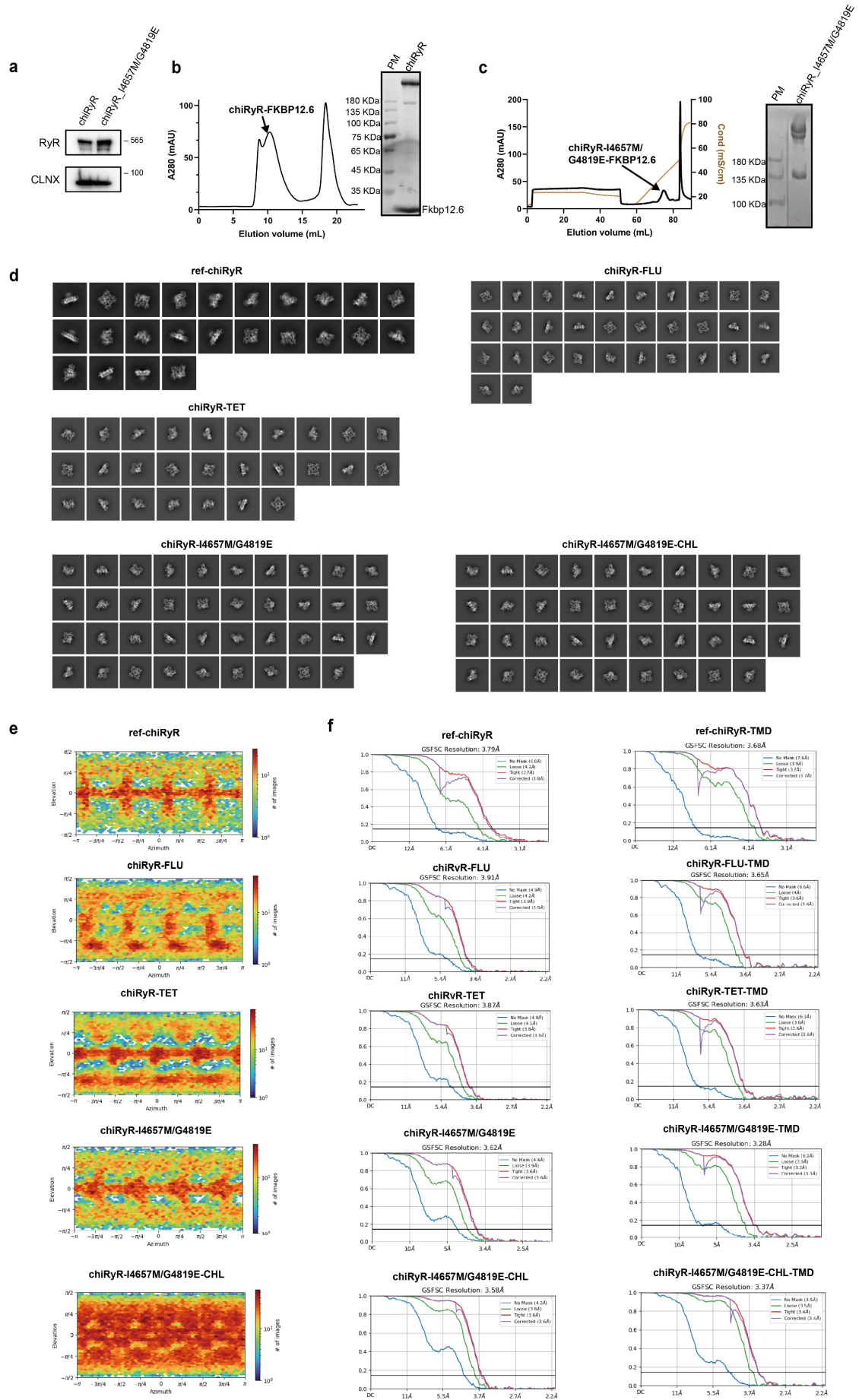


rRyR1	MGD - - GGE	- DEVQFLRTD	DEVVLQCSAT	VLKEQLKCL	AAEGFGNRLC	FLEPTSAQN	VPPDLAICCF	TLEQSLSVRA	LQEML - ANT	EAGVES - - S	93
sRyR1	MAEAEGGASE	QDDVSLRTE	DMVCLSCAT	- - - - GERVCL	AAEGFGNRRHC	FLENIAD - KN	IPPDLSQCVF	VIEQALSVRA	LQELVTAAGS	ETGKENLGKG	95
rRyR1	OGGHRHTLLY	GHAILLRHAH	SRMYLSCLTT	SRSMTDKLAF	DVYGLQEDATG	EACWWTMHPA	SKQRSEGEKV	RVGDDLLVLS	VSSERYLHLS	TASGELQ - VD	192
sRyR1	TGSGHRHTLLY	GNAILLRHLN	SDMYLACLST	SSSQ - DKLAF	DVYGLQEHSGQ	EACWWTLHPA	SKQRSEGEKV	RVGDDLLVLS	VATERYLH - T	TKENEVSIVN	193
rRyR1	ASFMQTLWNM	NP - - - - ICSC	CEEGYVTGGH	VLRFLFHGMD	ECLTI - SAAD	SDQRRRLVY	EGGAVCTHAR	SLWRLEPLRI	SWSGSHLRWG	QPLRIRHVTT	287
sRyR1	ASFHVTHWSV	QPYGTGISRM	KYGVYVFGGD	VLRFFHGG - D	ECLTIPTSTWT	KDGGQNIIVY	EGGSVMSQAR	SLWRLELART	KWAGGFINWY	HPMRIRHITT	292
rRyR1	GRYLALEDQ	GLVVVDACKA	HTKATSF CFR	VSKE - KLDTA	PKRDVEGMCP	PEIKYGESLC	FVQHVASGLW	LTAAAPDPKA	LRLG - VLKKK	ATLHQEGHMD	385
sRyR1	GRYLVNDQN	ELYLVSREEA	TTASCACFLR	QEKDDQKQVL	EDKDLEVIGA	PIIKYGDSTV	IVQHSETGLW	LSYKSYETKK	KGVGKVEEQ	AILHEEGKMD	392
rRyR1	DALFLTRCQ	EESQAARMIH	STAGLYNQFI	KGLDSFSGKP	RGSGPPAGPA	LPTEAVILSL	QDLIGYFEPF	SEELQHEEKQ	SKLRSLRNRQ	SLFOEEGMLS	485
sRyR1	DGLDFRSRQE	EESRTARVIR	KCSLSLTFKI	NGLETQENR	RHSMFFA - - S	VNLGEMVMCL	EDLINYFAQP	DEDMHEEKQ	NKFRALNRNQ	DLFQEEGLIN	490
rRyR1	LVLNCIDRLN	VYTTAAHFAE	Y - AGEAAAE	WKEIVNLLYE	LLASLIRGNR	ANCALF - - ST	NLDWVYSKLD	RLEAS - - SG	ILEVLVCLVI	ESPEVLNTIQ	579
sRyR1	LILEADIKIN	VITSQGLFAG	FLAGDESGQS	WEMISGYLYQ	LLAAIIKGNH	TNCAQFANSN	RLNWLFSRLG	S - QASGEGTG	MLDVLHCVLI	DSPEALNMMR	589
rRyR1	ENHIKSIISL	LDKHGRNHKV	LDVLSCLVCV	NGVAVRNQD	LITENLLPQR	ELLQTINLIN	VYTSIRPNIF	VGRAEGSTQY	GKWYFEVMDV	EVPFPLTAQA	679
sRyR1	DEHIKVIISL	LEKHGRDPKV	LDVLSCLCVG	NGVAVRSSQN	NICDYLLPGK	NLLQTLALVD	HVSSVRPNIF	VGRVEGSAYV	RKWYFEVTMD	HIIEK - TTHMM	688
rRyR1	THLRVGWALT	EGSYPPYGGG	EGWGGNGVGD	DLYSYGFDGL	HLWTGHVARP	V - - TSPGQHL	LAPEDVVSCC	LDLSVPSISF	RINGCPVQGV	FEAFNLGCLF	777
sRyR1	PHLRIGWANT	TGYVPYPPGG	EKWGGNGVGD	DLYSYGFDGA	YVWSGGRKTQ	VNRTHAEFPY	IRKGDVIGCA	LDLTVPIINF	MFNGVTVTGS	FTNFLNLCMF	788
rRyR1	FPVVSFSAGV	KYRFLGGRH	GFKFLPPPG	YAPCHEAVLP	RERLRLEPIK	EYRREGPRGP	HLVGPSCRLS	HTDFVCPVVD	TVQIVLPPHL	ERIREKLAEN	877
sRyR1	FPVISCSSKL	SCRFLGGH	GRLRYAAPEG	YSLPVSLLP	QQILSLPECF	YFGNIAKRA -	- LAGPPLVQD	DTAFVPTVVD	TLQIPLPSYV	EQIRDKLAEN	886
rRyR1	IHELWALTRI	EQGWYGPVR	DDNKRHLHPL	VNFHSLPEPE	RNYNLQMSGE	TLKTLLALGC	HVGMADAKAE	DNLKKTCLP -	KTYMMSNGYK	PAPLDLSHVR	976
sRyR1	IHEMWAMNKI	EAGWYVGIQR	DDLHLHPCFL	VPFERLPPAE	KRYDIQLAVQ	TLKTLLALGY	YISL - - DKPP	ARIRNRYLPN	EPPMGSNGYK	PAPLDLSAVT	984
