

## Supplementary information

### Predicted STOP codon locations in *PCSK9*

TGA canonical

TGA new

Full-length *PCSK9*

Exon 12

GTGACCGTGGCCTGCGAGGAGGGCTGGACCCTGACTGGCTGCAGTGCCCTCCCTGGGACC  
TCCCACGTCCTGGGGGCCTACGCCGTAGACAACACGTGTGTAGTCAGGAGCCGGGACGTC  
AGCACTACAGGCAGCACACCAGCGAAGGGGCCGTGACAGCCGTTGCCATCTGCTGCCGGAGC  
CGGCACCTGGCGCAGGCCTCCCAGGAGCTCCAGTGA CAGCCCCATCCCAGGATGGGTGTC

Exclusion of exon 2

Exon 12

GTGACCGTGGCCTGCGAGGAGGGCTGGACCCTGACTGGCTGCAGTGCCCTCCCTGGGACC  
TCCCACGTCCTGGGGGCCTACGCCGTAGACAACACGTGTGTAGTCAGGAGCCGGGACGTC  
AGCACTACAGGCAGCACACCAGCGAAGGGGCCGTGACAGCCGTTGCCATCTGCTGCCGGAGC  
CGGCACCTGGCGCAGGCCTCCCAGGAGCTCCAGTGA CAGCCCCATCCCAGGATGGGTGTC

Exclusion of exon 8

Exon 12

GTGACCGTGGCCTGCGAGGAGGGCTGGACCCTGACTGGCTGCAGTGCCCTCCCTGGGACC  
TCCCACGTCCTGGGGGCCTACGCCGTAGACAACACGTGTGTAGTCAGGAGCCGGGACGTC  
AGCACTACAGGCAGCACACCAGCGAAGGGGCCGTGACAGCCGTTGCCATCTGCTGCCGGAGC  
CGGCACCTGGCGCAGGCCTCCCAGGAGCTCCAGTGA CAGCCCCATCCCAGGATGGGTGTC

Exclusion of exon 9

Exon 10

GCCCAAGGGGGCAAGCTGGTCTGCCGGGCCACAACGCTTTTGGGGGTGA GGGTGTCTAC  
GCCATTGCCAGGTGCTGCCTGCTACCCCAGGCCAACTGCAGCGTCCACACAGCTCCACCA  
GCTGAGGCCAGCATGGGGACCCGTGTCCACTGCCACCAACAGGGCCACGTCCTCACAG

Exclusion of exon 10

Exon 11

GCTGCAGCTCCCCTGGGAGGTGGAGGACCTTGGCACCCACAAGCCGCCTGTGCTGAGGC

CACGAGGTCAGCCCAACCAGTGCCTGGGCCACAGGGAGGCCAGCATCCACGCTTCCTGCT  
GCCATGCCCCAGGTCTGGAATGCAAAGTCAAGGAGCATGGAATCCCGGCCCTCAGGAGC  
AG

Exclusion of exon 11 (cryptic splicing)

Exon 11

GCTGCAGCTCCCACTGGGAGGTGGAGGACCTTGGCACCCACAAGCCGCCTGTGCTGAGGC  
CACGAGGTCAGCCCAACCAGTGCCTGGGCCACAGGGAGGCCAGCATCCACGCTTCCTGCT  
GCCATGCCCCAGGTCTGGAATGCAAAGTCAAGGAGCATGGAATCCCGGCCCTCAGGAGC  
AG

Exon 12

GTGACCGTGGCCTGCGAGGAGGGCTGGACCCTGACTGGCTGCAGTGCCCTCCCTGGGACC  
TCCCACGTCCTGGGGGCCTACGCCGTAGACAACACGTGTGTAGTCAGGAGCCGGGACGTC  
AGCACTACAGGCAGCACCAAGCGAAGGGGCCGTGACAGCCGTTGCCATCTGCTGCCGGAGC  
CGGCACCTGGCGCAGGCCTCCAGGAGCTCCAGTGAAGCAGCCCCATCCAGGATGGGTGTC  
TGGGGAGGGTCAAGGGCTGGGGCTGAGCTTTAAATGGTTCCGACTTGTCCCTCTCTCAG  
CCCTCCATGGCCTGGCACGAGGGGATGGGGATGCTTCCGCCTTCCGGGGCTGCTGGCCT  
GGCCCTTGAGTGGGGCAGCCTCCTTGCTGGAACACTCACTCACTCTGGGTGCCTCCTCCC

### **Amino acid composition and predicted MW of PCSK9**

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

MGTVSSRRSWWPLPLLLLLLLLLLPAGARAQEDDGDYEELVLALRSEEDGLAEAPEHGT  
TATFHRCADPWRLPGTYVVVLKEETHLSQSERTARRLQAQAARRGYLTKILHVFHGLLP  
GFLVKMSGDLLELALKLPHVDYIEEDSSVFAQSIPWNLERITPPRYRADEYQPPDGGSLV  
EYLLDTSIQSDHREIEGRVMVDFENVPEEDGTRFHRQASKCDSHGTHLAGVSGRDAG  
VAKGASMRSLRVLNQCQKGTVSGTLIGLEFIRKSQLVQPVGPLVLLPLAGGYSRVLNAA  
CQRLARAGVVLVTAAGNFRDDACLSPASAPEVITVGATNAQDQPVTGLTGTNFGRCVD  
LFAPGEDIIGASSDCSTCFVSQSGTSQAAAHVAGIAAMMLSAEPELTLAELRQLIHFS  
KDVINEAWFPEDQRVLTPLNVAALPPSTHGAGWQLFCRTVWSAHS GPTRMATAVARCAPD  
EELLSCSSFSRSGKRRGERMEAQGGKLVCRAHNAFGGEGVYAIARCCLLPQANCSVHTAP  
PAEASMGTRVHCHQQGHVLTGCSSHWEVEDLGTHKPPVLRPRGQPNQCVGHREASIHASC  
CHAPGLECKVKEHGIPAPQEQVTVACEEGWTLTGCSALPGTSHVLGAYAVDNTCVVRSRD  
VSTTGSTSEGAVTAVAICCRSRHLAQASQELQ

