Supplemental Material

Supplemental Figure Legends

Supplemental Figure S1. ***2DG decreased ATP levels in living cells.*** MCF-7 cells were transfected with pcDNA-cATeam or pcDNA-mut cATeam. The signals for cellular ATP levels are shown in the presence or absence of 2 mM 2DG, as described in the Materials and Methods section.

Supplemental Figure S2. ***Compound C inhibited phosphorylation of ACC in cells.*** After pre-treatment with or without 10 μM compound C, an inhibitor of AMPK kinase activity, for 1 h, MCF-7 cells were cultured 2 h in the presence or absence of 2DG at the indicated concentrations, lysed, and analyzed by Western blotting with antibodies against phosphorylated (phosphorylated Ser-79) and total ACC. Although the phosphorylation of ACC was increased by 2DG treatments within 2 h, pretreatment with compound C abolished the increase of ACC phosphorylation. The positions of protein markers with defined molecular weights are indicated on the right side of the figure. The relative intensities of bands are shown under the panels.

Supplemental Figure S3. ***AMPK-specific siRNA knocks down AMPK.*** Three days after transfection of control (control) or AMPKα1/2 siRNA (siAMPK), MCF-7 cells were cultured 2 h in the presence or absence of 2DG at the indicated concentrations. Cells were lysed and analyzed by Western blotting to detect AMPKα and β-actin. The signals for AMPKα were reduced by the siRNA specific for AMPKα1/2. The relative

intensities of bands are shown under the panels.

Supplemental Figure S4. ***Compound C does not affect levels of H3K36me2 in rDNA promoter.*** MCF-7 cells were cultured 1 h in the presence or absence of 10 μM compound C, and ChIP analyses were performed to detect H3K36me2 (A), H3K36me3 (B), and KDM2A (C) in the rDNA promoter. The results of the histone marks were expressed % of input normalized by histone H3. The results of KDM2A were expressed as % of input. The results show that compound C did not affect the levels of H3K36me2, H3K36me3, and KDM2A in rDNA promoter. The primer used to detect the fragments of the rDNA promoter (H0; rDNA from +1 to +155 from the transcriptional start site) was previously described (1).

