Supplementary Data

The CPVT mutation R33Q disrupts the N-terminus structural motif that regulates reversible calsequestrin polymerization

Naresh C. Bal, Ashoke Sharon, Subash C. Gupta, Nivedita Jena, Sana Shaikh, Sandor Gyorke and Muthu Periasamy^{*}

LEGENDS TO SUPPLEMENTARY FIGURES

Figure S1. Multiple Sequence alignment of calsequestrin from residue 60 to C-terminus. There are dispersed patches of highly conserved residues throughout the molecule, which might be important for Ca²⁺ buffering and dynamic polymerization and depolymerization. The labeling is same as in the figure 1 in the text. Accession numbers are as follows: NP_001133632 (Salmo, Atlantic salmon), CAG00977 (Tetradon), NP 001002682 (Danio, zebrafish), BAG49513 (Solea, Senegalese sole), AAH80039 (Xenopus, Western clawed frog), NP 033944 (Mus), NP 058827 (Rattus), NP_001223 (Homo), XP_513677 (Pan, chimpanzee), Q5RAN9 (Pongo, orangutan), NP 001095161 (Oryctolagus), NP 001030451 XP 001101353 (Macaca). (Bos),XP 001500410 (Eqgus), XP 001363916 (Monodelphis, opossum), NP 989857 (Gallus), NP 033943 (Mus CASQ1), NP 001222 (Homo CASQ1), XP 002130664 (Ciona, sea squirt), and XP 001677823 (Caenorhabditis).

Figure S2. Conformation of CASQ2 mutants at 0 mM CaCl₂. Far UV CD spectra of cluster #1 (A) and cluster #2 (B) mutants. None of the mutations affected conformation of the protein in absence of Ca, the concentration at which these proteins are expected to be present predominantly in monomeric state. Hence these point mutations had no effect on protein conformation at monomeric level.

Figure S3. Thermal stability of CASQ2 mutants. Far UV CD spectra of cluster #1 (A) and cluster #2 (B) mutants at temperatures indicated in °C. Mutants of Cluster #1 behave more like R33Q mutant while cluster #2 mutants behave more like WT protein. This suggests that cluster #1, which is highly conserved, is indispensable for Ca^{2+} -induced CASQ2 polymerization (supported by figure 3 in text and S3 below); while cluster #2 is dispensable and it is less conserved in evolution.

Figure S4. Ca²⁺/EGTA-induced structural changes as analyzed by CD spectra (A) cluster #1 mutants; Charge alteration in cluster #1 leads to loss of reversible polymerization. (B) Cluster #2 mutants; Single charge neutralization of cluster #2 'E39A' could not affect polymerization-depolymerization behavior. In contrast, double charge neutralization of cluster #2 'K40A-K43A' shows altered CD spectra in presence of 5 mM CaCl₂, effect was intermediate between WT and R33Q. Interestingly however, K40A-K43A could regain native conformation upon Ca²⁺-chelation with EGTA.

Figure S5. Polymerization dynamics of WT and mutant CASQ2 as analyzed by turbidimetric assay: As shown in figure 3C and 3D, WT-CASQ2 can undergo rapid aggregation and resolubilization at physiological $[Ca^{2+}]$ (~2.0 mM) and at ~2.5 mM EGTA concentration respectively. However, mutations in cluster #1 alter this bidirectional transition. Replotting of data from figure 3 reveals Ca²⁺/EGTA mediated transition of WT (A), R33Q (B), D29A-D32A (C), and K31A-K33A (D). The percentage of protein aggregate at 0 mM EGTA is highest before EGTA mediated chelation. The calcium induced aggregation and disaggregation (by EGTA) curve for the WT protein is qualitatively similar to the mathematical calculation by Restrepo et al, 2008. However, mutation in the crucial cluster #1 shifts the transition to the right deterring buffering-polymerization dynamics.

