THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 290, NO. 27, pp. 16607–16618, July 3, 2015

SET7/9 Enzyme Regulates Cytokine-induced Expression of Inducible Nitric-oxide Synthase through Methylation of Lysine 4 at Histone 3 in the Islet β Cell*

Received for publication, April 28, 2015, and in revised form, May 18, 2015 Published, JBC Papers in Press, May 20, 2015, DOI 10.1074/jbc.M115.661777

Kyoko Fujimaki[‡], Takeshi Ogihara[‡1], David L. Morris[§], Hisanobu Oda[¶], Hitoshi Iida[‡], Yoshio Fujitani[‡‖], Raghavendra G. Mirmira[§‡‡§§¶¶], Carmella Evans-Molina[§‡‡§§¶¶2], and Hirotaka Watada[‡‖‖]**

*From the [‡]Department of Metabolism and Endocrinology, Centers for [‖‖]Molecular Diabetology and [‖]Therapeutic Innovations in Diabetes, and **Japan Science and Technology Agency-Core Research for Evolutionary Science and Technology Program, Juntendo University Graduate School of Medicine, Tokyo 113-8421, Japan, [‡‡]Departments of Biochemistry and Molecular Biology, [§]Medicine, and [§§]Cellular and Integrative Physiology and the [¶¶]Herman B. Wells Center for Pediatric Research, Indiana University School of Medicine, Indianapolis, Indiana 46202, and [¶]Department of Gastrointestinal and Medical Oncology, National Hospital Organization Kyushu Cancer Center, Fukuoka 811-1395, Japan*

Background: SET7/9 regulates expression of NF-κB target genes in non-β cells.
Results: SET7/9 knockdown attenuated cytokine-induced expression of inducible nitric-oxide synthase and apoptosis in β cells.
Conclusion: SET7/9 regulates *Nos2* expression through methylation of H3K4 in β cells.
Significance: SET7/9 contributes to proinflammatory cytokine signaling in the pancreatic β cell.

SET7/9 is an enzyme that methylates histone 3 at lysine 4 (H3K4) to maintain euchromatin architecture. Although SET7/9 is enriched in islets and contributes to the transactivation of β cell-specific genes, including *Ins1* and *Slc2a*, SET7/9 has also been reported to bind the p65 subunit of nuclear factor κB in non-β cells and modify its transcriptional activity. Given that inflammation is a central component of β cell dysfunction in Type 1 and Type 2 diabetes, the aim of this study was to elucidate the role of SET7/9 in proinflammatory cytokine signaling in β cells. To induce inflammation, βTC3 insulinoma cells were treated with IL-1β, TNF-α, and IFN-γ. Cytokine treatment led to increased expression of inducible nitric-oxide synthase, which was attenuated by the diminution of SET7/9 using RNA interference. Consistent with previous reports, SET7/9 was co-immunoprecipitated with p65 and underwent cytosolic to nuclear translocation in response to cytokines. ChIP analysis demonstrated augmented H3K4 mono- and dimethylation of the proximal *Nos2* promoter with cytokine exposure. SET7/9 was found to occupy this same region, whereas SET7/9 knockdown attenuated cytokine-induced histone methylation of the *Nos2* gene. To test this relationship further, islets were isolated from SET7/9-deficient and wild-type mice and treated with IL-1β, TNF-α, and IFN-γ. Cytokine-induced *Nos2* expression was reduced in the islets from SET7/9 knock-out mice. Together, our findings suggest that SET7/9 contributes to *Nos2* transcription and proinflammatory cytokine signaling in the pancreatic β cell through activating histone modifications.

Glucose homeostasis is maintained in response to nutrient intake through finely tuned insulin secretion from the pancreatic β cells that is exquisitely coupled to the demands of insulin-sensitive organs, including muscle, liver, and adipose tissue. Impairments in glucose homeostasis result in the development of hyperglycemia and diabetes mellitus of which there are two main forms. Although the etiologies of Type 1 diabetes (T1D)[3] and Type 2 diabetes (T2D) are distinct, increasing evidence suggests a primary role for β cell dysfunction arising from inflammation in both disorders. T1D occurs as a result of loss of self-tolerance, activation of innate and adaptive immunity, and immune cell infiltration into islets, all eventually culminating in T cell-mediated destruction of the pancreatic β cell (1, 2). In contrast, T2D arises from impaired peripheral insulin sensitivity and progressive β cell dysfunction. Recently, T2D-associated metabolic dysfunction has been linked to macrophage

* This work was supported, in whole or in part, by National Institutes of Health Grants R01 DK093954 (to C. E.-M.) and F32 DK091976 and K01 DK100515 (to D. L. M.). This work was also supported by Japan Society for the Promotion of Science KAKENHI Grants 23591324 and 26461345 and the Suzuken Memorial Foundation (both to T. O.) and Veterans Affairs Merit Award I01BX001733 (to C. E.-M.). H. W. has received lecture fees from Asteras, Astrazeneca, Boehringer Ingelheim, Daiichi Sankyo Inc., Eli Lilly and Co., Kissei Pharmaceutical Co., Kowa Pharmaceutical Co., Kyowa Hakko Kirin Co., Merck Sharp and Dohme, Novartis Pharmaceuticals, Novo Nordisk Pharma, Ono Pharmaceutical Co., Mitsubishi Tanabe Pharma, Sanofi-Aventis, Sanwakagaku Kenkyusho, and Takeda Pharmaceutical Co. and research funds from Asteras, Astrazeneca, Bristol-Myers Squibb, Boehringer Ingelheim, Daiichi Sankyo Inc., Dainippon Sumitomo Pharma, Eli Lilly, Johnson and Johnson, Kissei Pharmaceutical Co., Kowa Pharmaceutical Co., Kyowa Hakko Kirin Co., Merck Sharp and Dohme, Mitsubishi Tanabe Pharma, Mochida Pharmaceutical Co., Novartis Pharmaceuticals, Novo Nordisk Pharma, Pfizer, Sanwakagaku Kenkyusho, Sanofi-Aventis, and Takeda Pharmaceutical Co.

[1] To whom correspondence may be addressed: Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan. Tel.: 81-3-5802-1579; Fax: 81-3-3813-5996, E-mail: take@juntendo.ac.jp.

[2] To whom correspondence may be addressed: Indiana University School of Medicine, 635 Barnhill Dr., MS 2031A, Indianapolis, IN 46202. Tel.: 317-274-4145; Fax: 317-274-4107; E-mail: cevansmo@iu.edu.

[3] The abbreviations used are: T1D, Type 1 diabetes; T2D, Type 2 diabetes; H3K4, histone 3 at lysine 4; iNOS, inducible nitric-oxide synthase; H3, histone 3; TBP, TATA-binding protein; qRT-PCR, quantitative RT-PCR; RIPA, radioimmune precipitation assay.

infiltration of a variety of metabolic tissues, including the pancreatic islets (3–5). Local and systemic elevations of proinflammatory cytokines lead to the activation of deleterious signaling cascades within the β cell, including those regulated by NF-κB, JAK, and STAT1 (6, 7). The *NOS2* gene is a key downstream target of NF-κB, leading to translation of inducible nitric-oxide synthase (iNOS) and the catalysis of L-arginine to produce nitric oxide (NO). In both T1D and T2D, NO-supplied reactive oxygen species contribute to mitochondrial dysfunction, impacting cellular energy status, glucose-stimulated insulin secretion, and ultimately β cell survival (8–13). Because the inflammatory response responsible for NO generation could be a potential target to treat diabetes mellitus, an improved understanding of the transcriptional pathways that regulate iNOS production is needed.

Gene transcription is regulated epigenetically through alterations in patterns of DNA methylation and covalent histone modifications that either promote or restrict the accessibility of components of the transcriptional machinery to gene promoters (14, 15). SET7/9 is a SET (Su(var)3–9, Enhancer-of-zeste, Trithorax) domain-containing enzyme that exhibits methyltransferase activity and promotes open chromatin architecture and target gene expression through methylation of histone 3 at lysine 4 (H3K4) (16). In addition to its activity as a histone methyltransferase, SET7/9 is also known to methylate lysine residues of non-histone proteins, including TAF10, pRB, p53, and the estrogen and androgen receptors, where SET7/9-mediated methylation has been shown to regulate target protein stability and/or activity (17–21). Previously, we have shown that SET7/9 is enriched in rodent and human islets and methylates H3K4 in a number of β cell-specific genes, including *Ins1* and *Slc2a2*. Furthermore, mice with attenuated islet SET7/9 expression exhibited glucose intolerance and impaired glucose-stimulated insulin secretion (22, 23). However, several groups have also shown that SET7/9 contributes to inflammation through interactions with components of the NF-κB signaling cascade. In monocytes, SET7/9 forms a complex with NF-κB and is recruited specifically to the *CCL2* and *TNFA* promoters where it methylates H3K4 (24). In mouse embryonic fibroblast cells, SET7/9 has also been shown to methylate Lys-37 of the p65 subunit of NF-κB and up-regulate NF-κB transcriptional activity (25). In contrast, in human osteosarcoma cells, p65 is methylated at lysine residues 314 and 315, leading to its ubiquitination and degradation and subsequent down-regulation of NF-κB activity (26). Therefore, the effects of SET7/9 on NF-κB activity remain controversial. Moreover, at present, the role of SET7/9 in the pathogenesis of islet inflammation has not been explored.

In this report, we investigate the role of SET7/9 in cytokine-induced inflammatory gene expression and β cell apoptosis. Our results show that SET7/9 interacts with NF-κB and is recruited to and enhances cytokine-induced H3K4 methylation of the *Nos2* promoter. Diminution of SET7/9 attenuates cytokine-induced iNOS expression as well as apoptosis in a murine insulinoma cell. Furthermore, we show that cytokine-induced *Nos2* expression was reduced in islets isolated from SET7/9 knock-out mice compared with wild-type mice. Together, these data suggest a novel role for SET7/9 in the regulation of proinflammatory β cell gene expression.

