Lack of Phospholipase A_2 Receptor Increases Susceptibility to Cardiac Rupture after Myocardial Infarction

Hideto Mishina[1], Kazuhiro Watanabe[1], Shun Tamaru[1], Yosuke Watanabe[1], Daisuke Fujioka[1], Soichiro Takahashi[1], Koji Suzuki[1], Takamitsu Nakamura[1], Jun-ei Obata[1], Kenichi Kawabata[1], Yasunori Yokota[2], Osamu Inoue[3], Makoto Murakami[4], Kohji Hanasaki[2] and Kiyotaka Kugiyama[1]

[1] Department of Internal Medicine II, University of Yamanashi, Faculty of Medicine, Chuo, Yamanashi, Japan; [2] Shionogi Research Laboratories, Shionogi & Co. Ltd., Osaka, Japan; [3]Department of Clinical and Laboratory Medicine, Faculty of Medicine, University of Yamanashi, and; [4] Lipid Metabolism Project, the Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan.

Running title: PLA_2 Receptor and Myocardial Infarction

Subject codes:
[108] Other myocardial biology
[145] Genetically altered mice,
[151] Ischemic biology - basic studies

Address correspondence to:
Kiyotaka Kugiyama
Department of Internal Medicine II
University of Yamanashi
Faculty of Medicine
1110 Shimokato, Chuo, 409-389
JAPAN
Tel: +81-55-273-9590
Fax: +81-55-273-6749
kugiyama@yamanashi.ac.jp

In November 2013, the average time from submission to first decision for all original research papers submitted to *Circulation Research* was 14.6 days.

ABSTRACT

Rationale: Recent evidence indicates that the biological effects of secretory phospholipase A_2s ($sPLA_2$) cannot be fully explained by their catalytic activities. A cell surface receptor for $sPLA_2$ (PLA_2 receptor 1 [PLA_2R]) and its high affinity ligands (including $sPLA_2$-IB, -IIE and -X) are expressed in the infarcted myocardium.

***Objective*:** This study asked whether PLA_2R might play a pathogenic role in myocardial infarction (MI), using mice lacking PLA_2R ($PLA_2R^{-/-}$).

***Methods and Results*:** MI was induced by permanent ligation of the left coronary artery. $PLA_2R^{-/-}$ mice exhibited higher rates of cardiac rupture after MI compared with PLA_2R wild-type ($PLA_2R^{+/+}$) mice (46% vs. 21%, respectively, $P = 0.015$). $PLA_2R^{-/-}$ mice had a 31% decrease in collagen content and a 45% decrease in the number of α-SMA-positive fibroblasts in the infarcted region compared with $PLA_2R^{+/+}$ mice. PLA_2R was primarily found in myofibroblasts in the infarcted region. $PLA_2R^{-/-}$ myofibroblasts were impaired in collagen-dependent migration, proliferation and activation of focal adhesion kinase in response to $sPLA_2$-IB. Binding of $sPLA_2$-IB to PLA_2R promoted migration and proliferation of myofibroblasts through functional interaction with integrin β1 independent of the catalytic activity of $sPLA_2$-IB. In rescue experiments, the injection of $PLA_2R^{+/+}$ myofibroblasts into the infarcted myocardium prevented post-MI cardiac rupture and reversed the decrease in collagen content in the infarcted region in $PLA_2R^{-/-}$ mice.

***Conclusions*:** PLA_2R deficiency increased the susceptibility to post-MI cardiac rupture through impaired healing of the infarcted region. This might be partly explained by a reduction in integrin β1-mediated migratory and proliferative responses of $PLA_2R^{-/-}$ myofibroblasts.

Keywords:
Phospholipase A_2 receptor, acute myocardial infarction, myofibroblasts, integrin, collagen, cardiac rupture

Nonstandard Abbreviations and Acronyms:

PLA_2	Phospholipases A_2
$sPLA_2$	secretory PLA_2
PLA_2R	phospholipase A_2 receptor 1
CRDs	carbohydrate-recognition domains
LV	left ventricle
MI	myocardial infarction
LVDd	LV end-diastolic diameter
LVDs	LV end-systolic diameter
LVFS	LV fractional shortening
ELISA	enzyme-linked immunosorbent assay
TGF	transforming growth factor
CTGF	connective tissue growth factor
MMP	matrix metalloproteinase
TUNEL	Terminal deoxynucleotidyl transferase dUTP nick end labeling
FAK	focal adhesion kinase
SPR	surface plasmon resonance

INTRODUCTION

Secretory phospholipases A_2s ($sPLA_2$) play critical roles in various physiological and pathophysiological conditions, including inflammation and tissue injury.[1-8] It has long been believed that the biological effects of $sPLA_2$ are attributable to their catalytic activity, i.e., the hydrolysis of membrane phospholipids. We previously showed that several $sPLA_2$s play a pathogenic role in myocardial infarction (MI) in animal studies using mice that lacked group V or X $sPLA_2$ or in clinical studies.[6-8] However, a recent multicenter clinical trial showed that a non-selective inhibitor of $sPLA_2$ enzymatic activity (varespladib) did not reduce the risk of recurrent cardiovascular events and significantly increased the risk of MI in patients with acute coronary syndrome.[9] Thus, the precise role of $sPLA_2$ enzymatic activities in pathogenesis of MI remains to be determined.

Two decades ago, we and others discovered a cell surface receptor for $sPLA_2$-IB (phospholipase A_2 receptor 1; PLA_2R).[10, 11] PLA_2R, a so-called M-type PLA_2 receptor, is a type I transmembrane glycoprotein with a molecular mass of 180 kDa. It is composed of a large extracellular portion consisting of an N-terminal cysteine-rich region, a fibronectin-like type II domain, a tandem repeat of eight carbohydrate-recognition domains (CRDs) and a short intracellular C-terminal region.[3, 4, 10-13] Three of the CRD-like domains (CRDs 3 to 5) are responsible for $sPLA_2$ binding in mouse PLA_2R.[3, 4, 12] PLA_2R belongs to the C-type lectin family, having homology with the macrophage mannose receptors, Endo-180 and DEC-205.[13] Among $sPLA_2$ isozymes, mouse PLA_2R has a high affinity to $sPLA_2$-IB, -IIA, -IIE, -IIF and -X.[10, 12, 14] A previous in vitro study using over-expression of PLA_2R in cultured cells suggested that binding of $sPLA_2$ to PLA_2R might mediate some of the physiological effects of $sPLA_2$s independent of the catalytic activities of $sPLA_2$.[15] However, the cytoplasmic tail of PLA_2R is very short and does not seem to contain any specific signaling motifs other than an internalization motif on the basis of its sequence.[10-13, 16] Thus, the mechanisms by which PLA_2R mediates the biological effects of $sPLA_2$ remain unclear. We previously developed a mouse line that lacks PLA_2R. In our previous reports [6] and preliminary observations, we found that PLA_2R and its ligands, $sPLA_2$-IB, -IIE and -X, were expressed in the ischemic myocardium of mice and humans. However, the role of PLA_2R in myocardial ischemia is unknown. Using mice that lack PLA_2R, this study now reveals that PLA_2R plays a pathogenic role in post-MI cardiac rupture and that PLA_2R functionally interacts with integrin β1 in myofibroblasts.

METHODS

An expanded Methods section describing all materials and procedures is available in the Online Data Supplement.

Mice.

The experimental protocol was approved by the University of Yamanashi Animal Care and Use Committee (approval reference no. 19-35), and procedures were carried out in accordance with the *Guide for the Care and Use of Laboratory Animals*, 8th edition, 2011, U.S. National Institutes of Health. Details regarding the generation and characterization of $PLA_2R^{-/-}$ mice (systemically deficient in PLA_2R) were described in our previous reports.[17,18] $PLA_2R^{-/-}$ male mice with a C57BL/6J background from F15 to 18 (11 to 12 weeks old, 25 to 30 g) were used in the present study. The littermates of the wild-type ($PLA_2R^{+/+}$) males served as a control group.

RESULTS

Mice deficient in PLA_2R.

$PLA_2R^{-/-}$ mice appeared healthy and revealed no significant pathology in major tissues, as reported previously.[17, 18] At baseline (10 weeks old), there were no significant differences between the $PLA_2R^{+/+}$ and $PLA_2R^{-/-}$ mice in terms of body weight, heart weight, heart rate, blood pressure or complete blood counts in the peripheral circulation (Online Table I), confirming earlier findings.[17, 18]

Survival rates, cardiac rupture rates, infarct size and echocardiographic data after MI.

There were no operative deaths within 24 hr after MI or the sham operation. The survival rate up to 10 days after MI was significantly lower in $PLA_2R^{-/-}$ than in $PLA_2R^{+/+}$ mice (46% vs. 72% in the respective genotypes, $P = 0.016$; Figure 1A). A post-mortem histological examination showed that most of these mice died as a result of left ventricular (LV) free wall cardiac rupture as evidenced by bleeding and perfusion leakage from the infarcted region. The cardiac rupture was more prevalent in $PLA_2R^{-/-}$ mice than in $PLA_2R^{+/+}$ mice (Figure 1B). A typical example of the cardiac rupture is shown in Online Figures IA and IB. The rates of death not due to cardiac rupture were similar in the two mouse genotypes (7% vs. 8%, respectively, $P = 0.8$). For the period extending from 11 days to 4 weeks after MI, survival rates reached plateaus in both groups (data not shown).

A separate experiment showed that the infarct sizes / LV 24 hr after MI were similar in the two mouse genotypes (Figure 1C). Also, the areas at risk /LV were similar in $PLA_2R^{-/-}$ mice and $PLA_2R^{+/+}$ mice ($58 \pm 5\%$ vs. $62 \pm 5\%$, respectively, $P = 0.32$). Heart rates and blood pressures in the conscious state using the tail-cuff method were similar between the two mouse genotypes at 3 and 7 days after MI (Online Table II). Echocardiography in the surviving mice at 3 and 7 days after MI showed an increase in LV end-diastolic diameter (LVDd) and LV end-systolic diameter (LVDs) and a decrease in % LV fractional shortening (LVFS) compared with the sham-operated mice in both mouse groups. However, these parameters were similar in the two mouse genotypes at each time point examined (Online Table II). Thus, blood pressure, infarct size and LV dysfunction might not account for the relatively higher prevalence of cardiac rupture in $PLA_2R^{-/-}$ mice after MI.