Supplementary Figures

	60	70	80	90	100	110	120
Salmo_CASQ2	PQPAH	GRQKQLQMTE	LVLELTAQ	LEDKDIGEGMV	DSQKDAKVA	KKLGLEE.E	G <mark>SLYI</mark> F <mark>K</mark> DD <mark>RVIEFDG</mark>
Tetraodon_CASQ2	PIPAN	GL <mark>QKR</mark> FQMT <mark>E</mark> I	LVLELTAQ	/LENKDIG <mark>F</mark> GMV	DSLKDAKVA	KKLGLEE.V	G <mark>SLYV</mark> F <mark>K</mark> DN <mark>RVIEFD</mark> G
Danio_CASQ2	PPPAA	EL <mark>QKQ</mark> LH L T <mark>E</mark> I	LVLELAAQ	/LEEKDIGEGMV	DSQ <mark>K</mark> DAKVA	KKLGLHE.E	G <mark>SVYI</mark> F <mark>K</mark> DD <mark>RVIEFD</mark> G
Solea_CASQ2	PIPDN	EHQROHQMTEI	LVLELAAQ	/TEEKDIGEGMV	DSHKDAKVA	KKLGLEE.V	G <mark>SVYI</mark> F <mark>K</mark> AD <mark>RVIEYD</mark> G
Xenopus_CASQ2	PPPGD	GAORRLHLTEN	V LELTAQ	/LERKQIGEGLI	DPKKNSKIA	KKLGCSE.E	G <mark>SLYI</mark> F <mark>K</mark> ED <mark>NVIEFD</mark> G
Mus_CASQ2	PVSSD	VS <mark>OKO</mark> FQLKE	IVLELVAQ	/LEHKNIGEVMV	DSRKEAKLA	KRLGFSE.E	G <mark>SLYV</mark> L <mark>K</mark> GD <mark>RTIE</mark> FD <mark>G</mark>
Rattus_CASQ2	PVSSD	VACKOFQLKE	IVLELVAQ	/LEHKNIGEVMV	DSRKEAKLA	KRLGFSE.E	3 <mark>SLYV</mark> L <mark>K</mark> GG <mark>RTIEFD</mark> G
Homo_CASQ2	PVSSD	VTOKOFQLKE	IVLELVAQ	/LEHKAIGEVMV	DAKKEAKLA	KKLGFDE.E	G <mark>SLYI</mark> LKGD <mark>RTIEFD</mark> G
Pan_CASQ2	PVSSD	VACKOFQLKE	IVLELVAQ	/LEHKAIGEVMV	DAKKEAKLA	KKLGFDE.E	G <mark>SLYI</mark> LKGD <mark>RTIE</mark> FDG
Pongo_CASQ2	PVSSD	VACKOFQLKE	IVLELVAQ	/LEHKAIGEVMV	DAKKEAKLA	KKLGFDE.E	S <mark>SLYI</mark> L <mark>K</mark> GD <mark>RTIEFD</mark> G
Macaca_CASQ2	PVSSD	VACKOFQLKE	IVLELVAQ	/LEHKAIGEVMV	DAKKEAKLA	KKLGFDE.K	G <mark>SLYV</mark> LKGD <mark>RTIEFD</mark> G
Oryctolagus_CASQ2	PVSAD	VACKOFQLKE	IVLELVAQ	/LEHKEIGEVMV	DAKKEAKLA	KKLGFDE.E	G <mark>SLYI</mark> LKGD <mark>RTIEFD</mark> G
Bos_CASQ2	PLSSD	VVQKQFQLKE	IVLELVAQ	/LEHKDIGEVMV	DAKKEAKLA	KKLGFDE.E	S <mark>SLYI</mark> L <mark>K</mark> GD <mark>RTIEFD</mark> G
Eqqus_CASQ2	PVSSD	VAQKOFQLKE	IVLELVAQ	/LEHKDIGEVMV	DAKKEAKLA	KKLGFDE.E	G <mark>SLYI</mark> L <mark>K</mark> GD <mark>RTIE</mark> FD <mark>G</mark>
Monodelphis_CASQ2	PVSSD	VS <mark>OKO</mark> FQMKE	IVLELAAQ	/LEHKDIGFAMV	DAKKEAKLA	KKLGFDE.E	S <mark>SLYI</mark> L <mark>K</mark> GD <mark>RTIEFD</mark> G
Gallus_CASQ2	PVSSD	VSOKOFOMTEN	MVLELAAQ	/LEPRSIGEGMV	DSKKDAKLA	KKLGLVE.E	G <mark>SLYV</mark> F <mark>K</mark> EE <mark>RLIE</mark> FD <mark>G</mark>
Mus CASQ1	PPEDD	ASOROFEMEEI	LILELAAQ	/LEDKGVGFGLV	DSEKDAAVA	KKLGLTE.EI	D <mark>SVYV</mark> FKGD <mark>EVIEYD</mark> G
Homo_CASQ1	PPEDD	ASOROFEMEEI	LILELAAQ	/LEDKGVGFGLV	DSEKDAAVA	KKLGLTE.VI	D <mark>SMYV</mark> F <mark>K</mark> GD <mark>EVIEYD</mark> G
Ciona_CASQ	HDAND	YLAKOWQLTEI	EMLELAAQ	TEREGVGEGVV	DLEKDKKLA	EKLDKTE.A	G <mark>AIYA</mark> YKAG <mark>HSVE</mark> FD <mark>G</mark>
