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"**Wong A, Grubb DR, Cooley N, Luo J, Woodcock EA. Regulation of autophagy in cardiomyocytes by Ins(1,4,5)P(3) and IP(3)-receptors. J Mol Cell Cardiol. 2013; 54: 19-24.**"

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Regulation of autophagy in cardiomyocytes by Ins(1,4,5)P_3 and IP_3-receptors

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Summary

Autophagy is a process that removes damaged proteins and organelles and is of particular importance in terminally differentiated cells such as cardiomyocytes, where it has primarily a protective role. We investigated the involvement of inositol(1,4,5)*tris*phosphate ($Ins(1,4,5)P_3$) and its receptors in autophagic responses in neonatal rat ventricular myocytes (NRVM). Treatment with the IP_3-receptor (IP_3-R) antagonist 2-aminoethoxydiphenyl borate (2-APB) at 5 or 20 μmol/L resulted in an increase in autophagosome content, defined as puncta labeled by antibody to microtubule associated light chain 3 (LC3). 2-APB also increased autophagic flux, indicated by heightened LC3II accumulation, which was further enhanced by bafilomycin (10 nmol/L). Expression of $Ins(1,4,5)P_3$ 5-phosphatase (IP_3-5-Pase) to deplete $Ins(1,4,5)P_3$ also increased LC3-labeled puncta and LC3II content, suggesting that $Ins(1,4,5)P_3$ inhibits autophagy. The IP_3-R can act as an inhibitory scaffold sequestering the autophagic effector, beclin-1 to its ligand binding domain (LBD). Expression of GFP-IP_3-R-LBD inhibited autophagic signaling and furthermore, beclin-1 co-immunoprecipitated with the IP_3-R-LBD. A mutant GFP-IP_3-R-LBD with reduced ability to bind $Ins(1,4,5)P_3$ bound beclin-1 and inhibited autophagy similarly to the wild type sequence. These data provide evidence that $Ins(1,4,5)P_3$ and IP_3-R act as inhibitors of autophagic responses in cardiomyocytes. By suppressing autophagy, IP_3-R may contribute to cardiac pathology.

Key words: cell survival, beclin-1, IP_3-5-phosphatase, autophagosome, IP_3-R, $Ins(1,4,5)P_3$, FoxO1.

Introduction

Chronic diseases of the myocardium, including heart failure and valvular heart disease commonly display an increased expression of IP_3-receptors (IP_3-R) [1-4]. No functional significance of this increase has been established, although arrhythmia [5] and hypertrophic growth [6] have been suggested as possible consequences. Another possibility is that IP_3-R influence autophagy in the diseased myocardium and thereby contribute to disease progression.

Cardiomyocytes *in vivo* and in cell culture undergo autophagy, in addition to the well characterized hypertrophic and apoptotic responses. Given that cardiomyocytes are replaced with only minimal efficiency at best, apoptosis is considered to be always detrimental to the myocardium. In contrast, the role of autophagy is less clear, and it is likely that autophagy plays a complex role whereby moderate levels of autophagy protect the myocytes by removal of damaged proteins and organelles, but excessive levels contribute to cardiomyocyte death [7, 8]. With this in mind, it is important to define the pathways that culminate in autophagy from different initiating stimuli and to understand the mechanisms by which autophagy can be inhibited.

Autophagy in cardiomyocytes is heightened by glucose depletion as well as by ischemia and post-ischemic reperfusion and there is evidence that different pathways are involved under these different conditions. The pathway best characterized in cardiomyocytes involves inhibition of the mTOR, itself an inhibitor of autophagy, by AMP-activated protein kinase (AMPK) [9, 10]. AMPK is activated by nutrient, especially glucose, depletion and by ischemia and both of these initiate autophagic responses [10, 9]. Under conditions of post-ischemic reperfusion, however, autophagy is

activated by mechanism independent of AMPK and mTOR, instead involving increased availability of beclin-1 [10]. Beclin-1 forms complexes with class-III PI3kinase (PI3K), Bcl-2 and nutrient activated factor-1 (NAF-1), and in some tissues, these are scaffolded onto IP_3-R [11]. Beclin-1 bound onto this complex is inactive in promoting autophagy, but, once released, beclin-1 initiates autophagy by facilitating the formation of autophagic vesicles [12]. Disruption of the beclin-1-containing complex by IP_3-R antagonists promotes the release of beclin-1 and initiates autophagy [13]. In addition to the role of inhibitory scaffold, IP_3-R, in some cell types, act as inhibitors of autophagy by facilitating mitochondrial Ca^{2+} uptake to sustain energy metabolism [14].

Whilst a role for IP_3-R in autophagy has been established in neuronal cells [15, 16], there is currently no information concerning a possible involvement in autophagy in heart. This is important because there is increasing evidence that autophagy is activated in failing myocardium, a condition associated with heightened IP_3-R expression [1, 17] As autophagy is protective under these conditions [18], there is a possibility that the increased IP_3-R expression in heart failure might contribute to disease progression by limiting autophagy.

Methods

Preparation of neonatal rat ventricular myocytes (NRVM). Studies were approved by the Alfred Medical Research and Education Precinct Animal Ethics Committee. Ventricular myocytes were prepared from 1-2 day old Sprague-Dawley rats using repeated pancreatin/collagenase digestion, followed by separation from non-myocytes using discontinuous Percoll gradients as described previously [19]. Treatments were initiated 1 day after isolation.

Constructs and adenoviruses. Adenoviruses expressing FLAG-tagged IP_3-5-phosphatase (INPP5A, 43 kDa type 1) have been described previously [20]. The GFP-tagged IP_3-R(1) ligand binding domain (human IP_3-R(1) 224-605; GFP-IP_3-R-LBD) [21] was provided by Dr Tamas Balla (National Institutes of Child Health, Bethedsa, MD). Mutations in GFP-IP_3-R-LBD (K579Q and R582Q, GFP-IP_3-R-LBD-QQ) were generated using GeneTailor^{TM} (Invitrogen, #12397-014) [22]. Adenoviruses were prepared using the Gateway® procedure (Invitrogen), propagated on HEK-293 cells and purified using CsCl gradients. Viruses were used at a multiplicity of infection of 30-50 per cell.

