

Figure S1: DUX4-fl mRNA expression in biopsies detected at 55 PCR cycles. Polyadenylated DUX4-fl mRNA was detected from biopsy mRNA using nested RT-PCR (25 cycles followed by 30 nested cycles). DUX4-fl mRNA was expressed in 22 out of 45 FSHD biopsies and 1 out of 34 control biopsies (Figure 3; cohort 03). Sequencing confirmed spliced DUX4-fl mRNA expression (arrows). One of three independent replicate experiments is shown.

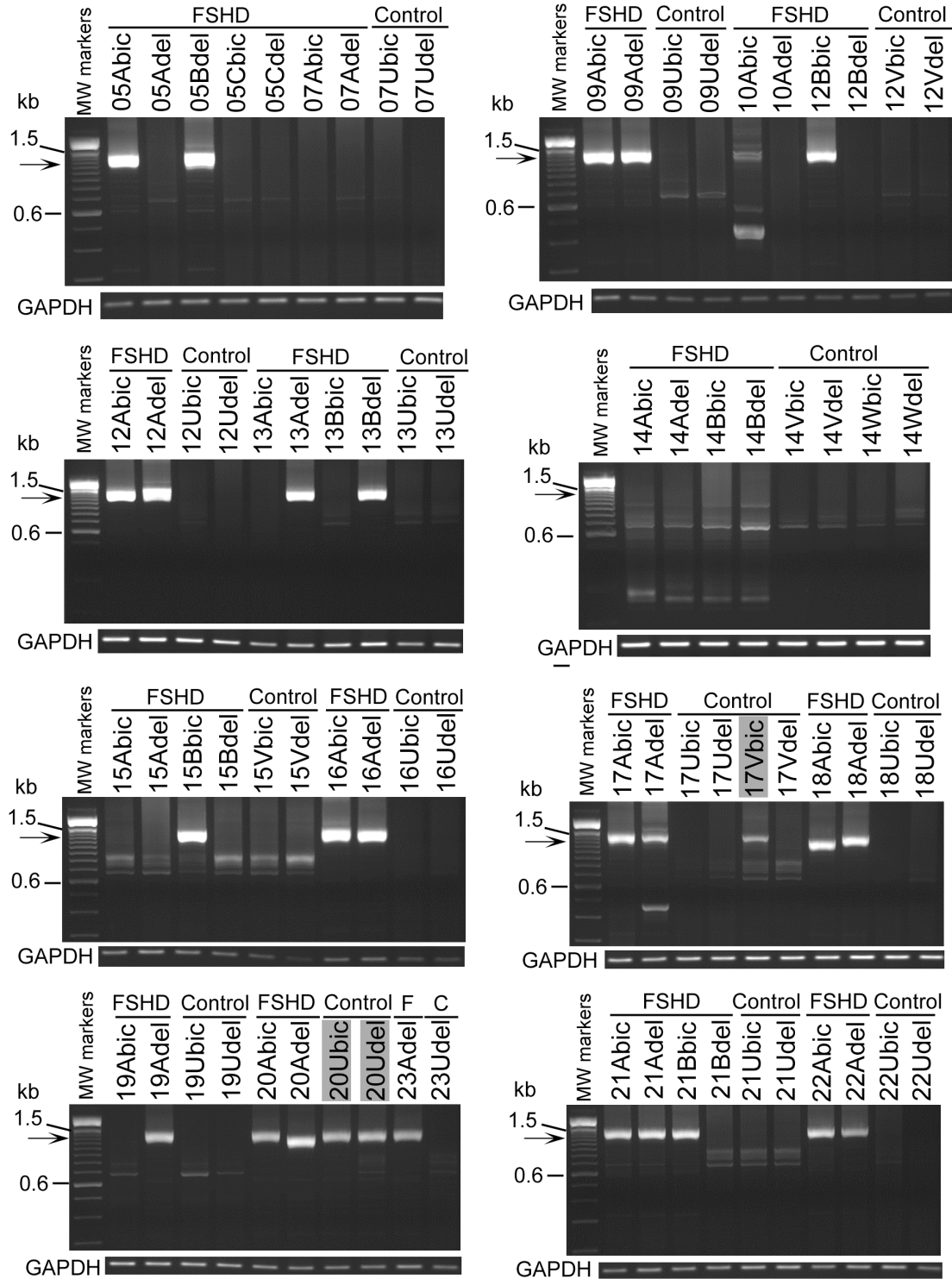


Figure S2: Polyadenylated DUX4-fl expression in biopsies detected at 65 PCR cycles. Nested RT-PCR (25 cycles followed by 40 nested cycles) from biopsy mRNA. Sequencing confirmed spliced DUX4-fl mRNA expression (arrows) was detectable in 3 out of 33 unaffected biopsies (gray boxes; 17Vbic, 20Ubic, 20Udel) from two control individuals (17U, 20U) and 26 out of 44 FSHD biopsies. Gray shading indicates DUX4-fl expression in control biopsies. One of two independent replicate experiments is shown.

