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Supplementary Materials for

Strontium ions protect hearts against myocardial ischemia/reperfusion injury

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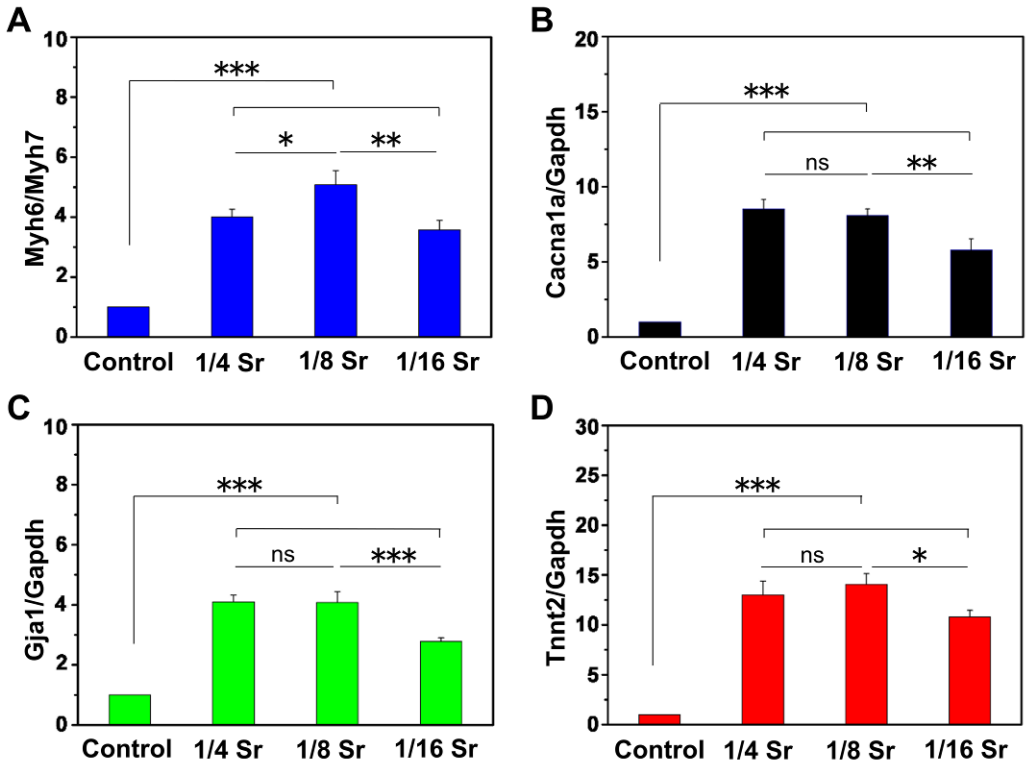


Fig. S1. Sr ions enhance the cardiac genes expression in NRCMs after 10 days treatment *in vitro*. qRT-PCR analysis of the expression of cardiac-specific genes including (**A**) Myh6/Myh7, (**B**) Cacna1a, (**C**) Gja1, and (**D**) Tnnt2 in NRCMs cultured with Sr ions at various concentrations. All data were presented as means ± SEM. The experiments were conducted in triplicate. One-way ANOVA was used for statistical analyses. **P* < 0.05, ***P* < 0.01, ****P* < 0.001; ns: no statistical significance.

