l-Asparaginase regulates mTORC1 activity via a TSC2-dependent pathway in pancreatic beta cells

Seike, Masako ; Asahara, Shun-ichiro ; Inoue, Hiroyuki ; Kudo, Michiyo ; Kanno, Ayumi ; Yokoi, Aisha ; Suzuki, Hirotaka ; Kimura-Koyanagi,…

(Citation)
Biochemical and Biophysical Research Communications,652:121-130

(Issue Date)
2023-04-16

(Resource Type)
journal article

(Version)
Accepted Manuscript

(URL)
https://hdl.handle.net/20.500.14094/0100482075

L-Asparaginase Regulates mTORC1 Activity via a TSC2-dependent Pathway in Pancreatic Beta Cells

Masako Seike[1], Shun-ichiro Asahara[1*], Hiroyuki Inoue[2], Michiyo Kudo[2], Ayumi Kanno[1], Aisha Yokoi[1], Hirotaka Suzuki[1], Maki Kimura-Koyanagi[1], Yoshiaki Kido[2], Wataru Ogawa[1]

[1]Division of Diabetes and Endocrinology, Department of Internal Medicine, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe, Hyogo 650-0017, Japan; Email: koto0125@med.kobe-u.ac.jp (M.S.), asahara@med.kobe-u.ac.jp (S.A.), akanno@med.kobe-u.ac.jp (A.K.), hirany@med.kobe-u.ac.jp (A.Y.), shirotak@med.kobe-u.ac.jp (H.S.), koyanagi@med.kobe-u.ac.jp (M.K.-K.), ogawa@med.kobe-u.ac.jp (W.O.)

[2]Division of Medical Chemistry, Department of Metabolism and Diseases, Kobe University Graduate School of Health Sciences, 7-10-2 Tomogaoka, Suma-ku, Kobe, Hyogo 654-0142, Japan; Email: h.inoue310@gmail.com (H.I.), 1483815m.kudo@gmail.com (M.K.), kido@med.kobe-u.ac.jp (Y.K.)

*Corresponding author: Shun-ichiro Asahara

Division of Diabetes and Endocrinology, Department of Internal Medicine, Kobe University Graduate School of Medicine, 7-5-1 Kusunoki-cho, Chuo-ku, Kobe, Hyogo 650-0017, Japan

Tel.: +81-78-382-5861; Fax: +81-78-382-2080; E-mail: asahara@med.kobe-u.ac.jp

Abstract

Eif2ak4, a susceptibility gene for type 2 diabetes, encodes GCN2, a molecule activated by amino acid deficiency. Mutations or deletions in GCN2 in pancreatic β-cells increase mTORC1 activity by decreasing Sestrin2 expression in a TSC2-independent manner. In this study, we searched for molecules downstream of GCN2 that suppress mTORC1 activity in a TSC2-dependent manner. To do so, we used a pull-down assay to identify molecules that competitively inhibit the binding of the T1462 phosphorylation site of TSC2 to 14-3-3. L-asparaginase was identified. Although L-asparaginase is frequently used as an anticancer drug for acute lymphoblastic leukemia, little is known about endogenous L-asparaginase. L-Asparaginase, which is expressed downstream of GCN2, was found to bind 14-3-3 and thereby to inhibit its binding to the T1462 phosphorylation site of TSC2 and contribute to TSC2 activation and mTORC1 inactivation upon TSC2 dephosphorylation. Further investigation of the regulation of mTORC1 activity in pancreatic β-cells by L-asparaginase should help to elucidate the mechanism of diabetes and insulin secretion failure during anticancer drug use.

Keywords

Pancreatic β-cell, TSC2, L-Asparaginase, mTORC1 activity, 14-3-3, GCN2

Introduction

In recent years, the number of patients with type 2 diabetes has increased dramatically worldwide, becoming a major problem due to the associated increase in cardiovascular complications and cancer risk. The primary pathophysiological factors of type 2 diabetes are insulin resistance and insulin insufficiency. Insufficient insulin secretion has been shown to be important, particularly in East Asians. Using various animal models, we previously reported that decreased pancreatic β-cell mass plays an important role in the development of type 2 diabetes [1-3]. Decreased expression of the non-coding RNA Kcnq1ot1—which is expressed in the gene region of *Kcnq1*, one of the candidate genes for type 2 diabetes mellitus—has also been shown to cause decreased pancreatic β-cell mass and impaired glucose tolerance, but only when the mutation is inherited from the father [4].

We also reported the mechanism by which the eukaryotic translation initiation factor 2α kinase 4 (*Eif2ak4*) gene—which was reported to be a susceptibility gene for type 2 diabetes in 2009 [5]—causes the development of type 2 diabetes: the *Eif2ak4* gene encodes general control nonderepressible 2 (GCN2), which is activated by amino acid deficiency in the cell [6]. In systemic GCN2-deficient mice, pancreatic β-cell mass was found to be decreased by a high-fat diet (HFD), although the phenotype was not affected by normal diet feeding. Similar results were observed in mice lacking pancreatic β-cell-specific GCN2 [7]. GCN2 in pancreatic islets is not activated by a normal diet but is activated by a HFD. This was thought to be due to decreased intracellular amino acid concentrations in pancreatic β-cells because insulin synthesis in pancreatic β-cells is enhanced by a HFD [8].

The decreased pancreatic β-cell volume in HFD-fed GCN2-deficient mice may be due to GCN2 deficiency in pancreatic β-cells, which induces constitutive mechanistic target of

---

Abbreviations: PCR, polymerase chain reaction; SEM, standard error of the man; siRNA, small interfering RNA

rapamycin complex 1 (mTORC1) activation. We previously reported that mice with constitutive mTORC1 activation in pancreatic β-cells show increased insulin secretory capacity and increased pancreatic β-cell volume at a young age but decreased pancreatic β-cell volume and insulin secretory failure at an old age [9]. In addition, we demonstrated that this is due to decreased insulin signaling and impaired autophagy by negative feedback in pancreatic β-cells [10, 11]. Decreased insulin signaling has also been observed in islets of HFD-fed GCN2-deficient mice, along with increased mTORC1 activity [7]. In a recent report, we found that GCN2 in pancreatic β-cells suppresses mTORC1 activity in a tuberous sclerosis complex 2 (TSC2)-independent manner via enhanced Sestrin2 expression during a high-fat diet [7]. In other words, in islets of HFD-fed GCN2-deficient mice, mTORC1 is activated because GATOR2, which suppresses mTORC1, is repressed by decreased Sestrin2 expression [12].

Although we have elucidated the GCN2-mediated TSC2-independent regulation of mTORC1 by Sestrin2 in pancreatic β-cells upon a high-fat diet, the existence of a TSC2-dependent mTORC1 regulatory mechanism in the absence of GCN2 should also be investigated. We believe that TSC2 plays a particularly important role in pancreatic β-cells. We previously reported that pancreatic β-cell-specific TSC2-deficient mice exhibit a marked phenotype [9] characterized by hypoglycemia and hyperinsulinemia at young ages but progressive hyperglycemia and hypoinsulinemia at old ages, suggesting that a mechanism to maintain TSC2 inactivation in the regulation of mTORC1 activity may be very important. In the present study, we investigated how GCN2 deficiency regulates mTORC1 activity via TSC2-dependent pathways in pancreatic β-cells during a high-fat diet challenge.

Materials and Methods

Mice

Eif2ak4 generalized knockout mice (Accession No. CDB0538K) were generated as previously described (http://www2.clst.riken.jp/arg/Methods.html) [7]. Primers (forward and reverse, respectively) for genotyping generalized *Eif2ak4* null mice were P1 (5′-GTGGGTATGT GCACATGTGG ATAC-3′) and P2 (5′-CTTCCCTTTC CACTCCCAAA ATG-3′) for the wild-type allele and P3 (5′-CGCCTTCTTG ACGAGTTCTT CTG-3′) and P2 for the targeted allele, which yielded 453-bp and 665-bp products, respectively.

The mice were maintained on a 12-h light/12-h dark cycle. Normal chow diet and HFD were obtained from CLEA Japan and Oriental Bio, respectively, and the mice were fed the respective diet from weaning. All experiments were performed with male mice. The study followed the ARRIVE guidelines and the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Animal Ethics Committee of Kobe University Graduate School of Medicine.

Cell culture and treatment

INS-1 cells were kindly provided by Professor Kazuya Yamagata (Kumamoto University, Japan) and maintained in RPMI 1640 medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum.

Isolation of pancreatic islets

Pancreatic islets were isolated by collagenase digestion (Collagenase P; Roche) and Histopaque (Sigma-Aldrich) density gradient centrifugation as previously described [3, 4, 7].

Transfection with small interfering RNA

INS-1 cells were transfected with small interfering RNAs (siRNAs) targeting GCN2, ATF4, and Sestrin2 (SMARTpool; Dharmacon) as previously described [3].

Preparation of 14-3-3β expression vector

14-3-3β cDNA was synthesized using Prime Star Max Premix (TakaRa) with MIN6 total RNA as template. 14-3-3β cDNA synthesis primers were as follows: forward, 5′-TAGGATCCAC ATGGGCTGCA GCTATGAAG-3′, and reverse, 5′-TAGGATCCTT AGTTCTCTCC CTCTCCAG-3′. After insertion into the BamHI site of pBlueScript, the 14-3-3β cDNA was inserted into the multiple cloning site of pFN21A HaloTag CMV Flexi Vector (Promega).

Immunoblot analysis

Lysates of isolated islets or INS-1 cells were prepared as previously described [11]. The lysates were probed with antibodies against the following proteins: phospho-TSC2 (Thr^{1462}) (#2971S), TSC2 (#4308S), phospho-S6 kinase (Thr^{389}) (#9206S), S6 kinase (#2217S), phospho-S6 ($Ser^{235/236}$) (#2211S), S6 (#2217S), phospho-4EBP1 ($Thr^{37/46}$) (#9459S), 4EBP1 (#9452S) (all from Cell Signaling Technology), and β-actin (#A5316-100UL) (Sigma-Aldrich). Band intensity on immunoblots was quantitated with the use of Multi Gauge software (Fujifilm).

Mass spectrometry analysis

Samples were separated by SDS-PAGE and stained with silver nitrate. Next, the target protein band was excised from a sample lane, followed by in-gel digestion with 10 μg/ml sequencing grade modified trypsin (Promega) overnight at 37°C [13]. The digested peptides were eluted with 0.1% formic acid and were subjected to LC-MS/MS analysis, which was performed on a LCMS-IT-TOF (Shimadzu) interfaced with a nano reverse-phase liquid chromatography system (Shimadzu). LC separation was performed using a Pico Frit Beta

Basic C18 column (New Objective) at 300 nl/min. Peptides were eluted using gradients of 5%–40% acetonitrile in 0.1% formic acid and sprayed directly into the mass spectrometer. The heated capillary temperature and electrospray voltage were set at 200°C and 2.5 kV, respectively. MS/MS data were acquired in the data-dependent mode by LCMS solution software (Shimadzu) and were converted to a single text file (containing the observed precursor peptide *m/z*, the fragment ion *m/z*, and intensity values) by Mascot Distiller (Matrix Science). The file was analyzed using Mascot (Matrix Science) MS/MS Ion Search to search for and assign the obtained peptides using the SwissProt database. The search parameters were set as follows: database, SwissProt 2014_09; taxonomy, all or *Rattus norvegicus*; enzyme, trypsin (one missed cleavage allowed); variable modifications, carbamidomethyl (C), oxidation (M), and propionamide (C); peptide tolerance, ±0.05 Da; and MS/MS tolerance, ±0.05 Da. For protein identification, the criteria were as follows: (1) Mascot scores above the statistically significant threshold ($P < 0.05$), and (2) at least one top-ranked unique peptide matching the identified protein.

