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Supplementary Materials for

An oral antisense oligonucleotide for PCSK9 inhibition

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

(available at stm.sciencemag.org/cgi/content/full/13/593/eabe9117/DC1)

Data file S1 (Microsoft Excel format). Transgenic mouse, Fig. 1 and fig. S2.
Data file S2 (Microsoft Excel format). Wild-type mouse Pcsk9 and lipid concentrations, table S2.
Data file S3 (Microsoft Excel format). Wild-type mouse triglyceride secretion, fig. S3.
Data file S4 (Microsoft Excel format). Monkey PK, Fig. 2.
Data file S5 (Microsoft Excel format). Human PCSK9 and LDL cholesterol, Fig. 3.
Data file S6 (Microsoft Excel format). Rat PK and knockdown, Fig. 4 and table S3.
Data file S7 (Microsoft Excel format). Dog PK, Fig. 5 and fig. S4.
Data file S8 (Microsoft Excel format). Monkey PCSK9 and LDL cholesterol, Fig. 6.

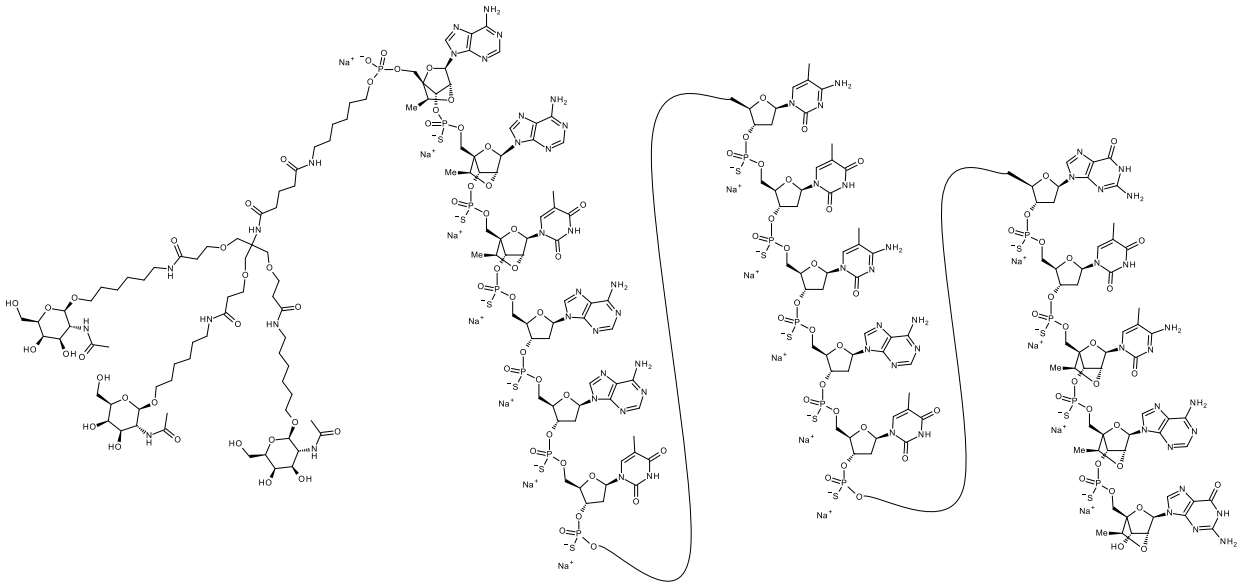

Fig. S1. Chemical structure of AZD8233 as sodium salt.