Caenorhabditis_CASQ	VEEDD	ELDOYE(LOLSAQ	MTKRGYNFYTV	NTTKEHRLR	KOEEVEKGEI	D T I H V Y K D G Y K I E Y N G
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	130	140	150	160	170	180	190
Salmo_CASQ2	EL	SADTLVEFLLDVLET	PVELISNPN	ELRAFERMEE	DIRLIGYFKGE	DS.YYKAFQE	ASERFQPYIKFFA
Tetraodon_CASQ2	EF	SSDTLVEFLLDVLEI	PV EMINNAN	ELRAFERMEE	DIRLIGYFKGE	DS.HFRAFQK	A SELFQPYIKFFA
Danio_CASQ2	LP	SADTLVEFLLDLLE	D <mark>PV</mark> EIIDNAI	ELRAFDRMEE	DIKLIGFFKSQ	ESEHYLAFQE	AAEQFQPFIKFFA
Solea_CASQ2	LL	SANTLVEFLLDLLEF	E <mark>PV</mark> EVIGNAI	ELRAFDRMEE	DIKLIGFFKSE	DSDHFDAFKE	A A E Q F H P Y I K F Y A
Xenopus_CASQ2	EM	AADVIVDFLLDLMEI	PVEVINSKA	AELKVLDHMEE	EMKLIGYFKGE	DSEYYKDYEE	AAEHFHPFIKFFA
Mus_CASQ2	EF	AADVLVEFLLDLIEI	O <mark>PV</mark> EIVNNKI	LEVQAFERIEL	QTKLLGFFKNE	DSEYYKAFQE	AAEHFQPYIKFFA
Rattus_CASQ2	EF	AADVLVEFLLDLIET	P V EIVNNKI	EVQAFERIED	QIKLLGFFKNE	DSEYYKAFQE	AAEHFQPYIKFFA
Homo_CASQ2	EF	AADVLVEFLLDLIEI	PVEIISSKI	LEVQAFERIEL	YIKLIGFFKSE	DSEYYKAFEE	AAEHFQPYIKFFA
Pan_CASQ2	EF	AADVLVEFLLDLIEI	PVEIISSKI	LEVQAFERIED	YIKLIGFFKSE	DSEYYKAFEE	AAEHFQPYIKFFA
Pongo CASQ2	EF	AADVLVEFLLDLIEI	PVEIISSKI	EVQAFERIED	YIKLIGFFKSG	DSEYYKAFEE	A A E H F O P Y I K F F A
Macaca_CASQ2	EF	AADVLVEFLLDLIET	PVEIISSKI	LEVQAFERIEL	HIKLIGFFKSE	DSEYYKAFEE	AAEHFOPYIKFFA
Oryctolagus_CASQ2	EF	AADVLVEFLLDLIEI	PVEIINSKI	EVQAFERIED	HIKLIGFFKSA	DSEYYKAFEE	AAEHFOPYIKFFA
Bos CASQ2	EF	AADVLVEFLLDLIEI	PVEIINSKI	LEVQAFERIED	HIKLIGFFKSE	ESEHYKAFEE	AAEHFOPYIKFFA
Eggus CASO2	EF	AADVLVEFLLDLIEI	PVEIINSKI	EVOAFERIED	OIKLIGFFKSC	DSEYYKAFEE	AAEHFOPYIKFFA
Monodelphis_CASQ2	EL	AADVLVEFLLDLIET	PVEVINSKI	LEVQAFERIDE	FIKLIGFFKSE	DSEHYKAFEE	AAEHFÕPYIKFFA
Gallus_CASQ2	EL	ATDVLVEFLLDLLEI	PVEVINSKI	ELQAFDOIDD	EIKLIGYFKGE	DSEHYKAFEE	AAEHFOPYVKFFA
Mus_CASQ1	EF	SADTLVEFLLDVLEI	PVELIEGE	RELOAFENIEL	EIKLIGYFKSK	DSEHYKAYED	AAEEFHPYIPFFA
Homo_CASQ1	EF	SADTIVEFLLDVLED	PVELIEGE	RELQAFENIED	EIKLIGYFKSK	DSEHYKAFED	AAEEFHPYIPFFA
Ciona_CASQ	QR	STDVLVEFVLELDEY	P V EEINSK	EVQGFRR. DE	STKVIGYFESN	TASGYDEFVD	AAHDFQPVISFYA
Caenorhabditis_CASQ	VR	DPETF <mark>V</mark> SWLMDIPDI	PVTIINDE	DLEEFENMDD	ECVRIIGYFEP	GSVALKEFEE	AAEDFMGEIEFFA

	200	210	220	230 24	0 250	260
Salmo_CASQ2	TEDKATAKHL	SL.KMNEVNF	YEPFMEEPAIL	P. GRQLSEMEIVE	FVHQHKRATLRKLR	AEDMFETWEDDL
Tetraodon_CASQ2	TFDKSMAKHL	SL.KMNEVNF	YEPFMEEPAIL	P.GRPLSEMEIVE	FVKQHRRATLRKLR	AENMFETWEDDL
Danio_CASQ2	TFEKSVAKEL	TL.KMNEVDF	YEPFMEEPVTI	P.DKPHSEELVA	FISEHRRPTLRKLK	AEDMFETWEDDL
Solea_CASQ2	TFEKSVAKEL	TL.KMNEVDF	YEPFMEEPVTI	P.GKPLSEEDIVE	FINEHRRPTLRKLR	AEDMFETWEDDI
Xenopus_CASQ2	TFEKSVAKAL	SL.KMNEVDF	YEPFMEEPVTI	P.DKPYSEEELVI	FIHKHKRATLRKLR	PEDMFETWEDDL
Mus_CASQ2	TFDKAVAKKL	SL.KMNEVGF	YEPFMDEPNVI	P.NKPYTEEELVE	FVKEHQRPTLRRLR	PEDMFETWEDDL
