

Supplementary Information for

Programmable production of bioactive extracellular vesicles in vivo to treat myocardial infarction

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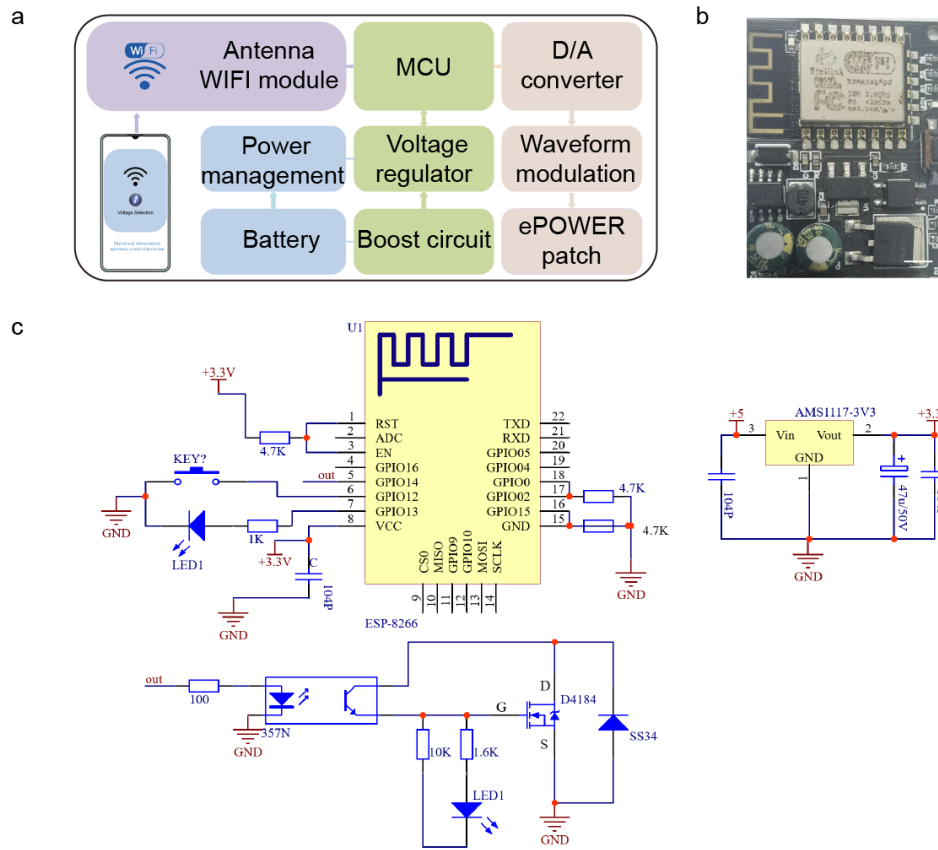
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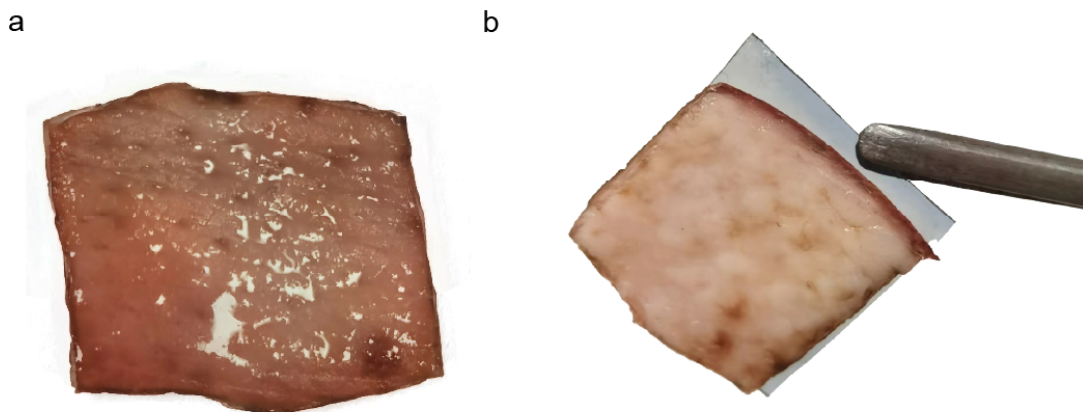
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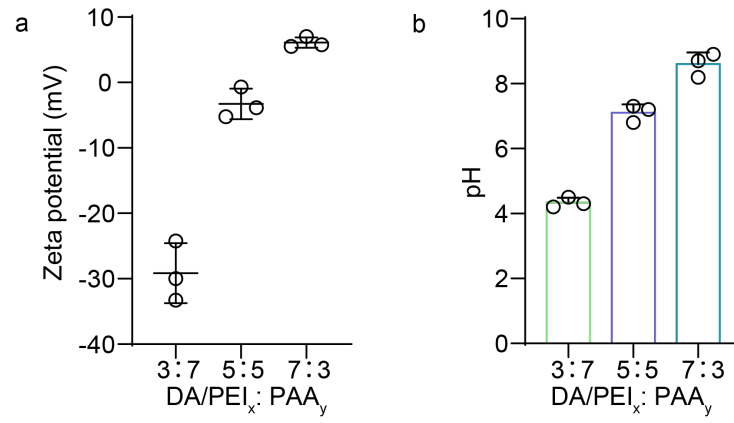
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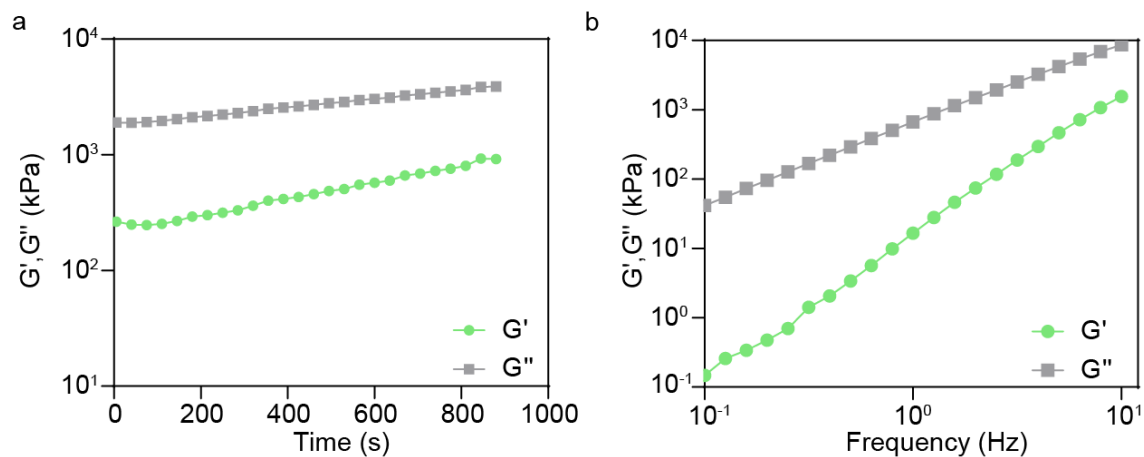
Supplementary Fig. 1. Wireless control module. **(a)** Schematic diagram of ePOWER, **(b)** PCB integrated with MCU and antenna (Scale bar, 5 mm) and **(c)** Partial circuit diagrams and component information from the PCB in **(b)**.



