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Title**In vitro and in vivo cardioprotective and metabolic efficacy of vitamin E TPGS/Apelin**

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Short Title: TPGS/apelin in cardio metabolic disorder

1 **Abstract**

2 **Aims:** Apelin and vitamin E have been proposed as signaling molecules, but their synergistic role is
3 unknown. The aim of this work was to develop vitamin E TPGS/Apelin system to test their
4 cardioprotective and metabolic efficacy in vitro and in vivo. **Methods:** FDA-approved surfactant D- α -
5 tocopheryl polyethylene glycol 1000 succinate (TPGS-1000) and Apelin complex were characterized
6 by physico-chemical methods (CMC determination, dynamic light scattering and circular dichroism).
7 *In vitro* studies were carried out on H9C2 cardiomyoblasts and isolated murine cardiomyocytes. In
8 vivo studies were performed in isoproterenol- and high-fat diet-induced cardiac remodeling models
9 in mice. **Results:** We found that vitamin E TPGS/Apelin provide cardioprotective and metabolic
10 efficacy in vitro and in vivo. In vitro studies revealed that vitamin E TPGS/Apelin reduces hypoxia-
11 induced mitochondrial ROS production in cultured cardiomyocytes and H9C2 cardiomyoblasts. In
12 addition, vitamin E TPGS/Apelin confers apoptotic response to hypoxic stress in cells. In a mouse
13 model of isoproterenol-induced cardiac injury, TPGS is not able to affect cardiac remodeling,
14 however combination of vitamin E TPGS and Apelin counteracts myocardial apoptosis, oxidative
15 stress, hypertrophy and fibrosis. Furthermore, combination treatment attenuated obesity-induced
16 cardiometabolic and fibrotic remodeling in mice. **Conclusion:** Together, our data demonstrated the
17 therapeutic benefits of vitamin E TPGS/Apelin complex to combat cardiovascular and metabolic
18 disorders.

19

20

21 **Keywords: cardiac hypertrophy, obesity, oxidative stress, surfactant**

22 **Introduction**

23 The global prevalence of obesity and cardiovascular diseases has markedly increased over the past
24 several decades in both developed and developing nations [1]. Apelin is a highly conserved peptide
25 from the adipokine family and a key regulator of cardiovascular and metabolic status [2]. Apelin
26 exerts strong inotropic actions [3] and regulates cardiac hypertrophy in response to pathological
27 stress, activating the antioxidant enzyme catalase [4]. Apelin was also shown to reduce
28 cardiomyocyte cell death [5,6] and fibrotic remodeling of the heart [7]. Moreover, it has been
29 reported by us and others that apelin improves diabetic cardiomyopathy phenotype [8–10],
30 promotes survival of transplanted mesenchymal cells [11] and that apelin plasma levels are increased
31 in obese patients after myocardial infarction [5], opening new therapeutic avenues for the treatment
32 of cardiovascular and metabolic disorders.

33 Vitamin E is a fat-soluble antioxidant vitamin whose deficiency results from several chronic diseases
34 associated with fat malabsorption, such as cystic fibrosis, Crohn's disease, chronic cholestasis,
35 pancreatic insufficiency or gastric bypass in obese patients [12]. Antioxidant vitamins are commonly
36 prescribed to patients after cardiac transplant, in order to limit oxidative stress and therefore
37 transplant rejection [13]. Nevertheless, the clinical efficacy of vitamin E supplementation is not fully
38 established [14–16]. In recent years, interest has grown on D- α -tocopheryl polyethylene glycol 1000
39 succinate (TPGS-1000), a water-soluble derivative of natural vitamin E. TPGS-1000 is formed by the
40 esterification of D- α -tocopheryl succinate with polyethylene glycol (PEG), resulting in a hydrophilic
41 polar PEG head and a lipophilic tocopheryl tail, providing TPGS-1000 with the amphipathic properties
42 of surfactants [17]. TPGS-1000 is a GRAS (generally regarded as safe)-listed supplement and is FDA-
43 approved as a drug solubilizer. Recently, it has been shown to be effective in several anti-cancer drug
44 delivery systems, including doxorubicin [18] and curcumin [19], improving their therapeutic value.
45 Moreover, it is widely used as a water-soluble vitamin E supplement for people with impaired uptake
46 or chronic liver diseases, in particular in children with chronic cholestasis [20] and short bowel

47 syndrome [21]. These disorders may affect cardiac function and predispose to the development of
48 cardiomyopathy [22,23]. More recently, the effect of TPGS-1000 as a vitamin E alternative has been
49 investigated in a rat model of cardiac transplant [24], opening new perspectives on the use of its
50 surfactant properties in the field of cardiovascular diseases. In this context, combination of TPGS-
51 1000 and apelin is a promising approach to combat complex or multifactorial disorders including
52 obesity and heart failure. However, ~~to~~to date, there is no study on the interactions of TPGS-1000
53 and apelin. In this study we characterized TPGS-1000/Apelin complex to test their cardioprotective
54 benefits in vitro and in vivo. We found that TPGS-1000/Apelin complex counteracts adverse
55 cardiometabolic and fibrotic remodeling in mice. In addition, in vitro studies revealed that TPGS-
56 1000/Apelin complex confers protection against hypoxia-induced cell death and oxidative stress.

57

58 **Materials and Methods**

59 **Reagents**

60 [Pyr¹]-Apelin 13 (stated as apelin in the text) was purchased from Genecust Europe. D- α -tocopheryl
61 polyethylene glycol 1000 succinate (TPGS-1000), isoproterenol hypochloride and Hoechst 34580
62 were obtained from Sigma-Aldrich (France).

63 **Cell culture and treatments**

64 The H9C2 rat embryonic cardiomyoblastic cell line was cultured in DMEM medium (Life technologies)
65 supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin (Life technologies) in a
66 37°C, 5% CO₂ incubator. Primary cardiomyocytes were isolated from adult C57BL6/J mice hearts as
67 previously described [25]. Cell viability was assessed using MTT (3-(4,5-Dimethyl-2-thiazolyl)-2,5-
68 diphenyl-2H-tetrazolium bromide) according to manufacturer's instructions (Sigma). For hypoxic
69 treatment, cells were pretreated for 30 min with apelin 10⁻⁷M as described [5], TPGS-1000 or
70 apelin+TPGS-1000 and subjected to normoxia (5% CO₂, 21% O₂, balance N₂) or hypoxia in a hypoxic

71 chamber (5% CO₂, 1% O₂, balance N₂) for 2 hours for ROS production measurement and for 16 hours
72 followed by a reoxygenation period of 4 hours for apoptosis evaluation. Quantification was
73 performed as described [26].

74 **Evaluation of apoptosis and ROS production**

75 H9C2 apoptosis was assessed by nuclear staining with 5µg/ml Hoechst 34580 for 20min. Cells were
76 then live-imaged on a wide-field Leica microscope. Pyknotic nuclei were counted and expressed as
77 percentage of total nuclei. Apoptosis level on heart cryosections and cells was assessed using
78 DeadEnd Fluorometric TUNEL system according to manufacturer's instructions (Promega).
79 Mitochondrial superoxide levels on heart cryosections and cells were assessed with MitoSOX Red
80 fluorescent probe (Life Technologies) and imaged by confocal microscopy as described [26].

81 **Cell lysate and Western-blotting**

82 Proteins from H9C2 cells were extracted using RIPA buffer supplemented with proteases inhibitors
83 and western-blotting was performed as described before [5]. Antibodies used are as follows: cleaved-
84 Caspase 3 from Cell Signaling Technology and RhoGDI from Santa Cruz.

