Supporting Information

García-López et al. 10.1073/pnas.1018213108

SI Material and Methods.

Luciferase Assay. Two-day-old flies were fed 250 μM ABP1 or 0.12% DMSO (1). Luciferase activity was measured according to the Luciferase Assay System (Promega) protocol with a Victor Wallac 1420 Multilabel Counter (Perkin Elmer Lifesciences), with minor modifications.

Reverse Transcription-PCR. Total RNA was extracted from approximately 30 whole flies or mouse hind limb TA (approximately 40 mg) with TriReagent (Sigma). Reverse transcription, primer sequences, and PCR conditions are described (Table S3 and ref. 1).

RNA Synthesis. CUG60 was synthesized by subcloning 60 CTG repeats (2) into pBlueScript II SK (+/−). Linearized plasmid (1 μg) was transcribed using the T7 promoter (MEGAscript kit, Ambion). Other RNAs were purchased from Metabion (Table S3).

Peptide Synthesis and Purification. Synthesis employed solid-phase N-(9-fluorenyl) methoxycarbonyl chemistry. Purification employed preparative reversed-phase high-pressure liquid chromatography with a mediterranea sea (3) column (Teknokroma). Identification employed matrix-assisted laser desorption ionization-time-of-flight mass spectrometry with a 4700 Proteomics Analyzer (Applied Biosystems).

Circular Dichroism. CUG60 spectra of RNA (1 μM in binding buffer), incubated with different concentrations of ABP1 or MblZF were measured (10 measurements averaged) at 10 °C with a J-810 spectropolarimeter (Jasco).

Toxicity in Wild-Type Mice. Peptide (0.5 μg, 1 μg, 10 μg, and 100 μg in saline) was injected intramuscularly into the right TA of five-week-old *FVB* mice ($n = 6$ per group). Animals were sacrificed one month later, and total blood from the heart was analyzed using standard toxicity assays.

Statistics. Two-tailed Student's t tests were used for comparisons between two samples ($\alpha = 0.05$). Multiple-comparisons of PS-SCL screen results were described (1) ($\alpha = 0.05$). GraphPad Prism 5 software was used for comparing regression slopes in the tryptophan quenching experiments.

1. Garcia-Lopez A, et al. (2008) Genetic and chemical modifiers of a CUG toxicity model in *Drosophila*. *PLoS One* 3:e1595.
2. Miller JW, et al. (2000) Recruitment of human muscleblind proteins to (CUG)(n) expansions associated with myotonic dystrophy. *EMBO J* 19:4439–4448.
3. Mulders SA, et al. (2009) Triplet-repeat oligonucleotide-mediated reversal of RNA toxicity in myotonic dystrophy. *Proc Natl Acad Sci USA* 106:13915–13920.

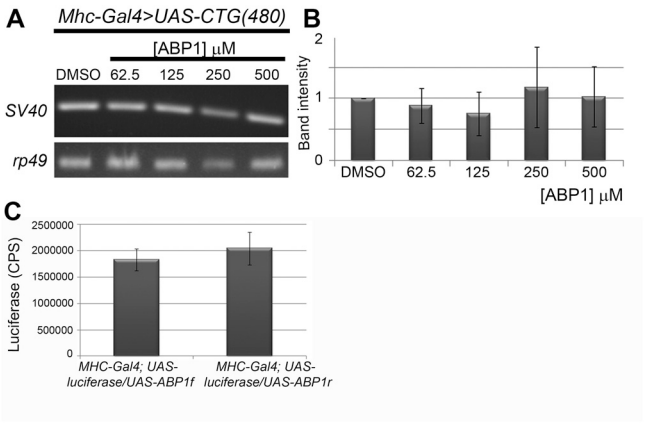

Fig. S1. ABP1 does not affect the levels of $i(CUG)_{480}$ transcripts. (*A*) Semiquantitative RT-PCR of flies expressing $i(CUG)_{480}$ in the fly musculature fed with different concentrations of ABP1. (*B*) Statistical analysis of the intensity of the bands (normalized to rp49 and to DMSO) did not reveal significant differences between DMSO-treated and peptide-treated flies ($\alpha = 0.05$, Student's t test). PCRs were performed with 30 cycles. *rp49* was used as a cDNA template loading control. (*C*) ABP1r transgenic expression did not reduce the expression of Luciferase reporter compared to ABP1f. Importantly, ABP1r suppressed $i(CUG)_{480}$-induced phenotypes in the fly eye and muscle, whereas ABP1f did not. The average Luciferase light emission is shown for 24 replicates.

