Supplementary Material

Induction of myocardial infarction

Mice were anesthetized by intraperitoneal injection of pentobarbital (70 mg/kg). In the supine position, endotracheal intubation was performed. Mice were ventilated with a small animal respirator (Minivent type 845; tidal volume = 0.2 ml, rate = 100 breaths/min). Proper ventilation was confirmed by chest expansion and pink tongue color. Surgical procedures were carried out under a stereo microscope (Zeiss 2000C). The chest was opened via a left thoracotomy. The pectoralis major muscle was cut transversely. The fourth intercostal space was entered using blunt dissection, and a retractor was inserted and opened. The left coronary artery was identified as a pink vessel, and a 7-0 prolene suture was placed around the artery 1-2 mm below the left auricle. The electrocardiogram (ECG) was monitored continuously. Permanent occlusion of the left coronary artery resulted from its ligation with the suture. Myocardial ischemia was confirmed by pallor in heart color and ST-segment elevation. For animals undergoing a sham operation, the suture was placed in an identical location but not tied. The chest cavity was closed in layers with a 6-0 silk suture. Once spontaneous respiration resumed, the endotracheal tube was removed. The animals were monitored until fully conscious. After they were returned to their cages, standard chow and water were provided. HET mice were randomly divided into three groups. Akt Inhibitor (AI) [2.5 mg/kg], anti-IL-10R1 antibody (RA) (5μg in 200 μl saline) (BioLegend, San Diego, CA, USA), or normal mouse IgG was separately given to one group of mice via intraperitoneal injection, once a day, for 5 days, starting before the mice were returned to the animal facility. In a separate experiment, WT and HET mice were observed for 4 weeks after myocardial infarction.

Measurement of myocardial infarct size and changes in LV geometry

Two methods were used to measure myocardial infarct size, because of the degrees of left ventricular remodeling. Mice were subjected to MI for 24 h or 5 days. Hearts were collected and stored at -80 ^{0}C for overnight. Since no living tissue was detected in the infarcted area, Evans blue was not used. After freezing, hearts were transected into five pieces with a blade, followed by incubation with 1.5% triphenyltetrazolium chloride (TTC) for 15 min at 37 ^{0}C. Cardiac sections were photographed with a digital camera, and they were weighed individually. The infarcted area (white) and noninfarcted areas (red) of each section were measured by computerized planimetry (Imagine J; National Institutes of Health, Bethesda, MD, USA). Infarct size was calculated as a percentage of LV = total weight

of white tissue to weight of LV. To determine changes in LV geometry after 5-day MI, the ratio of the thickness of interventricular septum (Se) to the diameter of LV section was measured. Because of the distribution of the septum in the heart, only the middle three sections were assessed. Their averages are presented as a percentage of Se/LV. To measure myocardial infarct size 4 weeks after MI, hearts were separated from the chest wall and transected at the ascending aorta. After freezing, hearts were cut into five pieces and incubated with 1.5% TTC for 15 min, scars stained white. The infarcted LV wall was identified as thin white tissue. To consider the variation induced by sectioning, we measured the length of infarcted LV wall and the entire endocardial circumference of each section. Infarct size was calculated as a percentage of total infarcted LV wall length to the sum of circumferences.

PTEN overexpression by adenoviral gene transfer

Mice were anesthetized by intraperitoneal injection of pentobarbital (70 mg/kg). In the supine position, endotracheal intubation was performed. Mice were ventilated with a small animal respirator (Minivent type 845; tidal volume = 0.2 ml, rate = 100 breaths/min). Proper ventilation was confirmed by chest expansion and pink tongue color. Surgical procedures were carried out under a stereo microscope (Zeiss 2000C). The chest was opened via a left thoracotomy. The pectoralis major muscle was cut transversely. The fourth intercostal space was entered using blunt dissection, and a retractor was inserted and opened. The heart was exposed. A dose of 10^8 plaque-forming units (p.f.u)/30 μl of *Pten* adenoviruses (*adPten*) containing human *Pten* expressing sequence or empty vectors (*adNull*) (Vector Biolabs, Philadelphia, PA, USA) was injected into the left ventricular wall at three equidistant points by a 30G syringe. Five days later, hearts were isolated for protein analysis and measurement of mononuclear cell infiltration.

