

Supplementary Information

Development of green fluorescent protein-based cAMP indicators for covering a wide range of cAMP concentrations

Sohei Hiasa^a, Takeru Fujimori^a, Saki Aiki^b, Hiroshi Ueda^c, Takashi Tsuboi^{b*}, Tetsuya Kitaguchi^{c*}

^aSchool of Life Science and Technology, Department of Life Science and Technology, Tokyo Institute of Technology 4259 Nagatsuta-cho, Midori-ku, Yokohama-shi, Kanagawa, 226-8501, Japan

^bDepartment of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902, Japan

^cLaboratory for Chemistry and Life Science, Institute of Innovative Research, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama-shi, Kanagawa, 226-8503, Japan,

* E-mail: kitaguct-gfp@umin.ac.jp, takatsuboi@bio.c.u-tokyo.ac.jp

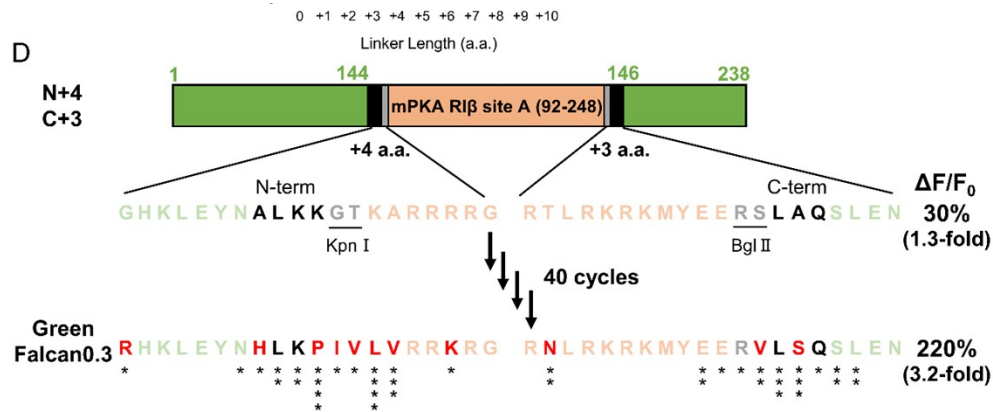


Table S1. Comparison of Single-FP based fluorescent cAMP indicator

Sensor name	Type	Ex/Em (nm)	Dynamic range (fold)	EC ₅₀ (μM)	Reference
Flamindo	Turn-off	506/521	2.0	2.1	11
Flamindo2	Turn-off	504/523	4.0	3.2	12
Pink Flamindo	Turn-on	567/590	4.2	7.2	13
cADDiS	Turn-off	488/525*	1.4	NA	15
	Turn-on	488/525*	1.3	NA	
cAMPr	Turn-on	488/509	1.7	NA	16
gCarvi	Turn-on	504/523	2.0	1.5	17
G-Flamp1	Turn-on	490/510	13	2.2	18

* Wavelength used in the measurement

NA: Not applicable



Supplementary Figures

Figure S1. Screening by the crude Green Falcan protein (A-C) Diagram for (A) GFP, mouse cAMP-dependent protein kinase (PKA) type I-beta regulatory subunit (mPKA RIβ), and the indicators under development, and the screening results of various constructs with modified linker lengths at (B) the C-terminus and (C) the N-terminus. Responses to 100 μM cAMP are shown as ΔF/F₀. (D) The amino acid

sequences around the linker and introduced point mutations. Asterisks indicate where random mutations were performed, and red letters indicate mutated amino acids.

