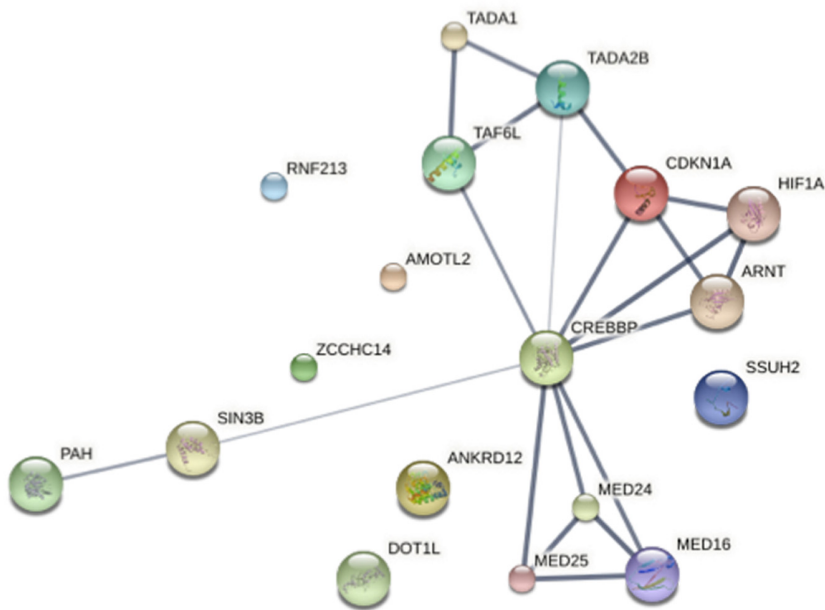
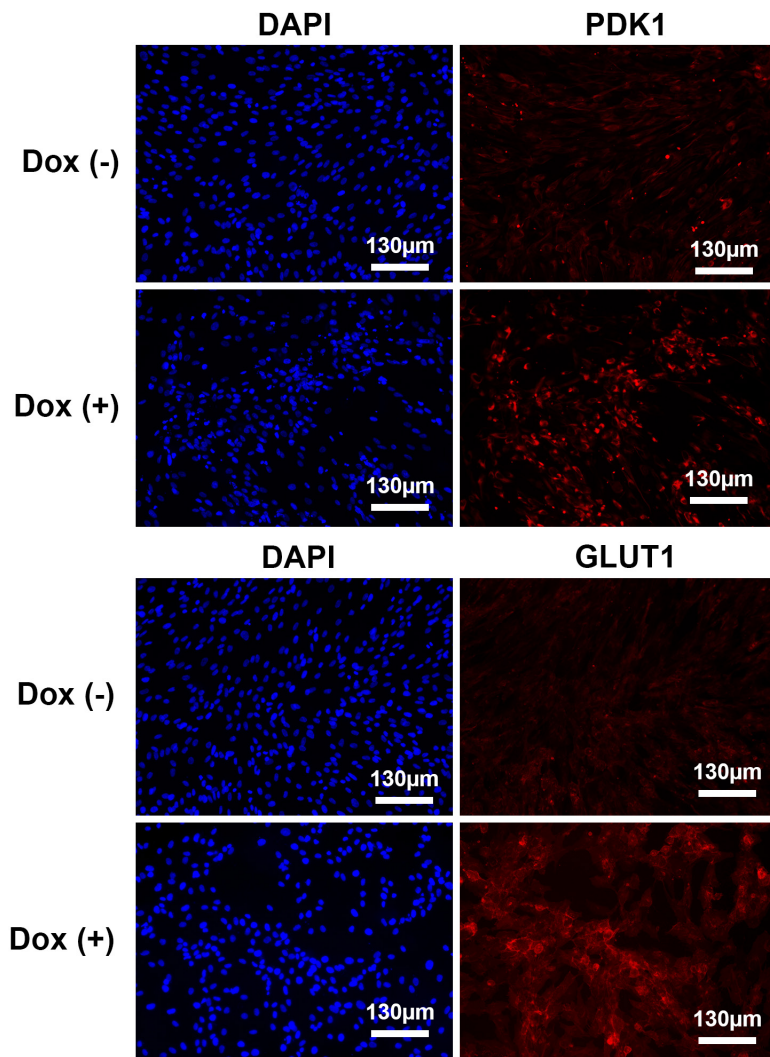


