Supplementary figure legends

Figure S1. *Phosphorylation of PKCζ at the IS formed between T_H cells and EBV-transformed B cells.* (**A**) EBV-B cells, either unpulsed (upper panels) or TSST-1 pulsed (lowed panels) and loaded with CMTMR-Orange (red) were conjugated with $CD4^+V\beta2^+$ T cells. After 30 minutes at 37°C, cells were fixed, permeabilized and stained for the phosphorylated form of PKCζ (p-PKCζ) (green and pseudo-colour scale) and phosphotyrosines (p-Tyr) (blue). Results are from one representative experiment out of three. (**B**) Measurement of p-PKCζ in T cells by FACS analysis. EBV-B cells either unpulsed (green) or TSST-1 pulsed (red) were conjugated with $CD4^+V\beta2^+$ T cells. After 30 minutes, cells were fixed, permeabilized and stained with an antibody against p-PKCζ or an isotype control (blue). Results are from one representative experiment out of four.

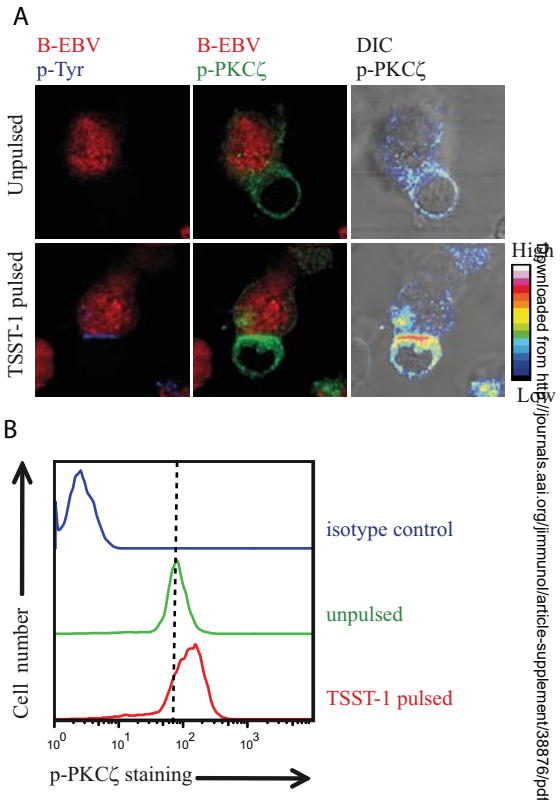
Figure S2. *Inhibition of PKCζ function affects the polarization of T_H cell Golgi apparatus towards DC.* (**A**) TSST-1 pulsed DC (red) were conjugated with $CD4^+V\beta2^+$ T cells either untreated or pre-treated with PKCζ-PS and washed. After 6 hours at 37°C, cells were fixed, permeabilized and stained for GM130. (**B**) Distances of Golgi apparatus from the center of the T cell/DC contact site were measured using the Profile function of the Zeiss software. Each dot corresponds to a T cell/ DC conjugate. 138 conjugates with unpulsed DC, 143 conjugates with pulsed DC and 135 with pulsed DC plus PKCζ-PS were scored in three independent experiments. Statistical significance of difference between groups was evaluated by an unpaired Mann-Whitney test using the GraphPad Prism software. *** $P < 0.0001$. Bar = 5 μm.