Confocal microscopy. NRVM were plated onto gelatinized glass bottom confocal dishes (MatTek, USA) and infected with adenovirus or treated, as indicated. After 24 h, NRVM were fixed with PFA (4% w/v paraformaldehyde, 0.1 M Pipes pH 6.8) before permeabilization with Saponin buffer (0.05% w/v, 0.1 M Pipes pH 6.8). The plates were washed in PBS, and incubated with anti-LC3 antibodies (abcam #ab58610) diluted in PBA (PBS + BSA 1% w/v). Plates were then further washed in PBS, and incubated with anti-rabbit-AlexaFluor-633 (Invitrogen). The samples were then further washed in PBS,

and mounted using Vectashield mounting media (Abacus ALS, Australia). Images were captured using a Zeiss Meta-510 LSM (excitation 633nm, emission at 647nm). LC3-labled puncta were counted in individual cells (4-5/field, 20 fields per experiment, 3 experiments total) and values averaged. Each value represents a total of 200 cells counted.

Western blotting.

For evaluation of LC3-II accumulation, NRVM, treated as described, were incubated in the presence or absence of bafilomycin (10 nmol/L) for 4 h to inhibit autophagy downstream of the autophagosome [23]. Proteins were separated by SDS-PAGE using 16% gels and were transferred to PVDF membranes (Immobilon^{Psq}). Antibodies were used at the following dilutions; LC3 (Cell Signaling # 2775, 1/500), tubulin (abcam, #ab6046, 1/5000). HRP-conjugated secondary antibodies and ECL plus (Amersham Life Sciences) were used to detect proteins of interest. Images were captured and data analysed using a BioRad ChemiDoc^{TM} XRS+ imaging system. Expression of p62 was measured using 10% gels, and anti-p62 antibody (1/1000, Sigma #P0067). Data were normalized relative to tubulin.

Co-immunoprecipitation of beclin-1 and GFP-IP$_3$-R-LBD or GFP-IP$_3$-R-LBD-QQ

NRVM were washed and lysed in RIPA buffer (50 mmol/L pH 7.8, 150 mmol/L NaCl, 0.1% SDS, 0.5% deoxycholate, 1% NP40 and protease inhibitors (Roche)) and centrifuged. Supernatants were pre-cleared with Protein A Sepharose (IP beclin-1) or Protein G Sepharose (IP GFP). Primary antibody, anti-GFP (Cell Signaling #2555 1/100) or anti-beclin-1 (Cell Signaling #3738 1/100) was added for 2 h at 4^{o}C and then Protein A- or Protein G-Sepharose was added and incubated overnight. Pellets were washed 3

times in RIPA buffer containing protease inhibitors and subjected to SDS-PAGE on 9% gels, western blotting and developed with anti-GFP (1/1000) or anti-beclin-1 (1/500) antibodies.

Results

The IP_3-R blocker, 2-aminoethyoxyphenyl borate (2-APB), increases LC3-stained puncta, LC3-II generation and p62 content.

Macroautophagy involves the encapsulation of damaged proteins and organelles within a membrane structure, an autophagosome, enriched in microtubule-associated light chain 3 (LC3) subsequent to addition of phosphatidylethanolamine to generate LC3II. Thus, autophagy is commonly measured by the accumulation of autophagosomes identified as LC3-stained puncta or by the generation of LC3II from LC3I. Using these criteria, we examined the effect of the IP_3-R antagonist, 2-aminoethoxyphenyl borate (2-APB), on autophagy in NRVM. FoxO1 is an established autophagic effector in NRVM [24] and we treated cells with adenovirus expressing constitutively active FoxO1 (Ad-CA-FoxO1) as a positive control. NRVM were treated with 2-APB (2-APB, 5 or 20 μmol/L, [25]) for 24 h. Cells were fixed and stained using anti-LC3 antibodies to examine the formation of autophagosomes (punctate LC3 staining). As shown in Fig. 1A, addition of 2-APB (5 or 20 μM) caused the appearance of LC3-labeled vesicles. Autophagosomes were quantified as the average number of clearly defined puncta per cell (Fig. 1B). Data from CA-FoxO1-treated cells are included for comparison because FoxO1 is a well established autophagic factor in cardiomyocytes [24].

We also measured the effect of 2-APB (5 and 20 μM) on the generation of LC3II from LC3I. These experiments were performed in the presence and absence of bafilomycin (10 nmol/L), an inhibitor of autolysosome formation, to ensure that increased LC3II reflected increased generation rather than inhibition of autophagic flux downstream [23]. 2-APB at 5 and 20 μM increased LC3II as shown in Fig. 1C & 1D.

Bafilomycin further increased the response, validating that 2-APB increased flux through the autophagic pathway.

In addition to LC3-II, p62 (sequestosome 1/SQSTM1) associates with autophagosomes and is subsequently degraded in the autolysosome [26]. Content of p62 often decreases during the autophagic process, except in cardiomyocytes where increased p62 content has been reported in response to several different autophagic stimuli [27-29]. In agreement with these studies, p62 expression increased after 24 h treatment with 2-APB (Fig. 2A&B). As a positive control, we examined p62 expression after treatment with Ad-CA-FoxO1 for 24 h, to induce autophagy [9]. As found after 2-APB treatment, expression of CA-FoxO1 caused an increase in p62 expression (Fig. 2C&D).

Depletion of Ins(1,4,5)P_3 increases LC3-stained puncta, LC3-II generation and p62 content.

