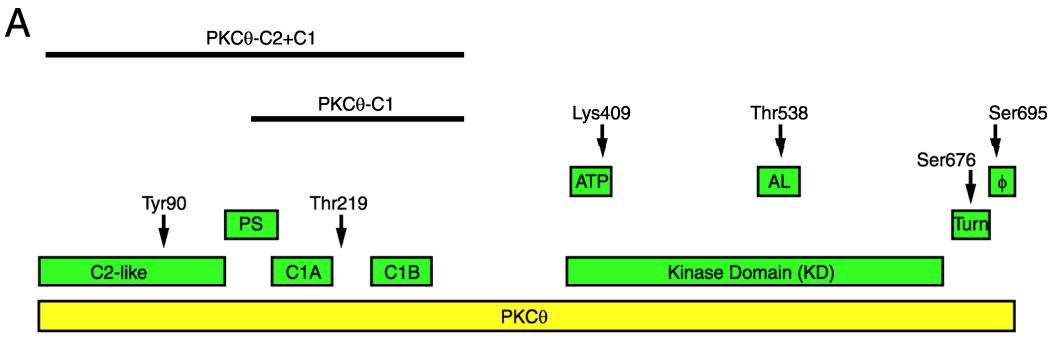
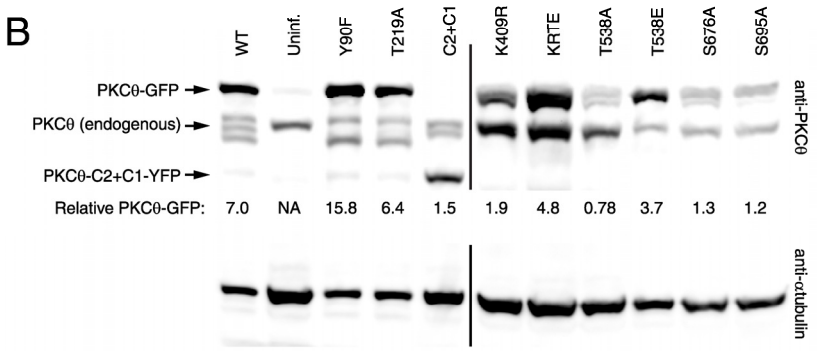
## Supplemental Figure Legends



