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## Supplemental information

# HFD-induced TRAF6 upregulation promotes liver cholesterol accumulation and fatty liver development via EZH2-mediated miR-429/PPARα axis

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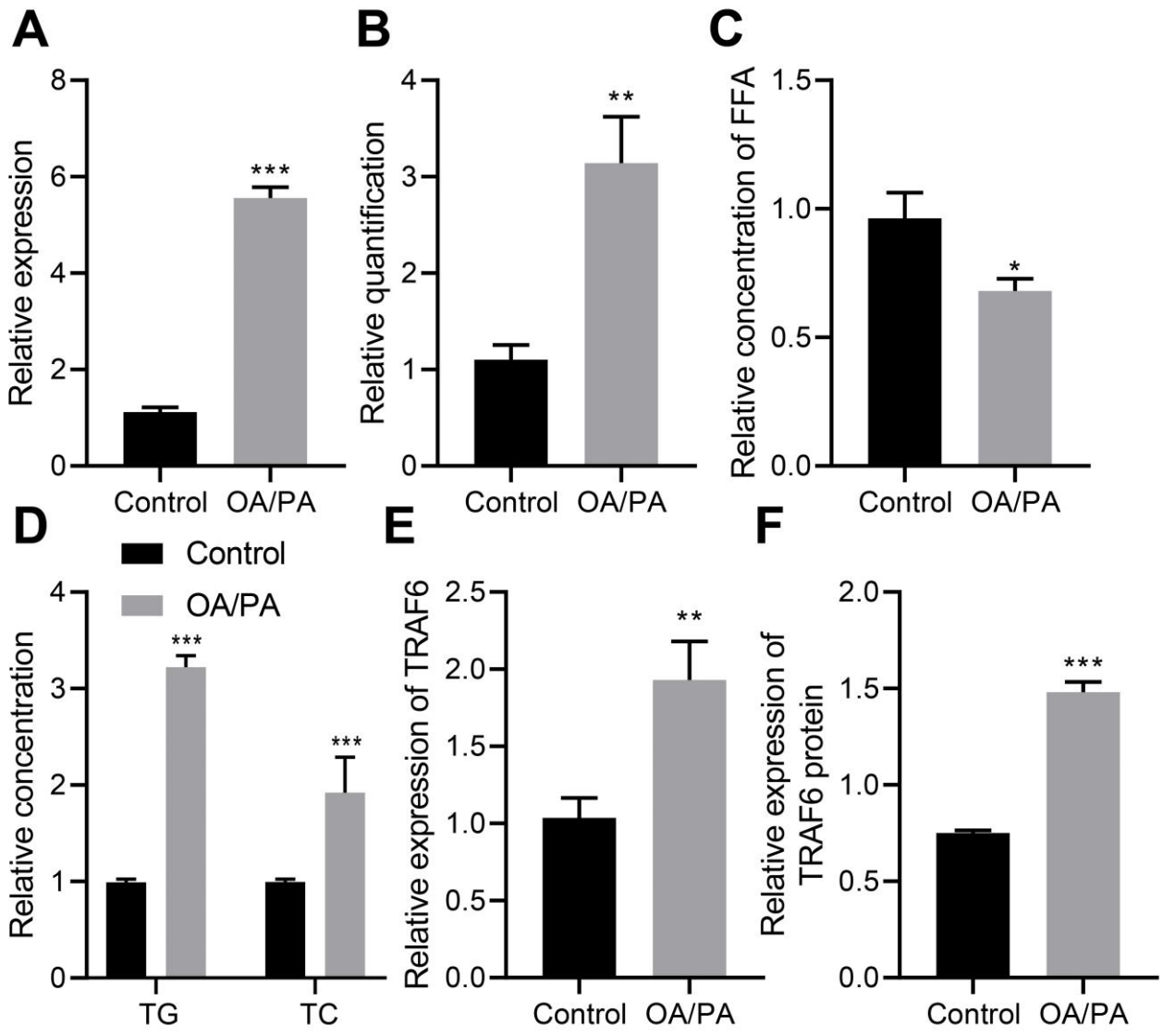
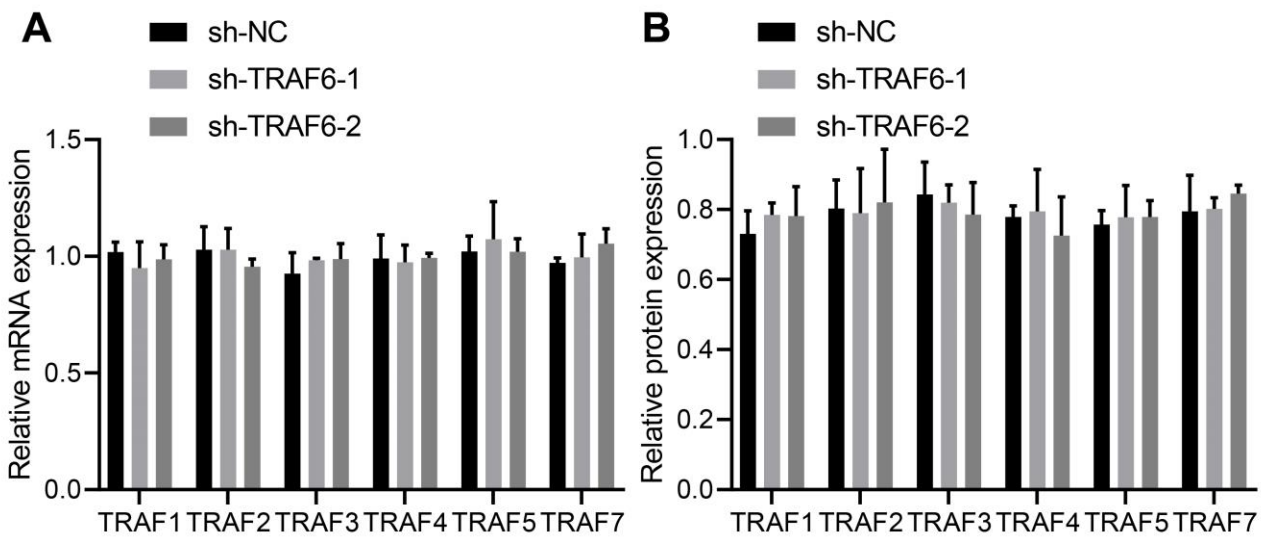
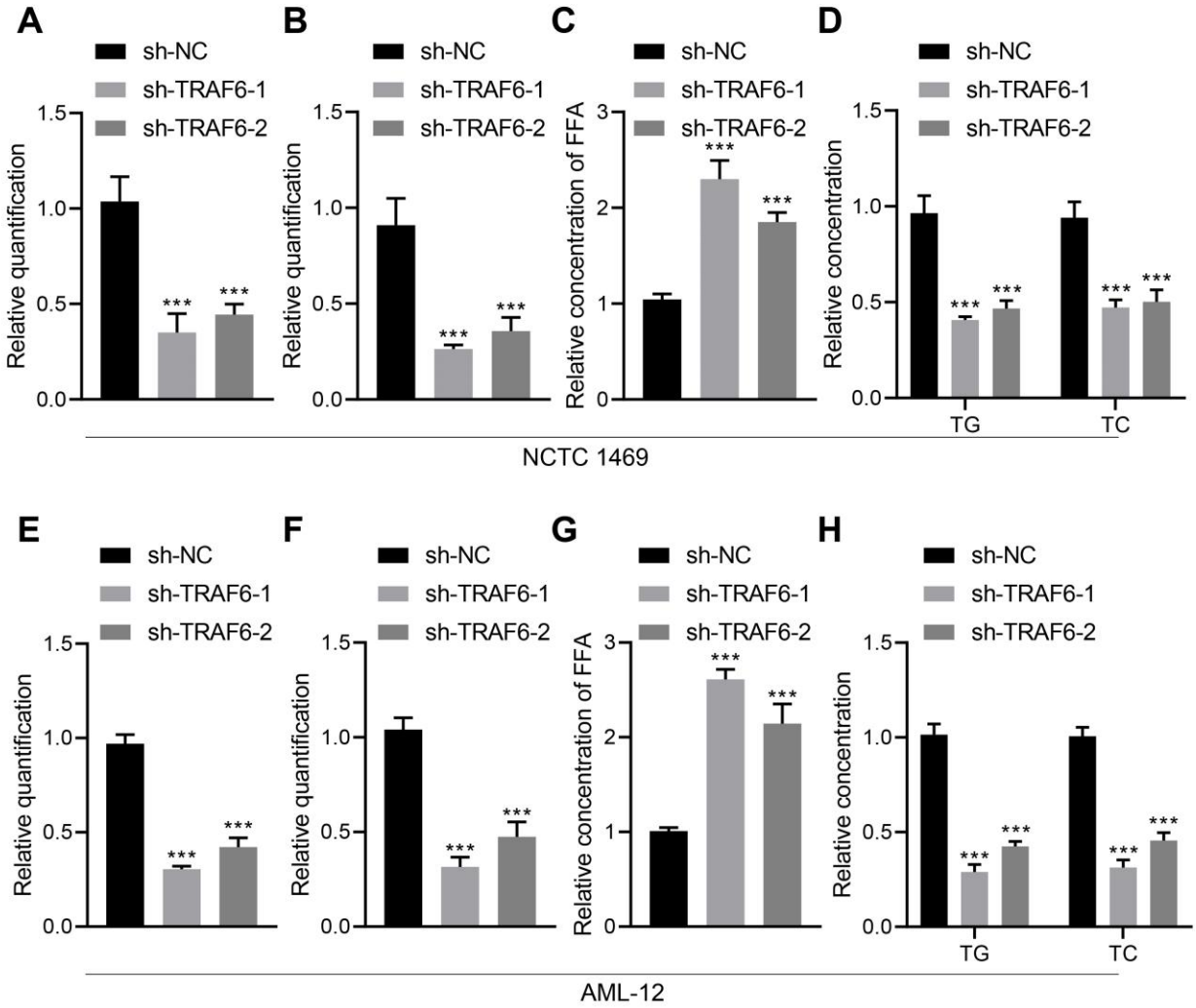


