

#### $\alpha$ -Actinin-1 promotes activity of the L-type Ca<sup>2+</sup> Channel Ca<sub>v</sub>1.2

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#### **Appendix Figure S1**

Species		Pore			C-t	erminus			
		IVS6		EF-1	nand:++ ++ +	+ + ++	++ ++ +		
Human	Cav1.1	AVFYFISFYMLCAFLIIN	ILFVAVIM	NFDYLTRDWSILGP	HHLDEFKAIWAEY	DPEAKGRIKHL	DVVTLLRRIQPP:	LGFGKFCPHRVA	ACKRLV <mark>G</mark> MNMP
Human	Cav1.2	AVFYFISFYMLCAFLIIN	ILFVAVIN	NFDYLTRDWSILGP	HHLDEFKRIWAEY	DPEAKGRIKHL	DVVTLLRRIQPP	LGFGKLCPHRVA	ACKRLVSMNMP
Human	Cav1.3	A <mark>IV</mark> YFISFYMLCAFLIIN	ILFVAVIM	NFDYLTRDWSILGP	HHLDEFKRIW <mark>S</mark> EY	DPEAKGRIKHL	DVVTLLRRIQPP:	LGFGKLCPHRVA	ACKRLV <mark>A</mark> MNMP
Human	Cav1.4	A <mark>IA</mark> YFISFFMLCAFLIIN	ILFVAVIM	NFDYLTRDWSILGP	HHLDEFKRIW <mark>S</mark> EY	dp <mark>g</mark> akgrikhl	DVVALLRRIQPP:	LGFGKLCPHRVA	ackrlv <mark>a</mark> mnmp
Monkey	Cav1.2	AVFYFISFYMLCAFLIIN	ILFVAVIM <mark>I</mark>	ONFDYLTRDWSILGP	HHLDEFKRIWAEY	DP <mark>E</mark> AKGRIKHL	DVVTLLRRIQPP:	LGFGKLCPHRVA	ACKRLVSMNMP
Cow	Cav1.2	AVFYFISFYMLCAFLIIN	ILFVAVIM	NFDYLTRDWSILGP	HHLDEFKRIWAEY	DPEAKGRIKHL	DVVTLLRRIQPP:	LGFGKLCPHRVA	ACKRLVSMNMP
Rabbit	Cav1.2	AVFYFISFYMLCAFLIIN	ILFVAVIM <mark>I</mark>	NFDYLTRDWSILGP	HHLDEFKRIWAEY	DPEAKGRIKHL	DVVTLLRRIQPP:	LGFGKLCPHRVA	ACKRLVSMNMP
Rat	Cav1.2	AVFYFISFYMLCAFLIIN	LFVAVIM	NFDYLTRDWSILGP	HHLDEFKRIWAEY	DPEAKGRIKHL	DVVTLLRRIQPP	LGFGKLCPHRVA	ACKRLVSMNMP
Mole Rat	Cav1.2	AVFYFISFYMLCAFLIIN	ILFVAVIM	NFDYLTRDWSILGP	HHLDEFKRIWAEY	DPEAKGRIKHL	DVVTLLRRIQPP:	LGFGKLCPHRVA	ACKRLVSMNMP
Mouse	Cav1.2	AVFYFISFYMLCAFLIIN	ILFVAVIM <mark>I</mark>	NFDYLTRDWSILGP	HHLDEFKRIWAEY	DPEAKGRIKHL	DVVTLLRRIQPP:	LGFGKLCPHRVA	ACKRLVSMNMP
Zebrafish	Cav1.2	A <mark>I</mark> FYF <mark>V</mark> SFYMLCAFLIIN	ILFVAVIM	ONFDYLTRDWSILGP	HHLDEFKRIWAEY	DPEAKGRIKHL	DVVTLLRRIQPP:	LGFGKLCPHRVA	ACKRLVSMNMP
Fly	Cav1.2	YFISFYVLC <mark>S</mark> FLIIN	ILFVAVIM	NFDYLTRDWSILGP	HHLDEF <mark>IRLWS</mark> EY	DP <mark>D</mark> AKGRIKHL	DVVTLLRKI <mark>S</mark> PP:	lgfgklcphr <mark>m</mark> #	ACKRLVSMNMP
Sea urchin		A <mark>YV</mark> YFISFY <mark>SI</mark> C <mark>S</mark> FLIIN	ILFVAVIM	NFDYLTRDWSILGP	HHLDEF <mark>VRQWSE</mark> F	dp <mark>dat</mark> grikhl	DVVTLLRSI <mark>S</mark> PP	LGFGKLCPHR <mark>I</mark> A	ACKRLV <mark>T</mark> MNMP
Snail		A <mark>IP</mark> YFISF <mark>LL</mark> L <mark>VA</mark> F <mark>FV</mark> LN	MFVGVVVI	ONFDYLTRDWSILGP	HHLDEF <mark>VRL</mark> WSEY	DPEAKGRIH <mark>YT</mark>	D <mark>MYEM</mark> LR <mark>NME</mark> PP	VGFGK <mark>K</mark> CP- <mark>YKI</mark>	A <mark>Q</mark> RLVSMNMP
C. elegans		A <mark>IV</mark> YFISF <mark>F</mark> MLC <mark>S</mark> FL <mark>V</mark> IN	ILFVAVIM	NFDYLTRDWSILGP	HHL <mark>E</mark> EF <mark>V</mark> RLWSEY	DP <mark>D</mark> AKGRIKHL	DVVTLLRKI <mark>S</mark> PP	LGFGKLCPHR <mark>L</mark> A	ACKRLVSMNMP
