**SUPPLEMENTARY INFORMATION**

**Functional TCR Retrieval From Single Antigen-Specific Human T Cells Reveals**

**Multiple Novel Epitopes**

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| **Supplementary Item and Number** | **Title** |
| Supplementary Table S1 | Primers used in this study |
| Supplementary Table S2 A/B | Coverage of TCR-α (A) and TCR-β (B) variable genes with clonotype-specific primers |
| Supplementary Table S3 | Characteristics of cloned TCRs |
| Supplementary Figure S4 | Flow cytometric sorting ofpp65-specific CD8+ T cells from CMV-seropositive donor ID3 after one week of expansion |
| Supplementary Table S5 | Synthetic peptides used in this study |
| Supplementary Figure S6 | Functionality of TCRCD8-NY#5 in CD4+ and CD8+ T cells |
| Supplementary Figure S7 | Functionality of NY-ESO-1-specific CD4-TCRs in CD8+ T cells |
| Supplementary Table S8 | HLA haplotypes from healthy donors and NSCLC patients |
| Supplementary Methods | Detailed materials and methods |

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|  | 5'-3' nucleotide sequence | Restriction site | TAa  [°C] |
| 1. **Primers for SMART-based cDNA synthesis and amplification** | | | |
| dT-Primer long | AAGCAGTGGTATCAACGCAGAGTACT30VN | RsaI | - |
| TS-short | AAGCAGTGGTATCAACGCAGAGTACGCrGrGrG | RsaI | - |
| TS-PCR | AAGCAGTGGTATCAACGCAGAGT | - | 65 |
| 1. **Primers for control of the SMART-Process** | | | |
| TCRbC\_ctr\_s | AGGCCTGGGGTAGAGCAGACTG | - | 64 |
| TCRbC\_ctr\_as | GYAATCCTTTCTCTTGACCATGGC | - |
| 1. **TRAV clonotype-specific primers** | | | |
| A1 | TATGCGGCCGCCACCATGTGGGGAGYTTTCCTTCTYTATG | NotI | 64 |
| A3 | TATGCGGCCGCCACCATGGCTTTGCAGAGCACTCTGGGGGC | NotI | 68 |
| A4 | TATGCGGCCGCCACCATGGCCTCTGCACCCATCTCGATGC | NotI | 68 |
| A5 | TATGCGGCCGCCACCATGAGGCAAGTGGCGAGAGTGATCGTG | NotI | 64 |
| A6 | TATGCGGCCGCCACCATGAAGACATTTGCTGGATTTTCGTTC | NotI | 66 |
| A7 | TATGCGGCCGCCACCATGGAGTCATTCCTGGGAGGTGTTTTG | NotI | 60 |
| A9 | TATGCGGCCGCCACCATGCTCCTGTTGCTCATACCAGTGC | NotI | 68 |
| A10 | TATGCGGCCGCCACCATGCTCCTGCTGCTCGTCCCAGYGYTC | NotI | 68 |
| A11 | TATGCGGCCGCCACCATGCTCCTGGWGCTYATCCCACTGCTG | NotI | 68 |
| A17 | TATGCGGCCGCCACCATGAACTATTCTCCAGGCTTAGTATC | NotI | 60 |
| A18 | TATGCGGCCGCCACCATGAAAAAGCATCTGACGACCTTCTTG | NotI | 64 |
| A20 | TATGCGGCCGCCACCATGAWATCCTTGAGAGTTTTACTRGTG | NotI | 64 |
| A22 | TATGCGGCCGCCACCATGATGAAATCCTTGAGAGTTTTACTG | NotI | 64 |
| A23 | TATGCGGCCGCCACCATGACATCCATTCGAGCTGTATTTATATTC | NotI | 62 |
| A24 | TATGCGGCCGCCACCATGGCAGGCATTCGAGCTTTATTTATG | NotI | 64 |
| A25 | TATGCGGCCGCCACCATGTCACTTTCTAGCCTGCTGAAGG | NotI | 66 |
| A27 | TATGCGGCCGCCACCATGAAGCCCACCCTCATCTCAGTGC | NotI | 66 |
| A28 | TATGCGGCCGCCACCATGGAAACTCTCCTGGGAGTGTCTTTG | NotI | 66 |
| A30 | TATGCGGCCGCCACCATGCTGACTGCCAGCCTGTTGAGGGC | NotI | 68 |
| A31 | TATGCGGCCGCCACCATGGAGAAAATGTTGGAGTGTGCATTC | NotI | 62 |
| A32 | TATGCGGCCGCCACCATGGAGACCCTCTTGGGCCTGCTTATCC | NotI | 64 |
| A33 | TATGCGGCCGCCACCATGAAGAGGATATTGGGAGCTCTGCTG | NotI | 66 |
| A34 | TATGCGGCCGCCACCATGGACAAGATCTTAGGAGCATC | NotI | 64 |
| A35 | TATGCGGCCGCCACCATGGAGAAGAATCCTTTGGCAGCCCCATTAC | NotI | 64 |
| A36 | TATGCGGCCGCCACCATGCTACTCATCACATCAATGTTGGTC | NotI | 64 |
| A38 | TATGCGGCCGCCACCATGAAGTTGGTGACAAGCATTACTG | NotI | 68 |
| A39 | TATGCGGCCGCCACCATGGTCCTGAAATTCTCCGTGTCCATTC | NotI | 64 |
| A41 | TATGCGGCCGCCACCATGGCCATGCTCCTGGGGGCATCAGTG | NotI | 64 |
| A46 | TATGCGGCCGCCACCATGGAGACTGTTCTGCAAGTACTCCTAGG | NotI | 60 |
| A47 | TATGCGGCCGCCACCATGCTCCTTGAACATTTATTAATAATC | NotI | 60 |
| A48 | TATGCGGCCGCCACCATGATGAAGTGTCCACAGGCTTTACTAGC | NotI | 60 |
| A50 | TATGCGGCCGCCACCATGACACGAGTTAGCTTGCTGTGGGC | NotI | 68 |
| A51 | TATGCGGCCGCCACCATGGCATGCCCTGGCTTCCTGTGGGC | NotI | 66 |
| A52 | TATGCGGCCGCCACCATGAAGAAGCTACTAGCAATGATTCTGTGG | NotI | 60 |
| A53 | TATGCGGCCGCCACCATGAACTCCTCTCTGGACTTTCTAATTCTG | NotI | 60 |
| TRACex1\_as | [Phos]TTAGAGTCTCTCAGCTGGTACACGGCAG | - | - |

