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Structural determination of the phosphorylation domain of the ryanodine receptor

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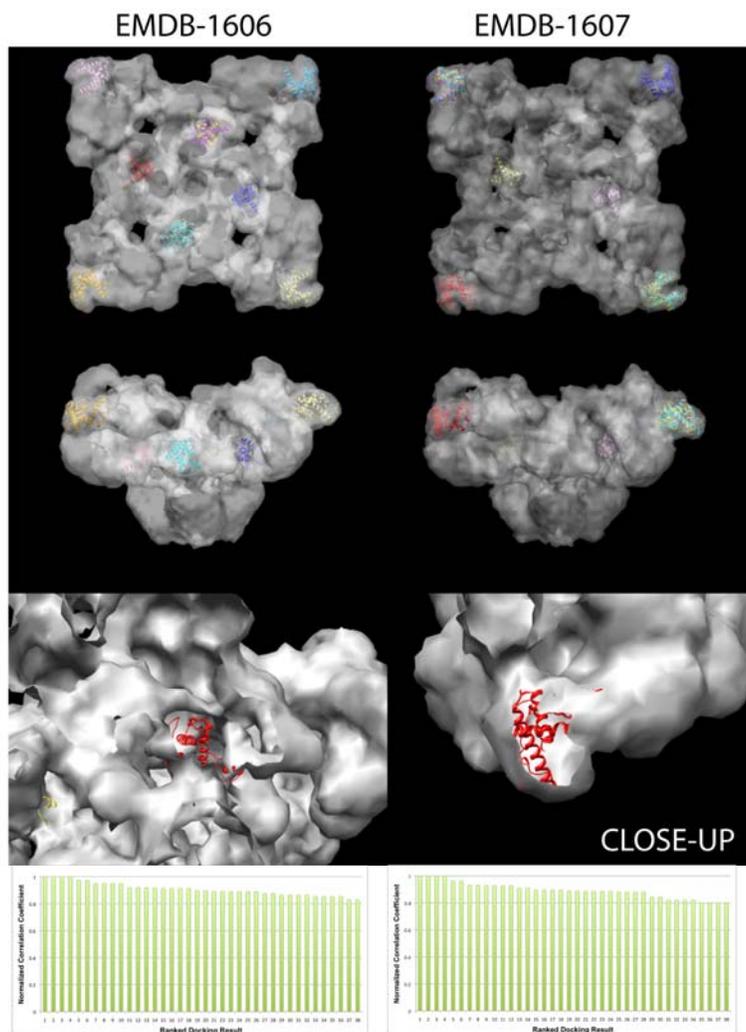
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Supplemental Figure 1: C3 construct sequence analysis