Jellyfish		A <mark>YL</mark> Y <mark>FM</mark> SFY <mark>MI</mark> C <mark>S</mark> FLIIN	LFVAVIM	ONFDYLTRDWSILG <mark>A</mark>	HHL <mark>E</mark> E <mark>YV</mark> RIWAEYI	dpea <mark>s</mark> gr <mark>m</mark> kh <mark>v</mark>	D <mark>IVSMLK</mark> RIEPP	lgfgk <mark>c</mark> cphr <mark>e</mark> a	ACKRLVSMNM <mark>M</mark>
				C-te	erminus				
				Pre-IQ Doma	erminus	1	IQ DO	MAIN	]
Human	Cav1.1	LNSDGTV <mark>T</mark> FNAFLFALVR	TAL <mark>K</mark> IKTE	Pre-IQ Doma CGNFEQANEELRAII	erminus	QV <mark>I</mark> PP <mark>I</mark> G-DDE	<u>IQ DO</u> VTVGKFYATFLI	<b>MAIN</b> QE <mark>H</mark> FRKF <mark>M</mark> KR <mark>Q</mark> E	EYYGYRP
Human Human	Cav1.1 Cav1.2	LNSDGTV <mark>T</mark> FNATLFALVR LNSDGTVMFNATLFALVR	TAL <mark>K</mark> IKTE	Pre-IQ Doma Pre-IQ Doma CGN <mark>F</mark> EQANEELRAII CGNLEQANEELRAII	rminus iin KKIWKRTSMKLLD KKIWKRTSMKLLD	QV <mark>I</mark> PP <mark>I</mark> G-DDE QVVPPAG-DDE	<u>IQ DO</u> VTVGKFYATFLI VTVGKFYATFLI	<b>MAIN</b> 2E <mark>H</mark> FRKF <mark>M</mark> KR <mark>Q</mark> E 2EYFRKFKKRKE	EYYGYR DGLVGKP
Human Human Human	Cav1.1 Cav1.2 Cav1.3	LNSDGTV <mark>T</mark> FNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR	TAL <mark>K</mark> IKTE TALRIKTE TAL <mark>K</mark> IKTE	Pre-IQ Doma Pre-IQ Doma CGNFEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAVI	rminus in KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD	CV <mark>I</mark> PP <mark>I</mark> G-DDE CVVPPAG-DDE CVVPPAG-DDE	IQ DO VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI	<b>MAIN</b> QE <mark>H</mark> FRKF <mark>M</mark> KR <mark>Q</mark> E QEYFRKFKKRKE Q <mark>D</mark> YFRKFKKRKE	E <mark>YYGYR</mark> P QGLVGKP 2GLVGK <mark>Y</mark>
Human Human Human Human	Cav1.1 Cav1.2 Cav1.3 Cav1.4	LNSDGTV <mark>T</mark> FNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVTFNATLFALVR	TAL <mark>K</mark> IKTE TALRIKTE TALRIKTE TSLKIKTE	Pre-IQ Doma Pre-IQ Doma CGNFEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAVI CGNLEQANCELRIVI	erminus iin KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRMKQKLLD	CVIPPIG-DDE CVVPPAG-DDE CVVPPAG-DDE CVVPPAG-DDE EVIPP <mark>PD-EEE</mark>	IQ DO VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI	MAIN QE <mark>H</mark> FRKF <mark>M</mark> KRQE QEYFRKFKKRKE QDYFRKFKKRKE QDYFRKF <b>RR</b> RKE	EYYGYR DGLVGKP DGLVGK <mark>Y</mark> KGL <mark>L</mark> G <mark>ND</mark>
Human Human Human Human Monkey	Cav1.1 Cav1.2 Cav1.3 Cav1.4 Cav1.2	LNSDGTV <mark>T</mark> FNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVTFNATLFALVR LNSDGTVTFNATLFALVR	TAL <mark>K</mark> IKTE TALRIKTE TAL <mark>K</mark> IKTE TSLKIKTE TALRIKTE	Pre-IQ Doma Pre-IQ Doma CGNFEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAVI CGNLEQANCELRIVI CGNLEQANEELRAII	erminus in KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRMKQKLLD KKIWKRTSMKLLD	CVIPPIG-DDE CVVPPAG-DDE CVVPPAG-DDE EVIPP <mark>PD-EEE</mark> CVVPPAG-DDE	IQ DO VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI	MAIN QE <mark>H</mark> FRKF <mark>M</mark> KRQE QEYFRKFKKRKE QDYFRKFKKRKE QDYFRKF <b>RR</b> RKE QEYFRKFKKRKE	EYYGYR DGLVGKP DGLVGK <mark>Y</mark> KGL <mark>L</mark> G <mark>ND</mark> DGLVGKP
