# SRC-2 orchestrates polygenic inputs for fine-tuning glucose homeostasis

Tiffany Fleet[a,b], Bin Zhang[b], Fumin Lin[b], Bokai Zhu[b], Subhamoy Dasgupta[b], Erin Stashi[b], Bryan Tackett[c], Sundararajah Thevananther[b,c], Kimal I. Rajapakshe[b], Naomi Gonzales[b], Adam Dean[b], Jianqiang Mao[b], Nikolai Timchenko[d], Anna Malovannaya[b,e], Jun Qin[b,e], Cristian Coarfa[b], Francesco DeMayo[b,f], Clifford C. Dacso[b,g], Charles E. Foulds[b], Bert W. O'Malley[a,b,f,1,2], and Brian York[b,f,1,2]

[a]Interdepartmental Department in Translational Biology and Molecular Medicine, Baylor College of Medicine, Houston, TX 77030-3411; [b]Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX 77030-3411; [c]Department of Pediatrics, Gastroenterology, Hepatology & Nutrition, Baylor College of Medicine, Houston, TX 77030-3411; [d]Department of Surgery, University of Cincinnati College of Medicine, Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229-3026; [e]Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX 77030-3411; [f]Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX 77030-3411; and [g]Department of Medicine, Baylor College of Medicine, Houston, TX 77030-3411

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**Despite extensive efforts to understand the monogenic contributions to perturbed glucose homeostasis, the complexity of genetic events that fractionally contribute to the spectrum of this pathology remain poorly understood. Proper maintenance of glucose homeostasis is the central feature of a constellation of comorbidities that define the metabolic syndrome. The ability of the liver to balance carbohydrate uptake and release during the feeding-to-fasting transition is essential to the regulation of peripheral glucose availability. The liver coordinates the expression of gene programs that control glucose absorption, storage, and secretion. Herein, we demonstrate that Steroid Receptor Coactivator 2 (SRC-2) orchestrates a hierarchy of nutritionally responsive transcriptional complexes to precisely modulate plasma glucose availability. Using DNA pull-down technology coupled with mass spectrometry, we have identified SRC-2 as an indispensable integrator of transcriptional complexes that control the rate-limiting steps of hepatic glucose release and accretion. Collectively, these findings position SRC-2 as a major regulator of polygenic inputs to metabolic gene regulation and perhaps identify a previously unappreciated model that helps to explain the clinical spectrum of glucose dysregulation.**

Steroid Receptor Coactivator 2 | SRC-2 | glucose homeostasis | glucokinase | polygenic disease

Glucose homeostasis is a tightly regulated biological process requiring intricate communication between multiple tissues of the body. Central to this process are the essential functions of the liver that protect the organism during the feeding-to-fasting transition. The feeding-responsive regulation of glucose homeostasis by the liver illustrates how humoral metabolic cues control the delicate balance of glucose in the body. Insulin produced by the endocrine pancreas is released prandially and drives the simultaneous uptake, catabolism, and storage of excess glucose. Conversely, glucagon is released during conditions of fasting in response to low blood sugar, which acts primarily on the liver to initiate glycogenolysis and gluconeogenesis. Together, both of these pathways function to release glucose for use by obligate glucose-using tissues such as the brain and red blood cells.

At the molecular level, transcriptional activation and repression are key cellular events in the feeding-to-fasting transition that fine tunes cellular programmatic shifts between glycolysis and gluconeogenesis. The transcriptional activation of glucose storage and utilization programs and the repression of glucose-producing programs during feeding are ultimately the result of signaling cascades that instruct the actions of transcriptional machinery. A similar process occurs during fasting whereby glucose-producing genes are actively transcribed and glucose-using and storage programs are repressed. This fine balance of activation and repression is often perturbed in diseases of glucose metabolism such as type 2 diabetes mellitus (T2DM), insulin-resistant fatty liver, and various glycogenopathies (1). To enhance our understanding of the molecular pathogenesis of these diseases, it is necessary to define the transcriptional machinery that is responsive to metabolic signals.

Published studies from our laboratory, as well as others, have established Steroid Receptor Coactivator 2 (SRC-2) as a powerful pleiotropic metabolic modulator (2). In adipose tissue, SRC-2 is a key regulator of lipolytic (white fat) and thermogenic gene programs (brown fat) (3). In muscle, SRC-2 facilitates mitochondrial function (skeletal) (4) and promotes fatty acid utilization (cardiac) (5, 6). In the liver, SRC-2 was identified as an essential coactivator for brain and muscle Arnt-like 1 (BMAL1) for synchronizing peripheral and central circadian functions (7). Additional hepatic roles for SRC-2 have highlighted its importance for maintaining fasting glycemia through coactivation of retinoic acid receptor-related orphan receptor (RORα) (8).

Recently published work from our laboratory showing that coregulators form hormonally responsive transcriptional complexes suggests that platform coactivators such as SRC-2 may differentially influence transcriptional complexes in response to metabolic cues (9, 10). The mapping of protein complexes that provide the foundation for the coregulator "complexome" further

## Significance

**Maintenance of glucose concentrations within a homeostatic range is essential for preserving the function of glucose-sensitive tissues. Perturbations in the mechanisms that control this homeostasis give rise to a continuum of glucopathologies associated with aberrant carbohydrate metabolism. Here we show Steroid Receptor Coactivator 2 (SRC-2) to be an integral coregulator that couples gene output with energetic demand by stabilizing and amplifying transcriptional complexes. This study highlights the collective importance of transcriptional coregulators for coordination of gene expression events and may provide insight for understanding components of polygenic diseases such as type 2 diabetes mellitus.**

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[1]B.W.O. and B.Y. contributed equally to this work.

[2]To whom correspondence may be addressed. Email: york@bcm.edu or berto@bcm.edu.

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supports this notion (9). The dynamic interaction of multiple protein networks is collectively referred to as the "interactome," and it is acutely responsive to cellular signaling cues (10). Despite these conceptual advances, the way that energetic signals (i.e., fasting and feeding) alter the transcriptional complexome and expression of reparative metabolic gene programs remains largely undefined.

We propose that dynamic coregulator complex composition is a fundamental mechanism for the transcriptional switch from glycolysis to gluconeogenesis. To interrogate this hypothesis, we have developed a DNA pull-down technique coupled with mass spectrometry (MS) to evaluate the impact of metabolic fluctuations on coregulator complex formation on two opposing rate-limiting genes regulating glucose metabolism [i.e., Glucokinase (*Gck*) and Glucose-6-phosphatase (*G6pc*)]. Congenic ablation or acute knockdown of *SRC-2* leads to a marked reduction in *Gck* and *G6pc* gene expression. Consistent with these findings, we show that *SRC-2*$^{KO}$ mice are hypoglycemic during the fasted state, yet surprisingly display postprandial hyperglycemia. Using our newly developed DNA pull-down system, we identified metabolically sensitive, SRC-2–dependent coregulator complexes on the *Gck* and *G6pc* gene promoters. Validation of these complex components identified an interaction of SRC-2 with the transcription factor (TF) C/EBPα on the *Gck* promoter during the fed state. Perturbing this interaction resulted in alterations in glucose homeostasis. From a broader perspective, our findings implicate SRC-2 as a pleiotropic factor that coordinates polygenic inputs for fine-tuning metabolic gene transcription through establishment of promoter-specific coregulator complexes. The technical advance highlighted in this study combined with our findings on the activating and repressive coregulator complexes that are sensitive to the actions of SRC-2 provide insight into how whole animal energetics coordinately direct metabolic transcriptional events. Conceptually, these findings establish SRC-2 as a model for polygenic disease by identifying a previously unappreciated mechanism by which metabolic cues integrate a hierarchy of transcriptional inputs that precisely govern glucose availability.

