AVIDITY BIOSCIENCES

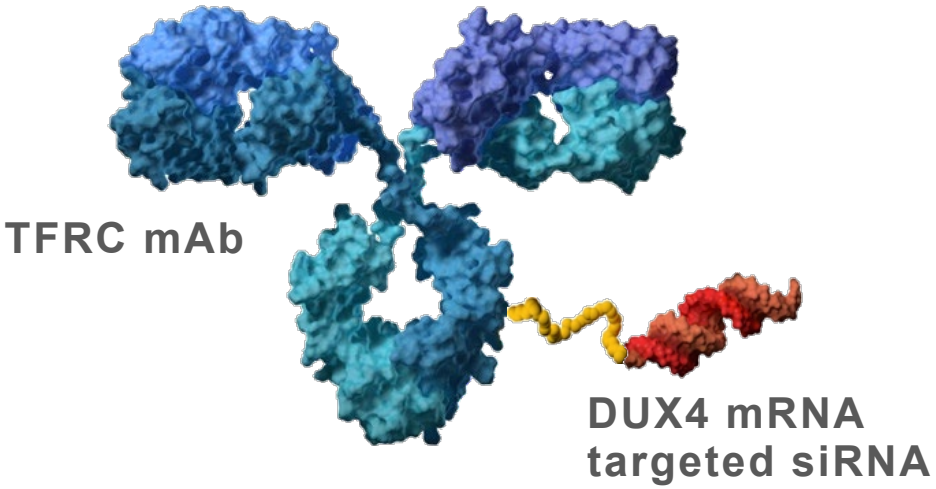
FSHD Society IRC Annual Meeting 2021

DUX4 siRNA Optimization for the Development of an Antibody Oligonucleotide Conjugate (AOC™) for the Treatment of FSHD

Presenter: Dr. Barbora Malecova

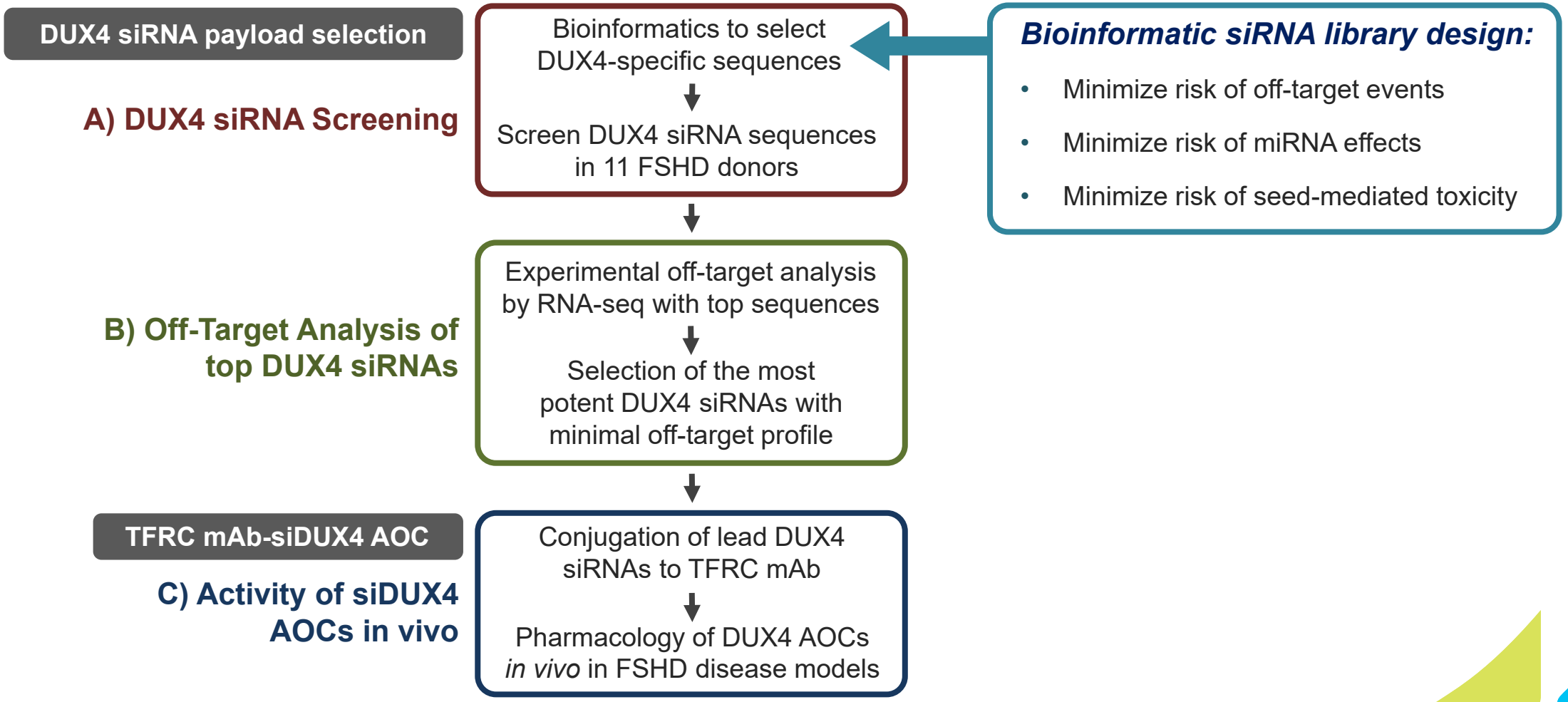
Avidity's AOC Targets DUX4 mRNA for Degradation and Eliminates the Cause of FSHD

