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SUPPLEMENTARY INFORMATION

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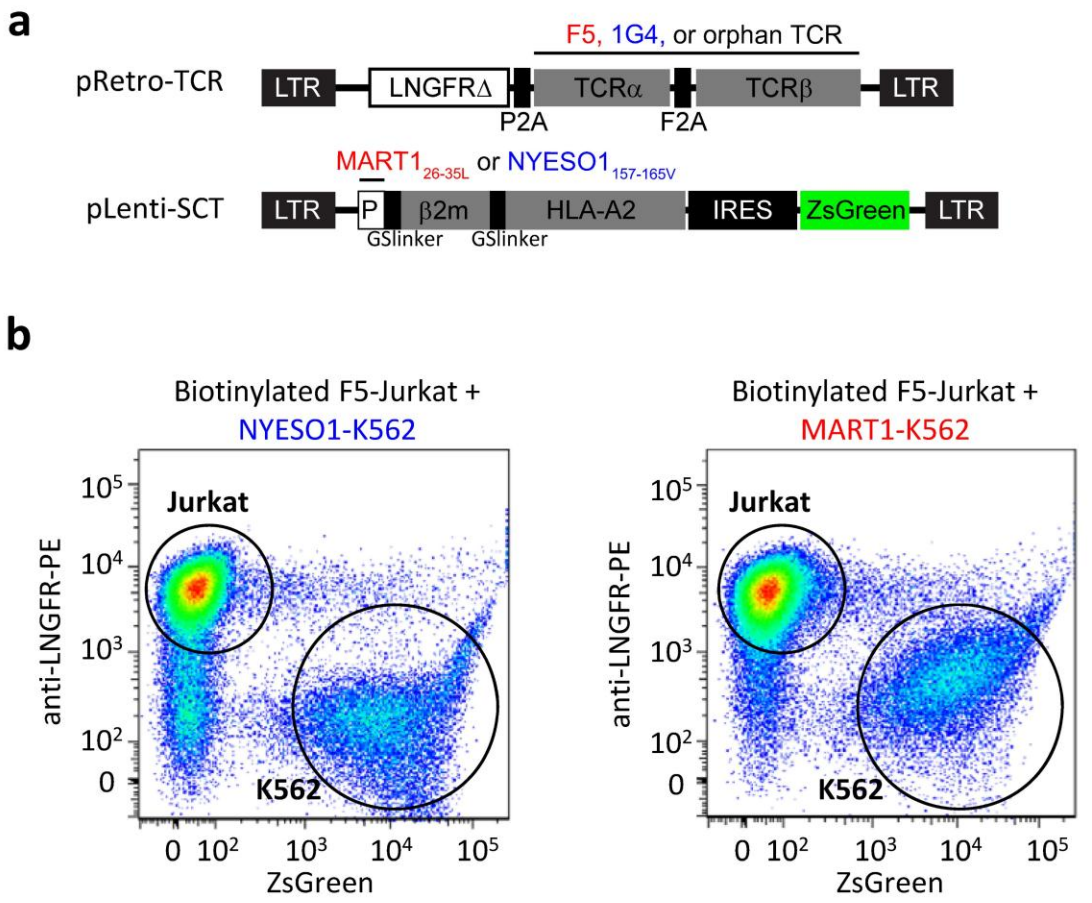
T cell antigen discovery via trogocytosis

Guideng Li[1,2,3,12*], Michael T. Bethune[1,12*], Stephanie Wong[1], Alok V. Joglekar[1], Michael T. Leonard[1], Jessica K. Wang[1], Jocelyn T. Kim[4], Donghui Cheng[5], Songming Peng[6], Jesse M. Zaretsky[7], Yapeng Su[1,6], Yicheng Luo[1], James R. Heath[6,7,8], Antoni Ribas[7,9,10,11], Owen N. Witte[5,7,11] and David Baltimore[1,8*]