**Supplementary Table S1.** Primers used in this study**.**

aTA: Annealing temperature

**Supplementary Table S1 (continued).** Primers used in this study

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| 1. **TRBV clonotype-specific primers** | | | | |
| Primer | 5'-3' nucleotide sequence | | Restriction site | TAa  [°C] |
| B2 | TATGCGGCCGCCACCATGGATAYCTGGCTCSTMTGCTGGG | | NotI | 66 |
| B5 | TATGCGGCCGCCACCATGGGCTGCAGGCTGCTCTGCTGTGCG | | NotI | 68 |
| B8 | TATGCGGCCGCCACCATGGGCTCCAGGCTGCTCTGTTGG | | NotI | 66 |
| B14 | TATGCGGCCGCCACCATGGGCCCYGGGCTCCTCTGCTGGG | | NotI | 68 |
| B15 | TATGCGGCCGCCACCATGGGACCCAGGCTCCTCTTCTGGGC | | NotI | 66 |
| B20 | TATGCGGCCGCCACCATGAGMATCRGSCTCCTGTGCTGTG | | NotI | 66 |
| B23 | TATGCGGCCGCCACCATGAGCCTCGGGCTCCTGTGCTGTG | | NotI | 66 |
| B28 | TATGCGGCCGCCACCATGGGCACMAGGCTCCTCTKCTGGG | | NotI | 66 |
| B30 | TATGCGGCCGCCACCATGGGYACCAGTCTCCTATGCTGGGTG | | NotI | 68 |
| B33 | TATGCGGCCGCCACCATGRGCACCAGSCTYCTCTGCTGGATG | | NotI | 68 |
| B36 | TATGCGGCCGCCACCATGGGCTKCAGGCTCCTCTGCTRTGTG | | NotI | 68 |
| B38 | TATGCGGCCGCCACCATGGGCACMAGGSTSTTCTTCTATG | | NotI | 66 |
| B42 | TATGCGGCCGCCACCATGGSYACCAGGCTCCTCTGCTGKGTG | | NotI | 66 |
| B45 | TATGCGGCCGCCACCATGGACTCCTGGACCTTCTGCTGTG | | NotI | 66 |
| B46 | TATGCGGCCGCCACCATGGACTCCTGGACCCTCTGCTGTG | | NotI | 66 |
| B48 | TATGCGGCCGCCACCATGCTTAGTCCTGACCTGCCTGAC | | NotI | 66 |
| B49 | TATGCGGCCGCCACCATGGTTTCCAGGCTTCTCAGTTTAG | | NotI | 66 |
| B50 | TATGCGGCCGCCACCATGGGTCCTGGGCTTCTCCACTGG | | NotI | 66 |
| B51 | TATGCGGCCGCCACCATGAGCCCAATATTCACCTGCATC | | NotI | 62 |
| B53 | TATGCGGCCGCCACCATGGACACCAGAGTACTCTGCTGTGC | | NotI | 64 |
| B54 | TATGCGGCCGCCACCATGAGCAACCAGGTGCTCTGCTGTG | | NotI | 66 |
| B55 | TATGCGGCCGCCACCATGCTGCTGCTTCTGCTGCTTCTG | | NotI | 66 |
| B59 | TATGCGGCCGCCACCATGGCCTCCCTGCTCTTCTTCTGTGG | | NotI | 66 |
| B60 | TATGCGGCCGCCACCATGACTATCAGGCTCCTCTGCTACATGG | | NotI | 66 |
| B62 | TATGCGGCCGCCACCATGGGCCCCCAGCTCCTTGGCTATG | | NotI | 66 |
| B63 | TATGCGGCCGCCACCATGGGAATCAGGCTCCTCTGTCGTG | | NotI | 66 |
| B64 | TATGCGGCCGCCACCATGCTGAGTCTTCTGCTCCTTCTCC | | NotI | 66 |
| TRBCex1\_as | [Phos]GGCTCAAACACAGCGACCTCGGGTG | | - |  |
| 1. **Primers for the construction of pST1-vectors** | | | | |
| Primer | | 5'-3' nucleotide sequence | Restriction  site | |
| αC blunt\_s | | TATGGATCCGATATCCAGTGACAACTCTGTC | BamHI; EcoRV | |
| βC1\_2\_blunt\_s | | TATGGATCCGATATCGGTGTGCCTGGCCAC | BamHI; EcoRV | |
| αC\_AsiSI\_as | | TATGGATCCGCGATCGCCTCAGCTGGACCACAGCC | BamHI; AsiSI | |
| β1C\_stop\_AsiSI\_as | | TATGGATCCGCGATCGCTCAGAAATCCTTTCTCTTGAC | BamHI; AsiSI | |
| β2C\_stop\_AsiSI\_as | | TATGGATCCGCGATCGCTCAGCCTCTGGAATCCTTTCTC | BamHI; AsiSI | |
| B2M\_HindIII-Kozak-s | | TATAAGCTTGCCACCATGTCTCGCTCCGTGGCCTTAG | HindIII | |
| B2M-BamHI-as | | TATGGATCCTTACATGTCTCGATCCCACTTAAC | BamHI | |