ANTIBODY OLIGONUCLEOTIDE CONJUGATE (AOC)

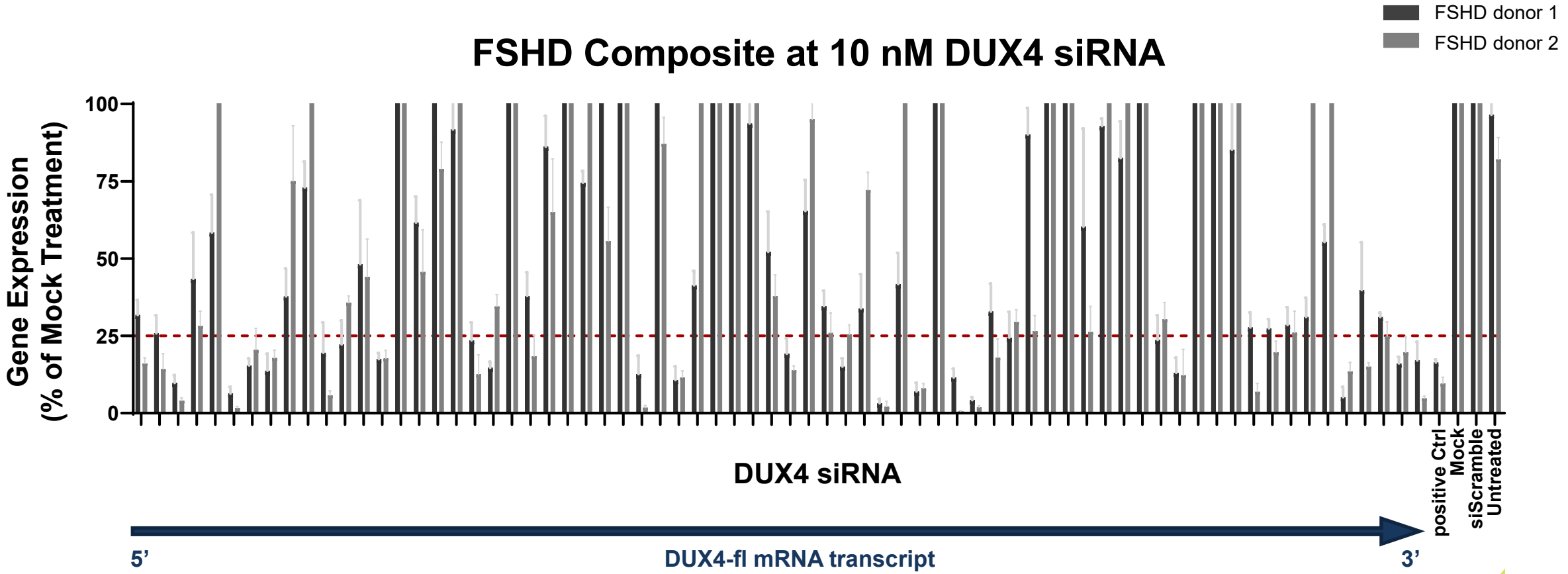


- AOCs represent a new class of therapeutics allowing delivery of oligonucleotides to target tissues
- Avidity's AOCs combine proven and safe technologies of monoclonal antibodies and oligonucleotides
  - Specificity of targeting with
  - Potency & precision of oligonucleotides
  - Targets tissues with potent and durable agents
- We optimized each of component of AOCs and engineered the molecules to maximize activity, durability, and safety
  - TFRC mAb: Optimized through engineering to be effector function null, epitope selection for optimal activity, highly efficient delivery to muscle
  - Linker: Enhanced for safety and durability, Optimized ratio of oligonucleotides to antibodies
  - DUX4-targeted siRNA: Engineered to withstand lysosomal enzymes, Selected for potency and specificity and modified to diminish off-target effects

Avidity Selected the Lead DUX4 siRNAs Payloads as Therapeutic Candidates for the Treatment of FSHD

