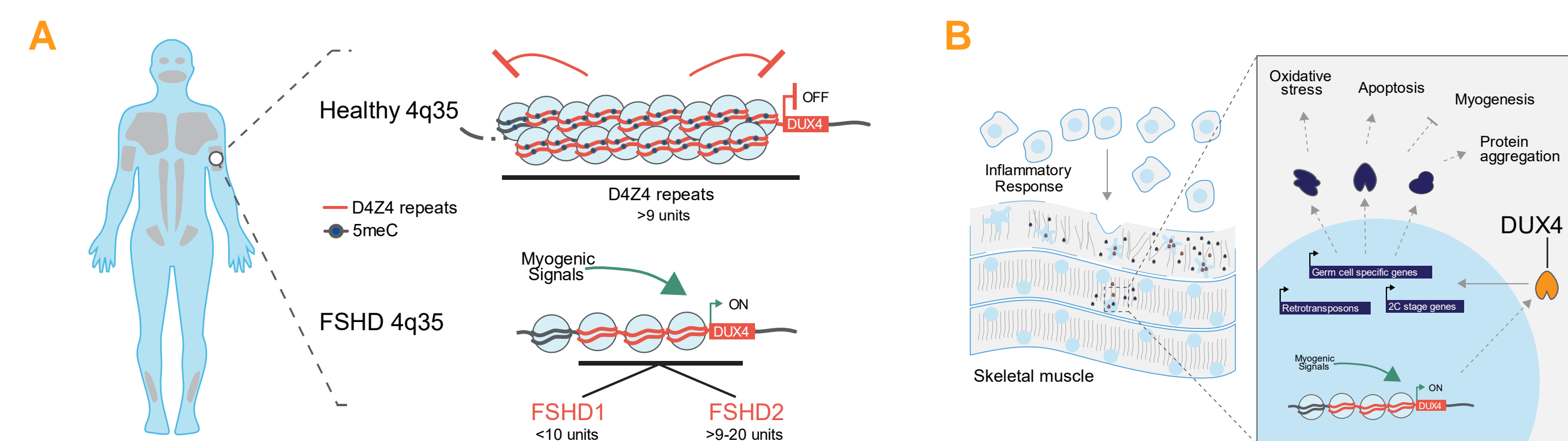


Abstract

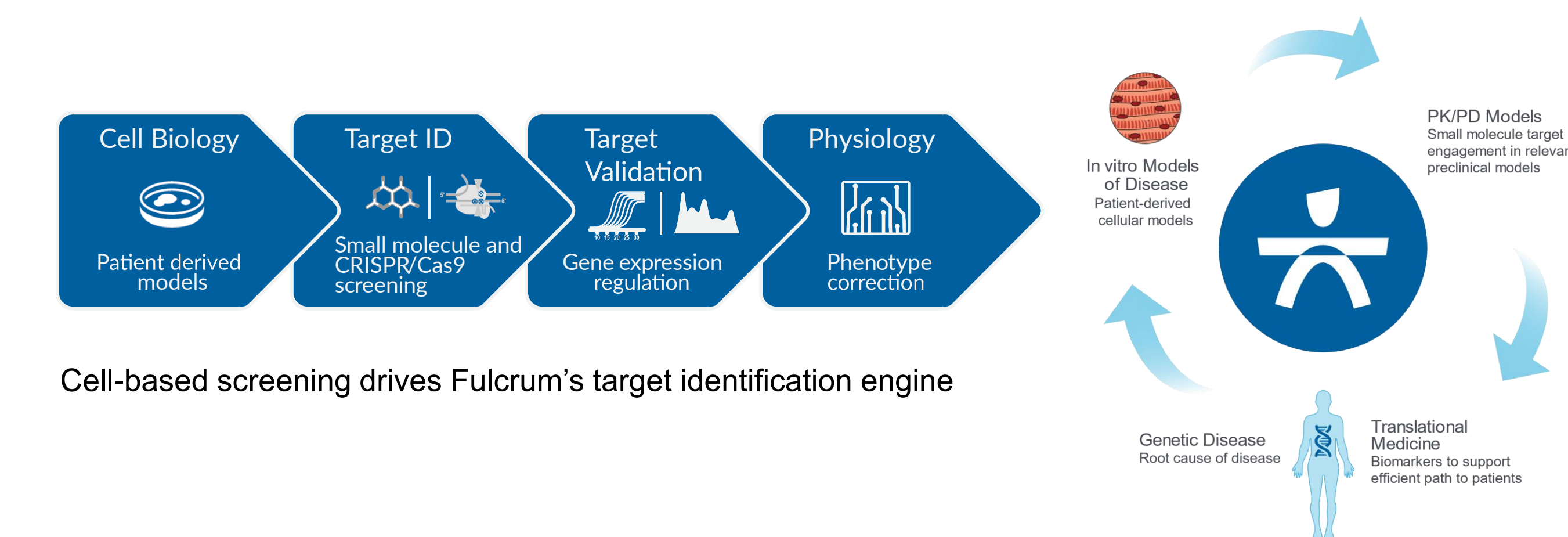
- FSHD is caused by the loss of repression at the D4Z4 locus leading to DUX4 expression in skeletal muscle, activation of its early embryo transcriptional program and muscle fiber death.
- While some progress toward understanding the signals driving DUX4 expression has been made, the factors and pathways involved in the transcriptional activation of this gene remain largely unknown.
- Using optimized myotube culture conditions, we identified losmapimod and investigated its efficacy in FSHD1 and FSHD2.
- We observed that losmapimod treatment results in reduction of DUX4 expression, activity and cell death in FSHD patient-derived myotubes with minimal impact on myogenesis.
- RNA-seq studies revealed that only a small number of genes were differentially expressed after treatment with losmapimod, ~80% of these are targets of DUX4.
- Fulcrum Therapeutics has selected losmapimod, a specific p38 α / β inhibitor, for clinical development.

