

## SUPPLEMENTAL FIGURE LEGENDS

**Figure S1. T cells express all BBSome core components.** (A) Real-time quantitative PCR (RT-qPCR) of the BBSome subunits *BBS-1*, *-2*, *-4*, *-5*, *-7*, *-8*, *-9* and *-18* in Jurkat cells, primary T cells and BJ-5at cells ( $n \geq 3$ ). (B) Immunofluorescence analysis of BBS4 in 30-min conjugates of SEE-pulsed Ramos cells (APCs) and SYFP-BBS4-expressing Jurkat cells co-stained for  $\gamma$ -tub. Size bar, 5  $\mu$ m.

**Figure S2. Representative images of the BBS1 co-localization analyses and of conjugates stained for PCNT and CD3 $\zeta$ .** (A) Immunofluorescence analysis of BBS1-GFP expressing Jurkat cells co-stained for CEP290 (pericentrosomal marker), Rab11 (recycling endosomes) and Rab7 (late endosomes). The histograms on the right show the intensity profiles along the white lines within the selected area in the overlay images for each channel. The raw pixel intensity signals were normalized to maximum intensity pixel of each channel (% max grey value). The quantification using Manders' coefficient of the colocalization of BBS1-GFP with these markers is shown in Fig.1C (10 cells/sample,  $n \geq 3$ ). Representative images (medial optical sections) are shown. (B) Immunofluorescence analysis of PCNT and CD3 $\zeta$  localization in 15-min conjugates of control (ctr) and BBS1KD (J KD) Jurkat cells with Raji cells (APCs) in the absence or presence of SEE ( $n=3$ ). Size bar, 5  $\mu$ m.

**Figure S3. Characterization of the Jurkat cell lines and primary T cells used in this study.** (A) Immunoblot analysis of BBS1 in lysates of Jurkat cells transduced with lentiviral particles containing a non-targeting control shRNA (ctr) or a shRNA specific for BBS1 (J KD) (*left*), or in lysates of control (ctr) primary and BBS1 KO (T KO) primary T cells gene-edited by CRISPR-Cas9 technology (*middle*), or in lysates of control (ctr) and BBS1 KO (J KO) Jurkat cells gene-edited by CRISPR-Cas9 technology (*right*) ( $n \geq 3$ ). Representative

immunoblots are shown. The migration of molecular mass markers is shown for each filter. The quantification of the relative protein expression normalized to actin is shown (mean $\pm$ SD, n $\geq$ 3, paired two-tailed Student's *t*-test). **(B)** Flow cytometric analysis of CD3 $\epsilon$  in Jurkat cells transduced with lentiviral particles containing a non-targeting control shRNA (ctr) or a shRNA specific for BBS1 (J KD) (*left*), in control (ctr) and BBS1 KO (T KO) primary T cells (*middle*), or control (ctr) and BBS1 KO (J KO) Jurkat cells (*right*). The means of mean fluorescence intensities (MFIs) measured in multiple experiments are reported in each representative FACS profile (n $\geq$ 3). **(C)** Real-time quantitative PCR (RT-qPCR) of the BBSome subunits *BBS-1*, *-2*, *-4*, *-5*, *-7*, *-8*, *-9* and *-18* in Jurkat cells transduced with lentiviral particles containing a non-targeting control shRNA (ctr) or a shRNA specific for BBS1 (J KD). **(D)** Immunoblot analysis of BBS1 in lysates of control (ctr) or BBS1 KO (J KO) Jurkat cells. Both ctr and J KO were transfected with the empty vector (ctr+GFP, J KO+GFP), while J KO were also transfected with the same vector encoding wild-type BBS1 (J KO+BBS1-GFP) (n=3). The migration of molecular mass markers is shown for each filter. The quantification of the relative protein expression normalized to actin is shown on the right. **(E)** Flow cytometric analysis of GFP in control (ctr) Jurkat cells either untransfected (neg ctr) or transfected with the empty vector (ctr+GFP), and BBS1 KO (J KO) Jurkat cells transfected with either the empty vector (J KO+GFP) or the same vector encoding wild-type BBS1 (J KO+BBS1-GFP) (mean $\pm$ SD, n=3). Cells were co-stained with the cell viability dye propidium iodide (PI) analyzed 24 h after transfection. The percentages (%) of GFP<sup>+</sup>/PI<sup>-</sup> cells are shown in each representative dot plot. The data are expressed as mean $\pm$ SD. \*\*\*P $\leq$ 0.001; \*\*P $\leq$ 0.01.

**Figure S4. Representative images of non-activated T cell conjugates and masks for image analyses.** **(A)** Immunofluorescence analysis of  $\gamma$ -tubulin, CD3 $\zeta$  and p-Tyr in conjugates of control (ctr) and BBS1KD (J KD) Jurkat cells with Raji B cells (APCs) in the

absence of SEE ( $n \geq 3$ ). Quantifications are shown in Fig.2C,H,3A. **(B)** Immunofluorescence analysis of PCNT in conjugates of control (ctr) and BBS1KO (T KO) primary T cells with Raji B cells (APCs) in the absence of SAg. Conjugates were co-stained for nesprin-2. Quantifications are shown in Fig.2D,E. **(C) Top**, Representative image of primary T cells conjugated with Raji cells (APCs) for 15 min and co-stained with anti-nesprin-2 and anti-PCNT antibodies. The T cell was magnified to depict the parameters used for quantification. A line from the center of the centrosome (PCNT) forming an angle of  $90^\circ$  with the tangent to the nuclear membrane (nesprin-2) was drawn to measure the distance between nucleus and centrosome ( $\mu\text{m}$ ) in primary T cells. In cells showing a weak nesprin-2 staining the nucleus-centrosome distance was further confirmed by overlaying the PCNT fluorescence to the DIC image, in which the nuclear contour is easily identified. *Bottom*, Representative image of Jurkat cells conjugated with Raji cells (APCs) for 15 min and triple stained for PCNT, Rab5 and F-actin. The Jurkat cell has been magnified to depict the parameters used for quantification. A  $2\text{-}\mu\text{m}$  diameter circle centered around the centrosome of Jurkat cells (or  $1.2\text{-}\mu\text{m}$  for primary T cells) indicates the centrosomal area used for the colocalization analyses shown in Fig.4B,D;6I;7A-D,G;S5F,G;S7J. The endosome-enriched ring corresponding to a  $4.5\text{-}\mu\text{m}$  diameter circle around the centrosome indicates the area used for the colocalization analyses shown in Fig.5E,F; S6C,D. An endosome-enriched ring corresponding to a  $4.5\text{-}\mu\text{m}$  diameter circle around the centrosome, from which a  $2\text{-}\mu\text{m}$  diameter circle (centrosomal area) was excluded, indicates the area used for the colocalization analyses shown in Fig.S6B. **(D)** Immunofluorescence analysis of CD3 $\zeta$  and p-Tyr in conjugates of control (ctr) and BBS1KO (T KO) primary T cells with Raji B cells (APCs) in the absence of SAg ( $n=3$ ). Quantifications are shown in Fig.2I,3B. **(E-G)** Immunofluorescence analysis of p-ZAP-70 **(E)**, p-LAT **(F)** and F-actin **(G)** in conjugates of control (ctr) and BBS1KD (J KD) Jurkat cells with Raji B cells (APCs) in the absence of SEE ( $n=3$ ). Quantifications are shown in Fig.3C,D,5C. **(H)** Immunofluorescence analysis of