Experimental Procedures

Antibodies and Materials—Monoclonal antibodies against SET7/9 were obtained from Epitomics (5131-1) and LifeSpan BioSciences (LS-C138726). Polyclonal antibodies against dimethyl-H3 Lys-4 (07-030), monomethyl-H3 Lys-4 (07-436), and iNOS (06-573) were obtained from Millipore. Polyclonal antibodies against p65 (ab7970) and TATA-binding protein (TBP) (ab63766) were obtained from Abcam. A polyclonal antibody against cleaved caspase-3 (9661) and a monoclonal antibody for p53 (2524) were from Cell Signaling Technology. Anti-FLAG® M2 affinity gel was obtained from Sigma-Aldrich. Mouse TNF-α, mouse IL-1β, and mouse IFN-γ were obtained from PeproTech.

Cell Culture and Cytokine Treatment—βTC3 mouse insulinoma cells were maintained in high glucose Dulbecco's modified Eagle's medium supplemented with 15% horse serum, 2.5% fetal bovine serum (FBS), and 1% penicillin/streptomycin. MIN6 mouse insulinoma cells were maintained in high glucose Dulbecco's modified Eagle's medium supplemented with 15% FBS, 10 mM HEPES, and 1% penicillin/streptomycin. βTC3 cells were treated with or without a mixture of cytokines that included 5 ng/ml IL-1β, 10 ng/ml TNF-α, and 100 ng/ml IFN-γ.

RNA Interference—Stealth RNAi™ siRNAs against *Setd7* (si-Set7/9) or non-targeting sequences (si-scramble) were purchased from Life Technologies and transfected into βTC3 cells and MIN6 cells using Lipofectamine RNAiMAX transfection reagent (Life Technologies) according to the manufacturer's instructions. Ninety-six hours after transfection, cells were treated with or without a cytokine mixture for the indicated times. siRNA sequences used were as follows: si-Set7/9, 5′-CCUGGACGAGGAGACAGUCAUUGAU-3′; si-scramble, 5′-UAAAUGUACUGCGCGUGGAGAGGAA-3′.

Quantitative RT-PCR (qRT-PCR)—βTC3 cells (7×10^5) were seeded in 6-well plates, transfected with si-Set7/9 or si-scramble, and treated with cytokines 96 h after transfection. Total RNA was isolated from βTC3 cells using the RNeasy® kit (Qiagen) and subjected to cDNA synthesis using SuperScript III reverse transcriptase (Invitrogen) according to the manufacturer's instructions. PCR mixtures were prepared using Fast SYBR Green Master Mix (Life Technologies), and qRT-PCR was performed using the 7300 real time PCR system (Applied Biosystems). Forward and reverse primers used for quantitative RT-PCR are listed in Table 1.

Immunoblot Assays—For isolation of total protein, βTC3 cells were washed twice with PBS and then lysed by adding RIPA buffer containing 140 mM NaCl, 20 mM Tris, 1 mM EDTA, 50 mM NaF, 2 mM $MgCl_2$, 1 mM PMSF, 25 units/ml Benzonase, and 1× Halt™ protease-phosphatase inhibitor mixture. Cytosolic and nuclear fractions were prepared as described previously (27). Approximately 20 μg of total protein, 20 μg of the cytosolic fraction, or 10 μg of the nuclear fraction were separated in 12% SDS-polyacrylamide gels, transferred to a polyvinylidene fluoride membrane, and incubated with antibodies against SET7/9, iNOS, p65, cleaved caspase-3, p53, β-actin, or TBP. Bound primary antibodies were detected with peroxidase-

TABLE 1
The primer sequences for quantitative PCR

Gene	Forward sequences	Reverse sequences
Primers for RT-PCR		
Tbp	GCTGCAGTCATCATGAGAATAAGAG	CACCATGTTCTGGATCTTGAAGT
Setd7	GGCCGGTCCATTCAGCTCTCC	GCAGGGCCAGGGCGTTTACA
Nos2	CACCACAAGGCCACATCGGATT	CCGACCTGATGTTGCCATTGTT
Tnfa	GGCAGGTCTACTTTGGAGTCATTGC	ACATTCGAGGCTCCAGTGAATTCGG
Sod2	CTCCCGGCACAAGCACAGCC	GCGCGTTAATGTGTGGCTCCA
Bax	TCTCCGGCGAATTGGAGATG	CGTGTCCACGTCAGCAATCA
Pten	CTGGGCTCTGGACCATACAC	AGCAGCCAATCTCTCGGATG
Tnfrsf6	AGAAGATGCACACTCTGCGA	GGTATTCTGGGTCAGGGTGC
Cdkn1a	CAGCTCAGTGGACTGGAAGG	CTGTCTCACCACCAAGGACC
Primers for ChIP assay		
β-gal	TCAATCCGCCGTTTGTTCCCAC	TCCAGATAACTGCCGTCACTCCAAC
Proximal *Nos2*	TCACTCAGCACAGCCCATCCACT	ATGGAAGGCAAGCTGTGGGCA
Nos2 −5 kb	TCCTTGCTCTGAAGGGCATC	GCTCACAGACCACGGATGAA
Nos2 +1 kb	CGCCAAGGAGAAGCCACATA	TTGGGAGTCATCACGCATCC
Proximal *Tnfa*	GCCAGCCAGCAGAAGCTCCC	GCGCCTTGGGCCAGTGAGTG
Tnfa −5 kb	AGTGCTCAGAACCCTAAGCC	CATGTGTGAGCCCCTGAGTC
Tnfa +1 kb	TCGAGCCAGGCTGAGAAAAG	GCAGCTACCCACACTTCACT
Proximal *Sod2*	ACACGCAAACCTGCGACGTG	AGCCAAGGCTCTGCTGCTGGT
Sod2 −5 kb	GGAGCATGCTAGCTTCTGGA	CTTCCCAGTCACCGTTGTCA
Sod2 +1 kb	AGCTAGCATTGGTGGTGGTC	AAACTCTCCTGAAGCCACGT
Alb	TGGGAAAACTGGGAAAACCATC	CACTCTCACACATACACTCCTGCTG

coupled secondary mouse or rabbit antibodies and IRDye®-conjugated secondary mouse or rabbit antibody. Immunoblots were visualized using the Odyssey (LI-COR Biosciences), LAS-3000 (Fuji Film) with ECL-Plus™ (Amersham Biosciences), or a SuperSignal West Pico system (Pierce).

Flow Cytometry—Approximately 1×10^6 βTC3 cells were treated with the cytokine mixture for 20 h, single cell-suspended using trypsin/EDTA, and washed twice with PBS. Apoptosis analysis was performed using an Annexin V-FITC/PI Apoptosis Detection kit (Merck Millipore) according to the manufacturer's instructions. Fluorescence-labeled cells were analyzed using a BD FACSCalibur HG™ flow cytometer and BD CellQuest Pro software version 6.0 (BD Biosciences). βTC3 cells treated without cytokines were used as a negative control. βTC3 cells treated with 150 μM hydrogen peroxide were used for the gating of Annexin V to detect apoptotic cells.

Co-immunoprecipitation—A/G Mag Sepharose (GE Healthcare) was preincubated with anti-SET7/9 antibody or mouse IgG, transferred into 500 μl of RIPA buffer containing 500 μg of total protein, incubated at 4 °C for 2 h, and washed three times with RIPA buffer. Approximately 1×10^7 βTC3 cells were seeded in a 10-cm dish for 24 h before transfection. Cells were next transfected with 10 μg of an expression plasmid for FLAG-SET7/9 (16) or an empty vector using Effectene (Qiagen) according to the manufacturer's instructions. Total protein was obtained using RIPA buffer, immunoprecipitated with anti-FLAG M2 affinity gel at 4 °C for 2 h, and washed three times with RIPA buffer. Eluted proteins were subjected to immunoblotting as described previously.

Chromatin Immunoprecipitation Assays—Chromatin immunoprecipitation (ChIP) assays were performed as described previously (28). Briefly, $\sim 2 \times 10^7$ βTC3 cells were treated with 1% formaldehyde to cross-link protein to DNA. Chromatin was next fragmented by sonication using a Q500 Sonicator (QSonica) and then immunoprecipitated with anti-monomethyl-H3 Lys-4 or anti-dimethyl-H3 Lys-4 antibodies or normal rabbit serum. DNA fragments extracted from immunoprecipitated protein and DNA complexes were subjected to quantitative PCR as described. Forward and reverse primers used for PCR are listed in Table 1.

Mouse Experiments and Islet Isolation—*Setd7*-deficient mice were established and provided by the laboratory of Dr. Danny Reinberg (New York University). Animal studies were performed under protocols approved by the Indiana University School of Medicine Animal Care and Use Committee. Islets were isolated following collagenase digestion of the pancreas as described previously (29) and maintained in RPMI 1640 medium supplemented with 10% FBS and 1% penicillin/streptomycin. Islets were treated with or without a mixture of cytokines for 4 h and then harvested for mRNA isolation.

Statistical Analysis—All data are presented as the means ± S.E. Student's *t* tests were used for comparisons involving two conditions. Differences between groups were analyzed for significance using one-way analysis of variance with multiple comparisons and a Tukey-Kramer post-test. Statistical significance was assumed at $p < 0.05$.

