Yin Yang 1 Promotes Hepatic Gluconeogenesis Through Upregulation of Glucocorticoid Receptor

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Gluconeogenesis is critical in maintaining blood glucose levels in a normal range during fasting. In this study, we investigated the role of Yin Yang 1 (YY1), a key transcription factor involved in cell proliferation and differentiation, in the regulation of hepatic gluconeogenesis. Our data showed that hepatic YY1 expression levels were induced in mice during fasting conditions and in a state of insulin resistance. Overexpression of YY1 in livers augmented gluconeogenesis, raising fasting blood glucose levels in C57BL/6 mice, whereas liver-specific ablation of YY1 using adenoviral shRNA ameliorated hyperglycemia in wild-type and diabetic *db/db* mice. At the molecular level, we further demonstrated that the major mechanism of YY1 in the regulation of hepatic glucose production is to modulate the expression of glucocorticoid receptor. Therefore, our study uncovered for the first time that YY1 participates in the regulation of hepatic gluconeogenesis, which implies that YY1 might serve as a potential therapeutic target for hyperglycemia in diabetes. *Diabetes* 62:1064–1073, 2013

Hepatic gluconeogenesis is essential for maintenance of blood glucose levels in a normal range in a state of prolonged fasting. The rate of hepatic glucose production is tightly controlled by the key enzymes, including phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phophatase (G6Pase) (1,2). On the other hand, dysregulation of gluconeogenesis is critically responsible for fasting hyperglycemia in type 2 diabetes. Indeed, the expression or activity of these enzymes is significantly increased in diabetic human and rodents (3,4).

In a state of fasting, pancreatic glucagon and adrenal glucocorticoids are secreted to upregulate gluconeogenesis and increase hepatic glucose production (5). Glucagon increases *PEPCK* and *G6Pase* gene expression via cAMP/cAMP-dependent protein kinase (PKA) and cAMP-responsive element binding (CREB)/CREB-regulated transcriptional coactivator 2 (CRTC2), whereas glucocorticoids signal is conveyed via the action of glucocorticoid receptor (GR),

a member of nuclear hormone receptor superfamilies (6–8). It is known that GR stimulates gluconeogenesis by directly upregulating *PEPCK* and *G6Pase* gene expression. GR response elements are found in both promoters of these two key enzymes (9). Besides, recent studies also underscore the importance of GR in the regulation of other important gluconeogenic genes, such as *CREBH*, an endoplasmic reticulum–bound transcription factor (10). However, the molecular determinants of hepatic GR expression remain largely unexplored. Indeed, hepatic GR overexpression is displayed in diabetic *db/db* mice, while deletion of GR using specific antisense oligonucleotides markedly improves hyperglycemia in these mice (11,12), indicating that targeting molecules that regulate GR expression could also be a new option for therapeutic intervention for hyperglycemia.

Yin Yang 1 (YY1) is a ubiquitous transcription factor of the polycomb group protein family, which is widely expressed in various tissues and binds to CCATNTT consensus sequences to activate or silence gene transcription via chromatin modification (13,14). It has been shown that YY1-null mice die during embryonic development around implantation, suggesting its specific roles in the regulation of cell proliferation and differentiation (15). Besides, the findings of YY1 overexpression in multiple cancer cells suggest that YY1 might play an important role in the cancer development and progression (14,16). Moreover, YY1 was able to enhance adipocyte differentiation through upregulation of C/EBPα, implicating its potential significance in obesity (17). YY1 was also reported to repress insulin/IGF-signaling activation (18). As a result, skeletal muscle–specific YY1 knockout mice exhibited glucose tolerance improvement and insulin-signaling activation (18). In addition, a recent study uncovered that YY1 might be a candidate gene responsible for body weight, blood glucose, cholesterol, and free fatty acid levels (19). However, whether YY1 is involved in the regulation of hepatic glucose homeostasis remains unexplored.

In the current study, we showed that hepatic YY1 expression was induced in C57BL/6 mice during fasting and in a state of insulin resistance. YY1 promoted hepatic glucose production via transcriptional upregulation of the GR. Moreover, liver-specific ablation of YY1 results in the reduction of blood glucose levels in wild-type and diabetic *db/db* mice. Therefore, our data support the notion that YY1 is a crucial regulator of hepatic gluconeogenesis. YY1 could be a therapeutic target for fasting hyperglycemia in diabetes.

RESEARCH DESIGN AND METHODS

C57BL/6 and *db/db* mice, 10–12 weeks of age, were purchased from Shanghai Laboratory Animal Company (Shanghai, China). All mice were raised in a temperature- and light-controlled environment with a 12-h light (0800–2000 h) and 12-h dark (2000–0800 h) cycle. Animal experiments were performed

during the light cycle. Blood glucose was measured using a portable blood glucose meter (LifeScan, Johnson & Johnson). Plasma levels of insulin and corticosterone were determined using commercial kits from Millipore. Pyruvate tolerance tests (PTTs) were performed in a state of fasting for 16 h, with injection of 1.5 g/kg i.p. sodium pyruvate in saline. The animal protocol was reviewed and approved by the animal care committee of Shanghai Jiao Tong University School of Medicine.

Adenovirus. Adenovirus-expressing murine YY1 (Ad-YY1) or GFP (Ad-GFP) was constructed by Invitrogen (Shanghai, China) with a full-length YY1 or GFP cDNA coding sequence. Overexpression of hepatic YY1 or GFP was achieved by means of tail-vein injection of viruses (4×10^9 plaque-forming units) in normal C57BL/6 mice. For silencing of YY1 or GR expression, adenoviruses expressing YY1 or GR short hairpin RNA (shRNA) were generated using pAD_BLOCK_IT_DEST vectors (Invitrogen). The sense sequence for the YY1 shRNA is 5′-GGGAGCAGAAGCAGGTGCAGA-3′. The sense sequences for GR shRNA is 5′-AGAAATGACTGCCTTACTA-3′, which was kindly provided by Dr. Stephan Herzig (German Cancer Research Center, Heidelberg, Germany) (20). A nucleotide loop (TCAAGACT) was placed between the sense and antisense sequences. All viruses were purified by the cesium chloride method and dialyzed in phosphate-buffered saline containing 10% glycerol prior to animal injection.

Reagents, cell culture, and small interfering RNA. Dexamethasone and forskolin were obtained from Sigma-Aldrich. Human embryonic kidney cells (HEK293T cells) and hepatocellular carcinoma cells (HepG2 cells) were cultured in Dulbecco's modified Eagle's medium (Invitrogen), supplemented with 10% FBS (Gibco), 100 units/mL penicillin, and 100 µg/mL streptomycin (Gibco).

All small interfering RNAs (siRNAs) were chemically synthesized by GenePharma (Shanghai, China). The siRNA sequences for targeting YY1 were as follows: 5′-GGGAGCAGAAGCAGGUGCAGAU-3′ (steroid receptor co-activator-1 [SRC-1]), 5′-GACAUUGACAGCUUGAGUGUA-3′ (S1), and 5′-CUUCAACUACAGCAGCGAUUAC-3′ (S2). As negative control, an siRNA sequence targeting luciferase was used: 5′-CGUACGCGGAAUACUUCGA-3′.

Transfection and dual-luciferase reporter assay. YY1 and GR promoters were amplified from the mouse genomic DNA template and inserted into pGL4.15 empty vectors (Promega). Mutant promoters were generated using a PCR mutagenesis kit (Toyobo). All of the transient transfections were conducted using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. For luciferase reporter assays, cells were seeded in 24-well plates and transfected with the indicated plasmids. *Renilla* luciferase pRL-SV40 (Promega) was used to normalize the luciferase activity, which was further measured using the dual-luciferase reporter assay system (Promega).

