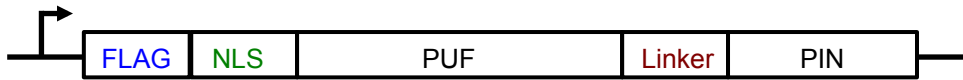


Supplementary Figure 1

a



b

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FLAG      NLS      PUF
ASRE-(C) DYKDDDDKPKKKRKVGRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGNFYIQLKLERATPAERQLVFNEILQAAYQLMVDVFGSYVIRKFFEFGSLEQKLALAERIRGHVLSLALQM
ASRE-(G) DYKDDDDKPKKKRKVGRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSYFIRLKLERATPAERQLVFNEILQAAYQLMVDVFGSYVIEKFFEFGSLEQKLALAERIRGHVLSLALQM
ASRE-(U) DYKDDDDKPKKKRKVGRSRLLEDFRNNRYPNLQLREIAGHIMEFSQDQHGSRFIELKLERATPAERQLVFNEILQAAYQLMVDVFGNYVIQKFFEFGSLEQKLALAERIRGHVLSLALQM
*****. **.*****

ASRE-(C) YGSYVIEKALEFIPSDQQNEMVRELDGHVLCVKDQNGNHVVQKCIQVQPSLQFIDAFKGGVFALSTHPYGSYVIRRIEHLCPDQTLPIEELHQHTEQLVQDQYGSYVIEHVLEH
ASRE-(G) YGNRVIQKALEFIPSDQQNEMVRELDGHVLCVKDQNGNHVVQKCIQVQPSLQFIDAFKGGVFALSTHPYGSRVIERIEHLCPDQTLPIEELHQHTEQLVQDQYGNVVIQHVLEH
ASRE-(U) YGSYVIRKALEFIPSDQQNEMVRELDGHVLCVKDQNGSYVVEKCIQVQPSLQFIDAFKGGVFALSTHPYGNRVIQRILEHLCPDQTLPIEELHQHTEQLVQDQYGSYVIRHVLEH
**.*.*.*****.:.*.*****.*****.*****.*****.*****

ASRE-(C) GRPEDKSKIVAEIRGNVLSQHKFASNYVVKCVTHASRTERAVLIDEVCTMNDGPHSALYTMMDQYASYVVRKIDVAEPGQRKIVMHKIRPHIATLRKYTYGKHILAKLEKYYMKNG
ASRE-(G) GRPEDKSKIVAEIRGNVLSQHKFASVYVVRKCVTHASRTERAVLIDEVCTMNDGPHSALYTMMDQYASYVVEKIDVAEPGQRKIVMHKIRPHIATLRKYTYGKHILAKLEKYYMKNG
ASRE-(U) GRPEDKSKIVAEIRGNVLSQHKFASNVVEKCVTHASRTERAVLIDEVCTMNDGPHSALYTMMDQYANYVVQKIDVAEPGQRKIVMHKIRPHIATLRKYTYGKHILAKLEKYYMKNG
*****. **.*****

ASRE-(C) VDLGVDTANGSQMELEIRPLFLVPDTNGFIDHLASLARLLESRKYILVVPLIVINELDGLAKGQETDHRAGGYARVVQEKARKSIEFLEQRFSRDSCLRALTSRGNESIAFRSEDI
ASRE-(G) VDLGVDTANGSQMELEIRPLFLVPDTNGFIDHLASLARLLESRKYILVVPLIVINELDGLAKGQETDHRAGGYARVVQEKARKSIEFLEQRFSRDSCLRALTSRGNESIAFRSEDI
ASRE-(U) VDLGVDTANGSQMELEIRPLFLVPDTNGFIDHLASLARLLESRKYILVVPLIVINELDGLAKGQETDHRAGGYARVVQEKARKSIEFLEQRFSRDSCLRALTSRGNESIAFRSEDI
*****