Results

SET7/9 Regulates Cytokine-induced iNOS Expression and Apoptosis in β Cells—To investigate the role of SET7/9 in inflammatory signaling in the β cell, the gene encoding SET7/9 (*Setd7*) was first knocked down using RNA interference. *Setd7* mRNA levels were reduced by ~75%, whereas protein levels were reduced by 60% in βTC3 cells treated with an siRNA against *Setd7* (si-Set7/9) compared with those treated with an siRNA for a nonspecific target (si-scramble) (Fig. 1, *A* and *B*). Next, βTC3 cells were treated with a mixture of proinflammatory cytokines that included 10 ng/ml TNF-α, 5 ng/ml IL-1β, and 100 ng/ml IFN-γ. Consistent with previous reports using rodent islets and rat insulinoma cell lines (30, 31), cytokine treatment led to increased expression of NF-κB target genes, including *Nos2*, *Tnfa*, and *Sod2* (Fig. 1*B*). Diminution of SET7/9 expression attenuated cytokine-induced *Nos2* mRNA expression as well as elevations in iNOS protein levels (Fig. 1, *B* and *C*). Interestingly, SET7/9 knockdown had no effect on the NF-κB target genes *Tnfa* and *Sod2*. To rule out potential off-target

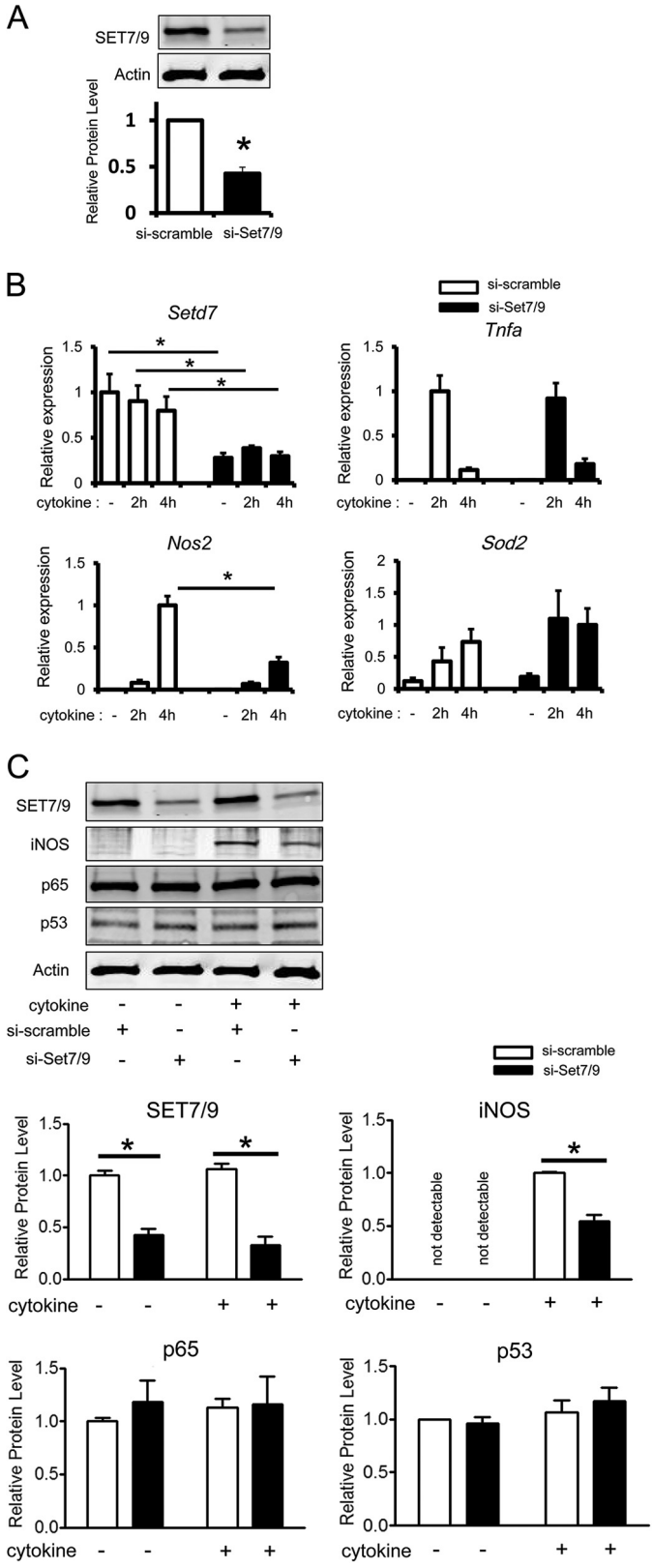


FIGURE 1. **SET7/9 knockdown attenuates cytokine-induced *Nos2* expression in mouse βTC3 cells.** *A*, βTC3 cells were transiently transfected with a non-targeting siRNA (si-scramble) or si-RNA against *Setd7* (si-Set7/9) and then harvested for immunoblot analysis. The expression level of SET7/9 was normalized to β-actin expression ($n = 4$). *B*, 96 h after transfection with si-scramble or si-Set7/9, βTC3 cells were stimulated with or without a mixture of cytokines (5 ng/ml IL-1β, 10 ng/ml TNF-α, and 100 ng/ml IFN-γ) for the indicated times. Total RNA was isolated, and expression of NF-κB target genes was determined by qRT-PCR. The expression level of mRNA was normalized to *Tbp* expression. Data represent the average of four independent transfections. *C*, 96 h after transfection with si-scramble or si-Set7/9, βTC3 cells were stimulated with or without cytokines for 4 h. Total protein was isolated and subjected to immunoblotting for SET7/9, iNOS, p65, p53, and β-actin, and the expression level of each protein was normalized to β-actin expression. Data represent the average of three independent transfections. *, the indicated comparisons are significantly different ($p < 0.05$). Results are displayed as the means ± S.E. (*error bars*).

effects of si-Set7/9, we transfected different sequences of siRNA for *Setd7* (si-Set7/9B), and results were similar (Fig. 2*A*). Furthermore, we performed SET7/9 depletion in a second mouse β cell line, MIN6 cells, and confirmed that SET7/9 knockdown led to a preferential inhibition of cytokine-induced expression of *Nos2* (Fig. 2*B*).

To determine whether SET7/9 knockdown protected against cytokine and nitric oxide-induced β cell apoptosis, βTC3 cells were stained with Annexin V, and apoptotic cells were counted using flow cytometry. Treatment with cytokines for 20 h increased apoptotic cells, whereas SET7/9 knockdown significantly attenuated cytokine-induced apoptosis (si-Set7/9, 15.37 ± 1.02% *versus* si-scramble, 26.87 ± 1.12%) (Fig. 3, *A* and *B*). Furthermore, cytokine treatment increased expression of cleaved caspase-3, an effect that was partially reversed by knockdown of SET7/9 (Fig. 3*C*). Because p53 is a crucial regulator of apoptosis that has been shown to be stabilized by SET7/9-induced lysine methylation (19, 32), we next evaluated the expression of p53 and its downstream targets. Although SET7/9 knockdown decreased cleaved caspase-3 levels, expression of p53 (Fig. 1*C*) or p53 target genes, including *p21* (gene symbol *Cdkn1a*), *Pten*, *Fas* (gene symbol *Tnfrsf6*), and *Bax*, was not decreased 4 h after cytokine treatment (Fig. 3*D*).

SET7/9 Translocates into the Nucleus in Response to Cytokine Treatment—Yang *et al.* (26) have shown that SET7/9 methylates the p65 subunit of NF-κB, leading to p65 ubiquitination and degradation. To evaluate whether SET7/9 regulates NF-κB protein stability in β cells, total protein from βTC3 cells was isolated before and after cytokine treatment, and immunoblotting for the p65 subunit of NF-κB was performed. SET7/9 knockdown had no effect on total p65 protein levels in βTC3 cells (Fig. 1*C*), indicating that SET7/9 does not regulate NF-κB protein stability in β cells.

SET7/9 has also been shown to shuttle between the nucleus and cytosol in response to various stimuli (33, 34). To investigate the effect of cytokines on SET7/9 intracellular distribution, SET7/9 content was evaluated in total, nuclear, and cytosolic fractions isolated from βTC3 cells before and after cytokine treatment. Consistent with a previous study performed in THP1 cells (24), p65 translocated into the nucleus in response to cytokine treatment, and this was unaffected by SET7/9 knockdown (Fig. 4, *A* and *B*). In response to cytokine treatment, SET7/9 significantly increased in the nuclear fraction and decreased in the cytosolic fraction, indicating that pro-inflammatory cytokines induced translocation of SET7/9 into the nucleus (Fig. 4, *A* and *C*). SET7/9 is known to form a complex with p65 in non-β cells (24–26). To test this relationship in β cells, immunoprecipitation experiments were performed. Results showed that p65 was co-immunoprecipitated using anti-SET7/9 antiserum in βTC3 cells (Fig. 5*A*).

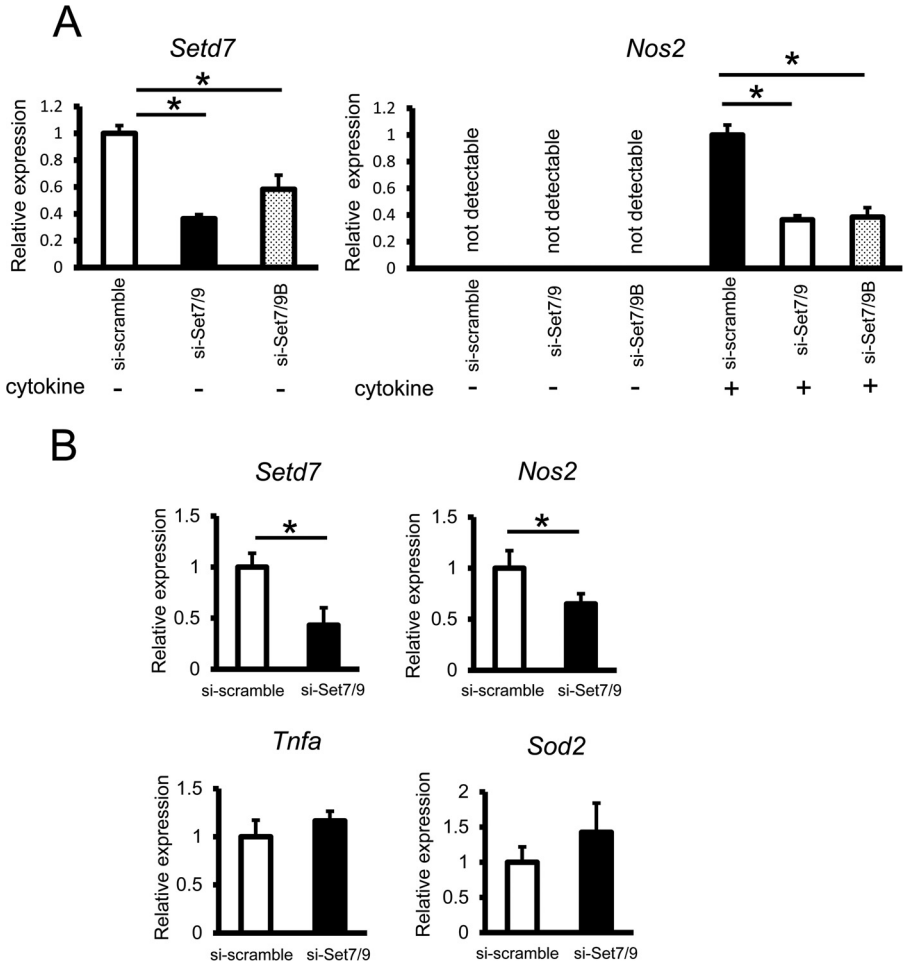


FIGURE 2. **SET7/9 knockdown attenuates cytokine-induced *Nos2* expression in mouse MIN6 cells.** *A*, 96 h after transfection with si-scramble, si-Set7/9, or si-Set7/9B (5′-AUAAGGGUCUGGAAGGAGAGCAUCG-3′), βTC3 cells were treated with or without cytokines (5 ng/ml IL-1β, 10 ng/ml TNF-α, and 100 ng/ml IFN-γ) for 4 h. Total RNA was isolated and subjected to qRT-PCR. *B*, after transfection with si-scramble or si-Set7/9, MIN6 cells were treated with a mixture of cytokines for 4 h. Total RNA was isolated, and the expression of NF-κB target genes was determined by qRT-PCR. The expression level of RNA was normalized to *Tbp* expression. Data represent the average of four independent transfections. * indicates a statistical difference ($p < 0.05$) compared with βTC3 cells transfected with si-scramble. Results are displayed as the means ± S.E. (*error bars*).