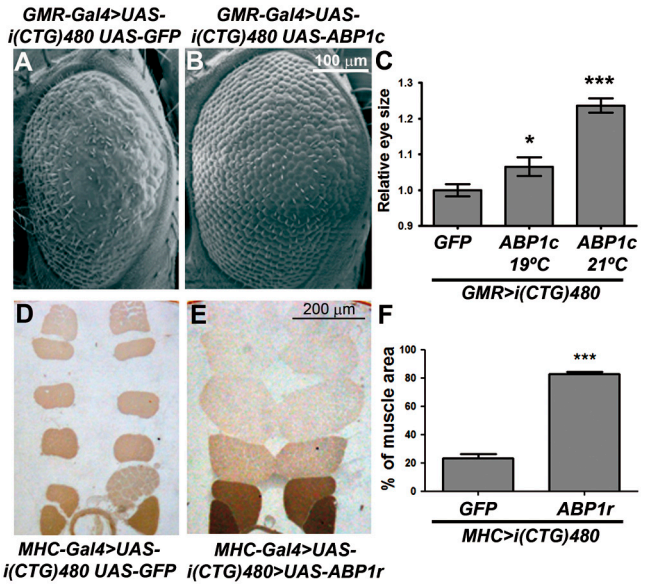

Fig. S2. Transgenic expression of ABP1 using the Gal4/UAS system suppressed $i(CTG)_{480}$ toxicity. (*A* and *B*) Representative scanning electron micrographs of *Drosophila* eyes after coexpression of (*A*) *UAS-*$i(CTG)_{480}$ and *UAS-green fluorescent protein* (GFP; control) or (*B*) *UAS-*$i(CTG)_{480}$ and *UAS-ABP1c* at 21 °C ($n = 9$) with the *GMR-Gal4* driver. Expression of *UAS-*$i(CTG)_{480}$ caused roughness and reduced eye size. This was significantly reversed with *UAS-ABP1c* expression at 19 °C and 21 °C (*C*). Five independent *UAS-ABP1r* or *UAS-ABP1c* transgenic lines showed similar effects. (*D* and *E*) Representative bright field microscopy images of transversal sections of resin-embedded adult IFM of *MHC-Gal4 Drosophila* after coexpression of *UAS-*$i(CTG)_{480}$ and *UAS-GFP* (control) or *UAS-*$i(CTG)_{480}$ and *UAS-ABP1r* (or *UAS-ABP1c*). ABP1 expression suppressed histological defects in the IFM causing a 3.5-fold increase in muscle area ($n = 7$; F).

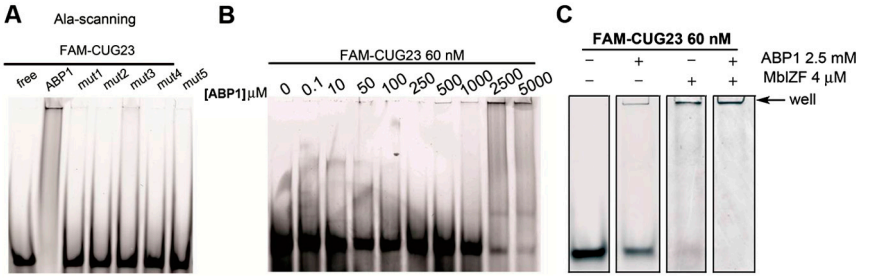

Fig. S3. RNA binding and specificity of ABP1. (*A*) Electrophoretic mobility shift assay showing that mutant derivatives of ABP1, by alanine scanning mutagenesis (2.5 mM), did not bind to CUG-repeat RNA (60 nM). (*B*) A similar experiment showed that ABP1 binding to FAM-CUG23 (60 nM) responded to peptide concentration, starting between 250–500 μM. The binding increased proportionally to the peptide concentration. (*C*) Coaddition of ABP1 and Mbl1ZF did not increase the amount of free RNA compared to ABP1 or MblZF alone (lane 4 compared to lanes 2 and 3, respectively). In contrast, the binding of ABP1 and MblZF to the CUG RNA seemed to be additive. However, because in all cases the complexes formed got retained in the well no firm conclusions could be drawn from these experiments. Note that high concentrations of peptide (or MblZF protein) were needed in all fluorescent electrophoretic mobility gel shift assays to detect binding to the CUG RNAs, which may account for the high molecular weight complexes formed upon interaction that were retained in the well of the gels, as similarly described in other unrelated studies (1). Because in our nonfluorescent experiments an RNA : peptide molar ratio lower than 1 : 1 was sufficient to detect interaction it is possible that the fluorophore used in our fluorescent assays hindered the binding. Supporting this notion, Nt conjugation of ABP1 with carboxyfluorescein completely abolished the ability of the peptide to bind CUG-repeat RNA.