1. Facioscapulohumeral Muscular Dystrophy (FSHD)

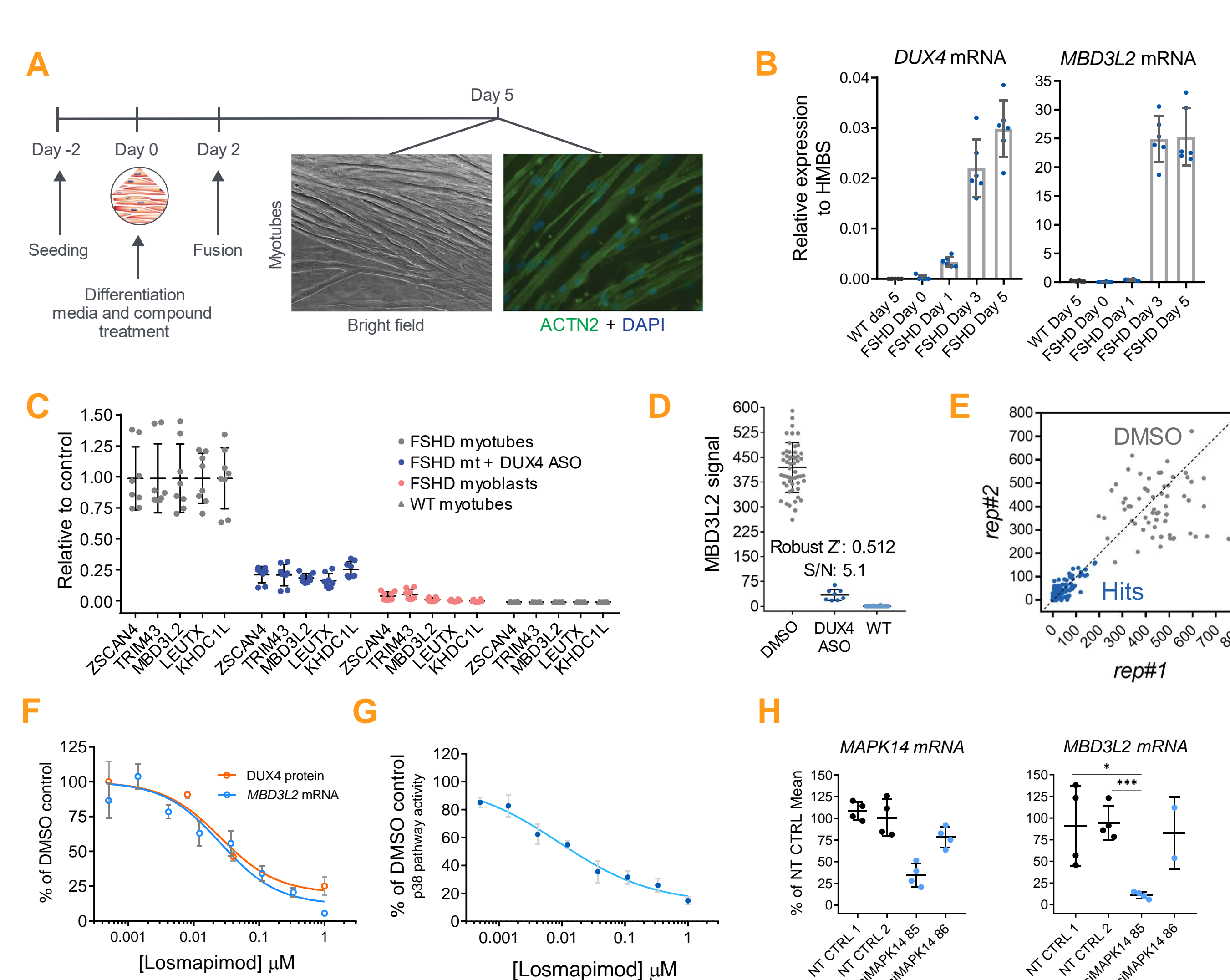


- (A) Schematic of the loss in gene repression caused by contraction of D4Z4 repeats that leads to DUX4 expression in the muscle of FSHD patients.
- (B) Schematic describing the downstream consequences of DUX4 expression in skeletal muscle.

