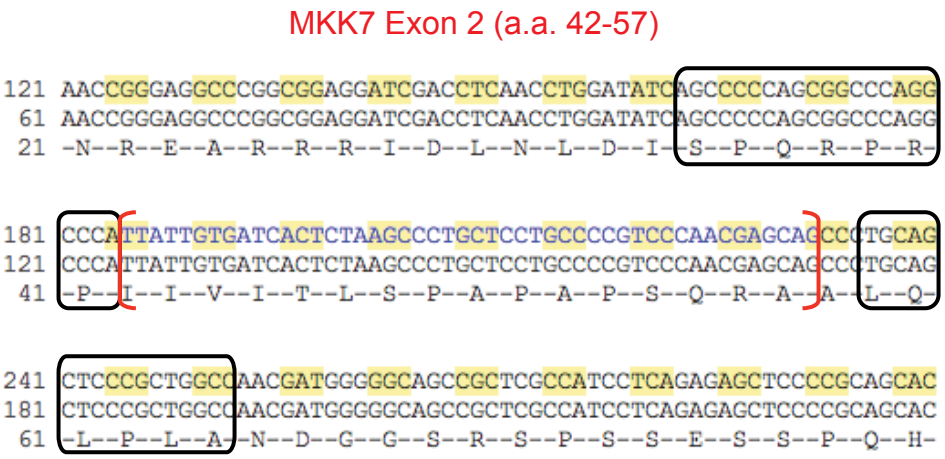
A



B

MAPK docking site consensus: $(R/K)_{3\text{-}5}(X)_{1\text{-}5}(\Phi\text{-}X\text{-}\Phi)$

Basic Hydrophobic

C

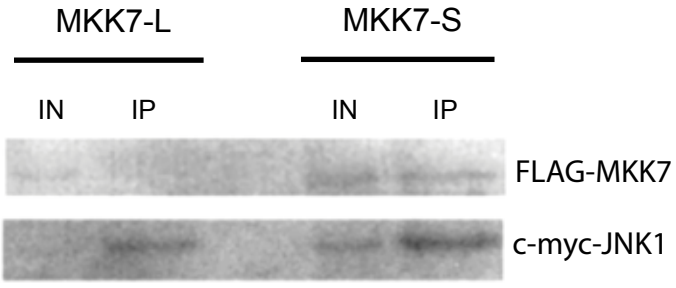


Figure S1. Details of MKK7 docking site. (A) The nucleotide and encoded protein sequence of MKK7 Exon 2 (red brackets) and two halves of the second docking site (black boxes). (B) Consensus sequence of MAPK docking sites. (C) Western blot of FLAG-tagged MKK7-L and MKK7-S co-associated with c-myc-tagged JNK1 following immunoprecipitation with anti-c-myc from lysates of co-transfected HEK293 cells. Lysate input (IN) and precipitated material (IP) are both shown.

