Supplemental Methods

In vitro trimming by rmERAP1 and peptide analysis by HPLC

For *in vitro* digestion experiments 50 µM peptide was digested with 3 or 6 ng of purified recombinant mouse ERAP1 (R&D Systems) in 20 µl digestion buffer at 37°C. All peptides were synthesized at the Institute of Biochemistry, Charité-Universitätsmedizin Berlin. Digestion buffer contained 25 mM Tris ph 7.5 (AppliChem), 150 mM NaCl (AppliChem), and 0.5 µl Albumin Fraction V (AppliChem). The reaction was stopped at the indicated time points with 0.5% trifluoroacetic acid (TFA). High performance liquid chromatography (HPLC) analysis of the digestion products was performed on a System Gold High Performance Liquid Chromatograph (Beckman Coulter) equipped with a 125 Solvent Module and 166 Detector. Samples were separated on a MICRA NPS ODS-I polymeric C-18 bonded phase 1.5 µm column (33 x 4.6 mm, Eprogen). The analysis involved a constant flow rate of 0.5 ml/min and an injection volume of 20 µl. The gradient mobile phase system was comprised of solvent A (0.1% TFA in water) and solvent B (0.1% TFA acid in water/acetonitrile [10/90, v/v]). The initial mobile phase was 15% B. Subsequently, solvent B was increased to 45% over 35 minutes. Peptides were identified by comparison to synthesized peptide standards. All the calculations including data acquisition and graphics were done with 32 Karat Software (version 5).

Peptide translocation assay

TAP transport assays were performed in Raji cells as described in Fischbach et al. (2009) [1]. Raji cells were sourced from a cryopreserved aliquot in liquid nitrogen. A single cryovial was thawed for use and propagated. Raji cells were not re-authenticated. Mycoplasma testing was performed with the Venor®GeM OneStep Kit (Minerva). Raji cells were mycoplasma negative. In brief, Raji cells were permeabilized with 0.25 µg/ml Streptolysin O (Abcam) for 15 min at 4°C. Peptide transport was performed in duplicates for 2 x 10^5 cells in 50 µl PBS supplemented with 1 mM ADP/ATP (Merck) and 10 mM $MgCl_2$ (AppliChem) and in the presence of 10 nM fluorescein-labelled peptide (C4: RRYC^{F}KSTEL, E5: EPGYC^{F}NSTD,

NST: RRYQNST^{F}L, SAINNYAQC^{F}L, VSAINNYAQC^{F}L, RVSAINNYAQC^{F}L, VVYDFLKC^{F}; SVVYDFLKC^{F}, DSVVYDFLKC^{F}) for 15 min at 37°C. The reaction was stopped by addition of 150 µl PBS supplemented with 20 mM EDTA (AppliChem). Fluorescence of the samples was measured on a BD FACSCalibur Cell Analyzer (BD Biosciences). Samples were analyzed using FlowJo 7.6.5.

Histology and immunohistochemistry

Serial sections (1-2 µm) of formalin-fixed livers of WT (*Erap1*$^{+/+}$) x LoxP-TAg x Alb-Cre and *Erap1*$^{-/-}$ x LoxP-TAg x Alb-Cre mice were mounted on slides and stained with hematoxylin and eosin. For immunostaining, consecutive slides were subjected to a heat-induced epitope retrieval step before incubation with rabbit anti–SV40 TAg antibody (v-300, Santa Cruz) or rat anti-mouse Ki-67 antibody (TEC-3, Dako). For detection, the Streptavidin-AP Kit (K5005, Dako) alone or biotinylated donkey anti-rat (Dianova) or rabbit anti-rat (Dako) secondary antibodies were used followed by the Streptavidin-AP Kit or the EnVision Peroxidase Kit (K 4010, Dako). Alkaline phosphatase (AP) and peroxidase were developed by Fast Red or diaminobenzidine chromogenic substrates, respectively. Images were acquired using a fluorescence microscope (AxioImager Z1) equipped with a CCD camera (AxioCam MRm) and processed with ApoTome and ZEN software (Carl Zeiss MicroImaging Inc.).

