Supporting Information

Aoki et al. 10.1073/pnas.1204638109

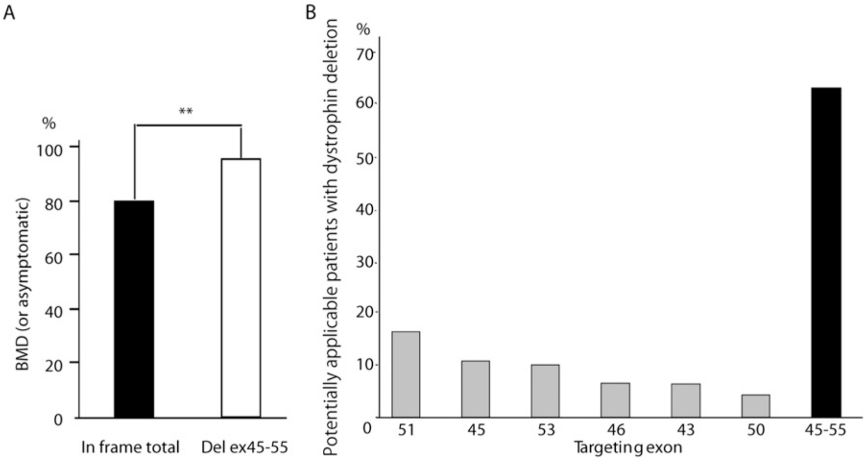

Fig. S1. Applicability of antisense-mediated exon skipping targeting each exon and exon 45–55 block skipping. (*A*) Percentage of DMD or BMD patients with in-frame deletion mutations in the *DMD* gene based on the Leiden database (1). Among 27 patterns of in-frame deletions in the *DMD* mutation hotspot (exons 45–55), deletion exons 45–55 (Del ex45–55) leads to among the mildest phenotype (highest BMD or asymptomatic percentage). The χ^2 test is used to calculate *P* values. $^{**}P < 0.01$. (*B*) Percentage of applicable patients of the *DMD* deletion mutations with exon-skipping targeting each exon. Applicability of exon-skipping targeting each exon is quite limited with 17% of the *DMD* deletion mutations at most in the case of exon 51 skipping. Exon 45–55 skipping can potentially treat ~63% of them.

1. Aartsma-Rus A, Van Deutekom JC, Fokkema IF, Van Ommen GJ, Den Dunnen JT (2006) Entries in the Leiden Duchenne muscular dystrophy mutation database: An overview of mutation types and paradoxical cases that confirm the reading-frame rule. *Muscle Nerve* 34:135–144.

