nature methods

SUPPLEMENTARY INFORMATION

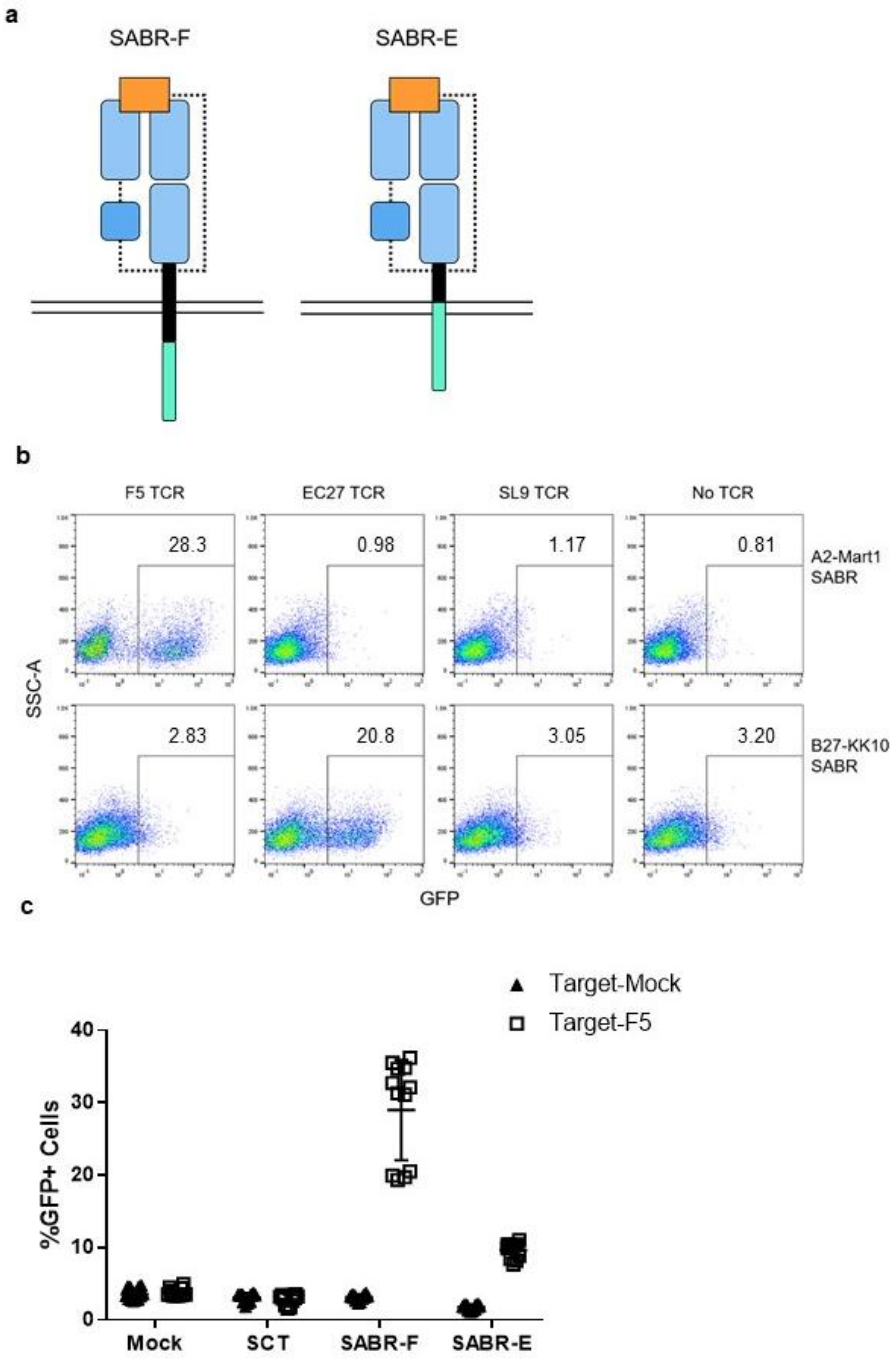
https://doi.org/10.1038/s41592-018-0304-8

In the format provided by the authors and unedited.

