Lp-PLA 2 Antagonizes Left Ventricular Healing After Myocardial Infarction by Impairing the Appearance of Reparative MacrophagesCLINICAL PERSPECTIVE

Lp-PLA$_2$ antagonizes left ventricular healing after myocardial infarction by impairing the appearance of reparative macrophages

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Background: Healing after myocardial infarction (MI) involves the biphasic accumulation of inflammatory Ly-6C^{high} and reparative Ly-6C^{low} monocytes/macrophages. Excessive inflammation disrupts the balance between the two phases, impairs infarct healing, and contributes to left ventricle remodeling and heart failure. Lipoprotein-associated phospholipase A_2 (Lp-PLA_2), a member of the phospholipase A_2 family of enzymes, produced predominantly by leukocytes, participates in host defenses and disease. Elevated Lp-PLA_2 levels associate with increased risk of cardiovascular events across diverse patient populations, but the mechanisms by which the enzyme elicits its effects remain unclear. This study tested the role of Lp-PLA_2 in healing after MI.

Methods and Results: In response to MI, Lp-PLA_2 levels markedly increased in the circulation. To test the functional importance of Lp-PLA_2, we generated chimeric mice whose bone marrow-derived leukocytes were Lp-PLA_2-deficient (*bmLp-PLA$_2^{-/-}$*). Compared to WT controls, *bmLp-PLA$_2^{-/-}$* mice subjected to MI had lower serum levels of inflammatory cytokines TNFα, IL-1β and IL-6, and decreased number of circulating inflammatory myeloid cells. Accordingly, *bmLp-PLA$_2^{-/-}$* mice developed smaller and less inflamed infarcts with reduced numbers of infiltrating neutrophils and inflammatory Ly-6C^{high} monocytes. During the later, reparative phase, infarcts of *bmLp-PLA$_2^{-/-}$* mice contained Ly-6C^{low} macrophages with a skewed M2-prone gene expression signature, increased collagen deposition, fewer inflammatory cells, and improved indices of angiogenesis. Consequently, the hearts of *bmLp-PLA$_2^{-/-}$* mice healed more efficiently, as determined by improved left ventricle (LV) remodeling and ejection fraction.

Conclusions: Lp-PLA_2 augments the inflammatory response after myocardial infarction and antagonizes healing by disrupting the balance between inflammation and repair, providing a rationale for focused study of ventricular function and heart failure after targeting this enzyme acutely in MI.

Myocardial infarction (MI) is a leading cause of death worldwide[1]. Although the case fatality rate of MI has declined, survival with development of long-term left ventricular dysfunction due to cumulative ischemic myocardial damage has added to the growing epidemic burden of chronic heart failure. The human, social, and economic consequences of chronic ischemic cardiomyopathy present a major challenge and unmet medical need. Patients who initially survive MI must overcome a major obstacle: ischemia damages the heart, and effective cardiac repair likely requires a precise balance between removal of debris and formation of a scar that is compatible with heart function. MI survivors frequently develop heart failure; while many therapeutics in current use have proven beneficial, the high residual morbidity and mortality presents an urgent problem that requires a better understanding of the disease's pathophysiology.

Over the last several years, neutrophils, monocytes and macrophages have emerged as consequential to the inflammatory and healing process that occurs after MI[2]. We now understand that ischemic injury triggers the accumulation of these myeloid cells in the infarcted myocardium[3, 4]. Shortly after onset of ischemia, large numbers of neutrophils and inflammatory Ly-6C^{high} monocytes infiltrate the infarcted myocardium and produce IL-1β, IL-6, and TNFα. Within 4-5 days, Ly-6C^{high} monocytes give rise to Ly-6C^{low} reparative macrophages[3], which potentiate healing via VEGF, TGFβ, and IL-10. The two phases comprising inflammatory Ly-6C^{high} monocyte recruitment and reparative Ly-6C^{low} macrophage differentiation are essential to post-MI recovery; their perturbation (i.e., in the context of co-morbidities) leads to impaired heart function and heart failure[3, 4].

Lipoprotein-associated phospholipase A2 (Lp-PLA$_2$), a member of the phospholipase A$_2$ family of enzymes, hydrolyses glycerophospholipids. The ensuing enzymatic reactions frequently generate metabolic signaling molecules with a multitude of biological actions. For example, by hydrolysing phosphotidylcholine at C2 of the glycerol backbone, Lp-PLA$_2$ produces lysophosphatidylcholine (lysoPC)[5], which fosters oxidative stress, affects vascular smooth muscle cell proliferation, and increases tissue accumulation of macrophages[6]. A number of observational studies showed that Lp-PLA$_2$ levels correspond with future cardiovascular events such as acute myocardial infarction and sudden cardiac death.[7-9] While preliminary studies reported reduced development of advanced coronary atherosclerosis[10] or stabilization of the necrotic core size[11] with selective inhibition of Lp-PLA$_2$ by the inhibitor darapladib, two recently completed phase III trials did not provide evidence in favor of inhibiting Lp-PLA$_2$ in cardiovascular disease[12]. Neither study focused on left ventricular function nor chronic heart failure endpoints. Moreover, it remains unclear whether Lp-PLA$_2$ participates in the inflammatory and reparative phases that characterize the innate immune response shortly after MI; these pathways likely influence left

ventricular remodeling and the development of chronic ischemic cardiomyopathy. This study sought to evaluate whether Lp-PLA_2 participates in infarct healing and HF after MI.

Methods

For further details, see the online Supplemental Material.

Animals and animal experiments. 8-10 weeks old Female C57BL6/J (WT) were purchased from the Jackson Laboratory (Bar Harbor, ME). Lp-PLA_2-deficient mice (*Lp-$PLA_2^{-/-}$*) were kindly provided by Glaxosmithkline Pharmaceuticals Ltd (King of Prussia, PA 19112). All protocols were approved by the Animal Review Committee at Massachusetts General Hospital. C57BL6/J mice were lethally irradiated and reconstituted with WT and *Lp-$PLA_2^{-/-}$* bone marrow to generate respective chimeric mice. The chimeras had normal leukocyte counts and exhibited no obvious abnormalities, consistent with the Lp-PLA_2-deficient mice[13]. Myocardial infarction (MI) was induced by permanent ligation of the left anterior descending artery. We observed no differences in mortality between the groups.

Lp-PLA2/PAF acetyl-hydrolase activity assay. The PAF hydrolase activity assay was performed as previously described[14] with modifications using [^{3}H]PAF (Platelet Activating Factor, 1-O-Hexadecyl-[Acetyl-^{3}H(N)]-, Hexadecyl PAF) as a substrate. Unlabeled PAF (1-O-Hexadecyl-2-O-acetyl-sn-glycero-3-phophorylcholine) was purchased from Enzo Life Sciences, 1-O-Hexadecyl-2-O-[Acetyl-^{3}H(N)]-, Hexadecyl PAF, [acetyl-^{3}H]-, (250μCi (9.25MBq) was purchased from Perkin Elmer, and Bio-Safe II was purchased from Research Products International Corp., Mount Prospect, IL.

Histology. Murine hearts were embedded in Tissue-Tek O.C.T compound (Sakura Finetek) and prepared for sectioning and staining.