rRyR1	LTPAQTTLLD	RLAENGHNWV	ARDRVAQGW	YSYAVQDIPAR	RNRLVYPYRL	LDEATKRSNR	DSLCAQVRTL	LGVGYNIEFP	DQEP - SQV - - -	- ENQSRWRDV	1072
sRyR1	LTPKMDELVD	QLAENTHNLW	ARERIQQGW	YGLNEDPDMH	RSPHLVYPYK	VDDAIKKNR	DTASETVRTL	LVYGNLDLFP	TGEJHEALL	EASKQQAQEF	1084
rRyR1	RIFRAEKSYT	VQSGRWYFEF	EAVTIGEMRV	GWARPELRPD	VELGADELAY	VFNHGRQRW	HLG - SEPFR	PWQSGDVVGC	MIDLTTENTII	FTLNGEVLMS	1171
sRyR1	RTYRAEKNYA	VSGSKWYFEF	EILTAGPMRV	GWAHADMAPG	MMLGQDENSW	AFDGYNSLKY	HGGASDTFGL	QLKVGDIYGC	FLDVTBQITIS	FSLNGELIMD	1184
rRyR1	DSGSETAFRE	IEIGDGFLPV	CSLPGQGVGH	NLNGQDVSSL	RFFAICGLQE	GFEFFAINMQ	RPVTTWFSKS	LPQFEPYPPE	- HPHYEVARM	DGTVDTPPCL	1270
sRyR1	ALGETTFAD	VQ - GDNFVA	CTLGVGQKAR	LTYGQDVNTL	KYFTTCGLQE	GYPEFCVNMK	RDVTHWYTKD	QPIFENTDEM	IDTRIDVTRI	PAGSDTPPCL	1283
rRyR1	RLAHTWGSQ	NSLVEMFLFR	LSLPVQFHQH	FRCTA - - - -	FIDEAEKARR	WWEIKERQQI	LMKEAVEAQM	PAHIDQIMRS	GAT - - - - -	- - - - - PLA	1311
sRyR1	KISHINTFET	EK - ANWEFLR	LSLPVICHNE	FIDEAEKARR	WWEIKERQQI	LMKEAVEAQM	PAHIDQIMRS	GFTMNDIKGL	HYEDNQEEVP	SSKVRQPSR	1382
rRyR1	PP - - - - -	- - - - - GLQ	PP - - - - - A	EDEARAAE - -	- - - - - PDP	DYENLRRSAG	GWGEAEGGKE	GTAKEGTPGG	TPQP - - GVEA	QPVRAE - - - N	1375
sRyR1	PPRKGSMTRG	VSYQNNYLQ	PGQVNGMHR	TSEAEAMAKYE	LGAQNLSPDD	KKDKGRSFPF	KFRFSKRGES	SDRAKSRSKS	TPDPFSDTEV	SPEGRPRPN	1482
rRyR1	EKDATTENK	K - - - - -	RGFLFKAKKA	AMMTQPPATP	ALPR - - - - -	- LPHDVVPAD	NRD - DPETI	LNTTTYVYSV	RVFAGQEPSC	VWVGWVTPDY	1457
sRyR1	PQIKVSAQAN	RYNGMNRAPS	RTNLYGSQVG	LNSNAQMATP	TQDRKQMTS	TLAQSTTETV	GNEIFDAECL	KLINEYFYGV	RIFPGQDPTH	VYIGWVTTQY	1582
rRyR1	HQHDMMFDSL	KYRAVVTVMG	DEQGNVHSSL	KCSNICYMVWG	GDF - - - VSPG	QQGRISHTDL	VIGCLVDLAT	GLMTFTTANGK	ESNTFFQVEP	NTKLFFAVFV	1554
sRyR1	HLHSKDFVNS	DDYDRVVEN	NRQSCYMVRA	DELYNEVMAE	ATAKQASQGM	FIGCSYDST	GTYSFTCEGK	DTFSFKKMEP	ETKLFFAIFV	1682	
rRyR1	LPTHQNVIOF	ELGKQKNIMP	LSAAMFLSER	KNPAPQCPRP	LEVOMLMPVS	WSRMPNHFLQ	VETRAGERL	GWAVQCQDPL	TMALHIPEE	NRCMDILELS	1654
sRyR1	EATSKELIQI	ELGRSTSLP	LSAAVLPTSD	KHYIPIQFPP	LKYQCLKPHQ	WARVPNQSLL	VHALKLSDIR	GWSMLCEDAV	SMALHIPEE	DRCIDILELI	1782
rRyR1	ERLDLQRFHS	HTLRLYRVC	ALGNRRVAHA	LCSHVDQAQL	LHALEDAHLP	GPLRAGYDDL	LISIHLESAC	RSRRSMLSEY	IVPLTPETRA	ITLFFPPGRKG	1754
sRyR1	EMDKLLSFHS	HTLTLYAALC	YQSNRYRAHA	LCTHYDQKQL	LYAIQSQYMS	GPLRQGFYDL	LIALHLESAC	TTMEACKNEF	VIPGLPELKA	LYEDPD - - -	1878
rRyR1	GNARRHGLPG	VGVTSLRRP	HHFSPPCFVA	ALPAAGVAEA	PARLSPAIPL	EALRDKALRM	LGEAVRDGGQ	HARDPPVGSVS	EFQFVYVPLKL	VSTLLVMGIF	1854
sRyR1	- - - MGHSLSR	LQ - TESVRPQ	MKMTDI - - -	- - - AESITDI	SNLYSPYFPL	EYVREFVMAQ	LAEAVETNQV	HNRPVVGGSN	ENLFLPLIKL	VDRLLLVGMM	1967