T cell antigen discovery via signaling and antigen-presenting bifunctional receptors

Alok V. Joglekar[1]*, Michael T. Leonard[1], John D. Jeppson[1], Margaret Swift[1], Guideng Li[1,2,3], Stephanie Wong[1], Songming Peng[4], Jesse M. Zaretsky[5], James R. Heath[4,6], Antoni Ribas[5,6,7,8], Michael T. Bethune[1] and David Baltimore[1,6]*

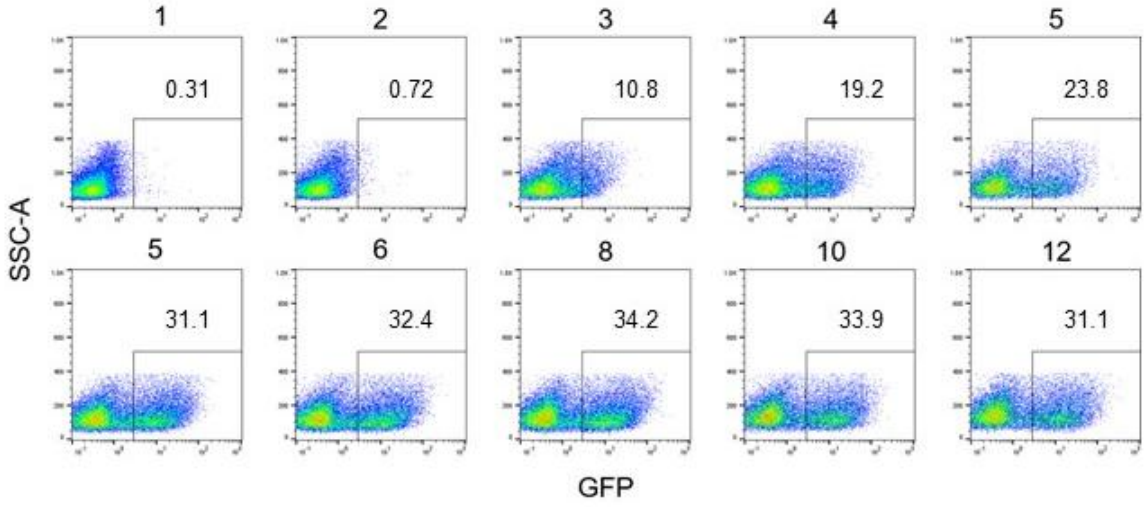
[1]Department of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, USA. [2]Center of Systems Medicine, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China. [3]Suzhou Institute of Systems Medicine, Suzhou, China. [4]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA, USA. [5]Department of Molecular and Medical Pharmacology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA. [6]Parker Institute for Cancer Immunotherapy (PICI), University of California, Los Angeles and California Institute of Technology, Pasadena, CA, USA. [7]Division of Hematology & Oncology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA. [8]Department of Surgery, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA. *e-mail: alokvj@caltech.edu; baltimo@caltech.edu



Supplementary Figure 1

Variants of SABR constructs.

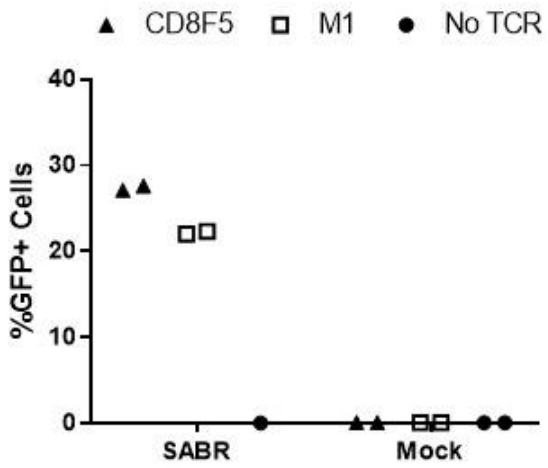
a. Schematics showing the SABR-F and SABR-E constructs. SABR-F contains the transmembrane domain from HLA (black rectangle), whereas SABR-E contains the transmembrane domain from CD3ζ (teal rectangle). The two horizontal lines indicate two leaflets of the plasma membrane. **b.** Representative flow cytometry plots showing GFP expression in coculture assays from Fig 1b. The experiments were performed at *n* = 3 biologically independent cell culture replicates. **c.** GFP expression in coculture assays comparing SABR-F and SABR-E. The lines and error bars indicate mean ± s.d. from *n* = 12 biologically independent cell culture replicates.



Supplementary Figure 2

Time course of GFP expression induced by SABRs.

