



**Figure S4.** Phylogenetic profiles of D $\beta$  and J $\beta$  RSSs. **(A)** Comparative analysis of 12RSSs from the human and mouse J $\beta$ 1 (left) and J $\beta$ 2 (right) clusters. RSSs are depicted as sequence logos. PWM are represented at individual nt position by the letter's height. **(B)** Cross-species comparison between human, mouse, cow and opossum D $\beta$ 1 (top) and D $\beta$ 2 (bottom) 12- and 23RSSs. The conservation rate of the separate RSS elements across the analyzed species is indicated for each type of RSSs. **(A and B)** Heptamer and nonamer nt matching those in the consensus motifs [d(CACAGTG) and d(ACAAAAACC)] are in red.

All functional human and mouse J $\beta$  and D $\beta$  RSSs were extracted from the IMGT database (<http://imgt.cines.fr/>). The cow and opossum D $\beta$  RSSs were recovered in four steps: (1) the TCR $\beta$  locus in these two species was localized by Blast using three representative mouse V $\beta$  gene segments and the mouse C $\beta$  regions to query the cow and opossum ensembl databases (<http://www.ensembl.org>); (2) the localization of TCR $\beta$  orthologues was confirmed using Blastz (<http://mulan.dcode.org>) to match these putative V $\beta$ - and C $\beta$ -contiguous loci (*i.e.*, within the sequences comprised between +700kb to -150kb upstream and downstream of the

cow and opossum C $\beta$  regions) to those in the mouse TCR $\beta$  sequences; (3) the D $\beta$ 1 and D $\beta$ 2 gene segments were defined within these regions using Blast2seq software (<http://bioweb.pasteur.fr/seqanal/interfaces/bl2seq.html>) and the mouse homologous sequences as a reference; (4) the mouse, cow and opossum sequences were re-aligned using ClustalW (<http://www.ebi.ac.uk/clustalw/>) in order to eventually annotate the D $\beta$  segments and cognate RSSs. The J $\beta$  conservations were computed over all human and mouse J $\beta$  segments. Therefore, D $\beta$  and J $\beta$  conservations are not comparable because of differences in methodology. Graphic representations of nucleotide conservation used Weblogo software (<http://weblogo.berkeley.edu>).