Supplementary information for

MATE-Seq: Microfluidic Antigen-TCR Engagement Sequencing

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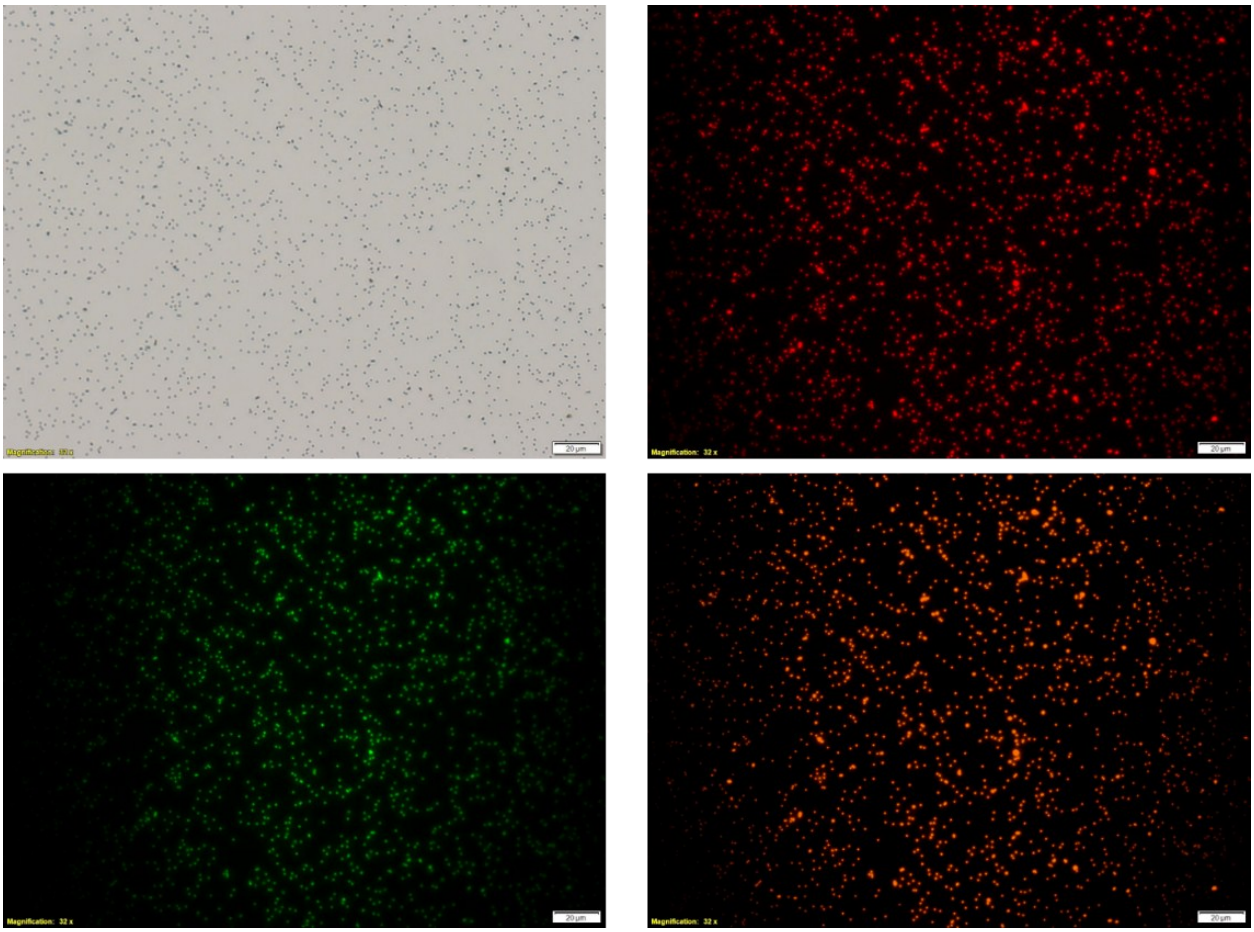
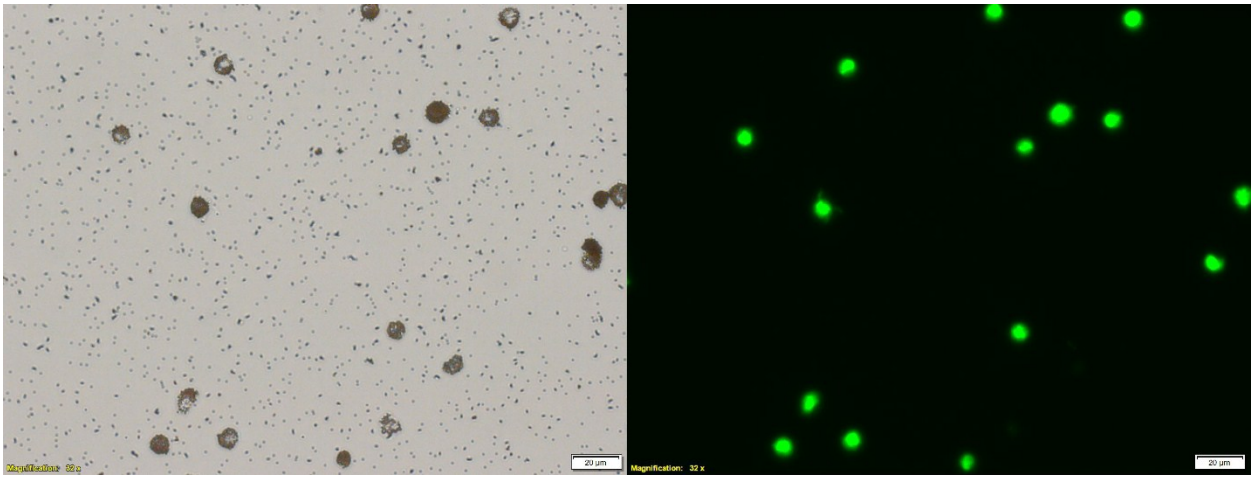
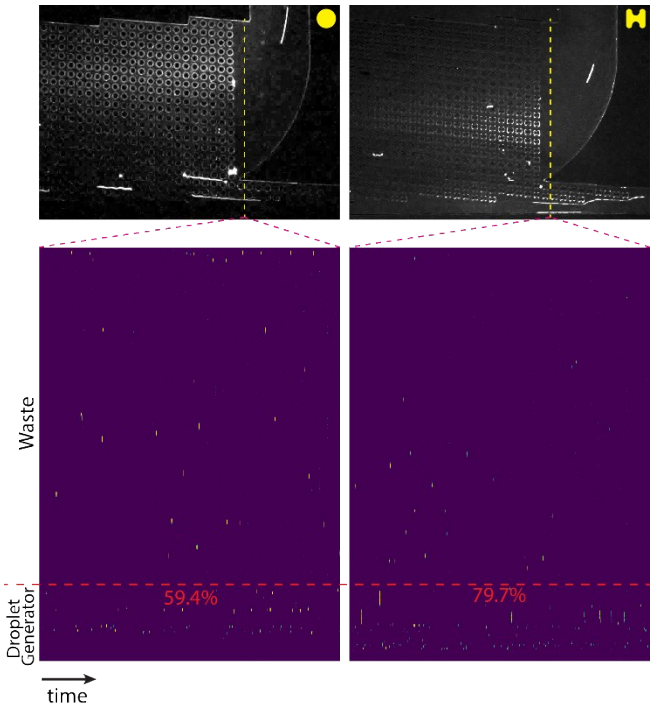


