Supplementary Information

Materials and methods

Mice and animal procedures

All experiments with mice were performed according to protocols approved by the Institutional Animal Care and Use Committees of Children's Hospital Boston and Shanghai Institute for Biological Science, Chinese Academy of Sciences. *Wt1*$^{CreERT2/+}$, *Rosa26*$^{mTmG/+}$ mice were described previously [1]. For embryonic studies, noon of the day of the vaginal plug was defined as E0.5. Tamoxifen (Sigma) was administered at 1 mg / 10g body weight by gavage to preganant mice at E10.5 to induce Cre. For adult mice, 4 mg tamoxfen was administered twice each week for 2-3 weeks to induce Cre.

Myocardial infarction was performed one week after Tam administration by ligation of the left anterior descending coronary artery as previously described [2]. Briefly, mice were anaesthetized with isofluorane blended with oxygen. A 20-G catheter was connected to mouse ventilator and inserted into trachea under the help of forceps. We administered 100 μl 0.1% lidocaine under the chest skin before chest open. After chest was opened, the left anterior descending artery was ligated with 7-0 silk suture. Before closing chest cavity and muscle, we overinflated the lungs to assist in removal of air in the chest cavity. Buprenorphine was administered at 0.1 mg/kg post MI at every 12 hours for two days. Survival rate one week after MI was 70-75%. TB4 (RegeneRx) was administered intraperitoneally (i.p.) at 300 μg in PBS each day for 7 days.

Cell isolation and culture

E14.5 embryonic heart or adult heart were dissociated and digested into single cells using collagenase as described previously [3]. Briefly, hearts were incubated in digestion solution (0.08% collagenase IV (Sigma) and 0.05% trypsin (Invitrogen) in HBSS) at 37°C with gentle

rocking. Supernatant was removed every 5-7 minutes and neutralized with horse serum. After 5-7 rounds of digestion, dissociated cells were pelleted and washed once with PBS. The cells were then passed through a 70 micron filter and the single cell suspensions were FACS separated into GFP+ or GFP- populations as described previously [4]. For subsequent culturing, FACS-purified cells were pelleted and resuspended in DMEM or mesenchymal stem cell growth medium (MSCGM, Lonza) supplemented with 10% FBS and 1% penicillin, streptomycin and glutamine. Cells were cultured in 24 well plates coated with 1% gelatin. After overnight culture in a 37°C CO2 incubator, the cells were used for immunostaining.

qRT-PCR

Isolated cells were collected in Trizol (Invitrogen), and RNA was purified according the Invitrogen Trizol protocol. RNA was reverse transcribed into cDNA using Superscript III (Invitrogen) and oligo (dT) primers. For quantitative RT-PCR, Sybr Green detection chemistry was used. Expression values are relative to internal control *Gapdh*. *Gapdh* forward, TTGTCTCCTGCGACTTCAAC, reverse, GTCATACCAGGAAATGAGCTTG; *Wt1* forward, AGACACACAGGTGTGAAACCA, reverse, ATGAGTCCTGGTGTGGGTCT; *Tbx18* forward, AACAGAATGGGTTTGGAAGC, reverse, ACTTGTGTTGCCTTGCTTTG; *Msln* forward, TGGACAAGACCTACCCACAA, reverse, TGGTGAGGTCACATTCCACT.

Immunostaining and immunohistochemistry

Immunostaining was done as previously described [5]. For each heart, transverse sections were collected at 6 different level below the ligature. Each level, we collected 3 different slides for further analysis. Attached cells or tissue slides were washed with PBS twice and fixed in 4% PFA at 4°C for 10 minutes, followed by PBS wash twice. Slides for immunostaining were first blocked by 5% normal donkey serum and 0.1% Triton X-100 in PBS (PBSST) for half an hour,

followed by antibodies (1:100 in PBSST) incubation at 4°C overnight. After washing three times, slides were stained with Alexa-conjugated secondary antibody (Invitrogen) and incubated at room temperature for half an hour. After washing three times, the cells were mounted in VectorShield (Vector lab). For weaker signals, we used the ABC system (Vector) to amplify the signal and developed it with DAB or the tyramide signal amplification system (PerkinElmer). Fluorescent images were acquired on a Fluoview FV1000 confocal microscope (Olympus). The antibodies were obtained from the following sources: PECAM, BD Biosciences; WT1, GATA4, TBX18, procollagen I, HSP47, and DDR2, Santa Cruz Biotechnology; cardiac troponin T (TNNT2), Lab Vision; sarcomeric α-Actinin, smooth muscle α actin, and fibronectin, Sigma; calponin, Millipore; FSP1, Dako; Pro-Col1A1, Desmin, Biomeda; Collagen III, Southern Biotech; GFP, Invitrogen. TUNEL was performed with the Roche in situ cell death detection kit.

