SAMP1/TMEM201 Promotes Hepatic Gluconeogenesis by PKA/CREB Pathway

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Abstract

Background & Aims: Hepatic gluconeogenesis is aberrantly activated in type 2 diabetes (T2DM), contributing to fasting hyperglycemia. While the PKA/CREB signaling axis is a central regulator, the precise mechanisms fine-tuning this pathway, particularly at the level of the nuclear compartment, remain incompletely understood. SAMP1, also known as Transmembrane Protein 201 (TMEM201), is an inner nuclear membrane protein. Its potential role in metabolic regulation, especially in hepatic glucose production, is entirely unexplored. This study aimed to investigate the function and mechanism of SAMP1 in regulating hepatic gluconeogenesis.

Methods: The expression of SAMP1 was examined in the livers of diabetic (db/db) mice. Gain- and loss-of-function studies were conducted in vivo using hepatocyte-specific SAMP1 overexpression and knockdown mice delivered via AAV8 vectors, and in vitro in primary mouse hepatocytes using adenoviral vectors. Glucose and pyruvate tolerance tests were performed. Molecular mechanisms were probed using co-immunoprecipitation, Western blotting, and pharmacological inhibition.

Results: Hepatic SAMP1 expression was significantly upregulated in db/db mice. Forced overexpression of SAMP1 in hepatocytes enhanced gluconeogenic gene expression (Pck1, G6pc) and glucose output upon glucagon stimulation, and exacerbated hyperglycemia in mice. Conversely, genetic knockdown of SAMP1 attenuated gluconeogenesis both in vitro and in vivo. Mechanistically, SAMP1 physically interacted with the catalytic subunit of PKA. This interaction facilitated the phosphorylation of the transcription factor CREB, leading to the transcriptional activation of gluconeogenic genes. The pro-gluconeogenic effect of SAMP1 was abolished by the CREB phosphorylation inhibitor KG-501.

Conclusions: Our findings identify the inner nuclear membrane protein SAMP1/TMEM201 as a novel critical enhancer of hepatic gluconeogenesis. It functions as a scaffold that interacts with PKA to potentiate the PKA/CREB signaling cascade. Targeting SAMP1 may represent a promising therapeutic strategy for mitigating excessive hepatic glucose output in T2DM.

Keywords: SAMP1; TMEM201; Gluconeogenesis; PKA; CREB; Type 2 Diabetes.

Introduction

Hepatic gluconeogenesis, the process by which the liver synthesizes glucose, is central to maintaining fasting blood glucose levels. In Type 2 Diabetes Mellitus (T2DM), this pathway becomes hyperactivated, leading to excessive glucose production and contributing significantly to fasting hyperglycemia, a hallmark of the disease[1]. The regulation is complex, involving hormonal signals, key transcription factors, and enzymatic activities.

The canonical pathway involves hormones like glucagon, which, during fasting, activates the PKA/CREB signaling cascade. This leads to the phosphorylation of the transcription factor CREB (cAMP response element-binding protein) and the de-repression of its coactivator CRTC2 (CREB regulated transcription coactivator 2)[2]. This complex then binds to the promoters of key gluconeogenic genes, such as PCK1(encoding Phosphoenolpyruvate carboxykinase, PEPCK) and G6PC (encoding Glucose-6-phosphatase, G6Pase), driving their expression. Insulin normally counteracts this process, but insulin resistance in T2DM blunts this effect[3].

SAMP1, also known as Transmembrane Protein 201 (TMEM201), is an integral inner nuclear membrane protein that has garnered significant research interest due to its role in cellular structure maintenance and its implications in cancer and angiogenesis[4-6]. The protein's location at the nuclear envelope positions it as a potential key regulator of nuclear integrity and cellular signaling pathways, with emerging evidence linking

its dysfunction to broader systemic effects. In this article, we discovered that this protein may play a significant role in liver gluconeogenesis and the PKA signaling pathway.

Results

1. SAMP1/TMEM201 Expression is Upregulated in the Livers of Diabetic Models

To investigate the potential role of SAMP1 in metabolic disorders, we first examined its expression in the livers of leptin receptor-deficient (db/db) induced mouse models of type 2 diabetes (Fig.1A). Immunoblotting analysis revealed that SAMP1 protein levels were significantly elevated in this model compared to their respective controls (Fig.1B). This upregulation suggests a potential link between SAMP1 and the dysregulated metabolic state characteristic of diabetes.