Flow cytometry and flow assisted cell sorting. Antibodies used for flow cytometry are listed in the online Supplemental Material. Data were acquired on a BD LSRII and analyzed with FlowJo. Cells were sorted with BD AriaII.

Reverse transcription PCR. RNA was isolated from sorted cells with the RNeasy Micro Kit (Qiagen). Quantitative real-time TaqMan PCR was run on a 7500 PCR thermal cycler (Applied Biosystems).

Magnetic resonance imaging (MRI). MRI was carried out on days 1 and 21 after permanent coronary ligation as described perviously[3]. We obtained cine images of the left ventricular short axis by using a 7 Tesla horizontal bore Pharmascan (Bruker) and a custom-built mouse cardiac coil (Rapid Biomedical). Late gadolinium enhancement was performed on day 1 to determine infarct size. Acquisition was done as

described previously[15]. Images were analyzed using the software Segment (http://segment.heiberg.se).The end-diastolic volume (EDV), end-systolic volume (ESV), ejection fraction (EF), left ventricle volume (LVM), heart rate and cardiac output (CO) were measured.

Statistics: Results are shown as mean ± SEM. Unpaired Student's t-test was applied to evaluate differences between two groups. One-way ANOVA with post-hoc Tukey's multiple comparisons test was performed when comparing more than two groups between days, because different mice were sacrificed on each time points for organ harvest. P-values of 0.05 and less denote significant changes.

Results

Expression of Lp-PLA$_2$ after MI and its role on healing

To elucidate whether Lp-PLA$_2$ participates in healing after MI, we first measured Lp-PLA$_2$ expression and serum activity in steady state and after MI. Lp-PLA$_2$ mRNA expression by RT-PCR increased in the infarcts of wild type (WT) mice as early as 1 day after MI (**Fig. 1A**), suggesting Lp-PLA$_2$ may participate in myocardial ischemic injury. Concordantly, Lp-PLA$_2$ activity was also increased shortly after MI (**Fig. 1B**). To define the role of Lp-PLA$_2$ in hematopoietic cells in the pathophysiology of acute MI, we generated chimeric mice by irradiating and reconstituting WT mice with bone marrow either from WT or *Lp-PLA$_2^{-/-}$* (*bmLp-PLA$_2^{-/-}$*) mice. In comparison to WT mice, *bmLp-PLA$_2^{-/-}$* mice had lower Lp-PLA$_2$ activity at steady state, and this activity did not change after MI (**Fig. 1B**). These findings establish leukocytes as major sources of Lp-PLA$_2$ in response to MI. We then evaluated infarct healing and demonstrated a significant decrease in infarct size in *bmLp-PLA$_2^{-/-}$* mice 7 days after MI compared to WT (**Fig. 1C, D**).

Lp-PLA$_2$ influences systemic inflammation

Coronary occlusion stimulates an inflammatory response characterized by cytokine and chemokine production, and leukocyte recruitment to the heart. Because Lp-PLA$_2$ participates in inflammation, we assessed the effect of Lp-PLA$_2$ deficiency after MI. Serum concentrations of inflammatory cytokines TNFα, IL-1β and IL-6 increased dramatically in WT mice on day 1 after MI, and eventually declined to undetectable amounts on day 7. In contrast, *bmLp-PLA$_2^{-/-}$* mice showed only moderately elevated TNFα and IL-6, and negligibly increased IL-1β, demonstrating a diminished inflammatory response in the absence of Lp-PLA$_2$ (**Fig. 2A**). Time-course profiling of circulating leukocytes after MI revealed that both WT and *bmLp-PLA$_2^{-/-}$* mice augmented inflammatory myeloid cells (neutrophils and Ly-6C^{high} monocytes), but compared to WT mice, *bmLp-PLA$_2^{-/-}$* mice had fewer neutrophils and Ly-6C^{high} cells in blood at days 3 and 7, indicating Lp-PLA$_2$ contributes to the systemic inflammation after MI (**Fig. 2B, C**).

Lp-PLA$_2$ impairs the appearance of reparative macrophages

The myocardium displays a biphasic monocyte and macrophage response during MI[3, 4]. In the first phase, inflammatory Ly-6C^{high} monocytes infiltrate the ischemic myocardium from the blood and participate in inflammation. In the second phase, reparative Ly-6C^{low} macrophages contribute to collagen deposition and scar formation. We profiled leukocytes in the myocardium in the steady state and 1, 3 and 7 days after MI in both WT and *bmLp-PLA$_2^{-/-}$* mice. The infarcts of both strains accumulated neutrophils, which peaked on day 1, Ly-6C^{high} monocytes, which peaked on day 3, and Ly-6C^{low} macrophages, which peaked on day 7 (**Fig. 3A, B**). This finding agrees with our previous observations[3, 4]. Yet, for nearly every peak, infarcts of *bmLp-PLA$_2^{-/-}$* mice accumulated only half the number of cells (neutrophils on day 1 and Ly-6C^{high} monocytes on day 3) compared to WT controls, consistent with our observations in the blood (**Fig. 2**), and affirming that Lp-PLA$_2$ aggravates inflammation.

Aside from determining the number of cells that accumulate (quantity), macrophage activity (quality) is an essential measure of the cells' impact on inflammation and repair. We asked whether Lp-PLA$_2$ shapes macrophage function by measuring expression of signature M1/M2 genes in sorted cardiac macrophages. In comparison to WT macrophages, *Lp-PLA$_2^{-/-}$* macrophages exhibited higher expression of mRNAs that encode M2-associated genes (Arg, IL-10, CD36, Fizz) and lower levels of those corresponding to M1-associated genes (MMP-3, MMP-9, TLR9, TLR4) (**Fig. 3C**). These data are consistent with the idea that lysophosphotidylcholine, a product of Lp-PLA$_2$, potentiates an M1-like macrophage phenotype.[16] Together, these data show that Lp-PLA$_2$ promotes recruitment of inflammatory myeloid cells and delays the appearance of reparative macrophages in the ischemic myocardium.

Lp-PLA$_2$ retards healing after myocardial infarction

The differences in leukocyte recruitment between WT and *bmLp-PLA$_2^{-/-}$* mice prompted us to determine whether the absence of Lp-PLA$_2$ affects the repair of the ischemic myocardium. To this end, we profiled myeloid cell infiltration, extracellular matrix deposition, neovascularization, and smooth muscle cell accumulation by immunohistochemistry (**Fig. 4**). Compared with WT controls, the myocardium of *bmLp-PLA$_2^{-/-}$* mice accumulated fewer myeloid CD11b$^+$ cells, indicating less severe inflammation. Infarcts of *bmLp-PLA$_2^{-/-}$* mice also had larger regions of extracellular matrix deposition, as evidenced by higher percentage of collagen I$^+$ areas (24% versus 32%,), larger CD31$^+$ areas (5% vs 11%), suggesting improved neovascularization of the heart, but no changes in the number of smooth muscle actin$^+$ myofibroblasts (α-SMA+ area). Collectively, the results of histologic examination demonstrate more effective healing in the absence of Lp-PLA$_2$. These results demonstrate that Lp-PLA$_2$ inhibits the resolution of inflammation after MI.