RNA isolation and real-time PCR. Total RNA was isolated from tissues or cells using TRIzol (Invitrogen) according to the manufacturer's instructions. For quantification of the transcripts of the interest genes, quantitative real-time PCR was performed using a SYBR Green Premix Ex Taq (Takara Bio, Otsu, Japan) on Light Cycler 480 (Roche, Basel, Switzerland). The sequences of all used primers are available on request.

Western blotting. Tissues and cells were lysed in radioimmunoprecipitation buffer containing 50 mmol/L Tris-HCl, 150 mmol/L NaCl, 5 mmol/L MgCl₂, 2 mmol/L EDTA, 1 mmol/L NaF, 1% NP40, and 0.1% SDS. Western blots were performed using antibodies against YY1 (Santa Cruz Biotechnology), GR (Santa Cruz Biotechnology), PEPCK (Santa Cruz Biotechnology), G6Pase
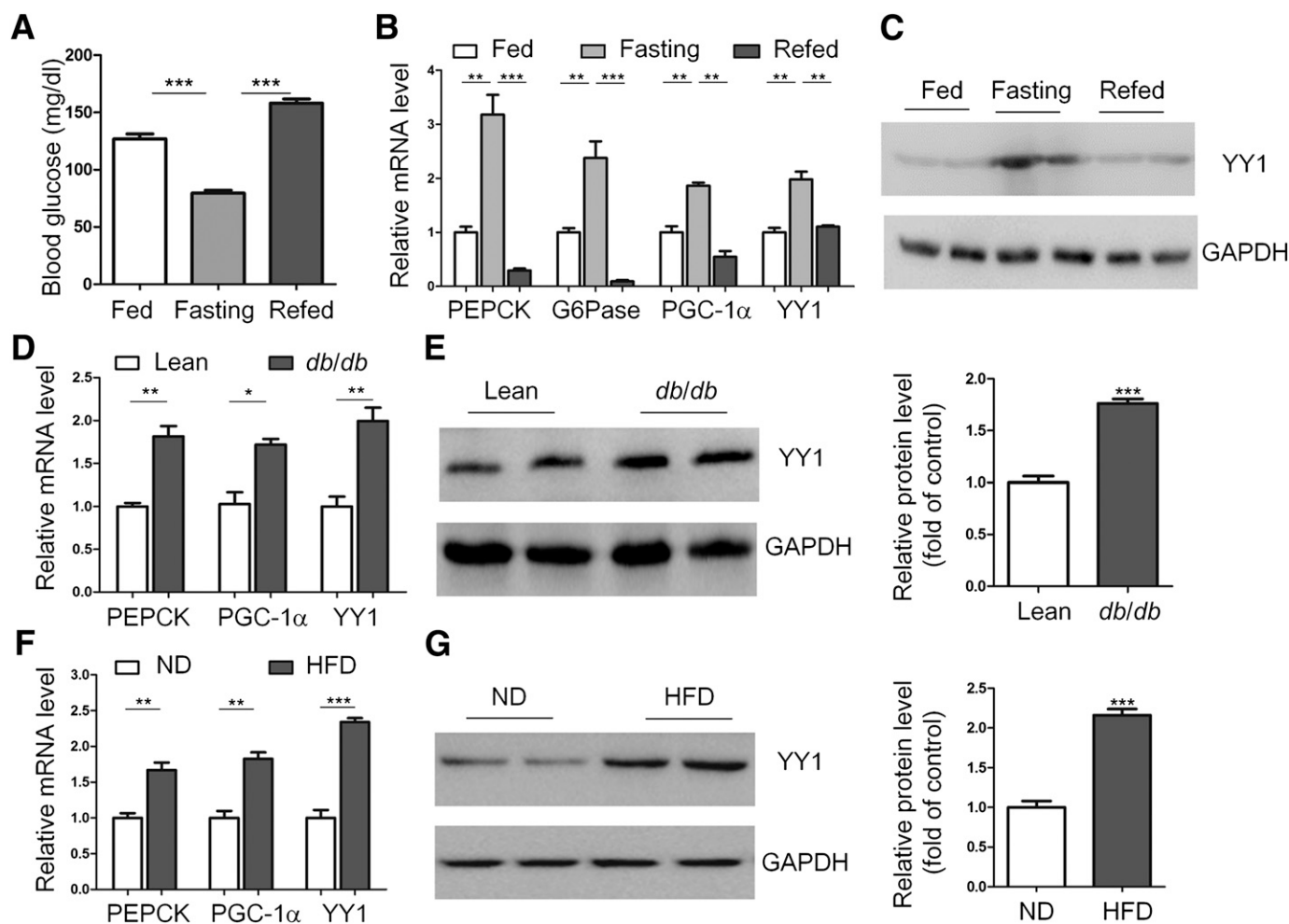
FIG. 1. Induction of hepatic YY1 expression during fasting and in a state of insulin resistance. *A*: Blood glucose in C57BL/6 mice under fed or 24-h fasted or 24-h fasted/6-h refed conditions (*n* = 5). *B*: Real-time PCR analysis of hepatic YY1 and gluconeogenic gene expression in mice as in *A*. *C*: Western blot analysis for YY1 protein levels in mice as indicated above. *D* and *E*: Real-time PCR (*D*) and Western blotting (*E*) analysis of hepatic YY1 or gluconeogenic gene expression in either lean or *db/db* mice under 16-h fasting (*n* = 6). *F* and *G*: mRNA (*F*) and protein (*G*) levels of YY1 in livers from mice with high-fat diet (HFD)-induced obesity undergoing 16 h fasting. *n* = 6–7. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ND, normal diet; PGC-1α, peroxisome proliferator–activated receptor γ coactivator-1α.

(Abcam), glyceraldehyde-3-phosphate dehydrogenase (Cell Signaling Technology), Flag (Cell Signaling Technology), and GFP (Cell Signaling Technology). Protein expression levels were quantitated with the use of ImageJ.

Chromatin immunoprecipitation. A chromatin immunoprecipitation (ChIP) assay kit was used (Upstate Biotechnology). In brief, lysates from HepG2 cells or homogenized mouse liver nuclear lyses were fixed with formaldehyde. DNA was sheared to fragments at 200–1,000 bp using sonication. The chromatins were incubated and precipitated with antibodies against YY1, SRC-1 (Santa Cruz Biotechnology), acetylated histone H3 (Cell Signaling Biotechnology), or IgG (Santa Cruz Biotechnology).

Statistics. All values are presented as means ± SEM. Statistical differences were determined by a Student t test. Statistical significance is displayed as $P <$ 0.05, $P < 0.01$, or $P < 0.001$.

RESULTS

Hepatic YY1 expression is increased during fasting and in obese mice. To evaluate the potential involvement of YY1 in hepatic gluconeogenesis, we measured its expression levels in mouse liver. YY1 expression was significantly induced during fasting conditions and reduced upon refeeding, which was in parallel with a characteristic regulatory pattern for gluconeogenesis (Fig. 1A–B). Western blotting analysis also showed the alternate pattern of YY1 protein in the fasting-to-fed transition (Fig. 1C). Hepatic YY1 was also upregulated in *db/db* mice and mice with high-fat diet–induced obesity, in which gluconeogenesis was markedly enhanced (Fig. 1D–G). These results indicated that YY1 might be involved in the transcriptional regulation of hepatic gluconeogenesis.

