



HAL
open science

Chemical inhibition of SUMOylation activates the FSHD locus

Alice Nordlinger, Loéva Morin, Alexandra Andrieux, Jean Philippe Trani, Pierre Perrin, Nathalie Eudes, Anne Bigot, Anne Dejean, Frédérique Magdinier

► **To cite this version:**

Alice Nordlinger, Loéva Morin, Alexandra Andrieux, Jean Philippe Trani, Pierre Perrin, et al.. Chemical inhibition of SUMOylation activates the FSHD locus. *Scientific Reports*, inPress, <10.1038/S41598-025-33624-0>. <hal-05454310>

HAL Id: hal-05454310

<https://amu.hal.science/hal-05454310v1>

Submitted on 2 Feb 2026

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons CC BY 4.0 - Attribution - International License



OPEN Chemical inhibition of SUMOylation activates the FSHD locus

Alice Nordlinger^{1,2}, Loéva Morin³, Alexandra Andrieux^{1,2}, Jean Philippe Trani³, Pierre Perrin³, Nathalie Eudes³, Anne Bigot⁴, Anne Dejean^{1,2,5} & Frédérique Magdinier^{3,5}✉

Facioscapulo humeral muscular dystrophy (FSHD) is a progressive and debilitating muscle disease for which no cure currently exists. In the majority of cases, FSHD is associated with the contraction of the D4Z4 macrosatellite repeat array at the 4q35 locus, leading to the inappropriate activation of *DUX4*, normally expressed during early embryogenesis. In FSHD, the genetic contraction is accompanied by hypomethylation of the D4Z4 array. Although a connection between DNA hypomethylation and *DUX4* expression has been suggested, the precise mechanisms that regulate *DUX4* transcription remain incompletely defined. The post-translational modification by SUMO was shown previously to repress the expression of *Dux*, the *DUX4* homolog, in mouse embryonic stem cells. Based on these findings, we explored here the contribution of SUMOylation in the regulation of *DUX4* in human muscle cells. We demonstrate that TAK-981 (subasumstat), a selective SUMOylation inhibitor, promotes transcriptional reprogramming of the 4q35 locus and induces *DUX4* expression. Importantly, this activation occurs independently of changes in DNA methylation or SMCHD1 ATPase activity. Our findings identify SUMOylation inhibition as a novel regulatory process driving *DUX4* expression. This work uncovers the importance of SUMOylation in the epigenetic control of the 4q35 locus and *DUX4* transcription, providing a potential therapeutic strategy to modulate *DUX4* expression in FSHD.

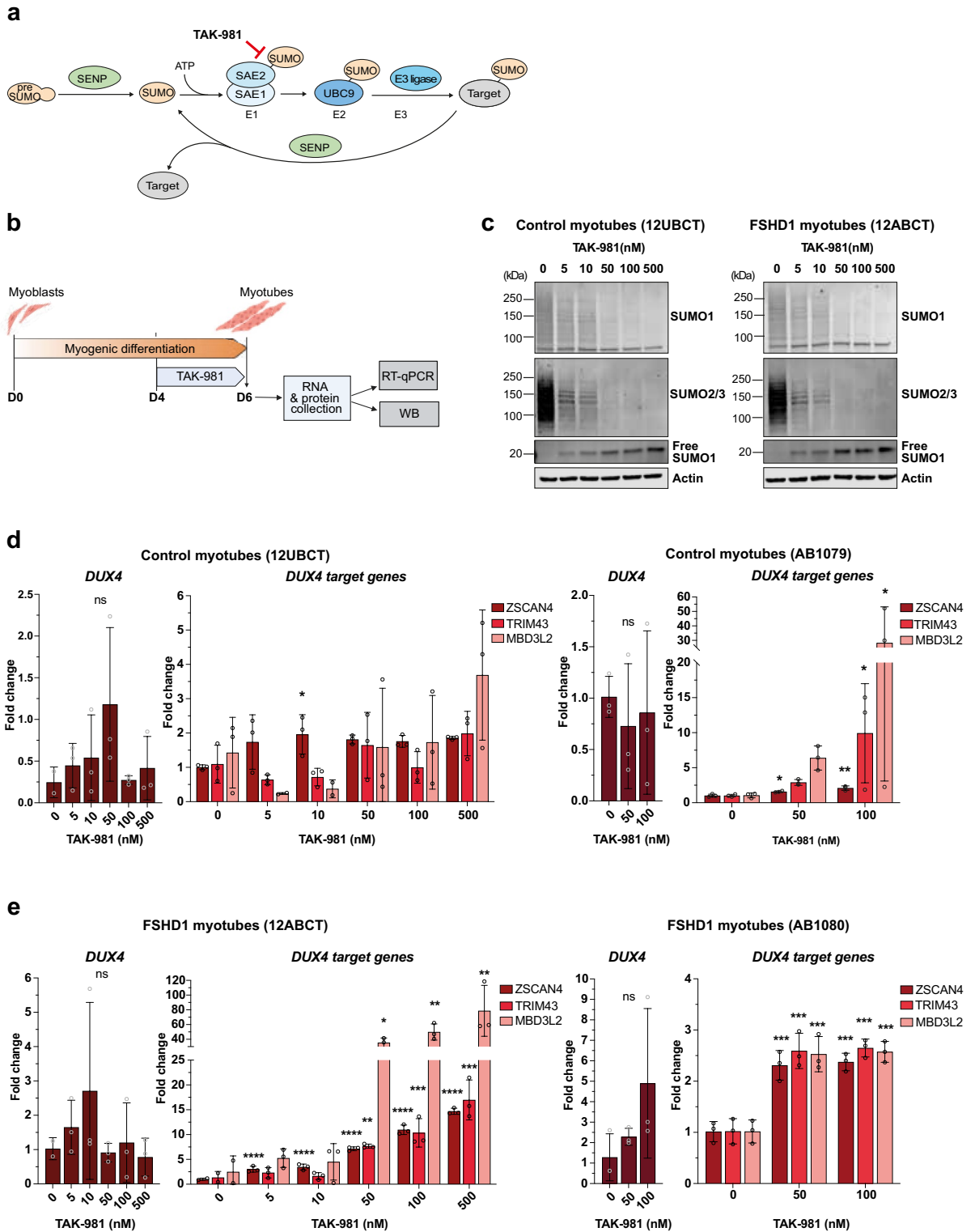
Keywords SUMOylation, SUMO, SMCHD1, FacioScapuloHumeral Dystrophy, D4Z4, DUX4, Muscle

SUMOylation is a reversible post-translational modification that regulates protein functions through covalent attachment of small ubiquitin-like modifier (SUMO) proteins to their protein substrates in eukaryotic cells^{1,2}. SUMO proteins exist as three main paralogues in mammals, SUMO1, SUMO2 and SUMO3, with SUMO2 and 3 sharing high similarities. SUMOylation is a dynamic energy-dependent process involving unique E1 and E2 enzymes and a series of E3 ligases. The reversible aspect of SUMOylation is ensured by action of specific SUMO proteases called SENPs (Fig. 1a). The SUMO pathway, which predominantly targets transcription factors and chromatin-associated proteins^{3–6}, is a key regulator of nuclear processes and cell identity. In particular, SUMOylation was reported to regulate the expression of *Dux* in mESCs, with loss of SUMOylation leading to derepression of *Dux* and transition to a totipotent-like state^{7–10}. In mESCs, SUMOylation-dependent silencing of *Dux* was shown to involve Prc1.6 and Kap/Setdb1 recruitment onto the *Dux* locus⁷, as well as proper Dppa2/Dppa4^{8,9} and histone H1 SUMOylation¹⁰.

The mouse *Dux* and human *DUX4* transcription factors are involved in the zygotic genome activation (ZGA) during the 2C–4C stage in mouse and 4C–8C stage in human embryos, respectively^{11,12}. In human cells, *DUX4* is encoded by the D4Z4 macrosatellite element that is located in the subtelomeric region of the 4q arm (4q35 locus)¹³. Each D4Z4 element is approximately 3.3 kilobases in size and forms large tandemly repeated array structures at the subtelomeric 4q35 and 10q26 loci^{14,15}. Smaller size or partial D4Z4-like sequences are scattered in heterochromatin regions throughout the genome, in particular at the p arm of acrocentric chromosomes. At 4q35 and 10q26 loci, the number of repeat units can vary between individuals, typically ranging from 11 to over 100¹⁶. These arrays are highly GC-rich and present with heterochromatin features such as DNA methylation, leading to epigenetic repression^{17–19}.

