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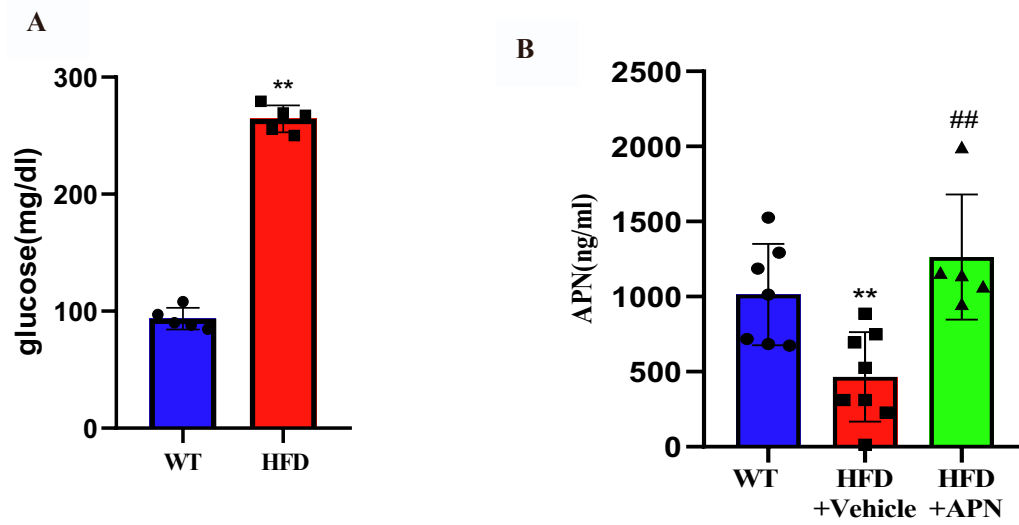
## **Supplemental information**

### **Adiponectin-mediated promotion of CD44 suppresses diabetic vascular inflammatory effects**

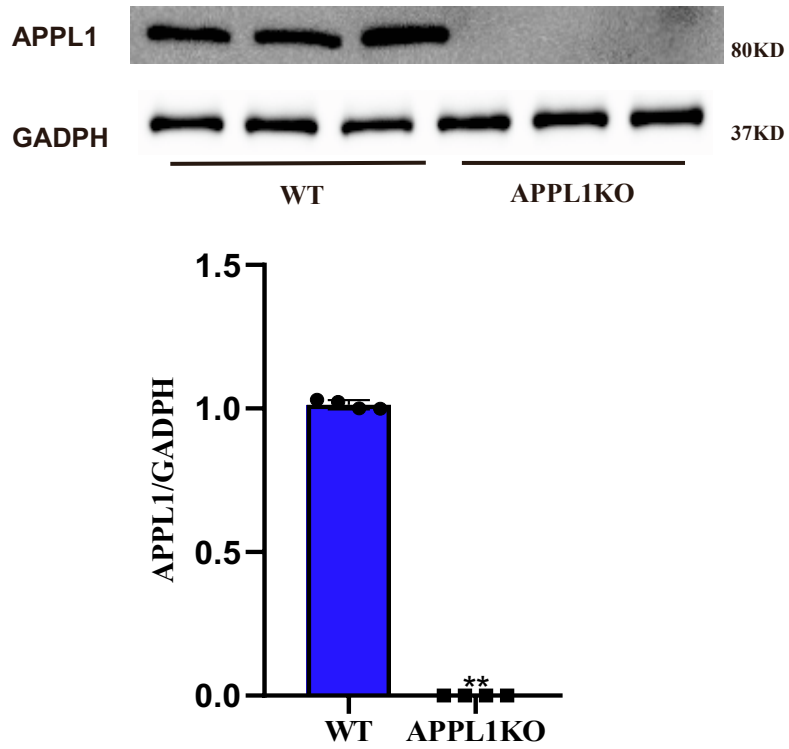
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## **SUPPLEMENTAL INFORMATION**

Supplemental Figures and Figure Legends



**Figure S1. Type 2 diabetic mice model, Related to STAR Methods.** WT mice were fed with HFD for 12 weeks to induce type 2 diabetes mice model. **A.** The fasting glucose level (N=5, \*\* $P < 0.01$  vs WT). **B.** The plasma Adiponectin level (Data are represented as mean  $\pm$  SEM. N=5-8, \*\* $P < 0.01$  vs WT, ## $P < 0.01$  vs HFD+Vehicle).



**Figure S2. Identification of APPL1 knock out mice, Related to Figure1.** Western blot examined the expression of APPL1 proteins in aortic vessels of WT/APPL1KO mice (Data are represented as mean±SEM. N=4, \*\* $P$ <0.01 vs WT).