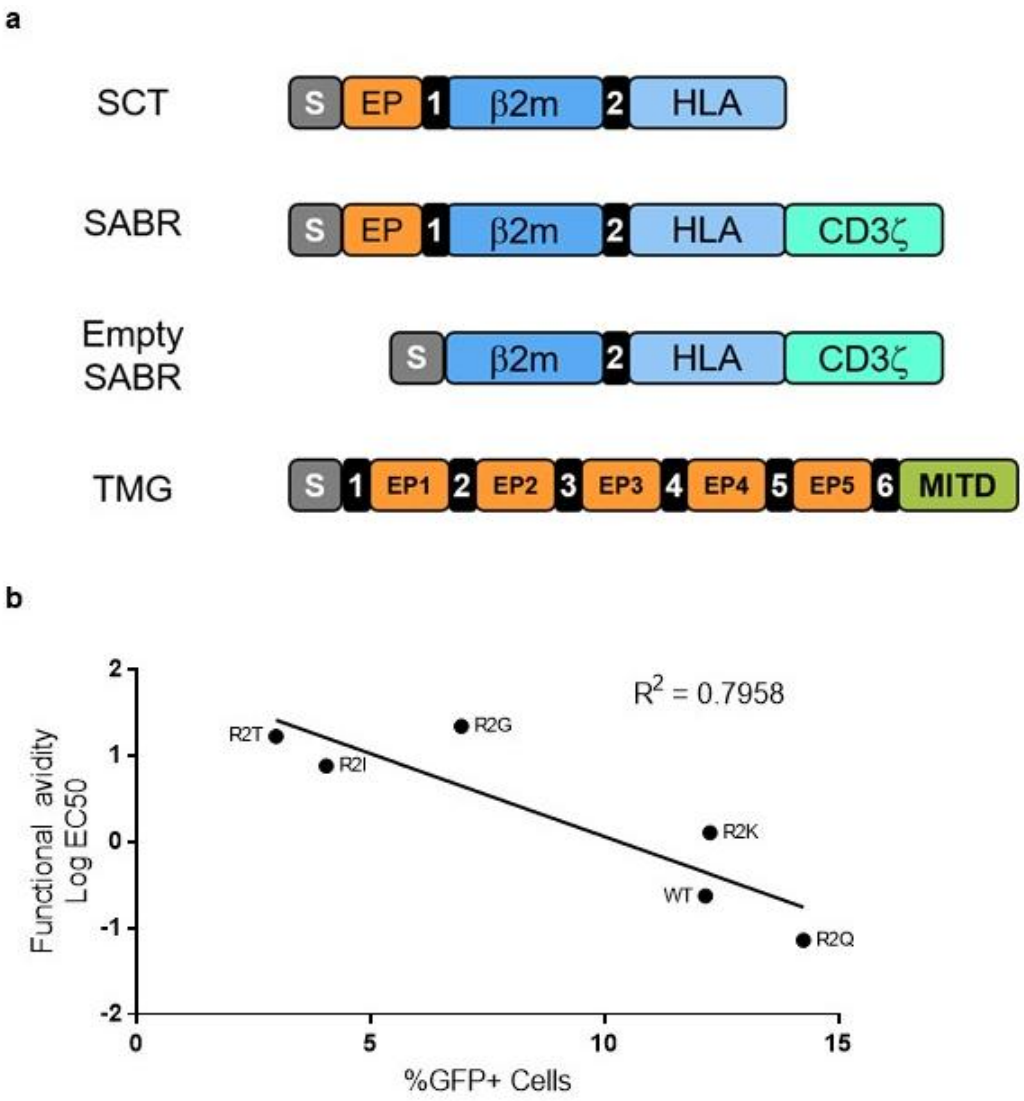
Representative flow cytometry plots from the F5+A2-MART1 experiment enumerated in Fig. 1e are shown. The rectangle in the right bottom corners shows the gate for counting GFP^+ cells. The time at which each sample was collected is shown as hours. The frequency of cells in the GFP^+ gate is indicated as a percentage.



Supplementary Figure 3

Recognition of low-affinity TCRs by SABRs.