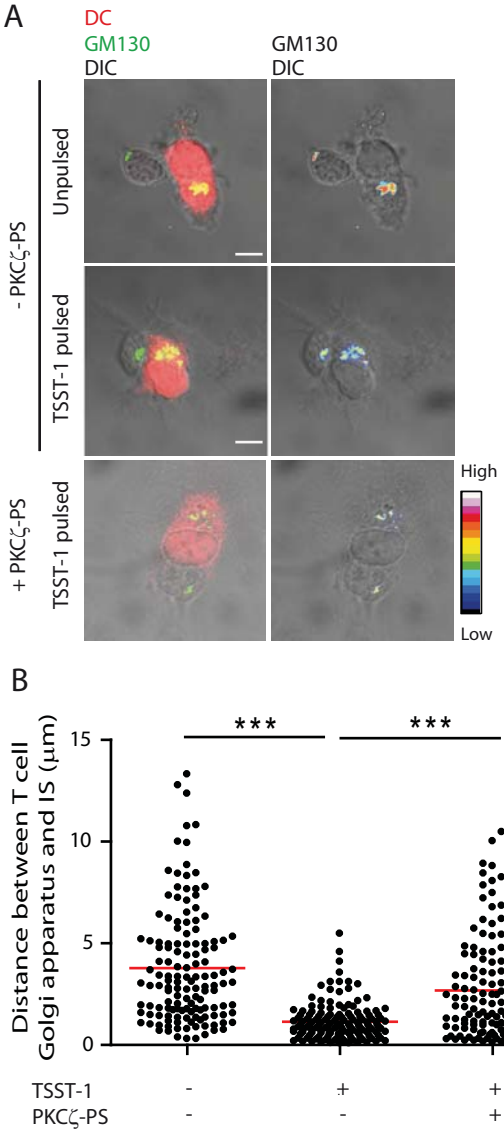
Figure S3. *T cell activation and TCR/CD3ζ enrichment at the IS are unaffected by inhibition of PKCζ.* (**A**) Indo-1-loaded $CD4^+V\beta2^+$ T cells were either untreated or pre-treated with

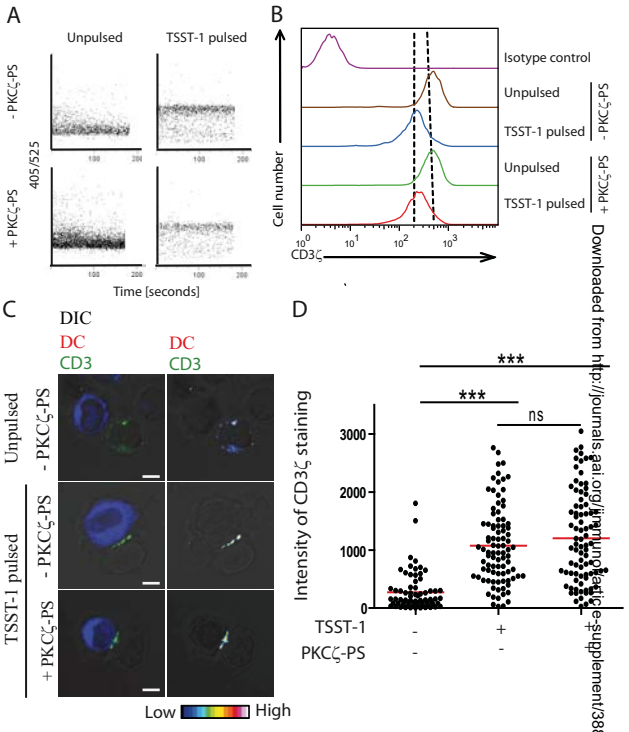
PKCζ-PS. After washing, T cells were conjugated with DC either unpulsed or TSST-1 pulsed. After 30 minutes of conjugation cell were gently re-suspended and $[Ca^{2+}]_i$ in T cells was measured on a LSRII flow cytometer (Becton Dickinson). (**B**) $CD4^+V\beta2^+$ T cells either untreated (brow, blue) or pre-treated (green, red) with PKCζ-PS were conjugated with TSST-1 pulsed (blue, red) or unpulsed DC (brown, green). After 4 hours of co-culture, cells were stained with anti-CD3 mAb or an isotype control (magenta). (**C**) DC, either unpulsed (upper panels) or TSST-1 pulsed (middle and lower panels) and loaded with BODIPY 630 (blue) were conjugated with $CD4^+V\beta2^+$ T cells pre-treated (lower) or not (upper and middle panels) with PKCζ-PS. After 10 minutes at 37°C, cells were stained with an anti-CD3ζ mAb (green and pseudo-color) **(D)** Intensity of CD3ζ staining was measured using the linescan function of MetaMorph software. The synaptic area (IS) was defined as in Fig. 1. Each dot corresponds to a T cell/DC conjugate. 69 conjugates with unpulsed DC, 91 with TSST-1 pulsed DC and 83 with TSST-1 pulsed DC + PKCζ-PS were scored in three independent experiments. Statistical significance of difference between groups was evaluated by an unpaired Mann-Whitney test using the GraphPad Prism software. Bar = 5µm.

