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Supporting Online Material for

Regulation of CD45 Alternative Splicing by Heterogeneous Ribonucleoprotein, HnRNPLL

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Supporting Online Material

Materials and Methods

Virus production

By rearraying LKO.1 shRNA constructs supplied as glycerol stocks by the RNAi Consortium (TRC), Broad Institute (*1*), we generated a human lentiviral shRNA library directed against splicing regulators. Minipreps and virus production were performed as described in the TRC online protocols.

Cell Culture

JSL1 cells were maintained in 5% FCS, 1% L-glutamine RPMI. BJAB and BL41 cells were maintained in 10% FCS, 1% L-glutamine RPMI.

Cloning

HnRNPL and hnRNPLL cDNA (Open Biosystems) were cloned into an IRES-GFP containing lentiviral vector (gift from B. North) or pMSCV-puro FLAG (Clontech). The empty constructs and hnRNP encoding constructs were transfected into 293T cells along with VSV-G and gag/pol encoding constructs to produce virus.

Cell line Infection and Flow Cytometry

Screen: JSL1 cells were plated in 96 well round-bottom plates at 10,000 cells per well. 5 ul of virus plus 8 ug/ml polybrene was added per well and the plates were spun at 2250 rpm for 90 minutes. The supernatants were removed and fresh media was added. Puromycin was added at a final concentration of 5 ug/ml on day 2. PMA was added at a final concentration of 10 nM on day 8. Cells were stained with CD45RO-APC antibody (custom-made BD-Pharmingen) and fixed with 2% formaldehyde on day 10. Flow cytometry was performed on a FACScalibur (Becton Dickinson). A candidate gene was defined as a hit if two or more shRNAs increased or decreased CD45RO expression, in duplicate, by more than one standard deviation from the mean (Tables S1, S2). Most of the hits produced moderate variations from the mean on a log scale (Fig. S1a), but hnRNPLL knockdown had a striking effect (Fig. 1C).

Secondary: JSL1, BL41 and BJAB cells were infected with hnRNPLL or hnRNPL knockdown and expression viruses. $5x10^5$ cells were plated in 125 ul media plus 25 ul virus and a final concentration of 8 ug/ml polybrene in a 24 well plate. Cells were spun at 2250 rpm for 90 minutes and the media was replaced with fresh media. 5 ug/ml puromycin was added on day 2 and the cultures were expanded in puromycin containing medium. The cells were stained for cell-surface CD45 isoform expression approximately two weeks later with CD45RO-APC (custom-made BD-Pharmingen) or CD45RA-PE (Ebioscience) antibody and flow cytometry was performed on a FACScalibur.

Quantitative RT-PCR

RNA was isolated with the Qiagen RNeasy Mini Kit and reverse transcription was performed with SuperScript II (Invitrogen) according to the manufacturer's directions. PCR was performed in the presence of SYBR green reagent (Applied Biosystems) and amplification was performed on an iCycler (BioRad). Cycle thresholds were normalized to GAPDH levels and fold enrichments were set to the normalized uninfected or unstimulated value where applicable.

Western Blots

Cells were lysed in RIPA buffer (50mM Tris, pH 8, 150 mM NaCl, 1% NP40, 0.1% SDS, 0.5% sodium deoxycholate, protease inhibitors). 50 ug of protein was loaded for anti-hnRNPLL blots and 15 ug for anti-hnRNPL blots per lane on a 12.5% SDS-PAGE gel. Western Blot was performed with anti-hnRNPL (Aviva Biosystems), anti-hnRNPLL antibodies (Aviva Biosystems), or anti-p65 RelA (Santa Cruz) antibodies. Anti-RelA immunoblotting served as a loading control for the Western blots.

Immunoprecipitation

Cells were lysed in 1% NP-40 lysis buffer (150mM Tris, pH 7.4, 150 mM NaCl, 1% NP40, 10% glycerol, 2mM EDTA, 2mM EGTA, protease inhibitors). 500 ug of total lysate was loaded onto anti-Flag-M2 agarose beads (Sigma) in a 1 ml volume. The immunoprecipitations were incubated at 4°C overnight. After washing in 1xTBS, samples were eluted by boiling at 95°C for 5 minutes in 1x Laemmli loading buffer. The immunoprecipitated samples and 5% inputs were resolved on a 12.5% acrylamide gel. Western blot was performed with anti-hnRNPL antibody (Aviva Biosystems).

