



Supplementary Materials for

Structure of the STRA6 receptor for retinol uptake

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This PDF file includes:

Figs. S1 to S14
Tables S1 to S3
Captions for Movies S1 to S3

Other Supplementary Materials for this manuscript includes the following:

Movies S1 to S3

Table S1: Data collection and validation statistics for STRA6-CaM cryo-EM structure.

Dataset	<i>Danio rerio</i> STRA6-CaM
Pixel size (Å)	1.255
Number of grids	2
Number of micrographs (total)	2613
Number of micrographs (final)	1657
Number of picked particles	317416
Final number of particles	56615
Box size (pixels)	200
Symmetry	C2
Resolution, overall	3.9
Est. accuracy rotations (degrees)	2.49
Est. accuracy translations (pixels)	0.72
Applied B-factor (sharpening)	-113.68
Molprobit clashscore	5.23
Ramachandran favored (%)	94.03
Ramachandran allowed (%)	5.28
Ramachandran outliers (%)	0.69
Rotamer outliers (%)	0.16

Table S2: Data collection and refinement statistics for CaM-CaMBP2 complex crystal structure. Values in the highest resolution shell (1.801-1.739) are given in parentheses.

Wavelength	0.97919
Resolution range	25.37 - 1.739 (1.801 - 1.739)
Space group	C 1 2 1
Unit cell	156.5, 37.2, 35.7; 90, 94.4, 90
Total reflections	67579 (3076)
Unique reflections	21134 (2084)
Multiplicity	3.2 (2.6)
Completeness (%)	98.8 (97.8)
Mean I/sigma(I)	6.7 (1.2)
Wilson B-factor	18.29
R-merge	0.095 (0.658)
R-meas	0.131 (0.882)
CC1/2	0.986 (0.609)
Reflections used in refinement	21098 (2083)
Reflections used for R-free	1026 (115)
R-work	0.2279 (0.3448)
R-free	0.2672 (0.4032)
Number of non-hydrogen atoms:	1504
macromolecules	1364
ligands	10
Protein residues	172
RMS(bonds)	0.007
RMS(angles)	0.88
Ramachandran favored (%)	100
Ramachandran allowed (%)	0
Ramachandran outliers (%)	0
Rotamer outliers (%)	0
Molprobrity clashscore	2.97
Average B-factor:	32.35
macromolecules	32.06
ligands	30.25
solvent	35.52
Number of TLS groups	5

Table S3: STRA6 MWS missense mutations

Mutation	zSTRA equivalent position	Structural element	Reference
P90L	P77	TM1TM2L	Pasutto, F. <i>et al.</i> , Am. J. Human Gen. 2007
P293L	P272	JM1TM6L	Pasutto, F. <i>et al.</i> , Am. J. Human Gen. 2007
T321P	T300	TM6	Pasutto, F. <i>et al.</i> , Am. J. Human Gen. 2007
T644M	T613	CaMBP2	Pasutto, F. <i>et al.</i> , Am. J. Human Gen. 2007
R655C	R626	α CT	Pasutto, F. <i>et al.</i> , Am. J. Human Gen. 2007
G217E	A194	TM5	White, T. <i>et al.</i> , Mol. Vis. 2008
Q438R	Q417	TM8	Chassaing, N. <i>et al.</i> , Human Mut. 2009
R638P	R609	CaMBP2	Chassaing, N. <i>et al.</i> , Human Mut. 2009
D560H/R655H	D539/R626	JML/ α CT	Segel, R. <i>et al.</i> , Am. J. Med. Gen. 2009
G304K	F283	TM6	Casey, J. <i>et al.</i> , Human Mut. 2011
R408Q	R387	TM7TM8L	Slavotinek, A.M. <i>et al.</i> , Clin. Genet. 2015
G339S	G318	TM6TM7L (lid helix)	Kawaguchi, R., <i>et al.</i> , J. Biol. Chem. 2008

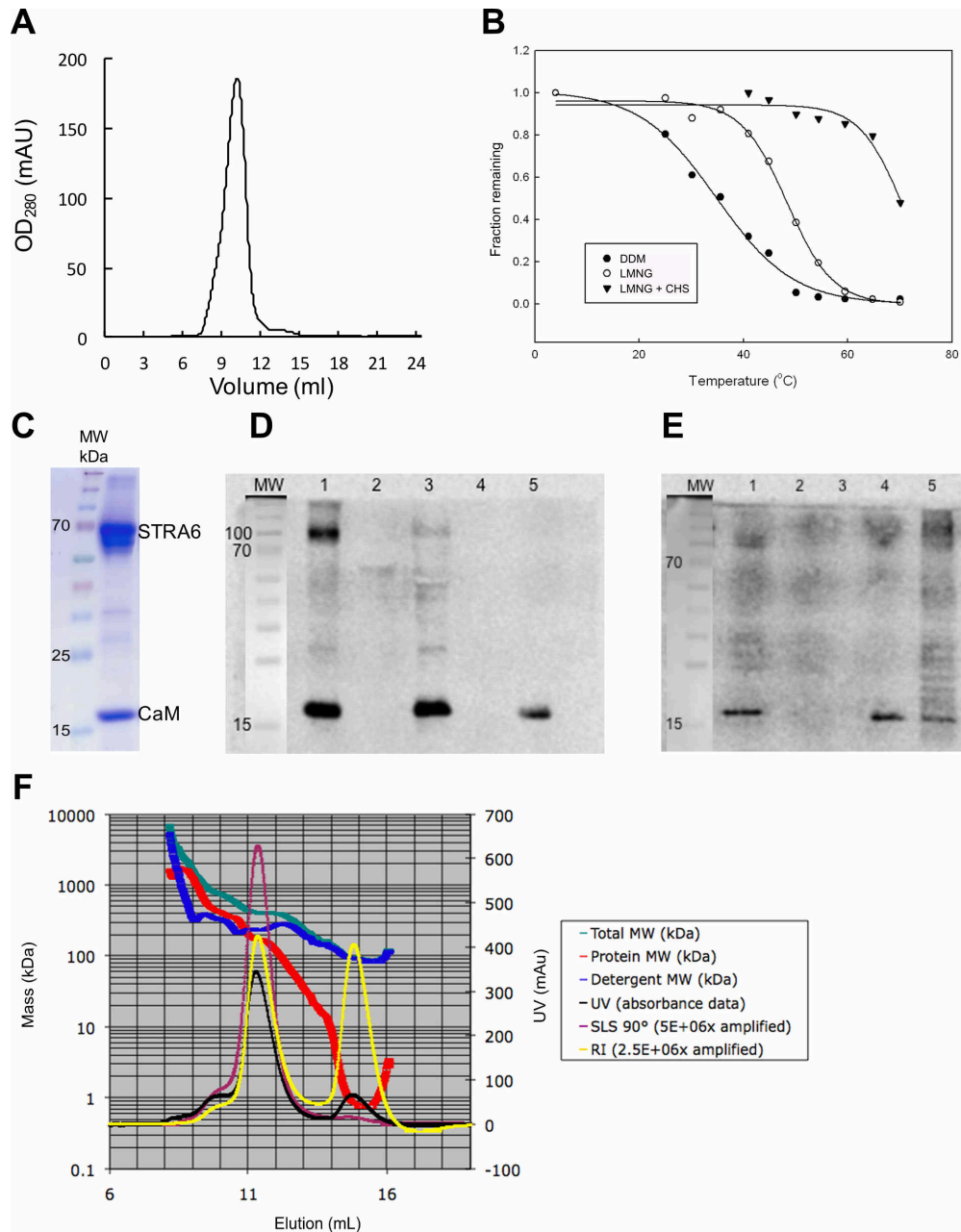


Fig. S1. Purification and biochemical characterization of zebrafish STRA6. (A) Elution profile from size exclusion chromatography of STRA6 purified in buffer containing LMNG and CHS. (B) Denaturation curves of STRA6 purified in β DDM (black circles), LMNG (MNG, white circles) and LMNG/CHS (MNG+CHS, black triangles). The fraction of folded protein remaining is plotted against a control sample maintained at 4 °C. (C) Purified STRA6 analyzed on SDS-PAGE denaturing gel, stained by Coomassie blue. (D) Immunoblot of STRA6-CaM complex purified from transiently transfected HEK cells. Probed with anti-CaM antibody. Lanes as follows: (1): zebrafish STRA6-CaM purified from insect cells and reconstituted in amphipol; (2): Pulldown from HEK cells transfected with control plasmid pFM1.2; (3): Pulldown from HEK cells transfected with expression plasmid for zebrafish STRA6; (4): Empty; (5):

Purified recombinant human CaM (5ng). **(E)** Immunoblot of STRA6-CaM complex immunoprecipitated using an anti-STRA6 antibody from native zebrafish tissue. Probed with anti-CaM antibody. Lanes as follows: (1): zebrafish STRA6 immunoprecipitated using antibodies against the recombinant protein purified from insect cells; (2): Immunoprecipitation using control antibody; (3): Empty; (4) Purified recombinant human CaM; (5): Supernatant from immunoprecipitation (5 μ g total protein). **(F)** Oligomerization state of STRA6 in a solution containing β DDM, determined by multi angle light scattering coupled to refractive index and UV measurements. The estimated molecular weight of the protein component is ~200 kDa (left peak), consistent with STRA6 being a dimer in solution associated with two molecules of CaM. The peak to the right corresponds to excess β DDM in micelles of ~100 kDa.