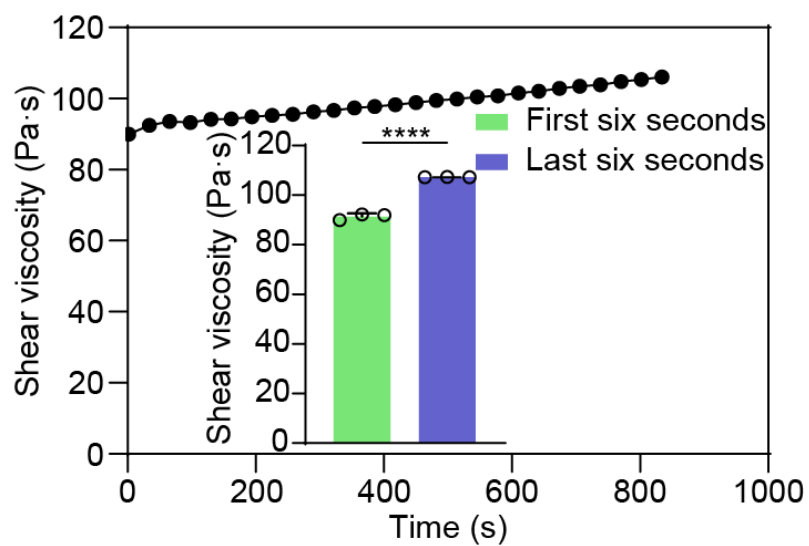
Supplementary Fig. 2. (a) Wet pig skin tissue for shear testing and (b) Display of combining tissue with ePOWER patch using adhesive.



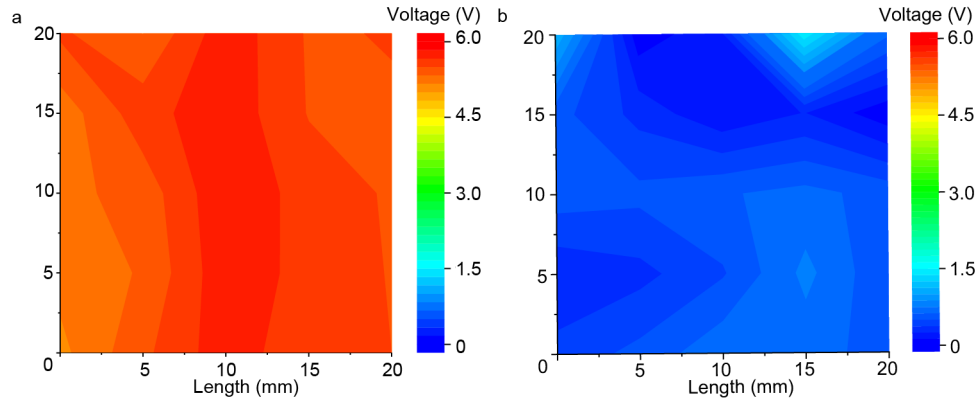
Supplementary Fig. 3. Zeta potential (a) and pH (b) of adhesive powder (15 mg) in deionized water (1.5 mL) (n = 3). Data are shown as the mean ± standard deviation.



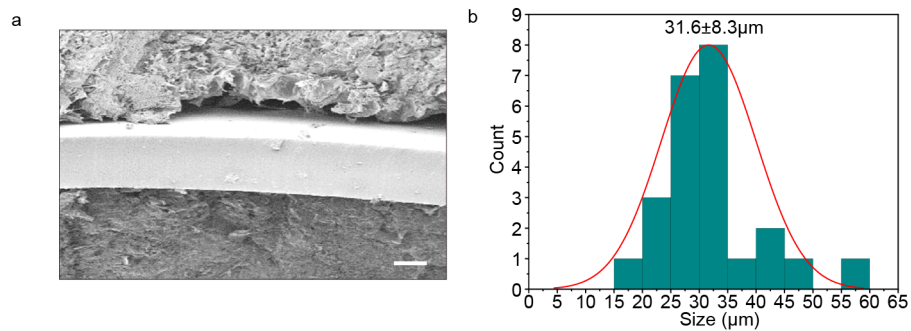
Supplementary Fig. 4. Rheological Analysis of DA/PEI₅/PAA₅ Adhesive. (a) Storage modulus (G') and loss modulus (G'') as a function of time for DA/PEI₅/PAA₅ adhesive. Strain=1%, frequency=1Hz, temperature=25°C and (b) Storage modulus (G') and loss modulus (G'') as a function of frequency for DA/PEI₅/PAA₅ adhesive. Strain=1%, frequency= 10^{-1} - 10^1 Hz, temperature=25 °C.