Reverse transcription and real-time polymerase chain reaction analysis

Total RNA was isolated from mouse islets or INS-1 cells with the use of an RNeasy kit (Qiagen). Real-time polymerase chain reaction (PCR) analysis was performed with SYBR Green reagent (Promega) and an ABI 7900 sequencer (Life Technologies) as previously described [4]. The abundance of target mRNAs was normalized to that of cyclophilin mRNA as the invariant control.

Statistical analysis

Quantitative data are presented as means ± standard error of the mean (SEM), and differences between means were assessed with Student's *t*-test (two-tailed). A P value < 0.05 was

considered statistically significant.

Results

TSC2 is inactivated by phosphorylation of T1462 in the islets of HFD-fed GCN2-deficient mice

We recently reported that mTORC1 activity was enhanced in the islets of HFD-fed GCN2-deficient mice [7]. Confirmation of TSC2 phosphorylation (T1462) in these islets revealed increased phosphorylation compared with HFD-fed wild-type mice (Fig. 1A). Further examination of downstream molecules to confirm mTORC1 activity revealed enhanced phosphorylation of ribosomal protein S6 kinase 1 (S6K1), S6, and eIF4E binding protein 1 (4EBP1) (Fig. 1B, C, D) [7], suggesting that GCN2 deficiency induces TSC2 inactivation and that there is a mechanism of mTORC1 activity enhancement via the TSC2 pathway other than the downregulation of Sestrin2 expression.

[Insert Fig. 1 near here.]

14-3-3 regulates mTORC1 activation via maintenance of TSC2 (T1462) phosphorylation in pancreatic β-cells

TSC2 is phosphorylated by Akt downstream of the phosphatidylinositol-3 kinase (PI3K) pathway. Meanwhile, a 14-3-3-mediated mechanism for maintaining phosphorylation has been reported [14]. 14-3-3 contributes to signal transduction by binding to phosphorylation sites, and it has also been shown to bind to the T1462 phosphorylation site of TSC2 to stabilize its phosphorylation. T1462 phosphorylation of TSC2 inactivates TSC2, which in turn activates mTORC1. However, experiments using mouse embryo fibroblasts revealed that T1462 dephosphorylation of TSC2 was induced when the binding rate of TSC2 to 14-3-3 decreases because of increased REDD1 (regulated in development and DNA damage

response 1) expression and enhanced binding to 14-3-3 under hypoxic conditions [15]. Because REDD1 expression is regulated by activating transcription factor 4 (ATF4) downstream of protein kinase R-like endoplasmic reticulum kinase (PERK), which is encoded by the *EIF2ak3* gene [16], we hypothesized that its expression could also be induced by GCN2 activation. Therefore, we initially predicted that reduced expression of REDD1 in islets of HFD-fed GCN2-deficient mice would maintain the binding of 14-3-3 protein to the TSC2 phosphorylation site, thereby inducing increased mTORC1 activity through TSC2 inactivation. However, no difference in REDD1 expression was observed in islets of HFD-fed GCN2-deficient mice or in a GCN2-knockdown rat pancreatic β-cell line compared with the control group [7]. Therefore, we hypothesized that, in addition to REDD1, there is another molecule whose expression is regulated downstream of GCN2 and that this molecule regulates mTORC1 activity in pancreatic β-cells by competing with TSC2 in the binding of 14-3-3 (Fig. 2A). To test this hypothesis, we generated a 14-3-3 expression vector, and INS-1 cells, a rat pancreatic β-cell line, were used to examine the effect of 14-3-3 expression on TSC2 phosphorylation. The results showed that TSC2 phosphorylation was maintained in INS-1 cells overexpressing 14-3-3 protein, indicating that 14-3-3 contributes to the maintenance of TSC2 phosphorylation (Fig. 2B).

[Insert Fig. 2 near here.]

L-Asparaginase is regulated downstream of GCN2 and binds to 14-3-3

To further identify the particular molecule expressed downstream of GCN2 signaling, we performed experiments in INS-1 cells under three different conditions: “normal condition,” “amino acid–deficient condition,” and “GCN2-deficient condition under amino acid deficiency” (Fig. 2C). Then, the molecules binding to 14-3-3 only under amino acid deficiency, in which GCN2 is activated, were comprehensively analyzed by proteome

analysis (Table 1). Five molecules were identified that bind in the GCN2-activated condition but not in the GCN2-deficient condition: ATP-binding cassette subfamily C member 5 (ABCC5), RecQ-like helicase 5 (RECQL5), L-asparaginase, heat shock protein 90 β (HSP90β), and signal induced proliferation associated 1 like 2 (SIPA1L2). Because the expression of these molecules is thought to be induced under GCN2 activity, we first performed gene expression analysis in the pancreatic β-cell line under the amino acid–deficient condition (in which GCN2 is activated). The results revealed increased expression of L-asparaginase and ABCC5 (Fig. 3A). We previously found that GCN2 was phosphorylated (i.e., activated) upon glucose stimulation of a pancreatic β-cell line [7]. Here, when INS-1 cells were glucose-stimulated, only L-asparaginase was found to be upregulated (Fig. 3B). Subsequently, in vivo analysis was performed using wild-type mice. Gene expression analysis using isolated islets from HFD-loaded and normal diet–fed mice, in which GCN2 in islets is activated, showed increased expression of L-asparaginase and SIPA1L2 (Fig. 3C). In contrast, gene expression analysis in islets of HFD-fed GCN2-deficient mice showed that only L-asparaginase was significantly downregulated (Fig. 3D). These results suggest that L-asparaginase is the binding molecule for 14-3-3, whose expression is regulated downstream of GCN2.

[Insert Fig. 3 and Table 1 near here.]

Endogenous L-asparaginase expression is regulated by GCN2-ATF4 signaling in pancreatic β-cells

Next, to compare the expression of endogenous L-asparaginase in different tissues of individual mice, the expression levels of L-asparaginase in the brain, liver, kidney, myocardium, spleen, and islets of wild-type mice fed a normal diet were measured by quantitative real-time PCR. The results showed that L-asparaginase expression was highest in

the myocardium, followed by pancreatic islets (Fig. 3E). Subsequent comparison of glucose-stimulated GCN2-knockdown INS-1 cells with controls showed that L-asparaginase expression was significantly decreased in GCN2-knockdown cells compared with controls (Fig. 3F). Furthermore, when INS-1 cells with siRNA knockdown of ATF4—whose expression is regulated downstream of GCN2—were used, L-asparaginase expression was also significantly decreased (Fig. 3G). These results suggest that L-asparaginase is endogenously expressed in the body and that its expression is regulated downstream of GCN2–ATF4 signaling in pancreatic β-cells.

Decreased L-asparaginase expression causes mTORC1 activation in pancreatic β-cells

To further confirm that L-asparaginase actually affects mTORC1 activity, we performed L-asparaginase knockdown in INS-1 cells, using L-asparaginase siRNA. L-Asparaginase-knockdown INS-1 cells were established, in which L-asparaginase expression was reduced to about 30% (Fig. 4A). We evaluated mTORC1 activity in this group of cells. The results showed elevated phosphorylation of S6K1, S6, and 4EBP1, which are downstream of mTORC1, confirming that mTORC1 activity was increased (Fig. 4B, C, and D).

These results suggest that in pancreatic β-cells with deficient or persistently inactive GCN2, L-asparaginase expression is also reduced with decreased ATF4 expression, resulting in lower binding of 14-3-3 to L-asparaginase. This further suggests that the T1462 phosphorylation of TSC2 and 14-3-3 binding are increased. Finally, we postulate a mechanism of mTORC1 activation by suppression of TSC2 (Fig. 4E).

[Insert Fig. 4 near here.]

Discussion

Under diabetic conditions, pancreatic β-cells are dysfunctional and decrease in number,

further aggravating glucose intolerance. Numerous mechanisms have been reported to cause this “pancreatic β-cell failure,” including endoplasmic reticulum stress, oxidative stress, and autophagy disorders [17-19]. Stress-exposed pancreatic β-cells lead to decreased pancreatic β-cell volume through decreased proliferative capacity and increased apoptosis. mTOR, a serine-threonine kinase, is activated by growth factors such as insulin and nutrients such as amino acids to form complexes such as mTORC1 and mTORC2 [20]. mTOR is a molecule that regulates proliferation and growth in various cells, and its function in pancreatic β-cells is very important. We previously generated mice specifically lacking TSC2, a molecule that inhibits mTOR, in pancreatic β-cells (βTSC2$^{-/-}$). We reported that βTSC2$^{-/-}$ mice exhibit increased pancreatic β-cell volume, hyperinsulinemia, and hypoglycemia at young ages, but markedly decreased pancreatic β-cell volume, hypoinsulinemia, and hyperglycemia at old ages [9]. Constitutive activation of mTORC1 has been found to lead to decreased proliferation and increased apoptosis of pancreatic β-cells. Yuan et al. reported that mTORC1 activity is increased in islets of type 2 diabetic patients [21], suggesting that it may be the cause of the pancreatic β-cell failure in diabetic conditions.

Recently, we discovered that mutations in the *Eif2ak4* gene, one of the susceptibility genes for type 2 diabetes, enhance mTORC1 activity in pancreatic β-cells [7]. We reported that the loss of GCN2 in pancreatic β-cells increases mTORC1 activity by downregulating the expression of Sestrin2, which has the function of suppressing mTORC1. This mechanism is regulated by a TSC2-independent pathway. However, here, we found that the T1462 phosphorylation site of TSC2 is phosphorylated in islets of HFD-fed GCN2-deficient mice, suggesting that both TSC2-independent and -dependent pathways exist for mTORC1 activation by GCN2 deficiency. In this study, we examined the TSC2-dependent pathway. REDD1 is a molecule whose expression is regulated by ATF4 downstream of PERK, an eIF2α kinase [16], and binds to 14-3-3 in mouse embryo fibroblasts under hypoxic

conditions. It was previously reported that this competitively inhibits the binding of the TSC2 T1462 phosphorylation site to 14-3-3, thereby suppressing mTORC1 activity via inhibition of TSC2 inactivation [15]. We hypothesized that a similar phenomenon may occur downstream of GCN2 because REDD1 expression is also regulated by ATF4. However, in our study, GCN2 loss did not alter REDD1 expression. Therefore, we hypothesized that a molecule other than REDD1 is regulated downstream of GCN2 and that this molecule competitively inhibits the binding of TSC2 to 14-3-3. We searched for molecules downstream of GCN2 that bind to 14-3-3 by proteome analysis and found five molecules: ABCC5, SIPA1L2, RECQL5, HSP90β, and L-asparaginase.

ABCC5 is a gene encoding a protein called multidrug resistance-associated protein 5 (MRP5), which has been reported to function as a transporter in the brain and placenta [22]. SIPA1L2 is a member of neuronal RapGAPs and regulates Rap activity [23]. RECQL5 is an important molecule in DNA replication that contributes to genome stabilization [24]. These three molecules have not been shown to play roles in pancreatic β-cells and have not been found to be associated with diabetic pathophysiology or mTORC1 activity.

However, HSP90β acts as a chaperone that stabilizes unfolding proteins and that has been used as a target molecule for anticancer drugs [25]. It has also been reported that HSP90 protein levels in the serum of type 1 diabetic patients are increased, suggesting its possible application as a novel biomarker for the development of type 1 diabetes [26].