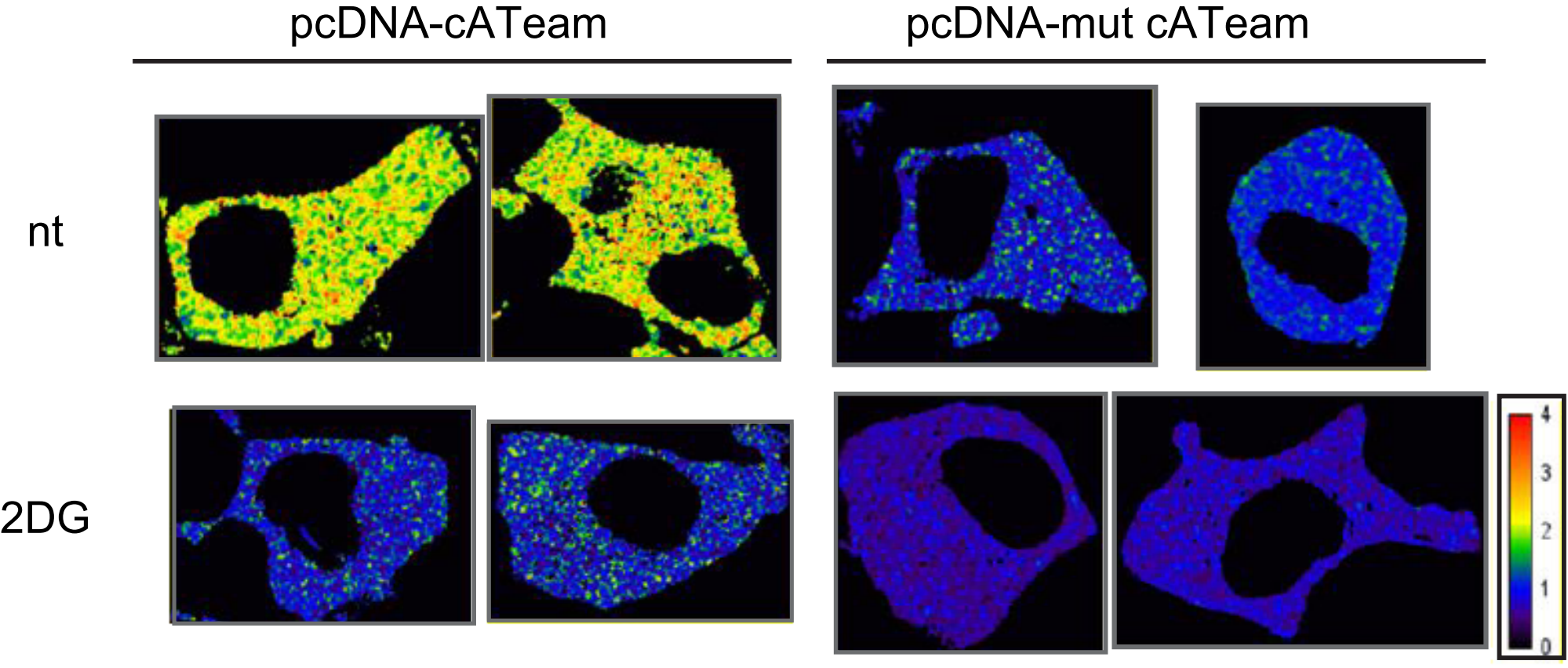
Supplemental Figure S5. ***TIF-IA is detected in rDNA promoter and compound C did not affect levels of TIF-IA in the rDNA promoter.*** MCF-7 cells were cultured 1 h in the presence or absence of 10 μM compound C and 2 mM 2DG, and ChIP analyses were performed to detect TIF-IA in the rDNA promoter (H0) and the rDNA gene body region (H13). The results were expressed as % of input. The primers used to detect the fragments of rDNA promoter (H0; rDNA from +1 to +155 from the transcriptional start site) and the gene body region of rDNA (H13; rDNA from +12885 to +12970 from the transcriptional start site) were described previously (1).

Supplemental Figure S6. ***Effects of a KDM2A knockdown and 2DG treatment on cellular ATP levels, phosphorylation of AMPK, and rRNA transcription.*** (A) Three days after transfection of control siRNA (control) or KDM2A siRNA (siKDM2A or

