Project number: AoE/M-05/12

RESEARCH GRANTS COUNCIL
AREAS OF EXCELLENCE (AoE) SCHEME

Completion Report on Funded Project

Project start date: *1 Jan 2014*
Project completion date: *31 Dec 2021*

1. Project Title: Centre for Organelle Biogenesis and Function

2. Names and Academic Affiliations of Project Team Members[#]

Project team member	Name / Post	Unit / Department / Institution	Average number of hours per week spent on this project in the whole project period
Project Coordinator (PC)	JIANG, Liwen	School of Life Sciences, CUHK	14
Co-Principal Investigator(s)	XIA, Jun	Division of Life Science, HKUST	4
	BANFIELD, David K	Division of Life Science, HKUST	4
	XIA, Yiji	Department of Biology, HKBU	4
	YAO, Xiaoqiang	School of Biomedical Science, CUHK	4
	WONG, Kam-bo	School of Life Sciences, CUHK	6
	LAM, Hon-ming	School of Life Sciences, CUHK	4
Co-Investigator(s)	CHAN, Raymond Hon-	Department of Mathematics,	2

	fu	CityU	
	KWAN, Kin-ming	School of Life Sciences, CUHK	4
	CHAN, Edwin Ho-yin	School of Life Sciences, CUHK	4
	BLU, Thierry	Department of Electronic Engineering, CUHK	4
	ZHANG, Jianhua	Faculty of Science, HKBU	2
	GE, Wei	Faculty of Health Sciences, University of Macau	2
	KANG, Byung-Ho	School of Life Sciences, CUHK	6
	CHYE, Mee-len	School of Biological Sciences, HKU	2
	HE, Junxian	School of Life Sciences, CUHK	4
	GUO, Yusong	Division of Life Science, HKUST	4
Collaborators	SCHEKMAN, Randy	Department of Molecular & Cell Biology, Uni. Of California at Berkeley, USA	N.A.
	ZHANG, Qifa	National Key Laboratory of Crop Genetic Improvement, Huazhong Agricultural University, China	N.A.
	LIN, Hongxuan	National Key Laboratory of Plant Molecular Genetics, Shanghai Institute of Plant Physiology and Ecology, Chinese Academy of Sciences, China	N.A.
	LIU, Chang-jun	Biology Department, Brookhaven National Laboratory, USA	N.A.

Please highlight the approved changes in the project team composition and quote the date when the UGC/RGC granted approval of such changes. For changes in the project team composition, please submit a separate request, together with the justification and the curriculum vitae of the new member(s), to the RGC three months prior to the intended effective date of the change.

3. Project Objectives

Summary of objectives addressed/achieved:

Objectives*	Percentage achieved	Remarks**
1. **Project A : Golgi Dynamics, Biogenesis and Function**	100%	On Schedule
2. **Project B : TGN as a Multiple**	100%	On Schedule

Objectives*	Percentage achieved	Remarks**
Sorting Station		
3. **Project C : EXPO and UPS**	100%	On Schedule
4. **Other AoE-related Projects**	N.A.	N.A.

* *Please highlight the approved changes in objectives and quote the date when the UGC/RGC granted approval of such changes.*

** *Please provide reasons for significantly slower rate of progress than originally planned.*

6. Research Highlights and Outputs

Exclusive Summary of our AoE Project (2014-2021):

1) We have achieved our **AoE mission** by establishing and developing a world-leading research Centre on Organelle Biogenesis and Function for promoting research and education in Hong Kong and beyond.
2) We have achieved our **AoE goals** of A) conducting world-leading research in the field of organelle biogenesis and function in important biological pathways related to human health and agriculture with significant contributions in the field; and B) gaining global reputation via generating publications in top journals, and through interactions and collaboration with nationally and internationally renowned scientists.
3) We have addressed and completed all the original Objectives of this project and beyond with excellent research progress annually, exciting new findings and research outputs. We have published 278 articles in international journals (e.g. ***Plant Cell, Plant Physiology, Molecular Plant, Nature Plants, Nature Communications, Autophagy, Cell, PNAS,*** and ***Science***) with AoE funding and joint efforts of PC/Co-PIs/national/international Collaborators. **(Appendix V)**
4) With the support of the AoE, 3 CRF-Equipment grants and CUHK matching fund (>**HK$80M** Equipment fund), we have established several state-of-the-art shared platforms in the CUHK-allocated **453m^2** AoE LAB for promoting advanced collaborative research in organelle biogenesis and function, including 1) 3D TEM in 2015, 2) Cryo-EM/ET (electron tomography) in 2019, 3) Cryo-FIB (Focused Ion Beam)/CLEM(correlative light-electron microscopy) in 2021, and 4) Integrated/Advanced live-cell imaging platform [including SRM (super-resolution microscopy), (Lattice) Light-Sheet, Two-Photon, and TIRF] in 2022/2023. **(Appendix XVI)**
5) School of Life Sciences (SLS) of CUHK has recruited Prof. KANG Byung-ho (3D TEM expert) and Prof. LAU Wilson Chun Yu (Cryo-EM expert) in 2015/2016. We have since developed our AoE advanced shared platforms with in-house expert support into an international-leading hub for advanced Life Science Research and major grants application with excellent track record.
6) The AoE project and advanced shared platforms have played significant roles in promoting local/national/international collaborative research with excellent records. **(Appendix IV)**
7) The PC/Co-PIs have received many prestigious awards such as Croucher Senior Research Fellowship and RGC Senior Research Fellow. **(Appendix X)**
8) The AoE has been an excellent Education/Training Centre as we have trained 92 postgraduate students (RPGs) with very good research outputs (**Appendix XI**), and more than 15 PhD/Postdoc have become independent PIs in Universities after their study/research in the AoE.
9) As an Education/Training Centre, our AoE has organized 8 Annual AoE Symposium on Organelle Biogenesis and Function, 6 Joint Symposia, and 4 Croucher Summer Courses or Croucher Advanced Study Institute for training young scientists (**Appendix III**). We have also organized 57 Research Seminars delivered by international speakers. (**Appendix IX**)
10) As an Education Centre, our AoE Centre has been awarded 16 CUHK Teaching Development Grants and produced >90 Teaching Videos (on advanced technologies and AoE-derived articles) for freely download from AoE website for training and teaching purposes. (**Appendix XII**)
11) For Future Development and Long-Term Sustainability, we will build on our well-established AoE advanced platforms and technologies to a) further promote national/international collaboration, and b) attract major collaborative research funding in the near future. Indeed, we are working collaboratively to develop a new AoE of "***Institute of Integrative Biology***" in addressing the cellular mechanisms of abiotic stress responses in model organisms including plants as well as their potential impact on crop improvement and environmental sustainability.

In the next pages, we will highlight some of our most exciting research accomplishments from this AoE project and advanced shared platforms that are achieved by our AoE PC/Co-PIs/Collaborators/Associates in using various model organisms. (see **Appendix XV** for details)

6.1 What are the most exciting research accomplishments of the project?

A. Novel Functions of EMP as Ion Channel in Animal Model.

We have cloned TM9SF4, the human homolog of *Arabidopsis* EMP4 (a Golgi-localized integral membrane protein), and demonstrated the ion channel properties of TM9SF4 in animal cells. More importantly, we have identified novel functions of this protein in autophagy, ER stress and inflammation. Intriguingly, we found that an N-terminal fragment of TM9SF4 can directly bind to F-actin to promote cofilin-mediated F-actin disassembly. Knockdown of TM9SF4 reduces cell migration and invasion in ovarian cancer cells. In vivo, knockdown of TM9SF4 completely abolishes the tumor growth and metastasis in athymic nude mice. Our findings provide novel mechanistic insights into TM9SF4-mediated regulation of actin dynamics in ovarian cancer cells. These exciting findings have been published in Nature Communications 2022; Cell Death & Differentiation 2018; Oncogene 2019 and Cellular and Molecular Gastroenterology and Hepatology 2022.

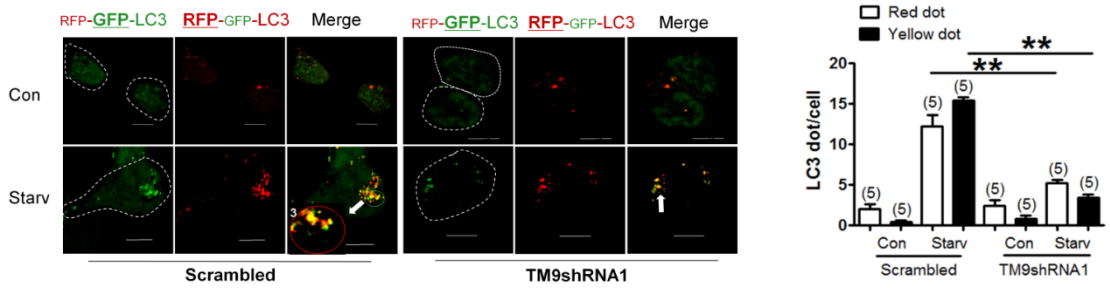

Figure 1. Knockdown of TM9SF4 using lenti-TM9SF4-shRNA1 inhibited the starvation-induced autophagic flux in HEK293 cells. HEK293 cells were transfected with a tandem reporter RFP-GFP-LC3. Shown are the effect of TM9SF4-shRNA1 on the formation of autophagosome (yellow dots in merged images) and autolysosome (red dots in merged images) with representative images (**left**) and data summary (**right**). From Sun *et al.*, 2018 Cell Death and Differentiation.

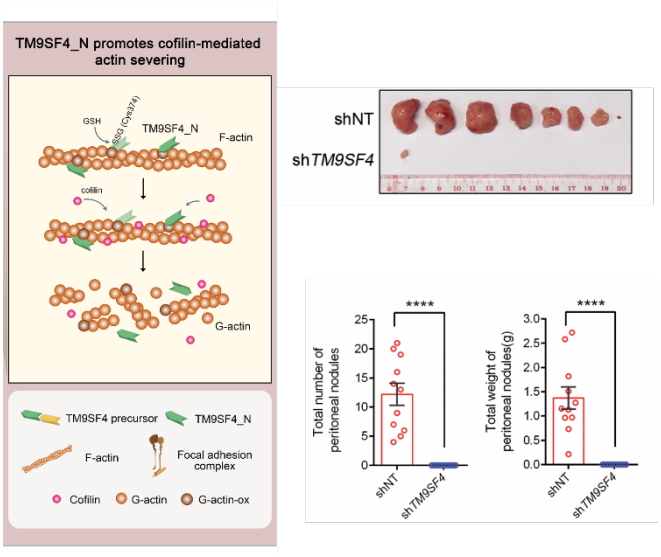

Figure 2. Left: A schematic model showing that TM9SF4 binds to F-actin, induces actin oxidation and change the stability of actin, consequently promoting cofilin-mediated actin depolymerization. Right: This TM9SF4-mediated actin disassembly affects growth and metastasis of ovarian cancer with representative images of tumor sizes (right upper) and data summary (right lower). From Meng *et al.*, 2022 Nature Communications.