In this study, L-asparaginase, which has been used as an anticancer drug for acute lymphocytic leukemia, was identified as a molecule that binds to 14-3-3 downstream of GCN2. L-Asparaginase is an enzyme that hydrolyzes asparagine to aspartate and exerts its antitumor effect by depleting intracellular asparagine [27, 28]. It has also been recently shown that L-asparaginase suppresses mTORC1 activity by decreasing asparagine levels in HeLa cells [29]. That is, L-asparaginase may suppress mTORC1 through a TSC2-dependent

pathway via binding to 14-3-3 and a TSC2-independent pathway via decreased asparagine concentration.

The role of L-asparaginase in vivo has not been fully analyzed beyond its antitumor effect, but it is expected to exert various functions via regulation of mTORC1 activity. Further functional analysis of L-asparaginase in pancreatic β-cells and in glucose metabolism is expected to elucidate its relationship with the pathogenesis of diabetes mellitus.

Acknowledgments

This research was supported by a Grant-in-Aid for Creative Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology (MEXT; 20K08860 to S.A.); a Grant-in-Aid for Creative Scientific Research from MEXT (20K08906 to M.K.K.); and a Grant-in-Aid for Creative Scientific Research from MEXT (21K08579 to Y.K.). The sponsor had no role in study design, in the collection, analysis, or interpretation of data, in the writing of the report, or in the decision to submit the article for publication.

Conflicts of Interest

The authors declare no conflicts of interest.

References

1. N. Hashimoto, Y. Kido, T. Uchida, S. Asahara, Y. Shigeyama, T. Matsuda, A. Takeda, D. Tsuchihashi, A. Nishizawa, W. Ogawa, Y. Fujimoto, H. Okamura, K.C. Arden, P.L. Herrera, T. Noda, M. Kasuga, Ablation of PDK1 in pancreatic beta cells induces diabetes as a result of loss of beta cell mass, Nat. Genet. 38 (2006) 589–593. https://doi.org/10.1038/ng1774.

2. T. Matsuda, Y. Kido, S. Asahara, T. Kaisho, T. Tanaka, N. Hashimoto, Y. Shigeyama, A. Takeda, T. Inoue, Y. Shibutani, M. Koyanagi, T. Hosooka, M. Matsumoto, H. Inoue, T.

Uchida, M. Koike, Y. Uchiyama, S. Akira, M. Kasuga, Ablation of C/EBPbeta alleviates ER stress and pancreatic beta cell failure through the GRP78 chaperone in mice, J. Clin. Invest. 120 (2010) 115–126. https://doi.org/10.1172/jci39721.

3. S. Asahara, Y. Shibutani, K. Teruyama, H.Y. Inoue, Y. Kawada, H. Etoh, T. Matsuda, M. Kimura-Koyanagi, N. Hashimoto, M. Sakahara, W. Fujimoto, H. Takahashi, S. Ueda, T. Hosooka, T. Satoh, H. Inoue, M. Matsumoto, A. Aiba, M. Kasuga, Y. Kido, Ras-related C3 botulinum toxin substrate 1 (RAC1) regulates glucose-stimulated insulin secretion via modulation of F-actin, Diabetologia 56 (2013) 1088–1097. https://doi.org/10.1007/s00125-013-2849-5.

4. S. Asahara, H. Etoh, H. Inoue, K. Teruyama, Y. Shibutani, Y. Ihara, Y. Kawada, A. Bartolome, N. Hashimoto, T. Matsuda, M. Koyanagi-Kimura, A. Kanno, Y. Hirota, T. Hosooka, K. Nagashima, W. Nishimura, H. Inoue, M. Matsumoto, M.J. Higgins, K. Yasuda, N. Inagaki, S. Seino, M. Kasuga, Y. Kido, Paternal allelic mutation at the Kcnq1 locus reduces pancreatic β-cell mass by epigenetic modification of Cdkn1c, Proc. Natl. Acad. Sci. U. S. A. 112 (2015) 8332–8337. https://doi.org/10.1073/pnas.1422104112.

5. K. Miyake, W. Yang, K. Hara, K. Yasuda, Y. Horikawa, H. Osawa, H. Furuta, M.C.Y. Ng, Y. Hirota, H. Mori, K. Ido, K. Yamagata, Y. Hinokio, Y. Oka, N. Iwasaki, Y. Iwamoto, Y. Yamada, Y. Seino, H. Maegawa, A. Kashiwagi, H.Y. Wang, T. Tanahashi, N. Nakamura, J. Takeda, E. Maeda, K. Yamamoto, K. Tokunaga, R.C.W. Ma, W.Y. So, J.C.N. Chan, N. Kamatani, H. Makino, K. Nanjo, T. Kadowaki, M. Kasuga, Construction of a prediction model for type 2 diabetes mellitus in the Japanese population based on 11 genes with strong evidence of the association, J. Hum. Genet. 54 (2009) 236–241. https://doi.org/10.1038/jhg.2009.17.

6. T.E. Dever, A.G. Hinnebusch, GCN2 whets the appetite for amino acids. Mol. Cell 18 (2005) 141–142. https://doi.org/10.1016/j.molcel.2005.03.023.

7. A. Kanno, S.I. Asahara, A. Furubayashi, K. Masuda, R. Yoshitomi, E. Suzuki, T. Takai, M. Kimura-Koyanagi, T. Matsuda, A. Bartolome, Y. Hirota, N. Yokoi, Y. Inaba, H. Inoue, M. Matsumoto, K. Inoue, T. Abe, F.Y. Wei, K. Tomizawa, W. Ogawa, S. Seino, M. Kasuga, Y. Kido, GCN2 regulates pancreatic β cell mass by sensing intracellular amino acid levels, JCI Insight 5 (2020) e128820. https://doi.org/10.1172/jci.insight.128820.

8. A. Kanno, S.I. Asahara, K. Masuda, T. Matsuda, M. Kimura-Koyanagi, S. Seino, W. Ogawa, Y. Kido, Compensatory hyperinsulinemia in high-fat diet-induced obese mice is associated with enhanced insulin translation in islets, Biochem. Biophys. Res. Commun. 458 (2015) 681–686. https://doi.org/10.1016/j.bbrc.2015.02.024.

9. Y. Shigeyama, T. Kobayashi, Y. Kido, N. Hashimoto, S. Asahara, T. Matsuda, A. Takeda, T. Inoue, Y. Shibutani, M. Koyanagi, T. Uchida, M. Inoue, O. Hino, M. Kasuga, T. Noda, Biphasic response of pancreatic beta-cell mass to ablation of tuberous sclerosis complex 2 in mice, Mol. Cell. Biol. 28 (2008) 2971–2979. https://doi.org/10.1128/mcb.01695-07.

10. M. Koyanagi, S. Asahara, T. Matsuda, N. Hashimoto, Y. Shigeyama, Y. Shibutani, A. Kanno, M. Fuchita, T. Mikami, T. Hosooka, H. Inoue, M. Matsumoto, M. Koike, Y. Uchiyama, T. Noda, S. Seino, M. Kasuga, Y. Kido, Ablation of TSC2 enhances insulin secretion by increasing the number of mitochondria through activation of mTORC1, PLoS One 6 (2011) e23238. https://doi.org/10.1371%2Fjournal.pone.0023238.

11. A. Bartolomé, M. Kimura-Koyanagi, S. Asahara, C. Guillén, H. Inoue, K. Teruyama, S. Shimizu, A. Kanno, A. García-Aguilar, M. Koike, Y. Uchiyama, M. Benito, T. Noda, Y. Kido, Pancreatic β-cell failure mediated by mTORC1 hyperactivity and autophagic impairment, Diabetes 63 (2014) 2996–3008. https://doi.org/10.2337/db13-0970.

12. L. Chantranupong, R.L. Wolfson, J.M. Orozco, R.A. Saxton, S.M. Scaria, L. Bar-Peled, E. Spooner, M. Isasa, S.P. Gygi, D.M. Sabatini, The Sestrins interact with GATOR2 to negatively regulate the amino-acid-sensing pathway upstream of mTORC1, Cell Rep. 9

(2014) 1–8. https://doi.org/10.1016/j.celrep.2014.09.014.

13. N. Hatano, T. Hamada, Proteome analysis of pitcher fluid of the carnivorous plant Nepenthes alata, J. Proteome Res. 7 (2008) 809–816. https://doi.org/10.1021/pr700566d.

14. S.D. Shumway, Y. Li, Y. Xiong, 14-3-3beta binds to and negatively regulates the tuberous sclerosis complex 2 (TSC2) tumor suppressor gene product, tuberin, J. Biol. Chem. 278 (2003) 2089–2092. https://doi.org/10.1074/jbc.c200499200.

15. M.P. DeYoung, P. Horak, A. Sofer, D. Sgroi, L.W. Ellisen, Hypoxia regulates TSC1/2-mTOR signaling and tumor suppression through REDD1-mediated 14-3-3 shuttling, Genes Dev. 22 (2008) 239–251. https://doi.org/10.1101%2Fgad.1617608.

16. H.O. Jin, S.K. Seo, Y.S. Kim, S.H. Woo, K.H. Lee, J.Y. Yi, S.J. Lee, T.B. Choe, J.H. Lee, S. An, S.I. Hong, I.C. Park, TXNIP potentiates Redd1-induced mTOR suppression through stabilization of Redd1, Oncogene 30 (2011) 3792–3801. https://doi.org/10.1038/onc.2011.102.

17. D.R. Laybutt, A.M. Preston, M.C. Akerfeldt, J.G. Kench, A.K. Busch, A.V. Biankin, T.J. Biden, Endoplasmic reticulum stress contributes to beta cell apoptosis in type 2 diabetes. Diabetologia 50 (2007) 752–763. https://doi.org/10.1007/s00125-006-0590-z.

18. Kaneto H, Kawamori D, Matsuoka TA, Y. Kajimoto, Y. Yamasaki, Oxidative stress and pancreatic beta-cell dysfunction, Am. J. Ther. 12 (2005) 529–533. https://doi.org/10.1097/01.mjt.0000178773.31525.c2.

19. C. Ebato, T. Uchida, M. Arakawa, M. Komatsu, T. Ueno, K. Komiya, K. Azuma, T. Hirose, K. Tanaka, E. Kominami, R. Kawamori, Y. Fujitani, H. Watada, Autophagy is important in islet homeostasis and compensatory increase of beta cell mass in response to high-fat diet, Cell Metab. 8 (2008) 325–332. https://doi.org/10.1016/j.cmet.2008.08.009.

20. S.G. Kim, G.R. Buel, J. Blenis, Nutrient regulation of the mTOR complex 1 signaling pathway, Mol. Cells 35 (2013) 463–473. https://doi.org/10.1007/s10059-013-0138-2.

21. T. Yuan, S. Rafizadeh, K.D.D. Gorrepati, B. Lupse, J. Oberholzer, K. Maedler, A. Ardestani, Reciprocal regulation of mTOR complexes in pancreatic islets from humans with type 2 diabetes, Diabetologia 60 (2017) 668–678. https://doi.org/10.1007/s00125-016-4188-9.

22. I.G. Chambers, P. Kumar, J. Lichtenberg, P. Wang, J. Yu, J.D. Phillips, M.A. Kane, D. Bodine, I. Hamza, MRP5 and MRP9 play a concerted role in male reproduction and mitochondrial function, Proc. Natl. Acad. Sci. U. S. A. 119 (2022) e211617119. https://doi.org/10.1073/pnas.2111617119.

23. M. Andres-Alonso, M.R. Ammar, I. Butnaru, G.M. Gomes, G.A. Sanhueza, R. Raman, P. Yuanxiang, M. Borgmeyer, J. Lopez-Rojas, S.A. Raza, N. Brice, T.J. Hausrat, T. Macharadze, S. Diaz-Gonzalez, M. Carlton, A.V. Failla, O. Stork, M. Schweizer, E.D. Gundelfinger, M. Kneussel, C. Spilker, A. Karpova, M.R. Kreutz, 15SIPA1L2 controls trafficking and local signaling of TrkB-containing amphisomes at presynaptic terminals, Nat. Commun. 10 (2019) 5448. https://doi.org/10.1038/s41467-019-13224-z.