aTA: Annealing temperature

**Supplementary Table S1 (continued).** Primers used in this study

|  |  |  |
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| 1. **Primers for human HLA class I and II molecules** | | |
| Primer | 5'-3' nucleotide sequence | Restriction  site |
| HLA-A\_s | [Phos]GCCACCATGGCCRTCATGSCKCCCCGAAC | - |
| HLA-B\_s\_1 | [Phos]GCCACCATGCTGGTCATGGCGCCCCGAAC | - |
| HLA-B\_s\_2 | [Phos]GCCACCATGCGGGTCACGGCGCCCCGAAC | - |
| HLA-B\_s\_3 | [Phos]GCCACCATGCGGGTCACGGAGCCCCGAAC | - |
| HLA-C\_s\_1 | [Phos]GCCACCATGCGGGTCATGGCGCCCCGAAC | - |
| HLA-C\_s\_2 | [Phos]GCCACCATGCGGGTCATGGCGCCCCGAGC | - |
| HLA-C\_s\_3 | [Phos]GCCACCATGCGGGTCATGGCGCCCCAAGC | - |
| HLA-DPA1\_s | [Phos]GCCACCATGCGCCCTGAAGACAGAATGTTCC | - |
| HLA-DPB1\_s | [Phos]GCCACCATGATGGTTCTGCAGGTTTCTGC | - |
| HLA-DQA1\_s | [Phos]GCCACCATGATCCTAAACAAAGCTMTGCTG | - |
| HLA-DQB1\_s | [Phos]GCCACCATGTCTTGGAARAAGKCTTTGCGGATC | - |
| HLA-DRA\_s | [Phos]GCCACCATGGCCATAAGTGGAGTCCCTGTG | - |
| HLA-DRB\_s\_1 | [Phos]GCCACCATGGTGTGTCTGARGYTCCCTGG | - |
| HLA-DRB\_s\_2 | [Phos]GCCACCATGGTGTGTCTSARGCYSCCTGG | - |
| HLA-A\_as | TATTATGCGATCGCTCACACTTTACAAGCTGTGAGRGAC | AsiSI |
| HLA-B\_as | TATTATGCGATCGCTCAAGCTGTGAGAGACACATCAGAGC | AsiSI |
| HLA-C\_as\_1 | TATTATGCGATCGCTCAGGCTTTACAAGCGATGAGAGACTC | AsiSI |
| HLA-C\_as\_2 | TATTATGCGATCGCTCAGGCTTTACAAGTGATGAGAGACTC | AsiSI |
| HLA-DPA1\_as\_1 | TATTATGCGATCGCTCACAGGGTCCCCTGGGC | AsiSI |
| HLA-DPA1\_as\_2 | TATTATGCGATCGCTCACAGGGGCCCCTGGGC | AsiSI |
| HLA-DPB1\_as | TATTATGCGATCGCTTATGCAGATCCTCGTTGAACTTTC | AsiSI |
| HLA-DQA1\_as | TATTATGCGATCGCTCACAAKGGCCCYTGGTGTCTG | AsiSI |
| HLA-DQB1\_as\_1 | TATTATGCGATCGCTCAGTGCAGAAGCCCTTTCCGAC | AsiSI |
| HLA-DQB1\_as\_2 | TATTATGCGATCGCTCAGTGCAGRAGCCCTTTCTGAC | AsiSI |
| HLA-DQB1\_as\_3\* | TATTATGCGATCGCTCAGTGCAGAAGCCCTGCTGGTG | AsiSI |
| HLA-DRA\_as | TATTATGCGATCGCTTACAGAGGCCCCCTGCGTTCTG | AsiSI |
| HLA-DRB\_as\_1 | TATTATGCGATCGCTCAGCTCAGGAATCCTGTTGG | AsiSI |
| HLA-DRB\_as\_2 | TATTATGCGATCGCTCAGCTCAGGAATCCTCTTGG | AsiSI |
| HLA-DRB\_as\_3 | TATTATGCGATCGCTCAGCTCAGCAGTCCTTTTGG | AsiSI |
| HLA-DRB\_as\_4 | TATTATGCGATCGCTCAGCTCAAGAGTCCTGTTGG | AsiSI |
| HLA-DRB\_as\_5 | TATTATGCGATCGCTCAGCTCACGAGTCCTGTTGG | AsiSI |
| HLA-DRB\_as\_6 | TATTATGCGATCGCTCAGCTCAGGAGTCCTGTTGG | AsiSI |
| HLA-DRB\_as\_7 | TATTATGCGATCGCTCAGTTCAGGAGTCCTGTTGG | AsiSI |
| HLA-DRB3\_in\_s | GAAGCTCCCTGGAGGCTCCAGCTTGG | - |

