

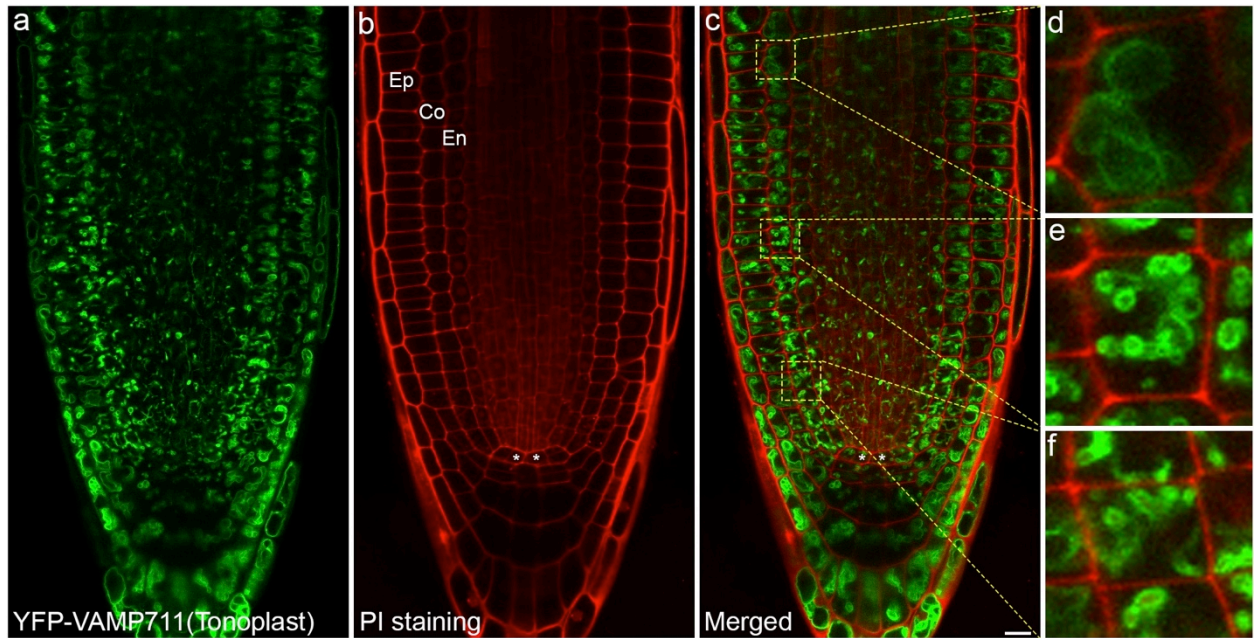
In the format provided by the authors and unedited.

A whole-cell electron tomography model of vacuole biogenesis in *Arabidopsis* root cells

Yong Cui^{1,8*}, Wenhan Cao^{1,8}, Yilin He^{1,8}, Qiong Zhao¹, Mayumi Wakazaki², Xiaohong Zhuang¹, Jiayang Gao¹, Yonglun Zeng¹, Caiji Gao^{1,6}, Yu Ding^{1,7}, Hiu Yan Wong¹, Wing Shing Wong¹, Ham Karen Lam¹, Pengfei Wang¹, Takashi Ueda³, Marcela Rojas-Pierce⁴, Kiminori Toyooka², Byung-Ho Kang¹ and Liwen Jiang^{1,5*}

¹School of Life Sciences, Centre for Cell & Developmental Biology and State Key Laboratory of Agrobiotechnology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong, China. ²RIKEN Center for Sustainable Resource Science, Yokohama, Japan. ³Division of Cellular Dynamics, National Institute for Basic Biology, Okazaki, Japan. ⁴Department of Plant and Microbial Biology, North Carolina State University, Raleigh, NC, USA. ⁵The Chinese University of Hong Kong Shenzhen Research Institute, Shenzhen, China. ⁶Present address: Guangdong Provincial Key Laboratory of Biotechnology for Plant Development, School of Life Sciences, South China Normal University, Guangzhou, China. ⁷Present address: Department of Food Science & Technology, School of Science and Technology, Jinan University, Guangzhou, China. ⁸These authors contributed equally: Yong Cui, Wenhan Cao, Yilin He.