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

MGTVSSRRSWWPLPLLLLLLLLLLPAGARAQEDEDGDYEELVLALRSEEDGLAEAPEHGT  
TATFHRCALKLPHVDYIEEDSSVFAQSIPWNLERITPPRYRADEYQPPDGGSLV  
EVYLLDTSIQSDHREIEGRVMVTD FENVPEEDGTRFHRQASKCDSHGTHLAGVVSGRDAG  
VAKGASMRLRVLNQCQKGT VSGTLIGLEFIRKSQLVQPVGPLVLLPLAGGYSRVLNAA  
CQLRARAGVVLVTAAGNFRDDACLYSPASAPEVITVGATNAQDQPVT LGTLGTNFGRCVD  
LFAPGEDIIGASSDCSTCFVSQSGTSQAAAHVAGIAAMMLSAEPELTLAELRQRLIH FSA  
KDVINEAWFPEDQRVLT PNLVAALPPSTHGAGWQLFCRTVWSAHS GPTRMATAVARCAPD  
EELLSCSSF SRSGKRRGERMEAQGGKLV CRAHNAFGGEGVYAIARCCLLPQANCSVHTAP  
PAEASMGTRVHCHQQGHVLTGCSSHWEVEDLGTHKPPVLRPRGQPNQCVGHREASIHASC  
CHAPGLECKVKEHGIPAPQE QVTVACEEGWTLTGCSALPGTSHVLGAYAVDNTCVVRSRD  
VSTTGSTSEGAVTAVAICCRSRHLAQASQELQ

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

MGTVSSRRSWWPLPLLLLLLLLLLPAGARAQEDEDGDYEELVLALRSEEDGLAEAPEHGT  
TATFHRCAKDPWRLPGTYVVVLKEETHLSQSERTARRLQAQAARRGYLTKILHVFHGLLP  
GFLVKMSGDLLELALKLPHVDYIEEDSSVFAQSIPWNLERITPPRYRADEYQPPDGGSLV  
EVYLLDTSIQSDHREIEGRVMVTD FENVPEEDGTRFHRQASKCDSHGTHLAGVVSGRDAG  
VAKGASMRLRVLNQCQKGT VSGTLIGLEFIRKSQLVQPVGPLVLLPLAGGYSRVLNAA  
CQLRARAGVVLVTAAGNFRDDACLYSPASAPEVITVGATNAQDQPVT LGTLGTNFGRCVD  
LFAPGEDIIGASSDCSTCFVSQSGTSQAAAHVAGWQLFCRTVWSAHS GPTRMATAVARCAPD  
EELLSCSSF SRSGKRRGERMEAQGGKLV CRAHNAFGGEGVYAIARCCLLPQANCSVHTAP  
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CHAPGLECKVKEHGIPAPQE QVTVACEEGWTLTGCSALPGTSHVLGAYAVDNTCVVRSRD  
VSTTGSTSEGAVTAVAICCRSRHLAQASQELQ

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

MGTVSSRRSWWPLPLLLLLLLLLLPAGARAQEDEDGDYEELVLALRSEEDGLAEAPEHGT  
TATFHRCAKDPWRLPGTYVVVLKEETHLSQSERTARRLQAQAARRGYLTKILHVFHGLLP  
GFLVKMSGDLLELALKLPHVDYIEEDSSVFAQSIPWNLERITPPRYRADEYQPPDGGSLV  
EVYLLDTSIQSDHREIEGRVMVTD FENVPEEDGTRFHRQASKCDSHGTHLAGVVSGRDAG  
VAKGASMRLRVLNQCQKGT VSGTLIGLEFIRKSQLVQPVGPLVLLPLAGGYSRVLNAA

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

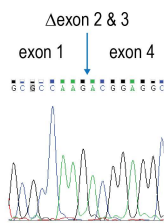
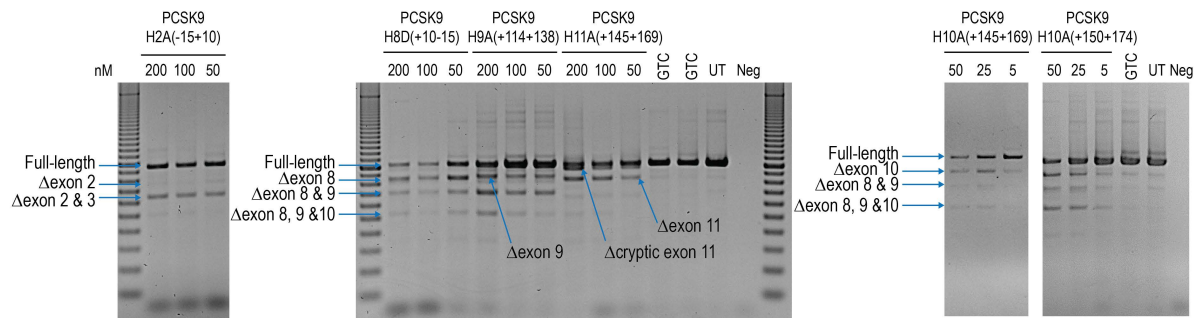
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TATFHRC AKDPWRLPGTYVVVLKEETHLSQSERTARRLQAQAARRGYLTKILHVFHGLLP  
GFLVKMSGDLLELALKLPHVDYIEEDSSVFAQSIPWNLERITPPRYRADEYQPPDGGSLV  
EVYLLDTSIQSDHREIEGRVMVTFENVPEEDGTRFHRQASKCDSHGTHLAGVVSGRDAG  
VAKGASMRSLRVLNQCQKGT VSGTLIGLEFIRKSQLVQPVGPLVLLPLAGGYSRVLNAA  
CQRLARAGVVLVTAAGNFRDDACLYSPASAPEVITVGATNAQDQPVTLGTLGTNFGRCVD  
LFAPGEDIIGASSDCSTCFVSQSGTSQAAAHVAGIAAMMLSAEPELTLAELRQRLIHFS  
KDVINEAWFPEDQRVLT PNLVAALPPSTHGAGWQLFCRTVWSAHS GPTRMATAVARCAPD  
EELLSCSSFSRSGKRRGERMEAAAPTGRWRTLAPTSRLC