Representative publications (*, Corresponding Authors; Bold/Blue, AoE PIs/Collaborators/Associates):

Meng Z, Li Z, Xie M, Yu H, **Jiang L*** and **Yao X*** (2022) TM9SF4 is an F-actin disassembly factor that promotes tumor progression and metastasis. *Nature Communications* 13(1):5728.

Xie M, Mak JW, Yu Y, Cheng CT, Chan HC, Chan TT, Lau LH, Wong MT, Ko WH, **Jiang L** and **Yao X*** (2022) TM9SF4 is a crucial regulator of inflammation and ER stress in inflammatory bowel disease. *Cellular and Molecular Gastroenterology and Hepatology* 14(2):245-270.

Sun L, Meng Z, Zhu Y, Lu J, Li Z, Zhao Q, Huang Y, **Jiang L** and **Yao X*** (2018) TM9SF4 is a novel factor promoting autophagic flux under amino acid starvation. *Cell Death and Differentiation* 25:368-79.

B. Structural Basis of VSR-cargo Recognition and Protein Sorting in Plants.

With joint efforts of structural and plant cell biologists, we have made significant advances in understanding endomembrane trafficking from the perspective of structural biology. Our work have revealed how soluble cargo proteins are recognized and sorted to the vacuoles by vacuolar sorting receptors (VSRs) (Luo *et al*., 2014; Tsao *et al*., 2022). The protease-associated (PA) domain of *Arabidopsis* VSR1 recognizes the residues preceding the NPIR motif of the sequence-specific vacuolar sorting determinant (ssVSD) of aleurain, a cysteine protease targeted to the lytic vacuoles (Luo *et al*., 2014). Binding of cargo induces a 180° swivel motion of the C-terminal tail of the PA domain that relocates the Central domain of VSR1 to synergize the recognition of ssVSD (Luo *et al*., 2014) (**Figure 3A**). The PA domain is also responsible for recognizing the C-terminal VSD (ctVSD) of cruciferin 1, a 12S globulin seed storage protein targeted to the protein storage vacuoles (Tsao *et al*., 2022). Both ssVSD and ctVSD bind to the cargo-binding loop consisting of a conserved motif of $_{95}$RGDCYF$_{100}$ (**Figure 3B**). Based on the structural insights obtained, we were able to use protein engineering to convert the C-terminal sequence of cruciferin 2 to a ctVSD that is recognized and sorted by VSR1 to the vacuoles (Tsao *et al*., 2022).

We also identified an atypical interaction between autophagy adaptor protein SH3P2 and ATG8. We determined the NMR structure of *Arabidopsis* ATG8F (Sun *et al*., 2021). Structural comparison revealed structural changes in the region near the W- and L- sites off ATG8 upon binding of a canonical AIM (ATG8-Interacting Motif) ligand (**Figure 3C**). NMR chemical shift and mutagenesis experiments show that the interaction between ATG8 and SH3P2 differs from that between ATG8 and NBR1. Our results suggest that ATG8 could switch protein-binding partners from SH3P2 to NBR1 for selective autophagy (Sun *et al*., 2021). Teams in the AoE project also determined the cryo-electron microscopy structure of Arabidopsis ATG9 at 7.8 Å (Lai *et al*., 2020) (**Figure 3D**). We show, for the first time, ATG9 forms a homotrimer, which was disturbed by P521A substitution in one of the transmembrane helices.

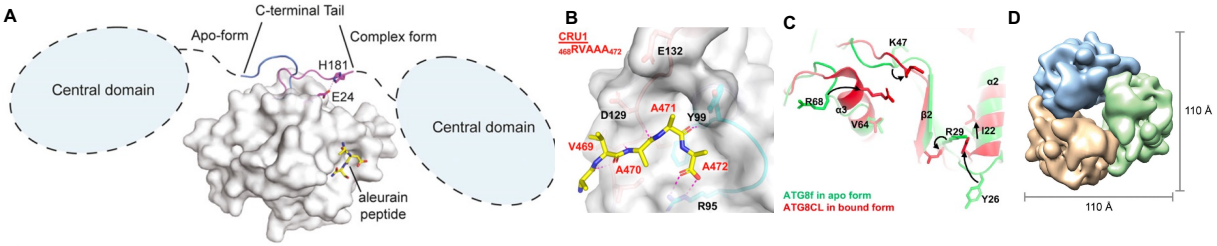

Figure 3. (A) Conformational changes in VSR1-PA upon binding the ssVSD of aleurain. (B) Crystal structure of VSR1-PA in complex with ctVSD of cruciferin 1. (C) Conformational changes in ATG8 upon binding an AIM. (D) Cryo-EM structure of Arabidopsis ATG9.

Representative publications (*, Corresponding Authors; Bold/Blue, AoE PIs/Collaborators/Associates):

Lai LTF, Yu C, Wong JSK, Lo HS, Benlekbir S, **Jiang L** and **Lau WCY*** (2020) Subnanometer resolution cryo-EM structure of Arabidopsis thaliana ATG9. *Autophagy* 16(3):575-583.

Luo F, Fong YH, Zeng Y, Shen J, **Jiang L** and **Wong KB*** (2014) How Vacuolar Sorting Receptor Proteins Interact with Their Cargo Proteins: Crystal Structures of Apo and Cargo-Bound Forms of the Protease-Associated Domain from an *Arabidopsis* Vacuolar Sorting Receptor. *Plant Cell* 26(9): 3693-708.

Sun S, Feng L, Chung KP, Lee KM, Cheung HH, Luo M, Ren K, Law KC, **Jiang L**, **Wong KB** and **Zhuang X*** (2021) Mechanistic insights into an atypical interaction between ATG8 and SH3P2 in *Arabidopsis thaliana. Autophagy* 18(6):1350-1366.

Tsao HE, Lui SN, Lo AHF, Chen S, Wong HY, Wong CK, **Jiang L** and **Wong KB*** (2022) Structural insights into how vacuolar sorting receptors recognize the sorting determinants of seed storage proteins. *Proceedings of the National Academy of Sciences of the United States of America* 119(1):e2111281119.

C. Protein Sorting in the Secretory Pathway in Mammalian Cells.

With joint efforts of cellular and proteomic biologists, we have developed a large scale vesicle formation assay in combination with quantitative mass spectrometry analysis to profile proteins associated with transport vesicles. Using this approach, we revealed cargo proteins that depend on atlastin, a GTPase that connects the endoplasmic reticulum (ER) tubules, to be exported out of the ER. We also revealed novel cytosolic proteins that are associated with vesicle membranes in a GTP-dependent manner and regulate anterograde trafficking. Moreover, we revealed specific clients of two cargo receptors, ERGCI53 and SURF4. These results indicate that the vesicle formation assay in combination with quantitative mass spectrometry analysis is a robust and powerful tool to systematically reveal cargo proteins that depend on a specific factor to be packaged into vesicles.

In addition, we revealed a novel SURF4-proteoglycan relay mechanism that regulates sorting and secretion of sonic hedgehog (Shh) (Huang *et al.*, 2021). Shh plays important roles in various developmental processes in metazoan, including embryonic patterning, stem cell maintenance and tissue homeostasis. However, how newly synthesized Shh is secreted remains largely unknown. Here we demonstrated that secretion of Shh occurs through four steps (**Figure 5**): 1) A cargo receptor, SURF4, interacts with the CW motif of Shh to package ShhN into COPII vesicles; 2) After ShhN is delivered to the Golgi, proteoglycans compete with SURF4 to bind the CW motif of Shh; 3) The released SURF4 is retrieved to the ER by COPI vesicles; and 4) Association of proteoglycan with ShhN is critical for exporting ShhN out of the TGN. Our studies have thus provided new insights into an important question in cell biology: how do cargo receptors capture their clients in one compartment, then disengage at their destination.

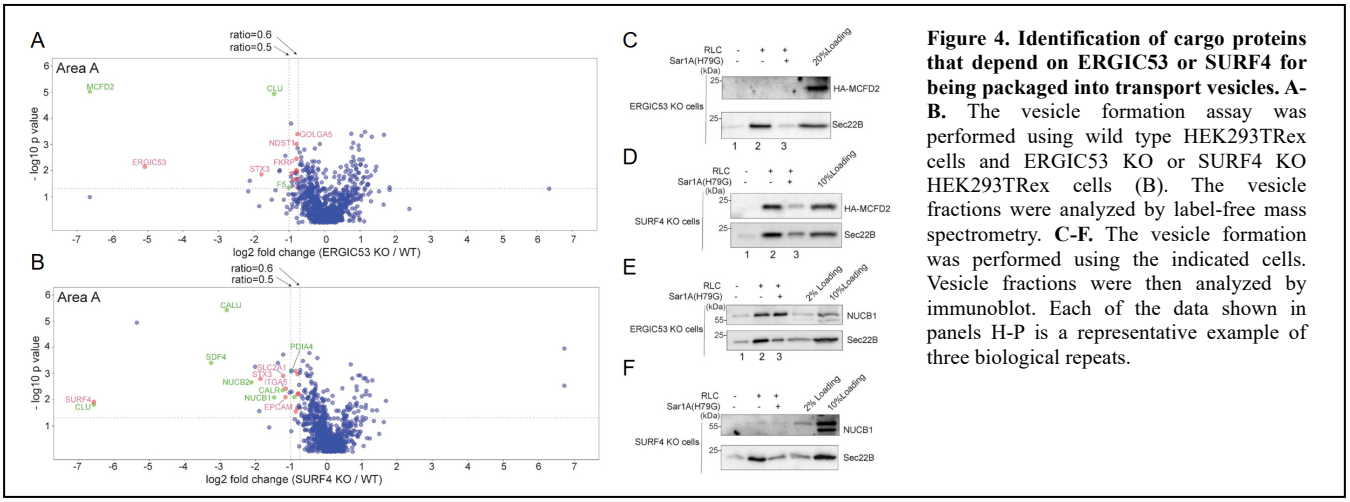

Figure 4. Identification of cargo proteins that depend on ERGIC53 or SURF4 for being packaged into transport vesicles. A-B. The vesicle formation assay was performed using wild type HEK293TRex cells and ERGIC53 KO or SURF4 KO HEK293TRex cells (B). The vesicle fractions were analyzed by label-free mass spectrometry. **C-F.** The vesicle formation was performed using the indicated cells. Vesicle fractions were then analyzed by immunoblot. Each of the data shown in panels H-P is a representative example of three biological repeats.

