
Supplementary information

Structures of human $\gamma\delta$ T cell receptor–CD3 complex

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Structures of human $\gamma\delta$ T cell receptor–CD3 complex

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Supplementary Methods

Amino acid sequences of the constructs used in this study

TCR δ 1:

MDMRVPAQLLGLLLLWLSGARCMDYKDDDDKGGSETGAQKVTQAQSSVSM
PVRKAVTLNCLYETSWWSYYIFWYKQLPSKEMIFLIRQGSDEQNAKSGRYSV
NFKKAAKSVALTISALQLEDYSAKYFCALGDPGGLNTDKLIFGKGTRVTVEPRS
QPHTKPSVFVMKNGTNVACLVKEFYPKDIRINLVSSKKITEFDPAIVISPSGKYN
AVKLGKYEDSNSVTCSVQHDNKT VHSTDFEVKTDSTDHVKPKETENTKQPSK
SCHKPKAIVHTEKVNMMSLTVLGLRMLFAKTAVAVNFLTAKLFFL

TCR γ 5:

MDMRVPAQLLGLLLLWLSGARCMDYKDDDDKGGSETGSSNLEGGTKSVTRP
TRSSAEITCDLTVINAFYIHWYLHQEGKAPQRLLYYDVSNKDVLESGLSPGK
YYTHTPRRWSWILIRNLIENDSGVYYCATWDRGNPKTHYYKKLFGSGTTLV
VTDKQLDADVSPKPTIFLPSIAETKLQKAGTYLCLLEKFFPDVIKHWQEKKS
N TILGSQEGNTMKTNDTYMKFSWLTVPKSLDKEHRCIVRHENNKNGVDQEII
FPPIKTDVITMDPKDNCSKDANDTLLLQLTNTSAYMYLLLLLKSVMYFAITCC
LLRRTAFCCNGEKS

TCR δ 2:

MDMRVPAQLLGLLLLWLSGARCMDYKDDDDKGGSETGAIELVPEHQTVPVSI
GVPATLRCSMKGEAIGNYYINWYRKQTQNTMTFIYREKDIYGPFGKDNFQGD
IDIAKNLAVLKILAPSERDEGSYYCACDTLGMGGEYTDKLIFFGKGTRVTVEPR
SQPHTKPSVFVMKNGTNVACLVKEFYPKDIRINLVSSKKITEFDPAIVISPSGKY
NAVKLGKYEDSNSVTCSVQHDNKT VHSTDFEVKTDSTDHVKPKETENTKQPS
KSCHKPKAIVHTEKVNMMSLTVLGLRMLFAKTAVAVNFLTAKLFFL

TCR γ 9:

MDMRVPAQLLGLLLLWLSGARCMDYKDDDDKGGSETGAGHLEQPQISSTKT
LSKTARLECVVSGITISATSVYWYRERPGEVIQFLVSISYDGTVRKESGIPSGKF
EVDRIPESTSTLTIHNVEKQDIATYYCALWEAQQELGKKIKVFGPGTKLIITDK
QLDADVSPKPTIFLPSIAETKLQKAGTYLCLLEKFFPDVIKHWQEKKSNTILGS
QEGNTMKTNDTYMKFSWLTVPKSLDKEHRCIVRHENNKNGVDQEIIFFPIKT
DVITMDPKDNCSKDANDTLLLQLTNTSAYMYLLLLLKSVMYFAITCCLLRR
TAFCCNGEKS

CD3 ϵ :

MQSGTHWRVLGLCLLSVGVWGQDGNEMGGITQTPYKVSISGTTVILTCPQY
PGSEILWQHNDKNIGGDEDDKNIGSDEHLSLKEFSELEQSGYYVCYPRGSKP
EDANFYLYLRARVCENCMEMDVMSVATIVVDICITGGLLLLVYYWSKNRKA
KAKPVTRGAGAGGRQRGQNKERPPVPNPDIYPIRKGQRDLYSGLNQRR

CD3 γ :

MEQGKGLAVLILAILLQGTLAQSIKGNHLVKVYDYQEDGSVLLTCDAEAKNI
TWFKDGKMIGFLTEDKKKWNLGSNAKDPRGMYQCKGSQNKSKPLQVYYRM
CQNCIELNAATISGFLFAEIVSIFVLAVGVYFIAGQDGVQRQSRASDKQTLLPND
QLYQPLKDREDDQYSHLQGNQLRRN

CD3 δ :

MEHSTFLSGLVLATLLSQVSPFKIPIEELEDRLFVN CNTSITWVEGTVGTLLSDI
TRLDLGKRILDPRGIYRCNGTDIYKDKESTVQVHYRMCQSCVELDPATVAGII
VTDVIATLLLALGVFCFAGHETGRLSGAADTQALLRNDQVYQPLRDRDDAQY
SHLGGNWARNK

CD3 ζ :

MKWKALFTAAILQAQLPITEAQSFGLLDPKLCYLLDGILFIYGVILTALFLRVK
FSRSADAPAYQQGQNQLYNELNLGRREEYDVLDRRGRDPEMGGKQRRKN
PQEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDAL
HMQALPPRAAAWSPQFEKGGGSGGGSGGSAWSPQFEK

Soluble CD1d:

MDMRVPAQLLGLLLLWLSGARCEVPQRLFPLRCLQISSFANSSWTRTDGLAW
LGELQTHSWNSDSTVRSCLKPWSQGTFSQQWETLQHIFRVYRSSFTRDVKE
FAKMLRLSYPLELQVSAGCEVHPGNASNNFFHVAFQGKDILSFQGTWEPTQE
APLWVNLAIQVLNQDKWTRETVQWLLNGTCPQFVSGLLESGLKSELKKQVKP
KAWLSRGPSGPGRLLLLVCHVSGFYKPVVVKWMRGEQEQQGTQPGDILPN
ADETWYLRATLDVVAGEAAGLSCRVKHSSLEGQDIVLYWGGSHHHHHHHH

CD1d:

MDMRVPAQLLGLLLLWLSGARCEVPQRLFPLRCLQISSFANSSWTRTDGLAW
LGELQTHSWNSDSTVRSCLKPWSQGTFSQQWETLQHIFRVYRSSFTRDVKE
FAKMLRLSYPLELQVSAGCEVHPGNASNNFFHVAFQGKDILSFQGTWEPTQE
APLWVNLAIQVLNQDKWTRETVQWLLNGTCPQFVSGLLESGLKSELKKQVKP
KAWLSRGPSGPGRLLLLVCHVSGFYKPVVVKWMRGEQEQQGTQPGDILPN
ADETWYLRATLDVVAGEAAGLSCRVKHSSLEGQDIVLYWGGSYTSMGLIALA
VLACLLFLLIVGFTSRFKRQTSYQGV

β_2m :

MDMRVPAQLLGLLLLWLSGARCIQRTPKIQVYSRHPAENGKSNFLNCYVSGF
HPSDIEVDLLKNGERIEKVEHSDLSFSKDWSFYLLYTEFTPTKDEYACRVN
HVTLSQPKIVKWDRDM

BTN2A1:

MDMRVPAQLLGLLLLWLSGARCQFIVVGPTDPILATVGENTTLRCHLSPEKNA
EDMEVRWFRSQFSPAVFVYKGGREERTEEQMEEYRGRTTFVSKDISRGSVALVI
HNITAQENGTYRCYFQEGRSYDEAILHLVVAGLGSKPLISMRGHEDGGIRLECI
SRGWYPKPLTVWRDPYGGVAPALKEVSMPPADGLFMVTTAVIIRDKSVRNMS
CSINNTLLGQKKESVIFIPESFMPSPCAVALPIIVVILMIPIAVCIYWINKLQKE
KKILSGEKEFERETREIALKELEKERVQKEEELQVKEKLQEELRWRRTFLHAV
DVVLDPDTAHPDLFLSEDRRSVRRCPFRHLGESVPDNPERFDSQPCVLGRESF
ASGKHYWEVEVENVIEWTVGVCRDSVERKGEVLLIPQNGFWTLEMHGQY
RAVSSPDRILPLKESLCRVGVFLDYEAGDVSYFYNMRDRSHIYTCPRSAFSPVVR
PFFRLGCEDSPIFICPALTGANGVTVP EEGTLHRVGTHQSLGSSGMDYKDDD
DK

BTN3A1 :

MDMRVPAQLLGLLLLWLSGARCQFSVLGSPGPILAMVGEDADLPCHLFPTMS
AETMELKWVSSSLRQVVNVYADGKEVEDRQSAPYRGRTSILRDGITAGKAAL
RIHNVTASDSGKYLCYFQDGDIFYEKALVELKVAALGSDLHVDVKGYKDGGI
HLECRSTGWYPQPIQWSNNKGENIPTVEAPVVADGVGLYAVAASVIMRGSS
GEGVSCTIRSSLLGLEKTASISIADPFFRSAQRWIAALAGTLPVLLLLLGGAGYF
LWQQQEEKKTQFRKKKREQELREMAWSTMKQEQQSTRVKLLEELRWRSIQYA
SRGERHSAYNEWKKALFKPADVILDPKTANPILLVSEDQRSVQRAKEPQDLPD
NPERFNWHYCVLGCESFISGRHYWEVEVGDRKEWHIGVCSKNVQRKGWVK
MTPENGFWTMGLTDGNKYRTLTEPRTNLKLPKPPKVGVLVDYETGDISFYN
AVDGSHIHTFLDVSFSEALYPVFRILTLEPTALTICPAGSSGHHHHHHHHH

ECD BTN2A1:

MDMRVPAQLLGLLLLWLSGARCQFIVVGPTDPILATVGENTTLRCHLSPEKNA
EDMEVRWFRSQFSPAVFVYKGGREERTEEQMEEYRGRTTFVSKDISRGSVALVI
HNITAQENGTYRCYFQEGRSYDEAILHLVVAGLGSKPLISMRGHEDGGIRLECI

SRGWYPKPLTVWRDPYGGVAPALKEVSMPDADGLFMVTTAVIIRDKSVRNMS
CSINNTLLGQKKESVIFIPESFMPSVSGTSGSGGGLNDIFEAQKIEWHEHHHHH
H

TCR δ 2 (C α /C $P\alpha$):

MDMRVPAQLLGLLLLWLSGARCMDYKDDDDKGGSETGAIELVPEHQTPVPSI
GVPATLRCSMKGEAIGNYYINWYRKQTQNTMTFIYREKDIYGPGFKDNFQGD
IDIAKNLAVLKILAPSERDEGSYYCACDTLGMGGEYTDKLIFGKGTRVTVEPRI
QNPDPVYQLRDSKSSDKSVCLFTDFDSQTNVSQSKSDVYITDKTVLDMRS
MDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPSPESSCDVKLVEKSFETDT
NLNMMSLTVLGLRMLFAKTAVNFLTAKLFFL

TCR γ 9 (C β 2/C $P\beta$ 2):

MDMRVPAQLLGLLLLWLSGARCMDYKDDDDKGGSETGAGHLEQPQISSTKT
LSKTARLECVVSGITISATSVYWYRERPGEVIQFLVSISYDGTVRKESGIPSGKF
EVDRIPESTSTLTIHNVEKQDIATYYCALWEAQQELGKKIKVFGPGTKLIITDL
KNVFPPKVAVFEPSEAEISHTQKATLVCLATGFYPDHVELSWVNGKEVHSG
VSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQNPRNHFRCQVQFYGLSENDE
WTQDRAKPVTQIVSAEAWGRADCGFTSESYQQLTNTSAYMYLLLLLKSVV
YFAITCCLLRRTAFCCNGEKS

TCR γ 9 (C γ 2):

MDMRVPAQLLGLLLLWLSGARCMDYKDDDDKGGSETDAGHLEQPQISSTKT
LSKTARLECVVSGITISATSVYWYRERPGEVIQFLVSISYDGTVRKESGIPSGKF
EVDRIPESTSTLTIHNVEKQDIATYYCALWEAQQELGKKIKVFGPGTKLIITDK
QLDADVSPKPTIFLPSIAETKLQKAGTYLCLLEKFFPDVIKIHWEKKSNTILGS
QEGNTMKTNDTYMKFSWLTVPKSLDKEHRCIVRHENNKNGVDQEIIFFPIKT
DVTTVDPKYNYSKDANDVITMDPKDNCSKDANDTLLLQLTNTSAYMYLLL
LLKSVVYFAITCCLLRRTAFCCNGEKS

TCR γ 5 (C γ 2):