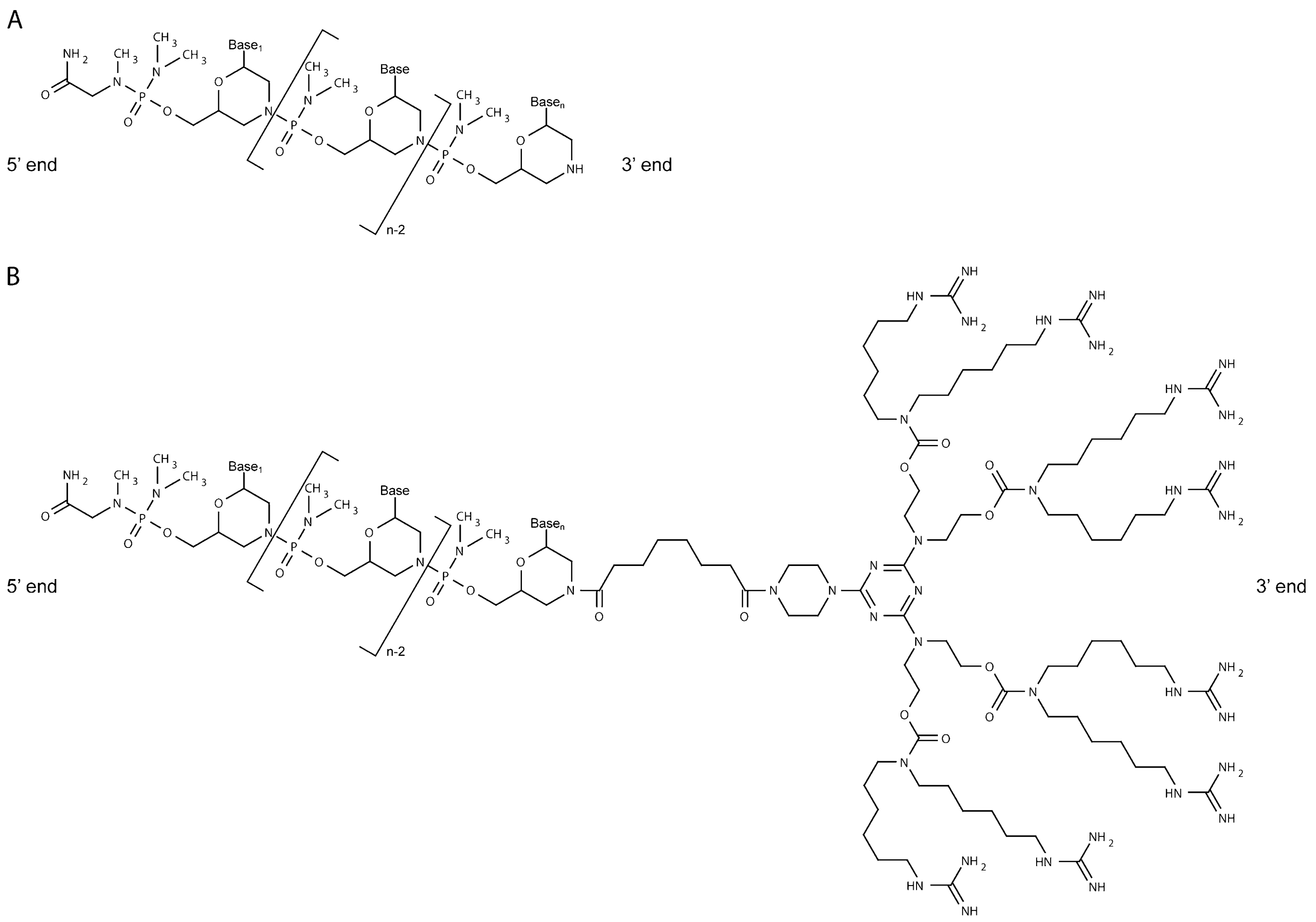

Fig. S2. Antisense chemistry and modifications for multiple exon skipping in *mdx52* mice. (*A*) Morpholino. (*B*) Vivo-morpholino.