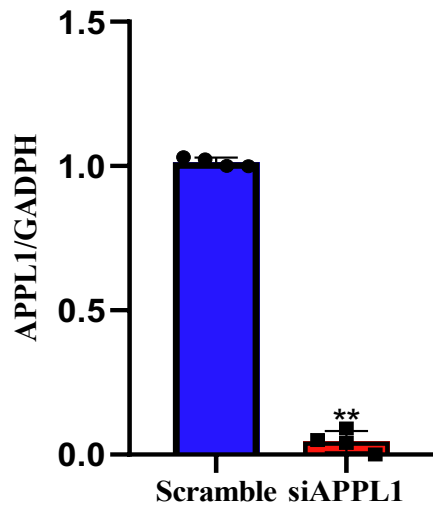
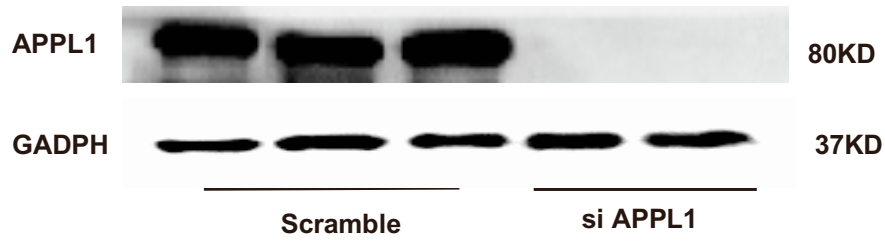
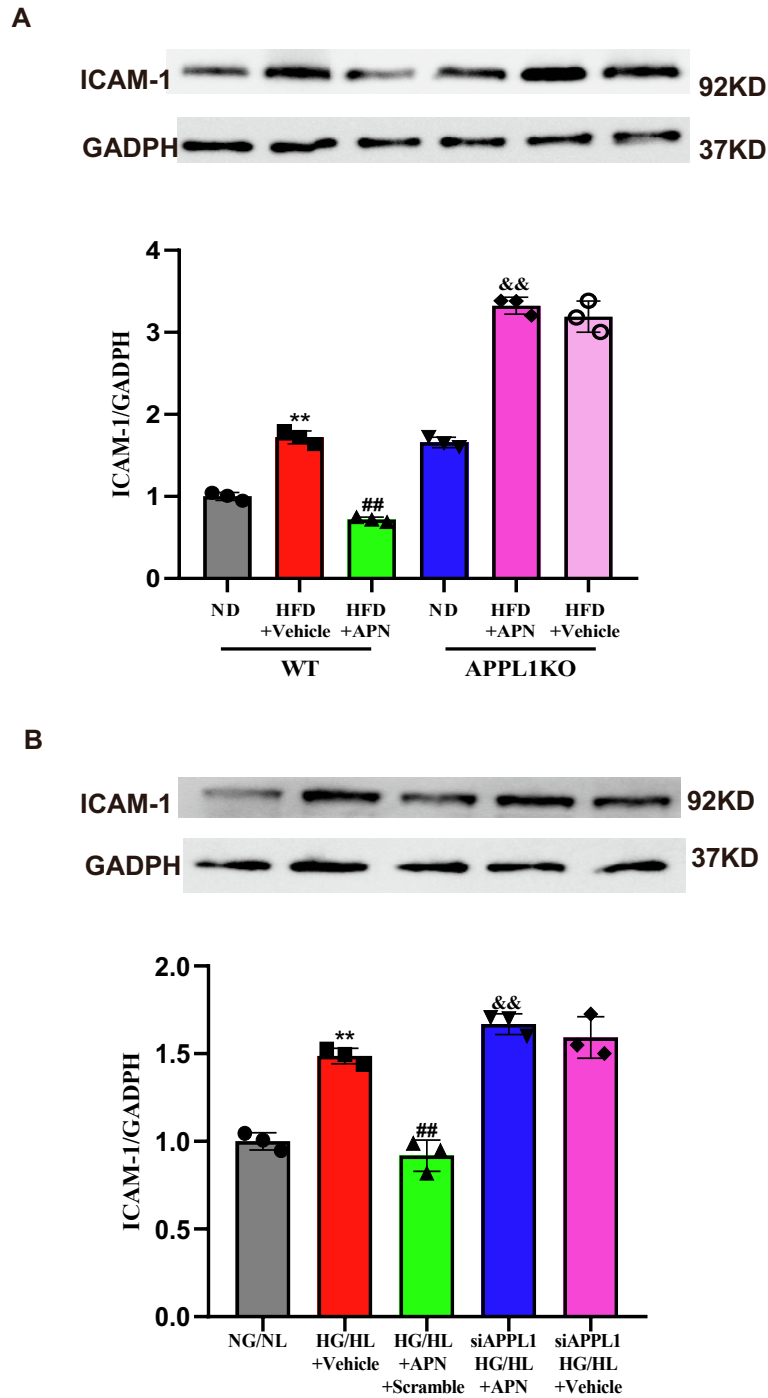


Figure S3. Western blot examined the expression of APPL1 in HUVEC after NC siRNA or siAPPL1 transfection, Related to Figure1. (Data are represented as mean±SEM. N=4, \*\* $P$ <0.01 vs Scramble).



**Figure S4. The expression of ICAM-1 in vivo and in vitro, Related to Figure1. A.** The expression of ICAM-1 in WT or APPL1<sup>-/-</sup> mice (N=3, HFD+vehicle vs. ND; ##*P* < 0.01 vs. HFD+vehicle; &&*P* < 0.01 vs. Scramble+HFD+APN); **B.** The expression of ICAM-1 in HUVEC (Data are represented as mean±SEM. N=3, HG/HL vs. NG/NL; ##*P* < 0.01 vs. HG/HL+vehicle; &&*P* < 0.01 vs. Scramble+HG/HL+APN).

