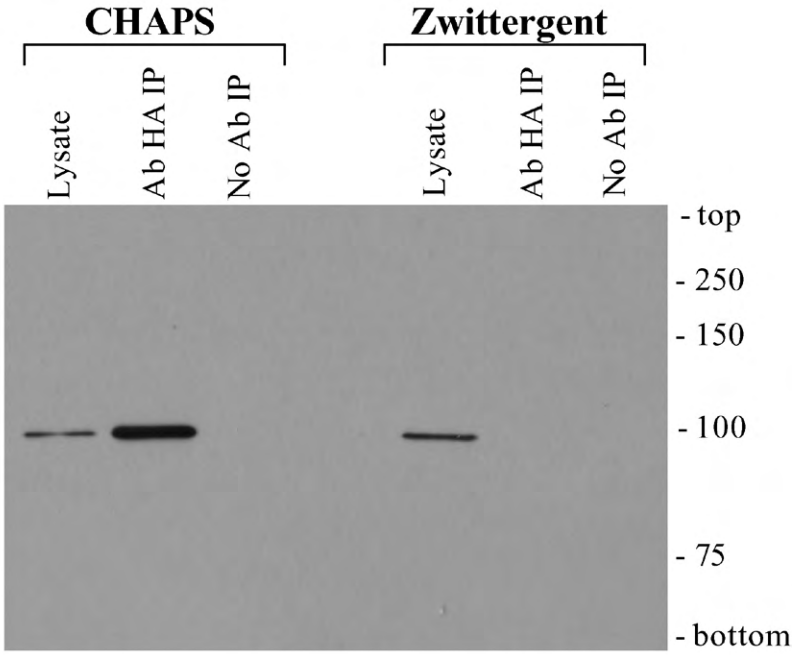

Fig. S1. RyR2 N-terminus self-interaction is abolished by zwittergent 3-14. Co-immunoprecipitation assays from HEK293 cell lysate co-expressing cMyc-tagged (BT4L) and HA-tagged (AD4L) RyR2 residues 1-906, pre-treated with 10 mM DTT. AD4L was immunoprecipitated with Ab^{HA} from either CHAPS- or zwittergent 3-14-solubilized HEK293 lysate and the presence of associated BT4L was analyzed by SDS-PAGE (6% gel) and immunoblotting using Ab^{cMyc}. Cell lysate, $1/50^{th}$ of the volume processed in IP samples, was also included to serve as molecular weight standard.

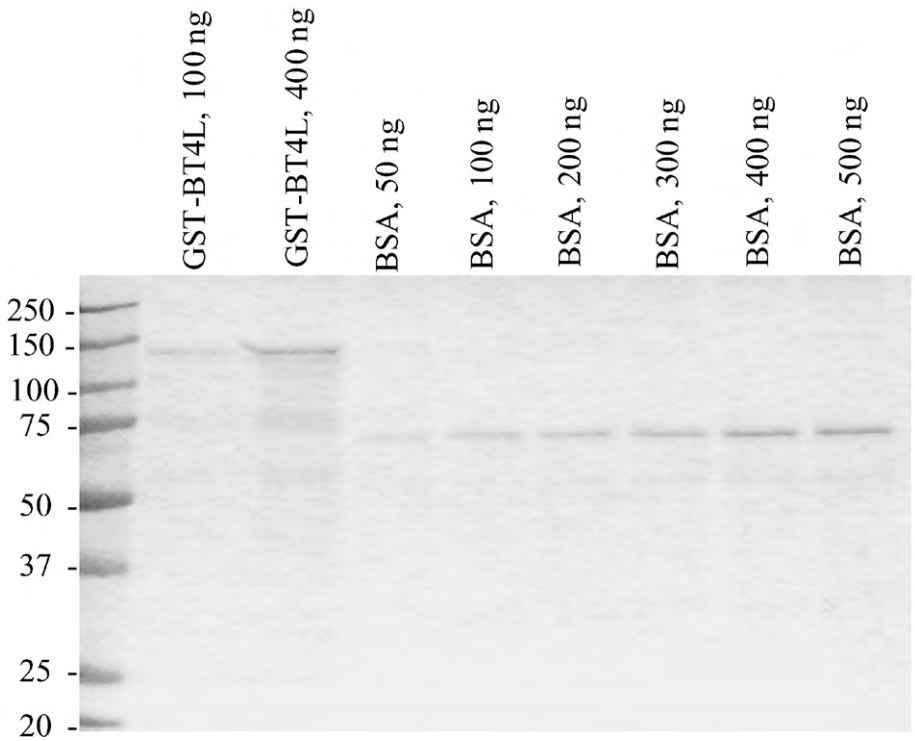

Fig. S2. Bacterially expressed and purified RyR2 N-terminal fragment (GST-BT4L). Coomassie-stained SDS-PAGE gel of purified GST-BT4L together with known amounts of bovine serum albumin (BSA).