## Materials and Methods

Complete details of materials and methods are described in *SI Materials and Methods*.

**Animal Experiments.** All vertebrate animal experiments were performed in accordance with the Animal Care Research Committee at Baylor College of Medicine.

**ChIP-Quantitative PCR (qPCR).** Liver tissue was isolated and flash frozen from WT or *SRC-2* knockout mice as indicated. Chromatin was isolated using the SimpleChIP Enzymatic Chromatin IP Kit (Cell Signaling) and performed per the manufacturer's suggestion. Different antibodies were used at 2 μg per reaction. qPCR was performed with gene-specific primers and SyberGreen technology (Applied Biosystems) with normalization to total input DNA. ChIP-qPCR primer sequences are listed in Table S1.

**DNA Pull-Down Assays.** DNA pull-downs were performed as previously described (10). Briefly, 60 μL of Dynabeads M280 streptavidin (Life Technologies) beads were pelleted with standard magnetic racks (Life Technologies), and 4 μg of biotinylated *Gck* or *G6pc* DNA were bound to the Dynabeads in 150 μL D-PBS by rotation for ∼1 h at 4 °C. Biotinylated *Gck* and *G6pc* DNAs were synthesized by PCR using 5′-biotinylated primers (as described in *SI Materials and Methods*) and mouse genomic DNA subcloned into either pCR2-Topo or pCR4-Topo. Bead-immobilized DNAs were then washed, and the final PBS removed. Nuclear extract (NE) was thawed on ice and clarified by centrifugation at 4 °C for 10 min. We added NE (1 mg) to resuspend beads, and reactions were incubated with rotation at 4 °C for 1.5 h. Protein complexes were cross-linked with 1% formaldehyde for 10 min at room temperature (RT) and quenched with 1× glycine for 5 min at RT. Beads were washed using ice-cold buffers [two washes in 100 mM NaCl, 0.5 mM EDTA, 20 mM Tris-HCl (pH 8.0), 0.5% Nonidet P-40 (10), followed by one wash in D-PBS]. After PBS removal, beads were resuspended in 20–30 μL 5× SDS sample buffer (Thermo Fisher). After boiling, protein samples were loaded on 4–15% (wt/vol) Mini-PROTEAN TGX Precast gels (Bio-Rad) for immunoblotting or on NuPage gels (8% Bis–Tris in Mops buffer; Invitrogen) for preparative separation-gel slice excision for MS.

**Protein Identification by MS.** For MS analysis, the entire DNA pull-down reaction was scaled up twofold and was separated on 1D SDS/PAGE. After staining proteins in SDS/PAGE gels with Coomassie blue, gel lanes were sliced into different bands and in-gel digested overnight at 37 °C with trypsin. After digestion, peptides were extracted twice in 200 μL of acetonitrile with resuspension in 20 μL of 2% (wt/vol) formic acid before a second extraction, dried in a Savant SpeedVac, and dissolved in a 5% (wt/vol) methanol/0.1% formic acid solution. The samples were loaded through a 2 cm C18 trap followed by 1 h 0–30% (wt/vol) acetonitrile gradients on a 10 cm C18 column (packed in-house with Reprosil-Pur Basic C18 3 μm beads; Dr. Maisch GmbH) and measured online with the Thermo Orbitrap Velos or QExactive instruments (Thermo Scientific). The raw data were searched with Proteome Discoverer 1.3 Mascot engine against a human RefSeq database using lenient 1%/5% restricted/relaxed peptide-specific matches FDR (false discovery rate), and the data were further grouped into gene products that were assigned homology and identification quality groups using an in-house developed algorithm. All protein gene products chosen for follow-up in this study were required to have at least one identification where a spectral match passing <1% FDR and >20 ion score or <5% FDR and >30 ion score thresholds was present. The amount of each gene product was estimated with a label-free intensity-based absolute quantification (iBAQ) approach (as the sum of peptide areas normalized to the theoretical tryptic peptide potential) (11) and reported as a fraction of total protein iBAQ amount per experiment (in $10^{-5}$ units for visual comprehension). All MS datasets described in this study are available online at epicome.org/index.php/msprojects/src2metabolismresource.

## Results

**SRC-2 Dynamically Regulates Glycemia via Opposing Transcriptional Programs.** Previous characterization of *SRC-2*$^{KO}$ mice revealed fasting hypoglycemia accompanied with increased liver glycogen stores (8). These phenotypes result from the aberrant expression of *G6pc* in the absence of SRC-2, which coactivates RORα on the *G6pc* promoter. In addition to fasting hypoglycemia, we found that *SRC-2*$^{KO}$ mice surprisingly displayed postprandial hyperglycemia (Fig. 1*A*). In line with these data, fasted and refed *SRC-2*$^{KO}$ mice also show disturbances in glycogen storage/utilization as well as lipid deposition, consistent with aberrant glucose metabolism in the feeding-to-fasting transition (Fig. S1 *A* and *B*). These findings point to an overarching role for SRC-2 in the dynamic regulation of glucose homeostasis. Supporting this notion, integration of SRC-2 liver cistromic and transcriptomic data revealed glycolysis/gluconeogenesis as the top ontology of the candidate genes controlled by SRC-2 (Fig. 1*B* and Fig. S1*C*). Additionally, integration of metabolomic signatures from livers of *SRC-2*$^{WT}$ and *SRC-2*$^{KO}$ mice further specifies glycolysis/gluconeogenesis as the top candidate pathway controlled by SRC-2 (Fig. 1*C*). Our laboratory has demonstrated by ChIP-Seq that SRC-2 has a preference for the proximal promoter of its target genes (7). Analysis of the hepatic SRC-2 cistrome identified SRC-2 binding sites in the promoter regions of multiple glycolytic and gluconeogenic genes, suggesting SRC-2 may coordinately regulate gene programs that control glycemia (Fig. 1*D*).

To determine the enzymes in the glucose metabolism pathways that may be responsible for the glycemic phenotypes of the *SRC-2*$^{KO}$ mice, we performed a qPCR analysis for genes of the glycolytic and gluconeogenic programs in *SRC-2*$^{WT}$ and *SRC-2*$^{KO}$ mice that were fasted for 24 h or fasted and then refed for 3 h. This qPCR screen revealed significant decreases in the expression of multiple glycolytic and gluconeogenic target genes (Fig. 1*E*). We also tested the effects of *SRC-2* ablation on genes encoding the liver-specific glucose transporters (i.e., *Glut1*, *Glut2*, *Glut4*), but the mild alterations in these genes are unlikely to fully explain the glycemic phenotypes of the *SRC-2*$^{KO}$ mice (Fig. S1*D*). Ablation of *SRC-2*, however, resulted in a significant reduction of *Gck* expression during the fed state, whereas the expression of *G6pc* was decreased under both fasted and refed conditions. These changes are consistent with the effects of SRC-2 on fasting versus

