SUPPLEMENTARY DATA SET

Calmodulin confers calcium sensitivity to the stability of the distal intracellular assembly domain of Kv7.2 channels

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Running title: Reciprocal calmodulin-tetramerization influences the C-terminal domain of Kv7.2 channels

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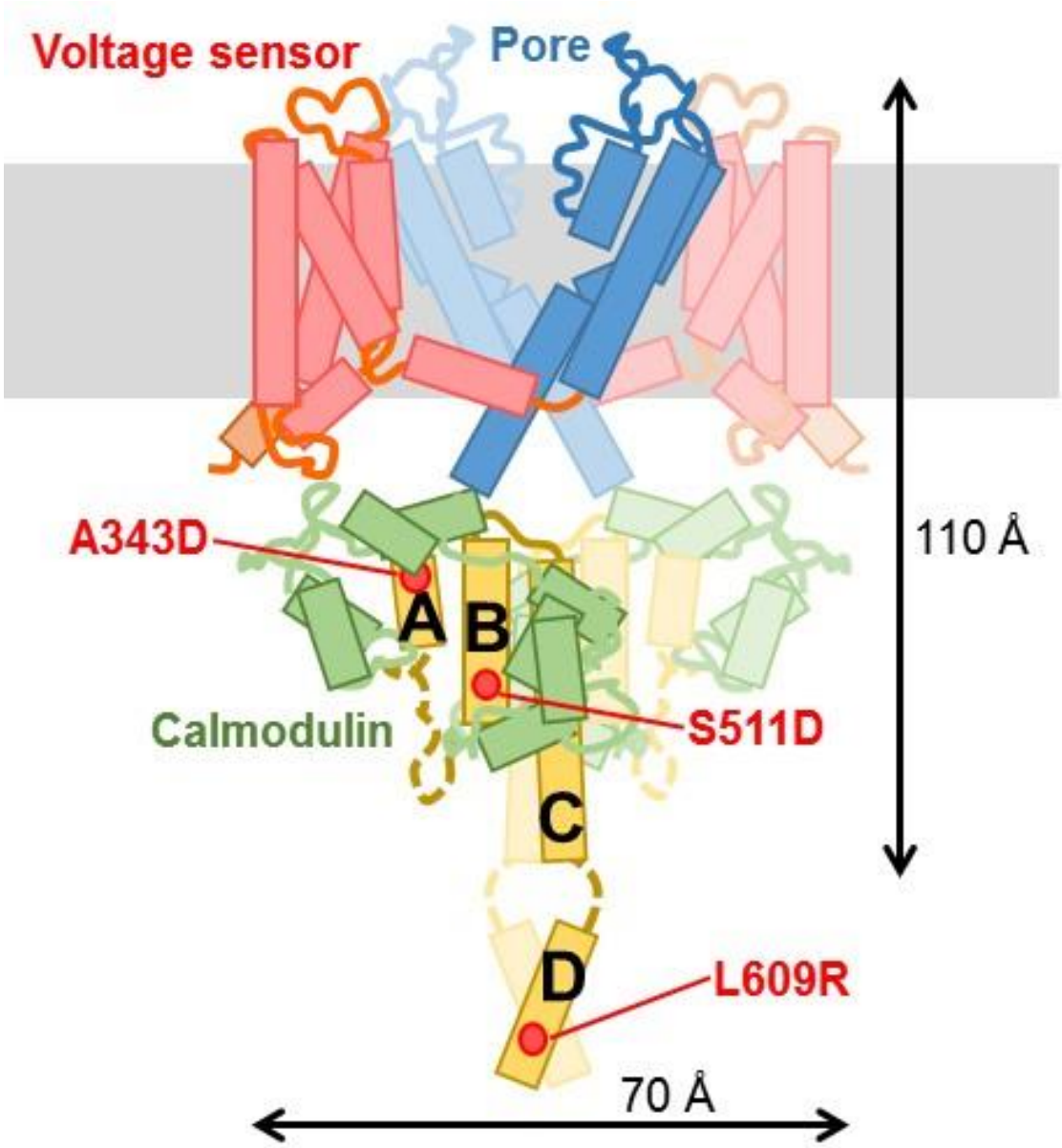
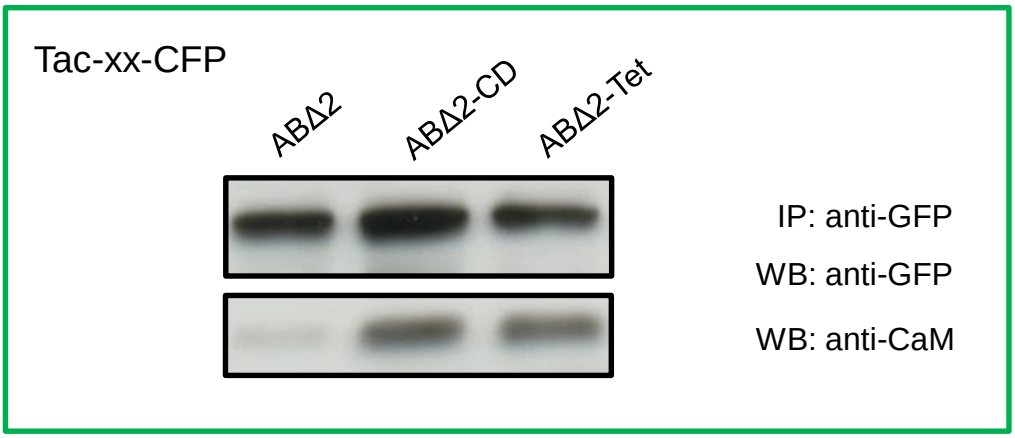
Keywords: calmodulin, coiled-coil, KCNQ, M-current

