Supporting Information for

Original article

Compound Danshen Dripping Pill inhibits hypercholesterolemia/atherosclerosis-induced heart failure in ApoE and LDLR dual deficient mice *via* multiple mechanisms

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Received 11 August 2022; received in revised form 19 September 2022; accepted 10 October 2022

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Supporting Information

1. Supplemental materials and methods

1.1. Analysis of KDM4A/E kinase activity

1.1.1. Materials

Trimethylated histone H3 peptide-biotin JMJD2A (KDM4A) or JMJD2E (KDM4E) (1–350) was purchased from Sangon Biotechnology (Shanghai, China). The activity of KDM4A or KDM4E is 0.084 or 0.14 pmole/min/μg. Anti-histone H3 (dimethyl K9) antibody was purchased from BPS Bioscience (Shanghai, China). L-Ascorbic acid, *α*-ketoglutaric acid sodium salt and ammonium iron (II) sulfate hexahydrate were purchased from Sigma-Aldrich (Shanghai, China). Streptavidin-D2 and protein A-Eu cryptate were purchased from Cisbio (Wuhan, China).

1.1.2. Compound's information

Entry	Compound name	Stock Conc.	Solvent	Start Conc.
1	CDDP	200 mg/mL	DMSO	1 mg/mL
Ref	2,4-Pyridine dicarboxylicacid	200 μmol/L	DMSO	1000 nmol/L

Notes: 2,4-Pyridine dicarboxylicacid (PDCA) was used as a positive control. All test samples were prepared in dimethyl sulfoxide (DMSO). The start concentration for PDCA and CDDP was 1000 nmol/L and 1 mg/mL, respectively. Ten concentration points of each compound were obtained by diluting it from the start concentration at 3-fold with PBS.

1.1.3. Assay procedure

a) Transfer 40 nL compound diluted solution into each well of the assay plate using Echo 550.

b) Seal the assay plate and centrifuge it at 100 × *g* for 1 min.

c) Prepare 2 × KDM4 enzyme solution and add it to each well (4 μL/well) of the assay plate.

d) Seal the assay plate and equilibrate it at room temperature for 30 min.

e) Prepare 2 × KDM4 substrate solution and add it to each well (4 μL/well) of the assay plate.

f) Seal the assay plate and equilibrate it at room temperature for 60 min.

g) Prepare 2 × detection solution by mixing 2.5 μL protein-A-Eu, 0.5 μL anti-histone H3 (dimethyl K9) antibody and 1.5 μL streptavidin-d2, and adding the reaction buffer up to 2 mL. Add 2 × detection solution to each well (8 μL/well) of the assay plate.

h) After 1 h reaction, read the plate to obtain the ratio of fluorescence at 665 nm to that at 615 nm with the Envision plate reader.

1.1.4. Data analysis

Relative ratio (RR): The relative ratio [(ratio 665 nm/615 nm – ratio of background)] is calculated for each well.

% Inhibition: The inhibition (%) is calculated based on the following formula:

$$\%|Inhibiton = \left[1 - \frac{RR_{cmpd} - \overline{RR}_{positive}}{\overline{RR}_{vehicle} - \overline{RR}_{positive}}\right] \times 100$$

$\overline{RR}_{positive}$: The average RR for the positive controls across the plate.

$\overline{RR}_{vehicle}$: The average RR for negative controls across the plate.

Calculation of IC_{50} and plotting the effect-dose curves: The IC_{50} was calculated by fitting % inhibition values and log of compound concentrations to nonlinear regression (dose response–variable slope) with Graphpad 8.0.

Y = Bottom + (Top – Bottom)/(1 + 10^((LogIC_{50} – X) × Hill Slope)

X: $\log_{10}$ of inhibitor concentration; Y: % inhibition

2. Supplemental tables

Table S1 The information of antibodies used in this study.

Antibody	Supplier	Cat no.
BMP3	ABclonal Technology	A6877
NRF2	ABclonal Technology	A0674
HRP–β-Actin	ABclonal Technology	AC028
IL-1β	ABclonal Technology	A11370
p-P65	ABclonal Technology	AP0475
SFRP4	ABclonal Technology	A6409
SFRP5	ABclonal Technology	A16734
SOD2	ABclonal Technology	A19576
WIF1	ABclonal Technology	A12969
DKK2	Affinity	DF12942
NKD1	Affinity	AF4646
Anti-histone H3 (ChIP Grade)	Abcam	ab1220
KDM4A	Proteintech	24943-1-AP
P65	Proteintech	10745-1-AP
LDLR	Proteintech	10785-1-AP
ATGL	Santa Cruz	sc-365278
APOE	Santa Cruz	sc-98574
FASN	Santa Cruz	sc-20140
HSL	Santa Cruz	sc-74489
TNF-α	Santa Cruz	sc-52746

Table S2 The results of the statistical analysis of band density for Western blots in Figs. 3–6.

Fig. 3

Fig. 3F [*P < 0.05, **P < 0.01, ***P < 0.001 *vs.* Lane 2; ^P < 0.05; ^^P < 0.01, ^^^P < 0.001 *vs*. Lane 6; ` = 4]

Gene	Group					
	WT	Ctrl	LST	HST	CDDP	CST
WIF1	100±21***	52±8	49±3^^^	84±11*	109±4***	105±12***
SFRP5	100±37**	34±6	50±9^^	85±11*	80±14*	109±6***
BMP3	100±6*	47±8	152±28**	132±17***	102±12*	187±34***
β-catenin	100±23***	210±36	164±10	160±6*	124±9***	144±12**
SFRP4	100±24*	64±4	66±12^	101±4*	96±2*	96±4*
NKD1	100±35*	22±7	60±20^^^	78±26	172±26***	200±45***
DKK2	100±13*	68±15	100±9*	101±10*	144±14***	116±10**
Lane	1	2	3	4	5	6

Fig. 4

Fig. 4B [*P < 0.05, **P < 0.01, ***P < 0.001 *vs.* Lane 2; ^P < 0.05, ^^P < 0.01 *vs*. Lane 6; n = 4]

Gene	Group					
	WT	Ctrl	LST	HST	CDDP	CST
KDM4A	100±16**	282±51	244±29	286±79	101±72**	151±15*
P65	100±22***	376±84	322±30^^	225±34*	221±48*	159±47***
p-P65	100±19***	492±25	185±37***	150±33***	112±28***	147±64***
IL-1β	100±14**	156±14	146±10^	111±21*	112±19*	103±19**
TNF-α	100±13***	260±19	165±41**^	117±34***	117±38***	72±16***
Lane	1	2	3	4	5	6

Fig. 4G [*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *vs.* Lane 2; $n = 3$]

Cell	Group			
	OGD	–	+	+
	CDDP	–	–	+
H9c2	KDM4A	100±2*	140±19	72±12**
	P65	100±4	100±11	102±6
	p-P65	100±4**	196±10	122±16*
	IL-1β	100±19*	187±7	133±16**
	TNF-α	100±3***	153±23	78±6***
iPSC-CM	KDM4A	100±1*	149±26	50±4**
	P65	100±18	98±8	91±5
	p-P65	100±18*	188±11	77±3**
	IL-1β	100±12*	148±14	72±4*
	TNF-α	100±10**	192±19	76±26**
	Lane	1	2	3

Fig. 4H [**$P < 0.01$, ***$P < 0.001$ *vs.* Lane 2; ^^$P < 0.01$, ^^^$P < 0.001$ *vs.* Lane 5; $n = 3$]

RAW 264.7					
OGD	–	+	+	+	+
CDDP	–	–	–	+	+
PDCA	–	–	+	–	+
KDM4A	100±4 ***	169±8	110±5 ***	100±1 ***^^	106±8 ***
P65	100±9	101±5	99±6	103±5	100±11
p-P65	100±9 ***	176±4	146±1 ***^^	114±3 ***	124±1 ***
IL-1β	100±4 **	131±4	99±5 **	95±5 **	80±10 ***
TNF-α	100±7 ***	242±4	187±4 ***	89±7 ***^^^	140±22 ***
Lane	1	2	3	4	5

Fig. 4J [*$P < 0.05$, **P < 0.01, ***$P < 0.001$ *vs.* Lane 2; ^$P < 0.05$, ^^$P < 0.01$ *vs.* Lane 5; $n = 3$]

RAW 264.7					
OGD	–	+	+	+	+
CDDP	–	–	–	+	+
si*KDM4A*	–	–	+	–	+
si*Ctrl*	+	+	–	+	–
KDM4A	100±10 **	151±8	106±1 *	76±7 ***	85±11 ***
P65	100±7	102±4	101±2	106±7	101±8
p-P65	100±6 ***	189±10	164±6 **^^	150±10 **^	124±4 ***
IL-1β	100±4 ***	234±10	170±16 *	130±19 *^	117±23 ***
TNF-α	100±4 **	140±6	100±3 **	77±14 ***	76±8 ***
Lane	1	2	3	4	5

Fig. 5

Fig. 5D [*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *vs.* Lane 2; ^$P < 0.05$, ^^$P < 0.01$ *vs*. Lane 6; $n = 4$]

Gene	Group					
	WT	Ctrl	LST	HST	CDDP	CST
NRF2	100±11 *	66±5	80±10 ^	96±9	135±21 ***	117±12 **
CAT	100±3 *	48±4	82±4 ^^	115±19 *	138±40 ***	151±18 ***
SOD2	100±20 *	49±4	72±4 ^	100±16 *	120±34 **	131±15 ***
Lane	1	2	3	4	5	6

Fig. 5G [*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *vs.* Lane 2; $n = 3$]