MDMRVPAQLLGLLLLWLSGARCMDYKDDDDKGGSETGSSNLEGGTKSVTRP
TRSSAEITCDLTVINAFYIHWYHLHQEGKAPQRLLYDVSNKDVLESGLSPGK
YYTHTPRRWSWILILRNLIENDSGVYYCATWDRGNPKTHYYKKLFGSGTTLV
VTDKQLDADVSPKPTIFLPSIAETKLQKAGTYLCLLEKFFPDVIKIHWEKKSNTILGS
QEGNTMKTNDTYMKFSWLTVPKSLDKEHRCIVRHENNKNGVDQEIIFFPIKT
DVTTVDPKYNYSKDANDVITMDPKDNCSKDANDTLLLQLTNTSAYMY
YLLLLLKSVVYFAITCCLLRRTAFCCNGEKS

TCR δ 2 (F283A/F290A/F291A (AAA)):

MDMRVPAQLLGLLLLWLSGARCMDYKDDDDKGGSETGAIELVPEHQTPVPSI
GVPATLRCSMKGEAIGNYYINWYRKQTQNTMTFIYREKDIYGPGFKDNFQGD
IDIAKNLAVLKILAPSERDEGSYYCACDTLGMGGEYTDKLIFGKGTRVTVEPR
SQPHTKPSVFMKNGTNVACLKVEFYPKDIRINLVSSKKITEFDPAIVISPSGKY
NAVKLKGYEDSNSVTCSVQHDNKT VHSTDFEVKTDSTDHVKPKETENTKQPS
KSCHKPKAIVHTEKVNMMSLTVLGLRMLFAKTAVNALLTAKLAAL

TCR δ 2 (T $M\alpha$ (I254-S273)):

MDMRVPAQLLGLLLLWLSGARCMDYKDDDDKGGSETGAIELVPEHQTPVPSI
GVPATLRCSMKGEAIGNYYINWYRKQTQNTMTFIYREKDIYGPGFKDNFQGD
IDIAKNLAVLKILAPSERDEGSYYCACDTLGMGGEYTDKLIFGKGTRVTVEPR
SQPHTKPSVFMKNGTNVACLKVEFYPKDIRINLVSSKKITEFDPAIVISPSGKY
NAVKLKGYEDSNSVTCSVQHDNKT VHSTDFEVKTDSTDHVKPKETENTKQPS
KSCHKPKAIVHTEKVNMMSLTVLGLRILLK VAGFNLLMTLRWSS

ALOD4:

MRGSHHHHHHGMASMTGGQQMGRDLYDDDDDKDPCAKMTLDHYGAYVAQ
FDVSWDEFTFDQNGKEVLTHKTWEGSGKDCKTAHYSTVIPLPPNSKNIVARE
ATGLAWEWWRITINEQNVPLTNEIKVSIGGTTLYPTATISH

ALOD4-mutant:

MRGSHHHHHHGMASMTGGQQMGRDLYDDDDDKDPCAKMTLDHYGAYVAQ
FDVSWDEFTFDQNGKEVLTHKTWEGSGKDCKTAHYSTVIPLPPNSKNIVARE
ATGLAWEWWRITINEQNVPLTNEIKVSIGAAAAATATISH

ECD TCR δ 1:

MDMRVPAQLLGLLLLWLSGARCMDYKDDDDKGGSETGAQKVTQAQSSVSM
PVRKAVTLNCLYETSWWSYYIFYWKQLPSKEMIFLIRQGSDEQNAKSGRYSV
NFKKAASKSVALTISALQLEDSAKYFCALGDPGGLNTDKLIFGKGTRVTVPEPR
QPHTKPSVFVMKNGTNVACLVEFYPKDIRINLVSSKKITEFDPAIVISPSGKYN
AVKLGKYEDSNSVTCSVQHDNKT VHSTDFEVKTDSGSHHHHHH

ECD TCR γ 5:

MDMRVPAQLLGLLLLWLSGARCMDYKDDDDKGGSETGSSNLEGGTKSVTRP
TRSSAEITCDLTVINAFYIHWYLHQEGKAPQRLLYDVSNKDVLESGLSPGK
YYTHTPRRWSWILRLNLIENDSGVYYCATWDRGNPKTHYYKKLFGSGTTLV
VTDKQLDADVSPKPTIFLPSIAETKLQKAGTYLCLLEKFFPDVIKHWQEKKS
N TILGSQEGNTMKTNDTYMKFSWLTVPKSLDKEHRCIVRHENNKNGVDQEII
PPIKTDVAAAWSHPPQFEKGGGSGGGSGGSAWSPQFEK

CD3 γ -T2A-CD3 ϵ -P2A-CD3 δ -E2A-CD3 ζ :

MEQGKGLAVLILAILLQGTLAQSIKGNHLVKVYDYQEDGSVLLTCDAEAKNI
TWFKDGKMIGFLTEDKKKWNLGSNAKDPRGMYQCKGSQNKSKPLQVYYRM
CQNCIELNAATISGFLFAEIVSIFVLAVGVYFIAGQDQVRQSRASDKQTLLPND
QLYQPLKDREDDQYSHLQGNQLRRNGSGEGRGSLLTCGDVEENPGPMQSGT
HWRVLGLCLLSVGWVGQDGNEEMGGITQTPYKVSISGTTVILTCQYPGSEIL
WQHNDKNIGGEDDDKNIGSDEDHLSLKEFSELEQSGYYVCYPRGSKPEDANF
YLYLRARVCENCMEMDVMSVATIVIVDICITGGLLLL VYYWSKNRKAKAKPV
TRGAGAGGRQRGQNKERPPPVPNPDIYEPKRGQRDLYSGLNQRIGSGATNFS
LLKQAGDVEENPGPMEHSTFLSGLVLATLLSQVSPFKIPIEELEDVFNVCNTSI
TWVEGTVGTLLSDITRLDLGKRILDPRGIYRCNGTDIYKDKESTVQVHYRMC
QSCVELDPATVAGIIVTDVIATLLALGVFCFAGHETGRLSGAADTQALLRNDQ
VYQPLRDRDDAQYSHLGGNWARNKSGGQCTNYALLKLAGDVESNPGPMKW
KALFTAAILQAQLPITEAQSFGLLDPKLCYLLDGILFIYGVILTALFLRVKFSRSA
DAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEG
LYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQA
LPPRAAAWSPQFEKGGGSGGGSGGSAWSPQFEK

