

Supplementary information

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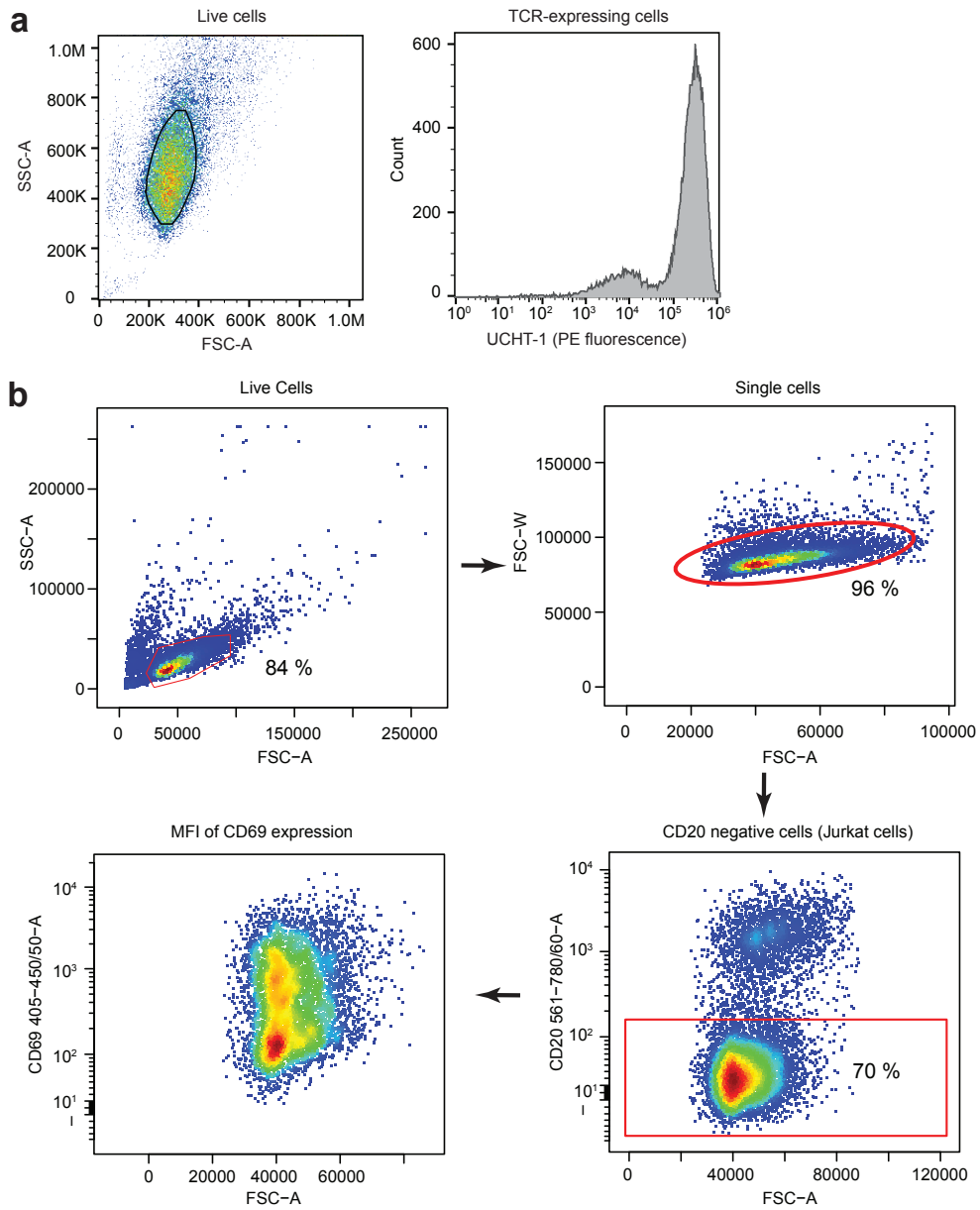
Supplementary information

Structure of a fully assembled $\gamma\delta$ T-cell antigen receptor

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Supplementary Figure 1

Supplementary Table 1



Supplementary Figure 1 | Gating strategies for flow cytometry. a, Representative gating strategy for surface molecule staining of the Jurkat T-cell lines. **b**, Representative gating strategy for measurements of CD69 expression in the T-cell activation assay.

