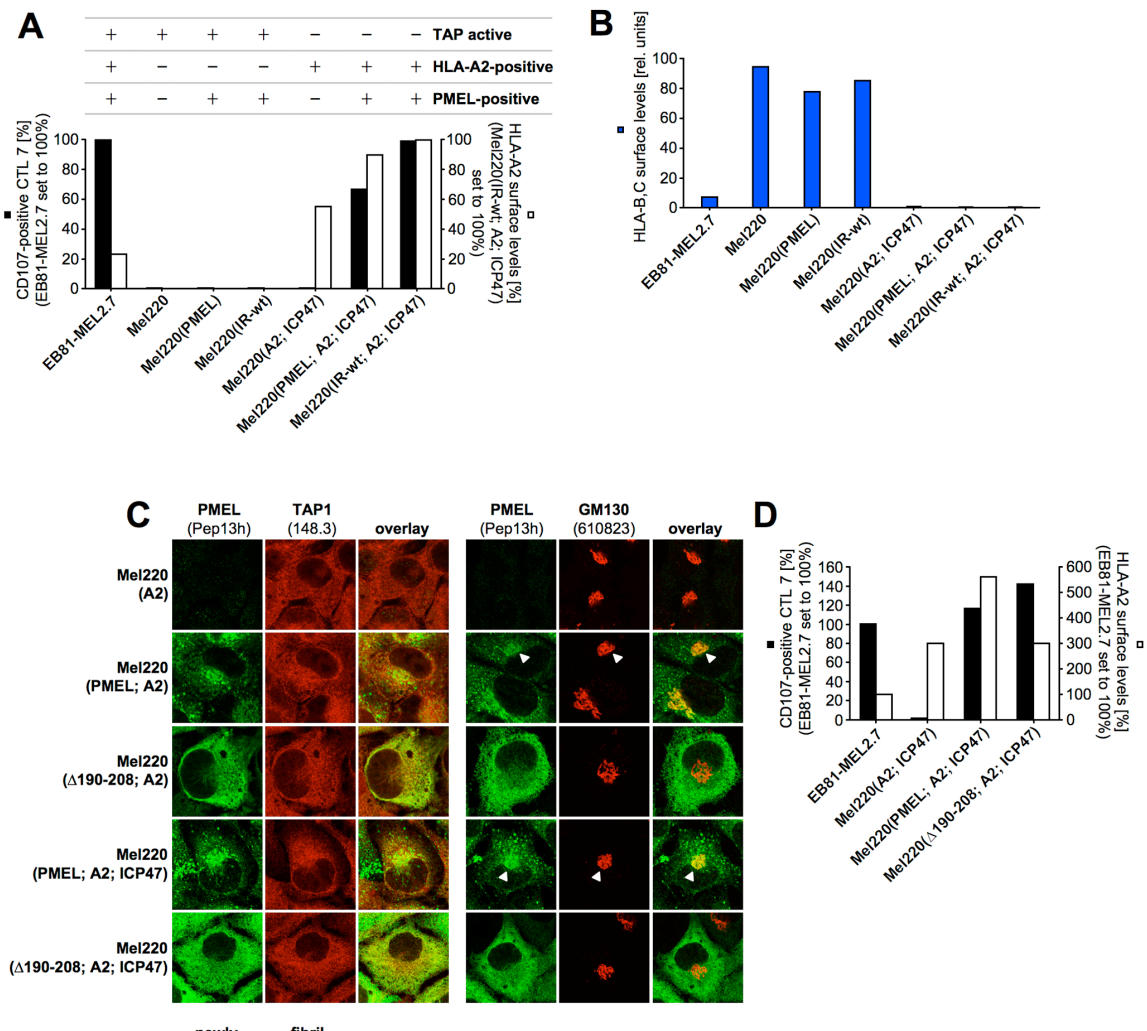
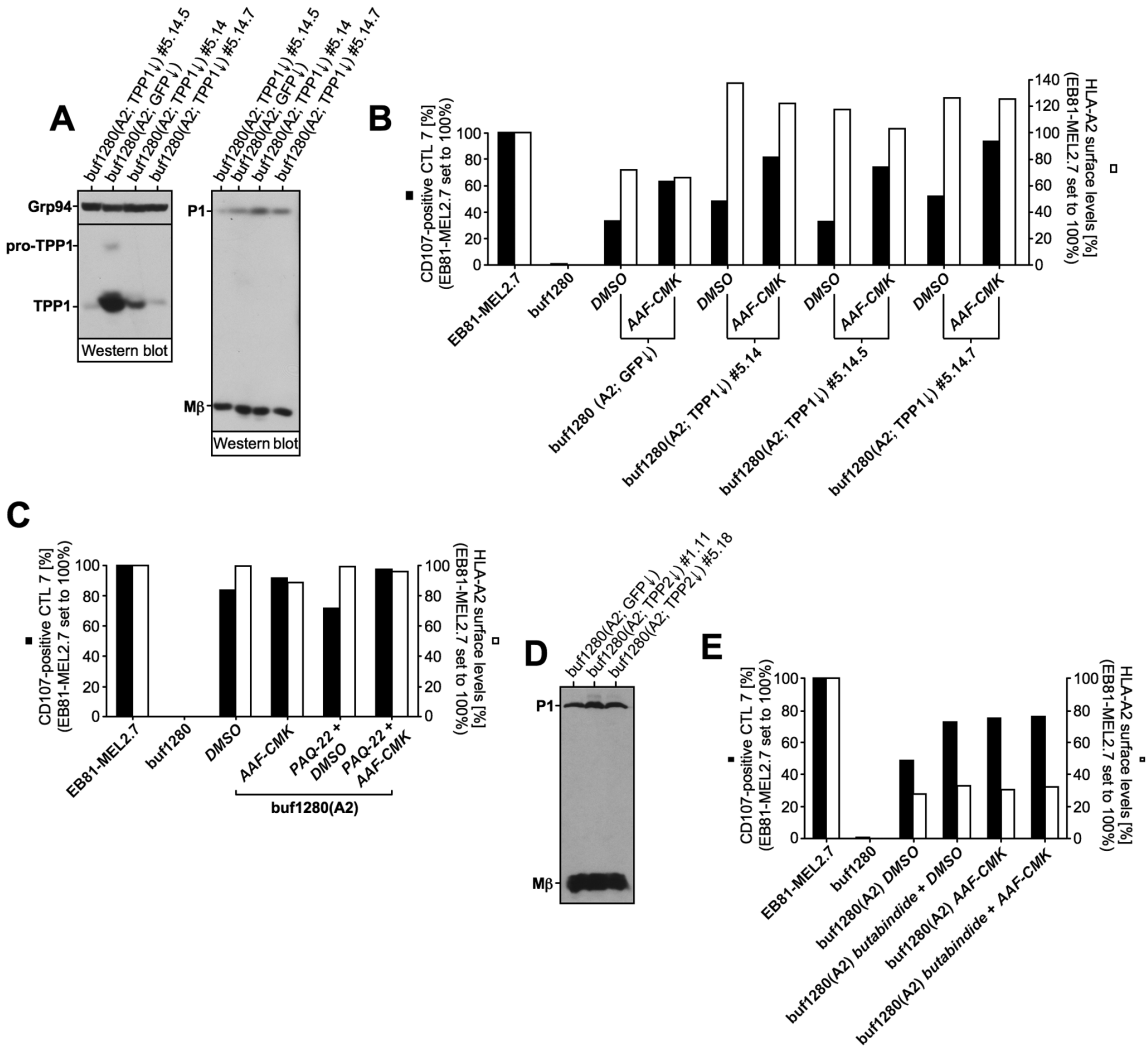
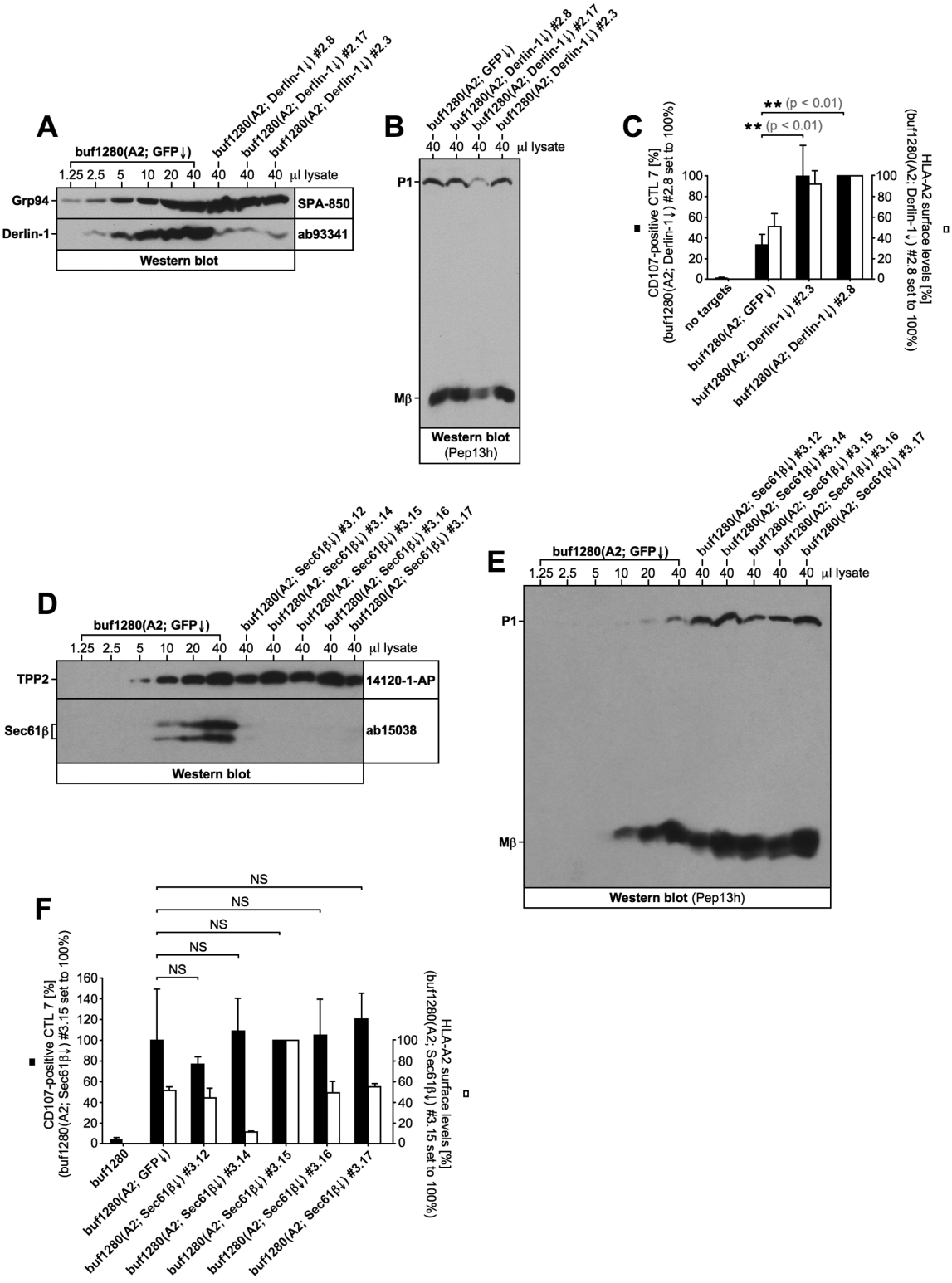


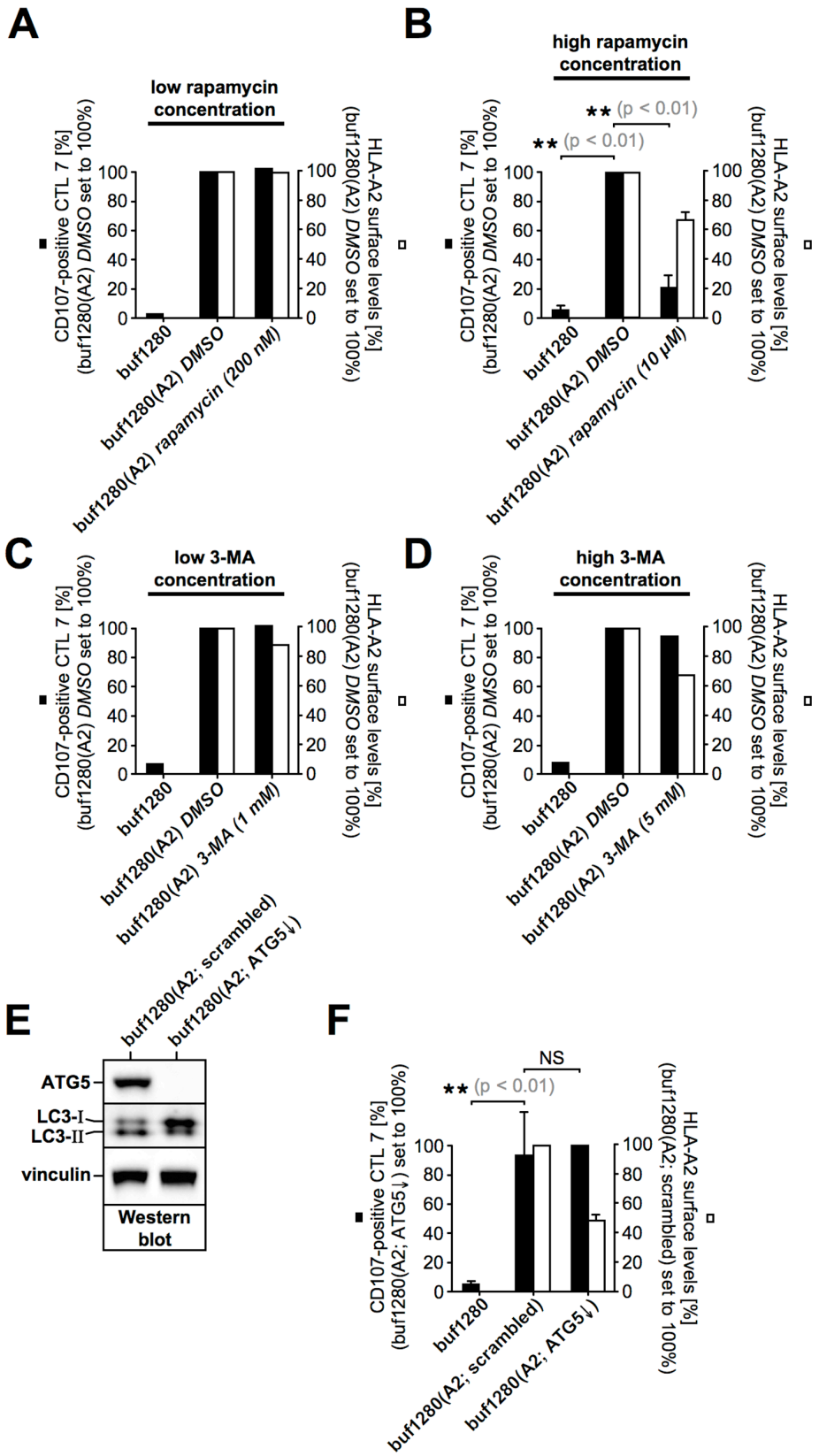
Supplemental Figure S1, TAP-independent PMEL$_{209-217}$ presentation does not require ER export of PMEL or melanosomal fibril formation (A) T cell activation of clone CTL7 was measured in response to autologous tumor cells (EB81-MEL2.7) or various stably transduced Mel220-derived cell lines. As indicated, some of the Mel220 cell lines express wildtype PMEL or PMEL mutant IR-wt, which does not form