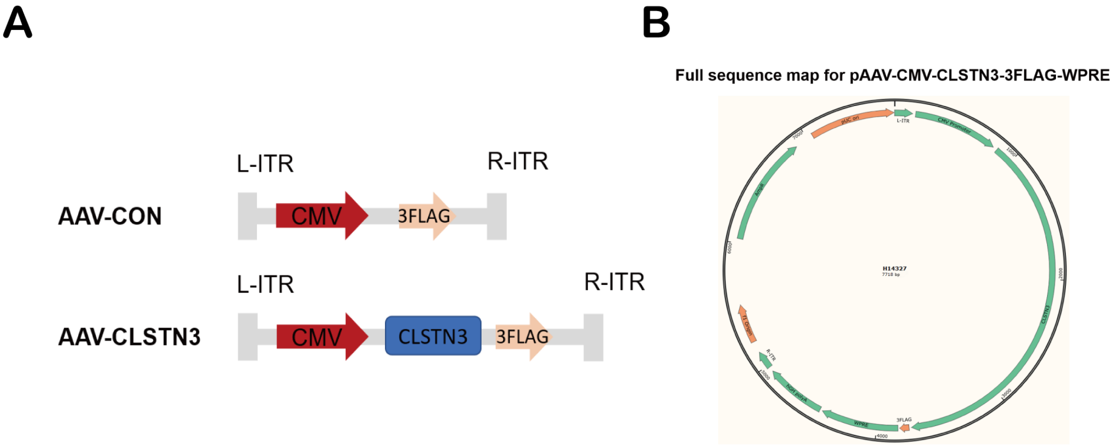
Supplementary Figures and figure legends

Figure S1



C

Coding sequence for CLSTN3 gene

```
ATGACCCTCCTGCTGCTGCCCCTTCTGCTGGCCTCTCTGCTCGCGTCCTGCTCCTGTAACAAAGCCAACAAGCACAAGCCATGGATTGAGGC
AGAGTACCAGGGCATCGTCATGGAGAATGACAACACGGTCCTACTGAATCCACCACTCTTTGCCTTGGACAAGGATGCCCCGCTGCGCTATG
CAGGTGAGATCTGCGGCTTCCGGCTCCATGGGTCTGGGGTGCCCTTTGAGGCTGTGATCCTTGACAAGGCGACAGGAGAGGGGCTGATCC
GGGCCAAGGAGCCTGTGGACTGCGAGGCCCAGAAGGAACACACCTTCACCATCCAGGCCTATGACTGTGGCGAGGGCCCCGACGGGGCC
AACACCAAGAAGTCCCACAAGGCCACTGTGCATGTGCGGGTCAACGATGTGAACGAGTTTGCCCCAGTGTTTGTGGAACGGCTGTATCGTG
CGGCTGTGACAGAGGGGAAGCTGTACGATCGCATCCTGCGGGTGGAAGCCATTGACGGTGACTGCTCCCCCCAGTACAGCCAGATCTGCTA
CTATGAGATTCTCACACCCAACACCCCTTTCCTCATTGACAATGACGGGAACATTGAGAACACAGAGAAGCTGCAGTACAGTGGTGAGAGGC
TCTATAAGTTTACAGTGACAGCTTATGACTGTGGGAAGAAGCGGGCAGCAGATGATGCTGAGGTGGAGATTCAGGTGAAGCCCACCTGTAAA
CCCAGCTGGCAAGGCTGGAACAAAAGGATCGAATATGCACCAGGTGCTGGGAGCTTGGCTTTGTTCCCTGGTATCCGCCTGGAGACCTGTG
ATGAACCACTCTGGAACATTCAGGCCACCATAGAGCTGCAGACCAGCCATGTGGCCAAGGGCTGTGACCGTGACAACTACTCAGAGCGGGC
GCTGCGGAAACTCTGTGGTGCTGCCACTGGGGAGGTGGATCTGTTGCCCATGCCTGGCCCCAATGCCAACTGGACAGCAGGACTCTCGGT
GCACTACAGCCAGGACAGCAGCCTGATCTACTGGTTCAATGGCACCCAGGCTGTGCAGGTGCCCCTGGGTGGCCCCAGTGGGCTGGGCTC
TGGGCCCCAGGACAGCCTCAGTGACCACTTCACCCTGTCCTTCTGGATGAAGCATGGCGTAACTCCCAACAAGGGCAAGAAGGAAGAGGAA
ACCATCGTATGTAACACTGTCCAGAATGAGGACGGCTTCTCTCACTACTCGCTGACTGTCCACGGCTGTAGGATTGCCTTCCTCTACTGGCCC
CTGCTTGAGAGTGCCCGCCCAGTCAAGTTCCTCTGGAAGCTGGAGCAGGTCTGTGATGATGAGTGGCACCACTACGCTCTGAACCTCGAGT
TCCCCACAGTCACACTCTATACCGACGGCATCTCCTTCGACCCTGCCCTCATCCATGACAATGGCCTCATCCACCCACCCCGAAGGGAGCCT
GCTCTCATGATTGGGGCCTGCTGGACTGAGGAGAAGAACAAAGAGAAGGAAAAGGGAGACAACAGTACAGACACCACCCAAGGAGACCCTT
TGTCGATCCACCACTACTTCCATGGCTACCTGGCTGGTTTCAGCGTGCGCTCAGGTCGCCTGGAGAGCCGCGAGGTCATCGAGTGCCTCTA
TGCATGTCGGGAGGGGCTGGACTATAGGGATTTCGAGAGCCTGGGCAAAGGCATGAAGGTCCACGTGAACCCCTCACAGTCCCTGCTCACC
CTGGAGGGGGATGATGTGGAGACCTTCAACCATGCCCTGCAGCATGTGGCTTACATGAACACTCTGCGCTTTGCCACGCCCGGCGTCAGGC
CCCTGCGCCTCACCACTGCTGTCAAGTGCTTCAGCGAAGAGTCCTGCGTCTCCATCCCTGAAGTGGAGGGCTACGTGGTCGTCCTTCAGCC
TGACGCCCCCCAGATCCTGCTGAGTGGCACTGCTCATTTTGCCCGCCCAGCTGTGGACTTTGAGGGAACCAACGGCGTCCCTTTGTTCCCT
GATCTTCAAATCACCTGCTCCATTTCTCACCAGGTGGAGGCCAAAAAGGATGAGAGTTGGCAGGGCACAGTGACAGACACACGCATGTCGG
ATGAGATTGTGCACAACCTGGATGGCTGTGAAATTTCTCTGGTGGGGGATGACCTGGATCCCGAGCGGGAAAGCCTGCTCCTGGACACAAC
CTCTCTGCAGCAGCGGGGGCTGGAGCTCACCAACACATCTGCCTACCTCACTATTGCTGGGGTGGAGAGCATCACTGTGTATGAAGAGATCC
TGAGGCAGGCTCGTTATCGGCTGCGACACGGAGCTGCCCTCTACACCAGGAAGTTCCGGCTTTCCTGCTCGGAAATGAATGGCCGTTACTC
CAGCAATGAATTCATCGTGGAGGTCAATGTCCTGCACAGCATGAACCGGGTTGCCCACCCCAGCCACGTGCTCAGCTCCCAGCAGTTCCTG
CACCGTGGTCACCAGCCCCCGCCTGAGATGGCTGGACACAGCCTAGCCAGCTCCCACAGAAACTCCATGATACCCAGCGCCGCAACCCTCA
TCATTGTGGTGTGCGTGGGCTTCCTGGTGCTCATGGTCGTCCTGGGCCTGGTGCGCATCCATTCCCTTCACCGCCGCGTCTCAGGGGCCG
GCGGGCCTCCAGGGGCCTCCAGTGACCCCAAGGACCCAGACCTCTTCTGGGATGACTCAGCTCTCACCATCATTGTGAACCCCATGGAGTC
CTACCAGAATCGGCAGTCCTGTGTGACGGGGGCTGTTGGGGGCCAGCAGGAGGATGAGGACAGCAGTGACTCGGAGGTGGCCGATTCCC
CCAGCAGCGACGAGAGACGCATCATCGAGACCCCCCCACACCGCTACTAA
```

Figure S1: The detailed information for AAV-mediated *CLSTN3* overexpression. (A) Diagrammatic representation of AAV-CLSTN3 and the empty viral vector AAV-CON used in the study. (B) The full sequence map for pAAV-CMV-CLSTN3-3FLAG-WPRE vector. (C) The coding sequence for human *CLSTN3* gene.

Figure S2

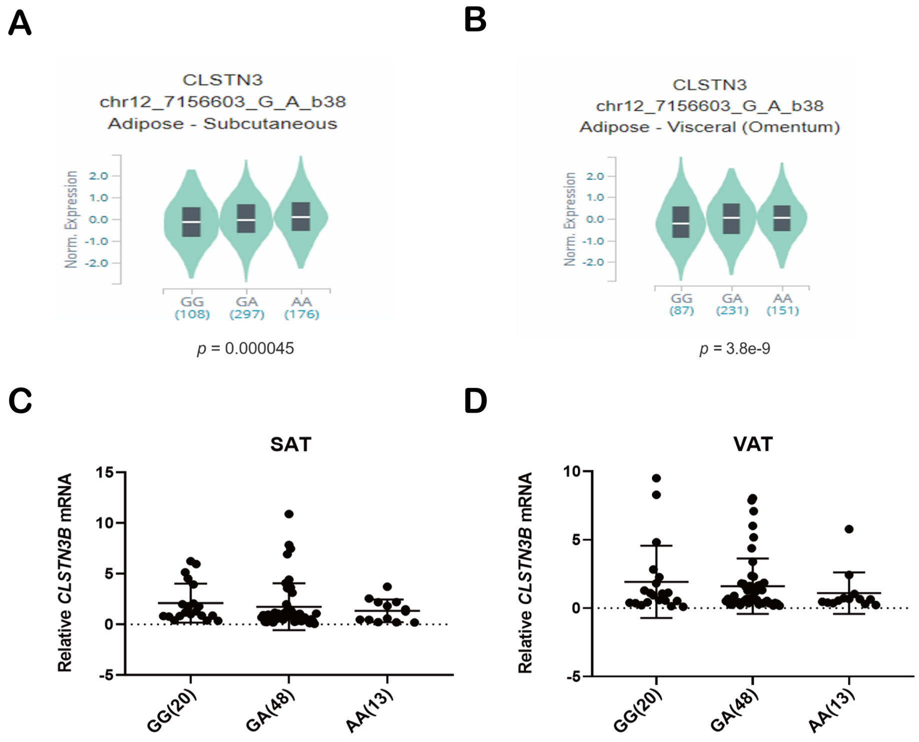


Figure S2: Association of SNP rs7296261 in the *CLSTN3* locus with gene expression in human adipose tissue. (A and B) *CLSTN3* mRNA expression in human subcutaneous (A) and visceral (B) adipose tissue from Genotype-Tissue Expression (GTEx, https://www.gtexportal.org) database. Individuals were grouped according to the genotyping for rs7296261, including GG, GA and AA genotype carriers. The number of three groups was indicated and *p*-value was calculated by GTEx Consortium. (C and D) *CLSTN3B* mRNA expression in SAT (C) and VAT (D) from 81 obese participants, grouped by GG (n = 20), GA (n = 48) and AA (n = 13) genotypes. Data are expressed as mean ± SD.

Figure S3

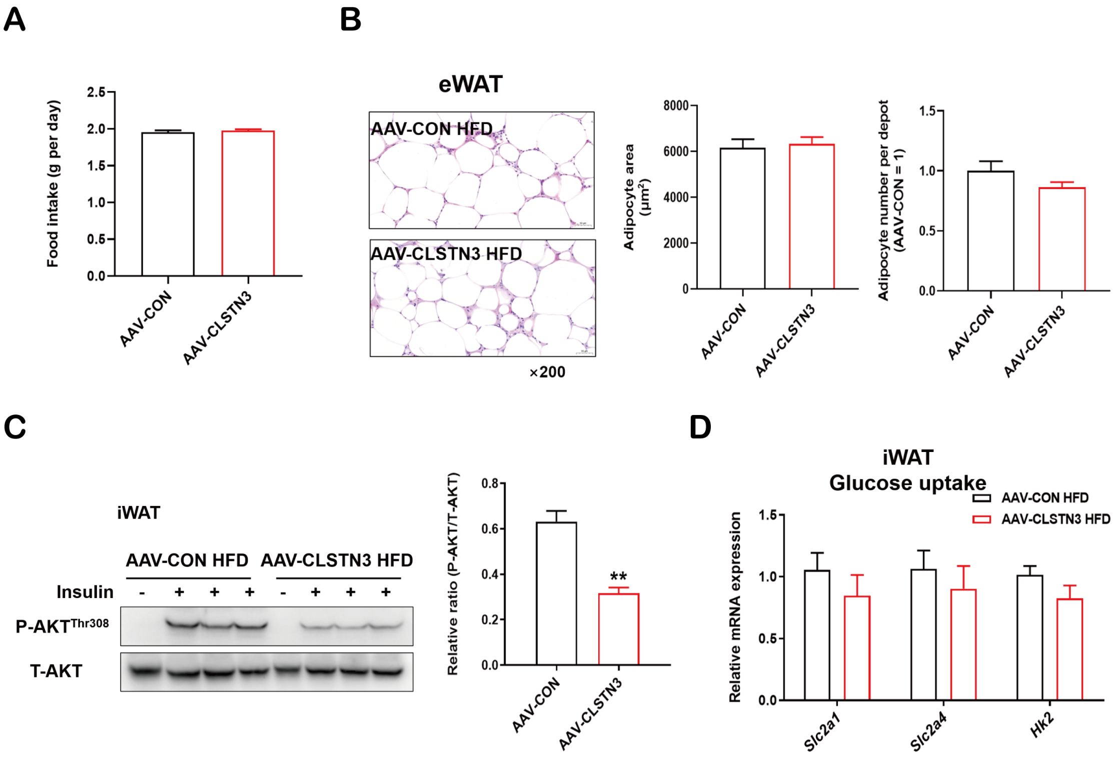


Figure S3: *CLSTN3* overexpression deteriorates diet-induced iWAT dysfunction. (A) Food intake of AAV-CON and AAV-CLSTN3 mice under HFD feeding (n = 6). (B) Representative H&E staining images of eWAT sections at 15-week HFD, and its quantification of adipocyte size and number. Scale bar, 50 μm. (C) Representative immunoblot analysis for the expression of phosphorylated AKT Thr308 (P-AKT^{Thr308}) and total AKT (T-AKT) in iWAT from HFD-fed AAV-CON and AAV-CLSTN3 mice upon insulin stimulation, and the quantification for the ratio of P-AKT to T-AKT expression. (D) Quantitative PCR analysis of mRNA expression of genes (*Slc2a1*, *Slc2a4*, and *Hk2*) associated with glucose uptake in iWAT from both groups upon 15-week HFD (n = 9). Data are shown as mean ± SEM of biologically independent samples, and **p<0.01.

Figure S4

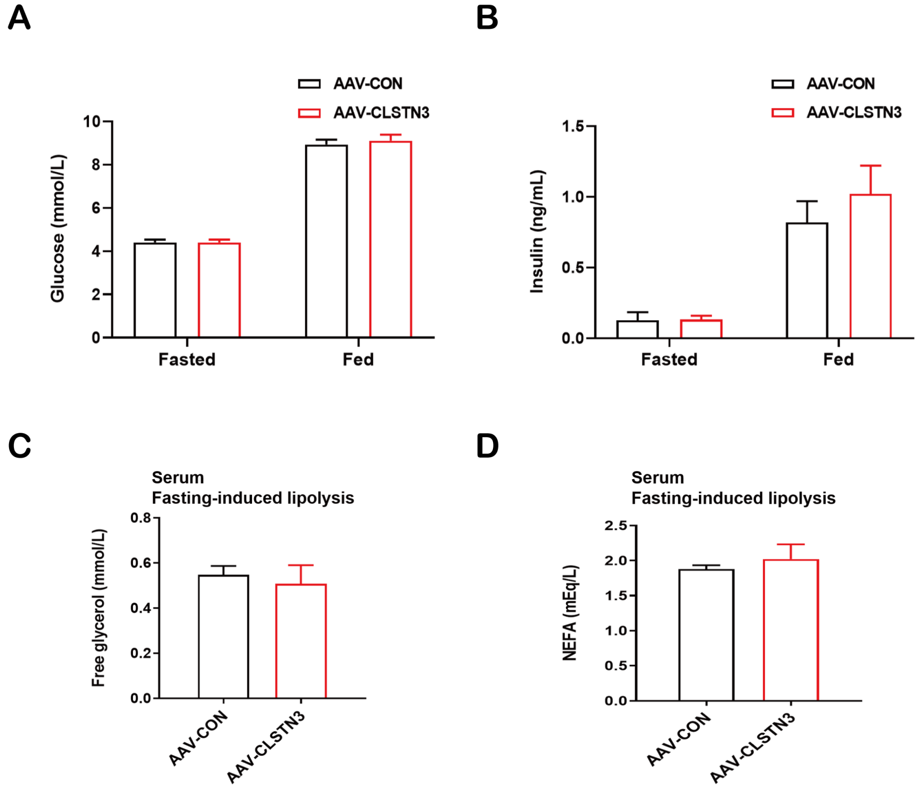


Figure S4: *CLSTN3* overexpression has no effect on fasting-induced lipolysis. (A and B) Blood glucose (A) and serum insulin (B) levels in AAV-CON and AAV-CLSTN3 under fasted or fed state (n = 8). (C and D) Free glycerol (C) and NEFA (D) levels in serum from both groups of mice under fasting condition (n = 5). Data are shown as mean ± SEM of biologically independent samples.

Supplementary Tables

Table S1: Anthropometric and clinical characteristics of 2,386 individuals from Shanghai obesity study (SHOS), grouped by GG, GA, and AA genotype carriers

	GG (n = 659)	GA (n = 1,180)	AA (n = 547)	*P*-value
Sex (male%)	0.46	0.44	0.45	0.7045
Age (year)	51 (46,57)	52 (47,57)	51.39 (46,57)	0.6524
BMI (kg/m^2)	24.24 (21.68,26.42)	24.06 (21.8,26.56)	24.74 (22.38,26.69)	0.023
WC (cm)	83 (76,90)	83 (75.5,90)	84.24 (77,90.5)	0.0671
Fat mass (kg)	17.5 (14.2,22.4)	18.2 (14.3,22.6)	19.67 (15.13,23.18)	0.0863
Body fat (%)	27.5 (23.2,32.7)	28.5 (24.13,33.9)	29.4 (24.1,33.9)	0.025
Nonfat mass (kg)	44.6 (39.2,52.2)	43.7 (38.7,51.35)	46.08 (39.4,52.3)	0.9661
SBP (mmHg)	122 (113.33,132.67)	123.33 (116,133.33)	123.62 (112,131.42)	0.6954
DBP (mmHg)	79.33 (72.67,84.67)	80 (74,86.67)	79.85 (73.33,86)	0.6147
SFA (cm^2)	153.07 (113.2,198.53)	153.85 (117.6,199.95)	167.22 (117.95,206.59)	0.1214
VFA (cm^2)	75.51 (49.98,107.85)	76.49 (50.62,111.2)	85.9 (54.51,115.65)	0.0936
Fasting plasma glucose (mmol/L)	5.37 (4.94,5.95)	5.33 (4.94,5.9)	5.74 (4.95,5.9)	0.7187
Fasting insulin (mU/L)	9.23 (7,12.42)	9.71 (7.11,12.85)	10.65 (6.96,13.43)	0.1929
HbA1c (%)	5.7 (5.4,6)	5.7 (5.4,6.1)	5.85 (5.4,6.1)	0.5572
HOMA-IR	2.34 (1.73,3.29)	2.4 (1.79,3.28)	2.84 (1.71,3.43)	0.5451
HOMA-β	111.83 (75.92,147.95)	111.19 (77.58,148.58)	128.99 (78.38,150.51)	0.3022
Total cholesterol (mmol/L)	5 (4.4,5.73)	5.11 (4.48,5.81)	5.28 (4.48,5.82)	0.0465
Triglyceride (mmol/L)	1.3 (0.91,1.86)	1.32 (0.91,2)	1.78 (0.92,1.96)	0.1529
HDL-c (mmol/L)	1.4 (1.19,1.66)	1.4 (1.2,1.63)	1.44 (1.2,1.63)	0.8666
LDL-c (mmol/L)	2.9 (2.38,3.65)	3.08 (2.46,3.83)	3.21 (2.48,3.85)	0.0382

P-values indicate statistic difference between GG and AA genotype carriers. Data are shown as median (interquartile range, 25-75%). SBP, systolic blood pressure; DBP, diastolic blood pressure; SFA, subcutaneous fat area; VFA, visceral fat area; HOMA-IR, homeostatic model assessment for insulin resistance; HOMA-β, homeostatic model assessment for β-cell function.

Table S2: Clinical characteristics of 81 obese participants in eQTL analysis, grouped by GG, GA, and AA genotype carriers

Characteristic	GG	GA	AA	*P*-value
Sex (male/female)	9/11	13/35	5/8	-
Age (year)	36.5 (28.25,44)	36 (27.25,53.25)	39 (34,49)	0.1879
BMI (kg/m^2)	34.8 (30.08,41.01)	34 (30.64,38.24)	34.68 (30.49,40.06)	0.8745
SBP (mmHg)	132.5 (120,145)	130 (120,140)	128 (120,145)	0.485
DBP (mmHg)	84.5 (80,90)	85 (80,90)	80 (77,90)	0.3386
Triglyceride (mmol/L)	1.97 (1.31,2.67)	1.5 (1.26,2.12)	1.45 (1.11,3.4)	0.4462
HDL-c (mmol/L)	0.97 (0.87,1.1)	1.06 (0.93,1.18)	0.96 (0.87,1.21)	0.6269
LDL-c (mmol/L)	3.3 (2.68,4.2)	3.24 (2.54,3.71)	3.1 (2.55,3.69)	0.2183
Fasting plasma glucose (mmol/L)	5.54 (4.56,6.89)	6.01 (4.92,7.66)	7.56 (6.15,11.35)	0.0362
HbA1c (%)	6.05 (5.4,6.88)	5.95 (5.53,8.08)	7.8 (6.25,10.05)	0.0035
2-hour plasma glucose (mmol/L)	8.4 (5.89,10.73)	9.92 (7.58,14)	11.2 (8.71,14.48)	0.071

P-value indicates statistic difference between GG and AA genotype carriers. Data are presented as median (interquartile range, 25-75%).