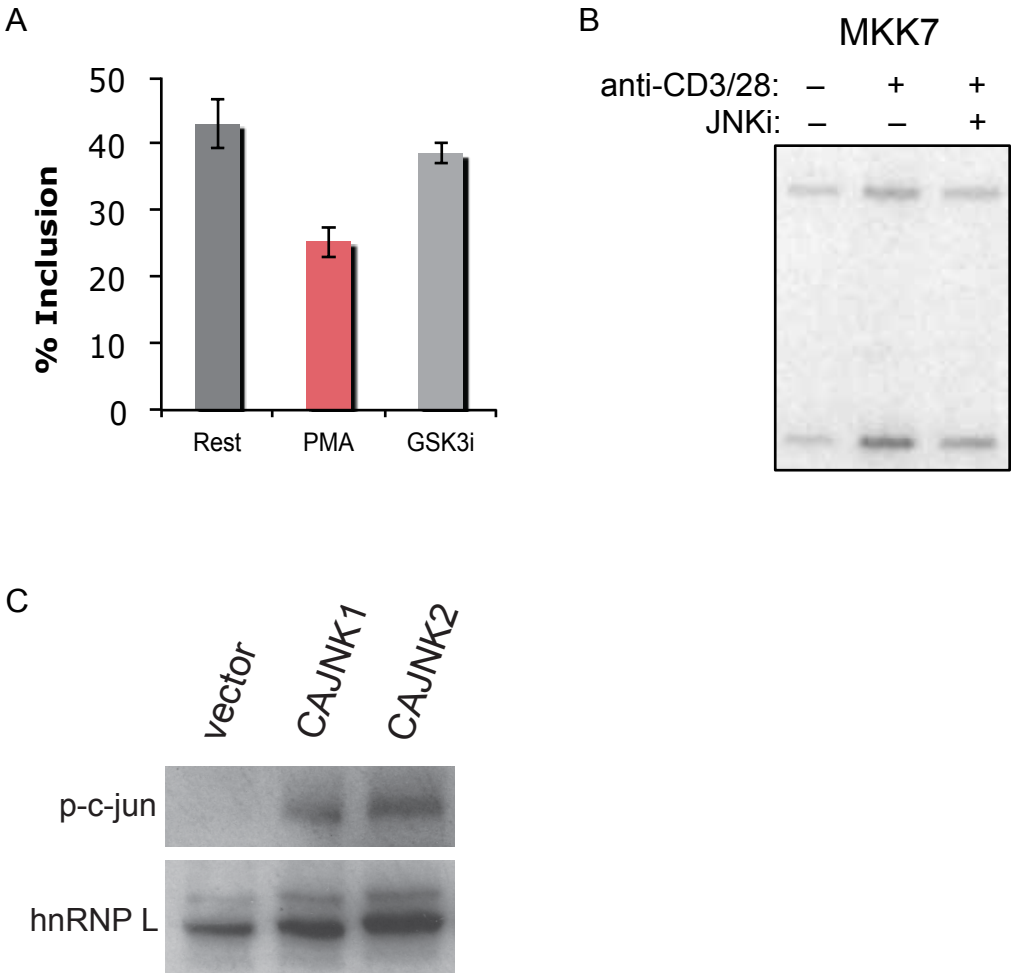


Figure S2. Further data on signaling requirements for MKK7 alternative splicing. (A) Inclusion of MKK7 exon 2 in Jurkat cells left unstimulated (resting), stimulated with PMA, which inhibits GSK3, or in which GSK3 is directly inhibited with the small molecule SB216763. (B) Representative RT-PCR gel of splicing of MKK7 in human CD4+ T cells treated with (+) and without (-) anti-CD3/CD28 stimulating antibodies in the absence (-) or presence (+) of the JNK inhibitor SP600125. (C) Western blot of phospho-c-Jun, as a marker for activity of CAJNK1 and CAJNK2 in transfections shown in Figure 3E.

A

MKK7 sequences contained in MKK7 minigenes

CCTCCTCGTTTATGATTTGATTTCTTTTCTTTTGGACGAATCGGTCGTTT **Up-intron**
CTGTTGTGATTTATCGTGGTGTTGTTTTTTTCTTCCTTTTCCCCATCCAG
TTATTGTGATCACTCTAAGCCCTGCTCCTGCCCCGTCCCAACGAGCAG **Exon2**
GTACCAGCCTTTTTATCATCGCTGCTTGGACATCTGCACCATTGACCTA
ACCGCTGCCCCGGCCGCAGAATGGCGTCC **Down-intron**