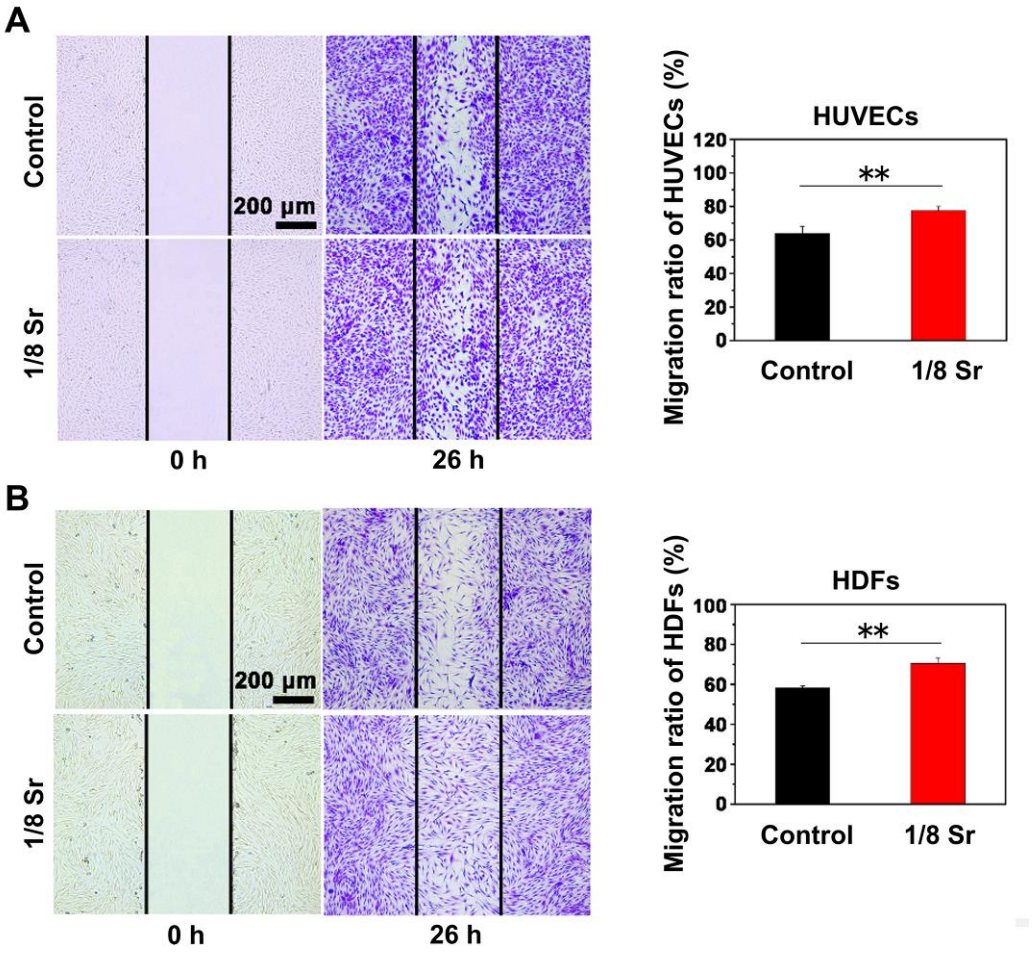


Fig. S2. Sr ions stimulate the migration of HUVECs and HDFs. The effect of Sr ions in cell migration was evaluated in a scratch wound healing assay. Images and quantitative analysis of mono layer cultivated HUVECs (**A**) and HDFs (**B**) in the medium supplemented with Sr ions after scratching (0 h) and healing for 26 h. Experiments were conducted in triplicate. All data were presented as means ± SEM. Unpaired t test was used for statistical analyses. $^{**}P < 0.01$.

