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1 **Right coronary artery ligation in mice:**  
2 **A novel method to investigate right ventricular dysfunction and**  
3 **biventricular interaction.**

4  
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25

26 **Abstract**

27 Right ventricular (RV) dysfunction can lead to complications following acute inferior  
28 myocardial infarction (MI). However, it is unclear how RV failure after MI contributes to left-  
29 sided dysfunction is unclear. The aim of this study was to investigate the consequences of  
30 right coronary artery (RCA) ligation in mice. RCA ligation was performed in  
31 C57BL/6JRj mice (n=38). The cardiac phenotypes were characterized using high-resolution  
32 echocardiography performed up to 4 weeks post-RCA ligation. Infarct size was measured  
33 using 2,3,5-triphenyltetrazolium chloride (TTC)-staining 24h post-RCA ligation and the  
34 extent of the fibrotic area was determined 4 weeks after MI. RV dysfunction was confirmed  
35 24h post RCA ligation by a decrease in the tricuspid annular plane systolic excursion  
36 ( $p<0.001$ ) and RV longitudinal strain analysis ( $p<0.001$ ). Infarct size measured *ex-vivo*  
37 represented  $45.1\pm 9.1\%$  of RV free wall. RCA permanent ligation increased RV/LV area ratio  
38 ( $p<0.01$ ). Septum hypertrophy ( $p<0.01$ ) was associated with diastolic septal flattening. During  
39 the 4 weeks post-RCA ligation, the LV ejection fraction was preserved, yet it was associated  
40 with impaired LV diastolic parameters ( $E/e'$ , global strain rate during early diastole).  
41 Histological staining after 4 weeks confirmed the remodelling process with a thin and fibrotic  
42 RV. This study validates that RCA ligation in mice is feasible and induces right ventricular  
43 heart failure associated with development of LV diastolic dysfunction. Our model offers a  
44 new opportunity to study mechanisms and treatments of RV/LV dysfunction after MI.

45 **NEW & NOTEWORTHY:**

46 RV dysfunction frequently causes complications after acute inferior MI. How RV failure  
47 contributes to left-sided dysfunction is elusive because of the lack of models to study  
48 molecular mechanisms. Here, we created a new model of MI by tying permanently the RCA

49 in mice. This model offers a new opportunity to unravel mechanisms underlying RV/LV  
50 dysfunction and evaluate drug therapy.

51 **Keywords:**

52 Right ventricular infarction model, diastolic dysfunction, cardiomyocytes

53

54 **Classifications:**

55 Pathophysiology; Heart failure

56

## 57 **Introduction**

58 Long considered a simple pipe feeding the pulmonary arteries, the right ventricle (RV) is now  
59 acknowledged as a main actor of the heart. Despite an increasing interest for its role in pulmonary  
60 artery hypertension, injuries following RV infarction have been neglected. RV myocardial infarction  
61 (MI) is a specific event, which rarely occurs on its own. RV MI complicates 30 to 50% of inferior left  
62 ventricular (LV) infarction and is recognized in this situation as a major prognostic factor (13, 18, 28).  
63 RV infarction is associated with a higher incidence of conduction disturbance, ventricular arrhythmias,  
64 cardiogenic shock and short-term death (19). Despite the fact that the RV MI patient presents a better  
65 recovery than LV MI (1, 29), the mechanisms of RV dysfunction and remodelling remain unclear (32).  
66 Recent studies have shown that diastolic and systolic ventricular interactions are negatively influenced  
67 by the RV regional inhomogeneity and prolongation of contraction. This right-to-left ventricular  
68 interaction, integrated into the concept of biventricular interdependence (3), is notably linked to septal  
69 wall sharing, RV architecture participation in LV work and reduction of LV preload by RV  
70 inefficiency (15). In addition, RV ischemia could directly impair left ventricular contractility (5).  
71 However, the mechanisms that determine whether RV failure contributes to left-sided dysfunction are  
72 not well defined. Tantalizingly, small animal models of RV infarction induced by RCA ligation are  
73 currently unavailable, as the most common rodent model used by researchers to address MI  
74 remodelling is the permanent occlusion of the left descending coronary artery. In contrast to large  
75 animal models, (14, 21, 26) a mouse model of RV infarction should be more effective to study  
76 molecular mechanisms of RV dysfunction and remodeling after MI. The aim of this study was to  
77 develop and validate a mouse model of RV infarction through right coronary artery (RCA) ligation  
78 and investigate its consequences on LV function.

79

## 80 **Methods**

### 81 ***In vivo* right ventricular ischemia mice model**

82 Seventy-seven male C57BL/6JRj mice were anesthetized by 2% isoflurane inhalation with analgesia  
83 (buprenorphine 0.1 mg/kg s.c), and ventilated by orotracheal intubation (minivent, Harvard apparatus,  
84 USA). A specific procedure was designed in order to minimize the size of the thoracotomy and to limit

85 bleeding during RCA ligation, especially during the delicate maneuver of revealing the RCA by  
86 maintaining the right atrium. A small incision (2 cm) was made through the skin over the right chest  
87 (Figure 1 A). After dissection, cauterization and retraction of the pectoral major and minor muscle, the  
88 fourth intercostal space was exposed (Figure 2B). An incision was made at the fourth intercostal space  
89 with a cauterization tool to open the pleural membrane (Figure 1C). The right side of the heart was  
90 exposed and a sterile compress was used to maintain the right atrium (Figure 1D-E). The RCA was  
91 sutured, and ligated at a site  $\approx$ 3-5 mm from its origin using a 9-0 nylon suture (Figure 1 F-H) (n=43).  
92 Ischemia was verified by the sudden regional paleness of the myocardium and ST elevation (Figure 1  
93 I). The thoracotomy site was closed while increasing positive end expiratory pressure. Only RCA  
94 ligation was omitted in the sham procedure (n=34). A movie of this new method of MI available in the  
95 Online Data Supplement.

96 The Animal Care and Use Committees of the University of Montpellier (CEEA-LR-1435-13129)  
97 approved all animal experiments. Mice were housed in a pathogen-free facility and handled in  
98 accordance with the principles and procedures outlined in the ARRIVE guidelines (20) and in the  
99 *American Journal of Physiology* guidelines for experimental models of myocardial ischemia and  
100 infarction (23). In order to minimize the number of animals used per experiment in our study, we used  
101 only male mice. Moreover, this investigation conformed to the guidelines for ethical care of  
102 experimental animals of the European Union (2010/63/EU).

103

#### 104 **Echocardiography and speckle tracking analysis**

105 High-resolution echocardiography (VisualSonics/Fujifilm, Canada with a MS550D ultrasound probe  
106 40 MHz) was performed under anaesthesia by 2% isoflurane inhalation at 37°C, ECG and respiratory  
107 rate were monitored.

108 The mitral valve leaflet was visualized and mitral flow (Mitral valve (MV) flow and MV tissular  
109 Doppler) was assessed at long axis b-mode view by placing the transducer on the left lateral chest wall  
110 were recorded using pulse wave Doppler. Wall thicknesses, end-systolic and end-diastolic LV  
111 dimensions were measured according the *American Physiological Society* guidelines as applied to  
112 mice (24). LV wall thickness was measured at the level of intraventricular septum and posterior wall.

113 LV volume was calculated from Simpson's method of disks and ejection fraction determined from the  
114 formula  $(LV \text{ end-diastolic-end-systolic volume}) / (LV \text{ end-diastolic volume})$ . Longitudinal strain  
115 analysis was performed under long axis view, whereas circumferential strain analysis was performed  
116 under short axis view.

