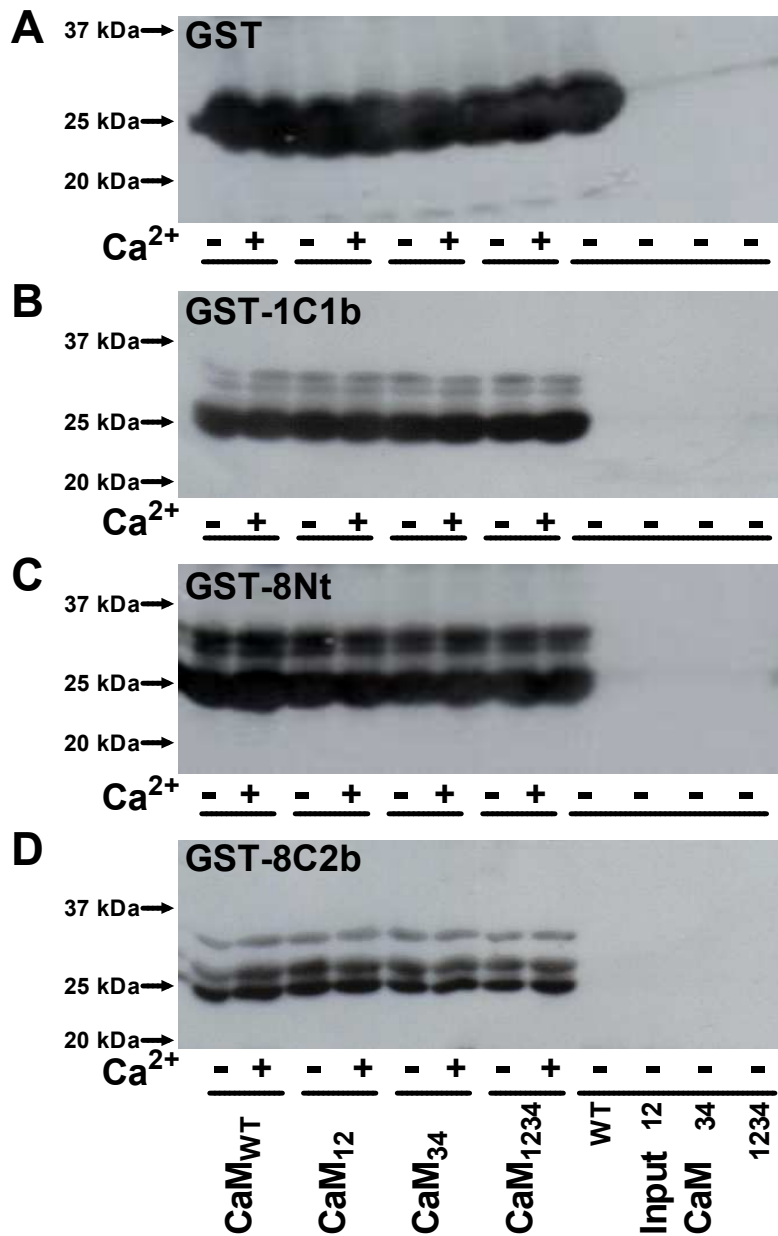
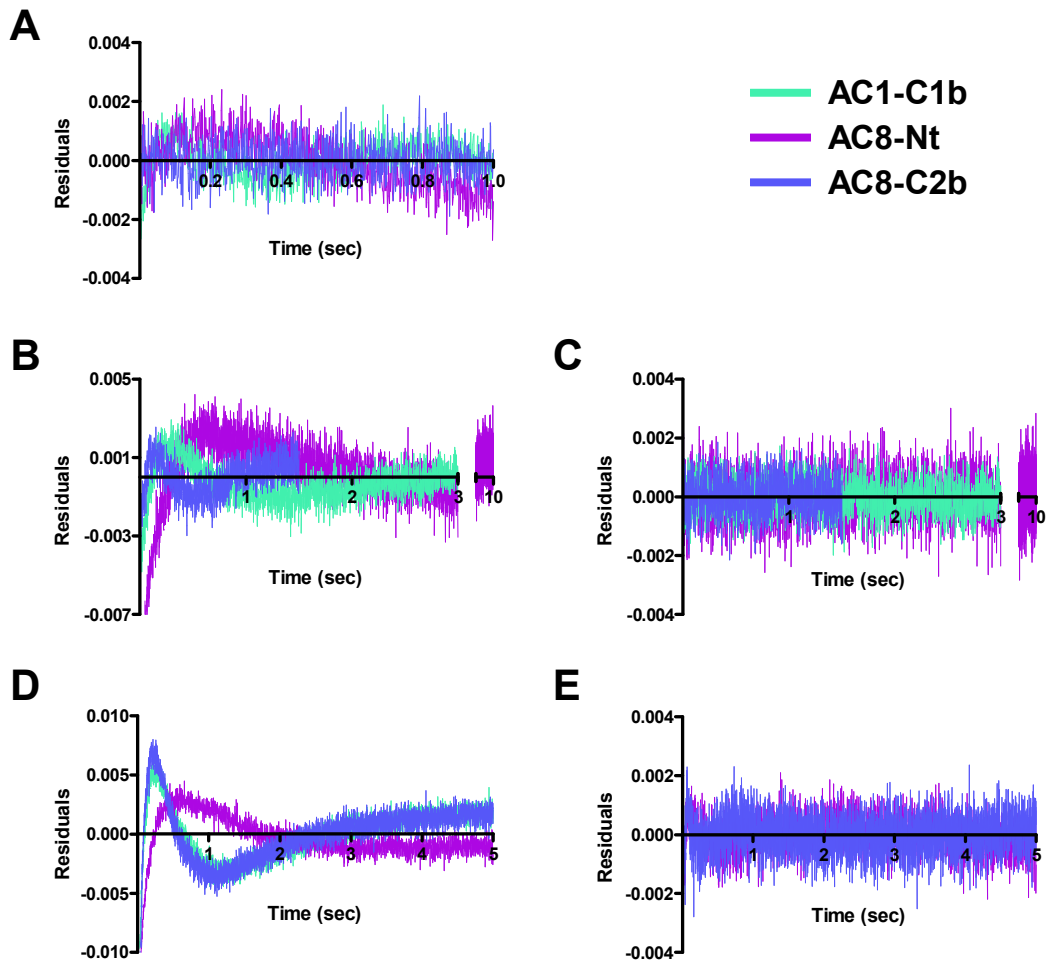