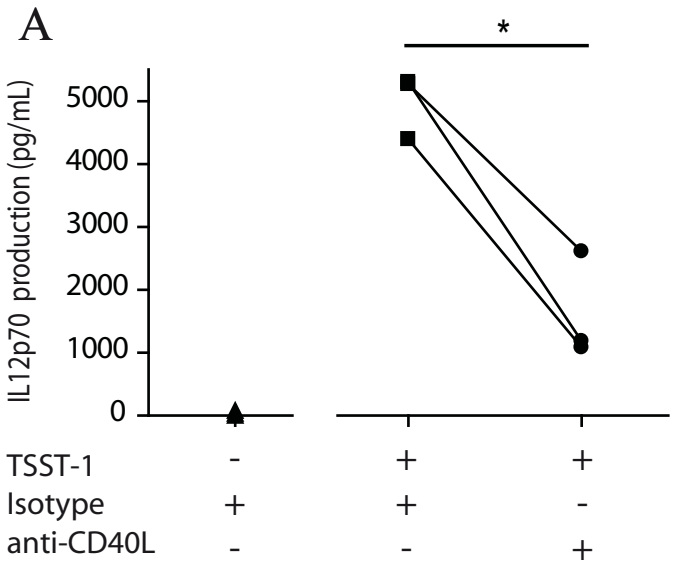
Figure S4 *CD40/CD40L interaction is required for IL-12 production by DC.* DC either unpulsed (triangles) or pulsed with TSST-1 (squares and circles) were co-cultured overnight with $CD4^+V\beta2^+$ T cells in the presence of an anti-CD40L or an isotype control mAb. IL-12p70 secretion was measured by CBA in supernatants in three independent experiments each one performed in duplicate. Statistical significance of difference between groups was evaluated by an unpaired Mann-Whitney test using the GraphPad Prism software. * P = 0.0154.

Figure S5. *Inhibition of PKCζ affects T_H cell polarization towards the DC offering the strongest stimulus.* **(A)** DC, either pulsed with 0.1 ng/ml TSST-1 (cyan) or pulsed with 10 ng/ml TSST-1 (red) were conjugated for 10 minutes with $CD4^+V\beta2^+$ T cells that were pre-treated with PKCζ-PS. Cells were fixed, permeabilized and stained for p-PKCζ (green in the first panel and pseudo-color in second panel) and for α-tubulin (blue in the third and pseudo-color in the fourth panel). **(B)** Intensity of p-PKCζ staining and distances of MTOC from the center of the T cell/DC contact site were measured as in Fig 6 D. 70 three-cell conjugates were scored in three independent experiments. The graph shows the distance in μm of the T cell MTOC from the two synapses (*x* axis) versus the intensity of p-PKCζ at the two synapses (*y* axis). Bar = 5 μm.









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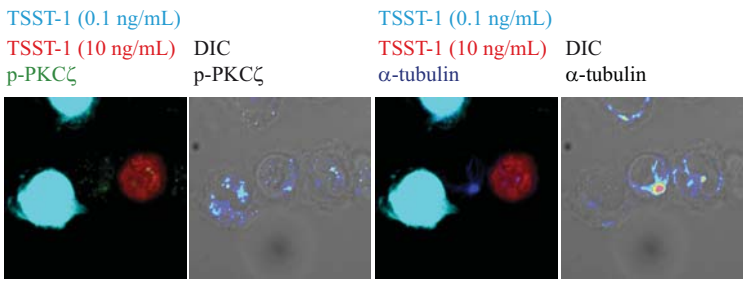

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