

## Appendix for Structure of RyR1 in native membranes

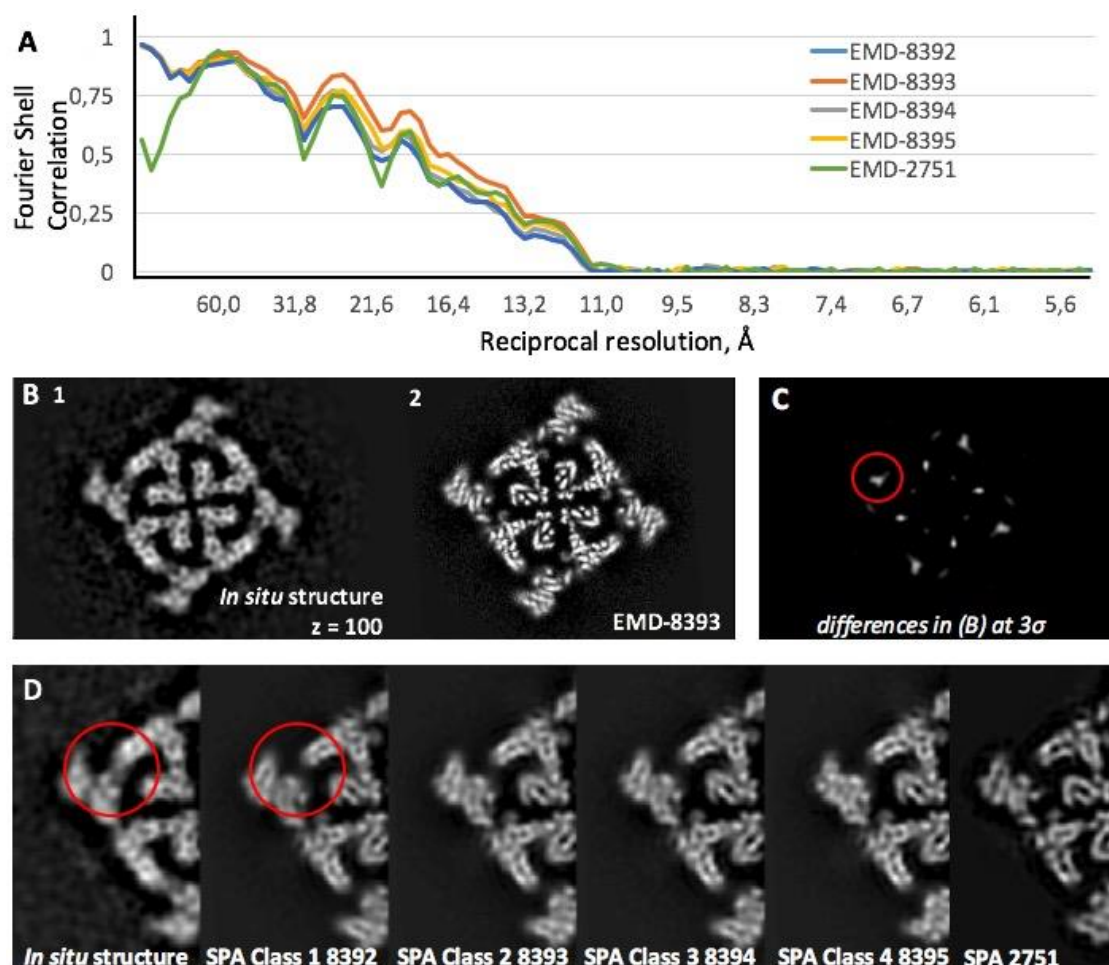
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### Appendix Table S1. Statistics of data collection and data processing

