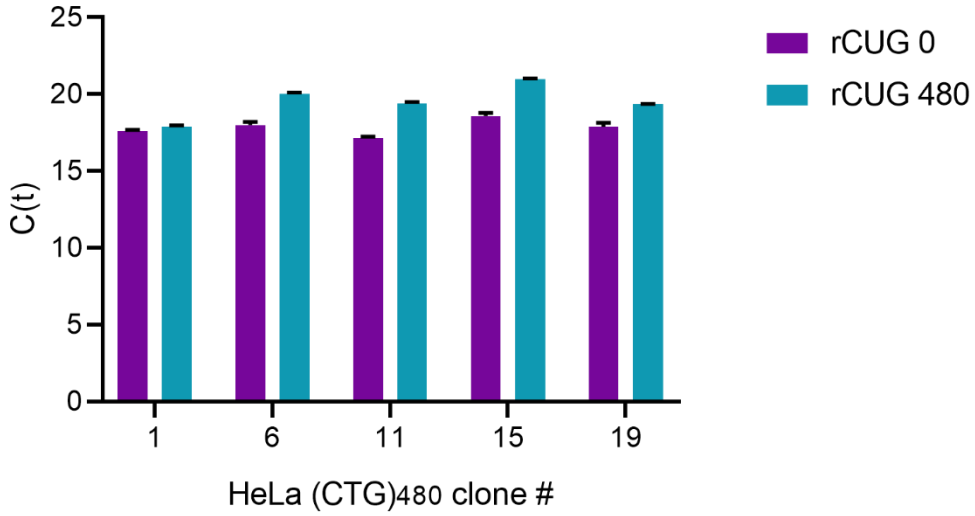
Supplementary figures

A



B

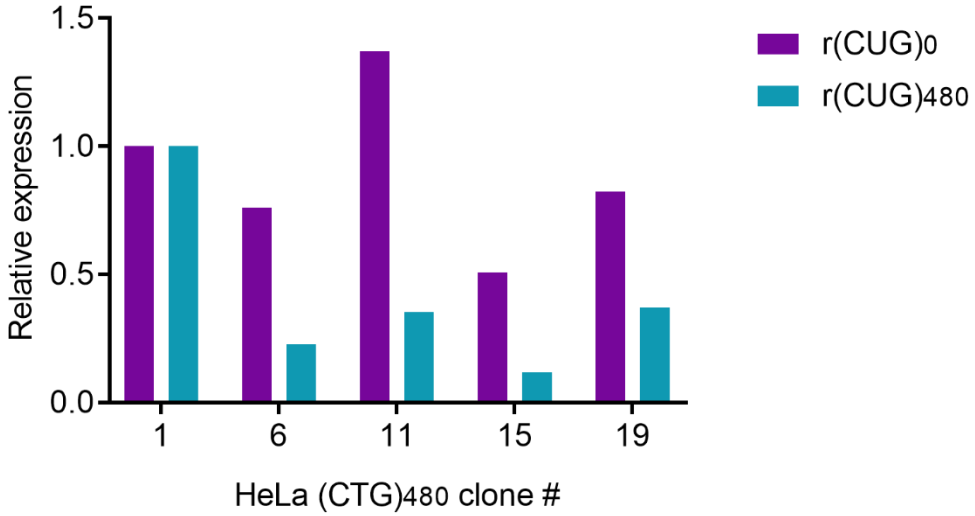


Fig. S1.

Expression of r(CUG)0 and r(CUG)480 in stable HeLa (CTG)480 clones. Multiplex RT-qPCR performed on RNA extracted from cultured HeLa (CTG)480 clonal lines simultaneously probing for r(CUG)0 and r(CUG)480 plotted by (*A*) C(t) values (mean of 3 technical replicates ± s.d.) or (*B*) Mean relative expression normalized to clone 1.

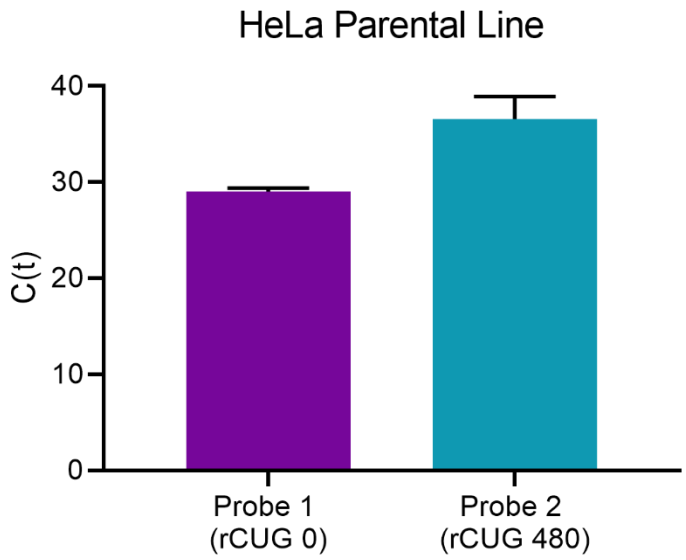


Fig. S2.