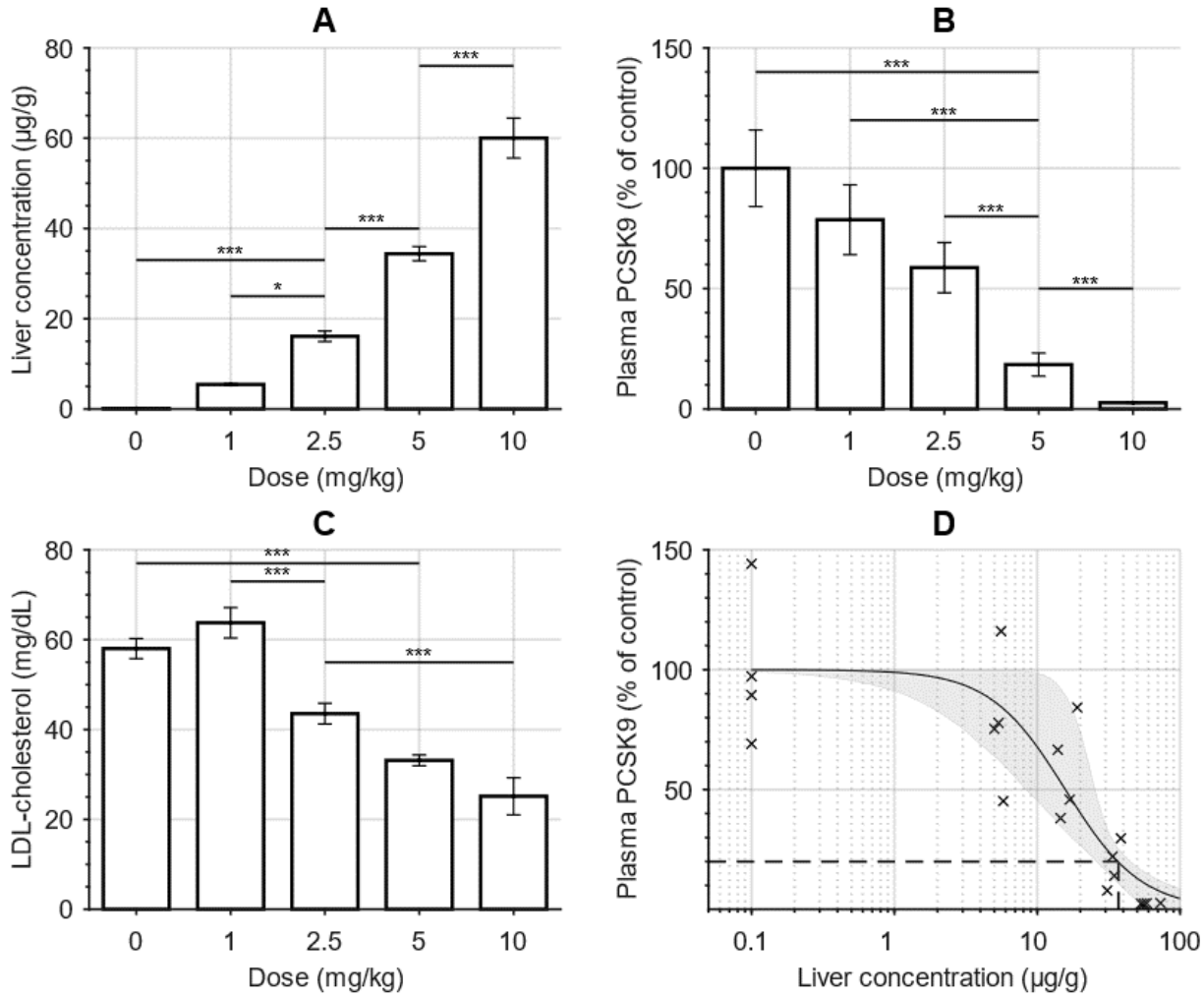

Fig. S2. The non–GalNAc-conjugated parent of AZD8233, ION 848833, in a 4-week dose-response study in transgenic mouse overexpressing human PCSK9. (**A**) Liver exposure according to dose. **B-C**. Human plasma PCSK9 (**B**) and LDL-cholesterol (**C**) in response to doses. (**D)** Plasma PCSK9 versus liver exposure (crosses), and model fit (solid line) with grey shaded area indicating 5th and 95th percentiles. Liver exposure of about 39 µg/g resulted in 80% reduction of PCSK9 (dashed lines). The study included 6 dose groups, with N=4 mice per group. In **A-C**, error bars denote standard error of the mean, and the horizontal bars indicate the significant differences between treatments (*$P < 0.05$; ***$P < 0.005$) for Tukey's honestly significant difference test

