Supplemental information for:

A Toxic RNA Catalyzes the Cellular Synthesis of Its Own Inhibitor, Shunting It to Endogenous Decay Pathways

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SUPPLEMENTAL TABLES AND FIGURES

Table S1. Sequences of primers used for PCR (related to STAR Methods).			
Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')	Purpose
MAP4K4	CCTCATCCAGTGAGGAGTCG	TGGTGGGAGAAATGCTGTATGC	RT-PCR
IR	CCAAAGACAGACTCTCAGAT	AACATCGCCAAGGGACCTGC	RT-PCR
CNBP i1 3'' SS	GAACTTTCAGTGGTTTAATGCTG	CCGCAGTTATAGCAGGCTTC	RT-PCR
GAPDH	AAGGTGAAGGTCGGAGTCAA	AATGAAGGGGTCATTGATGG	qPCR
CNBP Intron1	ATTCCAAGGTTGGTTGAAGC	AACCCAAACCAATGAAGCTG	qPCR
CNBP mature mRNA	AAACTGGTCATGTAGCCATCAAC	AATTGTGCATTCCCGTGCAAG	qPCR
MBNL1	TTCATCCACCCCCACATTTA	TTGGCTAGTTGCATTTGCTG	qPCR

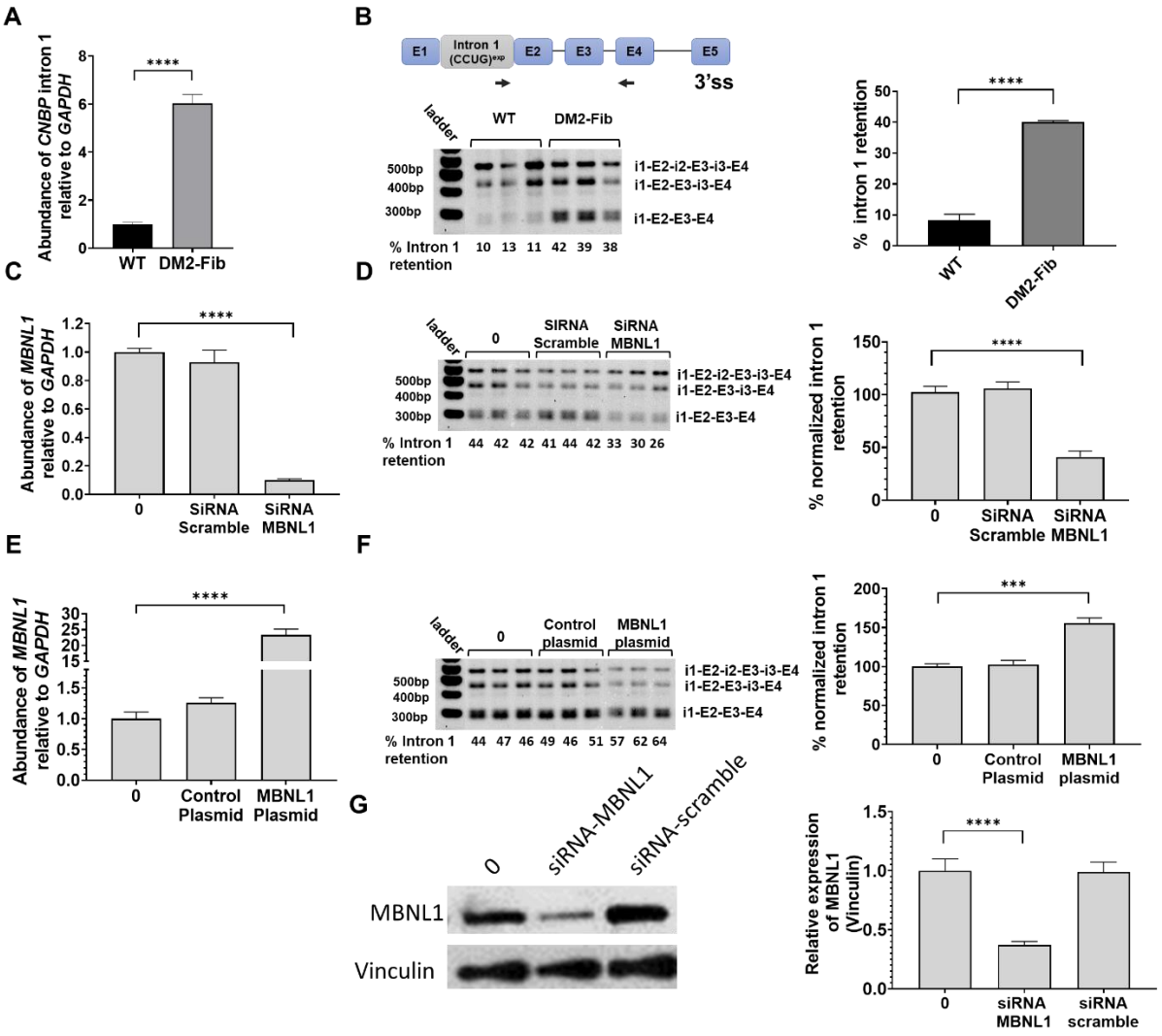

Figure S1: *MBNL1*'s effect on *CNBP*-intron 1 retained species in DM2 patient-derived fibroblasts (related to Figure 1). (*A*) Abundance of *CNBP* intron 1 in WT fibroblasts compared to DM2 fibroblasts, as evaluated by RT-qPCR. (*B*) *CNBP* intron 1 retention levels in WT fibroblasts compared to DM2 fibroblasts, as evaluated by RT-PCR (left) and its quantification (right). Schematic of the *CNBP* i1 3′ss (2 primers - arrows) RT-PCR assay. *i* indicates intron and *E* indicates Exon. (*C, D*) Effect of MBNL1 knock-down on *CNBP* isoforms using an siRNA in DM2 fibroblasts. (*C)* Relative abundance of *MBNL1* measured by RT-qPCR. (*D*) Representative gel image of *CNBP* intron 1 retention levels as measured by RT-PCR (left) and its quantification (right). (*E, F*) MBNL1 knock-in using a plasmid encoding MBNL1 in DM2 fibroblasts. (*E*) Relative abundance of *MBNL1* as measured by RT-qPCR. (*F*) Representative gel image of the effect of MBNL1 overexpression (knock-in) on *CNBP* isoforms by transfecting DM2 fibroblasts with a plasmid encoding MBNL1, as measured by RT-PCR (left) and its quantification (right). (*G*) Effect of siRNA *MBNL1* on expression of MBNL1 protein, determined by Western blot. Error bars represent SD. ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001, as determined by a one-way ANOVA (n = 3).

