Supporting Information

From Bench to Bedside: Improving the Clinical Safety of GalNAc-siRNA Conjugates Using Seed-Pairing Destabilization

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Sequences of siRNAs

Table S1. siRNA duplexes used in these studies

Duplex	mRNA Target	Passenger	Sequence (5' to 3')	Guide	Sequence (5' to 3')
D1	*Ttr*	P1	a●a●cagu*G*u*UCU*ugcucuauaaL	G1	u●*U*●aua*G*agcaaga*A*c*A*cuguu●u●u
D2	*Ttr*	P1	a●a●cagu*G*u*UCU*ugcucuauaaL	G2	u●*U*●au**A***G*agcaaga*A*c*A*cuguu●u●u
D3	*Ttr*	P1	a●a●cagu*G*u*UCU*ugcucuauaaL	G3	u●*U*●aua**G**agcaaga*A*c*A*cuguu●u●u
D4	*Ttr*	P1	a●a●cagu*G*u*UCU*ugcucuauaaL	G4	u●*U*●aua*G***A**gcaaga*A*c*A*cuguu●u●u
D5	*Ttr*	P1	a●a●cagu*G*u*UCU*ugcucuauaaL	G5	u●*U*●aua*G*a**G**caaga*A*c*A*cuguu●u●u
D6	*Hao1*	P2	g●a●augu*G*aa*AG*ucaucgacaaL	G7	u●*U*●guc*G*a*UG*acuu*U*c*A*cauuc●u●g
D7	*Hao1*	P2	g●a●augu*G*aa*AG*ucaucgacaaL	G8	u●*U*●gu**C***G*a*UG*acuu*U*c*A*cauuc●u●g
D8	*Hao1*	P2	g●a●augu*G*aa*AG*ucaucgacaaL	G9	u●*U*●guc**G**a*UG*acuu*U*c*A*cauuc●u●g
D9	*Hao1*	P2	g●a●augu*G*aa*AG*ucaucgacaaL	G10	u●*U*●guc*G***A***UG*acuu*U*c*A*cauuc●u●g
D10	*Hao1*	P2	g●a●augu*G*aa*AG*ucaucgacaaL	G11	u●*U*●guc*G*a**T***G*acuu*U*c*A*cauuc●u●g
D11	*Ttr*	P1	a●a●cagu*G*u*UCU*ugcucuauaaL	G6	u●*U*●a**t**a**g**a**g**caaga*A*c*A*cuguu●u●u
D12	*Hao1*	P2	g●a●augu*G*aa*AG*ucaucgacaaL	G12	u●*U*●g**t**c**g**a**t***G*acuu*U*c*A*cauuc●u●g
ALN-HBV	HBV	P3	g●u●gu*G*c*ACU*ucgcuucacaL	G13	u●*G*●uga*A*g*CG*aagu*G*c*A*cac●u●u
ALN-HBV02	HBV	P3	g●u●gu*G*c*ACU*ucgcuucacaL	G14	u●*G*●uga**A**g*CG*aagu*G*c*A*cac●u●u
D13	HBV	P3	g●u●gu*G*c*ACU*ucgcuucacaL	G14	u●*G*●uga*A***G***CG*aagu*G*c*A*cac●u●u
D14	*Ttr*	P1	a●a●cagu*G*u*UCU*ugcucuauaaL	G15	u●*U*●au(A)*G*agcaaga*A*c*A*cuguu●u●u
D15	*Ttr*	P1	a●a●cagu*G*u*UCU*ugcucuauaaL	G16	u●*U*●aua(G)agcaaga*A*c*A*cuguu●u●u
D16	*Ttr*	P1	a●a●cagu*G*u*UCU*ugcucuauaaL	G17	u●*U*●aua*G*(A)gcaaga*A*c*A*cuguu●u●u
D17	*Ttr*	P1	a●a●cagu*G*u*UCU*ugcucuauaaL	G18	u●*U*●aua*G*a(G)caaga*A*c*A*cuguu●u●u
D18	*Hao1*	P2	g●a●augu*G*aa*AG*ucaucgacaaL	G19	u●*U*●gu(C)*G*a*UG*acuu*U*c*A*cauuc●u●g
D19	*Hao1*	P2	g●a●augu*G*aa*AG*ucaucgacaaL	G20	u●*U*●guc(G)a*UG*acuu*U*c*A*cauuc●u●g
D20	*Hao1*	P2	g●a●augu*G*aa*AG*ucaucgacaaL	G21	u●*U*●guc*G*(A)*UG*acuu*U*c*A*cauuc●u●g
D21	*Hao1*	P2	g●a●augu*G*aa*AG*ucaucgacaaL	G22	u●*U*●guc*G*a(U)*G*acuu*U*c*A*cauuc●u●g

Italicized uppercase, lowercase, uppercase bold underlined, lowercase bold underlined, and uppercase letters in parentheses represent 2'-F, 2'-OMe, (*S*)-GNA, 2'-deoxy, and 2'-5'-linked ribose sugar modifications, respectively, to Adenosine, Cytosine, Guanosine, Uridine, and Thymidine. "L" represents the tri-N-acetylgalactosamine ligand. Phosphorothioate linkages are indicated by the "●" symbol.

Dose response of selected siRNAs in rats

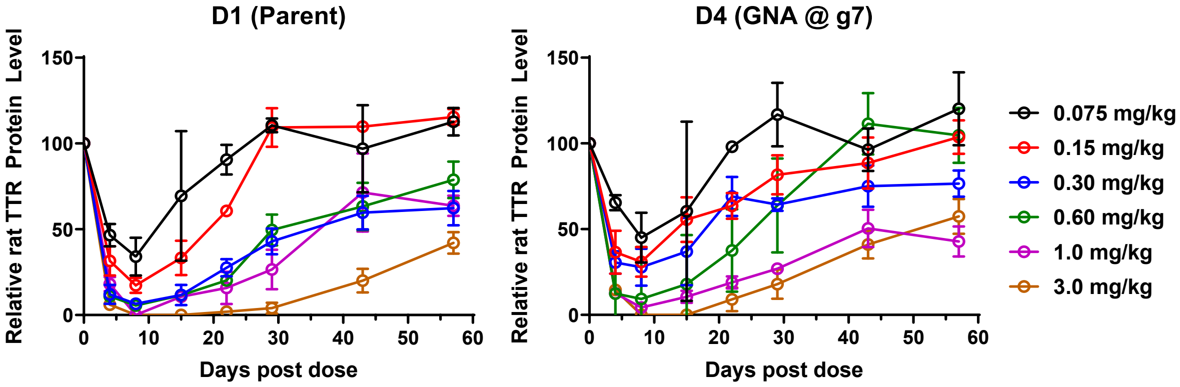

Figure S1. Knockdown of serum TTR protein in rats (n=3) with parent and GNA-modified siRNAs across the specified doses.

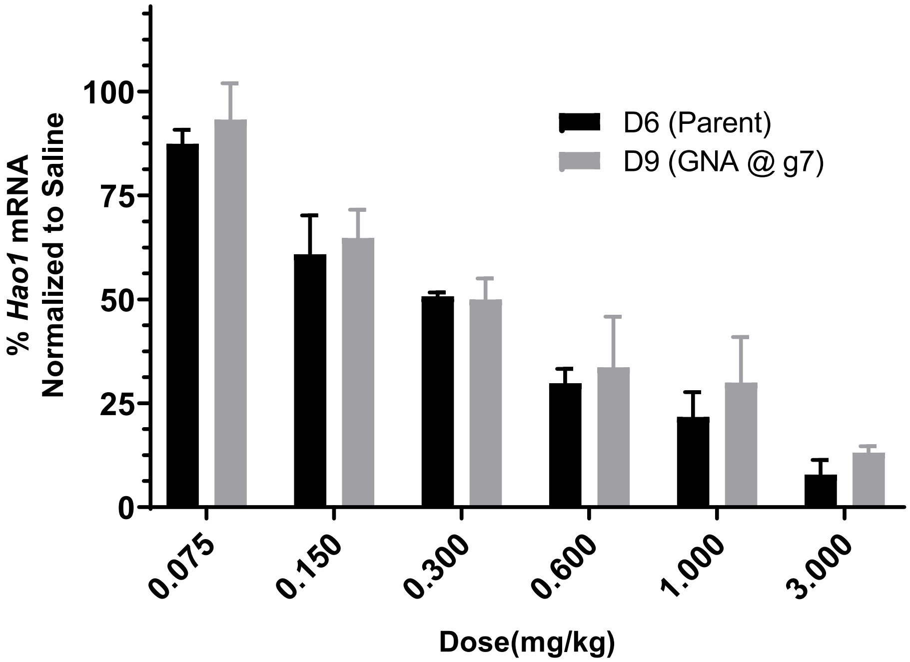

Figure S2. Knockdown of liver *Hao1* mRNA in rats (n=3) with parent and GNA-modified siRNAs across the specified doses.

Metabolism identification and test article quantification in mouse liver

Table S2. Identification of Guide Strand Metabolites from Selected siRNAs in Mouse Liver (n=1)

siRNA	Observed MW	Identified Fragment (5' to 3')	Nucleotides in Fragment (5' to 3')	% of Total Strand
D1	7652.163	u●*U*●aua*G*agcaaga*A*c*A*cuguu●u●u	1-23	55
	7316.153	u●*U*●aua*G*agcaaga*A*c*A*cuguu●u	1-22[a]	45
D4	7580.144	u●*U*●aua*G***A**gcaaga*A*c*A*cuguu●u●u	1-23	47
	7244.128	u●*U*●aua*G***A**gcaaga*A*c*A*cuguu●u	1-22[a]	41
	5566.906	**A**gcaaga*A*c*A*cuguu●u●u	7-23	6
	5230.896	**A**gcaaga*A*c*A*cuguu●u	7-22	6
D11	7588.166	u●*U*●a**t**a**g**a**g**caaga*A*c*A*cuguu●u●u	1-23	57
	7252.151	u●*U*●a**t**a**g**a**g**caaga*A*c*A*cuguu●u	1-22	43
D6	7534.054	u●*U*●gucGa*UG*acuu*U*c*A*cauuc●u●g	1-23	47
	7159.012	u●*U*●gucGa*UG*acuu*U*c*A*cauuc●u	1-22	53
D9	7462.034	u●*U*●guc*G***A***UG*acuu*U*c*A*cauuc●u●g	1-23	35
	7086.991	u●*U*●guc*G***A***UG*acuu*U*c*A*cauuc●u	1-22	57
	5456.814	**A***UG*acuu*U*c*A*cauuc●u●g	7-23	4
	5081.774	**A***UG*acuu*U*c*A*cauuc●u	7-22	4
D12	7508.112	u●*U*●g**t**c**g**a**t***G*acuu*U*c*A*cauuc●u●g	1-23	45
	7133.069	u●*U*●g**t**c**g**a**t***G*acuu*U*c*A*cauuc●u	1-22	55

Italicized uppercase, lowercase, uppercase bold underlined, and lowercase bold underlined represent 2'-F, 2'-OMe, (*S*)-GNA, and 2'-deoxy modifications, respectively, to Adenosine, Cytosine, Guanosine, Uridine, and Thymidine. Phosphorothioate linkages are indicated by the "●" symbol. Percent of strand is calculated based on TIC intensity summed for all metabolites of a given strand. [a] Metabolite is isobaric with the 2-23 fragment, but the proposed 3'-metabolite is more likely.

