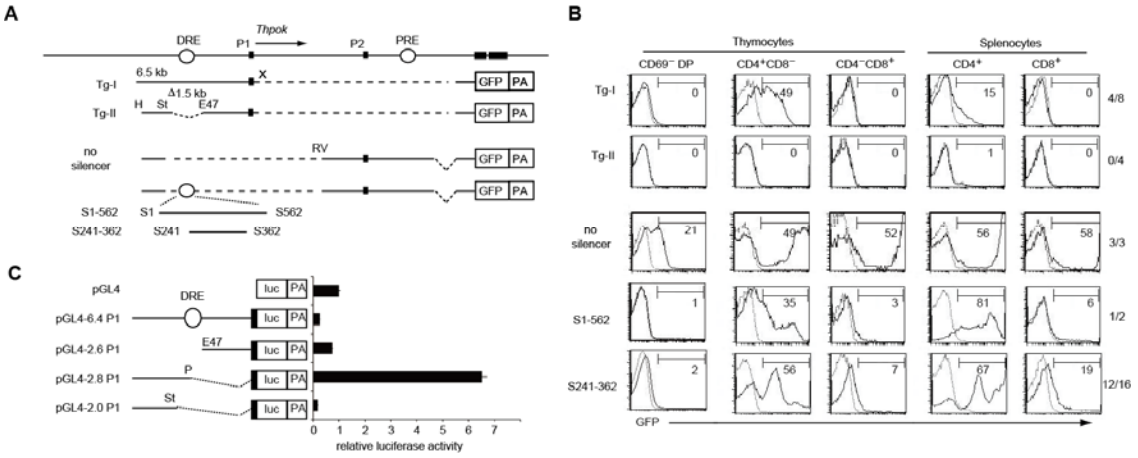
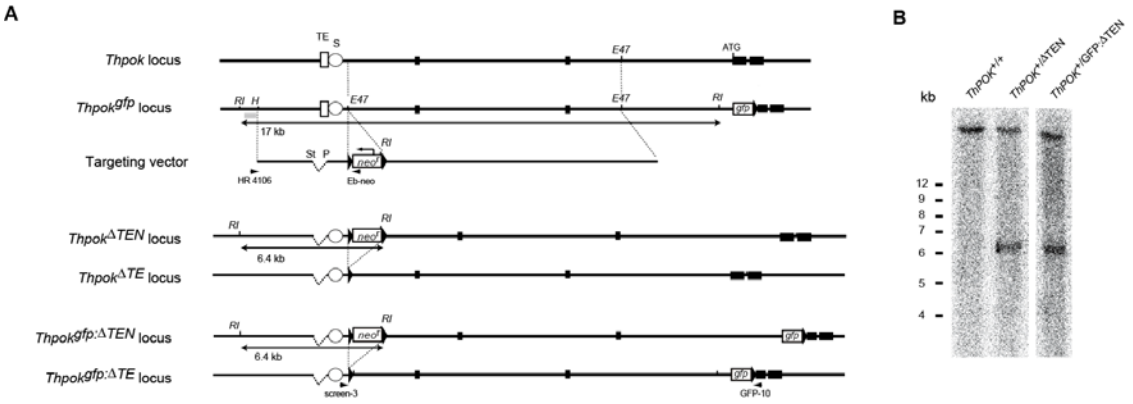


Supplemental Figure S1. Characterization of enhancer sequences in the DRE. *A*. Schematic structure of the murine *Thpok* locus is shown at the top. Black boxes represent exons. Circles represent the distal regulatory region (DRE) and the proximal regulatory region (PRE). Schematic structures of GFP reporter transgenes are indicated. Silencer activity in long S1-562 or short S241-362 fragment is tested in a reporter construct that is driven by a genomic fragment covering 2.0 kb upstream and 3.4 kb downstream region of the P2 promoter. *B.* Histograms showing the GFP expression in the indicated T cell subsets from one representative transgenic founder for each construct. The dashed line indicates non-transgenic littermate control. Numbers in the histogram indicate the percentage of GFP^+ cells. The numbers of transgenic founders expressing GFP among the total transgenic founders are indicated at the right. *C*. Reporter transfection assay. Schematic structures of reporter constructs are shown at the left. Black box, white circle, *luc* and PA represent exon Ia of the *Thpok* gene, DRE, *luciferase* gene and SV40 polyA signal, respectively. The luciferase activity of each construct relative to control pGL4 vector is shown as mean. Data shown are one out of two independent experiments. The restriction sites shown are Eco47III (E47), EcoRV(RV), HindIII (H), PstI (P), StuI (St) and XhoI(X).



Supplemental Figure S2. Targeting strategy for deleting the thymic enhancer (TE) by homologous recombination in ES cells. *A*. Structure of the wild-type *Thpok*, *Thpok*^{gfp}, a targeting vector, targeted *Thpok*$^{\Delta TEN}$, *Thpok*$^{\Delta TE}$, *Thpok*$^{gfp:\Delta TEN}$ and *Thpok*$^{gfp:\Delta TE}$ loci. The targeting vectors shown were designed to delete the StuI-PstI region containing core thymic enhancer. Exons and loxP sequences are indicated as black boxes and black triangles, respectively. Open box marked as TE and circle marked as S represent the thymic enhancer and the Thpok silencer respectively. The DNA probe used in Southern blot analysis is indicated as a gray box. The restriction sites shown are Eco47III (E47), EcoRI (RI), HindIII (H), PstI (P) and StuI (St). In order to determine whether the wild-type *Thpok or Thpok*^{gfp} allele underwent homologous recombination with the targeting vector, targeted ES clones were screened by PCR for the recombination event between the loxP site downstream of the *gfp* gene and the loxP sites upstream of the *neo*^{r} gene upon retroviral Cre recombinase transduction. Arrowheads represent primers used to screen homologous recombination (HR-4106 and Eβ-neo) and to screen target allele (screen-3 and GFP-10). The neomycin resistant (*neo*^{r}) gene was removed in ES cells by transient transfection of an expression vector encoding Cre recombinase. *B*. Southern blots of ES cell clones to confirm homologous recombination.