2. Fulcrum's approach to target identification and validation

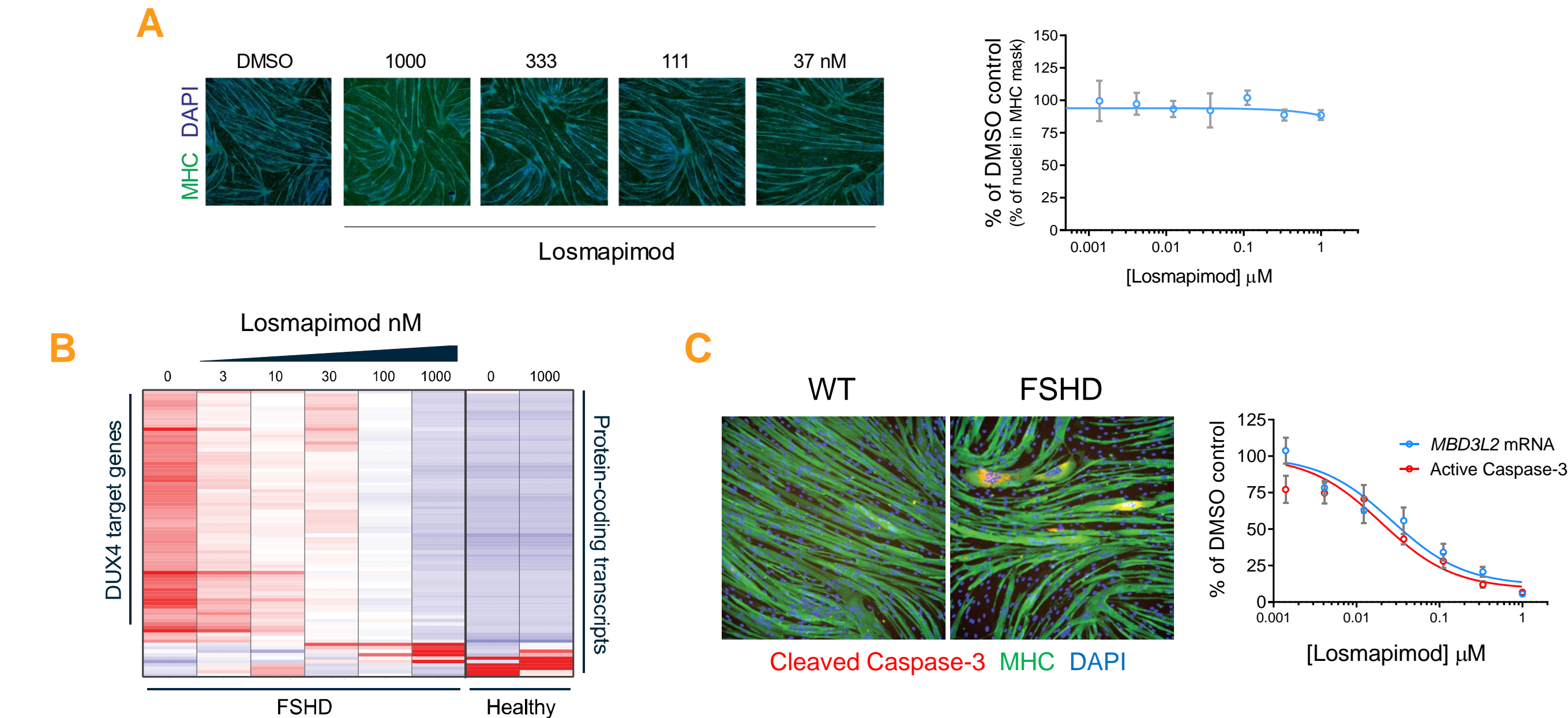


3. Identification of a drug target that inhibits DUX4 expression



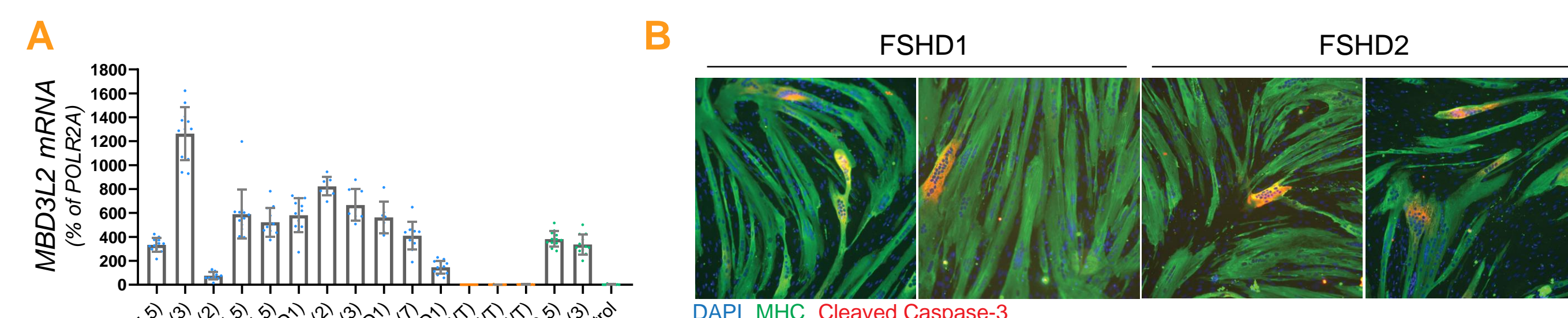
- (A) Cell-based assay schematic. (B) Expression of DUX4 and DUX4 target gene during FSHD myotube differentiation in vitro. (C) DUX4 target gene selection for HTS. (D) 96-well format assay allows for identification of targets modulating the expression of DUX4. (E) Hits identified showed high correlation in between biological replicates. (F) Losmapimod reduces expression of DUX4 in a concentration dependent manner in FSHD myotubes. (G) Losmapimod reduces p38 alpha/beta activity in FSHD myotubes. (H) P38a knockdown reduces activity of DUX4 in FSHD myotubes.

4. Losmapimod reduces DUX4 and its downstream consequences



- (A) Losmapimod treatment does not affect in vitro differentiation of myotubes. (B) RNA-seq analysis of myotubes indicates that losmapimod selectively inhibits DUX4 and its downstream program expression in a concentration dependent manner with minimal impact across the transcriptome of FSHD myotubes. <100 differentially expressed genes (abs(FC)>4; FDR<0.001) (C) Losmapimod reduces cleaved caspase-3 signal detected in FSHD myotubes indicating reduction of cell death.