Myocardial expression of PLA_2R, $sPLA_2$-IB, -IIE and –X.

In $PLA_2R^{+/+}$ mice, the expression of myocardial PLA_2R mRNA and protein was increased in infarcted regions compared with sham-operated hearts, with a peak at 7 days after MI (Figure 1D and 1G). Expression of myocardial mRNAs of $sPLA_2$-IB, $sPLA_2$-IIE and $sPLA_2$-X (high affinity ligands of murine PLA_2R) was increased in infarcted regions compared with sham-operated hearts (Figure 1E and 1F, Online Figure IC). The mRNA expression levels of these $sPLA_2$s in infracted regions were similar in $PLA_2R^{-/-}$ mice and $PLA_2R^{+/+}$ mice (Figure 1E and 1F, Online Figure IC). The myocardial protein contents of $sPLA_2$-IB and $sPLA_2$-IIE were increased in infarcted regions in both mouse genotypes as determined by immunoblot analysis and enzyme-linked immunosorbent assay (ELISA), respectively (Figure 1H and 1I). The expression of $sPLA_2$-IB protein in the infarcted region was greater in $PLA_2R^{-/-}$ mice than $PLA_2R^{+/+}$ mice (Figure 1H), whereas that of $sPLA_2$-IIE was similar in both genotypes (Figure 1I). $sPLA_2$-X protein was undetectable in whole homogenates of the infarcted regions by immunoblotting and ELISA probably because of its very low concentration (data not shown). In the myocardial homogenates of infarcted regions of $PLA_2R^{-/-}$ mice, ELISA showed that protein concentrations of $sPLA_2$-IB and $sPLA_2$-IIE were less than 5 nmol/L, a range that was insufficient to elicit fatty acid release from the cell membrane through their enzymatic activities (Online Figure II). Also, there was no significant production of eicosanoids in cultures of cardiomyocytes, neutrophils and macrophages after incubation with

sPLA$_2$-IB at the concentration of 100 nmol/L (Online Figure III). These results indicated that the enzymatic activity of sPLA$_2$-IB and -IIE was unlikely to have a major role in the phenotypes of the infarcted regions in mice hearts.

PLA$_2$R was expressed in the infarcted regions and colocalized with myofibroblasts in PLA$_2$R$^{+/+}$ mice.

Immunohistochemical analysis of PLA$_2$R$^{+/+}$ mice showed the presence of immunoreactivities of PLA$_2$R, sPLA$_2$-IB, sPLA$_2$-IIE and α-smooth muscle actin (SMA) in the infarcted regions (Figure 2A – 2D), whereas they were only weakly detected in the remote region of the myocardium (Figure 2E – 2H) and in the sham-operated myocardium (data not shown). The immunofluorescence microscopic images showed that the immunoreactivity of PLA$_2$R was co-localized with α-SMA-positive cells (Figure 2I – 2L) or vimentin-positive cells (Online Figure IV). Within the infarcted regions, sPLA$_2$-IB and -IIE were mainly co-localized with neutrophils and CD68-positive cells (Figure 2M – 2T). Using cultures of cardiac fibroblasts and cardiomyocytes from PLA$_2$R$^{+/+}$ mice, RT-PCR study confirmed that PLA$_2$R mRNA was abundantly present in cardiac myofibroblasts but only faintly present in cardiomyocytes (data not shown). In flow cytometric analysis of cell suspensions isolated from the infarcted region of PLA$_2$R$^{+/+}$ myocardium, PLA$_2$R was expressed on α-SMA$^+$ cells but not on CD31$^+$ cells (endothelial cells), CD45$^+$ cells (leukocytes), CD11b$^+$ cells (monocytes/macrophages) or Ly-6G$^+$ cells (granulocytes) (Online Figure VA, VB). Moreover, PLA$_2$R was not expressed in cultures of bone marrow-derived mast cells from PLA$_2$R$^{+/+}$ mice (Online Figure VC, VD). Thus, PLA$_2$R expression was restricted in myofibroblasts among cardiac cells in the infarcted myocardium.

PLA$_2$R expression was not increased in response to cytokines, growth factors, collagen VI or extra domain A-fibronectin in cultures of cardiac fibroblasts (Online Figure VI). The mechanism that regulates PLA$_2$R expression in myofibroblasts remains unclear.

Collagen content, prevalence of myofibroblasts and inflammatory cells and the expression of profibrogenic genes in the infarcted region.

Cardiac healing requires the recruitment of myofibroblasts and inflammatory cells followed by fibrous tissue formation in the infarcted region.[19, 20] Thus, collagen content and the prevalence of myofibroblasts and inflammatory cells in the infarcted region were examined in surviving mice at 3 and 7 days after MI. The hydroxyproline assay showed that the collagen content in the infarcted region was increased in the surviving mice in both genotypes at 7 days after MI compared with sham-operated mice, but the increase was less in PLA$_2$R$^{-/-}$ mice than in PLA$_2$R$^{+/+}$ mice (Figure 3A). The number of α-SMA-positive cells and the expression of α-SMA mRNA in the infarcted region of the surviving mice at 3 days after MI were lower in PLA$_2$R$^{-/-}$ mice than PLA$_2$R$^{+/+}$ mice (Figure 3B, 3C and 3D). Prevalence of α-SMA-positive cells exhibiting Ki-67 immunoreactivity in the infarcted area was lower in PLA$_2$R$^{-/-}$ hearts than in PLA$_2$R$^{+/+}$ hearts (Figure 3E and 3F), indicating that proliferative activity of myofibroblasts was decreased in the infarcted regions of PLA$_2$R$^{-/-}$ hearts. The recruitment of inflammatory cells in the infarcted region after MI was comparable between the two mouse genotypes (Figure 3G – 3J). Thus, the infarcted region in PLA$_2$R$^{-/-}$ mice was characterized by a decreased collagen content and lower prevalence and proliferative activity of myofibroblasts. However, the prevalence of neutrophils and monocytes/macrophages was similar in PLA$_2$R$^{-/-}$ mice and PLA$_2$R$^{+/+}$ mice.

Next, the expression of genes related to fibrous tissue formation in the infarcted regions at 3 days after MI was examined. The mRNA expression of profibrogenic molecules, including transforming growth factor (TGF)-β1 and -β2, procollagen I and III and connective tissue growth factor (CTGF) was increased in the infarcted region of the surviving mice after MI in both mouse genotypes, but the increase was less in PLA$_2$R$^{-/-}$ mice than in PLA$_2$R$^{+/+}$ mice (Figure 4A – 4E). These results were consistent with the

lower collagen content in the infarcted region in $PLA_2R^{-/-}$ than in $PLA_2R^{+/+}$ mice.

Expression of mRNA of pro-inflammatory and chemoattractant molecules, eicosanoids levels, matrix metalloproteinase (MMP) activity and microvessel density in the infarcted region.

Next, the expression of genes related to the recruitment of fibroblasts and inflammatory cells in the infarcted regions after MI was examined. The mRNA expression of pro-inflammatory and chemoattractant molecules, including IL-1β, IL-6, TNFα, MCP-1, CXCL2, in the infarcted region of the surviving mice at 3 days after MI was increased in both mouse genotypes, whereas CXCL12 expression was decreased. The expression levels in the infarcted region did not differ significantly between the two groups (Online Figure VII, A - F). Also, eicosanoids levels were increased in similar fashions in the infarcted myocardium in both mouse genotypes (Online Figure VIII). Thus, the levels of these proinflammatory and/or chemoattractant factors and eicosanoids probably did not contribute profoundly to the lower prevalence of myofibroblasts in the infarcted region in $PLA_2R^{-/-}$ mice than in $PLA_2R^{+/+}$ mice. There was not a significant difference between $PLA_2R^{-/-}$ and $PLA_2R^{+/+}$ mice in the increase in activities of MMP-2 and -9 in the infarcted region of the surviving mice at 3 days after MI (Online Figure VII, G and H). Microvessel densities were similar in $PLA_2R^{-/-}$ and $PLA_2R^{+/+}$ mice (Online Figure VII, I and J). Thus, it is unlikely that MMP-2 and -9 activities and the prevalence of neovascularization in the infarcted region account for the higher prevalence of cardiac rupture in $PLA_2R^{-/-}$ relative to $PLA_2R^{+/+}$ mice.

Impaired migration and proliferation in cultures of myofibroblasts isolated from $PLA_2R^{-/-}$ myocardium.

As shown above, the prevalence and the proliferative activity of myofibroblasts in the infarcted region were decreased in $PLA_2R^{-/-}$ mice. This finding prompted us to examine the role of PLA_2R in the migration and proliferation of isolated myofibroblasts in vitro. In Boyden chamber experiments, cultured myofibroblasts from $PLA_2R^{-/-}$ hearts and $PLA_2R^{+/+}$ hearts failed to migrate on plane membrane filters (PBS coating, as a control) (Figure 5A). However, $PLA_2R^{-/-}$ and $PLA_2R^{+/+}$ myofibroblasts did migrate on filters precoated with collagen I, laminin, vitronectin or fibronectin (Figure 5A and 5B). Importantly, the migration on filters precoated with collagen I was less in $PLA_2R^{-/-}$ than $PLA_2R^{+/+}$ myofibroblasts. In contrast, the migratory activities on filters precoated with the other extracellular matrix components were similar in the two myofibroblast genotypes (Figure 5A and 5B). When $sPLA_2$-IB was added to the medium in the lower chamber, collagen-dependent migration of $PLA_2R^{+/+}$ myofibroblasts was significantly increased, but migration of $PLA_2R^{-/-}$ myofibroblasts was not enhanced (Figure 5A). Similar results were observed in an experiment using a catalytically-inactive $sPLA_2$-IB (obtained by pretreatment with bromophenylacylbromide; BPB) (Figure 5A). $sPLA_2$-IB did not increase the migration of $PLA_2R^{+/+}$ myofibroblasts on filters precoated with laminin, vitronectin or fibronectin (Figure 5B). It is known that integrins mediate the migration and proliferation of fibroblasts in response to collagen and that the integrin β1 family has an important role in their interaction among the integrin families.[21] When a blocking antibody (Ab, clone Ha 2/5, BD Biosciences, San Jose, CA, USA) against integrin β1 was added to the medium in the upper chamber, collagen-dependent migration was inhibited in both myofibroblast genotypes in the presence or absence of $sPLA_2$-IB (Figure 5A). siRNA specific for integrin β1 exerted effects similar to the integrin β1 blocking antibody in addition to reducing the expression of integrin β1 protein (Online Figure IX). Thus, integrin β1 mediated the migration of myofibroblasts in response to collagen in either the presence or absence of $sPLA_2$-IB in both myofibroblast genotypes.