Western blot analysis

For Western blot analysis cells were lysed in 50 mM Tris-HCl pH 7.5 (AppliChem), 50 mM NaCl (AppliChem), 5 mM $MgCl_2$ (Merck), 0.1% Triton X-100 (Sigma) and protease inhibitor (cOmplete, Roche). 20 µg of total protein extracts were separated by SDS-PAGE (10% or 12.5.% polyacrylamide gels) and transferred onto nitrocellulose (Whatman) or PVDF membranes (Millipore). The membranes were incubated in 5% non-fat milk (AppliChem) supplemented with 0.4% Tween-20 (AppliChem) for 1 h. Primary antibodies recognizing Jak1 (#3344, Cell Signaling), Jak2 (#3230, Cell Signaling), Stat1 (#9172, Cell Signaling), Phospho-Stat1 (#9167, Cell Signaling), ERAP1 (clone 6H9, R&D Systems), TAP1 (sc-11465,

Santa Cruz), TAP2 (sc-11473, Santa Cruz), MECL1 (clone K6512), LMP2 (ab3328, Abcam), LMP7 (ab3329, Abcam), or β-Actin (C4, Santa Cruz) were diluted in 2% non-fat milk with 0.1% Tween-20 and were applied overnight at 4°C. The membrane was washed three times with PBS/0.4% Tween-20 followed by 1 h incubation with 0.2 µg/ml of the secondary antibodies: goat anti-rabbit IgG H&L chain specific peroxidase conjugate, rabbit anti-mouse IgG H&L chain specific peroxidase conjugate, or rabbit anti-goat IgG H&L chain specific peroxidase conjugate (Calbiochem) in 2% non-fat milk with 0.1% Tween-20. The ECL Prime Western Blotting Detection Kit (GE Healthcare) was used for detection. Membranes were analyzed with the Fusion FX System using FusionCapt Advance FX7 Software (Peqlab).

Flow cytometry

For flow cytometry, single cell suspensions of un-stilmulated and stimulated (100 U/ml recombinant mouse IFNγ, Roche) TAg^+ HCC cell lines were prepared. Antibody-staining was performed in PBS with 1% BSA (AppliChem) for 30 min on ice. Samples were stained with PE-conjugated anti-mouse MHC-I H2-D^b (clone 28-14-8, eBioscience), APC- conjugated MHC-I H2-K^b (clone AF6-88.5.5.3, eBioscience), PE- conjugated mouse IgG2b kappa isotype control (eBioscience), or APC- conjugated mouse IgG2a kappa isotype control (eBioscience).

For analysis of blood lymphocytes, EDTA-blood was collected in MiniCollect tubes (Greiner). 50 µl EDTA-blood were stained with 0.5 µl Alexa Fluor 647-conjugated anti-mouse CD8α antibody (BioLegend) for 30 min at 4°C. In some experiments blood samples were co-stained with PE-conjugated H2-D^b (SAINNYAQKL, Tet-I) or H2-K^b (anchor-modified VVYDFLKL, Tet-IV) tetramers (Beckman Coulter). Red blood cells were lysed in ACK lysis buffer containing 155 mM NH_4Cl (AppliChem), 10 mM $KHCo_3$ (Merck), and 0.1 mM Na_2EDTA (AppliChem) at ph 7.2. For lysis 500 µl ACK-buffer was added after antibody staining and samples were mixed for 1-2 min at room temperature. The reaction was stopped by adding 4.5 ml 1 x PBS supplemented with 3% FCS (Biochrom). White blood cells were re-suspended in 500 µl 1 x PBS. All samples were analyzed on a BD FACSCalibur Cell Analyzer (BD Biosciences).

Production of retrovirus supernatants and retroviral transduction

Cloning of the retroviral vectors pMP71-TCR-I and pMP71-TCR-IV was previously described [2]. Retroviral supernatants were generated by transfection of ≥70% confluent Plat-E ecotropic retroviral packaging cell line with pMP71-TCR-I or pMP71-TCR-IV plasmids using Lipofectamine 2000 Reagent (Invitrogen) according to the manufacturer's protocol. Plat-E cells were sourced from M. Leisegang (group of W. Uckert, Berlin, Germany) [3]. Plat-E cells were not re-authenticated, and were cultivated for a maximum of 15 passages after thawing. Mycoplasma testing was performed with the Venor®GeM OneStep Kit (Minerva). Plat-E cells were mycoplasma negative.

