Supporting Information

The cyclic nucleotide-binding homology domain of CNNM mediates dimerization and is required for Mg^{2+} efflux activity

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Supplemental Figures

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SEDYRDTVVKRKPASLMAPLKRKEEFSLFKVSDDEYKVTISPQLLLATQRF
            1                  2
LSREVDVFSPLRISEKVLLHLLKHPSVNQEVRFDESNRLATHHYLYQRSQP
                                    3
VDYFILILQGRVEVEIGKEGLKFENGAFTYYGVSALTVPSSVHQSPVSSLQ
                4                           5
PIRHDLQPDPGDTHSSAYCPDYTVRALSDLQLIKVTRLQYLNALLATRAQ
            67
NLPQSPENTDLQVIPGSQTRLLGEKTTTAAGSSHSRPGVPVEGSPGRNPGV
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Figure S1. Methionine substitutions tested for phasing of the CNNM3 CNBH domain. The amino acid sequence of human CNNM3 is shown for residues 453 to 707. The naturally occurring methionine is shown in red. Sites of methionine substitutions are shown in blue and numbered 1 to 7. The best diffracting crystals were obtained with methionines at sites 1, 3, 4, 5, 6, and 7.

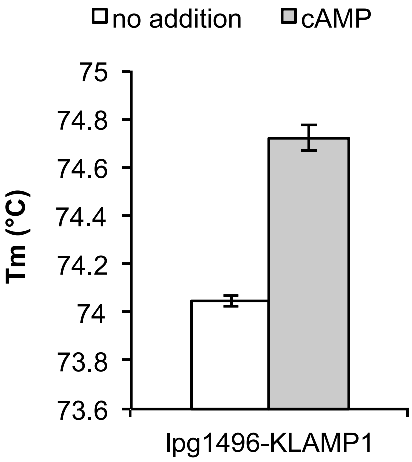

Figure S2. Thermal shift assays of positive control protein lpg1496-KLAMP1 in presence and absence of 1 mM cAMP. The melting temperature is increased by 0.7°C with addition of 1 mM cAMP. Each experiment was performed in triplicates. The error bars represent standard error.

