Silencing of *Inhbe* mRNA Using GalNAc-siRNAs Induces Durable Weight Loss in a Mouse Model of Diet-Induced Obesity

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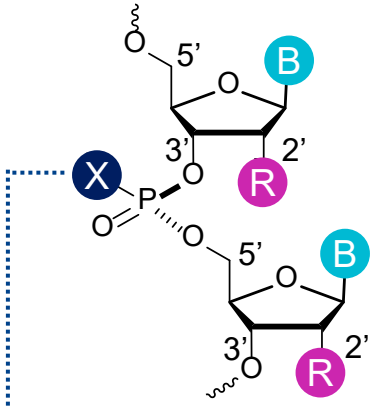
Disclosures

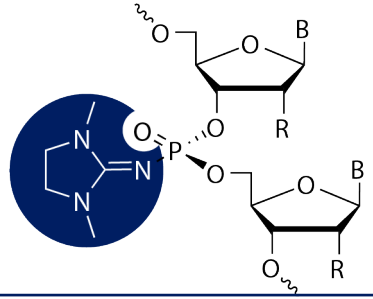
- **Naoki Iwamoto**, Wei Liu, Stearne Briem, Michael Byrne, Cynthia Caracta, Arindom Chatterjee, Jigar Desai, Frank Favaloro, Pachamuthu Kandasamy, Tomomi Kawamoto, Anthony Lamattina, Fangjun Liu, Ken Longo, Richard Looby, Khoa Luu, Subramanian Marappan, Jake Metterville, Padma Narayanan, Lola Owen, Qianli Pan, Erin Purcell-Estabrook, Priyanka Shiva Prakasha, Himali Shah, Stephany Standley, Kris Taborn, Snehlata Tripathi, Hailin Yang, Hsiu-Chiung Yang, Yuan Yin, Erik Ingelsson, and Chandra Vargeese are employees of Wave Life Sciences

Wave's ability to rationally design oligonucleotides enables access to unique disease targets

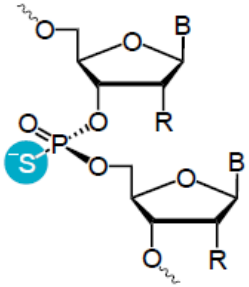
- **B** Base
- **R** 2'-Ribose
- **X** Stereochemistry and backbone modification



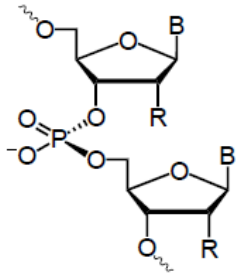
Phosphoryl guanidine (PN)



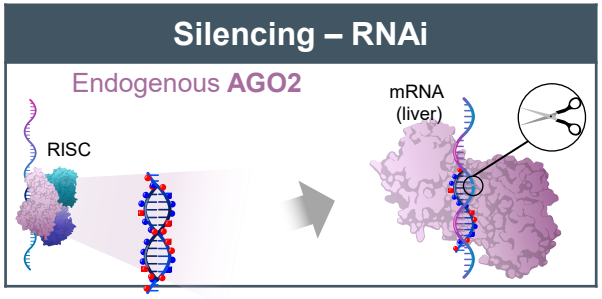
Phosphorothioate (PS)



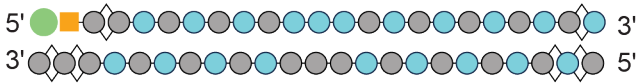
Phosphodiester (PO)



Stereochemistry of PS modifications impacts activity of GalNAc-conjugated siRNA in mice



