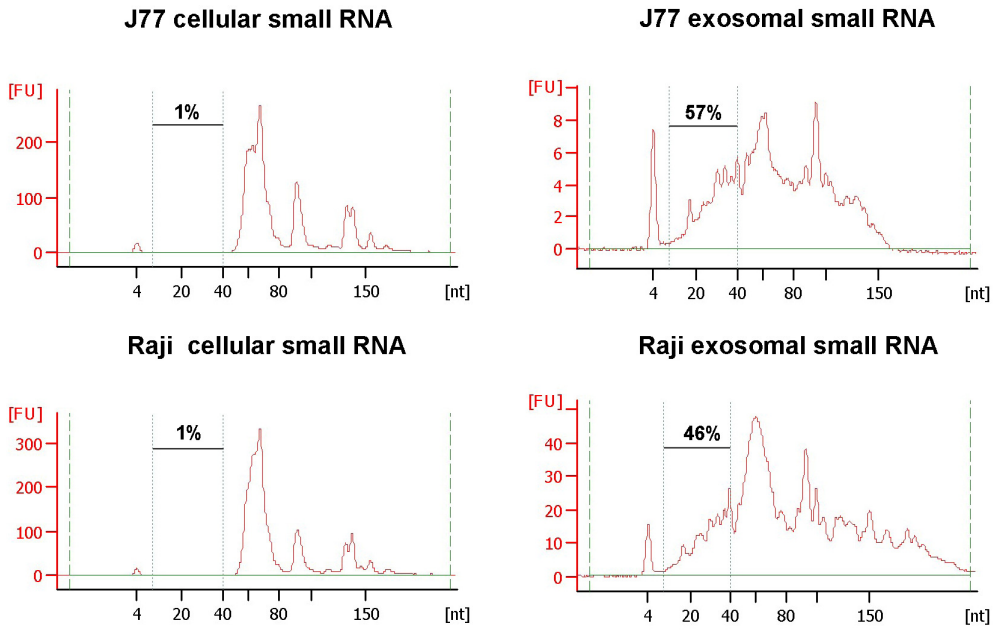
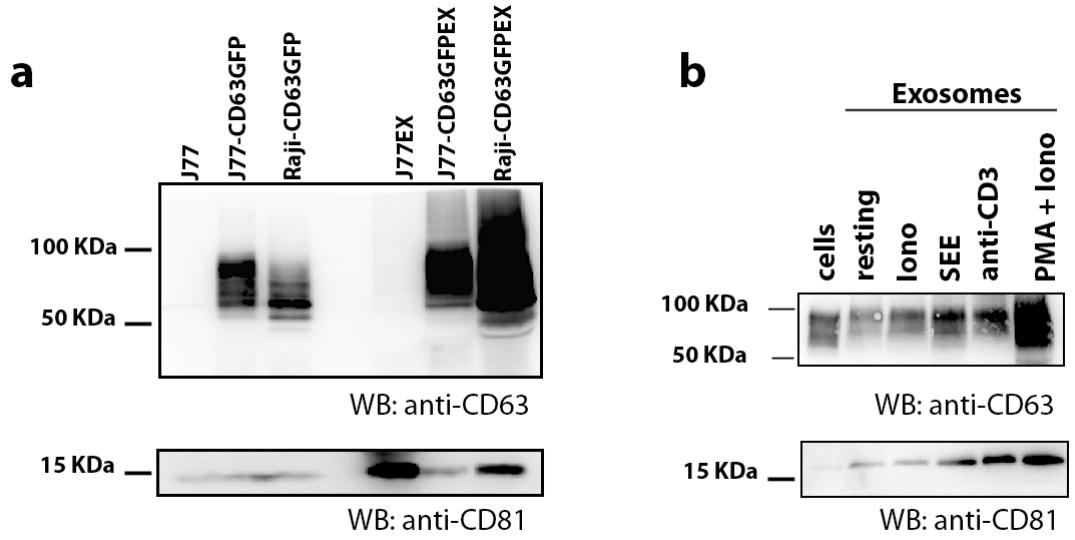
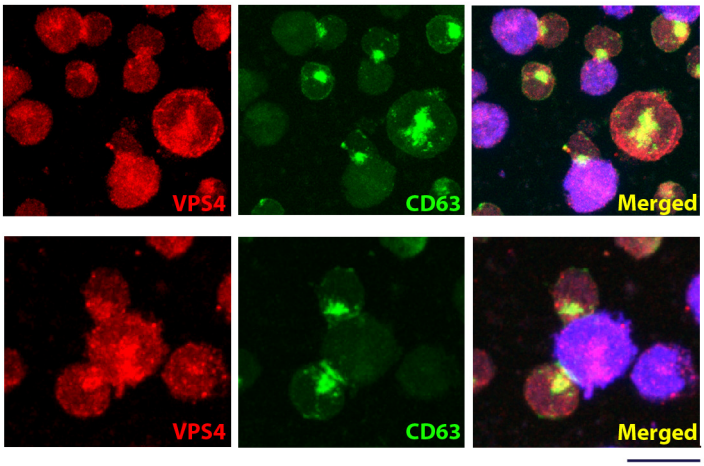


