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Supplemental information

GFAT2 mediates cardiac hypertrophy through HBP-O-GlcNAcylation-Akt pathway

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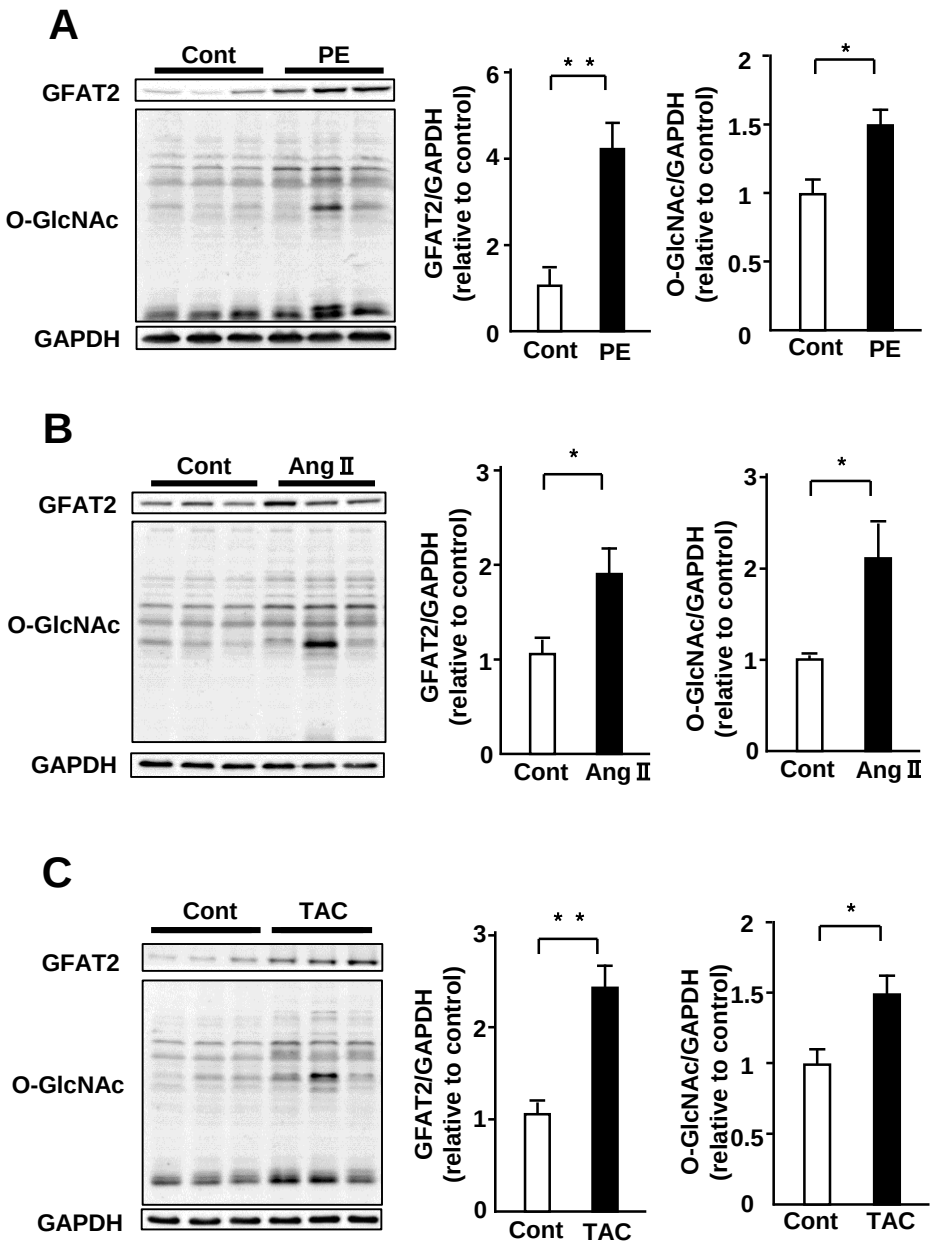

Fig. S1. GFAT2 is universally upregulated by hypertrophic stimuli. Related to Figure 1. **(A)** Representative immunoblots of GFAT2, protein O-GlcNAcylation, and GAPDH in PE (100 mg/kg body weight/day, 7 days)- or control vehicle (PBS)- treated C57B/6J mouse hearts. Quantitative analysis of GFAT2 and protein O-GlcNAcylation in PE- or control vehicle-treated C57B/6J mouse hearts (n=5).**(B)** Representative immunoblots of GFAT2, protein O-GlcNAcylation, and GAPDH in angiotensin II (Ang II, 1.44 mg/kg body weight/day, 7 days)- or control vehicle (PBS)- treated C57B/6J mouse hearts. Quantitative analysis of GFAT2 and protein O-GlcNAcylation in Ang II- or control vehicle-treated C57B/6J mouse hearts (n=5). **(C)** Representative immunoblots of GFAT2, protein O-GlcNAcylation, and GAPDH in C57B/6J mouse hearts 7 days after either sham or transverse aortic constriction (TAC) operation. Quantitative analysis of GFAT2 and protein O-GlcNAcylation in C57B/6J mouse hearts 7 days after either sham or TAC operation (n=5). *P<0.05, **P<0.01: post-hoc Tukey's comparison test.

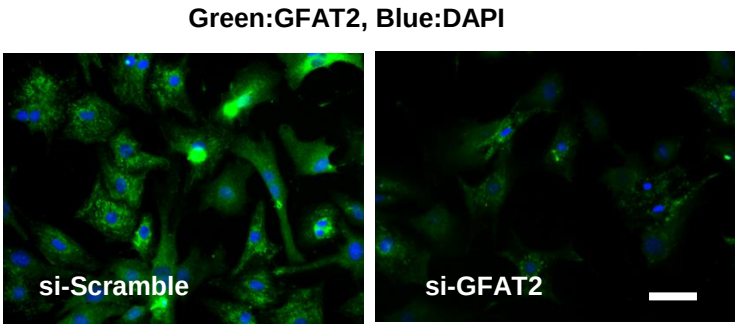

Fig. S2. Efficacy of GFAT2 knockdown in cardiomyocytes. Related to Figure 2. Image of immunostaining of GFAT2 in control and GFAT2 knocked-down NRVMs. Bar=50μm.

