

# The small GTPase Rab7 regulates antigen processing in B cells in a possible interplay with autophagy machinery

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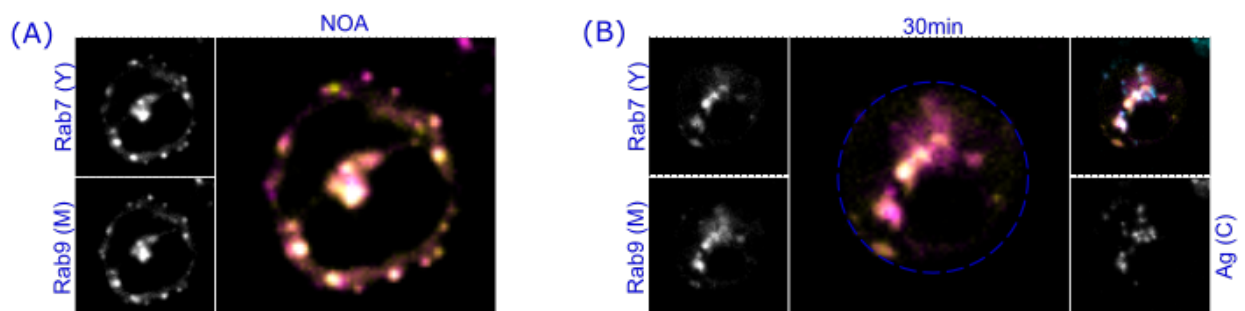
## SUPPLEMENTARY MATERIALS

### Supplementary Video S1.

A20D1.3 cells transiently expressing both EGFP-Rab7 (yellow) and mCherry-Rab9 (magenta) were imaged live with SDCM. The cells were imaged for 5 minutes with 806 ms frame capturing speed.

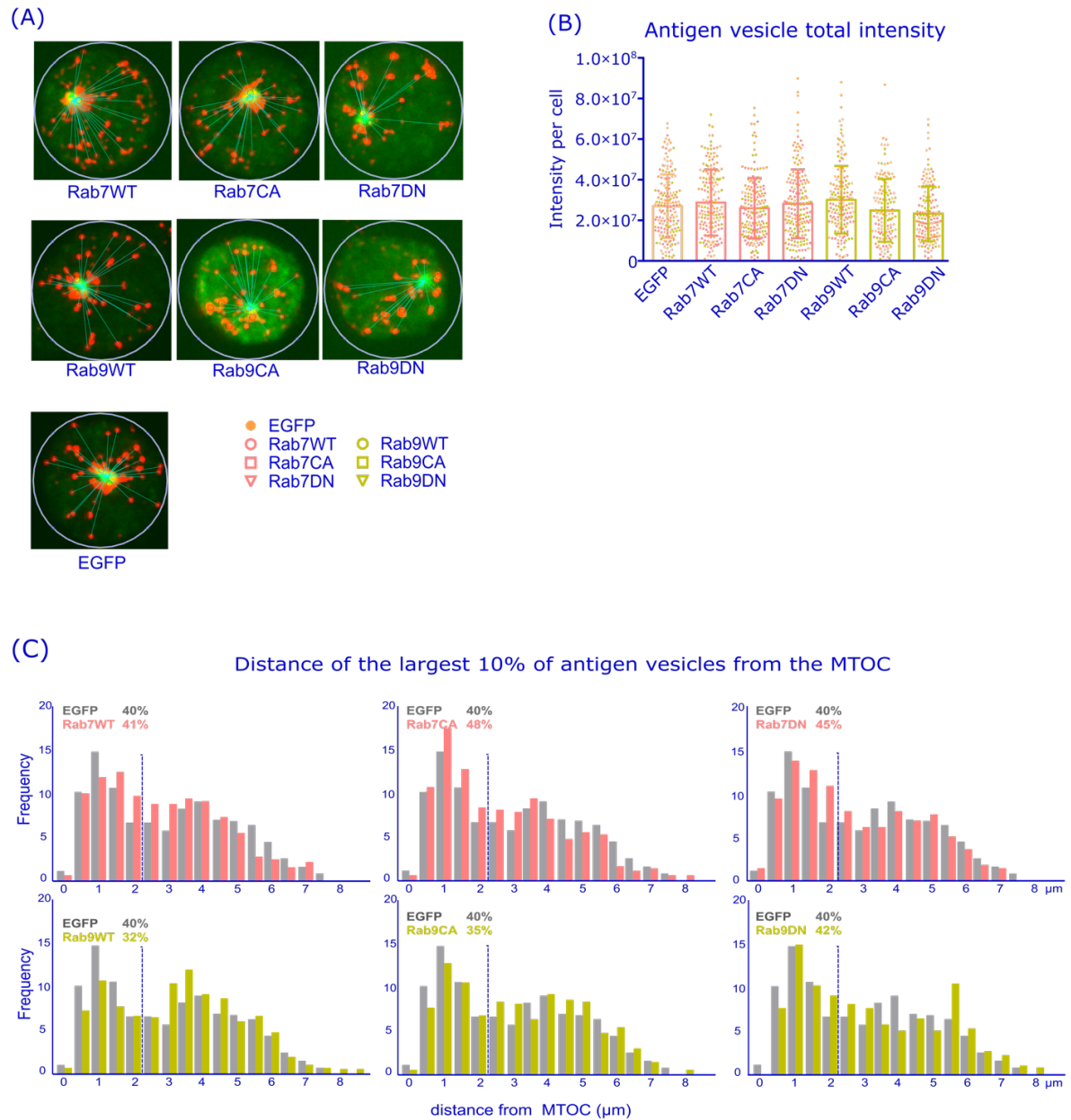
### Supplementary Video S2.

