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Supplemental Information

CRISPR-Mediated Base Conversion Allows Discriminatory Depletion of Endogenous T Cell Receptors for Enhanced Synthetic Immunity

Roland Preece, Andrea Pavesi, Soragia Athina Gkazi, Kerstin A. Stegmann, Christos Georgiadis, Zhi Ming Tan, Jia Ying Joey Aw, Mala K. Maini, Antonio Bertoletti, and Waseem Qasim

Supplementary Bioinformatics Methods:

Screening RNA-seq data for *in silico* predicted *TRBC* off-target:

Using the RNA-seq data, a customised bioinformatics methodology was used to investigate the frequency of 1,071 off-target predictions produced by Cas-OFFinder:

1) Off-targets sites were padded to cover 250bp around each site; 2) The file was converted to a .bed file including the chromosome number, with start and end positions, and strand orientation. 3) Using Homer_annotatepeaks the .bed file was annotated to include information on genomic positions (intronic, exonic, intergenic, 3' UTR, 5' UTR etc), gene names, Ensembl numbers and other related information. 4) File was abbreviated for further processing, and a header was created in file. 5) After including exonic regions only, 51 sites remained. 6) By removing duplicates already identified by Cas-OFFinder, 24 exonic sites were advanced. 7) Naïve Variant Caller was performed on RNAseq data previously aligned to human genome (version hg38) and .bed file including the 24 sites. 8) The resulting .vcf files were annotated for any variants using Variant Annotator with a minor allele frequency of 1. 9) Resulting tabular files were finalized on excel. 10) Step 7 resulted with 0 variants annotated. 11) To verify the results, the .vcf files from step 6 were cross-referenced to Integrated Genome Viewer for the aligned .bam files. 12) All files were filtered to include only C to T (or G to A if on anti-sense strand) changes. 13) Any variants found in untransduced (eTCR+/rTCR-) samples were excluded. 14) Remaining variants exhibited coverage of less than 100bp and in most cases less than 10bp, with low MAPQ. This suggested these 24 sites had low transcriptional activity and not likely to be critical to function.

Supplementary Table:

Table S1. Target-specific primer combinations for NGS on top 6 Benchling off-targets

Notes	Off-target Number	Sequence	Genomic Positions Analysed
Fwd primer	1	GAGGCAGGTCTTTCACATTCA	chr19:47,157,708-47,157,727
Rev primer	1	CAAAACGGAAGGAGGCATGT	chr19:47,157,708-47,157,727
Fwd primer	2	AATCTTCACAGCCCCTCCTC	chr9:124,276,868-124,276,887
Rev primer	2	AGTGCGGCCTCAGGTAGC	chr9:124,276,868-124,276,887
Fwd primer	3	GAGAGTGAACGAGGGTGTGG	chr20:33,016,052-33,016,071
Rev primer	3	CCACCTTCACCCCTCGAG	chr20:33,016,052-33,016,071
Fwd primer	4	GTGGTAAGTGTGAGCATTGCAG	chr6:151,587,120-151,587,139
Rev primer	4	TCAGTCCATACTTTCCCCGC	chr6:151,587,120-151,587,139
Fwd primer	5	GATACCTGCTGTCCCCTCTC	chr5:153,537,378-153,537,397
Rev primer	5	CAGAGGTAGATTGCTATGGACCT	chr5:153,537,378-153,537,397
Fwd primer	6	CTTATCCCAGCAGCACTTGG	chr17:45,118,095-45,118,114
Rev primer	6	TCTCTTCCTCCCACAACACC	chr17:45,118,095-45,118,114

Following an internally-built workflow, a 20bp window around the predicted off-target nucleotide position, was analysed to a minor allele frequency of 0.01%. Primer sequences are given in the 5'-3' orientation. Fwd: forward, Rev: reverse.