sp P21817 RYR1_HUMAN	2733	GNFDPRPVETLNVI IPEKLDSF INKFAEYTHE KWAFDKIQNNWSY GENID	2782
tr Q29105 Q29105_PIG	2734	GNFDPRPVETLNVI IPEKLDSF INKFAEYTHE KWAFDKIQNNWSY GENID	2783
sp P11716 RYR1_RABIT	2733	GNFDPRPVETLNVI IPEKLDSF INKFAEYTHE KWAFDKIQNNWSY GENVD	2782
tr Q80X16 Q80X16_MOUSE	2734	GNFDPRPVETLNVI IPEKLDSF INKFAEYTHE KWAFDKIQNNWSY GENID	2783
tr B4XW97 B4XW97_MELGA	2720	GNFDPKPVETLNVI IPEKLDGF INKYAEFTHE KWAFDKIQNNWTYGETVD	2769
tr O13054 O13054_MAKNI	2772	GNFDPKPVETNTNI IPERLDPF INRFAEYTHDKWAFDKIQNNWSYGEVLD	2821
tr A6P4B8 A6P4B8_DANRE	2755	GNFDPKPVETNTNI IPERLDAF INKYAEHTDKWAFDKIQNNWTYGEVLD	2804
		*****:**** *.:*****:*** **::**.*:****:*****:*** :*	
sp P21817 RYR1_HUMAN	2783	EELKTHPMLR PYKTFSEKDKEI YRWPIKESLKAMIAWEWTIEKAREGEEE	2832
tr Q29105 Q29105_PIG	2784	EELKTHPMLR PYKTFSEKDKEI YRWPIKESLKAMIAWEWTIEKAREGEEE	2834
sp P11716 RYR1_RABIT	2783	EELKTHPMLR PYKTFSEKDKEI YRWPIKESLKAMIAWEWTIEKAREGEEE	2832
tr Q80X16 Q80X16_MOUSE	2784	EELKTHPMLR PYKTFSEKDKEI YRWPIKESLKAMIAWEWTVEKAREGEEE	2834
tr B4XW97 B4XW97_MELGA	2770	EAKTHPMLR PYKTFSEKDKEI YRWPIKESLKAMIAWEWNVEKAREGEEE	2819
tr O13054 O13054_MAKNI	2822	ENAKTHPMLR PYKTFSEKDKEI YRWPIKESIKAMIAWEWNIEKARE-EEE	2870
tr A6P4B8 A6P4B8_DANRE	2805	ENAKTHPMLR PYKTFSEKDKEI YRWPIKESIKAMIAWEWTLEKARDGETE	2854
		*: *****:*****:***:**** :****: :*	
sp P21817 RYR1_HUMAN	2833	-KTEKKKTRK ISQSAQ-TYDPREGYNPQPPDL SAVTLSRELQAMAEQLAE	2880
tr Q29105 Q29105_PIG	2835	-KTEKKKTRK ISQSAQ-TYDAREGYNPQPPDL SGVTLSRELQAMAEQLAE	2881
sp P11716 RYR1_RABIT	2833	-RTEKKKTRK ISQTAQ-TYDPREGYNPQPPDL SGVTLSRELQAMAEQLAE	2880
tr Q80X16 Q80X16_MOUSE	2835	-KTEKKKTRK ISQTAQ-TYDPREGYNPQPPDL SVVTLSRELQAMAEQLAE	2881
tr B4XW97 B4XW97_MELGA	2820	-KAEKKKTRK ISQSAQATYDPS HGYNPQPPDL SGVTLSRELQAMAEQLAE	2868
tr O13054 O13054_MAKNI	2871	SEKKKATSRK ISQTAQATYDPS HGYSQPPI DI SHVALSRDLQSMAEQLAE	2920
tr A6P4B8 A6P4B8_DANRE	2855	VKTEVKATRK ISQTAQATYDPS QGYSQPVDL IGMALSRELQSMAEQLAE	2904
		. : :*****:*** ** . **.* ** * : :***:***:*****	
sp P21817 RYR1_HUMAN	2881	NYHNTWGRKK KQELEAKGGGTH PLLVPYDTLT AKEKARDREKAQE LLKFL	2930
tr Q29105 Q29105_PIG	2882	NYHNTWGRKK KQELEAKGGGTH PLLVPYDTLT AKEKARDREKAQE LLKFL	2931
sp P11716 RYR1_RABIT	2881	NYHNTWGRKK KQELEAKGGGTH PLLVPYDTLT AKEKARDREKAQE LLKFL	2930
tr Q80X16 Q80X16_MOUSE	2882	NYHNTWGRKK KQELEAKGGGSH PLLVPYDTLT AKEKARDREKAQE LLKFL	2931
tr B4XW97 B4XW97_MELGA	2869	NYHNTWGRKK KQELEAKGGGSH PLLVPYDTLT AKEKARDREKAQE LLKFL	2918
tr O13054 O13054_MAKNI	2921	NYHNTWGRKK KLELQAKGGGTH PLLVPYDTLT AKEKARDREKAQE LLKFL	2970
tr A6P4B8 A6P4B8_DANRE	2905	NYHNTWGRKK KQELEAKGGGTH PLLVPYDTLT AKEKARDREKAQD LLKFL	2954
		***** **.:*****:*****:*****:***** :****	
sp P21817 RYR1_HUMAN	2931	QMNGYAVTRG	2940
tr Q29105 Q29105_PIG	2932	QMNGYAVTRG	2941
sp P11716 RYR1_RABIT	2931	QMNGYAVTRG	2940
tr Q80X16 Q80X16_MOUSE	2932	QMNGYAVTRG	2941
tr B4XW97 B4XW97_MELGA	2919	QLNGYAVTRG	2928
tr O13054 O13054_MAKNI	2971	QLNGYAVTRG	2980
tr A6P4B8 A6P4B8_DANRE	2955	QLNGYAVTRG	2964
		*:*****	

Supplemental Figure 1: C3 construct sequence analysis. Sequence analysis of the RyR1 C3 construct (2733-2940) through several species indicates that this is a highly conserved region showing $\geq 82\%$ sequence homology through all seven species with 96% homology between rabbit and human RyR1. Red indicates known phosphorylation site Ser 2843.

