# Supplemental material

**Marijt et al., https://doi.org/10.1084/jem.20180577**

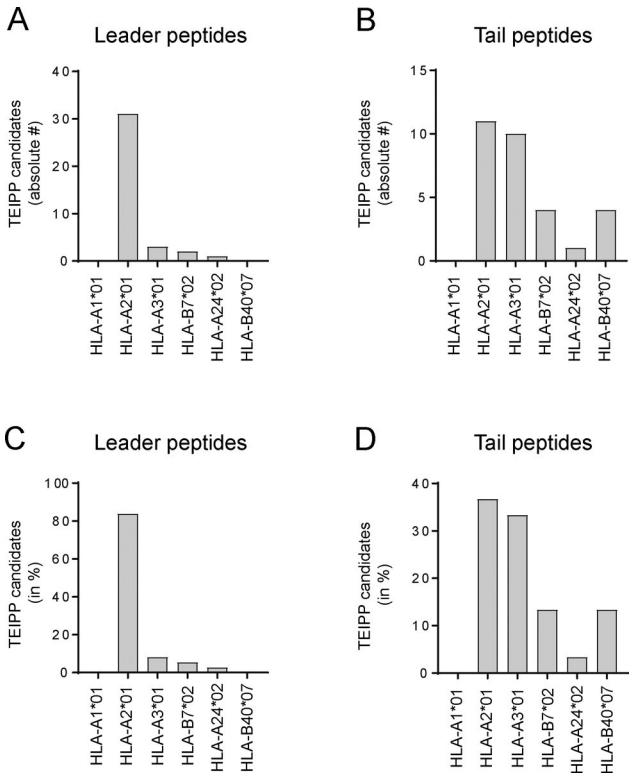
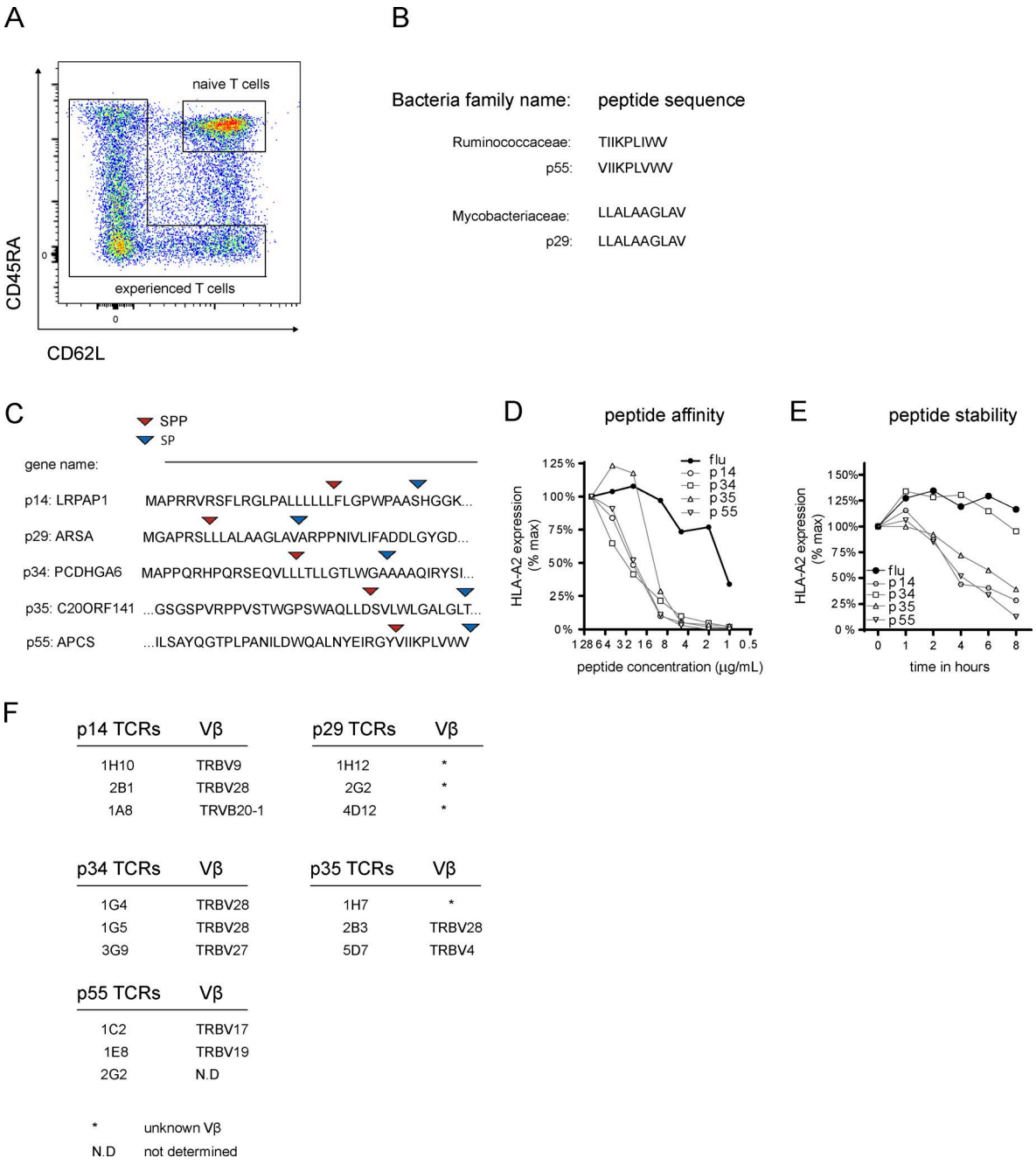