Human Human Human Human Monkey Cow	Cav1.1 Cav1.2 Cav1.3 Cav1.4 Cav1.2 Cav1.2	LNSDGTV <mark>T</mark> FNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVTFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR	TAL <mark>K</mark> IKTE TALRIKTE TALKIKTE TSLKIKTE TALRIKTE	Pre-IQ Doma Pre-IQ Doma CGNFEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAVI CGNLEQANEELRAII CGNLEQANEELRAII	Erminus in KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD	CVIPPIG-DDE CVVPPAG-DDE CVVPPAG-DDE EVIPP <mark>PD-EEE</mark> CVVPPAG-DDE CVVPPAG-DDE	IQ DO VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI	MAIN QE <mark>H</mark> FRKF <mark>M</mark> KRQ QEYFRKFKKRKE QDYFRKFKKRKE QDYFRKF <b>RR</b> RKE QEYFRKFKKRKE QEYFRKFKKRKE	EYYGYR DGLVGKP DGLVGK <mark>Y</mark> KGL <mark>L</mark> G <mark>ND</mark> DGLVGKP DGLVGKP
Human Human Human Human Monkey Cow Rabbit	Cav1.1 Cav1.2 Cav1.3 Cav1.4 Cav1.2 Cav1.2 Cav1.2	LNSDGTV <mark>T</mark> FNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVTFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR	TAL <mark>R</mark> IKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE	Pre-IQ Doma Pre-IQ Doma CGNFEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAVI CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII	Erminus in KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD	CVIPPIG-DDE CVVPPAG-DDE CVVPPAG-DDE EVIPPD-EE CVVPPAG-DDE CVVPPAG-DDE CVVPPAG-DDE	IQ DO VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI	MAIN QEHFRKFMKRQ QEYFRKFKKRKE QUYFRKFKRKE QEYFRKFKRKE QEYFRKFKKRKE QEYFRKFKKRKE QEYFRKFKKRKE	EYYGYR DGLVGKP DGLVGK <mark>Y</mark> KGL <mark>L</mark> GND DGLVGKP DGLVGKP
Human Human Human Monkey Cow Rabbit <b>Rat</b>	Cav1.1 Cav1.2 Cav1.3 Cav1.4 Cav1.2 Cav1.2 Cav1.2 Cav1.2	LNSDGTV <mark>T</mark> FNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVTFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR <b>LNSDGTVMFNATLFALV</b> R	TAL <mark>R</mark> IKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE	Pre-IQ Doma Pre-IQ Doma CGNFEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAVI CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII	Erminus in KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD	CVIPPIG-DDE VVPPAG-DDE VVPPAG-DDE VIPP <mark>PD-EEE</mark> VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE	IQ DO VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI	MAIN QEHFRKFMKRQ QEYFRKFKKRKE DYFRKFKRKE DYFRKFKRKE QEYFRKFKKRKE QEYFRKFKKRKE QEYFRKFKKRKE QEYFRKFKKRKE	EYYGYR DGLVGKP DGLVGK <mark>Y</mark> XGL <mark>L</mark> GND DGLVGKP DGLVGKP DGLVGKP