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

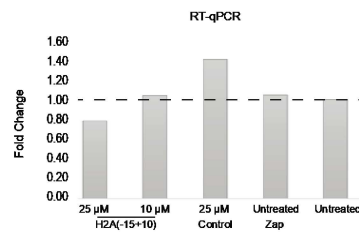
MGTVSSRRS WWPLPLLLLLLLLLLPAGARAQEDEDGDYEELVLALRSEEDGLAEAPEHGT  
TATFHRC AKDPWRLPGTYVVVLKEETHLSQSERTARRLQAQAARRGYLTKILHVFHGLLP  
GFLVKMSGDLLELALKLPHVDYIEEDSSVFAQSIPWNLERITPPRYRADEYQPPDGGSLV  
EVYLLDTSIQSDHREIEGRVMVTFENVPEEDGTRFHRQASKCDSHGTHLAGVVSGRDAG  
VAKGASMRSLRVLNQCQKGT VSGTLIGLEFIRKSQLVQPVGPLVLLPLAGGYSRVLNAA  
CQRLARAGVVLVTAAGNFRDDACLYSPASAPEVITVGATNAQDQPVTLGTLGTNFGRCVD  
LFAPGEDIIGASSDCSTCFVSQSGTSQAAAHVAGIAAMMLSAEPELTLAELRQRLIHFS  
KDVINEAWFPEDQRVLT PNLVAALPPSTHGAGWQLFCRTVWSAHS GPTRMATAVARCAPD  
EELLSCSSFSRSGKRRGERMEAQGGKLV CRAHNAFGGEGVYAIARCCLLPQANCSVHTAP  
PAEASMGTRVHCHQQGHVLTGCSSHWEVEDLGTHKPPVLRPRGQPNQCVGHREASIHAS  
CCHAPGDRGLRGGLDPDWLQCPPWDLPRPGGLRRRQHVC SQEPGRQHQRGRD  
SRCHLLPEPAPGAGLPGAPVTAPSQDGCLGRVKGWG

## Supplementary figures

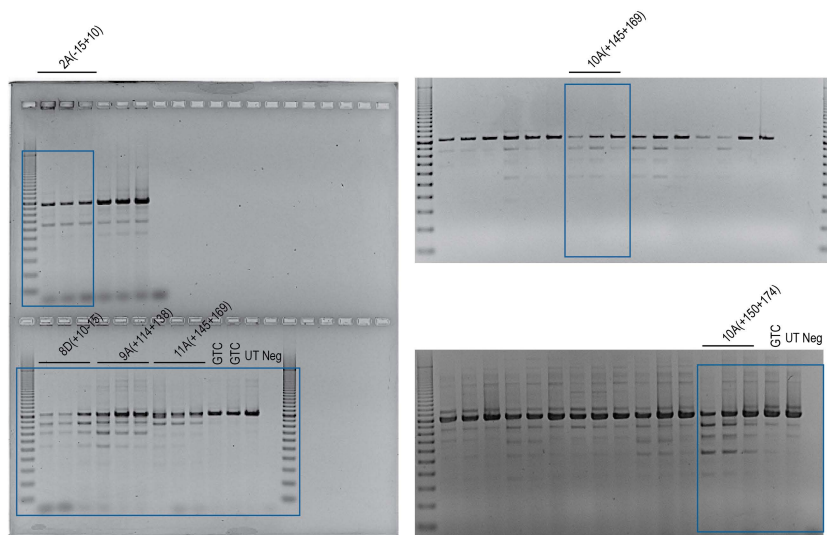
(a)



(b)



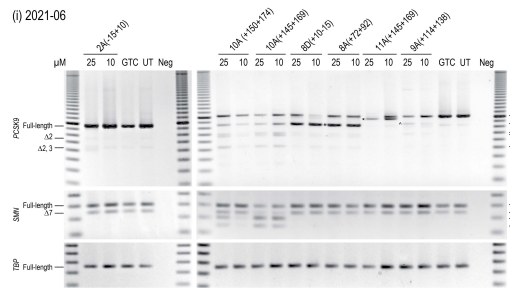
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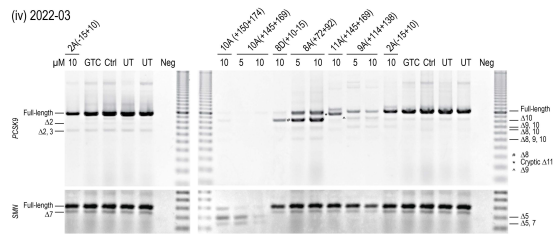
**Supplementary figure 1.** (a) RT-PCR analysis of *PCSK9* transcripts in Huh 7 cells after transfection with 2'OMe PS AOs targeting exon 2, 8, 9, 10 and 11. (b) RT-qPCR analysis of full-length *PCSK9* after treatment with exon 2 targeting PMO. Ctrl; samples treated with GeneTools control AO. UT; untreated.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