1 Ingmer H, Fong EL, Cohen SN (1995) Monomer-dimer equilibrium of the pSC101 RepA protein. *J Mol Biol* 250:309–314.

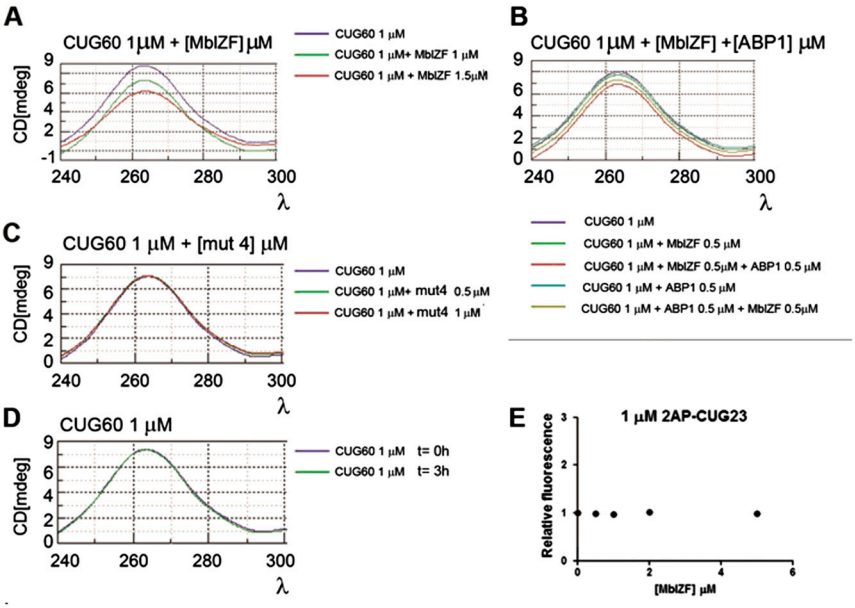

Fig. S4. MblZF and ABP1 bind to the RNA differently. Although both molecules reduced the CD signal of a CUG60 RNA (*A* and *B*), MblZF did not change the fluorescence emission of 2AP-CUG23 (*E*), indicating that binding of the protein did not destabilize the CUG hairpin. The reduction in the RNA CD signal was not caused by RNA degradation during the time-course of the experiment (*D*) and mutant peptide mut4 did not show any effect (*C*).

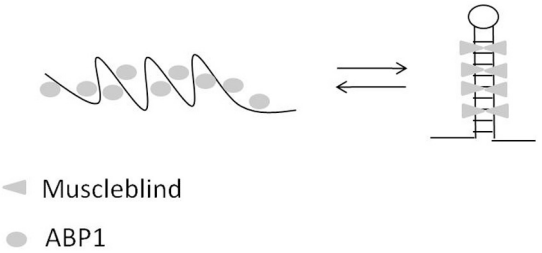

Fig. S5. Proposed mechanism of action of ABP1. The CUG RNA is proposed to exist in a chemical equilibrium between single (ss) and double stranded (ds) conformations. Muscleblind proteins are known to bind ds(CUG), whereas our in vitro and in vivo data suggest that ABP1 binds and stabilizes ss(CUG) RNA, displacing the equilibrium, and releasing sequestered Muscleblind. Both ds(CUG) and ss(CUG) RNA are assumed to coexist in vitro thus explaining that ABP1 and MblZF did not compete for the same binding site, but rather showed additive effects in polarization and gel shift assays.

Table S1. List of defined hexapeptides obtained by deconvolution of the PS-SCL

Sequence	Concentration assayed	Emerged treated/emerged control
Ac-cpyaqe-NH2	80 μM	0,3*
Ac-cpyawe-NH2	80 μM	-
Ac-cpytqe-NH2	80 μM	0,8
Ac-cpytwe-NH2	62 μM	-
Ac-cqyaqe-NH2	25 μM	2,0
Ac-cqyawe-NH2	25 μM	-
Ac-cqytqe-NH2	80 μM	1,4
Ac-cqytwe-NH2	57 μM	0,9
Ac-ppyaqe-NH2	80 μM	2,0
Ac-ppyawe-NH2 (ABP1)	80 μM	4,0*
Ac-ppytqe-NH2	80 μM	0,8
Ac-ppytwe-NH2	80 μM	3,0
Ac-pqyaqe-NH2	80 μM	0,8
Ac-pqyawe-NH2	40 μM	1,8
Ac-pqytqe-NH2	40 μM	0,5
Ac-pqytwe-NH2	38.5 μM	0,4