117 A four-chamber view was used to characterize the RV function with the Tricuspid annular plane  
118 systolic excursion (TAPSE) measurement. Speckle tracking analysis was used to study global and  
119 regional RV strain modification. We specifically used left atrium area, Isovolumic Relaxation Time  
120 (IVRT), peak early filling (E wave) and late diastolic filling (A wave) ratio (E/A), early filling (E) to  
121 early diastolic mitral annular velocity (E') (E/E' ratio), E wave and end diastolic strain rate ratio  
122 (E/SRe) to determine diastolic function index (33, 35, 36). Offline image analyses were performed  
123 using dedicated VisualSonics VevoLab 3.1.0 software.

124

#### 125 **Infarct size**

126 Triphenyltetrazolium chloride (TTC) staining was realized 24 hours post-MI. Five mice per group  
127 were euthanized with pentobarbital (300 mg/kg) and heparin (150 U) intraperitoneally and the heart  
128 was quickly excised, sliced into four 1.0-mm-thick sections perpendicular to the long axis of the heart.  
129 The sections were then incubated with 1% TTC at 37°C for 10 min and then scanned. The infarct area  
130 was measured using ImageJ software and myocardial infarct sizes were expressed as a percentage of  
131 the right ventricle area (n=7 /group).

132

#### 133 **Histology**

134 Four weeks after permanent RCA ligation, hearts were dissected, fixed for four hours in 4%  
135 paraformaldehyde (vol/vol) in PBS, washed in sucrose gradient, then embedded in OCT and  
136 cryosectioned. Hearts were observed under a Zeiss Apotome microscope. Fibrotic area was  
137 determined with wheat germ agglutinin-Cy3 (WGA-Cy3 from Sigma-Aldrich) as described  
138 previously (7, 25). To measure interventricular wall septum thickness, three sections from  
139 each heart were analyzed with ImageJ software. For each section, six different measurements

140 were taken along the septum, and results were represented as average of heart wall thickness  
141 of IVS (n=7 /group).

142

### 143 **Single-cell contractility**

144 Four weeks after surgery, single RV, LV and septal myocytes were isolated by enzymatic digestion  
145 from sham and RCA ligation heart. Hearts were rapidly excised after euthanasia (cervical dislocation)  
146 and submitted to enzymatic action (liberase) using a Langendorff perfusion system in order to disperse  
147 single rod-shaped left ventricular (LV) myocytes (10, 25). Only cardiomyocytes with clear edges and  
148 quiescent were used within 1–4 h of isolation for experiments. Unloaded cell shortening was measured  
149 (Sarcomere length, SL; IonOptix system, Hilton, USA) during field stimulation (1-ms current pulses at  
150 0.5 Hz, room temperature 22 °C ±2 °C, 1.8 mM external Ca<sup>2+</sup>). Data were analyzed using Ionwizard  
151 Software.

152

### 153 **Statistical analysis**

154 Results are expressed as mean ± SEM. Kaplan-Meier survival curves plot of the Sham (n = 24) and  
155 RCA ligation mice (n=33), where the outcome is time until 4 weeks (Log-rank Mantel-Cox test).  
156 Experimental groups were compared using the Mann-Whitney test for independent samples. A value  
157 of p<0.05 was considered significant. Analyses were performed using the GraphPad Prism 6 software.

158

## 159 **Results**

### 160 **Surgical procedure and survival analysis**

161 A total of 57 mice were used in the MI survival study. The average procedure time was 30.4±1.5 min  
162 for sham and 31.2±2.1 minutes for RCA ligation. Surgical approach differed from classical LCA  
163 ligation firstly in the right thoracic access, and especially in the delicate maneuver of revealing the  
164 RCA by maintaining the right atrium as shown in Figure 1 D-E and supplemental video 1.

165 During the peri-surgical period (from the beginning of surgery to 6 hours after), three mice died  
166 (12.5%) in the sham group and five mice died (15.1%) in the RCA ligation group (Figure 1 J).

167 Causes of death were bleeding (6 mice) and pneumothorax (2 mice). After the peri-surgical period, no  
168 mice died in the sham group and 2 mice (7.7%) died in the RCA ligation group (Figure 1 K). The  
169 reason for those deaths was not identified, yet we assume that this can be attributed to either cardiac  
170 arrhythmia or sudden cardiac arrest because autopsy of these mice showed no blood in the thoracic  
171 cavity. The overall survival rates (excluding surgical-related death) were 100% in sham group and  
172 74% if peri-surgical death was not included ( $p=0.133$ ).

173

#### 174 **RCA permanent occlusion induced RV dysfunction**

175 We used high-resolution ultrasound as a non-invasive method to measure RV function 24h hour, 1  
176 week and 4 weeks after permanent RCA occlusion. As shown in Figure 2 A, the RCA ligation group  
177 exhibited severely impaired and akinetic RV motion compared to sham mice (RV global longitudinal  
178 strain  $-12.7\pm 0.8$  vs.  $-8.8\pm 0.9$ ,  $p<0.001$ ) especially in the mid base part of free RV wall (Figure 2 B,  
179  $p<0.003$ ) while the apex longitudinal strain was progressively restored 4 weeks post-MI. In addition,  
180 the TAPSE was decreased (Figure 2 C) during the follow up. RV and RA were clearly dilated 24h  
181 post-surgery and even more after 28 days post-MI (Figure 2 D-E, Figure 4 E). Heart rates were similar  
182 among all of the groups ( $503.4 \pm 52.1$  vs.  $487.6 \pm 12.9$  bpm,  $p=0.367$ ). Overall, we observed an acute  
183 and global deterioration of RV function parameters after ligation of its main artery. We investigated  
184 single cell shortening, measured as a variation of SL (Figure 2 F-J). At rest, SL was unchanged (Panel  
185 F). However, a decreased in cell peak shortening ( $p<0.0001$ ) (Panel G) and late acceleration  
186 ( $p<0.0001$ ) (Panel H) and deceleration ( $p<0.0001$ ) (Panel I) were observed, reflecting impaired single  
187 cell contraction (Panel J). Consistent with the echocardiographic observations, histologic analysis with  
188 TTC staining confirmed systematic RV infarction in RCA-L group, which was absent in sham mice.  
189 Infarct size was homogeneous between RCA-L mice and represented less than 50% of the RV (Figure  
190 2 K). We performed histological assessment of RV infarction at 4 weeks post-MI as shown in Figure 2  
191 L. RCA-L group presented a thinner fibrotic RV free wall in nearly half of the RV (Figure 2 L-M). We  
192 did not identify major interstitial fibrosis in the septum or LV compared to Sham mice.

**193 Segmental behaviour in the LV: hypertrophy and hyperkinesia of the septum**

194 Between the left and the right ventricle, the septum appears as the keystone of bi-ventricular  
195 interaction (34). In our model, RV dysfunction and dilation caused leftward deviation of the  
196 interventricular septum (a D-shaped septum is shown in Figure 3 A) especially during diastole and  
197 respiratory phase (supplemental videos 2 and 4 for sham, compared to supplemental video 3 and 5 for  
198 RCA ligation mice). In addition, the septal wall was thickened by nearly 20% (Figure 2 N and Figure  
199 3 B) and septal contractility, estimated by circumferential strain (Figure 3 C) and strain rate (Figure 3  
200 D), was improved 4 weeks after RCA ligation compared to Sham mice. The peak of early diastolic  
201 strain rate was measured for septal segment (figure 3 E) and revealed a better septal relaxation index  
202 after RCA ligation compared to Sham mice ( $p<0.05$ ). We next decided to isolate septal ventricular  
203 cardiomyocytes and study their contractility (Figure 3 F-J). Septal cardiomyocytes from RCA Ligation  
204 mice exhibited higher peak rate of cell shortening compared to Sham cardiomyocytes ( $p<0.0004$ )  
205 (Panel G, J). In addition, velocity of shortening was improved ( $p<0.017$ ) (Panel I, J).