Conflict of Interest: The authors declare no competing financial interests



Suppl. Fig. 1. Cartoon representation of a KCNQ channel based on the flat projection of the atomic structure of Kv7.1 complexed with CaM[1]. The alpha helices are represented as rectangles and only two subunits of the tetramer are displayed. Some helices appear relatively shorter (e.g. Helix A) due to the projection of tilted helices on a surface. The approximate location of the helix A A343D and helix B S511D mutants that preclude CaM binding, and the helix D L609R that disrupts coiled-coil formation, are indicated. Because most of the linker between helices A and B is missing in the Kv7.1/CaM cryo-EM derived structure, this linker is only partially represented in this cartoon.

a



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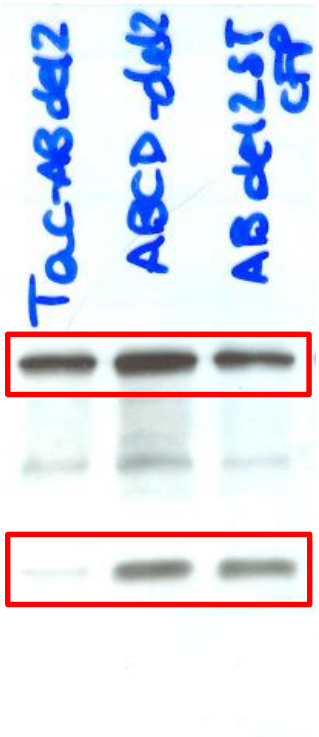
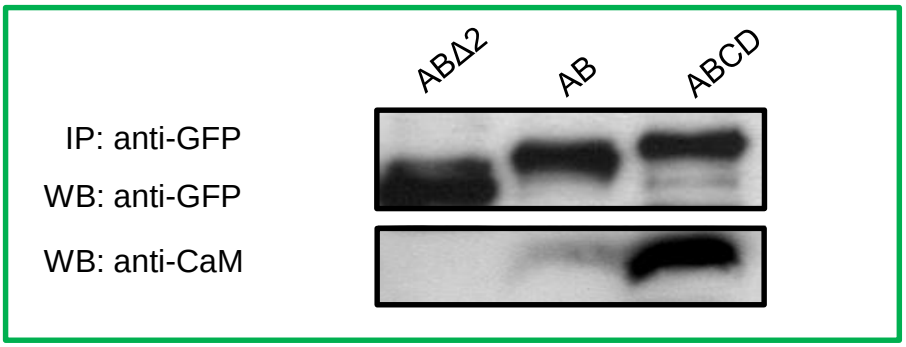


Suppl. Fig. 2. Full-length blots of the cropped images shown in Fig. 1A. (**a**) Fig. 1A of the manuscript contains images cropped from the full-length blots presented here (**b**).

