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Supplemental information

Characterization and modulation of anti-αβTCR antibodies and their respective binding sites at the βTCR chain to enrich engineered T cells

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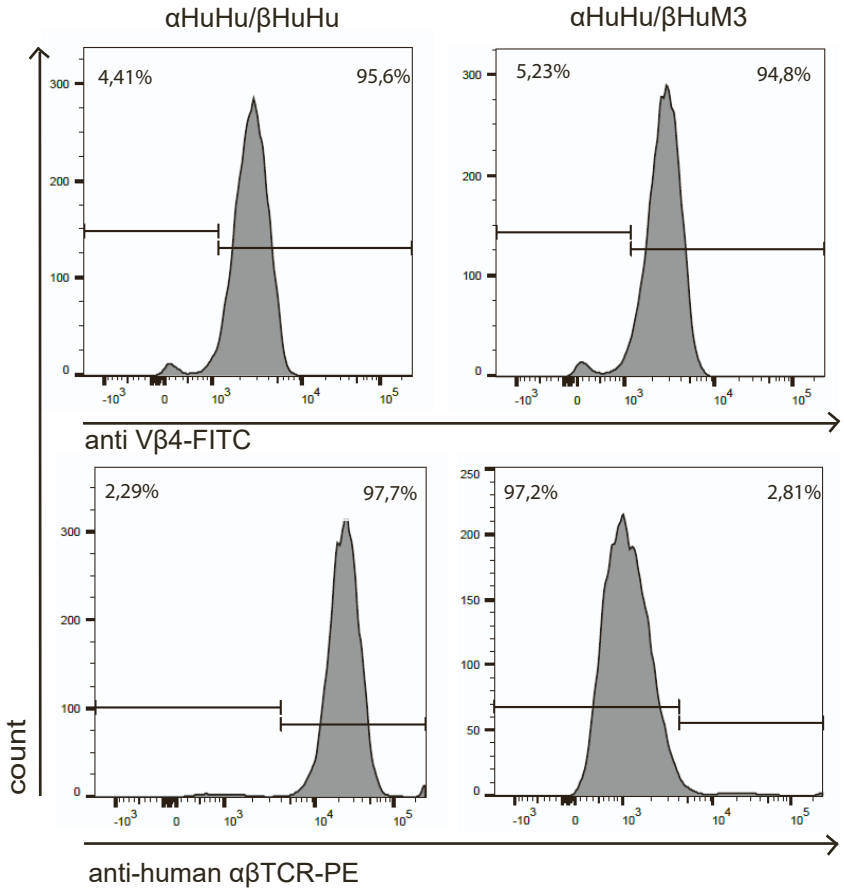
Supplemental Figure 1



Supplemental Figure 1. αhuhu/βhuM3 murinized TCR is expressed at the cell surface. Jurma cells were transduced with the αhuhu/βhuhu and αhuhu/βhuM3 murinized TCR after which TCR expression was confirmed with an anti-Vβ4 antibody (upper panel) and binding of the anti-human αβTCR antibody was assessed (lower panel), by flow cytometry. The data correspond to 2 independent experiments and a representative figure is shown.

Supplemental Figure 2

TCRα

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                    1                                                  
    1         11        21        31        41        51        61
Hu  IQNPDPAVYQ LRDSKSSDKS VCLFTDFDSQ TNVSQSKDSD VYITDKTVLD MRSMDFKSNS AVAWSNKSDF
Mu  ****E***** *K*PR*O*ST L********* I**PKTME*G TF******** *KA**S***G *I****OTS*

                2                   3
    71        81        91        101       111       121       131
Hu  ACANAFNNSI IPEDTFFPSP ESSCDVKLVE KSFETDTNLN FQNLSVIGFR ILLLKVAGFN LLMTLRLWSS
Mu  T*QDI*K--- -ETNATY**S DVP**AT*T* ******M*** ******M*L* ********** **********
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TCRβ