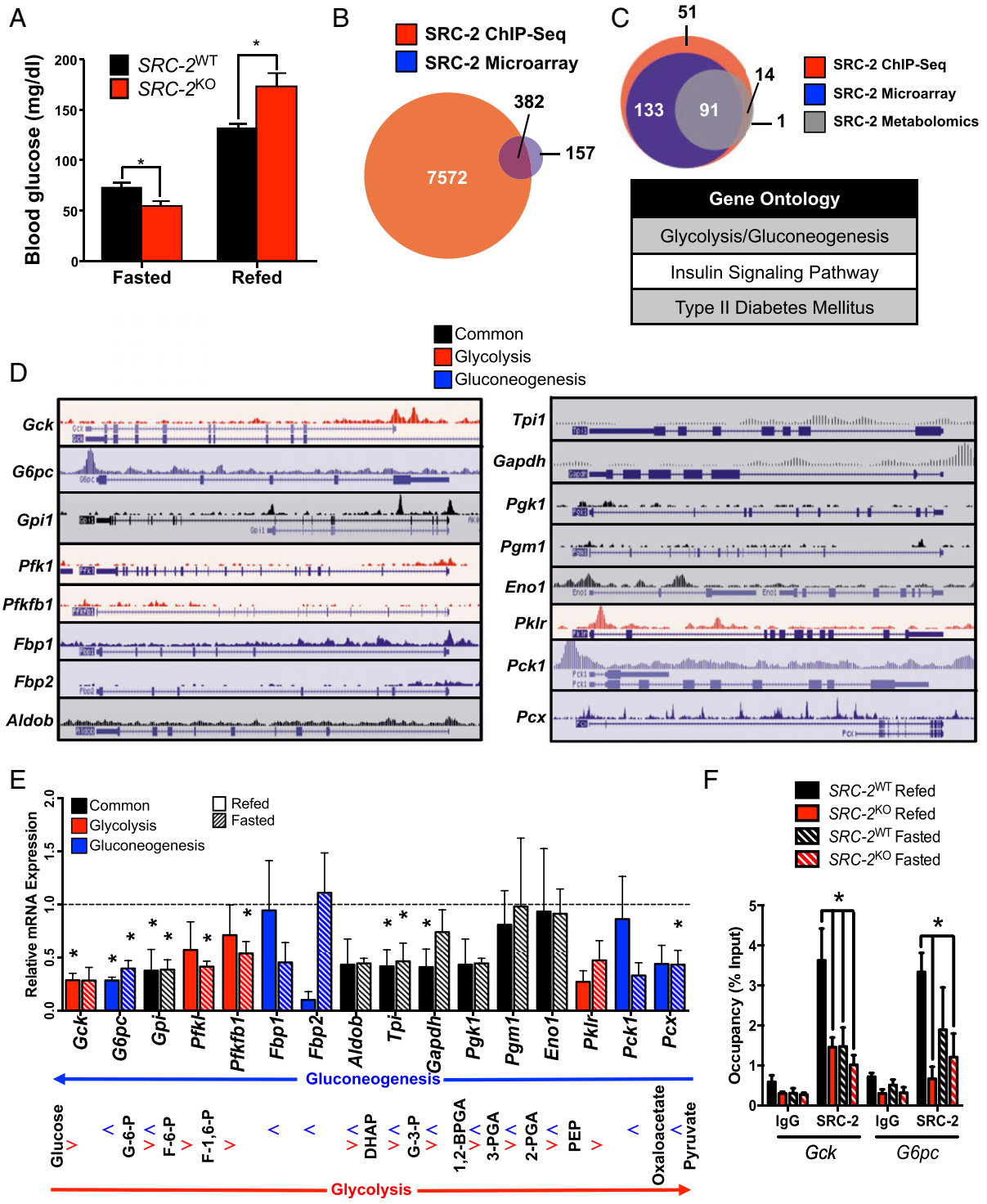


**Fig. 1.** SRC-2 dynamically regulates glycemia via opposing transcriptional programs. (*A*) Blood glucose (mg/dL) measurements of *SRC-2*$^{WT}$ and *SRC-2*$^{KO}$ mice ($n = 5$ each) after either an overnight fast or an overnight fast followed by a 6 h refeed. (*B*) Venn diagram representation of integrated hepatic SRC-2 ChIP-Seq binding sites with liver microarray data from *SRC-2*$^{KO}$ mice. (*C*) Venn diagram overlap of Kyoto Encyclopedia of Genes and Genomes (KEGG) gene ontologies from liver SRC-2 ChIP-Seq (red), *SRC-2*$^{KO}$ microarray (blue), and metabolomics data (gray). The top KEGG gene ontologies from the integration of these three ontological sets are listed in the accompanying table. (*D*) University of California, Santa Cruz (UCSC) genome browser representations of SRC-2 binding sites as determined by ChIP-Seq (7) on common (black) or unique glycolytic (red) or gluconeogenic (blue) gene promoters in wild-type mouse liver. (*E*) qPCR analysis of common (black) or unique glycolytic (red) or gluconeogenic (blue) genes in livers isolated from *SRC-2*$^{WT}$ and *SRC-2*$^{KO}$ mice ($n = 5$ each) after either a 24 h fast or a 24 h fast followed by a 3 h refeed. Data are represented as relative to wild-type gene expression for each feeding condition, which was set to 1. Accompanying the qPCR data is an outline of glycolysis and gluconeogenesis indicating where each gene product functions in those processes. (*F*) ChIP-qPCR analysis of SRC-2 occupancy on the gene promoters of *Gck* and *G6pc* in *SRC-2*$^{WT}$ and *SRC-2*$^{KO}$ mice ($n = 3$ each) after either a 24 h fast or a 24 h fast followed by a 3 h refeed. ChIP data are represented as a percent of the input, whereas all other data are graphed as the mean ± SEM. $^*P < 0.05$.

postprandial glycemia (Fig. 1*E*). Additionally, we found that *Gpi* and *Tpi* expression were decreased in the absence of SRC-2, however neither of these gene products represent rate-limiting steps of glycolysis or gluconeogenesis such as those controlled by *Gck* and *G6pc*. Therefore, we sought to explore the mechanism (s) by which SRC-2 controlled the expression of these two opposing rate-limiting stages of hepatic glucose entry and release.

To confirm the presence of SRC-2 on the *Gck* and *G6pc* promoters as suggested by the SRC-2 ChIP-Seq (Fig. 1*D*), we performed ChIP-qPCR using chromatin isolated from livers of *SRC-2*$^{WT}$ and *SRC-2*$^{KO}$ mice that were either fasted or fasted and then refed. We found that SRC-2 localized to the proximal promoter region of *Gck* in the refed state in *SRC-2*$^{WT}$ mice, and this recruitment is attenuated during fasting (Fig. 1*F*). These data verified the specificity of our SRC-2 antibody as we observed minimal recruitment of SRC-2 in the *SRC-2*$^{KO}$ livers (Fig. 1*F*). Somewhat unexpectedly, we found SRC-2 recruitment to the *G6pc* promoter to be highest during the refed state with no significant differences in SRC-2 recruitment in fasted versus refed conditions (Fig. 1*F*). These data suggest that SRC-2 may function differently in the regulation of *G6pc* and *Gck* such that feeding signals may recruit SRC-2 to the *Gck* promoter, whereas the recruitment and dismissal of SRC-2 to the *G6pc* promoter may be a part of a poised complex. Importantly, no recruitment of SRC-2 to the *UTR10* gene region was observed, which is consistent with our SRC-2 ChIP-Seq data (Fig. S1*E*) (5).

**SRC-2 Directly Controls the Cell-Autonomous Expression of *Gck* and *G6pc*.** To assess the cell-autonomous activity of SRC-2 on the promoters of *Gck* and *G6pc*, we knocked down *SRC-2* in primary hepatocytes using two unique siRNAs, which shows greater than 90% reduction in *SRC-2* mRNA (Fig. S2*A*). Following acute knockdown of *SRC-2*, *Gck* and *G6pc* expression were significantly decreased as measured by qPCR (Fig. 2*A*). To confirm SRC-2 activity on the *Gck* and *G6pc* promoters, we transfected HeLa cells with either a *Gck*– or *G6pc*–promoter–luciferase reporter construct in the presence or absence of HA-SRC-2. These data demonstrate that SRC-2 overexpression potentiates luciferase expression driven by both *Gck* and *G6pc* promoters, although the amount of HA–SRC-2 needed for maximal activation differs on these two promoters (Fig. 2*B*). Adenoviral overexpression of SRC-1, SRC-2, or SRC-3 revealed that SRC-2 confers the greatest increased expression of both *Gck* and *G6pc* relative to the other SRCs (Fig. S2*B*).