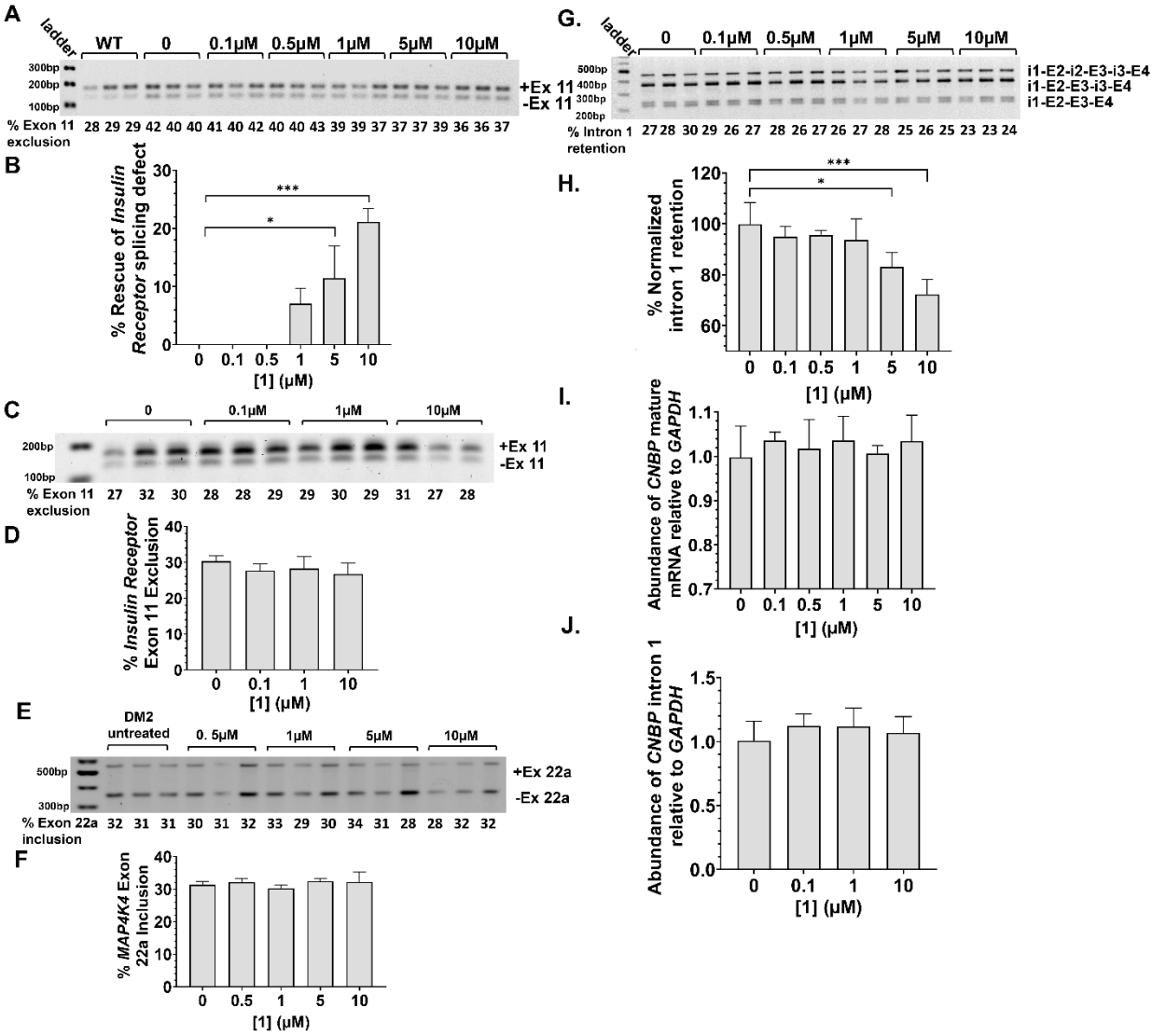

Figure S2: Effect of 1 treatment on DM2 and WT fibroblasts (related to Figure 3). (*A, B*) Ability of **1** to rescue aberrant splicing events in DM2 patient-derived fibroblasts. (*A*) Representative gel image of the alternative splicing of *Insulin Receptor* (*IR*) exon 11 in DM2 fibroblasts treated with **1**. (*B*) Quantification of RT-PCR analysis of the *IR* exon 11 splicing defect treated with **1**. (*C, D*) Evaluation of **1** in healthy fibroblasts. (*C*) Representative gel image of *IR* exon 11 splicing in WT fibroblasts treated with **2**. (*D*) Quantification of *IR* exon 11 splicing defect. (*E*) Representative gel image of *MAP4K4* exon 22a alternative splicing (non-MBNL1 regulated) in DM2 fibroblasts treated with **1**. (*F*) Quantification of *MAP4K4* exon 22a splicing. (*G, H*) Ability of **1** to rescue aberrant *CNBP* splicing in DM2 patient-derived fibroblasts. (*G*) Representative gel image of *CNBP* intron 1 retention in DM2 fibroblasts treated with **1**. (*H*) Quantification of RT-PCR analysis of *CNBP* intron 1 retention treated with **1**. (*I*) Effect of **1** on abundance of *CNBP* mature mRNA. (*J*) Evaluation of **1** in healthy fibroblasts. RT-qPCR analysis of *CNBP* intron 1 abundance in WT fibroblasts treated with **2**. Error bars represent SD. **P* < 0.5; ****P* < 0.001, as determined by a one-way ANOVA relative to 0 (n = 3).

