

SUPPLEMENTAL MATERIAL

Figure S1: Comparison of purine (R) versus pyrimidine (Y) 2' modifications in the absence of 5' phosphorylation of the guide strand. In vitro cell-based evaluation of mRNA knockdown (error bars represent standard deviation of 4 replicates). Unmodified ribose ("r") compared to 2'-deoxy ("d"), 2'-fluoro ("f") and 2'-methoxy ("m"). **(A)** ApoB (6981), **(B)** ApoB (9470), **(C)** ApoB (10127), **(D)** SSB (386), and **(E)** SSB (963) target sites evaluated. Single strands containing 2'F content exhibit the most pronounced mRNA knockdown of the various single strand modification patterns tested.

Figure S2: Comparison of purine (R) versus pyrimidine (Y) 2' modifications in the presence of 5' phosphorylation of the guide strand. In vitro cell-based evaluation of mRNA knockdown (error bars represent standard deviation of 4 replicates). Unmodified ribose ("r") compared to 2'-deoxy ("d"), 2'-fluoro ("f") and 2'-methoxy ("m"). **(A)** ApoB (6981), **(B)** ApoB (9470), **(C)** ApoB (10127), **(D)** SSB (386), and **(E)** SSB (963) target sites evaluated. Single strands containing 2'F content exhibit the most pronounced mRNA knockdown of the various single strand modification patterns tested.

Figure S3: Three abasic residues (3aba) at positions 1-3 of antisense strand decrease activity for both duplex and single strands. In vitro cell-based evaluation of mRNA knockdown (error bars represent standard deviation of 4 replicates). Unmodified ribose ("r") compared to 2'-deoxy ("d"), 2'-fluoro ("f") and 2'-methoxy ("m"). **(A)** ApoB (8786), **(B)** ApoB (6981), **(C)** ApoB (9470), **(D)** ApoB (10127), **(E)** SSB (386), and **(F)** SSB (963) target sites evaluated. All RNA oligos contain 5' phosphate (5'p).