Multiplex RT-qPCR performed on RNA extracted from the HeLa parental cell line from which the stable HeLa $(CTG)_{480}$ clones were derived, simultaneously probing for $r(CUG)_0$ and $r(CUG)_{480}$ plotted by C(t) values (mean of 3 technical replicates $\pm$ s.d.).

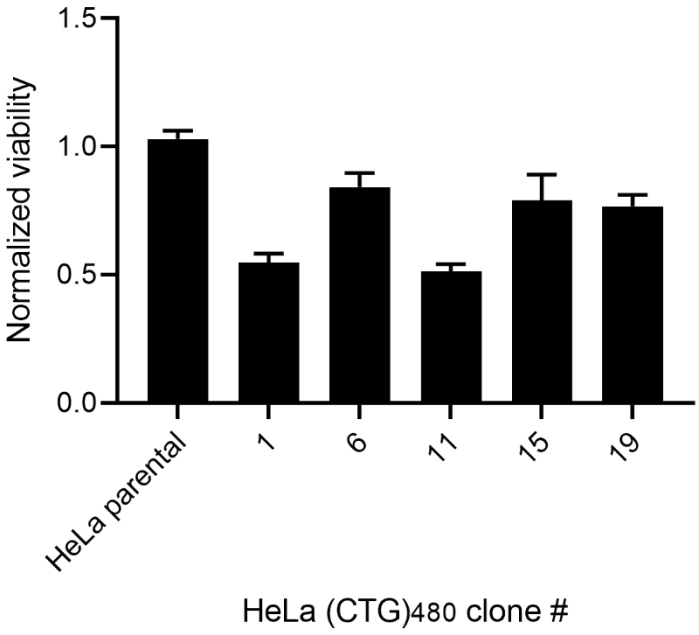


Fig. S3

Cell viability normalized to the HeLa parental line (mean + s.d. n=3 biological replicates).

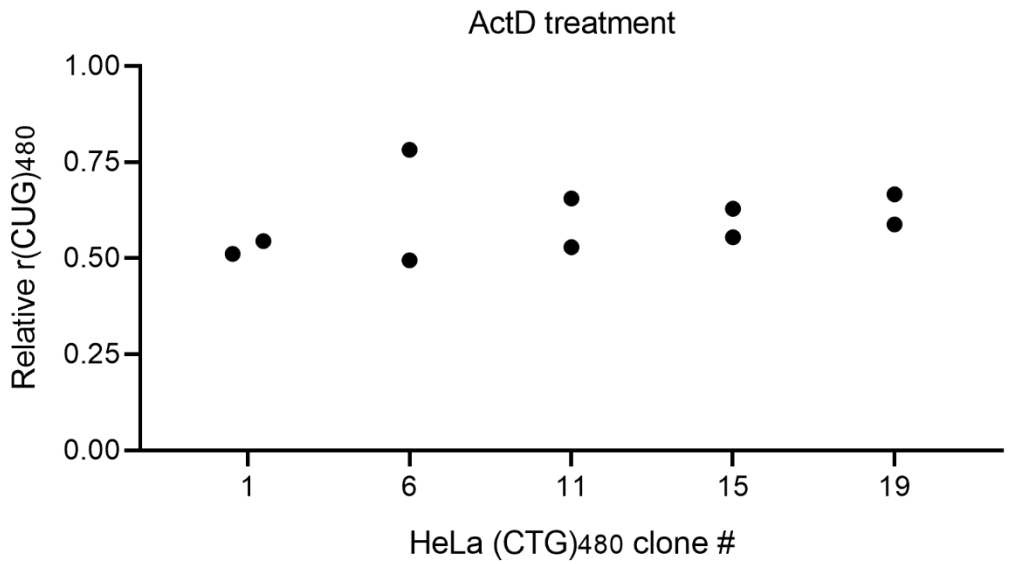


Fig. S4

HeLa $(CTG)_{480}$ clones were treated with ActD at 20 nM for ~ 24 hours and relative $r(CUG)_{480}$ levels normalized to $r(CUG)_0$ were determined using multiplex RT-qPCR (n=2 biological replicates).