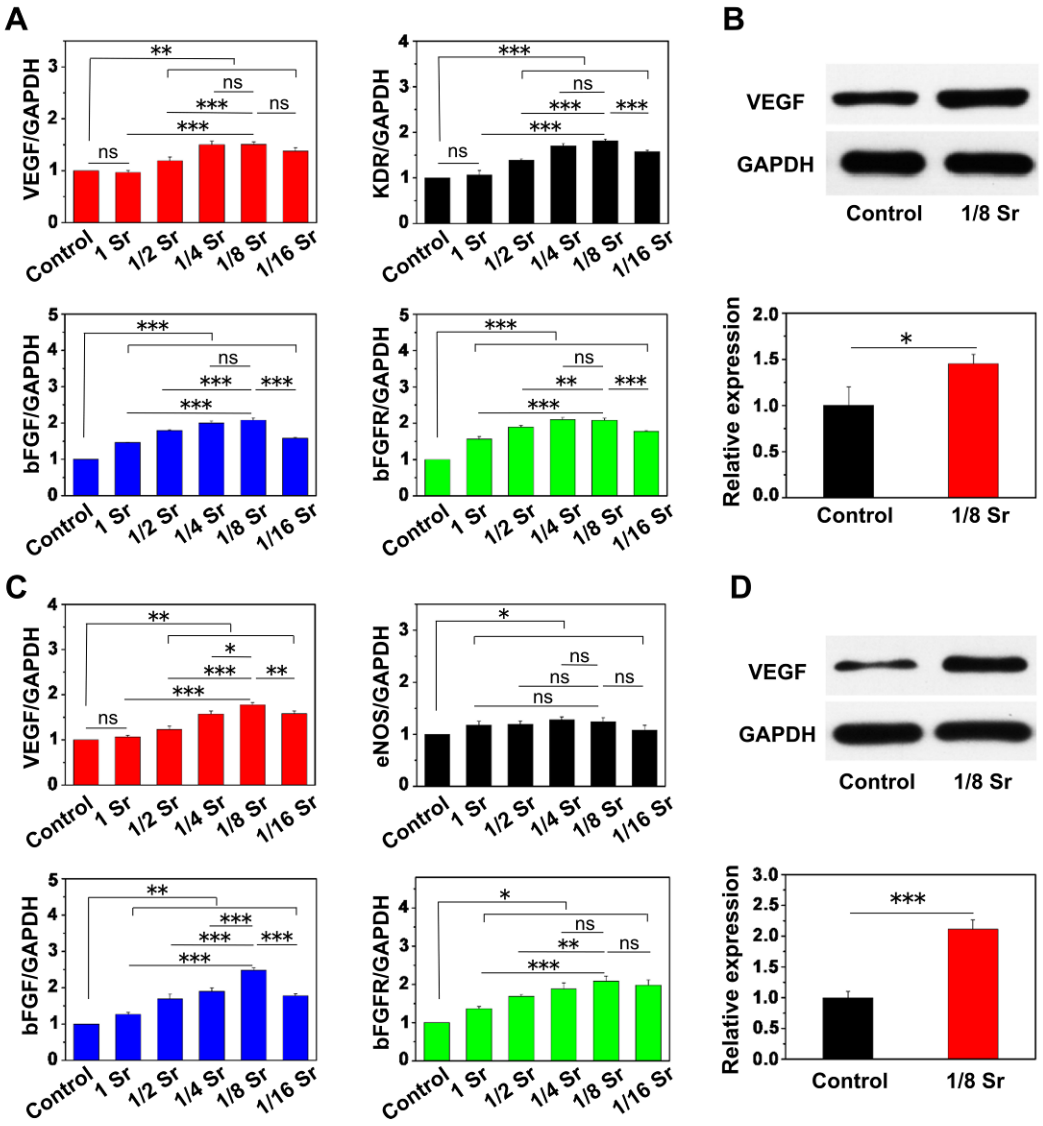


Fig. S3. Sr ions stimulate the expression of angiogenesis-related genes and protein in HUVECs and HDFs. (**A**) qRT-PCR analysis of the angiogenesis-related genes expression (VEGF, KDR, bFGF and bFGFR) in the HUVECs cultured with Sr ions at different concentrations for 3 days. (**B**) Western blot analysis of the VEGF expression in the HUVECs after cultured with the medium with or without Sr ions for 5 days. The experiments were conducted in triplicate. (**C**) qRT-PCR analysis of the angiogenesis-related genes (VEGF, eNOS, bFGF and bFGFR) expression in HDFs after cultured with Sr ions at different concentrations for 3 days. (**D**) Western blot analysis of the VEGF expression in HDFs cultured with the medium with or without Sr ions after 5 days. Experiments were conducted in triplicate. All data were presented as means ± SEM. An unpaired t test was used to compare between any two groups. One-way ANOVA was used to compare between three or more groups. *P < 0.05, **P < 0.01, ***P < 0.001; ns, no statistical significance.

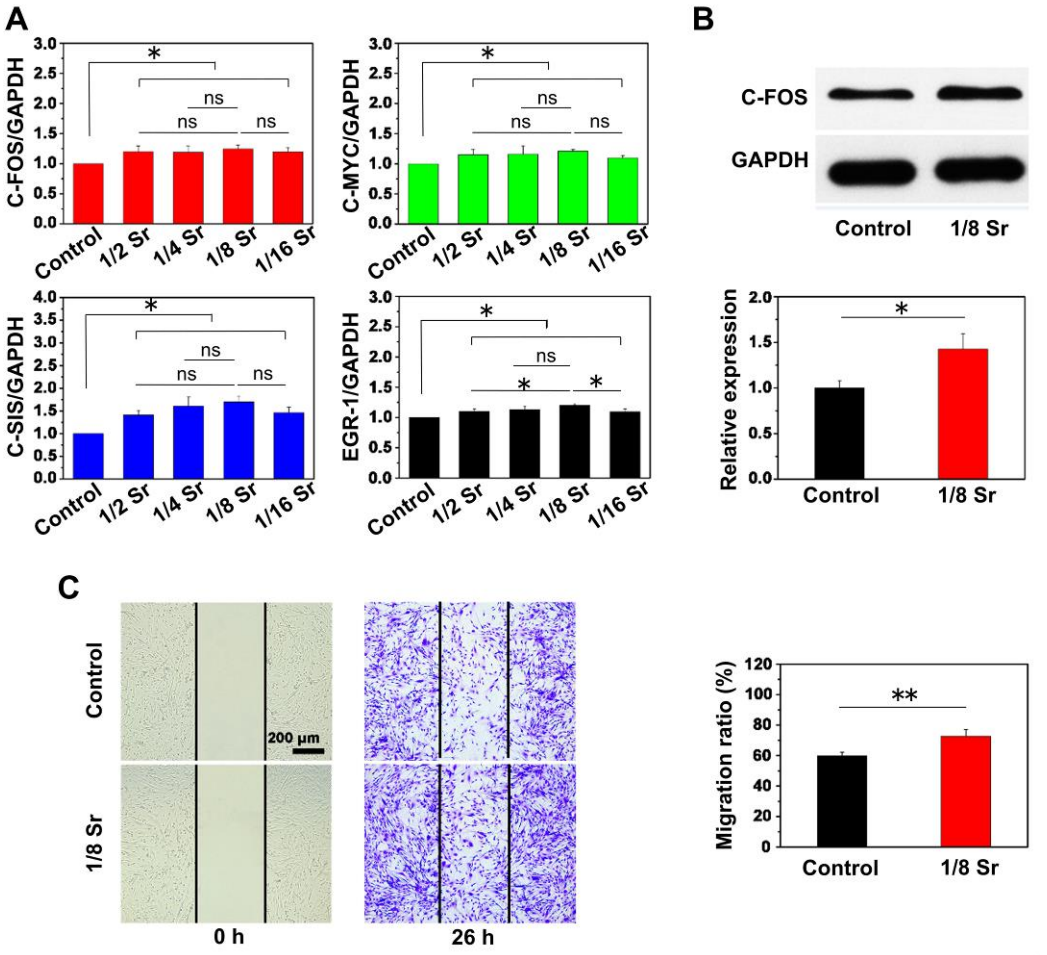


Fig. S4. Sr ions stimulate the expression of proliferation-related genes and migration of HUVSMCs. (**A**) qRT-PCR analysis of the expression of proliferation-related genes (C-FOS, C-MYC, C-SIS and EGR-1) in HUVSMCs cultured with different concentrations of Sr ion after 3 days. (**B**) Western blot analysis of the expression of proliferation related protein C-FOS in HUVSMCs cultured with the medium with or without Sr ions after 5 days. (**C**) Images and quantitative analysis of mono layer HUVSMCs in the medium supplemented with different concentrations of Sr ions after scratching (0 h) and healing for 26 h. Experiments were conducted in triplicate. All data were presented as means ± SEM. An unpaired t test was used to compare between any two groups. One-way ANOVA was used to compare between three or more groups; $*P < 0.05$, $**P < 0.01$; ns: no statistical significance.

