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Supplementary Materials for

A novel P300 inhibitor reverses DUX4-mediated global histone H3 hyperacetylation, target gene expression, and cell death

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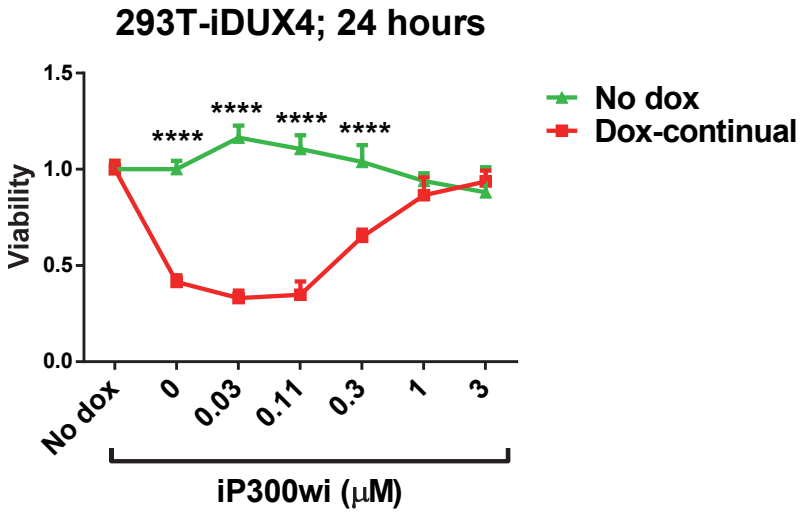


Fig. S1. iP300w protects 293T cells from DUX4-induced toxicity.

ATP assay at 24 hours on 293T-iDUX4 cells induced with 500 ng/mL doxycycline and treated with various concentration of iP300w.

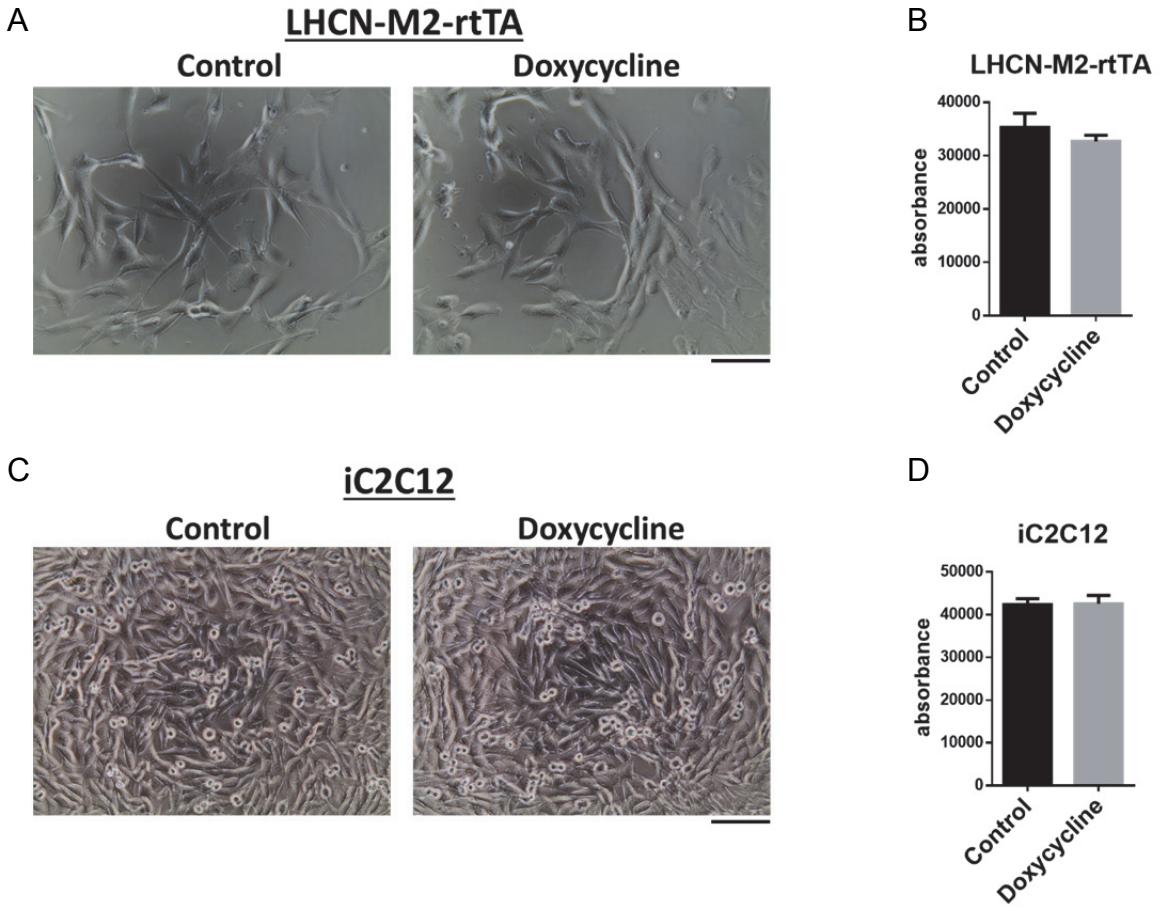
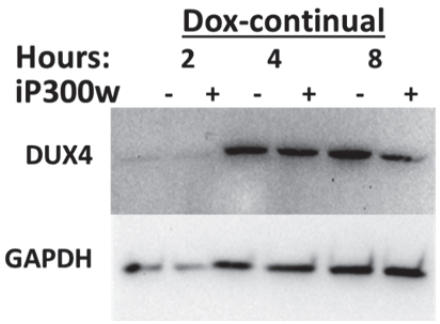


