Figure S1. Multiple Sequence Alignment of SLP-76 Orthologs, Related to Figure 1

A portion of a multiple sequence alignment of SLP-76 orthologs from 8 species, with identical residues highlighted in yellow. This region of SLP-76 includes the three known tyrosine phosphorylation sites and Gads-binding motif, both highly conserved. Other conserved motifs include the region surrounding Y173, and an adjacent proline motif, previously suggested to bind to the SH3 domain of PLC-γ1, Itk or Lck.
Figure S2. Identification of phospho-Y145 by MSMS, Related to Figure 2

MS and MSMS analysis of peptides derived from SLP76 with Y145 being phosphorylated. Shown is the MSMS analysis of the peptide DDADpYEPPPSN derived from SLP76 after digestion of the protein with endoproteinase Asp-N as described in Figure 2A. The sequence of the peptide together with its fragment ions that led to the unambiguous identification of the sequence and pY-145 is listed within the figure. The mass spectrum of the intact peptide is shown in the insert, as in Figure 2A. Of note, the mass spectrum of the intact reveals a second peptide with m/z 650.19 adjacent to phosphopeptide DDADpYEPPPSN (m/z 650.23). This peptide was selected for MSMS analysis together with actual phosphopeptide DDADpYEPPPSN, so that the corresponding MSMS spectrum is a mixture of fragment ions derived from both the peptides (m/z 650.19 and 650.23). However, we could not identify the sequence of this particular co-eluting peptide.
J14 cells were retrovirally infected with pMIGR-based viruses expressing the indicated alleles of SLP-76. Each reconstitution was performed at two different titers of virus, to create independent lines, which we denoted high- and low-titer cells. Two weeks following infection, cells from the low titer infection were sorted by FACS to remove uninfected cells. GFP and TCR expression were measured by FACS. All experiments in this study were repeated in both high- and low-titer cell lines, with similar results consistently observed at both levels of infection.
Figure S4. Different mutations that disrupt phosphorylation of Y173 have an equivalent effect on PLC-γ1 phosphorylation, Related to Figure 4C

J14 cells were retrovirally reconstituted with the indicated alleles of SLP-76, and sorted by FACS to obtain the populations shown in Figure S3. Lysates from 0.5*10^6 TCR-stimulated cells were probed with anti-phospho-PLC-γ1, then stripped and reprobed to demonstrate equivalent loading of PLC-γ1 in all lanes.
Figure S5. Sustained reduction in intracellular calcium upon mutation of Y173, Related to Figure 6A.

J14 cells stably infected with wild type or Y173F mutated SLP-76 were labeled with indo-1, aliquoted to a 96 well plate, and preheated to 37C. Ratiometric measurement of intracellular calcium was performed with a plate fluorimeter, as described in the legend to figure 4D, except that anti-TCR stimulant was added manually immediately prior to the first measurement, followed by thirty fluorescence measurements at two minute intervals, with the plate temperature maintained at 37C throughout. Shown are the average ratio obtained from triplicate measurements at each time point, with error bars indicating the standard deviation. Results are representative of at least three independent experiments.