B

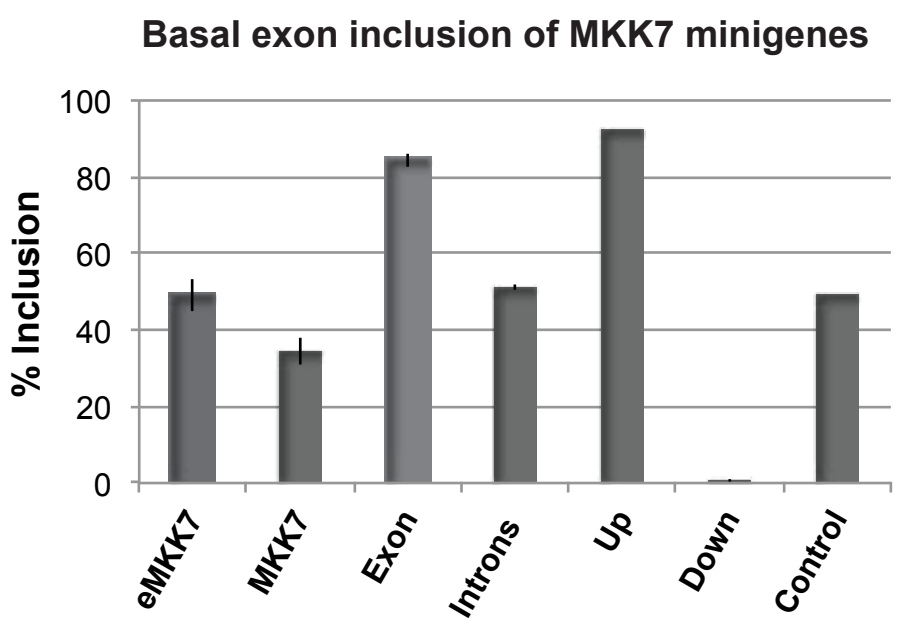


Figure S3. MKK7 sequences and minigene information. (A) Sequences of endogenous MKK7 that are included in the various minigenes. In black are the sequences of the upstream ~100 nucleotides and the downstream ~80 nucleotides introns flanking exon 2. In red are is the sequence of MKK7 exon 2. (B) Graph of average percent basal exon inclusion of MKK7 minigenes as determined by RT-PCR, n=3 and error bars represent standard deviation.