Furthermore, p65 was similarly detected in immunoprecipitated fractions by an anti-FLAG antibody in βTC3 cells transfected with a construct encoding FLAG-SET7/9 (Fig. 5*B*).

SET7/9 Contributes to the Methylation of Histone 3 at Lysine 4 at the Nos2 Promoter—Given the observed effects on *Nos2* expression, we hypothesized that SET7/9 may regulate chromatin structure in the *Nos2* gene promoter. Using ChIP analysis, we evaluated the H3K4 methylation status of three distinct regions of each NF-κB target gene. These regions included the proximal promoter region containing consensus sequences for NF-κB-binding (Fig. 6*A*), the distal promoter region (−5 kb), and the 3′ region of the transcriptional start site (+1 kb) that lacked the consensus sequences. Treatment of βTC3 cells with cytokines augmented monomethylation of the proximal promoter and the +1 kb region of *Nos2* as well as the proximal promoter region of *Tnfa* but had no effect on H3K4 monomethylation across the *Sod2* gene (Fig. 6, *B* and *D*). Interestingly, SET7/9 knockdown partially inhibited cytokine-induced H3K4 monomethylation of the *Nos2* but not the *Tnfa* or *Sod2* proximal promoters (Fig. 7, *A*–*C*), consistent with qRT-PCR results (Fig. 1*B*). Although we also evaluated the −5 kb region and the +1 kb region of each gene, SET7/9 knockdown did not impair monomethylation of these regions (Fig. 7, *A*–*C*).

Histone lysine residues may undergo mono-, di-, or trimethylation. These modifications may be regulated independently (35), and di- and trimethylation of H3K4 are strongly associated with actively transcribed genes (36, 37). To demonstrate further evidence of cytokine-induced changes in the chromatin architecture of the *Nos2* promoter, H3K4 dimethylation levels were next evaluated. Cytokine treatment led to increased levels of H3K4 dimethylation of the *Nos2* proximal promoter. Moreover, this effect was prevented by SET7/9 knockdown (Fig. 8, *A* and *B*).

Next, to assess for the presence of SET7/9 on the *Nos2* proximal promoter, βTC3 cells were transfected with an expression vector encoding FLAG-SET7/9 or an empty vector control. ChIP analysis was performed using anti-FLAG antibody. As shown in Fig. 8*C*, recovery of the proximal promoter of *Nos2* was increased in βTC3 cells transfected with SET7/9 compared with cells transfected with the control

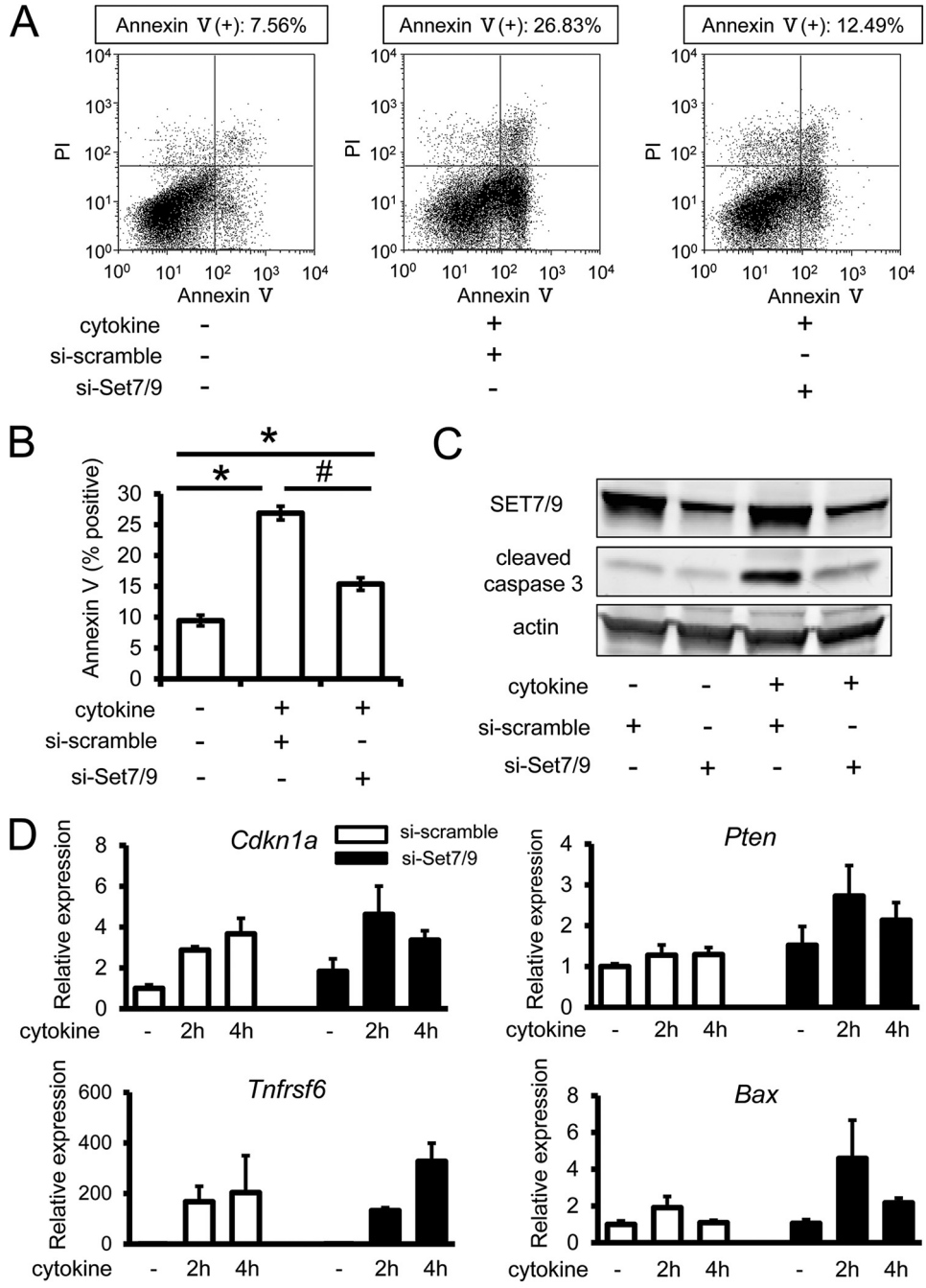


FIGURE 3. **SET7/9 knockdown inhibits cytokine-induced apoptosis in βTC3 cells.** *A*, after transfection with si-scramble or si-Set7/9, βTC3 cells were stimulated with or without cytokines for 20 h and then stained with Annexin V and propidium iodide (*PI*). Representative dot-blot analysis of flow cytometric analysis for each condition is shown, and the percentage of annexin V-positive apoptotic cells is indicated. *B*, data represent the average of six independent transfections. * indicates a statistical difference ($p < 0.05$) compared with βTC3 cells treated without cytokines. # indicates a statistical difference ($p < 0.05$) compared with βTC3 cells treated with a cytokine mixture and transfected with si-scramble. *C*, after transfection with si-scramble or si-Set7/9, βTC3 cells were treated with or without cytokines for 2 h and immunoblotted with antisera for SET7/9, cleaved caspase-3, and β-actin. *D*, after transfection with si-scramble or si-Set7/9, βTC3 cells were stimulated with or without cytokines for various times and harvested. The expression of p53 target genes was determined by qRT-PCR, and results were normalized to *Tbp* expression. Data represent the average of at least four independent transfections. Results are displayed as the means ± S.E. (*error bars*).

vector, indicating occupancy of SET7/9 on the *Nos2* promoter. Together, these results suggest that SET7/9 regulates *Nos2* transactivation through histone methylation of the *Nos2* gene.

Cytokine-induced Nos2 Expression Was Attenuated in Islets Isolated from SET7/9 Knock-out Mice—Finally, to investigate the role of SET7/9 in *Nos2* expression in pancreatic islets, the *Setd7* locus was deleted to generate total body SET7/9 knock-out mice. Previously, our group demonstrated that SET7/9 was expressed in liver, muscle, and islets (22). Therefore, we confirmed the absence of SET7/9 in those tissues (Fig. 9*A*). Islets were isolated from SET7/9 knock-out mice or wild-type controls and then treated with the mixture of cytokines. In response to cytokines, islets isolated from wild-type mice

