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# Gcn5 and PCAF Regulate *PPARγ* and *Prdm16* Expression To Facilitate Brown Adipogenesis

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**The acetyltransferase Gcn5 is critical for embryogenesis and shows partial functional redundancy with its homolog PCAF. However, the tissue- and cell lineage-specific functions of Gcn5 and PCAF are still not well defined. Here we probe the functions of Gcn5 and PCAF in adipogenesis. We found that the double knockout (DKO) of *Gcn5/PCAF* inhibits expression of the master adipogenic transcription factor gene *PPARγ*, thereby preventing adipocyte differentiation. The adipogenesis defects in *Gcn5/PCAF* DKO cells are rescued by ectopic expression of peroxisome proliferator-activated receptor γ (PPARγ), suggesting Gcn5/PCAF act upstream of PPARγ to facilitate adipogenesis. The requirement of Gcn5/PCAF for *PPARγ* expression was unexpectedly bypassed by prolonged treatment with an adipogenic inducer, 3-isobutyl-1-methylxanthine (IBMX). However, neither PPARγ ectopic expression nor prolonged IBMX treatment rescued defects in *Prdm16* expression in DKO cells, indicating that Gcn5/PCAF are essential for normal *Prdm16* expression. Gcn5/PCAF regulate *PPARγ* and *Prdm16* expression at different steps in the transcription process, facilitating RNA polymerase II recruitment to *Prdm16* and elongation of *PPARγ* transcripts. Overall, our study reveals that Gcn5/PCAF facilitate adipogenesis through regulation of *PPARγ* expression and regulate brown adipogenesis by influencing *Prdm16* expression.**

White adipose tissue (WAT) and brown adipose tissue (BAT) provide different functions in energy metabolism. WAT stores excess energy as fat and has hallmark features such as a large unilocular lipid droplet and sparse mitochondria. In contrast, BAT dispenses energy in the form of heat and features small multilocular lipid droplets and abundant mitochondria. Adipogenesis in WAT and BAT is controlled by a similar adipogenic transcriptional cascade (1, 2), in which peroxisome proliferator-activated receptor γ (PPARγ) serves as a master regulator. PPARγ is a member of the nuclear receptor superfamily of ligand-activated transcription factors, and it is both necessary and sufficient for adipocyte differentiation. Two PPARγ isoforms exist: PPARγ1 and PPARγ2. PPARγ1 is expressed in multiple cell types, whereas PPARγ2 is highly adipocyte specific. Both isoforms are strongly induced during adipogenesis. BAT-specific features are driven by additional factors, in particular Prdm16 (3). However, how Prdm16 expression is regulated is not well defined.

PPARγ expression is activated in the adipogenic transcriptional cascade by two transcription factors, C/EBPβ and C/EBPδ, which are immediately induced upon initiation of adipogenesis by cyclic AMP (cAMP)-activated cAMP response element-binding protein (CREB) and glucocorticoid-activated glucocorticoid receptor, respectively. C/EBPβ binds directly to the *PPARγ2* promoter to activate *PPARγ2* expression (4). C/EBPα is also important to adipogenesis, and it is also induced by C/EBPβ and C/EBPδ. PPARγ and C/EBPα positively regulate themselves and each other to form a feed-forward loop to promote terminal differentiation of adipocytes (5–8).

*PPARγ* expression during adipogenesis is regulated by alterations in histone modification patterns (7, 9). Mono- and dimethylation of histone H3 lysine 4 (H3K4me1/2) on *PPARγ* gene locus by MLL3/4 complexes facilitates, whereas dimethylation of histone H3 lysine 9 (H3K9me2) by G9a represses, *PPARγ* expression (10, 11). Adipogenesis also induces acetylation of histone H3 lysines 9 (H3K9ac) and 27 (H3K27ac) at the *PPARγ* gene locus (10, 12, 13). H3K27ac marks active enhancers and is catalyzed by histone acetyltransferases (HATs) CBP and p300 (14). Both CBP and p300 have been shown to be required for adipogenesis (15). However, it is still unclear whether the HATs responsible for H3K9ac regulate *PPARγ* expression and adipogenesis.

One HAT that acetylates histone H3 is Gcn5 (also known as Kat2a). Gcn5 is highly conserved across evolution in structure and enzymatic specificity (16), and mammals contain two highly homologous Gcn5-like paralogues, Gcn5 and PCAF (also known as Kat2b). Gcn5 regulates transcription as part of large multisubunit complexes, such as the SAGA complex, which is recruited to target gene loci by interactions with transcription factors, such as p53, c-myc, and E2F (17–19). In mouse embryonic fibroblasts (MEFs), Gcn5 and PCAF act redundantly for robust acetylation of H3K9. DKO of these HATs eliminates H3K9ac in MEFs (14).

Since Gcn5/PCAF-mediated H3K9ac is induced and correlates with chromatin opening and gene activation during adipogenesis (12, 20), we established immortalized *Gcn5/PCAF* DKO brown preadipocytes to probe the function of Gcn5/PCAF in adipogen-

