Supporting Information

Caged activators of artificial allosteric protein biosensors

Selvakumar Edwardraja, Zhong Guo, Jason Whitfield, Ignacio Retamal Lantadilla, Wayne A Johnston, Patricia Walden, Claudia E. Vickers, and Kirill Alexandrov

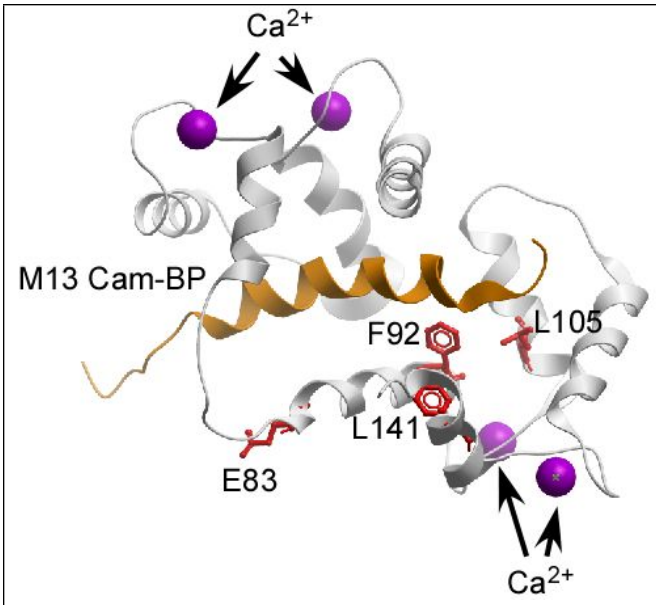

Figure S1. ***Design of calmodulin mutants with reduced affinity for CaM-BP.*** *A ribbon representation of NMR structure (PDB ID: 2bbm) of human Calmodulin complexed with M13 peptide was used for structure analysis. Calmodulin is displayed as white ribbon while the M13 peptide is colored in gold. Residues E83, F92, L105, F141 that form hydrophobic interactions with the peptide ligand are shown in ball and stick representation. All the Ca^{2+} atoms are colored in magenta.*

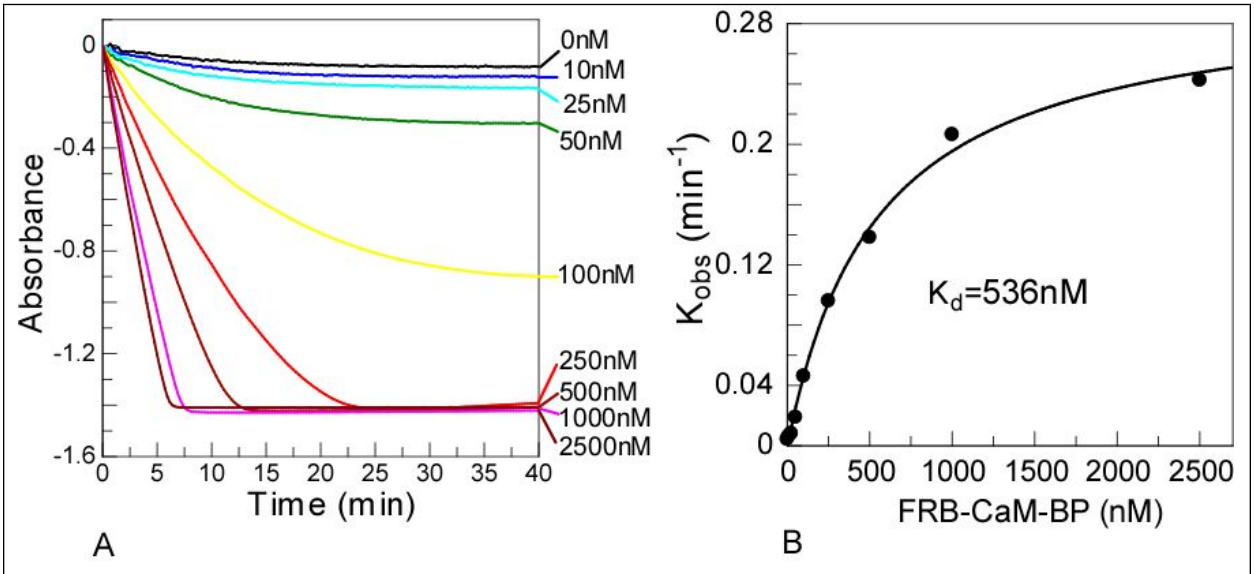

Figure S2. Activation of GDH activity of 10nM of CaM-GDH-FKBP in the presence of increasing concentrations of FRB-CaM-BP(low affinity). The activity of the enzyme was monitored by changes in absorption of electron accepting dye dichlorophenolindophenol in the presence of 0.6 mM electron

mediator phenazine methosulphate, 20mM of glucose and 1mM $CaCl_2$. The fit of the data led to a K_d value of 536 nM.

