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Autophagy During Left Ventricular Redilation After Ventriculoplasty: Insights from a Rat Model of Ischemic Cardiomyopathy

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Glossary of Abbreviations

αMHC	α myosin heavy chain
βMHC	β myosin heavy chain
BNP	brain natriuretic peptide
CI	cardiac index
FS	fractional shortening
LAD	left anterior descending artery
LC3	microtubule-associated protein light chain 3
LVDd	LV end-diastolic dimension
LVP	left ventriculoplasty
MI	myocardial infarction
3-MA	3-methyladenine

Abstract

Objectives: Myocardial autophagy has been recognized as an important factor in heart failure. It is not known whether changes in ventricular geometry by left ventriculoplasty influence autophagy in ischemic cardiomyopathy. We hypothesized that myocardial autophagy plays an important role in left ventricular (LV) redilation after ventriculoplasty.

Methods: Four weeks after ligation of the left anterior descending artery, ventriculoplasty or sham operation was performed. The animals were euthanized at 2 days (early) or 28 days (late) after the second operation. Ventricular autophagy was evaluated by protein expression of microtubule-associated protein light chain 3 (LC3) II, an autophagosome marker. Cardiomyocyte area was assessed by histological examination. LV function was evaluated by echocardiography. To examine the implications of autophagy, an autophagy inhibitor (3-methyladenine) was injected intraperitoneally for 3 weeks before sacrifice.

Results: The LV was reduced in size early and redilated late after ventriculoplasty. LV systolic function was improved early and later worsened after ventriculoplasty. LC3-II expression decreased early after ventriculoplasty and increased in the late period. Myocyte area increased from the early to late stage after ventriculoplasty. Autophagic inhibition exaggerated the increased myocyte hypertrophy and LV redilation.

Conclusions: In a rat model of myocardial infarction, autophagy decreased early after ventriculoplasty and increased again during LV redilation. These results provide new insights into the mechanism underlying the late failure of ventriculoplasty.

Central Picture Legend

Change in myocardial autophagy after left ventriculoplasty for ischemic cardiomyopathy.

Central Message

Myocardial autophagy decreases early after left ventriculoplasty and increases again during ventricular redilation, which might be one of the mechanisms for late failure of ventriculoplasty.

Perspective Statement

After left ventriculoplasty, myocardial autophagy decreases early and increases again during ventricular redilation, as an adaptive mechanism. Further studies are necessary to examine whether pharmacological interventions can modulate myocardial autophagy and maintain the early unloading effect of left ventriculoplasty in ischemic cardiomyopathy.

Introduction

Two main effects of left ventriculoplasty (LVP) have been described: reduction of LV volume and formation of an elliptical shape.[1] Despite the conceptual background, there is no clear evidence that LVP improves patient survival and clinical status. The STICH trial, a large clinical randomized study for ischemic cardiomyopathy, showed no beneficial effects of LVP in symptoms, exercise tolerance, and mortality.[2] In contrast, we have reported that LVP might be effective for selective patients.[3] However, the mechanism by which LVP affects biological properties in the myocardium has been poorly understood.

A previous investigation using a rat model of LVP has shown early decreasing effects of LVP on brain natriuretic peptide (BNP) gene expression.[4] However, the beneficial effects of LVP did not persist; myocardial BNP increased again following LV redilation late after LVP. However, the mechanism of LVP failure has not been fully elucidated. In recent years, “autophagy” (self-digestion system) has received attention as an important player in heart failure.

Myocardial autophagy has been reported to be activated in the failing heart,[5] cardiac hypertrophy,[6] and myocardial infarction (MI).[7] Autophagy is an intracellular degradation system, by which cytoplasmic materials, via autophagosome formation, are finally digested in the lysosome.[8] There are two important roles in autophagy: recycling degradation products for energy supply (amino acids and fatty acids) and control of intracellular quality. After MI, autophagy has been reported to protect against progression of LV dilation by increasing the energy supply to meet cellular demand in the face of

99 excessive wall stress and hypoxia.[7] In the present study, w**e** hypothesised that myocardial
100 autophagy plays an important role in LV redilation after LVP.
101
102 **Methods**
103 ***Experimental design***
104 Ten-week-old male Sprague-Dawley rats weighing 300–360 g were subjected to the
105 experimental protocol shown in **Fig. 1**. All rats were randomized into the following
106 groups: sham (sham/sham), MI/sham, and MI/LVP. The rats in the MI/sham group
107 underwent ligation of the left anterior descending artery (LAD) in the first operation and
108 only underwent thoracotomy in the second operation; the rats in the MI/LVP group
109 underwent LAD ligation in the first operation and LVP in the second operation. A 4-week
110 period was maintained between the procedures. The animals were euthanized at 2 days
111 ("early") or 28 days ("late") after the second operation. The mortality and number of rats
112 are indicated in **Fig. 1**. Transthoracic echocardiography was performed at the baseline,
113 before the second operation, and before euthanization. The heart and lung were excised
114 and weighed at the time of euthanization. All procedures were conducted according to the
115 Hokkaido University Manual for Implementing Animal Experimentation and were
116 consistent with the Guide for the Care and Use of Laboratory Animals, published by the
117 US National Institutes of Health (NIH publication No. 85-23, revised 1996). This study
118 was approved by the institutional animal ethics committee.
119
120 ***Myocardial infarction and left ventriculoplasty***

General anesthesia was induced by intramuscular ketamine (90 mg/kg) and xylazine (10 mg/kg). Following left lateral thoracotomy under ventilation, the proximal LAD was ligated with 7-0 polypropylene sutures (Ethicon, Somerville, New Jersey). The MI at the anterior LV wall was confirmed by akinesis through an echocardiographic examination before the chest was closed. Four weeks after the LAD ligation, the rats developed a large scar, but not an aneurysm, on the anterior LV wall with hypokinesis in the non-ischemic lesion (**Video 1**).

LVP was performed as reported previously under the same anesthesia as the MI.[9] Briefly, after ligation of the internal mammary arteries, a horizontal sternotomy was performed at the level of the fifth intercostal space to expose the entire heart. Thereafter, LVP was performed by plicating the akinetic scar area using three mattress sutures with pledgetted 6-0 polypropylene (Ethicon). Much attention was paid not to stitch on the scar tissue but on the viable myocardium. An over-and-over suture was then performed on the plicated scar tissue (**Video 1**).

Transthoracic echocardiography

Echocardiography was performed just before each operation and euthanization using a SONOS 5500 ultrasound system with a 12-MHz phased-array transducer (Philips Medical Systems, Andover, Massachusetts). After the rats were anesthetized and placed in the supine position, M-mode tracings were recorded by the long-axis view of the LV for the following parameters: LV end-diastolic dimension (LVDd) and end-systolic dimension, fractional shortening (FS). Cardiac index (CI: cardiac output divided by body

143 weight) was measured using pulse Doppler on the LV outflow tract.