The 16 peptides were tested at the highest concentration possible depending on the percentage of DMSO the stocks were diluted in. Two peptides showed significant differences in the number of emerged females compared to DMSO-treated controls: Ac-cpyaqe-NH2 (that enhanced the phenotype) and Ac-ppyawe-NH2 (ABP1, that suppressed it). "-" indicates the number of emerged females was 0 in treated tubes and controls.
*p-value < 0.05

Table S2. Peptides generated by alanine scanning mutagenesis

Name	Sequence
mut1	Ac-ppyawa-NH2
mut2	Ac-ppyaae-NH2
mut3	Ac-ppaawe-NH2
mut4	Ac-payawe-NH2
mut5	Ac-apyawe-NH2

The five peptides were tested in vivo at the highest concentration possible depending on the percentage of DMSO the stocks were diluted in.

Table S3. DNA and RNA oligos (5′ → 3′)

Primer	Primer sequence	Cycles	Annealing	Polymerase
Used for PCR reactions				
Rp49				
fRP49	ATGACCATCCGCCCAGCATAC	29	65 °C	Netzyme
rRP49	ATGTGGCGGGTGCGCTTGTTC			
SV40				
fSV40	GGAAAGTCCTTGGGGTCTTC	30	60 °C	Netzyme
rSV40	GGAACTGATGAATGGGAGCA			
Serca1				
fSerca1	CTCATGGTCCTCAAGATCTCAC	25	55 °C	AmpliTaq Gold
rSerca1	GGGTCAGTGCCTCAGCTTTG			
Tnnt3				
fTnnt3	TCTGACGAGGAAACTGAACAAG	25	55 °C	AmpliTaq Gold
rTnnt3	TGTCAATGAGGGCTTGGAG			
Capzb				
fCapzb	TCTGACGAGGAAACTGAACAAG	25	55 °C	AmpliTaq Gold
rCapzb	TGTCAATGAGGGCTTGGAG			
MblZF				
fMblZF	GGAATTCCATATGGCCAACGTTG			
rMblZF	CGGGATCCCGTTACTTGAGGGCCAAATGATT	10+25	65 °C	Pwo
	Used to generate the UAS-ABP1 transgenes			
UAS-ABP1f *				
fUAS-ABP1f	AATTCAAACACACCAAATCTTACAAAATGGGCGGCGGCCCCCCCTACGCCTGGGAGTAATAATAAT			
rUAS-ABP1f	GTTTGTGTGGTTTAGAATGTTTTACCCGCCGCCGGGGGGGATGCGGACCCTCATTATTATTAGATC			
UAS-ABP1r *				
fUAS-ABP1r	AATTCAAACACACCAAATCTTACAAAATGGGCGGCGGCGAGTGGGCCTACCCCCCCTAATAATAAT			
rUAS-ABP1r	GTTTGTGTGGTTTAGAATGTTTTACCCGCCGCCGCTCACCCGGATGGGGGGGGATTATTATTAGATC			
UAS-ABP1c *				
fUAS-ABP1c	AATTCAAACACACCAAATCTTACAAAATGGGCGGCCCCCCCTACGCCTGGGAGGGCGGCATGGGCGGCGAGTGGGCCTA CCCCCCCTAATAATAAT			
rUAS-ABP1c	CTAGATTATTATTAGGGGGGGTAGGCCCACTCGCCGCCCATGCCGCCCTCCCAGGCGTAGGGGGGGCCGCCCATTTTGT AAGATTTGGTGTGTTTG			
	Used in RNA binding assays			
FAM-CUG23	**FAM**-CUGCUGCUGCUGCUGCUGCUGCUGCUGCUGCUGCUGCUGCUGCUGCUGCUGCUGCUGCUGCUGCUG			
CUG23	CUGCUGCUGCUGCUGCUGCUGCUGCUGCUGCUGCUGCUGCUGCUGCUG CUGCUGCUGCUGCUG			
FAM-CUG4 †	**FAM**-GCUGCUGUUCGCUGCUG			
CUG4 †	GCUGCUGUUCGCUGCUG			
FAM-CUG23	CUGCUGCUGCUGCUGCUGCUGCU2APCUGCUGCUGCUGCUGCUGCUGCUGCUGCUGCUGCUGCUG			
DMPK	GAUCACAGACCAUUUCUU			
(CAG•CUG)4 †	GCAGCUGUUCGCAGCUG			
DMPK-CUG4 †	GAUCACAGACCAUUUCUUGCUGCUGUUCGCUGCUG			
	Used in DNA binding assays			
CUG4 †	GCTGCTGTTCGCTGCTG			
DMPK	GATCACAGACCATTTCTT			

*For the design of the transgenes the *Drosophila* codon use bias was taken into consideration. C was preferably used in synonym positions over other bases. When using C was not possible G was chosen.