85

86 **Animal studies**

87 The investigations conform to the Animal Care and Use Committee of the University of Toulouse,
88 INSERM/ENVY and to the guidelines of the European Convention for the Protection of Vertebrate
89 Animals used for Experimental and other Scientific Purposes (Council of Europe No 123, Strasbourg
90 1985). For isoproterenol induced cardiac hypertrophy, two-month-old C57BL6/J mice were
91 pretreated with one intraperitoneal injection of vehicle only (PBS), apelin (100µg/kg/d), TPGS-1000
92 (1.32µmol/kg/d) or the combinatory therapy. Apelin amount used here was as described before [5]
93 and the TPGS amount was set to maintain the Apelin:TPGS 1/20 ratio as for the in vitro studies and

94 physico-chemical characterizations. Mice were then treated with isoproterenol (i.p., 15mg/kg/d) in
95 the continuous presence of apelin, TPGS-1000, the combinatory therapy or vehicle only for 8
96 consecutive days. For obesity-linked cardiomyopathy, two-month old C57BL6/J mice were fed with
97 high fat diet (HFD, 45% fat, Research Diet D12451) for 14 months. HFD-fed mice were implanted with
98 AZELT osmotic minipumps to deliver vehicle only (PBS) or the combinatory therapy apelin+TPGS-
99 1000 for 28 days. Glucose tolerance test was performed on fasted mice by intraperitoneal injection
100 of glucose (1g/kg). Blood glucose levels from tail vein were monitored over time using a glucometer
101 (Accu-check, Roche Diagnostics). Echocardiographic studies were performed as described [26].

102 **Quantitative RT-PCR analysis**

103 Total RNAs were extracted from mice heart in Qiazol using FastPrep lysing matrix M tubes (MP
104 Biomedicals) and further purified using RNAeasy mini kit (Qiagen). Total RNAs were reverse
105 transcribed using superscript II reverse transcriptase (Invitrogen). Real time quantitative PCR was
106 performed as previously described (Alfarano et al., 2014) on a StepOnePlus realtime PCR system
107 (Thermo Fischer scientific). Primers used on this study are as follows: collagen I 5'-
108 TGTGTGCGATGACGTGCAAT-3' (forward) and 5'-GGGTCCCTCGACTCCTACA-3' (reverse); collagen III 5'-
109 AAGGCGAATTCAAGGCTGAA-3' (forward) and 5'- TGTGTTTAGTACAGCCATCCTCTAGA-3' (reverse) and
110 alpha-SMA 5'-GTCCCAGACATCAGGGAGTAA-3' (forward) and 5'-TCGATACTTCAGCGTCAGGA-3'
111 (reverse); PGC1- β 5'-TTGAGGTGTTCTGGTGGAGATTGTAG-3' (forward) and 5'-
112 GAAGGTGATAAAACCGTGCTTCTG-3' (reverse); ANP 5'-AGAGTGGGCAGAGACAGCAAA-3' (forward) and
113 5'-AAGGCCAAGACGAGGAAGAAG-3' (reverse); BNP 5'-GCACAAGATAGACCGGATCG-3' (forward) and
114 5'-CCCAGGCAGAGTCAGAAAC-3' (reverse).

115 **Morphology**

116 Hematoxylin-eosin (H/E) staining of heart cryosections was performed using standard method.
117 Myocytes hypertrophy was assessed by measuring cardiomyocyte cross sectional area on either H/E

118 or on fluorescent WGA stained cryosections. Cardiac fibrosis was assessed using sirius red staining
119 according to standard method or by fluorescent WGA staining according to [27].

120 **Statistical analysis**

121 Data are expressed as mean \pm SEM. Comparison between two groups was performed by Student's
122 two-tailed t-test while comparison of multiple groups was performed by one-way ANOVA followed
123 by a Bonferroni's post hoc test using GraphPad Prism version 5.00 (GraphPad Software, Inc).

124 **Results**

125 **Cumulative protection of vitamin E TPGS/Apelin against mitochondrial oxidative stress**

126 We first investigated the dose-dependent effects of TPGS-1000 on H9C2 cell viability. As shown in
127 Fig.1a, treatment with up to 2 μ M TPGS-1000 for 24h did not alter cell viability. However, higher
128 concentration decreased dramatically H9C2 cell viability, down to 25% for 20 μ M treatment.
129 Therefore, we used TPGS-1000 on H9C2 cells at 2 μ M final concentration throughout this study. In
130 order to evaluate the antioxidant properties of TPGS-1000, apelin and vitamin E TPGS/Apelin
131 complex, we measured mitochondrial O₂⁻ production by MitoSOX Red staining of living H9C2 cells
132 exposed to hypoxia. As shown in Fig.1b, while hypoxia treatment induced an excessive production of
133 mitochondrial ROS, treatment of cells with TPGS-1000 alone (Fig. 1b, HT) or apelin alone (Fig. 1b, HA)
134 totally abrogated mitochondrial O₂⁻ production induced by hypoxia. Interestingly, quantification
135 indicates a cumulative effect of vitamin E TPGS/Apelin (Fig. 1b, HTA). As undifferentiated H9C2
136 cardiomyoblasts may not represent cardiomyocytes, we investigated the effect of TPGS-1000 and
137 apelin on mitochondrial O₂⁻ production in primary cardiomyocytes isolated from adult mice. As
138 shown in Fig.1c, both apelin and TPGS-1000 strongly reduced mitochondrial O₂⁻ production induced
139 by hypoxia. Similarly to what we observed in H9C2 cells, the combinatory therapy showed a
140 cumulative anti-oxidant effect of TPGS1000 and apelin when added together (Fig. 1c).

141

142 As TPGS-1000 is a well-known surfactant commonly used to formulate molecules, we investigated
143 the physico-chemical properties of TPGS-1000 in the presence of apelin. Surface tension
144 measurements showed that the critical micelle concentration (CMC) of TPGS-1000 was affected by
145 the presence of apelin, decreasing from 10 μ M to 4 μ M (supplementary Fig. 1a). This suggests that
146 apelin slightly promotes the micellization of TPGS-1000. Moreover, some signs of a pre-aggregation
147 could be detected at very low concentrations, confirming this hypothesis. In contrast, dynamic light
148 scattering (DLS) measurements showed that apelin had no effect on the mean hydrodynamic radius
149 of TPGS-1000 micelles, remaining at 8nm (supplementary Fig. 1b). Moreover, using circular dichroism
150 spectroscopy we found that TPGS-1000 did not affect structuration of apelin: even if the profile was
151 slightly affected by the presence of the surfactant, it remained typical of a coil conformation
152 (supplementary Fig. 1c). Altogether these results indicate that, even if the two compounds might
153 interact weakly in the mixture, the main properties of TPGS-1000 and apelin remained conserved
154 without loss of activity.

155 **Vitamin E TPGS/Apelin counteracts hypoxia-induced apoptotic cell death in H9C2 cells**

156 Excessive ROS production is linked to mitochondrial damage, activation of apoptotic cascades and
157 ultimately cell death [28]. We next evaluated the effects of Vitamin E TPGS/Apelin complex on
158 hypoxia-induced apoptosis in H9C2 cardiomyoblasts. Apoptotic cell death was assessed either by
159 monitoring pyknotic nuclei (Fig. 2a and c) and by TUNEL staining (Fig. 2b and d). Whereas apelin was
160 potent to prevent hypoxia-induced apoptotic cell death, TPGS-1000 had no effect. Importantly,
161 treatment of cells with Vitamin E TPGS/Apelin complex significantly reduced the number of apoptotic
162 cells in H9C2 cells under hypoxia (Fig. 2a-e). This was further confirmed by monitoring the cleavage
163 of caspase 3, a bona fide marker of apoptotic cascade activation (Fig. 2e and f). This suggests that
164 Vitamin E TPGS/Apelin provide protection against apoptosis under stress conditions.