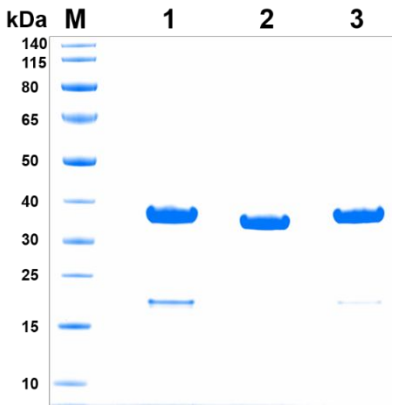

Figure S3. ***SDS–PAGE analysis of purified Caged-CaM-BP activators.*** M-molecular weight markers Lane 1, FRB-lowCaM-M13 Lane 2, FRB-wtCaM-M13, Lane 3 FRB-mediumCaM-M13.

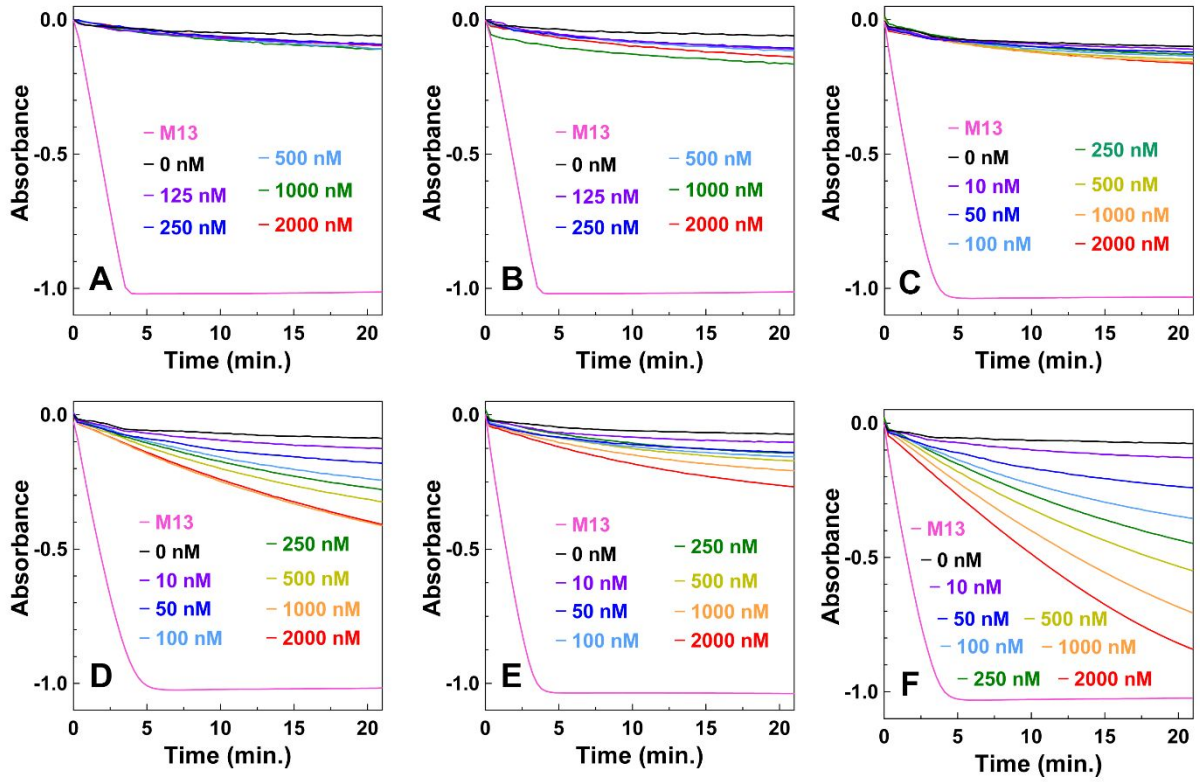

Figure S4. ***Activation of CaM-GDH reporter by caged-CaM-BP activators in two component rapamycin biosensors.*** (A) Changes in GDH activity of 10 nM solution of CaM-GDH-FKBP and increasing concentrations of FRB-wtCaM-M13 in the absence of rapamycin. (B) as in A but using FRB-medium affinity-CaM-M13, (C) Changes in GDH activity of 10 nM solution of CaM-GDH-FKBP and 1μM FRB-low-CaM-M13 in the absence of rapamycin, (D) (E) (F) as in A,B,C but in the presence of 0.5 μM

rapamycin. As a positive control 0.2 µM of CaM-BP M13 peptide was added to all experiments to activate the CaM-GDH-FKBP. The activity of the enzyme was monitored by changes in absorption of electron accepting dye dichlorophenolindophenol in the presence of 0.6 mM electron mediator phenazine methosulphate, 20mM of glucose and 1 mM $CaCl_2$.

