Supplementary Materials:

Fig. S1 The EACBE insulates the interactions between the left and the right DNA fragments. Quantification of the interactions of 4C data from the left viewpoint and the right viewpoint of EACBE was done with 4C-ker program.
**Fig. S2** The chromatin architecture stripes in non-T cells. A) Hi-C heatmap of mESC, NPC, and neuron cells with 10kb resolution of the region spanning from the proximal Vα region to around 300kb downstream of Cα. B) Hi-C heatmap of mESC-CTCF-auxin cells, in which CTCF was degraded, and control E14D0 cells. It was presented in Hi-C data browser (http://promoter.bx.psu.edu/hi-c/view.php). mESC, NPC and neuron: mouse embryonic stem cells, neural progenitors and cortical neurons from the developing mouse embryonic neocortex. E14D0: Mouse ES cell lines were derived from E14 strain. mESC-CTCF-auxin: CTCF depleted with auxin-inducible degron system for two days.
Fig. S3 EACBE deletion didn’t influence with thymocyte development and TCR expression on surface. A) Percentages of thymocyte subsets and B) DN subsets from 6-week-old wild type and EACBE⁻/⁻ mice were analyzed by using flow cytometry. Data represent mean ± SD of three independent experiments. C) Flow cytometry plot and D) Cell numbers of CD4⁺ and CD8⁺ lymphocytes in spleen of 6-week-old wild type and EACBE⁻/⁻ mice were analyzed by using flow cytometry. The flow cytometry dot plots show gating of CD4⁺ and CD8⁺ cells in CD3⁺ population. E) Flow cytometry showed the TCRβ and CD3 on cell surface. Data are two independent experiments.
**Fig. S4 Activation of EACBE-deleted T cells.** A) Flow cytometry of CD4+ and CD8+ T cell from spleen or lymph node of wild type and EACBE−/− mice before and after 24-hour plate-bound CD3/CD28 stimulate. Cell proportion of activated CD4+ and CD8+ T cells from B) spleen and C) peripheral lymph node after 24-hour stimulate. Data are representative of seven (spleen) or four (PLN, peripheral lymph node) independent experiments (one mouse per experiment). * P < 0.05 by two-side Student’s T test.
**Fig. S5 The repertoire of Tcrb and Tcrd in EACBE−/− thymocytes.** Relative clonotype numbers of A) Jβ, B) Vβ genes and C) heatmap of Vβ–Jβ rearrangements determined by deep sequencing of Tcrb transcripts amplified by 5’RACE of wild type and EACBE−/− mice respectively. Data are representative of two independent experiments. D) Vδ usage determined by high-throughput sequencing of Tcrb transcripts amplified by 5’RACE of wild type and EACBE−/− mice respectively. Data are mean ± SD of two experiments. * P < 0.05, ** P < 0.01 by two side Student’s T test.
Fig. S6 EACBE deletion didn’t influence thymocyte survival. A) Flow cytometry plot of apoptosis assay of thymocytes cultured on 0, 6 hours, 24 hours, and 48 hours in medium with 10% FBS. Data are representative of three independent experiments. B) Apoptosis cell percentage and C) survival cell percentage of thymocytes after 0-hour, 6-hour, 24-hour-s, and 48-hour cultures. The data represent mean of three experiments with normalization to the 0-hour.
Fig. S7 EACBE is involved in chromatin organization of the Tcra-Tcrd locus. A) H3K4me3 and H3K27ac ChIP-seq on the Actb gene locus in Rag2<sup>−/−</sup> (WT) and Rag2<sup>−/−</sup> x EACBE<sup>−/−</sup> (KO) DP thymocytes from anti-CD3 injected mice. Data are representative of two independent experiments. B) Heatmap and subtraction heatmap of 4Mb region on Chromosome 14. The 10 kb binned Hi-C data of DP thymocytes were generated from anti-CD3 injected EACBE<sup>+/−</sup> x Rag1<sup>−/−</sup> and EACBE<sup>−/−</sup> x Rag1<sup>−/−</sup> mice. 4C data normalized using 4C-ker program from C) EACBE right and D) INT viewpoint in CD3-stimulated-DP thymocytes of WT and EACBE<sup>−/−</sup> mice at Rag2<sup>−/−</sup> background. It was analyzed with two independent replicates. Filled circles highlight significant differences.
