

Electronic Supplementary Material (ESI) for Analyst

On-chip monitoring of skeletal myoblast transplantation for the treatment of hypoxia-induced myocardial injury

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1. Materials and reagents

RTV 615 polydimethylsiloxane (PDMS) prepolymer and curing agent were purchased from Momentive Performance Materials (Waterford, NY). The surface-oxidized silicon wafers were obtained from Shanghai Xiangjing Electronic Technology Ltd. (Shanghai, China). The AZ 50XT photoresist and developer were bought from AZ Electronic Materials (Somerville, NJ). The SU-8 2025 photoresist and developer were purchased from Microchem (Newton, MA). Collagen-I, acridine orange (AO), propidium iodide (PI), Hoechst 33258, and carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP) were bought from Sigma-Aldrich (MO, USA). The cell culture medium, fetal bovine serum (FBS), CellTracker Green CMFDA, CellTracker orange CMRA, trypsin, and TRITC-phalloidin were obtained from Gibco Invitrogen Corporation (CA, USA). The DEVD-NucView 488 caspase-3 assay kit and the JC-1 mitochondrial membrane potential detection kit were purchased from Biotium, Inc. (Hayward, CA). All solvents and other chemicals were purchased from local commercial suppliers and were of analytical reagent grade, unless otherwise stated. All aqueous solutions were prepared using ultra-purified water supplied by a Milli-Q system (Millipore).

2. Cell culture

Rat heart myocardium H9c2 cells and L6 skeletal myoblasts were obtained from the Chinese Academy of Sciences (Shanghai, China). The cells were cultured using Dulbecco's Modified Eagle Medium (DMEM, Invitrogen, NY, USA) supplemented with 10% FBS, 100 U/mL penicillin, and 100 µg/mL streptomycin in a humidified atmosphere of 5% CO₂ at 37 °C. The cells were normally passaged at a ratio of 1:2 every three days to maintain them in the exponential growth phase. When the cells reached confluence, they were harvested through trypsinization with 0.25% trypsin (Invitrogen) in Ca²⁺- and Mg²⁺-free Hank's balanced salt solution (CMF-HBSS) at 37 °C before use. Trypsinization was stopped by adding freshly supplemented DMEM. The cell suspension was centrifuged at 1000 rpm for 3 min. The cells were then resuspended in supplemented DMEM for further use.

3. Cellular viability assay

Cellular viability was analyzed using double staining with AO (Fluorescence was detected at 495/520 nm) and PI (Fluorescence was detected at 536/617 nm). Briefly, after rising cells with PBS thrice, 5 mg/L AO and 1 mmol/L PI were introduced into the cell culture wells. After incubation for 10 min and a final rinse, fluorescence images of the stained cells were acquired and quantitatively analyzed.^{s1}

4. Design and fabrication of the microfluidic device

The microfluidic device utilized for this study was designed using software AutoCAD (Autodesk, Inc., CA, USA) and fabricated using a multilayer soft lithography method.^{S2,S3} First, two kind of molds were produced by photolithographic processes to create the fluidic components (channel width: 300 μm , height: 35 μm ; cell culture chamber width: 300 μm , height: 35 μm , length: 3500 μm ; connection channel width: 150 μm , height: 35 μm , length: 200 μm) and control components (channel width: 75 to 150 μm ; height: 25 μm) to be embedded in corresponding layers of the PDMS matrix. To prepare the mold utilized for fabricating the fluidic components, a 35 μm thick positive photoresist (AZ 50XT) was spin-coated onto a silicon wafer. After ultraviolet (UV) light exposure, the fluidic components on the wafer were developed using an AZ 400K developer. The mold for the control channels was made by introducing a 25 μm thick negative photoresist (SU8-2025) pattern on a silicon wafer. To achieve reliable performance of each valve, the widths of the control channels were set to 150 μm in the sections where the valve modules were located.