rRyR1	GDEDVQKILK	MIPEVFTEE	EEEEEEEEEE	EEEEEEEEEE	KEEDEEEEEK	EDAEEEEEEA	PEGEKEDLEE	GLLQMKLPES	VKLQMCNLE	YFCDQELQHR	1954
sRyR1	RLHEDVKKLI	MTNPETW -	- - - - - DPS	- - - - - DPS	FDKEGKDEHR	K - - - - -	- - - - -	GLLHMKMAEG	AKLQMCYLLQ	HLNDILQRH	2028
rRyR1	VESLAFAER	YVDKLANQR	SRVALLMRAF	TMSAAETARR	TREFRSPQPE	QINMLLHFK -	- DEADEEDCP	LPEDIRQDLQ	DFHQDLLAHC	GIQL - - - - EG	2048
sRyR1	VEAIIAFAHD	FVGDQLQDQL	RRYTEIKQS -	DLPSAVAACK	TREFRCPPRE	QMNAILSFKH	LEEDKENCPC	CGEELIARMN	EFHESLMAHV	SLNALQEPDG	2127
rRyR1	EE - - - - -	- - - - -	- - - EEPET	SLSSRLRSL	ETVRLVKKKE	EKPEEELPAE	EKKPQSLQEL	VSHMVVRWAQ	EDYVQSPELV	RAMFSLLRHQ	2127
sRyR1	TENQPEAKP	GAFGKLYNII	NTYKELEEE	- - - - -	- - - - - KAI	DEPPKKT - E	EK - - - - FRKV	LIQTIYNWAE	ESQIETPKLV	REMFSLLYRQ	2205
rRyR1	YDGLGELLRA	LPRAYTISPS	SVEDTMSLLE	CLGQIRSLLI	VQMGPQEEEN	MIQSIGNIMN	NKVFYQHPNL	MRALGMHETV	MEVMNVVLG -	- - - - -	2216
sRyR1	YDAVAGELIRA	LEKTYVINAK	TKLDVAEMWV	GLSQIRALLP	VQMSQEEEL	MRKRLWKLVN	NHTFFQHPDL	IRVLRVHENV	MAYMNTLGR	RAQAQSDAQP	2305
rRyR1	- - - - - GGETK	EIRFP - KMVT	SCCRFLCYFC	RISRQNRQSM	FDHLSYLLN	SGIGLG - - M	QGSTPLDVAA	ASVIDNNELA	LALQEODLEK	VVSYLAGCGL	2307
sRyR1	ASPPVAEDSK	EKDTISEHMY	ACCRFLCYFC	RTGRQNRQAM	FDHFDLLEN	SNILSRPSL	RGSTPLDVAY	SLMENTELA	LALREHYLEK	IAYVLSRGLI	2405
rRyR1	QSCPMLLAKG	YPDIGWNPFC	GERYLDLFLR	AVFVNGESVE	ENANVVVRL	IRKPECFCGPA	LRGEGGSGLL	AAIEEAIRIS	EDPARDGPGV	RRDRRREHFG	2407
sRyR1	QSNSELVEKG	YPDLGWDPE	GERYLDLFLR	CVWVNGESVE	ENANVIRLL	IRRPECLGPA	LRGEG - EGLL	KAIVDANKMS	ERIAADR - - -	RKLREMEQEG	2500
rRyR1	- - - - - E	EPPEENRVHL	GHAIMSFYAA	LIDLLGRCAP	EMHLIQAGK	EALRIRAILR	SLVPLDDBIVG	LIISL - PLQI	PTLGKDGALV	QPK - MSASF	2494
sRyR1	DVNFSHPLPE	SDEDEDYIDT	GAILLFYCT	LVDLLGRCAP	DAGVIALGKN	ESLRARAILR	SLVPLEDLOG	VLSLRFITLN	PAAGEE - - -	RPKSDMPSGL	2596
rRyR1	VPDHKASMYL	FLDRVYGIEN	QDFLLHVLVD	GFLPDMRAAA	SLDTATFSTT	EMALALNRYL	CLAVLPLITK	CAPLFAGTEH	RAIMVDSMLH	TVYRLSRGRS	2594
sRyR1	IPGHKQSVGL	FLERVYGIET	QELFYKLLKE	AFLPDLRAAT	MLDRNDGCE	SMALSNRYI	GNSILPLLIK	HAYFYNEAEN	YASLLDATLH	TVYRLSKNRM	2696
rRyR1	LTKAQRDVIE	DCLMALCRYI	RPSMLQHLR	RLVFDVPIIN	EFAKMPKLK	TNHYERCWKY	Y - CLPTGWAN	FGVTSEELH	LTRKLFWGIF	DSLAKHKYDQ	2693
sRyR1	LTKGQREAVS	DFLVALTSA	QPSMLKLRL	KLVYDVSLS	EYTTVALRL	TLHYERCARK	YGSTGAGQGV	YGASSDEEK	LTMLFSNIF	DSLKSMDEYEP	2796
rRyR1	ELYRMAMPCL	CAIAGALPPD	Y - - - - VDAS	YSSKAIEKAT	VDAEGNFDPR	PVETLNVIIIP	EKLDSFINKF	AEYTHEKWAF	DKIQNNWSYG	ENVDEELKTH	2788
sRyR1	ELFGKALPCL	IAIGCALPPD	YLSKSNYDDE	FYKGEQVAGD	LD - NPQYDPP	PIINTSSVALN	NDLNTIVQKF	SEHYHDWAS	RKLENGWYVG	EGWSDSQKTH	2895

