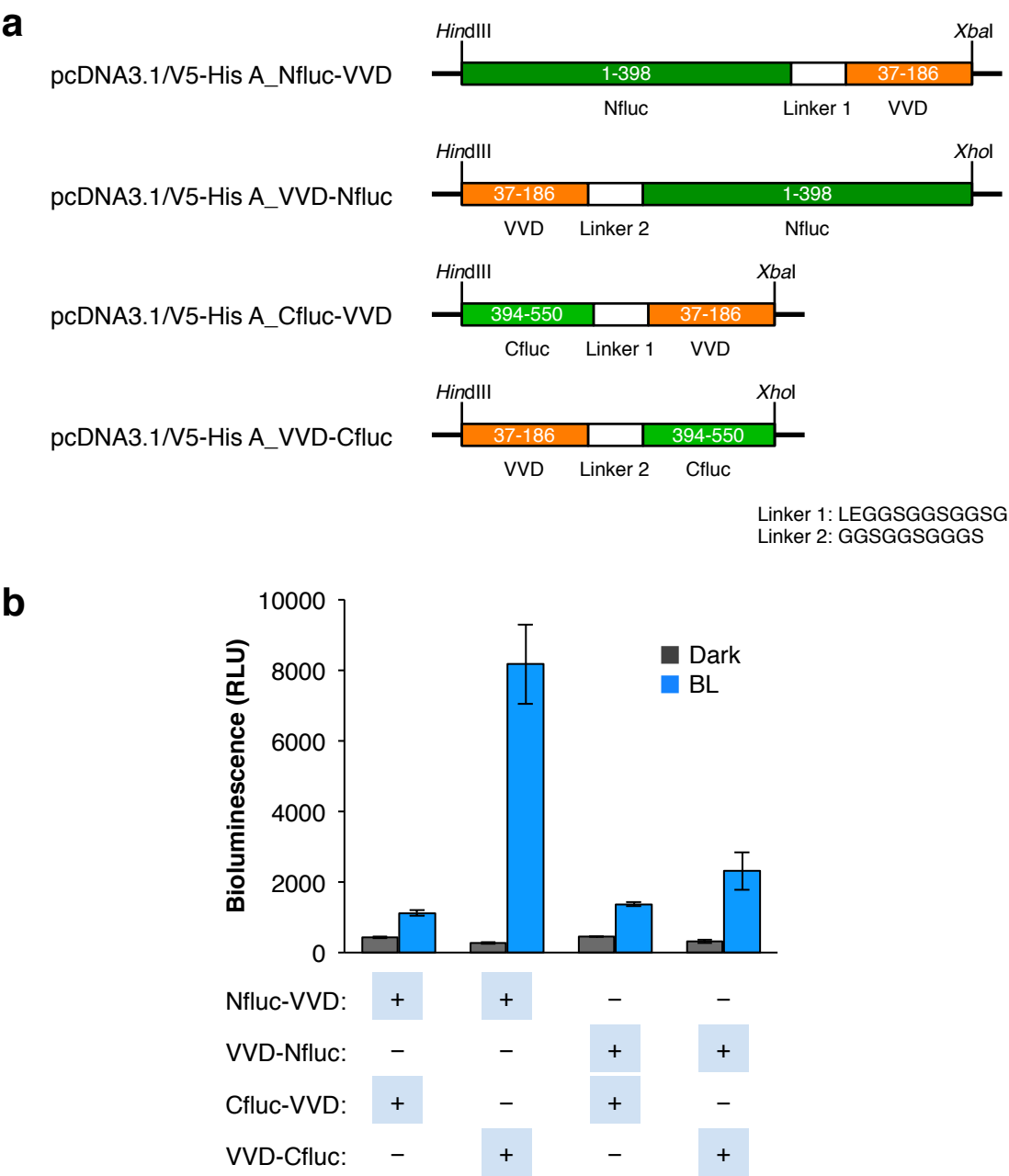
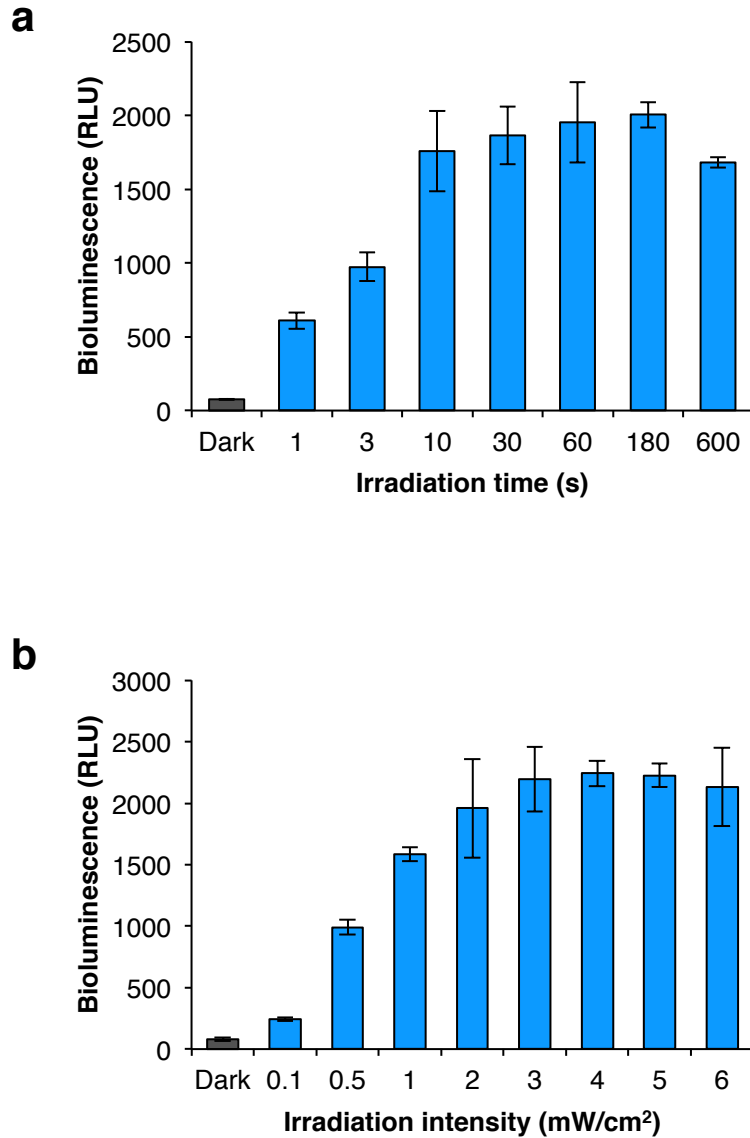