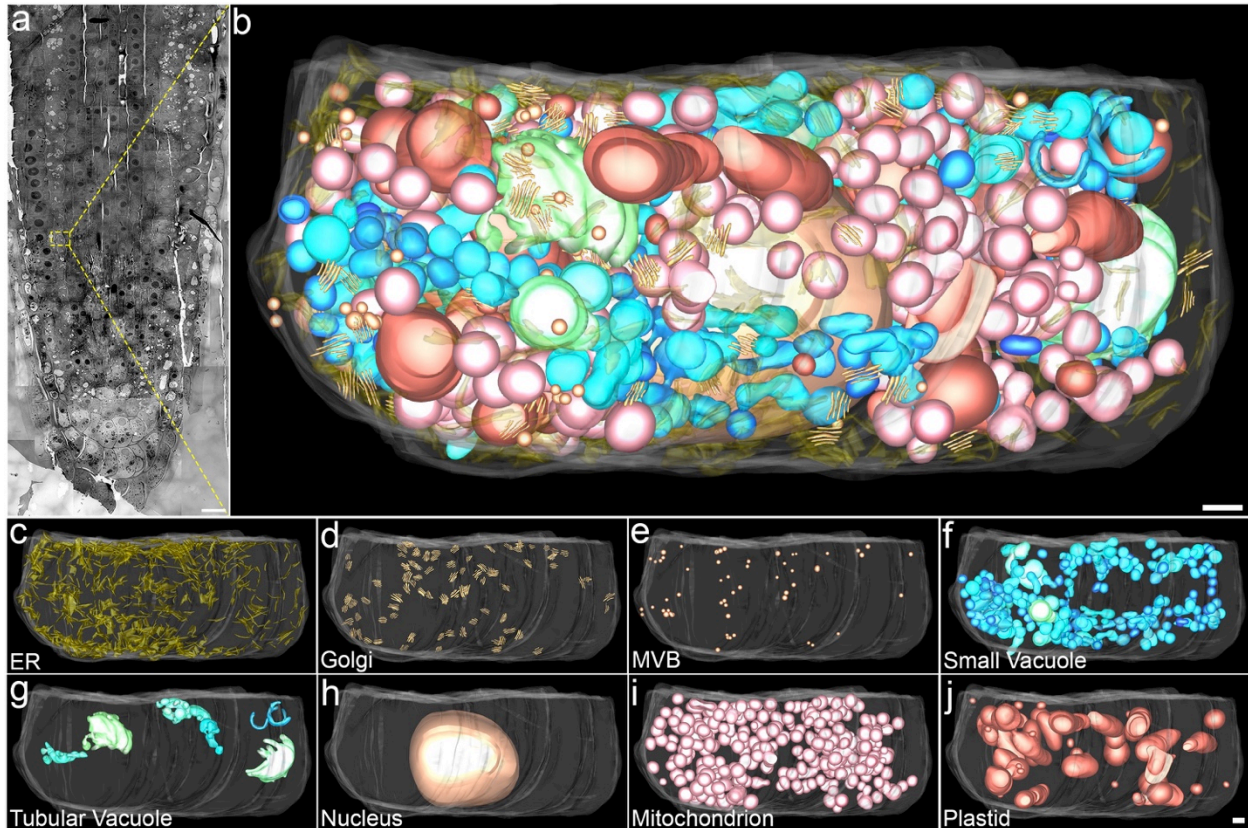
*e-mail: cuiyongwhu@gmail.com; ljjiang@cuhk.edu.hk



Supplementary Fig. 1

Full views of vacuole patterns in the root tip of a tonoplast marker line, YFP-VAMP711.

