>> Figure 1. Recruitment of SNX9 to the immunological synapse upon T cell activation

>Fig. 1 A,B: Images used in publication: Representative images of maximum intensity projections from Z-stacks of WT Jurkat T cells conjugated to SEE pulsed or non-pulsed antigen presenting cells.
-Original Data: Confocal Images
-Prism File: Mean percentage of SNX9 recruitment towards the synapse at indicated time points.
-Excel File

>Fig. 1 C: Images used in publication: Representative live images of WT Jurkat T cells activated on cover glass, expressing SNX9-mCherry and TCRζ-EGFP.
-Original Data: Confocal Images

>Fig. 1 D,E: Images used for paper: Representative images of 3D reconstruction and maximum intensity projections of wildtype Jurkat T cells expressing SNX9-EGFP either in resting or under different activating conditions.
-Original Data: Confocal Images
-Prism File: Mean number of SNX9-positive structures in T cells activated under different conditions.
-Excel File

>Fig. 1 F,G,H: Images used in publication: Representative live images of activated Jurkat T cells co-expressing SNX9-EGFP or -mCherry and either TCRζ-mCherry, Rab5-mCherry or CD28-EGFP.
-Original Data: Confocal Images
-Prism File: Quantification of the number of SNX9-EGFP/-mCherry, TCRζ-mCherry, Rab5-mCherry and CD28-EGFP positive vesicles in each frame of the z-stack.
- Average axial position where the maximum number of CD28, SNX9, TCRζ and Rab5 structures were identified.
-Excel File

>> Figure 2. Recruitment of SNX9 to CD28 clusters upon T cell activation
- Fig. 2 A,B,C: Images used in publication: Representative TIRF images of fixed WT Jurkat T cells expressing CD28WT-EGFP or CD28YF-EGFP.
-Original Data: Confocal Images
-Prism File: Fold fluorescent intensities of CD28WT/YF-EGFP (cyan) and anti-SNX9 (purple).
- Quantification of the percentage overlap between WT or YF CD28 and SNX9, or Rab5 and SNX9.

>Fig. 2 D Images used in publication: Representative TIRF live images of WT Jurkat T cells expressing SNX9-mCherry and CD28-EGFP.
-Original Data: TIRF time series

>Fig. 2 E: Images used in publication: Representative zoomed images of C, showing CD28-EGFP arriving at the immunological synapse first, recruiting SNX9-mCherry over time to the same microclusters.

-Original Data: TIFF time series

>Fig. 2 F: Images used in publication: Representative live images of activated Jurkat SNX9 KO#4 T cells expressing SNX9-WT-mCherry, SNX9-ΔSH3-mCherry or SNX9-ΔPX-mCherry.
-Original Data: Confocal Images

>Fig. 2 G,H: Images used in publication: Representative live images of activated SNX9 KO#4 T cells co-expressing CD28WT-EGFP and SNX9WT-mCherry, SNX9-ΔSH3-mCherry or SNX9-ΔPX-mCherry.
-Original Data: Confocal time series
-Prism File: Fold change of mean fluorescence intensity profiles of SNX9-WT-mCherry, SNX9-ΔSH3-mCherry or SNX9-ΔPX-mCherry intensity within CD28WT-EGFP microclusters compared to the mean fluorescence intensity profile of SNX9WT-mCherry within the cytosol.
-Excel File

>>Figure 3. Dynamics and persistence of SNX9 within CD28 clusters

>Fig. 3 A: Schematic of photoactivation of fluorescent proteins at the plasma membrane of cover glass activated cells with the photoactivated protein diffusing either though the cell membrane or through protein-clusters localized at the plasma membrane.

>Fig. 3 B,C: Images used in publication: Representative confocal time series of SNX9-PAmCherry repetitively photoactivated by 405 nm laser at the membrane region of interest in activated SNX9 KO#4 cells.
-Original Data: Confocal time series
-Prism File: Mean fluorescence intensity profiles of SNX9-PAmCherry intensity within CD28-EGFP clusters (blue) or within the cytosol (magenta).
-Excel File

>Fig. 3 D: Schematic of whole cell photoactivation in TIRF.