	Residues	Sequence
AC1-C1b	495-522	IKPAKRMKFKTVCYLLVQLMHCRKMFKA
GST-1C1b	481-548	SHRRKIFPGLILSDIKPAKRMKFKTVCYLLVQLMHCRKM FKAEIPFSNVMTCEDDDKRRALRTASEKL
AC8-NT	30-54	GSRPQRLLWQTAVRHITEQRFIHGH
GST-8Nt	1-77	MELSDVHCLSGSEELYTIHPTPPAADGGSGSRPQRLLWQ TAVRHITEQRFIHGHRRGGGGGSRKASNPAGSGPNHHA
AC8-C2b	1187-1211	YSLAAVVLGLVQSLNRQRQKQLLNE
GST-8C2b	1183-1248	LPGQYSLAAVVLGLVQSLNRQRQKQLLNENSNSGIIKSH YNRRTLLTPSGPEPGAQAEGTDKSDLP

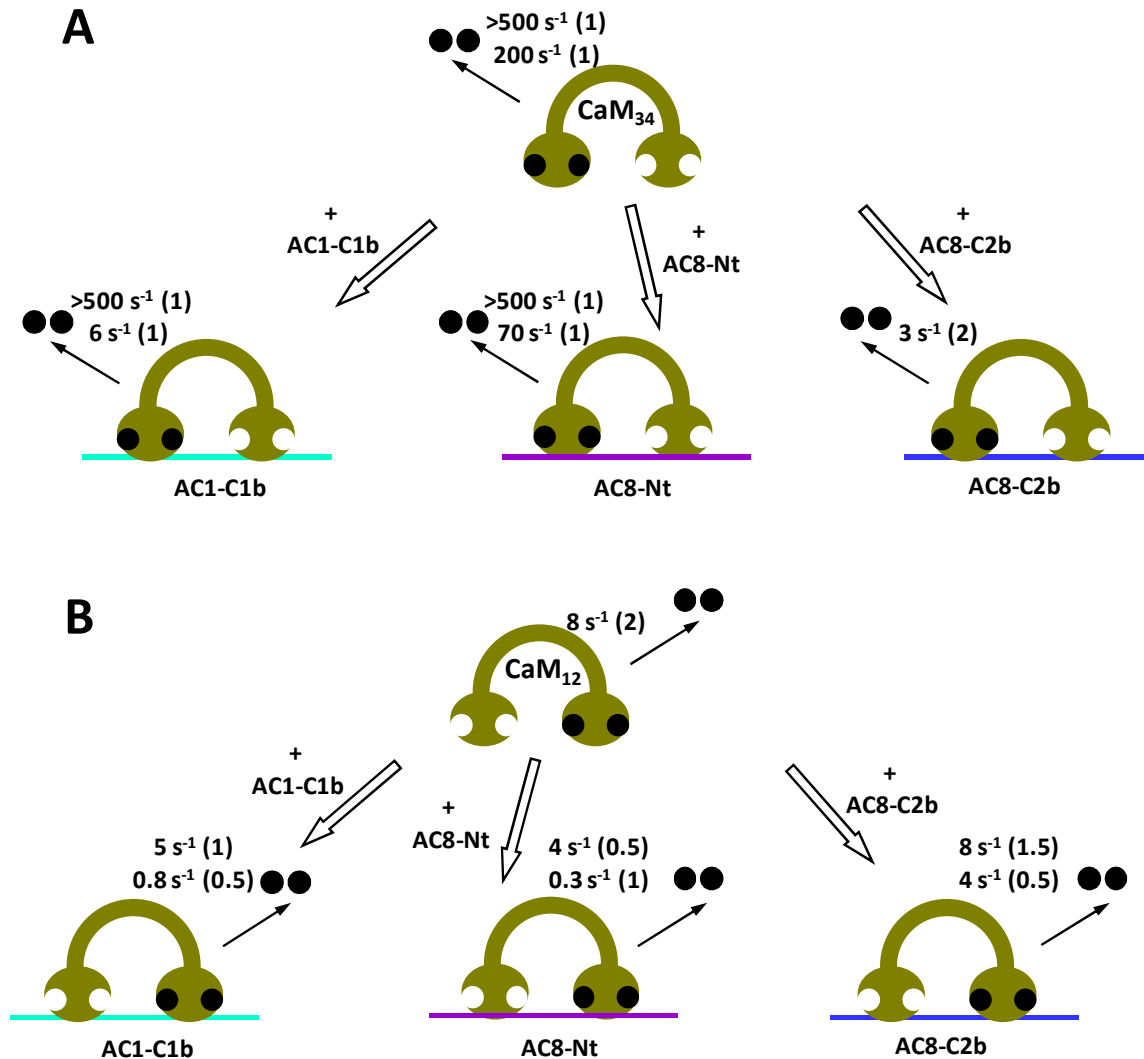
Supplemental Table 1. Sequences of the peptides and GST-fragments. Peptides (AC1-C1b, AC8-Nt, and AC8-C2b) used for *offline* nano-ESI-MS stopped-flow measurements and GST-fragments (GST-1C1b, GST-8Nt, and GST-8C2b) used for GST pull-down assays are shown. The residues correspond to bovine AC1 and rat AC8.



Supplemental Figure 1. GST expression from GST-pull down assay. The membranes from the Fig. 4 was stripped and re-plobed using GST antibody to show the amount of GST used in the assay. The molecular weights of GST (A), GST-1C1b (B), GST-8Nt (C), and GST-8C2b (D) are 26 kDa, 33.3 kDa, 34.5 kDa, and 33.5 kDa, respectively.