Rattus_CASQ2	TFDKGVAKKL	SL.KMNEVGF	YEPFMDEPSVI	P.NKPYTEEELVE	FVKEHQRPTLRRLR	PEDMFETWEDDL
Homo_CASQ2	TFDKGVAKKL	SL.KMNEVDF	YEPFMDEPIAI	P.NKPYTEEELVE	FVKEHQRPTLRRLR	PEEMFETWEDDL
Pan_CASQ2	TFDKGVAKKL	SL.KMNEVDF	YEPFMDEPIAI	P.NKPYTEEELVE	FVKEHQRPTLRRLR	PEEMFETWEDDL
Pongo_CASQ2	TFDKGVAKKL	SL.KMNEVDF	YEPFMDEPIAI	P.NKPYTEEELVE	FVKEHQRPTLRRLR	PEEMFETWEDDL
Macaca_CASQ2	TFDKGRVSSP	FL.GLHTS	PSTGSGWGV	E.WELLCDEFKQV	VCKIQEEAIGSV	TFDSFLIQEDDL
Oryctolagus_CASQ2	TFDKGVAKKL	SL.KMNEVDF	YEPFMDEPTPI	P.NKPYTEEELVE	FVKEHQRPTLRRLR	PEDMFETWEDDL
Bos_CASQ2	TFDKGVAKKL	SL.KMNEVDF	YEPFMDEPIAI	P.DKPYTEEELVE	FVKEHQRPTLRRLR	PEDMFETWEDDL
Eqqus_CASQ2	TFDKGVAKKL	SL.KMNEVHF	YEPFMDEPIAI	P.NKPYTEEELVE	FVKEHQRPTLRRLR	PEDMFETWEDDL
Monodelphis_CASQ2	TFDKGVAKKL	TL.KMNEVDF	YEPFMDEPIPI	P.DKPYTEEELVE	FVKEHQRPTLRRLR	PEDMFETWRQDV
Gallus_CASQ2	TFDKGVAKKL	GL.KMNEVDF	YEPFMDEPVHI	P.DKPYTEEELVE	FVKEHKRATLRKLR	PEDMFETWEDDM
Mus_CASQ1	TFDSKVAKKL	TL.KLNEIDF	YEAFMEEPMTI	P. DKPNSEEEIVS	FVEEHRRSTLRKLK	PESMYETWEDDL
Homo_CASQ1	TEDSKVAKKL	TL.KLNEIDF	YEAFMEEPVTI	P. DKPNSEEEIVN	IFVEEHRRSTLRKLK	PESMYETWEDDM
Ciona_CASQ	VFQKLLARQL	GLTELNQVDF	YEPYMKKSIVI	PGETPLDNTVIER	FVQEHKRATLRKLR	TMDMYETWEDDI
Caenorhabditis_CASQ	VVTSKWARKV	GLKRVGEVQM	RRPFEEDPLFA	P. TSADTEEFEE	WVEKNKEPVMQKLT	LDNYFNLWRDPE

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	270	280	290	300	310	
Salmo_CASQ2	DGIHIVAFAEI	E.EDPD <mark>G</mark> YEFLE	I <mark>L K D V A</mark> R D N T N N	P.ELSIVWI	DPD <mark>DF</mark> PLL	
Tetraodon_CASQ2	DGIHIVAFAEI	E.EDPDGYEFLE.	LKDVARDNTNN	IP.ELSIVWI	DPDDFPLVRFRPY	4FSNTCLNSSVLTWS
Danio_CASQ2	NGIHIVAPAEL	EDPDGFEFLE.	I KEVARDNIHN	P. DISIVWI		
Solea_CASUZ	DCTHIVAFAEI	EDEDGEEFLE.	TREVARDNIEL			
Mus CASO2	NGTHIVAFAE	SDPDGYEFLE	TKOVARDNTDN	IP DISTINT		
Rattus CASO2	NCTHIVAFAE	SDPDCVEFIE:	TKOVARDNTDN	IP DISTINT		
Homo CASO2	NGTHIVAFAER	SDPDGYEFLE	LKOVARDNTDN	IP DISTLWT		
Pan CASO2	NGTHIVAFAER	SDPDGYEFLE	LKOVARDNTDN	PDISTLWT	DPDDFPIT	
Pongo CASO2	NGIHIVAFAER	. SDPDGYEFLE	LKOVARDNTDN	P. DISILWI		
Macaca_CASQ2	NGIHIVAFAE	. SDPDGYEFLE	LKOVARDNTDN	IP. DLSILWI	DPD <mark>DF</mark> PLL	
Oryctolagus_CASQ2	NGIHIVPFAER	K.SDPD <mark>G</mark> YEFLE	I <mark>L K Q V A</mark> R D N T D N	IP.DLSIVWI	DPD <mark>DF</mark> PLL	
Bos_CASQ2	NGIHIVAFAE	R.SDPD <mark>G</mark> YEFLE:	I KQV <mark>A</mark> RDNTDN	IP.DLSIVWI	DPD <mark>D</mark> F <mark>PLL</mark>	
Eqqus_CASQ2	NGIHIVAFAE	R.SDPD <mark>G</mark> YEFLEI	I <mark>L K Q V A</mark> R D N T D N	IP.DLSIVWI	DPD <mark>D<mark>F</mark>PLL</mark>	
Monodelphis_CASQ2	LGSNMASDISH	K L C D P D <mark>G</mark> F E F L E I	I <mark>LKQVA</mark> RDNTNN	IP.DLSIVWI	DPD <mark>DF</mark> PML	
Gallus_CASQ2	EGIHIVAFAE	E.DDPD <mark>G</mark> FEFLEI	I KQV <mark>A</mark> RDNTDN	IP.DLSIVWI	DPD <mark>D</mark> F <mark>PLL</mark>	