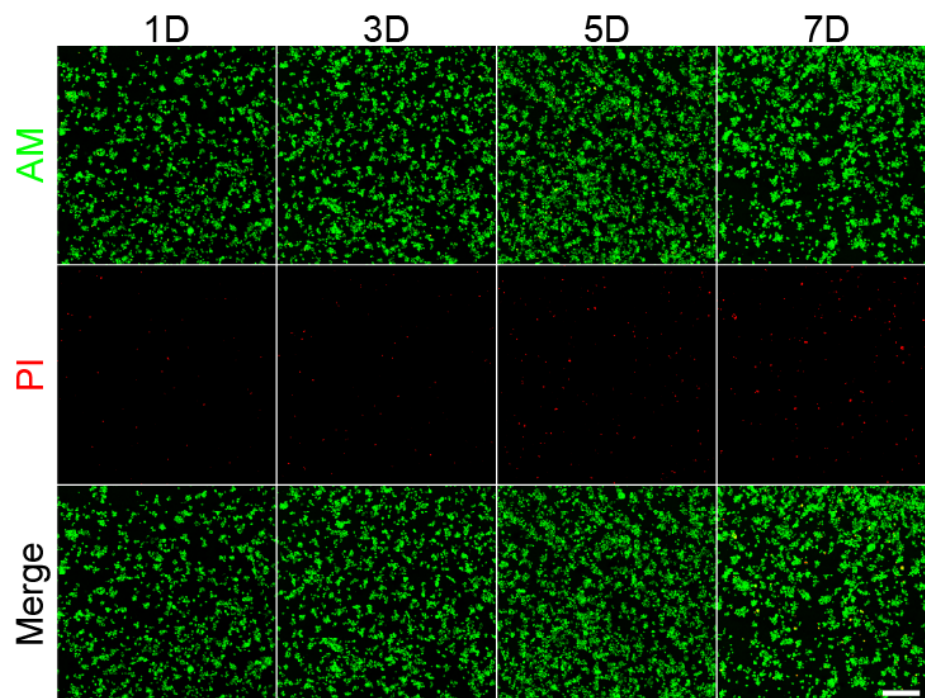
Supplementary Fig. 5. Shear viscosity varied as a function of time for the DA/PEI₅/PAA₅ adhesive (n = 3). Temperature=25 °C. Significances are presented by **** $P < 0.0001$. The data is represented by an average of $\pm$ SD.



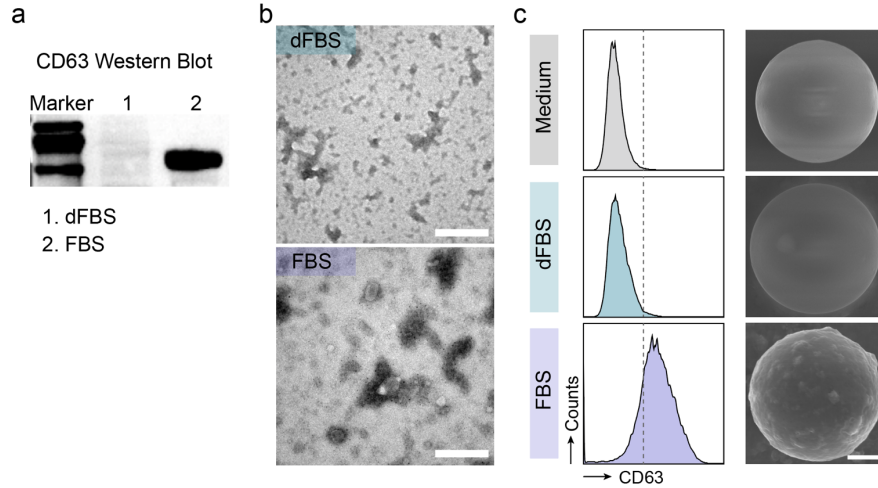
Supplementary Fig. 6. Voltage distribution map of ePOWER patch surface. **(a)** Voltage distribution before applying the adhesive layer. **(b)** Voltage distribution after covering the patch with adhesive layer. 25 points selected on the patch for voltage measurement. The color gradient represents the voltage levels, with higher voltages indicated in red.



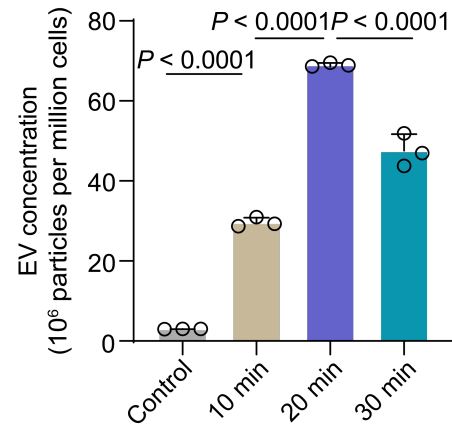
Supplementary Fig. 7. (a) Cross-section SEM image of ePOWER film (Scale bar, 50 μm) and **(b)** Pore size of the adhesive hydrogel layer. Quantitative analysis of SEM images of adhesive layers using ImageJ.



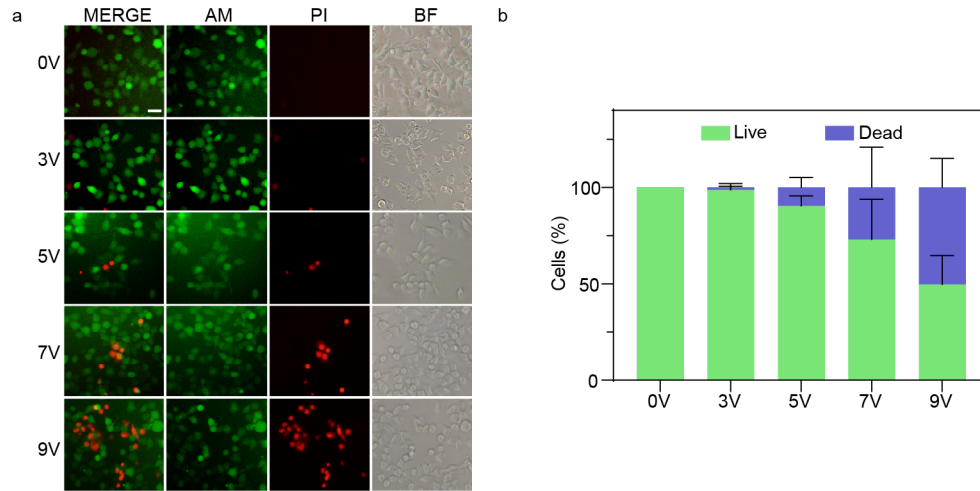
Supplementary Fig. 8. Viability of embedded cells in ePOWER patch. Confocal images depicting viable cells within the patch, stained with Calcein-AM (green), captured on different days of incubation (Scale bar, 100 μ m).