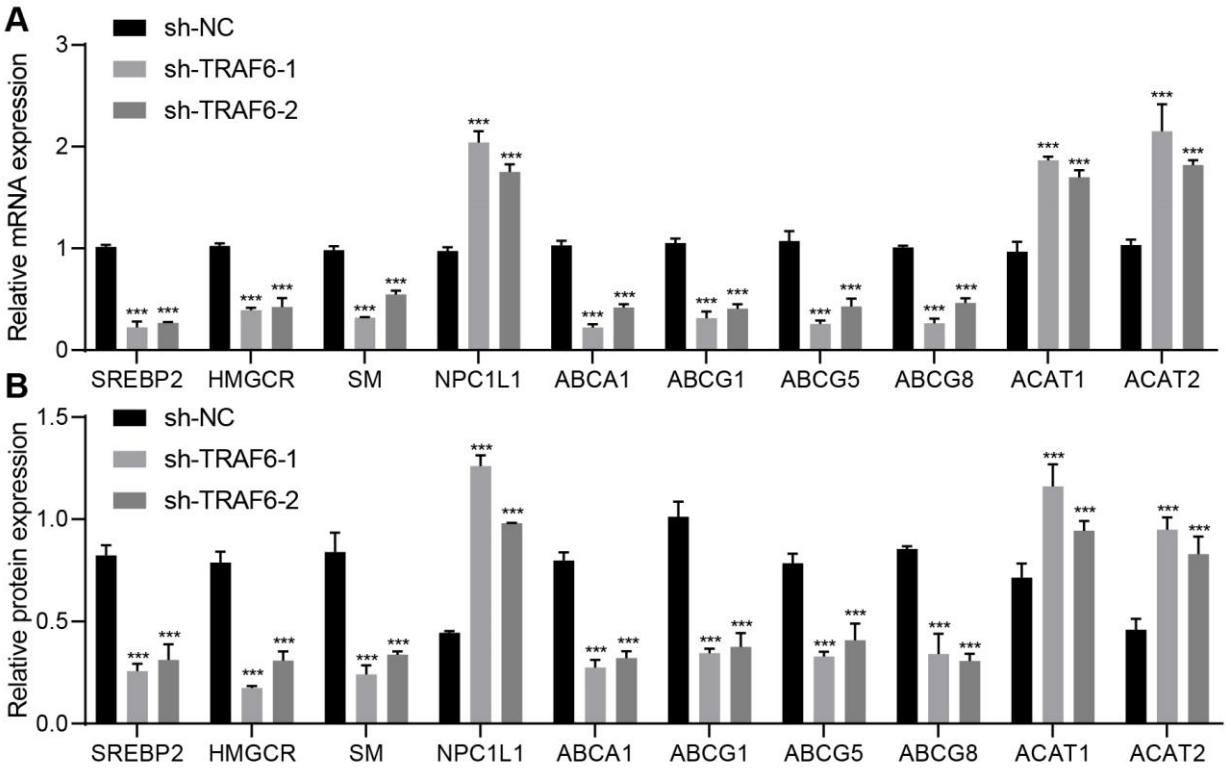

**Supplementary figure 1** TRAF6 is highly expressed in fatty liver caused by HFD. A, Filipin staining of cell model of fatty liver. B, Nile red staining of cell model of fatty liver. C, Statistical histogram of FFA amount in hepatocytes. D, Statistical histogram of TG and TC in hepatocytes. E, RT-qPCR determination of TRAF6 expression in cell model of fatty liver. F, Western blot analysis of TRAF6 expression in cell model of fatty liver. Measurement data were expressed as mean ± standard deviation, and data between two groups was tested using independent sample t test, and analyzed by repeated measures ANOVA and Bonferroni's test. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ *vs.* control. n=3 in the control group and OA/PA group.