Fig. S2. Doxycycline effect on morphology and viability of parent cell lines.

Morphology (A) and viability (B) evaluated by ATP assay of LHCN-rtTA cells treated with doxycycline (200 ng/mL) for 48 hours. Morphology (C) and viability (D) of iC2C12 treated with 500 ng/mL doxycycline for 48 hours.

A



B

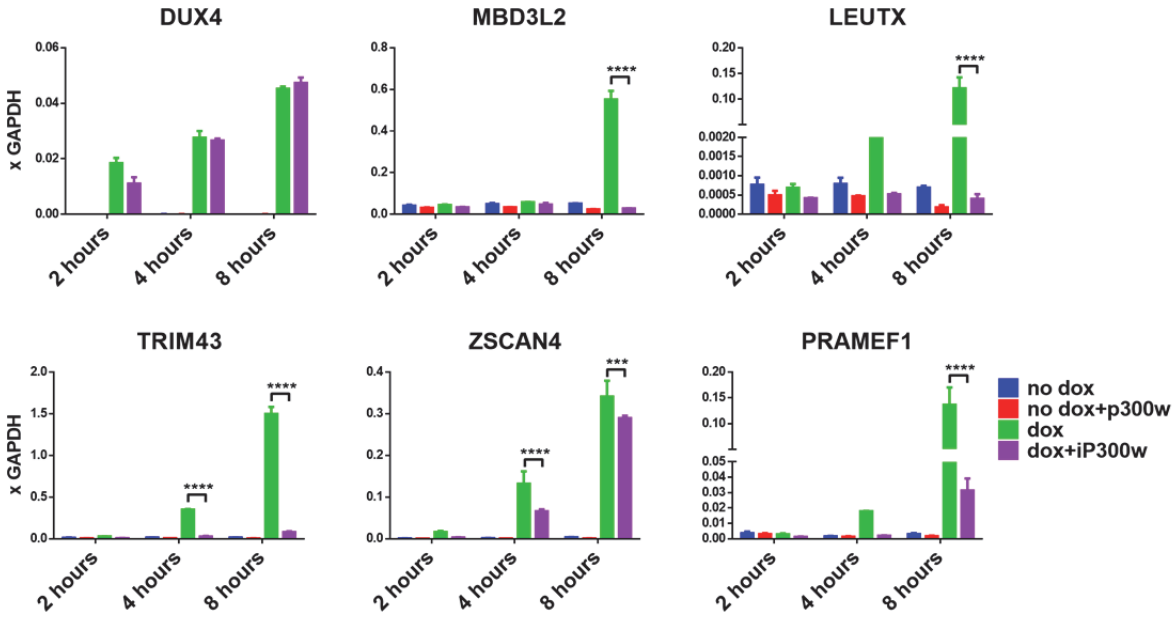


Fig. S3. iP300w inactivates induction of DUX4 target genes.

