SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. MB135-iDUX4 myoblasts as a model system to uncover secreted DUX4-induced proteins. (**A**) Level of *DUX4* and DUX4 target gene *KHDC1L* mRNA as measured by RT-qPCR in MB135-iDUX4 myoblasts left untreated (-DOX) or treated with doxycycline (+DOX) to induce *DUX4* transgene expression for 12 or 24 hours. (**B**) *DUX4* mRNA as measured by RT-qPCR in parental MB135 myoblasts and in MB135-iDUX4 myoblasts left untreated (-DOX) or treated with doxycycline (+DOX) for 24 hours. (**C**) Data replotted from Figures 1B and 1C showing ALPP protein levels (log2 normalized) as measured by Olink Proteomics assay in cell lysate and supernatant (Sup.) from MB135-iDUX4 myoblasts left untreated (-DOX) or treated with doxycycline (+DOX) to induce transgene expression for 24 hours following no transfection or transfection with non-targeting control siRNA. Error bars denote the standard deviation from the mean of three replicates, which are shown as individual data points. (**D**) *DUX4* and DUX4 target gene *ZSCAN4* mRNA levels measured by RT-qPCR in MB135-iDUX4 myoblasts treated with or without doxycycline for 24 hours following transfection with no (-), non-targeting control (siCTRL), or *DUX4* (siDUX4) siRNA. (**E**) ALPP protein levels (log2 normalized) as measured by Olink Proteomics assay in the cell lysate and supernatant (Sup.) of parental MB135 myoblasts treated with (+DOX) or without (-DOX) doxycycline for 24 hours. All data points in panels A, B, D, and E represent single replicates.

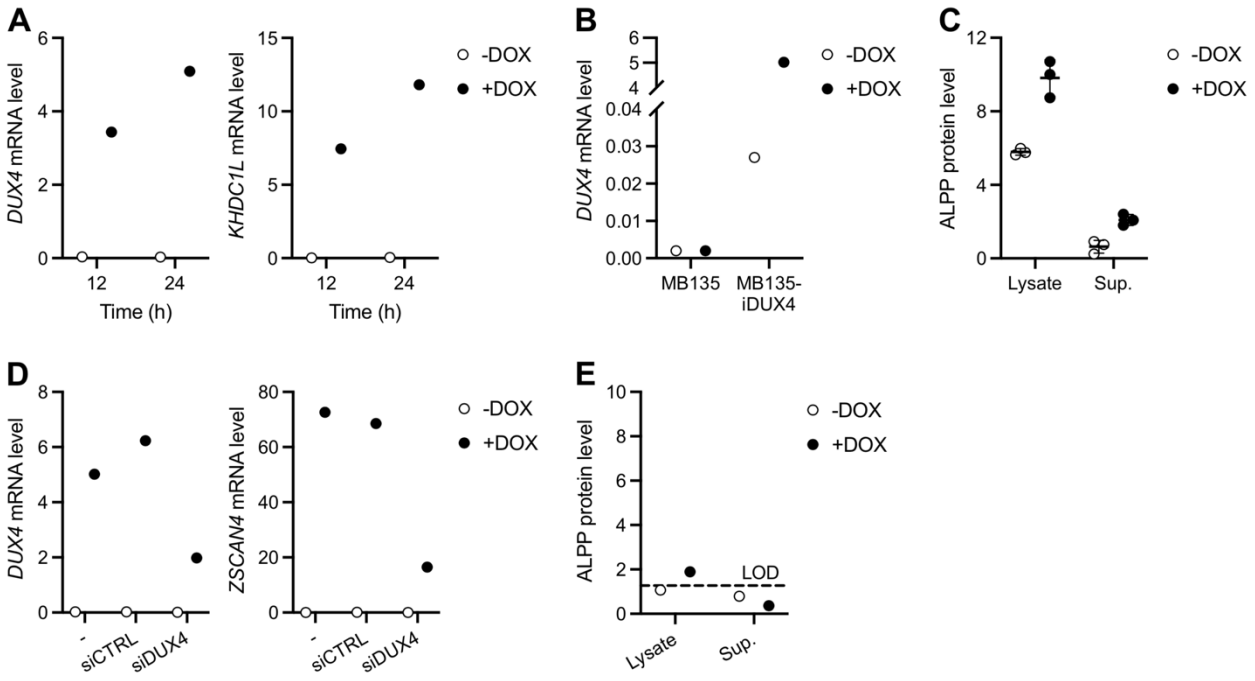
Supplementary Figure 2. Protein levels from cell culture studies. Separate heatmaps are shown for the Olink Proteomics Target 96 Oncology III and Target 96 Development panels for the (**A**) MB135-iDUX4 data, (**B**) MB135-iDUX4 siRNA data, and (**C**) FSHD versus control myotube data. NPX scores are on a log2 scale. Each replicate is displayed as a separate row. Sample type is indicated by columns in the left margin of each heatmap, with legends to the right of each heatmap. The DUX4 target proteins ALPP, CA2, and CRHBP are labelled in red in