Human Human Human Monkey Cow Rabbit <b>Rat</b> Mole Rat	Cav1.1 Cav1.2 Cav1.3 Cav1.4 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2	LNSDGTV <mark>T</mark> FNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVTFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR	TAL <mark>R</mark> IKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE	Pre-IQ Doma Pre-IQ Doma CGNFEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAVI CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII	Erminus in KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD	CVIPPIG-DDE VVPPAG-DDE VVPPAG-DDE VIPPIG-DE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE	IQ DO VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVG <b>FF</b> ATFLI VTVGKFYATFLI	MAIN QEHFRKFMKRQ QEYFRKFKKRKE DYFRKFKRKE QEYFRKFKRKE QEYFRKFKKRKE QEYFRKFKKRKE QEYFRKFKKRKE QEYFRKFKKRKE	EYYGYR DGLVGKP DGLVGK <mark>Y</mark> CGLVGKP DGLVGKP DGLVGKP DGLVGKP DGLVGKP
Human Human Human Monkey Cow Rabbit <b>Rat</b> Mole Rat Mouse	Cav1.1 Cav1.2 Cav1.3 Cav1.4 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2	LNSDGTV <mark>T</mark> FNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVTFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR	TAL <mark>R</mark> IKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE	Pre-IQ Doma Pre-IQ Doma CGNFEQANEELRAII CGNLEQANEELRAVI CGNLEQANEELRAVI CGNLEQANEELRAVI CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII	Erminus in KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD	CVIPPIG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE	IQ DO VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI	MAIN QEHFRKFMKRQ QEYFRKFKKRKE DYFRKFKRKE QEYFRKFKKRKE QEYFRKFKKRKE QEYFRKFKKRKE QEYFRKFKKRKE QEYFRKFKKRKE QUYFRFRFKKRKE	EYYGYR DGLVGKP DGLVGKY CGLUGKY DGLVGKP DGLVGKP DGLVGKP DGLVGKP DGLVGKP
Human Human Human Monkey Cow Rabbit <b>Rat</b> Mole Rat Mouse Zebrafish	Cav1.1 Cav1.2 Cav1.3 Cav1.4 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2	LNSDGTV <mark>T</mark> FNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVTFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR	TAL <mark>R</mark> IKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE	Pre-IQ Doma Pre-IQ Doma CGNFEQANEELRAII CGNLEQANEELRAVI CGNLEQANEELRAVI CGNLEQANEELRAVI CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII	Erminus in KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD	CVIPPIG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE	IQ DO VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI	MAIN QEHFRKFMKRQ QEYFRKFKKRKE DYFRKFKRKE QEYFRKFKKRKE QEYFRKFKKRKE QEYFRKFKKRKE QEYFRKFKKRKE QEYFRKFKKRKE QEYFRKFKKRKE QEYFRKFKKRKE	EYYGYR DGLVGKP DGLVGKY XGL <mark>LGND</mark> DGLVGKP DGLVGKP DGLVGKP DGLVGKP DGLVGKP