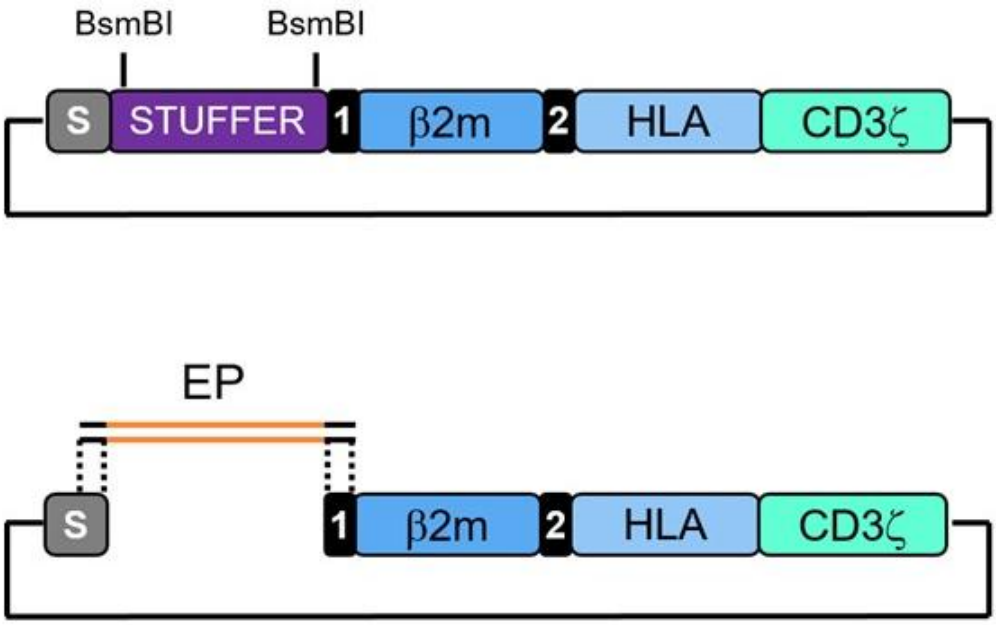
GFP expression in coculture assays using F5 and M1 TCRs with A2-MART1-SABR. The dots indicate individual values from $n = 2$ biologically independent cell culture replicates.



Supplementary Figure 4

Empty SABR vector constructs and recognition of low-affinity pMHC–TCR interactions.

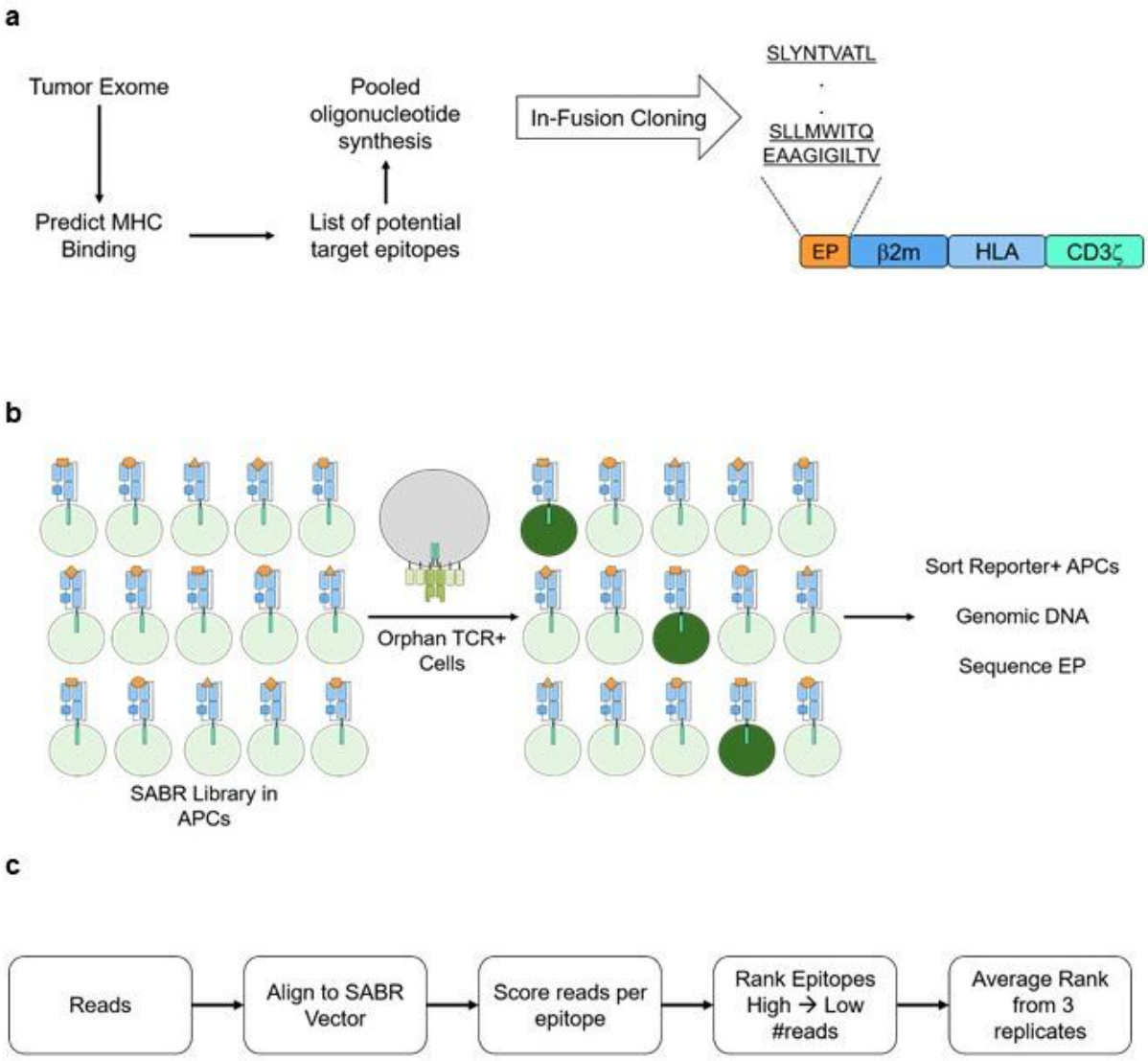
a. Schematic showing SCT, SABRs, empty SABRs, and TMGs. EP, epitope; S, signal sequence; MITD, MHC class I trafficking signal; numbers 1–6 indicate Gly–Ser linkers. **b.** Correlation of functional avidity of interaction of EC27 TCR with variants of KK10 peptides with their ability to initiate signal through SABRs. The indicated peptides are variants of the KK10 epitope. R2T, KTWIILGLNK; R2I, KIWIILGLNK; R2G, KGWIILGLNK; R2K, KKWIILGLNK; WT, KRWIILGLNK; R2Q, KQWIILGLNK.



