Supplemental material

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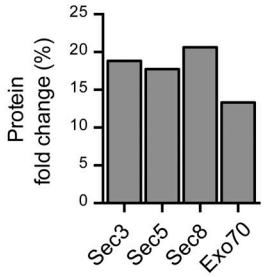
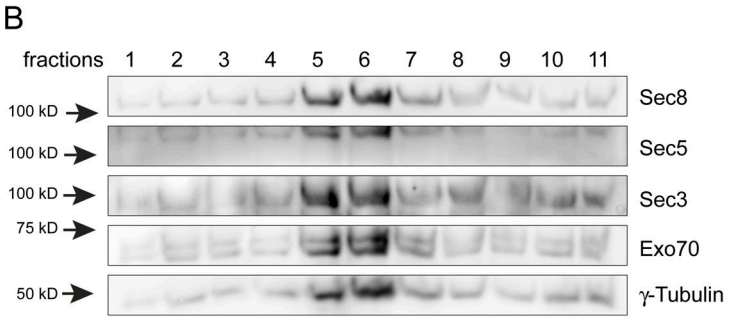
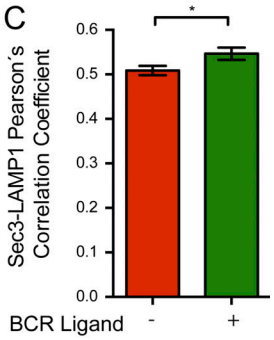
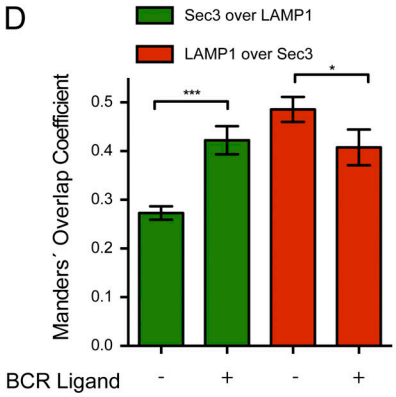
Protein	Activated/Resting			Peptide sets	
	Ratio	%	Adjusted *p-value*	Distinct	Used
Sec3	1.19	18.83	2.73E-04	2	4
Sec5	1.18	17.74	2.39E-03	6	15
Sec8	1.21	20.64	2.57E-04	8	11
Exo70	1.13	13.34	7.24E-06	6	11









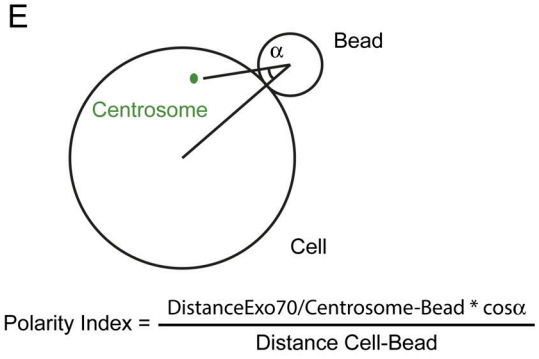


Figure S1. **Association of exocyst subunits with the centrosome and lysosomes.** Related to Fig. 1. **(A)** SILAC-based quantification of Exocyst subunits. Centrosomes purified from activated and nonactivated B cells were mixed 1:1, separated by SDS-PAGE, and analyzed by LC-MS/MS. To estimate activated/nonactivated ratio significance, a *t* test was performed with a Benjamini–Hochberg false discovery rate control threshold set to 0.05. **(B)** Western blot analysis of Exo70, Sec5, Sec8, and Sec3 distribution within purified centrosomal fractions of resting B cell lymphoma cells (as revealed by γ-tubulin immunoblotting). Representative of two independent experiments. **(C and D)** Pearson's (C) and Manders' (D) colocalization analysis of Sec3 and LAMP-1 were performed in Sec3-HA–transfected B cells activated with BCR ligand–positive or –negative beads. $n > 26$ cells from two independent experiments. Student's *T* test (C) or ANOVA followed by a Sidak's multiple comparison test (D) were performed as statistical tests. *, $P < 0.05$; ***, $P < 0.001$. Error bars are mean ± SEM. **(E)** Scheme depicting the method used to quantify centrosome and Exo70 polarity indexes.