**Supplementary Table S2 A.** Coverage of TCR-α variable genes with clonotype-specific primers.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
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| TRAV genes | | A1 | A2 | A3 | A4 | A5 | A6 | - | A7 | A8 | A9 | - | A10 | A11 | A12 | A13 | A14 | A15 | A16 | A17 | A18 | - | A19 | A20 | A21 | A22 | A23 | A24 | A25 | - | A26 | A27 | A28 | - | A29 | A30 | A31 | A32 | A33 | A34 | A35 | - |
| F | 1.1 | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 1.2 | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 2 |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F/P | 3 |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 4 |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 5 |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 6 |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 7 |  |  |  |  |  |  | - |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 8.1 |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 8.2 |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 8.3 |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 8.4 |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P | 8.5 |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 8.6 |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| ORF | 8.7 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 9.1 |  |  |  |  |  |  |  |  |  |  | - |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 9.2 |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 10 |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P | 11 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 12.1 |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 12.2 |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 12.3 |  |  |  |  |  |  |  |  |  |  |  |  |  | ● | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 13.1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 13.2 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 14/DV4 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P | 15 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 16 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 17 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 18 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | - |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 19 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 20 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 21 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 22 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 23/DV6 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 24 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 25 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 26.1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | - |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 26.2 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |
| F | 27 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |
| P | 28 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F/P | 29/DV5 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |
| F | 30 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | - |  |  |  |  |  |  |  |  |
| P | 31 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P | 32 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P | 33 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 34 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ○ |  |  |  |  |  |  |  |
| F | 35 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |
| F | 36 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |
| P | 37 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 38.1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |
| F | 38.2/DV8 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |
| F | 39 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |
| F | 40 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ○ |  |
| F | 41 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | - |

Rows represent TCR-α (TRAV) variable gene segments (IMGT nomenclature). F: functional gene; P: pseudogene; ORF: open reading frame. Columns list codes of clonotype-specific primers. As primers are partially degenerated with a maximum wobble of 3 bases and under the assumption that 100% complementarity of the oligonucleotide to a given TCR sequence results in an amplicon, the potential repertoire of up to 45 functional TRAV genes is covered by 35 primers. Closed circles: successful amplification of indicated V gene; open circles: V gene not discovered to date; hyphen: clonotype-specific primer for amplification of indicated V gene could not be established.

**Supplementary Table S2 B.** Coverage of TCR-β variable genes with clonotype-specific primers.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| TRBV genes | | B1 | B2 | B3 | B4 | B5 | B6 | B7 | B8 | B9 | B10 | B11 | B12 | B13 | B14 | B15 | B16 | B17 | B18 | B19 | B20 | B21 | B22 | B23 | B24 | B25 | B26 | B27 | - |
| P | 1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 2 | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 3.1 |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P | 3.2 |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 4.1 |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 4.2 |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 4.3 |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 5.1 |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P | 5.2 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| ORF | 5.3 |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 5.4 |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 5.5 |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 5.6 |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| ORF | 5.7 |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 5.8 |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 6.1 |  |  |  |  |  | ● | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F/P | 6.2 |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 6.3 |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 6.4 |  |  |  |  |  | ○ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 6.5 |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 6.6 |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| ORF | 6.7 |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 6.8 |  |  |  |  |  |  | ○ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 6.9 |  |  |  |  |  | ○ | ○ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| ORF | 7.1 |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 7.2 |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F/ORF | 7.3 |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F/P | 7.4 |  |  |  |  |  |  |  | ○ |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P | 7.5 |  |  |  |  |  |  |  | ● |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 7.6 |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 7.7 |  |  |  |  |  |  |  |  | ○ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 7.8 |  |  |  |  |  |  |  | ● |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 7.9 |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P | 8.1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P | 8.2 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 9 |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F/P | 10.1 |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 10.2 |  |  |  |  |  |  |  |  |  |  |  | ○ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 10.3 |  |  |  |  |  |  |  |  |  |  |  | ○ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 11.1 |  |  |  |  |  |  |  |  |  | ○ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 11.2 |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 11.3 |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P | 12.1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P | 12.2 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 12.3 |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 12.4 |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 12.5 |  |  |  |  |  |  |  |  |  |  |  |  | ○ |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 13 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 14 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |  |
| F | 15 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |  |  |
| F/P | 16 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ○ |  |  |  |  |  |  |  |  |  |
| ORF | 17 | ● |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 18 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |  |
| F | 19 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |  |
| F | 20.1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |  |
| P | 21.1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P | 22.1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| ORF | 23.1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 24.1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |  |
| F | 25.1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |  |
| P | 26 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| F | 27 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |  |
| F | 28 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |  |
| F | 29.1 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | ● |  |
| F/P | 30 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | - |
| P | A |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| P | B |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

Rows represent TCR-β (TRBV) variable gene segments (IMGT nomenclature). F: functional gene; P: pseudogene; ORF: open reading frame. Columns list codes of clonotype-specific primers. As primers are partially degenerated with a maximum wobble of 3 bases and under the assumption that 100% complementarity of the oligonucleotide to a given TCR sequence results in an amplicon, the potential repertoire of up to 48 functional TRBV genes is covered by 27 primers. Closed circles: successful amplification of indicated V gene; open circles: V gene not discovered to date; hyphen: clonotype-specific primer for amplification of indicated V gene could not be established.