Before fabricating the microfluidic device, both the fluidic and control molds were exposed to trimethylchlorosilane vapor for 3 min. A well-mixed PDMS pre-polymer (RTV 615 A and B in a 7 to 1 ratio, w/w) was poured onto the fluidic mold, which was placed in a Petri dish to yield a 5 mm thick fluidic layer. The PDMS pre-polymer (RTV 615 A and B in a 17 to 1 ratio, w/w) was also spin-coated onto the control mold (1,800 rpm, 60 s, ramp 15 s) to obtain the thin control layer. The thick fluidic layer and thin control layer were cured in an 80 °C oven for 50 min. After incubation, the thick fluidic layer was peeled off the mold, and holes were introduced into the fluidic layer for reagent and cell sample supply access as well as waste exclusion. The fluidic layer was then trimmed, cleaned, and

aligned onto the thin control layer. After baking at 80 °C for 120 min, the assembled layers were peeled off the control mold, and another set of holes was punched for access to control channels. These assembled layers were then placed on top of a glass slide (2,500 rpm, 30 s, ramp 15 s) coated with the PDMS pre-polymer (RTV 615 A and B in a 10 to 1 ratio, w/w) that had been cured for 12 min in the oven (80 °C). The microfluidic device was ready for use after baking at 80 °C for 72 h.

5. On-chip fluidic control test

For convenient monitoring of the mass transportation and distribution, the fluorescein solution was only loaded into a lateral channel and fluorescein-free solution (i.e., ultra-purified water) was loaded into the central channel, simulating the operation of on-chip coculture of H9c2 cells and L6 cells. When microvalves in PuS were opened, the solution immediately flowed from the lateral myoblast-cultured chamber into the central cardiomyocyte-cultured chamber, achieving the communication of two heterogeneous cells as indicated by fluorescein test (Fig. S1). Fluorescence optical density during the flow process was measured and analyzed, and the result showed that fluorescence intensity of the two culture chambers were almost equivalent after 36s, indicating timely communication.