Activation of cAMP-CREB signaling pathway upregulates YY1 expression. To elucidate a potential mechanism for YY1 upregulation, we treated HepG2 cells with stimuli known to mimic fasting signals, including forskolin, cAMP agonist, and glucagon. YY1 expression was significantly increased by forskolin (Fig. 2A and B) and glucagon (Fig. 2C), suggesting an involvement of a cAMP-PKA-CREB signaling pathway in this process. Indeed, H-89,
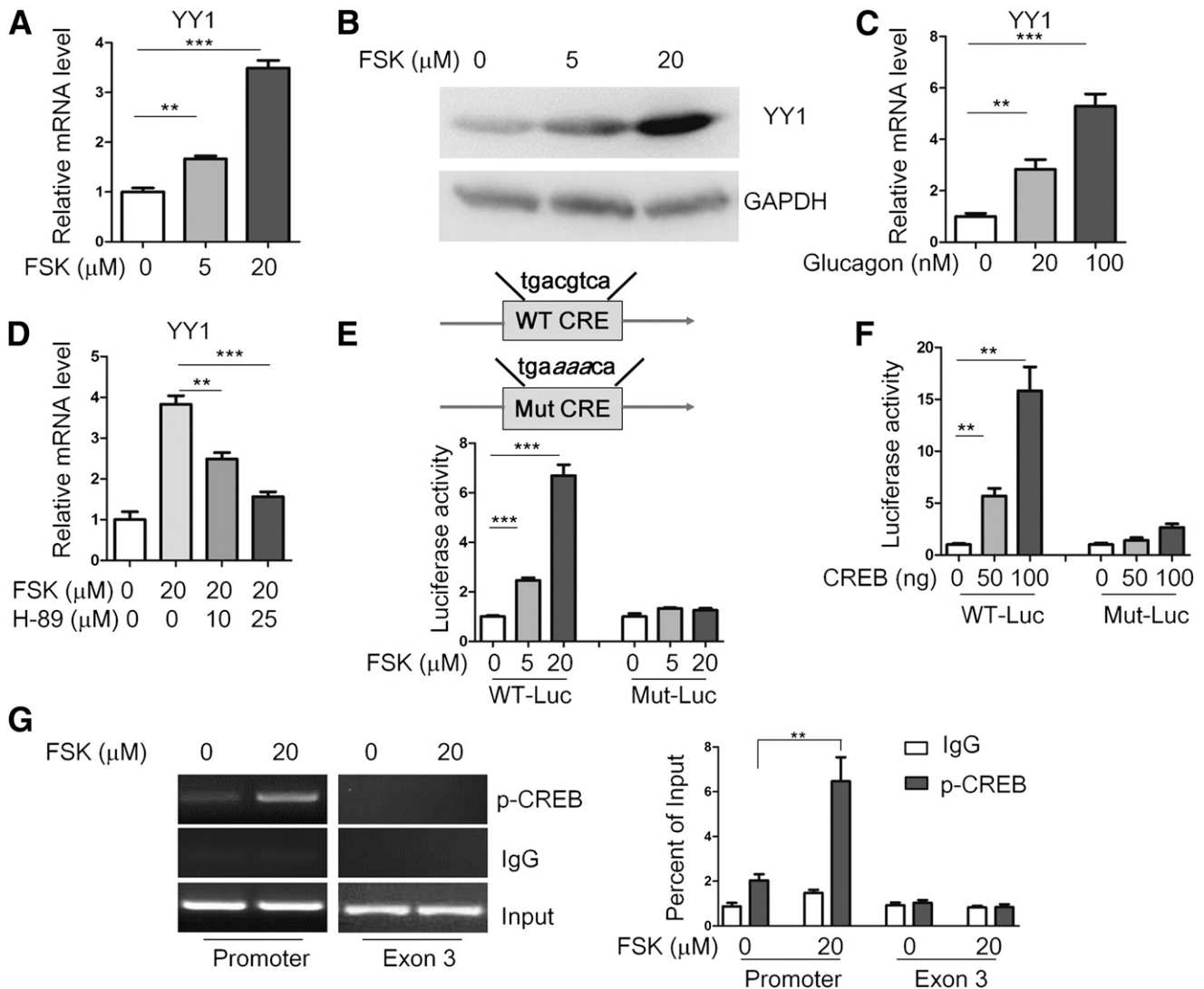

FIG. 2. cAMP-PKA-CREB signaling pathway upregulates YY1 expression. *A* and *B*: Real-time PCR (*A*) and Western blotting (*B*) analysis of YY1 in HepG2 cells treated with forskolin (FSK) as indicated. *C*: Real-time PCR analysis for YY1 expression in HepG2 cells treated with glucagon as indicated. *D*: Real-time PCR analysis of YY1 in HepG2 cells incubated with forskolin in the absence or presence of H-89. *E*: Identification of a CRE site in the mouse YY1 promoter. Luciferase (Luc) reporter assays were performed in HepG2 cells transfected with wild-type (WT) or mutant (Mut) promoters. *F*: Luciferase reporter assays in HepG2 cells transfected with CREB-expression plasmids. *G*: HepG2 cells were incubated in the absence or presence of forskolin for 2 h. Cells were then subjected to ChIP analysis by using anti–phosphorylated (p)-CREB (Ser[133]) antibody or control IgG quantified by real-time PCR (*right panel*). Exon 3 was used as a negative control. **$P < 0.01$, ***$P < 0.001$. CRE, cAMP response element.

a PKA inhibitor, significantly blocked the forskolin-mediated increase of YY1 mRNA levels (Fig. 2D). Moreover, we identified a classical cAMP response element in the promoter region of mouse *YY1* gene, which is located at bp -568 to -561 from the transcription start site. The luciferase reporter assays revealed an increased transcriptional activity by forskolin, while mutations of this region abrogated the increase in the luciferase activity (Fig. 2E). Furthermore, overexpression of CREB plasmids also led to a significant increase of the transcriptional activity for the wild-type but not the mutant promoter (Fig. 2F). The same results were also observed in HEK293T cells (data not shown). In addition, ChIP analyses were performed with anti–phosphorylated (phospho)-CREB (Ser[133]) antibody in HepG2 cells left untreated (control) or treated with forskolin. Phospho-CREB bound to the endogenous YY1 promoter region was dramatically increased after the treatment with forskolin (Fig. 2G). Collectively, these data indicate that cAMP-PKA-CREB signaling could participate in the upregulation of YY1 expression.

YY1 promotes hepatic gluconeogenesis. To determine whether YY1 participates in the regulation of gluconeogenesis in vivo, we generated recombinant adenoviruses expressing YY1 or GFP. Ad-YY1 was delivered to C57BL/6 mice via tail vein injection and dramatically overexpressed in the liver (Fig. 3A). Fasting plasma glucose levels were significantly higher in mice infected with Ad-YY1 compared with Ad-GFP controls (Fig. 3B). Plasma insulin concentrations were also elevated in mice with YY1 overexpression (Fig. 3C), while body weight, food intake, and body fat contents (Supplementary Fig. 1A–C) were not changed. Hepatic enzymes were not changed either (Supplementary Fig. 1D). PTT showed that plasma glucose levels were significantly increased in the mice with YY1 overexpression after pyruvate administration (Fig. 3D). Consistently, mRNA levels of PEPCK and G6Pase were markedly increased as well (Fig. 3E and F).