Mus_CASQ1	DGIHIVAFAE	E.ADPD <mark>G</mark> YEFLE	IKAVAQDNTEN	IP.DISIIWI	DPDDF <mark>PLL</mark>	
Homo_CASQ1	DGIHIVAFAEI	E. ADPDGFEFLE	ILKAVAQDNTEN	IP. DISTIWI	DPDDFPLL	
Ciona_CASQ	NDIHVVAFADI	. TDPEGFEFLQI	LKEIAHIHIDD	P.N.SIVWI		
Caenornabdicis_CASQ	EDERMILARVI	E ELI KEGRAMAA	LUKIADENSE	IAGI LEV		
		320 :	330 34	10	350	360
Salmo_CASQ2		320 : Wektek vniek	330 34 Poigvvvvtda	IQ Ndsvwldm	350 SNDED	360 A B B L B D W I B D V L S G
Salmo_CASQ2 Tetraodon_CASQ2	TT LVLSPPQLTT	320 WEKTFKVNLFK WEKTFKLDLFK	330 34 Poigvvnvtda Poigvvnvtda	IQ IDSV <mark>WLD</mark> M	350 SNDEDLP SNDEDLP	360 A E E E D WIEDVIS A E E E D WIEDVIS
Salmo_CASQ2 Tetraodon_CASQ2 Danio_CASQ2	 LVLSPPQLIT 	320 (WEKTEKVNLEK (WEKTEKLDLEK (WEMTEKVDLER	330 34 PQIG <mark>VVNVTDA</mark> PQIG <mark>VVNVTDA</mark> PQIG <mark>VVNVTDA</mark>	OSVWLDM ADSVWLDM ADSVWLDM	350 SNDEDLP SNDEDLP PNDDELPS	360 IAEELEDWIEDVLSG IAEELEDWIEDVLSG SAEELENWIEDVLSG
Salmo_CASQ2 Tetraodon_CASQ2 Danio_CASQ2 Solea_CASQ2	T LVLSPPQLT IP IP	320 WEKTEKVNLEK WEKTEKUDLER WEKTEKVDLER	330 34 PQIG <mark>VVNVTDA</mark> PQIGVVNVTDA PQIG <mark>VVNVTDA</mark> PQIG <mark>VINVTDA</mark>	DSVWLDM DSVWLDM DSVWLDM DSVWLEI DSVWLEI	350 SNDEDLP SNDEDLP PNDDELP EDEEDLP	360 TAEELEDWIEDVLSG TAEELEDWIEDVLSG SAEELENWIEDVLSG SAQELEDWIEDVLSG
Salmo_CASQ2 Tetraodon_CASQ2 Danio_CASQ2 Solea_CASQ2 Xenopus_CASQ2	LVLSPPQLIT IP VS	320 WEKTEKVNLFK WEKTEKULFK WEKTEKVDLFR WEKTEKVDLFR	330 34 PQIG <mark>VVNVTDA</mark> PQIGVVNVTDA PQIGVINVTDA PQIGVINVTDA PQIG <mark>VVNVTD</mark> A	Q DSVWLDM DSVWLDM DSVWLEI DSVWLEM DSVWMEM	350 SNDEDLP1 SNDEDLP1 PNDDELP5 EDEEDLP5 KDAEDLP5	360 TAEELEDWIEDVLSG TAEELEDWIEDVLSG SAEELENWIEDVLSG SAQELEDWIEDVLSG SADELEQWIEDVLSG
Salmo_CASQ2 Tetraodon_CASQ2 Danio_CASQ2 Solea_CASQ2 Xenopus_CASQ2 Mus_CASQ2	LVLSPPQLTT 	320 WEKTFKULLFK WEKTFKULLFR WEKTFKVULFR WEKTFHIDLFR WEKTFKIDLFK	330 34 PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA	Q DSVWLDM DSVWLEM DSVWLEI DSVWLEM DSVWMEM DSVWMEI	350 SNDEDLP1 SNDEDLP1 PNDDELP3 EDEDLP8 KDAEDLP8 KDAEDLP8	360 TAEELEDWIEDVLSG GAEELEDWIEDVLSG SAQELEDWIEDVLSG SADELEQWIEDVLSG TAEELEDWIEDVLSG
Salmo_CASQ2 Tetraodon_CASQ2 Danio_CASQ2 Solea_CASQ2 Xenopus_CASQ2 Mus_CASQ2 Rattus_CASQ2 Rattus_CASQ2	TT LVLSPPQLTT P VS VA VA	320 WEKTEKULFK WEKTEKULFK WEKTEKULFR WEKTEKILFR WEKTEKILFK	PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA	Q DSVWLDM DSVWLEI DSVWLEI DSVWLEM DSVWMEM DSVWMEI	350 SNDEDLP1 SNDEDLP1 PNDELP5 EDEEDLP5 KDAEDLP5 PDDDDLP1 PDDDDLP1	360 IAEELEDWIEDVLSG SAEELEDWIEDVLSG SAQELEDWIEDVLSG SADELEWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG
Salmo_CASQ2 Tetraodon_CASQ2 Danio_CASQ2 Solea_CASQ2 Xenopus_CASQ2 Mus_CASQ2 Rattus_CASQ2 Homo_CASQ2 Homo_CASQ2	TT LVLSPPQLTT IP VA VA VA	320 WEKTEKUDIFK WEKTEKUDIFK WEKTEKUDIFR WEKTEKUDIFR WEKTEKIDIFK WEKTEKIDIFK WEKTEKIDIFR	PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA	Q V V V L D M V V V L D M V V V L E I V V V L E M V V V L E M V V V L E M V V V L E I V V V V V L E I V V V V V V V V V V V V V V V V V V V	350 SNDEDLP1 SNDEDLP3 PNDDELP3 EDEEDLP3 PDDDDLP1 PDDDDLP1 PDDDDLP1	360 IAEELEDWIEDVLSG SAEELENWIEDVLSG SAQLEDWIEDVLSG SADELEQWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG
Salmo_CASQ2 Tetraodon_CASQ2 Danio_CASQ2 Solea_CASQ2 Xenopus_CASQ2 Mus_CASQ2 Rattus_CASQ2 Homo_CASQ2 Pan_CASQ2 Pango_CASQ2	LVLSPPQL T LVLSPPQL T P VS VA VA VA VA	320 WEKTEKLDLFK WEKTEKLDLFK WEKTEKVDLFR WEKTEKVDLFR WEKTEKIDLFR WEKTEKIDLFK WEKTEKIDLFR WEKTEKIDLFR WEKTEKIDLFR	PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA	IO V V V L D M V S V V L D M V S V V L E I V S V V L E M V S V V M E I V S V V M E I	350 SNDEDLP1 SNDEDLP3 EDEEDLP8 EDEEDLP8 PDDDLP1 PDDDLP1 PDDDLP1 PDDDLP1	360 TAEELEDWIEDVLSG SAELENWIEDVLSG SAELENWIEDVLSG SAELEDWIEDVLSG TAEELEDWIEDVLSG TAEELEDWIEDVLSG TAEELEDWIEDVLSG TAEELEDWIEDVLSG
Salmo_CASQ2 Tetraodon_CASQ2 Danio_CASQ2 Solea_CASQ2 Xenopus_CASQ2 Mus_CASQ2 Rattus_CASQ2 Pan_CASQ2 Pongo_CASQ2 Pongo_CASQ2 Pongo_CASQ2	LVLSPPQLT TP VS VA VA VA VA VA VA VA	320 WEKTFKVDLFR WEKTFKVDLFR WEKTFKVDLFR WEKTFKVDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR	330 34 PQIGVVNVTDA	Q DSVWLDM DSVWLEM DSVWLEM DSVWMEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI	350 SNDEDLP SNDEDLP PNDDELP EDEEDLP PDDDLP PDDDDLP PDDDDLP PDDDDLP PDDDDLP	360 IAEELEDWIEDVLSG SAEELEDWIEDVLSG SAEELEDWIEDVLSG SAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG
Salmo_CASQ2 Tetraodon_CASQ2 Danio_CASQ2 Solea_CASQ2 Xenopus_CASQ2 Mus_CASQ2 Mus_CASQ2 Homo_CASQ2 Pan_CASQ2 Pongo_CASQ2 Macaca_CASQ2 Macaca_CASQ2 Orvectolarus_CASQ2	TT LVLSPPQLIT 	320 WEKTFKVNLFK WEKTFKVDLFR WEKTFKVDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR	330 34 PQIGVVNVTDA	Q DSVWLDM DSVWLEM DSVWLEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI	350 SNDEDLP1 SNDEDLP1 PNDDELP2 EDEEDLP2 KDAEDLP2 PDDDDLP1 PDDDDLP1 PDDDDLP1 PDDDDLP1 PDDDDLP1 PDDDDLP1	360 TAEELEDWIEDVLSG SAEELEDWIEDVLSG SAEELEDWIEDVLSG SAEELEDWIEDVLSG TAEELEDWIEDVLSG TAEELEDWIEDVLSG TAEELEDWIEDVLSG TAEELEDWIEDVLSG TAEELEDWIEDVLSG
Salmo_CASQ2 Tetraodon_CASQ2 Danio_CASQ2 Solea_CASQ2 Xenopus_CASQ2 Mus_CASQ2 Mus_CASQ2 Homo_CASQ2 Pan_CASQ2 Pongo_CASQ2 Macaca_CASQ2 Oryctolagus_CASQ2 Pos CASQ2	LVLSPPQLIT 	320 WEKTFKVDLFR WEKTFKVDLFR WEKTFKVDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR	330 34 PQIGVVNVTDA	Q DSVWLDM DSVWLEM DSVWLEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI	350 	360 TAEELEDWIEDVLSG TAEELEDWIEDVLSG SADELEDWIEDVLSG SADELEQWIEDVLSG TAEELEDWIEDVLSG TAEELEDWIEDVLSG TAEELEDWIEDVLSG TAEELEDWIEDVLSG TAEELEDWIEDVLSG TAEELEDWIEDVLSG TAEELEDWIEDVLSG TAEELEDWIEDVLSG
