Potent, Durable mRNA Knockdown in Extrahepatic Tissues Using siRNAs With Novel Phosphoryl Guanidine Backbone Variants

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Forward-looking statements

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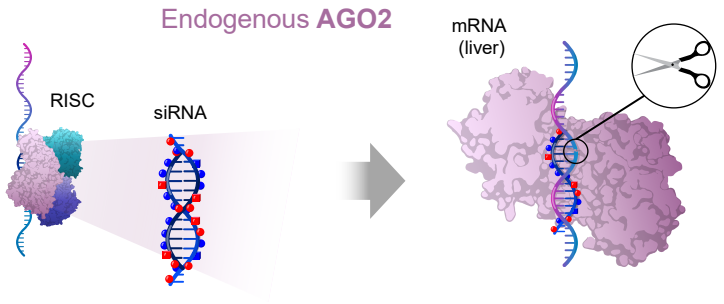
Disclosures

Wei Liu, Naoki Iwamoto, Subramanian Marappan, Himali Shah, Snehlata Tripathi, Erin Purcell-Estabrook, Khoa Luu, Anthony Lamattina, Qianli Pan, Fangjun Liu, Frank Favaloro, Arindom Chatterjee, Tomomi Kawamoto, Genliang Lu, Jake Metterville, Priyanka Shiva Prakasha, Hailin Yang, Yuan Yin, Lola Owen, Hui Yu, Michael Byrne, Pachamuthu Kandasamy, Chandra Vargeese

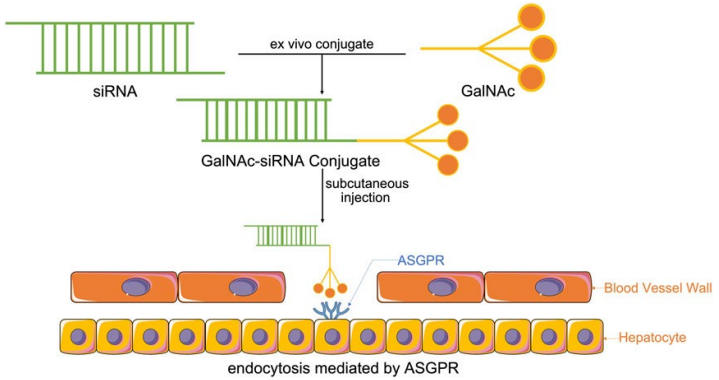
- All authors are employees of Wave Life Sciences

Oligonucleotide-directed gene silencing by RNA interference

siRNA induce mRNA degradation



GalNAc conjugation mediates hepatocyte delivery of siRNA[1]

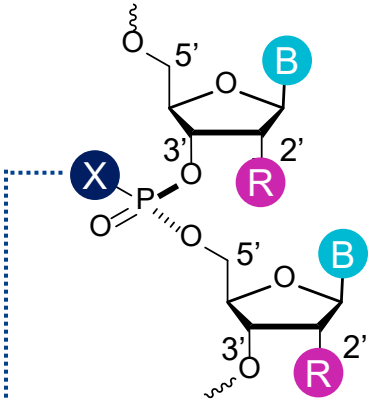


Extrahepatic tissue targeting remains a major challenge for the field

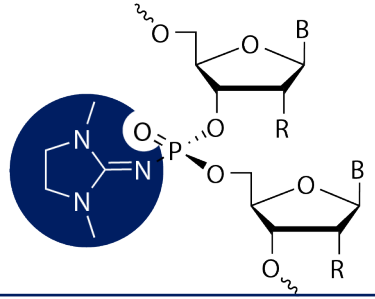
[1]Zhang L, Liang Y, Liang G, Tian Z, Zhang Y, Liu Z and Ji X (2022) Front. Pharmacol. 13:1090237. doi: 10.3389/fphar.2022.1090237. GalNac: N-Acetylgalactosamine