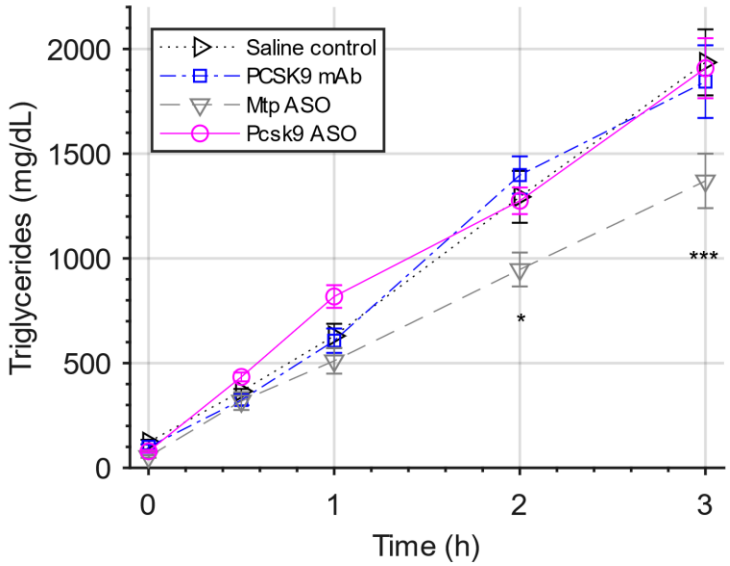

Fig. S3. Pcsk9 GalNAc ASO treatment did not reduce liver triglyceride secretion in wild-type mice fed with an HFD. Tyloxapol, a nonionic detergent that inhibits lipoprotein lipase and clearance of triglyceride from the plasma, was administrated intravenously following 3 weeks of treatments and plasma triglycerides were measured at baseline, and in time-series up to and including 3 h following Tyloxapol administration. GalNAc Pcsk9 ASO mouse surrogate (ION-866672, administered 5 mg/kg SC) or human PCSK9 monoclonal antibody (mAb) treatments were administered. Microsomal triglyceride transfer protein (Mtp) ASO was administered as the positive control. Values represent of N=7 mice per group. * (P<0.05) and *** (P<0.0001) denote statistically significant differences when compared to saline controls. Error bars denote standard error of the mean. Statistical significance was determined by 2-way ANOVA followed by Tukey's multiple comparisons test

