
SUPPLEMENTARY MATERIAL

Thymosin β4 released with functionalized self-assembling peptide activates epicardium and enhances repair of infarcted myocardium

Running head: Activating Epicardium by SAP-released Tβ4

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Table S1. The sequences of the primers

Symbol	Version	Sequence (5’ to 3’)	Length
bFGF	NM_008006.2	(F) GGCTGCTGGCTTCTAAGTG (R) CCAACTGGAGTATTTCCGTGA	101 bp
CD31	NM_001032378.2	(F) GCCAAGGCCAAACAGAAACC (R) CCATGTTCTGGGGGTCTTTAT	178 bp
CNN1	NM_009922.4	(F) CCCACAATCACCACCCGCACAACTA (R) TCATCTCCCCAAACCGTAACCCTAT	165 bp
cTnT	NM_001130174.2	(F) CGGGCGTTGGAAATAGATGA (R) TAGGGGTCAGGCAGAGTACT	165 bp
Cx43	NM_010288.3	(F) AAGGCGTGAGGGAAGTACCA (R) GGAGTAGGCTTGGACCTTGT	215 bp
GATA4	NM_001310610.1	(F) GGGATTCAAACCAGAAAACG (R) GCTGTGCCCATAGTGAGATG	198 bp
HGF	NM_001289458.1	(F) GGGACGGTATCCATCACT (R) TTCGTAGCGTACCTCTGG	186 bp
HIF-1α	NM_001313919.1	(F) TCGGCGAAGCAAAGAGTC (R) CCATCTGTGCCTTCATCTCA	182 bp
IGF-1	NM_001111274.1	(F) TGCCACATCACCGCAGGAT (R) CCACGCCAGGACCACTTT	200 bp
Mylk	NM_139300.3	(F) AAAAACCGTCTGGACTGCAC (R) TCACAGCATTGCCCGTTTTC	145 bp
Nkx2.5	NM_008700.2	(F) CAGTGGAGCTGGACAAAGCC (R) TAGCGACGGTTCTGGAACCA	217 bp
PDGF-BB	NM_011057.3	(F) TTAGCGGGCGAGTGAAGACG (R) GGGAGGACCTGGACAAGGGA	196 bp
Rock1	NM_009071.2	(F) AAGCTTTTGTTGGCAATCAGC (R) AACTTTCCTGCAAGCTTTTATCCA	129 bp
SCF	NM_001347156.1	(F) GTCATTGTTGGCTACGAG (R) CATAACACGAGGTCATCC	145 bp
SDF-1	NM_001012477.2	(F) GTCAGCCTGAGCTACCGA (R) GAAGGGCACAGTTTGGAG	103 bp
Tbx18	NM_023814.4	(F) GTGGAGTCATACGCATTCTGGA (R) GTGAGGATGTGTAGCAGGGACA	141 bp
Tcf21	NM_011545.1	(F) CATTCACCCAGTCAACCTGA (R) CCACTTCCTTCAGGTCATTCTC	71 bp
Vcl	NM_009502.4	(F) AACCAGCCAATGATGATGGC (R) TTGGCTGCTGCAATGATGTC	89 bp
VEGF	NM_001025250.3	(F) ACTATTCAGCGGACTCACCAG (R) TGAGGGAGTGAAGAACCAACC	171 bp
vWF	NM_011708.4	(F) GCGGTGTAAACGGACATCTC (R) ACAGGTTCGGGCATACTCAA	232 bp
WT1	NM_144783.2	(F) GCCTTCACCTTGCACTTCTC	186 bp

		(R) GACCGTGCTGTATCCTTGGT	
α-SMA	NM_007392.3	(F) GGCTCTGGGCTCTGTAAGG (R) CTCTTGCTCTGGGCTTCATC	149 bp
β-actin	NM_007393.5	(F) GGAGATTACTGCTCTGGCTCCTA (R) GACTCATCGTACTCCTGCTTGCTG	150 bp

