

## IMMUNOLOGY

# Pre-T cell receptors topologically sample self-ligands during thymocyte $\beta$ -selection

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Self-discrimination, a critical but ill-defined molecular process programmed during thymocyte development, requires myriad pre-T cell receptors (preTCRs) and  $\alpha\beta$ TCRs. Using x-ray crystallography, we show how a preTCR applies the concave  $\beta$ -sheet surface of its single variable domain ( $V\beta$ ) to “horizontally” grab the protruding MHC  $\alpha 2$ -helix. By contrast,  $\alpha\beta$ TCRs purpose all six complementarity-determining region (CDR) loops of their paired  $V\alpha V\beta$  module to recognize peptides bound to major histocompatibility complex molecules (pMHCs) in “vertical” head-to-head binding. The preTCR topological fit ensures that CDR3 $\beta$  reaches the peptide’s featured C-terminal segment for pMHC sampling, establishing the subsequent  $\alpha\beta$ TCR canonical docking mode. “Horizontal” docking precludes germline CDR1 $\beta$ - and CDR2 $\beta$ -MHC binding to broaden  $\beta$ -chain repertoire diversification before  $\alpha\beta$ TCR-mediated selection refinement. Thus, one subunit successively attunes the recognition logic of related multicomponent receptors.

**P**luripotent progenitors seed the thymus and undergo massive expansion in a precisely orchestrated manner (1). Proliferation at the early CD4<sup>+</sup>CD8<sup>-</sup> double-negative (DN) thymocyte stages (DN1, DN2, and DN3a) is driven by stem cell factor, interleukin-7, and Notch (2, 3). Productive T cell receptor (TCR)- $\beta$ -chain rearrangement and surface pre-T cell receptor (preTCR) expression begins a second stage of proliferation at DN3b with transitioning to DN4, immature single-positive thymocytes, and early CD4<sup>+</sup>CD8<sup>-</sup> double-positive (DP) thymocyte blasts. This second stage, referred to as  $\beta$ -selection, is required for  $\alpha\beta$  T-lineage commitment, allelic exclusion to restrict  $\beta$ -chain clonotypes to one per cell, and the development of thymocytes expressing  $\alpha\beta$ TCRs (4, 5).

PreTCRs each comprise a unique somatically rearranged TCR $\beta$  gene product disulfide-linked to an invariant transmembrane glycoprotein, pT $\alpha$ , which is thymocyte-specific. pT $\alpha$  lacks a ligand-binding variable domain, but has one membrane-proximal constant domain, which pairs with the  $\beta$ -chain constant domain (6). Although early studies suggested that preTCRs do not require ligands (7–10), instead signaling autonomously through site-specific self-oligomerization (11, 12), sequence analysis across mammalian species has refuted this model (13, 14). Notch signaling, in part, mimics preTCR signaling to explain this paradox (15). More recently, it has been shown that peptides bound to major histocompatibility complex molecules (pMHCs) are preTCR ligands, promoting thymocyte development through interactions with  $V\beta$  (16–20). After preTCR triggering, pT $\alpha$  transcription is shut off, Notch signaling is blunted, and TCR $\alpha$  transcription is initiated such that each pT $\alpha\beta$  heterodimer can be replaced by a TCR $\alpha\beta$  heterodimer (21). Consequently, the same  $\beta$ -chain is paired with an  $\alpha$ -chain at the major DP thymocyte stage. Each TCR $\alpha\beta$  clonotype creates a cognate pMHC ligand specificity with its  $V\alpha V\beta$  module. Desirable TCRs foster thymocyte survival, whereas autoreactive TCRs induce thymocyte apoptosis consequent to self-pMHC interaction (22).

Both the recognition logic for the obligate preTCR to  $\alpha\beta$ TCR transition and the impact of the preTCR-self-pMHC interaction on T-lineage repertoire formation have been elusive. In this report, we present crystal structures of a preTCR $\beta$  chain, the sole ligand-binding subunit of the preTCR, in complex with pMHC ligands, and accompanying functional data. The  $\beta$  chain used was N15 $\beta$  and its ligand

vesicular stomatitis virus octapeptide (VSV8) or variant bound to a truncated version of a class I MHC (MHC-I) K<sup>b</sup> molecule (called K<sup>b</sup>-t2) comprising the peptide-binding  $\alpha 1\alpha 2$  domains. To facilitate N15 $\beta$ -pMHC cocrystallization, we covalently linked N15 $\beta$  with K<sup>b</sup>-t2 (figs. S1 and S2). The representative structure of N15 $\beta$ -VSV8-K<sup>b</sup>-t2 revealed the topological fitting by which the concave C’C’CFG  $\beta$ -sheet face of the Ig-like N15V $\beta$  domain grabs the apex of the convex  $\alpha 2$  helical region of K<sup>b</sup>-t2 (Fig. 1A). Closed- and open-book surface views (Fig. 1B) as well as detailed sample-related analyses (figs. S2 to S5 and tables S1 to S3) further delineated this fitting.