**Supplementary Table S3.** Characteristics of cloned TCRs.

|  |  |  |  |
| --- | --- | --- | --- |
| **TCR** | **TCR-α chaina** | **TCR-β chaina** | **HLA restriction** |
| TCRCD8-CMV#1 | V1.2 J24\_2 C | V3.1 D2 J2.1 C2 | B\*3501 |
| TCRCD8-CMV#4 | V3 J43 C | V6.5 D1 J1.2 C1 | A\*0201 |
| TCRCD8-CMV#8 | V22 J58 C | V10.1 D J1.4 C1 | A\*0201 |
| TCRCD8-CMV#9 | V19 J26 C | V13 D2 J2.1 C2 | not done |
| TCRCD8-CMV#10 | V24 J49 C | V6.5 D1 J1.2 C1 | A\*0201 |
| TCRCD8-CMV#11 | V16 J36 C | V25.1 D1 J2.2 C2 | A\*0201 |
| TCRCD8-CMV#12 | V39 J58 C | V9 D2 J2.2 C2 | A\*0201 |
| TCRCD8-CMV#14 | V24 J21 C | V3.1 D2 J2.2 C2 | A\*0201 |
| TCRCD8-CMV#15 | V12.3 J43 C | V12.4 D1 J1.4 C1 | A\*0201 |
| TCRCD8-CMV#16 | V13.1\_2 J50 C | V25.1 J1.3 C1 | A\*0201 |
| TCRCD4-CMV#1 | V21 J43 C | V3.1 D1 J1.1 C1 | DRB1\*0701 |
| TCRCD4-CMV#3 | V8.6\_2 J37\_2 C | V6.1 D1 J1.2 C1 | DRB1\*0701 |
| TCRCD4-CMV#5 | V22 J49 C | V6.2 D2 J2.3 C2 | DRB1\*0701 |
| TCRCD8-NY#2 | V3 J28 C | V20.1\_2 J2.3 C2 | A\*6801 |
| TCRCD8-NY#5 | V24 J3 C | V7.6 D2 J2.2 C2 | B\*3508 |
| TCRCD8-NY#6 | V17 J47\_2 C | V12.3 D2 J2.1 C2 | B\*3508 |
| TCRCD8-NY#8 | V8.6\_2 J9 C | V28.1 D1 J1.1 C1 | B\*3508 |
| TCRCD8-NY#12 | V1.1 J23 C | V4.1 D2 J2.1 C2 | B\*0702 |
| TCRCD8-NY#13 | V5 J33 C | V5.5\_2 D1 J2.5 C2 | A\*6801 |
| TCRCD8-NY#15 | V12.2\_2 J53 C | V4.1 D2 J2.5 C2 | B\*3508 |
| TCRCD4-NY#1 | V22 J20 C | V9 D1 J1.1 C1 | DRB1\*0401 |
| TCRCD4-NY#3 | V12.3 J54 C | V11.2 D2 J2.2 C2 | DRB1\*0401 |
| TCRCD4-NY#5 | V8.4\_3 J48 C | V4.1 D1 J1.5 C1 | DRB1\*1101 |
| TCRCD4-NY#7b | V8.6\_2 J13\_2 C | V20.1 D2 J2.5 C2 | DRB1\*1101  DRB1\*1601 |
| TCRCD4-NY#10 | V9.2\_3 J42 C | V7.9\_3 D2 J2.7 C2 | DRB5\*0202 |
| TCRCD4-NY#11 | V8.1 J23 C | V11.2 D1 J1.2 C1 | DRB1\*1101 |
| TCRCD4-NY#13 | V21\_2 J24\_2 C | V7.9\_3 D1 J2.3 C2 | DRB5\*0202 |
| TCRCD4-NY#14b | V8.4\_3 J37\_2 C | V3.1 D2 J1.3 C1 | DRB3\*0201/02 |
| TCRCD4-NY#16 | V8.4\_3 J10 C | V20.1 D1 J1.5 C1 | DRB3\*0201 |
| TCRCD8-TPT#35 | V19 J17 C | V6.2/V6.3 D1 J1.2 C1c | B\*0702 |
| TCRCD4-TPT#4 | V14/DV4 J48 C | V29.1 D1 J1.2 C1 | DRB4\*0101 |
| TCRCD4-TPT#5 | V38.2/DV8 J40 C | V4.2 D2 J2.7 C2 | DRB1\*1401 |
| TCRCD4-TPT#6 | V12.3 J35 C | V5.4 D1 J1.3 C1 | DRB1\*1401 |
| TCRCD4-TPT#8b | V38.1 J45 C | V3.1 D1 J2.7 C2 | DRB3\*0201/0202 |
| TCRCD4-TPT#11 | V17 J27 C | V6.6\_2 D1 J2.3 C2 | DRB1\*0701 |
| TCRCD4-TPT#13 | V20\_2 J29 C | V19 D2 J2.1 C2 | DRB1\*1401 |
| TCRCD4-TPT#17 | V29/DV5 J49 C | V7.2 D1 J2.7 C2 | DRB5\*0202 |
| TCRCD4-TPT#27 | V13.1\_2 J45 C | V19 D1 J1.1 C1 | DRB3\*0301 |
| TCRCD4-TPT#33 | V29/DV5 J42 C | V24.1 D2 J2.1 C2 | DRB5\*0202 |
| TCRCD4-TPT#38 | V39 J18 C | V5.5\_2 D1 J1.4 C1 | DRB1\*1601 |
| TCRCD4-TPT#42 | V25 J10 C | V7.8 D2 J2.7 C2 | DRB1\*1301 |
| TCRCD4-TPT#45 | V13.2 J23 C | V20.1 D1 J1.2 C1 | DRB1\*1501 |
| TCRCD4-TPT#48 | V8.3 J43 C | V28 D1 J1.1 C1 | DRB1\*1501 |
| TCRCD4-TPT#49 | V38.1 J49 C | V19 D2 J2.2 C2 | DRB1\*1501 |
| TCRCD4-TPT#51 | V13.1\_2 J53 C | V14 D1 J1.1 C1 | DRB1\*1301 |
| TCRCD4-TPT#52 | V8.3 J54 C | V6.1 D2 J2.7 C2 | DRB1\*1501 |
| TCRCD4-TPT#55 | V38.2/DV8 J34 C | V5.1 J2.1 C2 | DRB1\*1301 |
| TCRCD4-TPT#57 | V8.1 J27 C | V5.1 D2 J2.7 C2 | DRB1\*1501 |
| TCRCD4-TPT#59 | V39 J49 C | V7.9\_3 D2 J2.4 C2 | DRB1\*1301 |
| TCRCD4-TPT#67 | V12.3 J9 C | V5.1 D2 J2.7 C2 | DRB1\*1501 |
| TCR CD4-TPT#76b | V8.3 J57 C | V19 D2\_2 J2.7 C2 | DQB1\*0602/DQA1\*0102  DQB1\*0602/DQA1\*0103  DQB1\*0603/DQA1\*0103 |
| TCRCD4-TPT#77 | V14/DV4\_3 J50 C | V20.1 D2 J2.2 C2 | DRB1\*1301 |
| TCRCD4-TPT#78 | V8.6\_2 J21 C | V2 D1 J1.6\_2 C1 | DRB1\*1301 |
| TCRCD4-TPT#82 | V38.2/DV8 J39 C | V19 D1 J2.7 C2 | DRB1\*1301 |
| TCRCD4-TPT#87 | V39 J31 C | V5.1 J2.6 C2 | DRB1\*1301 |
| TCRCD4-TPT#91 | V20\_2 J53 C | V6.1 D1 J2.7 C2 | DRB1\*1501 |