Further supporting our phenotypic data, quantitative metabolomic analysis of primary hepatocytes from *SRC-2*$^{WT}$ or *SRC-2*$^{KO}$ mice revealed a significant increase in glucose-6-phosphate/fructose-6-phosphate (G6P/F6P), consistent with the observed decrease of *G6pc* gene expression (Fig. 2*C* and Fig. S2*C*). Likewise, loss of *SRC-2* also led to a significant reduction in pyruvate due to low glycolytic flux (Fig. 2*C* and Fig. S2*C*), indicating the importance of SRC-2 for maintaining *Gck* expression to preserve the flow of glucose carbons. In line with the impaired expression of *G6pc* and *Gck*, we found that *SRC-2* ablation markedly reduced the levels of glucose produced from primary hepatocytes devoid of SRC-2 (Fig. 2*C*). Taken together, these data suggest that loss of SRC-2 is sufficient to perturb glucose entry due to impaired expression of *Gck*, although we cannot rule out the contribution of *G6pc* expression on glucose release that could partially account for the observed reduction in glucose levels in *SRC-2*$^{KO}$ primary hepatocytes.

To determine the cell-autonomous acute effects of SRC-2 on *Gck* or *G6pc* expression, *SRC-2* was either knocked down (siRNA) or overexpressed (adenoviral infection) in primary hepatocytes treated with the fasting mimetic forskolin or feeding mimetic insulin. Primary hepatocytes treated with siRNA targeting *SRC-2*, which has previously demonstrated an 80% reduction in SRC-2 protein expression (12), showed decreased mRNA expression of *SRC-2*, *Gck*, and *G6pc* (Fig. 2 *D* and *E* and Fig. S2*D*). As expected, forskolin treatment increased the expression of *G6pc* but failed to impact *Gck* expression (Fig. 2*D*). Knockdown of SRC-2 in hepatocytes treated with forskolin resulted in decreased expression of both *Gck* and *G6pc* relative to control (Fig. 2*D*). Hepatocytes treated with insulin showed increased expression of *Gck* and decreased expression of *G6pc* relative to vehicle controls (Fig. 2*E*). However, knockdown of SRC-2 decreased the expression of both *Gck* and *G6pc* upon insulin treatment (Fig. 2*E*).

Conversely, we used adenoviral overexpression of SRC-2 in primary hepatocytes to test its effect on the expression of *Gck* and *G6pc*. Ad–SRC-2 primary hepatocytes increased *SRC-2*, *Gck*, and *G6pc* in vehicle-treated hepatocytes relative to the Ad-GFP hepatocytes (Fig. 2 *F* and *G* and Fig. S2*E*). Treatment of Ad–SRC-2–expressing hepatocytes with forskolin resulted in increased expression of *G6pc* (Fig. 2*F*). This treatment, however, failed to overcome the increased expression of *Gck* with overexpression of SRC-2 (Fig. 2*F*). These data are highly consistent with the effect of siRNA knockdown of *SRC-2* whereby treatment with forskolin fails to suppress *Gck* expression. Treatment of Ad–SRC-2–expressing hepatocytes with insulin, however, resulted in increased expression of *Gck* relative to Ad-GFP insulin-treated hepatocytes (Fig. 2*G*). Similarly, insulin treatment of Ad–SRC-2 hepatocytes resulted in decreased expression of *G6pc* relative to the vehicle treatment, but the expression was still significantly increased relative to the Ad-GFP insulin-treated hepatocytes (Fig. 2*G*). Taken together, these studies confirm that SRC-2 localizes to, and transcriptionally coactivates, the *Gck* and *G6pc* promoters to regulate their dynamic expression in response to hormonal cues that recapitulate the feeding-to-fasting transition.

**SRC-2 Recruits Distinct Coregulator Complexes to Regulate *Gck* and *G6pc* Expression.** Published work from our laboratory has demonstrated that the SRCs participate in hormonally sensitive coregulator complexes on the promoter regions of an endogenous ERα target gene (10). We sought to determine if differential coregulator complex recruitment plays a mechanistic role in the dynamic regulation of *Gck* and *G6pc* by SRC-2. To that end, we performed DNA pull-down assays using endogenous *Gck* and *G6pc* promoter fragments that were biotinylated, bound to streptavidin magnetic beads, and incubated with liver NE isolated from *SRC-2*$^{WT}$ and *SRC-2*$^{KO}$ mice that were fasted for 24 h or fasted followed by a 3 h refeed (Fig. 3*A*). Immunoblotting and quantification confirmed the predominant recruitment of SRC-2 to the *Gck* and *G6pc* promoters (Fig. 3*B* and Fig. S3*A*). These data are congruent with the effects of SRC-2 to potentiate *Gck* and *G6pc*-luciferase activity whereby lower levels of SRC-2 recruitment to the *G6pc* promoter are required to coactivate gene expression compared with that of *Gck* (Fig. 2*B*). Using unbiased MS, we identified protein complexes that are recruited to the *Gck* or *G6pc* promoters in an SRC-2–dependent and/or metabolically sensitive manner (fasting versus refed) (Fig. 3*C* and table 1, hosted at epicome.org/index.php/msprojects/src2metabolismresource). Stratification of MS-identified proteins enriched on the *Gck* promoter during the fed state were dismissed in the fasted state (Fig. 3*C*, *Upper*). Conversely, proteins highly recruited to the *G6pc* promoter in the fasted state were subsequently reduced during the postprandial state (Fig. 3*C*, *Lower*). In general, proteins ontologically defined as TFs or transcriptional coregulators were among the most highly recruited and dynamic with respect to the metabolic state, further emphasizing the importance of these protein classes for controlling metabolic gene transcription (Fig. S3*B* and table 2, hosted at epicome.org/index.php/msprojects/src2metabolismresource). We also identified other regulatory classes of proteins that contain important enzymatic functions such as ADP ribosylases, kinases, and ubiquitin ligases