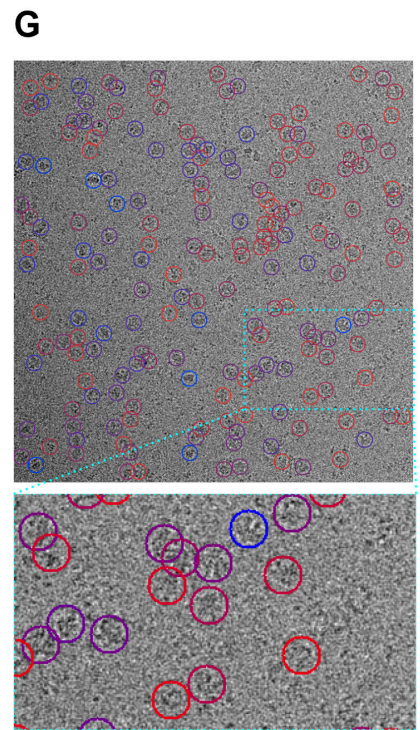
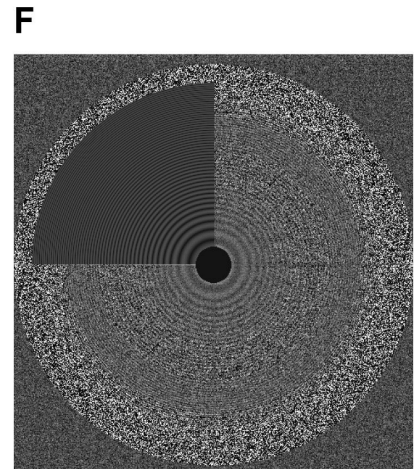
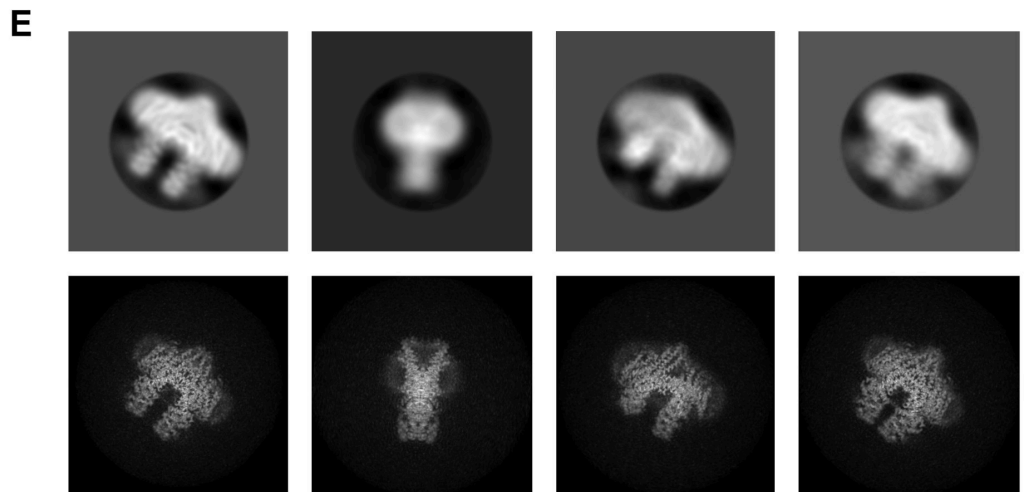
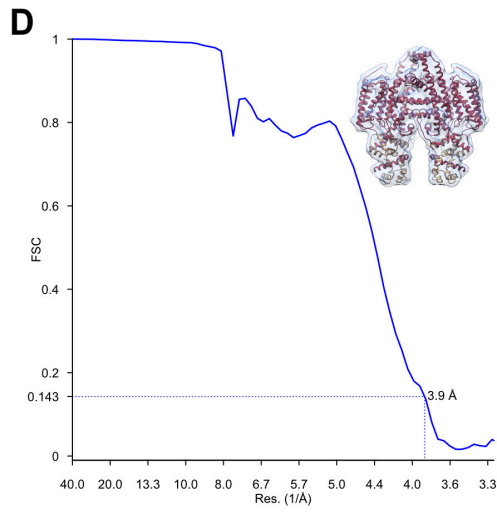
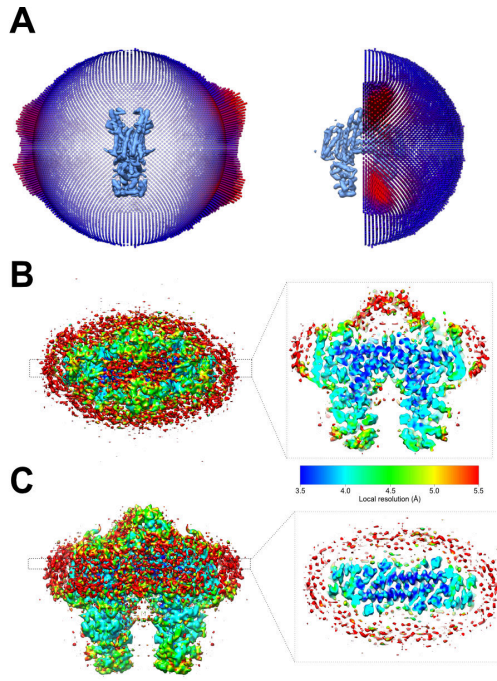


Fig. S2. Cryo-EM analysis of *Danio rerio* STRA6. (A) Orientation distribution of particles in the final C2-symmetric reconstruction. The reconstruction is positioned in the center of a sphere as reference, and a histogram displaying the frequency of each unique orientation is represented on the surface of the sphere, with the length of each cylindrical bar proportional to the population size of particles in the final reconstruction with that angular orientation. (B) Final reconstruction colored by local resolution, calculated using ResMap (68), viewed from the extracellular region. A central vertical slab through the TM region is represented at right. (C) As B, viewed parallel to the membrane, with a slab taken through the TM region displayed in the inset. (D) Fourier Shell Correlation plot of the final refined reconstruction. The resolution at which the curve drops below 0.143 is indicated, and a thumbnail of the mask used for FSC calculation is inset, overlaid on the atomic model. (E) Four representative 2D class averages (upper panels) with maximum intensity projections of the final reconstruction in matching orientations (lower panels) (F) Power spectrum of a representative stack, calculated as an average of the power spectra of the frames in the stack with a window size of 3 frames. The contrast transfer function, fit using CTFFIND4 to a resolution of 3.0 Å, is displayed in the upper left quadrant. (G) Representative micrograph with particles picked using *reliion_autopick* (prior to 2D and 3D classification) circled and colored by the autopick figure of merit. A magnified view of an indicated section of the micrograph is represented below the main panel.

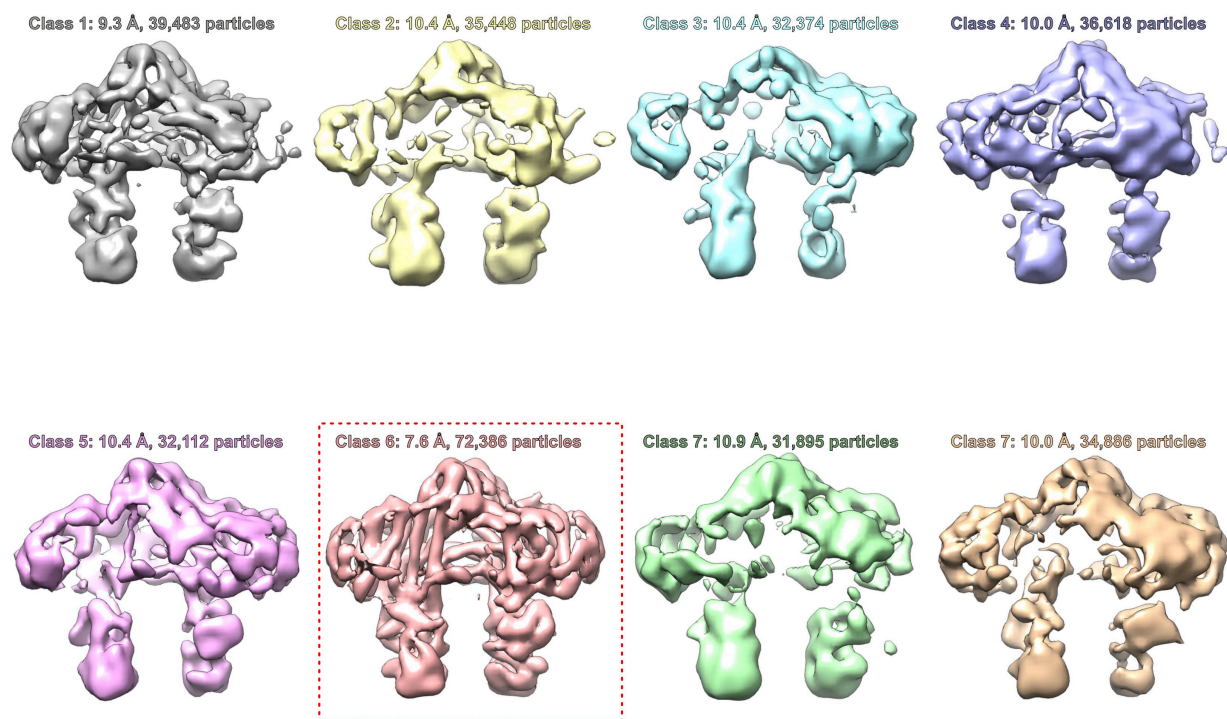


Fig. S3. Analysis of heterogeneity by 3D classification. The eight classes obtained from 3D classification with Relion 1.3 are viewed parallel to the membrane with the resolution where the SSNR of the class drops below 2.0, as well as the number of particles in each class, labeled above each panel. Class 6, which was chosen for high-resolution refinement, is enclosed within a dashed red box.