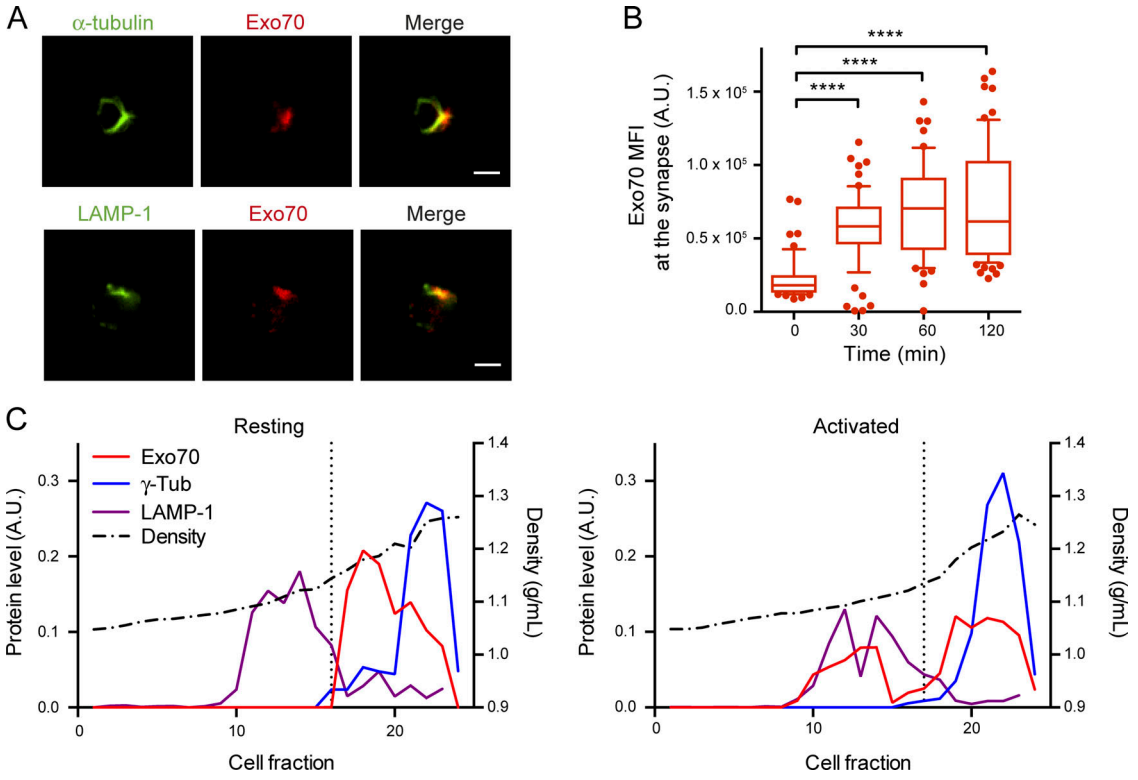


Figure S2. **Exo70 is found the centrosome of B cells and becomes associated to membrane fractions upon activation.** Related to Fig. 2. **(A)** Epifluorescence images of spleen isolated mouse primary B cells stained for Exo70 (red) and α-tubulin or LAMP-1 (green). Scale bar: 3 µm. **(B)** Quantification of the Exo70 MFI at the IS upon B cell activation. Exo70 accumulation at the synapse was quantified from $n > 52$ cells per time point from two independent experiments. Data are shown as box-and-whisker plots. The ends of whiskers represent the 10th and 90th percentile. ANOVA followed by a Sidak's multiple comparison test. ****, $P < 0.0001$. **(C)** Membrane flotation assay. Separation of Exo70, γ-tubulin, and LAMP-1 from activated cell homogenates in a discontinuous iodixanol gradient. The membrane-associated proteins are shown in fractions with a density <1.16 g/ml (dotted lines).