a



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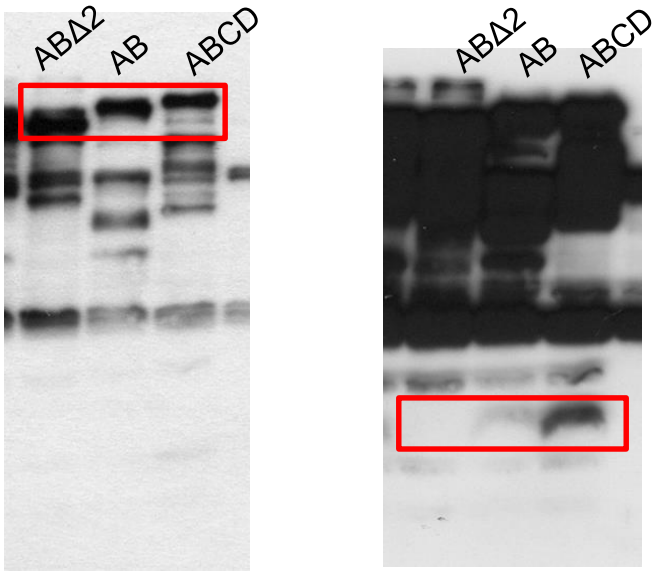
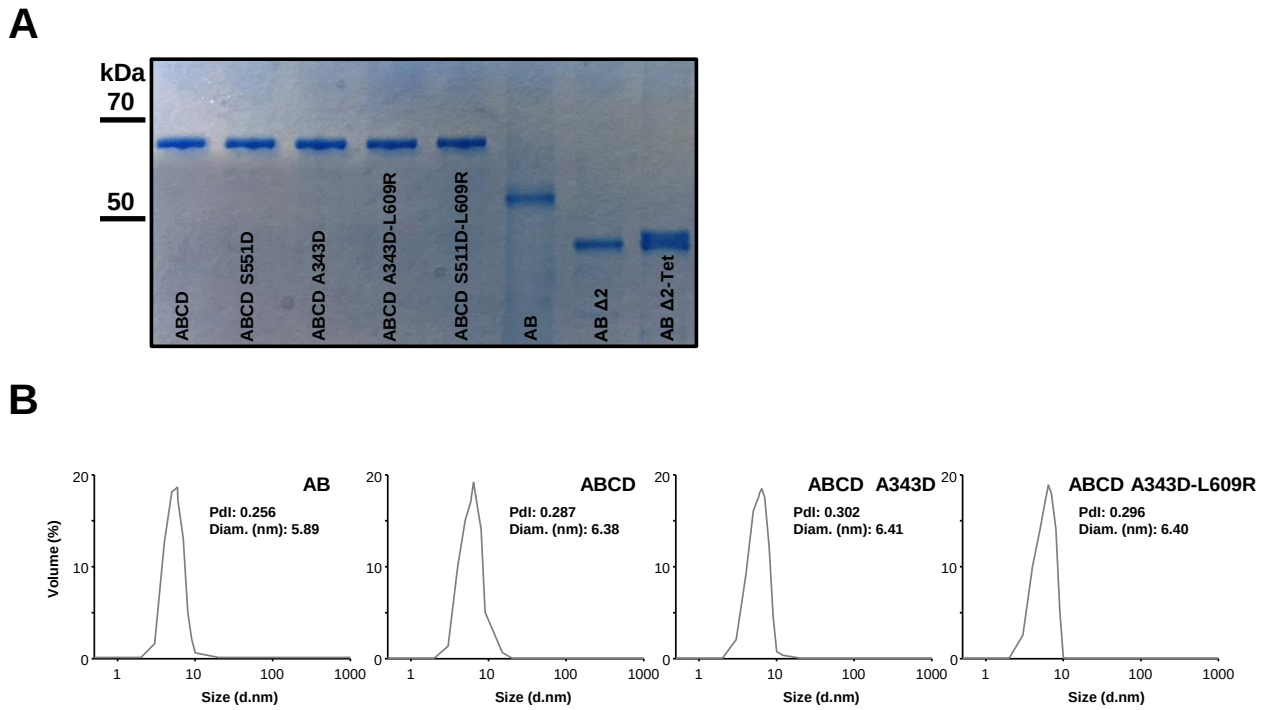
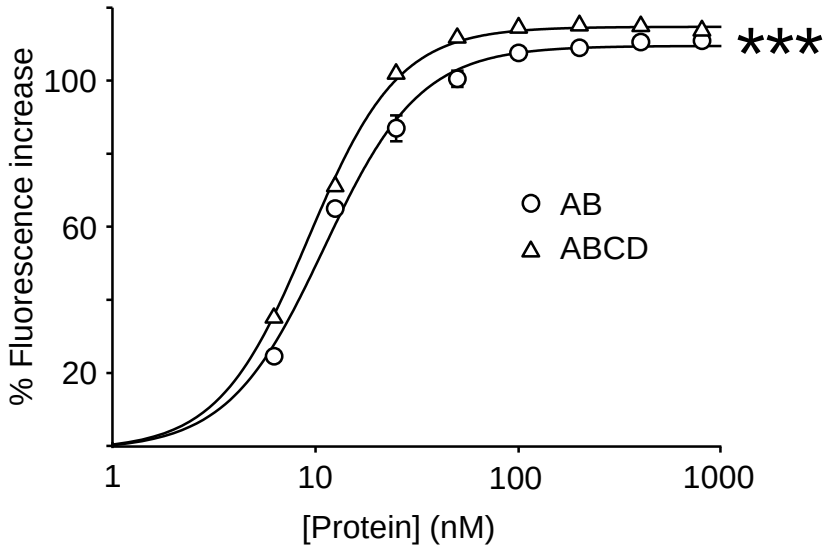