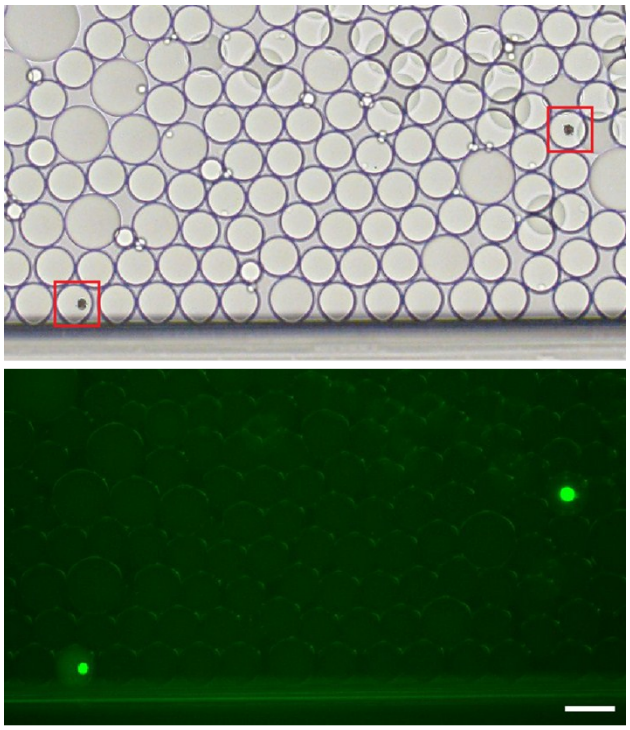
Supplementary Figure 1: Bright field and fluorescent micrographs of pNPs. The pNPs are labeled with three different DNA (PS1-PI-Cα, PS1-PI-Cβ, NP-DNA) at the same time. Complementary DNA for Cα (Red), Cβ (Green) and MHC-DNA (Yellow) are hybridized to the DNA on the pNP and give the correct fluorescent emission, indicating successful DNA labeling on the particles.



