RNA-binding protein hnRNPLL regulates mRNA splicing and stability during B-cell to plasma-cell differentiation

Xing Changa,b, Bin Li, and Anjana Raoa,b,d,e,1

Contributed by Anjana Rao, December 2, 2014 (sent for review July 20, 2014)

Posttranscriptional regulation is a major mechanism to rewire transcriptomes during differentiation. Heterogeneous nuclear RNA-binding protein LL (hnRNPLL) is specifically induced in terminally differentiated lymphocytes, including effector T cells and plasma cells. To study the molecular functions of hnRNPLL at a genomewide level, we identified hnRNPLL RNA targets and binding sites in plasma cells through integrated Photoactivatable-Ribonucleoside-Enhanced Cross-Linking and Immunoprecipitation (PAR-CLIP) and RNA sequencing. hnRNPLL preferentially recognizes CA dinucleotide-containing sequences in introns and 3′ untranslated regions (UTRs), promotes exon inclusion or exclusion in a context-dependent manner, and stabilizes mRNA when associated with 3′ UTRs. During differentiation of primary B cells to plasma cells, hnRNPLL mediates a genome-wide switch of RNA processing, resulting in loss of B-cell lymphoma 6 (Bcl6) expression and increased Ig production—both hallmarks of plasma-cell maturation. Our data identify previously unknown functions of hnRNPLL in B-cell to plasma-cell differentiation and demonstrate that the RNA-binding protein hnRNPLL has a critical role in tuning transcriptomes of terminally differentiating B lymphocytes.

RNA binding proteins | plasma cells | alternative splicing | PAR-CLIP | hnRN

In response to pathogen challenge, lymphocytes undergo activation and differentiation into effector cells to exert their physiological functions. This process is accompanied by dynamic changes in cellular transcriptomes and proteomes. Although many of these changes occur through alterations in gene transcription, posttranscriptional regulation of mRNA expression is also a major mechanism for rewiring the transcriptome and increasing proteome diversity during cell differentiation (1). Posttranscriptional regulation is mediated through diverse mechanisms of mRNA processing, including alternative splicing of pre-mRNA, 3′ UTR regulation, and translational control—all typically mediated through interactions between RNA-binding proteins (RBPs) and their target sequences on RNA (2). The accurate mapping of RBP-binding sites on mRNA is critical for elucidating the functions of RBPs and the mechanisms by which they regulate pre-mRNA processing.

The emergence of genome-wide approaches has greatly facilitated our ability to map RBP-binding sites on RNA and explore the mechanisms regulating mRNA alternative processing. RNA-protein interactions have been explored through RNA cross-linking and immunoprecipitation (RNA-CLIP), a method that uses UV irradiation to cross-link RNA to its binding proteins, followed by immunoprecipitation of selected RBPs and next-generation sequencing to identify RNAs bound to those RBPs at a genome-/transcriptome-wide level (3–7). A recent improvement known as PAR-CLIP (Photoactivatable-Ribonucleoside-Enhanced Cross-Linking and Immunoprecipitation) uses a photoactive ribonucleotide analog to increase the efficiency of RNA/protein cross-linking (8). A further advantage of PAR-CLIP is that the photomeres used to reverse-transcribe the cross-linked RNAs tend to introduce specific T-to-C mutations within or in the near vicinity of the RBP-binding sites, thus validating the specificity of RBP binding to coprecipitating RNAs and mapping RBP-binding sites on the validated RNAs at close to single-nucleotide resolution (8).

Heterogeneous nuclear RNA-binding proteins (hnRNPs) is the term applied to a collection of unrelated nuclear RBPs. hnRNPLL was identified through a targeted lentiviral shRNA screen for regulators of CD45RA to CD45RO switching during memory T-cell development (9) and independently through two separate screens performed by different groups for exclusion of CD45 exon 4 in a minigene context (10) and for altered CD44 and CD45R expression on T cells in N-ethyl-N-nitrosourea (ENU)-mutagenized mice (11). Mice with an ENU-induced mutant allele of Hnprll (which encodes hnRNPLL) showed defects in T-cell survival and homeostasis (11). hnRNPLL is up-regulated during T-cell activation; it also is highly expressed in plasma cells, where it regulates the switching between membrane and secreted Ig in a plasma cell line (12). However, the role of hnRNPLL during primary plasma cell differentiation is not known. Moreover, although exon arrays comparing wild-type and hnRNPLL-deficient T cells have provided a global view of hnRNPLL-mediated alternative splicing events in T cells (9, 11), such approaches are typically unable to discriminate direct and indirect effects, because splicing factors are well known to regulate the processing of mRNAs encoding other splicing factors (13, 14). Whether hnRNPLL is involved in RNA processing beyond inducing exon exclusion also remains to be determined. In this study, therefore, we generated a transcriptome-wide map of the direct sites of interaction of hnRNPLL with RNA, so as to increase our understanding of the roles of hnRNPLL in RNA alternative processing during lymphocyte differentiation.

Significance

Plasma cells produce immunoglobulin and provide long-lasting protective immunity. Differentiation of B cells to plasma cells is accompanied by major changes in gene expression, which are regulated at both transcriptional and posttranscriptional levels. We have used genome-wide methods to identify the binding sites and RNA targets of heterogeneous nuclear RNA-binding protein LL (hnRNPLL), whose expression is up-regulated during B-cell to plasma-cell differentiation. In addition to its recognized function in promoting exon splicing, hnRNPLL shapes the transcriptome of plasma cells by regulating exon inclusion and promoting mRNA stability. hnRNPLL binds to preferred sequences in RNA and is critical for complete plasma-cell differentiation, by mediating the down-regulation of B-cell-specific transcription factors and maximizing immunoglobulin production.

Author contributions: X.C. and A.R. designed research; X.C. performed research; X.C., B.L., and A.R. analyzed data; and X.C. and A.R. wrote the paper.

The authors declare no conflict of interest.

Data deposition: The sequence reported in this paper has been deposited in the Sequence Read Archive (SRA) database (accession no. SRP056204).

1To whom correspondence should be addressed. Email: arao@liai.org.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1422490112/-/DCSupplemental.

Author contributions: X.C. and A.R. designed research; X.C. performed research; X.C., B.L., and A.R. analyzed data; and X.C. and A.R. wrote the paper.

The authors declare no conflict of interest.

Data deposition: The sequence reported in this paper has been deposited in the Sequence Read Archive (SRA) database (accession no. SRP056204).