Figure S1

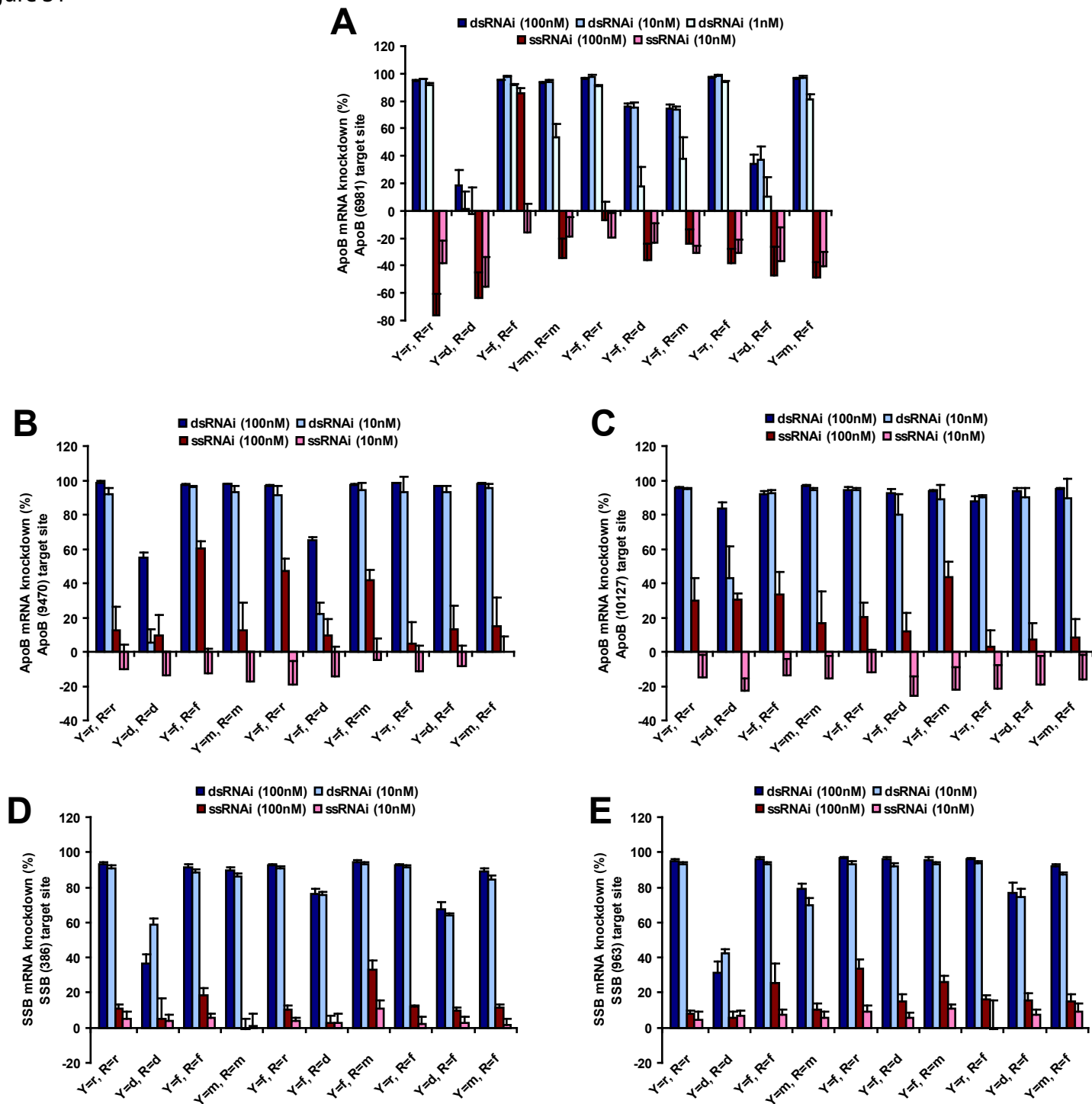


Figure S2

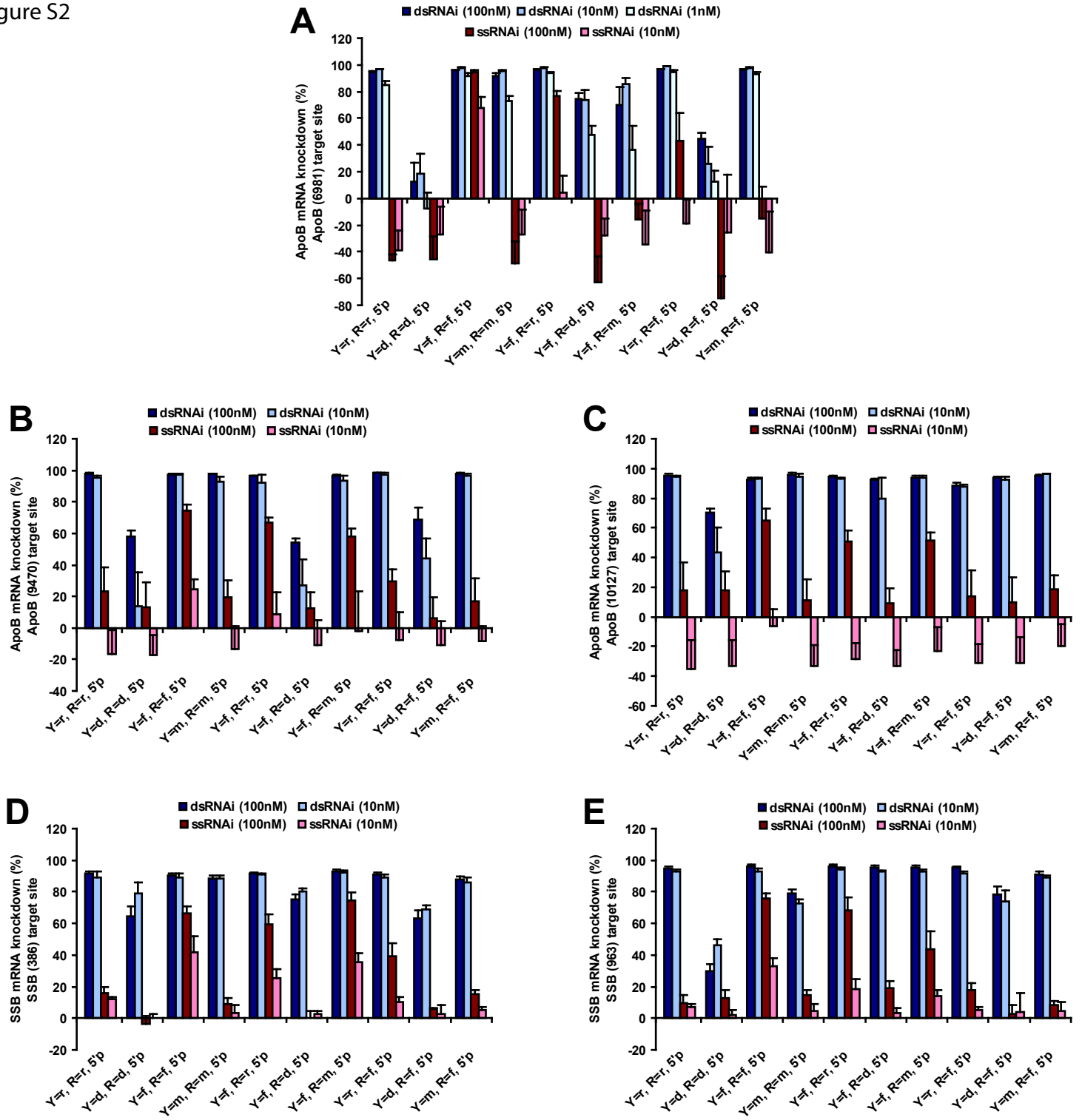
