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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☐	☒	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☐	☒	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	We used customized LabView software to collect the bond lifetime with various force ranges.
Data analysis	We used customized LabView software (version 8.6) to analyze the bond lifetime with various force ranges. For statistical analysis, we used Prism software GraphPad (version 5 & 7) for curve fitting, Student T-test, ANOVA, F-test analysis (Supplementary Table 3, 4, 5). We also used R (version: 3.4.2, with survival package) to determine catch or slip bonds criteria and to compare the differences in them (Supplementary Table 2).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample-size was pre-calculated. Our bond lifetime sample size was based on the pool data from BFP experiments which resulted in N=104-1351 for each ligand tested. All the sample size has been documented in Supplementary Table 1.
Data exclusions	Since adhesion frequency was lower than 20%, we were confident that over 95% of bond lifetime events were single bonds. However, because some events could have been multiple bonds we excluded bond lifetime events that were significantly longer (>10 sec) that could drift over long time (Chen et al., Journal of Biological Chemistry, 2010, 12;285(46):35967-78)
Replication	In order to test reproducibility, we had two independent people run the same condition which resulted in similar trend of force vs. bond lifetime plot.
Randomization	The frequency of bond lifetime event was randomized by sequential approach and retraction cycles between the biomembrane force probe and the thymocyte in the apposing micropipettes.
Blinding	We did not run blinding in our experiments due to complex in sample preparation. However, we had independent person other than the experimenter to analyze the data.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	We used PE-conjugated antibodies for site density measurement. Detail information is as following: anti-mouse Vα2 TCR monoclonal antibody (mAb) (B20.1, BD Pharmingen, San Jose, CA), anti-mouse CD4 (RM-45, eBioscience, San Diego, CA), anti-mouse CD8 (53-6.7, BD Pharmingen), anti-mouse OVA257-264 (SIINFEKL) peptide bound H2-Kb (25-D1.16, eBioscience), anti-mouse H2-Kb (AF6-88.5, BD Pharmingen), and β2 microglobulin (S19.8, Santa Cruz Biotechnology, Dallas, TX).
Validation	PE-conjugated rat IgG2a κ (eBioscience), mouse IgG2a (Santa Cruz Biotechnology), and hamster IgG3 λ1 (BD Pharmingen) were used as isotype controls to validate above antibodies.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	OT1 and 2C TCR transgenic mice (3-4 week old) were housed at the Emory University Department of Animal Resources facility and followed protocols approved by the Institutional Animal Care and Use Committee of Emory University. CD8.4 OT1 Rag-/-MHCII-/- (CD8.4) and WT OT1 Rag-/-MHCII-/- transgenic mice were housed at the NCI and OT1 transgenic mice homozygous for the CD3ζζ 6F knock-in mutation were housed in NICHD. CD8.4 and 6F mice were shipped from the NIH to the Zhu lab on the day of the experiment.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve samples collected from the field.