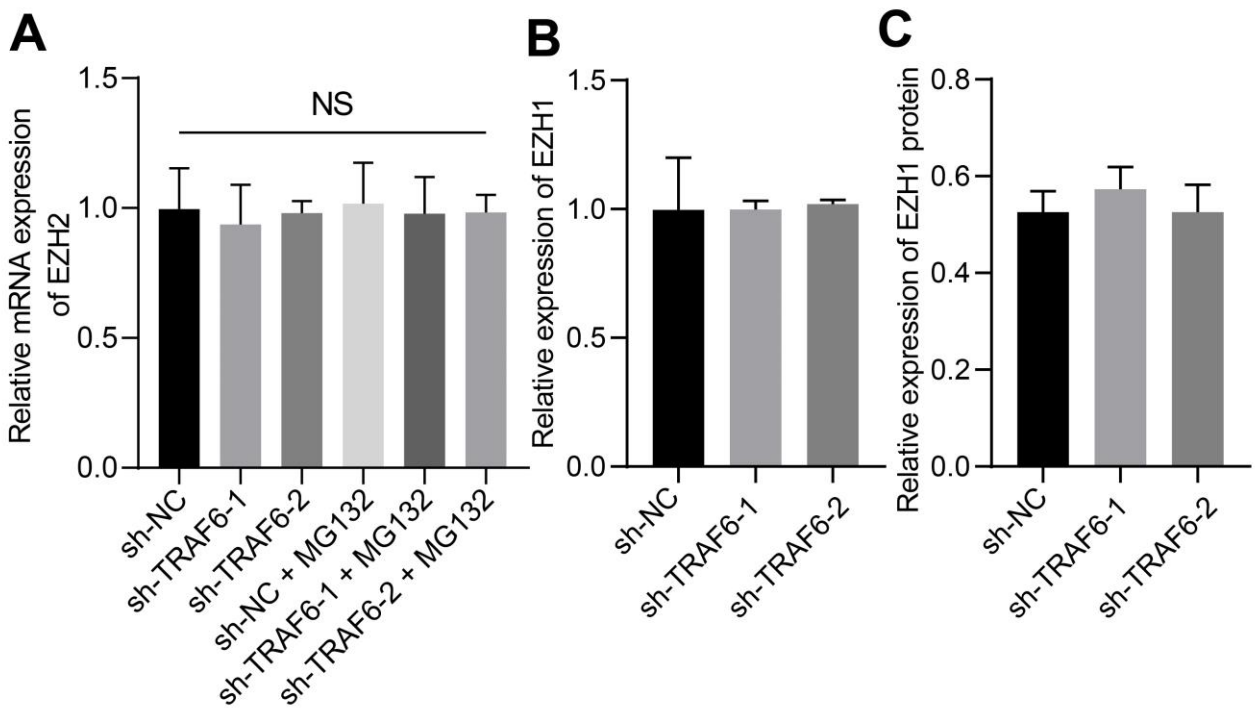

**Supplementary figure 2** Silencing TRAF6 affects the expression of other isoforms of TRAF6. A, RT-qPCR determination of TRAF1-5 and TRAF7 expression after sh-TRAF6-1 or sh-TRAF6-2 treatment. B, Western blot analysis of TRAF1-5 and TRAF7 expression after sh-TRAF6-1 or sh-TRAF6-2 treatment normalized to β-actin. Measurement data were expressed as mean ± standard deviation, independent sample t-test was used for comparison between two groups, and one-way ANOVA was used for comparison between multiple groups followed by Tukey's post-hoc test.



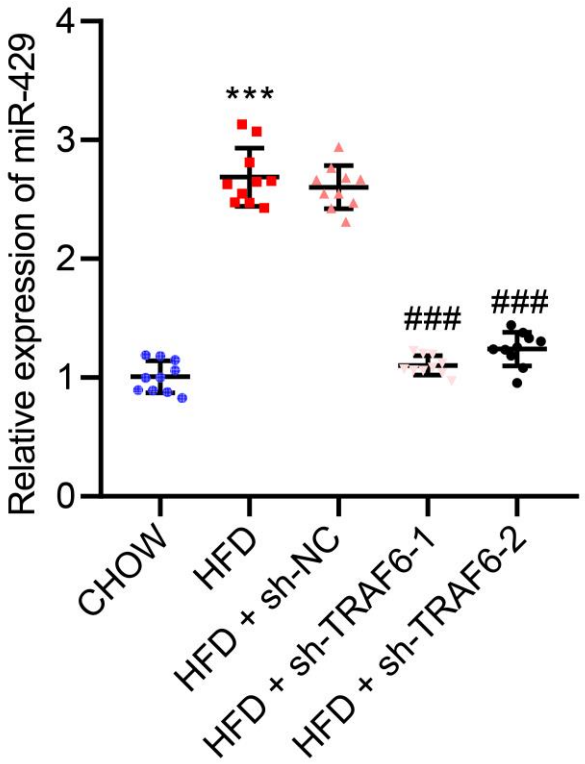

**Supplementary figure 3** TRAF6 knockdown inhibits the accumulation of TC in NCTC1649 and AML-12 hepatocytes. A, Statistical histogram of Filipin staining results of NCTC1649 hepatocytes. B, Statistical histogram of Nile red staining results of NCTC1649 hepatocytes. C, Statistical histogram of FFA amount in the NCTC1649 hepatocytes. D, Statistical histogram of TG and TC content in the NCTC1649 hepatocytes. E, Statistical histogram of Filipin staining results of AML-12 hepatocytes. F, Statistical histogram of Nile red staining results of AML-12 hepatocytes. G, Statistical histogram of FFA content in the AML-12 hepatocytes. H, Statistical histogram of TG and TC in the AML-12 hepatocytes. Measurement data were expressed as mean ± standard deviation. Data comparisons between two groups were performed by independent sample t-test, and data comparisons between multiple groups were performed by one-way ANOVA followed by Tukey's post-hoc test. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared with the sh-NC group.