TCR $\alpha\beta$  heterodimers “vertically” bind a pMHC molecule in a canonical mode, with  $V\alpha$  and  $V\beta$  domains contacting N- and C-terminal segments of a peptide, respectively (23, 24). By contrast, in the structure of the N15 $\beta$ -VSV8-K<sup>b</sup>-t2 complex, N15 $\beta$  bound to pMHC in a “horizontal” docking mode (Fig. 2A) with the CDR3 $\beta$  loop contacting the C terminus of the peptide. In the vertical N15 $\alpha\beta$ -VSV8-K<sup>b</sup> binding, all six complementarity-determining region (CDR) loops from both  $\alpha$  and  $\beta$  subunits made contact in a head-on fashion (Fig. 2A). The TCR $\alpha\beta$ -pMHC interface is generally flat with an  $\sim 1240$ - to  $2020$ - $\text{\AA}^2$  buried surface area (BSA) (23). Had a single TCR $\beta$  subunit assumed the same vertical binding to pMHC using its three CDR loops, the BSA would be only around  $900$ - $1000$   $\text{\AA}^2$ , considerably less than the average protein-protein interaction (PPI) value of  $1600 \pm 400$   $\text{\AA}^2$  (25). Horizontal topological fitting offered a BSA of  $1379$   $\text{\AA}^2$  (table S4). A composite view of the distribution of CDR loops and footprints of a preTCR $\beta$  versus TCR $\alpha\beta$  binding on the same K<sup>b</sup> molecule was then generated (Fig. 2B). Despite different approaches to pMHC (Fig. 2C), both preTCR and TCR $\alpha\beta$  were capable of associating with the same CD3 signaling subunits (fig. S6).

The inherent left-handed twist of the  $\beta$ -sheet platform of an MHC-I molecule caused the  $\alpha 1$  and  $\alpha 2$  helices on top of the sheet to form a convex point on the peptide-binding groove, most prominently for the  $\alpha 2$ -helix (Fig. 1A) (24). The twisted C’C’CFG  $\beta$  sheet of the N15 $\beta$  V-domain contoured as a concavity (Fig. 1A). Convex/concave topological fitting should be a feature of all preTCRs binding to pMHC. The binding-geometry positions CDR3 $\beta$  to contact the pMHC while orienting CDR1 $\beta$  and CDR2 $\beta$  loops away from the interface (Figs. 1A, 2C, and 3A and table S5). Only after TCR $\beta$  pairs with TCR $\alpha$  at the DP thymocyte stage does the resultant TCR $\alpha\beta$  heterodimer mediate potential CDR1 and CDR2 interactions with pMHC. The topological fitting skews N15 $\beta$  binding toward the peptide C-terminal segment in the MHC groove (Fig. 3B), suggesting that TCR $\alpha\beta$  canonical docking polarity onto pMHC (23, 24) is preset at the  $\beta$ -selection stage.

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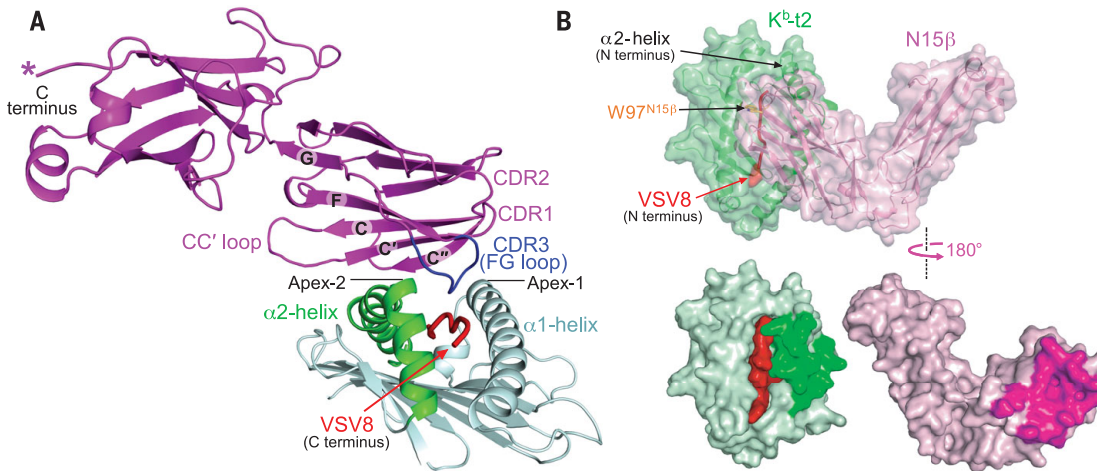
The C-terminal half of the bound peptide is the most structurally featured segment of pMHC-I ligands, as exemplified by overlaying four  $K^b$  bound peptides on the VSV8- $K^b$ -t2 structure (Fig. 3C). When loaded in an MHC-I molecule, a peptide's two termini are fixed in the groove by mainchain hydrogen bonds to conserved MHC residues (26). Beginning at the p4 residue, the peptides bulge out and diverge in backbone conformations (Fig. 3C). Topological fit enabled preTCRs to recognize this featured C-terminal backbone display, com-

