# Silencing *Inhbe* With a GalNAc-SpiNA siRNA Drives Tissue Remodeling and a Healthy Metabolic Profile

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SVP, Translational Medicine, Wave Life Sciences

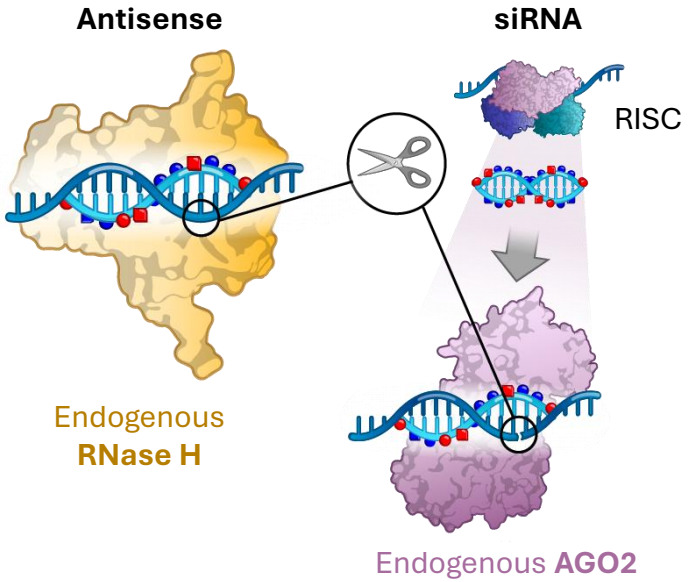
# Forward-looking statements

This document contains forward-looking statements. All statements other than statements of historical facts contained in this document, including statements regarding possible or assumed future results of operations, preclinical and clinical studies, business strategies, research and development plans, collaborations and partnerships, regulatory activities and timing thereof, competitive position, potential growth opportunities, use of proceeds and the effects of competition are forward-looking statements. These statements involve known and unknown risks, uncertainties and other important factors that may cause the actual results, performance or achievements of Wave Life Sciences Inc. (the "Company") to be materially different from any future results, performance or achievements expressed or implied by the forward-looking statements. In some cases, you can identify forward-looking statements by terms such as "may," "will," "should," "expect," "plan," "aim," "anticipate," "could," "intend," "target," "project," "contemplate," "believe," "estimate," "predict," "potential" or "continue" or the negative of these terms or other similar expressions. The forward-looking statements in this presentation are only predictions. The Company has based these forward-looking statements largely on its current expectations and projections about future events and financial trends that it believes may affect the Company's business, financial condition and results of operations. These forward-looking statements speak only as of the date of this presentation and are subject to a number of risks, uncertainties and assumptions, including those listed under Risk Factors in the Company's Form 10-K and other filings with the SEC, some of which cannot be predicted or quantified and some of which are beyond the Company's control. The events and circumstances reflected in the Company's forward-looking statements may not be achieved or occur, and actual results could differ materially from those projected in the forward-looking statements. Moreover, the Company operates in a dynamic industry and economy. New risk factors and uncertainties may emerge from time to time, and it is not possible for management to predict all risk factors and uncertainties that the Company may face. Except as required by applicable law, the Company does not plan to publicly update or revise any forward-looking statements contained herein, whether as a result of any new information, future events, changed circumstances or otherwise.

# Wave Life Sciences' best-in-class multimodal platform

## Silencing

Degradation of RNA transcripts to **turn off** protein production[1,2]

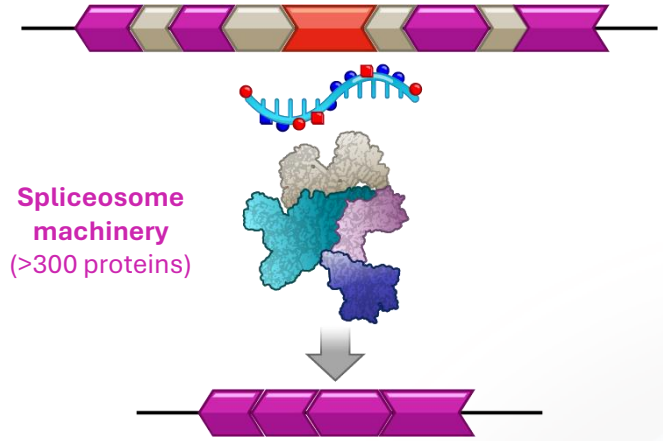



**WVE-003** (HD)

**WVE-007** (obesity)

## Splicing

Restoration of RNA transcripts to **turn on** protein production[3]

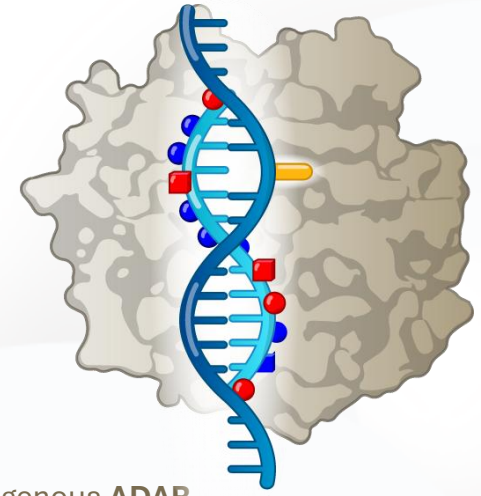



Restored **reading frame** for **functional protein**

**WVE-N531** (DMD)

## Editing

Efficient editing of RNA bases to **restore** or **modulate** protein production[4,5]




**WVE-006** (AATD),
**WVE-008** (PNPLA3 I148M liver disease)

AATD: alpha-1 antitrypsin deficiency; ADAR: adenosine deaminase acting on RNA; AGO2: Argonaute 2; DMD: Duchenne muscular dystrophy; HD: Huntington's disease; PNPLA3: patatin-like phospholipase domain-containing protein 3; RISC: RNA-induced silencing complex; RNase H: ribonuclease H

1. Iwamoto N et al., *Mol Ther Nucleic Acids.* 2024;35(3):102246; 2. Liu W et al., *Nucleic Acids Res.* 2023;51(9):4126-4147; 3. Kandasamy P et al., *Nucleic Acids Res.* 2022;50(10):5443-5466; 4. Lu G et al., *Nucleic Acids Res.* 2024;52(17):10068-10084; 5. Monian P et al., *Nat Biotechnol.* 2022;40(7):1093-1102

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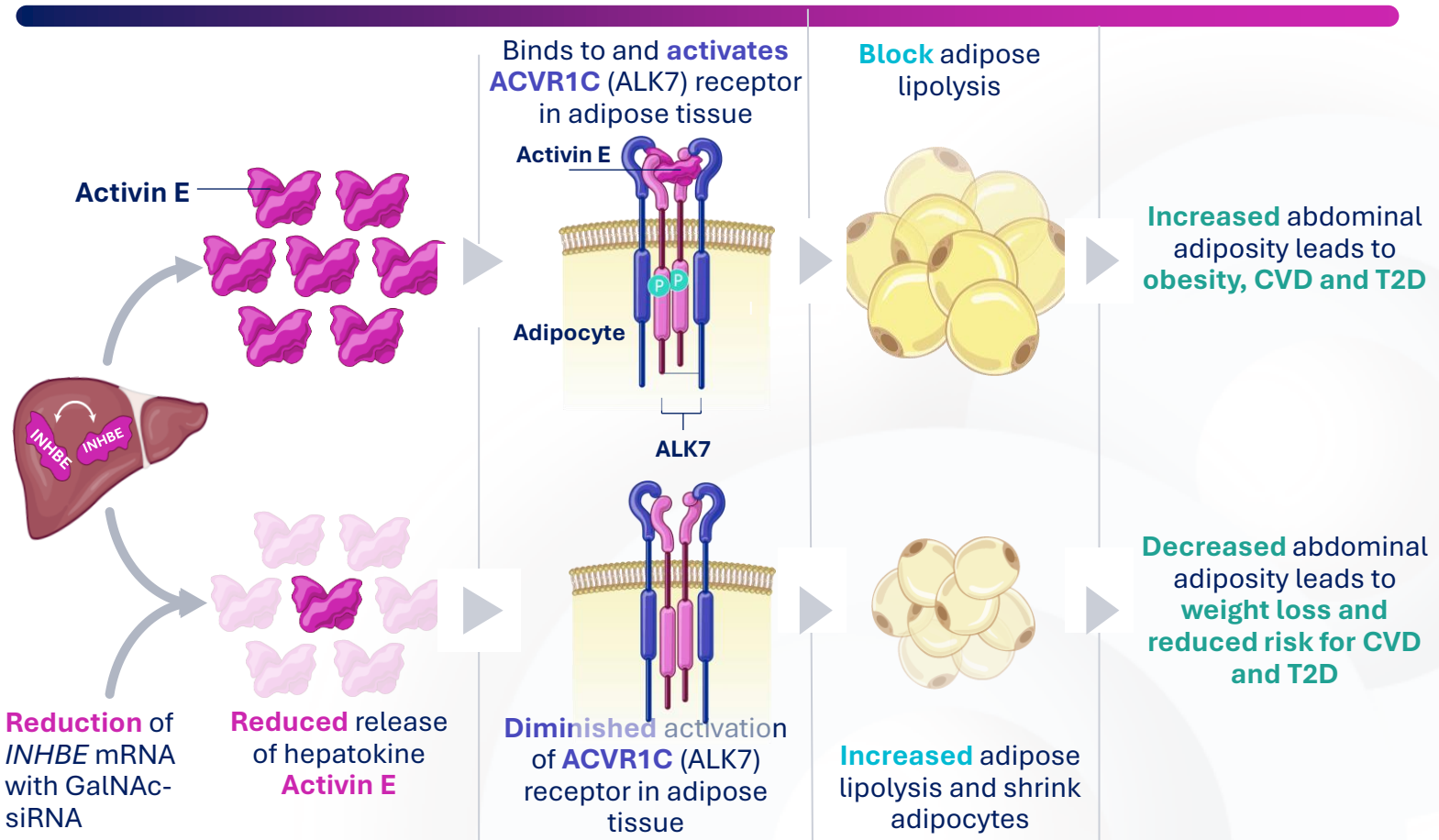
# Human genetic data demonstrate that heterozygous *INHBE* loss-of-function (LoF) carriers have lower visceral fat and a healthier metabolic profile

