Supplementary experimental procedures

MBNL1 RT-PCR

RNA was isolated from human myoblasts using Trizol Reagent (Invitrogen) and 2 µg of total RNA were reverse transcribed using M-MLV first-strand synthesis system according to the manufacturer's instructions (Invitrogen). Then, RT products were subjected to PCR amplification using the 2X ReadyMix reagent (ABgene, Thermo Scientific) and MBNL1 specific primers (Forward, ATTACAACCCGTGCCAATGT; Reverse, TTGTGGCTAGTCAGATGTTCG) under the following conditions: 5 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 55°C, 1 min at 72°C, followed by a final 10 min extension at 72°C. PCR products were resolved by electrophoresis using a 4% polyacrylamide gel and bands were stained with ethidium bromide. Each band was purified, ligated into a pCRII-TOPO vector (Invitrogen) and identified by DNA sequencing. Identification of endogenous isoforms obtained was also performed by co-migration on a 4% polyacrylamide gel with full-length MBNL1 variant constructs ($MBNL1_{38}$, $MBNL1_{40}$, $MBNL1_{41}$, $MBNL1_{42}$ and $MBNL1_{43}$) previously characterized (24) and amplified with the same primers.

Two-dimensional gel electrophoresis

Human myoblast cells were lyzed in 10 mM Tris 1% SDS buffer, sonicated and placed on an orbital shaker for 1h at 4°C. The homogenate was centrifuged at 12,000g for 10 min at 4°C and protein concentration was established in the supernatant using the BCA protein assay kit (Pierce). Sixty microgram of protein were then precipitated using methanol / chloroform. Briefly, Three volumes of chloroform, one volume of methanol and three volumes of H_2O were added to the protein lysate, mixed and spun at 10,000×g for 30 min at 4°C. The chloroform upper layer was removed and 3 volume of cold methanol was added. The resulting solution was centrifuged 20 min at 10,000×g and 4°C and the supernatant was removed and the protein pellet air-dried. The protein pellet was homogenized with 200 µl of a 2D buffer containing 7M urea, 2M thiourea, 2% CHAPS and 0.2% Pharmalytes 7-10 (G&E Healthcare). An IEF Strip pH 7-11 was rehydrated with the protein homogenate and a total of 16500 Volt hours was applied. For the second dimension, the IEF strip was equilibrated 3-times 15 min with an equilibration buffer containing 10 mM Tris-HCl pH 6.8, 5% SDS, 20% Glycerol, 20 mM DTT.

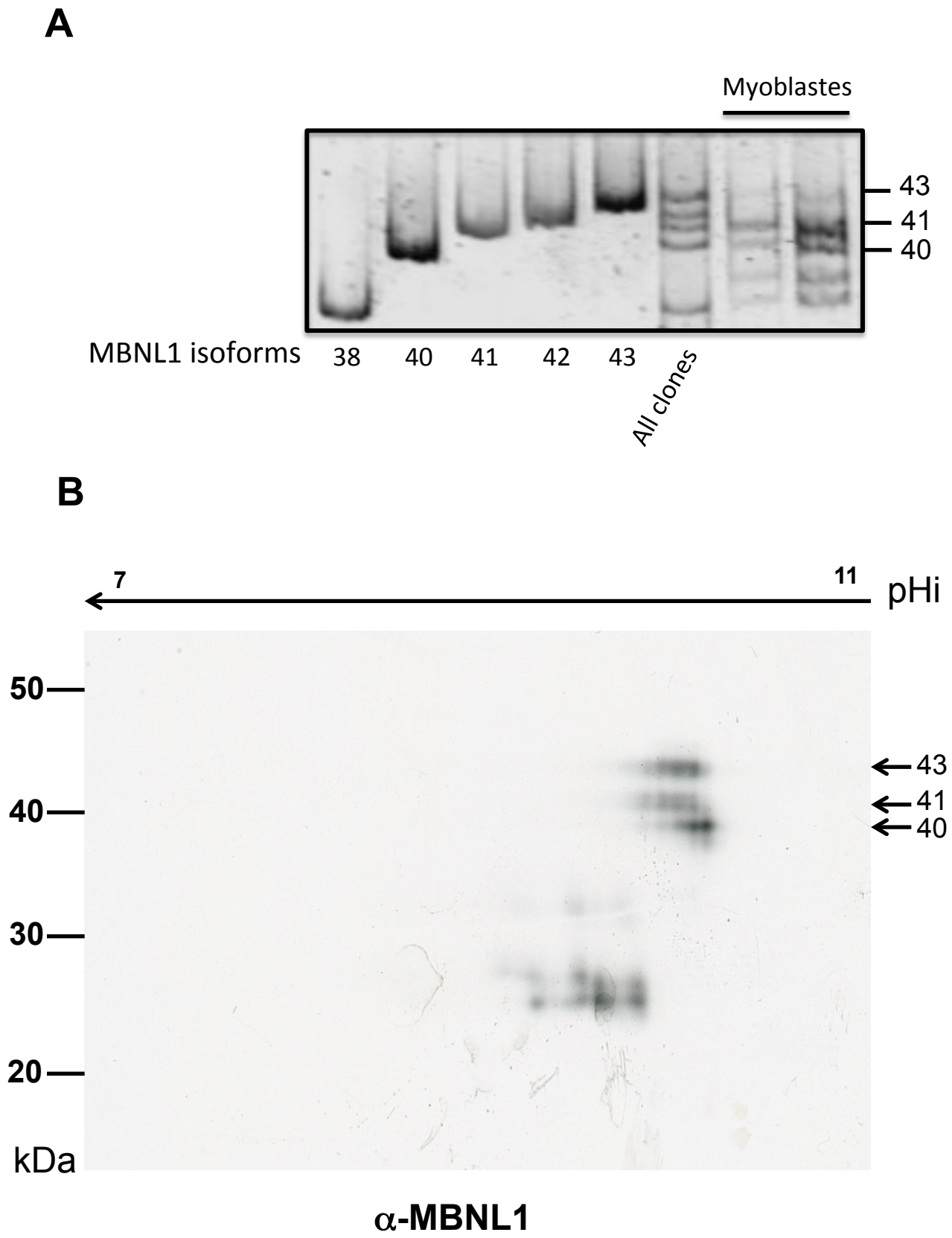
Supplementary Figure 1: MBNL1 isoforms expression in human myoblast cells

A. MBNL1 RNA isoforms were amplified using a couple of primers located in the exon 3 and exon 11 of MBNL1. The $MBNL1_{40}$ to $_{43}$ isoforms sequenced are indicated on the right. **B.** MBNL1 protein isoforms were separated by two-dimensional gel electrophoresis and followed by western-blotting with the MB1a monoclonal antibody (α-MBNL1). The putative MBNL1 proteins isoforms 40 to 43 are indicated on the right by arrows. The spots observed bellow 30 kDa might correspond to N-terminal catabolic products of MBNL1. The apparent molecular weights are indicated vertically on the left and the pH gradient (pHi) used is orientated horizontally from the basic on the right to the neutral on the left.

Supplementary Figure 2: Hill Plot of MBNL1 isoform equilibrium binding data

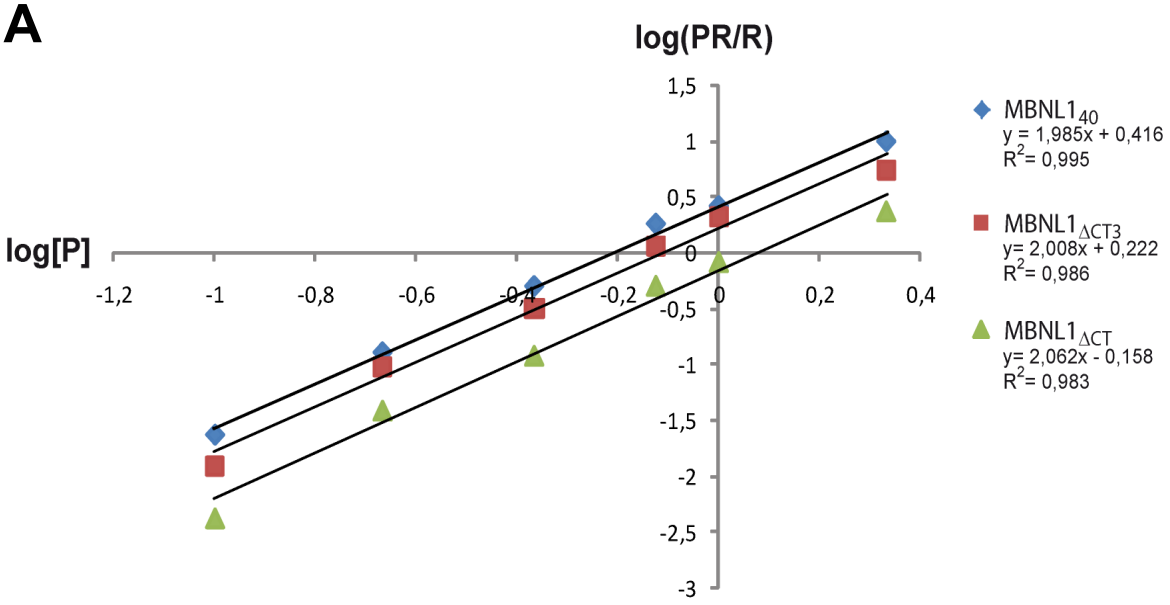
A. The Hill plot was constructed by representing the log (PR/R) versus log[P], where P = protein concentration; PR = bound RNA and R = free RNA. The equation of the best-fit line and the correlation factor for each series of data are indicated. **B.** The slope of the best fit line represents the Hill coefficient (n_h) and the intercept with the Y axis corresponds to –log(Kd). The average Kd and the standard deviation calculated from 3 independent experiments are indicated. The value of the Hill coefficient is an indication of the cooperativity: if $n_h = 1$, the binding is non-cooperative and all the sites are identical; if $n_h > 1$, the binding must be positively cooperative (there is an interaction between sites). The closer the value of n approaches the number of binding sites the more cooperative the system is. As two MBNL1 binding sites are present in hcTNT RNA used to perform the EMSA experiments, a n_h value of 2 indicates a total cooperativity for binding.

Supplementary figure 1



Supplementary Figure 2

A



B

	n_h	Kd (nM)
MBNL1$_{40}$	1,985	383 ± 50
MBNL1$_{\Delta CT3}$	2,008	599 ± 35
MBNL1$_{\Delta CT}$	2,062	1438 ± 20