Supporting Information

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SI Methods

Rap1 Pull-Down Assay. Cells were serum-starved for 48 h, incubated with drugs, lysed [1% Nonidet P-40, 25 mM Hepes (pH 7.4), 150 mM NaCl, 10% glycerol, 1 mM EDTA, 10 mM $MgCl_2$, 0.5 mM Na_3VO_4, 10 mM NaF, 1 μg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride] and centrifuged at 20,000 $\times$ *g* for 1 min. Supernatants were incubated with 15 μg of an agarose-Ral GDS-Rap binding domain conjugate for 45 min, beads were washed 3 times with lysis buffer, precipitates were separated by SDS/PAGE and analyzed by immunoblotting with anti-Rap1 mAb.

PKA Activity. PKA activity was determined by an enzyme-liked immunosorbent assay, using lysates of cardiac fibroblasts serum-starved for 48 h and stimulated with drugs of interest. Cells were then scraped into lysis buffer (20 mM Mops, 50 mM β-glycerol phosphate, 50 mM NaF, 1 mM $NaVO_4$, 5 mM EGTA, 2 mM EDTA, 1% Nonidet P-40, 1 mM DTT, and protease inhibitor mixture from Sigma), incubated for 10 min, and then passed through a 25-gauge needle 10 times. Homogenates were centrifuged at 15,000 $\times$ *g* for 15 min. PKA activity was measured in the resulting supernatant, using an assay kit (StressGen Biotechnologies) and with stimulation by 5 μM cAMP for 5 min for cells treated with PKA inhibitors; $>$ 95% of measured kinase activity resulted from PKA activation, as determined by pretreating lysates for 20 min with 10 μM PKA inhibitor (Wiptide).

Immunocytochemistry. Cardiac fibroblasts cultured on 12-mm glass coverslips were serum-starved for 48 h and then incubated for 1 h with media alone, Me-cAMP or Phe-cAMP. Cells were then fixed in 10% buffered formalin for 10 min, washed twice with PBS, and permeabilized in 0.1% Triton X-100/PBS for 10 min. The cells were washed twice with PBS/Tween 20 (0.1% Tween), incubated with 1% BSA/PBS/Tween for 20 min and then with primary antibodies for 16 h. After three washes with PBS/Tween, samples were incubated with secondary antibody for 1 h. After six washes with PBS/Tween, samples were incubated with DAPI for 20 min, washed 3 times and then mounted in gelvatol for microscopy.

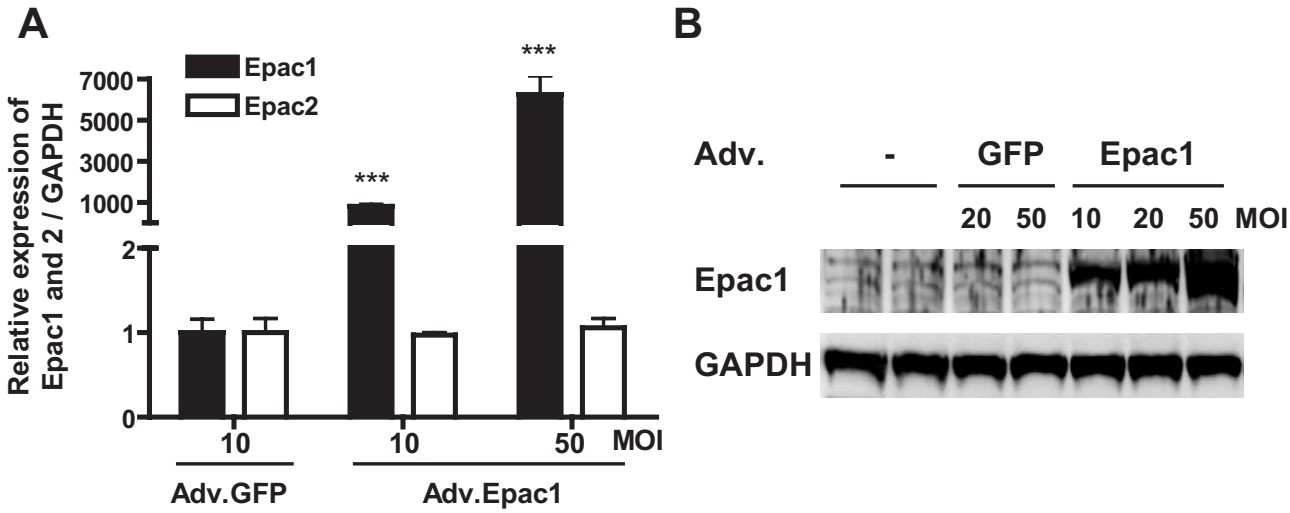

Fig. S1. Overexpression of Epac1 in cardiac fibroblasts. (*A*) Changes in the mRNA expression of Epac1 and Epac2 in cardiac fibroblasts incubated with adenovirus-Epac1 (Adv.Epac1) were assessed by quantitative RT-PCR. Values were normalized to GAPDH. Data are the fold-increase of adenovirus-mediated gene transfer of GFP (Adv.GFP) $n = 4$.***, $P < 0.001$ compared with Adv.GFP. MOI, multiplicity of infection. (*B*) Dose-dependent changes in the protein expression of Epac1 in cardiac fibroblasts that underwent gene transfer with Adv.Epac1 and Adv.GFP and were then analyzed by immunoblotting. GAPDH proteins are shown for the evaluation of protein loading.