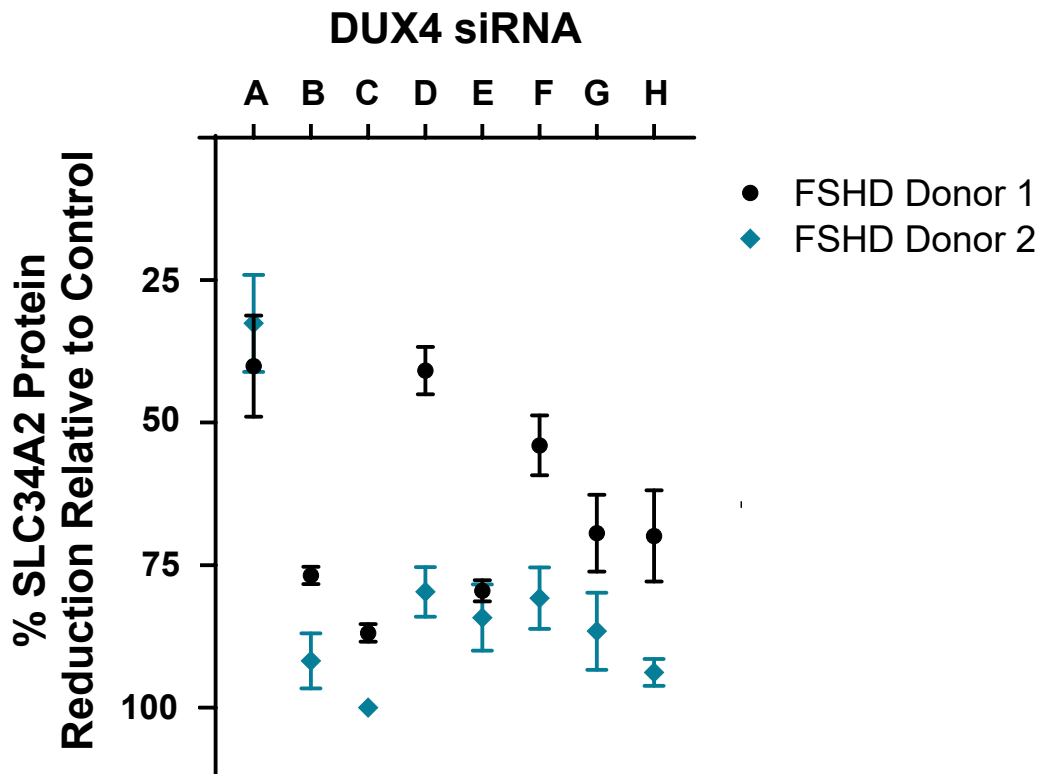


Active DUX4 siRNAs Were Identified by Screening in FSHD Donor Primary Myotubes



FSHD Composite is an average expression of 4 DUX4-target genes
(KHDC1L, LEUTX, MBD3L2, ZSCAN4) normalized to 2 HKGs (Transfection; N=4; mean -/+ SEM)

DUX4 siRNAs Reduced SLC34A2 Protein Expression in FSHD Patient-Derived Primary Myotubes



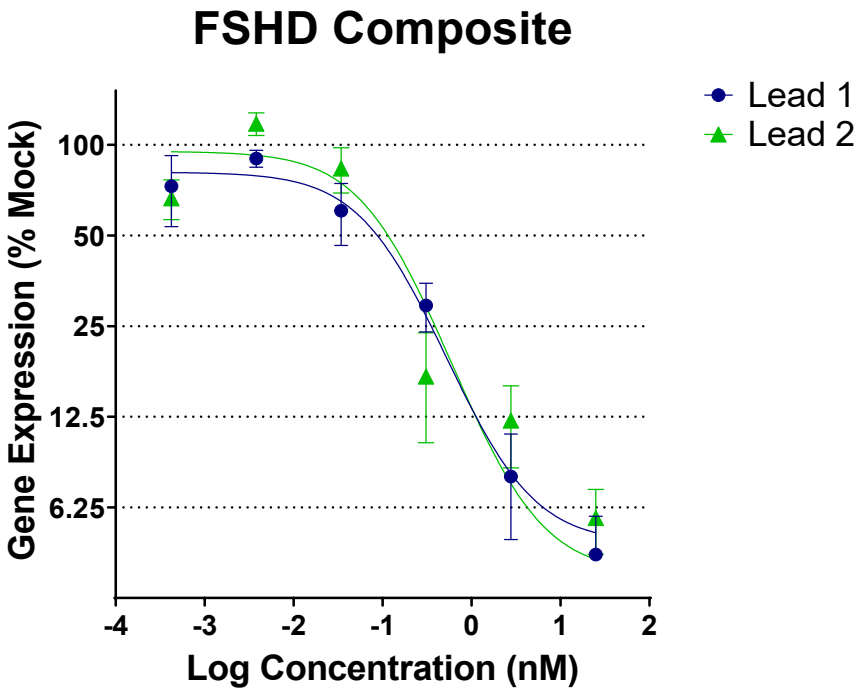
Conclusions:

- Immunofluorescent assay detected SLC34A2 protein expression in primary FSHD Patient donor myotubes
- Most of Avidity's Top 8 DUX4 siRNAs show a strong activity at 10 nM concentration in FSHD donors towards SLC34A2 protein expression downregulation (Transfection; N=4 per donor; mean -/+ SEM)

Avidity's DUX4 siRNA Lead Selection

Avidity's 2 lead DUX4 siRNAs were selected from the screening effort based on their potency assessed in 11 FSHD donor patient myotubes *in vitro*.

