Supporting Information

Mallory et al. 10.1073/pnas.1423695112

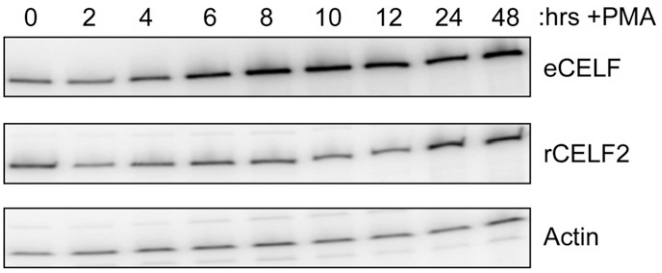

Fig. S1. RT-PCR analysis of the expression of endogenous and cDNA-derived CELF2 mRNA. Low-cycle RT-PCR of CELF2 mRNA derived from the endogenous gene (*Top*, eCELF2) or stably integrated cDNA (*Bottom*, rCELF2).

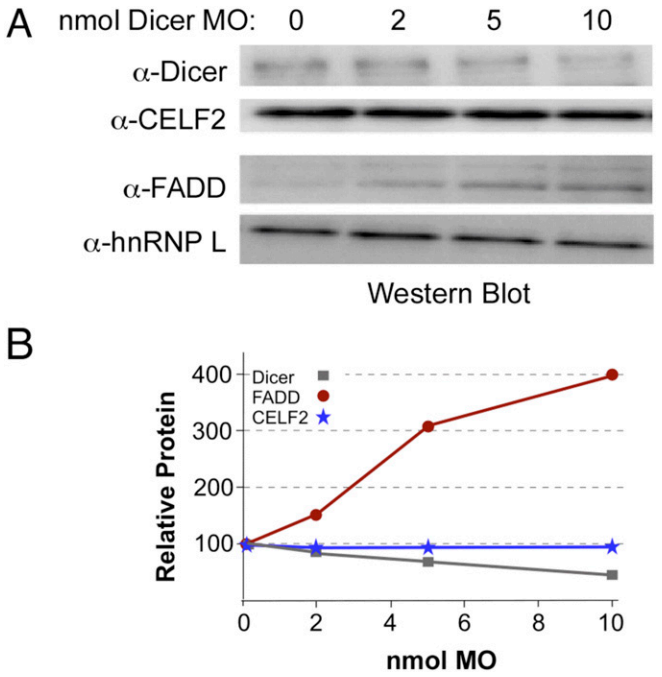

Fig. S2. CELF2 protein expression is unaffected by depletion of Dicer in JSL1 T cells. (*A*) Western blot for indicated proteins using lysates from JSL1 cells depleted of Dicer protein expression by treatment with a morpholino oligonucleotides. FADD serves as a positive control for a protein known to be under control of miRNAs/Dicer in T cells. hnRNP L serves as a loading control. (*B*) Quantification of two to three replicates of blots as shown in *A*.

Modeling CELF2 mRNA level

A simple model for the increase and eventual stabilization of CELF2 mRNA level can be derived as follows:

r(t) is the relative level of CELF2 mRNA level at time t after PMA stimulation.
p is the rate of transcription of new CELF2 mRNAs.
g is the rate of degradation of CELF2 mRNAs.

r(t) is related to p and g like:

$$dr(t) / dt = p - g*r(t)$$

The solution of this model is $r(t) = p/g + C*e^{-gt}$. In order for r(0) = 1 , set C = (1 – p/g) such that:

$$r(t) = p/g + (1 - p/g) * e^{-gt}$$

We observe a 4.5-fold increase in p and a 2.5-fold decrease in g from unstimulated to fully stimulated JSL1. This suggests a 4.5*2.5 = 11.25-fold increase in the steady state level of CELF2 mRNA.