Similarly, the proliferation rate of cultured myofibroblasts, as assessed by the [^{3}H]-thymidine incorporation assay, was increased on collagen-coated dishes (Figure 5C), but the increase was less in $PLA_2R^{-/-}$ myofibroblasts than that of $PLA_2R^{+/+}$ myofibroblasts. Both catalytically-active and -inactive $sPLA_2$-IB increased collagen-dependent proliferation of $PLA_2R^{+/+}$ myofibroblasts; those effects were not

seen in $PLA_2R^{-/-}$ myofibroblasts (Figure 5C). The anti-integrin β1 antibody (Ab) suppressed collagen-dependent proliferation in both myofibroblast genotypes in the presence and absence of $sPLA_2$-IB (Figure 5C).

There was no significant difference between frequencies of TUNEL-positive cells in $PLA_2R^{+/+}$ and $PLA_2R^{-/-}$ myofibroblast cultures after exposure to 100 μmol/L H_2O_2 for 12 hr (Online Figure X). Taken together, these data indicate that an impairment of the migratory and proliferative responses of myofibroblasts might contribute to the low prevalence of these cells in the infarcted region in $PLA_2R^{-/-}$ mice.

Low activation of focal adhesion kinase (FAK) in $PLA_2R^{-/-}$ myofibroblasts.

FAK is involved in the integrin-mediated migration and proliferation of myofibroblasts in response to collagen.[22] $sPLA_2$-IB did not activate FAK in myofibroblasts on non-coated plates (PBS) (Figure 5D). Phosphorylation of FAK was increased in myofibroblasts from both $PLA_2R^{+/+}$ and $PLA_2R^{-/-}$ mice on collagen-coated dishes (Figure 5D), but the increase was less in $PLA_2R^{-/-}$ myofibroblasts than $PLA_2R^{+/+}$ myofibroblasts. Both catalytically-active and -inactive $sPLA_2$-IB increased the collagen-dependent phosphorylation of FAK in $PLA_2R^{+/+}$ myofibroblasts, but not in $PLA_2R^{-/-}$ myofibroblasts (Figure 5D). Anti-integrin β1 antibody suppressed the collagen-dependent phosphorylation of FAK in both myofibroblast genotypes in the presence or absence of $sPLA_2$-IB (Figure 5D).

PLA_2R was colocalized with integrins in cultured myofibroblasts.

In flow cytometric analysis, the mean fluorescence intensities of β1 (CD29), α1 (CD49a) and α2 (CD49b) integrin subunits on the cell surface were similar in $PLA_2R^{-/-}$ and $PLA_2R^{+/+}$ myofibroblasts (Figure 6A–6C). Con-focal immunofluorescence images showed that PLA_2R was colocalized with integrin β1 on both the apical and the basal cell surfaces of $PLA_2R^{+/+}$ myofibroblasts cultured on collagen-coated plates (Figure 6D).

Interaction between PLA_2R and integrin $\alpha 2\beta 1$.

Microwell protein binding assays and surface plasmon resonance (SPR) analysis using recombinant proteins showed the direct binding of collagen I to either immobilized PLA_2R or immobilized integrin α2β1 (Figure 7A and Online Figure XIA, XID and XIE). $sPLA_2$-IB bound to the immobilized PLA_2R (Online Figure XIB). Microwell binding assays showed that PLA_2R had no significant direct binding activity to integrin α2β1, but PLA_2R had a binding activity to integrin α2β1 in the presence of collagen I (Figure 7B and Online Figure XIC). Similarly, SPR analysis showed that PLA_2R in combination with collagen I produced a binding signal to integrin α2β1, whereas PLA_2R alone failed to produce a binding signal (Figure 7C and 7D). In addition, the collagen I-mediated interaction between PLA_2R and integrin α2β1 as well as the binding between collagen I and integrin α2β1 were inhibited by a synthetic peptide with the sequence of GFOGER (Figure 7A, 7B and 7E), which is the integrin recognition motif in collagen I.[23] The GFOGER peptide inhibited the migratory response of $PLA_2R^{+/+}$ and $PLA_2R^{-/-}$ myofibroblasts on filters precoated with collagen I in the presence or absence of $sPLA_2$-IB (Figure 7F). These results indicated that PLA_2R interacted with integrin α2β1 in the presence of collagen I (Figure 7G), and that the inhibition of their interaction was associated with migratory dysfunction of myofibroblasts.

Comparison of expression of mRNA of profibrogenic molecules and α-SMA in cultures of fibroblasts.

$PLA_2R^{+/+}$ and $PLA_2R^{-/-}$ myofibroblasts on collagen-coated plates expressed similar amounts of mRNA for profibrogenic molecules including TGF-β1 and β2, procollagen I and III and CTGF at baseline (Online Figure XII). Their expression did not significantly change in response to $sPLA_2$-IB (100 nmol/L) in either genotype (Online Figure XII). The results indicated that PLA_2R might not affect the expression of these profibrogenic molecules in myofibroblasts. Thus, the decreased expression of profibrogenic molecules in the infarcted regions of $PLA_2R^{-/-}$ mice was likely explained by the low prevalence of myofibroblasts in the infarcted region and not a decreased ability of $PLA_2R^{-/-}$ myofibroblasts to produce these profibrogenic molecules.

The magnitude of the TGF-β-induced up-regulation of α-SMA in cultures of fibroblasts was similar in fibroblasts from $PLA_2R^{-/-}$ and $PLA_2R^{+/+}$ myocardium, suggesting that PLA_2R might not have a major role in myofibroblast transdifferentiation (Online Figure XIII).

Myocardial injection of $PLA_2R^{+/+}$ myofibroblasts into infarcted regions protected against cardiac rupture after MI in $PLA_2R^{-/-}$ mice.

We asked whether injection of $PLA_2R^{+/+}$ myofibroblasts into infarcted myocardial regions could prevent cardiac rupture after MI in $PLA_2R^{-/-}$ mice. There were no operative deaths within 24 hr after MI. Death due to cardiac rupture was less prevalent in the $PLA_2R^{-/-}$ mice treated with $PLA_2R^{+/+}$ myofibroblasts than those treated with $PLA_2R^{-/-}$ myofibroblasts (Figure 8A). The collagen content and the number of α-SMA-positive cells in the infarcted region of the surviving mice after MI were greater in the treatment with $PLA_2R^{+/+}$ myofibroblasts than that with $PLA_2R^{-/-}$ myofibroblasts (Figure 8B and 8C). Representative histological images are shown in Figure 8D – 8L. To evaluate the distribution of the injected fibroblasts in the infarcted area, fluorescent Qdot® nanocrystal-labeled myofibroblasts from $PLA_2R^{-/-}$ hearts or $PLA_2R^{+/+}$ hearts were intramuscularly injected. We found that the Qtracker fluorescence was co-localized with α-SMA-positive cells in the infarcted area, and the injected myofibroblasts pre-labeled with the fluorescent tracker were distributed diffusely in the area where α-SMA-positive cells were present (Figure 8M – 8P, Online Figure XIV). LVDd, LVDs and %LVFS on echocardiography in the surviving mice at 3 and 7 days after MI were similar in the treatments with $PLA_2R^{+/+}$ myofibroblasts and $PLA_2R^{-/-}$ myofibroblasts (Online Table III).

Binding, internalization and degradation of $sPLA_2$-IB in cultures of $PLA_2R^{+/+}$ myofibroblasts.

In agreement with our previous report,[18] the specific binding, internalization and degradation of ^{125}I-labeled $sPLA_2$-IB were found in cultures of $PLA_2R^{+/+}$ myofibroblasts (Online Figure XV).

DISCUSSION

The present study suggests that the decreased migratory and proliferative responses of $PLA_2R^{-/-}$ myofibroblasts could account for the reduced numbers of myofibroblasts, leading to the decreased expression of profibrogenic molecules in the infarcted region in $PLA_2R^{-/-}$ mice. The decreased migratory and proliferative responses of $PLA_2R^{-/-}$ myofibroblasts might therefore depress healing of the infarcted region, resulted in an elevated frequency of cardiac rupture after MI in $PLA_2R^{-/-}$ mice. This scenario is supported by the present results showing that cardiac rupture in $PLA_2R^{-/-}$ mice was significantly prevented by myocardial injection of $PLA_2R^{+/+}$ myofibroblasts into the infarcted region. This

treatment increased the collagen content and the number of myofibroblasts in the infarcted region.

The present experiments using cultures of myofibroblasts indicated that PLA_2R had a functional interaction with integrin β1 and that their interaction may mediate collagen-dependent migration and proliferation in response to $sPLA_2$-IB. This is supported by the microwell protein binding assays and SPR analysis showing that PLA_2R interacted with integrin α2β1 in the presence of collagen I. In line with these results, the inhibition of their interaction by synthetic peptide GFOGER was associated with suppression of the migratory response in cultures of myofibroblasts. Together, a potentially new mechanism underlying the biological effects of the $sPLA_2$-PLA_2R pathway can be proposed (Figure 7G). That is, PLA_2R might mediate collagen-dependent biological effects through its functional interaction with integrin β1. The binding of $sPLA_2$ to PLA_2R might modulate this interaction, promoting integrin signaling that induced migration and proliferation independent of the catalytic activity of $sPLA_2$. Previous studies [24-26] suggested that the fibronectin-like type II domain of PLA_2R might be involved in its interaction with integrin β1 via collagen I. However, there are many missing steps and uncertainties in this proposed mechanism, and it needs further studies.