Western blot (A) and RT-qPCR (B) analyses in LHCN-iDUX4 cells continually induced with doxycycline (200 ng/mL) and treated with 0.1 μM iP300w. Samples were analyzed at 2, 4 and 8 hours.

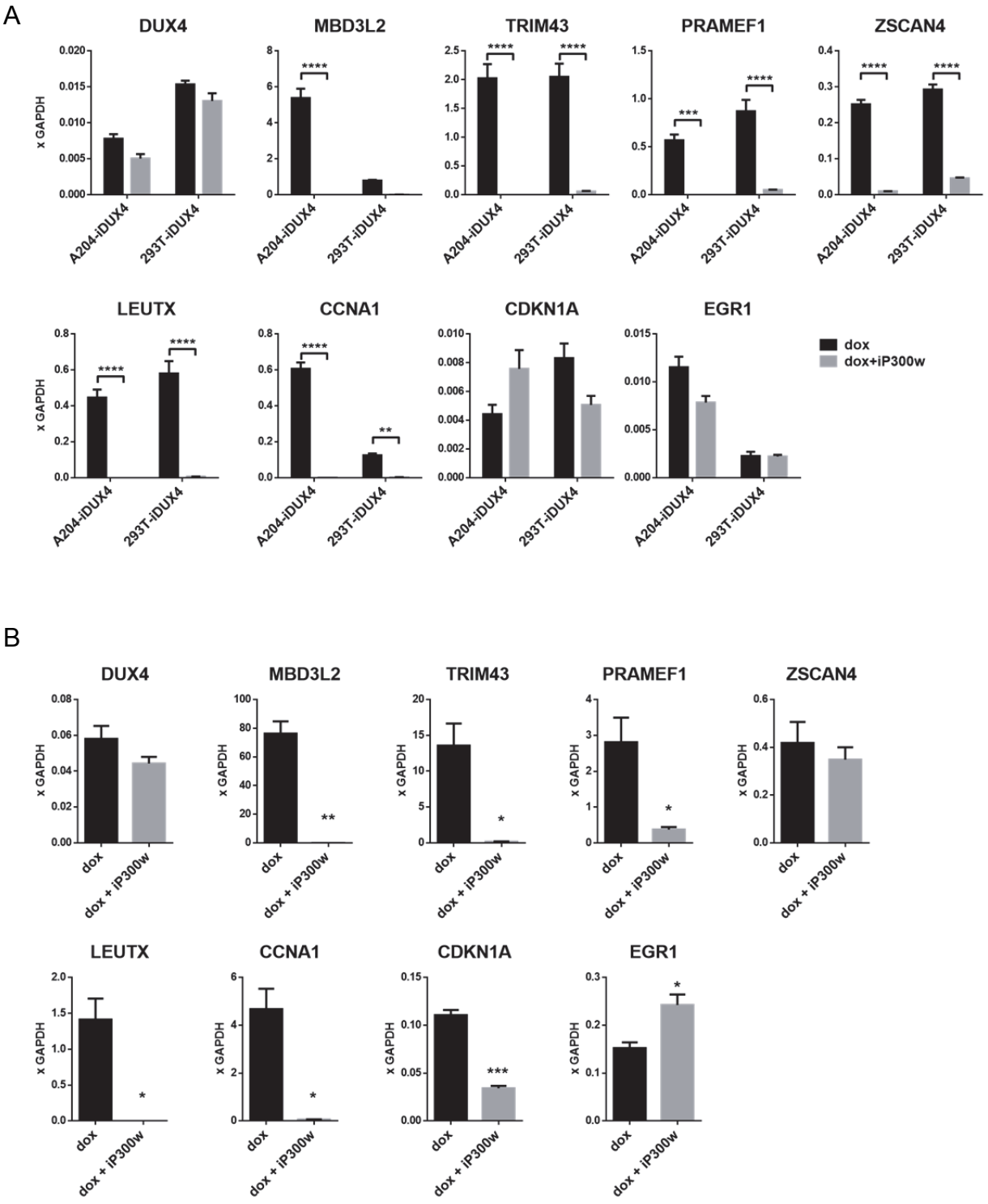


Fig. S4. iP300w inactivates induction of DUX4 target genes in different cell types.

(A) RT-qPCR for DUX4 and DUX4 target genes in non-myogenic cell lines A204 (A204-iDUX4) and 293T (293T-iDUX4) after 12 hours induction with 200 ng/mL doxycycline and 0.25 μM iP300w.