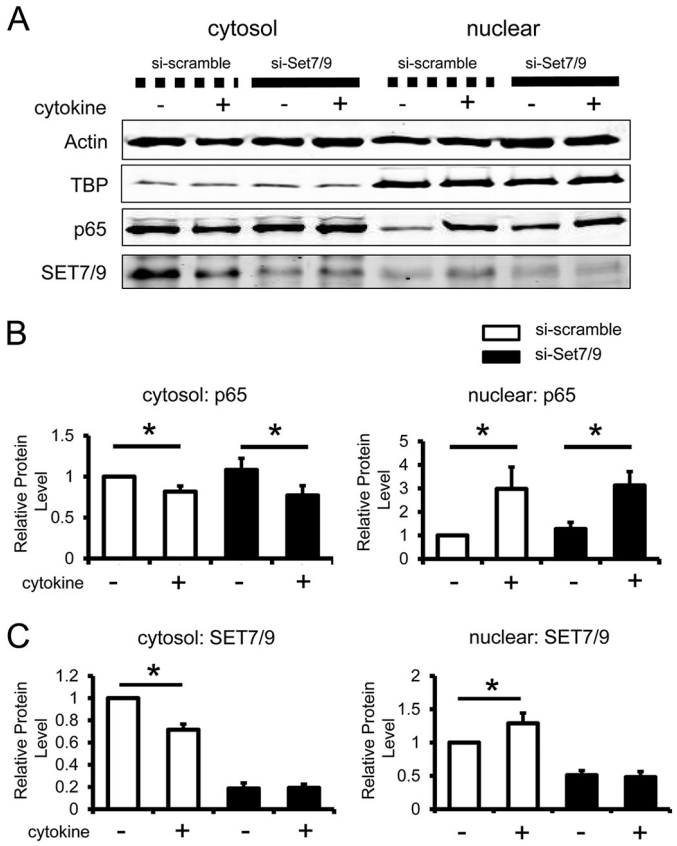


FIGURE 4. **SET7/9 translocates from the cytosol into the nucleus in response to cytokine treatment.** After transfection with si-scramble or si-Set7/9, βTC3 cells were stimulated with or without cytokines for 4 h and subjected to nuclear and cytosolic fractionation. *A*, panels show a representative immunoblot using antisera for SET7/9, p65, β-actin, and TBP in the cytosolic and nuclear fractions. *B* and *C*, cytosolic protein expression was normalized to β-actin levels, and nuclear protein expression was normalized to TBP levels. Relative protein levels are displayed as the means ± S.E. (*error bars*) ($n = 5$). * indicates a statistical difference ($p < 0.05$) compared with βTC3 cells treated without cytokines.

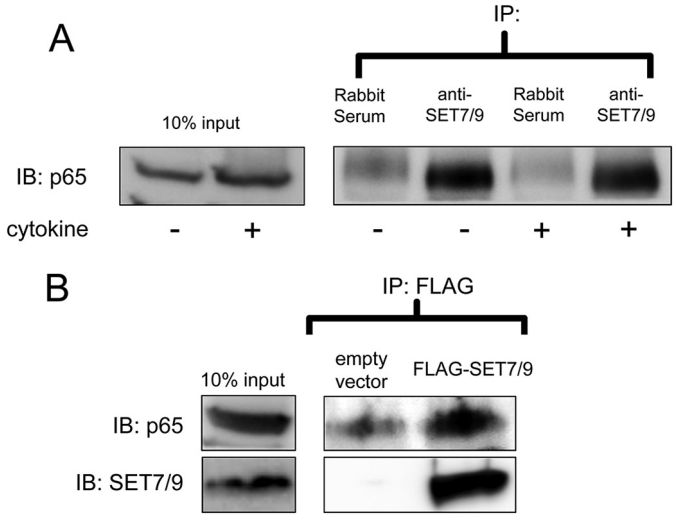


FIGURE 5. **SET7/9 forms a complex with p65.** *A*, after stimulation with or without cytokines for 4 h, βTC3 cells were harvested and immunoprecipitated with either rabbit serum or SET7/9 antiserum. Immunoprecipitated proteins were subjected to immunoblotting (*IB*) using a p65 antibody. *B*, βTC3 cells were transfected with either an empty vector or a vector encoding FLAG-SET7/9. Cells were harvested and immunoprecipitated (*IP*) with anti-FLAG antibody. Elutes were analyzed with antiserum for p65 or SET7/9. n = at least 3 independent experiments.

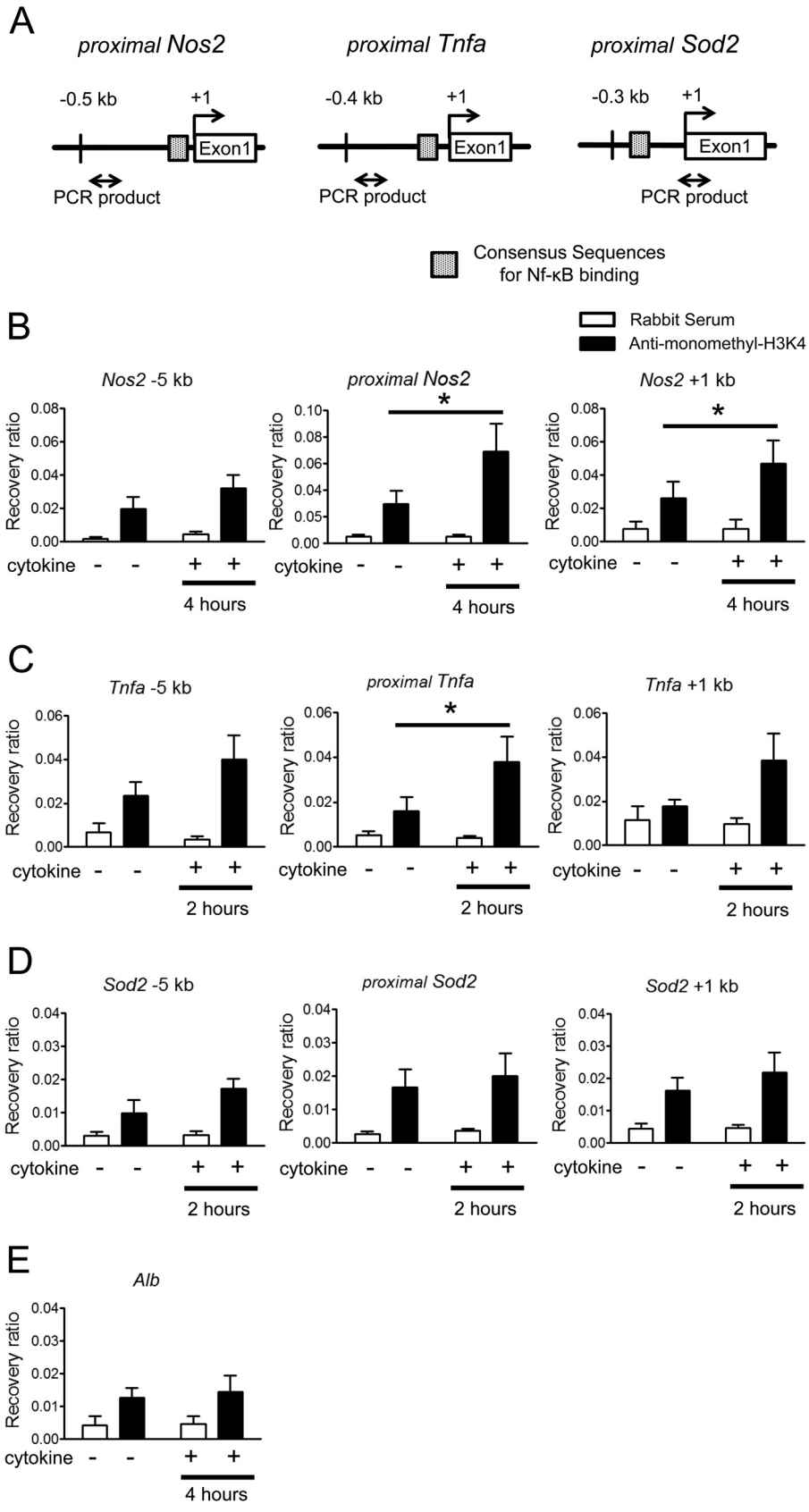
exhibited significant augmentation of *Nos2* expression, and this was partially attenuated in islets isolated from SET7/9 knock-out mice. In contrast, expression of *Tnfa* and *Sod2* was not different in wild-type and SET7/9 knock-out islets, consistent with results in MIN6 cells (Figs. 1*B* and 9*B*).

Discussion

SET7/9 is a monomethyltransferase that maintains euchromatin architecture through modification of H3K4 (16). We have previously demonstrated a role for SET7/9 in the regulation of H3K4 methylation at β cell-specific genes, including *Ins1* and *Slc2a2* (22). Several groups have also recently suggested a specific role for SET7/9 in inflammatory signaling in non-β cells wherein SET7/9 has been shown to physically interact with components of the NF-κB complex, undergo recruitment to specific NF-κB targets, and regulate target gene transcription (24–26). Inflammation is increasingly appreciated as a key contributor to islet β cell death and dysfunction in both T1D and T2D; however, the role of SET7/9 in β cell inflammatory signaling pathways has not been defined. In this study, we tested the hypothesis that SET7/9 modifies targets of NF-κB in the β cell. Our results showed that SET7/9 forms a complex with the p65 subunit of NF-κB and translocates into the nucleus to increase inflammatory gene transcription through H3K4 mono- and dimethylation of the *Nos2* promoter. SET7/9 also regulated cytokine-induced β cell apoptosis, whereas cytokine-induced *Nos2* expression was attenuated in islets isolated from SET7/9 knock-out mice.

Several groups have shown that SET7/9 also recognizes nonhistone protein substrates such that SET7/9 methylation may modulate both protein activity and/or stability. The p65 subunit of NF-κB contains several lysine residues that could serve as potential SET7/9 targets. In this regard, Ea and Baltimore (25) reported previously that SET7/9 methylation of the Lys-37 residue of p65 led to increased NF-κB-dependent gene expression. In contrast, Yang *et al.* (26) demonstrated that SET7/9-mediated methylation of the Lys-314 and Lys-315 residues of p65 triggers its ubiquitination and degradation, leading to decreased NF-κB function. To test the possibility that SET7/9 regulates inflammatory signaling in the β cell through p65 methylation, we endeavored to detect methylated lysine in immunoprecipitated p65 using an anti-pan-methylated lysine antibody. Using this approach, we could not find evidence that SET7/9 directly methylates p65 protein in βTC3 cells (data not shown). This finding is consistent with studies performed in monocytes demonstrating a primary role for SET7/9-mediated methylation of H3K4 at NF-κB gene targets (24). These discrepancies may certainly arise from differences in cell type or from the use of different provocative stimuli. For example, although HEK293 cells have the necessary machinery to produce COX-2 in response to proinflammatory cytokines, they fail to produce iNOS (38). Therefore, we cannot exclude the possibility that p65 methylation might contribute in part to cytokine-induced *Nos2* expression; mass spectrometric approaches could be used to investigate this in future studies.