206

**207 RCA ligation induced left ventricular diastolic dysfunction**

208 It is well known that RV failure and septal D-shaped is associated with LV dysfunction (6). We  
209 assessed, through echocardiography, the LV systolic and diastolic functions. LV ejection fraction and  
210 global longitudinal strain (systolic function) were preserved (Figure 4 A,B) over time despite RCA  
211 ligation. However, cardiac output decreased 4 weeks after RCA ligation (Figure 4 C), and was  
212 associated with a reduction of Left Ventricular Internal Size (LVID) in both systole and diastole in the  
213 RCA ligation group (Figure 4 D). Heart rate ( $503.4 \pm 52.1$  vs.  $487.6 \pm 12.9$  bpm,  $p=0.367$ ), thickness  
214 of left anterior wall (LVAW) and Left Ventricular Posterior Wall (LVPW) were similar between both  
215 groups (LVAWd:  $0.91 \pm 0.02$  mm Sham vs  $0.96 \pm 0.02$  mm RCA lig,  $p=0.115$ ; LVPWd:  $0.82 \pm 0.03$   
216 mm Sham vs.  $0.87 \pm 0.04$  mm RCA lig;  $p=0.07$ ).

217 Mice from the RCA Ligation group presented several characteristics for LV diastolic dysfunction,  
218 with an increased left and right atrium area (Figure 4 E), a prolonged IVRT (Figure 4 F), a reduction  
219 of E/A ratio (Figure 4G). The E/E' (Figure 4 H) and E/SRE ratios (Figure 4I) were both increased.

220 Contraction of LV cardiomyocytes from the RCA ligation group showed no major modification  
221 compared to that of cardiomyocytes isolated from Sham animals (Figure 4 J-N). Only a slight increase  
222 in the contraction time for maximal amplitude was evidenced between the two groups (Panel L and  
223 N).

224 Taken together, our results demonstrate that this model of MI achieved by RCA ligation in mice is  
225 feasible, inducing right ventricular dysfunction associated with septal adaptation and a flawed LV  
226 diastolic function.

227

## 228 **Discussion**

229 To our knowledge, this is the first description of a murine model of RCA ligation. The surgery  
230 provoked an important RV infarction without LV ischemic injuries. Mice presented RV systolic  
231 function breakdown and morphological alterations, with RV dilatation and inversion of RV/LV area  
232 ratio. In contrast to the LAD ligation procedure in mice (23, 25), the RCA is more difficult to access.  
233 The method to properly ligate properly the RCA is challenging because of the thickness of the RV  
234 wall and the proximity of the right atrium, which makes the surgical gesture critical. Despite necessary  
235 surgical training, the protocol is reliable with systematic infarction, reproductive, as well as safe with a  
236 low mortality. Mice can survive several weeks after ligation permitting longitudinal follow-up. RV  
237 infarction was associated with an echocardiographic pattern of RV failure. In our hands, achievement  
238 of RV infarct size (approximately 50% of the RV free wall) was consistent and comparable to *ex-vivo*  
239 model of global ischemia on isolated heart rats (2).

240 After 4 weeks follow-up, RCA ligation mice still displayed an impaired RV systolic function with  
241 reduced TAPSE and longitudinal strain, although these parameters were improved compared to their  
242 early assessment. This corroborates medical data concerning RV improvement after infarction (17).  
243 Within the RV, areas presented a distinct profile. Apex longitudinal strain recovered progressively  
244 while free wall longitudinal strain remained low. RV/LV area ratio was still increasing, but the  
245 dispersion of data evokes various remodelling profiles or status after infarction, or/and different  
246 infarction seriousness. Partial normalization of RV systolic parameters seen after 4 weeks follow-up  
247 corroborates observations about RV recovery following infarction in human and supports clinical

248 relevance of this model (17). Compared to LV infarction, the improvement of the RV function may be  
249 caused by lower afterload, better left to right collateral flow and systolo-diastolic perfusion. It is  
250 interesting to note that the RV is more resilient against ischemia than the LV. The right ventricle needs  
251 lower myocardial oxygen demand and a better oxygen supply and coronary perfusion throughout the  
252 cardiac cycle (30). The RV, unlike the LV, has an oxygen extraction reserve, which works as an  
253 additional defence mechanism against myocardial ischemia (8).

254 In our model, classical LV systolic parameters remained unchanged over time. This suggests there is  
255 no major ischemic remodelling process affecting LV systolic parameters. Our late segmental analysis  
256 reports a hyperkinetic and hypertrophic septum wall, contrary to the lateral wall. This could be part of  
257 the adaptive hypertrophic mechanism in reaction to RV failure. Diastolic dysfunction presents a  
258 complex pattern. First, there is a pattern of impaired relaxation, as described in models of diastolic  
259 dysfunction in mice, specifically those involving hypertrophy and pressure overload (35). Secondly,  
260 moderate increase of  $E/E'$  and  $E/SRE$  evokes an elevation of LV filling pressure, which is explained  
261 by two mechanisms: septal hypertrophy associated with fibrosis and dilatation of the RV within the  
262 restricted intra-pericardial space impairing LV filling. Acute and chronic RV dysfunction influence  
263 LV function because the two ventricles work in series but are anatomically arranged in parallel,  
264 sharing a common ventricular septum (11, 34). Under these conditions, during the end-diastolic phase,  
265 there is a leftward displacement of the interventricular septum producing distortion of the short-axis  
266 profile of the LV. It is worth to note that RV systolic dysfunction is present in 15% of human  
267 myocardial infarction (MI), mostly for patients with pluri-troncular coronary lesions. Moreover, RCA  
268 is the culprit coronary artery in one-third of MI (4). Interestingly, women have RV MI more frequently  
269 than men (27). Gender plays a major role in cardiovascular disease evolution, especially for HF with  
270 preserved ejection fraction that affects more women than men (9). This aspect warrants further  
271 investigation.

272 Overall, this study provides interesting and novel data about chronic remodelling after isolated RV  
273 infarction. There was a deficit of knowledge because isolated RV ischemia rarely occurs in human  
274 (12). We also unravelled development of LV diastolic dysfunction after RCA permanent ligation.  
275 Among the few studies in large animal models of chronic RV failure after RCA occlusion (5, 14, 16,

276 21, 22, 26, 31) only one reported that RV failure induces LV systolic dysfunction (5). We assumed  
 277 that different perfusion networks between animals and fewer collateral perfusion are responsible for  
 278 that differences. Large mammalian models (dogs, sheep, pigs) share many cardiovascular  
 279 characteristics with human, but have a high cost of housing and maintenance. In comparison, mice are  
 280 easy to handle and housing. In addition, murine models can be easily genetically modified (e.g. Knock  
 281 out, Knock in mice) allowing for the study of remodelling molecular mechanisms during RV failure.  
 282 In conclusion, we developed a new model of selective RV infarction, which allows the investigation of  
 283 RV adaptation during and after ischemia. This approach could provide valuable information for  
 284 preclinical mechanistic studies and drug therapy evaluation.