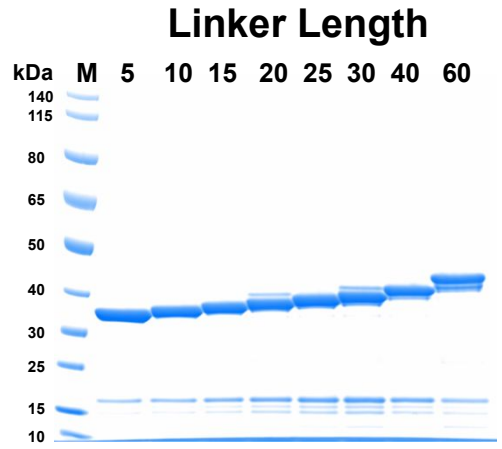

Figure S5. ***SDS analysis of purified recombinant FRB-lowCaM-CaM-BP proteins of different linker length.*** M-molecular weight marker. The number of residues between CaM-BP and CaM are indicated above each lane.

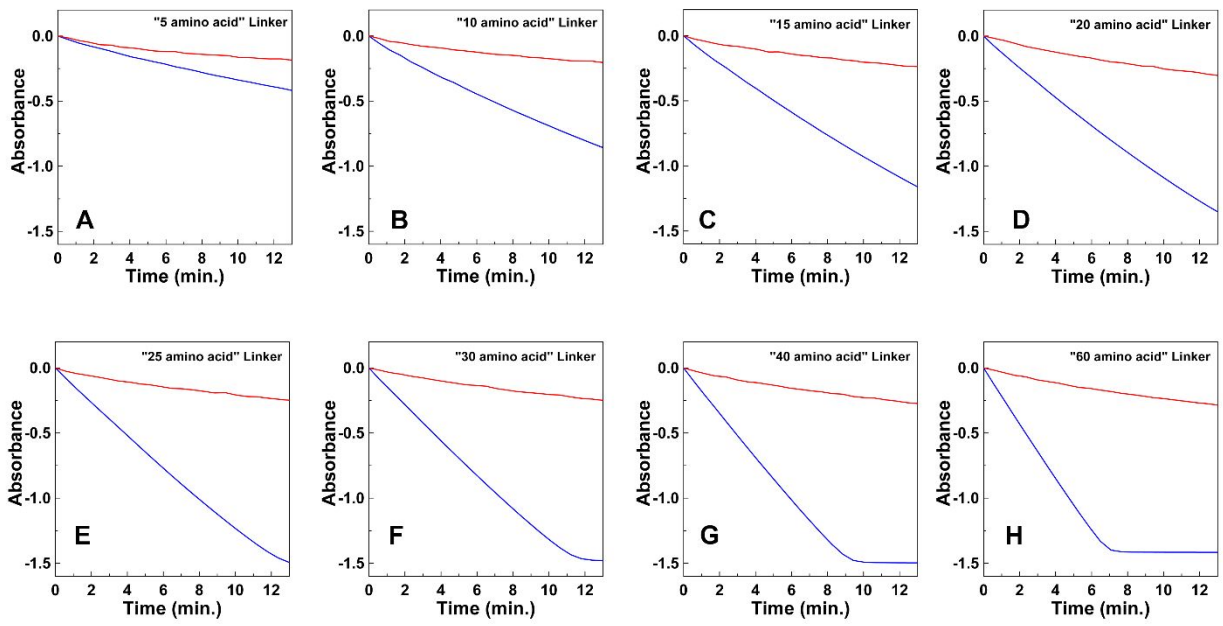

Figure S6. ***Testing caged-CaM-BP activators with different linkers in two component rapamycin biosensors based on CaM-GDH allosteric switch.*** The GDH activity of 10nM GDH-CaM-FKBP with different-linker caged activators 1µM low-affinity-CaM-BP in response to presence (blue) or absence (red) of 0.5 µM Rapamycin. The activity of the enzyme was monitored as in Figure S3.

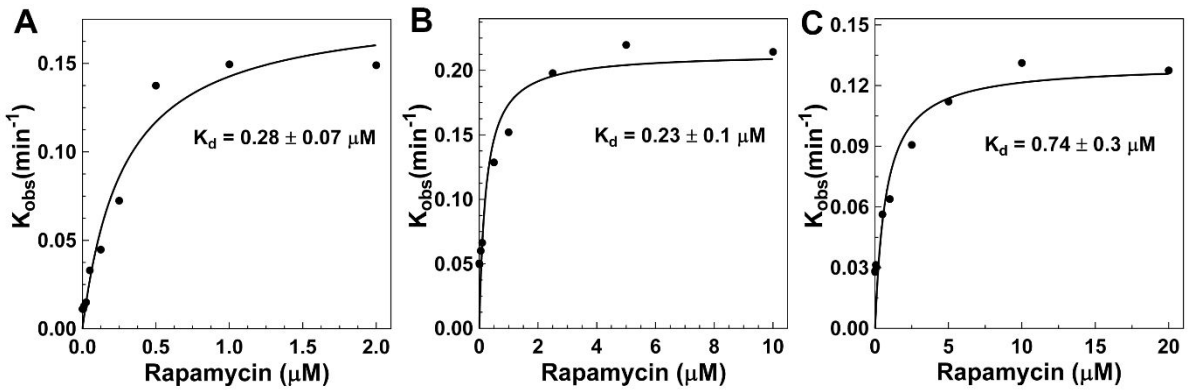

Figure S7. Fit of the data shown in figure 3 to a quadratic equation. The fit led to apparent K_ds of 0.28 ± 0.07 μM, 0.23 ± 0.1 μM and 0.74 ± 0.3 μM. As the experiments were performed at concentrations of the components orders of magnitude higher than their K_d, the obtained value reflects the concentration of the ligand in the reaction at which maximal response is achieved (active site titration).