The 4q35 locus is linked to type 1 Facioscapulo humeral Dystrophy (FSHD1, OMIM #158900), an autosomal dominant neuromuscular disease that is characterized by a progressive weakness and wasting of specific skeletal

¹Nuclear Organization and Oncogenesis Unit, Department of Cell Biology and Infection, Institut Pasteur, Université Paris Cité, 75015 Paris, France. ²INSERM, U993, 75015 Paris, France. ³Aix Marseille Univ, INSERM, Marseille Medical Genetics, 13005 Marseille, France. ⁴Inserm, Institut de Myologie, Centre de Recherche en Myologie, Sorbonne Université, IParis, France. ⁵Anne Dejean and Frédérique Magdinier : have contributed equally. ✉email: frederique.magdinier@univ-amu.fr



muscles of the face, shoulder girdle, and upper arms^{20,21}. In individuals with FSHD1, the D4Z4 repeat array on chromosome 4q35 is contracted to contain 1–10 repeat units, compared to the normal size range of 11–100 repeats in the unaffected population. This contraction is associated with D4Z4 DNA hypomethylation and chromatin opening enabling aberrant expression of the *DUX4* gene from the most distal D4Z4 repeat. Importantly, disease manifestation requires the presence of a permissive 4qA haplotype distal to the last D4Z4 unit, which provides a functional polyadenylation signal necessary for stable *DUX4* transcript production²². FSHD2 (5% of patients, OMIM #158901) is clinically similar to FSHD1 but arises from a digenic mechanism in the absence of D4Z4 array contraction. In approximately 80% of these FSHD2 patients, the disease is associated with pathogenic variants of *SMCHD1* and involves D4Z4 hypomethylation^{23,24}, allowing *DUX4* expression from a normally sized repeat array²⁵. In human somatic cells, experimental overexpression of *DUX4* activates the

◀ **Fig. 1.** Chemical inhibition of SUMOylation induces a coordinated *DUX4* program in FSHD1 patient-derived myotubes. **(a)** Representation of the reversible SUMOylation process. Before the first conjugation, a nascent SUMO precursor is proteolytically processed by a SUMO protease (SENP). Mature SUMO is then conjugated to a SAE1-SAE2 complex (E1) in an ATP-dependent manner. Next, activated SUMO is transferred to the E2 conjugating enzyme UBC9, before being conjugated to a target protein by the E3 ligase. SUMO conjugates can be cleaved from the target protein by a SENP, ensuring a dynamic and reversible SUMOylation process. TAK-981 inhibits SUMOylation by blocking activated SUMO at the catalytic site of SAE2. **(b)** Schematic representation of the experimental design for human hTERT-immortalized myoblasts²⁹. At confluence, control or FSHD1 immortalized myoblasts were differentiated for 6 days in the presence of 2% horse serum and treated with DMSO or TAK-981 at different concentrations from day 4 to 6 of differentiation. At day 6, RNA and proteins were collected for gene expression analysis by RT-qPCR and western blot experiments. **(c)** Immunoblots for SUMO1 and SUMO2/3 in myotubes from control (12UBCT) and FSHD1 (12ABCT) cells. Actin was used as a loading control. **(d)** *DUX4-fl* and *DUX4* target gene expression analyses in two cell lines of control myotubes (12UBCT and AB1079). Fold-changes were calculated relative to three housekeeping genes and normalized to the untreated (0) condition for each gene. Error bars represent mean \pm SD. $n = 3$. Significance: one-way ANOVA testing. ns: not significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$. **(e)** *DUX4-fl* and *DUX4* target gene expression analyses in myotubes obtained from two FSHD1 patients (12ABCT and AB1080). Fold-changes were calculated relative to three housekeeping genes and normalized to the untreated (0) condition for each gene. Error bars represent mean \pm SD. $n = 3$ independent experiments. Significance: one-way ANOVA testing. ns: not significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.

expression of more than 400 genes. This cascade of transcriptional activation has been associated with immune response, muscle inflammation and atrophy or inhibition of muscle regeneration²⁶.

Given the role of SUMOylation in the regulation of *Dux* in mESCs, we decided to evaluate whether the SUMO pathway may similarly regulate the expression of *DUX4* in human muscle cells and, in particular, in the context of FSHD. To this aim, we exploited two different cellular models in which we decreased the global cellular levels of SUMOylation using a selective chemical inhibitor to investigate the role of SUMOylation in the regulation of *DUX4* and the 4q35 region, as well as the possible involvement of SMCHD1 post-translational modifications²⁷. We show that TAK-981 (subasumstat), a first-in-class SUMOylation inhibitor²⁸, promotes transcriptional reprogramming at the 4q35 locus and induces *DUX4* expression, independently of changes in DNA methylation or SMCHD1 ATPase activity.

Materials and methods

Ethics declaration and cellular models

CDK4/hTERT immortalized myoblasts (12UBCT, 12ABCT) were described in²⁹ and obtained from the BBRI/Wellstone Institute. The 12ABCT cells were obtained from a FSHD1 patient carrying 5 D4Z4 units. The hTERT/CDK4-immortalized AB1080 and AB1079 lines were provided by the MyoLine platform of the Institute of Myology (Paris, France). Prior to hTERT/CDK4 immortalization, primary myoblasts were isolated from muscle samples anonymously provided by MyoBank, a tissue bank affiliated with EuroBioBank and authorized by the Ministry of Higher Education, Research and Innovation (AC-2019-3502). The AB1080 cell line was derived from paravertebral muscles of a FSHD1 patient carrying 6.3 D4Z4 units. The AB1079 cell line was obtained from the quadriceps of a 38 years old male. These cell lines were cultured in proliferative conditions in a Growth Medium composed of 4:1 volumes of Dulbecco's modified Eagle medium (DMEM, Gibco)/Medium 199 (Gibco), supplemented with 15% fetal bovine serum (Euroclone), 0.02 m HEPES buffer (Invitrogen), 1.4 mg L⁻¹ vitamin B12 (Sigma); 0.03 mg L⁻¹ ZnSO₄ (Fisher Scientific), 0.055 mg L⁻¹ dexamethasone (Sigma), 2.5 μ g L⁻¹ hepatocyte growth factor (Chemicon International) and 10 μ g L⁻¹ basic fibroblast growth factor (BioPioneer) in the presence of 1% Penicillin/streptomycin. Cells were tested for the presence of the PAS sequence and are all permissive for *DUX4* expression.