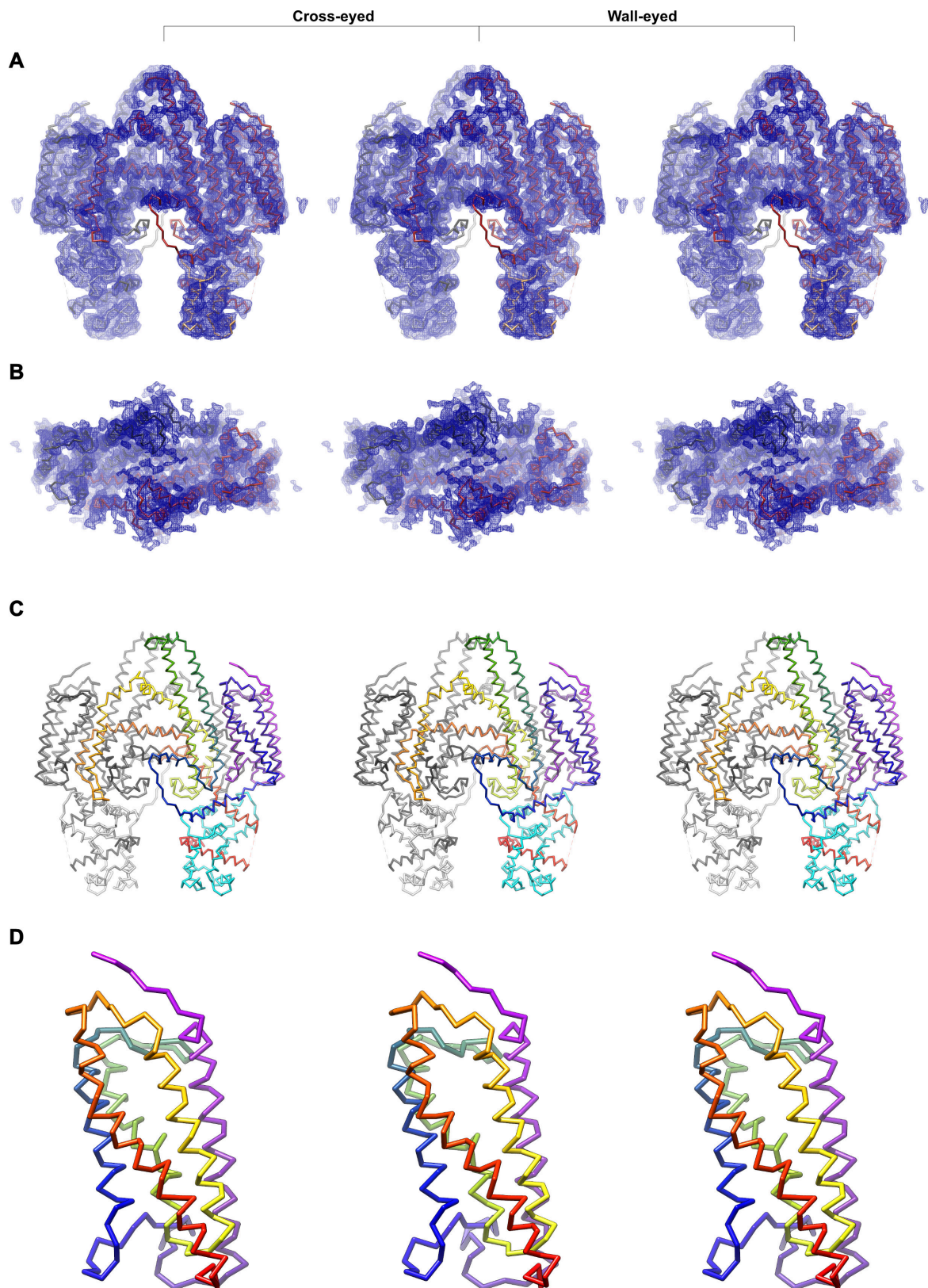


Fig. S4. STRA6 fold and global quality of the cryo-EM density map. All panels are represented in stereo, as indicated. All density maps are contoured at 6

X R.M.S. **(A & B)** Two views of the density maps are shown, together with the $C\alpha$ trace of the entire structure, in which one STRA6 protomer is displayed in dark red, and one CaM in yellow. **(C)** STRA6-CaM is depicted in licorice representation. One protomer of STRA6 is represented in spectral coloring, from violet N-terminus to red C-terminus. One protomer of CaM is colored cyan. **(D)** The TM1-TM5 bundle (residues 31-209) is depicted in licorice representation, in spectral coloring from violet (31) to red (209). The viewpoint is parallel to the membrane and to the IM helix axis. The N-terminal segment of STRA6, preceding TM1 is adjacent to the TM4-5 loop. The relative position of these two segments is consistent with the presence of a predicted disulfide bond between C31 and C171 (15). A distinctive feature of the NTD is that TM3 has a two-proline kink in the middle of the membrane, separating it into two segments (TM3a and TM3b) with an interhelical angle of 17°

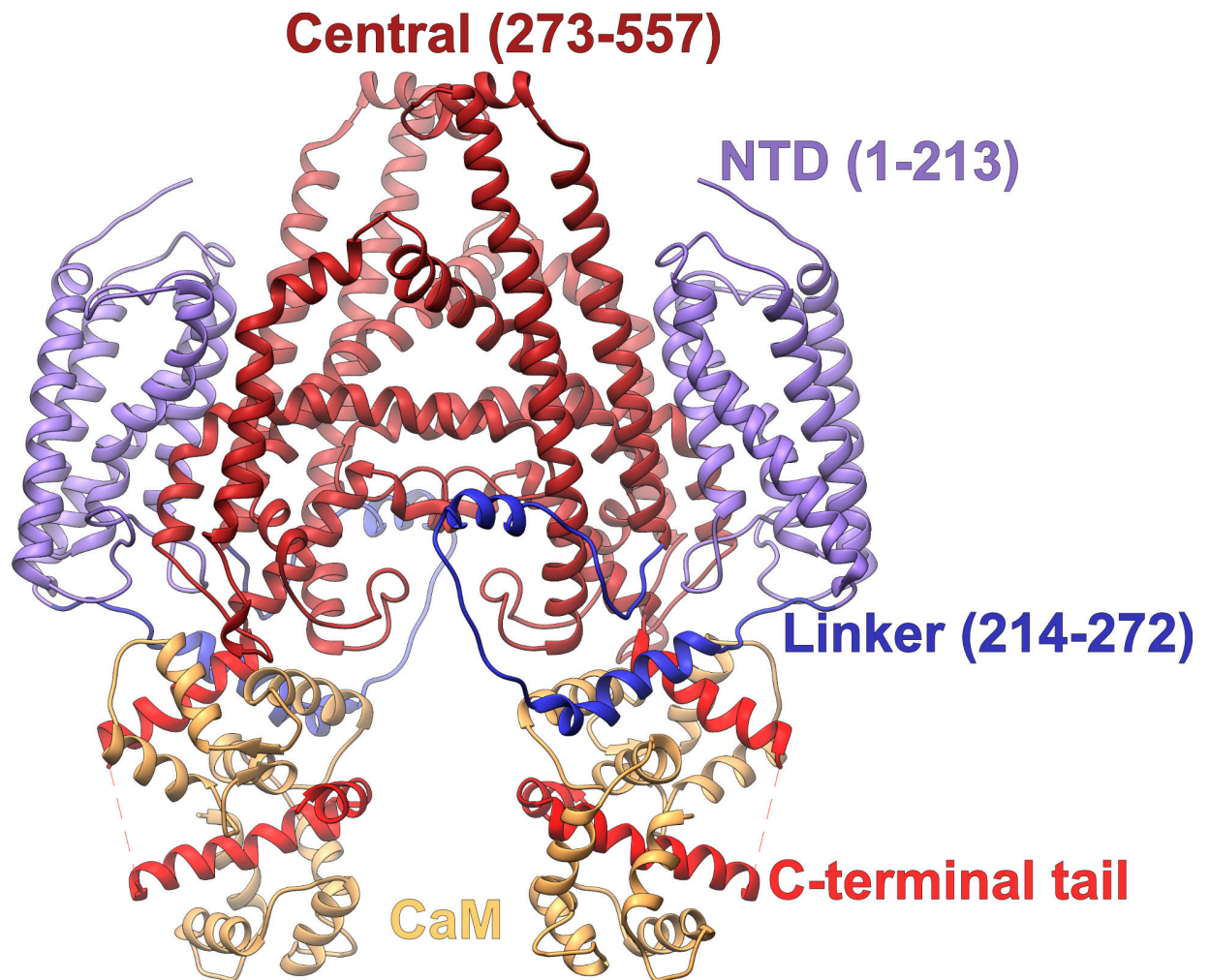


Fig. S5 Domain representation of the STRA6-CaM complex. Ribbon representation of the STRA6-CaM complex colored by domain, and labeled. Residue ranges are provided in parenthesis.

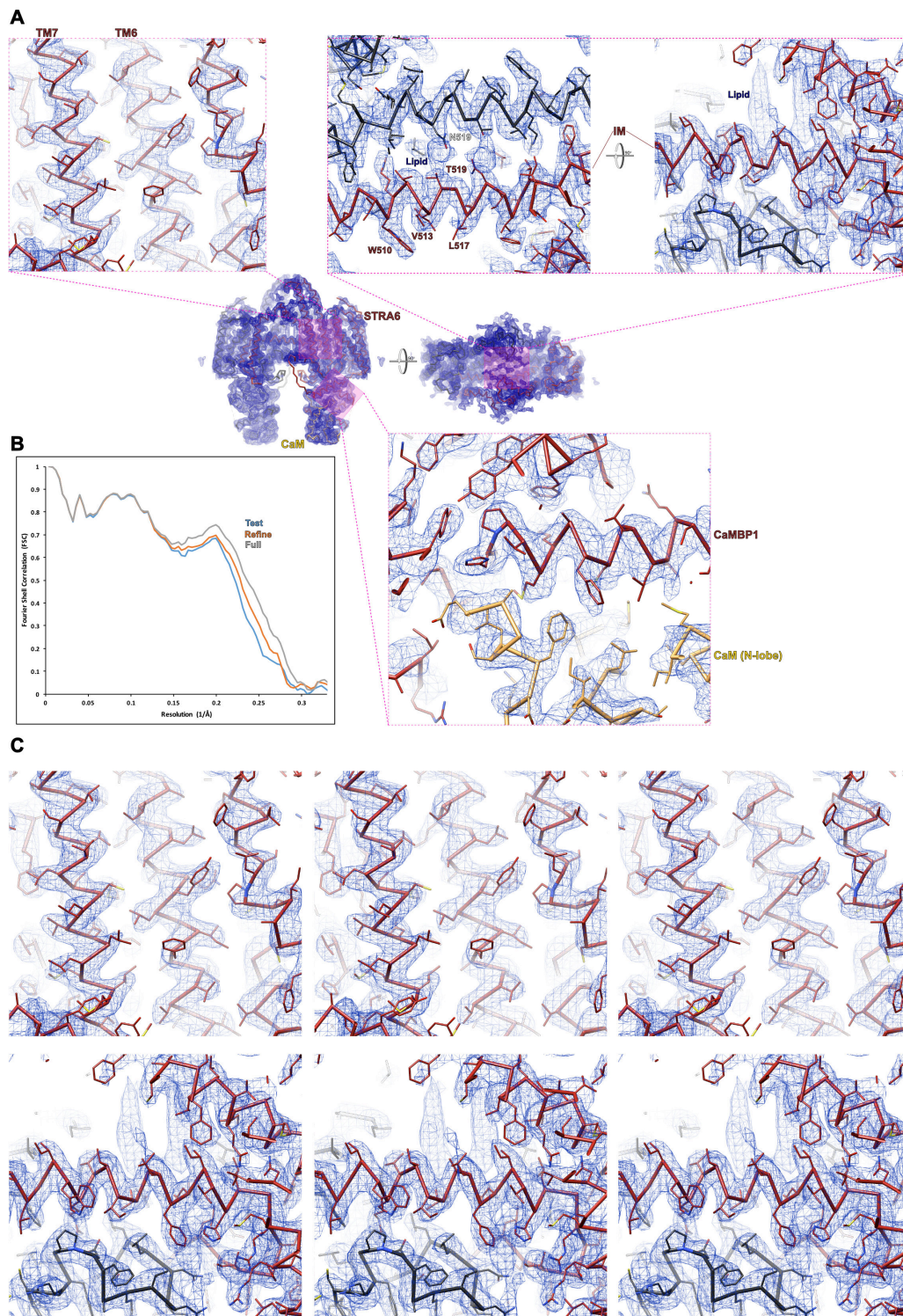


Fig. S6. Quality of the cryo-EM density map. (A) Two views of the STRA6-CaM complex are represented in the center and three insets are arranged around it, showing density quality in specific regions of the structure, with the structure represented as a $C\alpha$ trace with side chains in stick representation. (B) Model-map FSC curves calculated for the full map used for initial model building (gray); the test half-map withheld from the cross-validation refinement run (orange) and the

half-map used in the cross-validation refinement (blue). For details of model-map FSC calculations see Materials & Methods. Stereo views of two of the insets from (A) are shown in panel (C). Density maps are contoured at 6 X R.M.S.

