Supporting Information

Belizaire and Unanue 10.1073/pnas.0908583106

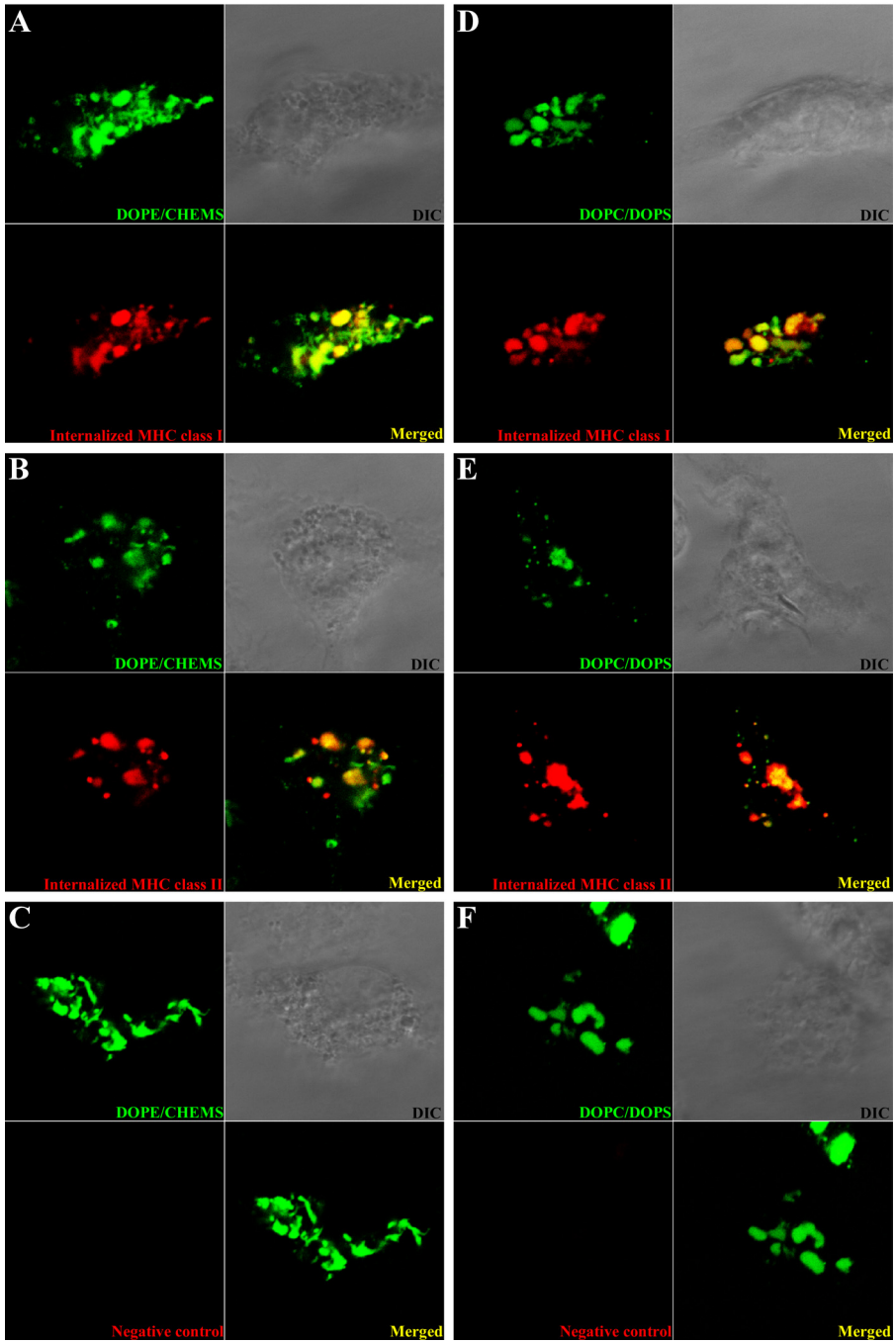

Fig. S1. Internalized MHC class I and II colocalize with dextrans in DOPC/DOPS and DOPE/CHEMS liposomes. Surface-bound MHC class I (*A* and *D*) and class II (*B* and *E*) antibodies on peritoneal Mϕs were internalized at 37 °C along with FITC-dextran encapsulated in DOPE/CHEMS (*A*–*C*) or DOPC/DOPS (*D*–*F*) liposomes. Irrelevant IgG (*C* and *F*) was used as a negative control for MHC internalization. Confocal images were acquired after 12 h of internalization. Similar results were obtained 1–2 h after internalization. (Magnification: ×63 oil, ×4 zoom.)

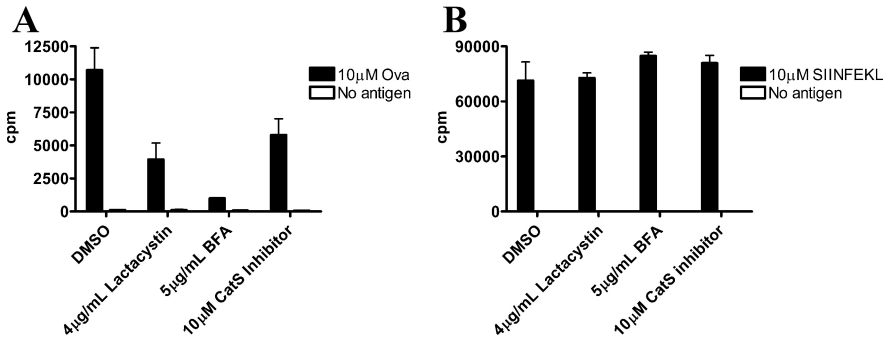