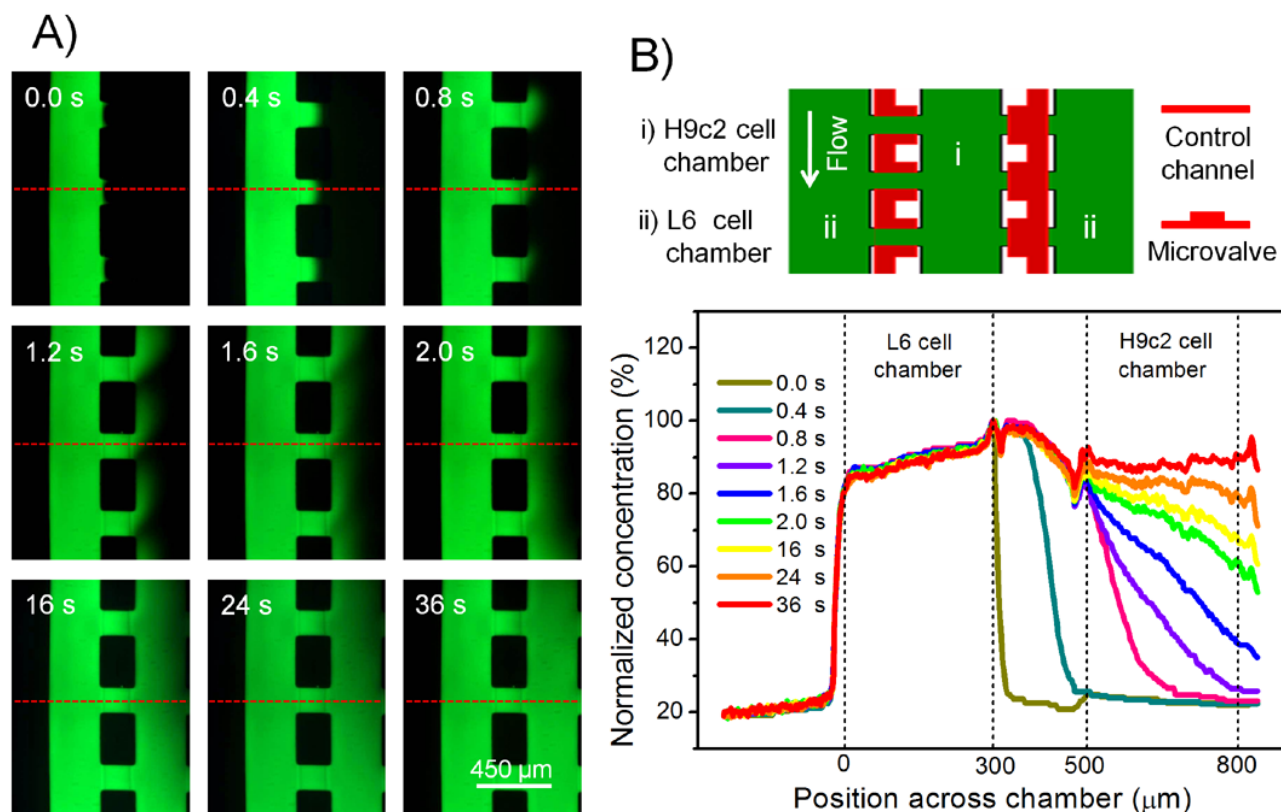


Fig. S1 Testing of on-chip fluidic control. (A) Time-lapse fluorescent images showing fluorescein solution flowed across the connected channel into myocardium H9c2 culture chambers when the microvalves in PuS were opened. (B) Normalized fluorescein concentration indicated by fluorescence intensity measured from the red dotted lines in the corresponding fluorescent images.

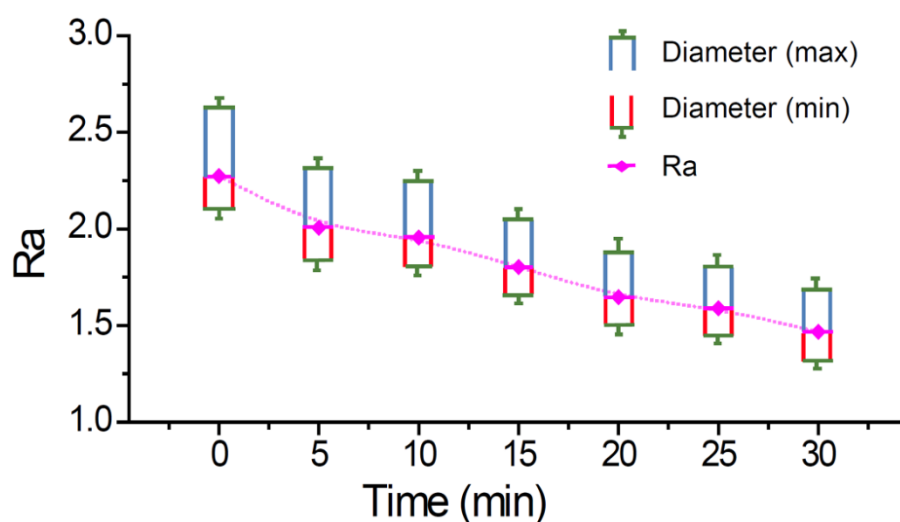


Fig. S2 The Ra value of FCCP-treated H9c2 cells. Ra was defined as the ratio between the long axis [or diameter (max)] and short axis [or diameter (min)] of cell. Data was given as means \pm SD.

6. Measurement of perinuclear density of actin

Actin filaments of normal cultured myocardial H9c2 cells were uniform and in alignment along the cellular axes. Whereas, after FCCP treatment, myocardial cells underwent a hypoxic condition and the actin filaments concentrated around the nuclei showing enhanced fluorescence intensity. The perinuclear density of actin (percentage) was defined by the ratio between the perinuclear area and the average area of corresponding cell. Method of calculating perinuclear density of actin was adapted from Webb et al.^{S4} Briefly, the Hoechst images were processed to select the nucleus using software Image-Pro® Plus 6.0 (Media Cybernetics). The perinuclear area is defined as an area of an X μm greater radius than the nucleus area, where X was an empirically given factor, such as 0 for normal cells, 6 for hypoxia-damaged cells, and 3.5 for cells cocultured with skeletal myoblast.

Perinuclear density of actin = Perinuclear area \times 100/Average area of corresponding cell.

7. Comparison of the effects of conditional culture medium of skeletal myoblast and normal culture medium on myocardial injury healing

To further figure out whether the repair process of myocardium H9c2 cells were caused by the paracrine signal secreted by L6 skeletal myoblast or the cell-to-cell interaction through coculture with L6 cells, we used conditional culture medium of L6 cells to substitute the paracrine signal and normal culture medium was used as control. The two kinds of cell culture media were respectively used to culture myocardium H9c2 cells. The result showed that no obvious change was found in the morphology and viability of H9c2 cells after introduction of the two kinds of media (Fig. S3-S6).