plementing the limited number of exposed peptide side chains to shape the  $\alpha\beta$ TCR repertoire as described below. A featured peptide conformation applied to MHC-II (fig. S7 and table S6) suggested analogous preTCR recognition. By contrast, certain nonclassical MHCs lack a regular peptide-binding groove (fig. S7).

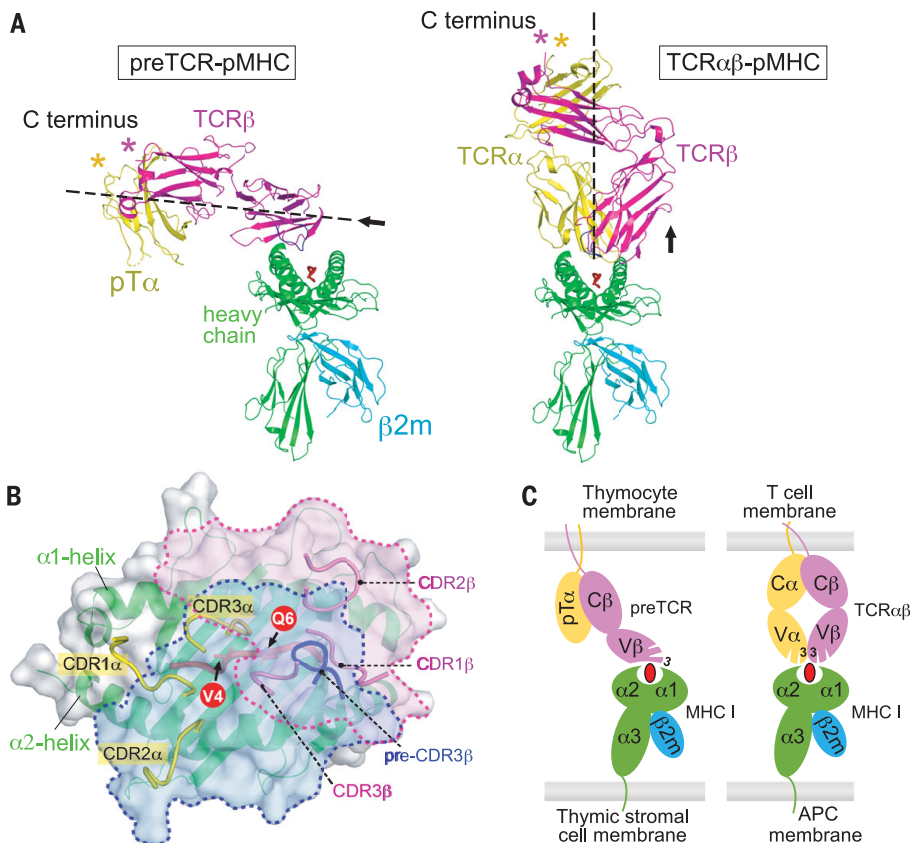
The N15 $\beta$ -VSV8- $K^b$ -t2 interface had two distinct areas (Fig. 3, A and D): (i) a large hydrophobic patch on the N15 $\beta$  C''C-face like the "palm" of a hand to contact the  $K^b$ -t2  $\alpha$ 2-helix and (ii) N15 $\beta$  CC' and FG (CDR3 $\beta$ )

loops like the "thumb" and "fingers," respectively. The thumb residue E42 on the N15 $\beta$  CC' loop formed a specific electrostatic interaction with  $K^b$ -t2 residues R157 and K131 as a binding register. Finger residues W97, E100, and Q101 on the CDR3 $\beta$  loop made extensive interactions with  $K^b$ -t2 at the  $\alpha$ 2 apex to facilitate CDR3 $\beta$  loop sampling of the VSV8 C-terminal segment. These two contact areas are likely a general feature for the preTCR-pMHC interaction.

