Quantitative studies of muscleblind proteins and their interaction with *TCF4* RNA foci support involvement in the mechanism of Fuchs' dystrophy

Ziye Rong[1,2], Jiaxin Hu[3,4], David R. Corey*[3,4], and V. Vinod Mootha*[1,2]

List of the material included:

FIGURE S1. Effect of depletion of MBNL1, MBNL2 or CUGBP1 on RNA foci in F35T corneal endothelial cells.

FIGURE S2. Effect of depletion of MBNL1, MBNL2 or CUGBP1 on RNA foci in F45SV corneal endothelial cells.

FIGURE S3. RNA foci are not affected by knockdown of *TCF4* in F35T patient-derived corneal endothelial cells.

FIGURE S4. Bioanalyzer results of the purified standard RNAs of *MBNL1* and *MBNL2* synthesized by *in vitro* transcription.

FIGURE S5. Representative standard curves for signal intensity of MBNL1 and MBNL2 proteins.

TABLE S1. Summary table of corneal endothelial tissues, FECD corneal endothelial cell lines (F35T, F45SV) and control corneal endothelial cell line (HCN19).

TABLE S2. Sequences of primers and probes for transcript copy number.

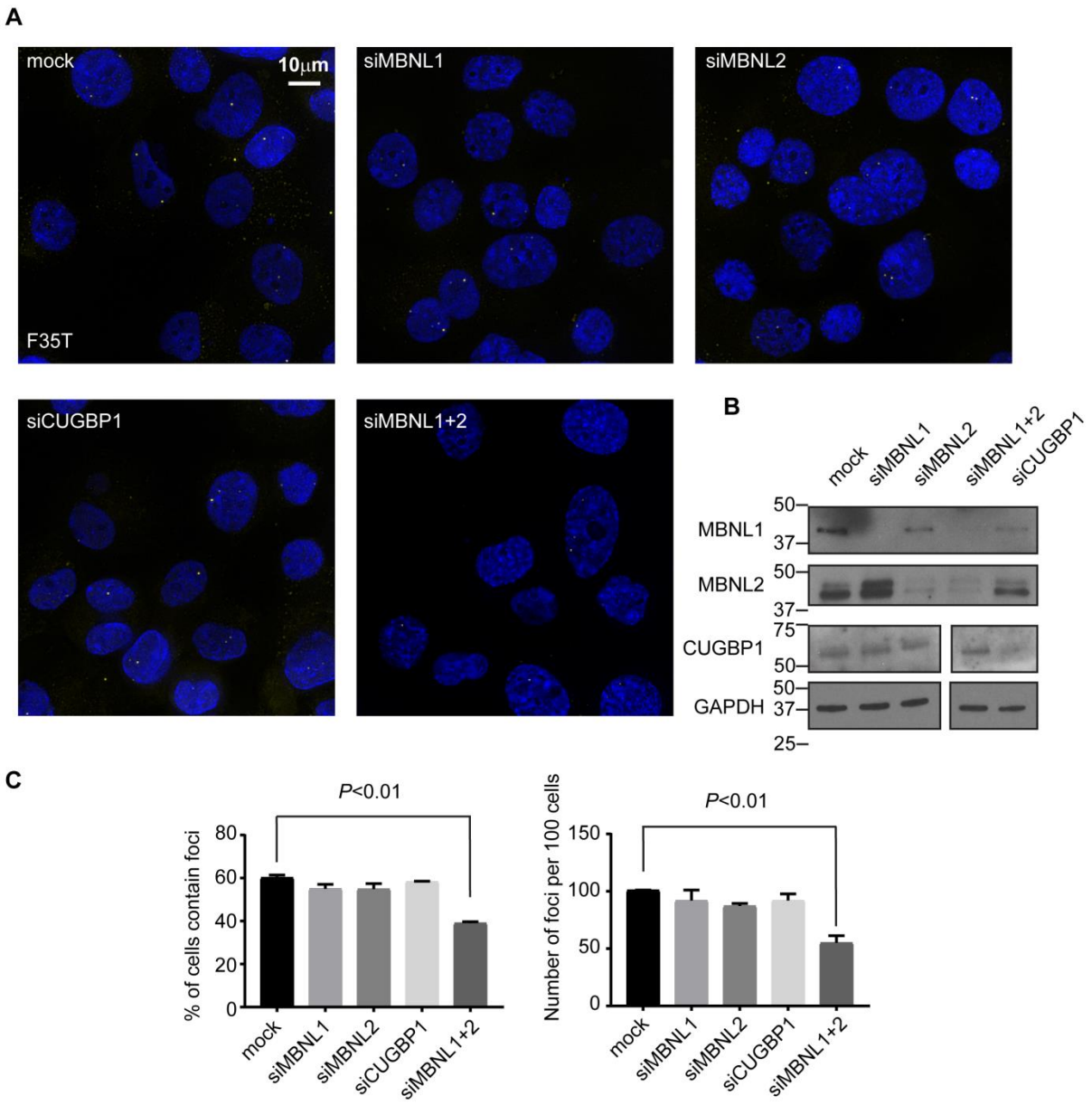

FIGURE S1. Effect of depletion of MBNL1, MBNL2 or CUGBP1 on RNA foci in F35T corneal endothelial cells. *A*, FISH images of CUG repeat RNA foci in F35T patient-derived corneal endothelial cells transfected with the indicated siRNA oligonucleotides. Different siRNA oligonucleotides targeting MBNL1 and MBNL2 were used in this experiment compared with those used in the experiment shown in Figure 2. *B,* Immunoblotting images of lysates from F35T cells showing siRNA depletion efficiency of targeted proteins. GAPDH was used as a loading control. *C*, Percentage of cells containing foci and number of foci per 100 cells are shown. Results are shown as the mean ± SD, n=2 independent experiments. *P*-value was obtained by t tests analysis of mock compared with siMBNL1+ siMBNL2. At least 100 cells were analyzed for each sample. *Scale bars* = 10 microns.

