SUPPLEMENTARY FIGURES

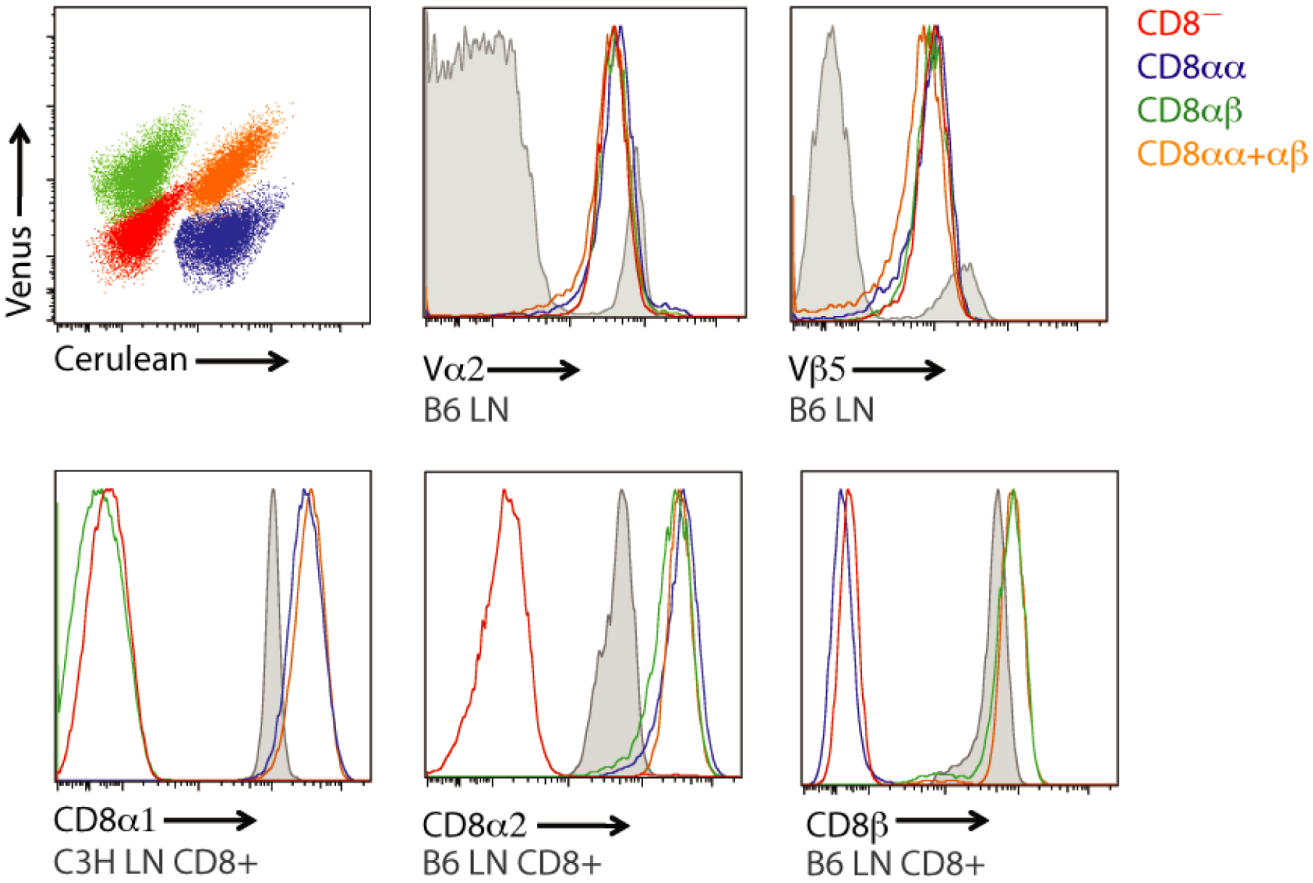

Figure S1

TCR and CD8 expression in transfected and sorted hybridoma cells. Upper panels: Left; epifluorescence profiles of cells expressing CD8αα-Cerulean (blue), CD8αβ-Venus (green), both (orange) or neither (red). Middle and right; expression of Vα2 and Vβ5 TCR subunits in hybridomas (color-coded as above) and CD8-gated B6 lymph node T cells (solid grey). Cells were stained with anti-Vα2 (PerCP-Cy5.5) or anti-Vβ5 (PE-conjugated) antibodies and analyzed by flow cytometry. The relative concentration of TCR per unit surface area was calculated (surface area of hybridomas was 5.8–6.4-fold that of primary LN T cells). The hybridomas had 7–9% of the concentration of TCR of the mature T cells. Lower panels: expression of CD8 subunits in the hybridomas and LN T cells (color coding as above). Based on anti-CD8α1-staining, the hybridomas expressed ~54% the concentration present on the LN T cells, but ~28% based on CD8β expression. The IgG anti-CD8β mAb was directly conjugated to the fluorophore, so is likely to give a more accurate estimate than the IgG anti-CD8α1 (biotinylated, developed with fluorescent streptavidin). The IgM anti-CD8α2, developed with labeled secondary Ab, was unsuitable for comparisons of CD8 surface density between cells of different sizes.