144

145 ***Catheter examination***

146 Through the right carotid artery, a 1.4-Fr micromanometer-tipped catheter (Millar,
147 Houston, Texas) was inserted into the ascending aorta and LV. The pressure data were
148 recorded using PowerLab (ADInstruments, Dunedin, NZ) and analyzed using LabChart
149 (ADInstruments). The heart rate, mean arterial blood pressure, LV end-diastolic pressure,
150 LV maximum, and minimum dPdt were measured. The time constant (Tau) was measured
151 as the time required for the pressure to decline to 50% of the value recorded at the
152 minimum dPdt.

153

154 ***Histological examination***

155 Ventricles were fixed in 10% paraformaldehyde in phosphate-buffered saline, embedded
156 in paraffin, and sectioned at 5-μm intervals. Hematoxylin-eosin staining and Masson's
157 trichrome staining were conducted using standard procedures at the mid-ventricular level.
158 The cardiomyocyte area was examined using hematoxylin-eosin-stained sections. About
159 100 randomly chosen oval-shaped cardiomyocytes with a nucleus in the septal lesion
160 (remote area) in each group were analyzed to measure the cross-sectional cardiomyocyte
161 area (μm^2) using ImageJ software (http://rsb.info.nih.gov/ij/) (NIH, Bethesda, Maryland).
162 The percentage of the fibrotic area compared to the whole tissue area was examined on
163 Masson's trichrome-stained sections. About 10 randomly chosen frames in the septal
164 lesion in each group were analyzed using ImageJ (NIH).

Gene expression of myosin heavy chain in the myocardium

In order to assess pathological hypertrophy, gene expression of the myosin heavy chain (MHC) was evaluated in each group at 28 days after the second operation. Myocardial total RNA was isolated from frozen tissue samples with a High Pure RNA Tissue Kit (Roche, Penzberg, Germany). The total RNA was reverse transcribed into cDNA with a Transcriptor First Strand cDNA Synthesis Kit (Roche). Quantitative real-time reverse transcription polymerase chain reaction (RT-PCR) was performed with FastStart Essential DNA Probes Master (Roche) and RealTime ready assay (Roche Assay ID, 501294 for *αMHC*; 500524 for *βMHC*). PCR amplification was performed in a volume of 20 μL using LightCycler Nano (Roche) under the conditions suggested by the manufacturer. The results were normalized to S29 transcription as a housekeeping gene, which was comparable among the groups.

Immunoblotting of microtubule-associated protein light chain 3

Myocardial autophagy was evaluated by protein expression of an autophagosome marker, microtubule-associated protein light chain 3 (LC3) II. A semidry western blot apparatus (Mini-PROTEAN Tetra Cell, BIO-RAD, CA, USA) was used for detecting the conversion from LC3-I (cytosolic form) to LC3-II (membrane-bound lipidated form). The amount of LC3-II usually correlates well with the number of autophagosomes. After sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12% Mini-PROTEANTGX^{TM}, BIO-RAD, CA, USA), the proteins were blotted to a polyvinyldine

deflouride membrane and incubated with primary (Anti-LC3B, abcam, Cambridge, UK) and secondary antibodies (Anti-rabbit IgG, Cell Signaling, MA, USA). The bands were semiquantified by chemiluminescence using JustTLC (Sweday, Sodra Sandby, Sweden). The membranes were then dyed with naphthol blue black solution to normalize the band intensity.

Autophagic inhibitor

To examine the implications of autophagy, a PI3-kinase inhibitor (3-methyladenine: 3-MA; Sigma-Aldrich, St. Louis, MO) was intraperitoneally injected at a dose of 15 mg/kg/day for 3 weeks before sacrifice in the MI/LVP + 3-MA group (**Fig. 1**).[10] Because autophagosome formation requires class III PI3-kinase activity, PI3-kinase inhibitors are the most commonly used pharmacological approach to inhibit autophagy.

Statistical analysis

All data are presented as median (interquartile range) for continuous variables. Statistical analysis was performed using non-parametric tests i.e. the Wilcoxon rank sum test, Kruskal-Wallis test, and Friedman's test. The Bonferroni correction was used for post hoc analysis. The differences were considered statistically significant at a value of $P < 0.05$. A post hoc power analysis was conducted using *G*Power*: assuming a 5% significance level and a two-sided test, the sample size had 99% power to detect effect size (r) of 0.77 and 0.82 (>0.5: large effect) between 2 days and 28 days for LVDd and LC3II, respectively. Statistical analysis was performed using JMP Pro version 14.0 software

(SAS Institute Inc., Cary, NC) and SPSS version 17.0 software (SPSS Inc. Chicago, Ill).

Results

Protein expression of LC3-II, an autophagosome marker

Fig. 2 shows the protein expression of the autophagosome marker LC3-II, early and late after LVP. In the MI/LVP group, LC3-II expression was lower than that in the MI/sham group in the early phase. The reduced LC3-II expression then increased late after LVP, whereas LC3-II did not change significantly from the early to late period in the MI/sham group. The increased LC3-II expression was reduced by the autophagic inhibitor, 3-MA.

Cardiac functional parameters

Fig. 3 shows LVDd and FS in the early and late phases after LVP. In the MI/LVP group, LVDd was smaller early after LVP than in the MI/sham group, which then increased late after LVP. The MI/LVP+3-MA group exhibited LV redilation which was the same as in the MI/sham group (**Fig. 3A**). FS was greater in the MI/LVP group than in the MI/sham group early after LVP, which then decreased late after LVP. In the MI/LVP+3-MA group, FS further decreased late after LVP (**Fig. 3B**). CI was not different between the MI/LVP and MI/sham groups early and late after LVP**,** whereas it was decreased only in the MI/LVP+3-MA group ($p<0.01$).

Table 1 shows the cardiac functional parameters by Millar catheter early and late after LVP. In both the MI/sham and MI/LVP groups, the maximum dPdt was smaller than that in the sham group. The LV end-diastolic pressure and Tau were higher in the

MI/sham and MI/LVP + 3-MA groups than those in the sham group, but not in the MI/LVP group late after LVP. From the early to late phase, the LV end-diastolic pressure and Tau increased only in the MI/LVP+3-MA group.

Histological examination

Fig. 4 shows the cardiomyocyte area and myocardial fibrosis determined by histological examination. In the MI/LVP group, the cardiomyocyte area was smaller than that in the MI/sham group in the late period. However, the myocyte area increased from the early to late stage after LVP. In the MI/LVP+3-MA group, myocyte area increased to the same degree as that in the MI/sham group (**Fig. 4B**). There was no significant difference in fibrosis among the MI groups (**Fig. 4D**).

Gene expression of myosin heavy chain in the myocardium

Though there was no significant difference in the gene expression of *αMHC* and *βMHC* among the groups, the *α/β MHC* ratio, a lower value of which indicates a relative increase in fetal gene, was significantly smaller in the MI/LVP+3-MA than in the MI/sham group (**Fig. 5**).

Body, heart, and lung weights early and late after LVP

Table 2 shows the body, heart, and lung weights early and late after LVP. The heart weights in the MI/LVP groups were excluded from the analysis due to presence of pledgets and additional scar tissue. Lung/body weight ratio in the late phase was greater

253 only in the MI/sham and MI/LVP+3-MA groups than in the sham group.

