Regulatory T (Treg) cells are engaged in sustaining immunological self-tolerance and homeostasis. While the transcription factor Foxp3 plays a key role in Treg cell development, a substantial portion of Treg-specific gene regulation and epigenetic modifications are progressively established irrespective of Foxp3. It is thus still obscure how Treg cell development initiates and progresses in the thymus. With recent findings of super-enhancers, which are associated with cell lineage determination in various cell types, we first identified Treg cell-specific super-enhancers (Treg-SEs). The activation of Treg-SEs developmentally began in Treg progenitor cells, preceding the expression of Foxp3, and facilitated the early induction of the associated genes. Furthermore, we identified the genome organizer Satb1 as one of the key factors regulating Treg-SE activation. At thymic CD4⁺CD8⁺ double positive stage, a portion of Satb1-binding regions retained closed chromatin status, but they afterward became open and forming Treg-SEs along Treg cell development. In addition, T cell-specific deletion of Satb1 showed prominent defects with Treg-SE activation at the precursor stage and subsequently hindered both Treg-specific epigenetic changes and gene regulations including Foxp3 expression. The consequent arrest of Treg cell differentiation resulted in the development of severe autoimmunity. Our results thus demonstrate that Satb1-dependent Treg-SE activation is prerequisite for the development of Treg cells, and that defects of chromatin configuration would contribute to the susceptibility to autoimmune diseases.