Supplementary Figure S1: A) Epigenetic profiles at the Cd3 loci. Each track gives the results of one ChIP-on-chip experiment for the histone modification specified within the ΔRag or ΔRagCD3 cell type. The arrows indicate the genomic location and transcriptional orientation of the corresponding genes. The positions of enhancer regions are indicated by a black rectangle and the associated epigenetic profiles are highlighted by a dotted rectangle. The genomic scales are also indicated. B) Genome-wide distribution and stage-specificities of enriched regions (peaks) for each epigenetic mark legends are figured as indicated in the legend of Figure 1B. C) Epigenetic profiles at the B-cell specific Ebf1 locus. D) Epigenetic profiles at two additional lymphoid-specific loci for which enhancer elements are known.
Supplementary Figure S2: Epigenetic profiles of bona fide, distal regulatory elements known to be activated via pre-TCR signaling (ChIP-on-chip data). A) Epigenetic profiles at the Cd8 locus. The name and location of the various Cd8 enhancers are indicated. B) Relative gain of H3K4 methylation at the indicated enhancer regions. The average ChIP-on-chip signal for each H3K4me mark was quantified in ΔRag and ΔRagCD3 thymocytes and the differences between the two cell populations were plotted as a percentage of the signal obtained in ΔRag thymocytes. C) Epigenetic profiles at the Cd4 locus. D) Epigenetic profiles of genomic regions that are located around the Eα enhancer. The 3' end of the Tcra locus is shown. For panels A, C and D, legends are as in Figure S1.
Supplementary Figure S3: ChIP-qPCR validation of H3K4 methylations at induced enhancers. ChIPs were performed in duplicate using independent biological samples from either ES cells or thymocytes from ΔRag and ΔRagCD3 mice as indicated. Values were normalized to the enrichment levels recorded at the Actb control promoter and expressed as percentages of the total input. The promoter of the Cd3e gene is shown as an example of promoter region. NR is an intergenic region that is not associated with H3K4 methylations. No significant values were obtained using isotypic IgG controls.
Supplementary Figure S4: H3K4 methylation profiles in mouse pre-pro-B and pro-B cells. ChIP-Seq data were obtained from Lin et al. (2010), and processed as described in Materials and Methods. A) Epigenetic profiles at three distinct loci for which distal regulatory regions have been characterized and are known to be induced during the pre-pro-B to pro-B cell transition. B) Epigenetic profiles at the Cd3e, Cd3d and Cd3g T-cell specific loci.
Supplementary Figure S5: Average profiles of H3K4 methylations at TSS-associated or intergenic regions displaying H3K4me1 and H3K4me3 overlapping peaks using either ChIP-on-Chip (A) or ChIP-Seq (B) data and centered on the middle of the H3K4me3 peak.
Supplementary Figure S6: ChIP-qPCR validation of the H3K4me1 and H3K4me3 enrichments at intergenic regions reported in Figures 4C and D (the name of the associated gene is indicated). ChIPs were performed in duplicate using independent biological samples from either ES cells or thymocytes from ΔRag and ΔRagCD3 mice as indicated. Values were normalized to the enrichment levels recorded in the Actb control promoter, and were expressed as percentages of the total input. No significant values were obtained using isotypic IgG controls.
A

Intergenic H3K4me1 domains

- 20% H3K4me1 only
- 45% + H3K4me2/3 (stable)
- 35% + H3K4me2/3 (dynamic)