254

255 **Discussion**

256 We demonstrated here that in a rat model of MI, myocardial autophagy decreased early
257 after LVP and increased again during LV redilation. Because pharmacological inhibition
258 of autophagy exaggerated the LV redilation, autophagy may play an important role in LV
259 redilation after LVP.

260

261 ***Pathology after MI in rats and humans***

262 There are several differences in myocardial pathology after MI between rats and humans.
263 First, the septal region is intact after ligation of LAD in rats because of good collaterals
264 from the right coronary system,[11] whereas both septum and free wall are usually affected
265 in humans. The risk area might be smaller in rats than in humans, which would influence
266 later LV remodeling and hypertrophy. Second, the distribution of the *MHC* isoforms is
267 different: *αMHC* is the predominant isoform in the rat ventricle, whereas *βMHC*
268 predominates in the human ventricle. Although the change in *MHC* gene expression
269 would be different between the species, the decrease in the α and β ratio in pathological
270 hypertrophy is consistent.[12] Myocyte hypertrophy seen in this model has been also found
271 in human studies late after MI.[11]

272

273 ***Differences in surgical techniques: Plication vs. excision of scar area***

274 In the current study, we performed LVP by plication of the akinetic scar area. In such a

small animal model, we cannot perform LVP to restore ventricular geometry by endoventricular patch plasty using cardiopulmonary bypass system (eg. Dor procedure in STICH trial). This plication technique might have induced too small an LV cavity. The LV volume was reduced by over 50% after LVP. In previous clinical studies, desired LV volume reduction ratio after LVP was more than 30-33%,[13,14] whereas in the STICH trial, the ratio was 19%.[2] Nonetheless, the drawback of the plication technique of the entire scar is that it is not possible to control the residual LV volume during surgery. Our model would be analogous to a recently developed less invasive LVP devise (Revivent TC system).[15] In both our model and the Revivent TC system, the LV anterior akinetic area can be excluded by linear plication. Moreover, both techniques do not require cardiopulmonary bypass, cardioplegic arrest, or a ventriculotomy.

Myocardial autophagy and left ventricular unloading

Myocardial autophagy has been reported to be activated in the failing heart,[5] cardiac hypertrophy,[6] and ischemic heart disease.[16] Whether myocardial autophagy can be "adaptive" or "maladaptive" depends on the pathology and stages of disease. Kassiotis and colleagues reported that in patients with advanced heart failure, mechanical unloading with a left ventricular assist device reduced the activation of autophagy.[17] They therefore insisted that autophagy might be adaptive in the failing heart.

In our recent study, reduction of LC3-II observed early after LVP may be related to the LV unloading by LVP. On the contrary, late after LVP, the reduced LC3-II expression was increased again, and was parallel to the LV redilation. We also showed that the

297 pharmacological inhibition of late autophagy exaggerated the pathological LV
298 hypertrophy and redilation. We thus speculate that LV unloading by LVP attenuates the
299 demand for myocardial hypertrophy and autophagy activation; however, late LV
300 redilation increases myocardial hypertrophy and triggers the reactivation of autophagy.
301 We therefore argue that myocardial autophagy may play an adaptive role in LV redilation
302 after LVP.
303
304 ***Limitations***
305 This study has some limitations. First, autophagic flux was not examined in the recent
306 study. Second, the animals were not administered any guideline-directed medicines.
307 Carvedilol, for instance, can also enhance autophagy in a rat model of MI.[18] Thus, the
308 current results cannot be directly applied to clinical practice in humans. Third, 3-MA has
309 several off-target effects; it can inhibit not only class III but also class I PI3K. Class I
310 PI3K is mainly activated via the insulin receptor, leading to AKT activation.[19] Thus, the
311 3-MA-mediated inhibition of class I PI3K might influence insulin signaling. Insulin
312 resistance is also one of the causes for myocyte hypertrophy and dysfunction. As 3-MA
313 was used 5 weeks after MI in the present study, it may not have influenced infarct size.
314 Nonetheless, we cannot exclude the negative off-target effects of 3-MA on myocardial
315 metabolism in the later phase. Concomitant use of wortmannin, another PI3K inhibitor,
316 should be considered in future studies. Forth, there would be selection bias because of the
317 high mortality especially after LVP; it is possible that autophagic activation was different
318 between the examined and dead animals after LVP.

Conclusions

In a rat model of MI, myocardial autophagy decreased early after LVP and increased during LV redilation late after LVP. Although the animal model does not replicate clinical pathogenesis, autophagy may play an important role in LV redilation after LVP.

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References

1. Matsui Y. Overlapping Left Ventricular Restoration. *Circ J*. 2009;Suppl A:13-18.
2. Jones RH, Velazquez EJ, Michler RE, Sopko G, Oh JK, O'Connor CM, et al. Coronary bypass surgery with or without surgical ventricular reconstruction. *N Engl J Med*. 2009;360:1705-1717.
3. Wakasa S, Matsui Y, Kobayashi J, Cho Y, Yaku H, Matsumiya G, et al. Estimating postoperative left ventricular volume : Identification of responders to surgical ventricular reconstruction. *J Thorac Cardiovasc Surg*. 2018;156:2088-2096.
4. Nishina T, Nishimura K, Yuasa S, Senri M, Nomoto T, Sakakibara Y, et al. Initial Effects of the Left Ventricular Repair by Plication May Not Last Long in a Rat Ischemic Cardiomyopathy Model. *Circulation*. 2001;104(suppl 1):I-241-I-245.
5. Takemura G, Miyata S, Kawase Y, Okada H, Maruyama R, Fujiwara H. Autophagic degeneration and death of cardiomyocytes in heart failure. *Autophagy*. 2006;2(3):212-214.
6. Elsässer A, Arnon E, Kostin S, Hein S, Schaper J, Bauer EP, et al. Progression From Compensated Hypertrophy to Failure in the Pressure-Overloaded Human Heart. *Circulation*. 2003;107(7):984-991.
7. Kanamori H, Takemura G, Goto K, Maruyama R, Tsujimoto A, Ogino A, et al. The role of autophagy emerging in postinfarction cardiac remodelling.

Cardiovasc Res. 2011;91(2):330-339.

8. Mizushima N, Komatsu M. Autophagy: Renovation of cells and tissues. *Cell*. 2011;147(4):728-741.

9. Asai H, Shingu Y, Yamakawa T, Niwano H, Wakasa S, Ooka T, et al. Left-Ventricular Plication Reduces Wall Stress and Cardiomyocyte Hypertrophy in a Rat Model of Ischemic Cardiomyopathy. *Eur Surg Res*. 2017;58(1-2):69-80.

10. Maeda H, Nagai H, Takemura G, Shintani-Ishida K, Komatsu M, Ogura S, et al. Intermittent-hypoxia induced autophagy attenuates contractile dysfunction and myocardial injury in rat heart. *Biochim Biophys Acta - Mol Basis Dis*. 2013;1832(8):1159-1166.

11. Olivetti G, Quaini F, Lagrasta C, Cigola E, Ricci R, Maestri R, et al. Cellular basis of ventricular remodeling after myocardial infarction in rats. *Am J Cardiol*. 1991;68:7-16.