A20D1.3 cells transiently expressing both EGFP-Rab7 (yellow) and mCherry-Rab9 (magenta) were activated with AlexaFluor647-labelled  $\alpha$ IgM F(ab')<sub>2</sub> fragments (cyan). The cells were imaged live with SDCM for 5 minutes starting at 25 minutes after the activation, with 980 ms frame capturing speed.



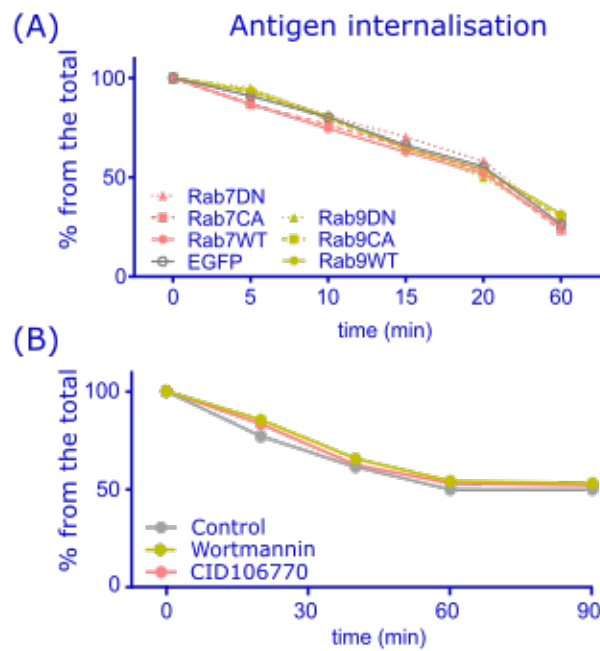
### Supplementary Figure S1. The localization of Rab7 and Rab9.

A20D1.3 cells transiently expressing both EGFP-Rab7 (yellow) and mCherry-Rab9 (magenta) were imaged live with SDCM. (A) The cells were either left non-activated or (B) activated for 30 minutes with AlexaFluor647-labelled  $\alpha$ IgM F(ab')<sub>2</sub> fragments (cyan). Imaging was carried out with 3D SDCM and representative planar images are shown.



