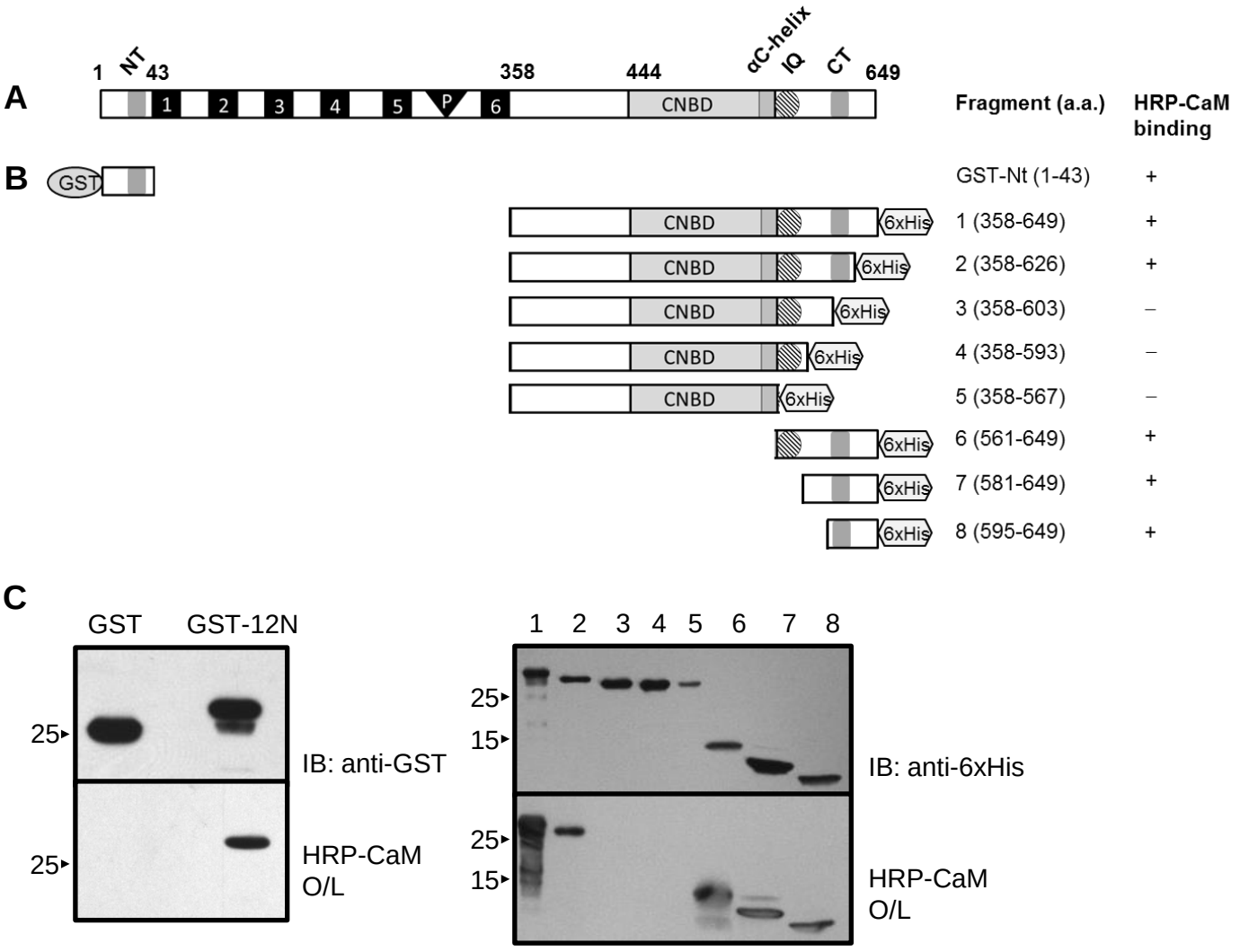
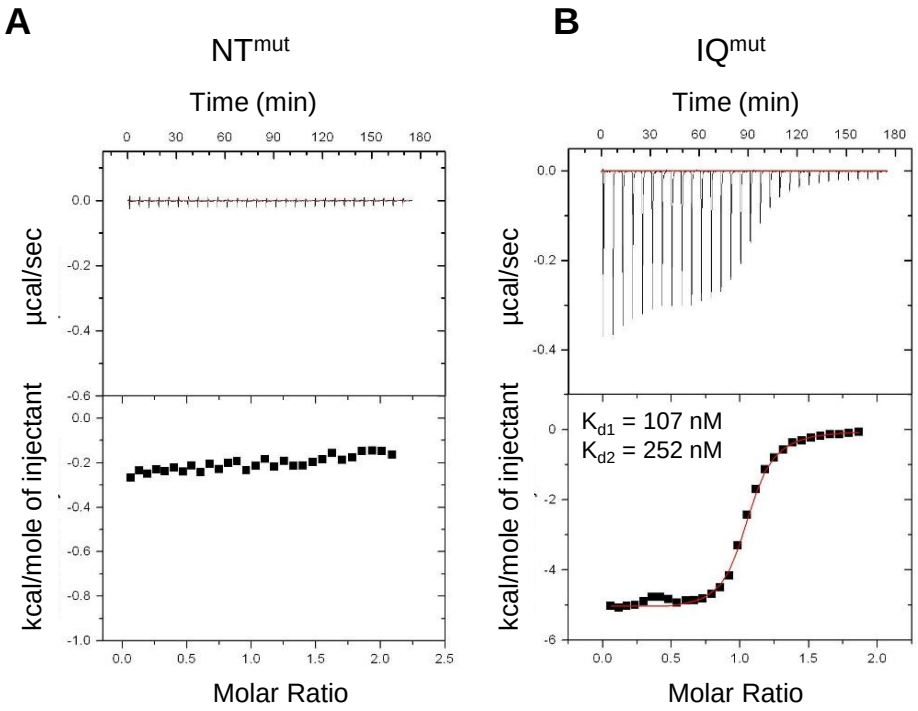