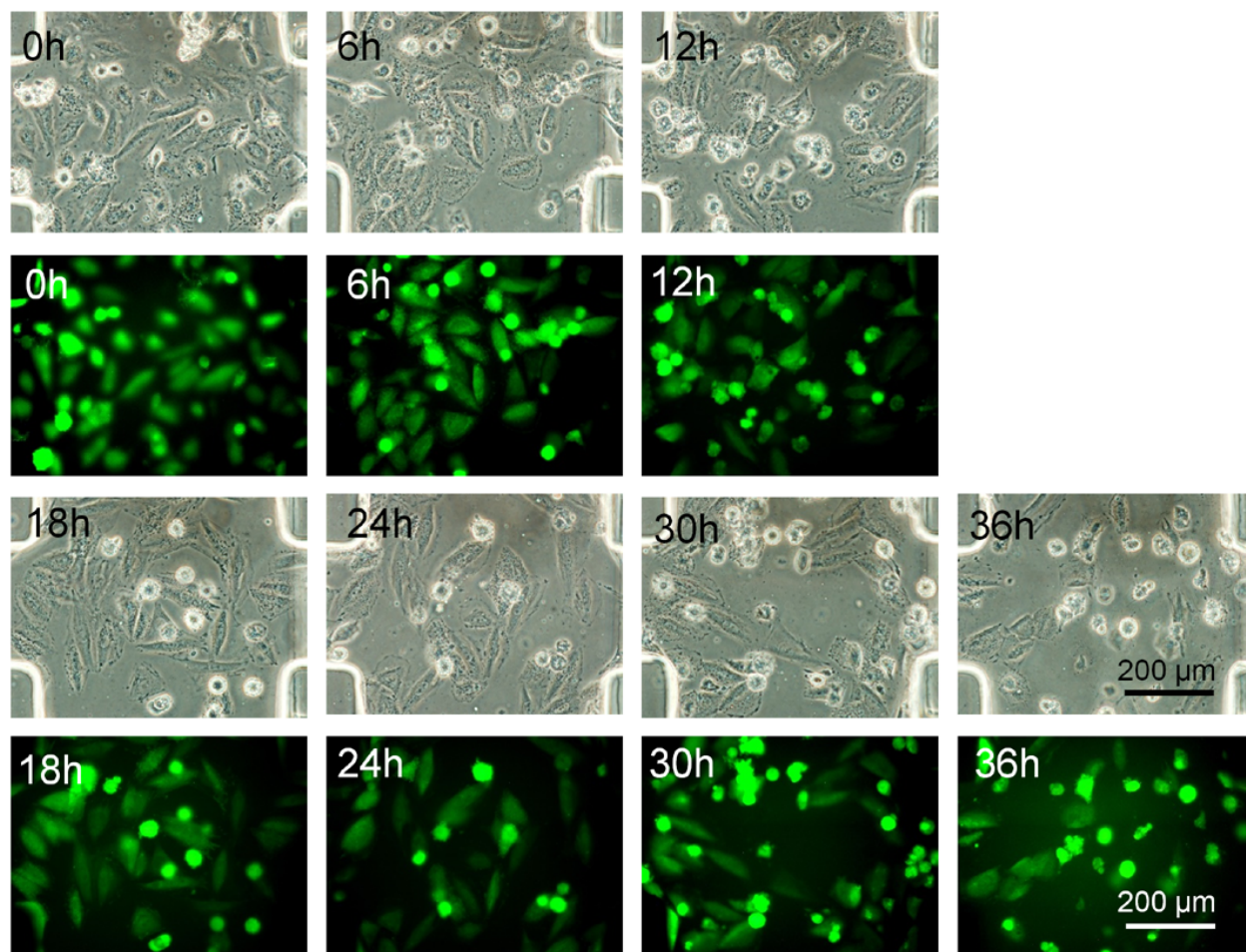


Fig. S3 Morphological changes of FCCP-treated myocardial cells after culture with conditional culture medium of L6 cells.

