Table S1. GCaMP2 mutants produced to increase GCaMP2 monomer:dimer ratio.

GCaMP2 mutant[a]	Fluorescent colonies/CFE[b]	Less dimer than T116V[c]	$\Delta F/F_0$
T116V (gfp T203V)	Yes	-	8.4 ± 0.54
A349R (cam A46R)	No	Not tested	Not tested
T196R, Y197N (gfp T38R, Y39N)	Yes	No	Not tested
T196E, Y197N (gfp T38E, Y39N)	Yes	No	Not tested
F136E (gfp F223E)	Yes	No	Not tested
F136R (gfp F223R)	Yes	Yes	4.4 ± 0.37
G87R (gfp G174R)	Yes	Yes	8.6 ± 0.32
G87E (gfp G174E)	Yes	Yes	Not tested
R81E (gfp R168E)	Yes	Yes	6.2 ± 0.16
R389E (cam R86E)	Yes	Yes	8.8 ± 0.8
K378W (cam K75W)	Yes	Yes	8.4 ± 0.23
V89E (gfp V176E)	Yes	No	Not tested
D305R (cam D2R)	Yes	No	Not tested

[a]All GCaMP2 mutants also contain the GCaMP2 brightness-enhancing mutation T116V (gfp T203V) (L. Tian *et al.*, in preparation). For clarity, numbering of all residues according to the published EGFP, Calmodulin and M13 peptide sequence is given in parentheses. "gfp" indicates that the mutation is present in the cpEGFP domain, "cam" indicates that the mutation is present in the calmodulin domain, and "linker" indicates a mutation in the cpEGFP-CaM linker. [b]Fluorescence of colonies and CFE was visualized using a Safe Imager blue light transilluminator (Invitrogen, USA). [c]The monomer:dimer ratio was qualitatively visualized by SEC analysis (Figure S4).

Table S2. Primers used for mutagenesis of GCaMP2.

