Supplementary Figure 1: CUG-RNA co-localizes with MBNL1 in nuclear foci, while cytoplasmic foci are not localized in PBs. Combined RNA-FISH/immunofluorescence (RNA-FISH/IF) on DM1 fibroblasts. CAG\textsubscript{10} fluorescent probes were used in combination with either MBNL1 antibody or antibody raised against indicated PB-factor. The inserts in the merged panels display enlarged representative foci. Scale bar equals 10 µm. A) Left panel displays RNA-FISH signal (inverted LUT). Middle panel displays MBNL1 IF-signal (inverted LUT), which is primarily nuclear. B) Left panels display RNA-FISH signal as distinct nuclear and cytoplasmic foci (inverted LUT). Arrows indicate cytoplasmic foci, which are enlarged in the merged pictures and shown as an insert. C) Increasing the exposure of DDX6 IF, indicates that DDX6 is transiently associated with nuclear CUG-foci. The three right panel are enlargements of nuclear focus.
Supplementary Figure 2: MBNL1 does not efficiently co-localize with DDX6 in DM1 fibroblasts. Co-immunofluorescence using MBNL1 and DDX6 antibodies. Scale bar equals 10 µm.
Supplementary Figure 3: Knockdown of RNA decay machinery increases PBs number and size without affecting cytoplasmic CUG-foci homeostasis. A) DM1 fibroblasts were transfected with siRNA targeting the human decapping enzyme hDCP2 or the major cytoplasmic 5’->3’ exo-nuclease Xrn1 and subjected to combined RNA–FISH/IF using hDCP1a antibody as a marker for PBs. Scale bar equals 10 µm B) Knockdown of DDX6 removes PBs and lowers the frequency of cytoplasmic CUG-foci. C) Cells (n>83) were scored for the frequency of cytoplasmic CUG-foci after DDX6 knockdown (DDX6) or control (Ctr). Error bars indicate SEM.
Supplementary Figure 4: Western blotting showing knockdown efficiency DDX6 siRNA#2 - transfected DM1 cells and RNA-FISH analysis on DM2 fibroblasts.  

A) Western blot using lysates from either WT or DM1 fibroblasts transfected with the indicated siRNAs and antibodies raised against DDX6 (upper) or HuR (lower; control).  

B) Panels display RNA-FISH signal combined with DAPI signal with inverted LUT to enable distinction of FISH signal in the nuclear or the cytoplasmic compartment. Upper panel: shows cells that have been transfected with DDX6 siRNA and lower panel: Control knockdown cells.  

C) Boxplot from a representative experiment, showing that the number of nuclear foci does not significantly change between the two cell populations (Student’s T-test, n>176). The experiment was repeated once with essentially the same result.
Supplementary Figure 5: Protein expression profiles. **A)** Western blot showing expression profile of DDX6 and PABP (control) in DM1 and WT fibroblasts. **B)** Expression profile of DDX6, MBNL1, CUG-BP1, DDX5, Staufen, LARP1 and HuR in FLAG-DDX6-transduced WT or DM1 fibroblasts. **C)** Expression profile of DDX6, Staufen, Myogenin and MBNL1 in MyoD-transduced WT or DM1 fibroblasts. Both Myogenin and Staufen proteins are upregulated upon differentiation, while DDX6 and MBNL1 remain constant.
Supplementary Figure 6: Lentiviral transduction with a vector containing a MyoD expression cassette induces myogenesis. A) RNA FISH/DAPI panels of multinuclear cells (not detected in non-transduced cells) demonstrating distinct nuclear and rare cytoplasmic foci as observed in fibroblasts. B) Immunofluorescence (IF) using mouse myogenin monoclonal antibody displays cytoplasmic and nuclear signal only in MyoD-virus transduced cells. Detection of overall myogenin expression levels by western blotting is assessed in Supplementary Figure 5. Scale bars equal 10 µm.
Supplementary Figure 7: qRT-PCR strategy to quantify defect of IR2 pre-mRNA splicing and restoration upon DDX6 overexpression. 

A) Primer sets were designed to amplify transcripts containing or lacking alternative exon 11

B) Quantification of triplicate qRT-PCR reactions demonstrating a significant increase in the inclusion of exon 11 in DM1 cells upon DDX6 overexpression compared to untreated or GFP-transduced. Error bars represent (SEM), n=3.
**Supplementary Figure 8**

**Supplementary Figure 8**: EMSA competition assay. Five fmol $^{32}$P body-labeled CUG-200 RNA was renatured and incubated with either no protein (lane 1) or 200 ng GST-DDX6 wt (lanes 2-10) using cold renatured CUG-200 RNA (lanes 3-6) at a 5-, 20-, 100- or 500-fold weighted excess. The DDX6:hot RNA complex is efficiently disrupted, while rRNA at the same level is less efficient (lanes 7-10).
Supplementary Figure 9: DDX6 expression levels. **A)** Expression levels of endogenous DDX6 and exogenous GFP-DDX6 or GFP-DDX6(DEAA) using increasing amounts of plasmid DNA for transfections as indicated, reveals similar expression levels. **B)** Comassie brilliant blue (GelCode blue) staining of immunoprecipitated FLAG-DDX6 (WT/DEAA) used for CUG-unwinding assay, showing similar expression and purification levels. **C)** Western blot of input and FLAG-IP fractions a rabbit anti-FLAG antibody.