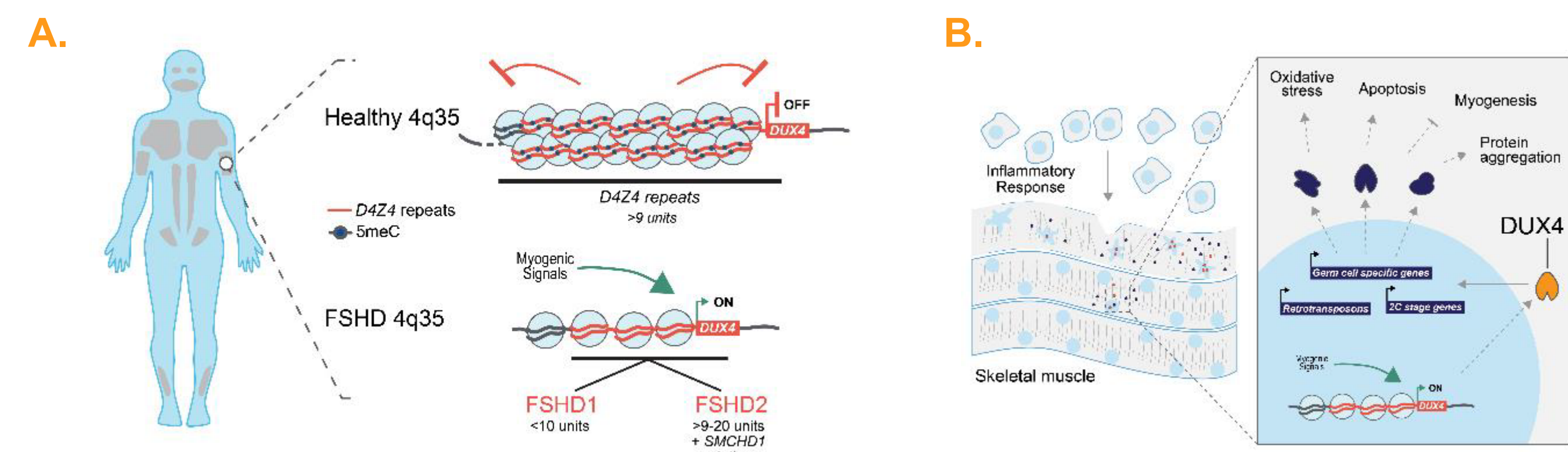


Abstract

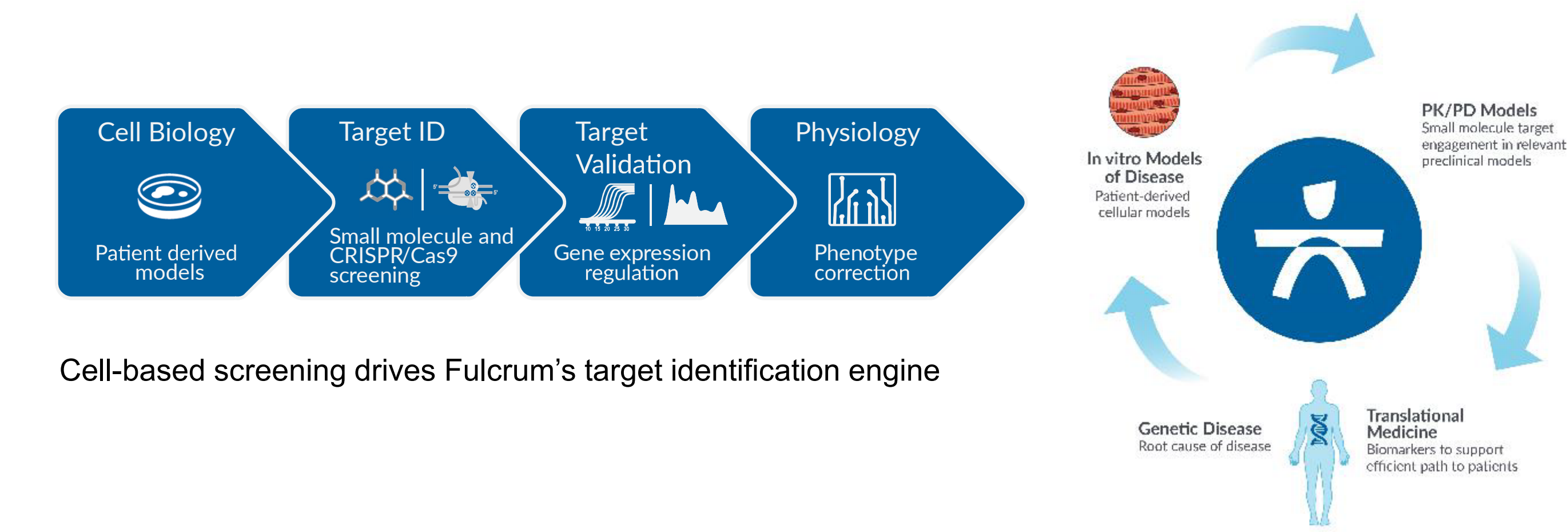
- FSHD is caused by the loss of repression at the D4Z4 locus leading to aberrant DUX4 expression in skeletal muscle, altered gene expression programming and muscle fiber death.
- Using optimized myotube culture conditions, we identified p38 MAPK as a key regulator of DUX4 expression.
- We observed that treatment with the p38 α/β inhibitor losmapimod 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, ~90% of these are targets of DUX4, with no negative impact on key drivers of myogenic programming.
- In vitro* findings highlight the potential of losmapimod for the treatment of FSHD, a condition that today has no approved therapies.