Cell	Group			
	OGD	–	+	+
	CDDP	–	–	+
H9c2	NRF2	100±6***	26±3	49±4**
	CAT	100±7***	42±7	76±6**
	SOD2	100±21**	26±14	57±12*
iPSC-CM	NRF2	100±17*	51±14	126±12**
	CAT	100±18*	59±6	126±11**
	SOD2	100±14**	55±1	106±16**
	Lane	1	2	3

Fig. 5H [*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *vs.* Lane 2; ^^$P < 0.01$, ^^^$P < 0.001$ *vs.* Lane 5, $n = 3$]

H9c2					
OGD	–	+	+	+	+
CDDP	–	–	–	+	+
PDCA	–	–	+	–	+
KDM4A	100±5**	127±8	98±3**	93±8**	90±1***
NRF2	100±7*	79±2	187±9***	180±3***	214±7***
CAT	100±3***	75±3	87±4*^^^	100±2**^^	127±10***
SOD2	100±16***	17±5	145±20***	118±8***	119±10***
Lane	1	2	3	4	5

Fig. 5J [*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *vs.* Lane 2; ^^^$P < 0.001$ *vs.* Lane 5; $n = 3$]

H9c2					
OGD	–	+	+	+	+
CDDP	–	–	–	+	+
PDCA	–	–	+	–	+
NRF2	100±5 *	82±2	183±1 ***	108±10 **^^^	197±1 ***
Lane	1	2	3	4	5

Fig. 5K [*$P < 0.05$, ***$P < 0.001$ *vs.* Lane 2; ^$P < 0.05$, ^^$P < 0.01$, ^^^$P < 0.001$ *vs.* Lane 5; $n = 3$]

H9c2					
OGD	–	+	+	+	+
CDDP	–	–	–	+	+
si*KDM4A*	–	–	+	–	+
si*Ctrl*	+	+	–	+	–
KDM4A	100±4 ***	202±8	115±7 ***	146±7 ***^	120±6 ***
NRF2	100±10 ***	22±1	86±4 ***	90±7 ***^^	116±4 ***^
CAT	100±4 ***	29±2	122±2 ***	137±4 ***^^	147±9 ***
SOD2	100±5 *	74±3	215±6 ***	174±4 ***^^^	383±8 ***^^^
Lane	1	2	3	4	5

Fig. 6

Fig. 6E [*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *vs.* Lane 2; $n = 4$]

Gene	Group					
	WT	Ctrl	LST	HST	CDDP	CST
FASN	100±12 **	150±11	107±20 *	112±10 *	88±13 ***	107±16 *
SCD1	100±22 ***	502±66	145±15 ***	150±30 ***	77±15 ***	96±37 ***
DGAT	100±5 ***	309±40	242±44	197±26 *	186±42 **	167±37 **
HSL	100±26 *	63±5	74±7	84±9	102±10 *	101±8 *
ATGL	100±3	85±5	162±24 *	259±10 *	258±41 ***	244±2 **
Lane	1	2	3	4	5	6

Fig. 6F [*P < 0.05, **P < 0.01, ***P < 0.001 *vs.* Lane 2; ^^^P < 0.001 *vs*. Lane 6; n = 4]

Gene	Group					
	WT	Ctrl	LST	HST	CDDP	CST
ABCG5	100±9	30±2	66±5	68±8	116±6	124±13
	***		***^^^	***	***	***
CYP7A1	100±13	54±6	66±14	388±8	84±10	151±9
	***		^^^	**	*	***
Lane	1	2	3	4	5	6

Table S3 The results of statistical analysis of band density for Western blots in Supplementary Figs. S4, S5 and S7.

Fig. S4 (Fig. S4C, D)**:** [*P < 0.05, **P < 0.01, ***P < 0.001 *vs.* Lane 2; n = 3]

Cell	Group			
	OGD	-	+	+
	CDDP	-	-	+
H9c2	SFRP4	100±18*	48±11	48±14*
	SFRP5	100±28*	39±6	93±12*
	BMP3	100±21	63±14	141±45*
	WIF1	100±11*	24±5	68±23*
	NKD1	100±15	70±8	173±29**
	β-catenin	100±34*	178±25	105±10*
	DKK2	100±6*	58±4	115±21**
iPSC-CM	SFRP4	100±8**	22±13	61±23
	SFRP5	100±20*	50±7	110±2**
	WIF1	100±23*	31±6	80±10*
	BMP3	100±21	63±14	141±45
	NKD1	100±11**	13±1	73±32*
	DKK2	100±9***	43±5	69±8*
	β-catenin	100±11*	12±1	28±2
	Lane	1	2	3

Fig. S5 (Fig. S5I) [*P < 0.05, **P < 0.01, ***P < 0.001 *vs.* Lane 2; ^P < 0.05, ^^P < 0.01 *vs.* Lane 5; n = 3]

RAW 264.7					
OGD	–	+	+	+	+
DSDF	–	–	–	+	+
PDCA	–	–	+	–	+
KDM4A	100±4**	125±4	95±3*	83±10***	72±4***
p-P65	100±1***	113±3	93±6***^^	67±1**^	93±3***
P65	100±10	102±2	103±4	96±12	108±4
IL-1β	100±6***	130±3	85±8*^	87±6***	96±1***
TNF-α	100±7**	134±2	111±5**	107±8***	74±1***
Lane	1	2	3	4	5

Fig. S5 (Fig. S5K) [***P < 0.001 *vs.* Lane 2; ^P < 0.05, ^^P < 0.01, ^^^P < 0.001 *vs.* Lane 5; n = 3]

RAW 264.7					
OGD	–	+	+	+	+
DSDF	–	–	–	+	+
si*KDM4A*	–	–	+	–	+
si*Ctrl*	+	+	–	+	–
KDM4A	100±2 ***	133±4	92±6 ***^^^	66±6 ***	61±1 ***
p-P65	100±1 ***	155±1	130±5 ***	120±2 ***^	135±3 ***
P65	100±1	105±2	95±2	103±3	95±8
IL-1β	100±4 ***	152±5	129±4 ***	111±3 ***^^	133±3 ***
TNF-α	100±4 ***	190±5	134±2 ***^^	144±6 ***^^^	106±8 ***
Lane	1	2	3	4	5

Fig. S5 (Fig. S5L) [*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *vs.* Lane 2; ^$P < 0.05$ *vs.* Lane 5; $n = 3$]

RAW 264.7					
OGD	–	+	+	+	+
XST	–	–	–	+	+
PDCA	–	–	+	–	+
KDM4A	100±14 ***	447±31	334±15 ***	268±14 ***^	344±8 **
p-P65	100±2 ***	205±12	146±9 ***^	177±4 *	178±3 *
P65	100±3	103±5	106±4	104±4	105±3
IL-1β	100±3 ***	180±3	96±10 ***	132±13 **^	87±17 ***
TNF-α	100±8 ***	303±16	203±10 ***	179±4 ***	210±14 ***
Lane	1	2	3	4	5

Fig. S5 (Fig. S5N) [*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *vs.* Lane 2; $n = 3$]

RAW 264.7					
OGD	–	+	+	+	+
XST	–	–	–	+	+
si*KDM4*A	–	–	+	–	+
si*Ctrl*	+	+	–	+	–
KDM4A	100±7 ***	186±1	169±1 *	139±8 ***^^	163±1 **
p-P65	100±8 ***	181±6	151±1 **	147±5 ***	162±5 *
P65	100±10	107±7	105±7	108±7	107±2
IL-1β	100±7 ***	146±1	120±3 *	111±11 **	125±3 *
TNF-α	100±3 ***	155±1	96±3 ***	86±6 ***	98±5.2 ***
Lane	1	2	3	4	5

Fig. S7 (Fig. S7C) [**$P < 0.01$, ***$P < 0.001$ *vs.* Lane 1; $n = 3$]

H9c2						
PDCA	0	0.25	0.5	1	2	4
NRF2	100±2	80±10	105±3	133±9**	139±10**	156±3***
KEAP1	100±3	104±7	88±5	75±2***	75±4**	90±3
Lane	1	2	3	4	5	6

Fig. S7 (Fig. S7D) [*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *vs.* Lane 2; ^$P < 0.05$, ^^$P < 0.01$, ^^^$P < 0.001$ *vs.* Lane 5; $n = 3$]

H9c2					
OGD	–	+	+	+	+
DSDF	–	–	–	+	+
PDCA	–	–	+	–	+
KDM4A	100±4**	126±8	87±9***^^	57±2***	48±2***
NRF2	100±9*	71±5	113±5*	161±6***^	131±9***
CAT	100±3***	30±1	66±2***	93±5***^	59±3***
SOD2	100±5*	74±10	77±3^^^	249±12***^^^	163±13***
Lane	1	2	3	4	5

Fig. S7 (Fig. S7E) [*$P < 0.05$, ***$P < 0.001$ *vs.* Lane 2; ^^^$P < 0.001$, *vs.* Lane 5; $n = 3$]

H9c2					
OGD	–	+	+	+	+
DSDF	–	–	–	+	+
PDCA	–	–	+	–	+
NRF2	100±4*	86±1	240±9***^^^	243±12***^^^	371±10***
Lane	1	2	3	4	5

Fig. S7 (Fig. S7G) [*$P < 0.05$, ***$P < 0.001$ *vs.* Lane 2; ^$P < 0.05$, ^^$P < 0.01$, ^^^$P < 0.001$ *vs.* Lane 5; $n = 3$]