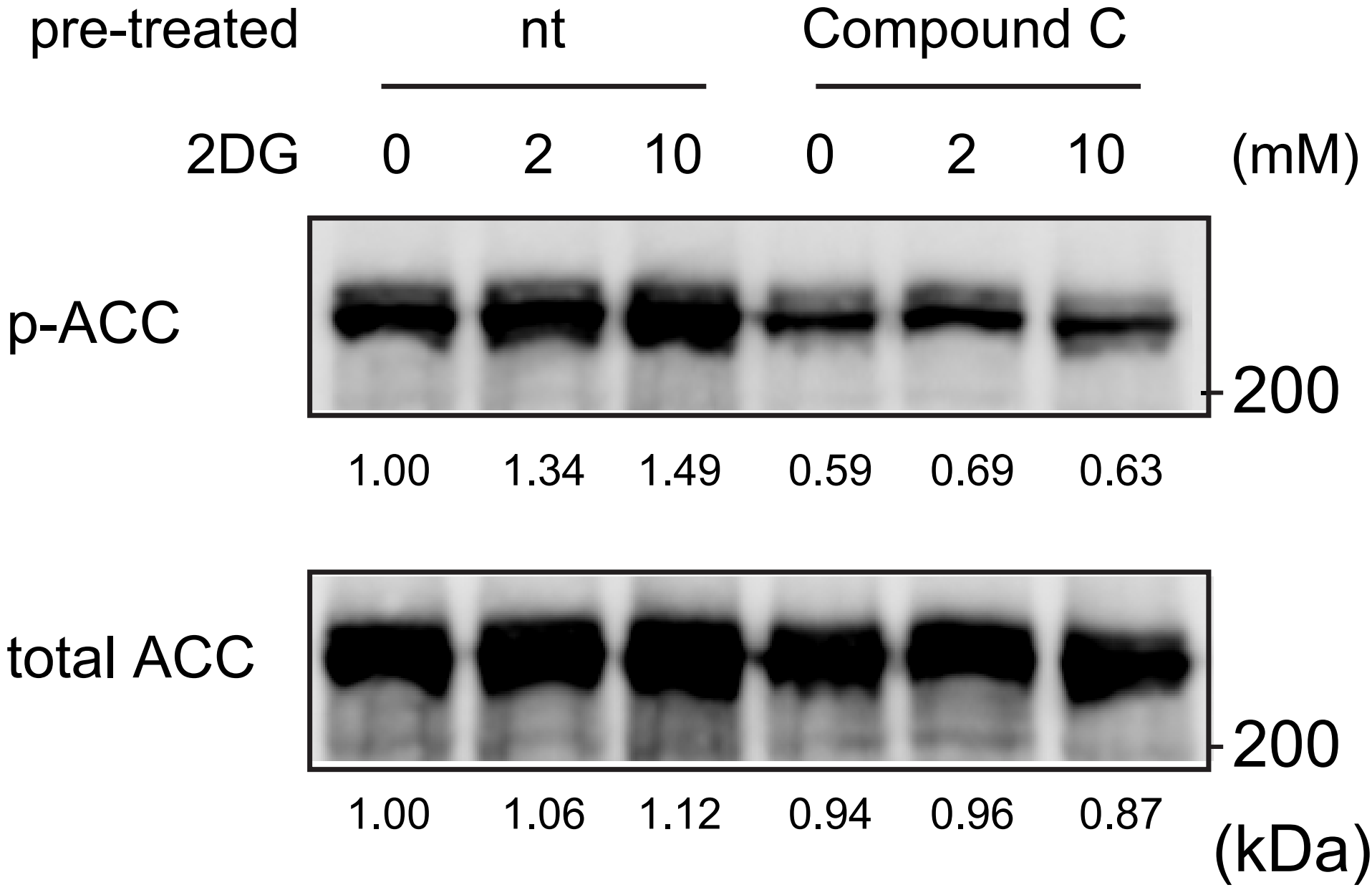
siKDM2A-w), cells were cultured 2 h in the presence of 2DG at the indicated concentrations, and the cellular ATP levels were detected by the luciferase assay system. The results were expressed as values relative to that with control siRNA in the absence of 2DG. The mean values with standard deviations (n=8) are indicated. *P<0.05. (B) Three days after transfection of siRNAs, cells were further cultured for 24 hours in the presence or absence of 2 mM 2DG and the cellular ATP levels were detected, and normalized by the DNA amounts detected with CyQUANT Direct Cell Proliferation Assay kit (Invitrogen #C35011) according to the manufacturer's instructions. (C) Phosphorylation of AMPK by treatment with 2DG. MCF-7 cells were cultured for the indicated hours in the presence or absence of 2 mM 2DG. Cells were lysed, and analyzed by Western blotting to detect phosphorylated AMPK (phosphorylated Thr-172), total AMPK. The relative intensities of bands are shown under the panels.