A series of previous studies raised the possibility that binding of $sPLA_2$ to PLA_2R might transmit signals that lead to various biological effects independent of the intrinsic enzymatic activities of $sPLA_2$.[15, 27, 28] However, the cytoplasmic tail of PLA_2R is very short and the sequence does not seem to contain any specific signaling motifs other than an internalization motif on the basis of its sequence.[10-13, 16] Our present results provide a new insight into the signaling role of PLA_2R, which might interact with integrin β1 and transmit integrin β1-mediated signals.

Consistent with our previous studies,[14, 18] the present experiments showed that PLA_2R binds to $sPLA_2$-IB and facilitates the internalization and degradation of $sPLA_2$-IB as a clearance receptor in cultures of $PLA_2R^{+/+}$ myofibroblasts. A lower clearance rate (rather than an increase in production) might partly account for the relatively higher levels of $sPLA_2$-IB protein in infarcted regions of $PLA_2R^{-/-}$ mice compared to $PLA_2R^{+/+}$ mice. However, $sPLA_2$-IB induced no hydrolytic activity against cultured myofibroblasts and elicited no production of eicosanoids in cultures of cardiomyocytes, macrophages and neutrophils at the higher concentrations than that observed in homogenates of infarcted myocardial regions in the present study. Also, mouse $sPLA_2$-IIE had no significant enzymatic activity. Thus, the enzymatic activities of $sPLA_2$-IB and -IIE in infarcted region were unlikely to affect the healing process of infarcted regions in the present study. $sPLA_2$-IB and -IIE might play a pathogenic role in MI as a ligand of PLA_2R.

The present findings might aid our understanding of the role of the $sPLA_2$-PLA_2R pathway in various pathological conditions including inflammation and cancer as well as ischemic tissue injury.[1, 29] Modulating the interaction between PLA_2R and integrin β1 might lead to novel therapeutic strategies for various pathological conditions in which $sPLA_2$ plays a role. Also, PLA_2R is a potential therapeutic target for fibrotic diseases in diverse organs. Blocking the interaction between PLA_2R and integrins could potentially prevent cardiac fibrosis due to pressure overload such as hypertension.

In conclusion, $PLA_2R^{-/-}$ mice were susceptible to cardiac rupture after MI. This might be explained by impaired healing of the infarcted region, probably due to the reduced recruitment of myofibroblasts in $PLA_2R^{-/-}$ mice. The interaction between PLA_2R and integrin β1 might mediate the effects of PLA_2R on the migratory and proliferative responses to collagen in myofibroblasts. $sPLA_2$-IB or -IIE, ligands of this receptor, might modulate this interaction independent of their catalytic activities. These results suggest a potentially new mechanism for the biological effects attributed to the $sPLA_2$-PLA_2R pathway.

ACKNOWLEDGMENTS
We gratefully acknowledge the technical assistance of A. Yazaki, C. Komatsu, M. Yoda and M. Tominaga.

SOURCES OF FUNDING
This work was supported by the Ministry of Education, Culture, Sports, Science, and Technology, Health, Tokyo, Japan (grants-in-aid for B2-19390209 and B-22390158).

DISCLOSURES
Yasunori Yokota and Kohji Hanasaki are employees of Shionogi Research Laboratories, Shionogi & Co., Ltd., (Osaka, Japan). They contributed to the development of PLA_2R deficient mice and a monoclonal antibody against PLA_2R, but they did not contribute to the study design or the data analysis. The other authors have no conflict of interest.

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FIGURE LEGENDS

Figure 1. Survival rates, frequencies of cardiac rupture after myocardial infarction (MI) and expression of mRNA and protein of PLA_2R and $sPLA_2$s in the infarcted region. A, Kaplan-Meier survival curves of $PLA_2R^{+/+}$ (n = 43) and $PLA_2R^{-/-}$ mice (n = 39) after MI. **P* = 0.016 by log-rank test. **B,** Prevalence of death due to cardiac rupture after MI. **C,** Infarct size/LV. **D,** PLA_2R mRNA levels after MI in the infarcted region. **E and F,** mRNA levels of $sPLA_2$-IB and -IIE (high affinity ligands to PLA_2R) at 3 days after MI in the infarcted region. Sham indicates sham-operated mice. Expression level of mRNA was quantified by real-time quantitative PCR and normalized to GAPDH mRNA expression. **G, H and I,** Protein levels of PLA_2R (**G**) and $sPLA_2$-IB (**H**) at 7 days after MI in the infarcted region were measured by immunoblot analysis. The protein levels are expressed relative to the β-tubulin levels. Protein concentrations of $sPLA_2$-IIE (**I**) at 7 days after MI in the supernatant of homogenates of infarcted regions were measured with ELISA kit. n = 7 - 12 in each experiment. n.s. = not significant. **P* < 0.05; †*P* < 0.05 compared with the respective genotypes after sham operation (Sham). +/+ denotes $PLA_2R^{+/+}$ mice and –/– denotes $PLA_2R^{-/-}$ mice.

Figure 2. Expression of PLA_2R, $sPLA_2$-IB and-IIE in the infarcted region: colocalization with α-SMA-positive cells or inflammatory cells assessed by immunohistochemical and immunofluorescence microscopy. A – H, Immunohistochemical light microscopic images of infarcted regions (upper panels, **A – D**) and remote areas (lower panels, **E – H**) at 3 or 7 days after MI in $PLA_2R^{+/+}$ mice using anti-PLA_2R antibody (**A and E**), anti-α-SMA antibody (**B and F**), anti-$sPLA_2$-IB antibody (**C and G**) or anti-$sPLA_2$-IIE antibody (**D and H**). Immunoreactivity of PLA_2R, α-SMA and $sPLA_2$-IB and -IIE was clearly observed in the infarcted regions but weakly in remote areas. Broken lines in panels A-D and I indicate boundaries between infarcted and non-infarcted region. **I – T,** Hematoxylin and eosin staining and confocal immunofluorescence microscopic images of infarcted regions of $PLA_2R^{+/+}$ mice. The infarcted regions were stained with antibodies for PLA_2R (green, **J**), α-SMA (red, **K**), $sPLA_2$-IB (green, **N**), neutrophils (red, **O**), $sPLA_2$-IIE (green, **R**) and CD68 (red, **S**). **L,** double-staining for PLA_2R and α-SMA. **P,** double-staining for $sPLA_2$-IB and neutrophils. **T,** double staining for $sPLA_2$-IIE and CD68. **I, M and Q,** Hematoxylin and eosin staining of infarcted regions. Blue rectangles in panels **I, M and Q** correspond to images of **J – L**, **N – P** and **R – T**, respectively. Arrow heads in panels **L**, **P** and **T** indicate co-localization. Scale bars in panels **A – H**, **I**, **J – L**, **M**, **N – P**, **Q**, **R – T** were 200 μm, 100 μm, 50 μm, 100 μm, 50 μm, 100 μm and 50 μm, respectively. Sections are representative of 5 mice.

Figure 3. Decreased collagen content and lower prevalence of myofibroblasts and similar prevalence of inflammatory cells in the infarcted regions in $PLA_2R^{-/-}$ mice compared with $PLA_2R^{+/+}$ mice. A, Collagen contents in the infarcted regions after MI were measured using the hydroxyproline assay. **B,** Number of α-SMA-positive cells at 3 days after MI. **C,** α-SMA mRNA levels at 3 days after MI. **D,** Hematoxylin and eosin (HE) staining and immunohistochemistry using anti-α-SMA antibody showed lower prevalence of α-SMA-positive cells in infarcted regions in $PLA_2R^{-/-}$ mice than $PLA_2R^{+/+}$ mice. Broken lines in HE staining indicate boundaries between infarcted and non-infarcted region. Scale bars in panels are 200 μm. **E,** Prevalence of α-SMA positive cells exhibiting Ki-67 immunoreactivity in the infarcted region was less in $PLA_2R^{-/-}$ myocardium than $PLA_2R^{+/+}$ myocardium. **F,** Representative immunofluorescence microscopic images showing cells with double-staining for α-SMA and Ki-67 in the infarcted region. Arrow heads indicate α-SMA positive cells exhibiting Ki-67 immunoreactivity. Scale bars in panels are 50 μm. The images were representative of 3 mice. **G,** Number of neutrophils (immuno-positive cells using anti-neutrophil antibody) at 3 days after MI. **H,** MPO activity after MI. **I,** Number of CD68-positive cells at 3 days after MI. **J,** CD68 mRNA levels at 3 days after MI. n = 6-8 mice in each experiment. **P* < 0.05; †*P* < 0.05 compared with the respective genotypes after sham operation.

n.s. denotes not statistically significant. +/+ denotes $PLA_2R^{+/+}$ mice and –/– denotes $PLA_2R^{-/-}$ mice.

Figure 4. Low expression of fibrogenic genes in the infarcted regions in $PLA_2R^{-/-}$ mice compared with $PLA_2R^{+/+}$ mice. A, TGF-β1 mRNA levels. **B,** TGF-β2 mRNA levels. **C,** Procollagen-I mRNA levels. **D,** Procollagen-III mRNA levels. **E,** CTGF mRNA levels. All samples were taken at 3 days after MI. n = 6-8 in each experiment. * $P < 0.05$, †$P < 0.05$ vs. compared with the respective sham-operated mice.

