Supplementary material

Hepatic NAD^+ deficiency as a therapeutic target for NAFLD in aging

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Supplementary Figure 1

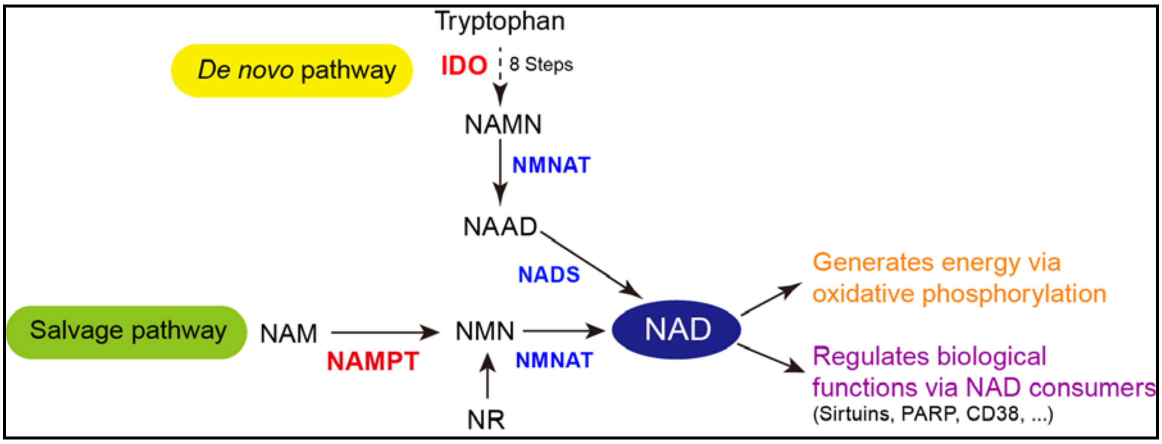
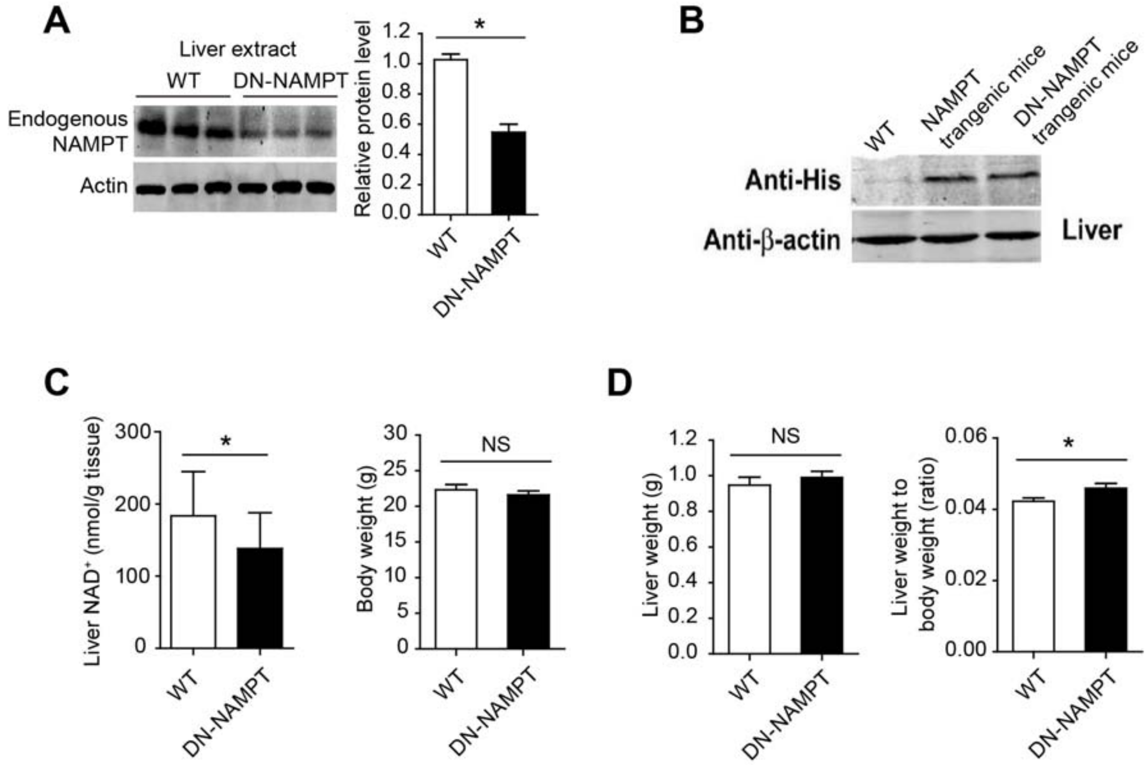