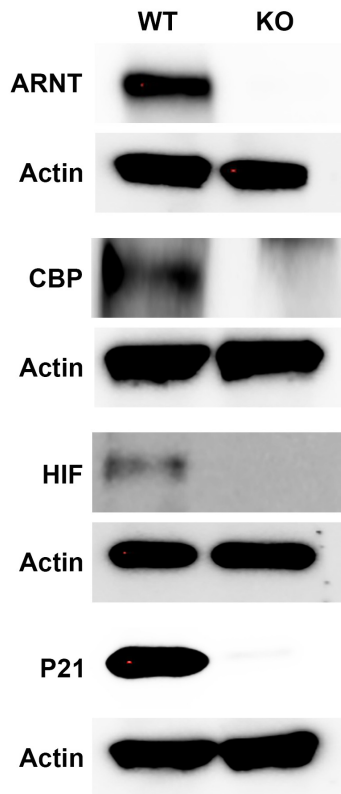
## Supplementary Materials and Methods



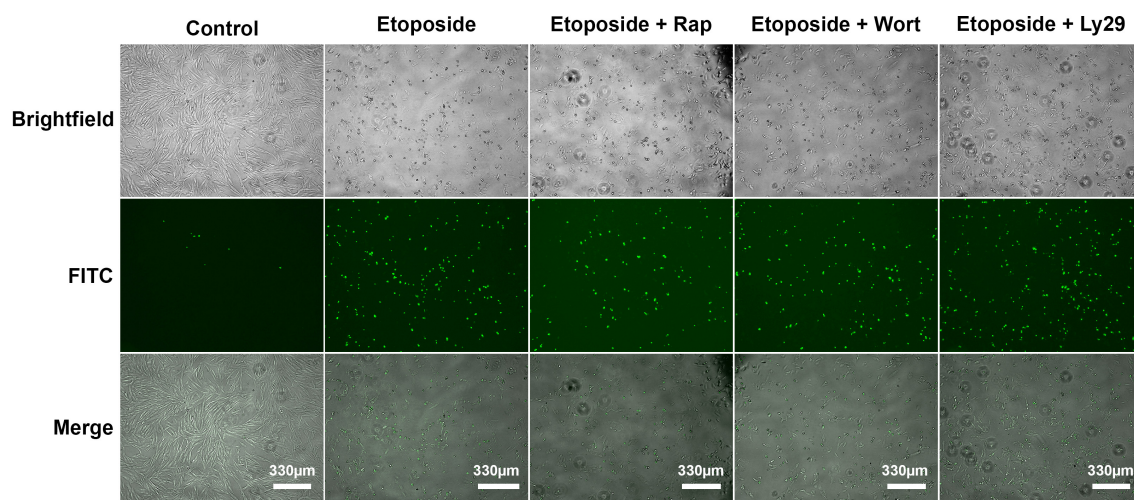
**Fig. S1. GeneMania functional associations between top screen hits.** Original output image generated by GeneMania's pathway analysis for significant ( $P < 0.01$ ) hits across two independent screens.



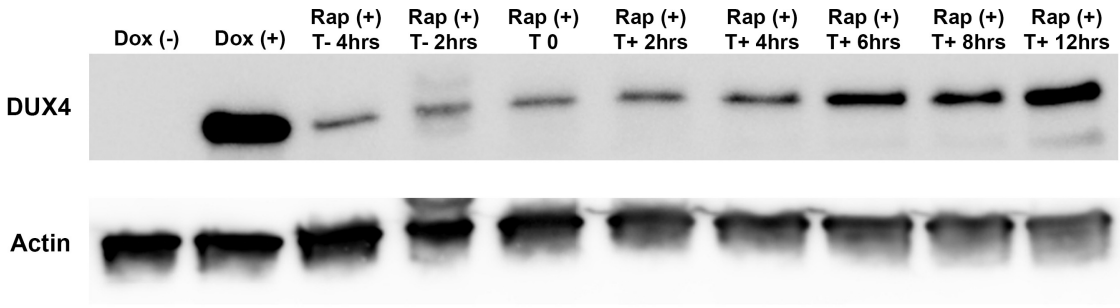
**Fig. S2. Changes in GLUT1 and PDK1 expression in the presence of DUX4.** Doxycycline (2 µg/mL) was added to MB135-DUX4i cells and fixed at 24hrs for immunofluorescence staining. Primary antibodies to GLUT1 and PDK1 (red) were applied with Alexa Fluor 555 secondary antibody and DAPI (blue) for nuclei counterstain.