NTD-A	59.7%
NTD-B	47.1%
NTD-C	51.5%
SPRY1	52.2%
RY1&2	53.4%
SPRY1	46.6%
SPRY2	29.0%
JSol	35.7%
BSol	48.5%



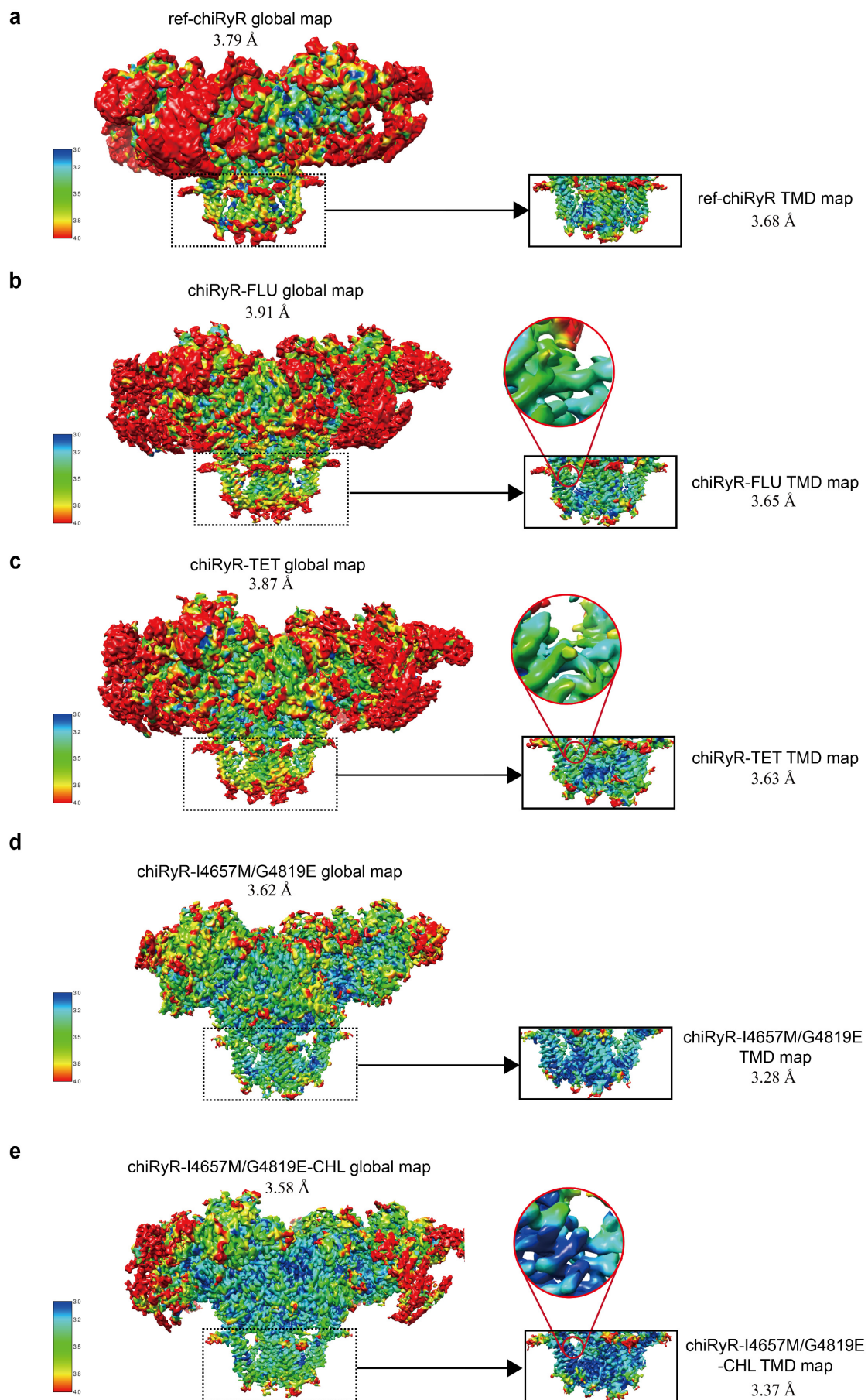
**Supplementary Figure 1. Sequence alignment between rRyR1 and Sf RyR.** Different domains are outlined in boxes of various colors, and the sequence identities of these domains are displayed on the right side. Amino acids that interact with diamides are marked with asterisks, while the resistance mutation sites are highlighted in purple.