Whereas the treatment of β cells with cytokines up-regulated the expression of a number of NF-κB target genes, including *Nos2*, *Tnfa*, and *Sod2*, deletion of SET7/9 uniquely attenuated


A
proximal Nos2
proximal Tnfa
proximal Sod2
-0.5 kb
+1
Exon1
PCR product
-0.4 kb
-0.3 kb
Consensus Sequences for Nf-κB binding
B
Rabbit Serum
Anti-monomethyl-H3K4
Nos2 -5 kb
proximal Nos2
Nos2 +1 kb
Recovery ratio
cytokine
4 hours
C
Tnfa -5 kb
proximal Tnfa
Tnfa +1 kb
2 hours
D
Sod2 -5 kb
proximal Sod2
Sod2 +1 kb
E
Alb

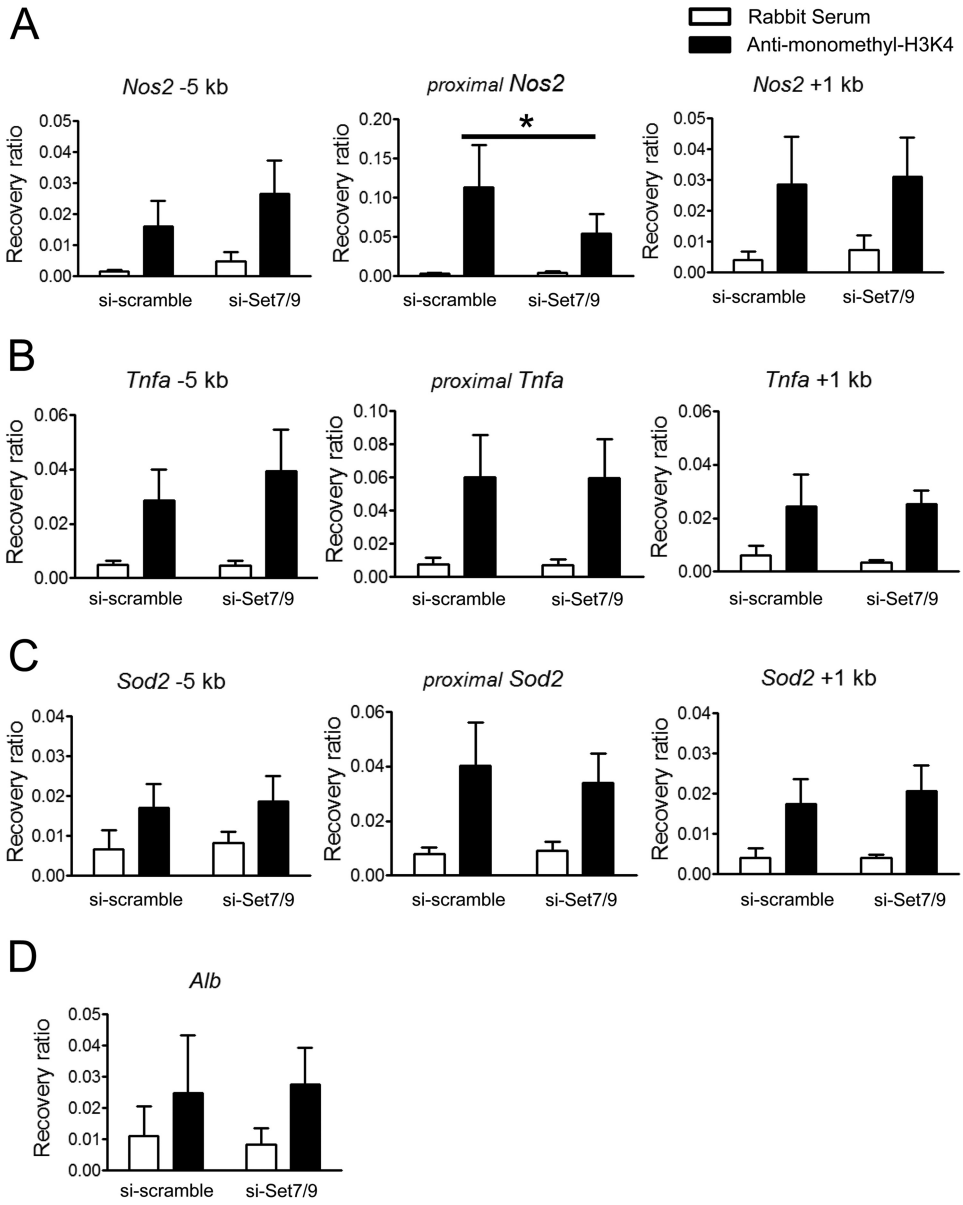


FIGURE 7. **SET7/9 knockdown inhibits cytokine-induced augmentation of H3K4 monomethylation of the *Nos2* gene.** βTC3 cells were transfected with si-scramble or si-Set7/9 and then treated with cytokines. ChIP assays were performed using an antibody for H3K4 monomethylation or normal rabbit serum. ChIP-enriched samples were analyzed by quantitative PCR using primers that amplified the proximal promoter region and the region −5 and +1 kb from the transcriptional start site of the *Nos2*, *Tnfa*, and *Sod2* genes. The proximal promoter region of the *Alb* gene was used as a negative control. Data represent the recovery ratio to input level, and n = at least 6 for each panel. *indicates statistical difference ($p < 0.05$) compared with βTC3 cells transfected with si-scramble. Results are displayed as the means ± S.E. (*error bars*).

the expression of *Nos2* in islets and cell lines. In agreement with these results, SET7/9 knockdown decreased methylation of H3K4 at the proximal promoter region of *Nos2* but not *Tnfa* or *Sod2*. Interestingly, the time frame of peak cytokine-induced up-regulation was different between each gene. For example, *Nos2* expression peaked about 4 h after cytokine treatment, whereas the maximal change in expression of *Tnfa* and *Sod2* was noted after 2 h after cytokine exposure. These results suggest that mechanisms regulating the expression of cytokine-responsive genes may be different. Indeed, methyltransferases other than SET7/9 may control chromatin architecture in the promoters of *Tnfa* and *Sod2*, and this could likewise be tested in future studies.

FIGURE 6. **Cytokine treatment augments H3K4 methylation of the *Nos2* gene.** *A*, schematic depiction of the proximal regions of the *Nos2*, *Tnfa*, and *Sod2* genes. The transcriptional start site of each gene is indicated as *+1*. The *arrows* indicate regions amplified by quantitative PCR in each ChIP assay. The *gray boxes* indicate consensus sequences for NF-κB binding. *B–E*, βTC3 cells were treated with or without cytokines for the indicated times, and ChIP assays were performed using an antibody for H3K4 monomethylation or normal rabbit serum. ChIP-enriched samples were analyzed by quantitative PCR using primers that amplified the proximal promoter region and the region −5 and +1 kb from the transcriptional start site of the *Nos2*, *Tnfa*, and *Sod2* genes. The proximal promoter region of the *Alb* gene was used as a negative control. Data represent the recovery ratio to input level, and n = at least 6 for each panel. * indicates statistical difference ($p < 0.05$) compared with βTC3 cells treated without cytokines. Results are displayed as the means ± S.E. (*error bars*).

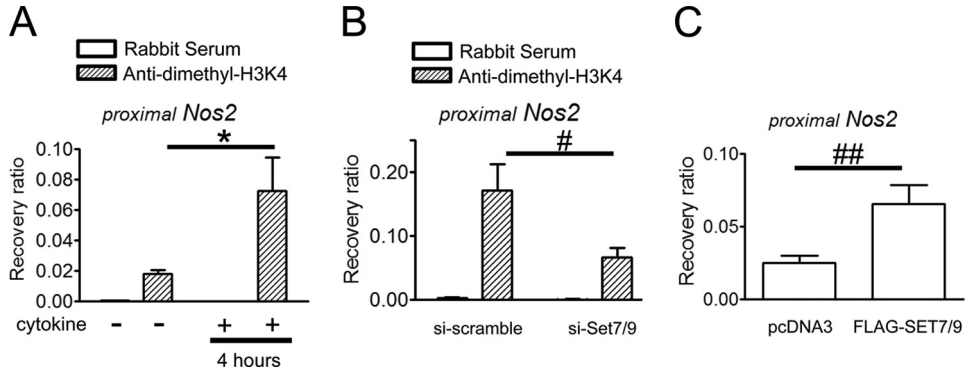


FIGURE 8. **SET7/9 knockdown inhibits cytokine-induced H3K4 dimethylation of the *Nos2* gene.** *A*, βTC3 cells were treated with or without cytokines for 4 h and subjected to ChIP assay using an antibody against dimethylated H3K4 or normal rabbit serum. ChIP-enriched samples were analyzed by quantitative PCR using primers that amplified the proximal promoter region of the *Nos2* promoter. *B*, βTC3 cells were transfected with si-scramble or si-Set7/9 and then treated with cytokines for 4 h. ChIP assays were performed using an antibody against dimethylated H3K4 or normal rabbit serum. *C*, following transfection with pcDNA3 or an expression vector encoding FLAG-SET7/9, βTC3 cells were treated with cytokines for 2 h, and ChIP assays were performed using an anti-FLAG antibody. Data represent the recovery ratio to input level, and n = at least 6 for each panel. * indicates statistical difference ($p < 0.05$) compared with βTC3 cells treated without cytokines. # indicates statistical difference ($p < 0.05$) compared with βTC3 cells transfected with si-scramble. ## indicates statistical difference ($p < 0.05$) compared with βTC3 cells transfected with pcDNA3. Results are displayed as the means ± S.E. (*error bars*).