Statistics

Intergroup differences were analyzed for statistical significance using Student's *t*-test, and $P < 0.05$ was considered to be significant. Values were displayed as mean ± standard error of the mean.

References

[1] Zhou B, Ma Q, Rajagopal S, Wu SM, Domian I, Rivera-Feliciano J *et al*. 2008. Epicardial progenitors contribute to the cardiomyocyte lineage in the developing heart. *Nature* 454 (7200): 109-13; Muzumdar MD, Tasic B, Miyamichi K, Li LLuo L. 2007. A global double-fluorescent Cre reporter mouse. *Genesis* 45 (9): 593-605.

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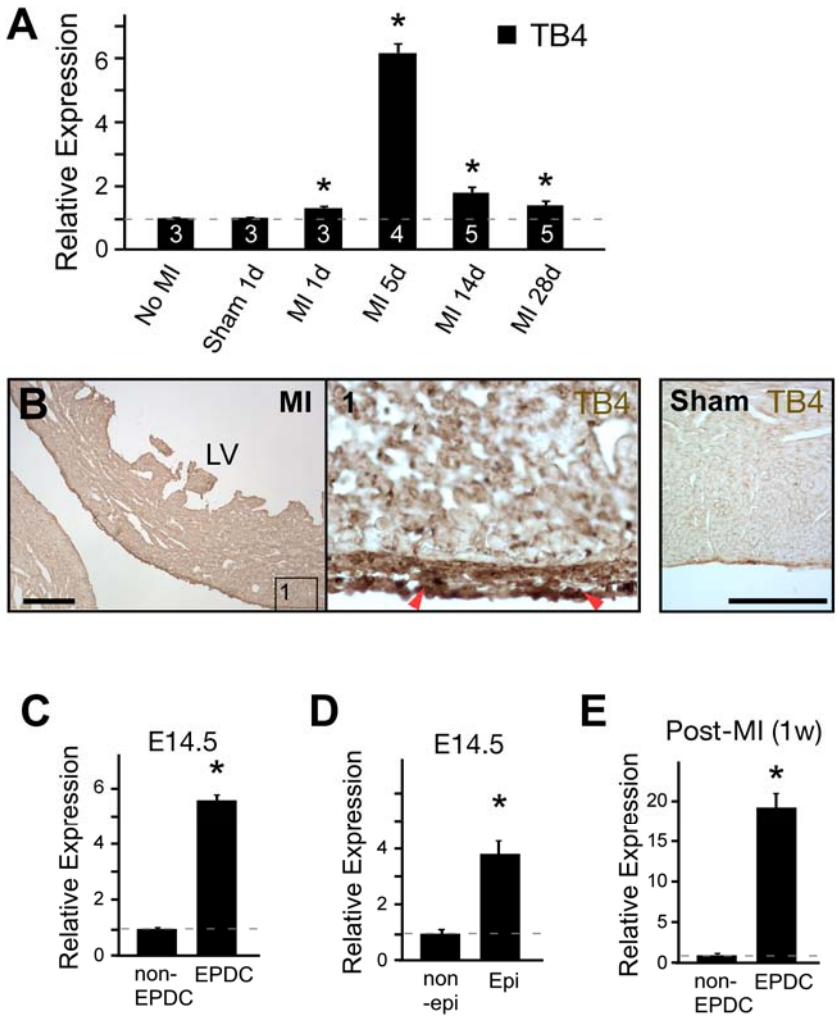
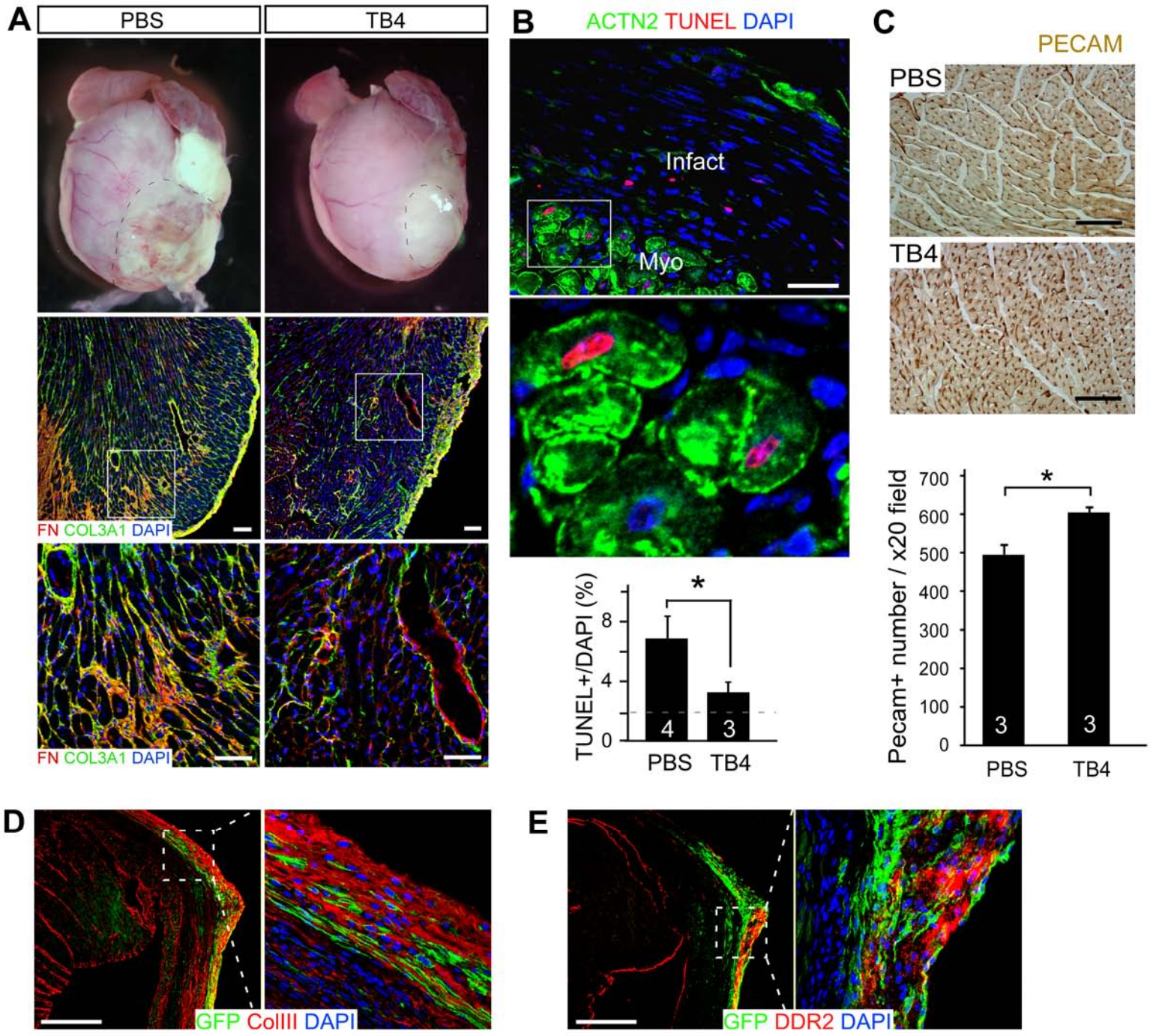
[3] Zhou B, Honor LB, He H, Ma Q, Oh JH, Butterfield C *et al*. 2011. Adult mouse epicardium modulates myocardial injury by secreting paracrine factors. *J Clin Invest* 121 (5): 1894-904.