## Silencing *INHBE* mRNA has the potential to recapitulate this profile

**Heterozygous LoF variants carries show:**

- ✓ **Lower abdominal obesity**
- ✓ **Reduced visceral fat**
- ✓ **Lower triglycerides**
- ✓ **Higher "good" cholesterol**
- ✓ **Lower ApoB**
- ✓ **Improved glucose control**
- ✓ **Less liver inflammation/fibrosis**
- ✓ **Less liver damage**
- ✓ **Lower risk of type 2 diabetes**
- ✓ **Lower risk of CHD**




Akbari et al. *Nat Commun.* 2022 Aug 23;13(1):4844; Deaton et al. *Nat Commun.* 2022 Jul 27;13(1):4319. Waist-to-hip ratio, BMI-adjusted: Visceral adipose volume (MRI), BMI adjusted; ACVR1C: activin A receptor type 1C; ALK7: activin receptor-like kinase 7; ApoB: apolipoprotein B; CHD: coronary heart disease; GalNAc: *N*-acetylgalactosamine; INHBE: Inhibin βE; T2D: type 2 diabetes

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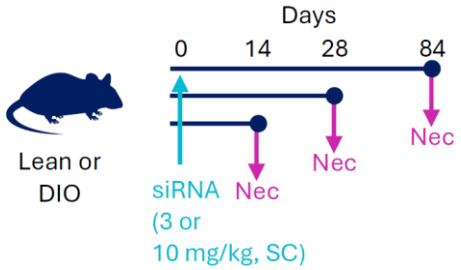
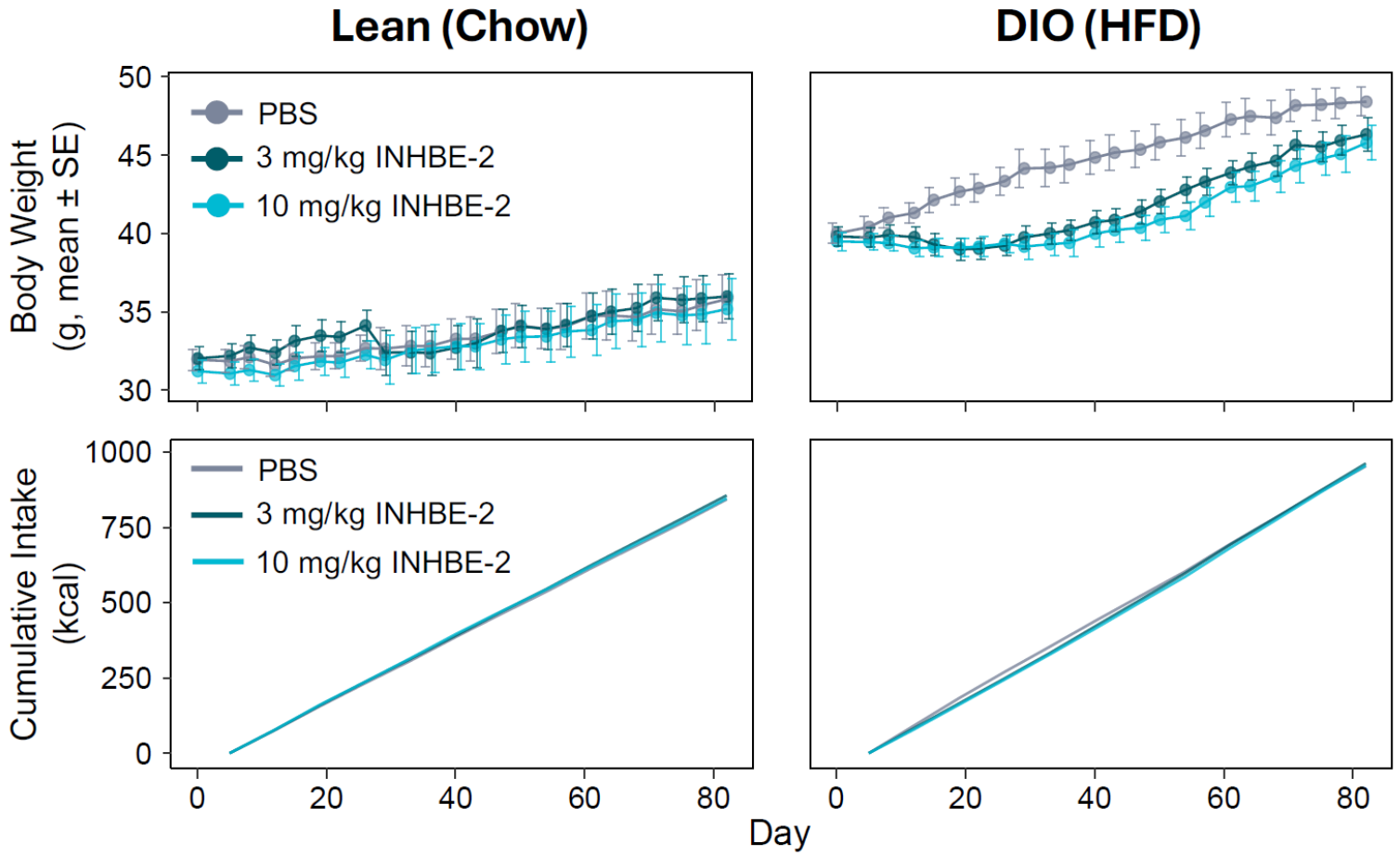
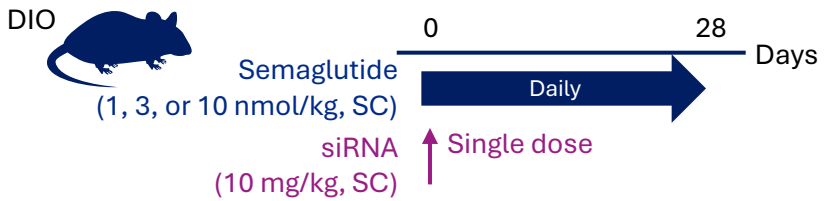
# *Inhbe*-silencing durably suppresses weight gain in DIO mice, but not in lean mice, without impacting final cumulative food intake



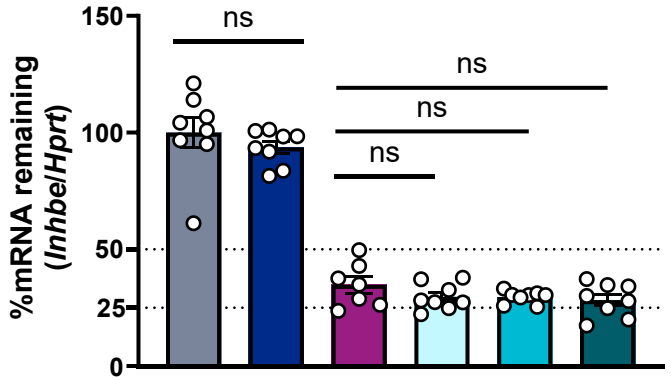

C57Bl6 mice were started on a regular chow diet (lean) or a high fat diet (HFD) to promote diet-induced obesity (DIO) at 6 weeks old. Mice received a single SC injection of 3 or 10 mg/kg INHBE-2 or or PBS (phosphate buffered saline, control). Mice were weighed twice weekly and sacrificed at D14, D28, or D84. Body weight ± SEM (n=8-24); Linear Mixed Effects ANOVA with post hoc comparisons of marginal treatment effects versus PBS per time point; * $p$ <0.05 compared to PBS; 3 mg/kg group and 10 mg/kg group were significantly different from PBS between D12-82 and D8-82, respectively. Food intake was measured each time HFD was changed, as weight of current food in the hopper versus previous weight.