Figure 5. Impaired migratory and proliferative responses and low phosphorylation status of FAK in cultured myofibroblasts from $PLA_2R^{-/-}$ myocardium. A, B, Migratory responses to human $sPLA_2$-IB or $sPLA_2$-IB that was catalytically-inactivated by pretreatment with bromophenylacylbromide (BPB) in the presence or absence of anti-integrin β1 antibody (Ab). Boyden chamber filters were coated with collagen-I, laminin, vitronectin, fibronectin or PBS (control). **C,** Proliferative responses to $sPLA_2$-IB or $sPLA_2$-IB catalytically-inactivated by BPB in the presence or absence of anti-integrin β1 antibody on 24-well culture plates coated with collagen-I or PBS. Proliferative activity was assessed by [^{3}H]-thymidine incorporation assay. **D,** The levels of phosphorylated FAK (p-FAK) were expressed as ratios of the intensities of the respective total protein bands. Upper panels show representative immunoblots of total and p-FAK. n = 6-8 in each experiment. * $P < 0.05$. †$P < 0.05$ vs. the respective genotypes of myofibroblasts on filters treated with PBS, #$P < 0.05$ vs. the respective genotypes on collagen in the absence of $sPLA_2$-IB and anti-integrin β1 antibody. β1-integrin Ab = anti-integrin β1 antibody.

Figure 6. PLA_2R was co-localized with integrins in cultured myofibroblasts from $PLA_2R^{+/+}$ myocardium. A - C, Flow cytometric analyses of integrin expression in cultured myofibroblasts using anti-β1 integrin (CD29) antibody (**A**), anti-α1 integrin (CD49a) antibody (**B**) or anti-α2 integrin (CD49b) antibody (**C**). The mean fluorescence intensities of β1, α1 and α2 integrin subunits are similar in the two myofibroblast genotypes. **D,** Con-focal immunofluorescence images of cultured myofibroblasts using anti-PLA_2R antibody. PLA_2R was colocalized with β1-integrin on both the apical and the basal cell surfaces of $PLA_2R^{+/+}$ myofibroblasts cultured on collagen-I-coated plates. Scale bars in panels **D** were 50 μm.

Figure 7. Interaction between PLA_2R and integrin α2β1. A and B, Microwell protein binding assays using recombinant proteins. n = 7 in each experiment. *p < 0.05. **A**, Biotinylated collagen I (100 nmol/L) was incubated with the immobilized integrin α2β1 in the presence or absence of synthetic peptide GFOGER (1 μmol/L) or the control peptide $(GPP)_{10}$ on microtiter plates. After washing, streptavidin-HRP and tetramethylbenzidine were added. The amount of collagen I bound to integrin α2β1 was quantified using an absorbance plate reader set at 450 nm. Biotinylated collagen I bound to the immobilized integrin α2β1, which was inhibited by GFOGER but not $(GPP)_{10}$. **B**, biotinylated PLA_2R bound to the immobilized integrin α2β1 in the presence of collagen I, which was inhibited by the peptide GFOGER. **C-E**, Representative sensorgrams of surface plasmon resonance analysis. **C and D,** PLA_2R alone (**C**) or in combination with collagen I (100 nmol/L) (**D**) was injected over the immobilized integrin α2β1. **E**, PLA_2R in combination with collagen I (100 nmol/L) with or without GFOGER was injected over the immobilized integrin α2β1. The arrows indicate the beginning and the end of injections. RU, response units. **F**, GFOGER inhibited migratory responses of cultured myofibroblasts in Boyden chamber experiments. n = 7 in each experiment. * $P < 0.05$. † $P < 0.05$ vs. $PLA_2R^{+/+}$ myofibroblasts in the absence of $sPLA_2$-IB and GFOGER. **G**, Schematic representation of potential mechanism for the interaction between PLA_2R and integrin β1.

Figure 8. Injection of $PLA_2R^{+/+}$ myofibroblasts into infarcted regions of the heart protected $PLA_2R^{-/-}$ mice against post-MI cardiac rupture. Cultured myofibroblasts (1.5×10^5 cells in 15 μL of PBS) from $PLA_2R^{-/-}$ hearts (n = 29) or $PLA_2R^{+/+}$ hearts (n = 32) or PBS as a vehicle (n = 11) were intramuscularly injected at 2 sites near the peri-infarcted zone of $PLA_2R^{-/-}$ mice 10 min after MI. **A,** Prevalence of cardiac rupture during 10 days after MI. **B,** Collagen content in infarcted regions measured by the hydroxyproline assay at 7 days after MI. **C,** Number of α-SMA-positive cells in the infarcted regions at 3 days after MI. *$P < 0.05$. †$P < 0.05$ vs. the $PLA_2R^{-/-}$ mice treated with PBS. n = 8 in each measurement. **D – H,** Hematoxylin and eosin (HE) staining (**D**), immunohistochemistry (**E**) and immunofluorescence images (**F – H**) using anti-PLA_2R antibody and anti-α-SMA antibody after injection of $PLA_2R^{+/+}$ myofibroblasts into $PLA_2R^{-/-}$ heart. Rectangle in panel **E** corresponds to panels **F – H**. Arrows in panel H indicate colocalization of PLA_2R immunoreactivity with α-SMA-positive cells. **I – L,** HE staining and immunohistochemistry after injection of $PLA_2R^{+/+}$ myofibroblasts (**I, J**) or $PLA_2R^{-/-}$ myofibroblasts (**K, L**) into $PLA_2R^{-/-}$ heart. Adjacent sections are shown in panels **D** and **E**, **I** and **J** and panels **K** and **L**. **M – P**, Microscopic images showing the distribution of injected myofibroblasts pre-labeled with fluorescent Qdot® nanocrystals in the infarcted area at 7 day after MI. **M**, HE staining, **N**, Fluorescence microscopic image showing the distribution of myofibroblasts pre-labeled with the fluorescent tracker in the infarcted area. **O** and **P**, Immunofluorescence images using anti-PLA_2R antibody and anti-α-SMA antibody, respectively. Rectangle in panel **M** corresponds to panels **N – P**. Same section was used for **N – P**, and adjacent sections were used for **M** and **N – P**. Scale bars in panels **D**, **E**, **I – L, M** and panels **F – H**, **N – P** were 200 μm and 50 μm, respectively. The images were representative of 3 mice.

Novelty and Significance

What Is Known?

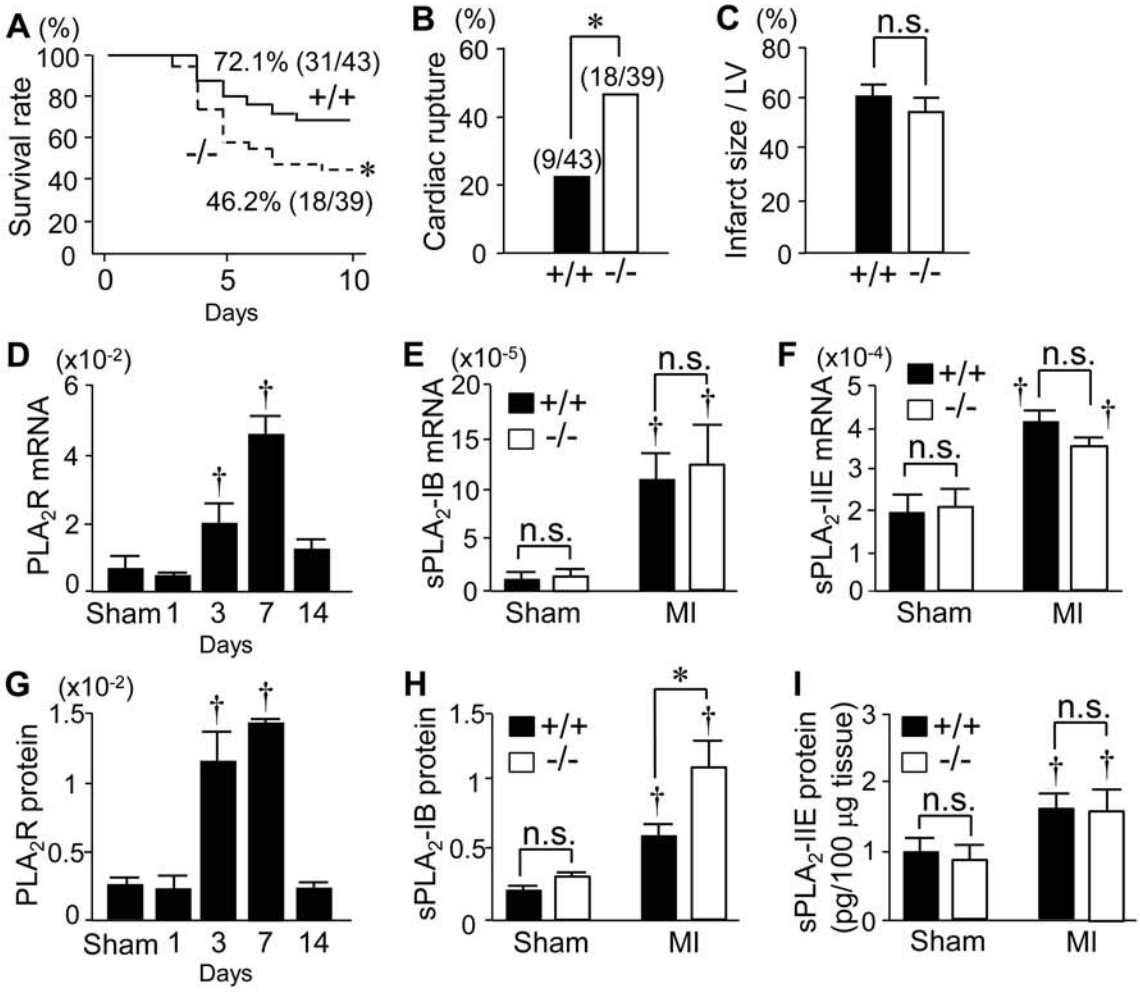
- Biological effects of secretory phospholipase A_2 ($sPLA_2$) cannot be fully explained by its catalytic activities.
- Binding of $sPLA_2$ to PLA_2 receptor 1 (PLA_2R) might mediate some of the biological effects of $sPLA_2$ independent of its catalytic activity.
- Cytoplasmic sequence of PLA_2R does not seem to contain any specific signaling motifs other than an internalization motif.

What New Information Does This Article Contribute?