(B) RT-qPCR for DUX4 target genes in RD-iDUX4 (rhabdomyosarcoma cell line) after 12 hours induction with 200 ng/mL doxycycline and 0.25 μM iP300w. Data are presented as mean ± SEM; ***p<0.001, ****p<0.0001, by t-test. Results are presented as relative expression to *GAPDH* (n=4).

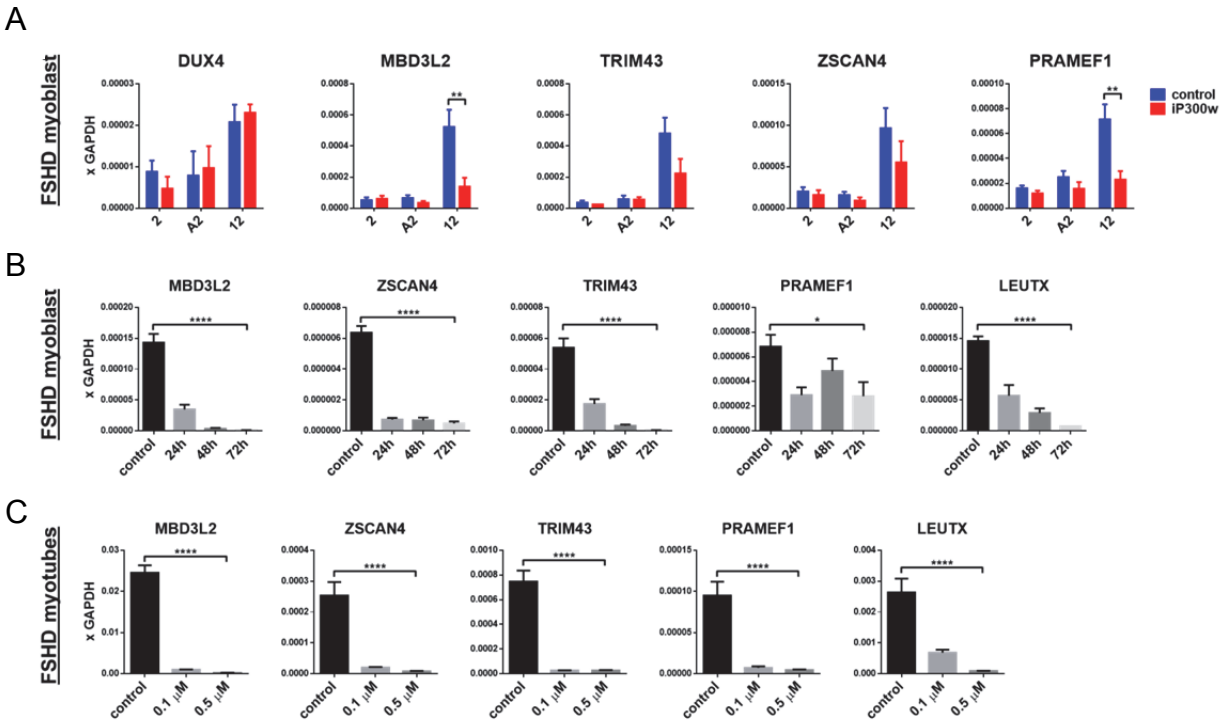


Fig. S5. Dose- and time-dependent DUX4 inactivation by iP300w in FSHD myoblasts.