24. P.L. Garcia, Y. Liu, J. Jiricny, S.C. West, P. Janscak, Human RECQ5beta, a protein with DNA helicase and strand-annealing activities in a single polypeptide. EMBO J. 23 (2004) 2882–2891. https://doi.org/10.1038%2Fsj.emboj.7600301.

25. K.W. Wen, B. Damania, Hsp90 and Hsp40/Erdj3 are required for the expression and anti-apoptotic function of KSHV K1, Oncogene 29 (2010) 3532–3544. https://doi.org/10.1038/onc.2010.124.

26. G.J. Ocaña, E.K. Sims, R.A. Watkins, S. Ragg, K.J. Mather, R.A. Oram, R.G. Mirmira, L.A. DiMeglio, J.S. Blum, C. Evans-Molina, Analysis of serum Hsp90 as a potential biomarker of β cell autoimmunity in type 1 diabetes, PLoS One 14 (2019) e0208456. https://doi.org/10.1371/journal.pone.0208456.

27. F.F. Becker, J.D. Broome, L-asparaginase: inhibition of early mitosis in regenerating rat

liver, Science 156 (1967) 1602–1603. https://doi.org/10.1126/science.156.3782.1602.

28. L.B. Sullivan, A. Luengo, L.V. Danai, L.N. Bush, F.F. Diehl, A.M. Hosios, A.N. Lau, S. Elmiligy, S. Malstrom, C.A. Lewis, M.G.V. Heiden, Aspartate is an endogenous metabolic limitation for tumour growth, Nat. Cell. Biol. 20 (2018) 782–788. https://doi.org/10.1038/s41556-018-0125-0.

29. A.S. Krall, P.J. Mullen, F. Surjono, M. Momcilovic, E.W. Schmid, C.J. Halbrook, A. Thambundit, S.D. Mittelman, C.A. Lyssiotis, D.B. Shackelford, S.R.V. Knott, H.R. Christofk, Asparagine couples mitochondrial respiration to ATF4 activity and tumor growth, Cell Metab. 33 (2021) 1013–1026. https://doi.org/10.1016/j.cmet.2021.02.001.

Figure legends

Figure 1. Phosphorylation of TSC2 (T1462) and mTORC1 signaling in islets of GCN2-deficient mice.

Quantitative data and representative blots from immunoblot analysis of phosphorylated (T1462) and total TSC2 (A) and mTORC1 signaling (B, C, and D) in islets of HFD-fed wild-type mice ($GCN2^{+/+}$) or HFD-fed GCN2-deficient mice ($GCN2^{-/-}$). Data are presented as means ± SEM of four independent populations from each group. *$P < 0.05$, **$P < 0.01$.

Figure 2. Search for molecules binding to 14-3-3 downstream of GCN2 signal.

(A) Hypothesis concerning the mTORC1 regulatory mechanism in pancreatic β-cells from GCN2-deficient mice and mechanism of the pull-down assay by HaloTag. (B) Quantitative data and representative blots in immunoblot analysis of phosphorylated (T1462) and total TSC2 using INS-1 cells in different situations, as described below. (C) Pull-down assay of molecules binding to 14-3-3 protein using INS-1 cells in three different situations. NC, normal condition; AD, amino acid–deficient condition; GDAD, GCN2-deficient condition with amino acid deficiency.

Figure 3. Quantification of candidate molecules for binding to 14-3-3 in cells and islets.

Quantitative real-time PCR analysis of each of the molecules shown in INS-1 cells under amino acid deprivation (A), in glucose-stimulated INS-1 cells (B), in islets of HFD-fed wild-type mice (C), and in islets of HFD-fed GCN2-deficient mice (D). Quantitative real-time PCR analysis of L-asparaginase mRNA expression in various organs (E), GCN2-knockdown INS-1 cells (GCN2-KD) (F), and ATF4-knockdown INS-1 cells (ATF4-KD) (G) or INS-1 cells transfected with scrambled siRNA (Control). Data are presented as means ± SEM of four independent populations from each group. *$P < 0.05$, **$P < 0.01$.

Figure 4. Analysis of mTORC1 activity in L-asparaginase-knockdown INS-1 cells.

(A) Quantitative real-time PCR analysis of L-asparaginase mRNA expression in L-asparaginase-knockdown INS-1 cells (Asparaginase-KD) or INS-1 cells transfected with scrambled siRNA (Control). Quantitative data and representative blots in immunoblot analysis of mTORC1 signaling (B, C, and D) in L-asparaginase-knockdown INS-1 cells (Asparaginase-KD) or INS-1 cells transfected with scrambled siRNA (Control). (E) A model for the regulation of mTORC1 activity by GCN2-mediated TSC2-dependent and -independent pathways in pancreatic islets. Data are presented as means ± SEM of four independent populations from each group. $*P < 0.05$, $**P < 0.01$.

Table 1. Molecules binding to 14-3-3 in INS-1 cells detected by pull-down assay under normal, amino acid–deficient, and GCN2-deficient conditions under amino acid deficiency.

Normal condition

Sample	#	SPROT ID	Protein name
1	1	MYH7_RAT	*Myosin-7*
	3	H4_RAT	Histone H4
	5	PNMA1_RAT	Paraneoplastic antigen Ma1 homolog
2	1	MYH7_RAT	Myosin-7
	4	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
3	1	MYH7_RAT	Myosin-7
	3	MYH4_RAT	Myosin-4
4	1	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
	2	MYPC_RAT	Myosin-binding protein C, cardiac-type
5	1	CLH1_RAT	Clathrin heavy chain 1
	2	PNMA1_RAT	Paraneoplastic antigen Ma1 homolog
6	1	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
7			no significant hit
8	1	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
9			no significant hit
10	1	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
	2	GRM8_RAT	Metabotropic glutamate receptor 8
	3	PNMA1_RAT	Paraneoplastic antigen Ma1 homolog
11			no significant hit
12	1	ATPA_RAT	ATP synthase subunit alpha, mitochondrial
	2	PHRF1_RAT	PHD and RING finger domain-containing protein 1
	3	TBB2A_RAT	Tubulin beta-2A chain
	4	TTL_RAT	Tubulin--tyrosine ligase
13	1	TBA1A_RAT	Tubulin alpha-1A chain

	2	EF1A1_RAT	Elongation factor 1-alpha 1
14	1	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
15	1	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
16	1	G3P_RAT	Glyceraldehyde-3-phosphate dehydrogenase
17	1	1433E_RAT	14-3-3 protein epsilon
	2	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
18			no significant hit
19			no significant hit
20	1	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
21	1	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
22	1	H2A1_RAT	Histone H2A type 1
	2	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
23	1	H4_RAT	Histone H4
	2	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
24	2	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1

SPROT ID, Swiss-Prot ID; MW, Molecular Weight

Amino acid-deficient condition

Sample	#	SPROT ID	Protein name
1	1	MYH6_RAT	Myosin-6
	2	MYH7_RAT	Myosin-7
	4	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
2	1	MYH7_RAT	Myosin-7
	4	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
3	1	MYH7_RAT	Myosin-7
	3	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
4	1	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
5	1	ASPG_RAT	N(4)-(Beta-N-acetylglucosaminyl)-L-asparaginase
6	1	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
7			no significant hit
8	1	H14_RAT	Histone H1.4

	2	HS90B_RAT	Heat shock protein HSP 90-beta
	3	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
	4	SI1L2_RAT	Signal-induced proliferation-associated 1-like protein 2
9	1	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
10	1	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
	2	ELMD3_RAT	ELMO domain-containing protein 3
	3	RECQ5_RAT	ATP-dependent DNA helicase Q5
11	1	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
	2	PNMA1_RAT	Paraneoplastic antigen Ma1 homolog
12	1	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
13	1	TBA1A_RAT	Tubulin alpha-1A chain
	2	RXFP1_RAT	Relaxin receptor 1
	3	H14_RAT	Histone H1.4
	4	ATPA_RAT	ATP synthase subunit alpha, mitochondrial
14	1	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
15			no significant hit
16	1	PNMA1_RAT	Paraneoplastic antigen Ma1 homolog
17	1	1433E_RAT	14-3-3 protein epsilon
	2	PHRF1_RAT	PHD and RING finger domain-containing protein 1
18	1	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
19			no significant hit
20	1	IMA6_RAT	Importin subunit alpha-6
21	1	H31_RAT	Histone H3.1
	2	PHRF1_RAT	PHD and RING finger domain-containing protein 1
	3	H2B1A_RAT	Histone H2B type 1-A
22	1	H2AZ_RAT	Histone H2A.Z
	2	PHRF1_RAT	PHD and RING finger domain-containing protein 1
	3	H2B1A_RAT	Histone H2B type 1-A
	4	MRP5_RAT	Multidrug resistance-associated protein 5
23	1	H4_RAT	Histone H4
	2	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1
	3	PNMA1_RAT	Paraneoplastic antigen Ma1 homolog

24	1	ASPG_RAT	N(4)-(Beta-N-acetylglucosaminyl)-L-asparaginase
	2	PHRF1_RAT	PHD and RING finger domain-containing protein 1
	3	PSMD1_RAT	26S proteasome non-ATPase regulatory subunit 1

SPROT ID, Swiss-Prot ID; MW, Molecular Weight

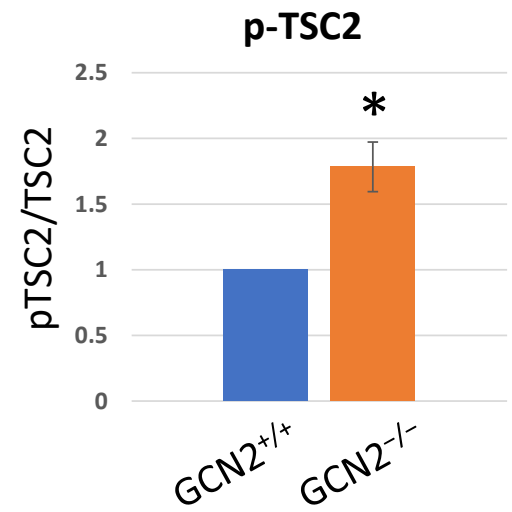
GCN2-deficient condition under amino acid deficiency

Sample	#	SPROT ID	Protein name
1	1	MYH7_RAT	Myosin-7
	2	MYH6_RAT	Myosin-6
	4	MAP1B_RAT	Microtubule-associated protein 1B
2	1	MYH7_RAT	Myosin-7
	2	MYH6_RAT	Myosin-6
3	1	MYH6_RAT	Myosin-6
	4	IMA6_RAT	Importin subunit alpha-6
	5	CAN10_RAT	Calpain-10
4			no significant hit
5	1	CLH1_RAT	Clathrin heavy chain 1
	2	ELMD3_RAT	ELMO domain-containing protein 3
6			no significant hit
7	1	ELMD3_RAT	ELMO domain-containing protein 3
8	1	HS90B_RAT	Heat shock protein HSP 90-beta
	2	H14_RAT	Histone H1.4
	3	CENPW_RAT	Centromere protein W
9	1	HSP7C_RAT	Heat shock cognate 71 kDa protein
	2	GRP78_RAT	78 kDa glucose-regulated protein
10			no significant hit
11			no significant hit
12			no significant hit
13	1	H14_RAT	Histone H1.4
	2	ATPA_RAT	ATP synthase subunit alpha, mitochondrial

