Antigen presentation via major histocompatibility complex class I (MHC I) molecules is essential to mount an adaptive immune response against pathogens and cancerous cells. To this end, the transporter associated with antigen processing (TAP) delivers snippets of the cellular proteome, resulting from proteasomal degradation, into the ER lumen. After peptide loading and editing by the peptide-loading complex (PLC), stable peptide-MHC I complexes are released for cell surface presentation. Since the process of MHC I trafficking is poorly defined, we established an approach to control antigen presentation by introduction of a photo-caged amino acid in the catalytic ATP-binding site of TAP. By optical control, we initiate TAP-dependent antigen translocation, thus providing new insights into TAP function within the PLC and MHC I trafficking in living cells. Moreover, this versatile approach has the potential to be applied in the study of other cellular pathways controlled by P-loop ATP/GTPases.
Antigen presentation via major histocompatibility complex class I (MHC I) molecules is one of the most elaborate defense strategies of the adaptive immune system against pathogens and tumor cells. A key player of this antigen presentation pathway is the heterodimeric transporter associated with antigen processing (TAP), which belongs to the superfamily of ATP-binding cassette (ABC) proteins. Each TAP subunit (TAP1/2, ABCB2/B3) can be divided into its core transporter domain with four helices N-terminally attached to each of the core subunits. Notably, TAP is part of the peptide-loading complex (PLC) located in the ER membrane and operates as a gatekeeper for the pool of antigenic peptides derived from cytosolic proteasomal degradation. To ensure MHC I antigen presentation, TAP transports peptides that have an overlapping length and sequence specificity for the interaction with MHC I molecules. In the ER lumen, longer peptides can be processed by the aminopeptidases ERAP1/2 to match with the MHC I peptide-binding pocket. The chaperones calreticulin and ERp57 assist in peptide loading by ensuring the correct assembly of MHC I molecules and the editing module, while tapasin is involved in peptide proofreading. The architecture of the native PLC illustrates the molecular synergy of antigen translocation and ER quality control, including specific MHC I chaperoning and editing. Optimally loaded peptide-MHC I complexes dissociate from the PLC and travel via the secretory pathway to the cell surface to elicit a CD8+ T-cell response. Although MHC I presentation is well studied, our knowledge on the trafficking of antigenic peptides and rate-limiting processes is still limited. Typically, antigen processing has been explored by gene silencing, gene editing, and gene knockouts, which are limited by compensation and feedback effects. Hence, new approaches to spatiotemporally control key steps in the MHC I surface presentation pathway are eagerly awaited.

Genetic encoding of unnatural amino acids (UAAs) allows the precise modulation of protein function within complex cellular processes. By incorporation of UAAs via suppression of the amber stop codon (TAG), target proteins can be site specifically modified. Utilization of photo-caged UAAs, such as amino acids with attached ortho-nitrobenzyl or coumarin moieties, enable light-convertible states. While the photocage initially renders the amino acid functionally inactive, illumination triggers uncaging and restores the native function. Thus, photo-caged UAAs are beneficial to gain control of protein activity within cellular pathways. However, the major bottleneck for the utilization of UAAs is the engineering of an orthogonal aminoacyl-tRNA synthetase with a cognate tRNA that efficiently incorporates the UAA into the nascent polypeptide chain. For the design of a photo-conditional TAP complex, we reasoned that the lysine residue in the Walker A motif of the canonical ATP-binding site would be best suited as it is critical for nucleotide binding. Thus, substitution of the lysine residue by a photo-caged UAA will initially block the coordination of the γ-phosphate of ATP and hence TAP-dependent antigen translocation. We utilized a lysine with a 6-nitropiperonyl cage, which can be cleaved off by illumination in the range of 365 nm. Via light control, the native lysine function can be restored to activate TAP and the entire PLC (Fig. 1).