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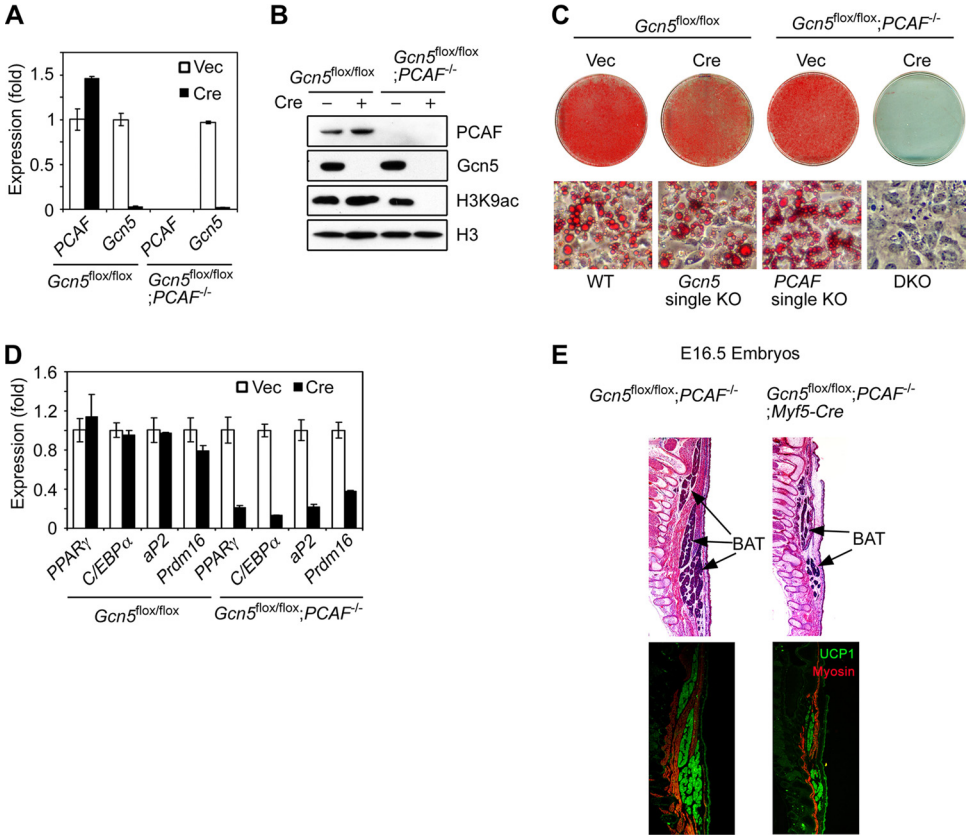


**FIG 1** Gcn5 and PCAF function redundantly to regulate adipogenesis. (A to D) Gcn5 and PCAF function redundantly for H3K9ac and adipogenesis in brown preadipocytes. Brown preadipocytes were isolated from *Gcn5*$^{flox/flox}$ and *Gcn5*$^{flox/flox}$; *PCAF*$^{-/-}$ mice and immortalized with SV40T prior to *Gcn5* deletion by retroviral Cre. Subconfluent cells were subjected to qRT-PCR analysis of *Gcn5* and *PCAF* mRNA (A) and immunoblotting with the antibodies indicated on the right (B). The cells were induced to undergo adipogenesis for 6 days, followed by Oil Red O staining (C) and qRT-PCR analysis of gene expression (D). (E) Gcn5 and PCAF are required for development of BAT *in vivo*. *Gcn5*$^{flox/flox}$; *PCAF*$^{-/-}$ males were crossed with *Gcn5*$^{flox/+}$; *PCAF*$^{-/-}$; *Myf5-Cre* females to generate *Gcn5*$^{flox/flox}$; *PCAF*$^{-/-}$; *Myf5-Cre* pups. Sagittal sections of E16.5 embryos were subjected to H&E staining (upper panels) and immunofluorescence staining with antibodies against myosin and UCP1 (lower panels). Myosin (red) and UCP1 (green) are specifically expressed in skeletal muscle and BAT, respectively.

esis. Our findings indicate that Gcn5/PCAF act redundantly during adipogenesis to regulate expression of both *PPARγ* and *Prdm16*. Interestingly, the requirement for Gcn5/PCAF for *PPARγ* expression and adipogenesis was bypassed by prolonged IBMX treatment. In contrast, *Prdm16* expression during adipogenesis was highly dependent on Gcn5/PCAF and was not bypassed by prolonged IBMX treatment. Mechanistically, Gcn5/PCAF modulate transcription elongation to regulate *PPARγ* expression, and modulate polymerase II (Pol II) recruitment to regulate *Prdm16* expression. Together, these results indicate that Gcn5/PCAF act at multiple steps to regulate adipocyte differentiation.

## MATERIALS AND METHODS

**Plasmids and antibodies.** Retroviral plasmids MSCVhygro-Cre and MSCVpuro-PPARγ2 have been described previously (21). The simian virus 40 (SV40) large T antigen-expressing retroviral plasmid pBABEneo-large T was obtained from Addgene (catalog no. 1780). Gcn5 and Gcn5 D608A mutant cDNAs were generated by PCR and inserted into MSCV-puro to generate MSCVpuro-Gcn5 and MSCVpuro-Gcn5-D608A. Primer sequences are available upon request.

Antibodies against C/EBPα (sc-61), C/EBPβ (sc-150X), C/EBPδ (sc-151), PPARγ (sc-7273), and β-actin (sc-47778) were from Santa Cruz. The Gcn5 (catalog no. 3305) and PCAF (catalog no. 3378) antibodies were from Cell Signaling. Antibodies to RNA Pol II (ab5408), S5P Pol II (ab5131), and S2P Pol II (ab5095) were from Abcam. The anti-H3K9ac (1328-1) antibody was from Epitomics. All chemicals were from Sigma unless otherwise indicated.