1To whom correspondence should be addressed. Email: arao@liai.org.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1422490112/-/DCSupplemental.
Plasma cells are terminally differentiated B lymphocytes that lose their B-cell characteristics and acquire the capacity to produce large quantities of antibodies. Plasma cells are the major source of antibodies for humoral immunity. The differentiation of plasma cells from B cells requires an extensive reorganization of transcriptional programs, a process mediated by two antagonistic transcription factors, B-cell lymphoma 6 (Bcl6) and B-lymphocyte–induced maturation protein 1 (Blimp1) (15). During plasma-cell differentiation, the differentiating B cells acquire plasma-cell–specific transcription factors, such as Blimp1 and X-box–binding protein 1 (Xbp1), and terminate the expression of B-cell–specific transcription factors, including Bcl6 and Pax5 (16). Plasma-cell differentiation is also accompanied by alteration of mRNA alternative processing: The mRNA encoding the transmembrane phosphatase CD45 undergoes alternative splicing to exclude exons 4–6, thus switching the CD45 protein from its highest-molecular-weight isoform, CD45RABC, to the lowest-molecular-weight isoform, CD45RO (17, 18). However, the role of posttranscriptional regulation in plasma-cell differentiation is less well characterized than the analogous process in T cells (1, 6, 9–11, 19).

In the B-cell lineage, hnRNPLL is minimally expressed at the naïve B-cell stage, but is up-regulated significantly after B-cell differentiation into plasma cells (12). In this study, we have carried out PAR-CLIP analysis of hnRNPLL in plasma cells and combined it with deep RNA sequencing (RNA-seq) to identify hnRNPLL-dependent regulatory events in plasma cells. We show that in plasma cells, hnRNPLL preferentially associates with CA-repeat RNA sequences in introns and 3′ UTRs and can either enhance or suppress the inclusion of alternative exons depending on its location relative to exon–intron junctions. Unexpectedly, we also found that the association of hnRNPLL with 3′ UTRs increases RNA stability. In the absence of hnRNPLL, the termination of Bcl6 expression and optimal Ig production in plasma cells were both compromised, indicating that RNA alternative processing mediated by hnRNPLL has an important role in plasma-cell development and function.

Results
PAR-CLIP Identifies hnRNPLL-Binding Sites on RNA of Plasmacytoma Cells
To systematically identify hnRNPLL-binding sites on RNA in vivo, we used the recently established PAR-CLIP technique (8) (outlined in Fig. S1). Briefly, we pulsed a plasmacytoma cell line, MPC11, with the photoreactive ribonucleoside analog 4-thio-
uracil (4-SU: Fig. S1A) for 12–14 h. The cells were then irradiated with UV light (365 nm) to induce covalent RNA–protein cross-linking, after which hnRNPLL and its cross-linked RNAs were immunoprecipitated with our previously generated anti-
hnRNPLL antibody (12). The proteins in the immunoprecipitate were resolved by SDS polyacrylamide gel electrophoresis (SDS/ PAGE), and the hnRNPLL/RNA complex was excised from the gel after visualization with an RNA dye, SYBR green II (Fig. 1A). The antibody pulled down the canonical isoform of hnRNPLL as well as another isoform of higher molecular weight (Fig. 1A), generated through the use of a noncanonical translational start site (CUG) located 5′ of the canonical ATG start codon (Fig. S2A). The longer hnRNPLL isoform was also expressed in primary plasma cells (Fig. S2C). Both isoforms were associated with RNA based on SYBR green staining; we focused on the more abundant canonical isoform for the following analyses. After the cross-linked RNAs were recovered from the hnRNPLL/RNA complex, they were li-
gated to RNA adaptors, converted into cDNA, and sequenced on the SOLiD high-throughput sequencing platform (Fig. S1B).

With this approach, in two independent experiments, we obtained >50 × 10^6 high-quality reads corresponding to hnRNPLL-biding RNAs, from which we derived ∼110,000 hnRNPLL-binding sites and ∼6,000 genes whose transcripts were bound by hnRNPLL (Table S1). More than 40% of the reads contained the T-to-C mutations characteristic of PAR-CLIP that are introduced through misincorporation by reverse transcriptase (8) (Fig. 1B), indicating high cross-linking efficiency in our assays. The most enriched Gene Ontology terms for transcripts bound by hnRNPLL were related to RNA processing (Fig. 1D), consistent with previous findings that hnRNPs largely regulate alternative processing of pre-mRNAs encoding splicing factors (13, 14). The hnRNPLL PAR-CLIP results were reproducible, with a large overlap between the hnRNPLL-binding transcripts identified in two independent experiments (Fig. 1C). Therefore, using the PAR-CLIP approach, we have successfully mapped the interaction sites of hnRNPLL on the RNAs to which it binds in plasmacytoma cells.

hnRNPLL Preferentially Binds (CA)n Motifs in Introns and 3′ UTRs of Pre-mRNA Transcripts
We examined the distribution of hnRNPLL-binding sites on mRNA transcripts. Based on Ref-seq annotations, >80% of hnRNPLL binding sites fell into the transcribed regions of annotated genes; ∼70% of these binding sites were located within introns, confirming that our PAR-CLIP experiments captured pre-mRNA (Fig. 2A). Predominant intronic binding is also consistent with the nuclear location of hnRNPLL and its established function as a regulator of pre-mRNA splicing. Un-
expectedly, however, 10% of the hnRNPLL-binding sites fell into 3′ UTR regions (Fig. 2A)—much higher than the proportion of 3′ UTRs in the genome (3%), suggesting an unappreciated function for hnRNPLL in regulating 3′ UTR activity.