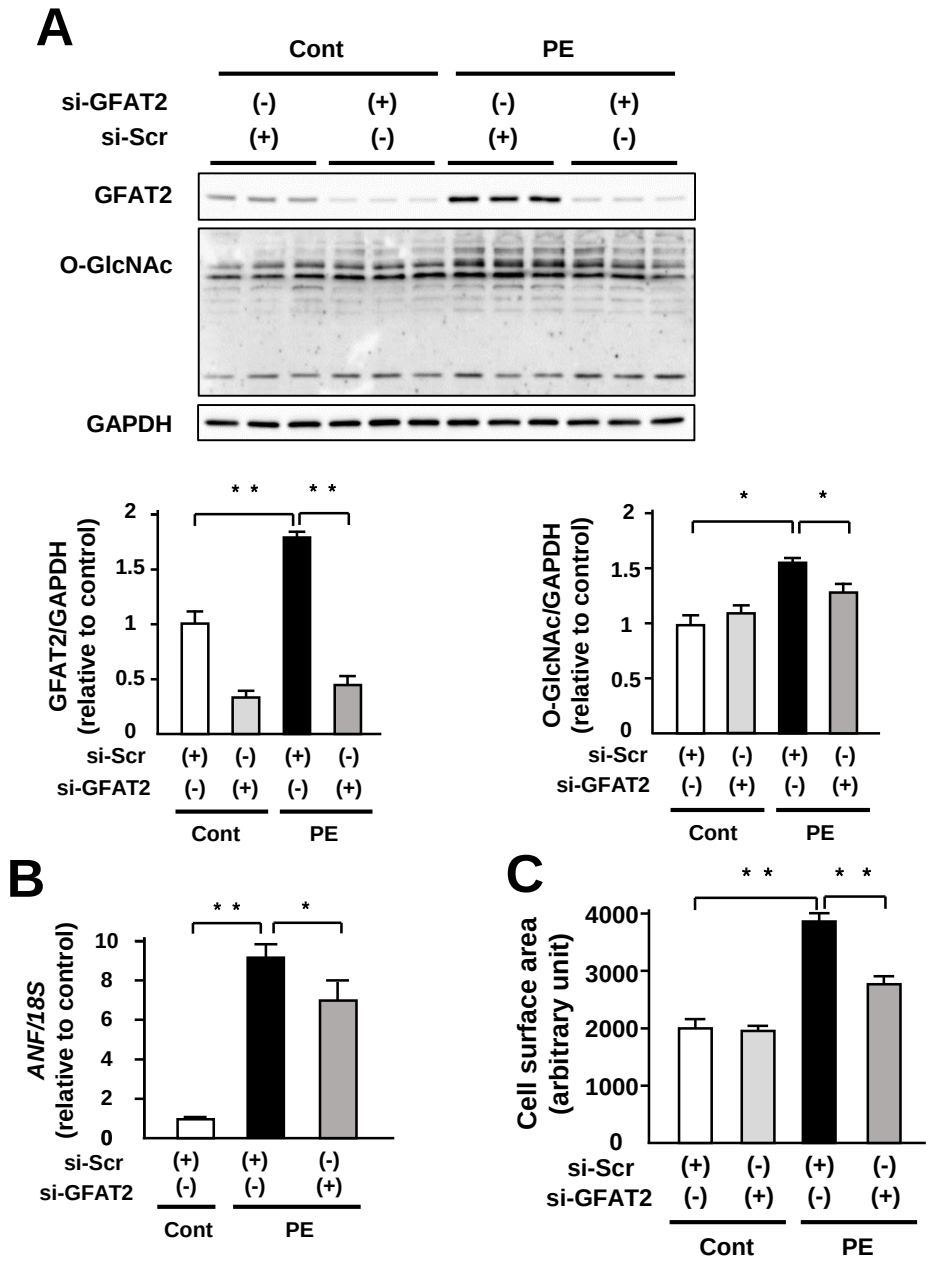

Fig. S3. Knockdown of GFAT2 attenuates protein O-GlcNAcylation and cardiomyocyte hypertrophy in response to α-adrenergic receptor stimulation. Related to Figure 2. (A) Representative immunoblots of GFAT2, protein O-GlcNAcylation, and GAPDH in NRVMs treated with indicated small interfering RNA (siRNA) in the presence or absence of phenylephrine (PE, 100 μM, 12 hours) (n=6). Quantitative analysis of GFAT2 and protein O-GlcNAcylation in NRVMs treated with indicated siRNA in the presence or absence of PE (100 μM, 12 hours) (n=6). **(B)** Expression of *atrial natriuretic factor (ANF)* and *18S* mRNA in NRVMs treated with indicated siRNA in the presence or absence of PE (100 μM, 12 hours) (n=6). **(C)** Cell surface area of NRVMs treated with indicated siRNA in the presence or absence of PE (100 μM, 12 hours) (n=150 cells in each group). *P<0.05, **P<0.01: post-hoc Tukey's comparison test.

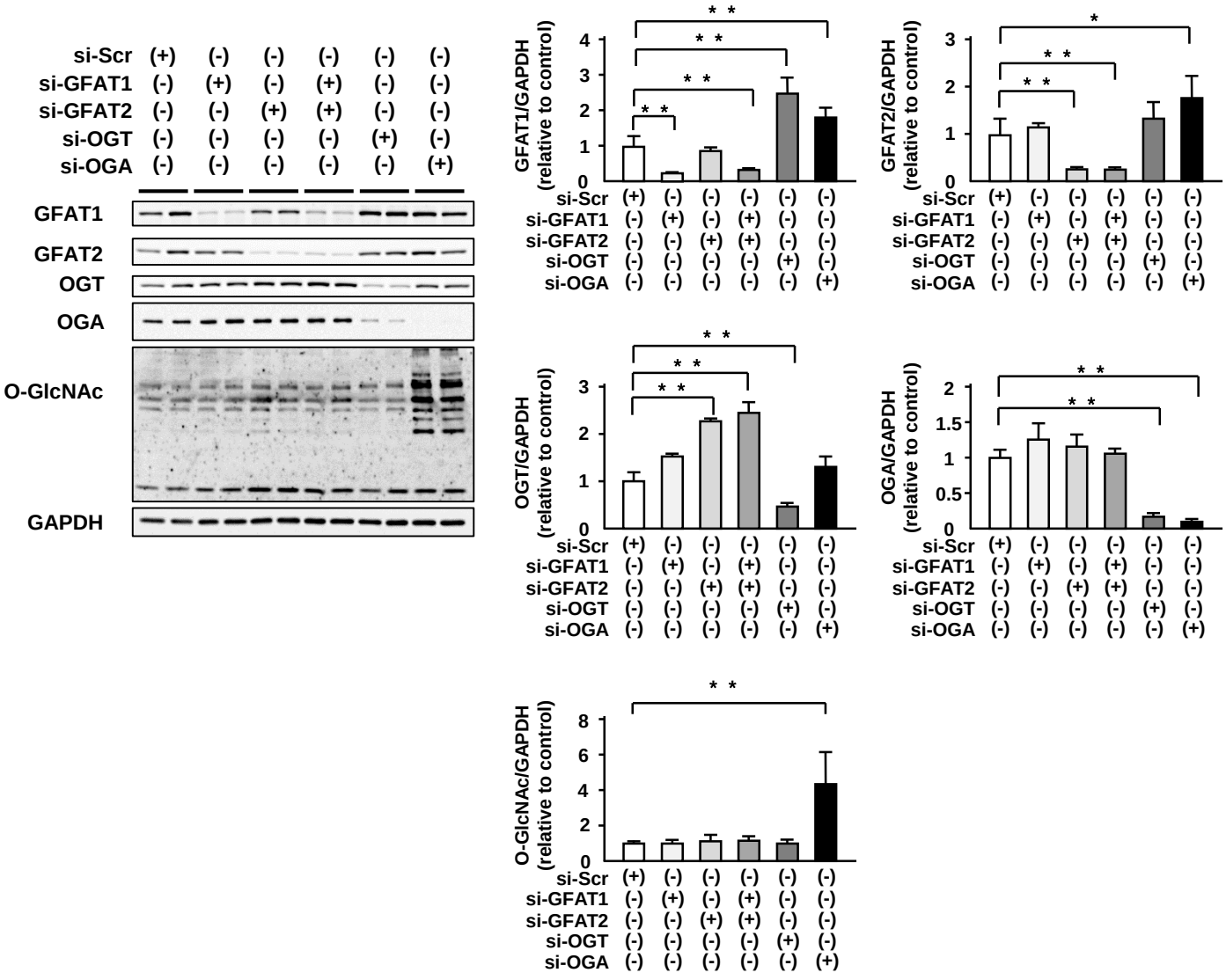

Fig. S4. The role of GFAT1, GFAT2, OGT, and OGA in protein O-GlcNAcylation in cardiomyocytes at baseline. Related to Figure 2. Representative immunoblots of GFAT1, GFAT2, OGT, OGA, protein O-GlcNAcylation, and GAPDH in NRVMs treated with indicated small interfering RNA (siRNA) at baseline. *P<0.05, **P<0.01: post-hoc Tukey's comparison test.

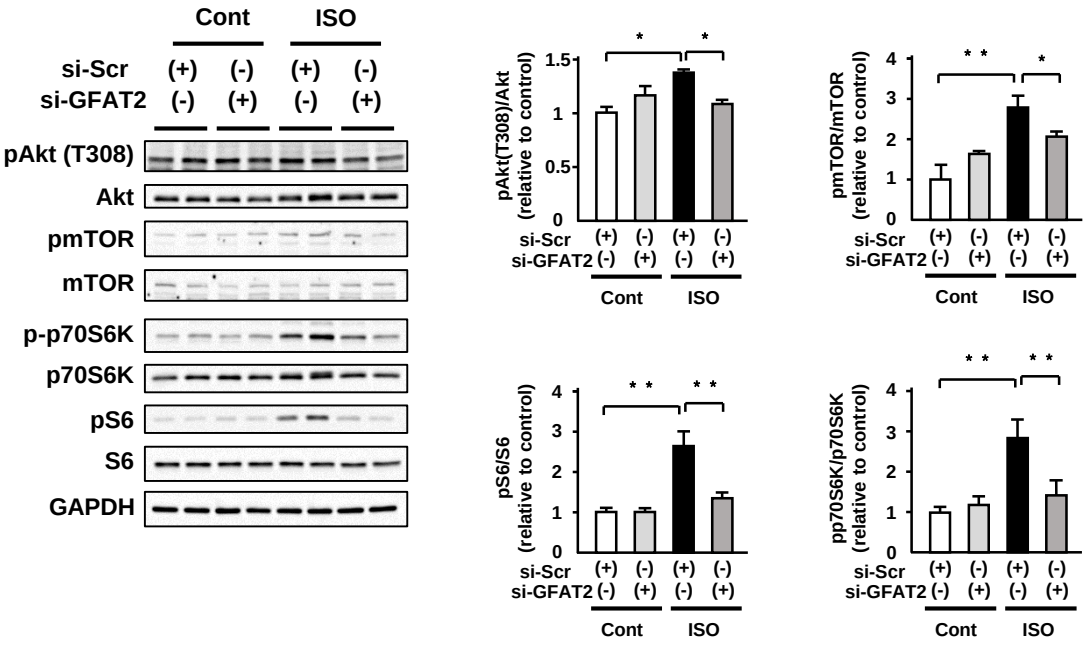

Fig. S5. The effect of GFAT2 knockdown on ISO-induced phosphorylation of Akt(T308), mTOR, p70S6K, and S6 in cardiomyocytes. Related to Figure 3. Representative immunoblots of Akt, phospho-Akt(T308), mTOR, phosphor-mTOR, p70S6K, phospho-p70S6K, S6, phosphor-S6, and GAPDH in NRVMs treated with indicated small interfering RNA (siRNA) in the presence or absence of ISO (1 μM, 12 hours). Quantitative analysis of phosphor-Akt(T308), phosphor-mTOR, phospho-p70S6K, and phosphor-S6 in NRVMs treated with indicated siRNA in the presence or absence of ISO (1 μM, 12 hours) (n=6). *P<0.05, **P<0.01: post-hoc Tukey's comparison test.