(a)

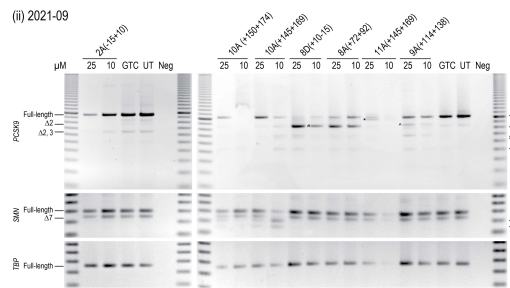
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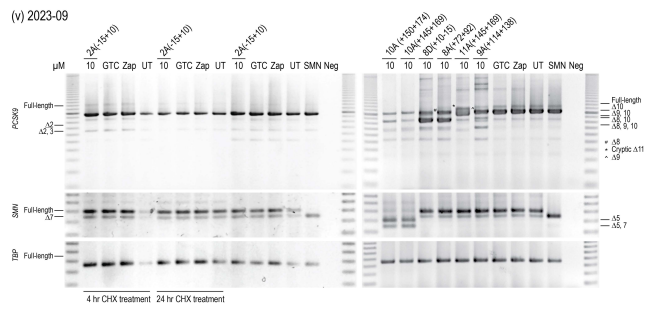
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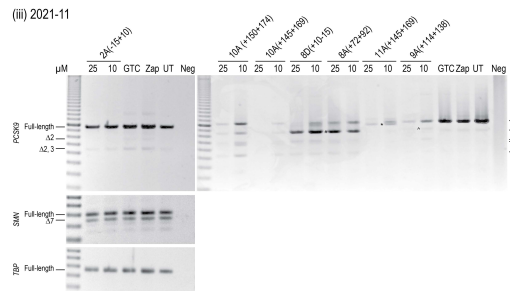
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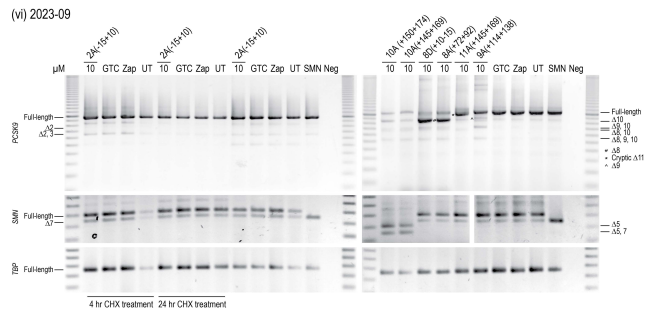
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(iii) 2021-11

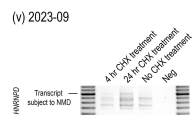


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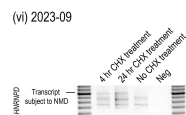


(b)

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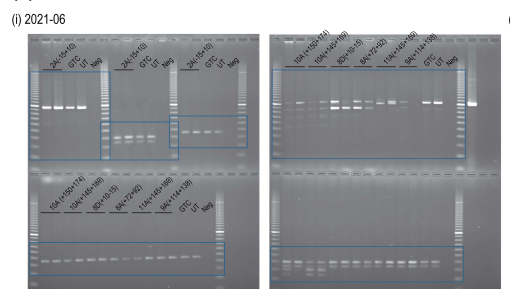


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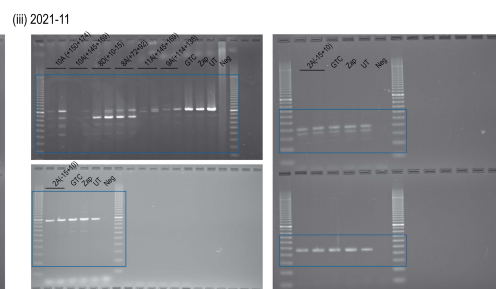


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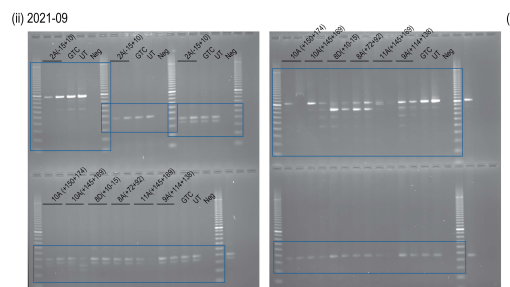
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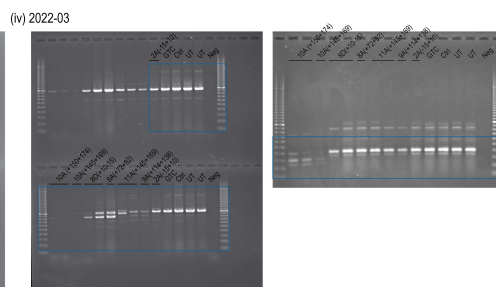
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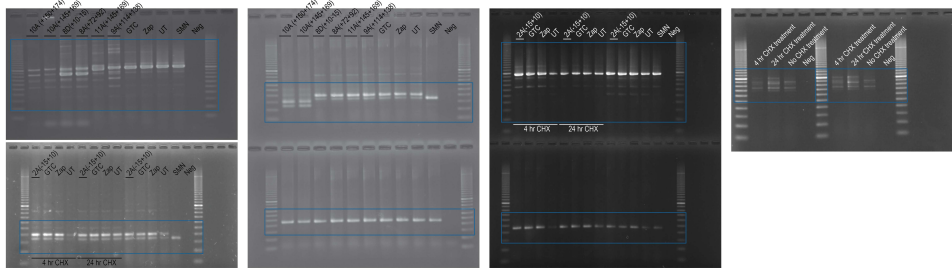


