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Supplementary Materials for

TCR/CD3-based synthetic antigen receptors (TCC) convey superior antigen sensitivity combined with high fidelity of activation

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This PDF file includes:

Figure S1

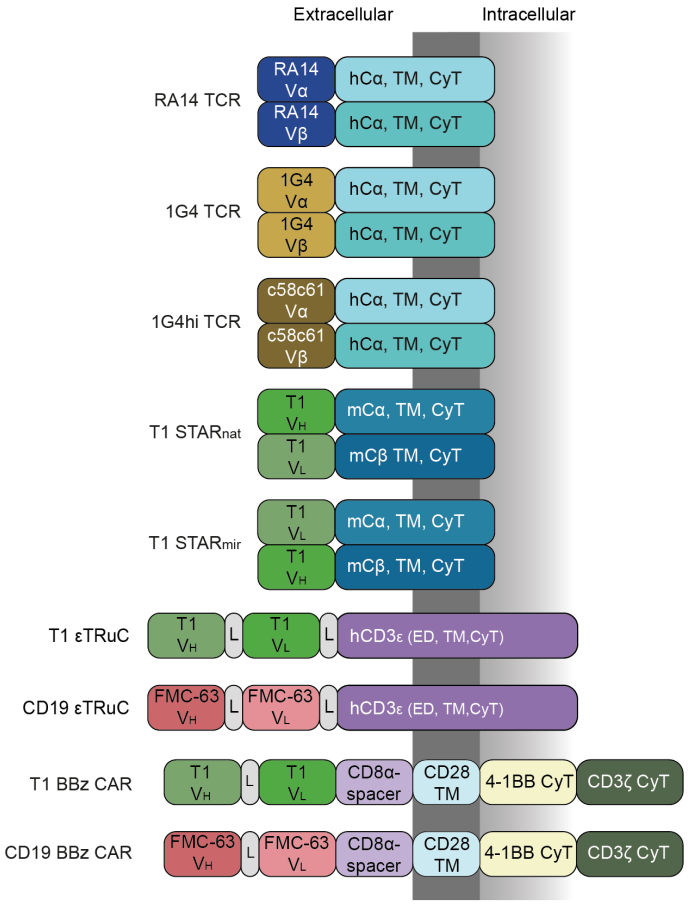

Extracellular
Intracellular
RA14 TCR
RA14 Vα
hCα, TM, CyT
RA14 Vβ
hCα, TM, CyT
1G4 TCR
1G4 Vα
hCα, TM, CyT
1G4 Vβ
hCα, TM, CyT
1G4hi TCR
c58c61 Vα
hCα, TM, CyT
c58c61 Vβ
hCα, TM, CyT
T1 STARnat
T1 VH
mCα, TM, CyT
T1 VL
mCβ TM, CyT
T1 STARmir
T1 VL
mCα, TM, CyT
T1 VH
mCβ, TM, CyT
T1 εTRuC
T1 VH
L
T1 VL
L
hCD3ε (ED, TM,CyT)
CD19 εTRuC
FMC-63 VH
L
FMC-63 VL
L
hCD3ε (ED, TM,CyT)
T1 BBz CAR
T1 VH
L
T1 VL
CD8α-spacer
CD28 TM
4-1BB CyT
CD3ζ CyT
CD19 BBz CAR
FMC-63 VH
L
FMC-63 VL
CD8α-spacer
CD28 TM
4-1BB CyT
CD3ζ CyT

Figure S1: Schematic illustration of TCRs, TCCs and CARs employed in this study. V_H = variable domain of the heavy chain, V_L= variable domain of the light chain, L = linker, Vα = variable domain of TCRα, Vβ = variable domain of TCRβ, hCα(β) = constant domain of human TCRα(β), mCα(β) = constant domain of murine TCRα(β), TM = transmembrane domain, CyT = cytoplasmic tail

Figure S2

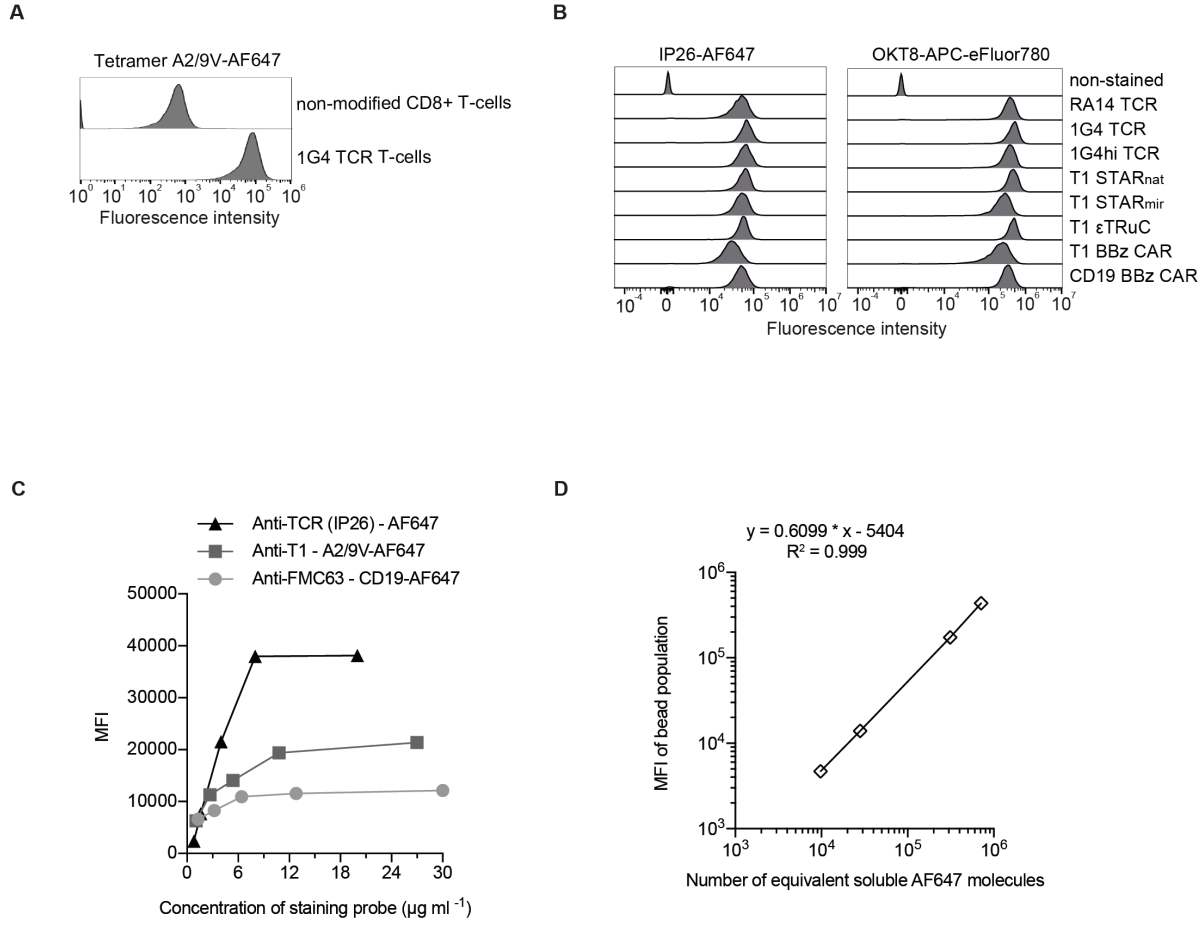

A
Tetramer A2/9V-AF647
non-modified CD8+ T-cells
1G4 TCR T-cells
Fluorescence intensity
B
IP26-AF647
OKT8-APC-eFluor780
non-stained
RA14 TCR
1G4 TCR
1G4hi TCR
T1 STARnat
T1 STARmir
T1 εTRuC
T1 BBz CAR
CD19 BBz CAR
Fluorescence intensity
C
Anti-TCR (IP26) - AF647
Anti-T1 - A2/9V-AF647
Anti-FMC63 - CD19-AF647
MFI
Concentration of staining probe (µg ml -1)
D
y = 0.6099 * x - 5404
R² = 0.999
MFI of bead population
Number of equivalent soluble AF647 molecules

Figure S2: Flow cytometric analysis of antigen receptor surface expression. (**A**) Surface staining of 1G4 TCRs via A2/9V-AF647 tetramers. (**B**) Engineered T-cells were labeled with TCR-reactive IP26-AF647 (left panel) and CD8-specific OKT8-APC-eFluor780 (right panel). (**C**) RA14 TCR T-cells, T1 BBz CAR T-cells and CD19 BBz CAR T-cells were stained as indicated with A2/CMV-AF647, A2/9V-AF647 and CD19-AF647 to determine probe concentrations required for label saturation. (**D**) Calibration curve of AF647 intensity values determined with the use of Quantum™ AF647 MESF beads.