aThe TCR V(D)J genes are indicated in IMGT nomenclature. V: variable; D: diversity; J: joining; C: constant.

bThe TCR recognizes an epitope in combination with more than one HLA allele.

cThe TCR beta gene is V6.2 or V6.3

**Supplementary Figure S4**

Supp Fig1.tif

**Supplementary Figure S4.** Flow cytometric sorting of pp65-specific CD8+ T cells from CMV-seropositive donor ID3 after one week of expansion.IFNy secreting CD8+ T cells were isolated after rechallenge with autologous pp65 RNA-transfected iDCs. Control: iDCs transfected with eGFP RNA.

**Supplementary Table S5.** Synthetic peptides used in this study

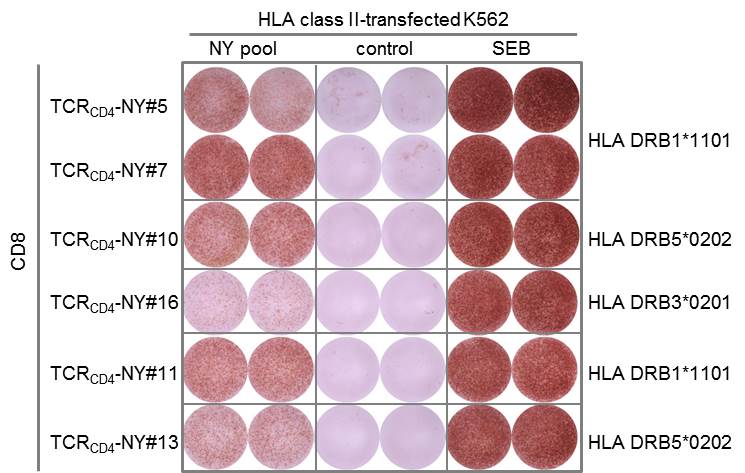
|  |  |
| --- | --- |
| Peptide | Sequence |
| NY-ESO-185-93 | SRLLEFYLA |
| NY-ESO-186-94 | RLLEFYLAM |
| NY-ESO-187-95 | LLEFYLAMP |
| NY-ESO-188-96 | LEFYLAMPF |
| NY-ESO-189-97 | EFYLAMPFA |
| NY-ESO-190-98 | FYLAMPFAT |
| NY-ESO-191-99 | YLAMPFATP |
| NY-ESO-192-100 | LAMPFATPM |
| NY-ESO-193-101 | AMPFATPME |
| TPTE185-193 | RNIPRWTHL |
| TPTE186-194 | NIPRWTHLL |
| TPTE187-195 | IPRWTHLLR |
| TPTE188-196 | PRWTHLLRL |
| TPTE189-197 | RWTHLLRLL |
| TPTE190-198 | WTHLLRLLR |
| TPTE191-199 | THLLRLLRL |
| pp65495-503 | NLVPMVATV |
| SSX-2241-249 | KASEKIFYV |
| tyr368-376 | YMDGTMSQV |

**Supplementary Figure S6**

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**Supplementary Figure S5. Functionality of TCRCD8-NY#5 in CD4+ and CD8+ T cells.** TCR-engineered CD4+ or CD8+ T cells were analyzed by IFNγ-ELISPOT for recognition of K562 cells transfected with individual HLA class I alleles and pulsed with NY-ESO-1 peptide pool. Negative control: HIV-gag peptide pool; positive control: SEB;

**Supplementary Figure S7**

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**Supplementary Figure S6. Functionality of NY-ESO-1-specific CD4-TCRs in CD8+ T cells.** TCR-engineered CD8+ T cells were analyzed by IFNγ-ELISPOT for recognition of K562 cells transfected with individual HLA class II alleles and pulsed with NY-ESO-1 peptide pool. Negative control: HIV-gag peptide pool;