Echocardiography

Before measurement, mice were habituated to handling on several occasions to reduce stress. Each mouse was gently held in the palm of the hand by securing the skin of the dorsal neck with the thumb and the index finger. In the supine position, the left hemithorax was shaved, and prewarmed hypoallergenic ultrasonic transmission gel was applied to the thorax. The transducer was gently placed on the chest wall to avoid reflex bradycardia. The mouse was held on a steady platform to achieve an angle for the transducer consistent with the left lateral decubitus position for imaging. *In vivo* cardiac function was assessed by transthoracic echocardiography (Acuson Sequoia

C256, 13MHz transducer; Siemens). The mouse heart was imaged in the two-dimensional mode in the parasternal short axis view at a papillary level. From this view, a M-mode cursor was positioned perpendicular to the interventricular septum, and the left ventricular posterior wall thickness and chamber dimensions were measured. Three or five sample recordings were obtained from each mouse. The LV end-diastolic diameter (LVEDD) and end-systolic diameter (ESD) were measured from a stable M-mode tracing. Fractional shortening (FS) was calculated as a percentage of [(LVEDD-LVESD)/LVEDD]. Studies and analyses were performed by investigators blinded to genotype or treatments.

Histological studies

Hearts were fixed with 10% buffered formalin, embedded in paraffin, and sectioned at 6 μm. Cardiac sections were mounted from warm water (40 ^{0}C) onto adhesive microscope slides. Sections were allowed to dry overnight at room temperature, followed by deparaffinization with xylene and rehydration. Three or more sections per heart were stained with hematoxylin and eosin (H&E) or Masson's trichrome. Mononuclear cell infiltration was assessed in cardiac sections by H&E staining. Mononuclear cells were counted in eight randomly selected fields (400x) from the peri-infarct area under a microscope. The numbers of mononuclear cells were averaged from two peri-infarct areas of each cardiac section. Each group comprised at least three hearts, and 48 fields were counted in each group. Fibrosis was assessed in the peri-infarct area by Masson's trichrome staining. Fibrotic blue area and whole peri-infarct area were measured using computerized planimetry (Image J). The fibrotic area was presented as a percentage of fibrotic area to the peri-infarct area. A total of 18 fields (100x) were counted in each group. The observer was blinded to the origin of the cardiac sections.

Immunohistochemistry

$CD45^{+}$ cells were detected in paraffin-embedded cardiac sections. After heating at 61°C, cardiac sections were deparaffinized in xylene and rehydrated. Antigen retrieval was performed by a heat-induced method with citrate buffer. The endogenous peroxidase activity was quenched with 3% (v/v) H_2O_2 in methanol. After blocking with 10% normal donkey serum, sections were incubated with primary anti-CD45 antibody (1:200) (BioLegend, San Diego, CA, USA), and then with a peroxidase-conjugated secondary antibody. Nuclei were counterstained with Mayer's hematoxylin. $CD45^{+}$ cells were counted in the same way as mononuclear cells.