Supplementary Figures:

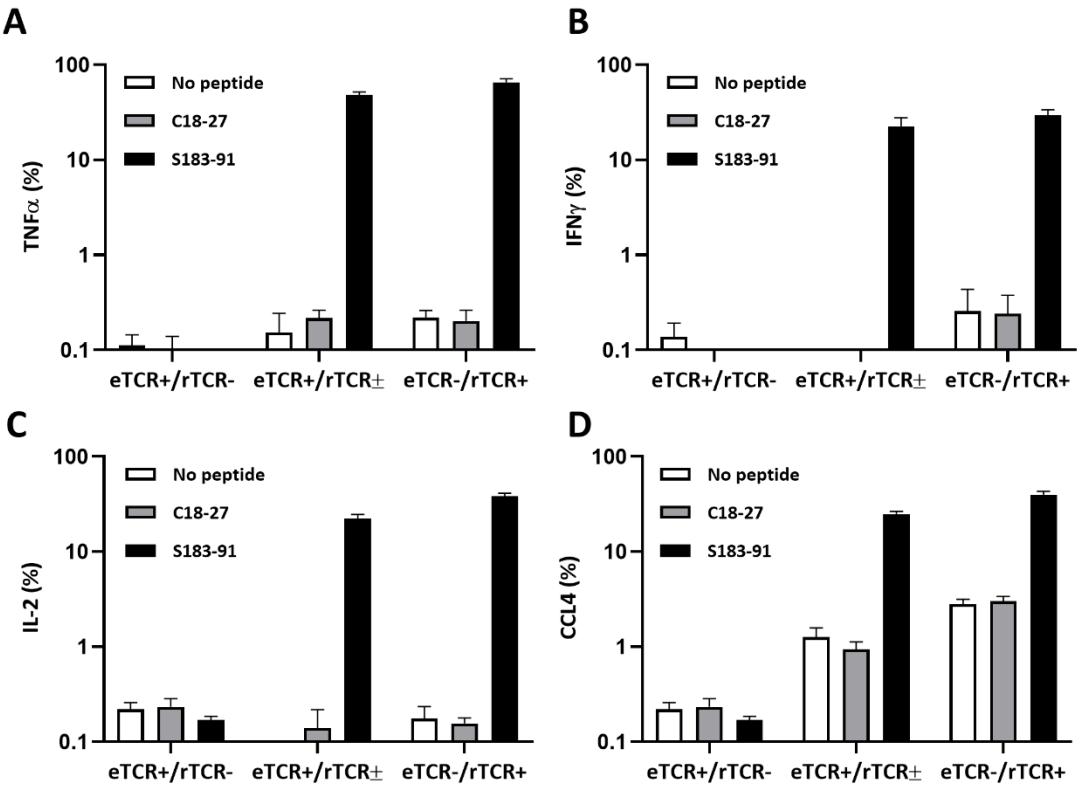

Figure S1: Cytokine responsiveness of effector T cells to HepG2 targets alone or pulses with either target (S183-91)/ control (C18-27) peptide.

Flow cytometry data quantifying TNFα (**A**), IFNγ (**B**), IL-2 (**C**), and CCL4 (**D**) cytokine responses of effector T cells against HepG2 targets alone (white) or pulsed with 1μM of either target HBV surface peptide (S183-91, FLLTRILTI, black), or irrelevant control HBV core peptide (C18-27 FLPSDFFPSV, grey) (n=3 biological replicates). Effector groups eTCR+/rTCR± and eTCR-/rTCR+ are gated on CD45+>CD3+>rTCR+CD8+; whereas unmodified eTCR+/rTCR- cells are gated on CD45+>CD3+>rTCR->CD8+. Error bars ± 1 SEM.