Improved heart function in the absence of Lp-PLA$_2$

To test whether inflammation mediated by Lp-PLA$_2$ after MI translated to impaired heart function, we performed magnetic resonance imaging (MRI) *in vivo* in WT and *bmLp-PLA$_2^{-/-}$* mice. In the steady state, we detected no differences in cardiac function between WT and *bmLp-PLA$_2^{-/-}$* mice. After permanent coronary artery ligation, the end-diastolic volume (EDV) and end-systolic volume (ESV), ejection fraction (EF), left ventricle volume (LVM), heart rate and cardiac output (CO) were measured in individual mice on day 1 and day 21 after MI. Late gadolinium enhancement was performed on day 1 to determine infarct size (**Fig. 5A, B and Table 1**). The infarct sizes were similar on day 1 in both groups, excluding a potential surgical bias (**Fig. 5B**). Although LVM increased similarly between the two groups on day 21, increased EDV was only observed in WT mice, indicating more favorable remodeling in mice lacking Lp-PLA$_2$. Moreover, compared with day 1, EF at day 21 diminished in WT mice but increased modestly in *bmLp-PLA$_2^{-/-}$* mice, suggesting improved recovery of heart function in the absence of Lp-PLA$_2$. Overall, the data show that Lp-PLA$_2$ aggravates LV remodeling and impairs LV function after MI.

Discussion

Recruitment of neutrophils and Ly-6C^{high} monocytes into the infarcted myocardium and the subsequent generation of reparative macrophages from Ly-6C^{high} monocytes contribute to necrotic debris clearance, matrix deposition, granulation tissue formation, and angiogenesis. Perturbations in the inflammatory response impair infarct healing and promote heart failure[17-19]. This study shows that deficiency of Lp-PLA$_2$ on hematopoietic cells attenuates systemic inflammation after MI, impairs leukocyte infiltration into infarcts, and enhances generation of reparative Ly-6C^{low} macrophages, leading to less adverse LV remodeling and improved recovery of LV function. Together, the data show that Lp-PLA$_2$ modulates inflammation after MI and suggest that targeting of Lp-PLA$_2$ might lessen LV dysfunction and the development of chronic heart failure following myocardial infarction.

Monocyte-derived macrophages can produce substantial Lp-PLA$_2$[20], and in the mouse Lp-PLA$_2$ is expressed almost exclusively by myeloid cells (www.immgen.org). Previous studies have identified macrophage Lp-PLA$_2$ expression at both the mRNA and protein levels in human and rabbit aortic lesions[21]. Plaques with characteristics of vulnerable and ruptured atheromata, but not early lesions, contain abundant Lp-PLA$_2$[22]. Therefore, extensive efforts have been taken to prevent atherosclerosis-related coronary heart disease by inhibiting Lp-PLA$_2$ and thus promoting plaque stability[12]. But Lp-PLA$_2$ may participate in coronary heart disease by modulating inflammation independent of effects on the plaque itself. For example, our data showed that Lp-PLA$_2$ rose dramatically during MI. As a phospholipase, increased Lp-PLA$_2$ hydrolyzes phospholipids of oxidatively damaged cells or lipoproteins. Lp-PLA2 hydrolyzes oxidized phosphatidylcholine, generating lysoPC and oxidized fatty acids that are capable of acting as monocyte chemoattractants as well as maintaining macrophages in a pro-inflammatory phenotype[6, 16, 23]. Lp-PLA2 can also contribute to inflammation by interfering with

phagocytic clearance of apoptotic cells, e.g. neutrophils, via cleavage and removal of oxidized phosphatidylserine, a known "eat-me" signal[24]. Consistent with this hypothesis, we found that *bmLp-PLA$_2^{-/-}$* mice had milder inflammatory responses as well as fewer neutrophils and Ly-6C^{high} monocytes accumulating in the myocardium. This reduced number of inflammatory leukocytes ameliorated adverse LV remodeling and improved heart function recovery. Therefore, our data strongly support a role for Lp-PLA$_2$ in increased risk of heart failure by directly promoting systemic and local myocardial inflammatory responses after MI, functions distinct from effects on the atherosclerotic plaques. Our experiments, performed with bone marrow chimeras, demonstrate an effect on MI healing with a ~80% knockdown of Lp-PLA$_2$, indicating this is a sufficient reduction to elicit positive effects. Future studies will need to elucidate in more detail the full scope of Lp-PLA$_2$ function not only on leukocyte behavior, but also on effects attributed to other cells in the heart, such as fibroblasts and endothelial cells.

Recently, two large multi-center phase III trials completed evaluation of the Lp-PLA2 inhibitor darapladib on the reduction of cardiovascular adverse events in over 28,000 patients with documented coronary heart disease. These two complementary trials covered both chronic and acute coronary heart disease and evaluated traditional coronary heart disease endpoints. Thus, these studies did not formally focus on LV function or long-term development of heart failure[25, 26]. The *S*tabilization *o*f p*L*aques us*I*ng *D*arapladib-*T*hrombolysis *i*n *M*yocardial *I*nfarction (SOLID-TIMI 52) trial enrolled patients within 30 days of acute coronary syndrome (ACS) and the *ST*abilization of *A*therosclerotic plaque *B*y *I*nitiation of darap*L*ad*I*b *T*herap*Y* (STABILITY) trial enrolled patients with stable chronic coronary heart disease. Despite promising preclinical results[10], both clinical trials concluded that direct inhibition of Lp-PLA$_2$ with darapladib failed to reduce major adverse cardiovascular events[12, 27]. Crucially, the trials did not test whether blockade of Lp-PLA$_2$ *per se* was beneficial: patients receiving darapladib or placebo were already receiving up to 4 different therapeutics against heart disease, including statins and ACE inhibitors. The trials therefore showed that under the specific parameters of the study, darapladib did not provide benefit above that afforded by current treatment regimens.

Our results provide an alternative explanation as to why the clinical trials might not have improved the tested end-points. Our data show that, as early as day 1 after MI, expression of Lp-PLA$_2$ was increased. In the absence of Lp-PLA$_2$, inflammatory leukocyte recruitment was blunted, yielding a smaller inflammatory response that correlated with improved healing and heart function 7 and 21 days later, respectively. Lp-PLA$_2$ might elicit its most detrimental effects in the acute phase after MI. If so, blocking the enzyme earlier than the SOLID or STABILITY trials might benefit endpoints related to LV function. Several human studies have shown data in support of the idea that healing of the infarcted myocardium involves the biphasic accumulation of monocytes and macrophages. In one study, a cohort of 36 patients monitored over two weeks after MI demonstrated a peak of circulating inflammatory CD16$^-$ monocytes

on day 2.6 after MI, followed by another peak of $CD16^+$ monocytes on day 4.8.[19] Because $CD16^-$ monocytes resemble inflammatory Ly-$6C^{high}$ monocytes whereas $CD16^+$ monocytes (and in particular $CD16^+$ $CD14^{dim}$) resemble Ly-$6C^{low}$ monocytes, these findings suggest that acute inflammation may likewise peak in humans within one week after MI. Immediate inhibition of Lp-PLA_2 might be the optimal time window for improving outcomes related to LV function. The SOLID trial, which enrolled patients within 30 days, did not test whether patients receiving darapladib within the first 3 days post MI benefited from treatment. Hence, the clinical trial findings do not argue against the role of Lp-PLA_2 in inflammation after MI.