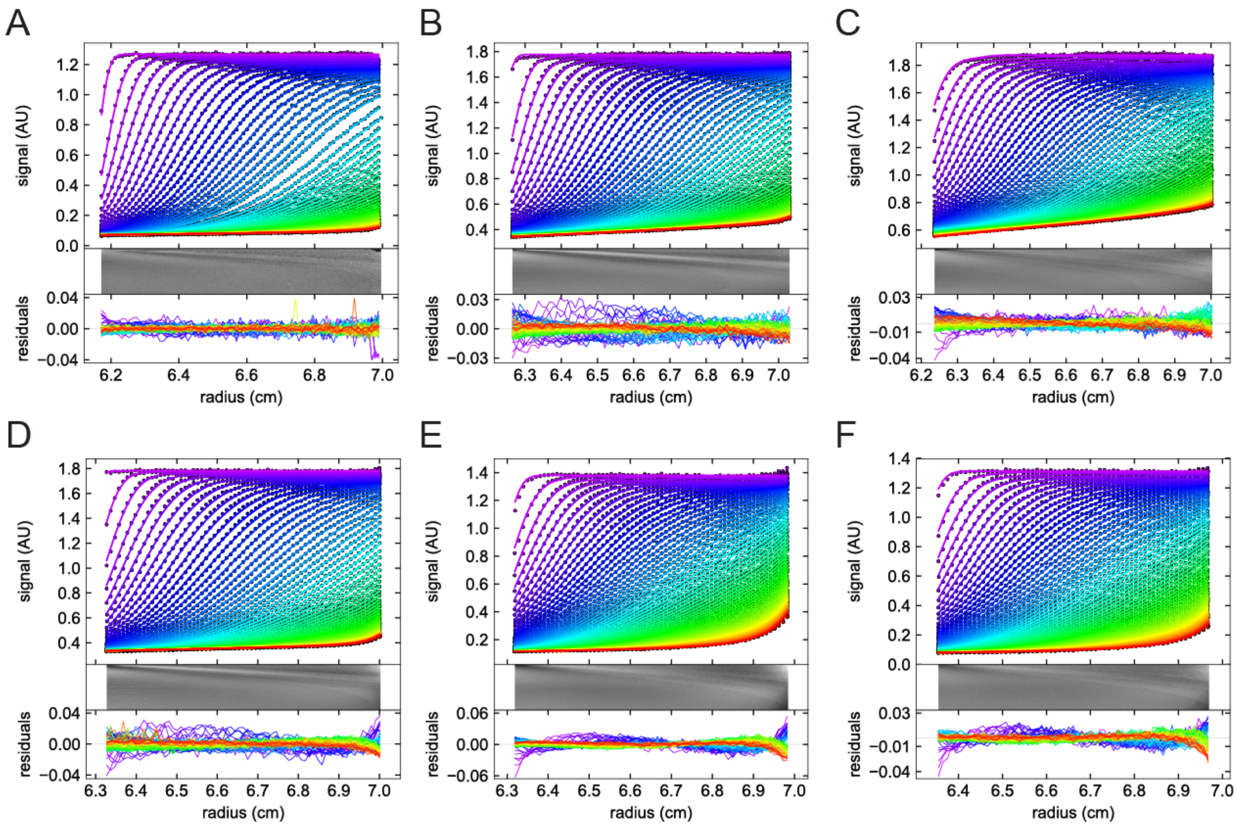

Figure S3. Representative sedimentation velocity AUC profile of 60 µM purified CNBH domains. (A) CNNM1 (B) CNNM2 (C) CNNM3 (D) CNNM4 (E) CNNM3 L575K (F) CNNM3 F577K

The absorbance of the sample at 280 nm and residuals are plotted against the radial position in the cell. One in every 15 scans is plotted.

Table S1. Thermal shift assay raw data

Protein	Condition	Tm (°C)				
		Replicate 1	Replicate 2	Replicate 3	Mean	Standard Error
CNNM1_CNBHD	no addition	48.20	48.02	48.11	48.11	0.05
CNNM1_CNBHD	cAMP	48.15	48.01	48.13	48.10	0.05
CNNM1_CNBHD	cAMP + $MgCl_2$	48.16	48.15	48.10	48.14	0.02
CNNM1_CNBHD	cGMP	48.17	48.04	48.01	48.08	0.05
CNNM1_CNBHD	cGMP + $MgCl_2$	48.16	48.18	48.29	48.21	0.04
CNNM2_CNBHD	no addition	50.23	49.94	50.10	50.09	0.09
CNNM2_CNBHD	cAMP	50.12	50.14	50.19	50.15	0.02
CNNM2_CNBHD	cAMP + $MgCl_2$	50.32	50.18	50.07	50.19	0.07
CNNM2_CNBHD	cGMP	50.34	50.21	50.13	50.23	0.06
CNNM2_CNBHD	cGMP + $MgCl_2$	50.39	50.30	50.32	50.34	0.03
CNNM3_CNBHD	no addition	49.49	49.48	49.43	49.47	0.02
CNNM3_CNBHD	cAMP	49.43	49.38	49.43	49.41	0.02
CNNM3_CNBHD	cAMP + $MgCl_2$	49.35	49.69	49.32	49.46	0.12
CNNM3_CNBHD	cGMP	49.54	49.32	49.43	49.43	0.07
CNNM3_CNBHD	cGMP + $MgCl_2$	49.38	49.35	49.67	49.47	0.10
CNNM4_CNBHD	no addition	52.46	52.53	52.36	52.45	0.05
CNNM4_CNBHD	cAMP	52.56	52.50	52.56	52.54	0.02
CNNM4_CNBHD	cAMP + $MgCl_2$	52.50	52.39	52.40	52.43	0.03
CNNM4_CNBHD	cGMP	52.65	52.64	52.53	52.61	0.04
CNNM4_CNBHD	cGMP + $MgCl_2$	52.58	52.64	52.63	52.62	0.02
lpg1496_KLAMP1	no addition	74.08	74.01	74.05	74.05	0.02
lpg1496_KLAMP1	cAMP	74.67	74.67	74.82	74.72	0.05

Table S2. SV-AUC sedimentation coefficients and estimated molecular weights

		Sedimentation coefficient (S)		Estimated MW (kDa)		RMSD	Theoretical MW (kDa)	
CNBH domain	[Protein] (μM)	Peak 1	Peak 2	Peak 1	Peak 2		Monomer	Dimer
CNNM1	60	2.52	3.68	28.1	52.9	0.0051	27.0	54.0
	30	2.62	3.68	30.2	46.7	0.0049		
	15	2.75	3.70	33.3	43.1	0.0043		
CNNM2	60	2.65	3.10	22.3	40.7	0.0053	22.7	45.4
	30	2.19	3.11	24.4	40.9	0.0047		
	15	2.11	3.04	25.8	41.1	0.0028		
CNNM3	60	2.76	-	44.2	-	0.0050	23.9	47.8
	30	2.70	-	37.1	-	0.0031		
	15	2.67	-	35.8	-	0.0025		
CNNM4	60	2.38	3.15	25.5	37.3	0.0053	25.0	50.0
	30	2.25	3.11	25.5	40.2	0.0043		
	15	2.24	3.06	25.3	35.6	0.0031		
CNNM3 L575K	60	2.08	-	20.7	-	0.0054	23.9	47.8
	30	2.04	-	21.5	-	0.0045		
	15	1.99	-	20.5	-	0.0031		
CNNM3 F577K	60	1.95	-	21.3	-	0.0053	23.9	47.8
	30	1.91	-	21.1	-	0.0035		
	15	1.90	-	20.7	-	0.0025		