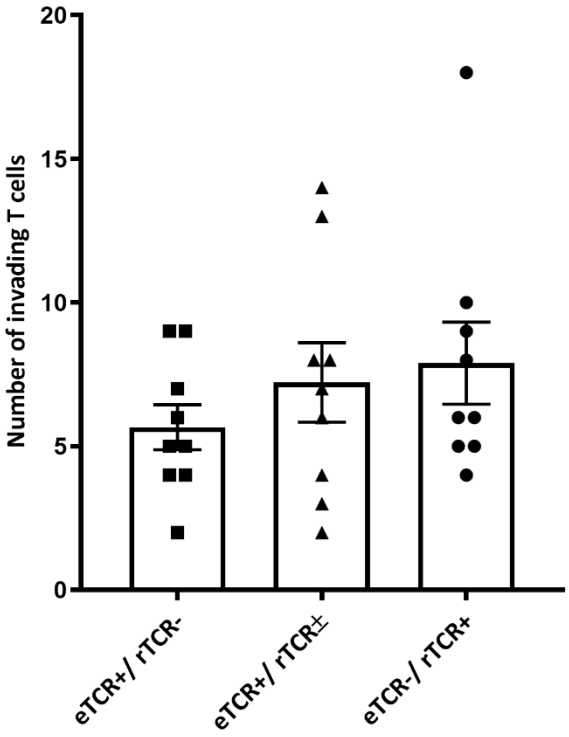

Figure S2: Effector T cell migration within a 3D microfluidics device. Quantification of effector T cells entering the gel region using imaris software. Each point represents a section of a 3D microfluidics device from n=3 technical replicate (3 sections analysed per device). Error bars ± 1 SEM.

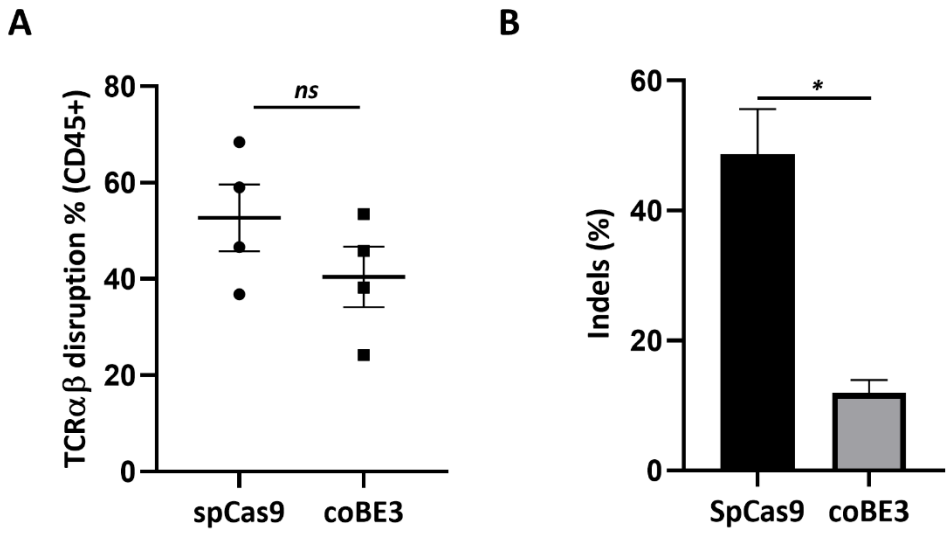

Figure S3: On-target genome editing comparison of *TRBC1/2* editing with coBE3 and SpCas9.

A) T cells isolated from n=4 healthy donors were transduced at MOI 5 with a lentiviral terminal-CRISPR vector expressing TRBC1/2 sgRNA, and an anti-CD19 chimeric antigen receptor (CAR) transgene. T cells were subsequently electroporated with either coBE3 or SpCas9 mRNA at 50µg/ml. Flow cytometry based analysis of TCRαβ disruption was gated on CD45+ and no significant difference between SpCas9 vs coBE3 disruption was uncovered. Error bars ± 1 SEM. Mann-Whitney U test, $p > 0.3$). **B)** NGS analysis on the whole cell population found notably higher indel formation at the *TRBC1/2* loci after SpCas9 compared to coBE3 editing ($p < 0.03$, Mann-Whitney U test; Error bars ± 1 SEM. Analysis run on unmodified control revealed an indel frequency of 1.9%.