**Supplementary Table S8.** HLA haplotypes from healthy donors and NSCLC patients

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **HLA class I** | | | **HLA class II** | | | |
| **ID** | **A** | **B** | **Cw** | **DRB1** | **DQA** | **DQB1** | **DRB** |
| 1 | A\*02/\*25 | B\*44/\*51 | Cw\*05/\*15 | DRB1\*0701/  \*1103 |  | DQB1\*0202/  \*0301 | DRB3/4 |
| 2 | A\*02/\*03 | B\*35/\*51 | Cw\*04/\*15 |  |  |  |  |
| 3 | A\*02/\*23 | B\*13/\*57 |  |  |  |  |  |
| 4 | A\*02 | B\*18/\*51 | Cw\*07 |  |  |  |  |
| 5 | A\*320101/  \*680101 | B\*070201/  \*350801 | Cw\*04010/  \*070201 | DRB1\*09/\*13 |  | DQB1\*03/\*06 | DRB3/4 |
| 6 | A\*010101/  \*240201 | B\*180101/  \*5108 | Cw\*1502 | DRB1\*040101/  \*040301 | DQA1\*030101 | DQB1\*030201 | DRB4\*010301 |
| 7 | A\*240301/  \*250101 | B\*070201/  \*4001 | Cw\*03040/  \*070201 | DRB1\*1101/  \*160101 | DQA\*010202 | DQB1\*030101/  \*050201 | DRB3\*020101/ DRB5\*0202 |
| 8 | A\*0201/  \*230101 | B\*3501/  \*4403 | Cw\*0401 | DRB1\*0701/ \*1401 | DQA1\*010401 | DQB1\*0202/  \*0503 | DRB4\*0101/  DRB3\*0201/  (\*020201) |
| 9 | A\*240201/  \*680201 | B\*390101/  \*530101 | Cw\*04010/  \*120301 | DRB1\*130201/  \*160101 | DQA1\*010202 | DQB1\*050201/  \*060401 | DRB3\*030101/ DRB5\*0202 |
| 10 | A\*010101/  \*240201 | B\*070201 | Cw\*07020/  \*060201 | DRB1\*130101/\*150101 | DQA1\*0102/  \*0103 | DQB1\*060201/  \*060301 |  |

**Supplementary Methods**

**PBMCs, monocytes and dendritic cells (DCs)**

PBMCs were isolated by Ficoll-Hypaque (Amersham Biosciences) density gradient centrifugation from buffy coats or from blood samples of lung cancer patients. The study was approved by the ethics committee at the Landesärztekammer Rheinland-Pfalz (Mainz, Germany) and from all donors informed consent for participating in this study was obtained.

HLA allelotypes were determined by PCR standard methods. Monocytes were enriched with anti-CD14 microbeads (Miltenyi Biotec). Immature DCs (iDCs) were obtained as described previously (31).

**RNA and tissue samples for fluidigm analysis**

RNAs of normal tissues were provided from commercial vendors. Tissue samples from lung cancer patients were obtained as human surplus materials during routine diagnostic or therapeutic procedures

and were stored at −80°C until use.

**Peptides and peptide pulsing of stimulator cells**

Pools of N- and C-terminally free 15-mer peptides with 11 amino acid overlaps corresponding to sequences of CMV-pp65, HIV-gag, TPTE or NY-ESO-1 (referred to as antigen peptide pool) were synthesized by standard solid phase chemistry (JPT Peptide Technologies GmbH) and dissolved in DMSO to a final concentration of 0.5 mg/ml. Nonamer peptides (Supplementary Table S5) were reconstituted in PBS 10% DMSO. For pulsing, stimulator cells were incubated for 1 h at 37 °C in culture medium using different peptide concentrations.

***In vitro* transcription (IVT) of RNA and transfer into cells**

Generation of IVT RNA was performed as described previously (31) and added to cells suspended in X-VIVO 15 medium (Lonza) in a precooled 4-mm gap sterile electroporation cuvette (Bio-Rad). Electroporation was performed with a Gene-Pulser-II apparatus (Bio-Rad) (T cells: 450 V/ 250 µF; IVSB T cells: 350 V/ 200 µF; Jurkat76: 300 V/300 µF; human DC: 300 V/ 150 µF; K562: 200 V/ 300 µF).

***In vitro* expansion of antigen-specific T cells**

2.5x106 PBMCs/well were seeded in 24-well plates (Costar), pulsed with peptide pool and cultured for 1 week in complete culture medium supplemented with 5% AB serum, 10 U/ml IL-2 and 5 ng/ml IL-7. For some experiments, CD8+ or CD4+ T cells were purified from PBMC by positive magnetic cell sorting (Miltenyi Biotec) and then expanded by co-culturing of 2x106 effectors with 3x105 autologous DCs either electroporated with antigen-encoding RNA or pulsed with the overlapping peptide pool for 1 week in complete medium supplemented with 5% AB serum, 10 U/ml IL-2 and 5 ng/ml IL-7.

**RNA extraction, SMART-based cDNA synthesis and unspecific amplification from sorted cells**

RNA from sorted T cells was extracted with the RNeasy Micro Kit (Qiagen). A modified BD SMART protocol was used for cDNA synthesis. First-strand synthesis was performed with Mint Reverse Transcriptase (Evrogen), *T-primer long* for priming of the reaction and *TS-short* (Eurogentec) introducing an oligo(riboG) sequence to allow for creation of an extended template by the terminal transferase activity of the reverse transcriptase and for template switch (33). First strand cDNA synthesized according to the manufacturer’s instructions was subjected to 21 cycles of amplification with Pfu Ultra Hotstart High-Fidelity DNA Polymerase (Stratagene) and *TS-PCR* primer (cycling conditions: 2 min at 95 °C for, 30 s at 94 °C, 30 s at 65 °C, 1 min at 72 °C for, final extension of 6 min at 72 °C). Amplification of TCR genes was controlled with TCR-β constant region-specific primers (*TCRβC\_ctr\_s, TCRβC\_ctr\_as*). Consecutive clonotype-specific Vα-/Vβ-PCRs were only performed if strong bands were detected.

First strand cDNA for the amplification of HLA class I or II sequences was synthesized with SuperScriptII Reverse Transcriptase (Invitrogen) and Oligo(dT) primer with 1-5 µg RNA extracted from patient-derived PBMCs.