Supplementary Table 1 | Sequences of the protein constructs used in this study

Name	Protein sequence
<p>1. G83.C4 SpyT-TCRγCD3$\delta\epsilon$_GFP2</p> <p>(For expression and purification of the Twin-StrepTag tagged complex, together with 2.)</p>	<p>MLLALALLAFLPPASQKMGRGVPHIVMVDAYKRYK SGGGSGENLYFQGGSGESGSGSSNLEGRTKSVTR PTGSSAVITCDLPVENAVYTHWYLHQEGKAPQRLLY YDSYNSRVVLESGISREKYHTYASTGKSLKFILENLIE RDSGVVYCATWDYKKLFGSGTTLVVDKQLDADVS PKPTIFLPSIAETKLQKAGTYLCLLEKFFPDVIKIHWQE KKSNTILGSQEGNTMKTNDTYMKFSWLTVPKSLDK EHRCIVRHENKNGVDQEIIFFPIKTDVITMDPKDNCS KDANDTLLLQLTNTSAYMYLLLLLLKSVVYFAITCCLL RRTAFCCNGEKSGSGQCTNYALLKLAGDVESNPGP MEHSTFLSGLVLATLLSQVSPFKIPIELEDRVFVNCN TSITWVEGTVGTLSDITRDLGKRILDPRGIYRCNGT DIYKDKESTVQVHYRMCQSCVELDPATVAGIIVTDVI ATLLLALGVFCFAGHETGRLSGAADTQALLRNDQVY QPLRDRDDAQYSHLGGNWARNGSGEGRGSLLTC GDVEENPGPMQSGTHWRVGLCLLSVGVWGDGN EEMGGITQTPYKVSISGTTVILTCPQYPGSEILWQH DKNIGGEDDDKNIGSDEDHLSLKEFSELEQSGYYVC YPRGSKPEDANFYLYLRARVCENCMEMDVMSVATIV IVDICITGLLLLVYYWSKNRKAKAKPVTRGAGAGGR QRGQNKERPPPVPNPDIYPIRKGQRDLYSGLNQRR IGDPPVATMVSKGEELFTGVVPIVELDGDVNGHKFS VSGEGEDATYGKLTCLKFICTTGKLPVPWPTLVTTLS YGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFF KDDGNYKTRAEVKFEGLTLVNRIELKIDFKEDGNIL GHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDG SVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALS SKDPNEKRDHMLLEFVTAAGITLGMDELYK</p>
