**Flow cytometry analysis of cell cycle.** T cells (5 x 10^5 cells per mL) were washed in PBS and resuspended in 2 mL of hypotonic staining solution (50 mg per mL propidium iodide, 0.1% sodium citrate, 0.1% Triton X-100, and 40 mg per mL RNAse). Cells were vortexed, and incubated at 4°C for 2 h before analysis in a FACSCalibur flow cytometer, using the ModfitLT software from BD Biosciences. Data were processed with CellQuest Pro 4.0.2 software (BD Biosciences).

**F-actin determination assay.** Determination of the levels of polymerized actin was performed as follow. Cells were fixed and permeabilized with 200 µL of CELLwash buffer (Becton Dickinson) for 10 minutes at 4°C and incubated for 30 minutes at 4°C with 5 µg/mL of Alexa 647-conjugated phalloidin. T cells were analyzed for phalloidin expression by flow cytometry using a FACScalibur flow cytometer (Becton Dickinson). Data were processed with CellQuest Pro 4.0.2 software (BD Biosciences).

**Time-lapse fluorescence confocal microscopy and immunofluorescence analysis.** As described in main text.
**Figure S1. Expression of C-term AKAP450-GFP construct by T cells.** (A) J77 cells were transfected with constructs encoding GFP or C-terminal AKAP450-GFP. Protein expression of endogenous AKAP450, GFP and total tubulin were determined by immunoblotting with specific antibodies. (B) Cell cycle profiles by flow-cytometry analysis of DNA content of sorted GFP cells and C-term AKAP450 cells-GFP. Solid peaks correspond to G0/G1 and G2/M cells, respectively. The area between two peaks indicates cells in S phase. Distribution of DNA content was quantified and represented in the table. (C) J77 cells transfected with C-term AKAP450-GFP were conjugated with SEE-stimulated Raji B cells, and were stained for gamma-tubulin (red). The cyan signal in the DIC image distinguishes the CMAC-preloaded APC (asterisk). Scale bars, 10 µm. (D) Histogram shows quantification of the number of total cell conjugates formed between J77 cells expressing GFP or C-term AKAP450-GFP and SEE-stimulated Raji B cells.

**Figure S2. Knockdown of AKAP450 in T cells.** J77 cells (A) and CH7C17 cells (B) were transfected with a control siRNA or AKAP450 siRNA. Protein expression of AKAP450 and total tubulin were determined by immunoblotting with specific antibodies.

**Figure S3. Actin polymerization was affected by overexpression of the C-terminal domain of AKAP450.** (A) T cells were stimulated with T3b (anti-human CD3) at the indicated times and stained for phalloidin; F-actin content in response to anti-CD3 was analyzed in T-cells by flow-cytometry. (B) Conjugates were formed between J77 cells (upper panel) or T lymphoblasts (lower panel) expressing GFP or C-term AKAP450-GFP and SEE-pulsed Raji B cells, and were stained for
phalloidin (red). Yellow arrows indicate the positions of immune synapses and the blue arrow indicates the position of the mislocalized MTOC in cells overexpressing C-term AKAP450-GFP. Asterisks in DIC images identify CMAC-loaded Raji APCs. Scale bars, 10 µm.

**Figure S4. Overexpression of the C-terminal domain of AKAP450 in J77 cells inhibits Golgi apparatus translocation to the IS.** J77 cells expressing GFP or C-term AKAP450-GFP were incubated with SEE-pulsed Raji B cells, and the Golgi apparatus was detected by immunostaining for giantin (red). Yellow arrows indicate the positions of immune synapses and the blue arrow indicates the position of the mislocalized MTOC in cells overexpressing C-term AKAP450-GFP. Asterisks in DIC images identify CMAC-loaded Raji APCs (cyan). Scale bars, 10 µm.
**SUPPLEMENTARY FIGURE 1**

**A**
- WB: AKAP450
- WB: GFP
- WB: Tubulin

**B**
- Propidium Iodide
- % PI-Positive Cells
  - GO/G1: 62.05, C-TERM: 61.51
  - G2/M: 19.16, C-TERM: 19.73

**C**
- C-term AKAP450-GFP
- γ-tubulin
- Merged
- DIC

**D**
- % Cell Conjugates
  - GFP
  - C-term
  - -SEE
  - +SEE
SUPPLEMENTARY FIGURE 3

A  
Jurkat J77  
\[ \text{Fold Induction} \]  
\[ \begin{array}{c} \text{Time - seconds (\(\alpha\)-CD3 stimulation)} \\ 0 \ 20 \ 60 \ 120 \ 300 \end{array} \]  
\[ \begin{array}{c} 0.9 \ 1.0 \ 1.1 \ 1.2 \ 1.3 \end{array} \]  
PBLs CD4+  
\[ \text{Fold Induction} \]  
\[ \begin{array}{c} \text{Time - seconds (\(\alpha\)-CD3 stimulation)} \\ 0 \ 20 \ 60 \ 120 \ 300 \end{array} \]  
\[ \begin{array}{c} 0.9 \ 1.0 \ 1.1 \ 1.2 \ 1.3 \end{array} \]  

B  

Control  
\[ \begin{array}{c} \text{GFP} \\ \text{Phalloidin} \\ \text{Merged} \\ \text{DIC} \end{array} \]  
\[ \begin{array}{c} \text{J77-Raji} \\ \text{C-term AKAP450-GFP} \end{array} \]  

Control  
\[ \begin{array}{c} \text{GFP} \\ \text{Phalloidin} \\ \text{Merged} \\ \text{DIC} \end{array} \]  
\[ \begin{array}{c} \text{T lymphoblasts-Raji} \\ \text{C-term AKAP450-GFP} \end{array} \]
SUPPLEMENTARY FIGURE 4

Control

C-term AKAP450-GFP