Supplementary Figure 2: Bright field and fluorescent micrographs of viability-stained NY-ESO-specific Jurkat cells pulldown by NY-ESO-specific pNPs.



Supplementary Figure 3: A movie screen capture of DLD sorting of viability-stained cells using a device with circular (left) or I-shaped pillars (right). As a cell passes the dotted yellow line in the device, the event is recorded as a spot on the corresponding kymograph, below. The fraction of cells that go to the droplet generator is the sorting efficiency of DLD.



Supplementary Figure 4: Viability-stained donor PBMCs after processing by MATE-seq without lysis reagent. Droplets with single cells are indicated by red boxes in the bright field micrograph at the top, and their viability is verified by the fluorescence at the bottom. Scale bar is 40 μm.

Supplementary Table 1: DNA sequences used for NP labeling

Name	DNA sequence
PS1-PI-Cα	/5PCBio/TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNNNN**XXXXXX**TCTCT CAGCTGGTACACGGC
PS1-PI-Cβ	/5PCBio/TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGNNNNNNNN**XXXXXX**GATGG CTCAAACACAGCGACCTC
NP-DNA	/5BiosG/AAAAAAAAACTATGTCGATACAAGTCAGATAGTTCAACTCGTTCACTATAA**CT GAATCCTCGGGATGCCTA**
N is either one of A, T, G or C **XXXXXX** is either one of ACTCTT, CGAGTC, TAGACG or TTCAGG, representing peptide identifiers for CMV, MHC-J, MART-1, or EBV, respectively. **CTGAATCCTCGGGATGCCTA** is the sequence that hybridizes to SAC-DNA	

