

Supplementary Material for

A Spatially Engineered Bilayer Microneedle Patch for Sequential Anti-apoptotic and Anti-fibrotic Therapy After Myocardial Infarction

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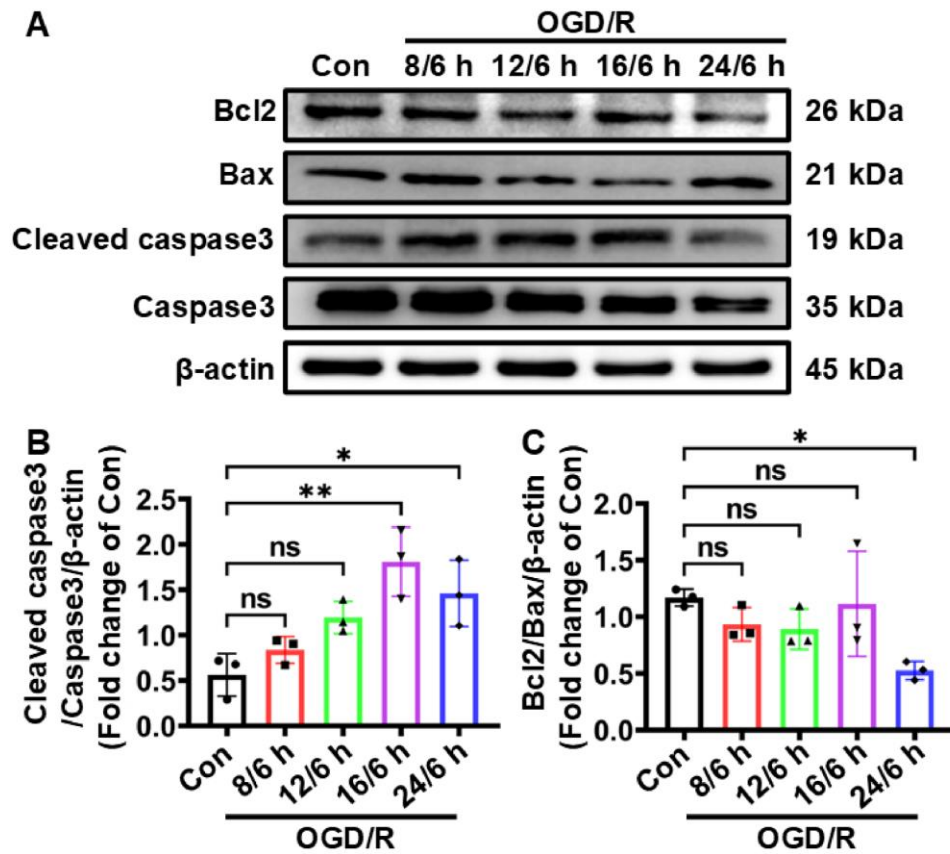
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23 This supplemental material includes Figure S10-S3, and Video S1-S2.

24 Supplemental Figure and Figure Legends



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26 **Figure S1. Establishment of an *in vitro* oxygen-glucose deprivation/reperfusion**
27 **model.**

28 (A-C) Western blotting and quantitative analysis of Bcl2, Bax, Cleaved caspase3, and
29 Caspase3 protein levels in NRCMs subjected to 8, 12, 16, or 24 h of oxygen-glucose
30 deprivation followed by 6 h of reoxygenation (n = 3).

31 Quantitative data were presented as mean ± SEM. Statistical significance was assessed
32 using one-way ANOVA followed by Tukey's post hoc test. Statistical significance was
33 indicated as * $P < 0.05$ and ** $P < 0.01$, while ns denoted no significant difference.