High Potency of the Lead DUX4 siRNAs in an *In Vitro* Concentration Response Assay in FSHD Patient-Derived Primary Myotubes



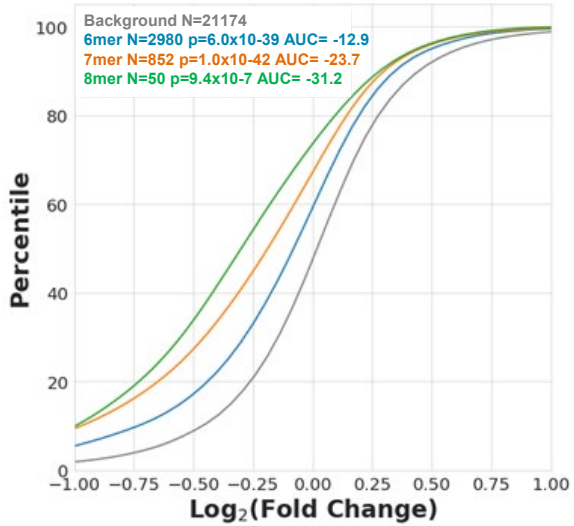
DUX4 siRNA	Emax (%)	IC50 (nM)
Lead 1	95.2	0.127
Lead 2	96.2	0.118

FSHD Composite: average expression of 4 DUX4 target genes LEUTX, MBD3L2, ZSCAN4, KHDC1L (Transfection; N=4; mean -/+ SEM)

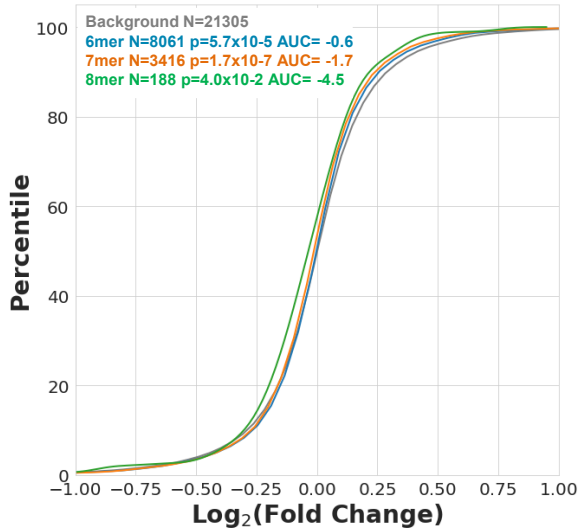
High Specificity of the Lead DUX4 siRNAs in FSHD Patient-Derived Primary Myotubes Assessed by RNA-seq

Cumulative distribution plots (Percentile) of transcriptome-wide differential gene expression (DE) of siRNA-treated FSHD donor myotubes vs control treatment (log2(Fold Change))

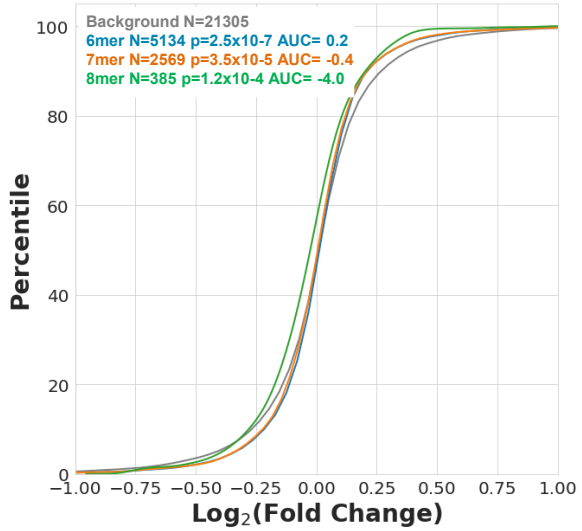
siRNA with a strong seed-mediated effect