The finding that the IP_3-R antagonist, 2-APB, induced autophagy in NRVM prompted us to question a possible involvement of the natural IP_3-R ligand, Ins(1,4,5)P_3. To directly assess the contribution of Ins(1,4,5)P_3, NRVM were treated with adenovirus expressing FLAG-IP_3-5-phosphatase (type 1, 43 kDa), a phosphatase that specifically dephosphorylates Ins(1,4,5)P_3 to Ins(1,4)P_2 [30] [20], a metabolite that does not bind IP_3-R [31]. As shown in Fig. 3 (A&B), IP_3-5-phosphatase expression increased autophagosome formation in NRVM, as indicated by an increase in the percentage of cells containing LC3 puncta.

IP_3-5-phosphatase expression increased the LC3II content ratio (Fig 3, C&D) and the content of p62 (Fig. 3E&F). Thus, depletion of Ins(1,4,5)P_3 is sufficient to cause autophagy, implying that Ins(1,4,5)P_3, itself, inhibits autophagy.

The IP_3-R ligand binding domain inhibits autophagy

IP_3-R have been shown to function as inhibitors of autophagy by scaffolding the pro-autophagic protein beclin-1 and its associated proteins, at the Ins(1,4,5)P_3 ligand binding domain (IP_3-R-LBD) [13, 32]. If this is the case in NRVM, then overexpressing IP_3-R-LBD would be expected to inhibit autophagy. NRVM were infected with Ad-GFP-IP_3-R-LBD for 24 h and this resulted in a reduction in LC3II, in the absence and in the presence of balifomycin (Fig. 4A&C). Thus, expressing the GFP-IP_3-R-LBD reduced autophagic signaling, supporting the hypothesis that IP_3-R act as autophagy-inhibitory scaffolds in cardiomyocytes. To examine the possibility that GFP-IP_3-R-LBD inhibited autophagy because of binding Ins(1,4,5)P_3 rather than beclin-1, similar experiments were performed using a mutant LBD that has reduced ability to bind Ins(1,4,5)P_3 ((K579Q and R582Q, LBD-QQ) [22]. Expressing GFP-IP_3-R-LBD-QQ at similar levels to GFP-IP_3-R-LBD (Fig. 4B) resulted in similar inhibition of LC3II accumulation, implying that binding of Ins(1,4,5)P_3 is not important for the anti-autophagic response to expression of GFP-IP_3-R-LBD (Fig. 4A&C).

The IP_3-R ligand binding domain binds beclin-1 in NRVM

The finding that expressing the IP_3-R-LBD effectively inhibited autophagic flux in NRVM is most easily explained by its acting as a scaffold binding beclin-1. To examine this directly, GFP-IP_3-R-LBD was expressed in NRVM, extracts immunoprecipitated with anti-beclin-1 or anti-GFP antibody. Beclin-1 immunoprecipitates were enriched in a GFP-fusion protein with a MW of 71 consistent with GFP-IP_3-R-LBD [21]. Lysate from GFP-IP_3-LBD-expressing cells is shown for reference. Similarly, GFP immunoprecipitates were enriched in a protein labeled by

beclin-1 antibody with a MW of 64 kDa, consistent with beclin-1 (Fig. 4D). Thus the exogenously expressed IP_3-R-LBD binds endogenous beclin-1. Similar experiments were conducted using NRVM expressing GFP-IP_3-R-LBD-QQ. As described for the wild type sequence, beclin-1 immunoprecipitates were enriched in GFP-IP_3-R-LBD-QQ (MW 71kDa) and GFP immunoprecipitates were enriched in beclin-1 (Fig. 4E). This demonstrates that the mutant LBD that does not bind Ins(1,4,5)P_3 retains the ability to associate with beclin-1. Taken together, these data support the hypothesis that expressing IP_3-R-LBD inhibits autophagic flux in NRVM by sequestration of beclin-1.

Discussion

Apoptosis, necrosis, and autophagy occur in cardiac myocytes during myocardial infarction, ischemia/reperfusion, and heart failure [33]. Apoptosis and necrosis are always considered to be detrimental to the heart, because of the limited potential of the myocardium for regeneration. In contrast, autophagy removes damaged proteins and organelles and can help the myocardium survive periods of energy deprivation, such as occur during an ischemic episode [7]. Restricting autophagy can worsen heart failure by facilitating the accumulation of denatured proteins and damaged mitochondria that contribute to further limitation of contractile efficiency [33]. For this reason, low levels of autophagy are considered beneficial to the myocardium [8], and inhibition of autophagy is damaging. Excessive levels of autophagy, however, could contribute to cardiomyocyte damage and subsequent death [34]. It is also possible that some types of autophagy are more pathological than others. For instance, autophagy initiated under ischemic conditions and mediated by AMPK and mTOR is considered beneficial, whereas that during reperfusion, involves beclin-1 and its associated proteins and has been suggested to contribute to tissue injury [10], although this interpretation has been challenged [7].

In the current paper, we have shown that Ins(1,4,5)P_3 and its receptors are involved in regulating autophagy in cardiomyocytes. Depletion of Ins(1,4,5)P_3 by overexpressing a selective phosphatase promoted autophagy, as did treatment with an IP_3-R antagonist (2-APB). IP_3-R have been demonstrated to act as inhibitory scaffolds for the autophagic effector beclin-1 and its associated proteins, Bcl-2 and NAF-1 [11]. The association with beclin-1 opposes autophagy by sequestering beclin-1 and reducing

downstream autophagosome formation. IP_3-R antagonists have been shown to induce autophagy in central neurons by promoting the dissociation of beclin-1 from the IP_3-R [13, 35]. The IP_3-R-LBD, by promoting beclin-1 sequestration, would be expected to inhibit autophagic signaling. Our data showed that a mutant LBD that binds beclin-1, but not Ins(1,4,5)P_3 [22], also inhibited autophagy, supporting the hypothesis that IP_3-R limit autophagy by sequestering beclin-1.

