SUPPLEMENTAL INFORMATION

Table S1. Comparison of ENTER with other platforms. Related to Figures 4, 6, and 7.

	DNA barcoded tetramer (TetTCR-seqHD)[57]	Cell reporter screen (T-scan[4], SABR[3] etc)	Display techniques (Yeast, phage etc)[7]	Engineered viral platform		
				RAPTR[56]	v-CARMA[11]	ENTER
Antigen-TCR pairing	✓	✓	✓	✓	n.d.	✓
# of antigens	10^2-10^3 known	10^5 known	10^8 unknown	10^2 known	n.a.	10^1-10^2 known
# of TCRs	10^{16} unknown	1-10 known	1-10 known	10^5 known	n.a.	10^{16} unknown
Gene expression	✓ 500 genes/cell	X	X	X	X	✓ 20,000 genes/cell
Apply to primary sample	✓	✓	X	n.a.	✓	✓
Antigen-specific cargo delivery	X	X	X	X	✓ T cell	✓ T cell and B cell
Cell type-specific cargo delivery	X	X	X	X	X	✓

n.d.: not done
n.a.: not available

Table S2. Comparison of ENTER pMHC virus and pMHC tetramer. Related to Figures 2, 6, and 7.

	ENTER pMHC virus	**DNA barcoded pMHC tetramer**
MHC expression system	Mammalian cell line: natural MHC glycosylation and proper folding in human cells	E. coli bacteria system: eukaryotic MHC post-translational modification and folding not possible
Sensitivity	More sensitive than pMHC tetramer on a molar basis per reagent	Sensitive under a high concentration
Throughput	12 pMHC ENTER viruses for single cell multi-omics profiling of antigen specificity, TCR clonality, and global transcriptomics.	1000 pMHC tetramers for only antigen specificity in bulk samples [58]. 100-250 pMHC tetramers for single cell multi-omics profiling of antigen specificity, TCR clonality, and targeted gene expression[57].
Barcode	2 copy of barcoded viral RNA naturally assembled per viral particle. ENTER-seq infer TCR affinity by analysis of pMHC binding per cell	Individual DNA barcode conjugation reaction may lead to uneven barcodes conjugated for tetramers. No analysis of pMHC binding per cell has been done using DNA barcoded pMHC tetramers.
Cost	$10 for 10 tests (directly apply to 10x genomics platform)	$1400 for 50 tests of commercial pMHC tetramer (not DNA barcoded) >$2000 for 50 tests of DNA barcoded pMHC tetramer/multimer
Procedure	1.One-step Gibson assembly cloning to insert DNA oligo sequence (encoding 9aa peptide) into pMHC plasmid vector.	***Option 1*** *(Purchase and mix reagents in a convenient but high-cost manner):* Purchase peptides (GenScript), DNA barcoded and fluorophore conjugated Klickmer (Immudex),

	2.Transfection of pMHC vector along with other ENTER vectors in HEK293T cells. 3.Collect supernatant containing viruses after 48h and 72h after transfection. 4.Viral concentration: add 1mL Lenti-X concentrator per 2mL supernatant, mix well and incubate at 4°C overnight, 2000Xg spin down for 45 min, resuspend pellet in 150uL DMEM medium (20X concentration)	and MHC-I monomers (immuneAware) (Total >$2000 for 50 tests). Mix peptides to MHC-I and Klickmer at specific ratios individually. ***Option 2*** *(Generate most reagents in house in a relatively low-cost but more complicated manner)* [59] 1. Generation of biotinylated MHC-I monomers: (1) Expression and purification of MHC-I light chain B2M and heavy chain using bacteria system (25 steps). (2) MHC in vitro refolding, biotinylation and purification (25 steps) 2. Generation of peptides by in vitro transcription and translation. 3. Generation of DNA barcoded fluorescent streptavidin. (1) Conjugation of DNA linker to commercial fluorescent labeled streptavidin ($500). (2) Annealing and overlap extension to conjugate DNA barcodes to complementary DNA linker 4. pMHC UV exchange of peptides, tetramerization, and purification (8-10 steps).
Specialized equipment	None	Size exclusion chromatography (HPLC) system with a gel-filtration column is required for purification of pMHC monomers.