The N15 $\beta$ -VSV8- $K^b$ -t2 interaction was somewhat degenerate (Fig. 3A and tables S3 and S5).



**Fig. 1. The structure of N15 $\beta$ -VSV8- $K^b$ -t2.** (A) Ribbon drawing of one representative structure of N15 $\beta$ -VSV8- $K^b$ -t2. The twisted concave C''C'CFG  $\beta$  sheet of the N15 $\beta$  V-domain topologically fits the protruding  $\alpha$ 2-helix of  $K^b$ -t2, with the N15 $\beta$  CDR3 loop reaching the C-terminal VSV8 segment. Note that CDR1 and CDR2 point away from pMHC. (B) Surface representation of the "closed-book" view (top panel) and "open-book" view (bottom panel) of N15 $\beta$ -VSV8- $K^b$ -t2.



**Fig. 2. Distinct pMHC-binding mode of preTCR versus TCR $\alpha\beta$ .** (A) Side-by-side ribbon drawings of the "horizontal" N15preTCR-VSV8- $K^b$  complex model (left) and "vertical" N15TCR $\alpha\beta$ -VSV8- $K^b$  complex structure (right) (31). The former was constructed by overlaying structures of human preTCR (PDB: 30F6) and mouse VSV8- $K^b$  (PDB: 1KPU) onto the current structure of N15 $\beta$ -VSV8- $K^b$ -t2. (B) Composite view of the footprints of a TCR $\alpha\beta$  (PDB: 2CKB) and the preTCR on the same  $K^b$  (gray surface and green ribbon). The  $\beta$ -chain footprints of TCR $\alpha\beta$  and preTCR are delineated by magenta and blue dashed lines, respectively. All CDR loops are displayed. The VSV8 is in red ribbon representation with its exposed residues V4 and Q6 shown. (C) Simplified illustration of pMHC binding of preTCR versus TCR $\alpha\beta$ . An atomic model comparison is provided in fig. S6. Only CDR3 is labeled. APC, antigen-presenting cell.

Hydrophobic interactions in the palm area were promiscuous. Although N15 $\beta$ -VSV8-K<sup>b</sup>-t2 potentially had seven maximal interfacial hydrogen bonds, only a few were conserved (Fig. 3A). On average, there should be  $9 \pm 5$  hydrogen bonds in a PPI to afford sufficient specificity (25), whereas the number in N15 $\beta$ -VSV8-K<sup>b</sup>-t2 is rather low, implying plasticity in the fit. W97 on the CDR3 $\beta$  loop tip had its large side chain distributing on either side of VSV8 (Fig. 3E and fig. S5), indicative of conformational variability. Transience may enable the CDR3 $\beta$  loop to sample myriad self-peptides. To investigate sampling directly, the N15 $\beta$  subunit was examined alone or with VSV8-K<sup>b</sup>-t in solution by NMR relaxation dispersion (Rex). The N15 $\beta$  W97 side-chain atom (W97N $\epsilon$ ) showed a Rex effect only upon addition of VSV8-K<sup>b</sup>-t (Fig. 3F and fig. S8), indicative of dynamic changes in the microsecond to millisecond time scale and consistent with ligand-dependent chemical exchange.

To examine the functional consequences of the observed preTCR interaction with pMHC, we seeded wild-type hematopoietic stem cells onto stroma expressing or lacking surface MHC (16) and followed thymocyte development (Fig. 4A). At DN3, both preTCR-independent (Notch-related) and preTCR-dependent cellular expansion occurs, whereas at DN4, signaling is dominated by the preTCR (27). Proliferation at the DN3 stage was observed at d9 independently of MHC expression, whereas progression to the DPsm stage only occurred robustly on the MHC<sup>+</sup> stroma (Fig. 4B and fig. S9). By d13, progression to the DPsm stage was maintained on the MHC<sup>+</sup> but strongly diminished on MHC<sup>-</sup> stroma. DN4 cell numbers were low across all experiments because of their short-lived but essential transitional nature (28).