D. rerio (A4IG66) /1-670
H. sapiens (Q9XK79) /1-667
L. oculatus (W5NCL1) /1-664
O. niloticus (I3KE07) /1-658
X. maculatus (M4AN84) /1-665
P. formosa (A0A087XK69) /1-665
A. mexicanus (W5LF12) /1-668
G. gallus (F1NSC4) /1-671
F. albicollis (U3KCM6) /1-645
M. domestica (F6X9H5) /1-684
R. norvegicus (Q4QR83) /1-670
M. musculus (070491) /1-670
H. glaber (G5BRG2) /1-662
C. porcellus (H0VUR2) /1-671
C. familiaris (J9NW10) /1-655
L. africana (G37653) /1-669
O. garnettii (H0X180) /1-707
P. anubis (A0A096MM01) /1-704
C. sabaueus (A0A09RNU1) /1-659
M. mulatta (F7GR11) /1-667
M. fascicularis (G7P935) /1-667
G. Gorilla (G3R6K2) /1-707
P. abelli (Q5R7B4) /1-667
B. taurus (F1N406) /1-668
P. alecto (L5JW03) /1-658
E. caballus (F626X7) /1-667

-----MSAETVN-NYDYSWYENALPTKAPV-----EVIPPCDPTADEGL 39
-----MSSQPA--GNQTSFGATE-DYSYSGWYDEPOGGEEL-OPE-----EVIPPCDPTADEGL 51
-----KAMASIDVPKDDSYEYVQLEPTTSP-----DPTLQCDPTADDDRL 43
-----MDYDPE--LDPLPSKIEP-----EVIPPCDPTADDDRL 31
-----MKDALV-DYEYD--LDPLPSKIEP-----ETIPPCDPTADDDRL 37
-----MKDALV-DYEYD--LDPLPSKIEP-----ETIPPCDPTADDDRL 40
-----MSAETGKDYDYSWYENALPTKPI-----EVIPPCDPTADDDGL 37
-----MAANNSGAHGSLLDSDYIYETAEPAP--QDDMFPSTIPEDDPTVSPRL 57
-----DNYLYESTESTP--QDDLYESTESTP--QDDLYESTESTP--QDDLYESTESTP 32
-----MDEILSLPFSASLCEVPEKMSLSLAA--ANQSS--GSE--DYSYSGWYDEPOGGEEL-OPE-----EVIPPCDPTADEGL 72
-----MESQASENGSTQSSGVD--DYS--SWYIEEPLGAEV-OPE-----GVNPLCOPTVAPPAL 51
-----MESQASENGSTQSSGVD--DYS--SWYIEEPLGAEV-OPE-----GVNPLCOPTVAPPAL 51
-----MSLSQAA--GNQTSFGAD--DYS--SWYDEPOGKEL-OPE-----GEVPSOPSPVAPPAL 50
-----MSLSQAA--ENQTSRAAD--DYS--SWYDEPLEDKEL-OPE-----GEVPSOPSPVAPPAL 50
-----MSSQAA--GNQTSAGAD--DYS--DNYLDEPODQEL--PAE--GAVPSCHPVLSPGL 49
-----MSSQAA--GNQTS--GATE--DYSYSGWYDEPPGEEP--KPE--RNVLSOTSPVAPPAL 57
-----IGDQGTGGTTRGSPQPAPWDLSPRSFRLKARVGGELKMSQAA--GNQTS--STG--DYS--DNYLDEPOGGEEL--OPD--GTVPSCHPSPVAPPAL 52
-----MCMCECAPIRRAHPLELTPGHEVDLGLCLFMDSQDQAQEEGKRRMSQPA--GNQTSFGATE--DYSYSGWYDEPOGGEEL-OPE-----GEVPSCHPSPVAPPAL 56
-----MSSQPA--GNQTSFGATE--DYSYSGWYDEPOGGEEL-OPE-----GEVPSCHPSPVAPPAL 51
-----MSSQPA--GNQTSFGATE--DYSYSGWYDEPOGGEEL-OPE-----GEVPSCHPSPVAPPAL 51
-----MSSQPA--GNQTSFGATE--DYSYSGWYDEPOGGEEL-OPE-----GEVPSCHPSPVAPPAL 51
-----MSSQPA--GNQTSFGATE--DYSYSGWYDEPOGGEEL-OPE-----GEVPSCHPSPVAPPAL 51
-----MGGKGGGDTTRGVLFPFPCQAQLSPRRAPRELKGGKRRMSQPA--GNQTSFGATE--DYSYSGWYDEPOGGEEL-OPE-----GEVPSCHPSPVAPPAL 50
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-----MSTQAA--GNQTSFGATE--DYSYSGWYDEPOGGEEL-OPE-----GLVPSOPSPVAPPAL 52
-----MSEAA--RMTQSSGTD--DYSYSGWYDEPOGGEEL-OPE-----GAVPSCHPSPVAPPAL 50
-----MSSQAT--GNQTSQAD--DYSYSGWYDEPPGQEL-OPE-----GVVPPCHPSPVAPPAL 51



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40 FHICIAAIIILVVMVLIILARRRQKI--SDNRQLTGLIIVFNLDHTQHKGLAVAVYGVLFCKLVGMVLSHHPLEPTKEVAN-----KEFWMLLALYLY 131
52 YHACLASLIIIVLLIILAVLRVRRQLWPCDVRGRGRLPVDVLAGDRPRAVPAVAVFVLSLCLLDPEDLFFLTLASAPSDQKTEAPRGAWKLGLGFY 154
44 YHCTIAAIIIVLLIILAVLRVRRQLWPCDVRGRGRLPVDVLAGDRPRAVPAVAVFVLSLCLLDPEDLFFLTLASAPSDQKTEAPRGAWKLGLGFY 138
32 YHICTVILVLLVLIILAVLRARMKV--GDRKGLPGLIPVFNLDHTQHKGLAVAVYGVLFCKLVGMVLSHHPLEPTKEVAN-----KEFWMLLALYLY 123
38 YHICTVILVLLIILAVLRARMKV--GDRKGLPGLIPVFNLDHTQHKGLAVAVYGVLFCKLVGMVLSHHPLEPTKEVAN-----KEFWMLLALYLY 129
38 YHICTVILVLLIILAVLRARMKV--GDRKGLPGLIPVFNLDHTQHKGLAVAVYGVLFCKLVGMVLSHHPLEPTKEVAN-----KEFWMLLALYLY 129
41 YHICTVILVLLIILAVLRARMKV--GDRKGLPGLIPVFNLDHTQHKGLAVAVYGVLFCKLVGMVLSHHPLEPTKEVAN-----KEFWMLLALYLY 132
58 YHCTMPTIILVLLIILAVLRVRRQLWPCDVRGRGRLPVDVLAGDRPRAVPAVAVFVLSLCLLDPEDLFFLTLASAPSDQKTEAPRGAWKLGLGFY 152
33 YHCTMPTIILVLLIILAVLRVRRQLWPCDVRGRGRLPVDVLAGDRPRAVPAVAVFVLSLCLLDPEDLFFLTLASAPSDQKTEAPRGAWKLGLGFY 127
73 YHCTMPTIILVLLIILAVLRVRRQLWPCDVRGRGRLPVDVLAGDRPRAVPAVAVFVLSLCLLDPEDLFFLTLASAPSDQKTEAPRGAWKLGLGFY 167
44 YHCTMPTIILVLLIILAVLRVRRQLWPCDVRGRGRLPVDVLAGDRPRAVPAVAVFVLSLCLLDPEDLFFLTLASAPSDQKTEAPRGAWKLGLGFY 138
52 YHACLAVLIIIVLLIILAVLRVRRQLWPCDVRGRGRLPVDVLAGDRPRAVPAVAVFVLSLCLLDPEDLFFLTLASAPSDQKTEAPRGAWKLGLGFY 154
51 YHACLAVLIIIVLLIILAVLRVRRQLWPCDVRGRGRLPVDVLAGDRPRAVPAVAVFVLSLCLLDPEDLFFLTLASAPSDQKTEAPRGAWKLGLGFY 153
50 YHACLAVLIIIVLLIILAVLRVRRQLWPCDVRGRGRLPVDVLAGDRPRAVPAVAVFVLSLCLLDPEDLFFLTLASAPSDQKTEAPRGAWKLGLGFY 152
51 YHACLAVLIIIVLLIILAVLRVRRQLWPCDVRGRGRLPVDVLAGDRPRAVPAVAVFVLSLCLLDPEDLFFLTLASAPSDQKTEAPRGAWKLGLGFY 153
51 YHACLAVLIIIVLLIILAVLRVRRQLWPCDVRGRGRLPVDVLAGDRPRAVPAVAVFVLSLCLLDPEDLFFLTLASAPSDQKTEAPRGAWKLGLGFY 153
93 YHACLAVLIIIVLLIILAVLRVRRQLWPCDVRGRGRLPVDVLAGDRPRAVPAVAVFVLSLCLLDPEDLFFLTLASAPSDQKTEAPRGAWKLGLGFY 154
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52 YHACLAVLIIIVLLIILAVLRVRRQLWPCDVRGRGRLPVDVLAGDRPRAVPAVAVFVLSLCLLDPEDLFFLTLASAPSDQKTEAPRGAWKLGLGFY 154
52 YHACLAVLIIIVLLIILAVLRVRRQLWPCDVRGRGRLPVDVLAGDRPRAVPAVAVFVLSLCLLDPEDLFFLTLASAPSDQKTEAPRGAWKLGLGFY 154
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52 YHACLAVLIIIVLLIILAVLRVRRQLWPCDVRGRGRLPVDVLAGDRPRAVPAVAVFVLSLCLLDPEDLFFLTLASAPSDQKTEAPRGAWKLGLGFY 154
53 YHCTMPTIILVLLIILAVLRVRRQLWPCDVRGRGRLPVDVLAGDRPRAVPAVAVFVLSLCLLDPEDLFFLTLASAPSDQKTEAPRGAWKLGLGFY 155
51 YHACLAVLIIIVLLIILAVLRVRRQLWPCDVRGRGRLPVDVLAGDRPRAVPAVAVFVLSLCLLDPEDLFFLTLASAPSDQKTEAPRGAWKLGLGFY 145
52 YHCTMPTIILVLLIILAVLRVRRQLWPCDVRGRGRLPVDVLAGDRPRAVPAVAVFVLSLCLLDPEDLFFLTLASAPSDQKTEAPRGAWKLGLGFY 155