5. DUX4 activity and apoptosis in primary myotubes

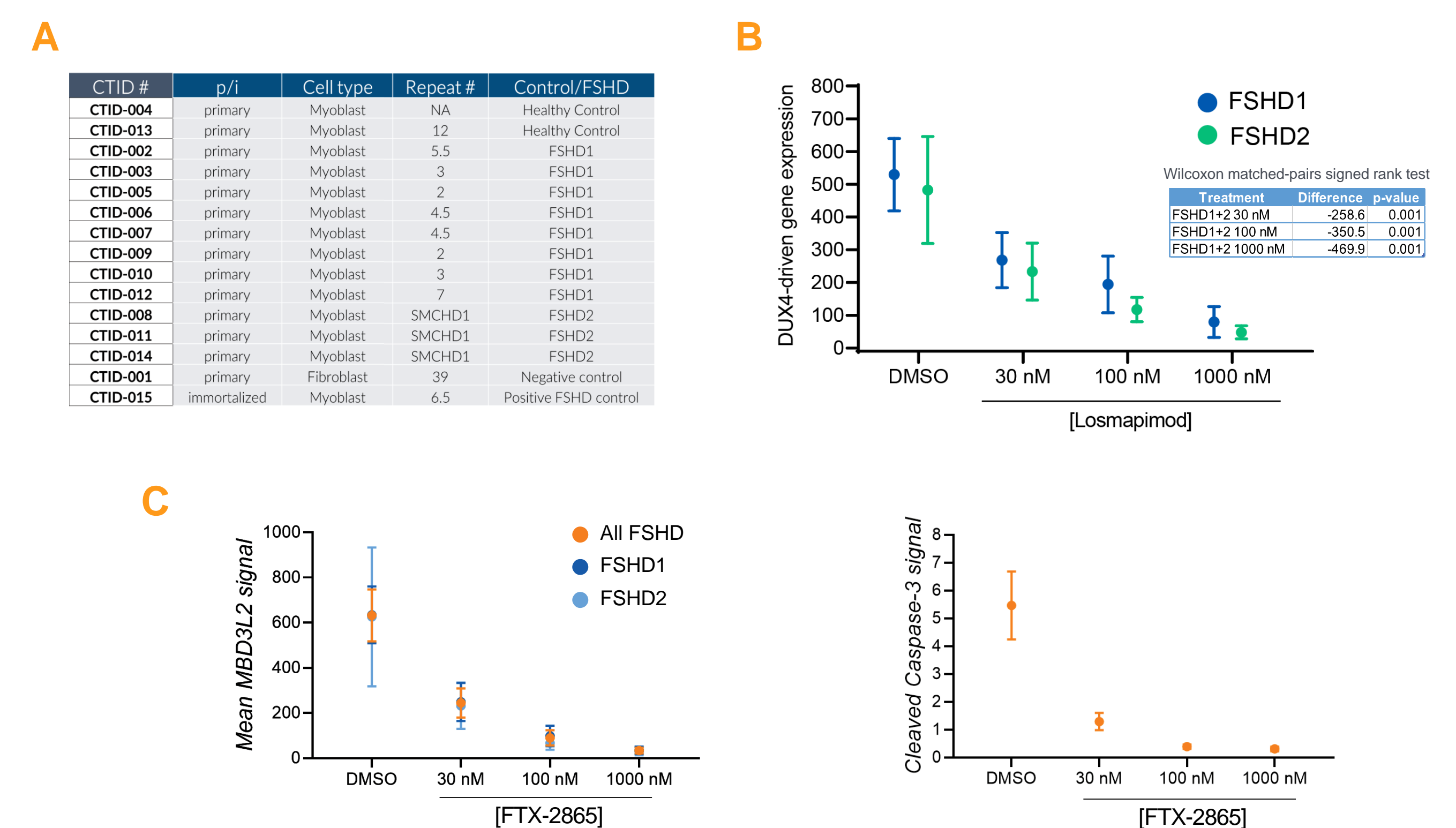


- (A) MBD3L2 expression across a variety of primary FSHD patient- and non-FSHD (WT) derived myotubes. (B) Detection of cleaved caspase-3 in primary FSHD1 and FSHD2 patient-derived myotubes.

6. Studying losmapimod efficacy across FSHD genotypes

"Preclinical trial in a dish"

- 11 primary FSHD cell lines: 8 FSHD1 and 3 FSHD2.
- 3 non-FSHD cells as negative control and one immortalized FSHD cell as positive control.
- Primary Efficacy Analysis: Wilcoxon matched-pairs signed rank test was performed between different treatment groups and vehicle group samples to determine significant differences.



- (A) Table describing the genotypes of cells used in this study. (B) MBD3L2 expression was reduced across all FSHD1 and FSHD2 patient-derived myotubes after Losmapimod treatment. (C) Inhibition of p38 α / β MAPK pathway results in reduction of DUX4 expression and inhibition of cell death in vitro across FSHD1 and FSHD2 genotypes.

6. Conclusions

- Using an in vitro model of FSHD, we identify novel regulators of DUX4 expression.
- Losmapimod is a pselective p38 α / β inhibitor that reduces DUX4 expression in FSHD myotubes.
- Further in vitro characterization demonstrates that the DUX4-driven gene expression and cell death are inhibited in FSHD myotubes exposed to losmapimod.
- Losmapimod reduces expression of DUX4 in all FSHD1 and FSHD2 genotypes tested.
- Reduction of DUX4 expression results in inhibition of cell death across all genotypes tested.