To identify RNA sequences that were recognized by hnRNPLL, we used the Meme-Chip de novo motif identification algorithm (20). (CA)n emerged as the most highly enriched sequence in both intronic and 3′ UTR binding sites of hnRNPLL (Fig. 2 B and C), indicating that hnRNPLL preferentially recognizes CA dinucleotide repeats on RNA. Interestingly, a systematic evolution
hnRNPLL recognizes tandem (CA) diribonucleotide repeats and other motifs in RNA. (A) Distribution of hnRNPLL-binding clusters among coding exons, introns, and 5’ and 3’ UTRs (Lower); the genomic ratios of the same elements are shown as a control (Upper). Note that the percentage of hnRNPLL-binding sites in 3’ UTR (10%) is much more than the genomic ratios of 3’ UTR (3%), 5’ (A) and C) hnRNPLL recognizes (C)(A)RNA motifs. Sequences of hnRNPLL-binding sites on either 3’ UTRs (B) or introns (C) were extracted and used as input for MEME-Chip motif identifier (20) to derive the most enriched sequences. The top three enriched motifs and their P values are depicted. (D and E) hnRNPLL-binding sites are enriched at 3’ splice sites (3’-SS), but not 5’ splice sites (5’-SS). The distance between the center of hnRNPLL-binding sites and 3’-SS (D) or 5’-SS (E) was calculated, and the distribution was plotted. The arrow in D depicts the enrichment of hnRNPLL clusters 5’ of the 3’-SS; the arrowhead in E shows lack of enrichment at the 5’ splice sites. (F) hnRNPLL-binding clusters were enriched at the proximal end of 3’ UTRs. As in D, the distance of hnRNPLL-binding sites relative to the start of 3’ UTRs was plotted. A.U., arbitrary units.

hnRNPLL Regulates RNA Splicing in a Context-Dependent Manner. To further examine how hnRNPLL regulates RNA processing, we defined as the percentage of transcripts containing the alternative splicing event (Fig. 2B). The highest proportion of hnRNPLL-mediated events involved changes in alternative first or last exon use or skipped exons, with tandem UTR choices also relatively frequent (Fig. 2B). Gene Ontology analysis of these hnRNPLL-mediated RNA alternative processing events suggests involvement of hnRNPLL in key plasma-cell functions, including protein translation, intracellular transport, and protein localization (Fig. S3A).

To further determine whether the location of hnRNPLL binding relative to an exon influenced exon inclusion/exclusion, we considered the combined data from RNA-seq and PAR-CLIP analyses. Considering only hnRNPLL-binding sites either 1 kb upstream or downstream of exons, we found that 29 of 100 (29%) exons with decreased inclusion (Δψ < −0.2) had hnRNPLL-binding sites within this window, compared with 54 of 150 (36%) exons showing increased inclusion (Δψ > 0.2). For exons whose inclusion was increased after hnRNPLL depletion (Δψ > 0.2), hnRNPLL-binding sites tended to occur 3’ of the exon. For example, hnRNPLL bound to a region 3’ of exon 2b of Pthf15 pre-mRNA (Fig. 3C), and its depletion was associated with increased inclusion of this exon (ψ score changed from 0.24 to 0.91, Fig. 3D; RT-PCR confirmation, Fig. 3E). In contrast, for exons whose inclusion was decreased after hnRNPLL depletion (Δψ < −0.2), hnRNPLL-binding sites tended to occur 5’ of the exon; for instance, hnRNPLL bound to a region 5’ of an alternative exon in Ube3a (Fig. 3F), and its depletion was associated with reduced inclusion of this exon (ψ score changed from 0.73 to 0.09; Fig. 3G and H). Analysis of all 250 exons provided a global confirmation of this observation (Fig. 3I and J). Therefore, we propose that hnRNPLL regulates splicing of cassette exons in a context-dependent manner: hnRNPLL binding 5’ of cassette exons tends to promote their inclusion, whereas hnRNPLL binding at 3’ of cassette exons tends to promote their exclusion.

We note that a relatively small percentage of hnRNPLL-regulated cassette exons contain hnRNPLL-binding sites nearby (within 1 kb either downstream or upstream), and in fact there is no significant difference in the number of hnRNPLL-binding sites upstream and downstream of these exons (Mann–Whitney test: P = 0.563 for Fig. 3F; P = 0.355 for Fig. 3J). However, this lack of significance does not necessarily mean that the remaining exons are indirectly affected by hnRNPLL, because many of them have hnRNPLL-binding sites at distal regions of introns (>1 kb away from exons). The significance of hnRNPLL binding at distal intronic regions remains to be determined.

hnRNPLL Binding at the 3’ UTR Increases mRNA Stability in Cells. Besides their enrichment in coding exons and introns, hnRNPLL-binding sites were also significantly enriched within 3’ UTRs in our PAR-CLIP analysis (Fig. 2A). Because the 3’ UTR is the most important cis-element that regulates RNA stability, we analyzed the expression of genes whose transcripts contained hnRNPLL-binding sites in their 3’ UTRs. Transcripts containing hnRNPLL-binding sites within their 3’ UTR regions showed significantly lower expression when hnRNPLL was depleted compared with transcripts lacking hnRNPLL binding. For instance, two of the most strongly down-regulated genes in hnRNPLL-depleted cells, Crip2 and Slx1b, both had strong and specific hnRNPLL
binding in their 3′ UTRs (Fig. 4 A and B). Analysis at the genome-wide level further supported such conclusions: For transcripts lacking hnRNPLL binding, the number of transcripts whose expression was significantly suppressed after hnRNPLL knockdown was similar to the number of transcripts whose expression was increased. In contrast, for transcripts that displayed hnRNPLL binding at their 3′ UTRs, more transcripts were suppressed than increased (Fig. 4C). Similarly, genes with hnRNPLL binding at their 3′ UTRs had significantly decreased expression after hnRNPLL depletion in contrast to the genes lacking hnRNPLL binding (Fig. 4D). Based on this analysis, we concluded that hnRNPLL binding at 3′ UTRs enhances mRNA stability in cells. Supporting its function in regulating 3′ UTR activity, we found that hnRNPLL was present both in the cytoplasm and in the nucleus (Fig. S3D).

Together, our results from our integrated genome-wide RNA-seq and PAR-CLIP analyses reveal a previously unidentified function of hnRNPLL in regulating mRNA stability, as well as a context-dependent role for hnRNPLL in pre-mRNA alternative splicing.

hnRNPLL Indirectly Suppresses Bcl6 Expression and Regulates Ig Production in Plasma Cells. Naïve B cells rapidly divide and differentiate into plasma cells in vitro after Toll-like receptor stimulation (24). We therefore used an in vitro LPS-induced plasma-cell differentiation assay to analyze hnRNPLL expression during plasma-cell differentiation. As expected, hnRNPLL was minimally expressed in naïve B cells, but was drastically up-regulated after three to four cycles of cell division after activation of B cells with LPS or anti-CD40 in the presence of the cytokines IL-4 and IL-5 (Fig. 5A and Fig. S4A, Upper). At day 5 after activation, hnRNPLL was most highly expressed in cells with plasma-cell phenotypes—i.e., cells expressing the plasma-cell marker CD138 and the transcription factor Blimp1—and cells with lower B220 (CD45RABC) expression. In contrast, activation of B cells with...
was significantly more abundant than in the control cells, whereas and RC exons and amplifying all of the splicing isoforms of (Fig. 5 C). CD45RA isoform remained up-regulated in the hnRNPLL-depleted cells. This change of B220 expression was mainly because the switch from the largest (B220, CD45RABC) to the smallest plasma cells. B-cell differentiation into plasma cells is accompanied by B-cell differentiation as judged by Blimp-1 and CD138 expression, B220 down-regulation, or hnRNPLL expression (Fig. S4A, Lower).