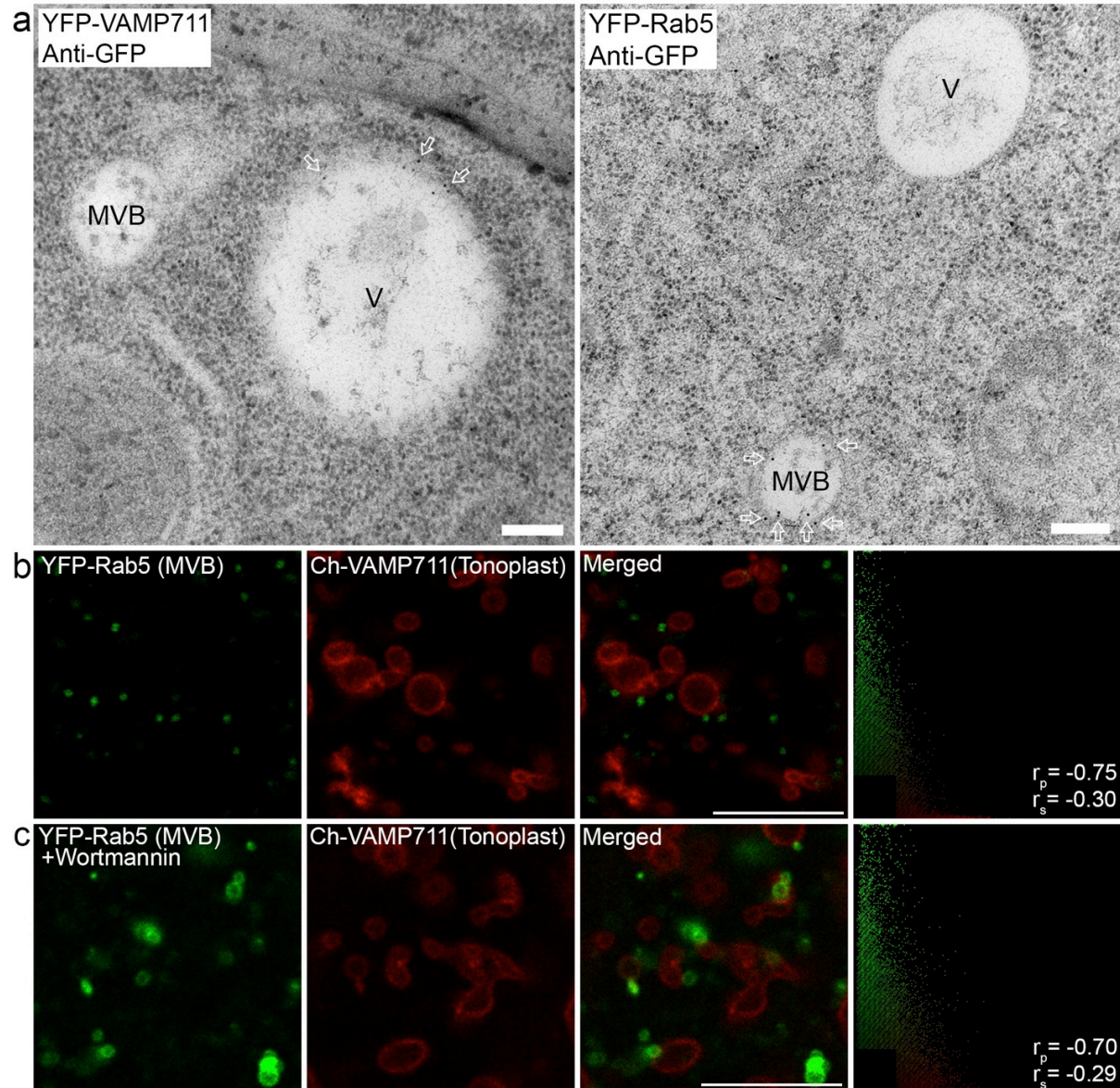
(a-f) Confocal microscopy images of the longitudinal root section of an *Arabidopsis* tonoplast marker line, YFP-VAMP711. (a) The tonoplast was labeled in green. (b) The cell borders were stained with PI (Propidium Iodide) in red. (c) In the merged graph, a close-up view (yellow-boxed regions) was taken for each of the three cortical cells. Cells were chosen based on their relative location to the quiescent center (QC) (as indicated by asterisks in b and c). (d-f) Three enlarged boxes of c, showing representative vacuole morphology in different developmental stages. Ep, epidermis; Co, cortex. En, endodermis. Scale bar, 10 μ m.



Supplementary Fig. 2

Whole-cell electron tomography analyses of various organelles in an early cortical cell show their morphology, distribution and relationship.