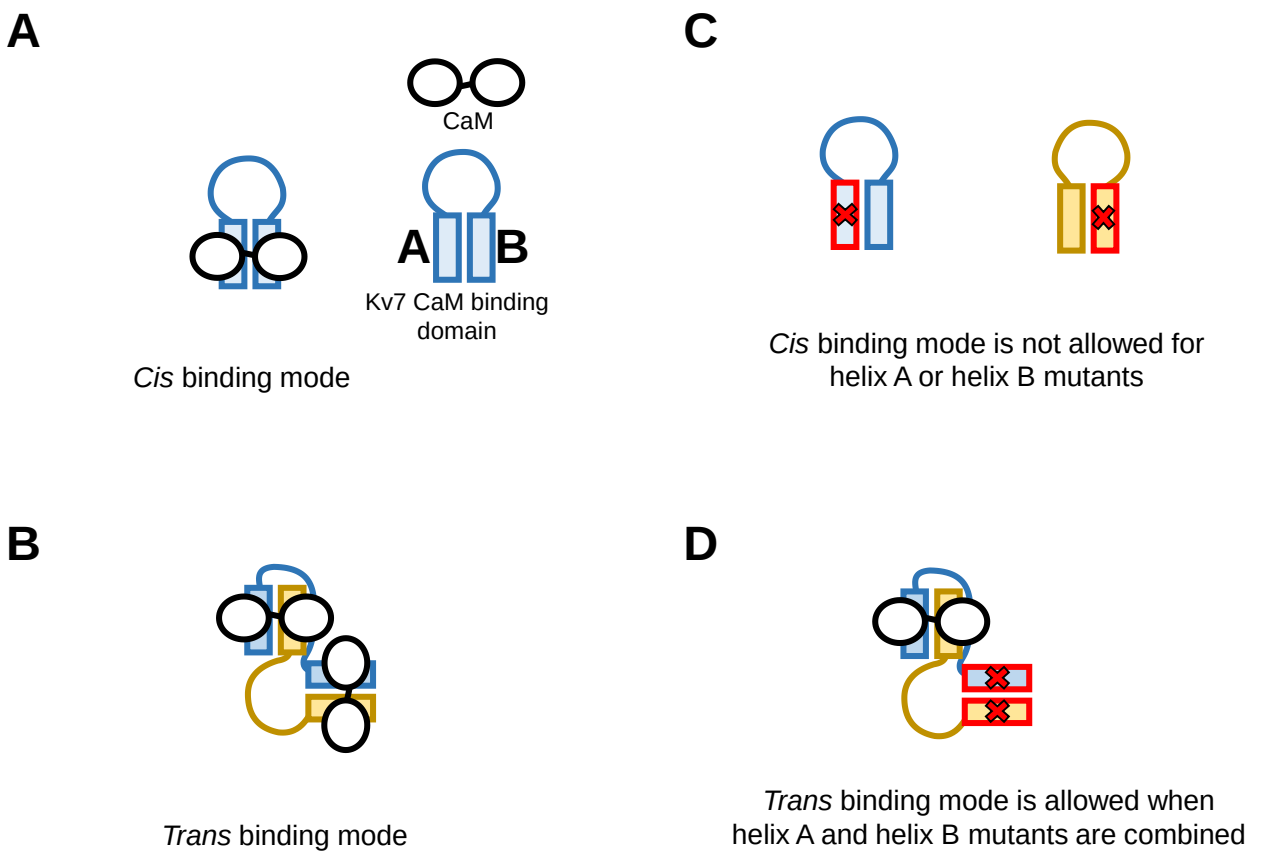
Suppl. Fig. 3. Full-length blots of the cropped images shown in Fig. 1C. (**a**) Fig. 1C of the manuscript contains images cropped from the full-length blots shown in **b** at two different exposures.