285

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290

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294

## 295 **Conflict of interest**

296 The authors have declared that no competing interests exist.

297

## 298 **References**

- 299 1. **Albulushi A, Giannopoulos A, Kafkas N, Dragasis S, Pavlides G, Chatzizisis YS.** Acute  
 300 right ventricular myocardial infarction. *Expert Rev Cardiovasc Ther* 16: 1–10, 2018.  
 301 2. **Andersen A, Povlsen JA, Bøtker HE, Nielsen-Kudsk JE.** Ischemic preconditioning reduces  
 302 right ventricular infarct size through opening of mitochondrial potassium channels. *Cardiology*  
 303 123: 177–180, 2012.  
 304 3. **Bernheim P.** De l'asystolie veineuse dans l'hypertrophie du coeur gauche par stenose  
 305 concomitante du ventricule droit. *Rev Med (Paris)* : 785–801, 1910.  
 306 4. **Bowers TR, O'Neill WW, Pica M, Goldstein JA.** Patterns of coronary compromise resulting  
 307 in acute right ventricular ischemic dysfunction. *Circulation* 106: 1104–1109, 2002.

- 308 5. **Brookes C, Ravn H, White P, Moeldrup U, Oldershaw P, Redington A.** Acute right  
309 ventricular dilatation in response to ischemia significantly impairs left ventricular systolic  
310 performance. *Circulation* 100: 761–767, 1999.
- 311 6. **Cativo Calderon EH, Mene-Afejuku TO, Valvani R, Cativo DP, Tripathi D, Reyes HA,**  
312 **Mushiyeve S.** D-Shaped Left Ventricle, Anatomic, and Physiologic Implications. *Case reports in*  
313 *cardiology* 2017: 4309165, 2017.
- 314 7. **Choquet C, Nguyen THM, Sicard P, Buttigieg E, Tran TT, Kober F, Varlet I, Sturny R,**  
315 **Costa MW, Harvey RP, Nguyen C, Rihet P, Richard S, Bernard M, Kelly RG, Lalevée N,**  
316 **Miquerol L.** Deletion of Nkx2-5 in trabecular myocardium reveals the developmental origins of  
317 pathological heterogeneity associated with ventricular non-compaction cardiomyopathy. *PLoS*  
318 *Genet* 14: e1007502, 2018.
- 319 8. **Crystal GJ, Pagel PS.** Right ventricular perfusion: physiology and clinical implications.  
320 *Anesthesiology* 128: 202–218, 2018.
- 321 9. **Duca F, Zotter-Tufaro C, Kammerlander AA, Aschauer S, Binder C, Mascherbauer J,**  
322 **Bonderman D.** Gender-related differences in heart failure with preserved ejection fraction. *Sci*  
323 *Rep* 8: 1080, 2018.
- 324 10. **Fazal L, Laudette M, Paula-Gomes S, Pons S, Conte C, Tortosa F, Sicard P, Sainte-Marie**  
325 **Y, Bissierier M, Lairez O, Lucas A, Roy J, Ghaleh B, Fauconnier J, Mialet-Perez J,**  
326 **Lezouale’h F.** Multifunctional mitochondrial epac1 controls myocardial cell death. *Circ Res*  
327 120: 645–657, 2017.
- 328 11. **Friedberg MK.** Imaging Right-Left Ventricular Interactions. *JACC Cardiovasc Imaging* 11:  
329 755–771, 2018.
- 330 12. **Goldstein JA.** Pathophysiology and management of right heart ischemia. *J Am Coll Cardiol* 40:  
331 841–853, 2002.
- 332 13. **Gorter TM, Lexis CPH, Hummel YM, Lipsic E, Nijveldt R, Willems TP, van der Horst**  
333 **ICC, van der Harst P, van Melle JP, van Veldhuisen DJ.** Right Ventricular Function After  
334 Acute Myocardial Infarction Treated With Primary Percutaneous Coronary Intervention (from the  
335 Glycometabolic Intervention as Adjunct to Primary Percutaneous Coronary Intervention in  
336 ST-Segment Elevation Myocardial Infarction III Trial). *Am J Cardiol* 118: 338–344, 2016.
- 337 14. **Haraldsen P, Lindstedt S, Metzsch C, Algotsson L, Ingemansson R.** A porcine model for  
338 acute ischaemic right ventricular dysfunction. *Interact Cardiovasc Thorac Surg* 18: 43–48,  
339 2014.
- 340 15. **Harjola V-P, Mebazaa A, Čelutkienė J, Bettex D, Bueno H, Chioncel O, Crespo-Leiro MG,**  
341 **Falk V, Filippatos G, Gibbs S, Leite-Moreira A, Lassus J, Masip J, Mueller C, Mullens W,**  
342 **Naeije R, Nordegraaf AV, Parissis J, Riley JP, Ristic A, Rosano G, Rudiger A, Ruschitzka**  
343 **F, Seferovic P, Sztrymf B, Vieillard-Baron A, Yilmaz MB, Konstantinides S.** Contemporary  
344 management of acute right ventricular failure: a statement from the Heart Failure Association  
345 and the Working Group on Pulmonary Circulation and Right Ventricular Function of the  
346 European Society of Cardiology. *Eur J Heart Fail* 18: 226–241, 2016.
- 347 16. **Hein M, Roehl AB, Baumert JH, Scherer K, Steendijk P, Rossaint R.** Anti-ischemic effects  
348 of inotropic agents in experimental right ventricular infarction. *Acta Anaesthesiol Scand* 53:  
349 941–948, 2009.
- 350 17. **Hoogslag GE, Haeck MLA, Velders MA, Joyce E, Boden H, Schalijs MJ, Bax JJ, Ajmone**  
351 **Marsan N, Delgado V.** Determinants of right ventricular remodeling following ST-segment  
352 elevation myocardial infarction. *Am J Cardiol* 114: 1490–1496, 2014.
- 353 18. **Horan LG, Flowers NC.** Right Ventricular Infarction: Specific Requirements of Management -  
354 American Family Physician [Online]. *Am Fam Physician*.  
355 <https://www.aafp.org/afp/1999/1015/p1727.html>.
- 356 19. **Jacobs AK, Leopold JA, Bates E, Mendes LA, Sleeper LA, White H, Davidoff R, Boland J,**  
357 **Modur S, Forman R, Hochman JS.** Cardiogenic shock caused by right ventricular infarction:  
358 a report from the SHOCK registry. *J Am Coll Cardiol* 41: 1273–1279, 2003.
- 359 20. **Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG.** Improving bioscience  
360 research reporting: the ARRIVE guidelines for reporting animal research. *PLoS Biol* 8:  
361 e1000412, 2010.