To generate gene-modified TCR-I or TCR-IV T cells, splenocytes were isolated from P14 x *Rag*$^{-/-}$ mice. Red blood cells were lysed with ACK-buffer. 1 x 10^6 cells per ml were cultured in 24-well plates in RPMI supplemented with 10% heat-inactivated FCS, 50 µg/ml gentamycin, 50 µM β-mercaptoethanol, 1x penicillin/streptamycin (Biochrom), 1 µg/ml functional grade purified anti-mouse CD3ε (clone 145-2C11, eBioscience), 0.1 µg/ml functional grade purified anti-mouse CD28 (clone 37.51, eBioscience) and 40 U/ml recombinant mouse IL-2 (Proleukin, Prometheus Laboratories) for 24 h at 37°C in a humidified 5% CO_2 incubator. After 24 h activation, media was removed. 1 ml retrovirus supernatant containing 10 µg/ml polybrene (Millipore) was added per well. The cells were spinoculated for 2 h at 800 x g at 32°C. After centrifugation, retrovirus supernatant was removed and 1 ml RPMI supplemented with 10% heat-inactivated FCS, 50 µg/ml gentamycin, 50 µM β-mercaptoethanol, 1x penicillin/streptamycin, and 40 U/ml rmIL-2 was added. Transduced cells were cultured for 24 h at 37°C in a humidified 5% CO_2 incubator. 24 h later a second transduction was performed. 24 h after the second transduction, the level of surface TCR expression was determined through co-staining of the transduced cells with Alexa Fluor 647-conjugated anti-mouse CD8α antibody and PE-conjugated H2-D^b (SAINNYAQKL) or H2-K^b (VVYDFLKL) tetramers. Samples were analyzed by flow cytometry. Transduction rates were between 70-90% and were comparable for TCR-I and TCR-IV.

T cell recognition assays and ELISA

For cancer cell recognition assays 5 x 10^4 TCR-I or TCR-IV T cells were co-cultured with titrated numbers (5 x 10^4-5 x 10^1) of un-stimulated or IFNγ-stimulated (100 U/ml recombinant mouse IFNγ, Roche) TAg^+ tumor cells. IFNγ-release was analyzed 24 h later by mouse IFNγ ELISA Set (#555138, BD Biosciences) according to the manufacturer's protocol.

***In vivo* cytotoxicity assay**

CTL activity *in vivo* was analyzed as previously described [4, 5]. Control mice were immunized one week before by i.p. injection of 1 x 10^7 16.113 cells in 200 µl PBS. TAg-I and TAg-IV-specific cytotoxicity was analyzed either separately or simultaneously. Splenocytes were isolated from C57BL/6 (CD45.2) mice and single cell suspensions were prepared. Following red blood cell lysis with ACK buffer for 2 min, splenocytes were washed twice with 1x PBS. Unloaded target cells were labelled with 0.075 µM $CFSE^{low}$ (eBioscience). TAg-I (SAINNYAQKL) or TAg-IV (VVYDFLKL) loaded splenocytes were labelled with 0.75 µM $CFSE^{high}$. Then, cells were washed three times with PBS and the different populations were mixed at 1:1 ratio, and 2 x 10^7 cells in 200 µl PBS were injected i.v. into the mice. 18 h later, spleens were isolated and $CFSE^+$ cells were analyzed by flow cytometry. For simultaneous analysis of TAg-I and TAg-IV $CD45.1^+$ unloaded target cells were labelled with 0.0075 µM $CFSE^{low}$, TAg-I-loaded target cells were labelled with 0.075 µM $CFSE^{int}$, and TAg-IV-loaded target cells were labelled with 0.75 µM $CFSE^{high}$. The cells were washed three times with PBS and the different populations were mixed at 1:1:1 ratio, and 2 x 10^7 cells in 200 µl PBS were injected i.v. into the mice. 18 h later, spleens were isolated and $CD45.1^+$ cells were separated according to their CFSE labelling by flow cytometry. The ratio of TAg-I or TAg-IV-loaded target cells/non-loaded target cells (e.g. $CFSE^{high}/CFSE^{low}$ or $CFSE^{int}/CFSE^{low}$) was analyzed for naive mice, control mice and treated mice. The specific cytolytic activity in control mice or treated mice was calculated as follows: percentage (%) of specific killing = (1

– [ratio of naive mice/ratio of control mice] × 100) or percentage (%) of specific killing = (1 – [ratio of naive mice/ratio of treated mice] × 100).

References

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