For the design of a photo-conditional TAP complex, we reasoned that the lysine residue in the Walker A motif of the canonical ATP-binding site would be best suited as it is critical for nucleotide binding. Thus, substitution of the lysine residue by a photo-caged UAA will initially block the coordination of the β- and γ-phosphate of ATP and hence TAP-dependent antigen translocation. We utilized a lysine with a 6-nitropiperonyl cage, which can be cleaved off by illumination in the range of 365 nm. Via light control, the native lysine function can be restored to activate TAP and the entire PLC (Fig. 1). In contrast to conventional knockout or knockdown approaches, which can be biased by complex compensation and feedback mechanisms, this photo-conditional system allows investigations on both, loss

**Fig. 1 Photo-conditional peptide loading complex.** a The conserved lysine of the TAP2 Walker A motif, which is crucial for ATP binding and hydrolysis in the canonical ATP-binding site, is replaced by a photo-caged lysine (PCK) using amber suppression. TAP function is arrested due to the photocage that prevents ATP binding. After illumination and release of the photocage, TAP and PLC function is restored, which activates peptide translocation, MHC I loading and trafficking. b Illumination triggers the uncaging of PCK and allows restoration of the native lysine residue.

![Diagram](https://example.com/diagram.png)
and gain of protein function, in the same genetic and cellular context. By establishing an in-cell system of a photo-conditional TAP complex, we developed an approach, which provides new insights into antigen processing within the PLC and MHC I trafficking. This tight spatiotemporal control has not been achieved in the ABC superfamily or antigen presentation and is the first application in cellular immunology. Importantly, this system can be transferred to other NTPases with a phosphate-binding loop (P-loop) and the conserved lysine residue, e.g., G-proteins, the AAA+ family, the ABC superfamily, the helicase superfamilies I, II, and III, thus expanding into a generic approach to explore various cellular pathways.

**Results**

**Designing a photo-conditional gatekeeper of antigen presentation.** The Walker A motif, also termed P-loop, is essential for the function of basically all ATP/GTPases, including the ABC transporter TAP. Here, we amber suppressed the conserved lysine residue in the Walker A motif of TAP2 (K509, GPNGSGKST), which is crucial for ATP binding and hydrolysis, thereby energizing the antigen translocation into the ER lumen. In addition to full-length TAP, we utilized also coreTAP, which lacks the extra N-terminal transmembrane TMD0 domains and codes solely for the core transporter unit. The coreTAP complex has been shown to be essential and sufficient for peptide binding and translocation. Although PLC assembly requires the TMD0s, coreTAP expression can still restore 50% of the MHC I surface expression in TAP-deficient cells. Our expression constructs encoded both TAP subunits, which were separated by an F2A ribosome-skipping site, derived from the foot-and-mouth disease virus, and comprised either full-length TAP or coreTAP (TAP and coreTAP, respectively) (Fig. 2a). Utilizing the F2A site for TAP1/2 co-expression has three advantages: (i) minimizing the DNA load for facilitated single transfection conditions, (ii) a stoichiometric translation of both TAP subunits, and (iii) an increased stability of the subunits during their biosynthesis as TAP1 serves as chaperone for the unstable TAP2 subunit. To simplify detection, coreTAP2 and TAP2 were C-terminally fused to mVenus.

We examined the expression of the coreTAP and TAP constructs in TAP2-deficient fibroblast cells (STF1-169), which have been generated from a Bare-Lymphocyte syndrome patient (Fig. 2b and Supplementary Fig. 1). CoreTAP or TAP with and without amber mutation was co-expressed with the wild-type pyrrolysyl-tRNA synthetase/tRNACUA pair (from now on abbreviated as wtPylRS) derived from *Methanosarcina mazei*. In the flow cytometric analysis both amber-suppressed TAP variants (from now on abbreviated as TAPTAG and coreTAPTAG) showed an ~30-fold higher expression compared to the background without Nε-Boc-L-lysine (BocK). Next, we analyzed expression and subunit separation of coreTAP and TAP on protein level (Fig. 2c). Apart from co-translationally processed coreTAP2mVenus and TAP2mVenus, we did not observe any unprocessed expression products by in-gel fluorescence. Notably, amber-suppressed coreTAP2mVenus and TAP2mVenus were only detected in the
presence of BocK, while immunoblotting revealed similar coreTAP1 and TAP1 expression level for all conditions. Expression of full-length TAP1 resulted also in coreTAP1 as a natural cleavage product. These results indicate both an efficient co-translational separation of the TAP subunits by the F2A site and successful amber suppression at position of the conserved lysine residue.