- 362 21. **Laster SB, Ohnishi Y, Saffitz JE, Goldstein JA.** Effects of reperfusion on ischemic right  
363 ventricular dysfunction. Disparate mechanisms of benefit related to duration of ischemia.  
364 *Circulation* 90: 1398–1409, 1994.
- 365 22. **Laster SB, Shelton TJ, Barzilai B, Goldstein JA.** Determinants of the recovery of right  
366 ventricular performance following experimental chronic right coronary artery occlusion.  
367 *Circulation* 88: 696–708, 1993.
- 368 23. **Lindsey ML, Bolli R, Cauty JM, Du X-J, Frangogiannis NG, Frantz S, Gourdie RG,  
369 Holmes JW, Jones SP, Kloner RA, Lefer DJ, Liao R, Murphy E, Ping P, Przyklenk K,  
370 Recchia FA, Schwartz Longacre L, Ripplinger CM, Van Eyk JE, Heusch G.** Guidelines for  
371 experimental models of myocardial ischemia and infarction. *Am J Physiol Heart Circ Physiol*  
372 314: H812–H838, 2018.
- 373 24. **Lindsey ML, Kassiri Z, Virag JAI, de Castro Brás LE, Scherrer-Crosbie M.** Guidelines for  
374 measuring cardiac physiology in mice. *Am J Physiol Heart Circ Physiol* 314: H733–H752,  
375 2018.
- 376 25. **Lucas A, Mialet-Perez J, Daviaud D, Parini A, Marber MS, Sicard P.** Gadd45 $\gamma$  regulates  
377 cardiomyocyte death and post-myocardial infarction left ventricular remodelling. *Cardiovasc*  
378 *Res* 108: 254–267, 2015.
- 379 26. **Malinowski M, Proudfoot AG, Eberhart L, Schubert H, Wodarek J, Langholz D, Rausch  
380 MK, Timek TA.** Large animal model of acute right ventricular failure with functional tricuspid  
381 regurgitation. *Int J Cardiol* 264: 124–129, 2018.
- 382 27. **Obradovic S, Dzudovic B, Djuric I, Jovic Z, Djenic N.** Women have right ventricular  
383 infarction more frequently than men. *Acta Cardiol* 70: 343–349, 2015.
- 384 28. **Ondrus T, Kanovsky J, Novotny T, Andrsova I, Spinar J, Kala P.** Right ventricular  
385 myocardial infarction: From pathophysiology to prognosis. *Exp Clin Cardiol* 18: 27–30, 2013.
- 386 29. **Popescu BA, Antonini-Canterin F, Temporelli PL, Giannuzzi P, Bosimini E, Gentile F,  
387 Maggioni AP, Tavazzi L, Piazza R, Ascione L, Stoian I, Cervesato E, Popescu AC, Nicolosi  
388 GL, GISSI-3 Echo Substudy Investigators.** Right ventricular functional recovery after acute  
389 myocardial infarction: relation with left ventricular function and interventricular septum motion.  
390 GISSI-3 echo substudy. *Heart* 91: 484–488, 2005.
- 391 30. **Rallidis LS, Makavos G, Nihoyannopoulos P.** Right ventricular involvement in coronary  
392 artery disease: role of echocardiography for diagnosis and prognosis. *J Am Soc Echocardiogr*  
393 27: 223–229, 2014.
- 394 31. **Ratliff NB, Peter RH, Ramo BW, Somers WR, Morris JJ.** A model for the production of  
395 right ventricular infarction. *Am J Pathol* 58: 471–480, 1970.
- 396 32. **Reddy S, Bernstein D.** Molecular Mechanisms of Right Ventricular Failure. *Circulation* 132:  
397 1734–1742, 2015.
- 398 33. **Rouhana S, Farah C, Roy J, Finan A, Rodrigues de Araujo G, Bideaux P, Scheuermann V,  
399 Saliba Y, Reboul C, Cazorla O, Aimond F, Richard S, Thireau J, Fares N.** Early calcium  
400 handling imbalance in pressure overload-induced heart failure with nearly normal left  
401 ventricular ejection fraction. *Biochimica et biophysica acta Molecular basis of disease* 1865:  
402 230–242, 2019.
- 403 34. **Saleh S, Liakopoulos OJ, Buckberg GD.** The septal motor of biventricular function. *Eur J*  
404 *Cardiothorac Surg* 29 Suppl 1: S126–38, 2006.
- 405 35. **Schnelle M, Catibog N, Zhang M, Nabeebaccus AA, Anderson G, Richards DA, Sawyer G,  
406 Zhang X, Toischer K, Hasenfuss G, Monaghan MJ, Shah AM.** Echocardiographic  
407 evaluation of diastolic function in mouse models of heart disease. *J Mol Cell Cardiol* 114: 20–  
408 28, 2018.
- 409 36. **Valero-Muñoz M, Backman W, Sam F.** Murine Models of Heart Failure with Preserved  
410 Ejection Fraction: a “Fishing Expedition”. *JACC Basic to translational science* 2: 770–789,  
411 2017.
- 412
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416 **Figures Panel**

417

418 **Figure 1: Photographs of various stages of the right coronary ligation surgical method.**

419 **Panel A through H**, see details in the *Methods* section describing each image. Arrow in F shows  
 420 RCA. **Panel I**: Representative ECG trace shows ST elevation after RCA ligation. **Panel J**: Kaplan-  
 421 Meier curve during the peri-surgical period in the sham group (black trace) and in the RCA ligation  
 422 group (Red trace) ( $p=0.914$ ; Log-rank Mantel-Cox test). **Panel K**: Kaplan-Meier curve after the peri-  
 423 surgical period in the sham group (black trace) and in the RCA ligation group (Red trace) ( $p=0.133$ ,  
 424 Log-rank Mantel-Cox test).

425

426 **Figure 2: RCA permanent occlusion induces subsequent RV Cardiac Dysfunction**

427 **Panel A**: RV global longitudinal strain ( $n=8$ /group) variation between Sham (black circle) and RCA  
 428 ligation (red triangle) mice during 4 weeks follow-up. **Panel B**: RV segmental longitudinal strain  
 429 ( $n=8$ /group) in the mid base free wall and RV apex variation between Sham (black circle) and RCA  
 430 ligation (red triangle) mice 4 weeks post-MI. **Panel C**: Tricuspid annular plane systolic excursion  
 431 (TAPSE) measurement between Sham (black circle  $n=16$ ) and RCA ligation (red triangle  $n=22$ ) mice  
 432 during 4 weeks follow-up. **Panel D**: RV/LV area ratio measurement, by echocardiography during  
 433 diastole, 24h post-surgery ( $n=16$  sham,  $n=22$  RCA ligation) and after 4 weeks between Sham (black  
 434 circle  $n=16$ ) and RCA ligation (red triangle  $n=22$ ). **Panel E**: Representative B-mode four chambers  
 435 view 4 weeks after RCA ligation. **Panel F**: Diastolic sarcomere length (SL) of cardiomyocytes  
 436 isolated from RV ( $n=16-35$  cells/group). **Panel G**: Maximal amplitude of SL shortening during  
 437 contraction of cardiomyocytes. **Panel H**: Contraction velocity. **Panel I**: Relaxation velocity. **Panel J**:  
 438 Contraction evoked by electrical field stimulation at 0.5 Hz as measured from SL shortening of single  
 439 intact RV cardiomyocytes 28 days after surgery in Sham (black line) and RCA ligation (red line)  
 440 (<sup>1</sup>Peak amplitude ( $\mu\text{m}$ ),  $p<0.0001$ ; <sup>2</sup>Velocity of shortening ( $\mu\text{m}/\text{sec}$ ),  $p<0.0001$ ; <sup>3</sup>Velocity of  
 441 lengthening ( $\mu\text{m}/\text{sec}$ ),  $p<0.0022$ ) ( $n=16-35$  cells/group). **Panel K**: Representative images and  
 442 quantification showing cardiac infarct size measured after 24h post RCA ligation. **Panel L**:  
 443 Representative images of WGA-Cy3 staining in mid-axis section from sham and RCA ligation hearts.

444 A white arrow indicate the location of the ligation. Scale 1mm. **Panel M:** Scar area quantification in  
445 the right ventricle in Sham (black circle n=7) and RCA ligation (red triangle n=7) heart. **Panel N:**  
446 Interventricular septum thickness measured in Sham (black circle) and RCA ligation (red triangle)  
447 hear after 4 weeks.

448 Results are expressed as mean  $\pm$  SEM. Experimental groups were compared using the Mann-Whitney  
449 test for independent samples.