<p>2. G83.C4 TCRδCD3$\zeta$$\gamma$_Strep2</p> <p>(For expression and purification of the Twin-StrepTag tagged complex, together with 1.)</p>	<p>MILTVGFSFLFFYRGTLCDKVTQSSPDQTVASGSEV VLLCTYDTVYSNPDLFWYRIRPDYSFQFVYGDNSR SEGADFTQGRFSVKHILTKAFHLVISPVRTEDSATY YCATRLWLGDPHTDKLIFGKGRVTVPEPRSQPHTKP SVFVMKNGTNAVACLKVEFYPKDIRINLVSSKKITEFDP AIVISPSGKYNAVKLGKYEDSNSVTCSVQHDNKT VHTDVEFKTDSTDHVKPKETENTKQPSKSKCHKPAI VHTKVNMMSLTVLGLRMLFAKTAVANFLLTAKLFFLG SGQCTNYALLKLAGDVESNPGPMKWKALFTAAILQA QLPITEAQSFGLLDPKLCYLLDGILFIYGVILTALFLRV KFSRSADAPAYQQGQNQLYNELNLRREEYDVL DKRRGRDPEMGGKQRRKNPQEGLYNELQKDKMAEA YSEIGMKGERRRGKGDGLYQGLSTATKDTYDALH MQALPPRSGEGRGSLLTCGDVEENPGPMEQKKG LAVLILAIILLQGTLAQSIKGNHLVKVYDYQEDGSVLLT CDAEAKNITWFKDGMIGFLTEDKKKWNLGSNAKIS RAGMYQCKGSQNKSKPLQVYYRMCQNCIELNAATD P GFLFAEIVSIFVLAVGVYFIAGQDGVQRSRASDKQTL LPNDQLYQPLKDREDDQYSHLQGNQLRRNGSGSA WSHPPQFEKGGGSGGGSSGSSAWSHPPQFEK</p>
<p>3. AF-7 TCR-α chain</p> <p>(For expression of the AF-7 TCR in Jurkat T-cells, together with 4.)</p>	<p>MWGVFLLYVSMKMGTTGQNIQDQPTTEMTATEGAIV QINCTYQTSGFNGLFWYQQHAGEAPTFLSYNVL DGL EEKGRFSSFLSRSGYSYLLKELQMKDSASYLCAV KDSNYQLIWGAGTKLIKPDIQNPDPVYQLRDSKSS DKSVCLFTDFDSQTNVSQSKDSDVYITDKTVLDMRS MDFKSNSAVAWSNKSDFACANAFNNSIIPEDTFFPS PESSCDVKLVEKSFETDTNLFQNLVIGFRILLKVA GFNLLMTRLRLWSS</p>
<p>4. AF-7 TCR-β chain</p> <p>(For expression of the AF-7 TCR in Jurkat T-cells, together with 3.)</p>	<p>MGILPSPGMPALLSLVSLLSVLLMGCVAETSYPYDVP DYAGGSGTSNAGVTQTPKFQVLKTGQSMTLQCAQD MNHNSMYWYRQDPGMGLRLIYYSASEGTTDKGEVP NGYNVSRNLNKRFLRLESAAPSQTSVYFCASSVWT GEGSGELFFGEGSRLTVLEDLKNVFPKAVFEPSE</p>