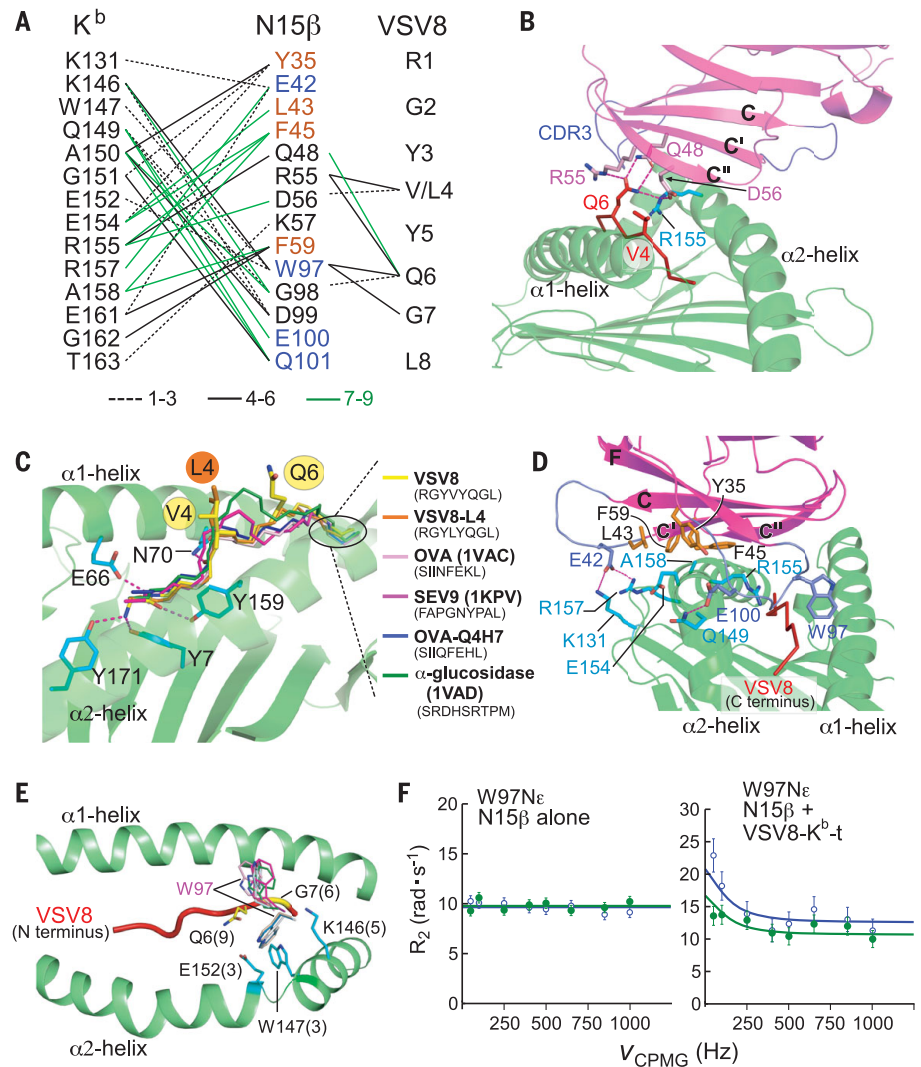
Because the preTCR is expressed on a substantial fraction of DN3 and DN4 thymocytes, we investigated whether the presence or absence of pMHC recognition by the preTCR affected functional preTCR $\beta$  clonotype representation (Fig. 4C). The DN3 subset expansion was essentially independent of pMHC presence on stroma with similar clonotype representations. By contrast, at the DN4 stage, when cells also expressed functional  $\beta$  chains without TCR $\alpha$  chains,  $\beta$ -chain clonotype diversity was 10-fold higher in the presence of MHC (Fig. 4C). Clonotype expansion on the MHC<sup>+</sup> stroma was caused by the inclusion of many distinct single-copy  $\beta$  chains absent from the MHC<sup>-</sup> stromal cultures (table S7). DN3 and DN4 thymocyte  $\beta$  chains harvested from both types of stroma showed comparable CDR3 length and hydrophobicity (fig. S10).

Finally, we studied stromal cells selectively expressing VSV8-K<sup>b</sup> (16) and stem cells from B6 *Rag2*<sup>-/-</sup> mice (lacking endogenous TCR  $\alpha$