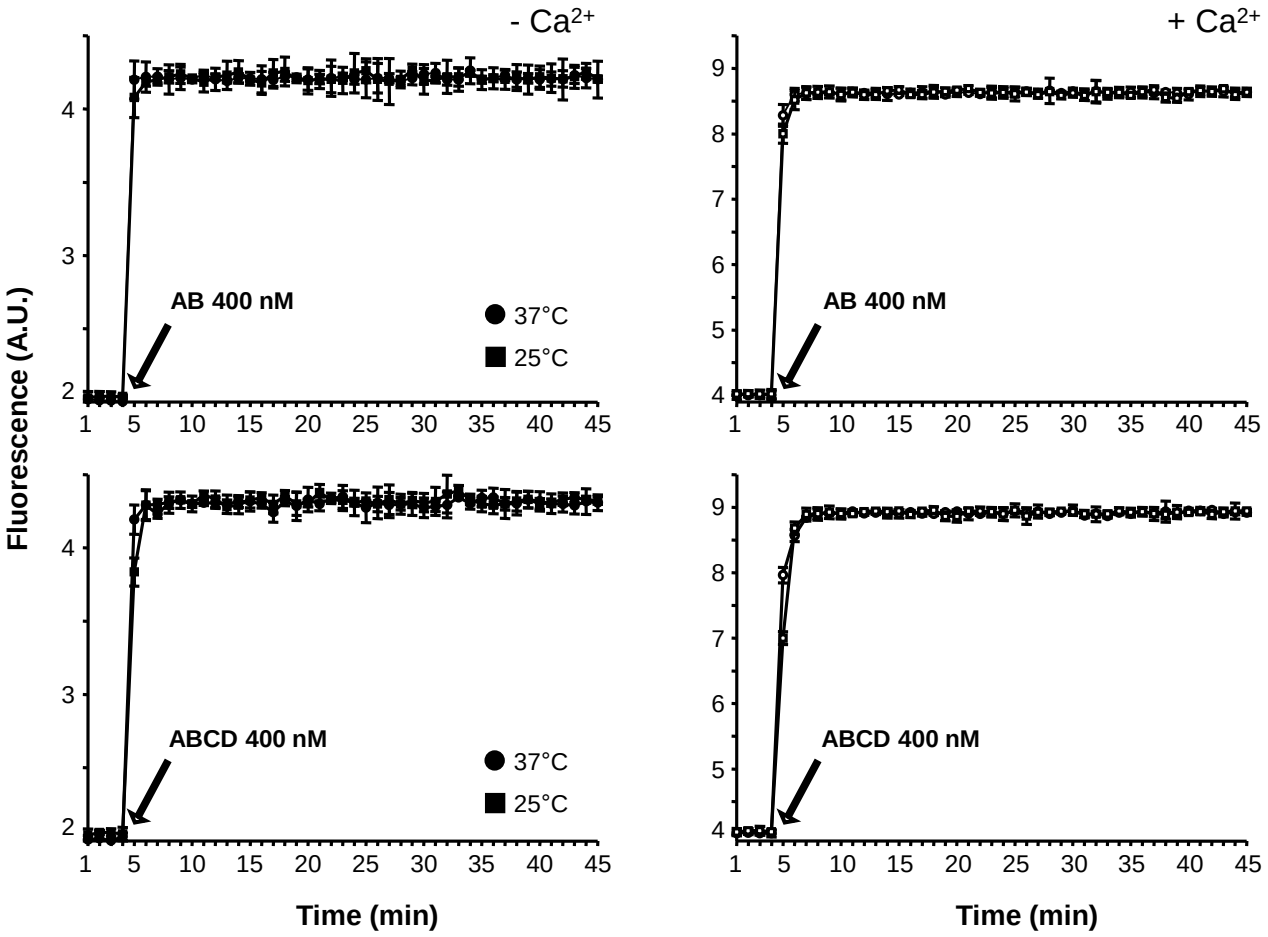
Suppl. Fig. 4. (**a**) SDS-PAGE analysis of the GST fusion proteins employed in this study (10% gel). (**b**) Representative dynamic light scattering (DLS) volume-weighted size distribution of 1 mg/ml of the indicated proteins. The buffer used contained Tris-HCl 20 mM and NaCl 100 mM [pH 7.5]. A low Polydispersity Index (PdI) was estimated from the apparent diameters assuming a globular protein shape and the unique peak argues against the presence of aggregates in these samples.