Salmo_CASQ2 Tetraodon_CASQ2 Danio_CASQ2 Solea_CASQ2 Xenopus_CASQ2 Mus_CASQ2 Mus_CASQ2 Homo_CASQ2 Pan_CASQ2 Pongo_CASQ2 Pongo_CASQ2 Oryctolagus_CASQ2 Bos_CASQ2 Ecqus CASQ2	LVLSPPQLTT 	320 WEKTFKUDLFR WEKTFKUDLFR WEKTFKUDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR	PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA	LOSVWLDM LDSVWLDM LDSVWLEI LDSVWLEM LDSVWMEI LDSVWMEI LDSVWMEI LDSVWMEI LDSVWMEI LDSVWMEI LDSVWMEI LDSVWMEI LDSVWMEI	350 SNDEDLP1 PNDELP2 PNDELP2 EDEEDLP2 KDAEDLP2 PDDDDLP1 PDDDDLP1 PDDDDLP1 PDDDDLP1 PDDDDLP1 PDDDDLP1 PDDDDLP1 PDDDDLP1 PDDDDLP1 PDDDDLP1 PDDDDLP1 PDDDDLP1	360 IAEELEDWIEDVLSG SAEELEDWIEDVLSG SAEELEDWIEDVLSG SAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG
Salmo_CASQ2 Tetraodon_CASQ2 Danio_CASQ2 Solea_CASQ2 Xenopus_CASQ2 Mus_CASQ2 Mus_CASQ2 Homo_CASQ2 Pan_CASQ2 Pongo_CASQ2 Pongo_CASQ2 Macaca_CASQ2 Oryctolagus_CASQ2 Eqqus_CASQ2 Eqqus_CASQ2	TT LVLSPPQLTT VS VS VA 	320 WEKTEKUDLFK WEKTEKUDLFR WEKTEKUDLFR WEKTEKIDLFR WEKTEKIDLFR WEKTEKIDLFR WEKTEKIDLFR WEKTEKIDLFR WEKTEKIDLFR WEKTEKIDLFR WEKTEKIDLFR WEKTEKIDLFR WEKTEKIDLFR WEKTEKIDLFR	PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA	Q DSVWLDM DSVWLEI DSVWLEI DSVWLEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI DSVWMEI	350 SNDEDLP SNDEDLP PNDELPS EDEEDLPS PDDDDLP PDDDDLP PDDDDLP PDDDDLP PDDDDLP PDDDDLP PDDDDLP PDDDDLP PDDDDLP PDDDDLP PDDDDLP PDDDDLP PDDDDLP PDDDDLP	360 IAEELEDWIEDVLSG SAEELEDWIEDVLSG SAEELEDWIEDVLSG SAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG
Salmo_CASQ2 Tetraodon_CASQ2 Danio_CASQ2 Solea_CASQ2 Xenopus_CASQ2 Mus_CASQ2 Rattus_CASQ2 Homo_CASQ2 Pan_CASQ2 Pongo_CASQ2 Pongo_CASQ2 Macaca_CASQ2 Cryctolagus_CASQ2 Eqqus_CASQ2 Eqqus_CASQ2 Gallus CASQ2	LVLSPPQLTT LVLSPPQLTP 	320 WEKTFKVDLFR WEKTFKVDLFR WEKTFKVDLFR WEKTFKVDLFR WEKTFKIDLFK WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFK WEKTFKIDLFK WEKTFKIDLFK WEKTFKIDLFK	PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA PQIGVVNVTDA	LOSVWLDM LDSVWLDM LDSVWLEI LDSVWLEI LDSVWMEI LDSVWMEI LDSVWMEI LDSVWMEI LDSVWMEI LDSVWMEI LDSVWMEI LDSVWMEI LDSVWMEI LDSVWMEI LDSVWMEI	350 SNDEDLP1 SNDEDLP3 PNDDELP3 PDDDLP1 PDDDLP1 PDDDLP1 PDDDLP1 PDDDLP1 PDDDLP1 PDDDLP1 PDDDLP1 PDDDLP1 PDDDLP1 PDDDLP1 PDDDLP1 PDDDLP1 PDDDLP1 PDDDLP1 PDDDLP1 	360 AEELEDWIEDVLSG AEELEDWIEDVLSG AEELEDWIEDVLSG AEELEDWIEDVLSG AEELEDWIEDVLSG AEELEDWIEDVLSG AEELEDWIEDVLSG AEELEDWIEDVLSG AEELEDWIEDVLSG AEELEDWIEDVLSG AEELEDWIEDVLSG AEELEDWIEDVLSG AEELEDWIEDVLSG AEELEDWIEDVLSG AEELEDWIEDVLSG