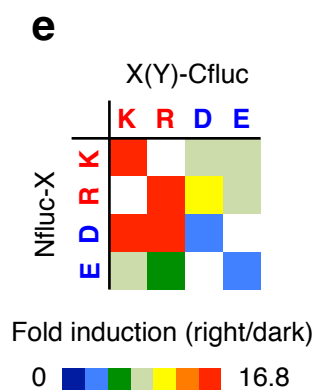
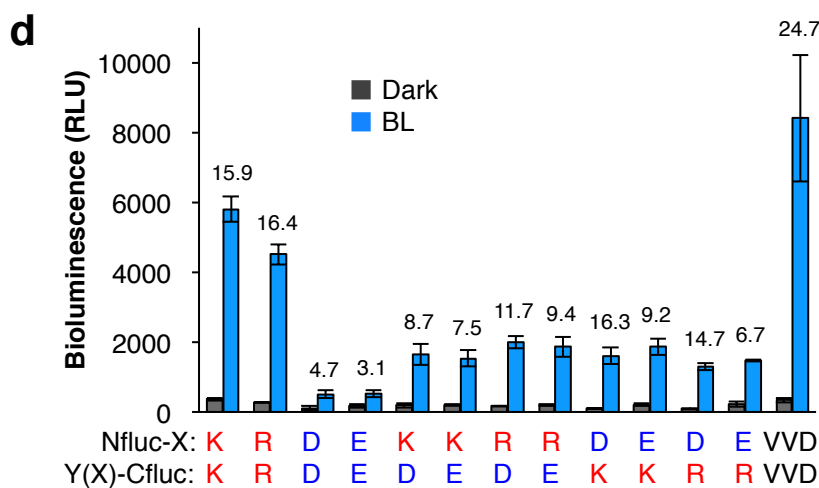
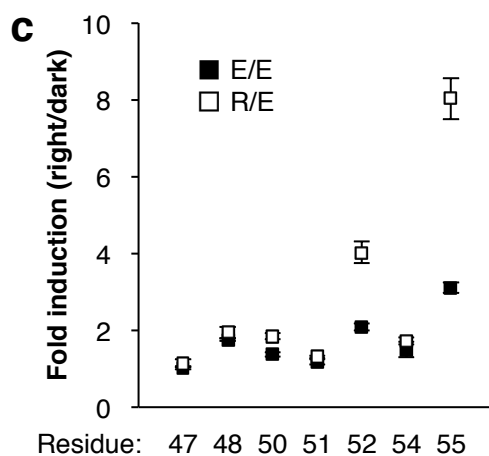
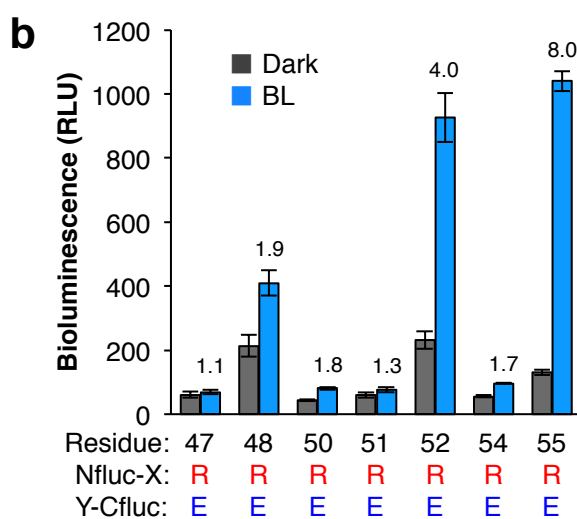
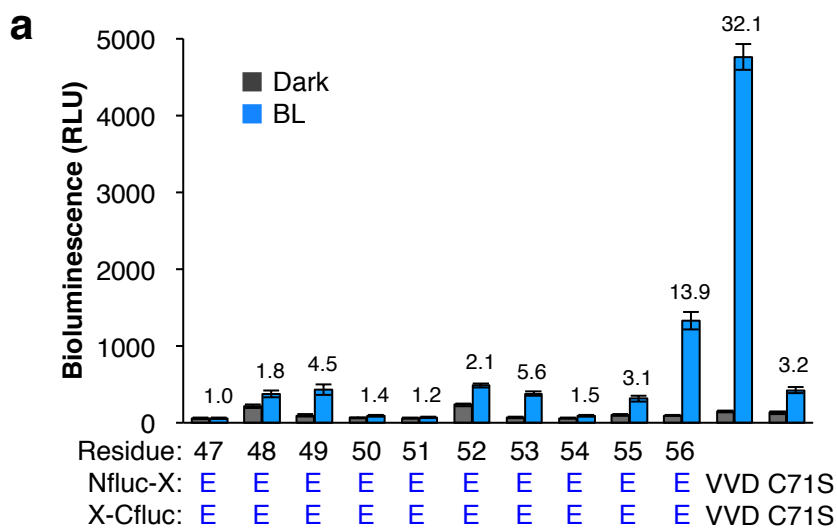
Supplementary Figures



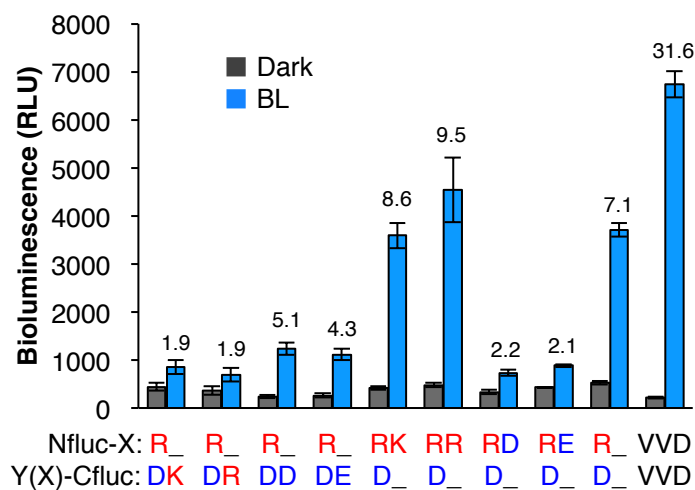
Supplementary Figure 1. A bioluminescence assay system for monitoring dimerization of VVD based on complementation of split-firefly luciferase fragments. (a) Schematic representations of domain structures of the fusion proteins tested in Supplementary Figure 1b. An N-half fragment (Nfluc: residues 1–398) and a C-half fragment (Cfluc: residues 394–550) of a split-firefly luciferase were separately tagged with VVD with different orientations. (b) Detecting blue light-dependent dimerization of VVD with split luciferase fragments. COS-7 cells co-expressing Nfluc-VVD or VVD-Nfluc along with Cfluc-VVD or VVD-Cfluc were irradiated with blue light (470 ± 20 nm, 3 mW cm^{-2}) for 30 s. The pair of Nfluc-VVD and VVD-Cfluc produced a larger bioluminescence signal than others in response to blue light. RLU, relative light unit. The result is means $\pm$ S.D. of three independent measurements.



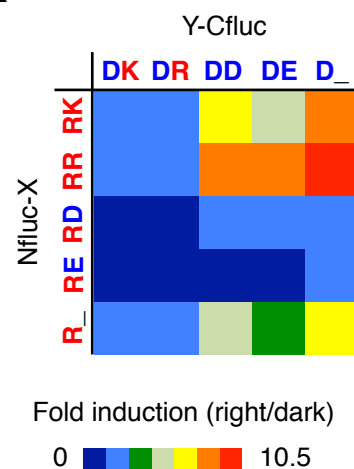
Supplementary Figure 2. Optimizing conditions for the irradiation to induce the VVD dimerization. (a) COS-7 cells expressing Nfluc-VVD and VVD-Cfluc were irradiated with blue light (470 ± 20 nm, 3 mW cm^{-2}) for 1, 3, 10, 30, 60, 180 and 600 s, respectively. The bioluminescence intensity reached the maximum level upon blue light irradiation with a power density at 3 mW cm^{-2} for 30 s. (b) COS-7 cells expressing Nfluc-VVD and VVD-Cfluc were irradiated for 30 s with 0.1, 0.5, 1, 2, 3, 4, 5 and 6 mW cm^{-2} blue light, respectively. The bioluminescence intensity reached the maximum level upon blue light irradiation with a power density at 3 mW cm^{-2} for 30 s. RLU, relative light unit. The results are means $\pm$ S.D. of three independent measurements.