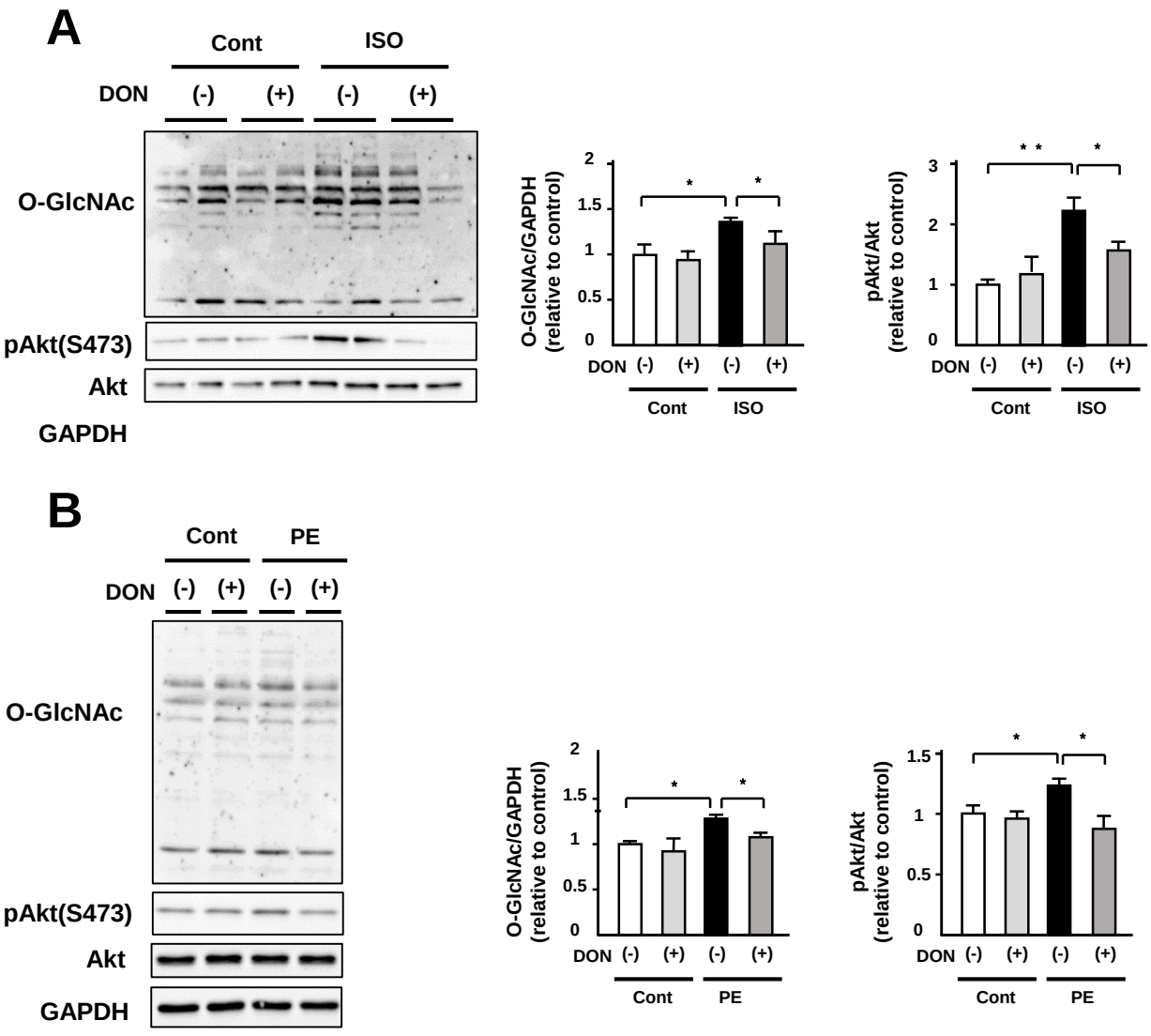

Fig. S6. The effect of DON on protein O-GlcNAcylation and phosphorylation of Akt(S473) in cardiomyocytes treated with ISO or PE. Related to Figure 3. (A) Representative immunoblots of protein O-GlcNAcylation, Akt, phosphor-Akt(S473), and GAPDH in NRVMs treated with DON (50 μM, 12 h) in the presence or absence of ISO (1 μM, 12 hours). Quantitative analysis of protein O-GlcNAcylation and phospho-Akt(S473) in NRVMs treated with DON in the presence or absence of ISO (1 μM, 12 hours) (n=6). (B) Representative immunoblots of protein O-GlcNAcylation, Akt, phospho-Akt(S473), and GAPDH in NRVMs treated with DON (50 μM, 12 h) in the presence or absence of PE (100 μM, 12 hours). Quantitative analysis of protein O-GlcNAcylation and phospho-Akt(S473) in NRVMs treated with DON in the presence or absence of PE (100 μM, 12 hours) (n=6). *P<0.05, **P<0.01: post-hoc Tukey's comparison test.

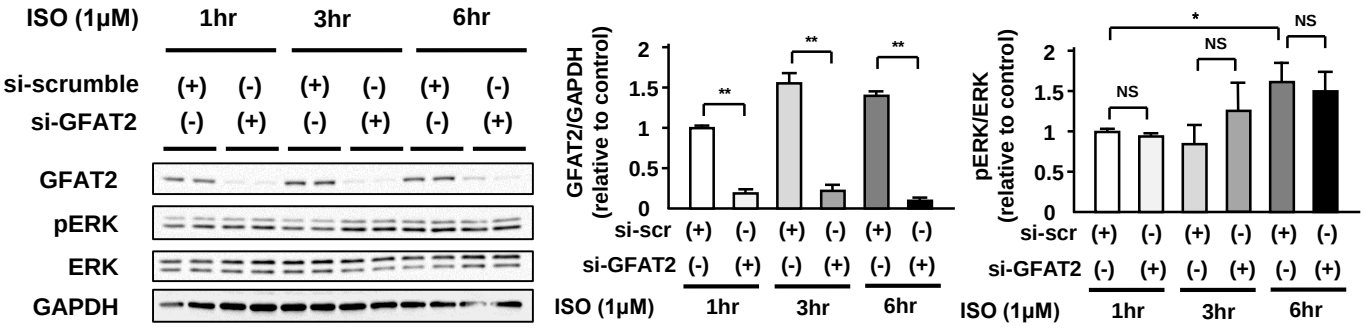

Fig. S7. Phosphorylation of ERK after ISO stimulation in cardiomyocytes. Related to Figure 3. (A) Representative immunoblots of GFAT2, ERK, phospho-ERK, and GAPDH in NRVMs treated with indicated small interfering RNA (siRNA) 1, 3, and 6 hours after ISO (1 μM) stimulation. Quantitative analysis of GFAT2 and phospho-ERK in NRVMs treated with indicated siRNA in the presence or absence of ISO (1 μM, 12 hours) (n=6). *P<0.05, **P<0.01: post-hoc Tukey's comparison test.

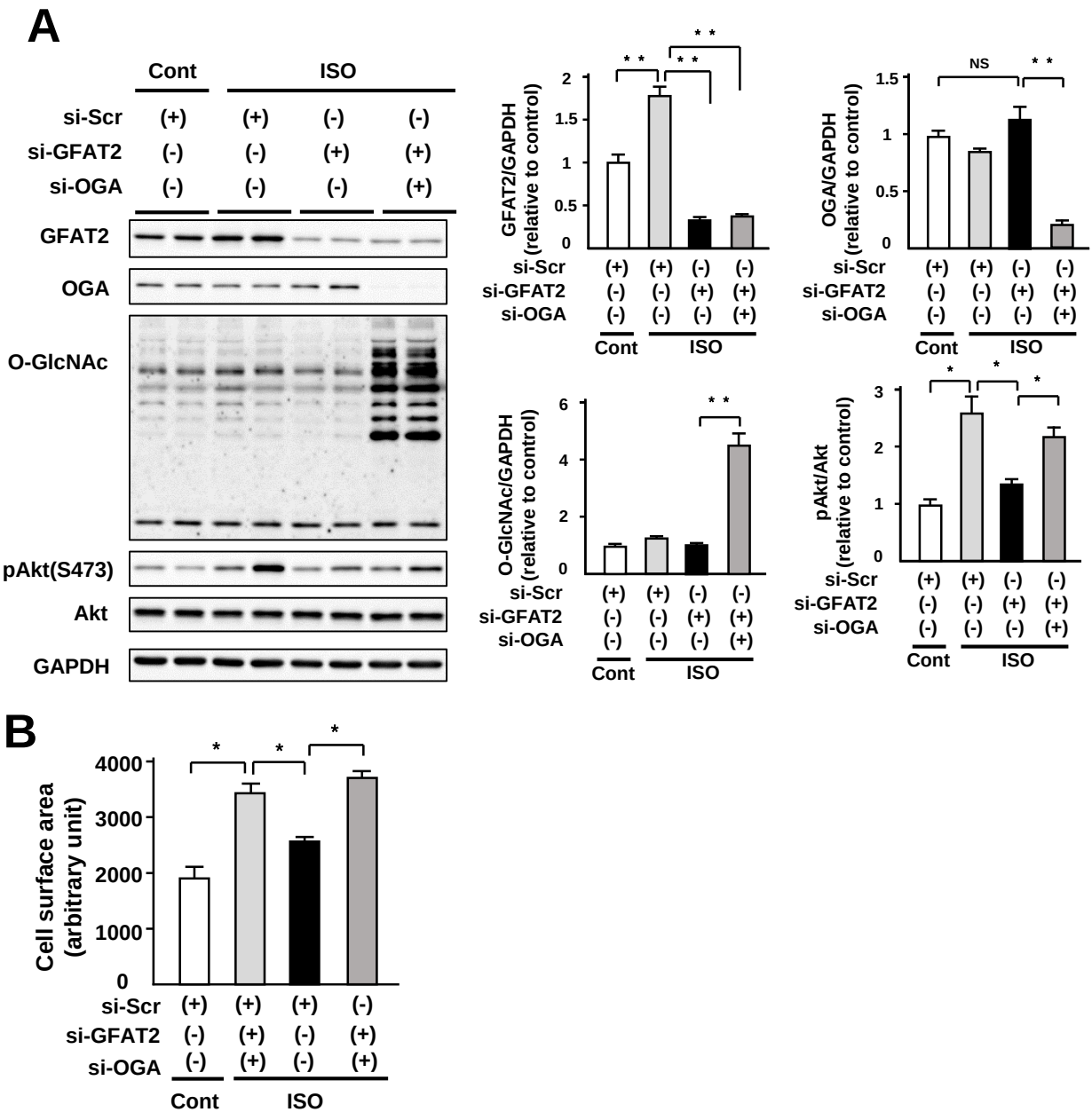

Fig. S8. Knockdown of OGA cancelled anti-hypertrophic effect by knockdown of GFAT2. Related to Figure 3. **(A)** Representative immunoblots of GFAT2, OGA, protein O-GlcNAcylation, Akt, phospho-Akt(S473), and GAPDH in NRVMs treated with indicated small interfering RNA (siRNA) in the presence or absence of ISO (1 μM, 12 hours). Quantitative analysis of GFAT2, OGA, protein O-GlcNAcylation and phospho-Akt(S473) in NRVMs treated with DON in the presence or absence of ISO (1 μM, 12 hours) (n=6). **(B)** Cell surface area of NRVMs treated with indicated siRNA in the presence or absence of ISO (1 μM, 12 hours) (n=150 cells in each group). *P<0.05, **P<0.01: post-hoc Tukey's comparison test.