control (ctr) and BBS1KD (J KD) Jurkat cells transfected with the LifeAct reporter and conjugated with Raji B cells (APCs) in the absence of SEE for 15 min (n=3). Quantifications are shown in Fig.5D. Size bar, 5  $\mu$ m.

**Figure S5. BBS1-deficient CD4<sup>+</sup> T cells form dysfunctional immune synapses. (A).** Immunoblot analysis of BBS1 in lysates of control (ctr) and BBS1 KO (CD4<sup>+</sup> KO) primary CD4<sup>+</sup> T cells gene-edited by CRISPR-Cas9 technology. A representative immunoblot is shown. The migration of molecular mass markers is indicated. The quantification of the relative protein expression normalized to actin is shown (mean $\pm$ SD, n=3, paired two-tailed Student's *t*-test). **(B-E)** Immunofluorescence analysis of 15-min conjugates of control (ctr) or BBS1 KO (CD4<sup>+</sup> KO) primary CD4<sup>+</sup> T cells with Raji cells (APCs) in the absence or presence of SAg. Conjugates were co-stained for PCNT and nesprin-2. Representative images (medial optical sections) are shown **(B)**. Quantification (%) of 15-min SAg-specific conjugates harboring PCNT staining at the IS ( $\geq$  25 cells/sample, n=3, unpaired two-tailed Student's *t*-test) **(C)**. Measurement of the distance ( $\mu$ m) of the centrosome (PCNT) from the T cell-APC contact site **(D)** ( $\geq$ 10 cells/sample, n=3, One-way ANOVA test) and from the nuclear membrane (nesprin-2) **(E)** ( $\geq$ 10 cells/sample, n=3, Kruskal-Wallis test) in 15-min conjugates of control (ctr) and BBS1 KO (CD4<sup>+</sup> KO) CD4<sup>+</sup> T cells with Raji cells (APCs) in the absence or presence of SAg. **(F)** Immunofluorescence analysis of centrosomal F-actin (phalloidin) in 15-min conjugates of control (ctr) or BBS1 KO (CD4<sup>+</sup> KO) primary CD4<sup>+</sup> T cells Raji cells (APCs) in the absence or presence of SAg. Conjugates were co-stained for the centrosomal marker PCNT. Quantification using Manders' coefficient of the weighted colocalization of PCNT with centrosomal F-actin (see Fig.S4C for mask generation) ( $\geq$  10 cells/sample, n=3, Kruskal-Wallis test). **(G)** Immunofluorescence analysis of 19S RP recruitment to the centrosome ( $\gamma$ -tubulin) in 1-min conjugates of control (ctr) or BBS1 KO (CD4<sup>+</sup> KO) primary CD4<sup>+</sup> T cells with Raji cells (APCs) in the absence or presence of SAg.

Quantification using Manders' coefficient of the weighted colocalization of 19S RP with the centrosome ( $\gamma$ -tubulin) (see Fig.S4C for mask generation) ( $\geq 10$  cells/sample,  $n=3$ , Kruskal-Wallis test). Size bar, 5  $\mu\text{m}$ . The data are expressed as mean $\pm$ SD. \*\*\*\* $P\leq 0.0001$ ; \*\* $P\leq 0.01$ ; \* $P\leq 0.05$ ; n.s., not significant.

**Figure S6. BBS1 deficiency does not affect endosomal F-actin during IS assembly.**

**(A)** Immunofluorescence analysis of endosomal F-actin in control (ctr) or BBS1 KD (J KD) Jurkat cells conjugated with Raji cells (APCs) in the absence or presence of SEE for 15 min and triple stained for PCNT, Rab5 and F-actin. Representative images are shown. **(B)** Colocalization analysis of F-actin with Rab5 in an endosome-enriched ring of 4.5- $\mu\text{m}$  diameter around the PCNT-labelled centrosome, from which a 2- $\mu\text{m}$  diameter circle (centrosomal area) was excluded (detailed in Fig.S4C) ( $\geq 10$  cells/sample,  $n=2$ , One-way ANOVA test). **(C,D)** Immunofluorescence analysis of endosomal WASH in control (ctr) and BBS1KD (J KD) Jurkat cells conjugated with Raji cells (APCs) in the absence or presence of SEE for 15 min and co-stained for the endosomal markers Rab5 **(C)** and Rab11 **(D)** (see Fig.S4C for mask generation). Colocalization of WASH on individual dots (*left*) and quantification of Rab5<sup>+</sup> or Rab11<sup>+</sup> dots positive for WASH (*right*) ( $\geq 10$  cells/sample, 15 dots/cell,  $n=2$ , Kruskal Wallis test or One-way ANOVA based on normality distribution of different data sets). Size bar, 5  $\mu\text{m}$ . The data are expressed as mean $\pm$ SD. n.s., not significant.