>Fig. 3 E,F: Images used in publication: Representative TIRF images of WT activated Jurkat T cells co-expressing CD28-EGFP and SNX9-PAmCherry photoactivated.
-Original Data: TIRF Images
-Prism File: Fold change of mean fluorescent intensity profiles of SNX9-PAmCherry intensity within CD28-EGFP microclusters compared to the cytosol.
-Excel File

>>Fig. 4 Part1. SNX9 defines tubules that are connected to CD28 clusters.
>Fig. 4 A-G: Images used in publication: Correlation of SNX9-mCherry transfected cell with the same cell re-registered by transmission electron microscopy.
-Original Data: Fluorescence confocal microscope Images
- Reconstructed movies and frames (Overlay of CD28-EGFP with stitched electron micrograph, overlay of SNX9-mCherry with stitched electron micrograph)
- Tiled micrographs of transmissive electron microscopy

>>Fig. 4 Part 2: SNX9 defines tubules that are connected to CD28 clusters. Raw and segmented data used in the publication.
-Tomogram and Tilt series
-Raw tilt series
-Reconstruction of Tilt data
-Segmentation

>>Figure 5. SNX9 promotes CD28 cluster stability.

>Fig. 5 A,B: Images used in publication: Representative live images of activated WT Jurkat and SNX9 KO#4 T cells expressing CD28WT-EGFP or CD28YF-EGFP with or without SNX9WT-mCherry.
-Original Data: Confocal Images
-Prism File: Number of CD28WT/YF-EGFP positive structures in Jurkat SNX9 KO T cells with or without SNX9WT-mCherry expression.

>Fig. 5 C: Images used in publication: Representative images at indicated time points of activated WT Jurkat T cells expressing SNX9-EGFP and CD28WT-PAmCherry or TCRζ-PAmCherry photoactivated within the dashed regions.
-Original Data: Confocal Images

>Fig. 5 D,E,F: Images used in publication: Representative images of activated WT and SNX9 KO#4 Jurkat T cells expressing CD28WT or YF-EGFP with or without SNX9-mCherry.
-Original Data: Confocal Images
-Prism File: (E)Increase in mean fluorescence intensity over time of bleached regions shown in (D). (F) Mean fluorescence intensity at t= 380 s from (E).
-Excel File

>>Figure 6. Surface levels and endocytosis of CD28 upon SNX9 knock-out.

>Fig. 6 -Fig. 6 A,B,C -Graphs: (A) Mean fluorescence intensity of resting and activated WT Jurkat, SNX9KO#3 and SNX9KO#4 T cells incubated with an antibody against CD28.
-(B) Mean fluorescence intensity of resting and activated Jurkat WT and SNX9KO#3 and SNX9KO#4 T cells stained with an antibody against CD28 and allowed to internalize CD28 complexes before removing any surface bound antibody.
-Original Data: Flow Cytometry Data
-Prism File: (A) Mean fluorescence intensity of CD28 surface expression in resting and activated Jurkat WT and SNX9KO#3 and SNX9KO#4 T cells.
-(B) Mean fluorescence intensity of CD28 intracellular expression in resting and activated Jurkat WT and SNX9KO#3 and SNX9KO#4 T cells.

>>Figure 7. SNX9 specifically contributes to CD28-mediated signalling.

>Fig. 7 A,B,C,D-Graphs: (A)Phosphorylation of CD28 and (C) TCRζ in activated WT Jurkat and SNX9KO T cells detected by phospho-specific antibodies in flow cytometry.

_(B) Normalized (WT = 100%) percentage of pCD28 and (D) pTCRζ in SNX9KO cells compared to WT Jurkat T cells.
-Original Data: Flow Cytometry Data
-Prism File: Dot Plots of phosphorylation of CD28 and TCRζ.