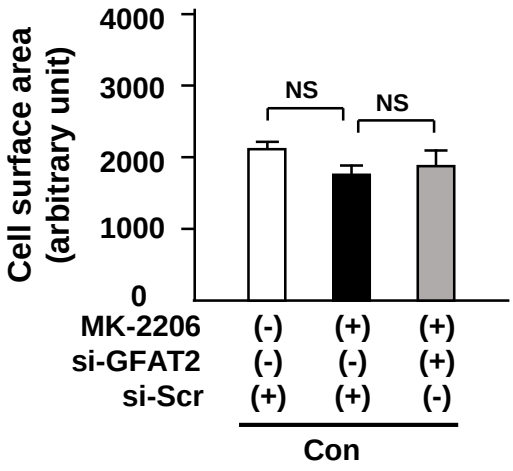

Fig. S9. The role of GFAT2 and Akt in cardiomyocyte surface area at baseline. Related to Figure 3. Cell surface area of NRVMs treated with GFAT2 siRNA and/or MK-2206 at baseline (n=150 cells in each group). *P<0.05, **P<0.01: post-hoc Tukey's comparison test.

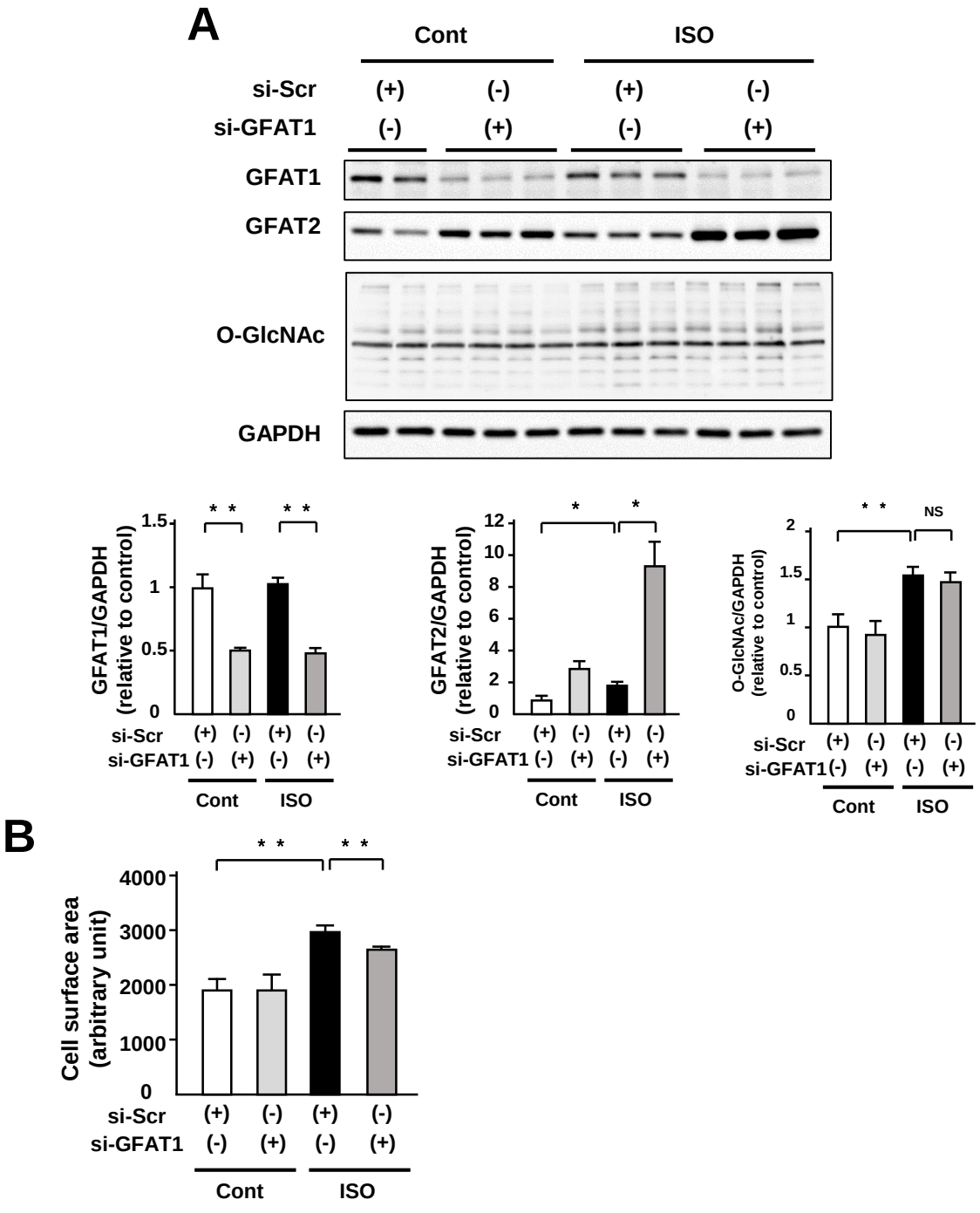

Fig. S10. The role of GFAT1 in ISO-induced protein O-GlcNAcylation and cardiomyocyte hypertrophy. Related to Figure 3. **(A)** Representative immunoblots of GFAT1, GFAT2, protein O-GlcNAcylation, and GAPDH in NRVMs treated with indicated small interfering RNA (siRNA) in the presence or absence of ISO (1 μM, 12 hours). Quantitative analysis of GFAT1, GFAT2 , and protein O-GlcNAcylation in NRVMs treated with indicated siRNA in the presence or absence of ISO (1 μM, 12 hours) (n=6). **(B)** Cell surface area of NRVMs treated with indicated siRNA in the presence or absence of ISO (1 μM, 12 hours) (n=150 cells in each group). *P<0.05, **P<0.01: post-hoc Tukey's comparison test.

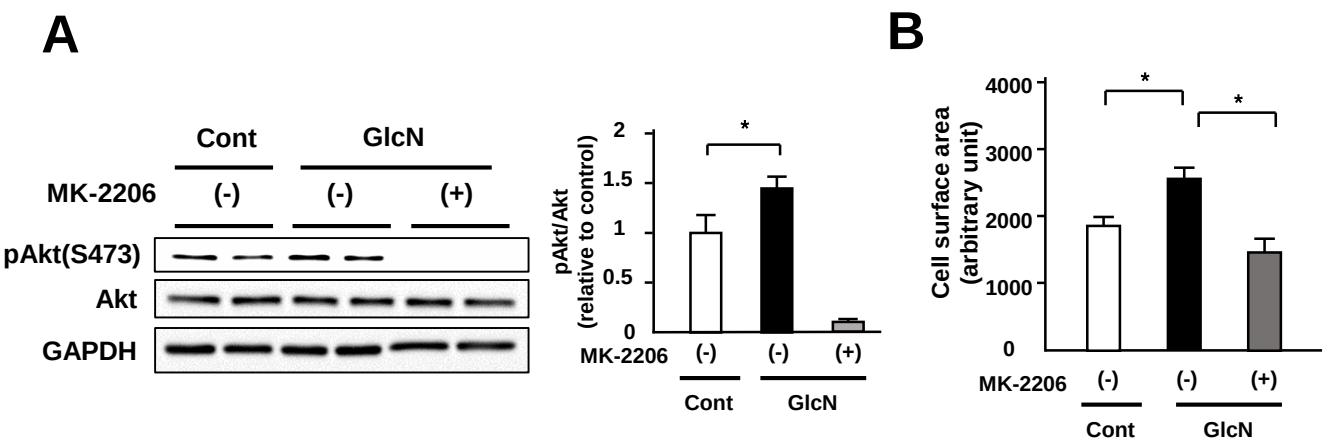

Fig. S11. Akt inhibition attenuated glucosamine-induced cardiomyocyte hypertrophy. Related to Figure 4. **(A)** Representative immunoblots of Akt, phospho-Akt(S473), and GAPDH in NRVMs treated with MK-2206 in presence or absence of glucosamine (50 mM, 48 hours). Quantitative analysis of phospho-Akt(S473) in NRVMs treated with MK-2206 in presence or absence of glucosamine (50 mM, 48 hours) (n=6). **(B)** Cell surface area of NRVMs treated with MK-2206 in presence or absence of glucosamine (50 mM, 48 hours) *P<0.05, **P<0.01: post-hoc Tukey's comparison test.