**Figure S7. Characterization of immune synapses formed by Jurkat and primary T cells treated with proteasome inhibitors.**

**(A)** Immunoblot analysis of ubiquitin (Ub) in lysates of control (ctr) Jurkat cells treated for 2 h with either carrier (DMSO) or the proteasome inhibitors MG132 and epoxomicin (Epox) ( $n=3$ ). Actin was used as loading control. **(B)** Viability (%) of control (ctr) Jurkat cells treated with either carrier (DMSO) or the proteasome

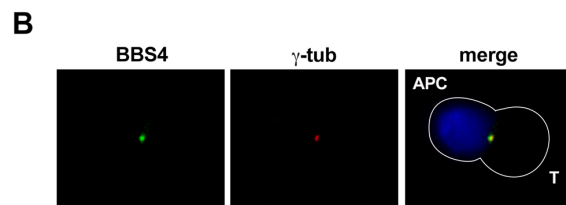
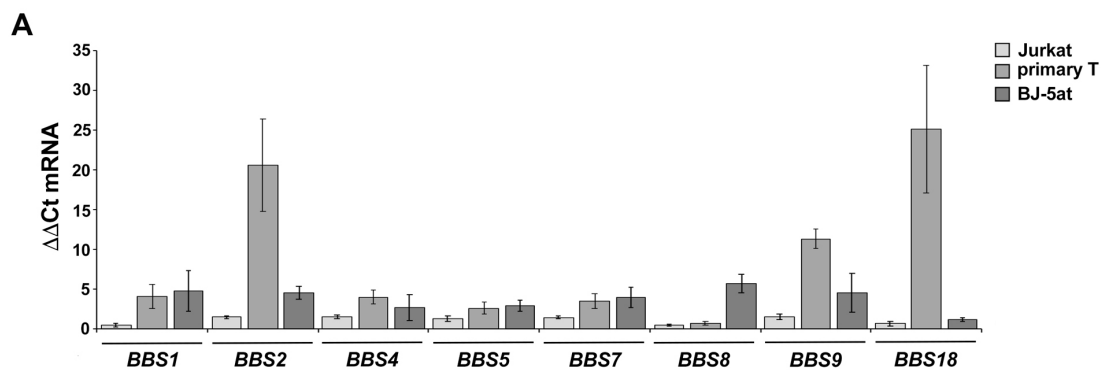
inhibitors MG132 and epoxomicin measured using trypan blue exclusion (n=3). **(C)** Flow cytometric analysis of CD3 $\epsilon$  in control (ctr) Jurkat cells treated for 2 h with either carrier or the proteasome inhibitors MG132 and epoxomicin (EpoX), either intact (*left*) or permeabilized (*middle*) to quantify surface and total CD3 $\epsilon$ , respectively. The data (mean $\pm$ SD) are expressed as MFI in intact cells (surface) and permeabilized cells (total). The ratio of MFI of the surface and total pools is plotted on the histogram on the right (n=3, unpaired two-tailed Student's *t*-test). **(D)** *Left*, Quantification (%) of 15-min SAg-specific conjugates formed by primary T cells, pre-treated with either carrier (DMSO) or the proteasome inhibitors MG132 or epoxomicin (EpoX), and showing PCNT staining at the IS ( $\geq 25$  cells/sample, n=3, unpaired two-tailed Student's *t*-test). *Right*, Measurement of the distance ( $\mu$ m) of the centrosome (PCNT) from the T cell-APC contact site in primary T cells, pre-treated with either carrier (DMSO) or the proteasome inhibitors MG132 or epoxomicin (EpoX), and conjugated with Raji cells (APCs) in the absence or presence of SAg for 15 min ( $\geq 10$  cells/sample, n=3, Kruskal-Wallis test). **(E)** Measurement of the centrosome-nucleus distance ( $\mu$ m) in primary T cells, pre-treated with either carrier (DMSO) or the proteasome inhibitors MG132 or epoxomicin (EpoX), and conjugated with Raji cells in the absence or presence of SAg for 15 min (see Fig.S4C for parameters used for quantification) ( $\geq 10$  cells/sample, n=3, Kruskal-Wallis test). **(F)** Measurement of the distance ( $\mu$ m) of the endosomal TCR-CD3 pool (eCD3 $\zeta$ ) from the T cell-APC contact site in primary T cells, pre-treated with either carrier (DMSO) or the proteasome inhibitors MG132 or epoxomicin (EpoX), and conjugated with Raji cells (APCs) in the absence or presence of SAg for 15 min ( $\geq 10$  cells/sample, n=3, Kruskal-Wallis test). **(G)** *Left*, Quantification (%) of 15-min SAg-specific conjugates formed by primary T cells, pre-treated with either carrier (DMSO) or the proteasome inhibitors MG132 or epoxomicin (EpoX), and showing CD3 $\zeta$  staining at the IS ( $\geq 25$  cells/sample, n=3, unpaired two-tailed Student's *t*-test). *Right*, Relative CD3 $\zeta$

fluorescence intensity at the IS primary T cells, pre-treated with either carrier (DMSO) or with the proteasome inhibitors MG132 or epoxomicin (Epox), and conjugated with Raji cells (APCs) in the absence or presence of SAg for 15 min ( $\geq 10$  cells/sample,  $n=3$ , Kruskal-Wallis test). The data are expressed as recruitment index, which is calculated as the *ratio* of CD3 $\zeta$  fluorescence intensity at the T cell-APC contact site to the total T cell area. **(H)** *Left*, Quantification (%) of 15-min SAg-specific conjugates formed by primary T cells, pre-treated with either carrier (DMSO) or with the proteasome inhibitors MG132 or epoxomicin (Epox), and showing p-Tyr staining at the IS ( $\geq 25$  cells/sample,  $n=3$ , unpaired two-tailed Student's *t*-test). *Right*, Relative p-Tyr fluorescence intensity at the IS in primary T cells, pre-treated with either carrier (DMSO) or the proteasome inhibitors MG132 or epoxomicin (Epox), and conjugated with Raji cells (APCs) for 15 min in the absence or presence of SAg for 15 min ( $\geq 10$  cells/sample,  $n=3$ , Kruskal-Wallis test). The data are expressed as recruitment index, which is calculated as the ratio of p-Tyr fluorescence intensity at the T cell-APC contact site to the total T cell area. **(I)** Quantification (%) of 5-min SEE-specific conjugates formed by control (ctr) Jurkat cells, pre-treated with either carrier (DMSO) or the proteasome inhibitors MG132 or epoxomicin (Epox), and showing p-Tyr staining at the IS ( $\geq 25$  cells/sample,  $n=2$ , unpaired two-tailed Student's *t*-test). **(J)** Quantification using Manders' coefficient of the weighted colocalization of PCNT with centrosomal F-actin (*left*) or  $\gamma$ -tubulin with centrosomal WASH (*right*) in primary T cells, pre-treated with either carrier (DMSO) or the proteasome inhibitors MG132 or epoxomicin (Epox), and conjugated with Raji cells (APCs) in the absence or presence of SAg for 15 min (see Fig.S4C for mask generation) ( $\geq 10$  cells/sample,  $n=3$ , Kruskal-Wallis test). **(K)** *Left*, Quantification (%) of 15-min SEE-specific conjugates formed by control (ctr) Jurkat cells, pre-treated with either carrier (DMSO) or the proteasome inhibitors MG132 or epoxomicin (Epox), and showing F-actin staining at the IS ( $\geq 25$  cells/sample,  $n=3$ , unpaired two-tailed Student's *t*-test). *Right*, Relative F-actin fluorescence intensity at the IS in Jurkat T cells, pre-treated with either carrier (DMSO) or