Supplemental Figure 2: Docking of the RyR domain into the cryo-EM maps of RyR



Locations of Top 10 Solutions

	EMDB-1606	EMDB-1607
Subregion 3:	1-6	9-10
Subregion 10:	7-10	1-8

Supplemental Figure 2: Docking of the RyR domain into the cryo-EM maps of RyR. Top ten docking solutions using cryo-EM maps of RyR in the open state (EMDB-1607) and the closed-state (EMDB-1606) are indicated by the RyR domain in various colours. The docked structures are shown from the top view (top panel), the side view (middle panel) and the close-up view (bottom panel). Low fitting contrast of these solutions are shown by plotting normalized correlation coefficients for the top ~40 docking solutions.

Supplemental Figure 3: C3 construct sequence analysis between RyR1 and RyR2

		$\alpha 1$		
RyR1	2733	GNFDP RPVETLNVIIP	EKLDSFINKFAEYTHEKWAFDKIQNNWSYGENVDEELKTHPMLR	2792
RyR2	2700	GNFN PQPVDTSNIIIP	EKLEYFINKYAEHSHDKWSMDKLANGWIYGEIYSDSSKIQPLMK	2759
RyR3	2599	GNFDPKPIINTINFSLP	EKLEYIVTKYAEHSHDKWACEKSQSGWKYGISLDENVKTHPLIR	2658
		:*:*:*:* * . :*: :*:****:****: :* . * ** . . . * :****:		
		$\alpha 2$	$\beta 1$	
RyR1	2793	PYKTFSEKDKIYRWPIKESL	KAMIAEWETIEKAREGEEERTEKKKTRKISQTAQTYDPR	2852
RyR2	2760	PYKLLSEKEKEIYRWPIKESL	KTMLAWGWRIER TREGDSMALYNRTRRISQTSQVSVDA	2819
RyR3	2659	PFKTLTEKEKEIYRWPARESL	KTMLAVGWTVERTKEGEALVQLRENEKLRVSQ--TSQG	2716
		: :*:****:****: :****:* * :****:****: . . . : . : .		
		$\alpha 3$		
RyR1	2853	EGYNPQPPDLSGVTL	SRELQAMAEQLAENYHNTWGRKKKQELEAKGGGTHPLLVPYDILT	2912
RyR2	2820	HGYS PRAIDMSNVTL	SRDLHAMAEMMAENYHNIWAKKKKLELESKGGGNHPLLVPYDILT	2879
RyR3	2717	NSYNPAPLDLSNVVLS	SRELQGMVEVVAENYHNIWAKKKKLELESKGGGSHPLLVPYDILT	2776
		..*.* *:* * .****:*. * . :**** * . :*** ** :****.*****		
		$\alpha 4$	$\beta 2$	
RyR1	2913	AKEKARDREKAQELLKFLQ	MNGYAVTRG 2940	
RyR2	2880	AKEKAKDREKAQDILKFLQ	INGYAVSRG 2907	
RyR3	2777	AKEKFRDREKAQDLFKFLQ	VNGVIVSRG 2804	
		**** :*****:****:*** * :**		

Supplemental Figure 3: C3 construct sequence analysis between RyR1 and RyR2. Amino acid sequence comparison between the RyR domain of RyR1 and the equivalent domain in RyR2 (2699-2907) showed 64% sequence homology between the two paralogs.

Supplemental Table 1. Diffraction data collection, processing and model refinement statistics

<i>Data collection and processing</i>	
Resolution	50-2.16 Å
R_{merge}^a	10.3% (84.6%) ^b
#Unique reflections	12353
Completeness	100% (100%)
Mean redundancy	9.2 (8.5)
$\langle I \rangle / \langle \sigma \rangle$	19.7 (2.0)
<i>Refinement</i>	
# Protein atoms	1407
# Solvent atoms	118
R_{cryst}^c	21.3%
R_{free}^d	26.5%
Estimated coordinate error ^e	0.3 Å
<i>Deviations from ideal stereochemistry</i>	
RMSD bonds	0.010 Å
RMSD angles	1.03°
<i>Ramachandran plot analysis^h</i>	
Core region	98.4%
Allowed region	1.6%
Generously allowed region	0
Disallowed region	0

Supplemental Table 2: CaMKII β peptides found in each mass spectrometry run for RyR1 C1-C3 constructs.