the lower margin. Dox-, untreated; Dox+, doxycycline-treated; lys, cell lysate; sup, cell supernatant.

Supplementary Figure 3. Control and FSHD cell lines differentiate into myotubes. mRNA level of myogenic genes *MYOG* and *CKM* as measured by RT-qPCR in two independent control and three independent FSHD myoblast cell lines differentiated into myotubes for 0, 2, 4, or 6 days.

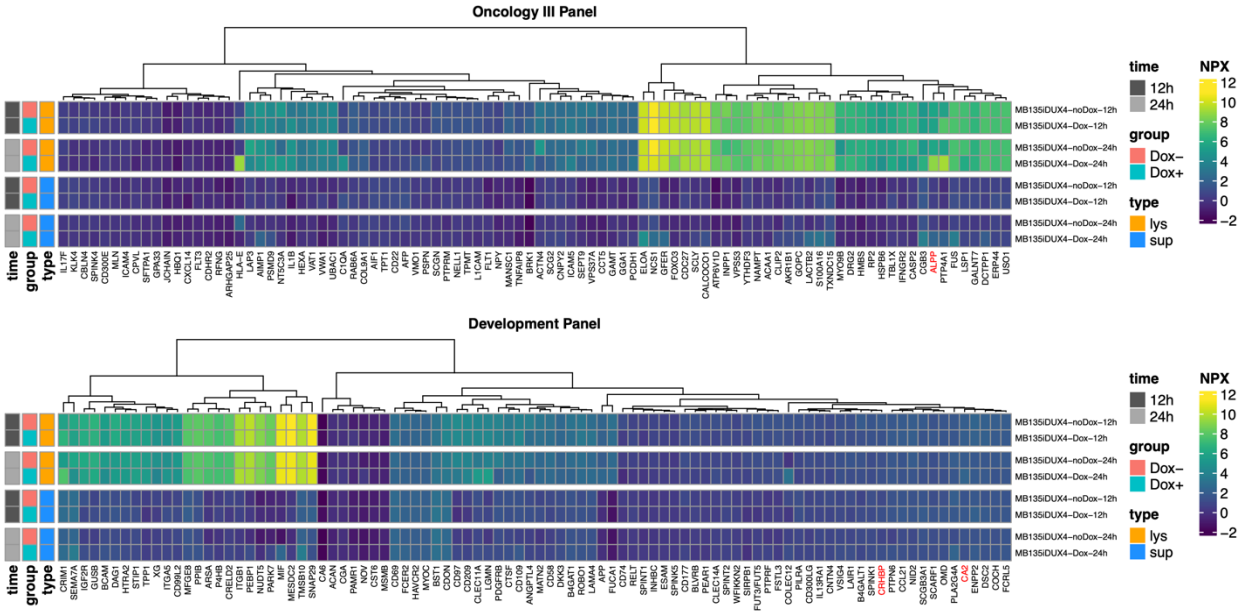
Supplementary Figure 4. Protein levels from serum study. Only the Olink Proteomics Target 96 Oncology III panel was used for serum analysis. NPX scores are on a log2 scale. Data is for n = 20 FSHD and n = 20 control individuals, each displayed as a separate row. Sample metadata (disease status, sex, age, CSS) is indicated by columns in the left margin of the heatmap, with legends to the right of the heatmap. (Missing values of CSS are shaded grey.) The DUX4 target protein ALPP is labelled in red in the lower margin, and is discussed in detail in the main text. The only proteins showing a significant difference associated with disease status and/or sex at FDR < 0.1, based on a LIMMA moderated F-test that is analogous to a two-way ANOVA, were HSPB6 and PSPN, both with FDR = 0.034. HSPB6 was up ~1.9-fold in FSHD versus control females with FDR = 0.021, and was up ~1.3-fold in FSHD versus control males but with non-significant FDR = 0.63. PSPN was up ~2.8-fold in males versus females with FDR = 0.0045, but without a significant interaction with FSHD status (FDR = 0.86). HSPB6, or Heat Shock Protein Family B (Small) Member 6, has recently been reported to be upregulated at the mRNA level in a transcriptomic study of Ant1-overexpressing mice [42], and overexpression of human ANT1 – a 4q35 gene involved in mitochondrial function – has also been associated with FSHD [43]. PSPN, or Persephin, is a secreted ligand of GDNF and TGF-beta proteins that is encoded on chromosome 19 and was also strongly elevated in male versus female plasma in a recent study using the Olink Proteomics platform [41].

