

Figure S3. Off-target effects. (A) miR-26a sgRNAs of off-target sites predicted using bioinformatics software. (B) miR-26b sgRNAs of off-target sites predicted using bioinformatics software. Red lowercase letters indicate mismatched bases. (C–E) T7EN1 digestion at the off-target site. (F) Location of off-target B-OT-2-4 in the genome.

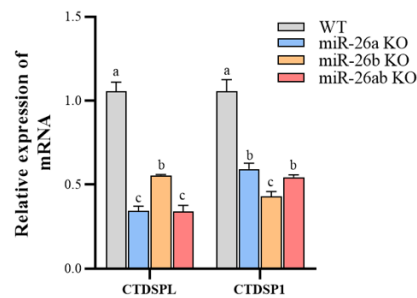


Figure S4. Expression of miR-26 host genes. WT, wild-type GMECs. miR-26a KO, miR-26a knockout GMECs. miR-26b KO, miR-26b knockout GMECs. miR-26ab KO, GMECs with co-knockout of miR-26a and miR-26b. Data are shown as mean \pm SD for three independent experiments. Lower case letters, $p < 0.05$.

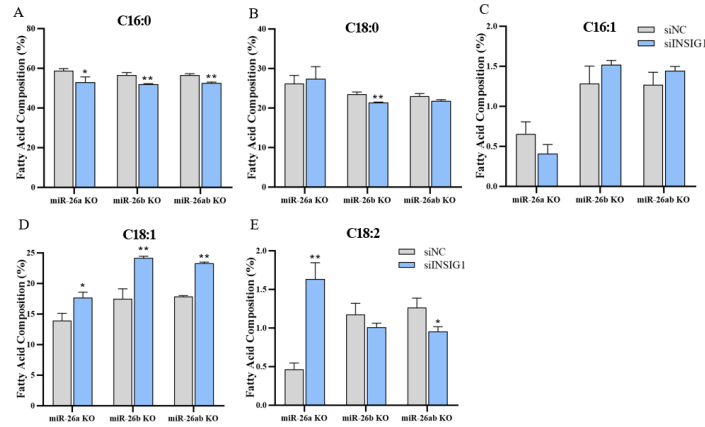


Figure S5. The percentage of fatty acid in knockout GMECs with inhibiting *INSIG1*. C16:0, palmitic acid. C18:0, stearic acid. C16:1, palmitoleic acid. C18:1, oleic acid. C18:2, linoleic acid. WT, wild-type GMECs. miR-26a KO, miR-26a knockout GMECs. miR-26b KO, miR-26b knockout GMECs. miR-26ab KO, GMECs with co-knockout of miR-26a and miR-26b. Data are shown as mean \pm SD for three independent experiments. *, $p < 0.05$. **, $p < 0.01$.

Table S1. Effects on fatty acid composition in knockout GMECs.

Fatty Acid (%)	WT	miR-26a KO	miR-26b KO	miR-26ab KO
C16:1	0.73 \pm 0.06	0.73 \pm 0.15	0.60 \pm 0.16	0.64 \pm 0.15
C16:0	50.51 \pm 2.04 ^b	52.75 \pm 0.70 ^b	58.15 \pm 0.51 ^a	58.76 \pm 2.57 ^a
C18:2	4.81 \pm 0.58 ^a	3.98 \pm 0.60 ^b	1.34 \pm 0.15 ^c	0.44 \pm 0.05 ^d
C18:1	18.65 \pm 0.64 ^a	14.31 \pm 0.62 ^b	14.01 \pm 1.53 ^b	12.24 \pm 0.5 ^c
C18:0	25.30 \pm 1.26	28.23 \pm 0.78	25.89 \pm 1.57	27.92 \pm 2.88
SFA	75.81 \pm 0.96 ^d	80.98 \pm 0.71 ^c	84.05 \pm 1.52 ^b	86.67 \pm 0.35 ^a
UFA	24.19 \pm 0.96 ^a	19.02 \pm 0.71 ^b	15.95 \pm 1.52 ^c	13.33 \pm 0.35 ^d

Statistical analysis among these four groups was assessed via one-way ANOVA with Duncan's test. Statistical significance is as follows: lowercase letters, $p < 0.05$, and the different lowercase letters represent a significant difference between any two groups. Data are shown as mean \pm SD. WT, the wild-type GMECs. miR-26a KO, the miR-26a knockout GMECs; miR-26b KO, the miR-26b knockout GMECs; miR-26ab KO, the miR-26a and miR-26b co-knockout GMECs.

Table S2. Effects of *INSIG1* silencing on fatty acid composition in knockout GMECs.