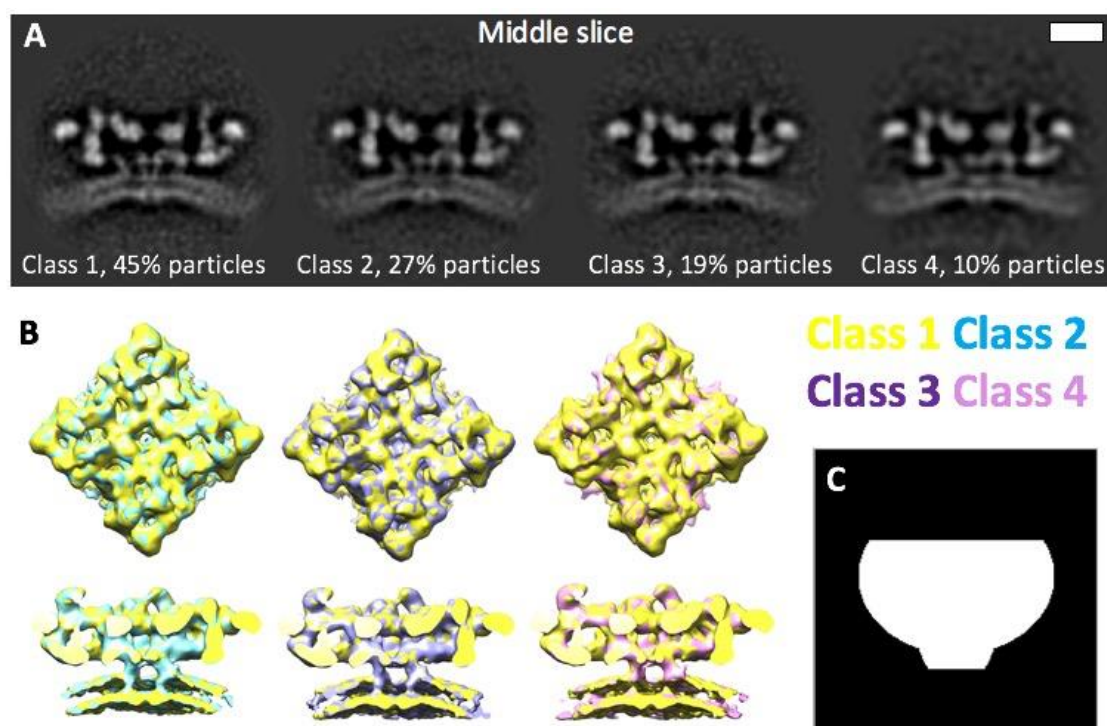
	EGTA / ApoRyR1, EMD-XXX	EGTA/apoR yR1-TT, EMD-XXX	ryRyR1, EMD-XXX	EDTA Class 1	EDTA Class 2	EDTA Class 3	EDTA Class 4	DDM+EDT A	DDM+1mM Ca2+	DDM+5mM Ca2+
Microscope	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios	Titan Krios
Voltage, Cs	300 / 2.7	300 / 2.7	300 / 2.7	300 / 2.7	300 / 2.7	300 / 2.7	300 / 2.7	300 / 2.7	300 / 2.7	300 / 2.7
Camera	Gatan K2 with a quantum GIF	Gatan K2 with a quantum GIF	Gatan K2 with a quantum GIF	Gatan K2 with a quantum GIF	Gatan K2 with a quantum GIF	Gatan K2 with a quantum GIF	Gatan K2 with a quantum GIF	Gatan K2 with a quantum GIF	Gatan K2 with a quantum GIF	Gatan K2 with a quantum GIF
Pixel Size	2.7 Å	2.7 Å	2.7 Å	2.7 Å	2.7 Å	2.7 Å	2.7 Å	2.7 Å	2.7 Å	2.7 Å
Total electron dose e-/Å <sup>2</sup>	68	68	68	68	68	68	68	68	68	68
Defocus range, um	4-5 um	4-5 um	1.5-5 um	4-5 um	4-5 um	4-5 um	4-5 um	4-5 um	4-5 um	4-5 um
Number of tomograms used	82	82	47	82	82	82	82	10	9	10
Number of particles in the final map	2547	205	890	1138	679	478	252	243	301	140
Symmetry of the map	C4	C4	C4	C4	C4	C4	C4	C4	C4	C4
Refinement method	independent half-set	non- independent	independen t half-set	non- independen t	non- independen t	non- independen t	non- independen t	non- independen t	non- independen t	non- independen t
Resolution, Å	12.6 / 12...15.5	38 at fsc = 0.5	17.5	15.4 at fsc=0.3	18 at fsc=0.3	18 at fsc=0.3	21.6 at fsc=0.5	25.7 at fsc = 0.5	27.7 at fsc = 0.5	30 at fsc = 0.5
Applied B- factor	-1200	0	0	0	0	0	0	0	0	0



**Appendix Figure S1. Structural similarities between the *in situ* structure and the previously reported high-resolution structures of purified RyRs.**

A. Fourier Shell Correlation between the mutually aligned *in situ* structure and the respective reconstructions of purified receptors by single particle cryo-EM [27, 28].