2 Overexpression of SAMP1 promotes Hepatic gluconeogenesis in Vitro

To assess the impact of SAMP1 on the regulation of gluconeogenesis in hepatocytes, we modulated SAMP1 expression using Adv-Vector and Adv-SAMP1 in Primary mouse hepatocyte and monitored glucose production changes in response to glucagon. Interestingly, overexpression of SAMP1 could alter glucose production in primary mouse hepatocyte following glucagon administration (Fig. 2A). Consistent with the results, Additionally, RT-PCR analyses confirmed the increased expression of G6PC, and PCK1 in the Adv-SAMP1 group, providing further support for our findings (Fig. 2B-2C). These results confirmed that SAMP1 promoted hepatic gluconeogenesis in vitro under glucagon stimulation

3 Overexpression of SAMP1 promotes Hepatic gluconeogenesis in Vivo

Hepatocyte-specific SAMP1 overexpression mice were obtained through tail vein injection of AAV8-TBG- SAMP1. The fed blood glucose was increased in the AAV8-TBG-SAMP1 group compared to the AAV8-TBG-SAMP1 group (Fig. 2D). Hepatocyte-specific overexpression of SAMP1 results in impaired GTT in mice (Fig. 2E). Furthermore, the gluconeogenesis-related gene was significantly activated in

liver. we observed a significant increase in the expression of the gluconeogenesis genes G6pc (Fig. 2F), and Pck1 (Fig. 2G). Collectively, these findings suggest that SAMP1/TMEM201 may play a role in hepatic gluconeogenesis and have significant implications in systemic glucose metabolism.

2. Genetic Knockdown of SAMP1 Attenuates Hepatic Gluconeogenesis

To assess the functional role of SAMP1 in glucose metabolism, we performed genetic knockdown of SAMP1 in primary mouse hepatocytes using adenoviruses expressing shRNA targeting SAMP1. Remarkably, SAMP1 deficiency led to a significant reduction in glucose output under glucagon-stimulated conditions (Fig. 3A). Consistent with this functional change, the mRNA expression of key gluconeogenic enzymes, glucose-6-phosphatase (G6pc) (Fig. 3B) and phosphoenolpyruvate carboxykinase (Pck1) (Fig. 3C), was markedly downregulated in SAMP1-knockdown hepatocytes (Fig. 3D). These results indicate that SAMP1 is required for efficient hepatic gluconeogenesis.

3. Hepatocyte-Specific SAMP1 Knockout Improves Glucose Homeostasis In Vivo

Next, AAV8-TBG-shSAMP1 was employed to interfere with the expression of SAMP1 in the liver of mice to observe its effect on hepatic gluconeogenesis. In the AAV8-TBG-shSAMP1 group, fed blood glucose levels were decreased (Fig. 3D), PTT results were improved (Fig. 3E), and the expression of PCK1 and G6PC in the liver were decreased compared to the AAV8-TBG-SAMP1 group (Fig. 3F,3G). Together, these findings confirmed that downregulating the expression of SAMP1 could reduce hepatic gluconeogenesis

4. SAMP1 Interacts with PKA and Regulates the CREB pathway

Given that SAMP1 is an inner nuclear membrane protein, we hypothesized it might function by regulating the compartmentalization of key metabolic regulators. Co-immunoprecipitation assays in mouse livers identified PKA as a novel SAMP1-interacting partner (Fig. 4A). Furthermore, cellular experiments

demonstrated that overexpression of SAMP1 promotes CREB phosphorylation (Fig. 4B), and SAMP1 knockdown significantly impaired the glucagon-induced CREB phosphorylation (Fig. 4C). Simultaneously, the CREB phosphorylation inhibitor KG501 could block the regulation by SAMP1 (Fig. 4D)

Disucussion

This study is the first to reveal the critical role of the inner nuclear membrane protein SAMP1/TMEM201 in hepatic gluconeogenesis. Our data consistently demonstrate that SAMP1 expression is upregulated in diabetic models and functions as a significant positive regulator of gluconeogenesis in both in vivo and in intraexperiment. More importantly, we have elucidated a previously unrecognized molecular mechanism: SAMP1 directly interacts with PKA, thereby promoting the phosphorylation of CREB and amplifying the transcriptional activation of gluconeogenic genes.