1. Facioscapulohumeral Muscular Dystrophy (FSHD)

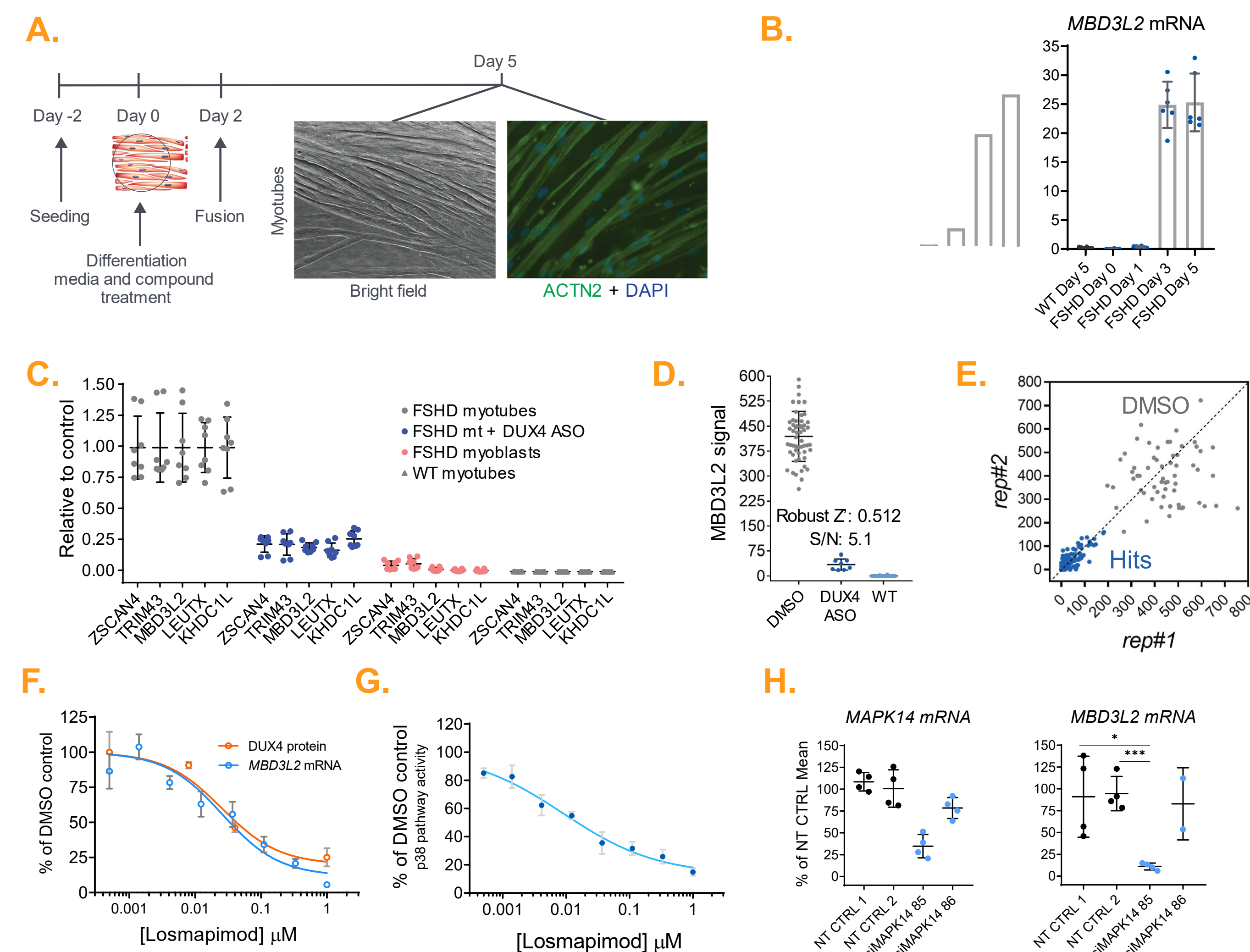


(A) Schematic of the loss in gene repression caused by contraction of D4Z4 repeats that leads to DUX4 expression in the muscle of FSHD1 patients. (B) Schematic describing the downstream consequences of DUX4 expression in skeletal muscle.