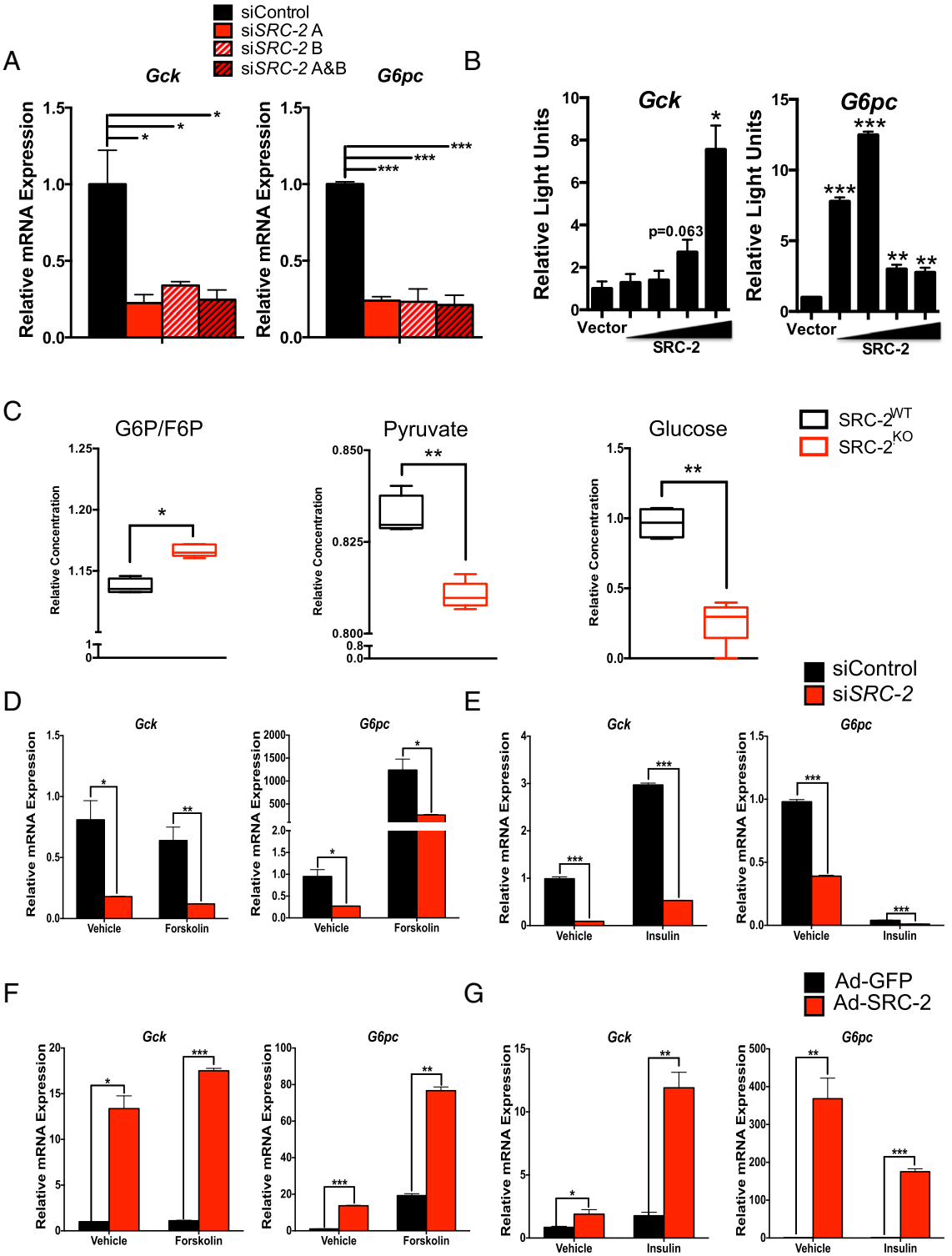


**Fig. 2.** SRC-2 directly controls the expression of *Gck* and *G6pc*. (*A*) qPCR analysis of *Gck* and *G6pc* gene expression in primary hepatocytes transfected with individual or combined siRNAs against *SRC-2*. (*B*) Transactivation assays in HeLa cells transfected with *Gck* or *G6pc* luciferase reporter constructs along with increasing amounts of SRC-2 expression vector. (*C*) Metabolomics analysis of glucose-6-phosphate/fructose-6-phosphate (G6P/F6P), pyruvate, and glucose in liver tissue from *SRC-2*$^{WT}$ and *SRC-2*$^{KO}$ mice. (*D*) qPCR analysis of *Gck* and *G6pc* expression in wild-type primary hepatocytes transfected with control siRNA or si*SRC-2* in the presence or absence of forskolin. (*E*) qPCR analysis of *Gck* and *G6pc* expression in wild-type primary hepatocytes transfected with control siRNA or si*SRC-2* in the presence or absence of insulin. (*F*) qPCR analysis of *Gck* and *G6pc* expression in wild-type primary hepatocytes infected with adenovirus expressing GFP or *SRC-2* in the presence or absence of forskolin. (*G*) qPCR analysis of *Gck* and *G6pc* expression in wild-type primary hepatocytes infected with adenovirus expressing GFP or SRC-2 in the presence or absence of insulin. Data are graphed as the mean ± SEM. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$.