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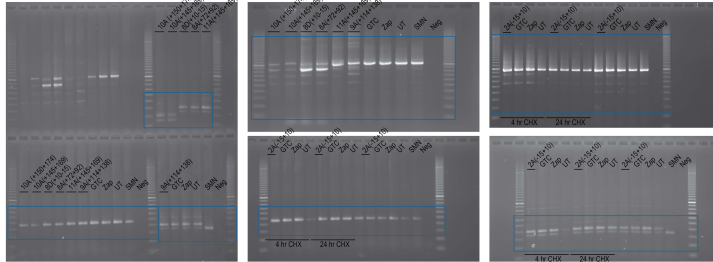


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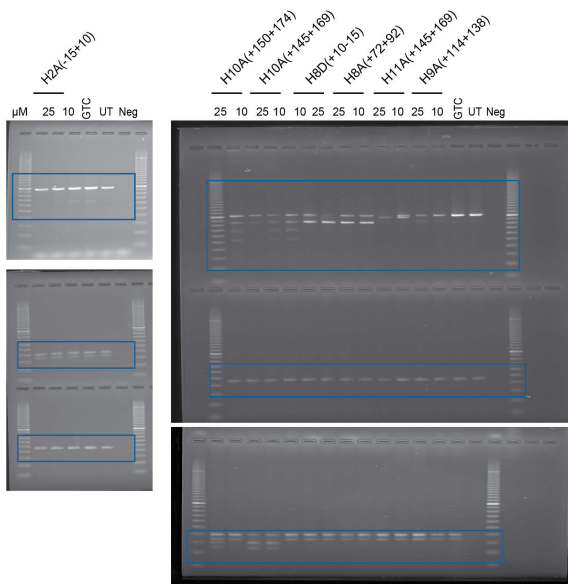


(vi) 2023-09

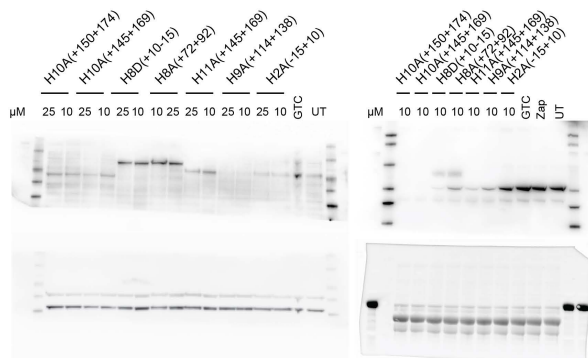


**Supplementary figure 2.** Dated replicates of phosphorodiamidate morpholino oligomer (PMO)-mediated exon skipping of proprotein convertase subtilisin kexin 9 (*PCSK9*) gene transcript. (a) Assessment of exon skipping from *PCSK9* transcripts in Huh-7 cells after treatment with PMO for three days using RT-PCR. Survival motor neuron (*SMN*) and TATA-box binding protein (*TBP*) transcripts were amplified as internal controls for RNA quality.  $\Delta$ ; removal of an exon, GTC; sample treated with Gene Tools control PMO, Zap; sample subject to Neon electroporation with no PMO, UT; untreated sample, Neg; RT-PCR without template added. (b) RT-PCR amplification of heterogeneous nuclear ribonucleoprotein D (*HNRNPD*) transcripts, as a positive control for inhibition of nonsense mediated decay (NMD) after cycloheximide (CHX) treatment. (c) Uncropped original gel images of panels (a) and (b).

(a)