TCR δ 3:

MDMRVPAQLLGLLLLWLSGARCMDYKDDDDKGGSETDKVTQSSPDQTVAS
GSEVVLLCTYDTVYSNPDLFWYRIRPDYSFQFVFYGDNSRSEGADFTQGRFS
VKHILTQKAFHLVISPVRTEDSATYYCAFTGLGDTSHADKLIFGKGTRVTVPEPR
SQPHTKPSVFVMKNGTNVACLVEFYPKDIRINLVSSKKITEFDPAIVISPSGKY
NAVKLGKYEDSNSVTCSVQHDNKT VHSTDFEVKTDSTDHVKPKETENTKQPS
KSCHKPKAIVHTEKVNMMSLTVLGLRMLFAKTAVVNFLLTAKLFFL

TCR γ 2:

MDMRVPAQLLGLLLLWLSGARCMDYKDDDDKGGSETGSSNLEGRTKSVIRQ
TGSSAEITCDLAEGSNGYIHWYLHQEGKAPQRLQYYDSYNSKVLESVSPG
KYYTYASTRNRLRLNLIENDFGVYYCATWDVKQNYKKLFGSGTTLVVT
DKQLDADVSPKPTIFLPSIAETKLQKAGTYLCLLEKFFPDVIKHWQEKKSNTI
LGSQEGNTMKTNDTYMKFSWLTVPKSLDKEHRCIVRHENNKNGVDQEII

PIKTDVITMDPKDNCSKDANDTLLLQLTNTSAYMYLLLLLLKSVVYFAITCCL
LRRTAFCNGEKS

TCR γ 3:

MDMRVPAQLLGLLLLWLSGARCMDYKDDDDKGGSETGSSNLEGRTKSVTRQ
TGSSAEITCDLTVTNTFYIHWYHQQEGKAPQRLLYYDVSTARDVLESGLSPGK
YYTHTPRRWSWILRLQNLIENDSGVYYCATWDGYYYKKLFGSGTTLVVTDK
QLDADVSPKPTIFLPSIAETKLQKAGTYLCLLEKFFPDVIKIHQQEKKSNILGS
QEGNTMKTNDTYMKFSWLTVPKSLDKEHRCIVRHENNKNGVDQEIIFFPIKT
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TAFCCNGEKS

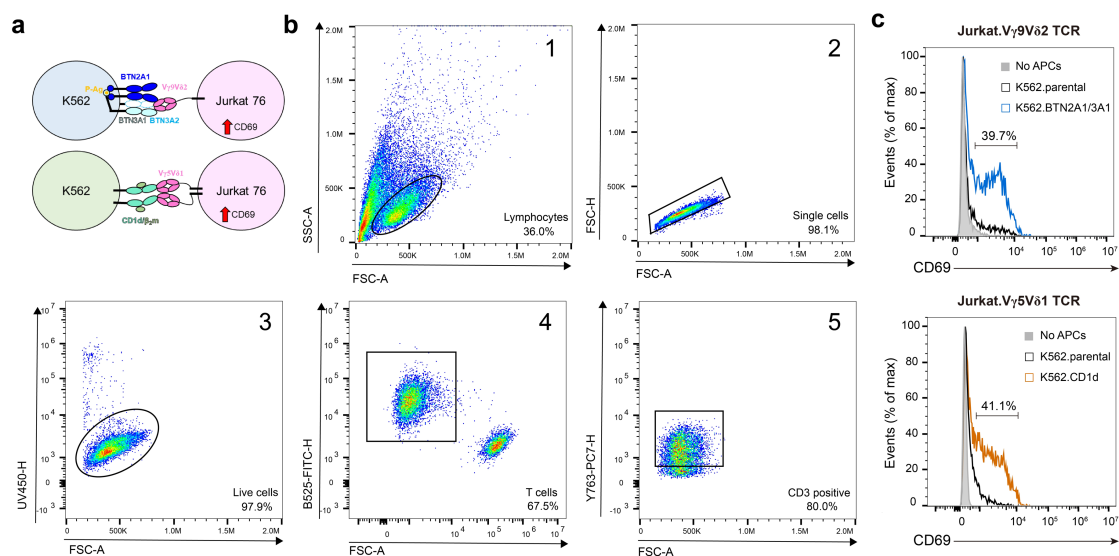
TCR γ 4:

MDMRVPAQLLGLLLLWLSGARCMDYKDDDDKGGSETGSSNLEGRTKSVIRQ
TGSSAEITCDLAEGSTGYIHWYHQQEGKAPQRLLYYDSYTSSVVLESGLSPGK
YDTYGSTRKNLRMILRNLIENDSGVYYCATWDGDYYKKLFGSGTTLVVTDK
QLDADVSPKPTIFLPSIAETKLQKAGTYLCLLEKFFPDVIKIHQQEKKSNILGS
QEGNTMKTNDTYMKFSWLTVPKSLDKEHRCIVRHENNKNGVDQEIIFFPIKT
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TAFCCNGEKS