# Activin E protein is lowered by *INHBE* SpiNA-GalNAc siRNA but minimally affected by semaglutide in mice



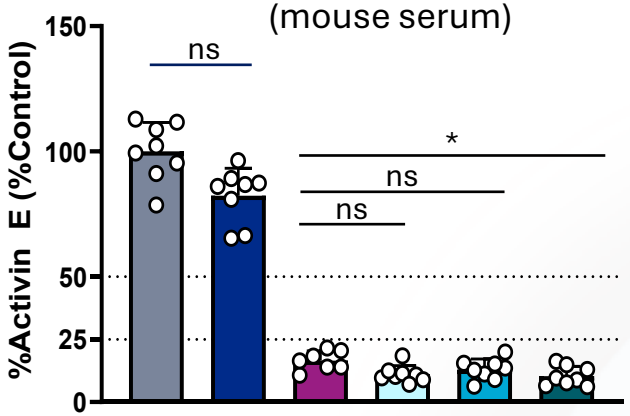

## *Inhbe* mRNA

(mouse liver)



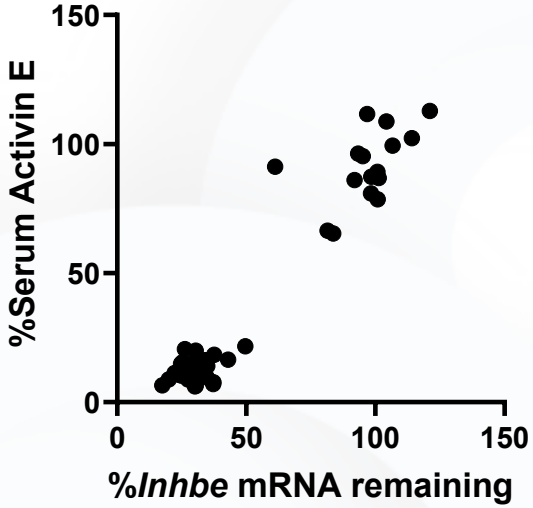

## Activin E protein

(mouse serum)




PBS
Semaglutide (10 nmol/kg)
INHBE-3
Semaglutide (1 nmol/kg) + INHBE-3
Semaglutide (3 nmol/kg) + INHBE-3
Semaglutide (10 nmol/kg) + INHBE-3

## *Inhbe* mRNA correlation with Activin E

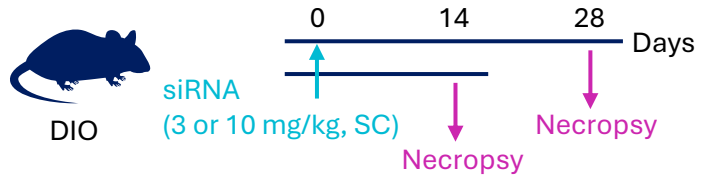


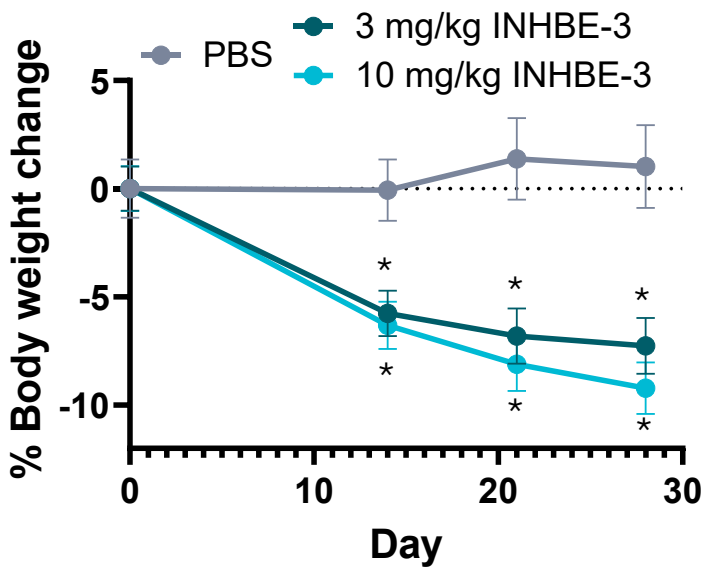

**Spearman coefficient = 0.77**

At 27 weeks (Day 0 [D0]), diet-induced obesity (DIO) mice received daily subcutaneous (SC) injections of PBS (for 28 days), a single 10 mg/kg dose of INHBE-3 siRNA ~~(stereopure interfering nucleic acid [SpiNA]; 10 mg/kg)~~, daily semaglutide (for 28 days), or a single dose of INHBE-3 + daily semaglutide (for 28 days). Mice were sacrificed on D28. *Inhbe* expression in liver was evaluated by RT-qPCR. Stats: Left, middle: Data analyzed by 1-way ANOVA followed by Tukey's HSD post hoc comparisons on log2-transformed data; * $p < 0.05$; ns, nonsignificant. n=7 or 8/group

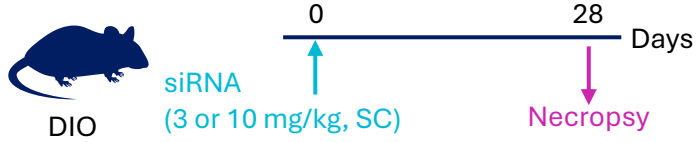
# Weight loss by a single dose of *INHBE* SpiNA-GalNAc siRNA is driven by fat loss with preservation of muscle