Human Human Human Monkey Cow Rabbit <b>Rat</b> Mole Rat Mole Rat Mouse Zebrafish Fly	Cav1.1 Cav1.2 Cav1.3 Cav1.4 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2	LNSDGTV <mark>T</mark> FNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVTFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR	TAL <mark>R</mark> IKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE	Pre-IQ Doma Pre-IQ Doma CGNFEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAVI CGNLEQANEELRAVI CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII	Erminus in KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD	CVIPPIG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE	IQ DO VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI	MAIN 2EHFRKFMKRQF 2EYFRKFKKKKE 2DYFRKFKRKE 2EYFRKFKKRKE 2EYFRKFKKRKE 2EYFRKFKKRKE 2EYFRKFKKRKE 2EYFRKFKKRKE 2EYFRKFKKRKE 2DYFRRFKKRKE 2DYFRRFKKRKE	EYYGYR DGLVGKP DGLVGKY CGLUGKY DGLVGKP DGLVGKP DGLVGKP DGLVGKP DGLVGKP DGLVGKP
Human Human Human Monkey Cow Rabbit <b>Rat</b> Mole Rat Mole Rat Mouse Zebrafish Fly Sea urchin	Cav1.1 Cav1.2 Cav1.3 Cav1.4 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2	LNSDGTVT FNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVTFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVLFNATLFALVR	TAL <mark>K</mark> IKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE TALRIKTE	Pre-IQ Doma Pre-IQ Doma CGNEEQANEELRAII CGNLEQANEELRAVI CGNLEQANEELRAVI CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII	Erminus in KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD	CVIPPIG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE	IQ DO VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI	MAIN 2EHFRKFMKROF 2EYFRKFKRKRO 2DYFRKFKRKRO 2EYFRKFKKRKE 2EYFRKFKKRKE 2EYFRKFKKRKE 2EYFRKFKKRKE 2EYFRKFKKRKE 2EYFRKFKKRKE 2DYFRRFKKRKE 2DYFRRFKKRKE 2DYFRRFKKRKE 2DYFRRFKKRKE	EYYGYR DGLVGKP DGLVGKY XGL <mark>IGND</mark> DGLVGKP DGLVGKP DGLVGKP DGLVGKP DGLVGKP DGLVGKP DGLVGKP
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Human Human Human Monkey Cow Rabbit <b>Rat</b> Mole Rat Mouse Zebrafish Fly Sea urchin Snail Worm Jellyfish	Cav1.1 Cav1.2 Cav1.3 Cav1.4 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2 Cav1.2	LNSDGTVT FNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR LNSDGTVMFNATLFALVR MNDGTVDFHATLFALVR	TALKIKTE TALKIKTE TALKIKTE TALKIKTE TALKIKTE TALKIKTE TALKIKTE TALKIKTE TALKIKTE TALKIKTE TALKIKTE	Pre-IQ Doma Pre-IQ Doma CGNFEQANEELRAII CGNLEQANEELRAVI CGNLEQANEELRAVI CGNLEQANEELRAVI CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAII CGNLEQANEELRAVI CGNIDDANSELRAVI CGNIDDANSELRAVI CGNIDDANSELRAVI CGNIDDANSELRAVI CGNIDDANSELRAVI CGNIDDANSELRAVI CGNIDDANSELRAVI CGNIDDANSELRAVI CGNIDEANEQLRSAI	KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD KKIWKRTSMKLLD	CVIPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE VVPPAG-DDE	IQ DO VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI VTVGKFYATFLI	MAIN 2EHFRKFMKROF 2EYFRKFKRKRO 2EYFRKFKRKRO 2EYFRKFKKRKE 2EYFRKFKKRKE 2EYFRKFKKRKE 2EYFRKFKKRKE 2EYFRKFKKRKE 2EYFRKFKKRKE 2EYFRFKKRKE 2EYFRFKKRKE 2EYFRFKKRKE 2EYFRFKKRKE 2EYFRFKKRKE 2EYFRFKKRKE	EYYGYR DGLVGKP DGLVGKP DGLVGKP DGLVGKP DGLVGKP DGLVGKP DGLVGKP DGLVGKP DGLVGKP DGLVGKP DGLVGKP DGLVGKP