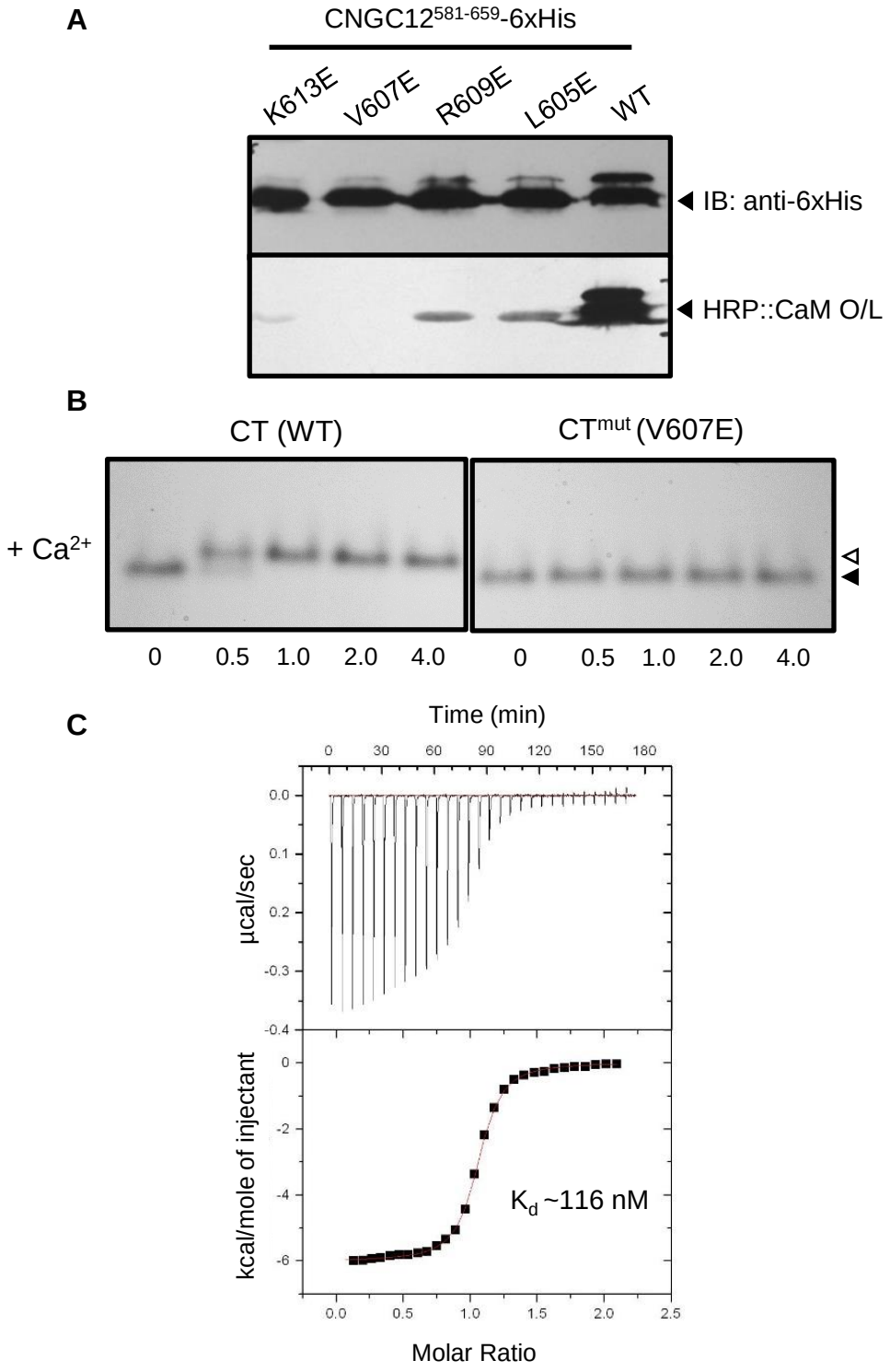