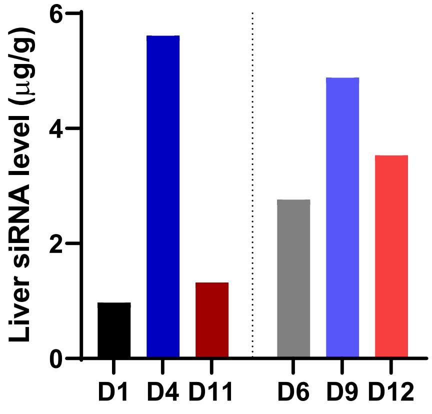

Figure S3. Level of guide strand detected in liver via RT-qPCR from various GalNAc-siRNAs seven days post a single dose of 10 mg/kg in mice (n=1).

***In vitro* off-target silencing of DNA-modified siRNAs**

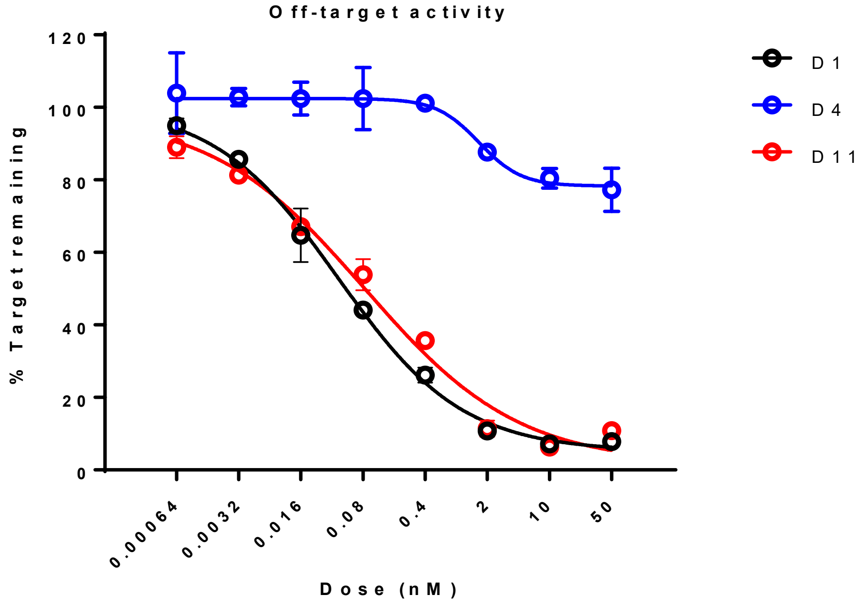

Figure S4. Activity of siRNAs targeting *Ttr* in the off-target reporter assay (n=4).

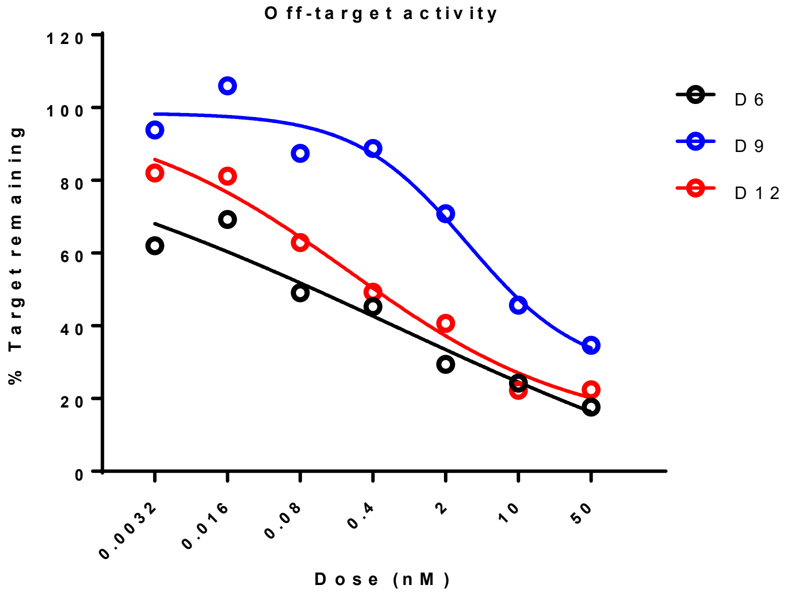

Figure S5. Activity of siRNAs targeting *Hao1* in the off-target reporter assay (n=4).

Additional single-dose knockdown data in mice

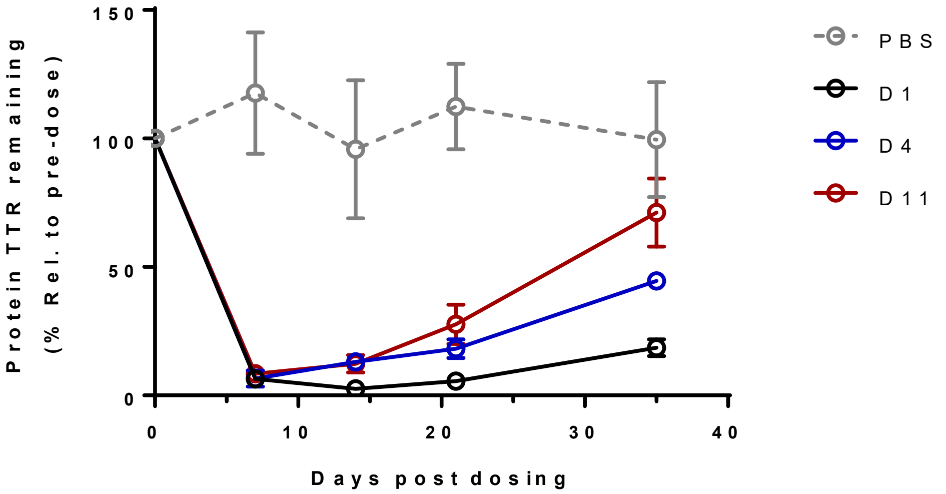

Figure S6. Knockdown of serum TTR protein in mice (n=3) with parent and modified siRNAs administered subcutaneously at 1 mg/kg.

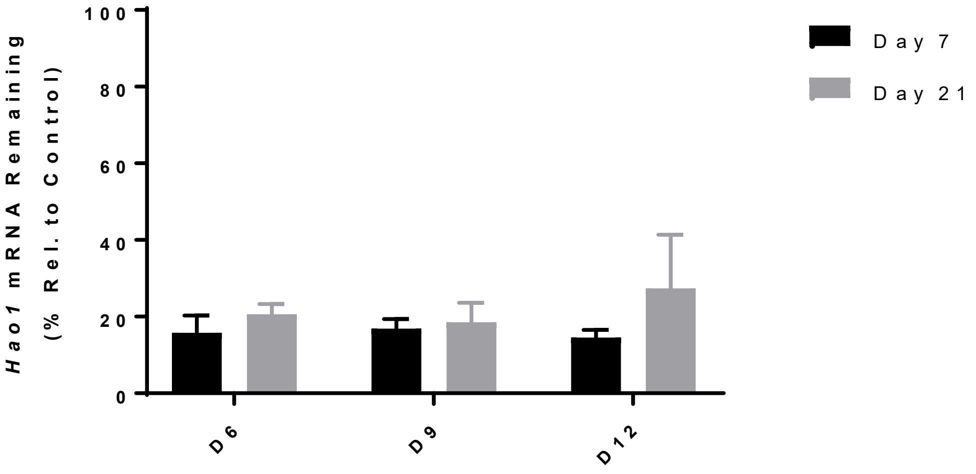

Figure S7. Knockdown of liver *Hao1* mRNA in mice (n=3) with parent and modified siRNAs administered subcutaneously at 1 mg/kg.

Clinical pathology graphs and tables

Results were generated for animals in four different rat toxicology studies (siRNAs dosed 3x weekly at specified dose):

1. **D1**, **D4**, **D6**, and **D9** @ 3, 10, and 30 mg/kg; 0.9% NaCl (#1) (n=4)
2. **D1** and **D4** @ 30, 60, and 120 mg/kg; 0.9% NaCl (#2) (n=4-5)
3. **D1**, **D4**, and **D11** @ 30 mg/kg; 0.9% NaCl (n=4)
4. **D6**, **D9**, and **D12** @ 30 mg/kg; **D6** and **D9** @ 60 and 120 mg/kg; 0.9% NaCl (#2) (n=4); Note that no animals from the 120 mg/kg cohort for **D6** successfully completed the study and the data is representative from those animals found moribund or sacrificed early.

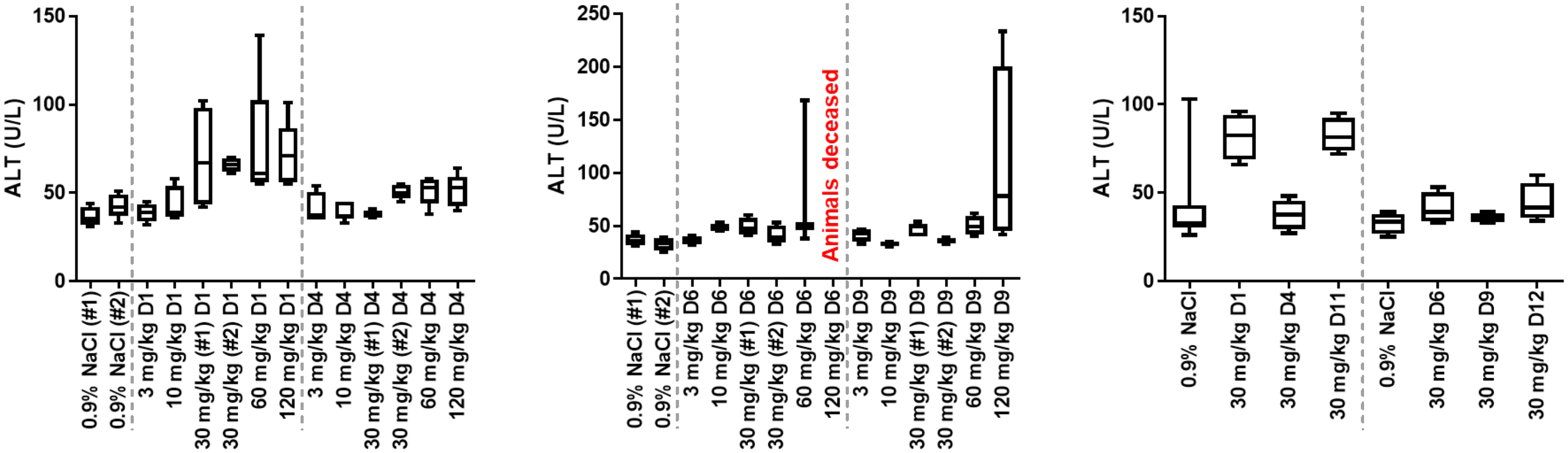

Figure S8. Alanine transaminase (ALT) levels in rats of *Ttr*- or *Hao1*-targeting siRNAs **D1**, **D4**, **D11**, **D6**, **D9**, and **D12** dosed 3x weekly at 3, 10, 30, 60, or 120 mg/kg (n=4-5). Levels for **D6** at 120 mg/kg were not able to be determined from animals that died prematurely.