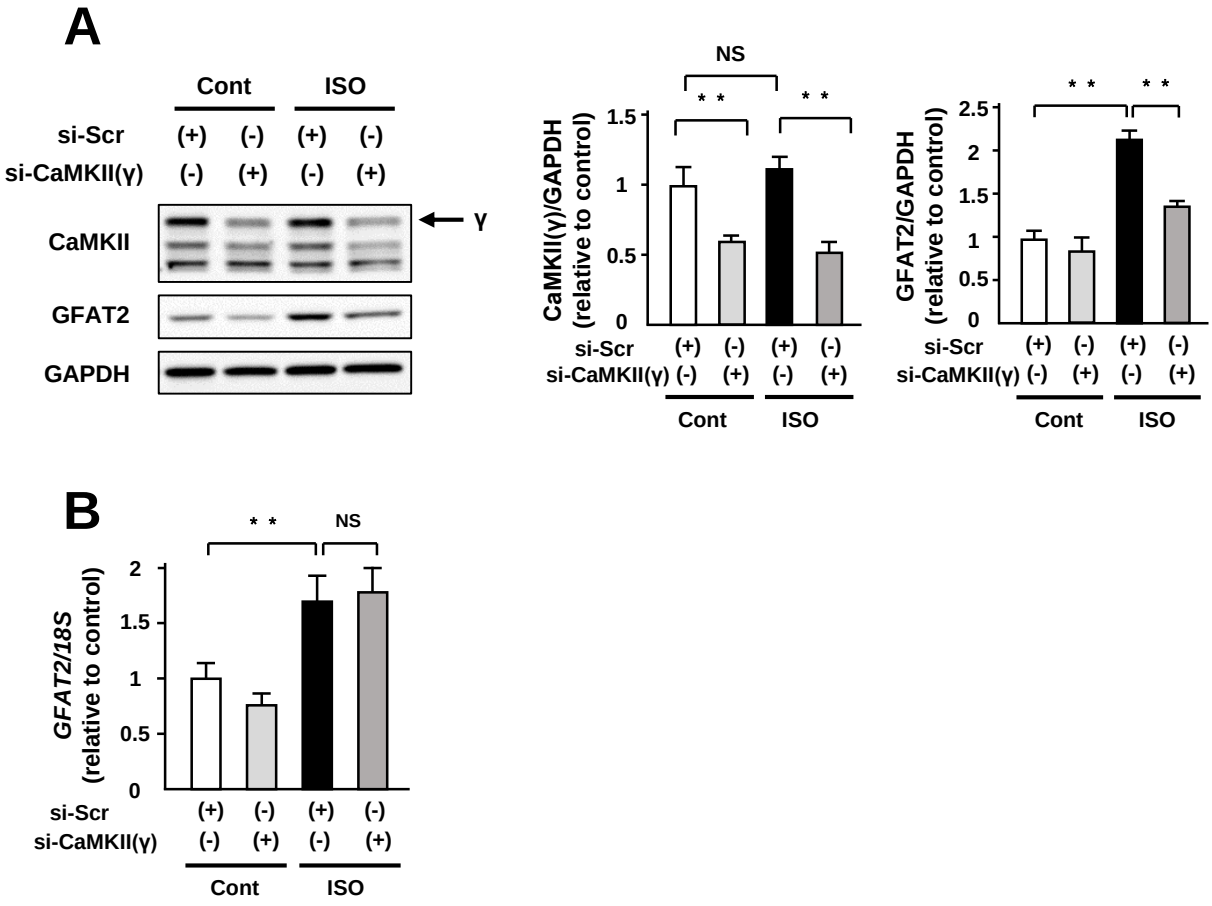

Fig. S12. CaMKIIγ positively regulates GFAT2 in cardiomyocyte hypertrophy. Related to Figure 5. **(A)** Representative immunoblots of CaMKII, GFAT2, and GAPDH in NRVMs treated with siRNA for CaMKIIγ in the presence or absence of ISO (1 μM, 12 hours). Quantitative analysis of protein CaMKIIγ and GFAT2 in indicated groups (n=5). (B) Expression of *GFAT2* and *18S* mRNA in NRVMs treated with siRNA for CaMKIIγ in the presence or absence of ISO (1 μM, 12 hours) (n=5). *P<0.05, **P<0.01: post-hoc Tukey's comparison test.

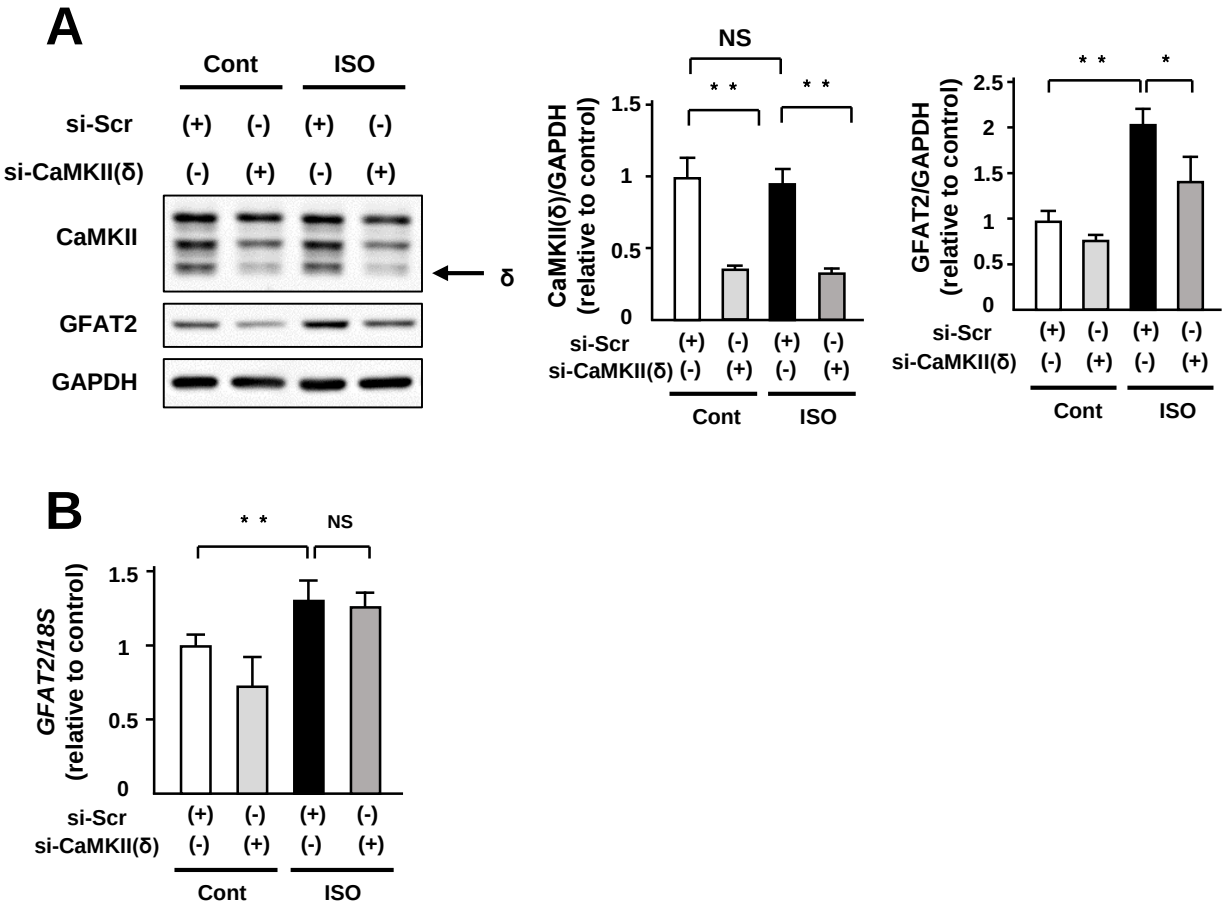

Fig. S13. CaMKIIδ positively regulates GFAT2 in cardiomyocyte hypertrophy. Related to Figure 5. **(A)** Representative immunoblots of CaMKII, GFAT2 and GAPDH in NRVMs treated with siRNA for CaMKIIδ in the presence or absence of ISO (1 μM, 12 hours). Quantitative analysis of protein CaMKIIδ and GFAT2 in indicated groups (n=5). **(B)** Expression of *GFAT2* and *18S* mRNA in NRVMs treated with siRNA for CaMKIIδ in the presence or absence of ISO (1 μM, 12 hours) (n=5). *P<0.05, **P<0.01: post-hoc Tukey's comparison test.

