Figure Legends.

Supplemental Movie S1, mitochondrial dynamics at the immune synapse.

J77 T cells transfected with mitoYFP were allowed to settle onto anti-CD3 plus anti-CD28 coated coverslips in Attofluor open chambers (Invitrogen) at 37ºC in a 5% CO2 atmosphere. Mitochondria located close to the activating surface were visualized with a Leica AM TIRF MC M, mounted on a Leica DMI 6000B microscope. Images were acquired (10 images per second) and processed with the accompanying confocal software (LCS; Leica).

Supplemental Figure 1. Mitochondria accumulation at the IS in J77 T cells conjugated with unpulsed or SEE-pulsed Raji B cells (S1A) or CH7C17 T cells conjugated with unpulsed or HA-pulsed Hom2 APCs. Quantification of mitochondria accumulation at the IS was performed as described in Materials and Methods from maximum projections of confocal Z stacks of conjugates preloaded with mitotracker orange. Data are means ± s.d. from 100-150 conjugates per condition from three independent experiments.

Supplemental Figure 2. Time course of Drp1 content in mitochondrial fractions of J77 and CH7C17 T cells conjugated with unpulsed or antigen-pulsed APCs during the indicated times. MnSOD was used as mitochondrial marker. Blots are representative of two independent experiments.

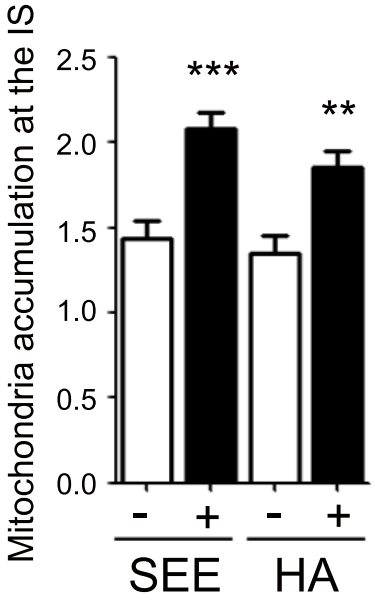
Supplemental Figure 3. **MTOC translocation facilitates mitochondria redistribution to the immune synapse.**

(**A**) Time-lapse confocal microscopy showing mitochondrial localization (mitotracker, red) in SEE-stimulated J77 T cells overexpressing p50GFP, which prevents MTOC translocation; mitochondrial localization in control cells is shown by mitoYFP expression. Arrows mark the MTOC (p50GFP, green). (**B**,**C**) Quantification of MTOC and mitochondrial accumulation at the IS calculated from maximal Z projections (see Methods). Data represent means ± s.d. of 100 conjugates from three experiments ($p<0.001$, ANOVA in **B** and $p<0.0001$, Mann Whitney in **C**)**.**

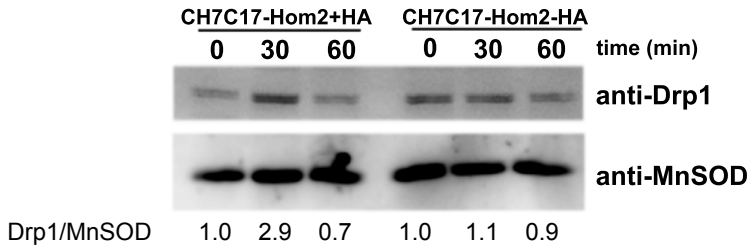
Supplemental Figure 4. Confocal video-microscopy of mitoYFP transfected J77 T cells loaded with the mitochondrial membrane potential dye TMRM and plated on an irrelevant Ab and PLL-coated coverslips. Images record the movement of individual mitochondria in a confocal plane at the surface. Right panel shows time course of mitoYFP and TMRM fluorescence intensity in the indicated mitochondria (regions of interest, ROI). Scale bar, 5μm.

Supplemental Figure 5. Intracellular calcium flux in control and Drp1-silenced J77 T cells activated with CD3 Ab (10 μg/ml). A representative experiment out of three is shown.