Table S2. The primary sequences of the self-assembling peptides

Code	Primary sequence
RADA16-I	Ac-RADARADARADARADA-$CONH_2$
RADA-RPR	Ac-$(RADA)_4$GGRPRHQGVMRGDS-$CONH_2$

Table S3. The antibodies used

Antibody	Dilution	Company	Application
Chicken anti-GFP	1:100	Novus Biologics, Littleton, CO, USA	Immunofluorescence
Rabbit anti-Aurora B	1:100	Sigma-Aldrich,Saint Louise, MO, USA	Immunofluorescence
Rabbit anti-CCR7	1:100	Abcam, Cambridge, MA, USA	Immunofluorescence
Rabbit anti-Cx43	1:100	Abcam, Cambridge, MA, USA	Immunofluorescence
Rabbit anti-GFP	1:50	Santa Cruz Biotech, Dallas, TX,USA	Immunofluorescence
Rabbit anti-LYVE-1	1:200	Novus Biologics, Littleton, CO, USA	Immunofluorescence
Rabbit anti-Tbx18	1:100	Abcam, Cambridge, MA, USA	Immunofluorescence
Rabbit anti-Tβ4	1:200	Santa Cruz Biotech, CA, USA	Immunofluorescence
Rabbit anti-WT1	1:100	Santa Cruz Biotech, CA, USA	Immunofluorescence
Mouse anti-CD31	1:100	Abcam, Cambridge, MA, USA	Immunofluorescence
Mouse anti-CD31	1:100	BDBiosciences, San Jose, CA, USA	Immunofluorescence
Mouse anti-CD68	1:100	Santa Cruz Biotech, Dallas, TX,USA	Immunofluorescence
Mouse anti-cTnT	1:200	Santa Cruz Biotech, CA, USA	Immunofluorescence
Mouse anti-Ki-67	1:200	Abcam, Cambridge,	Immunofluorescence

		MA, USA	
Mouse anti-α-SMA	1:100	Abcam, Cambridge, MA, USA	Immunofluorescence
Goat anti-chicken (conjugated with DyLight 488)	1:400	Novus Biologics, Littleton, CO, USA	Immunofluorescence
Goat anti-mouse (conjugated with Alexa Fluor 594)	1:400	Jackson, West Grove, PA, USA	Immunofluorescence
Goat anti-mouse (conjugated with Alexa Fluor 488)	1:400	Jackson, West Grove, PA, USA	Immunofluorescence
Goat anti-mouse (conjugated with Alexa Fluor 647)	1:400	Jackson, West Grove, PA, USA	Immunofluorescence
Goat anti-rabbit (conjugated with Alexa Fluor 488)	1:400	Jackson, West Grove, PA, USA	Immunofluorescence
Goat anti-rabbit (conjugated with DyLight 594)	1:400	Abcam, Cambridge, MA, USA	Immunofluorescence
Mouse anti-cTnT	1:1000	Santa Cruz Biotech, CA, USA	Western Blotting
Mouse anti-β-actin	1:4000	Sigma-Aldrich, Saint Louise, MO, USA	Western Blotting

