

Supplementary information

Predicted STOP codon locations in *PCSK9*

TGA canonical

TGA new

Full-length *PCSK9*

Exon 12

GTGACCGTGGCCTGCGAGGAGGGCTGGACCCTGACTGGCTGCAGTGCCCTCCCTGGGACC
TCCCACGTCCTGGGGGCCTACGCCGTAGACAACACGTGTGTAGTCAGGAGCCGGGACGTC
AGCACTACAGGCAGCACCAAGCGAAGGGGCCGTGACAGCCGTTGCCATCTGCTGCCGGAGC
CGGCACCTGGCGCAGGCCTCCCAGGAGCTCCAGTGAACAGCCCCATCCCAGGATGGGTGTC

Exclusion of exon 2

Exon 12

GTGACCGTGGCCTGCGAGGAGGGCTGGACCCTGACTGGCTGCAGTGCCCTCCCTGGGACC
TCCCACGTCCTGGGGGCCTACGCCGTAGACAACACGTGTGTAGTCAGGAGCCGGGACGTC
AGCACTACAGGCAGCACCAAGCGAAGGGGCCGTGACAGCCGTTGCCATCTGCTGCCGGAGC
CGGCACCTGGCGCAGGCCTCCCAGGAGCTCCAGTGAACAGCCCCATCCCAGGATGGGTGTC

Exclusion of exon 8

Exon 12

GTGACCGTGGCCTGCGAGGAGGGCTGGACCCTGACTGGCTGCAGTGCCCTCCCTGGGACC
TCCCACGTCCTGGGGGCCTACGCCGTAGACAACACGTGTGTAGTCAGGAGCCGGGACGTC
AGCACTACAGGCAGCACCAAGCGAAGGGGCCGTGACAGCCGTTGCCATCTGCTGCCGGAGC
CGGCACCTGGCGCAGGCCTCCCAGGAGCTCCAGTGAACAGCCCCATCCCAGGATGGGTGTC

Exclusion of exon 9

Exon 10

GCCCAAGGGGGCAAGCTGGTCTGCCGGGCCCACAACGCTTTTGGGGGTGAAGGGTGTCTAC
GCCATTGCCAGGTGCTGCCTGCTACCCCAGGCCAACTGCAGCGTCCACACAGCTCCACCA
GCTGAGGCCAGCATGGGGACCCGTGTCCACTGCCACCAACAGGGCCACGTCCTCACAG

Exclusion of exon 10

Exon 11

GCTGCAGCTCCCACTGGGAGG**TGG**AGGACCTTGGCACCCACAAGCCGCCTGTGCTGAGGC
CACGAGGTCAGCCCAACCAGTGCGTGGGCCACAGGGAGGCCAGCATCCACGCTTCCTGCT
GCCATGCCCCAGGTCTGGAATGCAAAGTCAAGGAGCATGGAATCCCGGCCCTCAGGAGC
AG

Exclusion of exon 11 (cryptic splicing)

Exon 11

GCTGCAGCTCCCACTGGGAGGTGGAGGACCTTGGCACCCACAAGCCGCCTGTGCTGAGGC
CACGAGGTCAGCCCAACCAGTGCGTGGGCCACAGGGAGGCCAGCATCCACGCTTCCTGCT
GCCATGCCCCAGGTCTGGAATGCAAAGTCAAGGAGCATGGAATCCCGGCCCTCAGGAGC
AG

Exon 12

GTGACCGTGGCCTGCGAGGAGGGCTGGACCCTGACTGGCTGCAGTGCCCTCCCTGGGACC
TCCCACGTCCTGGGGGCCTACGCCGTAGACAACACGTGTGTAGTCAGGAGCCGGGACGTC
AGCACTACAGGCAGCACCAGCGAAGGGGCCGTGACAGCCGTTGCCATCTGCTGCCGGAGC
CGGCACCTGGCGCAGGCCTCCCAGGAGCTCCAG**TGA**CAGCCCCATCCCAGGATGGGTGTC
TGGGGAGGGTCAAGGGCTGGGGC**TGA**GCTTTAAATGGTTCCGACTTGTCCCTCTCTCAG
CCCTCCATGGCCTGGCACGAGGGGATGGGGATGCTTCCGCCTTCCGGGGCTGCTGGCCT
GGCCCTTGAGTGGGGCAGCCTCCTTGCCTGGAACCTCACTCACTCTGGGTGCCTCCTCCC