[4] Zhou B, von Gise A, Ma Q, Hu YWPu WT. 2010. Genetic fate mapping demonstrates contribution of epicardium-derived cells to the annulus fibrosis of the mammalian heart. *Dev Biol* 338 (2): 251-61.

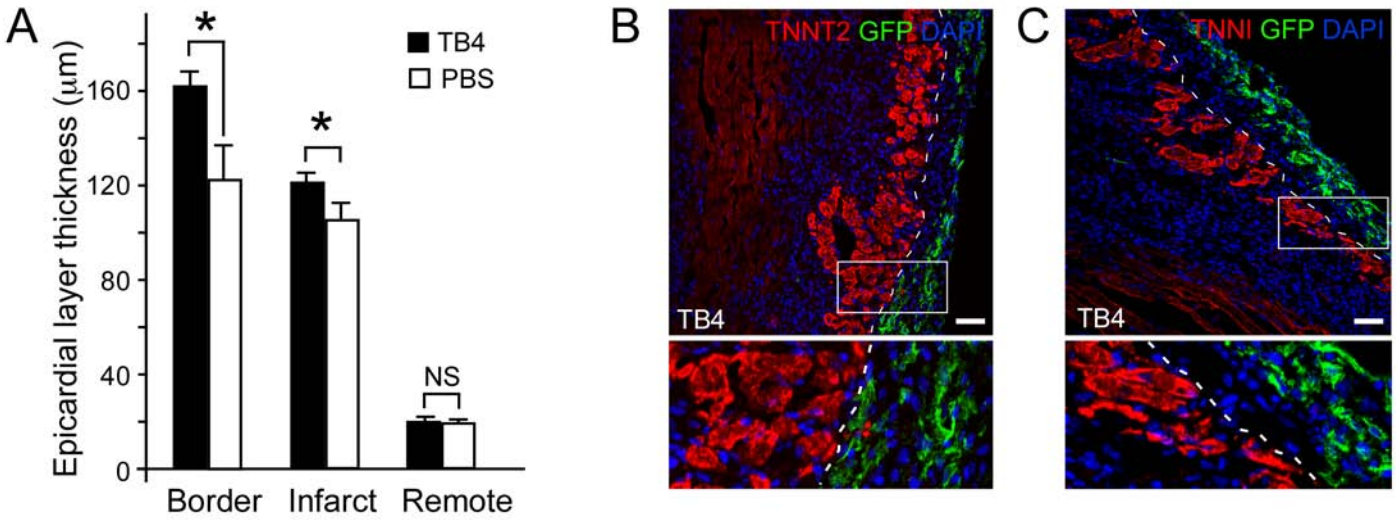
[5] Zhou B, Ma Q, Kong SW, Hu Y, Campbell PH, McGowan FX *et al*. 2009. Fog2 is critical for cardiac function and maintenance of coronary vasculature in the adult mouse heart. *J Clin Invest* 119 (6): 1462-76.



Suppl. Fig. 1. Endogenouse TB4 Expression in the normal and infarcted adult heart. (**A**) qRTPCR of heart samples showed TB4 was reactivated after cardiac injury, with peak around 5 days post myocardium infarction. n = 3 - 5 as indicated; **P* < 0.05. **(B)** TB4 Immunohistochemistry showed enriched TB4 expression epicardium 7 days after myocardium infarction (red arrowheads in insert 1). Bar = 500 μm. LV, left ventricle. **(C)** Enriched expression of TB4 in fetal EPDCs compared with non-EPDCs. qRTPCR of FACS-isolated populations from E14.5 *Wt1*$^{CreERT2/+}$;*Rosa26*$^{mTmG/+}$ embryonic ventricle. n = 3; **P* < 0.01. **(D)** Enriched expression of TB4 in fetal epicardial cells compared to non-epicardial cells. qRTPCR of FACS-isolated populations from E14.5 *Wt1*$^{GFPCre/+}$ embryonic ventricle. n = 3 - 4; **P* < 0.01. (**E**) Enriched expression of TB4 in adult post-MI EPDCs compared with non-EPDCs. qRTPCR of FACS-isolated populations from adult *Wt1*$^{CreERT2/+}$;*Rosa26*$^{mTmG/+}$ hearts 7 days post MI (n = 3).



Suppl. Fig. 2. TB4 and epicardium after MI. **(A)** TB4 reduced fibronectin (FN) and collagen III (COL3A1) immunoreactivity in post-MI myocardium. Bar = 50 μm. Representative of 3 separate hearts per group. **(B)** TUNEL assay showed that TB4 treatment after MI reduced the number of apoptotic cardiomyocytes in the border area. **P* < 0.05; n = 3 - 4. **(C)** Coronary vessel density detected by PECAM staining (brown) was significantly higher in the infarct border zone with TB4 treatment. n = 3, **P* < 0.05. Bar = 200 μm. (**D, E**) In peri-infarct myocardium treated with TB4, EPDCs (GFP+) were colocalized with fibroblast/fibrosis markers Collagen III and DDR2 (red). Bar = 200 μm.



Suppl. Fig. 3. Non-cardiomyocyte fate of epicardial cells after TB4 injection. (**A**) Quantitation of epicardial layer thickness in TB4 and PBS treated *Wt1*$^{CreERT2/+}$;*Rosa26*$^{mTmG/+}$ hearts. n = 4. *P< 0.05; NS, nonsignificant. (**B-C**) Immunostaining of cardiomyocyte markers cardiac troponin T (TNNT2) and cardiac troponin I (TNNI) with GFP, the genetic marker EPDCs, did not identify cells positive for both markers. White dotted line indicates the border between myocardium and the epicardial layer. Bar = 50 μm.