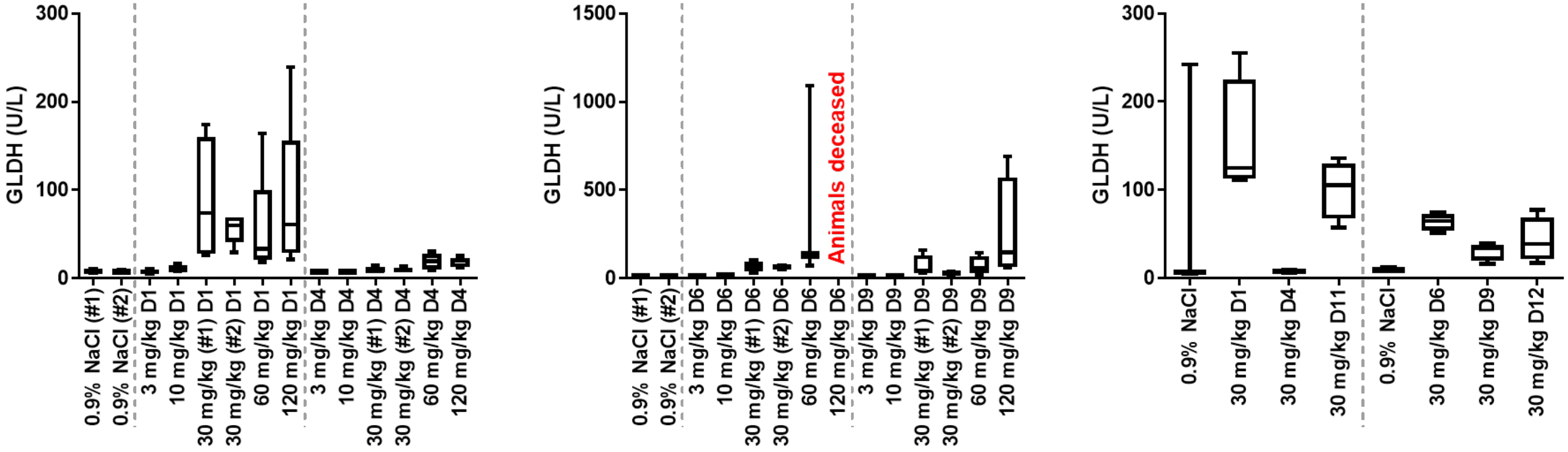

Figure S9. Glutamate dehydrogenase (GLDH) levels in rats of *Ttr*- or *Hao1*-targeting siRNAs **D1**, **D4**, **D11**, **D6**, **D9**, and **D12** dosed 3x weekly at 3, 10, 30, 60, or 120 mg/kg (n=4-5). Data for **D6** at 120 mg/kg was not collected from animals that died prematurely.

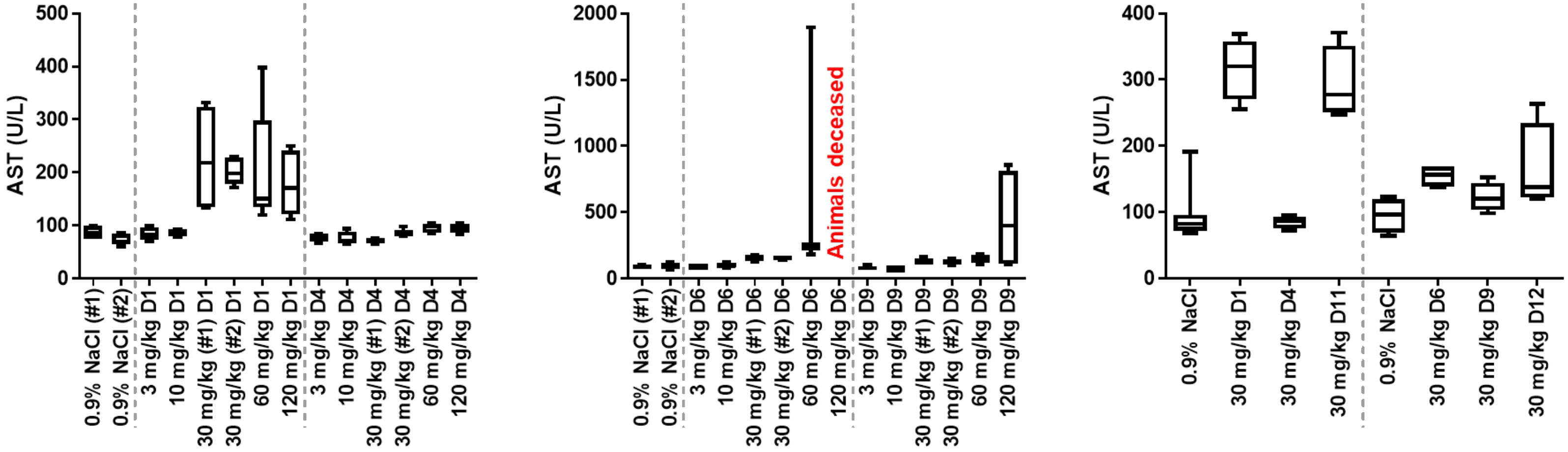

Figure S10. Aspartate aminotransferase (AST) levels in rats of *Ttr*- or *Hao1*-targeting siRNAs **D1**, **D4**, **D11**, **D6**, **D9**, and **D12** dosed 3x weekly at 3, 10, 30, 60, or 120 mg/kg (n=4-5). Data for **D6** at 120 mg/kg was not collected from animals that died prematurely.

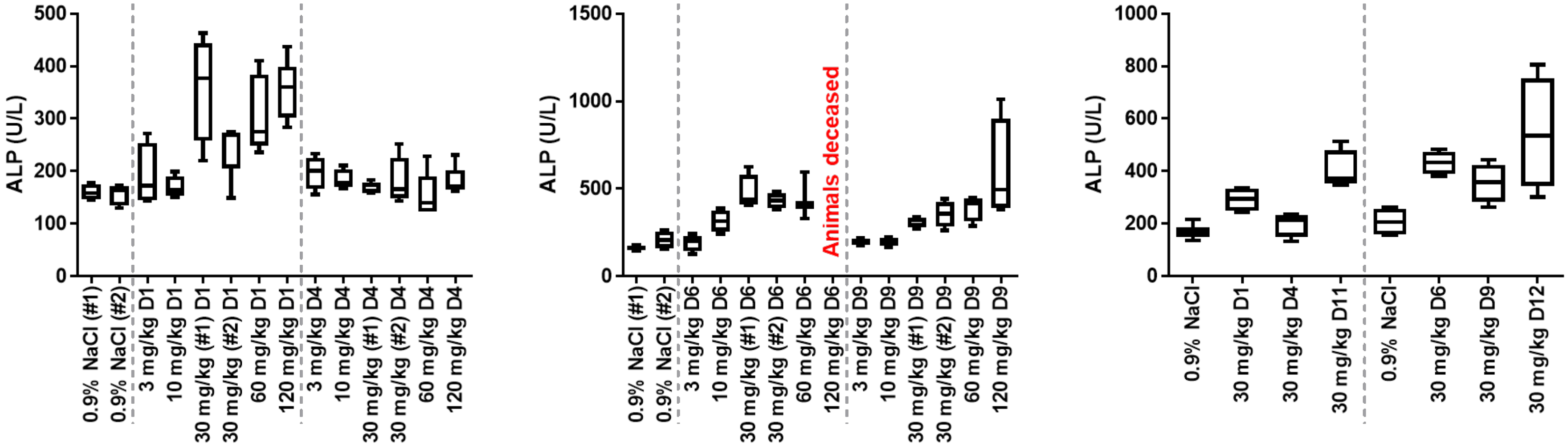

Figure S11. Alkaline phosphatase (ALP) levels in rats of *Ttr*- or *Hao1*-targeting siRNAs **D1**, **D4**, **D11**, **D6**, **D9**, and **D12** dosed 3x weekly at 3, 10, 30, 60, or 120 mg/kg (n=4-5). Data for **D6** at 120 mg/kg was not collected from animals that died prematurely.

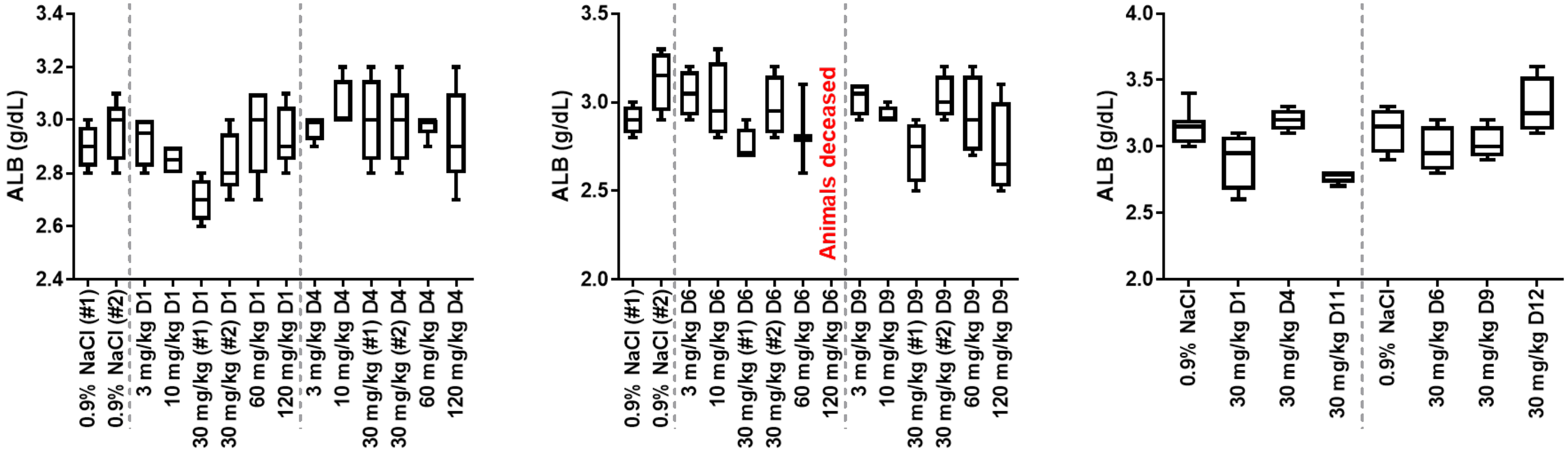

Figure S12. Albumin (ALB) levels in rats of *Ttr*- or *Hao1*-targeting siRNAs **D1**, **D4**, **D11**, **D6**, **D9**, and **D12** dosed 3x weekly at 3, 10, 30, 60, or 120 mg/kg (n=4-5). Data for **D6** at 120 mg/kg was not collected from animals that died prematurely.