450

451 **Figure 3: Segmental behaviour in the LV : Hypertrophy and hyperkinesia of the septum**

452 **Panel A:** Representative short axis view in Sham group (Top and supplementary video 4) and in RCA  
453 ligation group with a D-shaped septum (bottom and supplementary video 5). **Panel B:** Septum  
454 thickness measured by B-Mode short axis view during diastole (n=20-26/groups) 28 days after surgery  
455 in Sham (black circle) and RCA ligation (red triangle) mice. **Panel C:** Septum circumferential strain  
456 (n=21-26/group) 28 days after surgery in Sham (black circle) and RCA ligation (red triangle) mice.  
457 **Panel D:** Septum circumferential strain rate (n=21-26/group) 28 days after surgery in Sham (black  
458 circle) and RCA ligation (red triangle) mice. **Panel E:** Early diastole strain circumferential rate  
459 (n=15/groups) 28 days after surgery in Sham (black circle) and RCA ligation (red triangle) mice.  
460 **Panel F:** Diastolic sarcomere length (SL) of cardiomyocytes isolated from the septum (n=25-40  
461 cells/group). **Panel G:** Maximal amplitude of SL shortening during contraction of cardiomyocytes.  
462 **Panel H:** Contraction velocity. **Panel I:** Relaxation velocity. **Panel J:** Contraction evoked by electrical  
463 field stimulation at 0.5 Hz as measured from SL shortening of single septal cardiomyocytes 28 days  
464 after surgery in Sham (black line) and RCA ligation (red line) (<sup>4</sup>Peak amplitude ( $\mu\text{m}$ ),  $p<0.0004$ ;  
465 <sup>5</sup>Velocity of lengthening ( $\mu\text{m}/\text{sec}$ ),  $p<0.017$ ) (n=25-40 cells/group).

466 Results are expressed as mean  $\pm$  SEM. Experimental groups were compared using the Mann-Whitney  
467 test for independent samples.

468

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473 **Figure 4: RCA ligation induced left ventricular diastolic dysfunction**

474 **Panel A:** Ejection fraction (n=21-26/group) between Sham (black circle) and RCA ligation (red  
475 triangle) mice 4 weeks after RV MI. **Panel B:** LV global longitudinal strain (n=16/group) variation  
476 between Sham (black circle) and RCA ligation (red triangle) mice 4 weeks after RV MI. **Panel C:**  
477 Cardiac output measurement in Sham (black circle) and RCA ligation (red triangle) mice 4 weeks after  
478 RV MI (n=16/group). **Panel D:** left ventricle internal dimension during systole (LVIDs) and during  
479 diastole (LVIDd) measurement in Sham (black circle) and RCA ligation (red triangle) mice 4 weeks  
480 after RV MI (n=21-26/group). **Panel E:** left and right atrium area measurement during diastole in  
481 Sham (black circle) and RCA ligation (red triangle) mice 4 weeks after RV MI (n=9-17/group). **Panel**  
482 **F:** Isovolumic relaxation time (IVRT) measurement in Sham (black circle) and RCA ligation (red  
483 triangle) mice 4 weeks after RV MI (n=21-25/group). **Panel G:** E/A ratio measurement in Sham  
484 (black circle) and RCA ligation (red triangle) mice 4 weeks after RV MI (n=21-25/group). **Panel H:**  
485 E/E' ratio measurement in Sham (black circle) and RCA ligation (red triangle) mice 4 weeks after RV  
486 MI (n=16/group). **Panel I:** E/SRE ratio measurement in Sham (black circle) and RCA ligation (red  
487 triangle) mice 4 weeks after RV MI (n=16-26/group). **Panel J:** Diastolic sarcomere length (SL) of  
488 cardiomyocytes isolated from LV (n=22-28 cells/group). **Panel K:** Maximal amplitude of SL  
489 shortening during contraction of cardiomyocytes. **Panel L:** Contraction velocity. **Panel M:** Relaxation  
490 velocity. **Panel N:** Contraction evoked by electrical field stimulation at 0.5 Hz as measured from SL  
491 shortening of single intact LV 28 days after surgery in Sham (black line) and RCA ligation (red line)  
492 (<sup>6</sup>Velocity of shortening ( $\mu\text{m}/\text{sec}$ ),  $p < 0.001$ ) (n=22-28 cells/group).

493 Results are expressed as mean  $\pm$  SEM. Experimental groups were compared using the Mann-Whitney  
494 test for independent samples.

495

496 **SUPPLEMENTAL DATA**

497

498 **Supplemental video 1:** Surgical procedure video of RCA ligation in mice.499 **Supplemental video 2:** Four-chamber view video recorded in B-mode from Sham mice.

500 **Supplemental video 3:** Four-chamber view video recorded in B-mode from RCA ligation mice 4  
501 weeks after RV MI.

502 **Supplemental video 4:** Short axis view video recorded in B-mode from Sham mice.

503 **Supplemental video 5:** Short axis view video recorded in B-mode from RCA ligation mice 4 weeks  
504 after RV MI.