	AEISHTQKATLVCLATGFYPDHVELSWWWNGKEVHS GVSTDPQPLKEQPALNDSRYCLSSRLRVSATFWQN PRNHFRQCQVQFYGLSENDEWTQDRAKPVTQIVSAE AWGRADCGFTSESYQQGVLSATILYEILLGKATLYAV LVSALVLMAMVKRKDSRG
5. G83.C4 TCR-γ chain (For expression of the G83.C4 TCR in Jurkat T-cells, together with 6.)	MLLALALLAFLPPASQKTSYPYDVPDYAGGSGTSS SNLEGRTKSVTRPTGSSAVITCDLPVENAVYTHWYL HQEGKAPQRLLYYDSYNSRVVLESGISREKYHTYAS TGKSLKFILENLIERDSGVYYCATWDYKFLFGSGTTL VVTDKQLDADVSPKPTIFLPSIAETKLQKAGTYLCLLE KFFPDVIKIHVQEKKSNTILGSQEGNTMKTNDTYMKF SWLTVPEKSLDKEHRCIVRHENNKNGVDQEIIFPIK TDVITMDPKDNCSKDANDTLLLQLTNTSAYMYLLLL LKSVVYFAITCCLLRRTAFCCNGEKS
6. G83.C4 TCR-δ chain (For expression of the G83.C4 TCR in Jurkat T-cells, together with 5.)	MILTVGFSFLFFYRGTLCDKVTQSSPDQTVASGSEV VLLCTYDTVYSNPDLFWYRIRPDYSFQFVYGDNSR SEGADFTQGRFSVKHILTKQAFHLVISPVRTEDSATY YCATRLWLGDPHTDKLIFGKGTRVTVPEPSQPHTKP SVFVMKNGTNVACLVEKFPKDIRINLVSSKITEFDP AIVISPSGKYNAVKLGKYEDSNSVTCSVQHDNKT VH STDFEVKTDSTDHVKPKETENTKQPSKSKCHKPKAIV HTEKVNMMSLTVLGLRMLFAKTAVNFLLTAKLFFL
7. AF-7 V-α/C-δ chain (For expression of the chimeric AF-7 TCR in Jurkat T-cells, together with 8; C-δ chain sequence underlined.)	MWGVFLLYVSMKMGTTGQNIQPTTEMTATEGAIV QINCTYQTSGFNGLFWYQQHAGEAPTFLSYNVLDGL EEKGRFSSFLSRKGYSYLLKELQMKDSASYLCAV KDSNYQLIWGAGTKLIKPDSQPHTKPSVFMKNGTN <u>VACLVEKFPKDIRINLVSSKITEFDP AIVISPSGKYNAVKLGKYEDSNSVTCSVQHDNKT VHSTDFEVKTDSTDHVKPKETENTKQPSKSKCHKPKAIVHTEKVNMMSLTVLGLRMLFAKTAVNFLLTAKLFFL</u>
8. AF-7 V-β/C-γ chain (For expression of the chimeric AF-7 TCR in Jurkat T-cells, together with 7; C-γ chain sequence underlined.)	MGILPSPGMPALLSLVSLLSVLLMGCVAETSYPYDVP DYAGGSGTSNAGVTQTPKFQVLKTGQSMTLQCAQD MNHNSMYWYRQDPGMGLRLIYSAEGTTDKGEVP NGYNVSRNLNKRFLRLESAAPSQTSVYFCASSVWT GEGSGELFFGEGSRLTVLEDKQLDADVSPKPTIFLPS <u>IAETKLQKAGTYLCLLEKFFPDVIKIHVQEKKSNTILGSQEGNTMKTNDTYMKF SWLTVPEKSLDKEHRCIVRHENNKNGVDQEIIFPIKTDVITMDPKDNCSKDANDTLLLQLTNTSAYMYLLLLLKSVVYFAITCCLLRRTAFCCNGEKS</u>
9. G83.C4 V-γ/C-β chain (For expression of the chimeric G83.C4 TCR in Jurkat T-cells, together with 10; C-β chain sequence underlined.)	MLLALALLAFLPPASQKTSYPYDVPDYAGGSGTSS SNLEGRTKSVTRPTGSSAVITCDLPVENAVYTHWYL HQEGKAPQRLLYYDSYNSRVVLESGISREKYHTYAS TGKSLKFILENLIERDSGVYYCATWDYKFLFGSGTTL VVDLKNVFPKVAVFEPSEAEISHTQKATLVCLATG FYPDHVELSWWWNGKEVHSGVSTDPQPLKEQPALN <u>DSRYCLSSRLRVSATFWQNP RNHFRCQVQFYGLSENDEWTQDRAKPVTQIVSAEAWGRADCGFTSESYQQGVLSATILYEILLGKATLYAVLVSALVLMAMVKRKDSRG</u>
10. G83.C4 V-δ/C-α chain (For expression of the chimeric G83.C4 TCR in Jurkat T-cells, together with 9; C-α chain sequence underlined.)	MILTVGFSFLFFYRGTLCDKVTQSSPDQTVASGSEV VLLCTYDTVYSNPDLFWYRIRPDYSFQFVYGDNSR SEGADFTQGRFSVKHILTKQAFHLVISPVRTEDSATY YCATRLWLGDPHTDKLIFGKGTRVTVPEPQNPDP <u>VYQLRDSKSSDKSVCLFTDFDSQTNVSQSKSDVYITDKTVLDMRSMDFKSN SAVAWSNKSDFACANAFNN SIIPEDTFFPSPPESSCDVKLVEKSFETDTNLNFQNL SVIGFRILLKLVAGFNLLMTRLRLWSS</u>