Fig. S8 EACBE mediates interactions of the Eα with the genes in the downstream sub-TAD. 4C data normalized using 4C-ker program from EACBE right and INT viewpoint in CD3-stimulated-DP thymocytes of WT and EACBE−/− mice at Rag2−/− background. It was analyzed with two independent replicates. Filled circles highlight significant differences.
| Primers       | Sequence                                                                 | Use/figure |
|--------------|---------------------------------------------------------------------------|------------|
| musActbp F   | 5'-GCTGTGGGCTCTATAAAACC                                                   | F2B,C;F4B,C;F5D |
| musActbp R   | 5’-CAACGAAGGGAGCTGCAAAGAA                                               | F2B,C;F4B,C;F5D |
| MageA2C F    | 5'-AACGTTTTGTGAACGTCCTGAG                                                | F2C, F5B,C |
| MageA2B R    | 5’-GACGCCTCAGAAACAAATGGC                                                 | F2C, F5B,C |
| mus Ea F     | 5’-CTGACATGGGCAAACAGGTC                                                  | F2C; F4B,C |
| mus Ea R     | 5’-GTGGCCCGAGAGATCCTTAT                                                 | F2C; F4B,C |
| Ea50k/CBE50k F | 5’-AGGACCTTGCCACAACCTCTG                                                   | F2B; F5D |
| Ea50k/CBE50k R | 5’-GCTCTCCCTGAATCTGTG                                                   | F2B; F5D |
| ChIP TEAp F  | 5’-ATGGGAAAGGGAGGGGATGA                                                  | F4B,C |
| ChIP TEAp R  | 5’-GCTCAAGAGGACACTGGAAGG                                                 | F4B,C |
| ChIP Trav17-F | 5’-TCCCCAGTGACCACCTCTG                                                  | F4B,C |
| ChIP Trav17-R | 5’-TGTCCTGTTGTGAGTTCTCTG                                                  | F4B,C |
| ChIP Trav21-F | 5’-TGTCGGGTTGCTGCTTGAG                                                   | F4B,C |
| ChIP Trav21-R | 5’-AACCTTACCCAAGGCCAGAG                                                  | F4B,C |
| ChIP Trdv2-2F | 5’-TCCTGTTTGAAGGGTACAG                                                  | F4B,C |
| ChIP Trdv2-2R | 5’-AGCCTTTCAACAGAGAGG                                                   | F4B,C |
| ChIP Trdd1 F | 5’-TACGGCTGTGTTTCACTG                                                  | F4B,C |
| ChIP Trdd1 R | 5’-GCTCAAGAGGACACTGGAAGG                                                 | F4B,C |
| ChIP Trdj1 F | 5’-AGCTGCTGAGGTTTTGGAATG                                                | F4B,C |
| ChIP Trdj1 R | 5’-ATCCCTCAGACCTAACCAG                                                  | F4B,C |
| ChIP Trdj2 F | 5’-GCTGGTCCACAGACTGTTATCT                                               | F4B,C |
| ChIP Trdj2 R | 5’-AACCTTACCCAAGGCCAGAG                                                  | F4B,C |
| ChIP Trdv5 F | 5’-CTGGACTCTCTTTAACCACATC                                                | F4B,C |
| ChIP Trdv5 R | 5’-TCCCTGAGGATAGGTTACTACC                                                | F4B,C |
| ChIP Traj61 F | 5’-GCCATGACTGGAAGAGACTCAT                                                | F4B,C |
| ChIP Traj61 R | 5’-TCCATATTTTTGTTTTACTATTCTCCTGAG                                      | F4B,C |
| ChIP Traj58 F | 5’-TGGGCTCAAGCTGCTATTGG                                                  | F4B,C |
| ChIP Traj58 R | 5’-TGGACTGAGCTGATTTTGGAATG                                               | F4B,C |
| ChIP Traj47 F | 5’-GCTGGGAAACCATTGTTGAG                                                  | F4B,C |
| ChIP Traj47 R | 5’-CACCTTACCCAAGCTTTTGGT                                                | F4B,C |
| ChIP Traj18 F | 5’-AGAGCGGACAGAGACTGTT                                                  | F4B,C |
| ChIP Traj18 R | 5’-TATCTACACAGTGGCCAGGCC                                                 | F4B,C |
| ChIP Traj7 F  | 5’-GTGCTCTACAGACCTCCTACA                                                | F4B,C |
| ChIP Traj7 R  | 5’-AAAAACGACACATTTCCGCT                                                  | F4B,C |
| ChIP Traj2 F  | 5’-TCTAAGGAGTAGTAGGATGAGGC                                               | F4B,C |
| ChIP Traj2 R  | 5’-GGTCCCTTTCCTCCGGAATGTTA                                               | F4B,C |
| Ea 3’ F      | 5’-TCCCCAGGGGATACCTGTTA                                                  | F2B; F5D |
| Ea 3’ R      | 5’-ACCCCTTTTGCCATTTCTTAT                                                 | F2B; F5D |
| Dad1 CBE F   | 5’-CAGCACAGGTTGAGGAAGACA                                                 | F5D |
| Dad1 CBE R   | 5’-GACCAGGGGTTTCTTCTCAT                                                  | F5D |
| ACTB CTCF F  | 5’-ACGATGGGAGGGGGAATACAG                                                 | F5D; F6D; F6E |

Table S1: the primer sequences used in the paper.