H9c2					
OGD	–	+	+	+	+
DSDF	–	–	–	+	+
si*KDM4A*	–	–	+	–	+
si*Ctrl*	+	+	–	+	–
KDM4A	100±1 ***	164±9	132±10 ***	122±4 ***^	97±3 ***
NRF2	100±1 ***	26±1	107±3 ***^^	86±3 ***^	112±14 ***
CAT	100±7 ***	32±2	128±3 ***^^	113±4 ***	118±2 ***
SOD2	100±4 *	19±3	62±2 ***^^^	49±5 ***^^^	73±3 ***
Lane	1	2	3	4	5

Fig. S7 (Fig. S7H) [*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ *vs.* Lane 2; ^$P < 0.05$, ^^^$P < 0.001$ *vs.* Lane 5; $n = 3$]

H9c2					
OGD	–	+	+	+	+
XST	–	–	–	+	+
PDCA	–	–	+	–	+
KDM4A	100±4.6 *	128±4	96±8 *^	68±6 ***	66±13 ***
NRF2	100±6 *	69±3	110±5 **^^^	179±4 ***	198±16 ***
CAT	100±3 ***	25±3	65±9 **	74±9 ***	81±10 ***
SOD2	100±5 ***	41±1	118±5 ***	136±15 ***	138±13 ***
Lane	1	2	3	4	5

Fig. S7 (Fig. S7I) [*P < 0.05, **P < 0.01, ***P < 0.001 *vs.* Lane 2; ^^^P < 0.001 *vs.* Lane 5; n = 3]

H9c2					
OGD	–	+	+	+	+
XST	–	–	–	+	+
PDCA	–	–	+	–	+
NRF2	100±3 *	54±2	131±20 ***^^^	123±3 **^^^	223±17 ***
Lane	1	2	3	4	5

Fig. S7 (Fig. S7K) [***P < 0.001 *vs.* Lane 2; ^P < 0.05, ^^^P < 0.001 *vs.* Lane 5; n = 3]

H9c2					
OGD	–	+	+	+	+
XST	–	–	–	+	+
si*KDM4A*	–	–	+	–	+
si*Ctrl*	+	+	–	+	–
KDM4A	100±6 ***	198±9	88±1 ***	45± 3***^	95±8 ***
NRF2	100±12 ***	33±1	19±4 ***	73±4 ***^	93±1 ***
CAT	100±7 ***	17±1	136±4 ***^^^	141±4 ***^^^	193±4 ***
SOD2	100±5 ***	74±3	215±6 ***^^^	174±4 ***^^^	383±8 ***
Lane	1	2	3	4	5

Fig. S7 (Fig. S7L) [*P < 0.05, **P < 0.01, ***P < 0.001 *vs.* Lane 2; ^^P < 0.01, ^^^P < 0.001 *vs*. Lane 6; n = 4]

Gene	Group					
	WT	Ctrl	LST	HST	CDDP	CST
KEAP1	100±15 ***	283±51	244±29 ^^^	286±78 ^^	101±72 **	151±14 *
Lane	1	2	3	4	5	6

Fig. S7 (Fig. S7M) [*P < 0.05, ***P < 0.001 *vs.* Lane 2; ^^^P < 0.001 *vs.* Lane 5; n = 3]

H9c2					
OGD	–	+	+	+	+
CDDP	–	–	–	+	+
PDCA	–	–	+	–	+
KEAP1	100±3*	121±2	101±12*^^^	47±2***	40±2***
Lane	1	2	3	4	5

Fig. S7 (Fig. S7N) [**P < 0.01, ***P < 0.001 *vs.* Lane 2; ^^^P < 0.001, *vs.* Lane 5; n = 3]

H9c2					
OGD	–	+	+	+	+
DSDF	–	–	–	+	+
PDCA	–	–	+	–	+
KEAP1	100±3 **	125±1	59±5 ***^^^	73±8 ***^^^	15±1 ***
Lane	1	2	3	4	5

Fig. S7 (Fig. S7O) [**$P < 0.01$, ***$P < 0.001$ *vs.* Lane 2; ^$P < 0.05$ *vs.* Lane 5; $n = 3$]

H9c2					
OGD	–	+	+	+	+
XST	–	–	–	+	+
PDCA	–	–	+	–	+
KEAP1	100±1	129±5	60±5	90±12	63±4
	**		***	***^	***
Lane	1	2	3	4	5

Table S4 The sequences of primers for qRT-PCR analysis of mouse genes.

Genes	Primers	Sequence (5′ –3′)	Product (bp)
Bmp3	forward	ACTCCGTGAGACTGAGCCAA	106
	reverse	CCTGTCATAGAGCCACAGCATA	
Cat	forward	AGCGACCAGATGAAGCAGTG	181
	reverse	TCCGCTCTCTGTCAAAGTGTG	
Dkk2	forward	CTGATGCGGGTCAAGGATTCA	126
	reverse	CTCCCCTCCTAGAGAGGACTT	
Il-1β	forward	GCAACTGTTCCTGAACTCAACT	89
	reverse	ATCTTTTGGGGTCCGTCAACT	
Kdm4a	forward	GACATAGTGAGTCAGGACTGTCT	105
	reverse	GGCCACAAACTTAGCCCCATA	
Nkd1	forward	AGGAAAGGCATCGAGGAGTG	172
	reverse	TCGCTCAGTCTCTCCATTCTC	
Nrf2	forward	TCTTGGAGTAAGTCGAGAAGTGT	140
	reverse	GTTGAAACTGAGCGAAAAAGGC	
p65	forward	AGGCTTCTGGGCCTTATGTG	111
	reverse	TGCTTCTCTCGCCAGGAATAC	
Sfrp4	forward	AGAAGGTCCATACAGTGGGAAG	102
	reverse	GTTACTGCGACTGGTGCGA	
Sfrp5	forward	CACTGCCACAAGTTCCCCC	139
	reverse	TCTGTTCCATGAGGCCATCAG	
Sod2	forward	CAGACCTGCCTTACGACTATGG	113
	reverse	CTCGGTGGCGTTGAGATTGTT	
Tnf-α	forward	GACGTGGAACTGGCAGAAGAG	228
	reverse	TTGGTGGTTTGTGAGTGTGAG	
Wif1	forward	TCTGGAGCATCCTACCTTGC	106
	reverse	ATGAGCACTCTAGCCTGATGG	
Gapdh	forward	TGTGTCCGTCGTGGATCTGA	150
	reverse	TTGCTGTTGAAGTCGCAGGAG	

Table S5 The detailed information of 45 FDA-approved drugs for CHD.

Drug Name	Drug Bank ID	MoAs for CHD	SMILE
Benazepril	DB00542	ACE inhibitors	CCOC(=O)C(CCC1=CC=CC=C1)NC2CCC3=CC=CC=C3N(C2=O)CC(=O)O
Captopril	DB01197	ACE inhibitors	CC(CS)C(=O)N1CCCC1C(=O)O
Enalapril	DB00584	ACE inhibitors	CCOC(=O)C(CCC1=CC=CC=C1)NC(C)C(=O)N2CCCC2C(=O)O
Fosinopril	DB00492	ACE inhibitors	CCC(=O)OC(C(C)C)OP(=O)(CCCCC1=CC=CC=C1)CC(=O)N2CC(CC2C(=O)O)C3CCCCC3
Lisinopril	DB00722	ACE inhibitors	C1CC(N(C1)C(=O)C(CCCCN)NC(CCC2=CC=CC=C2)C(=O)O)C(=O)O
Perindopril	DB00790	ACE inhibitors	CCCC(C(=O)OCC)NC(C)C(=O)N1C2CCCCC2CC1C(=O)O
Quinapril	DB00881	ACE inhibitors	CCOC(=O)C(CCC1=CC=CC=C1)NC(C)C(=O)N2CC3=CC=CC=C3CC2C(=O)O
Ramipril	DB00178	ACE inhibitors	CCOC(=O)C(CCC1=CC=CC=C1)NC(C)C(=O)N2C3CCCC3CC2C(=O)O
Trandolapril	DB00519	ACE inhibitors	CCOC(=O)C(CCC1=CC=CC=C1)NC(C)C(=O)N2C3CCCCC3CC2C(=O)O
Azilsartan medoxmil	NA	Angiotensin II receptor blockers	CCOC1=NC2=C(N1CC1=CC=C(C=C1)C1=CC=CC=C1C1=NOC(=O)N1)C(=CC=C2)C(=O)OCC1=C(C)OC(=O)O1
Candesartan	DB13919	Angiotensin II receptor blockers	CCOC1=NC2=CC=CC(=C2N1CC3=CC=C(C=C3)C4=CC=CC=C4C5=NNN=N5)C(=O)O
Eprosartan	DB00876	Angiotensin II receptor blockers	CCCCC1=NC=C(N1CC2=CC=C(C=C2)C(=O)O)C=C(CC3=CC=CS3)C(=O)O
Olmesartan	DB00275	Angiotensin II receptor blockers	CCCC1=NC(=C(N1CC2=CC=C(C=C2)C3=CC=CC=C3C4=NNN=N4)C(=O)O)C(C)(C)O
Irbesartan	DB01029	Angiotensin II receptor blockers	CCCCC1=NC2(CCCC2)C(=O)N1CC3=CC=C(C=C3)C4=CC=CC=C4C5=NNN=N5