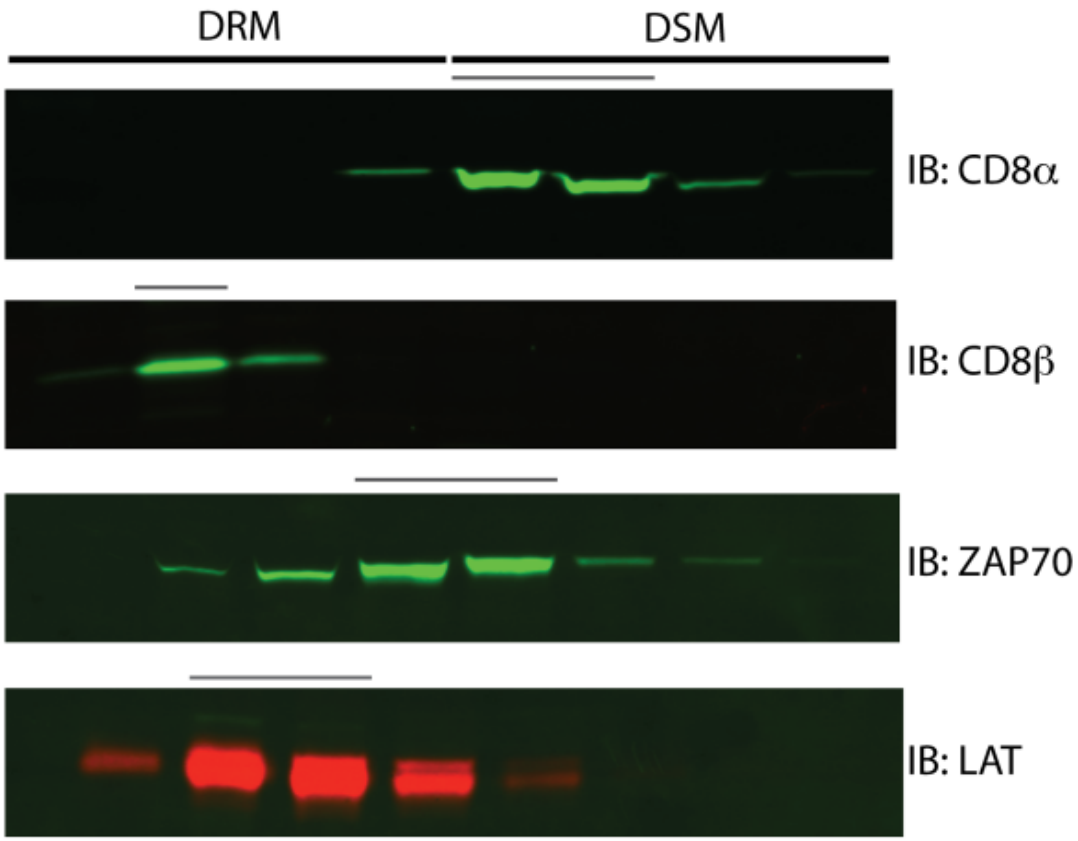

Figure S2
Expressed CD8α and CD8β correctly partition to lipid rafts. Postnuclear supernatants of cells expressing both CD8αα and CD8αβ were separated into fractions containing lipid rafts (DRM, detergent resistant membranes) and non-rafts (DSM, detergent soluble fractions) on Sephacryl columns (Krutikova et al, 2007; Radeva & Sharom, 2004). They were resolved by SDS-PAGE and analyzed by western blotting using antisera against CD8α and CD8β (from Dr. Rose Zamoyska, Edinburgh University), ZAP70 (BDBiosciences) and LAT (Cell Signaling Technology). Membranes were scanned and analyzed using Li-Cor Odyssey infrared imaging system. CD8β, but not CD8α mainly co-fractionates with lipid raft marker LAT. Fractions containing the bulk of each protein are labeled above the corresponding western blot.

Supplemental References

Krutikova MP, Krotov GI, Zgoda VG, Filatov AV (2007) Study of lipid rafts by gel filtration combined with preliminary staining with fluorescently labeled antibodies. *Biochemistry (Mosc)* **Suppl. A, 1:** 219-227

Radeva G, Sharom FJ (2004) Isolation and characterization of lipid rafts with different properties from RBL-2H3 (rat basophilic leukaemia) cells. *Biochem J* **380:** 219-230