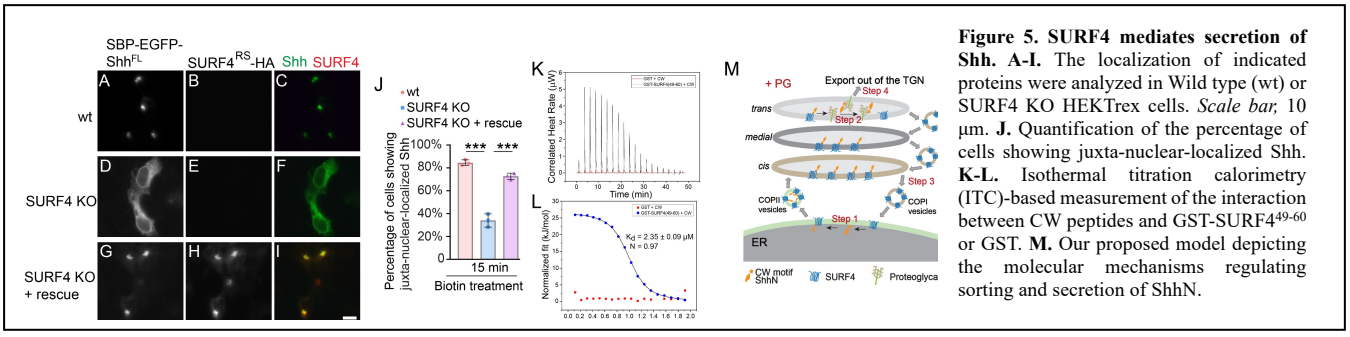

Figure 5. SURF4 mediates secretion of Shh. A-I. The localization of indicated proteins were analyzed in Wild type (wt) or SURF4 KO HEKTrex cells. *Scale bar,* 10 μm. **J.** Quantification of the percentage of cells showing juxta-nuclear-localized Shh. **K-L.** Isothermal titration calorimetry (ITC)-based measurement of the interaction between CW peptides and GST-SURF4$^{49\text{-}60}$ or GST. **M.** Our proposed model depicting the molecular mechanisms regulating sorting and secretion of ShhN.

Representative publications (*, Corresponding Authors; Bold/Blue, AoE PIs/Collaborators/Associates):

Niu L, Ma T, Yang F, Yan B, Tang X, Yin H, Wu Q, Huang Y, **Yao ZP**, Wang J, **Guo Y*** and Hu J* (2019) Atlastin-mediated membrane tethering is critical for cargo mobility and exit from the endoplasmic reticulum. *PNAS* 116: 14029-14038.

Huang Y, Yin H, Li B, Wu Q, Liu Y, Poljak K, Maldutyte J, Tang X, Wang M, Wu Z, Miller EA, **Jiang L**, **Yao Z*** and **Guo Y*** (2021) An in vitro vesicle formation assay reveals cargo clients and factors that mediate vesicular trafficking. *PNAS* 118(35):e2101287118.

Tang X, Chen R, Mesias VSD, Wang T, Wang Y, Poljak K, Fan X, Miao H, Hu J, Zhang L, Huang J, Yao S, Miller EA and **Guo Y*** (2022) A SURF4-to-proteoglycan relay mechanism that mediates the sorting and secretion of a tagged variant of sonic hedgehog. *PNAS* 119: e2113991119.

D. Autophagic Recycling of Mitochondria.

With joint efforts of international collaboration, we have determined molecular mechanisms underlying the selective recycling of damaged mitochondria by autophagy. Cryofixation of *C. elegans* and *Arabidopsis* samples that gave rise to electron microscopy samples preserved close to their native status was critical for visually differentiating damaged mitochondria from normal ones. It was possible to identify assembly intermediates of autophagosomes associated with damaged mitochondria and characterize structural alterations after mitochondria before and after being engulfed by autophagosomes. The mitochondria recycling processes in *C. elegans* and *Arabidopsis* were monitored with three-dimensional (3D) electron microscopy. The key findings are: 1) Paternal mitochondria that entered the oocyte during fertilization in *C. elegans* are degraded by their own endonuclease and the self-inactivated paternal mitochondria are removed selectively by autophagy. (**Figure 6 A-D**) (Zhou *et al.,* 2016); 2) Paternal mitochondria with self-inflicted damage and compromised maternal mitochondria in the *C. elegans* embryo compete for autophagic machinery, suggesting that paternal and maternal mitochondria are not intrinsically differentiated (Wang *et al*., 2016); 3) Depolarized mitochondria are selectively eliminated by autophagy in Arabidopsis cells and the mitophagy contributes to chloroplast biogenesis during the heterotroph-to-autotroph transition in seedlings (**Figure 6 E-G**) (Ma *et al*., 2021); and 4) We identified amphisomes, products from the fusion of autophagosomes and endosomes, in Arabidopsis, and they showed that they contribute to autophagic flux to vacuoles (Zhao *et al*., 2022).

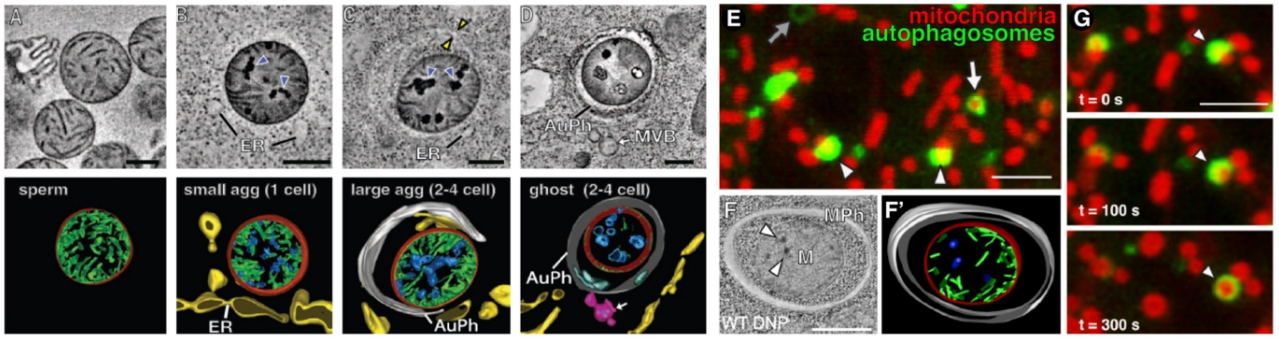

Figure 6. Selective autophagic removal of damaged mitochondria.
A-D. Electron tomography (ET) slice images and corresponding 3D models of a mitochondrion in a *C. elegans* spermatozoon (A) or a paternal mitochondrion in an N2 embryo (B-D) at the indicated stages. 3D models of autophagosomes (AuPh) and endoplasmic reticulum (ER) are shown. Mitochondrial membranes, cristae, and aggregates are colored red, green, and blue, respectively. Dark aggregates and autophagosome membranes are indicated with blue and yellow arrowheads, respectively. Scale bars: 300 nm.
E-G. Confocal laser scanning microscopy (E and G) and ET imaging (F) of mitophagy (i.e., autophagic removal of mitochondria) in Arabidopsis. The images were captured after depolarizing drugs were treated to Arabidopsis cells. In E and G, autophagosomes were visualized by YFP-ATG8e and MitoTracker Red, respectively. A mitochondrion completely enclosed in circularATG8e fluorescence is marked with a white arrow in E. G shows a time-lapse image set of a mitophagy event. YFP-ATG8e encircled a mitochondrion (arrowhead) over 5 min. Scale bars in E and G indicate 5 mm. F and F' are ET slice (F) and 3D model (F') images of the mitophagosome (MPh) and its cargo (M). Scale bar in F: 500 nm.

Representative publications (*, Corresponding Authors; Bold/Blue, AoE PIs/Collaborators/Associates):

Zhou Q, Li H, Li H, Nakagawa A, Lin JL, Lee, ES, Harry BL, Skeen-Gaar RR, Suehiro Y, William D, Mitani S, Yuan HS, **Kang BH*** and Xue D* (2016) Mitochondrial endonuclease G mediates breakdown of paternal mitochondria upon fertilization. *Science* 353: 394 – 399.

Wang Y, Zhang Y, Chen L, Liang Q, Yin XM, Miao L, **Kang BH*** and Xue D* (2016) Kinetics and specificity of paternal mitochondrial elimination in Caenorhabditis elegans. *Nature Communications* 7: 12569.

Ma J, Liang Z, Zhao J, Wang P, Ma W, Fernandez Andrade JA, Zeng Y, Grujic N, **Jiang L**, Dagdas Y* and **Kang BH*** (2021) Friendly mediates membrane depolarization-induced mitophagy in Arabidopsis. *Current Biology* 31: 1931-1944.

Zhao J, Bui MT, Ma J, Künzl F, Picchianti L, De La Concepcion JC, Chen Y, Petsangouraki S, Mohseni A, García-Leon M, Gomez MS, Giannini C, Gwennogan D, Kobylinska R, Clavel M, Schellmann S, Jaillais Y, Friml J, **Kang BH*** and Dagdas Y* (2022) Plant autophagosomes mature into amphisomes prior to their delivery to the central vacuole. *The Journal of Cell Biology* 221(12):e202203139.

E. Organelle Remodeling for Cell Differentiation or Stress Response in Plants.

Cell differentiation in multicellular organisms entails the proliferation, remodeling, rearrangements, and recycling of organelles in the cell. We have used the electron tomography setup for 3D TEM in AoE for the examination of membrane dynamics involved in organelle biogenesis and remodeling.

The organelle plasticity is well illustrated in the interconversions of distinct plastid types in plants. Plastids become chloroplasts in photosynthetic cells, while they transform into amyloplasts in storage cells to accumulate starch particles. Proplastids are a degenerated form of plastid in seeds that have the potential to become chloroplast during germination. We have examined changes in the plastid membrane architectures in differentiating plant cells using the 3D TEM equipment and investigated proteins regulating their inner membrane compartments. Our research has been focused on the transitions involving the proplastid, the etioplast (developmentally arrested plastids under darkness), and the chloroplast. We have also investigated the nuclear pore complex, of which structural modifications control the permeability required for plant defense against pathogens. Key findings are: 1) Assembly of photosynthetic thylakoids requires poly-ribosome binding to the thylakoid membrane and a dynamin-related protein-mediated thylakoid membrane fusion (**Figure 7** A-D) (Liang *et al*., 2017); 2) The paracrystalline structure of the prolamellar body in Arabidopsis etioplast matches cubic diamond symmetry and CURT1A family proteins play roles in the prolamellar body biogenesis and its conversion to thylakoids (**Figure 7 E-G**) (Liang *et al*., 2022); and 3) CPR5 is a component of the nuclear pore complex that undergoes cycles of conformational change and its structural switching is linked to the signaling of effector-triggered plant immunity and programmed cell death (**Figure 7 H-J**) (Gu *et al*., 2016).

