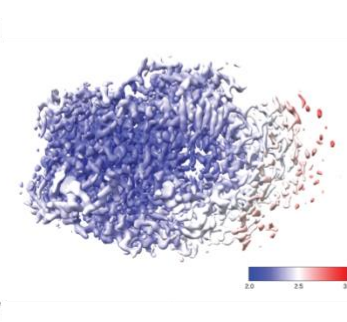
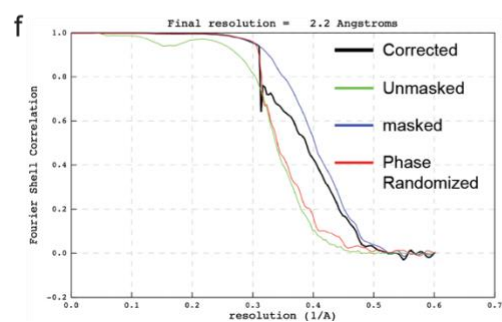
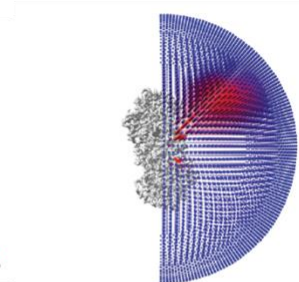
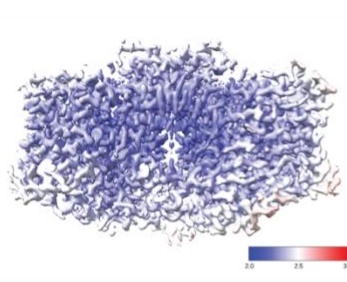
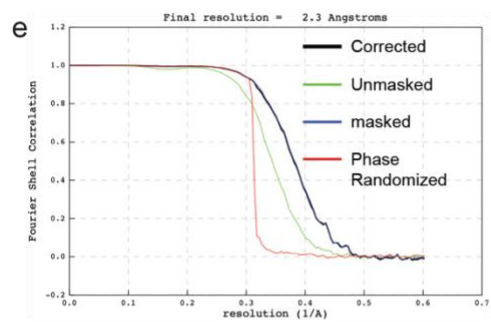
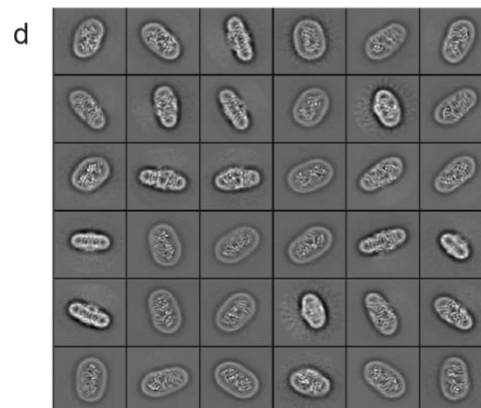
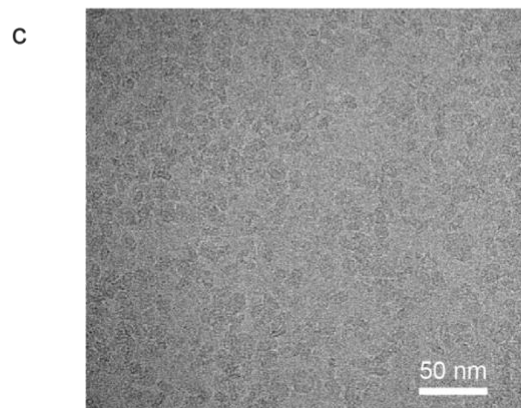
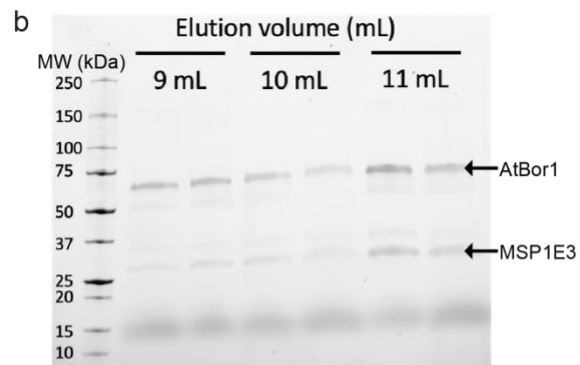
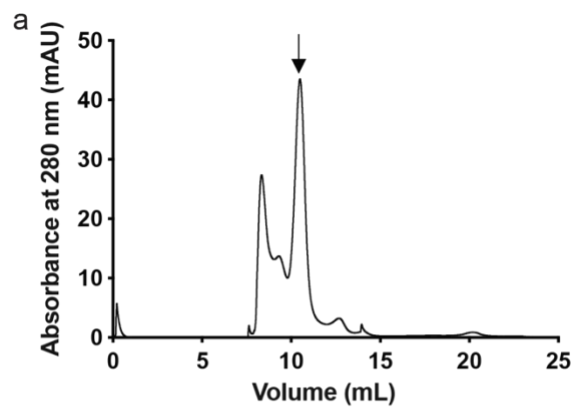
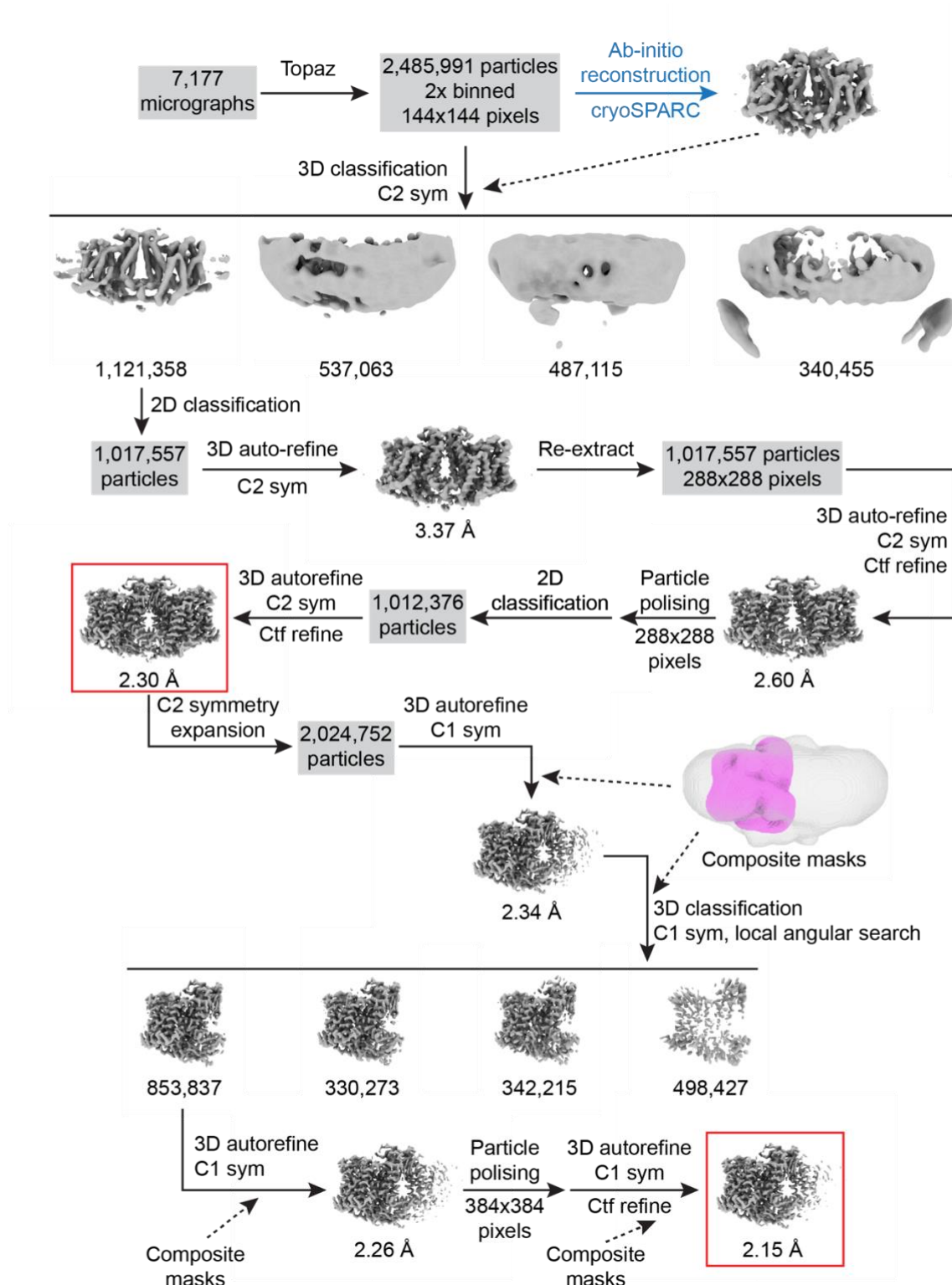
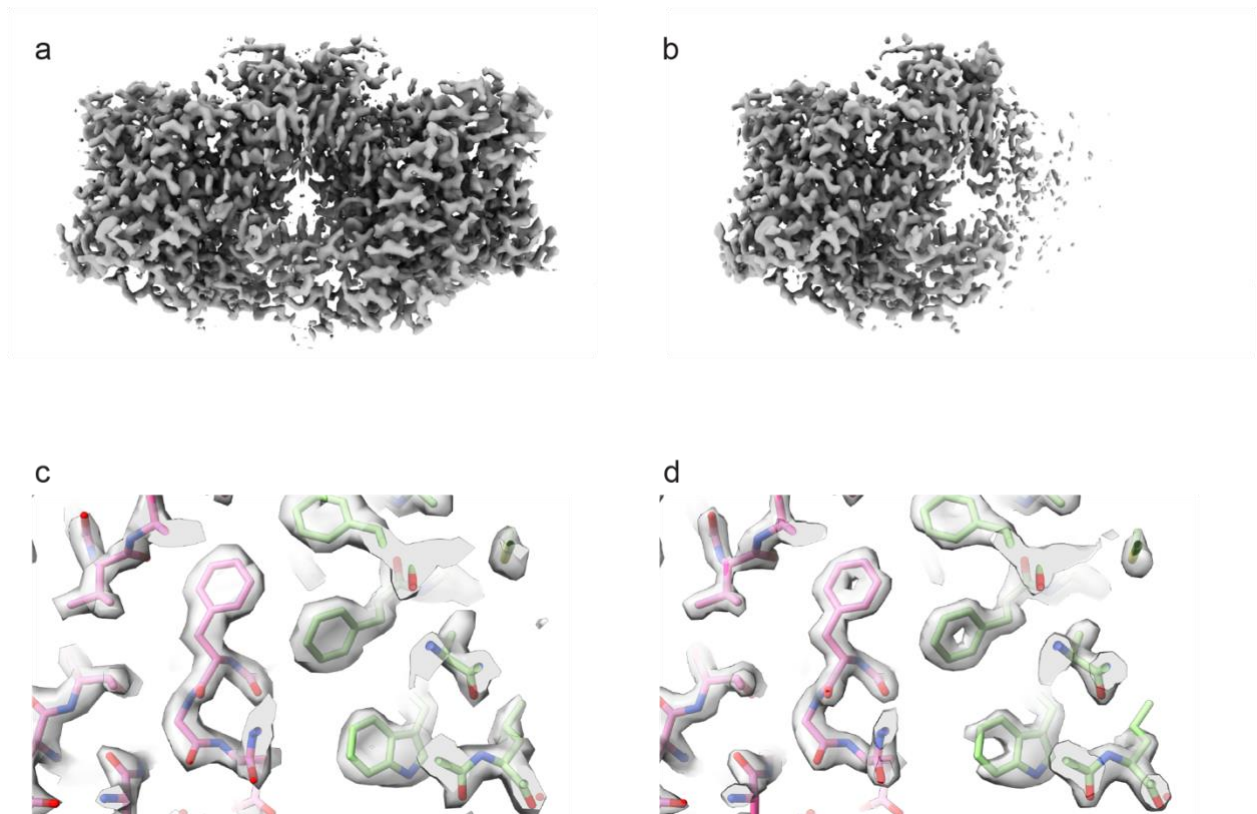