In summary, the data demonstrate that Lp-PLA_2 regulates the host response after MI through modulation of inflammation. By enhancing inflammation and impairing repair, the induced Lp-PLA_2 negatively regulates recovery of LV function. In addition to stimulating atherosclerotic plaque vulnerability, the effect of Lp-PLA_2 after MI reveals a novel role of this enzyme in modulating the myocardial response to ischemic injury. These mechanistic insights have implications for developing effective therapeutics against ischemic cardiomyopathy and chronic heart failure post MI.

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Disclosures

None.

References

1. Bloom DE, Cafiero ET, Jane-Llopis E, Abrahams-Gessel S, Bloom LR, Fathima S, Feigl AB, Gaziano T, Mowafi M, Pandya A, Prettner K, Rosenberg L, Seligman B, Stein AZ, Weinstein C. The Global Economic Burden of Noncommunicable Diseases. Geneva: World Economic Forum; 2011.
2. Swirski FK, Nahrendorf M. Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure. Science. 2013;339:161-166.
3. Hilgendorf I, Gerhardt LM, Tan TC, Winter C, Holderried TA, Chousterman BG, Iwamoto Y, Liao R, Zirlik A, Scherer-Crosbie M, Hedrick CC, Libby P, Nahrendorf M, Weissleder R, Swirski FK. Ly-6Chigh monocytes depend on Nr4a1 to balance both inflammatory and reparative phases in the infarcted myocardium. Circ Res. 2014;114:1611-1622.
4. Nahrendorf M, Swirski FK, Aikawa E, Stangenberg L, Wurdinger T, Figueiredo JL, Libby P, Weissleder R, Pittet MJ. The healing myocardium sequentially mobilizes two monocyte subsets with divergent and complementary functions. J Exp Med. 2007;204:3037-3047.

5. MacPhee CH, Moores KE, Boyd HF, Dhanak D, Ife RJ, Leach CA, Leake DS, Milliner KJ, Patterson RA, Suckling KE, Tew DG, Hickey DM. Lipoprotein-associated phospholipase A2, platelet-activating factor acetylhydrolase, generates two bioactive products during the oxidation of low-density lipoprotein: use of a novel inhibitor. Biochem J. 1999;338:479-487.
6. Shi Y, Zhang P, Zhang L, Osman H, Mohler ER, Macphee C, Zalewski A, Postle A, Wilensky RL. Role of lipoprotein-associated phospholipase A2 in leukocyte activation and inflammatory responses. Atherosclerosis. 2007;191:54-62.
7. Gerber Y, McConnell JP, Jaffe AS, Weston SA, Killian JM, Roger VL. Lipoprotein-associated phospholipase A2 and prognosis after myocardial infarction in the community. Arterioscler Thromb Vasc Biol. 2006;26:2517-2522.
8. Herrmann J, Mannheim D, Wohlert C, Versari D, Meyer FB, McConnell JP, Gossl M, Lerman LO, Lerman A. Expression of lipoprotein-associated phospholipase A(2) in carotid artery plaques predicts long-term cardiac outcome. Eur Heart J. 2009;30:2930-2938.
9. Oei HH, van der Meer IM, Hofman A, Koudstaal PJ, Stijnen T, Breteler MM, Witteman JC. Lipoprotein-associated phospholipase A2 activity is associated with risk of coronary heart disease and ischemic stroke: the Rotterdam Study. Circulation. 2005;111:570-575.
10. Wilensky RL, Shi Y, Mohler ER, Hamamdzic D, Burgert ME, Li J, Postle A, Fenning RS, Bollinger JG, Hoffman BE, Pelchovitz DJ, Yang J, Mirabile RC, Webb CL, Zhang L, Zhang P, Gelb MH, Walker MC, Zalewski A, Macphee CH. Inhibition of lipoprotein-associated phospholipase A2 reduces complex coronary atherosclerotic plaque development. Nat Med. 2008;14:1059-1066.
11. Serruys PW, Garcia-Garcia HM, Buszman P, Erne P, Verheye S, Aschermann M, Duckers H, Bleie O, Dudek D, Botker HE, von Birgelen C, D'Amico D, Hutchinson T, Zambanini A, Mastik F, van Es GA, van der Steen AF, Vince DG, Ganz P, Hamm CW, Wijns W, Zalewski A. Effects of the direct lipoprotein-associated phospholipase A(2) inhibitor darapladib on human coronary atherosclerotic plaque. Circulation. 2008;118:1172-1182.
12. White HD, Held C, Stewart R, Tarka E, Brown R, Davies RY, Budaj A, Harrington RA, Steg PG, Ardissino D, Armstrong PW, Avezum A, Aylward PE, Bryce A, Chen H, Chen MF, Corbalan R, Dalby AJ, Danchin N, De Winter RJ, Denchev S, Diaz R, Elisaf M, Flather MD, Goudev AR, Granger CB, Grinfeld L, Hochman JS, Husted S, Kim HS, Koenig W, Linhart A, Lonn E, Lopez-Sendon J, Manolis AJ, Mohler ER, Nicolau JC, Pais P, Parkhomenko A, Pedersen TR, Pella D, Ramos-Corrales MA, Ruda M, Sereg M, Siddique S, Sinnaeve P, Smith P, Sritara P, Swart HP, Sy RG, Teramoto T, Tse HF, Watson D, Weaver WD, Weiss R, Viigimaa M, Vinereanu D, Zhu J, Cannon CP, Wallentin L. Darapladib for preventing ischemic events in stable coronary heart disease. N Engl J Med. 2014;370:1702-1711.
13. Jiang Z, Fehrenbach ML, Ravaioli G, Kokalari B, Redai IG, Sheardown SA, Wilson S, Macphee C, Haczku A. The effect of lipoprotein-associated phospholipase A2 deficiency on pulmonary allergic responses in Aspergillus fumigatus sensitized mice. Respir Res. 2012;13:100.
14. Tew DG, Southan C, Rice SQ, Lawrence MP, Li H, Boyd HF, Moores K, Gloger IS, Macphee CH. Purification, properties, sequencing, and cloning of a lipoprotein-associated, serine-dependent phospholipase involved in the oxidative modification of low-density lipoproteins. Arterioscler Thromb Vasc Biol. 1996;16:591-599.
15. Courties G, Heidt T, Sebas M, Iwamoto Y, Jeon D, Truelove J, Tricot B, Wojtkiewicz G, Dutta P, Sager HB, Borodovsky A, Novobrantseva T, Klebanov B, Fitzgerald K, Anderson DG, Libby P, Swirski FK, Weissleder R, Nahrendorf M. In vivo silencing of the transcription factor IRF5 reprograms the macrophage phenotype and improves infarct healing. J Am Coll Cardiol. 2014;63:1556-1566.
16. Qin X, Qiu C, Zhao L. Lysophosphatidylcholine perpetuates macrophage polarization toward classically activated phenotype in inflammation. Cell Immunol. 2014;289:185-190.
17. Maekawa Y, Anzai T, Yoshikawa T, Asakura Y, Takahashi T, Ishikawa S, Mitamura H, Ogawa S. Prognostic significance of peripheral monocytosis after reperfused acute myocardial infarction:a possible role for left ventricular remodeling. J Am Coll Cardiol. 2002;39:241-246.
18. Panizzi P, Swirski FK, Figueiredo JL, Waterman P, Sosnovik DE, Aikawa E, Libby P, Pittet M, Weissleder R, Nahrendorf M. Impaired infarct healing in atherosclerotic mice with Ly-6C(hi) monocytosis. J Am Coll Cardiol. 2010;55:1629-1638.