A Green Falcan0.3

1	ATGGCCGAGGAAAAGCGACAGCGTGGATTCTGCGGATGCAGAGGAGGATGACTCTGATGTC	60
61	M A E E S D S V D S A D A E E D D S D V	120
121	TGGTGGGGTGGAGCGGACACAGACTACGCTGATGGCGGTGAAGACAAAGTAGTAGAAGTC	180
181	W W G G A D T D Y A D G G E D K V V E V	240
241	GCCGAAGAGGAGGAAGTGGCTGATGTTGAGGAAGAGGAAGCTGATGATGATGAGGATGTG	300
301	A E E E E V A D V E E E A D D D E D V	360
361	GAGGATGGGGACGAGGTGGAGGAGGAGGCCGAGGAGCCCTACGAAGAGGCCACCGAGAGA	420
421	E D D G D E V E E E A E E P Y E E A T E R	480
481	ACAAACGAGCACTGCCACCACCACCACAACCACCACCTGAGTCCGTGGAGGAGGTGGGATCC	540
541	T T S T A T T T T T T E S V E E V G S	600
601	ATGGTGAGCAAGGGCGAGGAGCTGTTTACCGGGGTGGTGCCCATCCAGGTCGAGCTGGAC	660
661	M V S K G E E L F T G V V P I Q V E L D	720
721	GGCGACGTAAACGGCCACAAGTTCAAGCTGCTCGGCGAGGGTGAGGGCGATGCCACCTAC	780
781	G D V N G H K F S V S G E G E G D A T Y	840
841	GGCAAGCTGACCCTGAAGTTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCACC	900
901	G K L T L K F I C T T G K L P V P W P T	960
961	CTCGTGACCACTGACCTACGGCGTGACGTCTTACGCCGCTACCCCGACCATGAAG	1020
1021	L V T T L T Y G V Q C F S R Y P D H M K	1080
1081	CAGCAGCACTTCTTCAAGTCCGCCATGCCCGAAGGCTACATCCAGGAGCGCACCATCTTC	1140
1141	Q H D F F K S A M P E G Y I Q E R T I F	1200
1201	TTCAAGGACGACCAACTACAAGACCCGCGCGAGGTGAAGTTTCGAGGGCGACACCTTG	1260
1261	F K D D G N Y K T R A E V K F E G D T L	1320
1321	GTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGCGGCAC	1380
1381	V N R I E L K G I D F K E D G N I L R H	1440
1441	AAGCTGGAGTACAACCATTTAAAGCCTATTGTTTTGGTGCGTCGCAAGCGGGCGGGTGTG	1500
1501	K L E Y N H L K P I V L V R R K R G G V	1521
	AGTGCTGAAGTCTACACTGAAGAAGATGCTGTCTCTACGTGAGGAAGGTCAATCCCAAG	
	S A E V Y T E E D A V S Y V R K V I P K	
	GACTATAAGACCATGACCGCTGGCCAAAGGCCATTTCTAAGAACGTGCTCTTTTCTCAC	
	D Y K T M T A L A K A I S K N V L F S H	
	CTGGACGACAAACGAGAGAAGTGACATATTTGACGCCATGTTTCTGTCACTCACATCGGT	
	L D D N E R S D I F D A M F P V T H I G	
	GGGGAACAGTCATACAGCAAGGGAATGAAGGAGATAATTTCTATGTGATTGACCAAGGA	
	G E T V I Q Q G N E G D N F Y V I D Q G	
	GAAGTAGATGTATATGTGAACGGGGGAATGGGTGACCAACATCAGTGAGGGGGGAAGTTTC	
	E V D V Y V N G E W V T N I S E G G S F	
	GGGAGCTGGCTCTCATCTACGGCACCCCGAGAGCGGCTACCGTGAAAGGCCAAGACGGAC	
	G E L A L I Y G T P R A A T V K A K T D	
	CTCAAGCTCTGGGGTATCGACCGTGACAGCTACAGGCGCATCCTCATGGGACGCAATCTG	
	L K L W G I D R D S Y R R I L M G R N L	
	AGGAAACGCAAGATGTATGAGGAGAGAGTGTATCTCAGTCACTGGAGAACGTCTATATC	
	R K R K M Y E E R V L S Q S L E N V Y I	
	AATGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCACAACATCGAG	
	N A D K Q K N G I K A N F K I R H N I E	
	GACGGCGGCGTGACGCTCGCCTACCACTACCAGCAGAACACCCCATCGGCGACGGCCCC	
	D G G V Q L A Y H Y Q Q N T P I G D G F	
	GTGCTGCTGCCGACAACCACTACCTGAGCGTGACGTCCATACTTTTCGAAAGACCCCAAC	
	V L L P D N H Y L S V Q S I L S K D P N	
	GAGAAAGCGCGATCACATGGTCTCTGCTGGAGTTCTGTGACCGCGCGGGGATCACTCTCGGC	