Amino acid composition and predicted MW of PCSK9

Full-length PCSK9 (precursor 74.286 kD, mature 57.273 kD)

MGTVSSRRSWWPLPLLLLLLLLLLPAGARAQEDDGDYEELVLALRSEEDGLAEAPEHGT
TATFHRCADPWRLPGTYVVVLKEETHLSQSERTARRLQAQAARRGYLTKILHVFHGLLP
GFLVKMSGDLLELALKLPHVDYIEEDSSVFAQSIPWNLERITPPRYRADEYQPPDGGSLV
EYLLDTSIQSDHREIEGRVMVTD FENVPEEDGTRFHRQASKCDSHGTHLAGVVSGRDAG
VAKGASMRLRLVLCQKGKTVSGTLIGLEFIRKSQLVQPVGPLVLLPLAGGYSRVLNAA
CQRLARAGVVLVTAAGNFRDDACLYSPASAPEVITVGATNAQDQPVTLGTLGTNFGRCVD
LFAPGEDIIGASSDCSTCFVSQSGTSQAAAHVAGIAAMMLSAEPELTLAELRQRLIHFS
KDVINEAWFPEDQRVLTPLNVAALPPSTHGAGWQLFCRTVWSAHSGPTRMATAVARCAPD
EELLSCSSFSRSGKRRGERMEAQGGKLVCRAHNAFGGEGVYAIARCCLLPQANCSVHTAP
PAEASMGTRVHCHQQGHVLTGCSSHWEVEDLGTHKPPVLRPRGQPNQCVGHREASIHASC
CHAPGLECKVKEHGIPAPQEQT VACEEGWTLTGCSALPGTSHVLGAYAVDNTCVVRSRD
VSTTGSTSEGAVTAVAICCRSRHLAQASQELQ

Exclusion of exon 2 (precursor 66.990 kD, mature 57.273 kD)

MGTVSSRRSWWPLPLLLLLLLLLLPAGARAQEDDEDGDYEELVLALRSEEDGLAEAPEHGT
TATFHRCALKLPHVDYIEEDSSVFAQSIPWNLERITPPRYRADEYQPPDGGSLV
EVYLLDTSIQSDHREIEGRVMVTD FENVPEEDGTRFHRQASKCDSHGTHLAGVVSGRDAG
VAKGASMRLRVLNQCQKGT VSGTLIGLEFIRKSQLVQPVGPLVLLPLAGGYSRVLNAA
CQRLARAGVVLVTAAGNFRDDACLYSPASAPEVITVGATNAQDQPVT LGTLGTNFGRCVD
LFAPGEDIIGASSDCSTCFVSQSGTSQAAAHVAGIAAMMLSAEPELTLAELRQR LIHFSA
KDVINEAWFPEDQRVLT PNLVAALPPSTHGAGWQLFCRTVWSAHSGPTRMATAVARCAPD
EELLSCSSFSRSGKRRGERMEAQGGKLV CRAHNAFGGEGVYAIARCCLLPQANCSVHTAP
PAEASMGTRVHCHQQGHVLTGCSSHWEVEDLGTHKPPVLRPRGQPNQCVGHREASIHASC
CHAPGLECKVKEHGIPAPQE QVTVACEEGWTLTGCSALPGTSHVLGAYAVDNTCVVRSRD
VSTTGSTSEGAVTAVAICCRSRHLAQASQELQ

Exclusion of exon 8 (precursor 67.966 kD, mature 50.953 kD)

MGTVSSRRSWWPLPLLLLLLLLLLPAGARAQEDDEDGDYEELVLALRSEEDGLAEAPEHGT
TATFHRCAKDPWRLPGTYVVVLKEETHLSQSERTARRLQAQAARRGYLTKILHVFHGLLP
GFLVKMSGDLLELALKLPHVDYIEEDSSVFAQSIPWNLERITPPRYRADEYQPPDGGSLV
EVYLLDTSIQSDHREIEGRVMVTD FENVPEEDGTRFHRQASKCDSHGTHLAGVVSGRDAG
VAKGASMRLRVLNQCQKGT VSGTLIGLEFIRKSQLVQPVGPLVLLPLAGGYSRVLNAA
CQRLARAGVVLVTAAGNFRDDACLYSPASAPEVITVGATNAQDQPVT LGTLGTNFGRCVD
LFAPGEDIIGASSDCSTCFVSQSGTSQAAAHVAGWQLFCRTVWSAHSGPTRMATAVARCAPD
EELLSCSSFSRSGKRRGERMEAQGGKLV CRAHNAFGGEGVYAIARCCLLPQANCSVHTAP
PAEASMGTRVHCHQQGHVLTGCSSHWEVEDLGTHKPPVLRPRGQPNQCVGHREASIHASC
CHAPGLECKVKEHGIPAPQE QVTVACEEGWTLTGCSALPGTSHVLGAYAVDNTCVVRSRD
VSTTGSTSEGAVTAVAICCRSRHLAQASQELQ

