Supplemental Data

ERAAP Synergizes with MHC Class I Molecules to Make the Final Cut in the Antigenic Peptide Precursors in the Endoplasmic Reticulum

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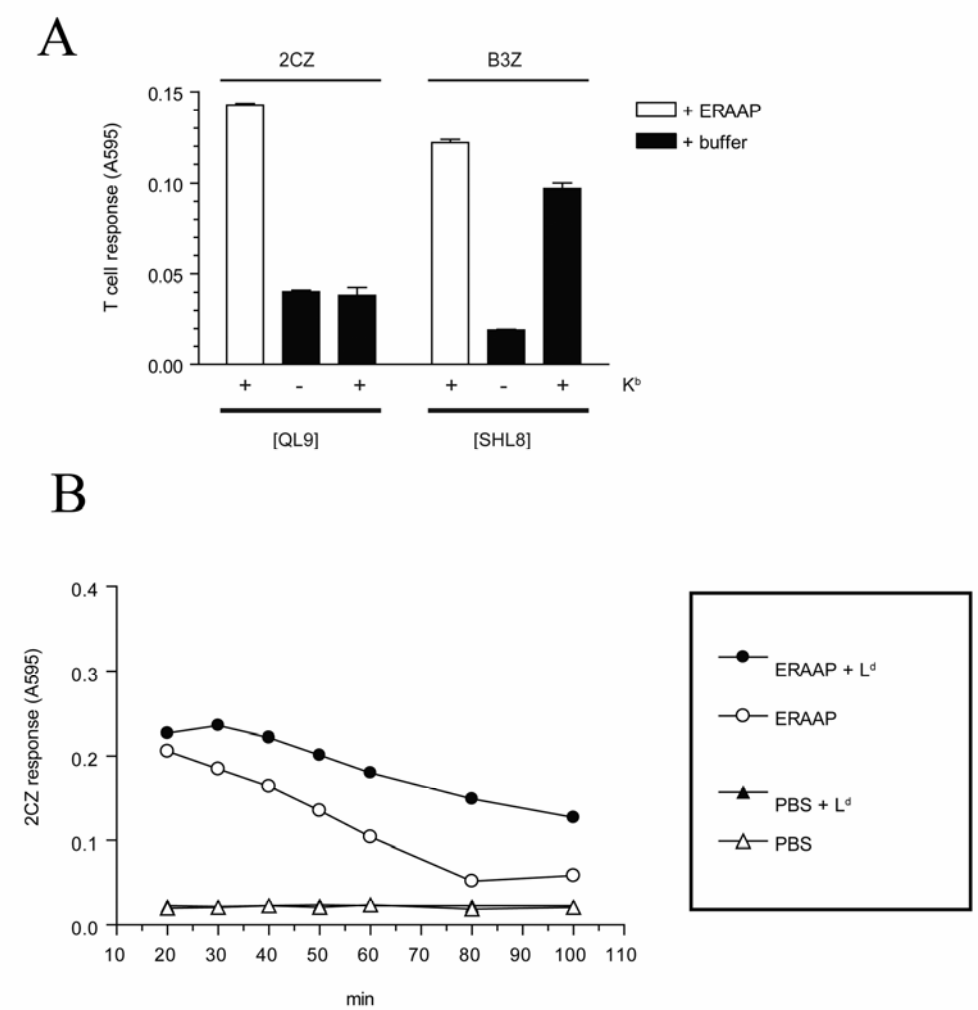

Figure S1. The Degredation of the Peptides by Recombinant ERAAP and Peptide-Specific Protection by MHC I

(A) Recombinant ERAAP trims both QL9 and SHL8 synthetic peptides, and K^b MHC protects SHL8 but not QL9 from degradation. Synthetic QL9 or SHL8 peptides were incubated with or without rERAAP, in the presence or absence of K^b-Ig fusion protein. The remaining QL9 or SHL8 peptides were measured by 2CZ or B3Z responses respectively in the presence of appropriate APCs.

(B) Kinetics of trimming the N-terminally extended X7[QL9] synthetic peptide. Recombinant L^d delays the peptide degradation by rERAAP. The X7[QL9] peptide was incubated with or wihout rERAAP, in the presence or absence of L^d-Ig fusion protein. Each reaction was stopped by adding acetic acid at indicated time points, and remaining QL9 peptides were detected in a T cell assay in the presence of APCs expressing L^d.