>Fig.7 E,F: Images used in publication: Representative immunofluorescence images of resting and activated WT and SNX9KO Jurkat T cells stained with an antibody against NFAT.
-Original confocal slides are not on this upload and available upon request.
-Original Data: Confocal z-Stacks
-Prism File: Integrated NFAT fluorescence intensity over the Hoechst-stained nucleus in WT and SNX9 KO Jurkat T cells activated or not with anti-CD3ε and CD28 antibodies.
-Excel File

>Fig. 7 G
-Prism File: WT Jurkat and SNX9KO T-cells were conjugated with SEE pulsed or non-pulsed Raji B cells and incubated together for 16 h in a 96 well plate to allow for IL-2 secretion. The supernatant was collected, and the amount of IL-2 present was determined by ELISA.

>>Figure Supplements

>Figure 1-figure supplement 1. SNX9 is recruited to the immunological synapse of primary T cells where it co-localises with CD28.
-Images used for paper: Representative images of maximum intensity projections of 5 Z-stacks of OT-II T cells expressing GFP-SNX9
-Original Data: Confocal Images
-Prism File: Mean recruitment of SNX9 to the synapse with and without peptide after 15 min of conjugation
-Excel File
>Figure 1-figure supplement 2. Worflow for identification of intracellular structures.

>Figure 2-figure supplement 1. Representative confocal images from three independent experiments of WT Jurkat T cells expressing SNX9-GFP and mCherry-Rab5, activated for 10 minutes.
>Figure 2-figure supplement 2. SNX9 is recruited into CD28 microclusters upon T cell activation.
-Prism File: Line Graphs of SNX9 and XD28 colocalization.
>Figure 2-figure supplement 3. SNX9 is recruited into CD28 microclusters upon T cell activation by antigen-presenting cells.
-Original Data: Confocal Images
>Figure 2-figure supplement 4. SNX9 expression in Jurkat WT and SNX9KO T cells.
 -Western blot
>Figure 2-video 1. Representative live cell movie showing SNX9 recruitment to CD28 clusters during T cell activation.
-Original Data: In Fig. 2 D

>Figure 3-figure supplement 1. Example of custom-made FIJI quantification of cluster versus cytosol.

Figure 3-figure supplement 2. Quantification approach used to count the number of PAmCherrry-positive vesicles after photoactivation.
Figure 3-video 1. Representative movie showing the dynamic localisation of SNX9 in CD28 clusters.
-Video: Confocal time series of SNX9 KO#4 cells expressing CD28-EGFP and SNX9-PA-mCherry and repetitively photoactivated by 405 nm laser at the membrane region of interest
- Original Data: Confocal time series

>Figure 4-figure supplement 1. Identification and quantification of tubular structure in correlative electron tomography.
Figure 4-video 1. Confocal slices of the WT Jurkat cell expressing CD28-EGFP and SNX9-mCherry used to overlaid on the EM pictures.
-Original Data: In Folder of Fig. 4
>Figure 4-video 2. Optical slices from a reconstructed tomogram of different z-depths demonstrating abundant tubules emerging from the plasma membrane.

>Figure 5-figure supplement 1. Fitting of the Fluorescence recovery after photobleaching (FRAP) curves for CD28 in WT, SNX9KO and SNX9 overexpressing Jurkat T cells.
Figure 5-figure supplement 2. SNX9 has no impact on the recovery of Lck fluorescence intensity after FRAP.
-Images used for paper:Confocal images of WT and SNX9KO Jurkat T cells expressing Lck10WT-EGFP and SNX9-mCherry, or only Lck10-EGFP, activated on anti-CD3ε and anti-CD28 coated glass surfaces.
-Original Data: Confocal time series
-Prism File:Increase in mean fluorescence intensity over time of bleached Lck10WT-EGFP regions in WT and SNX9KO Jurkat T cells.
Figure 5-video 1 Representative movie showing the diffusion of CD28 through clusters.
-Original Data: Confocal time series
Figure 5-video 2. Representative movie showing two-photon FRAP of CD28-EGFP clusters.
-Original Data: Confocal time series

>Figure 6-figure supplement 1. Flow cytometry gating strategy.
-Schematic: FSC and SSC dot plots were used to identify live T Lymphocytes, doublets were eliminated by using a pulse geometry gate with FSC-H and FSC-A.
>Figure 6-figure supplement 2. CD28 receptor internalisation compared to TCR.

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