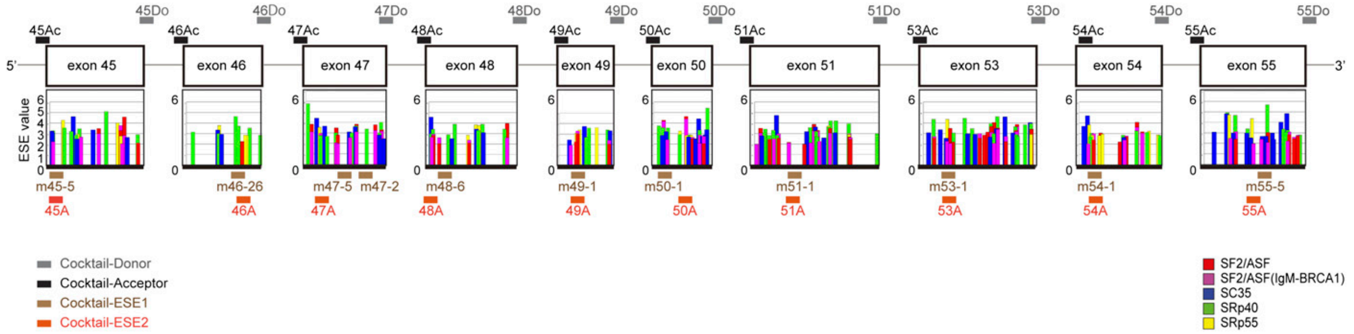

Fig. S3. Schematic outline of the vivo-morpholinos targeting exons 45–55 of murine dystrophin mRNA. Each AO targets either an exonic splicing enhancer (ESE) or the 5′ or 3′ splice site, indicated by gray, black, brown, or orange lines. The certainties of ESE sites according to ESEfinder 3.0 are indicated by colored boxes. Candidates for splicing enhancer-binding proteins are shown (red, SF2/ASF; purple, SF2/ASF (IgM-BRCA1); blue, SC35; green, SRp40; yellow, SRp55).