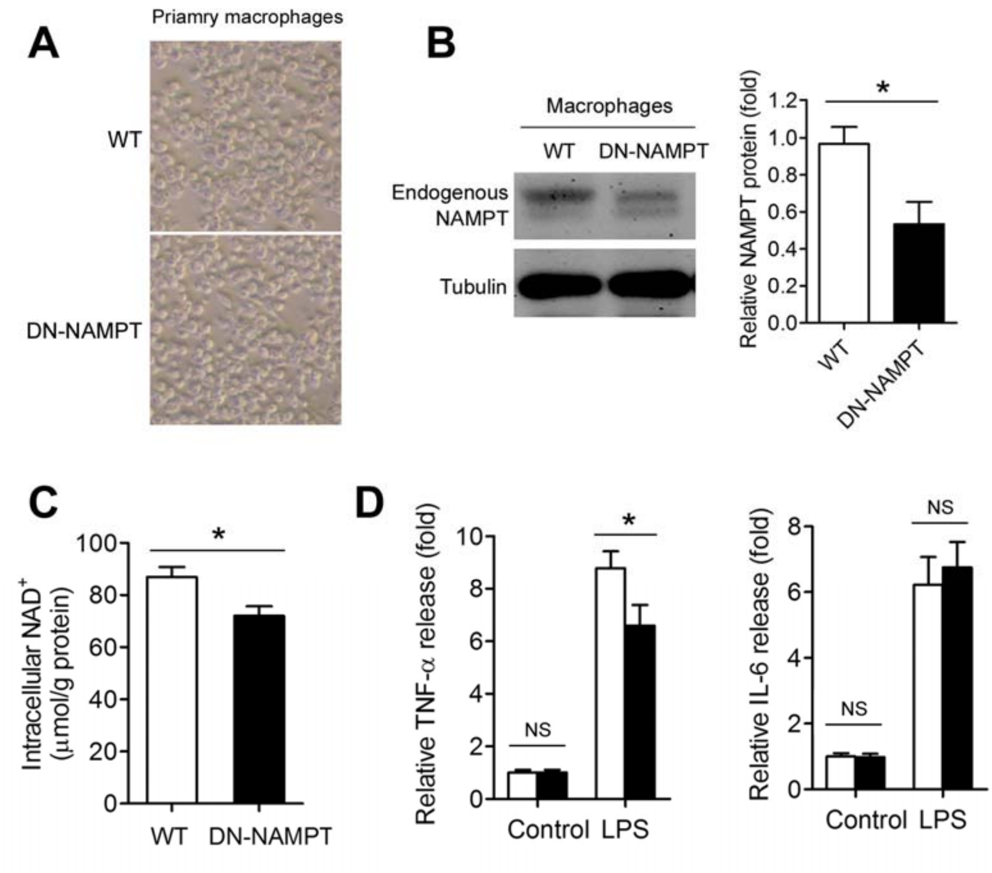
Illustration of NAD^+ *de novo* and salvage biosynthesis pathway. NAMPT is the step-limiting enzyme for salvage biosynthesis, whereas NADS and NMNAT are two important enzyme for NAD^+ *de novo* biosynthesis.

