Supplemental Table 1. DUX4 Mutagenesis Strategy.

Mutant Name	Mutation Type
Phosphorylation Mutants	
T5A	Phosphorylation deficient
T5E	Phosphorylation mimic
S7A	Phosphorylation deficient
S7E	Phosphorylation mimic
S9A	Phosphorylation deficient
S9E	Phosphorylation mimic
T10A	Phosphorylation deficient
T10E	Phosphorylation mimic
T27A	Phosphorylation deficient
T27E	Phosphorylation mimic
S29A	Phosphorylation deficient
S29D	Phosphorylation mimic
T99A	Phosphorylation deficient
T99E	Phosphorylation mimic
T102A	Phosphorylation deficient
T102E	Phosphorylation mimic
S104A	Phosphorylation deficient
S104E	Phosphorylation mimic
T106A	Phosphorylation deficient
T106E	Phosphorylation mimic
T5A, S7A	Phosphorylation deficient
T5E, S7E	Phosphorylation mimic
S29A, S31A	Phosphorylation deficient
S29D, S31D	Phosphorylation mimic

S29A, S31A, T106A	Phosphorylation deficient
S29D, S31D, T106D (Mutant 2)	Phosphorylation mimic
T27A, S29A, S31A	Phosphorylation deficient
T27E, S29E, S31E	Phosphorylation mimic
T99A, T102A, S104A, T106A	Phosphorylation deficient
T99E, T102E, S104E, T106E (Mutant 5)	Phosphorylation mimic
T5A, S7A, S9A, T10A	Phosphorylation deficient
T5E, S7E, S9E, T10E	Phosphorylation mimic
Phosphonull: T5A, S7A, S9A, T10A, T27A, S29A, S31A, T99A, T102A, S104A, T106A	Phosphorylation deficient
Phosophomimic: T5, S7, S9, T10, T27, S29, S31, T99, T102, S104, T106 D/E	Phosphorylation mimic
Methylation Mutants	
R35A	Methylation deficient
R35K	Basic charge conserved
R35L	Methylation mimic
R62A	Methylation deficient
R62K	Basic charge conserved
R62L	Methylation mimic
R71A	Methylation deficient
R71K	Basic charge conserved
R71L	Methylation mimic
R137A	Methylation deficient
R137K	Basic charge conserved
R137L	Methylation mimic
R236A	Methylation deficient
R236K	Basic charge conserved

R236L	Methylation mimic
Methyl_null basic: R35K, R62K, R71K, R137K, R236K	Basic charge conserved
Methyl_neutral: R35L, R62L, R71L, R137L, R236L	Methylation neutral
HOX1_methyl_null basic; HOX1 residues = R35, R62, R71; Methyl_null basic = R35K, R62K, R71K, R137K, R236K	Basic charge conserved
HOX1_methyl_mimic; HOX1 residues = R35, R62, R71; Methyl_mimic = R35L, R62L, R71L, R137L, R236L	Methylation mimic
Acetylation Mutants	
K265A	Acetylation deficient
K265Q	Acetylation mimic

DUX4 residues containing PTMs were mutated as indicated. Mutants were generated to mimic or prevent (null) the modification event, or to provide some conservation of the modified amino acid.

Supplemental Table 2. **Phosphorylation profile of DUX4 with serine/threonine kinases**.
A radiometric protein kinase filter-binding assay was used for measuring the kinase activity of 245 serine/threonine kinases. The activity value (raw counts of the kinase assay as measured in the filter plate assay), the normalized kinase autophosphorylation value, the median of three background values of the sample protein and the corrected activity value (raw value minus sample protein background) are reported in the table. The activity ratio value for each kinase describes the ratio between the activity of the particular kinase with the DUX4 protein and without the DUX4 protein. A ratio value of >3 may be considered as significant.

Supplemental Table 3. Proteins associated with the DUX4 complex in human myoblasts using the RIME assay.
RIME (Rapid Immunoprecipitation Mass Spectrometry of Endogenous Proteins) was carried out using an antibody against V5 tag and 100ug of chromatin from DUX4.V5 transfected human myoblasts to identify proteins that interact with DUX4 using mass spectrometry. The file includes a summary of the proteins enriched in the RIME analysis. Two independent experiments were performed (R1 and R2). The enriched protein list contains uniquely identified proteins for all samples after removing proteins present in the IgG negative control. Three lists were generated – two corresponding to the proteins identified uniquely in one of the two replicates, and one corresponding to proteins identified in both replicates. The file also includes a list of total spectrum counts for all proteins and peptides identified in all samples.