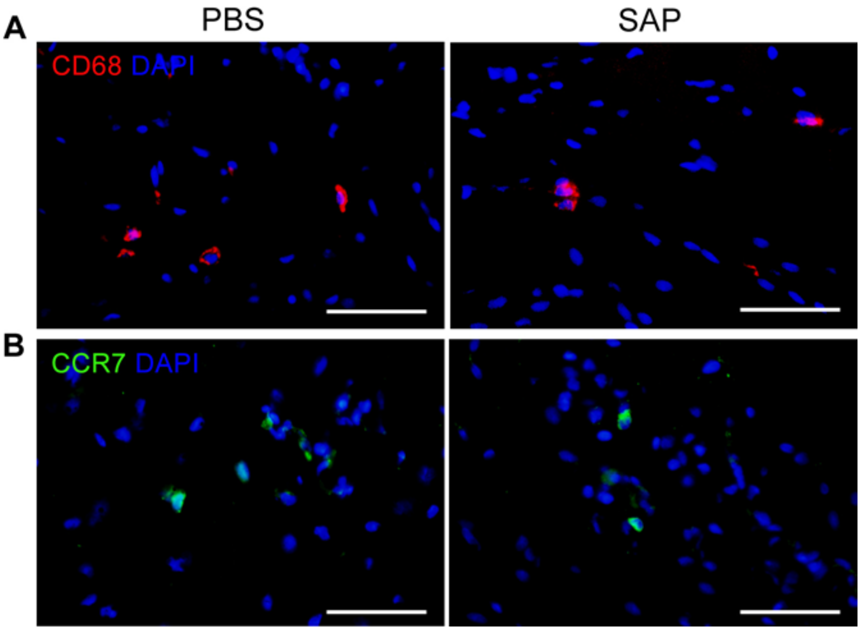

Figure S1. The distribution of CD68$^+$ or CCR7$^+$ immune cells at 1 week after

subcutaneous injection of the SAP. Immunostaining. Scale bar = 50 μm.

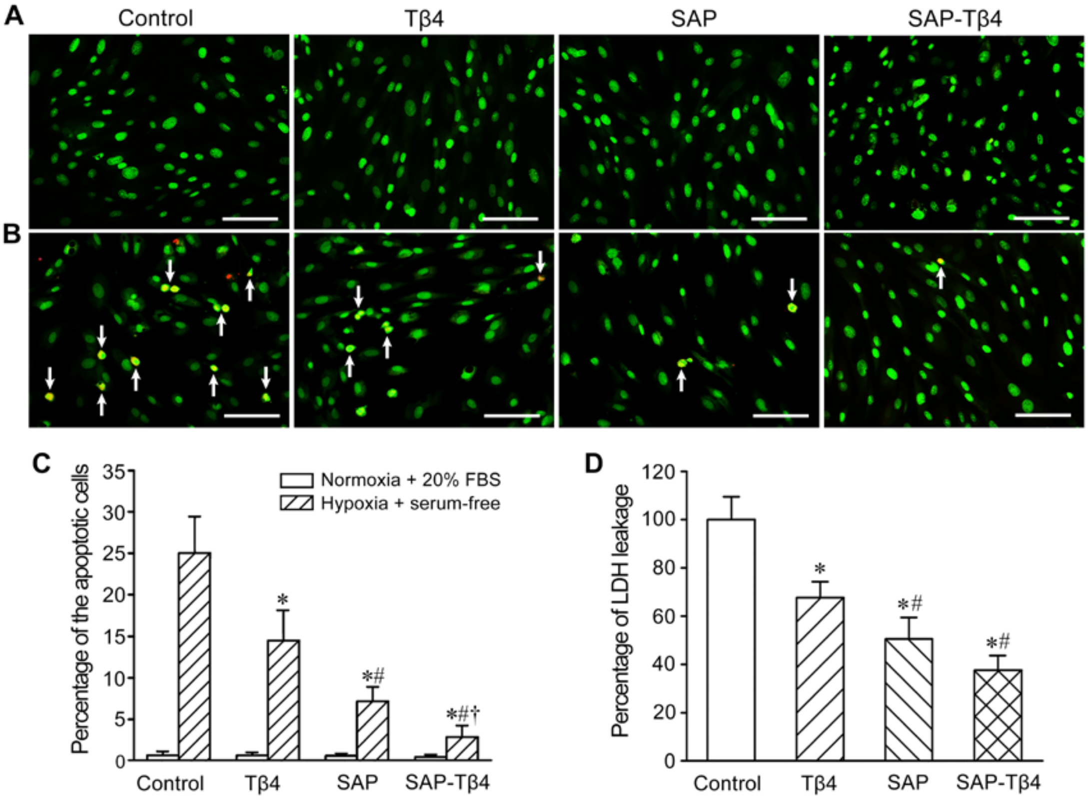

Figure S2. The protection of Tβ4 on survival of EPDCs. (A) EPDCs incubated in normoxic condition. **(B)** EPDCs treated with hypoxia (1% O_2) and serum-free for 12 h. Arrows indicate the apoptotic cells. EB/AO staining. Scale bar = 100 μm. **(C)** Statistical result of the number of the apoptotic cells. n = 5. **(D)** The concentration of LDH in the condition of hypoxia and serum deprivation. n = 5. **p* < 0.01 versus control group; #*p* < 0.05 versus Tβ4 group; †*p* < 0.05 versus SAP group.