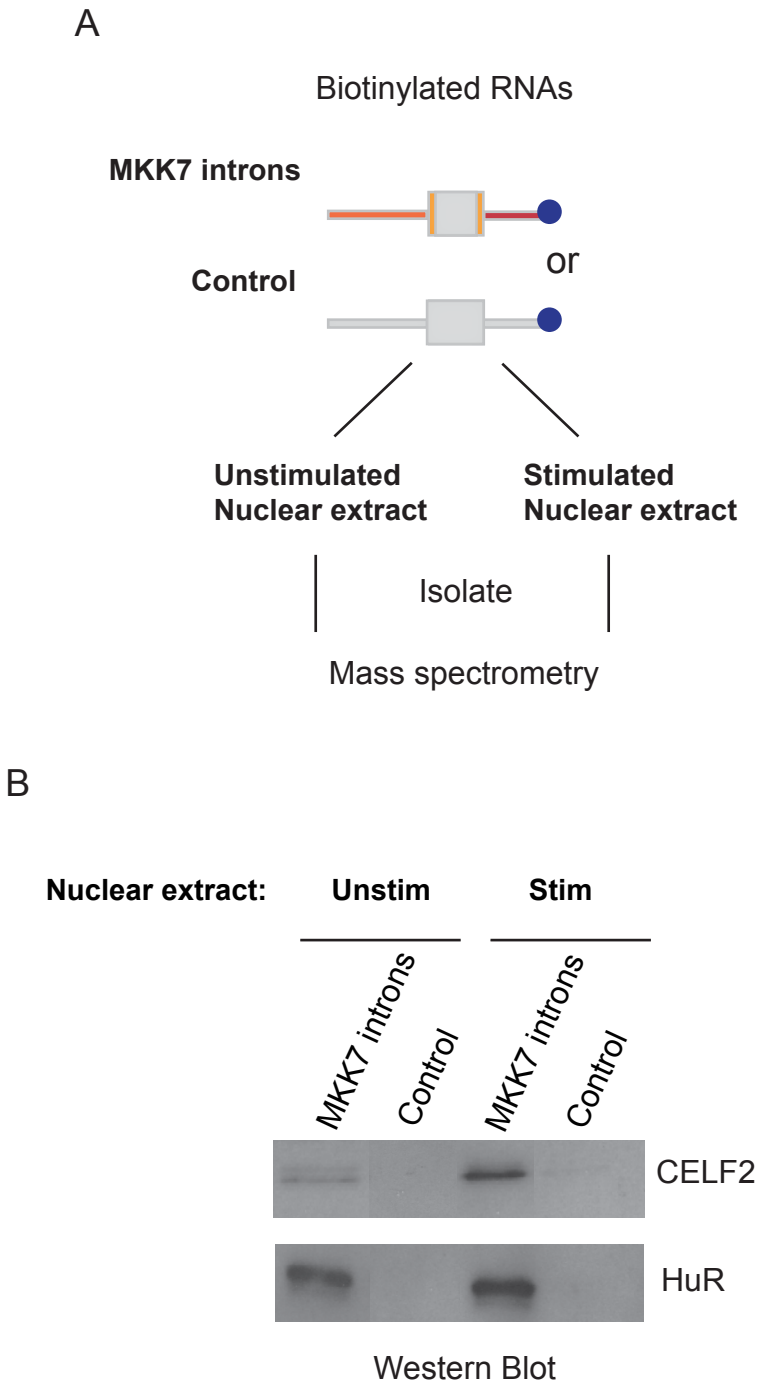


Figure S4. RNA affinity purification of MKK7 introns. (A) Schematic of RNA affinity purification experiment. RNA containing the MKK7 introns or the control RNA (from Fig 4A) were biotinylated, incubated with unstimulated or PMA stimulated nuclear extracts, and isolated by streptavidin-agarose. Following washing the entire reactions were submitted for mass spectrometry. (B) Western blot of CELF2 and HuR from RNA affinity purification of the MKK7 introns. See also Table S1.