YY1 upregulates GR expression. We next investigated the molecular basis for YY1 promotion of hepatic gluconeogenesis. Our mapping studies revealed no potential
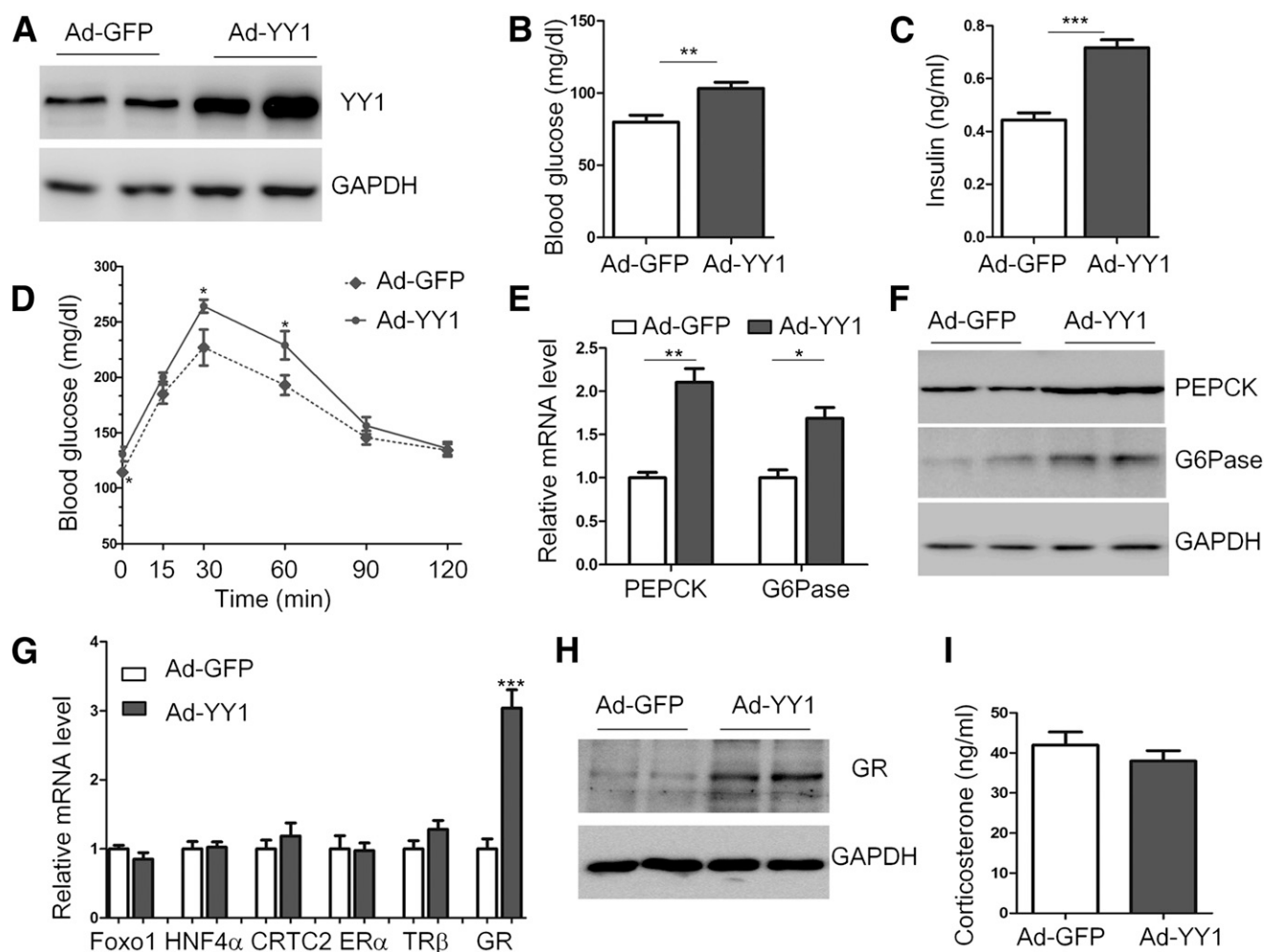

FIG. 3. YY1 overexpression promotes hepatic gluconeogenesis. *A*: Western blotting analysis for hepatic YY1 expression in C57BL/6 mice infected with Ad-GFP or Ad-YY1. *B* and *C*: Fasting blood glucose (*B*) and insulin (*C*) levels. Mice were fasted for 24 h, followed by measurements of fasting blood glucose levels. In addition, aliquots of blood (30 μL) were collected from individual mice for the determination of insulin levels. Data were obtained on day 5 after virus administration (*n* = 8). *D*: Blood glucose profiles of PTTs. Blood glucose was determined after a 16-h fast at day 9. *E* and *F*: Real-time PCR (*E*) and Western blotting (*F*) analysis of hepatic gluconeogenic genes (*PEPCK* and *G6Pase*) from mice livers. *G*: Gene expression levels of several transcription factors were determined by real-time PCR in livers from mice expressing YY1 and GFP (*n* = 8). *H*: Hepatic GR expression was analyzed by Western blotting in mice. *I*: Plasma corticosterone levels were assayed in mice expressing YY1 and GFP (*n* = 8). After a 24-h fast, mice were killed at day 14. Plasma and liver tissues were collected for further analysis (*A* and *E–I*). *P < 0.05, **P < 0.01, ***P < 0.001. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

YY1-response element in the promoters of PEPCK and G6Pase. Besides, YY1 overexpression could not stimulate their promoter activity in HEK293T cells (data not shown), suggesting that YY1 indirectly upregulates their expression. Thus, we analyzed the potential transcriptional factors that participate in regulation of gluconeogenesis. We found that GR expression was significantly increased in the liver with YY1 overexpression by real-time PCR and Western blotting (Fig. 3G and H). Plasma corticosterone levels were only slightly reduced, without reaching significance (Fig. 3I), which suggests that YY1 might augment hepatic glucocorticoid signaling. Indeed, mRNA levels of several known GR targets, including dual-specificity phosphatase 1, tyrosine aminotransferase, and IGFBP1 (21), were also induced by YY1 (Supplementary Fig. 1E).

To investigate whether YY1 could regulate GR expression, we further overexpressed YY1 in HepG2 cells in vitro using Ad-YY1 infection. The results showed that GR mRNA and protein levels were markedly increased by YY1 (Fig. 4A and B). In addition, PEPCK and G6Pase expressions were also upregulated in the presence of dexamethasone,

a classical GR agonist (Fig. 4C and D). Glucose output was correspondingly increased in YY1-overexpressed HepG2 cells treated with dexamethasone (Fig. 4E). Since HepG2 cells were derived from hepatocellular carcinoma and YY1 was shown to regulate tumor cell differentiation, we examined genes involved in cell differentiation such as hepatocyte nuclear factor 4 and albumin and found that they were not altered by YY1 overexpression (Supplementary Fig. 2A), suggesting that roles of YY1 in gluconeogenesis may be independent of cell-differentiation regulation. Moreover, to overcome the potential shortcomings of HepG2 cells, we performed YY1 overexpression in primary hepatocytes isolated from C57BL/6 mice. As shown in Supplementary Fig. 2B, YY1 could also upregulate PEPCK and G6Pase expression in the presence of dexamethasone in these cells.