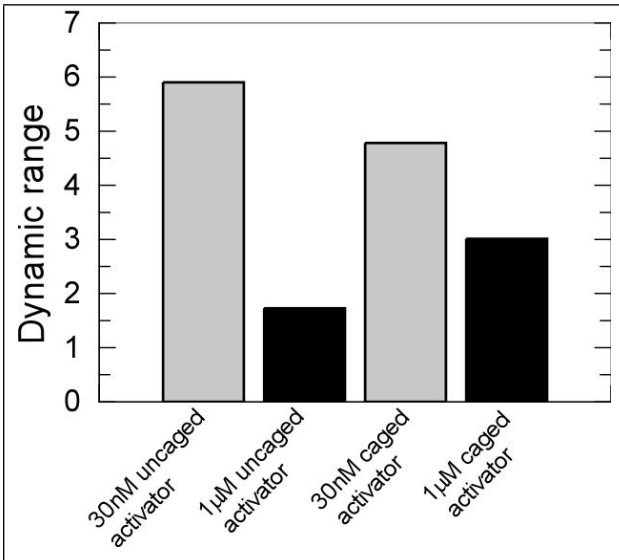

Figure S8. ***Dependence of the dynamic range of CaM-NanoLuc based rapamycin biosensor with caged or uncaged activator at different concentrations of the components.*** The reactions contained 10nM CaM-NanoLuc-FKBP fusion and either 30nM or 1μM FRB-CaM-BP or the corresponding concentrations of FRB-Medium affinity CaM-CaM-BP fusion. The reaction buffer contained 0.5mM $CaCl_2$ and Rapamycin was added to a final concentration of 50nM. DMSO was used as negative control. Reactions were incubated for 30 minutes prior to the addition of NanoLuc substrate (0.02μl substrate in 200μl reaction volume). The dynamic range was calculated as the fold signal difference in the absence and presence of rapamycin.

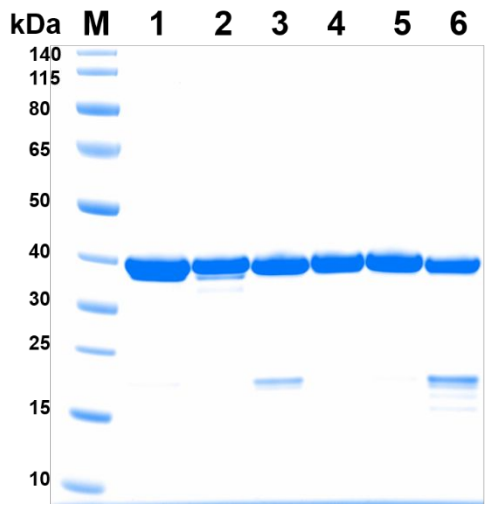

Figure S9. ***SDS PAGE analysis of purification of caged-CaM-BP activators.*** (Lane 1), FRB-lowCaM-N5A (Lane 2), FRB-lowCaM-iNOS (Lane 3) FRB-lowCaM-BP2, (Lane 4) FRB-lowCaM-BP3, (Lane 5) FRB-lowCaM-BP4 and (Lane 6) FRB-laCaM-BP11