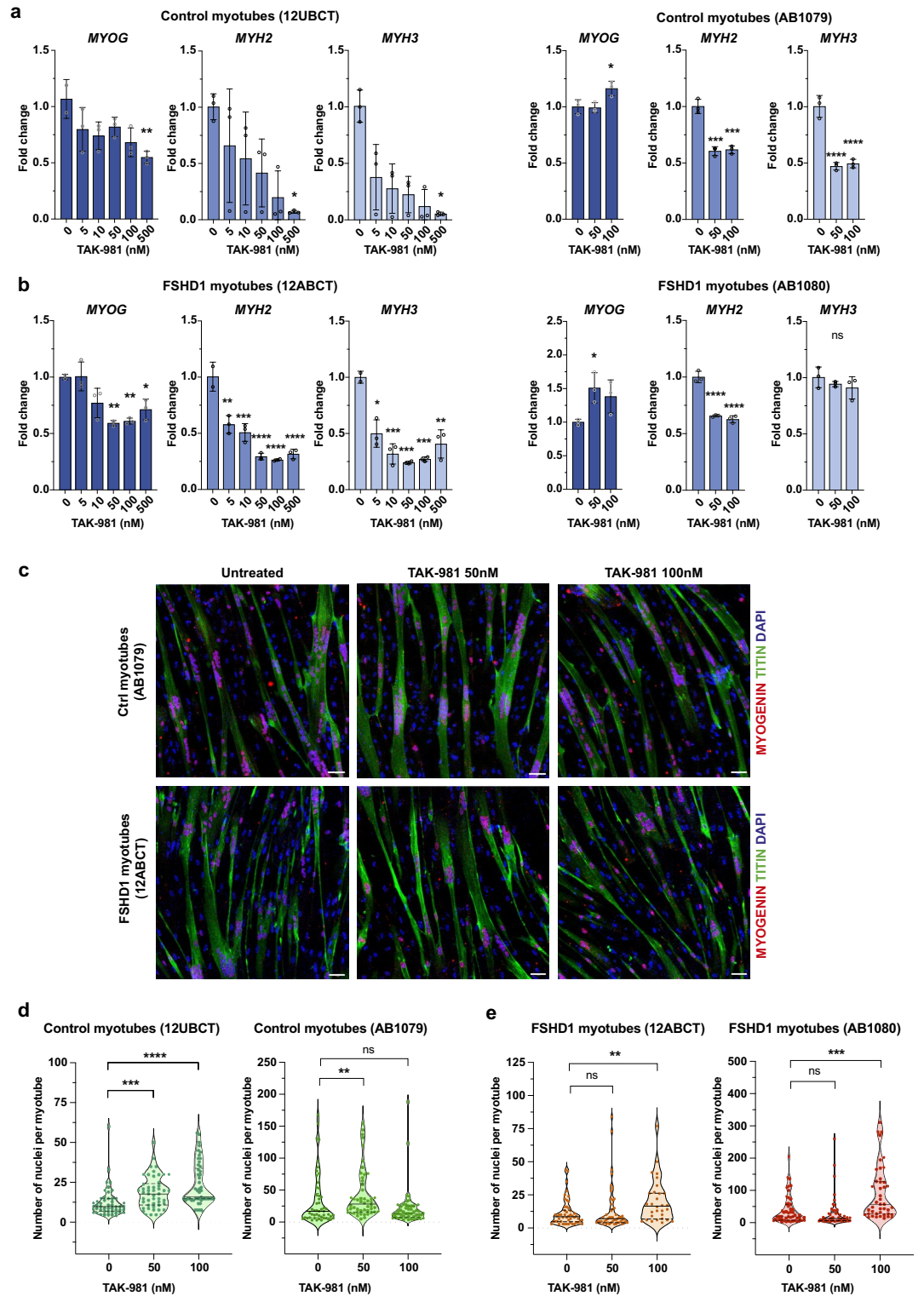
All hiPSC clones were generated from reprogramming of primary fibroblasts as described in³⁰. The C5 hiPSC clone (AG08498) was derived from human fibroblasts purchased from the Coriell Institute³¹. The 17706 hiPSC clones, 17706-L (long) and 17706-S (short, 2 D4Z4 units) were derived from a mosaic patient carrying one normal 4q35 allele and one shorter allele with 2 RU (FSHD1; female; age 56 at sampling). The 14,586 hiPSC clone was derived from a patient carrying two normal 4q35 alleles but one *SMCHD1* mutation (c.573A > C; p.Q193P; FSHD2; male; age 67 at sampling). hiPSC colonies were grown and expanded in mTeSR1 medium (Stemcell Technologies) on BD Matrigel (BD Biosciences, 354,277) coated dishes as described^{30,31}. Primary fibroblasts were provided by the Center for biological Resources (Department of Medical Genetics, La Timone Children's hospital) with the DC-2024-6282 (sample collection) and N°IE-2013-710 (ethical approval, Assistance Publique des hôpitaux de Marseille) accreditation numbers. Informed consent was obtained for all patients participating in the study or legal guardian for minor participants.

All other materials and methods are described in the supplemental information.

Results

SUMOylation inhibition activates the *DUX4* program in human myotubes

Given the repressive role of SUMOylation on *Dux* expression in mESCs, we explored the consequences of loss of SUMOylation in human muscle cells, in particular in the context of FSHD, which is characterized by aberrant expression of *DUX4* in skeletal muscle. To this end, we treated myotubes derived from hTERT/



CDK4-immortalized control and FSHD myoblasts with the TAK-981 SUMOylation inhibitor (henceforth called SUMOi) (Fig. 1a) and analyzed the expression of the pathogenic long *DUX4* transcript (*DUX4-fl*) and some of its target genes by RT-qPCR. TAK-981 covalently binds to the SUMO-E1 Activating Enzyme complex (SAE1) and prevents the activation and transfer of SUMO proteins to SUMO E2 UBC9 and downstream protein targets²⁸ (Fig. 1a). Immortalized myotubes were treated for 48 h (from day 4 to day 6 post-differentiation) with increasing doses of TAK-981 (0–500 nM, final concentration) and harvested for molecular analyses (Fig. 1b).

By western blotting, we observed a progressive and dose-dependent accumulation of free SUMO1 coupled with a decrease in SUMO1- and SUMO2/3-conjugated proteins in control and FSHD myotubes (Figs. 1c, S1a, b), attesting the efficacy of TAK-981 at tested concentrations. We next showed that SUMOylation inhibition is associated with a trend towards increased *DUX4-fl* expression, mainly in FSHD myotubes (Figs. 1d, e, S1c), with possible differences associated with the muscle-of-origin for available cell lines. Concomitantly, we observed a

◀ **Fig. 2.** Inhibition of SUMOylation impairs myogenic gene expression in control and FSHD1-patient derived myotubes. **(a)** *MYOG*, *MYH2* and *MYH3* expression analyses in control myotubes (12UBCT and AB1079). Fold-changes were calculated relative to three housekeeping genes and normalized to the untreated (0) condition for each gene. Error bars represent mean \pm SD. $n = 3$. Significance: one-way ANOVA testing. ns: not significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$. **(b)** *MYOG*, *MYH2* and *MYH3* expression analyses in myotubes obtained from FSHD1 patients (12ABCT and AB1080). Fold-changes were calculated relative to three housekeeping genes and normalized to the untreated (0) condition for each gene. Error bars represent mean \pm SD. $n = 3$. Significance: one-way ANOVA testing. ns: not significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$. **(c)** Immunostaining of differentiated control (AB1079) and FSHD1 (12ABCT) myotubes collected at day 6 after TAK-981 treatment at indicated concentrations (50 and 100 mM from day 4 to 6). Cells were stained with DAPI, anti-Titin and anti-Myogenin antibodies. Scale bar = 50 μm . **(d)** Quantification of nuclei per myotube for each immortalized myoblast line at day 6 after TAK-981 treatment at indicated concentrations. Each violin plot represents the distribution of individual nuclei count values. Black horizontal lines mark the median and dashed lines indicate quartiles. Data were compared using Kruskal–Wallis tests with Dunn’s correction for multiple comparisons. ns: not significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.

slight dose-dependent increase in the expression of some of the selected DUX4 target genes (*ZSCAN4*, *TRIM43*, *MBD3L2*) in control cells (12UBCT²⁹, AB1079). Increase in DUX4 target gene expression is more pronounced and statistically significant in FSHD cells (12ABCT²⁹, AB1080), in particular for high doses of TAK-981 (Fig. 1e). In addition, we noted a moderate but significant impact of SUMO_i on the expression of the *MyoG* myogenic marker in both control and FSHD cells, with a maximal effect at higher doses of TAK-981 (Fig. 2a, b). We also reported a dose-dependent decreased expression in *MyH2* and *MyH3*. Immunostaining of myofibers at Day6 with anti-Titin or anti-Myogenin antibodies showed no significant change in the proportion of myotubes in treated control or FSHD cells compared to mock-treated cells (Fig. 2c). Notably, SUMO_i-treated cells exhibited a significant increase in the fusion index, indicating that inhibition of SUMOylation does not impair but rather enhances muscle differentiation in both control and FSHD cells (Fig. 2d, e).

SUMOylation inhibition enhances DUX4 expression in hiPSC-derived muscle cells

Next, we investigated the impact of SUMO_i on *DUX4-fl* induction during muscle tissue differentiation. To this aim, we treated control and FSHD muscle cells derived from induced pluripotent cells (hiPSCs)³⁰ over a period of 15 days post-differentiation with daily change in cell culture medium supplemented with TAK-981 or DMSO alone (Fig. 3a). Different concentrations of TAK-981 were used and cells were collected at the end of the differentiation procedure (day 30, D30) for RNA and protein extraction. Efficiency of SUMOylation inhibition was verified by western blotting (Fig. S2a–c).