	Sample	Peptide Sequence	z	log e score	Parent Ion Mass
sp P28652 KCC2B_MOUSE	C2 run 1	n.d			
sp P28652 KCC2B_MOUSE	C2 run 2	n.d			
sp P28652 KCC2B_MOUSE	C2 run 3	AGAYDFPSPEWDTVTPEAK	2	-5.387216143	2080.95708
sp P28652 KCC2B_MOUSE	C3 Run 1	LHDSISEEGFHLYVFDLVTGGELFEDIVAR	3	-7.698970004	3407.722385
sp P28652 KCC2B_MOUSE	C3 Run 1	LHDSISEEGFHLYVFDLVTGGELFEDIVAR	3	-6.823908741	3407.708471
sp P28652 KCC2B_MOUSE	C3 Run 1	LHDSISEEGFHLYVFDLVTGGELFEDIVAR	3	-6.008773924	3407.719823
sp P28652 KCC2B_MOUSE	C3 Run 1	LHDSISEEGFHLYVFDLVTGGELFEDIVAR	4	-2	3407.718162
sp P28652 KCC2B_MOUSE	C3 Run 2	LHDSISEEGFHLYVFDLVTGGELFEDIVAR	3	-12.1079054	3407.720921
sp P28652 KCC2B_MOUSE	C3 Run 2	LHDSISEEGFHLYVFDLVTGGELFEDIVAR	3	-9.552841969	3407.725316
sp P28652 KCC2B_MOUSE	C3 Run 2	LHDSISEEGFHLYVFDLVTGGELFEDIVAR	3	-8.721246399	3407.72495
sp P28652 KCC2B_MOUSE	C3 Run 2	LHDSISEEGFHLYVFDLVTGGELFEDIVAR	3	-5.468521083	3407.7125
sp P28652 KCC2B_MOUSE	C3 Run 2	LHDSISEEGFHLYVFDLVTGGELFEDIVAR	4	-4.721246399	3407.70449
sp P28652 KCC2B_MOUSE	C3 Run 2	LHDSISEEGFHLYVFDLVTGGELFEDIVAR	4	-1.920818754	3407.719627
sp P28652 KCC2B_MOUSE	C3 Run 2	LHDSISEEGFHLYVFDLVTGGELFEDIVAR	4	-1.568636236	3407.705467
sp P28652 KCC2B_MOUSE	C3 Run 2	NLINQMLTINPAK	2	-2.244125144	1469.828417
sp P28652 KCC2B_MOUSE	C3 Run 3	ESSDSTNTTIEDEDAK	2	-2.853871964	1741.741259
sp P28652 KCC2B_MOUSE	C3 Run 3	LHDSISEEGFHLYVFDLVTGGELFEDIVAR	2	-10.22184875	3407.699512
sp P28652 KCC2B_MOUSE	C3 Run 3	LHDSISEEGFHLYVFDLVTGGELFEDIVAR	3	-7.853871964	3407.722019
sp P28652 KCC2B_MOUSE	C3 Run 3	LHDSISEEGFHLYVFDLVTGGELFEDIVAR	3	-6.292429824	3407.716892
sp P28652 KCC2B_MOUSE	C3 Run 3	LHDSISEEGFHLYVFDLVTGGELFEDIVAR	4	-2.207608311	3407.715965
sp P28652 KCC2B_MOUSE	C3 Run 4	LHDSISEEGFHLYVFDLVTGGELFEDIVAR	2	-10.34678749	3407.71953
sp P28652 KCC2B_MOUSE	C3 Run 4	LHDSISEEGFHLYVFDLVTGGELFEDIVAR	3	-10.16115091	3407.722754
sp P28652 KCC2B_MOUSE	C3 Run 4	LHDSISEEGFHLYVFDLVTGGELFEDIVAR	4	-1.387216143	3407.714256

Supplemental Table 2: CaMKII β peptides found in each mass spectrometry run for RyR1 C1-C3 constructs. The constructs were immobilised on His-link beads and incubated with mouse skeletal muscle lysate to allow the formation of protein interaction complexes between the RyR1 constructs and *in vivo* binding partners. Control spectra were obtained from beads only incubated with skeletal muscle lysate. All experiments were run in triplicate (n.d – none determined). Shown are charges (z), log e scores and parent ion mass for each identified peptide.