Figure S3

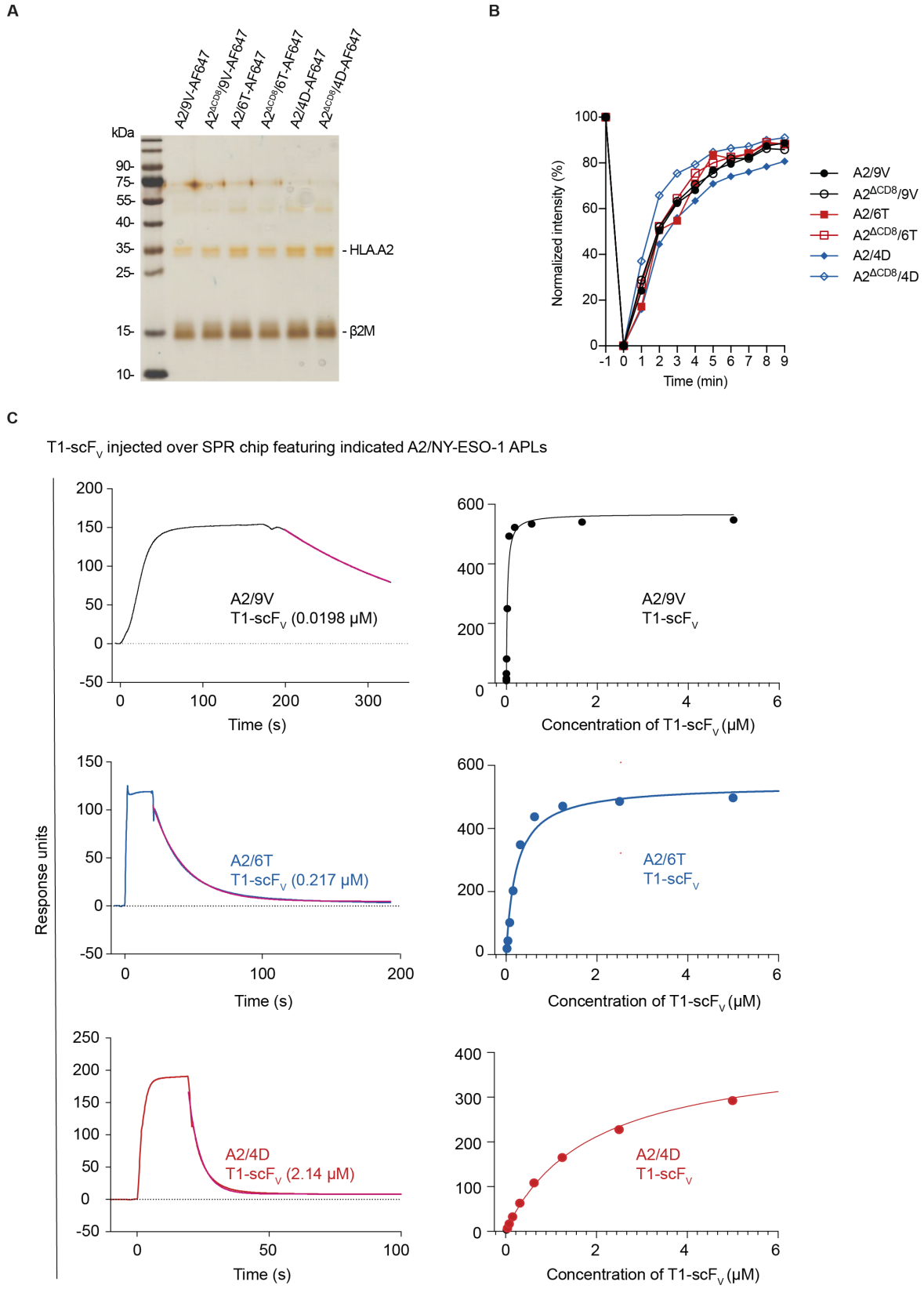

A
A2/9V-AF647
A2ΔCD8/9V-AF647
A2/6T-AF647
A2ΔCD8/6T-AF647
A2/4D-AF647
A2ΔCD8/4D-AF647
kDa
90-
75-
55-
40-
35-
25-
15-
10-
- HLA.A2
- β2M
B
Normalized intensity (%)
Time (min)
A2/9V
A2ΔCD8/9V
A2/6T
A2ΔCD8/6T
A2/4D
A2ΔCD8/4D
C
T1-scFV injected over SPR chip featuring indicated A2/NY-ESO-1 APLs
Response units
A2/9V
T1-scFV (0.0198 μM)
Time (s)
A2/9V
T1-scFV
Concentration of T1-scFV (μM)
A2/6T
T1-scFV (0.217 μM)
Time (s)
A2/6T
T1-scFV
Concentration of T1-scFV (μM)
A2/4D
T1-scFV (2.14 μM)
Time (s)
A2/4D
T1-scFV
Concentration of T1-scFV (μM)

Figure S3: SPR-based analysis and production of A2/NY-ESO-1 variants for SLB functionalization. (**A**) Analysis of apparent molecular weight via 12.5 % reducing SDS PAGE of all HLA-A2/NY-ESO-1 variants employed for SLB functionalization. (**B**) Fluorescence Recovery After Photobleaching (FRAP) was employed to determine the immobile fraction of SLB-anchored A2/NY-ESO-1 variants. Fluorescence intensities were normalized to the initial intensity values and plotted versus time. Data are representative of n=3 biological replicates. (**C**) SPR-based analysis of the interaction kinetics between the T1-scFV (solute) and HLA.A2 complexed with the altered peptide ligands (APLs) 9V-, 6T- and 4D-NY-ESO-1 as indicated (on the chip). Data are representative for one SPR measurement. Results are summarized in **table S1.**

Figure S4

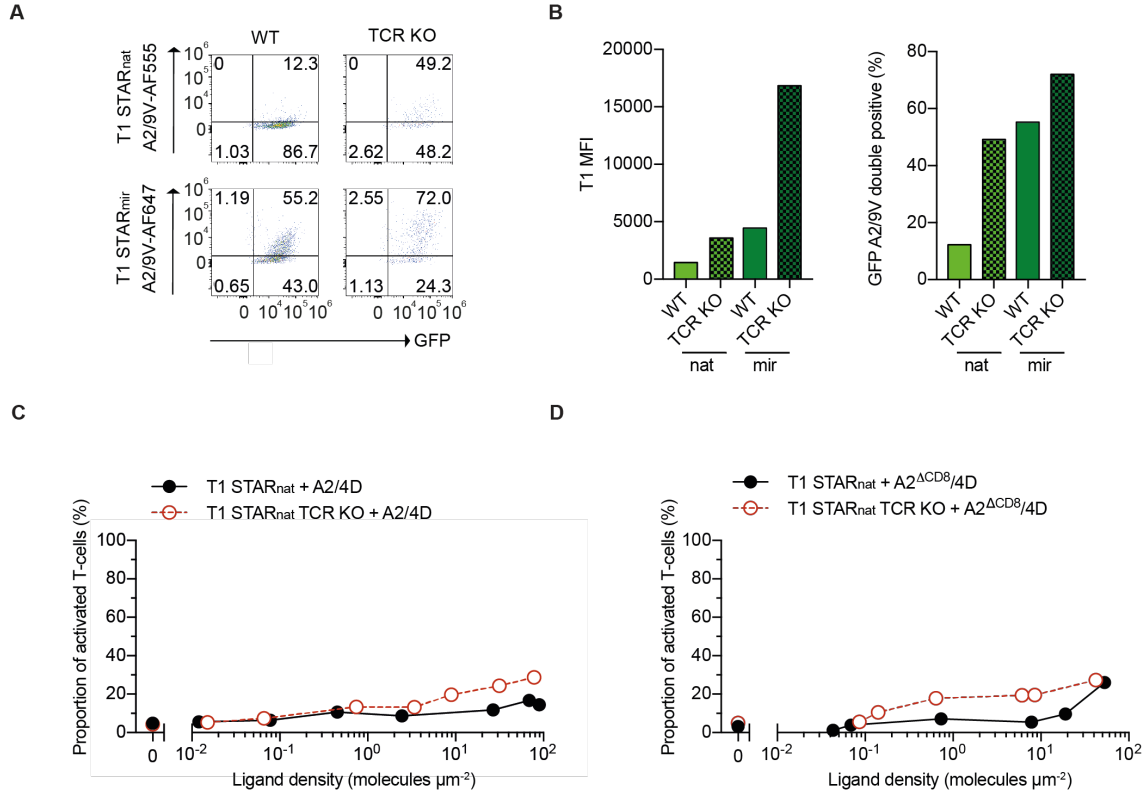

A
WT
TCR KO
T1 STARnat A2/9V-AF555
T1 STARmir A2/9V-AF647
0
12.3
1.03
86.7
49.2
2.62
48.2
1.19
55.2
0.65
43.0
2.55
72.0
1.13
24.3
GFP
B
T1 MFI
GFP A2/9V double positive (%)
WT
TCR KO
nat
mir
C
T1 STARnat + A2/4D
T1 STARnat TCR KO + A2/4D
Proportion of activated T-cells (%)
Ligand density (molecules µm-2)
D
T1 STARnat + A2ΔCD8/4D
T1 STARnat TCR KO + A2ΔCD8/4D
Proportion of activated T-cells (%)
Ligand density (molecules µm-2)

Figure S4: Surface expression of lentivirally introduced T1 $STAR_{nat}$ and T1 $STAR_{mir}$ improves substantially after CRISPR/Cas9-mediated genetic ablation of endogenous TCRαβ, but does not majorly affect the sensitivity of T1 $STAR_{nat}$ T-cells towards the A2/4D low affinity ligand. (**A**, **B**) Flow cytometric analysis of surface expression in TCR^{+} (WT) and TCR^{-} (KO) T-cells expressing T1 $STAR_{nat}$ and T1 $STAR_{mir}$ with the use of A2/9V-AF555 and A2/9V-AF647, respectively. (**C**, **D**) T1 $STAR_{nat}$ T-cell calcium response towards SLBs featuring ICAM-1 and A2/4D or $A2^{\Delta CD8}$/4D at indicated densities. Data are representative of one donor.