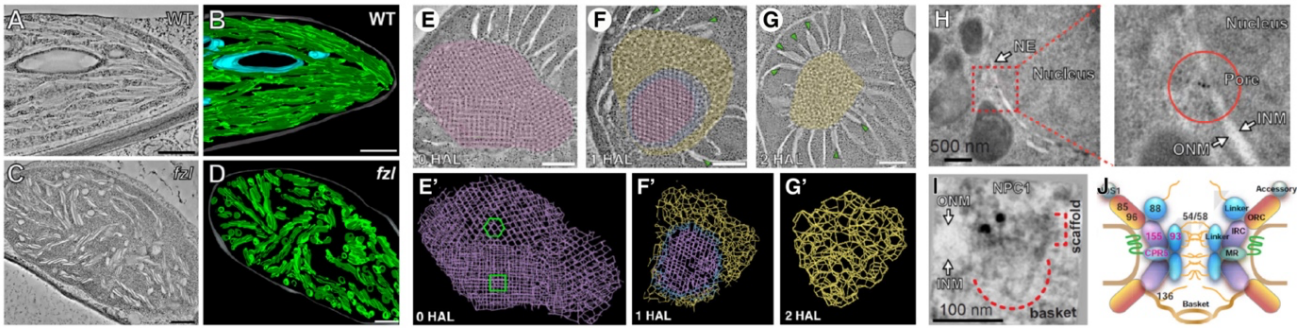

Figure 7. Structural dynamics of plant organelles determined by electron tomography (ET).
A-B, ET slice image (A) and 3D model image of a normal-sized wild-type (WT) chloroplast with typical thylakoid differentiation into stacked (grana) and unstacked domains. Green represents thylakoid membranes and blue represents starch grains. C-D, ET slice image and 3D model of a chloroplast (compare scale bars) with aberrant thylakoid membrane organization in an Arabidopsis *fzl* mutant. FZL is a dynamin-like protein, and thylakoid fusion is inhibited in the mutant. Scale bars: 500 nm.
E-G. The crystalline structure of Arabidopsis PLB and its decay during de-etiolation. The upper panels E, F, and G show ET images of 0, 1, and 2 hours after illumination (HAL), respectively. The lower panels are skeleton models of PLBs at each time point produced with automatic segmentation. Scale bars: 300 nm
H-J. Immunogold localization of CPR5 in the nuclear pore complex (H) and ET projection view (I) of a nuclear pore complex labeled with CPR5-specific gold particles. Immuno-ET indicated that CPR5 is a constituent of the scaffold in the nuclear pore complex (NPC). J shows the structural modules of NPC and the proposed position of CPR5 within the NPC. NE: nuclear envelope, ONM: outer nuclear membrane, INM: inner nuclear membrane.

Representative publications (*, Corresponding Authors; Bold/Blue, AoE PIs/Collaborators/Associates):

Liang Z, Lee K, Mai K, Osteryong K, Staehelin LA and **Kang BH*** (2018) Thylakoid-Bound Polysomes and a Dynamin-Related Protein, FZL, Mediate Critical Stages of the Linear Chloroplast Biogenesis Program in Greening Arabidopsis Cotyledons. *Plant Cell* 30:1476 – 1495.

Liang Z, Yeung WT, Ma J, Mai KKK, Liu Z, Chong YF, Cai X and **Kang BH*** (2022) Electron tomography of prolamellar bodies and their transformation into grana thylakoids in cryofixed Arabidopsis cotyledons. *Plant Cell* 34: 3830-3843.

Gu Y, Zebell SG, Liang Z, Wang S, **Kang BH** and Dong X* (2016) Nuclear Pore Permeabilization Is a Convergent Signaling Event in Effector-Triggered Immunity. *Cell* 166: 1526 - 1538.

F. Multiple Functions and Mechanisms of a Plant Unique ESCRT Component FREE1 in Plants.

We have identified and characterized a plant unique ESCRT component FREE1 (FYVE domain protein required for endosomal sorting 1) of multiple functions and mechanisms in plants (**Figure 8**): 1) MVB (multivesicular body)/ILV (intraluminal vesicle) and vacuole biogenesis (Gao *et al*., 2014); 2) Autophagosome-vacuole fusion (Gao *et al*., 2015; 3) Novel mechanisms of three SFO (Suppressors of FREE1) in regulating MVB biogenesis and function (Shen *et al*., 2018; Zhao *et al*. 2019; Zhu *et al*., 2022); 3) ABA (abscisic acid) signalling (Li et al., 2021); 4) LDs (lipid droplets) degradation in germinating seedlings (Huang *et al*., 2022); and 5) Autophagosome closure (Zeng *et al*., 2022).

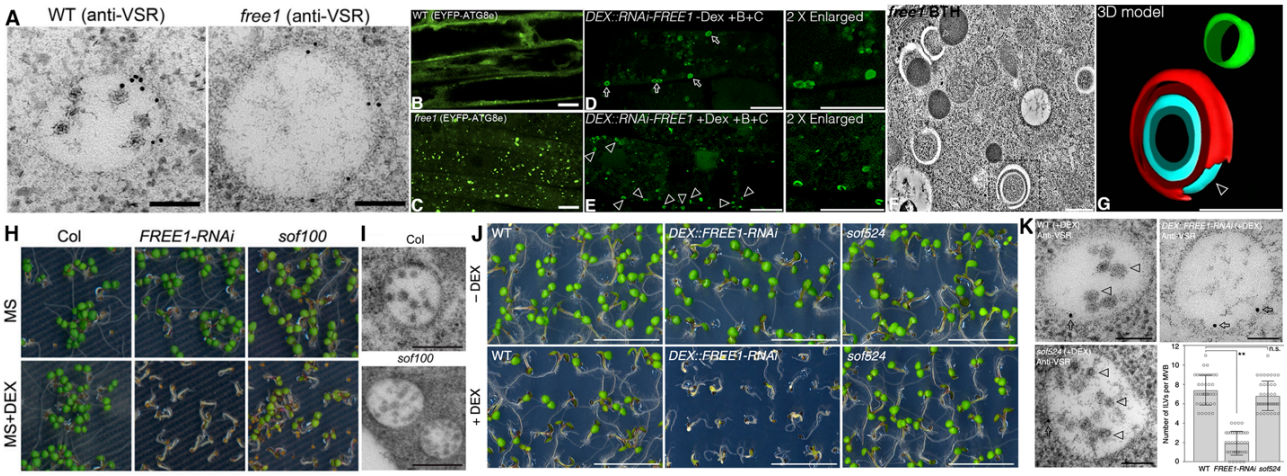

Figure 8. Multiple functions of in MVB/autophagy pathways and the SOFs (Suppressor Of FREE1).
A. Immunogold-TEM analysis of the defective of MVB/ILV biogenesis in *free1* mutants. Scale bar, 100 nm. (***Current Biology*** 2014) **B-C.** Confocal imaging analysis of the accumulation of autophagosome in *free1* mutants. Scale bar, 10 μm. (***PNAS*** 2015) **D-E.** Deconvolution and super-resolution analysis of the unclosed nature of the accumulated autophagosome in *free1* mutants. Arrowheads and arrows indicate examples of the unsealed and closed autophagosomal structures, respectively. Scale bar, 10 μm. (***Nature Communications*** 2022) **F-G.** 3D electron tomography (3D-ET) analysis of autophagosomal structures in homozygous *free1* mutant. Left panel, individual slice of the 3D-tomography. Right panel, 3D model of the tomography. Arrowheads indicate examples of the unsealed autophagosomal structures. Scale bars, 500 nm. (***Nature Communications*** 2022) **H-I.** Phenotypic analysis of the *sof100* and TEM analysis of MVB/ILV biogenesis in *sof100*. Scale bars, 500 nm. (***Plant Cell*** 2019) **J-K.** Phenotypic analysis of the *sof524* and TEM analysis of MVB/ILV biogenesis in *sof524*. Scale bars, 100 nm. (***Nature Communications*** 2018) MVB, multivesicular body; ILV, intraluminal vesicle.

Representative publications (*, Corresponding Authors; Bold/Blue, AoE PIs/Collaborators/Associates):

Gao C, Luo M, Zhao Q, Yang R, Cui Y, Zeng Y, **Xia J** and **Jiang L*** (2014) A unique plant ESCRT component, FREE1, regulates multivesicular body protein sorting and plant growth. *Current Biology* 24(21): 2556-2563.

Gao C, **Zhuang X**, Cui Y, Fu X, He Y, Zhao Q, Zeng Y, Shen J, Luo M and **Jiang L*** (2015) Dual roles of an Arabidopsis ESCRT component FREE1 in regulating vacuolar protein transport and autophagic degradation. *PNAS* 112(6): 1886-1891.

Gao C, **Zhuang X**, Shen J and Jiang L* (2017) Plant ESCRT Complexes: Moving Beyond Endosomal Sorting. *Trends in Plant Science* 22(11): 986-998.

Li H, Li Y, Zhao Q, Li T, Wei J, Li B, Shen W, Yang C, Zeng Y, Rodriguez P, Zhao Y, **Jiang L*,** Wang X* and Gao C* (2019) The Plant ESCRT Component FREE1 Shuttles to the Nucleus to Attenuate Abscisic Acid Signaling. *Nature Plants* 5(5):512-524.

Shen J*, Zhao Q, Wang X, Gao C, Zhu Y, Zeng Y and **Jiang L*** (2018) A Plant Bro1 Domain Protein BRAF Regulates Multivesicular Body Biogenesis and Membrane Protein Homeostasis. *Nature Communications* 9(1):3784.

Huang S, Liu Z, Cao W, Li H, Zhang W, Cui Y, Hu S, Luo M, Zhu Y, Zhao Q, Xie L, Gao C, Xiao S and **Jiang L*** (2022) The plant ESCRT component FREE1 regulates peroxisome-mediated turnover of lipid droplets in germinating Arabidopsis seedlings. *Plant Cell* 34(11):4255-4273.

Zeng Y, Li B, Huang S, Li H, Cao W, Chen Y, Liu G, Li Z, Yang C, Feng L, Gao J, Lo SW, Zhao J, Shen J, Gua Y, Gao C, **Dagdas Y** and **Jiang L*** (2022) The plant unique ESCRT component FREE1 regulates autophagosome closure. *Nature Communications* (In Press)

Zhao Q*, Shen J, Gao C, Cui Y, Wang Y, Cui J, Cheng L, Cao W, Zhu Y, Huang S, Zhou Q, Leong CK, Leung KP, **Chen X** and **Jiang L*** (2019) RST1 is a FREE1 suppressor that negatively regulates vacuolar trafficking in Arabidopsis. *Plant Cell* 31(9):2152-2168.