Exclusion of exon 9 (precursor 50.377 kD, mature 33.364 kD)

MGTVSSRRSWWPLPLLLLLLLLLLPAGARAQEDDEDGDYEELVLALRSEEDGLAEAPEHGT
TATFHRCAKDPWRLPGTYVVVLKEETHLSQSERTARRLQAQAARRGYLTKILHVFHGLLP
GFLVKMSGDLLELALKLPHVDYIEEDSSVFAQSIPWNLERITPPRYRADEYQPPDGGSLV
EVYLLDTSIQSDHREIEGRVMVTD FENVPEEDGTRFHRQASKCDSHGTHLAGVVSGRDAG

VAKGASMRSLRVLNCQGKGTVSGTLIGLEFIRKSQLVQPVGPLVVLLPLAGGYSRVLNAA
CQRLARAGVVLVTAAGNFRDDACLYSPASAPEVITVGATNAQDQPVTLGTLGTNFGRCVD
LFAPGEDIIGASSDCSTCFVSQSGTSQAAAHVAGIAAMMLSAEPELTLAELRQRLIHFS
KDVINEAWFPEDQRVLT PNLVAALPPSTHGA **GPRGQAGLPGPQRFWG**

Exclusion of exon 10 (precursor 56.210 kD, mature 39.197 kD)

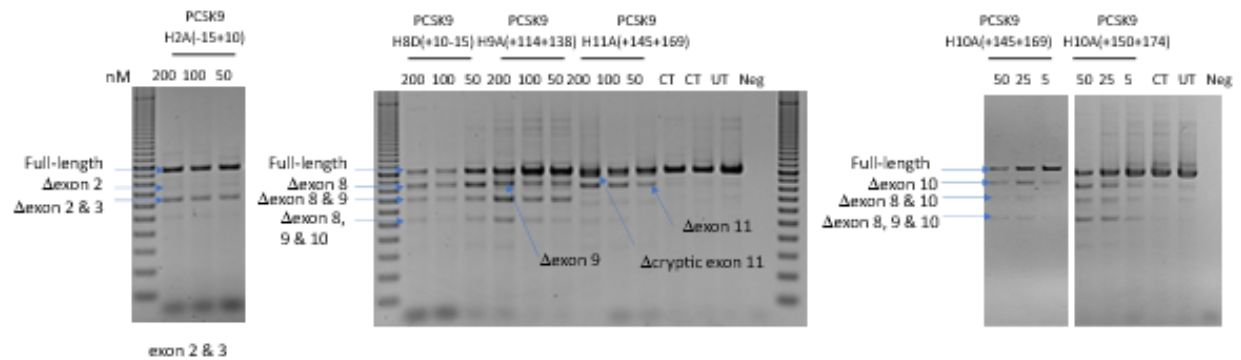
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VAKGASMRSLRVLNCQGKGTVSGTLIGLEFIRKSQLVQPVGPLVVLLPLAGGYSRVLNAA
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KDVINEAWFPEDQRVLT PNLVAALPPSTHGA GWQLFCRTVWSAHSGPTRMATAVARCAPD
EELLSCSSFSRSGKRRGERME **AAAPTGRWRTLAPTSRLC**

Exclusion of exon 11 (cryptic splicing) (precursor 74.846 kD, 57.833 kD)