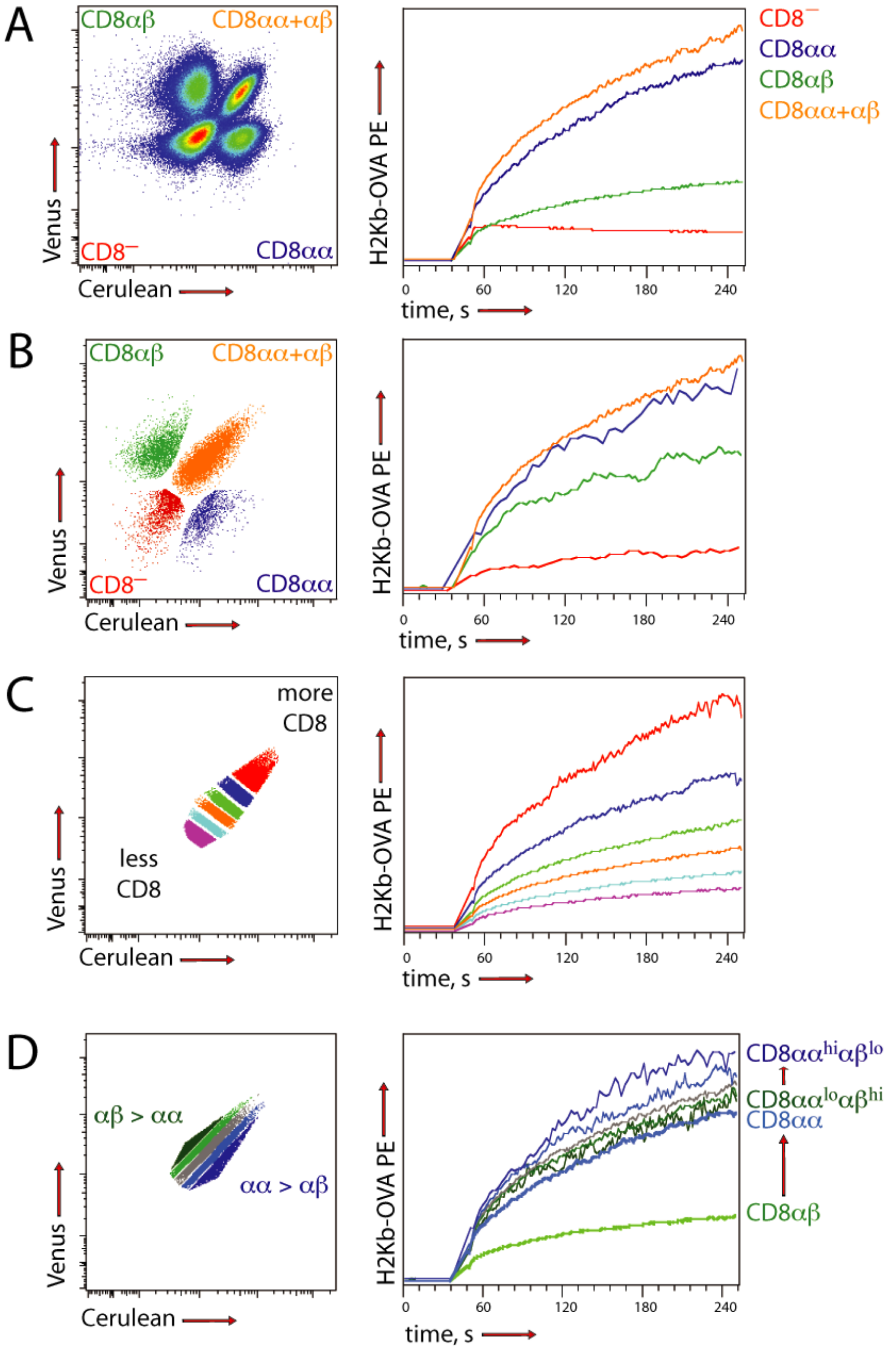

Figure S3

In vitro stimulation of hybridomas with PE labeled H2-K^{b}-OVA tetramers. **(A)** Left: Individual hybridoma clones are easily recognizable in mixed samples. Cells expressing CD8αα-Cerulean (bottom right quadrant), CD8αβ-Venus (top left), both (top right) or neither (bottom left) were mixed and analyzed by flow cytometry. Right: Real time tetramer binding. Mixed hybridomas were allowed to bind OVA tetramer for 3 min in a 37ºC flow cytometry chamber during analysis. Color coding is indicated. **(B)** Real time tetramer binding by hybridomas with differential expression of CD8αα or CD8αβ, due to loss in culture. Binding was done three weeks after sorting for Venus^{+}Cerulean^{+} (CD8αα plus CD8αβ) cells. Cells were gated as in A. **(C)** Tetramer binding is accelerated by increased coreceptor expression. The CD8αα^{+}CD8αβ^{+} population (orange in B) was subgated based on coreceptor expression level, and real time tetramer binding was plotted separately for each gate. Color coding of right panel is same as population colors of left panel. **(D)** Tetramer binding increases as the proportion of CD8αα increases relative to CD8αβ. The CD8αα^{+}CD8αβ^{+} cells from (C) were subgated for increasing ratio of one CD8 species to the other. Color coding of the CD8αα^{lo}αβ^{hi} to CD8αα^{hi}αβ^{lo} populations is as shown in the left panel. The CD8αα and CD8αβ tetramer-binding traces are from (A).