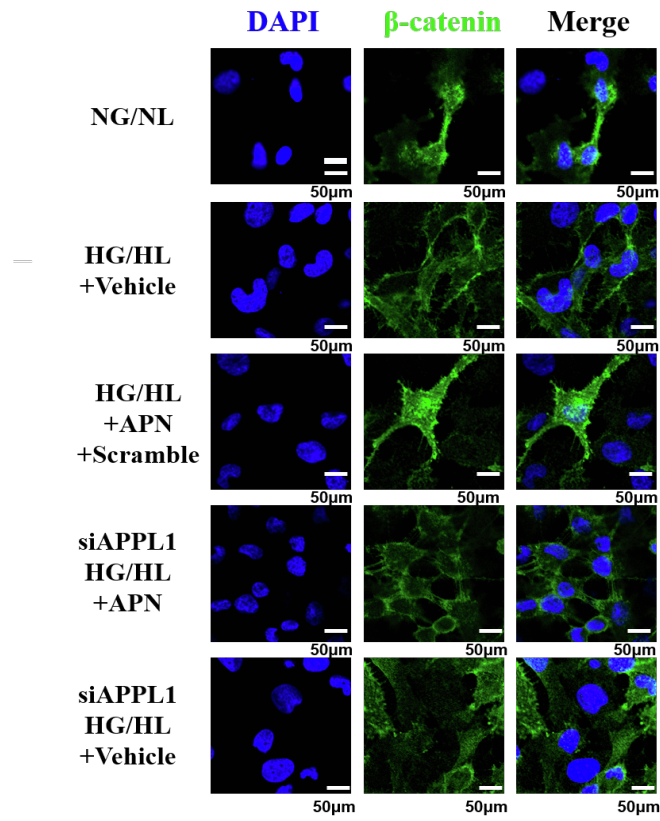
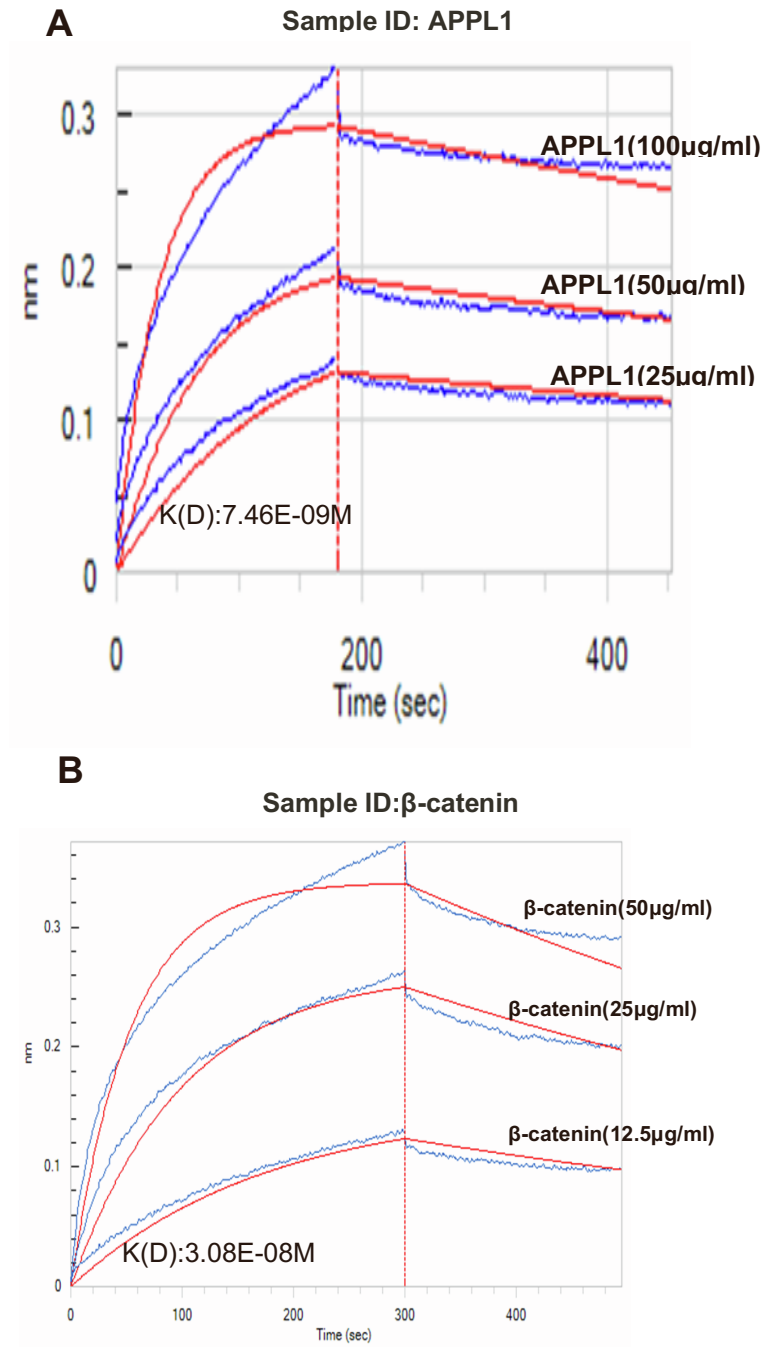
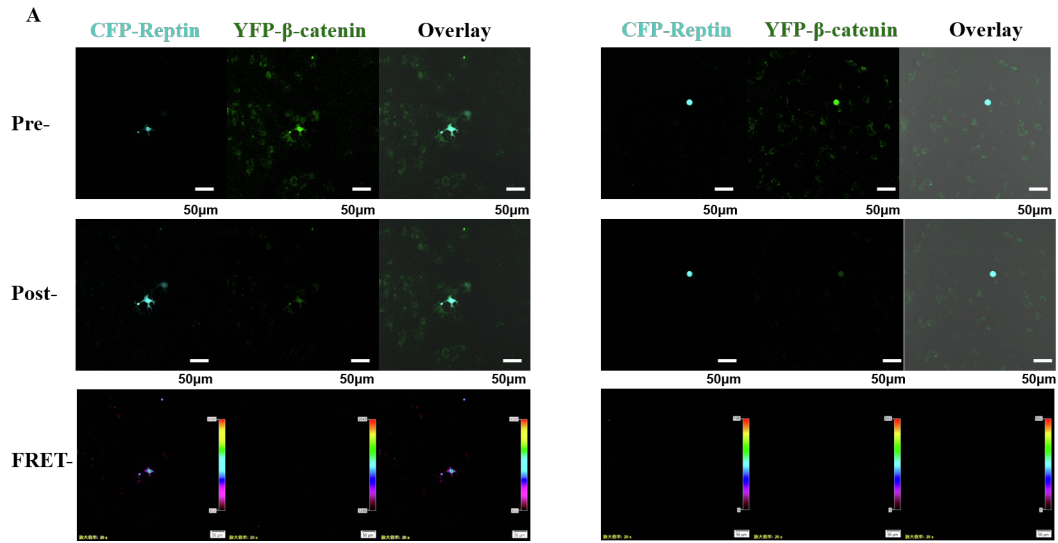