165 To further investigate how TPGS-1000/apelin reduced ROS production and apoptosis, we quantified
166 the expression level of mitochondrial biogenesis regulatory factor PGC1- β . As shown in Fig. 2g, we

167 found that long term hypoxic challenge dramatically reduced PGC1- β expression. Importantly, apelin,
168 but not TPGS-1000, restored PGC1- β level (Fig. 2g), suggesting that TPGS-1000, unlike apelin, is not
169 able to induce mitochondrial biogenesis.

170 **Effect of Vitamin E TPGS/Apelin on isoproterenol-induced cardiac injury in vivo**

171 In order to investigate the effect of Vitamin E TPGS/Apelin *in vivo*, we resorted to a chemically-
172 induced cardiac remodeling mouse model. Isoproterenol (ISO) is a beta-adrenergic agonist commonly
173 used *in vivo* to induce cardiac hypertrophy, ROS production and apoptosis [26]. As shown by
174 hematoxylin-eosin staining, chronic treatment with ISO induced cardiac hypertrophy (Fig. 3a, H/E).
175 These results were confirmed using the fluorescent probe wheat-germ agglutinin (Fig. 3a, WGA) and
176 quantification of cross-sectional cardiomyocyte area (Fig. 3b). Strikingly, treatment with Vitamin E
177 TPGS/Apelin complex or apelin attenuated ISO-induced cardiac hypertrophic response, while TPGS-
178 1000 treatment had no significant effects (Fig.3). Interestingly, TPGS-1000 did not alter apelin anti-
179 hypertrophic properties (Fig.3, ISO+TPGS+A). This was further confirmed by measuring the
180 expression levels of the hypertrophic markers ANP and BNP (Fig. 3c and d). Next we explored the
181 antioxidant properties of Vitamin E TPGS/Apelin complex versus apelin and TPGS-1000 *in vivo*. As
182 shown in Fig. 4a, both TPGS/Apelin complex and apelin markedly decreased ISO-induced myocardial
183 mitochondrial O₂⁻ production in cardiac tissue, while TPGS-1000 had no effect. To examine the anti-
184 apoptotic activity of TPGS/Apelin complex versus TPGS-1000 and apelin *in vivo*, we resorted to the
185 TUNEL-staining of apoptotic cells on heart cryosections. As shown in Fig. 4b, ISO-treatment induced
186 an increase in myocardial apoptotic cell death, which was prevented in vitamin E TPGS/Apelin-
187 treated or apelin-treated mice. TPGS-1000 treatment did not affect ISO-induced apoptotic cell death
188 in cardiac tissue in treated animals. Together, these data revealed anti-apoptotic activity of vitamin E
189 TPGS/Apelin in cardiac remodeling *in vivo*.

190 Excessive ROS production and massive loss of cardiomyocytes by apoptosis lead to the development
191 of cardiac fibrosis [29]. Therefore we examined the effects of vitamin E TPGS/Apelin on cardiac

192 fibrosis using Sirius red and fluorescent WGA staining in cardiac tissue from ISO-induced myocardial
193 injury. As shown in Fig. 5, treatment with ISO induced the development of a pronounced perivascular
194 and interstitial fibrosis in mice. While vitamin E TPGS/Apelin complex or apelin was able to efficiently
195 reduce cardiac fibrosis, TPGS-1000 had no anti-fibrotic activity in ISO-remodeled hearts (Fig. 5).

196 **Effect of vitamin E TPGS/Apelin on a HFD-induced diabetic cardiomyopathy**

197 We next examined the effects of vitamin E TPGS/Apelin on myocardial and metabolic remodeling in
198 mice exposed to HFD for 14 months. HFD-feeding resulted in the development of glucose
199 intolerance, insulin resistance (Fig. 6a and b) and increased body weight (Fig. 6c). Echocardiographic
200 analysis revealed left ventricular dysfunction as shown by the reduced ejection fraction (Fig. 6d) and
201 fractional shortening (Fig. 6e). Treatment with vitamin E TPGS/Apelin improved glucose tolerance in
202 HFD-fed mice (Fig.6a and b). The anti-glycemic activity of the vitamin E TPGS/Apelin was associated
203 with a reduction in body weight, although it did not reach statistical significance (Fig. 6c).
204 Importantly, vitamin E TPGS/Apelin treatment preserved cardiac function in obese mice as shown by
205 the increase in EF and FS (Fig. 6d and e). This was accompanied by a reduction of cardiac hypertrophy
206 as shown by cardiomyocytes cross-sectional area measurements (Fig. 7a), a decrease in myocardial
207 mitochondrial O₂⁻ production (Fig. 7b) and apoptosis (Fig. 7c). Interestingly, the combinatory therapy
208 is potent to reduce myocardial fibrosis in HFD-fed mice (Fig. 8a). These results were confirmed by
209 qPCR analysis of the expression of the myocardial pro-fibrotic markers α SMA, collagen I and collagen
210 III (Fig. 8b).

211 Taken together, our results show for the first time that vitamin E TPGS/Apelin behaves as a
212 competitive cardioprotective and metabolic modulator of myocardial remodeling processes.

213 **Discussion**

214 Apelin and vitamin E TPGS have been largely recognized for their antioxidant properties [4,17].
215 However, to date, there is no study regarding the interactions of vitamin E TPGS and apelin. In this

216 study we characterized vitamin E TPGS/Apelin complex to evaluate their cardioprotective benefits in
217 vitro and in vivo. We characterized the cardiometabolic efficacy of vitamin E TPGS/Apelin in the
218 progressive myocardial remodeling processes. In a mouse model of isoproterenol-induced cardiac
219 injury, vitamin E TPGS/Apelin counteracts myocardial apoptosis, oxidative stress, hypertrophy and
220 fibrosis. Furthermore, we provide the first evidence that vitamin E TPGS/Apelin attenuates obesity-
221 induced cardiometabolic and fibrotic remodeling in mice. In vitro studies reveal vitamin E
222 TPGS/Apelin-dependent antioxidant and anti-apoptotic reprogramming in cells under hypoxic stress.

223 We found that both TPGS-1000 and apelin protect cultured cardiomyoblasts and primary
224 cardiomyocytes against oxidative stress in hypoxic challenge in a cumulative manner. However, we
225 show that TPGS-1000 is ineffective to prevent apoptotic cell death as compared to apelin treatment.
226 This discrepancy between TPGS-1000 and apelin suggests that they may use separate
227 pathways/mechanisms to exert their antioxidant properties. Indeed, we and others previously
228 demonstrated that apelin, through the binding of its receptor AJP, 1/ triggers mitochondrial
229 biogenesis through activation of the transcription factors TFAM and NRF1 [8], 2/ activates the
230 antioxidant enzyme catalase [4], 3/ regulates the FoxO family of transcription factors [5,6] and 4/
231 activates the AMPK pathway [30]. On the other hand, TPGS-1000 is known to act through the
232 intracellular liberation of the vitamin E moiety, giving direct antioxidant action in cells, as previously
233 demonstrated in a rat cardiac transplant model [24]. Consistently, we found that TPGS-1000 is not
234 able to restore the expression of the mitochondrial biogenesis regulatory factor PGC1- β after long-
235 term hypoxic challenge. This could explain the lack of effect of TPGS-1000 in long-term challenges,
236 both in vitro and in vivo.

