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Supporting Information

for

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Anouk C. M. Platteel, Michele Mishto, Kathrin Textoris-Taube, Christin Keller, Juliane Liepe, Dirk H. Busch, Peter M. Kloetzel and Alice J. A. M. Sijts

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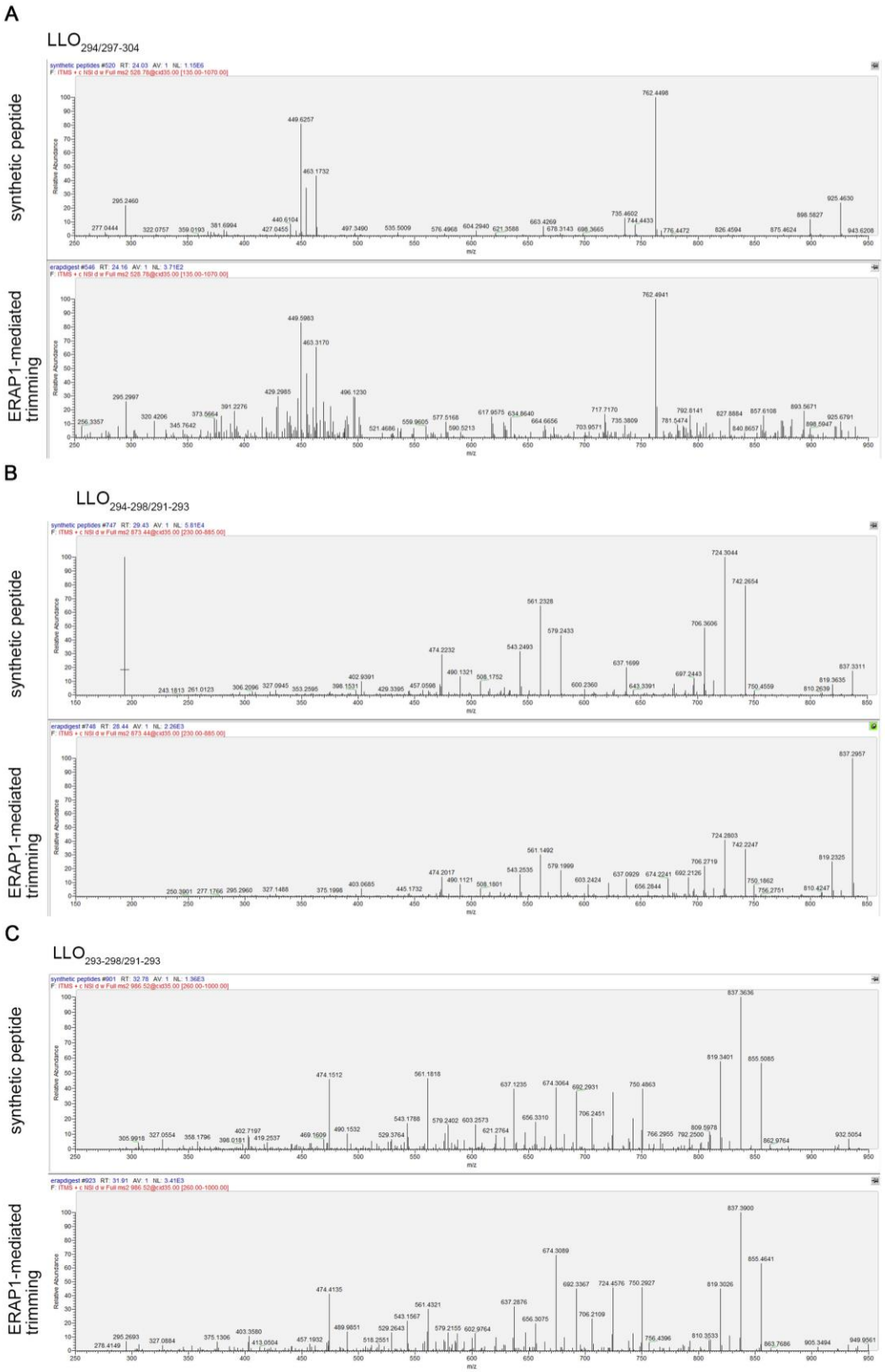
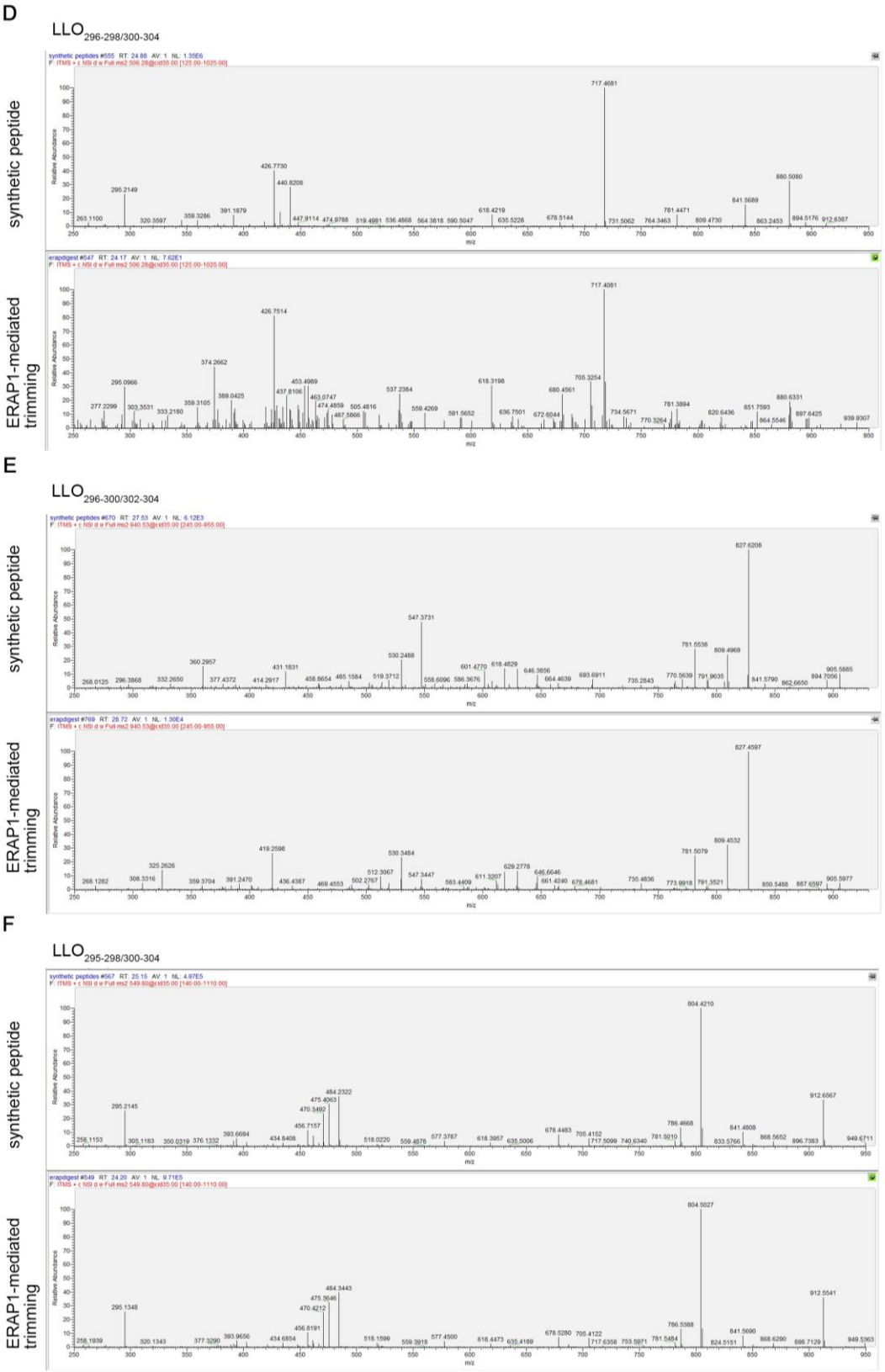
Supplemental Figure 1