Primary Cell Isolation and Infection

Peripheral blood lymphocytes were purified by spinning through a Ficoll gradient (Sigma). Isolated cells were washed twice with PBS and naïve CD4+ T cells were isolated by negative depletion (Dynal). The cells were plated in the presence of 20 U/ml IL-2 at 20,000 per well in 96 well flat-bottom plates that had been pre-coated with 2.5 ug/ml anti-CD3/anti-CD28. After 24 hours in culture, 20 ul of concentrated virus was added and the cells were spin infected at 2250 rpm for 90 minutes. The virus was removed and fresh media plus 20 U/ml IL-2 was added. 2.5 ug/ml puromycin was added to the LKO.1 transduced wells 24 hours later. Fresh media plus IL-2 was added every 2-3 days. Triplicate wells were counted to assess proliferation. Surface receptor staining was performed with CD45RO-APC (custom-made BD-Pharmingen), CD45RA-FITC (Ebioscience), and CD25-PE (Ebioscience) antibody and flow cytometry was performed on a FACScalibur. IRES-GFP- hnRNPLL transduced cells were analyzed on day 5 post-isolation. Knockdown cells were analyzed on day 7 post-isolation.

RNA-Immunoprecipitation

Cells were lysed in 1% NP-40 lysis buffer (150mM Tris, pH 7.4, 150 mM NaCl, 1% NP40, 10% glycerol, 2mM EDTA, 2mM EGTA, protease inhibitors). 2.7 mg of total lysate was loaded onto protein-A sepharose beads coupled to anti-FLAG-M2 (Sigma) and the volume was adjusted to 1 ml in lysis buffer including RNase-Out (2.5 U/ml), VRC (420 uM) and 20 mM EDTA. The immunoprecipitation reactions were incubated at 4°C for four hours and then washed in 1ml of cold lysis buffer. After adding an equal volume of lysis buffer, the samples were eluted at 50°C for 30 minutes. The beads were resuspended in Tri-reagent (Ambion) and total RNA and protein were isolated by ethanol and acetone precipitation, respectively. These protein fractions as well as 5% inputs from the initial lysates were loaded onto a 12.5% acrylamide gel for Western blot analysis. The RNA fractions were resuspended in 10 ul of poly-A tailing reaction mix (Ambion) and reverse transcription was performed with a mixture of random hexamers and oligo-dT (Ambion). PCR was performed on an iCycler (Bio-Rad) in the presence of SYBR green (Applied Biosystems) with primers that encompass CD45 exon 4 ESS1 (F-5'-GCAAAGATGCCCAGTGTTCCACTT-3', R-5'-TTCTCTTTCAAAGGTGCTTGCGGG-3'). Since pre-mRNA splicing occurs co-transcriptionally (*2*) and exons 4-6 are excluded from mature

CD45 transcripts in hnRNPLL-expressing cells, we chose PCR primers that bind within exon 4 to ensure accurate detection of the predicted targets of hnRNPLL rather than choosing primers that span separate exons in the unspliced precursor or the excised region. Cycle thresholds were normalized to CD45 transcript expression in total RNA isolated from 5% of the input fraction. The resulting values plotted in Fig. 3 thus represent 1/20th the pool of total CD45 transcripts.

Exon Array Analysis

RNA was isolated from 1) cord blood CD4+ T cells, 2) cord blood cells that had been activated with anti-CD3/anti-CD28 for 24 hours, 3) activated CD4+ peripheral T cells, 4) peripheral T cells infected with an shRNA directed against hnRNPLL and 5) peripheral T cells infected with an shRNA directed against GFP (as described above) with Qiagen RNeasy columns. The RNA was hybridized to Affymetrix human 1.0 ST exon arrays and the resulting CEL files were analyzed with XRAY^{TM} software (Biotique Systems). Transcripts showing statistically-significant alternative exon expression ($p < 0.01$) without a large change in overall gene expression were identified. To reduce false positives, exons that fell below a threshold for hybridization were excluded from the analysis, leading to analysis of ~7000 genes in each of the pairwise comparisons.

Supporting Figures

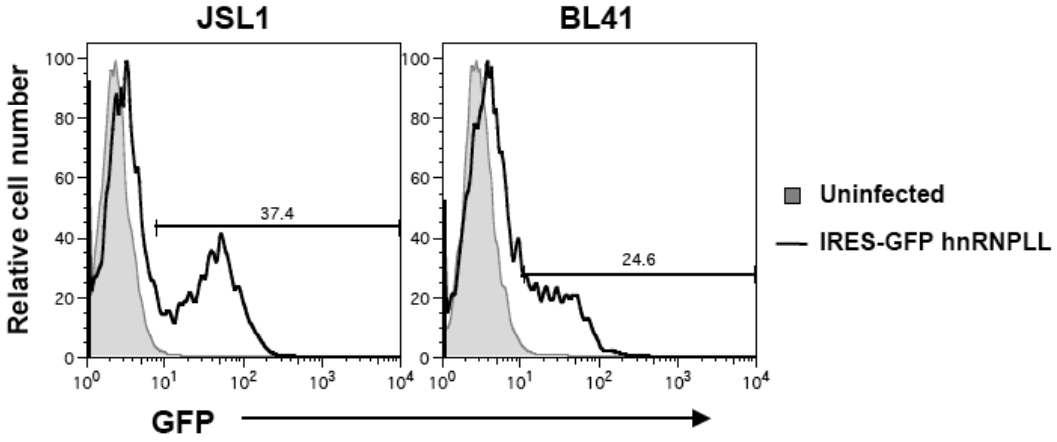

Fig. S1. JSL1 and BL41 cells were lentivirally transduced with a bicistronic vector encoding GFP and hnRNPLL. Transduction efficiency was determined by assessing GFP expression by flow cytometry. Approximately 35% of JSL1 and 25% of BL41 cells were successfully transduced.