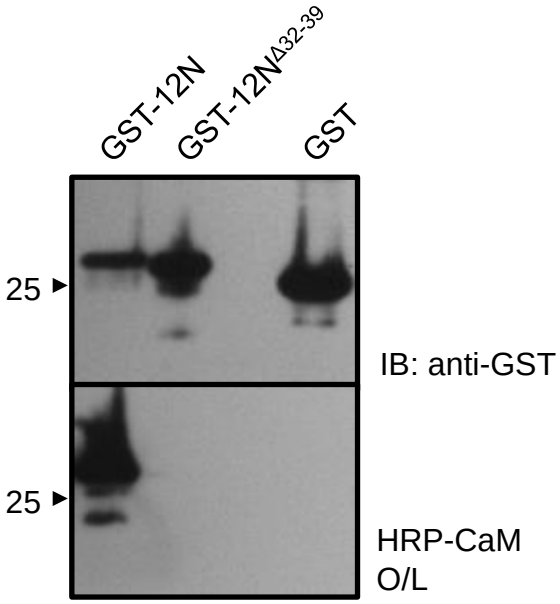
Supplemental Figure 1. N- and C-terminal regions of CNGC12 possess CaMBDs. (A), Domain organization of CNGC12. The location of six TM helices (1-6), pore-forming region (P), CNBD, αC-helix, and IQ motif were determined based on sequence alignment. Locations of the NT and CT motifs were determined in the present study. **(B)** Summary of *in vitro* CaM-binding assays shown in **(C)**. Fragments representing different regions of CNGC12 (as indicated by a.a. numbers) were recombinantly expressed as fusion proteins and subjected to HRP-CaM overlay assays. Fragments that produced a band in a HRP-CaM overlay (O/L) assay **(C)** are indicated with +. **(C)** Expression and CaM-binding of CNGC12 fusion proteins were determined via immunoblot (IB) (top panels) and HRP-CaM overlay (bottom panels), respectively.