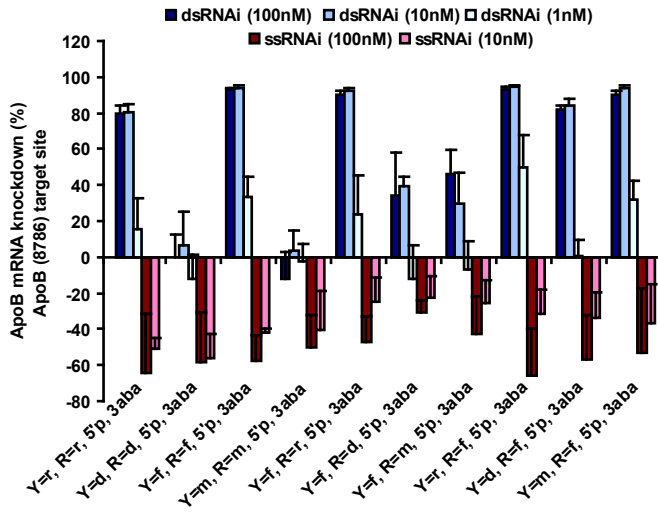
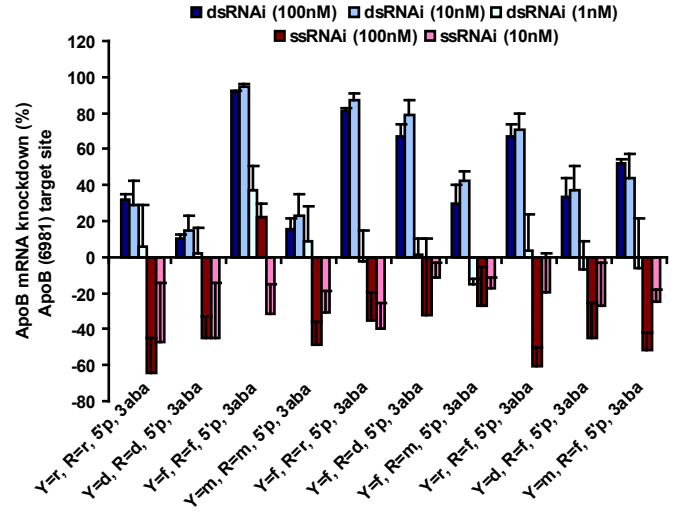