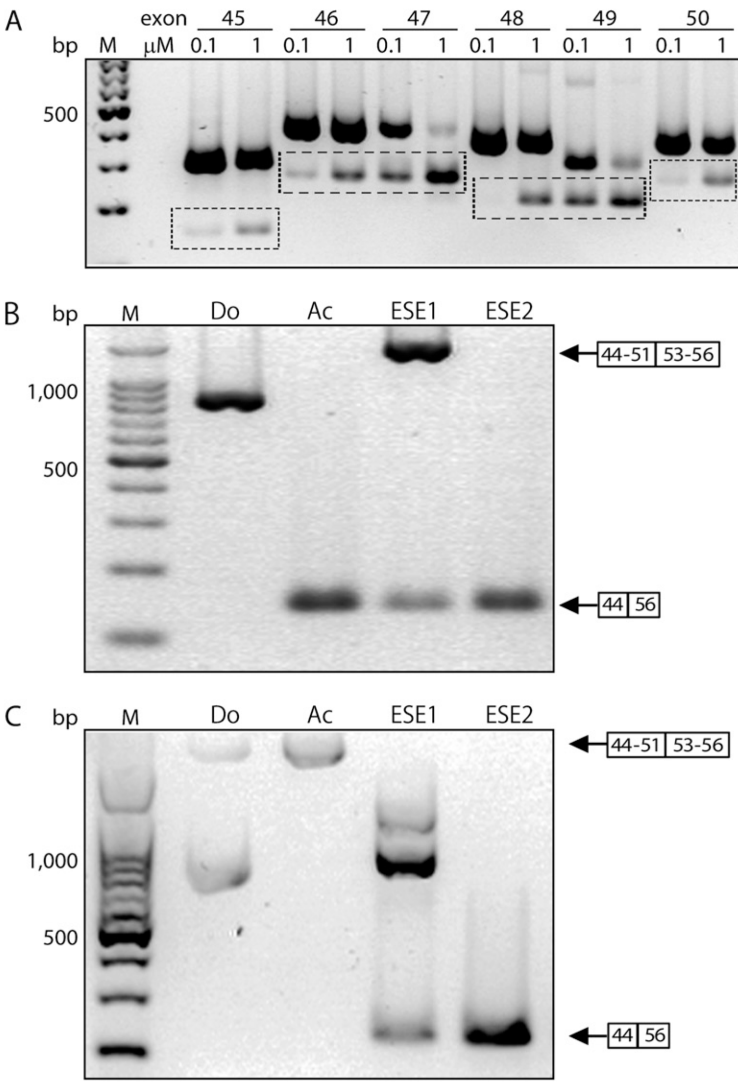

Fig. S4. Efficacy of single or 10 exon skipping targeting exons 45–55 in *H2K-mdx52* cells in vitro or in *mdx52* mice in vivo. (*A*) RT-PCR results after 0.1 or 1 μM of single vPMO (45A, 46A, 47A, 48A, 49A, 50A, 51A, 53A, 54A, or 55A) transfections into *H2K-mdx52* myotubes as indicated. Exon 45, 46, 47, 48, 49, 50, 51, 53, 54, or 55 skipping was detected by RT-PCR with a primer pair 44F1/46R, 45F/47R, 46F/48R, 47F/49R, 48F/50R, 49F/51R, 50F/53R, 51F/54R, 53F/56R1, or 54F/56R1, respectively. Skipped bands are surrounded by dotted frames. Representative data are shown. M, molecular marker. (*B*) RT-PCR results after 10 μM in total of mixture PMO transfections into *H2K-mdx52* myotubes as indicated. Exon 45–55 skipping was detected by RT-PCR with primers flanking exons 44 (44F) and 56 (56R). Do, mixture-donor; Ac, mixture-acceptor; ESE1, mixture-ESE1; ESE2, mixture-ESE2. Representative data are shown. (*C*) RT-PCR results 2 wk after mixture 10:00 PMO (10 μg in total) local injections into *mdx52* TA muscles as indicated. Exon 45–55 skipping was detected by RT-PCR with primers flanking exons 44 (44F1) and 56 (56R1). Do, mixture-donor; Ac, mixture-acceptor; ESE1, mixture-ESE1; ESE2, mixture-ESE2. Representative data are shown.

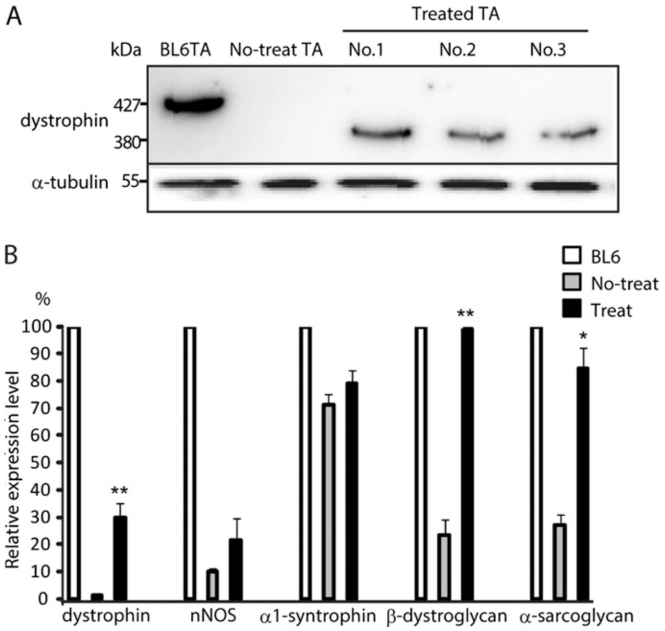

Fig. S5. Recovery of dystrophin and dystrophin-associated proteins with exon 45–55 skipping in *mdx52* mice by local vPMO injections. (*A*) Western blotting 2 wk after local injections with the mixture 10 vPMOs (1.5 μg in total, mixture-ESE2) to detect the expression of full-length dystrophin, 380-kDa quasidystrophin, and α-tubulin in TA muscles of WT (BL6TA), untreated (no-treat TA), and treated three different *mdx52* mice (treated TA). (*B*) Semiquantitative analysis of dystrophin, neuronal nitric oxide synthase (nNOS), α1-syntrophin, β-dystroglycan, and α-sarcoglycan expression after the mixture 10 vPMO (mixture-ESE2) injections. Data ($n = 4$) are presented as mean ± SEM $*P < 0.05$ versus untreated *mdx52* mice (no-treat), $**P < 0.01$ versus untreated *mdx52* mice.