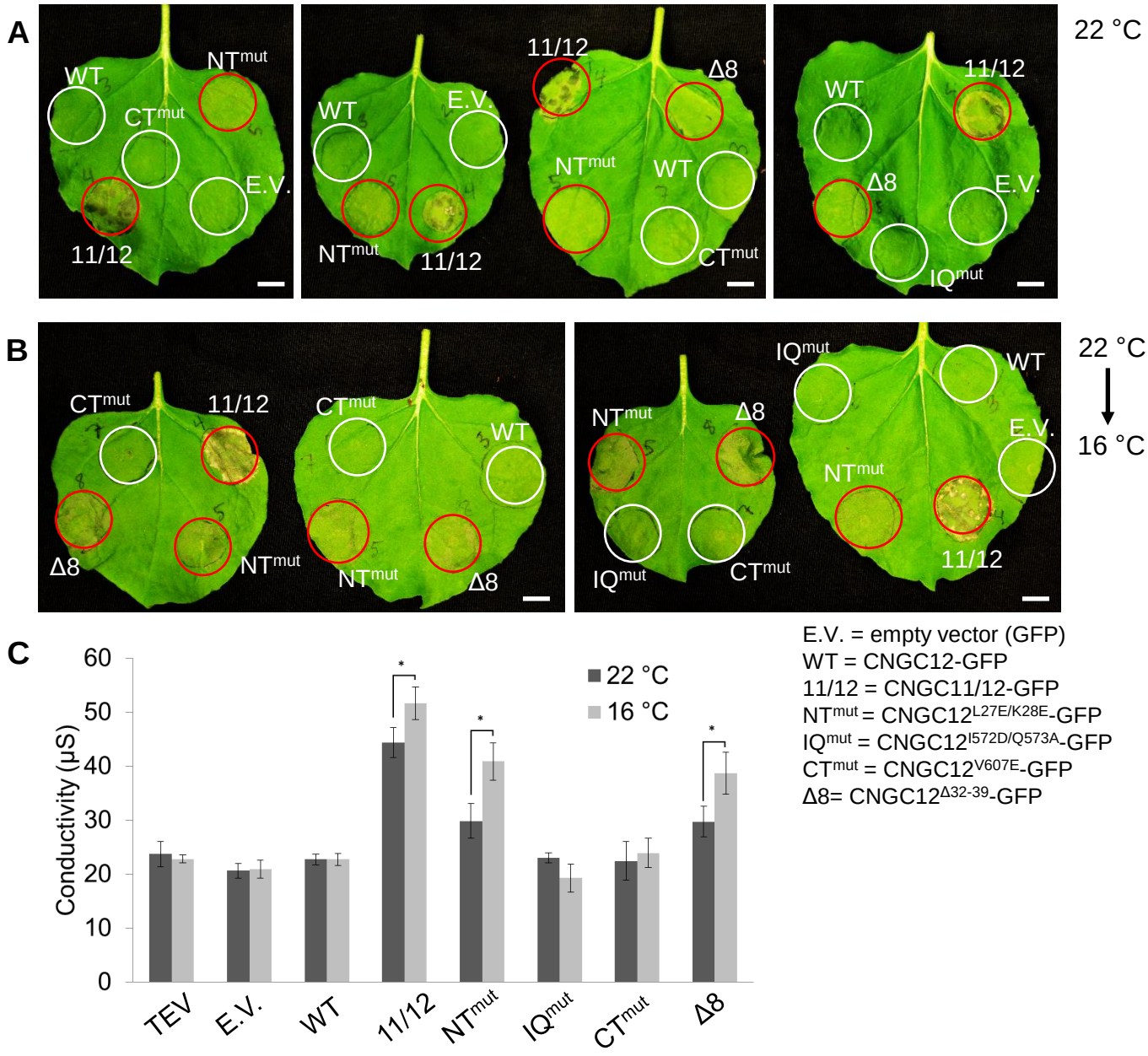
Supplemental Figure 2. ITC analysis of the NT^{mut} (L27E/K28E) and IQ^{mut} (I672D/Q573A) peptides. Data were collected, analyzed, and presented as outlined in Figure 3. **(A)** 150 µM NT^{mut} peptide was titrated into 16 µM CaM81. No binding was measured. **(B)** 140 µM IQ^{mut} peptide was titrated into 16 µM CaM81. The data were best fitted to a two-site model, with calculated Kd values of 107 and 252 nM.