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P. alecto (L5JW03) /1-658
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132 PTLYYPLAACGTLHNKGVYVLSLSSWTFHGLVWQKVDKPTPIYRYVAFVLSLPOIACLAFLAFVPLLLFKLQNTETANESDSSYYRDKVILK 234
155 AALYYPLAACATGAGTAAHLLSSTLSWAHLGVQWQRAECPQPKYRYVLSLASELLGLGFLVWVPLVRSFSRRTG--AGSKGQSSYSEYLRNLIC 256
139 PLYYPLAACGTLHNKGVYVLSLSSWTFHGLVWQKVDKPTPIYRYVLSLPOIACLAFLAFVPLLLFKLQNTETANESDSSYYRDKVILK 241
124 PLYYPLAACGTLHNKGVYVLSLSSWTFHGLVWQKVDKPTPIYRYVLSLPOIACLAFLAFVPLLLFKLQNTETANESDSSYYRDKVILK 232
130 PLYYPLAACGTLHNKGVYVLSLSSWTFHGLVWQKVDKPTPIYRYVLSLPOIACLAFLAFVPLLLFKLQNTETANESDSSYYRDKVILK 232
130 PTLYYPLAACGTLHNKGVYVLSLSSWTFHGLVWQKVDKPTPIYRYVLSLPOIACLAFLAFVPLLLFKLQNTETANESDSSYYRDKVILK 232
133 PLYYPLAACGTLHNKGVYVLSLSSWTFHGLVWQKVDKPTPIYRYVLSLPOIACLAFLAFVPLLLFKLQNTETANESDSSYYRDKVILK 235
153 PLYYPLAACATVGHRAAHLISLSSWTFHGLVWQKVDKPTPIYRYVLSLPOIACLAFLAFVPLLLFKLQNTETANESDSSYYRDKVILK 235
128 PVFYSPLAACGTPRHRLGYGAVLLAARCAHGLHRLDQKPTLPIYRYVLSLASELLGLGFLVWVPLVRSFSRRTG--AGSKGQSSYSEYLRNLIC 229
168 PLYYPLAACATVGHRAAHLISLSSWTFHGLVWQKVDKPTPIYRYVLSLASELLGLGFLVWVPLVRSFSRRTG--AGSKGQSSYSEYLRNLIC 229
155 PLYYPLAACATVGHRAAHLISLSSWTFHGLVWQKVDKPTPIYRYVLSLASELLGLGFLVWVPLVRSFSRRTG--AGSKGQSSYSEYLRNLIC 229
155 PLYYPLAACATVGHRAAHLISLSSWTFHGLVWQKVDKPTPIYRYVLSLASELLGLGFLVWVPLVRSFSRRTG--AGSKGQSSYSEYLRNLIC 229
146 PLYYPLAACATVGHRAAHLISLSSWTFHGLVWQKVDKPTPIYRYVLSLASELLGLGFLVWVPLVRSFSRRTG--AGSKGQSSYSEYLRNLIC 229
154 PLYYPLAACATVGHRAAHLISLSSWTFHGLVWQKVDKPTPIYRYVLSLASELLGLGFLVWVPLVRSFSRRTG--AGSKGQSSYSEYLRNLIC 225
153 PLYYPLAACATVGHRAAHLISLSSWTFHGLVWQKVDKPTPIYRYVLSLASELLGLGFLVWVPLVRSFSRRTG--AGSKGQSSYSEYLRNLIC 225
154 PLYYPLAACATVGHRAAHLISLSSWTFHGLVWQKVDKPTPIYRYVLSLASELLGLGFLVWVPLVRSFSRRTG--AGSKGQSSYSEYLRNLIC 225
195 AALYYPLAACATVGHRAAHLISLSSWTFHGLVWQKVDKPTPIYRYVLSLASELLGLGFLVWVPLVRSFSRRTG--AGSKGQSSYSEYLRNLIC 224
192 AALYYPLAACATVGHRAAHLISLSSWTFHGLVWQKVDKPTPIYRYVLSLASELLGLGFLVWVPLVRSFSRRTG--AGSKGQSSYSEYLRNLIC 224
147 AALYYPLAACATVGHRAAHLISLSSWTFHGLVWQKVDKPTPIYRYVLSLASELLGLGFLVWVPLVRSFSRRTG--AGSKGQSSYSEYLRNLIC 224
155 AALYYPLAACATVGHRAAHLISLSSWTFHGLVWQKVDKPTPIYRYVLSLASELLGLGFLVWVPLVRSFSRRTG--AGSKGQSSYSEYLRNLIC 226
155 AALYYPLAACATVGHRAAHLISLSSWTFHGLVWQKVDKPTPIYRYVLSLASELLGLGFLVWVPLVRSFSRRTG--AGSKGQSSYSEYLRNLIC 226
195 AALYYPLAACATVGHRAAHLISLSSWTFHGLVWQKVDKPTPIYRYVLSLASELLGLGFLVWVPLVRSFSRRTG--AGSKGQSSYSEYLRNLIC 226
155 AALYYPLAACATVGHRAAHLISLSSWTFHGLVWQKVDKPTPIYRYVLSLASELLGLGFLVWVPLVRSFSRRTG--AGSKGQSSYSEYLRNLIC 226
156 PLYYPLAACATVGHRAAHLISLSSWTFHGLVWQKVDKPTPIYRYVLSLASELLGLGFLVWVPLVRSFSRRTG--AGSKGQSSYSEYLRNLIC 227
146 PLYYPLAACATVGHRAAHLISLSSWTFHGLVWQKVDKPTPIYRYVLSLASELLGLGFLVWVPLVRSFSRRTG--AGSKGQSSYSEYLRNLIC 227
155 PLYYPLAACATVGHRAAHLISLSSWTFHGLVWQKVDKPTPIYRYVLSLASELLGLGFLVWVPLVRSFSRRTG--AGSKGQSSYSEYLRNLIC 226



D. rerio (A4IG66) /1-670
H. sapiens (Q9XK79) /1-667
L. oculatus (W5NCL1) /1-664
O. niloticus (I3KE07) /1-658
X. maculatus (M4AN84) /1-665
P. formosa (A0A087XK69) /1-665
A. mexicanus (W5LF12) /1-668
G. gallus (F1NSC4) /1-671
F. albicollis (U3KCM6) /1-645
M. domestica (F6X9H5) /1-684
R. norvegicus (Q4QR83) /1-670
M. musculus (070491) /1-670
H. glaber (G5BRG2) /1-662
C. porcellus (H0VUR2) /1-671
C. familiaris (J9NW10) /1-655
L. africana (G37653) /1-669
O. garnettii (H0X180) /1-707
P. anubis (A0A096MM01) /1-704
C. sabaueus (A0A09RNU1) /1-659
M. mulatta (F7GR11) /1-667
M. fascicularis (G7P935) /1-667
G. Gorilla (G3R6K2) /1-707
P. abelli (Q5R7B4) /1-667
B. taurus (F1N406) /1-668
P. alecto (L5JW03) /1-658
E. caballus (F626X7) /1-667

235 KKKPKTIS--SSTSKPKLFDRLDAVKSYYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 330
257 RKKLGS--S-YHTSKHGLSWARVLRHCITVPPQGRFLPLKLVLSATLTGTATVYVALLLVGVVTTQKVRAGVITDVSYLLAGGIVLVE-----DROEV 351
242 RKKLGS--S-SCYATLISARILLESYIYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 326
227 KKKPKTIS--SSTSKPKLFDRLDAVKSYYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 326
233 EKTSSKVSSTSDPKPKLQRTDAVRSYIYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 329
233 EKTSSKVSSTSDPKPKLQRTDAVRSYIYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 329
236 KKKPSQ--SSRPPKLSERIISDAFKSYIYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 328
256 RRLQKQ--S-TPKLEESLVSRLLVYCSYIYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 358
230 RKRGRK--S-SAKIEESLWSRIRSYLLSYIYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 329
271 RKRGRK--S-SVAVSPPFRILYFLSHHSRGRFLPLKLVLSATLTGTATVYVALLLVGVVTTQKVRAGVITDVSYLLAGGIVLVE-----DROEV 365
294 RKKLGS--S-YHTSKHGLSWARVLRHCITVPPQGRFLPLKLVLSATLTGTATVYVALLLVGVVTTQKVRAGVITDVSYLLAGGIVLVE-----DROEV 352
257 PKLSDCS--HPAKSRLSRAWAFSHHSYIYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 352
248 RKPQGG--R-SYHTKQSLRSRAWYFSRHYIYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 342
256 RKKPGWS--CHTSKQGLSRAWCSQRYIYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 351
255 RKKLAS--S-SRASKHGLSRAWYSRNYIYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 349
256 RKKLGS--S-SHTSKHGLSQAQWICFRHHIYIYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 350
295 RKKLET--S-SHTSKHGLSQAQWICFRHHIYIYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 389
294 RKKLGS--S-SHTSKHGLSQAQWICFRHHIYIYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 389
249 RKKLGS--S-SHTSKHGLSQAQWICFRHHIYIYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 343
257 RKKLGS--S-SHTSKHGLSQAQWICFRHHIYIYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 351
257 RKKLGS--S-SHTSKHGLSQAQWICFRHHIYIYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 351
297 RKKLGS--S-YHTSKHGLSQAQWICFRHHIYIYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 391
257 RKKLGS--S-SHTSKHGLSQAQWICFRHHIYIYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 351
258 RKKLGS--S-SHTSKHGLSQAQWICFRHHIYIYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 352
248 RKKLGS--S-SHTSKHGLSQAQWICFRHHIYIYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 342
257 RKKLGS--S-SHTSKHGLSQAQWICFRHHIYIYIPEDEFRFLKLAIVVVFIALYVMAILLISGVLPFLHIVRRVDENIAFLLAGNIIILN-----DROEV 351

(65). Residues are colored by their physicochemical properties using a modified version of the Zappo scheme: aliphatic residues (I, L, V, A & M) are in salmon, aromatics (F, W & Y) in orange, positively charged (K, R & H) in blue, negatively charged (D, E) in red, polars (S, T, N, Q) in green, G in magenta, P in cyan, C in yellow. Secondary structure elements are labeled and displayed above the alignment, in yellow. Solid and dotted indicate regions of the protein, which are visible and disordered in the structure, respectively.