Figure S5

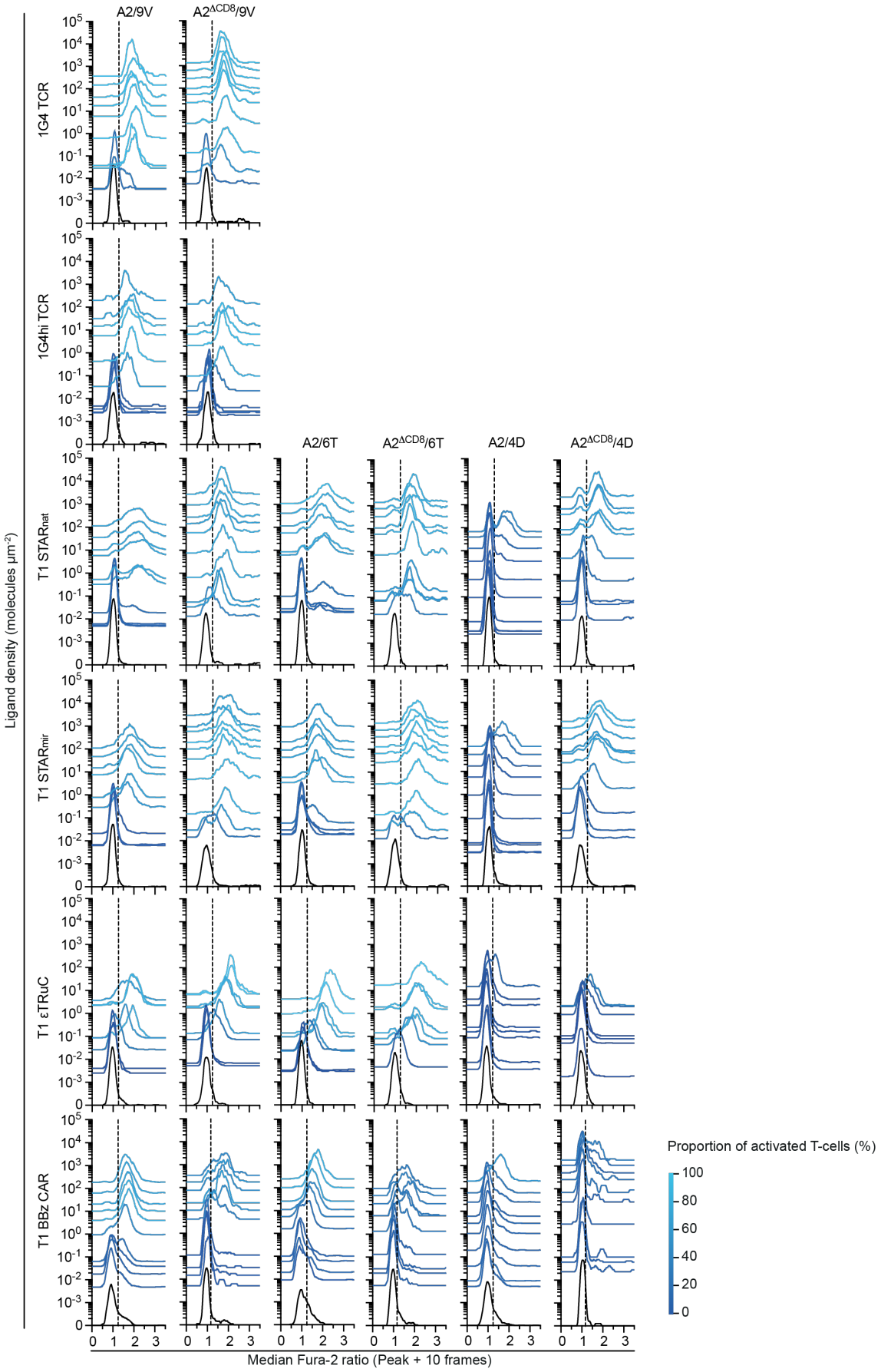

A2/9V
A2ΔCD8/9V
A2/6T
A2ΔCD8/6T
A2/4D
A2ΔCD8/4D
1G4 TCR
1G4hi TCR
T1 STARnat
T1 STARmir
T1 εTRuC
T1 BBz CAR
Ligand density (molecules µm-2)
Median Fura-2 ratio (Peak + 10 frames)
Proportion of activated T-cells (%)
100
80
60
40
20
0

Figure S5: Assessment of antigen sensitivity conveyed by NY-ESO-1-specific antigen receptor constructs. Population-based analysis of the calcium response of A2/NY-ESO-1-specific cells (one experiment shown) confronted with indicated A2/NY-ESO-1 variants at indicated densities (data refers to **Fig. 4** and **Fig. 5**). A2/NY-ESO-1-specific constructs were introduced by means of lentiviral transduction (T1 εTRuC) or CRISPR/Cas9-mediated knock in (1G4 TCR, 1G4hi TCR, T1 $STAR_{nat}$, T1 $STAR_{mir}$, T1 BBz CAR). Dashed lines indicate Fura-2 ratio thresholds above which cells were considered activated (i.e., 1.25 for 1G4 TCR T-cells, 1G4hi TCR T-cells, T1 $STAR_{nat}$ T-cells, T1 $STAR_{mir}$ T-cells, T1 εTRuC T-cells and 1.15 for T1 BBz CAR T-cells).

Figure S6

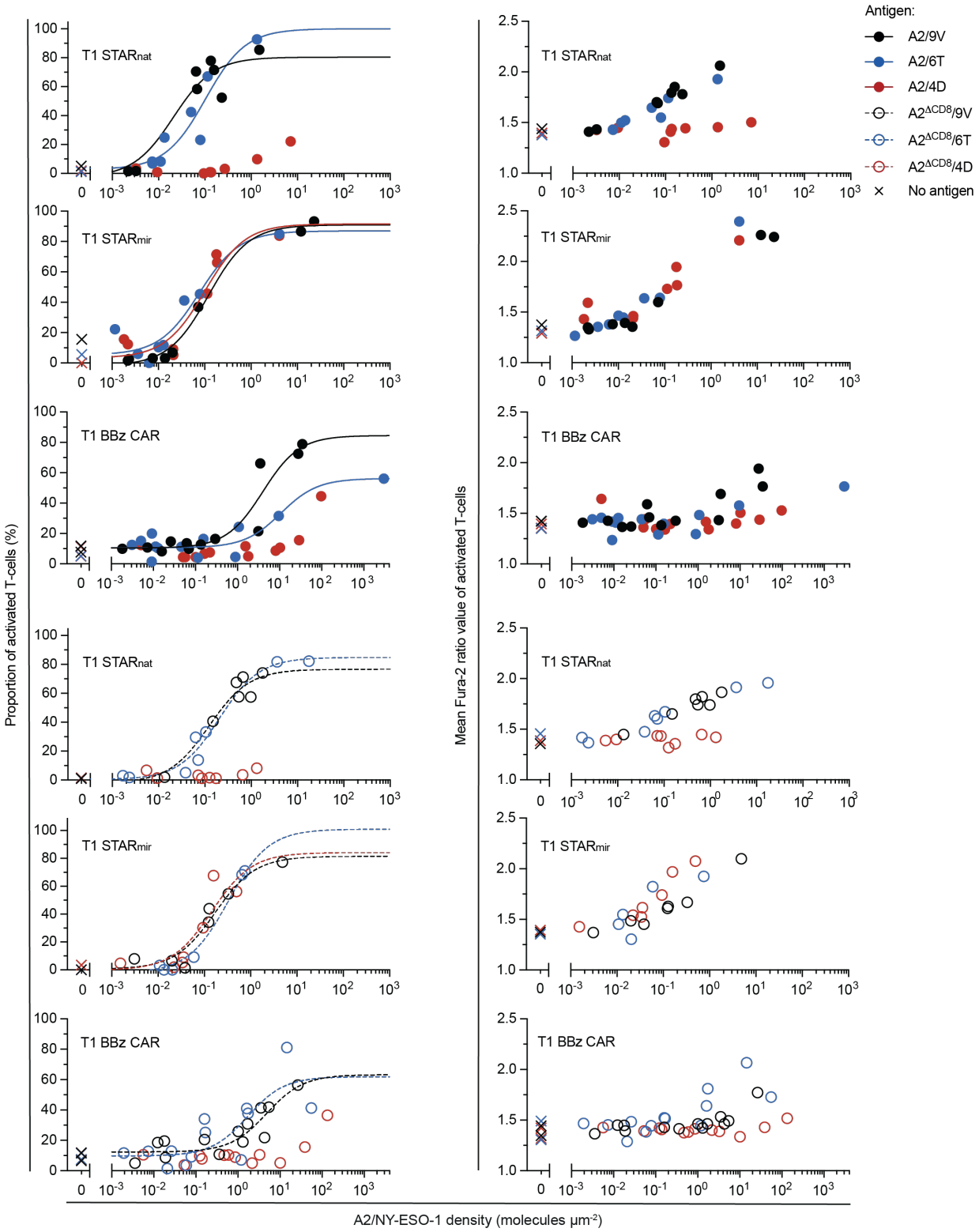

Antigen:
A2/9V
A2/6T
A2/4D
A2ΔCD8/9V
A2ΔCD8/6T
A2ΔCD8/4D
No antigen
T1 STARnat
T1 STARmir
T1 BBz CAR
Proportion of activated T-cells (%)
Mean Fura-2 ratio value of activated T-cells
A2/NY-ESO-1 density (molecules µm-2)

Figure S6: Assessment of calcium signaling of T-cells equipped with NY-ESO-1-specific TCCs and CARs. (**A**) Calcium dose-response of lentivirally transduced T1 $STAR_{nat}$ T-cells, T1 $STAR_{mir}$ T-cells and T1 BBz CAR T-cells which had been confronted with SLBs functionalized with A2/9V, A2/6T, and A2/4D at indicated densities. Data are representative of n=1-2 experiments with one to two donors. Data were fitted to a three-parameter dose response curve (equation (1)) to extract EC50 values and 95% confidence intervals as summarized in **Table 1.**