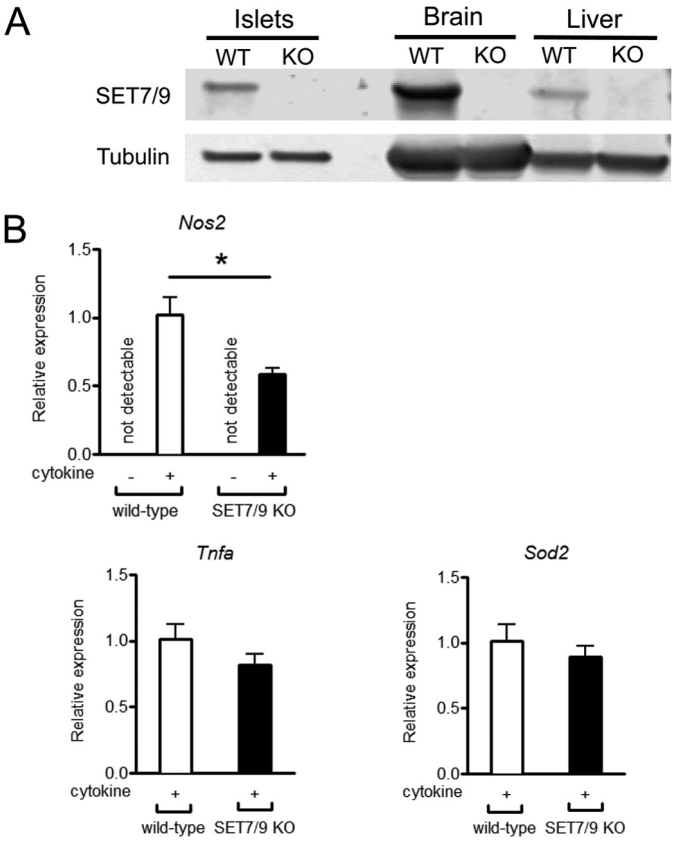


FIGURE 9. **Cytokine-induced *Nos2* expression is decreased in islets isolated from SET7/9 knock-out mice.** *A*, total protein was isolated from brain, liver, and islets from SET7/9 knock-out mice or wild-type controls and then subjected to immunoblotting for SET7/9 and tubulin. *B*, isolated islets from SET7/9 knock-out or wild-type mice were stimulated with or without cytokines for 4 h. Total RNA was isolated, and the expression of *Nos2*, *Tnfa*, and *Sod2* mRNA was determined by qRT-PCR and normalized to *Tbp* expression. Data represent the average of three independent experiments. * indicates statistical difference ($p < 0.05$) compared with islets isolated from wild-type mice. Results are displayed as the means ± S.E. (*error bars*).

Notwithstanding these limitations, our data show that SET7/9 knockdown attenuates cytokine-induced apoptosis in a β cell line. NO production by iNOS leads to a number of detrimental actions in the β cell, including mitochondrial dysregulation, Fas activation, dysregulated endoplasmic reticulum calcium, and activation of proapoptotic endoplasmic reticulum stress responses (8–10, 39–42). SET7/9 modulation of p53 signaling could contribute to this effect as p53 has been shown to be a protein target of SET7/9 in an osteosarcoma cell line (19, 32). Methylated p53 is stabilized and remains localized in the nucleus, preserving expression of downstream targets. We evaluated the expression of p53 and its target genes in βTC3 cells, including *Cdkn1a*, *Pten*, *Tnfrsf6*, and *Bax*. However, no alterations in their expression levels were observed with SET7/9 knockdown, suggesting that p53 methylation does not play a primary role in cytokine-induced apoptosis in our system.

Therefore, we propose a model for the role of SET7/9 in the regulation of *Nos2* expression in Fig. 10. As part of this model, SET7/9 binds p65 and is translocated into the nucleus in response to cytokine treatment. SET7/9 is recruited to the *Nos2* gene promoter where it methylates H3K4, induces an open chromatin conformation, and facilitates proinflammatory gene transcription. Previous studies have shown that SET7/9 has a variety of different targets, suggesting that SET7/9 could exert control of distinct pathways in the β cell depending on available binding partners. Previous studies have shown that SET7/9 forms a complex with PDX1 to induce euchromatin at genes related to glucose-stimulated insulin secretion (43, 44). Whereas SET7/9 maintains β cell function through activation of *Ins1* and *Slc2a2* via this pathway, SET7/9 also modulates the inflammatory response to cytokine signaling through an interaction with p65 and H3K4 methylation of the *Nos2* promoter. Whether SET7/9 should be activated or inhibited may therefore depend on the cellular context and prevailing conditions. A potential weakness of our study is that we were not able to recapitulate effects on apoptosis in SET7/9-deficient islets (data not shown), suggesting that germ line deletion of SET7/9 in a mouse model may not fully recapitulate the effects of acute SET7/9 knockdown. However, additional studies are needed to fully understand the therapeutic potential of targeting SET7/9

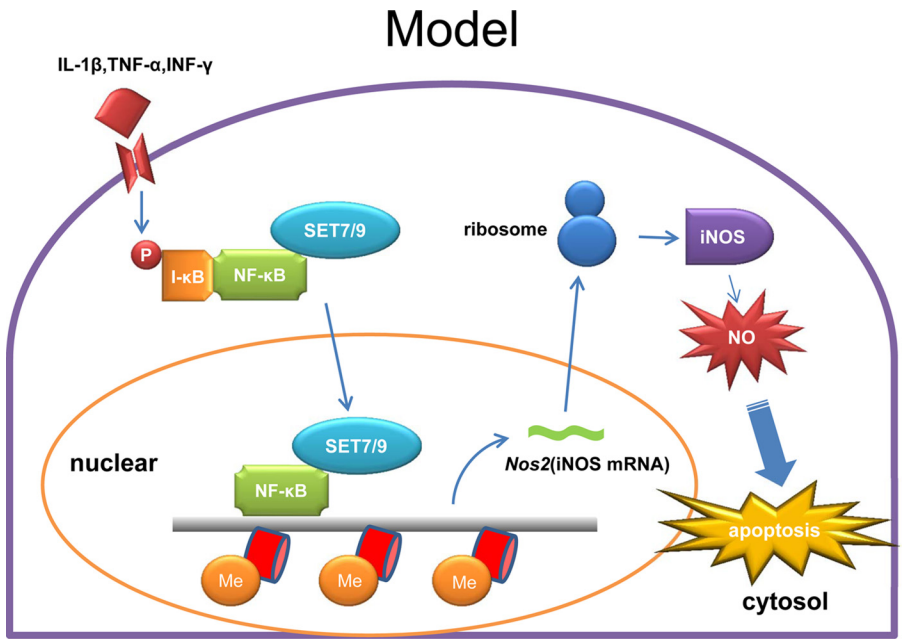


FIGURE 10. **An overall model describing SET7/9-mediated regulation of *Nos2* expression.** Within the pancreatic β cell, SET7/9 forms a complex with the p65 subunit of NF-κB. Proinflammatory cytokine treatment induces nuclear translocation of p65 and SET7/9 and recruitment of SET7/9 to the *Nos2* promoter. SET7/9 augments mono- and dimethylation of H3K4 in the *Nos2* gene, leading to increased iNOS expression and cytokine-induced apoptosis in the β cell.

as a means to regulate β cell inflammation in either T1D or T2D. Such studies may be facilitated by recent efforts to identify small molecule inhibitors of SET7/9 (45).

Author Contributions—T. O., C. E.-M., and H. W. designed the study. K. F., D. L. M., and H. I. performed and analyzed the experiments. H. O. contributed to the establishment of the SET7/9 knockout mouse. T. O., C. E.-M., H. W., Y. F., H. O., and R. G. M. analyzed the data. K. F., T. O., C. E.-M., and H. W. wrote the paper. All authors reviewed and approved the final version of the manuscript.

Acknowledgments—We thank Dr. Danny Reinberg (New York University) for establishing and providing Setd7-deficient mice. We gratefully acknowledge Naoko Daimaru and Hiroko Hibino for assistance of experiments.

References

1. Roep, B. O., and Tree, T. I. (2014) Immune modulation in humans: implications for type 1 diabetes mellitus. *Nat. Rev. Endocrinol.* **10,** 229–242
2. Eizirik, D. L., and Mandrup-Poulsen, T. (2001) A choice of death—the signal-transduction of immune-mediated β-cell apoptosis. *Diabetologia* **44,** 2115–2133
3. Donath, M. Y., Dalmas, É., Sauter, N. S., and Böni-Schnetzler, M. (2013) Inflammation in obesity and diabetes: islet dysfunction and therapeutic opportunity. *Cell Metab.* **17,** 860–872
4. Ehses, J. A., Perren, A., Eppler, E., Ribaux, P., Pospisilik, J. A., Maor-Cahn, R., Gueripel, X., Ellingsgaard, H., Schneider, M. K., Biollaz, G., Fontana, A., Reinecke, M., Homo-Delarche, F., and Donath, M. Y. (2007) Increased number of islet-associated macrophages in type 2 diabetes. *Diabetes* **56,** 2356–2370
5. Richardson, S. J., Willcox, A., Bone, A. J., Foulis, A. K., and Morgan, N. G. (2009) Islet-associated macrophages in type 2 diabetes. *Diabetologia* **52,** 1686–1688
6. Gysemans, C., Callewaert, H., Overbergh, L., and Mathieu, C. (2008) Cytokine signalling in the β-cell: a dual role for IFNγ. *Biochem. Soc. Trans.* **36,** 328–333
7. Eizirik, D. L., Colli, M. L., and Ortis, F. (2009) The role of inflammation in insulitis and β-cell loss in type 1 diabetes. *Nat. Rev. Endocrinol.* **5,** 219–226
8. Eizirik, D. L., Sandler, S., Hallberg, A., Bendtzen, K., Sener, A., and Malaisse, W. J. (1989) Differential sensitivity to β-cell secretagogues in cultured rat pancreatic islets exposed to human interleukin-1β. *Endocrinology* **125,** 752–759
9. Corbett, J. A., Wang, J. L., Hughes, J. H., Wolf, B. A., Sweetland, M. A., Lancaster, J. R., Jr., and McDaniel, M. L. (1992) Nitric oxide and cyclic GMP formation induced by interleukin 1β in islets of Langerhans. Evidence for an effector role of nitric oxide in islet dysfunction. *Biochem. J.* **287,** 229–235
10. Corbett, J. A., Wang, J. L., Sweetland, M. A., Lancaster, J. R., Jr., and McDaniel, M. L. (1992) Interleukin 1β induces the formation of nitric oxide by β-cells purified from rodent islets of Langerhans. Evidence for the β-cell as a source and site of action of nitric oxide. *J. Clin. Investig.* **90,** 2384–2391
11. Comens, P. G., Wolf, B. A., Unanue, E. R., Lacy, P. E., and McDaniel, M. L. (1987) Interleukin 1 is potent modulator of insulin secretion from isolated rat islets of Langerhans. *Diabetes* **36,** 963–970
12. Corbett, J. A., Sweetland, M. A., Wang, J. L., Lancaster, J. R., Jr., and McDaniel, M. L. (1993) Nitric oxide mediates cytokine-induced inhibition of insulin secretion by human islets of Langerhans. *Proc. Natl. Acad. Sci. U.S.A.* **90,** 1731–1735
13. Shimabukuro, M., Ohneda, M., Lee, Y., and Unger, R. H. (1997) Role of nitric oxide in obesity-induced β cell disease. *J. Clin. Investig.* **100,** 290–295
14. Li, B., Carey, M., and Workman, J. L. (2007) The role of chromatin during transcription. *Cell* **128,** 707–719
15. Berger, S. L. (2007) The complex language of chromatin regulation during transcription. *Nature* **447,** 407–412
16. Nishioka, K., Chuikov, S., Sarma, K., Erdjument-Bromage, H., Allis, C. D., Tempst, P., and Reinberg, D. (2002) Set9, a novel histone H3 methyltransferase that facilitates transcription by precluding histone tail modifications required for heterochromatin formation. *Genes Dev.* **16,** 479–489
17. Kouskouti, A., Scheer, E., Staub, A., Tora, L., and Talianidis, I. (2004) Gene-specific modulation of TAF10 function by SET9-mediated methylation. *Mol. Cell* **14,** 175–182
18. Munro, S., Khaire, N., Inche, A., Carr, S., and La Thangue, N. B. (2010) Lysine methylation regulates the pRb tumour suppressor protein. *Oncogene* **29,** 2357–2367