Fatty Acid (%)	miR-26a KO		miR-26b KO		miR-26ab KO	
	siNC	siINSIG1	siNC	siINSIG1	siNC	siINSIG1
C16:1	1.28 \pm 0.22	1.52 \pm 0.05	1.27 \pm 0.15	1.44 \pm 0.05	0.66 \pm 0.15	0.41 \pm 0.12
C16:0	56.60 \pm 1.25	51.88 \pm 0.33 **	56.55 \pm 0.72	52.49 \pm 0.56 **	58.78 \pm 1.04	52.88 \pm 2.76 *
C18:2	1.18 \pm 0.14	1.01 \pm 0.05	1.27 \pm 0.12	0.95 \pm 0.06 *	0.46 \pm 0.08	1.63 \pm 0.21 **
C18:1	17.48 \pm 1.63	24.17 \pm 0.28 **	17.89 \pm 0.12	23.28 \pm 0.22 **	13.92 \pm 1.19	17.70 \pm 0.86 *
C18:0	23.46 \pm 0.59	21.42 \pm 0.08 **	23.02 \pm 0.63	21.83 \pm 0.27	26.19 \pm 2.05	27.37 \pm 3.09
SF *	80.06 \pm 1.83	73.30 \pm 0.34 **	79.57 \pm 0.09	74.32 \pm 0.31 **	84.96 \pm 1.05	80.25 \pm 0.97 **
UF *	19.94 \pm 1.83	26.70 \pm 0.34 **	20.43 \pm 0.09	25.68 \pm 0.31 **	15.04 \pm 1.05	19.75 \pm 0.97 **

Differences between the two treatments were tested for significance using Student's *t*-test, where differences were considered to be statistically significant when $p < 0.05$ (* $p < 0.05$, ** $p < 0.01$). Data are shown as mean \pm SD. WT, the wild-type GMECs. miR-26a KO, the miR-26a knockout GMECs. miR-26b KO, the miR-26b knockout GMECs. miR-26ab KO, the miR-26a and miR-26b co-knockout GMECs. siNC, negative control of interfering RNA. siINSIG1, interfering RNA of *INSIG1*.

Table S3. Primers of PCR.

Primer	Primer Sequence (5' to 3')		Product Size (bp)
PCR-26a	F: GGCAGGCACTTGGGCATTTA	R: CCTTTGGGATGCTGCTTGGA	584
PCR-26b	F: CAGGTTCTCCACCAGTCC	R: GGTAACACCCACCGTCCC	420
A-OT-1-1	F: GTCCCACTTAAAGATTCCTACC	R: TTCAGCCTCAAACACTCCA	487
A-OT-1-2	F: CAAATGAGACCCAGCAAAG	R: GGCCAGCAAGTTAAGGATG	425
A-OT-1-3	F: GCCACTCAGAACCTCAAATGC	R: TCACTTTACCCAAGGTTGCCTT	429
A-OT-1-4	F: CTCTGCCTTCCAGCACATCA	R: CTCCGACTTTGCTCAGCTGAA	408
A-OT-1-5	F: CTGTCCTCCTCTTCCTCGGA	R: TGCCTTGTGTGGTCAAAGGTT	435
A-OT-1-6	F: TGCCAGACCTAAGCAATCT	R: TGGTGTGCCCTGTTAACGTTA	498
A-OT-2-1	F: CCACCCAGGCGGGAGTTGTA	R: TTACACGTAGGCAGGCCAGCTGGGG	481
A-OT-2-2	F: GCACCTGCCCAGTGCTAT	R: TGCGAGAAGGACCCCAAG	427
A-OT-2-3	F: TCCCAAACGTGCATTCTG	R: GGCCGGTTCTCACCAGTA	436
A-OT-2-4	F: ACGTCTAACCTCACTCCCATTT	R: AGCAGCCCAAGCAGAAAA	447
A-OT-2-5	F: GGTCAAGCCAGCATTCGT	R: GCGGCTCTTATGTCTCCTC	400
A-OT-3-1	F: TGTACCTGGTGCTCTTCT	R: CGTTCTACCTTACTGCGTGCCTGCG	316
A-OT-3-2	F: TCAGGGTATGCCATTGTG	R: GAGGGAAGTGAGTGCAGATA	444
A-OT-3-3	F: CCATCACTGCCTGGAACA	R: GAAAGGGAAAAGTGAAAGTGAAGTC	353
A-OT-3-4	F: GTCCGATCATTTCTTGTC	R: TCGTGTATCCCTTTTGTGTCATG	357
A-OT-3-5	F: TTCTTTGCGGGCTGAAAC	R: CCGAAGTACATCAGGAGGAT	329
B-OT-1-1	F: TTCCTGTCCCAAGGCATGAC	R: CCTTTCATCAGGCCACACTG	424
B-OT-1-2	F: TCTCAGCGCCTAACACAGTC	R: ACAGTCTCAGTGCAGAACCGG	433
B-OT-1-3	F: GACTGCTGCACACAACTG	R: AGCTAGGAGGGTGTGGATGTC	439
B-OT-1-4	F: ATGAAGGAGAAGCTGCCCAC	R: GTCTCCTTTGGGATGCTGCTT	473
B-OT-1-5	F: CGCCAAAAGTTACAAGGGGC	R: TGCCACTTCTCACTCTCAGCT	418
B-OT-2-1	F: AGATCCACTTCATCCCCGA	R: GTGCCTTCTCCGAGTCCAAAA	412
B-OT-2-2	F: CGAAAGTCCAACCTGCTTAAGGG	R: GGGATGGGCAGCATCCTTAAT	402
B-OT-2-3	F: GCAAGGTTGAGGGGTGCTAT	R: ACATTAATGCTGCAGGGGCTT	489
B-OT-2-4	F: TACCCTTCCCTGCTATCCCC	R: CGTCATCACCCAGAGACCTGT	425
B-OT-2-5	F: CAGACAGCAGAAGCCTGACA	R: GAATAGGCCACAGGTTTGGA	454
B-OT-2-6	F: GAGCTTGGGTTTGCTTTGCA	R: GGGATGTGTGTCGCTCTGTTT	417
B-OT-3-1	F: AGGCAGGAACATTTGTGAACA	R: CACTGACCCAAAACCGGAGTA	429
B-OT-3-2	F: ATTAGTGGCAGCGCTTCTGT	R: CGTCCCGTCACTTTCCTTGAG	412
B-OT-3-3	F: GAGGAGTGCATGTTCCCCAA	R: AGGGCAGAGAACATCCCTCTG	437
B-OT-3-4	F: AGCCTTGCTCCATCCTGAAC	R: CACATGTACGCCTCCCTTTC	491
B-OT-3-5	F: CTTCCGCCTCTTCGAGCTG	R: GTCACCGTCGAGAAGACCTGG	498