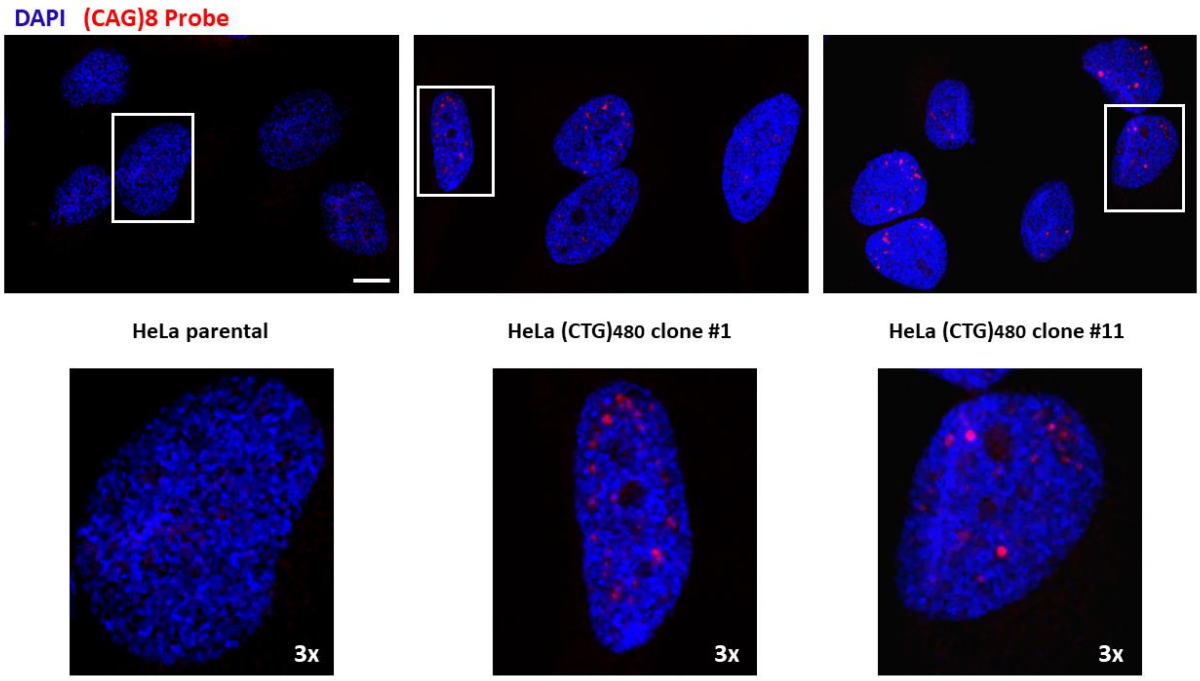


Fig. S5

Fluorescence *in situ* hybridization for $rCUG_{EXP}$ foci using a Cy3-$(CAG)_8$ probe and counterstained with DAPI for nuclei. Scale bar = 10 μm.

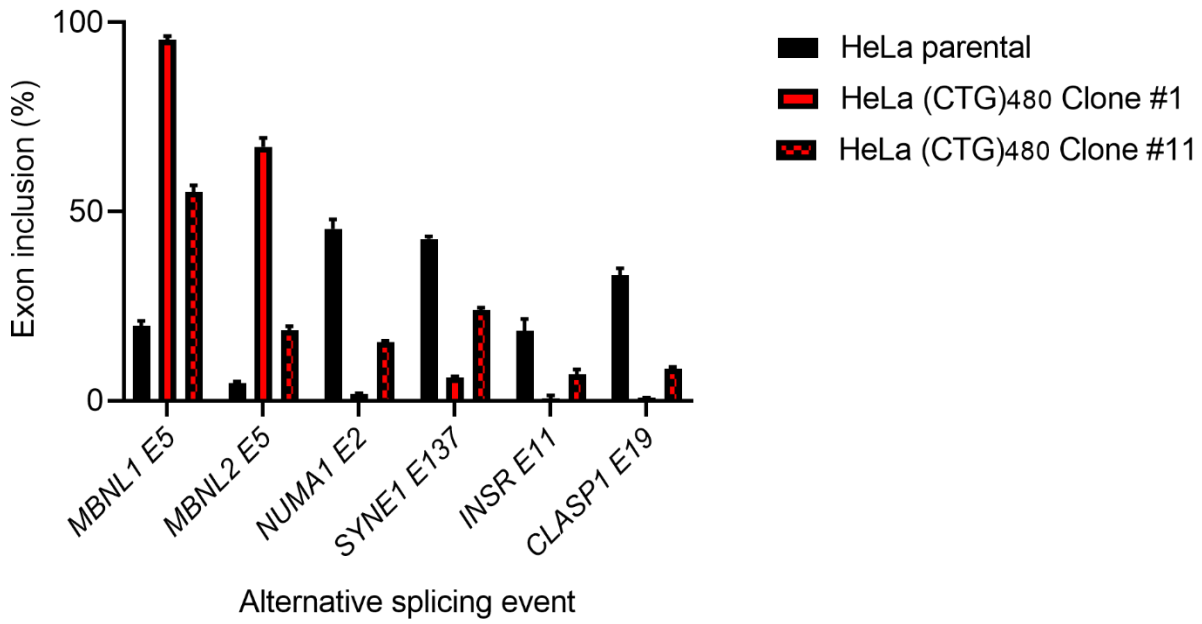


Fig. S6

RT-PCR isoform analysis of the indicated alternative cassette exon events comparing HeLa (CTG)480 clones #1 and 11 to the parental HeLa cell line (mean ± s.d. n=4 biological replicates).