Figure S3

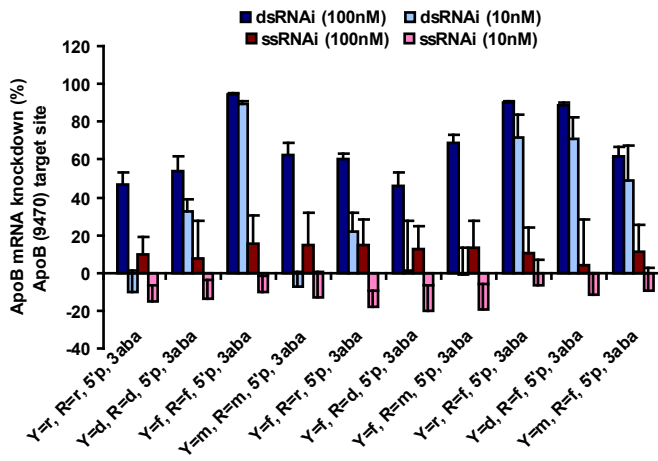
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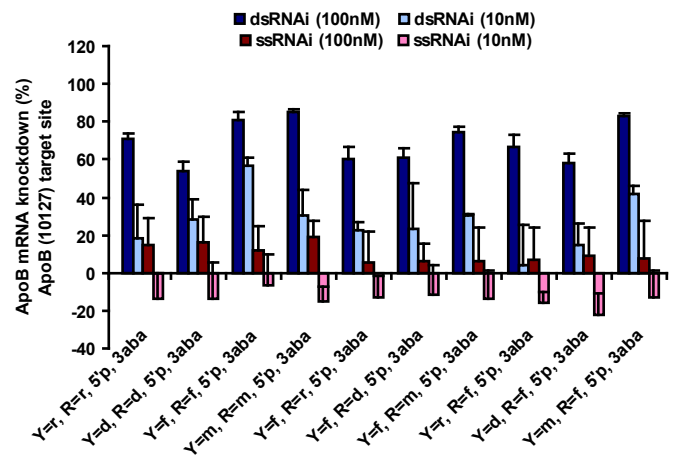
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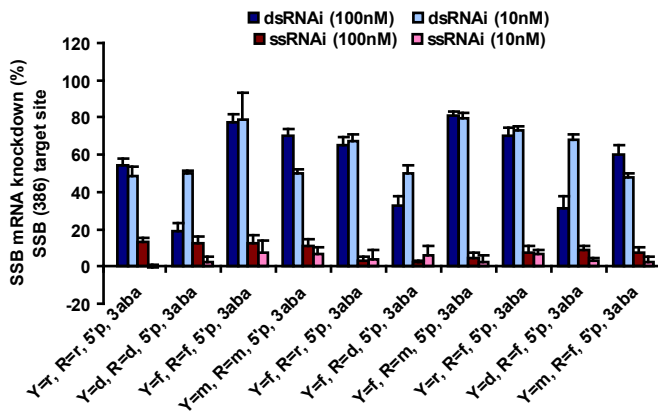
C



D



E



F

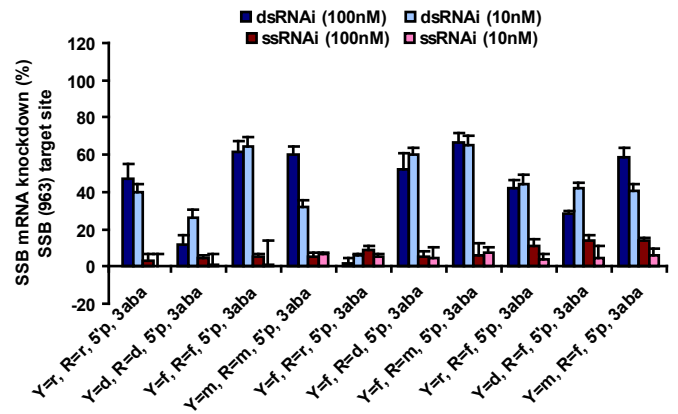


Table S1: In vitro potency and mRNA knockdown (at 10nM) measured in the Hepa1-6 mouse hepatocyte derived cell line. Cells transfected with RNAi Max.