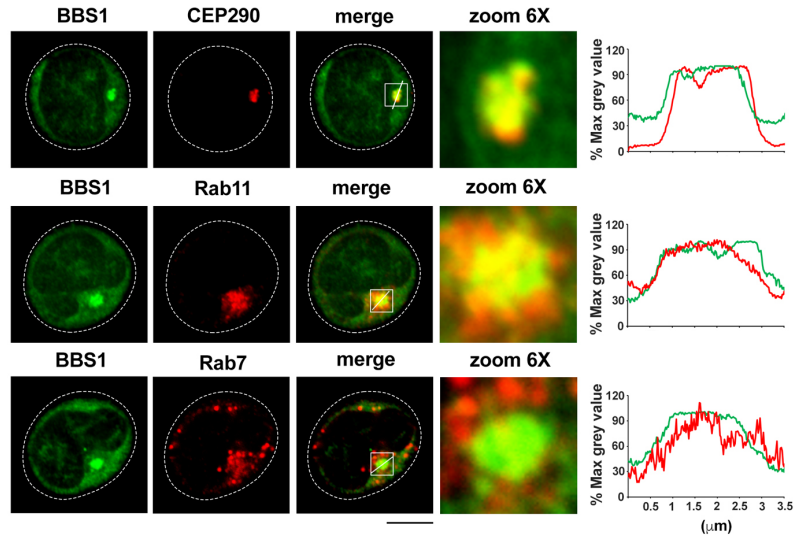
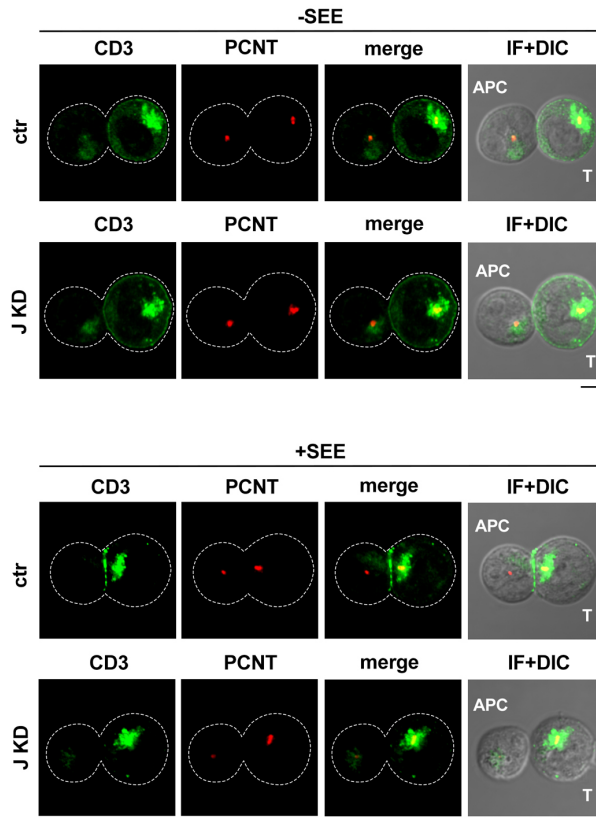
the proteasome inhibitors MG132 or epoxomicin (Epox), and conjugated with Raji cells in the absence or presence of SEE for 15 min ( $\geq 10$  cells/sample,  $n=3$ , Kruskal-Wallis test). The data are expressed as recruitment index, which is calculated as the ratio of F-actin fluorescence intensity at the T cell-APC contact site to the total T cell area. (L) Immunoblot analysis of ubiquitin (Ub) in lysates of control (ctr) and BBS1KD (J KD) Jurkat cells. Actin was used as loading control ( $n \geq 3$ ). The migration of molecular mass markers is indicated. \*\*\*\* $P \leq 0.0001$ ; \*\*\* $P \leq 0.001$ ; \*\* $P \leq 0.01$ ; \* $P \leq 0.05$ ; n.s., not significant.