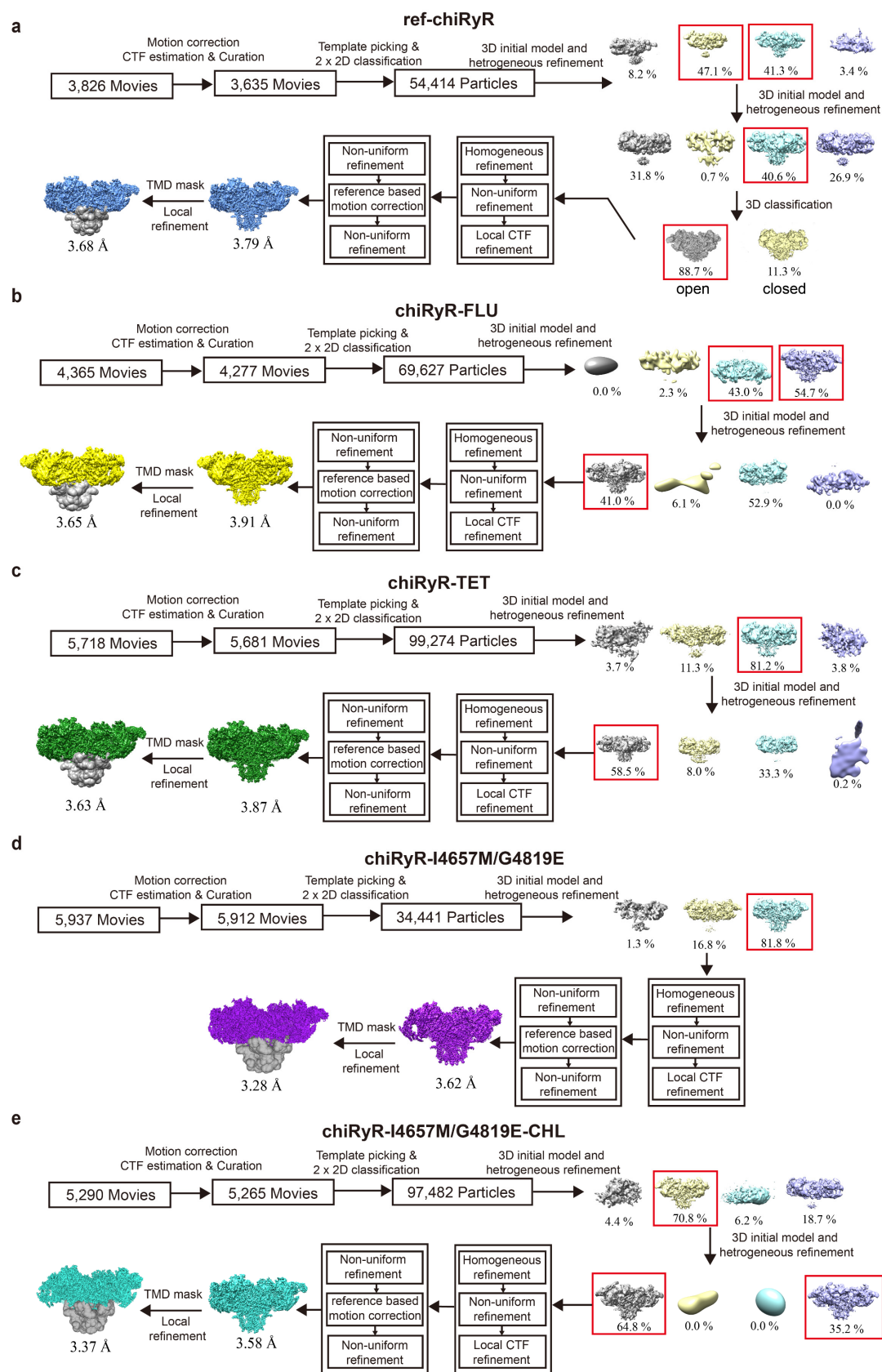


**Supplementary Figure 2. Purification and cryo-EM analysis of chiRyRs.** **a** Western blot showing the expression of chiRyR and chiRyR-I4657M/G4819E in HEK293 cells. CLNX protein was used as the internal control. Source data are provided as a Source Data file. **b** Purification of chiRyR. The elution profile of chiRyR by gel-filtration chromatography using a Superose 6 Increase column is shown in the left panel, and SDS-PAGE showing purified chiRyR is in the right panel. Source data are provided as a Source Data file. **c** Purification of chiRyR- I4657M/G4819E. The elution profile of chiRyR-I4657M/G4819E by anion exchange chromatography using a HiRes Q 5/50 column is shown in the left panel, and SDS-PAGE showing purified chiRyR-I4657M/G4819E is in the right panel. **d** Representative two-dimensional class averages for ref-chiRyR, chiRyR-FLU, chiRyR-TET, chiRyR-I4657M/G4819E, and chiRyR-I4657M/G4819E-CHL. **e** The orientation distributions of the particles used for the final reconstructions. **f** Summary of the Fourier shell correlation (FSC) curves generated by CryoSPARC for global refinement (left) and TMD local refinement (right).