fibrils in melanosomes (Leonhardt et al, 2010). Moreover, some Mel220 cell lines co-express HLA-A2 (A2) and the TAP inhibitor ICP47. Presentation of PMEL$_{209-217}$ requires expression of both PMEL and HLA-A2. CTL7 activation (*black bars*) and HLA-A2 surface levels on target cells (*white bars*) are depicted as in Fig. 1B. **(B)** Unlike HLA-A2, HLA-B,C alleles are highly TAP-dependent. To demonstrate ICP47-mediated TAP-inhibition in the Mel220 system, HLA-B,C surface levels on target cells were determined by flow cytometry (antibody 4E) and are shown as a bar diagram. **(C)** Immunofluorescence labeling of the indicated Mel220 derivative cell lines expressing wildtype PMEL or the ER-retained PMEL mutant Δ190-208 (Leonhardt et al, 2010) and additionally stably transduced with either HLA-A2 (A2) alone or with both HLA-A2 and the TAP inhibitor ICP47. Cells were labeled with antibodies against TAP (148.3) (here used as an ER marker), GM130 (610823) (a Golgi marker), and PMEL (Pep13h specifically recognizes newly synthesized but not mature PMEL (Leonhardt et al, 2013)). Note that PMEL mutant Δ190-208 is exclusively localized to the ER, while wildtype PMEL distributes in later secretory and endocytic compartments partially overlapping with GM130 staining (*white arrowheads*). **(D)** T cell activation of clone CTL7 was measured in response to autologous tumor cells (EB81-MEL2.7) or various stably transduced Mel220-derived cell lines. As indicated, some of the Mel220 cell lines express wildtype PMEL or the ER-retained PMEL mutant Δ190-208 (Leonhardt et al, 2010). All Mel220 cell lines co-express HLA-A2 (A2) and the TAP inhibitor ICP47. CTL7 activation (*black bars*) and HLA-A2 surface levels on target cells (*white bars*) are depicted as in Fig. 1B. **(E)** Immunofluorescence labeling of the indicated Mel220 derivative cell lines co-expressing HLA-A2 (A2) with either wildtype PMEL or the signal sequence-deleted PMEL mutant ΔSS. Cells were labeled with antibodies against post-Golgi PMEL (HMB45) (the antibody detects a sialylated epitope and mostly recognizes mature fibrillar PMEL) and antibodies recognizing the N-terminus of newly synthesized PMEL (PMEL-N). Note that cytosolic PMEL is not labeled with antibody HMB45, because the protein is not sialylated.



