Mice completely lacking immunoproteasomes display major alterations in antigen presentation.

Authors:

Eleanor Z Kincaid¹, Jenny W Che¹, Ian York², Hernando Escobar³, Eduardo Reyes-Vargas³, Julio C. Delgado³, Raymond M Welsh¹, Margaret L. Karow⁴, Andrew J. Murphy⁴, David M. Valenzuela⁴, George D. Yancopoulos⁴ and Kenneth L Rock¹

¹Department of Pathology, UMass Medical School. Worcester, MA
²CDC/NCIRD, Influenza Division-Molecular Virology and Vaccine Branch, Atlanta GA.
³ARUP Institute for Clinical and Experimental Pathology, Department of Pathology, University of Utah School of Medicine, Salt Lake City, UT
⁴Regeneron Pharmaceuticals Inc., Tarrytown, NY 10591

Correspondence should be addressed to K.R. (kenneth.rock@umassmed.edu).
Steady-state ubiquitinylation in TKO splenocytes. Western blots of serial dilutions (20µg, 10µg and 5µg) of whole cell lysates from WT and TKO splenocytes. After probing with (a) anti-ubiquitin, blot was stripped with Millipore Re-blot and reprobed with (b) GAPDH as a loading control. (Representative of 2 experiments.)
**Supplementary Figure 2.**

**Cell numbers in WT and TKO thymus and spleen.** Organs were harvested from mice of the indicated strains. Thymocytes and splenocytes (after red blood cell lysis) were counted. Thymocytes were stained with anti-TCRβ, anti-CD4, and anti-CD8α. Splenocytes were stained with anti-B220, anti-CD3 molecular complex and anti-CD4, and anti-CD8α. Scatter plots indicate individual animals with mean +/- SD indicated. Bar graphs indicate mean +/- Asterisks indicate a significant difference from WT (P < 0.05, student’s t-test, n of 6 or 9 for each strain, combined from 2 experiments).
Supplementary Figure 3.

**Stability of pMHC I complexes on the cell surface of TKO splenocytes.** Splenocytes from WT and TKO animals were treated with 1 µl/ml GolgiPlug (BDBiocience) at 37°C for the indicated times before surface Ab staining and flow cytometry. Data is mean +/- SD of 3 animals from each strain, combined from 3 experiments. Splenocytes from each animal were treated and stained in triplicate, and the mean from each animal was used. H2-K\textsuperscript{b} or H2-D\textsuperscript{b} GMFI was normalized to time 0 (no treatment) for each strain in each experiment. No significant difference was found between WT and TKO samples (two-way ANOVA).

Supplementary Figure 4.

**CD4 responses are normal in LCMV-infected TKO animals.** WT and TKO animals were infected with LCMV, and 9 days later, spleen lymphocytes were analyzed by *in vitro* restimulation followed by surface and intracellular staining. Mean and SD of 18 animals, combined from 4 experiments.
**Supplementary Figure 5.**

**WT hosts do not reject TKO splenocytes.** WT mice were injected with WT and KO cells differentially labeled with CFSE. Mice were bled at the indicated times, and the relative killing of WT and TKO cells was assessed. Mean ±SD from 14 mice, 3 experiments.
Supplemental Table 1. WT H2-D\textsuperscript{p} peptides.

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**Note:** The table lists peptides with mass-to-charge ratios and accession numbers, along with their corresponding protein entry names.
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Supplemental Table 3. WT H2-Kb peptides.

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