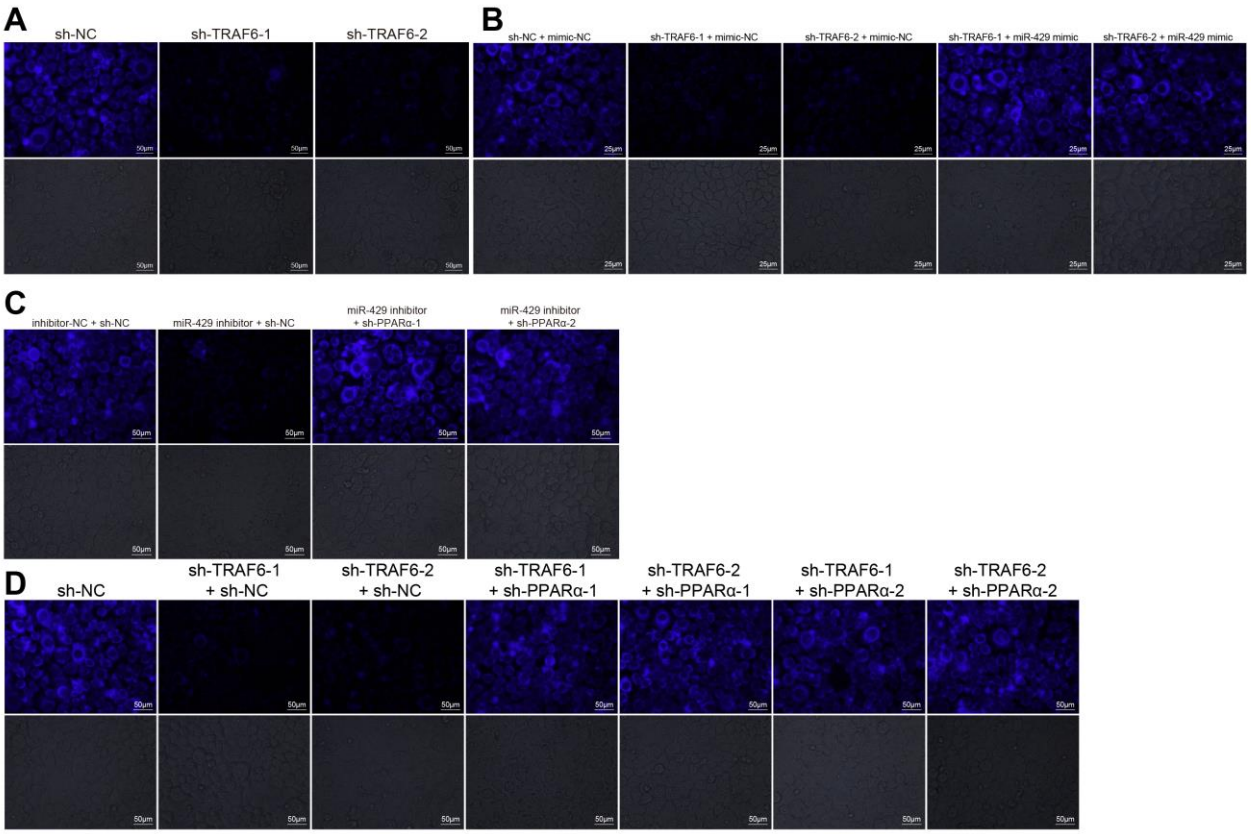

**Supplementary figure 4** TRAF6 regulates the genes related to cholesterol homeostasis. A, RT-qPCR determination of the expression of genes related to cholesterol homeostasis. B, Western blot analysis of the expression of genes related to cholesterol homeostasis. Measurement data were expressed as mean ± standard deviation. Data comparisons between two groups were performed by independent sample t-test, and data comparisons between multiple groups were performed by one-way ANOVA followed by Tukey's post-hoc test. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared with the sh-NC group.