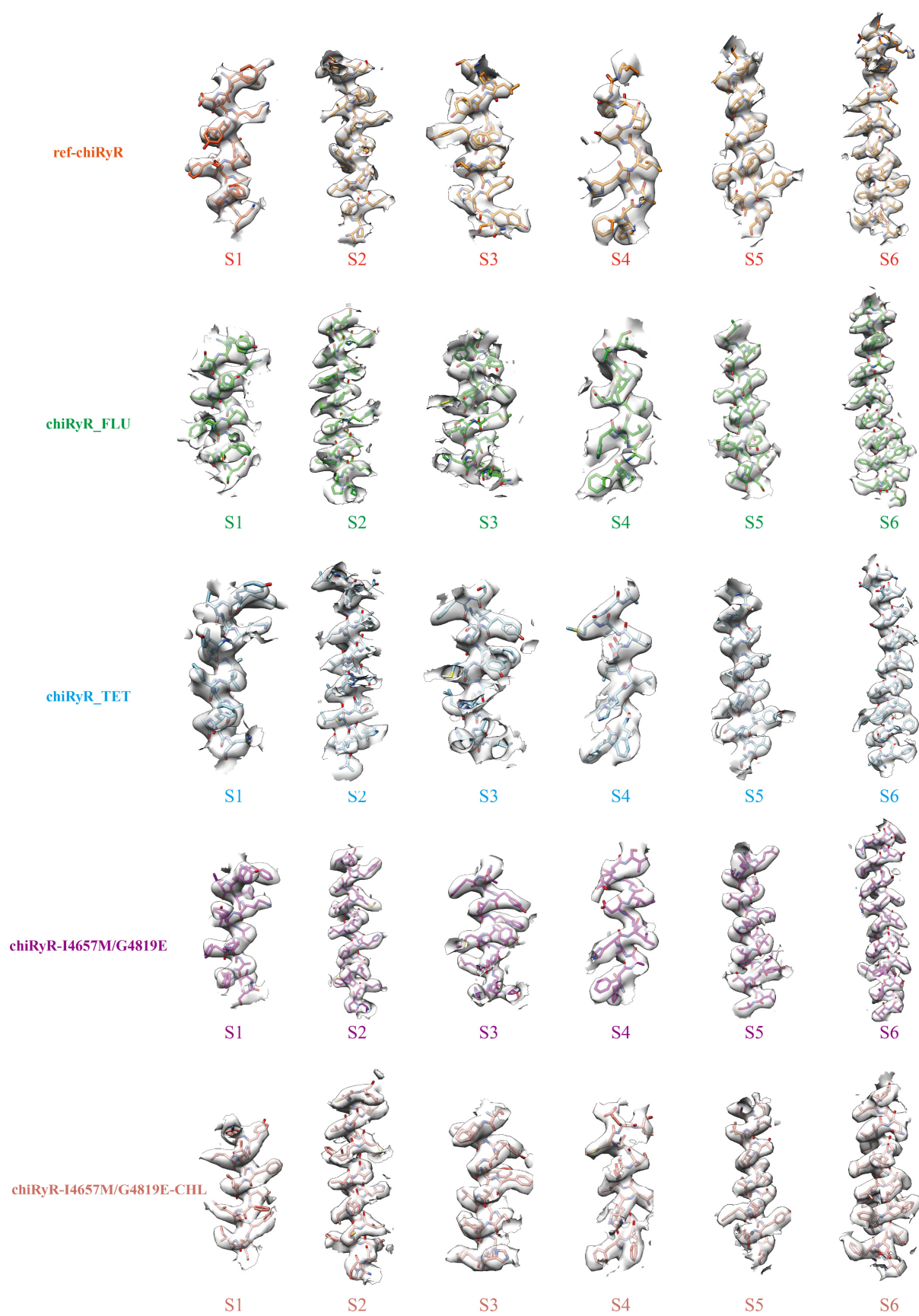


**Supplementary Figure 3. Local resolution estimates.** Local resolution estimation is shown by colored global and locally refined maps for ref-chiRyR (a), chiRyR-FLU (b), chiRyR-TET (c), chiRyR-I4657M/G4819E (d), and chiRyR-I4657M/G4819E-CHL (e). The color bar indicates resolution in angstroms (Å). The ligands in chiRyR-FLU, chiRyR-TET, and chiRyR-I4657M/G4819E-CHL are shown in zoomed-in insets.

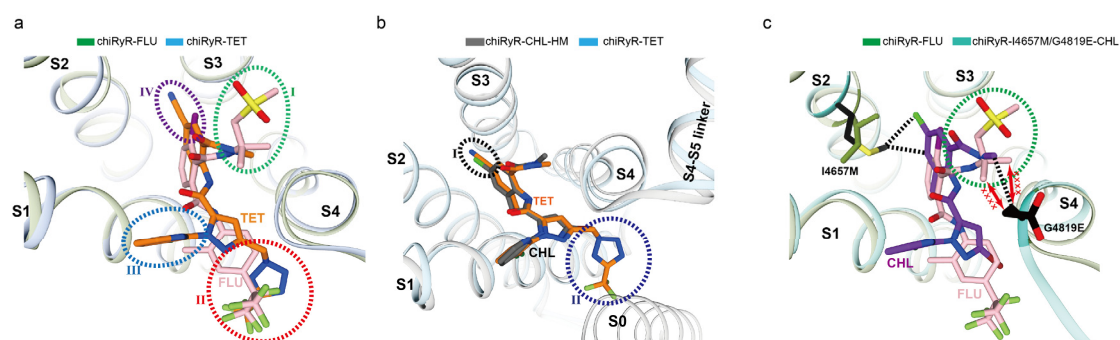


**Supplementary Figure 4. Cryo-EM processing pipeline.** Cryo-EM data collection, 3D classification, and refinement schemes for ref-chiRyR (a), chiRyR-FLU (b), chiRyR-TET (c), chiRyR-I4657M/G4819E (d), and chiRyR-I4657M/G4819E-CHL (e).

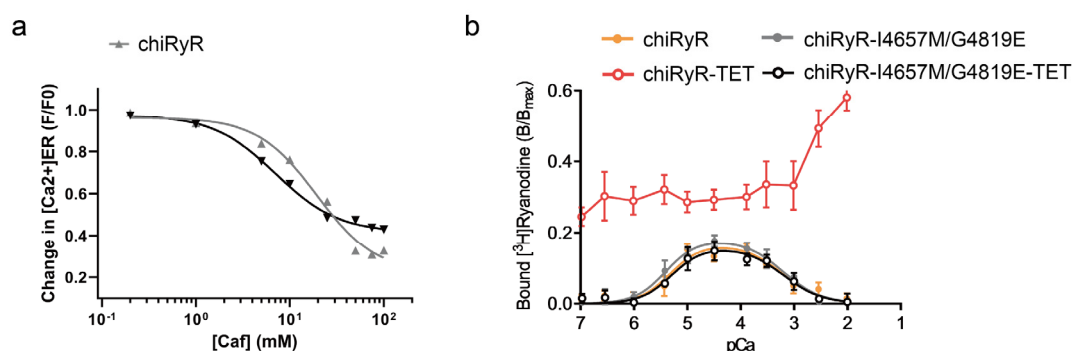




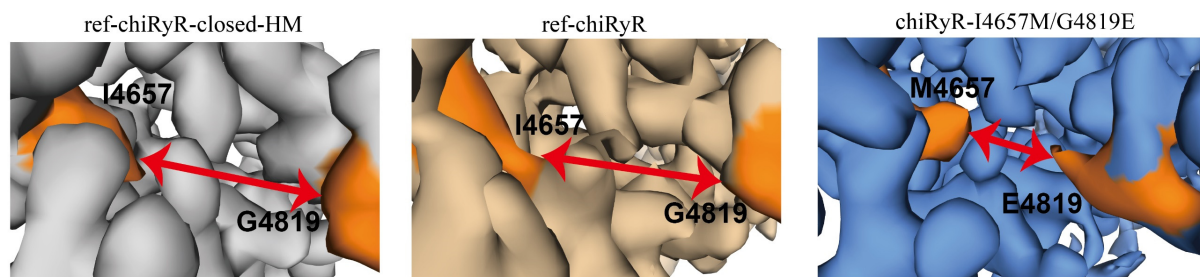
**Supplementary Figure 5. The density of the S1-S6 helices from different chiRyRs.**



**Supplementary Figure 6. Comparison of the diamide binding poses.** **a** The comparison of the diamide binding poses between chiRyR-FLU and chiRyR-TET reveals four major differences. **b** The comparison of the diamide binding poses between chiRyR-CHL (chiRyR-CH-HM: a homology model based on rRyR1-CHL (PDB ID: 6M2W)) and chiRyR-TET reveals two major differences. **c** The comparison of the diamide binding poses between chiRyR-FLU and chiRyR-I4657M/G4819E-CHL shows that FLU would have clash with the Glu4819.