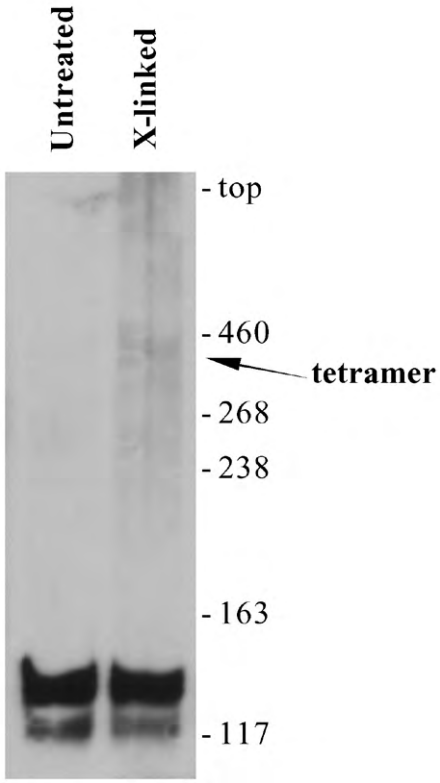

Fig. S3. Chemical cross-linking of purified GST-BT4L shows tetramer formation. Purified GST-BT4L was reacted with 0.0025% glutaraldehyde for 10 min and analyzed by SDS-PAGE (4% gel) and immunoblotting using Ab^{GST}.

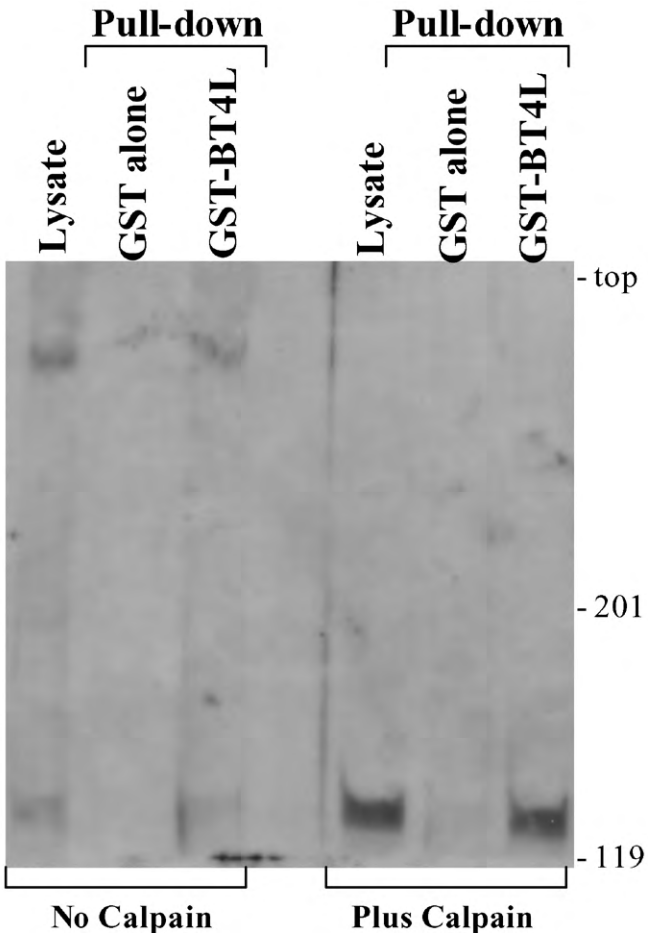

Fig. S4. GST pull-down assay shows BT4L interaction with native RyR2. Pig cardiac SR vesicles (500 μg), treated with or without 20 units of calpain-2 for 2 min, were solubilized overnight in buffer (20mM Tris, 150 mM NaCl, 0.4% CHAPS, 2 mM DTT, pH 7.4) and the insoluble material removed by centrifugation. Solubilised SR proteins were mixed with 100 nM GST-BT4L or 1 μM GST alone captured on glutathione-sepharose 4B beads, and incubated overnight at 4°C. Beads were washed twice and eluted proteins were analyzed by SDS-PAGE (4% gel) and immunoblotting using Ab^{D2}. Lysate (~25μg) was also included.