The central finding of this research lies in the direct binding between SAMP1/TMEM201 and PKA. The conventional view holds that the regulation of the cAMP/PKA signaling pathway occurs primarily in the cytoplasm and via second messengers. Our results introduce a nuclear membrane protein into this classical pathway, proposing a new dimension of spatial regulation. As an inner nuclear membrane protein, SAMP1 may provide a scaffolding platform for PKA in the perinuclear region. This interaction could potentially enhance the efficiency of PKA-mediated phosphorylation of its nuclear substrates, such as CREB, or influence the localization and activity of PKA itself. This aligns with the emerging trend emphasizing the importance of subcellular spatial localization in metabolic signaling. However, the precise mechanism by which SAMP1 regulates PKA's activity and function—whether by sequestering PKA at the nuclear envelope or facilitating its proximity to specific substrates—requires further investigation. Our findings carry significant pathophysiological implications. The observed upregulation of SAMP1 in the T2DM state suggests that it may be a contributing factor driving the excessive

gluconeogenic activity in the context of hepatic insulin resistance. Increased SAMP1 expression could sensitize hepatocytes to signals from gluconeogenic hormones like glucagon, thereby exacerbating hyperglycemia. Consequently, SAMP1 is not only a novel regulatory factor but also a potential therapeutic target. Developing small-molecule inhibitors that specifically disrupt the SAMP1-PKA interaction could pave the way for novel therapies aimed at selectively inhibiting hepatic gluconeogenesis while avoiding the side effects associated with systemic, complete blockade of PKA signaling.

Certainly, this study has limitations. First, the alteration of SAMP1 expression in the livers of human T2DM patients and its clinical relevance need further validation. Second, it remains unclear whether SAMP1 interacts with other nuclear signaling molecules or transcriptional coregulators involved in energy metabolism beyond PKA. Future research employing proteomic approaches may uncover additional interacting partners of SAMP1. Furthermore, exploring whether SAMP1 itself is subject to transcriptional or post-translational regulation by nutritional status, hormones, or cellular stress represents an intriguing line of inquiry.In summary, our work establishes SAMP1/TMEM201 as a key regulator of hepatic gluconeogenesis. By directly binding to PKA and enhancing CREB phosphorylation, it intricately links nuclear membrane structure with metabolic signaling pathways. These findings not only deepen our understanding of the mechanisms underlying hyperglycemia in diabetes but also identify a potential target for novel therapeutic strategies.

Materials And Methods

Male C57BL/6 mice (8-week-old) and male db/db mice (8-week-old) were acquired from Charles River Laboratories. To generate mice model featuring hepatocyte-specific overexpression and knockdown of the SAMP1, Adeno-associated virus 8 (AAV8)-TBG- SAMP1or AAV8-TBG-sh SAMP1 were administered via tail vein into C57BL/6 or db/db mice, respectively. 4 weeks post AAV8 injection.

Animals were housed under humane conditions with controlled temperature of 22-24° C, relative humidity of 40-60%, a 12-hour light/dark cycle from 7:00 to 19:00, and ad libitum access to food and water. The above-mentioned mice were sacrificed at 12 weeks of age, blood and tissue samples were collected for further experiments. Animal studies were reviewed and approved by the IRCCS Institutial Care and Use Committee, and were in accordance with the Animal Welfare Act and ARRIVE guidelines.

Glucose and pyruvate tolerance tests

pyruvate tolerance tests (PTT) were conducted in C57BL/6 (4 weeks after viral injection). For the PTT, mice were injected intraperitoneally with sodium pyruvate (2g/kg) after overnight fasting (5:00 P.M. to 9:00 A.M.), and blood glucose levels were measured at 0, 30-, 60-, 90-, and 120-minutes post-injection.

Cell culture and treatment

Mouse primary hepatocytes (MPHs) were isolated from 6-week-old male C57BL/6 mice as previously reported[7], and cultured in DME/F12 medium. Cells were incubated at 37°C in a 5% CO2 incubator. For transfection experiments, MPHs were transfected with Adv-*SAMP1* or Adv-sh*SAMP1* for 48 hours and then subjected to subsequent experiments.

Glucose production assay

Primary hepatocytes were washed three times with PBS and then incubated in glucose and phenol red-free DMEM containing 20 mM sodium lactate and 2 mM sodium pyruvate for 4 hours. Simultaneously, saline, glucagon (100nM, HY-P0082, MedChemExpress), were administered separately to observe the effects of these treatments on glucose production. Glucose levels in the culture medium were measured using a glucose assay Kit (MAK181, Sigma-Aldrich), and the total glucose production was adjusted for protein content.