19. Chuikov, S., Kurash, J. K., Wilson, J. R., Xiao, B., Justin, N., Ivanov, G. S., McKinney, K., Tempst, P., Prives, C., Gamblin, S. J., Barlev, N. A., and Reinberg, D. (2004) Regulation of p53 activity through lysine methylation. *Nature* **432,** 353–360
20. Subramanian, K., Jia, D., Kapoor-Vazirani, P., Powell, D. R., Collins, R. E., Sharma, D., Peng, J., Cheng, X., and Vertino, P. M. (2008) Regulation of estrogen receptor α by the SET7 lysine methyltransferase. *Mol. Cell* **30,** 336–347
21. Ko, S., Ahn, J., Song, C. S., Kim, S., Knapczyk-Stwora, K., and Chatterjee, B. (2011) Lysine methylation and functional modulation of androgen receptor by Set9 methyltransferase. *Mol. Endocrinol* **25,** 433–444
22. Deering, T. G., Ogihara, T., Trace, A. P., Maier, B., and Mirmira, R. G. (2009) Methyltransferase Set7/9 maintains transcription and euchromatin structure at islet-enriched genes. *Diabetes* **58,** 185–193
23. Maganti, A. V., Maier, B., Tersey, S. A., Sampley, M. L., Mosley, A. L., Özcan, S., Pachaiyappan, B., Woster, P. M., Hunter, C. S., Stein, R., and Mirmira, R. G. (2015) Transcriptional activity of the islet β cell factor Pdx1 is augmented by lysine methylation catalyzed by the methyltransferase Set7/9. *J. Biol. Chem.* **290,** 9812–9822
24. Li, Y., Reddy, M. A., Miao, F., Shanmugam, N., Yee, J. K., Hawkins, D., Ren, B., and Natarajan, R. (2008) Role of the histone H3 lysine 4 methyltransferase, SET7/9, in the regulation of NF-κB-dependent inflammatory genes. Relevance to diabetes and inflammation. *J. Biol. Chem.* **283,** 26771–26781
25. Ea, C. K., and Baltimore, D. (2009) Regulation of NF-κB activity through lysine monomethylation of p65. *Proc. Natl. Acad. Sci. U.S.A.* **106,** 18972–18977
26. Yang, X. D., Huang, B., Li, M., Lamb, A., Kelleher, N. L., and Chen, L. F. (2009) Negative regulation of NF-λB action by Set9-mediated lysine methylation of the RelA subunit. *EMBO J.* **28,** 1055–1066
27. Sadowski, H. B., Shuai, K., Darnell, J. E., Jr., and Gilman, M. Z. (1993) A common nuclear signal transduction pathway activated by growth factor and cytokine receptors. *Science* **261,** 1739–1744
28. Chakrabarti, S. K., James, J. C., and Mirmira, R. G. (2002) Quantitative assessment of gene targeting *in vitro* and *in vivo* by the pancreatic transcription factor, Pdx1. Importance of chromatin structure in directing promoter binding. *J. Biol. Chem.* **277,** 13286–13293
29. Stull, N. D., Breite, A., McCarthy, R., Tersey, S. A., and Mirmira, R. G. (2012) Mouse islet of Langerhans isolation using a combination of purified collagenase and neutral protease. *J. Vis. Exp.* **67,** e4137
30. Eizirik, D. L., Cagliero, E., Björklund, A., and Welsh, N. (1992) Interleukin-1β induces the expression of an isoform of nitric oxide synthase in insulin-producing cells, which is similar to that observed in activated macrophages. *FEBS Lett.* **308,** 249–252
31. Corbett, J. A., Kwon, G., Misko, T. P., Rodi, C. P., and McDaniel, M. L. (1994) Tyrosine kinase involvement in IL-1β-induced expression of iNOS by β-cells purified from islets of Langerhans. *Am. J. Physiol. Cell Physiol.* **267,** C48–C54
32. Ivanov, G. S., Ivanova, T., Kurash, J., Ivanov, A., Chuikov, S., Gizatullin, F., Herrera-Medina, E. M., Rauscher, F., 3rd, Reinberg, D., and Barlev, N. A. (2007) Methylation-acetylation interplay activates p53 in response to DNA damage. *Mol. Cell. Biol.* **27,** 6756–6769
33. Evans-Molina, C., Robbins, R. D., Kono, T., Tersey, S. A., Vestermark, G. L., Nunemaker, C. S., Garmey, J. C., Deering, T. G., Keller, S. R., Maier, B., and Mirmira, R. G. (2009) Peroxisome proliferator-activated receptor γ activation restores islet function in diabetic mice through reduction of endoplasmic reticulum stress and maintenance of euchromatin structure. *Mol. Cell. Biol.* **29,** 2053–2067
34. Okabe, J., Orlowski, C., Balcerczyk, A., Tikellis, C., Thomas, M. C., Cooper, M. E., and El-Osta, A. (2012) Distinguishing hyperglycemic changes by Set7 in vascular endothelial cells. *Circ. Res.* **110,** 1067–1076
35. Black, J. C., Van Rechem, C., and Whetstine, J. R. (2012) Histone lysine methylation dynamics: establishment, regulation, and biological impact. *Mol. Cell* **48,** 491–507
36. Bernstein, B. E., Kamal, M., Lindblad-Toh, K., Bekiranov, S., Bailey, D. K., Huebert, D. J., McMahon, S., Karlsson, E. K., Kulbokas, E. J., 3rd, Gingeras, T. R., Schreiber, S. L., and Lander, E. S. (2005) Genomic maps and comparative analysis of histone modifications in human and mouse. *Cell* **120,** 169–181
37. Barski, A., Cuddapah, S., Cui, K., Roh, T. Y., Schones, D. E., Wang, Z., Wei, G., Chepelev, I., and Zhao, K. (2007) High-resolution profiling of histone methylations in the human genome. *Cell* **129,** 823–837
38. Xu, L., Han, C., Lim, K., and Wu, T. (2008) Activation of cytosolic phospholipase A2α through nitric oxide-induced *S*-nitrosylation. Involvement of inducible nitric-oxide synthase and cyclooxygenase-2. *J. Biol. Chem.* **283,** 3077–3087
39. Stassi, G., De Maria, R., Trucco, G., Rudert, W., Testi, R., Galluzzo, A., Giordano, C., and Trucco, M. (1997) Nitric oxide primes pancreatic β cells for Fas-mediated destruction in insulin-dependent diabetes mellitus. *J. Exp. Med.* **186,** 1193–1200
40. Kono, T., Ahn, G., Moss, D. R., Gann, L., Zarain-Herzberg, A., Nishiki, Y., Fueger, P. T., Ogihara, T., and Evans-Molina, C. (2012) PPAR-γ activation restores pancreatic islet SERCA2 levels and prevents β-cell dysfunction under conditions of hyperglycemic and cytokine stress. *Mol. Endocrinol.* **26,** 257–271
41. Chan, J. Y., Cooney, G. J., Biden, T. J., and Laybutt, D. R. (2011) Differential regulation of adaptive and apoptotic unfolded protein response signalling by cytokine-induced nitric oxide production in mouse pancreatic β cells. *Diabetologia* **54,** 1766–1776
42. Evans-Molina, C., Hatanaka, M., and Mirmira, R. G. (2013) Lost in translation: endoplasmic reticulum stress and the decline of β-cell health in diabetes mellitus. *Diabetes Obes. Metab.* **15,** Suppl. 3, 159–169
43. Francis, J., Chakrabarti, S. K., Garmey, J. C., and Mirmira, R. G. (2005) Pdx-1 links histone H3-Lys-4 methylation to RNA polymerase II elongation during activation of insulin transcription. *J. Biol. Chem.* **280,** 36244–36253
44. Ogihara, T., Vanderford, N. L., Maier, B., Stein, R. W., and Mirmira, R. G. (2009) Expression and function of Set7/9 in pancreatic islets. *Islets* **1,** 269–272
45. Barsyte-Lovejoy, D., Li, F., Oudhoff, M. J., Tatlock, J. H., Dong, A., Zeng, H., Wu, H., Freeman, S. A., Schapira, M., Senisterra, G. A., Kuznetsova, E., Marcellus, R., Allali-Hassani, A., Kennedy, S., Lambert, J. P., Couzens, A. L., Aman, A., Gingras, A. C., Al-Awar, R., Fish, P. V., Gerstenberger, B. S., Roberts, L., Benn, C. L., Grimley, R. L., Braam, M. J., Rossi, F. M., Sudol, M., Brown, P. J., Bunnage, M. E., Owen, D. R., Zaph, C., Vedadi, M., and Arrowsmith, C. H. (2014) (R)-PFI-2 is a potent and selective inhibitor of SETD7 methyltransferase activity in cells. *Proc. Natl. Acad. Sci. U.S.A.* **111,** 12853–12858