Molecules and Cells





Supplementary Fig. S1. Analysis of PPARγ promoter activity by PPARγ expression. (A) Schematic diagram of the mutant PPARγ2 promoter luciferase reporter. For the construction of luciferase reporter, the promoter fragments of PPARγ2 (PPARγ-Luc, from nt -2,983 to +68) was amplified by PCR from the murine genomic DNA and subcloned into the pGL3-basic vector (Promega). The putative transcription factor binding sites are illustrated, and the nucleotide positions were numbered based on the transcriptional start site. PPRE, PPARγ responsive element; C/EBP, CCAAT/enhancer binding protein; SP1, specificity protein 1; AP-2, activator protein 2. (B) Analysis of the putative PPRE elements on the PPARγ2 promoter. Two putative PPRE consensus sequences were present at nt -2,185 (5'-aTTtctAaggtca-3') and nt -897 (5'-aggGcaAaggCct-3'). The putative PPRE consensus sequences were mutated by site-directed mutagenesis (mut1: 5'-cTTcacAtagaga-3' and mut2: 5'-cttGagAtaaCag-3'). Reporter plasmids (PPARγ-Luc [0.1 μ g] and pcDNA3.1/His/LacZ [0.1 μ g]) were cotransfected with epitope-tagged expression plasmids (Flag-PPARγ [0.5 μ g]) into 293T cells. The expression of transfected plasmids was analyzed by immunoblotting against anti-epitope antibodies. β-Actin was used as the loading control. **P* < 0.05; ***P* < 0.01; n.s., not significant.

Negative Effect of TDAG51 on $\ensuremath{\text{PPAR}}_\gamma$ Activity Sumi Kim et al.



Supplementary Fig. S2. Analysis of promoter activity by PPAR_Y or TDAG51 expression. (A) Inhibition of PPAR_Y-induced transcriptional activation by TDAG51. (Left panel) Schematic diagram of the luciferase reporter of the PPAR_Y2 promoter (PPAR_Y-Luc). The putative transcription factor binding sites are illustrated, and the nucleotide positions were numbered based on the transcriptional start site. PPRE, putative PPAR_Y responsive element; C/EBP, CCAAT/enhancer binding protein; SP1, specificity protein 1; AP-2, activator protein 2. (Right panel) The inhibitory effect of TDAG51 on PPAR_Y-induced PPAR_Y-Luc reporter activity. Reporter plasmids (PPAR_Y-Luc [0.1 μ g] and pcDNA3.1/His/LacZ [0.1 μ g]) were cotransfected with epitope-tagged expression plasmids (Flag-PPAR_Y [0.5 μ g] and/or Myc-TDAG51 [0.1 and 0.3 μ g]) into 293T cells. The expression of transfected plasmids was analyzed by immunoblotting against anti-epitope antibodies. β-Actin was used as the loading control. (B) Analysis of TDAG51 promoter activity by PPAR_Y. (Left panel) A schematic diagram of the luciferase reporter of the TDAG51 promoter (P_{T51}-3K-Luc) is shown in the left panel. The putative transcription factor binding sites are illustrated, and the nucleotide positions were numbered based on the transcriptional start site. NF- κ B, nuclear factor kappa B; CREB, cAMP response element-binding protein; AP-1, activator protein 1. (Right panel) The effect of PPAR_Y on TDAG51 promoter activity. Reporter plasmids (P_{T51}-3K-Luc [0.1 μ g] and pcDNA3.1/His/LacZ [0.1 μ g]) were cotransfected with Flag-tagged PPAR_Y on TDAG51 promoter activity. Reporter plasmids (P_{T51}-3K-Luc [0.1 μ g] and pcDNA3.1/His/LacZ [0.1 μ g]) were cotransfected with Flag-tagged PPAR_Y on TDAG51 promoter activity. Reporter plasmids (P_{T51}-3K-Luc [0.1 μ g] and pcDNA3.1/His/LacZ [0.1 μ g]) were cotransfected with Flag-tagged PPAR_Y expression plasmids (Flag-PPAR_Y [0.1 and 0.3 μ g]) into 293T cells. The expression of transfected plasmids was analyzed by