237 In vivo, we found that vitamin E TPGS/Apelin counteracts cardiac hypertrophy, oxidative stress and
238 cardiomyocyte cell death induced by isoproterenol, culminating in reducing fibrotic remodeling of
239 the heart. However, we demonstrated that TPGS-1000 alone did not provide protection against
240 myocardial hypertrophy, oxidative stress, apoptosis and activation of fibrotic reprogramming of the

241 myocardium. One might hypothesize that a poor bioavailability of TPGS-1000 in cardiac tissue could
242 explain this lack of effect. Although TPGS-1000 is well tolerated in vivo and has a good bioavailability,
243 previous studies suggested that it has a weak tropism for cardiac tissue [31]. Our physico-chemical
244 characterization shows that apelin slightly decrease the CMC of TPGS-1000 in solution, from 10 μ M to
245 4 μ M, not reaching the non-toxic concentration of 2 μ M. Nevertheless, we found here that chronic
246 treatment with TPGS-1000 in mice failed to protect the heart against ISO-induced cardiac
247 remodeling. Moreover, this is consistent with the fact that TPGS-1000 did protect neither
248 cardiomyoblasts nor cardiomyocytes against H/R-induced apoptotic cell death in long term
249 challenge.

250 TPGS-1000 is widely used for its surfactant properties, namely as a solubilizer, emulsifier and
251 bioavailability enhancer to increase drug delivery [32]. The initial aim of this study was to combine
252 apelin with TPGS-1000 in order to improve apelin cardioprotective effects. Our physico-chemical
253 characterization shows that apelin slightly modify the CMC of TPGS-1000 in solution, from 10 μ M to
254 4 μ M. Although apelin clearly decreases TPGS-1000 CMC, it does not reach the non-toxic
255 concentration of 2 μ M in vitro. More work needs to be done in order to decrease TPGS-1000 CMC in a
256 concentration range compatible with cardiomyoblasts viability. Moreover, a recent study confirmed
257 the cytotoxicity of TPGS-1000, showing that it induces cell cycle arrest and even apoptosis on breast
258 cancer cells [33], and thus care must be taken for its use in drug delivery.

259 Furthermore, in a mouse model of obesity-induced cardiomyopathy, we found that vitamin E
260 TPGS/Apelin counteracts obesity-induced myocardial and metabolic abnormalities. Specifically,
261 chronic treatment with TPGS/Apelin attenuated HFD-mediated cardiomyocyte death and cardiac
262 dysfunction. In addition, we found that vitamin E TPGS/Apelin improved glucose tolerance in HFD-fed
263 mice, suggesting that TPGS/Apelin modulates pathological remodeling of the diabetic heart in obese
264 conditions. The development of treatments involving combinations of drugs is a promising approach
265 to combat complex or multifactorial disorders including obesity and heart failure. This study provides

266 the first evidence for the therapeutic benefits of vitamin E TPGS/Apelin to prevent cardiovascular and
267 metabolic complications in obesity-linked heart failure. However, a limitation of this study is the
268 absence of the apelin-treated HFD-fed mice. Nevertheless, we previously reported apelin-mediated
269 cardioprotective responses to myocardial injury in HFD-induced obesity [5,6]. In isoproterenol-
270 treated mice, apelin and vitamin E TPGS/Apelin complex have similar results, suggesting that the
271 addition of vitamin E TPGS exerts no further benefit. The potential benefit of vitamin E TPGS/Apelin
272 complex need to be further addressed in different experimental models of heart failure, such as
273 transverse aortic constriction or ischemia-reperfusion.

274 In summary, our findings demonstrated the protective effects of vitamin E TPGS/Apelin on adverse
275 tissue reprogramming of the myocardium and metabolic defects in physiopathological context.
276 Moreover, we revealed that oxidative stress and apoptotic cell death, key components in the
277 development of cardiac injury, are attenuated by vitamin E TPGS/Apelin in both isoproterenol- and
278 HDF-induced myocardial remodeling. These findings may lead to novel approaches for the treatment
279 of adverse cardiovascular and metabolic complications linked to heart failure.

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283 **Disclosures**

284 The authors have no conflicts of interest to declare.

285 **Supplementary material**

286 Supplementary methods and figures are provided separately.

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Figure Legends

Fig. 1: Vitamin E TPGS/Apelin attenuates mitochondrial ROS production in H9C2 cells and cardiomyocytes .

a. Rat H9C2 cardiomyoblasts were treated with increasing concentration of TPGS-1000 for 24h and cell viability was assessed by MTT staining. **b-c.** H9C2 cells (**b**) or cardiomyocytes isolated from adult mice (**c**) were pretreated for 30 min in the presence of apelin (A), TPGS-1000 (T) or the combinatory therapy (TA), and subjected to hypoxia (H) or kept in normoxia (N) for 2h. Mitochondrial O₂⁻ production was assessed using MitoSOX red fluorescent probe. Cells were fixed and imaged by confocal microscopy. Scale bar is 10µm. Quantification if shown on the right panels as mean±/-SEM. ***p<0.001 vs. N; \$\$\$p<0.001 vs. H; ##p<0.01 between indicated conditions.

Fig. 2: Vitamin E TPGS/Apelin prevents apoptotic responses to hypoxic stress. a.

H9C2 cells were treated with apelin (A), TPGS-1000 (T) or TPGS-1000+apelin (TA) for 30min before being submitted to hypoxia (16h) followed by 4h re-oxygenation (H) or kept in normoxia (N). Cell nuclei were stained with Hoechst-34580 and pyknotic nuclei were counted. **b.** Cells were treated as in **a** and apoptosis was assessed using TUNEL staining (in green). Cell nuclei are stained with DAPI (in blue). Arrowheads highlight TUNEL-positive cells. Bar is 20µm. **c-d.** Quantification of pyknotic nuclei from **a** and TUNEL-positive cells from **b**. **e.** Cell lysates were probed with the indicated antibodies to assess caspase 3 cleavage. **f.** Quantification of caspase 3 cleavage from **e**. **g.** Expression level of PGC1b was assessed by RT-qPCR. Results are presented as mean±/-SEM from 3-6 independent experiments. ***p<0.001 and *p<0.05 as compared with N; \$\$\$p<0.001, \$\$p<0.01 and \$p<0.05 as compared with H; ns, non significant.

Fig.3: Vitamin E TPGS/Apelin inhibits ISO-induced hypertrophic remodeling in vivo. a.

Heart cryosections from mice treated as indicated were stained with hematoxylin/eosin (H/E) or with fluorescent WGA (in green). Bar is 25µm for H/E and 50µm for WGA. **b-d.** Cardiomyocytes cross-sectional area (**b**), ANP (**c**) and BNP (**d**) levels are shown as mean±/-SEM. n=3-5 mice per group.

***p<0.001, **p<0.01 and *p<0.05 as compared with control; \$\$\$p<0.001, \$\$p<0.01 and \$p<0.05 as

compared with ISO-treated mice.

Fig.4: Antioxidant and anti-apoptotic properties of vitamin E TPGS/Apelin in cardiac remodeling in vivo.

a. Oxidative stress was assessed in heart cryosections from mice treated as indicated. Bar is 50µm. **b.** Myocardial apoptosis was assessed using TUNEL staining (in green) on heart cryosections. Cell nuclei are stained with DAPI in blue. Arrowheads highlight TUNEL-positive cells. Bar is 50µm. Quantifications are shown on the right panels as mean±SEM. ***p<0.001 and *p<0.05 vs. Control; \$p<0.05 vs. ISO-treated mice. ns, non-significant.

