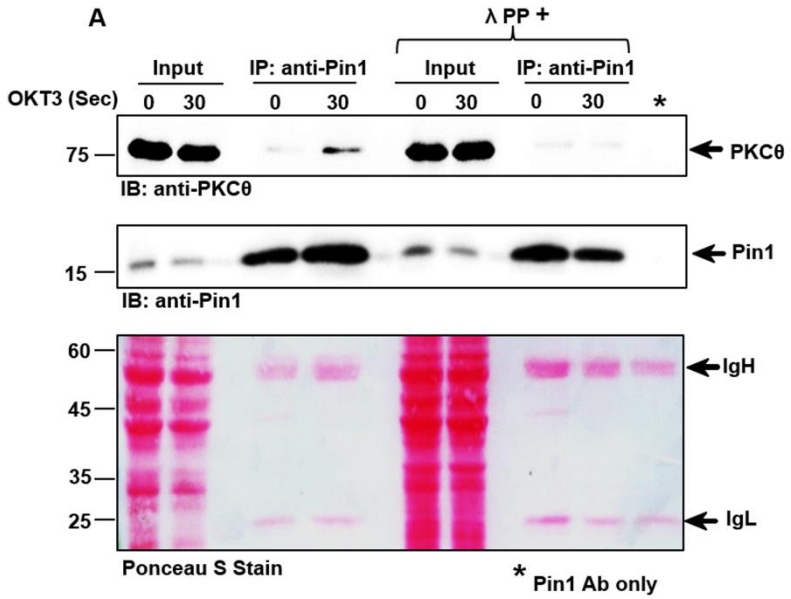
SUPPLEMENTARY INFORMATION

FIGURE S1



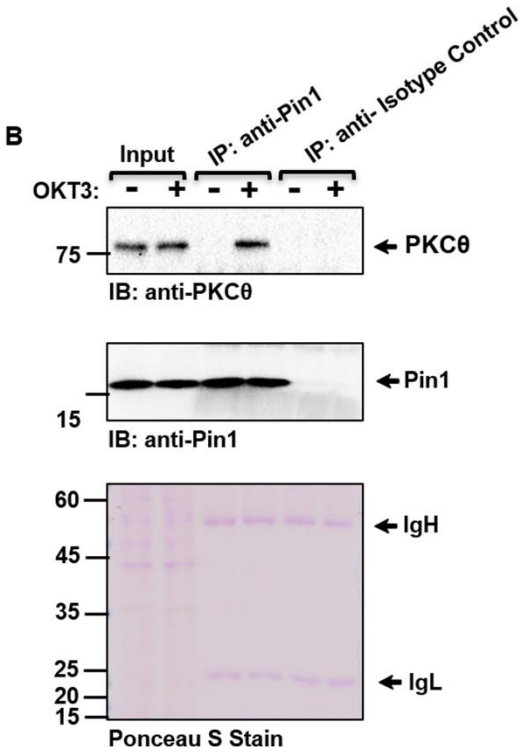
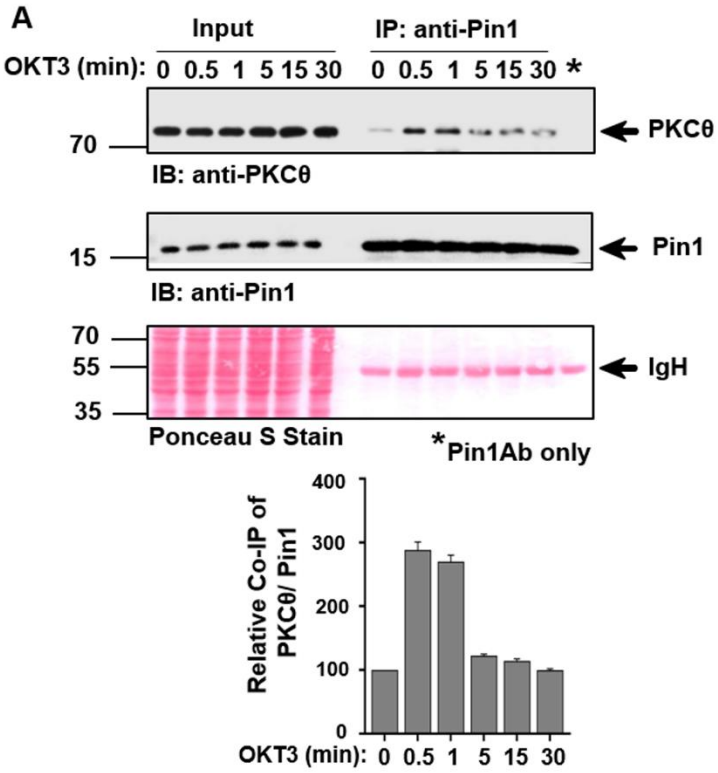