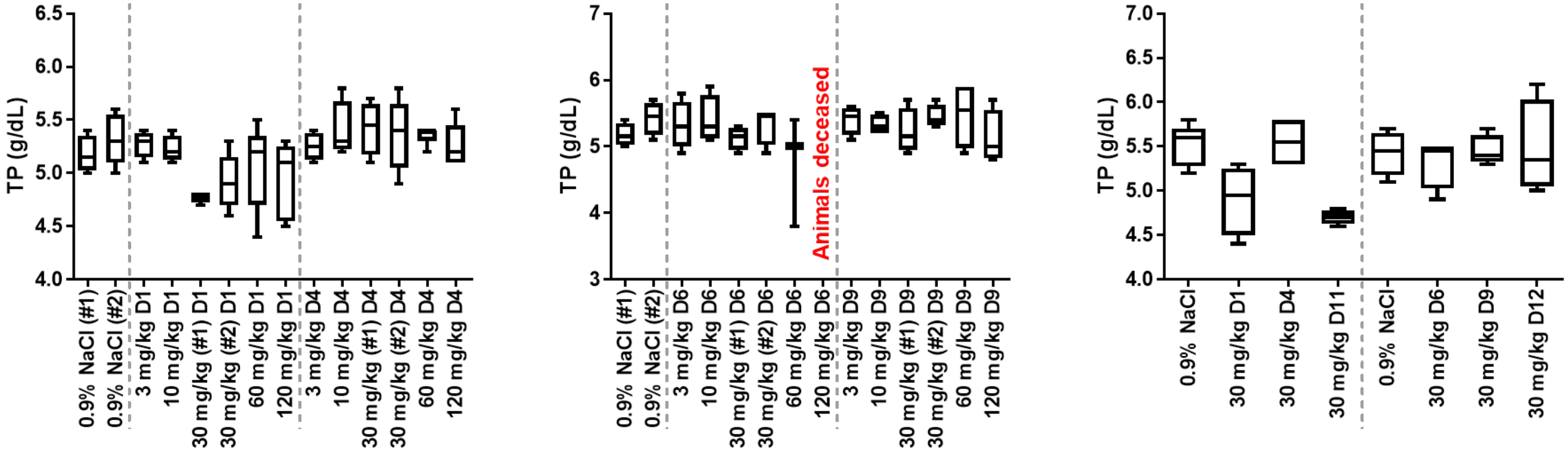

Figure S13. Total protein (TP) levels in rats of *Ttr*- or *Hao1*-targeting siRNAs **D1**, **D4**, **D11**, **D6**, **D9**, and **D12** dosed 3x weekly at 3, 10, 30, 60, or 120 mg/kg (n=4-5). Data for **D6** at 120 mg/kg was not collected from animals that died prematurely.

Table S3. Tabulated clinical pathology data for *Ttr*-targeting siRNAs (n=4-5).

				D1						**D4**					
		0.9% NaCl	0.9% NaCl	3 mg/kg	10 mg/kg	30 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Parameter	ALT	37 ± 5	43 ± 7	39 ± 5	43 ± 10	70 ± 29	66 ± 4	76 ± 36	71 ± 18	41 ± 9	42 ± 6	38 ± 2	51 ± 4	51 ± 8	51 ± 9
	GLDH	8 ± 2	7 ± 1	7 ± 2	11 ± 4	87 ± 71	56 ± 17	55 ± 61	86 ± 88	7 ± 1	8 ± 1	9 ± 3	9 ± 2	19 ± 9	18 ± 6
	AST	87 ± 14	76 ± 11	84 ± 13	87 ± 7	225 ± 105	202 ± 25	203 ± 112	179 ± 61	79 ± 8	75 ± 13	72 ± 5	86 ± 7	94 ± 9	94 ± 9
	ALP	160 ± 15	155 ± 19	190 ± 60	170 ± 21	359 ± 101	245 ± 54	308 ± 73	353 ± 57	198 ± 32	184 ± 19	166 ± 11	183 ± 44	153 ± 44	181 ± 28
	ALB	2.9 ± 0.1	3.0 ± 0.1	2.9 ± 0.1	2.9 ± 0.1	2.7 ± 0.1	2.8 ± 0.1	3.0 ± 0.2	2.9 ± 0.1	3.0 ± 0.1	3.1 ± 0.1	3.0 ± 0.2	3.0 ± 0.1	3.0 ± 0	2.9 ± 0.2
	TP	5.2 ± 0.2	5.3 ± 0.2	5.3 ± 0.1	5.2 ± 0.1	4.8 ± 0	4.9 ± 0.3	5.1 ± 0.4	4.9 ± 0.4	5.3 ± 0.1	5.4 ± 0.3	5.4 ± 0.3	5.4 ± 0.3	5.4 ± 0.1	5.3 ± 0.2

Values represent the mean +/- standard deviation for all animals in the study

Table S4. Tabulated clinical pathology data for selected siRNAs at 30 mg/kg (n=4).

		Ttr				*Hao1*			
		0.9% NaCl	**D1**	**D4**	**D11**	0.9% NaCl	**D6**	**D9**	**D12**
Parameter	ALT	42 ± 25	82 ± 13	38 ± 9	83 ± 10	33 ± 6	41 ± 9	36 ± 3	44 ± 11
	GLDH	36 ± 83	154 ± 68	8 ± 1	101 ± 33	9 ± 2	64 ± 10	31 ± 10	43 ± 25
	AST	96 ± 40	316 ± 47	85 ± 10	293 ± 55	95 ± 26	155 ± 17	123 ± 22	165 ± 67
	ALP	170 ± 25	292 ± 44	199 ± 46	401 ± 76	207 ± 53	432 ± 43	355 ± 74	544 ± 213
	ALB	3.2 ± 0.1	2.9 ± 0.2	3.2 ± 0.1	2.8 ± 0	3.1 ± 0.2	3.0 ± 0.2	3.0 ± 0.1	3.3 ± 0.2
	TP	5.5 ± 0.2	4.9 ± 0.4	5.6 ± 0.3	4.7 ± 0.1	5.4 ± 0.3	5.3 ± 0.3	5.5 ± 0.2	5.5 ± 0.5

Values represent the mean +/- standard deviation for all animals in the study

Table S5. Tabulated clinical pathology data for *Hao1*-targeting siRNAs (n=4).

		D6								**D9**					
		0.9% NaCl	0.9% NaCl	3 mg/kg	10 mg/kg	30 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg	3 mg/kg	10 mg/kg	30 mg/kg	30 mg/kg	60 mg/kg	120 mg/kg
Parameter	ALT	37 ± 5	33 ± 6	37 ± 4	49 ± 4	49 ± 8	41 ± 9	85 ± 72	n.d.	42 ± 6	33 ± 2	45 ± 7	36 ± 3	50 ± 9	108 ± 87
	GLDH	8 ± 2	9 ± 2	6 ± 1	19 ± 9	64 ± 30	64 ± 10	430 ± 572	n.d.	7 ± 1	7 ± 1	66 ± 62	31 ± 10	71 ± 53	260 ± 295
	AST	87 ± 14	95 ± 26	86 ± 9	100 ± 20	155 ± 24	155 ± 17	772 ± 974	n.d.	83 ± 16	70 ± 4	131 ± 22	123 ± 22	153 ± 34	441 ± 383
	ALP	160 ± 15	207 ± 53	192 ± 49	315 ± 62	477 ± 100	432 ± 43	445 ± 136	n.d.	195 ± 19	196 ± 23	312 ± 29	355 ± 74	392 ± 75	595 ± 288
	ALB	2.9 ± 0.1	3.1 ± 0.2	3.1 ± 0.1	3.0 ± 0.2	2.8 ± 0.1	3.0 ± 0.2	2.8 ± 0.3	n.d.	3.0 ± 0.1	2.9 ± 0.1	2.7 ± 0.2	3.0 ± 0.1	2.9 ± 0.2	2.7 ± 0.3
	TP	5.2 ± 0.2	5.4 ± 0.3	5.3 ± 0.4	5.4 ± 0.4	5.1 ± 0.2	5.3 ± 0.3	4.7 ± 0.8	n.d.	5.4 ± 0.2	5.3 ± 0.2	5.2 ± 0.3	5.5 ± 0.2	5.5 ± 0.5	5.1 ± 0.4

Values represent the mean +/- standard deviation for all animals in the study

Microscopic liver findings in rats

Results were generated for animals in four different rat toxicology studies (siRNAs dosed 3x weekly at specified dose):

1. **D1**, **D4**, **D6**, and **D9** @ 3, 10, and 30 mg/kg; 0.9% NaCl (#1) (n=4)
2. **D1** and **D4** @ 30, 60, and 120 mg/kg; 0.9% NaCl (#2) (n=5)
3. **D1**, **D4**, and **D11** @ 30 mg/kg; 0.9% NaCl (n=4)
4. **D6**, **D9**, and **D12** @ 30 mg/kg; **D6** and **D9** @ 60 and 120 mg/kg; 0.9% NaCl (#2) (n=4); Note that no animals from the 120 mg/kg cohort for **D6** successfully completed the study and the data is representative from those animals found moribund or sacrificed early.

Table S6. Microscopic liver findings after treatment with *Ttr*-targeting siRNAs (n=4-5).

		D1						**D4**					
Dose	**0.9% NaCl**	**3**	**10**	**30**	**30**	**60**	**120**	**3**	**10**	**30**	**30**	**60**	**120**
Degeneration			1	2-3	2	1-3	1-3						
Increased mitoses				2-3	2-3	1-2	2-3				1		1
Necrosis, coagulative				1			1-2						
Necrosis, single cell			1	1-2	1-2	1-2	1			1	1	1	1
Vacuolation, hepatocellular		1	1-2	2	2	1-3	1-3	1	1	1	1	1	1
Hyperplasia, oval cell							2-3						
Karyomegaly						1	1-2						

The range of severity grade for each microscopic finding if present was graded on a scale of 1-4; (1 = minimal, 2 = mild, 3 = moderate, and 4 = marked).

Table S7. Microscopic liver findings after treatment with selected siRNAs at 30 mg/kg (n=4).