A photo-caged lysine in the canonical ATP-binding site blocks TAP function. For the specific incorporation of photo-caged lysine (PCK), we modified the M. mazei wtPylRS by mutating four residues (M276F, A302S, Y306C, L309M), which had been shown to enable PCK incorporation in the M. barkeri PylRS. This engineered PylRS/tRNACUA pair was named optPylRS. By co-expression of coreTAP1TAG with optPylRS in the presence of different PCK concentrations, we examined the minimal concentration of PCK required to generate a photo-conditional TAP complex (Supplementary Fig. 2). We observed that for efficient amber suppression the PCK concentration can be decreased to 20 nM, which is substantially low compared to commonly applied UAA concentrations. This careful optimization prevented not only wasting of the precious UAA but also minimized potential cytotoxic effects at high PCK concentrations. To verify the specific incorporation of PCK by optPylRS, we co-expressed coreTAP1TAG with optPylRS or wtPylRS in TAP2-deficient cells and added either PCK, BocK, or no UAA. As control, amber-free coreTAP was co-expressed with optPylRS in the presence of PCK to assess the side effects of the UAA supplementation (Fig. 3a). Notably, only the co-expression of the coreTAP1TAG construct with optPylRS in the presence of PCK or wtPylRS in the presence of BocK resulted in coreTAP1TAG synthesis. No other combination of coreTAP1TAG and PylRS with or without UAA led to the production of amber-suppressed coreTAP2. Hence, these PylRS/UAA combinations were exclusively utilized for PCK and BocK incorporation and abbreviated from now on as coreTAP1TAG/PCK and coreTAP1TAG/BocK. Compared to the amber-free construct, both amber suppression systems reached similar protein levels of 30 to 40% (Fig. 3a). Consequently, incorporation of the photo-caged...
lysine in the Walker A motif of coreTAP2 by optPylRS is efficient and specific for PCK.

To analyze the stoichiometric assembly of a photo-conditional TAP complex, coreTAP/PCk, and coreTAP TAG/PCk were expressed in HEK293-F cells to achieve high transfection efficiencies and expression levels for isolation of the TAP1/2 complex. After orthogonal purification via His6- tag at TAP2 and streptavidin-binding peptide (SBP)-tag at TAP1, TAP complexes with a 1:1 stoichiometry were obtained, indicating a complete translation of the F2A constructs and assembly of the TAP subunits (Fig. 3b). Consistent to the expression levels in TAP-deficient cells, the yield of amber-suppressed coreTAP TAG/PCk is 25% of the amber-free construct. Next, we examined whether the site-specific UAA incorporation in the canonical ATP-binding site prevents TAP-dependent presentation of peptide-MHC I complexes on the cell surface (Fig. 3c). The MHC I surface expression was monitored by flow cytometry using a conformational specific anti-HLA-A, B, C antibody (W6/32). CoreTAP TAG was either expressed in the presence of PCk or BoaK, while amber-free coreTAP/PCk served as control. Both amber-suppressed coreTAP variants displayed only 10% of MHC I surface expression compared to amber-free coreTAP (Fig. 3c), demonstrating that the incorporated PCK efficiently prevents MHC I trafficking via TAP inhibition.