(A) RT-qPCR for DUX4 and DUX4 target genes in three different FSHD myoblast clonal cell lines (2, A2, and 12) at stage of proliferation (myoblast) 12 hours after treatment with 0.25 μM iP300w, (n=9).

(B) RT-qPCR for DUX4 target genes in FSHD myoblast (cell line 12) at stage of proliferation after 3 days treatment with 0.1 μM iP300w.

(C) RT-qPCR for DUX4 target genes in FSHD myotubes (cell line 12) after 24 hours treatment with 0.1 and 0.5 μM iP300w. Data are presented as mean ± SEM; ***p<0.001, ****p<0.0001, by one-way ANOVA. Results are presented as relative expression to *GAPDH* (n=3).

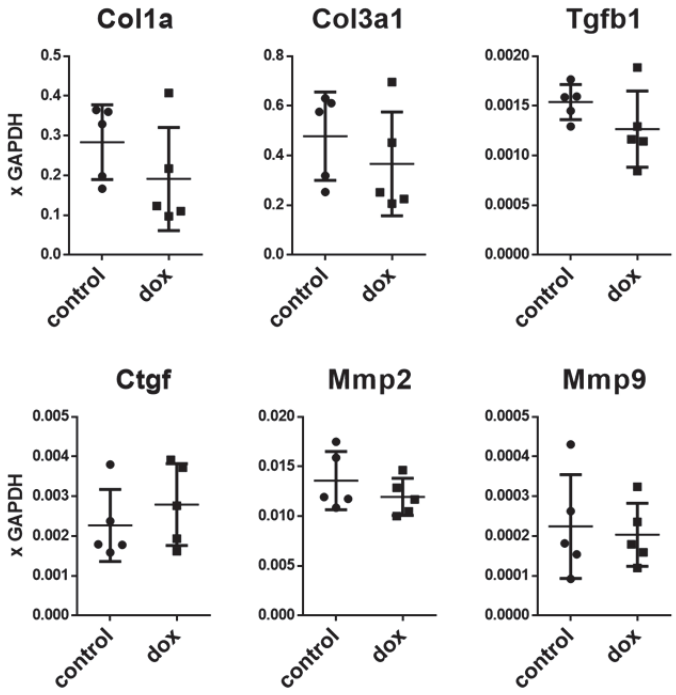


Fig. S6. Effect of doxycycline on profibrotic gene expression in wild-type mice.

RT-qPCR on RNA isolated from gastrocnemius at day 12 from WT mice treated with 5 mg/kg doxycycline daily. Data are presented as mean ± SEM. Results are presented as relative expression to *GAPDH* (n=5).