Figure S5. Representative images of  $\beta$ -catenin immunostaining in HUVECs localization, Related to Figure 2.

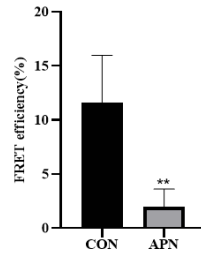


**Figure S6. Functional identification of Reptin, Related to Figure 3.** (A, B) Bio-layer interferometry (BLI) technique was performed to detect the binding and affinity of APPL1/ $\beta$ -catenin for Reptin. The real time binding curves are shown as blue lines while the red lines indicate the global fits generated by fitting the blue line using 1:1 Langmuir binding model with mass transport limitation using the Scrubber software.





**B**



**Figure S7. FRET Analysis was employed to determine the interaction of the Reptin- $\beta$ -catenin complex after APN treatment, Related to Figure 3. A.** Representative images of FRET analysis between CFP-Reptin and YFP- $\beta$ -catenin in HUVECs. The left group of images obtained from control HUVECS and the right group of images from APN-stimulated (5  $\mu$ g/ml for 2h) cells. **B.** Summarized results of detected FRET efficiency between Reptin and  $\beta$ -catenin, APN in HUVECs with or without treatment. n = 3 batches of cells, Data are represented as mean $\pm$ SEM. \*\* P < 0.05 vs. control group.

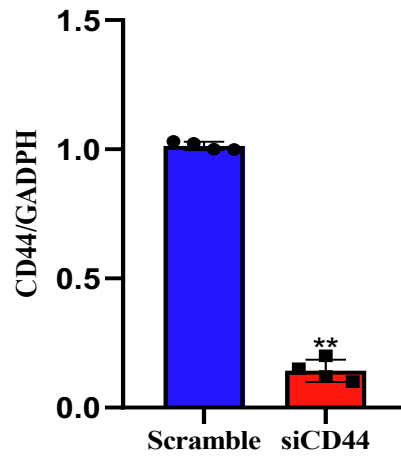
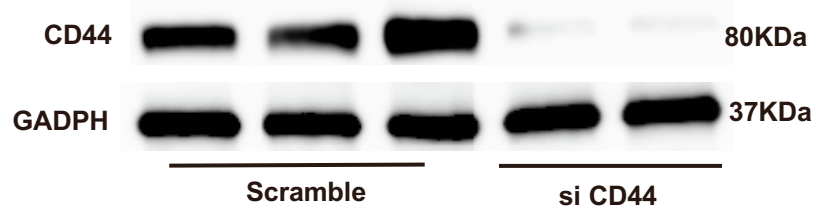


Figure S8. Western blot examined the expression of CD44 in HUVEC after NCsiRNA or siCD44 transfection, Related to Figure 5. (Data are represented as mean±SEM. N=4, \*\* $P < 0.01$  vs Scramble)

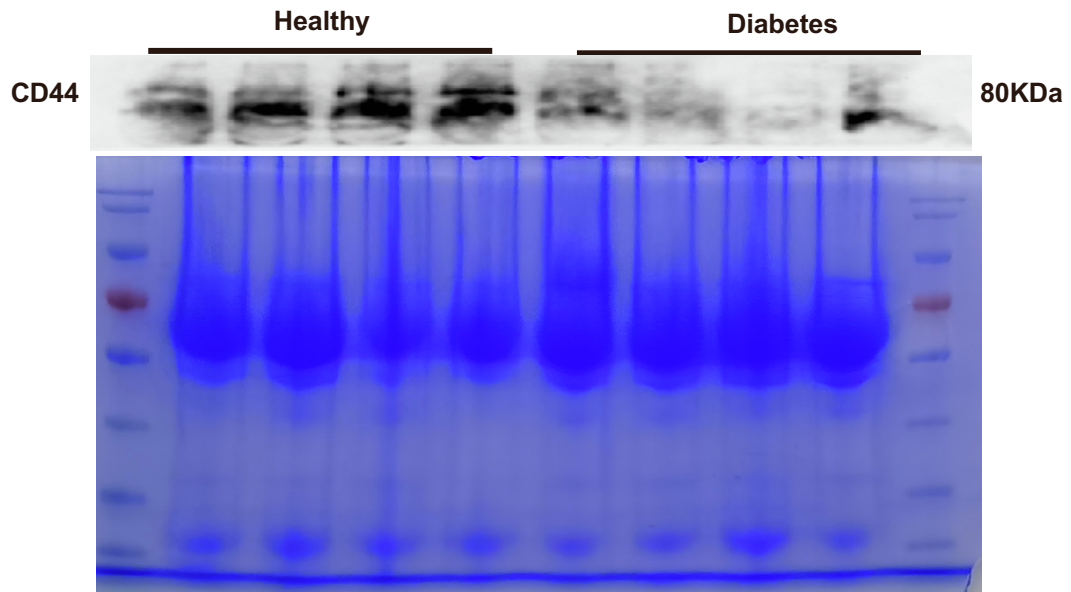
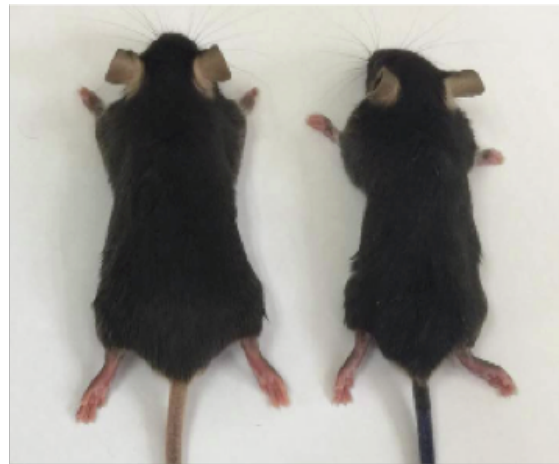