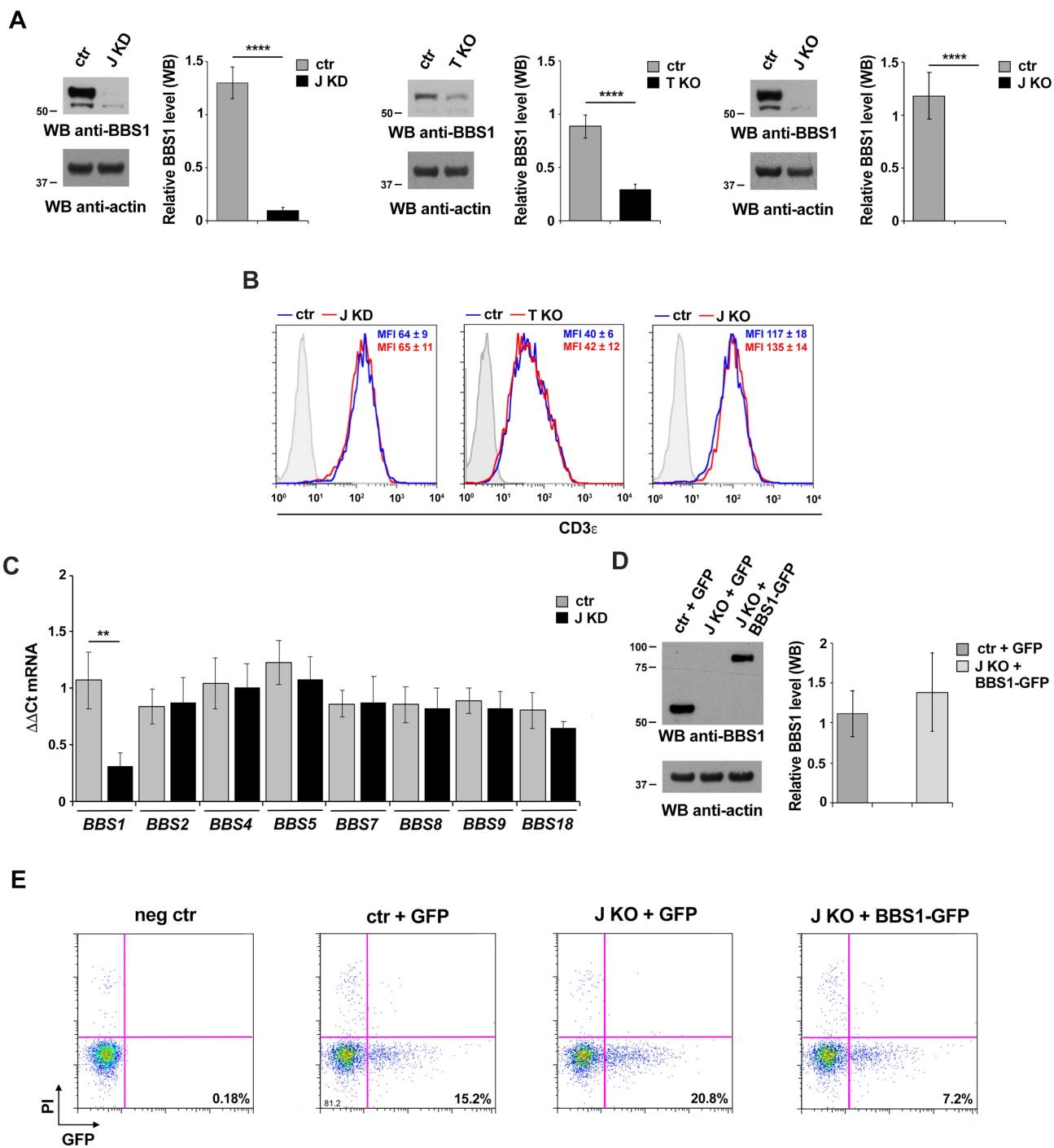
**Figure S8. Representative images of conjugates stained for K48 Ub and PCNT and of conjugates formed with ciliobrevin-treated T cells. (A).** Representative images of K48-linked ubiquitin (K48 Ub) staining in 1-min conjugates of control (ctr) and BBS1KD (J KD) Jurkat cells with SEE-loaded or unloaded Raji cells (APCs) that had been seeded on poly-L-lysine-coated slides. Conjugates were co-stained for PCNT. The time course analysis of centrosomal K48 Ub is shown in Fig.7D. **(B)** Representative images of 19S RP staining in 1-min conjugates of control (ctr) Jurkat cells treated with either carrier (DMSO) or 50  $\mu\text{M}$  ciliobrevin D (Cilio D) during conjugate formation with SEE-loaded or with unloaded Raji B cells (APCs) that had been seeded on poly-L-lysine-coated slides. Conjugates were co-stained for  $\gamma$ -tubulin. The percentage of SEE-specific conjugates harboring  $\gamma$ -tubulin staining at the IS was  $65 \pm 4$  and  $25 \pm 5$  in 15-min conjugates formed by Jurkat cells treated with either carrier (DMSO) or Cilio D, respectively. Quantification of the centrosomal 19S RP at different time points is shown in Fig.7G. Size bar, 5  $\mu\text{m}$ .