Fig.5: Anti-fibrotic properties of vitamin E TPGS/Apelin in ISO-challenged hearts.

Heart cryosections from mice treated as indicated were stained with fluorescent WGA (in green) or with Sirius red (SR) to highlight fibrotic area. Bar is 100µm for WGA and 25µm for SR. Quantification of fibrotic area is shown on the right. **p<0.01 and *p<0.05 vs. Control; \$p<0.05 vs. ISO-treated mice.

Fig.6: Vitamin E TPGS/Apelin ameliorates cardiometabolic profile in obese mice. **a.** Glucose-tolerance test was performed on 6h-fasted non-obese (ND), obese (HFD) or obese mice treated with TPGS-1000+apelin (HFD+TPGS+A). **b.** Area under the curve of glucose-tolerance test from **a.** **c.** Body weight of mice. **d-e.** Echocardiographic measures of ejection fraction (**d**, EF) and fractional shortening (**e**, FS) in mice. n=3-6 mice per group. **p<0.01 and ***p<0.001 vs. ND; \$p<0.05 vs. HFD mice.

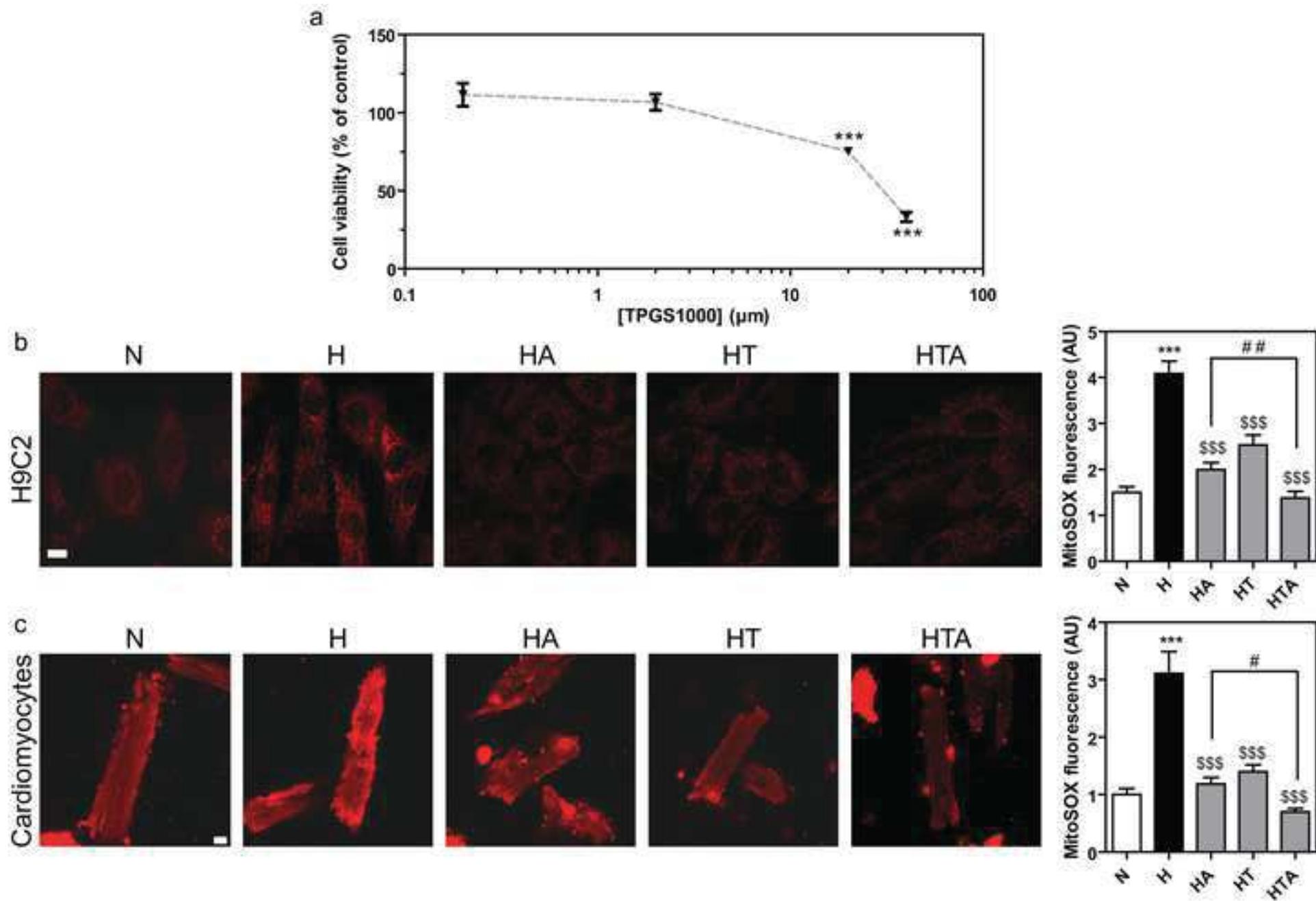
Fig.7: Vitamin E TPGS/Apelin reduces cardiac hypertrophy, ROS production and apoptosis in obese mice.

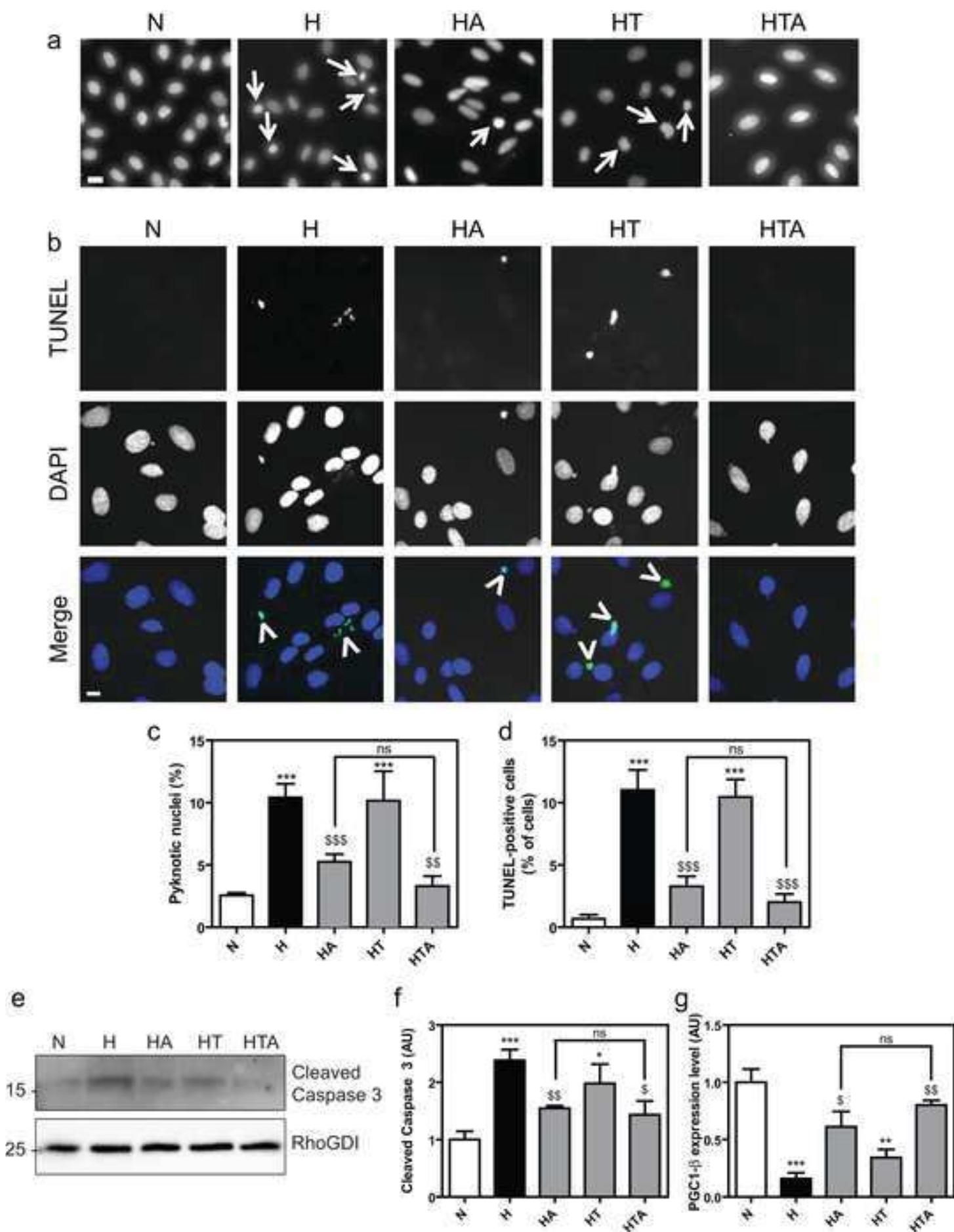
a. Heart cryosections from mice treated as in Fig.6 were stained with hematoxylin/eosin (H/E). Bar is 100µm. Quantification of myocyte cross-sectional area is shown on the right panel. **b.** ROS production was assessed on heart cryosections from mice treated as indicated. Bar is 50µm. **c.** Myocardial apoptosis was assessed using TUNEL staining (in green). Cell nuclei are stained with DAPI (in blue). Arrowheads highlight TUNEL-positive cells. Bar is 20µm. **p<0.01 and ***p<0.001 vs. ND; \$\$p<0.01 and \$\$\$p<0.001 vs. HFD.