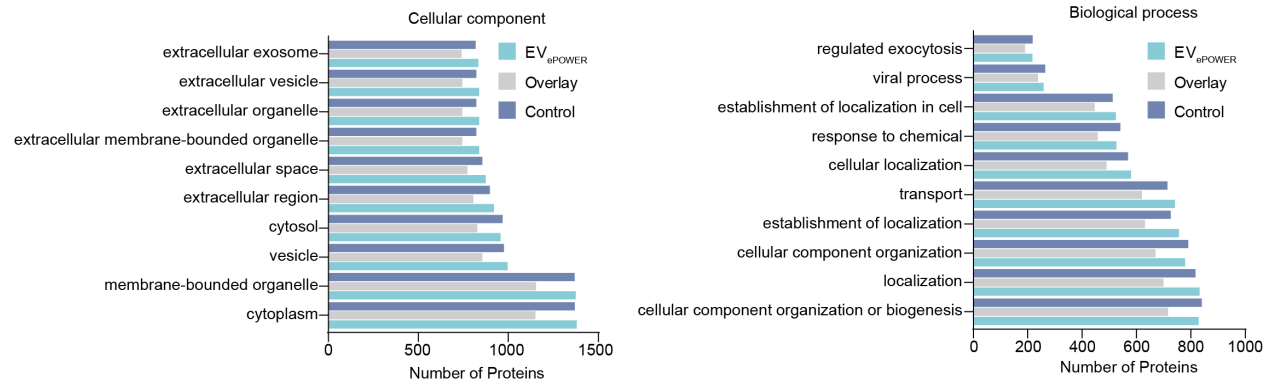
Supplementary Fig. 9. The analysis of dFBS. (a) The western blot analysis of dFBS and FBS. (b) The TEM analysis of dFBS and FBS. The samples were negative stained with 1% uranyl acetate. Scale bar, 200 nm. (c) The representative flow cytometry analysis (left) and SEM images (right) of EVs immuno-captured by anti-CD9 functionalized beads. CD9 antibody beads were incubated with different mediums and then detected with CD63 antibody. Scale bar, 500 nm.



Supplementary Fig. 10. The relative amount of EVs generated at different durations of the stimulation (n=3). The data is represented by an average of $\pm$ SD.



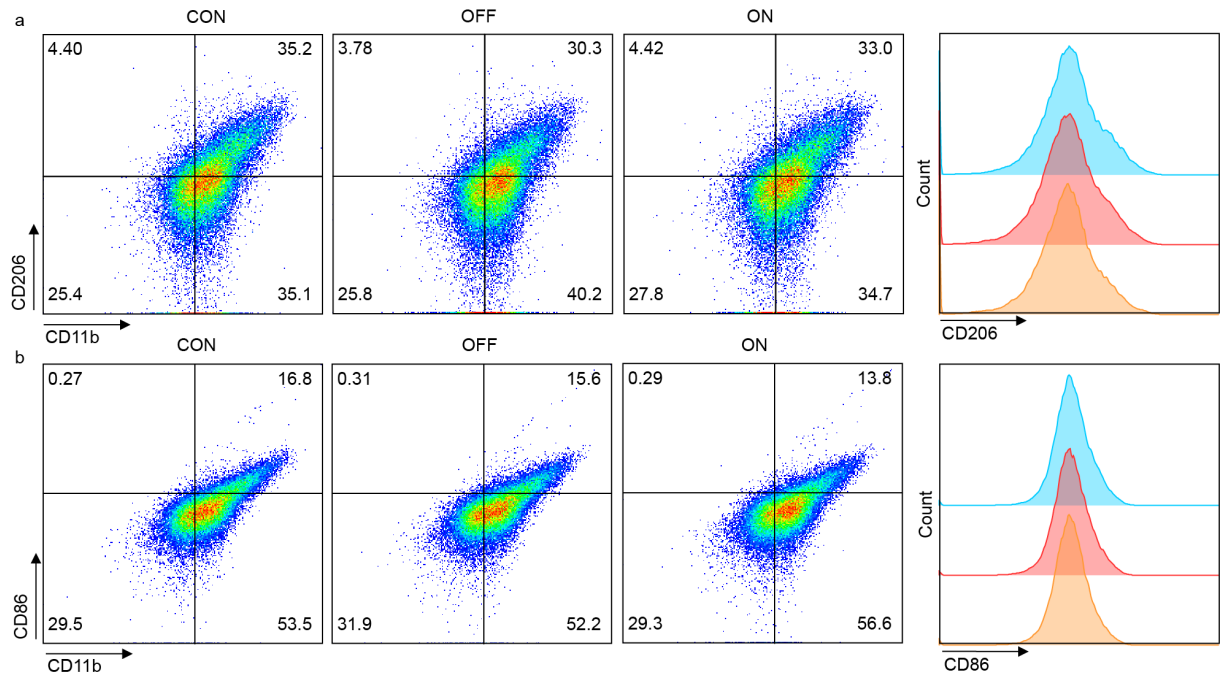
Supplementary Fig. 11. Voltage tolerance test of cells on ePOWER patch. **(a)** Fluorescence staining of M2 cells on the surface of patch stimulated by different voltages (green: live cells; red: dead cells; Scale bar, 100 μ m) and **(b)** Quantitative diagram of live and dead staining ($n = 3$).



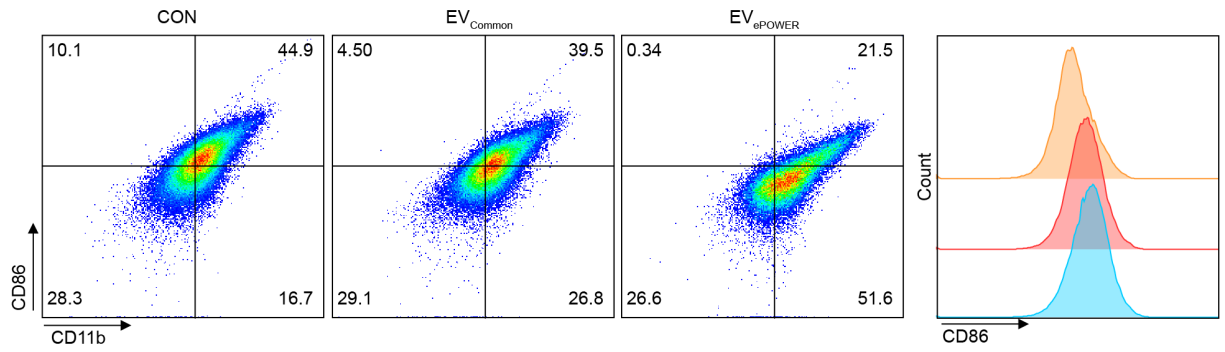
Supplementary Fig. 12. Biomass spectrometry identification analysis detected the number of proteins with corresponding cellular components and molecular functions in EV_{ePOWER} (light blue) and EV_{Common} (dark blue). The proteins co-expressed by both are represented in gray.