In control cells, we observed a robust increase in *DUX4-fl* expression at the highest dose of TAK-981 (Fig. 3b), associated with increased expression of selected DUX4 target genes, *MBD3L2* and *LEUTX* in particular. For FSHD1, we first compared the effect of SUMO_i on the transcriptional activation of *DUX4-fl* and DUX4 target gene in hiPSC-derived muscle tissue for a mosaic patient for which healthy (Fig. 3c) and diseased (Fig. 3d) isogenic clones were isolated³⁰. In both contexts, we observed a dose-dependent increase in *DUX4-fl* expression together with a significant increase in the expression of DUX4 target genes that is more consistent in FSHD cells (17706-S) and observed at low doses of TAK-981 (Fig. 3c, d). Similarly, expression of the *MyoD* (a DUX4 target) and *MyoG* myogenic factor and expression of Myosin genes (*MyH2*, *MyH3*) were decreased in both control and FSHD cells, at low doses of TAK-981 (Fig. 3e, f).

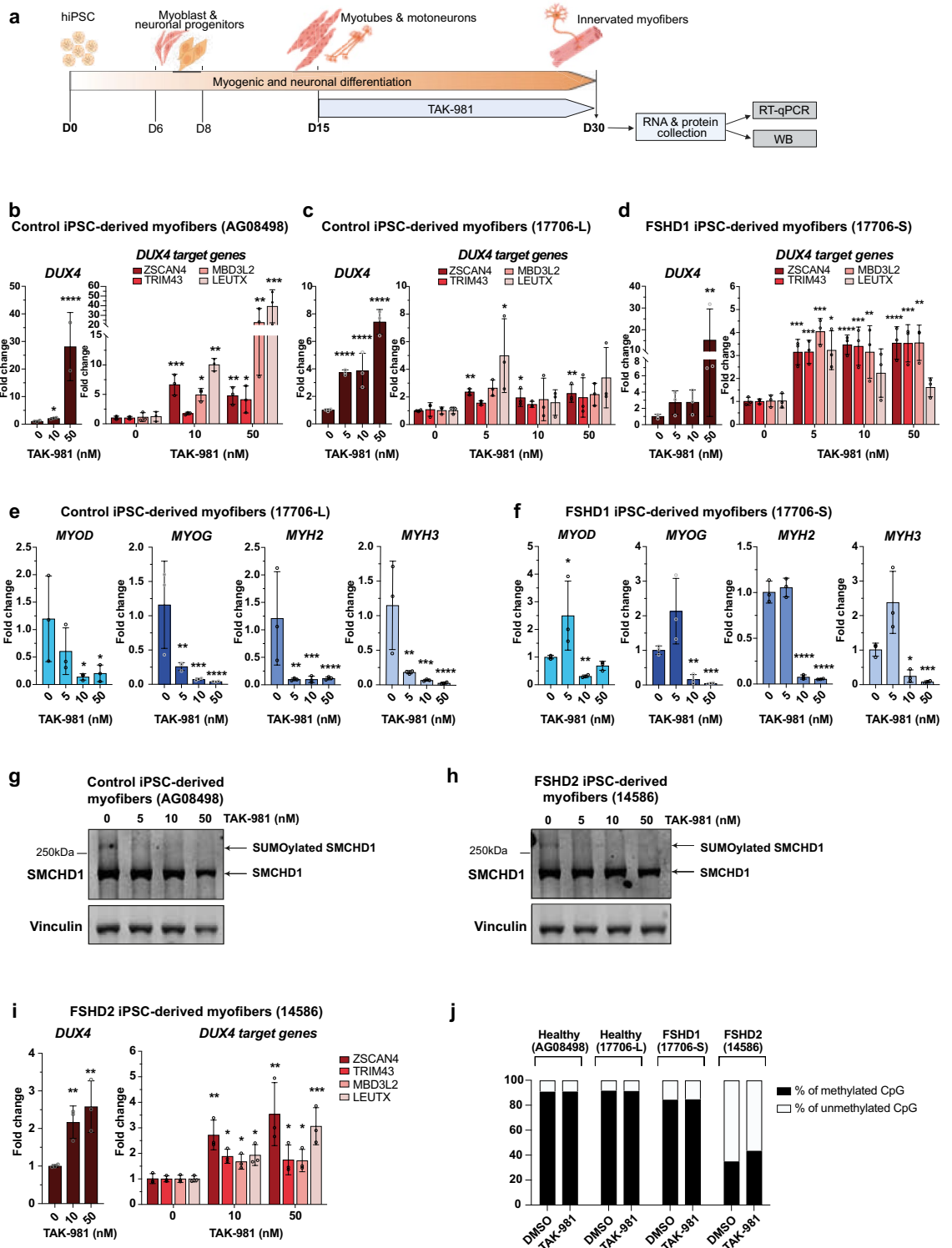
Impact of loss of SUMOylation on SMCHD1 and SMCHD1-deficient cells

The SMCHD1 protein contains six principal lysine residues (K784, K1373, K1496, K1958, K1976, K2002) modified by SUMO2/3²⁷. In hiPSC-derived myofibers, SUMOylated SMCHD1 was detected as an upper band by western blotting (Fig. 3g, h). This upper band is no longer detected after TAK-981 treatment in either controls or FSHD cells³⁰, showing that SMCHD1 SUMOylation is decreased upon TAK981 treatment.

We next assessed whether SMCHD1 loss of function in SMCHD1-deficient cells together with loss of SUMOylation might exacerbate *DUX4-fl* induction. We treated FSHD2 hiPSC-derived muscle cells from D15 to D30 of differentiation with SUMO_i using hiPSC from a FSHD2 patient carrying a pathogenic *SMCHD1* variant that abrogates the protein ATPase activity³⁰ (Fig. 3i). *DUX4* and its target genes exhibited expression profiles similar to those found in FSHD1 patient-derived myofibers (Fig. 3d, i). In addition, the presence of a loss of function variant in *SMCHD1* does not seem to affect SUMOylation of the protein in basal conditions in FSHD2 hiPSC-derived myofibers (Fig. 3h). We concluded that SUMOylation depletion promotes *DUX4-fl* expression in both control and FSHD cells, regardless of SMCHD1 catalytic activity.

SUMOylation inhibition does not impact D4Z4 methylation profile

As the D4Z4 macrosatellite is differentially methylated at the DNA level between non-affected individuals and FSHD patients, we next investigated whether *DUX4* induction upon TAK-981 resulted from D4Z4 hypomethylation. To this aim, hiPSC-derived muscle of two controls (healthy donor and healthy clone isolated from the mosaic patient), one FSHD1 patient (diseased clone from the mosaic patient) and one FSHD2 patient were exposed to the SUMOylation inhibitor from D15 to D30 post-differentiation as described (Fig. 3a). Genomic DNA was extracted at the end of the treatment and D4Z4 methylation level was analyzed by sodium bisulfite sequencing at the DR1 region which is hypomethylated in FSHD patients compared to non-affected



individuals^{23,24}. In all conditions, we compared cells treated at a final concentration of 50 nM TAK-981 to cells treated with DMSO only. Inhibition of SUMOylation does not impact the level of methylation at DR1 in all tested hiPSC derived-myofibers (Fig. 3j), suggesting that TAK-981-induced activation of *DUX4* expression does not involve the hypomethylation of the repeat.