RT-PCR

Total RNA was extracted from animal tissues or cells using TRIzol reagent (9109, Takara). Reverse transcription was carried out using the ScriptSure Reverse Transcription Kit (G3326, Takara). Subsequent quantitative PCR reactions were performed using the Takara SYBR Green qPCR Kit (6215A, Takara) and measurements were conducted on a Jena Real-Time PCR System (Analytik Jena). The expression levels of target genes were normalized to those of GAPDH.

Western blot analysis

Total proteins were isolated from animal tissues or cells using the RIPA lysis buffer (P0013B, Roche), supplemented with a phosphatase inhibitor or protease inhibitor cocktail (4693132001, 4906837001, Roche). Protein concentration was determined using the BCA Protein Assay Kit. Equal protein amounts were separated by 10% SDS-PAGE and transferred to PVDF membranes (IPVH00010, Millipore). Membranes were blocked using a blocking solution, followed by overnight incubation at 4°C with primary antibodies. After three TBST washes, the membranes were incubated with HRP-conjugated secondary antibodies at room temperature for 1 hour. Membrane visualization was done using the Jena Imaging System (Analytik Jena).

Statistical Analysis

All the quantified data are expressed as the means ± SD. An unpaired Student's t-test was performed to analyze the differences between the two groups. For multiple groups, one-way ANOVA was carried out, followed by the Bonferroni post hoc test. Statistical analyses were performed with GraphPad Prism 9.0 software. A value of $p<0.05$ was considered significant.

Contribution

Yunayuan Luo: Writing- original draft, Data curation, Software, Methodology,

Elisa Schena: Conceptualization, Funding acquisition, Supervision, Writing – review & editing

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Conflicts of Interest

The authors declare no conflict of interest.

Reference

1. Barroso, E., Jurado-Aguilar, J., Wahli, W., Palomer, X., and Vázquez-Carrera, M. (2024). Increased hepatic gluconeogenesis and type 2 diabetes mellitus. Trends Endocrinol Metab 35, 1062-1077. 10.1016/j.tem.2024.05.006.

2. Yang, M., Pan, M., Huang, D., Liu, J., Guo, Y., Liu, Y., and Zhang, W. (2023). Glucagon Promotes Gluconeogenesis through the GCGR/PKA/CREB/PGC-1α Pathway in Hepatocytes of the Japanese Flounder Paralichthys olivaceus. Cells 12. 10.3390/cells12071098.

3. Hatting, M., Tavares, C.D.J., Sharabi, K., Rines, A.K., and Puigserver, P. (2018). Insulin regulation of gluconeogenesis. Annals of the New York Academy of Sciences 1411, 21-35. 10.1111/nyas.13435.

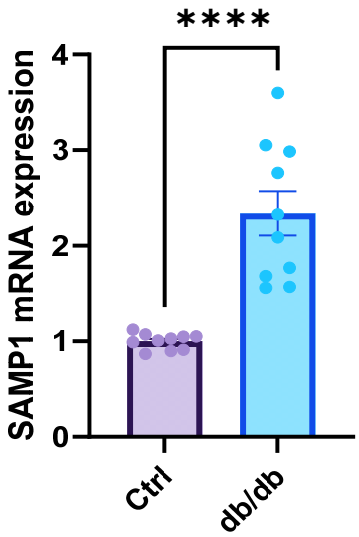
4. Kong, Y., Zhang, Y., Wang, H., Kan, W., Guo, H., Liu, Y., Zang, Y., and Li, J. (2022). Inner nuclear membrane protein TMEM201 promotes breast cancer metastasis by positive regulating TGFβ signaling. Oncogene 41, 647-656. 10.1038/s41388-021-02098-5.

5. Chen, D., Lou, Y., Lu, J., Fan, X., Zhu, Q., and Sun, H. (2023). Characterization of the Clinical Significance and Immunological Landscapes of a Novel TMEMs Signature in Hepatocellular Carcinoma and the Contribution of TMEM201 to Hepatocarcinogenesis. Int J Mol Sci 24. 10.3390/ijms241210285.

6. Zhang, Y., Kong, Y., Guo, H., Liu, Y., Zang, Y., and Li, J. (2022). Inner nuclear membrane protein TMEM201 maintains endothelial cell migration and angiogenesis by interacting with the LINC complex. J Mol Cell Biol 14. 10.1093/jmcb/mjac017.