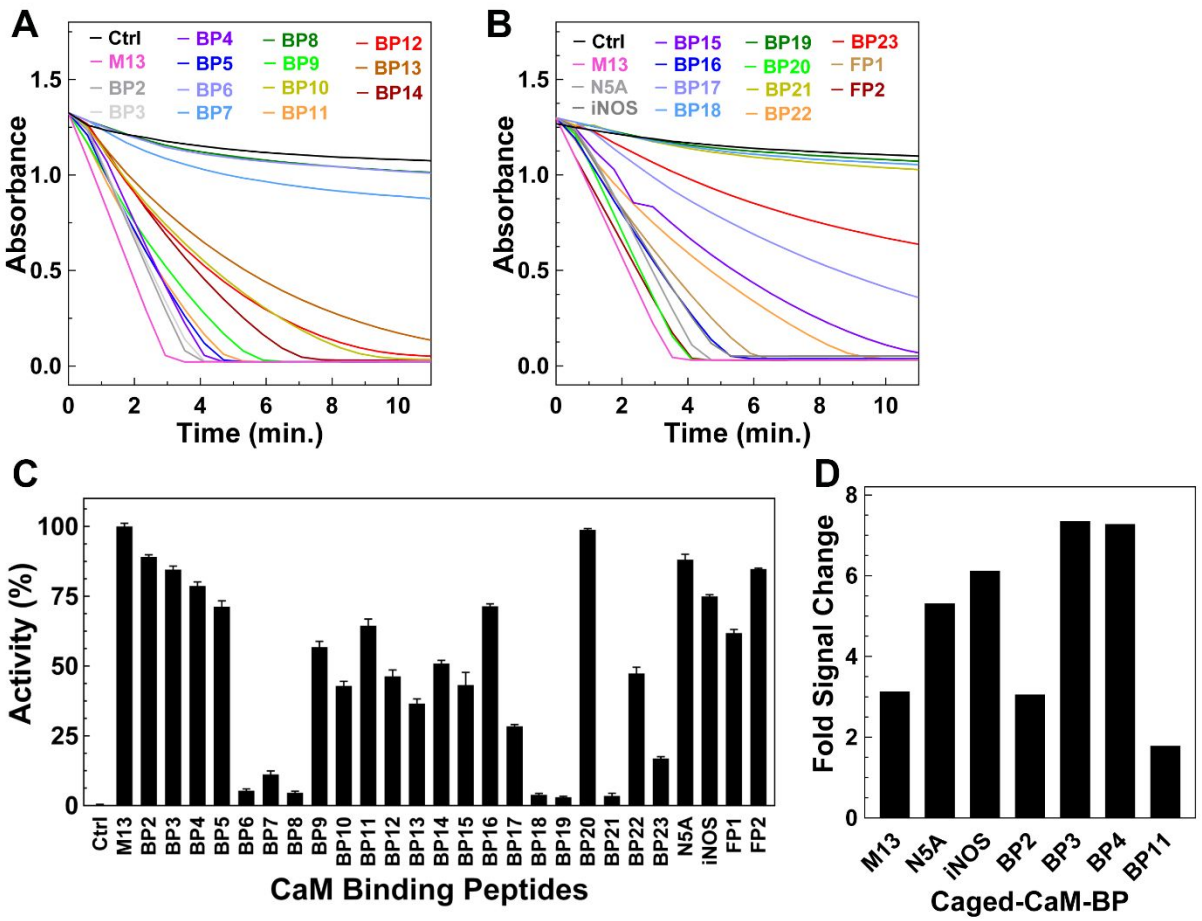

Figure S10. ***Analysis of the ability of different CaM-BPs to activate CaM-GDH.*** (A) and (B) 1 µM solution of CaM-GDH was incubated with 1 µM of the respective peptide for 10 minutes and the reactions were triggered by the addition of glucose and changes in absorption were monitored for 10 minutes. (C) The slope (K_{obs}) of the obtained curve was a used as measure of activity. The activity in the absence of

CaM-GDH was considered as zero and activity of M13 peptide (BP1) was taken as 100% and the data for both data sets scaled. (D) Activation of 10nM CaM-GDH-FKBP with 1μM different FRB-lowCaM-CaM-BPs constructs. The fold change represents the increase in signal level between the background activity and the maximal observed signal level at saturating concentrations of rapamycin.