Table S1: Ventricular parameters before and after MI

	BS		2 WS		4 WS	
	WT	HET	WT	HET	WT	HET
Sample size, n	13	13	9	11	12	16
BW, g	22.3 ± 0.6	22.7 ± 0.5	23.1 ± 0.6	23.3 ± 0.6	24.4 ± 0.4	25.3 ± 0.6
HW, mg	120 ± 10	114 ± 4	164 ± 4	149 ± 3*	192 ± 3	166 ± 2†
HR, beats/min	724 ± 9	729 ± 10	692 ± 11	704 ± 10	656 ± 15	714 ± 10†
HW/BW, mg/g	5.4 ± 0.5	5.1 ± 0.3	7.0 ± 0.3	6.3 ± 0.2*	7.9 ± 0.2	6.6 ± 0.2†
IVSD, mm	0.88 ± 0.03	0.86 ± 0.03	0.56 ± 0.03	0.69 ± 0.05*	0.48 ± 0.02	0.63 ± 0.05*
IVSS, mm	0.88 ± 0.03	1.03 ± 0.08	0.61 ± 0.04	0.89 ± 0.09†	0.54 ± 0.03	0.78 ± 0.09*
LVEDD, mm	2.9 ± 0.3	2.9 ± 0.3	5.0 ± 0.2	4.1 ± 0.3*	5.4 ± 0.3	4.1 ± 0.2†
LVESD, mm	1.1 ± 0.0	1.2 ± 0.1	4.1 ± 0.2	3.1 ± 0.3†	4.8 ± 0.3	3.3 ± 0.3†

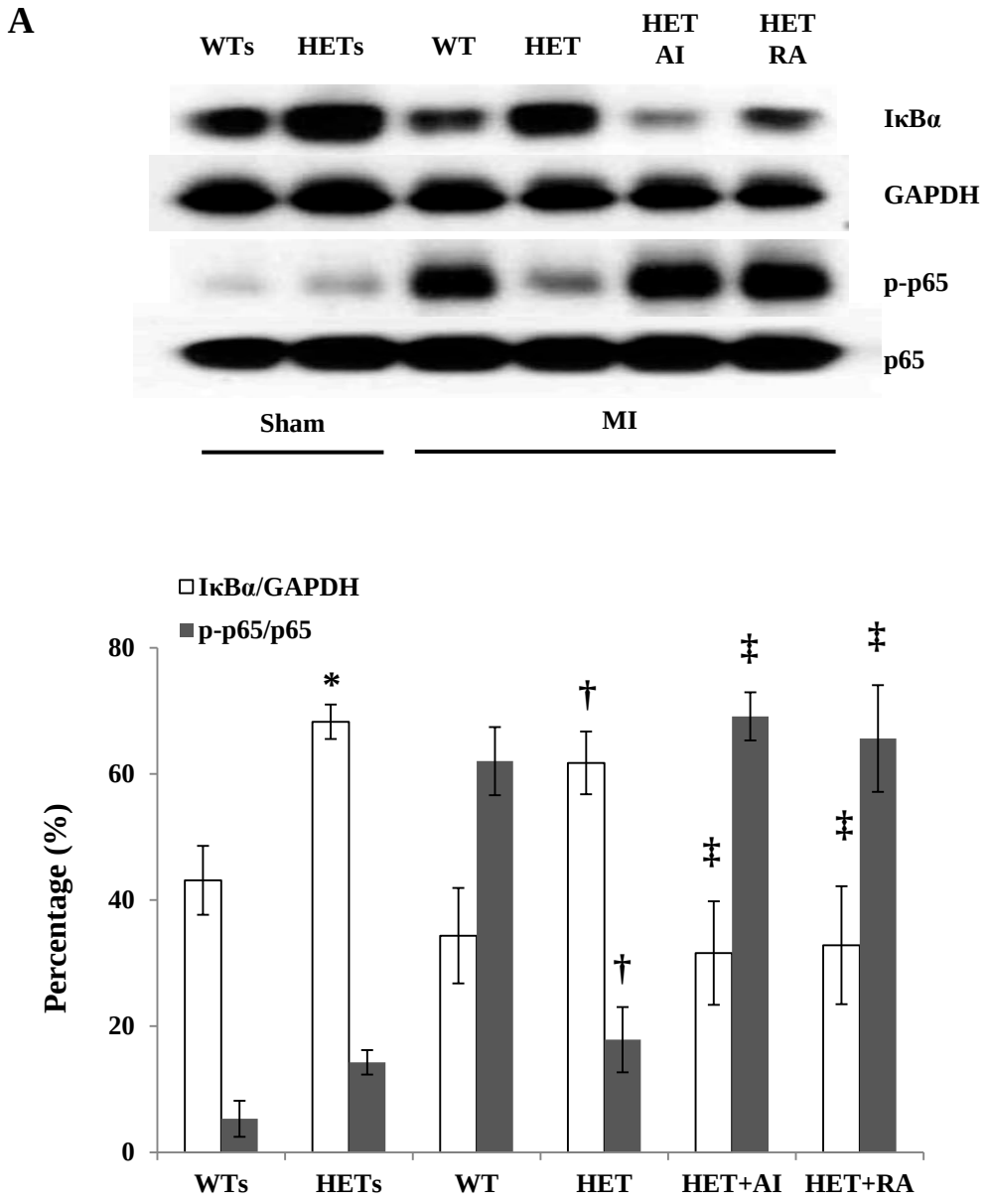
BW, body weight; HW, heart weight; HR, heart rate; IVSD, interventricular septal thickness at diastole; IVSS, interventricular septal thickness at systole; LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter. FS, fractional shortening. BS, basal levels; WS, weeks. *: $p<0.05$ vs. WT; †: $p<0.01$ vs. WT.