DUX4 siRNA – Lead 1



DUX4 siRNA – Lead 2



Background DE distribution of genes with a seed match to non-targeted siRNAs

DE distribution of genes with a 6mer seed match to siRNA within their 3'UTR

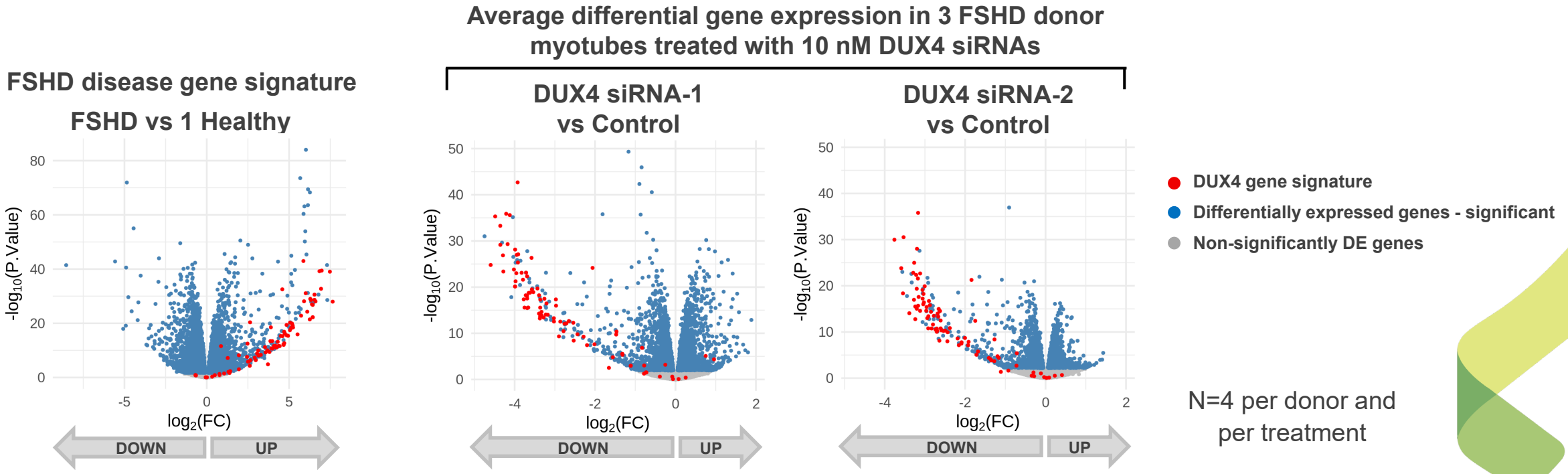
DE distribution of genes with a 7mer seed match to siRNA within their 3'UTR

DE distribution of genes with a 8mer seed match to siRNA within their 3'UTR

Conclusions:

The lead DUX4 siRNAs demonstrate a minimal seed-mediated effect on differential gene expression, and therefore a minimal potential to induce an unwanted off-target effects.

Silencing of FSHD Disease Gene Signature with Lead DUX4 siRNAs in FSHD Patient-Derived Primary Myotubes Assessed by RNA-seq