	0.9% NaCl	**D1**	**D4**	**D11**	**0.9% NaCl**	**D6**	**D9**	**D12**
Hyperplasia, bile duct		1						
Increases mitoses		1-2		1-3		2	1	2-3
Infiltration, mixed cell				1	1	2-3	1	2
Karyomegaly				1	n.d.	n.d.	n.d.	n.d.
Necrosis, single cell, hepatocellular		1-2		1-2		1-3	1	2
Vacuolation, hepatocellular		2-3	1	3-4	1	2-3	1-2	3-4

The range of severity grade for each microscopic finding if present was graded on a scale of 1-4; (1 = minimal, 2 = mild, 3 = moderate, and 4 = marked). n.d. = not determined

Table S8. Microscopic liver findings after treatment with *Hao1*-targeting siRNAs (n=4).

		D6						**D9**					
Dose	**0.9% NaCl**	**3**	**10**	**30**	**30**	**60**	**120**	**3**	**10**	**30**	**30**	**60**	**120**
Change, cytoplasmic, eosinophilic						1-2	2						2
Hyperplasia, bile duct						2-3	2-3						1-2
Hyperplasia, Kupffer cell				1-2			2-3						
Hyperplasia, oval cell						1-4	1						1-2
Hypertrophy, hepatocellular						2-3	2						
Increased mitoses				1	2	3	1-3			1	1	1	1-2
Necrosis						3-4	2-4						2-3
Necrosis, single cell				2-3	1-3	2-3	2			1	1	1-2	1-2
Vacuolation, hepatocellular	1	1	1-2	2-3	2-3	2-3	1-2	1	1	1-2	1-2	1-2	1-2

The range of severity grade for each microscopic finding if present was graded on a scale of 1-4; (1 = minimal, 2 = mild, 3 = moderate, and 4 = marked).

Therapeutic index calculation

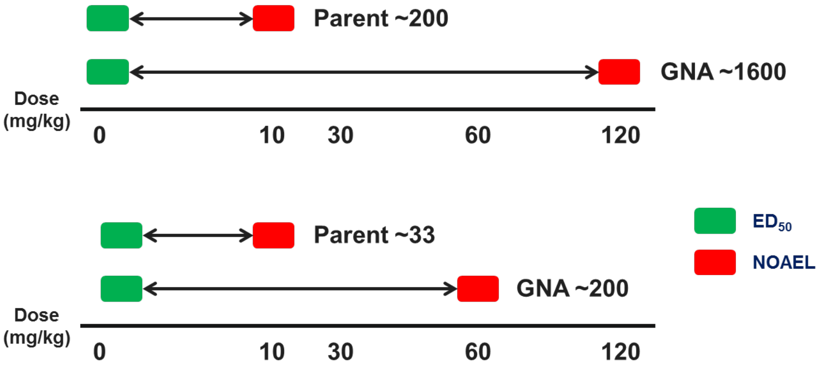

Figure S14. Therapeutic indices in rats as determined by ratio of ED_{50} dose and NOAEL (n=4-5). The ED_{50} values for *Ttr*-targeting siRNAs were 0.05 mg/kg (**D1**, parent) and 0.075 mg/kg (**D4**, GNA). The NOAEL values were 10 mg/kg (**D1**, parent) and >120 mg/kg (**D4**, GNA). The ED_{50} values for *Hao1*-targeting siRNAs were 0.3 mg/kg for both parent **D6** and GNA **D9**. The NOAEL values were 10 mg/kg (**D6**, parent) and 60 mg/kg (**D9**, GNA).

***In vivo* RNAseq MA plots, CDF plots, and DEG counts**

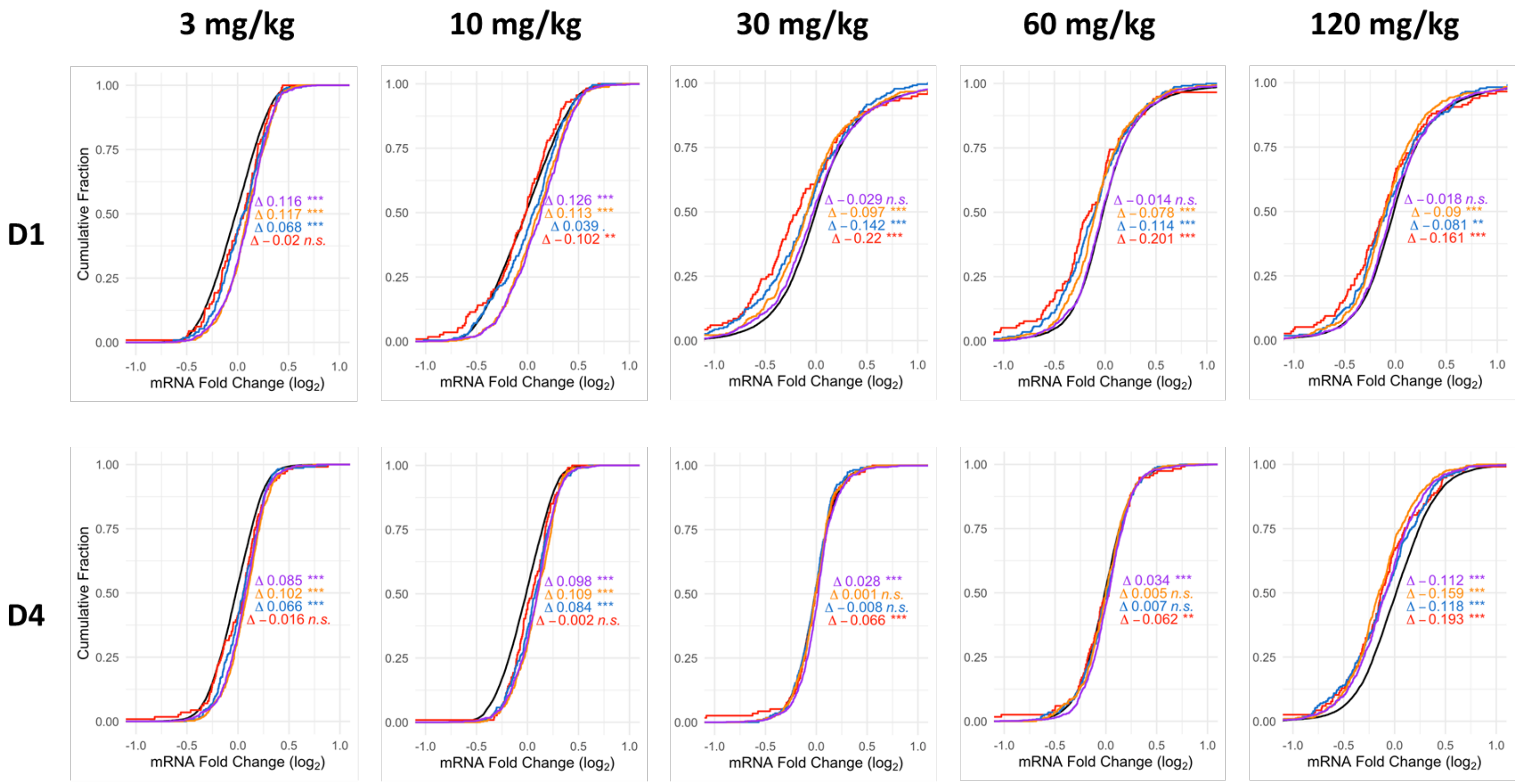

Figure S15. Cumulative distribution function (CDF) plots relative to control after treatment with the designated *Ttr*-targeting siRNAs **D1** and **D4** dosed 3x weekly at the specified dose (n=4-5). Each colored line represents the impact of different types of seed matches on the cumulative dysregulation of gene transcripts; black = background, purple = mer6, yellow = mer7-A1, blue = mer7-m8, red = mer8.

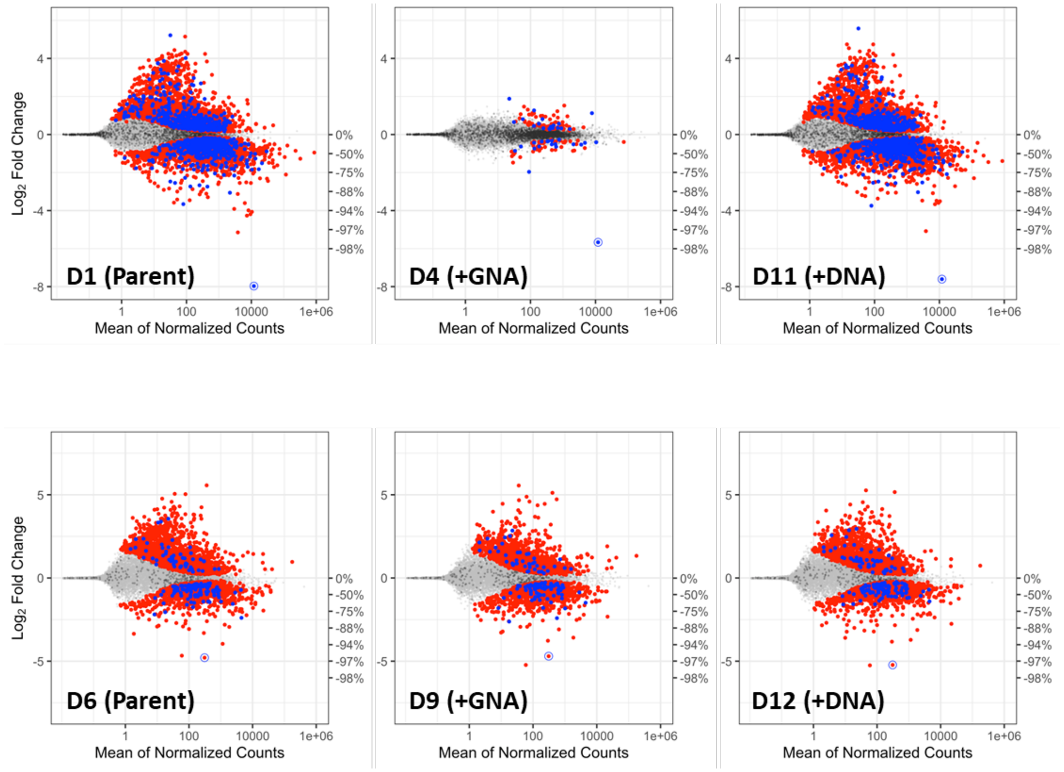

Figure S16. Gene dysregulation in rats following weekly dosing of GalNAc-siRNAs targeting *Ttr* (**D1**, **D4**, **D11**) or *Hao1* (**D6**, **D9**, **D12**). Rats were administered three weekly doses of 30 mg/kg, and frozen livers were collected 24 hours post last dose for RNAseq analysis (n=4). Each dot shown represents a gene transcript in the rat transcriptome, their average read count, and the level of change in expression compared to the control group dosed with 0.9% NaCl. Whereas grey dots represent gene transcripts which were not determined to be differentially expressed after siRNA treatment relative to the control, the blue and red dots represent differentially expressed transcripts (false discovery rate <0.05) with or without a canonical miRNA match (8mer, 7mer-A1, 7mer-m8) to the guide seed region, respectively. On-target knockdown is represented by the circled dot.