Figure S2


A
Input
IP: anti-Pin1
OKT3 (min): 0 0.5 1 5 15 30 0 0.5 1 5 15 30 *
70
PKCθ
IB: anti-PKCθ
15
Pin1
IB: anti-Pin1
70
55
35
IgH
Ponceau S Stain
*Pin1Ab only
Relative Co-IP of PKCθ/ Pin1
400
300
200
100
0
OKT3 (min): 0 0.5 1 5 15 30

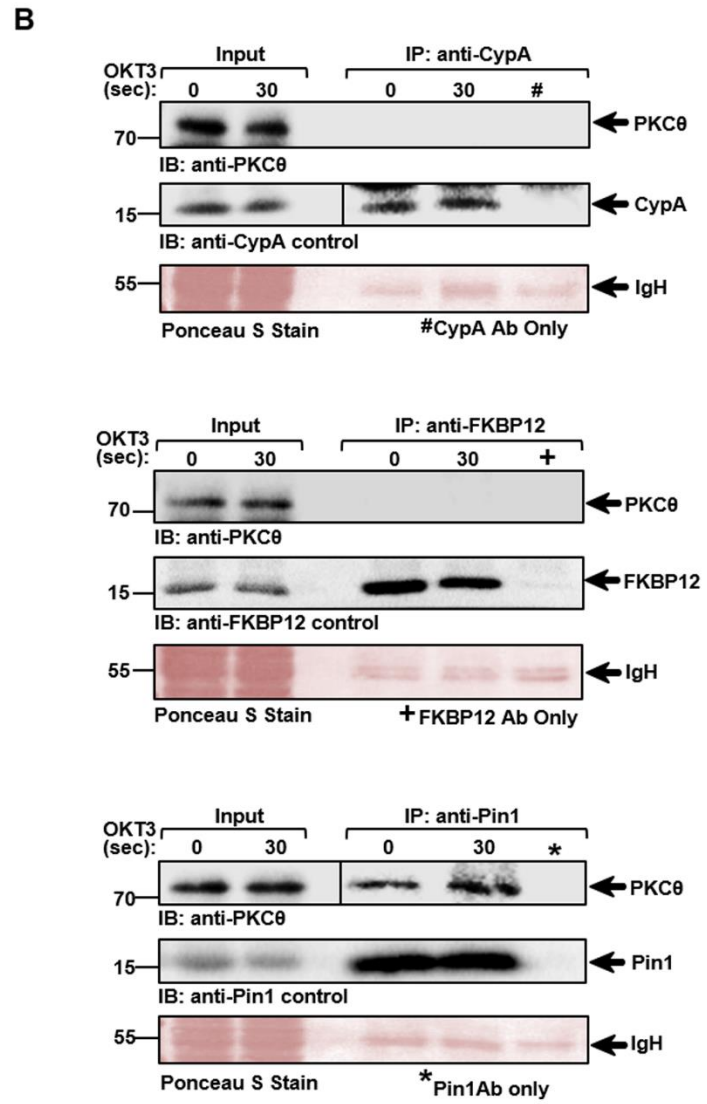

B
Input
IP: anti-CypA
OKT3 (sec): 0 30 0 30 #
70
PKCθ
IB: anti-PKCθ
15
CypA
IB: anti-CypA control
55
IgH
Ponceau S Stain
#CypA Ab Only
Input
IP: anti-FKBP12
OKT3 (sec): 0 30 0 30 +
70
PKCθ
IB: anti-PKCθ
15
FKBP12
IB: anti-FKBP12 control
55
IgH
Ponceau S Stain
+FKBP12 Ab Only
Input
IP: anti-Pin1
OKT3 (sec): 0 30 0 30 *
70
PKCθ
IB: anti-PKCθ
15
Pin1
IB: anti-Pin1 control
55
IgH
Ponceau S Stain
*Pin1Ab only