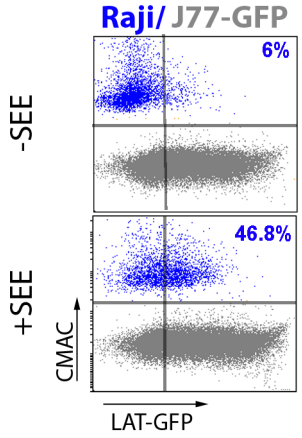
Supplementary Figure S1. Exosomes are enriched in small RNA. RNA from immune cells and their exosomes was assessed with the Agilent 2100 Bioanalyzer. The percentage of small RNAs (<30 nucleotides) was higher in exosomes than in their donor cells. FU, fluorescence units; nt, nucleotides.



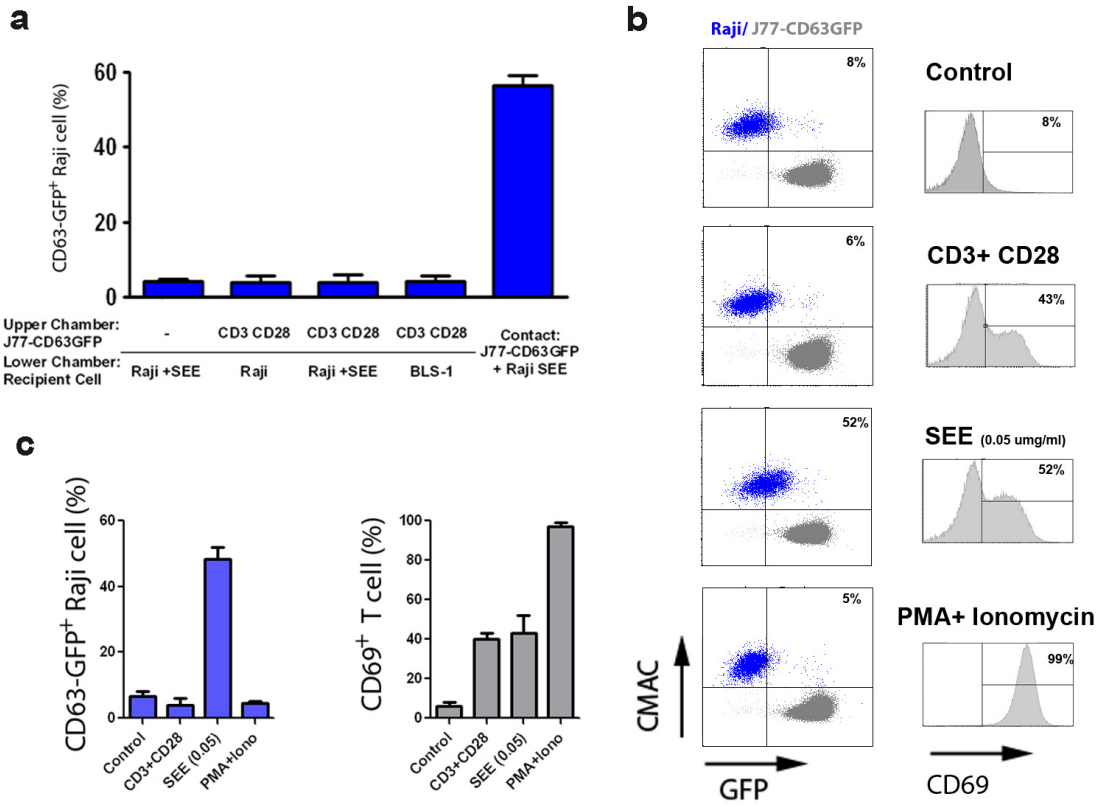
Supplementary Figure S2. Exosomes contain CD63-GFP (a) J77, J77-CD63GFP and Raji-CD63GFP cells were cultured in exosome-depleted medium for 24 h and released exosomes were purified from supernatant by ultracentrifugation. Both whole cell and exosomes (denoted as Ex) lysates from each cell type were analyzed by immunoblotting for the presence of CD81 and CD63 tetraspanins. **(b)** J77-CD63GFP cells were cultured in exosome-depleted medium for 24 h under resting conditions or with the indicated activation stimuli (Ionomycin (Iono), *Staphylococcus* enterotoxin superantigen-E (SEE), Phorbol myristate acetate (PMA) or anti-CD3). Released exosomes were purified from supernatant by ultracentrifugation and analyzed by immunoblot for the presence of CD81 and CD63 tetraspanins