Involvement of Ins(1,4,5)P_3 in autophagy has largely been implied from the autophagic activity of Li^+ and IP_3-R antagonists. However, heightened expression of IP_3-3-kinase (to increase Ins(1,4,5)P_3 metabolism) in cultured cell models was shown to facilitate removal of protein aggregates by an autophagic process, providing more direct evidence that Ins(1,4,5)P_3 can be inhibitory to autophagic pathways [15]. IP_3-3-kinase generates Ins(1,3,4,5)P_4, the precursor of the higher inositol phosphates (IP_4 to IP_7) [36, 37], some of which, particularly IP_6 and IP_7, have been implicated in autophagy [38, 39]. This complicates interpretation of data from studies where Ins(1,4,5)P_3 is removed by phosphorylation to Ins(1,3,4,5)P_4. In our studies, Ins(1,4,5)P_3 was metabolised by dephosphorylation using the 43 kDa (type 1) IP_3-5-phosphatase and this enzyme yields only inactive metabolites.

There are different views on the relevance of Ins(1,4,5)P_3 and IP_3-R in hearts and cardiomyocytes [40, 41]. Even though cardiomyocytes express receptors, G proteins and PLC subtypes, generation of Ins(1,4,5)P_3 is slow and weak compared with responses in other cell types. Furthermore expression of IP_3-R is low and any Ins(1,4,5)P_3-induced Ca^{2+} responses are small and localized [42]. Ins(1,4,5)P_3 generation increases acutely under conditions of post-ischemic reperfusion [43] and chronically in dilated fibrosed

atrial tissue from human and mouse [44]. Autophagy is increased in both of these circumstances, primarily as a protective mechanism [45, 46, 47]. It is possible that Ins(1,4,5)P_3 functions as an endogenous autophagy inhibitor under these pathological conditions. If this is so, the heightened Ins(1,4,5)P_3 could contribute to a worsening of disease progression and PLC inhibitors might prove beneficial therapeutically.

Acknowledgments

This work was supported by Grants-in Aid from the National Health and Medical Research Council (NHMRC) of Australia, #526621, #526623, as well as the Australian National Heart Foundation #GM08-3695. EAW is a Principal Research Fellow of the NHMRC #586621. Supported, in part, by the Victorian Government's Operational Infrastructure Support Program. We thank Monash medical Imaging for the provision of confocal microscopy facilities.

Disclosures

None of the authors has any conflict of interest to disclose.

Legends to Figures

Figure 1. ***IP_3-receptor blockade with 2- aminoethoxyphenyl borate (2-APB) increases LC3-stained puncta and LC3-II in NRVM.*** NRVM were treated with 2-APB, 5 or 20 μmol/L, for 24 h. **A.** Cells were stained with LC3 antibody followed by secondary antibody (green) and LC3 staining visualized using confocal microscopy. FoxO1 is a positive control. **B.** Average number of LC3-labeled puncta per cell. Values shown are mean ± SEM (n=3), * $p<0.05$ relative to control (Kruskal-Wallis One Way ANOVA on ranks). **C.** Western blot showing LC3-I and LC3-II in samples with and without pre-treatment with bafilomycin (10 nmol/L), +B or –B, respectively. Tubulin serves as loading control. **D**. Averaged values for LC3-II content (no bafilomycin) relative to tubulin from 4 experiments. Values shown are relative LC3II expression, relative to control, mean ± SEM, * $p<0.05$ relative to control.

Figure 2. ***p62 expression is heightened by 2-APB and FoxO1.*** **A**. Western analysis showing the effect of 2-APB (5 or 20 μmol/L) for 24 h on p62 expression in NRVM. **B.** Average values for p62 expression relative to tubulin after 2-APB treatment, mean +/- SEM, (n=4), * $p<0.05$ relative to control. **C**. Western analysis showing the effect of expressing FoxO1 on p62 expression in NRVM. **D**. Average values for p62 expression relative to tubulin after FoxO1 treatment, mean +/- SEM, n=4. * $p<0.05$ relative to control virus.

Figure 3. ***IP_3-5-phosphatase increases autophagy of NRVM.*** NRVM were treated with adenovirus expressing IP_3-5-phosphatase (IP_3-Pase) for 24 h. **A**. Cells were stained with LC3 antibody followed by secondary antibody (green) and LC3 staining was visualised by confocal microscopy. **B.** Average number of LC3-labeled puncta per cell. Values shown are mean ± SEM. * P<0.05 relative to control (Kruskal-Wallis One Way ANOVA on ranks). **C.** Western analysis showing LC3II and LC3I expression and the effect of bafilomycin (10 nmol/L) '*B*'. Tubulin is the loading control. **D**. LC3II/tubulin ratios (no bafilomycin) averaged from 3 experiments. Values are LC3II/tubulin, mean ± SEM. * p <0.05 relative to control. **E**. Western analysis showing p62 expression in control and IP_3-5-phosphatase overexpressing cells. **F**. p62/tubulin ratios averaged from 3 experiments. Values are fold increase over control, mean ± SEM. * p <0.05 relative to control.