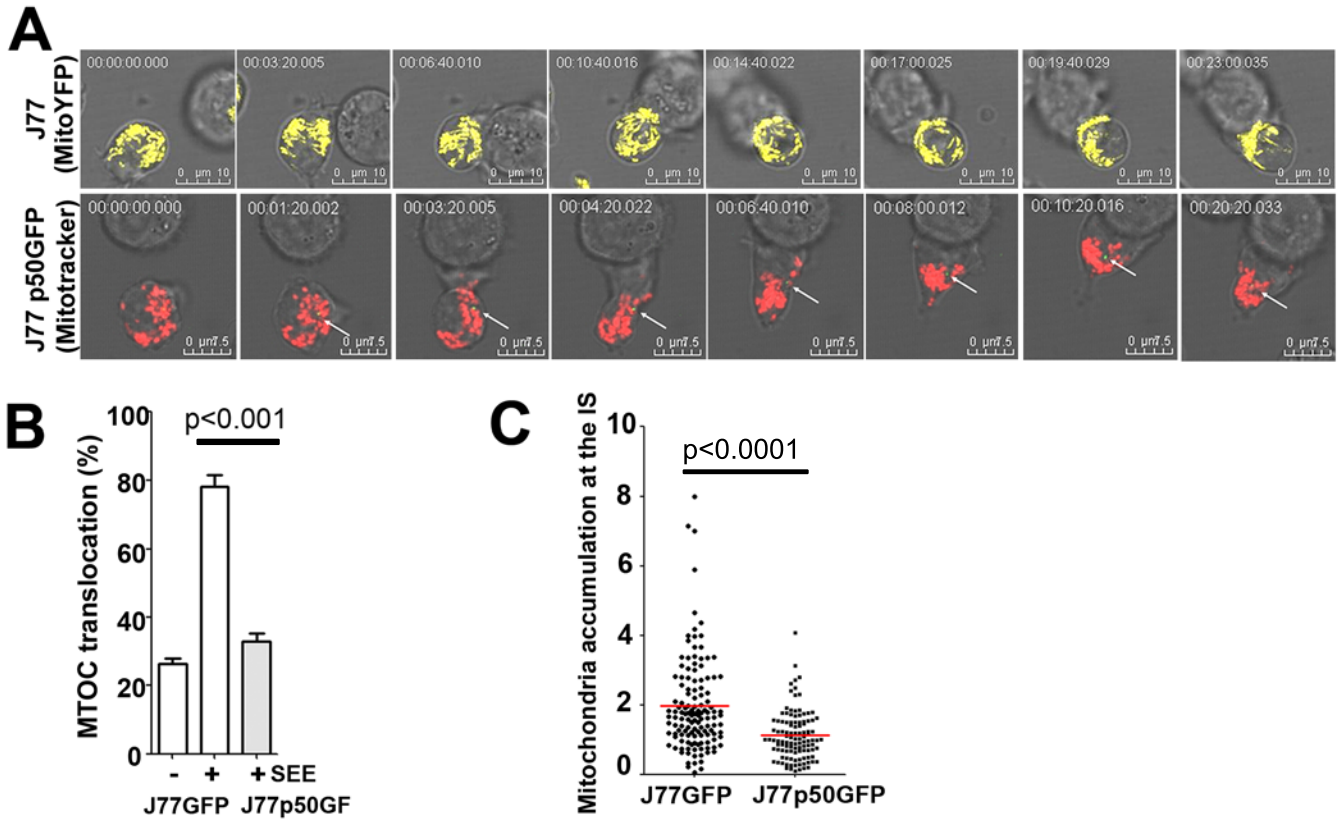
Supplemental Figure 1



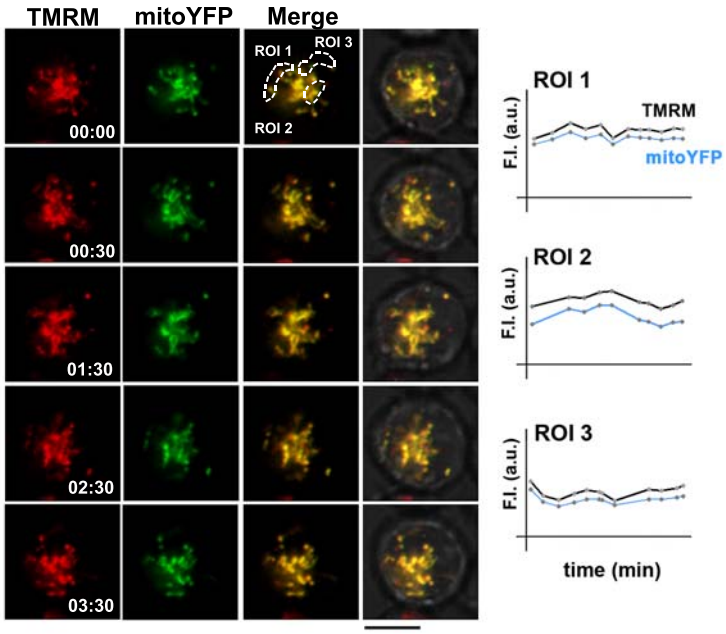
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5