| Gene   | Forward Primer | Reverse Primer |
|--------|---------------|----------------|
| ACTB   | 5'-TGATAGTTCGCCATGGATGAC | F5D; F6D; F6E |
| CTCF   | 5'-GAGCTCAAGTACTCCAGAG | F5D; F6D; F6E |
| Dad1_p | 5'-GTATCCGAAGTCACCGTGTT | F5D; F6D; F6E |
| Abdh4_p| 5'-TCAAGTTCTCCGAGATGAG | F5D; F6D; F6E |
| Prmt5_p| 5'-CCCAGATTCAGCTCTCCAGT | F5D; F6D; F6E |
| Ajuba_p| 5'-GAGCTCAAGTACTCCAGAG | F5D; F6D; F6E |
| Cdh24_p| 5'-TCAAGTTCCTCCGAGATGAG | F5D; F6D; F6E |
| Prmt5_p| 5'-CCCAGA TTCAGCCTTCCAGT | F5D; F6D; F6E |
| Ajuba_p| 5'-GAGCTCAAGTACTCCAGAG | F5D; F6D; F6E |
| Cdh24_p| 5'-TCAAGTTCCTCCGAGATGAG | F5D; F6D; F6E |

| DNA Jα usage |
|---------------|
| Trav12F | 5'-GCCAGCAGTCTCTCCATC | F3D |
| Trav13F | 5'-AGGAGCTCGAGCTCTTTTGG | F3D |
| Trav14F | 5'-GGTGGATCTGAGCCACCTACT | F3D |
| Trav17F | 5'-CCAGCCCCATTTGAGCGACT | F3F |
| Trav19F | 5'-CACCTACAGCTCCACGCTT | F3D |
| Trav21F | 5'-CACCTACGCTCCACAGCTT | F3F |
| Traj61R | 5'-ATGAGCTCTCAGTCACTGG | F3D,F |
| Traj57R | 5'-AGCCTACTCAGCTCTTTTGG | F3D,F |
| Traj53R | 5'-GGAGTCACAGTTAAGAGAGTTCC | F3D |
| Traj49R | 5'-GGAATTGCAAGTCAAACCTTGGTTCC | F3D |
| Traj40R | 5'-TGATGATTCTGCTCCAAAGACG | F3D |
| Traj37R | 5'-AAATGAGCAATAGCGAGCAG | F3D |
| Traj31R | 5'-GGCTCCCATACACCAAGAAG | F3D |
| Traj17R | 5'-TGATGCCGCTCTCCTCTTTC | F3D |
| Traj2R  | 5'-GCGCCGAGGCTGCAATGGT | F3D |
| Actb-pF | 5'-CGCCATAGTGATGACATTCG | F3D |
| Actb-pR | 5'-CGCCATAGTGATGACATTCG | F3D |

| 5' RACE |
|---------|
| 5' PCR IIA A501 | 5'-GAGCTCAAGTACTCCAGAG | F3A,B |
| 5' PCR IIA A502 | 5'-TGCTAAGTGACAGCTGTGGTG | F3A,B |
| 5' PCR IIA A503 | 5'-TGTTTCTTAAGTGACAGCTGTGGTG | F3A,B |
| 5' PCR IIA A504 | 5'-TAAGACACAACAGTGATGGATCAACGCA | F3A,B |
| 5' PCR IIA A505 | 5'-CTAATCGAAAGCAAGCTGTGGTG | F3A,B |
| 5' PCR IIA A506 | 5'-CTAATCGAAAGCAAGCTGTGGTG | F3A,B |
| 5' PCR IIA A507 | 5'-TAAGTTCCAGTGTCAGGTGGATCAACGCA | F3A,B |
| 5' PCR IIA A508 | 5'-TAAGCTTAAAGGAGTGGATCAACGCA | F3A,B |
| TRAC-R (N701) | 5'-GAGCTCAAGTACTCCAGAG | F3A,B |
| TRBC-R (N701) | 5'-GAGCTCAAGTACTCCAGAG | S3A,B |
| TRDC-R (N701) | 5'-GAGCTCAAGTACTCCAGAG | S5D |
| TRAC-R (N702) | 5'-GAGCTCAAGTACTCCAGAG | F3A,B |
| TRBC-R (N702) | 5'-GAGCTCAAGTACTCCAGAG | S5A,B |
| TRDC-R (N702) | 5'-GAGCTCAAGTACTCCAGAG | S5D |
TRAC-R (N703)  5’-AGGCAGAAACACAGCAGGTTCTGGGTTC  F3A,B
TRBC-R (N703)  5’-AGGCAGAAAGTGAGTACATTTCTCAG  S5A,B
TRDC-R (N703)  5’-AGGCAGAAAGAACAGATGGTTGGGCG  S5D
TRAC-R (N704)  5’-TGCTGAGCACAGCAGGTTCTGGGTTC  F3A,B