Figure S3

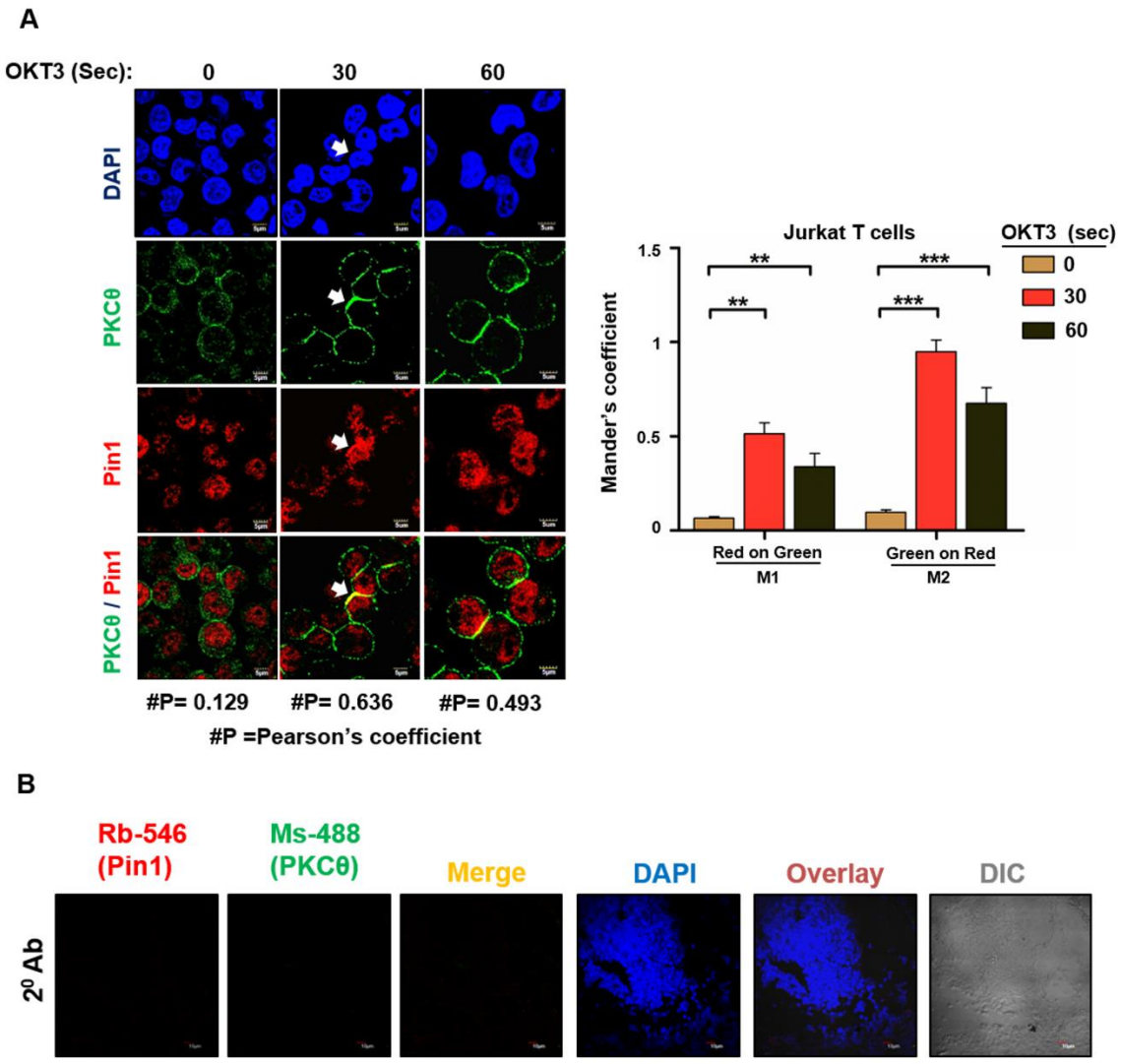


Fig. S1. Pin1-PKCθ complexes undergo dissociation in the presence of protein phosphatases. A. Jurkat TAg cells were stimulated for the indicated time intervals with anti-CD3 mAb (OKT3). Whole-cell lysates (WCL; Input), and immunoprecipitates (IP) were treated with λ-phosphatase (λ PP). Following incubation, the samples were subjected to immunoblotting (IB) as indicated. Membrane staining with Ponceau S monitored the equal loading of proteins in all lanes. Molecular weight markers (in kDa) are indicated on the left and arrows mark the position of the indicated protein bands. IgH, Ig heavy chain; IgL, Ig light chain. Results are representative of three independent experiments. **B.** Jurkat T cells were stimulated with OKT3 mAbs for 1 min. Cell lysates were then subjected to immunoprecipitation using rabbit anti-Pin1 and rabbit anti-IgG isotype control mAbs. The

samples were then subjected to SDS-PAGE and sequential immunoblotting with anti-PKCθ and anti-Pin1 mAbs. Membrane staining with Ponceau S monitored the equal loading of proteins in all lanes. Molecular weight markers (in kDa) are indicated on the left and arrows mark the position of the indicated protein bands. Results are representative of three independent experiments. IgH, Ig heavy chain; IgL, Ig light chain.