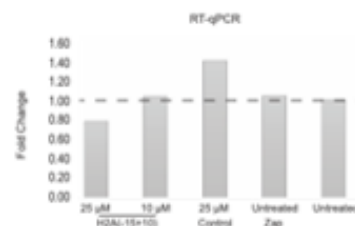
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TATFHRCADPWRLPGTYVVVLKEETHLSQSERTARRLQAQAARRGYLTKILHVFHGLLP
GFLVKMSGDLLELALKLPHVDYIEEDSSVFAQSIPWNLERITPPRYRADEYQPPDGGSLV
EYVLLDTSIQSDHREIEGRVMVTD FENVPEEDGTRFHRQASKCDSHGTHLAGVVSGRDAG
VAKGASMRSLRVLNCQGKGTVSGTLIGLEFIRKSQLVQPVGPLVVLLPLAGGYSRVLNAA
CQRLARAGVVLVTAAGNFRDDACLYSPASAPEVITVGATNAQDQPVTLGTLGTNFGRCVD
LFAPGEDIIGASSDCSTCFVSQSGTSQAAAHVAGIAAMMLSAEPELTLAELRQRLIHFS
KDVINEAWFPEDQRVLT PNLVAALPPSTHGA GWQLFCRTVWSAHSGPTRMATAVARCAPD
EELLSCSSFSRSGKRRGERME AQGGKLV CRAHNAFGGEGVYAIARCCLLPQANCSVHTAP
PAEASMGTRVHCHQQGHVLTGCSSHWEVEDLGTHKPPVLRPRGQPNQCVGHREASIHAS
CCHAPG **DRGLRGGLDPDWLQCPPWDLPRPGGLRRRQHVC SQEPGRQH YRQHQRGRD**
SRCHLLPEPAPGAGLPGAPVTAPSQDGCLGRVKGWG

Supplementary figures

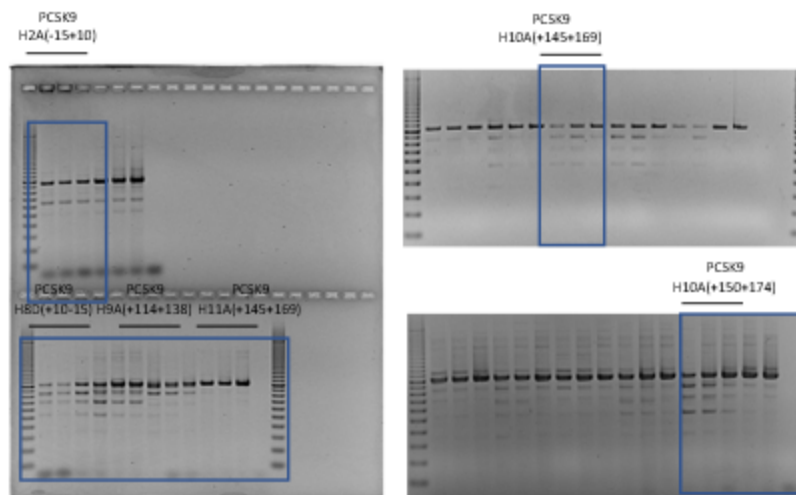
(a)