Zhu Y, Cao W, Huang S, Ji C, Zhang W, Zhao Q, Trujillo M, Shen J and **Jiang L*** (2022) The plant-unique DRIF1 coordinates with retromer to regulate membrane protein homeostasis. *Plant Cell* (Under Revision)

G. Whole-Cell Electron Tomography (ET) Models of Vacuole Biogenesis in Plants.

We have performed the first whole-cell electron tomography (ET) analysis on vacuole formation in *Arabidopsis* root cells and stomatal cells, respectively, resulting in the first whole-cell ET models of vacuole biogenesis with nano-meter resolution in 3D in plant cells, serving as the basis for future study to understand the underlying mechanisms of vacuole formation in plants. In our study, the whole-cell electron tomography analysis first identified unique small vacuoles (SVs; 400–1,000 nm in diameter) as nascent vacuoles in early developmental cortical cells as well as meristemoids and guard mother cells. These SVs contained intraluminal vesicles (ILVs) and were mainly derived/matured from multivesicular body (MVB) fusion. In addition, the whole-cell vacuole models and statistical analysis on root cells of different vacuole developmental stages demonstrated that central vacuoles were derived from MVB-to-SV transition and subsequent fusions of SVs. Further ET analysis on mutants defective in MVB formation/maturation or vacuole fusion demonstrated that central vacuole formation required functional MVBs and membrane fusion machineries. These exciting findings have been published in Nature Plants 2019; Trends in Plant Sciences 2020, and Plant Physiology 2022. Besides root and stomatal development, vacuoles also exist and rapidly change their morphology during various cellular processes in plants, including pollen development and germination as well as embryogenesis. Our future studies will use the whole-cell ET analysis to further illustrate vacuole dynamics and formation, the well-coordinated cycles of vacuole fusion and fission processes and vacuolar convolution during these important biological processes in plants.

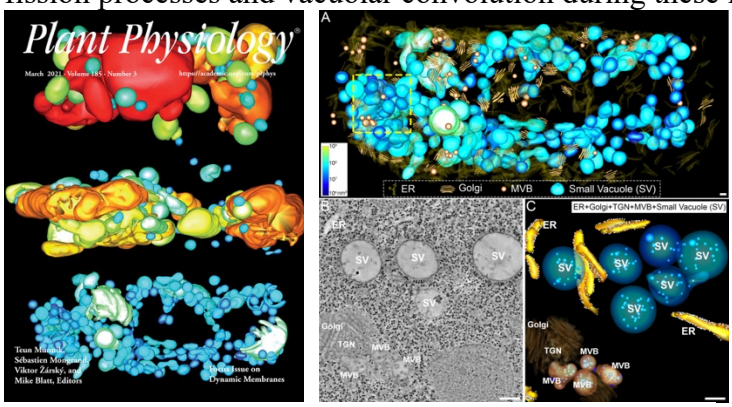

Figure 9. Vacuole biogenesis in plant root cortex. Left: Vacuoles are gradually increasing in size along an apical-to-basal developmental gradient in the root cortex in whole-cell electron tomography analyses. The models were built and colour coded based on the vacuole volumes (cover story). Right: Whole-cell electron tomography analysis of SVs in relationship with other organelles in a cortical cell of early developmental stage. ER, endoplasmic reticulum; MVB, multivesicular body; SV, small vacuole.

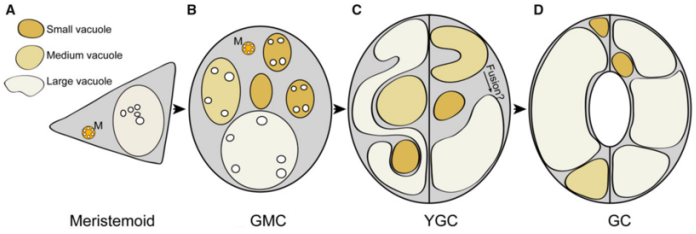

Figure 10. Working model of vacuole formation and distribution in Arabidopsis stomatal lineage cells. Distribution and morphology of vacuoles are based on the whole-cell ET analysis of individual stomatal lineage cells in this study, including meristemoid (A), GMC (B), YGC (C), and GC (D). M, multivesicular body.

Representative publications (*, Corresponding Authors; Bold/Blue, AoE PIs/Collaborators/Associates):

Cao W, Li Z, Huang S, Shi Y, Zhu Y, Lai MN, Lok PL, Wang X, Cui Y and **Jiang L*** (2022) Correlation of vacuole morphology with stomatal lineage development by whole-cell electron tomography. *Plant Physiology* 188(4):2085-2100.

Cui Y*, Zhao Q, Hu S and **Jiang L*** (2020) Vacuole Biogenesis in Plants: How Many Vacuoles, How Many Models? *Trends in Plant Science* 25(6): 538-548.

Cui Y*, Cao W, He Y, Zhao Q, Wakazaki M, **Zhuang X**, Gao J, Zeng Y, Gao C, Ding Y, Wong HY, Wong WS, Lam HK, Wang P, Ueda T, Rojas-Pierce M, Toyooka K, **Kang BH** and **Jiang L*** (2019) A whole-cell electron tomography model of vacuole biogenesis in Arabidopsis root cells. *Nature Plants* 5(1):95-105.

Cui Y, Zhao Q, Gao C, Ding Y, Zeng YL, Ueda T, Nakano A and **Jiang L*** (2014) Activation of the Rab7 GTPase by the MON1-CCZ1 complex is essential for PVC-to-vacuole trafficking and plant growth in Arabidopsis. *Plant Cell* 26(5): 2080-97.

H. Distinct Populations and Functions of COPII Vesicles in Plants.

With joint efforts with local/international collaborators, we have previously identified a unique Coat Protein Complex II (COPII) homologue pair AtSar1a/AtSec23a for selective ER protein export (Zeng *et al*., 2015). Furthermore, we have recently 1) established a plant-based COPII *in vitro* reconstitution system (Li *et al*., 2022) and characterized a specific population of giant COPII vesicles for tough cargos (e.g. membrane transporters) export upon abiotic stresses (Li *et al*., 2021); and 2) discovered the function of a specific AtSar1D-AtRabD2a nexus and COPII population in modulating autophagosome biogenesis in plants (Zeng *et al*., 2021). Our future studies will explore and illustrate the diverse functions of distinct COPII vesicles populations in stress-response pathways in plants.

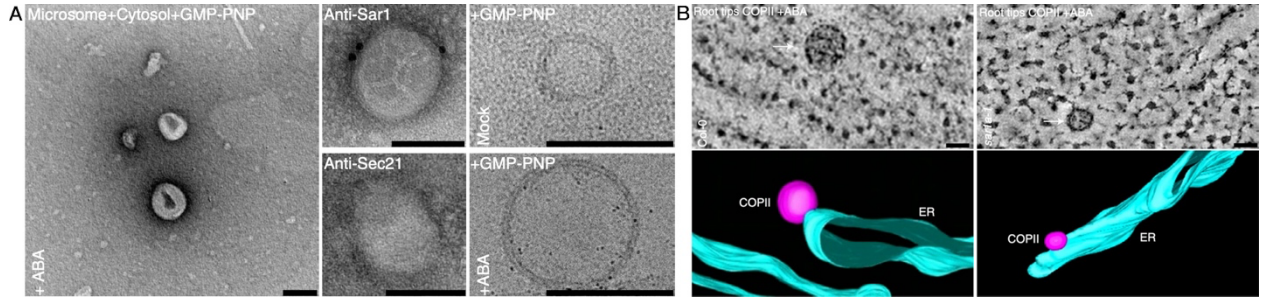

Figure 11. Identification of AtSar1a-dependent giant COPII vesicles forming under ABA treatment. A *In vitro* reconstituted giant COPII vesicles upon ABA treatment visualized by (immunogold labelling) negative staining and cryogenic-EM. Scale bars, 100 nm. **B** *In vivo* visualization of COPII vesicles in 4 day old wild type (Col-0)or *sar1a-1 Arabidopsis* root tips with ABA treatment by 3D-EM tomography. Scale bars, 50 nm. White arrows indicates COPII vesicles.

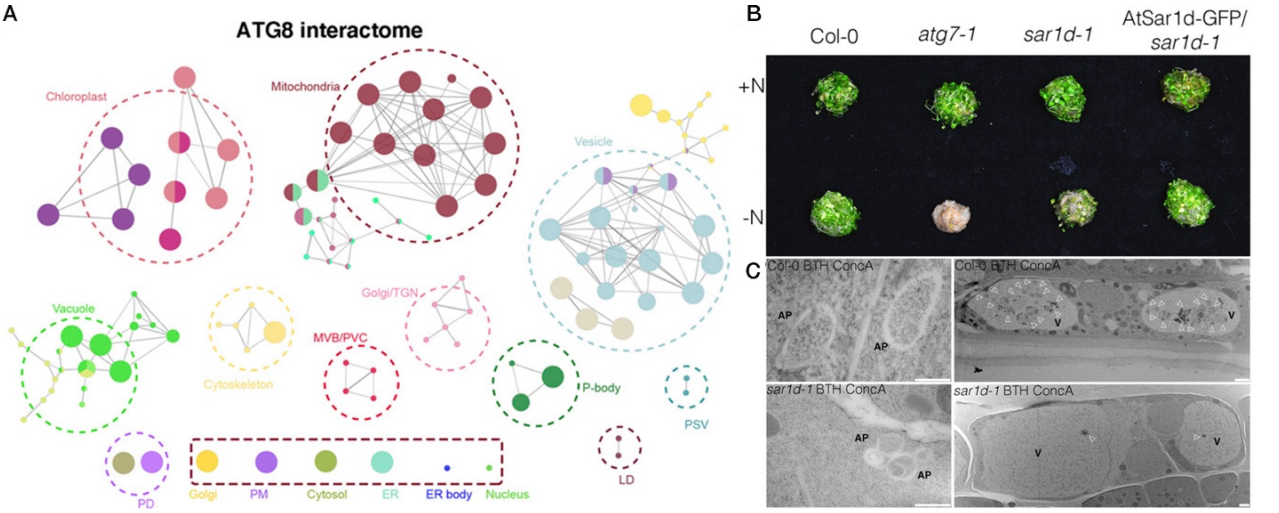

Figure 12. Characterization of functions of AtSar1d in autophagic flux. A GFP-trap and liquid chromatography–tandem mass spectrometry analysis followed by Gene Ontology enrichments and the ClueGO network analysis of ATG8e interactors based on cellular components. PD, plasmodesmata; PM, plasma membrane; TGN, trans-Golgi network; ER, endoplasmic reticulum; LD, lipid droplet; PSV, protein storage vacuole; MVB/PVC, multivesicular body/prevacuolar compartment. **B** *sar1d* mutant exhibits nutrient starvation-related phenotypes. **C** TEM analysis of showing accumulation of autophagic bodies (arrowheads) in 5 day old Col-0 or *sar1d* mutant *Arabidopsis* root tips upon autophagy induction. Scale bars, 500 nm. AP, Autophagosome; V, Vacuole.

Representative publications (*, Corresponding Authors; Bold/Blue, AoE PIs/Collaborators/Associates):

Zeng Y, Chung KP, Li B, Lai CM, Lam SK, Wang X, Cui Y, Gao C, Luo M, **Wong KB**, **Schekman R** and **Jiang L*** (2015) Unique COPII component AtSar1a/AtSec23a pair is required for the distinct function of protein ER export in *Arabidopsis thaliana*. *PNAS* 112(46): 14360–14365.