target	mod type	In Vitro Efficacy (24 hrs)		
		EC50 (nM)	EC50 (R ²)	% KD (10nM)
ApoB(8786)	duplex (2'OH, 5'p)	0.015	0.992	98
ApoB(8786)	single (2'OH, 5'p)	na	na	3
ApoB(8786)	duplex (2'F, 5'p)	0.015	0.999	97
ApoB(8786)	single (2'F, 5'p)	1.188	0.990	91
ApoB(6981)	duplex (2'OH, 5'p)	0.098	0.992	94
ApoB(6981)	single (2'OH, 5'p)	na	na	6
ApoB(6981)	duplex (2'F, 5'p)	0.042	0.997	97
ApoB(6981)	single (2'F, 5'p)	0.890	0.988	89

Table S2: In vivo knockdown measured in mouse livers. Percent knockdown, error (standard deviation), and number of mice per group (N) are shown.

target	mod type	study	dose	day 3			day 2			day 7			day 14			guide strand
				KD (%)	KD (SD)	N	KD (%)	KD (SD)	N	KD (%)	KD (SD)	N	KD (%)	KD (SD)	N	
ApoB (8786)	duplex (2'OH, 5'p)	study 1	3mpk	97	1	5										p;rU;rU;rA;rA;rG;rA;rG;rA;rA;rG;rC;rC;rU;rU;rA;rC;rU;rG;rG;mU;mU
ApoB (8786)	single (2'OH, 5'p)	study 1	3mpk	14	4	5										p;rU;rU;rA;rA;rG;rA;rG;rA;rA;rG;rC;rC;rU;rU;rA;rC;rU;rG;rG;mU;mU
ApoB (8786)	duplex (2'F, 5'p)	study 1	3mpk	98	1	5										p;fU;fU;fA;fA;fG;fA;fG;fA;fA;fG;fC;fC;fU;fU;fA;fC;fU;fG;fG;mU;mU
ApoB (8786)	single (2'F, 5'p)	study 1	3mpk	74	6	5										p;fU;fU;fA;fA;fG;fA;fG;fA;fA;fG;fC;fC;fU;fU;fA;fC;fU;fG;fG;mU;mU
ApoB (6981)	duplex (2'OH, 5'p)	study 1	3mpk	85	5	5										p;rU;rU;rG;rA;rU;rC;rU;rA;rA;rA;rU;rG;rC;rA;rU;rU;rG;rU;rG;mU;mU
ApoB (6981)	single (2'OH, 5'p)	study 1	3mpk	6	12	5										p;rU;rU;rG;rA;rU;rC;rU;rA;rA;rA;rU;rG;rC;rA;rU;rU;rG;rU;rG;mU;mU
ApoB (6981)	duplex (2'F, 5'p)	study 1	3mpk	97	0	5										p;fU;fU;fG;fA;fU;fC;fU;fA;fA;fA;fU;fG;fC;fA;fU;fU;fG;fU;fG;mU;mU
ApoB (6981)	single (2'F, 5'p)	study 1	3mpk	61	6	5										p;fU;fU;fG;fA;fU;fC;fU;fA;fA;fA;fU;fG;fC;fA;fU;fU;fG;fU;fG;mU;mU
ApoB (8786)	duplex (2'F, 5'p)	study 2	6mpk				98	1	5	97	0	5	95	1	5	p;fU;fU;fA;fA;fG;fA;fG;fA;fA;fG;fC;fC;fU;fU;fA;fC;fU;fG;fG;mU;mU
ApoB (8786)	single (2'F, 5'p)	study 2	6mpk				91	1	5	77	4	5	34	14	5	p;fU;fU;fA;fA;fG;fA;fG;fA;fA;fG;fC;fC;fU;fU;fA;fC;fU;fG;fG;mU;mU
ApoB (6981)	duplex (2'F, 5'p)	study 2	6mpk				97	1	5	97	1	5	95	1	5	p;fU;fU;fG;fA;fU;fC;fU;fA;fA;fA;fU;fG;fC;fA;fU;fU;fG;fU;fG;mU;mU
ApoB (6981)	single (2'F, 5'p)	study 2	6mpk				84	8	3	71	3	3	19	4	4	p;fU;fU;fG;fA;fU;fC;fU;fA;fA;fA;fU;fG;fC;fA;fU;fU;fG;fU;fG;mU;mU