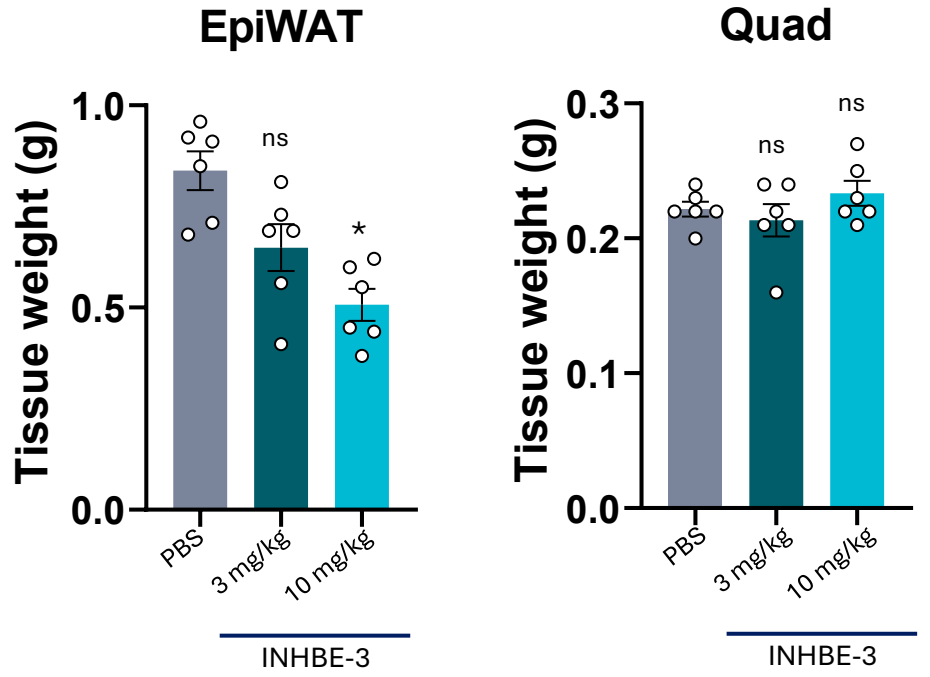
## Body weight




## Tissue weight

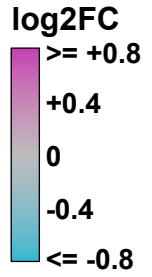


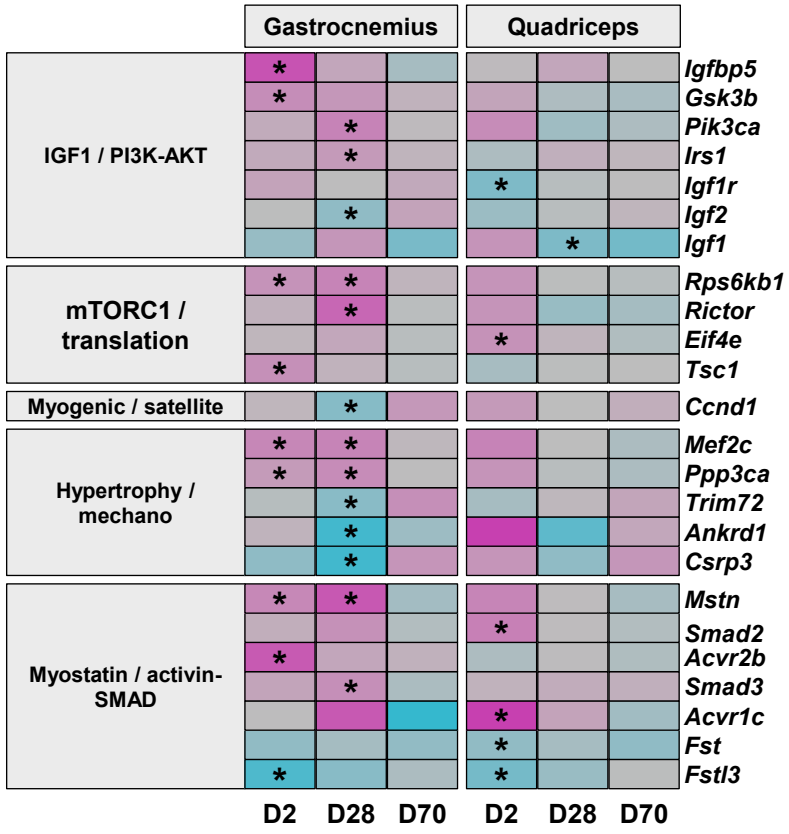

At ~25 weeks old (Day 0 [D0]), diet-induced obesity (DIO) mice received a single subcutaneous (SC) injection of 3 or 10 mg/kg INHBE-3 siRNA or PBS (control). Left, mean body weight change (%) from D0 ± SEM (n=12-18). Stats: Linear Mixed Effects ANOVA with post hoc comparisons of marginal treatment effects vs. PBS per time point; * $p < 0.05$. Right, a single epididymal white adipose tissue (epiWAT) fat pad and quadriceps muscle (Quad) were collected and weighed on D28. Stats: Mean weight (g) ± SEM, Linear Mixed Effects ANOVA with post hoc comparisons of marginal treatment effects vs. PBS per tissue type; * $p < 0.05$, ns, nonsignificant

# *Inhbe*-silencing inhibits expression of genes associated with muscle atrophy, with milder effect on muscle growth

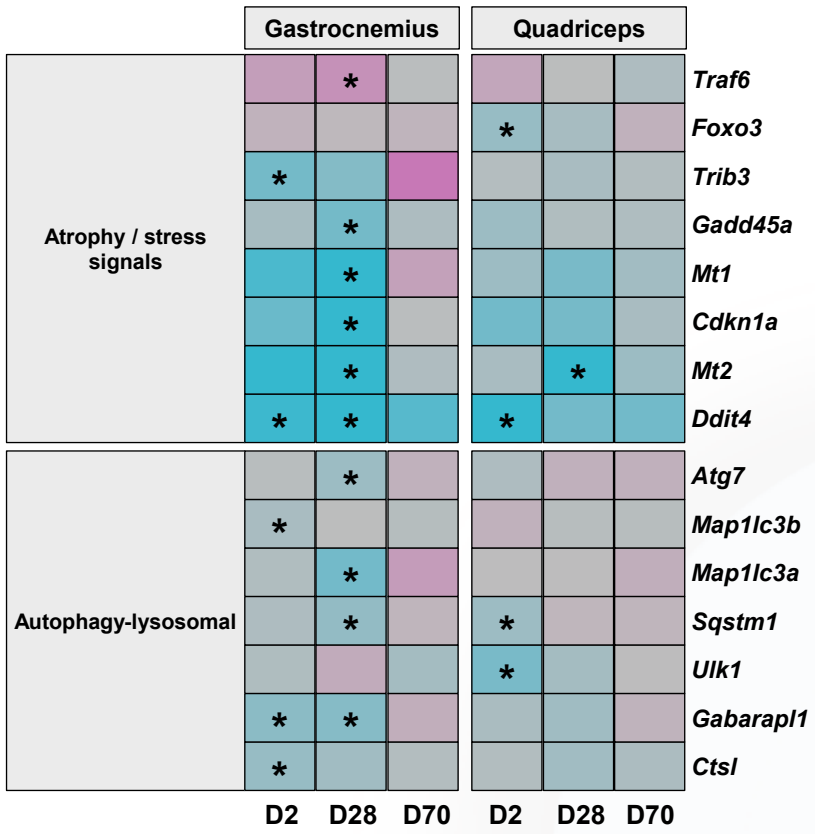
## INHBE-3 lowers atrophy, stress & proteosome proteolysis signaling, and mildly affects muscle growth



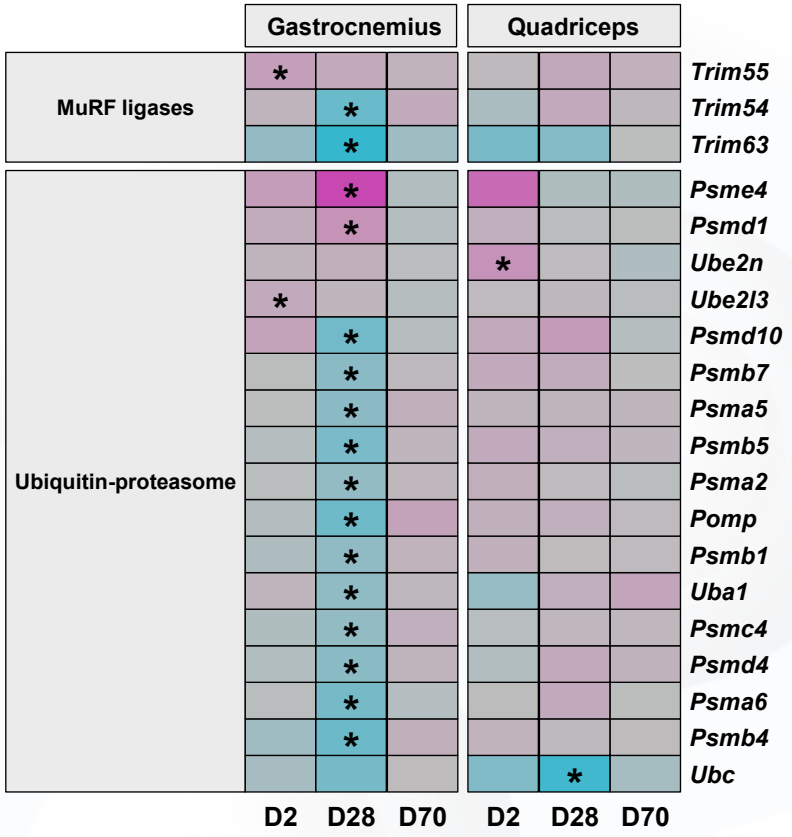

### Muscle Growth Signaling




### Atrophy, Stress, Autophagy




### Proteosome proteolysis




At 23 weeks of age, diet-induced obesity mice received a single subcutaneous injection of 10 mg/kg INHBE-3 siRNA or PBS, and animals were sacrificed on Day 2 (D2), D28, and D70 (n=9-10/group). Gastrocnemius and quadriceps were collected from sacrificed mice at each time point. Raw mRNA sequencing reads were aligned to the mm10 genome (mus musculus). Subsequently, differential gene expression analysis was conducted using the DESeq2 package. Genes are those drawn in the three-muscle heatmaps, restricted to genes with complete data in both muscles. Directional statistics are a two-sided sign test over the plotted cells. * = padj < 0.05.