Supplementary Table 2: Vα-gene-specific primers for cloning TCRα genes

TRAV gene	α ID	Vα
TRAV1-1*01	5'-TACAGGAAGCCTCAGCA	GGACAAAGCCTTGAGCAGCCCTC-3'
TRAV1-2*01	5'-TACAGGAAGCCTCAGCA	GGACAAAACATTGACCAGCCCACTG-3'
TRAV2*01	5'-TACAGGAAGCCTCAGCA	AAGGACCAAGTGTTTCAGCCTTCCAC-3'
TRAV3*01	5'-TACAGGAAGCCTCAGCA	GCTCAGTCAGTGGCTCAGCCGGA-3'
TRAV4*01	5'-TACAGGAAGCCTCAGCA	CTTGCTAAGACCACCCAGCCCATC-3'
TRAV5*01	5'-TACAGGAAGCCTCAGCA	GGAGAGGATGTGGAGCAGAGTCTTTTCC-3'
TRAV6*01	5'-TACAGGAAGCCTCAGCA	AGCCAAAAGATAGAACAGAATTCCGAGGC-3'
TRAV6*03	5'-TACAGGAAGCCTCAGCA	GAGGCCCTGAACATTCAGGAGGG-3'
TRAV7*01	5'-TACAGGAAGCCTCAGCA	GAAAACCAGGTGGAGCACAGCCC-3'
TRAV8-1*01	5'-TACAGGAAGCCTCAGCA	GCCCAGTCTGTGAGCCAGCATAACC-3'
TRAV8-2*01	5'-TACAGGAAGCCTCAGCA	GCCCAGTCGGTGACCCAGCTTG-3'
TRAV8-2*02	5'-TACAGGAAGCCTCAGCA	GCCCAGTCGGTGACCCAGCTTAG-3'
TRAV8-3*01	5'-TACAGGAAGCCTCAGCA	GCCCAGTCAGTGACCCAGCCTG-3'
TRAV8-4*06	5'-TACAGGAAGCCTCAGCA	CTCTTCTGGTATGTGCAATACCCCAACC-3'
TRAV8-4*07	5'-TACAGGAAGCCTCAGCA	GTTGAACCATATCTCTTCTGGTATGTGCAATACC-3'
TRAV8-6*01	5'-TACAGGAAGCCTCAGCA	GCCCAGTCTGTGACCCAGCTTGAC-3'
TRAV8-7*01	5'-TACAGGAAGCCTCAGCA	ACCCAGTCGGTGACCCAGCTTG-3'
TRAV9-1*01	5'-TACAGGAAGCCTCAGCA	GGAGATTCAGTGGTCCAGACAGAAGGC-3'
TRAV9-2*01	5'-TACAGGAAGCCTCAGCA	GGAAATTCAGTGACCCAGATGGAAGG-3'
TRAV9-2*02	5'-TACAGGAAGCCTCAGCA	GGAGATTCAGTGACCCAGATGGAAGG-3'
TRAV10*01	5'-TACAGGAAGCCTCAGCA	AAAAACCAAGTGGAGCAGAGTCCTCAGTC-3'
TRAV11*01	5'-TACAGGAAGCCTCAGCA	CTACATACACTGGAGCAGAGTCCTTCATTCC-3'
TRAV12-1*01	5'-TACAGGAAGCCTCAGCA	CGGAAGGAGGTGGAGCAGGATCC-3'
TRAV12-2*01	5'-TACAGGAAGCCTCAGCA	CAGAAGGAGGTGGAGCAGAATTCTGG-3'
TRAV12-2*03	5'-TACAGGAAGCCTCAGCA	GGACCCCTCAGTGTTCCAGAGGG-3'
TRAV12-3*01	5'-TACAGGAAGCCTCAGCA	CAGAAGGAGGTGGAGCAGGATCCTG-3'
TRAV13-1*02	5'-TACAGGAAGCCTCAGCA	GGAGAGAATGTGGAGCAGCATCCTTC-3'
TRAV13-2*01	5'-TACAGGAAGCCTCAGCA	GGAGAGAGTGTGGGGCTGCATCTTC-3'
TRAV14/DV4*01	5'-TACAGGAAGCCTCAGCA	GCCCAGAAGATAACTCAAACCCAACCAG-3'
TRAV14/DV4*04	5'-TACAGGAAGCCTCAGCA	CAGAAGATAACTCAAACCCAACCAGGAATG-3'
TRAV16*01	5'-TACAGGAAGCCTCAGCA	GCCCAGAGAGTGACTCAGCCCGA-3'
TRAV17*01	5'-TACAGGAAGCCTCAGCA	AGTCAACAGGGAGAAGAGGATCCTCAGG-3'
TRAV18*01	5'-TACAGGAAGCCTCAGCA	GGAGACTCGGTTACCCAGACAGAAGG-3'
TRAV19*01	5'-TACAGGAAGCCTCAGCA	GCTCAGAAGGTAACTCAAGCGCAGACTG-3'
TRAV20*01	5'-TACAGGAAGCCTCAGCA	GAAGACCAGGTGACGCAGAGTCCC-3'
TRAV21*01	5'-TACAGGAAGCCTCAGCA	AAACAGGAGGTGACGCAGATTCCTGC-3'
TRAV22*01	5'-TACAGGAAGCCTCAGCA	GGAATACAAGTGGAGCAGAGTCCTCCAG-3'
TRAV23/DV6*01	5'-TACAGGAAGCCTCAGCA	CAGCAGCAGGTGAAACAAAGTCCTCA-3'
TRAV23/DV6*04	5'-TACAGGAAGCCTCAGCA	CAGCAGGTGAAACAAAGTCCTCAATCTTTG-3'