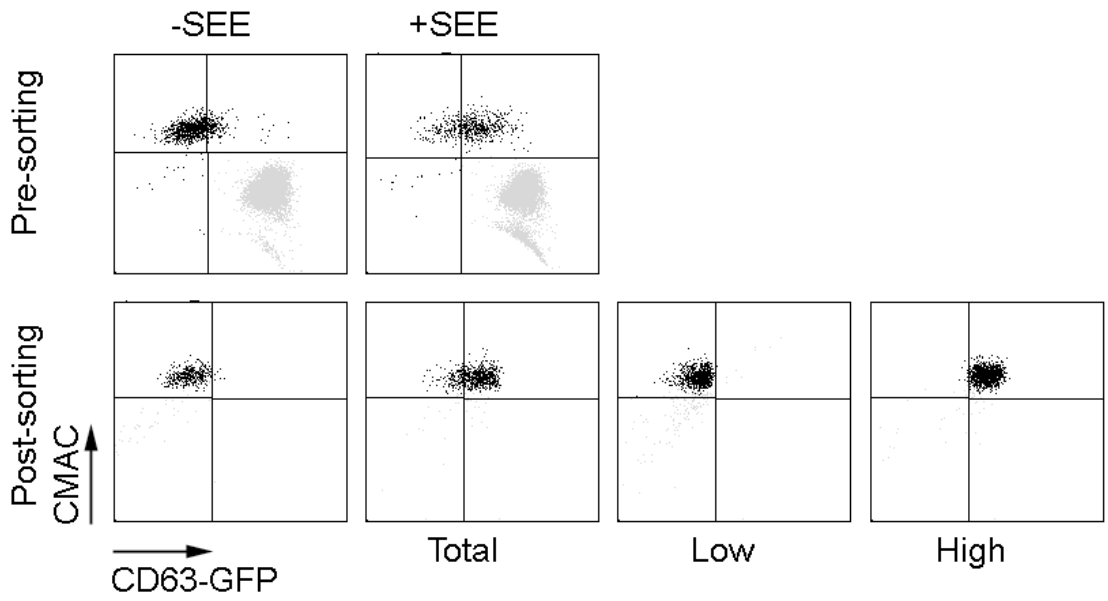
Supplementary Figure S3. T cell multivesicular bodies polarize to the immune synapse. J77-CD63-GFP cells were conjugated with SEE-primed Raji cells (blue). After 30 min, cells were fixed and stained for the ESCRT machinery component VPS4 (red). Images show maximal projections of confocal images. Scale bar: 10 μm.