Figure S7

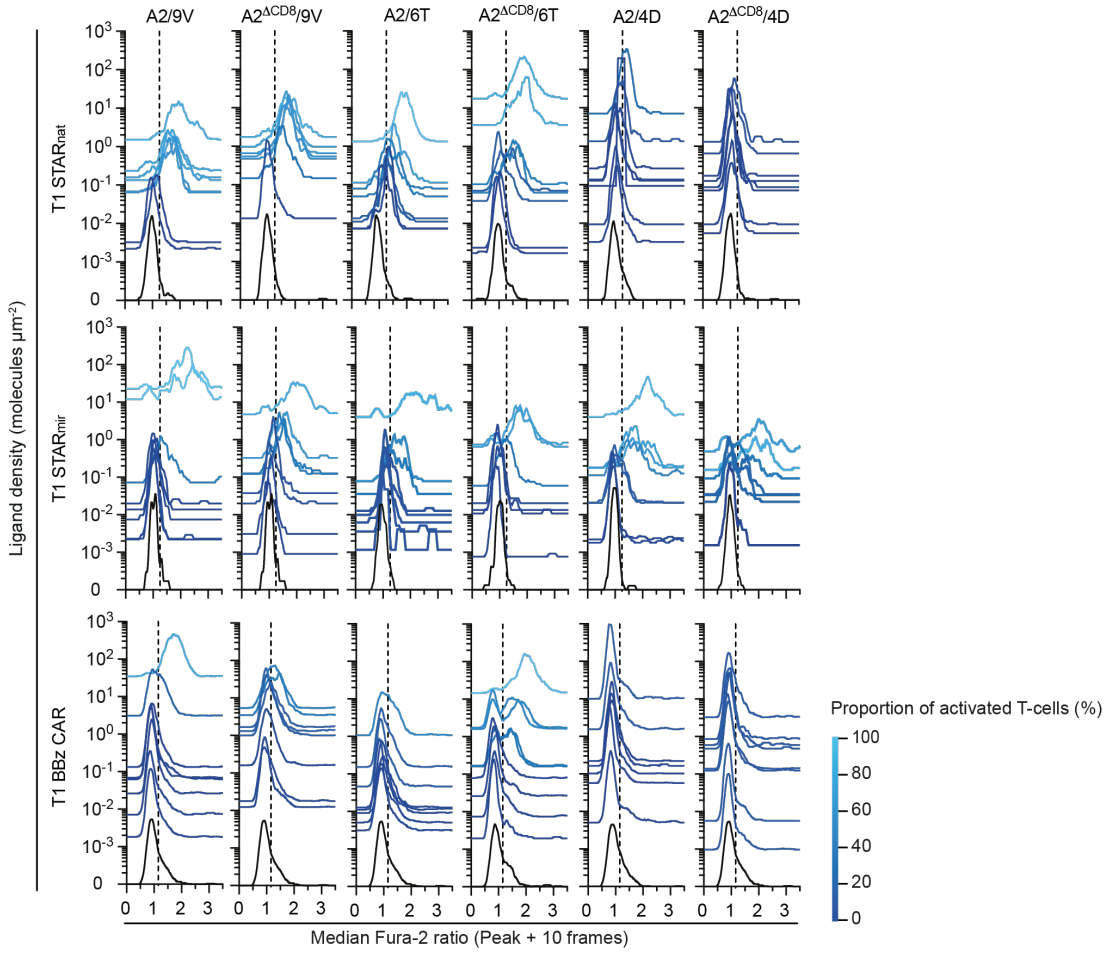
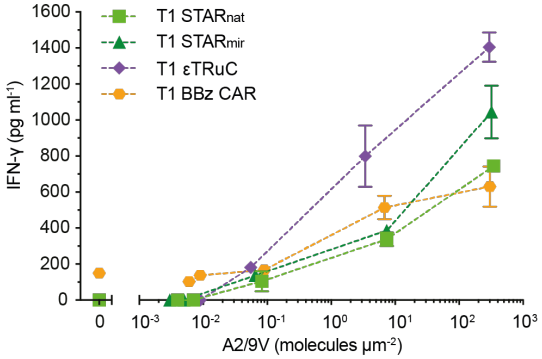

A2/9V
A2ΔCD8/9V
A2/6T
A2ΔCD8/6T
A2/4D
A2ΔCD8/4D
T1 STARnat
T1 STARmir
T1 BBz CAR
Ligand density (molecules µm-2)
Median Fura-2 ratio (Peak + 10 frames)
Proportion of activated T-cells (%)
100
80
60
40
20
0

Figure S7: Assessment of antigen sensitivity conveyed by lentivirally transduced NY-ESO-1-specific antigen receptor constructs. Population-based analysis of the calcium response of A2/NY-ESO-1-specific cells (of one donor) confronted with indicated A2/NY-ESO-1 variants at indicated densities (refer to **fig. S6**). Dashed lines indicate Fura-2 ratio threshold above which cells were considered activated (i.e., 1.25 for T1 $STAR_{nat}$ T-cells, T1 $STAR_{mir}$ T-cells and 1.15 for T1 BBz CAR T-cells).

Figure S8

A


T1 STARnat
T1 STARmir
T1 εTRuC
T1 BBz CAR
IFN-γ (pg ml-1)
A2/9V (molecules µm-2)

B

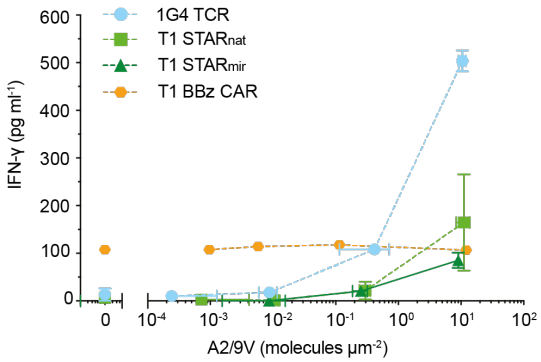

1G4 TCR
T1 STARnat
T1 STARmir
T1 BBz CAR
IFN-γ (pg ml-1)
A2/9V (molecules µm-2)

Figure S8: IFN-γ secretion by engineered T-cells responding to SLBs decorated with ICAM-1 and antigen at indicated densities. IFN-γ secreted into the media of engineered T-cells stimulated via SLB-resident A2/9V present at indicated densities. A2/NY-ESO-1-specific constructs were introduced by means of lentiviral transduction (**A**) or CRISPR/Cas9-mediated knock in (**B**). Data were pooled from n=1 (A) and n=2 (B) biological replicates each containing n=2 technical duplicates. Statistics: Mean and s.e.m. of n=1-2 biological replicates.

Figure S9

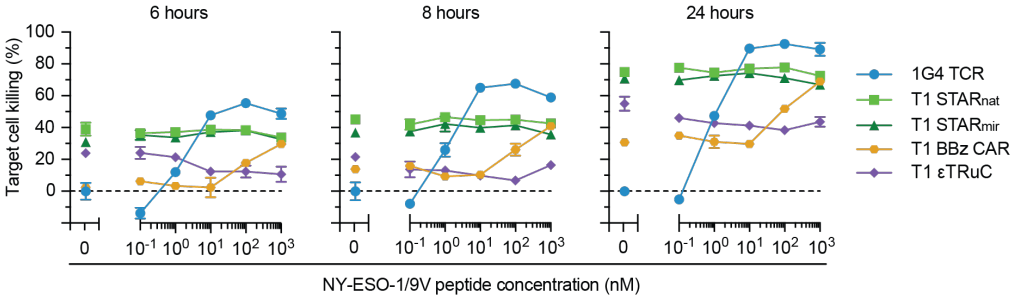

6 hours
8 hours
24 hours
Target cell killing (%)
100
80
60
40
20
0
0
10-1 100 101 102 103
NY-ESO-1/9V peptide concentration (nM)
1G4 TCR
T1 STARnat
T1 STARmir
T1 BBz CAR
T1 εTRuC

Figure S9: *Ex vivo* cytotoxic capacity of engineered T-cells with high antigen receptor expression levels. A2/NY-ESO-1-specific constructs (refer to **Fig. 3B-D**) were introduced by means of lentiviral transduction (T1 εTRuC, high expressors) or CRISPR/Cas9-mediated knock in (1G4 TCR, T1 $STAR_{nat}$, T1 $STAR_{mir}$, T1 BBz CAR). Indicated effector cells were cocultured at a ratio of 1:1 and for indicated times with HLA-A2-, CD80-, and luciferase-expressing K562 feeder cells, which had been pre-pulsed with the NY-ESO-1 peptide derivative 9V at indicated concentrations. Statistics: Mean ± s.e.m. from technical duplicates of one donor. Data are representative for n=2 biological replicates.

Table S1

Analyte	Ligand name	Peptide	Ka (mol^{-1} s^{-1})	Kd (s^{-1})	KD (M)	Half-life (s)	Temp
RA14 TCR	A2/CMV	NLVPMVATV	9.60E+04	8.00E-01	8.30E-06	0.87	37 °C
1G4 TCR	A2/9V	SLLMWITQV	5.34E+04	7.80E-02	1.46E-06	8.89	37 °C
1G4hi TCR	A2/9V	SLLMWITQV	1.17E+06	8.26E-05	7.07E-11	8391.61	37 °C
	A2/6T	SLLMWTTQV	8.64E+05	7.14E-02	8.27E-08	9.71	37 °C
T1 scFv	A2/9V	SLLMWITQV	2.32E+05	4.60E-03	1.98E-08	150.68	37 °C
	A2/6T	SLLMWTTQV	1.64E+05	3.56E-02	2.17E-07	19.47	37 °C
	A2/4D	SLLDWITQV	1.09E+05	2.33E-01	2.14E-06	2.97	37 °C
FMC63 scFv	CD19		2.10E+05	6.80E-05	3.00E-08	10193.34	25 °C

Table S1. Binding constants for indicated receptor-ligand pairs at indicated temperatures as determined by surface plasmon resonance.

SUPPLEMENTARY MATERIAL:

Gene and protein sequences of constructs employed in this study

Data S1: c58c61 1G4hi TCR T2A copGFP (GenBank: PP746584)