We further examined the function of hnRNPLL in plasma-cell differentiation in vitro using ShRNA to deplete Hnrpol. Briefly, purified naïve B cells were activated with LPS overnight and infected with retrovirus containing scrambled shRNA (ShCtrl) or two shRNA clones against Hnrpol (ShHLL1 and ShHLL2). Five days after infection, transduced cells were identified by GFP expression and analyzed for plasma-cell markers. As shown in Fig. 5B, both shRNAs efficiently reduced hnRNPLL expression in CD138+ plasma cells.

B-cell differentiation into plasma cells is accompanied by a switch from the largest (B220, CD45RABC) to the smallest (CD45RO) isoform of CD45 (17). In cells transduced with either of the two shRNAs against Hnrpol, expression of B220 remained higher than in control shRNA-transduced cells (Fig. 5C, Upper Left). This change of B220 expression was mainly because the CD45RA isoform remained up-regulated in the hnRNPLL-depleted cells (Fig. 5C, Upper Right), whereas expression of the CD45RB or CD45RC isoforms was affected at minimal level (Fig. 5C, Lower). This flow-cytometric analysis was confirmed by RT-PCR, using PCR primers flanking the alternative RA, RB, and RC exons and amplifying all of the splice isoforms of CD45 (Fig. 5D and Fig. S5A). In the hnRNPLL-depleted cells, the PCR product corresponding to B220 (CD45RABC) isoform was significantly more abundant than in the control cells, whereas the PCR products of the other spliced isoforms were much less. Thus, hnRNPLL selectively mediates the exclusion of CD45 exon 4, encoding CD45RA, during plasma-cell differentiation.

Plasma-cell differentiation is regulated by an elaborate transcriptional network mediated by two reciprocally regulated transcription factors, Bcl6 and Blimp1 (16, 25). Bcl6 is highly expressed in germinal center (GC) B cells and promotes their proliferation and survival. During plasma-cell differentiation, expression of Bcl6 has to be terminated to complete differentiation while Blimp1 expression is induced. hnRNPLL-depleted plasma cells, generated after 6 d of LPS stimulation in vitro, showed significantly higher expression of Bcl6 mRNA compared with wild-type plasma cells, suggesting that hnRNPLL acts to suppress Bcl6 expression in plasma cells (Fig. 5E). Consistent with elevated Bcl6 expression, hnRNPLL-depleted plasma cells expressed a higher level of the proliferation marker Ki-67, indicating that hnRNPLL-depleted plasma cells maintained their proliferation potential compared with control shRNA-transduced cells (Fig. S4B). However, Xbp1 and Blimp1, two other key transcription factors in plasma cells, showed no change in their mRNA expression in hnRNPLL-depleted cells (Fig. 5E); similarly, hnRNPLL depletion did not change the efficiency of initial plasma-cell differentiation as determined by acquisition of the plasma-cell marker CD138 (Fig. 5F).

Together, these results indicate that hnRNPLL controls certain, but not all, aspects of commitment to the plasma-cell lineage in vitro. One of the main properties of plasma cells is their ability to produce large amounts of Ig. In the presence of IL-4, plasma cells mainly produce the Ig IgG1 isofrom. Although hnRNPLL depletion did not affect CD138 expression, we found that hnRNPLL-depleted cells produced significantly less IgG1 than the control cells, as determined either by intracellular staining (Fig. 5G) or by ELISA (Fig. 5H). Class-switching to IgG1, however, was comparable between control shRNA-expressing and hnRNPLL-depleted cells, as determined by percentage of IgG1-producing cells (Fig. 5G). The reduced production of IgG1 was not caused by diminished transcription, because mRNA for IgG1 was comparable between control and hnRNPLL-depleted cells (Fig. 5E).

In summary, we found that hnRNPLL deficiency was dispensable for the initial steps of transition of differentiating B cells into plasma cells, such as up-regulation of CD138. In contrast, hnRNPLL was required at later stages for complete commitment of differentiating B cells to the plasma-cell lineage, including the CD45 isoform switch, the down-regulation of Bcl6, and exit from the cell cycle, and the capacity to produce a large quantity of immunoglobulins.

hnRNPLL Plays an Important Role in Determining the Plasma-Cell Transcriptome. To identify hnRNPLL-mediated alternative splicing events during B-cell to plasma-cell differentiation, we purified naïve B cells, in vitro-differentiated CD138+ plasma cells stably expressing control (scramble) shRNA and plasma cells stably expressing two independent hnRNPLL shRNAs (ShHLL1 or ShHLL2), and performed RNA-seq. Using the MISO algorithm, we identified 422 RNA cassette exons whose splicing patterns were significantly changed between B cells and sh-Control plasma cells (Δψ > 0.2 or < −0.2) and then asked to what extent these splicing events were mediated by hnRNPLL. Fig. 6A shows a heat map of ψ values of these plasma-cell–specific splicing events in the indicated cell populations (Table S2). The unsupervised hierarchical cluster analysis revealed two clear clusters in which splicing patterns were consistently reversed in hnRNPLL-depleted plasma cells. Within these alternative splicing events, exclusion of CD45 exon 4 (RA) was one of the top hits, as expected (Fig. S5B). Notably, hnRNPLL depletion increased the inclusion of Irf4 exon 8 in plasma cells, to a degree similar to that observed in naïve B cells (Fig. 6B); hnRNPLL has a binding site immediately downstream of this exon in MPC11 cells (Fig. 6D), suggesting that this Irf4 splicing event in plasma cells is directly dependent on hnRNPLL.
confirmed binding of hnRNPLL to Irf4 transcripts in activated B cells (Fig. S5C). Given the importance of IRF4 in regulating plasma-cell differentiation and function (26, 27), hnRNPLL-regulated alteration in Irf4 splicing may contribute to certain aspects of plasma-cell differentiation as described above.

We further analyzed gene-expression changes in hnRNPLL-depleted plasma cells. As expected from Fig. 5E, Bcl6 expression was higher in hnRNPLL-depleted cells than in control cells (Fig. 6C). Gene Ontology analysis revealed that the most significantly enriched gene set terms in hnRNPLL-depleted plasma cells were genes that are up-regulated from plasmablast to plasma cells ($P < 10^{-6}$; Table S3), further supporting the notion that hnRNPLL mediates terminal differentiation of plasma cells. Diminished expression of genes in this category was further validated by real-time PCR (Fig. 6D).