Reference for Supplemental Material

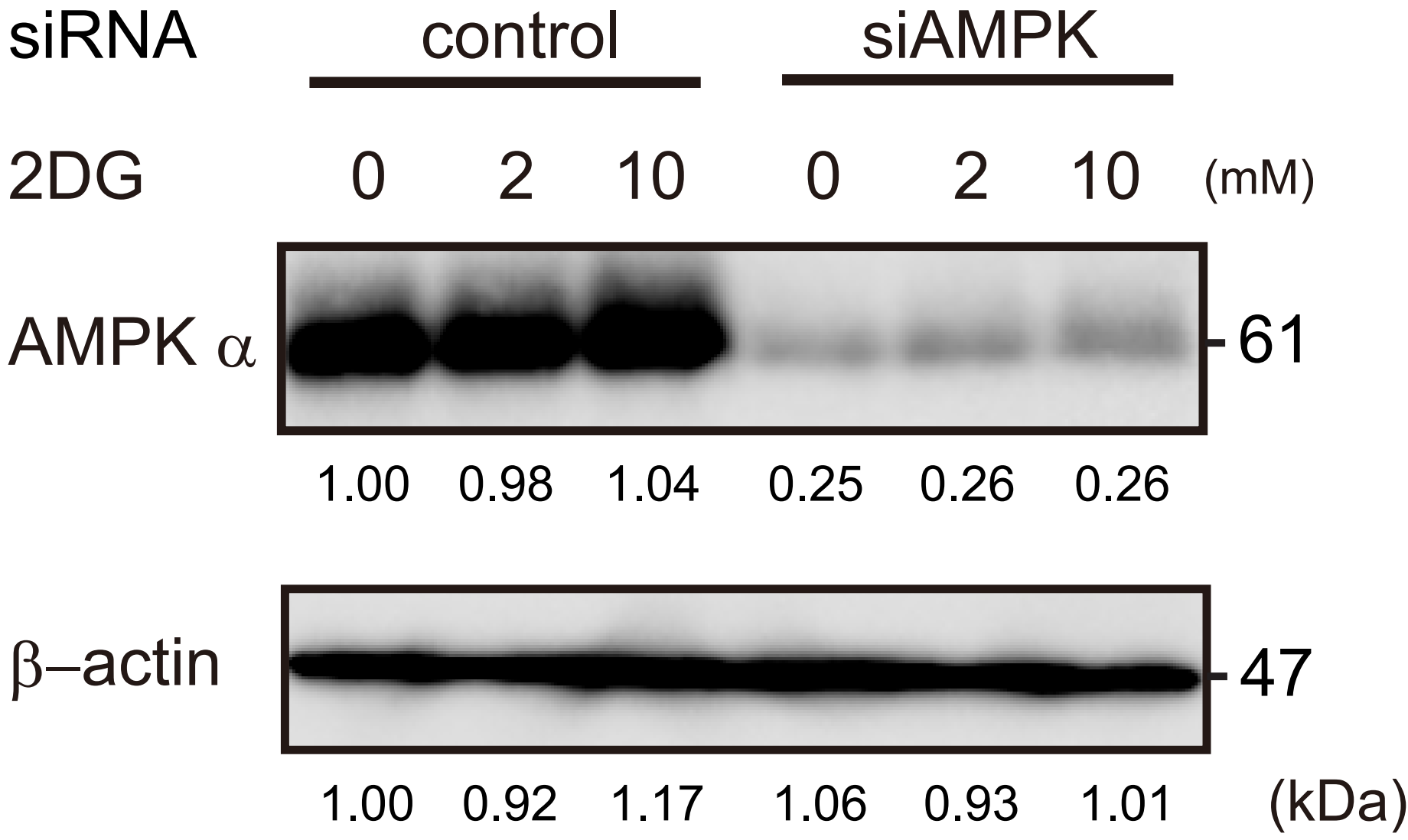
1. **Tanaka Y, Okamoto K, Teye K, Umata T, Yamagiwa N, Suto Y, Zhang Y, Tsuneoka M.** JmjC enzyme KDM2A is a regulator of rRNA transcription in response to starvation. EMBO J **29**:1510-1522., 2010.