Supplementary Figure 2



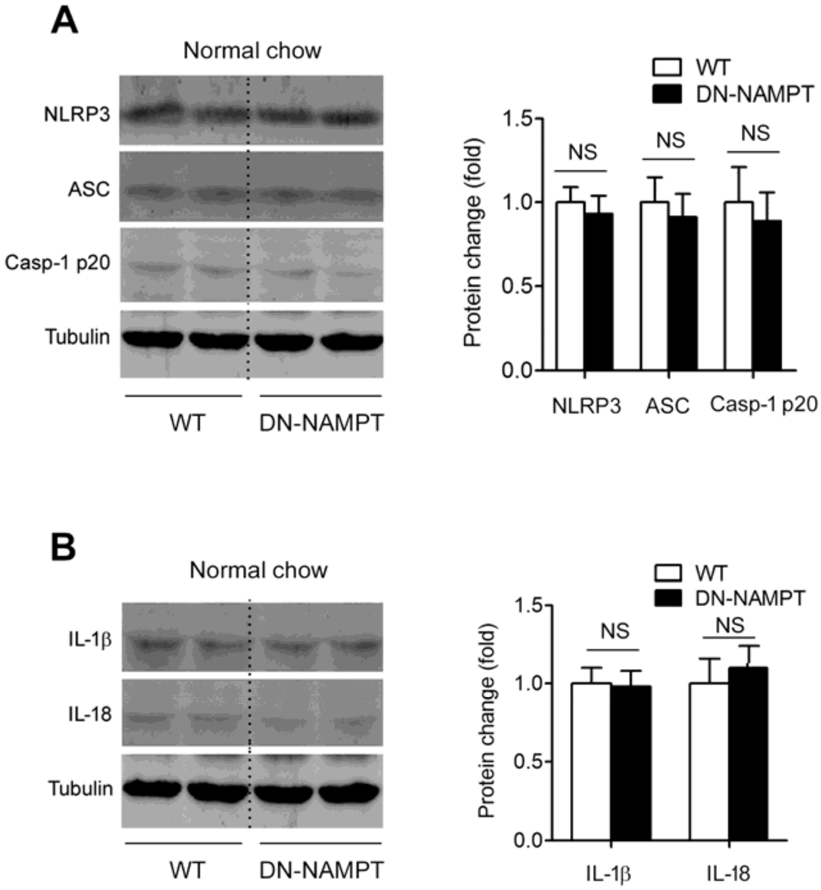
Decline of NAD^+ pool in DN-NAMPT mice. (A) Endogenous NAMPT protein expression in WT and DN-NAMPT mice. **P* < 0.05 by Student's t-test. n = 8 for each group. (B) Detecting his-tag in WT, NAMPT and DN-NAMPT transgenic mice. (C) Liver NAD^+ levels in liver tissues of WT and DN-NAMPT mice. *P < 0.05 by Student's t-test. n = 8 for each group. (D) Body weight, liver weight and liver/body weight ratio in WT and DN-NAMPT mice. *P < 0.05 by Student's t-test. n = 8 for each group.