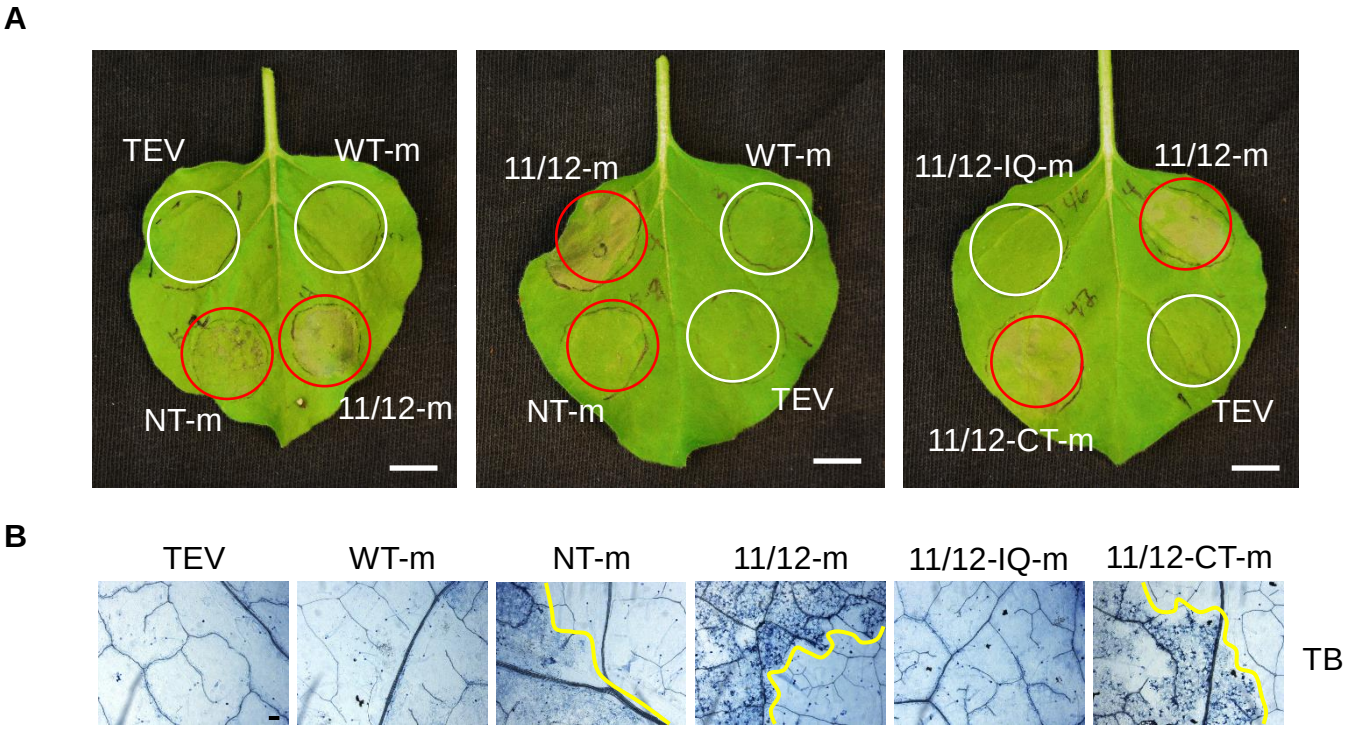
Supplemental Figure 3. The V607E mutation disrupts CaM-binding to the CT motif. **(A)** C-terminal fragments corresponding to a.a. 581-649 of CNGC12 featuring individual point mutations were expressed as 6xHis-tagged recombinant proteins and assayed for HRP-CaM-binding. **(B)** Ca^{2+}/CaM ND-PAGE assay with CT and Ctmut peptides. Closed and open arrows indicate the migration of CaM and CaM-peptide complex, respectively. Numbers indicate the molar ratio of peptide:CaM. **(C)** ITC analysis of CT^{mut} peptide binding to Ca^{2+}/CaM. 150 µM CT^{mut} peptide was titrated into 16 µM CaM81, and data were analyzed as described in Figure 3. A K_d value of 116 nM was calculated from measurements, which corresponds to an approximately 16-fold decrease in affinity compared to the WT CT peptide.