To further confirm this regulation in an independent setting, we adopted short interfering RNA (siRNA) to knockdown YY1 in HepG2 cells. YY1 deficiency led to a dramatic downregulation of GR expression (Fig. 4F). The silence of YY1 also led to a decrease of PEPCK and G6Pase expressions in the presence of dexamethasone (Fig. 4G
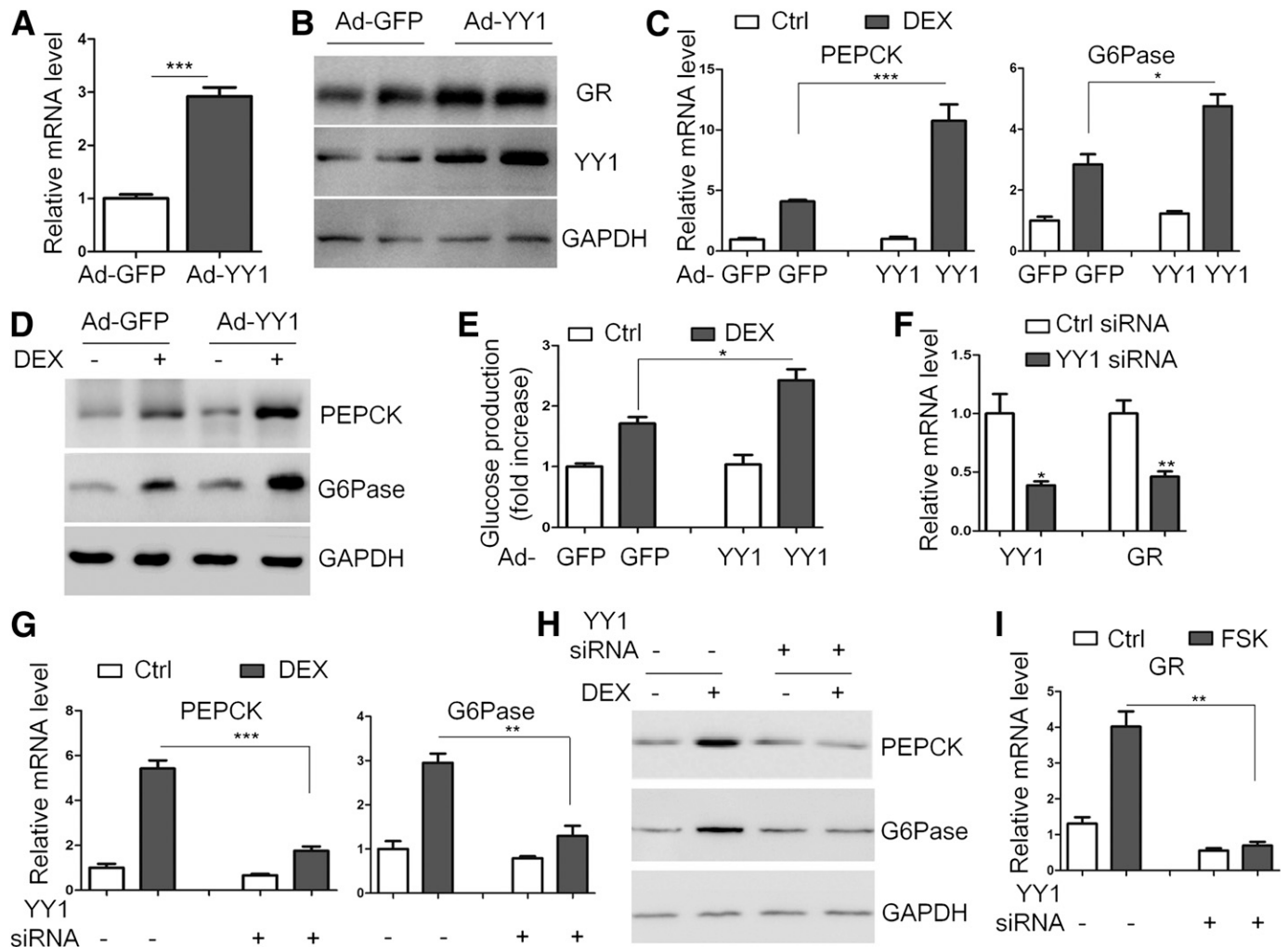

FIG. 4. YY1 controls the expression of the GR gene. A and B: Real-time PCR (A) and Western blotting (B) analysis of GR expression in HepG2 cells transfected with YY1 and GFP. C and D: PEPCK and G6Pase expression levels were determined in HepG2 cells in the presence or absence of dexamethasone (DEX) (10 nmol/L). E: Measurement of glucose production in HepG2 cells overexpressing YY1 and GFP in the presence or absence of dexamethasone. F: Real-time PCR analysis of YY1 and GR in HepG2 cells transfected with YY1 and control (Ctrl) siRNA oligos. G and H: PEPCK and G6Pase expression levels were determined in HepG2 cells in the presence or absence of dexamethasone (10 nmol/L). I: GR mRNA levels in HepG2 cells transfected with control or YY1 siRNA oligos in the absence or presence of forskolin (20 μmol/L). *P < 0.05, **P < 0.01, ***P < 0.001. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

and *H*). In contrast, YY1 knockdown had little impact on the glucagon-induced PEPCK and G6Pase expression in HepG2 cells or C57BL/6 mice (Supplementary Fig. 3*A* and *B*), suggesting that YY1 deficiency did not affect the glucagon-cAMP-PKA-CREB signaling pathway, consistent with our findings that YY1 is a downstream, not an upstream, target of cAMP-PKA-CREB signaling. Moreover, as YY1 was a target of cAMP-PKA-CREB signaling, we observed that GR mRNA levels were also induced in HepG2 cells with forskolin (Fig. 4*I*), which is consistent with previous reports (22). However, YY1 siRNA treatment

blocked the upregulation of GR by forskolin (Fig. 4*I*). Taken together, our data provide evidence supporting a direct control of YY1 on the gene expression of GR.

