Alonso et al., http://www.jcb.org/cgi/content/full/jcb.201202137/DC1

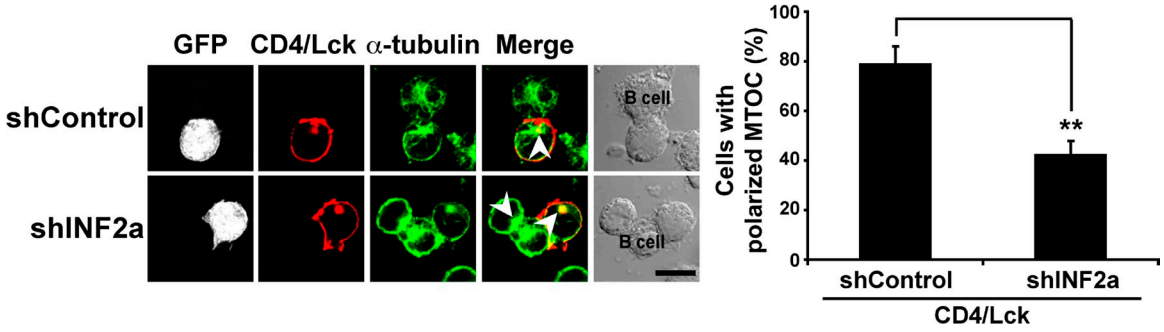


Figure S1. **Forced expression of Lck at the plasma membrane in INF2 KD cells does not correct MTOC reorientation.** Jurkat cells were cotransfected with the DNA construct expressing CD4/Lck chimera and a DNA construct coexpressing GFP and shINF2a or shControl. Cells were conjugated to SEE-loaded APCs for 15 min. Cells were fixed, permeabilized, and stained for total α-tubulin. The cells cotransfected with both plasmids were detected with antibodies to the murine CD4 ectodomain, which recognizes the CD4/Lck chimera, and the GFP fluorescence, which identifies the cells expressing the indicated shRNA. The arrowheads indicate the position of the MTOC of the T cells. The histogram represents the percentage of T cells positive for CD4/Lck expression with polarized MTOC. At least 40 T cells were analyzed. Data are summarized as means ± SEM from three independent experiments (error bars; **, $P < 0.01$). Bar, 5 µm.