Figure S1. **Prediction results of HLA-I binding of TEIPP neoantigen candidates.** TEIPP neoantigen candidate peptides ($n$ = 65) were aligned according to HLA binding specificity using a web-based algorithm. **(A and B)** Absolute number of TEIPP candidates with predicted binding in HLA superclasses for leader peptides and tail peptides. **(C and D)** Percentage of total TEIPP candidates with predicted binding in HLA superclasses for leader peptides and tail peptides.

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| p14 TCRs | Vβ |
|---|---|
| 1H10 | TRBV9 |
| 2B1 | TRBV28 |
| 1A8 | TRVB20-1 |

| p29 TCRs | Vβ |
|---|---|
| 1H12 | * |
| 2G2 | * |
| 4D12 | * |

| p34 TCRs | Vβ |
|---|---|
| 1G4 | TRBV28 |
| 1G5 | TRBV28 |
| 3G9 | TRBV27 |

| p35 TCRs | Vβ |
|---|---|
| 1H7 | * |
| 2B3 | TRBV28 |
| 5D7 | TRBV4 |

| p55 TCRs | Vβ |
|---|---|
| 1C2 | TRBV17 |
| 1E8 | TRBV19 |
| 2G2 | N.D |

* unknown Vβ
N.D not determined

Figure S2. **Characterization of TEIPP T cells and cognate antigen. (A)** Flow cytometry gating strategy for flow sorting PBMCs into experienced T cells and naive T cells. **(B)** Overview of bacterial peptide sequences with TEIPP peptide homology. **(C)** Gene name, peptide sequence, and predicted peptidase cleavage sites (SP [signal peptidase; blue triangle] and SPP [signal peptide peptidase; red triangle]) of p14, p29, p34, p35, and p55. **(D)** pHLA peptide affinity was measured by a cellular-based assay. Percentage of maximum HLA-I expression was determined by flow cytometry to calculate EC50 values. **(E)** pHLA peptide stability was measured by calculating EC50 values of maximum HLA-I expression over time. **(D and E)** Representative data of four independent experiments are shown. **(F)** Multiple T cell clones with different Vβs for the different specificities were isolated. Shown are the T cell clone name and corresponding T cell receptor β variable gene name, determined by using a TCR Vβ Screening Kit analyzed by flow cytometry.