Zeng Y, Li B, Ji C, Feng L, Niu F, Deng C, Chen S, Lin Y, Cheung KCP, Shen J, **Wong KB** and **Jiang L*** (2021) A unique AtSar1D-AtRabD2a nexus modulates autophagosome biogenesis in *Arabidopsis thaliana*. *PNAS* 118(17): e2021293118.

Li B, Zeng Y, Cao W, Zhang W, Cheng L, Yin H, Wu Q, Wang X, Huang Y, **Lau WCY**, **Yao ZP**, **Guo Y*** and **Jiang L*** (2021) A distinct giant coat protein complex II vesicle population in *Arabidopsis thaliana*. *Nature Plants* 7(10):1335-1346.

Li B, Zeng Y, Lo SW, **Guo Y** and **Jiang L*** (2022) *In vitro* reconstitution of COPII vesicles from *Arabidopsis* suspension cells. *Nature Protocols* (In Press)

I. Molecular Mechanism of Autophagosome Biogenesis in Plants.

With joint efforts of collaboration, we have utilized the 3D electron tomography (ET) for the first time to reveal the autophagosome formation from the endoplasmic reticulum (ER) in *Arabidopsis* plants, demonstrating that the ER is an essential platform for autophagosome formation. We have revealed the unique roles of plant autophagy-related 9 (ATG 9), SNARE proteins VAMP724/726, and Rho GTPase signaling for autophagosome formation (**Figure 13**) (Zhuang *et al*., 2017; 2018; He *et al*., 2022; Lin *et al*., 2021). Moreover, we have illustrated the underlying mechanisms for a plant-specific adaptor protein SH3P2 in binding with ATG8, and uncovered the interaction between the ER contact sites localized oxysterol-binding protein–related protein 2A (ORP2A) and ATG8 in regulating autophagosome formation from the ER contact sites (**Figure 14**) (Sun *et al*., 2021; Ye *et al.,* 2022). These exciting new findings have provided novel insights into the emerging model for autophagosome formation in plants, which greatly facilitate our understanding of the regulatory mechanisms in autophagosome formation.

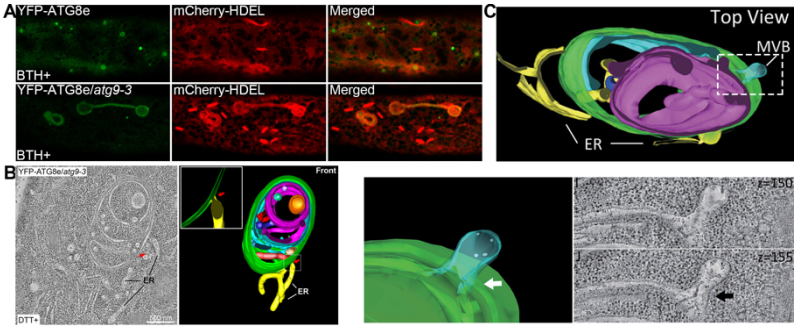

Figure 13. Roles of ATG9 and VAMP724/726 in plant autophagosome formation. A-B. Abnormal autophagosome tubules structures are accumulated with direct connection to the ER in *atg9* mutant. **C.** Direct connections for autophagic tubule with the ER membranes and endosomes in root cells of vamp724-1 vamp726-1 mutant upon autophagy induction.

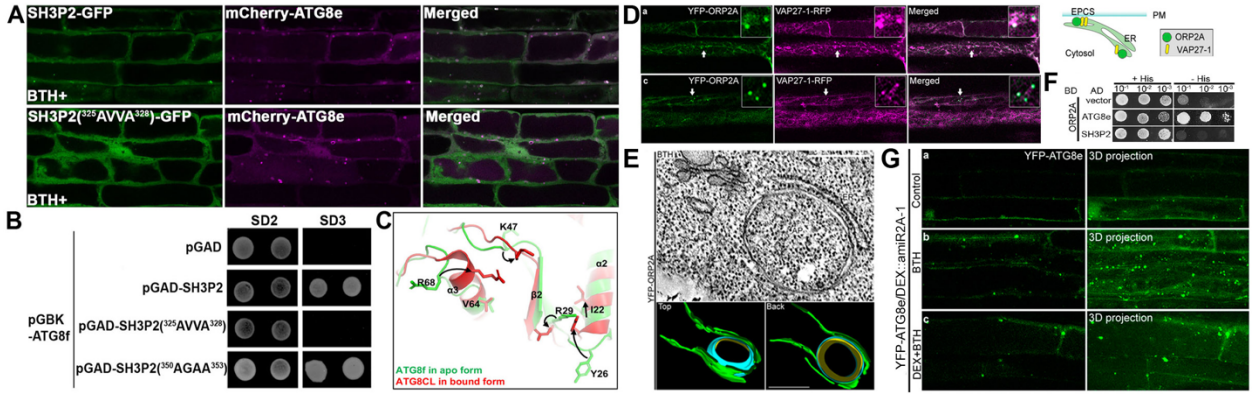

Figure 14. Novel plant ATG8-interacting regulators in plant autophagy. A-C. Identification of interaction motif essential for a plant unique adaptor protein SH3P2 binding to ATG8. **D-G.** Membrane contact sites localized ORP2A interacts with ATG8, and is required for plant autophagosome formation from the ER contact sites.

Representative publications (*, Corresponding Authors; Bold/Blue, AoE PIs/Collaborators/Associates):

He Y, Gao J, Luo M, Gao C, Lin Y, Wong HY, Cui Y, **Zhuang X** and **Jiang L*** (2022) VAMP724 and VAMP726 are involved in autophagosome formation in Arabidopsis thaliana. *Autophagy* (In Press) doi: 10.1080/15548627.2022.2127240

Lin Y, Zeng Y, Zhu Y, Shen J, Ye H and **Jiang L*** (2021) Plant Rho GTPase signaling promotes autophagy. *Molecular Plant* 14(6):905-920.

Sun S, Feng L, Chung KP, Lee KM, Cheung HH, Luo M, Ren K, Law KC, **Jiang L**, **Wong KB** and **Zhuang X*** (2022) Mechanistic insights into an atypical interaction between ATG8 and SH3P2 in Arabidopsis thaliana. *Autophagy* 18: 1350-1366.

Ye H, Gao J, Liang Z, Lin Y, Yu Q, Huang S and **Jiang L*** (2022) Arabidopsis ORP2A mediates ER-autophagosomal membrane contact sites and regulates PI3P in plant autophagy. *PNAS* 119: e2205314119

Zhuang X, Chung KP, Cui Y, Lin W, Gao C, **Kang BH** and **Jiang L*** (2017) ATG9 regulates autophagosome progression from the endoplasmic reticulum in *Arabidopsis*. *PNAS* 114: E426-E435.

Zhuang X, Chung KP, Luo M and **Jiang L*** (2018) Autophagosome Biogenesis and the Endoplasmic Reticulum: A Plant Perspective. *Trends in Plant Science* 23: 677-692.

J. Electron Tomography (ET) Analysis of EXPO and EV in Plants.

In eukaryotic cells, extracellular vesicles (EVs) play important roles in mediating intercellular communication via delivering biomolecules to extracellular space. However, there has been a long debate about the existence and functional role of extracellular vesicles in plants. We have previously identified and characterized a novel double-membrane organelle termed EXPO (exocyst-positive organelle) in mediating an unconventional protein secretion (UPS) pathway in plants (Wang *et al*., 2010; Ding *et al.,* 2012), EXPO, which represents the first type of EVs identified and characterized in plants, also involved in the autophagic pathway in plants (Lin *et al*., 2015; Ji *et al*., 2020). More recently, we have utilized 3D Electron Tomography (ET) combined with immunogold-TEM approaches to study the nature and identity of EVs *in vivo* in various types of plant cells/tissues. We have identified three major types of EVs according to their morphology: Type 1 EVs (electron-transparent, 30-200nm in diameter), Type 2 EVs (contain ribosomes, 200-500nm in diameter), and Type 3 EVs (contain ribosomes and small vesicles, 500-2000nm in diameter). Further immunogold-TEM analysis has distinguished the EXPO from other types of EVs in term of size, shape and cargo contents in the leaf cells. These findings provide a basis for future studying the biogenesis and biological functions of plant EVs. We have also developed and used cryo-ET technology in studying plant organelles (Liu *et al*., 2021)

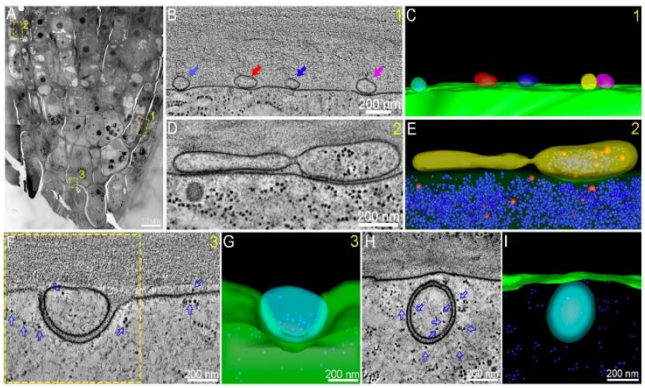

Figure 15. 3D tomographic analysis reveals the existence of EXPO or EXPO-like EVs outside the plasma membrane in Arabidopsis roots.
(A) A low-magnification electron microscopy image of a WT Arabidopsis root tip is shown. Scale bars, 10 μm
(B-I) Tomographic slices and their corresponding 3D models show different types of EV with various size, shapes and cargo contents. Scale bars, 200 nm.

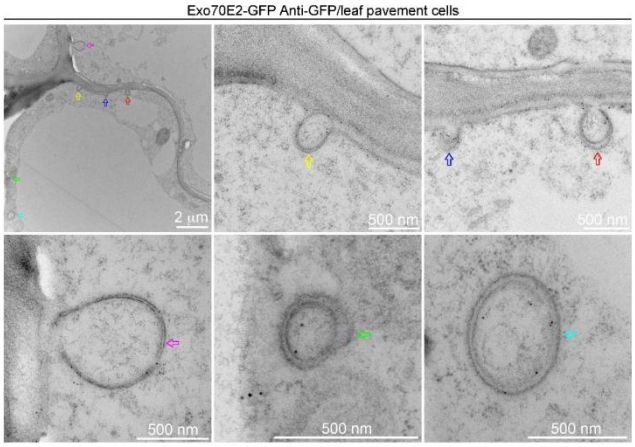

Figure 16. Immuno-gold labeling of EXPO-like EVs in leaf pavement cells.
Immunogold electron microscopy analysis of ultra-thin sections cut from high-pressure frozen/freeze-substituted samples of Exo70E2-GFP leaf cells. GFP antibodies labeled double membrane EXPO in leaf pavement cells. Scale bars, 500 nm.