Stereorandom reference format 1



Backbone configuration

PO

PS stereorandom

2'-sugar modifications

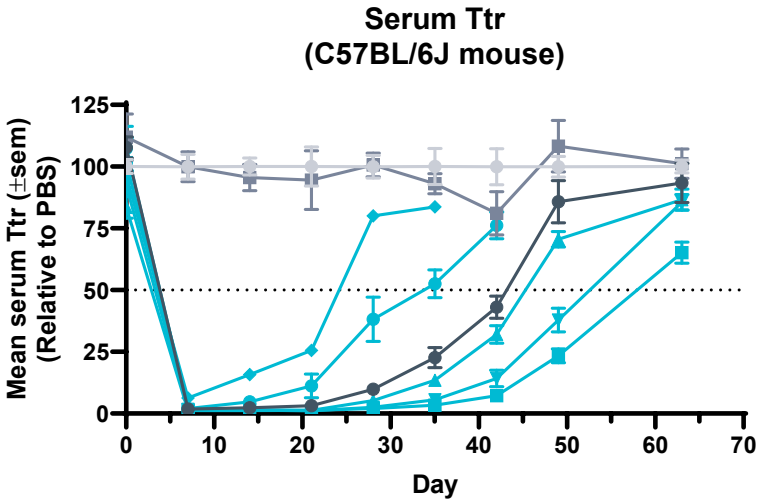
2'-OMe

2'-F

GalNAc conjugation

Triantennary GalNAc (Hepatocyte-targeting ligand)

Linker



PBS

NTC

Stereorandom Reference format 1

TTR-4

TTR-5

TTR-6

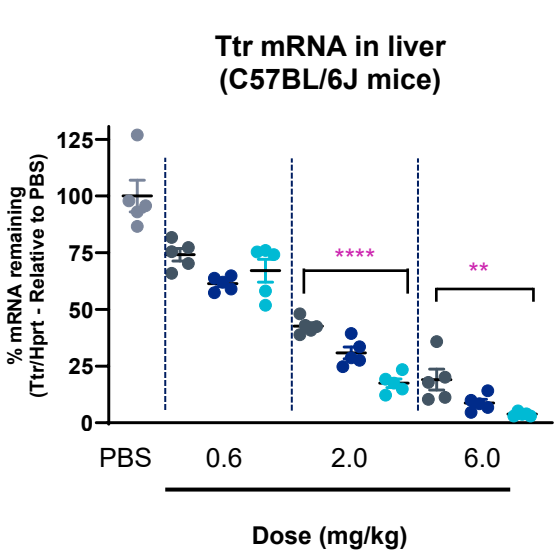
TTR-7

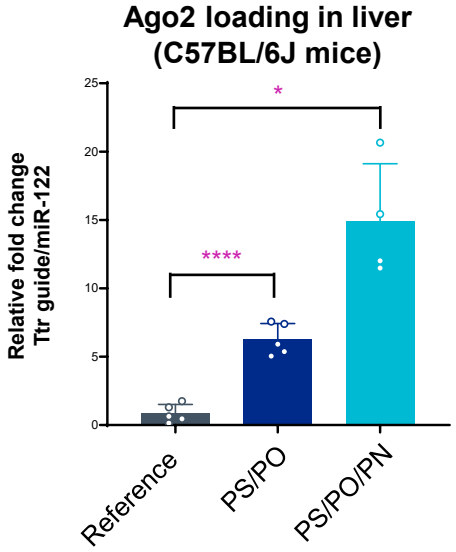
TTR-18

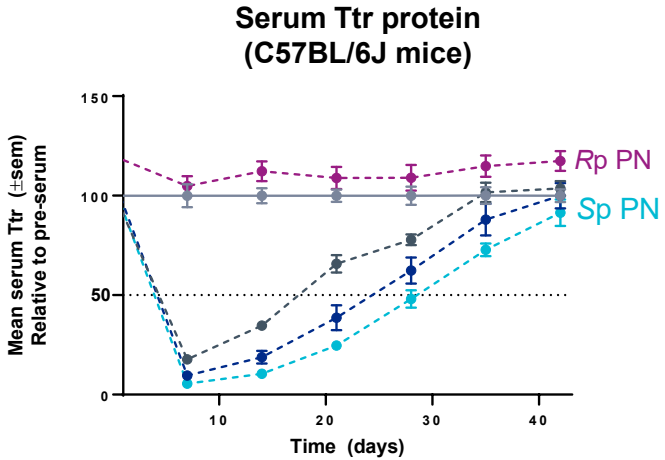
Stereopure siRNAs

C57BL/6J mice were treated with 6 mg/kg of the indicated siRNA or PBS by subcutaneous (SC) injection on day 0. Serum Ttr protein levels were assessed each week by ELISA through the end of the experiment. Liu et al., 2023 Nuc Acids Res. 51(9):4126. GalNAc: *N*-acetylgalactosamine; siRNA: small interfering RNA

Incorporation of PN chemistry improves GalNAc-siRNA potency and durability in mice in part by an Ago2 loading advantage