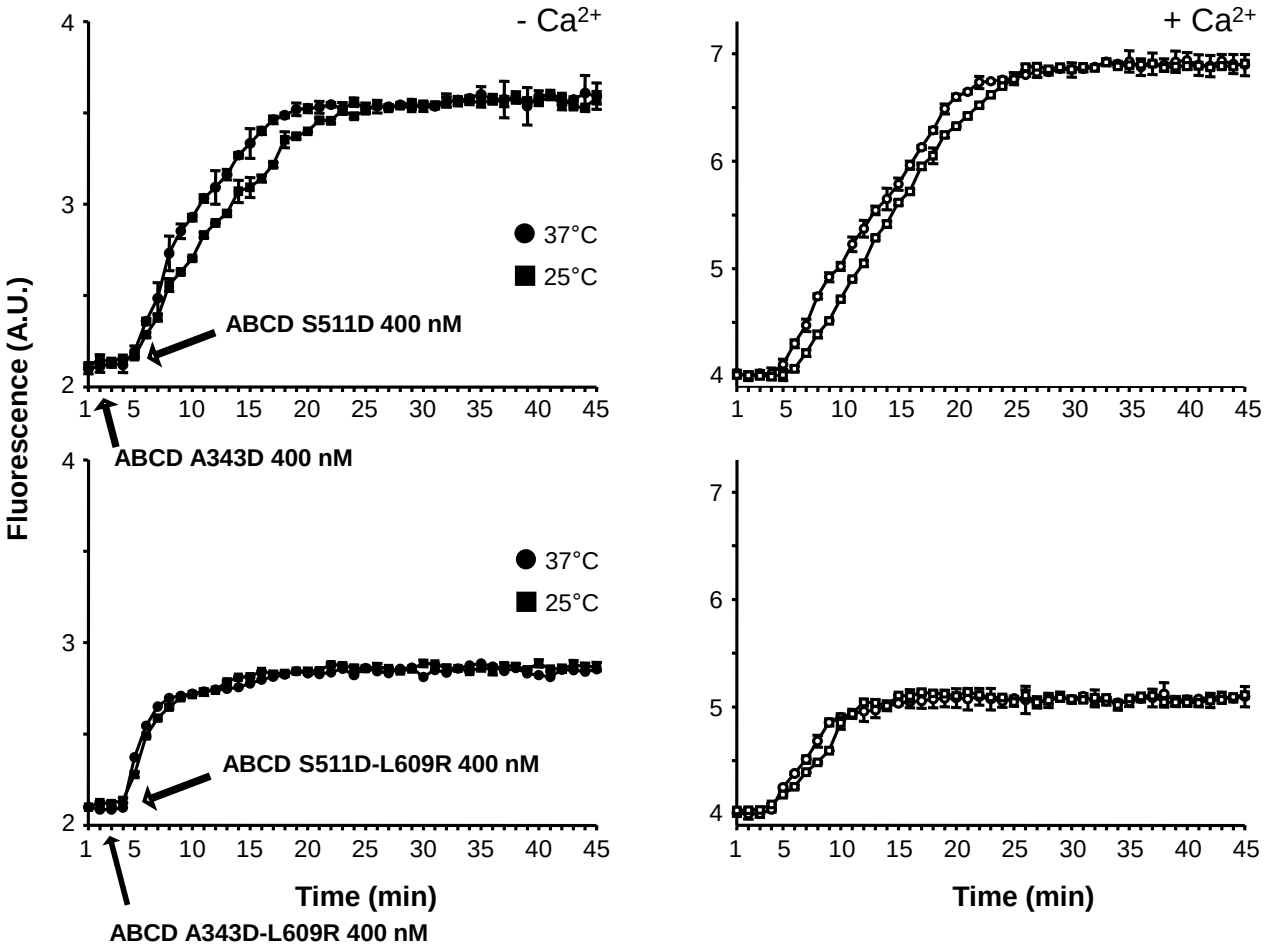
Suppl. Fig. 5. Concentration-dependent D-CaM (12.5 nM) fluorescence enhancement in the absence of Ca^{2+} (10 mM EGTA) for the indicated proteins. The lines are the result of fitting a Hill equation to the data. The EC_{50} (nM) was 11.0 ± 0.5 for AB and 9.1 ± 0.1 for ABCD. The asterisks indicate significantly different values of maximal fluorescence (*** = $p < 0.001$). The data represent the means ± SEM from 4 or more independent experiments: the data for AB is taken from[2] and that for ABCD from[3].