Optical control of antigen translocation. After verifying TAP inhibition by PCK incorporation in the canonical ATP-binding site, we examined whether the function of photo-conditional TAP is restored by illumination. To this end, we monitored the TAP-dependent peptide transport into the ER using an ultra-sensitive single-cell assay in TAP2-deficient cells expressing either coreTAP TAG/PCk or TAP TAG/PCk. Fluorescently labeled peptides were delivered into the cytosol after semi-permeabilization of the plasma membrane by the Streptococcus pyogenes toxin streptolysin O (SLO) (Fig. 4a). After optimization, we achieved 90% semi-permeabilization of the plasma membrane (Supplementary Fig. 3). By flow cytometry, we followed the antigen translocation of the peptide epitope RRYQNSTC8kxasfluor647L (NST AF647), which is retained in the ER lumen after N-core glycosylation (Fig. 4b, c and Supplementary Fig. 4). Cells expressing amber-free coreTAP or TAP were used as positive controls. Without illumination, these cells displayed an ATP-dependent peptide accumulation. Under the same conditions, cells expressing coreTAP TAG or TAP TAG in the presence of PCk or BoaK did not show ATP-dependent peptide transport above background (mVenus mock control). Notably, after uncaging of TAP by illumination, we observed a light-induced peptide transport. TAP TAG/PCk and coreTAP TAG/PCk restored TAP-dependent transport activity to 75% and 32%, respectively, compared to the amber-free constructs (Fig. 4b, c). These results are in line with a lower expression of the amber-suppressed variants compared to the amber-free constructs (Figs. 2, 3, Supplementary Figs. 1, 3) and demonstrate that the function of the antigen translocation complex TAP can be controlled in a precise manner via a single photo-caged amino acid.

Light-triggered MHC I trafficking and surface presentation. Encouraged by the finding that TAP-dependent antigen translocation can be controlled by light, we investigated whether this approach can be utilized to understand how PLC activation is coupled to MHC I trafficking and antigen presentation. To this end, we monitored peptide–MHC I complexes emerging at the cell surface after illumination. The MHC I surface expression was analyzed by flow cytometry using an HLA-A, B, C-specific antibody as described above. As first reference, we used an ‘acid wash’ and determined an appropriate timescale of 4 h for the analysis of newly surfaced peptide–MHC I complexes (Supplementary Fig. 5). Light activation of both, coreTAP TAG/PCk and TAP TAG/PCk, induced a significant increase in MHC I surface expression compared to non-illuminated cells, while MHC I presentation was not altered immediately after illumination (Fig. 5a, b). Light activation of full-length TAP TAG resulted in a slightly higher MHC I surface level compared to coreTAP TAG (Fig. 5a, b), reflecting that photo-conditional full-length TAP promotes the optimal assembly of the PLC34. Interestingly, even before illumination, TAP TAG expression led to an elevated MHC I surface presentation compared to coreTAP TAG, indicating that photo-conditional TAP TAG can assemble a symmetric PLC and thereby promote the MHC I loading of TAP-independent epitopes. By following the light-induced MHC I surface presentation of coreTAP TAG/PCk- and TAP TAG/PCk-expressing cells over time, we observed a gradual increase with a lag phase of 1 h (Fig. 5c, d). Based on the optical control of TAP-dependent antigen processing, we can temporally separate PLC assembly from peptide translocation and MHC I trafficking. These results demonstrate the precise control of MHC I antigen presentation by photo-conditional TAP but also the potential impact of pre-assembled multi-subunit complexes and cellular pathways synchronized via light.

Discussion

Here, we describe an approach for the spatiotemporal analysis of TAP-dependent peptide translocation, loading, and MHC I trafficking via light control. We genetically encoded the photo-caged lysine PCK in the Walker A motif of TAP2, which is essential for the peptide-transport function of TAP within the PLC. The site-specific incorporation of PCK required an orthogonal system, co-expressing the PCK optimized aminoacyl-tRNA synthetase optPylRS, its cognate tRNA, and amber-suppressed TAP. Photo-conditional TAP was exclusively expressed in the presence of optPylRS and PCK, demonstrating specific expression of the amber-suppressed TAP2 subunits (Fig. 3a). In contrast to previously reported photo-caged UAA approaches30,31,37, PCK was sufficiently incorporated if supplied at nanomolar concentrations (Supplementary Fig. 2). Thus, we could minimize possible cytotoxic effects and express photo-conditional TAP under near-physiological conditions. To gain new insights into TAP function within the PLC, we selected constructs coding for full-length TAP or coreTAP separated by an F2A ribosome-skipping site to produce both TAP subunits in a native 1:1 stoichiometry (Fig. 3b). By utilizing a single-cell-based peptide translocation assay combined with monitoring MHC I surface expression, we demonstrated that PCK incorporation in the canonical ATP-binding site blocks TAP function (Figs. 3c and 4b, c). Illumination triggered antigen translocation into the ER lumen (Fig. 4b, c), followed by MHC I trafficking and antigen surface presentation (Fig. 5). Light activation of both, photo-conditional full-length TAP and coreTAP, induced a gradual accumulation of newly surfaced MHC I molecules. These results are consistent with previous findings demonstrating that an asymmetric PLC can be assembled on endogenous full-length TAP1 and coreTAP34. However, illumination of full-length TAP led to a slightly higher surface MHC I presentation compared to coreTAP. Interestingly, this trend for an elevated MHC I level of full-length TAP expressing cells had already been seen before light activation. This result illustrates the important function of TAP as a PLC scaffold and indicates beneficial effects of PLC assembly even without TAP-dependent antigen translocation.