Supplementary Figure 3



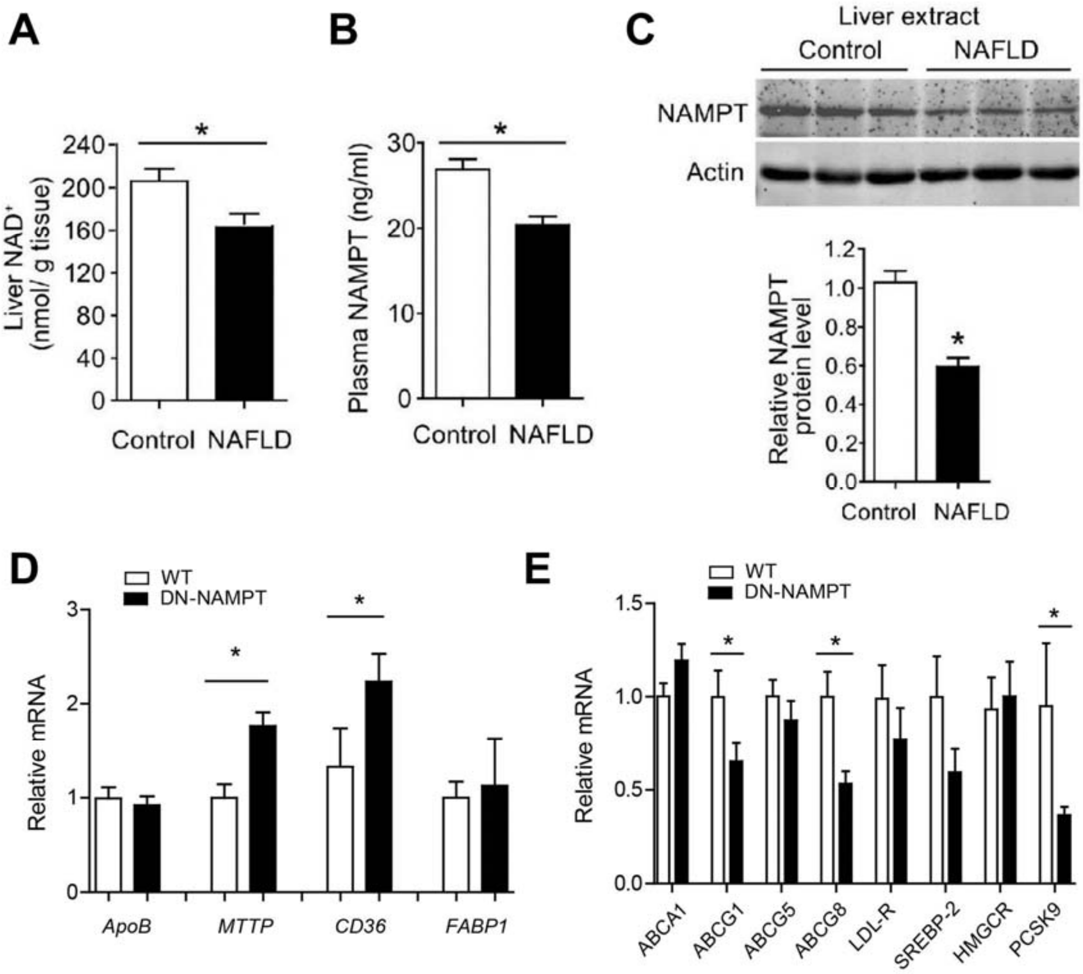
Macrophages isolated from WT and DN-NAMPT mice. (A) Representative images of isolated and cultured primary macrophages from WT and DN-NAMPT mice. (B) Endogenous NAMPT detection using a specific antibody against full-length NAMPT. $^*P < 0.05$ by Student's t-test. n = 6 for each group. (C) Intracellular NAD^+ levels in macrophages isolated from WT and DN-NAMPT mice. $^*P < 0.05$ by Student's t-test. n = 6 for each group. (D) Determination of TNF-α and IL-6 release from macrophages isolated from WT and DN-NAMPT mice. $^*P < 0.05$ by Student's t-test. n = 6 for each group. NS, no significance.

Supplementary Figure 4



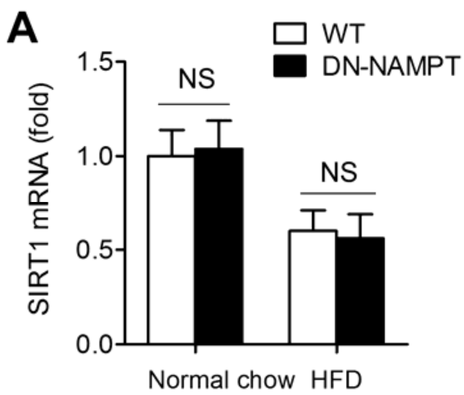
NLRP3 inflammasome pathway in livers of WT and DN-NAMPT mice under normal chow. (A) Representative images of isolated and cultured primary macrophages from WT and DN-NAMPT mice. (B) Endogenous NAMPT detection using a specific antibody against full-length NAMPT. **P* < 0.05 by Student's t-test. n = 6 for each group. (C) Intracellular NAD^+ levels in macrophages isolated from WT and DN-NAMPT mice. **P* < 0.05 by Student's t-test. n = 6 for each group. (D) Determination of TNF-α and IL-6 release from macrophages isolated from WT and DN-NAMPT mice. **P* < 0.05 by Student's t-test. n = 6 for each group. NS, no significance.