- Interaction of PLA_2R with integrin β1 induced migration and proliferation of myofibroblasts through integrin β1-mediated signal transduction.
- Binding of $sPLA_2$-IB to PLA_2R promoted the functional interaction with integrin β1 independent of its catalytic activity.
- PLA_2R null mice were susceptible to cardiac rupture after myocardial infarction (MI) through impaired healing of the infarcted region due to the depressed recruitment of myofibroblasts.

Recent studies indicat that binding of $sPLA_2$ to PLA_2R might transmit signals that lead to various biological effects independent of the intrinsic enzymatic activities of $sPLA_2$. However, the cytoplasmic tail of PLA_2R is short and the sequence does not contain specific signaling motifs other than an internalization motif. Thus, the mechanisms by which PLA_2R mediates the biological effects of $sPLA_2$ are unclear. This study shows that PLA_2R interacts with integrin β1 and transmitted integrin β1-mediated signals in cultured myofibroblasts. The binding of $sPLA_2$-IB to PLA_2R modulated this interaction to promote migration and proliferation of myofibroblasts independent of catalytic activity of $sPLA_2$. Consistent with these in vitro results, in vivo data using PLA_2R null mice showed that PLA_2R had a protective role against post-MI cardiac rupture through recruitment of myofibroblasts in the infarcted region. These findings reveal a new mechanism for the biological effects of the $sPLA_2$-PLA_2R pathway that could potentially contribute to the pathogenesis of MI. The modulation of the interaction between PLA_2R and integrin β1 could be a novel therapeutic strategy for the treatment of pathological conditions in which $sPLA_2$s play a role.

Figure 1


A
(%)
Survival rate
72.1% (31/43)
+/+
-/-
*
46.2% (18/39)
Days
B
(%)
Cardiac rupture
*
(18/39)
(9/43)
+/+ -/-
C
(%)
Infarct size / LV
n.s.
+/+ -/-
D
(x10-2)
PLA2R mRNA
Sham 1 3 7 14
Days
E
(x10-5)
sPLA2-IB mRNA
+/+
-/-
n.s.
n.s.
Sham MI
F
(x10-4)
sPLA2-IIE mRNA
+/+
-/-
n.s.
n.s.
Sham MI
G
(x10-2)
PLA2R protein
Sham 1 3 7 14
Days
H
sPLA2-IB protein
+/+
-/-
n.s.
*
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I
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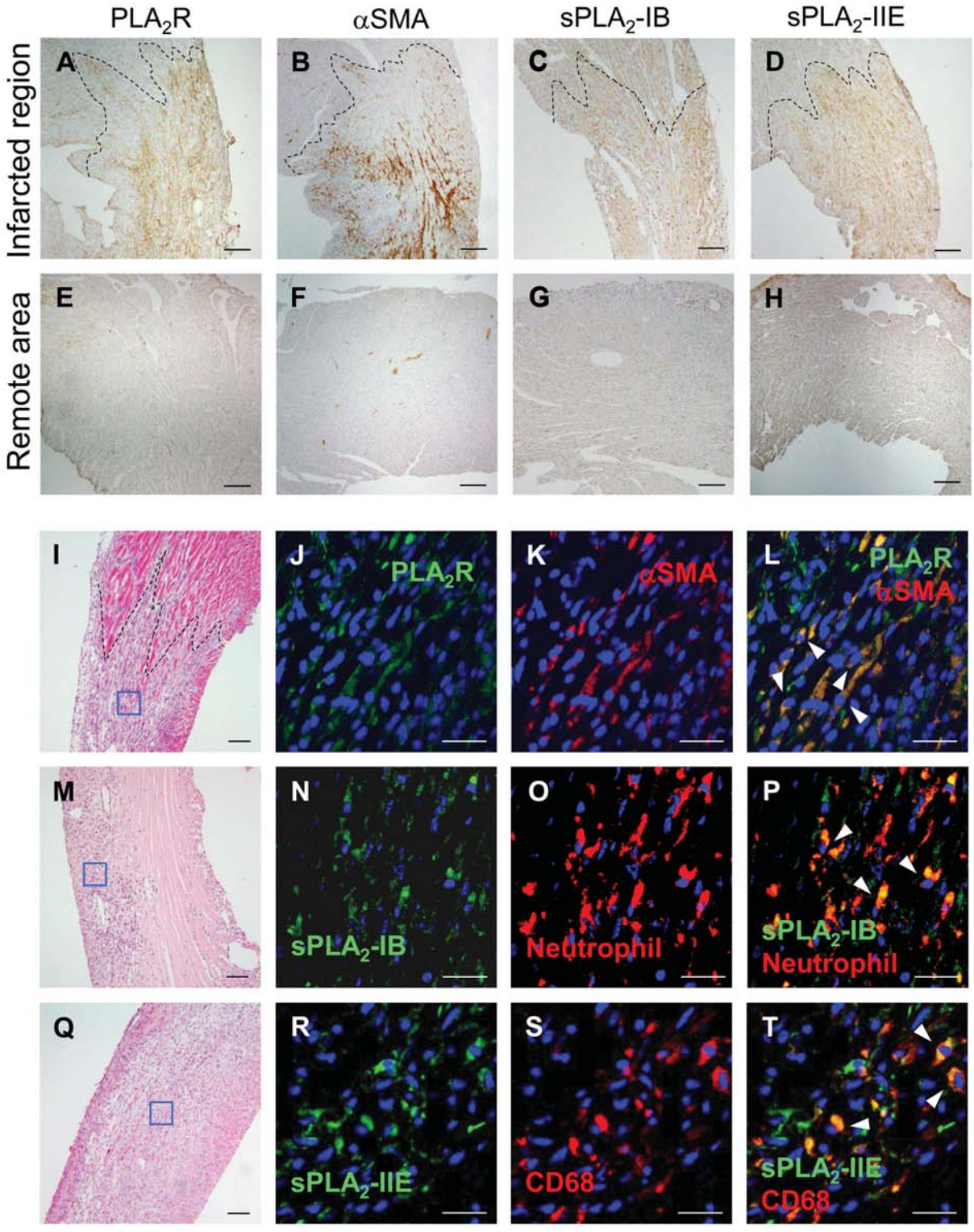
(pg/100 μg tissue)
+/+
-/-
n.s.
n.s.
Sham MI

Figure 2


PLA2R
αSMA
sPLA2-IB
sPLA2-IIE
Infarcted region
Remote area
A
B
C
D
E
F
G
H
I
J
PLA2R
K
αSMA
L
PLA2R
αSMA
M
N
sPLA2-IB
O
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CD68
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CD68

Figure 3

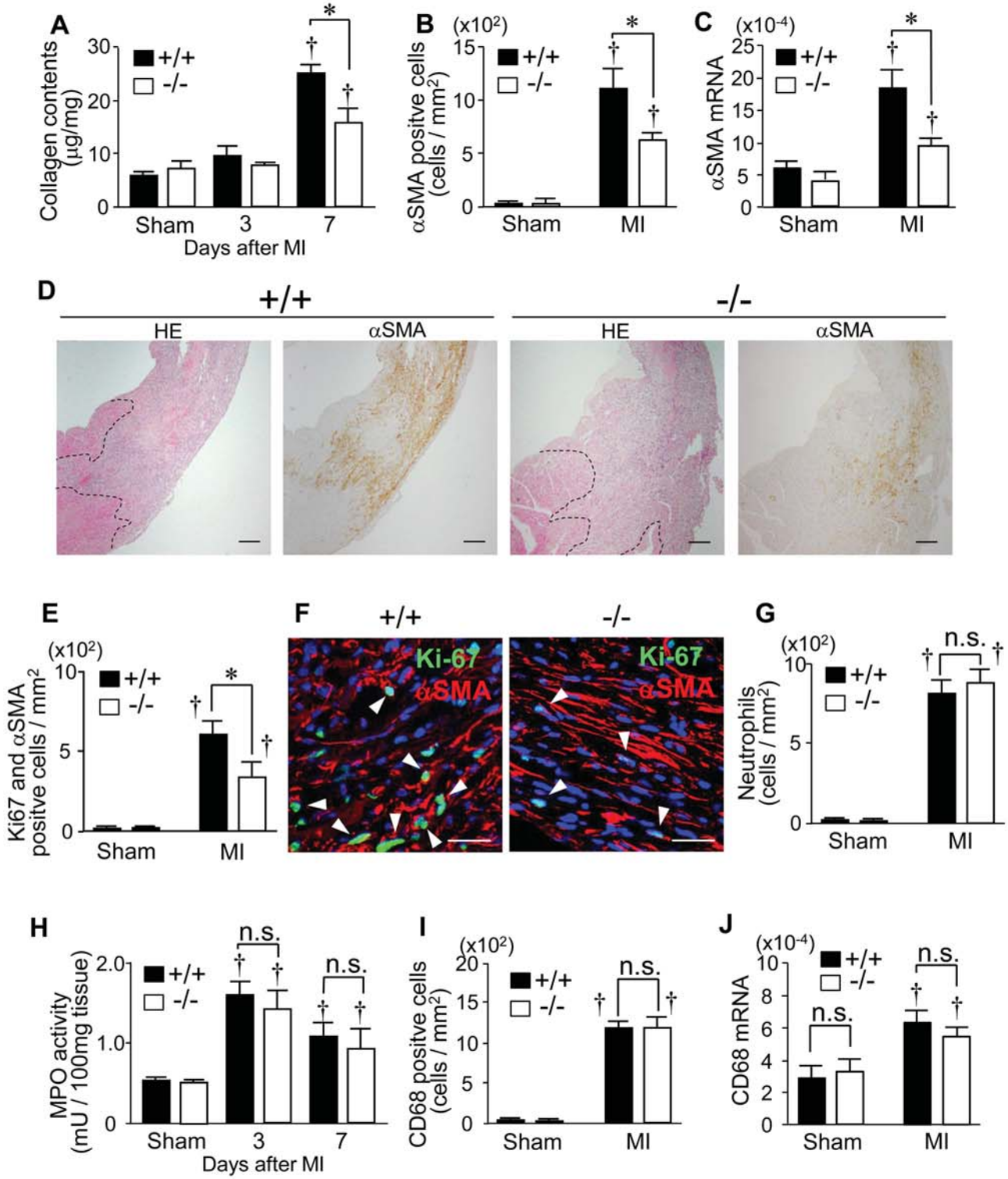

A
Collagen contents (μg/mg)
+/+
-/-
Sham
3
7
Days after MI
B
(x10²)
αSMA positve cells (cells / mm²)
Sham
MI
C
(x10⁻⁴)
αSMA mRNA
Sham
MI
D
+/+
-/-
HE
αSMA
HE
αSMA
E
(x10²)
Ki67 and αSMA positive cells / mm²
Sham
MI
F
+/+
-/-
Ki-67
αSMA
Ki-67
αSMA
G
(x10²)
Neutrophils (cells / mm²)
n.s.
Sham
MI
H
MPO activity (mU / 100mg tissue)
n.s.
n.s.
Sham
3
7
Days after MI
I
(x10²)
CD68 positve cells (cells / mm²)
n.s.
Sham
MI
J
(x10⁻⁴)
CD68 mRNA
n.s.
n.s.
Sham
MI