19. Tsujioka H, Imanishi T, Ikejima H, Kuroi A, Takarada S, Tanimoto T, Kitabata H, Okochi K, Arita Y, Ishibashi K, Komukai K, Kataiwa H, Nakamura N, Hirata K, Tanaka A, Akasaka T. Impact of heterogeneity of human peripheral blood monocyte subsets on myocardial salvage in patients with primary acute myocardial infarction. J Am Coll Cardiol. 2009;54:130-138.
20. Tsimikas S, Tsironis LD, Tselepis AD. New insights into the role of lipoprotein(a)-associated lipoprotein-associated phospholipase A2 in atherosclerosis and cardiovascular disease. Arterioscler Thromb Vasc Biol. 2007;27:2094-2099.
21. Hakkinen T, Luoma JS, Hiltunen MO, Macphee CH, Milliner KJ, Patel L, Rice SQ, Tew DG, Karkola K, Yla-Herttuala S. Lipoprotein-associated phospholipase A(2), platelet-activating factor acetylhydrolase, is expressed by macrophages in human and rabbit atherosclerotic lesions. Arterioscler Thromb Vasc Biol. 1999;19:2909-2917.
22. Kolodgie FD, Burke AP, Skorija KS, Ladich E, Kutys R, Makuria AT, Virmani R. Lipoprotein-associated phospholipase A2 protein expression in the natural progression of human coronary atherosclerosis. Arterioscler Thromb Vasc Biol. 2006;26:2523-2529.
23. Zalewski A, Macphee C. Role of lipoprotein-associated phospholipase A2 in atherosclerosis: biology, epidemiology, and possible therapeutic target. Arterioscler Thromb Vasc Biol. 2005;25:923-931.
24. Tyurin VA, Balasubramanian K, Winnica D, Tyurina YY, Vikulina AS, He RR, Kapralov AA, Macphee CH, Kagan VE. Oxidatively modified phosphatidylserines on the surface of apoptotic cells are essential phagocytic 'eat-me' signals: cleavage and inhibition of phagocytosis by Lp-PLA2. Cell Death Differ. 2014;21:825-835.
25. O'Donoghue ML, Braunwald E, White HD, Serruys P, Steg PG, Hochman J, Maggioni AP, Bode C, Weaver D, Johnson JL, Cicconetti G, Lukas MA, Tarka E, Cannon CP. Study design and rationale for the Stabilization of pLaques usIng Darapladib-Thrombolysis in Myocardial Infarction (SOLID-TIMI 52) trial in patients after an acute coronary syndrome. Am Heart J. 2011;162:613-619.e1.
26. White H, Held C, Stewart R, Watson D, Harrington R, Budaj A, Steg PG, Cannon CP, Krug-Gourley S, Wittes J, Trivedi T, Tarka E, Wallentin L. Study design and rationale for the clinical outcomes of the STABILITY Trial (STabilization of Atherosclerotic plaque By Initiation of darapLadIb TherapY) comparing darapladib versus placebo in patients with coronary heart disease. Am Heart J. 2010;160:655-661.
27. O'Donoghue ML, Braunwald E, White HD, Lukas MA, Tarka E, Steg PG, Hochman JS, Bode C, Maggioni AP, Im K, Shannon JB, Davies RY, Murphy SA, Crugnale SE, Wiviott SD, Bonaca MP, Watson DF, Weaver WD, Serruys PW, Cannon CP, Steen DL. Effect of darapladib on major coronary events after an acute coronary syndrome: the SOLID-TIMI 52 randomized clinical trial. JAMA. 2014;312:1006-1015.

Figure Legends

Figure 1: Lp-PLA$_2$ activity and mRNA expression during myocardial infarction. A, mRNA levels quantified by Realtime PCR in wild-type (WT) mice at the indicated time points after MI. Day 0 represents the steady state mice. Results are presented as mean ± SD, ** $p \leq 0.01$, n = 5-10 per group. **B**, Plasma Lp-PLA$_2$ activity in chimera WT and *bmLp-PLA$_2^{-/-}$* mice at steady state and in *bmLp-PLA$_2^{-/-}$* mice at indicated time points after MI. ** $p \leq 0.01$, n = 5-10 per group. **C**, Quantification of infarct size on day 1 and 7 after MI in WT and *bmLp-PLA$_2^{-/-}$* mice (left). * $p \leq 0.05$, n = 4-6 per group. **D,** Representative images of infarct size on day 7 after MI were shown in both WT and *bmLp-PLA$_2^{-/-}$* mice.

Figure 2: LP-PLA$_2$ moderately affects systemic inflammation. A, ELISA analysis of plasma levels of TNFα, IL-1β and IL-6 in wild-type (WT) and *bmLp-PLA$_2^{-/-}$* mice at the indicated time points after MI. **B,** Representative flow cytometric images of leukocyte profiling of blood in wild-type (WT) and *bmLp-PLA$_2^{-/-}$* mice, **C,** Quantification of total leukocytes, neutrophils, Ly-6C^{high} monocytes, Ly-6C^{low} monocytes in the blood. Results in one of three experiments with similar patterns are presented as mean ± SEM, * $p \leq 0.05$, ** $p \leq 0.01$ (WT vs. *bmLp-PLA$_2^{-/-}$*) vs. n = 5 per group.

Figure 3: Attenuated inflammatory response in *bmLp-PLA$_2^{-/-}$* myocardial infarct tissue. A, Representative images for flow cytometric analysis of MI tissue cell suspensions at the indicated time points after MI in WT and *bmLp-PLA$_2^{-/-}$* mice. **B,** Flow cytometry based quantification of neutrophil, monocyte and MΦ (macrophage) numbers in MI tissue of WT versus *bmLp-PLA$_2^{-/-}$* mice before and 1, 3, and 7 days post MI. Results in one of three experiments with similar patterns are presented as mean ± SEM, * $p \leq 0.05$, n = 4 per group. **C,** Gene expression profiling of WT and *bmLp-PLA$_2^{-/-}$* macrophages sorted from MI tissue 7 days after permanent LAD ligation. Results are presented as mean ± SEM percent change of marker expression in *Lp-PLA$_2^{-/-}$* compared to WT control mice, * $p \leq 0.05$, ** $p \leq 0.01$, n = 5 per group.

Figure 4: Improved cardiac remodeling in *bmLp-PLA$_2^{-/-}$* mice 7 days after myocardial infarction. Immunohistochemical staining of MI tissue for CD11b, Collagen 1 (Col1), CD31, and non-vascular smooth muscle alpha actin (SMA) in wild-type (WT) and *bmLp-PLA$_2^{-/-}$* chimeric mice 7 days post MI Quantification of 10 randomly selected fields of view per mouse (5 mice). Results are presented as mean ± SEM, * $p \leq 0.05$, *** $p \leq 0.001$, n =50 fields of view per group.