F: forward primer. R: reverse primer.

Table S4. All qPCR primers.

Gene	GenBank ID	Primer Sequence (5' to 3')	Product Size (bp)	Efficiency
miR-26a-5p	/	F: CGTTCAAGTAATCCAGGATAGGCT	/	2.01
miR-26a-3p	/	F: GGCCTATTCTCGGTACTTGCACG	/	1.96
snRNA U6	/	F: CTCGCTTCGGCAGCACATA	/	2.10
ACACA	XM_018064174.1	F: CTCCAACCTCAACCACTACGG R: GGGGAATCACAGAAGCAGCC	171	2.15
ACSS2	XM_018057751.1	F: GGCGAATGCCTCTACTGCTT R: GGCCAATCTTTCTCTAATCTGCTT	100	2.13
AGPAT6	XM_018041984.1	F: AAGCAAGTTGCCCATCCTCA R: AAAGTGTGGCTCCAATTTCA	101	2.30

CTDSP1	XM_018059509.1	F: CCAAAGCCCAGGACTCGGACAAGA R: GGACCACCCCATCAATCTCCACAGG	126	2.12
CTDSPL	XM_018066968.1	F: TGACCAGAGGCAGATCATTCCCATAACC R: TGTGGCCGCTTCAGCACATACACC	213	2.06
DGAT1	XM_018058728.1	F: CCACTGGGACCTGAGGTGTC R: GCATCACCACACACCAATTCA	101	1.94
DGAT2	XM_018058853.1	F: CATGTACACATTCTGCACCGATT R: TGACCTCCTGCCACCTTTCT	100	2.10
FASN	NM_001285629.1	F: GGGCTCCACCACCGTGTTCCA R: GCTCTGCTGGGCCTGCAGCTG	226	2.08
FADS2	XM_018043056.1	F: AAAGGGTGCCTCTGCCAACT R: ACACGTGCAGCATGTTTACA	101	2.06
GPAM	XM_005698479.3	F: ATTGACCCTTGGCACGATAG R: AACAGCACCTTCCCACAAAG	188	2.02
INSIG1	NM_001286088.1	F: AGCCTCACAAGTTCAAGCG R: ACAGTGCTGCTAATGTCAAGG	132	2.05
PPARG	NM_001285658.1	F: CCTTCACCACCGTTGACTTCT R: GATACAGGCTCCACTTTGATTGC	145	1.93
SCD1	NM_001285619.1	F: CCATCGCCTGTGGAGTCAC R: GTCGGATAAATCTAGCGTAGCA	257	2.02
SREBP1A	NM_001285755.1	F: CTGCTGACCGACATAGAAGACAT R: GTAGGGCGGGTCAAACAGG	81	2.00
SREBP1c	NM_001285755.1	F: ACGCCATCGAGAAACGCTAC R: GTGCGCAGACTCAGGTTCTC	181	2.04
SREBP2	XM_018048827.1	F: CGGCGTGATCGTGCTGAGCGTC R: CCGACAGGCAGGTTTGCAGGTTGG	173	2.03
UXT	XM_005700842.2	F: TGTGGCCCTTGGATATGGTT R: GGTTGTCGCTGAGCTCTGTG	101	2.06

F: forward primer. R: reverse primer.