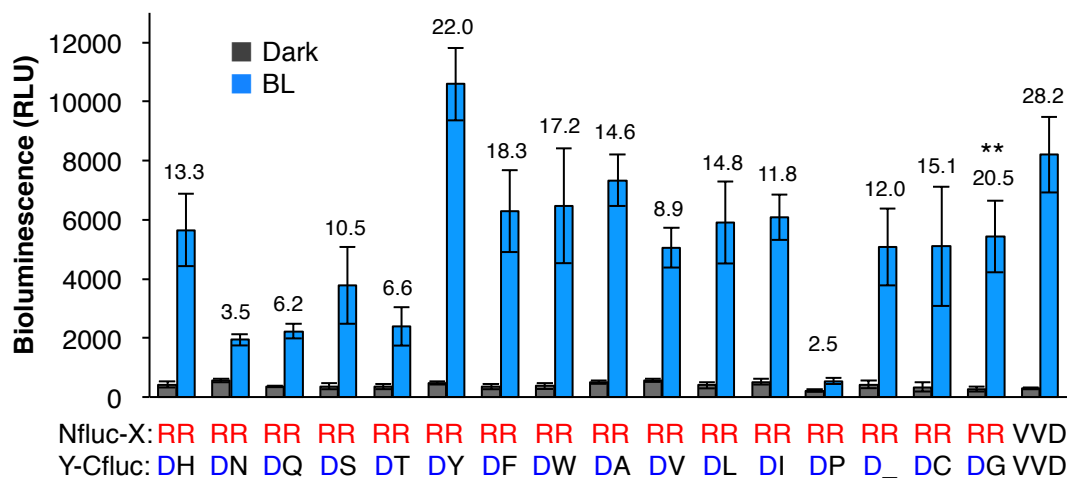
j



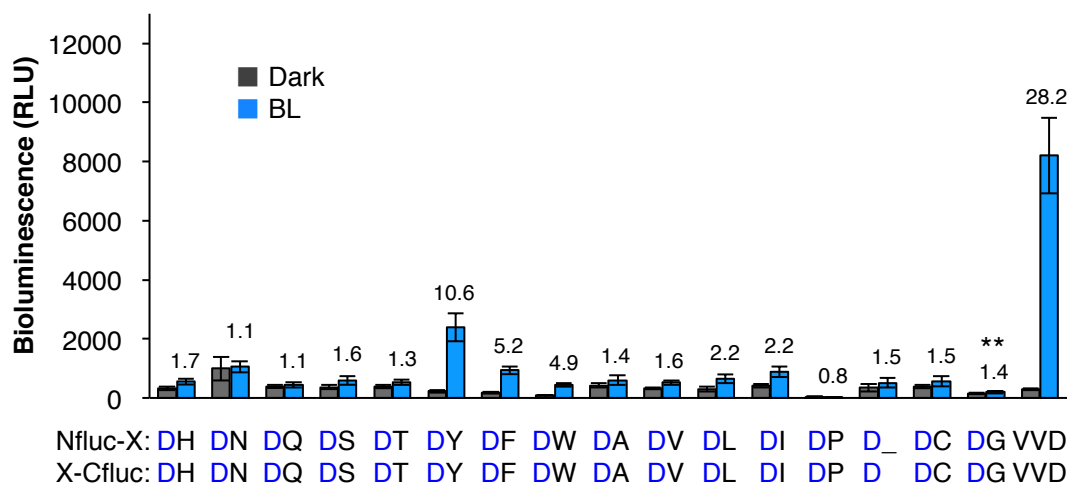
k



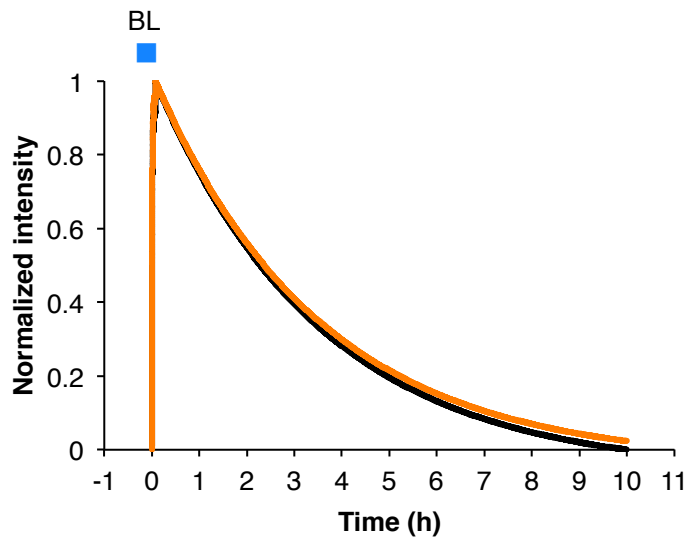
l



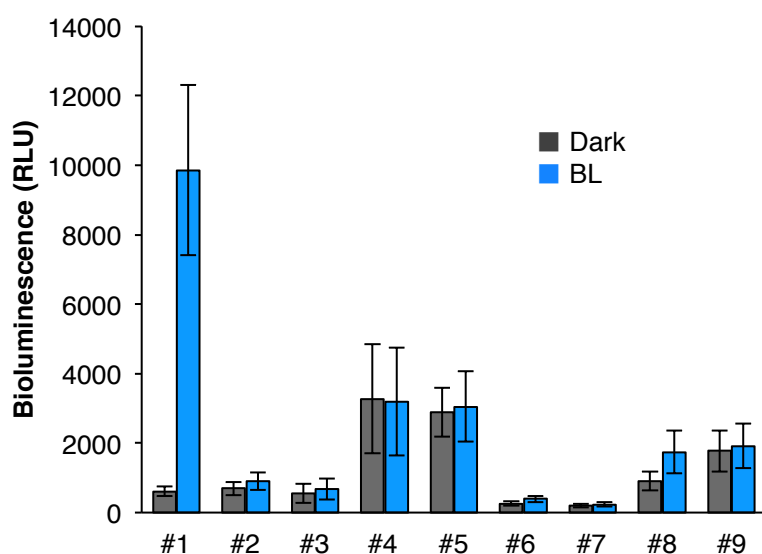
m



Supplementary Figure 3. Electrostatic interactions-based discrimination of the hetero-dimer from the homo-dimer. (a) Homo-dimerization of VVD variants, having single substitutions with glutamic acid at amino acid residues from 47 to 56, respectively. Nfluc-X and X(Y)-Cfluc (X and Y stand for VVD variants) were co-expressed in COS-7 cells. The cells were irradiated with blue light (470 ± 20 nm, 3 mW cm^{-2}) for 30 s, and bioluminescence measurements of the live cells were then performed. The fold induction (light/dark) of bioluminescence under the light condition to that under the dark condition is shown above each white bar. (b) Hetero-dimerization of each of the arginine variants and each of the glutamic acid variants, both having single substitutions at amino acid residue 47, 48, 50, 51, 52, 54 or 55. (c) A comparison of the fold induction (light/dark) between the homo-dimerization (closed circle) and the hetero-dimerization (open circle) of the VVD variants having single substitutions of amino acid residue 47, 48, 50, 51, 52, 54 or 55. The single substitution of Ile52 or Met55 was more effective to differentiate the fold inductions (right/dark) between the hetero-dimerization and the homo-dimerization than others. (d, f) Homo-dimerization and hetero-dimerization of VVD variants having single substitution with a charged amino acid at amino acid residue 55 (d) or 52 (f). In the case of Met55, its single substitution with a negatively charged amino acid suppressed homo-dimerization of the VVD variants, and hetero-dimerization was promoted between the variants with a negatively charged amino acid and those with a positively charged amino acid (d). However, single substitution of Met55 with a positively charged amino acid did not inhibit homo-dimerization of the VVD variants. This is in contrast to the case of substitution of Ile52 (f). The single substitution of Ile52 suppressed not only homo-dimerization of the VVD variants with a negatively charged amino acid but also that with a positively charged amino acid. In addition, the single substitution of Ile52 was effective to promote hetero-dimerization of the VVD variants with a negatively charged amino acid and those with a positively charged amino acid. Among the VVD variants with single substitutions of Ile52, the pair of the arginine variant (I52R) and the aspartic acid variant (I52D) was more effective to promote the hetero-dimerization than others. (e, g) Heat maps of the fold induction (light/dark) shown in Supplementary Figure 3d,f, respectively. (h) Homo-dimerization of VVD variants having double substitutions with a charged amino acid at amino acid residues 52 and 55. Two-letter code with R, K, D and E represents each of VVD variants with arginine, lysine, aspartic acid and glutamic acid at amino