Supplemental Figure 2. Residuals from the fit of Ca^{2+} dissociation from CaM. Residuals from a single-exponential fit (A, B, and D) or a double-exponential fit (C and E) of Ca^{2+} dissociation from CaM34 (A) from Fig. 6A, CaM12 (B and C) from Fig. 6B, or CaMWT (D and E) from Fig. 7A are shown.



Supplemental Figure 3. Summary showing the effect of AC1-C1b, AC8-Nt, and AC8-C2b on the rate constants for Ca²⁺ dissociation from CaM mutants. A, Ca²⁺ dissociates from the N-lobe of peptide-free CaM₃₄ with a rate constant of >500 s⁻¹ and 200 s⁻¹. When CaM₃₄ binds to AC1-C1b, the Ca²⁺-dissociation rate from one EF-hand of the N-lobe is decreased to 6 s⁻¹, without affecting the rate of other EF-hand. Ca²⁺ release from CaM₃₄ is still very fast in the presence of AC8-Nt. The rate constant for Ca²⁺ dissociation is dramatically reduced when CaM₃₄ is in complex with AC8-C2b, to 3 s⁻¹. B, Ca²⁺ dissociates from the C-lobe of peptide-free CaM₁₂ with a rate constant of 8 s⁻¹. When CaM₁₂ binds to AC1-C1b, the Ca²⁺-dissociation rate from

one EF-hand of the C-lobe is decreased to 0.8 s^{-1} , without affecting the rate of other EF-hand considerably. The rate constant for Ca^{2+} dissociation is dramatically reduced when CaM12 is in complex with AC8-Nt, to 4 s^{-1} and 0.3 s^{-1} . Ca^{2+} release from CaM12 is hardly influenced in the presence of AC8-C2b.