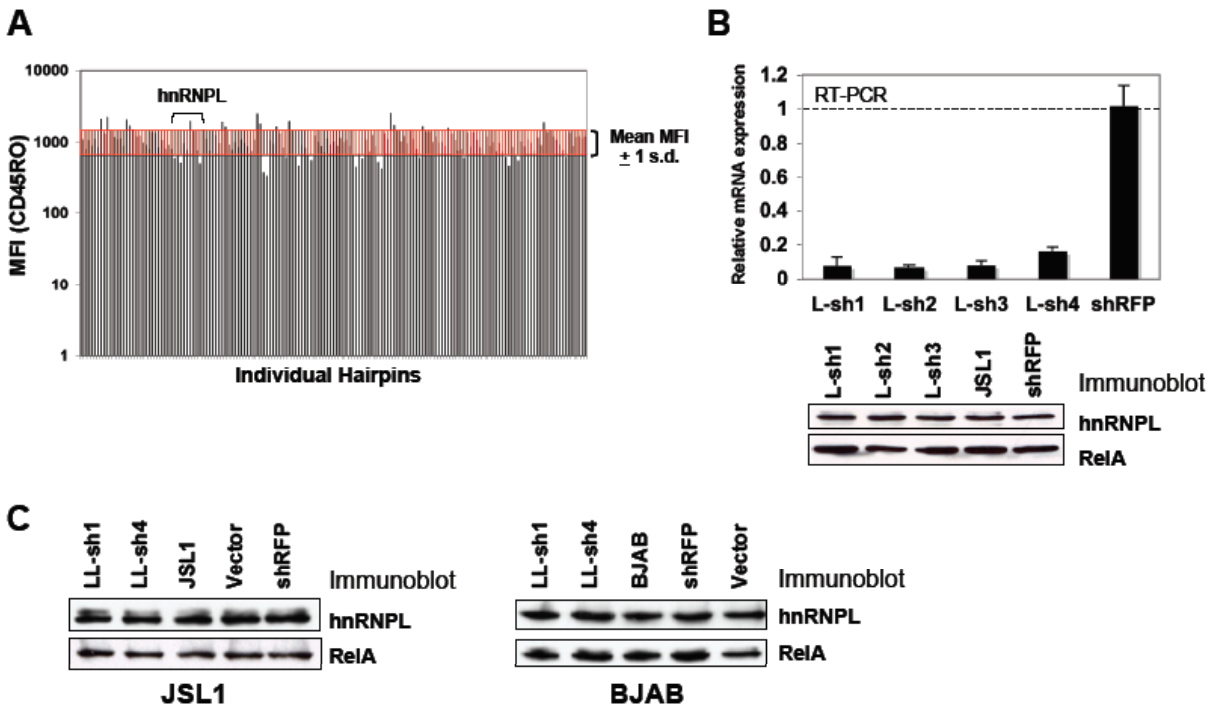

Fig. S2. HnRNPL knockdown was ineffective in JSL1 cells. **(A)** JSL1 cells were transduced with lentiviral shRNAs, PMA stimulated and stained for CD45RO cell surface expression in 96 well plate format. The mean fluorescence intensities (MFIs) of CD45RO expression for the individual hairpins in duplicate were plotted on a log scale. Data from a single 96-well plate are shown. One standard deviation from the mean is indicated in red. HnRNPL did not meet the criteria of being identified as a hit in our screen. **(B)** JSL1 cells stably expressing shRNAs directed against hnRNPL were produced. (top) Quantitative RT-PCR indicated that all four hairpins effectively knocked down hnRNPL transcripts. (bottom) Western blot with anti-hnRNPL antibody showed that in spite of reduced transcript expression, hnRNPL was not depleted at the protein level. **(C)** HnRNPL expression in JSL1 cells stably expressing shRNAs directed against hnRNP LL was determined by Western blot. HnRNPL expression was not altered by knockdown of hnRNPLL in JSL1 and BJAB cells.