Supplemental Figure 4. The Δ8 mutation (CNGC12 a.a. 32-39) disrupts the CaM-binding to the N-terminus of CNGC12. The N-terminus (representing CNGC12 a.a. 1-43) of WT or mutant CNGC12 was expressed recombinantly and assayed for CaM-binding with 50 nM HRP-CaM in the presence of 1 mM $CaCl_2$.

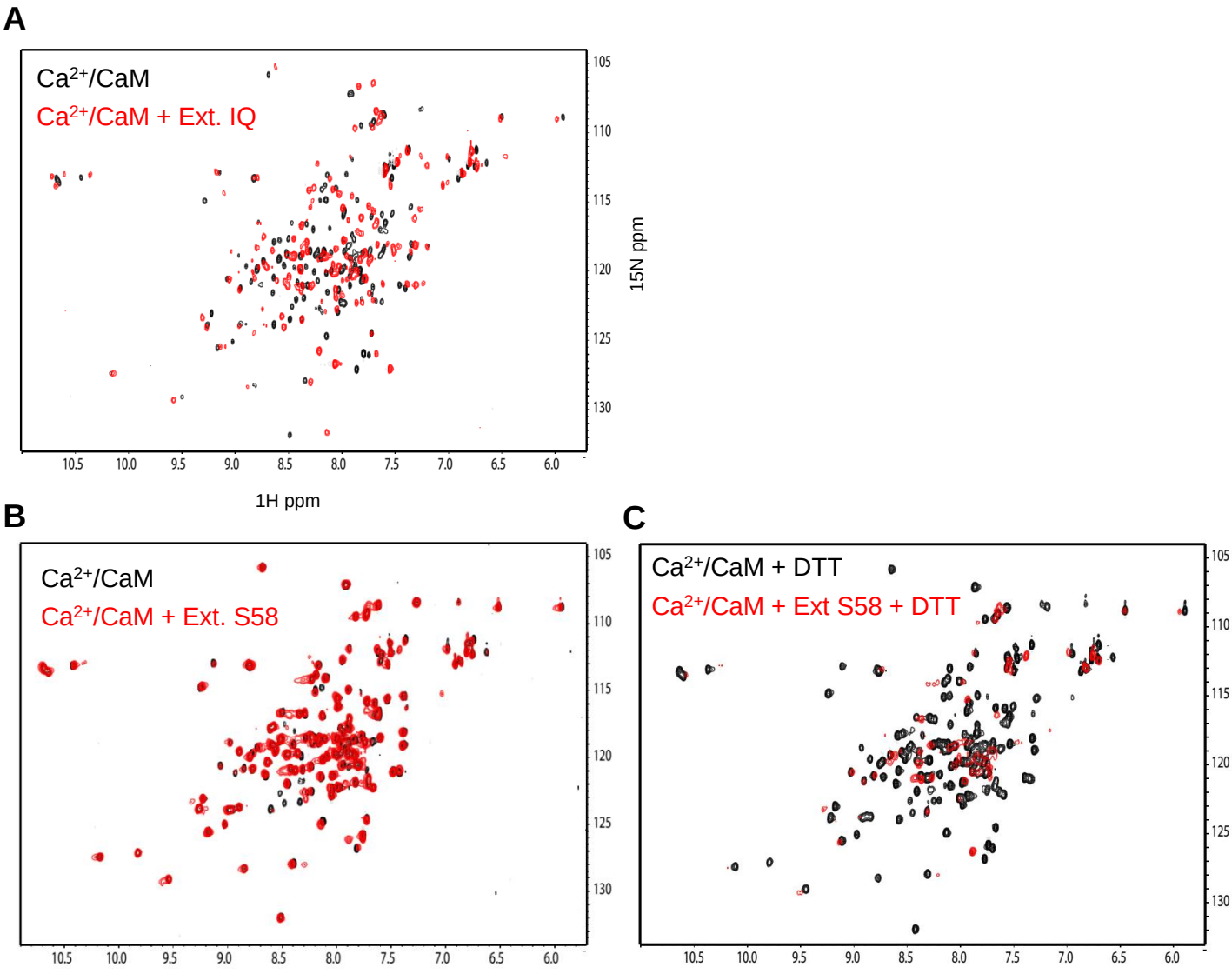


Supplemental Figure 5. PCD induction by NT mutants is increased at lower temperature. *N. benthamiana* leaves were infiltrated at 22 °C and left at 22 °C or shifted to 16 °C 24 hpi. Areas were infiltrated with HC-Pro from TEV alone (TEV) or co-infiltrated with GFP-tagged constructs labeled as indicated in the legend. (**A** and **B**) *N. benthamiana* leaves 4 dpi at 22 °C **(A)** or 16 °C **(B)**. Scale bars = 1 cm. **(C)** Conductivity of regions expressing CNGC11/12 or NT mutants (NTmut or Δ8) was significantly increased at 16 °C relative to 22 °C (* represents $p<0.05$, student's t-test). Values shown are averages of three replicates (error bars = standard deviation).