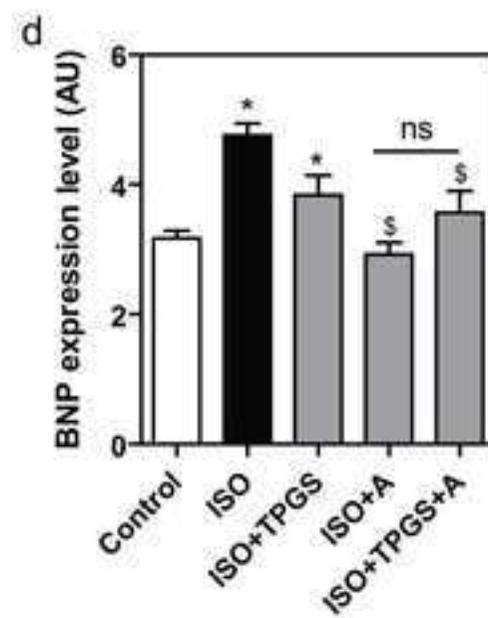
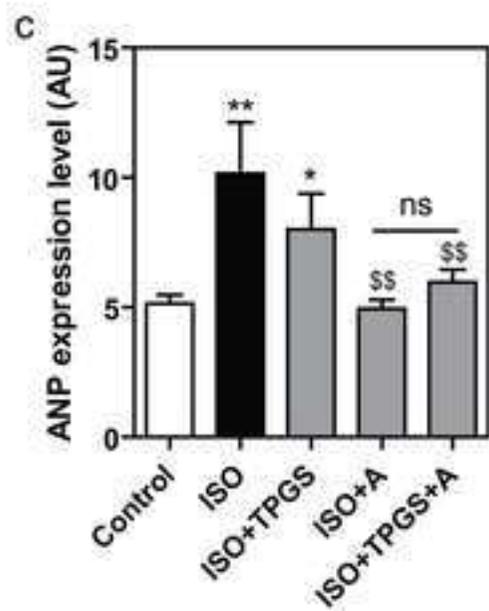
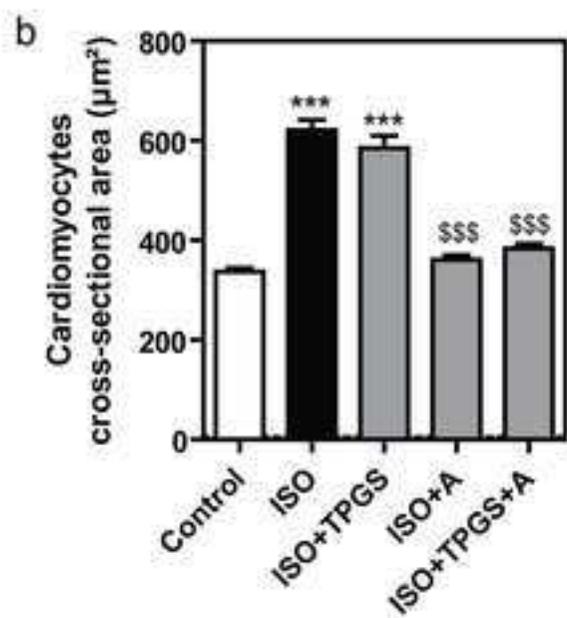
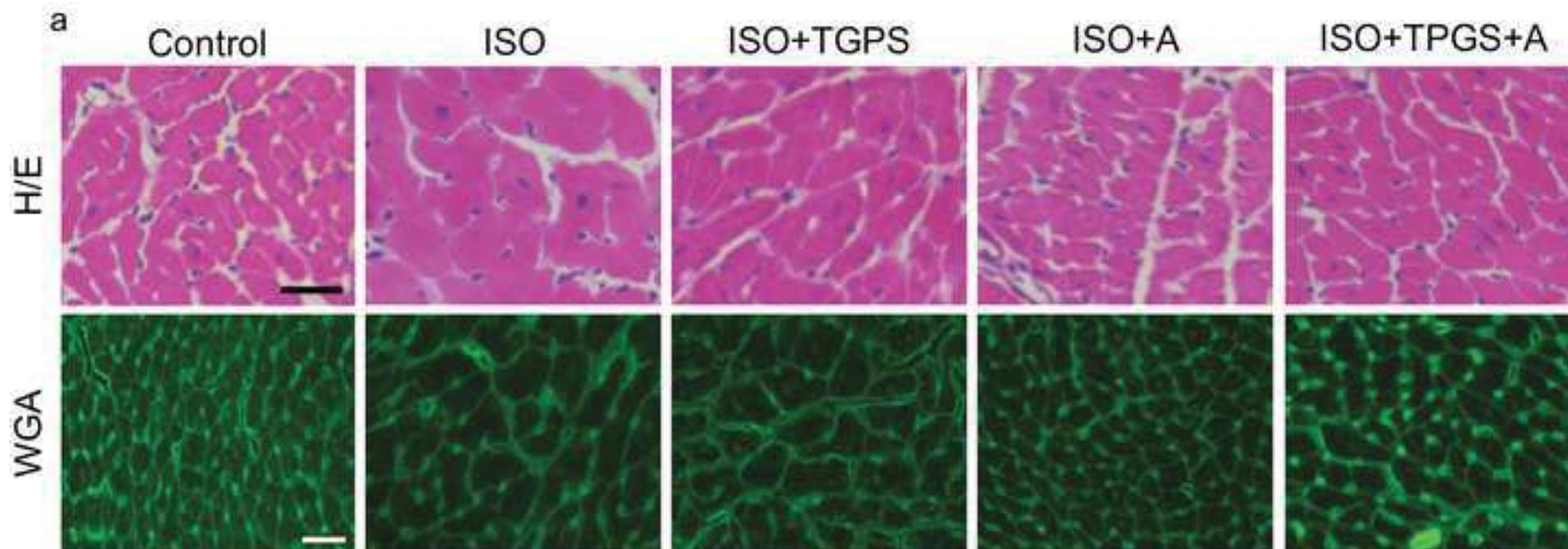
Fig.8: Vitamin E TPGS/Apelin reduces myocardial fibrosis in obese mice.