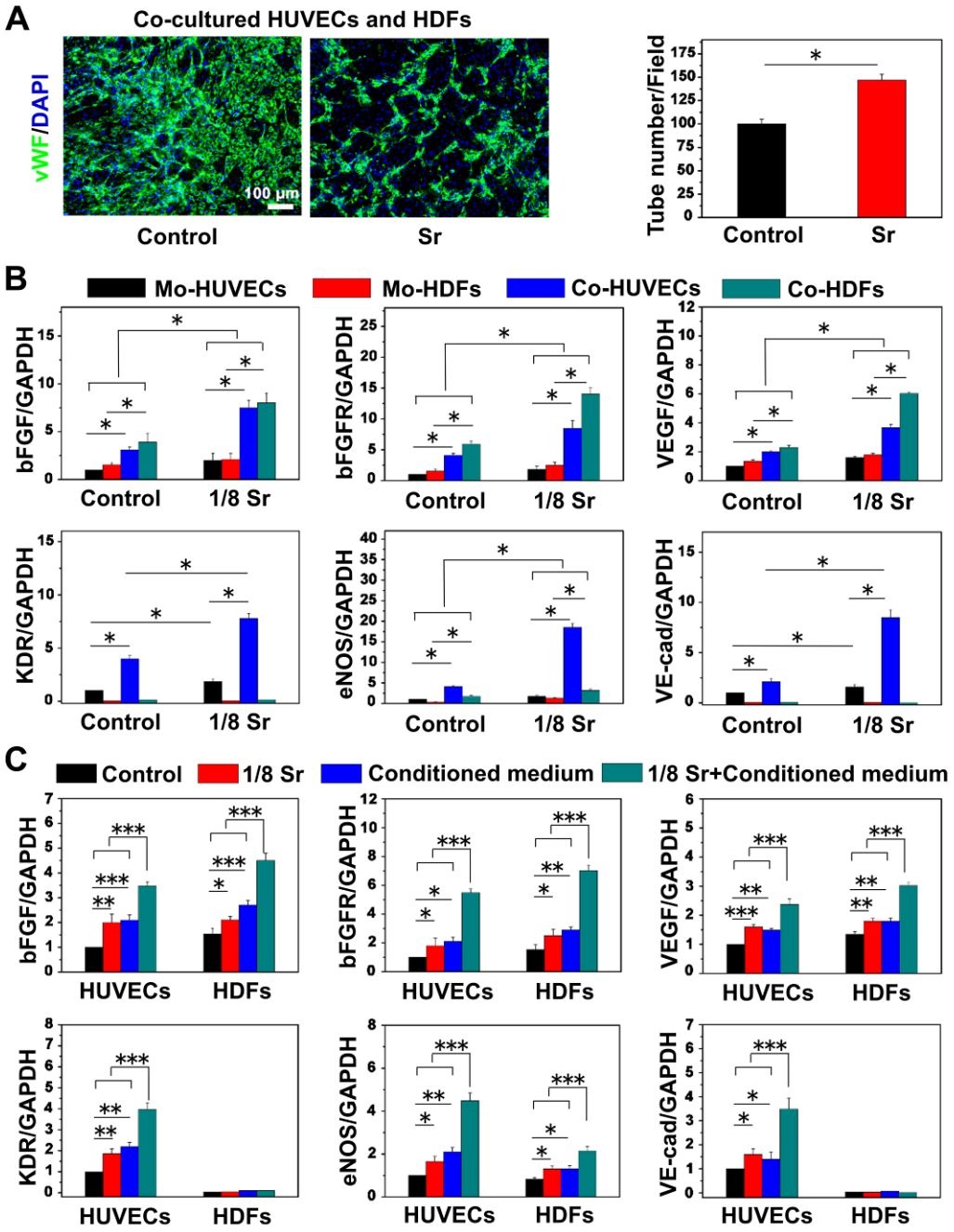
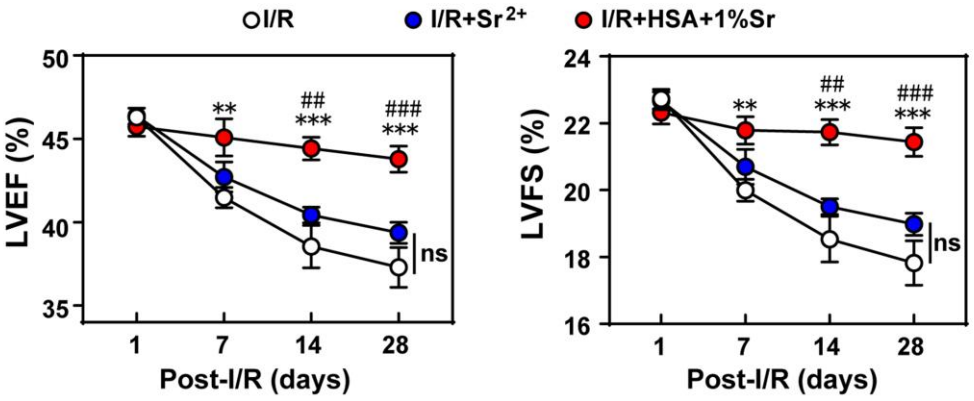