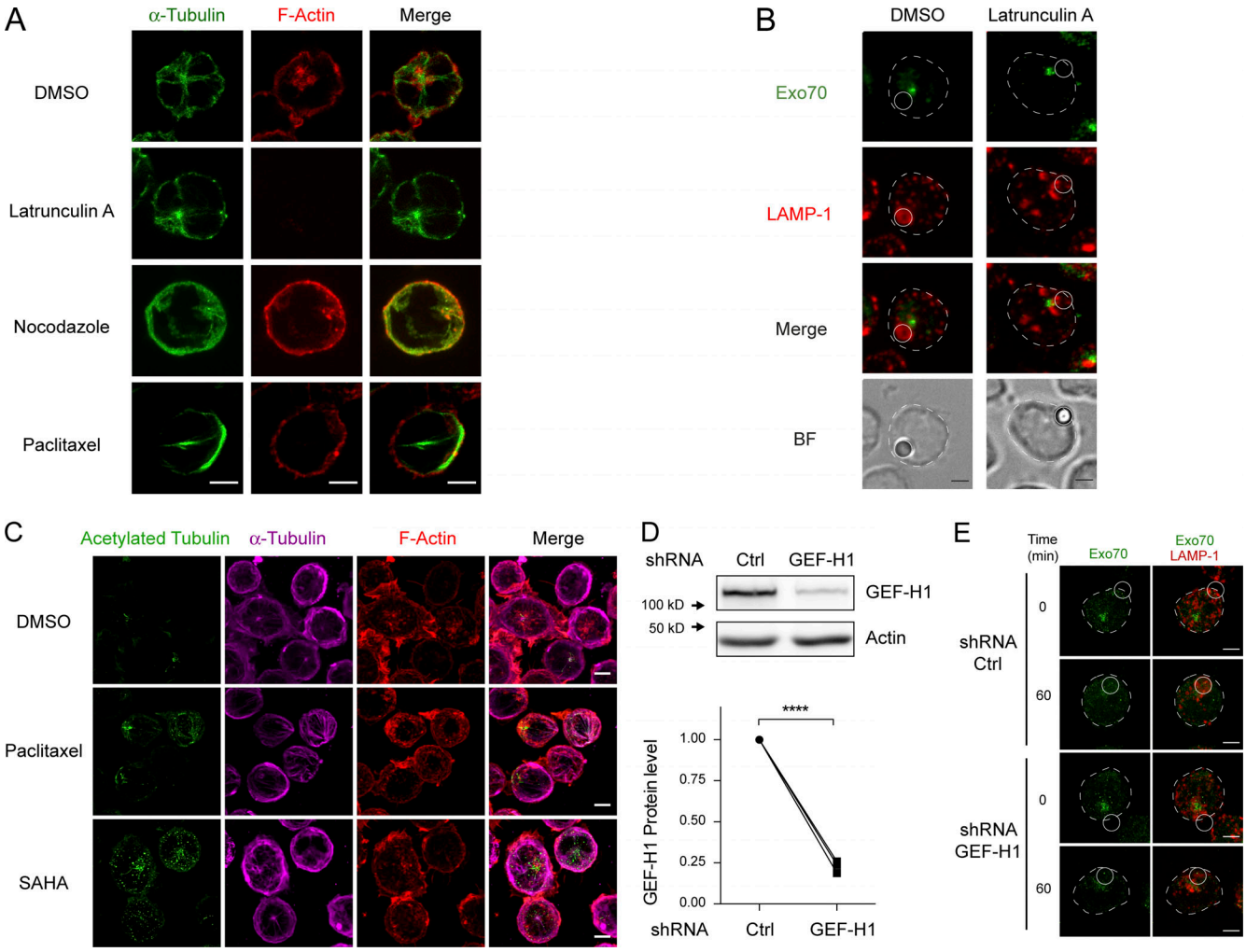


Figure S3. **Actin and microtubule cytoskeleton disruption does not change the localization of Exo70.** Related to Figs. 3 and 4. **(A)** Effects of drugs used to perturb cytoskeleton dynamics in B cells. Single-plane confocal images of B cells treated for 60 min with DMSO, 5 µM Latrunculin A, 10 µM nocodazole, or 20 µM paclitaxel, fixed, and stained for α-tubulin (green) and F-actin (red). Scale bar: 3 µm. **(B)** Z-projections of confocal images of B cells pretreated with DMSO or Latrunculin A for 60 min, activated with BCR ligand$^+$ beads for 60 min, and stained for Exo70 (green) and lysosomes (LAMP-1, red). Representative images of two independent experiments. Scale bar: 3 µm. **(C)** Effects of microtubule stabilizing drugs on microtubule acetylation. B cells were seeded on poly-L-lysine–coated coverslips and treated for 30 min with paclitaxel (20 µM), SAHA (1 µM), or DMSO, fixed, and stained for acetylated tubulin (green), α-tubulin (red), and F-actin (phalloidin, magenta). Scale bar: 5 µm. **(D)** Silencing of GEF-H1 expression. Top: Western blot showing GEF-H1 and actin levels from control or GEF-H1–silenced cells. Bottom: Quantification of GEF-H1 levels normalized to actin levels. n = 3 independent experiments. ANOVA followed by a Sidak's multiple comparison test: ****, $P < 0.0001$. **(E)** Effect of GEF-H1 silencing on the localization of Exo70. Representative confocal images of control or GEF-H1–silenced cells in resting conditions or activated with BCR ligand$^+$ coated beads, fixed, and stained for Exo70 (green) and LAMP-1 (red). Scale bar: 3 µm.