	E K R D H M V L L E F V T A A G I T L G	
	ATGGACGAGCTGTACAAGTAA	
	M D E L Y K *	

B Green Falcan1

1	ATGGCCGAGGAAAGCGACAGCGTGGATTCTGCGGATGCAGAGGAGGATGACTCTGATGTC	60
61	M A E E S D S V D S A D A E E D D S D V	120
121	TGGTGGGGTGGAGCGGACACAGACTACGCTGATGGCGGTGAAGACAAAGTAGTAGAAGTC	180
181	W W G G A D T D Y A D G G E D K V V E V	240
241	GCCGAAGAGGAGGAAGTGGCTGATGTTGAGGAAGAGGAAGCTGATGATGATGAGGATGTG	300
301	A E E E E V A D V E E E E A D D E D V	360
361	GAGGATGGGGACGAGGTGGAGGAGGAGGCCGAGGAGCCCTACGAAGAGGCCACCGAGAGA	420
421	E D G D E V E E A E E P Y E E A T E R	480
481	ACAACCAGCACTGCCACCACCACCAACCACCACTGAGTCCGTGGAGGAGGTGGGATCC	540
541	T T S T A T T T T T T T E S V E E V G S	600
601	ATGGTGAGCAAGGGCGAGGAGCTGTTTACCGGGGTGGTGCCCATCCAGGTCGAGCTGGAC	660
661	M V S K G E E L F T G V V P I Q V E L D	720
721	GGCGACGTAAACGGCCACAAGTTTCAGCGTGTCCGGCGAGGGTGAGGGCGATGCCACCTAC	780
781	G D V N G H K F S V S G E G E G D A T Y	840
841	GGGAGCTGACCCTGAAGTTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCCAC	900
901	G K L T L K F I C T T G K L P V P W P T	960
961	CTCGTGACACCTGACCTACGGCGTGCAGTCTTCAGCCGCTACCCGACACATGAAAG	1020
1021	L V T T L T Y G V Q C F S R Y P D H M K	1080
1081	CAGCACGACTTCTTCAAGTCCGCCATGCCGAAGGCTACATCCAGGAGCGCACCATCTTC	1140
1141	Q H D F F K S A M P E G Y I Q E R T I F	1200
1201	TTCAAGGACGACGGCAACTACAAGACCCGCGCGAGGTGAAGTTTCGAGGGCGACACCTG	1260
1261	F K D D G N Y K T R A E V K F E G D A T L	1320
1321	GTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGCGGCAC	1380
1381	V N R I E L K G I D F K E D G N I L R H	1440
1441	AAGCTGGAGTACAACCATTTAAGCCTATTGTTTGGTGCGTCGCAAGCGGGCGGTGTG	1500
1501	K L E Y N H L K P I V L V R R K R G G V	1521
	AGTCTGAAGTCTACACTGAAGAAGATGCTGTCTCCTACGTGAGGAAGGTCAATCCCAAG	
	S A E V Y T E E D A V S Y V R K V I P K	
	GACTATAAGACCATGACCGCGCTGGCCAAAGGCCATTTCTAAGAACGTGCTCTTTTCTCAC	
	D Y K T M T A L A K A I S K N V L F S H	
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	L D D N E R S D I F D A M F P V T H I G	
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	G E T V I Q Q G N E G D N F Y V I D Q G	
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	E V D V Y V N G E W V T N I S E G G S F	
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	G E L A L I Y G T P R H A T V K A K T D	
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	R K R K M Y E E R V L S Q S L E N V Y I	
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	N A D K Q K N G I K A N F K I R H N I E	
	GACGGCGGGCGTGCAGCTCGCCTACCACTACCAGCAGAACACCCCATCGGCGACGGCCCC	
	D G G V Q L A Y H Y Q Q N T P I G D G P	
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	V L L P D N H Y L S V Q S I L S K D P N	
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	E K R D H M V L L E F V T A A G I T L G	
	ATGGACGAGCTGTACAAGTAA	
	M D E L Y K *	