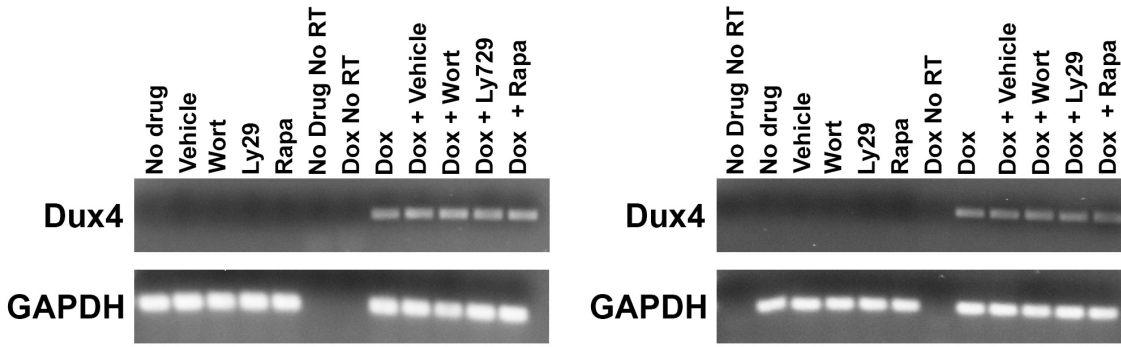
**Fig. S3. Western blot protein quantification of single gene knockouts.** For generation of single gene knockouts, guide sequences targeting each gene were designed and cloned into the lenti-CRISPR\_v2 plasmid. Lentivirus was produced and used to transduce the MB135-DUX4i line. After puromycin selection for 7 days, clonal populations were grown out and harvested for protein quantification using standard western blotting protocols.



**Fig. S4. Effect of inhibitors on etoposide-induced cell death.** Etoposide (Abcam, #ab120227, 150  $\mu$ M) was used to induce cell death in MB135-DUX4i cells in the presence and absence of inhibitor compounds (rapamycin, wortmannin and LY294002). At 48 hrs, CellEvent Caspase 3/7 FITC reagent was added to each well to visualize cell death under a fluorescent microscope.