Since MHC I trafficking and turnover is still poorly characterized, we utilized an ‘acid wash’ as first reference of the MHC I restoration at the cell surface40. However, it is important
**Fig. 4 Antigen translocation triggered by light.**

*a* Schematic representation of TAP-dependent peptide translocation. TAP2-deficient STF1-169 cells expressing photo-conditional TAP were illuminated and semi-permeabilized with streptolysin O (SLO). Peptide translocation was performed at 37 °C in the presence of fluorescently labeled NSTAF647 peptide and ADP or ATP. *b* Peptide translocation of TAP2-deficient STF1-169 cells expressing coreTAP or coreTAP variants without and with illumination was monitored by flow cytometry. mVenus expression served as mock control. The dotted lines represent the mode FI of the transported peptide of the corresponding amber-free counterpart (black) and the photo-conditional variants before (gray) and after illumination (red). Peptide transport after illumination was determined by normalizing the mean FI (± SEM, n = 3, biologically independent samples) of transported peptide to the corresponding amber-free variant in presence of ATP. FI fluorescence intensity.
to note that the intracellular pools of MHC I are not affected, and peptide–MHC I complexes can still continuously traffic to the cell surface. In contrast, our approach to control TAP and PLC function by light offers the advantage to trigger peptide translocation and MHC I loading in a spatiotemporal manner and provides a well-synchronized starting point of MHC I trafficking.

Combined with activation via confocal laser illumination, photo-conditional TAP has the potential to open avenues for studies on various intracellular MHC I pools and antigen presentation pathways in different cells. However, achieving levels of the amber-free variants in functional assays might not be possible, as we showed by the partial restoration of the peptide translocation activity (Fig. 4b, c) and MHC I surface presentation (Fig. 5c, d). Besides the reduced expression levels compared to their amber-free counterparts, a possible explanation for this result is that active TAP, which is exclusively generated by illumination, is subject to protein turnover. After light activation, de novo TAP2 expression is prematurely terminated by the amber stop codon. Thus, degradation of light-activated TAP, depending on the half-life of the variants, might be relevant for experiments that have to be monitored over long periods of time.

Conventional methods to investigate complex cellular processes such as antigen processing and presentation utilize inhibitors or conditional knockdown/knockout of key players. Nevertheless, both strategies are incapable to restore function of the target protein and, therefore, do not represent an ideal approach. As inhibitors allow the arrest of protein activity while maintaining the native cellular environment, we previously reported a method to arrest and re-activate TAP via a photo-conditional variant of the viral inhibitor ICP47. However, this approach has one major drawback: the photo-conditional immune suppressor is a synthetic cell-impermeable compound, which requires delivery into the cytosol. In contrast, the genetic encoding of an initially arrested TAP enables studies on the loss of function within an assembled PLC as well as antigen translocation and MHC I presentation after light activation.