siRNA ID	guide strand	mod pattern	ds		ds		ss		ss			
			100nM %KD	100nM STDEV	10nM %KD	10nM STDEV	ds 1nM %KD	ds 1nM STDEV	100nM %KD	100nM STDEV	ss 10nM %KD	ss 10nM STDEV
SSB963-108	p;dT;dT;dT;fA;dT;dT;dC;fA;dC;dC;fA;dT;fG;fA;dT;dT;mU;mU	Y=d, R=f, 5'p	78.67	4.68	73.80	7.24			2.75	5.67	3.88	12.33
SSB963-109	p;mU;mU;mU;mU;fA;mU;mU;mU;mC;fA;mC;mC;fA;rU;fG;fA;mU;mU;mU;mU	Y=m, R=f, 5'p	91.11	2.07	89.08	1.47			8.13	2.87	4.69	5.65
SSB963-110	rU;rU;rU;rA;rU;rU;rC;rA;rC;rA;rU;rG;rA;rU;rU;mU;mU	Y=r, R=r	95.16	0.69	93.16	0.95			7.91	1.95	4.44	4.51
SSB963-111	dT;dT;dT;dA;dT;dT;dC;dA;dC;dC;dA;dT;dG;dA;dT;dT;mU;mU	Y=d, R=d	31.27	6.55	42.39	2.50			5.85	3.18	6.61	3.42
SSB963-112	fU;fU;fU;fA;fU;fU;fU;fC;fA;fC;fC;fA;fU;fG;fA;fU;fU;mU;mU	Y=f, R=f	95.96	1.02	93.42	0.98			25.26	11.11	7.26	3.25
SSB963-113	mU;mU;mU;mA;mU;mU;mU;mC;mA;mC;mC;mA;rU;mG;mA;mU;mU;mU;mU	Y=m, R=m	79.33	2.98	69.90	3.97			10.47	3.23	5.38	4.06
SSB963-114	fU;fU;fU;rA;fU;fU;fC;rA;fC;fC;rA;rG;rA;fU;fU;mU;mU	Y=f, R=r	96.51	0.94	93.43	1.43			33.75	5.20	9.15	3.51
SSB963-115	fU;fU;fU;dA;fU;fU;fC;dA;fC;fC;dA;fU;dG;dA;fU;fU;mU;mU	Y=f, R=d	95.96	1.10	92.24	1.55			15.19	4.00	5.84	2.86
SSB963-116	fU;fU;fU;mA;fU;fU;fC;mA;fC;fC;mA;fU;mG;mA;fU;fU;mU;mU	Y=f, R=m	95.72	1.60	93.28	1.30			25.87	3.96	11.19	2.26
SSB963-117	rU;rU;rU;rA;rU;rU;rC;rA;rC;rA;rU;fG;fA;rU;rU;mU;mU	Y=r, R=f	96.07	0.70	93.72	0.96			15.91	2.41	-1.03	16.33
SSB963-118	dT;dT;dT;fA;dT;dT;dC;fA;dC;dC;fA;dT;fG;fA;dT;dT;mU;mU	Y=d, R=f	76.93	5.75	74.70	4.25			15.86	4.08	7.44	3.18
SSB963-119	mU;mU;mU;fA;mU;mU;mU;mC;fA;mC;mC;fA;rU;fG;fA;mU;mU;mU;mU	Y=m, R=f	91.78	1.47	87.07	1.46			15.27	3.77	8.93	4.73
SSB963-120	p;aba;aba;aba;rU;rA;rU;rU;rC;rA;rC;rA;rU;rG;rA;rU;rU;mU;mU	Y=r, R=r, 5'p, 3aba	47.26	7.81	39.80	4.23			3.20	3.40	0.20	6.28
SSB963-121	p;aba;aba;aba;dT;dA;dT;dT;dC;dA;dC;dC;dA;dT;dG;dA;dT;dT;mU;mU	Y=d, R=d, 5'p, 3aba	12.07	4.88	26.16	4.08			4.58	1.26	1.02	5.50
SSB963-122	p;aba;aba;aba;fU;fA;fU;fU;fC;fA;fC;fA;fU;fG;fA;fU;fU;mU;mU	Y=f, R=f, 5'p, 3aba	61.82	5.24	64.71	5.07			5.21	1.85	0.83	12.77
SSB963-123	p;aba;aba;aba;mU;mA;mU;mU;mC;mA;mC;mC;mA;rU;mG;mA;mU;mU;mU;mU	Y=m, R=m, 5'p, 3aba	60.06	4.16	32.24	2.99			5.38	2.30	6.61	0.92
SSB963-124	p;aba;aba;aba;fU;rA;fU;fU;fC;rA;fC;fC;rA;fU;rG;rA;fU;fU;mU;mU	Y=f, R=r, 5'p, 3aba	1.88	2.87	5.72	1.27			8.64	2.57	5.17	1.84
SSB963-125	p;aba;aba;aba;fU;dA;fU;fU;fC;dA;fC;fC;dA;fU;dG;dA;fU;fU;mU;mU	Y=f, R=d, 5'p, 3aba	52.10	8.61	60.40	3.50			5.24	2.74	4.37	6.01
SSB963-126	p;aba;aba;aba;fU;mA;fU;fU;fC;mA;fC;fC;mA;fU;mG;mA;fU;fU;mU;mU	Y=f, R=m, 5'p, 3aba	66.52	4.83	65.16	5.04			5.88	6.29	7.54	2.60
SSB963-127	p;aba;aba;aba;rU;fA;rU;rU;rC;rA;rC;rA;rU;fG;fA;rU;rU;mU;mU	Y=r, R=f, 5'p, 3aba	41.97	4.22	44.11	5.05			11.16	3.66	4.05	2.74
SSB963-128	p;aba;aba;aba;dT;fA;dT;dT;dC;fA;dC;dC;fA;dT;fG;fA;dT;dT;mU;mU	Y=d, R=f, 5'p, 3aba	28.25	1.56	42.17	2.76			13.97	3.04	4.85	5.91
SSB963-129	p;aba;aba;aba;mU;fA;mU;mU;mU;mC;fA;mC;mC;fA;rU;fG;fA;mU;mU;mU;mU	Y=m, R=f, 5'p, 3aba	58.65	4.84	40.77	3.28			13.96	1.31	5.99	3.43