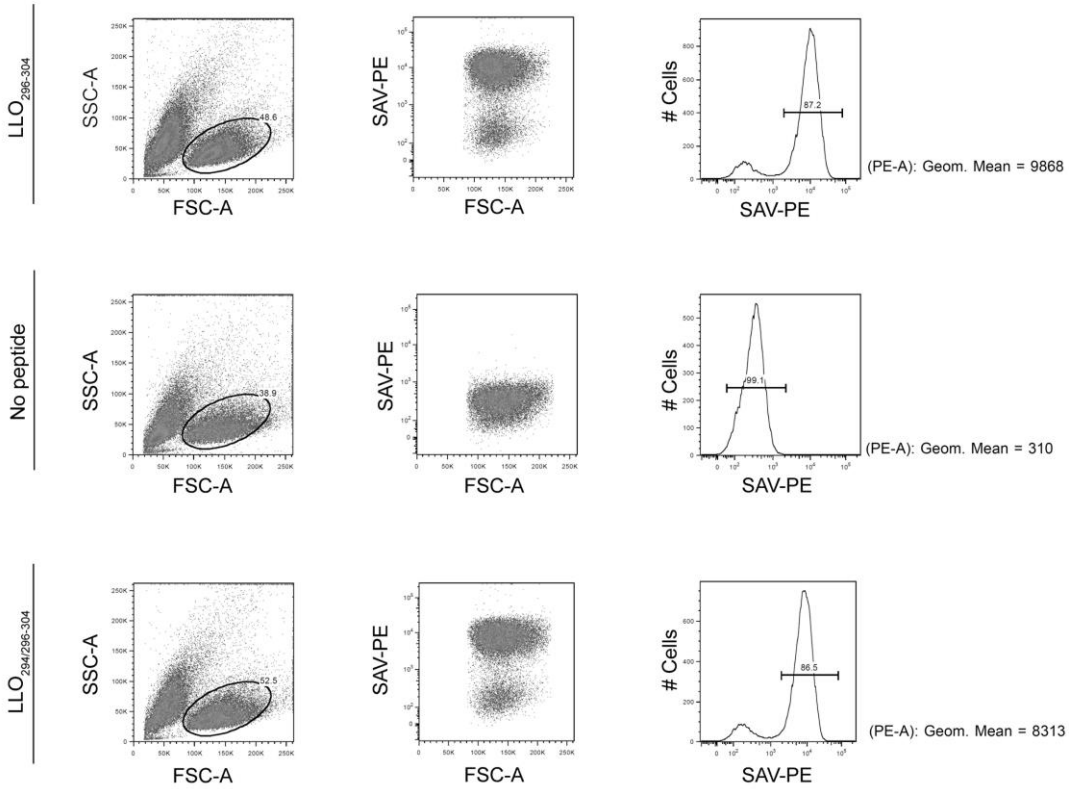




Orbitrap MS/MS spectra of N-terminally trimmed spliced peptides by recombinant ERAP1. A-F) Identification of products of the trimming by recombinant ERAP1 of the $LLO_{291-317}$-derived spliced N-terminal elongated precursors produced *in vitro* by 20S proteasomes is reported. (**A**) ESI fragment ion spectrum (MS/MS) of the doubly charged