Supplementary Figure 5

Strategy to clone custom oligonucleotides into SABR vectors.

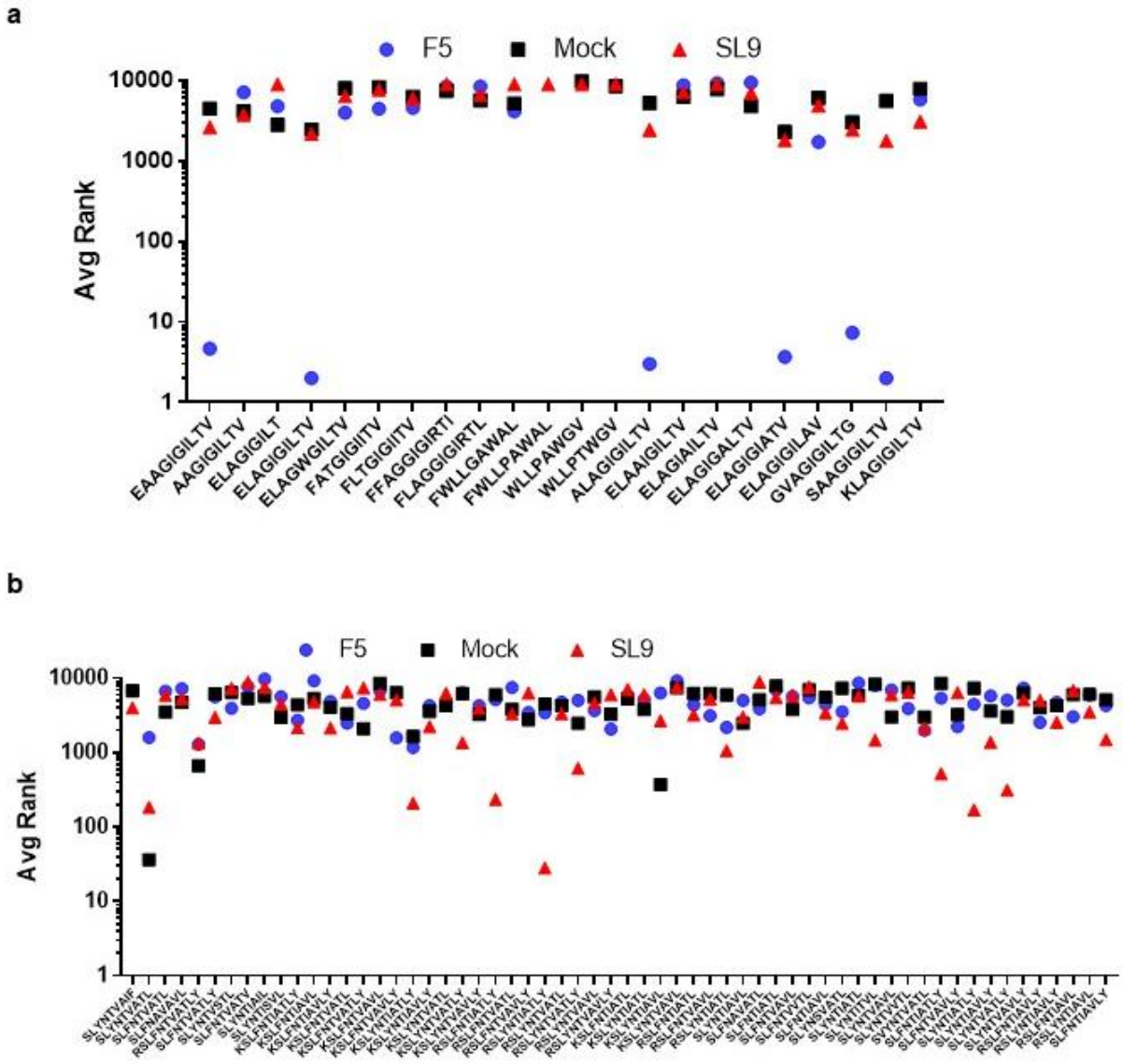
SABR vector constructs with a stuffer fragment showing BsmBI sites (top), and cloning strategy using double-stranded oligonucleotides with encoding the epitope flanked by overlaps.