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

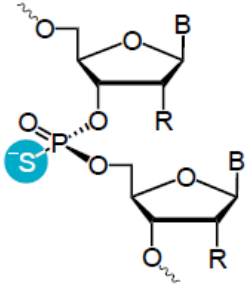
- **B** Base
- **R** 2'-Ribose
- **X** Stereochemistry and backbone modification



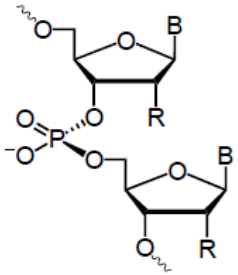
Phosphoryl guanidine (PN-1)



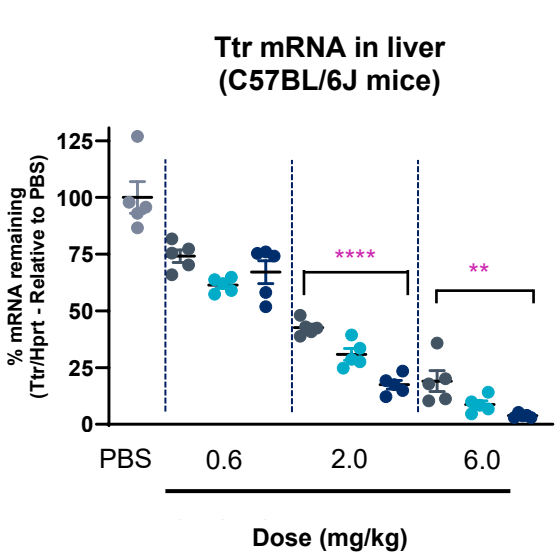
Phosphorothioate (PS)

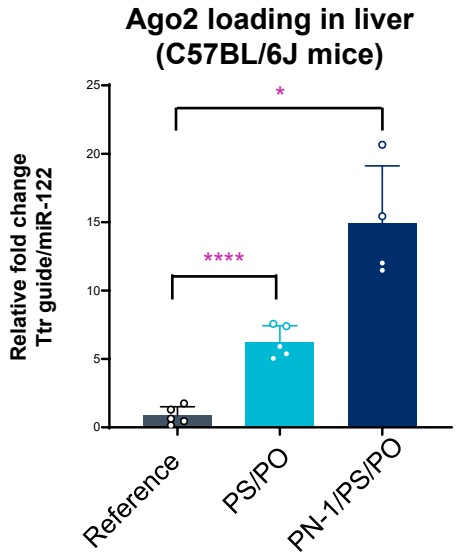


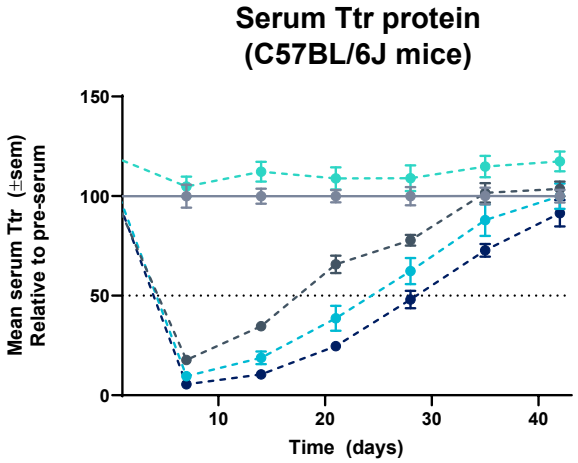
Phosphodiester (PO)



Incorporation of PN chemistry improves GalNAc-siRNA potency and durability in mice