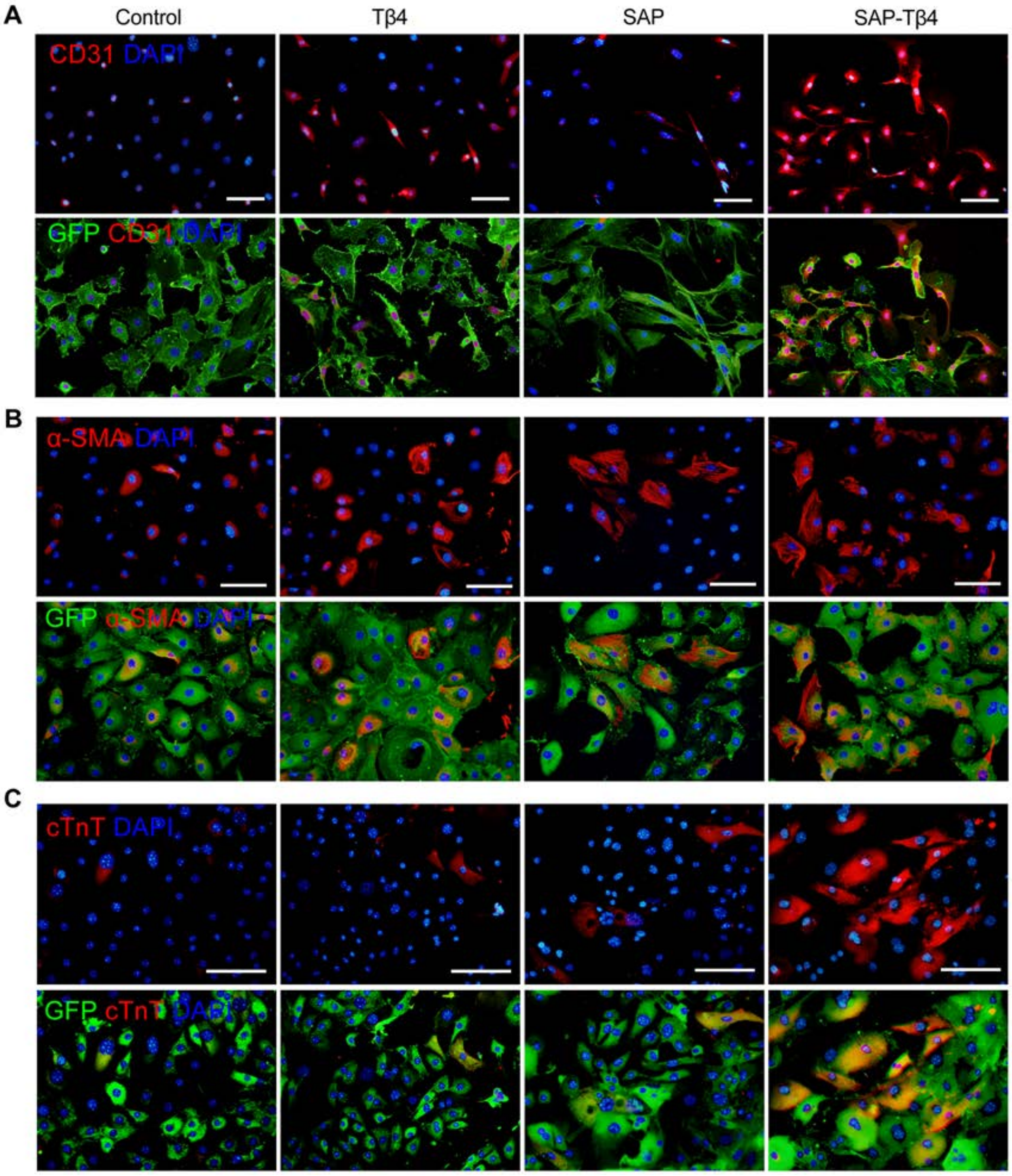

Figure S3. SAP-released Tβ4 promotes differentiation of EPDCs towards cardiovascular cells. (A) CD31$^+$ cells differentiated from EPDCs. **(B)** α-SMA$^+$ cells differentiated from EPDCs. **(C)** cTnT$^+$ cells differentiated from EPDCs. Immunostaining. Scale bar = 50 μm.