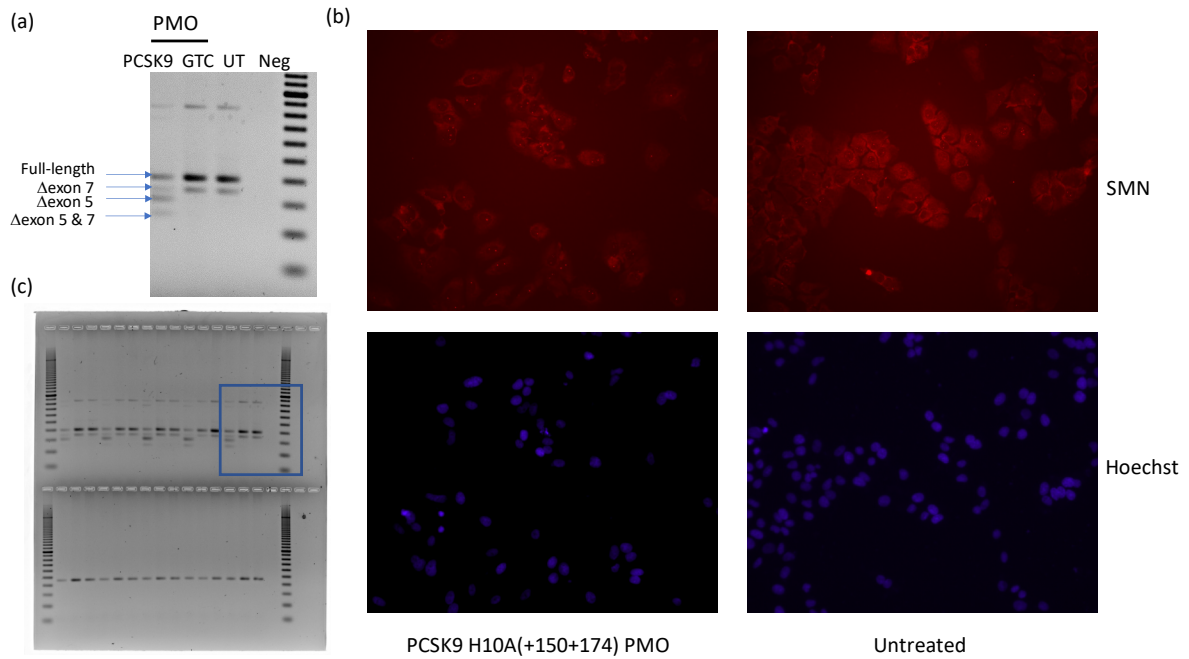
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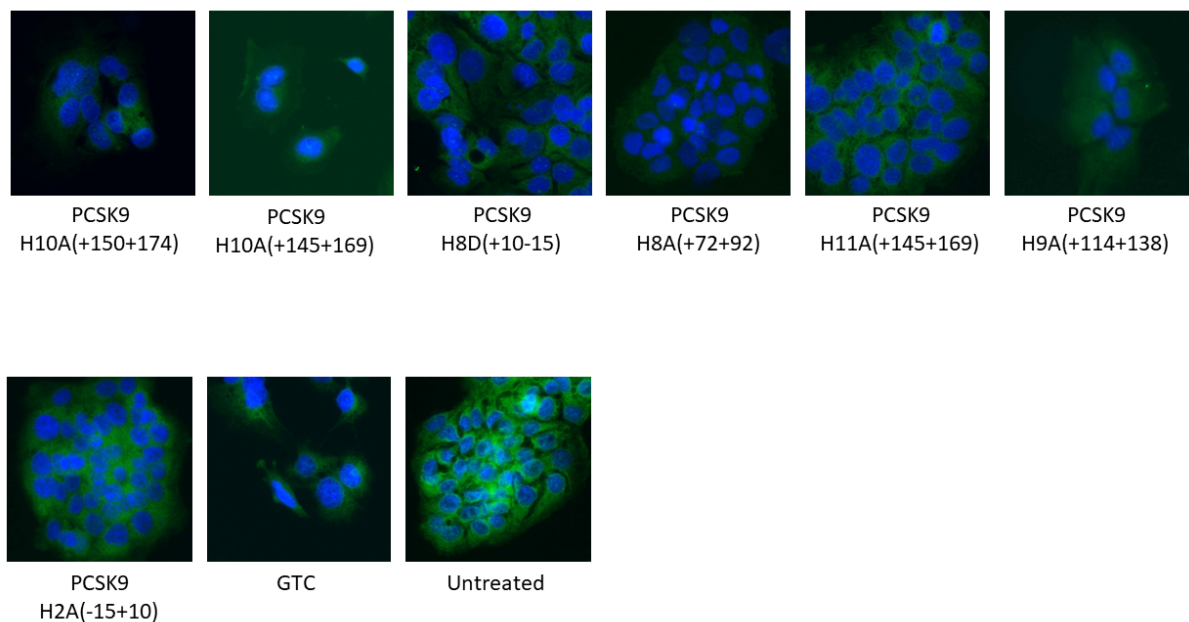
(c)



Supplementary figure 1. (a) RT-PCR analysis of *PCSK9* transcripts in Huh 7 cells after transfection with 2'OMe PS AOs targeting exon 2, 8, 9, 10 and 11. (b) RT-qPCR analysis of full-length *PCSK9* after treatment with exon 2 targeting PMO. Ctrl; samples treated with GeneTools control AO. UT; untreated. Neg; RT-PCR without template. (c) Original gels for panel (a).



Supplementary figure 2. Assessment of gem formation after treatment with PCSK9 PMO H10A(+150+174). (a) RT-PCR analysis of *SMN* transcripts in Huh 7 cells after treating with PCSK9 PMO for 48 hours. GTC: sample treated with GTC PMO, UT; untreated. Neg; RT-PCR without template. (b) Analysis of gems after immunolabelling of SMN (red) and nuclei (blue). (c) Original gel for panel (a).