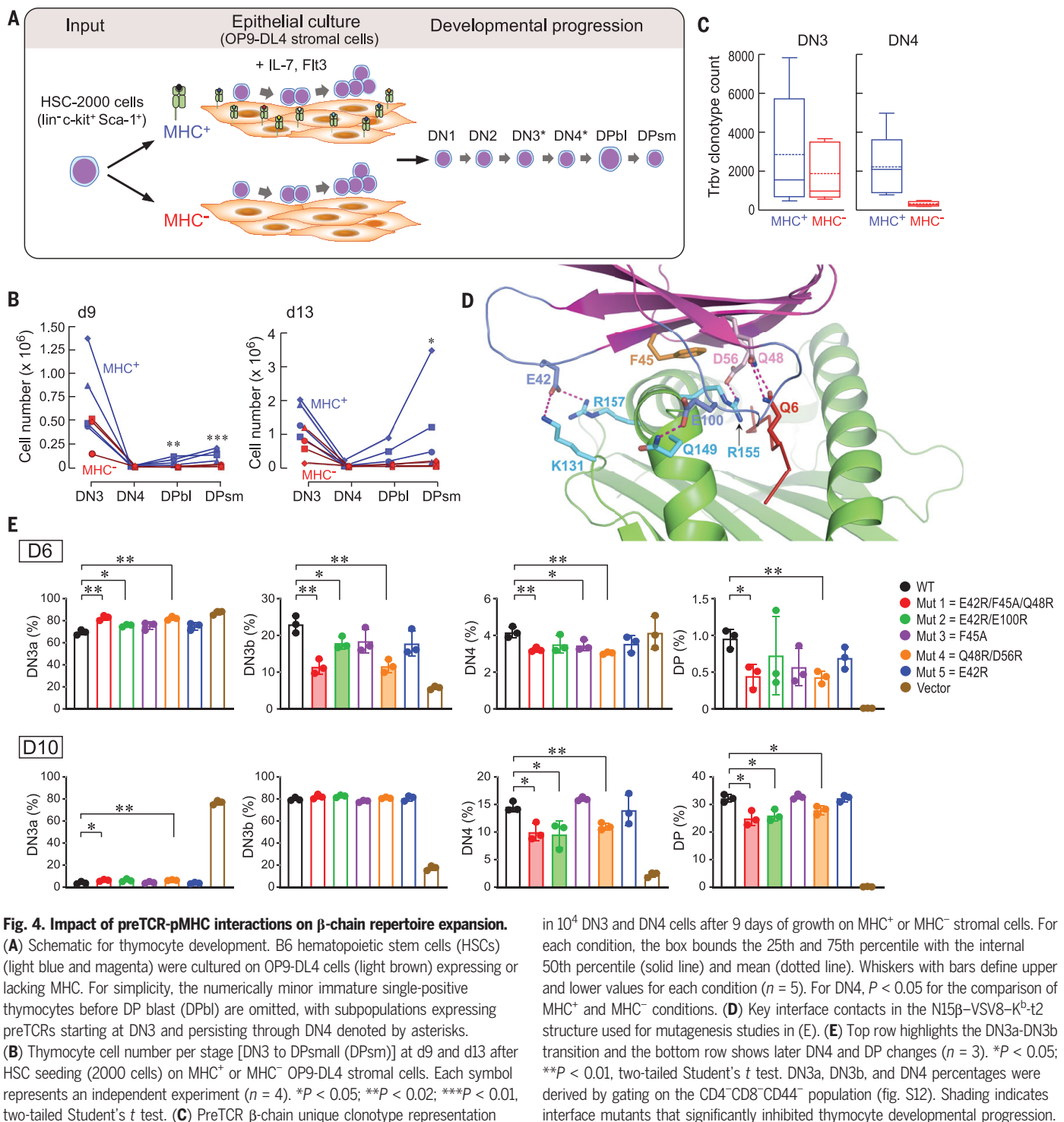
and  $\beta$  chains) as retroviral transduction recipients. Either wild-type N15 $\beta$  (wtN15 $\beta$ ) or one of five N15 $\beta$  chains mutated at interface residues in the N15 $\beta$ -VSV8-K<sup>b</sup>-t2 structure (Fig. 4D) were transduced, purified by fluorescence-activated cell sorting, and cultured (figs. S11 to S13). All mutant constructs expressed surface preTCRs at levels comparable to wild-type (fig.

S14). On day 6, capturing the DN3a to DN3b transition, or day 10, highlighting DN4 and DP development, mutants 1, 2, and 4 significantly blocked developmental progression (Fig. 4E and fig. S15), confirming the crystallographically defined interaction site.

Like the  $\alpha\beta$ TCR, the preTCR is a mechanosensor in which bioforces load and enhance