Together, these data indicate that hnRNPLL mediates a large number of alternative splicing events during plasma-cell differentiation, which together promote the transition from plasmablasts into terminally differentiated plasma cells.

**hnRNPLL Regulates B-Cell Homeostasis and Plasma-Cell Differentiation in Vivo.** To further examine the function of hnRNPLL in B lymphocytes in vivo, we used shRNAs against hnRNPLL to diminish its expression in primary B cells. Briefly, we reconstituted μMT mice, which lack endogenous B cells, with wild-type bone marrow cells transduced with retrovirus containing either sh-hnRNPLL-IRE5-GFP (ShLL) or sh-Scramble-IRE5-GFP (ShCtrl). Six weeks after reconstitution, GFP+ cells were analyzed. As expected, ~90% of B cells in the spleen of the chimeric mice expressed GFP and were derived from the donor bone marrow cells (Fig. 7A), whereas
there were very few GFP⁺ cells in other lineages (Fig. 7A). Real-time PCR analysis revealed 50% reduction of hnRNPL expression after shRNA-mediated depletion in B cells. Therefore, in the chimeric mice, only B cells specifically have reduced hnRNPL expression.

Notably, in the mice given hnRNPL-depleted bone marrow, both the percentages and the numbers of B cells were significantly reduced compared with those of control mice (~40% and ~50% reduction, respectively) (Fig. 7A). The proportion of mature B cells (IgMlowIgDhi) in the spleen was also significantly less in the hnRNPL-depleted mice (Fig. S6). However, analysis of pro- and pre-B cells in the bone marrow did not reveal significant changes between hnRNPL-depleted and control mice, indicating that hnRNPL promotes B-cell homeostasis and/or maturation in the periphery.

To further analyze hnRNPL functions in plasma-cell differentiation and function, we immunized the chimera mice with 4-hydroxy-3-nitrophenyl conjugated to keyhole limpet hemocyanin (NP-KLH) emulsified with Ribi’s adjuvant. Three weeks after immunization, total plasma cells in the bone marrow were shown to express CD138, a cell surface marker of plasma cells (Fig. 7B). NP-specific plasma cells were further identified with a NP-specific ELISpot assay (Fig. 7C). NP-specific and total plasma cells in the depleted mice were both present in lower numbers than their counterparts in the control mice. In contrast, the frequency of NP-specific antibody-secreting cells in the spleen was comparable between hnRNPL-depleted and control mice. Thus, hnRNPL plays a positive role in plasma-cell differentiation and/or homeostasis in vivo, consistent with what we observed in the in vitro differentiation assay.

Discussion

The splicing factor hnRNPL is highly expressed in terminally differentiated lymphocytes, including plasma cells and effector/memory T cells. In this study, we used PAR-CLIP in the MPC11 plasma cell line to generate a global map of hnRNPL-binding sites on RNA. Our data reveal that hnRNPL primarily binds CA repeat elements and, to a lesser extent, other C-, A-, and T-rich motifs, in the introns and 3’ UTRs of mRNAs. We documented that, depending on the location of its binding sites on mRNA, hnRNPL can regulate multiple steps of RNA processing, including exon inclusion, exon exclusion, and RNA stability. We also show that hnRNPL plays an important role in the terminal differentiation of plasma cells. Although hnRNPL is dispensable for the initial transition of B cells to the plasma-cell lineage, it has a key role in ensuring that differentiating B cells acquire the full repertoire of plasma-cell properties, including the CD45 isoform switch, termination of Bcl6 expression, exit from the cell cycle, and maximal Ig production. Together, our data indicate that hnRNPL-mediated RNA processing is essential for the optimal differentiation of B cells into plasma cells and for optimal plasma-cell function.

Fig. 6. hnRNPL mediates global splicing changes during plasma-cell differentiation. (A) hnRNPL is required for a large cohort of alternative splicing changes in plasma cells. Heat map shows the unsupervised hierarchical clustering of plasma-cell–specific splicing changes in B cells, control plasma cells (Sh-scramble), or plasma cells with hnRNPL depletion (ShLL1 and ShLL2). We first used the MISO algorithm to determine plasma-cell–specific splicing changes of cassette exons (n = 422, ΔΨ > 0.2 or < −0.2 between B cells and control plasma cells), and then calculated FDR values of these alternative splicing events in hnRNPL-depleted plasma cells. Two clusters labeled on the left represent hnRNPL-dependent exon inclusion (n = 66) and exon exclusion (n = 38), respectively, during plasma-cell differentiation. Only when both shRNAs against hnRNPL had similar effects were exons defined as hnRNPL-dependent (highlighted with black boxes). *Casette exons whose splicing in plasma cells was reverted by only one of two shRNAs against hnRNPL. (B) hnRNPL mediates a switch of Irf4 splice isoforms during B-cell to plasma-cell differentiation. (B, Top) hnRNPL bound immediately to 3’ of Irf4 exon 8 based on PAR-CLIP analysis in MPC11 cells. The binding sites are indicated on the top track with red rectangles, the bottom track depicts the Ref-seq annotation, and the alternative exon is boxed. (B, Middle) IGV genome browser view of RNA-seq data from B cells (brown), plasma cells with ShCtrl (green), or ShRNA to hnRNPL (blue). Numbers of junctional reads are indicated. Note: In the ShCtrl plasma cells, 92 junctional reads indicated skipping of exon 8 but such events were completely absent in B cells or ShLL plasma cells. (B, Bottom) Irf4 splicing was analyzed with RT-PCR in plasma cells with either shCtrl or sh hnRNPL. The result was quantified with the Agilent 2200 TapeStation, and ratios of each isoform are indicated. (C) RNA-seq reveals gene expression change between control plasma cells and hnRNPL-deficient plasma cells. Dot plots show RNA-seq analysis comparing gene expression between plasma cells with ShLL1 and ShCtrl. (D) Expression of a group of genes elevated during plasmablasts to plasma-cell transition (Table S3) was further examined by real-time PCR.
There is a growing appreciation of the importance of posttranscriptional regulation in lymphocyte differentiation and gene expression (1). The alternative splicing of CD45RA to CD45RO is one of the hallmarks of memory T-cell differentiation (28). The stability of ICOS mRNA, which is governed by the RBPs Roquin-1 and -2, restrains the differentiation of follicular helper T cells (29–33). The RBPs Zfp36l1 and Zfp36l2 suppress Notch1 mRNA stability, regulating normal thymocyte differentiation and preventing malignant transformation (34). However, most of these studies lack a genome-wide view, an important consideration given the complexity of the RNA-processing network and the need to differentiate between direct and indirect effects caused by the absence of an RBP.