12. Krenz M, Robbins J. Impact of beta-myosin heavy chain expression on cardiac function during stress. *J Am Coll Cardiol*. 2004;44(12):2390-2397.

13. Michler RE, Rouleau JL, Al-Khalidi HR, Bonow RO, Pellikka PA, Pohost GM, et al. Insights from the STICH trial: Change in left ventricular size after coronary artery bypass grafting with and without surgical ventricular reconstruction. *J Thorac Cardiovasc Surg*. 2013;146(5):1139-1145.e6.

14. Isomura T, Hoshino J, Fukada Y, Kitamura A, Katahira S, Kondo T, et al. Volume reduction rate by surgical ventricular restoration determines late outcome in ischaemic cardiomyopathy. *Eur J Heart Fail*. 2011;13(4):423-431.

15. Chiariello GA, Nesta M, Bruno P, Pasquini A, Ferraro F, Cammertoni F, Marano R, Colizzi C, Farina P, D'Amario D, Trani C MM. Total Surgical Plication of Left Ventricular Aneurysm Using the BioVentrixRevivent Myocardial Anchoring System. *Innovations*. 2019;14(4):369-373.

16. Kim S-J, Matsui Y, Yan L, Ge H, Vatner SF, Masurekar M, et al. Autophagy in chronically ischemic myocardium. *Proc Natl Acad Sci*. 2005;102(39):13807-13812.

17. Kassiotis C, Bailai K, Wellnitz K, Vela D, Gong M, Salazar RS, et al. Markers of autophagy are downregulated in failing human heart after mechanical unloading. *Circulation*. 2009;120(SUPPL. 1):191-198.

18. Zhang JL, Lu JK, Chen D, Cai Q, Li TX, Wu LS WX. Myocardial autophagy variation during acute myocardial infarction in rats: the effects of carvedilol. *Chin Med J (Engl)*. 2009;122(19):2372-2379.

19. Wu YT, Tan HL, Shui G, Bauvy C, Huang Q, Wenk MR, et al. Dual role of 3-methyladenine in modulation of autophagy via different temporal patterns of inhibition on class I and III phosphoinositide 3-kinase. *J Biol Chem*. 2010;285(14):10850-10861.

Figure legends

Figure 1. Experimental protocol. We performed MI for 170 rats, and 54 rats died after MI (mortality, 32%). Of the remaining 116 rats, 25 and 91 were categorized into the MI/sham and MI/LVP groups, respectively. In the MI/LVP group, 50 rats were lost after LVP (mortality, 55%). Three additional rats died during the period of 3-MA injection. LVP, left ventriculoplasty; 3-MA, 3-methyladenine; MI, myocardial infarction; TTE, transthoracic echocardiography.

Figure 2. Protein expression of LC3-II, an autophagosome marker, early (2-day group) and late (28-day group) after LVP. (A) representative bands; (B) normalized band analysis. The upper and lower borders of the box represent the upper and lower quartiles, respectively. The middle horizontal line represents the median. The upper and lower whiskers represent the maximum and minimum values of non-outliers, respectively. The P values are Bonferroni-adjusted. * $p < 0.05$, ** $p < 0.01$; $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs.* 2 days. LC3, microtubule-associated protein light chain 3, LVP, left ventriculoplasty; 3-MA, 3-methyladenine; MI, myocardial infarction.

Figure 3. Serial changes in echocardiographic parameters early (2-day group) and late (28-day group) after LVP: LVDd (A), FS (B). The time points of measurements were day 0, 28, 30 in the 2-day group and day 0, 28, 56 in the 28-day group. The upper and lower borders of the box represent the upper and lower quartiles, respectively. The middle horizontal line represents the median. The upper and lower whiskers represent the

412 maximum and minimum values of non-outliers, respectively. The P values are
413 Bonferroni-adjusted. *p < 0.05, ** p < 0.01; $^{\#}$ p < 0.05, $^{\#\#}$ p < 0.01 *vs*. 2-day group; † p <
414 0.05, †† p < 0.01 *vs*. day 0; $^{\$}$ p < 0.05 *vs*. day 28. FS, fractional shortening; LVDd, left
415 ventricular end-diastolic dimension; LVP, left ventriculoplasty; 3-MA, 3-methyladenine;
416 MI, myocardial infarction.
417
418 **Figure 4.** Cardiomyocyte hypertrophy and myocardial fibrosis. (A) Representative
419 photomicrographs of hematoxylin-eosin-stained sections in the septal lesion. (B)
420 Cardiomyocyte area in each group. (C) Representative photomicrographs of Masson's
421 trichrome-stained sections in the septal lesion. (D) Myocardial fibrosis in each group.
422 Scale bars in A and C: 50 μm. The upper and lower borders of the box represent the upper
423 and lower quartiles, respectively. The middle horizontal line represents the median. The
424 upper and lower whiskers represent the maximum and minimum values of non-outliers,
425 respectively. The P values are Bonferroni-adjusted. * p < 0.05, ** p < 0.01; $^{\#}$ p < 0.05, $^{\#\#}$
426 p < 0.01 *vs*. 2 days. LVP, left ventriculoplasty; 3-MA, 3-methyladenine; MI, myocardial
427 infarction.
428
429 **Figure 5.** *α/β MHC* ratio late after LVP. The upper and lower borders of the box represent
430 the upper and lower quartiles, respectively. The middle horizontal line represents the
431 median. The upper and lower whiskers represent the maximum and minimum values of
432 non-outliers, respectively. The P values are Bonferroni-adjusted. * p < 0.05. LVP, left
433 ventriculoplasty; 3-MA, 3-methyladenine; MHC, myosin heavy chain; MI, myocardial

infarction.

Figure 6 (graphical abstract).

Myocardial autophagy decreased early after LV plasty and increased during LV redilation late after LV plasty, which might be one of the mechanisms of late LV redilation. LV, left ventricle; 3-MA, 3-methyladenine; MI, myocardial infarction.

Video legend

Video 1. Surgical procedures of MI and LVP. LAD, left anterior descending artery; LVP, left ventriculoplasty; MI, myocardial infarction.

Table 1. Cardiac functional parameters by Millar catheter early and late after LVP