**Isolation of primary preadipocytes, immortalization, virus infection, and adipogenesis assays.** These experiments have been described previously (11). Briefly, primary brown preadipocytes were isolated from interscapular brown adipose tissues of newborn pups and immortalized with retrovirus pBABEneo expressing SV40 large T antigen. The immortalized cells were routinely cultured in Dulbecco modified Eagle medium (DMEM) plus 10% fetal bovine serum (FBS). For adipogenesis assays, brown preadipocytes were cultured in differentiation medium (DMEM plus 10% FBS, 0.1 μM insulin, and 1 nM T3) for 4 days before induction of adipogenesis. At day 0, overconfluent brown preadipocytes were treated with differentiation medium supplemented with an adipogenic cocktail consisting of 0.125 mM indomethacin, 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), and 2 μg/ml dexamethasone (DEX). Two days later, cells were changed to the differentiation medium alone. At day 6 postinduction, cells were analyzed by Oil Red O staining or quantitative reverse transcription-PCR (qRT-PCR) analysis of gene expression.

**Immunoblotting, qRT-PCR, ChIP, and quantitative PCR.** Immunoblotting, qRT-PCR using SYBR green assays, and chromatin immunoprecipitation (ChIP) assays were performed as previously described (14). To determine the *Gcn5* KO efficiency in cells with ectopic Gcn5, genomic DNA was extracted. The *Gcn5* genomic DNA was quantified with SYBR

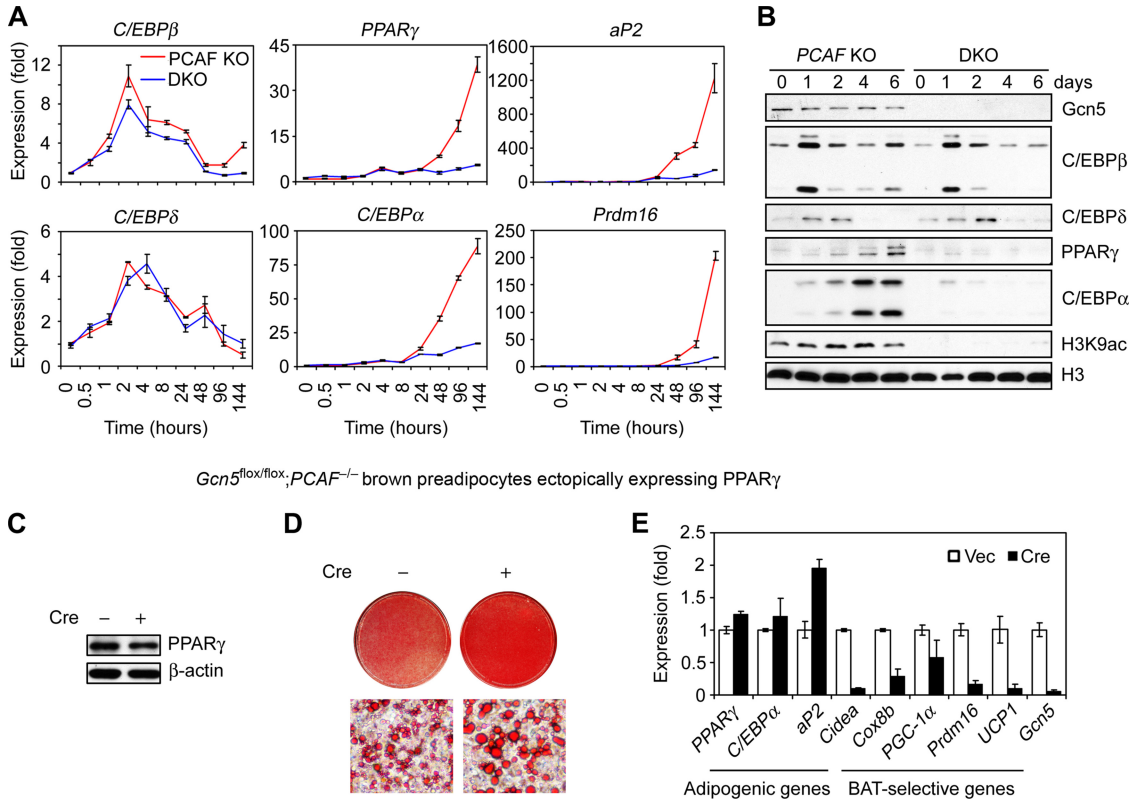


**FIG 2** Gcn5 and PCAF act upstream of PPARγ during adipogenesis. (A and B) Gcn5 and PCAF act downstream of C/EBPβ/δ but upstream of PPARγ during adipogenesis. Retroviral Vec- or Cre-infected *Gcn5*$^{flox/flox}$; *PCAF*$^{-/-}$ brown preadipocytes were induced to undergo adipogenesis, and samples were collected at the indicated times for qRT-PCR analysis of gene expression (A) and immunoblotting with the antibodies indicated on the right (B). (C to E) Gcn5 and PCAF are dispensable for ectopic PPARγ-induced adipogenesis. *Gcn5*$^{flox/flox}$; *PCAF*$^{-/-}$ brown preadipocytes were sequentially infected with retroviral PPARγ2 and retroviral Cre. Ectopic PPARγ2 proteins in subconfluent cells were detected by immunoblotting (C). The cells were induced to undergo adipogenesis. Six days later, the cells were subjected to Oil Red O staining (D) and qRT-PCR analysis of gene expression (E).

green assays using standard curve and relative quantitation with *GAPDH* genomic DNA as control. The data are presented as means ± the standard deviations.

**Histology and immunofluorescence staining.** E16.5 embryos dissected by Cesarean section were analyzed by hematoxylin and eosin (H&E) and immunofluorescence staining on paraffin sections as described previously (10). All mouse works were approved by the Animal Care and Use Committee of National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health.