<sup>11</sup>Lymnea stagnalis, <sup>12</sup>Caenorhabditis elegans <sup>13</sup>Cyanea lamarkii

Color code of residues: Different from Cav1.2

Important for α-actinin binding Important for  $\alpha$ -actinin and CaM binding

Important for apoCaM binding

# Appendix Figure S1. Amino acid sequence alignment of the membrane proximal portion of the C-terminal tail of L-type Ca<sup>2+</sup> channels.

Bold refers to reference sequence (rat  $\alpha_1 1.2$ ), turquoise to divergency in amino acid sequence, red to residues K1647 and Y1649, which are important for  $\alpha$ -actinin binding only, green to I1654, which is important for both,  $\alpha$ -actinin and apoCaM binding, and yellow to F1658 and K1662, which are important for apoCaM binding only. Amino acid sequences were derived from species according to the numerical labeling<sup>1-13</sup>. Data were extracted and compiled from uswest.ensembl.org.

#### **Appendix Figure S2**



#### Appendix Figure S2. Isothermal titration calorimetry of apoCaM added to IQ peptide.

a, Change of heat resulting from incremental addition of apoCaM (100  $\mu$ M stock solution) into IQ peptide (10  $\mu$ M) during ITC titration at 27°C in 50 mM HEPES (pH 7.4), 100 mM KCl, 0.05 mM EGTA and 1 mM MgCl<sub>2</sub>.

b, A binding isotherm of apoCaM binding to IQ peptide was derived from the integrated heat at each injection after subtracting a blank titration (to remove heat of dilution). The binding isotherm for apoCaM binding to IQ exhibited no detectable heat signal above the noise level, consistent with low fractional binding under ITC conditions and/or low enthalpy caused by the relatively weak binding affinity ( $K_d = 10 \mu M$ , see Fig. 2e and Table 4).

c, Fluorescence polarization for binding of apoCaM (0.015 to 256  $\mu$ M) to fluorescein-labeled WT IQ peptide (1.0  $\mu$ M) in the presence of 100 mM KCl (black squares) versus zero KCl (red dots) at room temperature. The buffer was the same as in (a) except that KCl was excluded in the zero KCl sample. The data were fit to a one-site model (dotted lines) with K<sub>d</sub> = 10  $\mu$ M in the presence of 100 mM KCl and K<sub>d</sub> = 2.6  $\mu$ M in the absence of KCl.

#### **Appendix Figure S3**







# Appendix Figure S3. Cav1.2 mutations that affect α-actinin-1 binding do not impair voltage sensitivity of channel activation.