SUPPLEMENTARY FIGURE 1



SUPPLEMENTARY FIGURE 2

A



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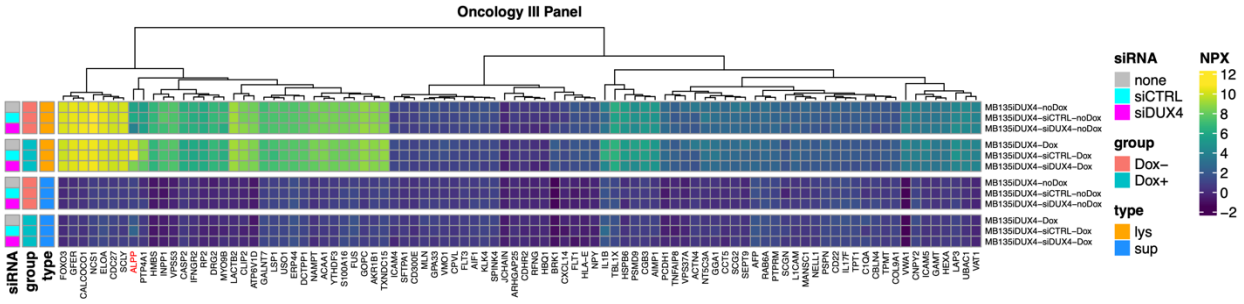