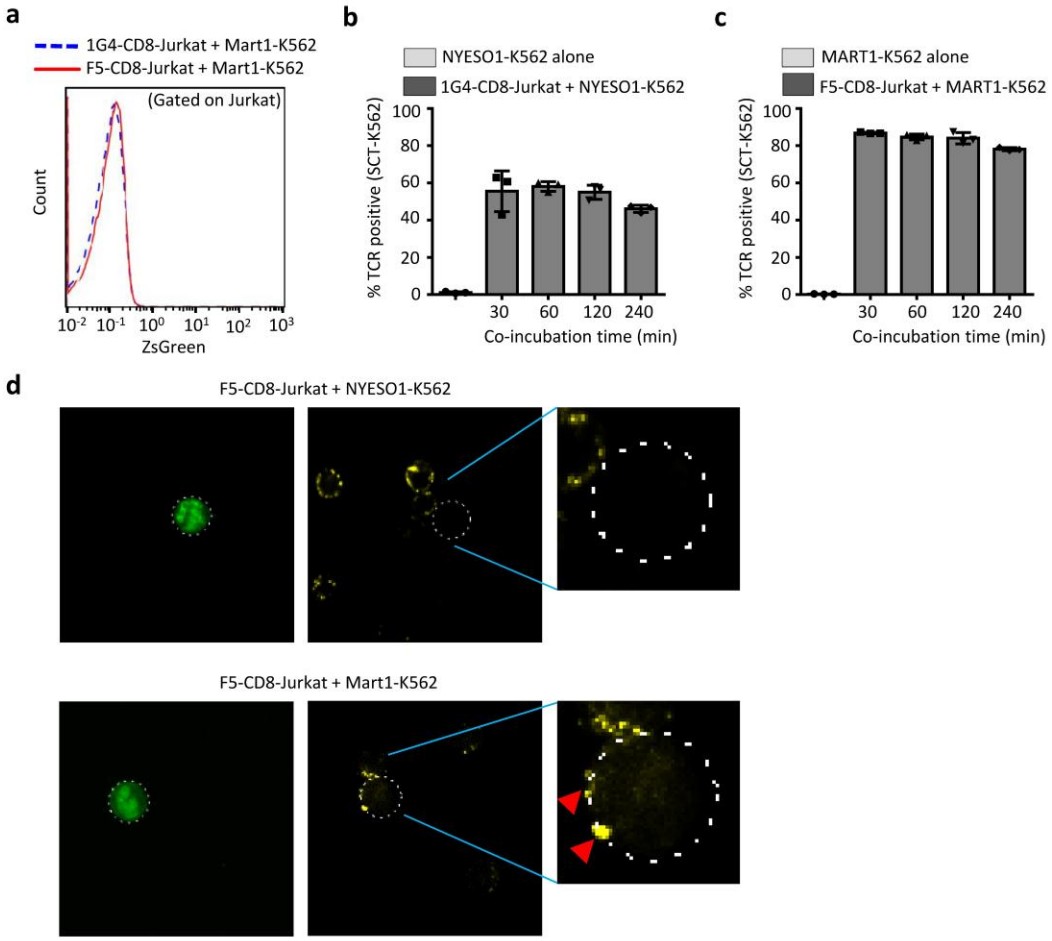
[1]Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, USA. [2]Center of Systems Medicine, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing, China. [3]Suzhou Institute of Systems Medicine, Suzhou, China. [4]Division of Infectious Diseases, Department of Medicine, University of California, Los Angeles, Los Angeles, CA, USA. [5]Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, Los Angeles, CA, USA. [6]Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA, USA. [7]Department of Molecular and Medical Pharmacology, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA. [8]Parker Institute for Cancer Immunotherapy (PICI) Center, California Institute of Technology, Pasadena, CA, USA. [9]Division of Hematology & Oncology, Department of Medicine, and Division of Surgical Oncology, Department of Surgery, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA, USA. [10]Jonsson Comprehensive Cancer Center, University of California, Los Angeles, Los Angeles, CA, USA. [11]Parker Institute for Cancer Immunotherapy (PICI) Center, University of California, Los Angeles, Los Angeles, CA, USA. [12]These authors contributed equally: G. Li, M. T. Bethune. *e-mail: lgd@ism.cams.cn; mbethune@pactpharma.com; baltimo@caltech.edu



Supplementary Figure 1

Establishment of Jurkat cells expressing F5 TCR or 1G4 TCR and K562 cells expressing single-chain trimer (SCT) of HLA-A2/MART1$_{26-35(A27L)}$ or HLA-A2/NYESO1$_{157-165(C165V)}$.

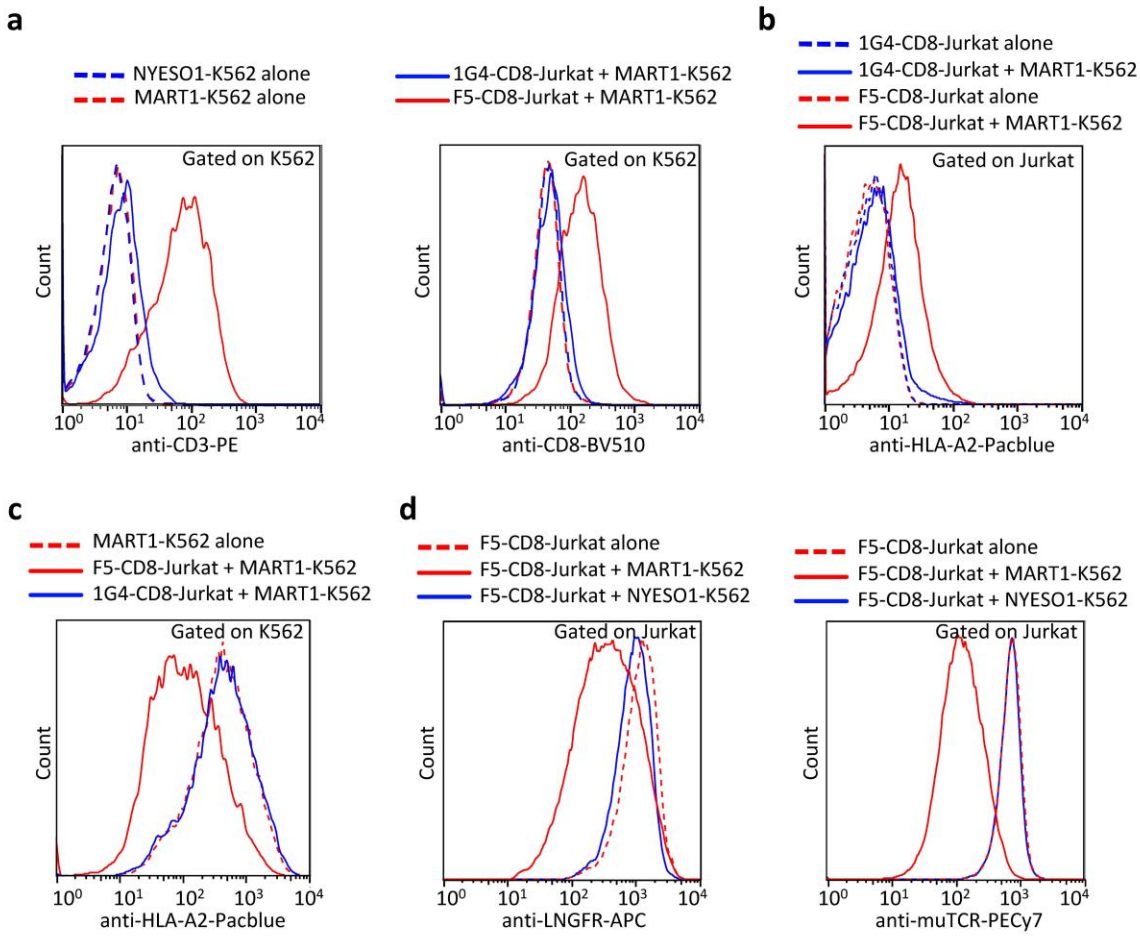
(**a**) A retroviral vector co-delivered F5 TCR or 1G4 TCR genes carrying either human or murine TCR constant regions together with LNGFRΔ, a transduction marker comprising low-affinity nerve growth factor receptor with the intracellular domain truncated, to Jurkat cells. A lentiviral vector co-delivered an SCT containing MART1 or NYESO1 peptide with ZsGreen as a transduction marker to K562 cells. SCTs are composed of a single polypeptide chain with a linear composition of antigenic peptide, β2-microglobulin, and HLA-A2 domains via flexible GS linkers. (**b**) Resolution via flow cytometry of Jurkat and K562 cells. The Jurkat T cells and K562 cells were resolved by gating on the LNGFR^{+} population and the ZsGreen^{+} population, respectively.