Representative publications (*, Corresponding Authors; Bold/Blue, AoE PIs/Collaborators/Associates):

Cui Y*, Gao J, He Y, and **Jiang L*** (2020) Plant Extracellular Vesicles. *Protoplasma* 257(1):3-12.

Ji CY, Zhou J, Guo R, Lin Y, Kung CH, Hu S, Ng WY, **Zhuang X** and **Jiang L*** (2020) AtNBR1 is a selective autophagic receptor for AtExo70E2 in Arabidopsis. *Plant Physiology* 84(2):777-791.

Lin Y, Ding Y, Wang J, Shen J, Kung CH, **Zhuang X**, Cui Y, Yin Z, **Xia Y**, **Lin H**, **Robinson DG** and **Jiang L*** (2015) Exocyst-Positive Organelles and Autophagosomes Are Distinct Organelles in Plants. *Plant Physiology* 169(3):1917-32.

Liu Z, Gao J, Cui Y, Klumpe S, Xiang Y, **Erdmann PS** and **Jiang L*** (2021) Membrane Imaging in the Plant Endomembrane System. *Plant Physiology* 185: 562–576.

Wang J, Ding Y, Wang JQ, Hillmer S, Miao YS, Lo SW, Wang XF, Robinson DG and **Jiang L*** (2010) EXPO: an Exocyst-positive Organelle distinct from Multivesicular Endosomes and Autophagosomes, mediates Cytosol to Cell Wall Exocytosis in Arabidopsis and tobacco cells. *Plant Cell* 22(12): 4009-4030.

Ding Y, Wang J, Wang JQ, Stierhof YD, Robinson DG and **Jiang L*** (2012) Unconventional Protein Secretion. *Trends in Plant Science* 17(10): 606-615.

K. New Mechanisms in Regulating Neuron Development.

With joint efforts of collaboration, we have identified LIM-homeodomain transcription factors Lhx1 and Lhx5 playing a critical role in regulating the dendritic development of Purkinje cells. Mutant mice lacking Lhx1 and Lhx5 in postnatal Purkinje cells display ataxic phenotypes due to dendritic defects found in the Purkinje cells. These findings show that Lhx1 and Lhx5 can transcriptionally activate an F-actin regulatory protein, Espin, which in turns controls F-actin localization in the Purkinje cell dendrites. Proper F-actin localization facilitates the elongation and branching of dendrites and the maturation of dendritic spines in neurons. Moreover, we demonstrated that overexpression of Espin could rescue the dendritic defects of Purkinje cells in the mutant mice *in vitro* (Lui *et al*., 2017). This is the first report showing that transcriptional factors can directly regulate an F-actin regulatory element to control cytoskeleton organization in Purkinje cells. More recently, We have also revealed that the signaling strength of bone morphogenetic protein (BMP) determined the temporal identity transition of neural progenitors in the cerebellum and identified SOX9 transcription factor as an essential regulatory factor of choroid plexus function for normal central nervous system functioning (Ma *et al*., 2020; Vong *et al*., 2021).

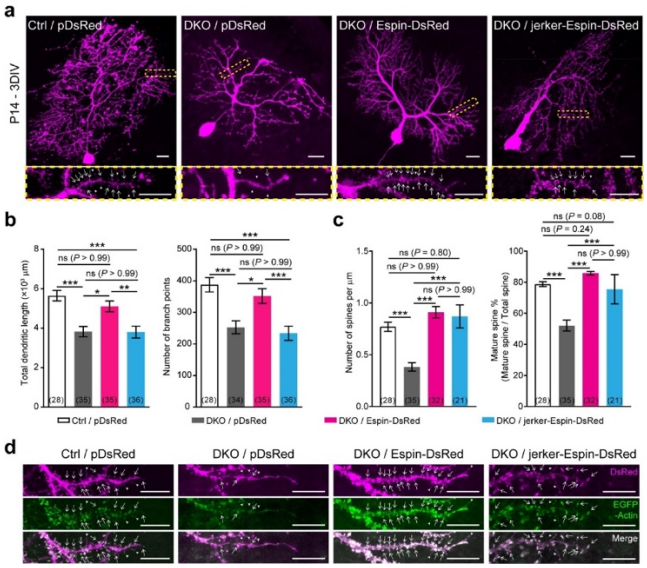

Figure 17. Overexpression of Espin rescues the dendritic defects in the Purkinje cells (PCs) of the DKO mutants ex vivo. (**a**) Representative PCs from P14 cerebellum slice cultures of the DKO mutants transfected with pDsRed or Espin-DsRed or jerker-Espin-DsRed and harvested at 3 DIV. Note that the dendritic trees of the mutant PCs transfected with Espin-DsRed became more extensive and had more mature spines. (**b**) Bar graphs showing the total dendritic length (left) and the branching number (right) of transfected PCs. The dendrite elongation and branching of the mutant PCs restored to the controls' level after transfection of Espin-DsRed. (**c**) Bar graphs showing the density of dendritic spines (left) and the percentage of mature spines (right). Spinogenesis and spine maturation of the mutant PCs significantly increased after transfection of Espin-DsRed or jerker-Espin-DsRed. (**d**) Distal dendrites of PCs from the controls or the mutants co-transfected with EGFP-Actin and pDsRed or Espin-DsRed or jerker-Espin-DsRed. White arrows point to the mature spines while arrowheads point to the immature spines. Espin overexpression can restore the spine maturation in dendrite of DKO PC.

Representative publications (*, Corresponding Authors; Bold/Blue, AoE PIs/Collaborators/Associates):

Lui NC, Tam WY, Gao C, Huang JD, Wang CC, **Jiang L**, Yung WH and **Kwan KM*** (2017) Lhx1/5 control dendritogenesis and spine morphogenesis of Purkinje cells via regulation of Espin. *Nature Communications* 8:15079.

Ma TC, Vong KI and **Kwan KM*** (2020) Spatiotemporal Decline of BMP Signaling Activity in Neural Progenitors Mediates Fate Transition and Safeguards Neurogenesis. *Cell Reports* 30(11): 3616-3624.

Vong KI, Ma TC, Li B, Leung TCN, Nong W, Ngai SM, Hui JHL, **Jiang L** and **Kwan KM*** (2021) SOX9-COL9A3-dependent regulation of choroid plexus epithelial polarity governs blood-cerebrospinal fluid barrier integrity. *PNAS* 118(6):e2009568118.

6.2 What was the added value of the AoE funding, rather than standard project grant funding?
(For example, could this work have been achieved with other funding scheme, such as the General Research Fund or Collaborative Research Fund? If not, why?)

This 8-years long-term AoE project has provided a unique and necessary platform for us to achieve the followings that cannot be achieved with any other RGC funding:

1) We have brought together an excellent team of Co-PIs and national/international collaborators to work on interdisciplinary projects more efficiently;
2) We have built an international-leading Centre with **453m^2** Lab Space and advanced shared platforms;
3) We have attracted additional major collaborative funding (e.g. CRF and RIF) for collaborative research and establishment of advanced platforms;
4) The AoE Centre has serviced as a platform for discussion and promotion of further collaborative research;
5) The AoE Centre has serviced as an Education Centre for training young scientists for their scientific career development;
6) We have established an international-leading research Centre on Organelle Biogenesis and Function;
7) This AoE Centre and its established advanced shared platforms will continue to serve as an internationally recognized hub for collaborative research and research excellence in Hong Kong, Great Bay Area and beyond.

6.3 If the project has not met its original objectives, why?

All original objectives have been achieved.

6.4 (a) Please provide details of each peer-reviewed journal publication arising directly from this project by completing the table at **Annex** *(one for each publication)*.

We have published 278 articles (**Annex and Appendix V**).

(b) Please provide details of recognised international conference(s) in which paper(s) related to this project was/were delivered by completing the following table:
(Please attach a copy of each conference abstract)

We have 136 conferences abstracts of recognised international conferences (**Appendix VII**).

(c) UGC/RGC funding should have been acknowledged in all publication(s)/conference papers listed in (a) and (b) above. If no acknowledgement has been made in any of the publications/papers, please indicate and provide explanations.

N.A.

6.5 To what extent this project has strengthened inter-institutional collaborations and other partnerships?

Since 2014, the RGC-AoE Centre has served as a platform for attracting and promoting local/national/international collaboration with excellent track records (e.g. Joint Publications,

Major Grant Applications and Student Training). In addition to local research collaboration among PIs from the major Universities in Hong Kong (CUHK, HKU, HKUST, HKBU, PolyU and CityU), we have also established collaborative research nationally (e.g. CAAC, CAS, NJAU, HZAU in China), and internationally (e.g. UC Berkeley, Caltech, University of Massachusetts, University of Toronto, University of Valencia, CRSIC Spain and University of Bonn). (**Appendix IV)**.

6.6 Research students trained (registration/awards):

We have 92 trained PhD students. Due to space limitation, details of students are included in **Appendix XI**.

6.7 Specific products (e.g. software or netware, instruments or equipment developed):

N.A.

6.8 Other education activities and/or training programmes developed:

N.A.

6.9 Please highlight any deliverables indicated in the project implementation timetable endorsed by the UGC/RGC which have not been covered or achieved as per sections 6.1 to 6.8 above, and explain/ elaborate.

All our deliverables have been achieved successfully.

6.10 Please elaborate how the PC has played his/her role in coordinating and managing the project.

Since 2014, we have held 49 AoE meetings (including AoE board meetings and AoE group meetings) hosted by the PC. The PC and AoE team members have discussed various AoE-related issues timely, including AoE annual report, fund distribution, AoE equipment charging, training and management, problems and timely solutions. The PC also plays important role in coordinating and discussing collaboration among different Universities of various AoE research projects. The PC also organized the Annual AoE Symposium and AoE Joint Symposium with national/international Institutions. The PC also demonstrated a role model in applying and attracting additional collaborative grants (e.g. CRF-Equipment, CRF and RIF) for the establishment of the advanced shared platforms and further collaborative research under the AoE Centre platforms since 2014.