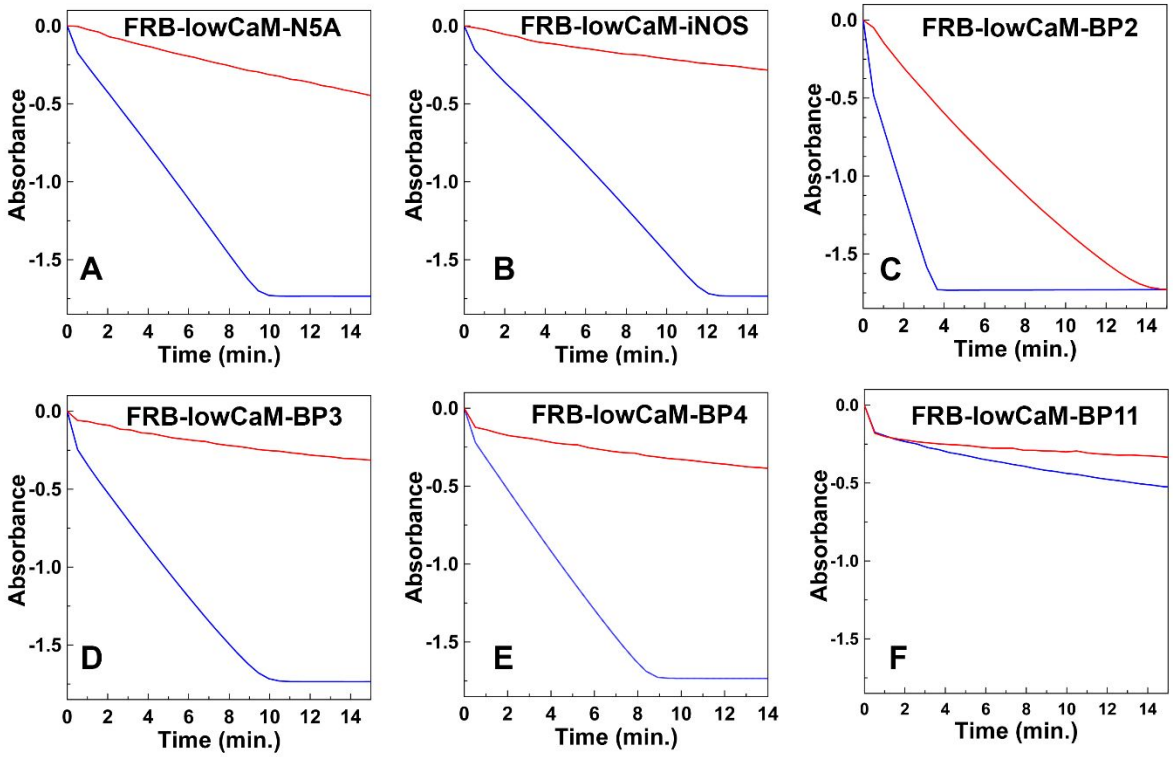

Figure S11. ***Activation of CaM-GDH based rapamycin sensor using different caged peptides.*** The GDH activity of 10nM CaM-GDH-FKBP with 1μM caged activators in response to presence (blue) or absence (red) of 0.5 μM of rapamycin. The GDH assay was performed as in Figure 2C. The fold change represents the increase in signal level between the background activity and the maximal observed signal level at saturating concentrations of rapamycin.

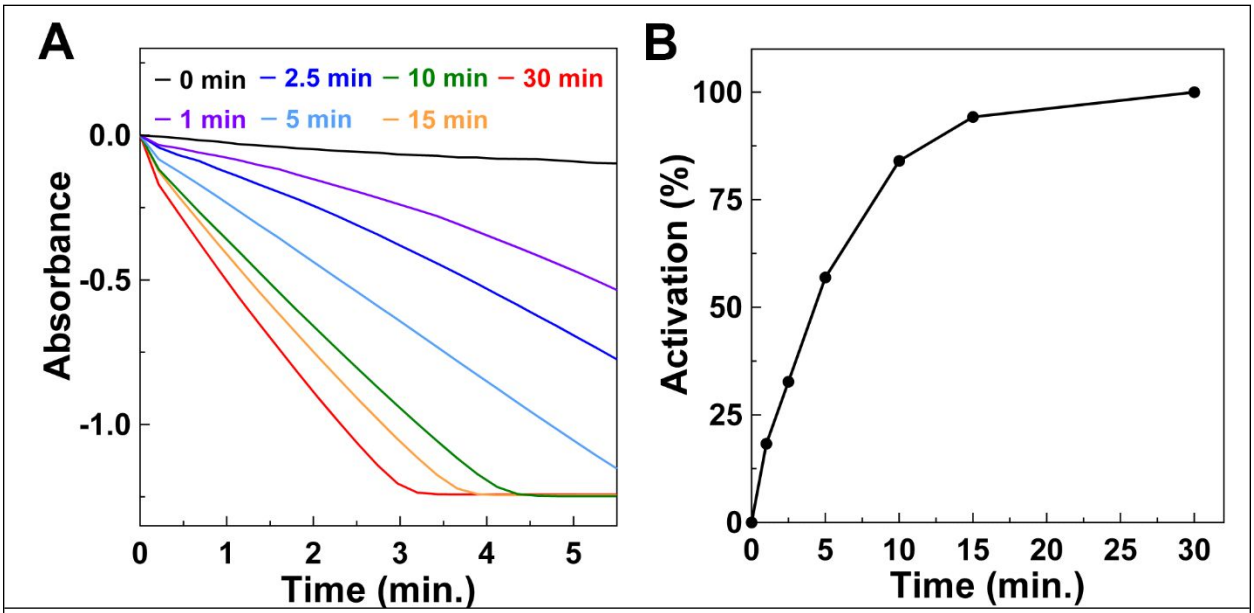

Figure S12. ***Analysis of CaM-GDH chimera activation by iNOS peptide.*** (A) Time trace of GDH activity of 1μM solution of CaM-GDH mixed with 1μM of iNOS peptide and incubated for indicated periods of time after which time the saturating amounts of glucose, electron mediator and reporter dye were added to the reaction. (B) Plot of the data from the experiment shown in (A).

Supplementary Table 1. Summary of protein sequences used in this study. The sequences are colored according to the functional elements.

FRB-wtCaM-12L-M13	MAHHHHHHSSGTRVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQ AYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISGGSGGSGSGSGGSGGTEEQIAEFKEAF SLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADGNGTIDFPEFLTMMARKMKDTDSEE EIREAFRVFDKDGNGYISAAELRHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEFVQMMTAGGS GGSGSGSGGKRRWKKNFIAVSAANRFKKISSSGAL
FRB-medCaM-12L-M13	MAHHHHHHSSGTRVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQ AYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISGGSGGSGSGSGGSGGTEEQIAEFKEAF SLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADGNGTIDFPEFLTMMARKMKDTDSEE EIREAFRVADKDGNGYISAAELRHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEAVQMMTAGGS GGSGSGSGGKRRWKKNFIAVSAANRFKKISSSGAL
FRB-lowCaM-12L-M13	MAHHHHHHSSGTRVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQ AYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISGGSGGSGSGSGGSGGTEEQIAEFKEAF SLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADGNGTIDFPEFLTMMARKMKDTDSES EIREAFRVADKDGNGYISAAEARHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEAVQMMTAGGS GGSGSGSGGKRRWKKNFIAVSAANRFKKISSSGAL
FRB-lowCaM-5L-M13	MGHHHHHHSSGTRVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQ AYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISGGSGGSGSGSGGSGGTEEQIAEFKEAF

	SLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADGNGTIDFPEFLTMMARKMKDTDSES EIREAFRVADKDGNGYISAAEARHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEAVQMMTAGGG GSKRRWKKNFIAVSAANRFKKISSSGAL
FRB-lowCaM-10L-M13	MGHHHHHHSSGTRVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQ AYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISGGSGGSGSGSGGSGGTEEQIAEFKEAF SLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADGNGTIDFPEFLTMMARKMKDTDSES EIREAFRVADKDGNGYISAAEARHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEAVQMMTAGGG GSGGGGSKRRWKKNFIAVSAANRFKKISSSGAL
FRB-lowCaM-15L-M13	MGHHHHHHSSGTRVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQ AYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISGGSGGSGSGSGGSGGTEEQIAEFKEAF SLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADGNGTIDFPEFLTMMARKMKDTDSES EIREAFRVADKDGNGYISAAEARHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEAVQMMTAGGG GSGGGGSGGGGSKRRWKKNFIAVSAANRFKKISSSGAL
FRB-lowCaM-20L-M13	MGHHHHHHSSGTRVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQ AYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISGGSGGSGSGSGGSGGTEEQIAEFKEAF SLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADGNGTIDFPEFLTMMARKMKDTDSES EIREAFRVADKDGNGYISAAEARHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEAVQMMTAGGG GSGGGGSGGGGSGGGGSKRRWKKNFIAVSAANRFKKISSSGAL
FRB-lowCaM-25L-M13	MGHHHHHHSSGTRVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQ AYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISGGSGGSGSGSGGSGGTEEQIAEFKEAF SLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADGNGTIDFPEFLTMMARKMKDTDSES EIREAFRVADKDGNGYISAAEARHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEAVQMMTAGGG GSGGGGSGGGGSGGGGSGGGGSKRRWKKNFIAVSAANRFKKISSSGAL
FRB-lowCaM-30L-M13	MGHHHHHHSSGTRVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQ AYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISGGSGGSGSGSGGSGGTEEQIAEFKEAF SLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADGNGTIDFPEFLTMMARKMKDTDSES EIREAFRVADKDGNGYISAAEARHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEAVQMMTAGGG GSGGGGSGGGGSGGGGSGGGGSGGGGSKRRWKKNFIAVSAANRFKKISSSGAL
FRB-lowCaM-40L-M13	MGHHHHHHSSGTRVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQ AYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISGGSGGSGSGSGGSGGTEEQIAEFKEAF SLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADGNGTIDFPEFLTMMARKMKDTDSES EIREAFRVADKDGNGYISAAEARHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEAVQMMTAGGG GSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSKRRWKKNFIAVSAANRFKKISSSGAL
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FRB-medCaM-M13	MAHHHHHHSSGTRVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQ AYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISGGSGGSGSGSGGSGGTEEQIAEFKEAF SLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADGNGTIDFPEFLTMMARKMKDTDSEE EIREAFRVADKDGNGYISAAELRHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEAVQMMTAGGS GGSETVRFQSGGSGGSGGSGSGSGGKRRWKKNFIAVSAANRFKKISSSGAL
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FRB-lowCaM-iNOS	MGHHHHHHSSGTRVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQ AYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISGGSGGSGSGSGGSGGTEEQIAEFKEAF SLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADGNGTIDFPEFLTMMARKMKDTDSES EIREAFRVADKDGNGYISAAEARHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEAVQMMTAGGS GGSETVRFQSGGSGGSGGSGSGSGGRREIPLKVLVKAVLFACMLMRK