Table S2: Left ventricular parameters at 5 days post-MI

	WT	HET	HET+AI	HET+RA	adNull	adPten
Sample size, n	7	6	6	6	8	6
BW, g	26.8 ± 0.4	26.2 ± 0.7	26.8 ± 0.6	26.8 ± 0.5	24.8 ± 0.4	24.7 ± 0.4
HW, mg	144 ± 4	162 ± 4*	151 ± 4	148 ± 3†	234 ± 3	239 ± 5
HR, beats/min	697 ± 15	714 ± 19	690 ± 16	703 ± 16	674 ± 22	571 ± 14$^{\#}$
HW/BW, mg/g	5.4 ± 0.1	6.2 ± 0.2*	5.6 ± 0.3	5.5 ± 0.1†	9.3 ± 0.2	9.6 ± 0.3
IVSD, mm	0.55 ± 0.01	0.82 ± 0.10*	0.50 ± 0.00†	0.54 ± 0.01†	0.60 ± 0.01	0.60 ± 0.01
IVSS, mm	0.65 ± 0.01	0.95 ± 0.14*	0.56 ± 0.00†	0.60 ± 0.01†	0.70 ± 0.01	0.70 ± 0.01
LVEDD, mm	4.4 ± 0.3	3.7 ± 0.2	5.0 ± 0.1†	4.4 ± 0.2	4.5 ± 0.3	4.3 ± 0.5
LVESD, mm	3.9 ± 0.4	2.4 ± 0.2*	4.5 ± 0.2†	3.8 ± 0.2†	3.8 ± 0.4	3.9 ± 0.5

AI, Akt Inhibitor; RA, an-IL-10 receptor antibody. *: $p<0.05$ vs. WT; †: $p<0.05$ vs. HET. $^{\#}$: $p<0.05$ vs. adNull.

Figure S1


A
WTs
HETs
WT
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HET AI
HET RA
IκBα
GAPDH
p-p65
p65
Sham
MI
IκBα/GAPDH
p-p65/p65
Percentage (%)
80
60
40
20
0
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WTs
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HET+AI
HET+RA