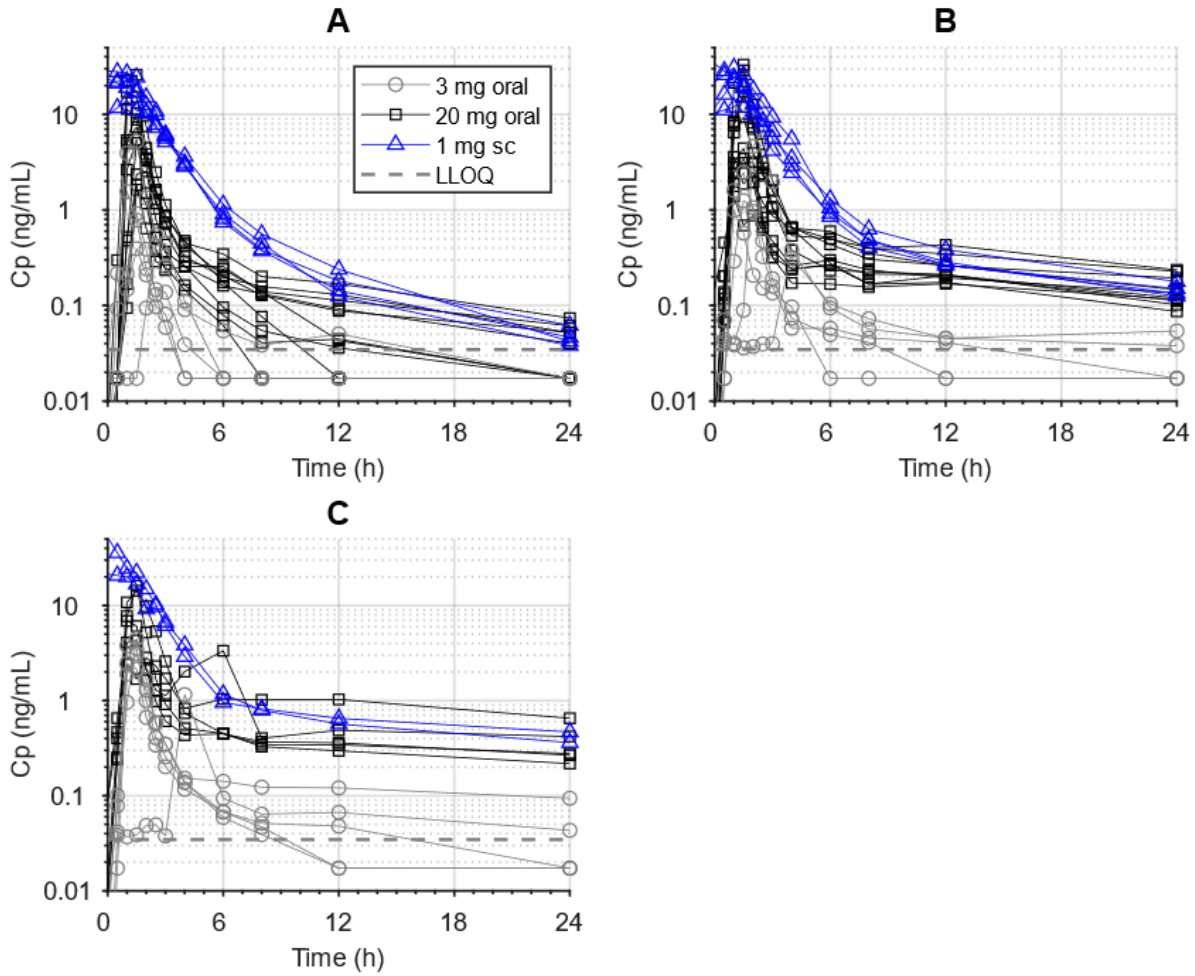

Fig. S4. Plasma concentration after repeated daily oral administration of AZD8233 tablets to dogs. A-C. Plasma concentration (Cp) time-courses during the first day (**A**), on day 8 (**B**) and on day 29 (**C**) of the study. The study included 3 dose groups, with N=4 for the subcutaneous group, N=5 for the 3 mg/day oral group, and N=10 for the 20 mg/day oral group. LLOQ=lower limit of quantification