TRAV24*01	5'-TACAGGAAGCCTCAGCA	ATACTGAACGTGGAACAAAGTCCTCAGTCAC-3'
TRAV25*01	5'-TACAGGAAGCCTCAGCA	GGACAACAGGTAATGCAAATTCCTCAGTACC-3'
TRAV26-1*01	5'-TACAGGAAGCCTCAGCA	GATGCTAAGACCACCCAGCCCCC-3'
TRAV26-1*02	5'-TACAGGAAGCCTCAGCA	GATGCTAAGACCACCCAGCCCACC-3'
TRAV26-2*01	5'-TACAGGAAGCCTCAGCA	GATGCTAAGACCACACAGCCAAATTCAATG-3'
TRAV27*01	5'-TACAGGAAGCCTCAGCA	ACCCAGCTGCTGGAGCAGAGCC-3'
TRAV29/DV5*01	5'-TACAGGAAGCCTCAGCA	GACCAGCAAGTTAAGCAAAATTCACCATC-3'
TRAV30*01	5'-TACAGGAAGCCTCAGCA	CAACAACCAGTGCAGAGTCCTCAAGC-3'
TRAV34*01	5'-TACAGGAAGCCTCAGCA	AGCCAAGAACTGGAGCAGAGTCCTCAG-3'
TRAV35*01	5'-TACAGGAAGCCTCAGCA	GGTCAACAGCTGAATCAGAGTCCTCAATC-3'
TRAV36/DV7*01	5'-TACAGGAAGCCTCAGCA	GAAGACAAGGTGGTACAAAGCCCTCTATCTC-3'
TRAV36/DV7*02	5'-TACAGGAAGCCTCAGCA	GAAGACAAGGTGGTACAAAGCCCTCAATC-3'
TRAV38-1*01	5'-TACAGGAAGCCTCAGCA	GCCCAGACAGTCACTCAGTCTCAACCAG-3'
TRAV38-1*04	5'-TACAGGAAGCCTCAGCA	GCCCAGACAGTCACTCAGTCCCAGC-3'
TRAV38-2/DV8*01	5'-TACAGGAAGCCTCAGCA	GCTCAGACAGTCACTCAGTCTCAACCAGAG-3'
TRAV39*01	5'-TACAGGAAGCCTCAGCA	GAGCTGAAAGTGGAACAAAACCCTCTGTTC-3'
TRAV40*01	5'-TACAGGAAGCCTCAGCA	AGCAATTCAGTCAAGCAGACGGGC-3'
TRAV41*01	5'-TACAGGAAGCCTCAGCA	AAAAATGAAGTGGAGCAGAGTCCTCAGAAC-3'