acid residues 52 and 55 (e.g., RK stands for I52R/M55K). I52R and I52D, having no substitution at amino acid residue 55, are depicted as R₋ and D₋, respectively. (i) Hetero-dimerization of VVD variants having double substitutions with a charged amino acid at amino acid residues 52 and 55. The double substitutions of both the residues 52 and 55 with a charged amino acid were not effective for the hetero-dimerization. (j) Hetero-dimerization between I52R or I52D and each of VVD variants having substitution with a charged amino acid at amino acid residue 55 of I52D or I52R. We found that I52R/M55R with arginine substitutions of both the residues 52 and 55 efficiently formed a hetero-dimer with I52D having no substitution of Met55. This implies that as the residue 55 of VVD variants having the I52D substitution, uncharged amino acids may be more promising compared with charged amino acids to achieve efficient hetero-dimerization with I52R/M55R. Thus we substituted Met55 of I52D with other fifteen uncharged amino acids, respectively, and examined the hetero-dimerization with I52R/M55R and the homo-dimerization in Supplementary Figure 3l,m. (k) A heat map of the fold induction shown in Supplementary Figure 3i,j. (l) Hetero-dimerization between I52R/M55R and each of VVD variants having substitution with an uncharged amino acid at amino acid residue 55 of I52D. (m) Homo-dimerization of VVD variants with double substitutions with an uncharged amino acid at amino acid residues 52 and 55. The results are means $\pm$ S.D. of three independent measurements. Single asterisks highlight I52R and I52D. Double asterisks highlight I52R/M55R and I52D/M52G, renamed pMag and nMag, respectively.

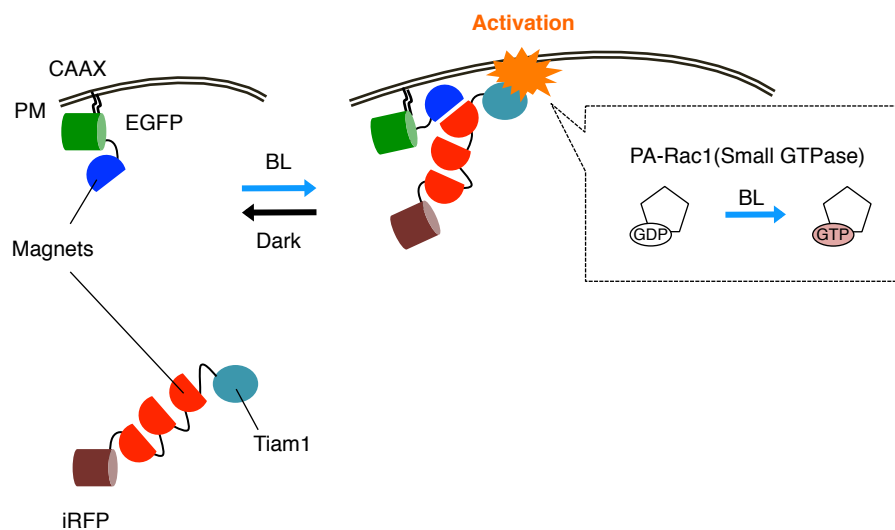


Supplementary Figure 4. Dissociation kinetics of the pMag–nMag hetero-dimer (black) and the VVD–VVD homo-dimer (orange). COS-7 cells expressing the pair of Nfluc-pMag and nMag-Cfluc or that of Nfluc-VVD and VVD-Cfluc were irradiated with blue light (470 ± 20 nm, 3 mW cm^{-2}) for 30 s. The homo-dimer of VVD dissociated with half-life $t_{1/2}$ of 2.0 h (rate constant, 9.5×10^{-5}), similar to the pMag–nMag hetero-dimer ($t_{1/2}$, 1.8 h, rate constant, 1.1×10^{-4}). The result represents typical experiments from three independent measurements.



	Nfluc-X	X(Y)-Cfluc
#1	pMag	nMag
#2	pMag	pMag
#3	pMagFast1	pMagFast1
#4	pMagFast2	pMagFast2
#5	pMagHigh1	pMagHigh1
#6	nMag	nMag
#7	nMagFast1	nMagFast1
#8	nMagFast2	nMagFast2
#9	nMagHigh1	nMagHigh1

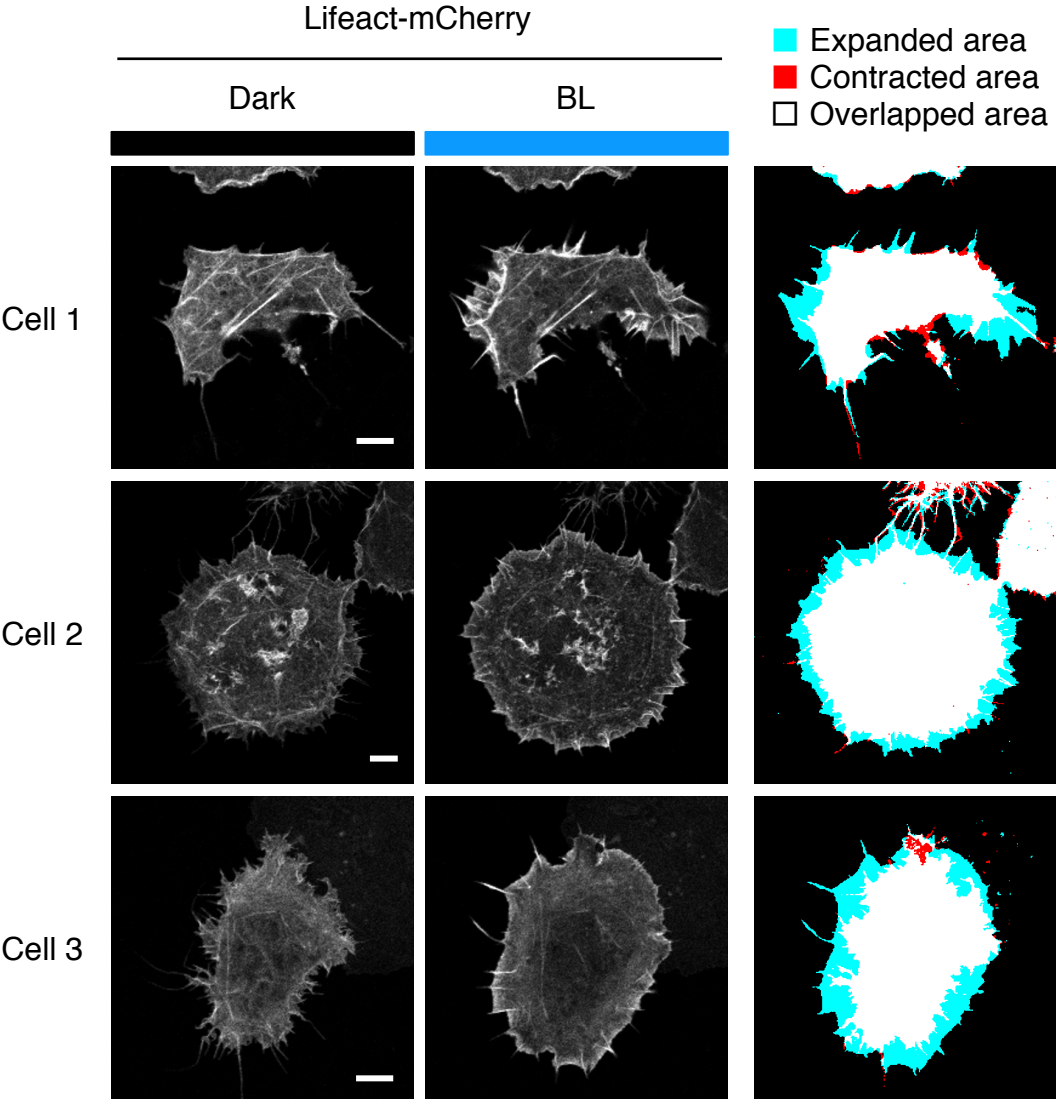
Supplementary Figure 5. Homo-dimerization of pMag variants and that of nMag variants. COS-7 cells expressing the Nfluc-X and X(Y)-Cfluc (X and Y stand for any one of Magnet variants shown in the figure) were irradiated with blue light (470 ± 20 nm, 3 mW cm^{-2}) for 30 s. The pMag variants and the nMag variants do not efficiently form their homo-dimers upon blue light irradiation. The results are means $\pm$ S.D. of three independent measurements.