Drug Name	Drug Bank ID	MoAs for CHD	SMILE
Losartan	DB00678	Angiotensin II receptor blockers	CCCCC1=NC(=C(N1CC2=CC=C(C=C2)C3=CC=CC=C3C4=NNN=N4)CO)Cl
Telmisartan	DB00966	Angiotensin II receptor blockers	CCCC1=NC2=C(N1CC3=CC=C(C=C3)C4=CC=CC=C4C(=O)O)C=C(C=C2C)C5=NC6=CC=CC=C6N5C
Valsartan	DB00177	Angiotensin II receptor blockers	CCCCC(=O)N(CC1=CC=C(C=C1)C2=CC=CC=C2C3=NNN=N3)C(C(C)C)C(=O)O
Aspirin	DB00945	Antiplatelet	CC(=O)OC1=CC=CC=C1C(=O)O
Clopidogrel	DB00758	Antiplatelet	COC(=O)C(C1=CC=CC=C1Cl)N2CCC3=C(C2)C=CS3
Warfarin	DB00682	Antithrombotic	CC(=O)CC(C1=CC=CC=C1)C2=C(C3=CC=CC=C3OC2=O)O
Atenolol	DB00335	Beta blocking agents	CC(C)NCC(COC1=CC=C(C=C1)CC(=O)N)O
Carvedilol	DB01136	Beta blocking agents	COC1=CC=CC=C1OCCNCC(COC2=CC=CC3=C2C4=CC=CC=C4N3)O
Labetalol	DB00598	Beta blocking agents	CC(CCC1=CC=CC=C1)NCC(C2=CC(=C(C=C2)O)C(=O)N)O
Metoprolol	DB00264	Beta blocking agents	CC(C)NCC(COC1=CC=C(C=C1)CCOC)O
Propranolol	DB00571	Beta blocking agents	CC(C)NCC(COC1=CC=CC2=CC=CC=C21)O
Timolol	DB00373	Beta blocking agents	CC(C)(C)NCC(COC1=NSN=C1N2CCOCC2)O
Amlodipine	DB00381	Calcium channel blockers	CCOC(=O)C1=C(NC(=C(C1C2=CC=CC=C2Cl)C(=O)OC)C)COCCN
Bepridil	DB01244	Calcium channel blockers	CC(C)COCC(CN(CC1=CC=CC=C1)C2=CC=CC=C2)N3CCCC3
Diltiazem	DB00343	Calcium channel blockers	CC(=O)OC1C(SC2=CC=CC=C2N(C1=O)CCN(C)C)C3=CC=C(C=C3)OC
Felodipine	DB01023	Calcium channel blockers	CCOC(=O)C1=C(NC(=C(C1C2=C(C(=CC=C2)Cl)Cl)C(=O)OC)C)C

Drug Name	Drug Bank ID	MoAs for CHD	SMILE
Nicardipine	DB00622	Calcium channel blockers	CC1=C(C(C(=C(N1)C)C(=O)OCCN(C)CC2=CC=CC=C2)C3=CC(=CC=C3)[N+](=O)[O-])C(=O)OC
Nifedipine	DB01115	Calcium channel blockers	CC1=C(C(C(=C(N1)C)C(=O)OC)C2=CC=CC=C2[N+](=O)[O-])C(=O)OC
Nisoldipine	DB00401	Calcium channel blockers	CC1=C(C(C(=C(N1)C)C(=O)OCC(C)C)C2=CC=CC=C2[N+](=O)[O-])C(=O)OC
Verapamil	DB00661	Calcium channel blockers	CC(C)C(CCCN(C)CCC1=CC(=C(C=C1)OC)OC)(C#N)C2=CC(=C(C=C2)OC)OC
Dihydropyridine	NA	Calcium channel blockers	C1C=CC=CN1
Nitroglycerin	DB00727	Dilated coronary artery	C(C(CO[N+](=O)[O-])O[N+](=O)[O-])O[N+](=O)[O-]
Isosorbide mononitrate	DB01020	Dilated coronary artery	C1C(C2C(O1)C(CO2)O[N+](=O)[O-])O
Isosorbide dinitrate	DB00883	Dilated coronary artery	C1C(C2C(O1)C(CO2)O[N+](=O)[O-])O[N+](=O)[O-]
Atorvastatin	DB01076	Lipid modifying agents	CC(C)C1=C(C(=C(N1CCC(CC(CC(=O)O)O)O)C2=CC=C(C=C2)F)C3=CC=CC=C3)C(=O)NC4=CC=CC=C4
Rosuvastatin	DB01098	Lipid modifying agents	CC(C)C1=NC(=NC(=C1C=CC(CC(CC(=O)O)O)O)C2=CC=C(C=C2)F)N(C)S(=O)(=O)C
Spironolactone	DB00421	Mineralocorticoid receptor antagonist	CC(=O)SC1CC2=CC(=O)CCC2(C3C1C4CCC5(C4(CC3)C)CCC(=O)O5)C
Eplerenone	DB00700	Mineralocorticoid receptor antagonists	CC12CCC(=O)C=C1CC(C3C24C(O4)CC5(C3CCC56CCC(=O)O6)C)C(=O)OC
Dapagliflozin	DB06292	SGLT2 inhibitor	CCOC1=CC=C(C=C1)CC2=C(C=CC(=C2)C3C(C(C(C(O3)CO)O)O)O)Cl
Empagliflozin	DB09038	SGLT2 inhibitor	C1COCC1OC2=CC=C(C=C2)CC3=C(C=CC(=C3)C4C(C(C(C(O4)CO)O)O)O)Cl
Canagliflozin	DB08907	SGLT2 inhibitor	CC1=C(C=C(C=C1)C2C(C(C(C(O2)CO)O)O)O)CC3=CC=C(S3)C4=CC=C(C=C4)F

Table S6 The information of 22 CDDP components including the bioactive equivalent components and the components detectable in patients' blood.

Component	Class	SMILE
Cryptotanshinone	Bioactive Equivalent Component	CC1COC2=C1C(=O)C(=O)C3=C2C=CC4=C3CCCC4(C)C
Dihydrotanshinone I	Bioactive Equivalent Component	CC1COC2=C1C(=O)C(=O)C3=C2C=CC4=C3C=CC=C4C
Ginsenoside-Rd	Bioactive Equivalent Component	CC(=CCCC(C)(C1CCC2(C1C(CC3C2(CCC4C3(CCC(C4(C)C)OC5C(C(C(C(O5)CO)O)O)OC6C(C(C(C(O6)CO)O)O)O)O)C)C)O)C)OC7C(C(C(C(O7)CO)O)O)O)C
Ginsenoside-Rh[1]	Bioactive Equivalent Component	CC(=CCCC(C)(C1CCC2(C1C(CC3C2(CC(C4C3(CCC(C4(C)C)O)C)OC5C(C(C(C(O5)CO)O)O)O)C)O)C)O)C
Lithospermic acid	Bioactive Equivalent Component	C1=CC(=C(C=C1CC(C(=O)O)OC(=O)C=CC2=C3C(C(OC3=C(C=C2)O)C4=CC(=C(C=C4)O)O)C(=O)O)O)O
Rosmarinic acid	Bioactive Equivalent Component	C1=CC(=C(C=C1CC(C(=O)O)OC(=O)C=CC2=CC(=C(C=C2)O)O)O)O
Salvianolic acid A	Bioactive Equivalent Component	C1=CC(=C(C=C1CC(C(=O)O)OC(=O)C=CC2=C(C(=C(C=C2)O)O)C=CC3=CC(=C(C=C3)O)O)O)O
Salvianolic acid B	Bioactive Equivalent Component	C1=CC(=C(C=C1CC(C(=O)O)OC(=O)C=CC2=C3C(C(OC3=C(C=C2)O)C4=CC(=C(C=C4)O)O)C(=O)OC(CC5=CC(=C(C=C5)O)O)C(=O)O)O)O
Salvianolic acid D	Bioactive Equivalent Component	C1=CC(=C(C=C1CC(C(=O)O)OC(=O)C=CC2=C(C(=C(C=C2)O)O)CC(=O)O)O)O
Salvianolic acid G	Bioactive Equivalent Component	C1=CC(=C(C=C1CC(C(=O)O)OC(=O)C=CC2=C(C(=C(C=C2)O)O)CC(=O)O)O)O
Tanshinone I	Bioactive Equivalent Component	CC1=CC=CC2=C1C=CC3=C2C(=O)C(=O)C4=C3OC=C4C
Tanshinone IIA	Bioactive Equivalent Component	CC1=COC2=C1C(=O)C(=O)C3=C2C=CC4=C3CCCC4(C)C
Borneol	Blood Component[1]	CC1(C2CCC1(C(C2)O)C)C
Caffeic acid	Blood Component[1]	C1=CC(=C(C=C1C=CC(=O)O)O)O
Isoborneol	Blood Component[1]	CC1(C2CCC1(C(C2)O)C)C
Notoginsenoside R[1]	Blood Component[1]	CC(=CCCC(C)(C1CCC2(C1C(CC3C2(CC(C4C3(CCC(C4(C)C)O)C)OC5C(C(C(C(O5)CO)O)O)OC6C(C(C(CO6)O)O)O)C)O)C)OC7C(C(C(C(O7)CO)O)O)O)C
Protocatechuic acid	Blood Component[1]	C1=CC(=C(C=C1C(=O)O)O)O
Tanshinone IIB	Blood Component[1]	CC1=COC2=C1C(=O)C(=O)C3=C2C=CC4=C3CCCC4(C)CO
Danshensu	Both[2]	C1=CC(=C(C=C1CC(C(=O)O)O)O)O
Ginsenoside-Rb[1]	Both[2]	CC(=CCCC(C)(C1CCC2(C1C(CC3C2(CCC4C3(CCC(C4(C)C)OC5C(C(C(C(O5)CO)O)O)OC6C(C(C(C(O6)C

Component	Class	SMILE
		O)O)O)O)C)C)O)C)OC7C(C(C(C(O7)COC8C(C(C(C(O8)CO)O)O)O)O)O)O)C
Ginsenoside-Rg[1]	Both[2]	CC(=CCCC(C)(C1CCC2(C1C(CC3C2(CC(C4C3(CCC(C4(C)C)O)C)OC5C(C(C(C(O5)CO)O)O)O)C)O)C)OC6C(C(C(C(O6)CO)O)O)O)C
Protocatechuic aldehyde	Both[2]	C1=CC(=C(C=C1C=O)O)O

[1]represent the CDDP components which are detectable in patients' blood after CDDP administration.