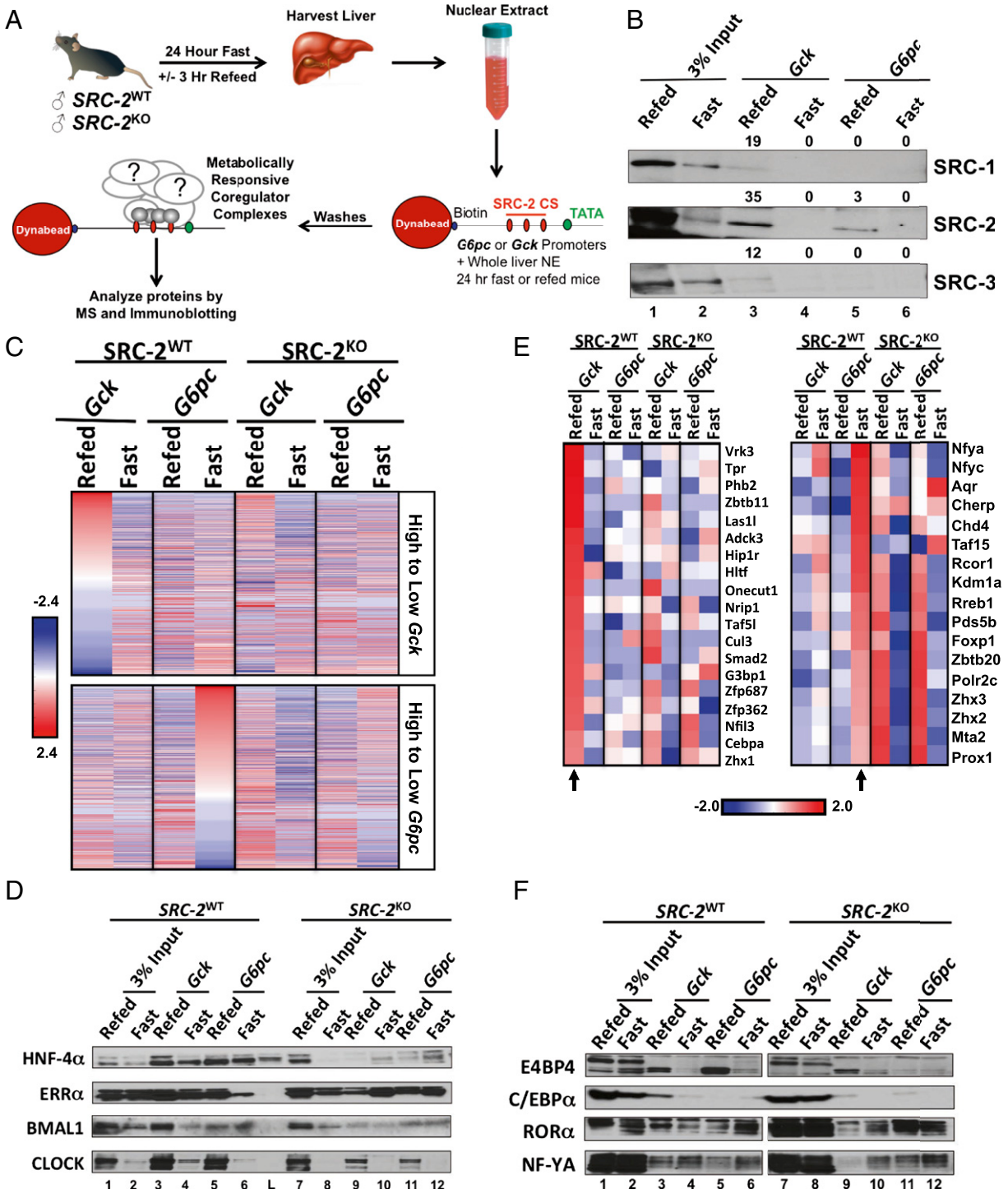


**Fig. 3.** SRC-2 interacts with distinct coregulator complexes to regulate *Gck* and *G6pc* expression. (*A*) Schematic of the DNA pull-down assay system. NE was isolated from livers of *SRC-2*$^{WT}$ or *SRC-2*$^{KO}$ male mice that were either fasted for 24 h or fasted for 24 h followed by a 3 h refeed. Biotinylated promoter fragments of *Gck* or *G6pc* genes were bound to magnetic Dynabeads and incubated with liver NE. Bound proteins were identified by unbiased MS. SRC-2 ChIP-Seq (CS) denotes cistromic locations of SRC-2 from our liver ChIP-Seq. (*B*) Immunoblot validation of SRC-1, SRC-2, and SRC-3 binding to *Gck* or *G6pc* promoters from fasted or refed wild-type liver NE. (*C*) Heat map representation of thresholded MS analysis of *Gck* and *G6pc* DNA pull-downs from liver NE from fasted or refed *SRC-2*$^{WT}$ or *SRC-2*$^{KO}$ mice. Proteins were stratified by those with the greatest input-normalized fraction of total (iFOT) signal on either the *Gck* promoter during the refed condition (*Top*) or the *G6pc* promoter in the fasted state (*Bottom*). (*D*) Immunoblot validation of candidate TFs identified from *C*. Experimental lanes are numbered; L denotes the protein marker. (*E*) Heat map representation of selected MS analysis of *Gck* and *G6pc* DNA pull-downs from liver NE from fasted or refed *SRC-2*$^{WT}$ or *SRC-2*$^{KO}$ mice. Proteins listed are enriched for binding to the *Gck* promoter in the refed state (*Left*) or the *G6pc* promoter in the fasted state (*Right*). Arrows indicate lanes used to determine enrichment. (*F*) Immunoblot validation of candidate complex components identified from stratification of *Gck* or *G6pc* promoter MS data in *E*. Lanes between pull-downs from *SRC-2*$^{WT}$ or *SRC-2*$^{KO}$ liver NE were cropped out of the same image to allow for consistent data representation, as this lane contained a protein marker. Heat maps use official gene names, and immunoblot data use common protein names.

that likely provide signaling inputs to these transcriptional complexes via posttranslational modifications (Fig. S3*B*) (13).

Consistent with the classical definition of a transcriptional coactivator as an amplifier and stabilizer of coregulator complexes (2, 9, 10, 14, 15), loss of SRC-2 strikingly reduced the recruitment of metabolically responsive proteins to both *Gck* and *G6pc* promoters (Fig. 3*C*). These findings highlight the collective importance of SRC-2 for establishing functional, biochemically stable TF–coregulator complexes on gene promoters that permit optimal transcriptional output in response to metabolic demand.

We confirmed the differential recruitment of top candidate TFs to both the *Gck* and *G6pc* promoters (i.e., HNF4α, BMAL1, CLOCK, and ERRα) via DNA pull-down followed by immunoblotting (Fig. 3*D* and Fig. S3*E*). Coomassie blue staining was used to confirm loading of NE inputs (Fig. S3*D*). Although these pleiotropic hepatic TFs demonstrated dependency on SRC-2 for stable recruitment to both *Gck* and *G6pc* promoters, the fact that they lack preferential metabolic recruitment to either promoter failed to offer a plausible mechanism for the metabolic-sensitive regulation of these two rate-limiting genes by SRC-2 (Fig. 3*D*). To identify candidate TFs that were recruited to either the *Gck* or *G6pc* promoters in response to fasting or refeeding, we stratified the DNA pull-down MS data to highlight proteins that preferentially localize to the *Gck* promoter in the refed state or the *G6pc* promoter in the fasted state (Fig. 3*E*, Fig. S3*C*, and table 3, hosted at epicome.org/index.php/msprojects/src2metabolismresource). Using immunoblot analysis, we confirmed that C/EBPα and E4BP4 were selectively recruited to the *Gck* promoter during the refed state (Fig. 3*F* and Fig. S3*F*). Consistent with published data, we found that RORα (8) was selectively recruited to *G6pc* during the fasted state, yet this effect was blunted in the absence of SRC-2 (Fig. 3*F* and Fig. S3*F*). Our immunoblot analysis also identified that NF-YA was preferentially recruited during the fasted state, although its recruitment was less dependent on SRC-2 (Fig. 3*F*).

**SRC-2 Selectively Coactivates C/EBPα to Promote *Gck* Expression.** As our laboratory has previously elucidated the mechanism by which SRC-2 coactivates RORα to facilitate *G6pc* expression during fasting (8), stratification of our MS data identified C/EBPα as a predominant TF by which SRC-2 may coactivate *Gck* gene transcription in the postprandial state (Fig. 3 *E* and *F* and Fig. S3*F*). C/EBPα is of particular interest as a putative SRC-2 interacting partner for coactivating *Gck* expression as the C/EBP motif was among the top predicted TF binding sites from our SRC-2 ChIP-Seq (7). Integration of hepatic SRC-2 (7) and C/EBPα (16) ChIP-Seq datasets revealed a robust cistromic overlap (~55% of SRC-2 peaks shared with C/EBPα) (Fig. 4 A, i). These overlapping binding sites corresponded to over 1,800 genes on which SRC-2 and C/EBPα share cistromic occupancy (Fig. 4 *A*, *ii*). Integration of published hepatic C/EBPα and SRC-2 cistromic data revealed identical binding sites for these two factors on the *Gck* promoter, providing further evidence that SRC-2 may cooperate with C/EBPα to drive *Gck* transcription (Fig. 4 *A*, *iii*).

To confirm the occupancy of C/EBPα on the *Gck* promoter, we performed ChIP-qPCR on liver chromatin prepared from *SRC-2*$^{WT}$ and *SRC-2*$^{KO}$ mice that were fasted or refed. We found that C/EBPα is enriched on the *Gck* promoter during the refed state, but not *UTR10*, and its occupancy is largely dependent upon SRC-2 (Fig. 4*B* and Fig. S4*A*). C/EBPα also was recruited to the *G6pc* promoter during refeeding but to a lesser extent than to the *Gck* promoter, suggesting that C/EBPα may bind these promoters with different affinities or perhaps is tethered to the *G6pc* promoter rather than direct binding (Fig. 4*B*). To determine the function of C/EBPα in mediating transcription of *Gck* or *G6pc*, we knocked down C/EBPα expression by siRNA in primary hepatocytes. Our results demonstrated that knockdown of C/EBPα with individual siRNAs or in combination results in significantly lower expression of *Cebpa*, *Gck*, and *G6pc* (Fig. 4*C* and Fig. S4*B*) (12, 17). As expected, knockdown of SRC-2 along with C/EBPα provided no additional inhibition of *Gck* or *G6pc* as it is likely C/EBPα is required for recruitment to either of these target gene promoters (Fig. 4*C* and Fig. S4*B*). To test whether C/EBPα is required for recruitment of SRC-2 to the *Gck* promoter, we knocked down C/EBPα with two unique siRNAs in primary hepatocytes and performed ChIP-qPCR with primers designed around the overlapping SRC-2 and C/EBPα ChIP-Seq peaks on the promoters of *Gck* and *G6pc* (Fig. 4 *A*, iii). We found that loss of C/EBPα selectively decreased the recruitment of SRC-2 to the *Gck* promoter, but not the *G6pc* promoter or the *UTR10* gene region (Fig. 4*D* and Fig. S4*C*). To further assess the ability of SRC-2 to preferentially coactivate C/EBPα on the *Gck* promoter, we performed luciferase assays with either *Gck* or *G6pc* promoters in the presence of SRC-2 and/or C/EBPα. We determined that SRC-2 synergistically coactivates C/EBPα on the *Gck* promoter but not the *G6pc* promoter (Fig. 4*E*). Additionally, titration of increasing amounts of C/EBPα into the luciferase assay was sufficient to dose-dependently potentiate luciferase expression from the *Gck* promoter while only marginally impacting *G6pc* promoter activity (Fig. S4*D*).

