SUPPLEMENTARY IMFORMATION

Regulation of mouse steroidogenesis by WHISTLE and JMJD1C through histone methylation balance

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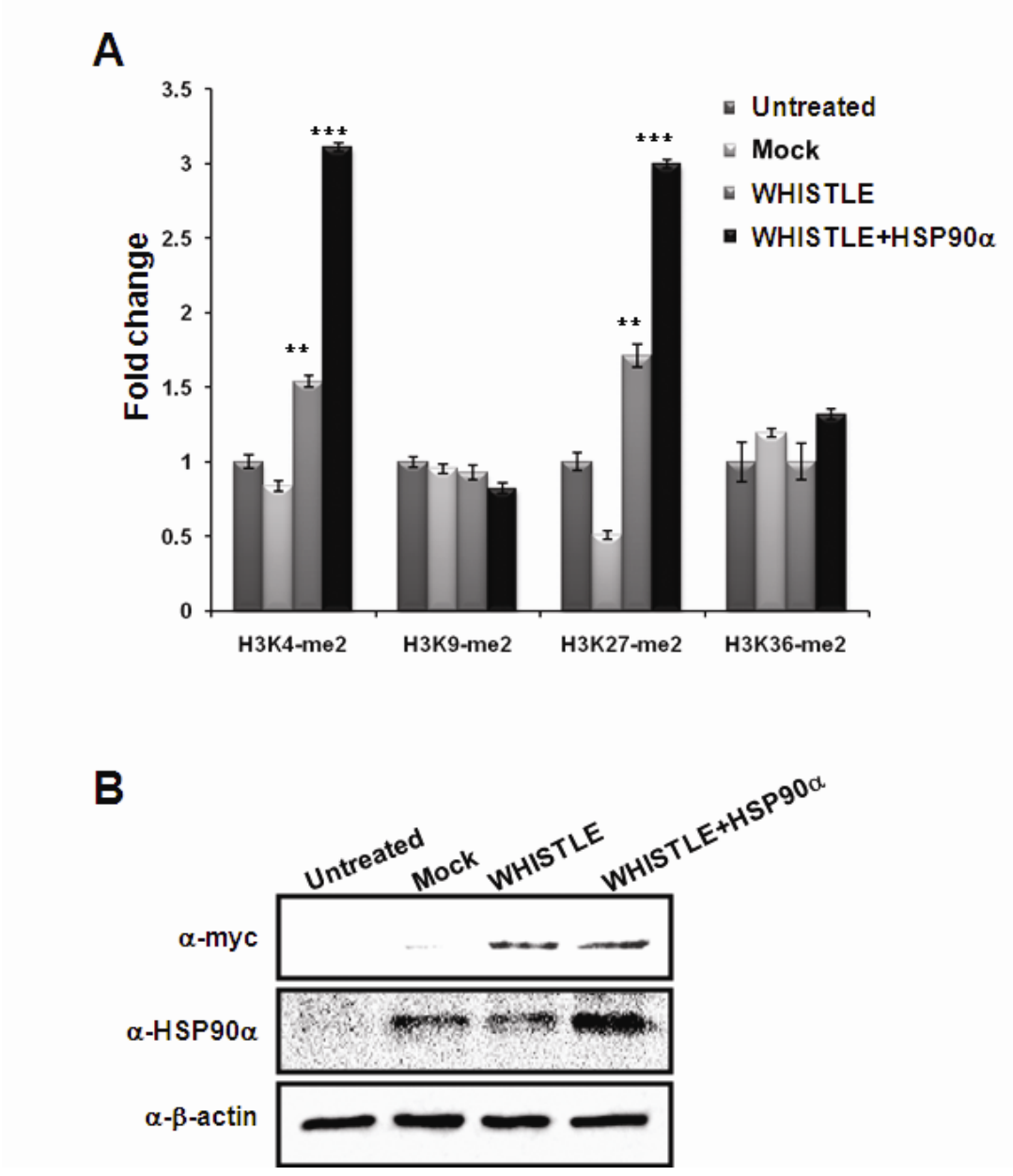
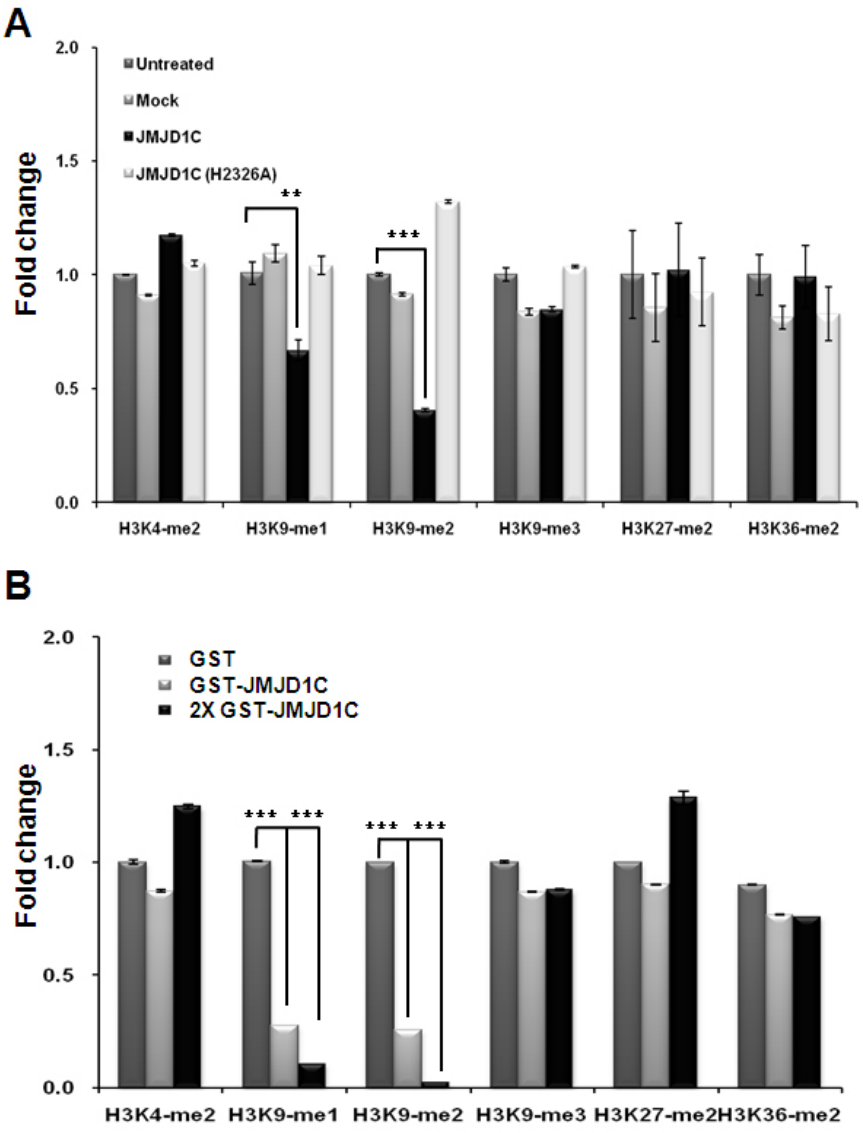
Supplementary Table S1