†To stabilize the shorter CUG repeats an ultrastable UUCG tetraloop was used (1).

1 Warf MB, Berglund JA (2007) MBNL binds similar RNA structures in the CUG repeats of myotonic dystrophy and its premRNA substrate cardiac troponin T. *RNA* 13:2238–2251.

Table S4. Toxicity analysis of ABP1 from *FVB* mice blood samples

	Ref.	DMSO 0.2%	ABP1 0.5 μg	ABP1 1 μg	DMSO 2%	ABP1 10 μg	ABP1 100 μg *
Kidney							
Bile acids †	<15	3.4 ± 0.4	3.7 ± 0.5	4.7 ± 0.9	2.9 ± 0.3	4.8 ± 0.7	3.3 ± 0.3
Urea†	17–28	45.2 ± 9.6 ‡	46.6 ± 4.9 ‡	36.1 ± 1.2 ‡	38.7 ± 4.6 ‡	54.2 ± 3.2 *	52.0 ± 3.3 *
Creatinine †	0.3–1	0.3 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
Muscle							
CPK §	50–500	203 ± 43	77.0 ± 18	40.5 ± 25	4923 ± 2012‡	4937 ± 1384‡	2461 ± 1036‡
Liver							
FA §	45–222	400 ± 25 ‡	399 ± 21 ‡	326 ± 15 ‡	371 ± 32 ‡	297 ± 16 ‡	343 ± 27 ‡
GGT §	0–12	1.0 ± 0.0	1.0 ± 0.0	1.6 ± 0.4	2.1 ± 0.5	0.7 ± 0.1	1.3 ± 0.4
GPT §	26–77	67.0 ± 7.0	67.2 ± 6.0	60.6 ± 7.1	70.3 ± 16.3	143 ± 32.4 *	75.7 ± 7.8

Toxicity of ABP1 on wild-type mice of the *FVB* strain was assessed by performing intramuscular injection of 0.5 μg, 1 μg (in 0.2% DMSO), 10 μg and 100 μg (in 2% DMSO) or saline with the corresponding amount of DMSO (controls) in the right hind limb *tibialis anterioris* (TA). Two out of five animals died 11 days after injection of 100 μg (†), which yields a dose close to the LD50. Injection of 10 μg caused mild alterations in kidney and liver functions (indicating that ABP1 was able to enter the blood stream) but did not affect muscle histology. Injection of 0.5 μg and 1 μg did not cause toxicity beyond DMSO effects. In all cases a visual autopsy of the animals was performed upon sacrifice, and no evident abnormalities were detected.

*Values higher than the respective DMSO controls.

†mcmol/L.

‡Values higher than the reference.

§U/L.

Table S5. Summary of the effect of ABP1 on DM1 phenotypes in *HSA^{LR}* mice

Dose 0.5 μg		Dose 10 μg				
Mouse	Histological improvement	Mouse	Histological improvement	*Serca* splicing improvement	*Tnnt3* splicing improvement	Clcn1 protein recovery
0.5_1	Yes ($p = 0.007$)	10_1	Yes ($p = 0.020$)	No ($p = 0.541$)	Yes	Yes
0.5_2	Yes ($p = 0.048$)	10_2	Yes ($p = 0.040$)	Yes ($p = 0.038$)	Yes	Yes
0.5_3	Yes ($p = 0.008$)	10_3	Yes ($p = 0.006$)	Yes ($p = 0.013$)	Yes	Yes
0.5_4	No ($p = 0.430$)	10_4	No ($p = 0.491$)	Yes ($p = 0.025$)	Yes	Yes
0.5_5	No ($p = 0.191$)	10_5	No ($p = 0.890$)	No ($p = 0.720$)	No	No

The effect of ABP1 intramuscular injection was studied in five five-week-old gender-matched *HSA^{LR}* mice. In every animal except #5, at least three out of the four phenotypes analyzed were reverted upon ABP1 treatment (10 μg; 1 month a/i) compared to the contralateral, DMSO-treated limb.