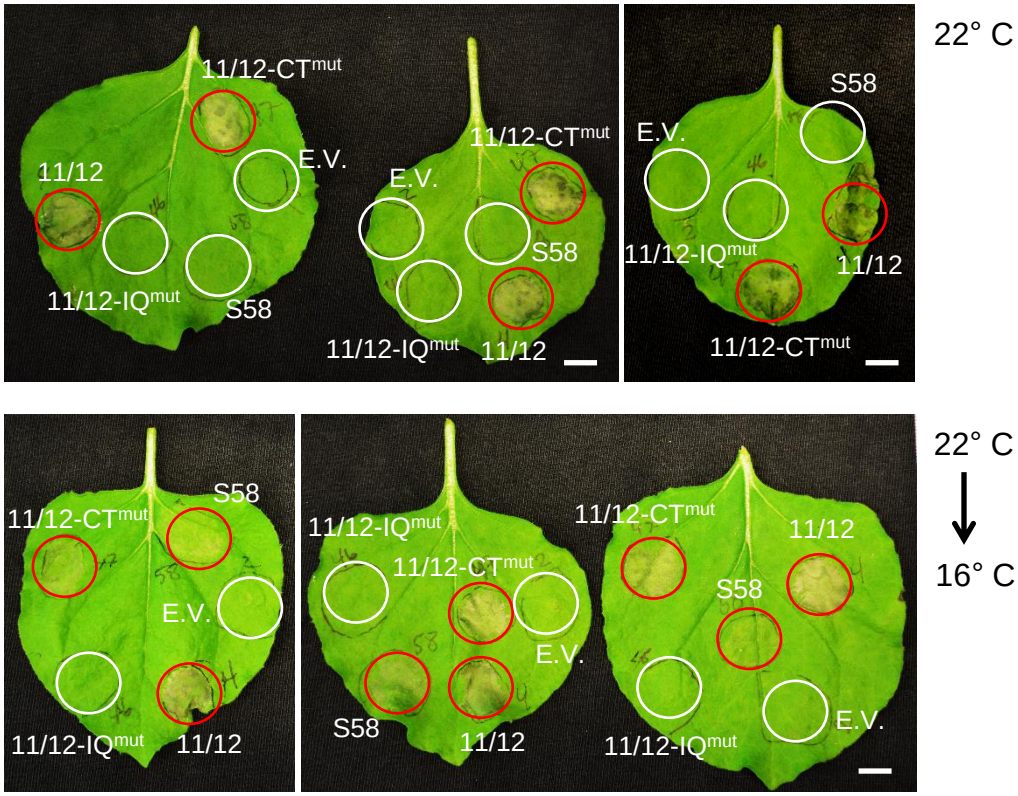


Supplemental Figure 6. Phenotypic analysis of myc-tagged CNGC12 construct expression in *N. benthamiana*.. Areas were infiltrated with the HC-Pro construct alone (TEV) or co-infiltrated with TEV and constructs: WT-m : CNGC12-myc, NT-m : CNGC12$^{L27E/K28E}$-myc, 11/12-m : CNGC11/12-myc, 11/12-IQ-m : CNGC11/12$^{I564D/Q565A}$-myc, 11/12-CT-m : CNGC11/12^{V599E}-myc. Experiments were performed as described for Figures 6 and 7, and results are shown 4 dpi. **(A)** Phenotypes of leaves 4 dpi. Red circles indicate areas showing visible signs of PCD. Scale bar = 1 cm. **(B)** Trypan blue (TB) staining for cell death. Yellow lines indicate the border of infiltrated vs uninfiltrated areas. Scale bar = 0.1 mm.

IQ peptide AAFFIQAAWRKHCKRKLSKTR
Ext. IQ peptide RLYSQQW**R**SWAAFFIQAAWRKHCKR
Ext. S58 peptide RLYSQQW**C**SWAAFFIQAAWRKHCKR



Supplemental Figure 7. The S58 mutant of CNGC11/12 (R577C) partially disrupts CaM-binding to the IQ motif. Spectra of 0.2 mM ^{15}N-CaM in 10 mM Tris-Cl, 150 mM NaCl, 10 mM $CaCl_2$, pH 7.5 in the absence **(A** and **B)** or presence of 5 mM DTT. Overlaid spectra are shown for CaM alone (black) and CaM in the presence of equimolar peptide, as indicated. **(A)** The WT extended peptide induces a shift in ^{15}N-CaM spectra indicative of interaction. **(B)** The S58 peptide induces only minor changes in the spectra of ^{15}N-CaM under normal conditions, suggesting this peptide cannot bind CaM; however binding can be partially restored under reducing conditions **(C)**.



Supplemental Figure 8. Low temperature does not restore PCD-induction by CNGC11/12-IQ^{mut}. *N. benthamiana* leaves were infiltrated at 22° C with Agrobacterium carrying constructs as labeled in figure legend. For temperature shift, plants were moved 24 hpi into a 16° C growth chamber. Images show leaves 3 dpi (48 h at 16° C). Areas showing PCD are circled in red. Scale bar = 1 cm.

Supplemental Table 1. CNGC12 CaMBD peptides. Postion refers to the a.a. sequence of CNGC12 corresponding to each peptide. Mutations are bolded.

Name	Position	Sequence
NT	16-40	VDGKLKSVRGRLKKVYGKMKTLENW
NT^{mut}	16-40	VDGKLKSVRGR**EE**KVYGKMKTLENW
IQ	568-588	AAFFIQAAWRKHCKRKLSKTR
IQ^{mut}	568-588	AAFF**DA**AAWRKHCKRKLSKTR
CT	600-623	NLASTLYVSRFVSKALQNRRKDTA
CT^{mut}	600-623	NLASTLY**E**SRFVSKALQNRRKDTA

All peptides used for biophysical analyses of CaM-binding (ITC or NMR) were synthesized to high purity (>90%) by GenScript (Piscataway, NJ, USA) or Peptide 2.0 (Chantilly, VA, USA).

Supplemental Table 2. Calculated parameters from ITC measurements. Values correspond to isotherms shown in Figure 3. The stoichiometry (*N*, no. sites/mol), binding constant (*K*, in cal/mol), heat change (*ΔH*, in cal/mol), and entropy change (*ΔS*, in cal/mol) are shown for each modeled binding site.