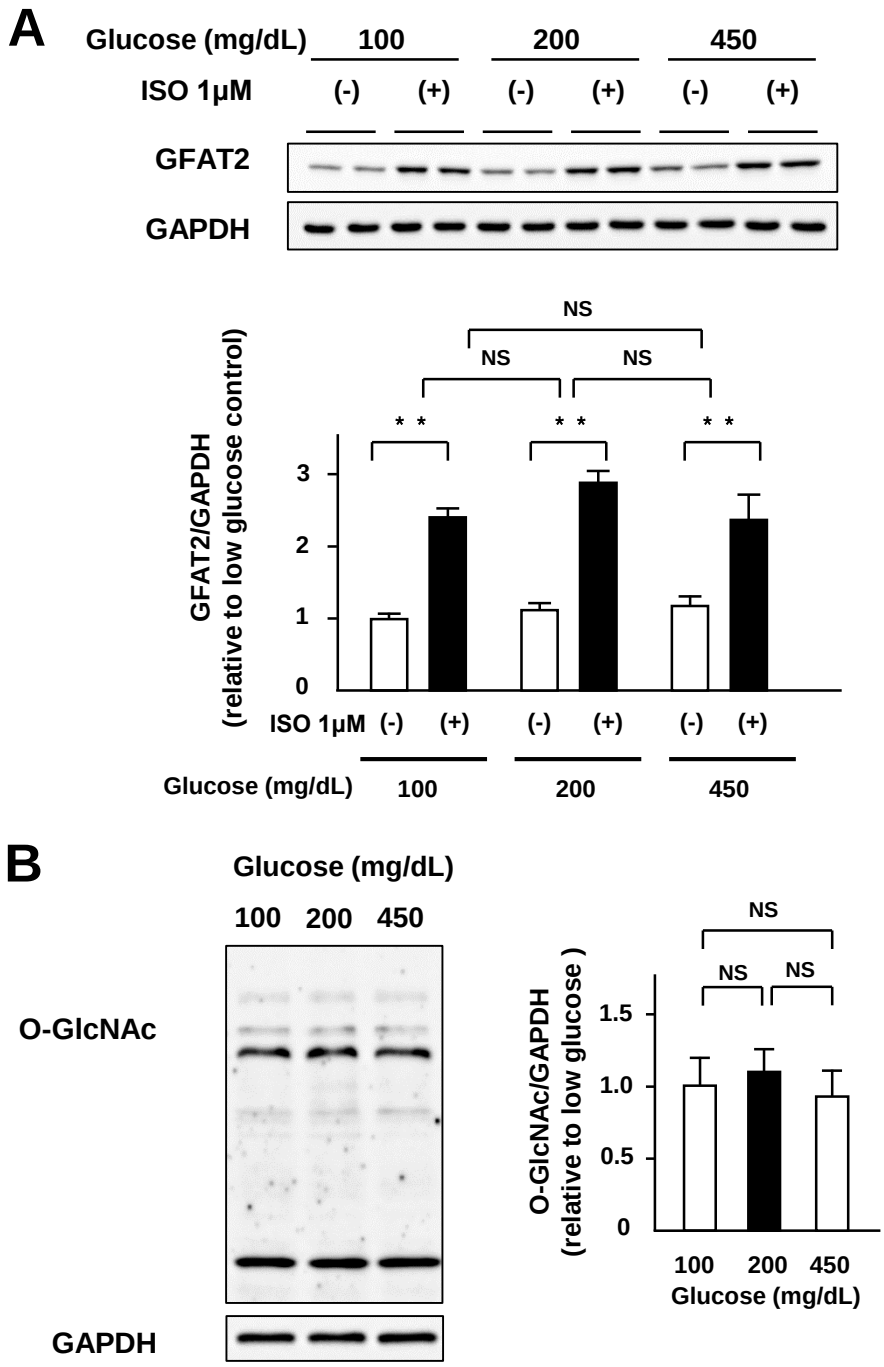

Fig. S14. Hyperglycemia does not affect GFAT2 in cardiomyocytes. Related to Figure 5. **(A)** Representative immunoblots of GFAT2 and GAPDH in NRVMs treated with glucose at multiple concentrations in the presence or absence of ISO (1 μM, 12 hours). Quantitative analysis of GFAT2 in NRVMs treated with glucose at multiple concentrations in presence or absence of ISO (1 μM, 12 hours) (n=6). **(B)** Representative immunoblots of protein O-GlcNAcylation and GAPDH in NRVMs treated with glucose at multiple concentrations. Quantitative analysis of protein O-GlcNAcylation in NRVMs treated with glucose at multiple concentrations in presence or absence of ISO (1 μM, 12 hours) (n=6). *P<0.05, **P<0.01: post-hoc Tukey's comparison test.

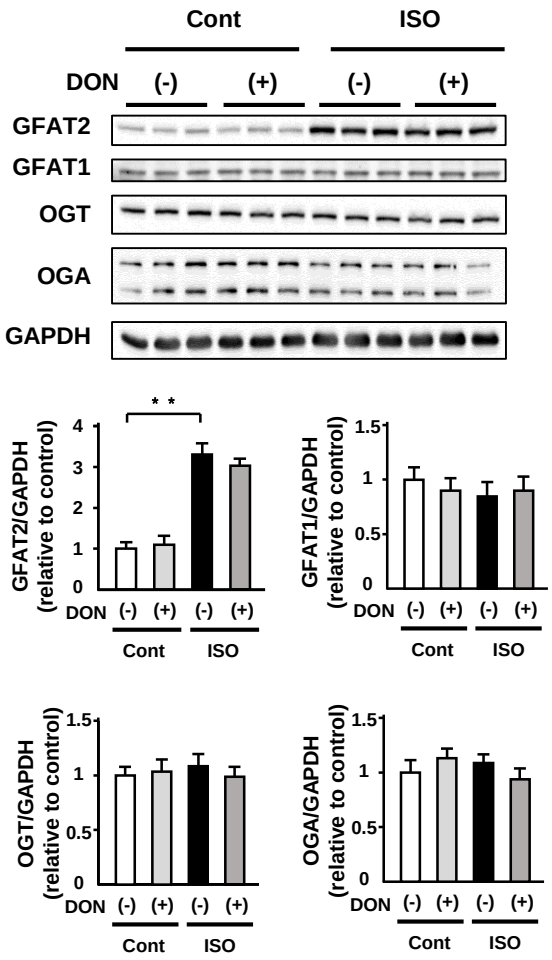

Fig. S15. DON does not alter GFAT1, GFAT2, OGT, and OGA in ISO-induced cardiac hypertrophy. Related to Figure 6. Representative immunoblots of GFAT2, GFAT1, OGT, OGA, and GAPDH in ISO (15 mg/kg body weight/day, 7 days)- or control vehicle (saline)-treated C57B/6J mouse hearts subjected to either DON (0.05 mg/kg body weight/day) or saline for 7 days. Quantitative analysis of GFAT2, GFAT1, OGT, and OGA in ISO- or control vehicle-treated C57B/6J mouse hearts subjected to either DON (0.05 mg/kg body weight/day) or saline for 7 days. (n=9). *P<0.05, **P<0.01: post-hoc Tukey's comparison test.

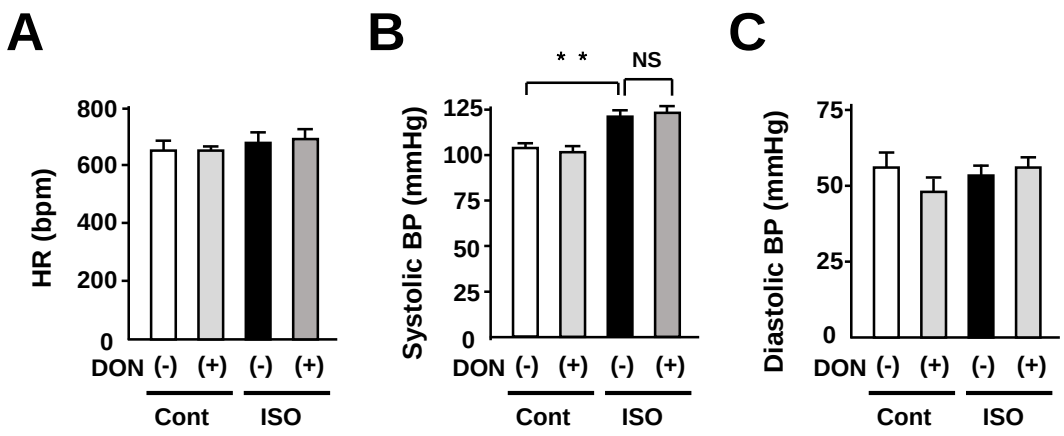

Fig. S16. Hemodynamics in mice treated with isoproterenol with or without DON. Related to Figure 6. **(A-C)** Heart rate, systolic blood pressure, and diastolic blood pressure in ISO (15 mg/kg body weight/day, 7 days)- or control vehicle(saline)- treated C57B/6J mouse hearts subjected to either DON (0.05 mg/kg body weight/day) or saline for 7 days. *P<0.05, **P<0.01: post-hoc Tukey's comparison test.