Supplementary Figure 2

Target cell trogocytosis occurs in an antigen-specific manner.

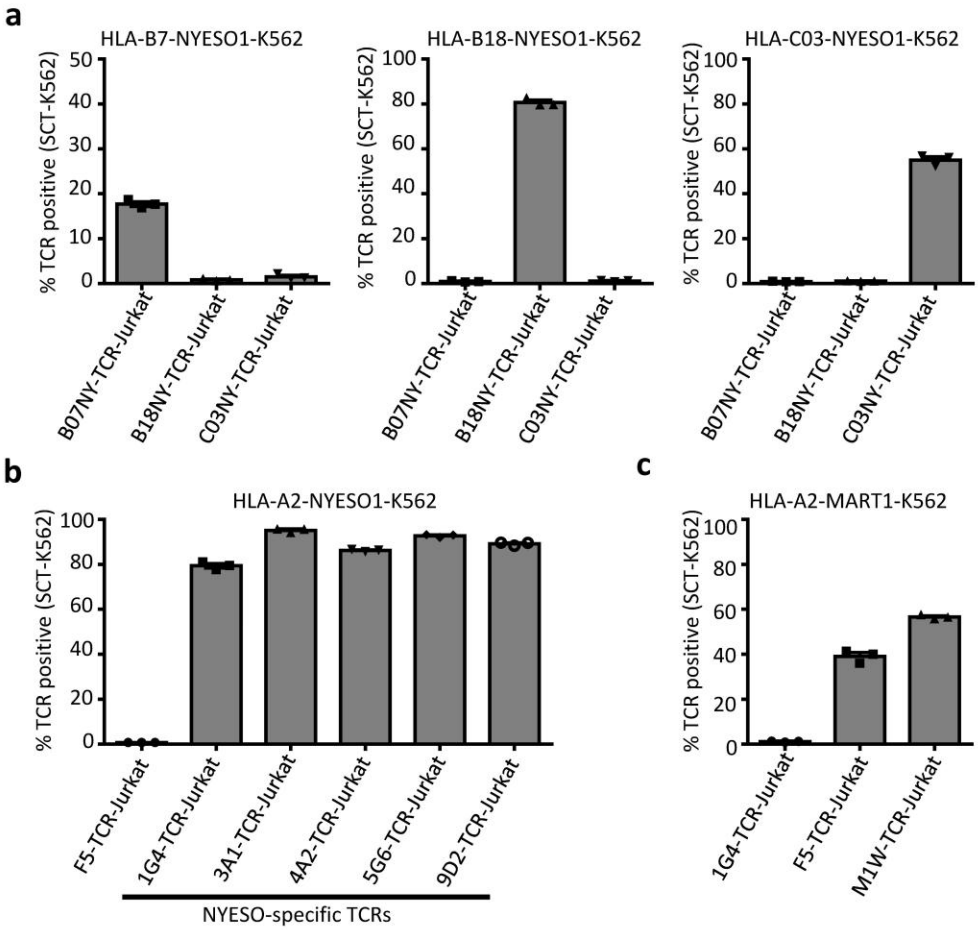
(**a**) ZsGreen cannot be transferred from K562 cells to Jurkat cells. Representative flow cytometry plot of Zsgreen level in F5-CD8-Jurkat or 1G4-CD8-Jurkat following co-incubation with MART1-K562. (**b,c**)The kinetics of trogocytosis for TCR–cognate pMHC interaction. Co-incubation of 1G4-CD8-Jurkat cells with NYESO1-K562 or F5-CD8-Jurkat cells with MART1-K562 for indicated time and trogocytosis was assessed using anti-muTCR antibody ($n = 3$). Data are presented as mean ± s.e.m. (**d**) Immunofluorescence staining of co-incubated F5-CD8-Jurkat with NYESO-K562 (Zsgreen^{+}) or MART1-K562 (Zsgreen^{+}) by MART1 pMHC dextramer (yellow). Data in this figure are representative of at least two independent experiments.