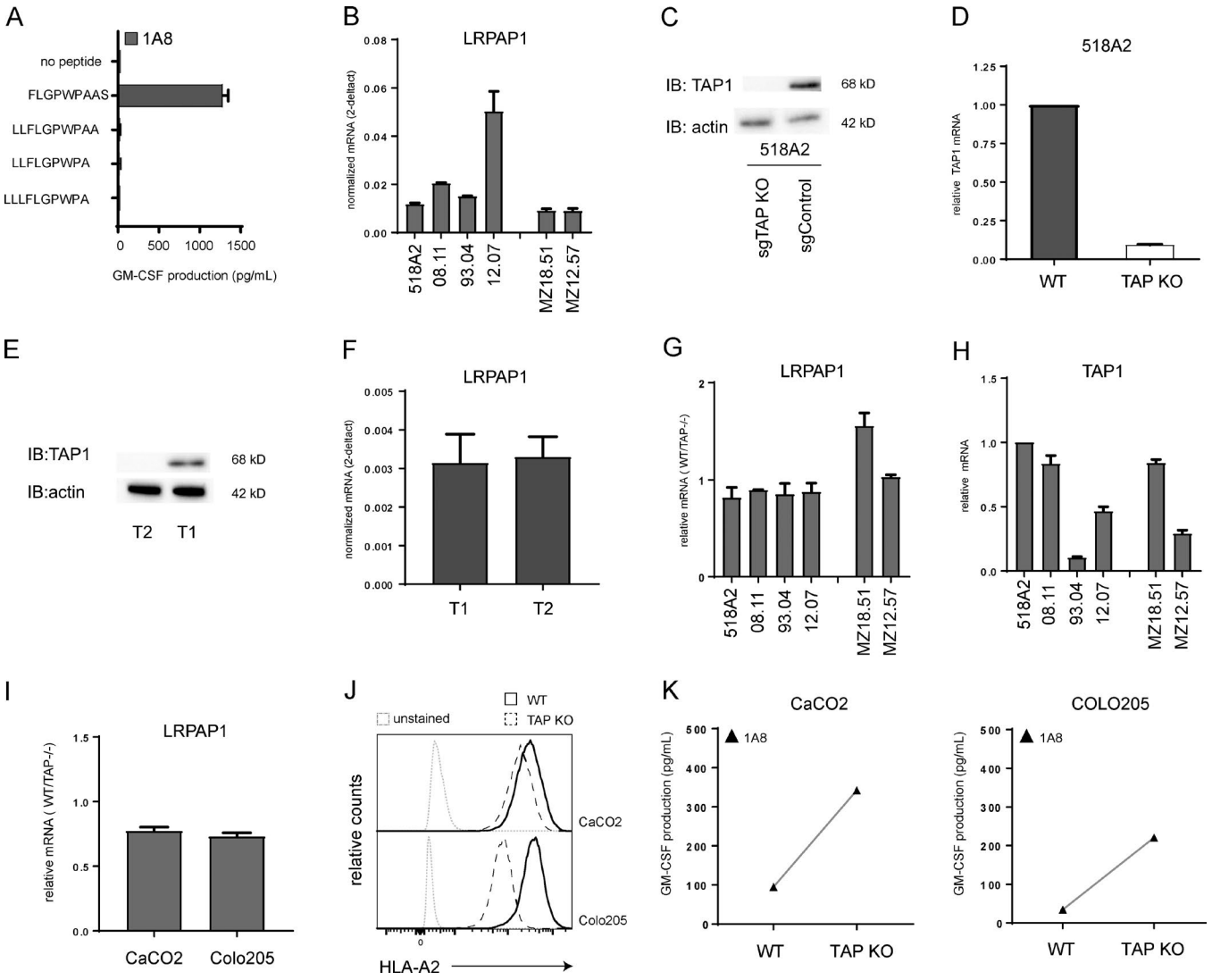


Figure S3. **mRNA and protein expression analysis on tumor panel. (A)** Peptide specificity of the LRPAP1$_{21-30}$-specific T cell clone 1A8 was assessed by testing different length variants of the p14 peptide at different locations of the signal peptide. Peptides with a predicted HLA-A*02:01 binding (IC50 < 500 nM) were tested. GM-CSF cytokine production was measured by ELISA. **(B)** *LRPAP1* mRNA levels of the tumor panel were determined by qPCR. **(A and B)** Data are shown as mean ± SD; *n* = 3. **(C)** TAP1 protein levels of the 518A2 skin melanoma cell line variants were measured using Western blot. No *TAP1* expression was observed in the 518A2 TAP KO variant. **(D)** mRNA expression of *TAP1* gene of the 518A2 skin melanoma cell line variants was determined by qPCR. Data are shown as mean ± SD; *n* = 2. **(E)** TAP1 protein levels of the T1 and T2 lymphoma were measured using Western blot. No TAP1 expression was observed in the T2 lymphoma. **(F)** *LRPAP1* mRNA expression levels of the T1 and T2 lymphoma were determined by qPCR. Both cell lines had equal LRPAP1 mRNA expression. Data are shown as mean ± SD; *n* = 2. **(G)** *LRPAP1* mRNA expression levels of the tumor panel were examined by qPCR. Relative mRNA gene expression of the TAP KO variant compared with WT variant was calculated. **(H)** Relative TAP1 mRNA levels of the tumor panel were determined by qPCR. **(G and H)** Data are shown as mean ± SD; *n* = 3. **(I)** LRPAP1 mRNA expression levels of two colon carcinoma cell lines were determined by qPCR. Equal levels of LRPAP1 mRNA expression were observed. Data are shown as mean ± SD; *n* = 2. **(J)** HLA-A*02:01 surface expression of the antigen-processing machinery–impaired colon carcinoma lines were measured by flow cytometry. **(K)** GM-CSF production of T cell clone 1A8 upon antigen recognition of two colon carcinomas (WT versus TAP KO). **(J and K)** Representative data of two independent experiments are shown.

