

Fig. A.1: The extended pedigree for Family 18 indicates FSHD through six generations.

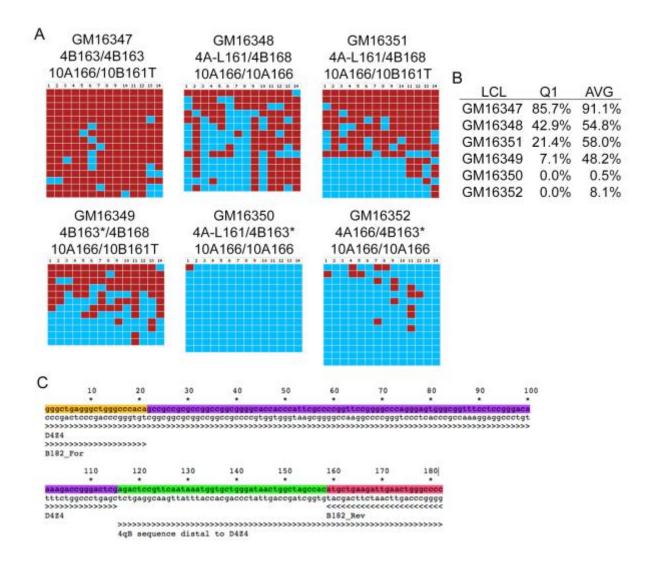


Fig. A.2: BSS sequencing of the 4qB subtelomere (BSSB). A) BSSB performed on lymphoblastoid cell lines from Family 36 shows that a contracted 4qB allele (4B163*) is hypomethylated. B) DNA methylation levels of the B-type subtelomeres on chromosome 4q and 10q. The percent DNA methylation for the first quartile and average total percent methylation are listed. C) Sequence amplified by the BSSB BS-PCR primers B182-For and B182-Rev contains 14 CpGs. All 4B and 10B alleles are amplified.

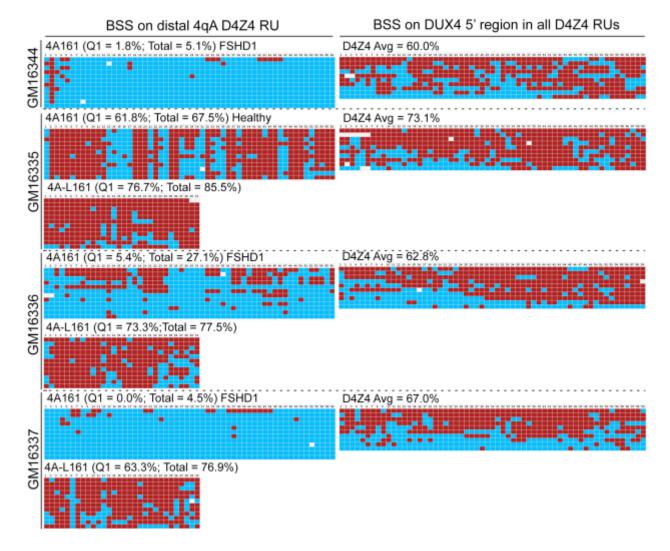
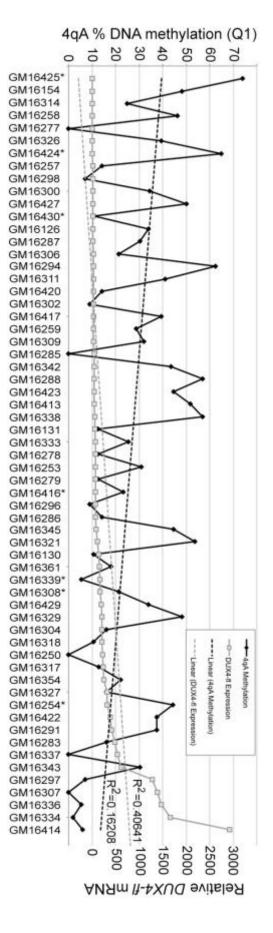


Fig. A.3: DNA methylation analysis of LCLs. BSS of Family 18, containing 3 FSHD1 LCLs and 1 heathy LCL, shows that DNA methylation signatures are conserved. A total of 56 CpGs of the distal-most D4Z4 DUX4 gene body (left column) or 59 CpGs of the DUX4 promoter (right column) were analyzed, depicted linearly from left to right, with methylated CpGs (red boxes), unmethylated CpGs (blue boxes), and missing CpGs (white boxes) indicated. Diagnostic FSHD1 methylation is defined as <35% methylation at the first quartile (Q1) of the DUX4 gene body; FSHD2 is defined as <25% average methylation of the DUX4 promoter and gene body. All three FSHD1 LCLs show FSHD1 levels of DNA methylation, while the healthy LCL line shows characteristic normal levels of DNA methylation. There is no indication of FSHD2 in these lines.



genetically FSHD1 subjects are indicated with an *. Overall across the whole cohort, DUX4-fl mRNA levels increase as DNA methylation levels decrease. the right. DNA % methylation (Q1) of the 4qA or A-L alleles is plotted on the y-axis to the left. The 7 clinically asymptomatic from the lowest to highest levels of DUX4-fl mRNA expression relative to GM16425 (expression = 1) and plotted on the y-axis to Fig. A.4: DUX4-fl expression imperfectly correlates with DNA hypomethylation. The FSHD1 samples are arranged left to right

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4A161 GTCTAGGCCCGGTGAGAGACTCCACACCGCGGA
4A166 GTCTAGGCCCGGTGAGAGACTCCACACAGCGGA
10A166 GCCTAGGCCCGGTGAGAGACTCCACACCGCGGA
GM16154 (4A161) GTCTAGGCCCGGTGAGAGACTCCACACAGCGGA
GM16126 (4A166) GTCTAGGCCCGGTGAGAGACTCCACACAGCGGA
GM16354 (4A166) GTCTAGGCCCGGTGAGAGACTCCACACAGCGGA
GM16254 (4A166) GTCTAGGCCCGGTGAGAGACTCCACACAGCGGA
GM16424 (4A166) GTCTAGGCCCGGTGAGAGACTCCACACAGCGGA
GM16425 (4A166) GTCTAGGCCCGGTGAGAGACTCCACACAGCGGA
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Fig. A.5: Sequencing confirms that the DUX4-fl mRNA expression from 5 FSHD1 members of Family 33 originates from the contracted 4A166 allele, as indicated by the presence of 4A166 specific SNPs in exon 2 of the RT-PCR product.