[2]represent the CDDP components which are bioactive equivalent component and detectable in patients' blood after CDDP administration.

3.Supporting figures

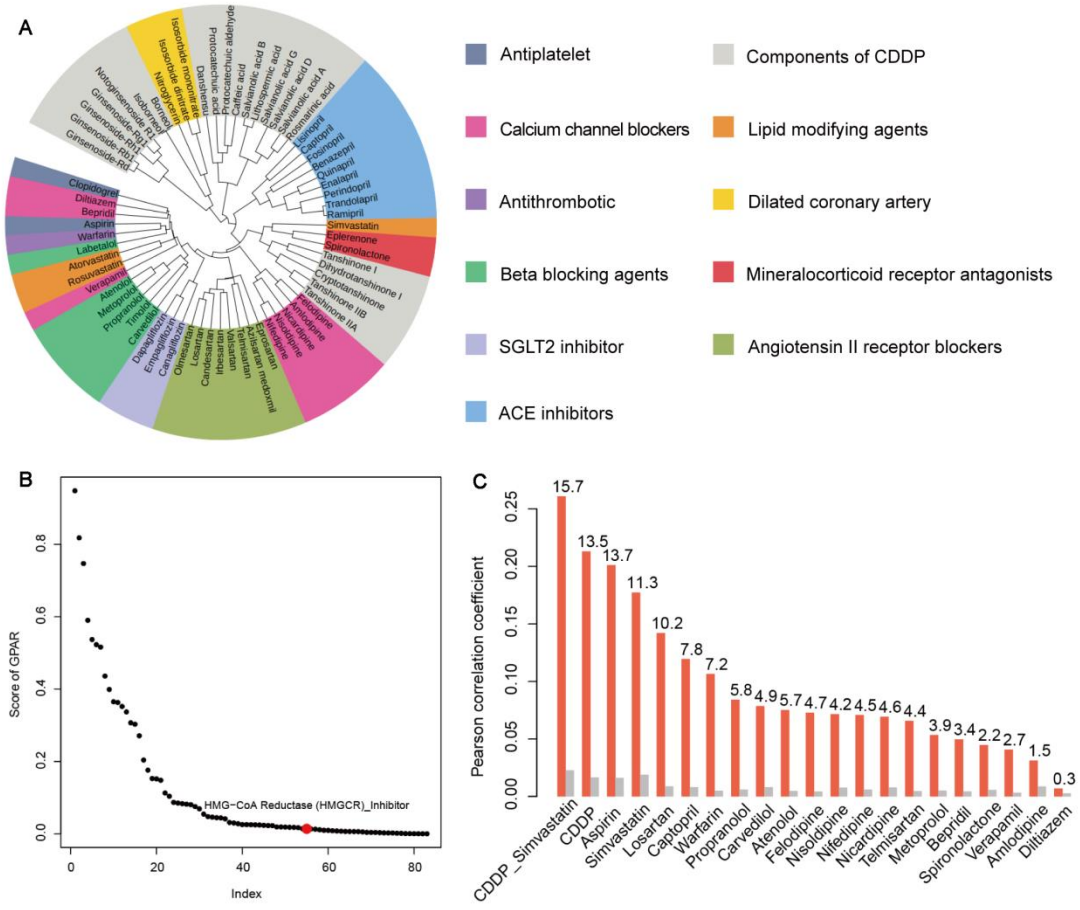

Figure S1 Identification of the pharmacological mechanisms of CDDP on CHD through computational systematic pharmacology. (A) Based on the similarity of chemical structure, the unsupervised hierarchical clustering of the CDDP components and FDA-approved drugs for CHD treatment was performed. Ten types of different mechanisms of action (MoAs) of drugs were highlighted by different colors, and CDDP components were marked by grey color. (B) Prediction of MoAs for CDDP using GPAR platform based on CDDP-regulated gene expression profiles. (C) Correlation between CDDP and FDA-approved drugs for CHD treatment was determined by calculating the Pearson correlation coefficient between targets of CDDP and FDA-approved drugs for CHD treatment and genes related to CHD. CDDP_Simvastain represents the union of targets between CDDP and simvastatin. The significance of correlation was evaluated by calculation of z-score and labeled on the top of each bar. CHD: coronary heart disease; GPAR: Genetic Profileactivity Relationship.

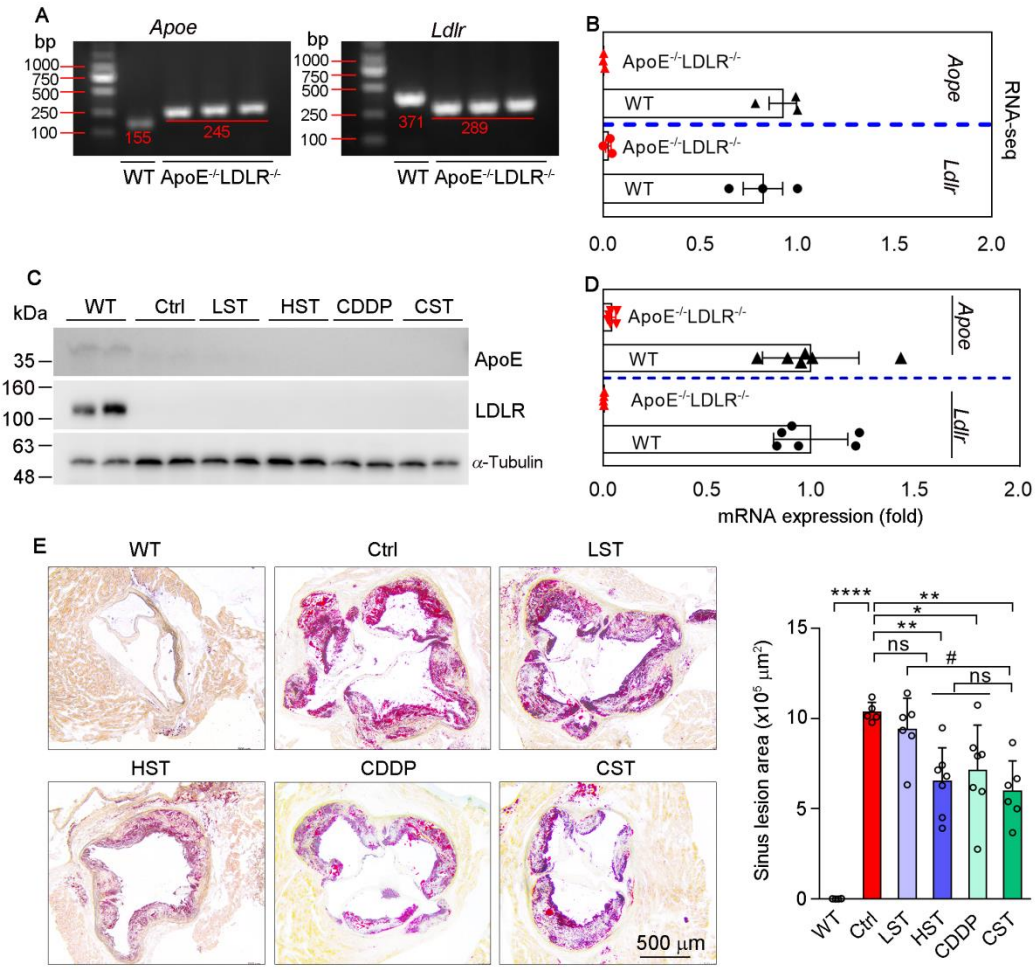

Figure S2 CDDP reduces HFD-induced atherosclerosis in ApoE$^{-/-}$LDLR$^{-/-}$ mice. (A) The genotype of ApoE$^{-/-}$LDLR$^{-/-}$ mouse was confirmed by PCR genotyping of tail DNA. (B) Expressions of ApoE and LDLR in WT and ApoE$^{-/-}$LDLR$^{-/-}$ mice were determined by RNA-seq with mouse heart samples (3/group). (C, D) Expressions of ApoE and LDLR protein or mRNA in heart samples were determined by Western blot or qRT-PCR (4–5/group). (E) Aortic root cross sections prepared from mice in Fig. 1 were conducted oil red O staining with quantitative analysis of lesion area (n = 5–7). The data are shown as the mean ± SEM. *P < 0.05, **P < 0.01, ****P < 0.0001; $^{\#}P$ < 0.05, ns: not significant between indicated groups.