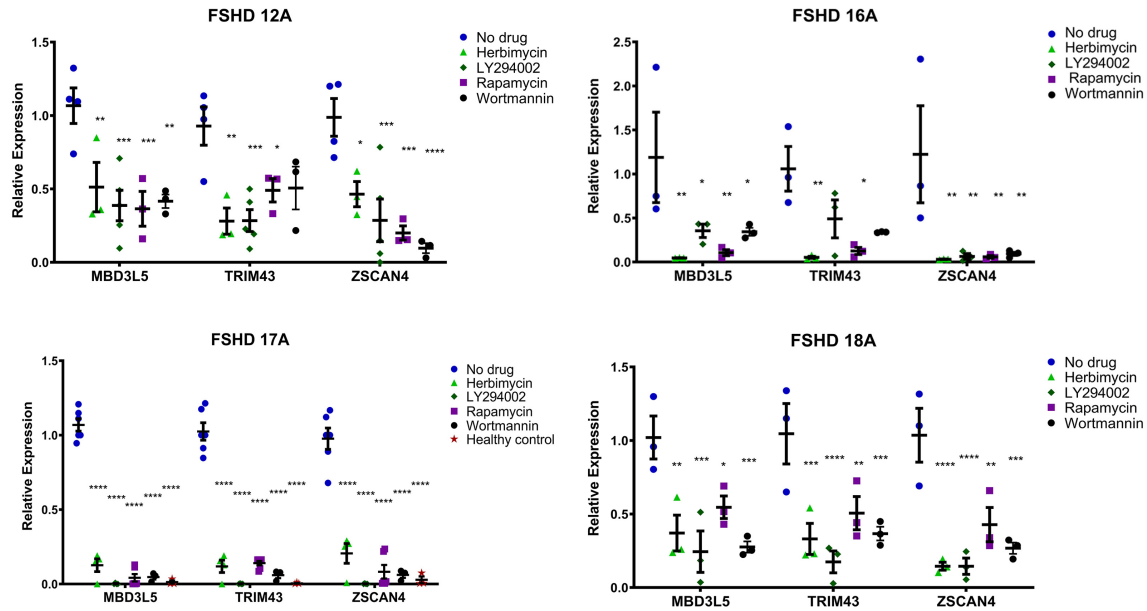


**Fig. S5. Western blot time-course of rapamycin dosing.** Rapamycin was added to MB135-DUX4i cells before and after inducing DUX4 expression, which resulted in varying amounts of DUX4 inhibition.



**Fig. S6. DUX4 transcript expression with inhibitor treatment.**

DUX4 transcript expression measured by RT-PCR on MB135-DUX4i cells with and without dox/drug treatment. GAPDH expression was measured in the same samples as a housekeeping gene control. Two independent drug treatments and RT-PCR reactions are shown above.



**Fig. S7. FSHD biomarker expression in four patient lines with inhibitor treatment.** Transcript of FSHD biomarkers MBD3L5, TRIM43, and ZSCAN4 in myotubes derived from four patient donor muscle biopsies (17A, 12A, 16A and 18A) in the presence of inhibitors compared to control (17U, shown in FSHD 17A plot). Myoblasts were differentiated to form myotubes for four days and test compounds were added overnight. The next day, cells were harvested for RNA extraction and cDNA synthesis. Quantitative PCR was performed using Taqman assays and expression was calculated by delta Ct relative to PPIA expression. Data are plotted as mean  $\pm$  SEM. Statistical analysis: One-way ANOVA and Tukey's multiple comparisons test were performed; \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , \*\*\*\* $P < 0.0001$ .

**Table S1. Oligo sequences for single gene knockout lines**

<b>Gene</b>	<b>Guide sequence for sgRNA cloning</b>
HIF1A	5'-CCTCACACGCAAATAGCTGA-3'
ARNT	5'-GACATCAGATGTACCATCAC-3'
CBP	5'-CAACTGTCGGAGCTTCTACG-3'
CDKN1A	5'-AGTCGAAGTTCCATCGCTCA-3'

**Table S2. Antibody concentrations.** WB = western blot, IF = immunofluorescence.

<b>Protein</b>	<b>Company</b>	<b>Species</b>	<b>Concentration</b>
HIF1A	Cell Signaling, 14179S	Rabbit monoclonal	1:1000 (WB)
HIF1A	ThermoFisher, PA1-16601	Rabbit polyclonal	1:100 (IF)
ARNT	Cell Signaling, 5537S	Rabbit monoclonal	1:1000 (WB)
CBP	Cell Signaling, 7389S	Rabbit monoclonal	1:1000 (WB)
CDKN1A	Cell Signaling, 2947S	Rabbit monoclonal	1:1000 (WB)
DUX4 (E55)	Abcam, ab124699	Rabbit monoclonal	1:1000 (WB)
DUX4 (P2B1)	Thermofisher, MA5-27584	Mouse monoclonal	1:400 (IF)
Beta-actin	Proteintech, 66009-2-1g,	Mouse monoclonal	1:10000 (WB)
GLUT1	Abcam, ab115730	Rabbit monoclonal	1:200 (IF)
PDK1	Abcam, ab202468	Rabbit monoclonal	1:1000 (IF)



**Table S3. Primer sequences used for RT-PCR**

<b>Primer</b>	<b>Sequence</b>
DUX4	Fwd: 5'-TAGGGGAAGAGGTAGACGGC-3' Rev: 5'-GGTTCCGGGATTCC-3'
GAPDH	Fwd: 5'-TGCACCACCAACTGCTTAGC-3' Rev: 5'-GGCATGGACTGTGGTCATGAG-3'