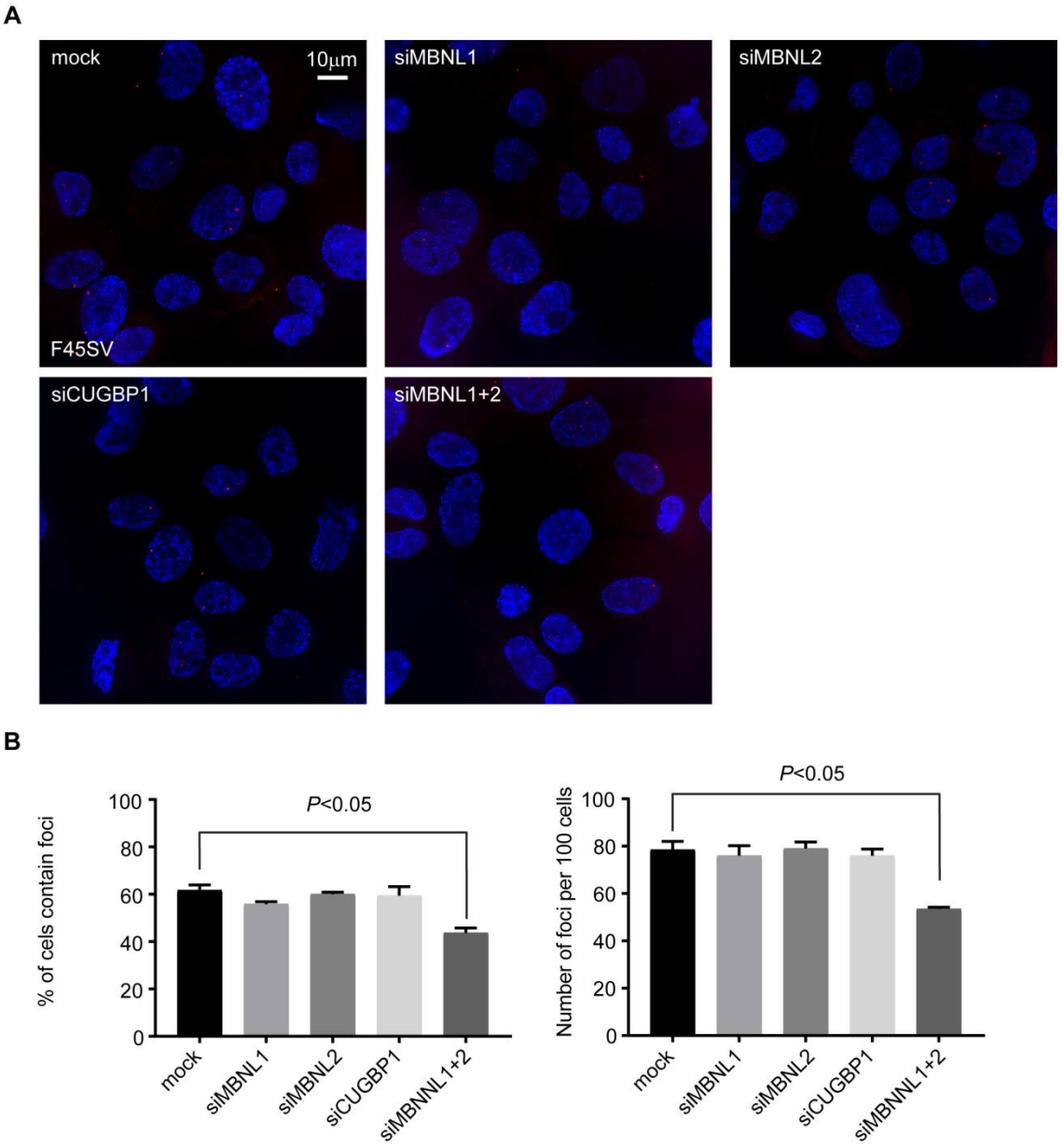

FIGURE S2. Effect of depletion of MBNL1, MBNL2 or CUGBP1 on RNA foci in F45SV corneal endothelial cells. *A*, FISH images of CUG repeat RNA foci in F45SV patient-derived corneal endothelial cells (16/1500 CTG18.1 alleles). Cells were transfected with the indicated siRNA oligonucleotides. *B*, Quantification of cells containing foci and number of foci per 100 cells in (B). *P*-value was obtained by t tests analysis of mock compared with siMBNL1+ siMBNL2. At least 100 cells were analyzed for each sample (n=2 independent experiments). *Scale bars* = 10 microns.