Fig. S3. Modeling of CELF2 mRNA expression based on observed regulation of transcription and stability demonstrates that the observed increases in transcription and stability are sufficient to account for the ~12-fold increase in mRNA expression that has been experimentally measured.

Key

--- = reported polyA cleavage site (Ensembl 8/8/14)
--- = proposed polyA recognition hexamer site
nn = experimentally validated polyA cleavage site (this study)
aaa = intron
AAA = exon
/ = exon-intron boundary

STOP

TGATCCTAACCCCAGAGGCTCCCTGCTCTCATTTTAGCTTTCTTAGG/gtaagtcccacgagccagcctgtctcaacagggaag 80

gcagaggaggaccacattgccaacttttaccaagagagacggttatttttacaataaggcctccatgtccccacccactt 160

PAS-i

ccctacccagtttgccataattaaaacttgggctactttgtttctattgagtaattcctcctccagttgtgccactgta 240

ttctcctttgttttgcgatttgaatctccttttacctttttttttaatttttttcattttgctttttttttaaagagaag 320

catatacacaaataaatggcatcttgagaatttaaaaggcaaacaaacaaacgctaatgtgcaattctaactctgaaacc 400

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PAS-1a

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GTGAAAAACATTGCTGCATTCATGCAAGACTGAAATTTATTATTAGACAAATTCATTATAGAAAAAACCTGTGGCAAAAA 1680

CGTTTCTTTCTTATTTTTTTTCTTTTCCTAAAACAGACTTGAAAGTATTATACAGGGATTGGCATTCTTCCCGGTCACTG 1760

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TACCTATATTTTATACTGTTTCAATGTACAGGAGAAAGGTTACTGTAAACTGTGTTATGTTGGTGCTTCTGTGAATTAAG 2160

PAS-1b

TTGTGGTTTCATCATGAGTCTTAATGTTCTTTGTTGATAAGACAAGTTTAGAATTGGTTTACTTATAATACAAAAAAAAAA 2240

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2400

Fig. S4. (Continued)

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2560
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2640
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2800
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3120
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3600
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5200
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5280
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Fig. S4. (Continued)

PAS-2

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5440
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5520
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5760
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PAS-3a
7520
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7600
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PAS-3b
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Fig. S4. Human CELF2 3′UTR sequence and annotation.