C

Green Falcan3

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1 ATGGCCGAGGAAAGCGACAGCGTGGATTCTGCGGATGCGAGGAGGATGACTCTGATGTC 60
  M A E E S D S V D S A D A E E D D S D V
61 TGGTGGGGTGGAGCGGACACAGACTACGCTGATGGCGGTGAAGACAAAGTAGTAGAAGTC 120
  W W G G A D T D Y A D G G E D K V V E V
121 GCCGAAGAGGAGGAAGTGGCTGATGTTGAGGAAGAGGAAGCTGATGATGATGAGGATGTG 180
  A E E E E V A D V E E E E A D D D E D V
181 GAGGATGGGGACGAGGTGGAGGAGGAGGCCGAGGAGCCCTACGAAGAGGCCACCGAGAGA 240
  E D G D E V E E E A E E P Y E E A T E R
241 ACAACCAGCACTGCCACCACCACCACAACCACCCTGAGTCCGTGGAGGAGGTGGGATCC 300
  T T S T A T T T T T T T E S V E E V G S
301 ATGGTGAAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCAGGTCGAGCTGGAC 360
  M V S K G E E L F T G V V P I Q V E L D
361 GCGCAGCTAAACGGCCACAAGTTTCAGCGTGTGCGCGAGGGTGAGGGCGATGCCACCTAC 420
  G D V N G H K F S V S G E G E G D A T Y
421 GGCACGCTGACCCTGAAGTTTCATCTGCACCACCAAGGCAAGCTGCCCGTGCCTGGCCACC 480
  G K L T L K F I C T T G K L P V P W P T
481 CTCGTGACCACCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCGACCACATGAAG 540
  L V T T L T Y G V Q C F S R Y P D H M K
541 CAGCAGCACTTCTTCAAGTCCGCCATGCCCCGAAGGCTACATCCAGGAGCGCACCATCTTC 600
  Q H D F F K S A M P E G Y I Q E R T I F
601 TTCAAGGACGACGGCAACTACAAGACC CGCGCGAGGTGAAGTTTCGAGGGCGACACCTTG 660
  F K D D G N Y K T R A E V K F E G D A T
661 GTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGCGGCAC 720
  V N R I E L K G I D F K E D G N I L R H
721 AAGTGGAGTACAACCATTAAAGCCTATTGTTTTGGTGCGTCGCAAGCGGGCGGTGTG 780
  K L E Y N H L K P I V L V R R K R G G V
781 AGTGCTGAAGTCTACACTGAAGAAGATGCTGTCTCTCAGTGAGGAAGGTCAATCCCAAG 840
  S A E V Y T E E D A V T S Y V R K V I P K
841 GACTATAAGACCATGACCGCGCTGGCCAAAGGCCATTTCTAAGAACGTGCTCTTTTCTCAC 900
  D Y K T M T A L A K A I S K N V L F S H
901 CTGGACGACAACGAGAGAAGTGACATATTTGACGCCATGTTTCTGTCTCACTCACATCGGT 960
  L D D N E R S D I F D A M F P V T H I G
961 GGGAAACAGTCATACAGCAAGGGGAATGAAGGAGATAATTTCTATGTGATTGACCAAGGA 1020
  G E T V I Q Q G N E G D N F Y V I D Q G
1021 GAAGTAGATGTATGTGAACGGGGAATGGGTGACCAACATCAGTGAGGGGGGAAGTTTC 1080
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1261 AATGCCGACAAGCAGAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCACAACATCGAG 1320