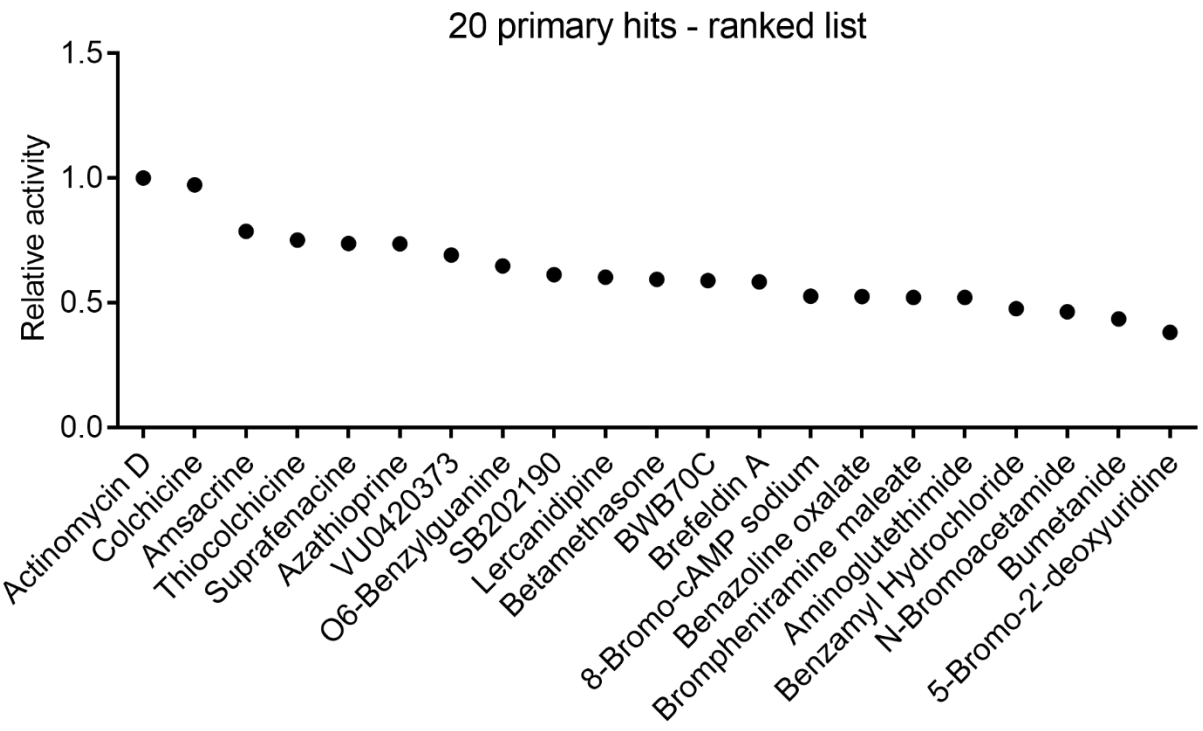


Fig. S7.

Ranked list of primary hits from LOPAC^{1280} screen. The 20 hits identified from the initial screen were re-screened at 1 μM along with Actinomycin D (ActD) at 20 nM and ranked relative to ActD (set to 1) in selective reduction of r(CUG)$_{480}$ levels compared to r(CUG)$_0$ (Relative activity).