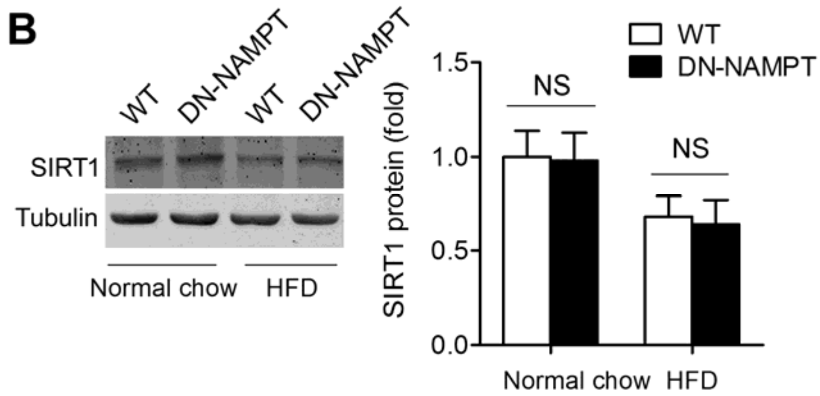
Supplementary Figure 5



Decline of NAD+ pool in HFD-induced NAFLD mice and DN-NAMPT mice and lipid profiles in HFD-fed WT and DN-NAMPT mice. (A) Liver NAD+ levels in liver tissues of HFD-induced NAFLD mice. The mice were fed with HFD for 16 weeks. *P < 0.05 by Student's t-test. n = 8 for each group. (B-C) Decline of NAMPT protein in plasma (B) and liver (C) of NAFLD mice. *P < 0.05 by Student's t-test. n = 8 for each group. (D-E) Expression of triglyceride and cholesterol efflux genes in HFD-fed WT and DN-NAMPT mice. **P* < 0.05 by Student's t-test. n = 8 for each group.