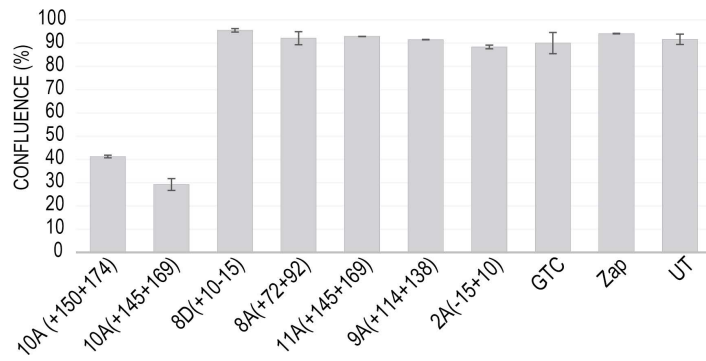


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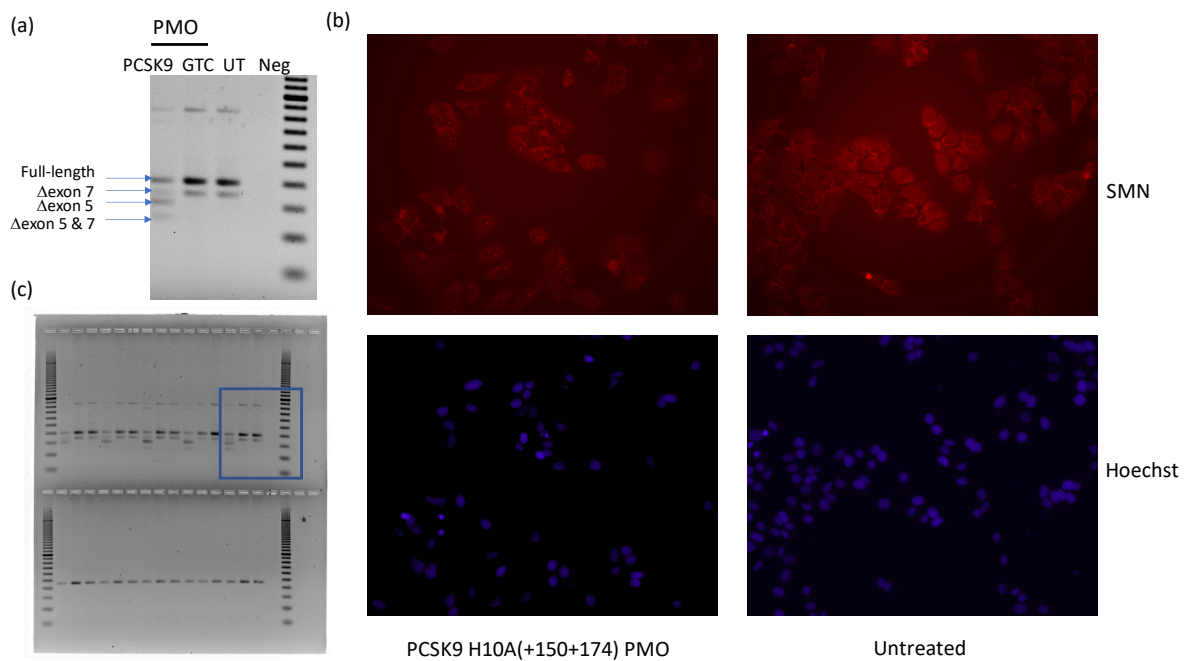


**Supplementary figure 3.** The uncropped original images for (a) Figure 1 and (b) Figure 2.



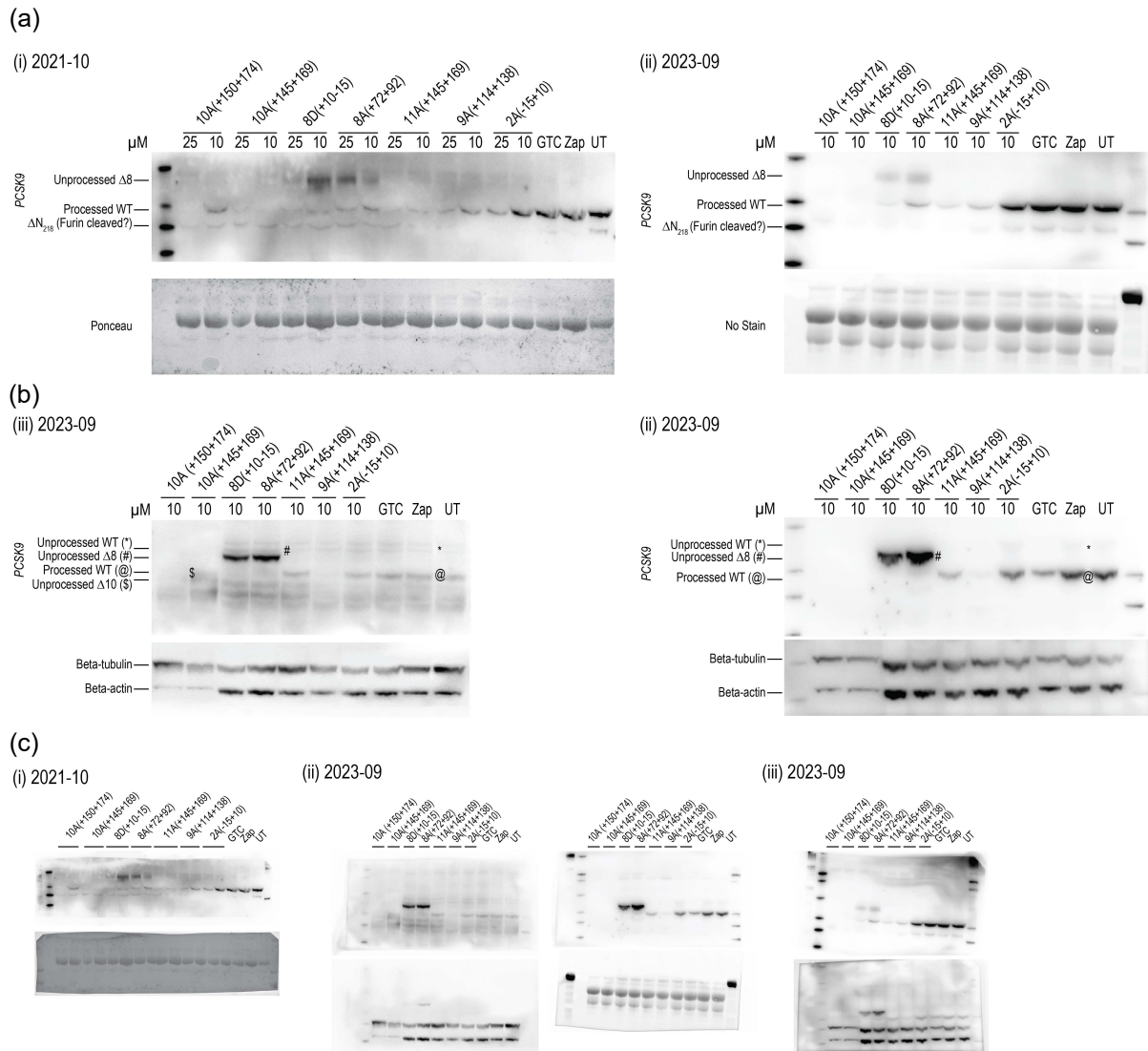


**Supplementary figure 4.** Assessment of confluence as a measure of cell death after treatment of Huh-7 cells with PCSK9 PMOs. Confluence was determined using the Incucyte® SX5 live-cell imaging system and provided software.