HEK293 cells were transfected with  $\alpha_1 1.2$ ,  $\alpha_2 \delta$ -1, and  $\beta_{2A}$  before whole cell patch recording in 20 mM Ba<sup>2+</sup>.

a, I-V curves. Currents were recorded upon depolarization from a holding potential of -80 mV to increasingly more positive potentials (insert on left). Shown are peak currents. Dashed lines indicate SEM. The WT Cav1.2 curve is reproduced in all graphs for the  $Ca_V 1.2$  mutants.

b, G-V curves. Tail currents  $(I_{Tail})$  were recorded upon repolarization to -50 mV following depolarization from a holding potential of -80 mV to increasingly more positive potentials. Shown are fitted curves in top panels and dot blots in bottom panels.

#### Appendix Figure S4



# Appendix Figure S4. Structural modeling addressing the function of Y1649 in the $\alpha_1$ 1.2 IQ motif and how the E851K mutation of $\alpha$ -actinin affects its binding to the IQ motif.

a, Y1649 stabilizes the  $\alpha$ -helical conformation of the  $\alpha_1 1.2$  IQ motif and thereby  $\alpha$ -actinin binding. Shown is a cartoon representation of  $\alpha_1 1.2$  IQ motif  $\alpha$ -helix (orange) and  $\alpha$ -actinin-1 EF3-EF4 (blue). Sidechains of key residues are depicted as stick representation and labeled. Y1649 is in close proximity to L1653, which is about one  $\alpha$ -helical turn downstream of Y1649. Stabilization of this  $\alpha$  helix ensures correct positioning of I1654, which is critical for binding to  $\alpha$ -actinin. This figure was created using UCSF Chimera (Pettersen et al., 2004).

b-d, The lysine residue in the E851K mutation of  $\alpha$ -actinin destabilizes the canonical EF hand structure. Conformers of the lysine residues when used to substitute E851 by itself or together with E847 in  $\alpha$ -Actinin-1 and of the glutamate residue when used to substitute K1647 in the IQ motif were calculated for the structure of the complex between the IQ motif of  $\alpha_1$ 1.2 and EF3\_EF4 of  $\alpha$ -Actinin-1 using the UCSF Chimera rotamer tool (Pettersen et al., 2004). The  $\alpha_1$ 1.2 IQ  $\alpha$ helix is shown in orange and the  $\alpha$ -actinin-1 EF3-EF4 in blue. Sidechains of key residues are depicted as stick representation and labeled. The lysine rotamers (thin sticks) are overlaid on top of the original E851 and E847 residues and the glutamate rotamers on top of the original K1647 residue.

b, Simulation of rotamers for the E851K mutation in  $\alpha$ -actinin-1 to show possible conformations of the lysine sidechain.

c, Simulation of rotamers for the E851K mutation in  $\alpha$ -actinin-1 and the K1647E mutation in  $\alpha_1$ 1.2 to show possible conformations of the lysine and glutamate sidechains.

d, Simulation of rotamers for the E847K and E851K mutations in  $\alpha$ -actinin-1 and the K1647E mutation in  $\alpha_1$ 1.2 to show possible conformations of the lysine and glutamate sidechains.

## Appendix Table S1. 95% Confidence intervals (CI) for surface labelling, Qon, and Po for WT and IQ mutant Cav1.2

Given are means<u>+</u>SEM and 95% CIs for experimental values. The number of experiments is shown in parenthesis. \*The biotinylation and flow cytometry data are based on data originally published by Tseng et al (2017).

Parameter	*Biotin- ylation (% of WT)	95% CI	*Flow Cytom etry (% of WT)	95% CI	Gating Current Qon (% of WT)	95% CI	P <sub>open</sub> (% of WT)	95% Cl
α1 <b>1.2 WT</b>	100±0 (16)	0-0	100±0 (7)	0-0	100±24 (18)	78- 122	100±2 0 (35)	87- 113
K1647A	61±5 (11)	52- 69	61±7 (7)	51- 71	35±9 (15)	26- 44	8±2 (12)	6-10
F1648A	94±7 (8)	85- 104	ND		ND		93±20 (14)	73- 114
Y1649A	66±5 (12)	61- 72	63±7 (6)	52 - 74	26±8 (13)	18- 35	15±5 (8)	8-22
I1654A	68±4 (10)	63- 73	61±8 (6)	49- 74	23±10 (13)	1 -34	10±5 (7)	3-17
Q1655A	85±5 (7)	78- 92	104±6 (4)	93- 116	ND		ND	
F1658A	ND		ND		76±16 (13)	59- 93	139±6 4 (12)	45- 121
K1662E	ND		ND		93±29 (17)	52- 134	120±3 2 (13)	86- 154

