

Description of Additional Supplementary Files

Supplementary Data 1. A complete list of all the peptide families identified in the MHC-II peptidome of the thymus. Each family represents a protein region (sequenced depicted) that was identified in three independent peptidome analyses.

Supplementary Data 2. A complete list of individual peptide sequences identified in the thymic MHC-II peptidome. This table contains individual MHC-II-bound peptides corresponding to peptide families shown in Supplementary Data 1. The peptides were merged from the three peptidome analyses.

Supplementary Data 3. Tissue localization of the source proteins for the identified MHC-II-bound peptides in the thymic peptidome. Tissue localization was analyzed by the DAVID tool. A total of 971 source proteins were assigned to different peripheral tissues as determined by $FDR < 0.05$.

Supplementary Data 4. PTAs identified in the thymic MHC-II peptidome correspond to transcripts shown to be regulated by Aire, Fezf2, and Chd4 in mTECs. The regulation by Aire, Fezf2, and Chd4 was assessed by correlating the source proteins of the MHC-II peptides in the thymic peptidome to genes that significantly diminished in mTECs deficient with each respective regulator, using the cut-off criteria in the original study (Tomofuji et al., Nature Immunology 2020). Each PTA was also mapped to different peripheral tissues using the DAVID tool, with a cut-off of $FDR < 0.05$. This table is a complete list of the data shown in Figure 4d.

Supplementary Data 5. Antibody information. The table summarizes the sources, catalogue numbers, and dilutions of antibodies used in this study.