Fig. S2. MHC class I presentation of soluble Ova protein utilizes nascent MHC molecules, proteasomal processing, and CatS. Peritoneal Mϕs from B6 mice were preincubated for 30 min with 4 μg/mL lactacystin, 5 μg/mL BFA, or 10 μM CatS inhibitor before addition of 10 μM soluble Ova (*A*) or SIINFEKL peptide (*B*) for 12–16 h. Cells were washed and cultured with SIINFEKL-specific CD8 T-cell hybridomas overnight, and IL-2 was measured in culture supernatants.

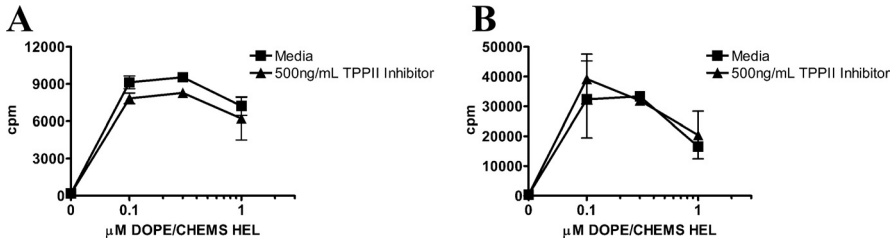

Fig. S3. Inhibition of TPPII activity does not affect MHC class I presentation of HEL in early endosomal liposomes. Peritoneal Mϕs from NOD mice were preincubated for 30 min with 500 ng/mL AAF-CMK before addition of HEL in DOPE/CHEMS liposomes for 12–16 h. Cells were washed and cultured with CD8 (*A*) or CD4 (*B*) T-cell hybridomas overnight, and IL-2 was measured in culture supernatants.