FRB-lowCaM-BP2	MGHHHHHHSSGTRVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQ AYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISGGSGGSGSGSGGSGGTEEQIAEFKEAF SLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADCNGTIDFPEFLTMMARKMKDTDSES EIREAFRVADKDGNGYISAAEARHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEAVQMMTAGGS GGSETVRFQSGGSGGSGGSGSGSGGEVTVGKFYATFLIQEYFRKFKKRKEQGLVGKPS
FRB-lowCaM-BP3	MGHHHHHHSSGTRVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQ AYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISGGSGGSGSGSGGSGGTEEQIAEFKEAF SLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADCNGTIDFPEFLTMMARKMKDTDSES EIREAFRVADKDGNGYISAAEARHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEAVQMMTAGGS GGSETVRFQSGGSGGSGGSGSGSGGHMGKIYAAMMIMEYYRQSKAKK
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FRB-lowCaM-BP11	MGHHHHHHSSGTRVAILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMMERGPQTLKETSFNQ AYGRDLMEAQEWCRKYMKSGNVKDLTQAWDLYYHVFRRISGGSGGSGSGSGGSGGTEEQIAEFKEAF SLFDKDGDGTITTKELGTVMRSLGQNPTEAELQDMINEVDADCNGTIDFPEFLTMMARKMKDTDSES EIREAFRVADKDGNGYISAAEARHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEAVQMMTAGGS GGSETVRFQSGGSGGSGGSGSGSGGADKLRAACIRIQKTIRGWLLRK
TAC-lowCaM-M13 (Tacrolimus sensor activator)	DVPLIPSQFAKAKSENFDKKGSDSEVNQEAKPEVKPEVKPETHINLKVSDGSSEIFFKIKKTTPLRR LMEAFAKRQGKEMDSLRFLYDGIRIQADQTPEDLDMEDNDIIEAHREQIGGGSGSGGAHHHHHHSSG TSEPKAIDPKLSTTDRVVKAVPFPPSHRLTAKEVFDNDGKPRVDILKAHLMKEGRLEESVALRIITE GASILRQEKNLLDIDAPVTVCGDIHGQFFDLMKLFEVGGSPANTRYLFLGDYVDRGYFSIECVLYLW ALKILYPKTLFLLRGNHECRHLTEYFTFKQECKIKYSERVYDACMDAFDCLPLAALMNQQFLCVHGG LSPEINTLDDIRKLDRFKEPPAYGPMCDILWSDPLEDFGNEKTQEHFTHNTVRGCSYFYSYPAVCEF LQHNNLLSILRAHEAQDAGYRMYRKSQTTGFPSLITIFSAPNYLDVYNNKAAVLKYENNVMNIRQFN CSPHPYWLPNFMDVFTWSLPFVGEKVTEMLVNVLNICSDDELGSEEDGSGSGSGGGNEASYPLEMCS HFDADEIKRLGKRFKKLDLDNSGSLSVEEFMSLPELQQNPLVQRVIDIFDTDGNGEVDFKEFIEGVS QFSVKGDKEQKLRFAFRIYDMDKDGYISNGELFQVLKMMVGNNLKDTQLQQIVDKTIINADKDGDGR ISFEEFCAVVGGLDIHKKMVVDVGGSGGSGSGSGGSGGTEEQIAEFKEAFSLFDKDGDGTITTKELG TVMRSLGQNPTEAELQDMINEVDADGNGTIDFPEFLTMMARKMKDTDSESEIREAFRVADKDGNGYI SAAEARHVMTNLGEKLTDEEVDEMIREADIDGDGQVNYEEAVQMMTAGGSGGSETVRFQSGGSGGSG GSGSGSGGKRRWKKNFIAVSAANRFKKISSSGAL

Table S2. ***Calmodulin binding peptides used in the study***

Peptide Name	Amino acid sequence	PDB structure/reference
BP1 (M13)	H-KRRWKKNFIAVSAANRFKKISSSGAL-OH	2BBM
BP2	H-EVTVGKFYATFLIQEYFRKFKKRKEQGLVGKPS-OH	2BE6_1 2BE6_2
BP3	H-HMGKIYAAMMIMEYYRQSKAKK-OH	3DVM_1
BP4	H-HMGKIYAAMMIMEAYRQSKAKK-OH	3DVM_2
BP5	H-EMRQKIRSHAHALLAANRFMDM-OH	4AQR
BP6	H-NMGQHLDVKLVPSSSYIKVVKAFHHSSLHE-OH	2KNE
BP7	H-GRNWKNFALVPLLRD-OH	3SUI
BP8	H-LTEIKKQIPQKEWDKVVNTPNSLEKQKGVTNLLIKYGIER-OH	1K90/1K93
BP9	H-IRNKIRAIGKMARVF-OH	2R28
BP10	H-HSMQALSWRKLYLSRAKLKA-OH	4EHQ

BP11	H-ADKLRAACIRIQKTIRGWLLRK-OH	2IX7
BP12	H-MQRAAITVQRYVRGYQARCYAKFLRRTK-OH	2IX7
BP13	H-EIRKILRNNLQKTRQRLRSYNRHTLVADPYEEAWNQMLLRRQK-OH	2YGG
BP14	H-RLSFKTVALLVLACVRMKRIAFY-OH	4DS7
BP15	H-IRIQKTIRGWLLRKRYL-OH	[1]
BP16	H-IPLKVLVKAVLFACMLMRKTMASR-OH	[1]
BP17	H-RQKFRNSVERVRLNMK-OH	[1]
BP18	H-KEMVRLKKLVAYWKEQ-OH	[1]
BP19	H-RFLLERALGNRRIGQF-OH	[1]
BP20	H-RRKLKAAVKAVVASSRLGSA-OH	[1]
BP21	H-AMNWRLSARNAARRDSVLAA-OH	[1]
BP22	H-IPSLATVILVKTMIRKRSFGNPF-OH	[1]
BP23	H-AMVIQRAFRRHLLQRSLKHA-OH	[1]
FP1	H-RRTLHKAIDTVRAINKLREG-OH	[2]
FP2	H-RRTLHKAVNIVRAINKLREG-OH	[2]
N5A	H-RWKKAFIAVSAANRFKKIS-OH	[3]
iNOS	H-RREIPLKVLVKAVLFACMLMRK-OH	[4]

Supplementary References:

(1) Ikura lab. CALMODULIN TARGET DATABASE http://calcium.uhnres.utoronto.ca/ctdb/ctdb/home.html.

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