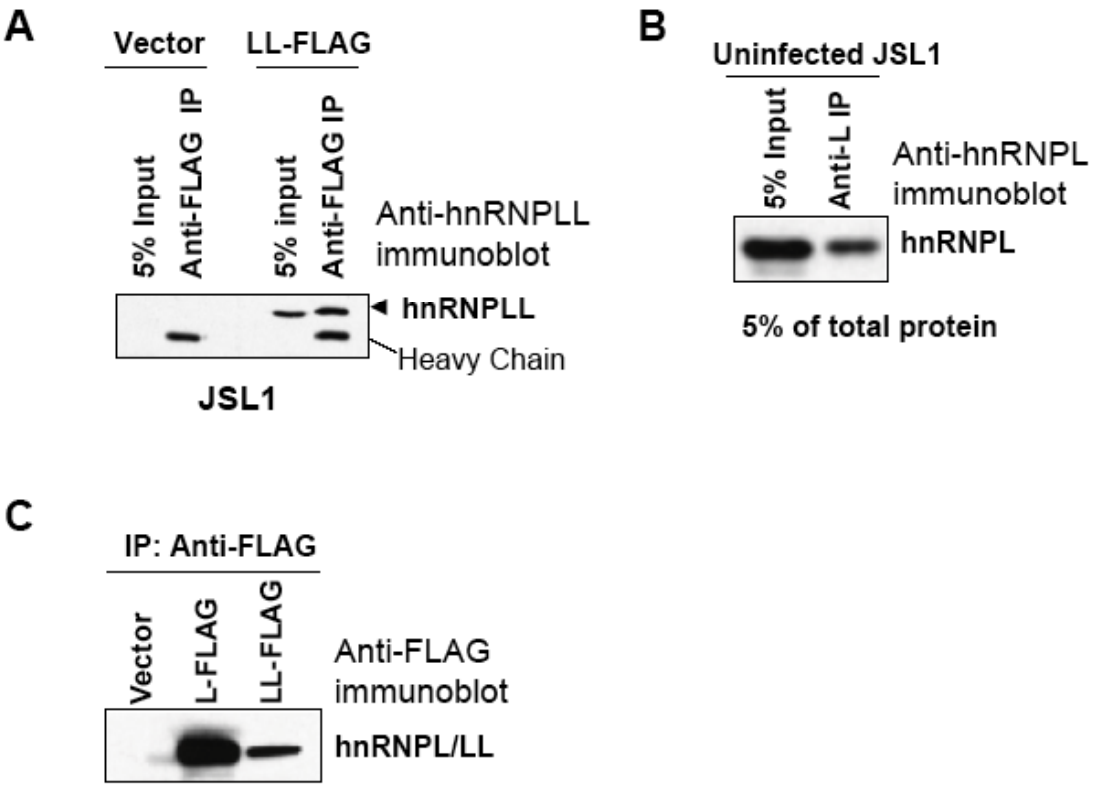

Fig. S3. Immunoprecipitation of hnRNPL and hnRNPLL from JSL1 cell lysates. **(A)** FLAG-tagged hnRNPLL (LL-FLAG) was immunoprecipitated from JSL1 cells with anti-FLAG antibody in the presence of RNase inhibitors. RNA was isolated for quantitative RT-PCR (Fig. 3E). Anti-hnRNPLL immunoblot of the protein fraction verified immunoprecipitation. HnRNPLL was detected in the 5% input and the immunoprecipitated fraction (IP) from JSL1 cells stably expressing LL-FLAG, but not in vector-transduced cells. **(B)** Endogenous hnRNP L was immunoprecipitated from JSL1 cells with anti-hnRNP L antibody in the presence of RNase inhibitors. RNA was isolated for quantitative RT-PCR (Fig. 3E). Anti-hnRNP L immunoblot of the protein fraction verified hnRNP L expression in the 5% inputs and in the immunoprecipitated sample. Given the high level of hnRNP L expression in the input and immunoprecipitated samples, only 5% of the total protein in each fraction was used for SDS-PAGE analysis. **(C)** FLAG-tagged hnRNPL (L-FLAG) or LL-FLAG were immunoprecipitated from stably expressing JSL1 cells. Western blot for FLAG protein indicated that L-FLAG was stably expressed at a higher level than LL-FLAG.