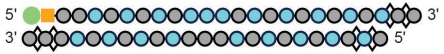




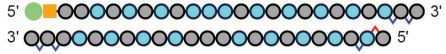


PBS

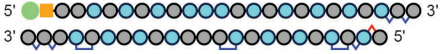
Reference compound



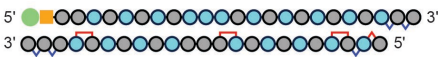
PS/PO backbone



PS/PO/PN(***Sp***) backbone



PS/PO/PN(***Rp***) backbone



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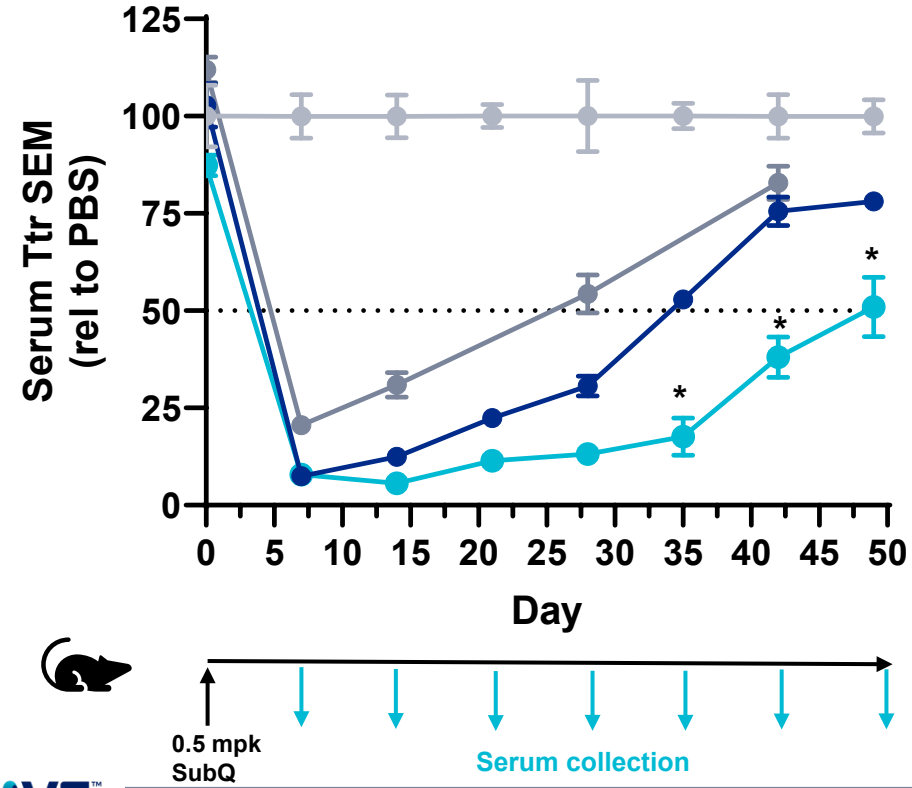
Left, C57BL/6J mice were treated with indicated dose of siRNA or PBS by SC injection on day 0. Liver *Ttr* mRNA levels were quantified by RT-PCR 1-week later. Stats: 2-way ANOVA ** p<0.01, **** p<0.0001. Middle, Ago2 loading was quantified by RT PCR from livers in panel A after 1 week of treatment with 2 mg/kg siRNA. Stats: Welch's 1-way ANOVA with post hoc comparisons * p<0.05, **** p<0.0001. Adapted from Liu et al., 2023 Nuc Acids Res. 51(9):4126. *Ttr:* Transthyretin; Ago2: Argonaute 2.