In conclusion, we established the optical control of TAP-dependent antigen processing while maintaining the native PLC assembly. Thus, photo-conditional PLC enables precise control of antigen translocation, peptide loading onto MHC I molecules, and surface presentation of peptide–MHC I complexes. In contrast to conventional knockout or knockdown approaches, TAP-dependent and TAP-independent antigen presentation can be dissected since the PLC, including MHC I assembly and peptide editing, remains intact. These advantages open avenues for new in vitro and in vivo studies in the field of antigen presentation, such as time-resolved investigations on PLC disassembly and MHC I trafficking in different presentation pathways. Moreover, this approach can be versatility transferred to various other ATP/GTPases, especially in the context of

**Fig. 5 Light activation of TAP synchronizes MHC I trafficking and surface presentation.** a coreTAPTAG/PCk and b TAPTAG/PCk was expressed in TAP2-deficient STF-169 cells. MHC I surface expression of the mVenus-positive cells was monitored before (w/o hv) and after illumination by flow cytometry using an APC-Fire750-labeled HLA-A, B, C-specific antibody (W6/32). The black dotted line represents the mean FI of non-illuminated cells. Mean FI was calculated (±SEM, n = 3, biologically independent samples) and a One-way ANOVA with Turkey’s multiple comparison test was performed. ns non-significant; ***, p < 0.0001. c coreTAPTAG/PCk- and d TAPTAG/PCk-dependent MHC I surface expression (± SEM, n = 3, biologically independent samples) up to 4 h after illumination. FI fluorescence intensity.
protein complex formation within cellular pathways. Hence, this system has the potential to answer key questions of intra- and intercellular processes that are essential for cell homeostasis and spatiotemporal compartmentalization of antigens.

**Methods**

**Synthesis of nitrophenyl caged lysine (PCK).** All reagents and solvents were of the highest grade available supplied by Fluka, Sigma Aldrich, Merck, or Carl Roth and used without further purification. Boc-Lys-OH was obtained from Iris Biotec.

Unless otherwise stated, all reactions were performed under argon atmosphere using dry solvents (Sigma Aldrich). Analytical thin-layer chromatography was performed on silica gel 60 plates with fluorescence indicator (254 nm, Merck KGA). Column chromatography was performed on silica gel 60 (40–63 μm, Macherey-Nagel GmbH & Co. KG) or silica gel C18 end-capped (0.035 mm, 200 mesh, Carl Roth) using solvents of technical grade.

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**Cell lines and culture.** The STF1-169 cell line (HLA-A*0301, HLA-B*1516, HLA-C*1402) was kindly provided by Dr. Henri de la Salle, University of Strasbourg, France, and cultured in DMEM (Gibco) supplemented with 10% fetal calf serum (FCS, Gibco) at 37 °C and 5% CO2. Adherent FreeStyle™© 293-F cells (HEK293-F cells) were cultured in DMEM (Gibco) supplemented with 10% FCS at 37 °C and 5% CO2 and in FreeStyle™© 293 Expression Medium (Gibco) at 37 °C, 8% CO2, and 125 rpm for suspension culture.

**Transfection of TAP2-deficient STF1-169 cells.** 1 × 106 STF1-169 cells per well were seeded in 12-well plates. After 16 h, cells were transfected stably and transiently using Lipofectamine LTX (Thermo Fisher Scientific). 1.5 µg DNA and 1.5 µl Plus reagent were mixed in 100 µl Opti-MEM (Gibco). 2.5 µl Lipofectamine LTX were mixed with 100 µl Opti-MEM. For transfection in a 6-well format, the number of cells and transfection mixture were applied. The two solutions were mixed and incubated at 30 min at RT. After medium exchange, the transfection solution was added dropwise. 5.5 h after transfection, the medium was exchanged for DMEM supplemented with 10% FCS. Cells used for incorporation of UAAs were further supplemented with 0.25 mM BocK or 20 nM PCK along with the medium change after transfection.

**Transfection of HEK293-F cells.** 1.5 × 106 HEK293-F cells were seeded in 300 ml FreeStyle® © 293 Expression Medium. After 24 h, cells were transfected using PEI. 300 µg DNA and 1.2 ml PEI (1 mg/ml) were dissolved separately in 10 ml Opti-MEM I (1X) + GlutaMAX™ reduced serum medium (Gibco). The solutions were mixed and incubated for 30 min at RT. After medium exchange, the transfection solution was added dropwise. 5.5 h after transfection, the medium was exchanged for DMEM supplemented with 10% FCS. Cells used for incorporation of UAAs were further supplemented with 20 nM PCK. The cells were harvested 30 h after transfection.

