ColXV promotes adipocyte differentiation via inhibiting DNA methylation and cAMP/PKA pathway in mice

Supplementary Materials

Supplementary Figure 1: The effect of HFD on body weight and food intake of chow and HFD fed mice. Mice were fed with HFD or chow diet for 9 weeks before collecting tissues. Body weight and food intake were recorded each week. Body weight (A) and average food intake (B) in HFD mice were compared with those in chow diet fed mice. n = 6. Data represent the mean ± SEM of three independent experiments. *P < 0.05.

Supplementary Figure 2: The effect of ColXV on metabolic parameters, serum lipid, differentiation and lipid metabolism related genes level. Mice fed with chow and HFD diet were intraperitoneal injected with Ad-Col15α1 or Ad-GFP. ColXV protein expressions in eWAT, iWAT, BAT, liver and muscle (A) were detected after adenovirus injection compared with that in corresponding tissue of mice without adenovirus injection. Food intake (B) serum lipids (C) TG (D) and FFA (E) isolated from eWAT were detected. Relative mRNA levels of adipogenic markers (F) in mice eWAT fed on HFD and chow diet after Col XV overexpression were detected by Quantitative Real-Time PCR. Protein activity of lipid metabolism related genes in eWAT of HFD and chow diet fed mice after Col XV overexpression (G) were detected by ELISA. n = 6. Data represent the mean ± SEM of three independent experiments. **P < 0.01, ***P < 0.001.
Supplementary Figure 3: ColXV overexpressed and interfered vector efficiency verification and effect of ColXV on lipolysis. Ad-Col15α1 and sh-Col15α1 or Ad-GFP infected adipocytes, and isolated RNA after 24 h or protein after 48 h for ColXV expression efficiency verification compared with negative control. Relative mRNA level (A) and protein level of ColXV (B) were determined. After infecting with Ad-Col15α1 and sh-Col15α1, relative mRNA level of FASN, ACCα, HSL and ATGL in mature adipocytes (C) and protein level (D) were measured, empty vector as control. \( n = 6 \). Data represent the mean ± SEM of three independent experiments. *\( P < 0.05 \).
Supplementary Figure 4: Prediction for transcription factors on ColXV promoter and the effect of CREB agonist on adipogenic and lipolysis gene expressions.  
(A) Prediction for transcription factors on ColXV promoter with Genomatix software analysis, CREB in adipose tissue was one of them. After infecting with Ad-Col15α1 and sh-Col15α1, CREB agonist Forskolin were added to adipocytes for 12 h, CREB mRNA (B) and C/EBPβ, PPARγ, FABP4 and ATGL mRNA levels (C) were detected. n = 6. Data represent the mean ± SEM of three independent experiments. *P < 0.05, **P < 0.01.
Supplementary Figure 5: The effect of ColXV on adipocyte differentiation compared with DNA methylation inhibition by 5-Aza-dC. Bisulphite sequencing analyses of the ColXV DNA. CpG methylation of ColXV promoter (A) and intron 1 (B) in adipocytes before or after differentiation (n = 3). (C) Adipocytes treated with 10 μM 5-Aza-dC or Ad-Col15α1 and sh-Col15α1, de novo methyltransferase (mtase) Dnmt3a and Dnmt3b activity was detected (n = 3). CpGs methylation of ColXV promoter (D) and intron 1 (E) in adipocytes were analysed after treating with 5-Aza-dC (n = 3). Data represent the mean ± SEM of three independent experiments. *P < 0.05, **P < 0.01.