TRBC-R (N704)  5’-TGCTGAGCGGTGGAGTCACAAGGCTCAG  S5A,B
TRDC-R (N704)  5’-TGCTGAGCGAAAACAGATGGTTGGGCG  S5D
TRAC-R (N705)  5’-CGACTCGAAGAAGAGTTGGGCG  S5D
TRBC-R (N705)  5’-CGACTCGAAGGTGGAGTCACAGGCTCAG  S5A,B
TRDC-R (N705)  5’-CGACTCGAAGAAAACAGATGGTTGGGCG  S5D
TRAC-R (N706)  5’-TCCTGAGCACACAGCAGGTTCTGGGTTC  F3A,B
TRBC-R (N706)  5’-TCCTGAGCGGTGGAGTCACAAGGCTCAG  S5A,B
TRDC-R (N706)  5’-TCCTGAGCGAAAACAGATGGTTGGGCG  S5D
TRAC-R (N707)  5’-GGACTCCTACACAGCAGGTTCTGGGTTC  F3A,B
TRBC-R (N707)  5’-GGACTCCTACGGTGGAGTCACAAGGCTCAG  S5A,B
TRDC-R (N707)  5’-GGACTCCTACAAAACAGATGGTTGGGCG  S5D
TRAC-R (N708)  5’-TAGGCATGACACAGCAGGTTCTGGGTTC  F3A,B
TRBC-R (N708)  5’-TAGGCATGGGTGGAGTCACAAGGCTCAG  S5A,B
TRDC-R (N708)  5’-TAGGCATGGAAAACAGATGGTTGGGCG  S5D
TRAC-R (N709)  5’-GGACTCCTACACAGCAGGTTCTGGGTTC  F3A,B
TRBC-R (N709)  5’-GGACTCCTACGGTGGAGTCACAAGGCTCAG  S5A,B
TRDC-R (N709)  5’-GGACTCCTACAAAACAGATGGTTGGGCG  S5D
TRAC-R (N710)  5’-TGCAGGCTGACACAGCAGGTTCTGGGTTC  F3A,B
TRBC-R (N710)  5’-TGCAGGCTGGGTGGAGTCACAAGGCTCAG  S5A,B
TRDC-R (N710)  5’-TGCAGGCTGGAAAACAGATGGTTGGGCG  S5D

DSB linker1  5’-GGCTTCGCCGATCTGAATTC  F3E
DSB linker2  5’-GTCATTCAAGATC  F3E
Linker primer  5’-CCGGGAGATCTGAATTCCAC  F3E
DSB Traj61 primer  5’-TCGGAGAGAGGAGTGCTG  F3E
DSB Traj61 probe  5’-TGAGGAACACGGAGTATCTC  F3E
DSB Traj27 primer  5’-ATGGCAGATAGAATGGAGCGG  F3E
DSB Traj27 probe  5’-CTGGCGGTGGAAAGACTATTG  F3E
DSB Traj18 primer  5’-CTGCGGCGGTTGAAAGACTATTG  F3E
DSB Traj18 probe  5’-TAAGCTCAGAGCGGACAGAA  F3E
DSB Traj6 probe  5’-GACCAATGGAAAGGAGGT  F3E
DSB Traj6 primer  5’-ATCAGACCAGACTGCTGCCCC  F3E
DSB Traj2 primer  5’-GTGGAGCTCCACAGCAGCAGCAG  F3E
DSB Traj2 probe  5’-GACCAATGGAAAGGAGGT  F3E
Cd14 F  5’-GCTCAAACTTTCAGAATCTACCGAC  F3E
Cd14 R  5’-AGTCAGTTCGTGGAGGGCGGAAATC  F3E

Germline transcription

GT-Trav17 F  5’-TGGAGCGGACTCAGCAGACAGGAAATCT  F4D
GT-Trav17 R  5’-CGTGCACAGAAGGTCTCAGG  F4D
GT-Trav19 F  5’-CCAGCCTTCAAGAGACAGCAGCAG  F4D
GT-Trav19 R  5’-AGGTCGGACTGACAGGTCCTTTTGT  F4D
GT-Trav21 F  5’-CGGCTGTGTACCACTGTATCTCAGCAGCAG  F4D
GT-Trav21 R  5’-CTGCGGCGGTTGAAAGACTATTG  F4D
GT-Trdj1 F  5’-ATCAGACCAGACTGCTGCCCC  F4D
GT-Trdj1 R  5’-ATCCCTCACCACTGCTCTTTC  F4D
GT-Trdv5 R  5’-AGTCAGTTCGTGGAGGGCGGAAATC  F4D
| Gene expression          | Primers                                      |
|--------------------------|----------------------------------------------|
| Actb mRNA F              | 5' - ACACCCGCCACCAAGTTCC                     |