Fig. S2. T cell activation-induced binding of Pin1 to PKCθ in Jurkat T cells is selective and transient. A. Jurkat TAg cells were stimulated for the indicated time intervals (0-30 min) with anti-CD3 mAbs (OKT3). Whole-cell lysates (Input) and Pin1 immunoprecipitates (IP) were subjected to immunoblotting (IB) as indicated. **B.** Comparative analysis of the association of PKCθ with representatives of the three different families of PPIases (CypA, FKBP12, and Pin1) in TCR/CD3-triggered Jurkat TAg cells. Membrane staining with Ponceau S monitored the equal loading of proteins in all lanes. Molecular weight markers (in kDa) are indicated on the left and arrows mark the position of the indicated protein bands. Results are representative of three independent experiments. IgH, Ig heavy chain.

Fig. S3. Pin1 colocalizes with PKCθ at the membrane of TCR/CD3-stimulated human Jurkat T cells. A. Confocal laser microscopy analysis of Pin1 and PKCθ distribution in OKT3-stimulated Jurkat T cells. Arrow indicates Pin1-PKCθ colocalization. Scale bar, 5μm. Quantification of red (Pin1) and green (PKCθ) colocalization was assessed using the JACoP plugin from ImageJ. #P indicates the Pearson's colocalization coefficient. Mander's coefficients indicate the fraction of red overlapping with green (M1) and green overlapping with red (M2), respectively. Mean$\pm$SEM (n=9), ****** P<0.001, *******P<0.0001 (one-way ANOVA, Tukey's Multiple Comparison Test). Data are representative of three independent experiments. **B.** A control experiment demonstrating the staining of C57BL/6J mouse thymic sections with fluorescently-labeled Alexa Fluor™546-conjugated goat anti-mouse and Alexa Fluor™488-conjugated goat anti-rabbit secondary Abs, to rule out the possible basal, non-specific staining. Scale bar equals 10 μm.