  N A D K Q K N G I K A N F K I R H N I E
1321 GACGGCGGCTGCACTCGCCTACCACCTACCAGCAGAACACCCCATCGGCGACGGCCCC 1380
  D G G V Q L A Y H Y Q Q N T P I G D G P
1381 GTGCTGCTGCCCGACAACCACTACCTGAGCGTGAGTCCATACTTTTGAAAGACCCCAAC 1440
  V L L P D N H Y L S V Q S I L S K D P N
1441 GAGAAAGCGGATCACATGGTCCTGCTGGAGTTCTGTGACCGCGCGGGATCACTCTCGGC 1500
  E K R D H M V L L E F V T A A G I T L G
1501 ATGGACGAGCTGTACAAGTAA 1521
  M D E L Y K *
```

D		Green Falcan10	
1	ATGGCCGAGGAAAGCGACAGCGTGGATTCTGCGGATGCAGAGGAGGATGACTCTGATGTC	60	
61	M A E E S D S V D S A D A E E D D S D V	120	
	TGGTGGGGTGGAGCGGACACAGACTACGCTGATGGCGGTGAAGACAAAGTAGTAGAAGTC		120
	W W G G A D T D Y A D G G E D K V V E V		
121	GCCGAAGAGGAGGAAGTGGCTGATGTTGAGGAAGAGGAAGCTGATGATGATGAGGATGTG	180	
	A E E E V A D V E E E E A D D D E D V		
181	GAGGATGGGGACGAGGTGGAGGAGGAGGCCGAGGAGCCCTACGAAGAGGCCACCGAGAGA	240	
	E D G D E V E E E A E E P Y E E A T E R		
241	ACAACCAGCACTGCCACCACCACCACCAACCACCCTGAGTCCGTGGAGGAGGTGGGATCC	300	
	T T S T A T T T T T T T E S V E E V G S		
301	ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCAGGTTCGAGCTGGAC	360	
	M V S K G E E L F T G V V P I Q V E L D		
361	GGCGACGTAAACGGCCACAAGTTTCAGCTGTCTCCGGCAGGGGTGAGGGCGATGCCACCTAC	420	
	G D V N G H K F S V S G E G E G D A T Y		
421	GGCAAGCTGACCTGAAGTTTCATCTGACCAACCGGCAAGCTGCCCGTGCCCTGGCCACC	480	
	G K L T L K F I C T T G K L P V P W P T		
481	CTCGTGACCACTTGACCTACGGCGTGCACTGCTTTCAGCCGCTACCCGACCACATGAAG	540	
	L V T T L T Y G V Q C F S R Y P D H M K		
541	CAGCAGCACTTCTTCAAGTCCGCCATGCCCCGAAGGCTACATCCAGGAGCGCATCTTC	600	
	Q H D G F F K S A M P E G Y I Q E R T I F		
601	TTCAAGGACGAGCGCAACTACAAGACCCGCGCCGAGGTGAAGTTTCGAGGGCGACACCTTG	660	
	F K D D G N Y K T R A E V K F E G D T Y		
661	GTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGCGGCAC	720	
	V N R I E L K G I D F K E D G N I L R H		
721	AAGCTGGAGTACAACCATTTAAAGCCTATTGTTTTGGTGCGTCGCAAGCGGGCGGTGTG	780	
	K L E Y N H L K P I V L V R R K R G G V		
781	AGTGCTGAAGTCTACACTGAAGAAGATGCTGTCTCTCTACGTGAGGAAGGTCTCCCAAG	840	
	S A E V Y T E E D A V S Y V R K V I P K		
841	GACTATAAGACCATGACCGCGCTGGCCAAAGGCCATTTCTAAGAACGTGCTCTTTCTC	900	
	D Y K T M T A L A K A I S K N V L F S H		
901	CTGGACGACAACGAGAGAAAGTGACATATTTGACGCCATGTTTCTGTCACTCACATCGGT	960	