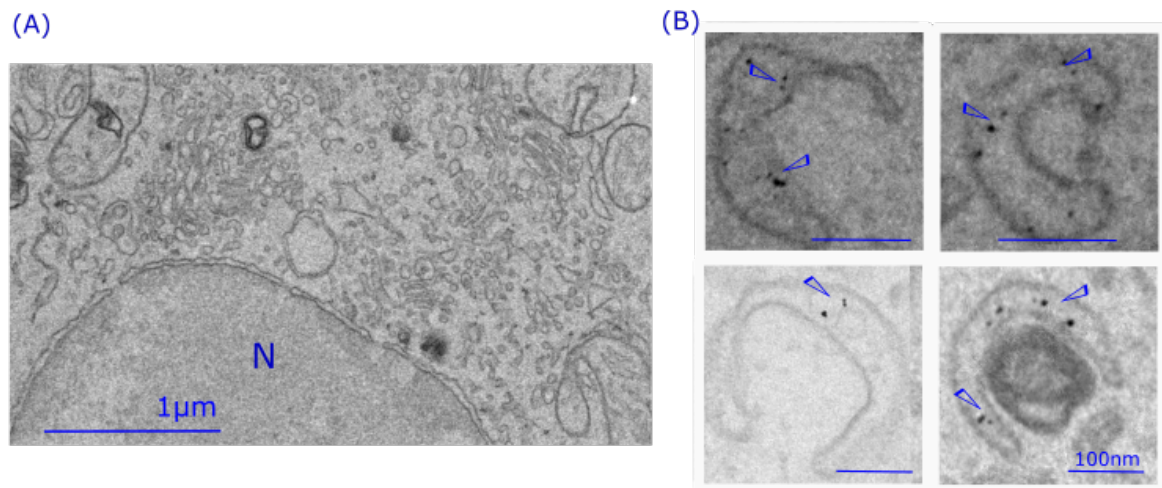
### Supplementary Figure S2. The antigen vesicle distance from the MTOC.

(A) A20D1.3 cells transiently expressing the different EGFP-Rab7/Rab9 constructs were activated with  $\alpha\text{IgM}$ -555 (red) for 60 min, PFA fixed and stained with anti-GFP (not shown) and anti-PCM-647 for MTOC (green). Cells were imaged with 3D SDCM and deconvoluted with Huygens. Green lines represent distance measurements in the following particle analyses (B,C; and Figure 3B,C). Square  $10 \times 10 \mu\text{m}$ , stacked Z-projections. (B) Total antigen intensity in the cells expressing different constructs. Data analyzed from  $>20$  cells from four individual experiments. Mean  $\pm$  SEM shown, dots represent cells (color coded for experiments). (C)  $>2500$  vesicles were pooled from the cells in (B). From the pooled vesicles, largest 10% ( $>250$  vesicles) were taken to the analysis and their distance from the MTOC (green lines in (A)) was determined with a MATLAB script (Hernández-Pérez et al. 2020). The data was binned on histograms with  $0.5 \mu\text{m}$  bin width (C).



**Supplementary Figure S3. Antigen internalization.**

**(A)** A20D1.3 cells transiently expressing the different EGFP-Rab7/Rab9 constructs were labelled with biotinylated  $\alpha$ IgM and incubated at 37 °C for different time points, after which they were stained on ice with AlexaFluor488-streptavidin to detect surface-resident  $\alpha$ IgM. The intensity was normalised to time point 0. The data is a mean from three independent experiments. **(B)** A20D1.3 cells were treated with 1  $\mu$ M Wortmannin or 60  $\mu$ M CID106770, or DMSO control were treated and analysed as in (A). The data is from one experiment.



**Supplementary Figure S4. Antigen localization in phagosome –like compartments**

A20D1.3 B cells were activated with  $\alpha$ IgM conjugated with 6 nm gold particles for 75 minutes after which they were processed for TEM. **(A)** Divergent endosomes in the perinuclear area (N = nucleus). Scale bar 1  $\mu$ m. **(B)** Gold-conjugated antigen (arrows) in phagosome-like structures. Scale bar 100 nm.