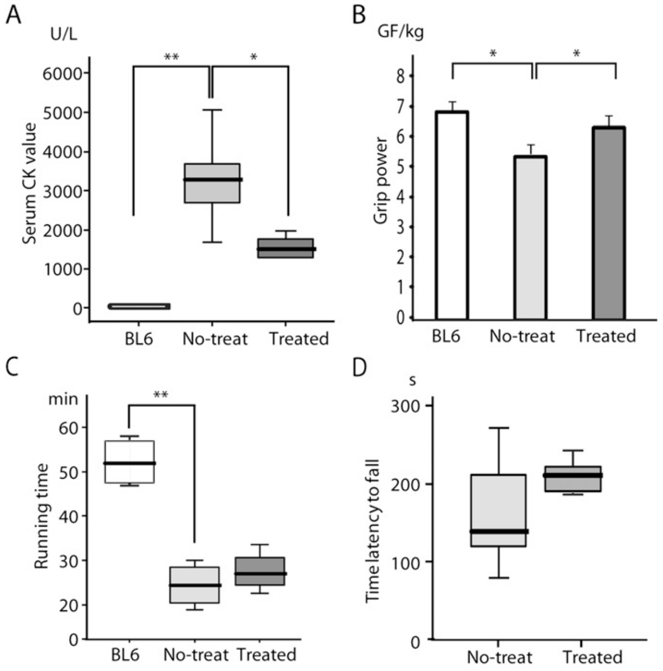

Fig. S6. Exon 45–55 skipped quasidystrophin ameliorates skeletal muscle function in *mdx52* mice. (*A*) Measurement of serum creatine kinase (CK) levels (IU/l). (*B*) Grip power test (GF/kg). (*C*) Treadmill performance (min). (*D*) Rotarod test (s). BL6, wild-type C57/BL6; no-treat, untreated *mdx52* mice; treated, treated *mdx52* mice with five times i.v. coinjections of 12 mg/kg/dose of vPMOs (mixture-ESE2). Data ($n = 4$) are presented as mean ± SD $*P < 0.05$; $**P < 0.01$.