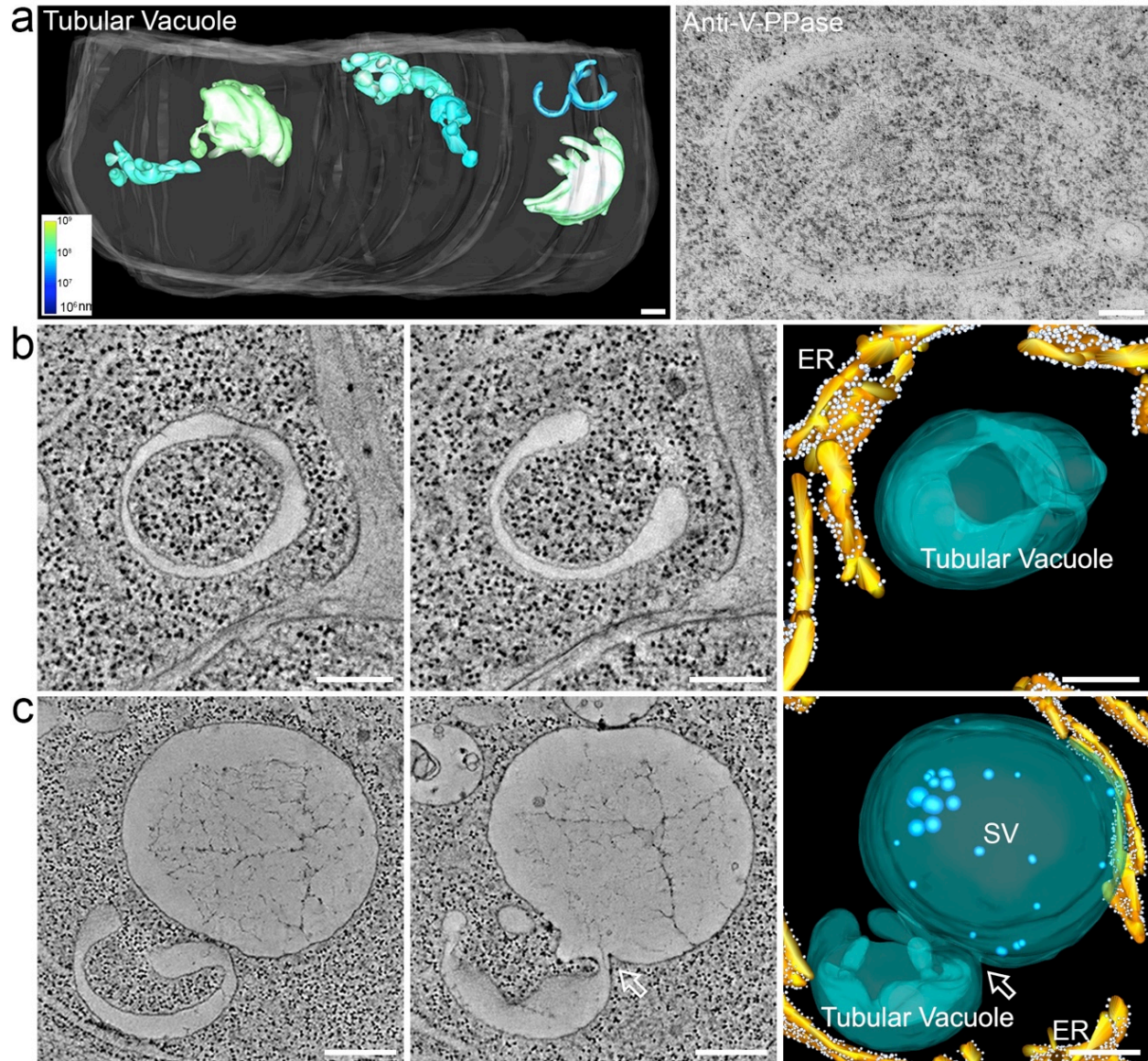
(a) An overview TEM image of a wild-type *Arabidopsis* root tip was shown. The yellow box indicated the early-stage cortical cell used for the whole-cell electron tomography analyses. Scale bar, 10 μm . (b-j) The whole-cell electron tomography models showed morphology and distribution of individual organelle including ER, Golgi, MVB, small vacuole, tubular vacuole, nucleus, mitochondrion and plastid. ER, endoplasmic reticulum; MVB, multivesicular body; Scale bar, 500 nm.



Supplementary Fig. 3

Immuno-EM and confocal microscopy analyses demonstrate that SVs and MVBs are separate organelles with different molecular markers.