Our study revealed unexpectedly diverse functions of hnRNPLL in posttranscriptional regulation. Specifically, hnRNPLL function appears to be highly dependent on the relative location of its binding site relative to cassette exons in pre-mRNA: When the binding site is 5′ of a cassette exon, hnRNPLL promotes exon inclusion; when it binds 3′ of a cassette exon, it promotes exon exclusion. In addition to binding to introns and exons, hnRNPLL also associates with 3′ UTRs of mRNAs, thereby promoting mRNA stability. Thus, hnRNPLL is a multifaceted RBP.

As observed for many other RBPs (1, 2, 6, 14), the motifs recognized by hnRNPLL are rather degenerate in their sequence. An interesting question is how, given this degeneracy, hnRNPLL achieves specific recognition of its target pre-mRNAs. In our PAR-CLIP analysis, sites of hnRNPLL binding often contained multiple independent T–C conversion events within a single binding cluster, suggesting that hnRNPLL might interact with RNA through multiple protein domains. Indeed, although the (CA)n motif was the most highly enriched in our analysis, other sequence motifs (CTTCC\textsuperscript{T,C}, CAT\textsuperscript{T,A}, CATT, TTATA\textsuperscript{T,A}, and an A-rich motif) were also significantly enriched, consistent with the fact that hnRNPLL contains three RRM domains at its N terminus. Further studies will determine whether the three RRM domains of hnRNPLL recognize distinct or similar RNA sequences in their target pre-mRNAs.

Although hnPNPLL appears to be the key hnRN involved in regulating the exclusion of CD45 exon 4 (encoding CD45RA) in activated T cells (9–11), a near relative, hnRNPL, is responsible for setting basal CD45 exon expression levels (1). hnRNPLL is structurally similar to hnRNPL; moreover, the (CA)n RNA motif we have identified here as recognized by hnRNPLL is similar to the motif identified through SELEX experiments as recognized by hnRNPL (6, 21, 35). Thus, these two related RBPs may have redundant as well as nonoverlapping functions, as confirmed by the overlap in their target genes (e.g., NEAT5) (this work and ref. 6). It will be interesting to examine further the shared vs. independent functions of hnRNPLL and hnRNPL in lymphocytes and other cell types (1). In the case of CD45, hnRNPL and hnRNPLL regulate pre-mRNA splicing by binding in part to the same RNA sequence and in part to distinct RNA sequences in the pre-mRNA (1). Additional studies at single nucleotide resolution in the same cells (i.e., using PAR-CLIP or i-CLIP) will reveal whether recognition of the same as well as distinct sequences is a general property of these related RBPs.

hnRNPL and hnRNPLL both bind 3′ UTRs in addition to introns, thereby increasing mRNA stability (this work and refs. 6, 30, and 37). In a well-studied example, hnPNPLL stabilizes VEGF-A mRNA through binding to a CA-rich element in the VEGF-A 3′ UTR (36, 37). Two mechanisms for this phenomenon have been documented: a riboswitch-like mechanism involving mutually exclusive binding of hnRNPL and the GAIT complex (37), and the ability of hnRNPL to block binding of miRNAs to the 3′ UTR (36) (reviewed in ref. 38). It will be interesting to ask whether similar mechanisms are responsible for the ability of hnRNPLL to mediate mRNA stabilization in differentiating B cells.

Depletion of hnRNPLL in B cells and bone marrow cells has diverse physiological effects on plasma-cell differentiation, implying that hnRNPLL controls numerous facets of the B-cell to plasma-cell switch, as also observed in activated T cells (9, 11). Certain early events of plasma-cell differentiation, such as CD138 up-regulation, were unaffected by hnRNPLL depletion; in contrast, later events—including Ig production and Bcl6 mRNA down-regulation—were clearly influenced by hnRNPLL. Our data show that hnRNPLL directly regulates pre-mRNA splicing of several proteins known to be important for plasma-cell differentiation, including Irf4 and Oct1; further characterization of the functions of these alternatively spliced isoforms in plasma cells will provide a deeper understanding of the role of hnRNPLL and posttranscriptional regulation in the B-cell to plasma-cell switch. In several other cases, however, the mechanism of regulation by hnRNPLL appeared to be indirect. For instance, consistent with our previous finding (12) that hnRNPLL binds IgG1 mRNA in a plasma-cell line, we found that hnRNPLL depletion resulted in decreased Ig production in primary plasma cells (Fig. 5H); however, the mRNA level of IgG1 was not significantly
changed after hnRNPLL depletion (Fig. 5E), suggesting that hnRNPLL may act directly or indirectly to control the translation rather than the level or stability of IgG1 mRNA.

Chimeric mice reconstituted with hnRNPLL-deficient bone marrow had reduced B-cell compartment in addition to reduced plasma-cell differentiation, indicating additional roles for hnRNPLL in regulating B-cell development and/or homeostasis, similar to its function in T cells (11). Further detailed analysis of B-cell development in hnRNPLL-deicient mice will help elucidate its functions in the various stages of B-cell development.

Previous studies of Bcl6, a transcription factor critical for GC responses (15, 16), have focused primarily on its transcriptional regulation through Blimpl1. In contrast, our data point to previously unidentified posttranscriptional mechanisms that govern Bcl6 expression. Instead of being down-regulated during B- to plasma-cell differentiation, as observed in control cells, Bcl6 mRNA expression was maintained at high levels in hnRNPLL-depleted cells. Again, however, Bcl6 mRNA was not directly bound by hnRNPLL, and the splicing patterns of Bcl6 pre-mRNA were unaltered in hnRNPLL-deleted cells, suggesting that hnRNPLL indirectly facilitates down-regulation of Bcl6 mRNA during plasma-cell differentiation. Since If4 directly suppresses Bcl6 transcription in GC B cells (39), one potential mechanism is that hnRNPLL regulates Bcl6 expression through alternative splicing of If4 transcripts. Posttranscriptional regulation of Bcl6 expression at the protein level may also be involved; an early study comparing resting human B cells versus tonsil GC B cells showed that Bcl6 mRNA levels were similar in these two cell types, whereas Bcl6 protein was much more highly expressed in GC B cells (40). Clearly, much remains to be elucidated regarding the posttranscriptional regulation of B-cell to plasma-cell differentiation and the potential role of hnRNPLL in this process.