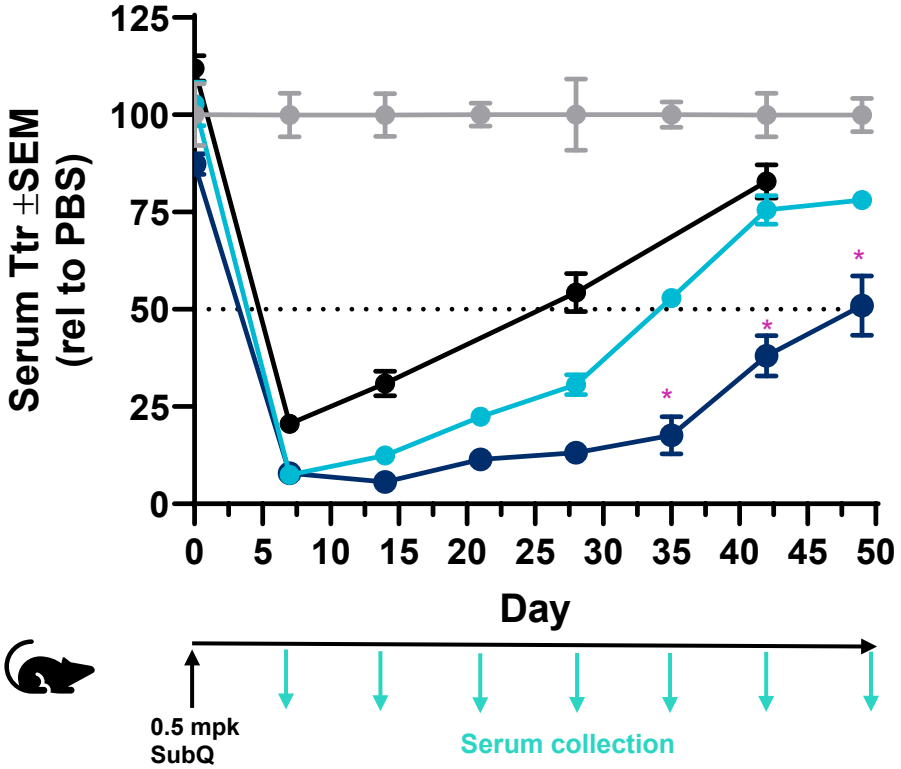




PBS
Reference compound
PS/PO backbone
PS/PO/PN-1 backbone
PS/PO/PN-1 backbone (non-optimal PN-1)

Left, C57BL/6J mice were treated with 0.6, 2, or 6 mg/kg of the indicated 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 * p<0.05, **** P<0.0001. Right: Serum Ttr assayed at time points indicated after 2mg/kg siRNA treatment (day 0). Liu et al., 2023 Nuc Acids Research

Wave's new design for GalNAc-siRNA demonstrates improved durability in mice



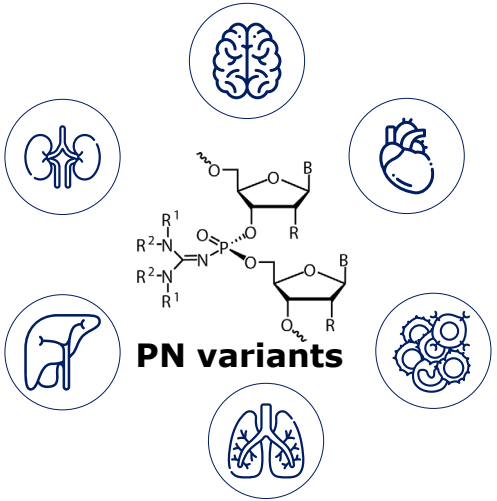
PBS
Reference siRNA[1]
Liu et al. NAR 2023 design siRNA[2]
New design siRNA

[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 Two-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

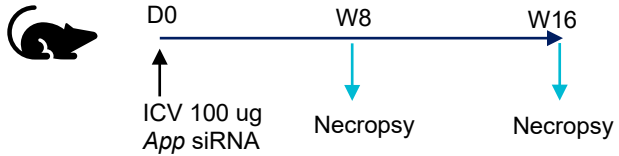
Wave's platform chemistry enables siRNA extrahepatic delivery