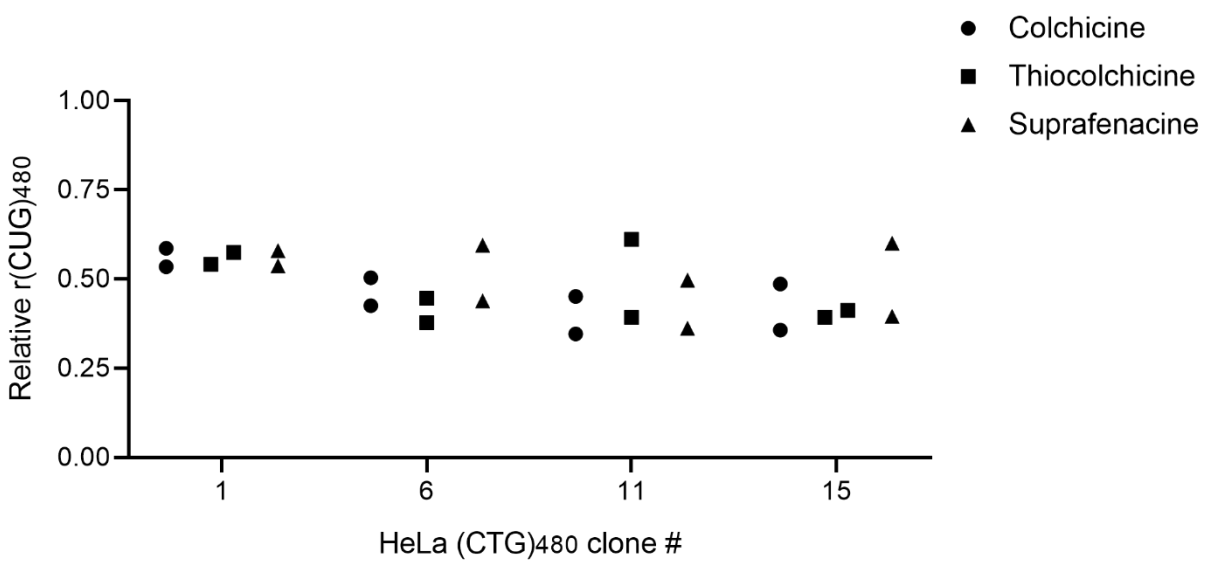
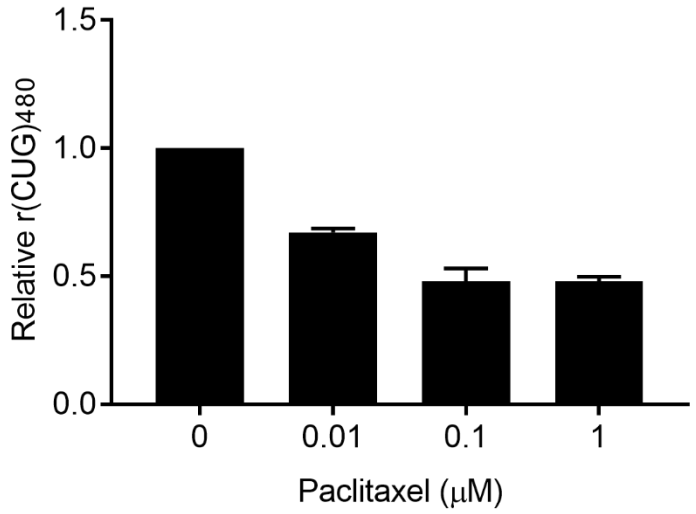


Fig. S8.

Clones 1, 6, 11 and 15 were re-screened with colchicine, thiocolchicine and suprafenacine at 1 μM for ~ 24 hours and relative $r(CUG)_{480}$ levels normalized to $r(CUG)_0$ were determined using RT-qPCR (n=2 biological replicates).

A



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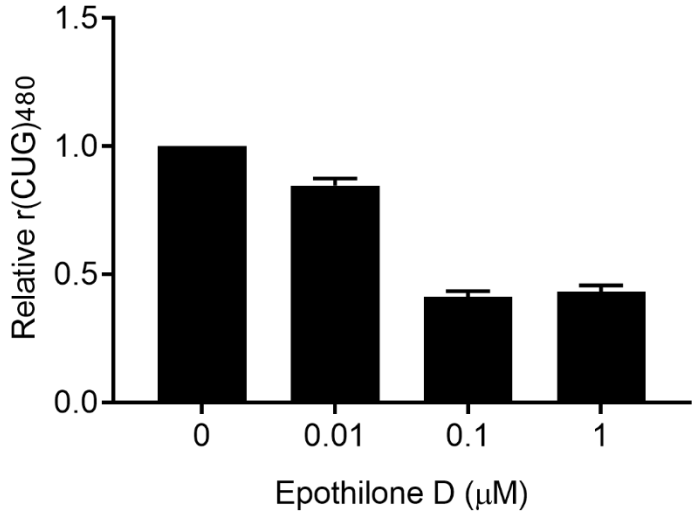


Fig. S9.

Effect of microtubule stabilizing agents (*A*) paclitaxel (Taxol) and (*B*) Epothilone D treatment on relative r(CUG)480 levels. HeLa (CTG)480 clone #19 was treated at the indicated concentrations, RNA was extracted and the relative r(CUG)480 levels (normalized to r(CUG)0) were determined using RT-qPCR (mean ± s.d. n=3 biological replicates).

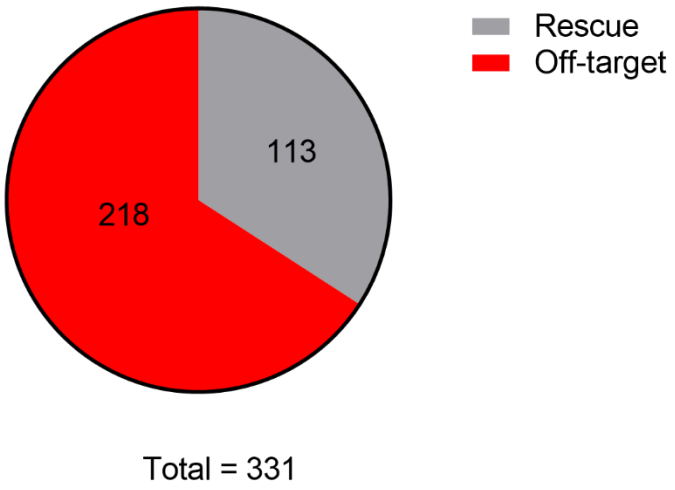


Fig. S10.