Materials and Methods

RNA-Seq. Total RNA was isolated with TRIzol (Life Tech). mRNA was selected from ∼10 μg of total RNA with the Ambion MicroPoly(A) Purist Kit or the Ribozero selection kit (Epibo). RNA fragmentation, cDNA conversion, and library preparation were performed by using SOLID RNA-seq library prep kit (Life Tech). RNA-seq was performed by using SOLID 4 or SOLID 5000 instruments by using paired-end (50 + 35 bp) sequencing chemistry.

Generation of MPC11 Cell Lines. MPC11 cells were transduced overnight with pLKO.1-shGFP, pLKO.1-sh-hnRNPLL-1, or pLKO.1-sh-hnRNPLL-2 lentivirus and polybrene according to standard protocols in a six-well plate format. After 18 h, medium was removed, and fresh medium with puromycin was added, with selection allowed to occur for 72 h.

PAR-CLIP. PAR-CLIP experiments were conducted as described (6). Briefly, MPC11 cells were pulsed with 4SU (100 μM; Sigma) overnight. After irradiation with UV 365 nm, hnRNPLL/RNA complexes were immunoprecipitated with in-house rabbit anti-hnRNPLL antibody and Protein A Dynal beads (Life Science Tech). After RNAse T1 treatment, the immunoprecipitates were resolved on 4–12% LDS gel with Mops buffer (Life Science Tech). RNA on the gel was visualized with SYBR green I staining on the PhosphorImager (GE).

DNA Extraction, cDNA Synthesis, and Quantitative Real-Time PCR. Total RNA was isolated with the RNeasy plus kit (Qiagen), and cDNA was synthesized by using SuperScript III reverse transcriptase (Life Tech). Quantitative PCR was performed by using the FastStart Universal SYBR Green Master mix (Roche) on a StepOnePlus real-time PCR system (Applied Biosystems). Gene expression was normalized to β-actin. Primers used for qPCR are listed in Table S4.

RNA Immunoprecipitation. B cells were activated with LPS, IL-4, and IL-5 for 4 d and were pulsed with 4SU as in the PAR-CLIP experiments. After irradiation, hnRNPLL/RNA complexes were purified, and RNA was extracted directly from Protein A Dynal beads with TRIZOL reagent. Purified RNA was converted into cDNA, and relative enrichment of target mRNA was determined after normalized to the input and rabbit IgG controls.

Bone Marrow Transplantation. Bone marrow cells were purified from wild-type mice treated with 5-fluourouracil 5 d earlier. The cells were cultured in DMEM with IL-3 (10 ng/ml), IL-6 (20 ng/ml), and Scf (50 ng/ml) and transduced with retrovirus containing the indicated shRNAs in the LMP vector, an murine stem cell virus-based retroviral vector containing an miR30-adapted shRNA backbone, and a phosphoglycerate kinase promoter-driven puromycin-GFP selection cassette (41), at 20 and 40 h after culture. Puromycin (2 μg/ml) was added to the culture on day 3. Cells were harvested on day 5, and 2–3 × 106 cells were i.v. injected into μtM mice that had been sublethally irradiated (400 rad) 1 d before cell transfer. Chimeric mice were analyzed 6–8 wk after transplantation.

B-Cell Isolation and In Vitro Differentiation. Naïve B cells were isolated from spleens of C57BL/6 mice. Briefly, splenocytes were treated with red blood cell lysis buffer (155 mM NH4Cl, 10 mM NaHCO3, and 0.1 mM EDTA), incubated with anti–CD43–PE–BD (Biocytin) (1:2000 for 30 min on ice, washed with PBS +1% FBS), and incubated with anti-PE microbeads (Miltenyi Biotech) for 20 min on ice. Naïve B cells were then purified by negative selection using a MACS LS column. Naive B cells were stimulated with 10 μg/ml LPS (Invivojogen), 10 ng/ml IL-4, and 10 ng/ml IL-5 in RPMI 1640 medium (10% FBS, 100 U/ml penicillin, 100 U/ml streptomycin, 50 μM β-mercaptoethanol, and 1% glucose). Where indicated, naïve B cells were labeled with Cell-Trace Violet (2 nM) before activation.

shRNA-Mediated hnRNPLL Depletion in Primary B Cells. B cells were activated as described above, and on days 2 and 3 after activation, cells were spin-infected with supernatant containing LMP-sh-Scramble, LMP-sh-hnRNPLL-1, or LMP-sh-hnRNPLL-2 retroviruses. Puromycin (2 μg/ml) was added into the culture on day 3, and, when indicated, CD138+ plasma cells were isolated by using anti-CD138–PE and anti-PE MACS beads.

Staining and Flow Cytometry. For cell surface staining, cells were washed with FACS buffer (1% FBS in PBS with 0.1% Na2EDTA), incubated with the indicated antibodies on ice for 30 min, washed two more times with FACS buffer, and fixed in 1% paraformaldehyde in PBS before being analyzed using BD LSRII or Fortessa machine (BD Biosciences). For intracellular staining, cells were surface stained, fixed/permeabilized with a Cytofix/Cytoperm kit (BD Bioscience), and stained with antibodies against the indicated antigens. Intracellular hnRNPLL staining was carried out by using our in-house hnRNPLL antibody, PE-anti-rabbit IgG (BD Biosciences), and the Foxp3 intracellular staining buffer set (BioLegend).

ELISPOT. To measure NP-specific antibody-secreting cells, Millipore MAHA 96-well filter plates were coated with NP-BSA (50 μg/ml) in PBS at 4 °C overnight. Plates were washed with PBS plus 0.05% Tween 20 twice and blocked with complete DMEM (10% FBS) at 37 °C for 2 h. A total of 1 × 105 bone marrow cells were seeded in duplicate in the first well, and two serial dilutions were performed. The plates were then incubated at 37 °C for 5 h. After incubation, plates were washed with PBS and PBS plus Tween 20 each three times, and 0.5 μg/ml biotin anti-mouse IgG was added into each well for overnight incubation at 4 °C. After washing with PBS plus Tween 20, the plates were incubated with Streptavidin–HRP and developed with BD ELISPOT AREC substrate set according to manufacturer’s instruction. The red dots, which were indicative of NP-specific antibody-secreting cells, were counted with computer-assisted image analysis (KS-ELISPOT reader; Zeiss).