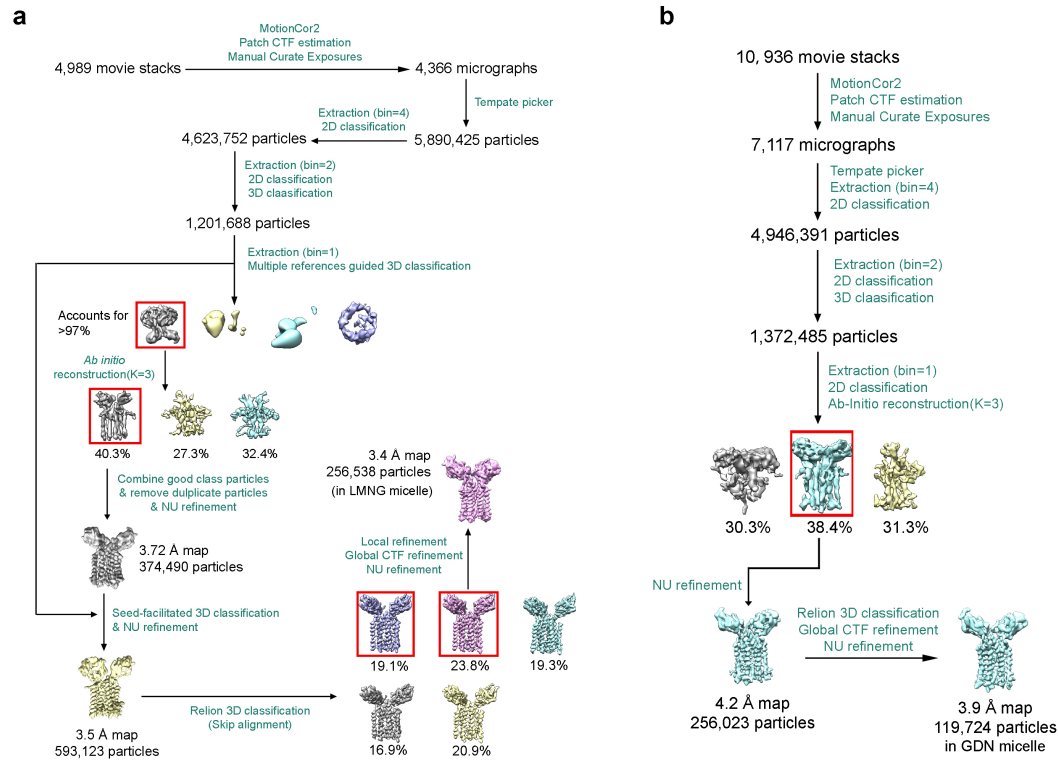
TCR γ 8:

MDMRVPAQLLGLLLLWLSGARCMDYKDDDDKGGSETGSSNLEGRTKSVTRP
TGSSAVITCDLPVENAVYTHWYHQQEGKAPQRLLYYDSYNSRVVLESGLSREK
YHTYASTGKSLKFIENLIERDSGVYYCATWDSSKLFGSGTTLVVTDKQLDAD
VSPKPTIFLPSIAETKLQKAGTYLCLLEKFFPDVIKIHQQEKKSNILGSQEGNT
MKTNDTYMKFSWLTVPKSLDKEHRCIVRHENNKNGVDQEIIFFPIKTDVITM
DPKDNCSKDANDTLLLQLTNTSAYMYLLLLLLKSVVYFAITCCLRRTAFC
NGEKS