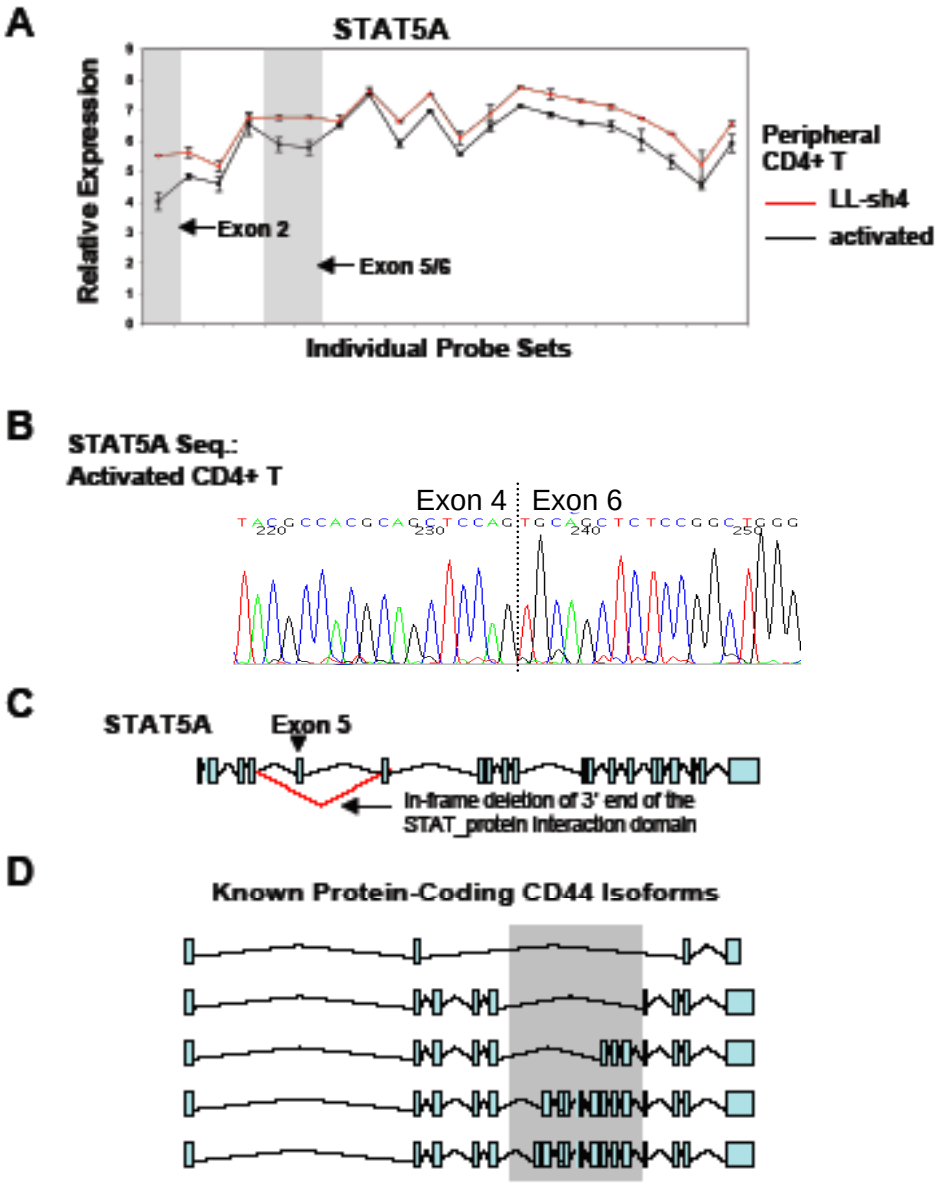

Fig. S4. Exon array isoform predictions. (A) Expression of STAT5A exons 2, 5 and 6 increases following hnRNPLL depletion (GEO Accession GSE11834). (B) Loss of STAT5A exon 5 was confirmed by RT-PCR and sequencing (direct splicing of exons 4 and 6). (C) Resulting transcripts would result in an in-frame deletion of the C-terminal part of the STAT_interaction domain. This domain is known to influence dimerization. (D) CD44 exons 6 through 14 undergo extensive alternative splicing to generate a number of protein coding isoforms.

Supporting Tables

Table S1. Hits with > 1 Standard Deviation Increase in CD45RO Expression (Negative Regulators)

Gene	Full Name/Function
EWSR1	Ewing Sarcoma Breakpoint Region 1
SF3B1	Splicing Factor, Component of U2
SRRM1	serine/arginine repetitive matrix 1
CPSF1	Cleavage and polyadenylation factor
SDCCAG10	serologically defined colon cancer antigen 10
PRPF4B	Precursor mRNA-processing factor 4
SNW1	SKI-interacting protein (SKIIP)
U2AF2	U2AF65
CRK7	Cdc2-related kinase with RS domain
FUBP1	Far Upstream Element-Binding Protein 1
VIM	intermediate filament subunit specific for mesenchymal cells
TPR	Translocated Promoter Region
ELAVL3	Embryonic Lethal, Abnormal Vision, Drosophila, Homolog-Like 3
NUMA1	Nuclear Mitotic Apparatus Protein 1
PRPF38B	Pre-mRNA processing factor
SNRPD2	Small Nuclear Ribonucleoprotein Polypeptide D2
BRUNOL5	Bruno-like 5, RNA binding protein
WBP11	WW domain-binding protein 11
MORG1	Mitogen-activated protein kinase organizer 1
PCBP2	Poly(rC)-binding protein 2 (Alpha-CP2) (hnRNP-E2)
PRPF3	PRP3 pre-mRNA processing factor 3 homolog
CDC5L	Cell division cycle 5-like protein
HNRPCL1	heterogeneous nuclear ribonucleoprotein C-like 1V
CTNNBL1	catenin, beta like 1

Table S2. Hits with >1 Standard Deviation Decrease in CD45RO Expression (Positive Regulators)