Gene Ontology Analysis. To determine the functions of significantly changed transcripts, genes with Log2 (shLshCtrl) > 1 or −1 and fragments per kilobases of transcript per million mapped reads (FPKM) > 0.5 in either control or sh-hnRNPLL samples were analyzed with DAVID, and all genes with FPKM > 0.5 were used as background.

Cluster Analysis of Alternative Splicing Events. The efficiency of splicing of casette exons was determined by using the MISO algorithm. Splicing events with ∆Ψ > 0.2 or < −0.2 between control plasma cells and B cells were selected. The Ψ values of these splicing events from B cells, control plasma cells, and plasma cells expressing two independent shRNAs against hnRNPLL were clustered by using the K-mean clustering approach.
ACKNOWLEDGMENTS. We thank Prof. Bjoern Peters (head of the La Jolla Institute for Allergy and Immunology Bioinformatics Core) for support, Jeremy Day for next-generation sequencing, Ryan Haste for technical support, and the LIAI bioinformatics core for constant support and feedback. This work was supported by J. Grant 3R01AI08725, AI040127, and RC4 AI092763 (to A.R.), and HL114093 (to Bjoern Peters and A.R.).

Supporting Information
Chang et al. 10.1073/pnas.1422490112

**Fig. S1.** Outline of the PAR-CLIP protocol adapted in this study. The chemical structure of 4-SU is depicted in A; the procedure for the PAR-CLIP experiments is shown in B.
Fig. S2. Mass-spectrometric analysis reveals an hnRNPLL isoform that uses an alternative start codon 5′ of the annotated start codon. (A) hnRNPLL was immunoprecipitated as described, and the higher-molecular-weight band was exercised and subjected to mass-spectrometric analysis. Green boxes indicate the peptides recovered from the 5′ UTR sequence of hnRNPLL cDNA. The predicted alternative start codon (CUG) and the canonical start codon (AUG) are indicated. (B) Diagram shows the gene structure of Hnrpll (the intron and exon lengths are not proportional) and the target sequences of two shRNAs. (C) Both hnRNPLL isoforms were expressed in plasma cells. B cells were activated with LPS+IL4+IL5 for 5 d and were transduced with either control shRNA or shRNA against hnRNPLL at days 2 and 3. Plasma cells (CD138+) were purified, and expression of hnRNPLL was analyzed by immunoblotting.
Fig. S3. RNA-seq reveals hnRNPLL-mediated alternative splicing events in MPC11 cells. (A) Gene Ontology analysis of hnRNPLL-mediated alternative splicing events. As in Fig. 3B, genes whose splicing patterns were significantly changed after hnRNPLL depletion were subject to Gene Ontology analysis. The top nine enriched biological processes were depicted. (B) As in Fig. 3F, hnRNPLL binding 5′ of a cassette exon in the Ptpn4 gene enhances its inclusion. (B, Left) Genome browser view of hnRNPLL-binding sites on Ptpn4 (from top to bottom are hnRNPLL PAR-CLIP reads, reads with T>C conversion, binding clusters, and Ref-seq annotation, respectively). The cassette exon is highlighted with a black box. (B, Right) RT-PCR analysis revealed that the top band, which includes the cassette exon, is decreased in intensity relative to the bottom band after hnRNPLL depletion. (C) As in Fig. 3C, hnRNPLL binding 3′ of a cassette exon of Nfat5 promotes its exclusion. (C, Right) RT-PCR analysis revealed that the top band, which includes the cassette exon, is increased in intensity relative to the bottom band after hnRNPLL depletion. (D) hnRNPLL is present in both the cytoplasm and the nucleus. MPC11 cells were fractionated into cytoplasmic and nuclear fractions, and the expression of hnRNPLL was analyzed by immunoblotting. GAPDH and Histone H3 were used as controls for cytoplasmic and nuclear proteins, respectively.
Fig. S4. hnRNPLL expression in activated B cells. (A) Naïve B cells were activated with either anti-CD40 (10 μg/mL) plus IL-4 (10 ng/mL) plus IL-5 (10 ng/mL) (Upper) or anti-IgM (10 μg/mL) (Lower). Five days after activation, expression of hnRNPLL was determined by intracellular staining together with Blimp1–YFP reporter, cell tracer, CD138, and B220. hnRNPLL is highly expressed in plasma cells generated by anti-CD40 or LPS stimulation (Fig. 5A), but less expressed in B cells activated with anti-IgM. (B) hnRNPLL-depleted plasma cells maintained their proliferative potential compared with ShCtrl cells, which down-regulate proliferation upon differentiation. Six days after LPS stimulation, cell proliferation marker Ki-67 was stained in GFP+CD138+ plasma cells either with control shRNA or two shRNAs against Hnrpll. Data are representative of three independent experiments.

Fig. S5. hnRNPLL mediates plasma-cell–specific alternative splicing during differentiation. (A) A diagram shows the exon structure of CD45. (B) hnRNPLL induces CD45RA splicing in plasma cells. Sashimi plots depict the junctional reads for CD45 RA, RB, and RC exons. From top to bottom are B cells, plasma cells with ShCtrl, and plasma cells with ShLL1 and ShLL2. (C) hnRNPLL binds to Irf4 mRNA in activated B cells. Isolated naïve B cells were activated with LPS plus IL-4 plus IL-5 for 3 d. The activated cells were pulsed with 4SU overnight and irradiated with 365 NM UV as in the PAR-CLIP experiments. The cell lysate was immunoprecipitated with either anti-hnRNPLL or control rabbit IgG. RNA associated with the immunoprecipitates was purified with TRIZOL from Dynal Beads and converted to cDNA. Enrichment of target mRNA was normalized to the rabbit IgG control. The data summarize two independent experiments.
Fig. S6. hnRNPLL promotes B-cell maturation in the periphery. (A) FACS plots depict immature B cells (IgM⁺IgD⁻) and mature B cells (IgM⁻IgD⁺) cells from the spleen of chimeric mice. The plots were gated on GFP⁺CD19⁺ cells. (B) Summary of percentage of IgM⁻IgD⁺ cells in the spleen of four mice from two independent experiments.

Table S1. Summary of mapping and clusters generated from the hnRNPLL PAR-CLIP experiments

Table S2. Ψ values of differentially spliced cassette exons during B-cell to plasma-cell differentiation in wild-type B cells, ShCtrl plasma cells, ShLL1 plasma cells, and ShLL2 plasma cells

Table S3. GO analysis of differentially expressed genes between control plasma cells and shLL-1 plasma cells

Table S4. Primers that were used in this study