## **Supporting Information**



Figure S1. Esculetin did not exert hepatic toxicity at the doses of 90 and 180 mg/kg/d. (A) The structure of esculetin. (B) The serum levels of liver enzymes alanine aminotransferase (ALT) and aspartate transaminase (AST) in HFD-fed mice with or without esculetin at indicated doses. Data points in graphs show individual mice (n = 7) over 2 experiments, analyzed by one-way ANOVA with Tukey's multiple comparisons test for ALT or Kruskal-Wallis with Dunn's multiple comparisons test for AST. (C) Hematoxylin and eosin (H&E) staining of hepatic tissue. (D) The heatmap of apoptosis-related genes from the liver. RNA sequencing was performed using the livers from HFD-fed mice with 0, 90, 180 mg/kg/d esculetin treatment (n = 3). One sample contains two mice. (E) Gene set enrichment analysis (GSEA) against apoptosis pathway is shown using the RNA sequencing data from the livers.

## Figure S2

Control
Esculetin



Figure S2. The multivariate statistical analysis of serum metabolites. (A) The *Pearson* correlation coefficients among the quality control (QC) samples. (B) Orthogonal projection to latent structures-discriminant analysis (OPLS-DA) score graphs. (C) Partial least squares discriminant analysis (PLS-DA) score graphs. Permutation test results of the OPLS-DA (D) and PLS-DA (E) model in the positive and negative ion modes. (F) The volcano plot of esculetin-induced significantly differentially expressed metabolites (DEMs) identified from positive and negative ion modes (n = 6). (G) Heatmap of lipid-associated metabolites. (H) Transcription levels of *Apoa1*, expressed as transcripts per kilobase million (TPM), were quantified by RNA-seq analysis of liver samples from high-fat diet-fed mice treated with a control or 90 mg/kg/day esculetin (n = 3).





**Figure S3. Quantitative and histological analysis of lipid metabolism and adipose tissue macrophage in eWAT with Esculetin treatment.** (**A**, **B**) Quantification of postprandial serum Cholesterol and Triglycerides. (**C**) Hematoxylin and eosin (H&E) staining and

immunohistochemistry (IHC) of F4/80 and CD36 in eWATs. Scale bar, 50  $\mu$ m. (**D**) The average adipocyte area per field was quantified. (**E**) F4/80<sup>+</sup> cells per field were quantified. (**F**) CD36<sup>+</sup> cells per field were quantified. Arrow indicated the positive signals. Data points represent individual fields (n = 9) from 3 mice across two independent experiments. (**G**) Representative flow cytometry plots of F4/80<sup>+</sup> CD11b<sup>+</sup> macrophage gating in CD11b<sup>+</sup> Lineage<sup>-</sup> cells from epididymal white adipose tissue (eWAT). The lineage markers used include CD19, TCR $\beta$ , Ly-6G, and Ly-6C. (**H**) Percentage of F4/80<sup>+</sup> CD11b<sup>+</sup> Lineage<sup>-</sup> macrophages among CD45<sup>+</sup> cells in eWAT. (**I**) Absolute counts of F4/80<sup>+</sup> CD11b<sup>+</sup> macrophages per mouse. Statistical analysis was performed using one-way ANOVA with Tukey's multiple comparisons test (A, D-F, H-I), and the Kruskal-Wallis test with Dunn's multiple comparisons test (B).



**Figure S4. Esculetin did not change the macrophages subsets from liver. (A)** Representative image of cytokine/chemokine antibody array using serum from HFD-fed mice treated with either

0 or 90 mg/kg/day of esculetin. One representative out of two similar experiments is displayed. One sample contains three mice. (**B**) Representative gating strategies of flow cytometry analysis. (**C**) The percentages of CD36<sup>+</sup> CD11b<sup>+</sup> cells in CD45<sup>+</sup> cells from liver. Data points represent individual mice (n = 6) over two experiments, analyzed by ANOVA with Tukey's multiple comparisons test. (**D**) Mean fluorescence intensity (MFI) of LipidTOX in CD36<sup>+</sup> CD11b<sup>+</sup> cells from liver. Data points represent individual mice (n = 6) over two experiments, analyzed by Kruskal-Wallis with Dunn's multiple comparisons test. (**E**) Representative flow cytometry of ATMs before and after sorting.