B-D. Panels showing slices through the *in situ* structure and the reported single particle cryo-EM structures of the purified receptors filtered to 15 Å for comparison. Panel C is a difference between the volumes in panels B1 and B2 at a contrast level of 3σ.

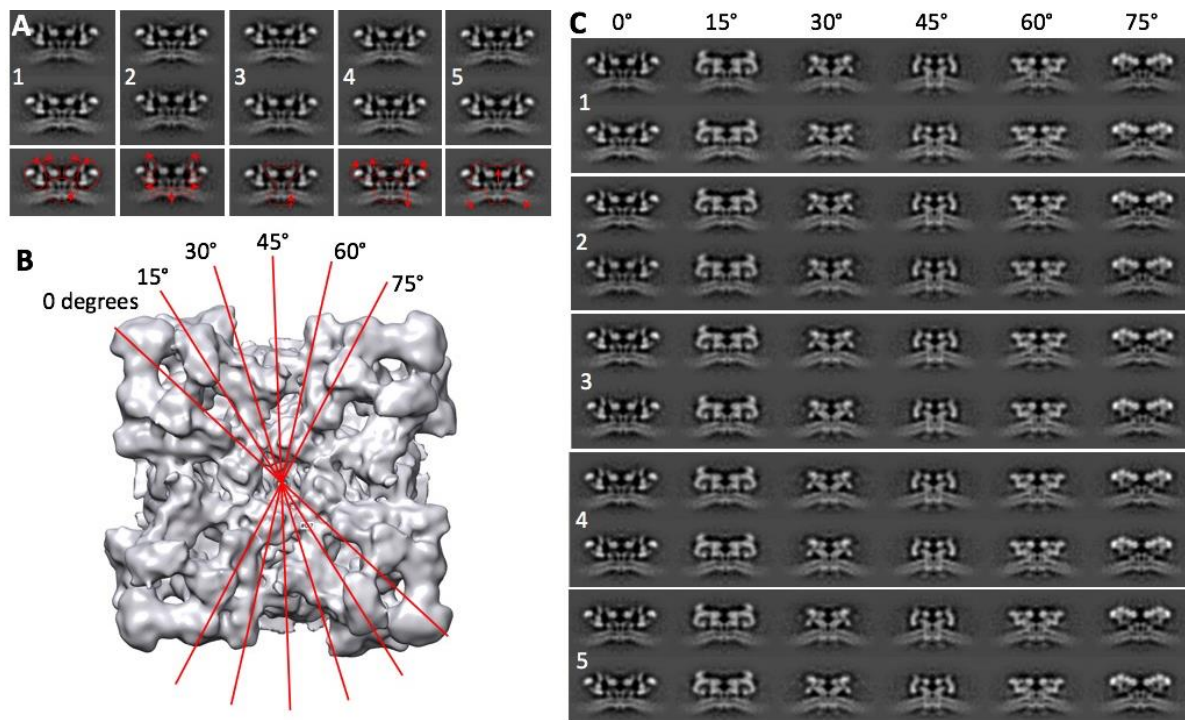


**Appendix Figure S2. Classification of apoRyR1 particles into four classes.**

A. Slices through the class averages at 15.4, 18, 18 and 27 Å respectively. Scale bar: 10 nm.

B. Isosurface representation of the differences between the classes viewing from the cytoplasmic side and in the central section through the volume-rendered representation.