Supplementary Figure 3

Trogocytosis can be tracked by multiple protein transfer.

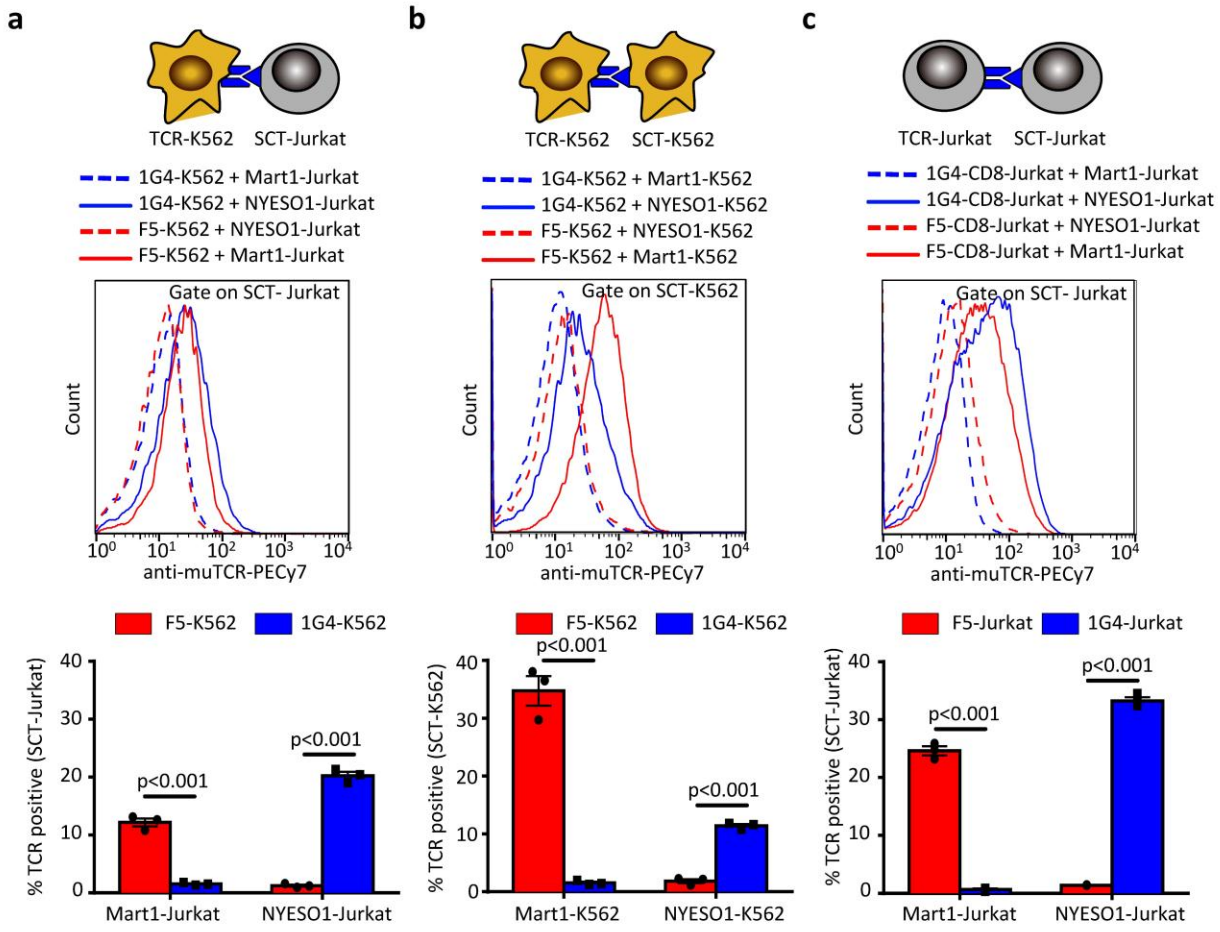
(**a)** Antigen-specific transfer of the T cell membrane proteins, CD3 and CD8, from CD8-expressing F5-Jurkat and 1G4-Jurkat cells to K562 cells ($ZsGreen^{+}$), as assessed by anti-CD3 and anti-CD8 antibodies (5:1 J:K). (**b**) Antigen-specific transfer of the K562 cell membrane protein, HLA-A2, from K562 cells to Jurkat cells ($ZsGreen^{-}$), as assessed by an anti-HLA-A2 antibody (5:1 J:K). **(c, d)** Concomitant reduction of membrane proteins from donor cells. Data are representative of three independent experiments.



Supplementary Figure 4

Trogocytosis occurs among various TCR and pMHC allele pairs.