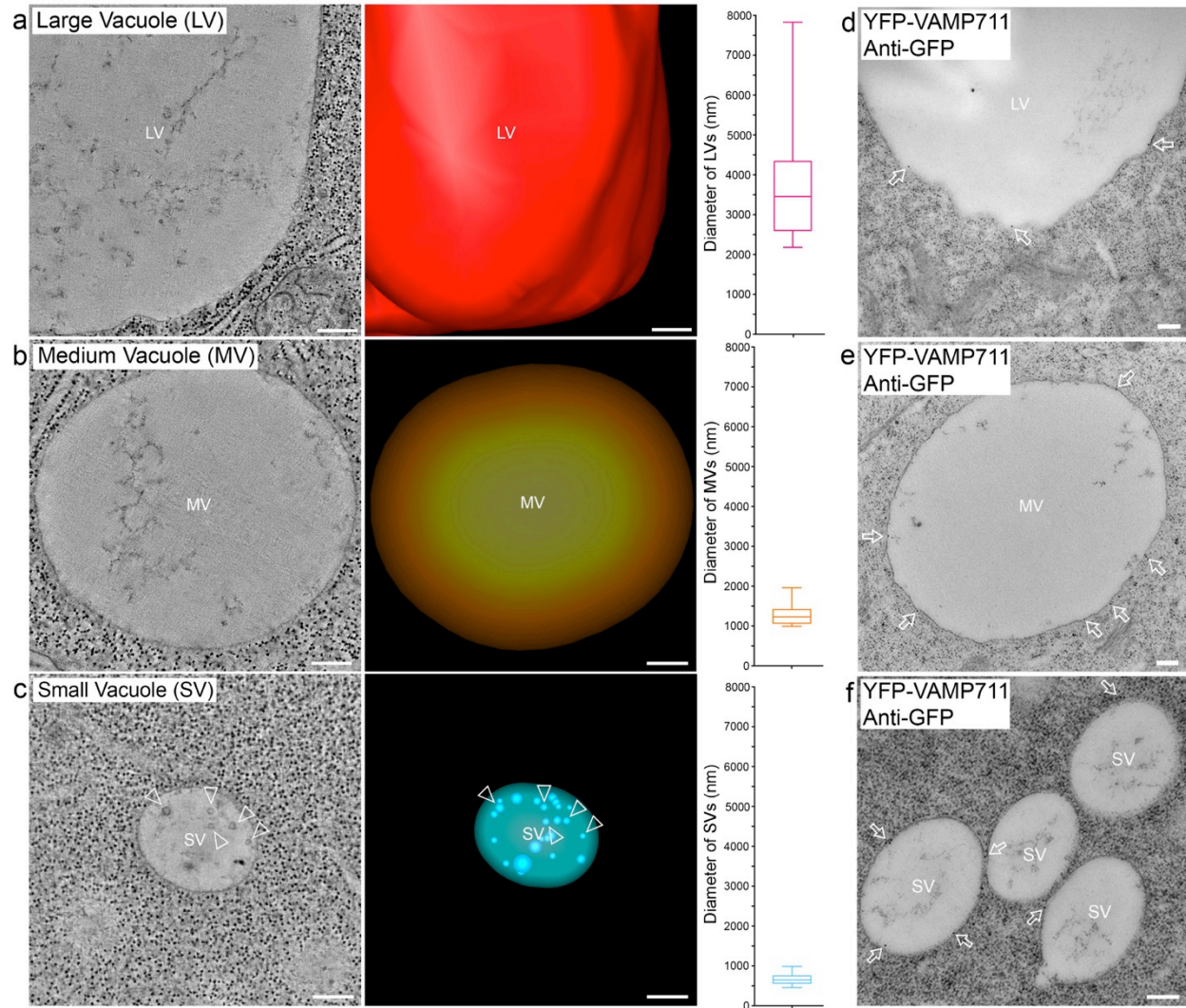
(a) Immunolabeling of the root in a tonoplast marker line, YFP-VAMP711 showed that the vacuole, instead of the MVB, was labeled by GFP antibodies (left panel), while in the root of the MVB marker line YFP-Rab5, only the MVB was labeled by GFP antibodies (right panel). The arrows indicated the labeled gold particles using anti-GFP. MVB, multivesicular body; V, vacuole. Scale bars, 200 nm. (b,c) Confocal images of root cells expressing the MVB marker, YFP-Rab5 (in green) and tonoplast marker, Ch-VAMP711 (in red) were presented. The small-size vacuoles were separate from MVBs regardless of wortmannin treatment. The right column showed the scatter plots and the corresponding linear Pearson correlation coefficient (r_p) and the nonlinear Spearman correlation coefficient (r_s), indicating the extent of colocalization with the value less than zero corresponds to a negative relationship. Scale bars, 10 μ m.



Supplementary Fig. 4

Whole-cell electron tomography analyses show the structure of tubular vacuoles in an early cortical cell.