Figure 4. ***IP_3-R ligand binding domain inhibits autophagy in NRVM***. NRVM were treated with adenovirus expressing GFP-tagged IP_3-R-LBD (LBD) or GFP-IP_3-R-LBD-QQ (QQ) for 24h. **A.** Western analysis showing LC3II expression and the effect of bafilomycin (10 nmol/L) *'B'*. **B**. Expression of GFP-IP_3-R-LBD and GFP-IP_3-R-LBD-QQ in NRVM. **C**. LC3II/tubulin ratios averaged over 3 experiments. Values are mean ± SEM (n=3), * p<0.05 relative to + bafilomycin control, † p<0.05 relative to no bafilomycin control. **D**. Beclin-1 associates with GFP-IP_3-R-LBD. NRVM were treated with Ad-GFP-IP_3-R-LBD for 24 h, extracts were immunoprecipitated with anti-GFP or anti-beclin-1 antibodies, as indicted. Western blots were developed with antibodies to beclin-1 or GFP, as shown on the figure. Extracts from cells expressing GFP-IP_3-LBD

are shown for reference (LBD). **E.** Beclin-1 associates with GFP-IP_3-R-LBD-QQ. NRVM were treated with Ad-GFP-IP_3-R-LBD-QQ for 24 h, extracts were immunoprecipitated with anti-GFP or anti-beclin-1 antibodies, as indicted. Westerns blots were developed with antibodies to beclin-1 or GFP, as shown on the figure. Extracts from cells expressing GFP-IP_3-LBD-QQ are shown for reference (QQ). NV no Ad-GFP-LBD, -Ab no antibody control, In is input. The experiment was performed 3 times with similar results. **D&E** arrows indicate Beclin-1, GFP-LBD or GFP-LBD-QQ.

References

[1] Marks AR. Cardiac intracellular calcium release channels: role in heart failure. Circ Res. 2000;87:8-11.

[2] Yamda J, Ohkusa T, Nao T, Ueyama T, Yano M, Kobayashi S, et al. Up-regulation of inositol 1,4,5 *tris*phosphate receptor expression in atrial tissue in patients with chronic atrial fibrillation. J Am Coll Cardiol. 2001;37:1111-9.

[3] Liang X, Xie H, Zhu PH, Hu J, Zhao Q, Wang CS, et al. Enhanced activity of inositol-1,4,5-*tris*phosphate receptors in atrial myocytes of atrial fibrillation patients. Cardiology. 2009;114:180-91.

[4] Anger M, Lompre AM, Vallot O, Marotte F, Rappaport L, Samuel JL. Cellular distribution of Ca^{2+} pumps and Ca^{2+} release channels in rat cardiac hypertrophy induced by aortic stenosis. Circulation. 1998;98:2477-86.

[5] Li X, Zima AV, Sheikh F, Blatter LA, Chen J. Endothelin-1-induced arrhythmogenic Ca^{2+} signaling is abolished in atrial myocytes of inositol-1,4,5-*tris*phosphate (IP_3)-receptor type 2-deficient mice. Circ Res. 2005;96:1274-81.

[6] Nakayama H, Bodi I, Maillet M, DeSantiago J, Domeier TL, Mikoshiba K, et al. The IP_3 receptor regulates cardiac hypertrophy in response to select stimuli. Circ Res. 2010;107;656-666 .

[7] Gottlieb RA, Mentzer RM. Autophagy during cardiac stress: joys and frustrations of autophagy. Annu Rev Physiol. 2010;72:45-59.

[8] Gottlieb RA, Finley KD, Mentzer RM, Jr. Cardioprotection requires taking out the trash. Basic Res Cardiol. 2009;104:169-80.

[9] Sengupta A, Molkentin JD, Yutzey KE. FoxO transcription factors promote autophagy in cardiomyocytes. J Biol Chem. 2009;284:28319-31.

[10] Matsui Y, Takagi H, Qu XP, Abdellatif M, Sakoda H, Asano T, et al. Distinct roles of autophagy in the heart during ischemia and reperfusion - Roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy. Circ Res. 2007;100:914-22.

[11] Chang NC, Nguyen M, Germain M, Shore GC. Antagonism of Beclin 1-dependent autophagy by BCL-2 at the endoplasmic reticulum requires NAF-1. Embo J. 2010;29:606-18.

[12] Luo S, Rubinsztein DC. Apoptosis blocks Beclin 1-dependent autophagosome synthesis: an effect rescued by Bcl-xL. Cell Death Differ. 2010;17:268-77.

[13] Vicencio JM, Ortiz C, Criollo A, Jones AW, Kepp O, Galluzzi L, et al. The inositol 1,4,5-*tris*phosphate receptor regulates autophagy through its interaction with Beclin 1. Cell Death Differ. 2009;16:1006-17.

[14] Cardenas C, Miller RA, Smith I, Bui T, Molgo J, Muller M, et al. Essential regulation of cell bioenergetics by constitutive $InsP_3$ receptor Ca^{2+} transfer to mitochondria. Cell. 2010;142:270-83.

[15] Williams A, Sarkar S, Cuddon P, Ttofi EK, Saiki S, Siddiqi FH, et al. Novel targets for Huntington's disease in an mTOR-independent autophagy pathway. Nat Chem Biol. 2008;4:295-305.

[16] Sarkar S, Rubinsztein DC. Inositol and IP_3 levels regulate autophagy: biology and therapeutic speculations. Autophagy. 2006;2:132-4.

[17] Shannon TR, Pogwizd SM, Bers DM. Elevated sarcoplasmic reticulum Ca^{2+} leak in intact ventricular myocytes from rabbits in heart failure. Circ Res. 2003;93:592-4.

[18] Nemchenko A, Chiong M, Turer A, Lavandero S, Hill JA. Autophagy as a therapeutic target in cardiovascular disease. J Mol Cell Cardiol. 2011;51:584-93.

[19] Sah VP, Hoshijima M, Chien KR, Brown JH. Rho is required for Gαq and α_1-adrenergic receptor signaling in cardiomyocytes - Dissociation of Ras and Rho pathways. J Biol Chem. 1996;271:31185-90.

[20] Vasilevski O, Grubb DR, Filtz TM, Yang S, McLeod-Dryden TJ, Luo J, et al. Ins(1,4,5)P_3 regulates phospholipase Cβ1 expression in cardiomyocytes. J Mol Cell Cardiol. 2008;45:679-84.

[21] Varnai P, Balla A, Hunyady L, Balla T. Targeted expression of the inositol 1,4,5-triphosphate receptor (IP_3R) ligand-binding domain releases Ca^{2+} via endogenous IP_3R channels. Proc Natl Acad Sci U S A. 2005;102:7859-64.