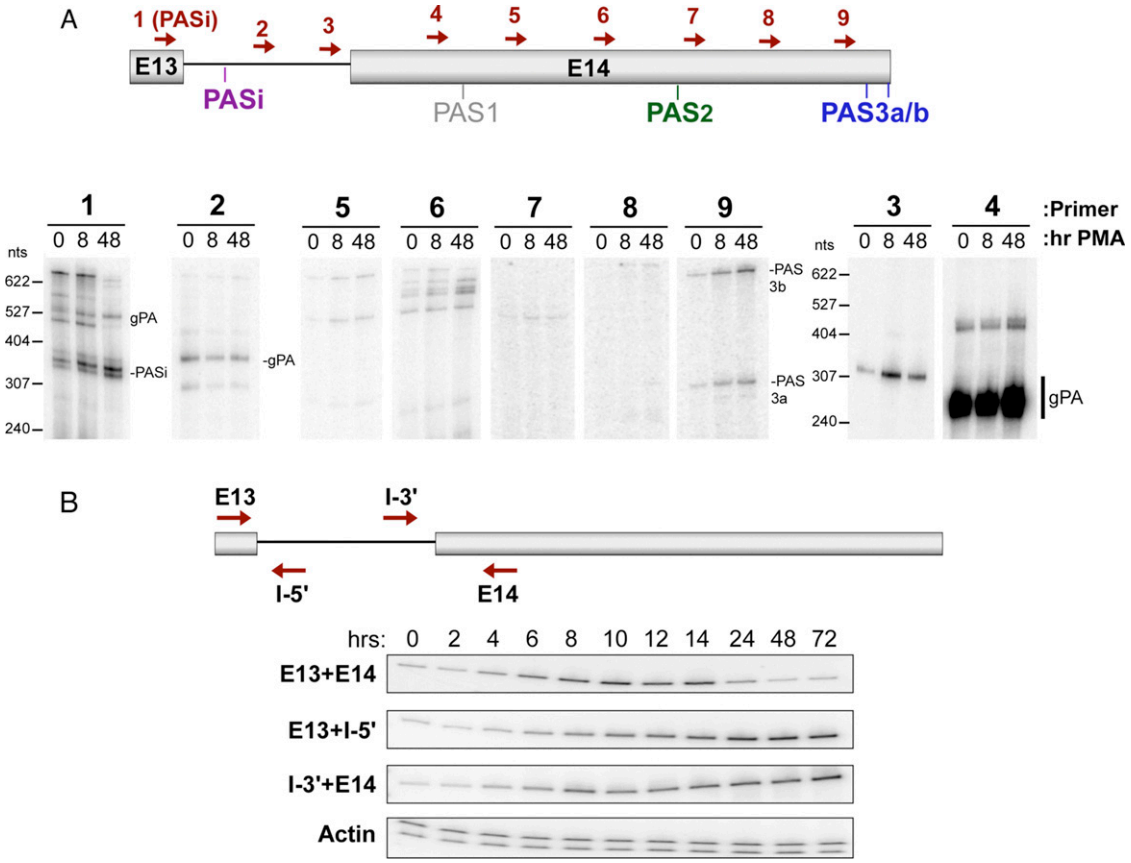

Fig. S5. Additional analysis of CELF2 3′UTR. (*A*) 3′ RACE using forward primers indicated on the schematic (*Upper*) to identify sites of cleavage/PAS along the entire 3′UTR of CELF2. Products derived from confirmed genuine polyadenylation sites are noted with a "PAS" label. Note that none of these primers are position to detect PAS2. Products that correspond to annealing of the reverse 3′ RACE primer to genomically encoded stretches of adenosines are noted by a "gPA." The prominent band observed with primer 4 could either be PAS1 or an extensive gPA; however, because we have no evidence for use of PAS1 in the Northern blot (Fig. 4*C*), we assign this as a gPA. Multiple faint bands detected with primers 5 and 6 most likely correspond to gPA, as no potential PAS is within 1 kb. (*B*) RT-PCR showing an increase in the retention of intron 13–14 in response to stimulation with PMA. Primers I-5′ and I-3′ interrogate the presence of RNA contain the 5′ and 3′ end of the intron retained with the adjoining exon. Primers Ex13–E14 interrogate the junction of exon 13 to exon 14 with the intron spliced out. The length of the intron precludes amplification from exon 13 to exon 14 across the retained intron. Actin is a loading control.