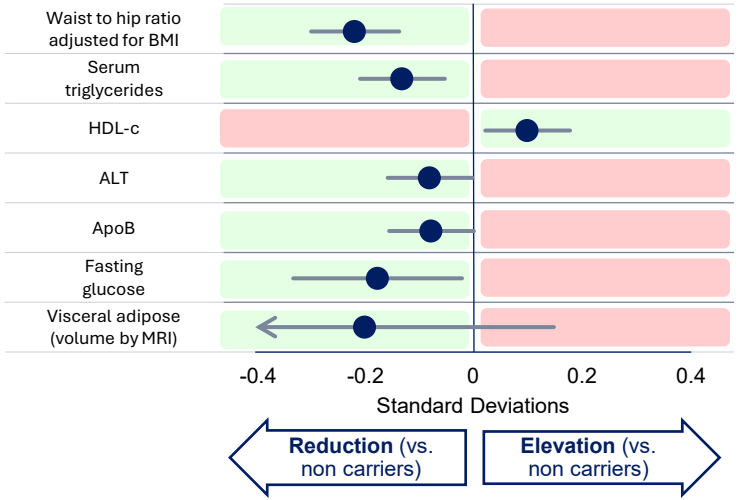
Wave's new design for GalNAc-siRNA improves durability following single administration in mice



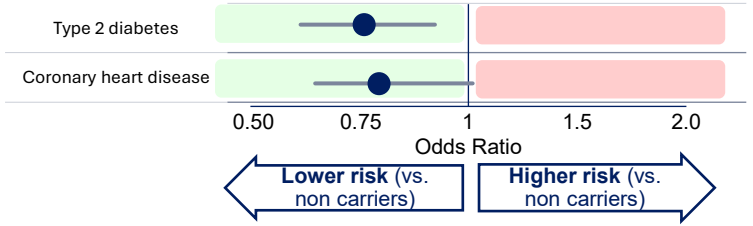
[1]Foster, DJ. et.al. Mol Ther. 2018, 26(3), 708. [2] Liu W et al., Nucl Acids Res. 2023 51(9) C57BL/6 mice administered PBS or 0.5 mg/kg of siRNA (subcutaneous). Stats: Mixed 2-way ANOVA followed by post hoc test comparing prior format siRNA vs. new format siRNA per day derived from linear mixed effects model * p< 0.0001

Human genetic data demonstrate that heterozygous *INHBE* LoF carriers have a healthy metabolic profile

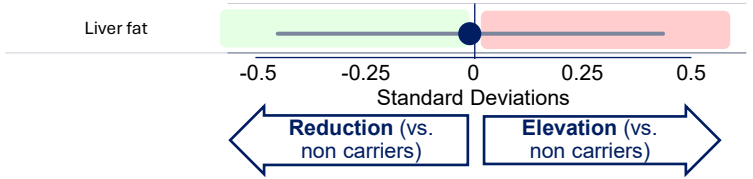
Heterozygous *INHBE* LoF carriers have favorable traits: lower abdominal obesity, lower triglycerides, higher HDL-c



Heterozygous *INHBE* LoF carriers have lower risk of Type 2 diabetes and CHD



Heterozygous *INHBE* LoF carriers have no difference in liver fat

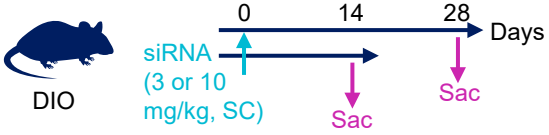


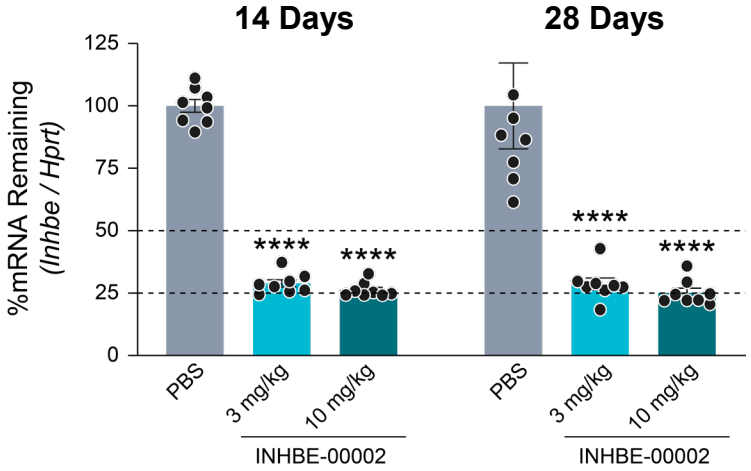
Silencing liver *INHBE* mRNA by ≥50% is expected to recapitulate the healthy metabolic profile of heterozygous *INHBE* loss of function (LoF) carriers