Transcript changes in *HSA*^{LR} mice treated with colchicine. **‘**Rescue’ were transcripts displaying reversion following colchicine-treatment of *HSA*^{LR} mice toward wild type mice (≥ 10% change, $P < 0.1$). ‘Off-target’ were those transcript changes resulting from colchicine treatment vs PBS control treatment of *HSA*^{LR} mice (≥ 10%, $P < 0.1$) that were not different between wild type and PBS control-treated *HSA*^{LR} mice.

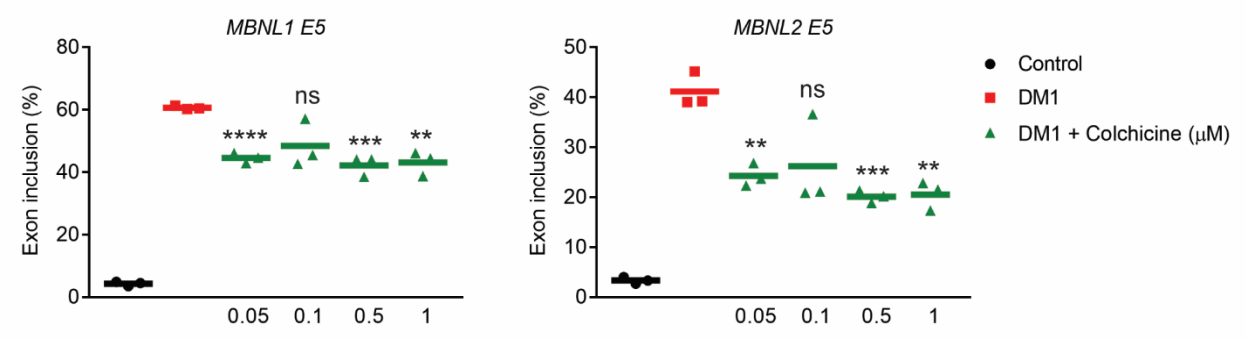


Fig. S11.

Effect of colchicine treatment on MBNL-dependent mis-splicing in differentiated DM1 myotubes. RT-PCR isoform analysis of the indicated alternative cassette exon events following treatment of DM1 myotubes with DMSO or colchicine at the indicated dose. (mean, n=3 biological replicates, ns = not significant, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$). Control myotubes treated with DMSO were used to determine wildtype alternative splicing levels (Control).

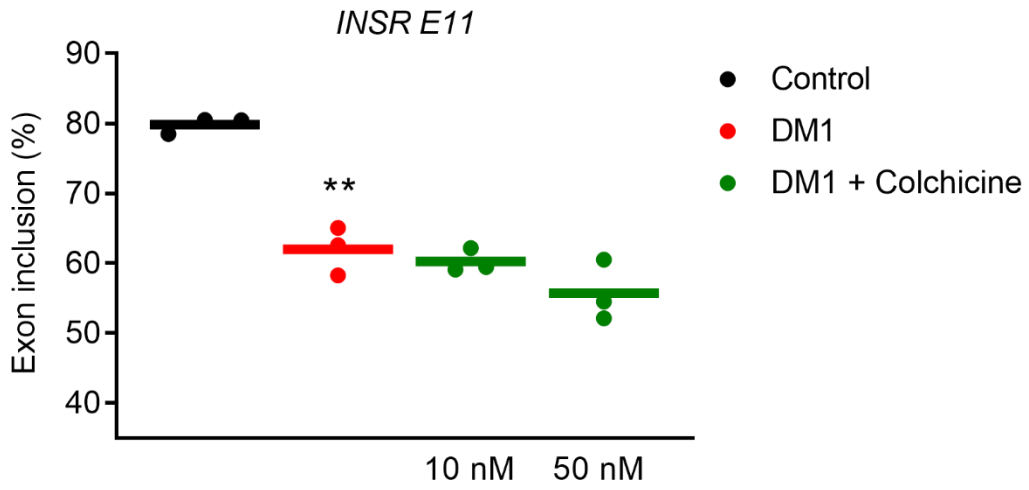


Fig. S12

Effect of colchicine treatment on mis-splicing of insulin receptor exon 11 in patient-derived fibroblasts. RT-PCR isoform analysis of the indicated alternative cassette exon events following treatment of DM1 myotubes with DMSO or colchicine at the indicated dose. (mean, n=3 biological replicates, $^{**}P < 0.01$). Control fibroblasts treated with DMSO were used to determine wildtype alternative splicing levels (Control).