## RESULTS

**Gcn5 and PCAF function redundantly to regulate adipogenesis.** To investigate the function of Gcn5 and PCAF in adipogenesis, primary white preadipocytes were isolated from *Gcn5*$^{flox/flox}$ or *Gcn5*$^{flox/flox}$; *PCAF*$^{-/-}$ mice, and *Gcn5* was deleted by adenovirus expressing Cre to generate *Gcn5* single-knockout (KO) or *Gcn5/PCAF* DKO cells (see Fig. S1A in the supplemental material). The cells were then induced to undergo adipogenesis by treatment with an adipogenic cocktail that included insulin, IBMX, and DEX. Individual deletion of either *Gcn5* or *PCAF* had little effect on adipogenesis. However, *Gcn5/PCAF* DKO inhibited lipid droplet formation and expression of adipogenic genes, such as *PPARγ* and *aP2* (see Fig. S1B and C in the supplemental material).

To obtain sufficient amounts of cells for mechanistic studies, brown preadipocytes from *Gcn5*$^{flox/flox}$ or *Gcn5*$^{flox/flox}$; *PCAF*$^{-/-}$ mice were immortalized with SV40 large T antigen (SV40T). The immortalized cells were infected with the retrovirus expressing Cre (retroviral Cre) to delete *Gcn5* (Fig. 1A). *Gcn5* deletion in *Gcn5*$^{flox/flox}$; *PCAF*$^{-/-}$ cells did not change cell morphology but did retard cell growth (see Fig. S1D and E in the supplemental material). Consistent with previous findings in MEFs (14), *Gcn5/PCAF* DKO dramatically reduced global levels of H3K9ac (Fig. 1B), indicating that Gcn5 and PCAF act redundantly as the major drivers of H3K9ac in brown preadipocytes. Similar to our observations in white preadipocytes, deletion of both *PCAF* and *Gcn*, but not *Gcn5* or *PCAF* alone strongly prevented lipid accumulation and expression of adipogenic genes, including *PPARγ*, *C/EBPα*, and *aP2*, as well as the BAT master regulator, *Prdm16* (Fig. 1C and D).

Although *PCAF* KO mice do not show any obvious, abnormal phenotype, *Gcn5* KO leads to early embryonic lethality (22). DKO embryos die even earlier (22), preventing analysis of BAT development in either *Gcn5* single-KO or DKO mice. To circumvent this lethality, we introduced a Myf5-driven Cre transgene into *Gcn5*$^{flox/flox}$; *PCAF*$^{-/-}$ mice to specifically delete *Gcn5* in precursor cells that develop to both brown fat and skeletal muscle tissues in the back (3). *Gcn5*$^{flox/flox}$; *PCAF*$^{-/-}$; *Myf5-Cre* pups died right after birth, likely due to skeletal muscle defects (data not shown). Importantly, these pups had much less BAT compared to their littermates (see Fig. S1F in the supplemental material). To further confirm defects in BAT development in these mice, sagittal sec-

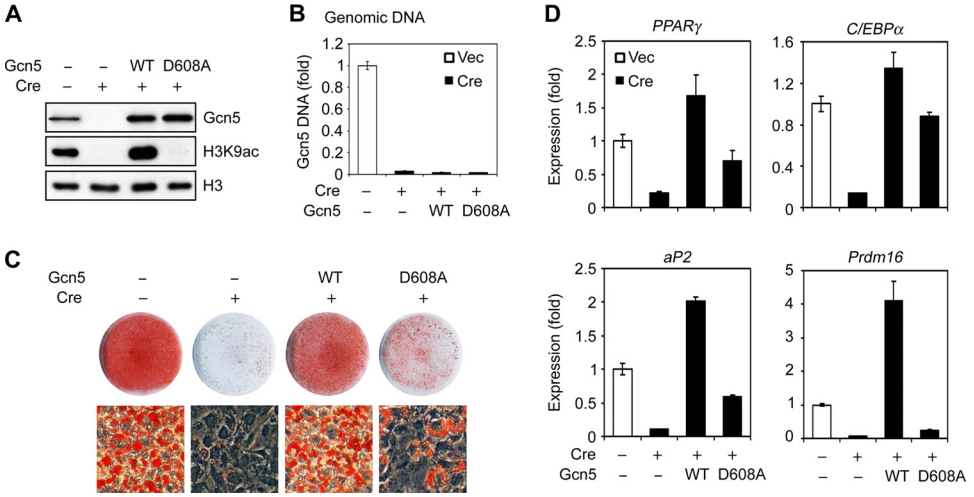


**FIG 3** Gcn5 catalytic activity is required for efficient adipogenesis. *Gcn5*$^{flox/flox}$; *PCAF*$^{-/-}$ brown preadipocytes were first infected with retroviruses expressing wild-type (WT) Gcn5 or an enzymatically dead Gcn5 mutant D608A prior to endogenous *Gcn5* deletion by Cre. In subconfluent cells, Gcn5, histone H3, and H3K9ac were analyzed by immunoblotting (A), and *Gcn5* knockout was confirmed by qPCR analysis of *Gcn5* genomic DNA (B). The cells were induced to undergo adipogenesis for 6 days, followed by Oil Red O staining (C) and qRT-PCR analysis of gene expression (D).

tions of E16.5 embryos were subjected to H&E and immunofluorescence staining of BAT- and skeletal muscle-specific markers, UCP1 and myosin, respectively. These results again reveal that BAT was reduced substantially in *Gcn5*$^{flox/flox}$; *PCAF*$^{-/-}$; *Myf5-Cre* pups compared to their littermate controls (Fig. 1E), indicating that Gcn5/PCAF are required for BAT development *in vivo*. Collectively, these results indicate that, Gcn5 and PCAF function redundantly to regulate adipocyte differentiation.