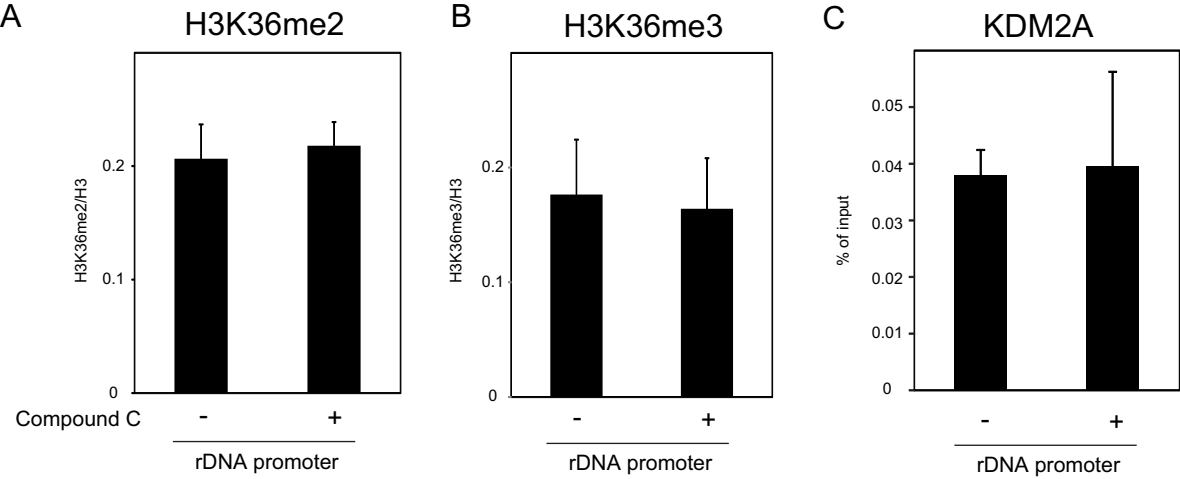
Supplemental Figure S1



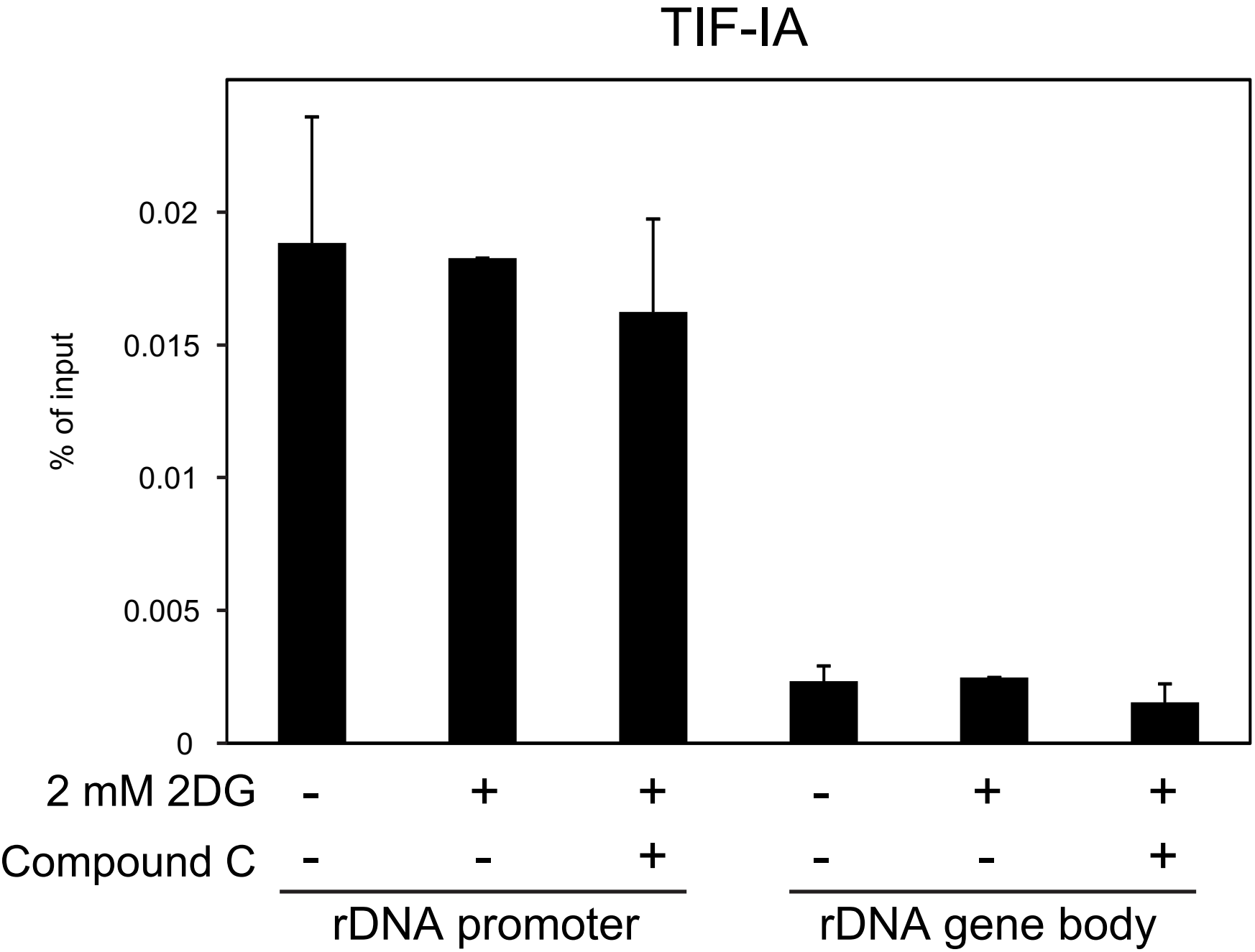