**Fig. 3. The interactions of N15 $\beta$  with VSV8-K<sup>b</sup>-t2.** (A) Interaction network of N15 $\beta$  with K<sup>b</sup>-t2 and VSV8 (4-Å cutoff). Black dashed lines, black solid lines, and green lines denote those interactions observed in 1 to 3, 4 to 6, or 7 to 9 complexes in three crystals, respectively. N15 $\beta$  residues in orange and blue are located in two distinct K<sup>b</sup>-t2-contacting regions, respectively [see (D)]. (B) N15 $\beta$  binding to the VSV8 peptide. Only the C-terminal part of VSV8, particularly V4 and Q6, are involved in the N15 $\beta$  contact. Dashed lines represent hydrogen bonds. (C) Two K<sup>b</sup>-binding octapeptides (OVA and OVA variant Q4H7) and nonapeptides (SEV9 and  $\alpha$ -glucosidase) are superimposed on VSV8-K<sup>b</sup>-t2. The backbone of N-terminal p1-p3 peptide residues is held in the binding-groove by hydrogen bonds to conserved MHC residues, whereas their protruding C-terminal residues diverge in backbone conformation. (D) Two distinct VSV8-K<sup>b</sup>-t2-binding areas on N15 $\beta$  are the "palm" in orange and the "thumb and fingers" in blue. Their interacting residues on K<sup>b</sup>-t2 are colored in cyan. (E) Conformational variability of W97 on the N15 $\beta$  CDR3 $\beta$  loop. Numbers in parentheses represent the frequency of each contact among the nine complexes. Note that the W97 side chain can position on either side of the VSV8 peptide. (F) <sup>15</sup>N relaxation dispersion profiles of W97 showing changes consistent with chemical exchange on ligand binding with addition of VSV8-K<sup>b</sup>-t. Blue circles and green points represent measured transverse relaxation (R<sub>2</sub>) at 700 and 600 MHz, respectively, at a given Carr-Purcell-Meiboom-Gill pulse frequency (V<sub>CPMG</sub>).



receptor-ligand bond lifetimes through the  $\beta$  subunit (16, 29). Linked preTCR structural transitions exhibit greater reversibility compared with those of TCR $\alpha\beta$ , reflecting unpaired V $\beta$  domain compliance. Facile PreTCR signaling (30) fosters  $\beta$ -chain repertoire formation, and T-lineage repertoire logic is programmed through alterations in pMHC-binding receptor structures. PreTCRs exploit a topological fit that

only permits CDR3 $\beta$  to interact with pMHC, supporting self-pMHC reactivity and reduced ligand specificity to favor  $\beta$ -clonotype inclusiveness. Subsequently, beginning in DP thymocytes, when TCR $\alpha$  replaces pT $\alpha$ , the same C $^{\prime}$ C $^{\prime}$  CFG  $\beta$ -sheet face of the V $\beta$  domain combines with that of the V $\alpha$  domain to form the V $\alpha$ -V $\beta$  module in the  $\alpha\beta$ TCR, which prevents horizontal pMHC binding. Instead, a vertical geometry

is mandated to foster greater specificity among pMHC interface interactions, testing all six CDRs through positive and negative selection. Laxity of pMHC binding permitted at the  $\beta$ -selection stage is eliminated, thereby augmenting self-versus-nonself discrimination. Successful passage through this gauntlet allows a diverse set of matured T-lineage cells to move into the periphery to discern and

confront in their vertical  $\alpha\beta$ TCR mode abnormal cells altered by pathogens or cancerous transformations.