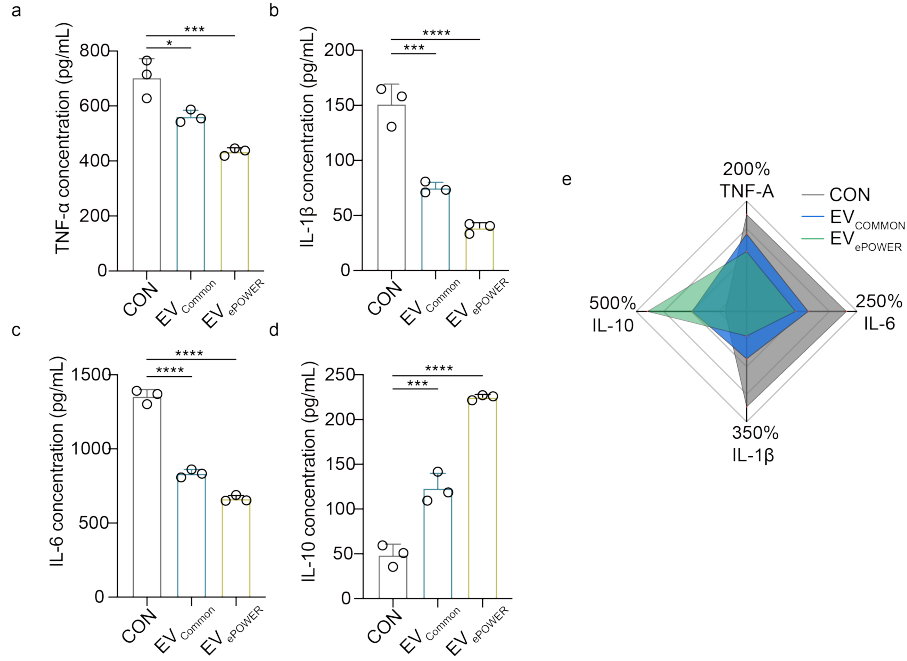
Supplementary Fig. 13. Bright-field image of macrophages. Compared with circular control macrophages with short pseudopodia, macrophages with long pseudopodia were elongated after polarization by EV_{Common} and EV_{ePOWER} (Scale bar, 20 μ m).



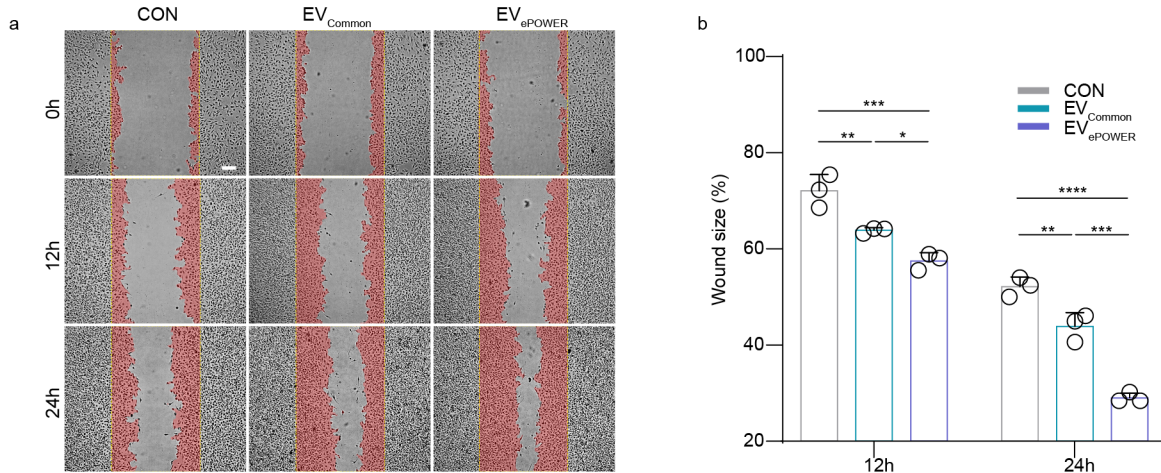
Supplementary Fig. 14. Phenotypic changes of macrophages before and after ePOWER stimulation. **(a)** Flow cytometry analysis and Histogram analysis of CD206 macrophages and **(b)** Flow cytometry analysis and Histogram analysis of CD86 macrophages.



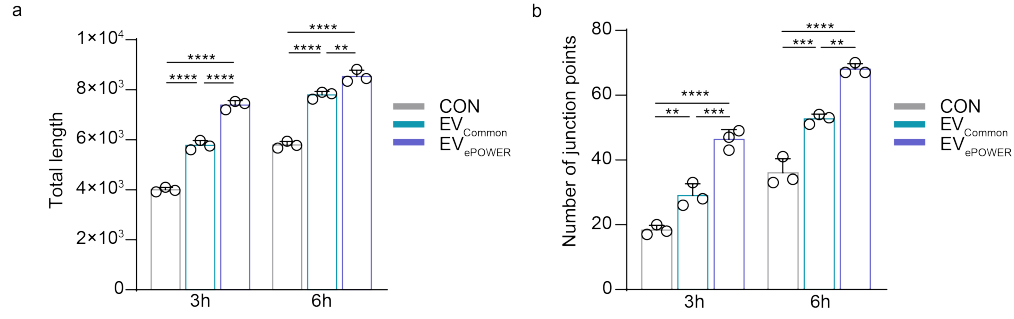
Supplementary Fig. 15. Flow cytometry analysis of the phenotype of co-cultured macrophages treated with EVs. Typical M2 phenotype marker CD86 was employed for flow cytometry assay.



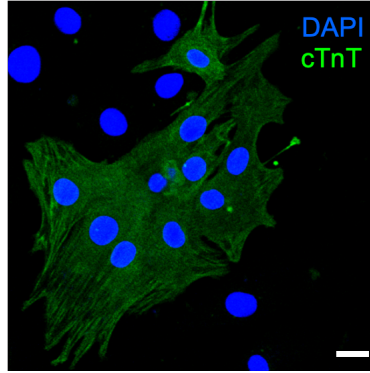
Supplementary Fig. 16. Relative protein expression of M1 and M2 macrophage markers in macrophages after various treatments ($n = 3$). **(a)** tumor necrosis factor- α (TNF- α), **(b)** interleukin-1 β (IL-1 β), **(c)** interleukin-6 (IL-6), **(d)** interleukin-10 (IL-10) and **(e)** Quantitative comparison chart of relative protein expression. Statistical differences were analyzed using one-way ANOVA with Turkey's multiple comparisons test. Significances are presented by $*P < 0.05$, $***P < 0.001$ and $****P < 0.0001$. The data is represented by an average of $\pm$ SD.