Loss of SUMOylation modulates expression of genes at the 4q35 locus

We then evaluated whether TAK-981-induced hypoSUMOylation only affects *DUX4* expression or also the expression of other genes at the 4q35 locus. We analyzed expression of four different genes within a 7 Mb range of the D4Z4 region (*WWC2*) and located in different Topologically Associated Domains (TADs)³². We included genes previously implicated in FSHD such as *DUX4c*³³, *FRG2*^{33–35}, *FRG1*³³ and *FAT1*³⁶ (Fig. 4a). The expression of the most distal gene *WWC2* is not impacted by TAK-981 treatment compared to mock-treated controls or FSHD

◀ **Fig. 3.** Loss of SUMOylation triggers *DUX4* activation in FSHD1 iPSC-derived myotubes. (a) Experimental design for hiPSC-derived myofibers. HiPSCs derived from a healthy donor or FSHD1 patient³⁰ were differentiated for 30 days as described^{53,54}. TAK-981 was added every day from day 15 to 30. Cell culture medium supplemented in DMSO or TAK-981 was changed every 24 h. At day 30, RNA and proteins were collected. (b–d) *DUX4-fl* and *DUX4* target gene expression analyses in control AG08498³⁰ (b), control 17,706-L³⁰ (c) or FSHD1 17,706-S³⁰ (d) hiPSC-derived myofibers. Healthy iPSC (17,706-L) and FSHD1 iPSC (17,706-S) are isogenic clones obtained from a mosaic patient (17,706). Fold-changes were calculated relative to three housekeeping genes and normalized to the untreated (0) condition for each gene. Error bars represent mean \pm SD. $n = 3$. Significance: one-way ANOVA testing. ns: not significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ (e–f) *MYOD*, *MYOG*, *MYH2* and *MYH3* expression analyses in the control 17706-L (e) and FSHD1 17706-S (f) hiPSC-derived myofibers. Fold-changes were calculated as above. Error bars represent mean \pm SD. $n = 3$. Significance: one-way ANOVA testing. ns: not significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$. (g–h) Immunoblots for the endogenous SMCHD1 protein in control AG08498 (g) and FSHD2 14,586 (h) hiPSC-derived myofibers treated with TAK-981 at indicated concentrations. Vinculin is used as a loading control. Arrows indicate SMCHD1 and its SUMOylated form. (i) *DUX4-fl* and *DUX4* target gene expression analyses in the FSHD2 hiPSC-derived myofibers (14586). Fold-changes were calculated as above. Error bars represent mean \pm SD. $n = 3$. Significance: one-way ANOVA testing. ns: not significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$. (j) Percentage of methylated (black) and unmethylated (white) CpG at the DR1 region located in the proximal part of the D4Z4 repeat. Methylation was determined for each CpG within the sequence of interest (31 CpGs). On the *x-axis*, histogram bars represent the mean percentage of methylated (black) or unmethylated (white) CpG of all CpG in the region of interest after sodium bisulfite sequencing analysis in control and FSHD hiPSC-derived myofibers after TAK-981 or DMSO treatment. This global methylation level was determined after sequencing of > 45,000 amplicons per condition.

immortalized myotubes (Fig. 4b, c). In contrast, we observed a robust and statistically significant upregulation of *FRG2* in all cell types at low doses of TAK-981 and of *FAT1* in both control and FSHD1 cells, whereas a more heterogeneous effect on *FRG1* expression as well as on *DUX4c* expression was observed depending on samples and doses of SUMOi (Fig. 4b–c).

We observed the same trend in hiPSC-derived muscle tissue when cells were treated during differentiation, in particular for *FRG2* and *FAT1*, both in controls and FSHD cells (Fig. 5a–d). These observations overall suggest that SUMOylation inhibition contributes to the relaxation of the 4q35 locus.

Discussion

Post-translational modification by SUMO acts to stabilize cell identity in a variety of contexts^{7,37–39}. In particular, hypoSUMOylation enhances pluripotency reprogramming in vitro and in vivo and suppression of SUMOylation promotes the spontaneous conversion of ESCs to a 2-cell-embryo-like (2C-like) state^{7,8,39}. This transition involves two distinct mechanisms. First, hypoSUMOylation activates transcription of genomic loci regulated by the H3K9 histone methyltransferases Setdb1 and Suv39H1 by inducing a global decrease in H3K9 trimethylation^{7,40–42}. Second, hypoSUMOylation leads to *Dux* derepression by interfering with the recruitment of Prc1.6 and Kap/Setdb1 at the *Dux* promoter⁷, together with impairing Dppa2/Dppa4 and histone H1 repressing activities^{1,9,10}.

Mouse *Dux* and human *DUX4* both encode a transcription factor that plays a key role in triggering zygotic genome activation (ZGA) in the early stages of embryogenesis^{11,12}. In human somatic cells, *DUX4* is normally silenced through epigenetic repression of the D4Z4 macrosatellite repeat array on chromosome 4q35, involving DNA methylation and repressive histone marks such as H3K9me3 and H3K27me3^{22,43}. In FSHD, ectopic expression of the long *DUX4* transcript (*DUX4-fl*) has been proposed as the main trigger leading to disease-associated muscle dysfunction²². Activation of *DUX4-fl* in a subset of muscle fibers (1/1000 nuclei) is subsequent to the reduction in the number of D4Z4 macrosatellite at the 4q35 locus in FSHD1²² or to the presence of a variant in *SMCHD1* in FSHD2²⁵, both leading to hypomethylation of D4Z4 and chromatin relaxation of the 4q35 locus. However, the mechanisms underlying burst of *DUX4* transcriptional activation in muscle remain unknown.

A large number of transcription factors and chromatin regulators are SUMOylated, and SUMOylation is generally associated with transcriptional repression^{3–6,27}. SUMO facilitates the recruitment of SUMO interacting motif (SIM)-containing proteins^{44,45} such as histone deacetylases or the Setdb1 histone methyltransferase. Interestingly, the SMCHD1 protein contains six main lysine residues (K784, K1373, K1496, K1958, K1976, K2002) that are modified by SUMOylation and 15 minor acceptor lysines containing the consensus sequence required for SUMOylation²⁷. Together, these findings point towards a role for SUMOylation in the regulation of the 4q35 locus and potentially of aberrant *DUX4* expression in the context of FSHD. However, nothing is currently known on how SUMOylation might regulate SMCHD1 function. To date, no changes in global SUMOylation or in the levels of SUMOylation pathway components have been detected in FSHD cells^{46,47}.

Recent development of a highly specific small molecule inhibitor of SUMOylation, TAK-981, proved to be a powerful tool for studying various facets of SUMOylation biology²⁸. Notably, TAK-981 is able to induce a strong spontaneous type I interferon response in myeloid cells⁴⁸, mirroring genetic inactivation of SUMOylation⁴⁹. In line with this finding, TAK-981 was shown to activate antitumor immune response in pre-clinical models and in the clinics⁵¹. Here, we show that short or prolonged treatment of muscle cells in vitro with TAK-981 activates the *DUX4* expression program in control cells and exacerbates *DUX4* activation in cells from FSHD patients. Using cells from a patient affected with FSHD2 and carrying a variant in *SMCHD1* altering the