	L D D N E R S D I F D A M F P V T H I G		
961	GGGAAACAGTCATACAGCAAGGGAATGAAGGAGATAATTTCTATGTGATTGACCAAGGA	1020	
	G E T V I Q Q G N E G D N F Y V I D Q G		
1021	GAAAGTAGATGTATATGTGAACGGGGAAATGGGTGACCAACATCAGTGAGGGGGGAAGTTTC	1080	
	E A D V Y V N G E W V T N I S E G G S F		
1081	GGGAGCTGGCTCTCATCTACGGCACCCCGACATTCTACCGTGAAGGCCAAGACGGAC	1140	
	G E L A L I Y G T P R H S T V K A K T D		
1141	CTCAAGCTCTGGGGTATCGACCGTGACAGCTACAGGCGCATCCTCATGGGACGCAATCTG	1200	
	L K L W G I D R D S Y R R I L M G R N L		
1201	AGGAAACGCAAGATGTATGAGGAGAGAGTGTATCTCAGTCACTGGAGAACGTCTATATC	1260	
	R K R K M Y E E R V L S Q S L E N V Y I		
1261	AATGCCGACAAGCAGAAAGAACGGCATCAAGGCGAACTTCAAGATCCGCCACAACATCGAG	1320	
	N A D K Q K N G I K A N F K I R H N I E		
1321	GACGGCGGGCTGCAGCTCGCCTACCACTACCAGCAGAACACCCCATCGGCGACGGCCCC	1380	
	D G G V Q L A Y H Y Q Q N T P I G D G P		
1381	GTGCTGGTGGCCGACAACCACTACCTGAGCGGTGCAAGTCCATACTTTTCGAAAGACCCCAAC	1440	
	V L L P D N H Y L S V Q S I L S K D P N		
1441	GAGAAGCGCGATCACAATGGTCTGCTGGAGTTCTGTGACCGCGCGGGATCACTCTCGGC	1500	
	E K R D H M V L L E F V T A A G I T L G		
1501	ATGGACGAGCTGTACAAGTAA	1521	
	M D E L Y K *		

Figure S2. DNA and amino acid sequences of Green Falcans (A-D) DNA and amino acid sequences of Green Falcan0.3 (A), 1 (B), 3 (C), 10 (D). Blue, green, orange, black and gray letters correspond to amino acid from APP, GFP, PKA, linker and restriction enzyme site, respectively. Asterisks showed stop codon.

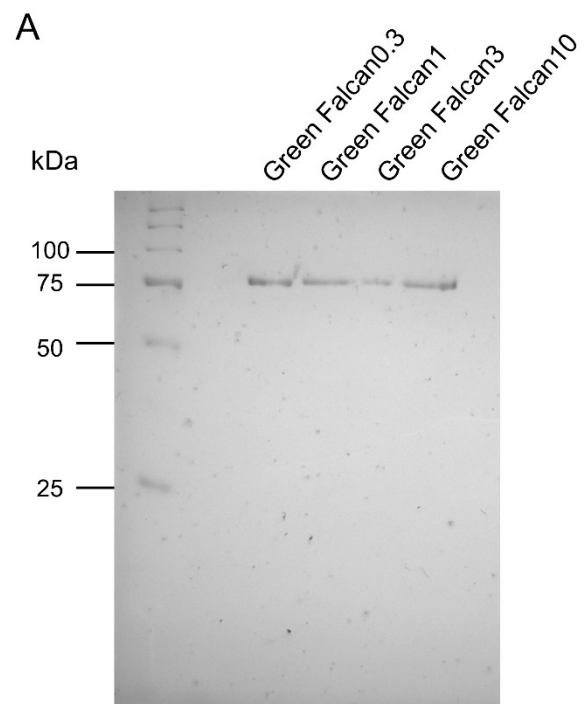


Figure S3. SDS-PAGE for purified Green Falcans protein (A) The image of SDS-PAGE for purified Green Falcans protein (1 μ g).

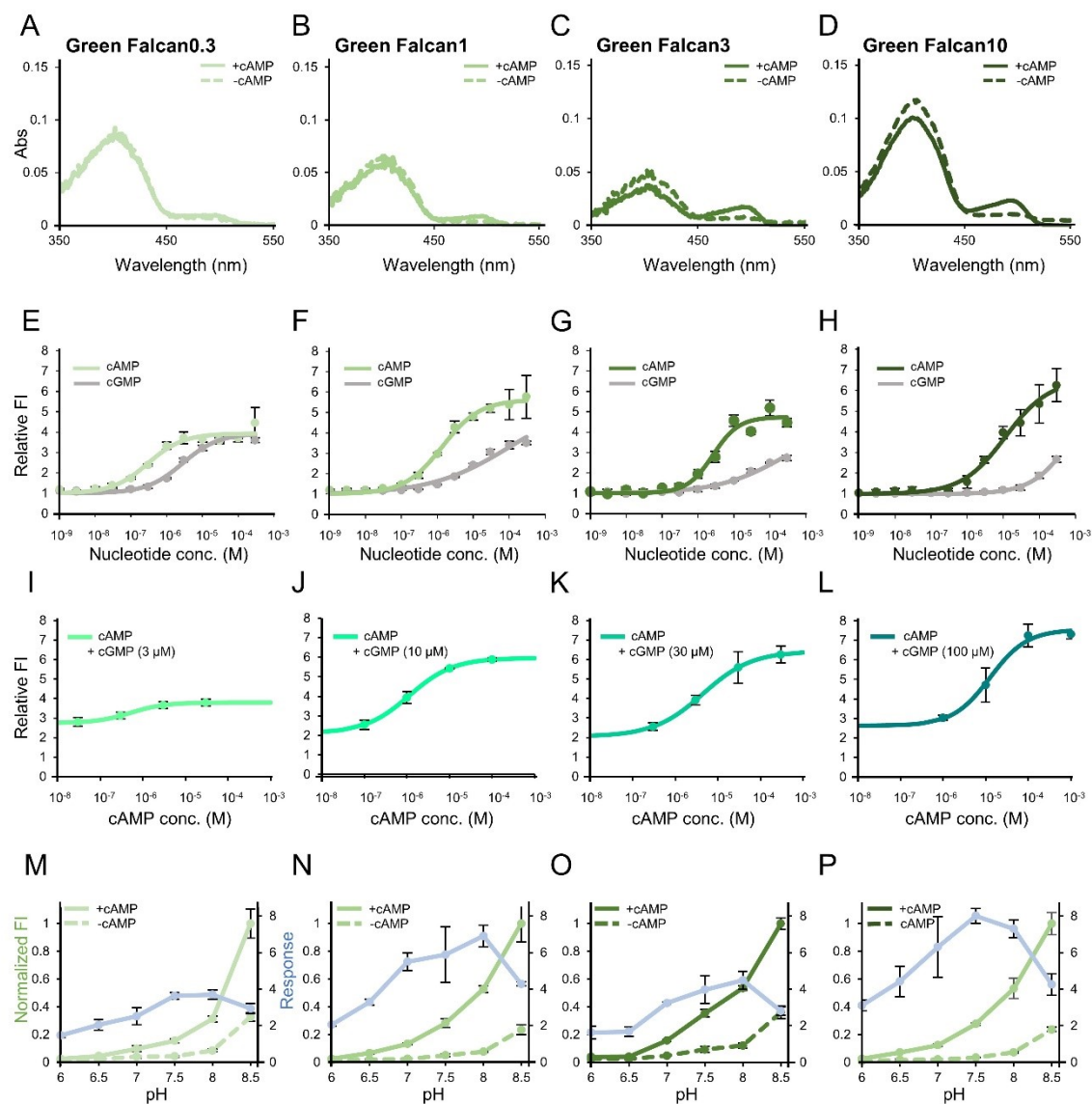
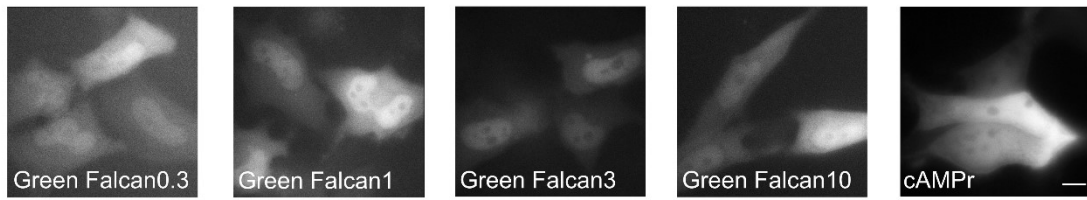


Figure S4. Property evaluation of Green Falcans (A-D) Absorption spectra of purified Green Falcans 0.3, 1, 3, and 10 in the presence (solid line) and absence (dashed line) of 100 μ M cAMP. (E-H) Dose-response curves of purified Green Falcans 0.3, 1, 3, and 10 for cAMP (green line) and cGMP (gray line). The FI was measured with excitation at 480 nm. The normalized FI was calculated by dividing by the FI of the peak in the absence of cAMP, and the minimum FI in fitting was normalized to 1. The data represent the means \pm standard deviation ($n=3$). (I-L) Dose-response curves of purified Green Falcans 0.3, 1, 3, and 10 for cAMP in the presence of cGMP (10-times of the EC_{50} value for cAMP). The FI was measured with excitation at 480 nm. The Relative FI was calculated by dividing by the FI of the peak before the addition of cAMP.