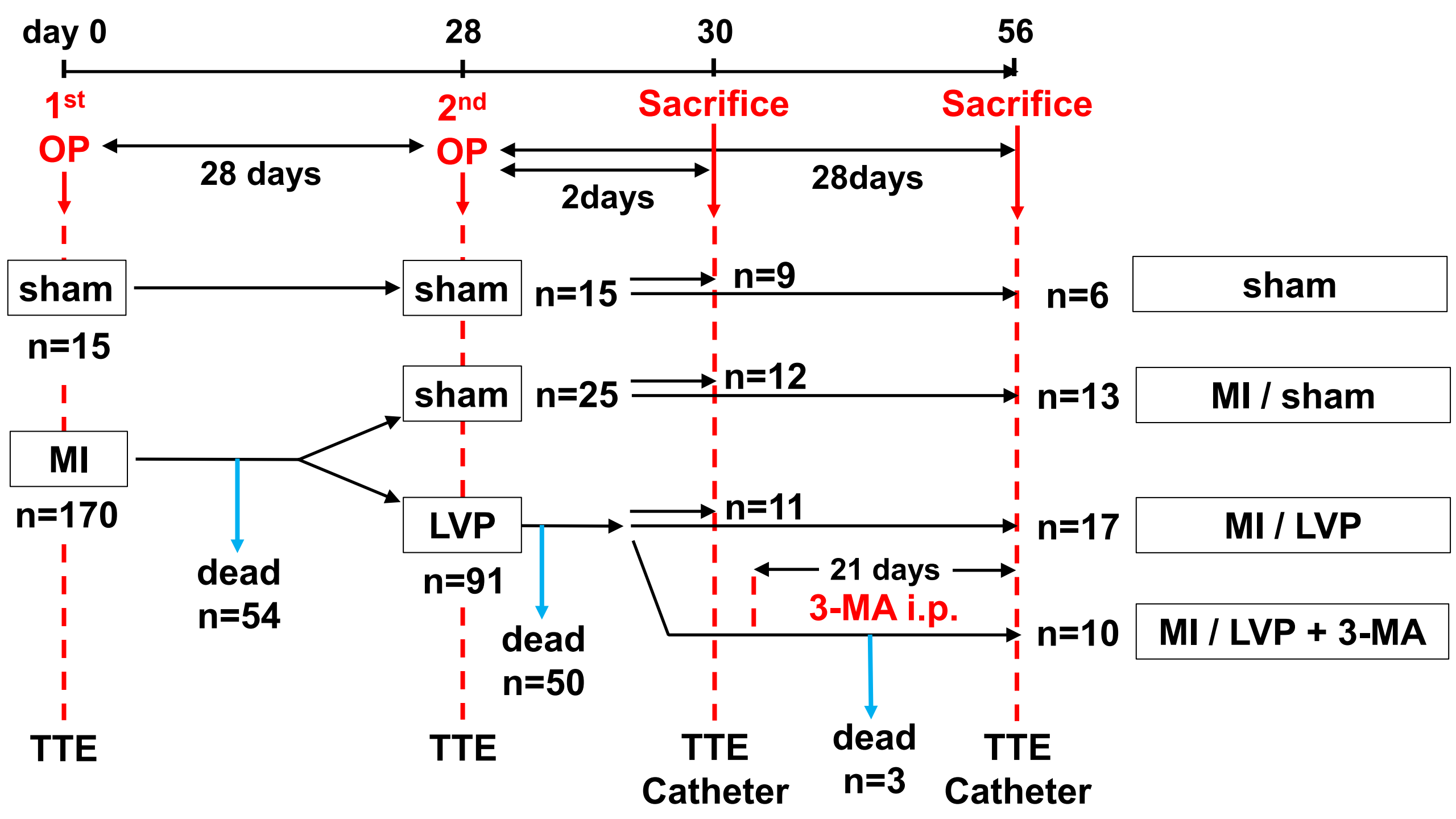
	2-day group			28-day group			
	sham n=9	MI/sham n=12	MI/LVP n=11	sham n=6	MI/sham n=13	MI/LVP n=17	MI/LVP + 3MA n=10
Heart rate (bpm)	270 (218-316)	291 (270-323)	318 (295-345)	279 (247-310)	243 (234-259)	263 (239-339)	232 (230-238)
mABP (mmHg)	77 (72-86)	80 (75-92)	80 (69-87)	100$^{\#\#}$ (93-102)	92$^{\#}$ (85-98)	84* (78-88)	84 (60-85)
LV developed pressure (mmHg)	102 (94-116)	95 (88-101)	97 (91-101)	123$^{\#}$ (112-123)	99 (91-112)	95 (89-105)	88 (72-89)
max dp/dt (mmHg/s)	8360 (7046-9552)	6371 (5367-6747)	6649 (6030-7182)	10133 (8491-11397)	6510* (5579-7391)	7123* (5992-8029)	5972 (3318-6306)
min -dp/dt (mmHg/s)	6128 (4423-7014)	4403 (4250-5129)	4322 (3601-4709)	6076 (5312-6839)	3884 (3075-4463)	4083 (3349-5054)	3184$^{\#}$ (3079-3195)
LVEDP (mmHg)	-2.3 (-8.1-2.2)	6.3 (2.4-9.6)	1.0 (-1.3-6.6)	0.2 (-0.8-1.0)	14.7$^{\#}$ ** (6.4-17.7)	5.2 (2.1-20.0)	12.2$^{\#}$ * (5.7-22.0)
Tau (ms)	7.4 (6.5-8.8)	9.3 (8.5-12.4)	8.3 (6.8-9.6)	7.6 (7.3-7.8)	11.3* (9.8-14.1)	8.9 (8.3-10.9)	13.4$^{\#\#}$ * (12.5-14.4)

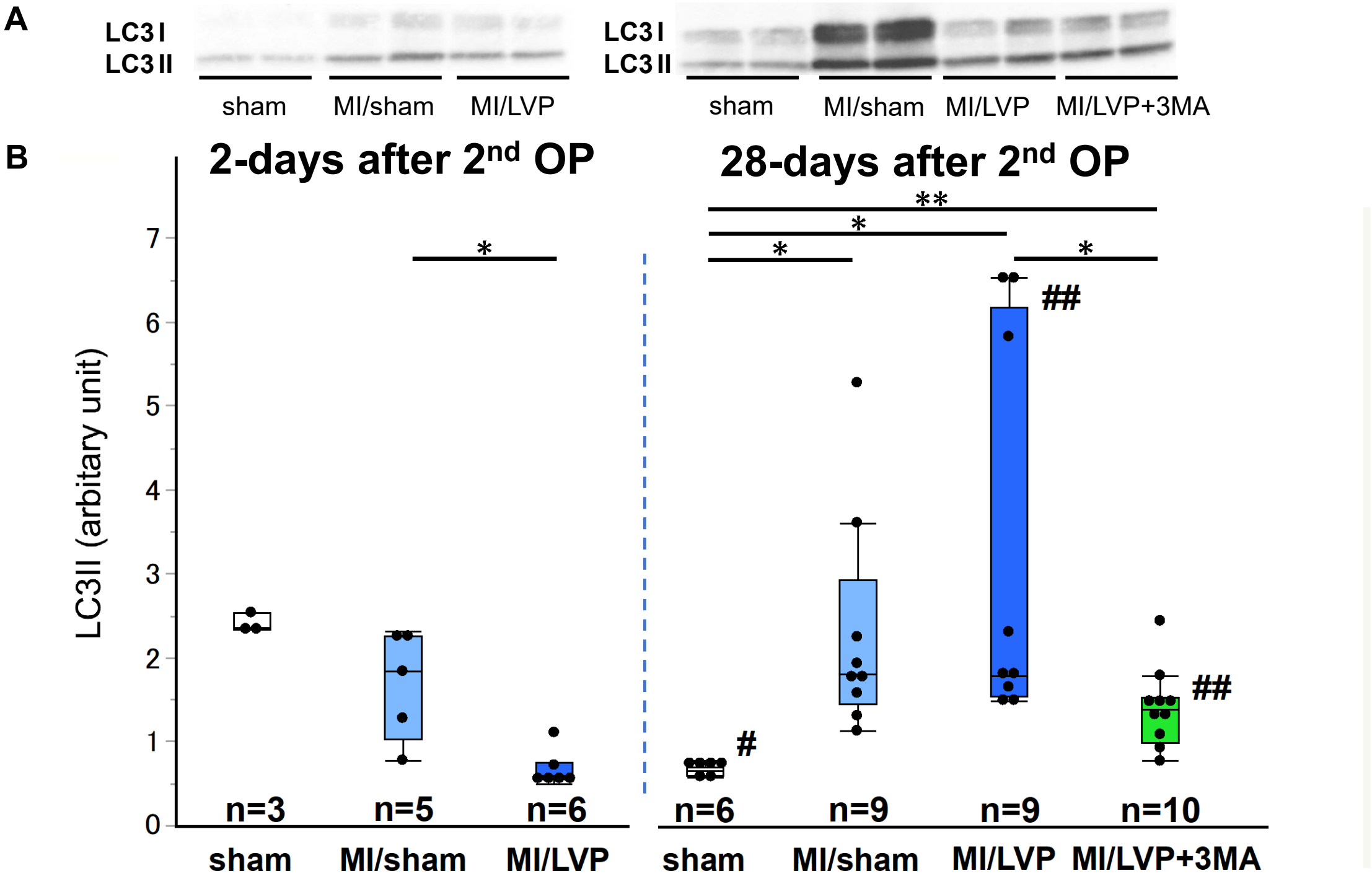
Values are median (first quartile – third quartile). LV, left ventricle; LVEDP, left ventricular end-diastolic pressure; LVP, left ventriculoplasty; 3-MA, 3-methyladenine; mABP, mean aortic pressure; MI, myocardial infarction. The P values are Bonferroni-adjusted. *p<0.05 vs sham; ** p<0.01 vs sham; $^{\#}$ p<0.05 vs 2-day group; $^{\#\#}$ p<0.01 vs 2-day group.