	3	TBB2A_RAT	Tubulin beta-2A chain
	4	TBA1A_RAT	Tubulin alpha-1A chain
	5	ATPB_RAT	ATP synthase subunit beta, mitochondrial
	6	EF1A1_RAT	Elongation factor 1-alpha 1
14			no significant hit
15	1	G3P_RAT	Glyceraldehyde-3-phosphate dehydrogenase
16	1	H14_RAT	Histone H1.4
17	1	1433E_RAT	14-3-3 protein epsilon
	2	TPM1_RAT	Tropomyosin alpha-1 chain
	3	PHRF1_RAT	PHD and RING finger domain-containing protein 1
18	1	RXFP1_RAT	Relaxin receptor 1
	2	1433Z_RAT	14-3-3 protein zeta/delta
	3	UBP1_RAT	Ubiquitin carboxyl-terminal hydrolase 1
19			no significant hit
20	1	RS25_RAT	40S ribosomal protein S25
21	1	H31_RAT	Histone H3.1
	3	H2B1A_RAT	Histone H2B type 1-A
	5	PCNP_RAT	PEST proteolytic signal-containing nuclear protein
	6	BSCL2_RAT	Seipin
22	1	H2AZ_RAT	Histone H2A.Z
	2	NCBP2_RAT	Nuclear cap-binding protein subunit 2
	3	PHRF1_RAT	PHD and RING finger domain-containing protein 1
	4	C56D2_RAT	Cytochrome b561 domain-containing protein 2
	6	CENPW_RAT	Centromere protein W
23	1	H4_RAT	Histone H4
24	1	IMA6_RAT	Importin subunit alpha-6

SPROT ID, Swiss-Prot ID; MW, Molecular Weight

A



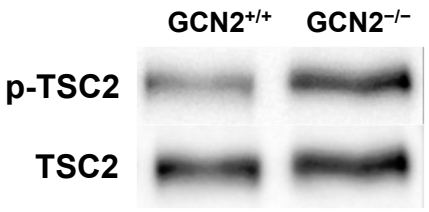


Fig. 1A

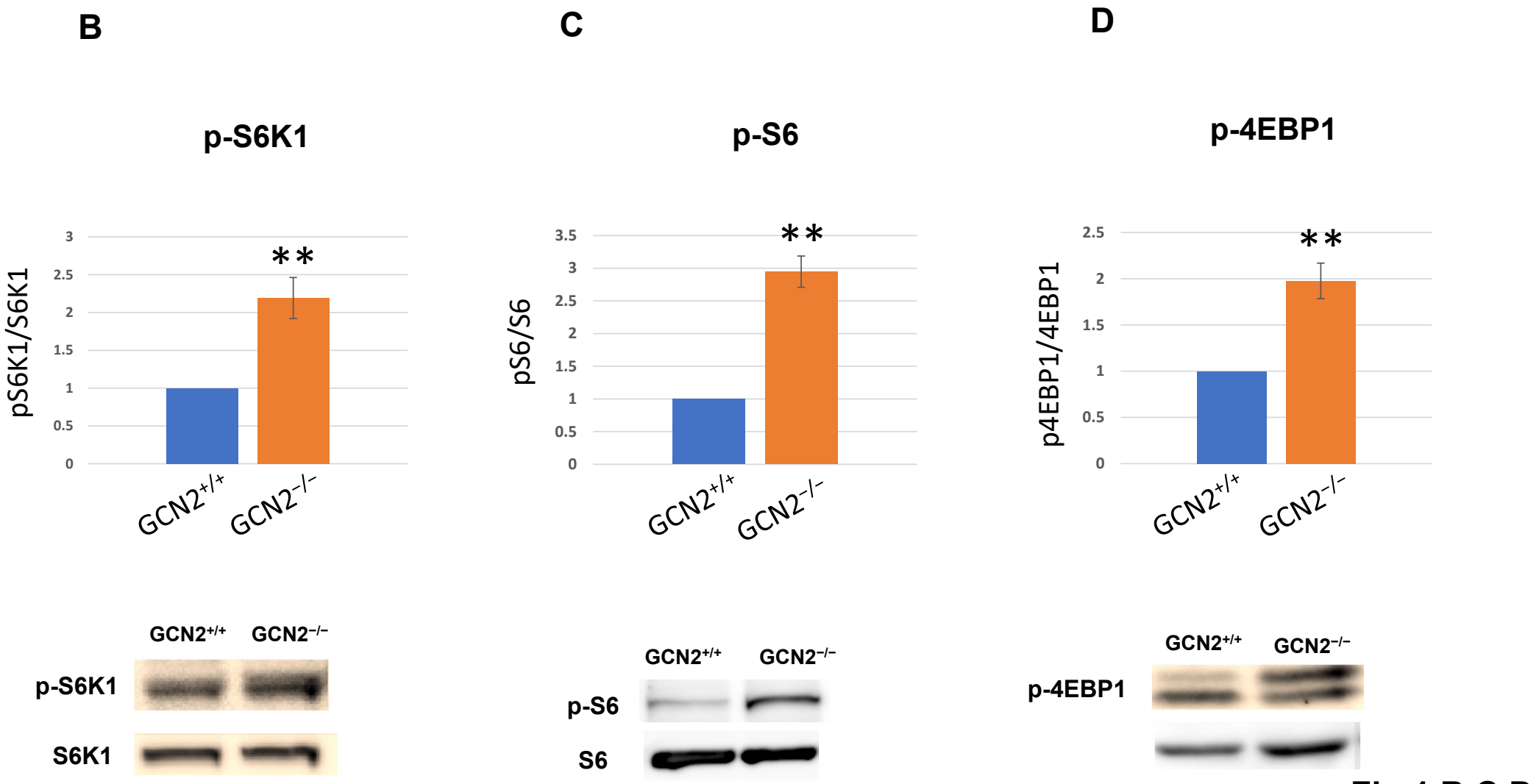

B
p-S6K1
pS6K1/S6K1
3
2.5
2
1.5
1
0.5
0
**
GCN2+/+
GCN2−/−
GCN2+/+
GCN2−/−
p-S6K1
S6K1
C
p-S6
pS6/S6
3.5
3
2.5
2
1.5
1
0.5
0
**
GCN2+/+
GCN2−/−
GCN2+/+
GCN2−/−
p-S6
S6
D
p-4EBP1
p4EBP1/4EBP1
2.5
2
1.5
1
0.5
0
**
GCN2+/+
GCN2−/−
GCN2+/+
GCN2−/−
p-4EBP1

Fig.1 B,C,D

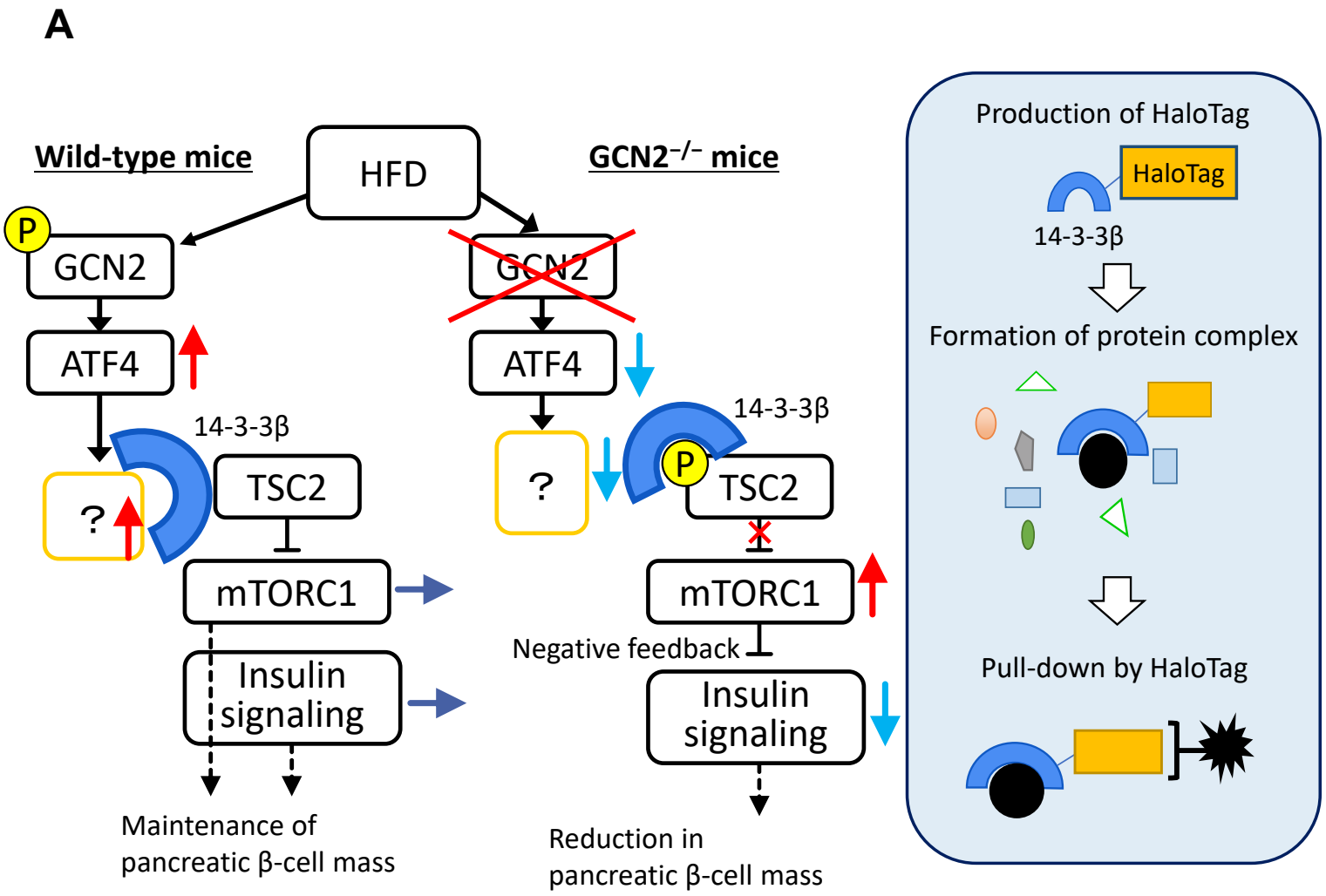
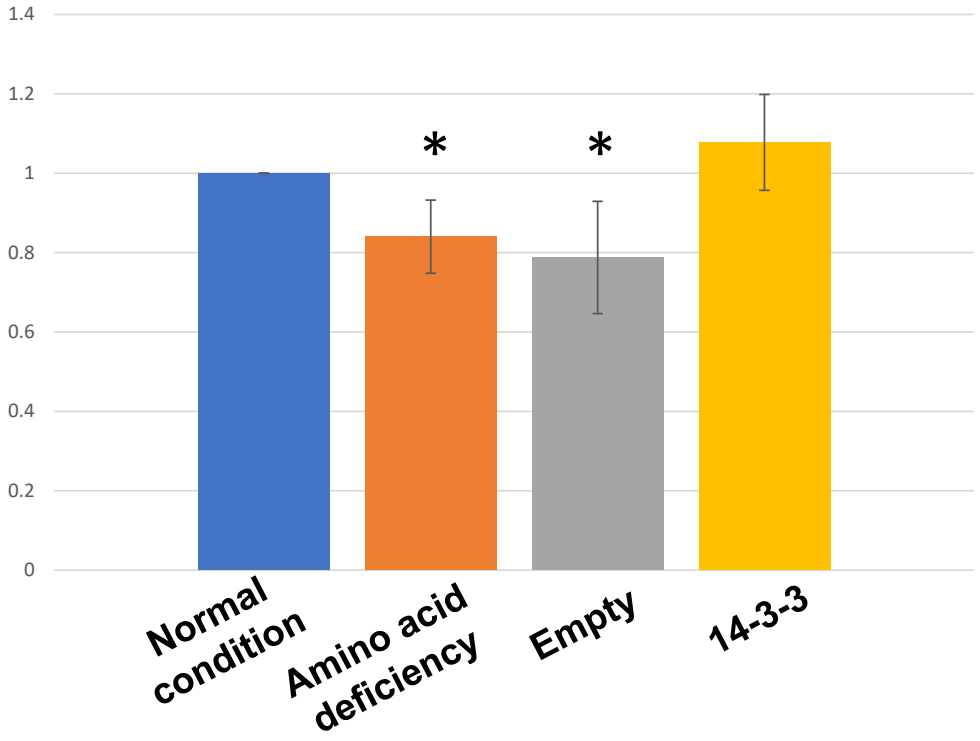