Supplementary Figure 6

SABR library screen for antigen discovery.

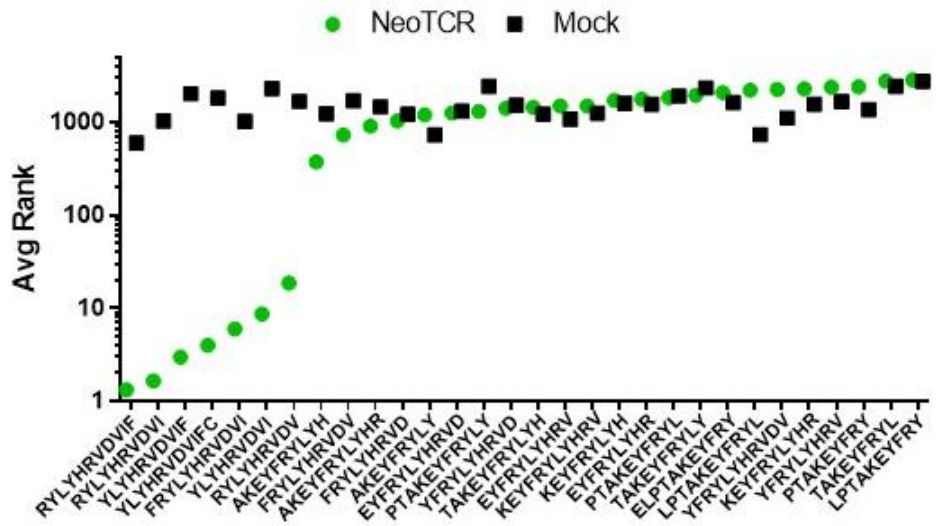
a. Schematic showing the pipeline to construct custom SABR libraries. EP, epitope. The left panel shows the procedure to obtain and synthesize a list of epitopes. The right panel shows the schematic of the SABR library. **b.** Schematic showing coculture experiment to select cells from SABR library that are recognized by an orphan TCR. Left panel shows a SABR library presenting numerous unique epitopes. The middle panel shows antigen-presenting cells (APCs) showing reporter expression induced by SABRs presenting the cognate epitope for the orphan TCR. Right panel shows processing of the selected cells. **c.** Flowchart showing the computational analysis pipeline.



Supplementary Figure 7

Enrichment of EAAGIGILTV and SLYNTVATL analogs in SABR library screen.