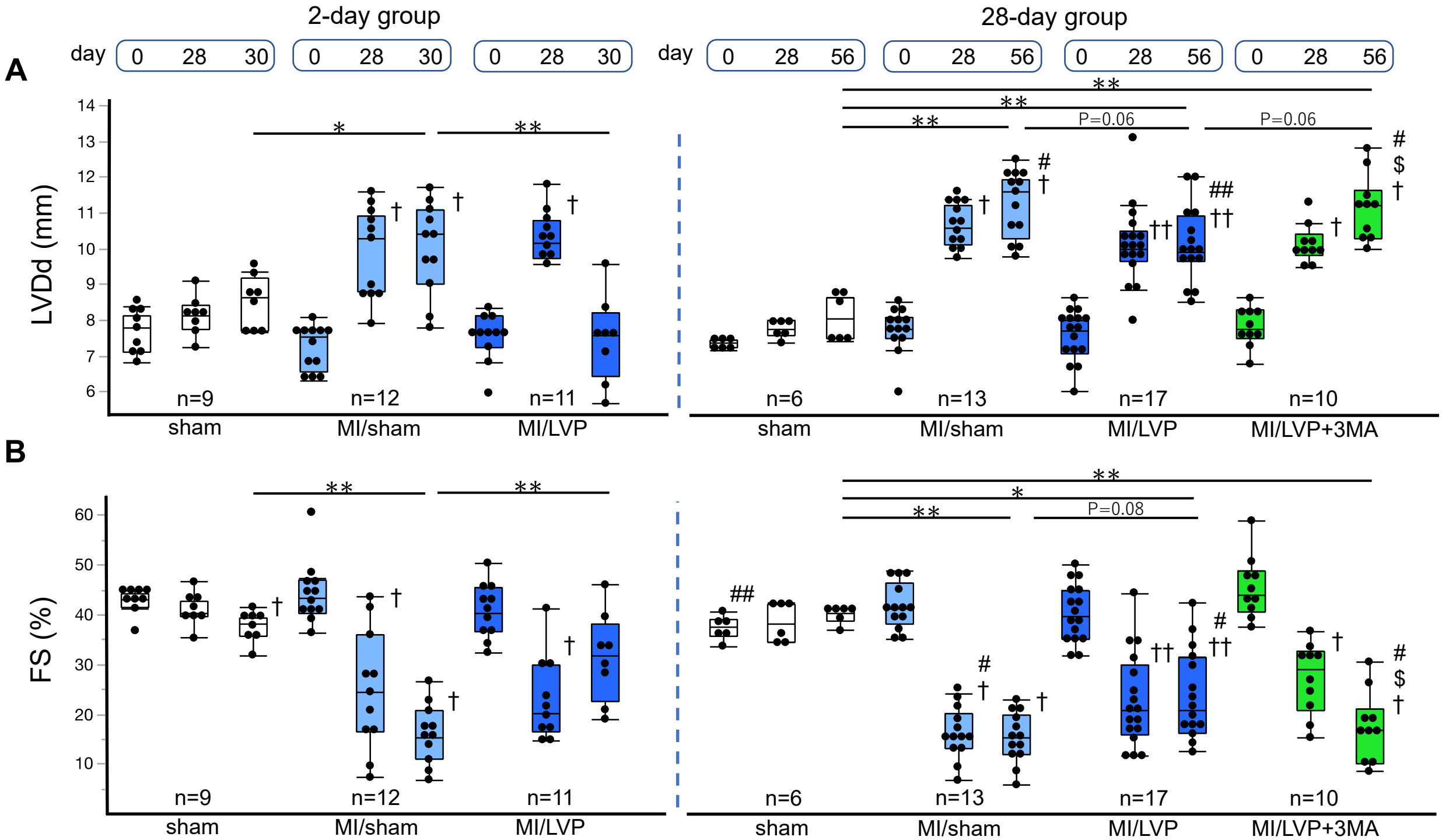
Table 2. Body, heart, and lung weights early and late after LVP