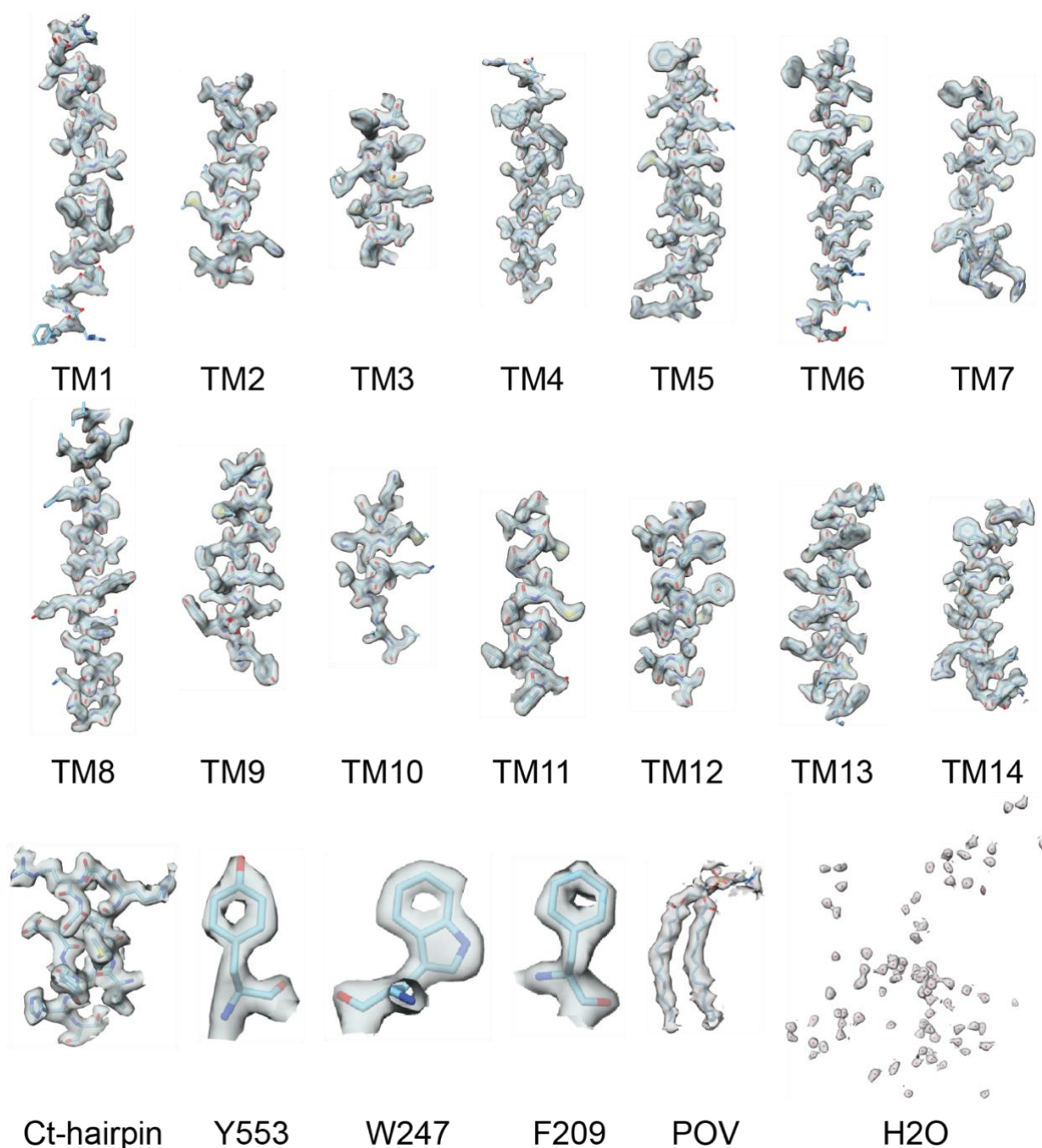
Supplementary Fig. 1 **Cryo-EM analysis of wild-type AtBor1 in lipid nanodiscs.** **a** Chromatographs of wild-type AtBOR1 in lipid nanodiscs. The arrowed peak was collected for cryo-EM study. **b** The SDS-PAGE of the peak fractions. Monomeric AtBor1 protein was observed clearly on the SDS-PAGE between 75 to 50 KDa and the MSP1E3 protein was observed on the SDS-PAGE at ~25KDa. **c** A representative electron micrograph of wild-type AtBor1 in lipid nanodiscs. **d** Representative two-dimensional class averages of the electron micrographs for wild-type AtBor1 in lipid nanodiscs. **e, f** Fourier Shell Correlation (FSC) curves (left), local resolution maps (middle), and particle orientation plots (right) of the 3D reconstruction of inward-autoinhibition dimer (**e**) and inward-autoinhibition protomer (**f**).



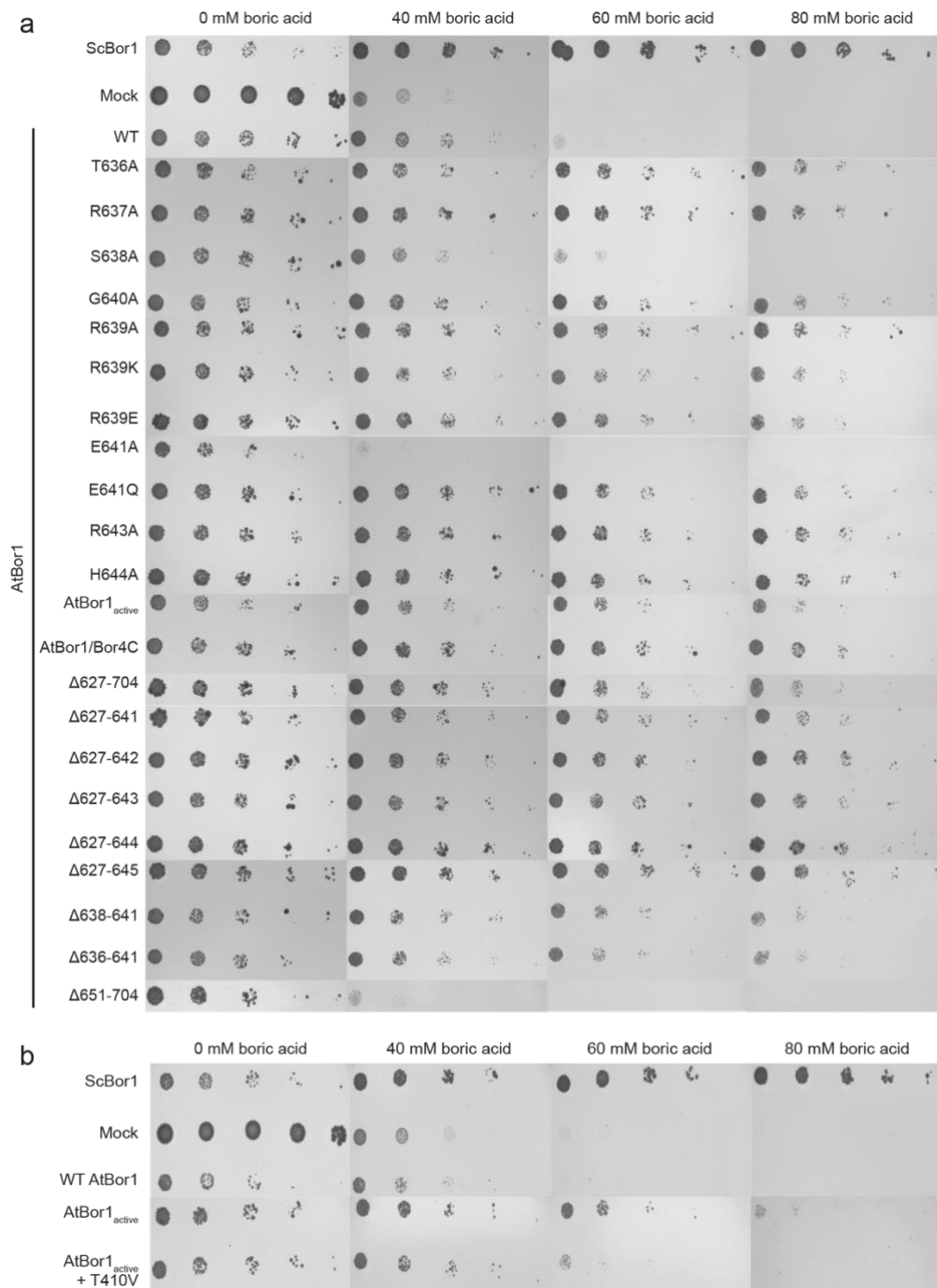
Supplementary Fig. 2 **Cryo-EM data processing workflow for wild-type AtBor1**. The 3D reconstructions are shown without B-factor sharpening. The reconstructions in red boxes are used for atomic model building.



Supplementary Fig. 3 **Comparison of the cryo-EM density maps of wild-type AtBor1 dimer and protomer.** **a, b** B-factor sharpened map of wild-type AtBor1 dimer (**a**) or protomer (**b**). **c, d** Close-up view of the same region in the density map of wild-type AtBor1 dimer (**c**) or protomer (**d**). The holes in some aromatic residues are clearly resolved in (**d**), suggesting an improved resolution.



Supplementary Fig. 4 **Cryo-EM maps for representative regions of wild-type AtBor1.** The cryo-EM maps for the TM1-TM14 helices, the Ct-hairpin region, the selected tyrosine (Y), tryptophan (W) and phenylalanine (F) residues, the POPC (POV) lipid and the water molecules of wild-type AtBor1.



Supplementary Fig. 5 **Complementation assay identifies the Ct hairpin inhibited the transporter activity of AtBor1.** **a, b** *S. cerevisiae* strain Y01169 (*Scbor1Δ*) were transformed with the Ct domain truncated or mutant AtBor1 cDNAs. Overnight liquid cultures of the transformants were adjust to an OD_{600nm} of 1.0 and serially diluted by 5 times, spotted on solid media containing 0 mM (control), 40 mM, 60 mM and 80 mM boric acid and incubated for 6 days. Galactose was used as carbon source to induce the expression of the AtBor1s.

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AtBor3 Q93Z13	-----	0
AtBor4 Q9XI23	-----	0
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AtBor2 Q9M1P7	-----	0
AtBor3 Q93Z13	-----	0
AtBor4 Q9XI23	-----	0
AtBor5 Q9SSG5	-----	0
AtBor6 Q3E954	-----	0
AtBor7 Q9SUU1	-----	0
OsBor1 Q2QNH0	-----	0
OsBor2 Q1ZYR7	-----	0
OsBor3 Q7X9F3	-----	0
OsBor4 Q1ZYR6	-----	0
TaBor1.1 A0A060PT44	-----	0
TaBor1.2 A0A060PVQ3	-----	0
TaBor1.3 W5FNG8	-----	0
ScBor1 P53838	-----MSNESTLVTVSRG--CTASDECAQA-L-----ERTNDEL	31
SLC4A11 Q8NBS3	ALVHQROLTMTVSHGFPVAPRTKERSTVSLPA-----	311
SLC4A1 AE1 P02730	SLEGFLDCSLVLPPTDAPSEQALLSLVPVQRELLRR-----RYQSSPAKPDSSFYKGL-----	362
SLC4A2 AE2 P04920	AINAFLDCSVLPPEVQGEELLRSVAHFQRMKKK-----REEQGRLLP---TGAGLEPKS-----A	655
SLC4A3 AE3 P48751	AISEFLDGSIVIPPEVEGRDLLRSVAAFQRELLRR-----RREREQTKVEMTTRGGYTA-----P	655
SLC4A4 NBCe1 Q9Y6R1	GIDEFLDEVIVLPPGEWDPAIRIEPPKSLPSSDKRNMYSGGENVQMGDTPHDG-GHGGGGH-----	430
SLC4A5 NBCe2 Q9BY07	GIDEFLDEVIVLPPGEWDPNIRIEPPKKVPSADKRKSVFSLAELGQMNGSVGGGG-GAPGGGNGGGGGGGGAGSGGA	467
SLC4A7 NBCn1 Q9Y6M7	GIDEFLDQVTVLPPGEWDPSIRIEPPKSVPSQEKRRIPV-----FHNGSTPTLG-ETPKEA-----	567
SLC4A8 NDCBE Q2Y0W8	GIDEFLDQVTVLPPGEWDPSIRIEPPKNVPSQEKRMMPG-----VPNGNVCHIE-QEP--H-----	437
SLC4A9 AE4 Q96Q91	ALDAFLDEVTVLPPGRWDPTARIPPPKCLPSQHKLLPSQQREIRG---PAVPRLT-----	362
SLC4A10 NBCn2 Q6U841	GIDEFLDQVTVLPPGEWDPSIRIEPPKNVPSQEKRRIPA-----VPNGTAAHGE-AEP--H-----	468

	H1a	H1b	1
AtBor1 Q8VYR7	-----MEETFPVPEGIKNDLGRLMCYKQDWTGGFKAGF----		RILAPTTYIFFASAIPVISFGE 56
AtBor2 Q9M1P7	-----MEETFPVPEGIKNDLGRLMCYKQDWTGGFKAGF----		RILAPTTYIFFASAIPVISFGE 56
AtBor3 Q93Z13	-----MDEAESFVPPQGIKQDVGRNLNCKYQDWISGLRAGF----		RILAPTTYIFFASAIPVISFGE 58
AtBor4 Q9XI23	-----MEEERVSSKRLFGIVADLAGRALCYKQDWVAGLRSGF----		GILAPTTYIFFASALPVIAFGE 61
AtBor5 Q9SSG5	-----MEEERVSSKRLFGIVADLAGRALCYKQDWVAGLRSGF----		GILAPTTYIFFASALPVIAFGE 61
AtBor6 Q3E954	-----MKSEGESGPFQGIIRDIEGRKCYKQDWIRGIKTGI----		RILAPTCYIFFASSLPVIAFGE 58
AtBor7 Q9SUU1	-----MGVVKFPFGGIIIDFNRRKCYKQDWLAENSGV----		RILAPTLYIFIASALPVIAFGE 56
OsBor1 Q2QNH0	-----MEESFVPLNGIKNDLGRLCYKQDWTGGFRAGI----		RILAPTTYIFFASAIPVISFGE 56
OsBor2 Q1ZYR7	-----MDLLRTPFGGVVADIEGRVANYKHVDVAGFRSGF----		RILAPTMYYIFFASALPVIAFGE 56
OsBor3 Q7X9F3	-----MEESFVPLNGIKNDLGRLCYKQDWTGGFRAGI----		RILAPTTYIFFASAIPVISFGE 56
OsBor4 Q1ZYR6	-----MTGTVKAPFEGVNDVGRGLSCYKQDWIDGFRGTGF----		RILAPTLYYIFFASALPVIAFGE 57
TaBor1.1 A0A060PT44	-----MEESFVPLNGIKNDVGRGLACYKQDWTGGFSAGI----		RILAPTTYIFFASAIPVISFGE 56
TaBor1.2 A0A060PVQ3	-----MEESFVPLNGIKNDVGRGLACYKQDWTGGFSAGI----		RILAPTTYIFFASAIPVISFGE 56
TaBor1.3 W5FNG8	-----MEESFVPLNGIKNDVGRGLACYKQDWTGGFSAGI----		RILAPTTYIFFASAIPVISFGE 56
ScBor1 P53838	DRESSVSERSDEESHEKLSRRFPTLGIIGIWLDDRIPIYKSDWVDAENY----		RVIPSIVDTYFNNLLPAIAFAQ 105
SLC4A11 Q8NBS3	-----HRHPEPPKCKDFVPPFGGIIREDIARREFPLDFTDGIIGKNKAVGYITTTTLTYIFFACLLPTIAFGS 379		
SLC4A1/AE1 P02730	-----DLNGGDDPDLQQTQGLFGLVLRDRIIRRYPYLLSDITDAFSP----		QVLAAVIFYFAALSPAITFGG 425
SLC4A2/AE2 P04920	QDKALLQMV-EAAGAAEDDPLRRTGRFPGGLLRDVRRRYPHYLSDFRDALDP----		QCLAAVIFYFAALSPAITFGG 728
SLC4A3/AE3 P48751	GKELSLELGG-SEATPEDDPLRTGSVFGGLVRDVRRRYPHYPSDLRDALHS----		QCVAAVIFYFAALSPAITFGG 728
SLC4A4/NBCe1 Q9Y6R1	-----GDCEELQRTGRFCGGLIKDKRKAPFFASDFYDALNI----		QALSAILFYLYLATVTNAITFGG 489
SLC4A5/NBCe2 Q9BY07	GGTSSGDDGEMPAMHEIGEELIWTGRFFGGLCLDKRKLPWFPSDFYDGFHI----		QSIISAILFYLYLCITNAITFGG 541
SLC4A7/NBCn1 Q9Y6M7	-----AHHAGPELQRTGRFGLGLIDIKRKAPFFLSDFKDALSL----		QCLASILFLYCACMSPVITFGG 628
SLC4A8/NDCBE Q2Y0W8	-----GGHSGPELQRTGRFGLGLVLDIKRKAPWYWSDFYDALSL----		QCLASFLFLYCACMSPVITFGG 498
SLC4A9/AE4 Q96Q91	-----SAEDRHRHGPHASPELQRTGRFGLGLIDVRRKVPWYPSDFLDALHL----		QCFSAVLYLYLATVTNAITFGG 432
SLC4A10/NBCn2 Q6U841	-----GGHSGPELQRTGRFGLGLIDIKRKAPYFWSDFYDALSL----		QCLASFLFLYCACMSPVITFGG 529

	1	2	3	4
AtBor1 Q8VYR7	QLERSTDGVLTAVQTLASTAICGMIHSIIIGGOLLILGVAEPTVIMYTFMFNFAKAPLGRDLFLAWSGWVCVWTALML 136			
AtBor2 Q9M1P7	QLERSTDGVLTAVQTLASTAICGMIHSIIIGGOLLILGVAEPTVIMYTFMFNFAKAPLGRDLFLAWSGWVCVWTALML 136			
AtBor3 Q93Z13	QLERSTDGVLTAVQTLASTAICGMIHSIIIGGOLLILGVAEPTVIMYTFMFNFAKAPLGRDLFLAWSGWVCVWTALML 138			
AtBor4 Q9XI23	QLSNDTEGALSTVETLASTALCGVIHSILGGOLLILGVAEPTVIMYVLYNFAIGRPGLGQLYLAWAANVCVWTALLL 141			
AtBor5 Q9SSG5	QLSNDTERSLSTVETLASTALCGVIHSILGGOLLILGVAEPTVIMYVLYNFAIGRPGLGQLYLAWAANVCVWTALLL 141			
AtBor6 Q3E954	QLSKHTGGALSAVETLASTSICGIIHAIFFGGOLLIVGVAEPTIIMYTYLYSFCISRPDIGRELYLAWVANVCVWTSVLL 138			
AtBor7 Q9SUU1	QLSRETDRSLGIAESLASTALCGIHSIVFGGOLLIVGVAEPTIIMYTYLYSFSKSPGLGQLYLAWAGWVCVWTAVAL 136			
OsBor1 Q2QNH0	QLERSTDGVLTAVQTLASTALCGIHSILGGOLLILGVAEPTVIMYTFMFNFAKAPLGRDLFLAWSGWVCVWTALML 136			
OsBor2 Q1ZYR7	QLSRETNGILTVEETLASTALCGIHSILGGOLLIVGVAEPTIIMYTYLYNFAKAPLGRDLFLAWSGWVCVWTALML 136			
OsBor3 Q7X9F3	QLERSTDGVLTAVQTLASTALCGIHSILGGOLLILGVAEPTVIMYTFMFNFAKAPLGRDLFLAWSGWVCVWTALML 136			
OsBor4 Q1ZYR6	QLSNDTDGALTVEETLASTAICGIIHSILGGOLLIVGVAEPTIIMYTYLYNFAKAPLGRDLFLAWSGWVCVWTALML 137			
TaBor1.1 A0A060PT44	QLERSTDGVLTAVQTLASTALCGIHSIVGGOLLILGVAEPTVIMYTFMFNFAKAPLGRDLFLAWSGWVCVWTALML 136			
TaBor1.2 A0A060PVQ3	QLERSTDGVLTAVQTLASTALCGIHSIVGGOLLILGVAEPTVIMYTFMFNFAKAPLGRDLFLAWSGWVCVWTALML 136			
TaBor1.3 W5FNG8	QLERSTDGVLTAVQTLASTALCGIHSIVGGOLLILGVAEPTVIMYTFMFNFAKAPLGRDLFLAWSGWVCVWTALML 136			
ScBor1 P53838	DMFDRDINSYGVNEVLLSSAMAGIVFGLGGOLPCLIVGVTPGISIFNYTVYEIINPLN----TSYFGFMFICMWSMIFH 181			
SLC4A11 Q8NBS3	LNDETDGAIQVQKTIAGQSIGGLLYALFSGGPLVILLTAPLALYIQVIRVICDDYD----LDFNSFYANTGLWNSFFL 455			
SLC4A1/AE1 P02730	LLGEKTRNQMGVSELLISTAVQGIILFALLGAQPLLIVGFGSGPLLVEEAFSFCETNG----LEYIVGRVWIGFWLILLV 501			
SLC4A2/AE2 P04920	LLGEKTDQLIGVSELIMSTALQGVVFCLLGAQPLLIVGFGSGPLLVEEAFSFCSSNH----LEYLVGRVWIGFWLVLFLA 804			
SLC4A3/AE3 P48751	LLGEKTEGLMGVSELIVSTAVGLVFLSLGAQPLLIVGFGSGPLLVEEAFSFCRAQD----LEYLTGRVWVGLWLVVFLV 804			
SLC4A4/NBCe1 Q9Y6R1	LLGDATDNMQGVLESFLGTAVSGAIFCLFAGQPLTILSSTGPVLVFERLLFNEKNN----FDYLEFRLWIGLWSAFLC 565			
SLC4A5/NBCe2 Q9BY07	LLGDATDNYQGVMSFLGTAMAGSLFCLFSGQPLTILSSTGPVLVFERLLFNEKNN----LDYMEFRLWIGLWSAFLC 617			
SLC4A7/NBCn1 Q9Y6M7	LLGEATEGRISAIESLFGASLTGIAYSLFAGQPLTILSSTGPVLVFERLLFNEKNN----LSYLSLRTSIGLWTSFCL 704			
SLC4A8/NDCBE Q2Y0W8	LLGEATEGRISAIESLFGASMTGIAYSLFAGQPLTILSSTGPVLVFERLLFNEKNN----LSYLSLRACIGLWTSFCL 574			
SLC4A9/AE4 Q96Q91	LLGDATDGAQGVLESFLGTAVAGAAFLMAGQPLTILSSTGPVLVFERLLFNEKNN----LDYLPFRLWIGLWSAFLC 508			
SLC4A10/NBCn2 Q6U841	LLGEATEGRISAIESLFGASMTGIAYSLFAGQPLTILSSTGPVLVFERLLFNEKNN----LSYLSLRASIGLWTSFCL 605			

4 H2 5 H3 6

R153 E157 F159 I163 F167

AtBor1|Q8VYR7 FVLAICGACSIINRFRVAGELFGLLIAMLFMOQAIGLVDEFRIPERENQKLKEFL-----PSW-- 196
 AtBor2|Q9M1P7 FVLAICGACSFINRFRVAGELFGLLIAMLFMOQAIGLVDEFAPAREDLKLVFEL-----PSW-- 196
 AtBor3|Q93Z13 FLLAVLGACTFINRFRVAGELFGLLIAMLFMOQAIGLVDEFVPGRTNPPSAEFQ-----PAW-- 198
 AtBor4|Q9XI23 FVMAILNTADIINRFRVAGELFGMLISVLFIQQAIGKGVSEFGMPKDEDSKLEKYK-----FEW-- 201
 AtBor5|Q9SSG5 FLMAIFNMAYIINRFRVAGELFGMLIAVLFLQQTIKGMVSEFRIPKGEDSKLEKYQ-----FEW-- 201
 AtBor6|Q3E954 ILLSIFNAGTIITRFRVAGELFGMLIAVLFLQEAIGLISEFHAPFIKMQETGKSH-----FLL-- 198
 AtBor7|Q9SUU1 MLLAMLNACNIISRFRVAGELFGMLITVLFIQEAIGLVDEFVPGRTNPPSAEFQ-----FQW-- 196
 OsBor1|Q2QNH0 FLLAILGACSIINRFRVAGELFGLLIAMLFMOQAIGLVDEFRIPERENRKALEFV-----SSW-- 196
 OsBor2|Q1ZYR7 FLLAMFNASNVISRFRVAGELFGMLITVLFQQAIGKGVSEFGMPKDEDSKLEKYK-----FQW-- 196
 OsBor3|Q7X9F3 FLLAILGACSIINRFRVAGELFGLLIAMLFMOQAIGLVDEFRIPERENRKALEFV-----SSW-- 196
 OsBor4|Q1ZYR6 FLLAMFNAAVINRFRVAGELFGMLITVLFQQAIGKGVSEFGMPKDEDSKLEKYK-----FQW-- 197
 TaBor1.1|A0A060PT44 FLLAVLGACSIINRFRVAGELFGLLIAMLFMOQAIGLVDEFRIPERENIKALQYI-----PSW-- 196
 TaBor1.2|A0A060PVQ3 FLLAVLGACSIINRFRVAGELFGLLIAMLFMOQAIGLVDEFRIPERENIKALQFV-----PSW-- 196
 TaBor1.3|W5FNG8 FLLAMFNAAVINRFRVAGELFGMLITVLFQQAIGKGVSEFGMPKDEDSKLEKYK-----PSW-- 196
 ScBor1|P53838 LVLAFTNAVCLLOVYVTFPCDIFGLFINVVYIQKGIQILTRQPSAKSGKS-----232
 SLC4A11|Q8NBS3 ALYAFFNLNLSVMSLFKRSTEEIIFALFISITFVLDVAVKGVKIFWKYYYGHYLD-DYHTKRTSSLVSLSG-----523
 SLC4A1|AE1|P02730 VLVVAFEGSFLVRIFSRVTFQEIFSFLISLIFIVETFSKLKIEQDHPLQKTYNINVMVP-----561
 SLC4A2|AE2|P04920 LLMVALEGSFLVRIFSRVTFQEIFAFLISLIFIVETFSKLKIEQDHPLQKTYNINVMVP-----G--GENMTWAG 873
 SLC4A3|AE3|P48751 LALVAEGSFLVRIFSRVTFQEIFAFLISLIFIVETFSKLKIEQDHPLQKTYNINVMVP-----G--GENMTWAG 865
 SLC4A4|NBCe1|Q9Y6R1 LILVATDASFLVQYETRTTEEGFSLISFIFIFIDAFKMIKGLADYYPINSNFKVGYNTLFSCTCVPPDPANISISNDTT--644
 SLC4A5|NBCe2|Q9BY07 LILVATDASFLVQYETRTTEEGFSLISFIFIFIDAFKMIKGLADYYPINSNFKVGYNTLFSCTCVPPDPANISISNDTT--697
 SLC4A7|NBCn1|Q9Y6M7 IVLVATDASSLVCIYTRTEEAFASLICIIFIVYEALEKLFIDGETYAFNMHNNLDKLTYSVCVTEPPNPNSETLAQW--782
 SLC4A8|NDCBE|Q2Y0W8 IVLVATDASSLVCIYTRTEEAFASLICIIFIVYEALEKLFIDGETYAFNMHNNLDKLTYSVCVTEPPNPNSETLAQW--652
 SLC4A9|AE4|Q96Q91 LVLVATEASVLVRYETRTTEEGFSLISFIFIFIDAFKMIKGLADYYPINSNFKVGYNTLFSCTCVPPDPANISISNDTT--582
 SLC4A10|NBCn2|Q6U841 IILVATDASSLVCIYTRTEEAFASLICIIFIVYEALEKLFIDGETYAFNMHNNLDKLTYSVCVTEPPNPNSETLAQW--683

6 R22

AtBor1|Q8VYR7 -----RFANGMFALVLSFGLLLTGLRSRKARSWRVYGT 228
 AtBor2|Q9M1P7 -----RFANGMFALVLSFGLLLTGLRSRKARSWRVYGT 228
 AtBor3|Q93Z13 -----VFANGMFGVLVSSGLLYTGLKSRKARSWRVYGT 230
 AtBor4|Q9XI23 -----LYTNGLLGLIFTFGLLYTALKSRKARSWRVYGT 233
 AtBor5|Q9SSG5 -----LYTNGLLGLIFTFGLLYTALKSRKARSWRVYGT 233
 AtBor6|Q3E954 -----LYANGLLAVIFSLGLLITALKSRKARSWRVYGT 230
 AtBor7|Q9SUU1 -----RYTNGLLAVIFSGLLYTALKSRKARSWRVYGT 228
 OsBor1|Q2QNH0 -----RFANGMFAIVLSFGLLLTGLRSRKARSWRVYGT 228
 OsBor2|Q1ZYR7 -----LYVNGLLGVIFSIGLLYTALKSRKARSWRVYGT 228
 OsBor3|Q7X9F3 -----RFANGMFAIVLSFGLLLTGLRSRKARSWRVYGT 228
 OsBor4|Q1ZYR6 -----AYVNGLLGIIFSMGLLYTAIRSRKARSWRVYGT 229
 TaBor1.1|A0A060PT44 -----RFANGMFAIVLSFGLLLTGLRSRKARSWRVYGT 228
 TaBor1.2|A0A060PVQ3 -----RFANGMFAIVLSFGLLLTGLRSRKARSWRVYGT 228
 TaBor1.3|W5FNG8 -----RFANGMFAIVLSFGLLLTGLRSRKARSWRVYGT 228
 ScBor1|P53838 -----VQDGFASVVVALVMTAFGLFFLFIYYPLFS 263
 SLC4A11|Q8NBS3 LGASL-----NASL-HTALNASFLASP-----TELPSATHSGQATAVLSLLIMLGLTWLGYTYLQFVKSPYLH 585
 SLC4A1|AE1|P02730 -----KPQGPLENTALLSLVLMAGTFFIFAMMLRKFKNSSYFP 598
 SLC4A2|AE2|P04920 ARPTLGPNG-----RSLAQSGQGKPRGQNTALLSLVLMAGTFFIFAMMLRKFKNSSYFP 928
 SLC4A3|AE3|P48751 LDAGLEPNG-----SALPPTGPPSPRNPNTALLSLIIMLGTFFIFAFFLAKFKNSREL 920
 SLC4A4|NBCe1|Q9Y6R1 LAPEYLPMTSSDMDYHNTTFDWAFLSKKECSYGGNVLGNNC-----NFVPDITLMSFILFLGTYTSSMALKKFKTSRYFP 720
 SLC4A5|NBCe2|Q9BY07 LAPDT--NASLYNLLNLTALDWSLLSKKECLSYGGNVLGNNC-----KFIPLDALMSFILFFGTYSMTLTLLKKFKTSRYFP 771
 SLC4A7|NBCn1|Q9Y6M7 -----KKDNITAHNISWRNLTVSECKKLRGVFLGSACGHHGPIYIPDVLFVFCVILFFTTFFLSSFLAQFKTKRYFP 852
 SLC4A8|NDCBE|Q2Y0W8 -----KDHNIIVTAEVHWNLTVSECKKLRGVFLGSACGHHGPIYIPDVLFVFCVILFFTTFFLSSFLAQFKTKRYFP 722
 SLC4A9|AE4|Q96Q91 TRPKDRDDI---VSMDLGLINASLLPPPECTRQGGHPRGPGC-----HTVPDIAFFSLLFLTSFFFAMALACVKTSREFF 655
 SLC4A10|NBCn2|Q6U841 -----RESNISASDIWENLTVSECKKLRGVFLGSACGHHGPIYIPDVLFVFCVILFFTTFFLSSFLAQFKTKRYFP 753

7 H4 8

AtBor1|Q8VYR7 GWLRSIADYGVPMLVLTGVSYSI---PA-GDVPK-GIPRRLFSNPNSPGAYGNWTVVKEMLD---VPIVYIIGAFIP 300
 AtBor2|Q9M1P7 GWLRSIADYGVPMLVLTGVSYSI---PT-GDVPK-GIPRRLFSNPNSPGAYGNWTVVKEMLD---VPIVYIIGAFIP 300
 AtBor3|Q93Z13 ENLRGFIADYGVPVMVVVMTCSYSI---PW-KSVVQ-GIPRRLFSNPNSPGAYGNWTVVKEMLD---VPIVYIIGAFIP 302
 AtBor4|Q9XI23 GWYRSFIADYGVPMLVVVMTALSFS---TP-SKLPS-GVPRRLFSPLPNDSPSLSHWTVVKEMLD---VSPGYIFAFAIP 305
 AtBor5|Q9SSG5 GCCRSFVADYGVPMLVVVMTALSFS---TP-SKLPS-GVPRRLFSPLPNDSPSLSHWTVVKEMLD---VSPGYIFAFAIP 305
 AtBor6|Q3E954 GWLRSFIADYGVPMLVLTGVSYSI---VP-SEVLP-SVPRRLFCPLPNEPASLYHWTVKEMLD---VPIVYIIGAFIP 302
 AtBor7|Q9SUU1 RWMRGFIADYGVPMLVLTGVSYSI---VP-RNLPE-GVPRRLFSPLPNDSPSLSHWTVVKEMLD---VPIVYIIGAFIP 300
 OsBor1|Q2QNH0 GWLRSFIADYGVPMLVLTGVSYSI---PY-GSVPK-GIPRRLFSNPNSPGAYGNWTVIRDMPN---VPLLVIIGAFIP 300
 OsBor2|Q1ZYR7 GWLRSFIADYGVPMLVLTGVSYSI---LP-KDVPS-GVPRRLFSPLPNESSSLQHWTVAKDLFS---VPPAYIFAAILP 300
 OsBor3|Q7X9F3 GWLRSFIADYGVPMLVLTGVSYSI---PY-GSVPK-GIPRRLFSNPNSPGAYGNWTVIRDMPN---VPLLVIIGAFIP 300
 OsBor4|Q1ZYR6 GWLRSFIADYGVPMLVLTGVSYSI---LP-SKIPS-GVPRRLFSPLPNEPSLQHWTVAKDLFS---VPPAYIFAAILP 301
 TaBor1.1|A0A060PT44 GWLRSFIADYGVPMLVLTGVSYSI---PH-DSVPK-GIPRRLFSNPNSPGAYGNWTVIKDMAQ---VPMYIIGAFIP 300
 TaBor1.2|A0A060PVQ3 GWLRSFIADYGVPMLVLTGVSYSI---PH-DSVPK-GIPRRLFSNPNSPGAYGNWTVIKDMAQ---VPMYIIGAFIP 300
 TaBor1.3|W5FNG8 GWLRSFIADYGVPMLVLTGVSYSI---PH-DSVPK-GIPRRLFSNPNSPGAYGNWTVIKDMAQ---VPMYIIGAFIP 300
 ScBor1|P53838 HIRTFISDYSIALSVLFWSSFTHFGGYLHDVFKKLPITAFPPFSKV-NRPQNTWLA-Y---EPIPVKDVFIAPF 336
 SLC4A11|Q8NBS3 PCVREILSDCALPIAVLAFSLISSHGF---REIEMSKFRY-----NPS-E---SPFAM-AQIQS---LSLRAVSGAMGL 648
 SLC4A1|AE1|P02730 GKLRVIGDFGVPIISILIMVLVDFF---IQDTYTOQLSVSPSGFSVTAPK-K---RGWVI-NPLGKSPFFVMMVASLLP 670
 SLC4A2|AE2|P04920 GKLRVIGDFGVPIISILIMVLVDFF---IEDTYTOQLSVSPSGFSVTAPK-K---RGWVI-NPLGKSPFFVMMVASLLP 1000
 SLC4A3|AE3|P48751 GKLRVIGDFGVPIISILIMVLVDFF---IEDTYTOQLSVSPSGFSVTAPK-K---RGWVI-NPLGKSPFFVMMVASLLP 992
 SLC4A4|NBCe1|Q9Y6R1 TTARKLISDFAILILSILIFCVDAI---VG-VDTPLILVPSSEKPTSP-N---RGWFI-NPLGKSPFFVMMVASLLP 787
 SLC4A5|NBCe2|Q9BY07 TVRSTISDFAVFLTIVMTVIDYL---VG-VSPKLVHVPSEKPTSP-N---RGWFI-NPLGKSPFFVMMVASLLP 838
 SLC4A7|NBCn1|Q9Y6M7 TVRSTISDFAVFLTIVMTVIDYL---VG-VSPKLVHVPSEKPTSP-N---RGWFI-NPLGKSPFFVMMVASLLP 919
 SLC4A8|NDCBE|Q2Y0W8 TVRSTISDFAVFLTIVMTVIDYL---VG-VSPKLVHVPSEKPTSP-N---RGWFI-NPLGKSPFFVMMVASLLP 789
 SLC4A9|AE4|Q96Q91 SVVRRGLSDFSSVLAILLCCGLDAF---LG-LATPKLMVPSSEKPTSP-N---RGWFI-NPLGKSPFFVMMVASLLP 722
 SLC4A10|NBCn2|Q6U841 TVRSTISDFAVFLTIVMTVIDYL---LG-LATPKLMVPSSEKPTSP-N---RGWFI-NPLGKSPFFVMMVASLLP 820

8 H5 9 10 B1 10-ext

D311 Q321 E323 F324 R327 R328 P359 P362 K366 K372

AtBor1|Q8VYR7 ASMAVLVYFFDESVAQLAQOKEFNLRKPPSSYHYDLLLLGFLTLMCGLLGVPPSNGVPIQSPMHTKSLATLYQLLNRL 380
 AtBor2|Q9M1P7 ATMAVLVYFFDESVAQLAQOKEFNLRKPPSSYHYDLLLLGFLTLMCGLLGVPPSNGVPIQSPMHTKSLATLYQLLNRL 380
 AtBor3|Q93Z13 ASMAVLVYFFDESVAQLAQOKEFNLRKPPSSYHYDLLLLGFLTLMCGLLGVPPSNGVPIQSPMHTKSLATLYQLLNRL 382
 AtBor4|Q9XI23 ALMIAGLYFFDESVAQLAQOKEFNLRKPPSSYHYDLLLLGFLTLMCGLLGVPPSNGVPIQSPMHTKSLATLYQLLNRL 385
 AtBor5|Q9SSG5 ALMIAGLYFFDESVAQLAQOKEFNLRKPPSSYHYDLLLLGFLTLMCGLLGVPPSNGVPIQSPMHTKSLATLYQLLNRL 385
 AtBor6|Q3E954 GVMVAGLYFFDESVAQLAQOKEFNLRKPPSSYHYDLLLLGFLTLMCGLLGVPPSNGVPIQSPMHTKSLATLYQLLNRL 382
 AtBor7|Q9SUU1 AIMIAGLYFFDESVAQLAQOKEFNLRKPPSSYHYDLLLLGFLTLMCGLLGVPPSNGVPIQSPMHTKSLATLYQLLNRL 380
 OsBor1|Q2QNH0 ATMAVLVYFFDESVAQLAQOKEFNLRKPPSSYHYDLLLLGFLTLMCGLLGVPPSNGVPIQSPMHTKSLATLYQLLNRL 380
 OsBor2|Q1ZYR7 ALMVAGLYFFDESVAQLAQOKEFNLRKPPSSYHYDLLLLGFLTLMCGLLGVPPSNGVPIQSPMHTKSLATLYQLLNRL 380
 OsBor3|Q7X9F3 ATMAVLVYFFDESVAQLAQOKEFNLRKPPSSYHYDLLLLGFLTLMCGLLGVPPSNGVPIQSPMHTKSLATLYQLLNRL 380
 OsBor4|Q1ZYR6 AVMVAGLYFFDESVAQLAQOKEFNLRKPPSSYHYDLLLLGFLTLMCGLLGVPPSNGVPIQSPMHTKSLATLYQLLNRL 381
 TaBor1.1|A0A060PT44 ATMAVLVYFFDESVAQLAQOKEFNLRKPPSSYHYDLLLLGFLTLMCGLLGVPPSNGVPIQSPMHTKSLATLYQLLNRL 380
 TaBor1.2|A0A060PVQ3 ATMAVLVYFFDESVAQLAQOKEFNLRKPPSSYHYDLLLLGFLTLMCGLLGVPPSNGVPIQSPMHTKSLATLYQLLNRL 380
 TaBor1.3|W5FNG8 ATMAVLVYFFDESVAQLAQOKEFNLRKPPSSYHYDLLLLGFLTLMCGLLGVPPSNGVPIQSPMHTKSLATLYQLLNRL 380
 ScBor1|P53838 GIFTILIFMDQITAVIVNRKELKKGAGYHLDLFWVAILMVICSLMALPWHYVAATVISIARIDSLKMETETS----- 407
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Protein	Sequence	Position
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OsBor4	PVRVNEQRVSNNLLQS--LLIAGCGVMPI--IQKIPTSVLWGYFAFMAIESLPGNQ--FWERTQLE	514
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SLC4A9/AE4	FLGIREQRVTGLVVF--ILTGASIFLAPV--LKFIEMPVLYGVFLYMGVAALSSIQ--FTNRVKLE	865
SLC4A10/NBCn2	FLGIREQRVTGLMIF--ILMGSSVFMTSI--LKFIEMPVLYGVFLYMGVSSLKGIO--FFDRILKLE	963

H8 H9 13 14 H10

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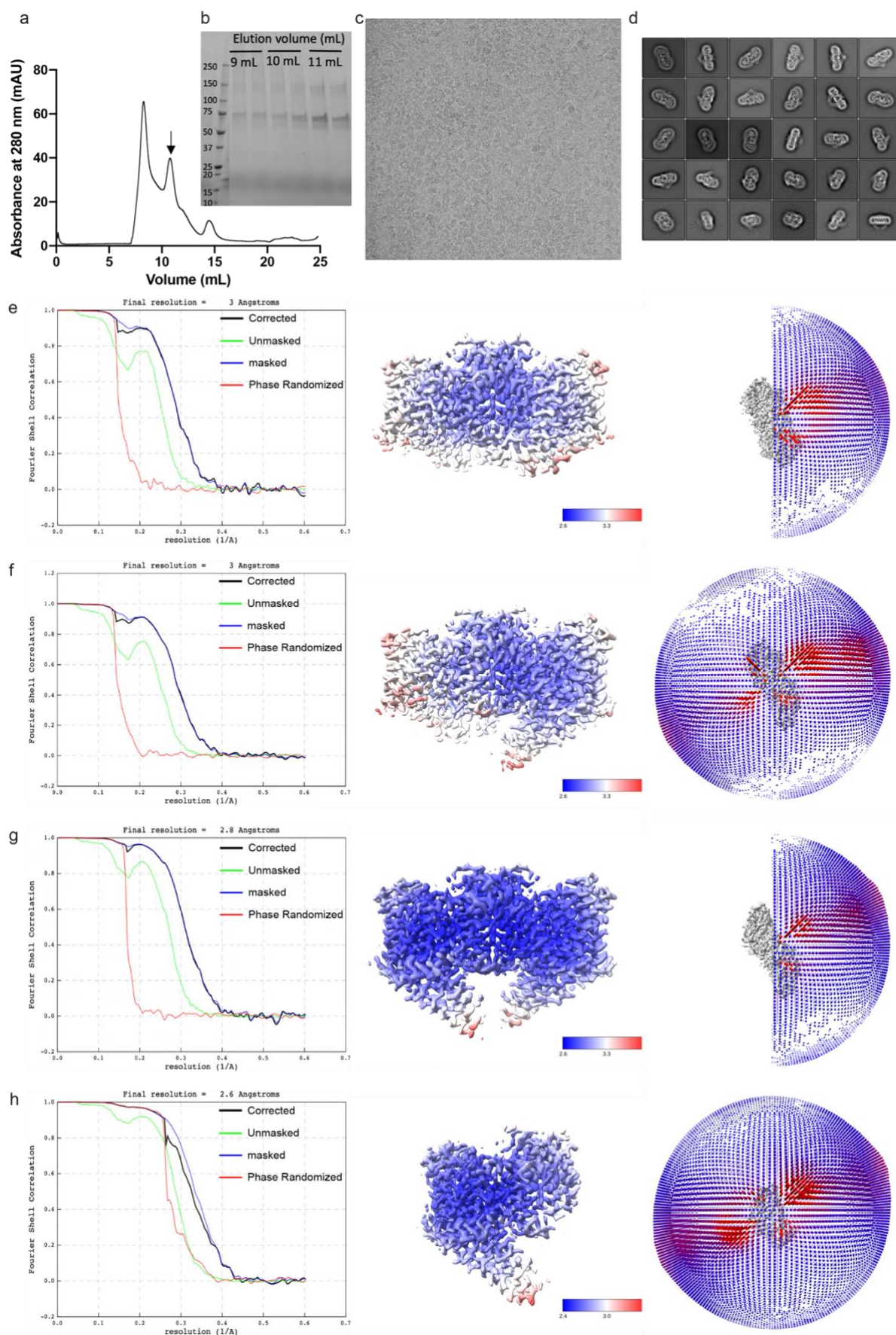
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 AtBor7|Q9SUU1 VLDSEYEEIMVGAQQRNSSFNGELREAHNIPLSVENSEDEFYDAEILDEI-TTSRGEIRKVSLLKGGGSG--STV 668
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 TaBor1.3|W5FNG8 DLDAAYEESPAIPFNLAQDIDVAL-----GRTQSAEILDDIVTSRGEIRKVSLLKGGGSG--STV 660
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 SLC4A2|AE2|P04920 CLDANEAEFVFD-----E-----REGVDEYNEMMPV----- 1241
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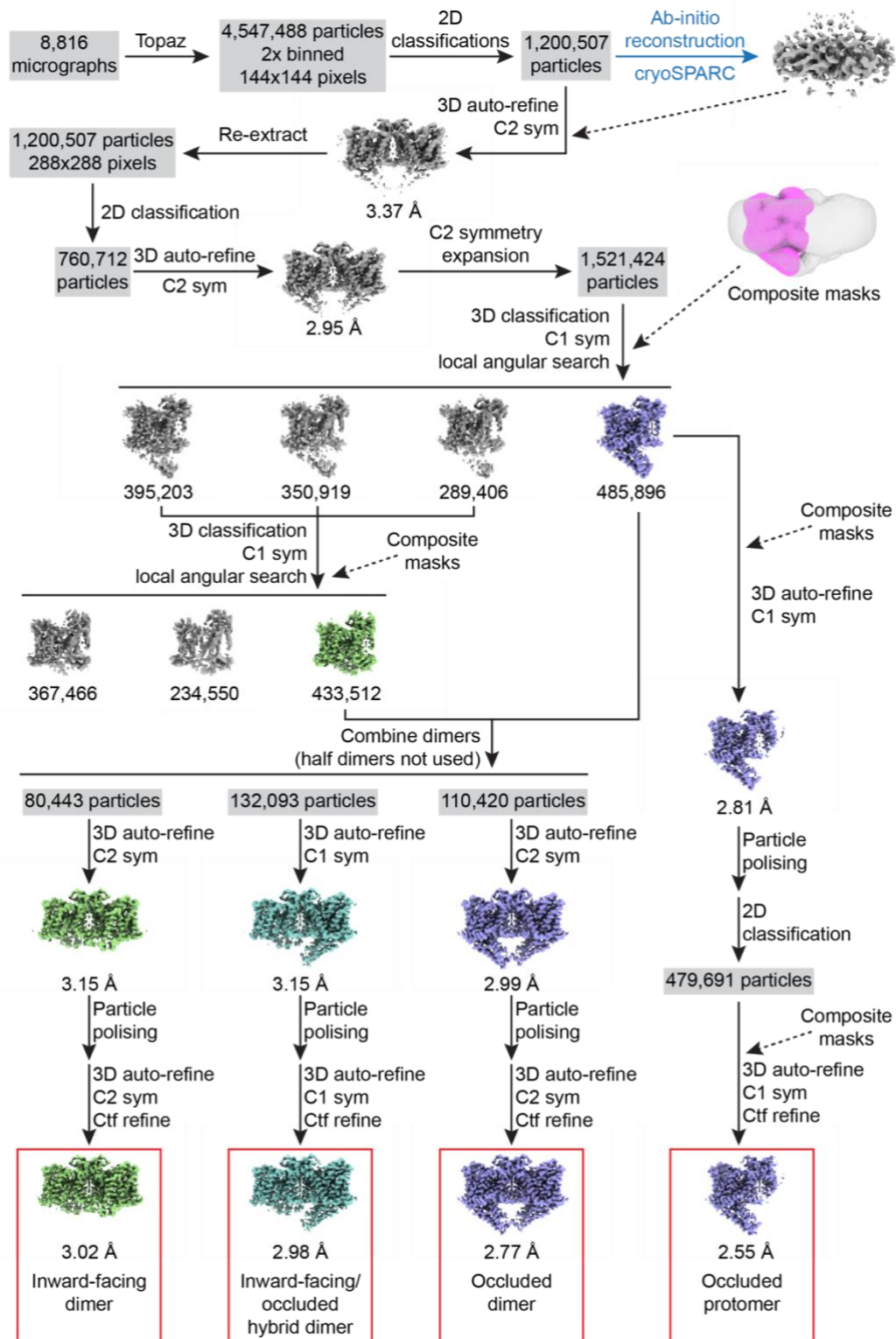
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AtBor7|Q9SUU1      -IYNHS----- 673
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SLC4A1/AE1|P02730   ----- 911
SLC4A2/AE2|P04920   ----- 1241
SLC4A3/AE3|P48751   ----- 1232
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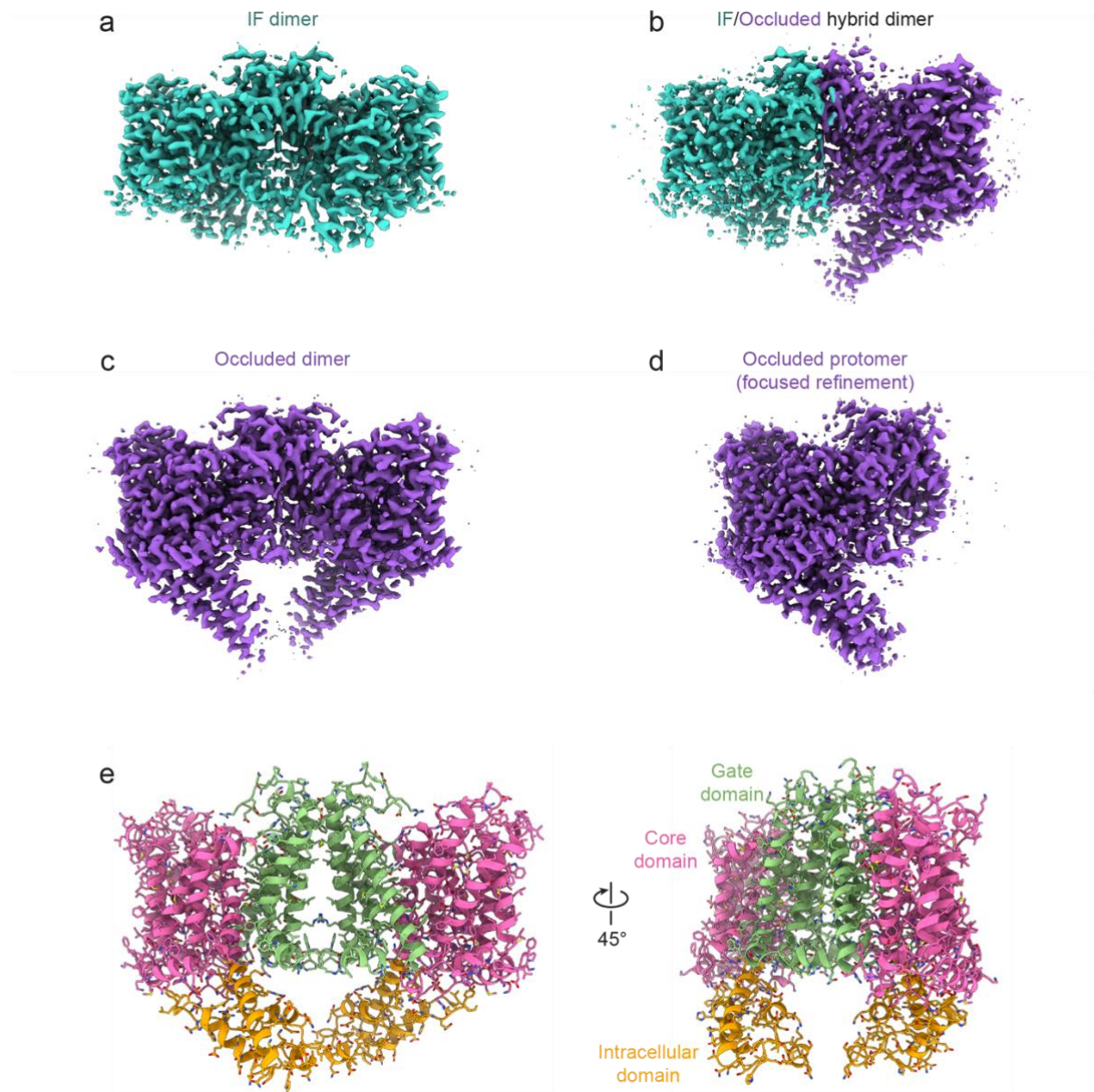
Supplementary Fig. 6 Sequence alignment of *Arabidopsis thaliana*, rice, wheat borate transporters and human SLC4 transporters. UniProt ID is shown after vertical slash. Secondary structural elements of AtBor1 are depicted above the sequence alignment. Dotted black lines represent unmodeled regions. Invariant and highly conserved residues are colored.



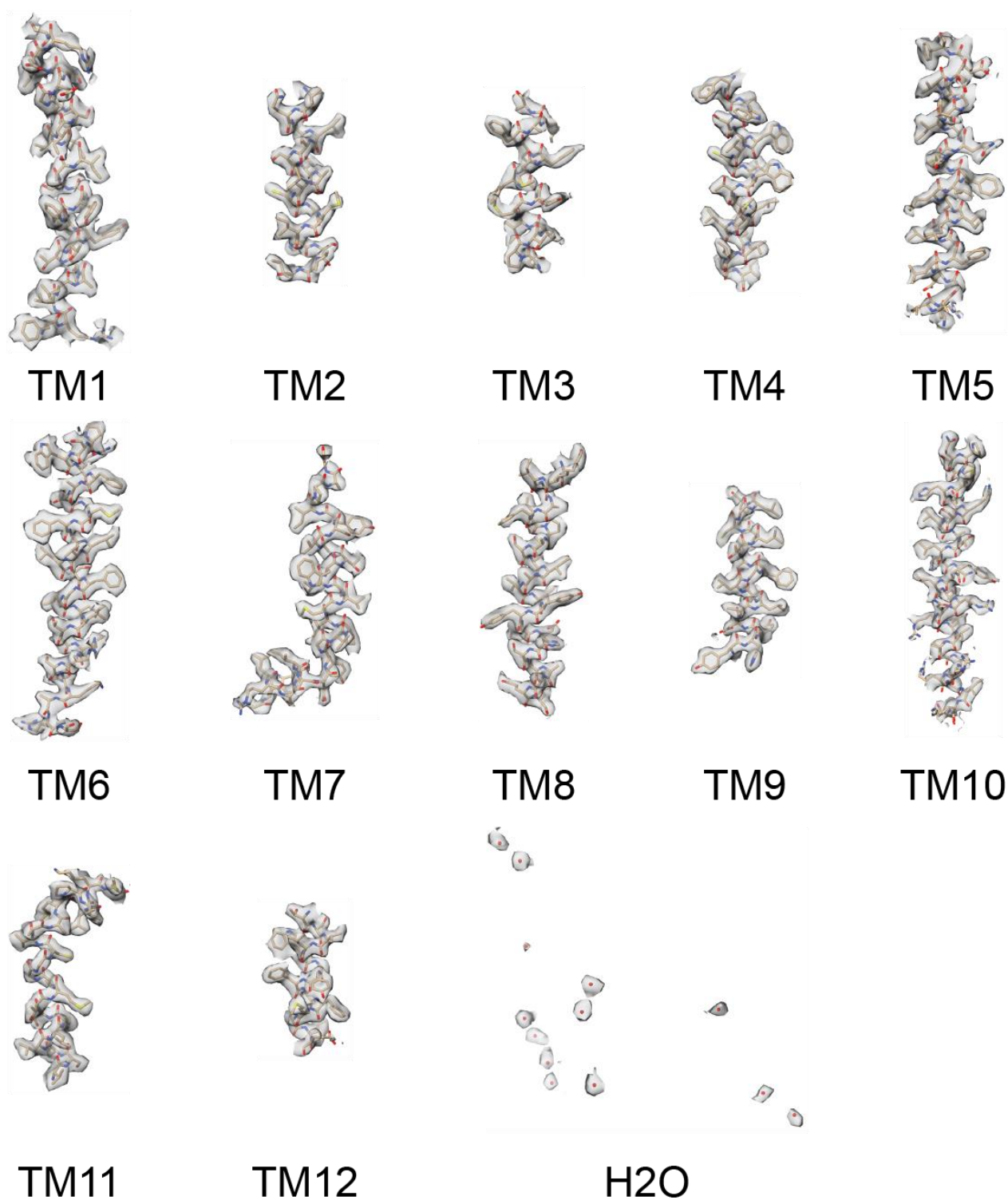
Supplementary Fig. 7 **Cryo-EM analysis of AtBor1_{active} in 0.01% LMNG.** **a** Chromatographs of AtBor1_{active} in 0.01% LMNG. The arrowed peak was collected for cryo-EM study. **b** The SDS-PAGE of the peak fractions. Monomeric AtBor1_{active} protein was observed clearly on the SDS-PAGE between 75 to 50 KDa. **c** A representative electron micrograph of AtBor1_{active} in 0.01% LMNG. **d** Representative two-dimensional class averages of the electron micrographs for AtBor1_{active} in 0.01% LMNG. **e-h** Fourier Shell Correlation (FSC) curves (left), local resolution maps (middle), and particle orientation plots (right) of the 3D reconstruction of inward-facing dimer (**e**), hybrid inward-facing/occluded dimer (**f**), occluded dimer (**g**), and occluded protomer (**h**).



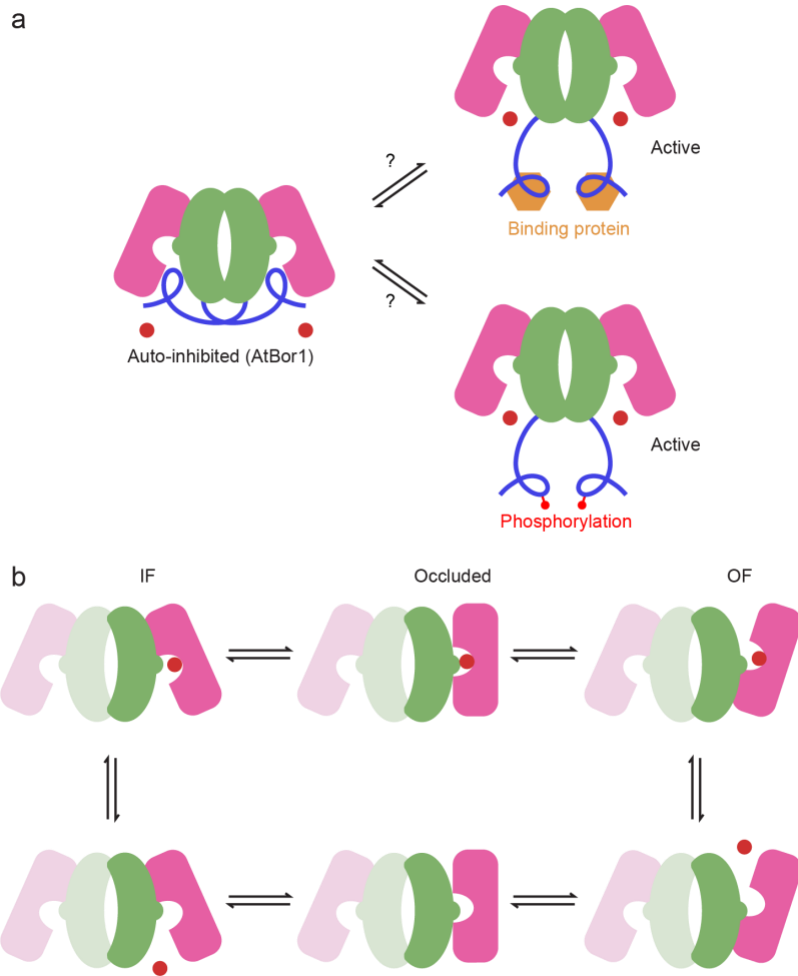
Supplementary Fig. 8 **Cryo-EM data processing workflow for AtBor1_{active}**. The 3D reconstructions are shown without B-factor sharpening. The reconstructions in red boxes are used for atomic model building.



Supplementary Fig. 9 **Structures of active AtBor1.** **a-d**, Cryo-EM density maps of active AtBor1 IF dimer (**a**), IF/occluded hybrid dimer (**b**), occluded dimer (**c**) or occluded protomer (**d**). **e** The atomic model of active AtBor1 occluded dimer.



Supplementary Fig. 10 **Cryo-EM maps for representative regions of AtBor1_{active}.** The cryo-EM maps for the TM1-TM14 helices and the water molecules of AtBor1_{active} in the occluded conformation.



Supplementary Fig. 11 **The molecular basis of the elevator transport mechanism and the activity regulation for the SLC4 transporters.** **a** Proposed mechanisms for the release of autoinhibition. The Ct domain (blue) either interacts with an intracellular binding partner (yellow) or undergoes phosphorylation, leading to the release of the autoinhibitory domain. This conformational change allows substrates (red dots) to access the substrate binding pocket in the Core domain (pink) from the intracellular site. **b** Schematic representation of conformational transitions. In the inward-facing (IF) state, the substrate access to the substrate binding pocket in the Core domain (pink) from the intracellular site. Next, the Core domain moves upwards through the occluded state to the outward-facing (OF) state, allowing the substrate to be released from the transporter. The Core domain then moves downwards through the occluded conformation back to the inward-facing state in preparation for the next transport activity. The Gate domain (green) remains rigid during the process and the transport transition in one protomer is completely independent to the other protomer.

Supplementary Table 1 **Cryo-EM data collection, refinement, and validation statistics**

	WT AtBor1 protomer in lipid nanodiscs (EMD-41185) (PDB 8TEG)	WT AtBor1 dimer in lipid nanodiscs (EMD-41186) (PDB 8TEH)
Data collection and processing		
Magnification		165,000×
Voltage (kV)		300
Electron exposure (e-/Å ²)		55
Defocus range (μm)		-0.8 to -2.5
Pixel size (Å)		0.83
Symmetry imposed	C1	C2
Initial particle images (no.)	2,485,991	2,485,991
Final particle images (no.)	853,837	1,012,376
Map resolution (Å)	2.15	2.30
FSC threshold	0.143	0.143
Map resolution range (Å)	2.1 – 3.0	2.2 – 5.2
Refinement		
Model resolution (Å)	2.1	2.3
FSC threshold	0.5	0.5
Map sharpening <i>B</i> factor (Å ²)	-48	-61
Model composition		
Non-hydrogen atoms	4,518	9,816
Protein residues	561	1,108
Ligands	0	24
Water	88	214
<i>B</i> factors (Å ²)		
Protein	27.98	28.28
Ligand	—	8.89
Water	27.17	26.87
R.m.s. deviations		
Bond lengths (Å)	0.007	0.003
Bond angles (°)	0.742	0.517
Validation		
MolProbity score	0.94	1.00
Clashscore	1.68	2.23
Poor rotamers (%)	1.05	0.43
Ramachandran plot		
Favored (%)	98.55	98.36
Allowed (%)	1.45	1.64
Disallowed (%)	0	0

Supplementary Table 2 Cryo-EM data collection, refinement and validation statistics

	AtBor1 _{active} occluded protomer (EMD-41188) (PDB 8TEJ)	AtBor1 _{active} occluded dimer (EMD-41190) (PDB 8TEL)	AtBor1 _{active} IF dimer (EMD-41191) (PDB 8TEM)	AtBor1 _{active} occluded/IF dimer (EMD-41192) (PDB 8TEN)
Data collection and processing				
Magnification			165,000×	
Voltage (kV)			300	
Electron exposure (e-/Å ²)			55	
Defocus range (μm)			-0.8 to -2.5	
Pixel size (Å)			0.83	
Symmetry imposed	C1	C2	C2	C1
Initial particle images (no.)	4,547,488	4,547,488	4,547,488	4,547,488
Final particle images (no.)	479,691	110,420	80,443	132,093
Map resolution (Å)	2.55	2.77	3.02	2.98
FSC threshold	0.143	0.143	0.143	0.143
Map resolution range (Å)	2.5 – 3.8	2.7 – 5.0	2.9 – 4.8	2.8 – 4.9
Refinement				
Model resolution (Å)	2.6	2.8	3.1	3.0
FSC threshold	0.5	0.5	0.5	0.5
Map sharpening <i>B</i> factor (Å ²)	-62	-58	-62	-60
Model composition				
Non-hydrogen atoms	3,925	7,806	7,992	7,872
Protein residues	494	988	1,010	996
Ligands	0	0	0	0
Water	22	0	0	0
<i>B</i> factors (Å ²)				
Protein	42.88	47.89	55.41	54.35
Ligand	—	—	—	—
Water	36.29	—	—	—
R.m.s. deviations				
Bond lengths (Å)	0.003	0.002	0.003	0.003
Bond angles (°)	0.513	0.489	0.557	0.518
Validation				
MolProbity score	1.74	1.45	1.59	1.36
Clashscore	2.78	3.10	3.52	3.70
Poor rotamers (%)	2.87	1.20	1.87	1.07
Ramachandran plot				
Favored (%)	95.26	95.77	96.39	96.94
Allowed (%)	4.74	4.23	3.61	3.06
Disallowed (%)	0	0	0	0

Supplementary Table 3 **List of oligonucleotides used in AtBor1 mutagenesis**

Mutations	Forward Primer	Reverse Primer
T636A	GTC ATG GCT AGA TCT AGA GGC GAG TTC AGA	AGA TCT AGC CAT GAC TTC GTC CAA GAT CTC
R637A	ATG ACC GCT TCT AGA GGC GAG TTC AGA CAC	TCT AGA AGC GGT CAT GAC TTC GTC CAA GAT
S638A	ACC AGA GCT AGA GGC GAG TTC AGA CAC	GCC TCT AGC TCT GGT CAT GAC TTC GTC
G640A	TCT AGA GCT GAG TTC AGA CAC ACC TCT TCC	GAA CTC AGC TCT AGA TCT GGT CAT GAC TTC
R639A	AGA TCT GCT GGC GAG TTC AGA CAC ACC	CTC GCC AGC AGA TCT GGT CAT GAC TTC GTC
R639K	AGA TCT AAG GGC GAG TTC AGA CAC ACC	CTC GCC CTT AGA TCT GGT CAT GAC TTC GTC
R639E	CAG ATC TGA GGG CGA GTT CAG ACA CAC C	TCGCCCTCAGATCTGGTCATGACTT CGTCC
E641A	AGA GGC GCT TTC AGA CAC ACC TCT TCC	TCT GAA AGC GCC TCT AGA TCT GGT CAT GAC
E641Q	AGA GGC CAG TTC AGA CAC ACC TCT TCC	TCT GAA CTG GCC TCT AGATCTGGTCATGAC
R643A	CGAGTTCGCTCACACCTCTTCCCC AAAGG	GTGTGAGCGAACTCGCCTCTAGATC TGG
H644A	G TTCAGAGCTACCTCTTCCCCAAA GGTTACC	GAGGTAGCTCTGAACTCGCCTCTAG ATCTGG
R637E/E641R/R643 E	G TCT AGA GGC AGA TTC GAG CAC ACC TCT TCC CCA AAG G	A ATC TGC CTC TAG ACT CGG TCA TGA CTT CGT CCA AGA TCT C
AtBor1/Bor4C	CTAGAGGCGAGTTGAAGGTTAGA ACCTCTTCCCCAAAGGTTACC	TCAACTCGCCTCTAGAGGTGGTGAC TTCGTCCAAGATCTCCAAATCG
Δ627-704	TGA TAA CTC GAG TCA TGT AAT TAG	GCC TGG GTA AGA GGT AGT AGA ACC
Δ627-641	TTC AGA CAC ACC TCT TCC CCA AAG	GCC TGG GTA AGA GGT AGT AGA ACC
Δ627-642	AGA CAC ACC TCT TCC CCA AAG GTT ACC	GCC TGG GTA AGA GGT AGT AGA ACC
Δ627-643	CAC ACC TCT TCC CCA AAG GTT ACC	GCC TGG GTA AGA GGT AGT AGA ACC
Δ627-644	ACC TCT TCC CCA AAG GTT ACC	GCC TGG GTA AGA GGT AGT AGA ACC
Δ627-645	TCT TCC CCA AAG GTT ACC TCT TCT TCC	GCC TGG GTA AGA GGT AGT AGA ACC
Δ638-641	TTC AGA CAC ACC TCT TCC CCA AAG	TCT GGT CAT GAC TTC GTC CAA GAT CTC
Δ636-641	TTC AGA CAC ACC TCT TCC CCA AAG	CAT GAC TTC GTC CAA GAT CTC CAA ATC
Δ651-704	CAAAGGTTTGATAACTCGAGTCA TGTAATTAGT	GTTATCAAACCTTTGGGGAAGAGGT GT
R637E/E641R/R643 E/T410V	ATG CAA GTC CCA TTG GTT TAC CAG CAG CCA	CAATGGGACTTGCATGTGGTGGTAA GCTTC