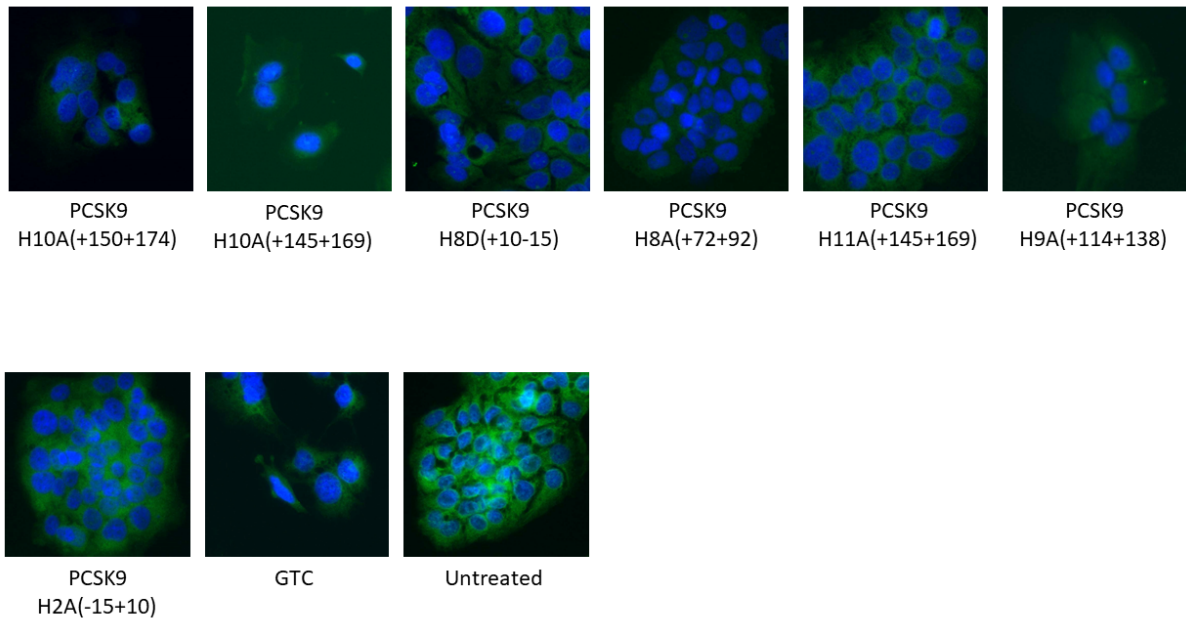


**Supplementary figure 5.** Assessment of gem formation after treatment with PCSK9 PMO H10A(+150+174). (a) RT-PCR analysis of *SMN* transcripts in Huh 7 cells after treating with PCSK9 PMO for 48 hours. GTC: sample treated with GTC PMO, UT; untreated. Neg; RT-PCR without template. (b) Analysis of gems after immunolabelling of SMN (red) and nuclei (blue). (c) Original gel for panel (a).