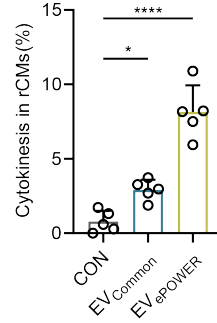
Supplementary Fig. 17. (a) Representative images of HUVEC migration by cell scratch assay at 0, 12 and 24h (scale bars, 200 μm) and (b) Quantification of HUVEC migration by cell scratch assay at 0, 12 and 24 h (n = 3). Statistical differences were analyzed using one-way ANOVA with Turkey's multiple comparisons test. Significances are presented by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$. The data is represented by an average of $\pm$ SD.



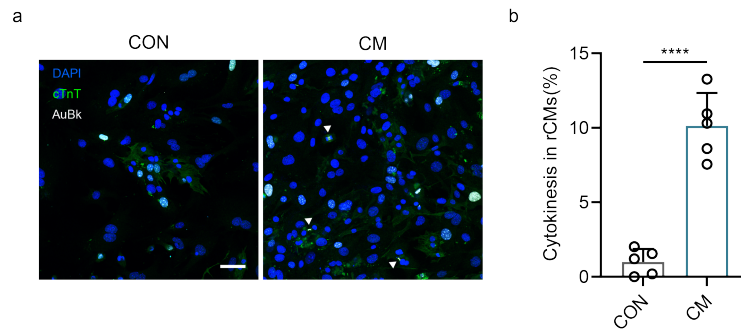
Supplementary Fig. 18. (a) Quantification of the total vascular length representing vascular formation capability in different EV treatment groups ($n = 3$) and (b) Quantification of the number of junction points in different groups ($n = 3$). Statistical differences were analyzed using one-way ANOVA with Turkey's multiple comparisons test. Significances are presented by $**P < 0.01$, $***P < 0.001$ and $****P < 0.0001$. The data is represented by an average of $\pm$ SD.



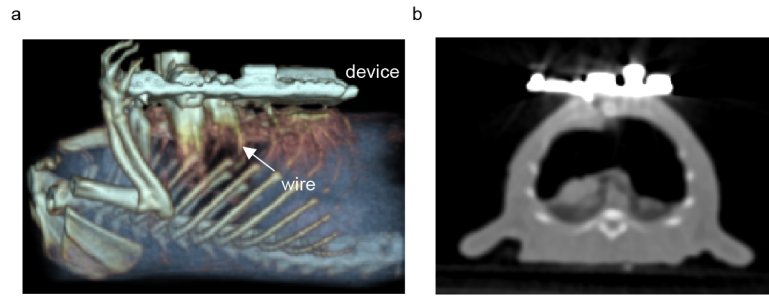
Supplementary Fig. 19. The immunofluorescence staining of the primary rat cardiomyocytes. Blue, DAPI; Green, Cardiac Troponin (cTnT). Scale bar, 10 μm .



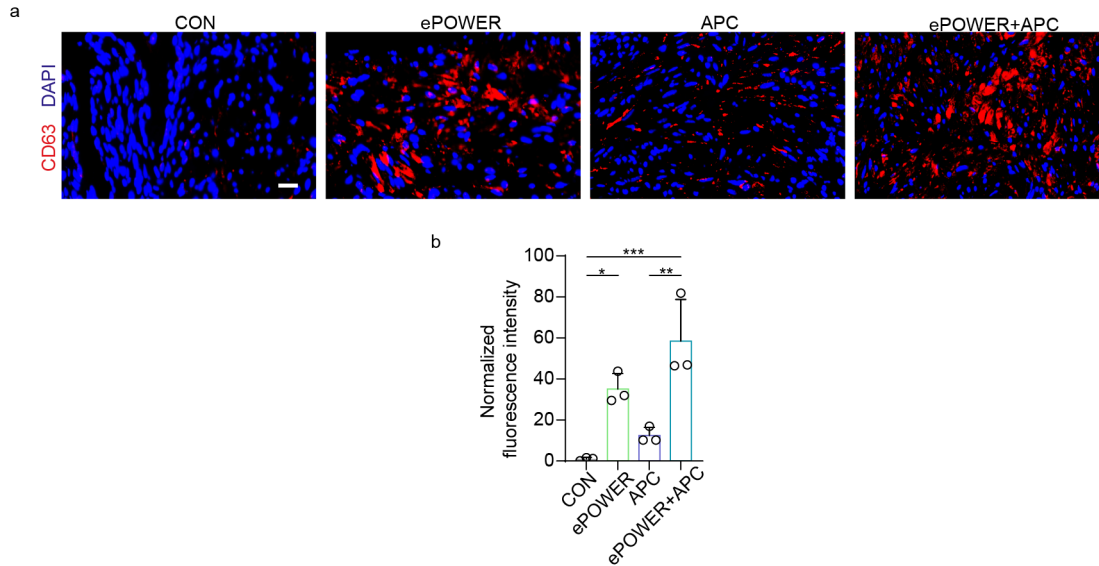
Supplementary Fig. 20. The analysis of Aurora B kinase (AuBk) expression at cleavage furrows following treatment with EV_{Common} or EV_{ePOWER} (n = 5). Significances are presented by * $P < 0.05$, **** $P < 0.0001$. The data was represented by an average of $\pm$ SD.