Akbari et al. Nat Commun. 2022 Aug 23;13(1):4844; Deaton et al. Nat Commun. 2022 Jul 27; 13(1):4319.
HDL-c: high-density lipoprotein cholesterol; ALT: alanine transaminase; ApoB: apolipoprotein B; CHD: coronary heart diesease

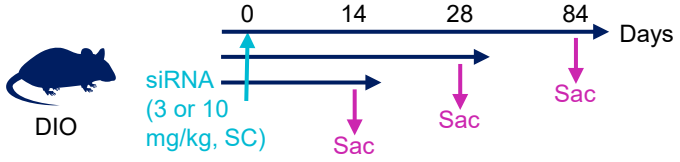
A single dose of INHBE GalNAc-siRNA supports durable weight loss in a mouse model of diet induced obesity (DIO)

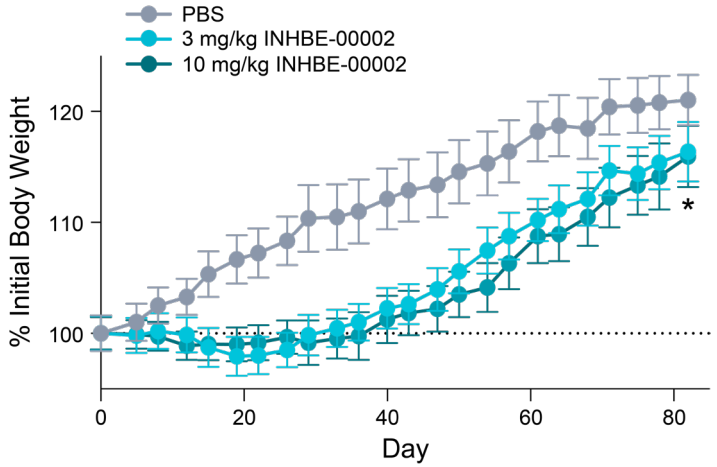
Inhbe mRNA Knockdown





Body Weight 84 Days

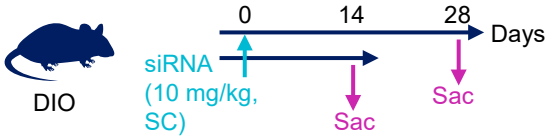


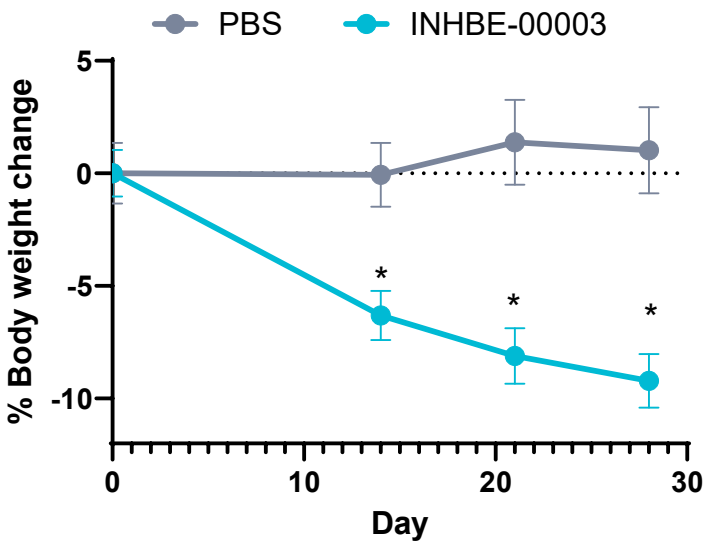


C57Bl6 DIO mice received a single SC injection of INHBE-00002 or PBS (control) on Day 0 (D0). Left: Inhbe expression in liver was evaluated by RT-qPCR on D4 or D28. Stats: mean ±SEM,% mRNA remaining relative to mean of PBS at D0; 2-way ANOVA, **** p<0.0001 vs. PBS per time point. Right: Mice were weighed twice weekly and sacrificed at D14, D28, or D84. Stats: Mean body weight change (%) relative to D0 ±SEM (n=8-24); Linear mixed effects ANOVA, * p<0.05 compared to PBS at timepoint; 3 mg/kg group and 10 mg/kg group were significantly different from PBS between D12-82 and D8-82, respectively.