Gene	Full Name/Function
SFPQ	Splicing Factor, Proline- and Glutamine-rich
HNRPD	Heterogeneous Nuclear Ribonucleoprotein D
SAFB	Scaffold Attachment Factor B
EE1AF1	Eukaryotic Translation Elongation Factor 1
DDX1	DEAD/H box RNA helicase
FIP1L1	FIP1-Like 1, resembles FIP1- involved in poly-A synthesis
BUB3	Budding Uninhibited by Benzimidazoles 3

PABPC4	Poly(A) Binding Protein, Cytoplasmic 4
PRCC	Papillary renal cell carcinoma
HNRPLL	Heterogeneous nuclear ribonucleoprotein L-like

Table S3. Genes displaying significant alternative exon usage in response to hnRNP LL knockdown in peripheral CD4+ T cells, but not in response to infection with an shRNA directed against GFP, as assessed by exon array analysis ($p<0.01$).

Gene	Gene-Annotation	Alternative Exon Use (p-value)
ADAM15	ADAM metallopeptidase domain 15 (metargidin)	2.91E-15
PTPRC	protein tyrosine phosphatase receptor type C	3.73E-15
WAC	WW domain containing adaptor with coiled-coil	4.39E-09
IL4I1	interleukin 4 induced 1	9.99E-09
HIST1H2BJ	histone cluster 1 H2bj	6.63E-08
COL1A1	collagen type I alpha 1	1.93E-07
CD44	CD44 molecule (Indian blood group)	4.60E-07
RAD54B	RAD54 homolog B (S. cerevisiae)	7.65E-07
HIST1H1C	histone cluster 1 H1c	2.60E-06
STAT5A	signal transducer and activator of transcription 5A	3.28E-06
SCD	stearoyl-CoA desaturase (delta-9-desaturase)	3.87E-06
SNRPA1	small nuclear ribonucleoprotein polypeptide A'	4.66E-06
ARHGEF3	Rho guanine nucleotide exchange factor (GEF) 3	5.31E-06
ARHGEF18	rho/rac guanine nucleotide exchange factor (GEF) 18	6.68E-06
CD53	CD53 molecule	8.65E-06
REXO1	REX1 RNA exonuclease 1 homolog (S. cerevisiae)	2.70E-05
HNRPLL	heterogeneous nuclear ribonucleoprotein L-like	5.06E-05
SNORA18	small nucleolar RNA H/ACA box 18	5.70E-05
HIST1H2AC	histone cluster 1 H2ac	6.03E-05
CHID1	chitinase domain containing 1	1.01E-04
CFLAR	CASP8 and FADD-like apoptosis regulator	1.02E-04
TXNDC5	thioredoxin domain containing 5	1.28E-04
FASN	fatty acid synthase	1.39E-04
USP36	ubiquitin specific peptidase 36	1.48E-04
PDE4A	phosphodiesterase 4A cAMP-specific	2.10E-04
PARG	poly (ADP-ribose) glycohydrolase	2.14E-04
MKI67	antigen identified by monoclonal antibody Ki-67	2.33E-04
NAB1	NGFI-A binding protein 1 (EGR1 binding protein 1)	2.73E-04
POLQ	polymerase (DNA directed) theta	2.87E-04
SMG6	Smg-6 homolog nonsense mediated mRNA decay factor	2.94E-04
EIF3S7	eukaryotic translation initiation factor 3 subunit 7 zeta 66/67kDa	3.22E-04
CCDC125	coiled-coil domain containing 125	3.43E-04
DUSP4	dual specificity phosphatase 4	3.88E-04
MT1H	metallothionein 1H	4.06E-04
PXDNL	peroxidasin homolog (Drosophila)-like	4.11E-04
TBL1XR1	transducin (beta)-like 1X-linked receptor 1	4.12E-04
CD3D	CD3d molecule delta (CD3-TCR complex)	4.59E-04
FXR2	fragile X mental retardation autosomal homolog 2	4.79E-04
PTPN2	protein tyrosine phosphatase non-receptor type 2	6.36E-04