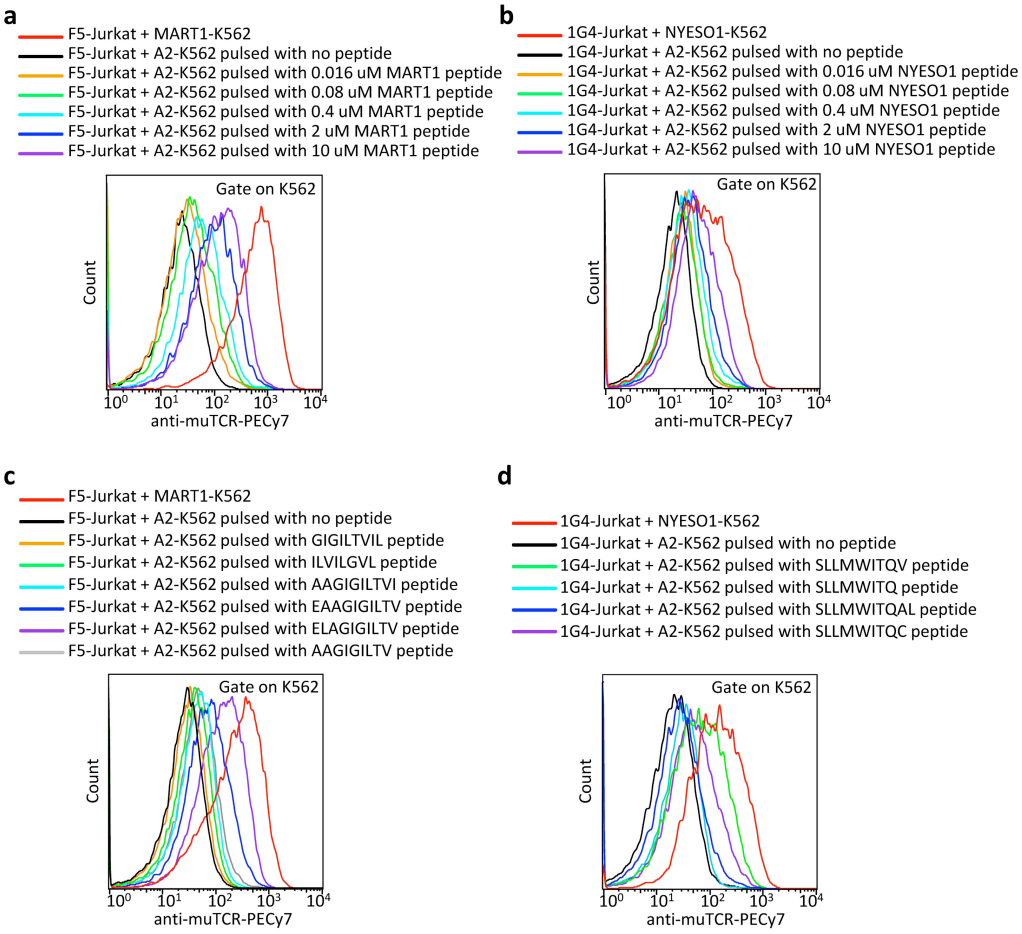
(**a**) Antigen-specific trogocytosis between Jurkat expressing B07-, B18- or C03-restricted NY-ESO-1-specific TCRs and K562 expressing their cognate antigens (10:1 J:K). (**b**) Comparison of trogocytosis capability 1G4-Jurkat cells or Jurkat cell expressing four other novel A2-restricted NYESO-specific TCRs with NYESO1-K562 cells (10:1 J:K). (**c**) Comparison of trogocytosis capability F5-Jurkat cells or Jurkat cell expressing low-affinity M1W-TCR with MART1-K562 cells (5:1 J:K). Data are presented as mean ± s.e.m. and are representative of two independent experiments.



Supplementary Figure 5

Antigen-specific TCR transfer occurs from donor cells to acceptor cells regardless of cell identity.

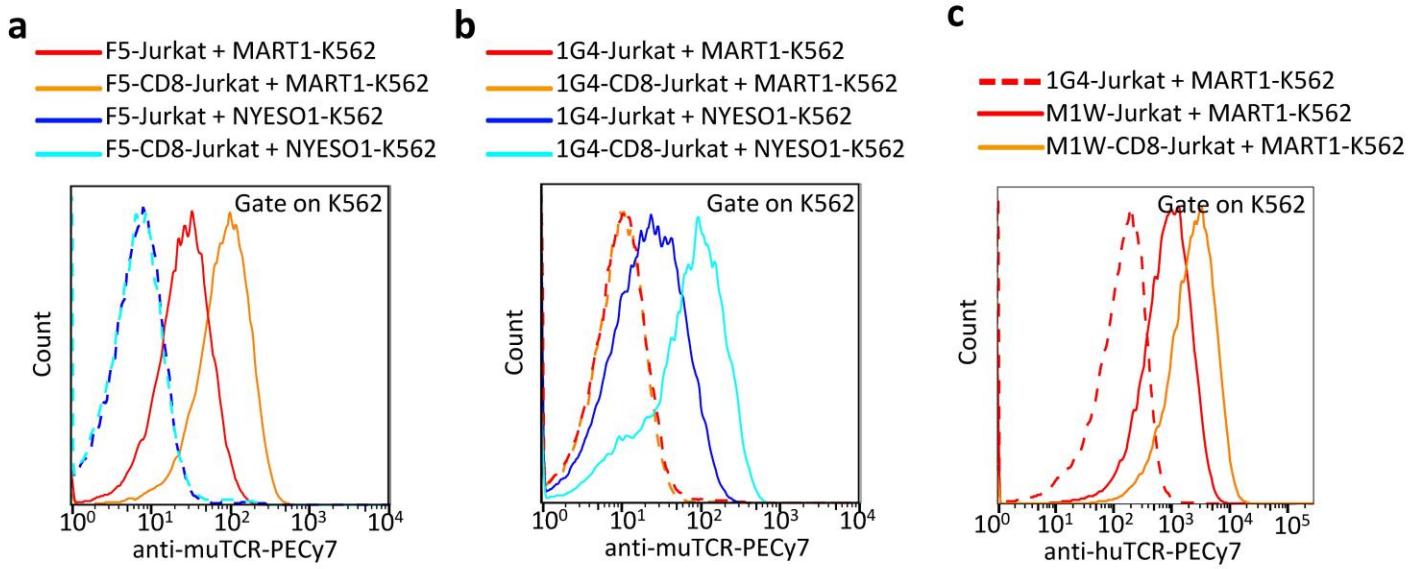
(**a)** Antigen-specific transfer of TCR after co-incubation of a 5:1 ratio of F5 or 1G4 TCR-K562 cells and MART1 or NYESO1 SCT-Jurkat cells (ZsGreen^{+}) as assessed by an anti-muTCR antibody. **(b**) Antigen-specific transfer of TCR after same-cell-type co-incubation of a 5:1 ratio of F5 or 1G4 TCR-K562 and MART1 or NYESO1 SCT-K562 (ZsGreen^{+}) as assessed by an anti-muTCR antibody. **(c)** Antigen-specific transfer of TCR after co-incubation of a 1:1 ratio of F5 or 1G4 TCR-Jurkat and MART1 or NYESO1 SCT-Jurkat (ZsGreen^{+}) as assessed by an anti-muTCR antibody. Mean and s.e.m. for each group is shown (n = 3). Data are representative of two independent experiments.