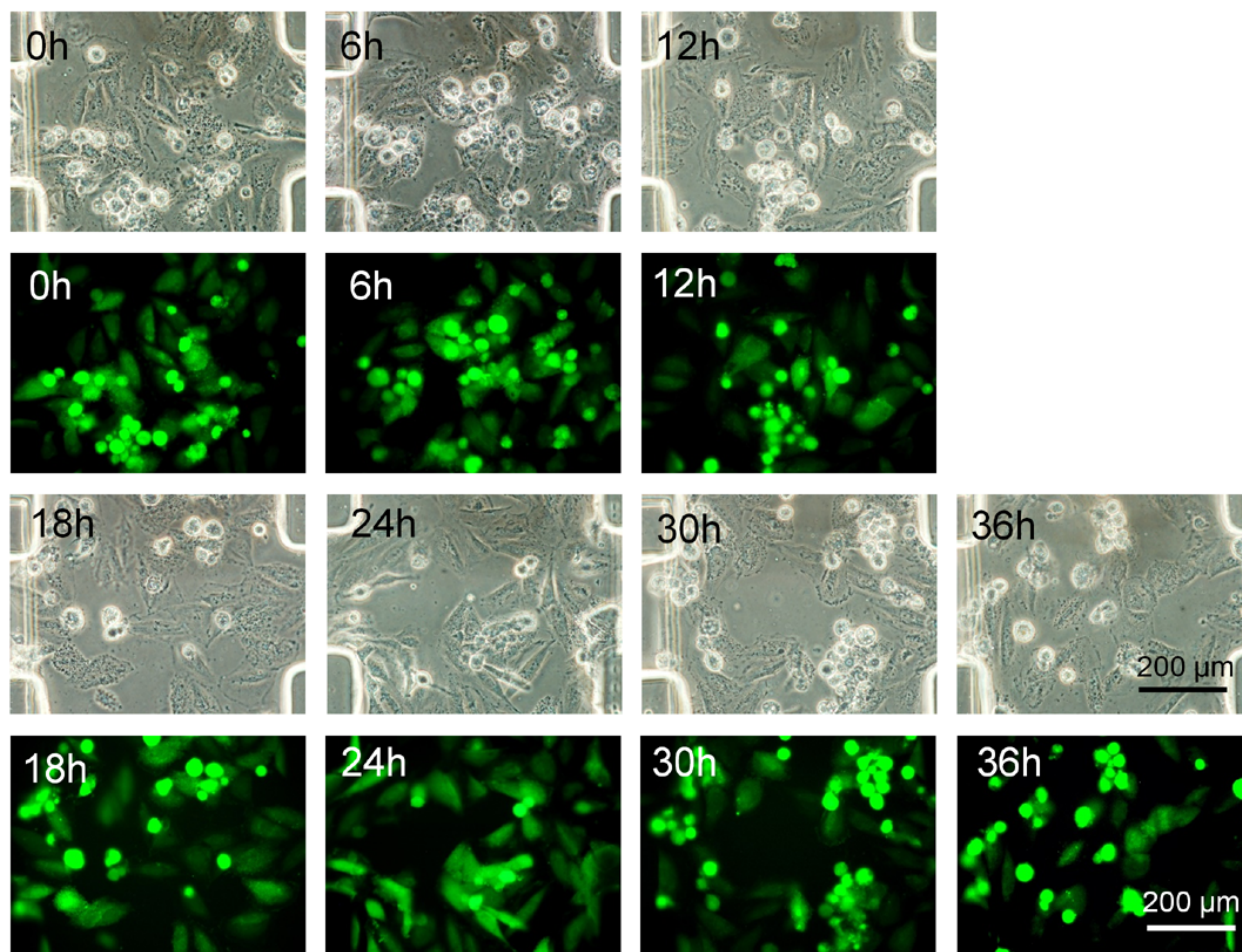


Fig. S4 Morphological changes of FCCP-treated myocardial cells after culture with normal cell culture medium.