YY1 upregulates GR expression through recruitment of SRC-1. Next, we focused on the molecular mechanism of YY1 regulation of GR transcription. Sequence analysis showed that the promoter region of mouse GR gene contained a potential YY1 binding site (approximately between −1,118 and −1,112 bp) (Fig. 5*A*), which is conserved among species from rats to human (data not shown). Luciferase report assay showed that the transcriptional activity of
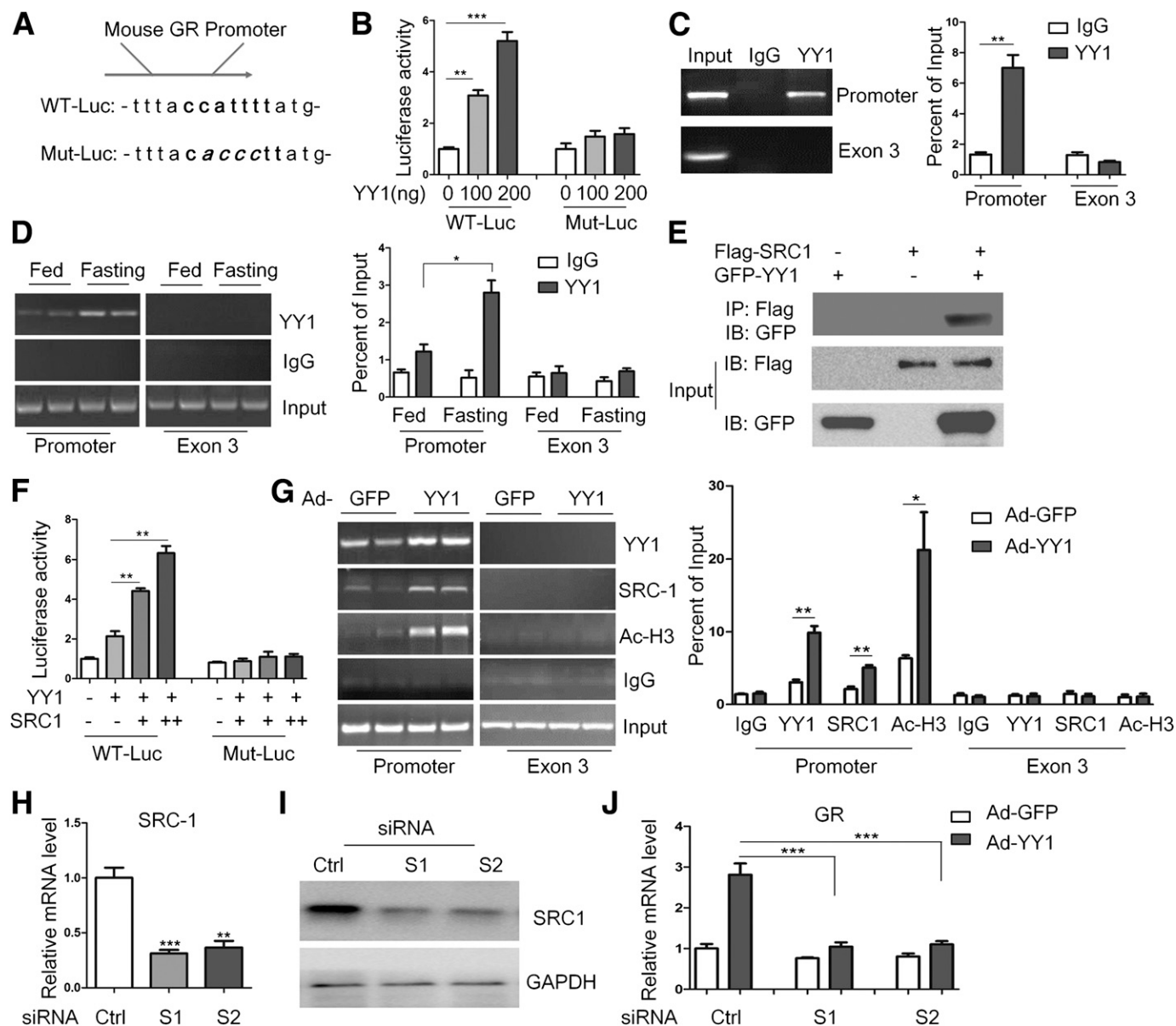
FIG. 5. Molecular mechanisms of the regulation of GR by YY1. *A*: A potential YY1-binding site in the promoter region of the mouse GR gene as indicated. *B*: Luciferase (Luc) assays of wild-type (WT) or mutant (Mut) mouse GR promoter in HepG2 cells transfected with YY1 expression plasmids. *C*: ChIP assays for representative YY1 binding to the GR promoter but not the exon 3 (*left panel*) and quantified by real-time PCR (*right panel*). HepG2 cells were seeded in 10-cm dishes. Forty-eight hours later, cells were >80% confluent and subjected to fixation, lysis, sonication, and incubation with YY1 antibody or IgG controls. *D*: ChIP assays showing the binding of YY1 to the GR promoter in C57BL/6 mice under fed or 24-h fasted conditions and quantified by real-time PCR (*right panel*) (*n* = 6). *E*: Coimmunoprecipitation (Co-IP) analysis of YY1 and SRC-1. HEK293T cells were transfected with the indicated plasmids and harvested for immunoprecipitation (IP) with Flag-M2 beads. IB, immunoblot. *F*: Luciferase assays of GR-promoter activity in HepG2 cells cotransfected with YY1 and SRC-1. *G*: ChIP assays with the indicated antibodies using nuclear lysates in livers from mice overexpressing Ad-YY1 and Ad-GFP (*left panel*) and quantified by real-time PCR (*right panel*) (*n* = 6). Ac-H3, acetyl histone H3. *H* and *I*: mRNA and protein levels of SRC-1 in HepG2 cells transfected with nonspecific control (Ctrl) siRNA or two specific siRNA oligos (S1 and S2). *J*: GR expression was analyzed by real-time PCR in HepG2 cells with SRC-1 deficiency and YY1 overexpression. *P < 0.05, **P < 0.01, ***P < 0.001.
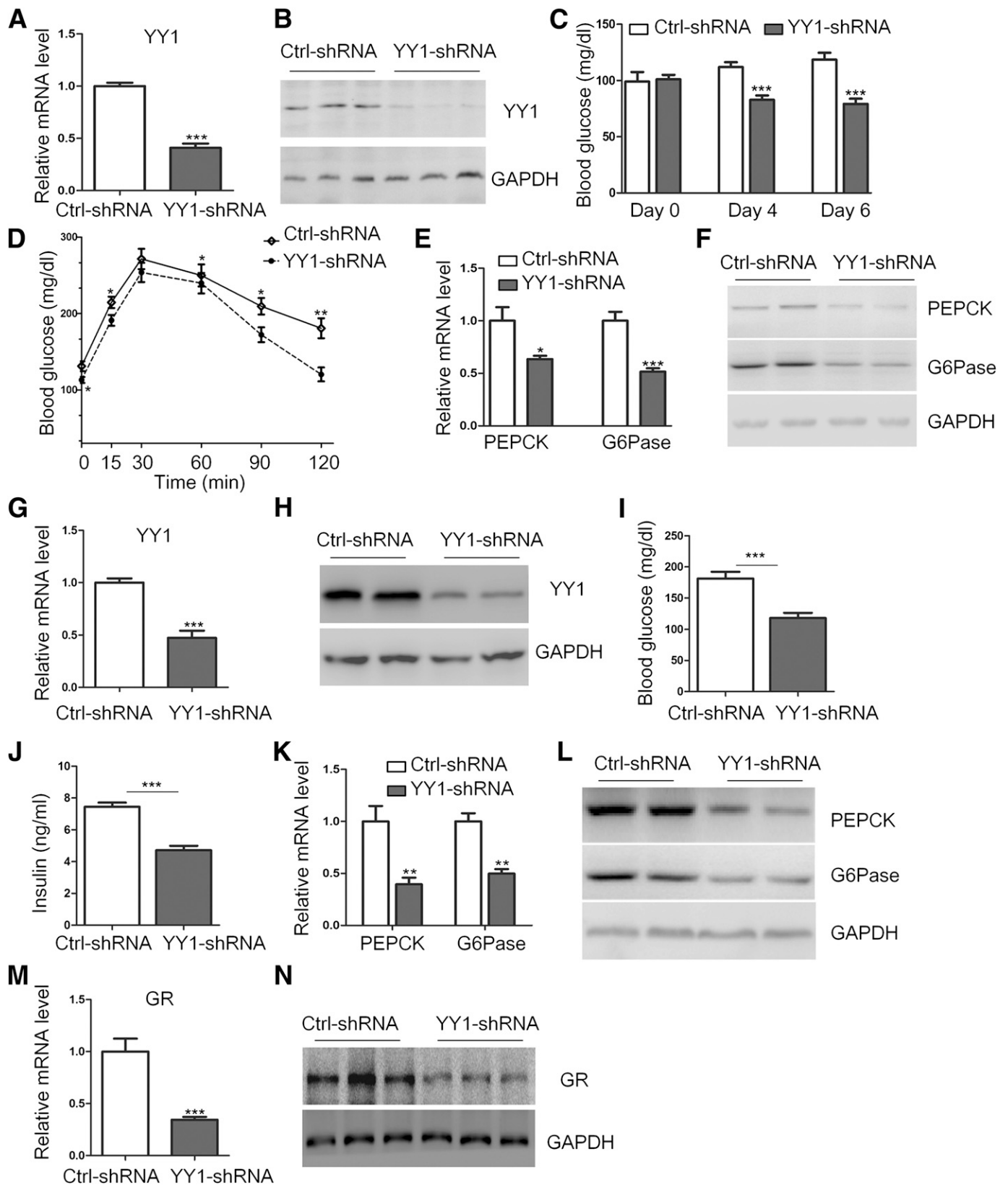
FIG. 6. YY1 deficiency ameliorates hepatic gluconeogenesis and hyperglycemia. *A* and *B*: Relative mRNA (*A*) and protein (*B*) levels of YY1 in C57BL/6 mice transfected with control or YY1-specific adenoviral shRNA (*n* = 6–7). *C*: Blood glucose concentrations from two groups of mice were measured under 24-h fasting conditions at the indicated date after virus injection (at 1400 h). For day 0, mice were fasted and then injected with adenoviral shRNA. Three hours later, blood glucose was determined. *D*: PTTs in mice infected with control or YY1 shRNA at day 10 after adenovirus injection. *E* and *F*: Relative mRNA (*E*) and protein (*F*) levels of PEPCK and G6Pase were determined in two groups of mice. After a 24-h fast, mice were killed at day 16. Liver tissues were collected for the analysis of gene expression (*A*, *B*, *E*, and *F*). *G* and *H*: Real-time PCR (*G*) and Western blotting (*H*) analysis of hepatic YY1 from mice after a 24-h fast. Male *db/db* mice were infected with adenoviral YY1 and control (Ctrl) shRNA (*n* = 8). *I* and *J*: Blood glucose (*I*) and insulin (*J*) levels were determined in two groups of mice after 24 h of fasting at day 5 after infection. *K* and *L*:

wild-type GR promoter was dramatically upregulated by YY1, whereas the transcriptional activity was abolished in the promoter bearing a mutation in YY1-binding sites (Fig. 5*B*). Furthermore, ChIP assays showed that YY1 could uniquely bind to GR promoter (Fig. 5*C*). Besides, the binding of YY1 to the GR promoter was also induced in mice after fasting for 24 h (Fig. 5*D*)

As a transcriptional factor, YY1 can recruit coactivators in the regulation of target gene expression (23). Previous studies demonstrated that SRC-1 could act as a coactivator for many transcription factors (24). We thus examined whether SRC-1 could be a coactivator for YY1 regulation of GR expression. As shown in Fig. 5*E*, YY1 could interact with SRC-1 by coimmunoprecipation. The transcriptional activity of GR promoter was further upregulated by cotransfection of SRC-1 and YY1 (Fig. 5*F*). Consistently, YY1 overexpression recruited much more SRC-1 to the GR promoter in the liver and acetylated histone H3 (Fig. 5*G*), a marker of actively transcribed genes. We further abolished SRC-1 expression using siRNA oligos in HepG2 cells (Fig. 5*H* and *I*). As expected, YY1-induced GR expression was significantly blunted by silence of SRC-1 (Fig. 5*J*). The above data demonstrated that YY1 upregulated GR expression through a recruitment of transcriptional coactivator complexes, including SRC-1.

Ablation of hepatic YY1 ameliorates gluconeogenesis and hyperglycemia. To further verify the physiological role of hepatic YY1 expression on glucose homeostasis, we generated adenoviral shRNA for YY1, which significantly inhibited YY1 mRNA and protein levels in C57BL/6 mice compared with nonspecific shRNA controls (Fig. 6*A* and *B*). Of interest, knockdown of YY1 caused a moderate decrease in blood glucose levels and hepatic glucose output in C57BL/6 mice (Fig. 6*C* and *D*). *PEPCK* and *G6Pase* gene expressions were reduced accordingly (Fig. 6*E* and *F*).

Next, to investigate whether suppression of hepatic YY1 could also affect glucose metabolism in pathological conditions, we silenced YY1 using adenoviral shRNA in *db/db* mice (Fig. 6*G* and *H*). Plasma glucose and insulin levels were significantly reduced in these mice with YY1 silence (Fig. 6*I* and *J*). In agreement, PEPCK and G6Pase expressions were decreased (Fig. 6*K* and *L*), while no significant changes in body weight or food intake were observed (Supplementary Fig. 4*A* and *B*). Consistently, mRNA and protein levels of GR were markedly decreased in the liver treated with YY1 shRNA (Fig. 6*M* and *N*). Taken together, these data suggest that acute ablation of hepatic YY1 would reduce gluconeogenesis in both physiological and pathological conditions.

YY1 promotion of hepatic gluconeogenesis depends on GR. If the regulatory effects of YY1 expression on glucose homeostasis rely mainly on the ability of YY1 to upregulate GR, it would be anticipated that YY1 overexpression should have no further effect on gluconeogenesis when GR is depleted in the livers. To evaluate this possibility, we introduced YY1 in the liver while simultaneously depleting GR with an adenovirus that expresses GR-targeting shRNA in C57BL/6 mice (Fig. 7*A* and *B*). Fasting plasma glucose and area under the curve by PTT were reduced in GR-depleted mice in the presence of YY1 overexpression (Fig. 7*C* and *D*), which indicates that YY1 cannot further increase blood

glucose levels in the absence of GR. In conclusion, PEPCK and G6Pase expressions were only upregulated in the YY1-overexpressing group and not in those with depletion of GR (Fig. 7*E* and *F*). Collectively, these results indicate that promotion of gluconeogenesis by YY1 primarily relies on GR upregulation (Fig. 7*G*).

DISCUSSION

Previously, YY1 was shown to bind with human GR promoter (25,26); however, its physiological relevance in gluconeogenesis remains unknown. In the current study, we characterized YY1 as a novel transcription factor involved in hepatic gluconeogenesis. We noticed that YY1 was induced during fasting at the transcriptional level via a cAMP-PKA-CREB–dependent mechanism. To unmask the impact of YY1 in the fasting glucose homeostasis in vivo, we adopted YY1 gain or loss of function in mouse models. Our data suggested a primary role for YY1 in the positive regulation of hepatic glucose production. Of interest, our results also revealed that the major role of YY1 during fasting is to control the gene expression of GRs (Fig. 7*G*).

The expression of gluconeogenesis enzymes including PEPCK and G6Pase could be activated by glucagon via cAMP/PKA and CREB because of CREB-response elements on their promoters (6,7). However, a variety of other transcription factors were also identified to play fundamental roles in the regulation of PEPCK or G6Pase, such as *CREBH, HNF4, FOXO1, FOXO6,* and *TR4* (10,27–30). Deficiency of any of these cluster genes resulted in fasting hypoglycemia and reduced expression of gluconeogenesis enzymes, suggesting that these transcription factors could not compensate each other and that all of them are required for the liver to fully activate expression of gluconeogenic genes.