Supplementary Figure 6

Histographic visualization of trogocytosis capability based on peptide dosing and variants.

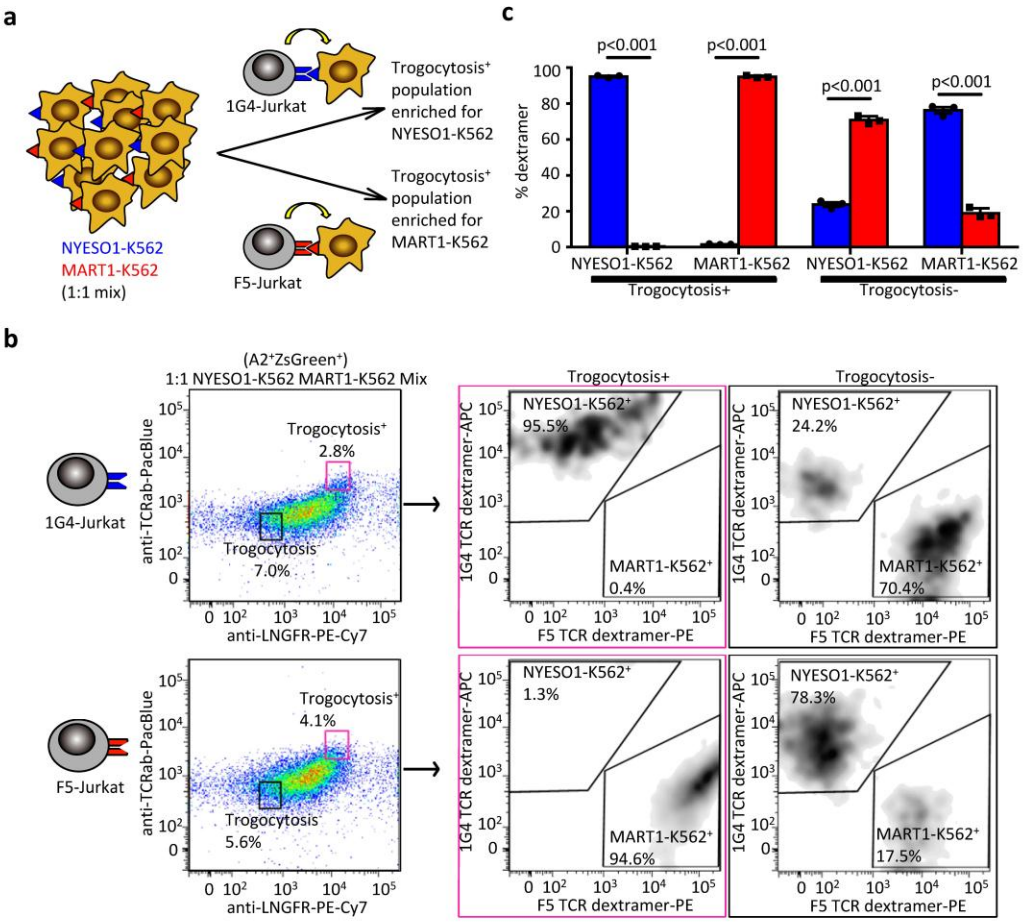
(a) Comparison of trogocytosis capability of murinized F5-Jurkat cells with A2-K562 cells loaded with different doses of cognate MART1 heteroclitic peptide (ELAGIGILTV). **(b)** Comparison of trogocytosis capability of murinized 1G4-Jurkat cells with A2-K562 cells loaded with different doses of cognate NYESO1 heteroclitic peptide (SLLMWITQV). **(c)** Comparison of trogocytosis capability of murinized F5-Jurkat cells with A2-K562 cells loaded with same dose of different MART1 peptide variants. **(d)** Comparison of trogocytosis capability of murinized 1G4-Jurkat cells with A2-K562 cells loaded with same dose of different NYESO1 peptide. Data are representative of two independent experiments.



Supplementary Figure 7

Target cell trogocytosis is enhanced by coexpression of CD8.