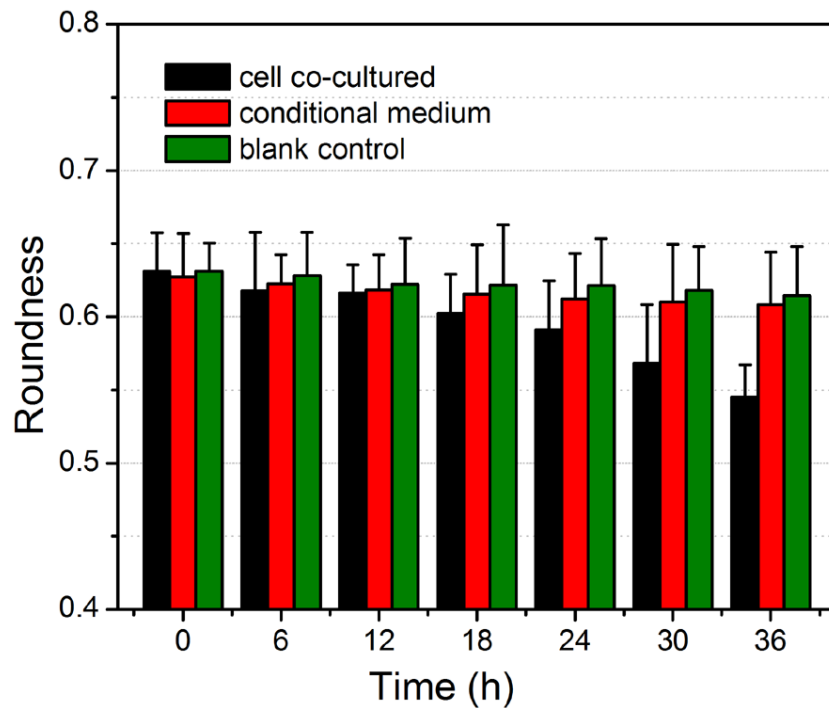


Fig. S5 The changes of cell roundness of hypoxia-injured H9c2 cells after coculture with L6 cells (marked with black), conditional cell culture medium (red) and normal cell culture medium (green) within 36 h. Cell roundness is the measure of how closely the shape of a cell approaches that of a circle. FCCP-treated H9c2 cells showed round morphology, indicated by large roundness, while healthy or repaired H9c2 cells showed spread morphology, indicated by small roundness. The results showed that conditional and normal cell culture medium did not significantly change the cell roundness, while after coculture with L6 cells, the cell roundness of H9c2 cells decreased pronouncedly, indicating cell restoration. Data are collected from three independent experiments with each has at least 420 cells counted and given as means \pm SD.

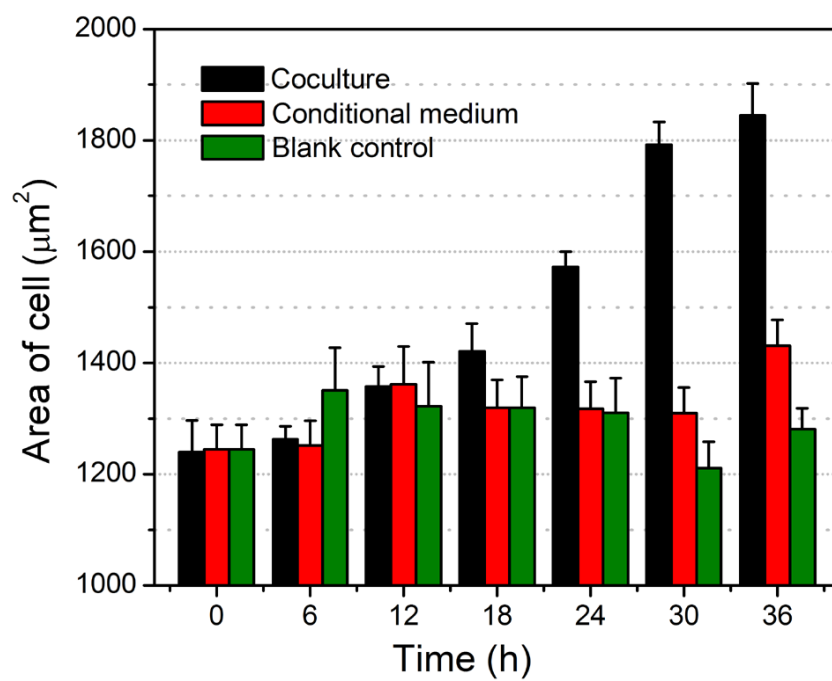


Fig. S6 Dynamic changes of cell area of hypoxia-treated H9c2 cells after culture with different three conditions for 36 h. Data are given as means \pm SD.

8. References for Supplementary information

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