Supplementary Table 3: Vβ-gene-specific primers for cloning TCRβ genes

TRBV gene	**β ID**	**Vβ**
TRBV1*01	5'-CAGGAGGGCTCGGCA	GATACTGGAATTACCCAGACACCAAAATACCTG-3'
TRBV2*01	5'-CAGGAGGGCTCGGCA	GAACCTGAAGTCACCCAGACTCCCAG-3'
TRBV3-1*01	5'-CAGGAGGGCTCGGCA	GACACAGCTGTTTCCCAGACTCCAAAATAC-3'
TRBV3-2*01	5'-CAGGAGGGCTCGGCA	GACACAGCCGTTTCCCAGACTCCA-3'
TRBV4-1*01	5'-CAGGAGGGCTCGGCA	GACACTGAAGTTACCCAGACACCAAAACAC-3'
TRBV4-1*02	5'-CAGGAGGGCTCGGCA	CACCTGGTCATGGGAATGACAAATAAGAAG-3'
TRBV4-2*01	5'-CAGGAGGGCTCGGCA	GAAACGGGAGTTACGCAGACACCAAG-3'
TRBV4-3*04	5'-CAGGAGGGCTCGGCA	AAGAAGTCTTTGAAATGTGAACAACATCTGGG-3'
TRBV5-1*01	5'-CAGGAGGGCTCGGCA	AAGGCTGGAGTCACTCAAACTCCAAGATATC-3'
TRBV5-1*02	5'-CAGGAGGGCTCGGCA	AGGGCTGGGGTCACTCAAACTCC-3'
TRBV5-3*01	5'-CAGGAGGGCTCGGCA	GAGGCTGGAGTCACCCAAAGTCCC-3'
TRBV5-4*01	5'-CAGGAGGGCTCGGCA	GAGACTGGAGTCACCCAAAGTCCCAC-3'
TRBV5-4*03	5'-CAGGAGGGCTCGGCA	CAGCAAGTGACACTGAGATGCTCTTCTCAG-3'
TRBV5-4*04	5'-CAGGAGGGCTCGGCA	ACTGTGTCCTGGTACCAACAGGCCCT-3'
TRBV5-5*01	5'-CAGGAGGGCTCGGCA	GACGCTGGAGTCACCCAAAGTCC-3'
TRBV5-8*01	5'-CAGGAGGGCTCGGCA	GAGGCTGGAGTCACACAAAGTCCCAC-3'
TRBV5-8*02	5'-CAGGAGGGCTCGGCA	AGGACAGCAAGCGACTCTGAGATGC-3'
TRBV6-1*01	5'-CAGGAGGGCTCGGCA	AATGCTGGTGTCACTCAGACCCCA-3'
TRBV6-4*01	5'-CAGGAGGGCTCGGCA	ATTGCTGGGATCACCCAGGCAC-3'
TRBV6-4*02	5'-CAGGAGGGCTCGGCA	ACTGCTGGGATCACCCAGGCAC-3'
TRBV7-1*01	5'-CAGGAGGGCTCGGCA	GGTGCTGGAGTCTCCCAGTCCCTG-3'
TRBV7-2*01	5'-CAGGAGGGCTCGGCA	GGAGCTGGAGTCTCCCAGTCCCC-3'
TRBV7-2*04	5'-CAGGAGGGCTCGGCA	GGAGCTGGAGTTTCCCAGTCCCC-3'
TRBV7-3*01	5'-CAGGAGGGCTCGGCA	GGTGCTGGAGTCTCCCAGACCC-3'
TRBV7-3*05	5'-CAGGAGGGCTCGGCA	TGGGAGCTCAGGTGTGATCCAATTTC-3'
TRBV7-4*01	5'-CAGGAGGGCTCGGCA	GGTGCTGGAGTCTCCCAGTCCC-3'
TRBV7-6*01	5'-CAGGAGGGCTCGGCA	GGTGCTGGAGTCTCCCAGTCTCCC-3'
TRBV7-9*01	5'-CAGGAGGGCTCGGCA	GATACTGGAGTCTCCCAGAACCCCAG-3'
TRBV7-9*03	5'-CAGGAGGGCTCGGCA	GATACTGGAGTCTCCCAGGACCCCAG-3'
TRBV7-9*04	5'-CAGGAGGGCTCGGCA	ATATCTGGAGTCTCCCACAACCCCAGAC-3'
TRBV7-9*07	5'-CAGGAGGGCTCGGCA	CACAACCGCCTTTATTGGTACCGACAG-3'
TRBV9*01	5'-CAGGAGGGCTCGGCA	GATTCTGGAGTCACACAAACCCCAAAGC-3'
TRBV10-1*01	5'-CAGGAGGGCTCGGCA	GATGCTGAAATCACCCAGAGCCCAAG-3'
TRBV10-2*01	5'-CAGGAGGGCTCGGCA	GATGCTGGAATCACCCAGAGCCCA-3'
TRBV10-2*02	5'-CAGGAGGGCTCGGCA	AAGGCAGGTGACCTTGATGTGTCACC-3'