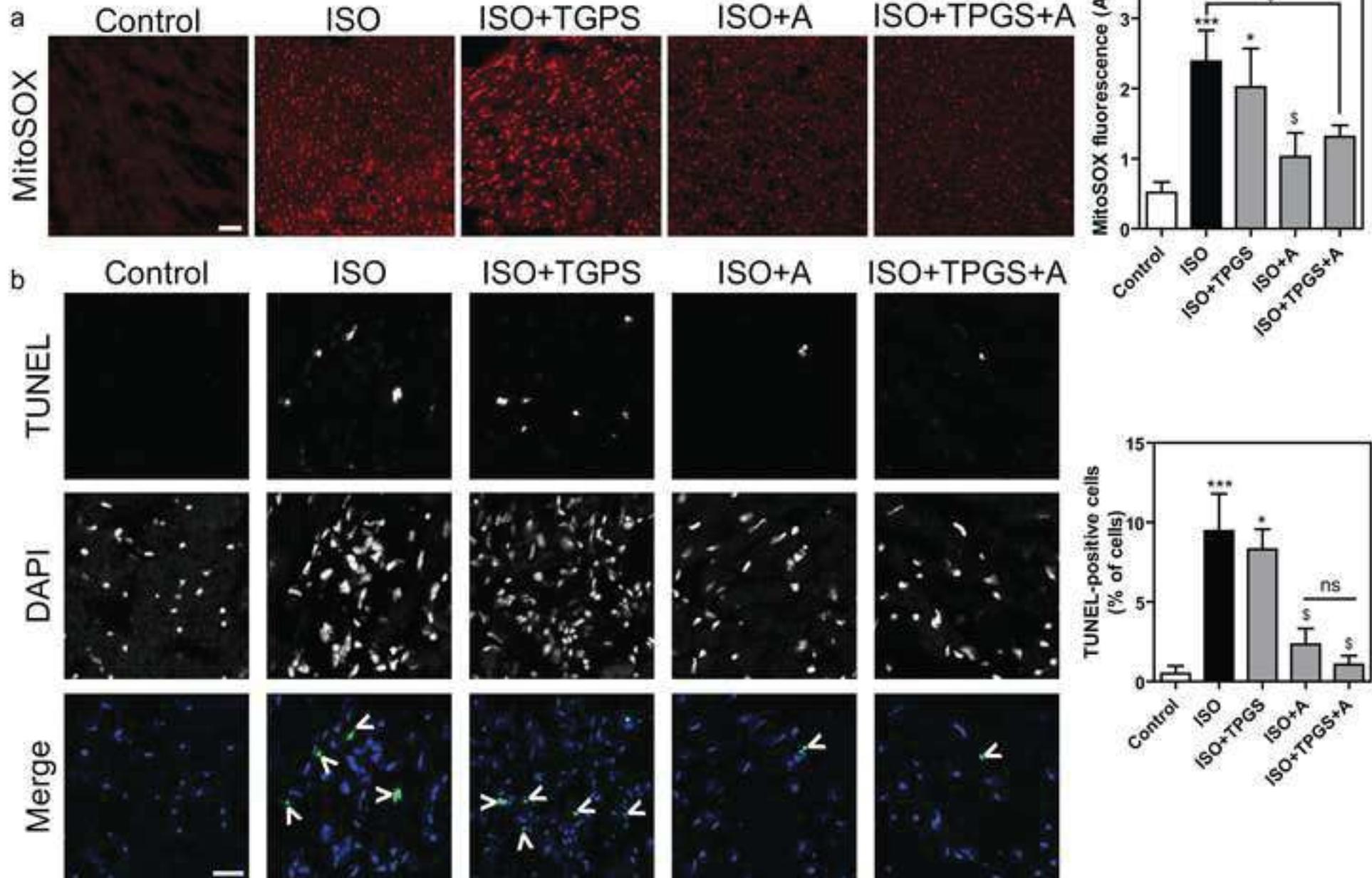
a. Mice heart cryosections were stained with fluorescent WGA or Sirius red (SR). Bar is 25µm for SR or 100µm for WGA.

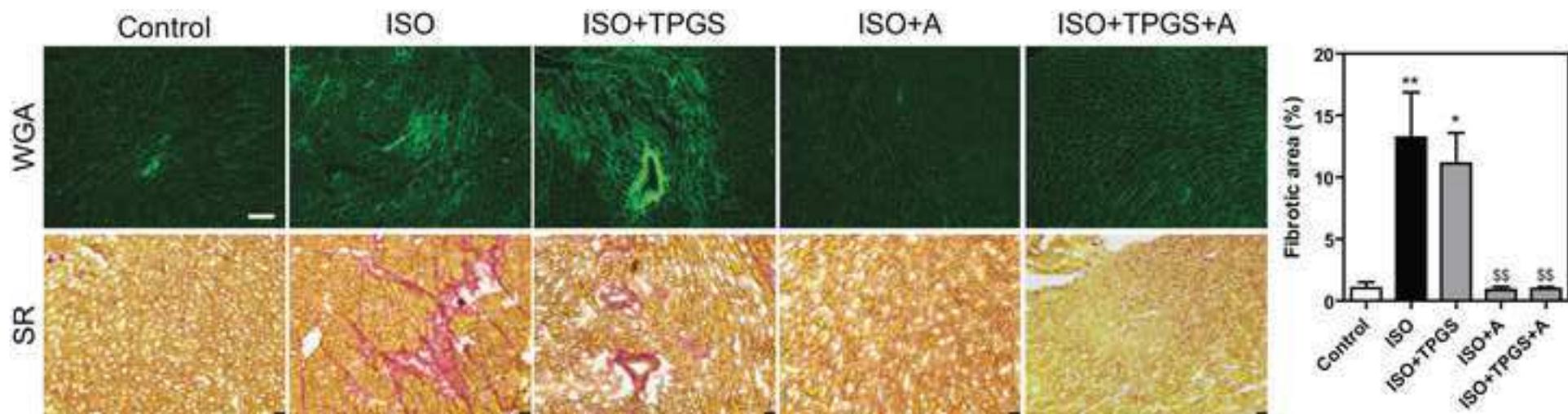
Quantification of fibrotic area is shown on the right. **b.** Expression level of α SMA, collagen I (Col I) and collagen III (Col III) was assessed by qPCR from cardiac tissue. * $p < 0.05$ and *** $p < 0.001$ vs. ND; $\$p < 0.05$ vs. HFD.