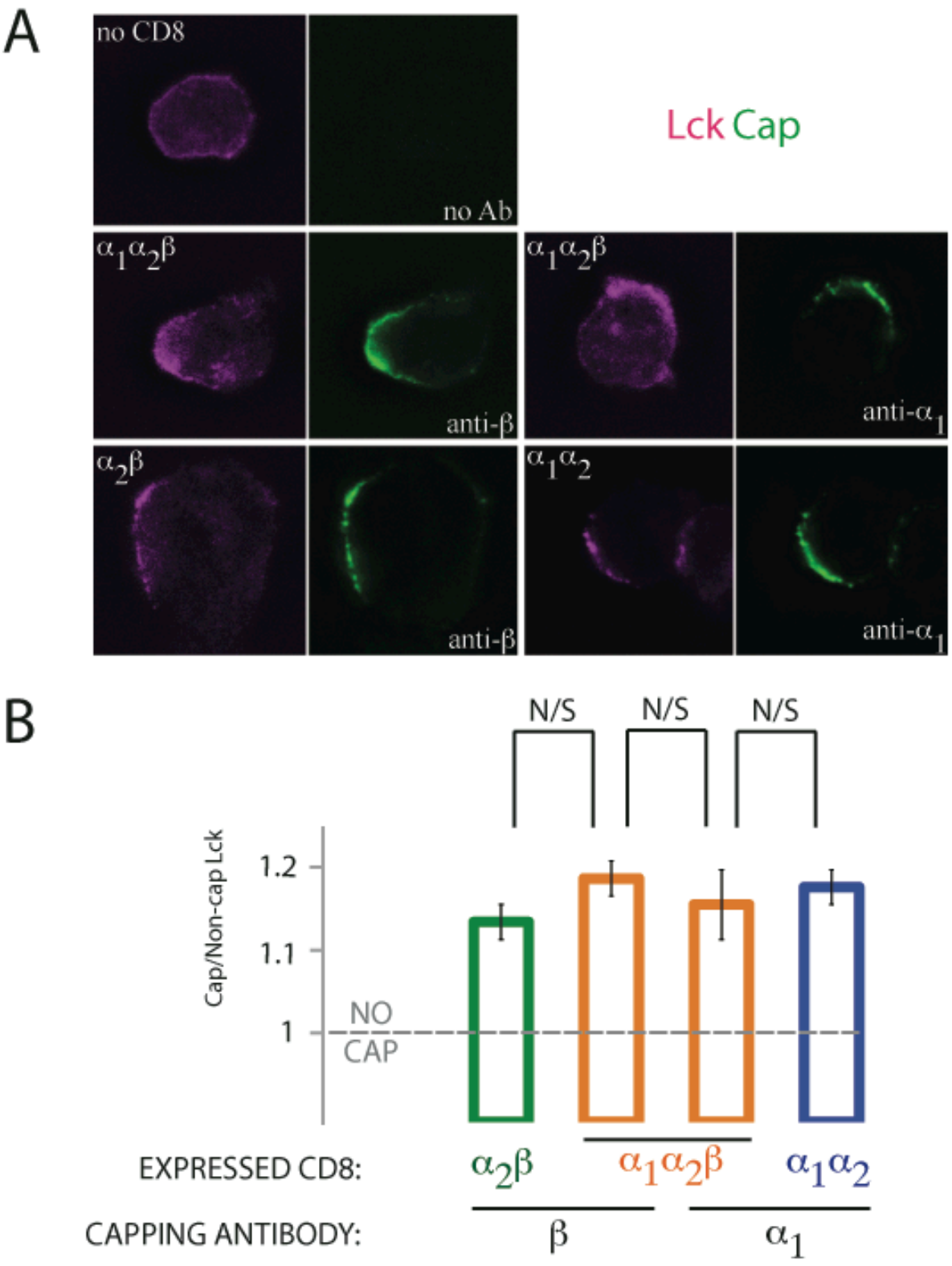

Figure S4

(A) Lck co-caps with both CD8αα and CD8αβ in dual-expressing cells. Hybridomas expressing no CD8, CD8αβ alone (α2β), CD8αα alone (α1α2), or both (α1α2β), were incubated with the indicated biotinylated antibody, and crosslinked with streptavidin-alexa594 (shown in green, anti-Lck staining in purple. **(B)** Ratio of Lck in the cap to uncapped regions of the cell surface demonstrates that Lck is not preferentially associated with CD8αα or CD8αβ in the cells expressing both species. Error bars are ±s.e.m. for n=15 for each cell type. N/S: not significant.

