 frames/sec. The pixel resolution was 150×150 µm², and the data was recorded and stored at intervals of 4–8 s.

For patch-clamp experiments, adult rat ventricular myocytes were isolated using the isolation method as previously described. The Na⁺ channel current (I_{Na}) was recorded in the whole-cell configuration from a holding potential of -100 mV to +40 mV in 5 mV increments and 20 ms in duration. The current recordings were obtained with an Axopatch 200B amplifier (Axon Instruments Inc, Foster City, CA, USA), controlled by a Clampex 10 and Digidata 1550 (Axon Instruments Inc.). Data were low pass-filtered at 5 kHz and sampled at 10 kHz.

REFERENCES