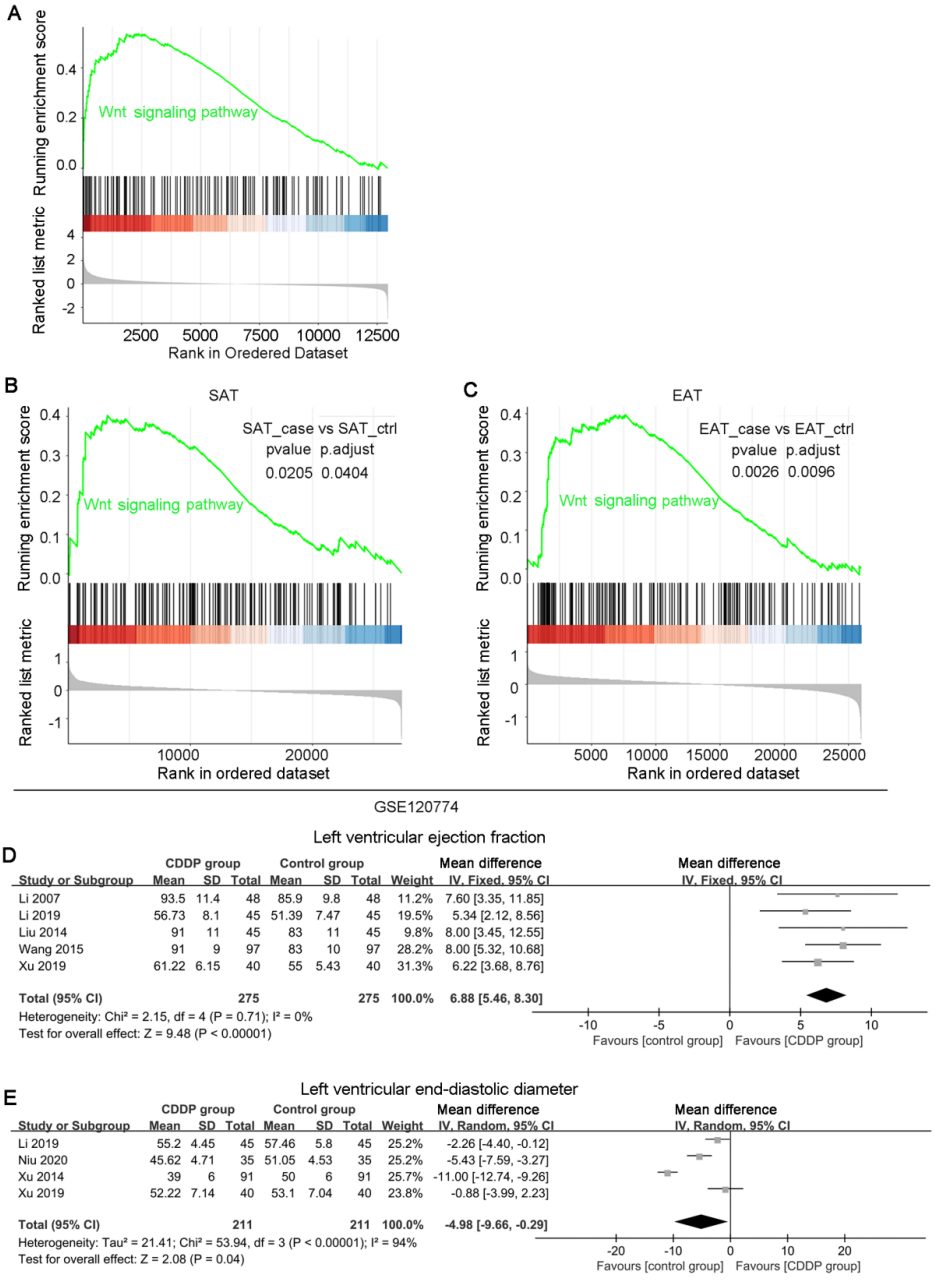

Figure S3 Protection against myocardial damage by CDDP is related to inactivating Wnt pathway in mice and CHD patients. GSEA analysis shows significant positive enrichment of Wnt signaling pathway gene set in expression profiles for HFD-fed ApoE$^{-/-}$LDLR$^{-/-}$ mice or CHD patients. (A) HFD-fed ApoE$^{-/-}$LDLR$^{-/-}$ mouse heart tissue; (B, C) Subcutaneous adipose tissue (SAT) from a site adjacent to the right coronary artery and epicardial adipose tissue (EAT) of CHD patients. (D, E) Meta-analysis of left ventricular ejection fraction, left ventricular end diastolic diameter of CHD patients receiving co-treatment of CDDP and statins. CHD: coronary heart disease; GSEA: gene set enrichment analysis.

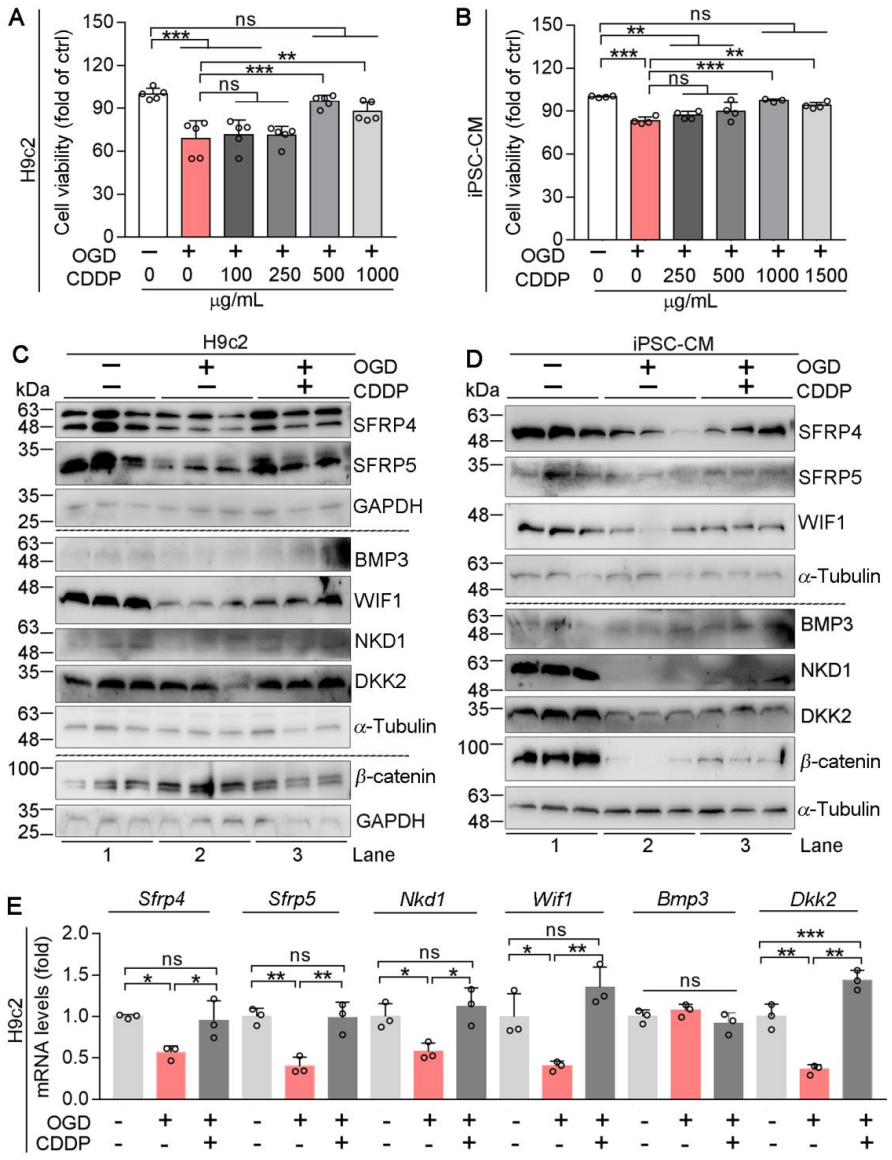

Figure S4 CDDP protects cardiomyocytes against OGD-induced damage by regulating Wnt pathway. H9c2 or iPSC-CM cells were cultured in normal condition (Ctrl), OGD condition (in a tank filled with a gas mixture of 95% N_2 plus 5% CO_2 at 37 °C and in serum-free medium lacking glucose) or OGD plus CDDP treatment at the indicated concentrations (A, B: CDDP at 500 μg/mL for H9c2 and 1000 μg/mL for iPSC-CM) for 12 h. After treatment, cells were used to conduct the following assays: cell viability by an assay kit (A, B: n = 4–5); expressions of Wnt-related proteins (BMP3, WIF1, SFRP4/5, NKD1, DKK2 and β-catenin) by Western blot (C, D: n = 3); and expressions of *Sfrp4/5*, *Nkd1*, *Wif1*, *Bmp3* and *Dkk2* mRNA in H9c2 cells by qRT-PCR (E: n = 3). The data are shown as the mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, ns: not significantly different between indicated groups.