| Actb mRNA R              | 3' - TACACCCGCCACCAAGTTCC                   |
| Dad1 mRNA F              | 5' - TGTGGGAGCAGCTCCATCCTAG                 |
| Dad1 mRNA R              | 3' - GTGTGGGAGCAGCTCCATCCTAG                |
| Abhd4 mRNA F             | 5' - TGGAGCAGCGATCCTCCAG                    |
| Abhd4 mRNA R             | 3' - CATCACCAGAGGGGTGCGAT                   |
| Prmt5 mRNA F             | 5' - GGTGTGGTGTTGCTTCCGATG                  |
| Prmt5 mRNA R             | 3' - GCCATCTCCCCACCAAGCAT                   |
| Ajuba mRNA F             | 5' - TGCTCTGCCCCCATAGATACCT                 |
| Ajuba mRNA R             | 3' - GTCTCTGCCCCCATAGATACCT                 |
| Cdh24 mRNA R             | 5' - CTGCTGGGGCTGCTGAGCCAG                 |
| Cdh24 mRNA F             | 3' - GGGCCAGATCCTCGCCAGGT                  |
| Acin1 mRNA F             | 5' - GATGAGACGCGCAGTCCCTCT                  |
| Acin1 mRNA R             | 3' - CCCGTTCCCGCAGCAAGCA                   |
| Homez mRNA F             | 5' - AGCAAGTGCTCATTTCCATTCC                 |
| Homez mRNA R             | 3' - AGCAAGTGCTCATTTCCATTCC                 |
| Pabpn1 mRNA F            | 5' - TCAAGAGCTGAGTCAGGAGGA                 |
| Pabpn1 mRNA R            | 3' - ACGTAGATAGACGGGCATCA                  |
| Ngdn mRNA F              | 5' - CACTGAGACAAAGTCTCGAGC                 |
| Ngdn mRNA R              | 3' - AGAGGCGCTTGGCCAGGATAG                 |
| 4C primer                |                                              |
| Ea-MboII up:             | 5' - TGGCGAAGATGAGTTGACCTTGTGATC           |
| Ea-NlaIII2 up:           | 5' - CAGGCAGAGAAGCTCTCGAGC                 |
| Ea-MboII I down:         | 5' - TGCCCATCATCCAGGTTGACATC               |
| Ea-NlaIII2 I down:       | 5' - CTGGGAGTTTGGCTCAGACCTCGT              |
| 4C TEAp MboI:            | 5' - ACACCTCTTTACACAGCTGTGATC              |
| 4C TEAp NlaIII:          | 5' - GCGTTCTGATTTCTCTGACTTTC               |
| 4C TARV17 MboI:          | 5' - CATTCCTCCAGGATTCAGTGTGATC             |
| 4C TARV17 NlaIII:        | 5' - GAAATGGAAAGGAGAAAGGGGT                |
| 4C INT MboI:             | 5' - GATCTCAACAAAGCAGCTGCTGATC             |
| 4C INT NlaIII:           | 5' - GTTTCCTGAGTTGGAGTAGC                  |
| 4C EACBE right Mbo1 up | 5' - GCCTGGGTCCTGCTAAGGATC | S7D |
|------------------------|-----------------------------|-----|
| 4C EACBE right Nla3 up:| 5' - GGCAGAGAGCTAGACAGATGTAGT | S7D |