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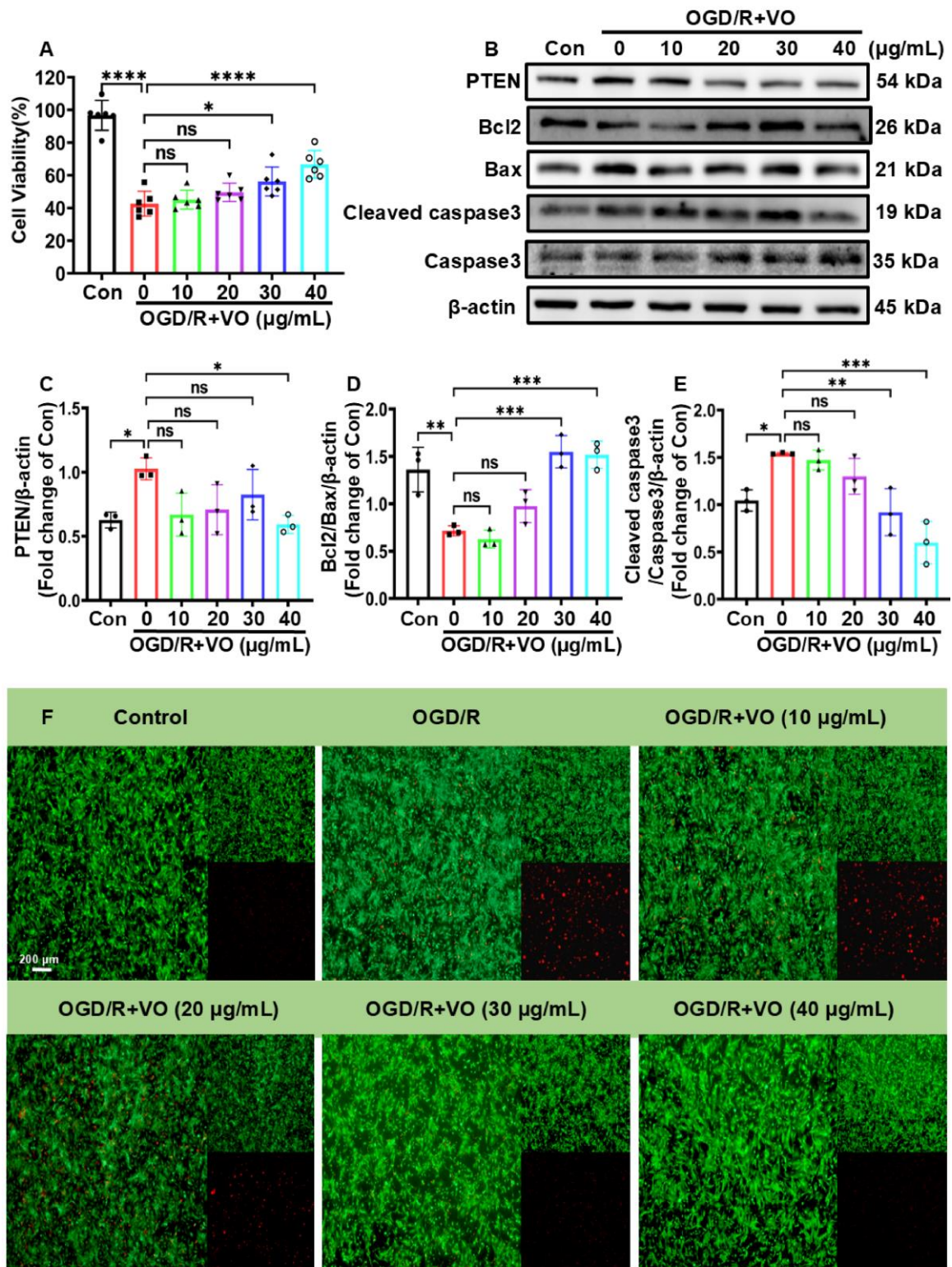


Figure S2. Therapeutic effects of VO-OHpic on NRCMs under OGD/R conditions.

(A) Cell viability of NRCMs detected by CCK-8 assay after treatment with VO-OHpic at concentrations of 10, 20, 30, 40 μg/ml for 24 h under OGD conditions, followed by

39 6 h of reoxygenation (n = 5).

40 **(B-E)** Western blotting and quantitative analysis of PTEN, Bcl2, Bax, Cleaved caspase3
41 and Caspase3 protein levels in NRCMs treated with VO-OHpic at concentrations of 10,
42 20, 30, 40 µg/ml for 24 h under OGD conditions, followed by 6 h of reoxygenation (n
43 = 3).