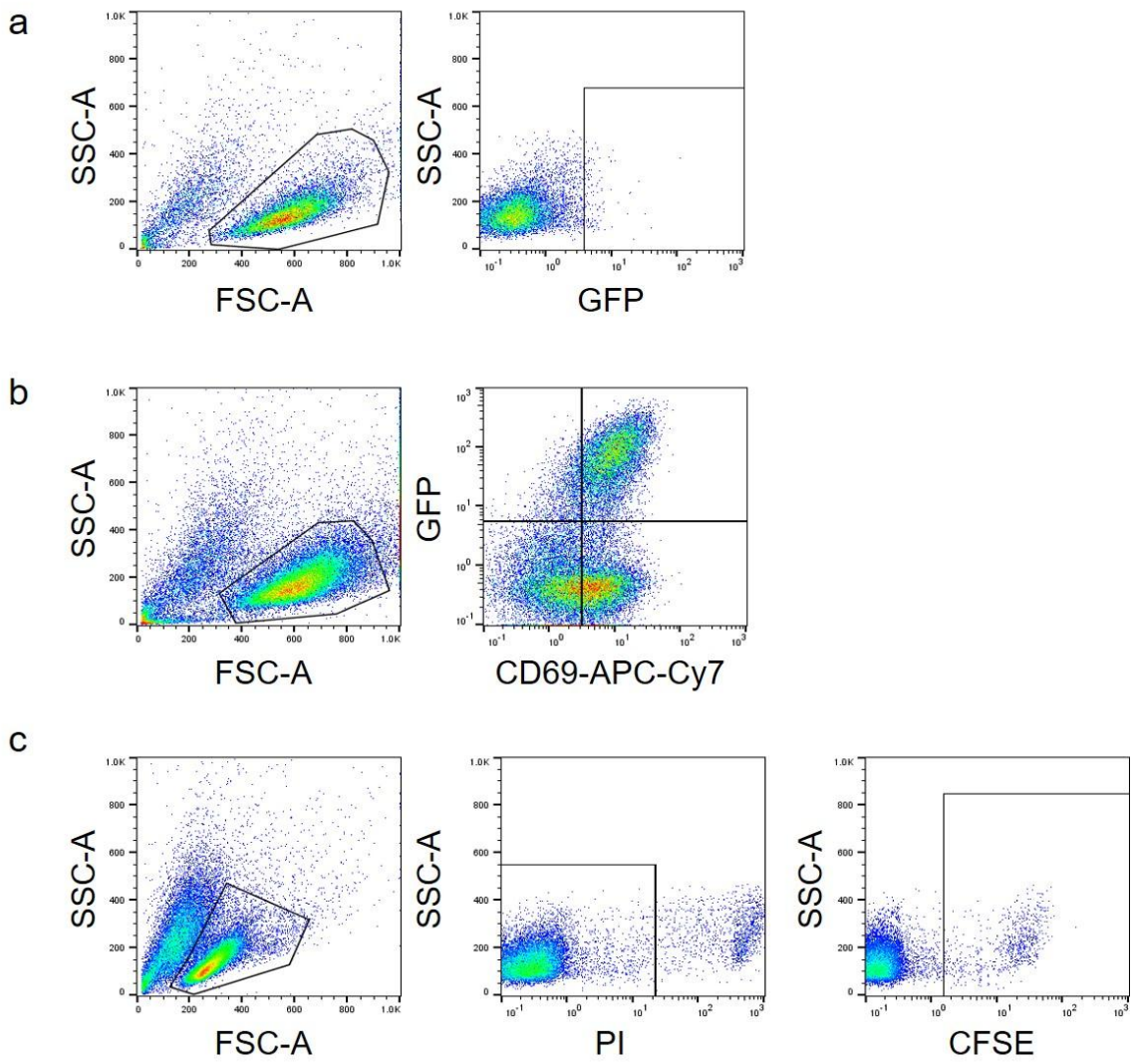
a. Average ranks for all the EAAGIGILTV analogs in the A2-SABR library. **b.** Average ranks for all the SLYNTVATL analogs in the A2-SABR library. The ranks were calculated as described in the manuscript. The data are averaged from three biologicallyindependent cell culture replicates.



Supplementary Figure 8

Enrichment of USP7 neoepitopes in SABR library screen.

Average ranks for all the USP7-derived epitopes in the NeoAg-SABR library. The data are averaged from three biologically independent cell culture replicates.



Supplementary Figure 9

Gating strategy used in flow cytometry.

a. Gating strategy used in coculture assays to measure GFP expression. **b.** Gating strategy used in coculture assays to measure GFP and CD69 expression. **c.** Gating strategy used in cytotoxicity assays.

Product Name	**Manufacturer / Source**	**Cat. No**
Jurkat Cells, Clone E6-1	ATCC, Manassas, VA	TIB-152
NFAT-GFP-Jurkat Cells	Provided by Arthur Weiss and Yvonne Chen	N/A
K562-A2.1+ cells	ATCC, Manassas, VA	CCL-243
GXR-B27+ cells	Provided by Bruce D. Walker	N/A
Primary T Cells	UCLA, CFAR Virology Core	N/A
HEK-293T Cells	ATCC, Manassas, VA	CRL-3216
RPMI 1640, 1X with L-glutamine	Corning™, Corning, NY	10-040-CV
10% Fetal Bovine Serum	Corning™, Corning, NY	35-015-CV
Penicillin-Streptomycin Solution, 100X	Corning™, Corning, NY	30-002-CI
G-418 Sulfate	Corning™, Corning, NY	30-234-CI
Immunocult™ CD3/28 T Cell Activator	StemCell Technologies™, Vancouver, Canada	10991
Human IL-2 IS, premium grade	MACS Miltenyi Biotec, Bergisch Gladbach, Germany	130-097-746
All indicated peptides	Synthesized by Pierce Thermo Fisher	N/A
DMEM, 1X with L-Glutamine, 4.5g/L Glucose and Sodium Pyruvate	Corning™, Corning, NY	10-013-CV
Clontech In-Fusion® HD Cloning Kit	Clontech, Takara Bio USA, Mountain View, CA	639650
BsmBI	New England BioLabs®, Inc, Ipswich, MA	R0580S
Clontech Stellar™ Competent Cells	Clontech, Takara Bio USA, Mountain View, CA	636763