Figure 4

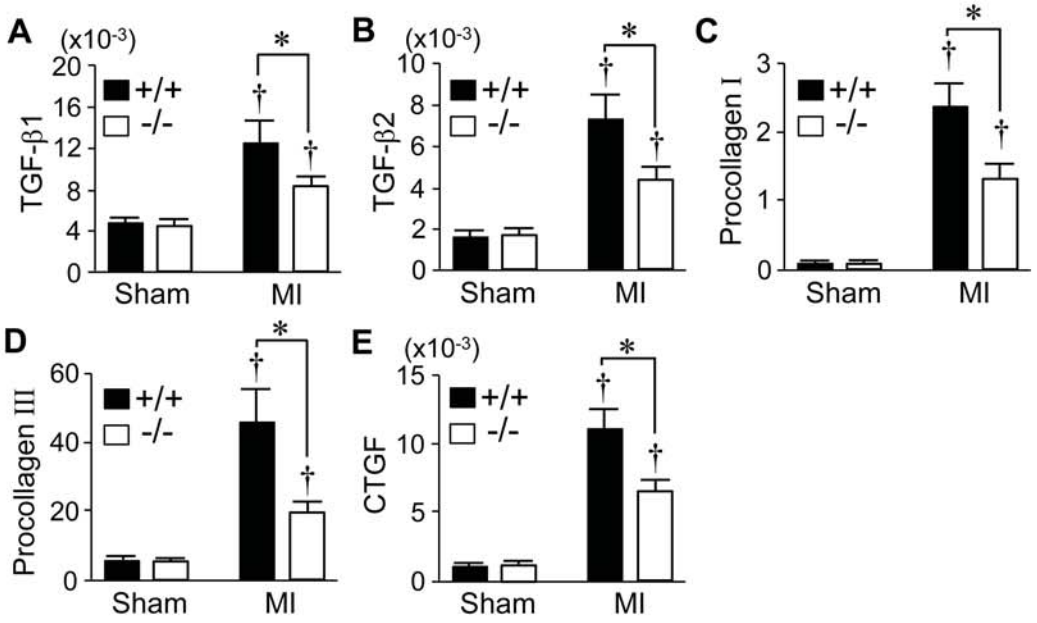
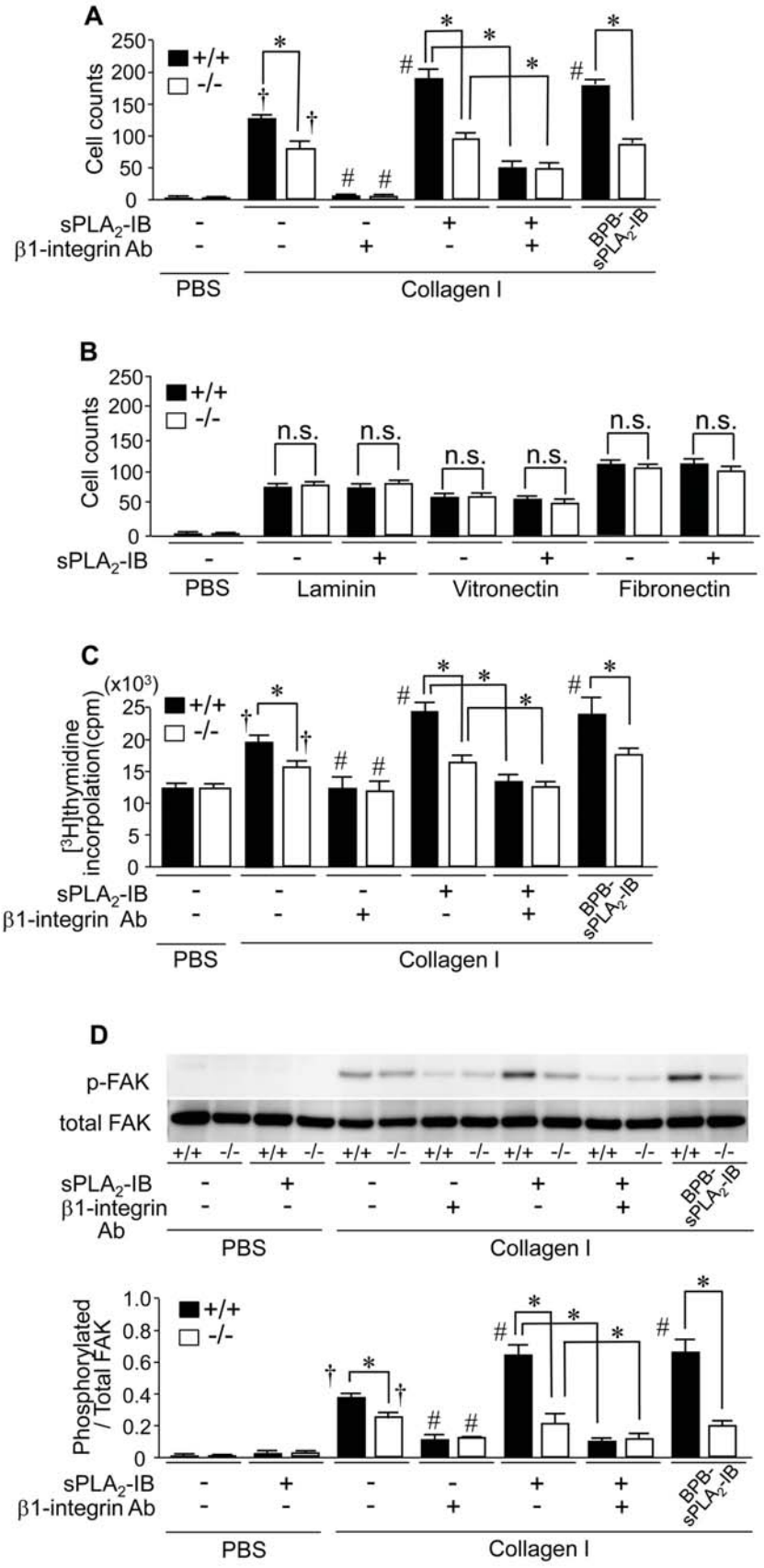

A
(x10-3)
TGF-β1
+/+
-/-
Sham
MI
B
(x10-3)
TGF-β2
C
Procollagen I
D
Procollagen III
E
(x10-3)
CTGF

Figure 5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Cell counts
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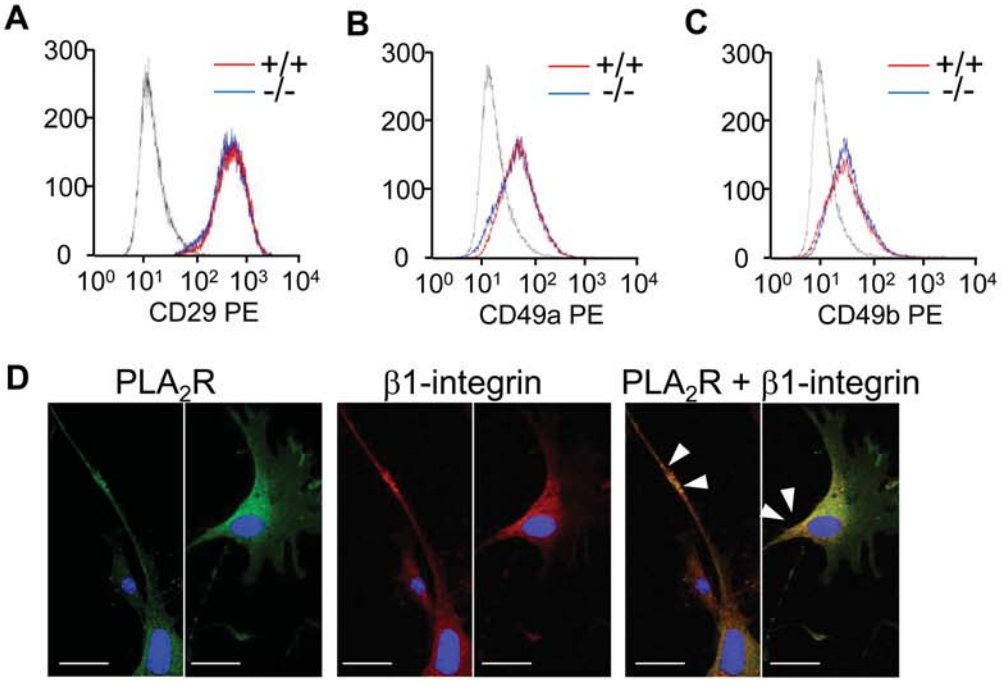
-/-
sPLA2-IB
β1-integrin Ab
BPB-sPLA2-IB
PBS
Collagen I
B
Cell counts
n.s.
sPLA2-IB
PBS
Laminin
Vitronectin
Fibronectin
C
[3H]thymidine incorpolation(cpm)
(x10³)
sPLA2-IB
β1-integrin Ab
BPB-sPLA2-IB
PBS
Collagen I
D
p-FAK
total FAK
sPLA2-IB
β1-integrin Ab
BPB-sPLA2-IB
PBS
Collagen I
Phosphorylated / Total FAK
sPLA2-IB
β1-integrin Ab
BPB-sPLA2-IB
PBS
Collagen I

