

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

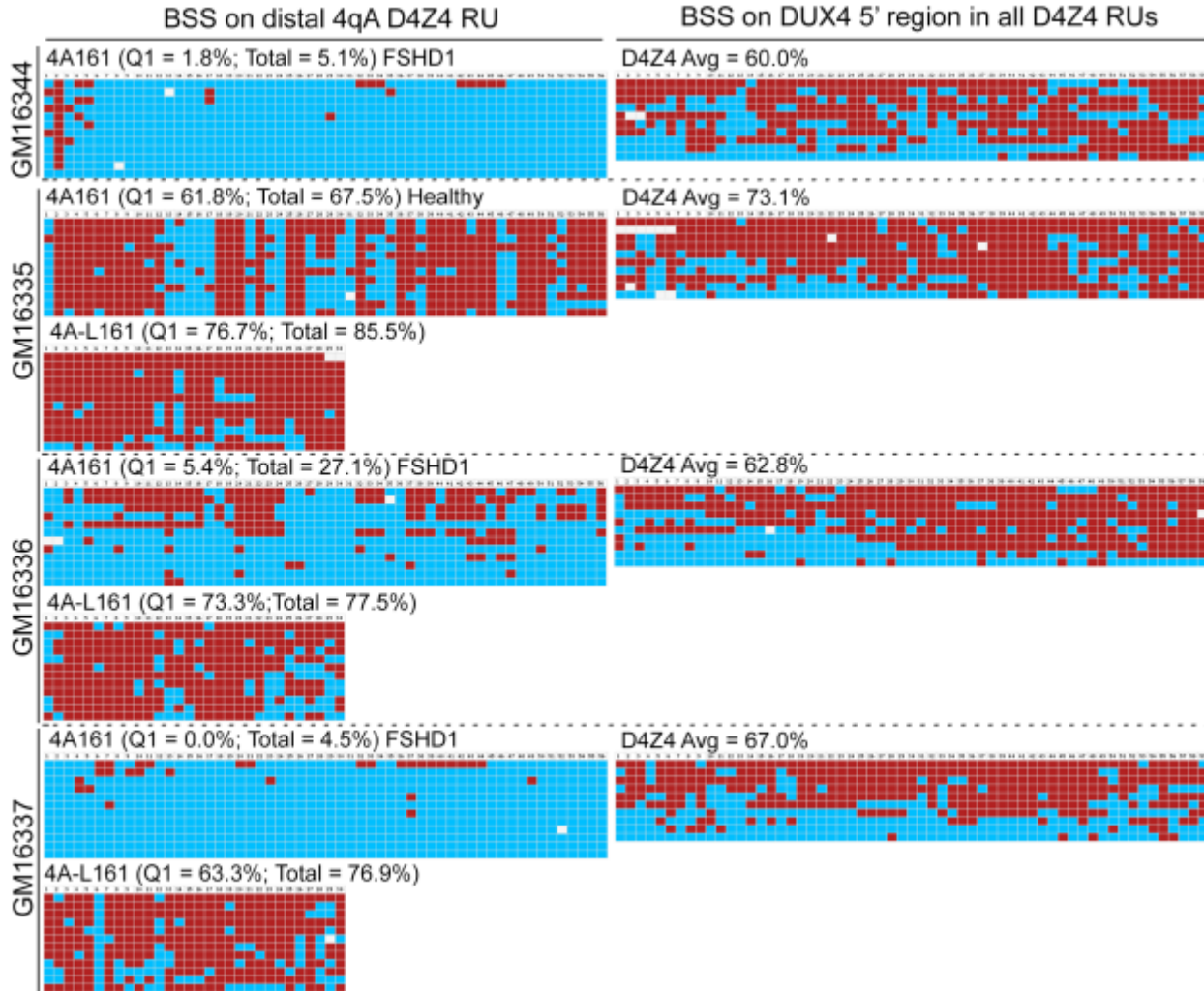


Fig. A.3: DNA methylation analysis of LCLs. BSS of Family 18, containing 3 FSHD1 LCLs and 1 healthy LCL, shows that DNA methylation signatures are conserved. A total of 56 CpGs of the distal-most D4Z4 *DUX4* gene body (left column) or 59 CpGs of the *DUX4* promoter (right column) were analyzed, depicted 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.

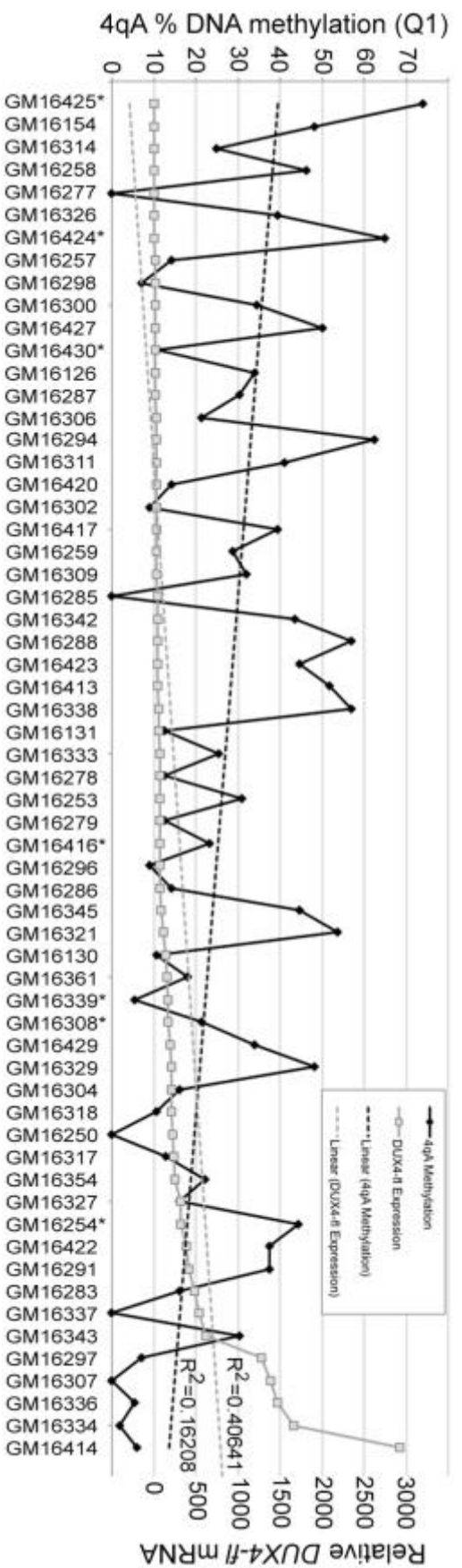


Fig. A.4: DUX4-fl expression imperfectly correlates with DNA hypomethylation. The FSHD1 samples are arranged left to right from the lowest to highest levels of DUX4-fl mRNA expression relative to GM16425 (expression = 1) and plotted on the y-axis to the right. DNA % methylation (Q1) of the 4qA or A-L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

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4A161 GTCTAGGCCCGGTGAGAGACTCCACACCGCGGA
4A166 GTCTAGGCCCGGTGAGAGACTCCACACAGCGGA
10A166 GCTAGGCCCGGTGAGAGACTCCACACCGCGGA
GM16154 (4A161) GTCTAGGCCCGGTGAGAGACTCCACACCGCGGA
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.