Parameter	NT + Ca^{2+}/CaM	IQ + Ca^{2+}/CaM	CT + Ca^{2+}/CaM	IQ + apoCaM
N	0.9485 ±0.002059	$_1$ 0.4767 ±0.03102 $_2$ 0.4879 ±0.0342	0.9487 ±0.001604	0.9861 ±0.003099
K	3.105E7 ±2.542E6	$_1$ 5.637E7 ±2.224E7 $_2$ 1.186E7 ±1.372E6	2.548E8 ±4.642E7	1.048E6 ±3.054E4
ΔH	-1.118E4 ±45.72	$_1$ -2328 ±409.4 $_2$ -6688 ±613.8	-9742 ±39.15	-8955 ±39.70
ΔS	-2.609	$_1$ 27.78 $_2$ 10.31	6.328	-1.995

Supplemental Table 3. Primers used for experiments.

Purpose	Gene	Name	Sequence (5'→3')
RT-PCR	*CNGC12* F11C10-14-F5		CGGGTGATACCAGAGTA
		F11C10-14-R3	GGAAGGCGAGAACCATT
	NbACTIN	NbACT_RT_F1	CATATGTAGGAGATGAAGCT
		NbACT_RT_R1	ATCAGTAAGGTCACGACCAG
	NbPR1a	NbPR1a_RT_F1	AATATCCCACTCTTGTCG
		NbPR1a_RT_R1	CCTGGAGGATCATAGTTG
	NbHSR203J	NbHSR203J_RT_F1	TGCGTCAGCCAAGCTGATTG
		NbHSR203J_RT_R1	CCGATAGGACCGCACGAAAC
Cloning	*AtCNGC12*	CNGC12_F_XbaI	TATCTAGAATGAATCTTCAGAGGAGAAAATTTG
		CNGC12_R_nostop_XbaI	TATCTAGAGCTTCAGCCTTTGCAAACTC
		CNGC12_SalI_F	TAGTCGACATGAATCTTCAGAGGAG
		CNGC12Nt_NotI_R	TAGCGGCCGCTTAAGTCTTCCTCCAG
		CNGC12-pET28m-F	AAGCTAGCTCTACTACTAGAGTAGAT
		CNFGC12_R_BaMHI	TTGGATCCTGCTTCAGCCTTTGC
		CNGC12_626_R	TTGGATCCGGAACAACCTGCTGTATC
		CNGC12_603_R	TTGGATCCTGATGCAAGATTGAGTTGC
		CNGC12_593_R	TTGGATCCAATATTCTCGTTGTCTCTTG
		CNGC12_567_R	TTGGATCCCCATGAGCGCCATTGCTG
		CNGC12_561_F_NheI	AAGCTAGCTCACAGCAATGGCGCTCATG
		CNGC12_581_F	AAGCTAGCAAAAGGAAGCTGTCTAAGAC
		CNGC12_595_F	AAGCTAGCCAAGGCACGCAACTCAATC
Mutagenesis	*AtCNGC12*	F-N-term.LK::EE	AAAATTGAAAAGTGTTAGAGGACGCGAGGAGAAGGTTTACGGGAAGATGAAAAC
		R-N-term.LK::EE	GTTTTCATCTTCCCGTAAACCTTCTCCTCGCGTCCTCTAACACTTTTCAATTTT
		CNGC12_IQ572DA_F	CATGGGCAGCATTCTTCGATGCAGCGGCATGGAGGAAAC
		CNGC12_IQ572DA_R	GTTTCCTCCATGCCGCTGCATCGAAGAATGCTGCCCATG
		CNGC12_L605E_F	CTCAATCTTGCATCAACGGAATACGTGTCCAGATTTGTG
		CNGC12_L605E_R	CACAAATCTGGACACGTATTCCGTTGATGCAAGATTGAG
		CNGC12_V607E_F	CTTGCATCAACGCTCTACGAGTCCAGATTTGTGTCCAAAG
		CNGC12_V607E_R	CTTTGGACACAAATCTGGACTCGTAGAGCGTTGATGCAAG
		CNGC12_R609E_F	TCAACGCTCTACGTGTCCGAATTTGTGTCCAAAGCTTTG
		CNGC12_R609E_R	CAAAGCTTTGGACACAAATTCGGACACGTAGAGCGTTGA
		CNGC12_K613E_F	CGTGTCCAGATTTGTGTCCGAAGCTTTGCAAAATCGACG
		CNGC12_K613E_R	CGTCGATTTTGCAAAGCTTCGGACACAAATCTGGACACG