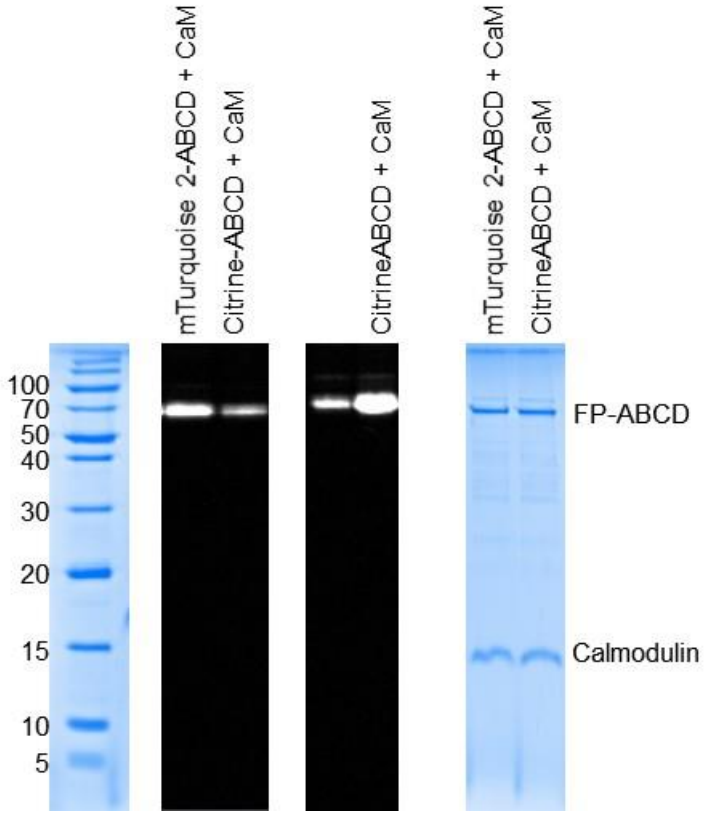
Suppl. Fig. 6. Hypothetical *cis*- and *trans*-calmodulin binding modes in the absence of helix D. (**a**) Representation of the *cis*-binding mode in which CaM embraces helices A and B (*boxes*) from the same subunit. The N-lobe engages helix B, whereas the C-lobe engages helix A[4-6]. (**b**) Illustration of CaM in the *trans*-binding mode, embracing helices A and B from two different subunits (note that CaM is embracing helices of different color). This arrangement has been observed in the crystallographic structure of the KCNQ1-AB/CaM complex[5]. (**c**) Mutations in either helix A (left) or helix B (right) preclude *cis*- (left) and *trans*- (right) binding. (**d**) In a mixture of helix A and helix B mutants *trans*-binding can occur, whereas *cis*-binding is precluded.



Suppl. Fig. 7. Time-course of the increase in 12.5 nM D-CaM fluorescence emission at 37 ºC and 25 ºC upon addition of GST-AB (top panels) and GST-ABCD (bottom panels) in the presence or absence (10 mM EGTA, left panels) of Ca^{2+} (3.9 μM free Ca^{2+}, right panels). Each point is the average of 4 independent experiments.