Figure 5: Lp-PLA$_2$ aggravates LV dysfunction after myocardial infarction.
A, Representative MRI images with late gadolinium enhancement (left panels) on day 1 after permanent LAD ligation, and end-systole (middle panels) and end-diastole (right panels) on day 21 of infarcted hearts from WT and *bmLp-PLA$_2^{-/-}$* mice, respectively. **B**, Quantification of individual changes (Δ) in heart parameters from day 1 to day 21 post MI (day 21 – day 1). Results are presented as mean ± SD percent change of marker expression in WT and *bmLp-PLA$_2^{-/-}$* mice, respectively. * $p \leq 0.05$, n $\geq$7 per group.

Figure 1

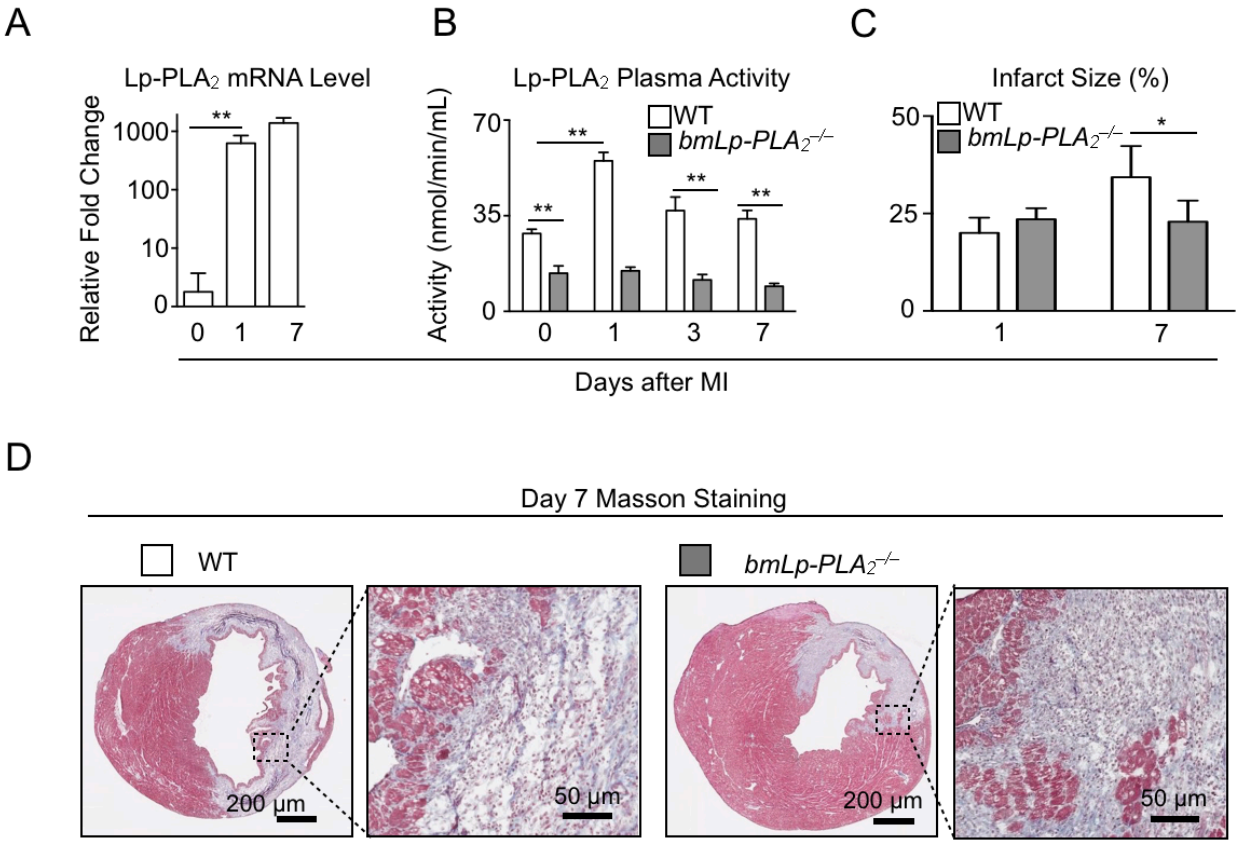

A
Lp-PLA2 mRNA Level
Relative Fold Change
1000
100
10
0
**
0 1 7
B
Lp-PLA2 Plasma Activity
Activity (nmol/min/mL)
70
35
0
WT
bmLp-PLA2−/−
**
**
**
**
0 1 3 7
C
Infarct Size (%)
50
25
0
WT
bmLp-PLA2−/−
*
1 7
Days after MI
D
Day 7 Masson Staining
WT
bmLp-PLA2−/−
200 μm
50 μm
200 μm
50 μm