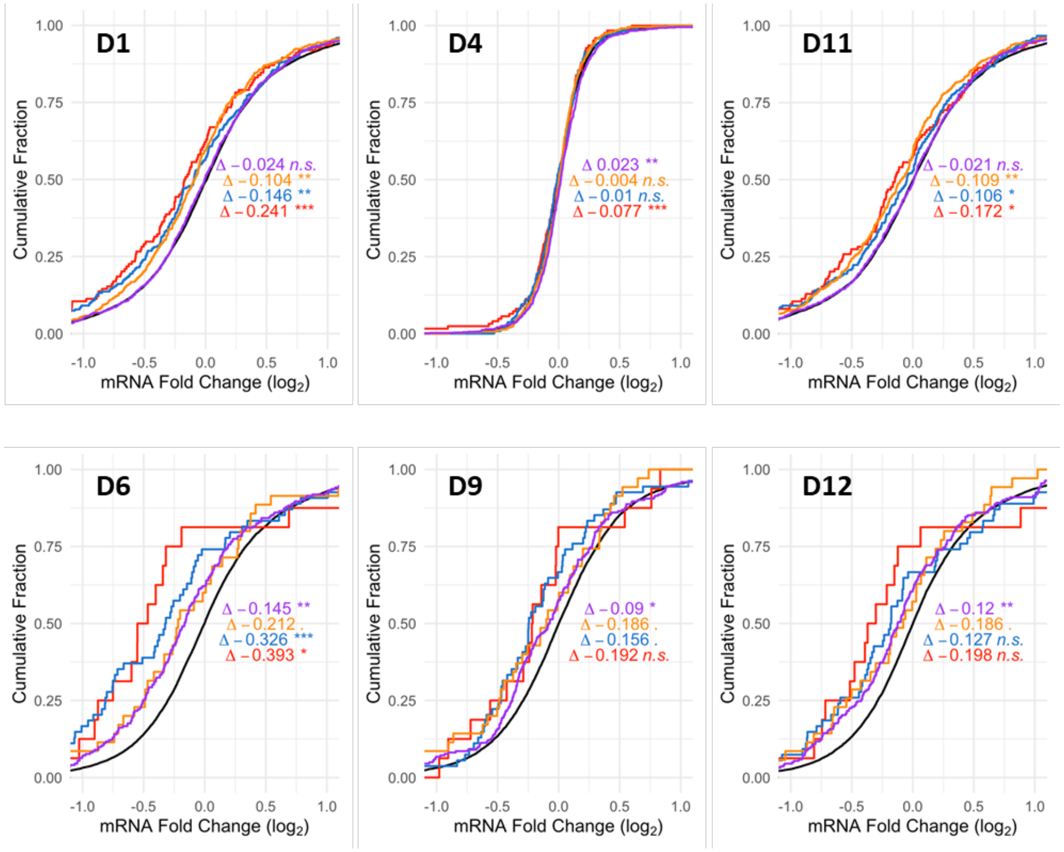

Figure S17. Cumulative distribution function (CDF) plots relative to control after treatment with the specified *Ttr*- and *Hao1*-targeting siRNAs dosed 3x weekly at 30 mg/kg (n=4). Each colored line represents the impact of different types of seed matches on the cumulative dysregulation of gene transcripts; black = background, purple = mer6, yellow = mer7-A1, blue = mer7-m8, red = mer8.

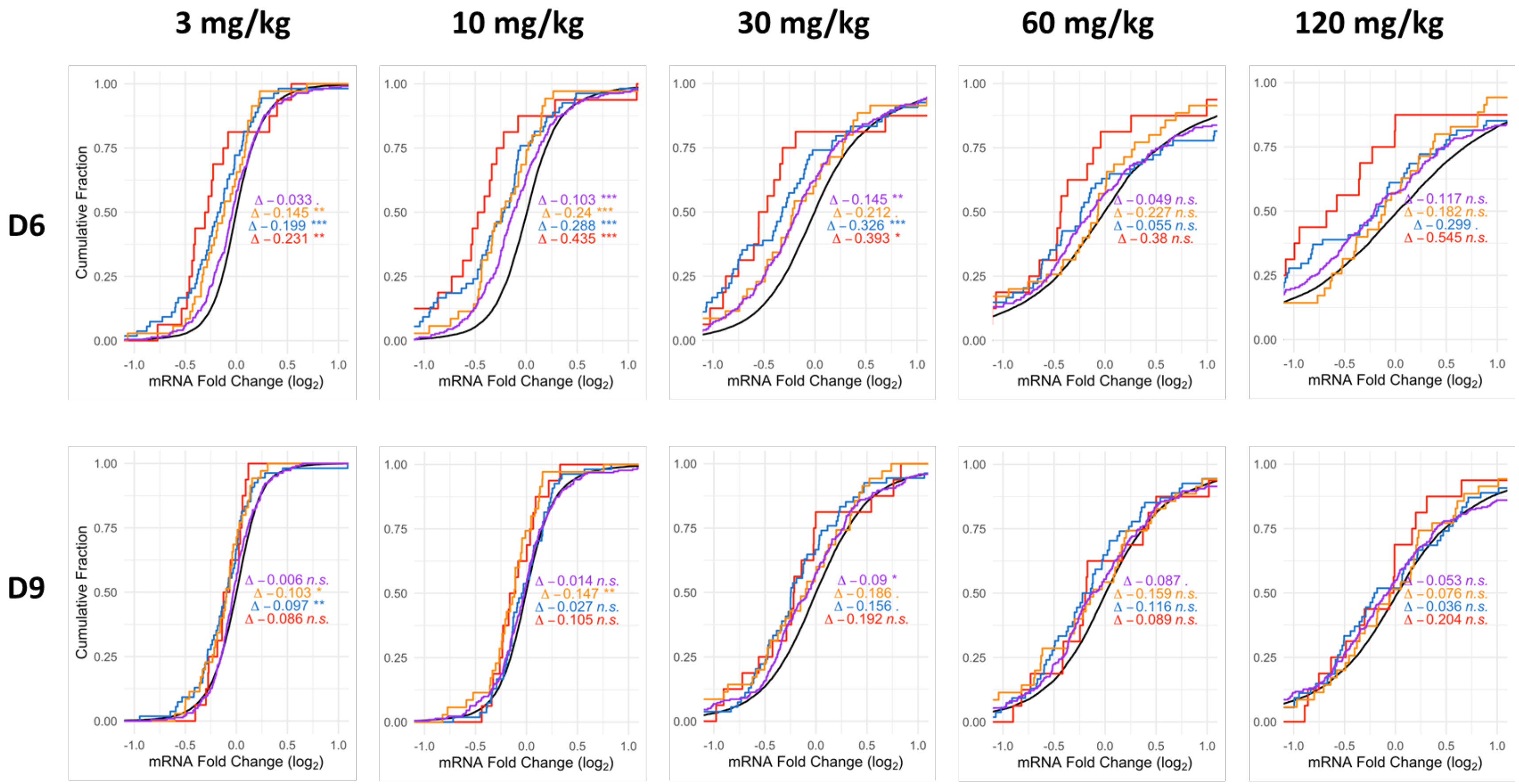

Figure S18. Cumulative distribution function (CDF) plots relative to control after treatment with the designated *Hao1*-targeting siRNAs **D6** and **D9** dosed 3x weekly at the specified dose (n=4). Each colored line represents the impact of different types of seed matches on the cumulative dysregulation of gene transcripts; black = background, purple = mer6, yellow = mer7-A1, blue = mer7-m8, red = mer8.

Table S9. Number of differentially expressed gene transcripts from *in vivo* RNAseq after treatment with the specified *Ttr*- or *Hao1*-targeting siRNAs dosed 3x weekly at the specified doses (n=4-5).

siRNA	**Target**		**#DEGs**				
		Seed Match	**3mg/kg**	**10mg/kg**	**30mg/kg**	**60mg/kg**	**120mg/kg**
D1	*Ttr*	Background	28	1003	1681	664	1204
		mer8	0	16	39	21	24
		mer7-m8	1	38	62	32	49
		mer7-A1	1	50	103	37	63
		mer6	7	95	186	62	116
D4	*Ttr*	Background	49	1	2	23	684
		mer8	2	0	0	2	11
		mer7-m8	1	0	0	4	31
		mer7-A1	5	0	0	2	46
		mer6	7	0	0	2	80
D6	*Hao1*	Background	70	791	3959	7436	8208
		mer8	1	5	10	12	14
		mer7-m8	5	16	29	41	41
		mer7-A1	0	7	18	23	24
		mer6	10	38	107	166	173
D9	*Hao1*	Background	3	294	3144	4521	6325
		mer8	0	0	6	8	8
		mer7-m8	0	0	21	26	34
		mer7-A1	0	1	15	22	18
		mer6	0	15	79	101	148

Number of DEGs estimated relative to saline controls 24 hours post last dose at a false discovery rate < 0.05.

Table S10. Magnitude of cumulative dysregulation of gene transcripts as measured in CDF plots after treatment with the specified *Ttr*- or *Hao1*-targeting siRNAs dosed 3x weekly at the specified doses (n=4-5).

siRNA	Target	Seed Match	CDF Shift 3mg/kg	10mg/kg	30mg/kg	60mg/kg	120mg/kg
D1	*Ttr*	mer8	-0.020	-0.102**	-0.220***	-0.201***	-0.161***
		mer7-m8	0.068***	0.039	-0.142***	-0.114***	-0.081**
		mer7-A1	0.117***	0.113***	-0.097***	-0.078***	-0.090***
		mer6	0.116***	0.126***	-0.029	-0.014	-0.018
D4	*Ttr*	mer8	-0.016	-0.002	-0.066***	-0.062**	-0.193***
		mer7-m8	0.066***	0.084***	-0.008	0.007	-0.118***
		mer7-A1	0.102***	0.109***	0.001	0.005	-0.159***
		mer6	0.085***	0.098***	0.028***	0.034***	-0.112***
D6	*Hao1*	mer8	-0.231**	-0.435***	-0.393*	-0.380	-0.545
		mer7-m8	-0.199***	-0.288***	-0.326***	-0.055	-0.299
		mer7-A1	-0.145**	-0.240***	-0.212	-0.227	-0.182
		mer6	-0.033	-0.103***	-0.145**	-0.049	-0.117
D9	*Hao1*	mer8	-0.086	-0.105	-0.192	-0.089	-0.204
		mer7-m8	-0.097**	-0.027	-0.156	-0.116	-0.036
		mer7-A1	-0.103*	-0.147**	-0.186	-0.159	-0.076
		mer6	-0.006	-0.014	-0.090*	-0.087	-0.053

Significance of each relative to background is indicated as follows *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Pharmacodynamics of GalNAc-siRNAs containing 2’-5’-RNA linkages

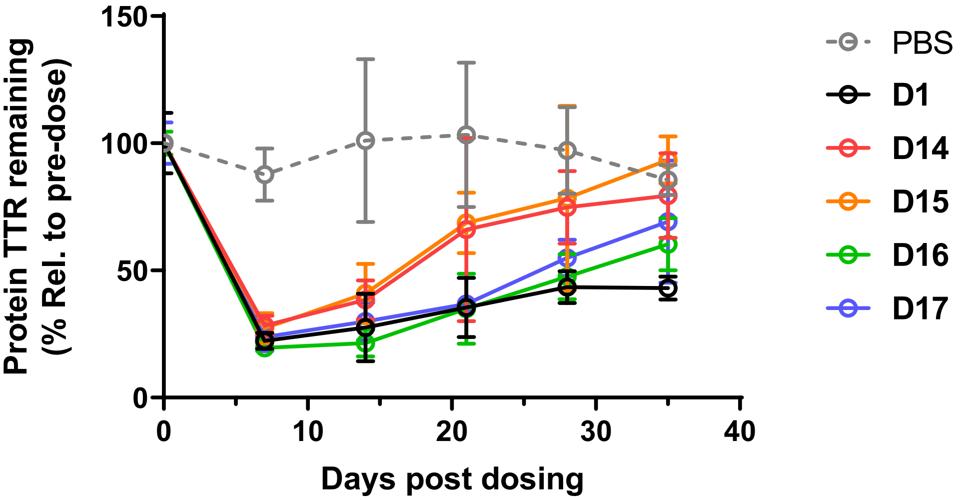

Figure S19. Knockdown of serum TTR protein after a single 0.5 mg/kg dose of parent or 2’-5’-RNA-modified siRNAs in mice (n=3).