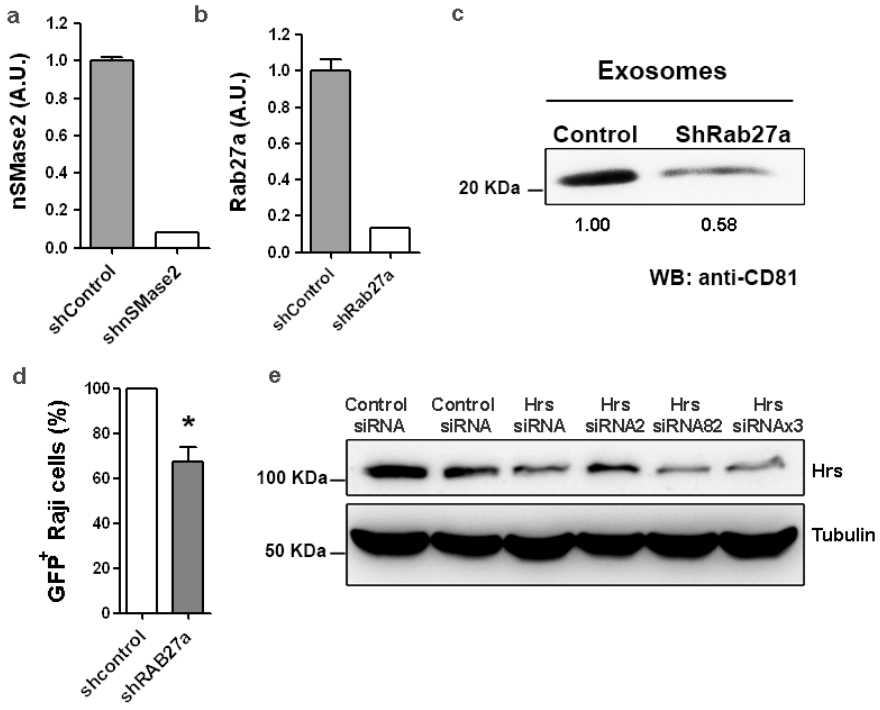
Supplementary Figure S4. LAT is transferred from T cell to APC. J77-LAT-GFP cells (donor) were conjugated with SEE-loaded or unloaded Raji cells (recipient; blue). Donor and recipient populations were analyzed by flow cytometry after 24 h coculture. Raji recipients acquired LAT-GFP in an antigen-dependent manner. Dot plot of one representative experiment is shown.



Supplementary Figure S5. Cell-Cell contact and T cell activation are not sufficient for the exosomal transfer (a) Transwell experiments were performed with donor and recipient cells placed in the upper and lower chambers as indicated. CD63-GFP signal was measured on the recipient cells after 16h by FACS, and compared with the signal from antigen-stimulated contact cultures at the same T-cell:APC ratio. **(b and c)** J77-CD63-GFP cells (donor) were conjugated with Raji cells (recipient; blue) in the presence of CD3 and CD28 Abs, SEE or PMA+Ionomycin. The cocultures were analyzed by flow cytometry 24 h later, donor cells for expression of the activation marker CD69 and recipients for the acquisition of CD63-GFP. A representative experiment is shown in **(b)** and the percentage ± s.e.m. of positive cells of 3 independent experiments in panel (c).



Supplementary Figure S6. Flow cytometry sorting strategy. J77-CD63-GFP cells over-expressing miR-335 were cocultured with non-loaded or SEE-loaded Raji cells (stained with CMAC) for 24 h, and the Raji cells were sorted by flow cytometry. To analyze only singlet cells, we employed a stringent multiparametric gating strategy based on FSC and SSC (pulse width and height). Raji cells were identified by CMAC staining. Representative dot plots of the cocultures before and after sorting are shown.



Supplementary Figure S7. Silencing of nSMase2, Hrs and RAB27a. (a) Real-time semiquantitative TaqMan RT-PCR was performed to confirm the silencing of nSMAse2 mRNA on J-335 transduced with shnSMase 2 or shControl. **(b)** Real-time semiquantitative TaqMan RT-PCR was performed to confirm the silencing of RAB27a mRNA on J-335 transduced with shRAB27a or shcontrol (n=3). **(c)** CD81 immunoblot of exosomes purified from equal numbers of cells transduced with control or Rab27a shRNA. Densitometric analyses were performed, and the ratio between the silenced and the control condition is shown. **(d)** FACS analysis of the CD63-GFP content of Raji recipient cells after coculture with J77-CD63GFP cells expressing shRab27a or shcontrol. Data are the percentage ± s.e.m. of Raji-GFP positive recipient cells relative to the control condition. n=5 independent experiments, p = 0.0069 (one sample t test). **(e)** Control RNA duplex or different siRNA against Hrs were delivered by electroporation into J77-CD63GFP cells. The efficiency of the knock-down was demonstrated by Western blotting (upper panel). Tubulin is shown as a loading control (lower panel).