Figure S9. Western blots and quantification of protein expression for CD44 in the serum of healthy and diabetic group, Related to Figure 5.

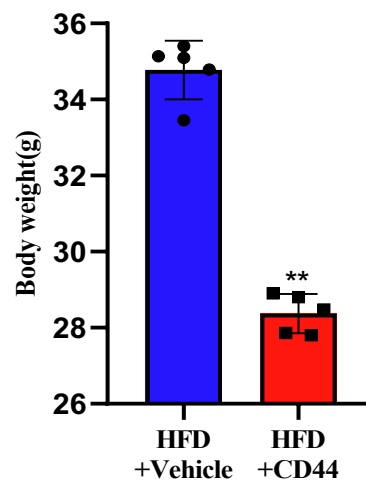
A



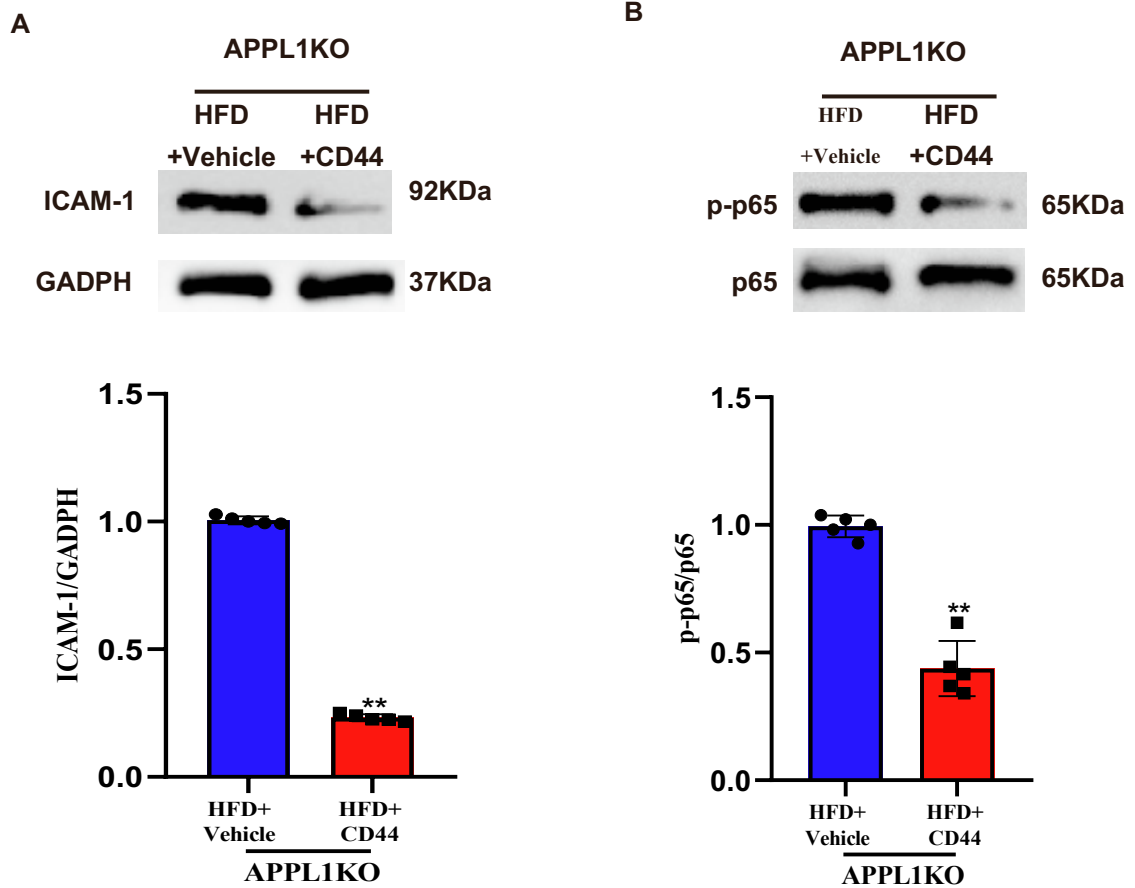
HFD  
+Vehicle

HFD  
+CD44

B



**Figure S10. The active domain of CD44 was administered to HFD animals, Related to Figure 6. A.** Representative gross morphology of HFD-fed WT mice. **B.** Statistics of heart weight/body weight (Data are represented as mean $\pm$ SEM. N=5, \*\* $P$  < 0.01 vs ND).