Supplemental Figure S2



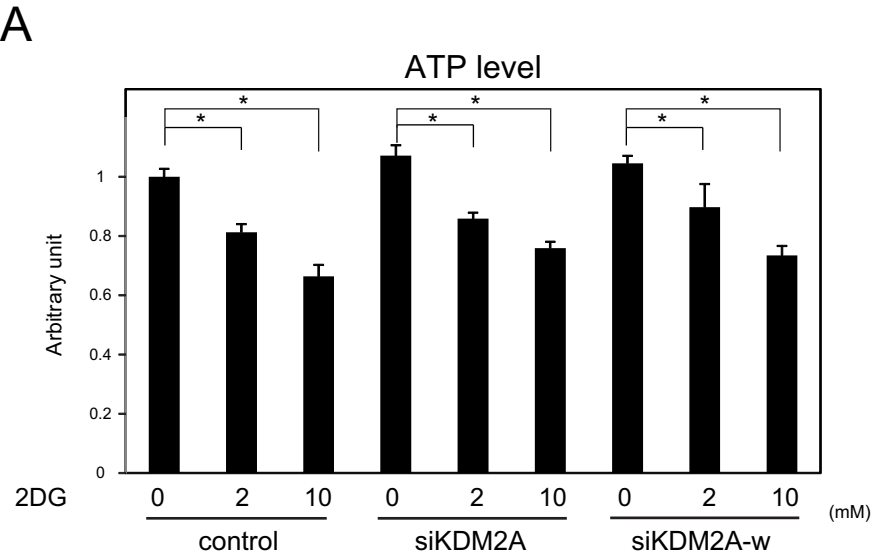
Supplemental Figure S3

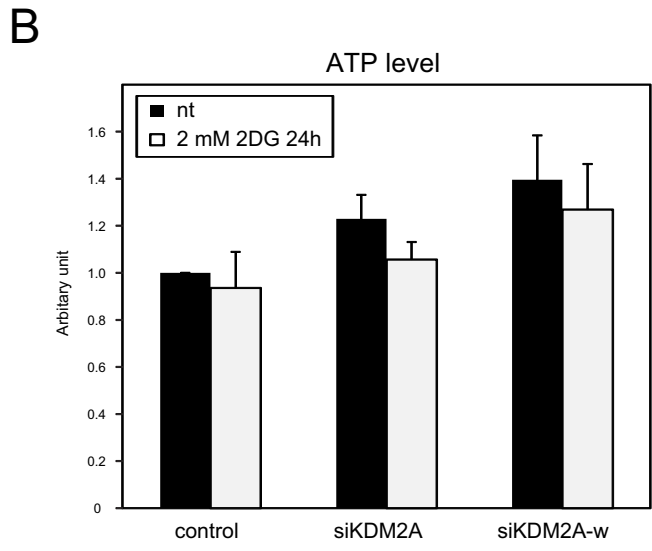