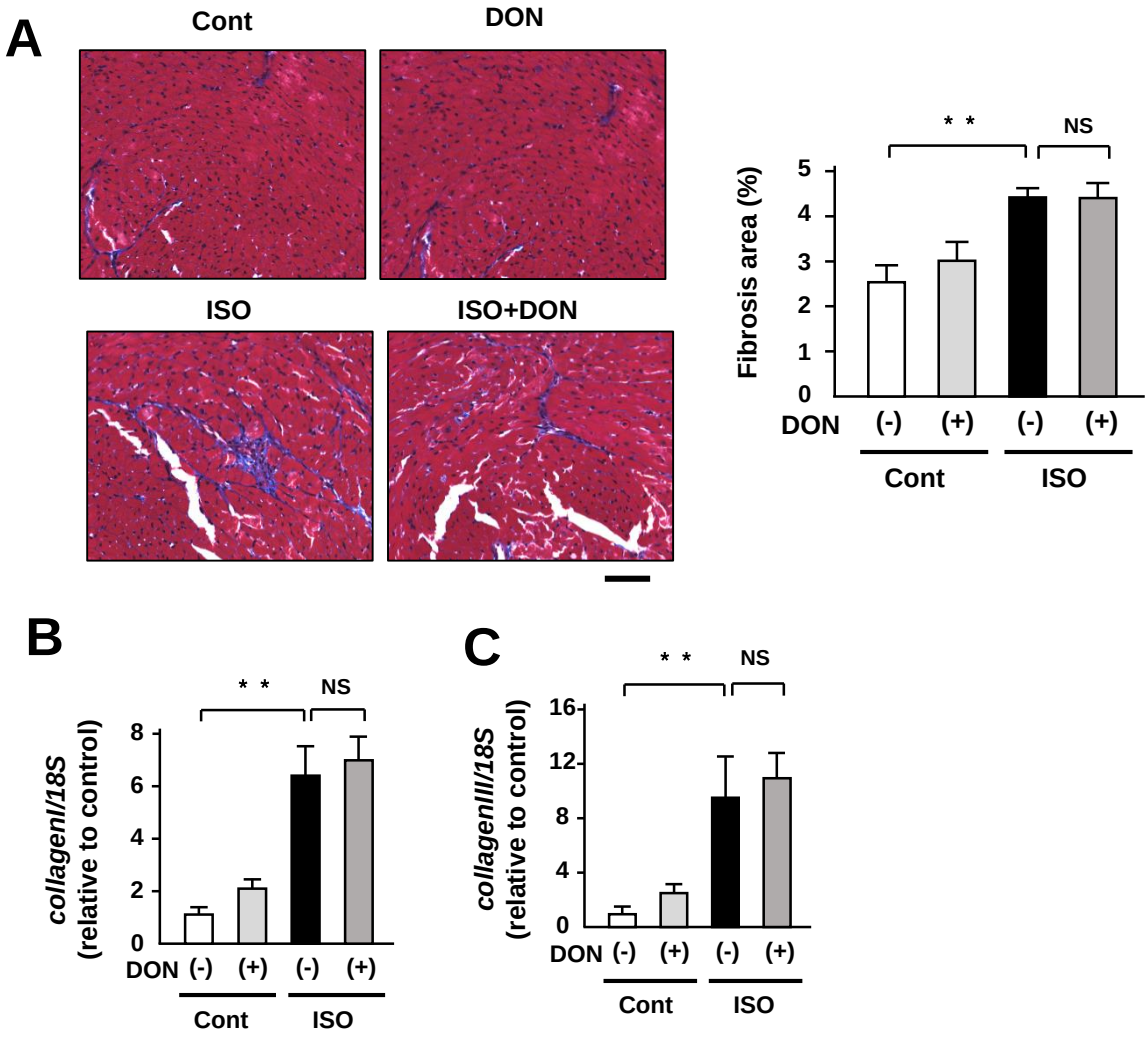

Fig. S17. Fibrosis in mouse hearts treated with isoproterenol with or without DON. Related to Figure 6. (A) Masson-trichrome-stained heart section in in ISO (15 mg/kg body weight/day)- or control vehicle (saline)- treated C57B/6J mouse hearts subjected to either DON (0.05 mg/kg body weight/day) or saline for 7 days. Interstitial fibrosis as assessed by fibrosis area in each group (n=6). Bar=50μm **(B and C)** Expression of *collagen I*, *collagen III*, and *18S* mRNA in the indicated mouse hearts (n=6). *P<0.05, **P<0.01: post-hoc Tukey's comparison test.

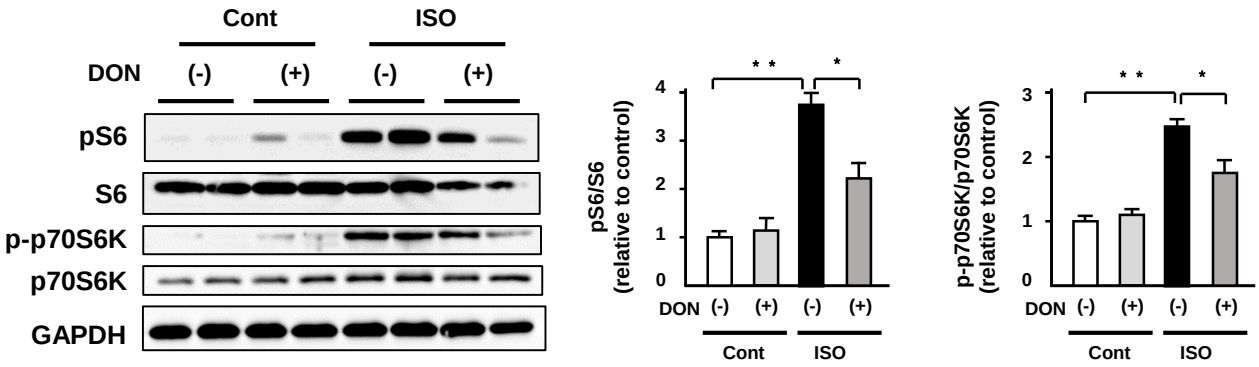

Fig. S18. DON attenuated ISO-induced phosphorylation of S6 and p70S6K in cardiomyocytes. Related to Figure 7. Representative immunoblots of S6, phospho-S6, p70S6K, phospho-p70S6K, and GAPDH in NRVMs treated with DON (50 μM, 12 h) in presence or absence of ISO (1 μM, 12 hours). Quantitative analysis of phospho-S6 and phospho-p70S6K in NRVMs treated with DON (50 μM, 12 h) in presence or absence of ISO (1 μM, 12 hours) (n=6). *P<0.05, **P<0.01: post-hoc Tukey's comparison test.

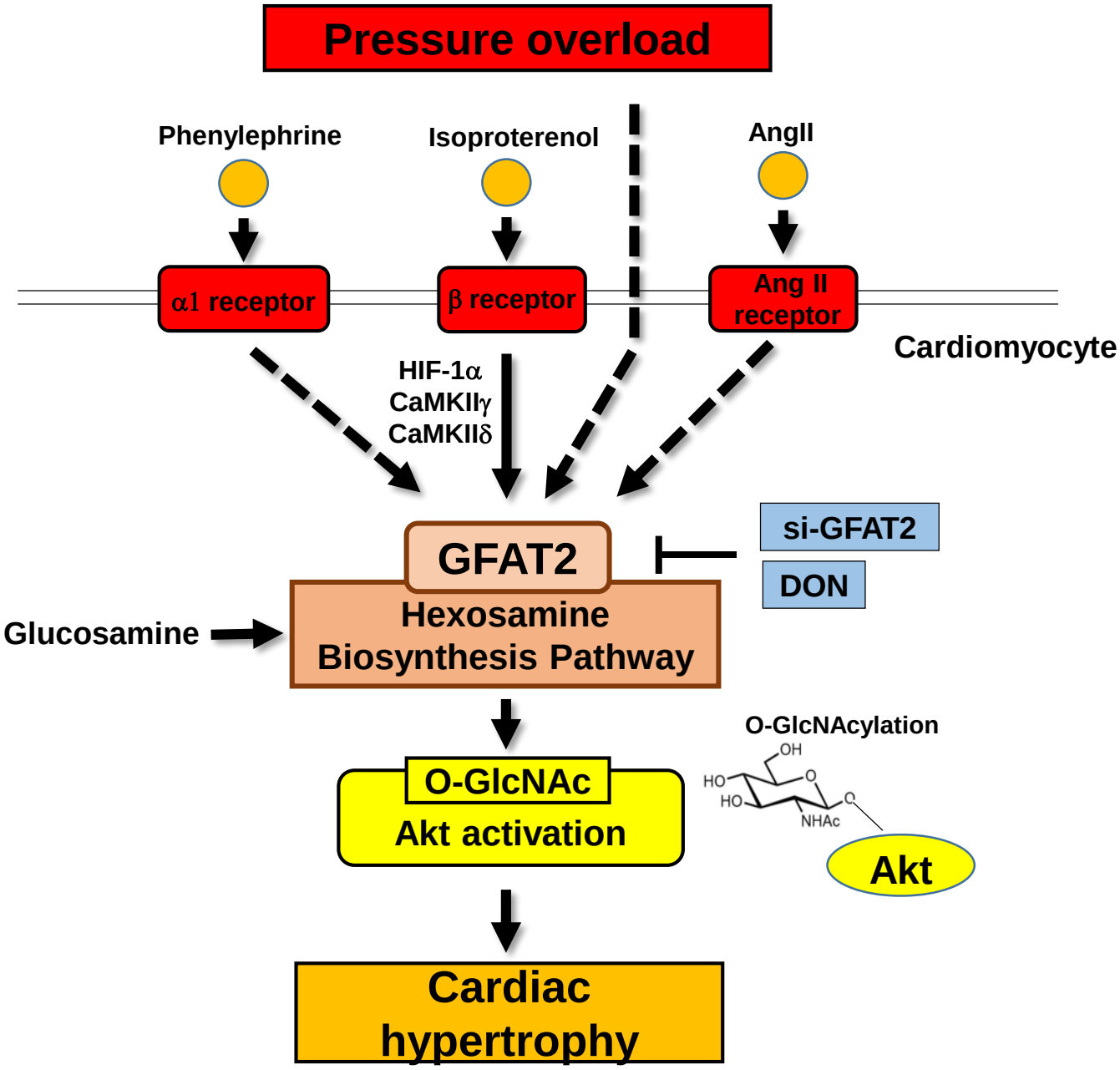

Fig. S19. A schematic representation of GFAT2-mediated signaling pathway in cardiac hypertrophy. GFAT2 mediates cardiac hypertrophy through protein O-GlcNAcylation and Akt activation. GFAT2 is regulated by HIF-1a, CaMKIIδ, and CaMKIIγ. GFAT2-O-GlcNac-Akt pathway is a potential target against cardiac hypertrophy.