Table S4: Passenger strands used in this study.

siRNA ID	passenger strand
APOB8786	iB;rC;rC;rA;rG;rU;rA;rA;rG;rG;rC;rU;rU;rC;rU;rC;rU;rU;rA;rA;mU;mU;iB
APOB6981	iB;rC;rA;rC;rA;rA;rU;rG;rC;rA;rU;rU;rU;rA;rG;rA;rU;rC;rA;rA;mU;mU;iB
APOB10127	iB;rU;rC;rA;rU;rC;rA;rC;rA;rC;rU;rG;rA;rA;rU;rA;rC;rC;rA;rA;mU;mU;iB
APOB9470	iB;rC;rU;rU;rU;rA;rA;rC;rA;rA;rU;rU;rC;rC;rU;rG;rA;rA;rA;rU;mU;mU;iB
SSB386	iB;rA;rC;rA;rA;rC;rA;rG;rA;rC;rU;rU;rU;rA;rA;rU;rG;rU;rA;rA;mU;mU;iB
SSB963	iB;rA;rA;rA;rU;rC;rA;rU;rG;rG;rU;rG;rA;rA;rA;rU;rA;rA;rA;rA;mU;mU;iB

Table S5: DNA primers used for 5'RACE (see Figure 6).

name	sequence	amplicon size*	ApoB site**
rev1	GGCATTGTGCTCACCGATCATTCTGCCTT	358	9153
rev1n	GAGCAGTGCCCGACCATTGGCTGTT	319	9114
rev2	CTCCGTGTAAGGCAAGTTAATTTTCAGGAATTGTT	712	9507
rev2n	CCAGGTTGGCATCTCCATTCATTCCTATA	659	9454
GeneRacer5'	CGACTGGAGCACGAGGACACTGA		
GeneRacer5' Nested	GGACACTGACATGGACTGAAGGAGTA		

* amplicon size calculated based on predicted cleavage site of ApoB (8786) siRNA

** site relative to NM009693