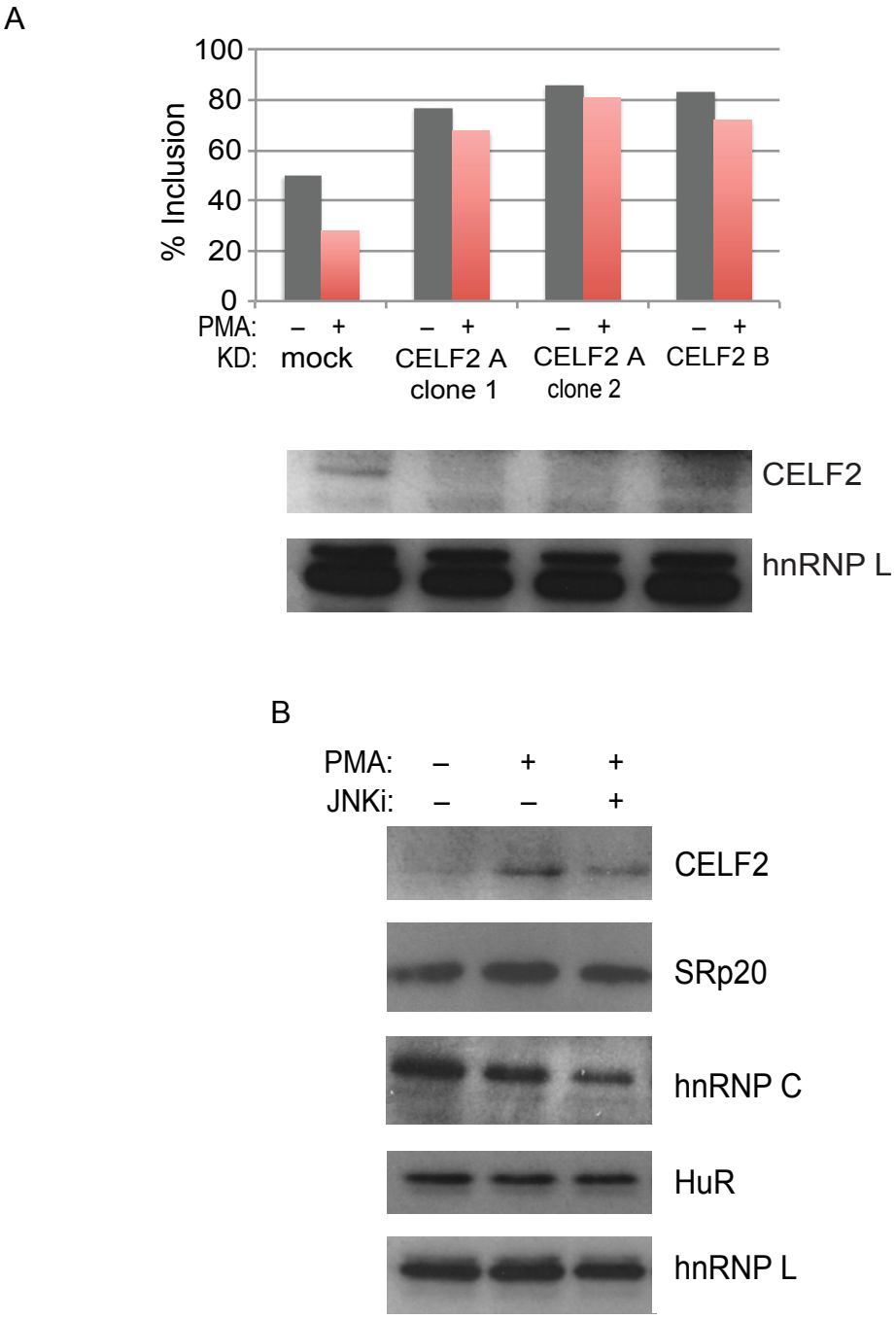


Figure S5. CELF2 protein expression. (A) RT-PCR analysis of independent Jurkat T lines depleted of CELF2 with two distinct shCELF2 targeting sequences CELF2 A and CELF2 B. The averages from these two targeting sequences are presented in Figure 5D. (B) Representative western blot analysis of total expression of proteins that bind MKK7 introns in Jurkat T cells that have been left unstimulated, activated with PMA or activated in cells that have been pre-treated the JNK inhibitor SP600125. See also Figure 5F.