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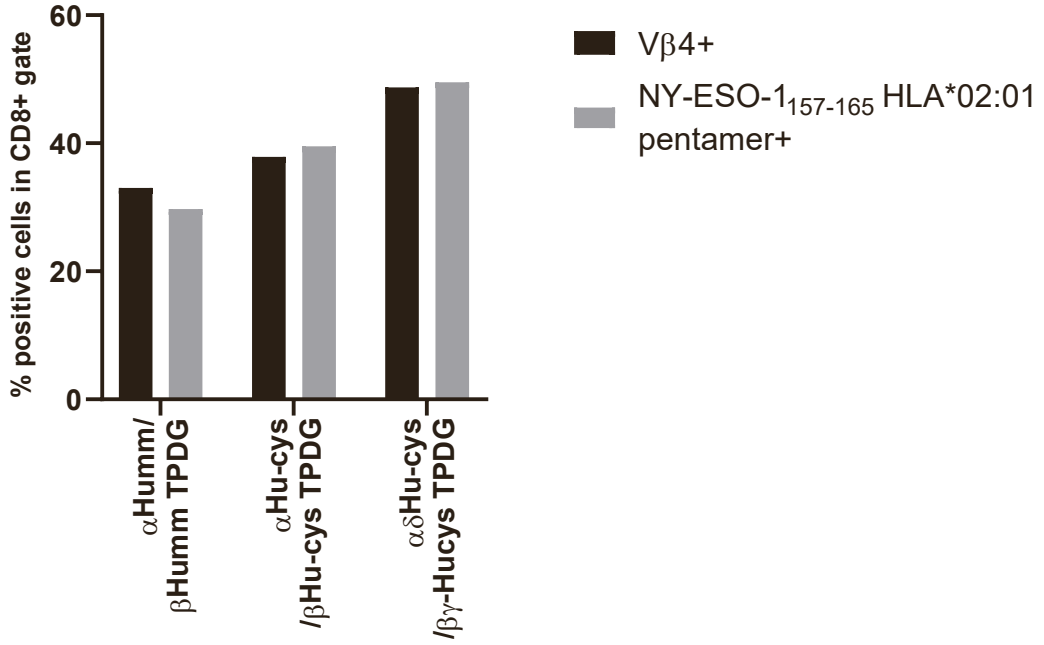
                    1                                                  2
    1         11        21        31        41        51        61
Hu  EDLKNVFPPE VAVFEPSEAE ISHTQKATLV CLATGFYPDH VELSWWVNGK EVHSGVSTDP QPLKEQPALN
Mu  ***R**T**K *SL****K** *ANK****** ***R**F*** ********** ********** *AY**---S*

                                    3
    71        81        91        101       111       121       131
Hu  DSRYCLSSRL RVSATFWQNP RNHFRCQVQF YGLSENDEWT QDRAKPVTQI VSAEAWGRAD CGFTSESYQQ
Mu  Y*-******* *******H** ********** H****E*K*P EGSP*****N I********* **I**A**H*