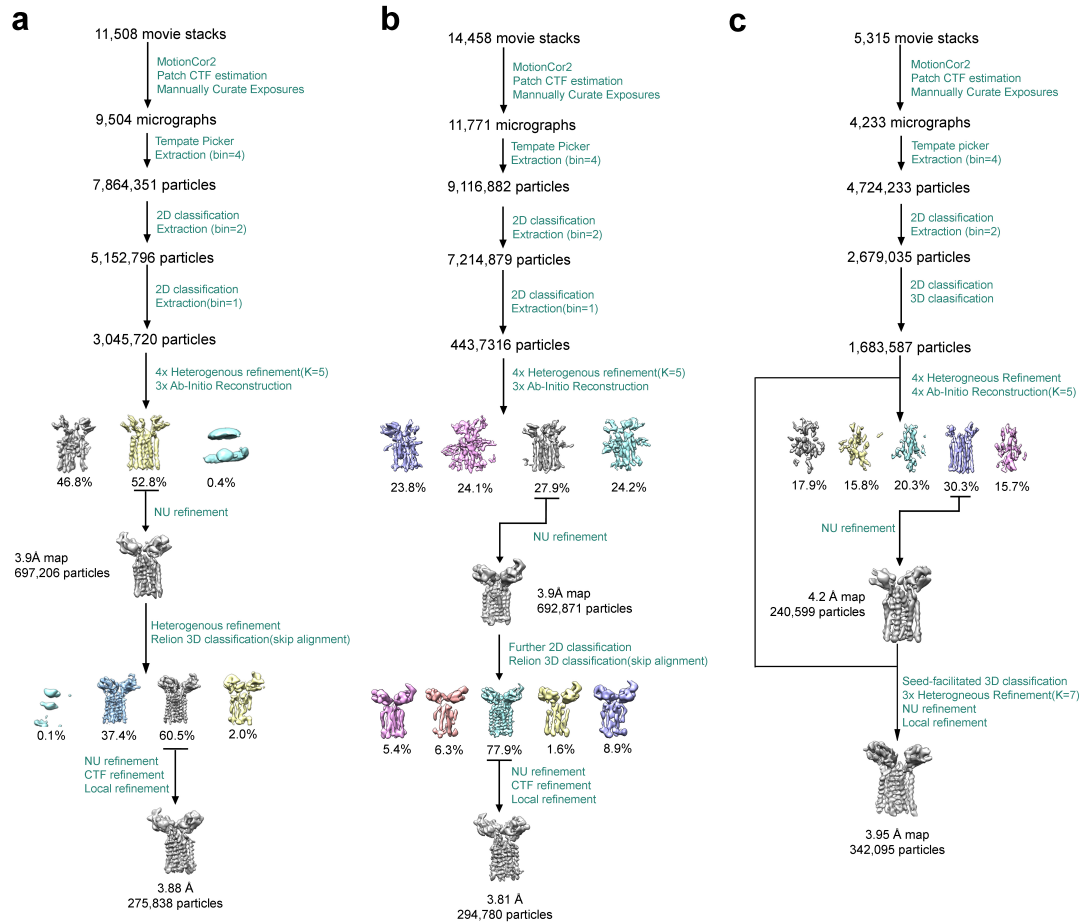
Supplementary Figures



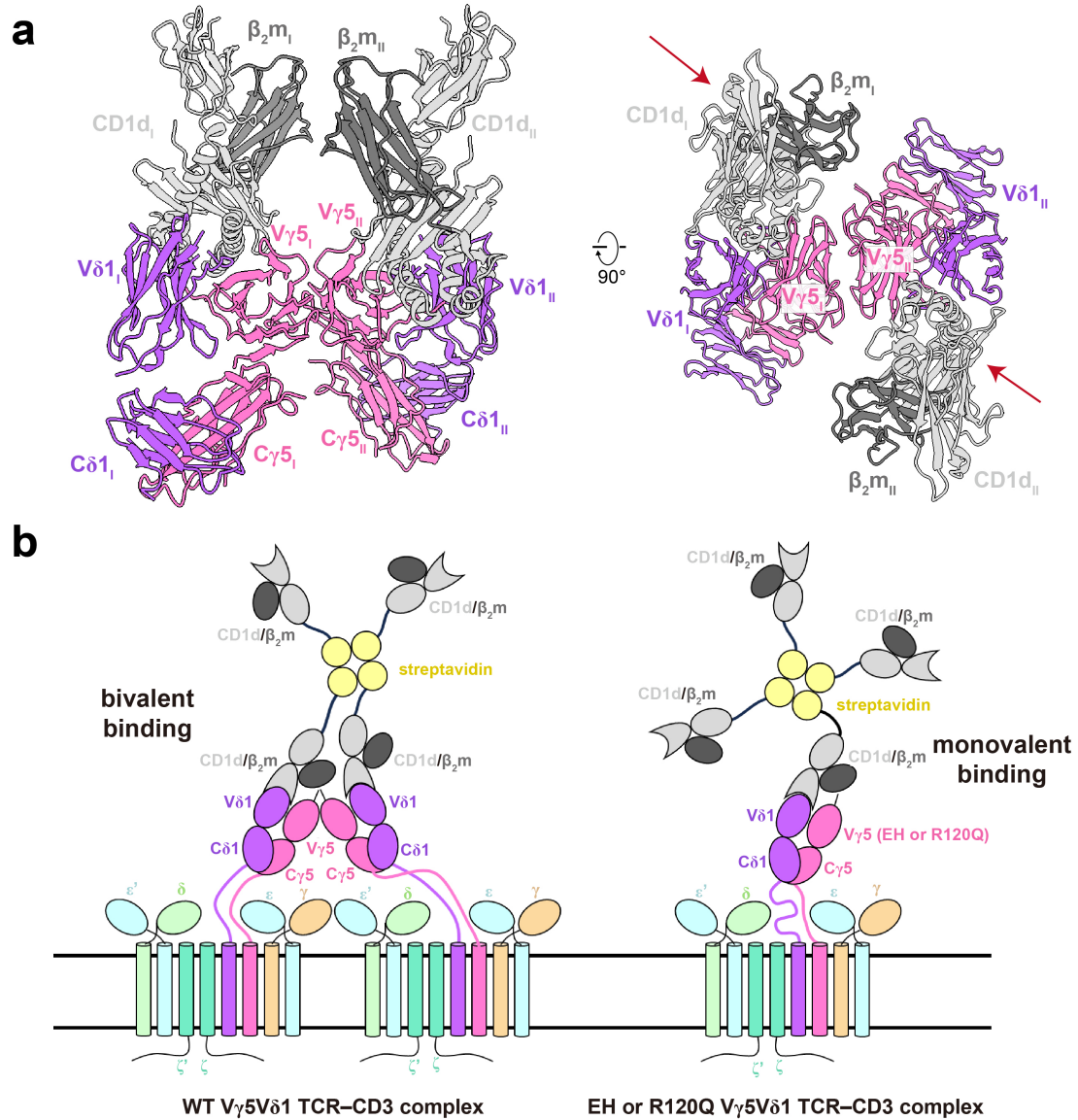
Supplementary Fig. 1 | Gating strategy used in the CD69 expression analysis and functional validation of Jurkat-76 cells stably expressing $\gamma\delta$ TCR of interest. **a**, A schematic diagram of the T cell activation assay. An upregulation of CD69 expression occurs upon the activation of Jurkat-76 cells. P-Ag: phosphoantigens. The endogenous BTN3A2 that facilitates V γ 9V δ 2 TCR activation is indicated by dotted blue circle. **b**, Selection of CD3⁺ Jurkat-76 cells through a series of five gates. The first gate is applied to remove cell debris, followed by the second gate to eliminate adhered cells. The third gate is applied to select live cells using DAPI. The fourth gate selects mGreenLantern⁺ cells, and the fifth gate employs anti-CD3 antibodies conjugated with PE-Cyanine7 dye to select CD3⁺ cells. **c**, Flow cytometry analysis of CD69 expression on G115 V γ 9V δ 2 (upper) or 9C2 V γ 5V δ 1 (lower) TCR transduced Jurkat-76 cells cocultured with K562 cells expressing BTN2A1/3A1 or CD1d or ZIM3–dCas9 (parental) or without K562 cells. Numbers in plots indicate percent of gated events. Data are representative of three independent experiments for all samples. APC: antigen-presenting cells.



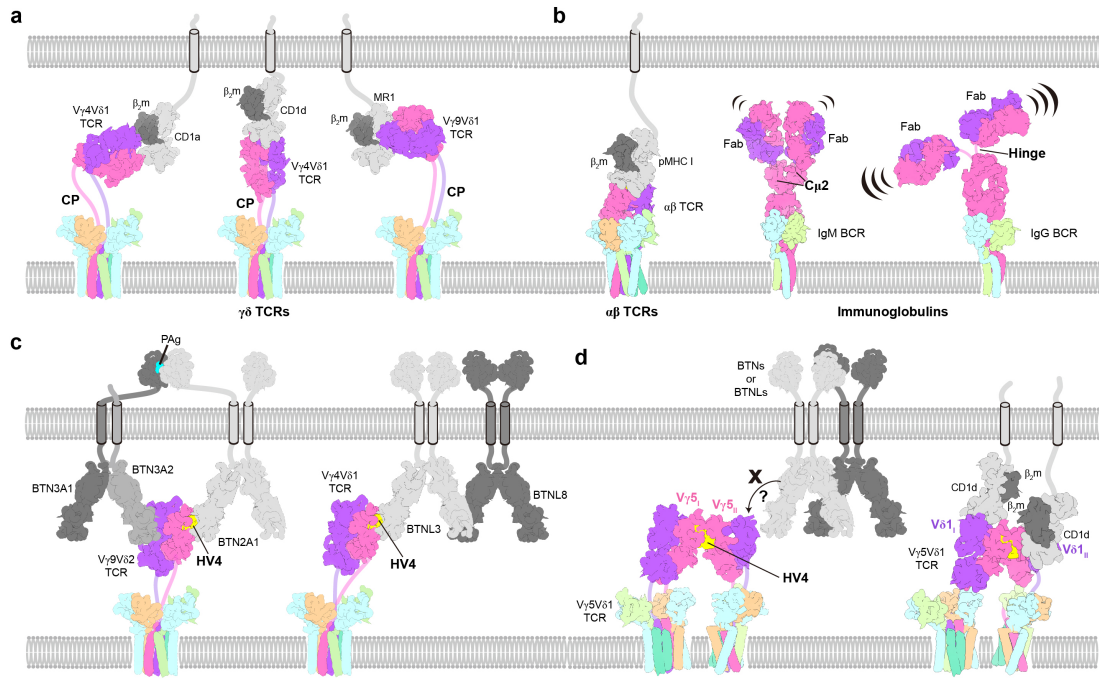
Supplementary Fig. 2 | The flowchart for cryo-EM data processing of the human V γ 9V δ 2 TCR-CD3 complex in LMNG or GDN detergent micelles. **a, Summary of the data processing procedure of the V γ 9V δ 2 TCR-CD3 complex. **b**, Summary of the data processing procedure of the V γ 9V δ 2 TCR-CD3 complex in GDN detergent micelles. Details are described in Methods. NU: non-uniform.**