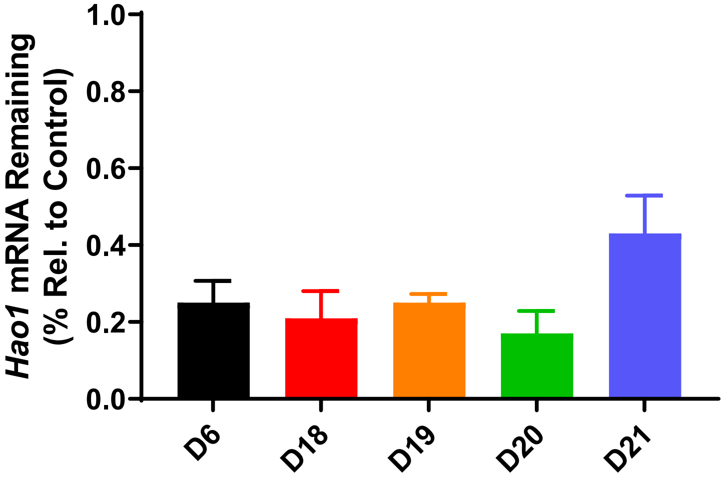

Figure S20. Knockdown of liver *Hao1* mRNA 7 days post a single 1 mg/kg dose of parent or 2’-5’-RNA-modified siRNAs in mice (n=3).

In vitro **off-target silencing of 2’-5’-RNA modified siRNAs**

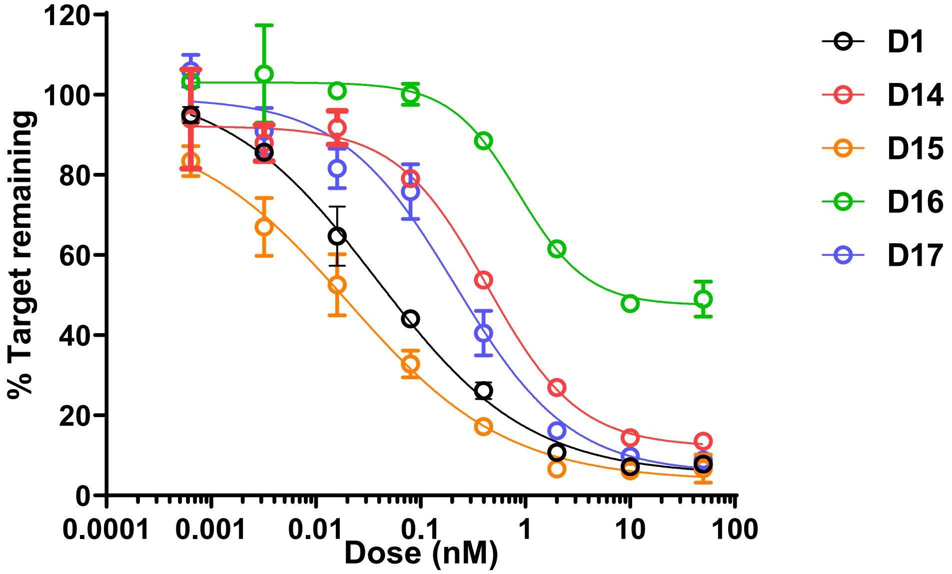

Figure S21. Activity of parent or 2’-5’-RNA-modified siRNAs targeting *Ttr* in the off-target reporter assay (n=4).

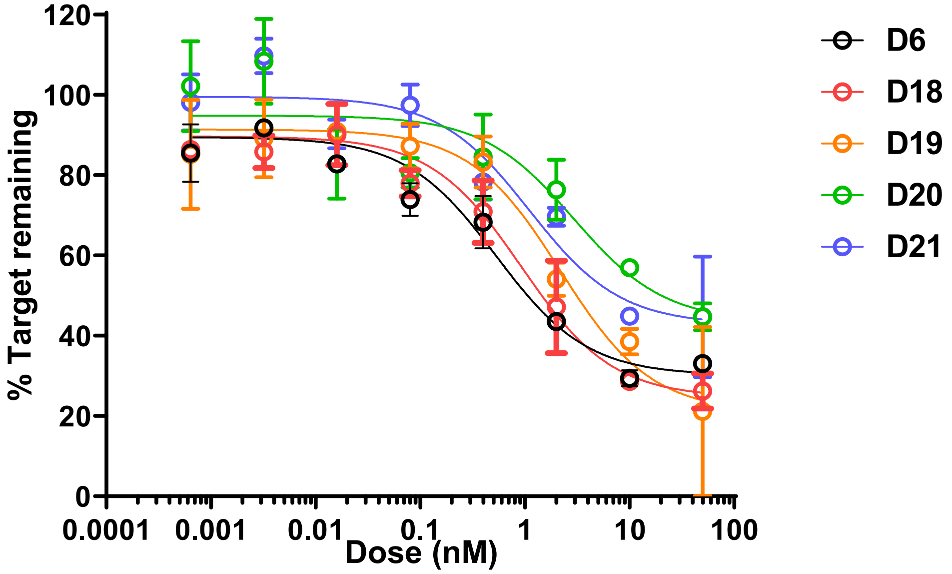

Figure S22. Activity of parent or 2’-5’-RNA-modified siRNAs targeting *Hao1* in the off-target reporter assay (n=4).

In vitro **CDF plots after treatment with 2’-5’-RNA modified siRNAs**

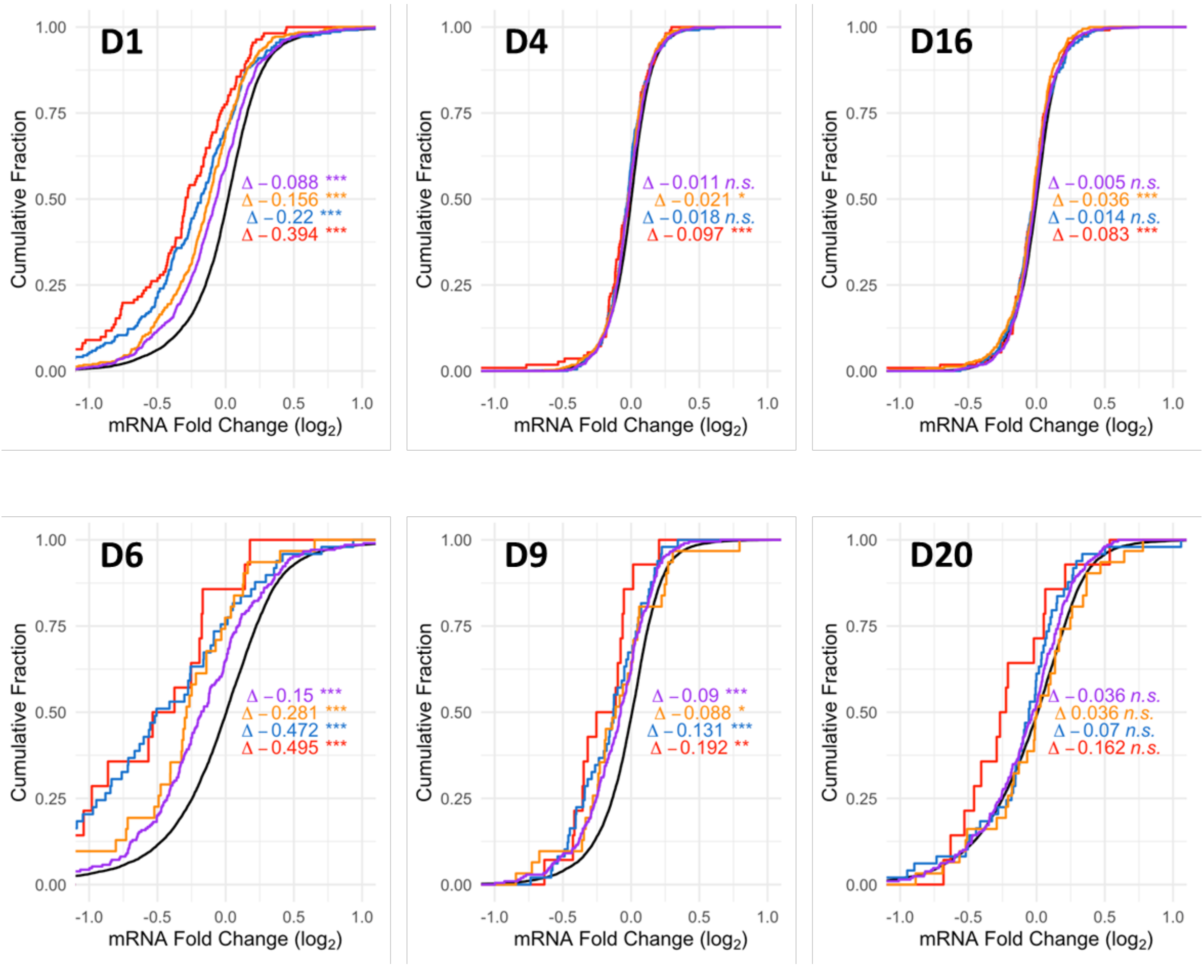

Figure S23. Cumulative distribution function (CDF) plots relative to control after transfection (n=4) with the designated *Ttr*- or *Hao1*-targeting siRNAs at a dose of 50 nM in primary rat hepatocytes. Each colored line represents the impact of different types of seed matches on the cumulative dysregulation of gene transcripts; black = background, purple = mer6, yellow = mer7-A1, blue = mer7-m8, red = mer8.