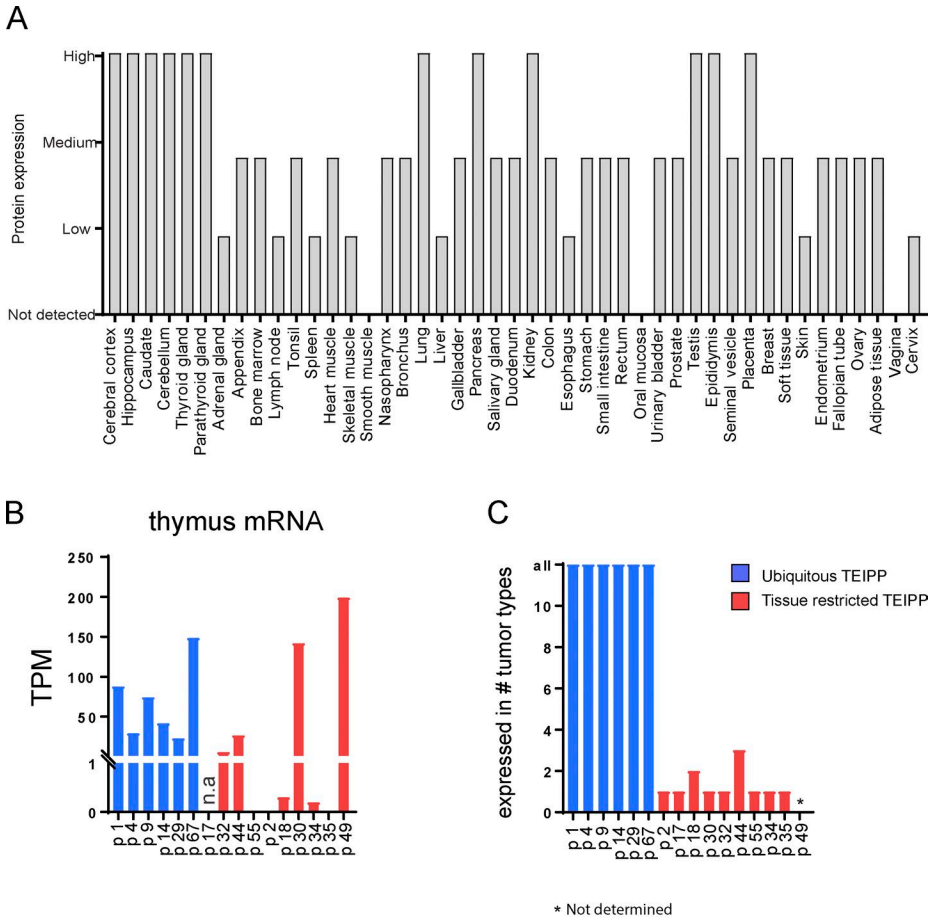


Figure S4. **Database analysis of TEIPP candidates. (A)** LRPAP1 protein expression in 44 different healthy tissues. Data collected from the Human Protein Atlas. **(B)** mRNA expression of 16 identified TEIPP antigens in the thymus, expressed in tags per million (TPM). Data collected from FANTOM5 datasets. n.a., not applicable. **(C)** mRNA expression data of the TEIPP antigens were of different tumor types and were extracted from the Cancer Genome Atlas database. Our list with 16 TEIPP antigens is categorized in two groups: ubiquitous TEIPP, all examined tumor types have mRNA expression of the respective TEIPP antigen (TPM > 5); and tissue-restricted TEIPP, mRNA expression was only detected in some tumor types (TPM > 5).

Table S1. **Overview of peptidome analyses from human tumor samples**

| Tissue type | Malignant/benign | *n* |
|---|---|---|
| Bone marrow mononuclear cells | Benign | 10 |
| Granulocytes | Benign | 2 |
| PBMC | Benign | 30 |
| Liver | Benign (adjacent to tumor) | 12 |
| Colon | Benign (adjacent to tumor) | 35 |
| Ovary | Benign (adjacent to tumor) | 4 |
| Kidney | Benign (adjacent to tumor) | 27 |
| Acute myeloid leukemia | Malignant | 19 |
| Breast cancer | Malignant | 2 |
| Chronic lymphocytic leukemia | Malignant | 32 |
| Chronic myeloid leukemia | Malignant | 16 |
| Glioblastoma | Malignant | 6 |
| Hepatocellular carcinoma | Malignant | 12 |
| Colorectal cancer | Malignant | 35 |
| Leiomyosarcoma | Malignant | 1 |
| Merkel cell carcinoma | Malignant | 1 |
| Meningioma | Malignant | 1 |
| Multiple myeloma | Malignant | 9 |
| Non–small cell lung cancer | Malignant | 1 |
| Ovarian cancer | Malignant | 35 |
| Peritoneal carcinomatosis | Malignant | 1 |
| Polycythemia vera | Malignant (pre-) | 10 |
| Renal cell carcinoma | Malignant | 31 |
| T cell acute lymphoblastic leukemia | Malignant | 2 |
| Cell lines | Malignant/immortalized | 17 |
| Total | Malignant | 231 |
| | Benign | 120 |