Primers for SMART-based cDNA synthesis and for control of this process are listed in Supplementary Table S1.

**Design of PCR primers for TCR amplification**

For design of TCR consensus primers, all 67 TCR-Vβ and 54 TCR-Vα chains (open reading frames and pseudogenes) as listed in the IMGT database (<http://www.imgt.org>) together with their corresponding leader sequences were aligned with the BioEdit Sequence Alignment Editor. Forward primers of 24 to 27 bp length with a maximum of 3 degenerated bases, a GC-content between 40-60% and a G or C at the 3’-end were designed to anneal to as many leader sequences as possible and equipped with a 15 bp 5’-extension (5'-TATGCGGCCGCCACCatg-3') featuring a NotI restriction enzyme site and Kozak sequence. Reverse primers were designed to anneal to the first exons of the constant region genes, with primer TRACex1\_as binding to sequences corresponding to aa 7 to 16 of Cα and TRBCex1\_as to aa 8 to 16 in Cβ1 and Cβ2. Both oligonucleotides were synthesized with a 5’ phosphate. Primers were bundled in pools of 2-5 forward oligonucleotides with identical annealing temperatures. Primers for amplification of TCR chains are listed in Supplementary Table S1.

**Design of PCR primers for HLA amplification**

HLA consensus primers were designed by aligning all HLA class I and II sequences listed on the Anthony Nolan Research Institute website (http://hla.alleles.org) with the BioEdit Sequence Alignment Editor. Forward primers of 23 to 27 bp length with a maximum of 3 degenerated but code-preserving bases annealing to as many as possible HLA sequences of one locus were equipped with a 5’-phosphate and Kozak sequence extension (5’-Ph-GCCACC). Reverse primers were designed analogously but without introduction of wobble bases and equipped with a 14 bp 5’extension (5’-TATTATGCGATCGC-3’) encoding an *AsiSI* restriction enzyme site. Primers for amplification of HLA molecules are listed in Supplementary Table S1.

**PCR amplification and cloning of HLA antigens**

HLA sequences were amplified according to the manufacturer’s instructions with 2.5 U Pfu polymerase from donor-specific cDNA using specific HLA class I or II sense and antisense primers. As transcription of DRB3 genes is at least five fold lower than that of DRB1 genes, amplification of DRB3 genes was conducted in two steps using inner primer *HLA-DRB3\_in\_s* (bp 12-37 of DRB3 genes, but not DRB1 genes) combined with antisense primer *HLA-DRB\_as\_1*, followed by a second round of PCR with primers *HLA-DRB\_s\_1* and *HLA-DRB\_as\_1.* PCR fragments were purified, *AsiSI*-digested and cloned into the *EcoRV-* and *AsiSI-*digested IVT vector. EciI- or SapI-sites within the inserts were mutated using QuikChange Site-Directed Mutagenesis Kits (Stratagene).

**Vectors for *in vitro* transcription**

All constructs are variants of the previously described pST1-sec-insert-2βgUTR-A(120)-Sap1 plasmid (29). To obtain plasmids encoding TCR chains, cDNA coding for TCR-α or TCR-β1/2 constant regions were cloned via *BamHI* sites into this backbone. Specific V(D)J PCR products were introduced into such cassettes via *NotI* and *EcoRV* sites to yield full length TCR chains (referred to as pST1-TCRα/β-2βgUTR-A(120)).

Analogously, individual HLA class I and II alleles were inserted into this backbone (referred to as pST1-HLA class I/II-2βgUTR-A(120) and pST1-β2M-2βgUTR-A(120)) using restriction sites *EvoRV/AsiSI* and *HindIII/BamHI*, respectively.

Primers for construction of pST1 vectors are listed in Supplementary Table S1. If not indicated otherwise, primers were purchased from Operon Biotechnologies, Cologne, Germany.

Plasmids coding for pp65 antigen of CMV (pST1-sec-pp65-MITD-2βgUTR-A(120)) and NY-ESO-1 (pST1-sec-NY-ESO-1-MITD-2βgUTR-A(120)) linked to a secretion signal (sec) and the MHC class I trafficking signal (MITD) were described previously (31). pST1-TPTE-MITD-2βgUTR-A(120) plasmid was constructed using a TPTE cDNA (NCBI Reference Sequence: NM\_199261.2) synthesized with corresponding restriction sites by a commercial provider (Geneart).

**Flow cytometric analyses**

Cell surface expression of transfected TCR genes was analyzed by flow cytometry using PE-conjugated anti-TCR antibodies against the appropriate variable region family or the constant region of the TCR-β chain (Beckman Coulter) and FITC- or APC-labeled anti-CD8/-CD4 antibodies (BD Biosciences). HLA antigens were detected by staining with FITC-labeled HLA class II-specific (Beckman Coulter) and PE-labeled HLA class I-specific antibodies (BD Biosciences). Flow cytometric analysis was performed on a FACS-Calibur analytical flow cytometer (BD Biosciences). Acquired data were analysed using version 7 of the FlowJo software (Tree Star).

**Cytotoxicity assay**

1x104 peptide-pulsed DCs or K562 cells transfected with luciferase-encoding IVT RNA were co-cultured with TCR-transfected T cells for 3 h. A reaction mixture containing D-Luciferin (BD Biosciences; final concentration 1.2 mg/ml) was added to the cells. One hour later luminescence was measured using a Tecan Infinite M200 reader (Tecan). Cell killing was calculated by measuring the reduction of total luciferase activity. Viable cells were measured by the luciferase-mediated oxidation of luciferin. Specific killing was calculated according to the following equation:

