# Science Advances

### Supplementary Materials for

## Cryo-EM structure and electrophysiological characterization of ALMT from *Glycine max* reveal a previously uncharacterized class of anion channels

Li Qin, Ling-hui Tang, Jia-shu Xu, Xian-hui Zhang, Yun Zhu, Chun-rui Zhang, Mei-hua Wang, Xue-lei Liu, Fei Li, Fei Sun, Min Su\*, Yujia Zhai\*, Yu-hang Chen\*

\*Corresponding author. Email: yuhang.chen@genetics.ac.cn (Y.-h.C.); yujia@ibp.ac.cn (Y. Zhai); minsu@genetics.ac.cn (M.S.)

> Published 2 March 2022, *Sci. Adv.* **8**, eabm3238 (2022) DOI: 10.1126/sciadv.abm3238

#### The PDF file includes:

Figs. S1 to S9 Tables S1 and S2 References

#### Other Supplementary Material for this manuscript includes the following:

Movies S1 to S3



#### Fig. S1. Family tree of the ALMT/QUACs.

The presentation was computed by the program COBALT from representative sequences, including thirteen *Arabidopsis* ALMTs, and two other ALMTs. The clustering of sequences into a subfamily was performed using PSI-BLAST at the different levels ( $E \le 10^{-160}$ ,  $E \le 10^{-180}$ ) with representative sequences, as detailed in the table S1. The numbers of the sequences in the family/subfamily are indicated in parentheses. The clade numbering in previous work (*60*) is also indicated, and the Clade6 is a new group.

60. I. Dreyer, J. L. Gomez-Porras, D. M. Riaño-Pachón, R. Hedrich, D. Geiger, Molecular evolution of slow and quick anion channels (SLACs and QUACs/ALMTs). *Front. Plant Sci.* **3**, 1–12 (2012).

	pre-TM TM1
GmQUAC1	80
AtQUAC1	MSNKVHVGSLEMEEGLSKTKWMVLEPSEKIKKIPKRLWNVGKEDPRRVIHALKVGLSLTLVSLLYLMEPL 70
AtALMT13	MGYKVEARSMEISMEDEDSRKKRKKGLNLPKKMKKILRNLWNVGKEDPRRVIHALKVGVALTLVSLLYLMEPF 73
AtALMT14	MSDRVHERSMG-MEEEGSTKNMKTKVLELPTKIKKILKNIWKVGKDDPRRVKHALKVGVSLTLVSLLYLMEPL 72
AtALMT10	89
AtALMT8	MDLNAQEKKAGFFQRLQDFPSKLKDDVTKRVKNVQKFAKDDPRRIIHSMKVGVALTLVSLLYYVRPL 67
AtALMT7	MEKVREIVREGRRVAKEDPRRVVHSFKVGLVLALVSSFYYYQPL 44
AtALMT1	MEKVREIVREGIRVGNEDPRRIIHAFKVGLALVLVSSFYYYQPFGPF 47
AtALMT2	MEKVREIVREGRRVGKEDPRRVVHAFKVGLALALVSSFYYYQPL 44
AtALMT6	84
AtalmT4	90
AtalmT5	MGGKMGSVPEQNTEKLLWQSSDVADSRDSKFRCCSWRALYEAPAKLYALGHSDRRKLYFSIKMGIALALCSFVIFLKEP 79
AtalmT3	MAAPKLESFRRGSMFDGSFRRGSMFDGSFRQSMRDRLILQSRGYSNVNDDDKTSVRCCSYSYFSDKITGVVKKLKDVLVTAWEMGTADPRKMIFSAKMGLALTLTSILIFFKIP 114
AtALMT9	MAAKQGSFRHGILEKRERLLSNNGFSDFRFTDIESNDLLENENCGRRTRLCCCCSCGNLSEKISGVYDDAKDVARKAWEMGVSDPRKIVFSAKIGLALTIVALLIFYQEP 110
	· · · · · · · · · · · · · · · · · · ·

		TM2			TM	3					TN	44					TM5				TM6			
	l	lllll	lll	ll	ععع	lee	lee	22	ll	lll	ll	فكفلا	ll		Q	2220	2220	2222	ll	222	2000	lll	lll	
GmQUAC1	FKGIGQSAI	WAVMTVVVVL	<mark>e</mark> ftagatlc	<mark>K</mark> GLN	RGLGT	LLAGLI	LAFLVO	GYIANAS	SDRVSQA	AIIIGA	AVFF	IGALATY	M <mark>r</mark> fipy	I <mark>KK</mark> NY	Y <mark>d</mark> Y	GLVIF	LLTFNI	ITVSSY	R <mark>LENVLKI</mark>	AHD <mark>f</mark>	VYTIAI	GCAVCI	LMSLL	215
AtQUAC1	FKGIGSNAI	WAVMTVVVL	<mark>e</mark> fsagatlc	KGLNI	RGLGT	LIAGSI	LAFFIE	EFVANDS	GKVLRA	IFIGT	AVFI	IGAAATY	I <mark>r</mark> fipy	I <mark>KK</mark> NY	Y <mark>d</mark> Y	GVVIFI	LLTFNI	ITVSSY	R <mark>VDSVINI</mark>	AHD <mark>F</mark>	FYTIAV	GCGICI	FMSLL	205
AtalmT13	FEGVGKNAL	WAVMTVVVL	<mark>e</mark> fsagatlr	KGLNI	RGLGT	LIAGSI	LAFFIE	EWVAIHS	GKILGO	GIFIGT	SVFT	IGSMITY	M <mark>r</mark> fipy	I <mark>KK</mark> NY	Y <mark>d</mark> Y	GMLVFI	LLTFNI	ITVSSY	R <mark>VDTVIKI</mark>	AHE <mark>F</mark>	LYTIGM	IGIGICI	FMSLL	208
AtALMT14	FKGIGNSAI	WAVMTVVVL	<mark>e</mark> fsagatlc	KGLNI	RGLGT	LIAGSI	LAFFIE	EFVANDS	GKIFRA	AIFIGA	AVFI	IGALITY	L <mark>r</mark> fipy	I <mark>KK</mark> NY	Y <mark>d</mark> Y	GMLIFI	LLTFNI	ITVSSY	R <mark>VDTVIKI</mark>	AHE <mark>F</mark>	FYTIAM	IGVGICI	LMSLL	207
AtalmT10	YDGVGGNAM	WAIMTVVVF	<mark>e</mark> snvgatfc	K CVN	RVVAT	ILAGSI	GIAVE	WVATQS	GK-AEV	FVIGC	SVFL	FAFAATY	S <mark>r</mark> fvps	F <mark>ka</mark> ri	F <mark>D</mark> Y	GAMIF	ILTFSI	VSVGGY	R <mark>VDKLVEI</mark>	JAQQ <mark>F</mark>	VSTIAI	GTSIC	LITVF	223
AtalmT8	YISFGVTGM	WAILTVVVVF	<mark>e</mark> ftvggtls	KGLN	RGFAT	LIAGAI	GVGA	/HLARFE	GHQGEF	PIVLGI	LVFS	LGAAATF	S <mark>r</mark> ffpi	RI <mark>KQ</mark> RY	Y <mark>d</mark> Y	GALIF	ILTFSF	VAISGY	RTDEILIM	1AYQ <mark>f</mark>	LSTILI	GGTICI	LVSIF	202
AtalmT7	YDSFGVNAM	WAVMTVVVF	<mark>E</mark> FSVGATLG	KGLN	RVAAT	LFAGGI	GIGA	HLASMS	GPTGEF	PILLAV	FVFV	QAALSTF	V <mark>r</mark> ffpi	RV <mark>KA</mark> RY	Y <mark>d</mark> Y	SLLIF	ILTFAI	ISVSGF	R <mark>EEQVVKI</mark>	.THK <mark>F</mark>	ISTVII	GGLSC	/IISIF	179
AtalmT1	TDYFGINAM	WAVMTVVVF	<mark>E</mark> FSVGATLG	KGLN	R <mark>GVAT</mark>	LVAGGI	GIGA	QLARLS	GATVER	PILLVM	LVFV	QAALSTF	V <mark>r</mark> ffpV	IV <mark>kt</mark> ki	F <mark>D</mark> Y	GILIF	ILTFAI	ISLSGF	RDEEIMDI	JAES <mark>F</mark>	LSTVVI	GGVSCI	LISIF	182
AtalmT2	YDNFGVNAM	WAVMTVVVF	<mark>e</mark> fsvgatlg	KGLN	RAVAT	LVAGGI	GIGA	HLASLS	GPTVEF	PILLAI	FVFV	LAALSTF	V <mark>r</mark> ffpi	RV <mark>KA</mark> RY	Y <mark>d</mark> Y	GVLIF	ILTFAL	ISVSGF	REDEILDI	JAHK <mark>F</mark>	LSTVIM	IGGVSC	/LISIF	179
AtalmT6	LHDASKYSV	WGILTVVVVF	<mark>e</mark> ysvgatlv	KGFNI	RAIGT	VSAGGI	LALGIA	ARLSVLS	SRD-FEÇ	QTIIIT	CIFL	AGFIASY	S <mark>K</mark> LHP#	M <mark>K-</mark> PY	Y <mark>E</mark> Y	AFRVF	LLTFCI	VLVSGN	NTGDFFSI	'AYY <mark>f</mark>	FLFIVV	GATTCI	LVVNIF	217
Atalmt4	LRDASKYAV	WAILTVVVVF	<mark>e</mark> ysigatlv	KGFNI	RAIGT	LSAGGI	LALGIA	ARLSVSF	AGE-FEE	LIIII	SIFI	AGFSASY	L <mark>K</mark> LYP <i>i</i>	M <mark>K-</mark> SY	Y <mark>E</mark> Y	AFRVF	LLTYCI	VLVSGN	NSRDFFSI	'AYY <mark>f</mark>	FLLILV	GAGICI	GVNIF	223
AtalmT5	LQDASKFAV	WAILTVVLIF	<mark>e</mark> yyvgatlv	KGFNI	RALGT	MLAGGI	LALGVA	AQLSVLA	AGE-FEE	VIIVI	CIFL	AGFGASY	L <mark>K</mark> LYAS	SM <mark>K-</mark> PY	Y <mark>e</mark> Y	AFRVFI	KLTYCI	VLVSGN	NSRDFLSI	'AYY <mark>f</mark>	ILLIGI	GATICI	LVNVF	212
Atalmt3	GLELSGHYL	WAILTVVVIF	<mark>E</mark> FSIGATFS	KGCNI	RGLGT	LSAGGI	LALGMS	SWISEMT	CGN-WAE	VFNAA	SIFV	VAFFATY	A <mark>k</mark> lyp:	M <mark>K-</mark> PY	Y <mark>E</mark> Y	GFRVFI	LLTYCY	VIVSGY	K <mark>TGEFMEI</mark>	'AVS <mark>F</mark>	FLLIAI	GASVGI	LIVNTC	247
AtalmT9	NPDLSRYSV	WAILTVVVVF	<mark>E</mark> FTIGATLS	KGFNI	RALGT	LSAGGI	LALGMA	AELSTLE	GD-WEE	IFCTL	SIFC	IGFLATF	M <mark>k</mark> lyps	SM <mark>K-</mark> AN	Y <mark>E</mark> Y	GFRVFI	LLTYCY	ILISGF	R <mark>TGQFIEV</mark>	/AIS <mark>F</mark>	FLLIAI	GAGVSI	GVNMF	243
	. :	*.::***:::	* *.*:	* *	* .*	: ** *	· • •	::			:*	. ::	::	• *	::*	. :*	**:	: :		: *	. : :	*	: :.	

	H1	H2	нз
			20202020202020
GmQUAC1	VFPNWSGEDLHNSTVYKLEGLAKSIEACVNEYFYGEIEGSG	YMKLSEDPIYKGYKAVLDSKSIDETLALHASWEP	rhsry- <mark>c</mark> hrfpwqqyvkvgavlrqfgytvvalhgclrteiqtprsvr 336
AtQUAC1	VFPIWSGEDLHKTTVGKLQGLSRSIEACVDEYFEEKEKEKT	DSKDRIYEGYQAVLDSKSTDETLALYANWEP	rhtlr- <mark>c</mark> hrfpcqqyvkvgavlrqfgytvvalhgclqteiqtprsvr 323
AtALMT13	FFPIWSGDDLHKSTITKLQGLSRCIEACVSEYFEEKLKDNETSD	SESDDEDLIYNGYNTVLDSKSADEALAMYAKWEP	rhtrr- <mark>c</mark> nkfpsqqyikvgsvlrkfgytvvalhgclqteiqtprsir 332
AtALMT14	VFPIWSGEDLHKSTVAKLQGLSYSIEACVNEYFEEEEKDEETSD	LSEDTIYNGYKTVLDSKSADEALAMYASWEP	rhtrh- <mark>C</mark> hrfpwkhyvkvgsvlrQfgytvvalhgclkteiQtprplr 328
AtalmT10	FCPIWAGSQLHRLIERNLEKLADSLDGCVAEYFKENEVSTNRNE	DENTNMKLQGFKCVLNSKGTEEAMANLARWEP	ahgsf- <mark>n</mark> frhpwklyvkigaamrrcayclenlsicinyeteapdqvk 345
Atalmt8	ICPVWAGEDLHKMIANNINKLAKYLEGFEGEYFQPEKI	SKETSSCVREYKSILTSKSTEDSLANLARWEP	GHGRF- <mark>R</mark> LRHPWKKYLKIAGLVRQCAVHLEILNGYVLSNDKAPQEFE 318
AtalmT7	VCPVWAGQDLHSLIASNFEKLSFFLADFGDKYCEVVENDGAKE	VDKRKKDFDNYKSVLNSKSNEESLANFAKWEP	GHGQF- <mark>R</mark> FRHPWKQYLAVGELIRQCAYRIHALNSYLNADNQVSVDIK 300
AtalmT1	VCPVWAGQDLHSLLASNFDTLSHFLQDFGDEYFEAREKGDYKV	VEKRKKNLERYKSVLDSKSDEEALANYAEWEP	phgqf- <mark>r</mark> frhpwkqyvavgallrqcayridalnsyinsdfqipvdik 303
Atalmt2	VCPVWAGQDLHSLLASNFDTLSHFLQEFGDEYFEATEDGDIKE	VEKRRRNLERYKSVLNSKSNEEALANFAKWEP	rhgqf- <mark>r</mark> frhpwrqylavgallrqsayridalnsninsdmqipmdik 300
AtALMT6	IFPIWAGEDLHKLVANNFKSVANSLEGCVNGYLQCVEYERVPSKILT	YQT-SDDPLYSGYRSAIQSTNQEESLLDFAIWEPH	phgpyr <mark>t</mark> fnhpwknyvklsgavrhcaftvmaihgcilseiqaapekr 344
AtALMT4	ILPIWAGEDLHKLVVKNFKSVANSLEGCVNGYLQCVEYERIPSKILT	YQA-SDDPLYSGYRSVVQSTSQEDSLLDFAVWEPH	phgpyk <mark>t</mark> fhhpwanyvklsgavrhcafmvmamhgcilseiqaapekr 350
AtalmT5	LFPIWAGEDLHKLVAKNFKNVANSLEGCVNGYLQCVEYERIPSKILT	YQA-SDDPLYSGYRSAVQSTSQEDSLLDFAIWEPH	PHGPYK <mark>T</mark> FNHPWKNYVKLSGAVRHCAFTVMAMHGCILSEIQASPEKR 339
Atalmt3	IYPIWAGEDLHNLVAKNFVNVATSLEGCVNGYLECVAYDTIPSRILV	YEAVAEDPVYSGYRSAVQSTSQEDTLMSFASWEPI	PHGPYK <mark>S</mark> FRYPWALYVKVGGALRHCAIMVMALHGCILSEIQAAEDRR 375
AtalmT9	IYPIWAGEDLHNLVVKNFMNVATSLEGCVNGYLRCLEYERIPSKILT	YQA-SEDPVYKGYRSAVESTSQEESLMSFAIWEPH	PHGPYK <mark>S</mark> FNYPWKNYVKLSGALKHCAFTVMALHGCILSEIQAPEERR 370
	. * *:*:** :: :: *	:. : * :::: * ***	** *::. ::: : : : : : .

	H4	H5				
		llllllll	_			
<i>Gm</i> QUAC1	AMFKDPCIRLAAEVSKVLIELSNSIRNRRHCS-	PEILSDHLHEALQDLNTAIKSQPRL <u>FLG</u>	PKHRHNQA <mark>T</mark> NMLKIA		<u>AAQVGQERHGK<mark>TS</mark>L<mark>S</mark>4</u>	426
AtQUAC1	ALFKDPCVRLAGEVCKALTELADSISNHRHCS-	PEILSDHLHVALQDLNSAIKSQPKLFLG	SNLHRHNNKHQNG <mark>S</mark> ISNNKHHQRN <mark>SS</mark> N	<mark>S</mark> GKDLNGDV <mark>S</mark> LQNTE <mark>T</mark> G <mark>T</mark> RKI <mark>T</mark>	E <mark>T</mark> G <mark>S</mark> RQGQNGAV <mark>S</mark> L <mark>S</mark> 4	447
AtALMT13	VLFKDPCVRLAGEICKVLSELSESIQNRRHCS-	SEILSDSLEAALKDLNSTIKSQPKLFLG	SNLH <mark>S</mark> NI <mark>T</mark> NKHLNGHV <mark>S</mark> YYI	NE <mark>T</mark> NSNGTV <mark>S</mark> YHNDNNTNGCVLGE <mark>T</mark> IEF	END <mark>T</mark> V <mark>S</mark> PLPLN <mark>S</mark> VV <mark>S</mark> L <mark>S</mark> 4	455
AtalmT14	GLFKDPCVRLAGEICKVLSELAASIRNRRHCS-	PEILSDSLQVALQDLNTAIKSQPKLFLG	<mark>SS</mark> QNGNV <mark>S</mark> QGN <mark>S</mark> GRHNPNVAV <mark>S</mark> QH:	INKD <mark>T</mark> NEAA <mark>S</mark> YQN <mark>T</mark> G <mark>T</mark> PRG	ERM <mark>S</mark> RFGPNV <mark>S</mark> F <mark>S</mark> 4	444
AtALMT10	NHFGEACMKLSSASSKILRELADMMKNTRKSS-	-KMDFLVFDMNSAVQELQETLKNVPIETNK	PEEVPSEEENK		4	413
AtALMT8	SKIQEPITTMSREVGEALKAIAKSIKTMRNDSA	ACVNAHIDNSKKAIKNLKIALKSSYPETY-			3	379
AtalmT7	KKLGEPLRRMSLESGKAMKEMSISLKKMTKPS-	-SSDLHVQNAKSACKSLTNLLNSGILKE			3	359
AtalmT1	KKLETPLRRMSSESGNSMKEMSISLKQMIKSS-	-SSDIHVSNSQAACKSLSTLLKSGILND			3	362
Atalmt2	KKIEEPLRRMSSESGKSMKEVSISLKNMTISS-	-SFDIHVVNSQSACKTLSTLLKSGILND			3	359
AtALMT6	QAFRHELQRVGNEGAKVLRLIGEKVEKMENL	-GPGEILNDVQRAAEELQMKIDSKSYLLVN	SESWAATKEKAEAEEYEEE	AHETKVIKSLSQIWDTN	NSSSNNQNPASG 4	451
AtalmT4	QAFRQELQRVGNEGAKVLRLFGEKVEKMEKLS-	PGNVLKDVQRAAEELQMKIDSNSFLLVN	SESWAAMKEKAEAEEAQQN	YHEAKDDESKVIQSLSQIWDNN	NNNPHHQNQHA 4	461
AtALMT5	HVFSNELRRVGNEGAKVLRLFGEKVEKMEKLSI	LSLGEILKDVQRAAEALQMKIDSKSYLLVN	SESWAAIKEQAEAEEAREN	DQEAKDDETKVIKSLSQIWDTN	NNNNHQS 4	449
Atalmt3	REFRNELQRVGIEGAKVLRYIGESLKKMEKLN-	-PIEDILYEIHQAAEELQSKIDKKSYLLVN	AKNWEIGNRPRVRDLTDEQKISN	LDSDLSRILAHKSQSEATLRPPKNWDD\	VTTAANLSSA 4	496
AtalmT9	QVFRQELQRVGVEGAKLLRELGEKVKKMEKLG-	-PV-DLLFEVHLAAEELQHKIDKKSYLLVN	SECWEIGNRATKESEPQELLSLEDSDP:	PENHAPPIYAFKSLSEAVLEIPPSWGEF	KNHREA 4	490
	: :. :: :	. * : * :				

		н6				H7				
		llll	_	ll	مععع	eeee	2225	222222		
GmQUAC1	SVKTDSSALLEWKTKRVSAEQTKESERKSLRPQ	L <mark>S</mark> KIA-ITS	le <mark>f</mark> s	EA <mark>l</mark> pe	' <mark>A</mark> AFASL	LVETVAK	LDLVI	EEVEELGRLA	<mark>C</mark> F <u>KEFIPGDEFVVTCQEPRVDVSQNHLPSHGVI</u>	<u>)</u> 537
AtQUAC1	SFRTDTSALMEYRRSFKNSNSEMSAAGERRMLRPQ	L <mark>S</mark> KIAVMTS	le <mark>f</mark> s	EA <mark>l</mark> pe	' <mark>A</mark> AFASL	LVEMVAR	LDNVI	EEVEELGRIA	<mark>S</mark> FKEYDNKRDQTADDVRCENPANVTISVGAAE-	- 560
AtalmT13	<mark>S</mark> lr <mark>S</mark> VKK <mark>S</mark> AA <mark>T</mark> GEKRRLRKQ	L <mark>S</mark> KIAVMKS	le <mark>f</mark> s	EA <mark>l</mark> pe	' <mark>a</mark> afasl	LVEMVAR	LDTVI	DEVEELGTIA	CFKEYDKTVEVRIENRLI	- 539
AtalmT14	RLRAD <mark>T</mark> LERR <mark>S</mark> AAA <mark>T</mark> NERKILRQQ	L <mark>S</mark> RIVVLTS	le <mark>f</mark> s	EA <mark>l</mark> pe	' <mark>a</mark> afasl	LVEMVAR	LDNVI	EEVEELGTIA	CFKDYDNNVDQKDVEVRVEKPADLVVGVE	- 543
AtalmT10		VDSEERTTS	MS <mark>L</mark> H	iev <mark>l</mark> pv	ATLVSL	LIENAAR	IQTAV	EAVDELANLA	DFEQDSKKKTGDNNTKQPPLSS	- 485
Atalmt8			KD <mark>L</mark> L	EI <mark>I</mark> PG	. <mark>V</mark> TMASI	LIEVVNC	VEKIY	EAVEEFSGLA	HFKETLDSKLSAEIGQHQLLHRGCVKPVLDGDN	446
AtalmT7			VE <mark>P</mark> L	EL <mark>V</mark> SI	LTAISL	LIDIINI	TEKII	ESLHELATAA	KFKNKIEHPLFSEKPKAKSFVSVRSIKCHDDHV	J 426
Atalmt1			VE <mark>P</mark> L	.QM <mark>I</mark> SI	MTTVSM.	LIDIVNI	TEKIS	ESVHELASAA	RFKNKMRPTVLYEKSDSGSIGRAMPIDSHEDHF	H 429
AtalmT2			VE <mark>P</mark> L	QM <mark>I</mark> SI	MTTVSL.	LIDIVNI	TEKIS	ESVHELASAA	<mark>K</mark> FKNKKKPSKSNSGSIGQAMPNKSHDDDI	2 422
AtalmT6	GGS	VVNETVYKV	YE <mark>S</mark> A	.SS <mark>l</mark> SI	ATFASL	LIEFVAR	LENLV	NAFEELSTKA	. <mark>D</mark> FRDPVPLNVVDQEGLWTTLMRLRRLRRYFGRS	3 560
AtalmT4	GRDSQLWISTESMMLRNRENWPSVSFIGGS	MINEIESKV	YE <mark>S</mark> A	.SS <mark>L</mark> SI	ATFASL	LIEFVAR	LQNIV	NAYEELSTKA	DFKEQVSETRI	- 548
AtalmT5	DGT	VVNEIECKV	YE <mark>S</mark> A	.SS <mark>L</mark> SI	ATFASL	LIEFVAR	LQNIV	NAFEELSTKA	<mark>G</mark> FKDAVDQIPKV	- 537
Atalmt3	TMLPYLQSRTMIHKQPSWPSRISITPGSMLQ	PPLGEPGKM	iye <mark>s</mark> a	.SN <mark>L</mark> SI	ATFASL	LIEFVAR	LENLV	NAYDELSVKA	<mark>n</mark> fkeavse	- 581
AtalmT9	LNHRPTFSKQVSWPARLVLPPHLETTNGA	SPLVETTKT	YE <mark>S</mark> A	.sa <mark>l</mark> si	. <mark>A</mark> TFASL	LIEFVAR	LQNVV	DAFKELSQKA	NFKEPEIVTTGTDVEFSGERVGLGQKIRRCFGN	4 598
			].	. :	: *:	*::	:	: .*:. *	*.:	

GmQUAC1		537
AtQUAC1		560
AtALMT13		539
AtALMT14		543
AtALMT10		485
AtALMT8	EKEDNSSCHVLITVHDEGYLPTATAKNVLGAEKTRVDIV	488
AtALMT7	VIIIEDDGNNDDTSKNDNGSKEVSIHEKHEDDDTHVDARCVSCGHTSVCVK	480
AtalmT1	VVTVLHDVDNDRSNNVDDSRGGSSQDSCHHVAIKIVDDNSNHEKHEDGEIHVHTLSNGHLQ	493
AtALMT2	HVVTILGDVDTSNNVDQSQSHGEISVDSCHHVTIKINDDDSIHDKNEDGDIHVHTNRVSCDHTNASDLLDSGVKKN	501
AtALMT6		560
AtALMT4		548
AtalmT5		537
AtalmT3		581
AtalmT9		598

Fig. S2. Structure-based sequence alignment for plant ALMT/QUACs.

Structure-based sequence alignments of thirteen *Arabidopsis* ALMT/QUACs and *Gm*ALMT12/QUAC1. The structure of *Gm*ALMT12/QUAC1 has been used to restrict sequence gaps to inter-helical segments, with the superior coils defining extents of the helical segments. The disordered regions in *Gm*ALMT12/QUAC1 (residues 1-35, 394-454 and 507-537) are indicated by underlines. The aligned sequences are as following: *Gm*ALMT12/QUAC1 (NP\_193531.1), *At*ALMT1 (NP\_172319.1), *At*ALMT2 (NP\_172320.1), *At*ALMT3 (NP\_173278.1), *At*ALMT4 (NP\_173919.1), *At*ALMT5(NP\_564935.1), *At*ALMT6 (NP\_179338.1), *At*ALMT7 (NP\_001324626.1), *At*ALMT8 (NP\_187774.1), *At*ALMT9 (NP\_188473.1), *At*ALMT10 (NP\_001319836.1), *At*ALMT13 (NP\_199472.1), *At*ALMT14 (NP\_199473.1).

The protein sequences are from three major groups: QF1A (*At*ALMT3/4/5/6/9) in red; QF2A (*At*ALMT1/2/7/8/10) in blue; QF2B (*Gm*ALMT12/QUAC1, *At*ALMT12/13/14) in black. Overall, the protein sequences in the QF2A have a shorter pre-TM region, and lack a disordered region and a domain-swapped helix found in the *Gm*ALMT12/QUAC1 structure.

Some key residues are highlighted or indicated as following:

1. The pore-lining positively charged R/K residues are highlighted in cyan (K109/R113/R158/K164/K165/R187/R198 in *Gm*ALMT12/QUAC1), and related interacting negatively-charged E/D residues are also highlighted in cyan (E100/D168 in *Gm*ALMT12/QUAC1).

2. The residues of mutagenesis for disrupting dimer interaction are highlighted in yellow (S461/F470/L474/A477 in GmALMT12/QUAC1).

3. Positively charged residues at the N-terminal pre-TM juxtamembrane helix are highlighted in grey.

4. Potential phosphorylation sites in the disordered regions of C-terminal CHD are highlighted in green.

5. The domain-swapped helix regions are indicated in a box (helix H6 in *Gm*ALMT12/QUAC1).

6. Other conserved motifs are indicated in boxes (W90, Y169, P218-N219-W220-S221-G222 and W288-E289-P290 in GmALMT12/QUAC1).



Fig. S3. The workflow for image processing of *Gm*ALMT12/QUAC1.



Fig. S4. Cryo-EM analysis of GmALMT12/QUAC1.

- (A) Representative micrograph of *Gm*ALMT12/QUAC1.
- (B) Representative 2D class averages of *Gm*ALMT12/QUAC1.
- (C) Local resolution electron density map of *Gm*ALMT12/QUAC1.
- (**D**) Euler angle distribution plot of particle projections in reconstruction of *Gm*ALMT12/QUAC1.
- (E) Fourier shell correlation (FSC) curve suggests an overall resolution at 3.5 Å, as estimated using the 0.143 cut-off criterion (dotted line).
- (F) Cross-validation of model to cryo-EM density map suggests a resolution at 3.5 Å, as estimated using the 0.5 cut-off criterion (dotted line). All line charts in (E) and (F) were prepared in GraphPad Prism.
- (G) Representative cryo-EM density map for the TM segments.



Fig. S5. Single channel analysis of malate regulation on GmALMT12/QUAC1.

(A and B) The representative current traces at +60 mV, before and after adding 2 mM L-malate to the *trans*- (A) or *cis*-(B) chamber. The chambers were filled with symmetrical solutions of 150 mM NaCl, and the purified  $GmALMT12/QUAC1^{NaCl}$  proteins were added to the *cis*-side.

(**C** and **D**) The regulation effect of malate on the *Gm*ALMT12/QUAC1<sup>NaCl</sup> in the NaCl solutions. The current amplitude (left) and open probability (right) analysis for the recordings, before (–) or after (+) addition of 2 mM L-malate to the *trans*- (C) or *cis*- (D) chamber, are shown (Data are mean  $\pm$  SEM, n=3 for each group).



#### Fig. S6. Analyses of the GmALMT12/QUAC1 pre-TM helix deletion mutant by TEVC recording.

(A) Representative current traces recorded at different voltages (from +60 mV to -180 mV in 20 mV decrement) in the external solutions of 30 mM L-malate for the *Gm*ALMT12/QUAC1 wild-type (upper) and  $\Delta$ 1-53 mutant (lower).

(B) Steady-state current-voltage (I<sub>ss</sub>-V) relations of the *Gm*ALMT12/QUAC1 wild-type and  $\Delta$ 1-53 mutant (Data are mean ± SEM, n≥6).



#### Fig. S7. Cross-linking experiments of the CHD proteins.

(A) Cross-linking of purified CHD proteins, wild-type (WT<sup>CHD</sup>) and A477E mutant (A477E<sup>CHD</sup>). Equal amount of proteins were incubated with 0.01% glutaraldehyde at 4°C for 3 hr. The reactions were quenched with 50 mM Tris-HCl (pH 8.0), and analyzed by 10% SDS-PAGE.

(**B**) Gradient fixation (GraFix) of the purified WT<sup>CHD</sup> and A477E<sup>CHD</sup> proteins. Equal amount of proteins were loaded onto the gradient of glycerol and glutaraldehyde, and ultra-centrifuged at 40,000 rpm, 4°C for 18 hr. The fractions of 0.5 ml were collected, and quenched with a final concentration of 80 mM glycine. The fractions were analyzed by 10% SDS-PAGE. In both experiments, the wild-type CHD results in more cross-linked dimer than that of A477E mutant under parallel conditions.





В



#### Fig. S8. Fluorescence and confocal imaging of the C-terminal GFP tagged GmALMT12/QUAC1.

(A) Representative fluorescence images of *Gm*ALMT12/QUAC1 wild-type (WT) and mutants ( $\Delta$ 460-467, S461D, W90F, A477E and A477E/W90F). The cRNAs fused with C-terminal GFP were injected into the *Xenopus laevis* oocytes. After ~48 hr expression at 18°C, the GFP fluorescence on the plasma membrane of oocytes was analyzed by a laser confocal microscope. (B) Mean fluorescence intensity measurements by using software ImageJ. A one-way ANOVA analysis was performed with a P value of 0.9704, showing no significant differences between them. (Data are mean ± SEM, n=3).



#### Fig. S9. Comparison of the cryo-EM structure and AF-structure.

(A) Superimposition of the cryo-EM structure (salmon) and AF-structure (grey). The superimposed  $C\alpha$  and their r.m.s.d values are indicated.

(B) The comparison of the cryo-EM structure (salmon) and AF-structure (grey). The TM3 and TM6 helices are shown in cartoon.

(C) The porcupine plot showing the motion across the AF-structure and the cryo-EM structure. The arrows indicate direction of motion and magnitude of the movements of the C $\alpha$  positions (from the AF-structure to the cryo-EM structure). The C $\alpha$  traces for the AF-structure are shown. The colors for the arrows are indicated as followings: red (outward motion), green (inward motion) and blue (static).

(**D**) The dihedral angle between the TMD and CHD interfaces in the AF-structure and the cryo-EM structure are 93.5° and 83°, respectively. The 10.5° rotation between these two states suggests that domain rearrangement occurs during conformational conversion. We speculate that the domain re-organization is coupled to channel gating.

(E) Plot of the pore radius as a function of the pore axis, cryo-EM structure (salmon) vs. AF-structure (grey).

#### Table S1

			1	
Family /Subfamily	Representative expansion sequence	GenBank ID	-log(E <sub>cut</sub> )	Sequence
QF1	Arabidopsis thaliana ALMT9	NP_188473.1	160	572
QF2	Arabidopsis thaliana ALMT2	NP_172320.1	160	1171
Others <sup>b</sup>				13
Total				1756
		-		
QF1A	Arabidopsis thaliana ALMT9	NP_188473.1	180	466
QF1B	Oryza sativa Japonica ALMT9	XP_015618041.1	180	106
Sub-total				572
QF2A	Arabidopsis thaliana ALMT2	NP_172320.1	180	805
QF2B°	Arabidopsis thaliana ALMT12/QUAC1	NP_193531.1	180	244
QF2C	Helianthus annuus ALMT4	XP_022041099.1	180	122
Sub-total				1171

#### PSI-BLAST analysis of ALMT/QUAC -related proteins<sup>a</sup>

<sup>a</sup> Procedures for the PSI-BLAST analysis of the ALMT/QUAC superfamily:

NCBI PSI-BLAST: Thirteen expansion sequences used to define the ALMT/QUAC superfamily were as following: AtALMT12/QUAC1 (NP\_193531.1), AtALMT1 (NP\_172319.1), AtALMT2 (NP\_172320.1), AtALMT3 (NP\_173278.1), AtALMT4 (NP\_173919.1), AtALMT5( NP\_564935.1), AtALMT6 (NP\_179338.1), AtALMT7 (NP\_001324626.1), AtALMT8 (NP\_187774.1), AtALMT9 (NP\_188473.1), AtALMT10 (NP\_001319836.1), AtALMT13 (NP\_199472.1) and AtALMT14 (NP\_199473.1). The AtALMT11 was not included, as it has only 152 amino acid in length, containing less than 6 TM and lacking the putative C-terminal helical domain.

The expansion sequences were run against the NCBI Protein Reference Sequence (refseq\_protein), in which the green plants (taxid:33090) and an E-value  $\leq 5 \times 10^{-3}$  were selected. Each sequence reached convergence at the 4<sup>th</sup> or 5<sup>th</sup> PSI-BLAST run, having generated ~2000 sequences. The resulted thirteen pools were merged to obtain a non-redundant set (2,061 sequences), to which some further purification was applied: i) removal of sequences not having a starting Met or containing non-amino acid X; ii) removal of sequences containing keyword "partial" in the sequence annotation; iii) removal of sequences <400 residues, unless 6 transmembrane segments confirmed by TMHMM 2.0. This resulted in a set of 1,843 sequences, which were subjected to the prediction of TM segments using THHMM 2.0. The sequences with fewer than 5 apparent TM helices were checked by *Gm*ALMT12/QUAC1 structure-based multiple sequence alignment with CLUSTAL\_W, and sequences obviously lacking some TM helices, or having deletion/insertion in the middle of TM or other helix in the C-terminal domain were removed. This reduced to a final pool of 1,756 sequences for family/subfamily classification.

#### ► Local PSI-BLAST:

The 1,756 identified sequences were used to establish a local PSI-BLAST database into which various sequences were expanded at different  $E_{cut}$  levels. We found that at  $E_{cut} = 10^{-160}$ , there were two distinct families plus several others. And subfamilies were divided at the level of  $E_{cut} = 10^{-180}$ .

<sup>b</sup>The other leftover sequences include 9 from *Physcomitrium patens*, 3 from *Selaginella moellendorffii*, and 1 from *Chlorella variabilis*.

<sup>c</sup>In QF2B, after removing redundant sequence >95% sequence identity, a set of 155 sequences was achieved. We further checked the sequences by GmALMT12/QUAC1 structure-based multiple sequence alignment with CLUSTAL\_W, and sequences obviously lacking some TM helix, or having deletion/insertion in the TM or helices in the C-terminal domain were removed. A final set of 137 sequences was achieved and used for sequence conservation analysis in Fig. 3C.

GMALMITZ/QUACT						
/ WOK						
EMD-32328						
Collection						
Titan Krios G2						
300						
Gatan K2 Summit						
20						
SerialEM						
1.04						
60						
32						
-1.2 ~ -2.2						
Reconstruction						
cryoSPARC 3.1 & Relion 3.0						
169,576						
C2						
189						
3.5						
ing & Refinement						
Coot						
PHENIX						
0.005						
0.692						
2.36						
Compesition						
820						
None						
lidation						
0						
9 5						
90.5						
2.01						

 Table S2

 Statistics of data colletion, image processing and model building

#### **REFERENCES AND NOTES**

- S. Meyer, J. Scholz-Starke, A. De Angeli, P. Kovermann, B. Burla, F. Gambale, E. Martinoia, Malate transport by the vacuolar *At*ALMT6 channel in guard cells is subject to multiple regulation. *Plant J.* 67, 247–257 (2011).
- S. Meyer, P. Mumm, D. Imes, A. Endler, B. Weder, K. A. S. Al-Rasheid, D. Geiger, I. Marten, E. Martinoia, R. Hedrich, *At*ALMT12 represents an R-type anion channel required for stomatal movement in *Arabidopsis* guard cells. *Plant J.* 63, 1054–1062 (2010).
- T. Sasaki, I. C. Mori, T. Furuichi, S. Munemasa, K. Toyooka, K. Matsuoka, Y. Murata, Y. Yamamoto, Closing plant stomata requires a homolog of an aluminum-activated malate transporter. *Plant Cell Physiol.* 51, 354–365 (2010).
- 4. A. De Angeli, J. Zhang, S. Meyer, E. Martinoia, *At*ALMT9 is a malate-activated vacuolar chloride channel required for stomatal opening in *Arabidopsis*. *Nat. Commun.* **4**, 1804 (2013).
- P. Kovermann, S. Meyer, S. Hörtensteiner, C. Picco, J. Scholz-Starke, S. Ravera, Y. Lee, E. Martinoia, The *Arabidopsis* vacuolar malate channel is a member of the ALMT family. *Plant J.* 52, 1169–1180 (2007).
- 6. T. Gutermuth, S. Herbell, R. Lassig, M. Brosché, T. Romeis, J. A. Feijó, R. Hedrich, K. R. Konrad, Tip-localized Ca<sup>2+</sup>-permeable channels control pollen tube growth via kinase-dependent R- and S-type anion channel regulation. *New Phytol.* 218, 1089–1105 (2018).
- 7. S. Herbell, T. Gutermuth, K. R. Konrad, An interconnection between tip-focused Ca<sup>2+</sup> and anion homeostasis controls pollen tube growth. *Plant Signal. Behav.* **13**, e1529521 (2018).
- T. Sasaki, Y. Yamamoto, B. Ezaki, M. Katsuhara, S. J. Ahn, P. R. Ryan, E. Delhaize, H. Matsumoto, A wheat gene encoding an aluminum-activated malate transporter. *Plant J.* 37, 645–653 (2004).
- 9. O. A. Hoekenga, L. G. Maron, M. A. Piñeros, G. M. A. Cançado, J. Shaff, Y. Kobayashi, P. R. Ryan, B. Dong, E. Delhaize, T. Sasaki, H. Matsumoto, Y. Yamamoto, H. Koyama, L. V. Kochian, *AtALMT1*, which encodes a malate transporter, is identified as one of several genes critical for aluminum tolerance in *Arabidopsis. Proc. Natl. Acad. Sci. U.S.A.* 103, 9738–9743 (2006).
- A. Ligaba, L. Maron, J. Shaff, L. Kochian, M. Piñeros, Maize ZmALMT2 is a root anion transporter that mediates constitutive root malate efflux. *Plant Cell Environ.* 35, 1185–1200 (2012).