**Figure S11. The protein expression of ICAM-1 and NF- $\kappa$ B pathway, Related to Figure 6.** Western blots and quantification of protein expression for ICAM-1 and NF- $\kappa$ B pathway in HFD-fed APPL1 KO mice (A, B) (Data are represented as mean $\pm$ SEM. N=5, \* $P$  < 0.05, \*\* $P$  < 0.01 vs. HFD+Vehicle).

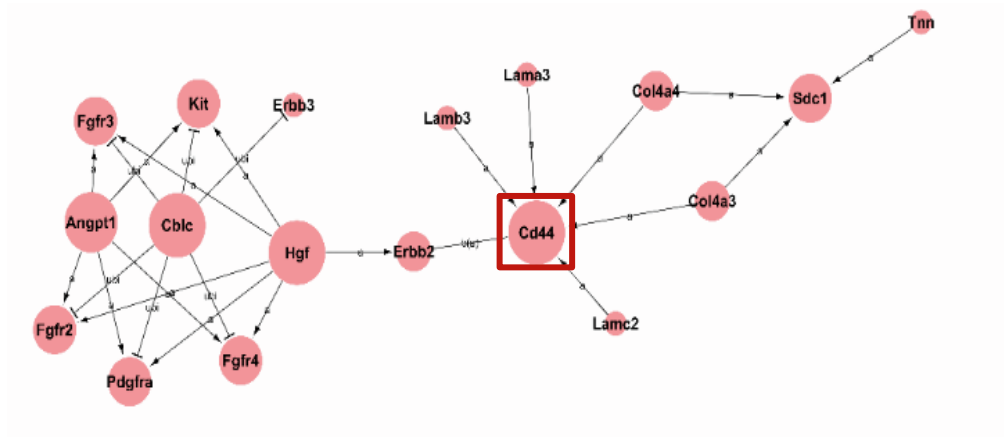
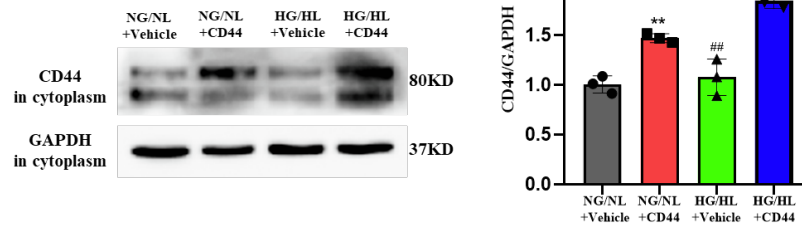
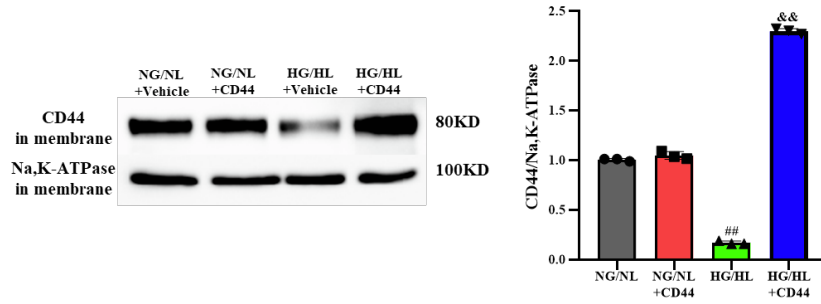
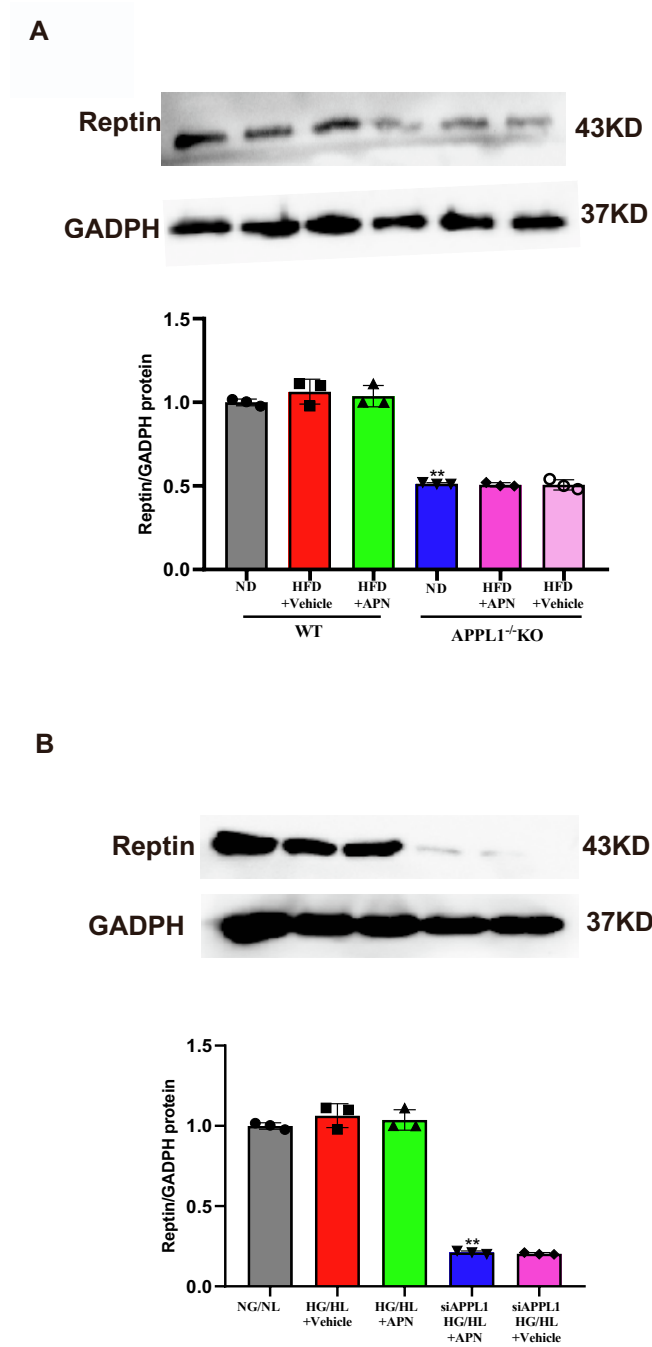