ASRE-(C) GQLGNNDDLILSCCLHYCKDKAKDFMPASKEEPIRLREVLLTDDRNLRVKALTRNVPVRDIPAFLTWAQVG
ASRE-(G) GQLGNNDDLILSCCLHYCKDKAKDFMPASKEEPIRLREVLLTDDRNLRVKALTRNVPVRDIPAFLTWAQVG
ASRE-(U) GQLGNNDDLILSCCLHYCKDKAKDFMPASKEEPIRLREVLLTDDRNLRVKALTRNVPVRDIPAFLTWAQVG
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Figure S1. Design of ASREs. (a) Schematic diagram of the ASRE construct. The different fragments or domain are not drawn to scales. (b) The amino acid sequences of ASREs. The colors of the sequences are the same as the panel a. The D1353 is labeled in red, mutation of this residue will reduce the activity of PIN domain.

Supplementary Figure 2

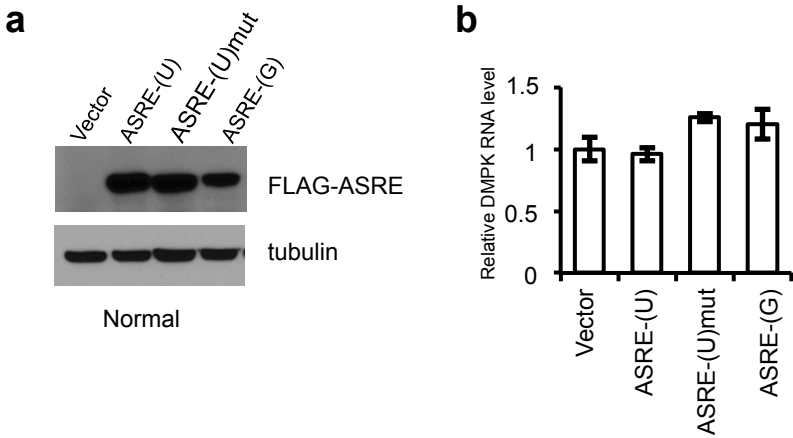


Figure S2. The expression of ASREs have no effect on DMPK mRNA in differentiated normal human fibroblasts. (a) The protein levels of ASREs were examined by Western blot to confirm the expression. (b) The mRNA levels of DMPK were examined with real-time RT-PCR. Same experimental conditions were used as in Figure 3 in main text.