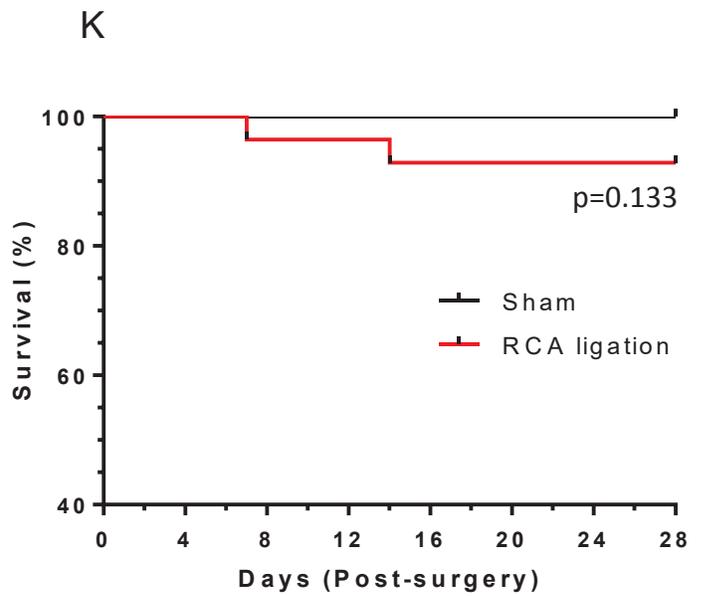
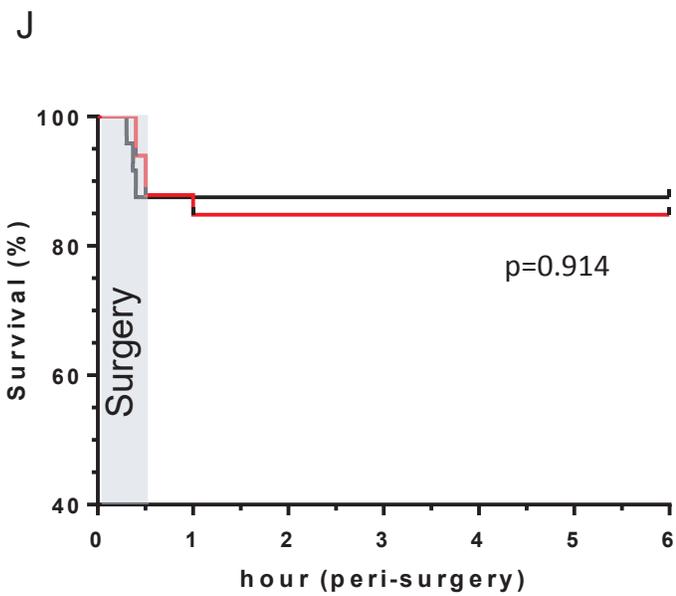
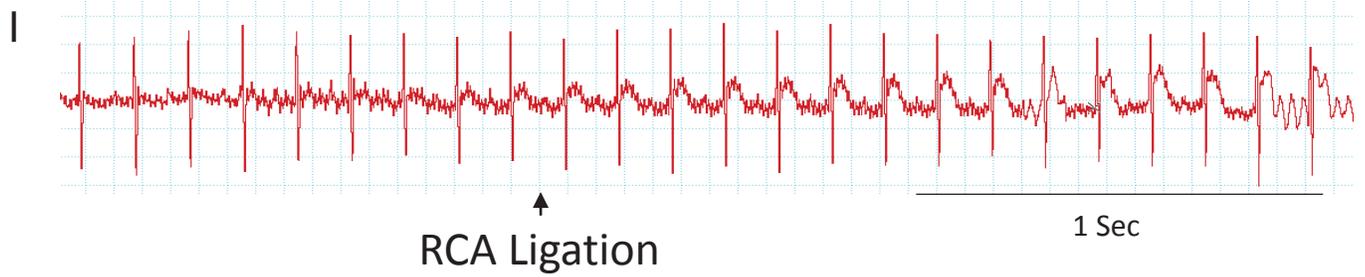
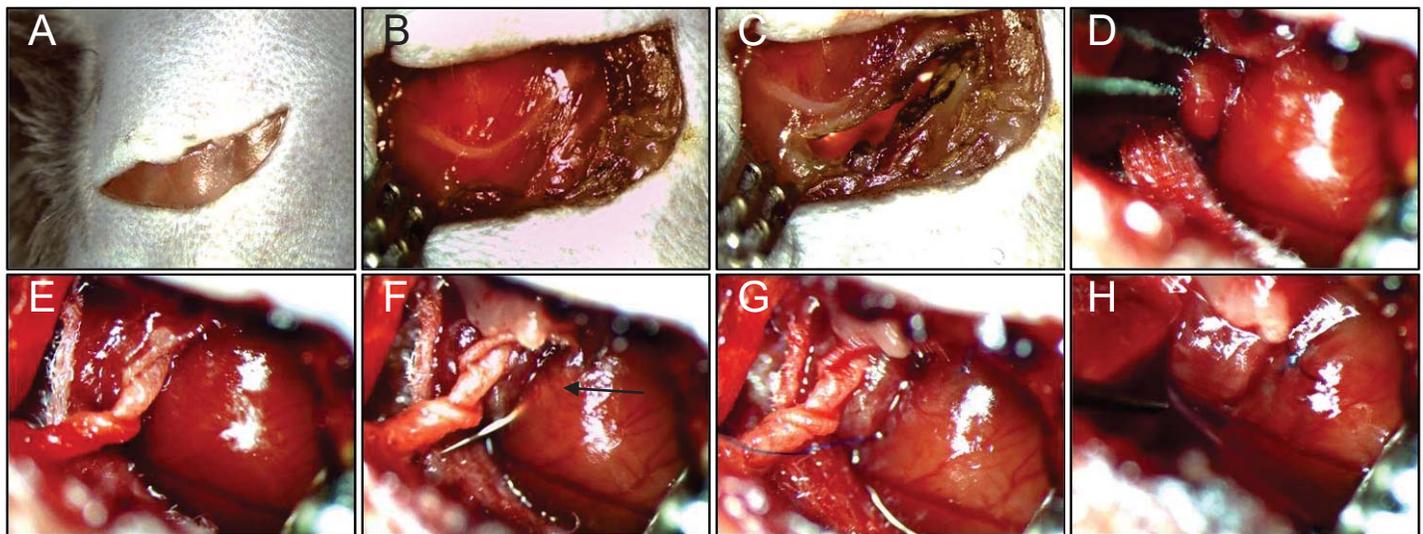