Additional PD and RNAseq data with HBV-targeting GalNAc-siRNAs

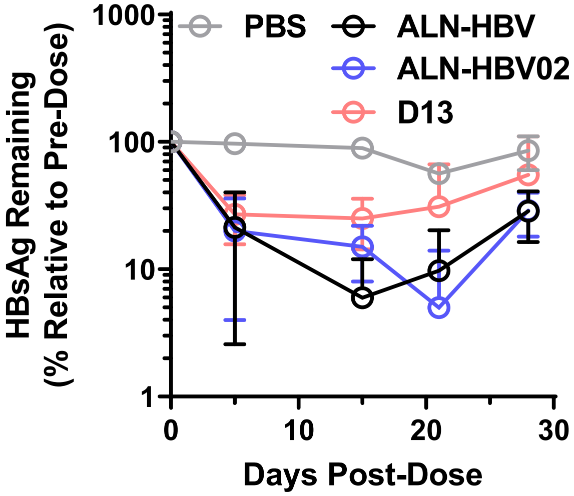

Figure S24. Evaluation of the pharmacodynamics after a single 1 mg/kg dose of HBV-targeting GalNAc-siRNAs in mice (n=3) transduced with HBV-AAV8. Serum HBsAg levels represent the average of all animals from a given cohort, each normalized to individual pre-dose serum HBsAg levels.

Table S11. Number of differentially expressed gene transcripts from *in vitro* RNAseq after treatment with **ALN-HBV** or **ALN-HBV02** at 10nM (n=4)

siRNA	Seed Match	#DEGs
ALN-HBV	Background	422
	mer8	57
	mer7-m8	121
	mer7-A1	72
	mer6	129
ALN-HBV02	Background	73
	mer8	10
	mer7-m8	17
	mer7-A1	20
	mer6	33

Number of DEGs estimated relative to mock controls post-transfection in HepG2.2.15 cells at a false discovery rate <0.05.

Table S12. Magnitude of cumulative dysregulation of gene transcripts as measured in the CDF plots after treatment with **ALN-HBV** or **ALN-HBV02** at 10nM (n=4)

siRNA	Seed Match	CDF Shift
ALN-HBV	mer8	-0.100***
	mer7-m8	-0.079***
	mer7-A1	-0.032***
	mer6	-0.013**
ALN-HBV02	mer8	-0.024***
	mer7-m8	-0.018***
	mer7-A1	-0.018***
	mer6	-0.009**

Significance of each relative to background is indicated as follows *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Overview of published hAgo2 structural data

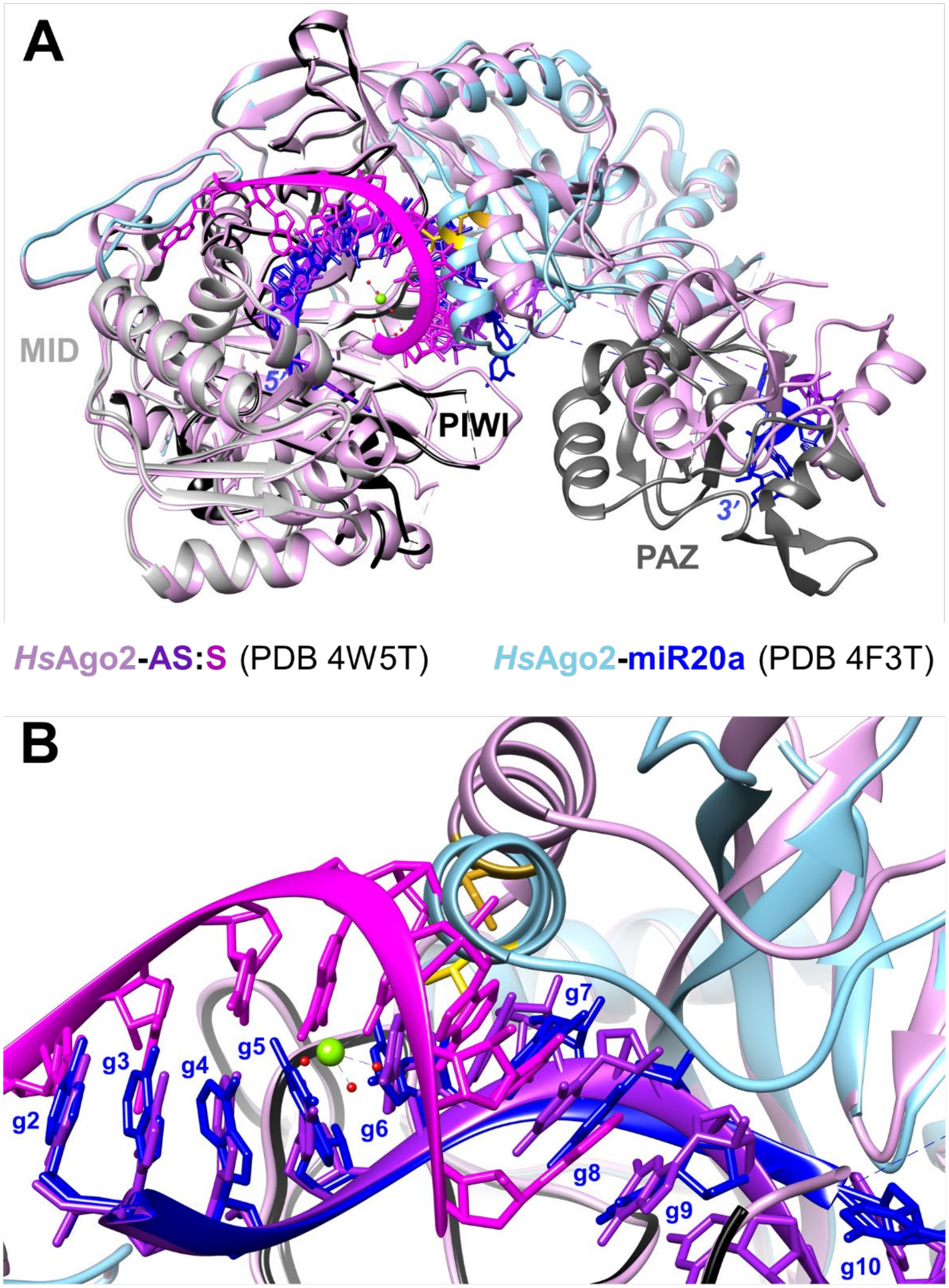

Figure S25. Overview of hAgo2 guide-only and guide-target structures. A) Global view of hAgo2 in guide-only (PDB ID 4F3T, (1)) and guide-target (PDB ID 4W5T, (2)) forms. B) Close up overlay of the two complexes, helix-7, and Ile365.

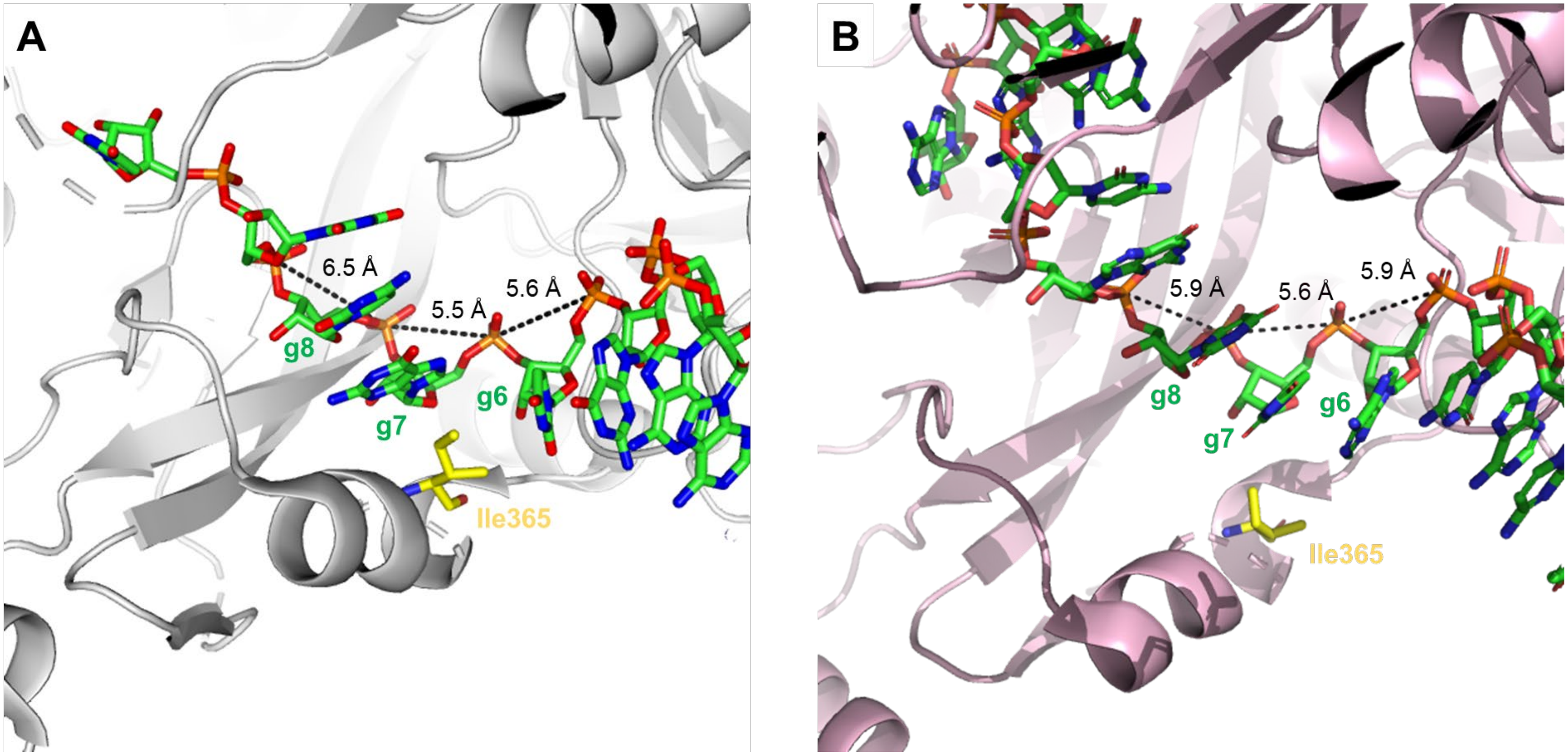

Figure S26. Overview of P-P distances between g5-g6, g6-g7, and g7-g8 in structures of Ago2 with guide only (A) or guide-target (B) complexes. Figures were generated using PyMol from PDB structures 4F3T (guide only, (1)) or 4W5T (guide-target, (2)).

References

1. Elkayam, E., Kuhn, C.-D., Tocilj, A., Haase, Astrid D., Greene, Emily M., Hannon, Gregory J. and Joshua-Tor, L. (2012) The Structure of Human Argonaute-2 in Complex with miR-20a. *Cell*, **150**, 100-110.
2. Schirle, N.T., Sheu-Gruttadauria, J. and MacRae, I.J. (2014) Structural basis for microRNA targeting. *Science*, **346**, 608-613.