Nucleotide sequence

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 121 accggccaga gcatgaccct gcagtgcgcc caggatatga accacgagta catgagctgg
 181 tacagacagg accctggcat gggcctgaga ctgatccact actctgtggc catccagacc
 241 accgacagag gcgaagtgcc caacggctac aacgtgtcca gaagcaccat cgaggacttc
 301 ccactgagac tgctgtctgc cgctcctagc cagaccagcg tgtacttttg tgccagcagc
 361 tacctgggca acaccggcga gctgtttttt ggcgagggca gcagactgac cgtgctggaa
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 541 gaactgtctt ggtgggtcaa cggcaaagag gtgcacagcg gcgtcagcac agatccccag
 601 cctctgaaag aacagcccgc tctgaacgac agccggtact gtctgtcctc cagactgaga
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 721 ggcctgagcg agaacgatga gtggacccag gatagagcca agcctgtgac acagatcgtg
 781 tctgccgaag cctggggcag agccgattgt ggctttacca gcgagagcta ccagcagggc
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 901 ctggtgtctg ccctggtgct gatggccatg gtcaagcgga aggacttttc tagaggcgcc
 961 gctgccggac cttctggacc tgatcctgcc accaatttca gcctgctgaa acaggccggc
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1081 caactgcaat gggtgtcctc caagcaagaa gtgactcaga tcccagccgc tctgagtgtg
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1441 ccctacattc agaacccga tcctgccgtg tatcagctga gagacagcaa gtccagcgac
1501 aagagcgtgt gcctgttcac cgacttcgac tcccagacca atgtgtccca gagcaaggac
1561 agcgacgtgt acatcaccga caagacagtg ctggacatgc ggagcatgga cttcaagagc
1621 aacagcgccg tggcctggtc caacaagagc gatttcgcct gcgccaacgc cttcaacaac
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1861 tccagcgaat tcgaaggatc cgcggccgct gagggcagag gaagtcttct aacatgcggt
1921 gacgtggagg agaatcccgg cccttccgga atggagagcg acgagagcgg cctgcccgcc
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```

```
2041 ggcggagagg gcacccccaa gcagggccgc atgaccaaca agatgaagag caccaaaggc
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2161 ggcacctacc ccagcggcta cgagaacccc ttcctgcacg ccatcaacaa cggcggctac
2221 accaacaccc gcatcgagaa gtacgaggac ggcggcgtgc tgcacgtgag cttcagctac
2281 cgctacgagg ccggccgcgt gatcggcgac ttcaaggtgg tgggcaccgg cttccccgag
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2581 accgagctgg gcatcgtgga gtaccagcac gccttcaaga cccccatcgc cttcgccaga
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2701 tctcgctag
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Protein sequence:

SP TCRβ P2A SP TCRα T2A GFP

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MACGSATMSI GLLCCAALSL LWAGPVNAGV TQTPKFQVLK TGQSMTLQCA QDMNHEYMSW
61         71         81         91         101        111
YRQDPGMGLR LIHYSVAIQT TDRGEVPNGY NVSRSTIEDF PLRLLSAAPS QTSVYFCASS
121        131        141        151        161        171
YLGNTGELFF GEGSRLTVLE DLKNVFPPEV AVFEPSEAEI SHTQKATLVC LATGFYPDHV
181        191        201        211        221        231
ELSWWVNGKE VHSGVSTDPQ PLKEQPALND SRYCLSSRLR VSATFWQNPR NHFRCQVQFY
241        251        261        271        281        291
GLSENDEWTQ DRAKPVTQIV SAEAWGRADC GFTSESYQQG VLSATILYEI LLGKATLYAV
301        311        321        331        341        351
LVSALVLMAM VKRKDFSRGA AAGPSGPDPA TNFSLLKQAG DVEENPGPME TLLGLLILWL
361        371        381        391        401        411
QLQWVSSKQE VTQIPAALSV PEGENLVLNC SFTDSAIYNL QWFRQDPGKG LTSLLLITPW
421        431        441        451        461        471
QREQTSGRLN ASLDKSSGRS TLYIAASQPG DSATYLCAVR PLLDGTYIPT FGRGTSLIVH
481        491        501        511        521        531
PYIQNPDPAV YQLRDSKSSD KSVCLFTDFD SQTNVSQSKD SDVYITDKTV LDMRSMDFKS
541        551        561        571        581        591
NSAVAWSNKS DFACANAFNN SIIPEDTFFP SPESSCDVKL VEKSFETDTN LNFQNLSVIG
601        611        621        631        641        651
FRILLLKVAG FNLLMTLRLW SSEFEGSAAA EGRGSLLTCG DVEENPGPSG MESDESGLPA
661        671        681        691        701        711
MEIECRITGT LNGVEFELVG GGEGTPKQGR MTNKMKSTKG ALTFSPYLLS HVMGYGFYHF
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721 731 741 751 761 771

GTYPSGYENP FLHAINNGGY TNTRIEKYED GGVLHVSFSY RYEAGRVIGD FKVVGTGFPE

781 791 801 811 821 831

DSVIFTDKII RSNATVEHLH PMGDNVLVGS FARTFSLRDG GYYSFVVDSH MHFKSAIHPS

841 851 861 871 881 891

ILQNGGPMFA FRRVEELHSN TELGIVEYQH AFKTPIAFAR SRAQSSNSAV DGTAGPGSTG

901

SR

Data S2: T1 STAR$_{nat}$ T2A copGFP (GenBank: PP746585)

Nucleotide sequence

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 121 tgtaccggca ccgaaagaga tgtgggcggc tacaactacg tgtcctggta tcagcagcac
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 361 accggcaccg acgtgaccgt ggaggatctg agaaatgtga ctccacccaa ggtctccttg
 421 tttgagccat caaaagcaga gattgcaaac aaacaaaagg ctaccctcgt gtgcttggcc
 481 aggggcttct tccctgacca cgtggagctg agctggtggg tgaatggcaa ggaggtccac
 541 agtggggtct gcacggaccc tcaggcctac aaggagagca attatagcta ctgcctgagc
 601 agccgcctga gggtctctgc taccttctgg cacaatcctc gaaaccactt ccgctgccaa
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2341 gtgctggtgg gcagcttcgc ccgcaccttc agcctgcgcg acggcggcta ctacagcttc
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2461 ggccccatgt tcgccttccg ccgcgtggag gagctgcaca gcaacaccga gctgggcatc
2521 gtggagtacc agcacgcctt caagaccccc atcgccttcg ccagatcccg cgctcagtcg
2581 tccaattctg ccgtggacgg caccgccgga cccggctcca ccggatctcg ctag

Protein sequence

SP T1 V_L mCβ T2A SP T1 V_H mCα T2A GFP

1 11 21 31 41 51
MGSWTLCCVS LCILVAKHTQ SELTQPRSVS GSPGQSVTIS CTGTERDVGG YNYVSWYQQH
61 71 81 91 101 111
PGKAPKLIIH DVIERSSGVP DRFSGSKSGN TASLTISGLQ AEDEADYYCW SFAGGYYVFG
121 131 141 151 161 171
TGTDVTVEDL RNVTPPKVSL FEPSKAEIAN KQKATLVCLA RGFFPDHVEL SWWVNGKEVH
181 191 201 211 221 231
SGVCTDPQAY KESNYSYCLS SRLRVSATFW HNPRNHFRCQ VQFHGLSEED KWPEGSPKPV
241 251 261 271 281 291
TQNISAEAWG RADCGITSAS YHQGVLSATI LYEILLGKAT LYAVLVSGLV LMAMVKKKNS
301 311 321 331 341 351
GSGEGRGSLL TCGDVEENPG PMMKSLRVLL VILWLQLSWV WSQEVQLLES GGGLVQPGGS
361 371 381 391 401 411
LRLSCAASGF TFSTYQMSWV RQAPGKGLEW VSGIVSSGGS TAYADSVKGR FTISRDNSKN
421 431 442 451 461 471
TLYLQMNSLR AEDTAVYYCA GELLPYYGMD VWGQGTTVTV SIQNPEPAVY QLKDPRSQDS
481 491 501 511 521 531
TLCLFTDFDS QINVPKTMES GTFITDKCVL DMKAMDSKSN GAIAWSNQTS FTCQDIFKET
541 551 561 571 581 591
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GSAAAEGRGS LLTCGDVEEN PGPSGMESDE SGLPAMEIEC RITGTLNGVE FELVGGGEGT
661 671 681 691 701 711
PKQGRMTNKM KSTKGALTFS PYLLSHVMGY GFYHFGTYPS GYENPFLHAI NNGGYTNTRI
721 731 741 751 761 771
EKYEDGGVLH VSFSYRYEAG RVIGDFKVVG TGFPEDSVIF TDKIIRSNAT VEHLHPMGDN
781 791 801 811 821 831
VLVGSFARTF SLRDGGYYSF VVDSHMHFKS AIHPSILQNG GPMFAFRRVE ELHSNTELGI
841 851 861 871 877

VEYQHAFKTP IAFARSRAQS SNSAVDGTAG PGSTGSR

Data S3: T1 STAR$_{mir}$ T2A copGFP (GenBank: PP746586)

Nucleotide sequence

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 181 ggcaagggcc tggagtgggt gtccggcatc gtgtccagcg gcggcagcac cgcctacgcc
 241 gacagcgtga agggcaggtt caccatcagc cgggacaaca gcaagaacac cctgtacctg
 301 cagatgaaca gcctgagagc cgaggacacc gccgtgtact actgtgccgg cgagctgctg
 361 ccctactacg gcatggatgt gtggggccag ggcaccaccg tgacagtgag cgaggatctg
 421 agaaatgtga ctccacccaa ggtctccttg tttgagccat caaaagcaga gattgcaaac
 481 aaacaaaagg ctaccctcgt gtgcttggcc aggggcttct tccctgacca cgtggagctg
 541 agctggtggg tgaatggcaa ggaggtccac agtggggtct gcacggaccc tcaggcctac
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 781 cgagcagact gtggaatcac ttcagcatcc tatcatcagg gggttctgtc tgcaaccatc
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 901 ctgatggcca tggtcaagaa aaaaaattcc ggcagcggcg agggcagagg aagtctgcta
 961 acatgcggtg acgtcgagga gaatcctgga cctatgatga agtccctgag ggtgctgctg
1021 gtgatcctct ggctgcagct gagctgggtg tggtcccagc agagcgagct gacccagccc
1081 agaagcgtgt ccggcagccc tggccagagc gtgaccatca gctgtaccgg caccgaaaga
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1381 gtgatccaga acccagaacc tgctgtgtac cagttaaaag atcctcggtc tcaggacagc
1441 accctctgcc tgttcaccga ctttgactcc caaatcaatg tgccgaaaac catggaatct
1501 ggaacgttca tcactgacaa atgcgtgctg gacatgaaag ctatggattc caagagcaat
1561 ggggccattg cctggagcaa ccagacaagc ttcacctgcc aagatatctt caaagagacc
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1681 gaaacagata tgaacctaaa ctttcaaaac ctgtcagtta tgggactccg aatcctcctg
1741 ctgaaagtag ccggatttaa cctgctcatg acgctgaggc tgtggtccag tgaattcgaa
1801 ggatccgcgg ccgctgaggg cagaggaagt cttctaacat gcggtgacgt ggaggagaat
1861 cccggccctt ccggaatgga gagcgacgag agcggcctgc ccgccatgga gatcgagtgc
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2101 ggctacgaga acccccttcct gcacgccatc aacaacggcg gctacaccaa cacccgcatc
2161 gagaagtacg aggacggcgg cgtgctgcac gtgagcttca gctaccgcta cgaggccggc
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2221 cgcgtgatcg gcgacttcaa ggtggtgggc accggcttcc ccgaggacag cgtgatcttc
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2341 gtgctggtgg gcagcttcgc ccgcaccttc agcctgcgcg acggcggcta ctacagcttc
2401 gtggtggaca gccacatgca cttcaagagc gccatccacc ccagcatcct gcagaacggg
2461 ggccccatgt tcgccttccg ccgcgtggag gagctgcaca gcaacaccga gctgggcatc
2521 gtggagtacc agcacgcctt caagaccccc atcgccttcg ccagatcccg cgctcagtcg
2581 tccaattctg ccgtggacgg caccgccgga cccggctcca ccggatctcg ctag

Protein sequence:

SP T1 V_H mCβ T2A SP T1 V_L mCα T2A GFP

1 11 21 31 41 51
MGSWTLCCVS LCILVAKHTE VQLLESGGGL VQPGGSLRLS CAASGFTFST YQMSWVRQAP
61 71 81 91 101 111
GKGLEWVSGI VSSGGSTAYA DSVKGRFTIS RDNSKNTLYL QMNSLRAEDT AVYYCAGELL
121 131 141 151 161 171
PYYGMDVWGQ GTTVTVSEDL RNVTPPKVSL FEPSKAEIAN KQKATLVCLA RGFFPDHVEL
181 191 201 211 221 231
SWWVNGKEVH SGVCTDPQAY KESNYSYCLS SRLRVSATFW HNPRNHFRCQ VQFHGLSEED
241 251 261 271 281 291
KWPEGSPKPV TQNISAEAWG RADCGITSAS YHQGVLSATI LYEILLGKAT LYAVLVSGLV
301 311 321 331 341 351
LMAMVKKKNS GSGEGRGSLL TCGDVEENPG PMMKSLRVLL VILWLQLSWV WSQQSELTQP
361 371 381 391 401 411
RSVSGSPGQS VTISCTGTER DVGGYNYVSW YQQHPGKAPK LIIHDVIERS SGVPDRFSGS
421 431 442 451 461 471
KSGNTASLTI SGLQAEDEAD YYCWSFAGGY YVFGTGTDVT VIQNPEPAVY QLKDPRSQDS
481 491 501 511 521 531
TLCLFTDFDS QINVPKTMES GTFITDKCVL DMKAMDSKSN GAIAWSNQTS FTCQDIFKET
541 551 561 571 581 591
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601 611 621 631 641 651
GSAAAEGRGS LLTCGDVEEN PGPSGMESDE SGLPAMEIEC RITGTLNGVE FELVGGGEGT
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PKQGRMTNKM KSTKGALTFS PYLLSHVMGY GFYHFGTYPS GYENPFLHAI NNGGYTNTRI
721 731 741 751 761 771
EKYEDGGVLH VSFSYRYEAG RVIGDFKVVG TGFPEDSVIF TDKIIRSNAT VEHLHPMGDN
781 791 801 811 821 831
VLVGSFARTF SLRDGGYYSF VVDSHMHFKS AIHPSILQNG GPMFAFRRVE ELHSNTELGI
841 851 861 871 877

VEYQHAFKTP IAFARSRAQS SNSAVDGTAG PGSTGSR

Data S4: T1 εTRuC T2A copGFP (GenBank: PP746587)

Nucleotide sequence

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2161 ggcatcgtgg agtaccagca cgccttcaag acccccatcg ccttcgccag atcccgcgct
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2221 cagtcgtcca attctgccgt ggacggcacc gccggacccg gctccaccgg atctcgctag

Protein sequence:

SP T1 scF$_V$ linker CD3ε T2A GFP

1 11 21 31 41 51
MLLLVTSLLL CELPHPAFLL IPMAEVQLLE SGGGLVQPGG SLRLSCAASG FTFSTYQMSW
61 71 81 91 101 111
VRQAPGKGLE WVSGIVSSGG STAYADSVKG RFTISRDNSK NTLYLQMNSL RAEDTAVYYC
121 131 141 151 161 171
AGELLPYYGM DVWGQGTTVT VSSAKTTPKL EEGEFSEARV QSELTQPRSV SGSPGQSVTI
181 191 201 211 221 231
SCTGTERDVG GYNYVSWYQQ HPGKAPKLII HDVIERSSGV PDRFSGSKSG NTASLTISGL
241 251 261 271 281 291
QAEDEADYYC WSFAGGYYVF GTGTDVTVLG QPKANPTGGG GSGGGGSGGG GSLEDGNEEM
301 311 321 331 341 351
GGITQTPYKV SISGTTVILT CPQYPGSEIL WQHNDKNIGG DEDDKNIGSD EDHLSLKEFS
361 371 381 391 401 411
ELEQSGYYVC YPRGSKPEDA NFYLYLRARV CENCMEMDVM SVATIVIVDI CITGGLLLLV
421 431 442 451 461 471
YYWSKNRKAK AKPVTRGAGA GGRQRGQNKE RPPPVPNPDY EPIRKGQRDL YSGLNQRRIE
481 491 501 511 521 531
FEGSAAAEGR GSLLTCGDVE ENPGPSGMES DESGLPAMEI ECRITGTLNG VEFELVGGGE
541 551 561 571 581 591
GTPKQGRMTN KMKSTKGALT FSPYLLSHVM GYGFYHFGTY PSGYENPFLH AINNGGYTNT
601 611 621 631 641 651
RIEKYEDGGV LHVSFSYRYE AGRVIGDFKV VGTGFPEDSV IFTDKIIRSN ATVEHLHPMG
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DNVLVGSFAR TFSLRDGGYY SFVVDSHMHF KSAIHPSILQ NGGPMFAFRR VEELHSNTEL
721 731 741 751 759
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Data S5: T1 BBz CAR T2A copGFP (GenBank: PP746588)

Nucleotide sequence

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2221 gggggcccca tgttcgcctt ccgccgcgtg gaggagctgc acagcaacac cgagctgggc
2281 atcgtggagt accagcacgc cttcaagacc cccatcgcct tcgccagatc ccgcgctcag
2341 tcgtccaatt ctgccgtgga cggcaccgcc ggacccggct ccaccggatc tcgctag

Protein sequence

SP T1 scF$_V$ CD8 stalk/CD28 TM/4-1BB CyT CD3ζ CyT T2A GFP

1 11 21 31 41 51
MLLLVTSLLL CELPHPAFLL IPMAEVQLLE SGGGLVQPGG SLRLSCAASG FTFSTYQMSW
61 71 81 91 101 111
VRQAPGKGLE WVSGIVSSGG STAYADSVKG RFTISRDNSK NTLYLQMNSL RAEDTAVYYC
121 131 141 151 161 171
AGELLPYYGM DVWGQGTTVT VSSAKTTPKL EEGEFSEARV QSELTQPRSV SGSPGQSVTI
181 191 201 211 221 231
SCTGTERDVG GYNYVSWYQQ HPGKAPKLII HDVIERSSGV PDRFSGSKSG NTASLTISGL
241 251 261 271 281 291
QAEDEADYYC WSFAGGYYVF GTGTDVTVLG QPKANPTLEN WSHPQFEKGG GGSPGTTTPA
301 311 321 331 341 351
PRPPTPAPTI ASQPLSLRPE ACRPAAGGAV HTRGLDFACD IYIWAPLAGT CGVLLLSLVI
361 371 381 391 401 411
TLYCKRGRKK LLYIFKQPFM RPVQTTQEED GCSCRFPEEE EGGCELRVKF SRSADAPAYQ
421 431 442 451 461 471
QGQNQLYNEL NLGRREEYDV LDKRRGRDPE MGGKPRRKNP QEGLYNELQK DKMAEAYSEI
481 491 501 511 521 531
GMKGERRRGK GHDGLYQGLS TATKDTYDAL HMQALPPREF EGSAAAEGRG SLLTCGDVEE
541 551 561 571 581 591
NPGPSGMESD ESGLPAMEIE CRITGTLNGV EFELVGGGEG TPKQGRMTNK MKSTKGALTF
601 611 621 631 641 651
SPYLLSHVMG YGFYHFGTYP SGYENPFLHA INNGGYTNTR IEKYEDGGVL HVSFSYRYEA
661 671 681 691 701 711
GRVIGDFKVV GTGFPEDSVI FTDKIIRSNA TVEHLHPMGD NVLVGSFART FSLRDGGYYS
721 731 741 751 761 771
FVVDSHMHFK SAIHPSILQN GGPMFAFRRV EELHSNTELG IVEYQHAFKT PIAFARSRAQ
781 791 798
SSNSAVDGTA GPGSTGSR

Data S6: 1G4wt TCR CRISPR construct (GenBank: PP746589)

Nucleotide sequence