Figure 6


A
B
C
300
200
100
0
+/+
-/-
10^0 10^1 10^2 10^3 10^4
CD29 PE
CD49a PE
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D
PLA_2R
β1-integrin
PLA_2R + β1-integrin

Figure 7

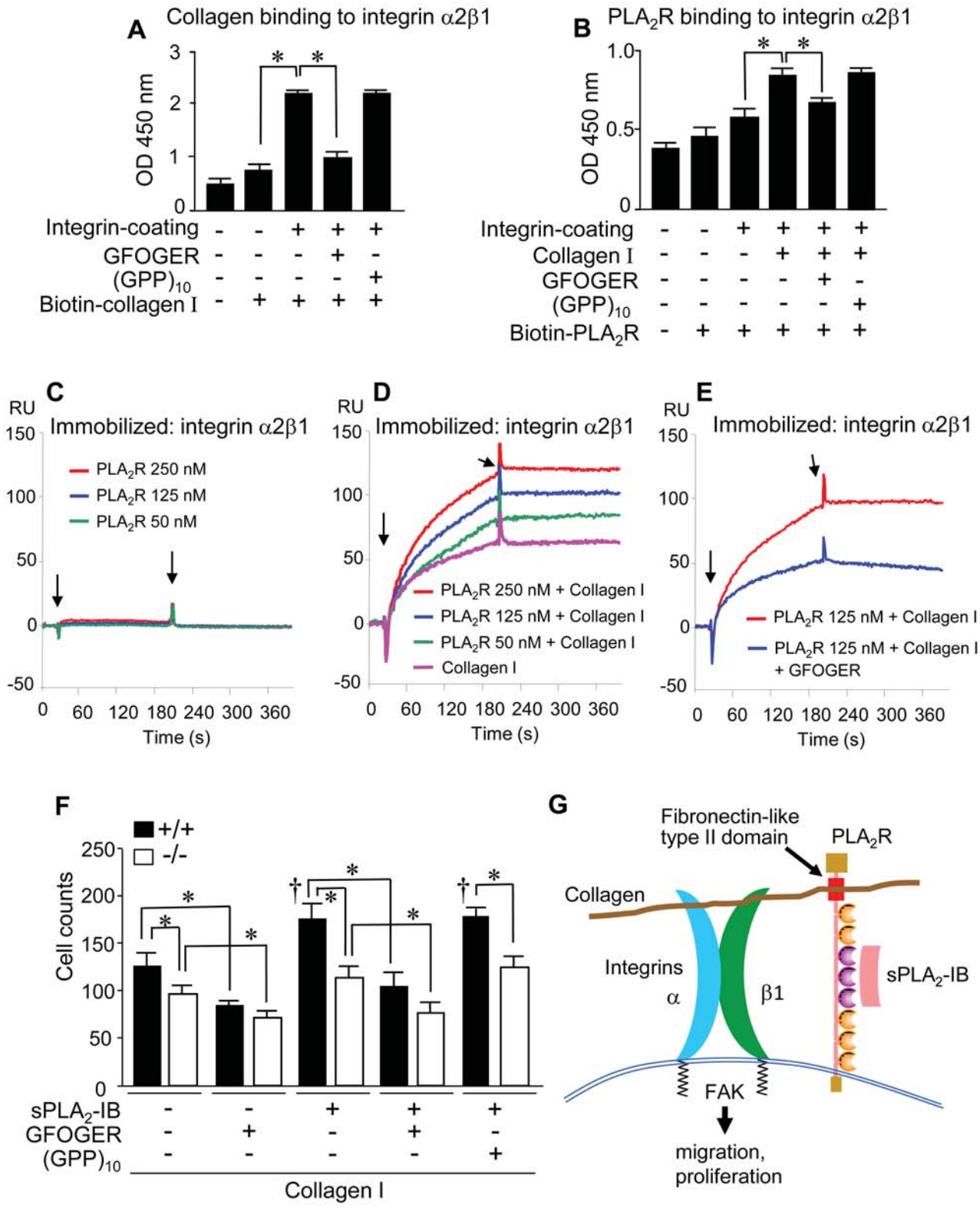
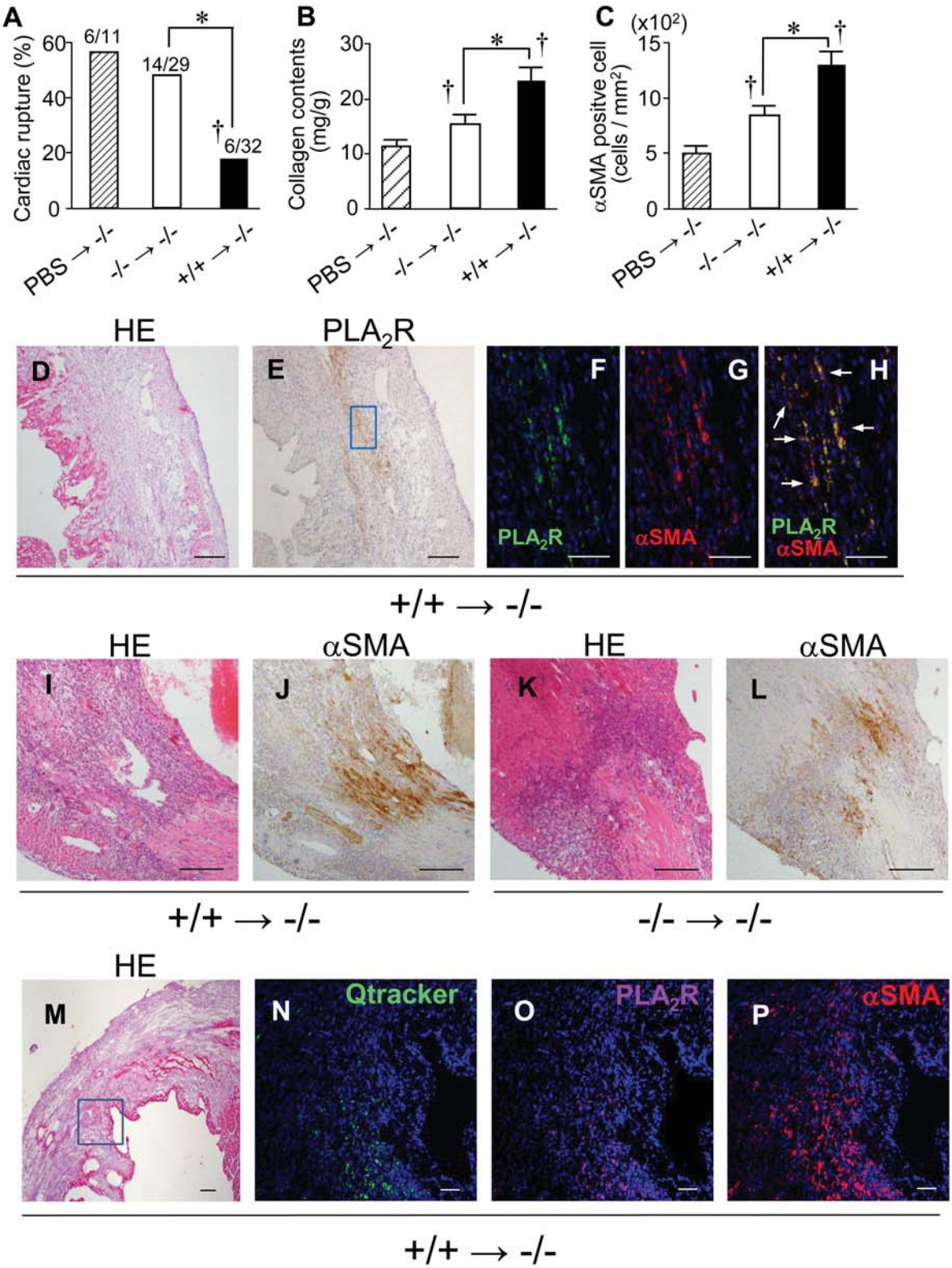

A
Collagen binding to integrin α2β1
OD 450 nm
Integrin-coating
GFOGER
(GPP)10
Biotin-collagen I
B
PLA2R binding to integrin α2β1
OD 450 nm
Integrin-coating
Collagen I
GFOGER
(GPP)10
Biotin-PLA2R
C
Immobilized: integrin α2β1
RU
PLA2R 250 nM
PLA2R 125 nM
PLA2R 50 nM
Time (s)
D
Immobilized: integrin α2β1
RU
PLA2R 250 nM + Collagen I
PLA2R 125 nM + Collagen I
PLA2R 50 nM + Collagen I
Collagen I
Time (s)
E
Immobilized: integrin α2β1
RU
PLA2R 125 nM + Collagen I
PLA2R 125 nM + Collagen I + GFOGER
Time (s)
F
+/+
-/-
Cell counts
sPLA2-IB
GFOGER
(GPP)10
Collagen I
G
Fibronectin-like type II domain
PLA2R
Collagen
Integrins
α
β1
sPLA2-IB
FAK
migration, proliferation

Figure 8


A
Cardiac rupture (%)
60
40
20
0
6/11
14/29
6/32
*
†
PBS → -/-
-/- → -/-
+/+ → -/-
B
Collagen contents (mg/g)
30
20
10
0
*
†
†
PBS → -/-
-/- → -/-
+/+ → -/-
C
αSMA positive cell (cells / mm²)
(x10²)
15
10
5
0
*
†
†
PBS → -/-
-/- → -/-
+/+ → -/-
HE
PLA2R
D
E
F
G
H
PLA2R
αSMA
PLA2R
αSMA
+/+ → -/-
HE
αSMA
HE
αSMA
I
J
K
L
+/+ → -/-
-/- → -/-
HE
M
N
Qtracker
O
PLA2R
P
αSMA
+/+ → -/-