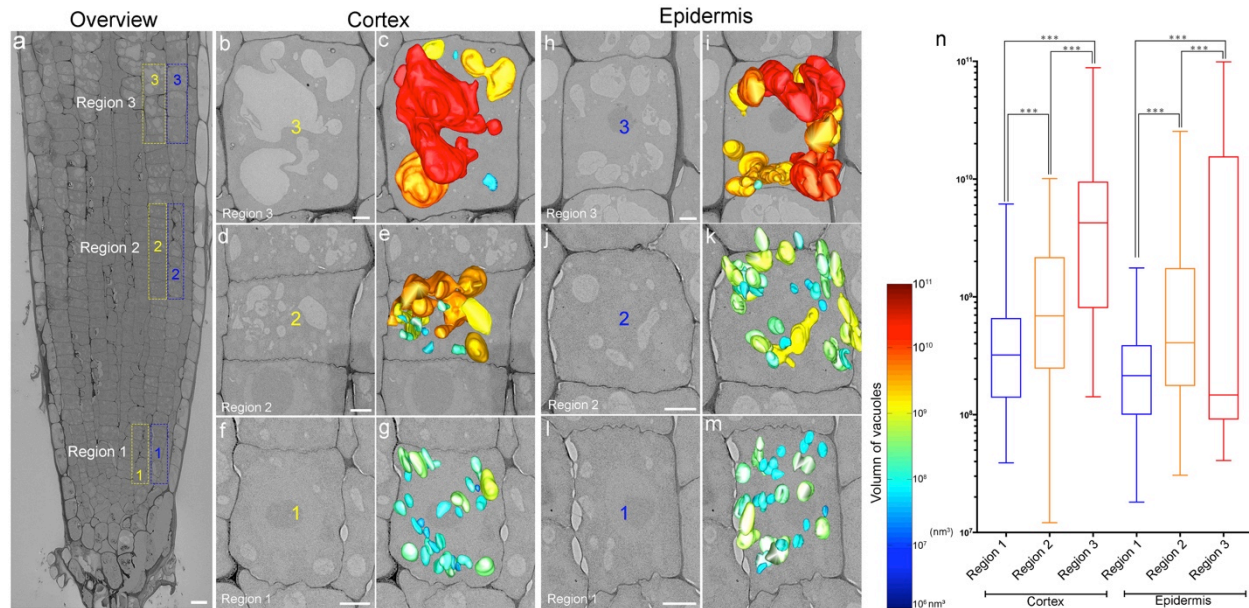
(a) 3D reconstruction of whole-cell electron tomography analyses of a cortical cell showed the morphology and distribution of tubular vacuoles (Left). The tubular structure was labeled by V-PPase (tonoplast marker) antibodies (Right). Scale bars, 500 nm. (b) The morphology of the simplest tubular structure was shown in the 3D model. It contained an autophagosome-like structure, which was separate with the rough ER (yellow). Scale bars, 200 nm. (c) The fusion of a tubular vacuole with an existing SV in early stage of vacuole formation was shown. The fusion part was indicated by arrows. The rough ER (yellow) with attached ribosomes (white) was also shown. ER, endoplasmic reticulum; SV, small vacuole. Scale bars, 200 nm.



Supplementary Fig. 5

3D tomography analyses of vacuoles of different sizes and demonstration of SVs containing ILVs in early cortical cells.