Figure S12. CD44 located in an important position in global signal transduction network of RNA sequence analysis, Related to Figure 4.

**A****B**

**Figure S13. The quantification of CD44 in the cells, Related to Figure6.** The expression of CD44 in cytoplasm (A) and membrane (B). (Data are represented as mean±SEM. N=3, \*\* $P < 0.01$  vs NG/NL, ## $P < 0.01$  vs NG/NL, && $P < 0.01$  vs HG/HL).



**Figure S14. The levels of Reptin in vivo and vitro, Related to Figure 3. (A, B)** Western blot was performed to analyze the expression levels of Reptin in vivo and vitro (Data are represented as mean $\pm$ SEM. N=3, \*\* $P$  < 0.01 vs ND or HG/HL).



**Table S1. The baseline characteristics of the patients and controls, Related to Figure 5**

	T2DM (n=74)	Control (n=30)	<i>P</i>
Age (years)	63(54-70)	56.5(47.75-67.25)	0.123
Male (n, %)	46/28	22/8	0.056
BMI (kg/m <sup>2</sup> )	24.50(21.90-26.25)	25.39(23.65-28.24)	0.115
SBP (mmHg)	127(116-140)	120(115-130)	0.110
DBP (mmHg)	74(70-80)	75(70-80.75)	0.727
FPG (mmol/L)	7.77±2.66	5.49±0.56	< 0.001
TG (mmol/L)	1.71±1.49	1.37±0.62	0.141
TC (mmol/L)	4.09(3.41-5.31)	4.18(3.42-5.19)	0.575
LDL-C (mmol/L)	2.47±1.04	2.58±0.87	0.665
HDL-C (mmol/L)	1.21±0.45	1.31±0.84	0.529
hs-CRP (mg/L)	2.34±2.68	2.87±4.44	0.446
Serum APN (ng/ml)	5550.963±2719.364	8255.391±3909.93	<0.001
Serum CD44 (ng/ml)	5.6750±3.743	14.8518±11.0819	<0.001

**Abbreviations:** BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; FPG: Fasting Blood Glucose; TG: Triglyceride; TC: Total Cholesterol; LDL-C: low-density lipoprotein; HDL-C: high-density lipoprotein; hs-CRP: high-sensitivity C-reactive protein; Serum APN: Serum Adiponectin.

**Table S2: Real Time PCR Primers, Related to STAR Methods.**

Gene Profile	Species	Genes	Forward primer (5'-3')	Reverse primer (5'-3')
House keeper	Mus musculus	Gapdh	CGTGCCGCCTGGAGAA	CCCTCAGATGCCTGCTTCAC
	Homo sapiens		AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
Inflammatory factors	Mus musculus	TNF- $\alpha$	GCGGTGCCTATGTCTCAGCC	CCCAATTCTCTTTTTGAGCC
	Homo sapiens		CTGCCCAATCCCTTTATT	CCCAATTCTCTTTTTGAGCC
	Mus musculus	IL-1 $\beta$	CCTGCAGCTGGAGAGTGTGGA	CCCATCAGAGGCAAGGAGGAA
	Homo sapiens		CCAGGGACAGGATATGGAGC	TTCAACACGCAGGACAGGTACAG
	Mus musculus	NLRP3	CTTCTGCACCCGGACTGTAACT	GAAGGCTGTGGTTGTGGGTCA
	Homo sapiens		GATCTTCGCTGCGATCAACA	GGGATTGAAACACGTCATTA
	Mus musculus	VCAM-1	AGACTGAAGTTGGCTCACAATTAAGAAG	AGTAGAGTGCAAGGAGTTCGGG
	Homo sapiens		GGAAGTGAATTAATTATCCAA	CTACACTTTTGATTTCTGTG
	Mus musculus	ICAM-1	GTCCGCTTCCGCTACCATCAC	GGTCCTTGCCTACTTGCTGCC
	Homo sapiens		GTCCCCCTCAAAGTCATCC	AACCCCATTCAGCGTCACCT
Transcription factors	Homo sapiens	COX2	CGCTCAGCCATACAGCAA	GAATCCTGTCCGGGTACAATC
	Homo sapiens	c-MYC	TCAAGAGGCGAACACACAAC	GGCCTTTTCATTGTTTTCAA