                    4
    141       151       161       171
Hu  GVLSATILYE ILLGKATLYA VLVSALVLMA MVKRKDSRG
Mu  ********** ********** ****G***** ***K*N*--
```

Supplemental Figure 2. Extensive homology between human and murine TCR chains. Sequence alignment of the Human (Hu) and Murine (Mu) TCR α (upper panel) or β (lower panel) constant chains. The three (TCRα) or four (TCRβ) TCR constant regions with clustered Hu-Mu sequence differences are indicated above the alingment.

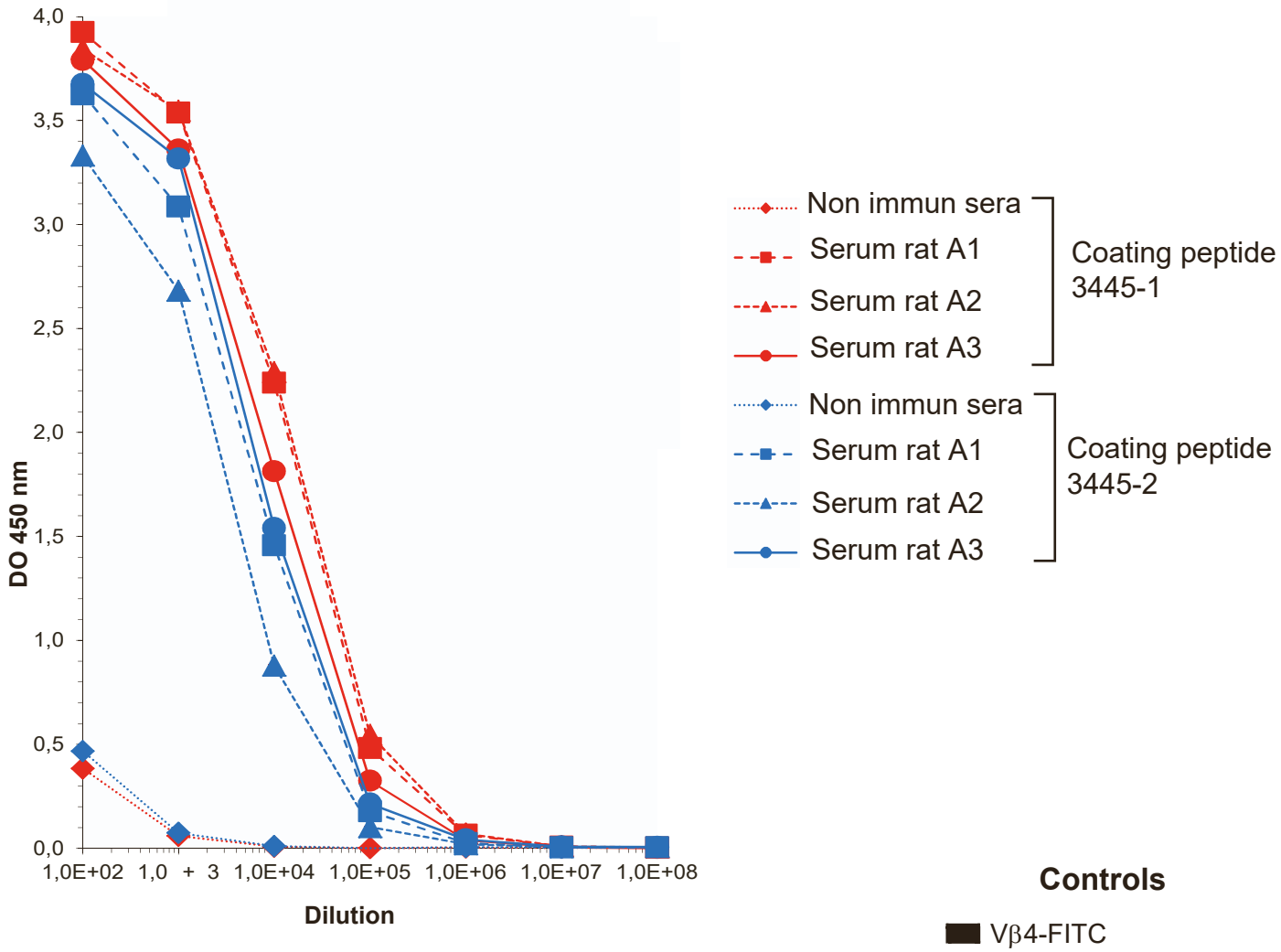
Supplemental Figure 3



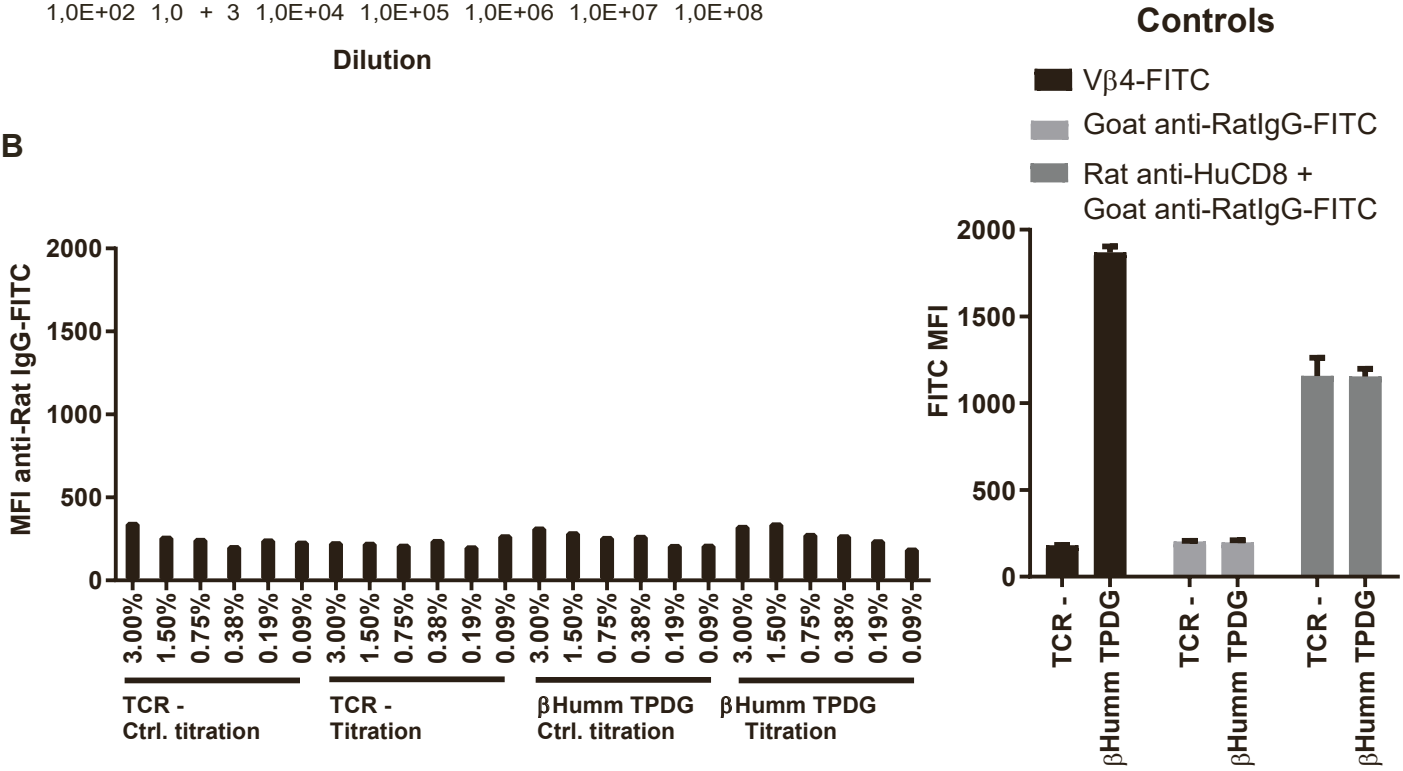
Supplemental Figure 3. Comparable efficacy of different strategies to induce preferential pairing of introduced α and βTCR chains. Primary αβT cells were transduced with the 3 differentially modified αβ TCRs and expression of the introduced βTCR was determined by an anti-Vβ4 antibody. Pairing of the introduced α and βTCR chains was assessed by staining with NY-ESO-1 pentamers. The data correspond to 2 independent experiments and a representative figure is shown.

Supplemental Figure 4

A



B



C