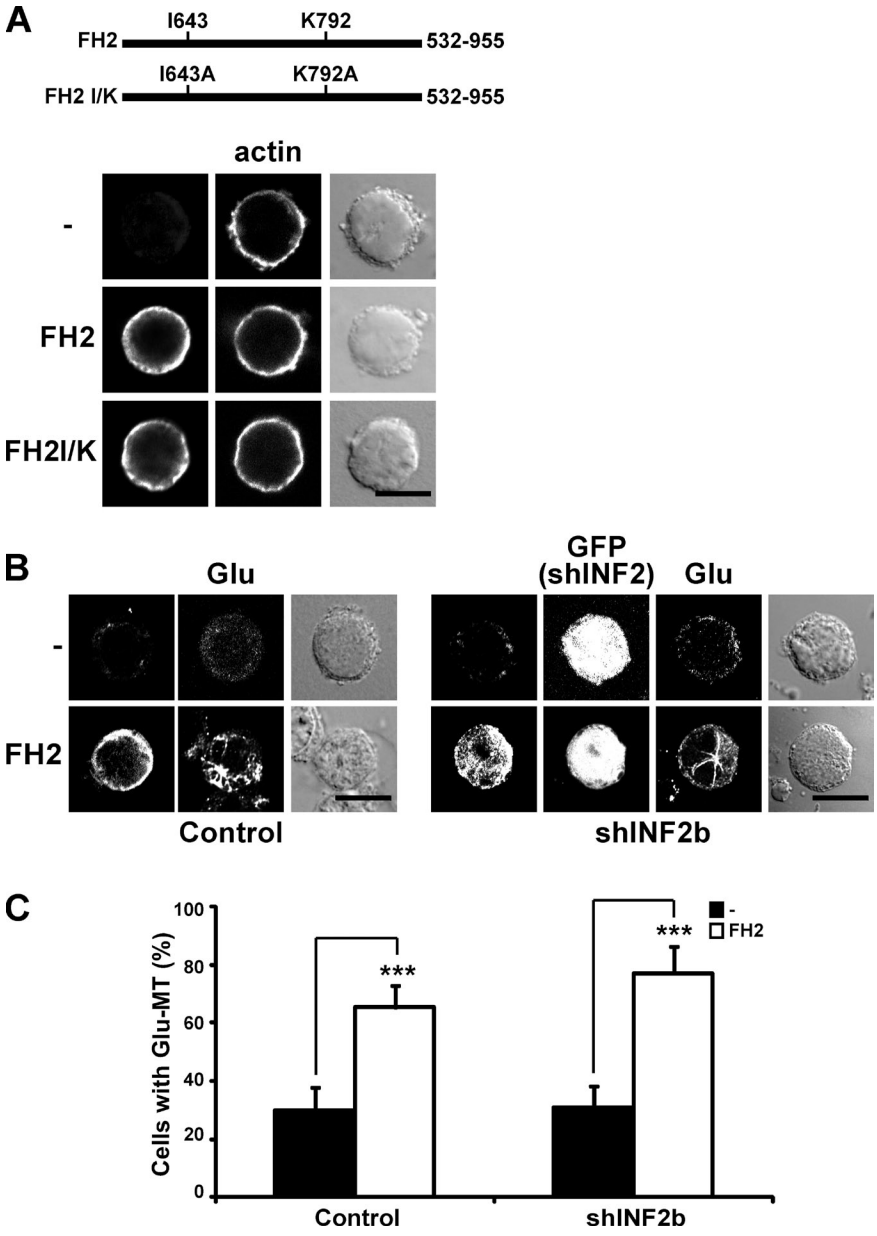


Figure S2. **The expression of the FH2 domain of INF2 induces the formation of Glu-MTs in resting T cells.** (A) Jurkat cells were transfected with constructs expressing INF2 FH2 or FH2 I/K, and were fixed with paraformaldehyde, permeabilized with 0.1% Triton X-100, and stained for the expressed FH2 fragment and F-actin. (B) Resting Jurkat cells expressing or not expressing the intact FH2 fragment of INF2 were transfected (right) or not transfected (left) with a DNA construct coexpressing GFP and shINF2b. Cells were stained for the expressed INF2 FH2 fragment and Glu-tubulin. (C) The histogram represents the percentage of T cells with Glu-MTs. At least 40 T cells were analyzed. Data in are summarized as means ± SEM from three independent experiments (error bars; ***, $P < 0.001$). Bars, 5 µm.

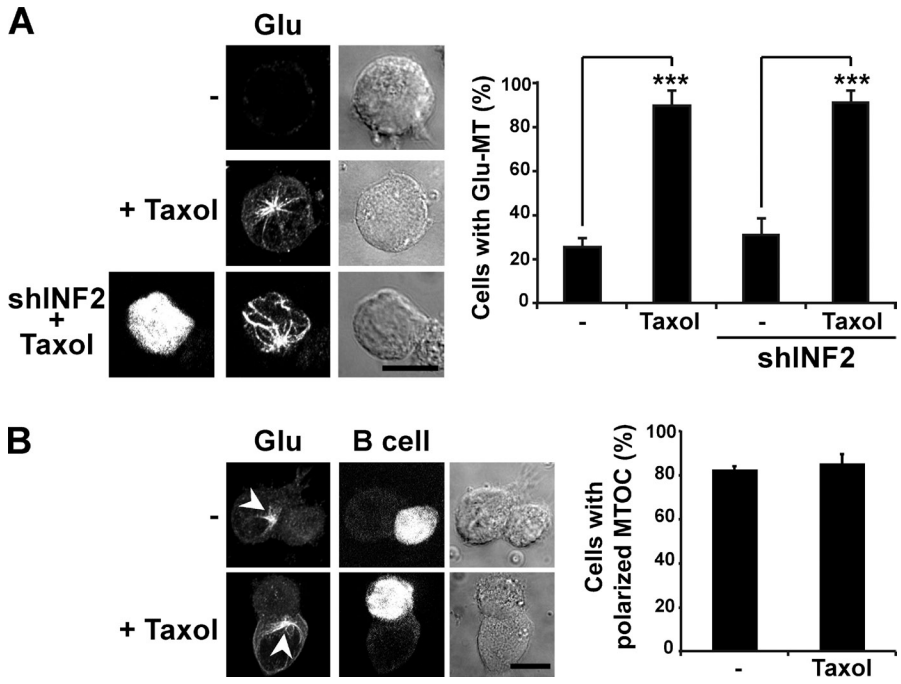
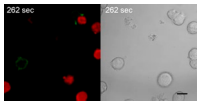
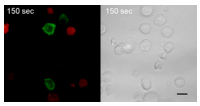