**Supplementary Figure 7. Functional characterization of chiRyR and chiRyR-I4657M/G4819E.** **a** The dose-dependent curves of caffeine show that the resistance mutations does not significantly change the caffeine sensitivity of chiRyR. The EC<sub>50</sub> values for chiRyR and chiRyR-I4657M/G4819E are 19.5 mM and 7.1 mM, respectively (Supplementary Table 4). Source data are provided as a Source Data file. **b** Ca<sup>2+</sup>-dependent [<sup>3</sup>H]ryanodine binding of chiRyR and chiRyR-I4657M/G4819E in the absence or presence of TET. The biphasic Ca<sup>2+</sup>-dependency of chiRyR-I4657M/G4819E shows no significant difference compared to that of chiRyR, with EC<sub>50</sub> values for Ca<sup>2+</sup> 5.0 ± 1.1 μM (chiRyR) and 4.4 ± 2.4 μM (chiRyR-I4657M/G4819E) and IC<sub>50</sub> values for Ca<sup>2+</sup> are 0.67 ± 0.37 mM (chiRyR) and 0.59 ± 0.19 mM (chiRyR-I4657M/G4819E) (mean ± SD, n=4). 1 μM TET can promote [<sup>3</sup>H]ryanodine binding in chiRyR but not in chiRyR-I4657M/G4819E. Source data are provided as a Source Data file.



**Supplementary Figure 8. Comparison of the diamide binding pockets between wild-type and resistant RyRs.** Comparison of the density maps of the diamide-binding pocket between the closed-state ref-chiRyR (ref-chiRyR-closed-HM: a homology model based on closed rRyR1(PDB ID: 5TAQ), ref-chiRyR and chiRyR-I4657M/G4819E-CHL. The density map of ref-chiRyR-closed-HM was generated by the molmap module in UCSF Chimera. The density maps are shown at the  $5\sigma$  level. The two resistance mutation sites are colored in orange, and the distances between them are indicated by red arrows.



**Supplementary Table 1. EC<sub>50</sub> values of diamide insecticides measured by time-lapse fluorescence assay using stable cell lines expressing RyRs from different species**

Diamide	RyR variants	EC <sub>50</sub> <sup>a</sup>	95% CI	R <sup>2</sup>
FLU	rRyR1	4.5 µM	2.2 µM– 14.0 µM	0.874
	<i>Sf</i> RyR	59.3 nM	45.6 nM – 76.2 nM	0.984
	chiRyR	25.1 nM	20.7 nM – 30.6 nM	0.996
TET	rRyR1	7.6 µM	5.8 µM – 10.0 µM	0.978
	<i>Sf</i> RyR	174.5 nM	130.2 nM - 224.7 nM	0.979
	chiRyR	46.1 nM	35.4 nM – 62.8 nM	0.935

a: n = 3.

**Supplementary Table 2. EC<sub>50</sub> values of diamide insecticides measured by time-lapse fluorescence assay using stable cell lines expressing different rRyR1 mutants**

Diamide	RyR variants	EC <sub>50</sub> <sup>a</sup>	95% CI	R <sup>2</sup>
FLU	rRyR1	4.5 µM	2.2 µM– 14.0 µM	0.874
	rRyR1 R4563K	3.9 µM	3.5 µM – 4.3 µM	0.995
	rRyR1 F4564Y	26.1 µM	21.6 µM – 33.4 µM	0.985
	rRyR1 C4657I	8.9 µM	8.2 µM – 9.6 µM	0.995
	rRyR1 L4792S	208.9 nM	172.7 nM – 250.4 nM	0.976
CHL	rRyR1	4.9 µM	4.5 µM – 5.4 µM	0.996
	rRyR1 R4563K	62.6 nM	52.8 nM – 74.0 nM	0.985
	rRyR1 F4564Y	555.7 nM	478.9 nM – 645.0 nM	0.993
	rRyR1 C4657I	167.3 nM	153.2 nM – 183.0 nM	0.994
	rRyR1 L4792S	44.8 nM	42.7 nM – 47.2 nM	0.994

a: n = 3.

**Supplementary Table 3. Cryo-EM data collection, refinement and validation statistics**

Protein	chiRyR			chiRyR-I4657M/G4819E	
Condition	5 mM caffeine	5 mM caffeine	5 mM caffeine	5 mM caffeine	5 mM caffeine
	2 mM ATP	2 mM ATP	2 mM ATP	2 mM ATP	2 mM ATP
	100 μM	100 μM	100 μM	100 μM	100 μM
	CaM1234	CaM1234	CaM1234	CaM1234	CaM1234
		50 μM FLU in	50 μM TET in		625 μM CHL
PDB ID	2% DMSO	2% DMSO	2% DMSO	2% DMSO	in 5% DMSO
	8XLF	8XJI	8XKH	8XLH	8Y40
EMDB ID	EMD-38447,	EMD-38398,	EMD-38417,	EMD-38448,	EMD-38446,
	EMD-60900	EMD-38551	EMD-38553	EMD-60899	EMD- 60901
	for TMD	for TMD	for TMD	for TMD	for TMD
Data collection and processing					
Microscope	FEI Titan Krios				
Detector	Gatan K3 direct electron detector				
Magnification	×22,500				
Volotage (kV)	300				
Electron exposure (e <sup>-</sup> /Å <sup>2</sup> )	50				
Defocus range (μm)	1.0-2.4				
Pixel size (Å)	1.06				
Initial particle images	54,414	69,627	99,274	34,441	97,482
Final particle images	19,505	27,906	47,206	28,189	69,016
Map resolution (Å)	3.79	3.91	3.87	3.62	3.58
FSC threshold	0.143				
Model building and refinement					
Model composition					
Protein atoms	119,048	119,880	119,330	124,576	123,204
Ligands	16	20	20	16	20
R.M.S. deviations					
Bond length (Å)	0.008	0.007	0.007	0.009	0
Bond angles (°)	0.974	0.904	0.882	1.045	1.169
Validation					
MolProbity score	2.22	2.02	2.37	2.58	2.41
Clashscore	6.03	9.28	10.21	11.15	11.26
Rotamer outliers (%)	0.62	0.98	1.13	1.28	1.96
Ramachandran plot					
Favored (%)	89.86	94.13	92.36	84.61	87.19
Allowed (%)	9.90	5.51	6.65	14.26	11.78
Outlier (%)	0.62	0.35	1.00	1.24	1.02

**Supplementary Table 4. EC<sub>50</sub> values of diamide insecticides and caffeine measured by time-lapse fluorescence assay using stable cell lines expressing wild-type or resistant RyRs**