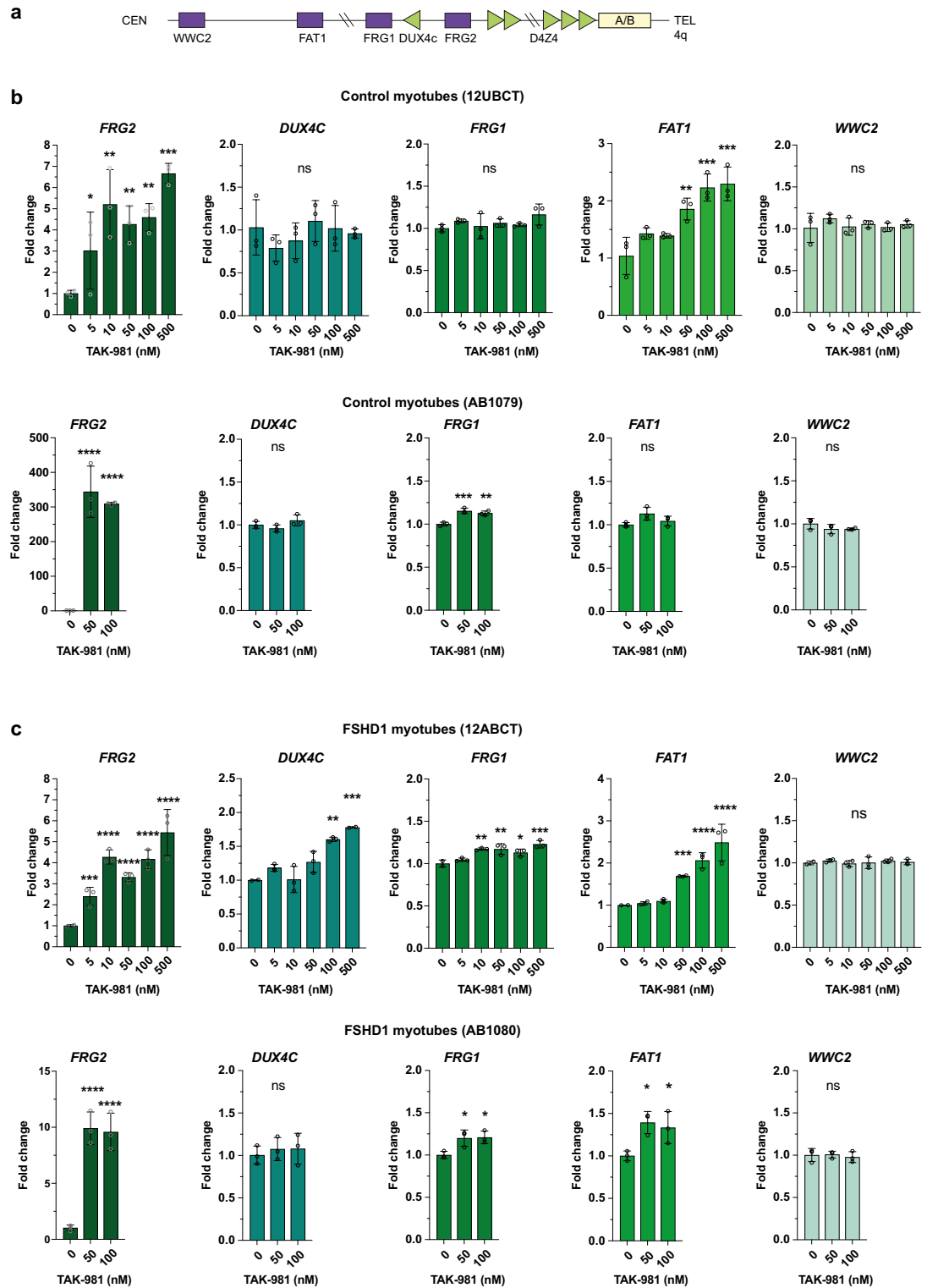


Fig. 4. SUMOylation inhibition affects expression of other genes of the *DUX4* locus in myotubes. (a) Schematic representation of human chromosome region 4q35. (b-c) *FRG2*, *DUX4c*, *FRG1*, *FAT1* and *WWC2* expression analyses in control myotubes 12UBCT and AB1079 (b) and in FSHD1 patient-derived myotubes 12ABCT and AB1080 (c). Fold-changes were calculated relative to three housekeeping genes and normalized to the untreated (0) condition for each gene. Error bars represent mean \pm SD. n = 3. Significance: one-way ANOVA testing. ns: not significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.

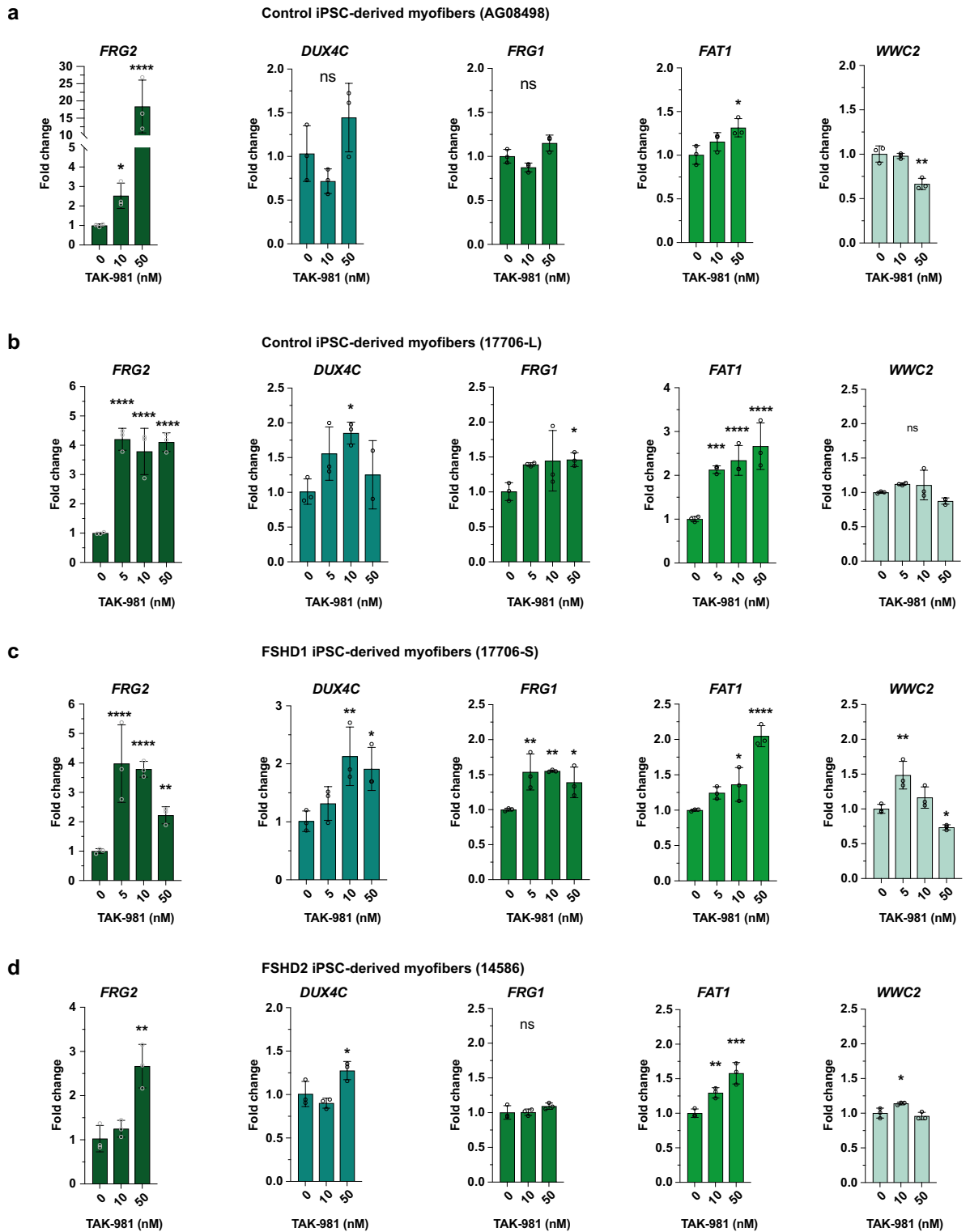


Fig. 5. Expressions of genes in *DUX4* locus are affected in hiPSC-derived myofibers. (a–d) *FRG2*, *DUX4c*, *FRG1*, *FAT1* and *WWC2* expression analyses in control AG08498 (a), control 17,706-L (b), FSHD1 17,706-S (c) and FSHD2 14,586 (d) hiPSC-derived myofibers. Fold-changes were calculated relative to three housekeeping genes and normalized to the untreated (0) condition for each gene. Error bars represent mean \pm SD. n = 3. Significance: one-way ANOVA testing. ns: not significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$.

functional activity of the ATPase domain and showing a marked decrease in D4Z4 methylation³⁰, we show that SMCHD1 loss of function and associated D4Z4 hypomethylation does not lead to increased TAK-981-induced *DUX4* activation beyond levels observed in TAK-981-treated FSHD1 cells. These data suggest that TAK-981-induced activation of *DUX4* expression is uncoupled from the D4Z4 methylation state.

In addition, we show that treatment with SUMOi increases expression of other genes located at the 4q35 locus such as the *FRG2* non-coding RNA and *FAT1*, that might also contribute to the FSHD phenotype^{33–36}. This suggests that SUMOylation inhibition contributes to a more global chromatin relaxation in the vicinity of the D4Z4 array that particularly affects genes located within the same TAD and that show variable long-distance interactions depending on the cell context³².