44 **(F)** Representative fluorescence images of NRCMs treated with VO-OHpic at
45 concentrations of 10, 20, 30, 40 µg/ml for 24 h under OGD conditions, followed by 6 h
46 of reoxygenation, after double staining with calcein AM and propidium iodide. Scale
47 bar: 200 µm.

48 Quantitative data were presented as mean ± SEM. Statistical significance was assessed
49 using one-way ANOVA followed by Tukey's post hoc test. Statistical significance was
50 indicated as * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$, while ns denoted no
51 significant difference.

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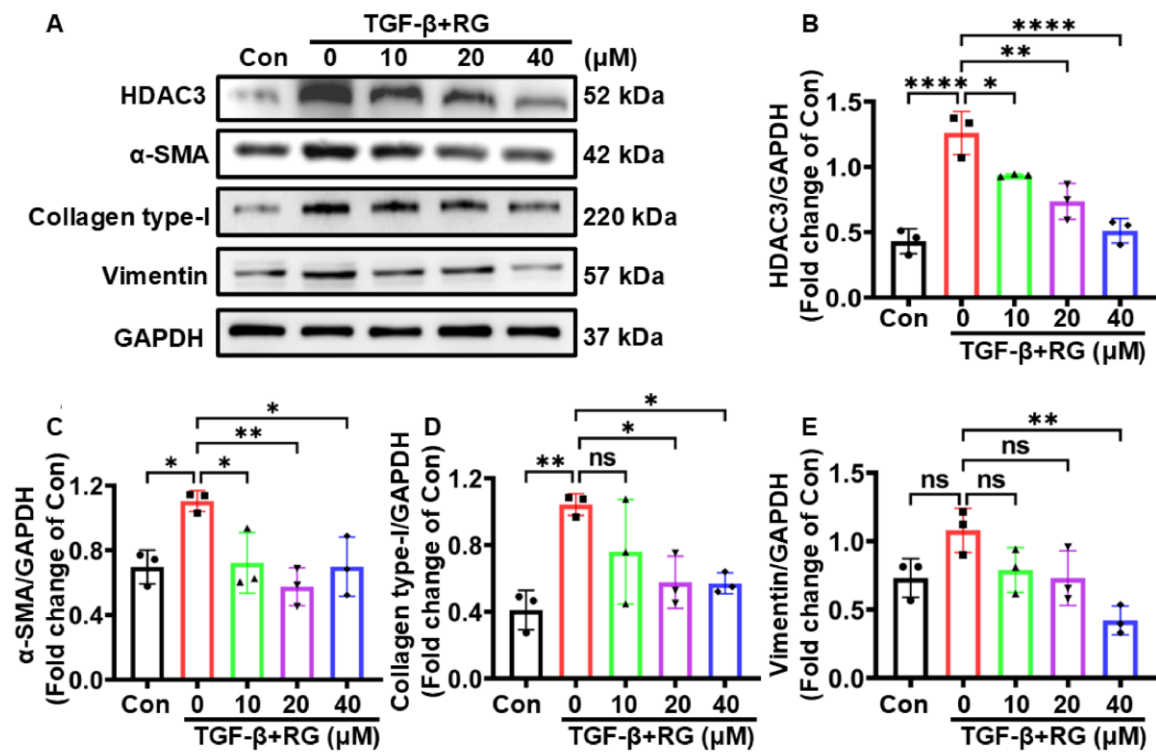


Figure S3. Anti-fibrotic effects of RGFP966 in NRCFs stimulated with TGF-β.

(A-E) Western blotting and quantitative analysis of HDAC3, α-SMA, collagen type I and vimentin protein levels in NRCFs treated with RGFP966 at concentrations of 10, 20, 40 μM for 48 h in the presence of 100 ng/ml of TGF-β (n = 3).

Quantitative data were presented as mean ± SEM. Statistical significance was assessed using one-way ANOVA followed by Tukey's post hoc test. Statistical significance was indicated as **P*<0.05, ***P*<0.01, ****P*<0.001, and *****P*<0.0001, while ns denoted no significant difference.

Video S1. Establishment of a myocardial infarction model in a rat.

Video S2. Implantation of GelMA-CSMA bilayer MN patch following MI in a rat.