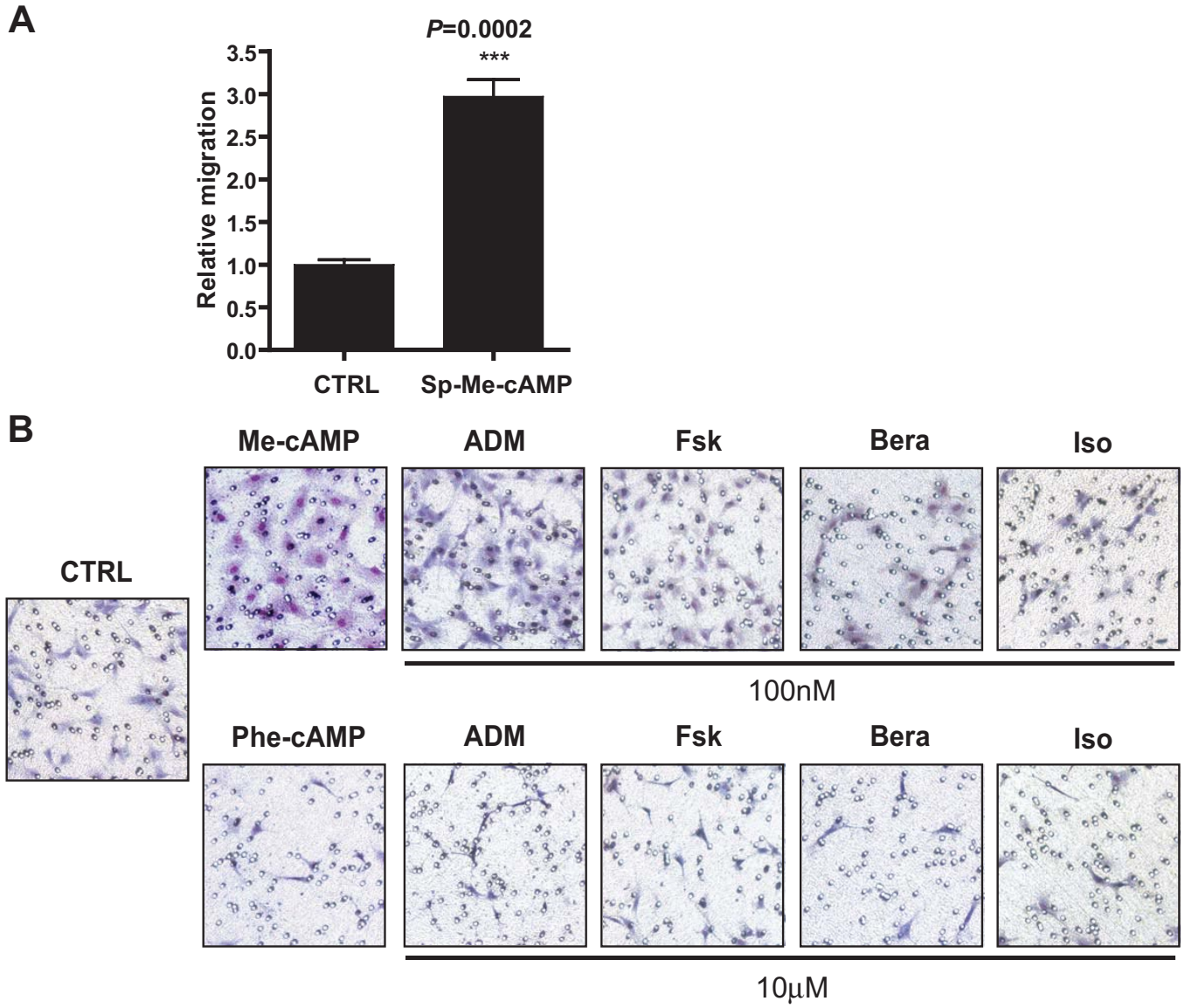

Fig. S2. The effect of Epac specific cAMP analog and different concentrations of cAMP-elevating agonists on cell migration. (*A*) Fibroblast migration was examined by using a modified Boyden chamber method in the absence (CTRL) or presence of Sp-Me-cAMP, an Epac-activator (50 μM). Data are shown as the fold-increase relative to control. $n = 6$. ***, $P = 0.0002$ compared with control. (*B*) Fibroblast migration in response to the indicated concentrations of adrenomedullin (ADM) forskolin (Fsk), beraprost (Bera), and isoproterenol (Iso) were measured by a modified Boyden chamber method. Cells that migrated (i.e., were on the bottom side of transwell filters) were stained with Hema 3.