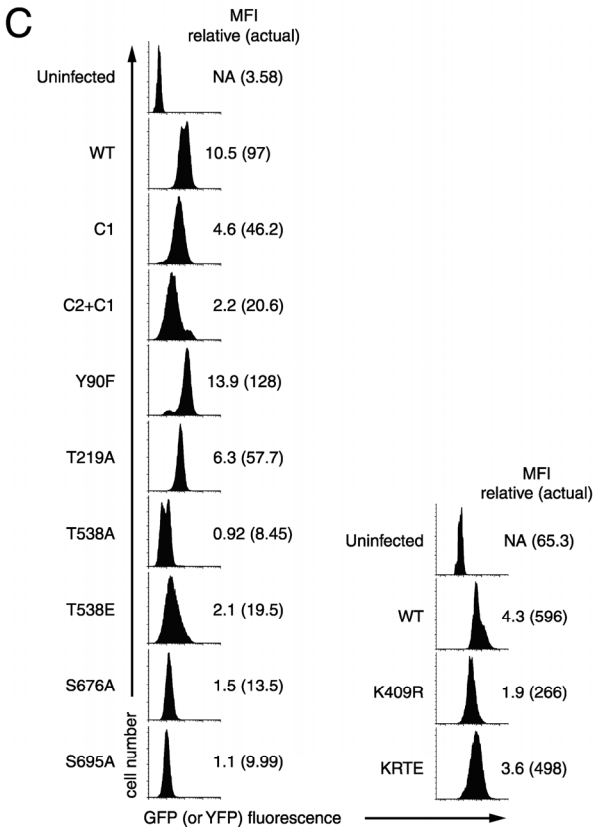



**Figure S1. PKCθ domains, constructs, and cell lines.** (A) Diagram showing details of PKCθ constructs used in this study. Individual PKCθ domains are indicated by boxes (green) above the box representing the entire ORF (yellow). Arrows indicate sites mutated in this study and horizontal lines indicate the PKCθ regions included in the C1 and C2+C1 constructs. PS, pseudosubstrate domain; C1A and C1B, diacylglycerol-binding C1 domains; ATP, ATP-binding site; AL, activation loop; Turn, turn motif; ϕ, hydrophobic motif. (B) Western blot showing levels of endogenous PKCθ and PKCθ-GFP (or YFP) protein in each D10 T cell line. Whole cell lysates from the indicated cell lines were separated by SDS-PAGE and probed with a polyclonal anti-PKCθ antibody. Relative levels of PKCθ-GFP/YFP proteins are shown below the anti-PKCθ blot. Line indicates position of C1-YFP lane, which was removed due to lack of reactivity with the anti-PKCθ antibody. Because degradation products of the ectopic PKCθ constructs co-migrate with endogenous PKCθ, accurate quantification of endogenous PKCθ within individual cell lines was not possible. Thus, numbers represent fold-expression relative to endogenous PKCθ in the uninfected D10 T cell line (defined as 1.0). Numbers also reflect normalization to α-tubulin for each lane (quantification of tubulin data is not shown. Note that the WT, Y90F, and T219A lysates were diluted 1:4 to keep all PKCθ-GFP/YFP intensities within a similar intensity range, to improve the accuracy of this quantification). (C) Flow cytometry quantification of PKCθ-GFP(or YFP) levels in D10 T cell clones. Intensity of GFP (or YFP) fluorescence was determined for each D10 T cell clone in this study. The left and right columns are data from distinct experiments. The actual median fluorescence intensity (MFI) is shown in parentheses and was calculated using FlowJo software. The numbers left of the actual MFI are MFI relative to endogenous PKCθ. Because (B) showed that the average of the PKCθ-GFP expression levels in the T538A and S695A constructs is approximately 1.0, we used the average GFP intensity from these constructs (9.22) as the estimated value for endogenous PKCθ expression. All other values in the left column were derived by dividing the measured MFI by 9.22, giving values that agree (within a factor of 2) with the western blotting data in (B). The values in the right column were determined by setting the relative PKCθ level in K409R to 1.9 (based on western blotting data in (B)) and using the equation of relative MFI = [(measured MFI) × 1.9]/266 for the remaining constructs. NA, not applicable.