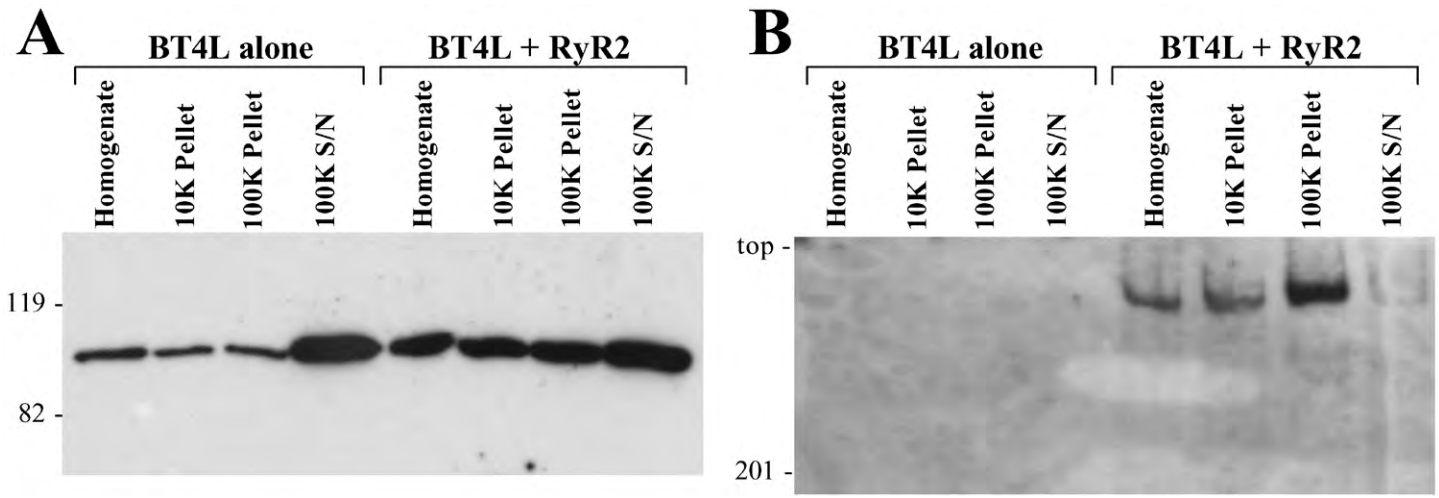

Fig. S5. BT4L associates with microsomal membranes upon co-expression with RyR2. Sub-fractionation of HEK293 cells expressing BT4L alone or RyR2 with BT4L, and immunoblotting using (BT4L) Ab^{cMyc} (A) or (RyR2) Ab^{1093} (B).

Table S1. Native RyR2 displays increased [^{3}H]ryanodine binding in the presence of GST-BT4L.

	GST	BT4L	GST	BT4L	GST	BT4L	GST	BT4L	GST	BT4L	GST	BT4L	GST	BT4L	GST	BT4L
Free [Ca^{2+}]	50nM		100nM		250nM		1µM		10µM		50µM		100µM		500µM	
fmol [^{3}H]ryanodine per mg SR	2.22	4.22	4.34	9.91	10.71	16.55	96.85	119.16	147.93	167.36	191.44	197.12	149.02	157.67	179.11	181.95
standard error	2.63	3.6	3.78	2.95	1.16	1.68	5.79	5.58	3.77	7.96	5.69	3.5	1.01	2.26	6.35	9.92

Table S1. Native RyR2 displays increased [^{3}H]ryanodine binding in the presence of GST-BT4L. [^{3}H]Ryanodine binding assays of pig cardiac SR incubated with 10nM GST-BT4L or 100nM GST alone, over a wide range of free Ca^{2+} concentrations. Data are expressed as mean value ± standard error of the mean.