[22] Walker SA, Kupzig S, Lockyer PJ, Bilu S, Zharhary D, Cullen PJ. Analyzing the role of the putative inositol 1,3,4,5-*tetrakis*phosphate receptor GAP1(IP4BP) in intracellular Ca^{2+} homeostasis. J Biol Chem. 2002;277:48779-85.

[23] Klionsky DJ, Abeliovich H, Agostinis P, Agrawal DK, Aliev G, Askew DS, et al. Guidelines for the use and interpretation of assays for monitoring autophagy in higher eukaryotes. Autophagy. 2008;4:151-75.

[24] Sengupta A, Molkentin JD, Paik JH, DePinho RA, Yutzey KE. FoxO transcription factors promote cardiomyocyte survival upon induction of oxidative stress. J Biol Chem. 2011;286:7468-78.

[25] Bilmen JG, Michelangeli F. Inhibition of the type 1 inositol 1,4,5-*tris*phosphate receptor by 2-aminoethoxydiphenylborate. Cell Signal. 2002;14:955-60.

[26] Komatsu M, Kageyama S, Ichimura Y. p62/SQSTM1/A170: Physiology and pathology. Pharmacol Res. 2012 (Epub Date 2012/07/13).

[27] Su H, Wang X. p62 Stages an interplay between the ubiquitin-proteasome system and autophagy in the heart of defense against proteotoxic stress. Trends Cardiovasc Med. 2011;21:224-8.

[28] Zheng Q, Su H, Ranek MJ, Wang X. Autophagy and p62 in cardiac proteinopathy. Circ Res. 2011;109:296-308.

[29] Qipshidze N, Tyagi N, Metreveli N, Lominadze D, Tyagi SC. Autophagy mechanism of right ventricular remodeling in murine model of pulmonary artery constriction. Am J Physiol Heart Circ Physiol. 2012;302:H688-96.

[30] Majerus PW, Kisseleva MV, Norris FA. The role of phosphatases in inositol signaling reactions. J Biol Chem. 1999;274:10669-72.

[31] Mourey RJ, Estevez VA, Marecek JF, Barrow RK, Prestwich GD, Snyder SH. Inositol 1,4,5-*tris*phosphate receptors - Labeling the inositol 1,4,5-*tris*phosphate binding site with photoaffinity ligands. Biochemistry. 1993;32:1719-26.

[32] Khan MT, Joseph SK. Role of inositol *tris*phosphate receptors in autophagy in DT40 cells. J Biol Chem. 2010;285:16912-20.

[33] Whelan RS, Kaplinskiy V, Kitsis RN. Cell death in the pathogenesis of heart disease: mechanisms and significance. Annu Rev Physiol. 2010;72:19-44.

[34] Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, et al. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell. 2005;122:927-39.

[35] Maiuri MC, Criollo A, Kroemer G. Crosstalk between apoptosis and autophagy within the Beclin 1 interactome. Embo J. 2010;29:515-6.

[36] Menniti FS, Oliver KG, Putney JW, Shears SB. Inositol phosphates and cell signaling - New views of InsP5 and InsP6. Trends Biochem Sci. 1993;18:53-6.

[37] Stephens LR, Hawkins PT, Stanley AF, Moore T, Poyner DR, Morris PJ, et al. myo-Inositol *pentakis*phosphates. Structure biological occurence and phosphorylation to myo-inositol *hexakis*phosphate. Biochem J. 1991;275:485-99.

[38] Anekonda TS, Wadsworth TL, Sabin R, Frahler K, Harris C, Petriko B, et al. Phytic acid as a potential treatment for alzheimer's pathology: evidence from animal and *in vitro* models. J Alzheimers Dis. 2011;23:21-35.

[39] Nagata E, Saiardi A, Tsukamoto H, Satoh T, Itoh Y, Itoh J, et al. Inositol *hexakis*phosphate kinases promote autophagy. Int J Biochem Cell Biol. 2011;42:2065-71.

[40] Kockskamper J, Zima AV, Roderick HL, Pieske B, Blatter LA, Bootman MD. Emerging roles of inositol 1,4,5-trisphosphate signaling in cardiac myocytes. J Mol Cell Cardiol. 2008;45:128-47.

[41] Woodcock EA, Matkovich SJ. Ins(1,4,5)P_3 receptors and inositol phosphates in the heart - evolutionary artefacts or active signal transducers? Pharmacol Ther. 2005;107:240-51.

[42] Wu X, Zhang T, Bossuyt J, Li XD, McKinsey TA, Dedman JR, et al. Local InsP_3-dependent perinuclear Ca^{2+} signaling in cardiac myocyte excitation-transcription coupling. J Clin Invest. 2006;116:675-82.

[43] Anderson K, Dart A, Woodcock E. Inositol phosphate release and metabolism during myocardial ischemia and reperfusion in rat heart. Circ Res. 1995;76:261-8.

[44] Woodcock EA, Grubb DR, Filtz TM, Marasco S, Luo JT, McLeod-Dryden TJ, et al. Selective activation of the "b" splice variant of phospholipase Cβ1 in chronically dilated human and mouse atria. J Mol Cell Cardiol. 2009;47:676-83.

[45] Sciarretta S, Hariharan N, Monden Y, Zablocki D, Sadoshima J. Is autophagy in response to ischemia and reperfusion protective or detrimental for the heart? Pediatr Cardiol. 2011;32:275-81.

[46] Gustafsson AB, Gottlieb RA. Autophagy in Ischemic Heart Disease. Circ Res. 2009;104:150-8.

[47] Chen MC, Chang JP, Wang YH, Liu WH, Ho WC, Chang HW. Autophagy as a mechanism for myolysis of cardiomyocytes in mitral regurgitation. Eur J Clin Invest. 2011;41:299-307.

