HDAC1 and 2 regulate endothelial VCAM-1 expression and atherogenesis by suppressing methylation of the *GATA6* promoter

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Figure S1

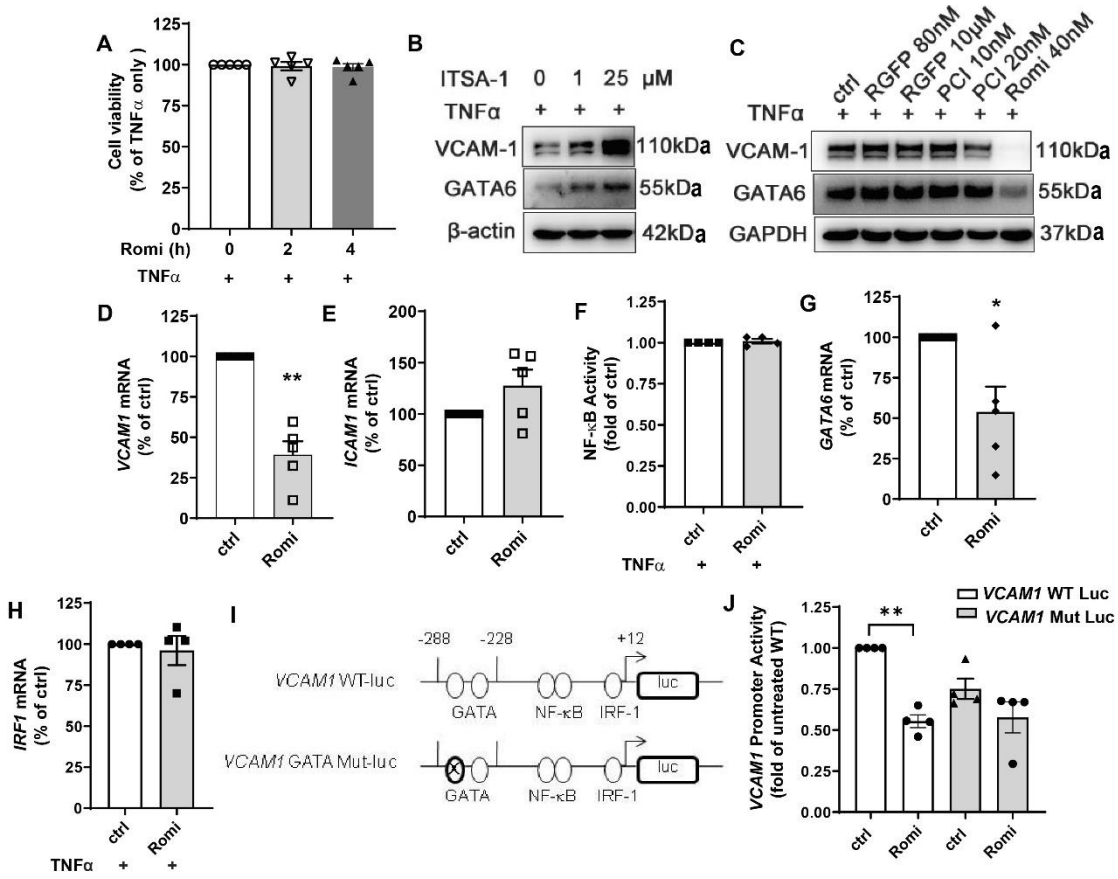

Figure S1. (A) HAEC were pretreated with Romi (40 nM) for 1 h, followed by another 2 h or 4 h con-incubation with TNFα (0.1 ng/mL). CCK-8 assay was applied to cell viability (n = 5). **(B)** HAEC were treated with HDAC activator ITSA-1 at indicated concentration, followed by TNFα stimulation prior to Western blot analysis. (**C**) HAEC were pretreated with HDAC3 inhibitor RGFP, HDAC8 inhibitor PCI or HDAC1/2 inhibitor Romi, followed by TNFα stimulation and Western blot analysis. (**D-E**) HAEC were treated with 40 nM Romidepsin (Romi) for 5 h followed by quantitative PCR to measure *VCAM1* (D) or *ICAM1* (E) mRNA (n = 5). (**F**) After treatment with 40 nM Romidepsin for 1 h, HAEC were stimulated with TNFα for 2 h, followed by dual-luciferase assay to detect the transcriptional activity of NF-κB (n = 4). (**G**) HAEC were treated with 40 nM Romidepsin (Romi) for 5 h followed by quantitative PCR to measure *GATA6* mRNA (n = 5). (**H**) After treatment with Romi (40 nM) and TNFα, *IRF1* mRNA was measured (n = 4). (**I**) pGL3 firefly luciferase (luc) constructs of the *VCAM1* promoter (-288/+12) were generated. Mutation (Mut) is depicted by an "X". (**J**) After