B

% of domains

Gained H3K4me2/3

Lost H3K4me2/3

C

Supplementary Figure S7 (Legend is on the next page).
Supplementary Figure S7: Epigenetic changes within distal regulatory regions correlate with transcriptional regulation (based on ChIP-on-chip data). A) Pie diagram indicating the rates of overlap between the H3K4me1 intergenic peaks observed in both ΔRag and ΔRagCD3 thymocytes and the H3K4me2/3 peaks found in either both (stable) thymocyte populations or in a single (dynamic) population. B) Percentage of dynamic H3K4me1 domains associated with either repressed (blue) or induced (red) genes, depending on whether they acquired or lost H3K4me2/3 peaks upon pre-TCR signaling. For this study, we selected the most differentially expressed genes (adjusted P val < 0.01) that were also present in our custom ChIP-on-chip array. C) Four examples of dynamic H3K4me1 domains associated with this type of regulated genes. The legend for the epigenetic profiles is as in Figure S1. The dynamic intergenic peak is indicated by an arrow. D) ChIP-qPCR validation of H3K4 methylations at intergenic regions reported in Figure S7C. ChIPs were performed in duplicate using independent biological samples from either ES cells or thymocytes from ΔRag and ΔRagCD3 mice as indicated. Values were normalized to the enrichment levels recorded within the Actb control promoter and were expressed as percentages of the total input. NR is an intergenic control not associated with H3K4 methylations. No significant values were obtained using isotypic IgG controls.
Supplementary Figure S8: A) Combinatorial H3 methylation at the dynamic H3K4me1 domains. Whether a peak for the indicated histone mark was always absent (white), always present (black), present only in ΔRag (blue) or ΔRagCD3 (red) thymocytes is shown. B-D) Enrichment in binding sites for stage-specific TFs. B) Enriched motifs found in the dynamic H3K4me1 domains that either gained or lost H3K4me2/3 peaks. Best matching transcription factor binding sites and corresponding e-values are indicated. A MEME motif list was generated for all observed H3K4me1/2/3 specific domains from either ΔRag or ΔRagCD3 thymocytes, using default parameters except that the number of motifs was set to 50 and their lengths set to a range of 5 to 15 nt. MEME results were clustered and annotated using the STAMP web-tools, Jaspar V3 and UniProbe Position Weight Matrices (PWM). Only matches showing a p-value ≤10^-3 were taken into account. Subsequently, MEME motifs matching the binding sites of TFs differentially expressed between ΔRag and ΔRagCD3 thymocytes were selected. C) Expression of the genes coding for the transcription factors SPIB and KLF7 in ΔRag or ΔRagCD3 thymocytes, based on our transcriptome data. D) Number of sites found for Motif A and B in the dynamic H3K4me1 domains that either lost or gained H3K4me2/3.
Supplementary Figure S9 (Figure Legend is on the next page).
Supplementary Figure S9: Validation of enhancer activity at H3K4me3-enriched intergenic regions. Luciferase assays were performed using the pGL3 promoter system (containing the SV40 promoter) in order to test for enhancer activity of selected regions. A-B) Examples of intergenic regions that were associated with H3K4me1 and H3K4me3 overlapping peaks (A) or H3K4me1 peaks only (B). The corresponding ChIP-Seq profiles for H3K4me1 and H3K4me3 in ΔRagCD3 thymocytes are shown. The cloned regions are indicated by blue boxes. C) Results of triplicate luciferase assays for the indicated constructs transfected into the EL4 T-cell line. Relative enhancer activity is indicated as the fold enrichment over the pGL3 promoter vector. The statistical significance is indicated (Student's t-Test).
Supplementary Figure S10: Pol II occupancy correlates with enhancer activity. A, C) Pol II occupancy at selected loci in ΔRag and ΔRagCD3 thymocytes. Known distal regulatory regions are highlighted. B) RT-qPCR analysis of the Dntt regulatory region (*) in panel A) in ΔRag and ΔRagCD3 thymocytes as performed in the presence (+) or absence (-) of reverse transcriptase. Relative expression are shown as a percentage of Hprt expression. D) Effect of inhibiting Pol II transcriptional elongation on H3K4me3 enrichment in proximal and distal regulatory regions as tested using the P5424 cell line. To verify the inhibition of transcriptional elongation, H3K36me3 ChIP-on-chip profiling performed from DMSO- (control) or KM05283-treated samples is also shown.
Supplementary Figure S11: A) Percentage of H3K4me1 domains that have acquired or lost H3K4me3 peaks and that were associated with Pol II peaks in ΔRag and ΔRagCD3 thymocytes. B) Average profiles for Pol II binding at intergenic (left panel) or TSS (right panel) genomic regions associated with peaks for H3K4me1 only, with peaks for H3K4me3 only, or with peaks for both histone marks. C) Heatmaps displaying the level of H3K4me3 (left panel) and Pol II (right panel) at intergenic regions associated with overlapping H3K4me1/me3 peaks (5078 regions).