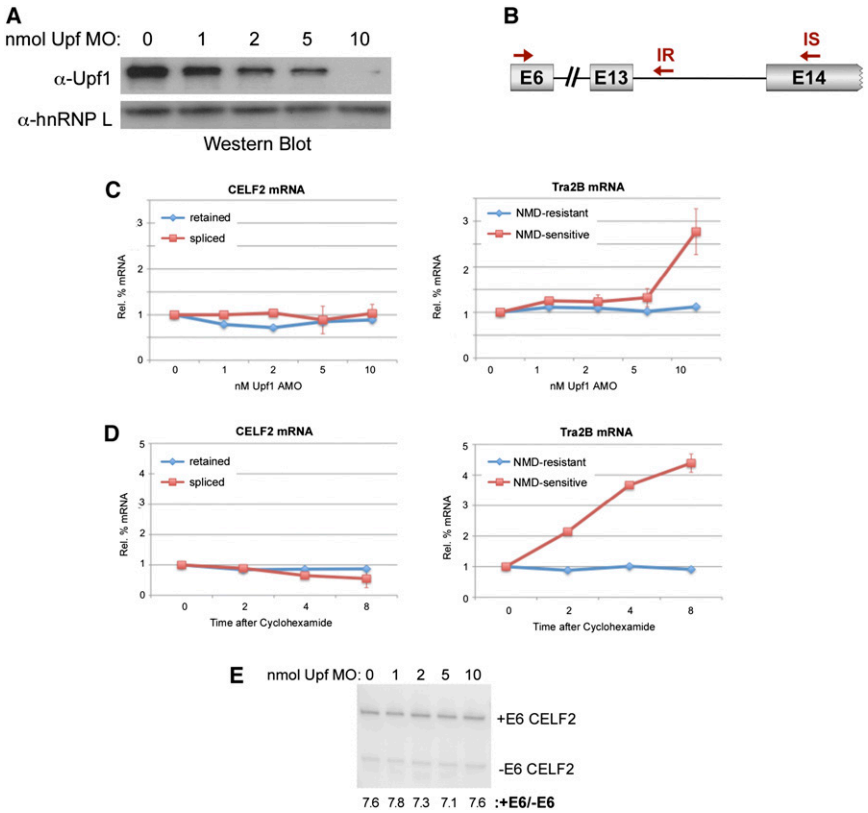

Fig. S6. Splicing of CELF2 3′UTR does not target message for NMD. (*A*) Western blot of Upf1 depletion by transfection with an antisense morpholino oligonucleotide (AMO). hnRNP L is used as a loading control. (*B*) Primers used to detect intron 13 retained (IR) and intron 13 spliced (IS) forms of CELF2. Upstream primer is specific for exon 6 to avoid potential ambiguity because of exon 6 skipping, although this does not appear to induce NMD (see *E*). (*C*, *Left*) Quantification of CELF2 messages with the intron retained (blue) and spliced (red) versions of the 3′UTR measured 60 h after Upf1 knock-down as in *A*. (*Right*) Parallel analysis of the NMD-resistant (blue) and NMD-sensitive (red) versions of Tra2B mRNA, used as a positive control for inhibition of NMD. (*D*, *Left*) Quantification of CELF2 messages with the intron retained (blue) and spliced (red) versions of the 3′UTR measured at indicated times after treatment with cycloheximide. (*Right*) Parallel analysis of the NMD-resistant (blue) and NMD-sensitive (red) versions of Tra2B mRNA, used as a positive control for inhibition of NMD. (*E*) RT-PCR with primers to exon 5 and 7 show no difference in exon 6 skipped product upon Upf1 KD, indicating that this splicing event does not trigger significant NMD in Jurkat cells.