Fig. S5. Sr ions stimulate paracrine-mediated angiogenesis effects of HUVECs and HDFs after 3 days co-culture. (**A**) Images and quantitative analysis of vWF-staining for the tube formation ability of co-cultured HUVECs with control medium and Sr ions containing medium (10 pictures for each group). An unpaired t test was used for statistical analyses. **P* < 0.05. (**B**) qRT-PCR analysis of the expression levels of angiogenesis-related genes (bFGF, bFGFR, VEGF, KDR, eNOS, and VE-cad) in HUVECs and HDFs that acquired from mono-cultured cells or co-cultured cells in each treatment group. Experiments were conducted in triplicate. An unpaired t test was used for statistical analyses. All data were presented as means ± SEM. **P* < 0.05 was considered statistically significant. Mo: mono-cultured, Co: Co-cultured. (**C**) qRT-PCR analysis of the expression levels of angiogenesis-related genes (bFGF, bFGFR, VEGF, KDR, eNOS, and VE-cad) in HUVECs treated with normal medium and conditioned medium from HDFs with or

without Sr ions as well as in HDFs treated with normal medium and conditioned medium from HUVECs with or without Sr ions. Experiments were conducted in triplicate. One-way ANOVA was used for statistical analyses. All data were presented as means ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

A



B

The concentration of Sr ions in post-I/R hearts

Treatments	Samples	1 day post-I/R	7 day post-I/R
I/R	Heart (μg/g)	1.040 ± 0.302	1.035 ± 0.204
	Serum (μg/mL)	4.010 ± 0.324	3.504 ± 0.430
I/R+Sr^{2+}	Heart (μg/g)	1.187 ± 0.399	1.207 ± 0.278
	Serum (μg/mL)	4.213 ± 0.510	3.928 ± 0.631
I/R+HSA+1%Sr	Heart (μg/g)	21.082 ± 4.003	16.055 ± 3.105
	Serum (μg/mL)	8.201 ± 0.346	5.510 ± 0.541