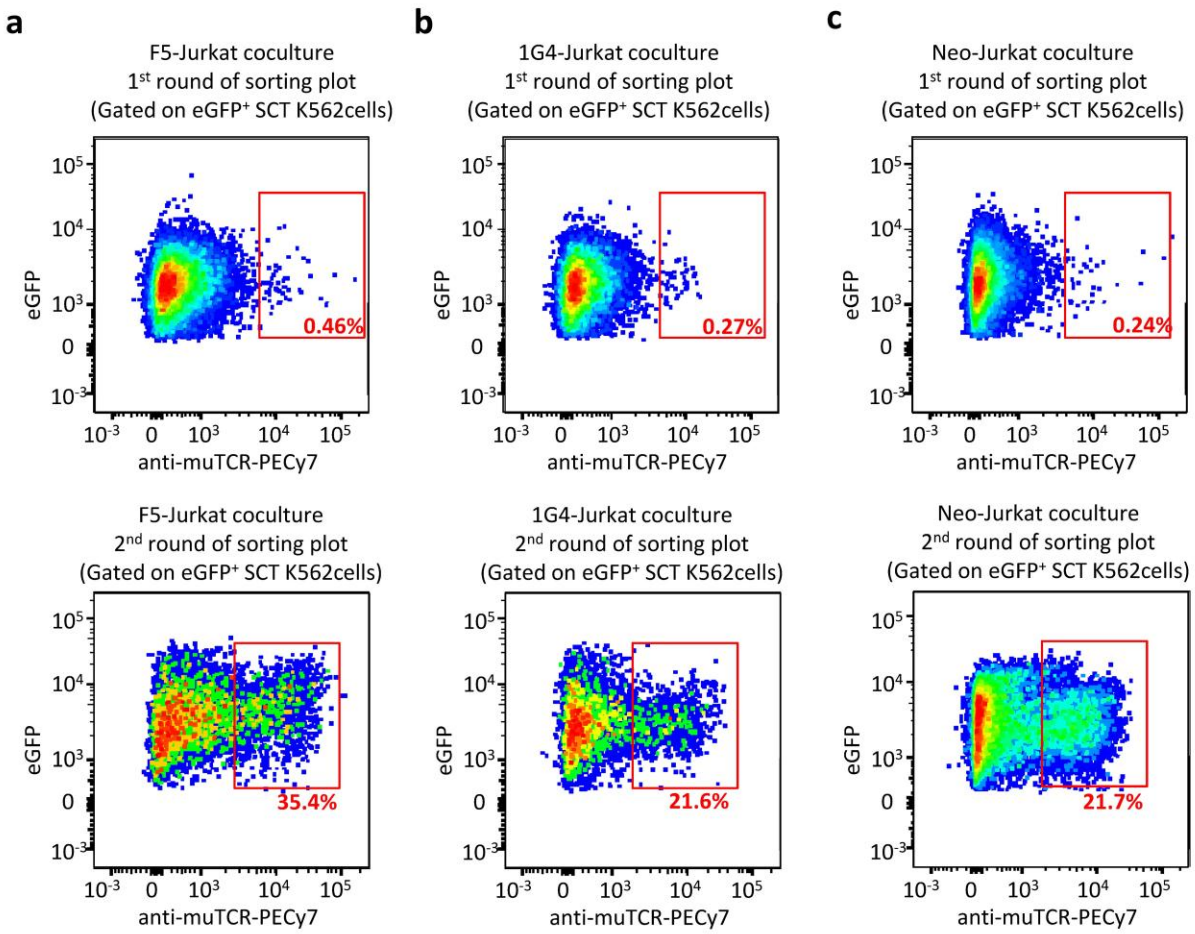
(a) Comparison of trogocytosis capability of $CD8^+$ or $CD8^-$ murinized F5-Jurkat cells with MART1-K562 or NYESO1-K562 cells. **(b)** Comparison of trogocytosis capability of $CD8^+$ or $CD8^-$ murinized 1G4-Jurkat cells with MART1-K562 or NYESO1-K562 cells. (**c**) Comparison of trogocytosis capability of $CD8^+$ or $CD8^-$ M1W-Jurkat cells with MART1-K562 cells. Data are representative of two independent experiments.



Supplementary Figure 8

Target cell trogocytosis resolves cognate antigen-expressing target cells from non-cognate antigen-expressing cells.