transfection and treated with Romi, the activity of the WT or Mut *VCAM1* promoter construct was measured by the dual-luciferase reporter system (n = 4). *p < 0.05; **p < 0.01; repeated measures one-way ANOVA followed by Dunnett's test (vs. ctrl, A, J); one sample *t*-test (D, E, F, G, H).

Figure S2

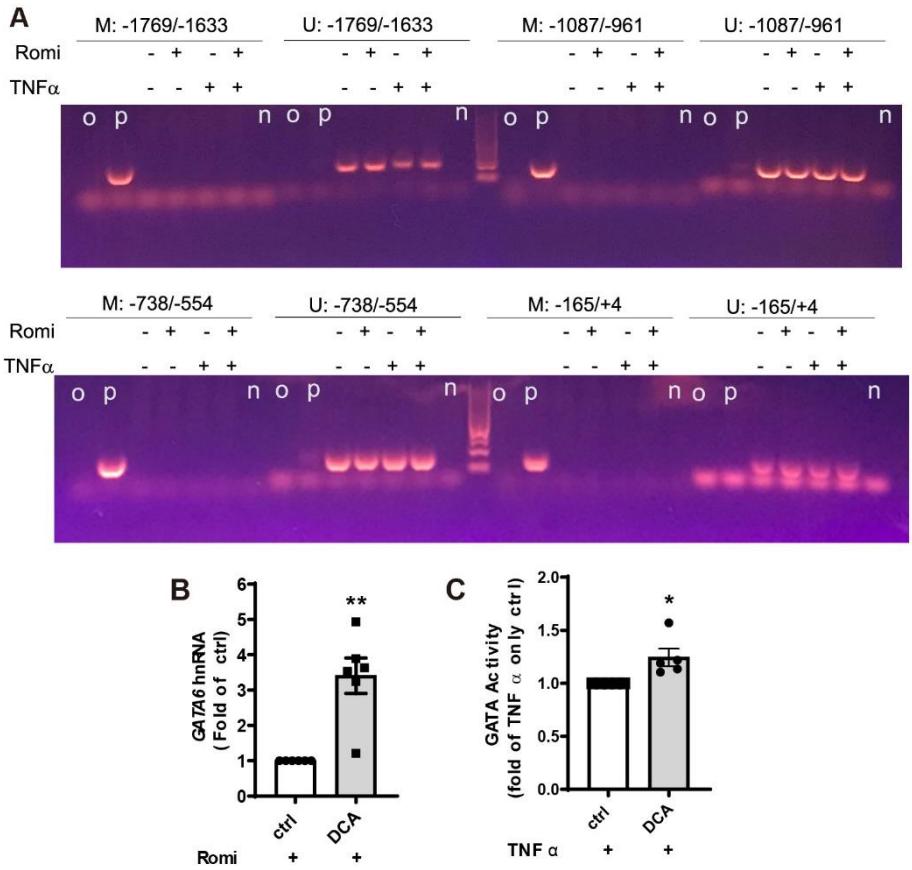

Figure S2. (A) After treatment, *GATA6* promoter methylation in CpG-rich regions -1769/-1633, -1087/-961, -738/-554 and -165/+4 was evaluated by agarose gel electrophoresis detection of MSP products with primers targeting the methylated (M) or unmethylated (U) cytosines. o, original DNA without bisulfite modification; p, positive control using M. SssI-treated peripheral blood leukocyte DNA; n, non-DNA negative control. Shown are representative images of two experiments. **(B)** HAEC were treated with Romidepsin alone or together with DCA (5 μM) followed by hnGATA expression measured as in 2E (n = 6). (**C**) HAEC were pretreated with DCA (5 μM) for 1 h followed by stimulation with TNFα. The transcriptional activity of GATA was examined by dual-luciferase assay (n = 5). *p < 0.05; **p < 0.01; one sample *t*-test (B, C).