C

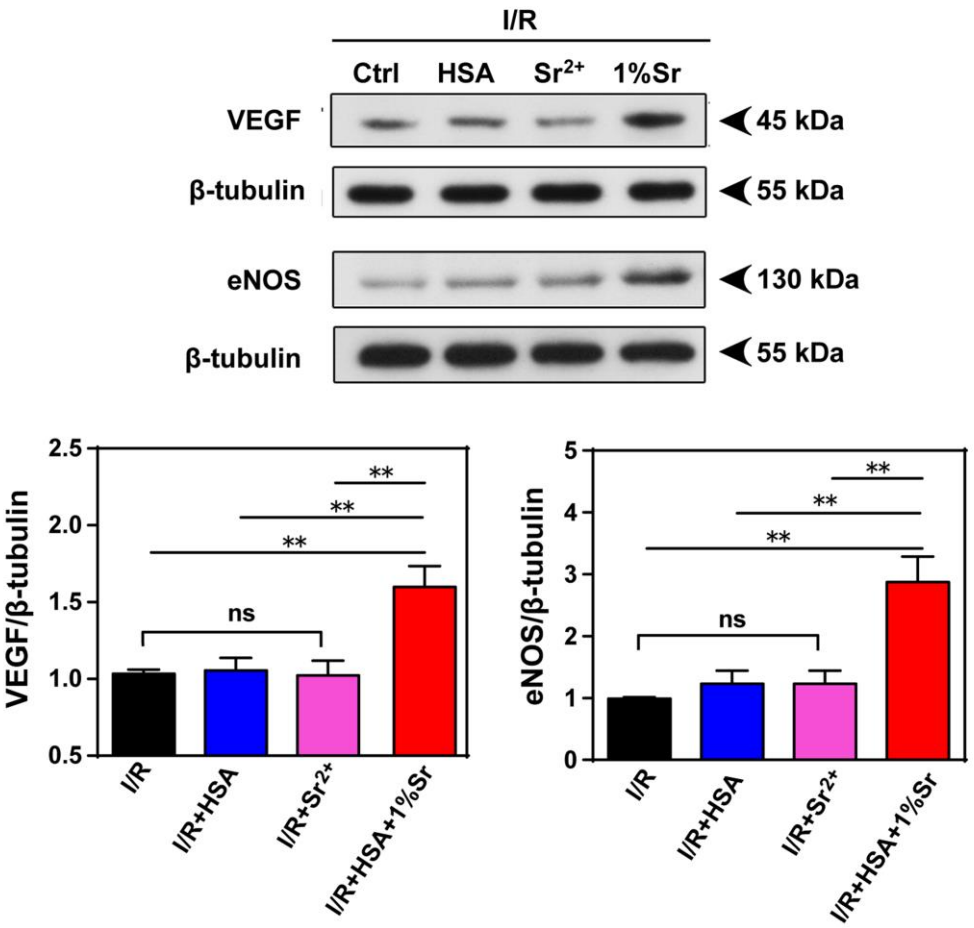


Fig. S6. The $SrCO_3$/HSA composite hydrogel was better than Sr ions-alone for the recovery of mice cardiac function post-I/R. (**A**) Cardiac function measured by the percentage of LVEF and LVFS at day 1, day7, day14, and day 28 post-I/R. n = 5-6 each. Comparisons were performed using two-way ANOVA. $^{**}P < 0.01$, $^{***}P < 0.001$ were considered statistically significant vs. I/R group, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. I/R + Sr^{2+} group. ns: no statistical significance. (**B**) The concentrations of Sr ions in heart and cardiac serum resected from the mice injected with Sr ions with or without hydrogel at day 1, and day 7 post-I/R. n = 3 each. (**C**) Representative and averaged western blot analysis for VEGF and eNOS in left ventricular heart tissues after treatment at day 7 post-I/R; n = 3 hearts each. Ctrl: I/R control; HSA: I/R + HSA; Sr^{2+}: I/R + Sr^{2+}; 1%Sr: I/R + HSA + 1%Sr. One-way ANOVA was used for statistical analyses. $^{**}P < 0.01$; ns: no statistical significance. All data were presented as means ± SEM.

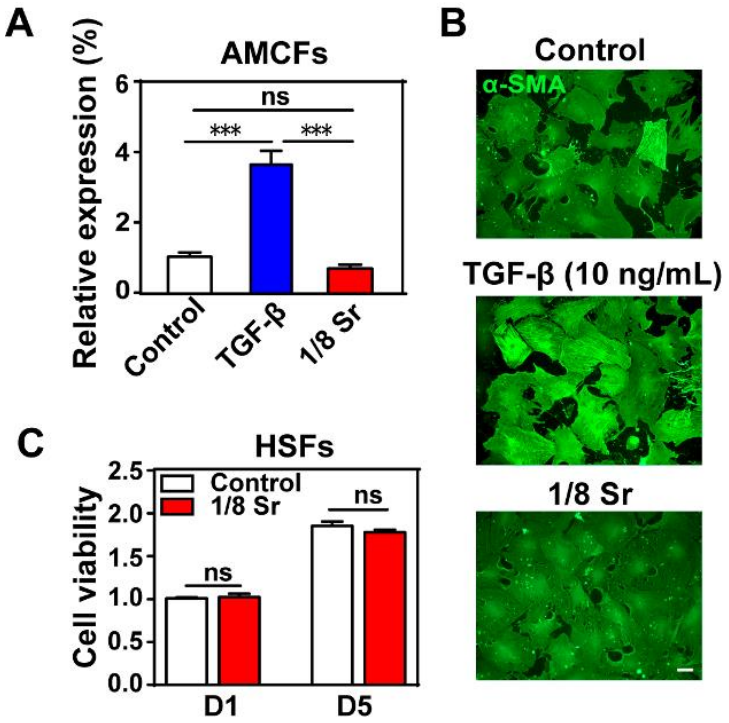


Fig. S7. Sr ions have few effects in the activation of AMVFs and proliferation of HSFs. (**A**) The determination of α-SMA mRNA expression levels by qRT-PCR; n = 4 plates of cells per condition. One-way ANOVA was used for statistical analyses (**B**) The representative images of α-SMA staining in AMVFs treated with TGF-β or Sr ions for 48 h, scale bar = 10 μm. (**C**) The viability of HSFs measured by CCK8 in the medium with or without Sr ions. Experiments were conducted in triplicate. Two-way ANOVA was used for statistical analyses. All data were presented as means ± SEM. ***$P < 0.001$; ns: no statistical significance.