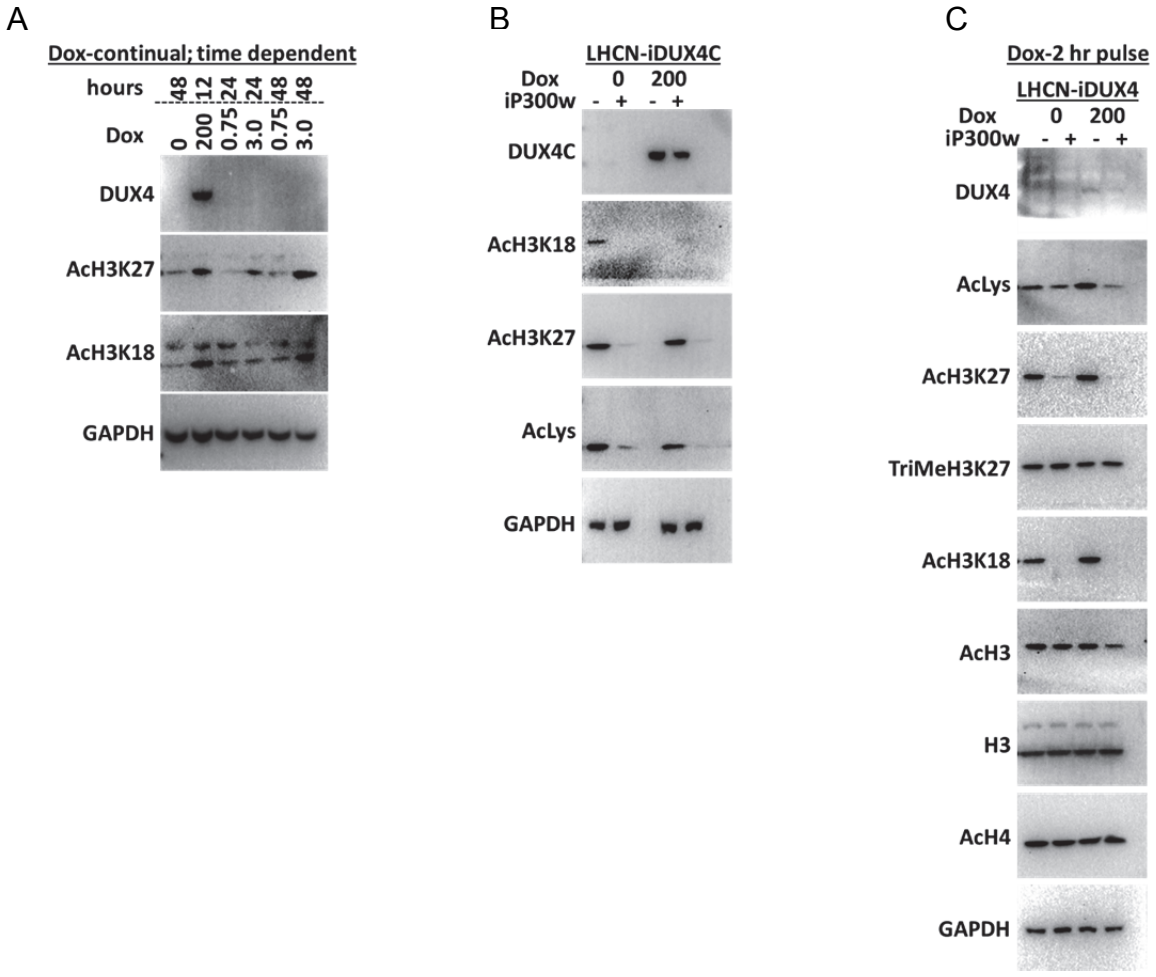


Fig. S7. Low levels of DUX4 induce H3 acetylation through p300 in human myoblasts.

(A) Western blot for acetylation of H3K27, H3K18 in LHCN-iDUX4 cells treated with low levels of doxycycline for 24 and 48 hours. Note that at this level of induction DUX4 is not detectable.

(B) Western blot for the markers of H3 acetylation in LHCN-iDUX4C cells continually induced with 200 ng/mL doxycycline and treated with 0.25 μM iP300w for 12 hours.

(C) Western blot for acetylation and methylation of H3 and H4 in LHCH-iDUX4 cells that were pulse-induced with 200 ng/mL doxycycline for 2 hours. Cells were treated with 0.25 μM iP300w after the doxycycline induction.

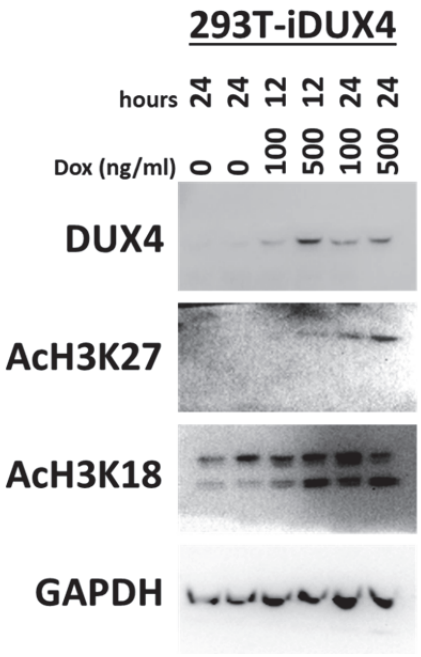


Fig. S8. DUX4 induces H3 acetylation in nonmyogenic cells.