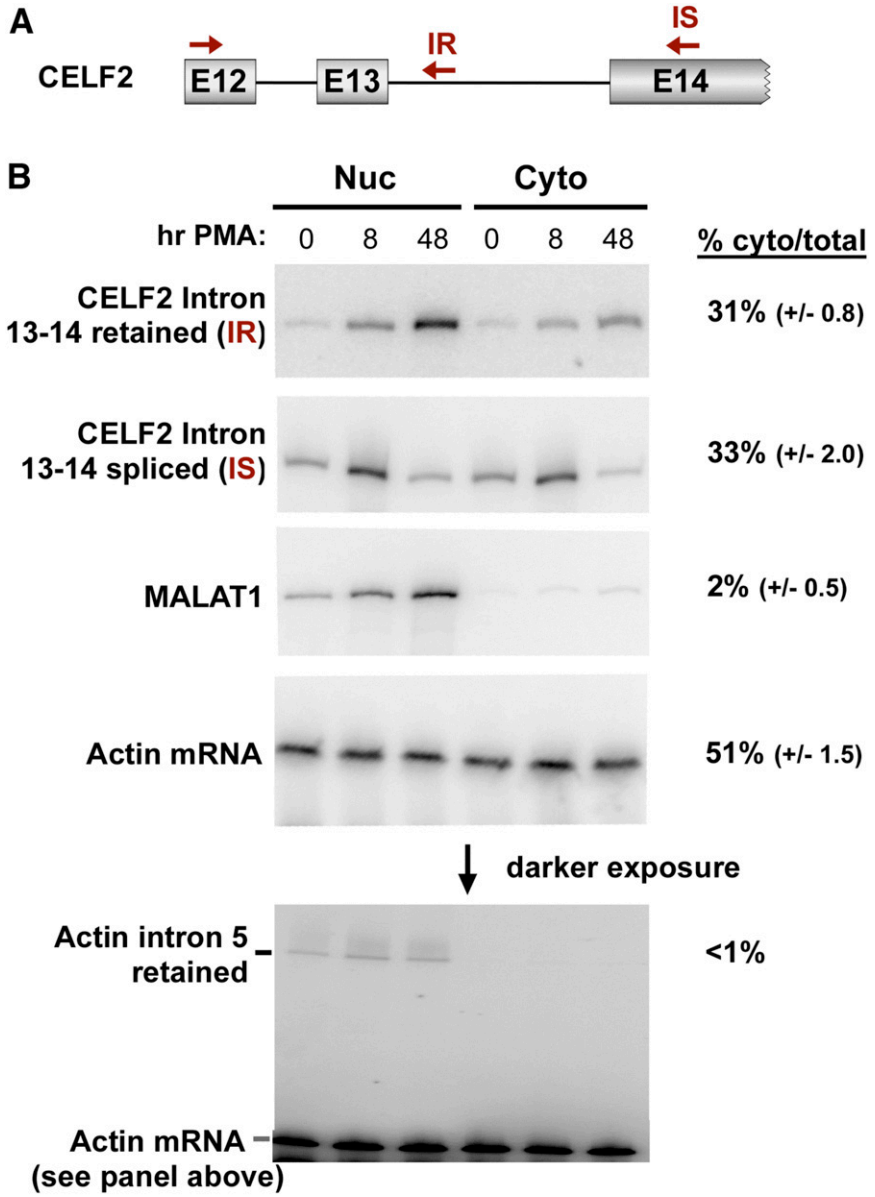

Fig. S7. CELF2 mRNA export to cytoplasm is independent of whether 3′UTR is spliced or retained. (*A*) Schematic of primers used to detect the intron retained (IR) and intron spliced (IS) versions of the CELF2 3′UTR. (*B*) RT-PCR analysis of indicated RNAs in nuclear and cytoplasmic pools. Purity of nucleo-cytoplasm was assessed by MALAT1, a strongly retained nuclear RNA (*Upper*). Strong retention of incompletely spliced versions of actin was also observed (*Lower*). In contrast, both the IR and IS versions of the CELF2 mRNA are observed in the cytoplasm with similar efficiencies, indicative that retention of intron 13–14 does not induce nuclear retention. Actin mRNA is used as a control of another mRNA efficiently exported from the nucleus. Percent cytoplasmic message was calculated at (cyto)/(ctyo+nuclear) from replicate experiments. The variation in the data is shown in parentheses.