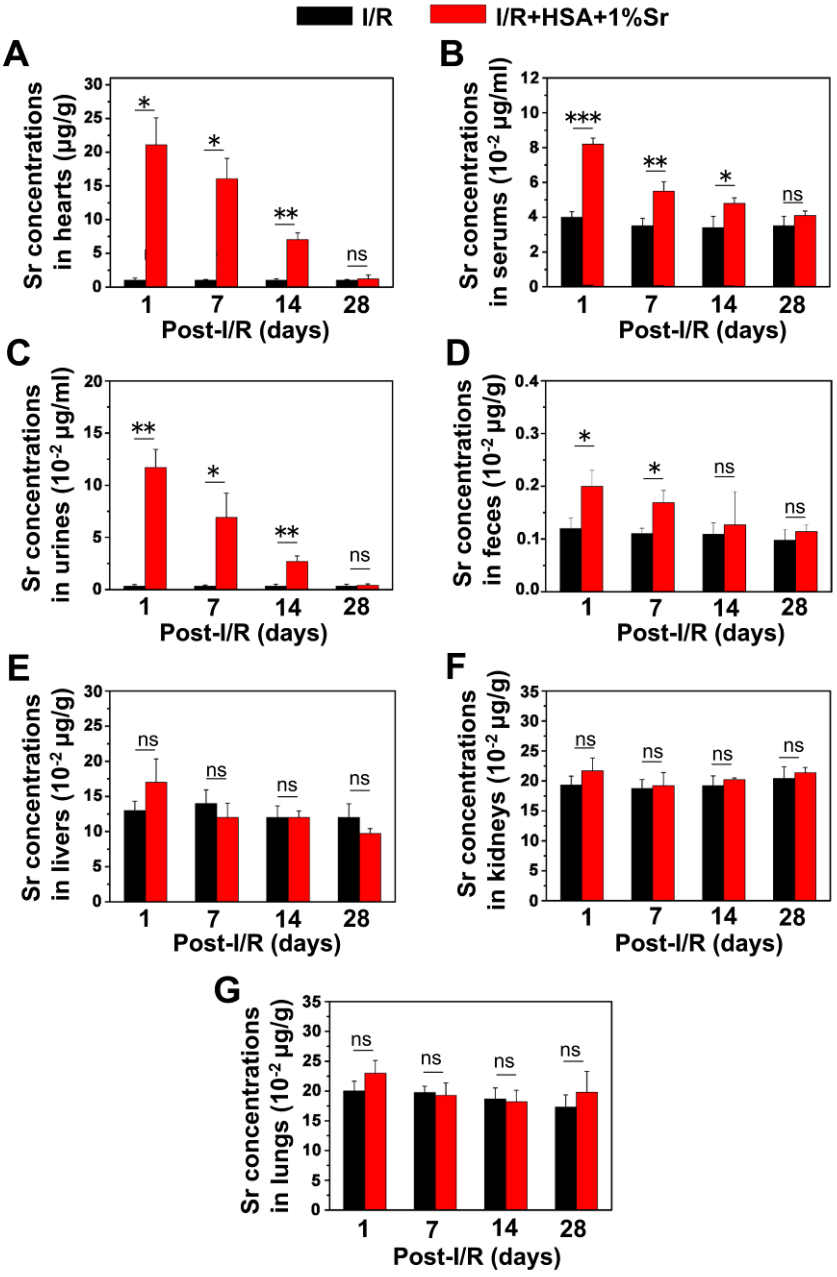


Fig. S8. Sr ions metabolize mainly through urine after myocardium injected. The concentrations of Sr ion in various organs/tissues resected from the mice injected with $SrCO_3$/albumin composite hydrogels at day 1, day 7, day 14, and day 28 post-I/R. (**A**) hearts, (**B**) cardiac serums, (**C**) urines, (**D**) feces, (**E**) livers, (**F**) kidneys and (**G**) lungs. n = 3 for each group and time point. All data are presented as means ± SEM. Comparisons were performed using two-way ANOVA, **P* < 0.05, ***P* < 0.01, ****P* < 0.001; ns: no statistical significance.