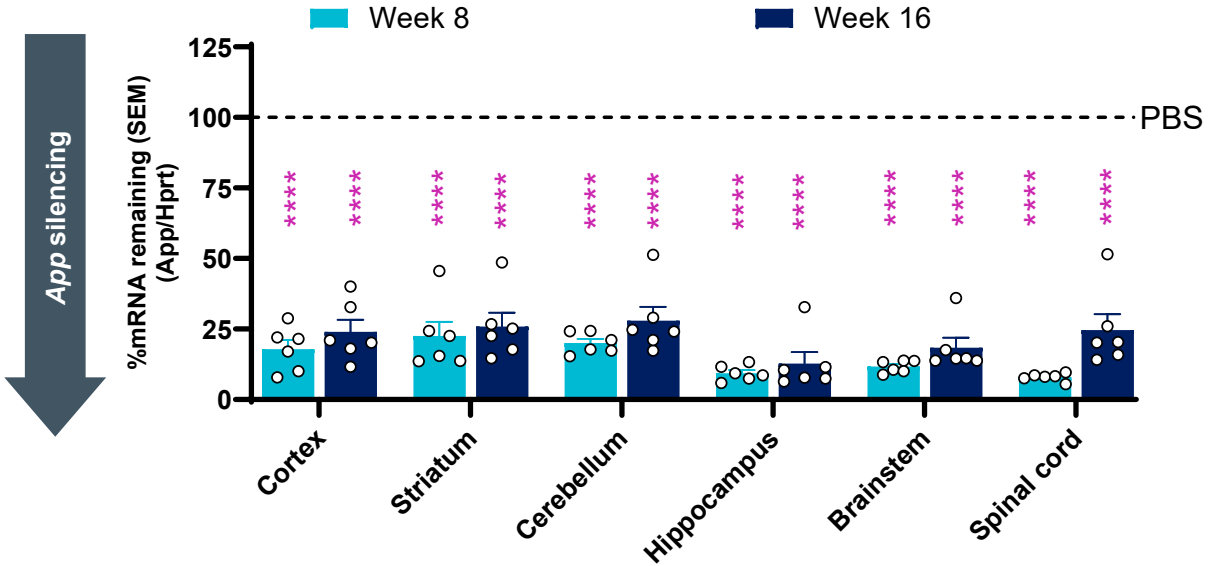
- Chemical impact of PN
  - Introduction of neutral pKa backbone linkages
  - Unique structural feature of PN, specifically guanidine, allows conjugation on oligonucleotide outside of 5' and 3' ends
  - Increased lipophilicity
  - Stereochemistry

- Extra-hepatic delivery
  - Titrating siRNA lipophilicity: tunable PNs (PN variants)
  - Maintaining high Ago2 loading and intracellular trafficking
  - Titrating plasma protein binding
  - Altered delivery, enhanced potency and durability in various tissues



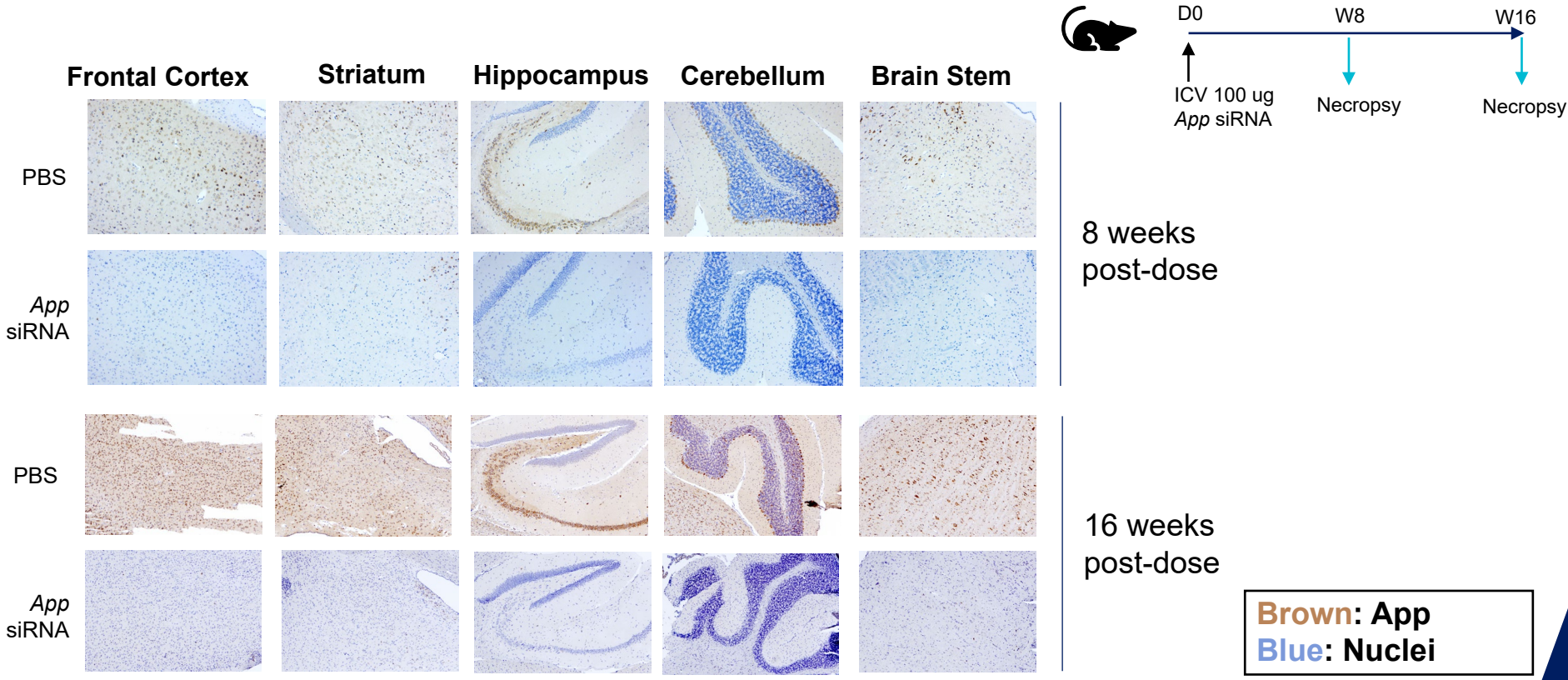
Single dose of siRNA with PN variant 2 (PN-2) linkages delivers broad, potent and durable CNS target engagement in mice





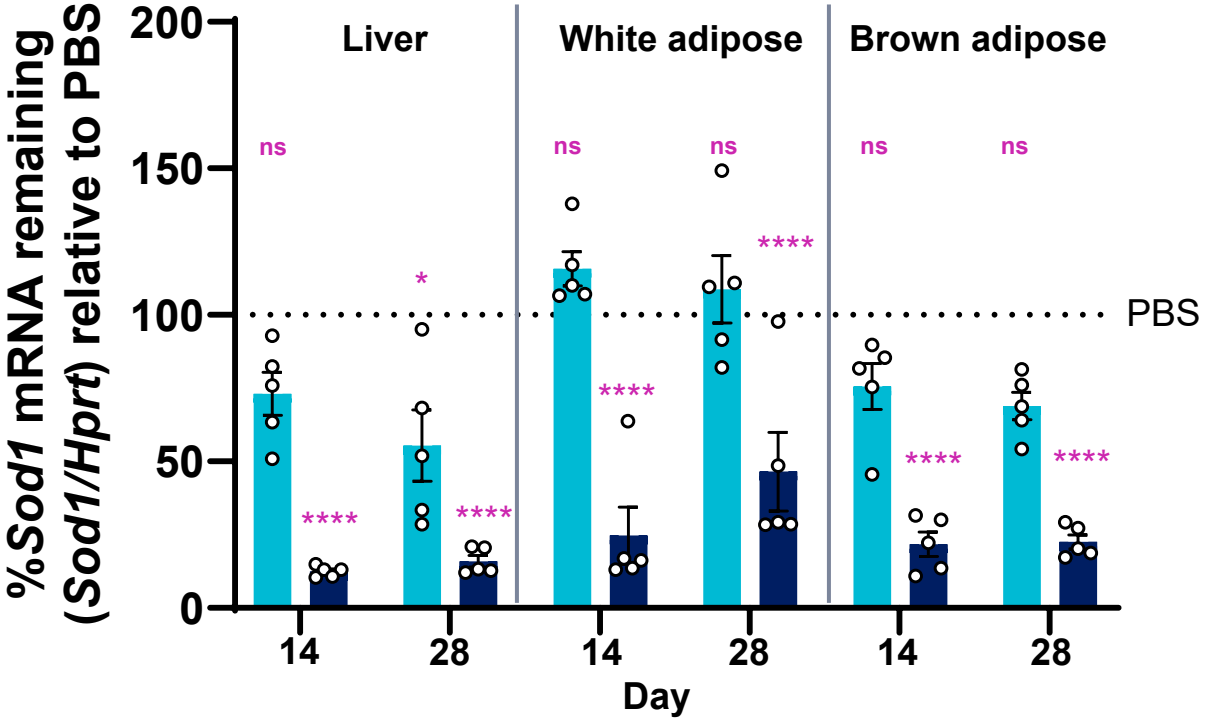
PBS (dotted line) or 100 µg of App siRNA administered ICV (n=7). PCR assays for RNA PD, relative fold changes of *App* to *Hprt* mRNA normalized to % of PBS; Stats: Three-way ANOVA followed by Bonferroni-adjusted post hoc test comparing condition to PBS (data not shown), **** P < 0.0001 compared to PBS.

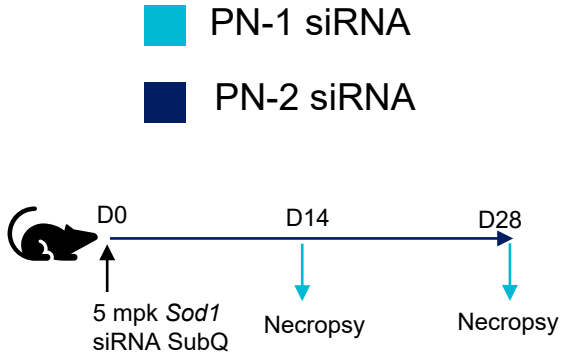
Single dose of siRNA incorporating PN-2 supports durable protein knockdown across the mouse CNS



C57BL/6 mice were administered PBS or a single 100 µg intracerebral ventricular (ICV) injection of siRNA directed against mouse App. Mice were sacrificed at 8 and 16 weeks. Immunohistochemical analysis of fresh frozen paraffin-embedded mouse brain tissue labeling App protein (Brown). Nuclei were counterstained with Hematoxylin (Color Blue). Representative images are shown, magnification 100X.

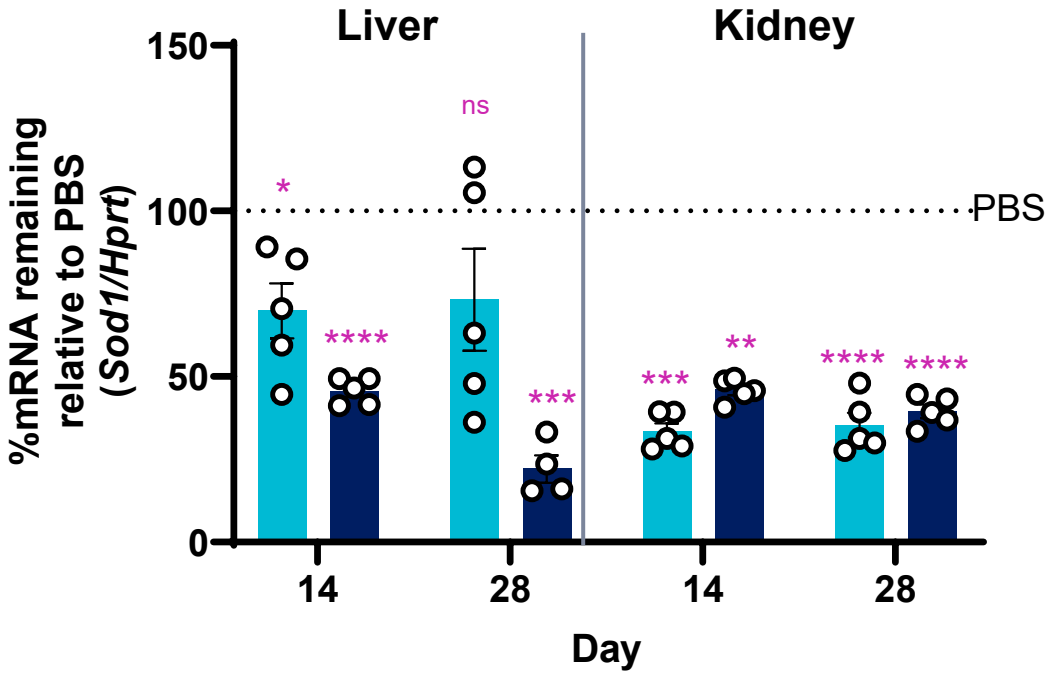
Tunable PN variants enhance single-dose potency and alter extra-hepatic delivery of non-GalNAc siRNA in mice

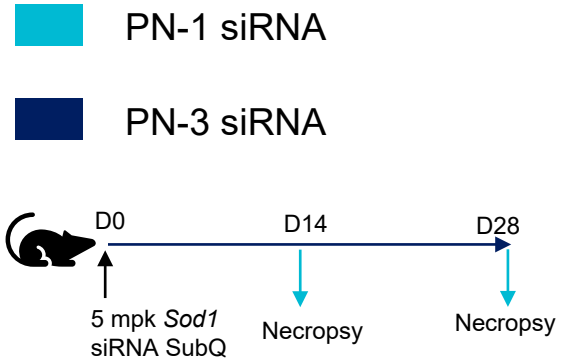




Stats: Data expressed as mean ±SEM, n=5; Three-way ANOVA followed by Bonferroni-adjusted post hoc test comparing condition to PBS (data not shown) * P < 0.05, **** P < 0.0001, ns nonsignificant; B6 mice administered PBS or 5 mg/kg of Sod1 siRNA (no GalNAc conjugate) subcutaneous injection (n=7). Taqman qPCR assays used for RNA PD, relative fold changes of *Sod1* to *Hprt* mRNA normalized to % of PBS group.

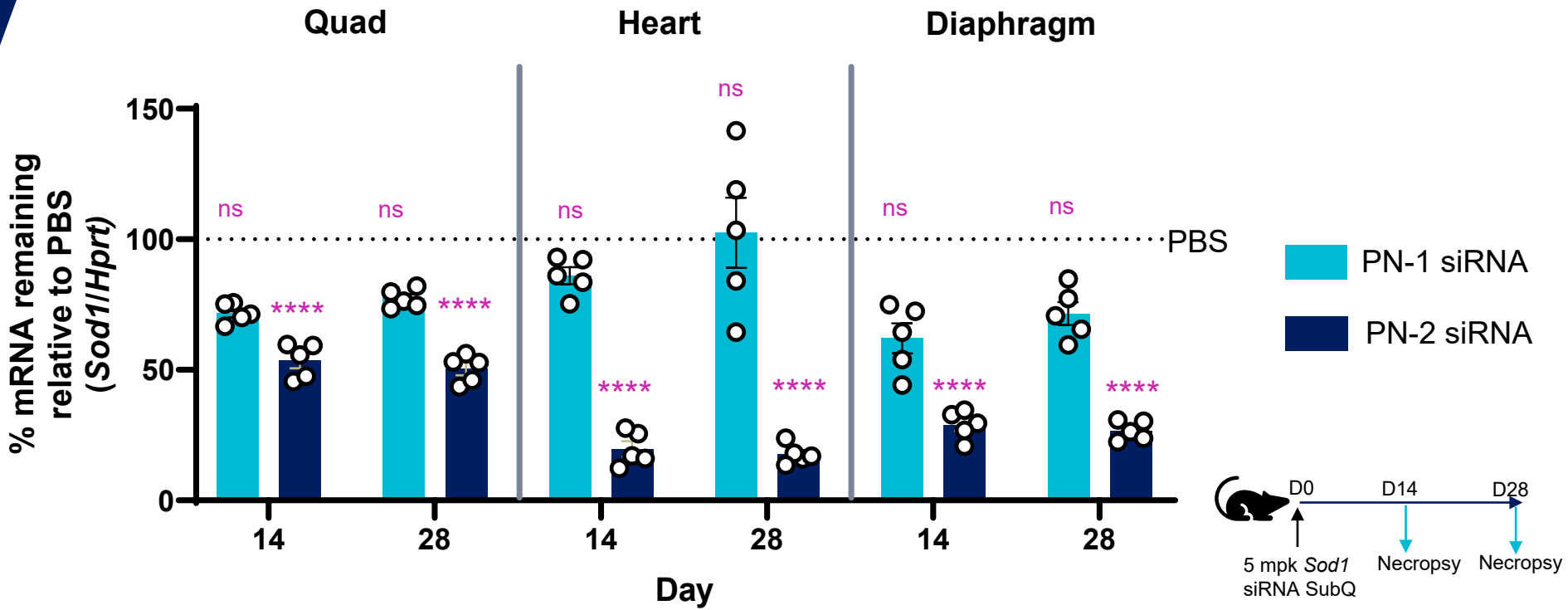
Tunable PN variants alter tissue delivery and target engagement of non-GalNAc siRNA after a single dose in mice





B6 mice administered PBS or 5 mg/kg of Sod1 siRNA by subcutaneous injection on day 0 (n=5) and euthanized 14 or 28 days after the administration. Taqman qPCR assays were used for RNA PD; relative fold changes of *Sod1* to *Hprt* mRNA were normalized to the percentage of PBS group. PBS values represented by dashed line; PBS data not shown. Stats: n=5, data presented as mean ± SEM ;Three-way white-adjusted ANOVA followed by Bonferroni-adjusted post hoc tests vs. PBS per tissue/day allowing unequal variance * P < 0.05, ** P < 0.01, *** P < 0.001, **** P < 0.0001; ns non-significant

Tunable PN variants enhance single-dose siRNA potency in mouse heart and muscle



B6 mice were administered PBS or 5 mg/kg of Sod1 siRNA (no GalNAc conjugate) by subcutaneous injection on day 0 (n=5). Mice were euthanized 14 or 28 days after the administration. Taqman qPCR assays were used for RNA PD, the relative fold changes of *Sod1* to *Hprt* mRNA were normalized to the percentage of PBS group. Data presented as mean ± SEM. PBS values represented by dashed line; PBS data not shown. Stats: Three-way ANOVA (treatment, tissue, day) followed by Bonferroni-adjusted post hoc tests vs. PBS per tissue/day **** P < 0.0001, ns nonsignificant.

Summary

- PRISM™, our discovery and drug development platform, enables the development of a new siRNA chemistry design that improves durability and leverages the chemical flexibility of PN linkages to improve potency and enhance extra-hepatic delivery in mice.
- Applying the new stereopure design to our GalNAc-siRNA conjugate improves knockdown durability in the mouse liver.
- An siRNA incorporating one PN variant supported potent, durable knockdown of mRNA and protein across mouse CNS tissues up to 16 weeks post single intracerebral ventricular (ICV) injection.
- PN variant linkages, which titrate siRNA lipophilicity, impact delivery, potency, and durability in various tissues.

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