```
   1 ctgcctttac tctgccagag ttatattgct ggggttttga agaagatcct attaaataaa
  61 agaataagca gtattattaa gtagccctgc atttcaggtt tccttgagtg gcaggccagg
 121 cctggccgtg aacgttcact gaaatcatgg cctcttggcc aagattgata gcttgtgcct
 181 gtccctgagt cccagtccat cacgagcagc tggtttctaa gatgctattt cccgtataaa
 241 gcatgagacc gtgacttgcc agccccacag agccccgccc ttgtccatca ctggcatctg
 301 gactccagcc tgggttgggg caaagaggga aatgagatca tgtcctaacc ctgatcctct
 361 tgtcccacag atatccagaa ccctgaccct gccgtgggca gcggcgccac caacttcagc
 421 ctgctgaagc aggccggcga cgtggaagag aaccccgggc ccatgagcat cggcctcctg
 481 tgctgtgcag ccttgtctct cctgtgggca ggtccagtga atgctggtgt cactcagacc
 541 ccaaaattcc aggtcctgaa gacaggacag agcatgacac tgcagtgtgc ccaggatatg
 601 aaccatgaat acatgtcctg gtatcgacaa gacccaggca tggggctgag gctgattcat
 661 tactcagttg gtgctggtat cactgaccaa ggagaagtcc ccaatggcta caatgtctcc
 721 agatcaacca cagaggattt cccgctcagg ctgctgtcgg ctgctccctc ccagacatct
 781 gtgtacttct gtgccagcag ttacgtcggg aacaccgggg agctgttttt tggagaaggc
 841 tctaggctga ccgtactgga ggacctgaaa aacgtgttcc cacccgaggt cgctgtgttt
 901 gagccatcag aagcagagat ctcccacacc caaaaggcca cactggtgtg cctggccaca
 961 ggcttctacc ccgaccacgt ggagctgagc tggtgggtga atgggaagga ggtgcacagt
1021 ggggtcagca cagacccgca gcccctcaag gagcagcccg ccctcaatga ctccagatac
1081 tgcctgagca gccgcctgag ggtctcggcc accttctggc agaacccccg caaccacttc
1141 cgctgtcaag tccagttcta cgggctctcg gagaatgacg agtggaccca agatagggcc
1201 aaacctgtca cccagatcgt cagcgccgag gcctggggta gagcagactg tggcttcacc
1261 tccgagtctt accagcaagg ggtcctgtct gccaccatcc tctatgagat cttgctaggg
1321 aaggccacct tgtatgccgt gctggtcagt gccctcgtgc tgatggccat ggtcaagaga
1381 aaggattcca gaggcggcag cggcgagggc agaggaagtc tgctaacatg cggtgacgtc
1441 gaggagaatc ctggacctat ggagaccctc ttgggcctgc ttatcctttg gctgcagctg
1501 caatgggtga gcagcaaaca ggaggtgaca cagattcctg cagctctgag tgtcccagaa
1561 ggagaaaact tggttctcaa ctgcagtttc actgatagcg ctatttacaa cctccagtgg
1621 tttaggcagg accctgggaa aggtctcaca tctctgttgc ttattcagtc aagtcagaga
1681 gagcaaacaa gtggaagact taatgcctcg ctggataaat catcaggacg tagtacttta
1741 tacattgcag cttctcagcc tggtgactca gccacctacc tctgtgctgt gaggcccaca
1801 tcaggaggaa gctacatacc tacatttgga agaggaacca gccttattgt tcatccgtat
1861 atccagaacc ctgaccctgc agtgtatcaa ttgcgcgata gcaagtctag cgataaatcc
1921 gtgtgtctgt ttactgactt cgactcacag acgaacgtca gtcagagcaa agacagcgac
1981 gtttacatta ccgataagac agtccttgat atgagatcca tggattttaa aagtaattct
2041 gcggttgctt ggtcaaataa gtccgatttc gcctgcgcca atgcttttaa taattccatc
2101 atccctgagg atacgttctt tccgtctcct gaaagttcct gtgatgtcaa gctggtcgag
2161 aaaagctttg aaacagatac gaacctaaac tttcaaaacc tgtcagtgat tgggttccga
```