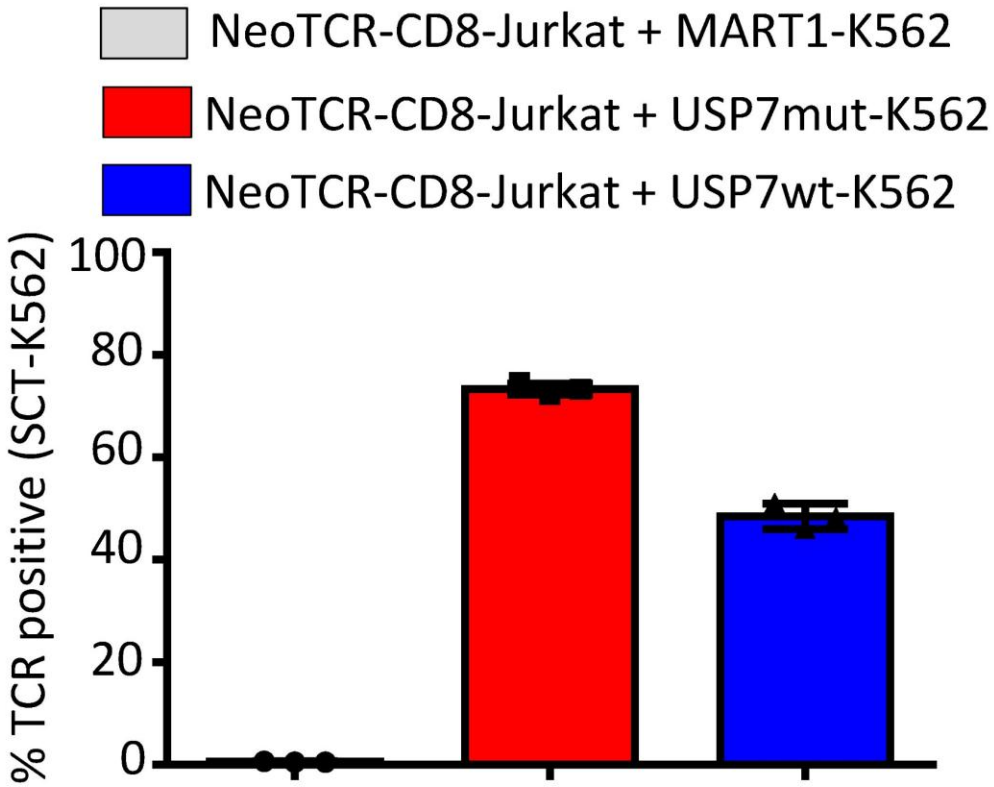
(**a**) Schematic of experiment. (**b**) Representative flow cytometry plots for a 1:1 mixture of NYESO1-K562 and MART1-K562 cells co-incubated with either 1G4-Jurkat (top) or F5-Jurkat (bottom) cells (10:1:1 Jurkat: NYESO1-K562:MART1-K562). The trogocytosis$^+$ and trogocytosis$^-$ populations were verified for antigen specificity by use of either F5 TCR or 1G4 TCR dextramer staining. Data are representative of two independent experiments. (**c**) Quantification of antigen-specific target cell in trogocytosis$^+$ and trogocytosis$^-$ populations via F5 TCR or 1G4 TCR dextramer staining ($n = 3$). Statistical analysis of quantification was performed using unpaired two-tailed Student's *t*-test. Data in **b, c** are presented as mean ± s.e.m. and are representative of two independent experiments.



Supplementary Figure 9

Flow cytometry plot for the first- and second-round sortings from co-incubation.

(**a,b,c**) F5-Jurkat, 1G4-Jurkat or neoTCR-Jurkat cells (E2-Crimson^{+}CD8^{+}) were co-incubated with K562 library cells and trogocytosis^{+} A2-SCT-K562 cells (E2-Crimson^{-}eGFP^{+}TCR^{+}) in red box were gated for sorting using FACS. Data are representative of two or three independent experiments.



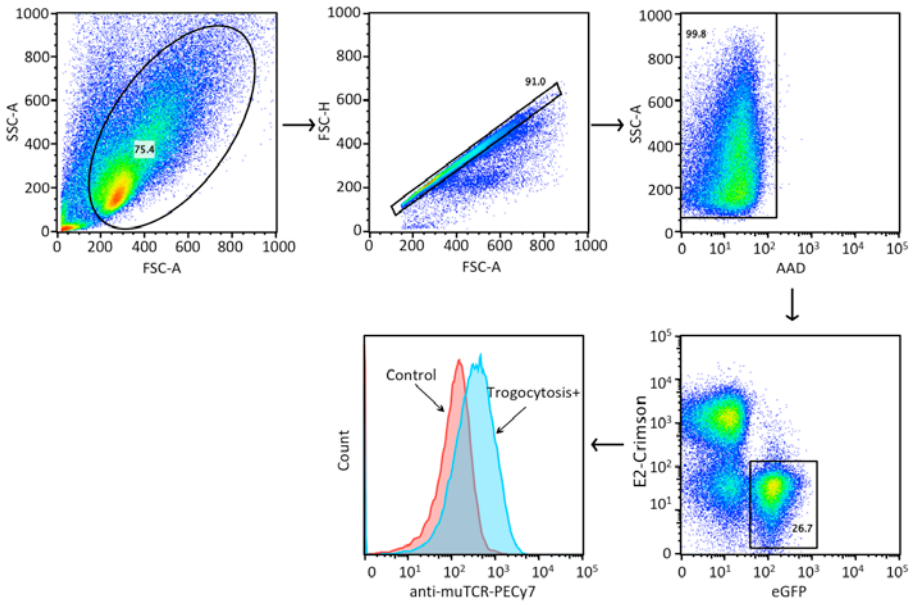
Supplementary Figure 10

Comparison of trogocytosis capacity of NeoTCR-CD8-Jurkat cells with MART1-K562, USP7mut-K562 or USP7wt-K562 cells.

The predicted affinity of mutUSP7 and wtUSP7 for the HLA-A2 binding groove by NetMHC are 33 nM and 7 µM, respectively. Data are presented as mean ± s.e.m. (n = 3) and are representative of two independent experiments.

SUPPLEMENTARY INFORMATION

Flow cytometry gating strategies.



Flow cytometric analysis of sorted trogocytosis$^+$ SCT-K562 cells following a validating co-incubation with F5-Jurkat or 1G4-Jurkat (2:1 J:K). The cells were first gated on FSC/SSC, followed by the exclusion of doublet events and dead cells (AAD$^+$). eGFP$^+$ K562 cells were then further gated for analysis of the transferred TCR level on the surface. These gating strategies were used for all library screening (n=3).