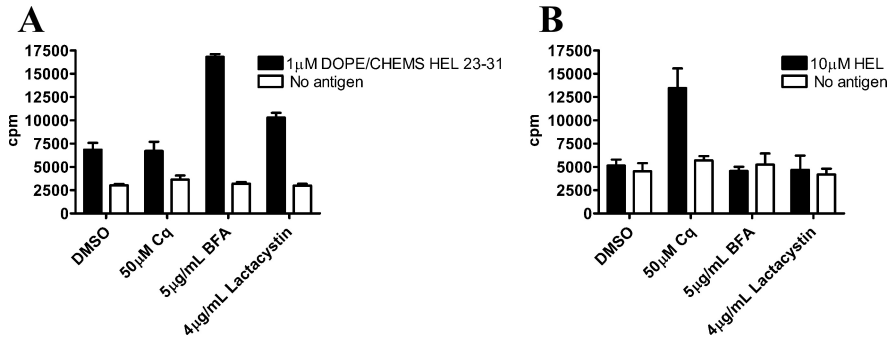

Fig. S4. Chloroquine (Cq) does not affect processing and MHC class I presentation of HEL 23–31 peptide encapsulated in acid-labile liposomes. Peritoneal Mϕs from NOD mice were preincubated for 30 min with 50 μM Cq, 5 μg/mL BFA, or 4 μg/mL lactacystin before addition of HEL 23–31 in DOPE/CHEMS liposomes (*A*) or soluble HEL (*B*) for 12–16 h. Cells were washed and cultured with CD8 T-cell hybridomas overnight, and IL-2 was measured in culture supernatants. (*B*) As a positive control, Cq treatment enables MHC class I presentation of soluble HEL.

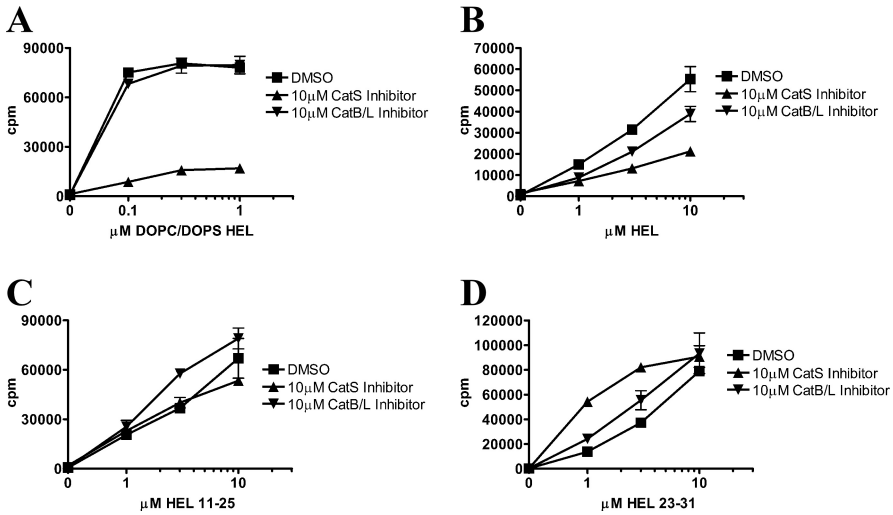

Fig. S5. Inhibition of CatS and CatB/L proteolytic activity blocks MHC class II presentation of HEL. Peritoneal Mϕs from NOD mice were preincubated for 30 min with 10 μM CatS or CatB/L inhibitor before addition of antigen dose curves for 12–16 h. Cells were washed and cultured with CD4 (*A*–*C*) or CD8 (*D*) T-cell hybridomas overnight, and IL-2 was measured in culture supernatants. (*C* and *D*) Cat inhibition has a minimal effect on peptide presentation to CD4 and CD8 T-cell hybridomas, respectively.

Table S1. MHC class I presentation of different exogenous antigens

	Antigen				
Treatment	Soluble Ova	Soluble HEL	DOPE/CHEMS HEL (early endosome)	DOPC/DOPS HEL (late endosome)	HEL 23–31
None	+	−	+	−	+
BFA	Diminished	−	Enhanced	−	+
Lactacystin/epoxomicin	Diminished	−	Enhanced	−	+
TPPII inhibition	n.d.	−	+	−	+
Chloroquine	n.d.	+	Diminished	+	+
CatS inhibition	Diminished	−	Enhanced	−	+
CatB/L inhibition	n.d.	−	+	−	+

+, presentation; −, no presentation; n.d., not determined