Table S3. List of TM domain sequence. Related to Figure 3 and STAR Methods

gene	transmembrane domain	cytoplasm domain
CD162(PSGL1)	LLAILILALVATIFFVCTVVL	AVRLSRKGHMYPVRN
LFA-1	YLYVLSGIGGLLLLLLIFIVL	YKVGFFKRNLK
CD62L	PLFIPVAVMVTAFSGLAFIIWLA	RRLKKGKKSKR
CD49d	IVIISSSLLLGLIVLLLISYVMWK	AGFFKRQYKS
CD43	GMLPVAVLVALLAVIVLVALLLL	WRRRQKRRT
HLA-A2	IVGIIAGLVLFGAVITGAVVAAVMW	RRKSSDRKG
VSV-G	FFFIIGLIIGLFLVLRVGIHLCI	KLKHTKKRQI
ICAM1	IVIITVVAAAVIMGTAGLSTYLY	NRQRKIKKYRL
HLA-Dra1	NVVCALGLTVGLVGIIIGTIFII	KGLRKSNAAERRGPL
PDGFR	GTVVVISAILALVVLTIISLIILIML	WQKKPR

Table S4. List of HLA peptide sequence. Related to Figure 2, 4, 5, 6, and 7

name	peptide	HLA allele	antigen
NY-ESO-$1_{157\text{-}165}$	SLLMWITQC	A0201	NY-ESO-1 (157-165)
NY-ESO-$1_{157\text{-}165}$ L3A	SLAMWITQC	A0201	NY-ESO-1 mutant peptide
NY-ESO-$1_{157\text{-}165}$ T7A	SLLMWIAQC	A0201	NY-ESO-1 mutant peptide
$pp65_{495\text{-}503}$	NLVPMVATV	A0201	CMV pp65 (495-503)
$pp65_{363\text{-}373}$	YSEHPTFTSQY	A0101	CMV pp65 (363-373)
$M1_{58\text{-}66}$	GILGFVFTL	A0201	Influenza M1 (58-66)
$IE1_{316\text{-}324}$	VLEETSVML	A0201	CMV IE1 (316-324)
$IE1_{81\text{-}89}$	VLAELVKQI	A0201	CMV IE1 (81-89)
$pp65_{14\text{-}22}$	VLGPISGHV	A0201	CMV pp65 (14-22)
$pp65_{120\text{-}128}$	MLNIPSINV	A0201	CMV pp65 (120-128)
$pp65_{188\text{-}196}$	FPTKDVALR	A0201	CMV pp65 (188-196)
$pp65_{325\text{-}333}$	QIFLEVQAI	A0201	CMV pp65 (325-333)
$pp65_{417\text{-}425}$	TPRVTGGGA	A0201	CMV pp65 (417-425)
$UL46_{100\text{-}108}$	CLLESVYTA	A0201	CMV UL46 (100-108)
$UL100_{200\text{-}208}$	TLIVNLVEV	A0201	CMV UL100 (200-208)
$US8_{74\text{-}82}$	GVLDAVWRV	A0201	CMV US8 (74-82)
$US150A_{152\text{-}161}$	ALWDVALLEV	A0201	CMV US150A (152-161)

Table S5. DNA Oligo sequences. Related to STAR Methods

name	Sequence	note
hs_TRAc_RT	gttgaaggcgtttgca	Reverse transcription (RT) primer for TCR alpha
nyeso_TRAc_RT	TCTTCTCAACGAGTTTAACGT	RT primer for NYESO-1 TCR alpha
HLA_nested_fw	ccaccatggcgacgggttca	For nested PCR to enrich HLA peptides
10x_5pRNA_Fw	ACACTCTTTCCctacacgacgctcttccgatct	For nested PCR to enrich HLA peptides
P7_Tru_HLA_fw	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTggttacaggagggctcggca	PCR to add Illumina adapters for HLA peptide library
P5_adapter	ACACTCTTTCCCTACACGACGCTCT	
nyeso_TRAc_rev	AACGTCACAGGAGCTTTCGGGA	PCR to enrich TCR alpha fragments for mixed cell lines
hs_TRAc_rev	TGAAGGCGTTTGCACATGCA	PCR to enrich TCR alpha fragments for mixed cell lines
P7_TRAc_nyeso_Rev	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTgggttttggatgtacggatgaac	Adding Illumina adapters for TCR library of mixed cell lines
P7_TRAc_hs_Rev	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTcagctggtacacggcagg	Adding Illumina adapters for TCR library of mixed cell lines
10xTSO_oligo	tCCCATATAAGAAAc	in Enter pHLA display vector

10xPCR_handle	AAGCAGTGGTATCAACGCAGAGTAC	in Enter vector; for cDNA amplification
Fas shRNA-1 sequence under U6	CCCTTGTGTTTGGAATTATAACTCGAGTTATAATTCCAAACACAAGGG	
Fas shRNA-2 sequence under U6	GCGTATGACACATTGATTAAACTCGAGTTTAATCAATGTGTCATACGC	
Fas shRNA-3 sequence under U6	CCTGAAACAGTGGCAATAAATCTCGAGATTTATTGCCACTGTTTCAGG	
Control shRNA sequence under U6	CCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG	
CRISPR guide target HLA-1	CGGCTACTACAACCAGAGCG	
CRISPR guide target HLA-2	AGATCACACTGACCTGGCAG	
CRISPR guide target HLA-3	AGGTCAGTGTGATCTCCGCA	