## Appendix Table S2. 95% Confidence intervals (CI) for surface biotinylation for Cav1.2 / $\alpha$ -actinin-1 charge inversion experiments

Given are means<u>+</u>SEM and 95% CIs (based on original data from this manuscript). The number of experiments is shown in parenthesis.

Parameter	Biotinylation (% )	95% CI
α1 <b>1.2 WT</b>	100±6 (7)	86- 114
α11.2 WT + α-Actinin WT	153±6 (7)	139- 167
α₁1.2WT+ α-Actinin WT	100±9 (5)	74- 126
α <sub>1</sub> 1.2 WT + α-Actinin EE/KK	48±9 (5)	24- 72
α11.2 K1647E + α-Actinin WT	60±5 (5)	47- 73
α11.2 K1647E + α-Actinin EE/KK	55±5 (4)	39- 71



## Appendix Table S3. 95% Confidence intervals (CI) for Po for Cav1.2 / $\alpha$ -actinin-1 charge inversion experiments

Given are means<u>+</u>SEM and 95% CIs (based on original data from this manuscript). The number of experiments is shown in parenthesis.

Parameter	Popen (%)	95% CI
α1 <b>1.2 WT</b>	100±20 (35)	87- 113
$\alpha_1$ 1.2 WT + $\alpha$ -Actinin WT	233±59 (23)	186 - 280
α <sub>1</sub> 1.2 WT+ α-Actinin EE/KK	55±22 (13)	32- 78
α11.2 K1647E + α-Actinin WT	100±18 (30)	87- 113
α11.2K1647E + α-Actinin EE/KK	147±28 (16)	120- 174

Cav1 2/	Used primers				
α-Actinin-1	Primer (Forward/Reverse; Bold type indicates mutated nucleotide)				
RtBr-Cav1.2-a1c-K1647E-Forw	5' – CGA GGT CAC AGT GGG C <b>G</b> A ATT CTA TGC CAC CTT C – 3'				
RtBr-Cav1.2-a1c-K1647E-Rev	5' – GAA GGT GGC ATA GAA TT <b>C</b> GCC CAC TGT GAC CTC G – 3'				
RtBr-Cav1.2-a1c-K1662-Forw	5' – CAA GAG TAC TTC AGG AAA TTC <b>G</b> A <b>A</b> AAG CGA AAA GAG CAG GGG CTG – 3'				
RtBr-Cav1.2-a1c-K1662-Rev	5' – CAG CCC CTG CTC TTT TCG CTT <b>T</b> T <b>C</b> GAA TTT CCT GAA GTA CTC TTG – 3'				
Hum-ACTN-1-E847K-Forw	5' – GAA CTA CAT TAC CAT GGA C <b>A</b> A <b>A</b> TT GCG CCG CGA GCT GCC ACC C – 3'				
Hum-ACTN-1-E847K-Rev	5' – GGG TGG CAG CTC GCG GCG CAA <b>T</b> T <b>T</b> GTC CAT GGT AAT GTA GTT C – 3'				
Hum-ACTN-1-E847KE851K-Forw	5' – CCA TGG AC <b>A</b> A <b>A</b> T TGC GC <b>A</b> G <b>AA</b> AGC TGC CAC CCG ACC AGG – 3'				
Hum-ACTN-1-E847KE851K-Rev	5' – CCT GGT CGG GTG GCA GCT TTC TGC GCA ATT TGT CCA TGG – 3'				

#### Appendix Table S4. Oligonucleotides used for QuikChange II mutagenesis.