Western blot for markers of acetylation in 293T-DUX4 cells induced with 200 ng/mL doxycycline and treated with 0.25 μM iP300w for 12 and 24 hours.

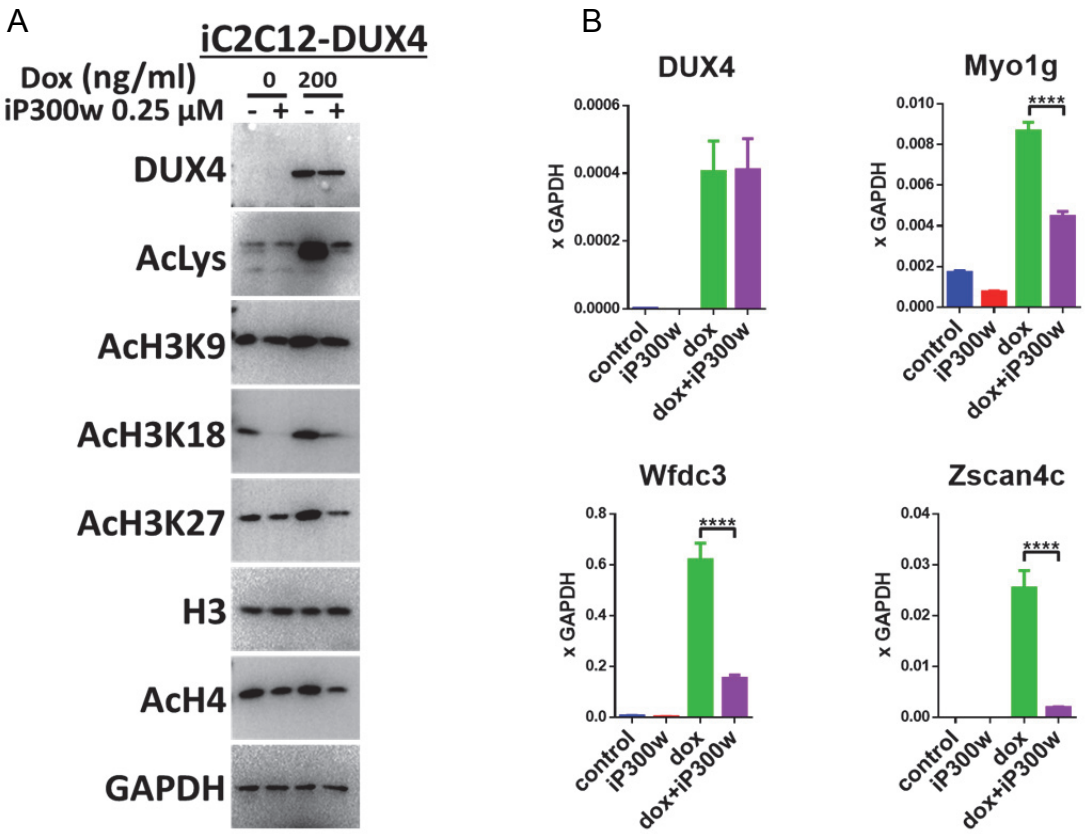


Fig. S9. Low levels of DUX4 induce H3 acetylation through p300 in mouse myoblasts.

(A) Western blot for markers of H3 acetylation in iC2C12-DUX4 cells induced with 200 ng/mL doxycycline and treated with 0.25 µM iP300w for 12 hours.

(B) RT-qPCR for DUX4 and DUX4 targets in iC2C12-DUX4 cells induced with 200 ng/mL doxycycline and treated with 0.25 µM iP300w for 12 hours. Data are presented as mean ± SEM; ****p<0.0001, by one-way ANOVA. Results are presented as relative expression to GAPDH
(n=3).