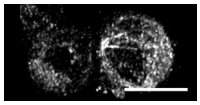
Figure S3. **Taxol treatment induces the formation of Glu-MTs in Jurkat cells.** (A) Normal or INF2 KD Jurkat cells were treated with 3 nM taxol for 18 h and stained for Glu-tubulin. The histogram represents the percentage of cells with Glu-MTs. (B) Jurkat cells treated with 3 nM taxol for 18 h were conjugated to SEE-loaded APCs. Cells were stained for Glu-tubulin. The arrowheads indicate the position of the MTOC of the T cells. The histogram represents the percentage of T cells with polarized MTOC. At least 40 T cells were analyzed in A and B. Data in A and B are summarized as means ± SEM from three independent experiments (error bars; ***, $P < 0.001$). Bars, 5 µm.



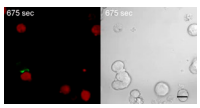
Video 1. **Video microscopy of actin of control Jurkat cells during conjugation to SEE-loaded APCs.** Jurkat cells expressing actin-GFP (green) were transfected with a DNA construct expressing shControl. Cells were then conjugated to SEE-pulsed Raji B cells that previously stained with CMTMR (red). Images from three sections were captured every 30 s using a laser-scanning confocal microscope (LSM510 META; Carl Zeiss). The movie is displayed at 5 frames/s and features a confocal section selected from the stacks. Bar, 5 µm.



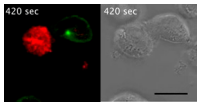
Video 2. **Video microscopy of actin of INF2 KD Jurkat cells during conjugation to SEE-loaded APCs.** Jurkat cells expressing actin-GFP (green) were transfected with a DNA construct expressing shINF2a. Cells were then conjugated to SEE-pulsed Raji B cells that previously stained with CMTMR (red). Images from three sections were captured every 30 s using a laser-scanning confocal microscope (LSM510 META; Carl Zeiss). The movie is displayed at 5 frames/s and features a confocal section selected from the stacks. Bar, 5 µm.



Video 3. **3D views of Glu-MTs in T cell–APC conjugates.** Conjugates formed by Jurkat cells with SEE-loaded Raji cells were stained for Glu-MTs. Images from different sections were captured using a laser-scanning confocal microscope (LSM510 META; Carl Zeiss). The images were deconvolved and reconstructed in 3D. The T cell and the B cell are labeled. Bar, 5 µm.



Video 4. **Video microscopy of tubulin of control Jurkat cells during conjugation to SEE-loaded APCs.** Jurkat cells expressing GFP-tubulin (green) were transfected with a DNA construct expressing shControl. Cells were then conjugated to SEE-pulsed Raji B cells that previously stained with CMTMR (red). Images from three sections were captured every 30 s using a laser-scanning confocal microscope (LSM510 META; Carl Zeiss). The movie is displayed at 5 frames/s and features a confocal section selected from the stacks. Bar, 5 µm.



Video 5. **Video microscopy of tubulin of INF2 KD Jurkat cells during conjugation to SEE-loaded APCs.** Jurkat cells expressing GFP-tubulin (green) were transfected with a DNA construct expressing shINF2a. Cells were then conjugated to SEE-pulsed Raji B cells that previously stained with CMTMR (red). Images from three sections were captured every 30 s using a laser-scanning confocal microscope (LSM510 META; Carl Zeiss). The movie is displayed at 5 frames/s and features a confocal section selected from the stacks. Bar, 5 µm.