GCaMP2 mutation	Primer
T116V (gfp T203V)	FW 5'-GACAACCACTACCTGAGCGTGCAGTCCAAACTTTCGAAAG-3' RV 5'-CTTTCGAAAGTTTGGACTGCACGCTCAGGTAGTGGTTGTC-3'
A349R (cam A46R)[a]	RV 5'-GATCATGTCCTGCAGCTCGCGTTCTGTGGGGTTCTG-3'
T196R,Y197N (gfp T38R,Y39N)	RV 5'-CTTCAGGGTCAGCTTGCCGTTGCGGGCATCGCCCTCACC-3'
T196E,Y197N (gfp T38E,Y39N)	RV 5'-CTTCAGGGTCAGCTTGCCGTTCTCGGCATCGCCCTCACC-3'
F136E (gfp F223E)	RV 5'-CCCGGCGGCGGTCACCTCCTCCAGCAGGACCATGTG-3'
F136R (gfp F223R)	FW 5'-CACATGGTCCTGCTGGAGCGGGTGACCGCCGCCGGG-3' RV 5'-CCCGGCGGCGGTCACGCGCTCCAGCAGGACCATGTG-3'
G87R (gfp G174R)	FW 5'CACAACATCGAGGACCGCGGCGTGCAGCTCGCC-3' RV 5'-GGCGAGCTGCACGCCGCGGTCCTCGATGTTGTG-3'
G87E (gfpG174E)	RV 5'-GGCGAGCTGCACGCCCTCGTCCTCGATGTTGTG-3'
R81E (gfpR168E)	FW 5'-CAAGGCGAACTTCAAGATCGAACACAACATCGAGGACGGC-3' RV 5'-GCCGTCCTCGATGTTGTGTTCGATCTTGAAGTTCGCCTTG-3'
R389E (cam R86E)	FW 5'-GACAGTGAAGAAGAAATTGAGGAAGCCTTCCGCGTGTTTGATAAGGATGGC-3' RV 5'GCCATCCTTATCAAACACGCGGAAGGCTTCCTCAATTTCTTCTTCACTGTC-3'
K378W (cam K75W)	FW 5'-ACAATGATGGCAAGATGGATGAAAGACACAGACAGT-3' RV 5'-ACTGTCTGTGTCTTTCATCCATCTTGCCATCATTGT-3'
V89E (gfpV176E)	RV 5'-GTAGTGGTAGGCGAGCTGCTCGCCGCCGTCCTCGAT-3'
D305R(cam D2R)	RV 5'-CTGCTCTTCAGTCAGTTGGCGACGCGTGTTGTACTC-3'
T303W (linker)	FW 5'-CACAAGCTGGAGTACAACTGGCGTGACCAACTGACTGAAG-3' RV 5'-CTTCAGTCAGTTGGTCACGCCAGTTGTACTCCAGCTTGTG-3'
T303Y (linker)	FW 5'-CACAAGCTGGAGTACAACTATCGTGACCAACTGACTGAAG-3' RV 5'-CTTCAGTCAGTTGGTCACGATAGTTGTACTCCAGCTTGTG-3'
T303R (linker)	FW 5'-CACAAGCTGGAGTACAACCGCCGTGACCAACTGACTGAAG-3' RV 5'-CTTCAGTCAGTTGGTCACGGCGGTTGTACTCCAGCTTGTG-3'
D381W (cam D78W)	FW 5'-GATGGCAAGAAAAATGAAATGGACAGACAGTGAAGAAGAAATTAG-3' RV 5'-CTAATTTCTTCTTCACTGTCTGTCCATTTCATTTTTCTTGCCATC-3'
D381Y (cam D78Y)	FW 5'-GATGGCAAGAAAAATGAAATACACAGACAGTGAAGAAGAAATTAG-3' RV 5'-CTAATTTCTTCTTCACTGTCTGTGTATTTCATTTTTCTTGCCATC-3'
D381R (cam D78R)	FW 5'-GATGGCAAGAAAAATGAAACGCACAGACAGTGAAGAAGAAATTAG-3' RV 5'-CTAATTTCTTCTTCACTGTCTGTGCGTTTCATTTTTCTTGCCATC-3'
R377W (cam R74W)	FW 5'-GTTCCTGACAATGATGGCATGGAAAATGAAAGACACAGAC-3' RV 5'-GTCTGTGTCTTTCATTTTCCATGCCATCATTGTCAGGAAC-3'
R377Y (cam R74Y)	FW 5'-GTTCCTGACAATGATGGCATACAAAATGAAAGACACAGAC-3' RV 5'-GTCTGTGTCTTTCATTTTGTATGCCATCATTGTCAGGAAC-3'
K380W (cam K78W)	FW 5'-CAATGATGGCAAGAAAAATGTGGGACACAGACAGTGAAGAAG-3' RV 5'-CTTCTTCACTGTCTGTGTCCCACATTTTTCTTGCCATCATTG-3'
K380Y (cam K78Y)	FW 5'-CAATGATGGCAAGAAAAATGTACGACACAGACAGTGAAGAAG-3' RV 5'-CTTCTTCACTGTCTGTGTCGTACATTTTTCTTGCCATCATTG-3'
R81E (gfp R168E)	FW 5'-CAAGGCGAACTTCAAGATCGAACACAACATCGAGGACGGC-3' RV 5'-GCCGTCCTCGATGTTGTGTTCGATCTTGAAGTTCGCCTTG-3'
R81A (gfp R168A)	FW 5'-CAAGGCGAACTTCAAGATCGCCCACAACATCGAGGACGGC-3' RV 5'-GCCGTCCTCGATGTTGTGGGCGATCTTGAAGTTCGCCTTG-3'
R81S (gfp R168S)	FW 5'-CAAGGCGAACTTCAAGATCAGCCACAACATCGAGGACGGC-3' RV 5'-GCCGTCCTCGATGTTGTGGCTGATCTTGAAGTTCGCCTTG-3'
A140W (gfp A227W)	FW 5'-CTGGAGTTCGTGACCGCCTGGGGGATCACTCTCGGCATG-3' RV 5'-CATGCCGAGAGTGATCCCCCAGGCGGTCACGAACTCCAG-3'
V219R (gfp V62R)	FW 5'-GTGCCCTGGCCCACCCTCCGCACCACCCTGACCTACGG-3' RV 5'-CCGTAGGTCAGGGTGGTGCGGAGGGTGGGCCAGGGCAC-3'
V219M (gfp V62M)	FW 5'-GTGCCCTGGCCCACCCTCATGACCACCCTGACCTACGG-3' RV 5'-CCGTAGGTCAGGGTGGTCATGAGGGTGGGCCAGGGCAC-3'
L120R (gfp L207R)	FW 5'-CTGAGCGTGCAGTCCAAACGCTCGAAAGACCCCAACGAG-3' RV 5'-CTCGTTGGGGTCTTTCGAGCGTTTGGACTGCACGCTCA-3'
L120Y (gfp L120Y)	FW 5'-CTGAGCGTGCAGTCCAAATACTCGAAAGACCCCAACGAG-3' RV 5'-CTCGTTGGGGTCTTTCGAGTATTTGGACTGCACGCTCAG-3'