## Figure S5



Figure S5. C/EBP $\beta$ -target genes were significantly enriched in esculetin-induced differentially expressed genes in livers. RNA sequencing was performed using the livers from HFD-fed mice with or without 90 mg/kg/d esculetin treatment (n = 3). One sample contains two mice. (A) Principal component analysis plot is shown. (B) Esculetin-induced significantly differentially expressed genes (DEGs) were used for enrichment analysis against TRRUST transcription factor gene sets. (C) The expression levels of *Cebpb* and *Cebpd* in livers were quantified by RNA-seq.





**Figure S6. Expression profiles of M1/M2 macrophage markers in postprandial high-fat dietinduced macrophages.** (**A**) Unsupervised clustering of ATMs using UMAP, where each dot represents a single cell, colored by cluster assignment, with separate groupings for control diet and high-fat diet conditions. (**B**) Violin plots showing the expression patterns of M1/M2 macrophage signature gene sets across different clusters and groups. (**C-F**) Dot plots and violin plots showing the cluster and group-specific expression profile of *Il1b*, *Cd86*, *Il10*, and *Cd163*, respectively.





Figure S7. Whole gel images of Western blot analysis.

## Supplementary Tables

Table S1. Key resources table

Reagent or resource	Identifier	Source
Antibodies		
Brilliant Violet 605 anti-mouse CD45	103155	Biolegend
Antibody		
Brilliant Violet 510 anti-mouse CD19	115545	Biolegend
Antibody		
Brilliant Violet 510 anti-mouse TCR $\beta$	109233	Biolegend
chain Antibody		
Brilliant Violet 510 anti-mouse Ly-6G	127633	Biolegend
Antibody		
Brilliant Violet 510 anti-mouse Ly-	108457	Biolegend
6G/Ly-6C (Gr-1) Antibody		
PerCP/Cyanine5.5 anti-mouse/human	101228	Biolegend
CD11b Antibody		
Alexa Fluor 700 CD36 Monoclonal	56-0362-82	Thermo Fisher
Antibody (HM36)		
Brilliant Violet 421 anti-mouse F4/80	123131	Biolegend
Antibody		
APC anti-mouse Tim-4 Antibody	130022	Biolegend
C/EBPB Rabbit pAb	A0711	Abclonal
Phospho-C/EBPB-T235 Rabbit pAb	AP1055	Abclonal
Anti -F4/80 Rabbit pAb	GB113373	Servicebio
GAPDH Mouse mAb	AC033	Abclonal
CD36 antibody	sc-7309	Santa Cruz
Goat anti-Rabbit IgG Secondary	GB23303	Servicebio
Antibody, HRP		
Goat anti-Rabbit IgG Secondary	A-11008	Thermo Fisher
Antibody, Alexa Fluor 488		
C/EBPB antibody (H-7) for ChIP	sc-7962	SCBT
Mouse IgG antibody	sc-52336	SCBT
Chemicals, kit and buffer		
Esculetin	D77970	ACMEC
Simvastatin	H20084420	Xinqi

Pitavastatin	H20193061	Salubris
Tween-80	T8360	Solarbio
Sulfo-N-succinimidyl oleate	HY-112847A	MedChemExpress
Puromycin dihydrochloride	HY-B1743A	MedChemExpress
Dimethyl sulfoxide	D8371	Solarbio
Methanol (LC-MS grade)	CAEQ-4-000306-4000	Anpel
Acetonitrile (LC-MS grade)	CAEQ-4-000308-4000	Anpel
Ammonium ethanoate (LC-MS grade)	73594	Sigma
Ammonium hydroxide (LC-MS grade)	60-046-886	Fisher Scientific
Dulbecco's Modified Eagle Medium	PM150210	Procell
Fetal Bovine Serum	164210-50	Procell
Penicillin and streptomycin	PB180120	Procell
DIL-ox-LDL	YB-0010	Yiyuan Biotechnologies
Collagenase D	11088866001	Roche
Liberase	5401127001	Roche
Dnase I	D5025	Lablead
Percoll	17089101	Cytiva
Prestained Protein marker	P1018	Lablead
WesternBright ECL HRP substrate	K-12045-D50	Advansta
FcR blocking reagent	130-092-575	Miltenyibiotec
Fixable Viability Dye eFluor 780	65-0865-14	Thermo Fisher
RPMI 1640	L210KJ	Basalmedia
		Technologies
Fetal bovine serum for flow cytometry	C4055L1050	Life-ilab
eBioscience Foxp3 / Transcription	00-5523-00	Invitrogen
Factor Staining Buffer Set		
IC fixation buffer	00-8222-49	Invitrogen
Lipid Tox Green Neutral Lipid Stain	H34475	Thermo Fisher
Trizol	15596026	Thermo Fisher
Nebnext Ultra RNA Library Prep Kit	E7530	New England Biolabs
for Illumina		
TruePrep DNA Library Prep kit V2 for	TD501	Vazyme
Illumina		
Qiagen PCR purification kit	28104	Qiagen
NEBNext Ultra II Q5 Master Mix	M0544	New England Biolabs