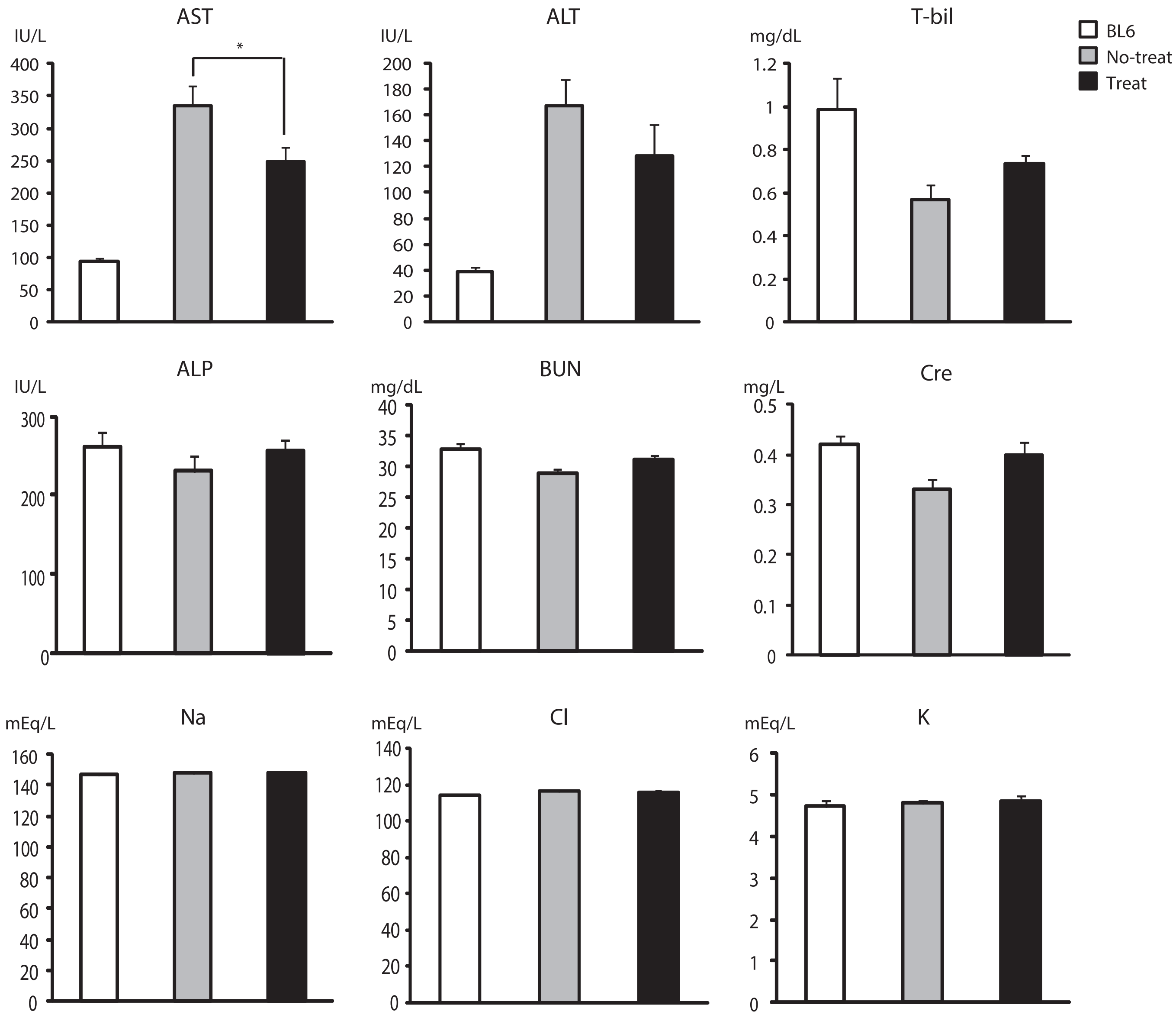

Fig. S7. Examination of adverse effects after systemic delivery of vPMOs. Blood tests after five times i.v. coinjections of 12 mg/kg/dose of vPMOs (mixture-ESE2) into *mdx*52 mice. Levels of serum enzymes aspartate transaminase (AST) (IU/L), alanine transaminase (ALT) (IU/L), total bilirubin (T-bil) (mg/dL), alkaline phosphatase (ALP) (IU/L), blood urea nitrogen (BUN) (mg/dL), creatinine (Cre) (mg/L), sodium (Na) (mEq/L), chloride (Cl) (mEq/L), and potassium (K) (mEq/L) among WT, untreated, and treated *mdx*52 mice. Data (n = 4) are presented as mean ± SEM.

Table S1. AO sequences against exons 45–55 of the *Dmd* gene of mice used in this study