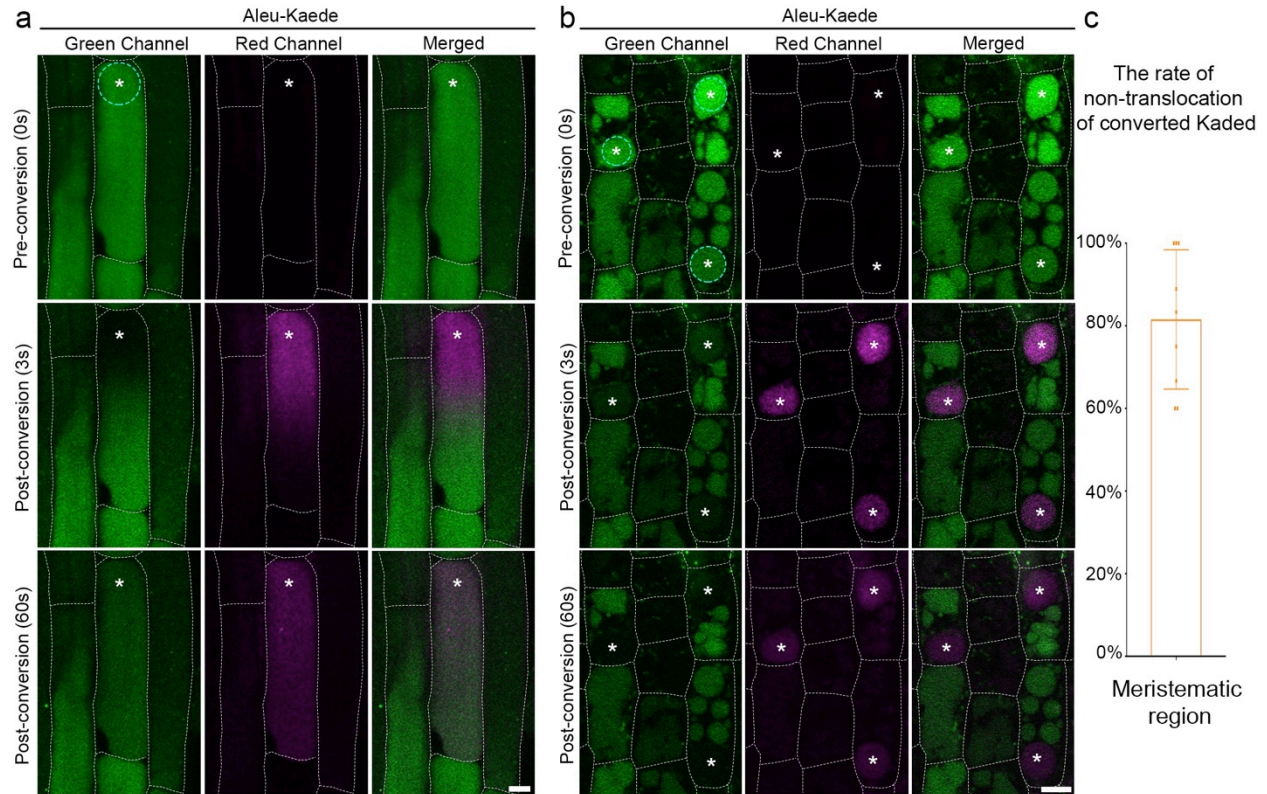
(a-c) Tomographic slices of vacuoles of different sizes with their 3D models in parallel. Vacuoles in cortical cells were classified into LVs (a), MVs (b) and SVs (c) based on their diameter. Diameter of vacuoles was defined as the longest distance between two sides through the vacuoles. In statistical charts, $n = 11$ for LVs, $n = 38$ for MVs and $n = 31$ for SVs. Boxes represent median values and first and third quartiles; whiskers represent minimum and maximum values. Notably, a considerable amount of ILVs existed in SVs, as indicated by arrowheads (c). Scale bars, 200 nm. (d-f) In the tonoplast marker line YFP-VAMP711, vacuoles of different sizes were all labelled with GFP antibodies (arrows) by immunogold detection. LV, large vacuole; MV, medium vacuole; SV, small vacuole. Scale bars, 200 nm.



Supplementary Fig. 6

Field emission scanning electron microscopy analyses demonstrate that vacuoles increase in size along an apical-to-basal developmental gradient in root cortex and epidermis.