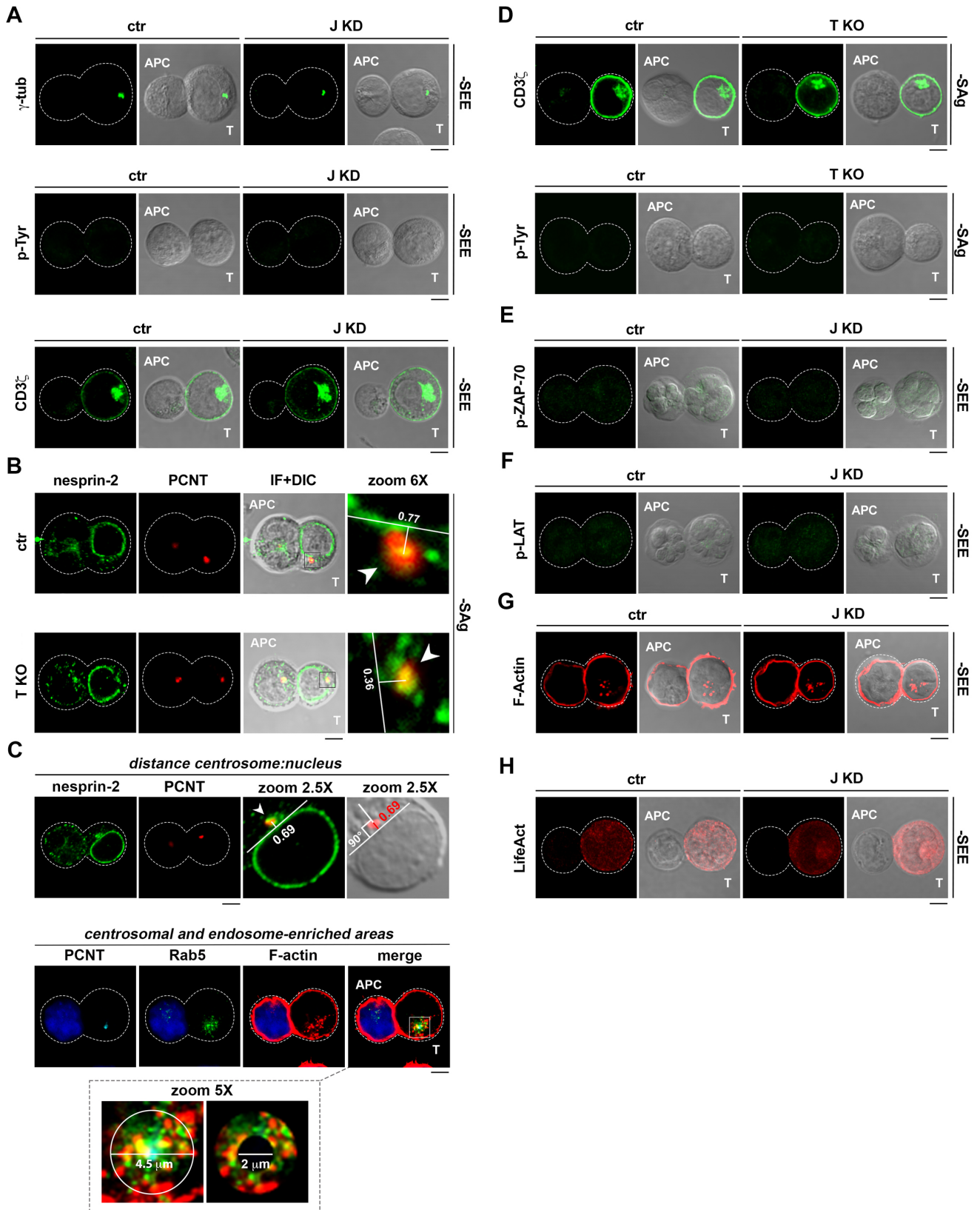


**FIGURE S1**

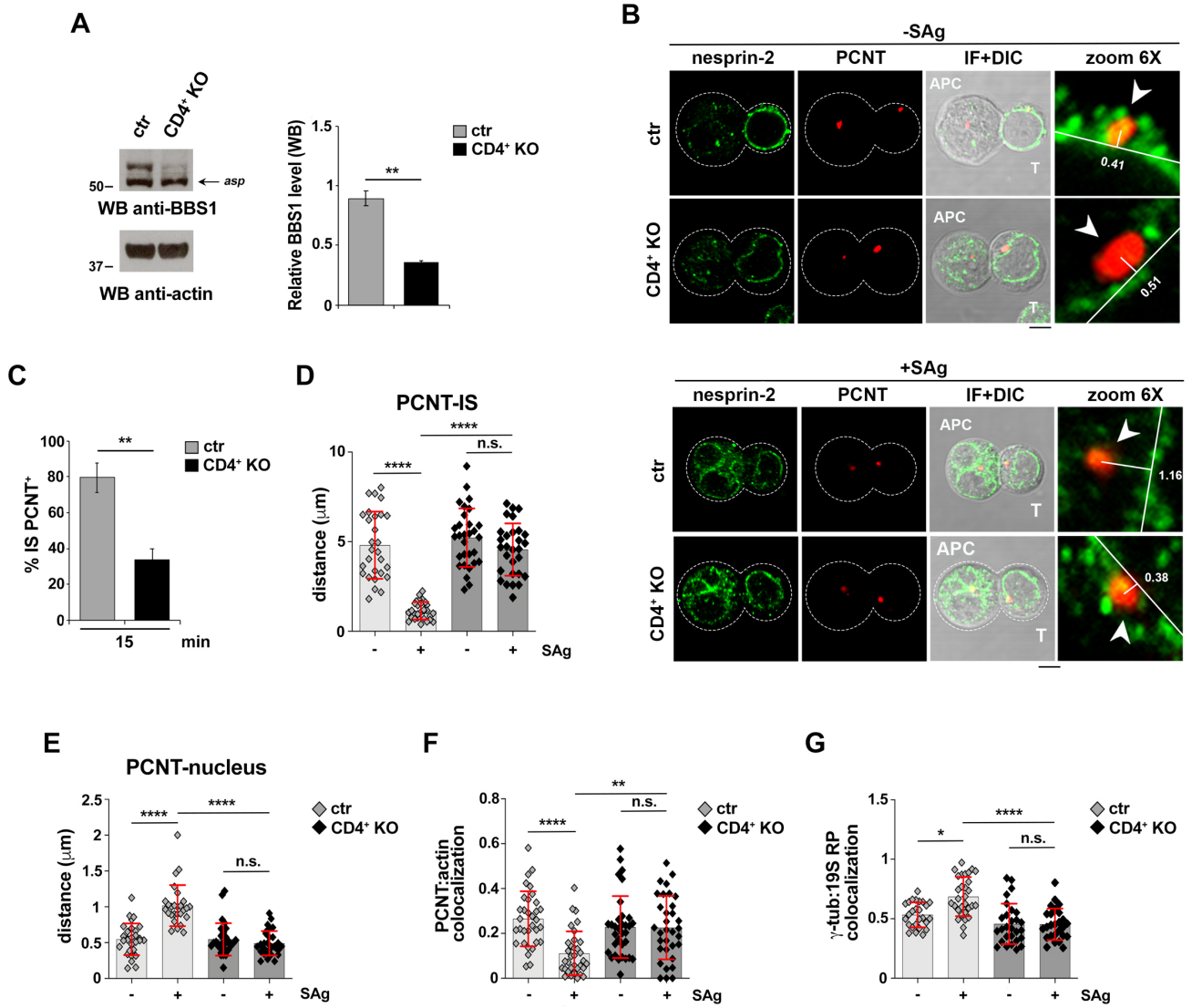
**A****B****FIGURE S2**



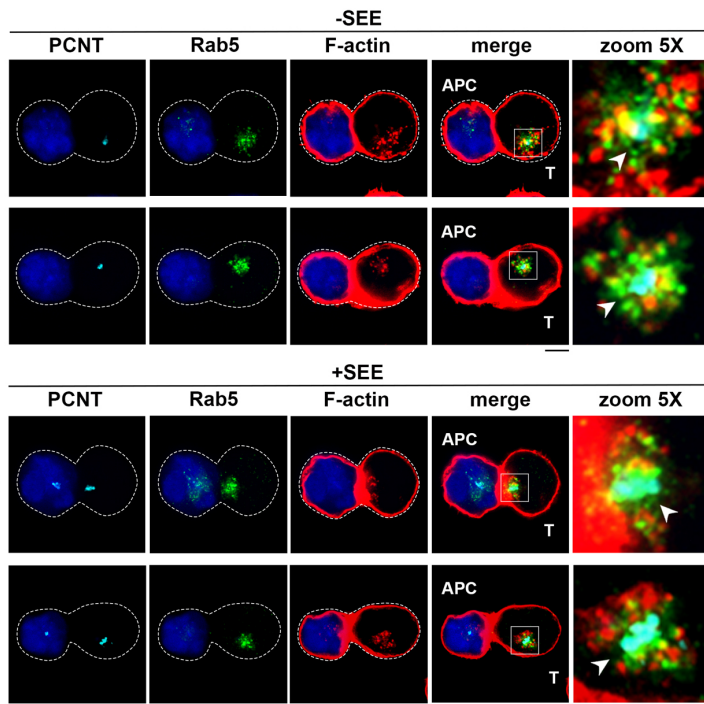
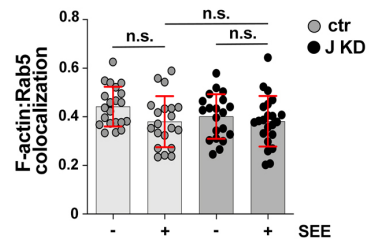
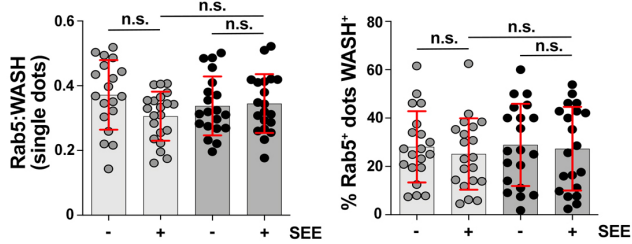
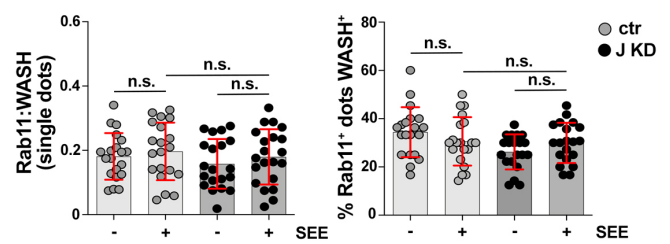
**FIGURE S3**