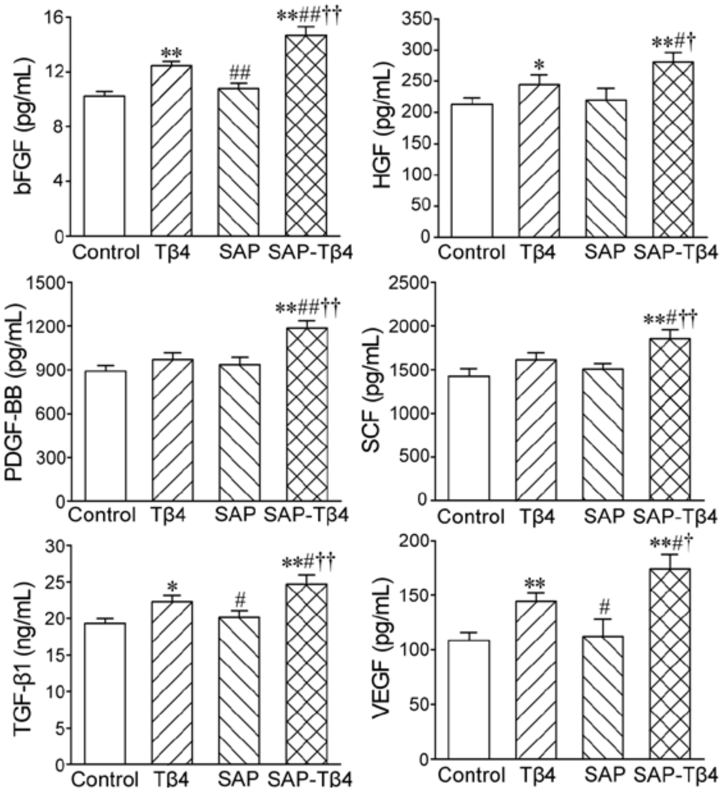

Figure S4. Paracrine factors secreted by EPDCs. The cells were incubated for one week. ELISA. *$p < 0.05$ and **$p < 0.01$ versus control group; #$p < 0.05$ and ##$p < 0.01$ versus Tβ4 group; †$p < 0.05$ and ††$p < 0.01$ versus SAP group. n = 3.