Supplementary Figure 6. A schematic diagram of a Magnet-based photoactivatable actuator for a small GTPase (Rac1). Upon blue light irradiation, a functional domain is recruited to the plasma membrane with the help of the present paired photoswitches, Magnets (red and blue). Here we demonstrated photoactivatable Rac1 using pMagFast2(3x) (red) and nMagHigh1 (blue). To develop photoactivatable Rac1, we used a guanine nucleotide exchanging factor (GEF) domain (residues 1033–1406) from mouse Tiam1.

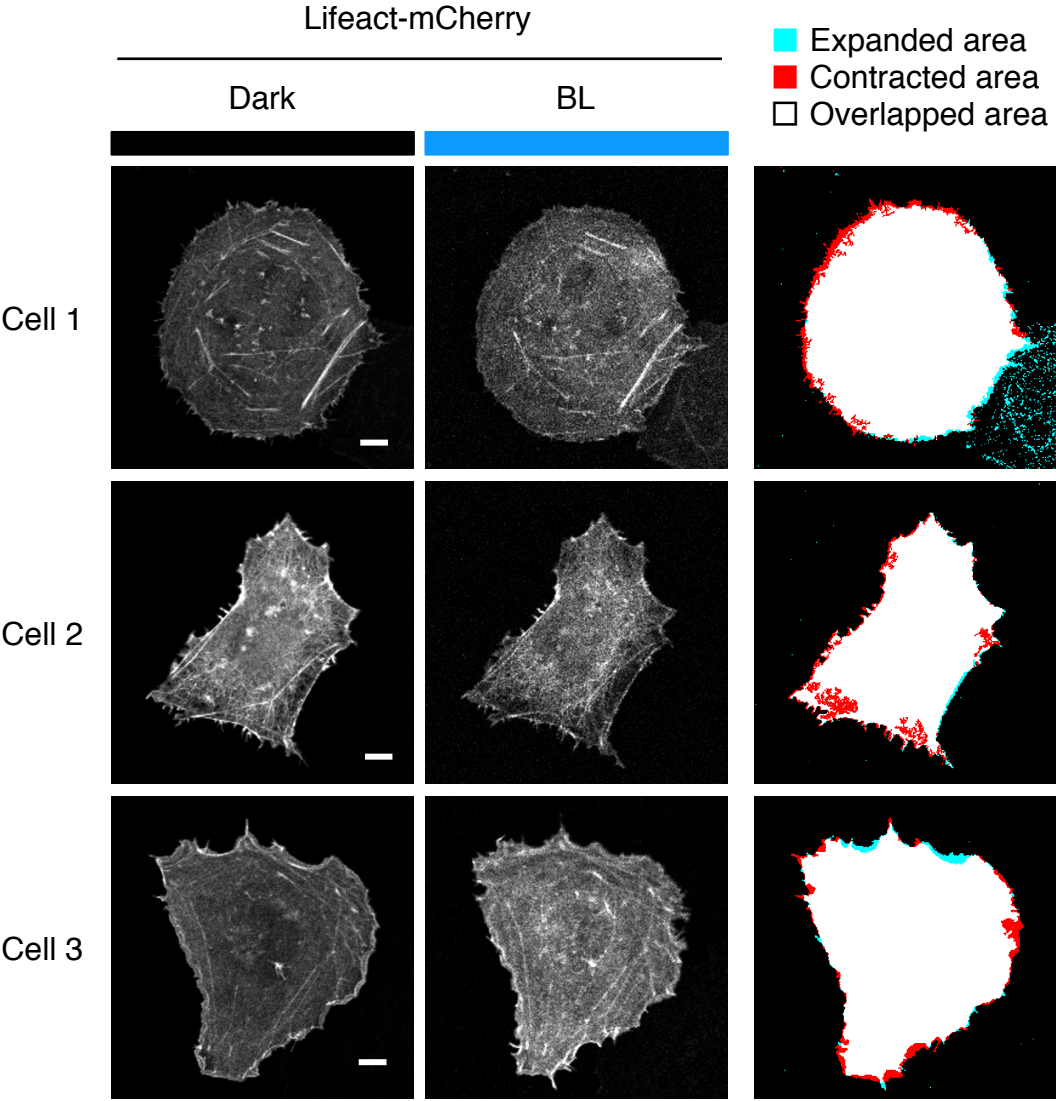
a

**Tiam1-pMagFast2(3x)-iRFP
+ nMagHigh1-EGFP-CAAX
+ Lifeact-mCherry**



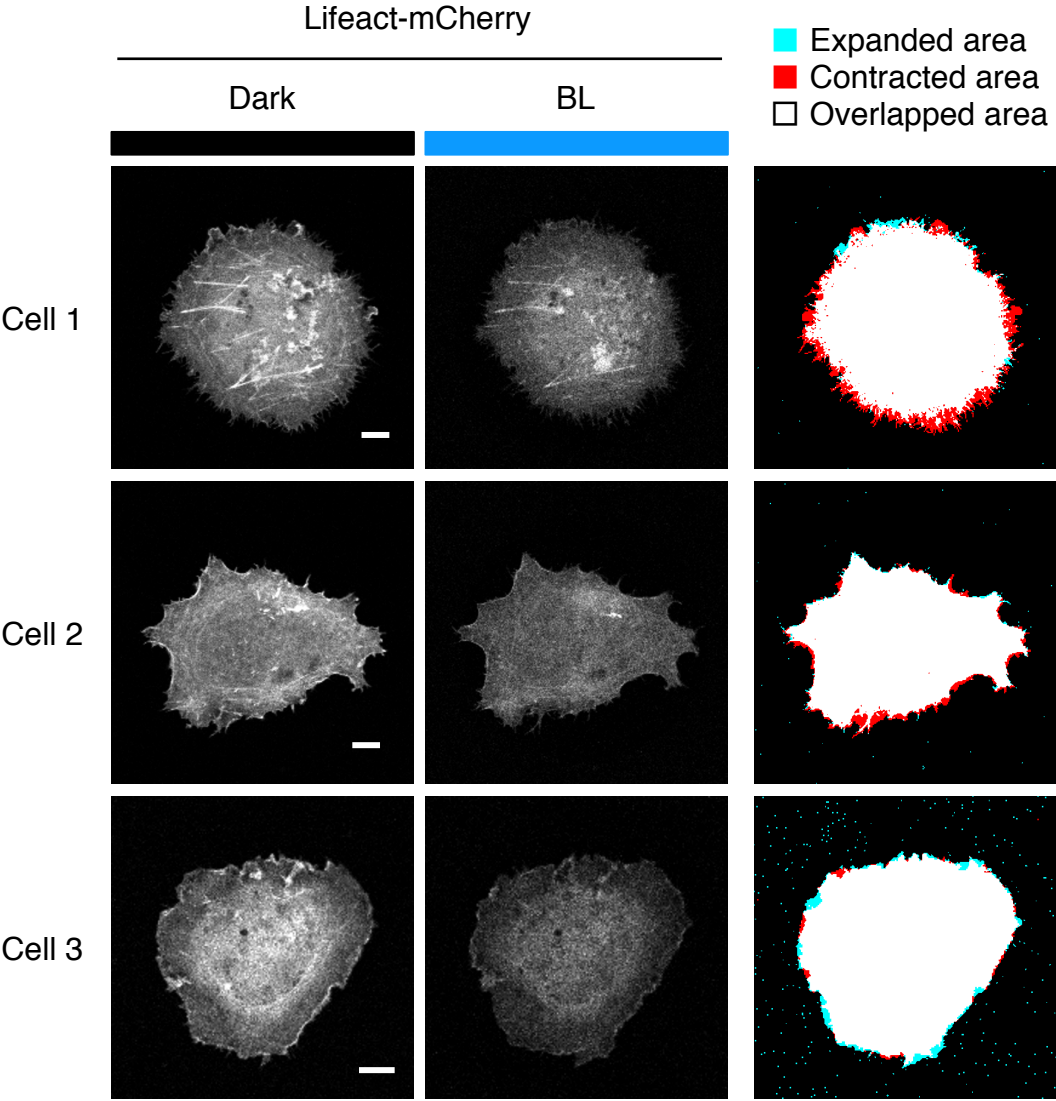
b

**Tiam1-pMagFast2(3x)-iRFP
+ nMagHigh1-EGFP-CAAX
+ Lifeact-mCherry
+ Rac1 inhibitor (100 μ M)**



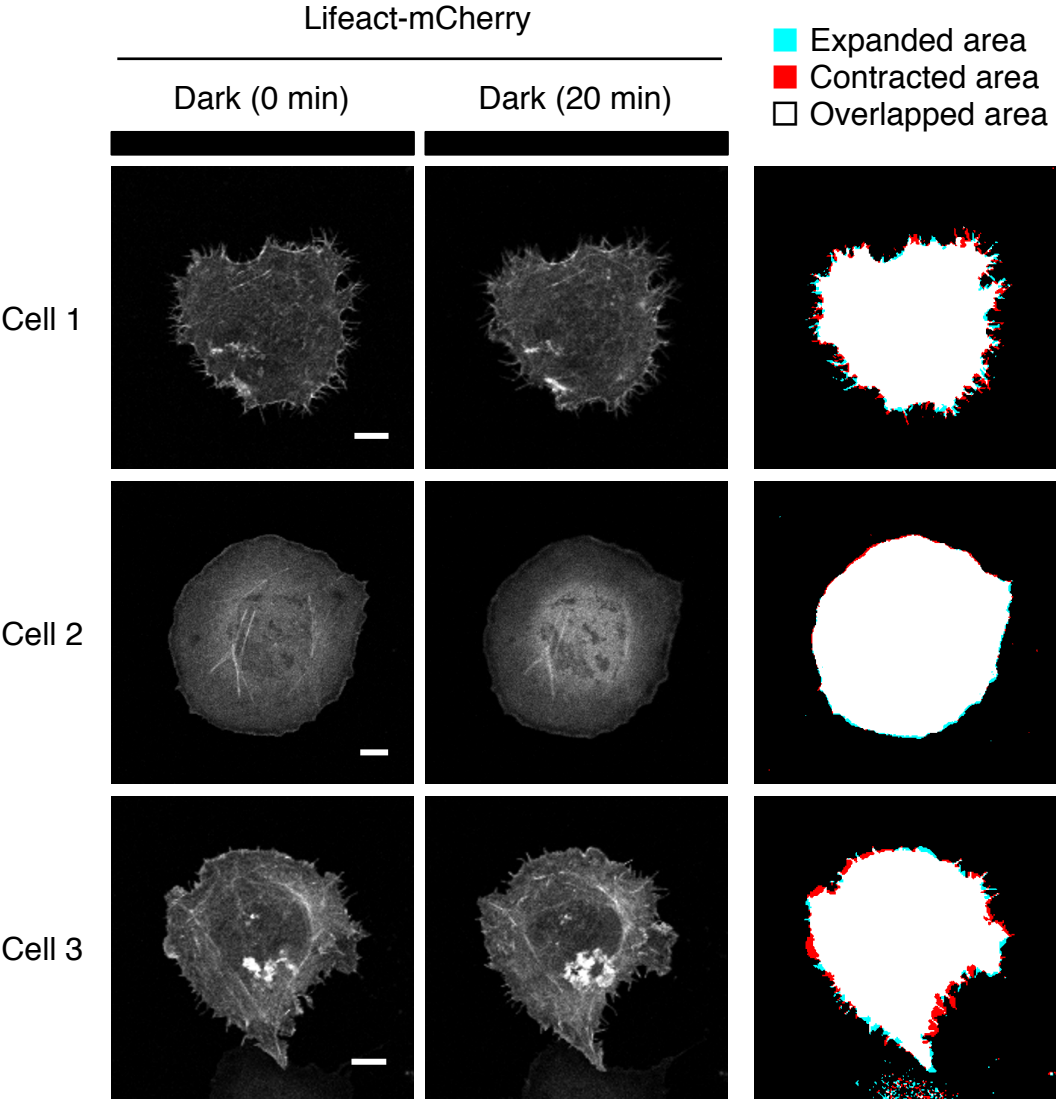
c

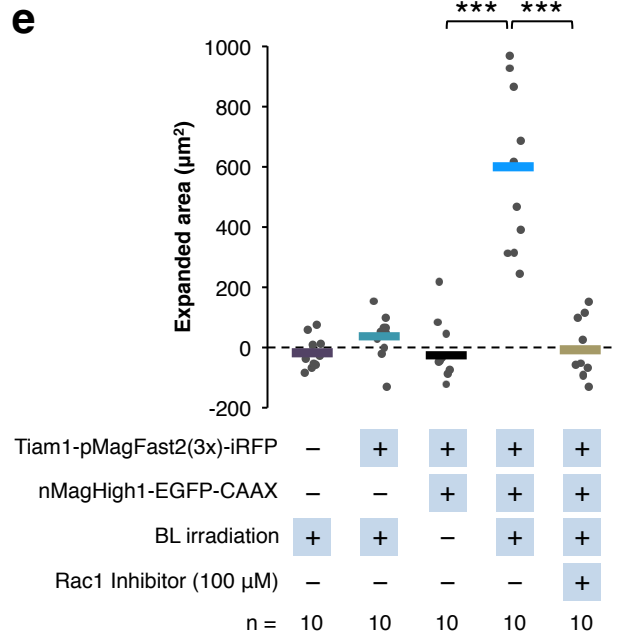
**Tiam1-pMagFast2(3x)-iRFP
+ Lifeact-mCherry**



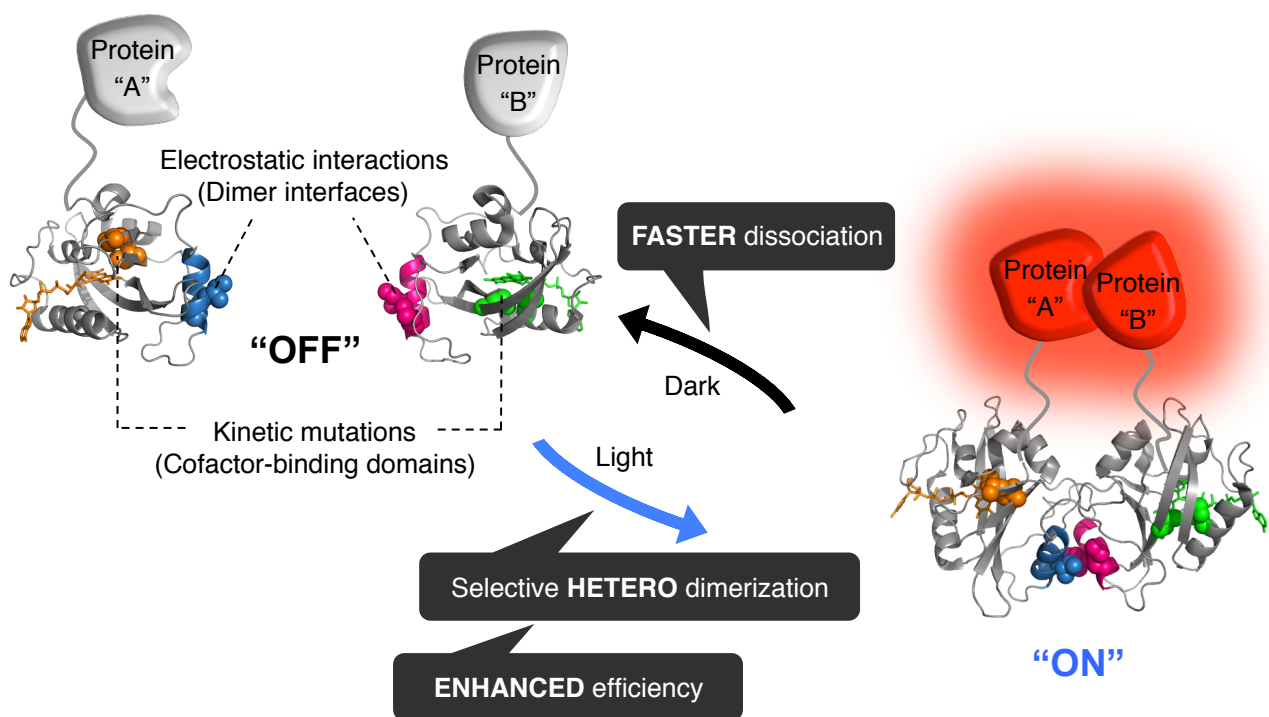
d

Tiam1-pMagFast2(3x)-iRFP
+ nMagHigh1-EGFP-CAAX
+ Lifeact-mCherry

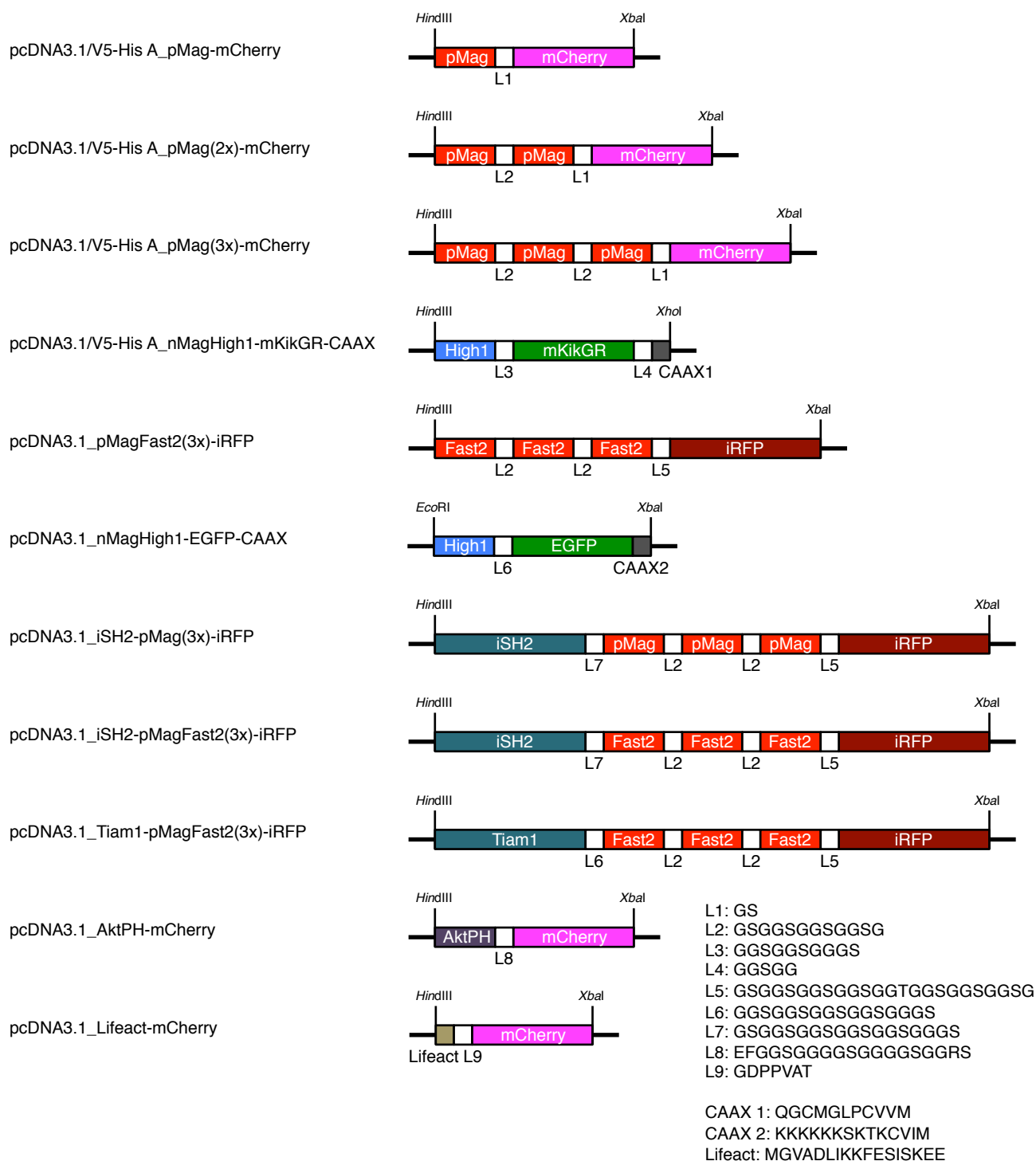




Supplementary Figure 7. Magnet-based photoactivatable Rac1. (a) Fluorescence images of COS-7 cells expressing nMagHigh1-EGFP-CAAX, Tiam1-pMagFast2(3x)-iRFP and Lifeact-mCherry before and 20 min after irradiation with blue light (Left and middle panels). Right panels show merged images of expanded area (cyan), contracted area (red) and overlapped area (white). (b) A potent inhibitor for Rac1-specific GEF Tiam1 (100 μ M) blocked the blue light-dependent cell expansion. (c) Fluorescence images of COS-7 cells expressing Tiam1-pMagFast2(3x)-iRFP and Lifeact-mCherry before and 20 min after irradiation with blue light. (d) Fluorescence images of COS-7 cells expressing nMagHigh1-EGFP-CAAX, Tiam1-pMagFast2(3x)-iRFP and Lifeact-mCherry at initiation time (0 min) and after elapsed time of 20 min in the dark condition. (e) Quantification of expanded area of the COS-7 cells expressing Magnet-based photoactivatable Rac1. The expanded area was calculated by subtracting cell area before blue light irradiation and from that after irradiation. Blue light irradiation was completed with a Multi-line Argon laser (488 nm) at 0.2 mW. Scale bars of all images, 10 μ m.



Supplementary Figure 8. Re-engineering of the nature of a fungal photoreceptor has created pairs of distinct photoswitches with selective hetero-dimerization properties, substantially enhanced dimerization efficiencies and accelerated dissociation kinetics, allowing spatially and temporally precise control of cellular proteins with blue light.