TMEM29	transmembrane protein 29	7.06E-04
CLK2	CDC-like kinase 2	7.32E-04
EIF4G3	eukaryotic translation initiation factor 4 gamma 3	7.32E-04
C16orf68	chromosome 16 open reading frame 68	7.36E-04
HSPA14	heat shock 70kDa protein 14	7.43E-04
HPCAL1	hippocalcin-like 1	1.04E-03
IFRD1	interferon-related developmental regulator 1	1.09E-03
GSTM2	glutathione S-transferase M2 (muscle)	1.24E-03
DUSP2	dual specificity phosphatase 2	1.29E-03
TRAF1	TNF receptor-associated factor 1	1.32E-03
YWHAZ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta polypeptide	1.36E-03
BCR	breakpoint cluster region	1.47E-03
C11orf59	chromosome 11 open reading frame 59	1.59E-03
YRDC	yrdC domain containing (E. coli)	1.62E-03
SPRYD3	SPRY domain containing 3	1.82E-03
PPP1CB	protein phosphatase 1 catalytic subunit beta isoform	1.84E-03
LATS1	LATS large tumor suppressor homolog 1 (Drosophila)	1.85E-03
STARD4	START domain containing 4 sterol regulated	2.22E-03
CHORDC1	cysteine and histidine-rich domain (CHORD)-containing 1	2.25E-03
RBM14	RNA binding motif protein 14	2.38E-03
PIK3IP1	phosphoinositide-3-kinase interacting protein 1	2.40E-03
CD2	CD2 molecule	2.46E-03
NEIL3	nei endonuclease VIII-like 3 (E. coli)	2.49E-03
CASC5	cancer susceptibility candidate 5	2.51E-03
EIF2B5	eukaryotic translation initiation factor 2B subunit 5 epsilon 82kDa	2.53E-03
C19orf48	chromosome 19 open reading frame 48	2.68E-03
CD80	CD80 molecule	2.76E-03
GPR114	G protein-coupled receptor 114	2.85E-03
BPNT1	3'(2') 5'-bisphosphate nucleotidase 1	2.91E-03
ZBTB7A	zinc finger and BTB domain containing 7A	3.09E-03
SEC31B	SEC31 homolog B (S. cerevisiae)	3.12E-03
TUBA1B	tubulin alpha 1b	3.29E-03
ARL17	ADP-ribosylation factor-like 17	3.30E-03
CENPF	centromere protein F 350/400ka (mitosin)	3.41E-03
CXorf40A	chromosome X open reading frame 40A	3.79E-03
SIRT5	sirtuin (silent mating type information regulation 2 homolog) 5	3.95E-03
ATAD5	ATPase family AAA domain containing 5	4.05E-03
WDR62	WD repeat domain 62	4.12E-03
C6orf167	chromosome 6 open reading frame 167	4.13E-03
DCLRE1B	DNA cross-link repair 1B (PSO2 homolog S. cerevisiae)	4.16E-03
LEPROT	leptin receptor overlapping transcript	4.23E-03
TMEM29	transmembrane protein 29	4.34E-03
TFE3	transcription factor binding to IGHM enhancer 3	4.44E-03
CG018	hypothetical gene CG018	4.55E-03
NAP1L1	nucleosome assembly protein 1-like 1	4.64E-03
WDR3	WD repeat domain 3	4.64E-03
DCC1	defective in sister chromatid cohesion homolog 1 (S. cerevisiae)	4.69E-03
TTC9C	tetratricopeptide repeat domain 9C	4.73E-03
ALG11	asparagine-linked glycosylation 11	4.76E-03
EMR2	egf-like module containing mucin-like hormone receptor-like 2	4.76E-03

PPAN-P2RY11	PPAN-P2RY11	4.82E-03
HEXIM1	hexamethylene bis-acetamide inducible 1	4.85E-03
SPATS2	spermatogenesis associated serine-rich 2	4.91E-03
HNRPK	heterogeneous nuclear ribonucleoprotein K	5.01E-03
FTL	ferritin light polypeptide	5.06E-03
L3MBTL3	l(3)mbt-like 3 (Drosophila)	5.17E-03
KIF15	kinesin family member 15	5.23E-03
MYST4	MYST histone acetyltransferase (monocytic leukemia) 4	5.24E-03
XBP1	X-box binding protein 1	5.41E-03
ZNF592	zinc finger protein 592	5.42E-03
SCP2	sterol carrier protein 2	5.60E-03
CD96	CD96 molecule	5.73E-03
CLSTN1	calsyntenin 1	6.01E-03
NUPL1	nucleoporin like 1	6.03E-03
TRIP10	thyroid hormone receptor interactor 10	6.03E-03
MAP3K12	mitogen-activated protein kinase kinase kinase 12	6.08E-03
EPHX1	epoxide hydrolase 1 microsomal (xenobiotic)	6.13E-03
FBXW7	F-box and WD repeat domain containing 7	6.42E-03
U2AF1L4	U2 small nuclear RNA auxiliary factor 1-like 4	6.43E-03
C1orf58	chromosome 1 open reading frame 58	6.44E-03
RUNX3	runt-related transcription factor 3	6.47E-03
AURKB	aurora kinase B	6.65E-03
LYCAT	lysocardiolipin acyltransferase	6.90E-03
USP20	ubiquitin specific peptidase 20	7.23E-03
FANCA	Fanconi anemia complementation group A	7.56E-03
SC5DL	sterol-C5-desaturase	7.64E-03
GSG2	germ cell associated 2 (haspin)	7.91E-03
PRKY	protein kinase Y-linked	8.09E-03
CDCP1	CUB domain containing protein 1	8.14E-03
CALM1	calmodulin 1 (phosphorylase kinase delta)	8.29E-03
TCOF1	Treacher Collins-Franceschetti syndrome 1	8.38E-03
CCL3	chemokine (C-C motif) ligand 3	8.52E-03
C12orf10	chromosome 12 open reading frame 10	8.72E-03
TNFSF8	tumor necrosis factor (ligand) superfamily member 8	8.79E-03
NUDCD1	NudC domain containing 1	8.99E-03
SYPL1	synaptophysin-like 1	9.25E-03
P4HA2	proline 4-hydroxylase alpha polypeptide II	9.34E-03
AAAS	achalasia adrenocortical insufficiency alacrimia (Allgrove triple-A)	9.42E-03
SBNO2	strawberry notch homolog 2 (Drosophila)	9.43E-03
ZNF398	zinc finger protein 398	9.62E-03
BIN1	bridging integrator 1	9.65E-03
CEP27	centrosomal protein 27kDa	9.70E-03
ZAK	sterile alpha motif and leucine zipper containing kinase AZK	9.71E-03