7. Charni-Natan, M., and Goldstein, I. (2020). Protocol for Primary Mouse Hepatocyte Isolation. STAR protocols 1, 100086. 10.1016/j.xpro.2020.100086.

A


****
SAMP1 mRNA expression
4
3
2
1
0
Ctrl
db/db

B

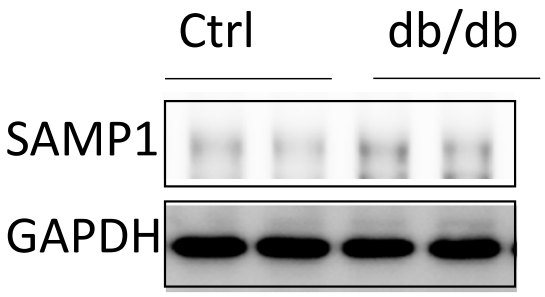

Ctrl
db/db
SAMP1
GAPDH

Fig. 1 Hepatic SAMP1 is upregulated in vivo.

A. SAMP1 mRNA level is increased in livers of db/db mice.

B. SAMP1 protein level is increased in livers of db/db mice.

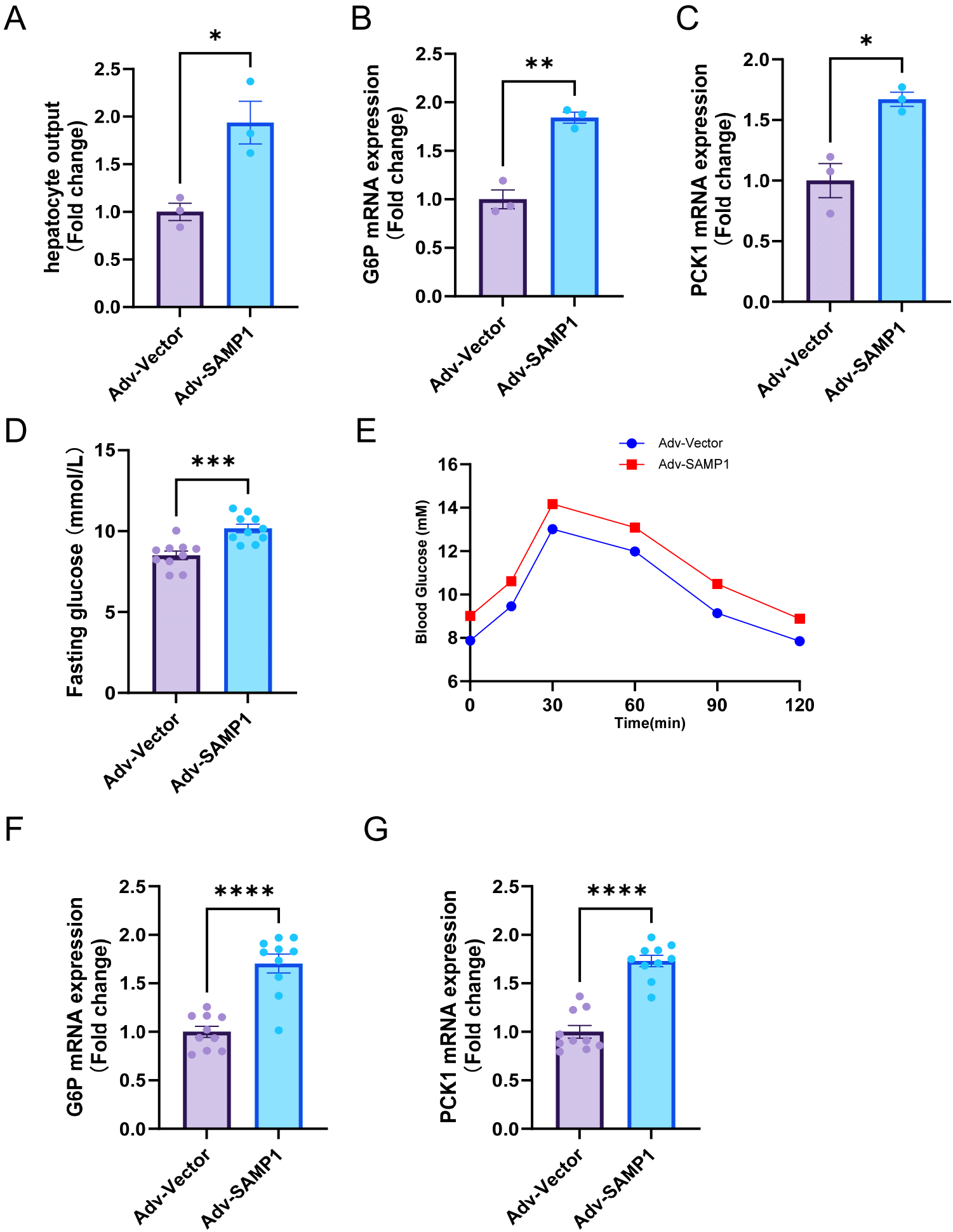

A
hepatocyte output (Fold change)
2.5
2.0
1.5
1.0
0.5
0.0
*
Adv-Vector
Adv-SAMP1
B
G6P mRNA expression (Fold change)
2.5
2.0
1.5
1.0
0.5
0.0
**
Adv-Vector
Adv-SAMP1
C
PCK1 mRNA expression (Fold change)
2.0
1.5
1.0
0.5
0.0
*
Adv-Vector
Adv-SAMP1
D
Fasting glucose（mmol/L）
15
10
5
0
***
Adv-Vector
Adv-SAMP1
E
Adv-Vector
Adv-SAMP1
Blood Glucose (mM)
16
14
12
10
8
6
0
30
60
90
120
Time(min)
F
G6P mRNA expression (Fold change)
2.5
2.0
1.5
1.0
0.5
0.0
****
Adv-Vector
Adv-SAMP1
G
PCK1 mRNA expression (Fold change)
2.5
2.0
1.5
1.0
0.5
0.0
****
Adv-Vector
Adv-SAMP1

Fig. 2 SAMP1 promotes gluconeogenesis in vitro and in vivo.