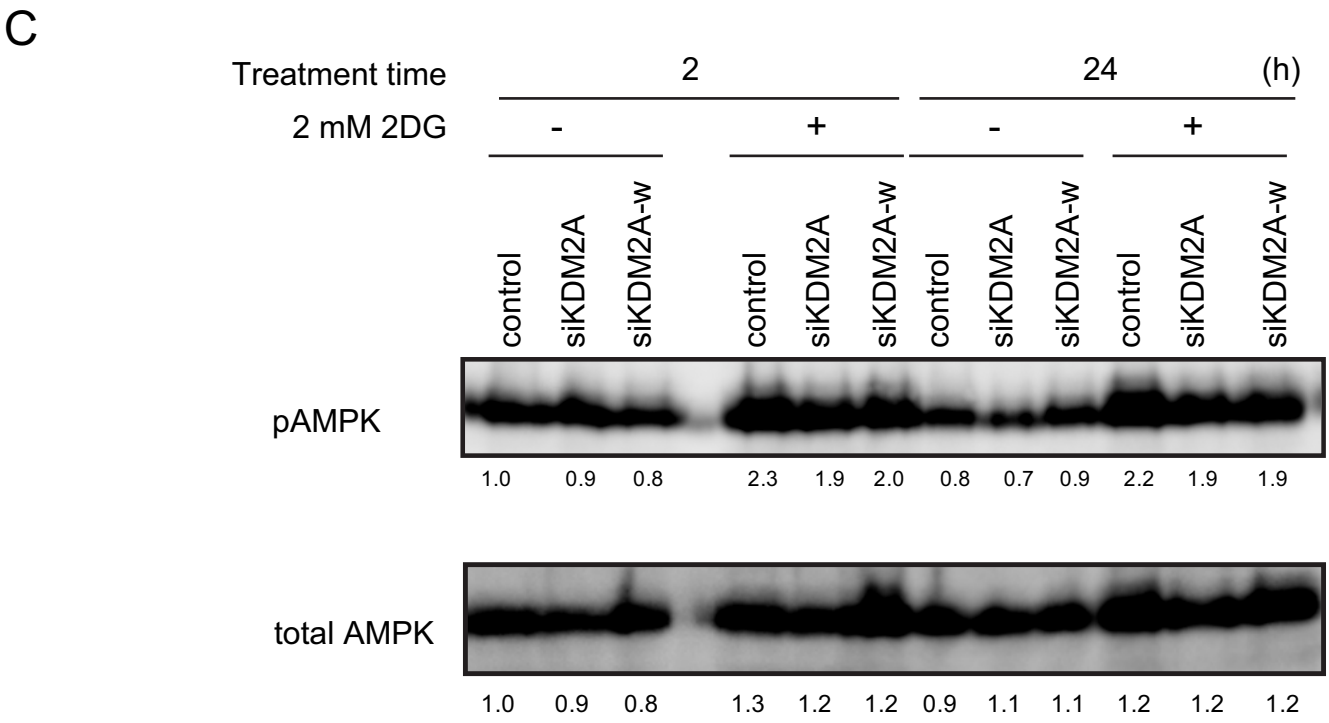
Supplemental Figure S4



Supplemental Figure S5







Supplemental figure S6