Supplementary figures

Figure S1. Organization of the chromosomal regions 4q35 and 10q26. **A.** D4Z4 repeats, MAR (Matrix attachment region), truncated D4Z4-repeat (D4Z4*) and nearby genes at 4q35 (*FRG2*, *DUX4c*, *TUBB4Q*, *FRG1*, *ANT1*) and 10q26 (*SYCE1*) are shown. Nucleotide numeration starts from the first nucleotide of the *FRG2* mRNA according to the human genome assembly GRCh37/hg19 (February 2009). **B.** Schematic alignment of two full-length 3.3 Kb D4Z4 repeats and the truncated D4Z4* repeat on chromosome 4 and the corresponding region at 10q26; % identity is shown; regions of homology "0-7" correspond to those in Supplementary alignment. Nucleotide numeration starts from the first nucleotide in the *FRG2* mRNA for chromosome 4 and *FRG2B* for chromosome 10.

Figure S2. Enhancer 170 forms two different complexes with proteins in nuclear extract. **A**. EMSA analysis of the HeLaS3 nuclear extract incubated with the ^{32}P-labeled fragment A in the presence of 3-, 10- , 30-, 100- or 300-fold excess of cold specific competitor; “-e”: no extract control. **B**. EMSA analysis of the differentiated C2C12 myoblasrs nuclear extract incubated with the ^{32}P-labeled fragment A in the presence of 10-, 30- or 100-fold excess of cold wild-type or mutated fragment A (Amut-all with mutations in EGR1, ZNF444, SP1 and KLF15 sites). **C**. EMSA analysis of HeLa S3 nuclear extract incubated with the ^{32}P-labeled fragment A in the presence of 10-, 30- or 100-fold excess of cold wild-type or mutant fragment A (Amut-all and Amut-E/Z with mutations in EGR1 and ZNF44 sites) and SP1-specific competitor (SP1b).

Figure S3. A. KLF15 activates the D4Z4 enhancer in various cell lines. Luciferase activity was measured in HeLa and iMyo cells co-transfected with the reporters pPro, pEA-Pro, pE170-Pro and the KLF15 plasmid. **B.** Consensus sequence of KLF15 and Sp1 binding sites. **C**. *FRG1*, *FRG2*, *ANT* and *KLF15* (endogenous and ectopic, see Materials and Methods for details) expression was measured using qRT-PCR in RD cells transiently transfected with the *KLF15* plasmid.*p-value <0.01 (Student's *t*-test). **D.** *DUX4c* expression was analyzed using semi-quantitative RT-PCR in proliferating iMyo cells transiently transfected with the *KLF15* plasmid or an empty vector control.

Controls with no reverse transcriptase (RT) are shown in lanes 2, 4, 6, 8, 10 and 12. As a positive control for *DUX4c* expression, RT-PCR was performed using total RNA from iMyo cells transfected with a plasmid expressing *DUX4c* under the control of its own promoter (tracks 9 and 10) or directly from a DUX4c plasmid (lane 12) using water as a negative control (lane 11).

Supplementary alignment. Alignment of the full-length 3.3 Kb D4Z4 repeats and truncated D4Z4* repeat on chromosome 4 and the corresponding region at 10q26. Nucleotide numbers start from the first nucleotide in the *KpnI* site in the chromosome 4-specific 3.3 Kb D4Z4 repeat. The regions of homology "0-7" correspond to those shown in Figure S1B.