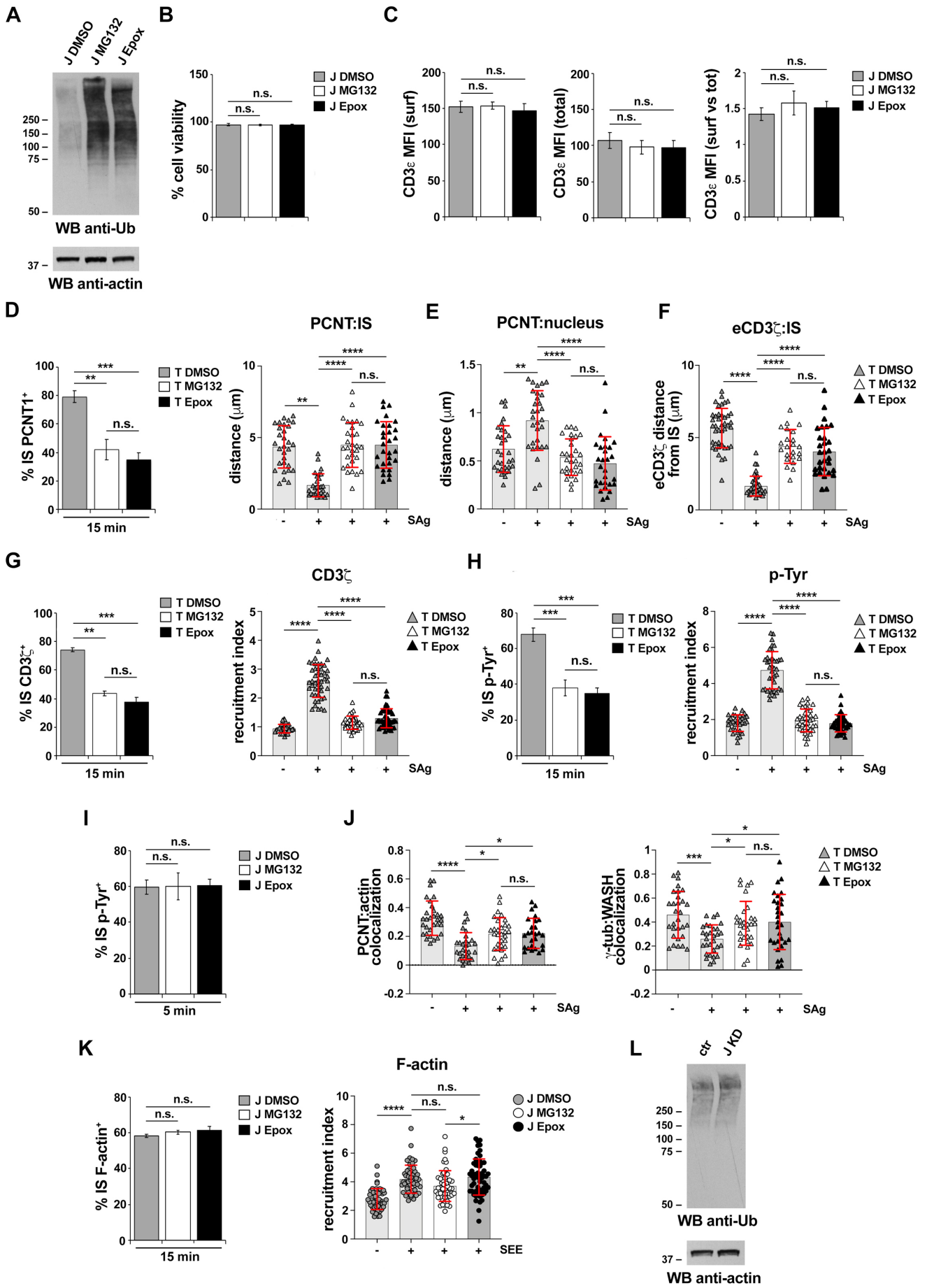


**FIGURE S4**



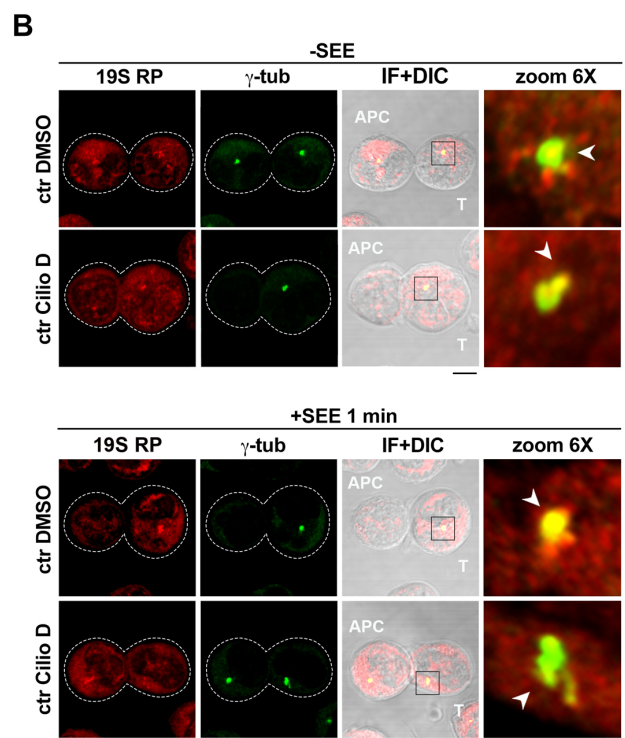
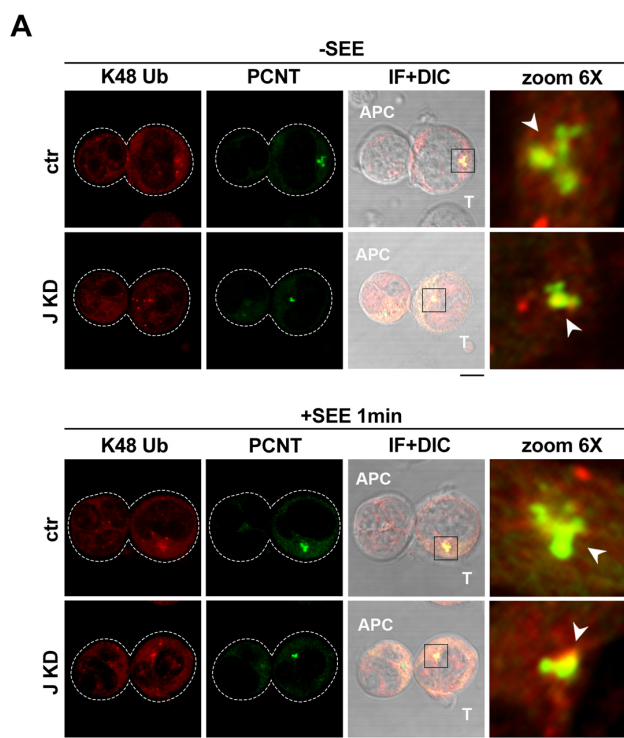
**FIGURE S5**