Supplementary Figure 3

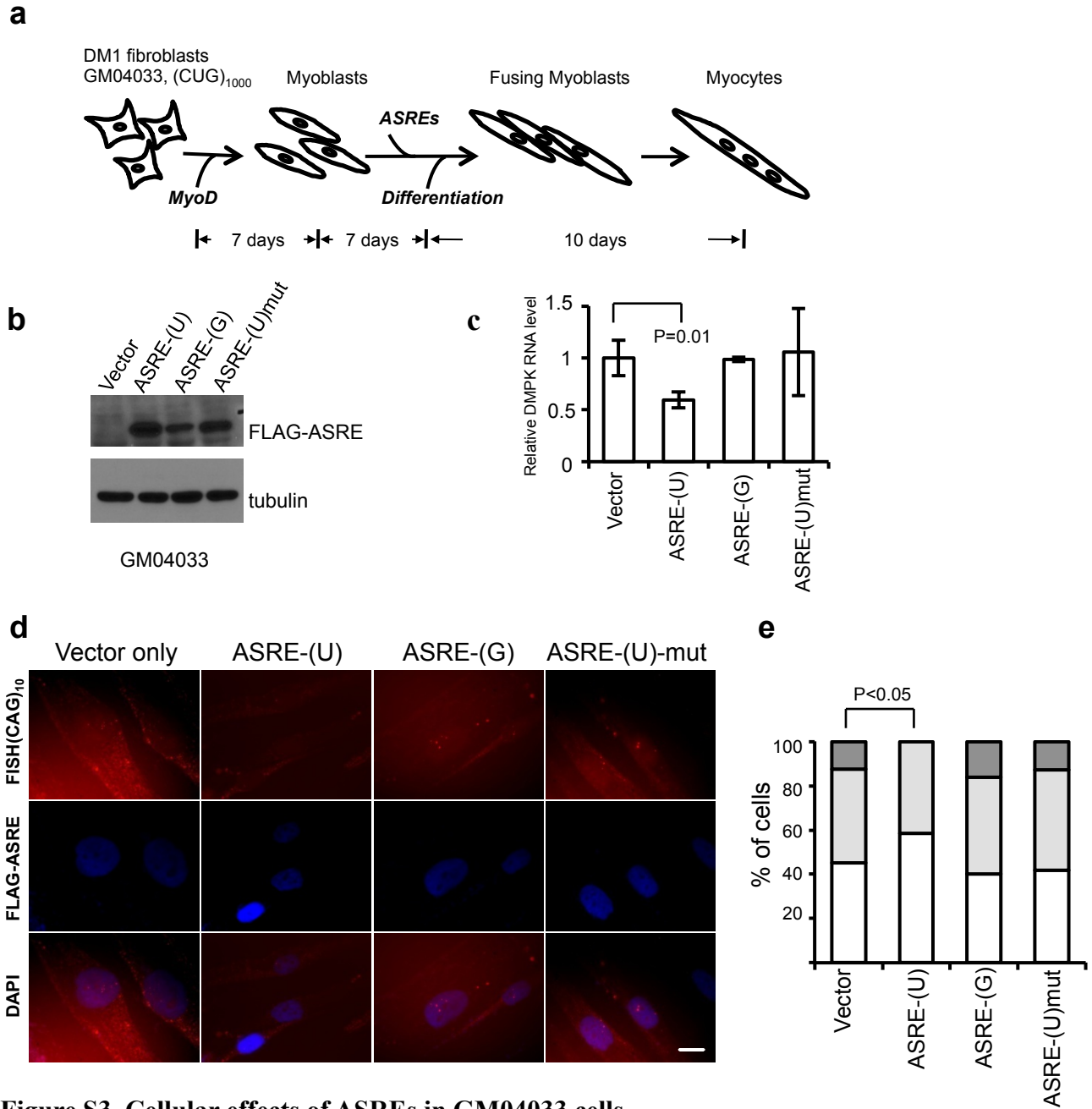


Figure S3. Cellular effects of ASREs in GM04033 cells.

(a) Schematic representation of ASRE treatment in DM1 myocytes derived from patient fibroblasts. The DM1 patient fibroblasts (GM04033) were stably transfected with human MyoD and further cultured for 7 days to convert them into myoblasts. The resulting cells were stably transfected with ASREs, after incubation for 7 days the myoblasts were induced into myocytes with differentiation medium, and the cells were harvest in another 10 days for further analyses. (b) The protein levels of ASREs were examined by western blot. As an internal control, the tubulin was detected. (c) The mRNA levels of DMPK were examined with real-time RT-PCR. Same experimental conditions were used as in Figure 3 in main text. (d) The (CUG)_n repeats in differentiated GM04033 cells were assayed by fluorescence in situ hybridization (FISH) using (CAG)₁₀ probes (red). The nuclei were stained with DAPI. Scale bar =10 μm. (e) The differentiated GM04033 cells transfected with vector or different ASREs were selected from random fields and counted for the number of nuclear foci. The percentage of cells with different number of nuclear foci were represented as histogram. From left to right, n=81, 54, 33 and 32.