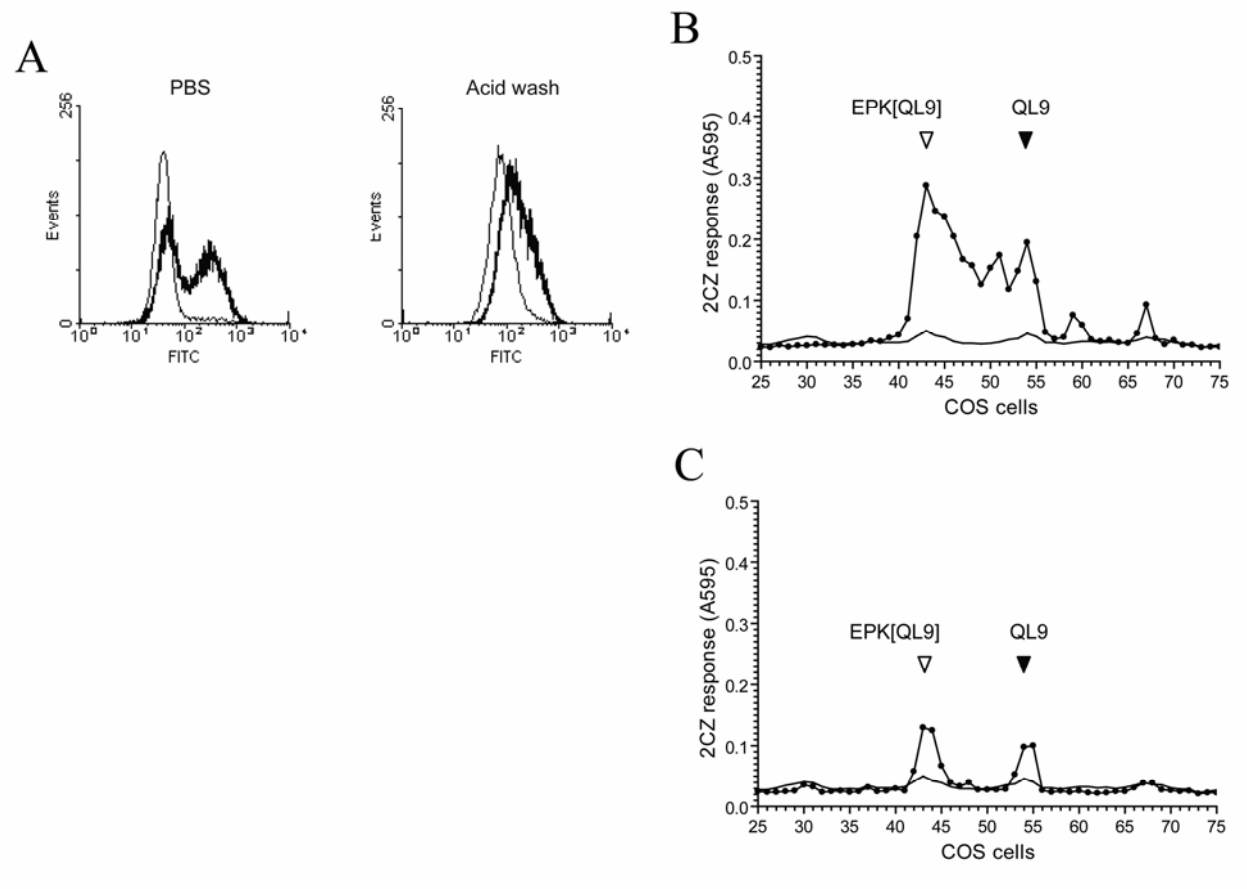

Figure S2. Intracellular L^d Is Bound to the Extended EPK[QL9] Peptide
The COS cells were transfected with ES-X3-EPK[QL9], L^d and ICP47. After two days the cells were treated with mild-acid and washed extensively before peptide extraction. Cell extraction, RP-HPLC and immunoprecipitation procedures were identical to those for Fig 7. (a) After the acid-wash the cells lost most of the L^d MHC from the cell surface as indicated by L^d staining by 28.14.8 monoclonal antibody. However, comparable profiles of peptides were found in (b) the total cell extract fractionated by RP-HPLC, as well as in (c) anti L^d (28.14.8) immunoprecipitate. All fractions were treated with trypsin before the T cell assay.