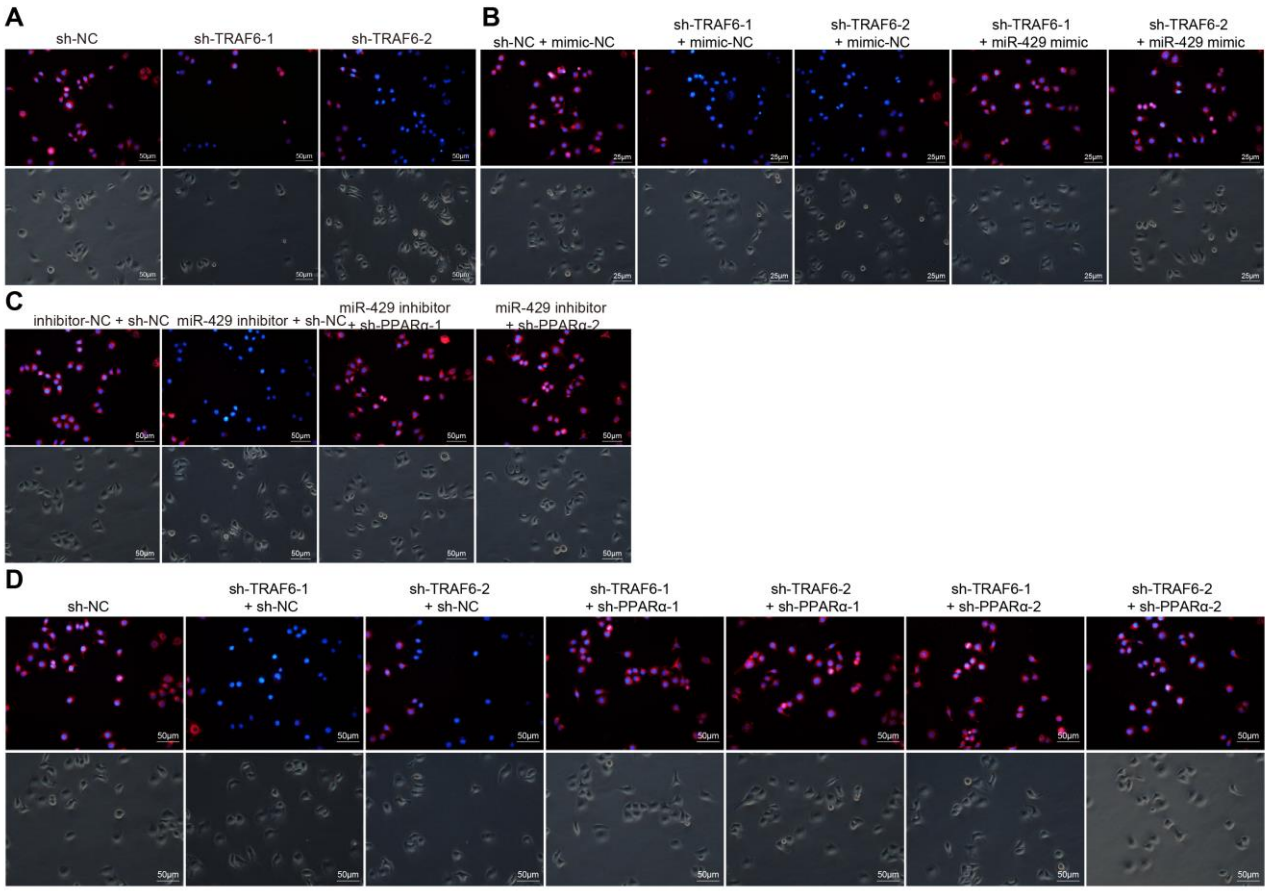

**Supplementary figure 5** A, RT-qPCR determination of EZH2 mRNA expression in the cells of each group and the statistical histogram. B, RT-qPCR determination of EZH1 expression after treatment of sh-EZH2-1 or sh-EZH2-2 treatment. C, Western blot analysis of EZH1 expression after treatment of sh-EZH2-1 or sh-EZH2-2 treatment. Measurement data were presented as the mean ± standard deviation. Data among two groups were compared using independent sample t-test, and data among multiple groups were compared using one-way ANOVA followed by Tukey's *post hoc* test, n = 3. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ compared with the sh-NC group.




**Supplementary figure 6** RT-qPCR determination of miR-429 expression in liver tissues of mice. Measurement data were presented as the mean ± standard deviation, and data among multiple groups were compared using one-way ANOVA followed by Tukey's *post hoc* test, n = 3. *** $p < 0.001$ *vs.* sh-NC, ### $p < 0.001$ *vs.* HFD + sh-NC.




**Supplementary figure 7** A, Filipin staining for hepatocytes treated with sh-NC or sh-TRAF6 (400 ×). B, Filipin staining for hepatocytes after different treatment (400 ×). C, Filipin staining for hepatocytes after different treatment (200 ×). D, Filipin staining for hepatocytes after different treatment (200 ×).




**Supplementary figure 8** A, Nile red staining results (400 ×). B, Nile red staining for hepatocytes (400 ×). C, Nile red staining (200 ×). D, Nile red staining after different treatment (200 ×).