8-fold EF-hand[b]	
E334Q,T329G	FW 5'-GACAAGGACGGGGATGGGGGCATAACAACCAAGCAGCTG-3' RV 5'-CAGCTGCTTGGTTGTTATGCCCCCATCCCCGTCCTTGTC-3'
D359G	FW 5'-GACATGATCAATGAAGTAGGCGCCGACGGTAATGGCAC-3' RV 5-GTGCCATTACCGTCGGCGCCTACTTCATTGATCATGTC-3'
E370Q	FW 5'-GGCACAATCGACTTCCCTCAGTTCCTGACAATGATGGC-3' RV 5'-GCCATCATTGTCAGGAACTGAGGGAAGTCGATTGTGCC-3'
D396G	FW 5'-GAAGCGTTCCGTGTGTTTGGCAAGGATGGCAATGGCTAC-3' RV 5'-GTAGCCATTGCCATCCTTGCCAAACACACGGAACGCTTC-3'
E407Q	FW 5'-GGCTACATCAGTGCAGCACAGCTTCGCCACGTGATGAC-3' RV 5'-GTCATCACGTGGCGAAGCTGTGCTGCACTGATGTAGCC-3'
D432G	FW 5'-GAAATGATCAGGGAAGCAGGCATCGATGGGGATGGTCAG-3' RV 5'-CTGACCATCCCCATCGATGCCTGCTTCCCTGATCATTTC-3'
E443Q	FW 5'-GGTCAGGTAAACTACGAACAGTTTGTACAAATGATGACA-3' RV 5'-CTGTCATCATTTGTACAAACTGTTCGTAGTTTACCTGACC-3'

Table S3. GCaMP2 mutants for GECI analysis

GCaMP2 variant[a]	$\Delta F/F_0$	Optimal $\lambda_{ex}/\lambda_{em}$	Absorbance Apo[a] $\lambda_{prot}/\lambda_{deprot}$	Absorbance Sat[a] $\lambda_{prot}/\lambda_{deprot}$
GCaMP2	4.5 ± 0.43	488/512	404/490	N.D.[b]/488
T116V (gfp T203V)	8.4 ± 0.54	498/515	403/501	399/498
Solvent access mutations				
T303W (linker)	4.2 ± 0.28	499/514	398/499	396/499
T303Y (linker)	11.0 ± 0.98	498/515	401/503	399/498
T303R (linker)	3.2 ± 0.41	498/515	400/502	400/498
D381W (cam D78W)	7.9 ± 0.78	498/514	401/503	400/498
D381Y (cam D78Y)	11.1 ± 0.69	498/515	401/503	401/498
D381R (cam D78R)	5.8 ± 0.39	498/515	401/503	399/498
R377W (cam R74W)	7.8 ± 1.26	498/515	400/501	396/499
R377Y (cam R74Y)	8.4 ± 0.62	498/515	403/502	399/499
K380W (cam K78W)	5.6 ± 0.57	499/515	403/501	396/499
K380Y (cam K78Y)	6.4 ± 0.08	498/515	403/502	395/499
Interface mutations				
R81E (gfp R168E)	6.5 ± 0.24	498/514	400/501	395/499
R81A (gfp R168A)	6.6 ± 0.67	499/514	401/501	395/499
R81S (gfp R168S)	9.0 ± 0.27	498/515	400/500	398/499
A140W (gfp A227W)	3.7 ± 0.05	498/514	401/503	400/498
Inner barrel mutations				
V219R (gfp V62R)	5.4 ± 0.30	490/510	399/492	399/492
V219M (gfp V62M)	8.5 ± 0.46	503/516	400/500	395/500
L120R (gfp L207R)	2.6 ± 0.15	495/514	396/504	396/498
L120Y (gfp L120Y)	6.5 ± 0.40	498/514	400/500	396/497

[a]All GCaMP2 mutants also contain the GCaMP2 brightness-enhancing mutation T116V (gfp T203V) (L. Tian *et al*., in preparation) as described in table 2. [a]Absorbance measured in presence and absence of calcium. Peak wavelengths for both protonated and deprotonated state are given. [b]Not determined due to peak convolution.