1    associated calsequestrin layers as defined in (c). The views in (a),(b),(c), and (e) are

2    looking up from the SR lumen. Scale bars equal 10 nm.

3

4    Figure 5.  Secondary density maxima in RyR class averages and possible tethers from the

5    SR membrane to calsequestrin and TT membrane. (a) Secondary maxima in the plane of

6    the SR membrane in the Class N average. Membrane-spanning domain of the RyR

7    template is rendered in yellow.  (b) Transverse slice through the Class N average (at

8    dashed line in (a)) showing faint density (white arrows) connecting an SR density (black

9    arrow) to calsequestrin  (black arrowhead).  (c) Cytoplasmic densities in average of the

10   subset of Class S with TT membranes nearby.  (d) Transverse slice through the TT

11   subaverage of Class S (at dashed line in (c)) showing faint density (white arrows)

12   connecting the cytoplasmic density (black arrows) to the TT membrane.  The planes

13   shown in (a) and (c) correspond to orthogonal slices in the direction of the black arrows

14   in (b) and (d). Scale bars equal 10 nm.

15

16   Figure 6.  Partial model for the triad junction "couplon" based on cryo-electron

17   tomography. Top: View from above the SR membrane (light blue) showing RyR

18   (yellow), calsequestrin (dark blue), secondary SR membrane densities (green), and

19   cytoplasmic TT-associated densities (pink).  Bottom: Side view of the couplon model

20   showing expected location of the TT membrane (black) and tethers (dashed lines)

21   inferred from the class averages in Figure 5.

22

23   Supplemental Figure 1. Location of the region within the subvolumes used for

24   multivariate statistical analysis (a,b) and the orientations of identified RyRs (c) and

1    quadrant subvolumes (d) relative to the electron beam. (a,b) Central slice of the global

2    average with surface rendering of a modified (yellow) and unmodified (blue mesh)

3    single-particle RyR superimposed. The RyR has been modified for underfocus and a

4    missing wedge directed about $15^\circ$ counter clockwise from the vertical. White dashed

5    boxes delineate the subvolume region that was used for classification. (c,d) Polar plots

6    showing the distribution of RyRs (c) and quadrants (d) as a function of the Euler angles

7    defined in Methods. In (c)   (radial direction) and   (rotational direction) are plotted for

8    the 49 RyRs. In (d)   (radial direction) and $\psi$ (rotational direction) are plotted for each

9    pseudo-monomer. In (c) the tilt-axis direction is vertical ( $=\pm90^\circ$). In (d) the beam

10    direction is vertical ($\psi = \pm90^\circ$). In (d) symbols represent Class N (triangles), Class S

11    (boxes) and quadrants that fell in neither class (crosses).