C. A mask used for multi-reference alignment and classification.

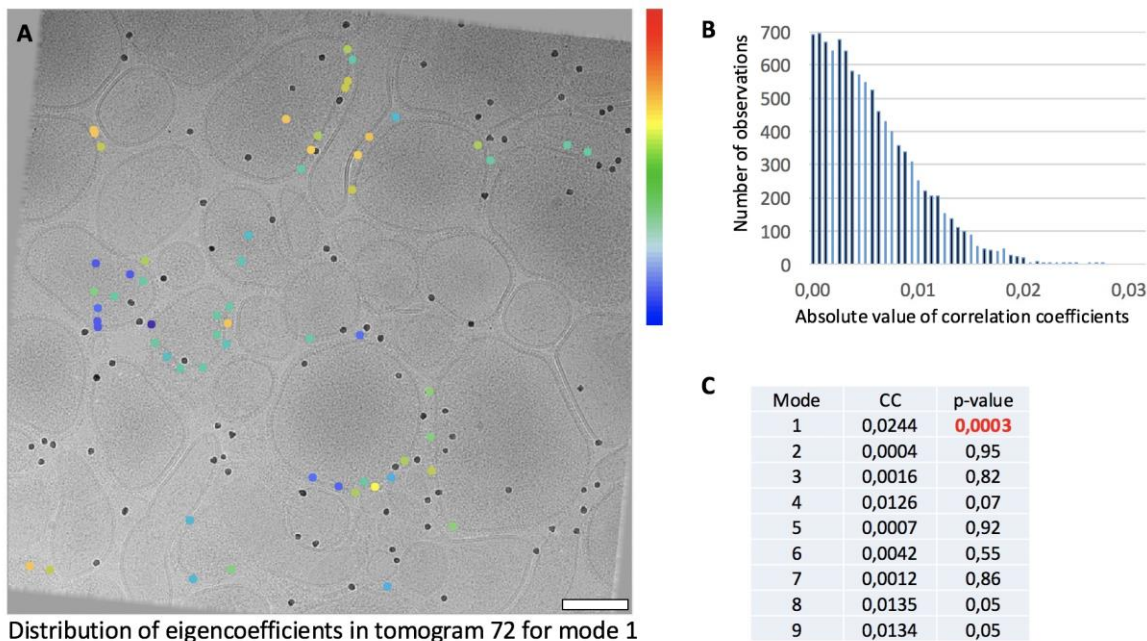


**Appendix Figure S3. Modes of structural variation of RyR1 *in situ*.**

A. Transitions between half-sets of each class summed according to their distribution in the first five modes.

B. The schematic at bottom-left shows the sections separated at 15° steps that are displayed in the panels at right. Movie EV2 toggles between the states in the top-left while movies EV3-EV7 demonstrate conformational modes from all the views.

C. The slices through the half-sets divided according to the modes as in (A) in six different directions with 15 degrees' step. The panels are the same as in movies EV3-EV7.

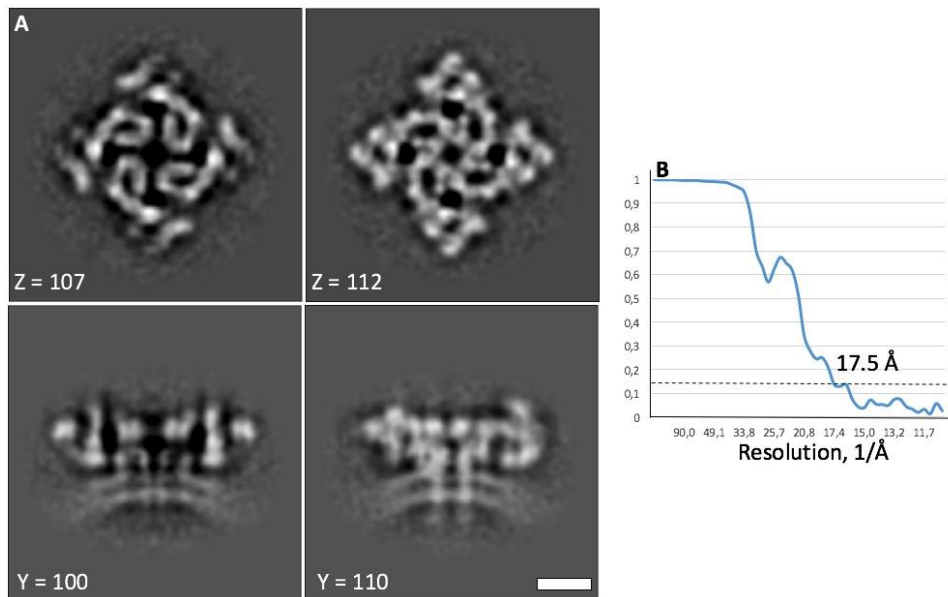


**Appendix Figure S4. Relation of spatial position and eigencoefficients from the structural modes presented in Fig 4.**

A. Locations of multiple RyR1s overlaid on the cryo-EM micrograph color-coded according to the eigencoefficients related to mode 1. Scale bar: 100 nm.

B. Distribution of correlation coefficients for particle-to-particle distances to a random variable with n=84062 measurements for 10 000 random initializations of the random variables.

C. A table with cross-correlation values between the distance between the particles and the difference between their eigencoefficients along the listed modes. P-values are derived from the simulations presented in panel B.



# **Appendix Figure S5. Structure of ryRyR1.**

A. Slices through the structure with the locations of the sections indicated by the Y or Z coordinates. Scale bar: 10 nm.

B. Fourier Shell Correlation between independently refined half-sets indicates a resolution of the reconstruction of 17.5 Å.