- A. De Angeli, U. Baetz, R. Francisco, J. Zhang, M. M. Chaves, A. Regalado, The vacuolar channel VvALMT9 mediates malate and tartrate accumulation in berries of Vitis vinifera. *Planta* 238, 283–291 (2013).
- 12. T. Rudrappa, K. J. Czymmek, P. W. Paré, H. P. Bais, Root-secreted malic acid recruits beneficial soil bacteria. *Plant Physiol.* **148**, 1547–1556 (2008).
- S. A. Ramesh, S. D. Tyerman, B. Xu, J. Bose, S. Kaur, V. Conn, P. Domingos, S. Ullah, S. Wege, S. Shabala, J. A. Feijó, P. R. Ryan, M. Gillham, GABA signalling modulates plant growth by directly regulating the activity of plant-specific anion transporters. *Nat. Commun.* 6, 7879 (2015).
- 14. H. Zhang, Y. Li, J. K. Zhu, Developing naturally stress-resistant crops for a sustainable agriculture. *Nat. Plants.* **4**, 989–996 (2018).
- 15. B. U. Keller, R. Hedrich, K. Raschke, Voltage-dependent anion channels in the plasma membrane of guard cells. *Nature* **341**, 450–453 (1989).
- 16. P. Dietrich, R. Hedrich, Interconversion of fast and slow gating modes of GCAC1, a Guard Cell Anion Channel. *Planta* **195**, 301–304 (1994).
- 17. J. I. Schroeder, B. U. Keller, Two types of anion channel currents in guard cells with distinct voltage regulation. *Proc. Natl. Acad. Sci. U.S.A.* **89**, 5025–5029 (1992).
- P. Mumm, D. Imes, E. Martinoia, K. A. S. Al-Rasheid, D. Geiger, I. Marten, R. Hedrich, C-terminus-mediated voltage gating of *Arabidopsis* guard cell anion channel QUAC1. *Mol. Plant* 6, 1550–1563 (2013).
- R. Hedrich, I. Marten, G. Lohse, P. Dietrich, H. Winter, G. Lohaus, H.-W. Heldt, Malatesensitive anion channels enable guard cells to sense changes in the ambient CO<sub>2</sub> concentration. *Plant J.* 6, 741–748 (1994).
- 20. K. Raschke, Alternation of the slow with the quick anion conductance in whole guard cells effected by external malate. *Planta* **217**, 651–657 (2003).
- 21. D. Imes, P. Mumm, J. Böhm, K. A. S. Al-Rasheid, I. Marten, D. Geiger, R. Hedrich, Open stomata 1 (OST1) kinase controls R-type anion channel QUAC1 in *Arabidopsis* guard cells. *Plant J.* 74, 372–382 (2013).
- 22. K. Luu, N. Rajagopalan, J. C. H. Ching, M. C. Loewen, M. E. Loewen, The malate-activated ALMT12 anion channel in the grass *Brachypodium distachyon* is co-activated by Ca<sup>2+</sup>/calmodulin. *J. Biol. Chem.* **294**, 6142–6156 (2019).
- 23. L. Holm, DALI and the persistence of protein shape. Protein Sci. 29, 128–140 (2020).

- 24. T. Furuichi, T. Sasaki, Y. Tsuchiya, P. R. Ryan, E. Delhaize, Y. Yamamoto, An extracellular hydrophilic carboxy-terminal domain regulates the activity of *Ta*ALMT1, the aluminum-activated malate transport protein of wheat. *Plant J.* **64**, 47–55 (2010).
- R. Dutzler, E. B. Campbell, M. Cadene, B. T. Chait, R. MacKinnon, X-ray structure of a ClC chloride channel at 3.0 Å reveals the molecular basis of anion selectivity. *Nature* 415, 287–294 (2002).
- 26. O. S. Smart, J. G. Neduvelil, X. Wang, B. A. Wallace, M. S. P. Sansom, HOLE: A program for the analysis of the pore dimensions of ion channel structural models. *J. Mol. Graph.* **14**, 354–360 (1996).
- 27. W. Peng, W. Wu, J. Peng, J. Li, Y. Lin, Y. Wang, J. Tian, L. Sun, C. Liang, H. Liao, Characterization of the soybean *Gm*ALMT family genes and the function of *Gm*ALMT5 in response to phosphate starvation. *J. Integr. Plant Biol.* **60**, 216–231 (2018).
- J. Jumper, R. Evans, A. Pritzel, T. Green, M. Figurnov, O. Ronneberger, K. Tunyasuvunakool, R. Bates, A. Žídek, A. Potapenko, A. Bridgland, C. Meyer, S. A. A. Kohl, A. J. Ballard, A. Cowie, B. Romera-Paredes, S. Nikolov, R. Jain, J. Adler, T. Back, S. Petersen, D. Reiman, E. Clancy, M. Zielinski, M. Steinegger, M. Pacholska, T. Berghammer, S. Bodenstein, D. Silver, O. Vinyals, A. W. Senior, K. Kavukcuoglu, P. Kohli, D. Hassabis, Highly accurate protein structure prediction with AlphaFold. *Nature* 596, 583–589 (2021).
- 29. F. Malcheska, A. Ahmad, S. Batool, H. M. Müller, J. Ludwig-Müller, J. Kreuzwieser, D. Randewig, R. Hänsch, R. R. Mendel, R. Hell, M. Wirtz, D. Geiger, P. Ache, R. Hedrich, C. Herschbach, H. Rennenberg, Drought-enhanced xylem sap sulfate closes stomata by affecting ALMT12 and guard cell ABA synthesis. *Plant Physiol.* **174**, 798–814 (2017).
- 30. O. Pantoja, Recent advances in the physiology of ion channels in plants. *Annu. Rev. Plant Biol.* **72**, 463–495 (2021).
- 31. J. I. Schroeder, S. Hagiwara, Cytosolic calcium regulates ion channels in the plasma membrane of *Vicia faba* guard cells. *Nature* **338**, 427–430 (1989).
- 32. R. Hedrich, H. Busch, K. Raschke, Ca<sup>2+</sup> and nucleotide dependent regulation of voltage dependent anion channels in the plasma membrane of guard cells. *EMBO J.* **9**, 3889–3892 (1990).
- 33. T. Vahisalu, H. Kollist, Y. F. Wang, N. Nishimura, W. Y. Chan, G. Valerio, A. Lamminmäki, M. Brosché, H. Moldau, R. Desikan, J. I. Schroeder, J. Kangasjärvi, SLAC1 is required for plant guard cell S-type anion channel function in stomatal signalling. *Nature* 452, 487–491 (2008).