Suppl. Fig. 8. Time-course of the increase in 12.5 nM D-CaM fluorescence emission at 37 ºC and 25 ºC in the complementation assay. Each point is the average of 3 independent experiments.



Suppl. Fig. 9. SDS-PAGE analysis of the purified FP-ABCD/Calmodulin complexes employed in this study (15% gel). Samples were not boiled to preserve the fluorescence of the FPs: first column, molecular weight markers; second column, gel fluorescence after excitation with a blue led; third column, fluorescence after excitation with a green led; fourth column, Coomassie blue staining.

Suppl. Table 1. Binding parameters obtained using D-CaM (12.5 nM) after fitting a Hill equation to the data

	- Ca^{2+} (EGTA 10 mM)			+ Ca^{2+} (3.9 µM)		
Protein	Maximal increase in fluorescence	EC_{50} (nM)	h (n)	Maximal increase in fluorescence	EC_{50} (nM)	h (n)
AB	111.0 ± 1.4	11.0 ± 0.5	1.6 ± 0.3 (6)	115.0 ± 2.1	27.1 ± 1.2	3.4 ± 0.5 (5)
ABCD	116.0 ± 0.3	9.1 ± 0.1	1.9 ± 0.1 (3)	118.0 ± 1.5	15.1 ± 0.6	2.5 ± 0.2 (3)
ABCD S511D	4.8 ± 0.2	N.D.	N.D. (3)	6.8 ± 0.2	N.D.	N.D. (3)
ABCD A343D	3.7 ± 0.2	N.D.	N.D. (3)	4.8 ± 0.3	N.D.	N.D. (3)
ABCD L609R	106.0 ± 1.7	30.8 ± 1.3	2.2 ± 0.2 (3)	110.0 ± 1.9	59.5 ± 2.1	2.8 ± 0.2 (3)
(ABCD S511D + ABCD A343D) **ABCD#**	76.0 ± 2.4	45.5 ± 3.8	1.5 ± 0.2 (3)	79.0 ± 3.4	78.5 ± 8.5	1.3 ± 0.1 (3)
ABCD S511D-L609R	4.3 ± 0.7	N.D.	N.D. (3)	4.8 ± 0.5	N.D.	N.D. (3)
ABCD A343D-L609R	5.1 ± 1.1	N.D.	N.D. (3)	4.3 ± 0.3	N.D.	N.D. (3)
(ABCD S511D-L609R + ABCD A343D-L609R) **ABCD#-L609R**	32.0 ± 1.1	119.0 ± 8.1	2.4 ± 0.3 (3)	28.0 ± 0.6	173.0 ± 6.2	2.8 ± 0.2 (3)
AB S511D	2.8 ± 0.3	N.D.	N.D. (4)	1.8 ± 0.2	N.D.	N.D. (4)
AB A343D	5.3 ± 0.3	N.D.	N.D. (4)	4.8 ± 0.3	N.D.	N.D. (4)
(AB S511D + AB A343D) **(AB#)**	33.0 ± 1.5	8.5 ± 1.1	1.9 ± 0.5 (4)	17 ± 1.1	22.9 ± 2.9	2.5 ± 0.7 (4)
AB Δ2	6.2 ± 0.4	38.2 ± 7.5	1.6 ± 0.4 (3)	6.3 ± 0.1	30.2 ± 2.7	1.6 ± 0.2 (3)
AB Δ2-Tet	88.0 ± 3.3	21.5 ± 0.5	2.1 ± 0.3 (3)	99.0 ± 5.9	41.4 ± 5.6	1.7 ± 0.3 (3)
ABΔ2CD	103.0 ± 1.9	17.8 ± 1.1	1.9 ± 0.2 (3)	101.0 ± 2.8	30.1 ± 2.2	2.4 ± 0.4 (3)

h, Hill coefficient; n, number of independent experiments; EC_{50}, concentration producing 50% of the maximal effect; n, number of independent experiments; N.D., not determined.

Please note that due to depletion of the free ligand, the EC_{50} is larger than the Kd and the values of the Hill coefficients are distorted. The interpretation of these coefficients is not straightforward.

Data for AB, AB S511D, AB A343D from[2], and data for ABCD and ABCD-L609R from[3].

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