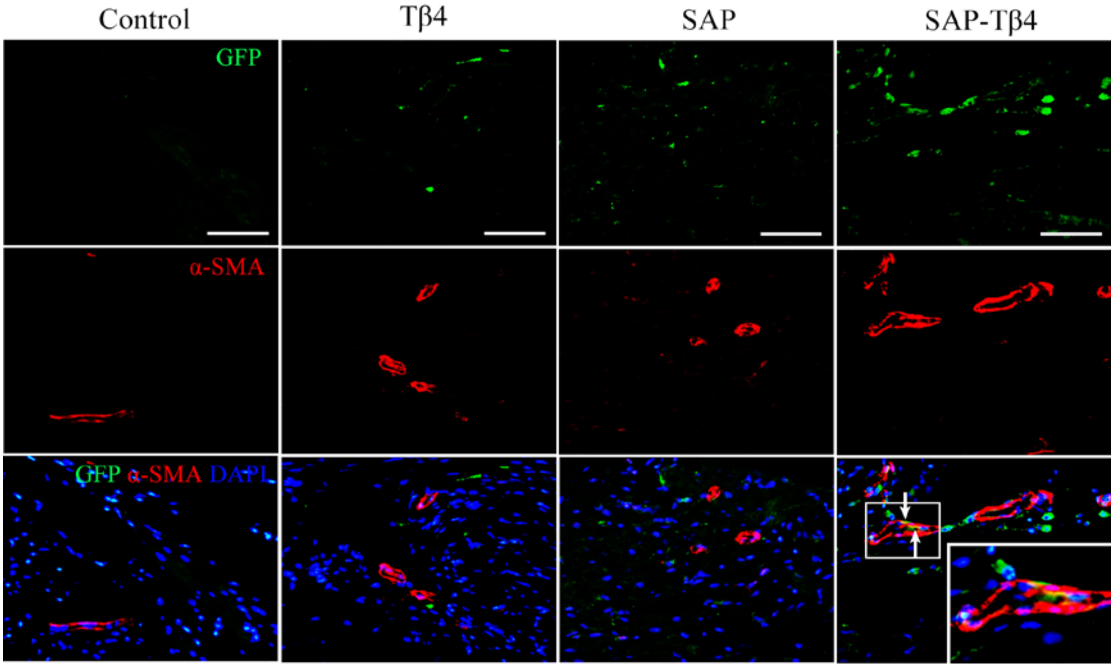

Figure S5. EPDCs differentiate into smooth muscle cells at 4 weeks after

implantation. α-SMA$^+$ cells differentiated from EPDCs (arrows) are located at the wall of the microvessels at the infarcted region. The large box is magnification of the small box. Immunostaining. Scale bar = 100 μm.

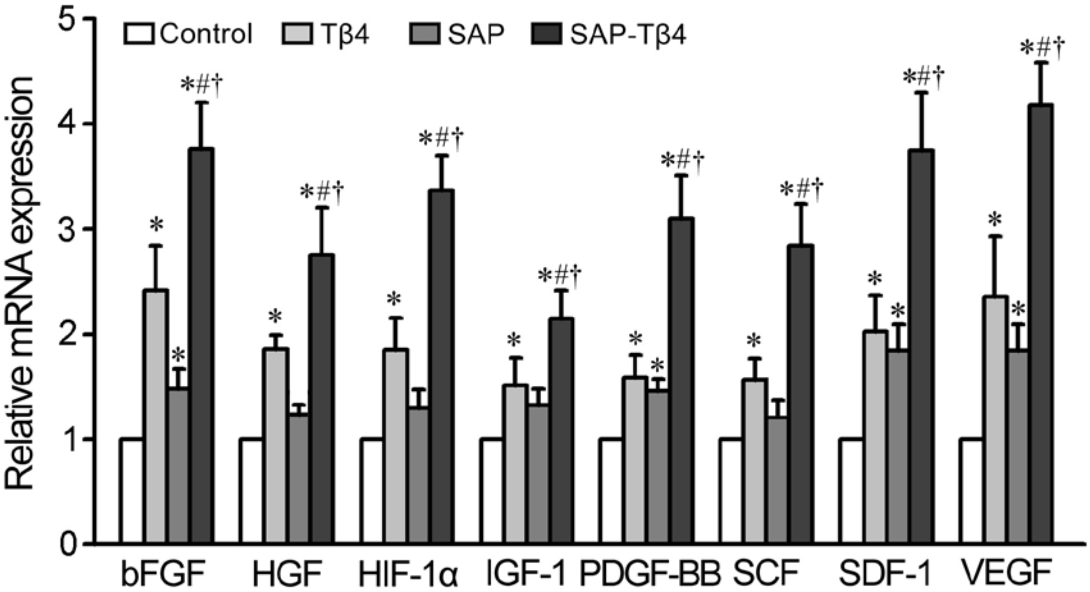

Figure S6. Expression of the genes in the infarcted myocardium at 1 week after implantation. qRT-PCR analysis. **p* < 0.05 versus control group; #*p* < 0.05 versus Tβ4 group; †*p* < 0.05 versus SAP group. n = 4.