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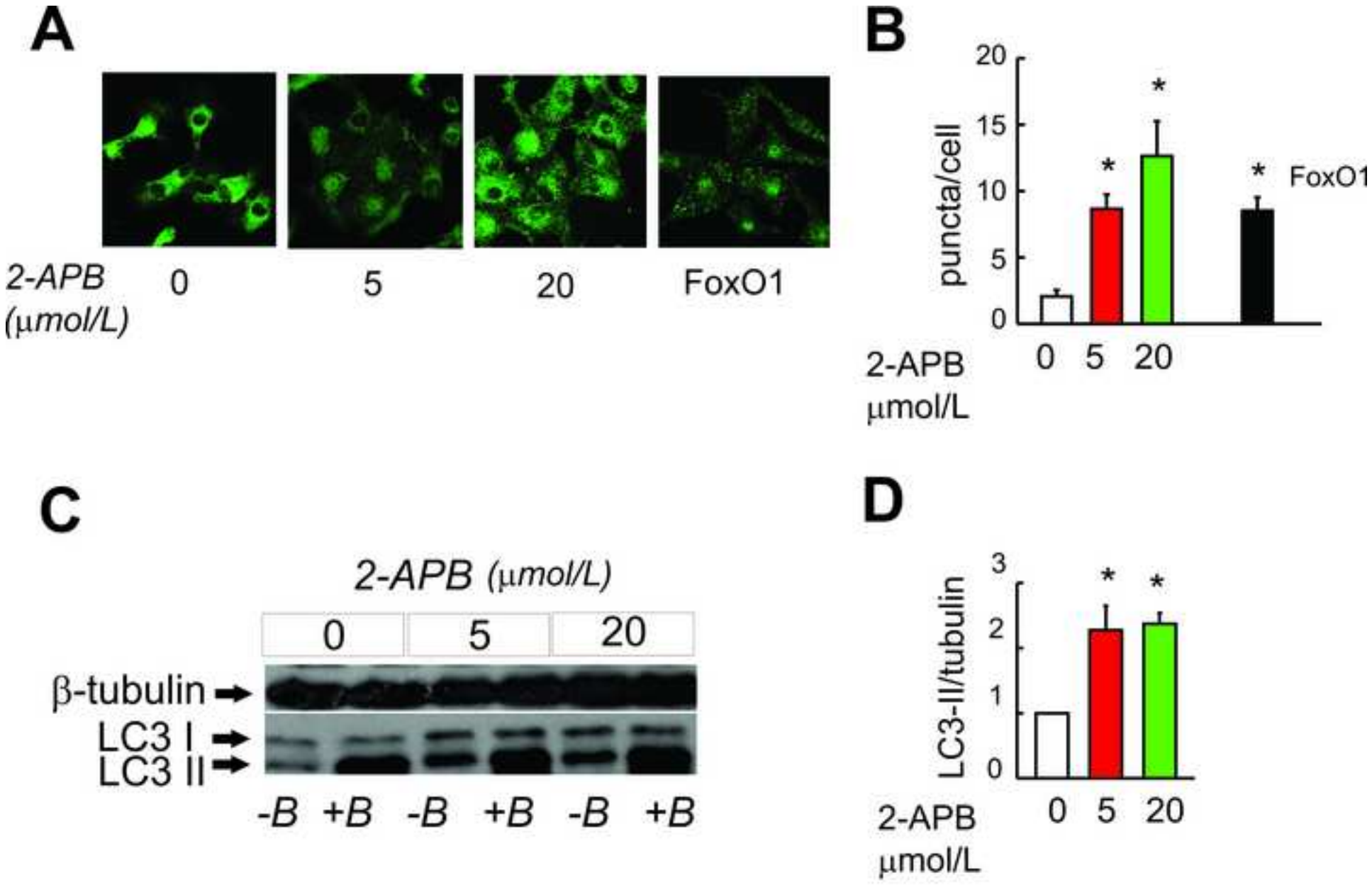

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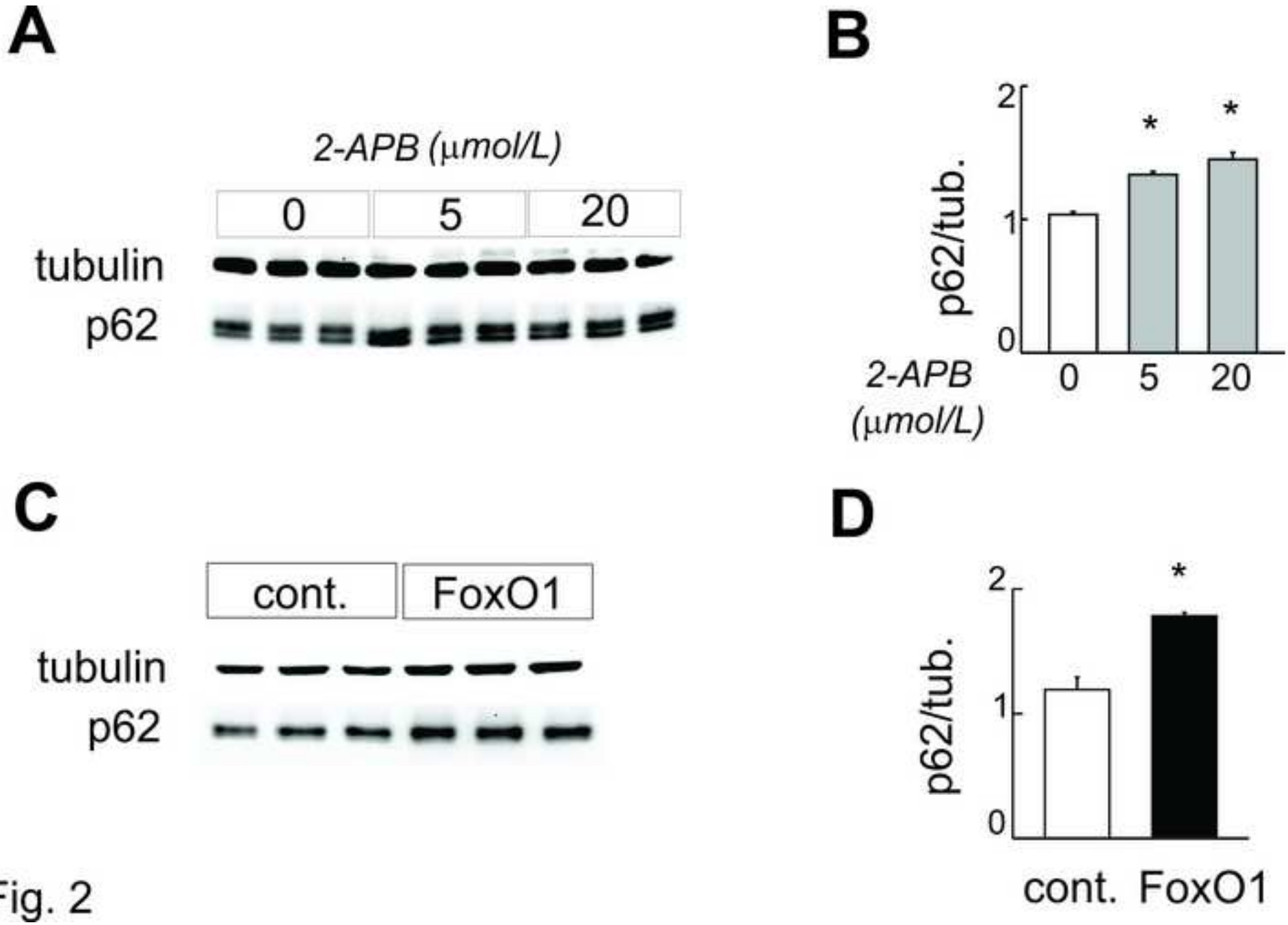