**Gcn5 and PCAF act upstream of PPARγ to facilitate adipogenesis.** To understand the mechanism by which Gcn5 and PCAF facilitate adipogenesis, we monitored induction of the adipogenic transcriptional cascade in DKO brown preadipocytes. We found that *C/EBP*β and *C/EBP*δ were induced normally in DKO cells, whereas the expression of *PPAR*γ and *C/EBP*α, as well as that of their downstream target gene *aP2*, was severely impaired by *Gcn5/PCAF* double deletion (Fig. 2A and B). *Prdm16* induction was also impaired in the DKO cells (Fig. 2A). Gcn5 protein levels were not significantly altered during adipogenesis in *PCAF* KO cells (Fig. 2B). These results suggest that Gcn5 and PCAF function upstream of PPARγ. To test this, we ectopically expressed PPARγ from a retroviral vector in *Gcn5*$^{flox/flox}$; *PCAF*$^{-/-}$ brown preadipocytes prior to *Gcn5* deletion by Cre (Fig. 2C). As shown in Fig. 2D and E, ectopic PPARγ stimulated adipogenesis well in both control and DKO cells, indicating that Gcn5/PCAF work upstream of PPARγ to facilitate adipogenesis.

**Gcn5 catalytic activity is required for efficient adipogenesis.** Gcn5 has both HAT activity-dependent and -independent functions (23, 24). To investigate the role of Gcn5 HAT activity in adipogenesis, wild-type (WT) Gcn5 or an enzymatically dead Gcn5 mutant (D608A) was transduced into *Gcn5*$^{flox/flox}$; *PCAF*$^{-/-}$ brown preadipocytes using retroviral vectors, followed by deletion of endogenous *Gcn5* by retroviral Cre (Fig. 3A and B). As expected, WT Gcn5 and PCAF, but not the Gcn5 D608A mutant, prevented loss of global H3K9ac after deletion of endogenous *Gcn5* (Fig. 3A; see Fig. S2A in the supplemental material). Upon induction of adipogenesis, ectopic expression of either WT Gcn5 or PCAF prevented defective adipogenesis in the DKO cells (Fig. 3C and D; see Fig. S2B and C in the supplemental material), confirming these HATs serve redundant functions during adipogenesis. Introduction of the Gcn5 D608A was less effective than wild-type Gcn5 in restoring lipid accumulation and adipogenic gene expression (Fig. 3C and D). Strikingly, ectopic overexpression of WT Gcn5 elevated *Prdm16* expression ~4-fold relative to expression seen in the presence of the wild-type endogenous protein, and ~16-fold relative to equally expressed ectopic Gcn5 D608A (Fig. 3A and D). These data suggest that Gcn5 has both HAT-dependent and -independent functions in adipogenesis and that *Prdm16* expression is highly dependent on Gcn5 HAT activity.

**Gcn5 and PCAF are required for *Prdm16* expression during brown adipogenesis.** Ectopic expression of PPARγ in *Gcn5/PCAF* DKO brown preadipocytes rescued adipogenesis but not *Prdm16* expression (Fig. 2E), indicating that Gcn5/PCAF are also involved in regulating *Prdm16* expression during brown adipogenesis. Unexpectedly, we found that prolonged treatment of DKO cells with IBMX rescued adipogenesis and expression of adipogenic genes such as *PPAR*γ, *C/EBP*α, and *aP2* (Fig. 4A and B; see Fig. S3A and B in the supplemental material). However, this treatment did not rescue expression of *Prdm16* (Fig. 4C).

Since Prdm16 is pivotal for development of brown adipocytes, we analyzed the effects of *Gcn5/PCAF* double deletion on expression of BAT- and WAT-specific genes (25) in differentiated cells after prolonged IBMX treatment (Fig. 4C and D). Prolonged IBMX treatment increased several BAT-selective genes in control cells, including *PPAR*α, *Cidea*, and *Dio2*, but rescued expression of only a few BAT-specific genes, such as *Eva1* and *Elovl3*, in DKO cells. Consistent with the requirement of Gcn5/PCAF for *Prdm16* expression, prolonged IBMX treatment of the DKO cells failed to rescue expression of Prdm16-dependent BAT-selective genes, including *Cidea*, *Cox8b*, *Dio2*, *Otop1*, *PGC-1*α, *PPAR*α, and *UCP1* (25–27) (Fig. 4C). The expression of the WAT-selective genes, *Psat1*, *Resistin*, and particularly *Serpin3ak*, was increased in DKO cells after prolonged IBMX treatment (Fig. 4D). Collectively, these