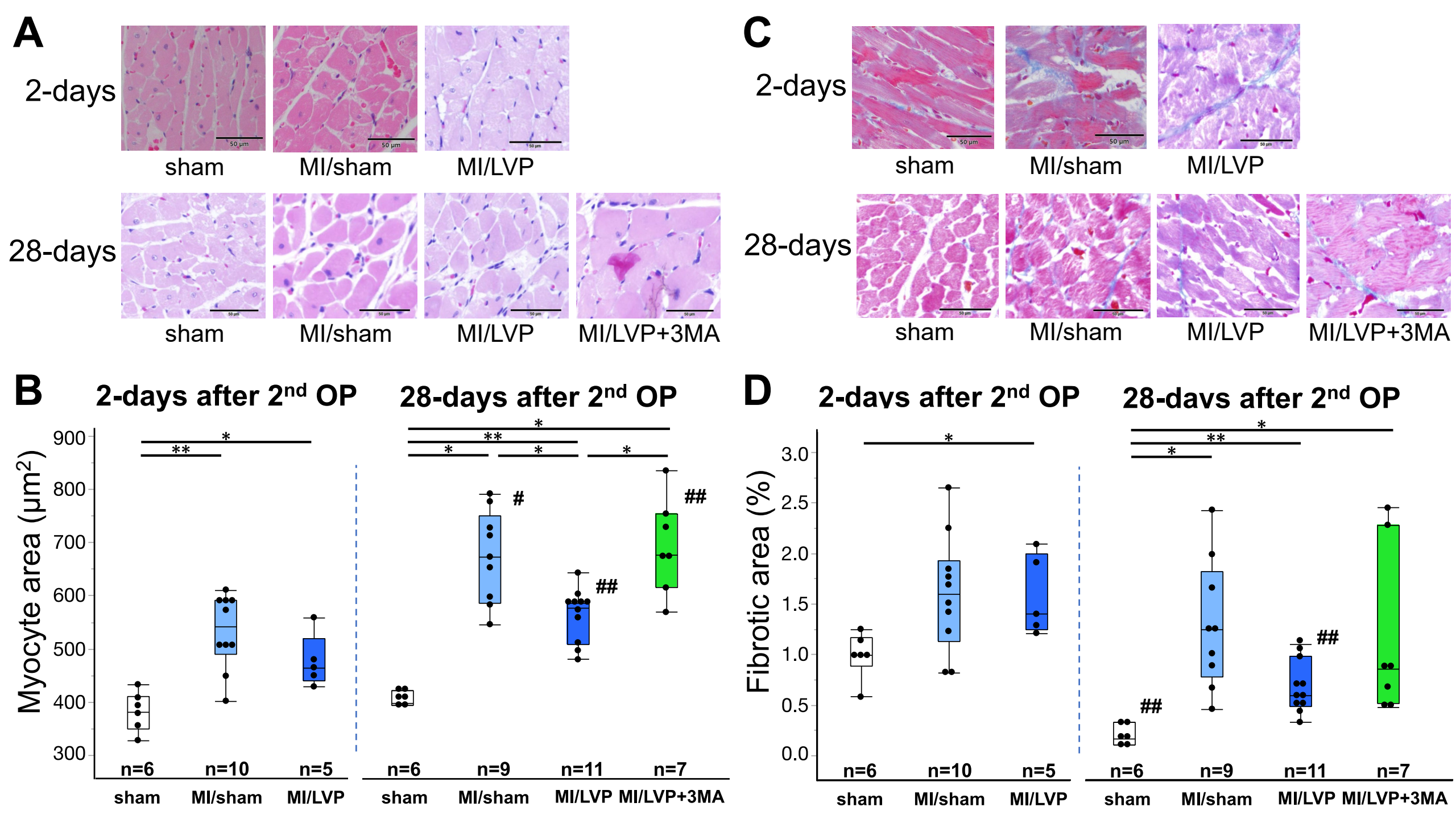
	2-day group			28-day group			
	sham n=9	MI/sham n=12	MI/LVP n=10	sham n=6	MI/sham n=13	MI/LVP n=17	MI/LVP + 3MA n=10
Body weight (g)	449 (425-463)	397* (384-400)	376** (354-412)	520# (503-538)	460## (445-500)	480## (430-520)	445# (423-485)
Heart weight (mg)	1223 (1119-1306)	1336* (1278-1500)	NA	1539## (1466-1632)	1732 (1464-1976)	NA	NA
Heart /body weight (mg/g)	2.7 (2.6-2.8)	3.4** (3.2-3.6)	NA	3.1 (3.0-3.2)	3.5 (3.1-4.1)	NA	NA
Atrial weight (mg)	155 (149-174)	184 (175-260)	276** (236-357)	255# (219-267)	327 (206-530)	256 (200-500)	461 (264-662)
Atrial/body weight (mg/g)	0.33 (0.33-0.37)	0.46** (0.46-0.65)	0.76** (0.57-0.92)	0.52 (0.39-0.57)	0.65 (0.48-1.07)	0.57 (0.39-0.99)	1.07 (0.66-1.47)
Lung weight (mg)	1478 (1447-1515)	1531 (1397-1989)	2171* (1701-2921)	1661## (1598-1681)	2305* (2008-2939)	1888 (1692-2585)	2239** (2034-3279)
Lung/body weight (mg/g)	3.3 (3.2-3.5)	3.9** (3.6-5.0)	6.0** (4.0-7.8)	3.1 (3.1-3.2)	5.1* (4.4-6.5)	3.9 (3.5-5.1)	5.6** (4.6-7.1)

Values are median (first quartile – third quartile). NA, not applicable; LVP, left ventriculoplasty; 3-MA, 3-methyladenine; MI, myocardial infarction.
The P values are Bonferroni-adjusted. * $p<0.05$ vs sham; ** $p<0.01$ vs sham; # $p<0.05$ vs 2-day group; ## $p<0.01$ vs 2-day group.