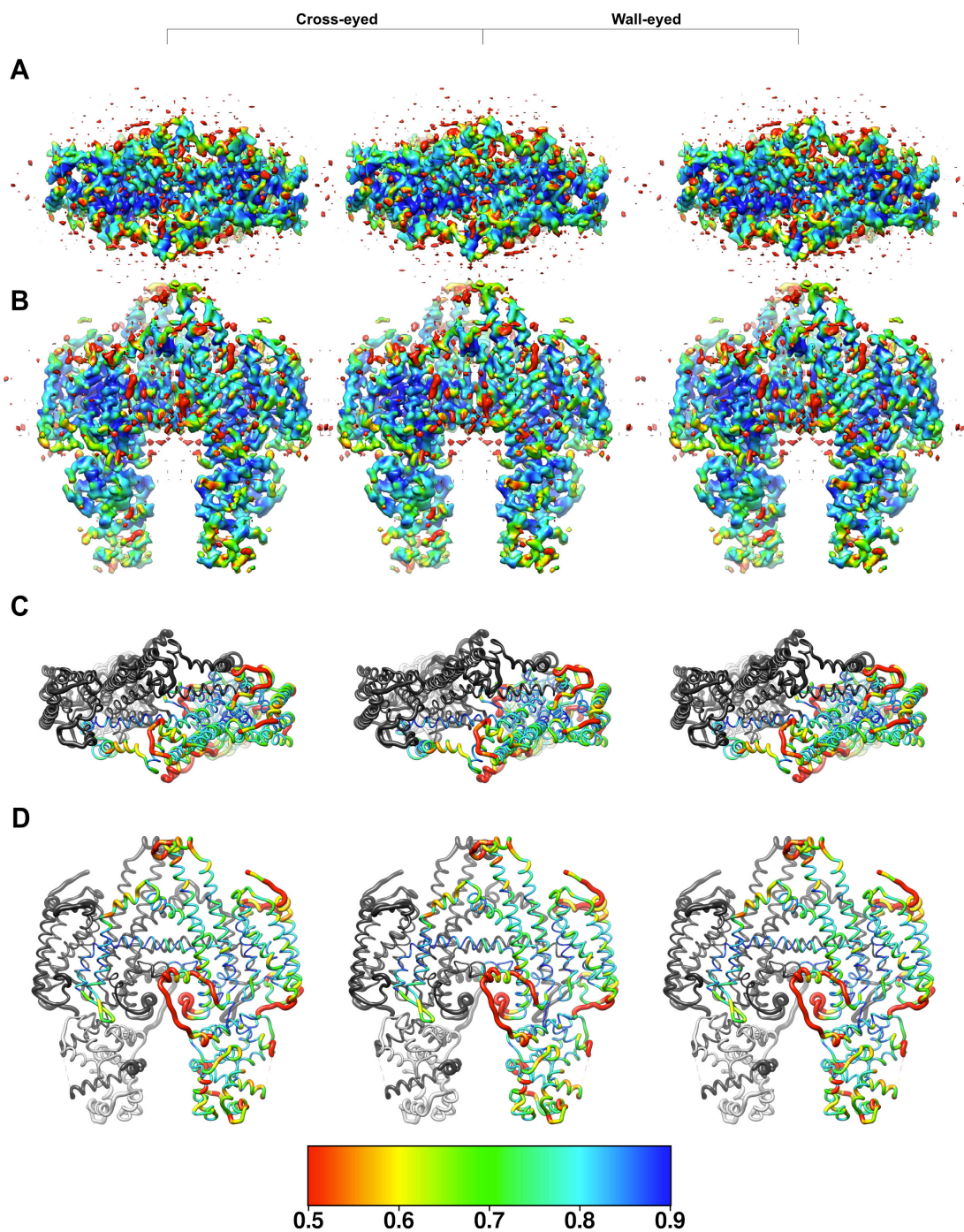


Fig. S8. Correlation between the STRA6 model and the density map. All panels are represented in stereo as indicated. The same color key is used for all panels and is reproduced at the bottom of the page. **(A and B)** Two views of the experimental density map, contoured at 6XR.M.S and colored by local correlation with a synthetic density map generated using *phenix.model_map* (version 1.10.1-dev-2427) from a model with B-factors estimated using *phenix.real_space_refine*. The synthetic map had the same sharpening B-factor applied as was used in post-processing of the cryo-EM map. Local correlation was calculated in a 5 voxel sliding window using the “*vop localcorrelation*” command in UCSF Chimera. **(C**

and **D)** Two views of the STRA6-CaM atomic model, with one protomer colored by the local correlation of each residue, averaged over the atomic positions. The radius of the backbone trace is inversely proportional to the correlation, such that a thicker trace indicates lower correlation with the experimental map.

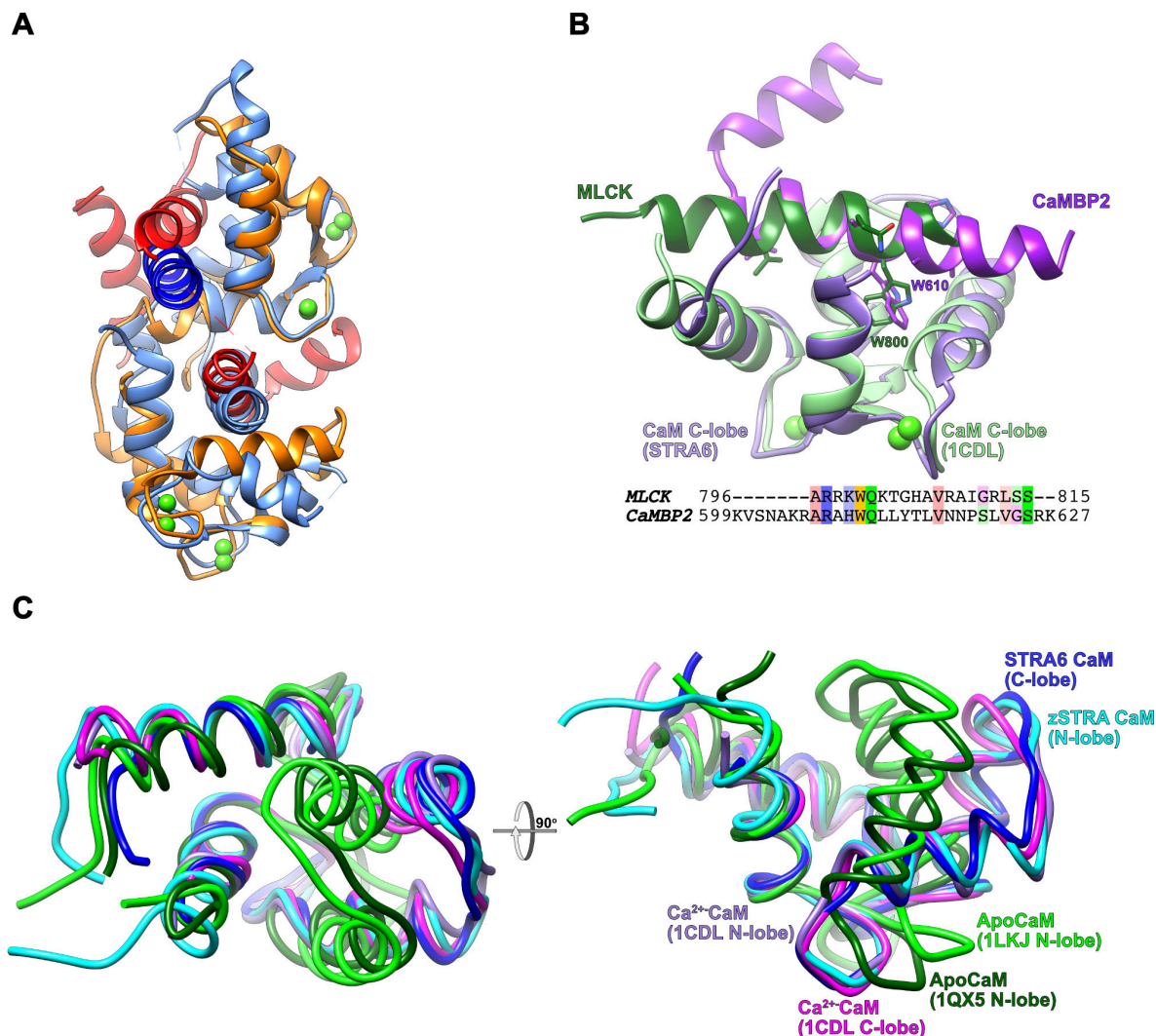
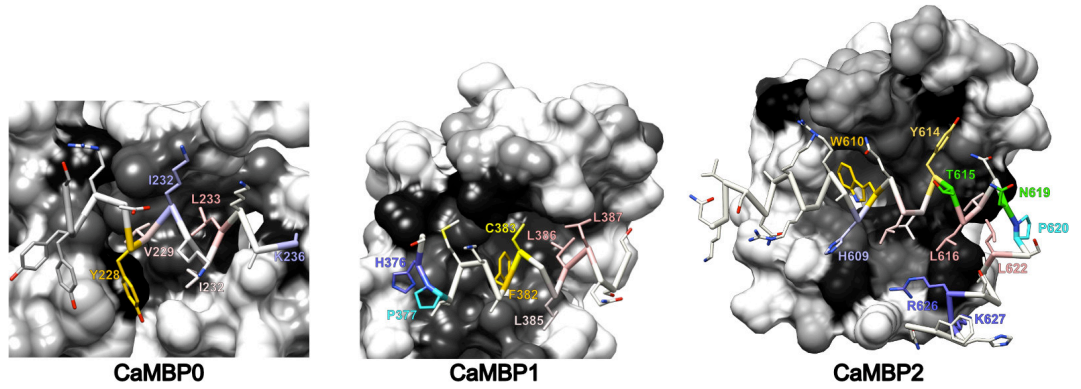


Fig. S9. Comparison of STRA6 bound CaM to other EF hand proteins and prior CaM structures. (A) The α trace of the structure of CaM (orange) bound to the three STRA6 peptides (red) is shown superimposed on the nearest match in the PDB (light/dark blue; PDB accession code: 3L19). Ca^{2+} -ions are represented as green spheres. (B) Superposition of the C-lobe of CaM bound to STRA6 CaMBP2, in light and dark purple respectively, with the complex of the MLCK target peptide and the C-lobe of Ca^{2+} -CaM (PDB accession code: 1CDL), in light and dark green respectively. Structurally homologous residues are shown in stick representation. A structure-based sequence alignment of the MLCK target peptide with CaMBP2 is shown below the structural superposition. (C) A superposition of the N- and C-lobes of CaM from the STRA6-CaM complex, displayed in cyan and blue respectively, with the N- and C-lobes from a Ca^{2+} -CaM structure (PDB accession code: 1CDL) in light purple and magenta respectively, and with two independent apo-CaM structures, the N-lobe of PDB accession code 1LKJ and the N-lobe of PDB accession code 1QX5, in light and dark green respectively.