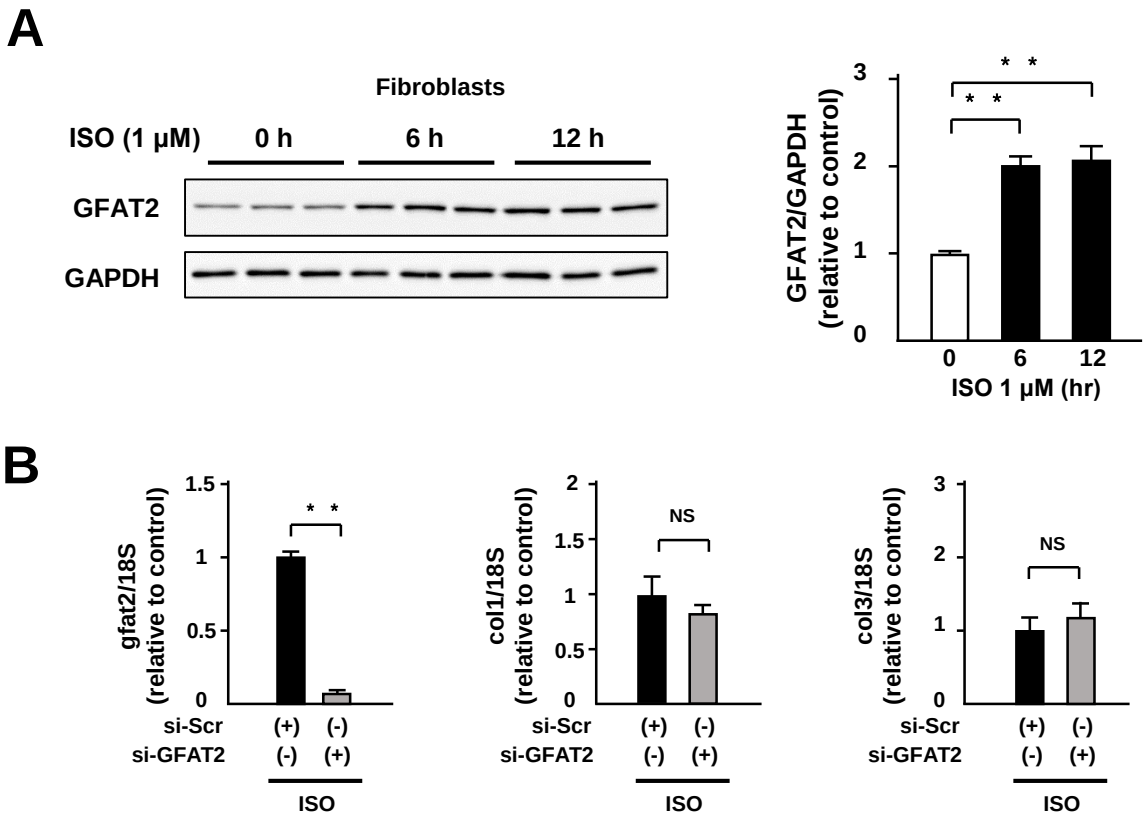

Fig. S20. Knockdown of GFAT2 did not affect collagen expression in fibroblasts. Related to Figure 2. **(A)** Representative immunoblots ofGFAT2 and GAPDH in cardiac fibroblasts at indicated time points after ISO (1 μM) administration. Quantitative analysis of GFAT2 in indicated groups (n=5). **(B)** Expression of *gfat2*, col1, and col3 mRNA in cardiac fibroblasts 12 hours after ISO (1 μM) administration. (n=5). *P<0.05, **P<0.01: post-hoc Tukey's comparison test.

Gene	Forward primer	Reverse primer
18S(rat)	5’- AAGTTTCAGCACATCCTGCGAGTA -3’	5’- TTGGTGAGGTCAATGTCTGCTTTC -3
ANF(rat)	5-TGACAGGATTGGAGCCCAGAG -3’	5’- TCGATCGTGATAGATGAAGACAGGA-3’
GFAT1(rat)	5’-GGCGACAGAGCTTTACCACCA-3’	5’-CACCAGCAAGGATGCCTTCA-3’
GFAT2(rat)	5’-CATCCGTGGCCTCAGATCTTTA-3’	5’-CTTCCAGGCATGTGGCATAGTTA-3’
18S(mus)	5’-TTCTGGCCAACGGTCTAGACAAC -3’	5’-CCAGTGGTCTTGGTGTGCTGA -3’
ANF(mus)	5’-TGACAGGATTGGAGCCCAGA-3’	5’-GACACACCACAAGGGCTTAGGA-3’
GFAT1(mus)	5’-GCAGAGCTAGACCCTCAGAGCAA -3’	5’-CCCAGCATTTAATACCTCAGCACAG-3’
GFAT2(mus)	5’-TCCTGAGCGTGATTCCACTCC-3’	5’-GCAGGTGACGACAGTCTTGTGATAG -3’
CollagenI(mus)	5’-GACATGTTCAGCTTTGTGGACCTC-3’	5’-GGGACCCTTAGGCCATTGTGTA-3’
CollagenIII(mus)	5’-CAGGAGCCAGTGGCCATAA-3’	5’-TCTCGACCTGGCTGACCATC-3’

Table S1. qPCR Primer Sequences. Related to STAR Methods.

	Cont	ISO	P value
Wall thickness (mm)	0.63±0.03	1.06±0.02	<0.01
LV diastolic diameter (mm)	3.53±0.13	3.66±0.14	0.52
LV systolic diameter (mm)	2.34±0.12	2.32±0.14	0.93
LVEF (%)	63.9±1.60	67.3±2.62	0.28
Heart weight / tibial length (mg/mm)	5.67±0.12	7.70±0.09	<0.01
LV weight / tibial length (mg/mm)	4.13±0.09	5.38±0.05	<0.01

Table S2. Echocardiographic data and organ weight in ISO-treated mice. Related to Figure 1. Isoproterenol (ISO, 15 mg/kg body weight/day) or vehicle (saline) was continuously infused into 8 to 9-week-old mice using a miniosmotic pump (model 1007D, Alzet) for 7 days (n=6). LV wall thickness, LV diastolic diameter, LV systolic diameter, and LVEF were evaluated by echocardiography. Heart weight and LV weight were adjusted by tibial length. Data shown as mean ± SEM. *P<0.05, **P<0.01: post-hoc Tukey's comparison test.

	Cont	PE	P value
Wall thickness (mm)	0.68±0.03	1.06±0.07	<0.01
LV diastolic diameter (mm)	3.83±0.10	3.16±0.04	<0.01
LV systolic diameter (mm)	2.68±0.07	2.00±0.06	<0.01
LVEF (%)	57.7±1.66	67.5±2.43	<0.01
Heart weight / tibial length (mg/mm)	5.42±0.11	7.87±0.61	<0.01
LV weight / tibial length (mg/mm)	3.75±0.08	5.17±0.26	<0.01

Table S3. Echocardiographic data and organ weight in PE-treated mice. Related to Figure 1. Phenylephrine (PE, 100 mg/kg body weight/day) or vehicle (saline) was continuously infused into 9 to 10-week-old mice using a miniosmotic pump (model 1007D, Alzet) for 7 days (n=5). LV wall thickness, LV diastolic diameter, LV systolic diameter, and LVEF were evaluated by echocardiography. Heart weight and LV weight were adjusted by tibial length. Data shown as mean ± SEM. *P<0.05, **P<0.01: post-hoc Tukey's comparison test.

	Cont	Ang II	P value
Wall thickness (mm)	0.69±0.01	0.92±0.09	<0.01
LV diastolic diameter (mm)	3.46±0.02	3.12±0.03	<0.01
LV systolic diameter (mm)	2.26±0.03	2.03±0.03	<0.01
LVEF (%)	64.7±1.39	65.6±0.95	0.62
Heart weight / tibial length (mg/mm)	5.22±0.16	6.18±0.08	<0.01
LV weight / tibial length (mg/mm)	3.83±0.12	4.67±0.06	<0.01

Table S4. Echocardiographic data and organ weight in Ang II-treated mice. Related to Figure 1. Angiotensin Ⅱ (Ang Ⅱ, 1.44 mg/kg body weight/day) or vehicle (saline) was continuously infused into 8 to 9-week-old mice using a miniosmotic pump (model 1007D, Alzet) for 7 days (n=5). LV wall thickness, LV diastolic diameter, LV systolic diameter, and LVEF were evaluated by echocardiography. Heart weight and LV weight were adjusted by tibial length. Data shown as mean ± SEM. *P<0.05, **P<0.01: post-hoc Tukey's comparison test.

	Cont	TAC	P value
Wall thickness (mm)	0.69±0.02	1.03±0.02	<0.01
LV diastolic diameter (mm)	3.57±0.16	3.64±0.15	0.52
LV systolic diameter (mm)	2.26±0.11	2.50±0.13	0.93
LVEF (%)	67.2±1.10	60.2±1.70	0.28
Heart weight / tibial length (mg/mm)	5.23±0.14	7.03±0.35	<0.01
LV weight / tibial length (mg/mm)	4.07±0.13	5.52±0.31	<0.01

Table S5. Echocardiographic data and organ weight in TAC-operated mice. Related to Figure 1. Aortic constriction was performed by ligation of the transverse thoracic aorta between the innominate artery and left common carotid artery with a 28-gauge needle using a 7-0 nylon suture in 8 to 9-week-old mice (n=5). Sham mice had their aorta mobilized, but no suture tightened (n=5). We performed our experiments after 7 days of TAC. LV wall thickness, LV diastolic diameter, LV systolic diameter, and LVEF were evaluated by echocardiography. Heart weight and LV weight were adjusted by tibial length. Data shown as mean ± SEM. *P<0.05, **P<0.01: post-hoc Tukey's comparison test.