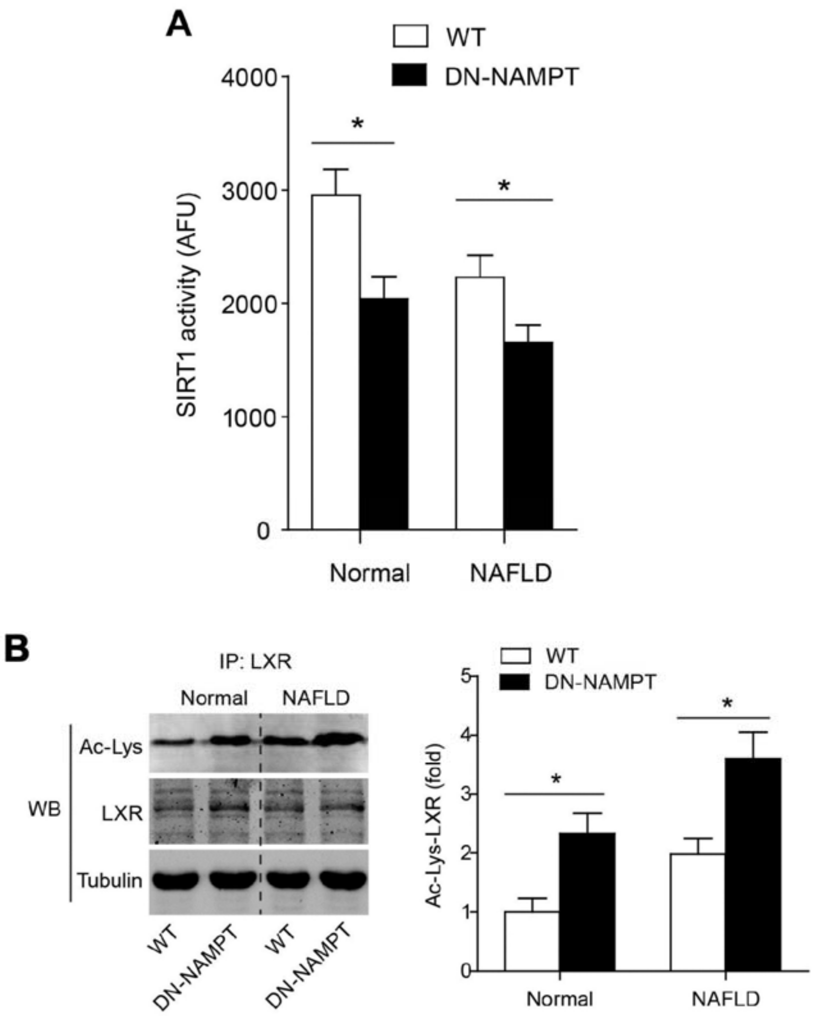
Supplementary Figure 6





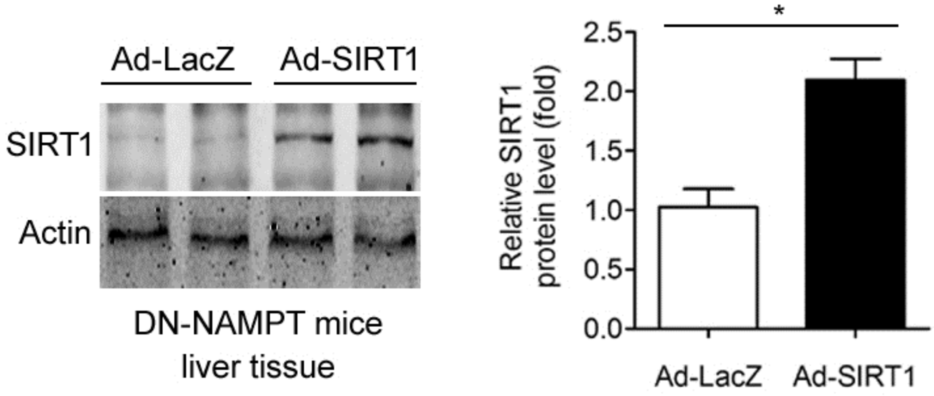
SIRT1 mRNA and protein levels in WT and DN-NAMPT mice under normal chow or HFD. (A) SIRT1 mRNA level in livers of WT and DN-NAMPT mice. NS, no significance. n = 6 for each group. (B) SIRT1 protein level in livers of WT and DN-NAMPT mice. NS, no significance. n = 6 for each group.

Supplementary Figure 7



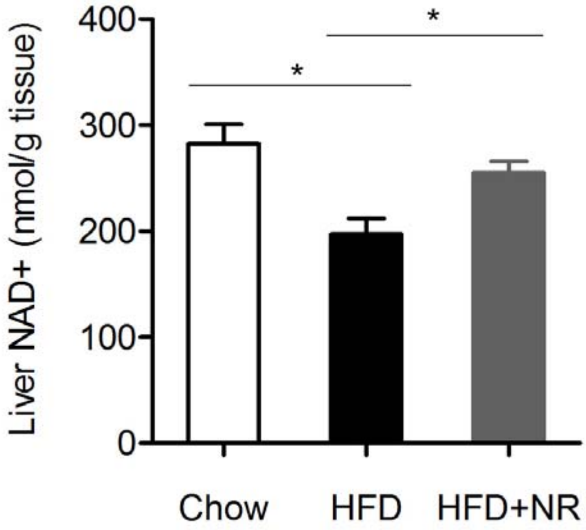
SIRT1 activity in WT and DN-NAMPT mice under normal chow or HFD. (A) SIRT1 activity in liver tissues of WT and DN-NAMPT mice under control and NAFLD conditions. *$P < 0.05$ by Student's t-test. n = 8 for each group. (B) Acetylation of LXR in liver tissues of WT and DN-NAMPT mice under control and NAFLD conditions. *$P < 0.05$ by Student's t-test. n = 6 for each group.