Table S4. Genes displaying significant alternative exon usage in response to hnRNPLL knockdown in peripheral CD4+ T cells and in response to activation of cord blood CD4+ T cells, but not in response to shGFP infection of peripheral CD4+ T cells, as assessed by exon array analysis ($p<0.01$).

Gene	Gene Annotation	Alternative Exon Use (p-value)
PTPRC	protein tyrosine phosphatase receptor type C	3.73E-15
WAC	WW domain containing adaptor with coiled-coil	4.39E-09
CD44	CD44 molecule (Indian blood group)	4.60E-07
RAD54B	RAD54 homolog B (S. cerevisiae)	7.65E-07
STAT5A	signal transducer and activator of transcription 5A	3.28E-06
SCD	stearoyl-CoA desaturase (delta-9-desaturase)	3.87E-06
SNRPA1	small nuclear ribonucleoprotein polypeptide A'	4.66E-06
SNORA18	small nucleolar RNA H/ACA box 18	5.70E-05
CFLAR	CASP8 and FADD-like apoptosis regulator	1.02E-04
TXNDC5	thioredoxin domain containing 5	1.28E-04
FASN	fatty acid synthase	1.39E-04
PARG	poly (ADP-ribose) glycohydrolase	2.14E-04
EIF3S7	eukaryotic translation initiation factor 3 subunit 7 zeta 66/67kDa	3.22E-04
PXDNL	peroxidasin homolog (Drosophila)-like	4.11E-04
EIF4G3	eukaryotic translation initiation factor 4 gamma 3	7.32E-04
HSPA14	heat shock 70kDa protein 14	7.43E-04
GSTM2	glutathione S-transferase M2 (muscle)	1.24E-03
DUSP2	dual specificity phosphatase 2	1.29E-03
TRAF1	TNF receptor-associated factor 1	1.32E-03
YWHAZ	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein zeta polypeptide	1.36E-03
CHORDC1	cysteine and histidine-rich domain (CHORD)-containing 1	2.25E-03
RBM14	RNA binding motif protein 14	2.38E-03
TUBA1B	tubulin alpha 1b	3.29E-03
ARL17	ADP-ribosylation factor-like 17	3.30E-03
SIRT5	sirtuin (silent mating type information regulation 2 homolog) 5	3.95E-03
TFE3	transcription factor binding to IGHM enhancer 3	4.44E-03
NAP1L1	nucleosome assembly protein 1-like 1	4.64E-03
ALG11	asparagine-linked glycosylation 11 homolog	4.76E-03
EMR2	egf-like module containing mucin-like hormone receptor-like 2	4.76E-03

PPAN-P2RY11	PPAN-P2RY11	4.82E-03
MYST4	MYST histone acetyltransferase (monocytic leukemia) 4	5.24E-03
CD96	CD96 molecule	5.73E-03
RUNX3	runt-related transcription factor 3	6.47E-03
USP20	ubiquitin specific peptidase 20	7.23E-03
C12orf10	chromosome 12 open reading frame 10	8.72E-03
TNSF8	tumor necrosis factor (ligand) superfamily member 8	8.79E-03

Supplementary Discussion

By using mouse models with conditional expression or deletion of hnRNPLL, the global program of hnRNPLL-mediated alternative splicing may be investigated in different cell types. For instance, B cells express high levels of B220 (CD45ABC) from the pro-B cell until the plasma cell stage, at which point they begin to express both CD45RO (*3*) and hnRNPLL (GEO profiles). The same strategy may be used to address some long-standing questions about the specific roles of long and CD45 isoforms in lymphocyte signaling (*4-6*). Assigning specific functions to the isoforms has been difficult since the expression level of CD45 is a critical parameter in its function (*7, 8*), yet basal CD45 expression is very high (*3, 4, 6*). Furthermore, even the short CD45 isoforms present in activated T cells appear to be excluded from TCR microclusters (*9*), potentially explaining why the activating effects of CD45 tend to predominate over its inhibitory effects (*3, 4*). Genetic manipulation of hnRNP expression, through its global effects on CD45 isoform expression, will facilitate our understanding of CD45 alternative splicing in a physiological context.

Supplementary References

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