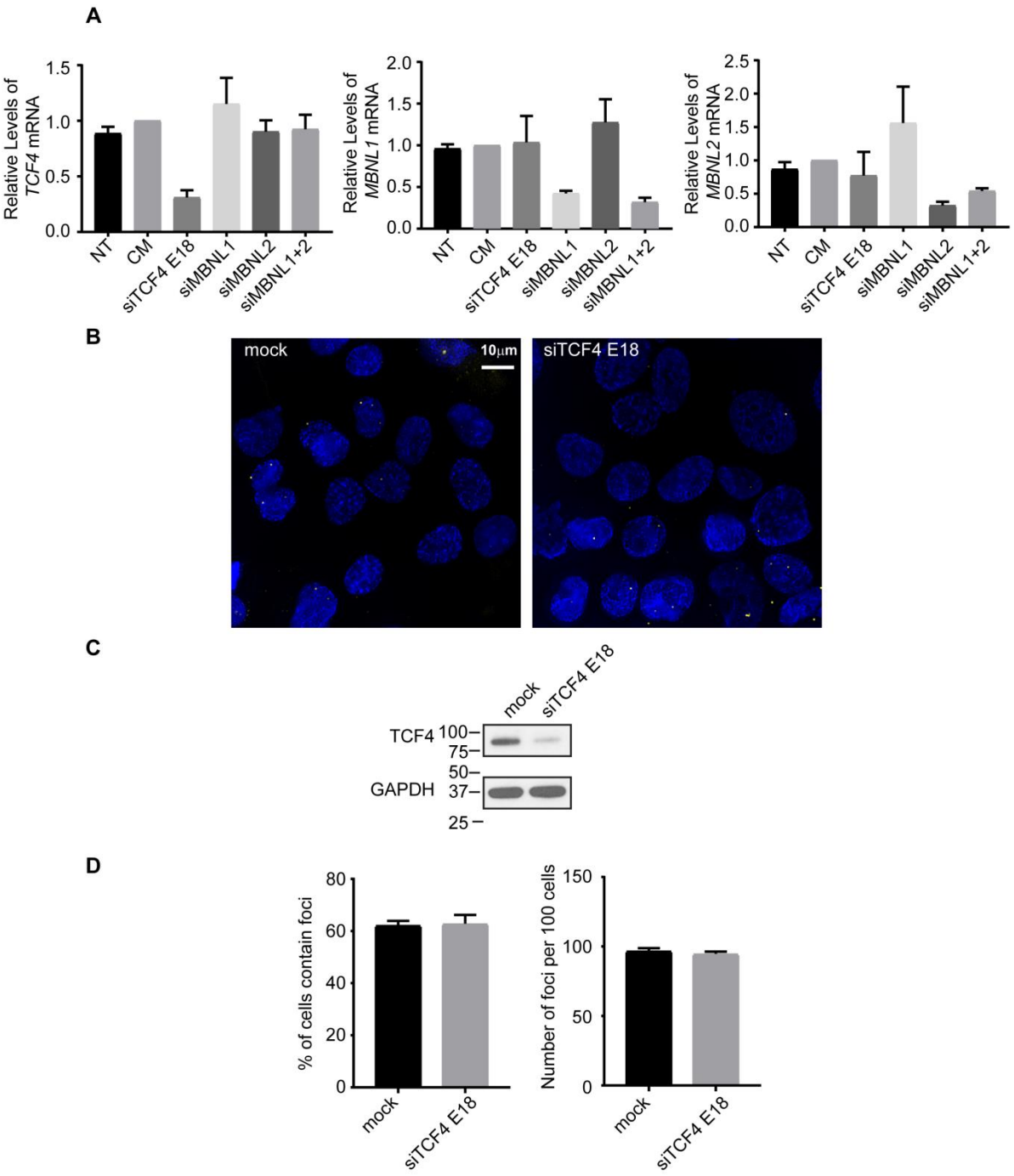

FIGURE S3. RNA foci are not affected by knockdown of *TCF4* in F35T patient-derived corneal endothelial cells. *A*, Real-time qPCR analysis showing knockdown of *TCF4*, *MBNL1* and *MBNL2* by siRNA. *TCF4* mRNA expression is not altered by knockdown of MBNL1 and/or MBNL2. NT, no treatment. CM, control siRNA without target. *B*, FISH images of CUG repeat RNA foci in F35T cells with siTCF4 E18 oligo. *C,* Immunoblotting images of lysates from F35T cells showing siRNA depletion efficiency of targeted protein. GAPDH was used as a loading control. *D*, Percentage of cells containing foci and number of foci per 100 cells are shown. Results are shown as the mean ± SD, n=2 independent experiments. At least 100 cells were analyzed for each sample. *Scale bars* = 10 microns.