Figure S3

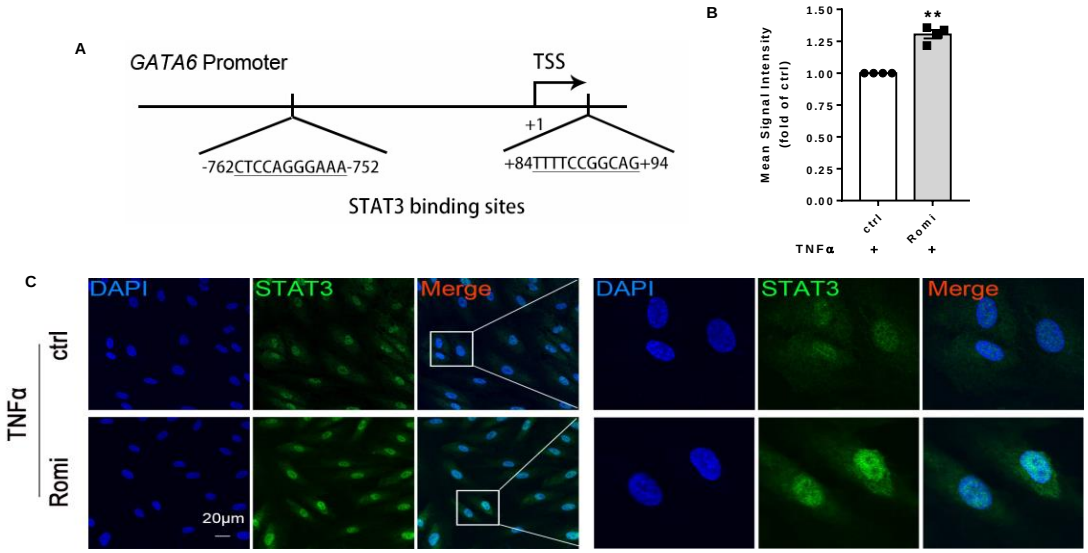

Figure S3. (A) Schematic illustrating two predicted STAT3-binding sites on human *GATA6* promoter. (**B-C**) HAEC were grown on coverslips and immunolabeled for STAT3. Images were obtained by a confocal laser scanning microscope (FV1200) and nuclear intensity quantified (B, n = 4). **C**, representative images. **p < 0.01; one sample *t*-test (B).

Figure S4

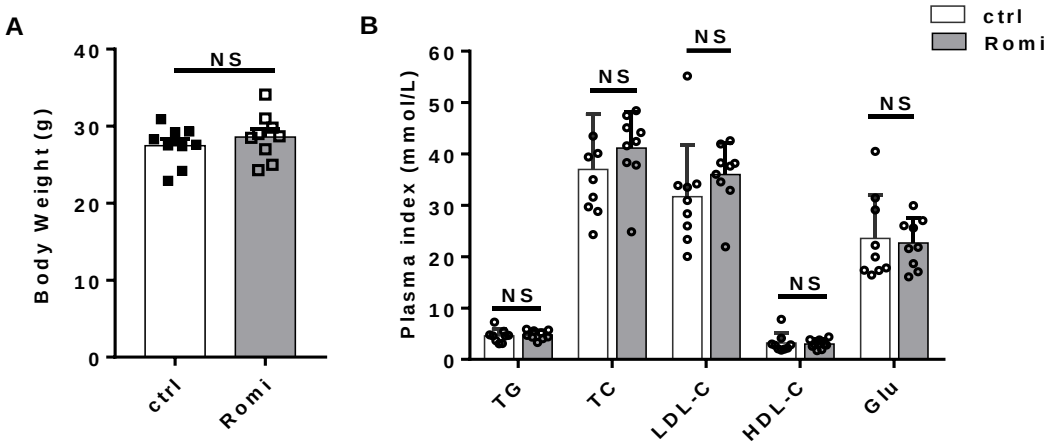

Figure S4. *Apoe*$^{-/-}$ male mice were i.p. injected with Romidepsin (Romi) or PBS (ctrl) and fed with a high-fat diet for 12 weeks (n = 9). Romi treatment caused no significant change in body weight (A), serum lipid or glucose levels (B). TG, triglycerides; TC, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; Glu, glucose. Two-tailed paired *t*-test (A, B).