- 34. J. Negi, O. Matsuda, T. Nagasawa, Y. Oba, H. Takahashi, M. Kawai-Yamada, H. Uchimiya, M. Hashimoto, K. Iba, CO<sub>2</sub> regulator SLAC1 and its homologues are essential for anion homeostasis in plant cells. *Nature* 452, 483–486 (2008).
- 35. S. Saji, S. Bathula, A. Kubo, M. Tamaoki, M. Kanna, M. Aono, N. Nakajima, T. Nakaji, T. Takeda, M. Asayama, H. Saji, Disruption of a gene encoding C4-dicarboxylate transporterlike protein increases ozone sensitivity through deregulation of the stomatal response in *Arabidopsis thaliana. Plant Cell Physiol.* **49**, 2–10 (2008).
- 36. D. Geiger, S. Scherzer, P. Mumm, A. Stange, I. Marten, H. Bauer, P. Ache, S. Matschi, A. Liese, K. A. S. S. Al-Rasheid, T. Romeis, R. Hedrich, Activity of guard cell anion channel SLAC1 is controlled by drought-stress signaling kinase-phosphatase pair. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21425–21430 (2009).
- S. C. Lee, W. Lan, B. B. Buchanan, S. Luan, A protein kinase-phosphatase pair interacts with an ion channel to regulate ABA signaling in plant guard cells. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21419–21424 (2009).
- 38. B. Brandt, D. E. Brodsky, S. Xue, J. Negi, K. Iba, J. Kangasjärvi, M. Ghassemian, A. B. Stephan, H. Hu, J. I. Schroeder, Reconstitution of abscisic acid activation of SLAC1 anion channel by CPK6 and OST1 kinases and branched ABI1 PP2C phosphatase action. *Proc. Natl. Acad. Sci. U.S.A.* 109, 10593–10598 (2012).
- 39. T. Maierhofer, M. Diekmann, J. N. Offenborn, C. Lind, H. Bauer, K. Hashimoto, K. A. S. Al-Rasheid, S. Luan, J. Kudla, D. Geiger, R. Hedrich, Site- and kinase-specific phosphorylation-mediated activation of SLAC1, a guard cell anion channel stimulated by abscisic acid. *Sci. Signal.* 7, ra86 (2014).
- Y.-N. Deng, H. Kashtoh, Q. Wang, G.-X. Zhen, Q. Li, L.-H. Tang, H.-L. Gao, C.-R. Zhang, L. Qin, M. Su, F. Li, X.-H. Huang, Y.-C. Wang, Q. Xie, O. B. Clarke, W. A. Hendrickson, Y.-H. Chen, Structure and activity of SLAC1 channels for stomatal signaling in leaves. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2015151118 (2021).
- 41. Y.-H. Chen, L. Hu, M. Punta, R. Bruni, B. Hillerich, B. Kloss, B. Rost, J. Love, S. A. Siegelbaum, W. A. Hendrickson, Homologue structure of the SLAC1 anion channel for closing stomata in leaves. *Nature* 467, 1074–1080 (2010).
- 42. S. F. Altschul, T. L. Madden, A. A. Schäffer, J. Zhang, Z. Zhang, W. Miller, D. J. Lipman, Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res.* 25, 3389–3402 (1997).
- 43. X.-H. Wang, M. Su, F. Gao, W. Xie, Y. Zeng, D. Li, X. Liu, H. Zhao, L. Qin, F. Li, Q. Liu, O. B. Clarke, S. M. Lam, G. Shui, W. A. Hendrickson, Y.-H. Chen, Structural basis for

activity of TRIC counter-ion channels in calcium release. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 4238–4243 (2019).

- 44. D. N. Mastronarde, Automated electron microscope tomography using robust prediction of specimen movements. *J. Struct. Biol.* **152**, 36–51 (2005).
- 45. C. Wu, X. Huang, J. Cheng, D. Zhu, X. Zhang, High-quality, high-throughput cryo-electron microscopy data collection via beam tilt and astigmatism-free beam-image shift. *J. Struct. Biol.* **208**, 107396 (2019).
- 46. J. Zivanov, T. Nakane, B. O. Forsberg, D. Kimanius, W. J. H. Hagen, E. Lindahl, S. H. W. Scheres, New tools for automated high-resolution cryo-EM structure determination in RELION-3. *eLife* 7, e42166 (2018).
- 47. A. Punjani, J. L. Rubinstein, D. J. Fleet, M. A. Brubaker, cryoSPARC: Algorithms for rapid unsupervised cryo-EM structure determination. *Nat. Methods* **14**, 290–296 (2017).
- 48. D. Asarnow, E. Palovcak, Y. F. Cheng, UCSF pyem v0.5 (2019); doi:10.5281/zenodo.3576630.
- 49. E. F. Pettersen, T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E. C. Meng, T. E. Ferrin, UCSF Chimera—A visualization system for exploratory research and analysis. *J. Comput. Chem.* 25, 1605–1612 (2004).
- 50. S. Q. Zheng, E. Palovcak, J.-P. Armache, K. A. Verba, Y. Cheng, D. A. Agard, MotionCor2: Anisotropic correction of beam-induced motion for improved cryo-electron microscopy. *Nat. Methods* 14, 331–332 (2017).
- 51. K. Zhang, Gctf: Real-time CTF determination and correction. *J. Struct. Biol.* **193**, 1–12 (2016).
- 52. K. Zhang, F. Sun, Gautomatch: An efficient and convenient gpu-based automatic particle selection program (2011); https://www2.mrc-lmb.cam.ac.uk/research/locally-developed-software/zhang-software/.
- D. Liebschner, P. V Afonine, M. L. Baker, G. Bunkóczi, V. B. Chen, T. I. Croll, B. Hintze, L.-W. Hung, S. Jain, A. J. McCoy, N. W. Moriarty, R. D. Oeffner, B. K. Poon, M. G. Prisant, R. J. Read, J. S. Richardson, D. C. Richardson, M. D. Sammito, O. V Sobolev, D. H. Stockwell, T. C. Terwilliger, A. G. Urzhumtsev, L. L. Videau, C. J. Williams, P. D. Adams, Macromolecular structure determination using X-rays, neutrons and electrons: Recent developments in PHENIX. *Acta Crystallogr. D. Struct. Biol.* **75**, 861–877 (2019).
- 54. A. Kucukelbir, F. J. Sigworth, H. D. Tagare, Quantifying the local resolution of cryo-EM density maps. *Nat. Methods* **11**, 63–65 (2014).

- 55. P. Emsley, B. Lohkamp, W. G. Scott, K. Cowtan, Features and development of Coot. *Acta Crystallogr. D Biol. Crystallogr.* **66**, 486–501 (2010).
- 56. F. Gabler, S.-Z. Nam, S. Till, M. Mirdita, M. Steinegger, J. Söding, A. N. Lupas, V. Alva, Protein sequence analysis using the MPI bioinformatics toolkit. *Curr. Protoc. Bioinformatics* 72, e108 (2020).
- 57. B. A. Barad, N. Echols, R. Y. R. Wang, Y. Cheng, F. Dimaio, P. D. Adams, J. S. Fraser, EMRinger: Side chain-directed model and map validation for 3D cryo-electron microscopy. *Nat. Methods* 12, 943–946 (2015).
- 58. E. F. Pettersen, T. D. Goddard, C. C. Huang, E. C. Meng, G. S. Couch, T. I. Croll, J. H. Morris, T. E. Ferrin, UCSF ChimeraX: Structure visualization for researchers, educators, and developers. *Protein Sci.* 30, 70–82 (2021).
- 59. G. Bi, M. Su, N. Li, Y. Liang, S. Dang, J. Xu, M. Hu, J. Wang, M. Zou, Y. Deng, Q. Li, S. Huang, J. Li, J. Chai, K. He, Y.-H. Chen, J.-M. Zhou, The ZAR1 resistosome is a calcium-permeable channel triggering plant immune signaling. *Cell* **184**, 3528–3541.e12 (2021).
- I. Dreyer, J. L. Gomez-Porras, D. M. Riaño-Pachón, R. Hedrich, D. Geiger, Molecular evolution of slow and quick anion channels (SLACs and QUACs/ALMTs). *Front. Plant Sci.* 3, 263 (2012).