**In-gel fluorescence and immunoblotting.** The transfected cells of one 6-well plate were harvested and lysed in 30 µl Pierce RIPA buffer (Thermo Fisher Scientific) supplemented with 1% benzoxane (Novagen, EMD Chemicals) and subsequently incubated for 2 h at RT. Sodium dodecyl sulfate (SDS)-buffer was added to a final concentration of 62.5 mM Tris/HC1, pH 6.8, 10 mM β-mercaptoethanol, 2% SDS, 0.02% bromophenol blue, and 10% glycerol. 30 µl of sample were loaded on a 10% SDS-PA-gel (Laemml) and run for 1 h at 125 V. In-gel fluorescence was detected using a Fusion FX (Vilber) at λex/λem 295/395 for mVenus detection. Protein samples used solely for immunoblotting were heated for 10 min at 62°C. Blotted PVDF membranes were blocked with 5% milk in TBS-T and incubated with anti-TAPI 1483 (hybridoma supernatant generated in-house, 1:1000) and anti-β-actin (Sigma, clone AC-74, E6000). Anti-mouse IgG (Fc specific)-Peroxidase conjugate (Sigma, 1:20000) was used as secondary antibody. For detection, membranes were incubated with Clarity Western ECL reagent (BioRad) or LumiGLO® Peroxidase Chemiluminescent Substrate Kit (seracare), and chemiluminescence was measured with a Fusion FX (Vilber). Quantification of signal intensities was done with ImageJ (1.52a).

**Purification of TAP.** For the orthogonal purification of coreTAP complexes, all steps were carried out on ice, and all buffers were adjusted to pH 7.4. The transiently and harvested HEK293-F cells were solubilized for 1.5 h in buffer 1 (20 mM HEPES/NaOH, 200 mM NaCl, 50 mM KCl, and 15% (v/v) glycerol) supplemented with 10 mM imidazole, 1× PI-mix HP (Serva), and 2% (w/v) glyco-digienin (GDN, Anatrace). The samples were centrifuged for 30 min at 12,000 × g and 4 °C. The proteins were bound to 200 µl Ni-NTA Sepharose 6 Fast Flow (GE Healthcare) for 2 h. The beads were washed twice for 15 min with buffer 1 supplemented with 10 mM imidazole and 0.05% GDN. To elute the proteins, the beads were incubated for 30 min in buffer 1 supplemented with 200 mM imidazole and 0.05% GDN. The eluate was used 3 h with 200 µl high-capacity streptavidin agarose resin (Thermo Fisher Scientific). The beads were washed twice for 15 min in buffer 1 supplemented with 0.05% GDN. Subsequently, bound proteins were eluted for 45 min in buffer 1 supplemented with 2.5 mM biotin and 0.05% GDN. The eluate was frozen in liquid nitrogen and stored at −80°C.

**Light activation.** Growth medium was replaced by preheated PBS (37 °C) supplemented with 10% FCS. Then, the culture plates (6- or 12-well) were placed on a transilluminator (312 nm, 12 mW/cm²) and illuminated three times for 2 min with 2 min breaks in between to prevent the medium from overheating. After illumination, PBS was replaced by DMEM supplemented with 10% FCS. The cells were cultured in the dark at 37 °C and 5% CO2 until further use.

**MHC I surface presentation.** MHC I surface expression was analyzed by flow cytometry. TAP2-deficient STF1-169 cells were harvested 18 to 24 h after transfection with different TAP constructs. All further steps were carried out on ice. The cells were washed in FACS-buffer (2% FCS in PBS, ice-cold) and

**Conclusions**

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centrifuged for 5 min at 300 × g at 4 °C. After discarding the supernatant, cells were blocked for 15 min by 5% BSA in FACS-buffer. Subsequently, the cells were washed twice with FACS-buffer and stained for 20 min with 0.5 µl APC/Fire750 and human HLA-A, B, C (clone W6/32; BioLegend) in 50 µl FACS-buffer. Finally, cells were washed and resuspended in FACS-buffer for analysis by flow cytometry. Data was recorded by a FACSComp II (BD) and processed using FlowJo V10 software.

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Correspondence and requests for materials should be addressed to R.Té.

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