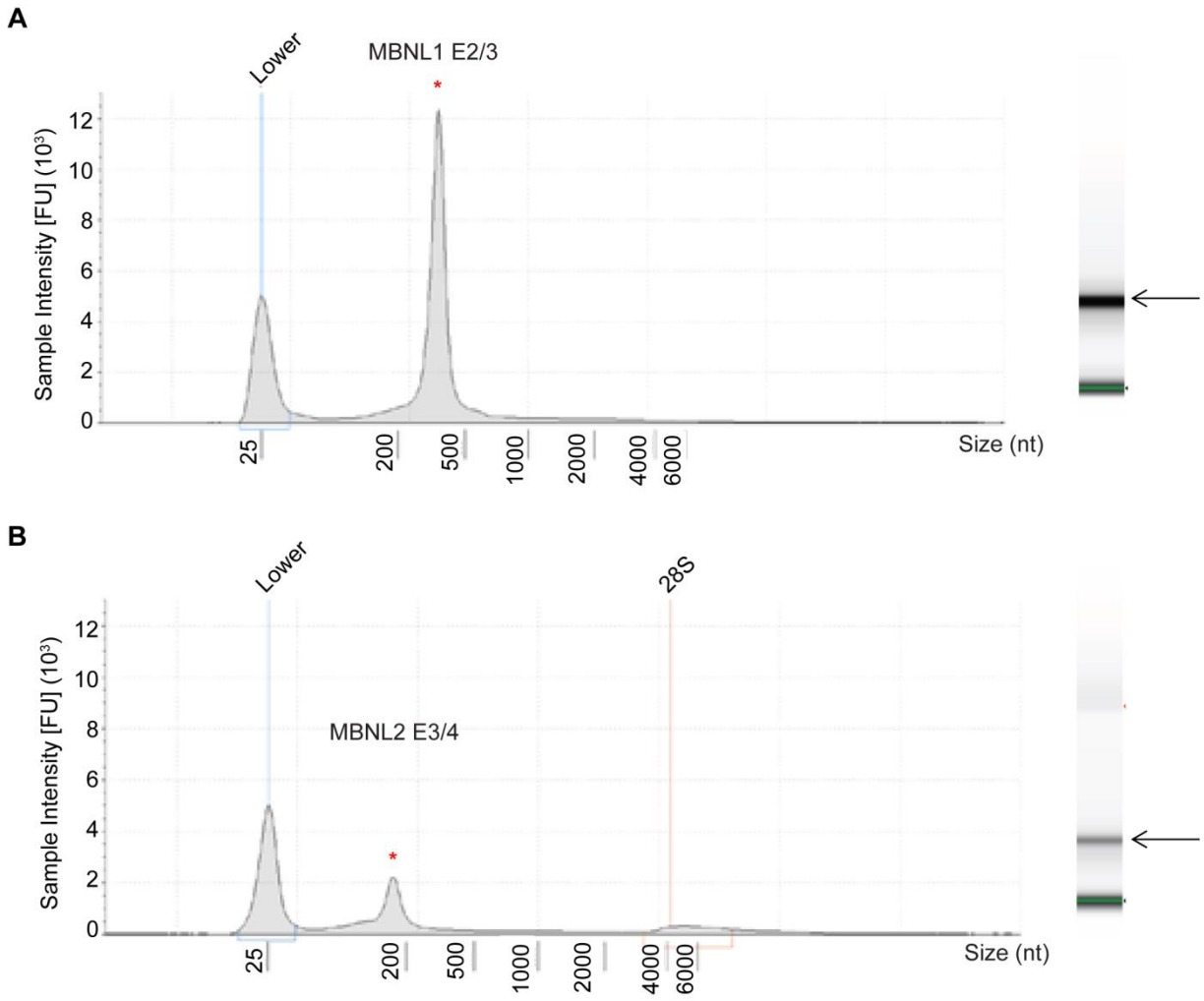

FIGURE S4. Bioanalyzer results of the purified standard RNAs of *MBNL1* and *MBNL2* synthesized by *in vitro* transcription. *A*, Standard RNA of *MBNL1* exon 2-3 mRNA. *B*, Standard RNA of *MBNL2* exon 3-4 mRNA.