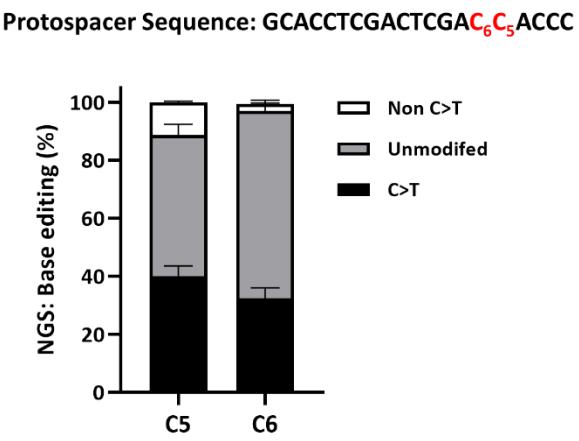

Figure S4: Next generation sequencing data analysis of on-target coBE3 C>T conversions. Summary of NGS data from n=3 healthy donors at cytosine positions 5 and 6 distal to the PAM, presented as C>T changes (black), non C>T changes (white) and unmodified (grey). Error bars ± 1 SEM.

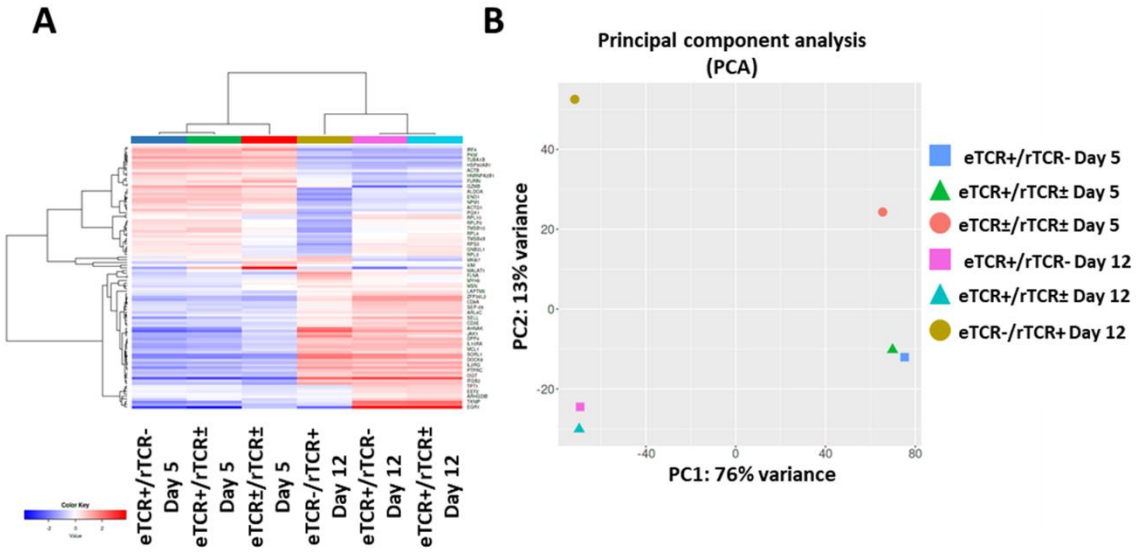

Figure S5: RNA transcriptomics of base edited T cells. PBMCs activated on day 0, T cells transduced with lentiviral TTRBC-S183-91 rTCR on day 1 and electroporated with coBE3 mRNA on day 4. RNA was extracted on days 5 and 12, including from samples enriched by depletion of T cells expressing residual eTCR **A)** Heatmap of top 100 most variable genes (iDEP9.1) at days 5 and 12 in untransduced cells (eTCR+/rTCR-), transduced and not-edited (eTCR+/rTCR±) and transduced, edited and purified (eTCR-/rTCR+) **B)** Principal Component Analysis demonstrating correlation between PC1 (76% variance) and tested samples over time, capturing the effects of activation and transduction and suggesting little perturbance from base editing activity.