Supplementary Figure 4

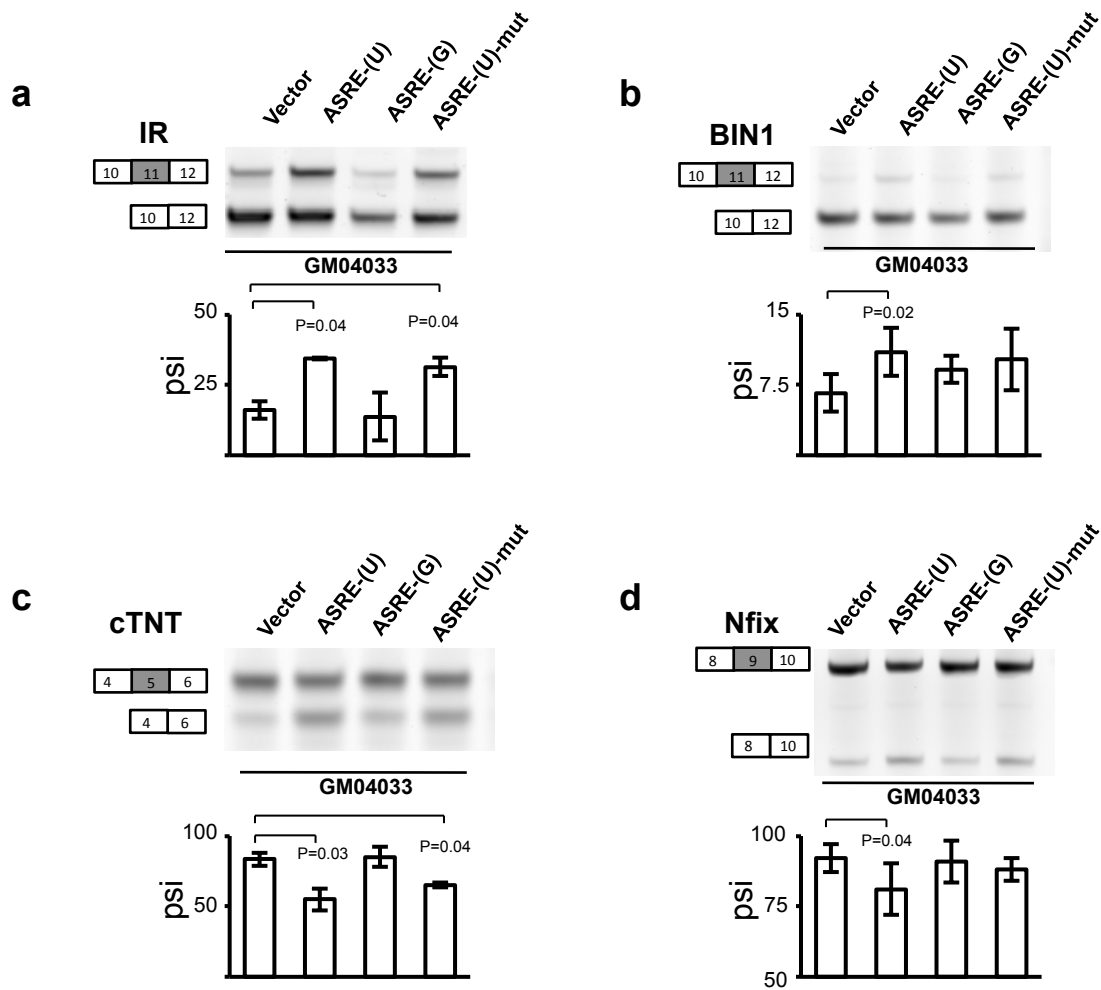


Figure S4. The reverse of aberrant alternative splicing by ASREs expression in GM04033 cells.

Semi-quantitative RT-PCR was used to detect different alternative splicing isoforms for IR (a), BIN1 (b), cTNT (c) and Nfix (d) in differentiated DM1 cells (GM04033). As the control, we used differentiated DM1 cells transfected with vector only. At least two independent experiments were carried out, with the means and s.d. of the percent-splice-in (psi) shown below a representative gel.

