Supplemental Information

Gcn5 and PCAF negatively regulate interferon β production through HAT-independent inhibition of TBK1

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Supplementary Materials and Methods

Plasmids, antibodies and chemicals
The Cre-expressing retroviral plasmids MSCVpuro-Cre and MSCVhygro-Cre have been described [1, 2]. Gal4-IRF3 plasmid, GAL4-responsive luciferase reporter UAS-Luc, IFNβ promoter-driven luciferase reporter IFNβ-Luc, and pcDNA3-FLAG-TBK1 were described [3]. Full length mouse Gcn5 cDNA, either WT, D608A [4] or NLSm, was cloned into retroviral vector MSCVpuro (Clontech). Full length human PCAF cDNA was amplified by PCR from MGC clone BC060823.1 and cloned into MSCVpuro. Gcn5 was also cloned into pEGFP vector (Clontech) to generate GFP-Gcn5 expression plasmid. FLAG- or myc-tagged Gcn5 were generated by PCR and cloned into pcDNA3 (Invitrogen). All plasmids were confirmed by DNA sequencing. Lentiviral shRNA constructs were from Sigma (Supplemental Table S3). All commercial antibodies are listed in Table S4. Anti-FLAG antibody-conjugated agarose (A2220) and mouse IgG agarose (A0919) were from Sigma. BX795 and Poly(I:C) were from InvivoGen.

Cell Culture, retroviral gene transfer, shRNA knockdown, peritoneal macrophage isolation
Unless indicated, all cells were routinely cultured in DMEM plus 10% FBS. Immortalized PCAF⁻/⁻;Gcn5⁺⁺/Δ MEFs were described [5]. Primary PCAF⁻/⁻;Gcn5⁺⁺/⁺⁺ brown preadipocytes were isolated and immortalized following an established protocol [2]. 293 cells expressing human TLR3 (293/TLR3) were from InvivoGen.

Retroviral infection of cells was done as described [1]. To package lentiviral shRNA constructs, 293FT cells (Invitrogen) were plated at a density of 3 x 10⁶ per 10-cm dish. The next day, cells were co-
transfected with 5 µg lentiviral shRNA construct, 3 µg psPAX2, 1 µg pMD2-G and 1 µg pCAG4-RTR2 using 20 µl Genjet (SignaGen). One day later, the cells were changed to 6 ml fresh medium. The next day, virus-containing media were collected, filtered through 0.45 µM membrane (Millipore), and 1:5 diluted in fresh medium. The diluted virus-containing media were supplemented with 16 µg/ml polybrene and used to infect the target cells. Two days later, infected cells were selected with 2 µg/ml puromycin for 1 week.

For peritoneal macrophage isolation, \( PCAF^{-/-}\);\( Gcn5^{\text{floxed/floxed}} \) mice were crossed with tamoxifen-inducible \( ER-Cre \) mice (Jackson 004682) to generate \( PCAF^{-/-}\);\( Gcn5^{\text{floxed/floxed}}\);\( Cre-ER \) mice. The adult mice were i.p. injected with 1ml 30% thioglycollate (TG) broth. After 5 days, peritoneal macrophages were harvested from peritoneal cavity and cultured in RPMI1640 plus 10% heat-inactivated FBS. All mouse works were approved by the Animal Care and Use Committee of NIDDK, NIH.

**Viruses**

VSV expressing GFP (VSV-GFP) was previously described [6]. For infection with VSV-GFP, cells were seeded at a density of 1.5 \( \times \) 10\(^5\) cells per well in 6-well plate. After overnight culture, cells were infected with indicated MOI of VSV-GFP in complete growth medium. At 24h post infection, cytopathic effects and GFP expression were observed under fluorescence microscopy. Virus titers were determined by a standard plaque assay in Vero cells [6].

For influenza virus replication experiments, 5 \( \times \) 10\(^5\) MEFs were re-suspended in 250 µl of MEM medium (pH6.6) and were infected with 0.5 MOI of influenza virus A/WSN/33 (WSN) [7] for 30 minutes at 37°C. Cells were washed, re-suspended in 1.2 ml complete medium (DMEM with 2% FBS), and cultured in 6-well plates for 2 days. Virus-containing media were collected and the TCID\(_{50}\) was determined by serial dilutions on MDCK cells.

**ELISA and luciferase assays**

MEFs were transfected with indicated concentration of poly(I:C) for 6h using FUGENE 6 (Roche). IFN\( \alpha/\beta \) levels in the conditioned media were determined using ELISA kits from PBL. To detect IFN\( \beta \) production in the absence of poly(I:C) treatment, cells were plated at a density of 1 \( \times \) 10\(^5\) per 10 cm dish and cultured for 3 - 4 days. The conditioned media were filtered through 0.45 µM membrane and concentrated by 30-
fold using Vivaspin 500 filter (Fisher NC9924359), followed by ELISA assay. Luciferase assays were done using a dual-luciferase reporter assay system as described [8].

**qRT-PCR, ChIP, ChIP-Seq, Spike-in RNA-Seq, and bioinformatics**

qRT-PCR using Sybr-Green assay and ChIP assays were performed as described [2, 5]. The sequences of quantitative PCR primers are listed in Table S5. Data are presented as means +/- s.d. Spike-in RNA-Seq in MEFs was done as previously described [9]. ChIP was done as described [5]. ChIP-Seq was done as described [10]. ChIP-Seq and RNA-Seq data sets were deposited in GEO database (accession no. GSE60969). Identification of ChIP-enriched regions in ChIP-Seq data was done using SICER [11], with window size 200bp, gap size 200bp, and FDR = 1E-3. Only reads on the ChIP-enriched regions are used for downstream analysis (Fig. 1A and Fig. S1).

To show the correlation between H3K9ac on TSS and gene expression levels, we selected three groups of genes (high, medium, and low) to represent different expression levels, and for each group we calculated the average profile of H3K9ac around the TSS of genes (Fig. 1A). The three groups of genes are defined as follows. We collected all the genes detected by RNA-Seq (i.e., genes with tag number no smaller than 10 in at least one of the samples) and ranked them by their expression levels in the retroviral Vec-infected cells. The top, medium and lowest 1000 genes are selected to represent the high-, medium- and low-expression genes. These three groups of genes were also used in Fig.S1B. The loss of both Gcn5 and PCAF leads to 19.3-fold decrease of total H3K9ac in cells [12]. As a result, we decrease the H3K9ac level in Cre-infected cells by 19.3-fold to allow direct comparison with the H3K9ac level in Vec-infected cells (Fig. S1C-I). GO analysis was carried out using DAVID [13].

**Protein purification, GST-pull down, co-immunoprecipitation, immunoblotting, kinase assays**

Purification of GST fusion proteins, GST-pull down, co-immunoprecipitation and immunoblotting were done as described [1]. FLAG-tagged TBK1 (F-TBK1) or F-TBK1 together with myc-tagged Gcn5 (myc-Gcn5) were transiently expressed and purified from 293T cells as previously described [14]. Protein kinase assays were done as described [15] with minor modifications. Briefly, purified proteins were added into kinase assay buffer containing 50 μM ATP and 0.3 μg substrate GST-IRF3 (380-427aa),
and incubated at 30°C for 30 min. The phosphorylation level of GST-IRF3 (380-427aa) was determined by immunoblotting.

Reference: