A novel Centyrin:siRNA targeting and delivery platform inhibits glycogen synthesis and reduces glycogen levels in skeletal and cardiac muscle in a mouse model of Pompe disease

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Philadelphia PA

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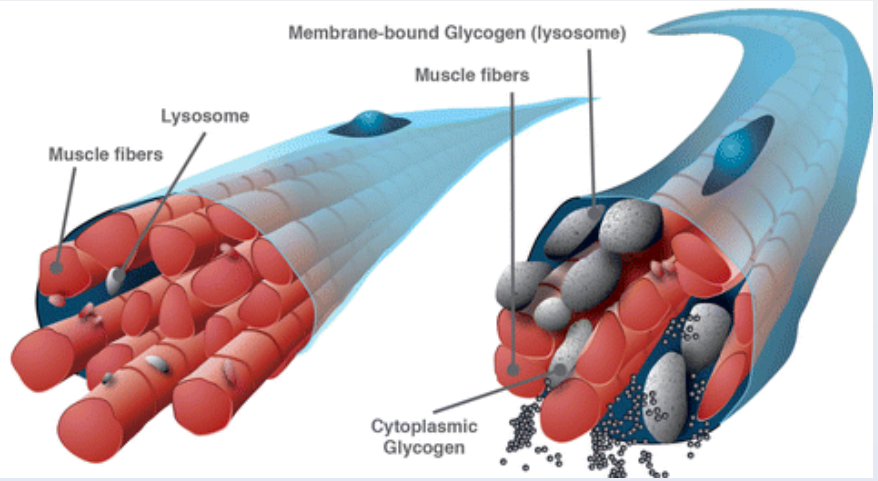
Pompe disease is a devastating rare neuromuscular disorder resulting from LOF mutations in acid alpha glucosidase (GAA)



Normal, Healthy Muscle

Pompe muscle with glycogen buildup in lysosomes

Symptoms include:

- Progressive muscle weakness
- Loss of mobility
- Respiratory distress
- Loss of independent ventilation
- Cardiomyopathy (infants only)

Approved Enzyme Replacement Therapy (ERT) is the standard of care; poor muscle uptake and immunogenicity limit efficacy

Alglucosidase alfa | Avalglucosidase alfa-ngpt | AT-GAA

Limited efficacy and durability

Black box warnings – anaphylaxis risk

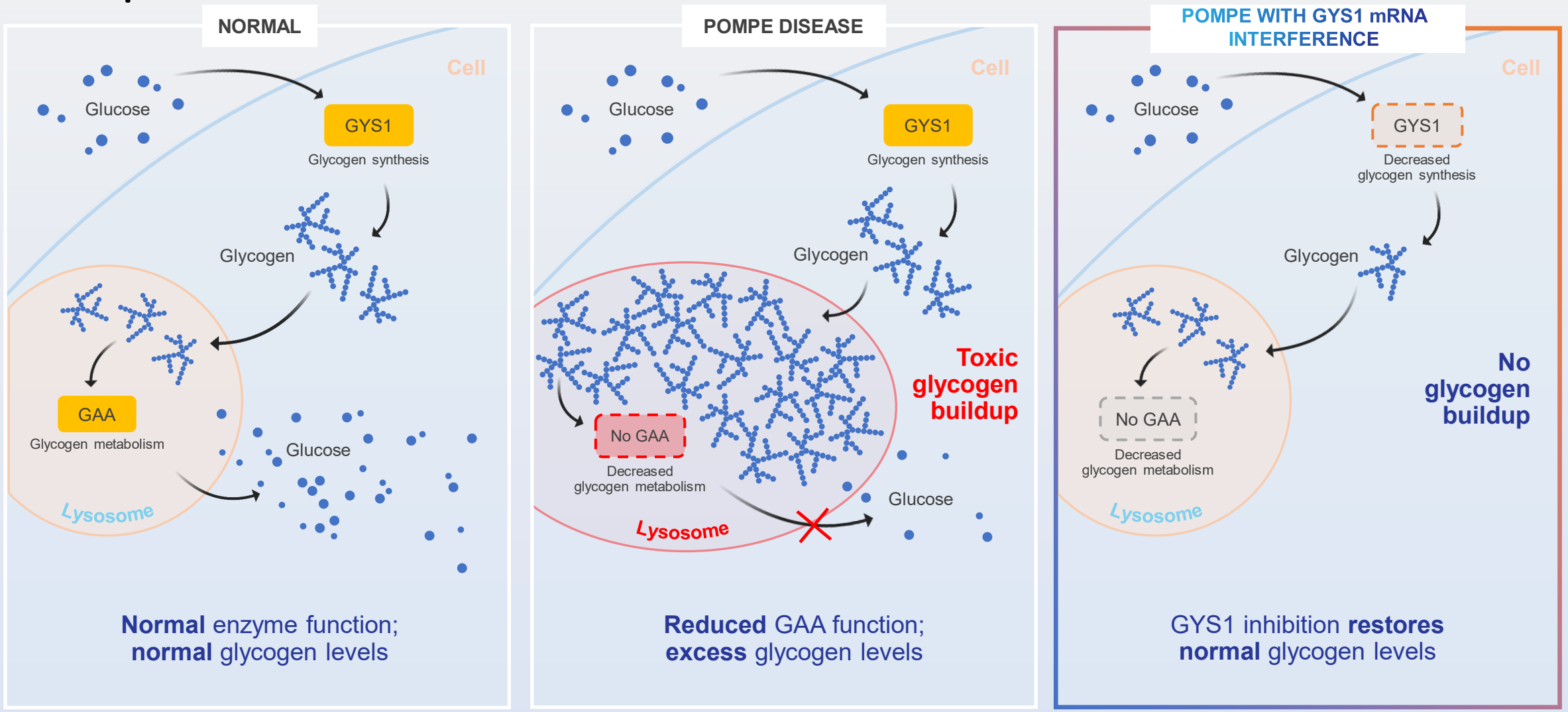
Patient inconvenience – biweekly long infusions

~7K DTx patients in US and EU-5

Newborn Screening driving increased DTx rate

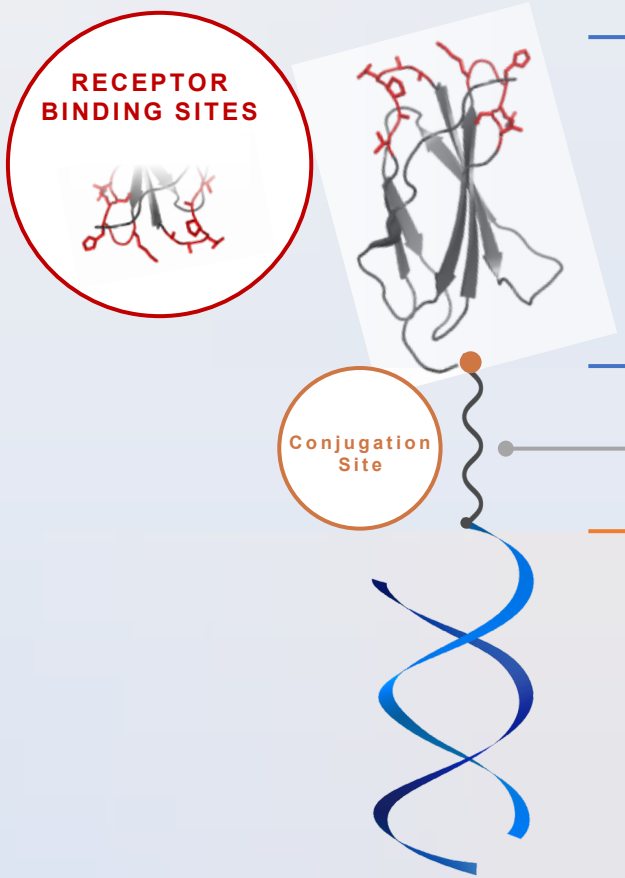
$1B+ growing market

Gys1 inhibition is a new approach to reducing toxic glycogen in Pompe disease



CD71 Centyrins* target siRNAs to tissues via receptor binding & internalization

CENTYRIN siRNA CONJUGATE



COMPONENTS

Centyrin
Receptor binding and internalization enables targeting to specific cells

CD71 (transferrin type 1 receptor) Centyrin enables muscle targeting

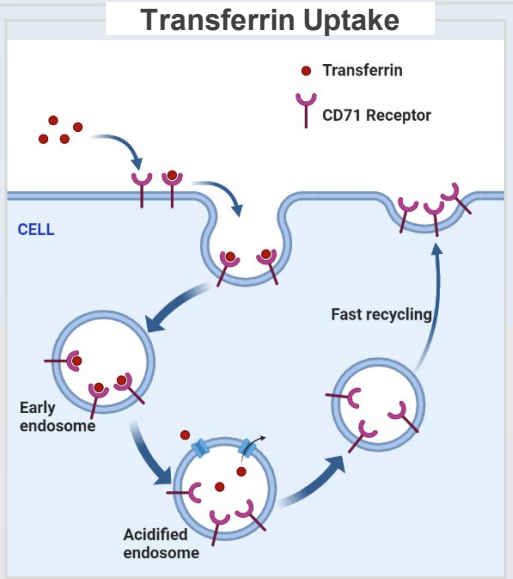
Linker
Clinically validated and non cleavable; attached at single conjugate site

siRNA
Inhibits production of target mRNA

Gys1 siRNA inhibits production of Gys1 enzyme required for glycogen synthesis

KEY FEATURES

- Small protein with high affinity / selectivity
- Improved therapeutic window and simplified manufacturing vs. mAbs



- Specific and highly potent
- Long PD effect

*Aro's proprietary platform for delivering oligonucleotides

Experimental design

- Mice
  - Disrupted *Gaa* ($6^{neo}/6^{neo}$)
  - WT on same genetic background
  - 12hr light/dark cycle
  - Food and water ad libitum

GAA activity was measured under standard conditions, pH 4.3 (15).

Tissue	Genotype		
	+/+	$6^{neo}/+$	$6^{neo}/6^{neo}$
Heart	10.6 ± 0.69	4.7 ± 0.5	0.11 ± 0.01
Muscle	17.2 ± 1.4	10.4 ± 1.2	0.11 ± 0.03
Brain	57.0 ± 4.5	16.4 ± 2.4	0.62 ± 0.04

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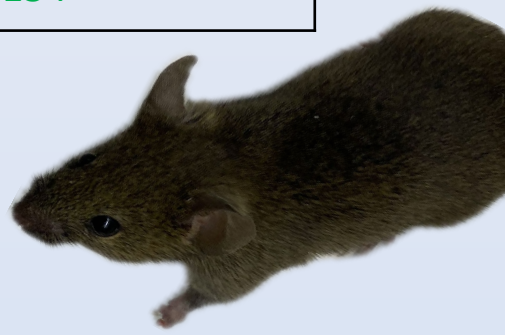
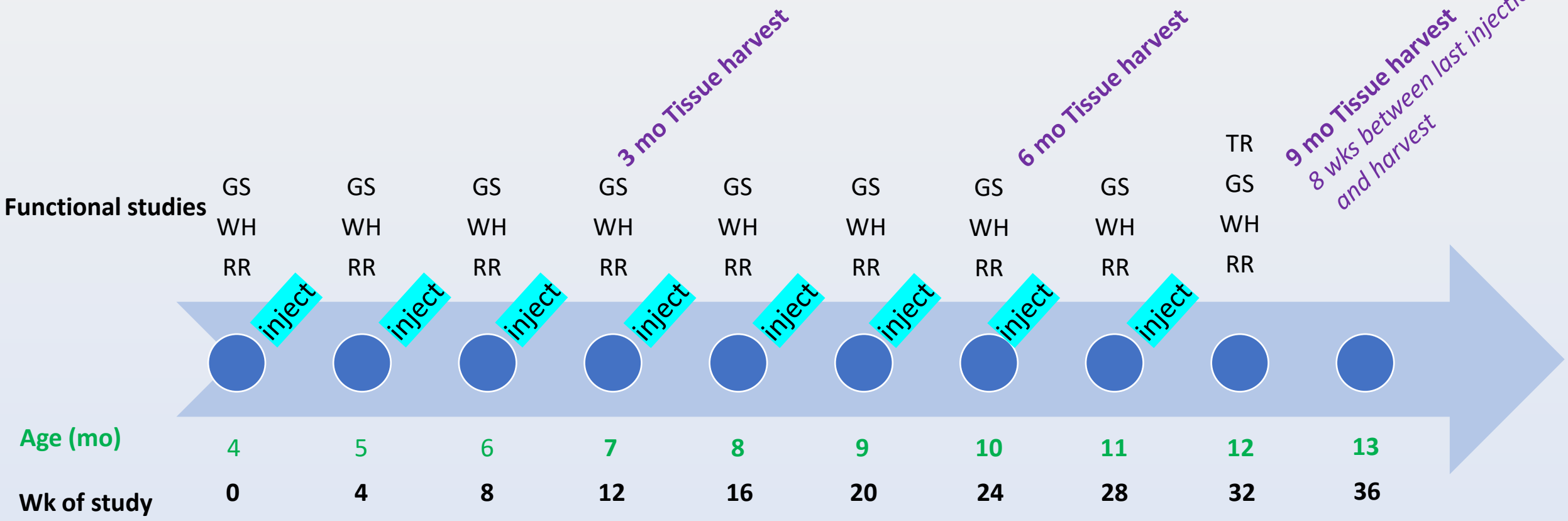
The Gaa KO represents the most severe Pompe patient phenotype

Treatment group	V1	V2	29 (active)	73 (Negative control)
Genotype	WT	*Gaa* $^{-/-}$	*Gaa* $^{-/-}$	*Gaa* $^{-/-}$
Treatment	Vehicle	Vehicle	ABXC-29 (*Gys1* murine siRNA conjugate)	ABXC-73 (scrambled siRNA conjugate)
Sample size	15 M, 15 F	9 M, 9 F	15 M, 15 F	15 M, 15 F

- Dosing
  - Four treatment groups with both M & F mice
  - Beginning at 4 months of age, treatment administered (via tail vein) every 28 days



Experimental timeline



Functional studies

Grip strength (GS)
Wirehang (WH)
Rotarod (RR)
Treadmill (TR)

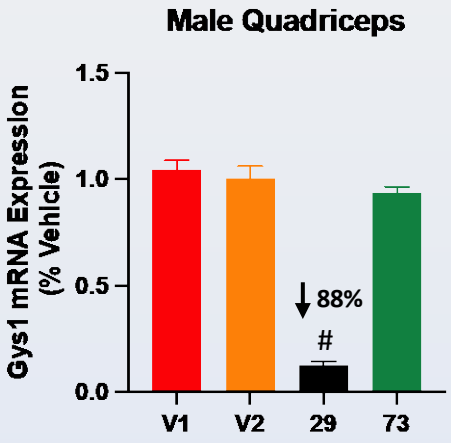
Tissue measurements

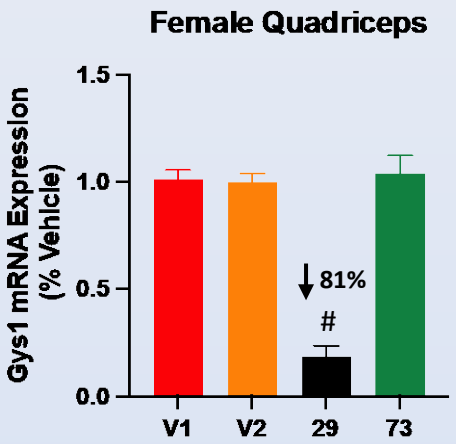
Glycogen concentration
Glycogen synthase enzymatic activity
GYS1 protein expression
GYS1 mRNA expression

ABXC-29 reduced *Gys1* mRNA levels in muscle (6 mo)

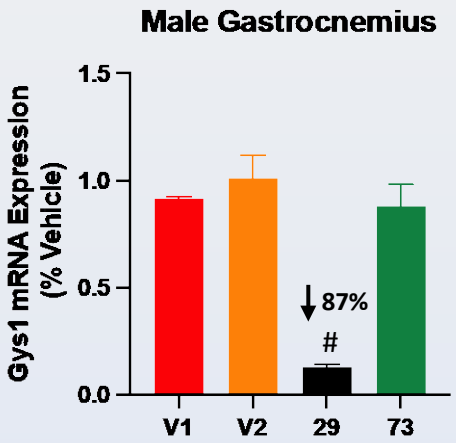
V1=Wildtype vehicle
V2=Pompe vehicle
29=Pompe Active
73=Pompe Neg. CTRL

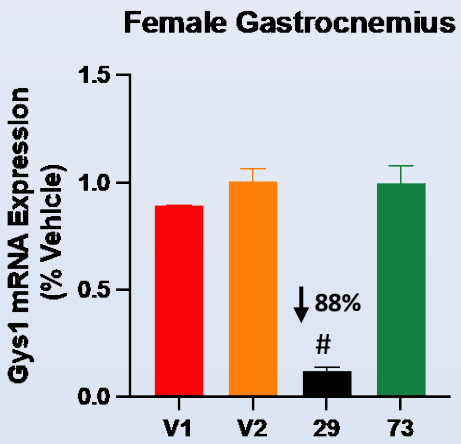
Quadriceps



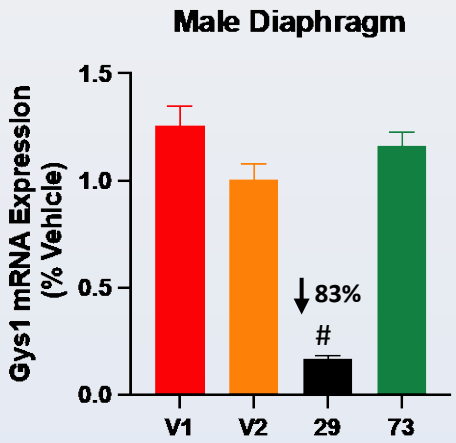


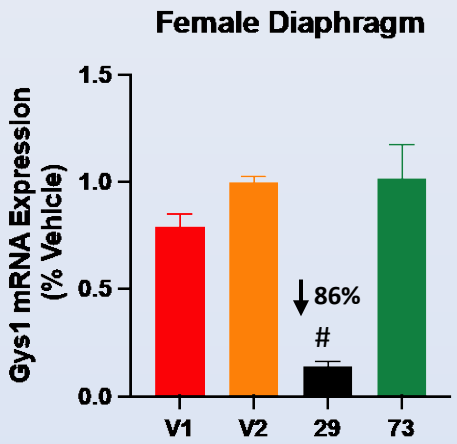
Gastrocnemius



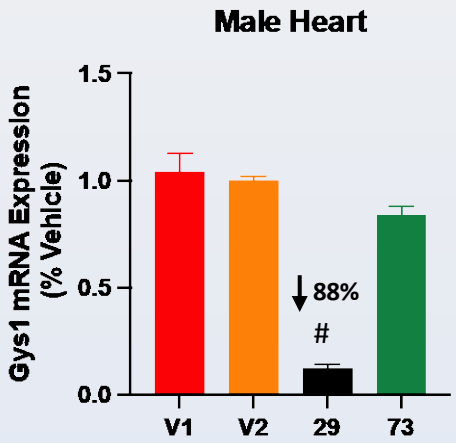


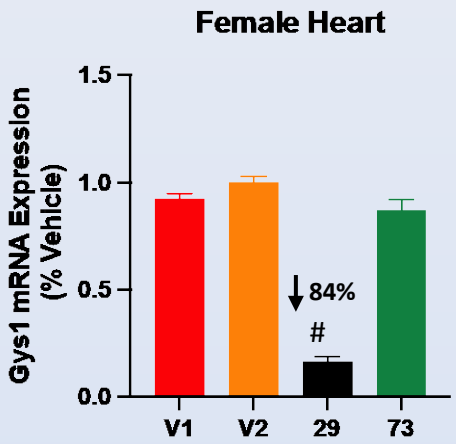
Diaphragm





Heart



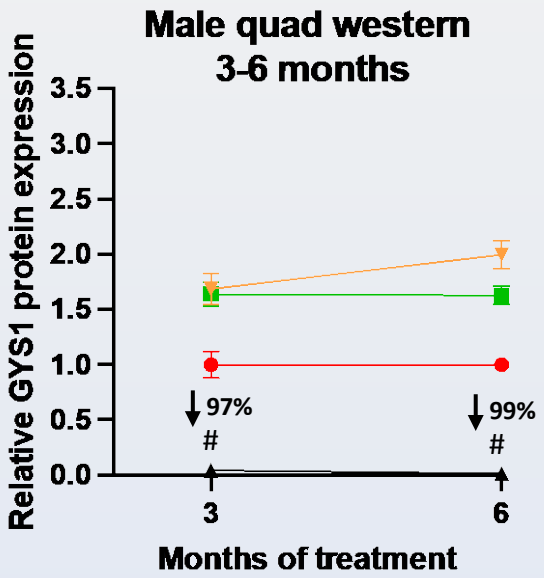


n=3
#p<0.05 vs V2

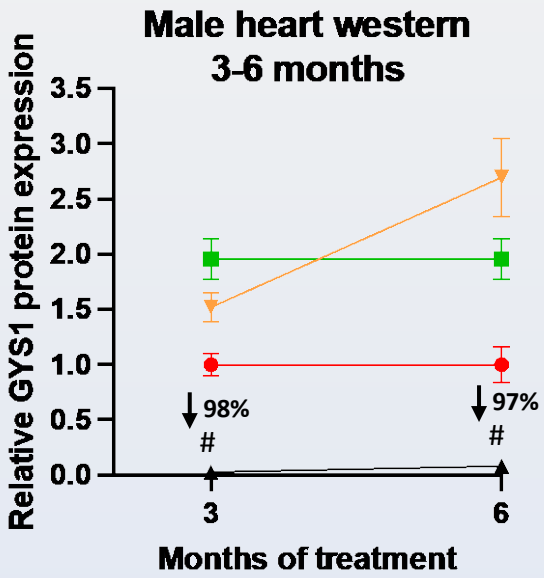
ABXC-29 reduced GYS1 protein levels in muscle

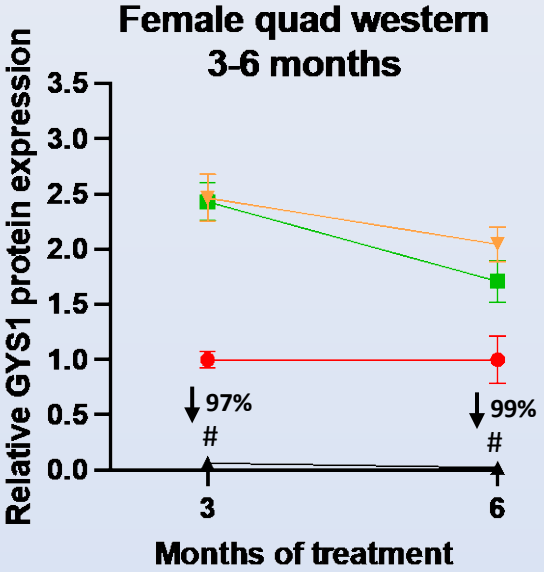
V1=Wildtype vehicle
V2=Pompe vehicle
29=Pompe Active
73=Pompe Neg. CTRL

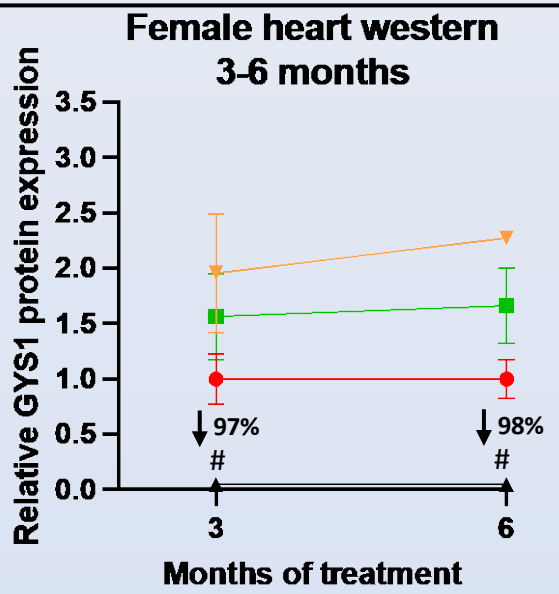
Quadriceps



Heart







n=3-5
#p<0.05 vs V2

ABXC-29 reduced GYS1 enzymatic activity levels in muscle

V1=Wildtype vehicle
V2=Pompe vehicle
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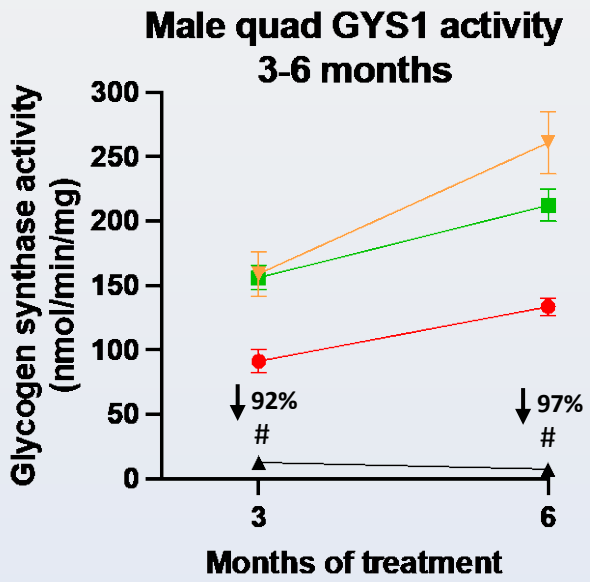
73=Pompe Neg. CTRL

Quadriceps



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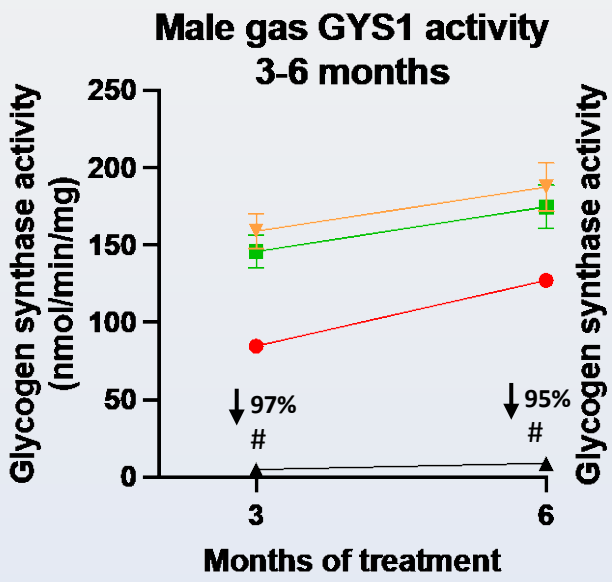
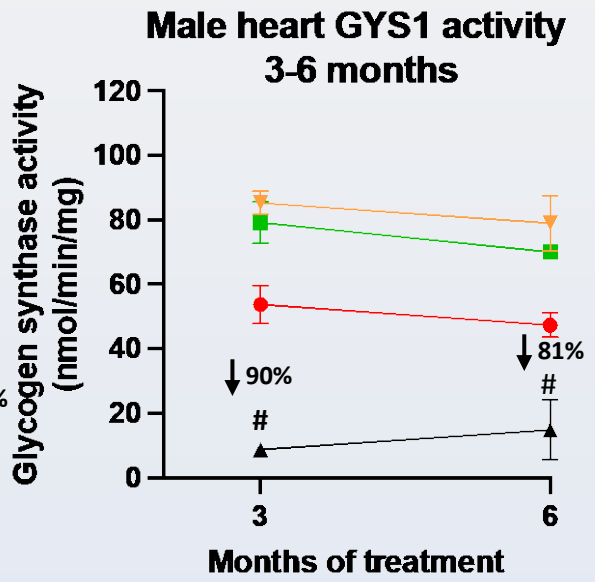
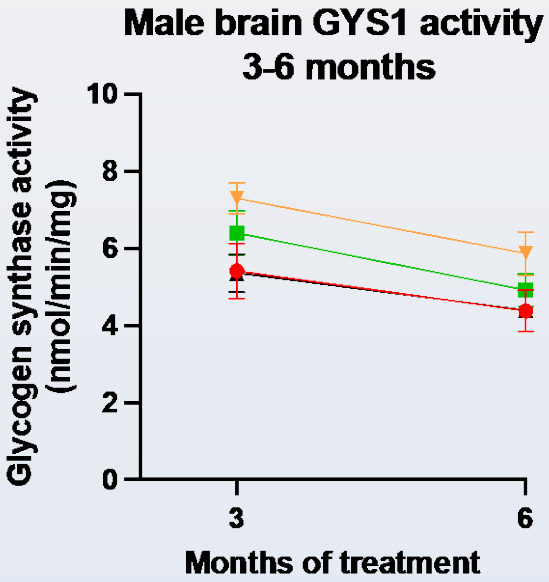
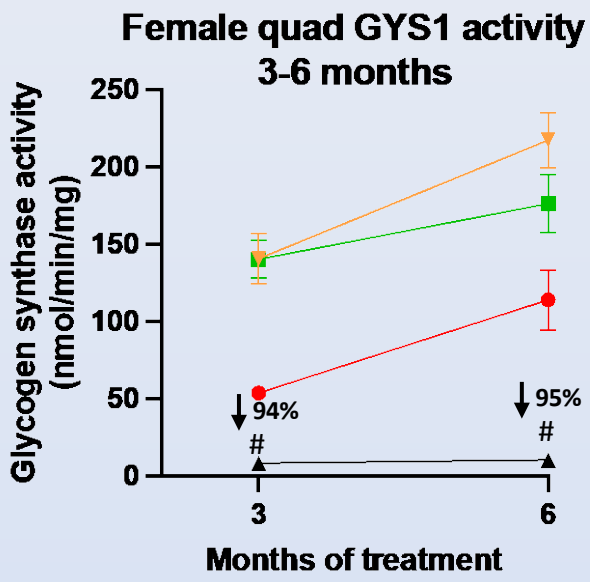


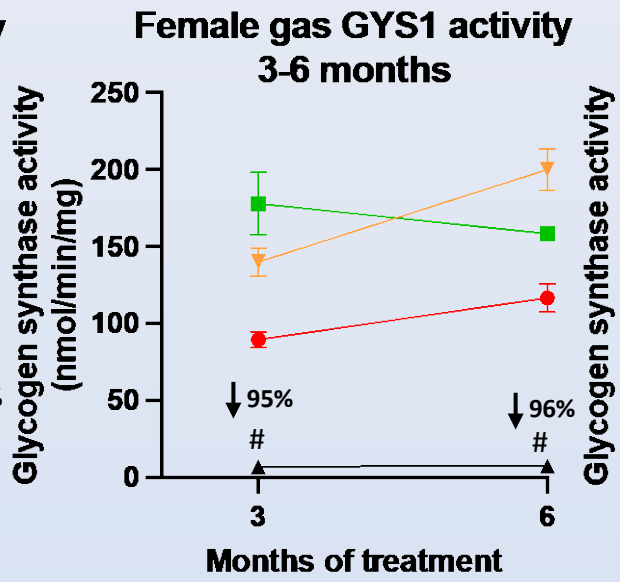
Heart

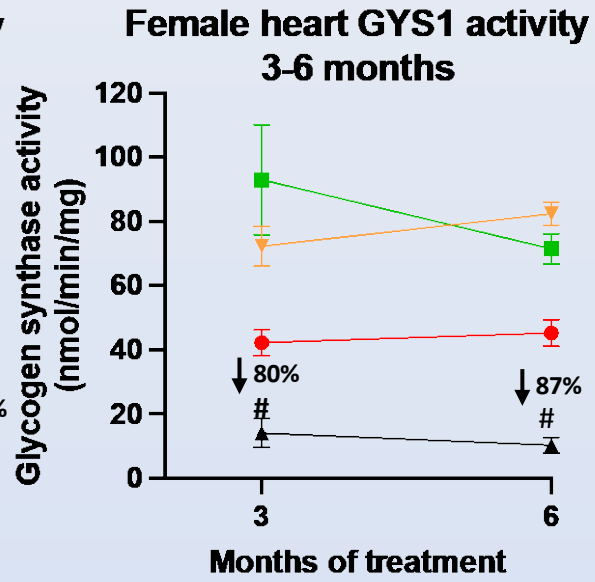


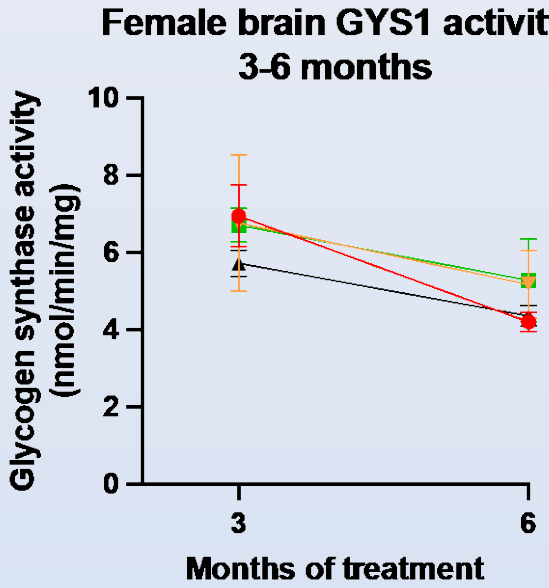
Brain











n=3-5
#p<0.05 vs V2

ABXC-29 reduced glycogen levels in muscle

-Biochemical measure of glycogen appears more sensitive vs histology

V1=Wildtype vehicle
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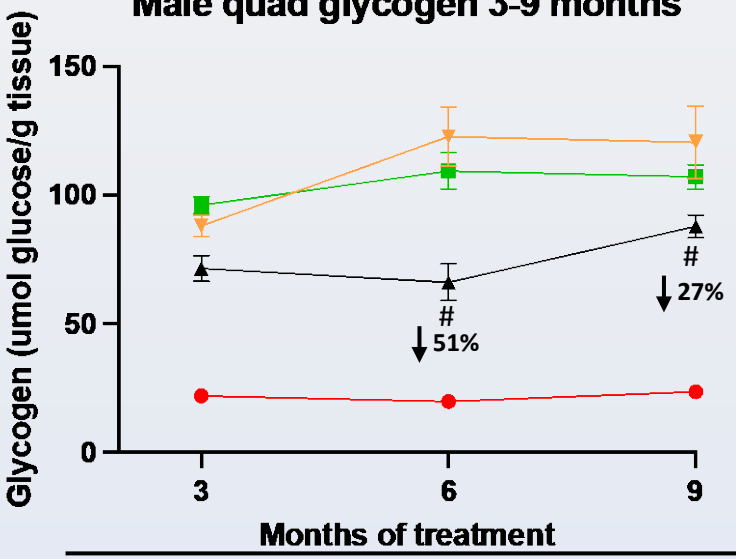
73=Pompe Neg. CTRL

Quadriceps



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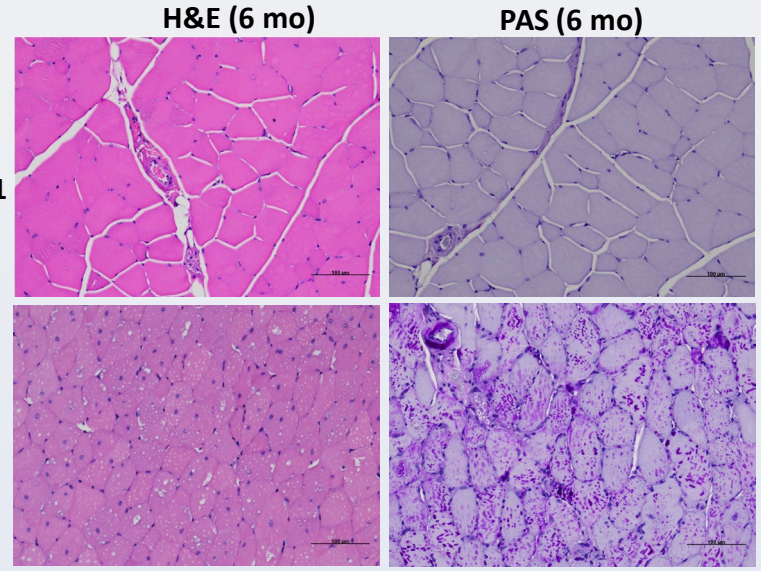
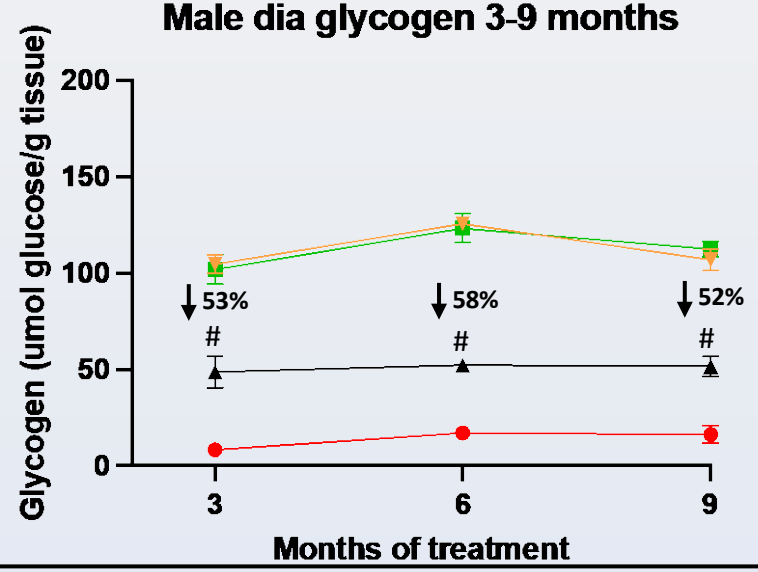
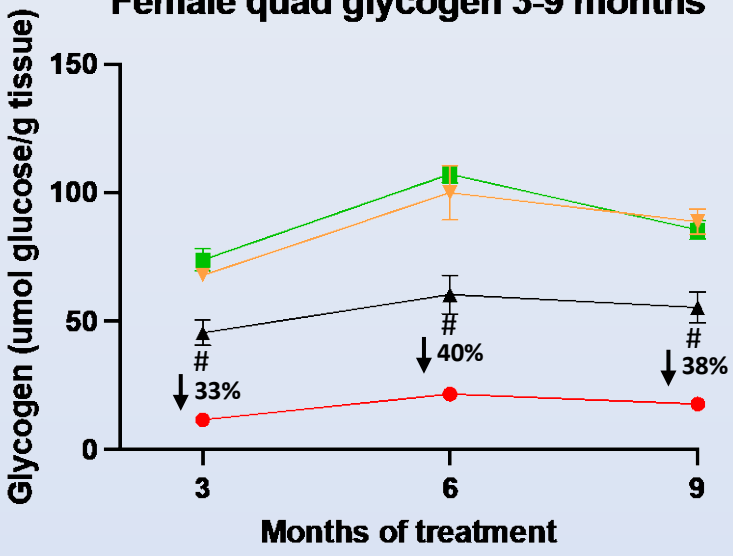
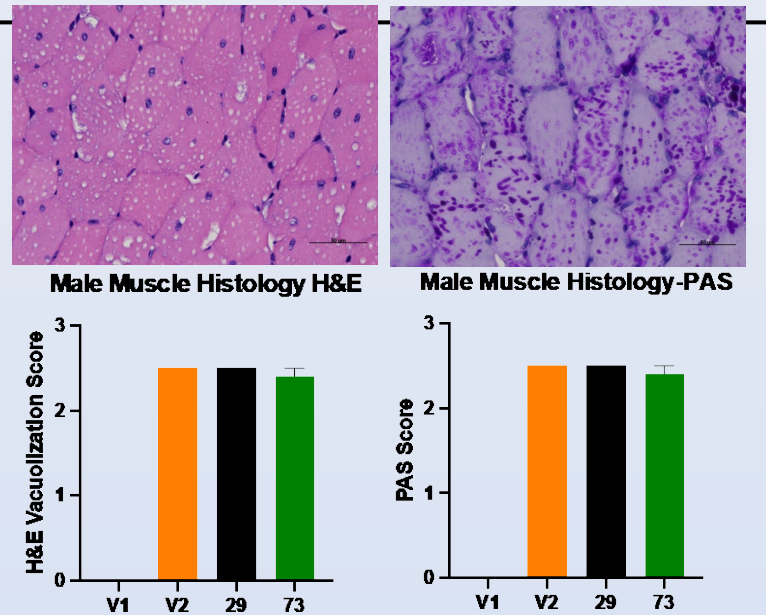
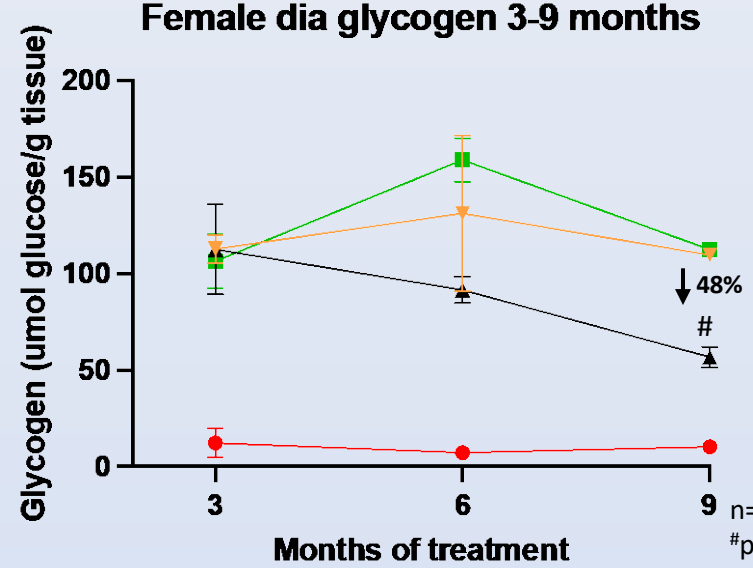


Diaphragm







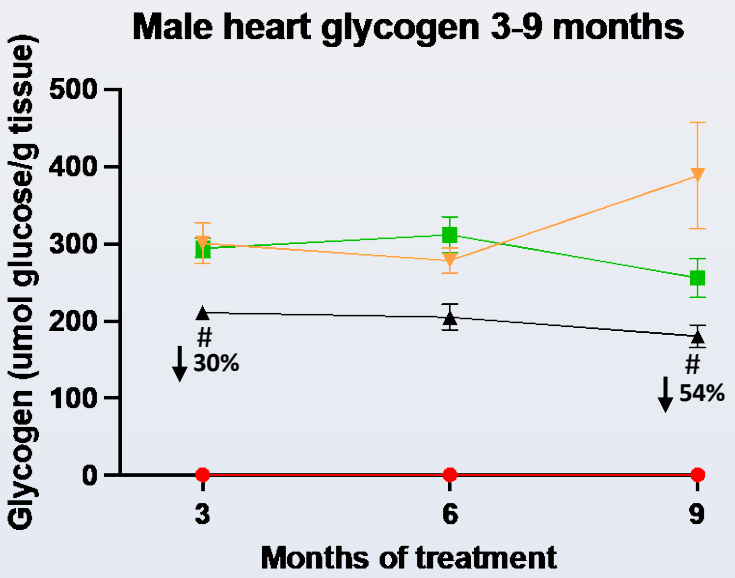


n=3-5
#p<0.05 vs V2

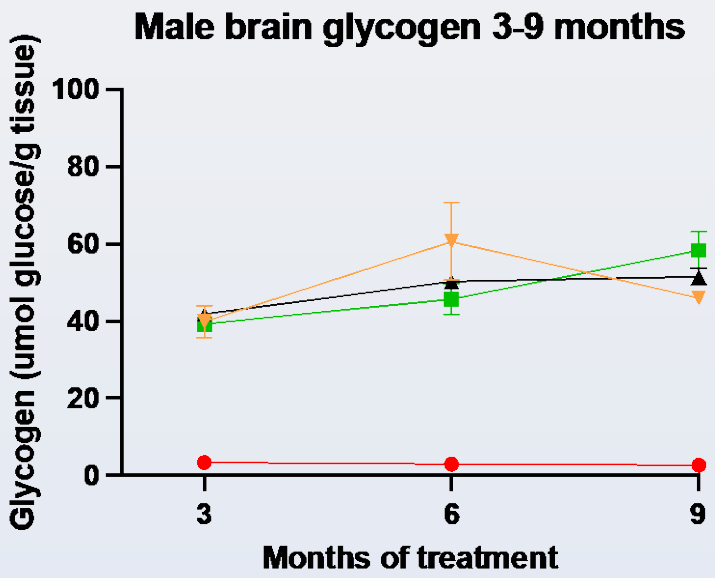
ABXC-29 reduced glycogen levels in heart but not brain or liver

V1=Wildtype vehicle
V2=Pompe vehicle
29=Pompe Active
73=Pompe Neg. CTRL

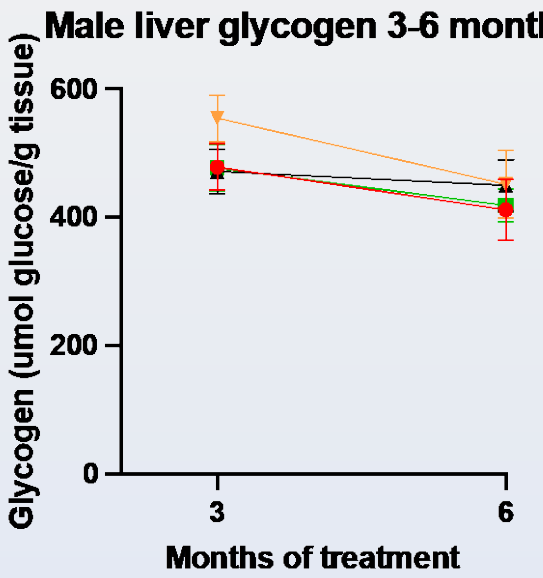
Heart

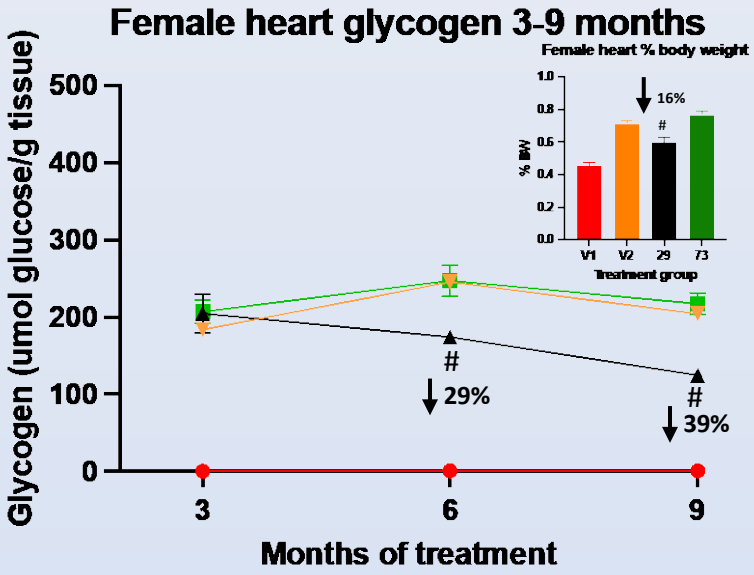


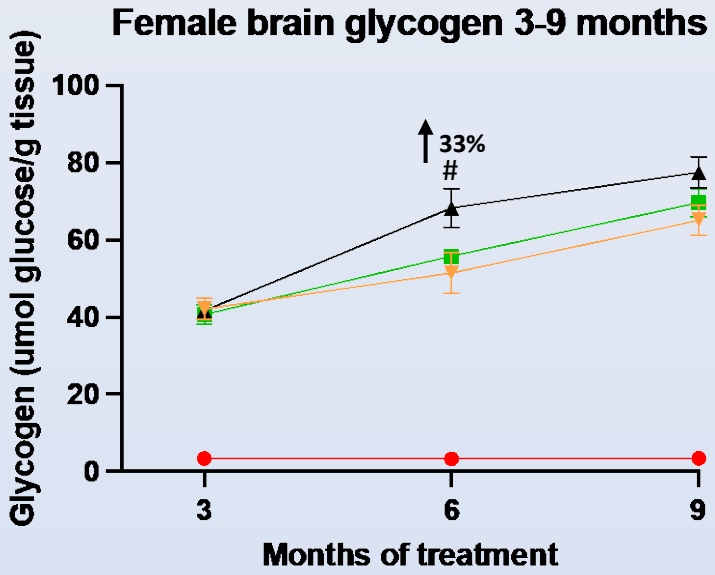
Brain

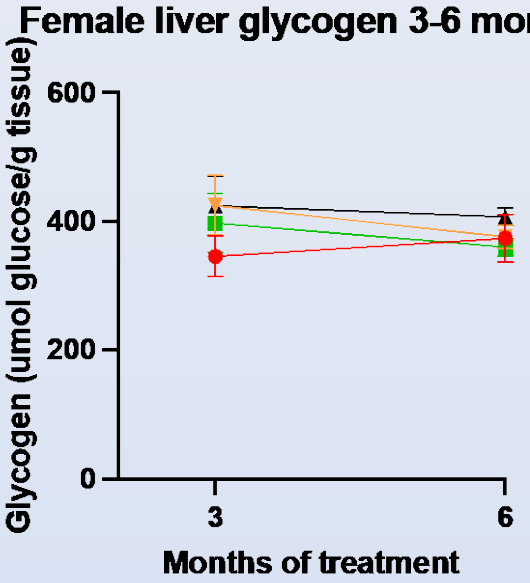


Liver





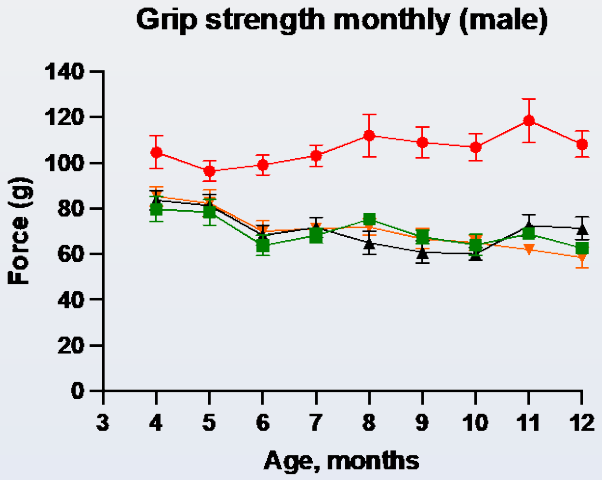


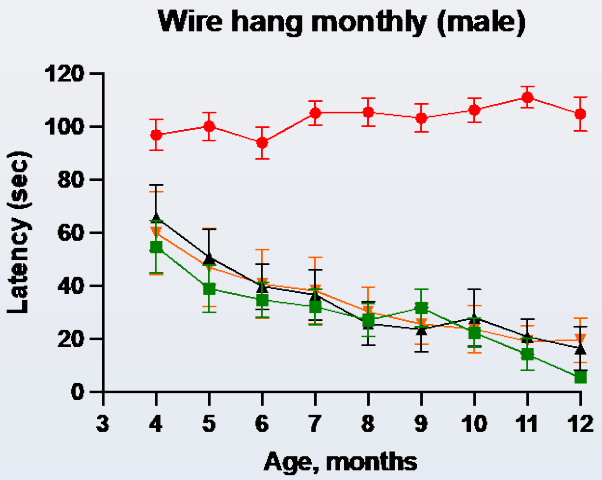


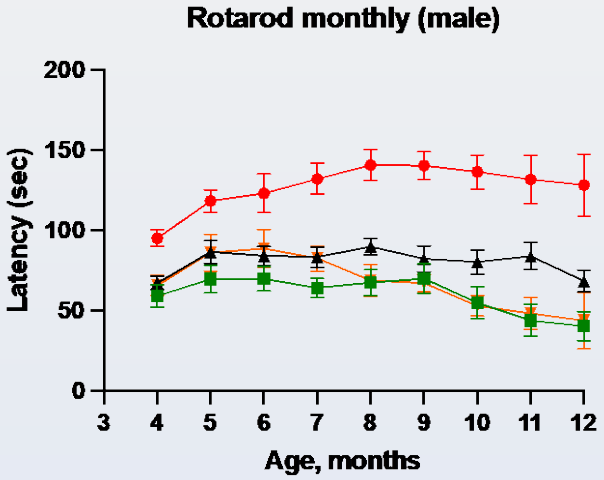
n=3-5
#p<0.05 vs V2

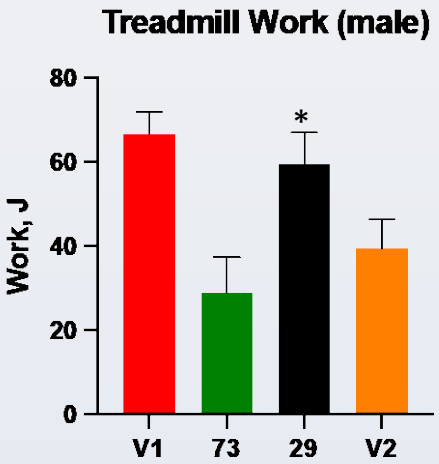
ABXC-29 does not mitigate GS, WH, or RR impairment but improves male treadmill performance

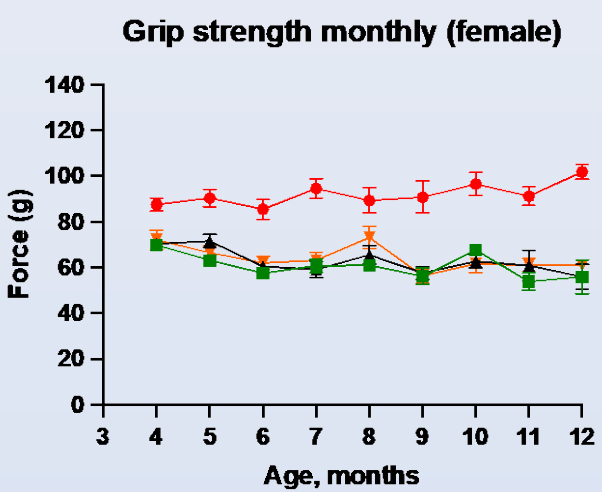
V1=Wildtype vehicle
V2=Pompe vehicle
29=Pompe Active
73=Pompe Neg. CTRL

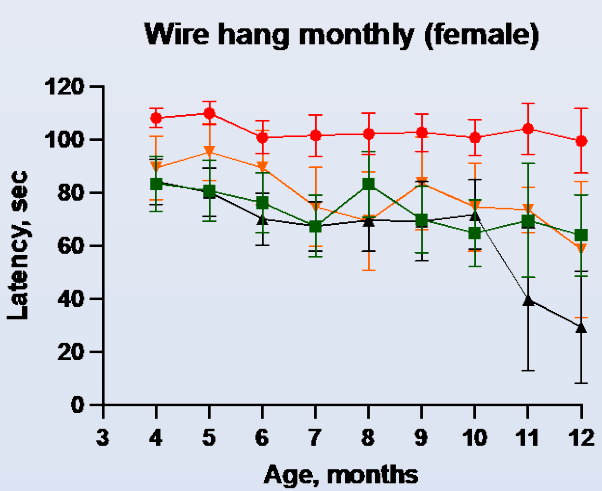


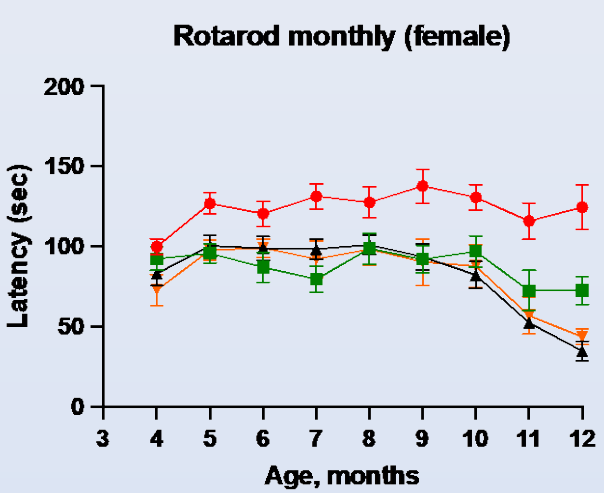


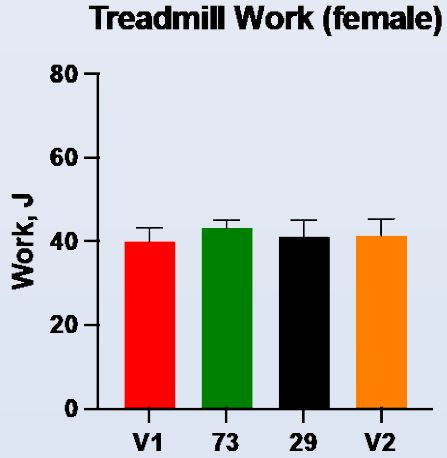












n=3-15
*p<0.05 vs 73

Summary

A Centyrin:*Gys1* siRNA conjugate is a promising modality for the treatment of patients with Pompe disease

- ABXC-29 reduced (≥80%) *Gys1* mRNA, GYS1 protein expression, and GYS1 enzymatic activity in skeletal and cardiac muscle from mice with Pompe disease
- ABXC-29 reduced (~30-60%) glycogen concentration in skeletal and cardiac muscle but not brain or liver from mice with Pompe disease
  - Time-, tissue- and sex-specific differences were observed
- ABXC-29 mitigated Pompe-disease associated impairment of treadmill performance in male mice
- ABXC-29 reduced cardiomegaly in female mice with Pompe disease

Acknowledgments

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