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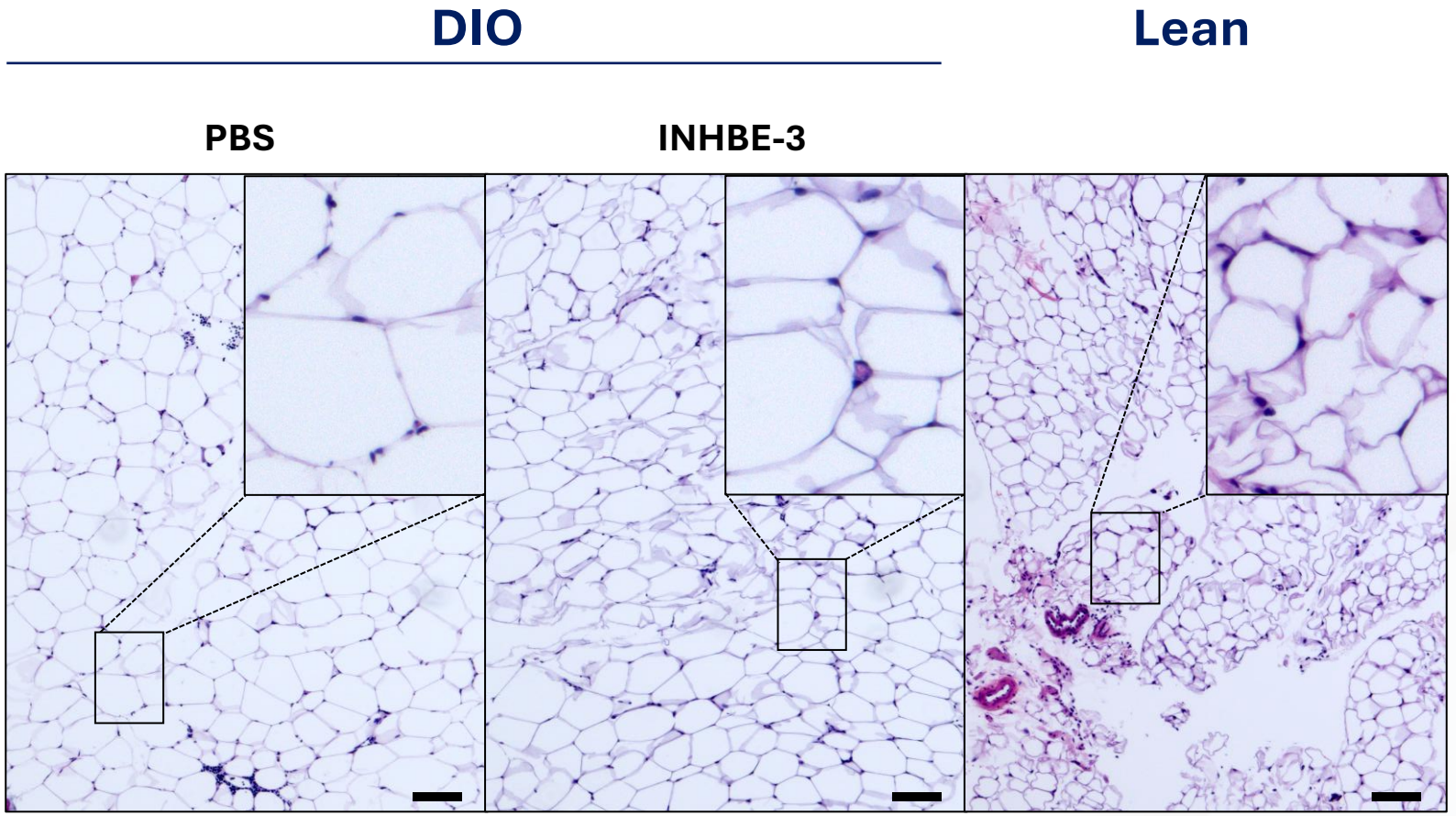
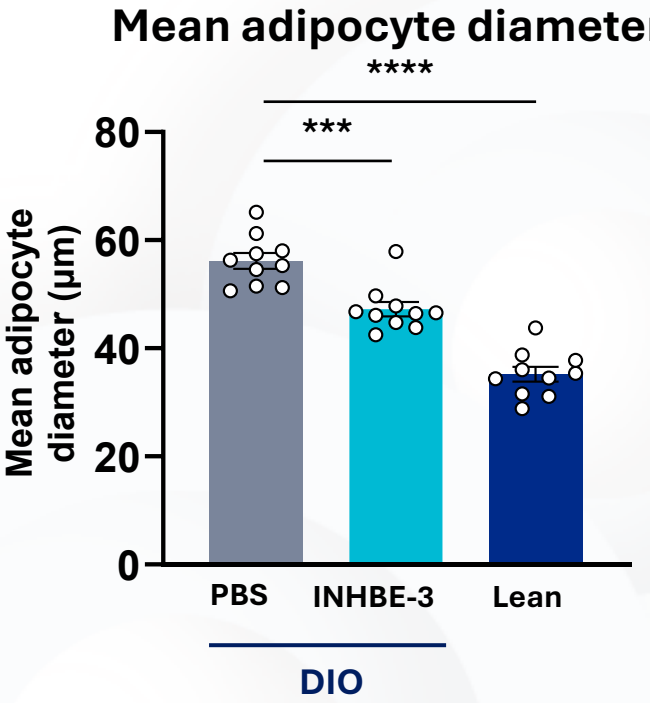
# *Inhbe* knockdown decreases adipocyte size in MesWAT of DIO mice



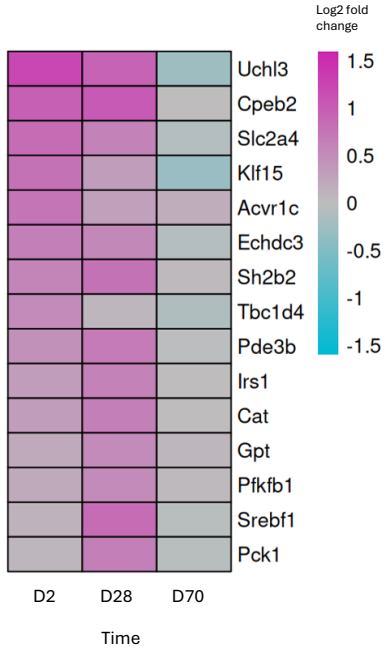



At 23 weeks old (Day 0 [D0]), diet-induced obesity (DIO) mice received a single subcutaneous (SC) injection of 10 mg/kg INHBE-3 siRNA or PBS. Lean mice were sacrificed on D0; INHBE-3- and PBS-treated mice were sacrificed on D28. Left, representative H&E-stained mesenteric white adipose tissue (MesWAT). Scale bar: 100 µm. Right, mean MesWAT adipocyte diameter (µm) ± SEM, calculated from H&E-stained sections, using the ImageJ Adiposoft plugin. One representative field analyzed per animal. Each point represents 1 animal. Stats: One-way ANOVA followed by Tukey HSD post hoc tests; not all comparisons are shown; *** $p < 0.001$, **** $p < 0.0001$

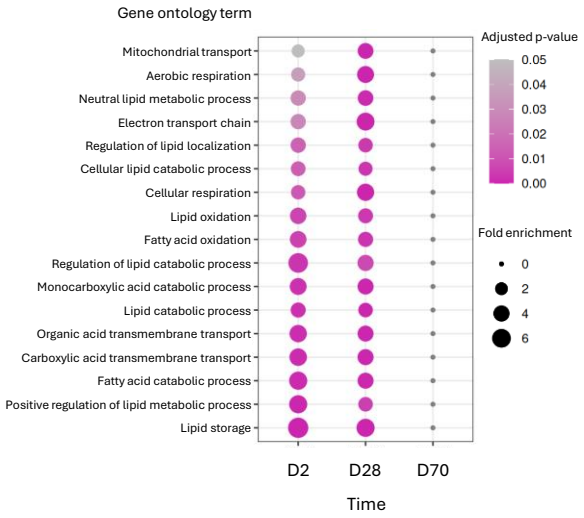
# INHBE-3 increases expression of genes associated with metabolic improvement and decreases expression of fibrotic genes in the subcutaneous (inguinal) fat

## Upregulation

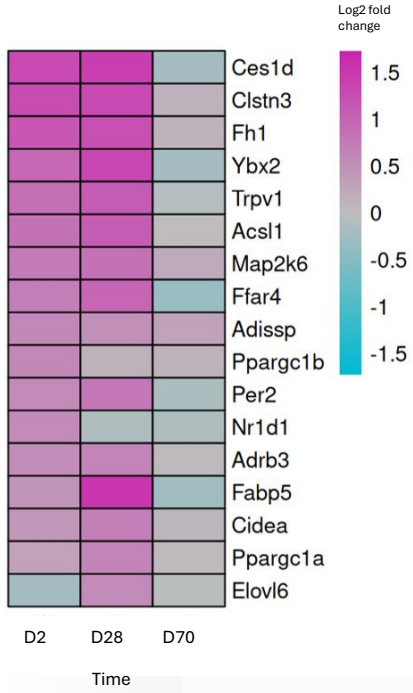
### Insulin sensitivity




### Fatty acid utilization for energy production

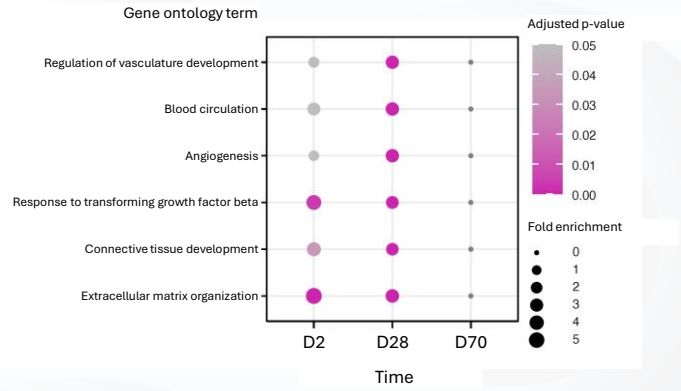



### Adaptive thermogenesis (beiging of white adipose)




## Downregulation

### Adipose remodeling and fibrosis pathways

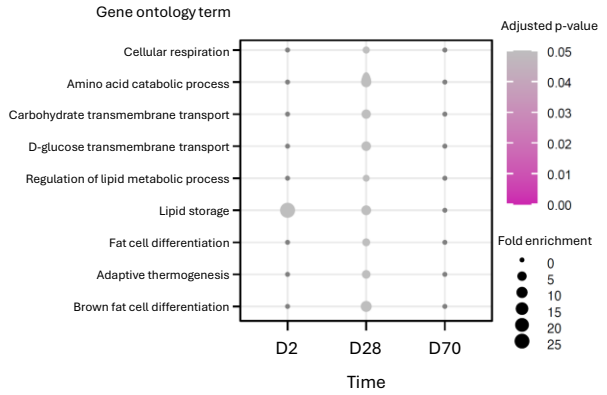



At 23 weeks of age, diet-induced obesity mice received a single subcutaneous injection of 10 mg/kg INHBE-3 siRNA or PBS, and animals were sacrificed on Day 2 (D2), D28, and D70 (n=9-10/group). Liver, inguinal fat, and mesenteric fat were collected from sacrificed mice at each time point. Raw mRNA sequencing reads were aligned to the mm10 genome (mus musculus). Subsequently, differential gene expression analysis was conducted using the DESeq2 package