Figure S1

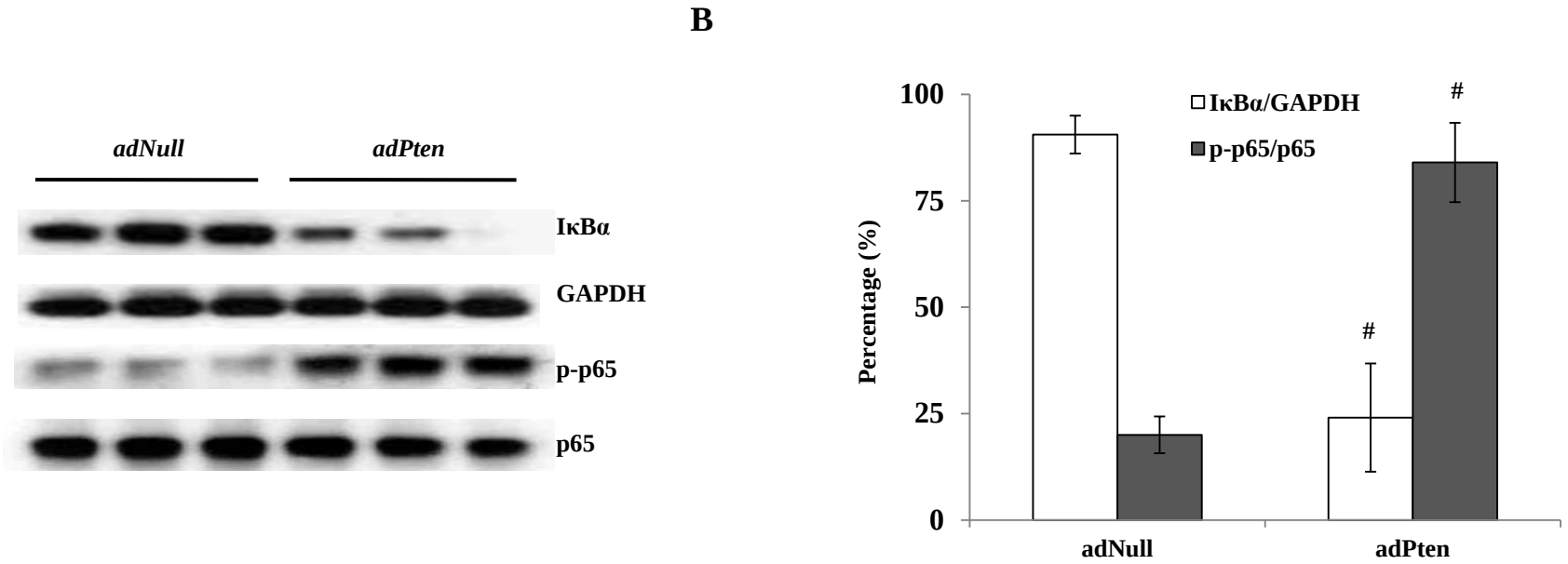

Fig. S1 PTEN promotes NF-κB activation in the infarcted heart Hearts were collected at 5 days post-MI. Cardiac tissues were isolated from the peri-infarct area. a IκBα protein levels were increased and p65 phosphorylation was decreased in HET mice. AI and RA reversed these effects in HET mice. b PTEN over-expression decreased IκBα protein levels and increased p65 phosphorylation in *adPten* mice. *: $p<0.05$ vs. WTs, †: $p<0.05$ vs. WT. ‡: $p<0.05$ vs. HET; #: $p<0.05$ vs. *adNull*. N=3-5.