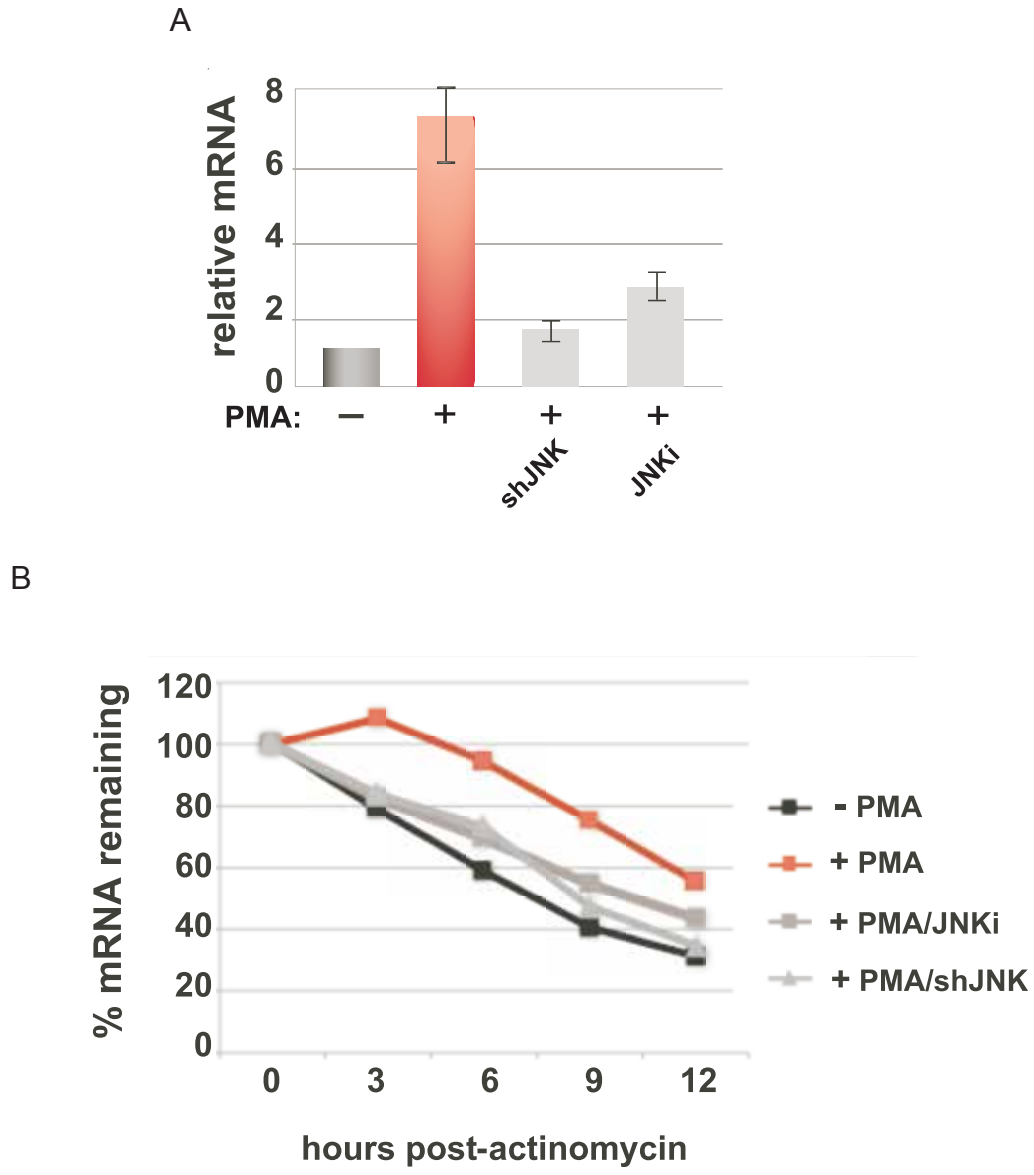


Figure S6. JNK signaling stabilizes CELF2 mRNA. Figure S6. (A) Quantification of total CELF2 mRNA normalized to actin in Jurkat T cells that have been left unstimulated, activated with PMA (48h) or activated in cells in which JNK is depleted (shJNK) or inhibited (JNKi, SP600125). Quantification was done by RT-PCR, n=3. (B) Quantification of CELF2 mRNA following actinomycin-induced inhibition of transcription in Jurkat T cells that have been left unstimulated, activated with PMA (48h) or activated in cells in which JNK is depleted (shJNK) or inhibited (JNKi, SP600125).

A

Gene Name	RASL-seq ΔPSI anti-CD3/28 (–/+ JNKi)	RT-PCR ΔPSI anti-CD3/28 (–/+ JNKi)	RT-PCR % Inc Rest	RT-PCR % Inc anti-CD3/28	RT-PCR % Inc anti-CD3/28 (–/+ JNKi)
CELF2	29	27	47	74	47
LEF1	-74	-19	98	79	98
LUC7L	-73	-62	52	5	67
FIP1L1	-45	-53	41	28	81
CCAR1	45	39	26	82	43
RNF34	29	16	12	21	5
ZDHHC20	-44	-33	20	10.5	44
CAMTA1	18	19	68	84	65
BAX	-43	-10	96	81	91
MTF2	-34	-19	68	59	79
PRPF3	-25	-21	99	77	98
RBM3	-55	-5.8	2	0.2	6
TRA2A	-79	-0.03	0.09	0.01	0.04
SMARCA4	-19	-1	9	6	7