The transcriptional activity of C/EBPα is influenced by phosphorylation that alters its interaction with coregulators (18). Insulin activation leads to PP2A-induced dephosphorylation of S193 on C/EBPα (19). Consistent with these findings, loss of the regulatory domain containing this serine residue was sufficient to increase *Gck* expression but failed to impact the expression of *G6pc* (18, 20). Supporting these data, mice harboring a congenic loss of function mutation (S193A) in C/EBPα displayed a marked increase in *Gck* expression but showed no effect on *G6pc* expression (Fig. 4*F*) (18). Conversely, both *Gck* and *G6pc* expression are elevated in mice carrying a constitutively active mutation (S193D) in C/EBPα (Fig. S4*E*). Taken together, these findings substantiate the importance of phosphorylation of C/EBPα at S193 for controlling the recruitment of coregulators like SRC-2 to specify target gene transcription.

## Discussion

Through the use of DNA pull-down technology coupled with the unbiased power of MS, we have elucidated two overarching roles for SRC-2 in the metabolic regulation of rate-limiting genes for maintenance of glucose homeostasis. First, our data demonstrate that SRC-2 dictates the composition of TF–coregulator complexes on the promoters of *Gck* and *G6pc* in the fasted and refed states. Second, SRC-2 is essential for establishing a functional threshold of transcriptional machinery required for meeting metabolic demand. Stratification, integration, and selective validation of candidate complex components lead to the concept of a metabolic switch whereby rate-limiting glycemic genes are transformed from a "basal" to an "activated" state in response to energetic cues (Fig. 5*A*).

Although our MS data clearly highlight the existence of a fasting–feeding switch for counterregulation of genes that control glucose homeostasis, we chose candidate TFs for our model that were most robustly responsive to these metabolic conditions (Fig. 3 *D* and *F* and table 4, hosted at epicome.org/index.php/msprojects/src2metabolismresource). During postprandial conditions, we identified an activated state complex on the *Gck* promoter that synergizes the activities of C/EBPα and SRC-2 to stabilize recruitment of the Mediator and Integrator complexes along with POL2 and components of the CCR4–NOT complex to drive *Gck* expression (Fig. 5 *A*, *Top*, Fig. S5, and table 4, hosted at epicome.org/index.php/msprojects/src2metabolismresource). In the fasted state, these components are dismissed, leading to the recruitment of members of the repressive BRAF–histone deacetylase (BHC) complex that maintains the gene in a basal state (Fig. 5 *A*, *Top*, Fig. S5, and table 4, hosted at epicome.org/index.php/msprojects/src2metabolismresource). Supporting our published

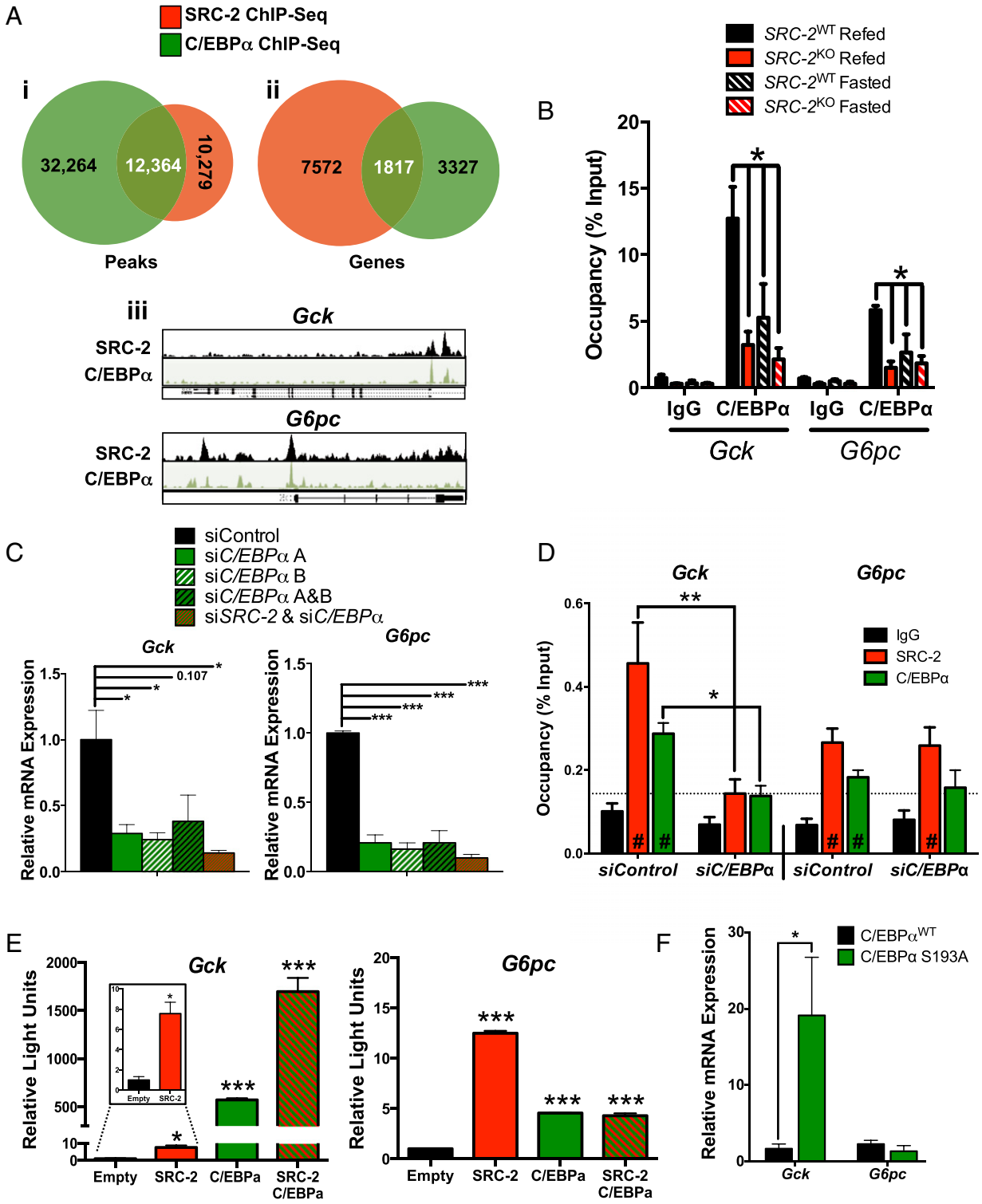


**Fig. 4.** SRC-2 coactivates C/EBPα function for selective regulation of *Gck* expression. (*A*, *i*) Venn diagram representation of integrated hepatic SRC-2 and C/EBPα ChIP-Seq binding sites in wild-type mice. (*A*, *ii*) Venn diagram representation of integrated hepatic SRC-2 and C/EBPα ChIP-Seq overlapping genes called from binding sites in wild-type mice. (*A*, *iii*) UCSC genome browser representations of overlapping SRC-2 and C/EBPα binding sites as determined by ChIP-Seq on the *Gck* and *G6pc* promoters. (*B*) Occupancy of C/EBPα on the *Gck* and *G6pc* promoter as determined by ChIP-qPCR. (*C*) qPCR analysis of *Gck* and *G6pc* expression in wild-type primary hepatocytes treated with si*SRC-2*, si*C/EBPα*, or both. (*D*) ChIP-qPCR analysis of SRC-2 and C/EBPα occupancy on the *Gck* and *G6pc* promoters in wild-type primary hepatocytes transfected with control siRNA or si*C/EBPα*. (*E*) Transactivation assays in HeLa cells transfected with *Gck* or *G6pc* luciferase reporter constructs along with overexpression of SRC-2, C/EBPα, or both. (*F*) qPCR analysis of *Gck* and *G6pc* expression in livers from mice harboring a congenic mutation in C/EBPα at serine 193 to alanine (S193A). ChIP data are represented as percent of input, whereas all other data are graphed as the mean ± SEM. **P* < 0.05, ***P* < 0.01, ****P* < 0.001. #, significance over IgG.