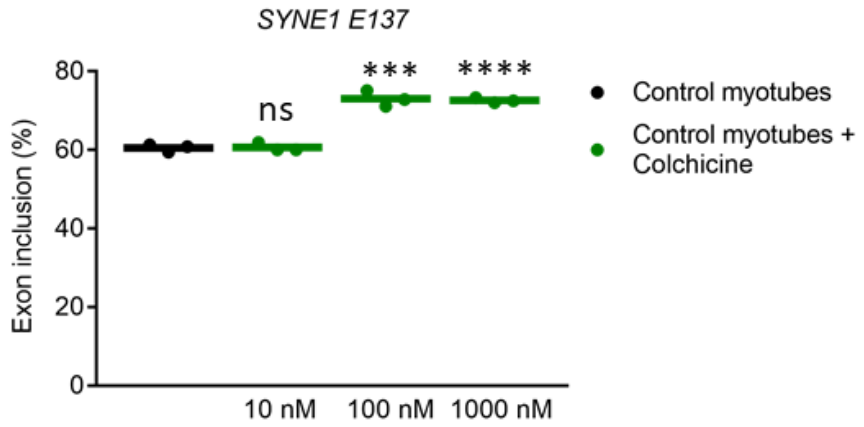
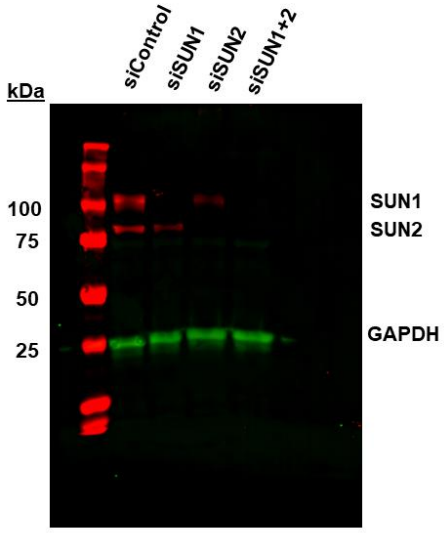


Fig. S13.

Effect of colchicine treatment on control myotubes for inclusion of *SYNE1* exon 137 (mean ± s.d. n=3 biological replicates, ns = not significant, ****P* < 0.001, *****P* < 0.0001).

A



B

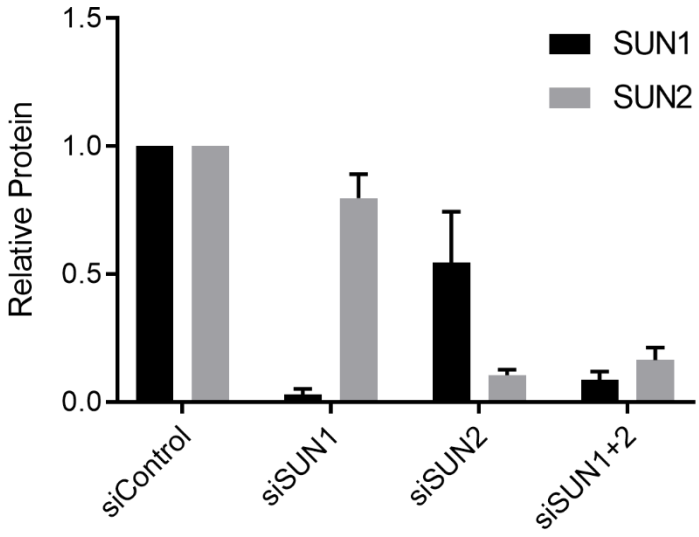


Fig. S14

(A) Representative Western blot probing for SUN1 and SUN2 proteins and GAPDH as a loading control. Protein lysates were obtained from HeLa (CTG)480 clone #19 that was treated with control siRNA (siControl), siRNA against SUN1 alone (siSUN1) or SUN2 alone (siSUN2) or SUN1 and SUN2 in combination (siSUN1+2) (B) Quantification of blots (mean ± s.d. n=4 biological replicates).