Notably, recent research by an independent group investigated the link between SUMOylation and *DUX4* regulation in FSHD, using ML-792, an alternative inhibitor of the SUMO-conjugation pathway, in cultured cells⁵². As observed in our cell models, the authors demonstrated that SMCHD1 is SUMOylated in both myoblasts and myotubes. Consistent with our observations, treatment with ML-792 induces *DUX4* expression and a variable decrease in myogenic gene expression. Their analysis of SMCHD1 mutants lacking SUMOylation sites revealed no significant impact on SMCHD1 protein stability, nuclear localization, dimerization, or chromatin binding at the D4Z4 locus. Similarly, our results on FSHD2-patient derived cells with a loss-of-function of SMCHD1 did not uncover a definitive role for SUMOylated SMCHD1 in regulating the 4q35 region, indicating that further functional studies are needed⁵².

Overall, using different control or FSHD muscle cells, we provide here a proof of concept of the effect of SUMOylation in the regulation of *DUX4* expression and epigenetic regulation of the 4q35 region. Our data suggest that manipulating SUMOylation, potentially through SENP deSUMOylase inhibition, may help silence inappropriate de-repression of *DUX4*, opening new avenues for investigating the contribution of SUMOylation in FSHD. Further studies will be essential to define the therapeutic relevance of such approaches.

Data availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Received: 4 August 2025; Accepted: 19 December 2025

Published online: 09 January 2026

References

- Vertegaal, A. C. O. Signalling mechanisms and cellular functions of SUMO. *Nat Rev Mol Cell Biol* **23**, 715–731. <https://doi.org/10.1038/s41580-022-00500-y> (2022).
- Seeler, J. S. & Dejean, A. SUMO and the robustness of cancer. *Nat Rev Cancer* **17**, 184–197. <https://doi.org/10.1038/nrc.2016.143> (2017).
- Rosonina, E. A conserved role for transcription factor sumoylation in binding-site selection. *Curr Genet* **65**, 1307–1312. <https://doi.org/10.1007/s00294-019-00992-w> (2019).
- Boulanger, M., Chakraborty, M., Tempe, D., Piechaczyk, M. & Bossis, G. SUMO and Transcriptional Regulation: The Lessons of Large-Scale Proteomic, Modificomic and Genomic Studies. *Molecules* **26**, <https://doi.org/10.3390/molecules26040828> (2021).
- Ryu, H. Y. & Hochstrasser, M. Histone sumoylation and chromatin dynamics. *Nucleic Acids Res* **49**, 6043–6052. <https://doi.org/10.1093/nar/gkab280> (2021).
- Cubenas-Potts, C. & Matunis, M. J. SUMO: a multifaceted modifier of chromatin structure and function. *Dev Cell* **24**, 1–12. <https://doi.org/10.1016/j.devcel.2012.11.020> (2013).
- Cossec, J. C. *et al.* SUMO Safeguards Somatic and Pluripotent Cell Identities by Enforcing Distinct Chromatin States. *Cell Stem Cell* **23**, 742–757 e748. <https://doi.org/10.1016/j.stem.2018.10.001> (2018).
- Yan, Y. L. *et al.* DPPA2/4 and SUMO E3 ligase PIAS4 oppositely regulate zygotic transcriptional program. *PLoS Biol* **17**, e3000324. <https://doi.org/10.1371/journal.pbio.3000324> (2019).
- Theurillat, I. *et al.* Extensive SUMO Modification of Repressive Chromatin Factors Distinguishes Pluripotent from Somatic Cells. *Cell Rep* **32**, 108146. <https://doi.org/10.1016/j.celrep.2020.108146> (2020).
- Sheban, D. *et al.* SUMOylation of linker histone H1 drives chromatin condensation and restriction of embryonic cell fate identity. *Mol Cell* **82**, 106–122 e109. <https://doi.org/10.1016/j.molcel.2021.11.011> (2022).
- De Iaco, A. *et al.* DUX-family transcription factors regulate zygotic genome activation in placental mammals. *Nat Genet* **49**, 941–945. <https://doi.org/10.1038/ng.3858> (2017).
- Hendrickson, P. G. *et al.* Conserved roles of mouse DUX and human DUX4 in activating cleavage-stage genes and MERVL/HERVL retrotransposons. *Nat Genet* **49**, 925–934. <https://doi.org/10.1038/ng.3844> (2017).
- Gabriels, J. *et al.* Nucleotide sequence of the partially deleted D4Z4 locus in a patient with FSHD identifies a putative gene within each 3.3 kb element. *Gene* **236**, 25–32 (1999).
- Deidda, G. *et al.* Physical mapping evidence for a duplicated region on chromosome 10qter showing high homology with the facioscapulohumeral muscular dystrophy locus on chromosome 4qter. *Eur J Hum Genet* **3**, 155–167 (1995).
- Hewitt, J. E. *et al.* Analysis of the tandem repeat locus D4Z4 associated with facioscapulohumeral muscular dystrophy. *Hum Mol Genet* **3**, 1287–1295 (1994).
- Nguyen, K. *et al.* Molecular combing reveals complex 4q35 rearrangements in Facioscapulohumeral dystrophy. *Hum Mutat* **38**, 1432–1441. <https://doi.org/10.1002/humu.23304> (2017).
- van Overveld, P. G. *et al.* Hypomethylation of D4Z4 in 4q-linked and non-4q-linked facioscapulohumeral muscular dystrophy. *Nat Genet* **35**, 315–317. <https://doi.org/10.1038/ng1262> (2003).
- Salsi, V., Magdinier, F. & Tupler, R. Does DNA Methylation Matter in FSHD? *Genes (Basel)* **11**, <https://doi.org/10.3390/genes11030258> (2020).
- Nikolic, A. *et al.* Interpretation of the Epigenetic Signature of Facioscapulohumeral Muscular Dystrophy in Light of Genotype-Phenotype Studies. *Int J Mol Sci* **21**, <https://doi.org/10.3390/ijms21072635> (2020).
- Padberg, G. W. & van Engelen, B. G. Facioscapulohumeral muscular dystrophy. *Curr. Opin. Neurol.* **22**, 539–542. <https://doi.org/10.1097/WCO.0b013e328330a572> (2009).
- Padberg, G. W., Lunt, P. W., Koch, M. & Fardeau, M. Diagnostic criteria for facioscapulohumeral muscular dystrophy. *Neuromuscul Disord* **1**, 231–234. [https://doi.org/10.1016/0960-8966\(91\)90094-9](https://doi.org/10.1016/0960-8966(91)90094-9) (1991).