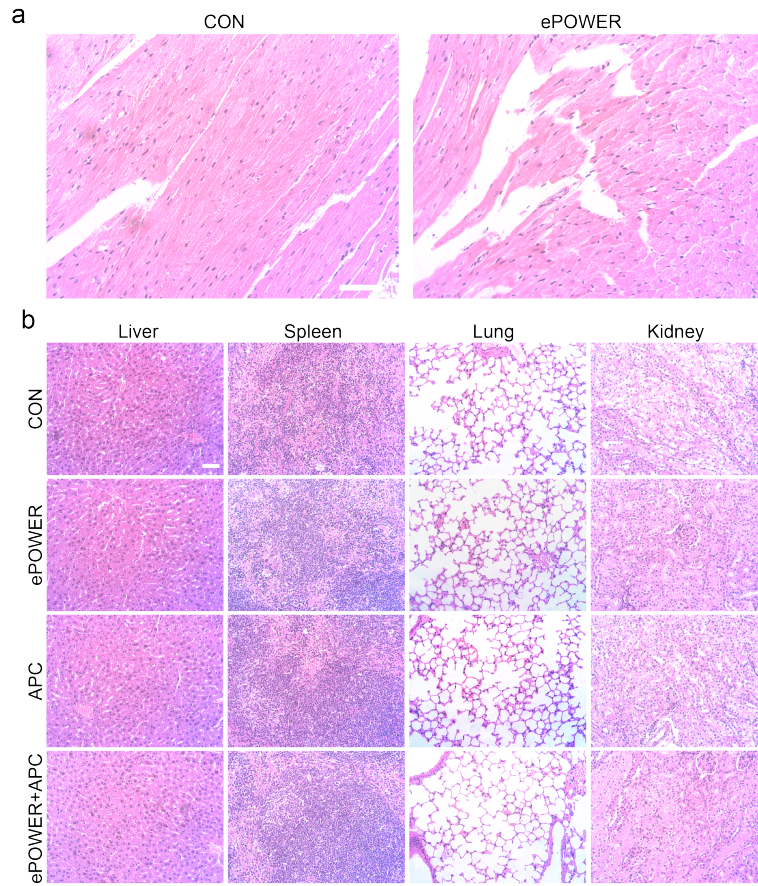
Supplementary Fig. 21. (a) Proliferation of primary rat cardiomyocytes at 12 hours following treatment with normal medium (CON group) or conditional medium (CM group) (n = 5), along with (b) the corresponding quantitative analysis of Aurora B kinase expression at cleavage furrows. (Scale bar, 50 μ m). Statistical differences were analyzed using unpaired two-tailed t-test. n = 5 per group; Significances are presented by **** $P < 0.0001$. The data is represented by an average of $\pm$ SD.



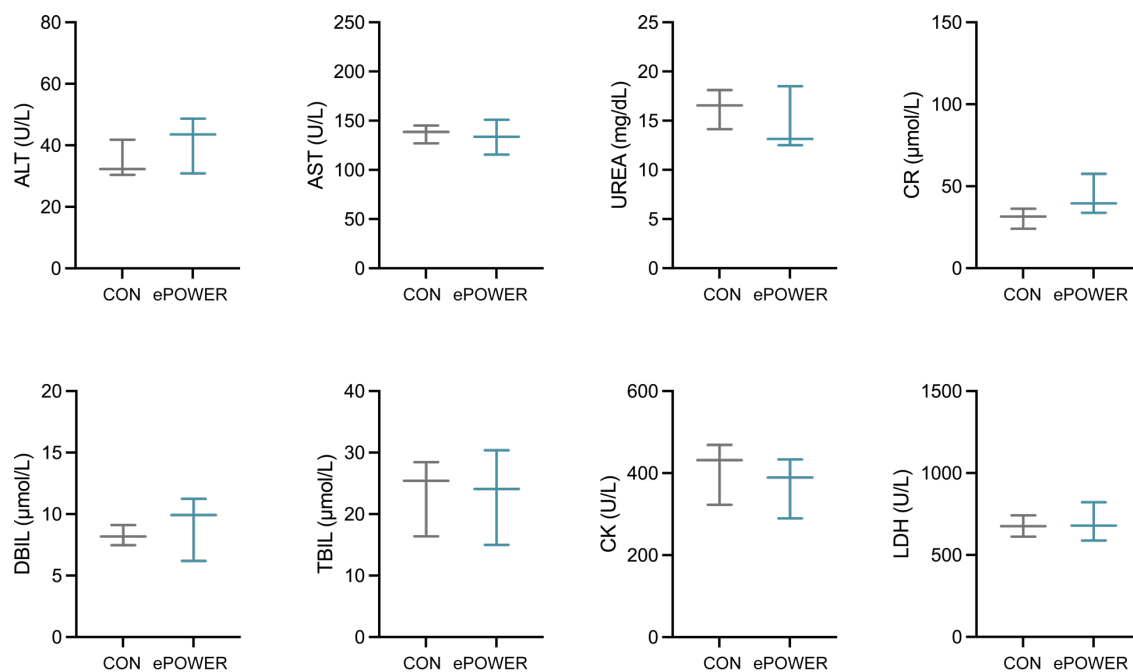
Supplementary Fig. 22. (a) Surgical photo of ePOWER patch pasted onto the heart using an adhesive layer, (b) The overall 3D reconstruction of animals after transplantation of ePOWER patch based on CT images and (c) Cross-sectional CT image.