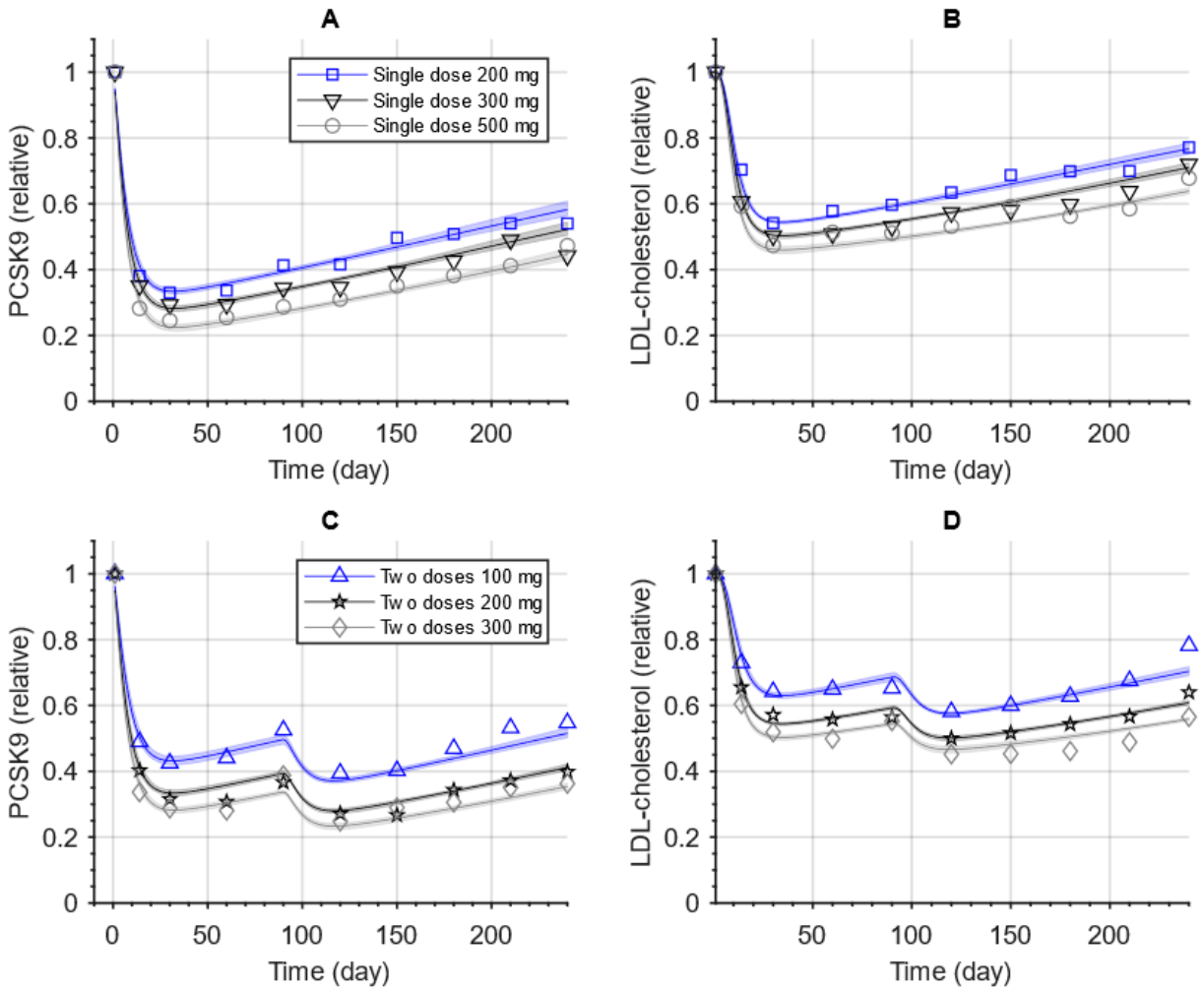

Fig. S5. Empirical PKPD model of PCSK9 and LDL cholesterol. Inclisiran Phase 2 data (41) were digitized, and mathematically represented by an empirical PKPD model. Plasma PCSK9 time course following single doses (**A**) and two doses (**C**). LDL cholesterol time course following single doses (**B**) and two doses (**D**). The model fits are indidated by solid lines and the shaded areas indicate the 5th and 95th percentiles of the point prediction

Table S1. Information about compounds used in the studies. 5'-THA-GN3 = Triantennary GalNAc-functionalised cluster

Compound	Chemistry	ASO sequence	MW free acid (g/mol)	Comment
AZD8233 (ION-863633)	3-10-3 cEt 5'THA-GN3	AAMeUAATMeCTMeCATGTMeCAG	6919.2	Human PCSK9 ASO
ION-848833	3-10-3 cEt	AAMeUAATMeCTMeCATGTMeCAG	5399.6	Unconjugated AZD8233
ION-704361	3-10-3 cEt 5'THA-GN3	ACCATGATACCACTTT	6883.3	Rat Malat-1
ION-866672	3-10-3 cEt 5'THA-GN3	AACTACAAAACCCTGC	6900.3	Mouse Pcsk9 ASO
ION-740133	3-10-3 cEt 5'THA-GN3	GGCCAATACGCCGTCA	6948.3	Mouse control ASO
ION-144477	5-10-5 20-mer phosphorothioate oligonucleotides with 2'- O-methoxyethyl groups at positions 1–5 and 15–20	CCCAGCACCTGGTTTGCCGT	7203.3	Mouse Mtp ASO
ION-556116	3-10-3 cEt	ACCATGATACCACTTT	5363.6	Unconjugated rat Malat-1