Figure 1

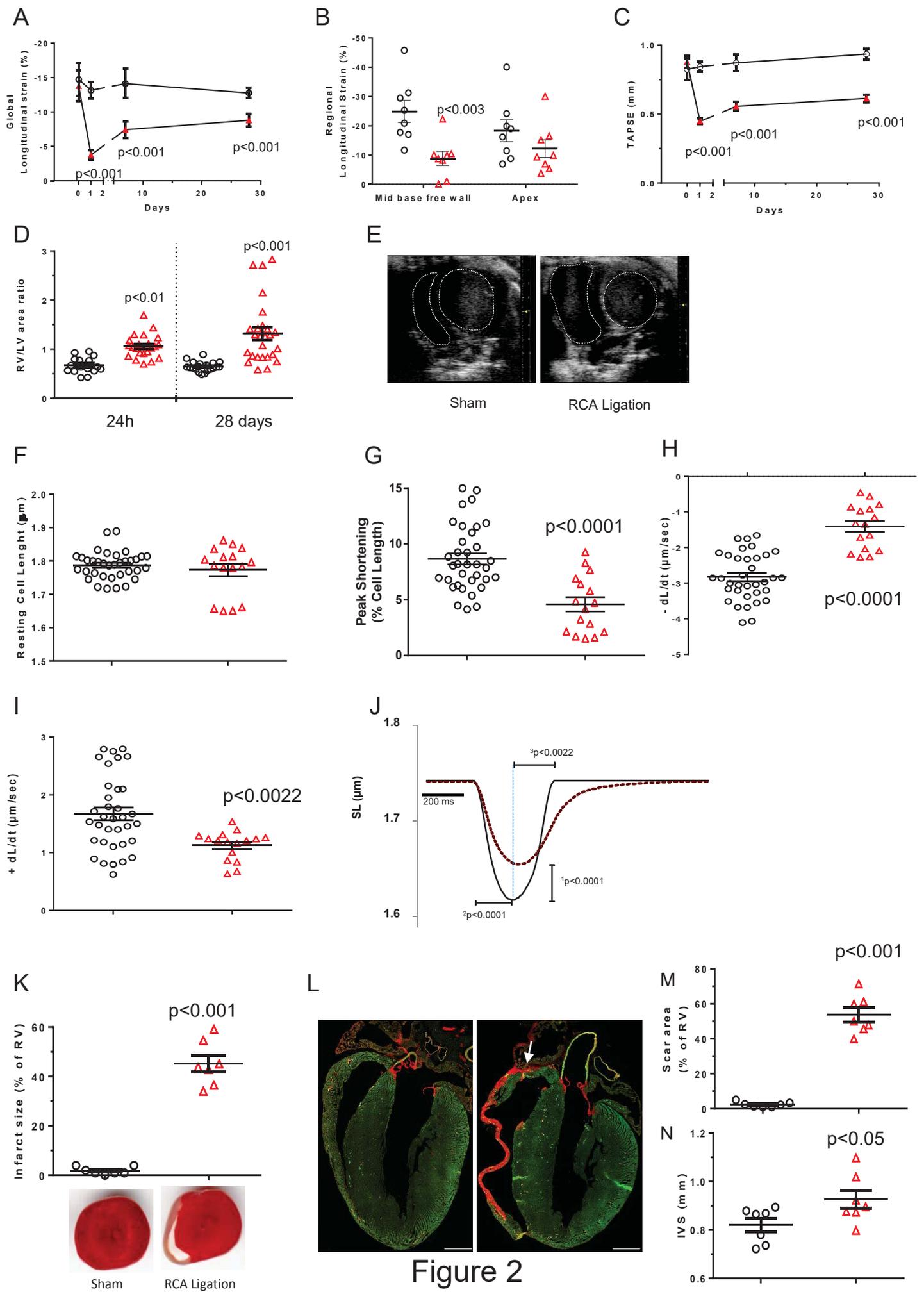


Figure 2

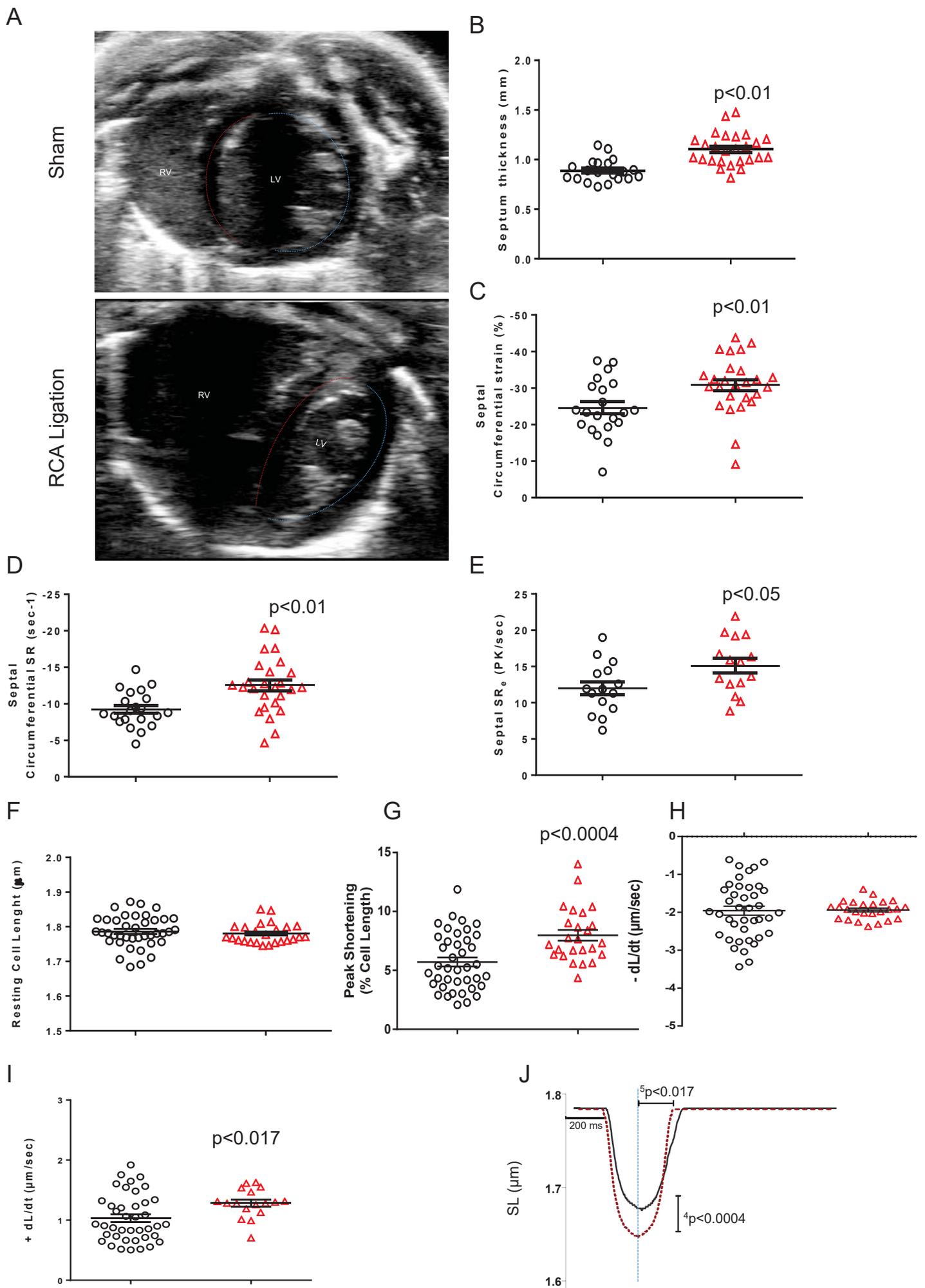


Figure 3

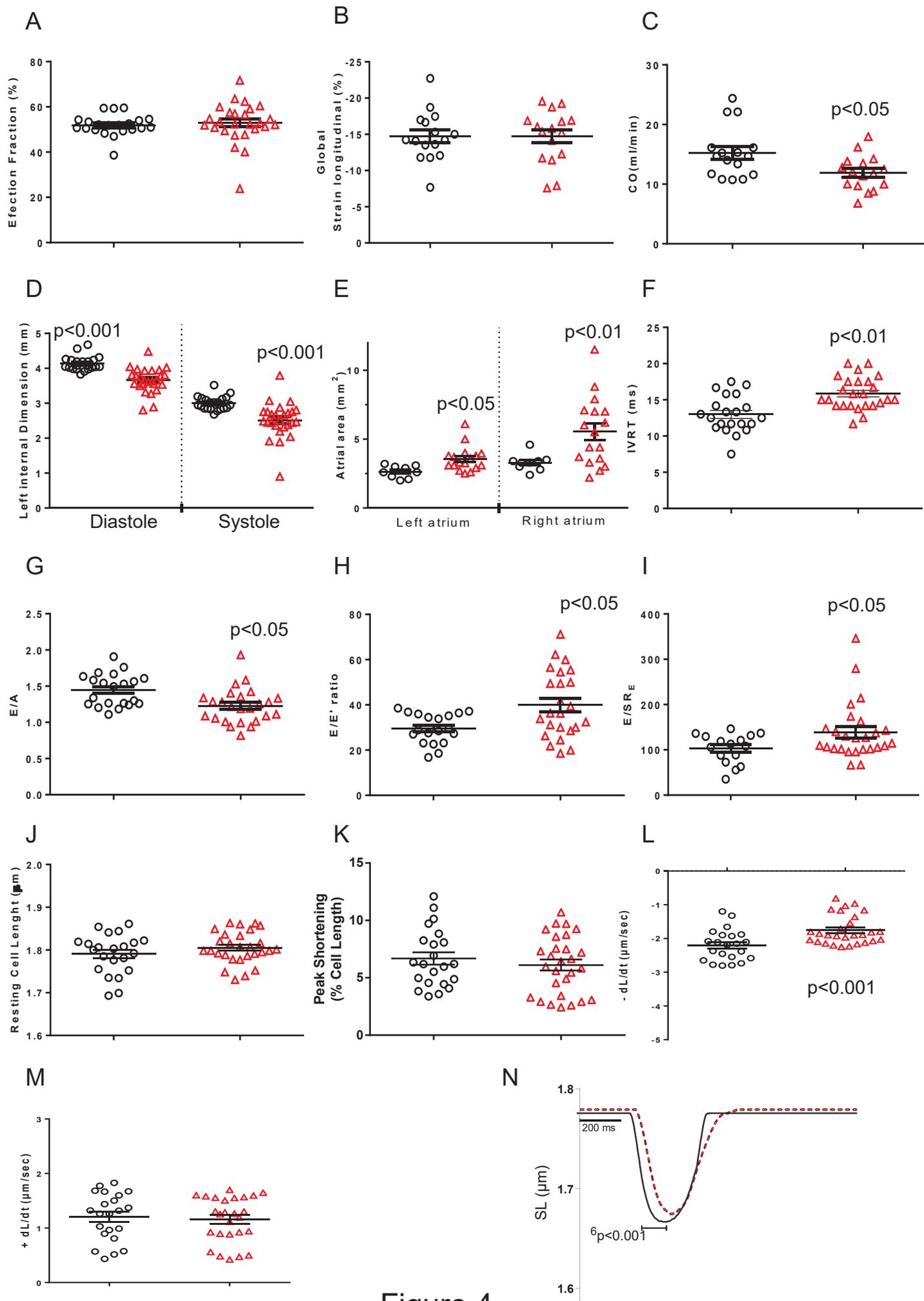


Figure 4