2. Fulcrum's approach to target identification and validation

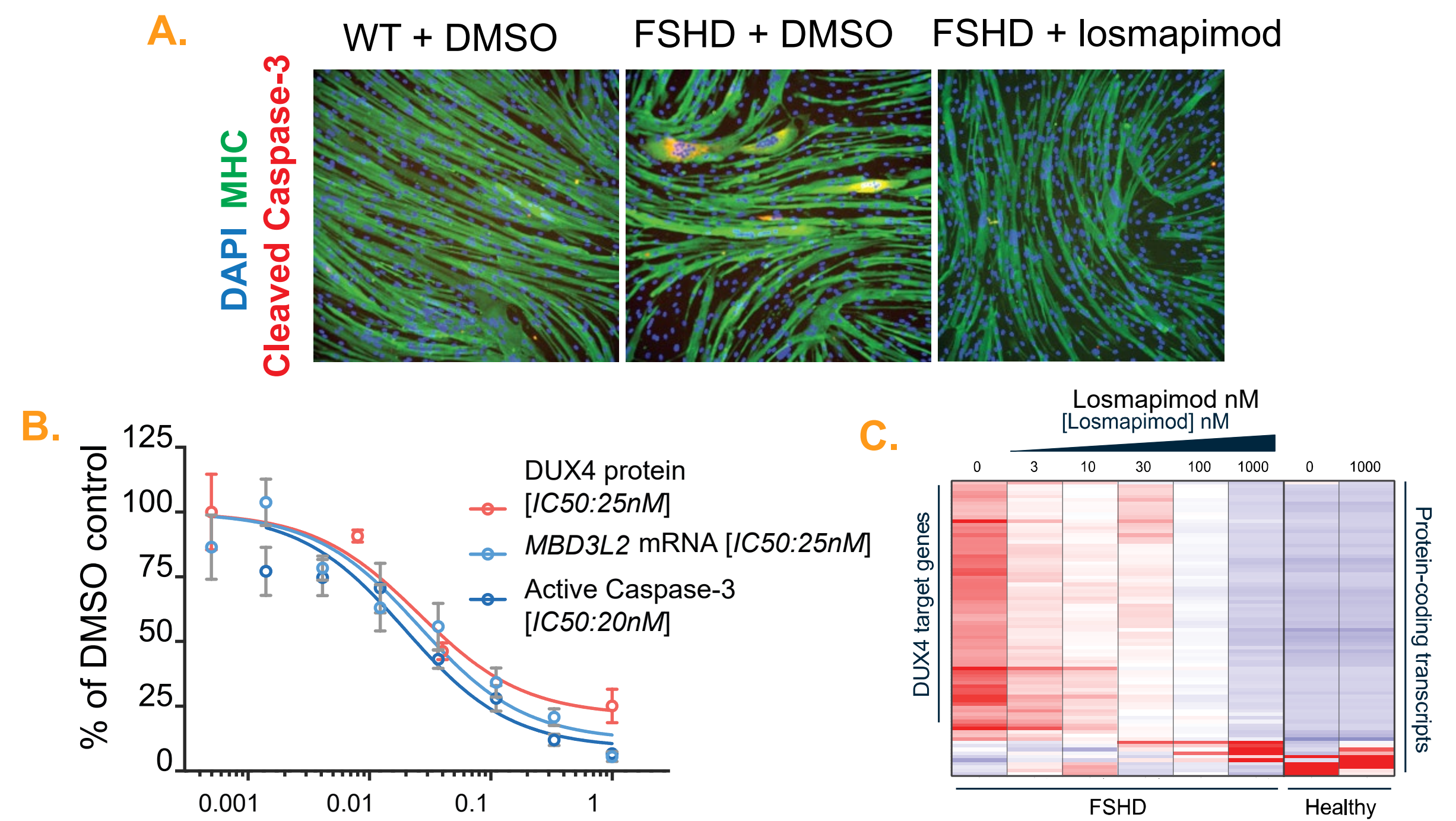


3. Identification of a drug target that inhibits DUX4 expression



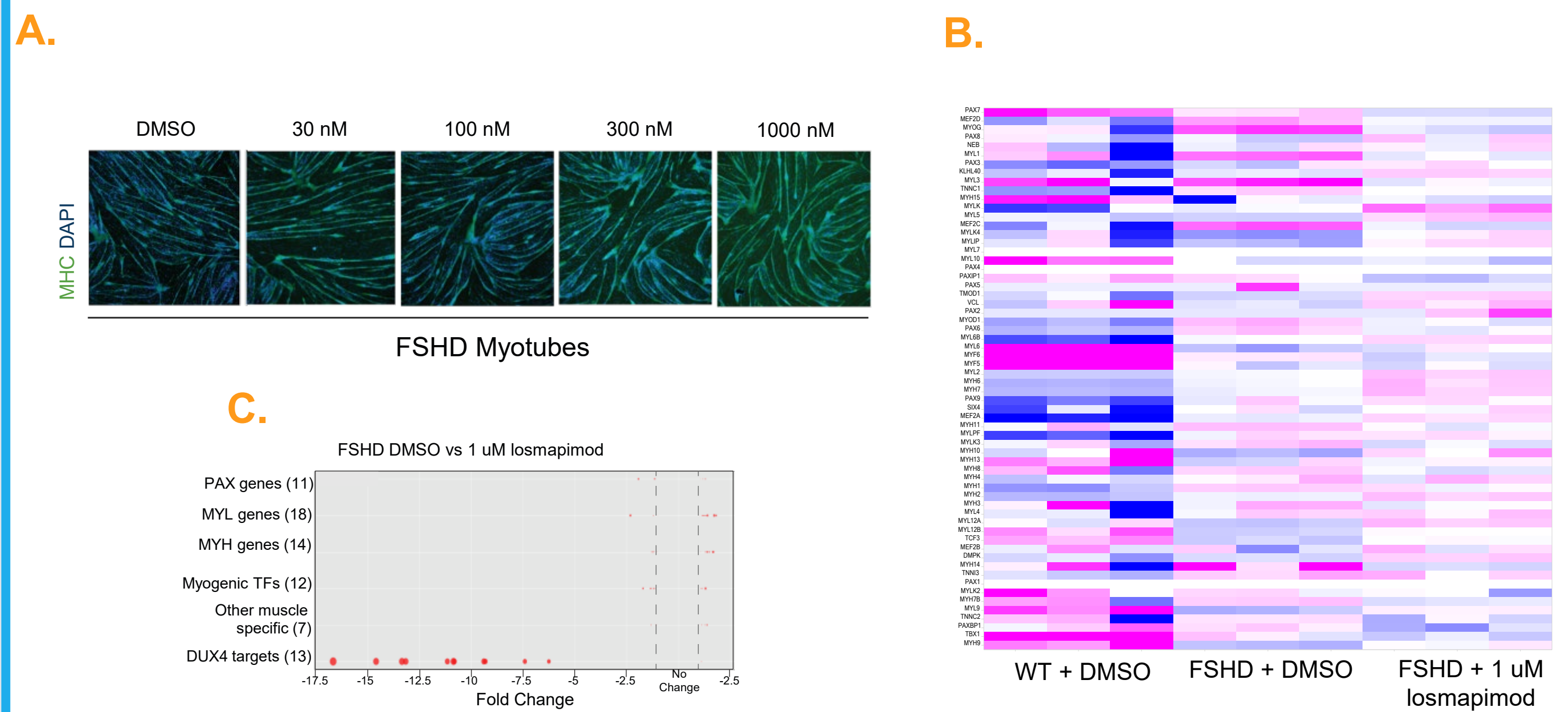
(A) Cell-based assay schematic. (B) Expression of DUX4 and DUX4 target gene *MBD3L2* during FSHD myotube differentiation *in vitro*. (C) DUX4 target gene selection for HTS. (D) 96-well format assay allows for identification of targets modulating the expression of DUX4. (E) Hits identified showed high correlation in between biological replicates. (F) Losmapimod reduces expression of DUX4 in a concentration dependent manner in FSHD myotubes. (G) Losmapimod reduces p38 α/β activity in FSHD myotubes. (H) P38 α knockdown reduces activity of DUX4 in FSHD myotubes.

