Supplemental FIGURE 1. PKC-θ constitutively associates with SAP. Thymocytes from CD2-SAP transgenic mice were stimulated with anti-CD3 and lysed. PKC-θ immunoprecipitated with human SAP but not the Ig control. TCL: Total cell lysates.

Supplemental FIGURE 2. Isolated GST proteins used in this paper (numbers above GST and fusion protein lanes indicate the volume in microliters loaded on the gel).

Supplemental FIGURE 3. GFP, GFP-SAP, GFP-SAP(R78A) or GFP-SAP(R55L) were introduced into WT and *SAP*$^{-/-}$ CD4^{+} T cells blasts via Amaxa. Representative example shown: top panel indicates cell size and bottom panel shows the percentage of GFP positive cells 4 h post-transfection.

Supplemental Figure 4: SAP associates with PKC-θ in the absence of Fyn. Thymocytes from WT and *Fyn*$^{-/-}$ mice were untreated or treated with *A,* 150 μM PV or *B,* anti-CD3, lysed and GST pull downs immunoblotted for PKC-θ. TCL: Total cell lysates.

Supplemental FIGURE 5. CD2-SAP transgenic CD4^{+} T cells produce elevated IL-4. *A,* SAP protein expression. Thymic and splenic cell lysates were immunoblotted for myc (transgenic SAP) and PKC-θ as a control. *B-C,* Thymocytes were assayed for (*B*) CD4 and CD8 expression (numbers in quadrants represent percentages of different cell populations) or (*C*) TCRβ chain expression on CD4 and CD8 single positive cells. *D-E,* RNA isolated from CD4^{+} T cells stimulated with anti-CD3 + anti-CD28 for 24 and 48 h was subjected to (*D*) real-time quantitative RT-PCR analysis for *T-bet*, *c-Maf* and *GATA-3*, normalizing to $\beta_2 m$ message or (*E)* northern analyses, probing for GATA-3 and β2M.

Supplemental Figure 6. Naïve CD2-SAP transgenic T cells demonstrate increased PKC-θ recruitment to the site of TCR engagement. *A-B,* WT and CD2-SAP CD4^{+} T cell cells were incubated with latex beads coated with anti-MHC (3μg/ml) or anti-TCR (3μg/ml) ± anti-CD28 (3μg/ml) and stained for *(A)* actin or *(B)* PKC-θ (representative example shown). Graphs represent the average percentage of cells scoring positive for enhanced staining at the site of T cell-bead interface, n=3 scoring a minimum of 30 bead conjugates each (* denotes bead). *C-D,* Naïve AND CD4^{+} T cell cells were permitted to conjugate to LPS-activated peptide pulsed B cells, then fixed and stained. Graphs represent the percentage of cells scoring positive for polarized *(C)* Lck or *(D)* PKC-θ (representative examples shown), n=3 scoring a minimum of 30 T: B cell conjugates (* denotes B cell).

Supplemental FIGURE 7. PKC-θ retroviral constructs improve cytokine production from *PKC-θ*$^{-/-}$ and *SAP*$^{-/-}$ CD4^{+} T cells. *A,* Negatively selected naïve WT and *PKC-θ*$^{-/-}$

$CD4^+$ T cells were stimulated and infected with the indicated retrovirus, viable $CD4^+$ T cells isolated and restimulated to evaluate cytokine production by ELISA: left panel, IL-2 and right panel, IL-4. *B,* Negatively selected naïve WT and *SAP*$^{-/-}$ $CD4^+$ T cells were stimulated and infected with the indicated retrovirus, viable $CD4^+$ T cells isolated and restimulated to evaluate cytokine production by ELISA: left panel, IL-4 and right panel, IFN-γ. *C,* WT OT-II and *SAP*$^{-/-}$ OT-II were retrovirally reconstituted with either Migr, Myr-PKC-θ or KA-PKC-θ in the presence of APC and peptide for 3 days. IL-2 expanded cells were sorted for GFP expression and rested for 24 h prior to B cell conjugation. Representative example shown: top panel indicates similar cell size while the bottom panel outlines GFP expression profiles.