Table S3: Primer sequences for quantitative PCR analysis

Primer sequences for human genes	
CLSTN3 (forward)	5’--3’ TGTGGAGACCTTCAACCATGC
CLSTN3 (reverse)	5’--3’ CCGTTGGTTCCCTCAAAGTC
CLSTN3B (forward)	5’--3’ GCCATCAGCTCTAAGGTCCG
CLSTN3B (reverse)	5’--3’ CCACAATGATGAGGGTTGCG
PPARG (forward)	5’--3’ AGCCTCATGAAGAGCCTTCCA
PPARG (reverse)	5’--3’ TCCGGAAGAAACCCTTGCA
LEP (forward)	5’--3’ TGCCTTCCAGAAACGTGATCC
LEP (reverse)	5’--3’ CTCTGTGGAGTAGCCTGAAGC
RPLP0 (forward)	5’--3’ AGCCCAGAACACTGGTCTC
RPLP0 (reverse)	5’--3’ ACTCAGGATTTCAATGGTGCC
Primer sequences for mouse genes	
Ucp1 (forward)	5’--3’ AGGCTTCCAGTACCATTAGGT
Ucp1 (reverse)	5’--3’ CTGAGTGAGGCAAAGCTGATTT
Ppargc1a (forward)	5’--3’ TATGGAGTGACATAGAGTGTGCT
Ppargc1a (reverse)	5’--3’ CCACTTCAATCCACCCAGAAAG
Cidea (forward)	5’--3’ GCCGTGTTAAGGAATCTGCTG
Cidea (reverse)	5’--3’ TGCTCTTCTGTATCGCCCAGT
Cox7a1 (forward)	5’--3’ CAGCGTCATGGTCAGTCTGT
Cox7a1 (reverse)	5’--3’ AGAAAACCGTGTGGCAGAGA
Clstn3b (forward)	5’--3’ CTCCGCAGGAACAGCAGCCC
Clstn3b (reverse)	5’--3’ AGGATAACCATAAGCACCAG
Adgre1 (forward)	5’--3’ TGACTCACCTTGTGGTCCTAA
Adgre1 (reverse)	5’--3’ CTTCCCAGAATCCAGTCTTTCC
Il6 (forward)	5’--3’ CCAGAGATACAAAGAAATGATGG
Il6 (reverse)	5’--3’ ACTCCAGAAGACCAGAGGAAAT
Nos2 (forward)	5’--3’ CAGAGGACCCAGAGACAAGC
Nos2 (reverse)	5’--3’ TGCTGAAACATTTCCTGTGC
Tnfa (forward)	5’--3’ GAGAAAGTCAACCTCCTCTCTG
Tnfa (forward)	5’--3’ GAAGACTCCTCCCAGGTATATG
Clec10a (forward)	5’--3’ TGAGAAAGGCTTTAAGAACTGGG
Clec10a (forward)	5’--3’ GACCACCTGTAGTGATGTGGG
Mrc1 (forward)	5’--3’ TGTGGTGAGCTGAAAGGTGA
Mrc1 (forward)	5’--3’ CAGGTGTGGGCGCAGGTAGT
Il10 (forward)	5’--3’ GCTCTTACTGACTGGCATGAG
Il10 (forward)	5’--3’ CGCAGCTCTAGGAGCATGTG
Hif1a (forward)	5’--3’ CAAGATCTCGGCGAAGCAA
Hif1a (reverse)	5’--3’ GGTGAGCCTCATAACAGAAGCTTT
Tgfb1 (forward)	5’--3’ CCTGCAAGACCATCGACATG
Tgfb1 (reverse)	5’--3’ TGTTGTACAAAGCGAGCACC
Col1a1 (forward)	5’--3’ GTGCTCCTGGTATTGCTGGT