Supplementary figure 3. Analysis of the distribution of intracellular PCSK9 protein isoforms after treating with PMOs to induce exon skipping. The name of the PMO is shown below. GTC; GeneTools control. Green; PCSK9, blue; nuclei.

Supplementary Table 1. Coordinates and sequences for 2'OMe PS AOs and PMOs

AO Names and Coordinates	2'OMe PS AOs	% Knockdown*	PMOs
PCSK9 H2A(-15+10)	UCCACGGAUCCUGGCCCAUGCAAG	61.5	TCCACGGATCCTGGCCCCATGCAAG
PCSK9 H2A(+37+61)	GCGAGAGGUGGGUCUCCUCCUUCAG	22.8	
PCSK9 H2A(+75+99)	AGCCUGGGCCUGCAGGCGGCGGGCA	33.0	
PXSK9 H2A(+110+134)	ACAUGCAGGAUCUUGGUGAGGUAUC	7.7	
PCSK9 H2A(+158+182)	AGGUCGCCACUCAUCUUCACCAGGA	36.6	
PCSK9 H2D(+10-15)	AAAAGGGUGGCUCACCAGCUCCAGC	20.5	
PCSK9 H8A(-5+20)	CAGCAUCAUGGCUGCAAUGCCUGGU	26.6	GATGACATCTTTGGCAGAGAA
PCSK9 H8A(+68+92)	GAUGACAUCUUUGGCAGAGAAGUGG	16.1	
PCSK9 H8A(+72+92)	GAUGACAUCUUUGGCAGAGAA	Not tested #	
PCSK9 H8A(+100+124)	CCCGCUGGUCCUCAGGGAACCAGG	11.8	
PCSK9 H8D(+10-15)	UGCCAUCCUGCUUACCUGCCCCAUG	80.0	
PCSK9 H9A(-2+23)	AGUCCUGCAAAACAGCUGCCAACCU	5.7	CGCCCCGCCGCTTCCCACTCCTGGA
PCSK9 H9A(+30+54)	UCCGUGUAGGCCCCGAGUGUGCUGA	0.0	
PCSK9 H9A(+60+84)	CUGGGGCGCAGCGGGCGACGGCUGU	24.0	
PCSK9 H9A(+85+109)	CUGGAGCAGCUCAGCAGCUCCUCAU	3.3	
PCSK9 H9A(+114+138)	CGCCCCGCCGCUUCCCACUCCUGGA	50.0	
PCSK9 H10A(-15+10)	CCCCUUGGGCCUAGAGUCAAGAC	28.7	GAGGACGTGGCCCTGTTGGTGGCAG CGTGGCCCTGTTGGTGGCAGTGGAC
PCSK9 H10A(+25+49)	CACCCCCAAAAGCGUUGUGGGCCCG	42.0	
PCSK9 H10A(+75+99)	GCAGUUGGCCUGGGGUAGCAGGCAG	63.3	
PCSK9 H10A(+70+94)	UGGCCUGGGGUAGCAGGCAGACCU	9.1	
PCSK9 H10A(+80+104)	ACGCUGCAGUUGGCCUGGGGUAGCA	15.8	
PCSK9 H10A(+150+174)	GAGGACGUGGCCUGUUGGUGGCAG	70.0	
PCSK9 H10A(+145+169)	CGUGGCCUGUUGGUGGCAGUGGAC	64.2	
PCSK9 H10D(+24-1)	CCUGUGAGGACGUGGCCUGUUGGU	56.5	
PCSK9 H11A(+1+25)	UCCACCUCCCAGUGGGAGCUGCAGC	24.5	
PCSK9 H11A(+46+70)	UGACCUCGUGGCCUCAGCACAGGCG	5.4	
PCSK9 H11A(+71+95)	CCUGUGGCCACGCACUGGUUGGGC	27.1	
PCSK9 H11A(+113+137)	CAGACCUGGGGCAUGGCAGCAGGAA	28.8	

PCSK9 H11A(+145+169)	GCCGGGAUUCCAUGCUCUUGACUU	50.0	GCCGGGATTCCATGCTCCTTGACTT
PCSK9 H11D(+5-20)	GGCCUCACGGGCCUCUUCACCUGCU	35.9	
GTC (GeneTools control)	CCUCUUACCUCAGUUACAAUUUAUA		CCTCTTACCTCAGTTACAATTATA

*% Full-length *PCSK9* transcript knockdown was calculated after treatment with 2'OMe PS AOs. # Not test as 2'OMe.