Figure S1. Stereo electron density for critical parts of the crystal structure. A stereo image (wall-eyed) of the 2Fo-Fc electron density map contoured at $1\sigma$ is shown for the P2 to P5 segment of the MBP$_{85-99}$ peptide, part of the CDR3$\alpha$ loop, and DQ$\beta$ His81.
Figure S2. Positioning of the $V_\alpha$ and $V_\beta$ domains of Hy.1B11 TCR on the MHC molecule. The centers of mass of both $V_\alpha$ and $V_\beta$ domains of Hy.1B11 TCR are shown as red spheres and compared with other human (HA1.7, Ob.1A12) and mouse (D10, B3K506, 1934.4) MHC class II restricted TCRs.
Figure S3. The MBP_{85-99} peptide binds in the same register to DQ1 and DR2. (a and b) The binding frame of the MBP_{85-99} peptide bound to DQ1 (a) and DR2 (b) is compared with MHC molecules as a ribbon representation and bound peptides as a stick model. Molecules are colored according to B-factor. (c) Superposition of the MBP_{85-99} peptide bound to DQ1 and DR2. The DQ1-bound peptide is shown in the red, the DR2-bound peptide in green. Residues in the P1 to P5 peptide segment critical for MHC binding and TCR recognition are labeled.
Figure S4. Specificity of surface plasmon resonance–based measurement of Hy.1B11 binding to DQ1–MBP<sub>85–99</sub>. 25 µM Hy.1B11 (line) or Ob.1A12 (triangle) TCR monomer were injected over a flow cell with immobilized DQ1-CLIP (550 RU), followed by a flow cell with immobilized DQ1-MBP<sub>85–99</sub> (550 RU) at a flow rate of 15 µl/min. TCR was injected for 1 min at 25°C. The signal from the DQ1-CLIP reference flow cell was subtracted from the DQ1-MBP<sub>85–99</sub> flow cell.
Figure S5. Equilibrium binding analysis of Hy.1B11 TCR mutants. Concentration series for equilibrium binding analysis of Hy.1B11 mutants measured by surface plasmon resonance (a–h). Nine serial dilutions of each mutant were injected over the DQ1-CLIP and DQ1-MBP85-99 flow cells, and the signal from the DQ1-CLIP control flow cell was subtracted from the signal of the DQ1-MBP85-99 flow cell. Noted affinity values represent a mean from two experiments.
Figure S6. Alignment of microbial peptides that stimulate the Hy.1B11 T cell clone with the MBP self-peptide sequence. The DQ1 anchor residues (P1 and P4) are indicated, and peptide positions important for Hy.1B11 TCR recognition (P2, P3, and P5) are highlighted.

Figure S7. Comparison of the placement of TCR-α germline encoded loops by the human autoimmune Ob.1A12 TCR and other MHC class II restricted TCRs. The position of the TCR-α CDR1 and CDR2 loops on the MHC class II β1 helix is compared among four TCRs, the human Ob.1A12 (a) and HA1.7 (b) TCRs and the murine B3K506 (c) and D10 (d) TCRs. The CDR1α and CDR2α loops, as well as their interacting MHC residues, are colored yellow and red, respectively, MHC molecules in blue, and peptides in gray. All other TCR CDR loops are colored green to visualize the overall positioning of the TCR on the pMHC surface.
**Table S1.** Data collection and refinement statistics (molecular replacement)

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<td><strong>Redundancy</strong></td>
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X-ray crystallographic data and model refinement statistics for the complex of Hy1B11 TCR and DQ1-MBP₂₁₋₃₂.
Table S2. Sequence of Hy.1B11 CDR3β loop is shorter than for most previously crystallized TCRs.

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Alignment of CDR3 regions of Hy.1B11 TCR (highlighted in red) with 29 other published TCRs. The CDR3 loops are in bold.