Salmo_CASQ2 Tetraodon_CASQ2 Danio_CASQ2 Solea_CASQ2 Xenopus_CASQ2 Mus_CASQ2 Rattus_CASQ2 Homo_CASQ2 Pan_CASQ2 Pongo_CASQ2 Macaca_CASQ2 Oryctolagus_CASQ2 Bos_CASQ2 Eqqus_CASQ2 Monodelphis_CASQ2 Gallus_CASQ2 Mus_CASQ1	LVLSPPQLT LVLSPPQLT P VS VA VA VA VA VA VA VA VA VA VA	320 WEKTFK WEKTFKVDLFR WEKTFKVDLFR WEKTFKVDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFR WEKTFKIDLFK WEKTFKIDLFK WEKTFKIDLFR WEKTFK	330 34 PQIGVVNVTDA	Q DSVWLDM DSVWLEM DSVWLEI DSVWMEI	350 	360 AEELEDWIEDVLSG AEELEDWIEDVLSG SAEELEDWIEDVLSG SAEELEDWIEDVLSG SAEELEDWIEDVLSG AEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG IAEELEDWIEDVLSG
Salmo_CASQ2 Tetraodon_CASQ2 Danio_CASQ2 Solea_CASQ2 Xenopus_CASQ2 Mus_CASQ2 Homo_CASQ2 Pan_CASQ2 Pongo_CASQ2 Pongo_CASQ2 Oryctolagus_CASQ2 Bos_CASQ2 Eqqus_CASQ2 Monodelphis_CASQ2 Mus_CASQ1 Homo_CASQ1	LVLSPPQL I T I P 	320 WEXTEKULFK WEXTEKULFR WEXTEKULFR WEXTEKULFR WEXTEKILFR	330 34 PQIGVVNVTDA	Q DSVWLDM DSVWLDM DSVWLEI DSVWLEI DSVWMEI	350 	360 TAEELEDWIEDVLSG TAEELEDWIEDVLSG SAEELEDWIEDVLSG SAEELEDWIEDVLSG TAEELEDWIEDVLSG
Salmo_CASQ2 Tetraodon_CASQ2 Danio_CASQ2 Solea_CASQ2 Xenopus_CASQ2 Mus_CASQ2 Mus_CASQ2 Homo_CASQ2 Pongo_CASQ2 Pongo_CASQ2 Pongo_CASQ2 Macaca_CASQ2 Oryctolagus_CASQ2 Bos_CASQ2 Equus_CASQ2 Equus_CASQ2 Gallus_CASQ2 Mus_CASQ1 Homo_CASQ1 Ciona_CASQ	LVLSPPQL I T 	320 WEXTFX W	330 34 PQIGVVNVTDA PQIGVNVTDA PQIGVNVTDA PQIGVNVTDA PQIGVNVTDA PQIGVNVTDA PQIGVNVTDA PQIGVNVTDA	LOSVWLDM LDSVWLDM LDSVWLEI LDSVWLEI LDSVWMEI	350 	360 TAEELEDWIEDVLSG SAEELEDWIEDVLSG SAEELEWIEDVLSG SAEELEWIEDVLSG TAEELEDWIEDVLSG

	370	380	390	400
Salmo_CASQ2	KVNTEDDDD	VKD	YDYDRVDREDRHH.	GDDHEDHGDYDDHDD
Tetraodon_CASQ2	RVNTEDDDE	ATNI		
Danio_CASQ2	TVNTEDDDD	. DDDI	DDDDDDDDDDN.	DDDDDDDDDDDDDD
Solea_CASQ2	KVNTEDDDD	.DDDI	DDDDDDEDDDD.	DDDDDDDDDDDDDDD
Xenopus_CASQ2	KVNTE <mark>DDD</mark> D	.DDDI	DDDDDDDDDDDDD.	DDDDDDDDDDDDDD
Mus_CASQ2	KINTEDDDN	.EDEI	DDGDDNDDDDDDI	DNDNSDEDNEDSDDDDD
Rattus_CASQ2	KINTEDDDN	.EDEI	DDGDNDNDDDDI	DDDDNSDEDNDDSDDDDD
Homo_CASQ2	KINTEDDD	.EDDI	DDD	NSDEEDNDDSDDDDD
Pan_CASQ2	KINTE <mark>DDD</mark> D	.EDDI	DDD	NSDEEDND.SDDDDD
Pongo_CASQ2	KINTEDDD	DEDI	DDD	NSDEEDNDDSDDDDD
Macaca_CASQ2	KINTEDDDE	.DDDI	DDD	NNSDEDNDDSDDDDD
Oryctolagus_CASQ2	KINTEDDDN	EDEI	DDDDNDDDDDDNG.	NSDEEDNDDSDEDDE
Bos_CASQ2	KINTEDDDN	DDE	DEDDDDDDD	NSDEEDNDDSDDDDE
Eqqus_CASQ2	KINTEDDDN	.EDEI	DDDDDDDDDDD	NSDEEDNDDSDDDDE
Monodelphis_CASQ2	KINTEDDDD	.DDGI	DDDDDDDDDDDDNS	SEEDDDDDDDDDDDDDDDDD
Gallus_CASQ2	KINTEDDDD	DDD		DDDDDDDDDDDDDDD
Mus_CASQ1	EINTEDDDD	. DDDI	DDDD	DDDDD
Homo_CASQ1	EINTEDDDD	. DDDI		
Ciona_CASQ	KINTEDDD DDFNI	DDA	EDDDDTDGDDSDSH	DGGADNDDDDDDDDP
Caenorhabditis_CASQ	<mark>SISLD</mark> DDDD	.DEPI	PPAP	PPTPKGKKSRK

Figure S1 continued