Figure 2

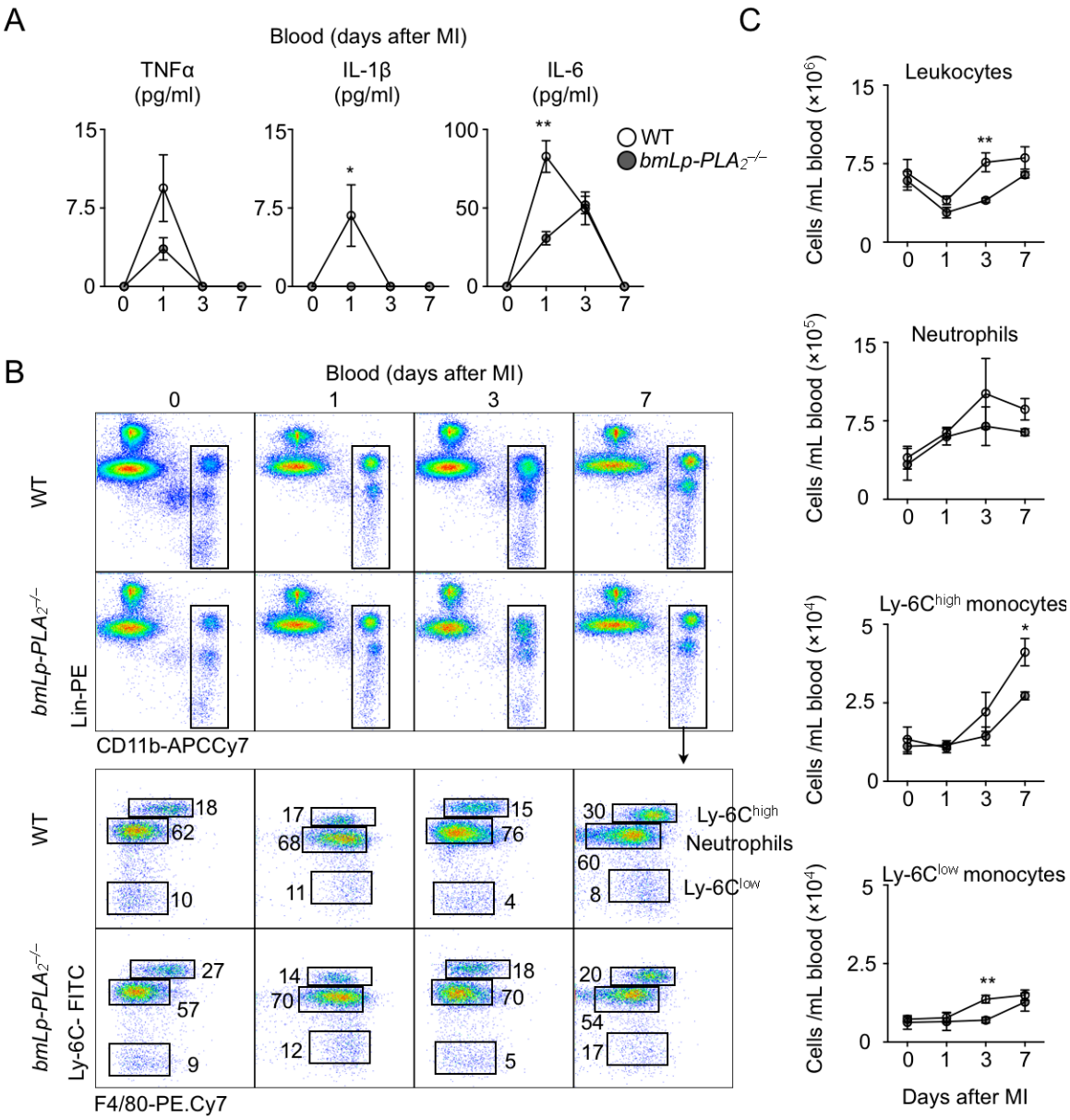

A
Blood (days after MI)
TNFα (pg/ml)
IL-1β (pg/ml)
IL-6 (pg/ml)
WT
bmLp-PLA2−/−
B
Blood (days after MI)
WT
bmLp-PLA2−/−
Lin-PE
CD11b-APCCy7
Ly-6C- FITC
F4/80-PE.Cy7
Ly-6Chigh Neutrophils
Ly-6Clow
C
Leukocytes
Cells /mL blood (×10^6)
Neutrophils
Cells /mL blood (×10^5)
Ly-6Chigh monocytes
Cells /mL blood (×10^4)
Ly-6Clow monocytes
Cells /mL blood (×10^4)
Days after MI

Figure 3

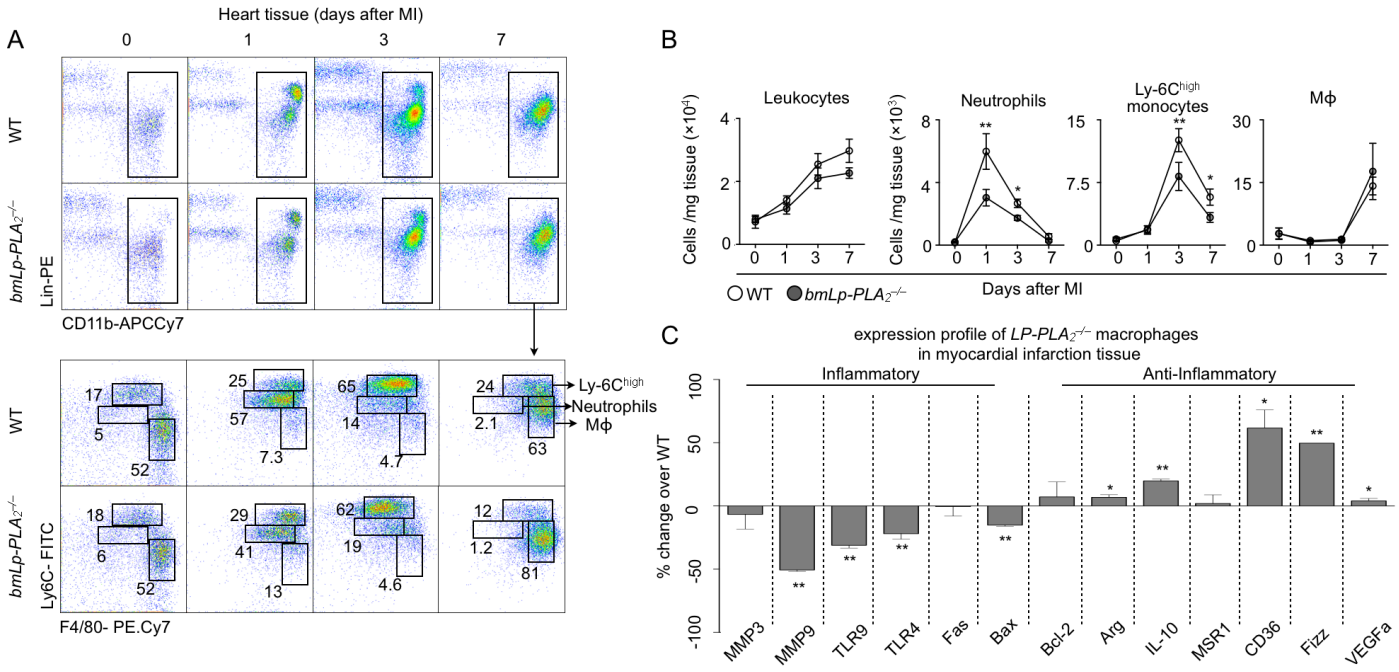

A
Heart tissue (days after MI)
0
1
3
7
WT
bmLp-PLA2−/−
Lin-PE
CD11b-APCCy7
Ly-6Chigh
Neutrophils
Mϕ
Ly6C- FITC
F4/80- PE.Cy7
B
Leukocytes
Neutrophils
Ly-6Chigh monocytes
Mϕ
Cells /mg tissue (×10⁴)
Cells /mg tissue (×10³)
WT
bmLp-PLA2−/−
Days after MI
C
expression profile of LP-PLA2−/− macrophages in myocardial infarction tissue
Inflammatory
Anti-Inflammatory
% change over WT
MMP3
MMP9
TLR9
TLR4
Fas
Bax
Bcl-2
Arg
IL-10
MSR1
CD36
Fizz
VEGFa