Zyppy™ Plasmid Miniprep Kit	Zymo Research, Irvine, CA	D4036
Carbenicillin (disodium)	Gold Biotechnology®, Saint Louis, MO	C-103-SL10
NucleoBond® Xtra Maxi EF Kit	Clontech, Takara Bio USA, Mountain View, CA	740424.50
TransIT®-293 Transfection Reagent	Mirus® Bio, LLC Madison, WI	MIR 2704
RetroNectin	Takara Bio Inc, CA	T100B
Gibco™ Opti-MEM™ I Reduced Serum Medium	Life Technologies, Thermo Fisher Scientific, Waltham, MA	31985-062
Millex®-HV Syringe Filter Unit, 0.45 µm, PVDF, 33 mm, gamma sterilized	EMD Millipore, Burlington, MA	SLHV033RS
MACSQuant® Analyzer 10	MACS Miltenyi Biotec, Bergisch Gladbach, Germany	130-096-343
APC/Cy7 anti-human CD69 Clone: FN50	BioLegend®, San Diego, CA	310913
BD FACSort™	Becton Dickinson, Franklin Lakes, NJ	
CFSE Cell Division Tracker Kit	BioLegend®, San Diego, CA	423801
Invitrogen™ PureLink™ Genomic DNA Mini Kit	Invitrogen™ Life Technologies, Thermo Fisher Scientific, Waltham, MA	K182001
KOD DNA Polymerase	EMD Millipore, Burlington, MA	71085
Macherey-Nagel NucleoSpin® Gel and PCR Purification Kit	Clontech, Takara Bio USA, Mountain View, CA	740609.250
2100 Bioanalyzer Instrument	Agilent, Santa Clara, CA	G2939BA
Ilumina® HiSeq 2500 Sequencing	Illumina® San Diego, CA	SY–401–2501

System		
TreeStar FlowJo® Flow Cytometric Data Analysis Software v10	FlowJo, LLC Ashland, Oregon	N/A
GraphPad Prism v7	GraphPad, San Diego, California	N/A

Supplementary Table 1:

Reagents used in this study and their ordering information.

Primer name	**Sequence (5'-3')**	**Purpose**
SS-Fwd	AGCTCCTCGAGATGGCGACGGGTTCAAG	SABR cloning
CD28-Overlap-HLA-A2-Rev	CCACCGCGAGACCTCTTGCTCCGCACTTTACA AGCTGTGAGAGACACA	SABR cloning
CD28-Overlap-HLA-B27-Rev	CCACCGCGAGACCTCTTGCTCCGAGCTGTGAG AGACACATCAGAGC	SABR cloning
CD28Intracell-Fwd	CGGAGCAAGAGGTCTCGC	SABR cloning
XhoI-CD3z-Rev	TTGACCTCGAGTCATCTTGGTGGCAGAGCC	SABR cloning
Oligo-Insert-Fwd	CAGGAGGGCTCGGCA	Cloning epitopes in SABRs
Oligo-Insert-Rev	GGACCCTCCGCATCC	Cloning epitopes in SABRs
Epitope-Oligo	CAGGAGGGCTCGGCA **NNN…NNN** GGATGCGGAGGGTCC	Cloning epitopes in SABRs
TruSeq-Univ-SCTfixed-F	AATGATACGGCGACCACCGAGATCTACACTCT TTCCCTACACGACGCTCTTCCGATCTGGCCTGC TTTGTTTGCC	High throughput sequencing
TruSeq-Read2-SCTfixed-R	GTGACTGGAGTTCAGACGTGTGCTCTTCCGAT CTCCTCCACCACCGCTACCTC	High throughput sequencing
Truseq-Adapter-Index	CAAGCAGAAGACGGCATACGAGAT **[index]** GTGACTGGAGTTCAGACGTGTGCTCTTCCGAT CT	High throughput sequencing

Supplementary Table 2: Primers used in this study

In the Epitope-Oligo, NNN…NNN indicates back-translated epitope

In the Truseq-Adapter-Index, [index] indicates the 6-nucleotide unique index used for sequencing.