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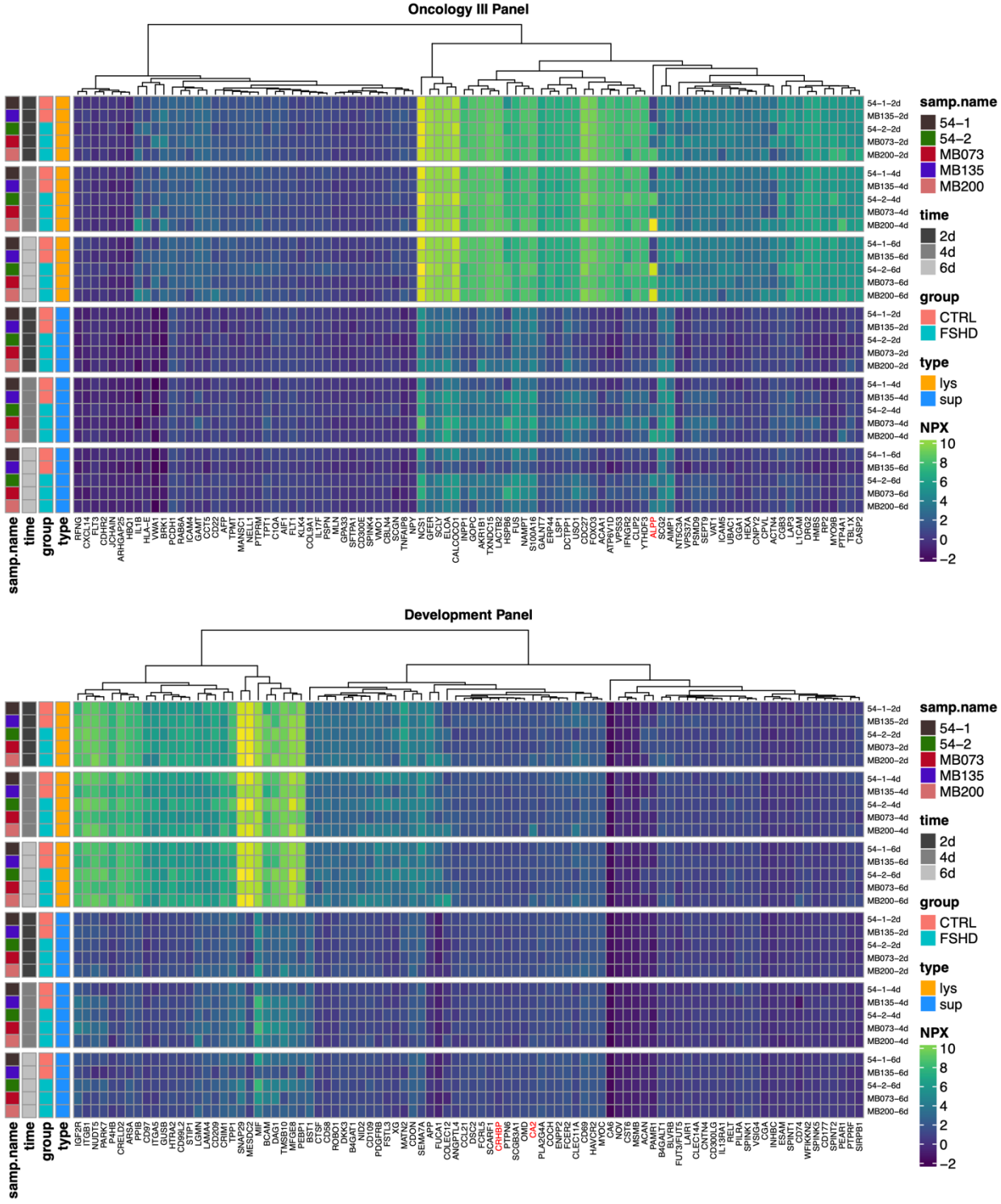
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Development Panel
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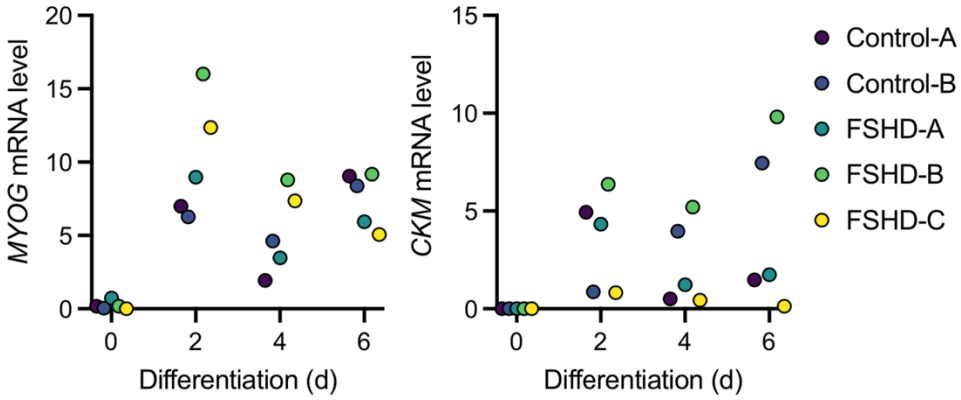
SUPPLEMENTARY FIGURE 3



SUPPLEMENTARY FIGURE 4