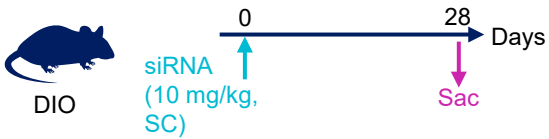
A single dose of INHBE GalNAc-siRNA leads to weight loss driven by fat loss without loss of muscle mass

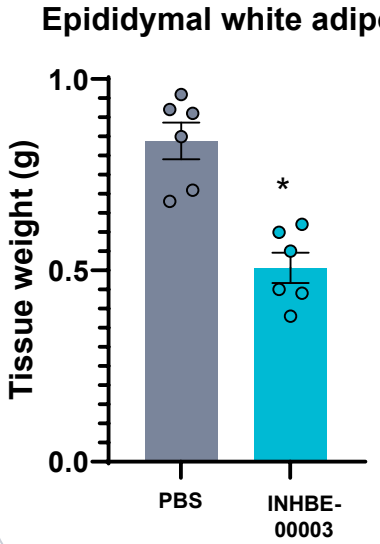
Body Weight

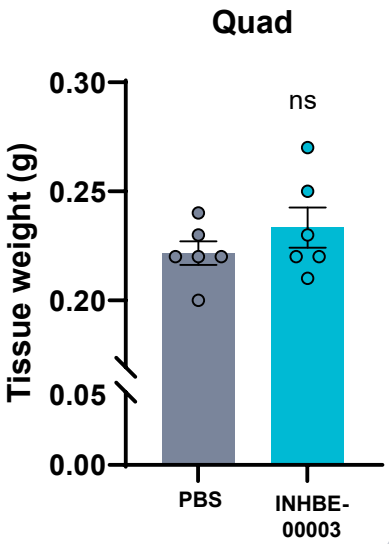




Tissue Weight







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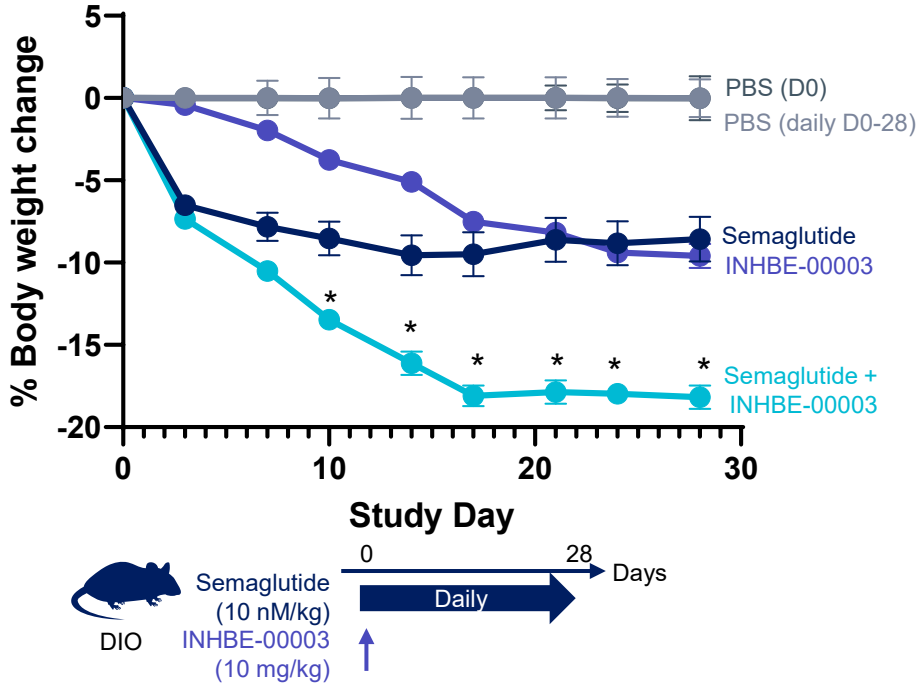
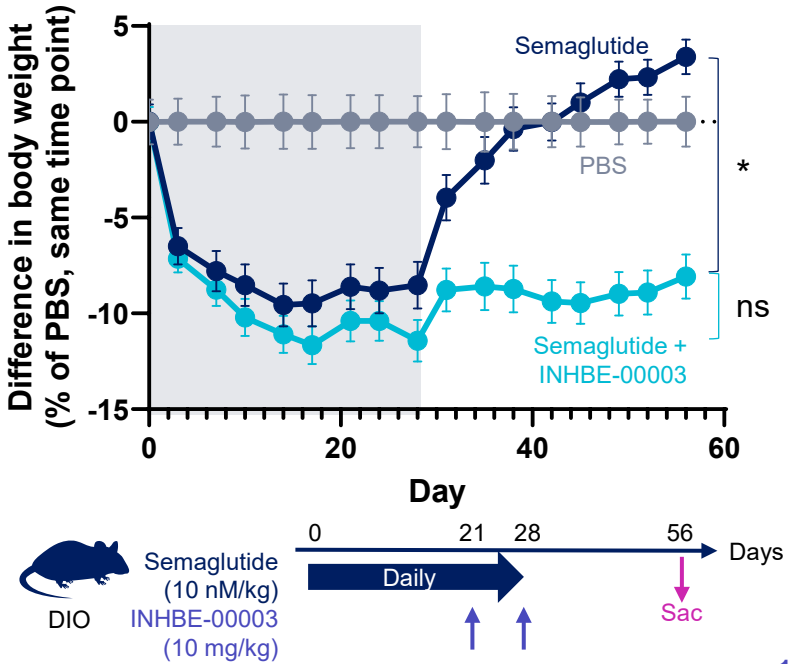
At ~25 weeks old (Day 0, D0), C57Bl6 DIO mice received a single injection of INHBE-00003 or PBS (control). Mice were sacrificed at D14 or D28. Left, Mean body weight change (%) from D0 ±SEM (n=12-18); Stats: linear mixed effects ANOVA with post hoc comparisons of marginal treatment effects versus PBS per timepoint; * p<0.05. Right, Epididymal visceral adipose (left) and quadriceps muscle (right) were collected and weighed on D28. Stats: Mean weight (g) ±SEM, Linear mixed effects ANOVA with post hoc comparisons of marginal treatment effects versus PBS per tissue type; * p<0.05; ns, nonsignificant.