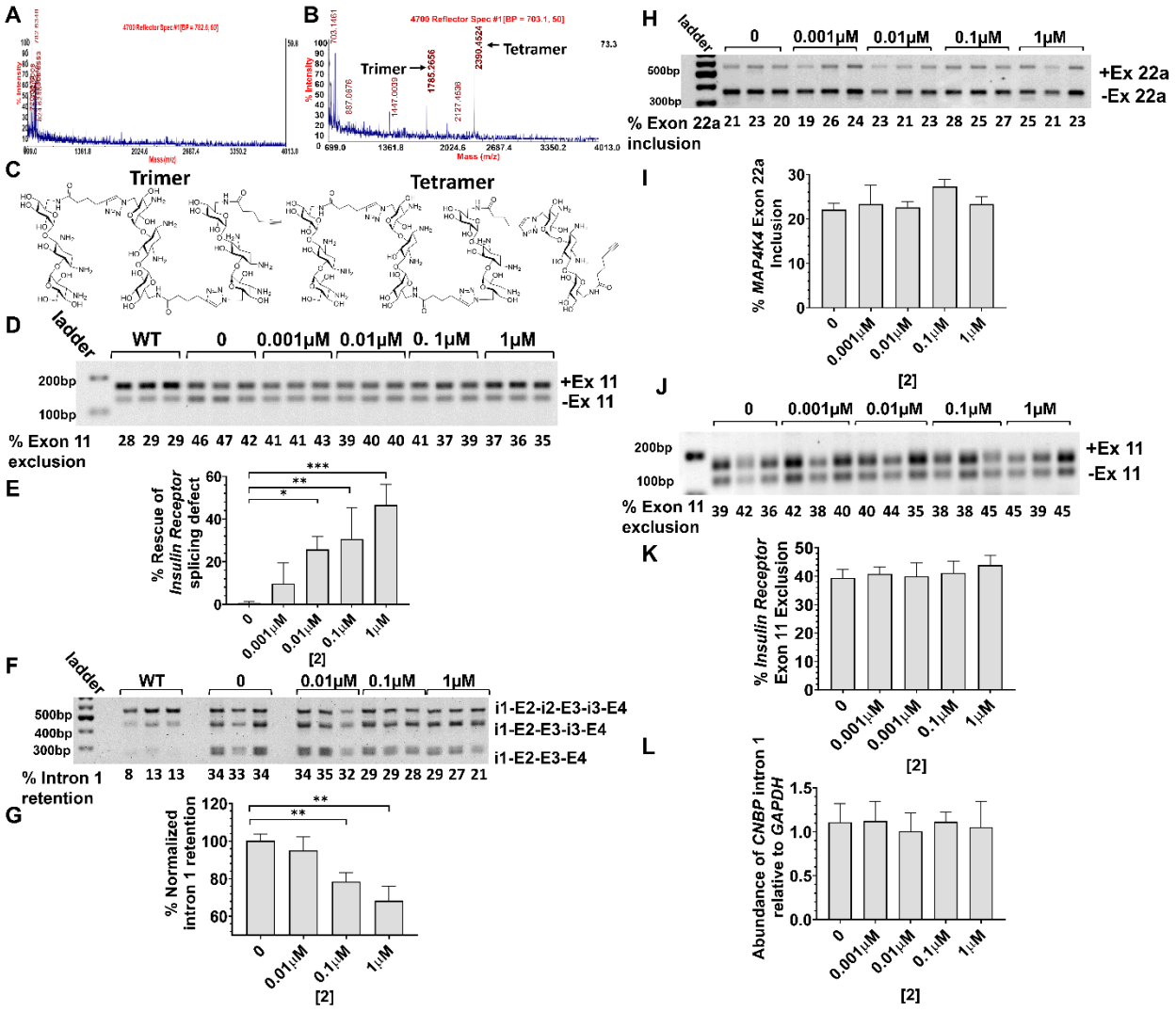

Figure S3. **Impact of 2 in DM2 and WT fibroblasts (related to Figure 4).** (*A, B, C*) Results of mass spectral analysis of *in cellulis* click reactions between **2** and **3** in DM2 and healthy fibroblasts. (*A*) MALDI MS spectrum of compounds isolated from healthy fibroblast cells. (*B*) MALDI MS spectrum of compounds isolated from DM2 fibroblasts, showing that a trimer and tetramer were formed. (*C*) Chemical structures of the *in cellulis* click products (trimer and tetramer). (*D, E, F, G, H, I*) Ability of **2** to rescue aberrant MBNL1-regulated splicing events in DM2 fibroblasts. (*D*) Representative gel image of *IR* exon 11 alternative splicing in DM2 fibroblasts treated with **2**. (*E*) Quantification of RT-PCR analysis of the *IR* exon 11 splicing defect upon treatment with **2**. (*F*) Representative gel image of *CNBP* intron 1 retention in DM2 fibroblasts treated with **2**. (*G*) Quantification of RT-PCR analysis of *CNBP* intron 1 retention in cells treated with **2**. (*H*) Representative gel image of *MAP4K4* exon 22a alternative splicing (non-MBNL1 regulated) treated with **2**. (*I*) Quantification of *MAP4K4* exon 22a splicing. (*J, K, L*) Evaluation of **2** in healthy (WT) fibroblasts. (*K*) Representative gel image of *IR* exon 11 alternative splicing in WT fibroblasts treated with **2**. (*L*) Quantification of *IR* exon 11 splicing defect. (*M*) RT-qPCR analysis of *CNBP* intron 1 abundance in WT fibroblasts treated with **2**. Error bars represent SD. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, as determined by a one-way ANOVA relative to 0 (n = 3).