sapiens			
Homo sapiens	Axin2	TACACTCCTTATTGGGCGATCA	TTGGCTAATCGTAAAGTTTTGGT
Homo sapiens	CyclinD1	GTGGCCTCTAAGATGAAGGAGA	GGAAGTGTTCAATGAAACGTGT
Homo sapiens	VEGFA	ACAGAACGATCGATACAGAA	AAAGATCATGCCAGAGTCTC
Homo sapiens	DKK1	CTGTCCTGAAAGAAGGTCAAGT	TGTGAAGCCTAGAAGAATTACTGG
Homo sapiens	MMP2	CCGTCGCCCATCATCAAGTT	CTGTCTGGGGCAGTCCAAAG
Homo sapiens	MMP9	GTGCTGGGCTGCTGCTTTGCTG	GTCGCCCTCAAAGGTTTGAAT
Homo sapiens	MMP7	GATGGGCCAGGAAACACGC	CCTAGACTGCTACCATCCGTCCA
Homo sapiens	CD44	AAGACATCTACCCCAGCA	GGTAGCAGGGATTCTGT
Homo sapiens	Notch2	GCAGGAGGTGGATGTGTTAG	CCAGGATCAGGGGTGTAGAG
Homo sapiens	OPN	ATCTCCTAGCCCCACAGAAT	CATCAGACTGGTGAGAATCATC
Homo sapiens	PPARD	AAGAGGAAGTGGCAGAGGCA	TGCCACCAGCTTCTTCTTCT

**Table S3 The differentially expressed down-regulated inflammatory genes in APN+HFD group, Related to Figure 1**

Gene Symbol	Description	Fold change		P value
		HFD+Vehicle (/ND)	HFD+APN (/HFD+Vehicle)	
ICAM1	intercellular adhesion molecule 1	7.58291	-7.8258	0.000724
VCAM1	Vascular cell adhesion molecule 1	2.65429	-2.22661	0.04771
IL-1 $\beta$	interleukin 1 beta	5.661808	-13.4898	0.003873
Tnfaip2	tumor necrosis factor, alpha-induced protein 2	2.135935	-2.36649	0.007669
Tnfaip3	tumor necrosis factor, alpha-induced protein 3	3.824305	-3.20331	0.005262
Tnfaip811	tumor necrosis factor, alpha-induced protein 8-like 1	2.677928	-3.07376	0.000693
NLRP3	NLR family, pyrin domain containing 3	4.814335	-6.74765	0.013253
Ccr2	chemokine (C-C motif) receptor 2	9.774162	-5.95368	0.000583
Ccl3	chemokine (C-C motif) ligand 3	7.401234	-4.08488	0.04108
Nfkbid	nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, delta	3.927666	-2.63233	0.029635
Il1f9	interleukin 1 family, member 9	20.00856	-29.0969	0.003301
Cx3cl1	chemokine (C-X3-C motif) ligand 1	2.597309	-2.58852	0.000111
Cxcl15	chemokine (C-X-C motif) ligand 15	3135.705	-1703.37	0.000247

**Table S4 The differentially expressed up-regulated transcription factors in APN+HFD group,  
Related to Figure 4**

Gene Symbol	Description	Fold change			P value
		HFD+Vehicle (/ND)	HFD+APN (/HFD+Vehicle)	APPL1KO +HFD+APN (/HFD+APN)	
Foxf2	forkhead box F2	-22.7681	37.97237	-37.97237	3.3E-19
TCF21	transcription factor 21	-32.5934	60.48744	-65.84932	1.55E-05
Sox2	SRY(sex determining region Y)-box 2	-22.8226	46.52984	-9.118387	0.000154
Foxa2	forkhead box A2	-380.522	769.2761	-5881.995	1.58E-08
Runx1	runt related transcription factor 1	-7.65333	12.33153	-7.869982	0.000393

**Table S5 The differentially activity of up-regulated transcription factors in APN+HFD group,  
Related to Figure 4.**

Gene Symb ol	Base- Mean NG/NL	Base- Mean HG/HL	Base- Mean HG/HL+ APN	Base- Mean siAPPL1+ HGHL+A PN	HFD +Vehicle (/ND)	Fold change	
						HFD+APN (/HFD +Vehicle)	APPL1KO +HFD+APN (/HFD+APN)
TCF/ LEF	55407.6 5	24933.1 5	93600.3 3	32034.33	0.4510418 98	1.7762659 33	0.084661379
C/EB P	40552.6 7	46748.6 7	36717.6 7	9559.667	0.7854298 36	1.1527822 23	0.26035605
POU 5F1	90513.8 6	24151.6 7	45078	31139	0.2668265 43	1.8664679 21	0.569198574
NFAT	245816. 7	21804	25960.6 7	24155	0.0887002 34	1.1971136 79	0.9304699
SMA D	4962.59 4	4005.66 7	23107.3 3	2946.333	0.8071382 89	5.7689010 94	0.127506419

**Table S6 The up-regulated transcription factors of Human Wnt/ $\beta$ -catenin Pathway in APN+HFD group, Related to Figure 4.**

Gene Symbol	CT-Mean NG/NL	CT-Mean HG/HL	CT-Mean HG/HL+APN	CT-Mean siAPPL1+HGHL+APN	HFD +Vehicle (/ND)	Fold change	
						HFD+APN (/HFD +Vehicle)	APPL1KO +HFD+APN (/HFD+APN)
NFAT C4	26.9199	27.0562	26.82877	28.58413	0.6634	1.257356	0.427362
TCF7 L2	24.5889	26.1285	24.69531	26.8994	0.2515	2.900103	0.313123
TP53	22.3409	23.4099	22.25045	24.25057	0.3485	2.398982	0.360675
JUN	24.3558	26.7100	20.64923	24.59492	0.1430	71.69086	0.093635