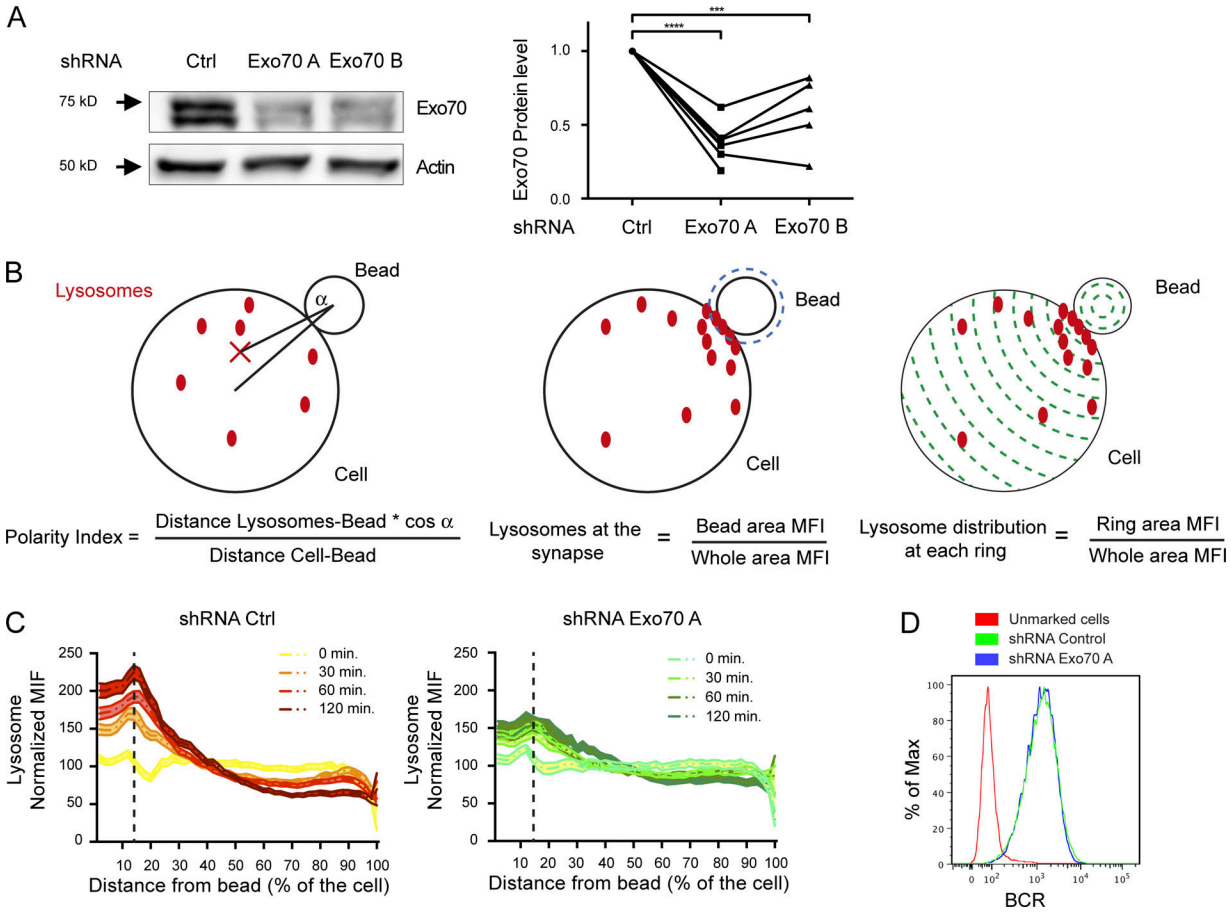


Figure S4. **Characterization of Exo70-silenced cells.** Supporting Fig. 5. **(A)** Left: Exo70 protein levels and actin levels from control or Exo70-silenced cells detected by Western blot. Right: Quantification of Exo70 levels normalized to actin levels. *n* = 5 independent experiments. ANOVA followed by a Sidak's multiple comparison test. ****, P < 0.0001; ***, P = 0.0003. **(B)** Scheme depicting the method used to quantify the lysosome polarity index (left), lysosome accumulation at the IS (middle), and the radial distribution of lysosomes (right). **(C)** Radial distribution of the lysosomes in control and Exo70-silenced cells after indicated times of activation, starting at the bead center to the maximum length of the cell. Dashed line represents bead–cell boundary. *n* > 70 cells per time from two independent experiments. **(D)** Quantification of BCR cell surface levels in control and Exo70-silenced cells by flow cytometry.