Supplementary Figure 5

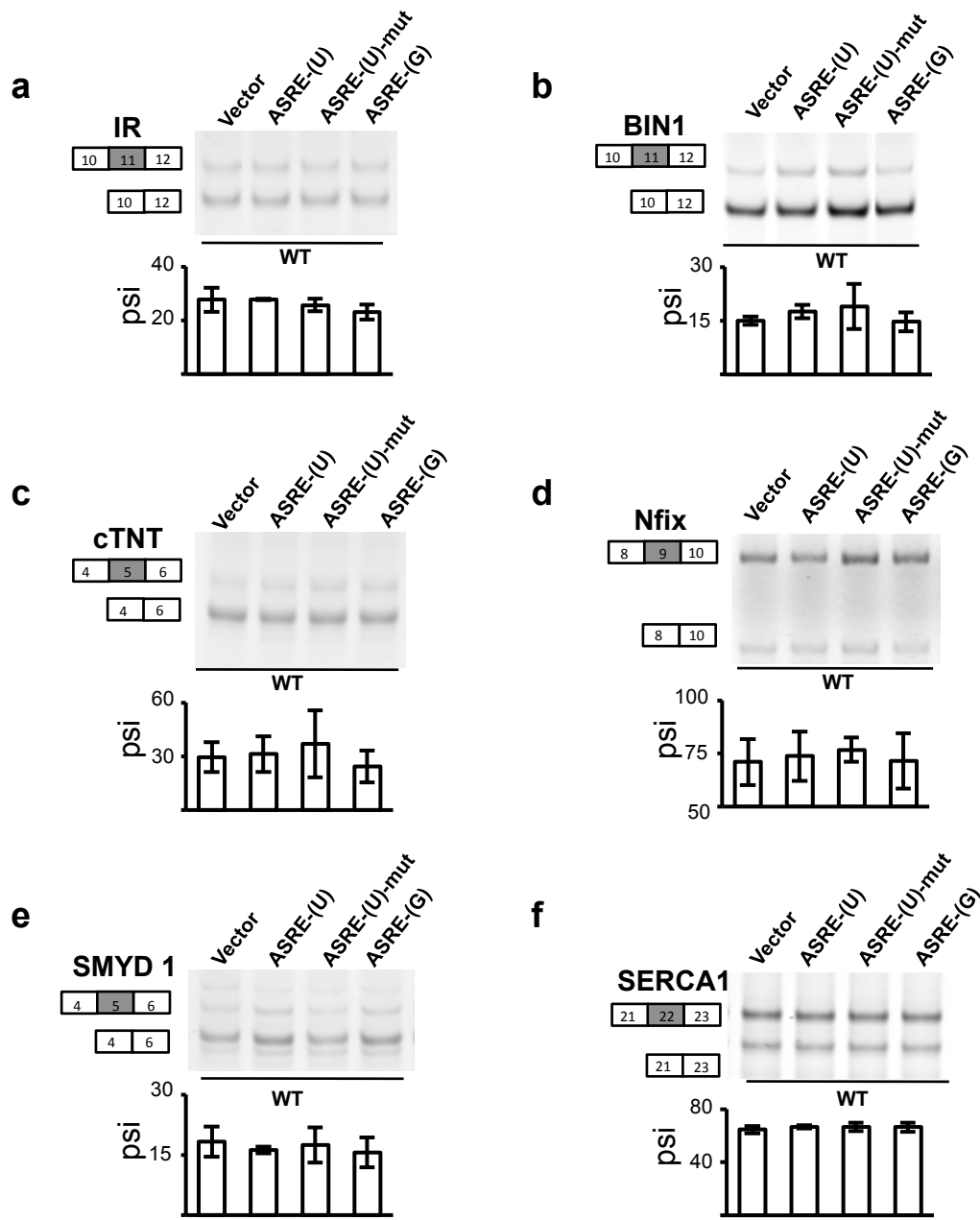


Figure S5. The expression of ASREs have no effect on alternative splicing in normal cells.

Semi-quantitative RT-PCR was used to detect different alternative splicing isoforms for IR (a), BIN1 (b), cTNT (c), Nfix (d), SMYD 1 (e) and SERCA1 (f) in differentiated normal human fibroblasts. At least two independent experiments were carried out, with the means and s.d of the percent-splice-in (psi) shown below a representative gel.