B

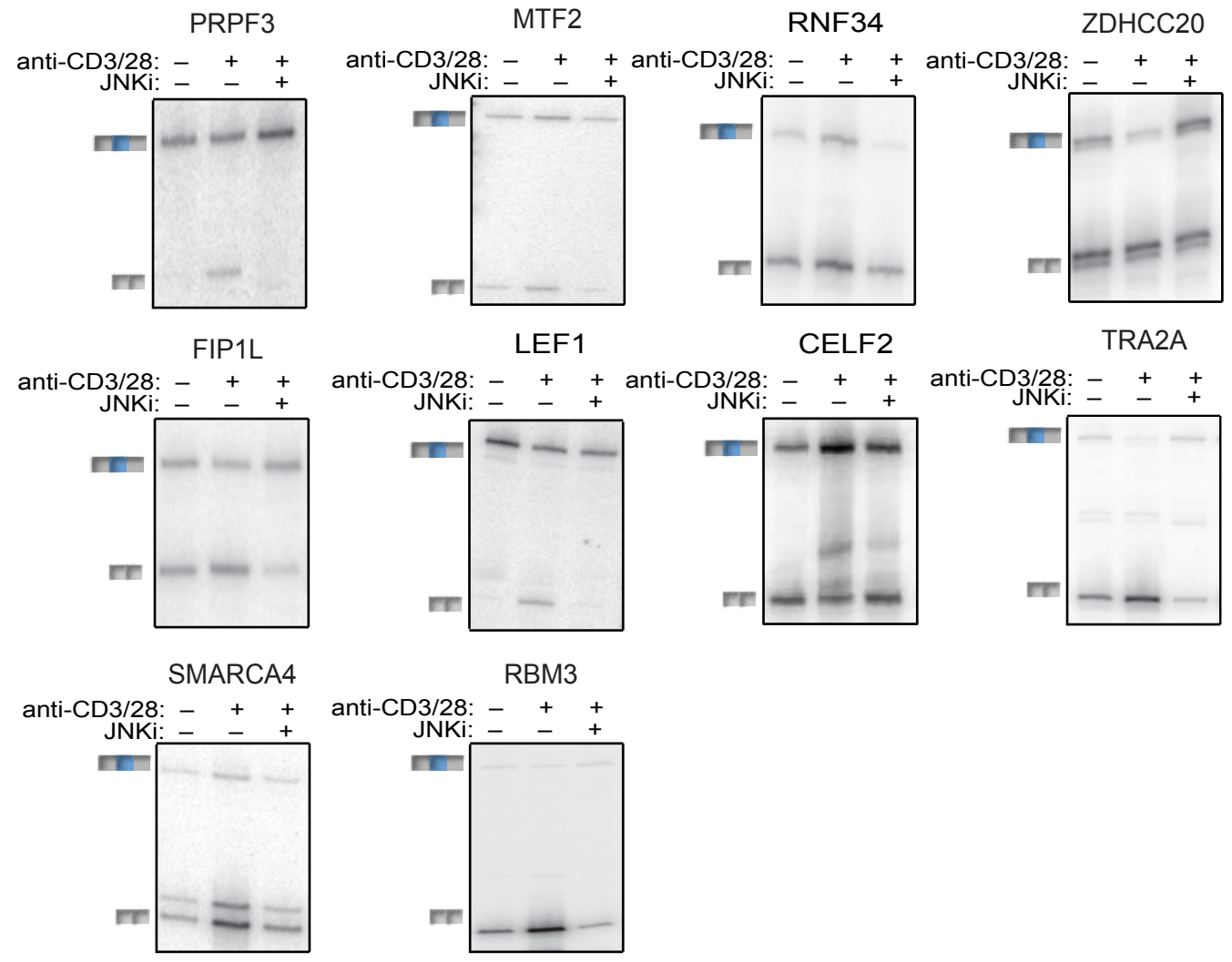


Figure S7. RASL-Seq and RT-PCR data on JNK-dependent splicing in primary human CD4+ T cells. Figure S7. RASL-Seq and RT-PCR data on JNK-dependent splicing in primary human CD4+ T cells. (A) Isoform expression change of 14 genes upon treatment of primary human CD4+ T cells with anti-CD3/CD28 in the presence or absence of the JNK inhibitor SP600125. Delta PSI values are calculated as the difference between percent expression of the longest isoform relative to total in cells treated with anti-CD3/CD28 alone minus that in cells treated with anti-CD3/CD28 in the presence of the JNK inhibitor. The absolute inclusion values calculated by RT-PCR are also given. (B) Representative RT-PCR gels for data shown in panel A. Final numbers presented in panel A are derived from triplicate RT-PCR experiments.