Figure S2

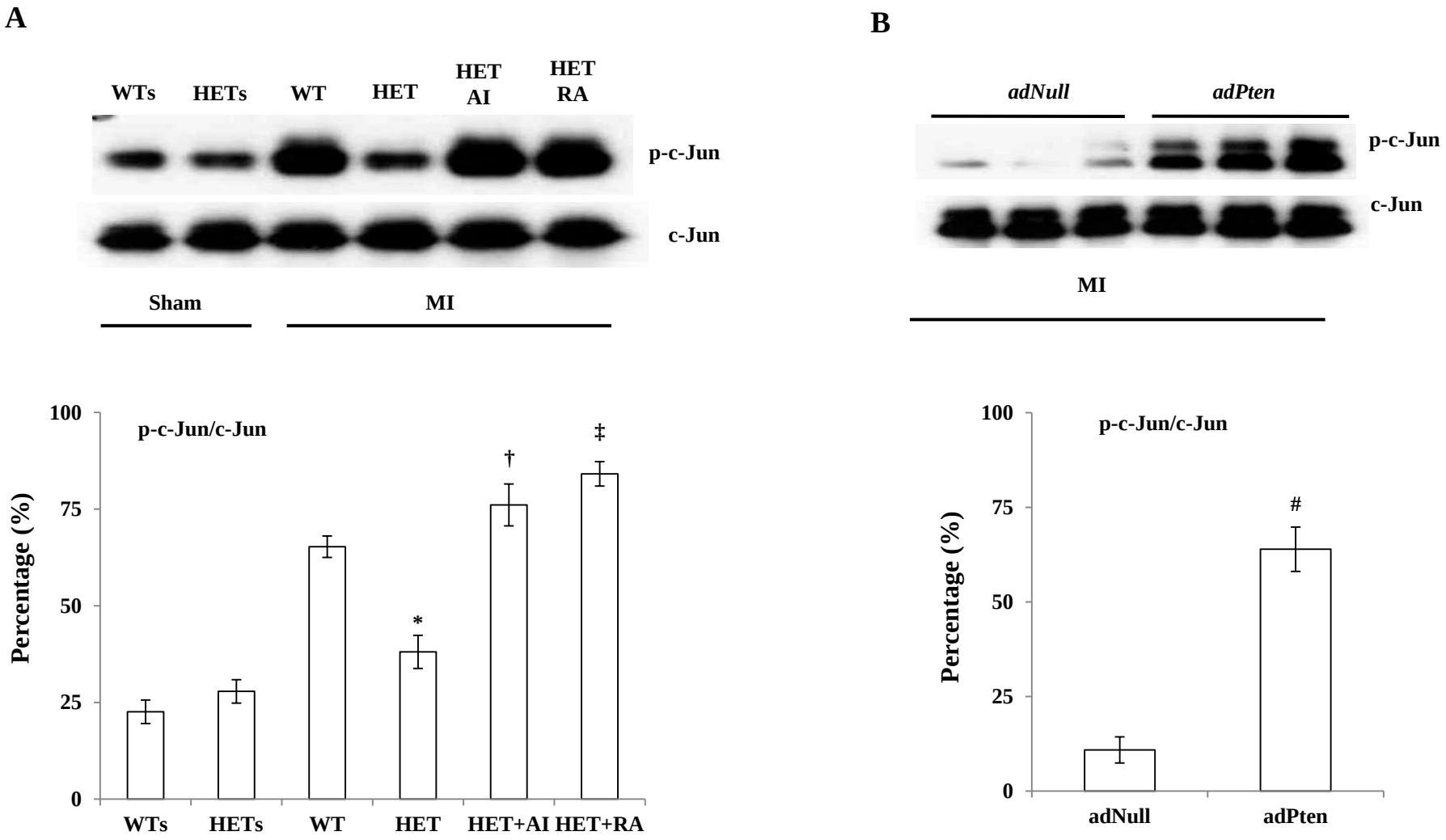

Fig. S2 PTEN increases c-Jun phosphorylation in the infarcted heart. a c-Jun phosphorylation was decreased in HET mice. AI and RA inhibited this effect in HET mice. b PTEN overexpression increased c-Jun phosphorylation in *adPten* mice. *: p<0.01 vs. WT, †: p<0.01 vs. HET. ‡: p<0.01 vs. HET; #: p<0.01 vs. *adNull*. N=3-5.