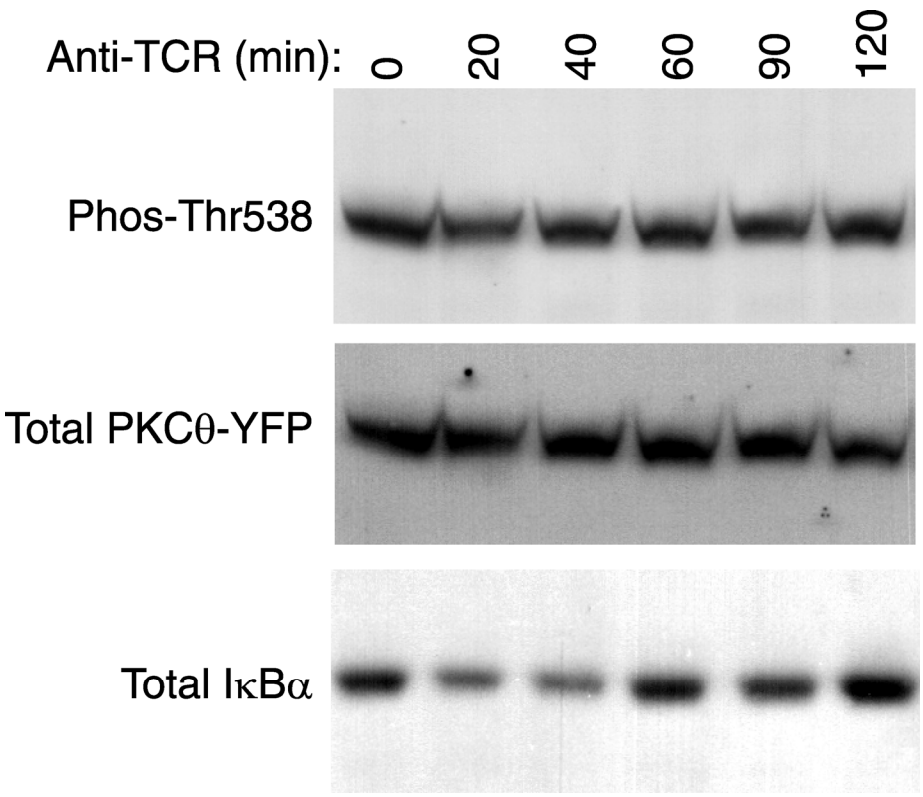



**Figure S2. Thr538 is constitutively phosphorylated in D10 T cells.** D10 T cells expressing PKCθ-GFP were stimulated for the indicated times with plate-bound anti-TCRβ (100 μg/mL). Whole cell lysates were separated by SDS-PAGE, and western blots were probed with anti-phospho-Thr538-PKCθ, anti-(total)-PKCθ, and anti-IκBα. Reduced levels of IκBα at 20 – 40 min confirm TCR activation of the PKCθ-dependent NF-κB pathway.

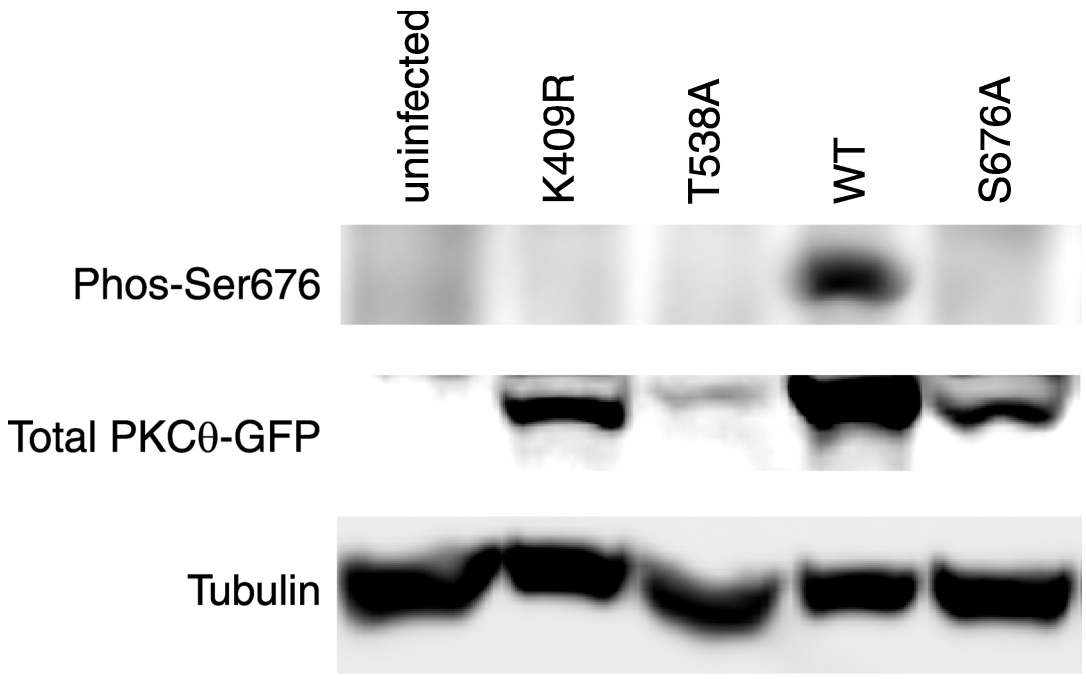



**Figure S3. Phosphorylation of Ser676 is not observed in D10 cells expressing the PKCθ ATP-binding domain mutant, K409R.** Whole cell lysates were prepared from D10 T cells stably infected with the indicated PKCθ-GFP retroviral constructs or with no construct (uninfected). Western blots were probed with anti-phospho-Ser676-PKCθ, anti-(total)-PKCθ, and anti-tubulin.

**Movie S01.** WT PKCθ efficiently translocates to and remains stably enriched at the IS.

**Movie S02.** PKCθ-KRTE is weakly recruited to the IS. Enrichment quickly spreads around the entire plasma membrane, consistent with poor retention of KRTE at the IS.

**Movie S03.** A second example of weak recruitment and poor retention of KRTE at the IS.