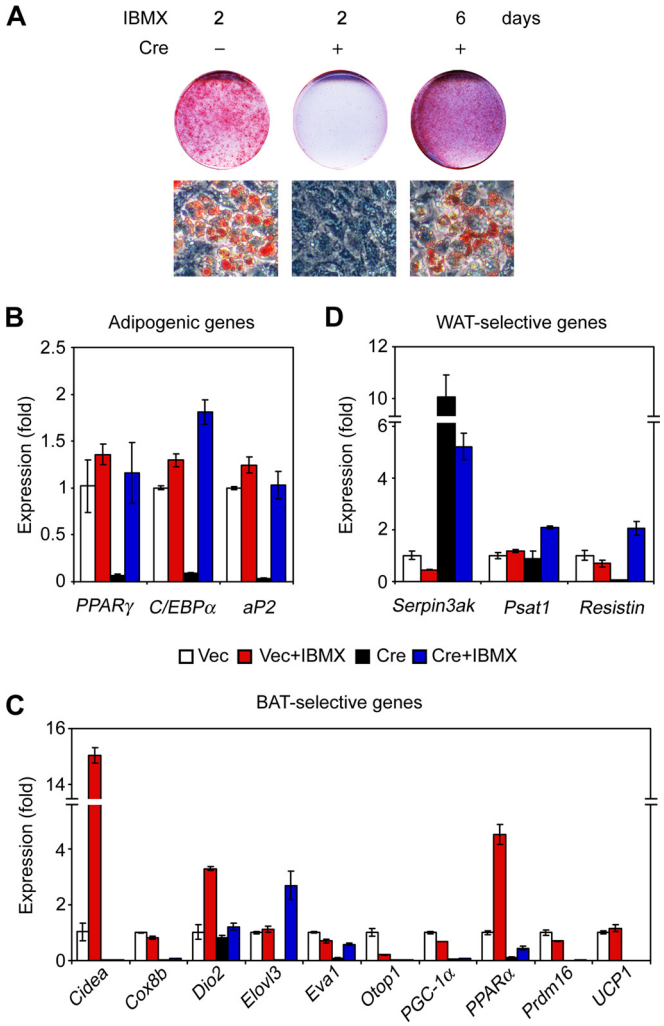


**FIG 4** Gcn5 and PCAF are required for *Prdm16* expression during brown adipogenesis. Retroviral Vec- or Cre-infected *Gcn5*$^{flox/flox}$; *PCAF*$^{-/-}$ brown preadipocytes were treated with the adipogenic cocktail to induce adipogenesis at day 0. At day 2, the adipogenic cocktail was removed and cells were treated with differentiation medium supplemented with or without 0.5 mM IBMX for 4 days. At day 6, cells were subjected to Oil Red O staining (A), and qRT-PCR analysis of adipogenic genes (B), BAT-selective genes (C), and WAT-selective genes (D).

data indicate that Gcn5 and PCAF are required for *Prdm16* expression during brown adipogenesis.

**Gcn5 and PCAF regulate different steps of *PPARγ* and *Prdm16* transcription.** Both PCAF and H3K9ac are enriched around transcription start sites (TSSs) of active genes (28, 29). PCAF and H3K9ac were gradually enriched at the *PPARγ2* and *Prdm16* genes during adipogenesis (Fig. 5A to D), and the increase in H3K9ac at these two genes was completely blocked by *Gcn5/PCAF* double deletion (Fig. 5B and D), a finding consistent with loss of the global H3K9ac in DKO cells. These results suggest PCAF and Gcn5 are set on *PPARγ2* and *Prdm16* genes to regulate their expression.

Gcn5/PCAF loss did not impair C/EBPβ induction during adipogenesis (Fig. 2A). To determine whether loss of these HATs affects C/EBPβ recruitment onto the *PPARγ2* gene, we performed ChIP assays for this factor to monitor its association with a C/EBPβ binding site located at kb −0.3 upstream of *PPARγ2* TSS (4). We found that C/EBPβ was greatly enriched at this site at day 2 of adipogenesis and that this enrichment decreased at days 4 and 6 (Fig. 5E). This pattern of C/EBPβ binding was not affected in the DKO cells. We next examined RNA Pol II association with *PPARγ2*. Phosphorylation on Serine 5 (S5P) and Serine 2 (S2P) of the Pol II C-terminal domain are markers of transcription initiation and elongation, respectively. Peaks of S2P Pol II are observed commonly at TSSs and at the 3′ ends of genes (30). Adipogenesis-induced enrichment of Pol II, S5P Pol II, and S2P Pol II had similar trends of enrichment as C/EBPβ at *PPARγ2* (Fig. 5E). *Gcn5/PCAF* double deletion did not change Pol II and S5P Pol II levels at the *PPARγ2* gene at days 2 and 4 but lowered enrichment of these factors at day 6 (Fig. 5E). S2P Pol II enrichment at the *PPARγ2* gene was not changed in the DKO cells at day 2 but was lowered at days 4 and 6, suggesting defective transcription elongation of *PPARγ2* at these time points. These data indicate that Gcn5/PCAF are required for efficient and sustained elongation of *PPARγ2* transcription.

In contrast, the enrichment of Pol II, S5P Pol II, and S2P Pol II near the TSS of the *Prdm16* gene (at kb +0.1), which increased first at day 4 in control cells with further increase at day 6, was diminished in the DKO cells (Fig. 5F). These results suggest that Gcn5/PCAF are required for Pol II recruitment onto the *Prdm16* gene.

Next, we investigated whether prolonged IBMX treatment stimulates transcription elongation of the *PPARγ2* gene in DKO cells, since it rescued *PPARγ2* expression in DKO cells. Prolonged treatment of IBMX enhanced C/EBPβ and Pol II recruitment at the *PPARγ* promoter in both control and DKO cells, and importantly, IBMX stimulated S2P Pol II enrichment at the *PPARγ* gene in DKO cells (Fig. 5G), which is consistent with elevated PPARγ expression in response to prolonged IBMX treatment (see Fig. S3C in the supplemental material). IBMX also enhanced Pol II enrichment on the *Prdm16* gene locus in control cells but not in DKO cells (Fig. 5H). These results indicate prolonged IBMX treatment rescues *PPARγ* expression in DKO cells by reactivating transcription elongation of this gene.