Accumulating evidence indicates that glucocorticoids play critical roles in the development of fasting hyperglycemia (31–33). During the fasting state or in obesity, glucocorticoid levels in circulation are significantly increased, which functions through binding to GR. GR is known to be a key player in glucose production, as liver-specific GR knockout in mice resulted in fasting hypoglycemia and also ameliorated hyperglycemia in streptozotocin-induced diabetes (34). Specific knockdown of GR expression in liver with antisense oligonucleotides or adenoviral shRNA also led to a significant attenuation of hyperglycemia and hepatic glucose overproduction in multiple diabetic rodents (12,20). Besides, a stronger activation of GR was important for the hyperglycemia in several diabetes models, including *db/db* mice. It was reported that hepatic GR expression was also upregulated in *db/db* mice (11). Therefore, GR has been identified as a promising target for treating fasting hyperglycemia and related metabolic syndrome (35). For example, GR antagonist RU486 and liver X receptor agonist T0901317 were shown to improve the phenotype of type 2 diabetes in *db/db* mice (11,36). At the molecular level, some of their antidiabetes action was mediated, at least in part, through inhibition of GR expression in hepatocytes (11,36). However, the molecular determinants of hepatic GR expression remain largely unexplored. Here, we provide

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mRNA (*K*) and protein (*L*) levels of PEPCK and G6Pase were determined in mice. *M* and *N*: mRNA (*M*) and protein (*N*) levels of GR were measured in mice. After a 24-h fast, *db/db* mice were killed at day 12. Liver tissues were collected for further analysis of gene expression (*G, H, K,* and *N*). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.
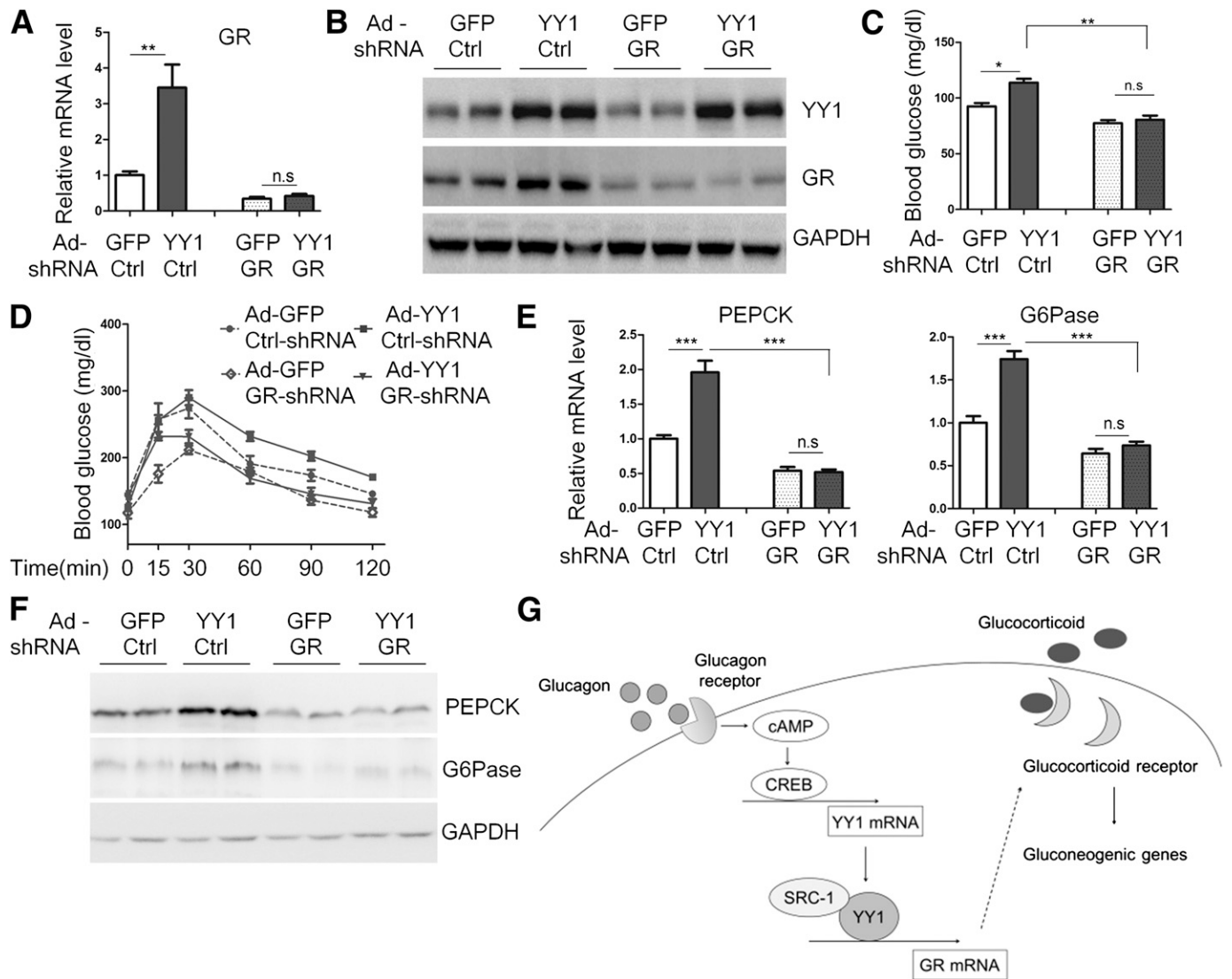
FIG. 7. GR-dependent YY1 action in the hepatic gluconeogenesis. *A* and *B*: Hepatic GR expression was determined in C57BL/6 mice overexpressing YY1 and GFP and combined with control (Ctrl) or GR shRNA adenoviruses ($n = 5$). *C*: Blood glucose levels were measured after a 24-h fasting from mice at day 5 after virus injection. *D*: PTTs in mice at day 10. *E* and *F*: Real-time PCR (*E*) and Western blotting (*F*) analysis of hepatic gluconeogenic gene expression in livers. After a 24-h fast, mice were killed at day 16. Liver tissues were collected for the analysis of hepatic gene expression (*A*, *B*, *E*, and *F*). *G*: Working model: under fasting condition, YY1 is upregulated by cAMP/PKA/CREB signaling and activates the mRNA transcription of GR through recruitment of SRC-1 coactivator complex, which results in the stronger activation of GR signaling to promote hepatic gluconeogenesis. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

strong evidence that YY1 overexpression promoted fasting glucose concentrations by upregulating GR. We proposed that the increased expression of GR enhanced the expression of gluconeogenic enzymes PEPCK and G6Pase without affecting the circulating glucocorticoid levels. In agreement, specific ablation of hepatic YY1 resulted in a decrease of blood glucose levels and gluconeogenic gene expression in normal mice. Furthermore, suppression of YY1 also improved fasting hyperglycemia in *db/db* mice, along with a reduction of GR expression. Therefore, our proposed YY1-GR signaling, together with other regulatory pathways, is essential for the functional integrity of the liver during fasting states.

Recent studies have demonstrated that YY1 could regulate the expression and activity of several nuclear receptors, such as peroxisome proliferator–activated receptor δ and androgen receptor (37,38). Interestingly, YY1 is shown to be able to recruit histone deacetylases or the coactivator proteins with acetylase activity to activate or repress expression of

many cellular and viral genes depending on its relative concentrations and cell-type–specific tissue factors (39,40). For example, YY1 was identified to regulate mitochondrial gene expression in muscle through binding to and recruiting peroxisome proliferator–activated receptor γ coactivator-1α (41). Likewise, here we demonstrate that YY1 could upregulate GR expression, at least in part through recruitment of SRC-1 coactivator. Indeed, previous reports proposed that SRC-1 also acted as a critical mediator of glucose homeostasis in the liver, in part by coactivation of C/EBPα (42). Thus, our data propose a mechanism for the induction of GR by YY1 and also provide an alternative fasting-mediated transcriptional route to modulate hepatic gluconeogenesis for SRC-1.

In summary, our results strongly implicate YY1 as a central transcriptional player orchestrating the gluconeogenic programs. Identification of the specific role for YY1 in hepatic gluconeogenesis would expand our knowledge to understand the important metabolic coordination for

proper blood glucose control and help to develop a potential treatment for metabolic disorders.

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