# INHBE-3 decreases expression of genes associated with inflammation and fibrosis in the visceral (mesenteric) fat

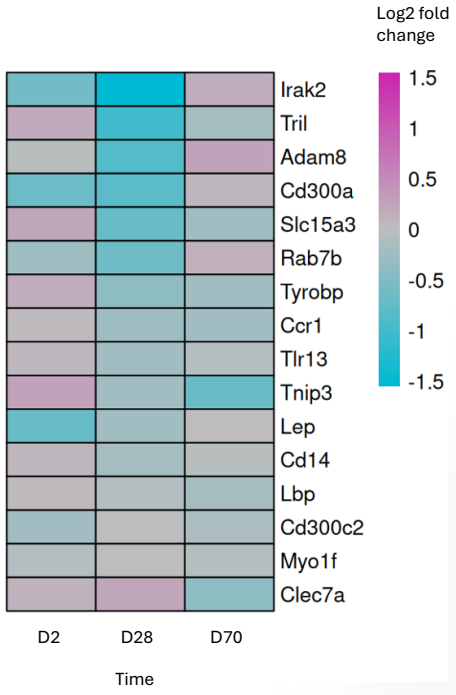
## Upregulation

### Glucose utilization, thermogenesis, and lipid metabolism (consistent with inguinal subcutaneous adipose)

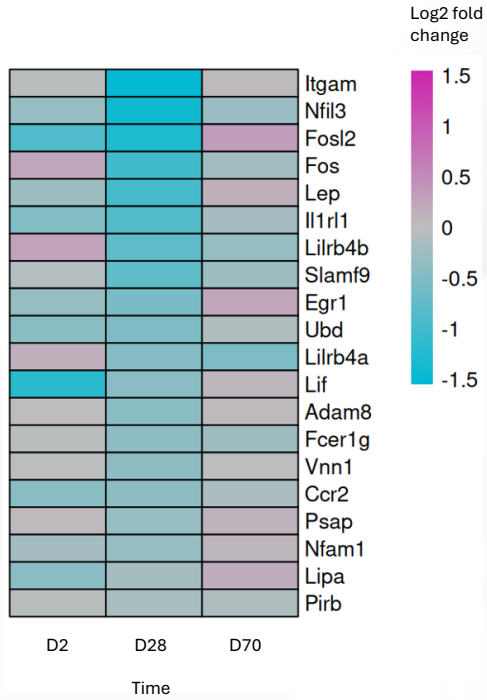



## Downregulation

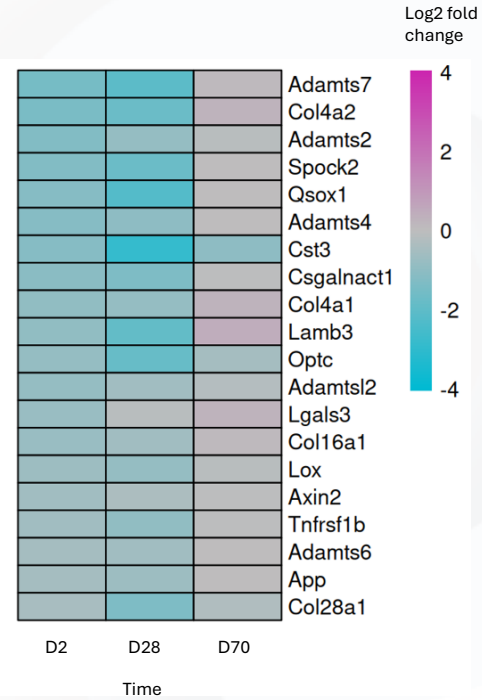
### Innate immunity




### Cytokine release



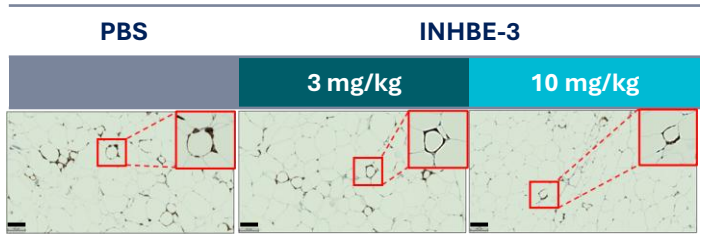

### Extracellular matrix remodeling (fibrosis)

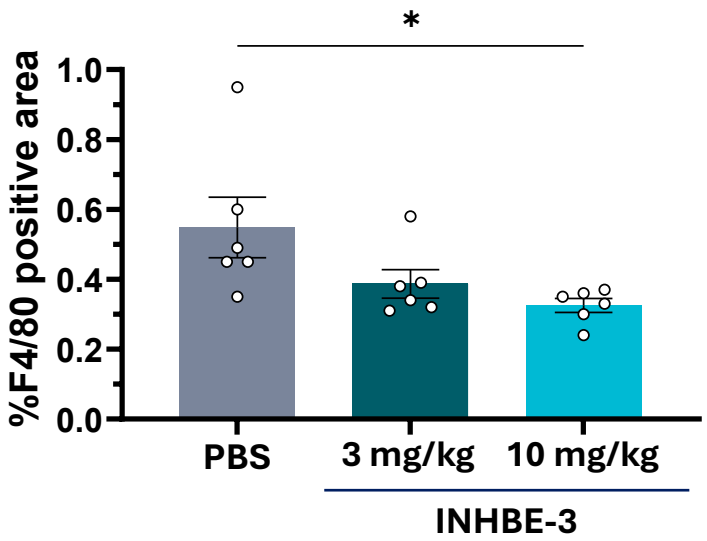



At 23 weeks of age, diet-induced obesity mice received a single subcutaneous injection of 10 mg/kg INHBE-3 siRNA or PBS, and animals were sacrificed on Day 2 (D2), D28, and D70 (n=9-10/group). Liver, inguinal fat, and mesenteric fat were collected from sacrificed mice at each time point. Raw mRNA sequencing reads were aligned to the mm10 genome (mus musculus). Subsequently, differential gene expression analysis was conducted using the DESeq2 package

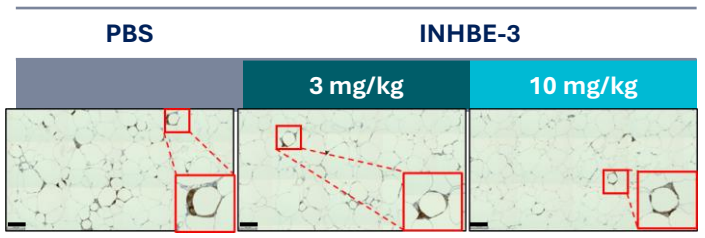
# *INHBE* SpiNA-GalNAc siRNA reduces proinflammatory macrophage recruitment in epiWAT of DIO mice

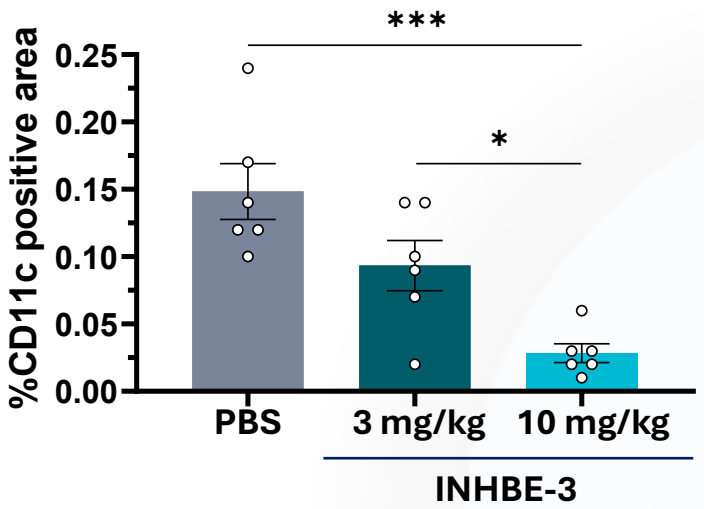
## Macrophages (MΦ) (F4/80)