4. Losmapimod reduces DUX4 activation and its downstream consequences



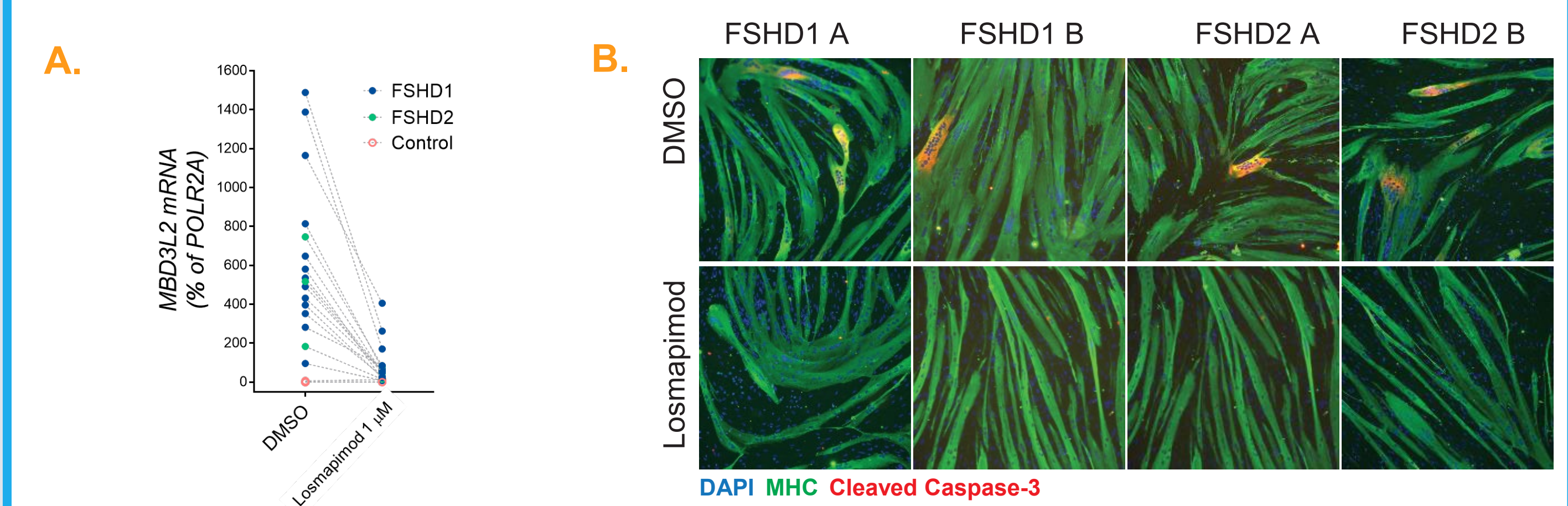
(A) FSHD myotubes show increased apoptosis as measured by ICC with activated (cleaved) caspase-3 staining compared to isogenic WT control. Treatment with 1 μ M losmapimod completely rescues apoptosis. (B) Quantification of ICC signal to measure cytoplasmic active caspase3 shows concentration-dependent apoptosis rescue upon treatment with losmapimod, with similar IC50 for target activity, DUX4 protein and DUX4 downstream gene expression. (C) 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. <math><100</math> differentially expressed genes (abs(FC)>4; FDR<0.001)

5. Losmapimod does not negatively impact myogenesis



(A) Treatment with losmapimod does not impact differentiation index quantified by ICC. (B) RNA-sequencing shows no dysregulation of key drivers of myogenic programming, comparing FSHD DMSO vs losmapimod treatment. (C) Visualization of changes in gene expression of selected families of genes shows no change in myogenic markers, only DUX4 downstream driven genes. Area of the circle represents significance in change of gene expression comparing DMSO and losmapimod treated myotubes.

6. Losmapimod results in downregulation of DUX4 expression and suppression of cell death across multiple FSHD1 and FSHD2 genotypes



(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 shows decreased apoptosis with losmapimod treatment.

6. Conclusions

- Using an *in vitro* model of FSHD, we identify novel regulators of aberrant DUX4 expression.
- Losmapimod is a selective p38 α/β inhibitor that reduces DUX4 expression in FSHD myotubes.
- Further *in vitro* characterization demonstrates that DUX4-driven gene expression and cell death are inhibited in FSHD myotubes exposed to losmapimod.
- Losmapimod does not negatively impact key drivers of myogenic programming.
- Reduction of DUX4 expression resulted in inhibition of cell death across all genotypes tested.
- in vitro* findings highlight the potential of losmapimod for the treatment of FSHD.