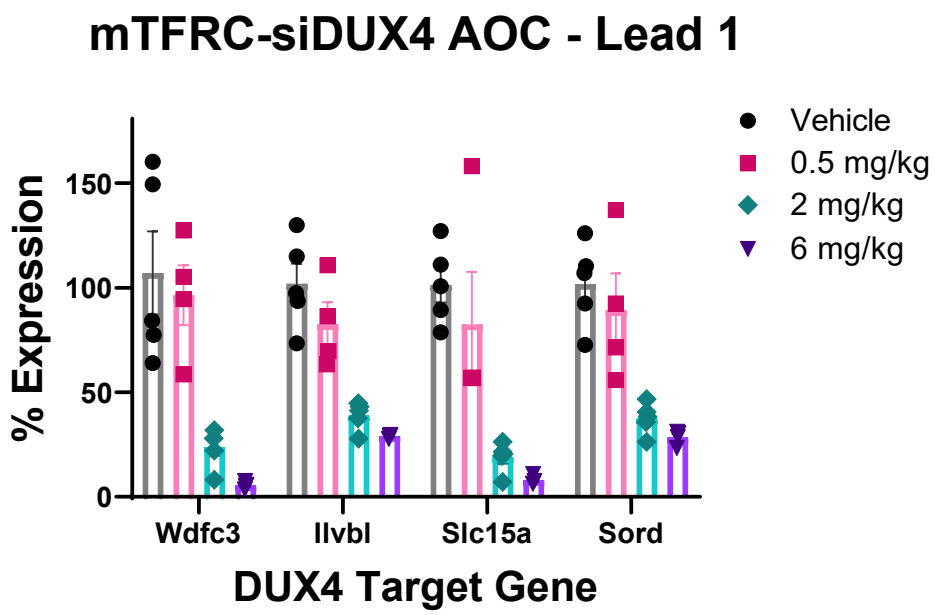


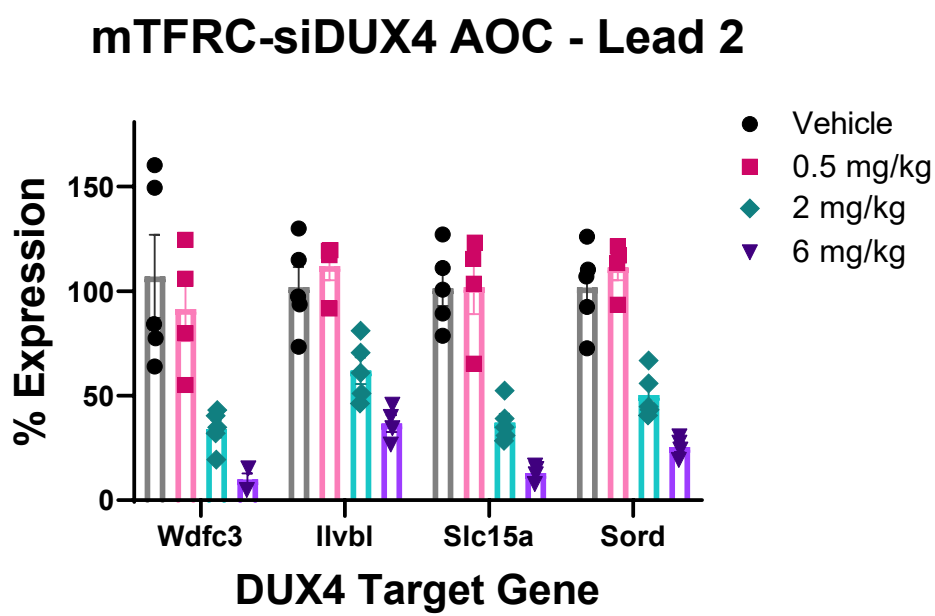
N=4 per donor and per treatment

Conclusions:

- DUX4 downstream gene signature was reported previously (Yao et al., Human Mol. Gen. 2014)
- Many DUX4 signature genes were confirmed to be strongly upregulated in FSHD donor primary myotubes
- Robust downregulation of DUX4 signature genes was observed with the lead DUX4 siRNAs in FSHD myotubes (e.g. ZSCAN4, LEUTX, MBD3L2, SLC34A2, PRAMEF12, CCNA1)

Lead DUX4 siRNAs Downregulate DUX4 Target Genes *In Vivo* in Muscles of FSHD Mouse Model in a Dose-Dependent Manner





N=4 or 5 per treatment;
mean -/+ SEM

***Conclusions*:**

- Dose-dependent downregulation of DUX4 target genes 3 weeks post single IV administration of DUX4 AOCs in Acta1-MCM;FLExDUX4 mouse model of FSHD
- Dose expressed as mg/kg of DUX4 siRNA within AOC

Characteristics of Avidity's Lead DUX4 siRNAs

- High potency *in vitro* in a variety of FSHD patient donor muscle cells
- Minimal seed-based off-target profile assessed transcriptome-wide in human muscle cells
- Robust *in vitro* efficacy – suppression of DUX4 gene signature in FSHD patient myotubes
- Dose-dependent activity *in vivo* in Acta1-MCM;FLExDUX4 FSHD mouse model

Avidity Biosciences is expecting the FSHD program to enter the clinic in 2022.

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