A
Wild-type mice
HFD
GCN2−/− mice
P
GCN2
GCN2
ATF4
ATF4
14-3-3β
?
TSC2
?
P
14-3-3β
TSC2
mTORC1
mTORC1
Negative feedback
Insulin signaling
Insulin signaling
Maintenance of pancreatic β-cell mass
Reduction in pancreatic β-cell mass
Production of HaloTag
HaloTag
14-3-3β
Formation of protein complex
Pull-down by HaloTag

Fig. 2A

B


1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
*
Normal condition
Amino acid deficiency
Empty
14-3-3

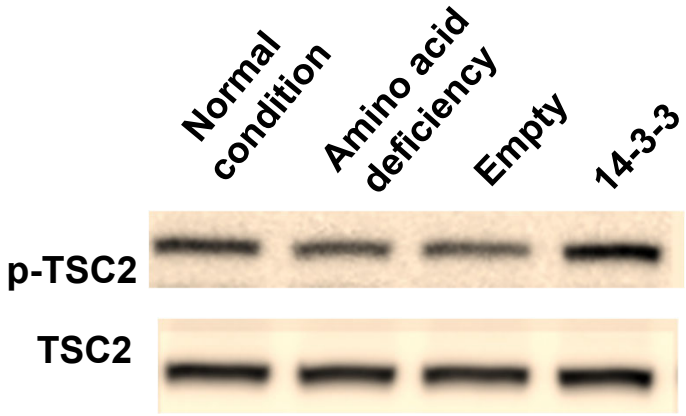

Normal condition
Amino acid deficiency
Empty
14-3-3
p-TSC2
TSC2

Fig. 2B

C

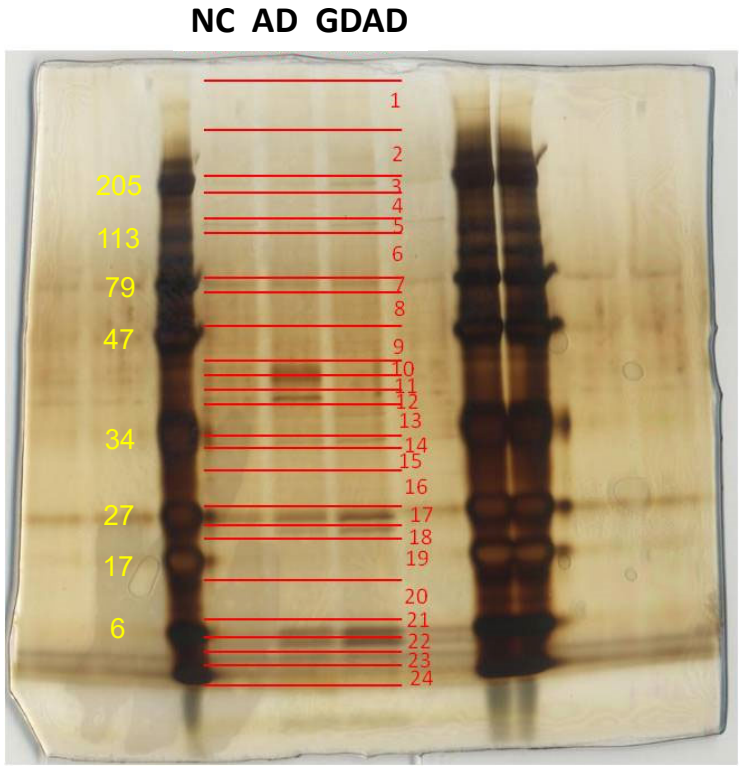
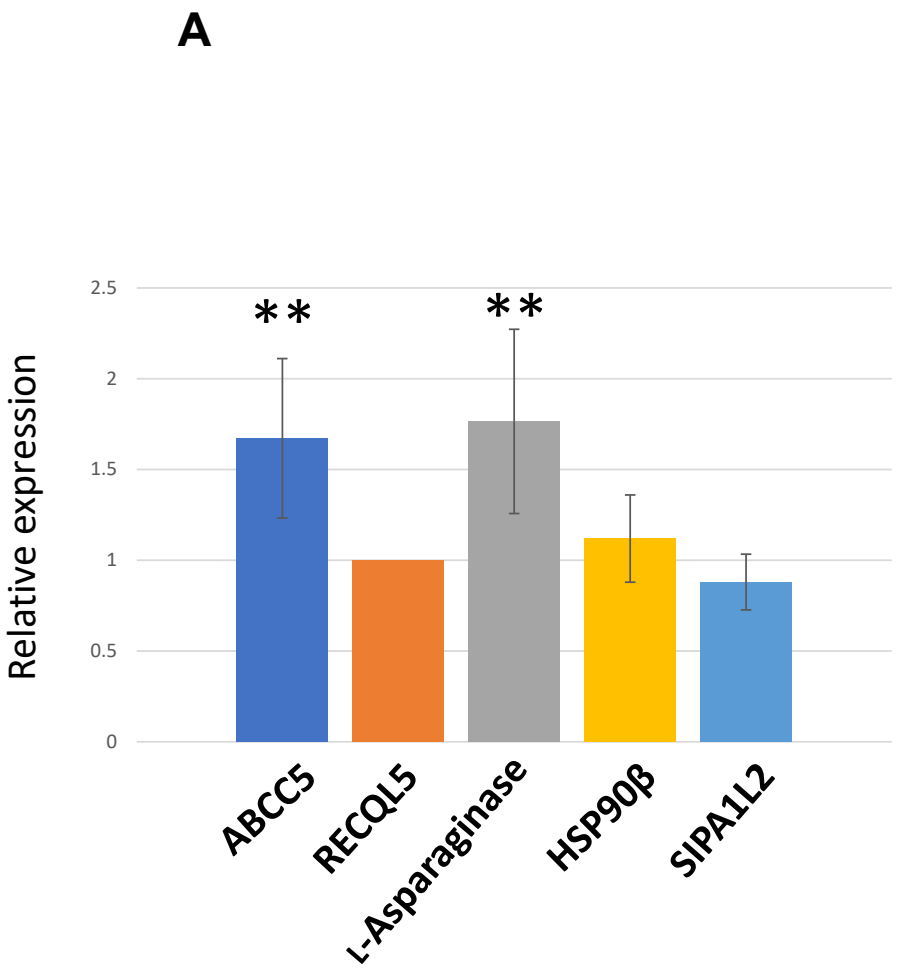

NC AD GDAD
205
113
79
47
34
27
17
6
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24

Fig. 2C


A
Relative expression
2.5
2
1.5
1
0.5
0
**
**
ABCC5
RECQL5
L-Asparaginase
HSP90β
SIPA1L2

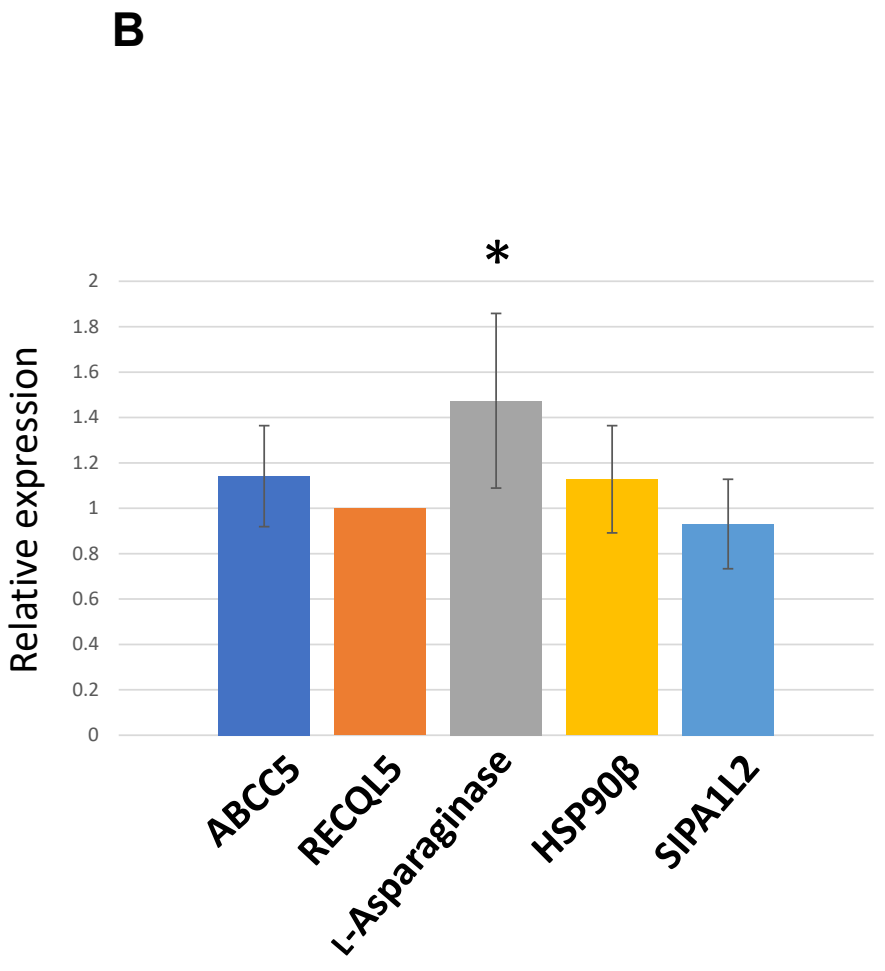
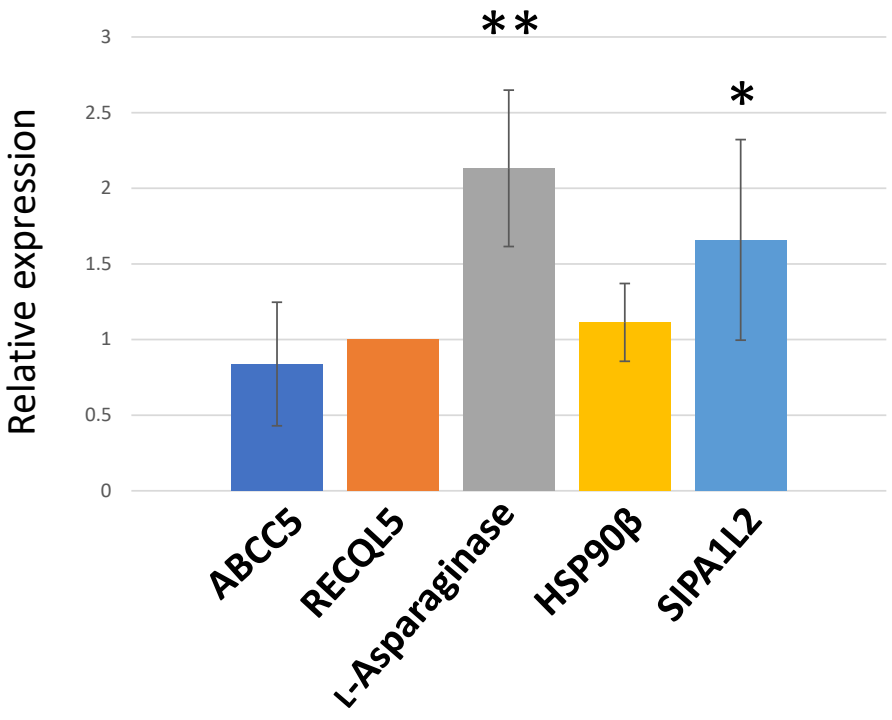