Supplementary Figure 8



Adenovirus-mediated SIRT1 overexpression in liver tissue of DN-NAMPT mice. Representative image and quantitative analysis of adenovirus-mediated SIRT1 overexpression in liver tissue of DN-NAMPT mice. *$P < 0.05$ by Student's t-test. n = 4 for each group.

Supplementary Figure 9



NR treatment enhances hepatic NAD^{+} level in HFD-fed mice. *P < 0.05 by Student's t-test. n = 6 for each group.

Supplementary Table1

Clinical information for the patients with hepatectomy

Group	Number	Age	Gender	Hepatectomy indication	Hepatitis B virus infection
Age < 45	1	43	Male	Hepatocellular carcinoma	+
	2	29	Male	Hepatocellular carcinoma	+
	3	36	Female	Hepatocellular carcinoma	+
	4	37	Male	Hepatocellular carcinoma	+
	5	42	Female	Hepatolithiasis	–
	6	45	Male	Hepatocellular carcinoma	+
Age > 60	7	62	Male	Hepatocellular carcinoma	+
	8	63	Female	Hepatocellular carcinoma	+
	9	65	Male	Hepatolithiasis	–
	10	66	Female	Hepatocellular carcinoma	+
	11	67	Male	Hepatocellular carcinoma	+
	12	71	Female	Hepatolithiasis	–

Supplementary Table2

Sequences of primers for PCR analysis

Gene	Forward Primer	Reverse Primer
ABCA1	GCTGCAGGAATCCAGAGAAT	CATGCACAAGGTCCTGAGAA
ABCG5	AGGGCCTCACATCAACAGAG	GCTGACGCTGTAGGACACAT
ABCG8	TCCGAGGAGAACAAGCTGTC	TCCGAGGAGAACAAGCTGTC
ABCG1	CCGATGTGAACCCGTTTCT	AGGCGGAGTCCTCTTCAGC
ApoB	TTGGCAAACTGCATAGCATCC	TCAAATTGGGACTCTCCTTTAGC
MTTP	GGAAGGCTTAATTGCAGCCA	TTCAGCCTTGTCCATCTGCAT
CD36	ATTAATGGCACAGACGCAGC	CCGAACACAGCGTAGATAGACC
FABP1	CAGAGCCAGGAGAACTTTCAG	GATTTCTGACACCCCCTTGATG
LDL-R	AGTGGCCCCGAATCATTGAC	CTAACTAAACACCAGACAGAGGC
SREBP-2	GCAGCAACGGGACCATTCT	CCCCATGACTAAGTCCTTCAACT
HMGCR	CATCATCCTGACGATAACGCG	AGGCCAGCAATACCCAGAATG
PCSK9	GAGACCCAGAGGCTACAGATT	AATGTACTCCACATGGGGCAA
α-SMA	GTTCAGTGGTGCCTCTGTCA	ACTGGGACGACATGGAAAAG
TIMP-1	AGGTGGTCTCGTTGATTCGT	GTAAGGCCTGTAGCTGTGCC
TGFβ-1	TTGCCCTCTACAACCAACACAA	GGCTTGCGACCCACGTAGTA
Pro-Col1α1	GACATCCCTGAAGTCAGCTGC	TCCCTTGGGTCCCTCGAC
GAPDH	GTATGACTCCACTCACGGCAAA	GGTCTCGCTCCTGGAAGATG