Table S2. Effects of 6-week subcutaneous administration (5 mg/kg, once weekly) of mouse Pcsk9 ASO, control ASO, or vehicle in C57BL/6J male mice on plasma Pcsk9 protein concentrations, liver Ldlr protein expression, and plasma and liver lipid concentrations. Values are presented as mean ± SEM. N=10/ group. *P* values were calculated using 1-way ANOVA followed by Tukey´s tests. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001 vs control ASO, #*P*<0.05, ##*P*<0.01, ####*P*<0.0001 vs vehicle. C=Cholesterol, TG= Triglycerides, and PL=Phospholipids

	Vehicle	Control ASO	Pcsk9 ASO
Plasma PCSK9 protein (ng/mL)	106.1±6.0	93.3±4.6	3.5±0.2$^{****, ####}$
Liver LDLR protein (ng/mg)	23.3±2,3	26.6±1.4	64.4±2.6$^{****, ####}$
Plasma cholesterol (mmol/L)	2.63±0.05	2.82±0.05##	1.87±0.02$^{****, ####}$
VLDL cholesterol (mmol/L)	0.08±0.01	0.08±0.01	0.10±0.01
IDL cholesterol (mmol/L)	0.06±0.00	0.05±0.01	0.04±0.00
LDL cholesterol (mmol/L)	0.18±0.01	0.15±0.01##	0.06±0.01$^{****, ####}$
HDL cholesterol (mmol/L)	1.77±0.08	1.68±0.08	1.22±0.05$^{***, ####}$
Plasma triglycerides (mmol/L)	0.57±0.04	0.59±0.04	0.63±0.04
VLDL triglycerides (mmol/L)	0.32±0.03	0.32±0.03	0.41±0.03
IDL triglycerides (mmol/L)	0.06±0.00	0.06±0.00	0.05±0.00
LDL triglycerides (mmol/L)	0.04±0.00	0.03±0.00##	0.01±0.00$^{****, ####}$
VLDL-PL (mmol/L)	0.06±0.01	0.07±0.01	0.08±0.01
IDL-PL (mmol/L)	0.03±0.00	0.03±0.00	0.02±0.00$^{*, #}$
LDL-PL (mmol/L)	0.08±0.01	0.06±0.00#	0.03±0.00$^{***, ####}$
HDL-PL (mmol/L)	1.20±0.07	1.14±0.08	0.83±0.04$^{**, ##}$
VLDL protein (mg/mL)	0.07±0.01	0.08±0.01	0.10±0.01
IDL protein (mg/mL)	0.04±0.00	0.03±0.00	0.03±0.00
LDL protein (mg/mL)	0.13±0.00	0.11±0.00	0.05±0.00$^{****, ####}$
HDL protein (mg/mL)	1.11±0.03	1.12±0.03	0.96±0.03$^{**, ##}$
VLDL (C+TG+PL)/protein	6.20±0.19	5.97±0.33	6.18±0.14
IDL (C+TG+PL)/protein	3.82±0.27	4.26±0.53	4.56±0.92
LDL (C+TG+PL)/protein	2.32±0.06	2.14±0.08	1.92±0.20
HDL (C+TG+PL)/protein	2.66±0.08	2.50±0.07	2.13±0.04$^{**, ####}$
Liver cholesterol (g/100g)	0.26±0.02	0.26±0.01	0.25±0.01
Liver triglycerides (g/100g)	1.48±0.11	1.59±0.13	1.49±0.13