B
Relative expression
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
ABCC5
RECQL5
L-Asparaginase
HSP90β
SIPA1L2

Fig. 3A,B

C


Relative expression
3
2.5
2
1.5
1
0.5
0
**
*
ABCC5
RECQL5
L-Asparaginase
HSP90β
SIPA1L2

D

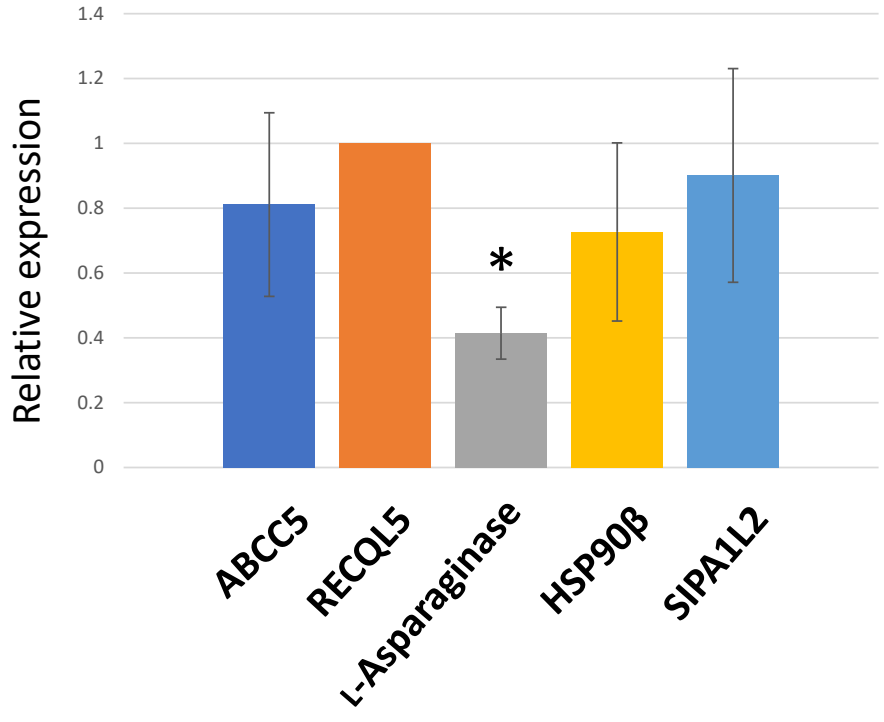

Relative expression
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
ABCC5
RECQL5
L-Asparaginase
HSP90β
SIPA1L2

Fig. 3C,D

A

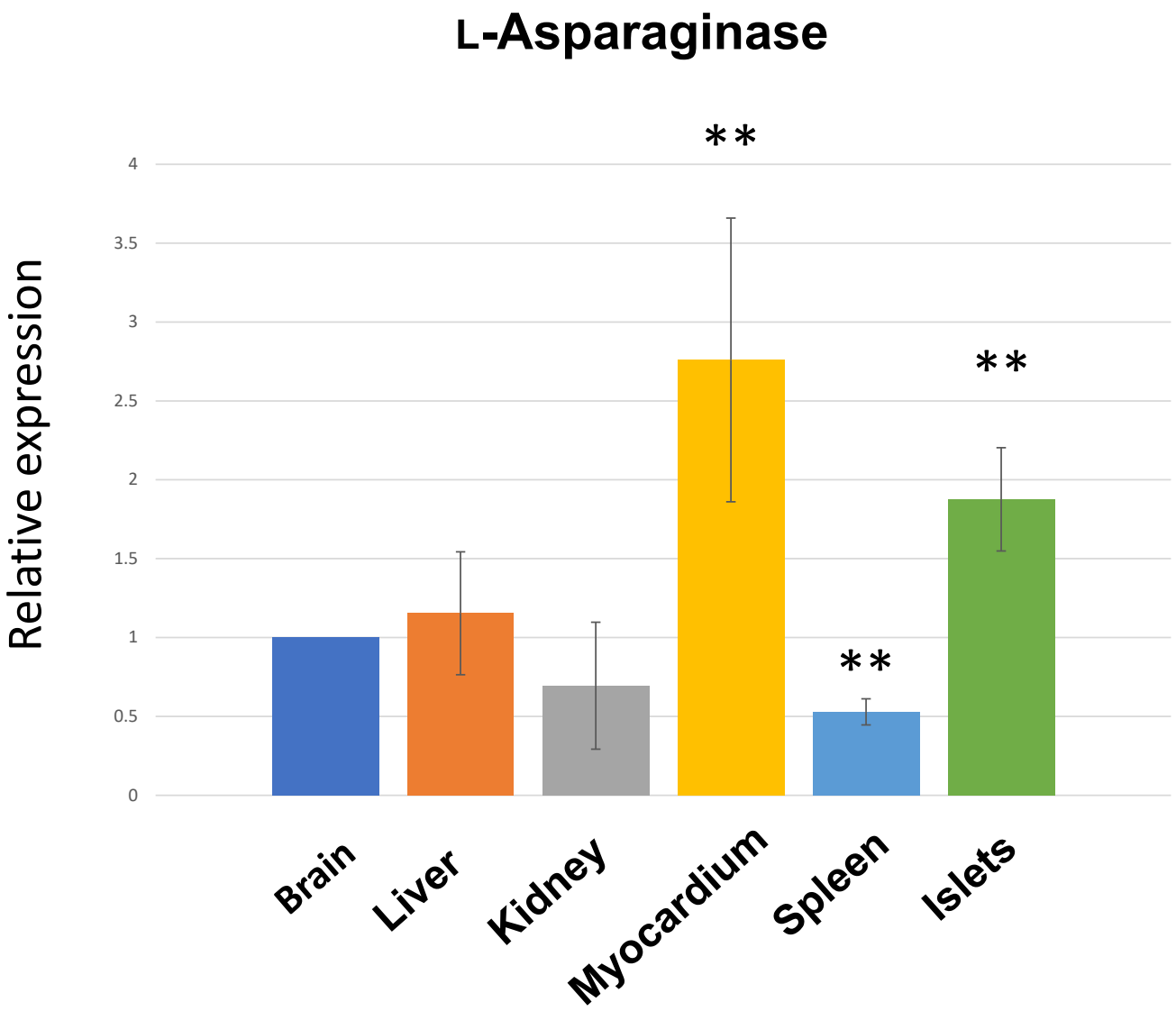

L-Asparaginase
Relative expression
4
3.5
3
2.5
2
1.5
1
0.5
0
**
**
**
Brain
Liver
Kidney
Myocardium
Spleen
Islets

Fig. 4A

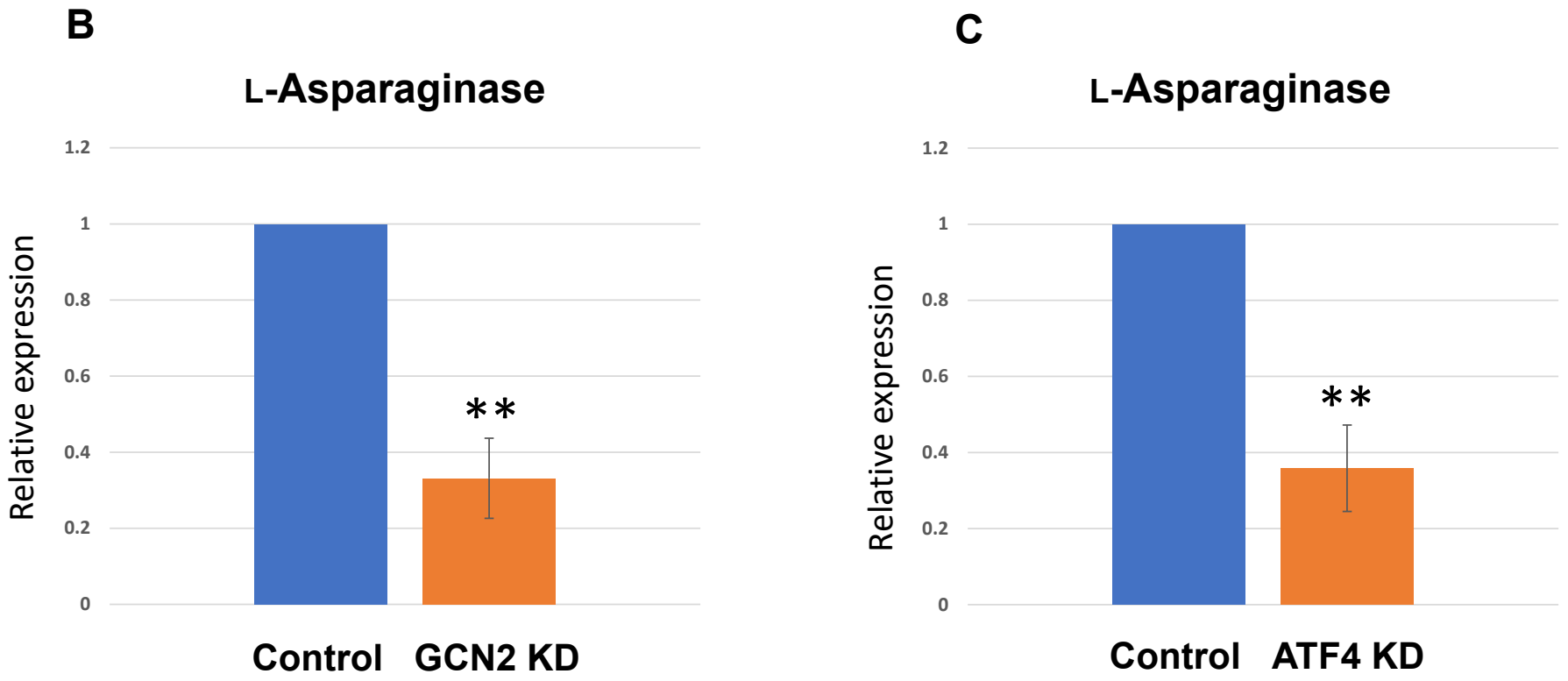

B
L-Asparaginase
Relative expression
1.2
1
0.8
0.6
0.4
0.2
0
**
Control
GCN2 KD
C
L-Asparaginase
Relative expression
1.2
1
0.8
0.6
0.4
0.2
0
**
Control
ATF4 KD