Supplemental Figure S2, The $PMEL_{209-217}$ epitope is degraded by TPP2, not by TPP1 or puromycin-sensitive amino peptidase (PSAP) (A) A buf1280(A2; GFP↓) control lysate was loaded on an SDS-PAGE alongside lysates derived from TPP1-silenced buf1280(A2; TPP1↓) clone #5.14 (shRNA construct #5), and clones #5.14.7 and #5.14.5 (both derivatives of clone #5.14 additionally transduced with shRNA construct #3). Expression of TPP1 (*left panel*) and PMEL (*right panel*) was analyzed by Western blotting. The PMEL ER form (P1) and the PMEL-Mβ fragment are labeled. **(B)** T cell activation of clone CTL7 was measured in response to autologous tumor cells (EB81-MEL2.7) or the TPP1-silenced buf1280(A2; TPP1↓) clones shown in Suppl. Fig. S2A. CTL7 activation (*black bars*) and HLA-A2 surface levels on target cells (*white bars*) are depicted as in Fig. 1B. **(C)** T cell activation of clone CTL7 was measured in response to autologous tumor cells (EB81-MEL2.7) or TAP-deficient buf1280(A2) cells treated with the puromycin-sensitive aminopeptidase (PSAP) inhibitor PAQ-22 (10 μM) or the tripeptidyl peptidase 2 inhibitor AAF-CMK (20 μM). CTL7 activation (*black bars*) and HLA-A2 surface levels on target cells (*white bars*) are depicted as in Fig. 1B. **(D)** A buf1280(A2; GFP↓) control lysate was loaded on an SDS-PAGE gel alongside lysates derived from TPP2-silenced clonal cell lines buf1280(A2; TPP2↓) #1.11 (shRNA construct #1) and #5.18 (shRNA construct #5). PMEL expression was analyzed by Western blotting. The PMEL ER form (P1) and the PMEL-Mβ fragment are labeled. **(E)** T cell activation of clone CTL7 was measured in response to autologous tumor cells (EB81-MEL2.7) or TAP-deficient buf1280(A2) cells treated with the TPP2 inhibitor AAF-CMK (20 μM) and/or the TPP2 inhibitor butabindide (100 μM). CTL7 activation (*black bars*) and HLA-A2 surface levels on target cells (*white bars*) are depicted as in Fig. 1B.



Supplemental Figure S3, PMEL$_{209-217}$ presentation is independent of Derlin-1 and Sec61β (A, B) The indicated amounts of a buf1280(A2; GFP↓) control lysate were loaded on an SDS-PAGE gel together with 40 µl of a lysate derived from Derlin-1-silenced buf1280(A2; Derlin-1↓) clones #2.3, #2.8, and #2.17 (shRNA construct #2). **(C)** CTL7 degranulation measured in response to buf1280(A2) cell lines stably transduced with Derlin1-specific shRNA construct #2 (buf1280(A2; Derlin-1↓) #2.3 and #2.8) or a GFP-specific control shRNA construct (buf1280(A2; GFP↓). Error bars represent the standard deviation from the mean of three independent experiments. A One-way ANOVA with Dunnett's post test was performed for statistical evaluation. **(D, E)** The indicated amounts of a buf1280(A2; GFP↓) control lysate were loaded on an SDS-PAGE gel together with 40 µl of a lysate derived from Sec61β-silenced buf1280(A2; Sec61β↓) clones #3.12, #3.14, #3.15, #3.16, and #3.17 (shRNA construct #3). **(F)** CTL7 degranulation measured in response to buf1280(A2) cell lines stably transduced with Sec61β-specific shRNA construct #3 (buf1280(A2; Sec61β↓) #3.12, #3.14, #3.15, #3.16, and #3.17) or a GFP-specific control shRNA construct (buf1280(A2; GFP↓). Error bars represent the standard deviation from the mean of three independent experiments. A One-way ANOVA with Dunnett's post test was performed for statistical evaluation (NS = not significant).



Supplemental Figure S4, TAP-independent PMEL$_{209-217}$ presentation is unlikely to depend on conventional macroautophagy (A-D) CTL7 degranulation is shown as in Fig. 1B. CTL7 activation (*black bars*) and HLA-A2 surface levels on target cells (*white bars*) are depicted in bar diagrams. The experiment depicted in Suppl. Fig. S4B was repeated three times. Error bars represent the standard deviation from the mean of these three independent experiments. A One-way ANOVA with Dunnett's post test was performed for statistical evaluation. **(E)** Western blot comparing equal amounts of lysates derived from buf1280(A2) cells stably transduced with an shRNA construct specifically targeting the key autophagy gene ATG5 or a scrambled control construct. **(F)** CTL7 degranulation measured in response to buf1280(A2) cell lines stably transduced with an ATG5-specific shRNA construct (buf1280(A2; ATG5↓)) or a scrambled control shRNA construct (buf1280(A2; scrambled). Error bars represent the standard deviation from the mean of three independent experiments. Surface-HLA-A2 levels are shown as white bars.