

Table S1

To simplify the labeling of the many different cell lines throughout this manuscript, we designate them as follows:

Cell line	Code name	Original name	Repeat number	REF	
XY WT hESCs	WT-ES-1	SZ-13	normal range	(AVITZOUR <i>et al.</i> 2014)	
	WT-ES-2	SZ-15			
	XX WT hESC	WT-ES-3			HES-123
	XY WT hESC	WT-ES-4			B-200
	XY WT hESC	WT-ES-5			SZ-FX C15B ¹
hESC with unmethylated full expansion at the FMR1	uFM-ES-1	HEFX	~200-650		
	uFM-ES-2 ²	SZ-FX7	~200-300		
	uFM-ES-3	SZ-FX C12B	~202, 300		
	uFM-ES-4	SZ-FX1			
hESC with premutation expansion at the FMR1	PM-ES-1	SZ-FX4	160 ³		
	PM-ES-2	SZ-FX14 C-11	107, 125 ³		
hESC with an expansion at the C9orf72	C9-ES-1	SZ-ALS1	270	(COHEN-HADAD <i>et al.</i> 2016)	
	C9-ES-2	SZ-ALS3	270		

¹ This cell line was used only as a wild type (WT) control for the *C9orf72* locus

² A female cell line with skewed inactivation of the normal allele (AVITZOUR *et al.* 2014).

³ Determined using Asuragen (Amplidex® PCR/CE *FMR1*).

⁴ Roughly estimated by Southern blot analysis

Table S2 (primers):

Method	Reaction	5' Primer (sequence 5'-3')	3' Primer (sequence 5'-3')	Tm	
DRIP	EGR1	GAACGTTCAGCCTCGTTCTC	GGAAGGTGGAAGGAAACACA	60	
	RPL13A	AATGTGGCATTTCCTTCTCG	CCAATTCGGCCAAGACTCTA	60	
	FMR1	GAGGGCTTCAGGTCTCCTTT	CAGTTGCCATTGTGATTGG	60	
	C9orf72	GCCTCCCCTATTAAGGTTTCG	TCTCACGAGGCTAGCGAAAT	60	
Colony bisulfite footprinting analysis	FMR1 5' R-loop boundary	GAGGGAAACAGCGTTGATCACGTG	TAACAACAACACCTCCATCACC*	56	
	FMR1 3' R-loop boundary	GAAGTTTTTTTTGATTTTGAGAGG*	CCAATGCTAGACCGGAAAAGAG	60	
	C9orf72 5' R-loop boundary	AGGAAAGAGAGGTGCGTCAA	CACACAACCTCTAAATCCAAAAC*	55	
	C9orf72 3' R-loop boundary	GTTTTGTTGGGGGAAGTT*	GGTGCATGGCAACTGTTGAATAG	55	
	FMR1 repeats sense with R-loops 1 [‡]	GAGGGAAACAGCGTTGATCACGTG	CCTCTCAAATCAAAAAAAAACTCC*	60	
	FMR1 repeats sense with R-loops 2	GGAACAGCGTTGATCACGTGACGTGGTTTC		60	
	FMR1 repeats (antisense and sense) 1 [‡]	GAGGGAAACAGCGTTGATCACGTG	CCTCTCGGAGTCGGAGAGGGGCTTC	60	
	FMR1 repeats (antisense and sense) 2	GGAACAGCGTTGATCACGTGACGTGGTTTC		61	
	C9orf72 repeats (antisense and sense)	TCAGAGAAATGAGAGGGAAAGTAAA	GACCTGATAAAGATTAACCGAAGAA	58	
	RPL13A	GATTGGACATTCGGAAGAGG	GGATGTAAAGCTCCGCAAAA	58	
Next-seq bisulfite footprinting analysis	FMR1 repeats [‡]	GAGGGAAACAGCGTTGATCACGTG	CCTCTCGGAGTCGGAGAGGGGCTTC	60	
		Adapter-GGAACAGCGTTGATCACGTGACGTGGTTTC	Adapter-CACCAGCTCCTCCATCTTCT	61	
	C9orf72 repeats [‡]	TCAGAGAAATGAGAGGGAAAGTAAA	GACCTGATAAAGATTAACCGAAGAA	58	
		Adapter-TCAGAGAAATGAGAGGGAAAGTAAA	Adapter-GACCTGATAAAGATTAACCGAAGAA	58	
	RPL13A [‡]	GATTGGACATTCGGAAGAGG	GGATGTAAAGCTCCGCAAAA	58	
		Adapter-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG	Adapter-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGA	58	
	SNP	C9orf72	GTTTTTCCCACCTCTCTCC	GGTGCATGGCAACTGTTGAATAG	56

* converted primers; [‡] hemi-nested reaction