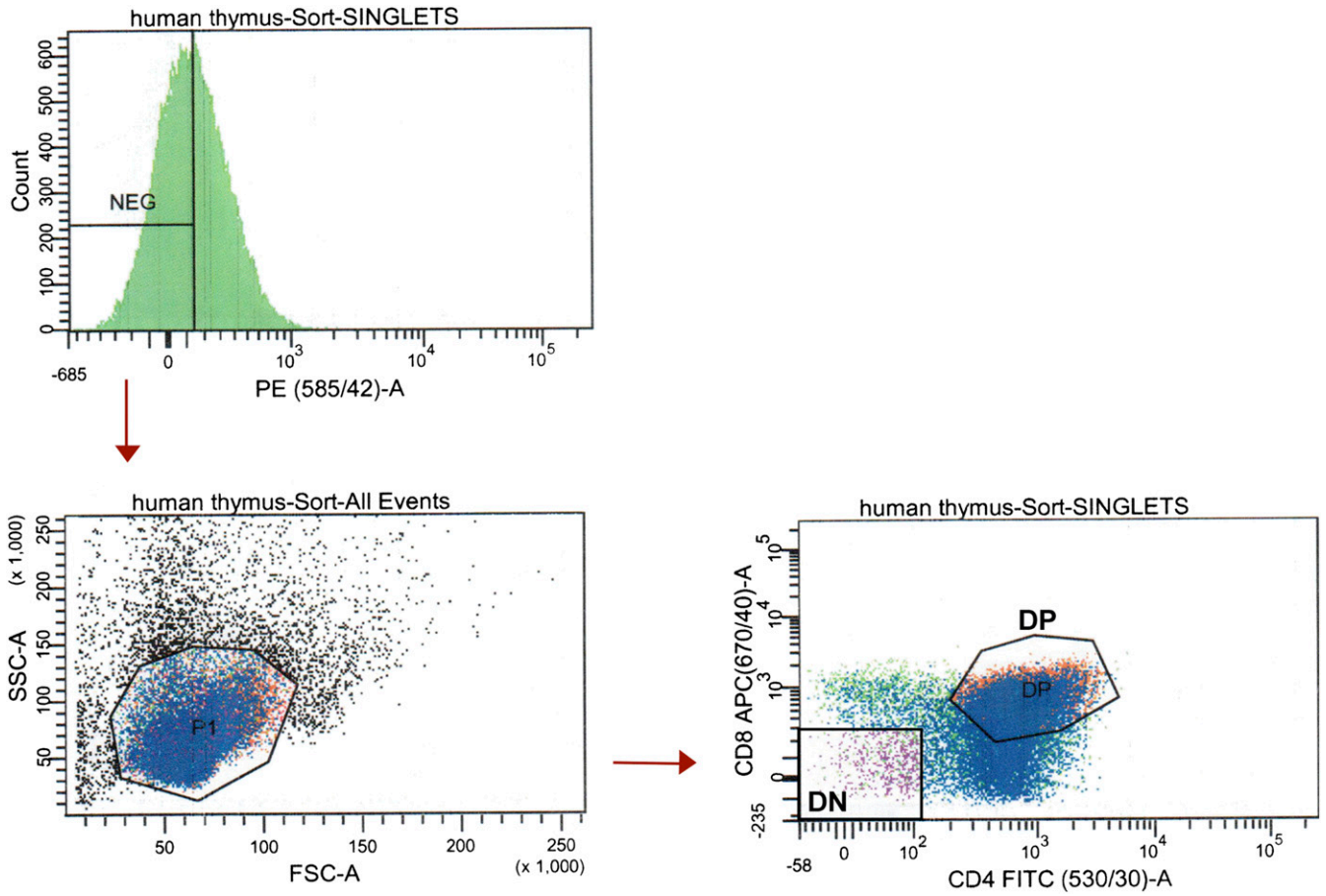

Fig. S8. Isolation of CD4/CD8 DP and DN thymocytes. Representative FACS plots of gating to isolate DP and DN thymocytes. Single-cell thymocytes suspensions were stained with anti–CD8-APC, anti–CD4-FITC, and a cocktail of antibodies to deplete nonthymocytes that were labeled with PE. Cells were first gated for PE^{-} (*Upper Left*), then by forward scatter/side scatter for live cells (*Lower Left*), and finally DN and DP cells were collected based on the gates shown (*Bottom Right*). Typical yields were $\sim 2 \times 10^6$ DP and 5×10^5 DN cells.