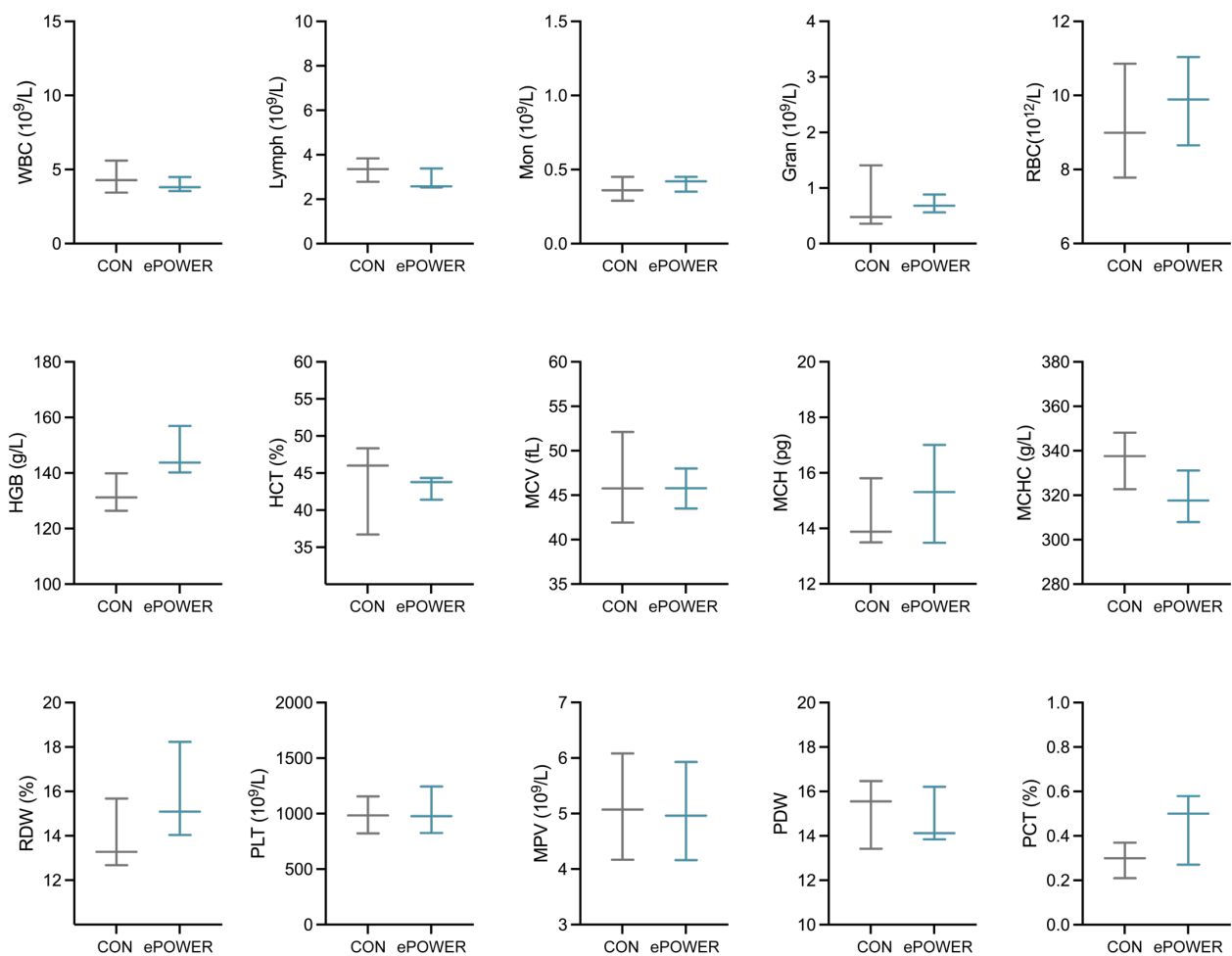
Supplementary Fig. 23. ePOWER treatment promotes the production and release of EVs effectively distributed in the MI region. (a) Representative fluorescence image of vesicular marker CD63 in cardiac tissue (scale bars, 20 μ m) and (b) Quantitative analysis of CD63 fluorescence signal. Statistical differences were analyzed using one-way ANOVA with a Tukey multiple comparisons test. $n = 3$ per group; significances are presented by $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. The data was represented by an average of $\pm$ SD.



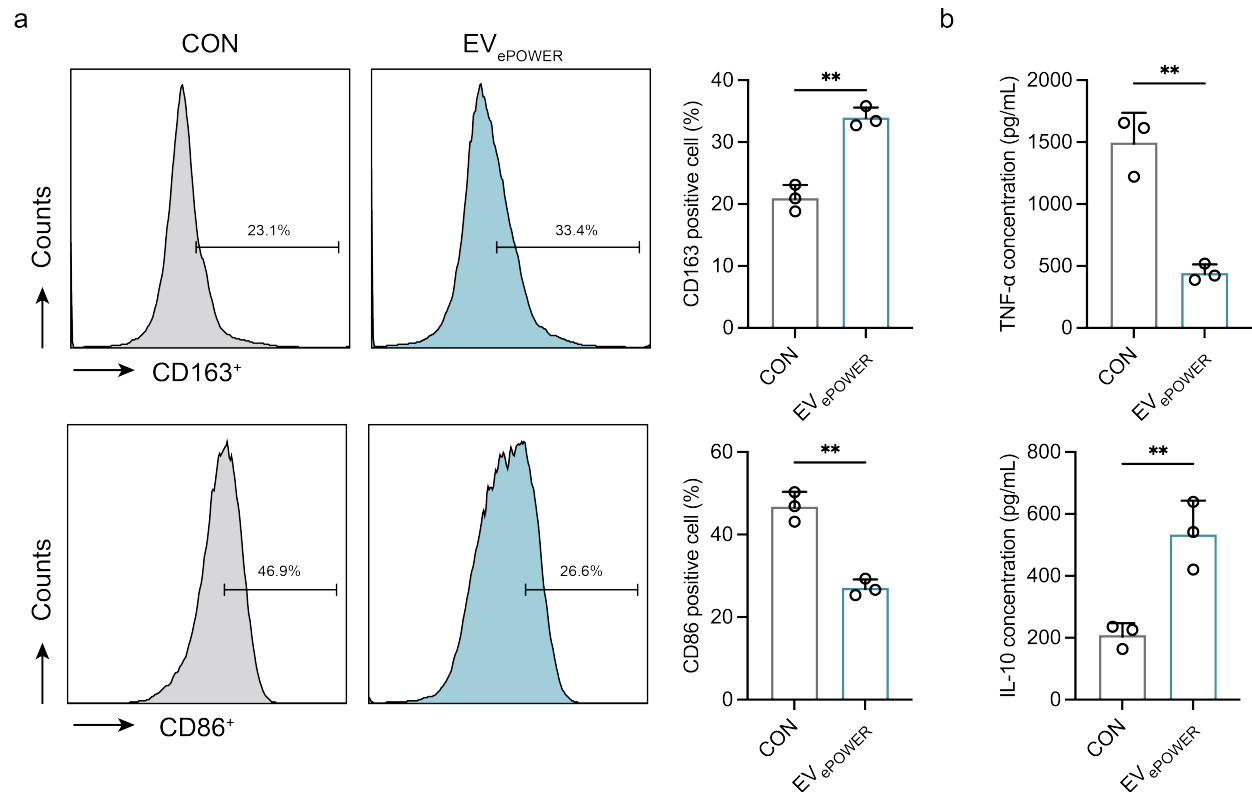
Supplementary Fig. 24. The biocompatibility of the ePOWER patch. **(a)** Hematoxylin and Eosin (H&E) of the cardiac tissue on day 28 ($n = 3$; Scale bar, 100 μm) and **(b)** Masson staining of the liver, spleen, lung and kidney on day 28 (Scale bar, 100 μm).



Supplementary Fig. 25. The blood chemistry for rats in CON group or ePOWER treatment group (n = 3).



Supplementary Fig. 26. Standard hematological safety tests for rats in CON group or ePOWER treatment group (n = 3).



Supplementary Fig. 27. Phenotypic changes in macrophages isolated from infarcted myocardial tissue following treatment with EV_{ePOWER}. The ePOWER system embedded with macrophages isolated from myocardial tissue. **(a)** Flow cytometry analysis of CD163 and CD86 expression on macrophages. **(b)** The analysis of TNF- α and IL-10 cytokine levels in EV_{ePOWER}-treated macrophages. Significances are presented by $**P < 0.01$. The data is represented by an average of $\pm$ SD.