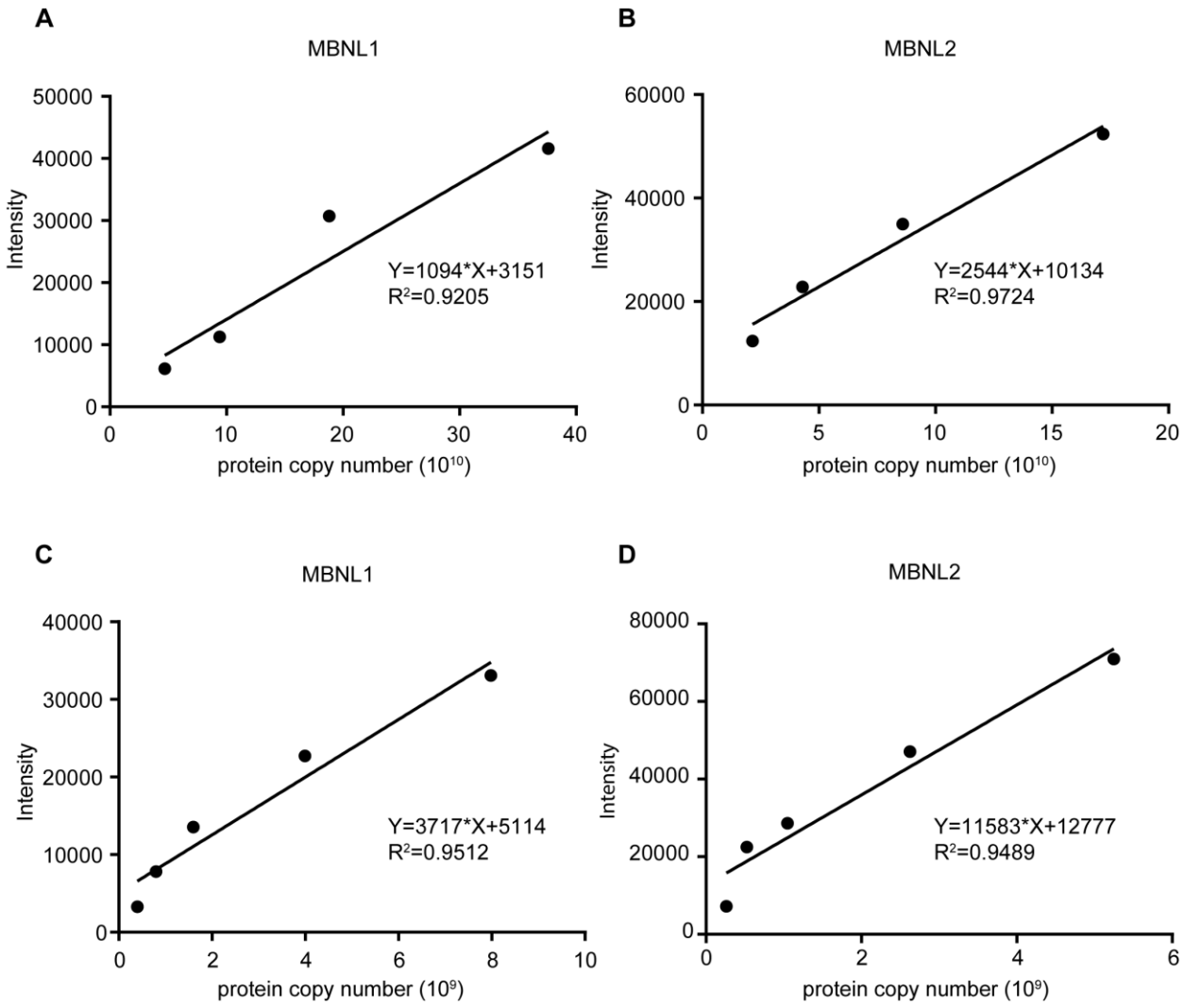

FIGURE S5. Representative standard curves for signal intensity of MBNL1 and MBNL2 proteins. A serial dilution of recombinant MBNL proteins were used to construct standard curves for signal intensity of MBNL1 and MBNL2 proteins in *A, B,* F35T patient-derived corneal endothelial cells. *C, D,* control corneal endothelial tissues

ID	Use	Age	CTG18.1 allele	DMPK CTG Repeat allele
CA004	MBNL co-localization	73	18, 100-120	12, 13
CA126		59	26, 87	13, 28
CA135		73	13, 84	12, 13
CA136		59	27, 51	12,14
AK010		77	18, >100	8, 12
W4056-17-001236	RNA copy number	63	16, 20	10, 12
2015-1477		71	13, 17	16, 16
W4056-16-000810		70	13, 19	10, 13
VVM527		72	33, 64	13, 21
VVM669		66	17, 84	10, 14
VVM670		50	12, 81	18, 21
W4056-18-003259	Protein copy number	72	12, 28	5, 13
W4056-18-002336		69	16, 18	5, 5
W4056-18-002344		16	25, 33	13, 21
