

Supplementary figures

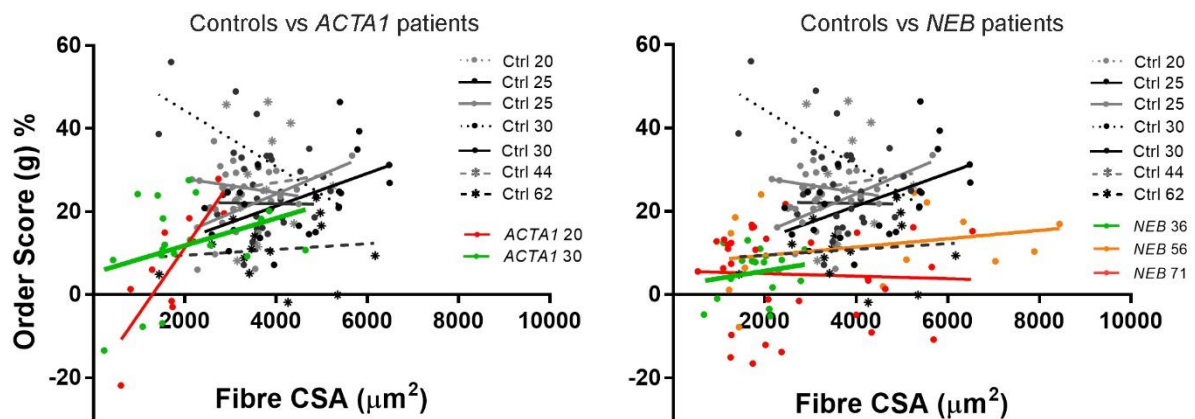


Fig S1. Relationship between nuclear spacing and muscle fiber size. Healthy control subjects and patients are denoted with their mutation and age. Order score (g), an algorithm to assess the regularity of nuclear spacing; a lower score indicates more irregular spacing and more nuclear clustering. This parameter was plotted against fibre cross-sectional area (CSA). Individual data points represent an individual skeletal muscle fibre. Left, regression lines for control subjects versus *ACTA1* patients; right, regression lines for control subjects versus *NEB* patients. For most controls and patients, either no correlation, or a weak positive correlation between fibre CSA and order score was observed. One patient (*ACTA1 20*) showed a positive correlation ($R^2 = 0.68$), indicating that larger fibres tended to be more ordered than small, although one control (*Ctrl 30*) showed a negative correlation ($R^2 = 0.47$), suggesting the reverse relationship.

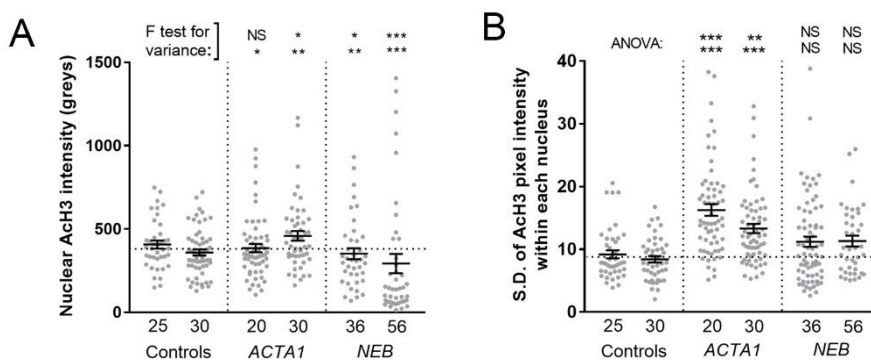


Fig S2. Altered chromatin organisation in patients with nemaline myopathy (Related to Fig 3H-J). (A) Mean acetylhistone H3 pixel intensity per nucleus (one data point per nucleus measured); F test for variance indicates that the variation in staining intensity between nuclei is significantly greater in patients than controls. (B) Standard deviation of pixel intensity within each nucleus, as a measure of staining variability within the nucleus (one data point per nucleus measured); patients frequently have more variable staining within each nucleus, possibly indicating irregularly packed regions of chromatin. 50+ nuclei were observed per subject across ~9 fibres, mean \pm SEM. * ($P < 0.05$), ** ($P < 0.01$), *** ($P < 0.001$).