Table S1. Primers used for indicated experiments

Experiment	Forward	Reverse
RT-PCR		
rCELF2	TCTGCTCGACAGCAGCACGCAGTG	ATTGTGCAGTGCATTCTGGGC
eCELF2	GGATTACAAGGATGACGACG	ATTGTGCAGTGCATTCTGGGC
Actin	CCCTGGCATTGCCGACAGG	GCCGATCCACACGGAGTACT
APP	CGTGGAGCTCCTTCCCGTGAATGG	CCCACCATGAGTCCAATGATTGCACC
CTTN	CGCCGTTGGCTTTGAGTATCAAGGC	CTTATCCATCCGATCCTTCTGCACC
FUS	GGCAGCGGTGGCTATGGACAGC	CGGAGTCATGACGTGATCCTTGG
MRPL42	CCAAAATTCGGGCGTACATGTGCAG	GTGTGTTCATATGGAATGTCCACAGAAGGG
NAB2	GGAGAACAGAGTCACCCTGAAATCC	GAGGTCCTGGGTCTGAAGACACC
OPA1	GTTCTCCGGAAGAAACGGCGTTTAG	TGATGAATGCCTTTGTCATCTTTCTGC
PPP1R12A	CCAAGCACCACATCAACACCAACAG	CTGTGTTGATCTTCTAGATTGTCTTGC
SRPK2	F1:GTCGTCCTCTTCAGAAAGGCCGGAG	TTGCAGTAGTCCGCAGGGTCCTCTTGC
	F2:CCGGAAAGTGCTGGCCATTCAGGC	
Tra2B	GCCTCCTTAAGGAAGGTGCAAGAGG	R1:GTCAAATGACGACTTCCGCATTTTCC
		R2:AGCGAGACCGTGACCGGGTATAATGC
CELF2-E5/7	GCAGATAGTGAAAAGTCCAACGCTGTGG	CCCAGGTGGCAGTGTTGAGCTGCTGC
CELF2-E6	TGCATCAGTCTCAGACCATGGAG	
CELF2-E12	CACAGGAATTTGGAGACCAGG	
CELF2-I13 (IR)		AAGTTGGCAATGTGGTCCTCCTCTGC
CELF2-E14 (IS)		GCTAGGCAAACGATGAACTAACGGGC
MALAT1	AGATTTCCCAAGCAGACAGC	ACCGCACAGCTCGGGCGAG
qPCR		
eCELF2	CCAGGGTAGGGCTGATAAGG	TGAGTGATCCAAAGCTCCGT
Actin	CCCTGGCATTGCCGACAGG	GCCGATCCACACGGAGTACT
3′ RACE		
1F (PASi)	AGGCCTCTCGAGCCTAACCCCAGAGGCTCCCTGC	
2F	AGGCCTCTCGAGCATAAATACATAAATAAAAAAGAAAGCCACAGGC	
3F	AGGCCTCTCGAGGATTTTGTTGCCGCTGCATAGATTCTGTG	
4F	AGGCCTCTCGAGGTTGGTGCTTCTGTGAATTAAGTTGTGG	
5F	AGGCCTCTCGAGCCCTGCATCTATCCTCTAAGTTGTTTCGG	
6F	AGGCCTCTCGAGGGTTATAGTTGGCTTGTGCTACTCTGG	
7F	AGGCCTCTCGAGCGATTCCACTTAAGTGAATATCTGATTTGG	
8F	AGGCCTCTCGAGGGAGGTAAAGTTTCTCACACTCAAGTCG	
9F	AGGCCTCTCGAGGGCTAAGGGTGTGATTTCTTTTTATTAGG	
PAS2	GGCTTGATTTCTTTTTTCCCTTTGCTTATATCTAGC	
PAS3	GCACTTGCTATCAGGAAACAATACTGG	

Dataset S1. Complete RASL-Seq data PMA and CELF2 knock-down

Dataset S1

Dataset S2. RASL-Seq data for ~200 PMA-responsive genes

Dataset S2

Dataset S3. RASL-Seq data for thymocytes

Dataset S3