Supplementary Figure 6

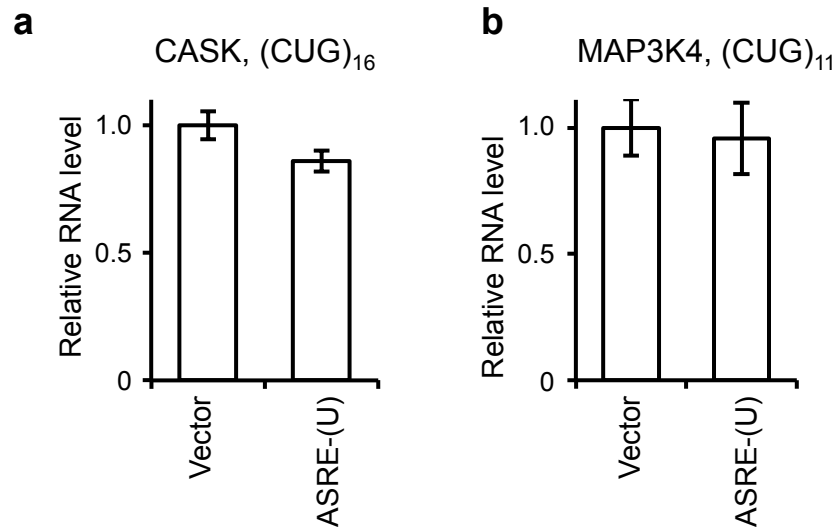


Figure S6. Effect of ASRE-(U) on other mRNA containing short CUG repeat.

Two human transcripts, CASK (a) and MAP3K4 (b), which contain (CUG)₁₆ and (CUG)₁₁ repeats respectively were measured with real time RT-PCR in differentiated GM04602 cells treated with control or ASRE-(U). The length of the repeat tract for each transcript is indicated. The mRNA level of GAPDH were measured as internal controls. The experiments were carried out 3 times, and the averages and s.d. were shown.

Supplementary Table 1: Primers used in detection of different mRNA isoforms

Primer Name	Primer sequence	Note
P1	GTTTCGCCGTTGTTCTGTC	Forward primer for DMPK exon 15
P2	TCGGAGCGGTTGTGAACT	Reverse primer for DMPK exon 15
P3	CCAAAGACAGACTCTCAGAT	Forward primer for IR exon 10
P4	AACATCGCCAAGGGACCTGC	Reverse primer for IR exon 12
P5	AGAACCTCAATGATGTGCTGG	Forward primer for BIN1 exon 10
P6	TCGTGGTTGACTCTGATCTCGG	Reverse primer for BIN1 exon 12
P7	ATAGAAGAGGTGGTGGAAGAGTAC	Forward primer for cTNT exon 4
P8	GTCTCAGCCTCTGCTTCAGCATCC	Reverse primer for cTNT exon 6
P9	AGCCCTGTTGATGACGTGTT	Forward primer for Nfix exon 8
P10	AGTGCAGGGCTGATGCTGT	Reverse primer for Nfix exon 10
P11	GTAGGCATCTTCCCCAACCT	Forward primer for SMYD1 exon 4
P12	CTGCTTCTTCAGCTGCCTCT	Reverse primer for SMYD1 exon 6
P13	ATCTTCAAGCTCCGGGCCCT	Forward primer for SERCA1 exon 21
P14	CAGCTCTGCCTGAAGATGTG	Reverse primer for SERCA1 exon 23
P15	CAGAGTTCGGCTGGTACAGT	Forward primer for CASK
P16	ACAGGACGAAGACTGAGTGC	Reverse primer for CASK
P17	TCTGCCGAGTCCTTCTGAAT	Forward primer for MAP3K4
P18	TTCAAAAGCGTCAATGTTGC	Reverse primer for MAP3K4