7. Awards and Recognition

7.1 Have any research grants been awarded that are directly attributable to the results obtained from this project?

Project Ref. No.	Project Title	Project Objectives	Capacity (PI / PC / Co-PI / Co-I)	Funding Source(s) and Amount (HK$)	Project Period
R4005-18	Plant Bioreactor for Pharmaceutical Proteins	1. To test and establish plant tissue culture expression systems for cost-effective large-scale production of selected important pharmaceutical proteins using our expertise and knowledge in protein trafficking and organelle biogenesis in plants. 2. To test the plant-derived pharmaceutical proteins for their bioactivity and biosafety using animal models. 3. To work closely with the two partners from the plant biotech companies in China and Korea to promote the newly developed platforms derived from this project for future application and commercialization of selected plant-derived proteins. 4. We will test and develop large-scale plant cell culture for large-scale production of selected pharmaceutical proteins (e.g. IDUA) and downstream processing for purification, as well as cost analysis for protein production.	PC	RGC RIF $5,000,000	30 Jun 19-29 Jun 24
C4002-21E	The First Integrated State-of- the-Art Live Cell Imaging Platforms to Timely Promote Interdisciplinary and Advanced Life Sciences Research	1. To Establish the First Integrated State-of-the-Art Live Cell Imaging Platforms to Timely Promote Advanced Life Sciences Research in Hong Kong; 2. To Promote Multidisciplinary and Interdisciplinary Collaborative Research using our Existing 3D	PC	RGC CRF $10,000,000	30 Jun 22-29 Jun 25

	in Hong Kong and Beyond	TEM and Cryo-EM/ET Systems as well as the Newly Established Advanced Live Cell Imaging Shared Platforms in Hong Kong and the Great Bay Area; 3. To Promote National and International Collaborative Research in Integrative Biology; 4. To Promote Research Excellence in Hong Kong.			
C4002-20W	Vacuole Biogenesis, Dynamics and Functions in Plants	1. Vacuoles in Arabidopsis Root Cells 2. Vacuoles in Arabidopsis Leaf Guard Cells 3. Vacuoles During Gametogenesis 4. State-of-the-Art: Development and Application of Cryo-EM/ET and Cryo-FIB	PC	RGC CRF $8,800,000	30 Jun 21-29 Jun 24
C4033-19E	The First Integrated State-of-the-Art Sample Preparation System for Cryo-Electron Microscopy/Tomography Analysis to Promote Advanced Cellular and Structural Biology Research in Hong Kong	1. To Establish an Integrated State-of-the-Art Sample Preparation System for Cryo-EM/ET Analysis to Promote Advanced Structural Cellular Biology Research in HK; 2. To Promote Multidisciplinary and Interdisciplinary Collaborative Research using the Existing 3D TEM and cryo-EM/ET Shared Facility in HK and Beyond; 3. To Promote National and International Collaborative Research in Cellular and Structural Biology; 4. To Promote Research Excellence in HK.	PC	RGC CRF $7,439,308	30 Jun 20 - 29 Jun 23
C4012-16E	The First Integrated cryo-EM and cryo-ET Shared Facility for Life Sciences Research in Hong Kong	1. To establish the First Integrated cryo-EM and cryo-ET Shared Facility for Life Sciences Research in Hong Kong 2. To Promote Multidisciplinary Research using the Integrated 3D Tomography TEM and Cryo-EM Shared Facility in Hong Kong and Beyond 3. To Promote National and International Collaborative Research in Structural and Organelle Biology 4. To Promote Research Excellence in Hong Kong	PC	RGC CRF $9,500,000	30 Jun 17 - 29 Jun 20

7.2 Have any project team members participated as invited speakers in or organisers of international conferences as a result of this project?

Our AoE teams members have delivered 136 invited talks. For examples, Jiang has given invited seminars in Australia, USA, Germany and China. Zhang has given invited seminars in UK and China. Due to space limitation, Please refer to **Appendix VII** for more information.

We have also organized 25 international conferences (**Appendix III**).

The PC Prof Jiang Liwen was the Director of the following conferences:

- AoE Symposium on Organelle Biogenesis and Function (2014-2021)
- Mini-Symposium on Plant Bioreactors for Pharmaceuticals and Vaccines (Nov 4, 2021)
- Croucher Summer Course 2019 "Frontier in Cellular Biology" (2019)
- Mini-Symposium on Cryo-Electron Microscopy (Jun 14, 2019)
- Croucher Advanced Study Institute (ASI) Symposium and Workshop "Frontier in Cell and Cellular Structural Biology Research" (Dec 17-19, 2018)
- Croucher Summer Course "Cell and Developmental Biology Research" (2013, 2015, 2017)
- Pre-CRF Symposium on Autophagy (Jan 7, 2017)
- AoE "Mini-Symposium on Cryo-EM and Organelle Biogenesis" (Jun 17, 2016)
- Croucher Advanced Study Institute (ASI) Symposium and Workshop "Frontier in Cell Biology Research" (Jan 4-8, 2012)

7.3 Have any project team members taken leadership positions in editorial boards, scientific and professional organisations?

Our AoE PC and Co-PIs have taken many leadership positions in editorial boards. For example, editors-in-chief of Plant Science (Jiang L), editors-in-chief of Sampling Theory in Signal and Image Processing (Blu T), senior editor of Journal of Integrative Plant Biology (Jiang L), associate editor of Frontiers in Neurodegeneration (Chan EHY) and associate editor of Journal of Experimental Botany (Zhang J).

Please refer to **Appendix XIV** for more information.

7.4 Any documentary proof of the application of technologies arising directly from this project?

N.A.

7.5 Other awards and recognitions as a result of this project (please specify):

The PC Prof Jiang Liwen has received the following awards:

- RGC Senior Research Fellow 2021
- Excellence Research Award 2015
- Croucher Senior Research Fellowship 2015-2016
- MoE Natural Science Award of China (2nd Class)- 2014 and 2017
- Choh-Ming Li Professor of Life Sciences 2014

The Co-I Prof Raymond Chan was awarded Fellow of the American Mathematical Society (2021).

The Co-I Prof Zhang Jianhua received the 2017 State Natural Science Award (Second Class) conferred by the State Council of China for "Regulation and physiological mechanism in enhancing the remobilization of assimilates to grain and grain filling in rice and wheat".

Please refer to **Appendix X** for more information.

8. Impacts

8.1 What are the current and expected impacts of the project in terms of its contribution to the local and regional economic and societal well-being (e.g. patent, technology transfer, collaboration with external organisations, etc.)?

A) **Basic Knowledge**: Organelle biogenesis and function is an important topic in cell biology as they are important in regulating growth and development. Our AoE research has put Hong Kong on the world map in this research field and beyond;
B) **Education**: Our AoE Centre has served as an important Education Centre for training young scientists for their scientific career development in Hong Kong and beyond;
C) **Shared Platforms**: We have built multiple advanced shared platform (e.g. 3D TEM, Cryo-EM/ET, Cryo-FIB/CLEM and Integrated Live Cell Imaging Systems) and technology with in-house expert support for promoting advanced and collaborative Life Sciences Research in Hong Kong and beyond;
D) **Potential Impacts**: Some of the basic knowledges on organelle biogenesis and function gained from our research are addressing important biological pathways related to human health/diseases and agriculture (e.g. stress responses), which will provide the needed basis in future development of drugs against diseases and plants/crops for stress tolerance. In addition, AoE members have one US Patent (on Cancer treatment) and two Invention Patents from China (on plants).
E) **Future Development**: We will build on our existing AoE advanced platforms and technologies to establish a new AoE of "***Institute of Integrative Biology***" in addressing the cellular mechanisms of abiotic stress responses in model organisms including plants as well as their potential impact on crop improvement and environmental sustainability.

8.2 Others (please specify):

N.A.

9. Sustainability of the Project

9.1 Whether there are new ideas evolved directly from this project?

Same for most scientific research, some of the exciting new findings derived from this AoE project and collaborative research have resulted in new ideas and research hypothesis being tested and developed timely in new grant applications including GRF, CRF, CRF-Equipment and RIF with great success (**Table in Session 7.1)**

9.2 Whether there are new projects evolved directly from this project?

With the joint efforts of PC and 17 Co-PIs, we have been awarded multiple collaborative grants (CRF, CRF Equipment and RIF), including the three CRF Equipment grants (**Table in Session 7.1**). Together with AoE/CRF-E and CUHK matching, we have established several state-of-the-art platforms for promoting collaborative research in Life Sciences and research excellence in Hong Kong and beyond, including A) The First 3D TEM system in Hong Kong in 2015; B) The First Cryo-EM/ET shared platform in 2019; C) The first Cryo-FIB/SEM and Cryo-CLEM systems in 2020; and D) The integrated advanced live-cell imaging platform in 2022/23.

9.3 Whether there are new collaborations developed directly from this project?

A) With the joint efforts of PC and 17 Co-PIs, we have been awarded two CRF Equipment grants (total HK$38M with CUHK matching) to establish a) The Cryo-EM/ET system in 2019; and b) The Cryo-FIB/CLEM system in 2021 for promoting NEW collaborative research (**Appendix XVI**);
B) With the establishment of these new platforms, we have also attracted new collaboration in the development and application of these new technologies, for example, in collaboration with Fondazione Human Technopole (Dr. ERDMANN Philipp), we have developed Cryo-FIB and Cryo-ET for analysis of pollen tube, allowing identification of transport vesicles in pollen tubes at their native state and an unprecedented resolution (Liu *et al*., 2020 Plant Physiology).

Please refer to **Appendix IV** for more details on AoE collaborations.

9.4 Please give details on how much money and from which sources has been obtained/requested for the specific purpose of continuing the work started under this project.

A) With the joint efforts of PC and 17 Co-PIs, we have recently been awarded a new CRF-Equipment grant (at HK$10M + 10M CUHK matching) for building an integrated advanced live-cell imaging platform in Hing Kong in 2022/2023 (**Appendix XVI**);
B) With the joint efforts of PC and 17 Co-PIs, we have submitted a preliminary AoE proposal on September 2022 to establish the "***Institute of Integrative Biology***" with a collaborative effort focusing on understanding the cellular mechanisms underlying abiotic stresses responses in plants (e.g. *Arabidopsis* and Rice) and other model organisms (e.g. Yeast and Mouse) as well as their potential applications for future development of abiotic stress-resistant plants/crops and drug development.

10. Statistics on Research Outputs

(Please ensure the statistics in this section are consistent with the information presented in other sections of this report.)

	Peer-reviewed journal publications	Conference papers	Scholarly, books, monographs and chapters	Patents awarded	Other research outputs (please specify)	
No. of outputs arising directly from this research project	271	136	7	3	Type	No.
					N.A.	

11. Public Access of Completion Report

(PCs of projects funded in 2010/11 academic year or onwards are required to release the completion reports to the public through the RGC website. Completion reports containing information such as abstracts in non-technical terms, objectives, research output including the list of conference papers / publications / journals and research findings and contact information of PCs should be open to public access.)

Please specify the information, if any, that cannot be provided for public access and give the reasons.

Information that cannot be provided for public access	Reasons

12. The Layman's Summary

(describe in layman's language the abstracts and research impact of the project.)

With the joint efforts of team members and collaborators, we have developed the AoE Centre into an internationally recognized hub for advanced research on organelle biogenesis and function. We have conducted world-leading research with exciting findings and significant contributions in the field. We have also established multiple state-of-the art advanced shared platforms with in-house expert support for promoting advanced and collaborative Life Sciences Research in Hong Kong and beyond. The AoE Centre has also played an important role in education and training young scientists for their scientific career development.