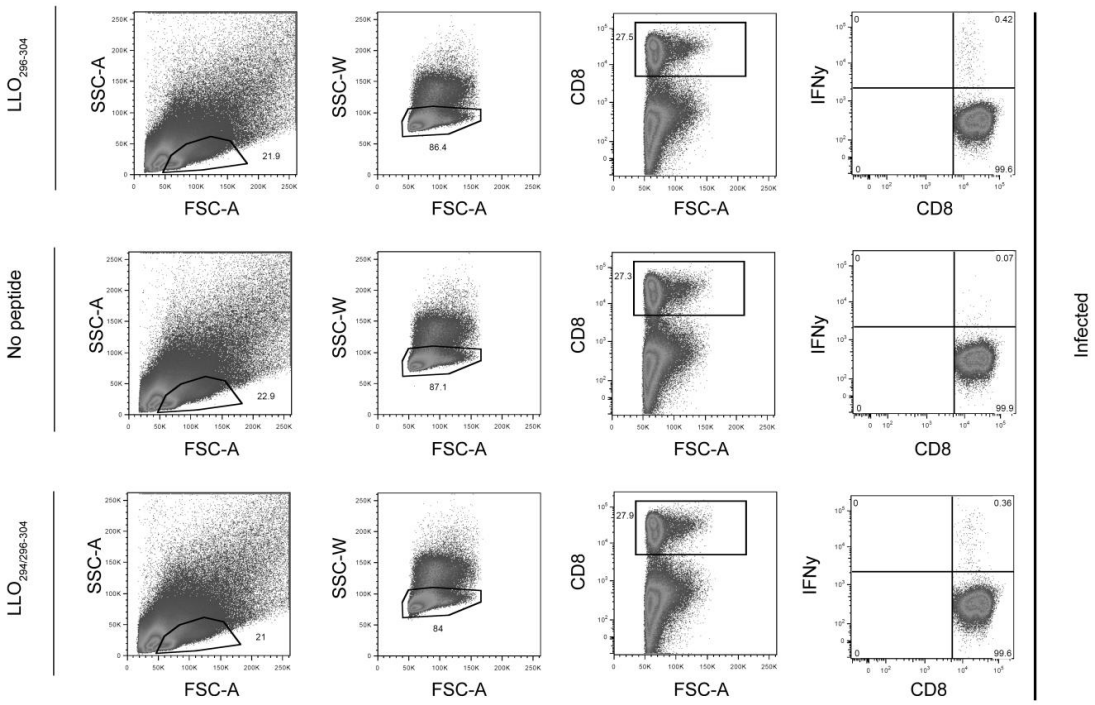
peptides $[M+2H]^{2+}$ $LLO_{294/297-304}$ [S][AYGRQVYL] (*m/z* 528.78, +2) (**B**) the singly charged ions $[M+H]^{1+}$ $LLO_{294-298/291-293}$ [SSVAY][AYI] (*m/z* 873.44, +1) (**C**) $LLO_{293-298/291-293}$ [ISSVAY][AYI] (*m/z* 986.52, +1) (**D**) $LLO_{296-298/300-304}$ [VAY][RQVYL] (*m/z* 506.28, +2) (**E**) $LLO_{296-300/302-304}$ [VAYGR][VYL] (*m/z* 940.52, +1) **(F)** $LLO_{295-298/300-304}$ [SVAY][RQVYL] (*m/z* 549.80, +2) in a synthetic peptide mixture (upper panels in **A-F**) or in a 4 h reaction with the N-terminally extended peptides (**A**) $LLO_{291-294/297-304}$ **(B, C)** $LLO_{291-298/291-293}$ (**D,F**) $LLO_{291-298/300-304}$ and (**E**) $LLO_{291-300/302-304}$ and ERAP1 (lower panels in **A-F**).

Supplemental Figure 2



Gating strategy. RMA-S cells were incubated overnight with synthetic peptide or without peptide. H-2K^b complexes (t = 0) were stained with a conformation-sensitive, biotin-conjugated anti-H-2K^b mAb and with PE-conjugated streptavidin. Immunofluorescence was measured using a FACS Canto II and analyzed with FlowJo software. Representative FACS plots and histograms including the MFI are shown for RMA-S cells loaded with $LLO_{296-304}$, $LLO_{294/296-304}$ or without peptide (t = 0).

Supplemental Figure 3



Gating strategy. C57BL/6 mice were intravenously infected with 2000 CFU *Listeria monocytogenes*. At day 7 post infection, splenocytes were harvested and re-stimulated *ex vivo* with $LLO_{296-304}$, $LLO_{294/296-304}$ or without peptide in the presence of monensin and then stained with fluorochrome-conjugated mAbs for CD8 cell surface expression and intracellular IFNγ. Representative FACS plots, analysed with FlowJo software, including percentages of $IFN\gamma^+$ and $IFN\gamma^-$ $CD8^+$ T cells are shown.