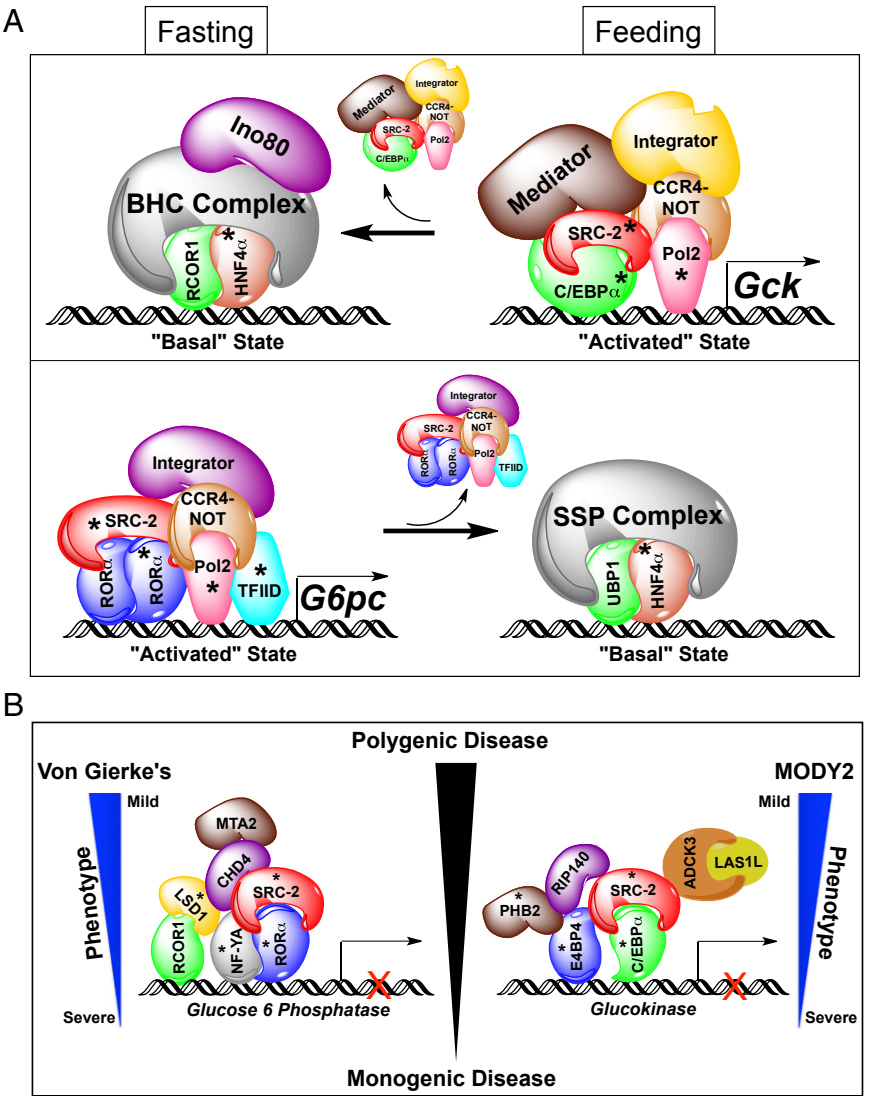


**Fig. 5.** Proposed model for transcriptional regulation of glucose homeostasis by SRC-2. (*A*) Schematic representation of SRC-2 regulation of the opposing enzymatic functions of *Gck* and *G6pc* in regulation of glucose homeostasis. (*B*) Schematic summary of protein complex components enriched on the *Gck* or *G6pc* promoters as identified by MS in the fasting versus feeding states. Asterisks indicate complex components analyzed by immunoblot. BHC, BRAF–histone deacetylase complex; SSP, stage selector protein complex.

data, during the fasted state RORα and SRC-2 function in concert with components of the Integrator complex, CCR4–NOT complex, POL2, and TFIID on the *G6pc* promoter to amplify its expression (8) (Fig. 5 *A*, *Bottom*, Fig. S5, and table 4, hosted at epicome.org/index.php/msprojects/src2metabolismresource). In the postprandial state, this activating machinery is replaced by members of the stage selector protein (SSP) complex that facilitates repression of *G6pc* transcription. These findings establish the importance of SRC-2 for dynamic TF–coregulator complex recruitment to modulate metabolic gene transcription.

Our findings raise the intriguing possibility that SRC-2 may serve as a model to explain a part of the spectrum of polygenic diseases such as T2DM (Fig. 5*B*). Genetic ablation of *SRC-2* represents a monogenic insult with polygenic consequences on the expression of nearly two-thirds of programmatic genes essential for glycolysis and gluconeogenesis (Fig. 1*E*). By definition, the milieu of transcriptional machinery recruited to either *Gck* in the fed state or *G6pc* during the fasted state represents the byproducts of polygenic inputs. Our findings suggest that a primary role of SRC-2 action is to stabilize metabolically responsive transcriptional complexes, thus establishing the set point for optimal gene output. As such, the repercussions of impaired SRC-2 function are amplified by perturbing complex formation on target gene promoters, resulting in blunted gene transcription in response to metabolic duress. Based on this logic, one might predict that mutations that diminish the function of a platform coregulator such as SRC-2 may explain a portion of the polygenic inputs to disorders of glucose regulation (Fig. 1*E*). In fact, these findings provide the most convincing evidence to date of our contention that coregulators like SRC-2 can serve as an entrée to understand a subset of polygenic diseases (21). It is not surprising that monogenic diseases give rise to the most severe phenotype, as is the case of *G6pc* for von Gierke's disease (22, 23) or *Gck* for maturity onset diabetes of the young (MODY2) (24, 25) where pathogenic mutations often lead to complete loss of gene function. However, our findings suggest that disturbances in coregulator function not only perturb TF binding but also dampen the recruitment of secondary and tertiary coregulators that fractionally contribute to gene output. Loss of SRC-2 function leads to impaired *Gck* and *G6pc*

expression, resulting in aberrant glucose homeostasis reminiscent of von Gierke's and MODY2 phenotypes (22–25).

To date, numerous genome-wide association studies and whole exome sequencing efforts have been insufficient to clarify definitive genetic contributions to polygenic diseases like T2DM (26–28). These shortcomings are understandable as these approaches use a phenotype-centric strategy wherein participants are selected that display a certain predefined definition of phenotype/disease (29, 30). In the case of T2DM, the disease spectrum ranges from simple hyperglycemia to a constellation of accompanying comorbidities defined as the metabolic syndrome (i.e., hypertension, dyslipidemia, abdominal obesity, insulin resistance/glucose intolerance, proinflammatory, and prothrombotic states). Given such heterogeneity in the clinical definition of T2DM, discovery of definitive genetic underpinnings that strongly correlate with the disease have remained elusive. The wealth of genetic sequencing information accumulated by these investigations provides a unique opportunity to evaluate whether small nucleotide polymorphisms or pathogenic mutations in genes like *SRC-2* correlate with patient phenotypes. Based on our findings of the expansive polygenic effects of SRC-2 disruption, the future integration of various sequencing datasets with matched transcriptomic analyses may permit a better understanding of how perturbations in pleiotropic factors like SRC-2 contribute to the phenotypic spectrum of diseases like T2DM.

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