C

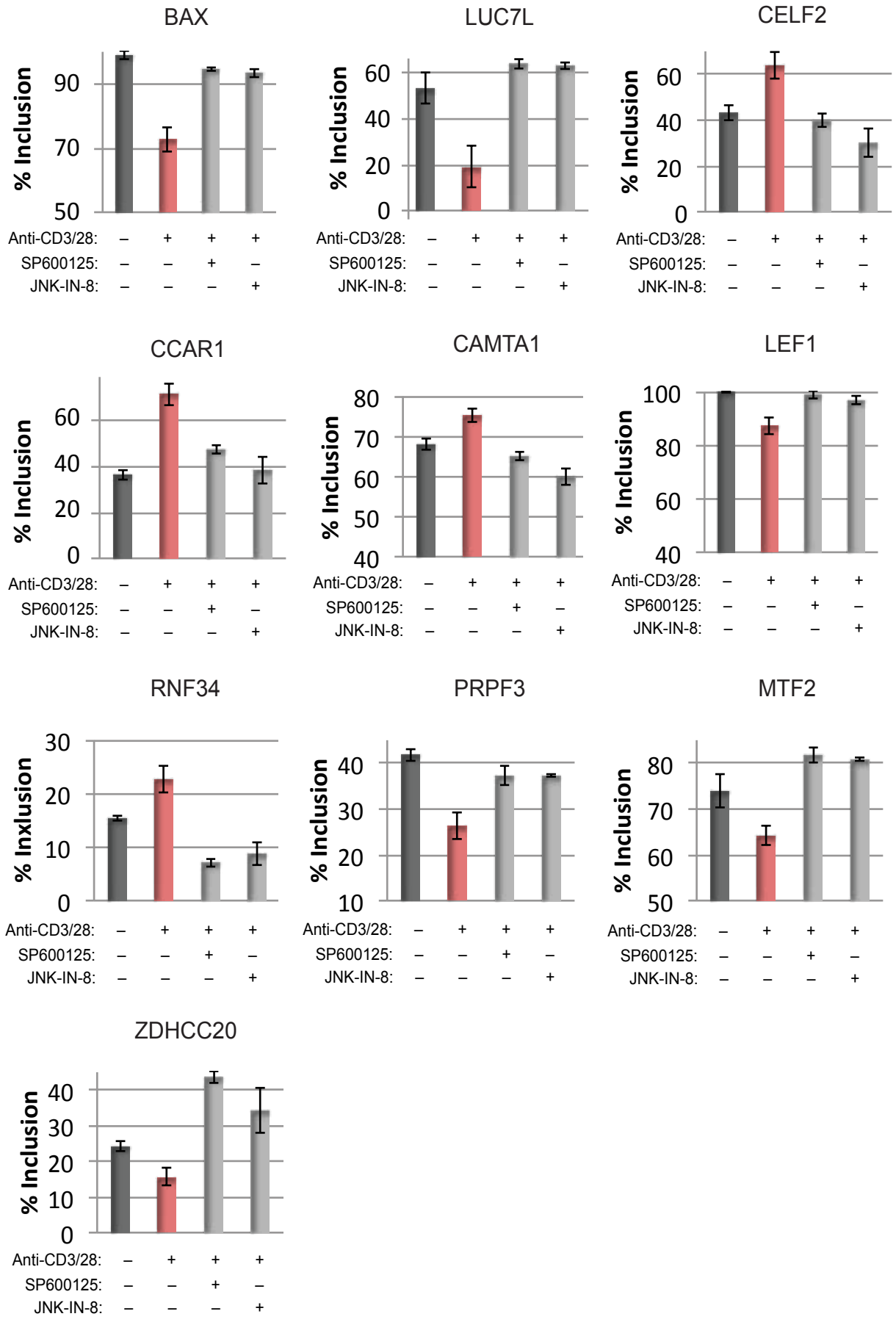


Figure S7. RASL-Seq and RT-PCR data on JNK-dependent splicing in primary human CD4+ T cells. (C) Graphs show the average percent inclusion and standard deviations from 3 primary human CD4+ T cell donors treated with anti-CD3/CD28 in the presence or absence of the JNK inhibitor SP600125 or JNK-IN-8**.**