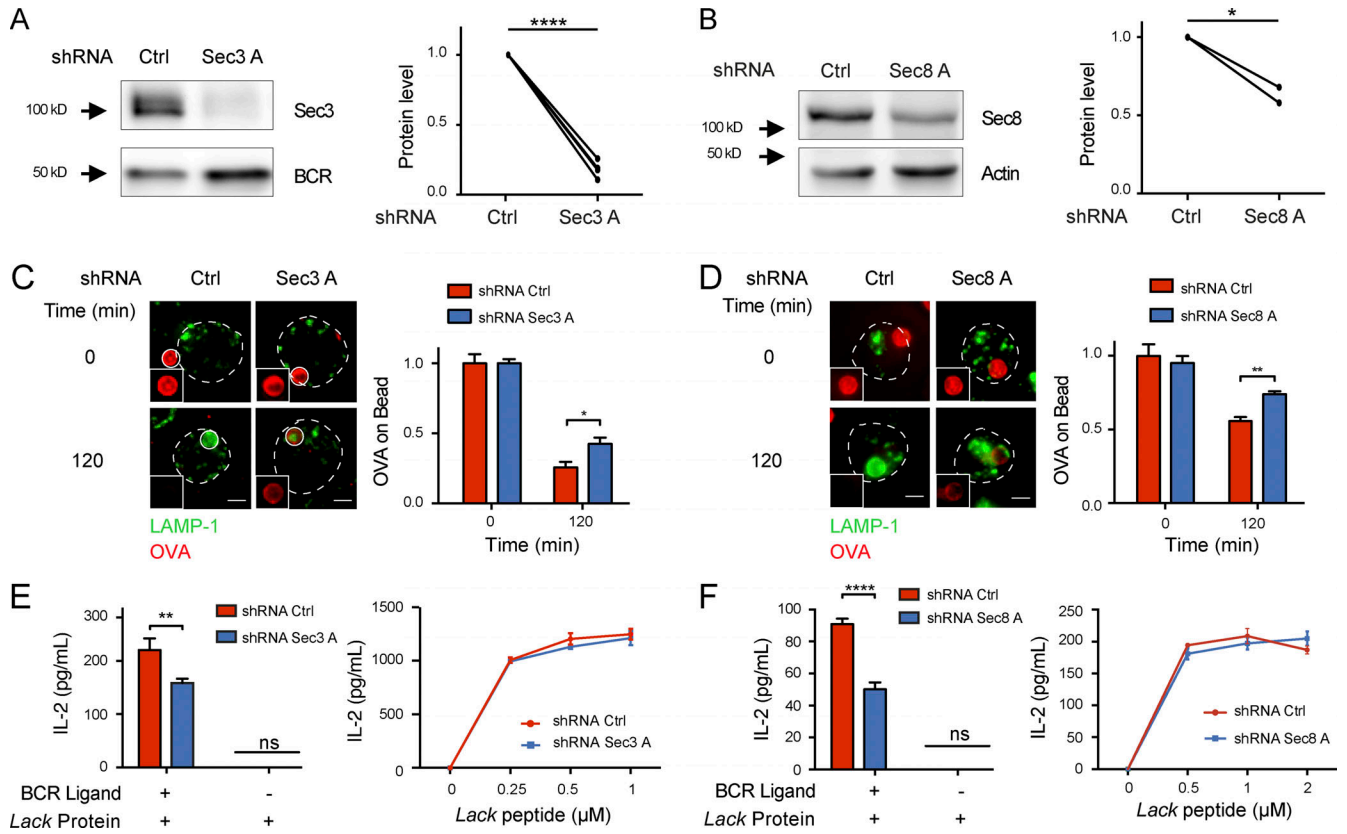
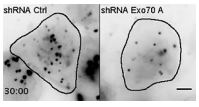


Figure S5. **Sec3- and Sec8-silenced B cells show impaired extraction and presentation of immobilized antigens.** Related to Fig. 7. **(A and B)** Sec3 or Sec8 protein levels from control or Sec3- or Sec8-silenced cells, respectively, detected by Western blot. Quantification of the Sec3 or Sec8 protein levels. Values were normalized to BCR or actin levels, respectively. Data pooled from three (Sec3), or two (Sec8) independent experiments. *t* test; ****, P < 0.0001; *, P = 0.0178. **(C and D)** Antigen extraction assay. Left: Representative epifluorescence images of control and Sec3-silenced (C) or Sec8-silenced (D) cells under resting conditions or activated BCR ligand$^+$ beads coupled to OVA for 120 min, fixed, and stained for OVA (red) and LAMP-1 (green). Cells are shown as Z-projections of a stack. Insets highlight the remaining OVA fluorescence intensity on the bead. Scale bar: 3 µm. Right: Quantification of the OVA fluorescence intensity remaining on the beads. Values were normalized by the initial fluorescence. Sec3: *n* > 25 cells per time point from two independent experiments; Sec8: *n* > 33 cells per time point from one experiment. ANOVA followed by Sidak's multiple comparison test. *, P = 0.008; **, P = 0.0017. **(E and F)** Left: Antigen presentation assay with control and Sec3-silenced (E) or Sec8-silenced (F) cells. ANOVA followed by Sidak's multiple comparison test. **, P = 0.0039; ****, P < 0.0001. Right: Peptide control for the cells used in each assay. Mean amounts of IL-2 are shown for a representative of three independent experiments performed in triplicate. Error bars are mean ± SEM.



Video 1. **Defective lysosome docking at the IS of Exo70-silenced cells.** Movement of LysoSensor Green stained lysosomes at the synapse of control (left) and Exo70-silenced (right) B cells plated on BCR ligand$^+$ slides. Images were acquired by TIRF microscopy at 1 image/s for 3 min. Scale bar: 3 µm.