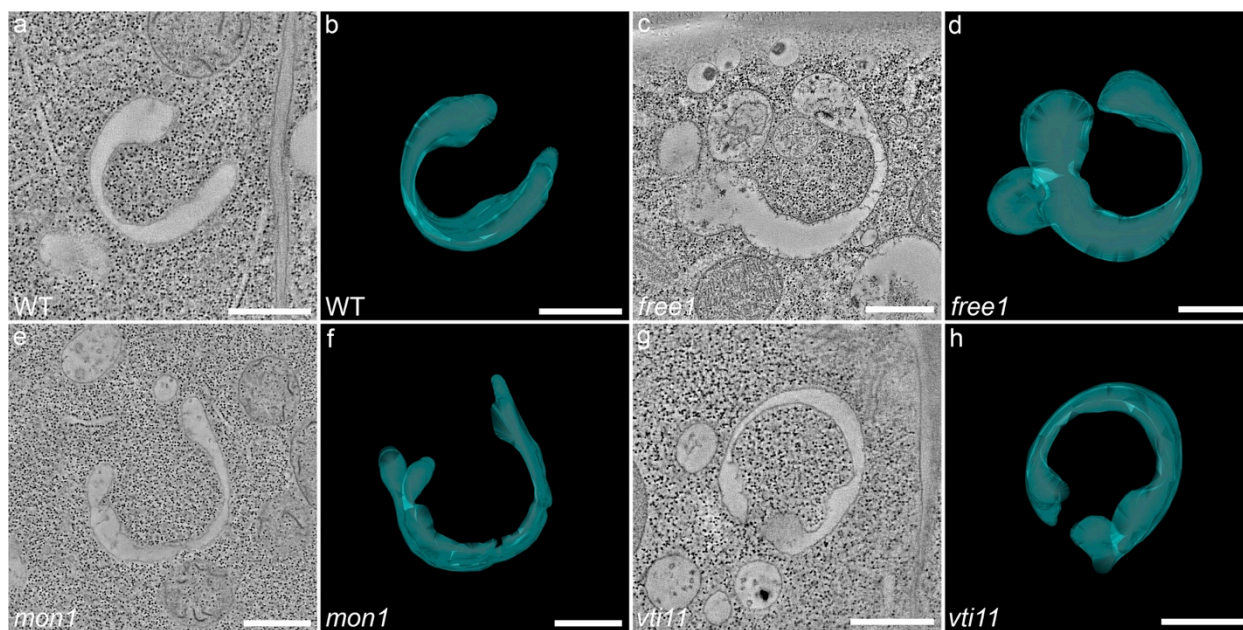
(a) An overview scanning electron microscopy image of a plant root under low magnification. Both cortical (yellow) and epidermal (blue) cells from three developmental regions (highlighted by yellow and blue dashed boxes) were used for vacuole remodeling analyses. Scale bar, 10 μm . (b-m) Representative scanning electron microscopy slices of cortical (b, d, f) and epidermal (h, j, l) cells for 3D reconstruction were shown. The corresponding 3D vacuole models were shown adjacent to their scanning electron microscopy images (c, e, g, i, k, m) and were later color-coded based on their volumes. Scale bars, 2 μm . (n) Statistical analysis of vacuole volumes of cells from three different regions in cortex and epidermis. ***: $P < 0.001$, two-sided Student's t-test. $n = 73$ for Region 1 in cortex, $n = 70$ for Region 2 in cortex, $n = 53$ for Region 3 in cortex, $n = 93$ for Region 1 in epidermis, $n = 75$ for Region 2 in epidermis and $n = 15$ for Region 3 in epidermis. Boxes represent median values and first and third quartiles; whiskers represent minimum and maximum values.



Supplementary Fig. 7

The photoconversion results demonstrate that vacuoles are separate organelles in meristematic cells.

(a,b) Transgenic *Arabidopsis* roots expressing photoconvertible Kaede-tagged vacuole marker Aleu were generated. Images before (top panel) and 3s (middle panel) and 60s post photoconversion (bottom panel) were shown. (a) In elongation region, the big central vacuoles were labeled by Aleu-Kaede in green. Upon excitation with 405-nm laser, the photoconverted red protein spreads from one side to the remaining part of the big central vacuole within 60s. (b) In the meristem, before bleaching, multiple Aleu-Kaede labeled vacuoles in green were observed. Upon excitation, vacuoles that were photo bleached turned red with photoconverted protein restricted inside individual vacuoles. The cyan cycles indicated the bleaching areas. The stars indicated the vacuoles that underwent photobleaching. The white dashed lines highlighted the cell boundary. (c) Statistical analysis showed the rate of non-translocation of converted Kaede to adjacent vacuoles in root meristematic region. Data were shown as mean $\pm$ s.d. $n = 9$. Scale bars, 10 μ m.



Supplementary Fig. 8

The formation of tubular vacuoles is not affected in *free1*, *mon1* and *vti11* mutants.

(a-h) Representative tomographic slices with their 3D models in parallel showed tubular vacuoles in WT (a,b), *free1* (c,d), *mon1* (e,f) and *vti11* (g,h) mutants. The formation of tubular structures was not affected in *free1*, *mon1* and *vti11* mutants, comparing with that in WT. Scale bars, 500 nm.

Supplementary Video 1. Whole-cell electron tomography analyses of SVs in relationship with other organelles in Cell 1.

Supplementary Video 2. 3D tomography analyses of detailed structures and relationship of ER, Golgi, TGN, MVBs and SVs.

Supplementary Video 3. 3D tomography analyses of fusion between MVBs and SVs, along with a transfer of ILVs.

Supplementary Video 4. Whole-cell electron tomography analyses of vacuoles in Cell 2.

Supplementary Video 5. Whole-cell electron tomography analyses of vacuoles in Cell 3.

Supplementary Video 6. 3D tomography analyses of vacuoles in WT and various mutants.