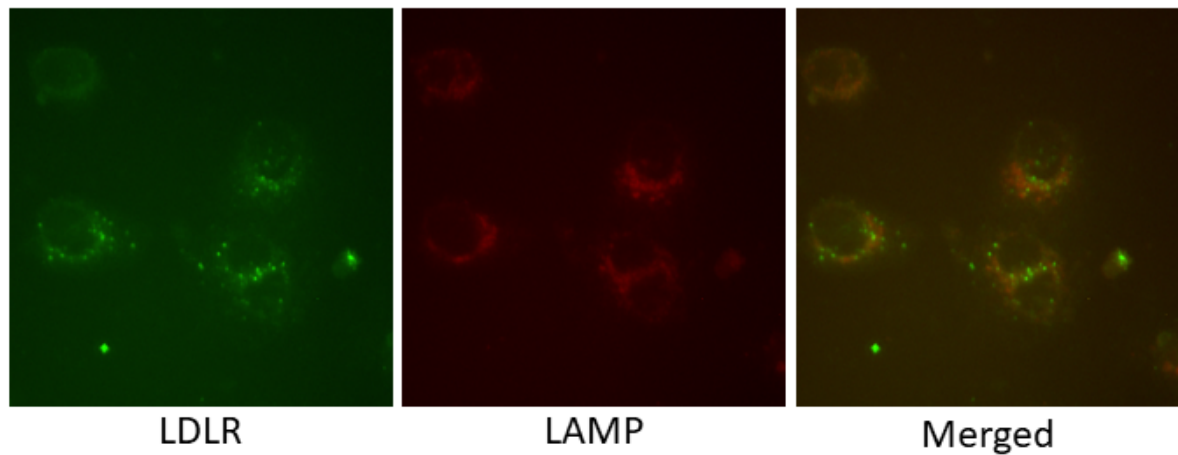




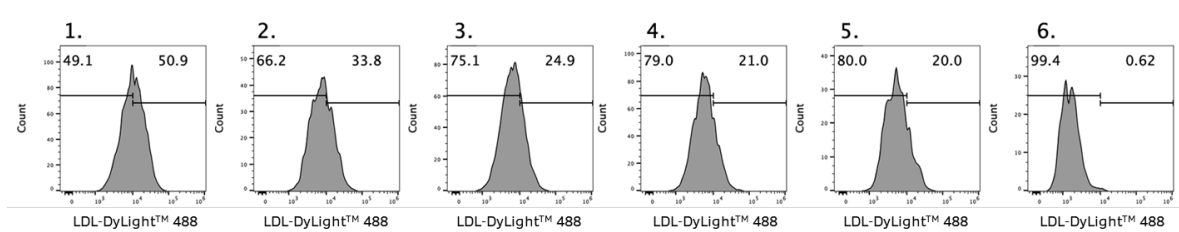
**Supplementary figure 6.** Dated replicates of PCSK9 protein isoform expression assessment in Huh-7 cells after treating with PMOs for three days. Western blot analysis of (a) secreted PCSK9 isoforms after targeted exon skipping and (b) intracellular PCSK9 protein isoforms. WT; wild-type, GTC; sample treated with Gene Tools control PMO, Zap; sample underwent Neon electroporation without any PMO, UT2; untreated sample. (c) Uncropped original images of panels (a) and (b).



**Supplementary figure 7.** 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 figure 8.** Analysis of LDLR and LAMP1 in Huh-7 cells treated with PMO H10A(+145+169) for three days.



1. PCSK9 siRNA (10 nM)    3. Negative control siRNA (10 nM)    5. Untreated  
 2. PCSK9 siRNA (5 nM)    4. Negative control siRNA (5 nM)    6. Untreated with no LDL

**Supplementary figure 9.** The LDL uptake of Huh-7 cells after treatment with siRNA for four days.

**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	
PCSK9 H2A(+110+134)	ACAUGCAGGAUCUUGGUGAGGUAUC	7.7	
PCSK9 H2A(+158+182)	AGGUCGCCACUCAUCUUCACCAGGA	36.6	
PCSK9 H2D(+10-15)	AAAAGGGUGGCUCACCAGCUCCAGC	20.5	
PCSK9 H8A(-05+20)	CAGCAUCAUGGCUGCAAUGCCUGGU	26.6	
PCSK9 H8A(+68+92)	GAUGACAUCUUUGGCAGAGAAGUGG	16.1	
PCSK9 H8A(+72+92)	GAUGACAUCUUUGGCAGAGAA	Not tested #	GATGACATCTTTGGCAGAGAA
PCSK9 H8A(+100+124)	CCCGCUGGUCCUCAGGGAACCAGG	11.8	
PCSK9 H8D(+10-15)	UGCCAUCCUGCUUACCUGCCCCAUG	80.0	TGCCATCCTGCTTACCTGCCCCATG
PCSK9 H9A(-02+23)	AGUCCUGCAAAACAGCUGCCAACCU	5.7	
PCSK9 H9A(+30+54)	UCCGUGUAGGCCCGAGUGUGCUGA	0.0	
PCSK9 H9A(+60+84)	CUGGGGCGCAGCGGGCGACGGCUGU	24.0	
PCSK9 H9A(+85+109)	CUGGAGCAGCUCAGCAGCUCCUCAU	3.3	
PCSK9 H9A(+114+138)	CGCCCCGCCGCUUCCACUCCUGGA	50.0	CGCCCCGCCGCTTCCCACTCCTGGA
PCSK9 H10A(-15+10)	CCCCUUGGGCCUUAGAGUCAAAAGAC	28.7	
PCSK9 H10A(+25+49)	CACCCCAAAAGCGUUGUGGGCCCG	42.0	
PCSK9 H10A(+75+99)	GCAGUUGGCCUGGGGUAGCAGGCAG	63.3	
PCSK9 H10A(+70+94)	UGGCCUGGGGUAGCAGGCAGCACCU	9.1	
PCSK9 H10A(+80+104)	ACGCUGCAGUUGGCCUGGGGUAGCA	15.8	
PCSK9 H10A(+150+174)	GAGGACGUGGCCUGUUGGUGGCAG	70.0	GAGGACGTGGCCCTGTTGGTGGCAG
PCSK9 H10A(+145+169)	CGUGGCCUGUUGGUGGCAGUGGAC	64.2	CGTGGCCCTGTTGGTGGCAGTGGAC
PCSK9 H10D(+24-01)	CCUGUGAGGACGUGGCCUGUUGGU	56.5	
PCSK9 H11A(+01+25)	UCCACCUCAGUGGGAGCUGCAGC	24.5	
PCSK9 H11A(+46+70)	UGACCUCGUGGCCUCAGCACAGGCG	5.4	
PCSK9 H11A(+71+95)	CCUGUGGCCACGCACUGGUUGGGC	27.1	
PCSK9 H11A(+113+137)	CAGACCUGGGCAUGGCAGCAGGAA	28.8	
PCSK9 H11A(+145+169)	GCCGGGAUCCAUGCUCCUUGACUU	50.0	GCCGGGATTCCATGCTCCTTGACTT
PCSK9 H11D(+05-20)	GGCCUCACGGGCCUCUUCACCUGCU	35.9	
GTC (GeneTools control)	CCUCUUACCUCAGUUACAAUUUAUA		CCTTTACCTCAGTTACAATTTATA

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