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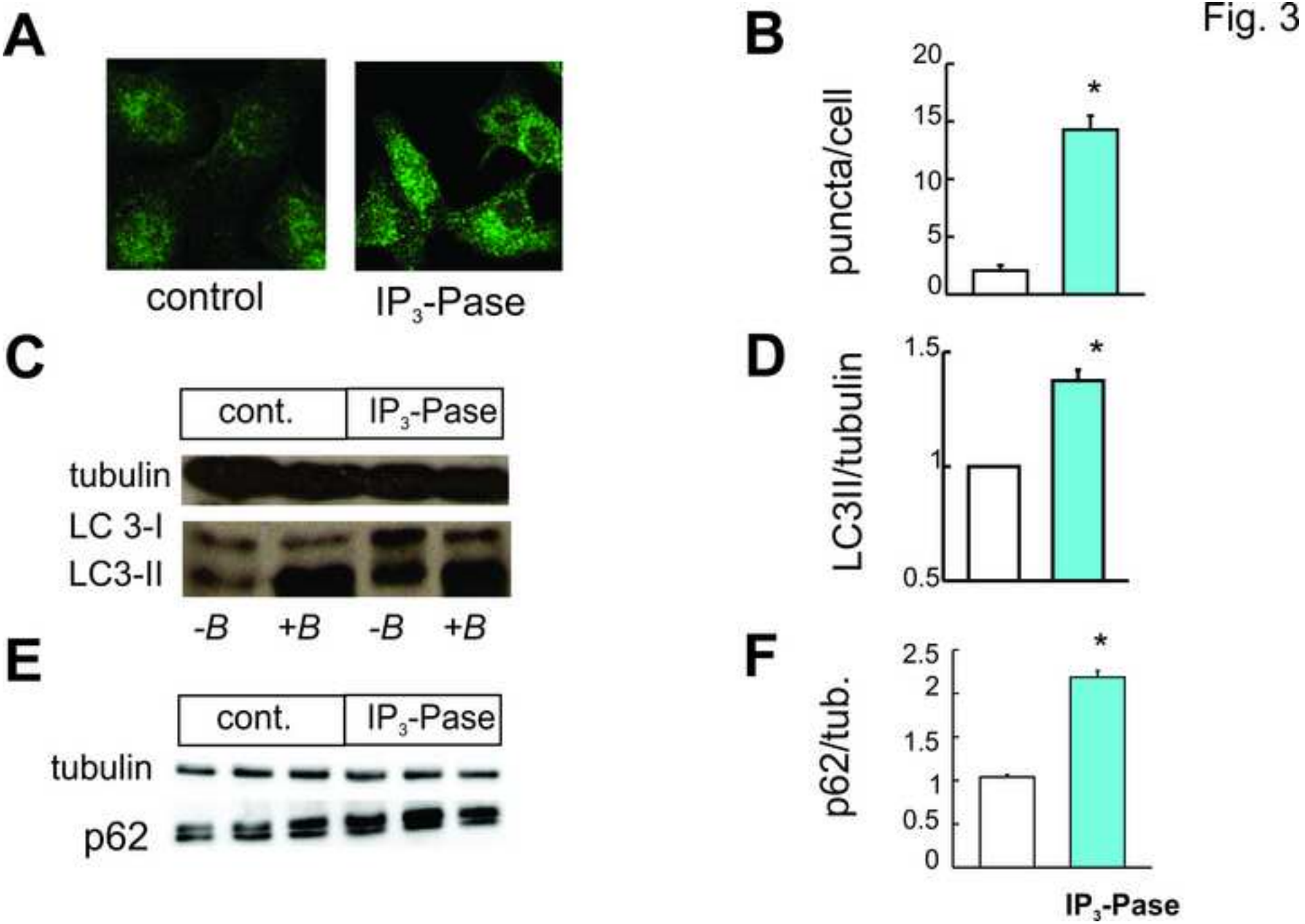

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Fig. 4