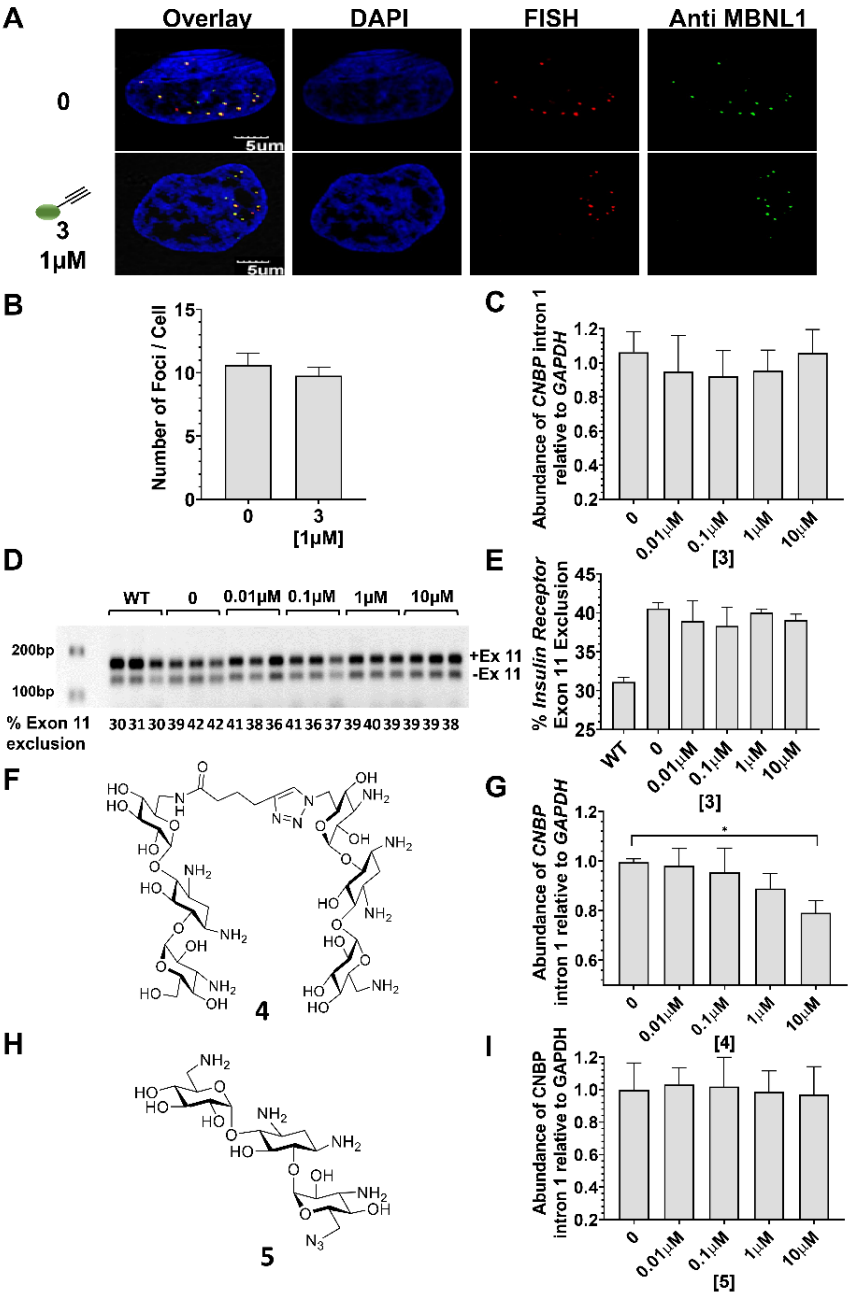

Figure S4: Evaluation of control compounds 3, 4 and 5 in DM2 fibroblasts (related to Figure 4). (*A, B*) Evaluation of nuclear foci using fluorescence *in situ* hybridization (FISH). (*A*) Representative images of r(CCUG)^{exp}-MBNL1 foci in DM2 fibroblasts treated with **3**. (*B*) Quantification of r(CCUG)^{exp}-MBNL1 foci/cell. Error bars represent SD (n=3 biological replicates; 40 nuclei counted per replicate). (*C*) RT-qPCR analysis of *CNBP* intron 1 abundance in DM2 fibroblasts treated with **3**. (*D*) Representative gel image of *IR* exon 11 alternative splicing in DM2 fibroblasts treated with **3**. (*E*) Quantification of *IR* exon 11 splicing. (*F*) Structure of compound **4**, the dimer formed by a reaction between two copies of **2**. (*G*) RT-qPCR analysis of *CNBP* intron 1 abundance in DM2 fibroblasts treated with **4**. (*H*) Structure of compound **5** (6"-azido kanamycin A). (*I*) RT-qPCR analysis of *CNBP* intron 1 abundance in DM2 fibroblasts treated with **5**. Error bars represent SD. *P < 0.05, as determined by a one-way ANOVA relative to 0 (n = 3).