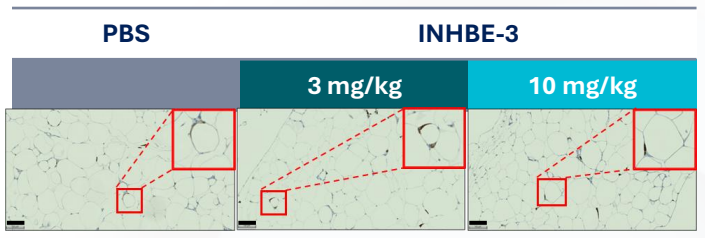



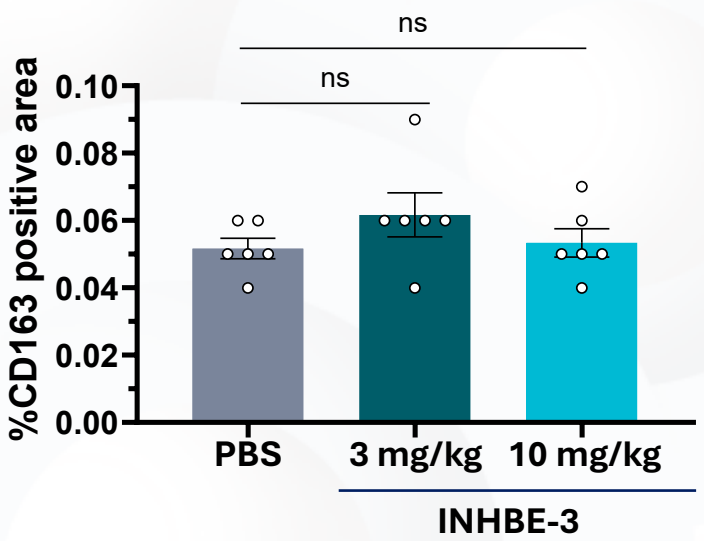

## Proinflammatory MΦ (CD11c)




## Anti-inflammatory (M2) MΦ (CD163)




At 25 weeks old (Day 0 [D0]), diet-induced obesity (DIO) mice received a single subcutaneous injection of 3 or 10 mg/kg INHBE-3 siRNA or PBS. Mice were sacrificed on D56. Top: representative images (5×) from F4/80-, CD11c-, or CD163-stained epididymal white adipose tissue (epiWAT) with values similar to the group mean. Scale bar: 100 µm. Bottom: mean ± SEM of % stain-positive area calculated from F4/80-, CD11c-, or CD163-stained sections from n=6 mice, using the HALO image analysis platform. Dots represent individual mice. Stats: one-way ANOVA with Tukey multiple comparison test (CD11c and CD163); Kruskal-Wallis test with Dunn's multiple comparisoin test (F4/80); *** $p < 0.001$, * $p < 0.05$, ns, nonsignificant

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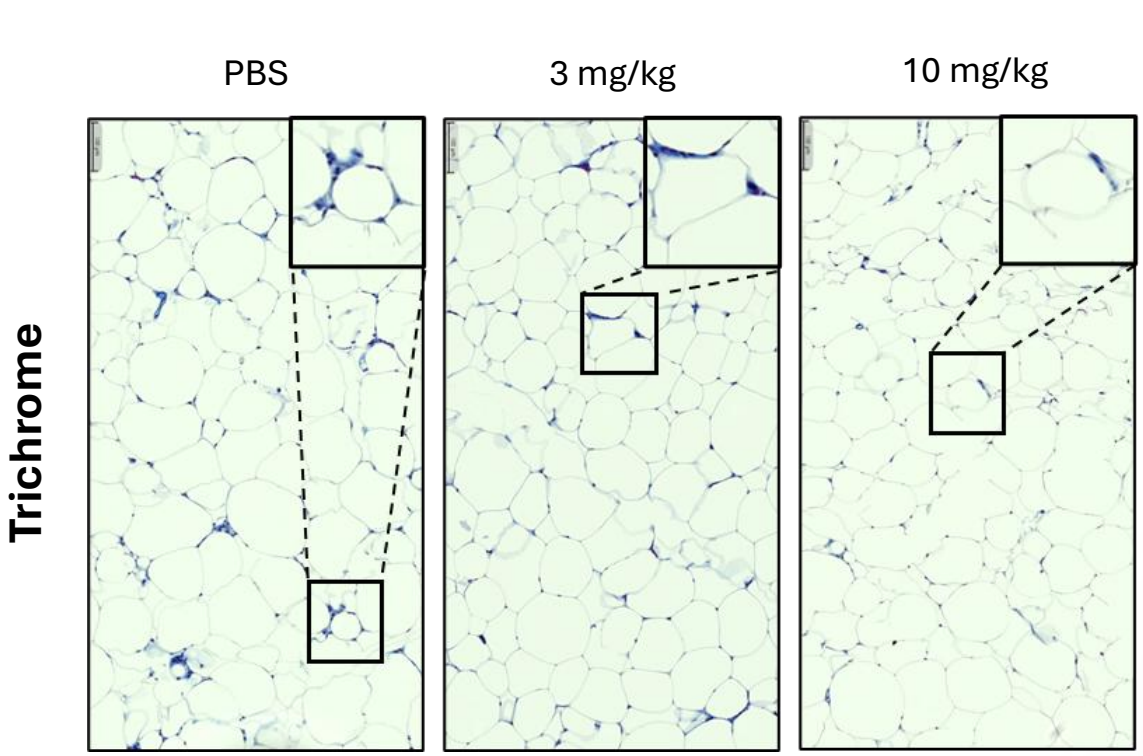
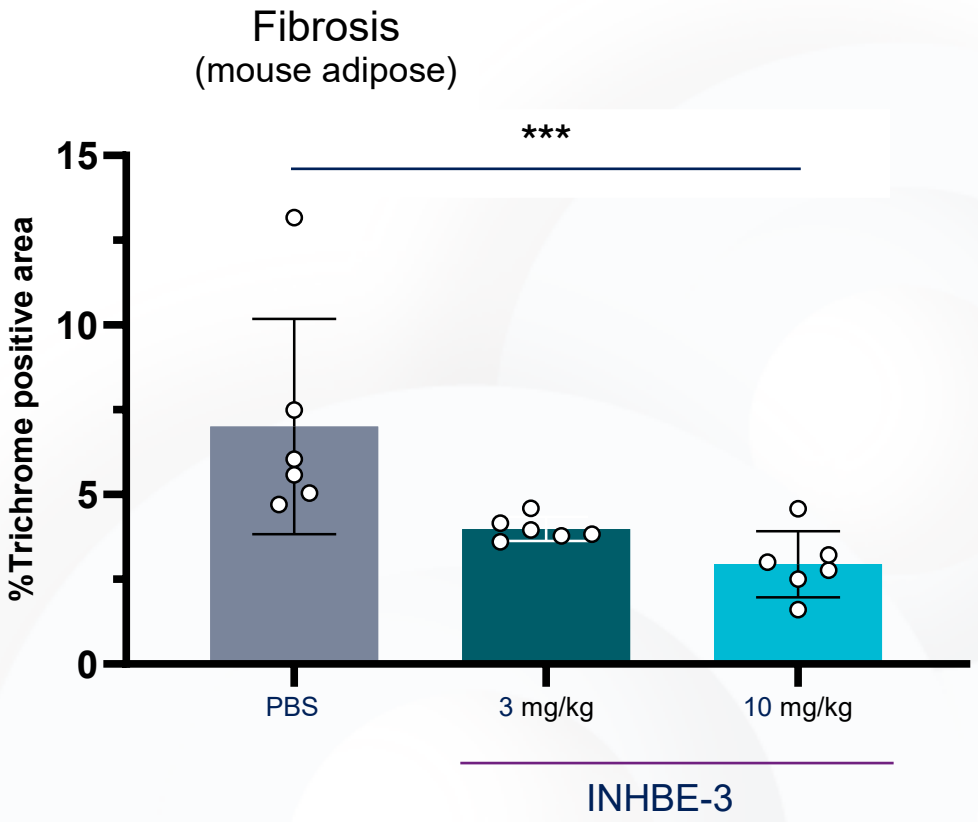
# A single dose of *INHBE* SpiNA-GalNAc siRNA reduces fibrosis by 58% in adipose tissue

## Collagen staining (trichrome) in mouse adipose (Day 56)




At 25 weeks old (Day 0 [D0]), diet-induced obesity (DIO) mice received a single subcutaneous (SC) injection of 3 or 10 mg/kg INHBE-3 siRNA or PBS. Mice were sacrificed on D56. Representative images (5×) from trichrome-stained epididymal white adipose tissue (epiWAT) with values similar to the group mean. Mean ± SEM of % stain-positive area calculated from trichrome-stained sections from n=6 mice, using the HALO image analysis platform. Dots represent individual mice. Kruskal-Wallis test with Dunn's multiple comparisons; *** $p < 0.001$