INHBE GalNAc-siRNA augments semaglutide-induced weight loss in DIO mice

~2x greater overall weight loss when added to GLP-1



Curtails weight regain after the cessation of GLP-1



DIO mice received daily SC injections of PBS or semaglutide (10 nmol/kg) for 28 days; PBS on Day 0; or daily semaglutide (10nmol/kg) D0-28 plus SC INHBE-00003 (10 mg/kg) on (left) D0 or (right) D21 and D28. Animals weighed 2x weekly. Stats: Mean weight difference as a % of PBS control on the same day (±SEM, n=10). Left, * $p < 0.05$ compared to semaglutide group. Right, * $p < 0.05$; ns, nonsignificant; comparisons of mariginal time point effects between D28 and D56 per treatment group.

Preclinical data support potential to use WVE-007 across multiple treatment settings

WVE-007 is an investigational INHBE GalNAc-siRNA in development for the treatment of obesity

Monotherapy	*Add-on to GLP-1s*	*Maintenance*
WVE-007 as a single agent	**WVE-007 in addition to GLP-1 therapy**	**WVE-007 for patients who stop treatment with GLP-1 therapy**
✓ Weight loss similar to semaglutide with a single dose ✓ No loss of muscle mass ✓ Reduction in fat mass	When administered as an add-on to semaglutide: ✓ A single dose of Wave's INHBE GalNAc-siRNA doubled the weight loss observed with semaglutide alone	✓ Curtailed rebound weight gain upon cessation of semaglutide