The data represent the means \pm standard deviation (n=3). (M-P) pH sensitivity of purified Green Falcen0.3, 1, 3, 10. The FI was measured with excitation at 480 nm. The normalized FI was calculated by dividing the FI in all conditions by the FI of each Green Falcen after the addition of cAMP in pH 8.5. Response was calculated by dividing the FI with cAMP by the FI without cAMP for each pH condition. The data represent the means \pm standard deviation (n=3).

A



B

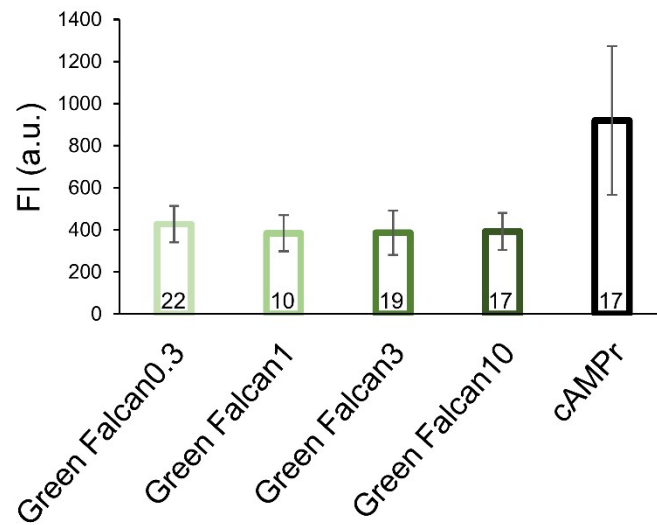


Figure S5. Basal fluorescence intensity of Green Falcans and cAMPr (A) Image of Green Falcans and cAMPr expressing HeLa cells. Scale bar represents 10 μ m. (B) Comparison of basal fluorescence intensity. The data represent the means \pm standard deviation. The numbers in bar graphs represent the number of cells showed 1.5 times higher than background, analyzed from three independent experiments.

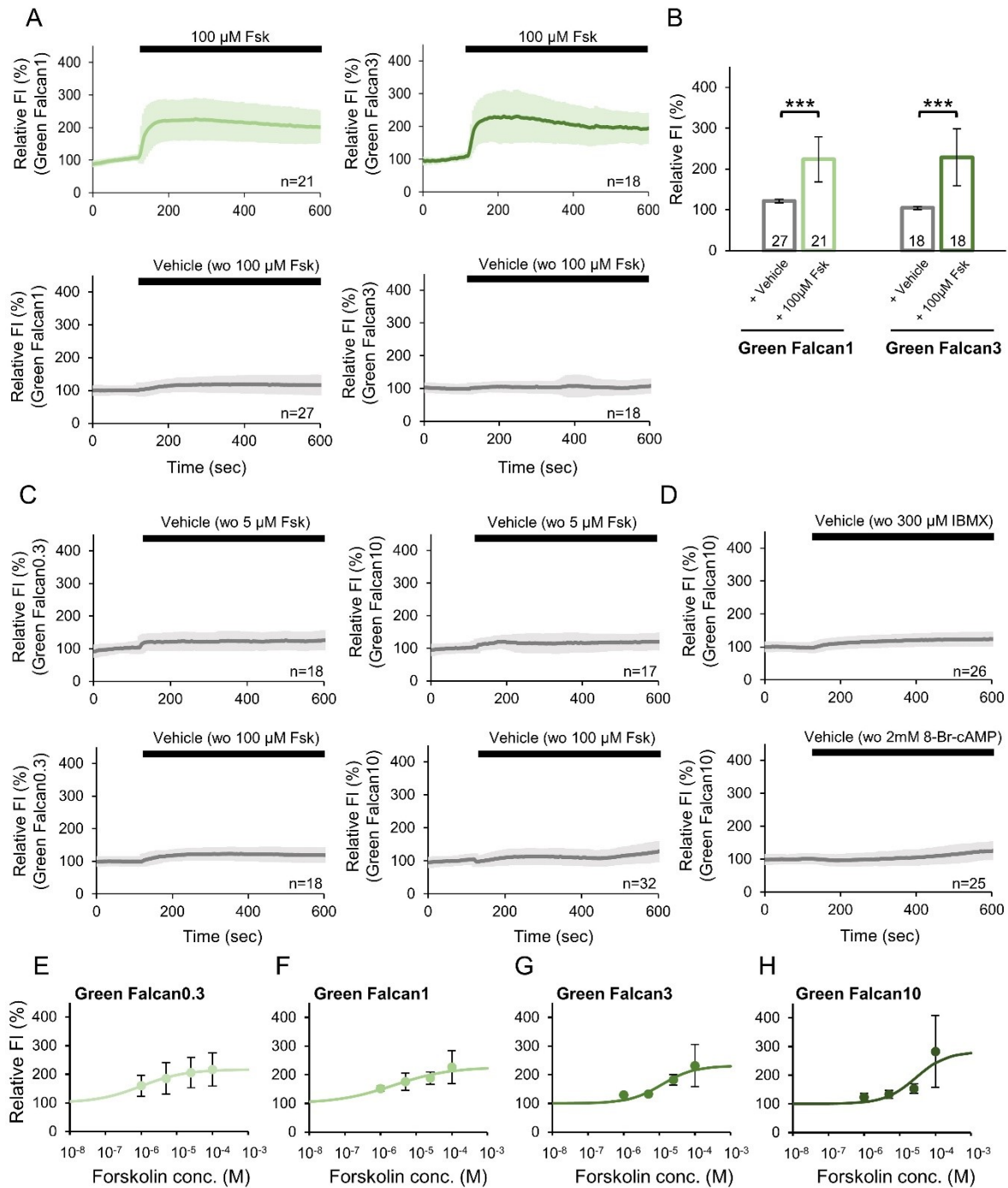


Figure S6. Live-cell imaging using Green Falcans (A) Time courses of fluorescence intensity (FI) in HeLa cells expressing Green Falcans1 and 3 in response to 100 μ M forskolin. Relative FI was calculated by dividing by the mean of the FI during the 2 min prior to administration. The data represent the means \pm standard deviation. The numbers in the lower right corner of the graphs represent the number of cells

analyzed from three independent experiments. (B) Comparison of relative FI at 2 min after the administration of DMSO (vehicle) or 100 μ M forskolin to HeLa cells expressing Green Falcans 1 and 3. The data represent the means \pm standard deviation. The numbers in bar graphs represent the number of cells analyzed from three independent experiments. *** $p < 0.001$. (C) Time courses of fluorescence intensity (FI) in HeLa cells expressing Green Falcans 0.3 and 10 in response to vehicle (wo 5, 100 μ M forskolin). Relative FI was calculated by dividing