TRBV11-1*01	5'-CAGGAGGGCTCGGCA	GAAGCTGAAGTTGCCCAGTCCCC-3'
TRBV11-2*01	5'-CAGGAGGGCTCGGCA	GAAGCTGGAGTTGCCCAGTCTCCCAG-3'
TRBV11-3*01	5'-CAGGAGGGCTCGGCA	GAAGCTGGAGTGGTTCAGTCTCCCAGA-3'
TRBV11-3*03	5'-CAGGAGGGCTCGGCA	GGTCTCCCAGATATAAGATTATAGAGAAGAAACAGC-3'
TRBV12-1*01	5'-CAGGAGGGCTCGGCA	GATGCTGGTGTTATCCAGTCACCCAGG-3'
TRBV12-2*01	5'-CAGGAGGGCTCGGCA	GATGCTGGCATTATCCAGTCACCCAAG-3'
TRBV12-3*01	5'-CAGGAGGGCTCGGCA	GATGCTGGAGTTATCCAGTCACCCC-3'
TRBV12-5*01	5'-CAGGAGGGCTCGGCA	GATGCTAGAGTCACCCAGACACCAAGG-3'
TRBV13*01	5'-CAGGAGGGCTCGGCA	GCTGCTGGAGTCATCCAGTCCCC-3'
TRBV14*01	5'-CAGGAGGGCTCGGCA	GAAGCTGGAGTTACTCAGTTCCCCAGC-3'
TRBV15*01	5'-CAGGAGGGCTCGGCA	GATGCCATGGTCATCCAGAACCCAAG-3'
TRBV16*01	5'-CAGGAGGGCTCGGCA	GGTGAAGAAGTCGCCCAGACTCCA-3'
TRBV17*01	5'-CAGGAGGGCTCGGCA	GAGCCTGGAGTCAGCCAGACCC-3'
TRBV18*01	5'-CAGGAGGGCTCGGCA	AATGCCGGCGTCATGCAGAAC-3'
TRBV19*01	5'-CAGGAGGGCTCGGCA	GATGGTGGAATCACTCAGTCCCCAAAG-3'
TRBV20-1*01	5'-CAGGAGGGCTCGGCA	GGTGCTGTCGTCTCTCAACATCCGAG-3'
TRBV20/OR9-2*01	5'-CAGGAGGGCTCGGCA	AGTGCTGTCGTCTCTCAACATCCGAG-3'
TRBV21-1*01	5'-CAGGAGGGCTCGGCA	GACACCAAGGTCACCCAGAGACCTAGAC-3'
TRBV21/OR9-2*01	5'-CAGGAGGGCTCGGCA	GACACCAAGGTCACCCAGAGACCTAGATTTC-3'
TRBV23-1*01	5'-CAGGAGGGCTCGGCA	CATGCCAAAGTCACACAGACTCCAGG-3'
TRBV24-1*01	5'-CAGGAGGGCTCGGCA	GATGCTGATGTTACCCAGACCCCAAG-3'
TRBV25-1*01	5'-CAGGAGGGCTCGGCA	GAAGCTGACATCTACCAGACCCCAAGATAC-3'
TRBV26*01	5'-CAGGAGGGCTCGGCA	GATGCTGTAGTTACACAATTCCCAAGACACAG-3'
TRBV26/OR9-2*01	5'-CAGGAGGGCTCGGCA	GATGCTGTAGTTACACAATTCTCAAGACACAGAATC-3'
TRBV27*01	5'-CAGGAGGGCTCGGCA	GAAGCCCAAGTGACCCAGAACCC-3'
TRBV28*01	5'-CAGGAGGGCTCGGCA	GATGTGAAAGTAACCCAGAGCTCGAGATATC-3'
TRBV29-1*01	5'-CAGGAGGGCTCGGCA	AGTGCTGTCATCTCTCAAAAGCCAAGC-3'
TRBV29-1*03	5'-CAGGAGGGCTCGGCA	ACGATCCAGTGTCAAGTCGATAGCCAAG-3'
TRBV30*01	5'-CAGGAGGGCTCGGCA	TCTCAGACTATTCATCAATGGCCAGCG-3'
TRBV30*04	5'-CAGGAGGGCTCGGCA	ACTATTCATCAATGGCCAGCGACCC-3'