## DISCUSSION

Our data suggest a model for Gcn5 and PCAF facilitation of brown adipogenesis via regulation of *PPARγ* and *Prdm16* expression (Fig. 6). PPARγ is known to be a master transcription factor that governs adipogenesis, whereas Prdm16 is a master regulator for development of brown adipocyte-specific features. Upon adipogenic stimulation, Pol II is recruited onto the *PPARγ* promoter by C/EBPβ and onto the *Prdm16* promoter by unknown transcription factors to activate expression of these genes. PPARγ and Prdm16 then drive cells differentiation into mature brown adipocytes. In the absence of Gcn5/PCAF, Pol II is still recruited to the *PPARγ* promoter via C/EBPβ, but Pol II elongation is inhibited, leading to inhibition of *PPARγ* transcription. Loss of these HATs prevents Pol II recruitment to the *Prdm16* promoter. Thus, adipogenesis is inhibited in DKO cells. In the absence of Gcn5/PCAF, prolonged IBMX treatment enhances C/EBPβ and Pol II recruitment to *PPARγ*, stimulating transcript elongation, but IBMX does not promote Pol II recruitment onto the *Prdm16* promoter. Consequently, IBMX restores expression of *PPARγ* but not *Prdm16* in DKO cells, resulting in their development into adipocytes that lack brown adipocyte molecular phenotype.

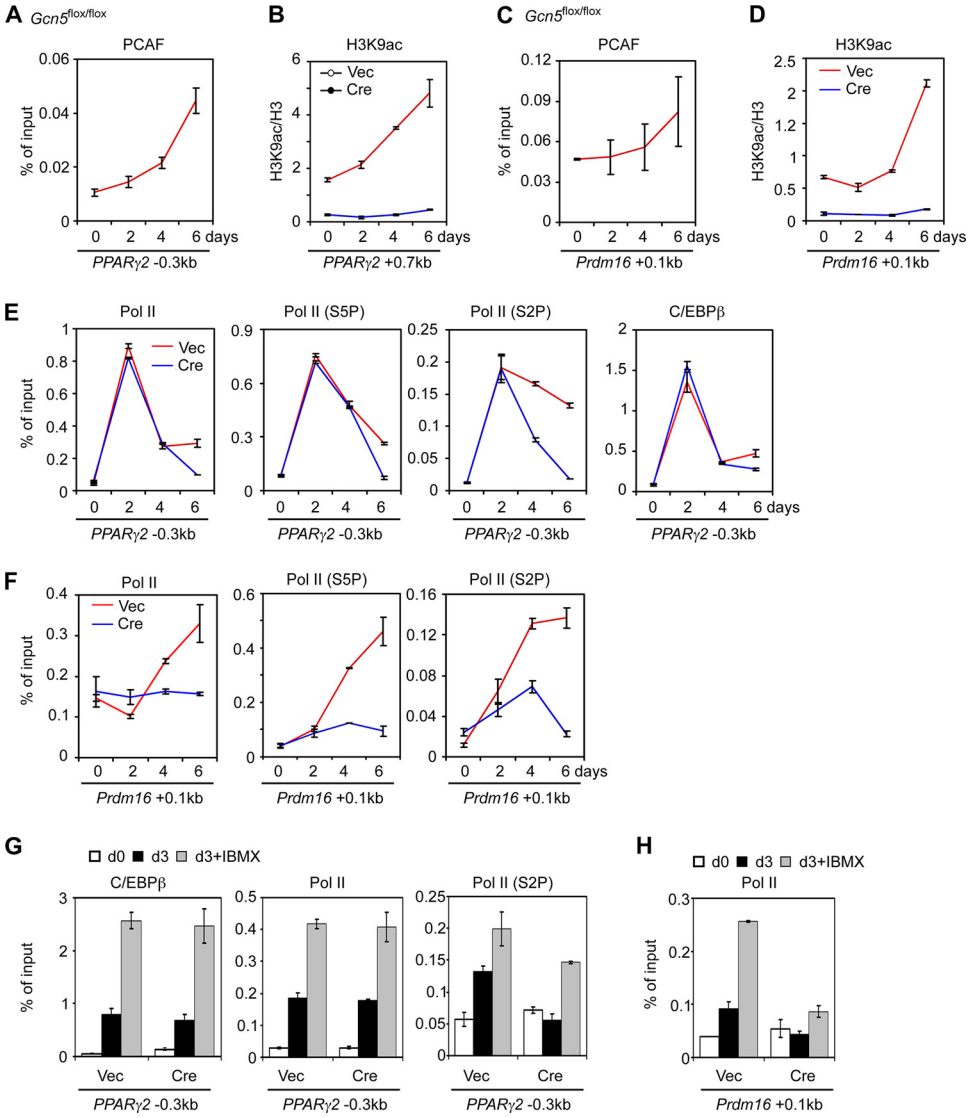


**FIG 5** Gcn5 and PCAF regulate different steps of *PPARγ* and *Prdm16* transcription. (A to D) Gcn5/PCAF acetylate H3K9 at *PPARγ2* and *Prdm16* genes during adipogenesis. $Gcn5^{flox/flox}$ brown preadipocytes (A and C) or retroviral Vec- or Cre-infected $Gcn5^{flox/flox}$; $PCAF^{-/-}$ brown preadipocytes (B and D) were induced for adipogenesis. ChIP assays were performed to analyze PCAF (A and C) and H3K9ac (B and D) at the indicated sites in the *PPARγ2* and *Prdm16* genes. (E and F) Gcn5/PCAF regulate different steps of *PPARγ* and *Prdm16* transcription. Retroviral Vec- or Cre-infected $Gcn5^{flox/flox}$; $PCAF^{-/-}$ brown preadipocytes were induced to undergo adipogenesis, and cells were collected at the indicated times for ChIP analysis of C/EBPβ, Pol II, Pol II (S5P), and Pol II (S2P) association at the indicated sites in the *PPARγ2* (E) and *Prdm16* (F) genes. (G and H) Prolonged IBMX treatment increases C/EBPβ and Pol II recruitment and reactivates transcription elongation of the *PPARγ2* gene in *Gcn5/PCAF* DKO cells but does not promote Pol II recruitment at the *Prdm16* gene. Retroviral Vec- or Cre-infected $Gcn5^{flox/flox}$; $PCAF^{-/-}$ brown preadipocytes were induced to adipogenesis. After removal of the adipogenic cocktail, the cells were treated with or without 0.5 mM IBMX for 1 more day and then collected for ChIP assays of C/EBPβ, Pol II, and Pol II (S2P) enrichments at indicated sites of the *PPARγ2* (G) and *Prdm16* (H) genes.

