**CD4+ T cell isolation and activation**

Human resting CD4+ T cells were isolated from peripheral blood monocytes using the Dynabeads Untouched Human CD4 T Cells kit (Invitrogen) as previously described ([Barski et al., 2007](#_ENREF_4)). CD4+ T cell activation was performed with the Dynabeads Human T-Activator CD3/CD28 for T-Cell Expansion and Activation kit (Invitrogen) by incubation at 37 °C for 18 hours. The cell morphology was checked under the microscope, and CD25, a cell surface marker, was used to confirm T cell activation by fluorescence assisted cell sorting (FACS). Mouse resting CD4+ T cells from LARP4 knock-out (KO, gift from Prof. Richard J. Maraia, NICHD, NIH) and wild-type (WT) mouse were isolated from mouse spleen using the Dynabeads Untouched mouse CD4 T Cells kit (Invitrogen) , followed by activation with CD3/CD28 antibody for a period of 18 hours.

**Bru-seq and BruChase-seq**

Bromouridine (Aldrich) was added to the media of resting and activated CD4+ T cell to a final concentration of 2 mM, and cells were incubated at 37 °C for 1h. Cells were then washed three times in PBS and either collected directly (nascent RNA, Bru-Seq) or chased in conditioned media containing 20 mM uridine for 30min or 2h at 37 °C (30min or 2-h-old RNA, BruChase-Seq). Total RNA was isolated by using TRIzol reagent (Invitrogen), and cytoplasmic RNA was isolated as described previously (Cell. 2012 July 20; 150(2): 279–290). Bru-labeled RNA was isolated from the total RNA or cytoplasmic RNA by incubation with anti-BrdU antibodies (BD Biosciences) conjugated to magnetic beads (Dynabeads, Goat anti-Mouse IgG; Invitrogen) under gentle agitation at room temperature for 1 h. For more detail, see the previously published paper (PNAS; Methods. 2014 May 1; 67(1): 45–54). 2 ul of 1:500 diluted ERCC RNA spike-in Mix was add to the equal mount of Isolated Bru-labeled RNA and then the mixture was used to prepare strand-specific DNA libraries by using the Illumina TruSeq Kit (Illumina) according to the manufacturer’s instructions. Sequencing of the cDNA libraries prepared from nascent RNA or 30min/2-h-old RNA was performed at the DNA Sequencing and Genomic Core, NHLBI, NIH by using the Illumina HiSeq. 3000 sequencer and the data sets used in this study are summarized in Table X.

**PA-seq**

Total RNA derived from human resting and activated CD4+ T cells. 10 μg of DNA-free total RNA was sheared into 200-300 nt fragments by heating (94°C for 3 minutes) with magnesium. Sheared RNA was precipitated by ethanol with GlycoBlue (Ambion) as a carrier. Reverse transcription was carried out using a modified oligo(dT) primer (5’ bio-TTTTTTTTTTTTTTTTdUT TTVN-3’, ’bio’ denotes duo biotin group, ‘dU’ stands for deoxyuricile, ‘V’ represents any nucleotide except T and ‘N’ denotes any nucleotide). Incubate the reverse transcription reaction at 42°C for 2 min before adding Superscript reverse transcriptase II (Invitrogen) to increase specificity. After second strand synthesis, Dynabeads MyOne C1 (Invitrogen) was used to pull down the resulting dsDNA. Incubate the beads with APex Heat-Labile Alkaline Phosphatase (Epicentre) to remove phosphate group, which enables strand specificity at the later PCR step since only the bottom strand cDNA can be ligated and thus amplified. To release dsDNA from MyOne beads, USER enzyme (NEB) was added. The released dsDNA was then end repaired, followed by adding an ‘A’ base at the ends. Illumina paired-end Y linker was ligated and size selected. A 16- cycle PCR was then carried out with Phusion Hot Start High-Fidelity DNA Polymerase (Finnzymes) to generate the final PA-seq libraries, which were sequenced at the DNA Sequencing and Genomic Core, NHLBI, NIH using Illumina HiSeq3000 platform.