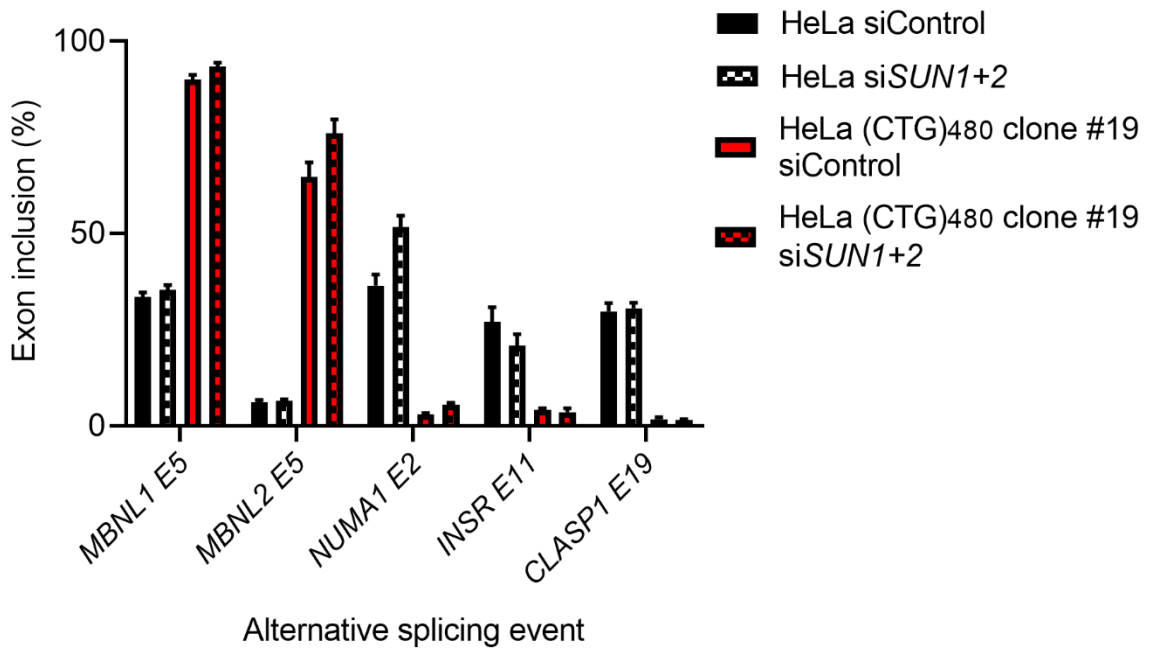


Fig. S15

RT-PCR isoform analysis of the indicated alternative cassette exon events in the HeLa parental cell line and the HeLa (CTG)480 clone #19 treated with a control siRNA (siControl) or siRNA against both *SUN1* and *SUN2* (si*SUN1+2*) (mean ± s.d. n=4 biological replicates).

Primers and probes

Primers/probes used for screening

Name	Sequence
HT_RT	5'-CTACACGACGCTCTTCCGATCTTCTTATCGAATGTCGGGGTCTCAGTGC
HT_Forward	5'-CGATCTCTGCCTGCTTACTC
HT_Reverse	5'-GTCGGAGGACGAGGTCAATAAA
HT_Probe1	/56FAM/AGAGCAGCG/ZEN/CAAGTGAGGAGG/3IABkFQ/
HT_Probe2	/5HEX/TGACGCAGC/ZEN/CACGTGAAGGTC/3IABkFQ/

Primers used for qPCR

Target	Forward Primer	Reverse Primer
Human *DMPK*	5'- CACGTTTTGGATGCACTGAGAC	5'- GATGGAGGGCCTTTTATTCGCG
Human *GAPDH*	5'- AATCCCATCACCATCTTCCA	5'- TGGACTCCACGACGTACTCA
Human *SUN1*	5'-TCAGCTTCGGTCAGAGACG	5'-TGGTGAAAGGCCATAAAGTCA
Human *SUN2*	5'-AAACTGCTGCTCGCATCC	5'GAGTCTTGCTGATGCTCTGCT

Primers used for RT-PCR splicing analysis.

Target	Forward Primer	Reverse Primer
Human *MBNL1* exon5	5'- AGGGAGATGCTCTCGGGAAAAGTG	5'- GTTGGCTAGAGCCTGTTGGTATTGG
Human *MBNL2* exon5	5'- ACAAGTGACAACACCGTAACCG	5'- TTTGGTAAAGGATGAAGAGCACC
Human *NUMA1* exon2	5'- AAGTATGAGGGTGCCAAGGT	5'- CTTCAGCTTCTGCTGCTGCA
Human *SYNE1* exon137	5'- GACAAAGATTTCTACCTCCGGGG	5'- CCCAGTTGTCGGATCTGTGACTC
Human *INSR* exon11	5'- CCTGTCCAAAGACAGACTCTCAGATCCTG	5'- GTCGAGGAAGTGTTGGGGAAAGC
Human *CLASP1* exon19	5'- CAAAGTCTCCTCATCTTCGGGCACG	5'- GCTGGGACTGTGAAACCACTTTAGC
Mouse *Atp2a1* exon22	5'- GCTCATGGTCCTCAAGATCTCAC	5'- GGGTCAGTGCCTCAGCTTTG
Mouse *Clcn1* exon7a	5'- TGAAGGAATACCTCACACTCAAGG	5'- CACGGAACACAAAGGCACTG
Mouse *Clasp1* exon13	5'- CAAATCTGTGTCGACGACAGGA	5'- GCTGAGACTGTGAAACCACTTTGG