22. Lemmers, R. J. et al. A unifying genetic model for facioscapulohumeral muscular dystrophy. *Science* **329**, 1650–1653. <https://doi.org/10.1126/science.1189044> (2010).
23. Roche, S. et al. Methylation hotspots evidenced by deep sequencing in patients with facioscapulohumeral dystrophy and mosaicism. *Neurol Genet* **5**, e372. <https://doi.org/10.1212/NXG.0000000000000372> (2019).
24. Hartweck, L. M. et al. A focal domain of extreme demethylation within D4Z4 in FSHD2. *Neurology* <https://doi.org/10.1212/WNL.0b013e31827f075c> (2013).
25. Lemmers, R. J. et al. Digenic inheritance of an SMCHD1 mutation and an FSHD-permissive D4Z4 allele causes facioscapulohumeral muscular dystrophy type 2. *Nat Genet* **44**, 1370–1374. <https://doi.org/10.1038/ng.2454> (2012).
26. Geng, L. N. et al. DUX4 activates germline genes, retroelements, and immune mediators: implications for facioscapulohumeral dystrophy. *Dev Cell* **22**, 38–51. <https://doi.org/10.1016/j.devcel.2011.11.013> (2012).
27. Hendriks, I. A. et al. Site-specific mapping of the human SUMO proteome reveals co-modification with phosphorylation. *Nat Struct Mol Biol* **24**, 325–336. <https://doi.org/10.1038/nsmb.3366> (2017).
28. Langston, S. P. et al. Discovery of TAK-981, a First-in-Class Inhibitor of SUMO-Activating Enzyme for the Treatment of Cancer. *J Med Chem* **64**, 2501–2520. <https://doi.org/10.1021/acs.jmedchem.0c01491> (2021).
29. Homma, S. et al. A unique library of myogenic cells from facioscapulohumeral muscular dystrophy subjects and unaffected relatives: family, disease and cell function. *Eur J Hum Genet* **20**, 404–410. <https://doi.org/10.1038/ejhg.2011.213> (2012).
30. Dion, C. et al. SMCHD1 is involved in de novo methylation of the DUX4-encoding D4Z4 macrosatellite. *Nucleic Acids Res* **47**, 2822–2839. <https://doi.org/10.1093/nar/gkz005> (2019).
31. Badja, C. et al. Efficient and cost-effective generation of mature neurons from human induced pluripotent stem cells. *Stem Cells Transl Med* **3**, 1467–1472. <https://doi.org/10.5966/sctm.2014-0024> (2014).
32. Gaillard, M. C. et al. Analysis of the 4q35 chromatin organization reveals distinct long-range interactions in patients affected with Facio-Scapulo-Humeral Dystrophy. *Sci Rep* **9**, 10327. <https://doi.org/10.1038/s41598-019-46861-x> (2019).
33. Gabellini, D., Green, M. R. & Tupler, R. Inappropriate gene activation in FSHD: a repressor complex binds a chromosomal repeat deleted in dystrophic muscle. *Cell* **110**, 339–348 (2002).
34. Salsi, V. et al. Nucleolar FRG2 lncRNAs inhibit rRNA transcription and cytoplasmic translation, linking FSHD to dysregulation of muscle-specific protein synthesis. *Nucleic Acids Res* **53**, <https://doi.org/10.1093/nar/gkaf643> (2025).
35. Puppo, F. et al. Identification of variants in the 4q35 gene FAT1 in patients with a facioscapulohumeral dystrophy-like phenotype. *Hum Mutat* **36**, 443–453. <https://doi.org/10.1002/humu.22760> (2015).
36. Caruso, N. et al. Deregulation of the protocadherin gene FAT1 alters muscle shapes: implications for the pathogenesis of facioscapulohumeral dystrophy. *PLoS Genet* **9**, e1003550. <https://doi.org/10.1371/journal.pgen.1003550> (2013).
37. Cossec, J. C. et al. Transient suppression of SUMOylation in embryonic stem cells generates embryo-like structures. *Cell Rep* **42**, 112380. <https://doi.org/10.1016/j.celrep.2023.112380> (2023).
38. Borkent, M. et al. A Serial shRNA Screen for Roadblocks to Reprogramming Identifies the Protein Modifier SUMO2. *Stem Cell Reports* **6**, 704–716. <https://doi.org/10.1016/j.stemcr.2016.02.004> (2016).
39. Cheloufi, S. et al. The histone chaperone CAF-1 safeguards somatic cell identity. *Nature* **528**, 218–224. <https://doi.org/10.1038/nature15749> (2015).
40. Maison, C., Bailly, D., Quivy, J. P. & Almouzni, G. The methyltransferase Suv39h1 links the SUMO pathway to HP1alpha marking at pericentric heterochromatin. *Nat Commun* **7**, 12224. <https://doi.org/10.1038/ncomms12224> (2016).
41. Maison, C., Quivy, J. P. & Almouzni, G. Suv39h1 links the SUMO pathway to constitutive heterochromatin. *Mol Cell Oncol* **3**, e1225546. <https://doi.org/10.1080/23723556.2016.1225546> (2016).
42. Ivanov, A. V. et al. PHD domain-mediated E3 ligase activity directs intramolecular sumoylation of an adjacent bromodomain required for gene silencing. *Mol Cell* **28**, 823–837. <https://doi.org/10.1016/j.molcel.2007.11.012> (2007).
43. Haynes, P., Bomsztyk, K. & Miller, D. G. Sporadic DUX4 expression in FSHD myocytes is associated with incomplete repression by the PRC2 complex and gain of H3K9 acetylation on the contracted D4Z4 allele. *Epigenetics Chromatin* **11**, 47. <https://doi.org/10.1186/s13072-018-0215-z> (2018).
44. Gonzalez-Prieto, R. et al. Global non-covalent SUMO interaction networks reveal SUMO-dependent stabilization of the non-homologous end joining complex. *Cell Rep* **34**, 108691. <https://doi.org/10.1016/j.celrep.2021.108691> (2021).
45. Lascorz, J., Codina-Fabra, J., Reverter, D. & Torres-Rosell, J. SUMO-SIM interactions: From structure to biological functions. *Semin Cell Dev Biol* **132**, 193–202. <https://doi.org/10.1016/j.semcd.2021.11.007> (2022).
46. Jagannathan, S., Ogata, Y., Gafken, P. R., Tapscott, S. J. & Bradley, R. K. Quantitative proteomics reveals key roles for post-transcriptional gene regulation in the molecular pathology of facioscapulohumeral muscular dystrophy. *Elife* **8**, <https://doi.org/10.7554/eLife.41740> (2019).
47. Moriggi, M. et al. Muscle Proteome Analysis of Facioscapulohumeral Dystrophy Patients Reveals a Metabolic Rewiring Promoting Oxidative/Reductive Stress Contributing to the Loss of Muscle Function. *Antioxidants (Basel)* **13**, <https://doi.org/10.3390/antiox13111406> (2024).
48. Lightcap, E. S. et al. A small-molecule SUMOylation inhibitor activates antitumor immune responses and potentiates immune therapies in preclinical models. *Sci Transl Med* **13**, eaba7791, <https://doi.org/10.1126/scitranslmed.aba7791> (2021).
49. Decque, A. et al. Sumoylation coordinates the repression of inflammatory and anti-viral gene-expression programs during innate sensing. *Nat Immunol* **17**, 140–149. <https://doi.org/10.1038/ni.3342> (2016).
50. Kumar, S. et al. Targeting pancreatic cancer by TAK-981: a SUMOylation inhibitor that activates the immune system and blocks cancer cell cycle progression in a preclinical model. *Gut* **71**, 2266–2283. <https://doi.org/10.1136/gutjnl-2021-324834> (2022).
51. Assouline, S. E. et al. Phase I/II Study of Subasumstat (TAK-981) in Combination With Rituximab in Relapsed/Refractory Non-Hodgkin Lymphoma. *Clin Lymphoma Myeloma Leuk* <https://doi.org/10.1016/j.clml.2025.04.020> (2025).
52. Goossens, R. et al. SUMOylation differentially regulates SMCHD1 complex formation and function in a genomic context-specific manner. *bioRxiv*, 2024.2010.2013.618066, <https://doi.org/10.1101/2024.10.13.618066> (2024).
53. Mazaleyrat, K. et al. Multilineage Differentiation for Formation of Innervated Skeletal Muscle Fibers from Healthy and Diseased Human Pluripotent Stem Cells. *Cells* **9**, <https://doi.org/10.3390/cells9061531> (2020).
54. Delourme, M., Brouqsault, N., Mazaleyrat, K. & Magdinier, F. Production of Innervated Skeletal Muscle Fibers Using Human Induced Pluripotent Stem Cells. *Methods Mol Biol* https://doi.org/10.1007/978-1-4939-9334-4_20 (2020).

Acknowledgements

We are grateful to Elise Jacquemet from bioinformatics and biostatistics hub of Institut Pasteur for assistance in statistical analyses, Agnès Marchio for help in the RT-qPCR settings and Jacob Seeler for helpful discussions. We also acknowledge the Myoline platform of the Institute of Myology for access to immortalized myoblast cells.

Author contributions

A.L., L.M., A.A., J.P.T., P.P., N.E performed the experiments A.B provided cellular model A.L analyzed the data, prepared the figures and edited the manuscript A.D and F.M, designed and supervised the study, obtained funding, wrote and edited the manuscript.

Funding

This work was supported by grants from ERC-AdG 'SUMiDENTITY', ANR (19-CE12-0011-01) and the Sjöberg Foundation to A.D, and from the Fondation pour la Recherche Médicale to F.M.

Declarations

Competing interests

The authors declare no competing interests.

Informed consent

Informed consent was obtained from all subjects. All methods were carried out in accordance with relevant guidelines and regulations.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-025-33624-0>.

Correspondence and requests for materials should be addressed to F.M.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2026