	CaMBP0	CaMBP1	CaMBP2
<i>D. rerio</i> (A4IGB6)	223 SYYRDVVKILKKK 236	375 SHPVMKAFCGLLQSS 390	599 KVS--NAKRARAHWQLLYTLVNNPSLVGSRK 627
<i>H. sapiens</i> (Q9BX79)	245 SYSEYLRNLLCRK 258	396 SHPAMTAFCSLLLQQA 411	626 KGARPGASRGRARWGLAYTLLEHPTLVQVFRK 656
<i>L. oculatus</i> (W5NCL1)	230 SYYKDVKEILKKK 243	381 SHPVMKAFCGVLLQSP 396	605 KLS--NSRRARARWLLLETLVNNPSLVASRK 633
<i>O. niloticus</i> (I3KE07)	215 SYYRDVVKMLNKK 228	368 SHPVMKAFCGMLLQSV 383	592 KVS--IAKRARRHWQLLYTLVNNPSLVGTRK 620
<i>X. maculatus</i> (M4AN84)	221 SYYRDVVKITLYEK 234	374 SHPVMKAFCGILLQSL 389	598 KVS--SAKRARMHWQLLYTLVNNPSLVGTRK 626
<i>P. formosa</i> (A0A087XK69)	221 SYYRDVVKISLYEK 234	374 SHPVMKAFCGILLQSL 389	598 KVS--SAKRARMHWQLLYTLVNNPSLVGTRK 626
<i>A. mexicanus</i> (W5LF12)	224 SYYRDVVKILKKK 237	373 SHPVMKAFCGILLQAP 388	597 KPV--SNGKRARGHWLLYTLVNNPSLVGSRK 626
<i>G. gallus</i> (F1NSC4)	244 GYYKYLKAVLSRR 257	401 SHPLMRAFCLLLLQPT 416	625 GGG--RLKRSRARWVWVAYTLLEHPSLMGCRK 653
<i>F. albicollis</i> (U3KCM6)	218 SYYRKYKAVLSRR 231	375 CHPLLKAFCFLLQPG 390	599 GRA--RIRRRARWVWVAYTLLEHPSLTASRK 627
<i>M. domestica</i> (F6X9H5)	259 SYEYLRHILSGK 272	410 SHPAMRAFCFLLQLP 425	638 NAT--RSRQSRIRWGLAYTLLEHPSLTFPRK 666
<i>R. norvegicus</i> (Q4QR83)	245 SYSEKYLKALLCPK 258	397 SHPGVIAFCALLLHVP 412	627 KGAGPKGSRGRARWGLAYTLLEHPSLQAFRK 657
<i>M. musculus</i> (O70491)	245 SYSEKYLKALLCPK 258	397 SHPGVIAFCALLLHVP 412	627 KGAGPKGSRGRARWGLAYTLLEHPSLQAFRK 657
<i>H. glaber</i> (G5BRG2)	236 RDSKYLRSLLCRK 249	387 SHPAMTAFCALLLEVQ 402	619 KAAGLRAGRSWARWGVAYTLLEHPPKKAARK 649
<i>C. porcellus</i> (H0VUR2)	244 SDTEEYKLSLLCRK 257	396 SHPAVTAFCALLLGAR 411	628 KAAGLRAGRSWARWGVAYTLLEHPPKKAARK 649
<i>O. garnettii</i> (J9NWI0)	243 SYSEDYLRLLCRK 256	394 SHPATTAFCFLLLRTR 409	619 RGVGSRARQGRARWGLAYTLLEHPPKKAARK 649
<i>L. africana</i> (G3T6S3)	244 SYSEYLRVLLRRK 257	395 SHPAVTAFCFLLLRGR 410	626 KGAGPRASHGRARWGLAYTLLEHPPKKAARK 649
<i>O. garnettii</i> (H0XI80)	283 RYSEYLRNLLCRK 296	434 SHPAMTAFCTLLLOAR 449	664 KGARPRVSRGRARWGLAYTLLEHPPKKAARK 694
<i>P. anubis</i> (A0A096NMQ1)	282 SYSEYLRNLLCRK 295	433 SHPAMTAFCTLLLOAR 448	663 KGARPRASRCRARWGLAYTLLEHPPKKAARK 693
<i>C. sabaetus</i> (A0A099RNU1)	237 SYSEYLRNLLCRK 250	388 SHPATTAFCFLLLOAR 403	618 KGARPRASRCRARWGLAYTLLEHPPKKAARK 648
<i>M. mulatta</i> (F7GR11)	245 SYSEYLRNLLCRK 258	396 SHPAMTAFCTLLLOAR 411	626 KGARPRASRCRARWGLAYTLLEHPPKKAARK 656
<i>M. fascicularis</i> (G7P935)	245 SYSEYLRNLLCRK 258	396 SHPAMTAFCTLLLOAR 411	626 KGARPRASRCRARWGLAYTLLEHPPKKAARK 656
<i>G. gorilla</i> (G3R6K2)	285 SYSEYLRNLLCRK 298	436 SHPAMTAFCSLLLQQA 451	666 KGARPRASRCRARWGLAYTLLEHPPKKAARK 696
<i>P. abelli</i> (Q5R7B4)	245 SYSEYLRNLLCRK 258	396 SHPAMTAFCSLLLQQA 411	626 KGARPRANRGRARWGLAYTLLEHPPKKAARK 656
<i>B. taurus</i> (FIN4Q6)	246 SYSEYLRLLCRK 259	397 SHPAMTAFCSLLLRTQ 412	625 KGARPRARQGRARWGLAYTLLEHPPKKAARK 655
<i>P. alecto</i> (L5JW03)	236 SYPEEYLRLLCRK 249	387 SHPAMITFCALLLRTQ 402	615 KGTGPRARQGRARWGLAYTLLEHPPKKAARK 645
<i>E. caballus</i> (F6Z6X7)	245 SYSEYLRLLCRK 258	396 SHPAMTAFCSLLLRTQ 411	624 KGARPRARHGRARWGLAYTLLEHPPKKAARK 654

Fig. S10. Molecular details of the STRA6-CaM interaction. Separate views of the three CaM-binding peptides of STRA6 represented as $\text{C}\alpha$ traces with side chains shown in stick, overlaid over a hydrophobic surface colored according to the Kyte-Doolittle hydrophobicity scale (71) from white (-4.5, most polar) to black (4.5, most hydrophobic). For CaMBP0, CaMBP1 is also represented as part of the hydrophobic surface, as it forms part of the CaMBP0-binding cleft.

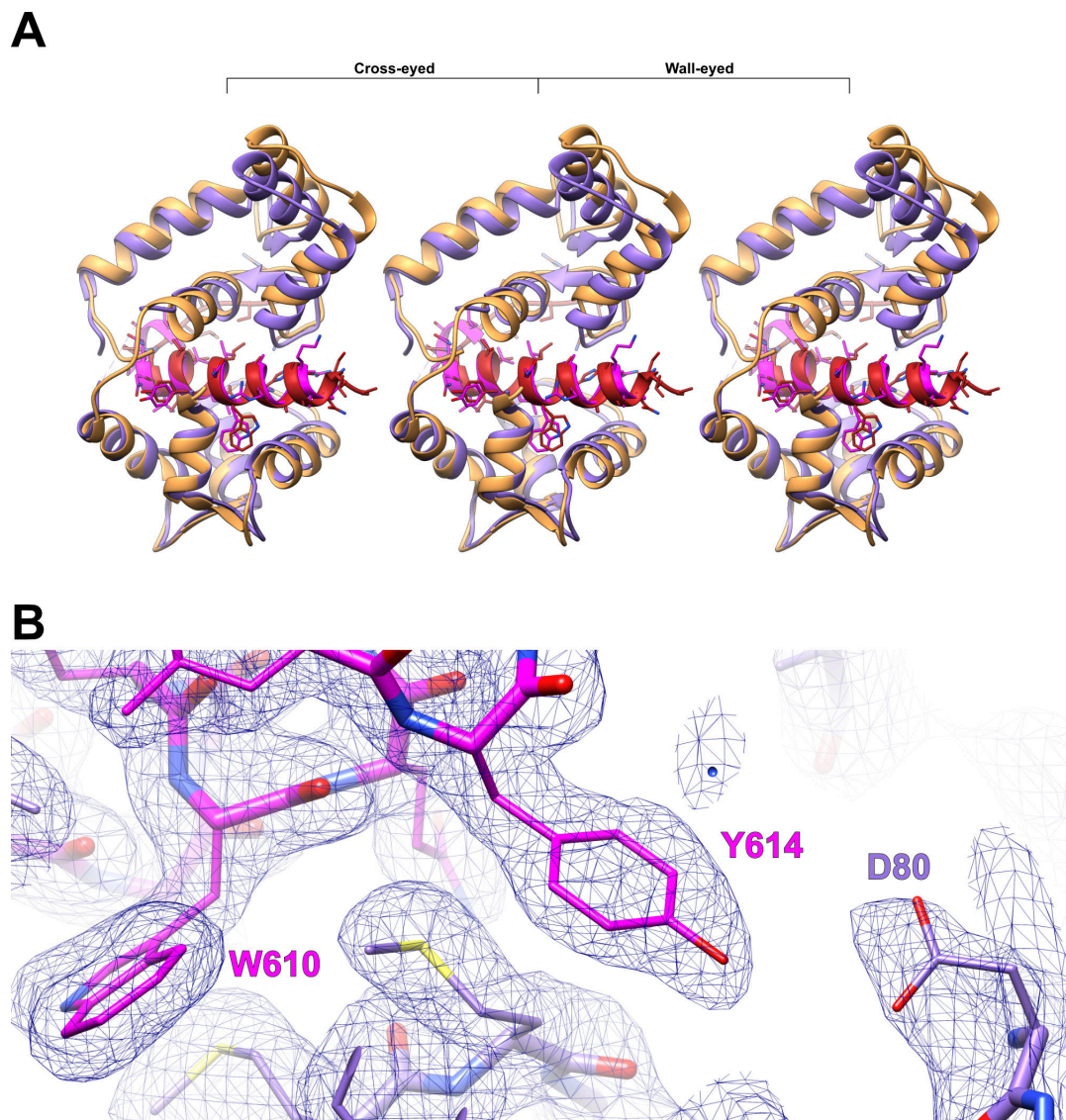


Fig. S11. Crystal Structure of CaM-CaMBP2. (A) Stereo representation of an overlay of the CaM-CaMBP2 crystal structure (in purple and magenta, respectively) and the corresponding region of the STRA6-CaM cryo-EM structure (in dark red, and gold, respectively). (B) Representative region of the 2Fo-Fc electron density map of the CaM-CaMBP2 crystal structure contoured at 1 x R.M.S.