Table S1: Summary of individual DUX4-fl assays

Control Subjects	RT-PCR Day 0	RT-PCR Day 4	RT-PCR Biopsy (55)	RT-PCR Biopsy (65)	DUX4-FL ICC	4qA (kb) <i>EcoRI/BlnI</i>
03Udel	-	-----	nd	nd	+	>112
03Ubic	-	-----	--	-	-	>112
07Udel	--	+ -	---	--	+	53
07Ubic	--	+ -	---	--	-	53
09Udel	--	-	---	--	-	>112
09Ubic	--	-	---	--	+	>112
12Udel	- + -	+ + +	---	--	+	>112
12Ubic	--	--	---	--	-	>112
12Vdel	nd	nd	---	--	nd	>112
12Vbic	nd	nd	---	--	nd	>112
13Udel ^a	nd	nd	---	--	nd	none
13Ubic ^a	nd	nd	---	--	nd	none
14Vdel	nd	nd	---	--	nd	60
14Vbic	nd	nd	---	--	nd	60
14Wdel ^a	nd	nd	---	--	nd	4qB only
14Wbic ^a	nd	nd	---	--	nd	4qB only
15Vdel ^a	-	---	--	--	-	4qB only
15Vbic ^a	-	---	--	--	-	4qB only
16Udel	---	+++	---	--	-	93
16Ubic	---	+++	---	--	+	93
17Udel	nd	nd	-----	--	nd	>112
17Ubic	nd	nd	-----	--	nd	>112
17Vdel	-----	- + - - - -	-----	--	+	90
17Vbic	-----	-----	- + - - - - -----	- +	-	90
18Udel ^a	---	---	---	--	-	4qB only
18Ubic ^a	---	---	---	--	-	4qB only
19Udel	nd	nd	---	--	nd	79
19Ubic	nd	nd	---	--	nd	79
20Udel	-----	+++++	---	- +	-	39
20Ubic	+ - + - - -	+ + + - - -	--- - + - - - -	- +	-	39
21Udel	nd	nd	---	--	nd	>112
21Ubic	nd	nd	---	--	nd	>112
22Udel	nd	nd	---	--	nd	>112
22Ubic	nd	nd	---	--	nd	>112
23Udel	nd	nd	---	--	nd	45

FSHD Subjects	RT-PCR Day 0	RT-PCR Day 4	RT-PCR Biopsy (55)	RT-PCR Biopsy (65)	DUX4-FL ICC	4qA (kb) Eco/Bln
03Adel	-	+ - + - +	nd	nd	-	20
03Abic	+ - +	- + + - +	++	+	+	20
05Adel	nd	nd	++-	--	nd	25
05Abic	nd	nd	---	-+	nd	25
05Bdel	+ - -	+++	+++	++	nd	25
05Cdel	nd	nd	++-	--	nd	25
05Cbic	---	+++	---	+-	-	25
07Adel	--+	---	---	--	+	29
07Abic	--	++-	---	--	+	29
09Adel	++	+	-+-	-+	+	25
09Abic	++	+	+-	++	+	25
10Adel	nd	nd	++-	+-	nd	24
10Abic	nd	nd	-++	++	nd	24
12Adel	+++	+++	+++	++	+	18
12Abic	+ -	- +	+ - -	++	+	18
12Bdel	nd	nd	- - +	+-	nd	18
12Bbic	nd	nd	+++	++	+	18
13Adel	nd	nd	+++	-+	nd	nd
13Abic	nd	nd	---	--	nd	nd
13Bdel	nd	nd	+ - +	++	nd	16
13Bbic	nd	nd	+ - +	--	nd	16
14Adel	nd	nd	---	--	nd	19
14Abic	nd	nd	---	--	nd	19
14Bdel	nd	nd	---	--	nd	19
14Bbic	nd	nd	---	--	nd	19


15Adel	-	--+	--	+-	-	28
15Abic	--+	---	++	+-	+	28
15Bdel	-	---	-+	--	+	28
15Bbic	-+	+ - +	+ -	--	+	28

16Adel	+++	+++	-++	-+	+	20
16Abic	--+--	+++	+++	++	+	20
17Adel	+++	+++	++++	++	+	19
17Abic	---	+++	++++ +++++	++	+	19
18Adel	+++	+++	-+-	++	+	21
18Abic	---	+++	-++	-+	+	21
19Adel	nd	nd	-+-	-+	nd	22
19Abic	nd	nd	---	--	nd	22
			++++--			
20Adel	+ - -	+++	-++	-+	+	20
20Abic	++-	+++	---	-+	+	20

21Adel	nd	nd	+++	++	nd	26
21Abic	nd	nd	-++	++	nd	26
21Bdel	nd	nd	+ - +	+ -	nd	26
21Bbic	nd	nd	- ++	++	nd	26
22Adel	nd	nd	++ -	++	nd	27
22Abic	nd	nd	---	- +	nd	27
23Adel	nd	nd	- - +	++	nd	18
27Adel	---	---	nd	nd	-	12
27Abic	---	+ + -	---	nd	-	12

27Bdel	+ - -	---	---	nd	-	12

27Bbic	---	---	---	nd	-	12
28Adel	+ - -	+++	---	nd	+	29
28Abic	- - +	+++	- ++	nd	+	29
28Bdel	- - +	+++	nd	nd	+	29
28Bbic	- + -	+++	+ - -	nd	+	29
29Adel	- ++	+++	- ++	nd	+	30
29Abic	+ - +	+++	+++ +++++	nd	+	30
29Bdel	+++	+++	---	nd	+	30
			+-----			
29Bbic	+++	+++	+ - -	nd	+	30
30Adel	---	+++	- + -	nd	nd	30
30Abic	---	+++	+++	nd	nd	30
30Bdel	+++	+++	---	nd	nd	32
30Bbic	+++	+++	---	nd	nd	32

 = DUX4-fl expression from unaffected subject

For RT-PCRs, each + or – reflects the result of one repetition

nd = not determined

^a no 4qA alleles detected

For ICC, cells are classified as + if one or more nucleus shows a positive immunostaining for DUX4-FL protein