Protein	MW (kDa)	Cellular Function
AHNAK	680	keratinocyte plasma membrane-associated protein
JMJD1C	285	Histone demethylase
Pumilio	110	Regulator during early embryogenesis
BCL6	95	Transcriptional repressor during lymphocyte differentiation
HSP90α	90	Cellular chaperone
Lamin A	70	Fibrous proteins providing structural function and transcriptional regulation
DDX5	68	RNA helicase
Vimentin	57	Lymphocyte adhesion and transcellular migration
NAP1L4	52	Nucleosome assembly
hnRNP	42	Nuclear RNA-binding proteins
TGIF5	35	Homeobox protein
Histone H3	15	Component of Histone octamer
Histone H2A	14	Component of Histone octamer

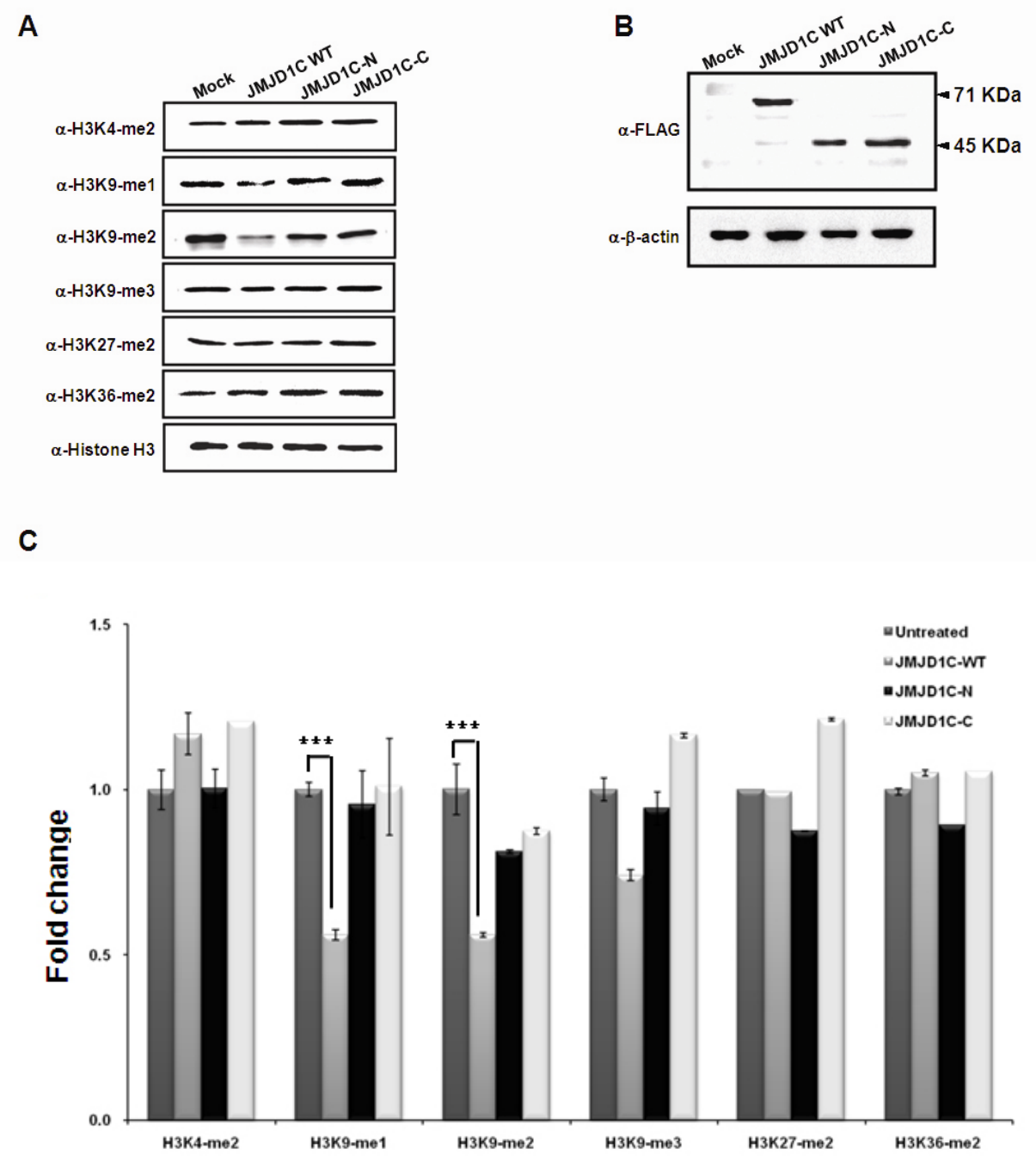
Supplementary Figure S1



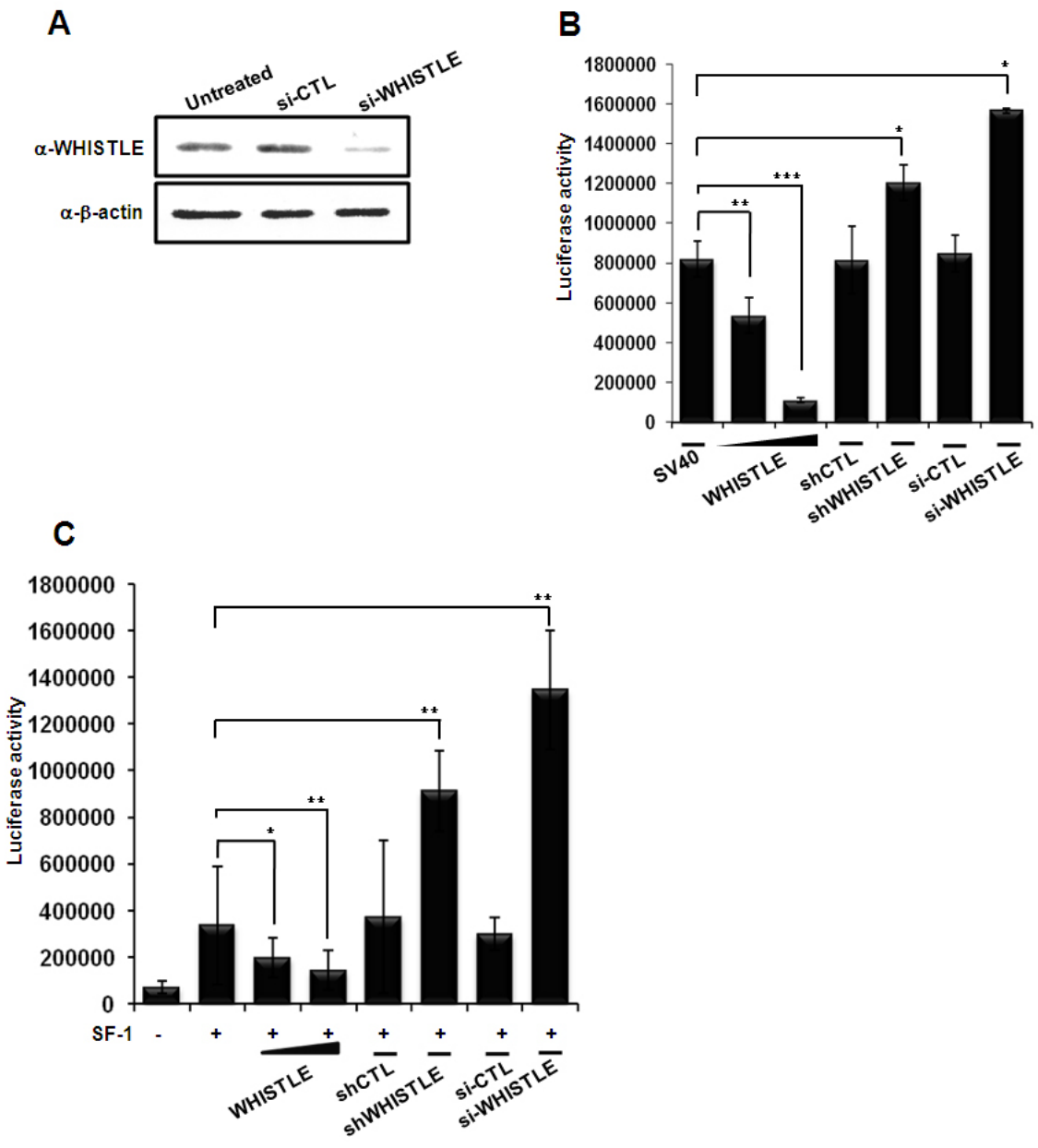
Supplementary Figure S2



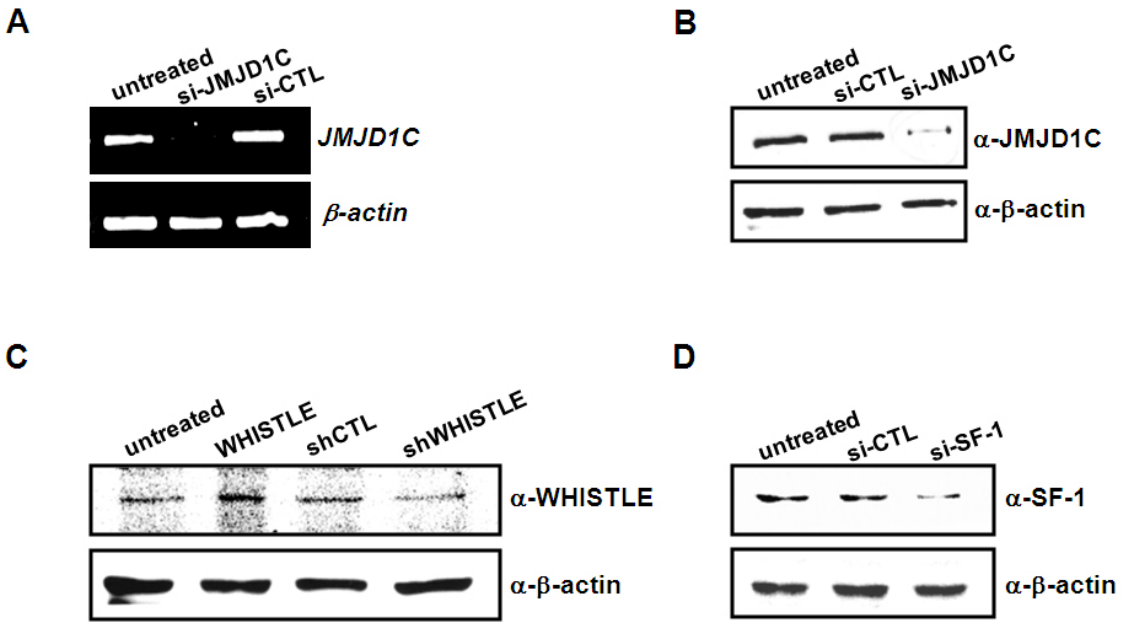
Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5



Supplementary Table and Figures Legends

Supplementary Table S1. Identified proteins in TAP purification. The partial list of WHISTLE interacting proteins identified by affinity purification with TAP system and LC-MS/MS.