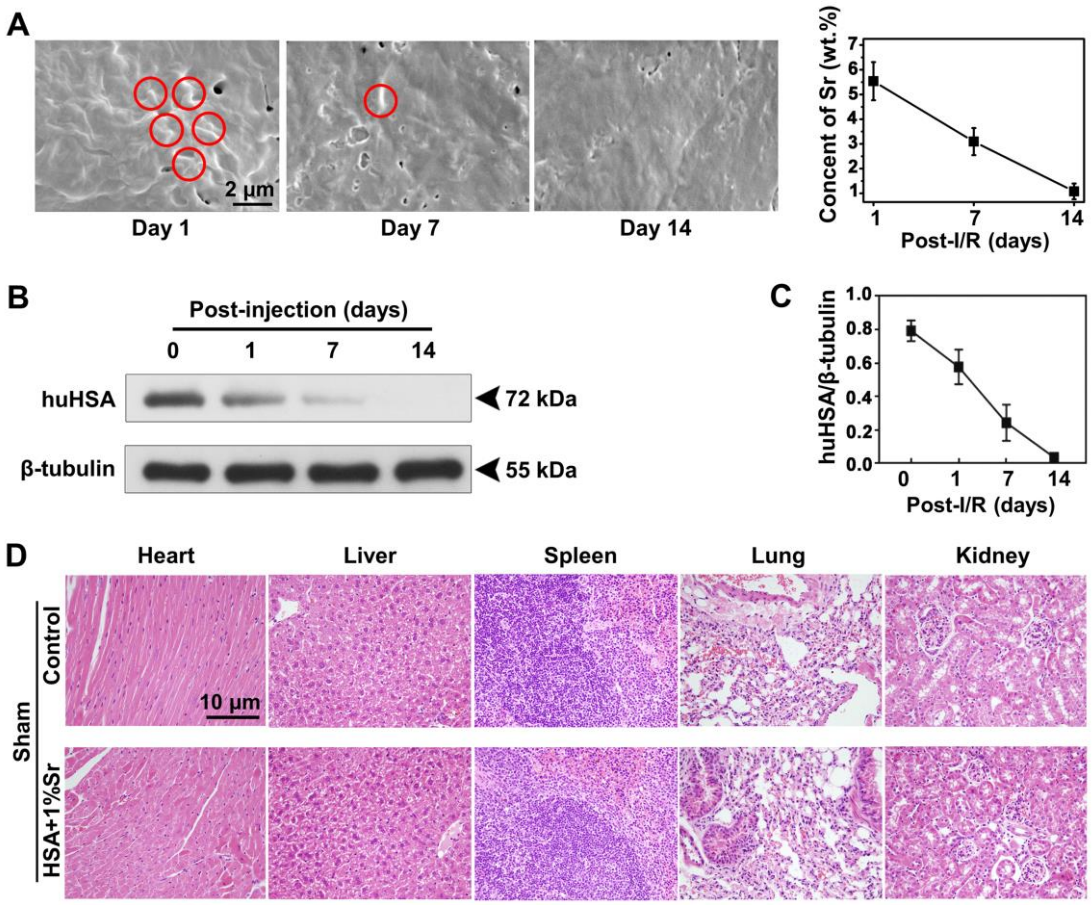


Fig. S9. The degradation analysis of $SrCO_3$/HSA composite hydrogels after injected into I/R hearts. (A) Representative SEM analysis for $SrCO_3$ particles (red circle) in left ventricular heart tissues after $SrCO_3$/albumin composite hydrogels treatment at day 1, 7, and 14 post-I/R and EDS analysis of contents in heart tissue samples obtained from 3 different heart slides in each group; n = 3 for each group and time point. **(B-C)** Representative and averaged western blot analysis for huHSA and β-tubulin in left ventricular tissues of the mice treated with $SrCO_3$/albumin composite hydrogels at day 1, day 7, and day 14 post-I/R; n = 3 for each group and time point. **(D)** Histopathological analysis of the main organs resected from the Sham mice with intramocardial injection of $SrCO_3$/albumin composite hydrogels at day 14; n = 3 each. All data are presented as means ± SEM.

Table S1

The concentrations of Sr ions and Ca ions in the culture medium supplemented with extracts of SrO and with serial dilutions for NRCMs, HUVECs, HDFs and HUVSMCs culture respectively (μg/mL)

	Control	1	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256
Sr^{2+} (NRCMs)	0	170.16	84.73	42.36	21.18	10.59	5.30	2.65	1.32	0.66
Ca^{2+} (NRCMs)	40.18	40.18	40.18	40.18	40.18	40.18	40.18	40.18	40.18	40.18
Sr^{2+} (HUVECs)	0	170.32	85.16	42.58	21.29	10.65	5.32	2.66	1.33	0.66
Ca^{2+} (HUVECs)	57.76	57.76	57.76	57.76	57.76	57.76	57.76	57.76	57.76	57.76
Sr^{2+} (HDFs)	0	169.46	84.73	42.36	21.18	10.59	5.30	2.65	1.32	0.66
Ca^{2+} (HDFs)	70.62	70.62	70.62	70.62	70.62	70.62	70.62	70.62	70.62	70.62
Sr^{2+} (HUVSMCs)	0	170.28	85.14	42.57	21.28	10.64	5.32	2.66	1.33	0.67
Ca^{2+} (HUVSMCs)	56.83	56.83	56.83	56.83	56.83	56.83	56.83	56.83	56.83	56.83

Table S2

Primer sequences for the genes observed in this study

Gene	Forward primer	Reverse primer
Myh6	TCAAGCGGGAGAACAAGAACCT	CTCCAGCTCGTGCACATTTTTAC
Myh7	ACCCCTACGATTATGCG	GTGACGTACTCGTTGCC
Cacna1a	CTCTTGCGGATGGACCTACC	GCTCCACCCTTTGCGATTTTGATA
Gja1	GCTATGACAAGTCTTTCCCA	CAGTTTCTCTTCCTTTCGCA
Tnnt2	ATGATGCATTTTGGGGGTTA	CAGCACCTTCCTCCTCTCAG
Gapdh	GGCATCGTGGAAGGGCTCAT	GGGATGACCTTGCCCACAG
VEGF	TATGCGGATCAAACCTCACCA	CACAGGGATTTTTCTTGTCTTGCT
KDR	CCCAGGCTCAGCATACAAAAAGAC	CCAGTACAAGTCCCTCTGTCCC
VE-cad	GGCTCAGACATCCACATAACC	CTTACCAGGGCGTTCAGGGAC
eNOS	TGTCCAACATGCTGCTGGAAATTG	AGGAGGTCTTCTTCCTGGTGATGCC
bFGF	CAATTCCCATGTGCTGTGAC	ACCTTGACCTCTCAGCCTCA
bFGFR	GACGGCTCCTACCTCAA	GCTGTAGCCCATGGTGTTG
PDGFB	CCTCATAGACCGCACCAAC	CGATCTTTCTCACCTGGACA
PDGFRB	AAGAACTGCGTCCACAGAGA	TAGAACTGCTCGTTCATGGG
C-FOS	ACTCCAAGCGGAGACAGACC	TGAGCTGCCAGGATGAACTC
C-MYC	GCCAGAGGAGGAACGAGCTA	TGGACGGACAGGATGTATGC
C-SIS	GATGATCTCCAACGCCTGCT	TCTTCCACGAGCCAAGCTCT
EGR-1	ACCCCTCTGTCTACTATTAAGGC	TGGGACTGGTAGCTGGTATTG
α-SMA	ACGAACGCTTCCGCTGC	GATGCCCGCTGACTCCAT

GAPDH	GATTTGGTCGTATTGGGCG	CTGGAAGATGGTGATGG