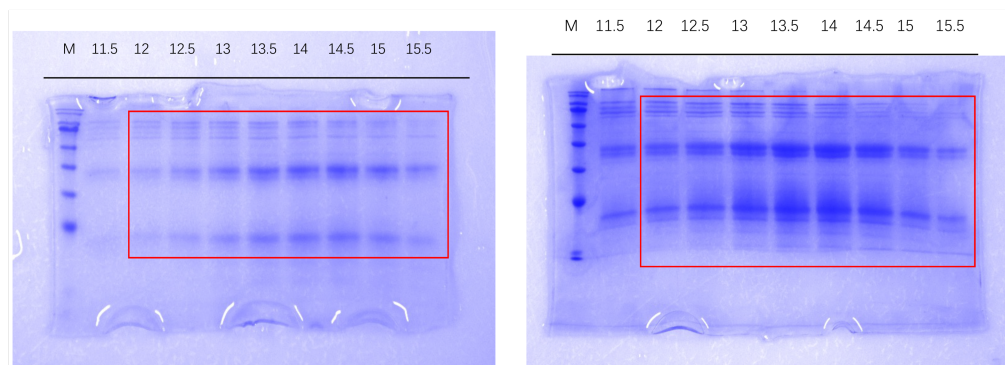
Supplementary Fig. 4 | The flowchart for cryo-EM data processing of the mutant V γ 9V δ 2 and mutant V γ 5V δ 1 TCR–CD3 complexes. a-c, Summary of the data processing procedure of the TM α V γ 9V δ 2 TCR–CD3 complex (a), AAA V γ 9V δ 2 TCR–CD3 complex (b), and EH V γ 5V δ 1 TCR–CD3 complex (c). Details are described in Methods. NU: non-uniform. TM α : replace TM δ (M273-L292) with TM α (I254-S273) in TCR δ 2. AAA: F283A/F290A/F291A in TCR δ 2. EH: Y106E/R120H in the V γ 5 domain.



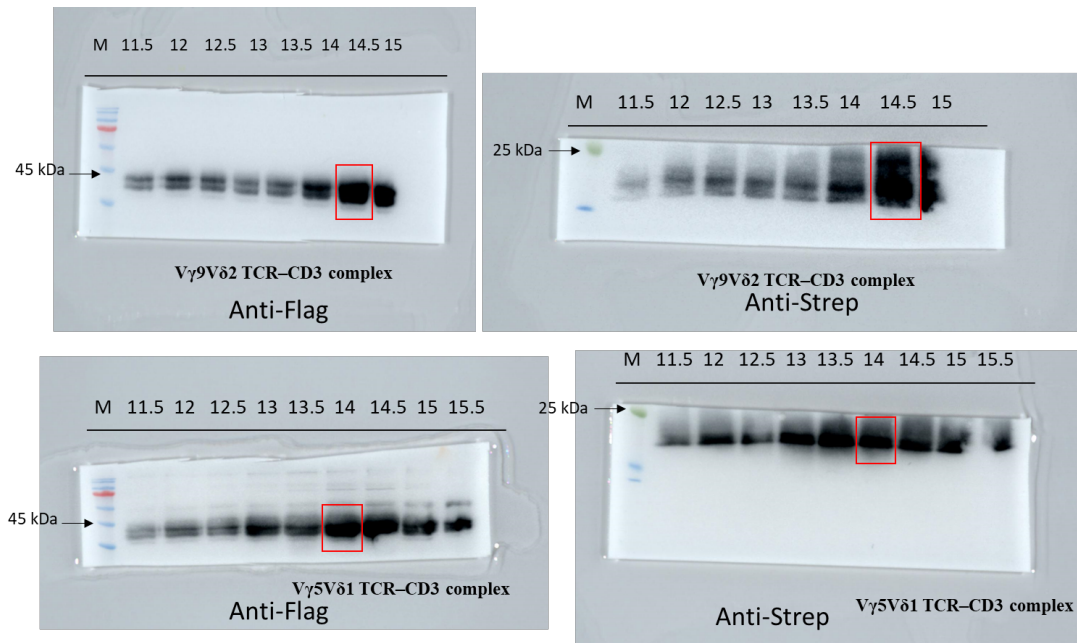
Supplementary Fig. 5 | V γ 5V δ 1 TCR-CD3 complex recognizes CD1d- β 2m in a bivalent binding manner. a, The structural superimposition between the CD1d- β 2m complex (gray, PDB: 4LHU) and the ECD of the V γ 5V δ 1 TCR-CD3 complex. The two docked CD1d- β 2m molecules are distanced from each other and do not exhibit steric hindrance. The CD1d- β 2m molecules are indicated by red arrows. Two perpendicular views are shown. **b**, A schematic diagram illustrates the binding of the dimeric V γ 5V δ 1 TCR-CD3 complex to CD1d- β 2m at a ratio of 1:2. However, for the EH mutant V γ 5V δ 1 TCR-CD3 complex, the binding mode occurs at a ratio of 1:1.