```
2221 atcctcctcc tgaaagtggc cgggtttaat ctgctcatga cgctgcggct gtggtccagc
2281 tgactagagc tcgctgatca gcctcgactg tgccttctag ttgccagcca tctgttgttt
2341 gcccctcccc cgtgccttcc ttgaccctgg aaggtgccac tcccactgtc ctttcctaat
2401 aaaatgagga aattgcatcg cattgtctga gtaggtgtca ttctattctg gggggtgggg
2461 tggggcagga cagcaagggg gaggattggg aagagaatag caggcatgct ggggatacca
2521 gctgagagac tctaaatcca gtgacaagtc tgtctgccta ttcaccgatt ttgattctca
2581 aacaaatgtg tcacaaagta aggattctga tgtgtatatc acagacaaaa ctgtgctaga
2641 catgaggtct atggacttca agagcaacag tgctgtggcc tggagcaaca aatctgactt
2701 tgcatgtgca aacgccttca acaacagcat tattccagaa gacaccttct tccccagccc
2761 aggtaagggc agctttggtg ccttcgcagg ctgtttcctt gcttcaggaa tggccaaggt
2821 tctgcccaga gctctggtca atgatg
```

Protein sequence

SP TCRβ T2A SP TCRα

```
1          11         21         31         41         51
MSIGLLCCAA LSLLWAGPVN AGVTQTPKFQ VLKTGQSMTL QCAQDMNHEY MSWYRQDPGM
61         71         81         91         101        111
GLRLIHYSVG AGITDQGEVP NGYNVSRSTT EDFPLRLLSA APSQTSVYFC ASSYVGNTGE
121        131        141        151        161        171
LFFGEGSRLT VLEDLKNVFP PEVAVFEPSE AEISHTQKAT LVCLATGFYP DHVELSWWVN
181        191        201        211        221        231
GKEVHSGVST DPQPLKEQPA LNDSRYCLSS RLRVSATFWQ NPRNHFRCQV QFYGLSENDE
241        251        261        271        281        291
WTQDRAKPVT QIVSAEAWGR ADCGFTSESY QQGVLSATIL YEILLGKATL YAVLVSALVL
301        311        321        331        341        351
MAMVKRKDSR GGSGEGRGSL LTCGDVEENP GPMETLLGLL ILWLQLQWVS SKQEVTQIPA
361        371        381        391        401        411
ALSVPEGENL VLNCSFTDSA IYNLQWFRQD PGKGLTSLLL IQSSQREQTS GRLNASLDKS
421        431        442        451        461        471
SGRSTLYIAA SQPGDSATYL CAVRPTSGGS YIPTFGRGTS LIVHPYIQNP DPAVYQLRDS
481        491        501        511        521        531
KSSDKSVCLF TDFDSQTNVS QSKDSDVYIT DKTVLDMRSM DFKSNSAVAW SNKSDFACAN
541        551        561        571        581        591
AFNNSIIPED TFFPSPESSC DVKLVEKSFE TDTNLNFQNL SVIGFRILLL KVAGFNLLMT
601   607
LRLWSS*
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Data S7: c58c61 1G4hi TCR CRISPR construct (GenBank: PP746590)

Nucleotide sequence

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   1 ctgcctttac tctgccagag ttatattgct ggggttttga agaagatcct attaaataaa
  61 agaataagca gtattattaa gtagccctgc atttcaggtt tccttgagtg gcaggccagg
 121 cctggccgtg aacgttcact gaaatcatgg cctcttggcc aagattgata gcttgtgcct
 181 gtccctgagt cccagtccat cacgagcagc tggtttctaa gatgctattt cccgtataaa
 241 gcatgagacc gtgacttgcc agccccacag agccccgccc ttgtccatca ctggcatctg
 301 gactccagcc tgggttgggg caaagaggga aatgagatca tgtcctaacc ctgatcctct
 361 tgtcccacag atatccagaa ccctgaccct gccgtgggca gcggcgccac caacttcagc
 421 ctgctgaagc aggccggcga cgtggaagag aaccccgggc ccatgtctat cggcctgctg
 481 tgttgtgccg ctctgtctct gctttgggcc ggacctgtta atgccggcgt gacccagaca
 541 cctaagttcc aggtgctgaa aaccggccag agcatgaccc tgcagtgcgc ccaggatatg
 601 aaccacgagt acatgagctg gtacagacag gaccctggca tgggcctgag actgatccac
 661 tactctgtgg ccatccagac caccgacaga ggcgaagtgc ccaacggcta caacgtgtcc
 721 agaagcacca tcgaggactt cccactgaga ctgctgtctg ccgctcctag ccagaccagc
 781 gtgtactttt gtgccagcag ctacctgggc aacaccggcg agctgttttt tggcgagggc
 841 agcagactga ccgtgctgga ggacctgaaa aacgtgttcc cacccgaggt cgctgtgttt
 901 gagccatcag aagcagagat ctcccacacc caaaaggcca cactggtgtg cctggccaca
 961 ggcttctacc ccgaccacgt ggagctgagc tggtgggtga atgggaagga ggtgcacagt
1021 ggggtcagca cagacccgca gcccctcaag gagcagcccg ccctcaatga ctccagatac
1081 tgcctgagca gccgcctgag ggtctcggcc accttctggc agaacccccg caaccacttc
1141 cgctgtcaag tccagttcta cgggctctcg gagaatgacg agtggaccca agatagggcc
1201 aaacctgtca cccagatcgt cagcgccgag gcctggggta gagcagactg tggcttcacc
1261 tccgagtctt accagcaagg ggtcctgtct gccaccatcc tctatgagat cttgctaggg
1321 aaggccacct tgtatgccgt gctggtcagt gccctcgtgc tgatggccat ggtcaagaga
1381 aaggattcca gaggcggcag cggcgagggc agaggaagtc tgctaacatg cggtgacgtc
1441 gaggagaatc ctggacctat ggaaacactg ctgggcctgc tgatcctgtg gctgcaactg
1501 caatgggtgt cctccaagca agaagtgact cagatcccag ccgctctgag tgtgcctgag
1561 ggcgaaaacc tggtcctgaa ctgcagcttc accgacagcg ccatctacaa cctgcagtgg
1621 ttcagacaag atcccggcaa gggactgaca agcctgctgc tgattacccc ttggcagaga
1681 gagcagacct ccggcagact gaatgccagc ctggataaga gcagcggccg gtctacactg
1741 tatatcgccg cttctcagcc tggcgatagc gccacatatc tgtgtgccgt cagacccctg
1801 ctggacggca catatatccc taccttggc agaggcacca gcctgatcgt gcacccctac
1861 atccagaacc ctgaccctgc agtgtatcaa ttgcgcgata gcaagtctag cgataaatcc
1921 gtgtgtctgt ttactgactt cgactcacag acgaacgtca gtcagagcaa agacagcgac
1981 gtttacatta ccgataagac agtccttgat atgagatcca tggattttaa aagtaattct
2041 gcggttgctt ggtcaaataa gtccgatttc gcctgcgcca atgcttttaa taattccatc
2101 atccctgagg atacgttctt tccgtctcct gaaagttcct gtgatgtcaa gctggtcgag
2161 aaaagctttg aaacagatac gaacctaaac tttcaaaacc tgtcagtgat tgggttccga
```

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2221 atcctcctcc tgaaagtggc cgggtttaat ctgctcatga cgctgcggct gtggtccagc
2281 tgactagagc tcgctgatca gcctcgactg tgccttctag ttgccagcca tctgttgttt
2341 gcccctcccc cgtgccttcc ttgaccctgg aaggtgccac tcccactgtc ctttcctaat
2401 aaaatgagga aattgcatcg cattgtctga gtaggtgtca ttctattctg gggggtgggg
2461 tggggcagga cagcaagggg gaggattggg aagagaatag caggcatgct ggggatacca
2521 gctgagagac tctaaatcca gtgacaagtc tgtctgccta ttcaccgatt ttgattctca
2581 aacaaatgtg tcacaaagta aggattctga tgtgtatatc acagacaaaa ctgtgctaga
2641 catgaggtct atggacttca agagcaacag tgctgtggcc tggagcaaca aatctgactt
2701 tgcatgtgca aacgccttca acaacagcat tattccagaa gacaccttct tccccagccc
2761 aggtaagggc agctttggtg ccttcgcagg ctgtttcctt gcttcaggaa tggccaaggt
2821 tctgcccaga gctctggtca atgatg
```