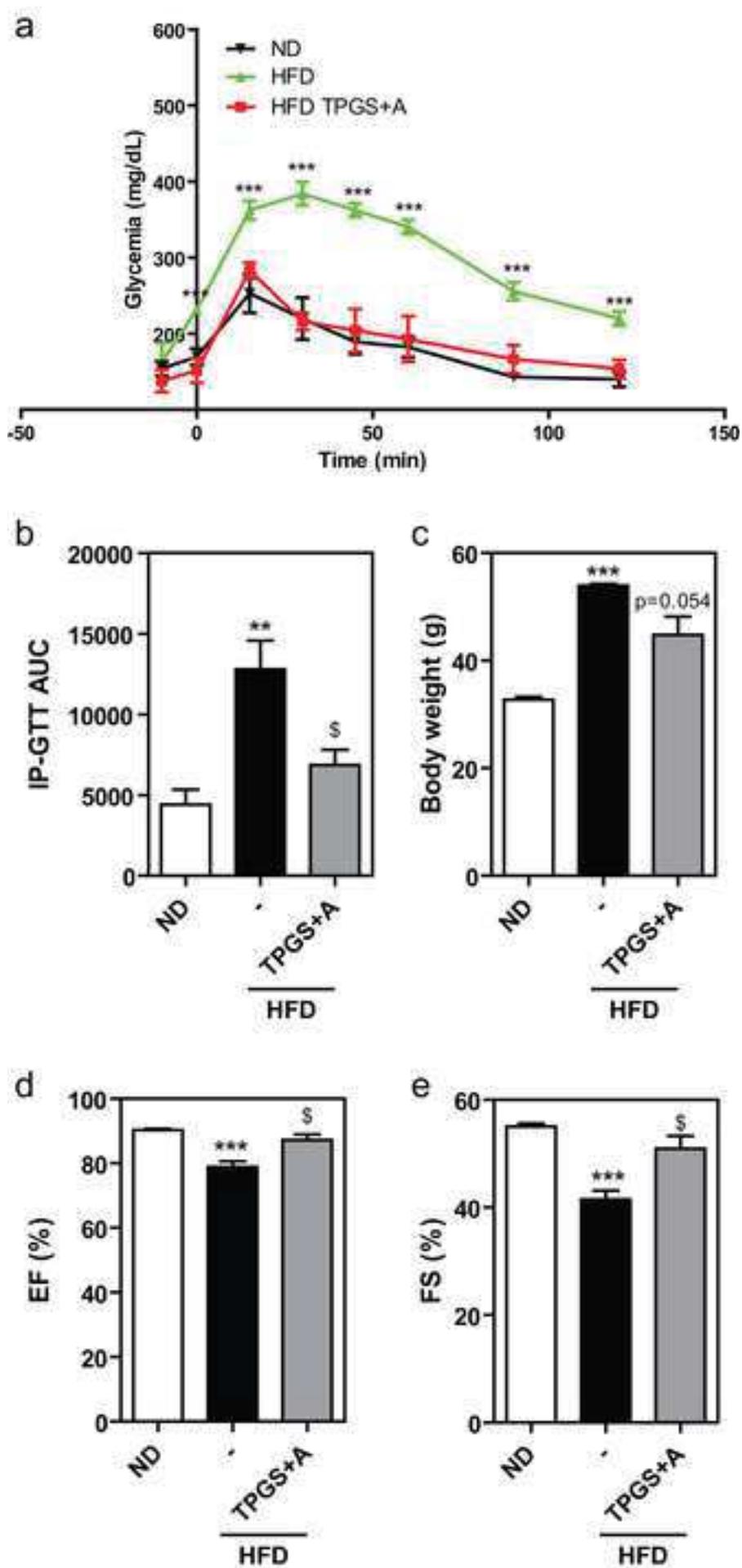


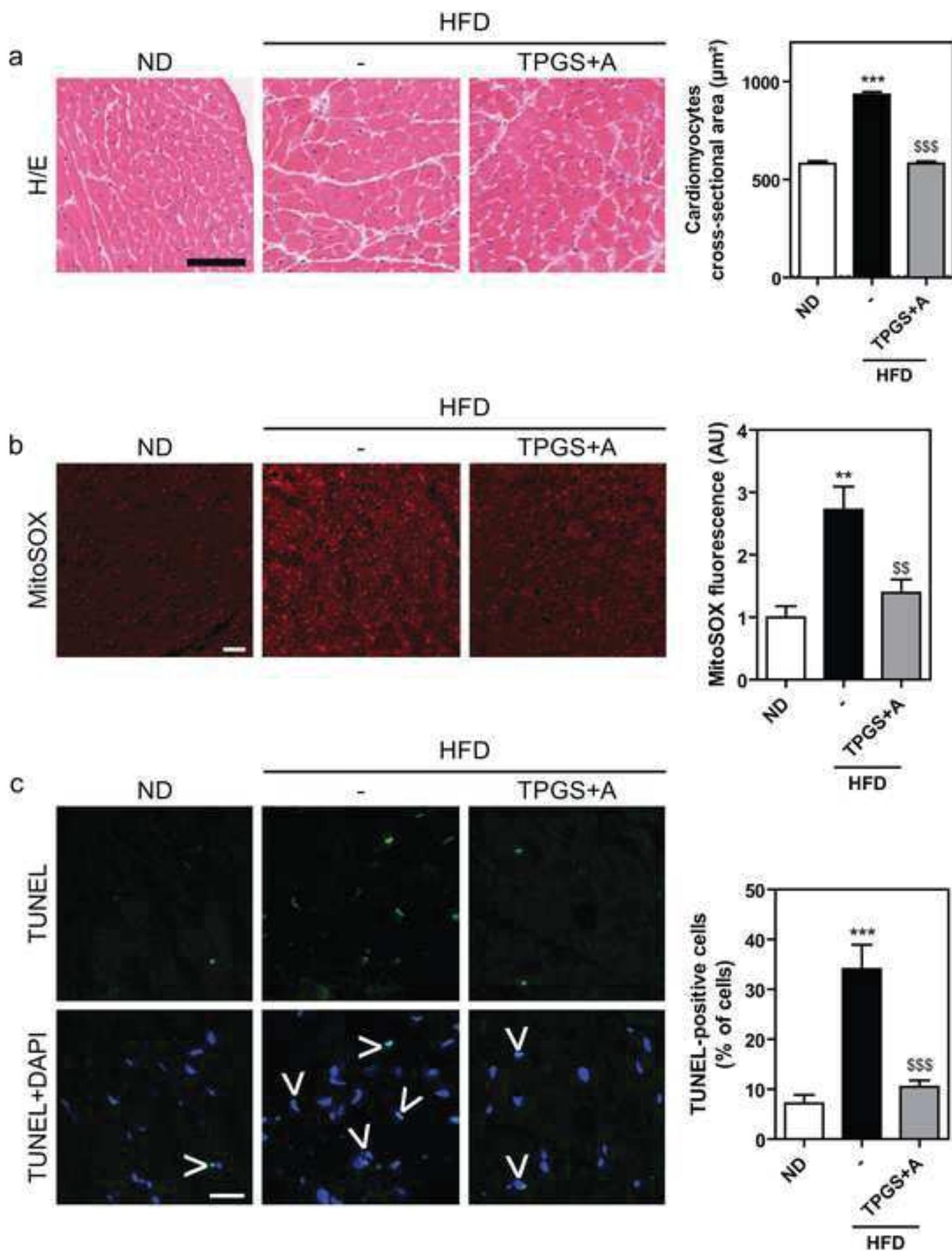


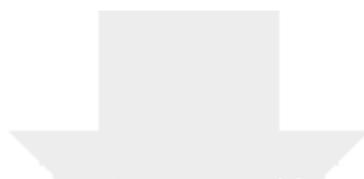












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