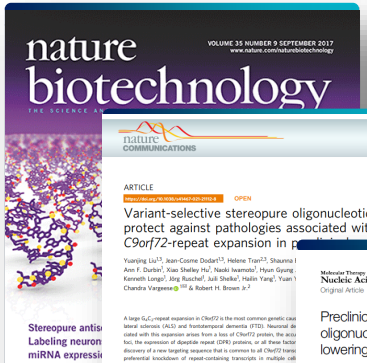
Summary

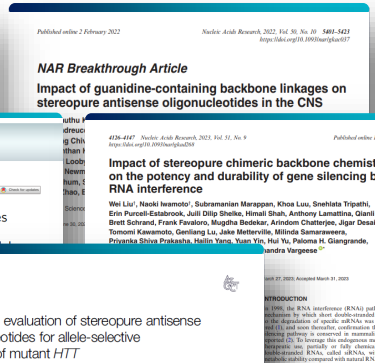
- PRISM, our discovery and drug development platform, enabled the development of a new siRNA chemical design that improves mRNA knockdown durability in mice.
- Applying the new chemical design to our GalNAc-siRNA improves knockdown durability in the mouse liver.
- INHBE GalNAc-siRNAs lower *Inhbe* mRNA expression, reduce body weight, and positively impact body composition in a mouse DIO model.
- When added on to semaglutide (a GLP-1) treatment, an INHBE GalNAc-siRNA doubled weight loss and reduced weight regain upon cessation of semaglutide in DIO mice.
- Wave is advancing WVE-007 as a novel, long acting, muscle sparing approach for obesity.
  - Preclinical data support potential to use WVE-007 across multiple treatment settings.

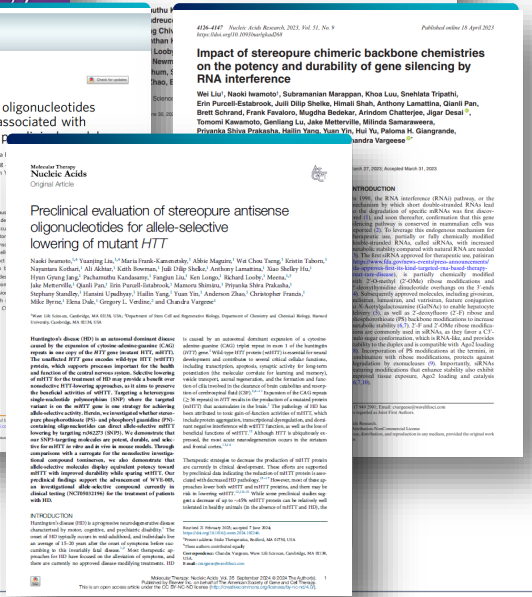
Acknowledgements

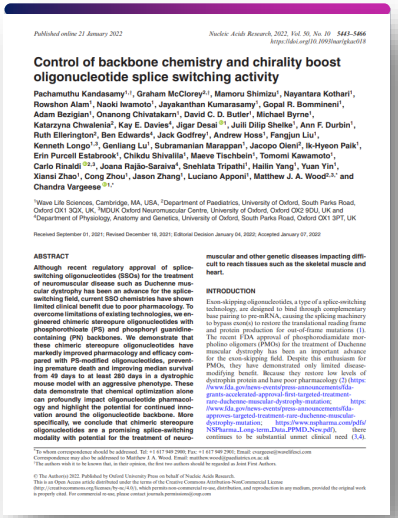
- Thanks to all colleagues and contributors from **Wave Life Sciences** and our collaborators.
- Nicole Neuman, Wave Life Sciences, assisted in preparing this presentation.

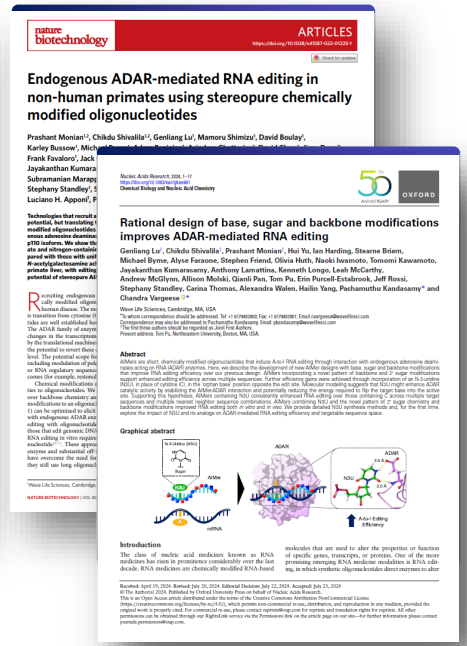
Silencing (RNase H and Ago2) | **Splicing** | **Editing**











Selected Wave publications: PMC10201370, PMC9092894, PMID 35256816, PMC9177980, PMC9178015, PMC7870851, PMC7804567, PMID 28829437, DOI: 10.1016/j.omtn.2024.102246; DOI: 10.1093/nar/gkae681; DOI: 10.1093/nar/gkae681