Supplementary Table 4: Primers for Enrichment PCR

Name	**DNA Sequence**
PS1 partial	5'-TCGTCGGCAGCGTCAGATG-3'
PS2-α ID	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTACAGGAAGCCTCAGCA-3'
PS2-β ID	5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAGGAGGGCTCGGCA-3'

Supplementary Table 5. Primers for Adapter Insertion PCR

Name	**DNA Sequence**
PS3-PS1	5'-AATGATACGGCGACCACCGAGATCTACAC[i5]TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'
PS4-PS2	5'-CAAGCAGAAGACGGCATACGAGAT[i7]GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'

Supplementary Table 6. Donor 1 TCR α and β genes and CDR3 amino acid sequences.

	CDR3	**Gene**
Run 1	CASSTVSGAPSEQFF	TRBV6-5*00
	CALYFFFGNEKLTF	TRAV16*00
	CLE*IME_SQGNLIF	TRAV4*00
	CAVEYGNQFYF	TRAV3*00
Run 2	CLE*IME_SQGNLIF	TRAV4*00
	CAVEYGNQFYF	TRAV3*00
	CASSLKT_NYEQYF	TRBV12-4*00
	CASNTGNQFYF	TRAV24*00
	CALYFFFGNEKLTF	TRAV16*00
	CLE*IME_S*GNLIF	TRBV6-5*00

Supplementary Table 7. Donor 2 TCR α and β genes and CDR3 amino acid sequences from FACS.

	α CDR3	**Gene**		**β CDR3**	**Gene**
α-1	CAFMTSG~ATNKLIF	TRAV38-1	β-1	CSVWSFGDRDGYTF	TRBV29-1
α-2	CAVNDRGSTLGRLYF	TRAV12-2	β-2	CASSSANYGYTF	TRBV12-3
α-3	CAVNFGGGKLIF	TRAV12-2	β-3	CASSSSPGLDNEQFF	TRBV7-6
α-4	CPREEGGSQGNLIF	TRAV26-1	β-4	CASSYITGTGSYGYTF	TRBV6-5
α-5	CAAAVETSGSRLTF	TRAV21	β-5	CSARDRIGNTIYF	TRBV20-1
α-6	CARNTGNQFYF	TRAV24			
α-7	CTSPNTNAGKSTF	TRAV26-1			

Supplementary Table 8. Donor 2 TCR α and β genes and CDR3 amino acid sequences from MATE-seq.

α CDR3	**Gene**	**FACS?**	**β CDR3**	**Gene**	**FACS?**
CPREEGGSQGNLIF	TRAV26-1	α-4	CASSSSPGLDNEQFF	TRBV7-6	β-3
CKASKIIF	TRAV3		CASSEGSWGSDTQYF	TRBV6-5	
CAVRGDYKLSF	TRAV1-2		CASSLGAGPYNEQFF	TRBV18	
CAVNDRGSTLGRLYF	TRAV12-2	α-2	CASSGPISSYNEQFF	TRBV6-1	
CIAQNNNDMRF	TRAV26-2		CAWSVSDLAKNIQYF	TRBV30	
			CASSLSFGTEAFF	TRBV6-4	
			CASSYPGTGIHGYTF	TRBV6-5	
			CASSLGPSSYEQYF	TRBV5-1	
			CASSEQAGGYGYTF	TRBV6-1	

Supplementary Movie 1 shows the removal of free pNP to waste and displacement of barcoded cells to the droplet generator, where individual cells are encapsulated in water-in-oil droplets with lysis RT-PCR mix.

Supplementary Movie 2 shows a fluorescent sequence of viability-stained cells at the output of a DLD array with circular posts.

Supplementary Movie 3 shows a fluorescent sequence of viability-stained cells at the output of a DLD array with I-posts.