**Calculation of intron retention index**

The intron retention index (IRI) of a gene is defined as the ratio of read density of shared intronic regions and that of shared exonic regions. The calculation is described in more details in our previous paper XX.

**genome-wide IRI**

To evaluate IRI genome wide, we define read density in intronic regions as the ratio of total number of intronic reads to the total length of intronic region. Similarly, read density in exonic regions is defined as the ratio of total number of intronic reads to the total length of intronic region. Genome-wide IRI is defined as the ratio of the read density in intronic region to the read density of in exonic region.

**IRI-high and IRI-low genes in resting T cell**

Expressed genes (RPKM>1) in resting T cell are ranked based on their IRI levels. IRI-high genes consist of top 1000 expressed genes with the highest IRI, while IRI-low genes consist of bottom 1000 expressed genes with the lowest IRI.

**Degradation model: beta1, beta2, overall beta, PST, nascent transcripts**

**Basic ingredients**

We used a first-degree model that directly connects RNA expression with transcription and degradation rates to fit the Bru-Seq and BruChase-Seq data. Formally, let α be the transcription rate (RNA/min\*cell), β the degradation rate (1/min), and X the expression level of a gene x (RNA/cell). The time evolution of X is determined by:

In BruChase-Seq, bromouridine is washed away after Bru-labeling so that we assume no labeled nascent transcripts are produced (). Meanwhile, we assume β is constant.

With the above assumptions, we can have an analytic solution for the differential equation:

Since expression level X is measured experimentally, we can use weighted least square fitting to estimate β for each gene:

**Model for IR-dependent RNA degradation**

Considering the effect of IR on transcriptional decay, we assume that the transcripts are a mixture of two types of RNAs: fully spliced and intron retained. They have potentially distinct degradation rates: β1 for fully spliced RNAs and β2 for intron retained RNAs. Letting X1 be the expression level of the fully spliced RNAs of a gene x and X2 the expression level of the intron retained RNAs of the gene x, we have:

They are associated with experimentally measured expression levels and IRI level as follows:

Similarly, we can use weighted least square fitting to estimate β1 and β2 for each gene with IR:

**overall beta, beta1 and beta2**

We use two alternative models to fit the degradation rate. The first model ignores the effect of IR and one single overall beta is estimated using equation (1). The alternative model assumes fully spliced transcripts and intron retained transcripts have different degradation rates, represented as beta1 and beta2 respectively. They can be estimated using equation (2). For the fully-spliced transcripts, differentially stabilized transcripts were selected using absolute beta1 difference ≥ 0.2 as a cutoff.

**PST**

Percentage spliced transcripts (PST) of gene is defined as the gene IRI level at 30 min in BruChase-Seq data. PST is used to approximate the splicing efficiency for a gene. For the fully-spliced transcripts, differentially splicing efficiency were selected using absolute percentage difference ≥ 5% as a cutoff.

**Nascent transcripts RPKM**

Nascent transcripts RPKM of a gene is defined as the RPKM of gene expression at 0 h in Bru-Seq data.

**Exon-exon / exon-intron junction reads**

We define a constitutive junction as an annotated splice junction that connects a shared intronic region and a shared exonic region. We consider a read to be exon-exon junction read if it covers the junction point and overlaps both sides for at least 8 bp. We consider a read to be exon-intron junction read if its coverage jumps over the junction point and overlaps the shared exonic region for at least 8 bp.

**Estimating beta1 and beta2 using junction reads**

Exon-exon junction reads (XEE) and exon-intron junction reads (XEI) can be used to approximate the fully spliced and intron retained transcripts respectively. By substituting into equation (2) we have:

**ERCC normalization**

ERCC RNA Spike-in molecules are a set of external RNAs with known concentrations that can only be found in bacteria. Equal amounts of ERCC are mixed with Bru-Seq and BruChase-Seq samples right before the sequencing step. The measured read counts of ERCC spike-in molecules can be used to normalize the RNA expression of different samples.