Table S3. Knockdown of *Malat-1* mRNA in liver and intestine after a single dose of rat-specific GalNAc-conjugated tool ASO targeting Malat-1 (ION-704361) with sodium caprate (300 mg/kg) as permeation enhancer after IJ administration to rats. Significant differences between treatments calculated by Tukey's honestly significant difference test are indicated by asterisks (***$P \leq 0.005$ versus control; all other comparions had $P > 0.05$). SEM=standard error of the mean

Dose of ION-704361 (mg/kg)	**N**	***Malat-1* mRNA in liver (relative expression) (mean±SEM)**	***Malat-1* mRNA in intestine (relative expression) (mean±SEM)**
0	2	1.1 (0.33)	1.0 (0.22)
2	4	0.58 (0.11)	2.1 (0.36)
20	4	0.088 (0.012)***	0.93 (0.23)
100	4	0.077 (0.012) ***	1.3 (0.24)

Table S4. LDL cholesterol, total cholesterol, triglycerides, and concentration of unconjugated AZD8233 in the liver in the 14-day tolerability study in monkeys.

ID	Dose (mg/d)	Time (d)	LDL-cholesterol (mmol/L)	LDL-colesterol (% of baseline)	Total cholesterol (mmol/L)	Total cholesterol (% of baseline)	Triglycerides (mmol/L)	Triglycerides (% of baseline)	Liver conc. (µg/g)
1001	28	Baseline	1.18	100	3.2	100	0.67	100	
		7	0.71	60	2.80	88	0.58	87	
		14	0.73	62	2.70	84	0.49	74	37
1501	28	Baseline	1.48	100	3.45	100	0.64	100	
		7	0.56	38	2.60	75	0.50	78	
		14	0.68	46	2.80	81	0.74	116	57
2001	42	Baseline	1.72	100	4.5	100	0.55	100	
		7	0.85	50	3.60	80	0.58	105	
		14	0.80	47	3.50	78	0.88	160	53
2501	42	Baseline	1.33	100	3.4	100	0.39	100	
		7	0.69	52	2.90	85	0.51	132	
		14	0.72	54	2.80	82	0.32	83	34
3001	56	Baseline	1.51	100	3.95	100	0.50	100	
		7	1.07	71	3.60	91	0.58	117	
		14	0.88	58	3.80	96	0.72	145	68
3501	56	Baseline	1.34	100	2.45	100	0.46	100	
		7	0.74	55	1.90	78	0.88	193	
		14	0.69	52	2.10	86	0.63	138	27

Table S5. Overview of animal studies and their purposes. SC=subcutaneous, IJ=intrajejunal

Species	**ASO**	**Administration (formulation)**	**Purpose**
Transgenic mouse expressing human PCSK9	AZD8233	Once weekly SC (solution)	To establish dose-PCSK9 and liver exposure-plasma PCSK9 relationship (Fig. 1)
C57BL/6J mouse	ION-866672 targeting mouse *Pcsk9*	Once weekly SC (solution)	To quantify endogenous *Pcsk9* knock-down effects on liver Ldlr expression and lipoproteins lipid composition (table S2)
Cynomolgus monkey	AZD8233	Once monthly SC (solution)	To quantify accumulation in liver and ASO half-life in liver (Fig. 2)
Cannulated rat	ION-704361 targeting rat *Malat-1*	Single IJ (solution) and single SC (solution with ASO and sodium caprate)	To quantify liver bioavailability (IJ versus SC) and target engagement (liver exposure-mRNA knockdown) for a rat-specific tool ASO (Fig. 4)
Cannulated rat	AZD8233	Single IJ (solution) and single SC (solution with ASO and sodium caprate)	To bridge the rat-specific tool ASO to AZD8233 by quantifying liver exposure and bioavailability (Fig. 4)
Dog	AZD8233	Once daily oral (tablet with ASO and sodium caprate)	To quantify liver exposure for oral administration of ASO tablets (Fig. 5)
Cynomolgus monkey	AZD8233	Once daily oral (tablet with ASO and sodium caprate)	To test tolerability and investigate target engagement upon oral administration (Fig. 6)