Name	Position	Sequence
Mixture-donor		
45Do	+7–18	AAG TCT CTG TCA CCC TAC CTC TTT C
46Do	+9–16	TAA TAA GGG AAA TTA CCT TGA CTT G
47Do	+12–13	GTT AAT ATC TAA CCT TTA TCC ACT G
48Do	+7–18	TAA GCA AGT GGT ACC CAC CTT TAT G
49Do	+6–19	CTA GAG GTT GCT TCA TTA CCT TCA C
50Do	+6–19	TGG GAC AAA ATG TAC TTA CAG GCT C
51Do	+10–15	TTG TTT TAT CCA TAC CTT CTG TTT G
53Do	+9–16	TAT GCT TGA CAC TAA CCT TGG TTT C
54Do	+12–13	AAG TAG TTC TTA CCT TTT ATG AAT G
55Do	+5–20	AGC TGA AAC ACT CAA CTT ACT TGC C
Mixture-acceptor		
45Ac	−19+6	GAG TTC CTG TAA GCC ATC AGA AGA C
46Ac	−17+8	CTT CAA TCC TGT ATT AAG AAT AAC A
47Ac	−17+8	GCC AGT AAC TGA AAC GGA CAA ATA C
48Ac	−15+9	TCT GGA GAC CTG AAA GGG AAA AAA
49Ac	−5+20	TGA ACT GTT ACT TCA ATC TCC TGG G
50Ac	−10+15	ATC TTC TAA CTT CCT CTT TAA TAG G
51Ac	−18+7	CTG GCA GCT AGT GTT TTT GAA AGA A
53Ac	−11+14	CTG AAT TCT TTC AAC TGG AAT AAA A
54Ac	+5+29	TGC CGT TGA CGG AGG TCT TTG GCC A
55Ac	−18+7	ACT TAC TCT GCA AAG GGA CAA ACA G
Mixture-ESE1		
m45-5	+6+30	TTG ACG CTG CCC AAT GCC ATC CTG G
m46-26	+88+112	CTG CTC ATC TCC AAG TGG AGT AAT A
m47-2	+101+125	TTG AGC TTC TTT TCA AGT TTA TCT T
m47-5	+63+87	TAT GGG AGC ACT TAC AAG TAC TGC T
m48-6	+23+47	AAG TGA ACC TCA AGC TCT CCT TGT T
m49-1	+25+49	AAA GCC TTT CCA CAT CCG CTT GTT T
m50-1	+11+35	GCC TCC CAC TCA GAC CTC AGA TCT T
m51-1	+68+92	CAA CAG CAA AGA AGA TGG CAT TTC T
m53-1	+45+69	CAT TCA ACT GTT GTC TCC TGT TCT G
m54-1	+21+45	CAC GTC TAC ACT TAT CTG CCG TTG A
m55-5	+104+128	AAG CGT CCT GTA GGA CAT TGG CAG T
Mixture-ESE2		
45A	+5+29	TGA CGC TGC CCA ATG CCA TCC TGG A
46A	+98+122	CTT TTA GCT GCT GCT CAT CTC CAA G
47A	+22+46	ATT GTT TTA GAA TTC CCT GGC GCA G
48A	−2+23	TTC TCA GGT AAA GCT CTG GAG ACC T
49A	+24+48	AAG CCT TTC CAC ATC CGC TTG TTT A
50A	+48+72	CTG CTT TGT CCT CAG CTC CCG AAG T
51A	+66+90	ACA GCA AAG AAG ATG GCA TTT CTA G
53A	+44+68	ATT CAA CTG TTG TCT CCT GTT CTG C
54A	+23+47	GCC ACG TCT ACA CTT ATC TGC CGT T
55A	+84+108	GCA GTT GTT TCT GCT TCC GTA ATC C

Table S2. Primer sequences used in this study

Name	Sequence
Forward primers	
43F	CCTCCATGGAAAAGGTGAAA
44F	CCTGAAAAC TGGGAACATGC
44F1	CAGTTGAAAAATGGCGACAC
45F	AGCGTCAAGCTGTTGTCAGA
46F	TGAAGAACAAAAGAATGTCTTGTCA
47F	GCGCCAGGGAATTCTAAAAC
48F	CAAGGAGAGCTTGAGGTTCAC
49F	AAACAAGCGGATGTGGAAAG
50F	TTTACTTCGGGAGCTGAGGA
51F	TGTCATCTCCAAACTAGAAATGC
53F	ATTCAGTGGGATGAGGTTCAA
54F	GCCAAAGACCTCCGTCAAC
Reverse primers	
46R	TAGCTGCTGCTCATCTCCAA
47R	CCACTGGAGATTTGTCTGTTTG
48R	GGTCCTGCCTGACTTGGTT
49R	CTTCACTGGCTGAGTGCTTG
50R	TCCTCAGCTCCCGAAGTAAA
51R	CTTCCAGATCACCCACCATC
53R	ACCTGTTCGGCTTCTTCCTT
54R	TCAGCAGAATAGTCCCGAAGA
56R	GGTGCTTCATCCGAACCTT
56R1	GTAACAGGGGTGCTTCATCC
57R	AACTGCTGGGAAATCACCAC