A. SAMP1 overexpression increases glucose production in mouse primary hepatocytes.

B. SAMP1 overexpression upregulates G6p mRNA levels in mouse primary hepatocytes.

C. SAMP1 overexpression upregulates Pck1 mRNA levels in mouse primary hepatocytes.

D. SAMP1 overexpression in mice increases fed blood glucose and impairs PTT.

E. SAMP1 overexpression in mice impairs PTT.

F. SAMP1 overexpression in mice upregulates G6p mRNA levels.

G. SAMP1 overexpression in mice upregulates Pck1 mRNA levels.

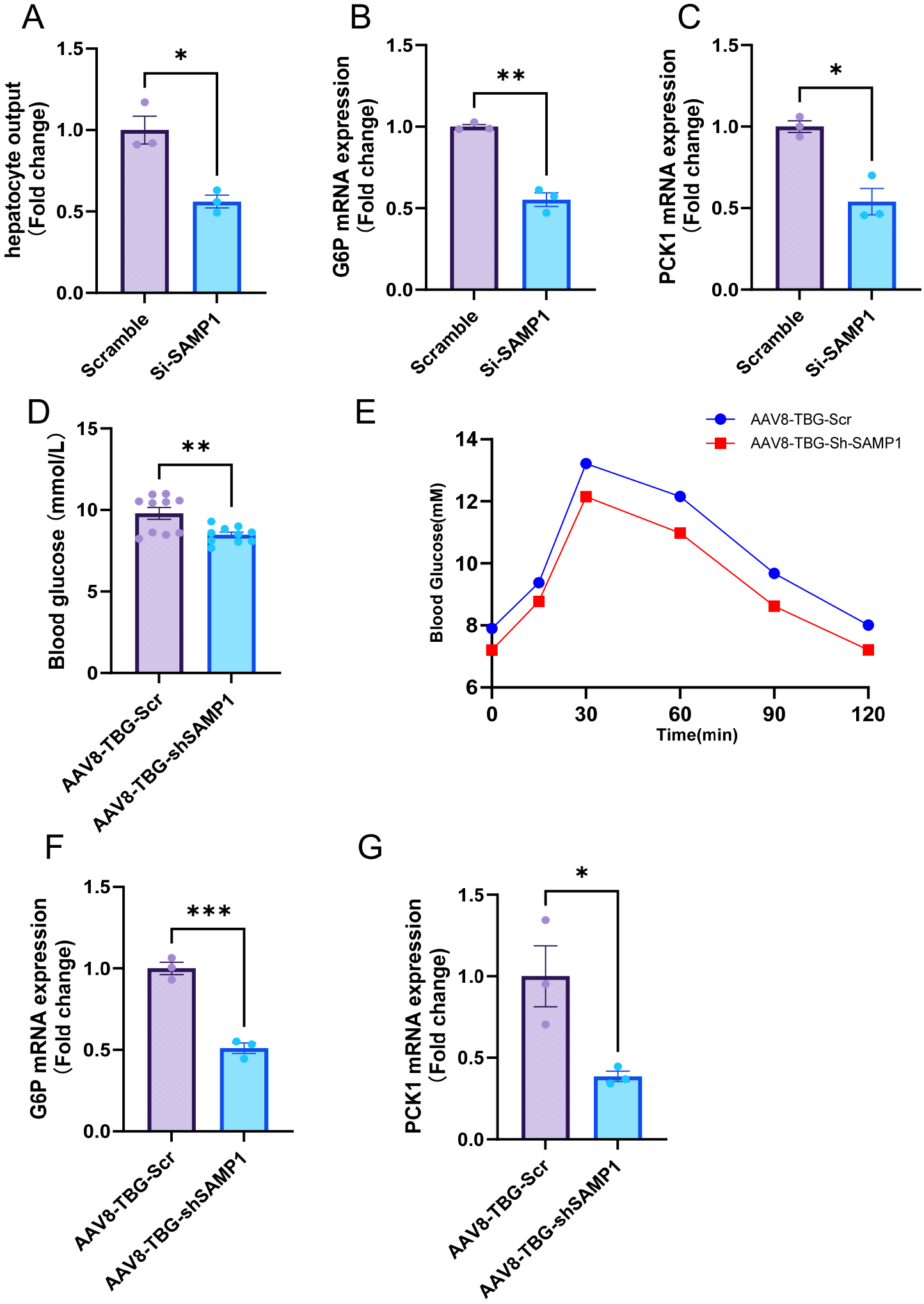

A
hepatocyte output
(Fold change)
1.5
1.0
0.5
0.0
*
Scramble
Si-SAMP1
B
G6P mRNA expression
(Fold change)
1.5
1.0
0.5
0.0
**
Scramble
Si-SAMP1
C
PCK1 mRNA expression
(Fold change)
1.5
1.0
0.5
0.0
*
Scramble
Si-SAMP1
D
Blood glucose（mmol/L）
15
10
5
0
**
AAV8-TBG-Scr
AAV8-TBG-shSAMP1
E
AAV8-TBG-Scr
AAV8-TBG-Sh-SAMP1
Blood Glucose(mM)
14
12
10
8
6
0
30
60
90
120
Time(min)
F
G6P mRNA expression
(Fold change)
1.5
1.0
0.5
0.0
***
AAV8-TBG-Scr
AAV8-TBG-shSAMP1
G
PCK1 mRNA expression
(Fold change)
1.5
1.0
0.5
0.0
*
AAV8-TBG-Scr
AAV8-TBG-shSAMP1

Fig. 3 Hepatocyte-specific SAMP1 knock-down inhibited hepatic gluconeogenesis in C57BL/6 mice.

A. SAMP1 knock-down decreases glucose production in mouse primary hepatocytes.

B. SAMP1 knock-down decreases G6p mRNA levels in mouse primary hepatocytes.

C. SAMP1 knock-down decreases Pck1 mRNA levels in mouse primary hepatocytes.

D.SAMP1 knock-down in mice decreases fed blood glucose.