Specifically, among the 92 ERCC spike in added, the read count for ERCC spike in with high concentration (C > 10, 44 out of 92) were added up. ERCC normalization factor is defined as below:

Reads per kilobase per million (RPKM) was calculated for genes in each time point and was further normalized by multiplying the corresponding ERCC normalization factor.

**Heatmap**

Heatmap shows upregulated genes are associated with the change in polII, PST, and beta1 upon activation. Relative change in polII RPKM, PST, beta1, and gene expression RPKM in the activation process are illustrated. Red represents increase and blue represents decrease. Orange rectangles denotes genes with significant change in one specific column.

**RNA-Seq analysis**

Differentially expressed genes were selected by requiring > 1.5 expression change and reads per kilobase per million (RPKM) >1 in the state with higher expression.

**ChIP-Seq analysis for Pol II data**

ChIP-Seq analysis for our Pol II data is described in our previous paper XX. Pol II level of a gene is defined as the RPKM of polII reads in the gene body region. Differentially Pol II occupancy genes were identified by requiring > 1.5 polII level change and RPKM > 0.1 in the activated state.

**PA-Seq analysis**

PA-Seq libraries were mapped to genome using Bowtie. UCSC genes from the iGenome human hg19 assembly (http://support.illumina.com/sequencing/sequencing\_ software/igenome.html) were used for gene annotation. Adrian was used for peak calling and the peaks identified in resting and activated T cells are merged. Combined peaks that locate in gene 3’UTR region are used for downstream analysis.

Specifically, for each gene, the two peaks with the largest number of read counts are named as proximal and distant peak, according to their distance to the stop codon. PA index of gene is defined as the fraction of reads that are in distant peak.

**Method for Page33 Figure A…………..**

S6A. Distribution of PA index in both resting and activated states.

S6B. Distribution of change in PA index upon activation for 3 gene sets: beta1 decreased genes, beta1 increased genes, beta1 unchanged genes.

**Sequence alignment / RNA-Seq analysis**

The sequencing quality of all libraries was assessed by FastQC v0.10.1 (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). RNA-Seq libraries were mapped to genome using Tophat (v2.1.0)45. UCSC genes from the iGenome mouse mm9 assembly and human hg19 assembly (http://support.illumina.com/sequencing/sequencing\_ software/igenome.html) were used for gene annotation for mouse and human data respectively. Reads directionality (strandness of reads) are determined by RSeQC (<http://rseqc.sourceforge.net/)>. Only uniquely mapped reads were used for downstream analyses.

**Gene ontology analysis**

Gene ontology analysis was carried out using the goatools (https://github.com/tanghaibao/goatools) package. The enriched GO terms are identified by requiring FDR > 0.05 (FDR correction with Benjamini/Hochberg)