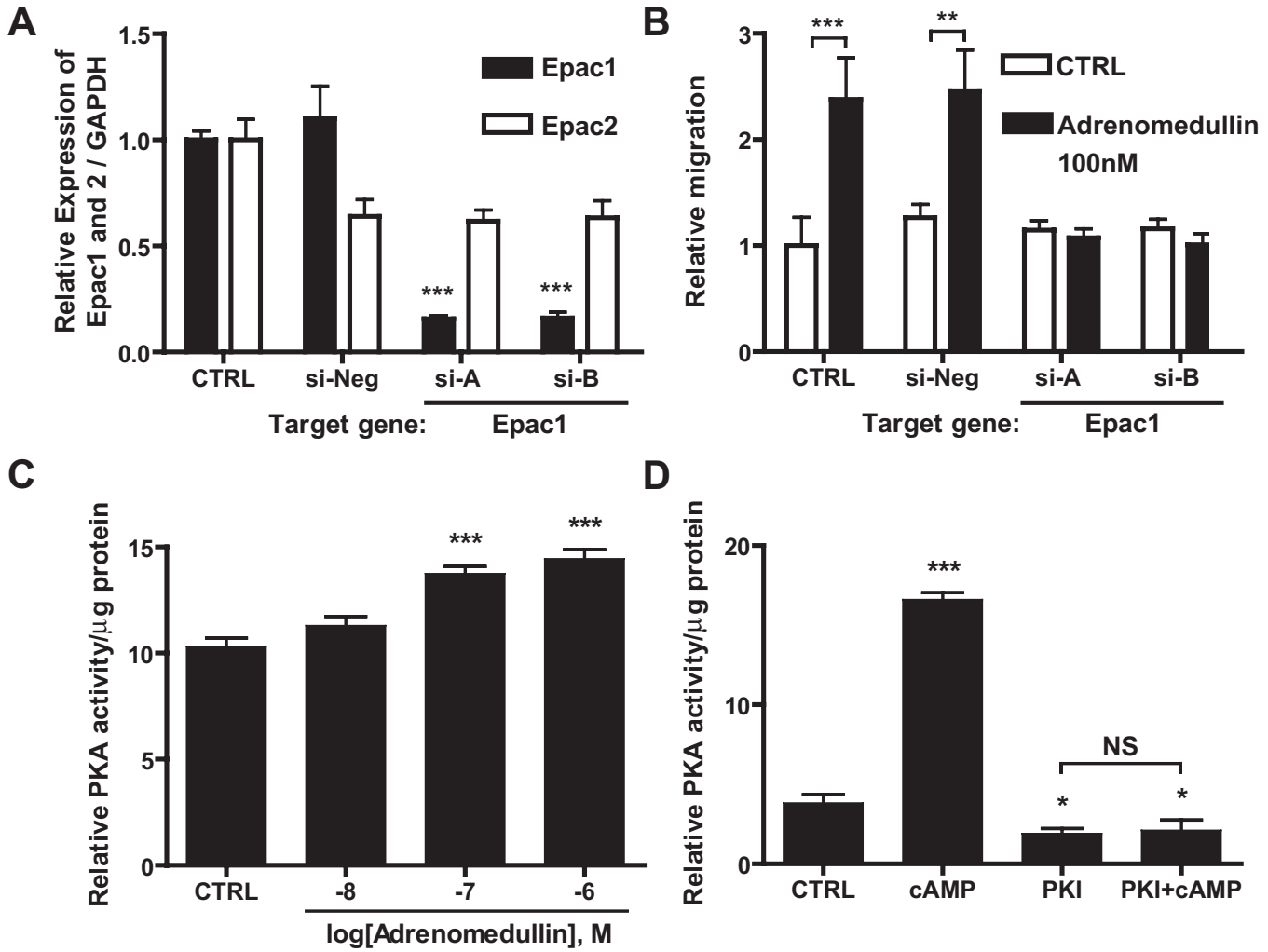

Fig. S3. Effect of inhibition of Epac1 and PKA on fibroblast migration. (*A*) Changes in the mRNA expression of Epac1 and Epac2 in cardiac fibroblasts treated with Epac1-targeted siRNA were assessed by quantitative RT-PCR. GAPDH was used to normalize RNA expression measurements. Data are the fold-increase from control. $n = 4–6$. ***, $P < 0.001$ compared with negative siRNA (si-Neg). (*B*) Cardiac fibroblast migration was examined in the absence (CTRL) or presence of either negative siRNA or Epac1-targeted siRNA. Cardiac fibroblasts were incubated with siRNA for 24 h and then with adrenomedullin for 16 h. $n = 4–8$; **, $P < 0.01$, ***; $P < 0.001$. (*C*) PKA activity was measured by using ELISA after 30 min incubation with adrenomedullin. Cardiac fibroblasts were placed in serum-free media for 48 h before the experiment. $n = 7$. ***, $P < 0.001$ compared with control (CTRL). (*D*) Cells were incubated with or without PKA inhibitor (PKI, Wiptide, 10 μM), then with cAMP (5 mM) for 30 min and assayed for PKA activity. $n = 6$; *, $P < 0.05$; ***, $P < 0.01$ compared with CTRL.

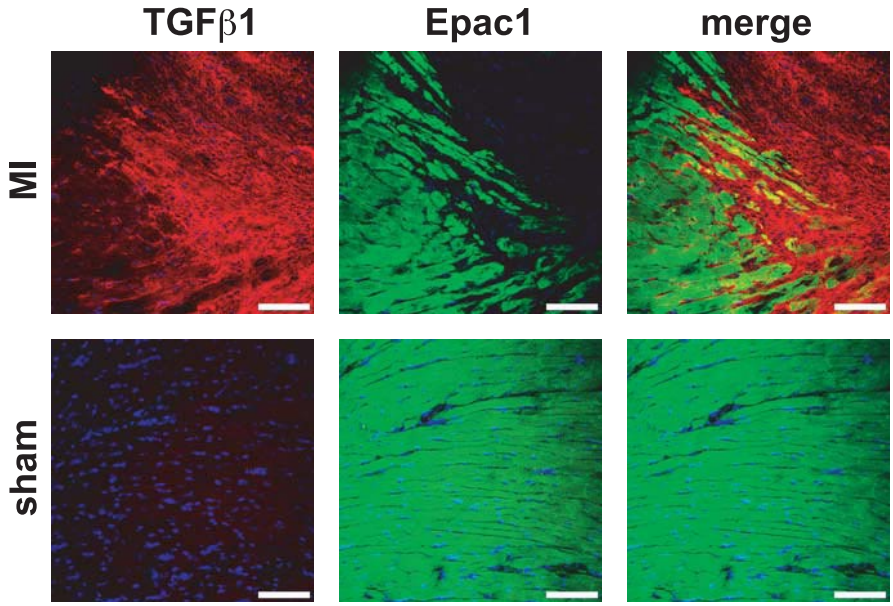