Col1a1 (reverse)	5’--3’ AAGGACCATCCCACTGTCTG
Col3a1 (forward)	5’--3’ GGGTTTCCCTGGTCCTAAAG
Col3a1 (reverse)	5’--3’ CCTGGTTTCCCATTTTCTCC
Col6a1 (forward)	5’--3’ GATGAGGGTGAAGTGGGAGA
Col6a1 (reverse)	5’--3’ CAGCACGAAGAGGATGTCAA
Slc2a1 (forward)	5’--3’ CAGTTCGGCTATAACACTGGTG
Slc2a1 (reverse)	5’--3’ GCCCCCGACAGAGAAGATG
Slc2a4 (forward)	5’--3’ GTGACTGGAACACTGGTCCTA
Slc2a4 (reverse)	5’--3’ CCAGCCACGTTGCATTGTAG
Hk2 (forward)	5’--3’ TGATCGCCTGCTTATTCACGG
Hk2 (reverse)	5’--3’ AACCGCCTAGAAATCTCCAGA
Pparg (forward)	5’--3’ TCGCTGATGCACTGCCTATG
Pparg (reverse)	5’--3’ GAGAGGTCCACAGAGCTGATT
Cebpa (forward)	5’--3’ CAAGAACAGCAACGAGTACCG
Cebpa (reverse)	5’--3’ GTCACTGGTCAACTCCAGCAC
Fabp4 (forward)	5’--3’ AAGGTGAAGAGCATCATAACCCT
Fabp4 (reverse)	5’--3’ TCACGCCTTTCATAACACATTCC
Acaca (forward)	5’--3’ CTCCCGATTCATAATTGGGTCTG
Acaca (reverse)	5’--3’ TCGACCTTGTTTTACTAGGTGC
Fasn (forward)	5’--3’ GGAGGTGGTGATAGCCGGTAT
Fasn (reverse)	5’--3’ TGGGTAATCCATAGAGCCCAG
Scd1 (forward)	5’--3’ CCGGAGACCCCTTAGATCGA
Scd1 (reverse)	5’--3’ TAGCCTGTAAAAGATTTCTGCAAACC
Lipe (forward)	5’--3’ GATTTACGCACGATGACACAGT
Lipe (reverse)	5’--3’ ACCTGCAAAGACATTAGACAGC
Pnpla2 (forward)	5’--3’ GACAGCTCCACCAACATCCA
Pnpla2 (reverse)	5’--3’ GCAAAGGGTTGGGTTGGTTC
Tfam (forward)	5’--3’ ATTCCGAAGTGTTTTTCCAGCA
Tfam (reverse)	5’--3’ TCTGAAAGTTTTGCATCTGGGT
Nrf1 (forward)	5’--3’ AGCACGGAGTGACCCAAAC
Nrf1 (reverse)	5’--3’ TGTACGTGGCTACATGGACCT
Nrf2 (forward)	5’--3’ CTTTAGTCAGCGACAGAAGGAC
Nrf2 (reverse)	5’--3’ AGGCATCTTGTTTGGGAATGTG
Hadh (forward)	5’--3’ TGCATTTGCCGCAGCTTTAC
Hadh (reverse)	5’--3’ GTTGGCCCAGATTTCGTTCA
Acadm (forward)	5’--3’ AGGGTTTAGTTTTGAGTTGACGG
Acadm (reverse)	5’--3’ CCCCGCTTTTGTCATATTCCG
Acaa2 (forward)	5’--3’ CTGCTACGAGGTGTGTTCATC
Acaa2 (reverse)	5’--3’ AGCTCTGCATGACATTGCCC
Cpt1b (forward)	5’--3’ GCACACCAGGCAGTAGCTTT
Cpt1b (reverse)	5’--3’ CAGGAGTTGATTCCAGACAGGTA
Cox4 (forward)	5’--3’ ATTGGCAAGAGAGCCATTTCTAC
Cox4 (reverse)	5’--3’ CACGCCGATCAGCGTAAGT

Cox5b (forward)	5'--3' TTCAAGGTTACTTCGCGGAGT
Cox5b (reverse)	5'--3' CGGGACTAGATTAGGGTCTTCC
Cox6a (forward)	5'--3' TCAACGTGTTCCTCAAGTCGC
Cox6a (reverse)	5'--3' AGGGTATGGTTACCGTCTCCC
36b4 (forward)	5'--3' AAGCGCGTCCTGGCATTGTCT
36b4 (reverse)	5'--3' CCGCAGGGGCAGCAGTGGT
Primer sequences for mtDNA copy number	
mt-Nd1 (forward)	5'--3' CAGCCGGCCCATTCGCGTTA
mt-Nd1 (reverse)	5'--3' AGCGGAAGCGTGGATAGGATGC
Rbm15 (forward)	5'--3' GGACACTTTTCTTGGGCAAC
Rbm15 (reverse)	5'--3' AGTTTGGCCCTGTGAGACAT

Table S4: Primary data of initial OCR value and protein content for mitochondrial respiration measurement

Sample	Basal OCR (pmol/min)	Maximal OCR (pmol/min)	Protein content (mg)
AAV-CON No.1	365.76	580.62	0.140
AAV-CON No.2	769.22	817.33	0.172
AAV-CON No.3	310.01	765.21	0.130
AAV-CON No.4	772.66	971.8	0.187
AAV-CON No.5	312.77	725.1	0.138
AAV-CLSTN3 No.1	439.03	591.06	0.137
AAV-CLSTN3 No.2	289.22	328.73	0.132
AAV-CLSTN3 No.3	310.23	361.53	0.114
AAV-CLSTN3 No.4	308.19	456.9	0.123
AAV-CLSTN3 No.5	413.51	427.48	0.134