W4056-18-002236	Distribution of MBNLs	56	14, 18	5, 13
W4056-18-002332		63	15, 26	5, 5
F35T		NA	22, 1500	5, 11
F45SV		45	16, 1500	5, 12
HCN19		19	19, 26	10, 13

TABLE S1. Summary table of corneal endothelial tissues, FECD corneal endothelial cell lines (F35T, F45SV) and control corneal endothelial cell line (HCN19).

primers and probes	Sequences
MBNL1 E2F1 SP6	5’-ATTTAGGTGACACTATAGAAGAGAGTTCCAGAGGGGGACT-3’
MBNL1 E3R1	5’-TTGGCTAGTTGCATTTGCTG-3’
MBNL2 E3F1 SP6	5’-ATTTAGGTGACACTATAGAAATCTTCACCCTCCGACACAC-3’
MBNL2 E4R1	5’-AACCCAACTCCAGGGGTTAC-3’
MBNL1 E2F2	5’-CTTCGAAAAGCTGCCAAGTT-3’
MBNL1 E3R2	5’-TTTTTAAATGTGGGGGTGGA-3’
MBNL2 E3F2	5’-GCCCAGCAGATGCAATTTAT-3’
MBNL2 E4R2	5’-CAGGGGTTACAGGTGCTAGG-3’
MBNL1 E2/3 probe	5’-AATGGACGAGTAATCGCCTG-3’
MBNL2 E3/4 probe	5’-CTTCATCCAGTGCCCACTTT-3’

TABLE S2. Sequences of primers and probes for transcript copy number.