Fig. S4. Epac1 expression is decreased after myocardial infarction in mice. Sections of cardiac intraventricular septa from mice subjected to myocardial infarction (MI) or sham operation (sham) were stained for TGFβ1 (red) and Epac1 (green), and nuclei were stained with DAPI (blue). The images of the border zone that abuts on areas of infarction are shown. (Scale bar, 100 μm.)

Table S1. Oligonucleotides used for real-time RT-PCR

Gene	Accession no.	Forward, 5′–3′	Position	Reverse, 5′–3′	Position	Size, bp
Epac1	NM_021690	tgggaatgtatctcctcagacc	1651–1672	ctgccatcacttccctcac	1823–1841	191
Epac2	XM_215985	gatgatgcacggatgatgg	2068–2086	actgcactgatgacttccttcac	2311–2333	266
Collagen Iα1	XM_213440	ctggcaagaacggagatgat	791–811	caccatccaaaccactgaaa	921–940	150
Collagen Iα2	NM_053356	aggtcttcctggagctgatg	1233–1252	acccacagggccttctttac	1406–1425	193
Collagen IIIα1	NM_032085	gctcgaggcaatgatggt	1056–1073	gtgtccttgtggtccaggtt	1210–1229	174
GAPDH	XM_214546	agaacatcatccctgcatcc	681–700	ggagacaacctggtcctcag	900–919	239

Table S2. siRNA sequences

Target gene		Forward, 5′–3′	Reverse, 5′–3′
Epac1	A	cca cag agc aug ugc aca a(tt)	uug ugc aca ugc ucu gug g(tg)
Epac1	B	gcu ccu gua ccg uca ucu c(tt)	gag aug acg gua cag gag c(tt)
Epac2	A	gac auu cau uga caa ucu a(tt)	uag auu guc aau gaa ugu c(tt)
Epac2	B	ccg uga aug uag uca uuu a(tt)	uaa aug acu aca uuc acg g(at)
Rap1		gca uuc cag acu uca aaa a(tt)	uuu uug aag ucu gga aug c(tg)