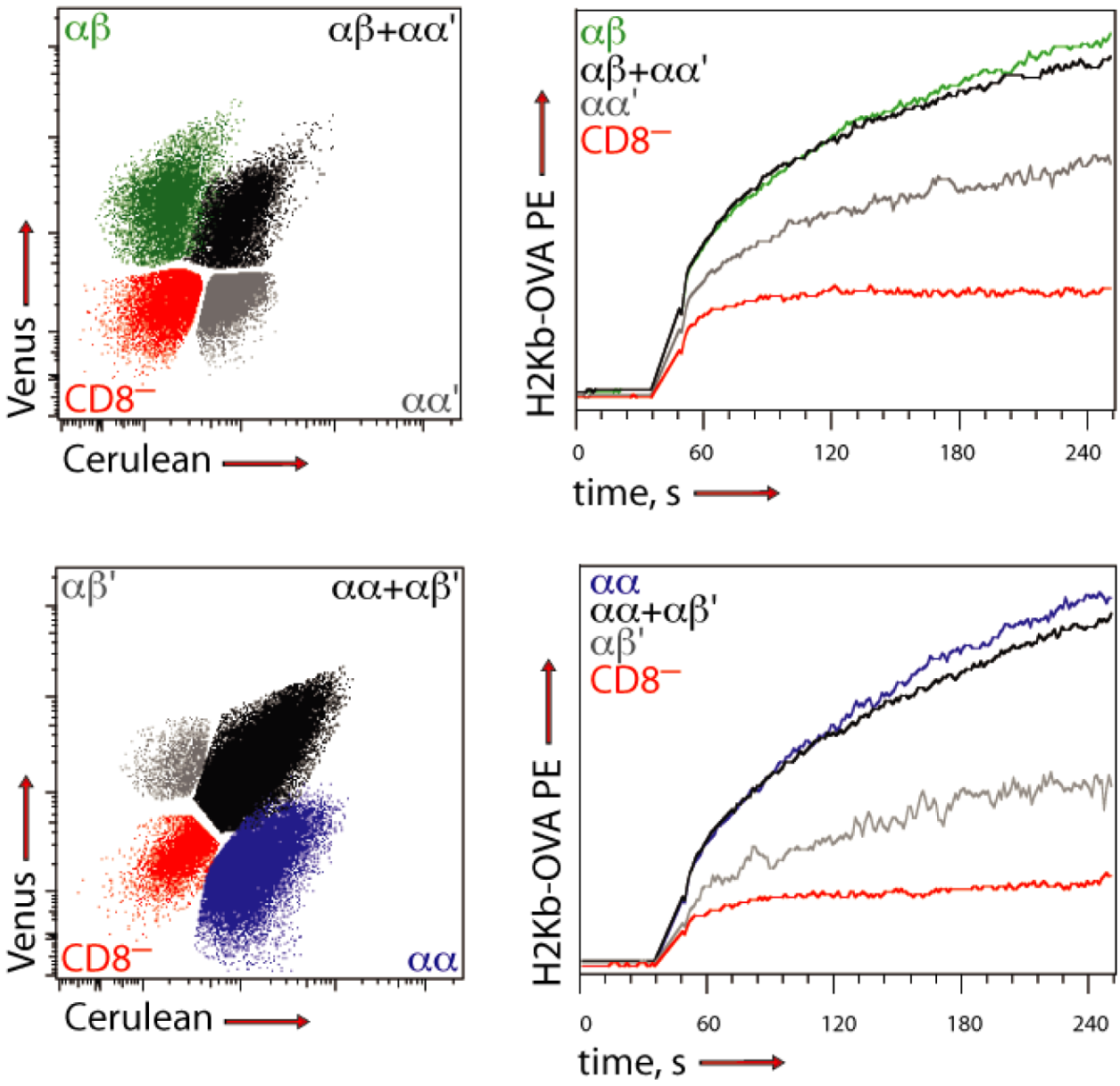

Figure S5

Co-expression of MHC class I binding mutant of CD8αα-Cerulean with binding-competent CD8αβ-Venus, or of class I binding mutant of CD8αβ-Venus with binding-competent CD8αα-Cerulean, does not affect the binding of PE labeled K^b-OVA tetramers. Experimental setup as in Fig. S3B. Top panel, tetramer binding to cells expressing CD8αβ-Venus (green) is compared with that of cells expressing both CD8αβ-Venus and MHC binding mutant of CD8αα-Cerulean (CD8αα': black), as well as CD8αα'-Cerulean alone (gray) and no CD8 (red). Bottom panel, tetramer binding to cells expressing CD8αα-Cerulean (blue) is compared with that of cells expressing both CD8αα-Cerulean and MHC binding mutant of CD8αβ-Venus (CD8αβ'-Venus: black), CD8αβ'-Venus alone (gray) and no CD8 (red).