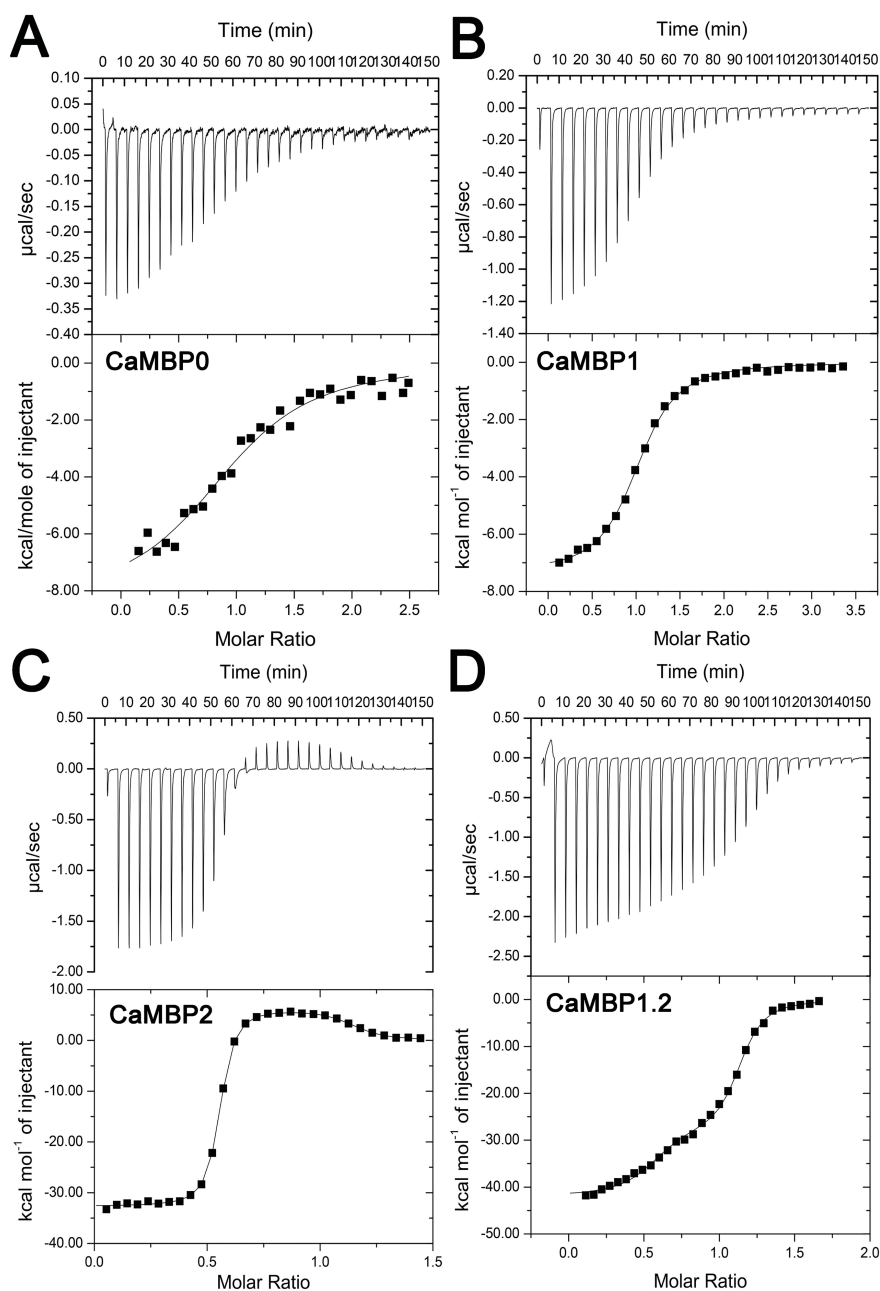


Fig. S12. ITC thermograms of STRA6 peptides – CaM interactions.

Representative ITC thermograms for the reversed titrations used to observe CaM interactions with STRA6 peptides: CaM interaction with (A) CaMBP0, (B) CaMBP1, (C) CaMBP2, and (D) CaMBP1.2 under calcium-loaded conditions. All experiments were performed in 20 mM HEPES, pH 7.4, 50 mM NaCl, 10 mM CaCl₂, 5 mM MgCl₂, and 0.5 mM TCEP at 37°C. CaMBP1 titrations into CaM were not performed, because the peptide aggregated at concentrations greater 0.1 mM in aqueous solutions due to its hydrophobicity, preventing its use as a titrant.

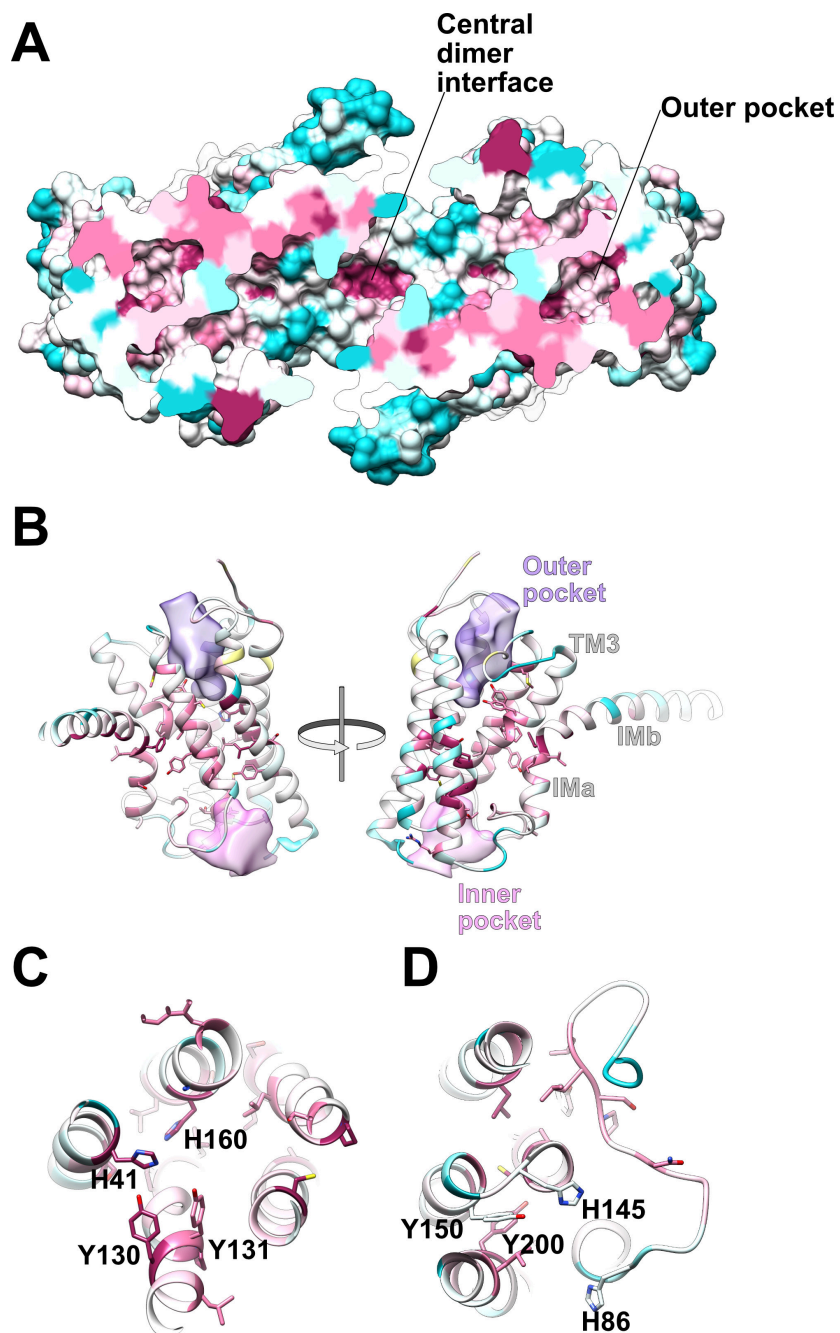


Fig. S13. Conservation of STRA6 within the outer cleft and pockets. (A) Slab in the membrane plane of the STRA6 conservation surface using the ConSurf server, viewed from the extracellular region. The STRA6 molecular surface is colored from cyan (least conserved) to maroon (most conserved). (B) Ribbon representation of the NTD, IMA and IMb colored by conservation as in (A), with conserved residues represented in stick, and the bounding surfaces of each pocket shown in purple and pink transparent surfaces for the outer and inner pockets, respectively. (C) Molecular details of conserved interactions within the outer pocket. (D) Molecular details of interactions within the outer pocket.

negatively charged (D, E) in red, polars (S, T, N, Q) in green, G in magenta, P in cyan, C in yellow. Secondary structure elements are labeled and displayed above the alignment, in yellow. Solid and dotted indicate regions of the protein, which are visible and disordered in the structure, respectively.

Movie S1. Density quality of STRA6. The STRA6-CaM complex is depicted in ribbon representation with one STRA6 protomer in spectral coloring (violet N-terminus to red C-terminus). The associated protomer of CaM is colored cyan. The density map is represented as blue mesh and contoured at 7 X R.M.S.

Movie S2. STRA6-CaM interactions. CaM is represented as a molecular surface colored by hydrophobicity according to the Kyte-Doolittle scale (71) from -4.5 (most polar) in light blue to 4.5 (most hydrophobic) in orange. The N-lobe of CaM is initially at the top of the screen. CaMBP0, 1 and 2 are depicted in ribbon representation with side chains shown in stick. The CaMBPs are colored by conservation from cyan (least conserved) to maroon (most conserved), based on the alignment presented in **Fig. 2** and **Fig. S7**.