#### REFERENCES AND NOTES

1. K. Shortman, M. Egerton, G. J. Spangrude, R. Scollay, *Semin. Immunol.* **2**, 3–12 (1990).
2. U. Koch et al., *J. Exp. Med.* **205**, 2515–2523 (2008).
3. H. R. Rodewald, M. Ogawa, C. Haller, C. Waskow, J. P. DiSanto, *Immunity* **6**, 265–272 (1997).
4. T. Kreslavsky et al., *Immunity* **37**, 840–853 (2012).
5. H. von Boehmer, *Cancer Immunol. Res.* **2**, 592–597 (2014).
6. C. Saint-Ruf et al., *Science* **266**, 1208–1212 (1994).
7. B. H. Koller, P. Marrack, J. W. Kappler, O. Smithies, *Science* **248**, 1227–1230 (1990).
8. M. J. Grusby et al., *Proc. Natl. Acad. Sci. U.S.A.* **90**, 3913–3917 (1993).
9. H. J. Fehling, A. Krotkova, C. Saint-Ruf, H. von Boehmer, *Nature* **375**, 795–798 (1995).
10. B. A. Irving, F. W. Alt, N. Killeen, *Science* **280**, 905–908 (1998).
11. S. S. Pang et al., *Nature* **467**, 844–848 (2010).
12. S. Yamasaki et al., *Nat. Immunol.* **7**, 67–75 (2006).
13. P. Smelty et al., *Proc. Natl. Acad. Sci. U.S.A.* **107**, 19991–19996 (2010).
14. B. Zhou et al., *Front. Immunol.* **2**, 5 (2011).
15. A. M. Michie et al., *Int. Immunol.* **19**, 1421–1430 (2007).
16. D. K. Das et al., *J. Biol. Chem.* **291**, 25292–25305 (2016).
17. R. J. Mallis, H. Arthanari, M. J. Lang, E. L. Reinherz, G. Wagner, *J. Biol. Chem.* **293**, 754–766 (2018).
18. R. J. Mallis et al., *Proc. Natl. Acad. Sci. U.S.A.* **112**, 8373–8378 (2015).
19. R. J. Mallis et al., *J. Biomol. NMR* **73**, 319–332 (2019).
20. X. Yang, R. A. Mariuzza, *Proc. Natl. Acad. Sci. U.S.A.* **112**, 8166–8167 (2015).
21. C. López-Rodríguez, J. Aramburu, R. Berga-Bolaños, *Cell. Mol. Life Sci.* **72**, 2305–2321 (2015).
22. L. Klein, B. Kyewski, P. M. Allen, K. A. Hogquist, *Nat. Rev. Immunol.* **14**, 377–391 (2014).
23. M. G. Rudolph, R. L. Starfield, I. A. Wilson, *Annu. Rev. Immunol.* **24**, 419–466 (2006).
24. J. H. Wang, E. L. Reinherz, *Immunol. Rev.* **250**, 102–119 (2012).
25. L. Lo Conte, C. Chothia, J. Janin, *J. Mol. Biol.* **285**, 2177–2198 (1999).
26. C. Zhang, A. Anderson, C. DeLisi, *J. Mol. Biol.* **281**, 929–947 (1998).
27. H. Hosokawa, E. V. Rothenberg, *Cold Spring Harb. Perspect. Biol.* **10**, a028621 (2018).
28. F. Vasseur, A. Le Campion, C. Pénit, *Eur. J. Immunol.* **31**, 3038–3047 (2001).
29. D. K. Das et al., *Proc. Natl. Acad. Sci. U.S.A.* **112**, 1517–1522 (2015).
30. Y. Feng, E. L. Reinherz, M. J. Lang, *Trends Immunol.* **39**, 596–609 (2018).
31. M. K. Teng et al., *Curr. Biol.* **8**, 409–414 (1998).

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organized, and supervised the project. E.L.R., J.H.W., and X.L. prepared the manuscript with input from all authors. R.M. and R.J.M. designed, cloned, refolded, and purified protein samples. X.L. produced crystals. X.L., K.T., and J.H.W. collected and processed diffraction data and determined and refined the structure models. X.L. deposited the structures. R.J.M., A.D., G.W., and H.A. performed NMR experiments. J.S.D.-C. performed thymic repertoire analysis. A.A. and P.W.T. performed cell and FACS experiments and animal breeding. J.H.W., X.L., K.T., and E.L.R. analyzed x-ray crystal structures. R.J.M., A.D., G.W., and H.A. analyzed NMR data. J.S.D.-C., A.A., P.W.T., and E.L.R. analyzed functionally experimental data. **Competing interests:** G.W. is a cofounder and has equity in the following companies: Enanta Pharmaceuticals, PIC therapeutics, Cellmig Biolabs, Skinap Therapeutics, Virtual Discovery, and Olaris Therapeutics. None of these companies is related to the topic of this manuscript. The remaining authors declare no competing interests.

**Data and materials availability:** All structural data have been deposited in the Protein Data Bank (<https://www.rcsb.org>) with PDB codes: 6WL2, 6WL3, 6WL4, 7J12. All other data associated with this study are in the main text or supplementary materials.

#### SUPPLEMENTARY MATERIALS

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Materials and Methods  
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Tables S1 to S8  
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## Pre-T cell receptors topologically sample self-ligands during thymocyte $\beta$ -selection

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### PreTCRs use horizontal docking geometry

The T cell receptor (TCR) recognizes peptide-bound major histocompatibility complex molecules (pMHCs) and consists of an  $\alpha$  chain in association with a  $\beta$  chain. Both chains have hypervariable complementarity-determining regions (CDRs) that inform whether a particular TCR can recognize a given pMHC. To successfully graduate from the thymus, aspiring  $\alpha\beta$ T cells must generate a functional TCR. During one early checkpoint in this process, the  $\beta$  chain is first paired with a preT  $\beta$  chain to form the preTCR. Li *et al.* used x-ray crystallography to visualize how preTCRs recognize pMHCs. They report that the CDR3 loop of the preTCR  $\beta$  chain contacts the pMHC with a distinctive lateral topography. This is in contrast to the established binding modality of mature TCRs, whereby all three CDR loops on both  $\alpha$  and  $\beta$  chains bind in a vertical orientation. These complexes help solve the mystery of how only functionally rearranged  $\beta$  chains using competent CDR3 loops can properly engage with pMHC at the preTCR stage.

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