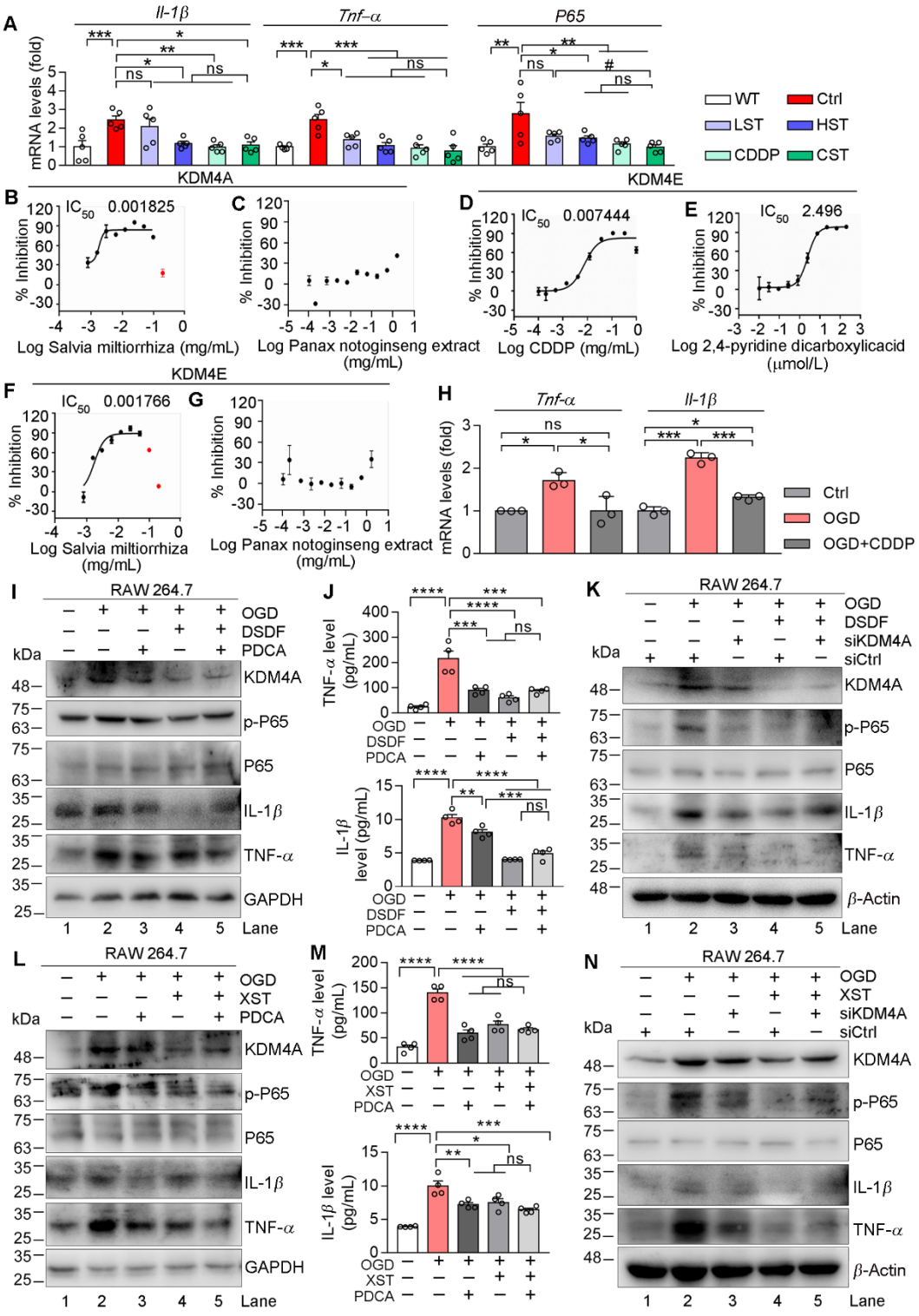

Figure S5 CDDP and CDDP components reduce inflammation by inhibiting KDM4A expression in OGD-induced cells or HFD-fed ApoE$^{-/-}$LDLR$^{-/-}$ mouse heart. (A) Expression of *Il-1β*, *Tnf-α* and *P65* mRNA in mouse heart was determined by qRT-PCR (n = 5). Inhibition of KDM4A (B, C) or KDM4E (D, F, G) activity by CDDP components was determined by an in *vitro* enzymatic activity assay system with PDCA

(a KDM4A chemical inhibitor) as a positive control (E). (H–N) H9c2 or RAW 264.7 cells were cultured in normal condition, OGD condition or OGD condition plus treatment of CDDP (500 μg/mL), DSDF (250 μg/mL), XST (500 μg/mL) or PDCA (1 μmol/L) as indicated for 12 h. After treatment, cells or treatment medium were used to conduct the following assays: mRNA expression of *Il-1β*, *Tnf-α* in H9c2 cells by qRT-PCR (H: $n = 3$); expression of KDM4A, P65, p-P65, IL-1β and TNF-α protein in RAW 264.7 cells by Western blot (I, K, L, N, $n = 3$); the levels of TNF-α and IL-1β in treatment medium collected from cells in Fig. S5I or S5L by ELISA assay kits (J or M, $n = 4$). The data are shown as the mean ± SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns: not significantly different between indicated groups.

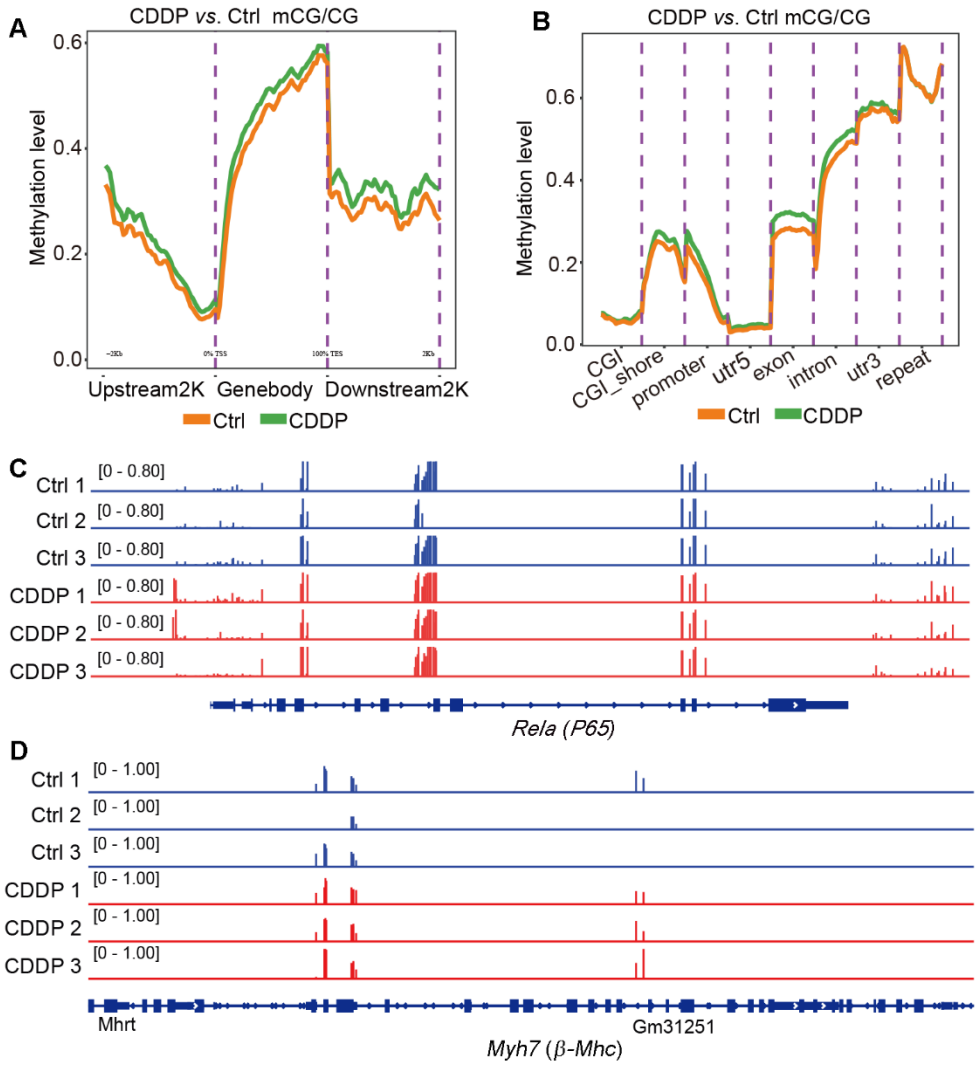

Figure S6 CDDP affects DNA methylation in mouse heart. At the end of experiment as indicated in Fig. 1A, mouse hearts were collected and used for DNA methylation sequencing (n = 3). (A, B) The characteristic of DNA methylation on a variety of annotated genomic regions. (C, D) Integrative Genomics Viewer (IGV) tracks displaying the characteristic of methylation for *Rela* (*P65*) and *Myh7* (*β-Mhc*) gene in the two groups, each was done in triplicate. Ctrl: HFD-fed ApoE$^{-/-}$LDLR$^{-/-}$ mice; CDDP: HFD-fed ApoE$^{-/-}$LDLR$^{-/-}$ mice received CDDP treatment.