Figure 4

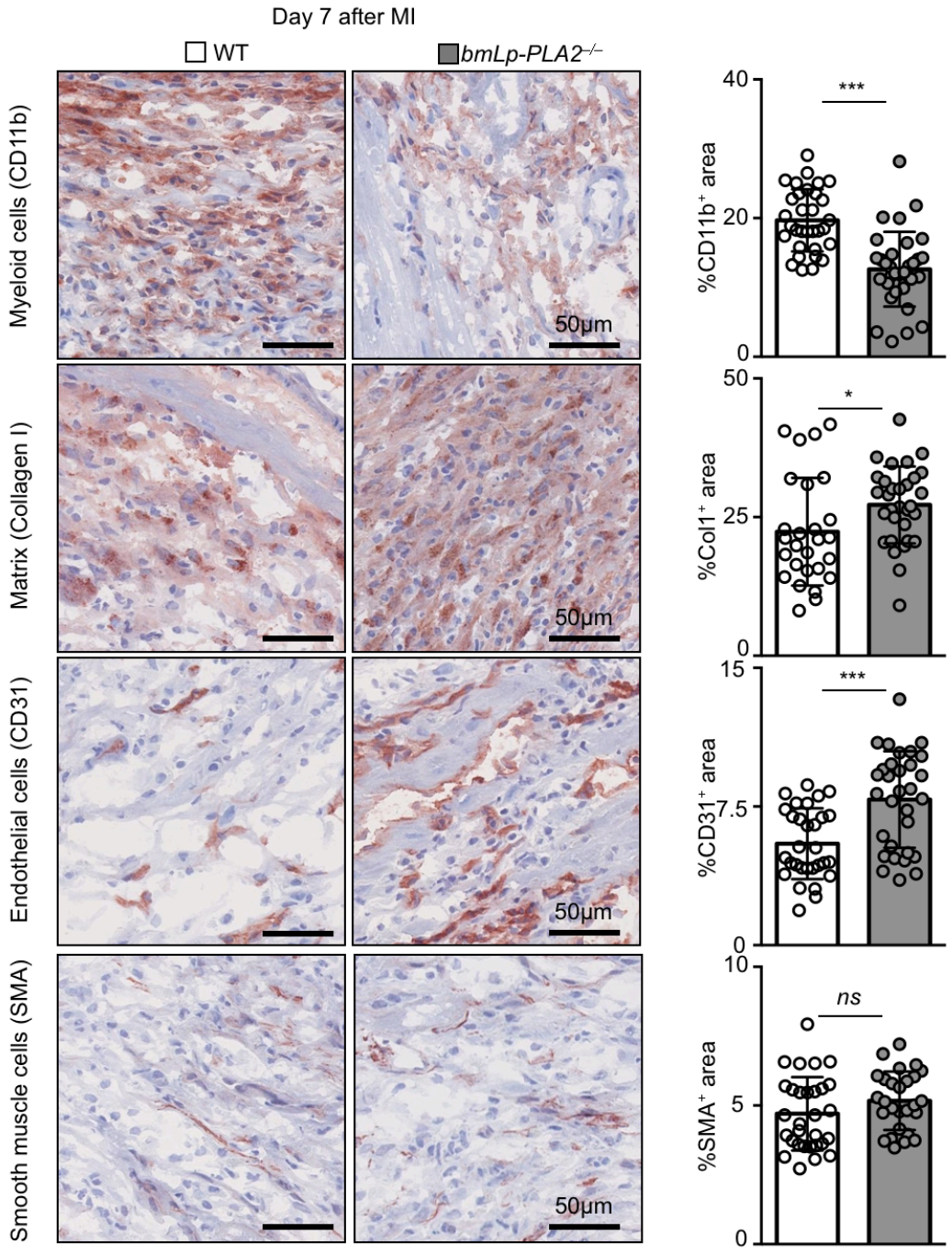
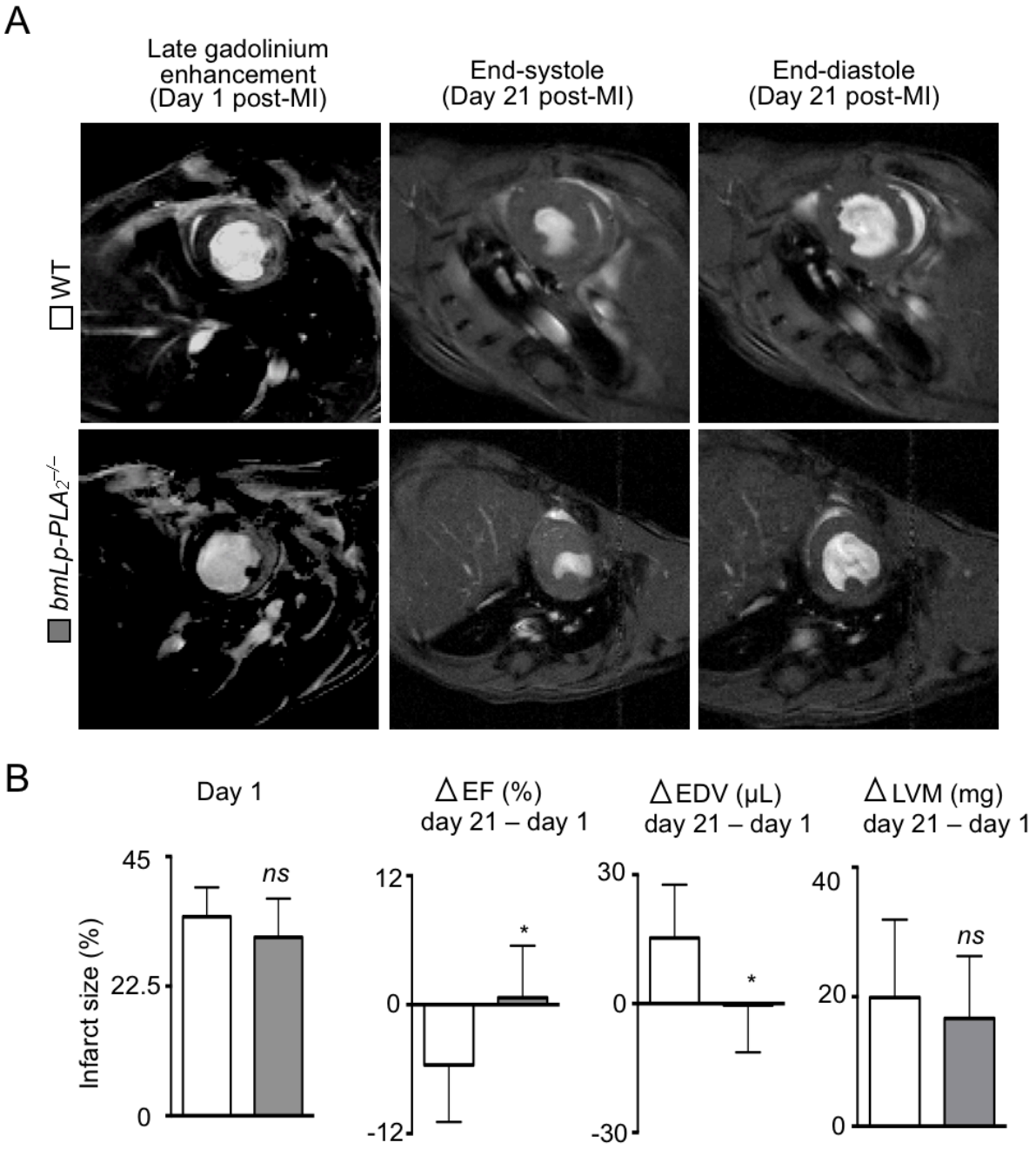

Day 7 after MI
WT
bmLp-PLA2−/−
Myeloid cells (CD11b)
Matrix (Collagen I)
Endothelial cells (CD31)
Smooth muscle cells (SMA)
50μm
%CD11b+ area
%Col1+ area
%CD31+ area
%SMA+ area
***
*
***
ns
40
20
0
50
25
0
15
7.5
0
10
5
0

Figure 5


A
Late gadolinium enhancement (Day 1 post-MI)
End-systole (Day 21 post-MI)
End-diastole (Day 21 post-MI)
WT
bmLp-PLA2-/-
B
Day 1
ΔEF (%) day 21 – day 1
ΔEDV (μL) day 21 – day 1
ΔLVM (mg) day 21 – day 1
Infarct size (%)
45
22.5
0
ns
12
0
-12
*
30
0
-30
*
40
20
0
ns