by the mean of the FI during the 2 min prior to administration. (D) Time courses of fluorescence intensity (FI) in HeLa cells expressing Green Falcans 10 in response to vehicle (300 μ M IBMX or 2 mM 8-Br-cAMP). Relative FI was calculated by dividing by the mean of the FI during the 2 min prior to administration. (E-H) Dose-response curves of all Green Falcans for the administration of forskolin. Each plot showed the maximum relative FI during imaging (10 min) after the administration of forskolin. For curve fitting of all Green Falcans, the minimum relative FI was normalized to 100, and maximum relative FI to maximum value. For Green Falcans 10 fitting, the hill coefficient was fixed to 1. The data represent the means \pm standard deviation (n=3).

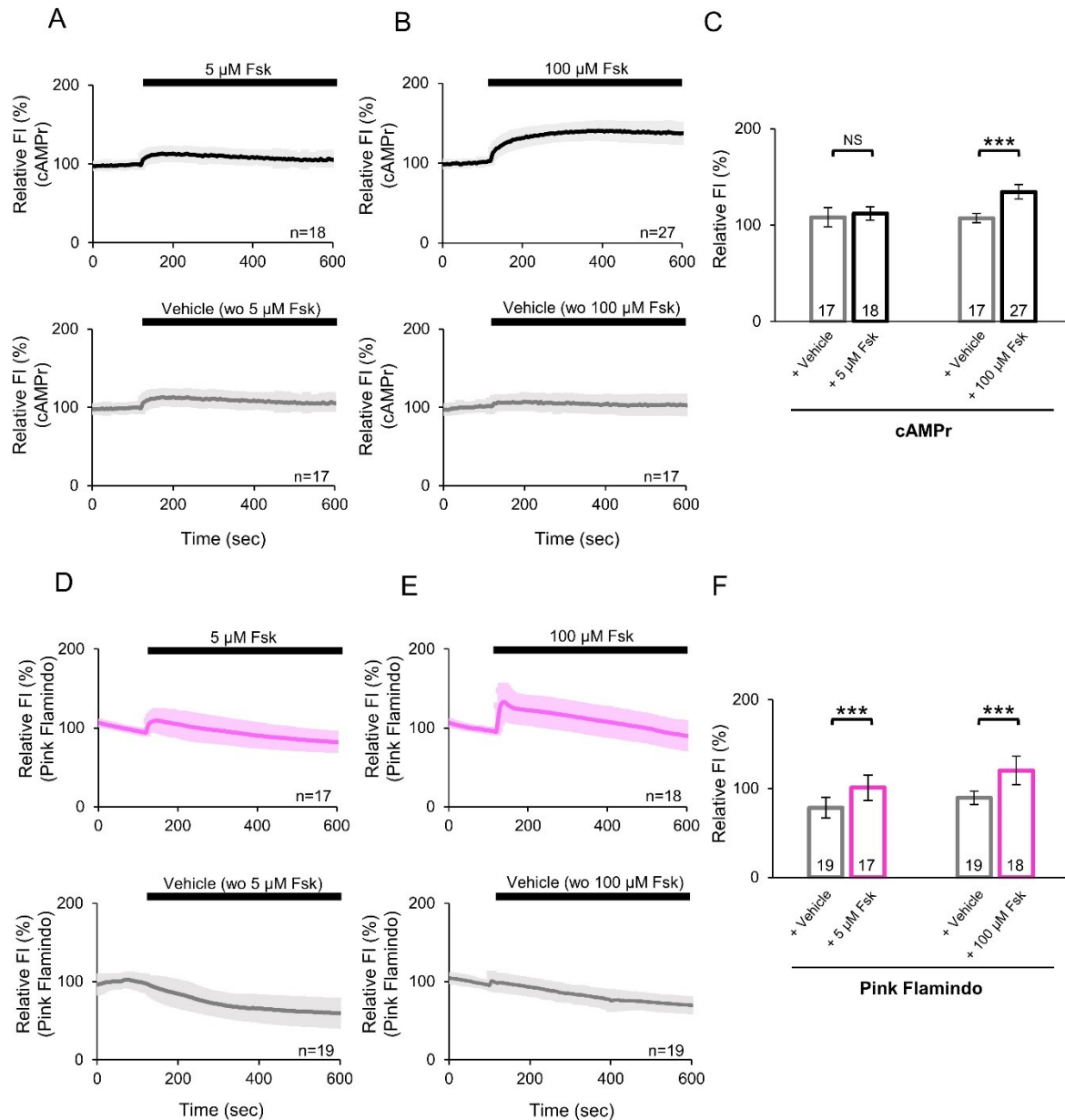


Figure S7. Live-cell imaging using other cAMP indicators (A, B) Time course of FI in HeLa cells expressing cAMPr in response to 5 μ M forskolin and DMSO (A), or 100 μ M forskolin and DMSO (B). The data represent the means \pm standard deviation. The numbers in the lower right corner of the graphs represent the number of cells analyzed from three independent experiments. (C) Comparison of relative FI at 2 min after the administration of DMSO (vehicle) or 5 μ M forskolin, and after that of DMSO (vehicle) or 100 μ M forskolin to HeLa cells expressing cAMPr. The data represent the means \pm standard deviation. The numbers in bar graphs represent the number of cells analyzed from three independent experiments.

*** $p < 0.001$. (D, E) Time course of FI in HeLa cells expressing Pink Flamindo in response to 5 μM forskolin and DMSO (D), or 100 μM forskolin and DMSO (E). The data represent the means \pm standard deviation. The numbers in the lower right corner of the graphs represent the number of cells analyzed from three independent experiments. (F) Comparison of relative FI at 2 min after the administration of DMSO (vehicle) or 5 μM forskolin, and after that of DMSO (vehicle) or 100 μM forskolin to HeLa cells expressing Pink Flamindo. The data represent the means \pm standard deviation. The numbers in bar graphs represent the number of cells analyzed from three independent experiments. *** $p < 0.001$.