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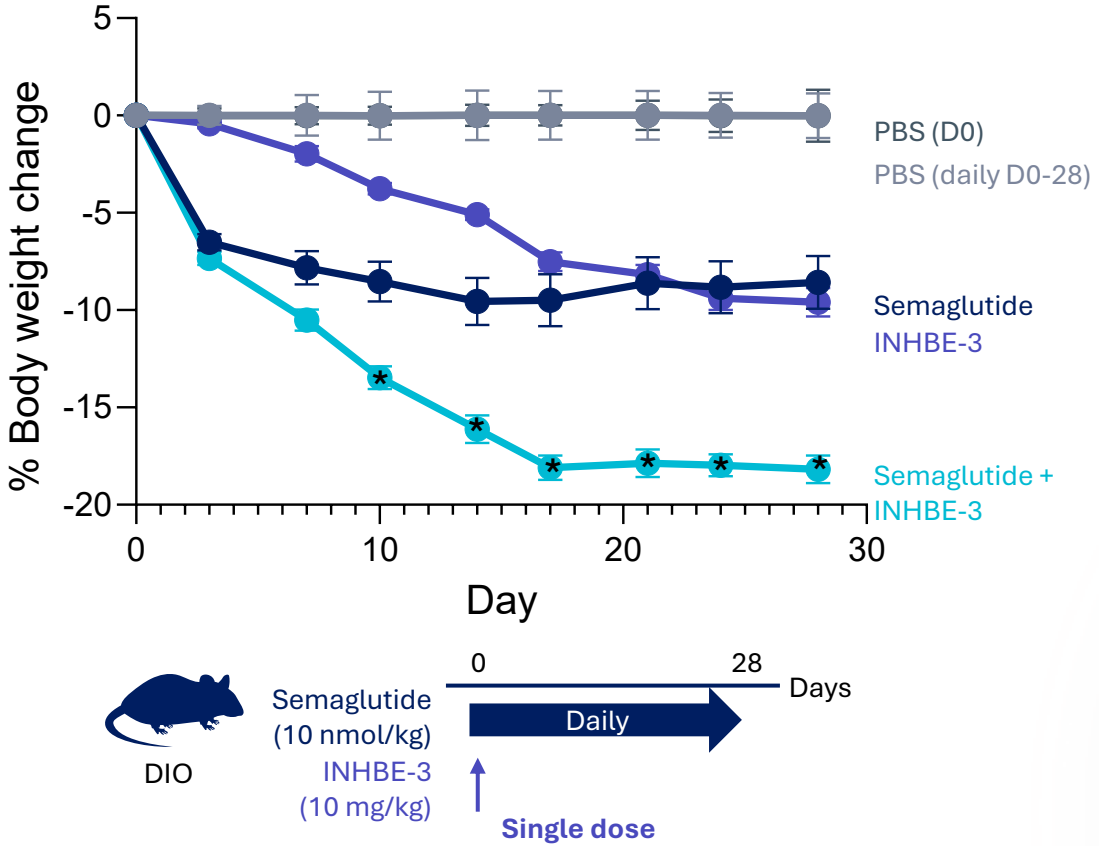
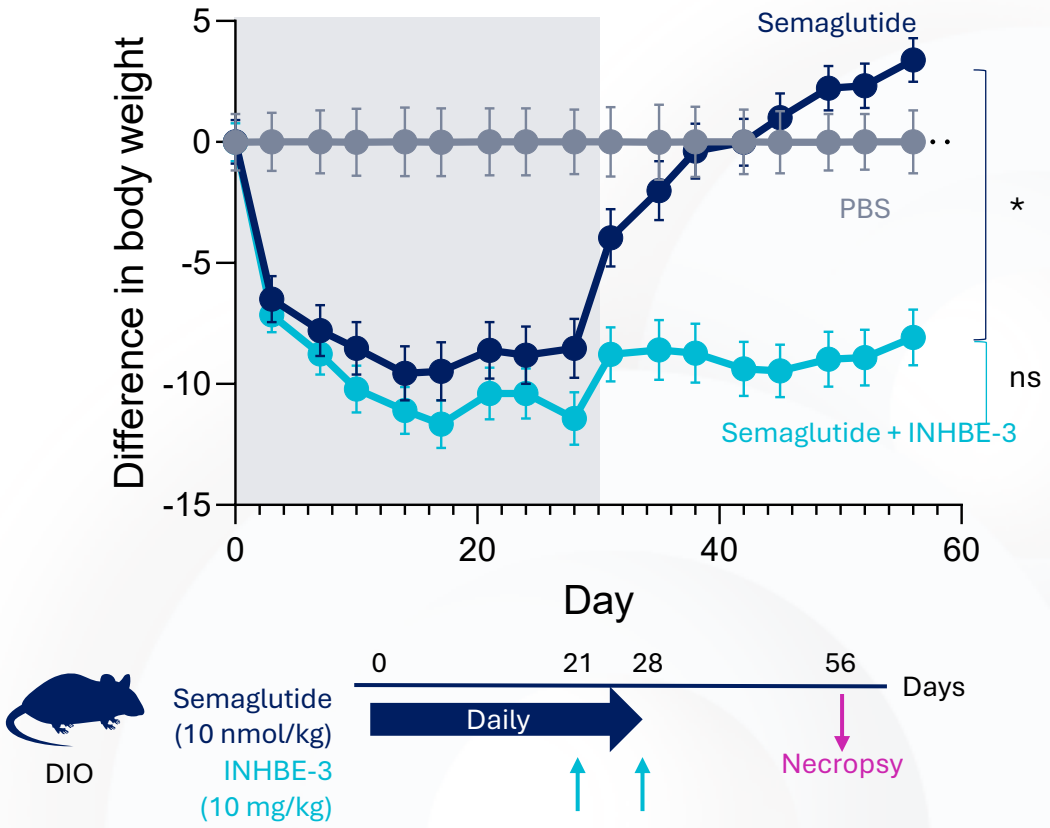
# Treatment with *INHBE* SpiNA-GalNAc siRNA augments semaglutide-induced weight loss and suppresses weight regain in DIO mice

## ~2x greater overall weight loss when added to semaglutide




## Curtails weight regain after the cessation of semaglutide




Stats: Data presented as mean difference in baseline-adjusted weight % relative to PBS control on same day ±SEM (diet-induced obesity [DIO] mice; n=10). Left, linear mixed effects ANOVA with post hoc comparisons of marginal treatment effects for semaglutide vs. semaglutide and INHBE-3 siRNA per time point; * $p < 0.05$ vs. semaglutide group. Right, linear mixed effects ANOVA with post hoc comparisons of marginal time point effects between Day 28 (D28) and D56 per treatment group; * $p < 0.05$; ns, nonsignificant

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# Summary

- **Silencing *INHBE* mRNA has the potential to treat obesity and associated metabolic diseases**
  - *INHBE* is a hepatokine that regulates adipocyte lipolysis
  - Human genetic data demonstrate that heterozygous *INHBE* loss-of-function carriers have lower visceral fat and a healthier metabolic profile

- **Preclinical data show that, in DIO mice, *INHBE* GalNAc-siRNAs:**
  - Lower liver *Inhbe* mRNA and serum Activin E protein
  - Do not affect the weight of lean (chow-fed) mice, nor have a significant impact on final cumulative food intake (chow or high-fat diets)
  - Induce weight loss mainly through reduction of fat mass, with preservation of muscle
  - Inhibit expression of genes associated with muscle atrophy, with a milder effect on genes related to muscle growth
  - Reduce adipocyte hypertrophy
  - Lead to adipose tissue remodeling, including robust changes in gene expression, reduced proinflammatory macrophages in adipose tissue, and reduced collagen (fibrosis)
  - Double weight loss when added on to semaglutide
  - Curtail weight regain upon cessation of semaglutide

**These preclinical data support *INHBE*-lowering as a means to improve body composition, including reducing visceral fat while preserving muscle, with a mechanism distinct from that of semaglutide**

Akbari et al. *Nat Commun.* 2022 Aug 23;13(1):4844; Deaton et al. *Nat Commun.* 2022 Jul 27;13(1):4319. DIO: diet-induced obesity; GalNAc: *N*-acetylgalactosamine; INHBE: Inhibin βE; SpiNA: stereopure interfering nucleic acid

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**Silencing** (RNase H and AGO2) | **Splicing** | **Editing**

AGO2: Argonaute 2; RNase H: ribonuclease H

Selected Wave publications shown above (left to right): Iwamoto N et al., *Nat Biotechnol*. 2017;35(9):845-851. Iwamoto N et al., *Mol Ther Nucleic Acids*. 2024;35(3):102246; Kandasamy P et al., *Nucleic Acids Res*. 2022;50(10):5401-5423;Liu W et al., *Nucleic Acids Res*. 2023;51(9):4126-4147; Kandasamy P et al., *Nucleic Acids Res*. 2022;50(10):5443-5466; Monian P et al., *Nat Biotechnol*. 2022;40(7):1093-1102; Lu G et al., *Nucleic Acids Res*. 2024;52(17):10068-10084. Full list of Wave publications: https://ir.wavelifesciences.com/events-publications/publications

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# Q A