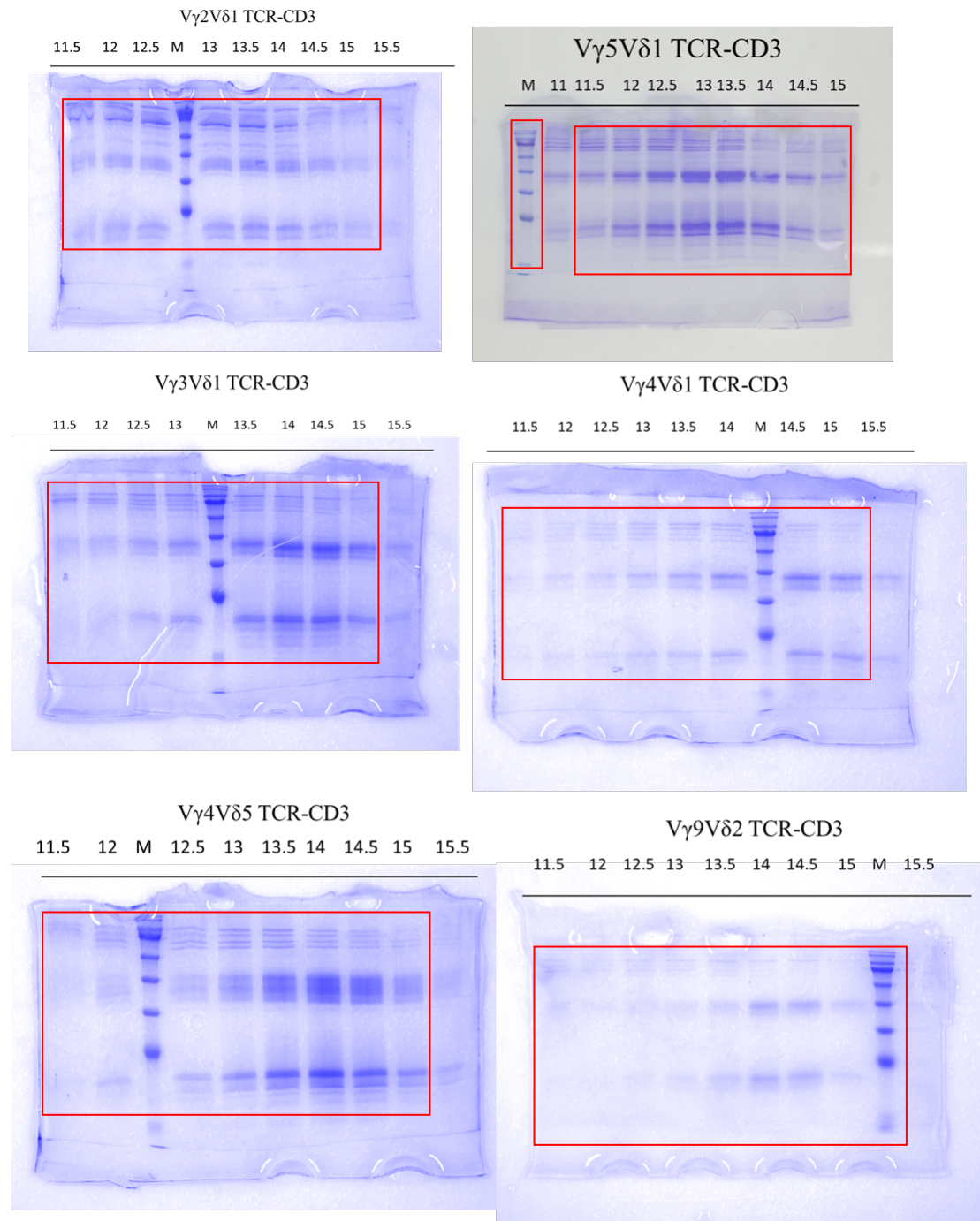
Supplementary Fig. 6 | Different ligand recognition modalities of the monomeric and dimeric $\gamma\delta$ TCRs, $\alpha\beta$ TCRs and BCRs. **a**, A schematic diagram showing the docking topologies among CO3 V γ 4V δ 1, DP10.7 V γ 4V δ 1, and G7 V γ 9V δ 1 TCR–CD3 with the ligands of CD1a– β_2 m, CD1d– β_2 m, and MR1– β_2 m. The exceptional flexibility of monomeric TCR ECDs and CPs permits the diverse docking topologies towards to their ligands. The figures were generated using the structures from Protein Data Bank (7RYN, 4MNG, and 6MWR). **b**, A schematic diagram showing the structural variation among $\alpha\beta$ TCR–CD3 complex, IgM and IgG B cell receptors (BCRs). The $\gamma\delta$ TCR–CD3 ECDs show similar conformational flexibility to BCR Fab domains in contrast to the rigid $\alpha\beta$ TCR–CD3 ECDs. The figures were generated using the structures from Protein Data Bank (7PHR, 7XQ8, and 7WSO). **c**, A schematic diagram showing the docking topologies of BTN2A1/3A1/3A2 and BTNL3/L8 with monomeric G115 V γ 9V δ 2 and LES V γ 4V δ 1 TCR–CD3 complexes via the germline-encoded HV4 region (yellow), respectively. The figures were generated using the structures from Protein Data Bank (8DFW, 8IGT, 8DFX, 7RYL, and 1HXM). **d**, A schematic diagram showing the docking topology of dimeric V γ 5V δ 1 TCR–CD3 complex to CD1d– β_2 m. The dimeric $\gamma\delta$ TCR–CD3 complex may not response to the BTNs or BTNLs or other potential ligands due to HV4 regions (yellow) being covered by V γ domain dimerization. The figures were generated using the structures from Protein Data Bank (4F9P, 8DFX, and 4LHU).



Supplementary Fig. 7 | Uncropped images for the Extended Data Figure 1c.



Supplementary Fig. 8 | Uncropped images for the Extended Data Figure 1d.



Supplementary Fig. 9 | Uncropped images for the Extended Data Figure 8b.

Supplementary Table S1 | The interactions within the V γ 5_I–V γ 5_{II} interface.

V γ 5 _I	V γ 5 _{II}	Interaction
R120	S92	Hydrogen bond
R120	T107/O	Hydrogen bond
R120	D94	Salt bridge
S92	R120	Hydrogen bond
T107/O	R120	Hydrogen bond
D94	R120	Salt bridge
R120	Y106	Cation- π
Y106	R120	Cation- π
Y106	Y106	π - π
E57	H108	Hydrogen bond
H108	E57	Hydrogen bond
H108	I118	Van der Waals effect
I118	H108	Van der Waals effect