Fig. 4B,C

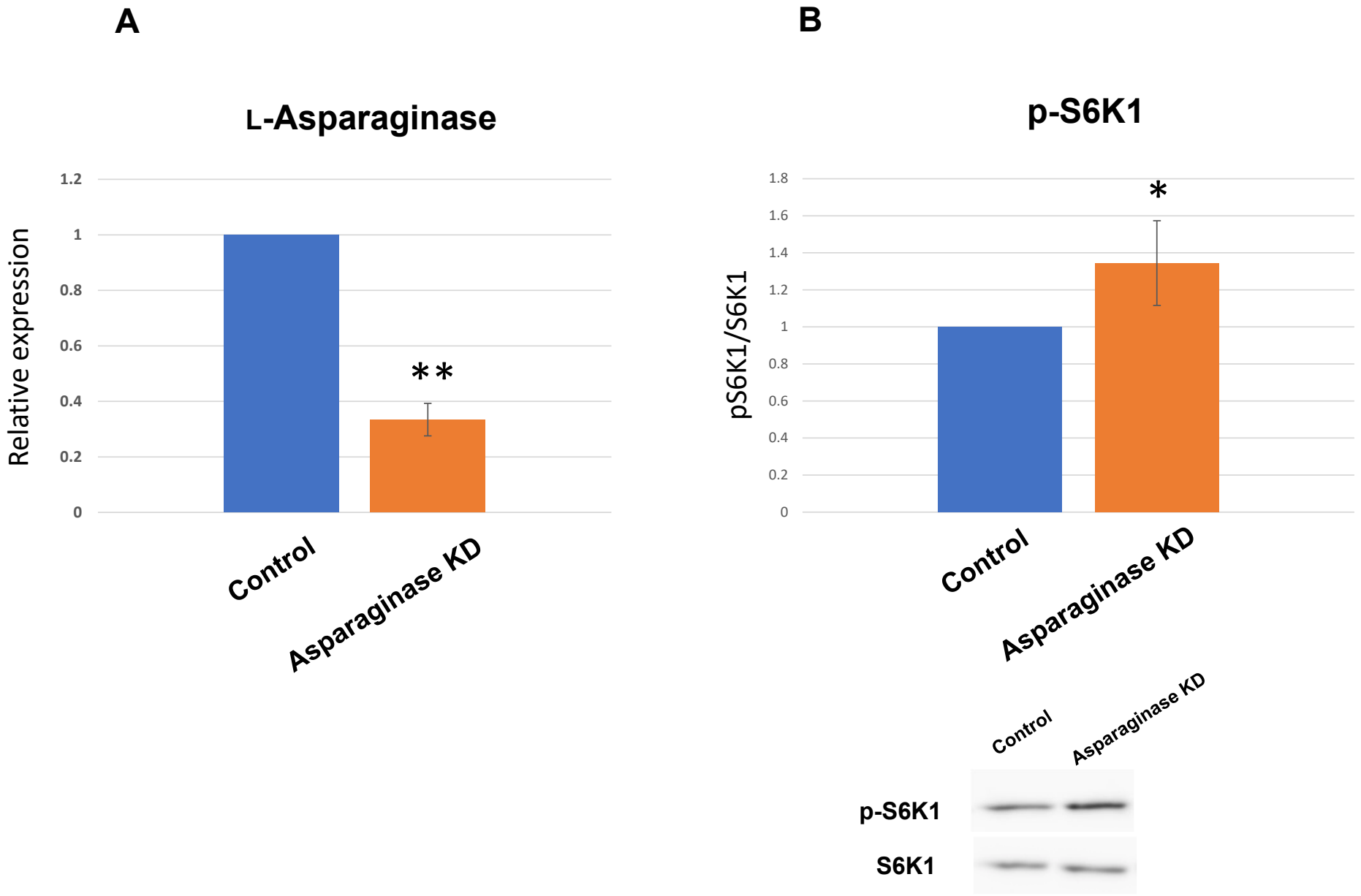

A
L-Asparaginase
Relative expression
1.2
1
0.8
0.6
0.4
0.2
0
**
Control
Asparaginase KD
B
p-S6K1
pS6K1/S6K1
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
Control
Asparaginase KD
Control
Asparaginase KD
p-S6K1
S6K1

Fig. 5A,B

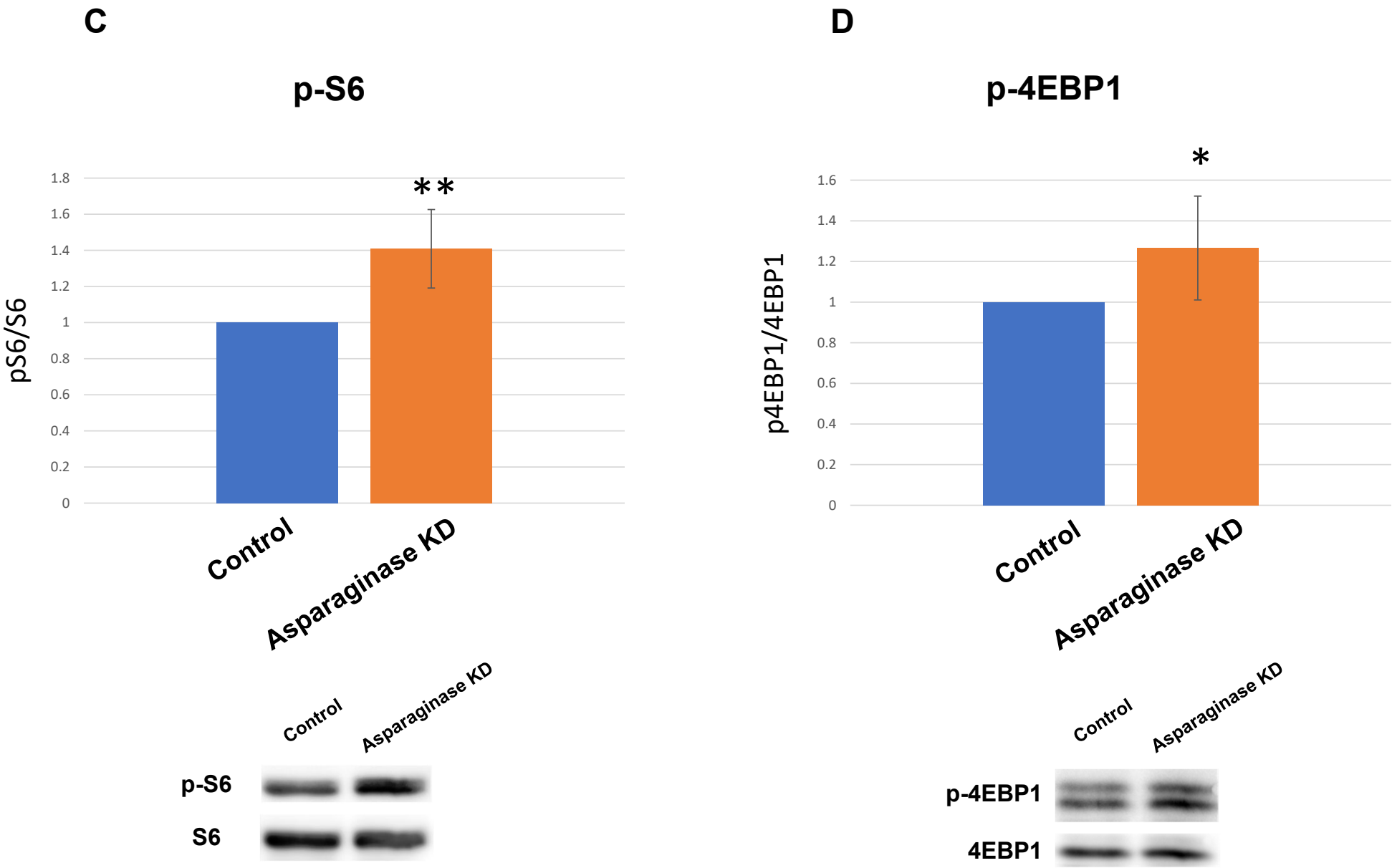

C
p-S6
pS6/S6
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
**
Control
Asparaginase KD
Control
Asparaginase KD
p-S6
S6
D
p-4EBP1
p4EBP1/4EBP1
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
Control
Asparaginase KD
Control
Asparaginase KD
p-4EBP1
4EBP1

Fig. 5C, D

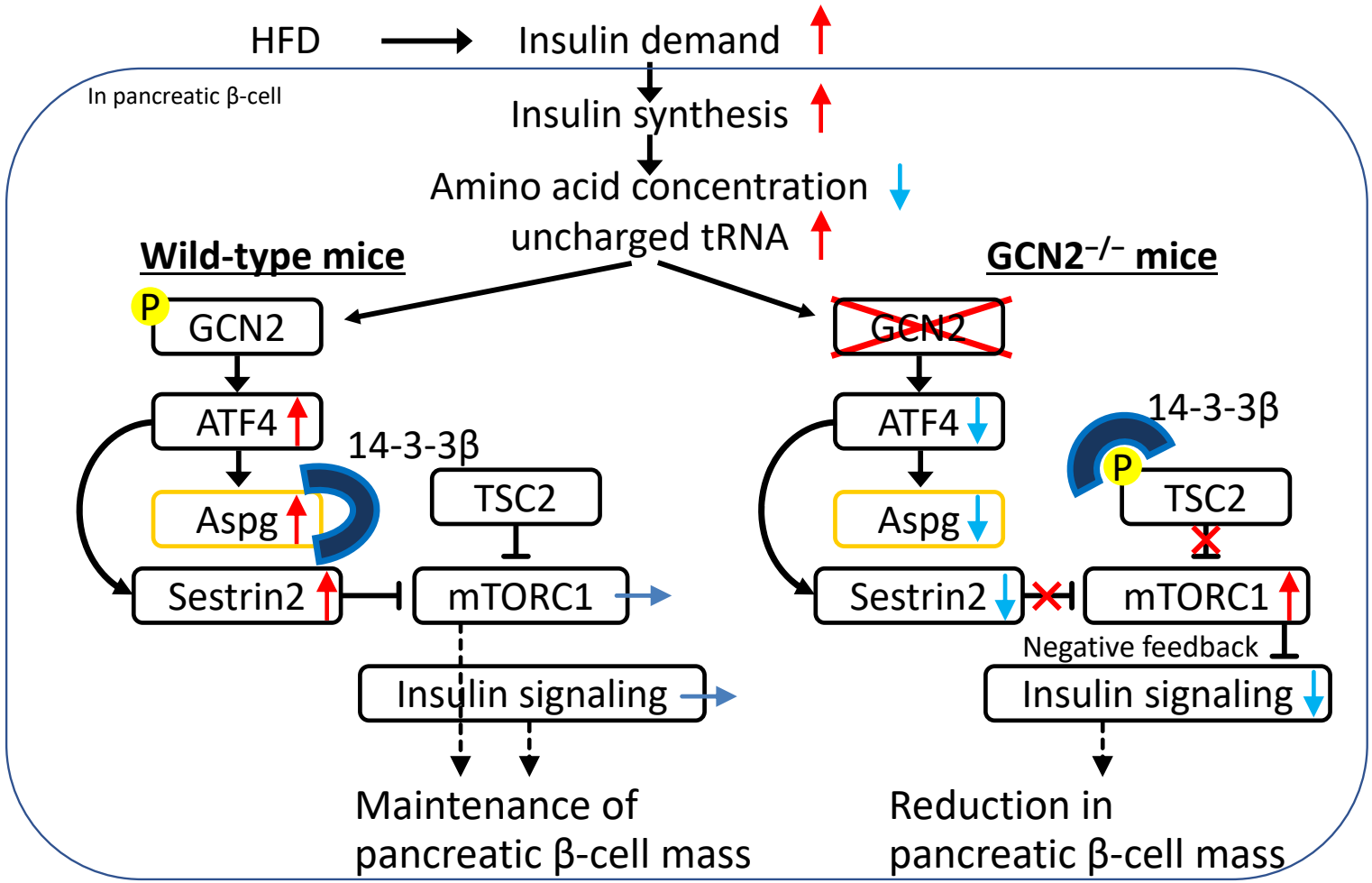

HFD
Insulin demand
In pancreatic β-cell
Insulin synthesis
Amino acid concentration
uncharged tRNA
Wild-type mice
GCN2−/− mice
P
GCN2
GCN2
ATF4
ATF4
14-3-3β
14-3-3β
P
Aspg
Aspg
TSC2
TSC2
Sestrin2
Sestrin2
mTORC1
mTORC1
Negative feedback
Insulin signaling
Insulin signaling
Maintenance of
pancreatic β-cell mass
Reduction in
pancreatic β-cell mass

Fig. 5E