**A****B****C****D****FIGURE S6**



**FIGURE S7**





**FIGURE S8**



## SUPPLEMENTARY TABLES

**Table S1. List of the primers used in this study**

Oligo name	Sequence	Description
common reverse primer	AGCACCGACTCGGTGCCACT	sgRNA production
GFP sgRNA	ttaatacgactcactataggGGGCGAGGAG CTGTTACCCGgttttagagctagaaatagc	sgRNA production
<i>BBS1</i> gRNA1	ttaatacgactcactataggGGGCTTTCGG TCATCACCAAGgttttagagctagaaatagc	sgRNA production
<i>BBS1</i> gRNA2	ttaatacgactcactataggGGATGCGCAC TACGACCCAAgttttagagctagaaatagc	sgRNA production
<i>BBS1</i> fw	AATTCGAAGTGGTTGGATGC	primer
<i>BBS1</i> rv	ATGTTGCTCCATGAGGAAGG	primer
<i>BBS2</i> fw	TCCCCTCTTGCGATTATTG	primer
<i>BBS2</i> rv	GTCACACAAGGCCAAGGAAT	primer
<i>BBS4</i> fw	ACCACTTCAACCAGCAAACC	primer
<i>BBS4</i> rv	GGCTTTGTGAACTGGGATGT	primer
<i>BBS5</i> fw	CGGATGCTTTTGTGGCTTAT	primer
<i>BBS5</i> rv	CCAAAGTCCCTGTAGGGTGA	primer
<i>BBS7</i> fw	CCAGTACGCAAGTGGGAAAT	primer
<i>BBS7</i> rv	CTTCCACCATTCCGTCATCT	primer
<i>BBS8</i> fw	AGAGGCAGCTGATGTCTGGT	primer
<i>BBS8</i> rv	GCGTGGTTGTTGTTGTTGAC	primer
<i>BBS9</i> fw	CCCCACATTCCTGTAGCAGT	primer
<i>BBS9</i> rv	AGAAGGATCTGTCCCAGGT	primer
<i>BBS18</i> fw	ACCATCTCGACTCACTGCAA	primer
<i>BBS18</i> rv	TGAGATTTAAGGGCTGGGCA	primer
<i>HPRT1</i> fw	AGATGGTCAAGGTCGCAAG	primer
<i>HPRT1</i> rv	GTATTCATTATAGTCAAGGGCATATC	primer

**Table S2. List of the antibodies used in this study**

<b>Antibody</b>	<b>Host Species</b>	<b>Cat.No.</b>	<b>Source</b>	<b>Dilution WB</b>	<b>Dilution IF</b>	<b>Dilution FC</b>
AF555 phalloidin		A34055	Invitrogen	-	1:100 1:50	-
anti-actin	mouse	MAB1501	Merck Life Science	1:10000	-	-
anti-BBS1	rabbit	ab166613	AbCam	1:1000	-	-
anti-CD3 $\epsilon$	mouse	317308	BioLegend	-	-	1:100
anti-CD3 $\zeta$	mouse	sc-1239	Santa Cruz	-	1:30	-
anti-CEP131	rabbit	ab99379	AbCam	-	1:300	-
anti-CEP290	rabbit	ab84870	AbCam	-	1:300	-
anti-dynein	mouse	MAB1618	Merck Life Science	1:500	-	-
anti-GM130	mouse	610822	BD	-	1:100	-
anti-GFP	mouse	A11120	Invitrogen	-	1:200	-
anti-GFP	rabbit	A11122	Invitrogen	1:1000	1:200	-
anti-nesprin 2	mouse	NBP2-59944	Novus	-	1:50	-
anti-p-Tyr	mouse	05-1050	Merck Life Science	-	1:100	1:400
anti-p-Tyr	rabbit	8954	Cell Signaling	-	1:100	-
anti-p-LAT	rabbit	3584	Cell Signaling	-	1:50	-
Anti-p-ZAP70	rabbit	2701	Cell Signaling	-	1:50	-
anti-PCNT	rabbit	ab4448	AbCam	-	1:200	-
anti-Rab5	mouse	610724	BD Biosciences	-	1:50	-
anti-Rab7	mouse	sc-376362	Santa Cruz	-	1:50	-
anti-Rab11a	rabbit	2413	Cell Signaling	-	1:50	-
Anti-RFP	rabbit	600-401-379	Rockland Immunochemicals	-	1:500	-
anti- $\gamma$ -tubulin	mouse	T6557	Merck Life Science	1:5000	1:200	-
anti- $\beta$ -tubulin	rabbit	15115	Cell Signaling	1:2000	-	-
anti-Ub	mouse	3936	Cell Signaling	1:500	-	-
anti-Ub	mouse	04-263	Merck Life Science	-	1:50	-

anti-K48 Ub	rabbit	05-1307	Merck Life Science	-	1:50	-
Anti-WASH1	mouse	SAB42 00552	Merck Life Science	-	1:100	-
anti-WASH	rabbit	PA5- 51731	Invitrogen	1:500	1:100	-
anti-19S RP	rabbit	ab140450	AbCam	1:2000	-	-
anti-19S RP	rabbit	ab3317	AbCam	-	1:100	-