

Table EV1 | Summary of data acquisition and image processing for cryo-ET data in this study.

Sample		WT	Opa1-OE	I-Opa1*	s-Opa1*	Opa1-KO
Cryo-FIB milling	Microscope	Aquilos Cryo-FIB, FEI – Thermo Fisher Scientific	Aquilos Cryo-FIB, FEI – Thermo Fisher Scientific	Aquilos Cryo-FIB, FEI – Thermo Fisher Scientific	Aquilos Cryo-FIB, FEI – Thermo Fisher Scientific	Aquilos Cryo-FIB, FEI – Thermo Fisher Scientific
	Acquisition settings	Titan Krios Gi3 FEI, Thermo Fisher Scientific	Titan Krios Gi3 FEI, Thermo Fisher Scientific	Titan Krios Gi3 FEI, Thermo Fisher Scientific	Titan Krios Gi3 FEI, Thermo Fisher Scientific	Titan Krios Gi3 FEI, Thermo Fisher Scientific
	Voltage (KeV)	300	300	300	300	300
	Detector	Gatan K3 IS	Gatan K3 IS	Gatan K3 IS	Gatan K3 IS	Gatan K3 IS
	Energy filter	Gatan BioQuantum K3	Gatan BioQuantum K3	Gatan BioQuantum K3	Gatan BioQuantum K3	Gatan BioQuantum K3
	Slit width (eV)	20	20	20	20	20
	Super-resolution mode	Yes	Yes	Yes	Yes	Yes
	Å/pixel	2.757	2.565	2.757	2.757	2.757
	Defocus (µm)	-3.5 to -5.0	-3.5 to -5.0	-3.5 to -5.0	-3.5 to -5.0	-3.5 to -5.0
	Acquisition scheme	-70/70, 2°, Dose-symmetric	-70/70, 2°, Dose-symmetric	-70/70, 2°, Dose-symmetric	-70/70, 2°, Dose-symmetric	-70/70, 2°, Dose-symmetric
	Total dose	~90 - 120	~90 - 180	~90 - 180	~90 - 120	~90 - 120
	Dose rate (e-/Å/sec)	~ 2.5 – 3.5	~ 2.5 – 3.5	~ 2.5 – 3.5	~ 2.5 – 3.5	~ 2.5 – 3.5
	Frame number	6	6	6	6	6
	Number of tomograms	33	7	27	22	11
Image processing	Frame alignment and dose weighting	<i>framealign</i> , IMOD	<i>framealign</i> , IMOD	<i>framealign</i> , IMOD	<i>framealign</i> , IMOD	<i>framealign</i> , IMOD
	Tilt series alignment	IMOD	IMOD	IMOD	IMOD	IMOD
	WBP	IMOD	IMOD	IMOD	IMOD	IMOD
	Denoising	Topaz	Topaz	Topaz	Topaz	Topaz
	3D-segmentation	Amira	Amira	Amira	Amira	Amira
	3D-rendering	Amira	Amira	Amira	Amira	Amira