Protein sequence

SP TCRβ T2A SP TCRα

```
1          11         21         31         41         51
MSIGLLCCAA LSLLWAGPVN AGVTQTPKFQ VLKTGQSMTL QCAQDMNHEY MSWYRQDPGM
61         71         81         91         101        111
GLRLIHYSVA IQTTDRGEVP NGYNVSRSTI EDFPLRLLSA APSQTSVYFC ASSYLGNTGE
121        131        141        151        161        171
LFFGEGSRLT VLEDLKNVFP PEVAVFEPSE AEISHTQKAT LVCLATGFYP DHVELSWWVN
181        191        201        211        221        231
GKEVHSGVST DPQPLKEQPA LNDSRYCLSS RLRVSATFWQ NPRNHFRCQV QFYGLSENDE
241        251        261        271        281        291
WTQDRAKPVT QIVSAEAWGR ADCGFTSESY QQGVLSATIL YEILLGKATL YAVLVSALVL
301        311        321        331        341        351
MAMVKRKDSR GGSGEGRGSL LTCGDVEENP GPMETLLGLL ILWLQLQWVS SKQEVTQIPA
361        371        381        391        401        411
ALSVPEGENL VLNCSFTDSA IYNLQWFRQD PGKGLTSLLL ITPWQREQTS GRLNASLDKS
421        431        442        451        461        471
SGRSTLYIAA SQPGDSATYL CAVRPLLDGT YIPTFGRGTS LIVHPYIQNP DPAVYQLRDS
481        491        501        511        521        531
KSSDKSVCLF TDFDSQTNVS QSKDSDVYIT DKTVLDMRSM DFKSNSAVAW SNKSDFACAN
541        551        561        571        581        591
AFNNSIIPED TFFPSPESSC DVKLVEKSFE TDTNLNFQNL SVIGFRILLL KVAGFNLLMT
601   607
LRLWSS*
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Data S8: RA14 TCR CRISPR construct (GenBank: PP746591)

Nucleotide sequence

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  61 agaataagca gtattattaa gtagccctgc atttcaggtt tccttgagtg gcaggccagg
 121 cctggccgtg aacgttcact gaaatcatgg cctcttggcc aagattgata gcttgtgcct
 181 gtccctgagt cccagtccat cacgagcagc tggtttctaa gatgctattt cccgtataaa
 241 gcatgagacc gtgacttgcc agccccacag agccccgccc ttgtccatca ctggcatctg
 301 gactccagcc tgggttgggg caaagaggga aatgagatca tgtcctaacc ctgatcctct
 361 tgtcccacag atatccagaa ccctgaccct gccgtgggca gcggcgccac caacttcagc
 421 ctgctgaagc aggccggcga cgtggaagag aaccccgggc ccatgagcat cggcctcctg
 481 tgctgtgcag ccttgtctct cctgtgggca ggtccagtga atgctggtgt cactcagacc
 541 ccaaaattcc aggtcctgaa gacaggacag agcatgacac tgcagtgtgc ccaggatatg
 601 aaccatgaat acatgtcctg gtatcgacaa gacccaggca tggggctgag gctgattcat
 661 tactcagttg gtgctggtat cactgaccaa ggagaagtcc ccaatggcta caatgtctcc
 721 agatcaacca cagaggattt cccgctcagg ctgctgtcgg ctgctccctc ccagacatct
 781 gtgtacttct gtgccagcag tcccgtgaca gggggcatct atggctacac cttcggttcg
 841 gggaccaggt taaccgttgt agaggacctg aaaaacgtgt tcccacccga ggtcgctgtg
 901 tttgagccat cagaagcaga gatctcccac acccaaaagg ccacactggt gtgcctggcc
 961 acaggcttct accccgacca cgtggagctg agctggtggg tgaatgggaa ggaggtgcac
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1081 tactgcctga gcagccgcct gagggtctcg gccaccttct ggcagaaccc ccgcaaccac
1141 ttccgctgtc aagtccagtt ctacgggctc tcggagaatg acgagtggac ccaagatagg
1201 gccaaacctg tcacccagat cgtcagcgcc gaggcctggg gtagagcaga ctgtggcttc
1261 acctccgagt cttaccagca aggggtcctg tctgccacca tcctctatga gatcttgcta
1321 gggaaggcca ccttgtatgc cgtgctggtc agtgccctcg tgctgatggc catggtcaag
1381 agaaaggatt ccagaggcgg cagcggcgag ggcagaggaa gtctgctaac atgcggtgac
1441 gtcgaggaga atcctggacc tatggagaag aatcctttgg cagccccatt actaatcctc
1501 tggtttcatc ttgactgcgt gagcagcata ctgaacgtgg aacaaagtcc tcagtcactg
1561 catgttcagg agggagacag caccaatttc acctgcagct tcccttccag caatttttat
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1801 cgcaacaccg gtaaccagtt ctattttggg acagggacaa gtttgacggt cattccaaat
1861 atccagaacc ctgaccctgc agtgtatcaa ttgcgcgata gcaagtctag cgataaatcc
1921 gtgtgtctgt ttactgactt cgactcacag acgaacgtca gtcagagcaa agacagcgac
1981 gtttacatta ccgataagac agtccttgat atgagatcca tggattttaa aagtaattct
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2101 atccctgagg atacgttctt tccgtctcct gaaagttcct gtgatgtcaa gctggtcgag
2161 aaaagctttg aaacagatac gaacctaaac tttcaaaacc tgtcagtgat tgggttccga
```

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2221 atcctcctcc tgaaagtggc cgggtttaat ctgctcatga cgctgcggct gtggtccagc
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2341 gcccctcccc cgtgccttcc ttgaccctgg aaggtgccac tcccactgtc ctttcctaat
2401 aaaatgagga aattgcatcg cattgtctga gtaggtgtca ttctattctg gggggtgggg
2461 tggggcagga cagcaagggg gaggattggg aagagaatag caggcatgct ggggatacca
2521 gctgagagac tctaaatcca gtgacaagtc tgtctgccta ttcaccgatt ttgattctca
2581 aacaaatgtg tcacaaagta aggattctga tgtgtatatc acagacaaaa ctgtgctaga
2641 catgaggtct atggacttca agagcaacag tgctgtggcc tggagcaaca aatctgactt
2701 tgcatgtgca aacgccttca acaacagcat tattccagaa gacaccttct tccccagccc
2761 aggtaagggc agctttggtg ccttcgcagg ctgtttcctt gcttcaggaa tggccaaggt
2821 tctgcccaga gctctggtca atgatg
```

Protein sequence

SP TCRβ T2A SP TCRα

```
1          11         21         31         41         51
MSIGLLCCAA LSLLWAGPVN AGVTQTPKFQ VLKTGQSMTL QCAQDMNHEY MSWYRQDPGM
61         71         81         91         101        111
GLRLIHYSVG AGITDQGEVP NGYNVSRSTT EDFPLRLLSA APSQTSVYFC ASSPVTGGIY
121        131        141        151        161        171
GYTFGSGTRL TVVEDLKNVF PPEVAVFEPS EAEISHTQKA TLVCLATGFY PDHVELSWWV
181        191        201        211        221        231
NGKEVHSGVS TDPQPLKEQP ALNDSRYCLS SRLRVSATFW QNPRNHFRCQ VQFYGLSEND
241        251        261        271        281        291
EWTQDRAKPV TQIVSAEAWG RADCGFTSES YQQGVLSATI LYEILLGKAT LYAVLVSALV
301        311        321        331        341        351
LMAMVKRKDS RGGSGEGRGS LLTCGDVEEN PGPMEKNPLA APLLILWFHL DCVSSILNVE
361        371        381        391        401        411
QSPQSLHVQE GDSTNFTCSF PSSNFYALHW YRWETAKSPE ALFVMTLNGD EKKKGRISAT
421        431        442        451        461        471
LNTKEGYSYL YIKGSQPEDS ATYLCARNTG NQFYFGTGTS LTVIPNIQNP DPAVYQLRDS
481        491        501        511        521        531
KSSDKSVCLF TDFDSQTNVS QSKDSDVYIT DKTVLDMRSM DFKSNSAVAW SNKSDFACAN
541        551        561        571        581        591
AFNNSIIPED TFFPSPESSC DVKLVEKSFE TDTNLNFQNL SVIGFRILLL KVAGFNLLMT
601   607
LRLWSS*
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Data S9: T1 STAR$_{nat}$ T2A copGFP CRISPR construct (GenBank: PP746592)

Nucleotide sequence

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   1 ctgcctttac tctgccagag ttatattgct ggggttttga agaagatcct attaaataaa
  61 agaataagca gtattattaa gtagccctgc atttcaggtt tccttgagtg gcaggccagg
 121 cctggccgtg aacgttcact gaaatcatgg cctcttggcc aagattgata gcttgtgcct
 181 gtccctgagt cccagtccat cacgagcagc tggtttctaa gatgctattt cccgtataaa
 241 gcatgagacc gtgacttgcc agccccacag agccccgccc ttgtccatca ctggcatctg
 301 gactccagcc tgggttgggg caaagaggga aatgagatca tgtcctaacc ctgatcctct
 361 tgtcccacag atatccagaa ccctgaccct gccgtgggca gcggcgccac caacttcagc
 421 ctgctgaagc aggccggcga cgtggaagag aaccccgggc ccatgggcag ctggaccctg
 481 tgttgcgtgt ccctgtgcat cctggtggct aagcacaccc agagcgagct gacccagccc
 541 agaagcgtgt ccggcagccc tggccagagc gtgaccatca gctgtaccgg caccgaaaga
 601 gatgtgggcg gctacaacta cgtgtcctgg tatcagcagc accccggcaa ggcccctaag
 661 ctgatcatcc acgacgtgat cgagagaagc agcggcgtgc ccgacagatt cagcggcagc
 721 aagagcggca acaccgccag cctgaccatc tctggcctcc aggccgagga cgaggccgac
 781 tactactgct ggagcttcgc cggcggatac tacgtgttcg gcaccggcac cgacgtgacc
 841 gtggaggatc tgagaaatgt gactccaccc aaggtctcct tgtttgagcc atcaaaagca
 901 gagattgcaa acaaacaaaa ggctaccctc gtgtgcttgg ccaggggctt cttccctgac
 961 cacgtggagc tgagctggtg ggtgaatggc aaggaggtcc acagtggggt ctgcacggac
1021 cctcaggcct acaaggagag caattatagc tactgcctga gcagccgcct gagggtctct
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Data S10: T1 STAR$_{mir}$ T2A copGFP CRISPR construct (GenBank: PP746593)

Nucleotide sequence

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Data S11: T1 BBz CAR T2A copGFP CRISPR construct (GenBank: PP746594)

Nucleotide sequence

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