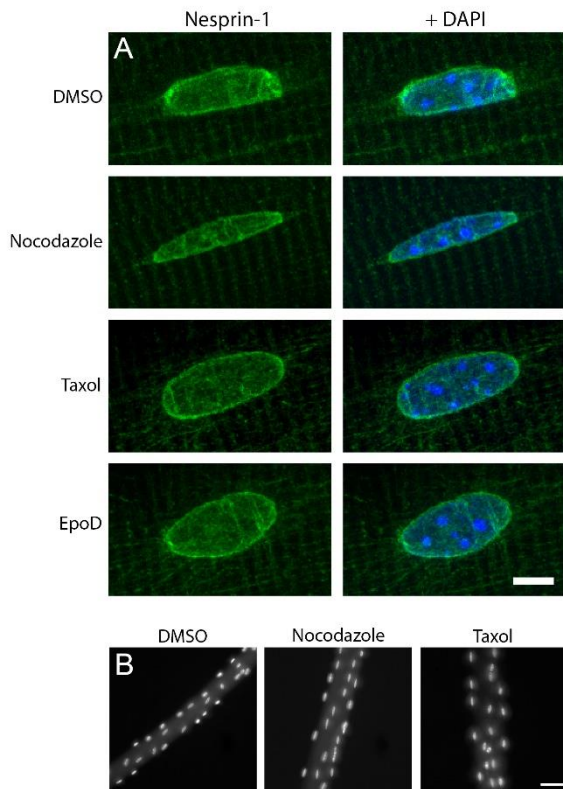


Fig S3. Nesprin-1 localisation and myonuclear spacing is unaffected by microtubule perturbations. (A) Typical myonuclei from mouse skeletal muscle fibres treated overnight with vehicle (DMSO), nocodazole, taxol or epothilone D. Nesprin-1 staining (green) and DAPI (blue). The localisation of nesprin-1 was not markedly affected by treatment with microtubule perturbing drugs. ~50 nuclei were observed per condition across 2-3 separate experiments. (B) Representative DAPI-stained images of muscle fibres treated with DMSO, nocodazole or taxol. No overt alterations to myonuclear spacing were observed in response to the drugs, even after 72 hours (~20 fibres observed across 2-3 experiments). Scale bars: 5 μ m (A); 50 μ m (B).

Gene	Age	M/F	Mutation (DNA)	Mutation (protein)	Source
<i>ACTA1</i>	20	M	c.16G>A	E6K	Copenhagen, Denmark
<i>ACTA1</i>	30	F	c.841T>C	Y281H	Genoa, Italy
<i>NEB</i>	36	F	c.2836-2A>G and c.5763+5G>A	Mutation in splice site	Copenhagen, Denmark
<i>NEB</i>	56	M	c.17234C>T and c.2271_22713del	R5745X and K7571del	Copenhagen, Denmark
<i>NEB</i>	71	F	c.508-7T>A and c.19097G>T	Mutation in splice site; and S6366I	Helsinki, Finland

Table S1. Patient muscle biopsy samples used for light microscopy.

Gene	Age	M/F	Mutation (DNA)	Mutation (protein)	Source
<i>NEB</i>	23	M	c.11164C>T and c.19097G>T	R3722* (nonsense) and S6366I	Helsinki, Finland
<i>NEB</i>	17	M	c.22249A>C and c.8392-8395 duplication	T7417P and R2799L frameshift	Helsinki, Finland
<i>NEB</i>	30	F	c.508-7T>A and c.19097G>T	Mutation in splice site and S6366I	Helsinki, Finland
<i>NEB</i>	2		c.17737-2A>T and c.21315delA	Mutation in splice site and R7105 frameshift	Milan, Italy
<i>ACTA1</i>	10		c.841T>C	Y281H	Milan, Italy
<i>ACTA1</i>	3		c.235A>G	T79A	Milan, Italy
<i>ACTA1</i>	4				London, UK

Table S2. Patient muscle biopsy samples used for electron microscopy.

Patient (mutation/age)	Chromatin density	Invaginations	Discontinuous and/or separation of nuclear membranes	Vacuolation of nuclear envelope
<i>NEB</i> 23	↑	+	++	+
<i>NEB</i> 17	↓↓	++	N	N
<i>NEB</i> 30	↑	+	N	+
<i>NEB</i> 2	↑	+	N	N
<i>ACTA1</i> 10	↓↓	N	N	N
<i>ACTA1</i> 3	↓↓	N	N	N
<i>ACTA1</i> 4	Only one nucleus image recorded			+

Table S3. Ultrastructural observations in myonuclei of nemaline myopathy patients. Categorisation is based on criteria described in **Figure 3**. For chromatin density, ↑ denotes an increase and ↓ a

decrease in density (respectively, an increase and reduction of heterochromatin). + indicates the presence of a given feature/observation. N denotes none observed. In all cases, two symbols indicates a particularly high incidence whereby the majority of observed myonuclei displayed the characteristic in question. 20 – 30 myonuclei observed in all patients, across multiple fibres and fields of view.