**Quantitative real-time PCR validation of the stability change for spliced transcripts**

We applied reverse transcription followed by quantitative polymerase chain reaction (qRT-PCR) to validate the stability change for spliced transcripts upon T cell activation. Primer pair in the upstream exon and downstream exon (intron between the two exons>1000bp) was used to detect the level of spliced transcripts. Primer pair in the upstream exon and downstream intron was used to reflect the intron retention signal. Primers were designed by Primer3 (version 0.4.0) and synthesized by IDT. Reverse transcription of 1 μg DNA-free total RNA was performed with SuperScript II reverse transcriptase (Invitrogen) in a 20 μl reaction, containing 1 x first strand synthesis buffer (Invitgrogen), 5 μM dNTP (Bioline), 0.5 μM oligo(dT) primer (5´-TTTTTTTTTTTTTTTTTTTTVN-3´; V=A/C/G and N=A/C/G/T), 10 mM DTT, 40 units of RNasin (Promega) and 200 units of SuperScript II Reverse Transcriptase (Invitrogen). RT reaction was incubated at 42°C for 2 min before adding reverse transcriptase. We then incubated the reaction at 42°C for 60 min and 75°C for 15 min.. Reverse transcription reaction for regular RT-PCR was also used for quantitative PCR (qPCR). The 20 μl qPCR reaction contains 0.5 μl RT reaction (cDNA template), 0.2 μM of the Forward primer and the Reverse primer and 10 μl 2 x SYBR Green Power Mix (ABI). The qPCR reaction was prepared in triplicates. Thermal cycling was carried out as follows: 95°C for 10 min; 40 cycles of 95°C for 15s, 52~58°C for 30s and 72°C for 30s. Annealing temperature was adjusted based on the gene-specific primers used. Melting curve module was included in the StepOnePlus Real-Time PCR system (Applied Biosystems). Primers for all the validated genes are listed in Table SX

**Chromatin Immunoprecipitation-qPCR validation of candidate genes**

Chromatin Immunoprecipitation (ChIP) coupled with qPCR assays were performed as described previously([Maunakea et al., 2013](#_ENREF_35)). Monoclonal anti-Pol II (ab76123, Abcam) antibody was used for immunoprecipitation. Primers fo*r NFKB1, PSMD7, IL2* and *STAT1* genes are listed in Table SX.

**Cross-Linking Immunoprecipitation-qPCR validation of RNA binding protein**

Cross-Linking Immunoprecipitation-qPCR (CLIP-qPCR) assays were performed as described previously (Methods Mol Biol. 2016 ; 1402: 11–17). Polyclonal anti-human LARP4 (A303-900A, Bethyl) antibody was used for immunoprecipitation. Primers for *NFKB1, PSMD7* and *CRKL* genes are listed in Table SX.

**Intracellular cytokine production in mouse resting and activated CD4+ T cells**

Supernatants of an intracellular cytokine assay of mouse resting and activated CD4+ T cells from the wild-type (WT) and LARP4 knock-out (KO) mouse were tested for 13 analytes (IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-13, IL-17A, IL-17F, IL-21, IL-22, IFNγ and TNFα) using the LEGENDplex™ Mouse Th Cytokine Panel (13-plex, BioLegend) according to the manufacturer’s instructions. Briefly, detection antibodies specific for the 13 analytes and 13 different fluorescence-encoded beads were added to the wells including serum (diluted two fold) or standard and then Incubated with shaking for 2 h at room temperature. Finally, streptavidin-PE was added, the samples were incubated for 30 min, and the beads were washed and analyzed by the flow cytometer.

**Statistical tests**

All statistical analyses were performed using python package Scipy (https://www.scipy.org/). One-sided Mann-Whitney U test was performed to test the significance of decrease in beta1, increase in PST, and elevation in nascent transcripts RPKM upon activation, respectively.

**LARP4**

eCLIP data in K562 cell line was downloaded from the ENCODE project (<https://www.encodeproject.org/)>. The binding strength of RBP in 3’ UTR region of a gene is estimated by summing the binding scores of all eCLIP peaks for that RBP that locates in the gene 3’UTR region. For each RBP, one-sided Mann-Whitney U test was performed to test the significance of difference between binding strength in beta1 decreased genes and in beta1 increased genes. LARP4 was identified as having the most distinct binding pattern between those two gene sets.

**ACCESSION NUMBERS:** Bru-seq, BruChase-seq, PA-seq andRNA-seq and ChIP-seq raw data can be found at the NCBI Sequence Read Archive upon manuscript acceptance (submission XXXXXXX, SRP058500). We also downloaded published datasets at the Gene Expression Omnibus with GEO number [GSE48138](http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE48138) and NCBI Sequence Read Archive with SRA number SRA000206.