```
                          91          101         111         121
Human                 QNP RNHFRCQVQF YGLSENDEWT QDRAKPVTQI V
11/11  (Murine)       H.. .......... H....E.K.P EGSP.....N I
2/11                  ... .......... .........P .G........ .
9/11                  H.. .......... H......K.P EGS......N I
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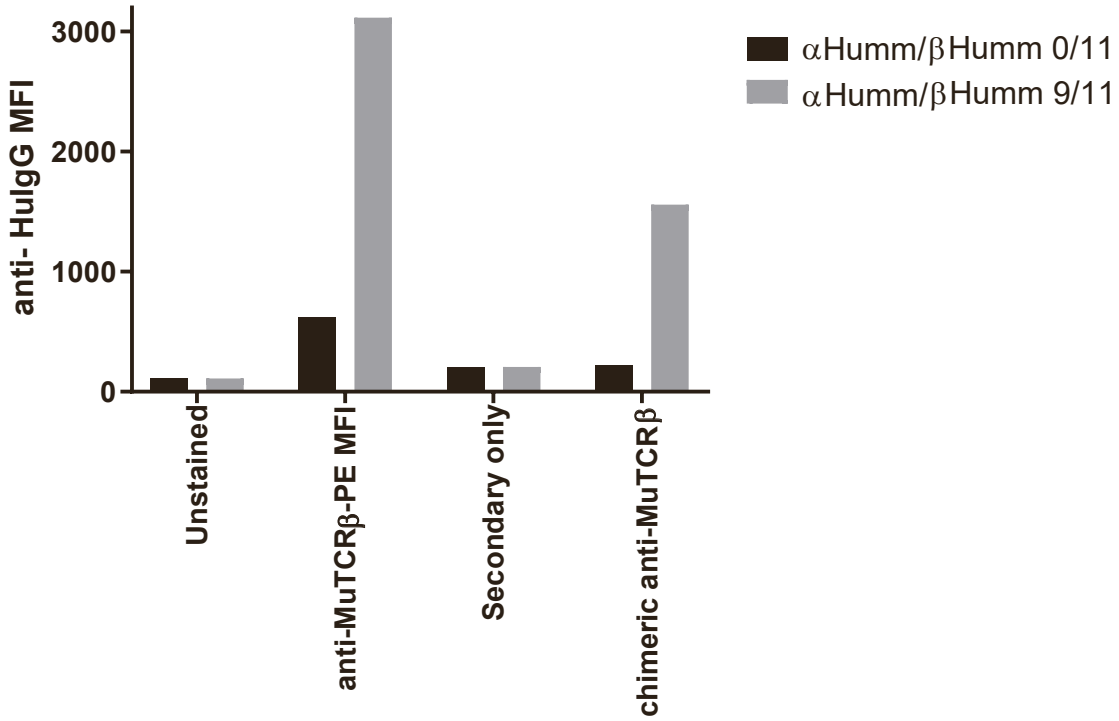
Supplemental Figure 4. Attempting to raise an antibody specific for the T110P+D112G murinized variant of the αβTCR by immunizing 3 Wistar rats with a human-mouse chimeric peptide. (A) Determining the presence of peptide-specific antibodies in the serum of the immunized rats. (B) Assessing the ability of the generated antibodies to bind surface-expressed TCRs. aHumm/βHumm TPDG transduced or non-transduced Jurkat-76 cells were incubated with the indicated percentage of rat serum, after which flow cytometry using anti-RatIgG-FITC was performed. In the controls panel, the functionality of this secondary antibody was confirmed by staining the Jurkat-76 cells with rat anti-HuCD8 followed by anti-RatIgG-FITC. Expression of the TCR was confirmed using anti-Vβ4-FITC. (C) Sequence alignment of the human and murine 3rd domain of the TCRβ chain and the constructed 2/11 and 9/11 murinized variants.