NovoNGS Index Kit for Illumina	N239	novoprotein
Qiagen MiniElute Reaction Celanup	28206	Qiagen
Kit		
RNA-direct SYBR Green Real time	QRT-201 100	TOYOBO
PCR Master Mix		
DAB staining kit	G1212	Servicebio
H&E staining kit	G1076	Servicebio
Human C/EBP $\beta$ recombinant protein	P17676	Raybiotech
Silicon-on-Sapphire sensing plate (11-	FmSOS1010046S2FT05US	MTI Corporation
02, R Plane)		
(3-aminopropyl) triethoxysilane	440140	Sigma-Aldrich
bis(sulfosuccinimidyl)suberate	S5799	Sigma-Aldrich
Pierce 16% Formaldehyde (w/v),	28908	Thermo Fisher
Methanol-free		
Dynabeads Protein G for	10003D	Thermo Fisher
Immunoprecipitation		
Cytokine Array C1 kit	AAM-CYT-1-8	Raybiotech
Hieff qPCR SYBR Green Master Mix	11204ES08	Yeansen
Software and Algorithms		
Xcalibur (v4.4)		Thermo Fisher
Proteowizard (v3.0)		
R (v4.2.1)		
XCMS package (v3.22.0)		
Biotreedb MS2 database (v2)		Gene Denovo
		Biotechnology
Ropls package (v1.32.0)		
Mesap package (v0.99.0)		
HISAT (v2.2.1)		
Featurecounts (v2.0.1)		
Limma v3.56.1		
Gseabase (v1.62.0)		
Clusterprofiler (v4.8.1)		
Trim-Galore (version 0.6.10)		
Bowtie2 (version 2.5.1)		
MACS2 (version 2.2.6)		

Sambamba (	version 1.01)		
HOMER (ve	rsion 4.10.4)		
Integrative C	enomics Viewer		
DeepTools (v	version 3.4.1)		
DESeq2			
Flow Jo (v.10	0.0.7)		Becton Dickinson
Prism 9			Graphpad
Seurat R pac	kage (v2.6)		
Mice and O	thers		
C57BL6J mi	ce		Gempharmatech
RAW 264.7	W 264.7 cell CL-0190		Procell
Lenticrispr v	nticrispr v2 plasmid 52961		Addgene
Control diet	rol diet D12450J		Research Diets
High fat diet	D1	2492	Research Diets
Oligos			
Primers	Fw		Rv
Cd36	GGACATTGAGATTCTTTTCCTCTG	GCAAAGGCA	TTGGCTGGAAGAAC
NcheI	CGGTATTTCTGGAGACAGTGCTG	GGTGTGTTGA	AGTCCAAAGCCTG
Cebpb	CAACCTGGAGACGCAGCACAAG	GCTTGAACAAGTTCCGCAGGGT	
Gapdh	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTCAG	
Cd36_ChIP			
P1	ACTTCCCAGATTCAGATGGAGC	AGGCAATC	GCTCTAACAGGC
P2	ACCCCATGCTGCTCTGCTAT	ACAGAGCA	TTGGGAGTTCCTC
Р3	AGCATATTGGGTGCTGACCA	CCAGGTAA	TCCACACTCCGT
gRNA			
Cebpb	GCTGCTTGAACAAGTTCCGC		