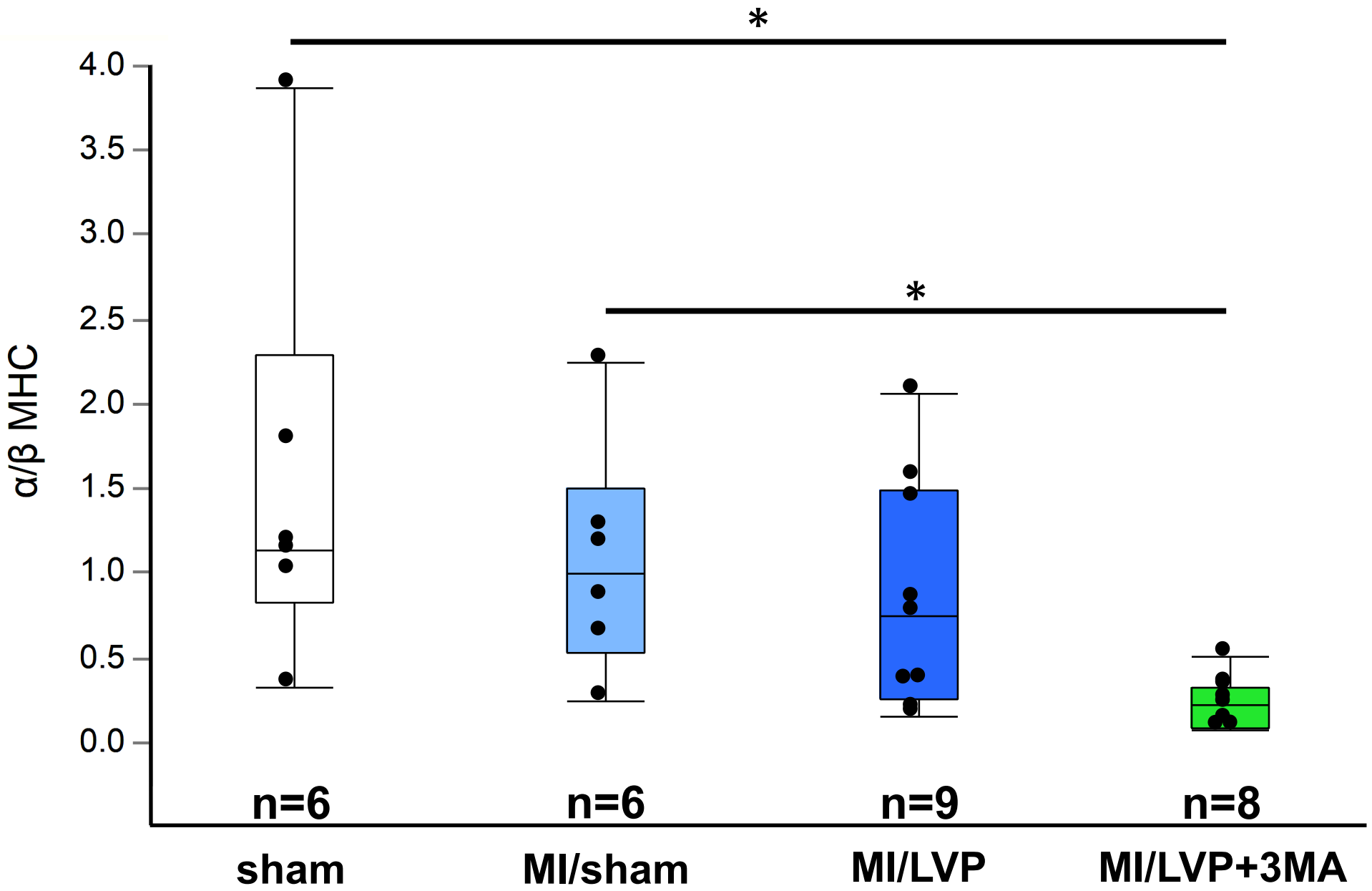

day 0
28
30
56
1st OP
2nd OP
Sacrifice
Sacrifice
28 days
2days
28days
sham
n=15
sham
n=15
n=9
n=6
sham
sham
n=25
n=12
n=13
MI / sham
MI
n=170
LVP
n=91
n=11
n=17
MI / LVP
dead
n=54
dead
n=50
21 days
3-MA i.p.
n=10
MI / LVP + 3-MA
dead
n=3
TTE
TTE
TTE
Catheter
TTE
Catheter

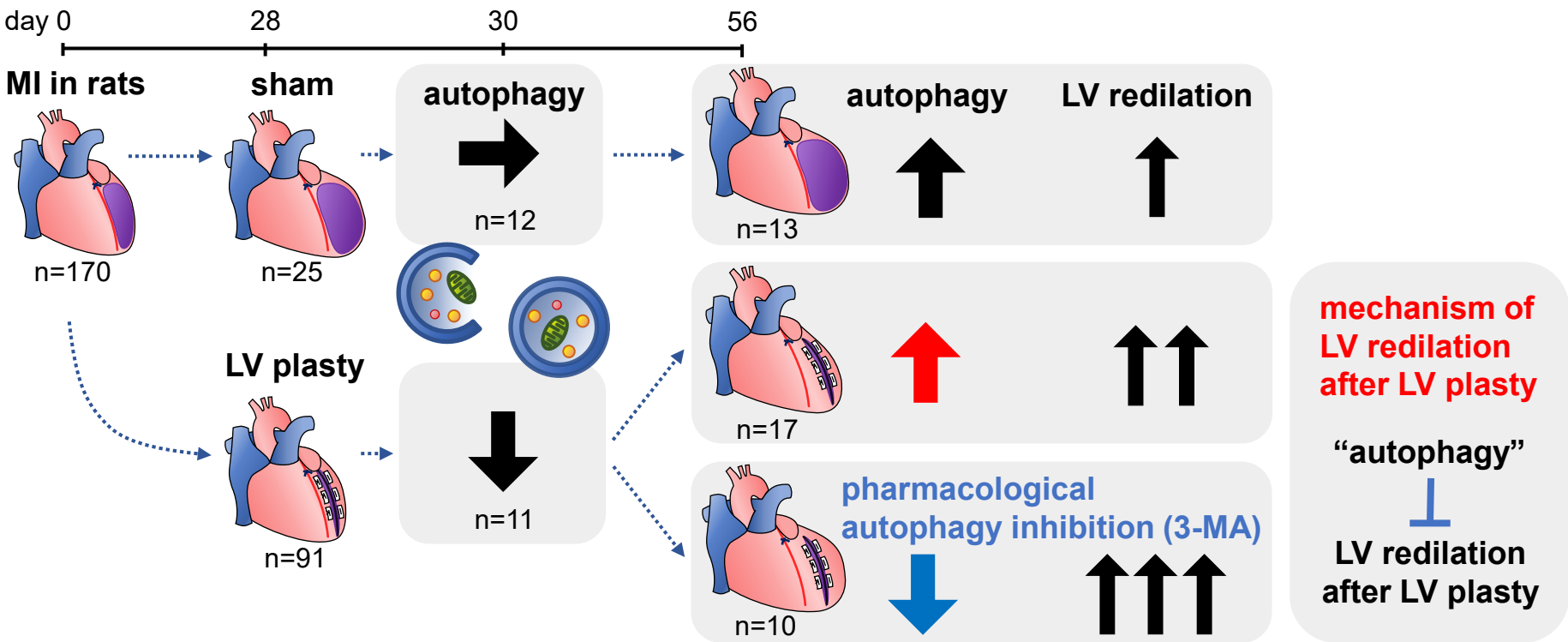

A
LC3 I
LC3 II
sham
MI/sham
MI/LVP
LC3 I
LC3 II
sham
MI/sham
MI/LVP
MI/LVP+3MA
B
2-days after 2nd OP
28-days after 2nd OP
LC3II (arbitary unit)
0
1
2
3
4
5
6
7
*
**
*
*
*
##
##
#
n=3
n=5
n=6
n=6
n=9
n=9
n=10
sham
MI/sham
MI/LVP
sham
MI/sham
MI/LVP
MI/LVP+3MA


2-day group
28-day group
A
day
0
28
30
56
LVDd (mm)
14
13
12
11
10
9
8
7
6
*
**
P=0.06
n=9
n=12
n=11
n=6
n=13
n=17
n=10
sham
MI/sham
MI/LVP
MI/LVP+3MA
B
FS (%)
60
50
40
30
20
10
P=0.08


A
2-days
sham
MI/sham
MI/LVP
28-days
sham
MI/sham
MI/LVP
MI/LVP+3MA
C
2-days
sham
MI/sham
MI/LVP
28-days
sham
MI/sham
MI/LVP
MI/LVP+3MA
B
2-days after 2nd OP
28-days after 2nd OP
Myocyte area (μm2)
900
800
700
600
500
400
300
n=6
n=10
n=5
n=6
n=9
n=11
n=7
sham
MI/sham
MI/LVP
sham
MI/sham
MI/LVP
MI/LVP+3MA
D
2-days after 2nd OP
28-days after 2nd OP
Fibrotic area (%)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
n=6
n=10
n=5
n=6
n=9
n=11
n=7
sham
MI/sham
MI/LVP
sham
MI/sham
MI/LVP
MI/LVP+3MA

28-days after 2nd OP




day 0
28
30
56
MI in rats
n=170
sham
n=25
LV plasty
n=91
autophagy
n=12
n=11
autophagy
LV redilation
n=13
n=17
pharmacological
autophagy inhibition (3-MA)
n=10
mechanism of
LV redilation
after LV plasty
“autophagy”
LV redilation
after LV plasty