Supplemental Figure 5



Supplemental Figure 5. Chimeric anti-MuTCRβ antibody binds to primary T cells expressing the murinized TCR containing 9 out of 11 murine residues in the 3rd domain of the β chain. Jurkat-76 cells were transduced with 2 different αβTCRs, containing 0/11 or 9/11 murine residues in the 3rd domain of the β chain, to assess binding of the newly generated chimeric and CDR grafted anti-MuTCRβ antibodies. As negative controls, unstained and secondary antibody only conditions were used. As a positive control, wild-type PE-conjugated anti-MuTCRβ was used The data correspond to 2 independent experiments and a representative figure is shown.

Supplemental Table 1. Differences between the eleven human-mouse non-homologous amino acids in the third domain of the β chain (βM3).

	Change in		
Mutation	Size	Charge	Hydrophobicity
$Q_{88}H$	-	Uncharged to positive charge	Hydrophilic to hydrophobic
$Y_{101}H$	-	Uncharged to positive charge	-
$N_{106}E$	-	Uncharged to negative charge	-
$E_{108}K$	**-**	**Negative to positive charge**	**Slightly more hydrophilic**
$T_{110}P$	**Less bulky**	Uncharged	**-**
$Q_{111}E$	-	Uncharged to positive charge	-
$D_{112}G$	**Less bulky**	**Negative charge to uncharged**	**Less hydrophilic**
$R_{113}S$	Less bulky	Positive charge to uncharged	-
$A_{114}P$	-	Uncharged	Less hydrophobic
$I_{120}N$	-	Uncharged	Hydrophobic to hydrophilic
$V_{121}I$	-	Uncharged	-