*Gcn5/PCAF* double deletion in preadipocytes caused severe defects in adipogenesis. These defects were rescued by ectopic expression of PPARγ, indicating these HATs act largely at the level of *PPARγ* expression. However, adipogenesis was also rescued by prolonged IBMX treatment of the DKO cells. The main function of IBMX in the adipogenic cocktail is to elevate intracellular cAMP concentrations, thereby activating CREB to induce *C/EBPβ* expression. cAMP also stimulates C/EBPβ phosphorylation (31), which is required for C/EBPβ binding to target genes (32). Therefore, prolonged exposure to IBMX likely stimulates C/EBPβ phosphorylation to enhance C/EBPβ binding to the *PPARγ2* promoter, consequently increasing Pol II recruitment and bypassing the need for Gcn5 and PCAF.

Gcn5 functions in transcription elongation have been reported previously in yeast. Yeast Gcn5 is predominantly localized to coding regions of highly transcribed genes (33), and it enhances transcription elongation by facilitating nucleosome eviction or other histone acetylation-dependent processes (34, 35). Our study provides evidence that Gcn5/PCAF are also involved in transcription elongation in mammalian cells. Previous work reporting that PCAF is distributed on both promoters and transcribed regions of active genes is also consistent with a role for this HAT in transcrip-

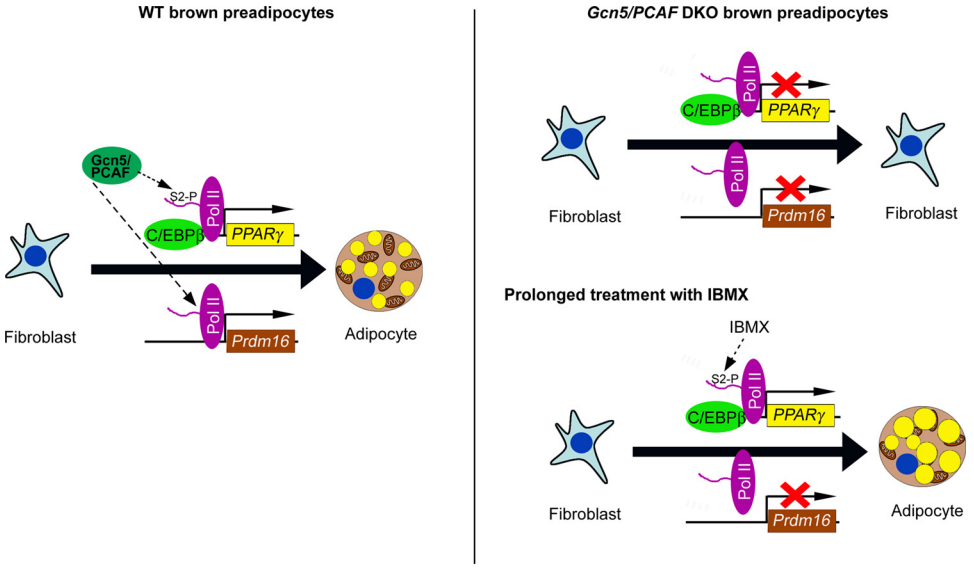


**FIG 6** Model for Gcn5 and PCAF regulating *PPARγ* and *Prdm16* expression to facilitate brown adipogenesis.

tion elongation (28). Gcn5/PCAF were reported to interact with transcription elongation factor p-TEFb (36), which may be recruited onto the *PPARγ* gene by Gcn5/PCAF to phosphorylate Pol II and trigger transcription elongation.

The transcriptional control of BAT development has received much attention over the last several years, since Prdm16 is the master transcription factor for BAT development, and furthermore, Prdm16 is required for browning of subcutaneous fat cells (27). Since Gcn5 and PCAF are important for *Prdm16* expression, these HATs are likely required not only for BAT development but also for browning of white adipocytes. Increased Prdm16 expression provides a potential strategy in the fight against the obesity epidemic. Prdm16 expression is regulated at both the mRNA and protein levels. miR-133 directly targets and downregulates *Prdm16* mRNA to inhibit brown adipocyte differentiation (37). The PPARγ agonist rosiglitazone stabilizes Prdm16 to induce a white-to-brown fat conversion (26). Before our study, little was known about how *Prdm16* is regulated at the transcriptional level. Gcn5/PCAF might mediate acetylation of either H3K9 or transcription factors/regulators to activate *Prdm16*. Both C/EBPβ and PGC-1α, which are involved in inducing expression of BAT-selective genes (38), are acetylated by Gcn5 to change their transcriptional activity (39, 40). Our findings might provide additional options for treatment of obesity. Modulation of Gcn5 HAT activity by small molecules or other agents might allow modulation of *Prdm16* expression, facilitating BAT development or white-to-brown fat conversion. Development of such therapeutic strategies could provide important steps forward in combating obesity and obesity-associated disorders.

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