Figure S3

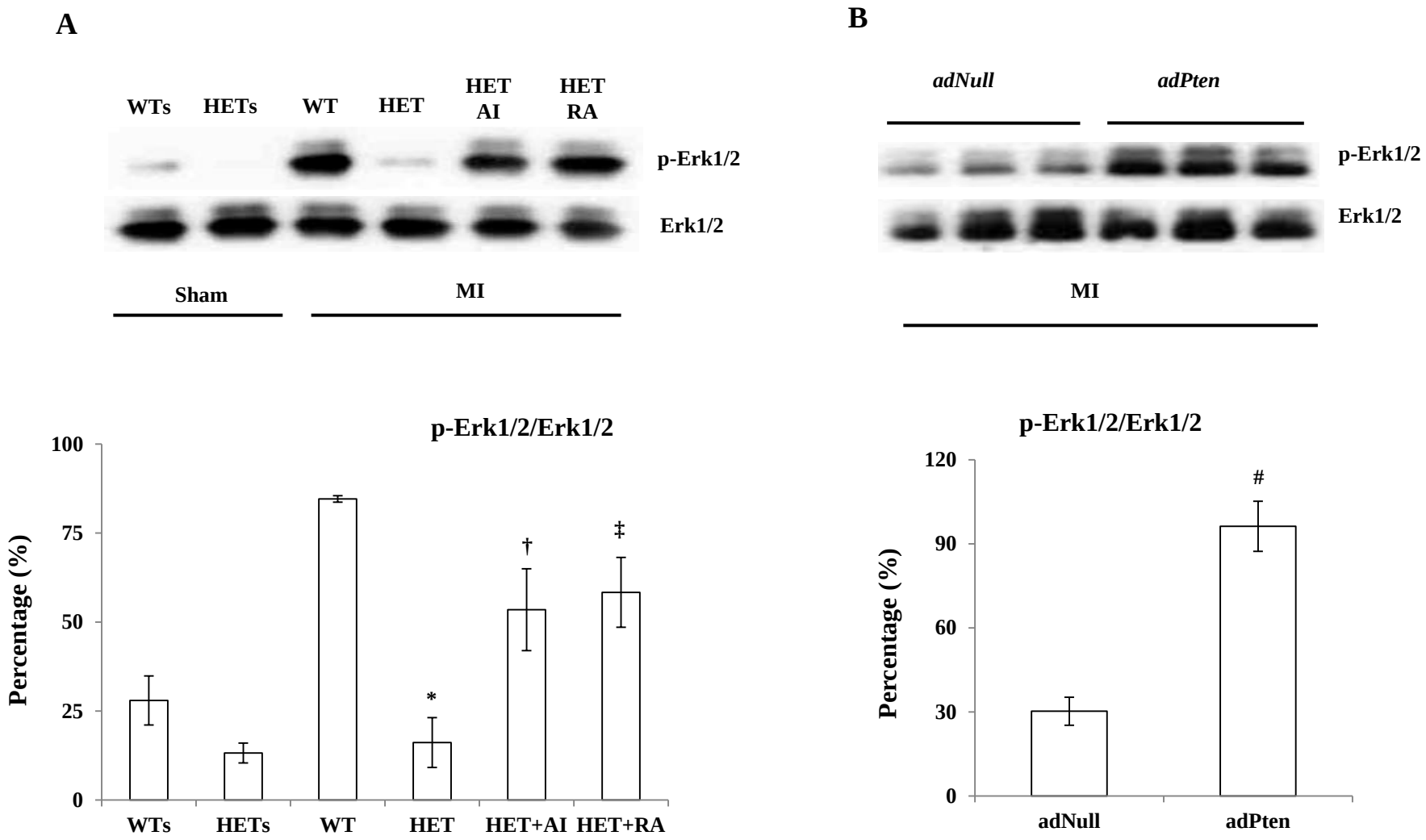

Fig. S3 PTEN regulates Erk1/2 phosphorylation in the infarcted heart. a Erk1/2 phosphorylation was decreased in HET mice. AI and RA inhibited this effect in HET mice. b PTEN overexpression increased Erk/12 phosphorylation in *adPten* mice. *: $p<0.01$ vs. WT, †: $p<0.05$ vs. HET. ‡: $p<0.05$ vs. HET; #: $p<0.05$ vs. *adNull*. N=3-5.