E. SAMP1 knock-down in mice showed improved PTT.

F. SAMP1 knock-down in mice downregulates G6p mRNA levels.

G. SAMP1 knock-down in mice downregulates Pck1 mRNA levels.

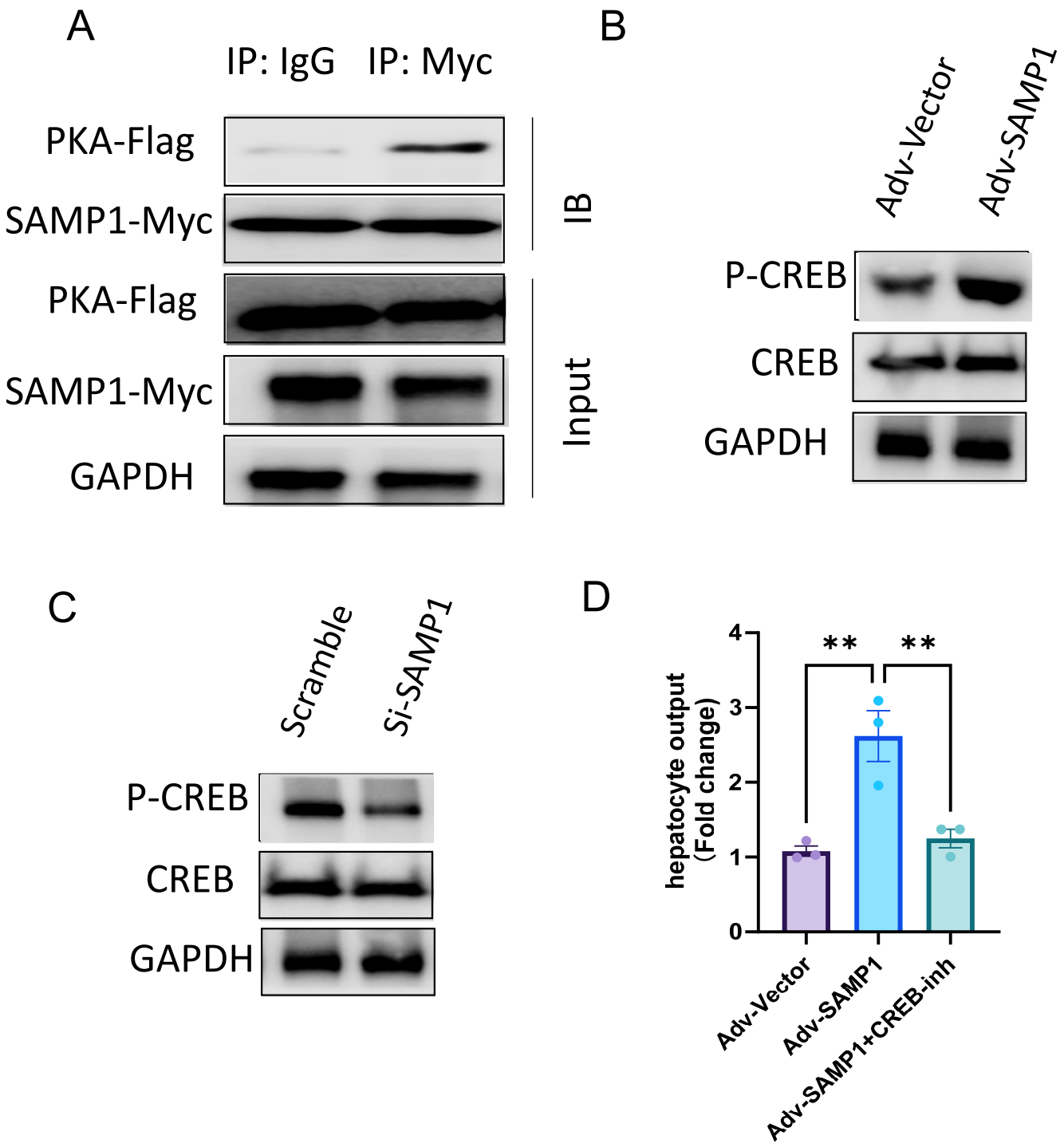

A
IP: IgG
IP: Myc
PKA-Flag
SAMP1-Myc
PKA-Flag
SAMP1-Myc
GAPDH
IB
Input
B
Adv-Vector
Adv-SAMP1
P-CREB
CREB
GAPDH
C
Scramble
Si-SAMP1
P-CREB
CREB
GAPDH
D
hepatocyte output
(Fold change)
0
1
2
3
4
**
**
Adv-Vector
Adv-SAMP1
Adv-SAMP1+CREB-inh

Fig. 4 Hepatocyte-specific SAMP1 knock-down inhibited hepatic gluconeogenesis in C57BL/6 mice.

A. Representative CO-IP analyses of the interaction between SAMP1 and PKA in 293T cells using exogenous Myc-SAMP1 and Flag -PKA.

B. Western blot analyses p-CREB (S133), CREB and GAPDH in mouse primary hepatocytes from Adv-Vector group and Adv-SAMP1 group.

C. Western blot analyses p-CREB (S133), CREB and GAPDH in mouse primary hepatocytes from Scramble and Si-SAMP1 group.

D. Glucose production in mouse primary hepatocytes stimulated by glucagon in mouse primary hepatocytes from Adv-Vector group, Adv-SAMP1 group, and Adv-SAMP1+CREB-inh.

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