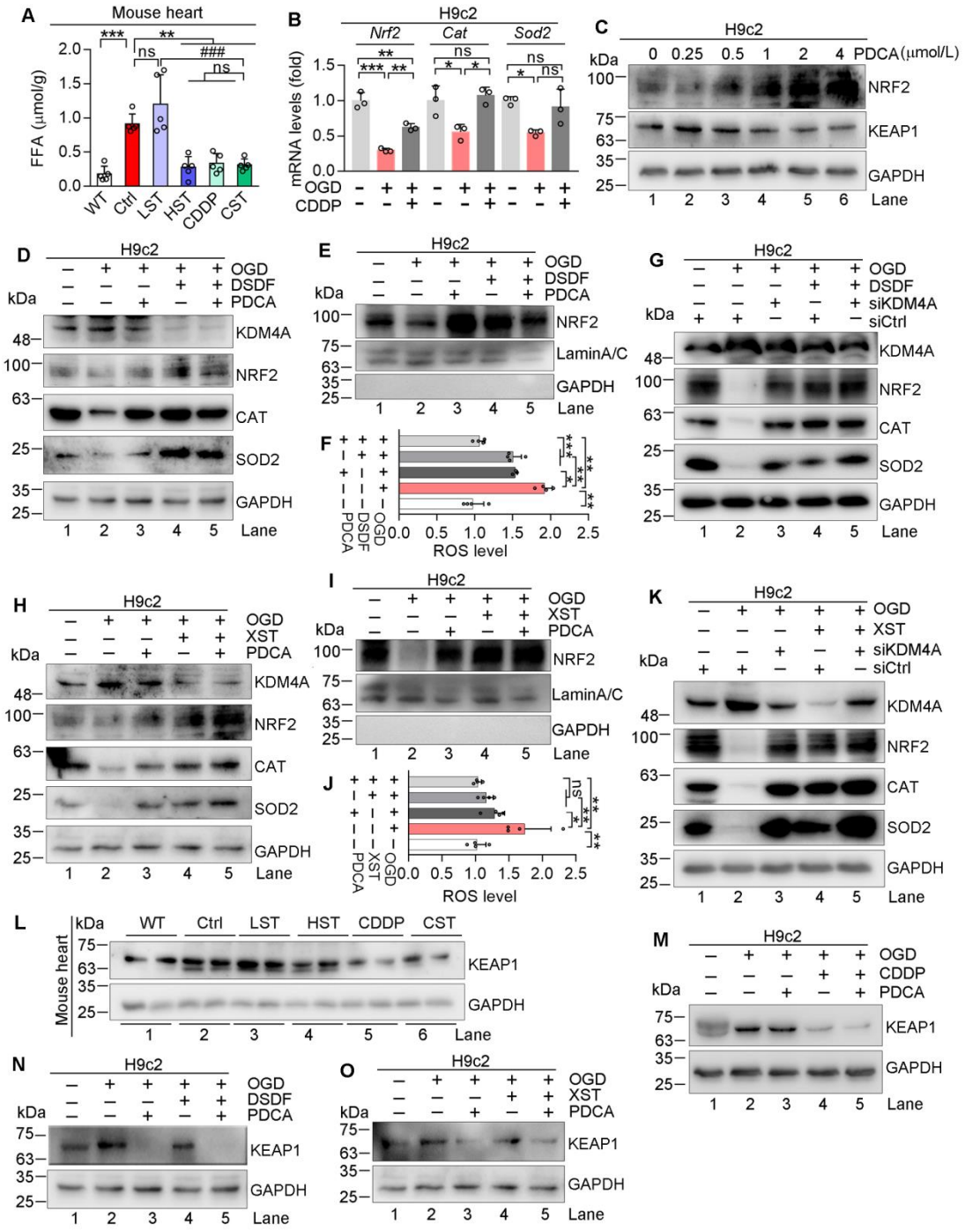

Figure S7 CDDP protects cardiomyocytes against OGD-induced damage by anti-oxidative stress. (A, L) FFA levels and KEAP1 expression in mouse heart were determined by the FFA assay kit (n = 5) and Western blot, respectively. (C) H9c2 cells cultured in normal condition were treated with PDCA at the indicated concentrations for 12 h; (B, D–K, M–O) H9c2 cells cultured in normal condition, OGD condition, OGD condition plus treatment of CDDP (500 μg/mL), DSDF (250 μg/mL), XST (500 μg/mL) and PDCA (1 μmol/L), or OGD condition plus si*Ctrl*/si*KDM4A* transfection

and DSDF or XST treatment as indicated for 12 h. After treatment, cells were used to conduct following assays: expression of *Nrf2*, *Cat* and *Sod2* mRNA by qRT-PCR (B: $n = 3$); expressions of NRF2, KEAP1, KDM4A, CAT and SOD2 protein in whole cellular extract (C, D, G, H, K, M–O) and NRF2 protein in nuclear extract (E, I) by Western blot ($n = 3$); ROS levels in cells in Fig. S7E and S7I by assay kits (F, J, $n = 4$). The data are shown as the mean ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; $^{\#\#\#}P < 0.001$, ns: not significantly different between indicated groups. FFA: free fatty acid; ROS: reactive oxygen species.

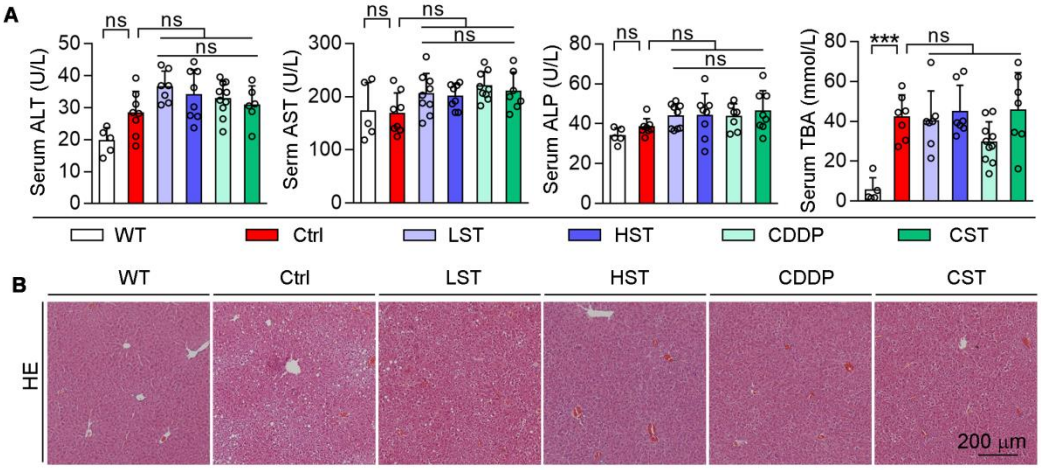

Figure S8 CDDP has no side effect on mouse liver. At the end of the 16-week treatment indicated in Fig. 1A, mouse serum and liver samples were used to conduct the following assays. (A) Levels of serum AST, ALT, ALP and TBA by assay kits ($n = 5–8$); (B) HE staining of mouse liver sections. The data are shown as the mean ± SEM. $^{***}P < 0.001$, ns, not significantly different between indicated groups. AST: Aspartate transaminase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; TBA: total bile acid.

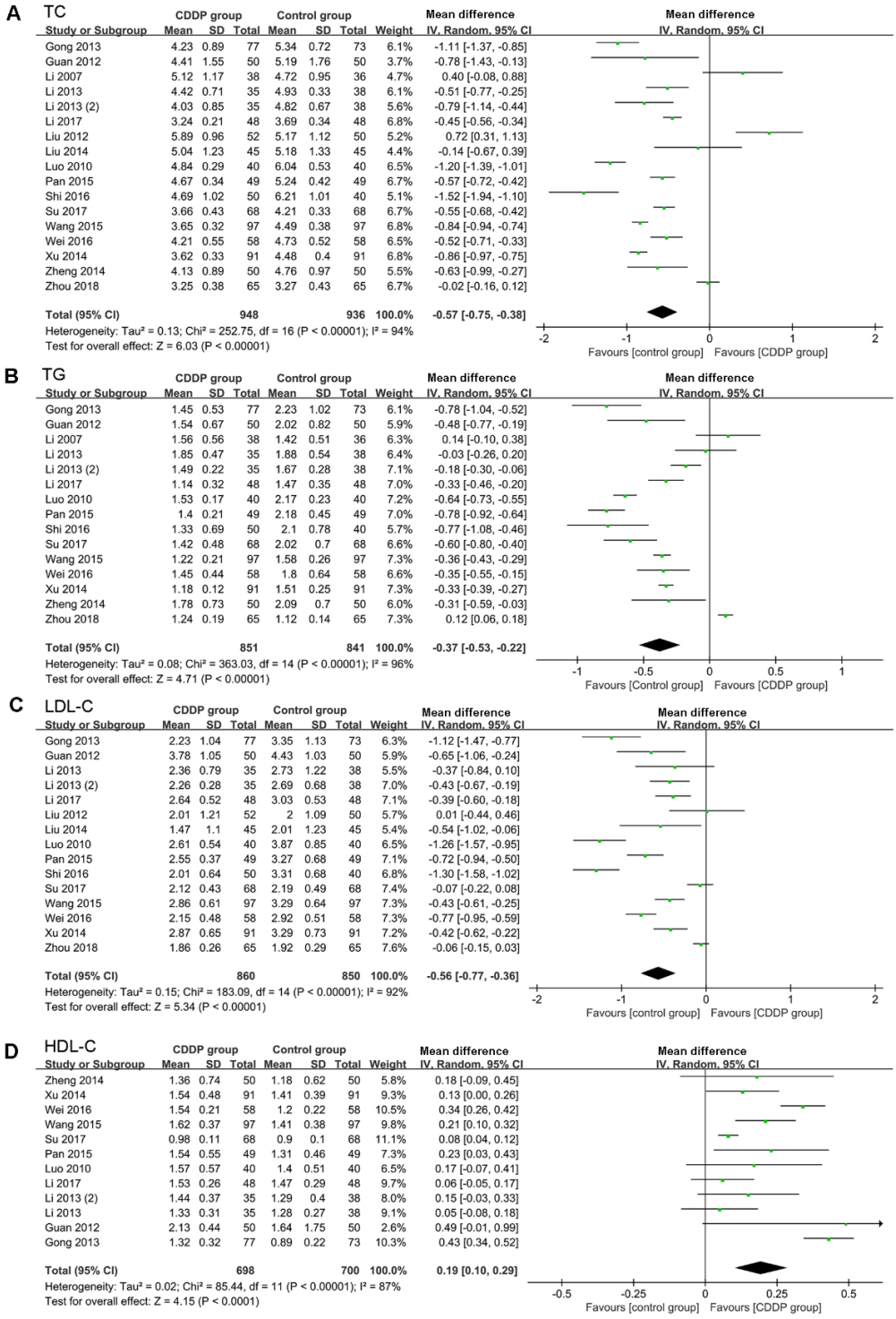

Figure S9 CDDP combined statins improves dyslipidemia better than statins alone in CHD patients. Meta-analysis of lipid profiles (TC, TG, LDL-C and HDL-C) of blood samples collected from CHD patients with treatment of CDDP combined statins (CDDP group) or statins alone (Control group). CHD: coronary heart disease; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TC: total cholesterol; TG: triglyceride.