Diamide	RyR variants	EC <sub>50</sub> <sup>a</sup>	95% CI	R <sup>2</sup>
FLU <sup>b</sup>	chiRyR	25.1 nM	20.1 nM – 30.6 nM	0.996
	chiRyR-I4657M/G4819E	> 300 µM	-	-
	SfRyR	59.3 nM	45.6 nM – 76.2 nM	0.984
	SfRyR_I4734M/G4891E	> 300 µM	-	-
CHL	chiRyR	1.6 nM	1.2 nM – 2.1nM	0.965
	chiRyR-I4657M/G4819E	14.6 µM	10.1 µM – 21.2 µM	0.893
	SfRyR	5.5 nM	5.3 nM – 5.7 nM	0.998
	SfRyR_I4734M/G4891E	9.1 µM	7.1 µM – 11.5 µM	0.982
Caffeine	chiRyR	19.5 mM	14.3 mM – 35.7 mM	0.983
	chiRyR-I4657M/G4819E	7.1 mM	4.6 mM – 11.7 mM	0.970

a: n = 3; b: Due to the solubility limit, the highest tested concentration of FLU was 300 µM.

**Supplementary Table 5. EC<sub>50</sub> values of diamide insecticides measured by [<sup>3</sup>H]Ryanodine binding assay**

	chiRyR <sup>a</sup>	chiRyR-I4657M/G4819E <sup>a</sup>
CHL	1.7 ± 0.3 nM	14.7 ± 1.6 µM
TET	11.1 ± 0.9 nM	87.8 ± 6.4 µM
FLU	4.0 ± 1.4 nM	-

a: n = 4.

**Supplementary Table 6. The LD<sub>50</sub> values of two diamide insecticides in different Cas9 *Drosophila melanogaster***

Diamide	<i>Drosophila</i>	LD <sub>50</sub> <sup>a</sup>	95% CI	R <sup>2</sup>
FLU*	M4758I	10.4 µM	8.4 µM - 12.8 µM	0.973
	G4915E	AA.	-	-
CHL	M4758I	127.9 nM	107.8 nM – 152.8 nM	0.977
	G4915E	1.6 mM	460.1 µm – 30.2 mM	0.711

a: n = 30; AA.: All alive.

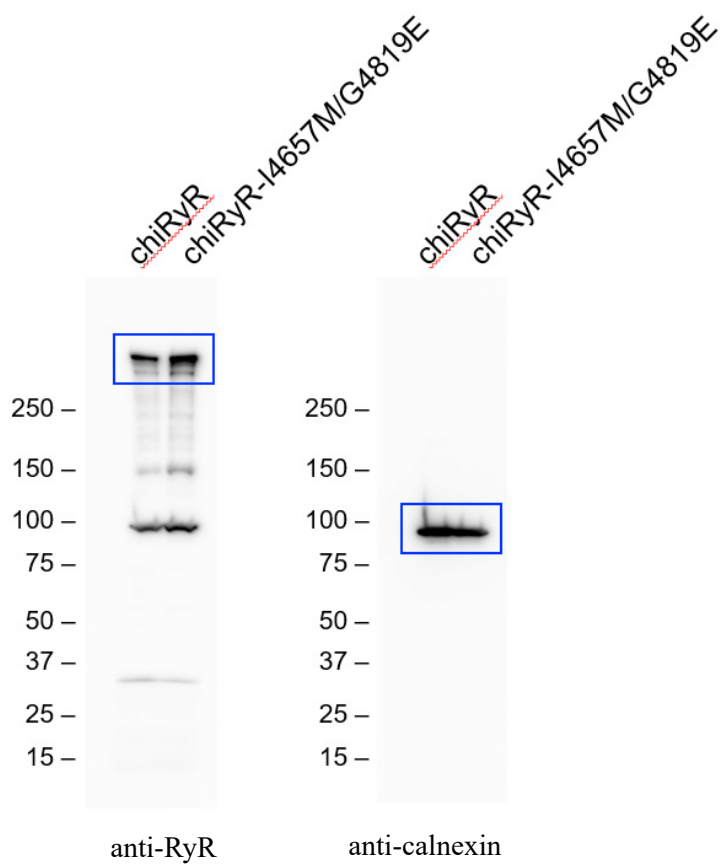


**Supplementary Table 7. Primers for rRyR1 and *Sf* RyR mutations**

Name	Sequence (5'→ 3')
rRyR1-R4563K-F	CTACACCCTGAAGTTCCTTGCC
rRyR1-R4563K-R	GGCAAGGAACTTCAGGGTGTAG
rRyR1-F4564Y-F	CACCCTGCGCTACCTTGCCCTCTTC
rRyR1-F4564Y-R	GAAGAGGGCAAGGTAGCGCAGGGTG
rRyR1-C4657I-F	GCCTTCCTCATCATCATCGG
rRyR1-C4657I-R	CCGATGATGATGAGGAAGGC
rRyR1-L4792S-F	G TTCCTGTACAGCGGCTGGTAC
rRyR1-L4792S-R	GTACCAGCCGCTGTACAGGAAC
rRyR1-I4657M-F	GTGGCCTTCCTCATGATCATCGGCTAC
rRyR1-I4657M-R	G TAGCCGATGATCATGAGGAAGGCCAC
rRyR1-G4819E-F	CATCGCCATGGAGGTCAAGACGCTG
rRyR1-G4819E-R	CAGCGTCTTGACCTCCATGGCGATG
<i>Sf</i> RyR-I4734M-F	GTGTCGCTCGCTATGCTCATCGGGT
<i>Sf</i> RyR-I4734M-R	ACCCGATGAGCATAGCGAGCGACAC
<i>Sf</i> RyR-G4891E-F	AGATGTCGCTGTCGAGTTCAAGACC
<i>Sf</i> RyR-G4891E-R	GGTCTTGA ACTCGACAGCGACATCT

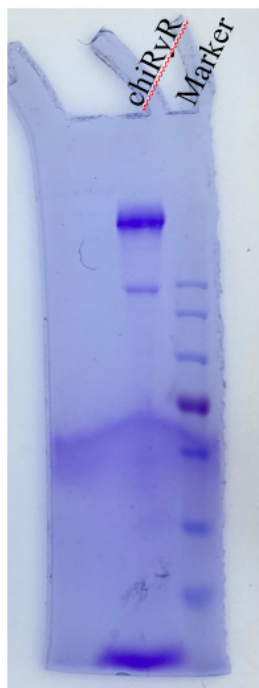
For Source Data

Supplementary Figure 2a uncropped blot



## For Source Data

Supplementary Figure 2a uncropped gel



Supplementary Figure 2c uncropped gel