Supplementary Figure S1. (**A**) *In vivo* HMTase assay immunoblot images (Fig. 2A) were analyzed quantitatively in bar graph by Image J program. Data were expressed as a fold change (relative to histone H3) and presented as means ± s.d of three independent experiments. $^{**}P < 0.01$ and $^{***}P < 0.001$, compared with histone H3 control. (**B**) Immunoblot analysis of the levels of transfected WHISTLE and HSP90α in NIH3T3 cells. Immunoblot analysis was performed using indicated antibodies.

Supplementary Figure S2. Quantification of demethylase activity of JMJD1C. (**A**) *In vivo* demethylase activites (Fig. 3B) were analyzed quantitatively in bar graph by Image J program. (**B**) *In vitro* demethylase activites (Fig. 3E) were analyzed quantitatively in bar graph by Image J program. Data were expressed as a fold change (relative to histone H3) and presented as means ± s.d of three independent experiments. $^{**}P < 0.01$ and $^{***}P < 0.001$, compared with histone H3 control.

Supplementary Figure S3. Demethylase activity with JMJD1C deletion mutants. (**A**) The deletion of the JmjC domain (JMJD1C-N) or ZF-like motif (JMJD1C-C) constructs were expressed in TM3 cells, and demethylation activities were analyzed via immunoblot analysis

using indicated antibodies. Anti-Histone H3 panel indicates the loading controls. (**B**) Immunoblot analysis of the expression levels of flag-JMJD1C WT, flag-JMJD1C-N and flag-JMJD1C-C. (**C**) Immunoblot images (Fig. S3A) were analyzed quantitatively in bar graph by Image J program. Data were expressed as a fold change (relative to Histone h3) and presented as means ± s.d of three independent experiments. ***$P < 0.001$, compared with histone H3 control.

Supplementary Figure S4. Regulatory effects of WHISTLE and si-WHISTLE on transcriptional activity of SF-1. (**A**) TM3 cells treated with si-WHSITLE were immunoblotted using anti-WHISTLE antibodies. (**B**) TM3 cells were transfected with pCMX-Gal4-SV40 and indicated DNA constructs and si-WHSITLE, and their cell extracts were assayed for luciferase activity. (**C**) TM3 cells were transfected with SF-1 and a three copy SF-1-RE-luc, and indicated DNA constructs and si-WHSITLE. All data are expressed as means ± s.d.; n = 5. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$, compared with untreated control.

Supplementary Figure S5. Immunoblot analysis with protein expression levels. (**A**) RT-PCR analysis of si-CTL and si-JMJD1C treated TM3 cells. (**B**) TM3 cells treated with si-JMJD1C were immunoblotted using anti-JMJD1C antibodies. (**C**) Immunoblot analysis of the levels of WHISTLE, shWHISTLE and sh-CTL transfected TM3 cells were shown. (**D**) TM3 cells treated with si-SF-1 and si-CTL were immunoblotted using anti-SF-1 antibodies.

SUPPLEMENTARY MATERIALS AND METHODS

Plasmids pFLAG-JMJD1C-C (ZF-like motif) and pFLAG-JMJD1C-N (JmjC domain) deletion mutant plasmids were genetated by PCR-based strategy. The siRNA against mouse WHISTLE (M-060989-01-0010) was purchased from Dharmacon.

Cell culture and transient transfections TM3 cells were maintained in DMEM medium supplemented with 5% fetal bovine serum (FBS) and 0.05 % antibiotics at 37 °C in a 5 % CO_2 atmosphere. For transient transfection, TM3 cells were seeded in 60 mm dishes with 5 X 10^5 numbers and transfected with pFLAG-JMJD1C, pFLAG-JMJD1C-N and pFLAG-JMJD1C-C using polyethylenimine (PEI).

RT-PCR

Total RNA samples from TM3 cells transfected with each indicated constructs and different mouse developmental stages were extracted by Trizol reagent (Invitrogen) according to the manufacture's instruction. Total RNA (1 μg) was used to synthesize the cDNA. The cDNA synthesis was primed with oligo-dT primer (Fermentas) and quantified cDNA was applied in of *JMJD1C* mRNA expression pattern analysis. The primer sequences were as follows ; JMJD1C : sense 5'-GAGGACTTCAAGGCC-3' and antisense 5'-AATTAGGTGTCTTCC-3'.