Supplementary Figure 9. Schematic representations of domain structures of constructs used in this study. L1-9, linker domains; CAAX1, a CAAX box motif (QGCMGLPCVVM) derived from C-terminus of N-Ras; CAAX2; a CAAX box motif (KKKKKKSKTKCVIM) derived from C-terminus of K-Ras; iSH2, an inter SH2 domain (residues 421–623) from bovine p85alpha-PI3kinase; Tiam1, a guanine nucleotide exchanging factor (GEF) domain (residues 1033–1406) from mouse Tiam1; AktPH, a PH domain (residues 1-164) derived from human Akt1.

a

pcDNA3.1/V5-His A_Nfluc-VVD

>Amino acid sequence

MGSEDAKNIKKGPAPFYPLEDGTAGEQLHKAMKRYALVPGTIAFTDAHIEVDITYAEYFEMSVRLAEAMKRYGLNTNHRIV
VCSENSLQFFMPVLGALFIGVAVAPANDIYNERELLSMGI SQPTVVVFSKKGLQKILNVQKKLP I IQKIIIMDSKTDYQG
FQSMYTFVTSHLPPGFNEYDFVPESFDRDKTIALIMNSSGSTGLPKGVALPHRTACVRFSHARDPIFGNQIIPDTAILS
VVPFHGFGMFTTLGYLICGFRVLMYRFEELFLRSLQDYKIQSALLVPTLFSFFAKSTLIDKYDLSNLHEIASGGAPLSKE
VGEAVAKRFHLPGIRQGYGLTETTSAILITPEGDDKPGAVGKVVPFPEAKVVDLDTGKTLGVNQRGELCVRGPMIMLEGG
SGSGSGSGHTLYAPGGYDIMGYL I QIMNRPNPQVELGPVDTSCALILCDLKQKDTPIVYASEAFLYMTGYSNAEVLGRNCRF
LQSPDGMVKPKSTRKYVDSNTINTMRKAIDRNAEVQVEVNFKNQGRFVNFLTMIPVRDETGEYRYSMGFQCETEGSGSG
SGGGSSRGPFEGKPIPNPLLGLDSTRTGHHHHHH*

>DNA sequence

ATGGGATCCGAAGATGCCAAAACATTAGAAGGGCCAGCGCCATTCTACCCACTCGAAGACGGGACCGCCGGCGAGCAG
CTGCACAAAACCATGAAGCGCTACGCCCTGGTGCCCGGCACCATCGCCTTTACCGACGCACATATCGAGGTGGACATTACC
TACGCCGAGTACTTCGAGATGAGCGTTCCGGCTGGCAGAAGCTATGAAGCGCTATGGGCTGAATACAAACCATCGGATCGTG
GTGTGCAGCGAGAATAGCTTGCACTTCTTCATGCCCGTGTGGGTGCCCTGTTTCATCGGTGTGGCTGTGGCCCCAGCTAAC
GACATCTACAACGAGCGCGAGCTGCTGAACAGCATGGGCATCAGCCAGCCACCGTCGTATTTCGTGAGCAAGAAAGGGCTG
CAAAAGATCCTCAACGTGCAAAAGAAGCTACCGATCATACAAAAGATCATCATCATGGATAGCAAGACCGACTACCAGGGC
TTCAAAGCATGTACACCTTCGTGACTTCCATTTGCCACCCGGCTTCAACGAGTACGACTTCGTGCCCGAGAGCTTCGAC
CGGGACAAAACCATCGCCCTGATCATGAACAGTAGTGCCAGTACCGGATTGCCCAAGGGCGTAGCCCTACCGCACCGCACC
GCTTGTGTCCGATTAGTCATGCCCGCAGCCCATCTTCGGCAACCAGATCATCCCCGACACCGCTATCCTCAGCGTGGTG
CCATTTACACAGGCTTCGGCATGTTACCAAGCTGGGCTACTTGATCTGCGGCTTTTCGGGTGCTGCTCATGTACCGCTTC
GAGGAGGAGCTATTCTTGCGCAGCTTGCAAGACTATAAGATTCAATCTGCCCTGCTGGTGCCACACTATTTAGCTTCTTC
GCTAAGAGCACTCTCATGACAAGTACGACCTAAGCAACTTGACAGAGATCGCCAGCGCGGGGCGCCGCTCAGCAAGGAG
GTAGGTGAGGCCGTGGCCAAACGCTTCACCTACCAGGCATCCGCCAGGGCTACGGCCTGACAGAAACAACCAGCGCCATT
CTGATCACCCCCGAAGGGGACGACAAGCCTGGCGCAGTAGGCAAGGTGGTGCCCTTCTTCGAGGCTAAGGTGGTGGACTTG
GACACCGGTAAAGACTGGGTGTGAACCAGCGCGGCGAGCTGTGCGTCCGTGGCCCCATGATCATGCTCGAGGGCGGCAGT
GGAGGGTCTGGTGGCAGCGGACATACTCTTTATGCCCCGGTGGATATGACATTATGGGATATCTGATTAGATCATGAAC
CGGCCAAACCCGAGGTGGAAGTGGGCCCCGTGGATACATCCTGCGCCTTGATTCTTTGTGACCTGAAACAGAAAGACACC
CCGATAGTTTACGCGAGTGAAGCCTTCCTCTACATGACAGGTTACAGCAACGCAGAGGTGCTGGGCCGGAATTGCCGTTT
CTGCAAAGCCCTGACGGCATGGTGAAGCCCAAGAGCACCCGGAAGTACGTGGATAGTAACACAATCAATACTATGCGCAAG
GCAATCGACAGGAATGCCGAGGTGAGGTTGAAGTAGTCAATTTTAAAAGAATGGACAGCGATTTGTTAATTTCTGACT
ATGATACCTGTTAGGGACGAAACAGGCGAGTATCGATACTCTATGGGATCCAGTGCGAAACAGAAAGGGGGTCTGGGGGG

AGCGGCGGGGATCCTCTAGAGGGCCCTTCGAAGGTAAGCCTATCCCTAACCCCTCTCCTCGGTCTCGATTCTACGCGTACC
GGTCATCATCACCATCACCATTGA

b

pcDNA3.1/V5-His A_VVD-Nfluc

>Amino acid sequence

MGLEGGSGGSGSGHTLYAPGGYDIMGYLIQIMNRPNPQVELGPVDTSCALILCDLKQKDTPIVYASEAFLYMTGYSNAEV
LGRNCRFLQSPDGMVKPKSTRKYVDSNTINTMRKAIDRNAEVQVEVNFKNQGRFVNFLTMIPVRDETGEYRYSMGFQCE
TEGGSGGSGGGSSEDAKNIKKGPAPFYPLEDGTAGEQLHKAMKRYALVPGTIAFTDAHIEVDITYAEYFEMSVRLAEAMKRY
GLNTNHRIVVCSENSLQFFMPVLGALFIGVAVAPANDIYNERELNSMGISQPTVVVFVSKGLQKILNVQKLPPIQKII
MDSKTDYQGFQSMYTFVTSHLPPGFNEYDFVPESFDRDKTIALIMNSSGSTGLPKGVALPHRTACVRFSHARDPIFGNQII
PDTAILSVPFHHGFMFTTLGYLICGFRVLMYRFEELFLRSLQDYKIQSALLVPTLFSFFAKSTLIDKYDLSNLHEIA
SGGAPLSKEVGEAVAKRFHLPGIRQGYGLTETTSAILITPEGDDKPGAVGKVVPFFEAKVVDLDTGKTLGVNQRGELCVRG
PMIMLESRGPFEGKPIPNPLLGLDSTRTGHHHHHH*

>DNA sequence

ATGGGACTCGAGGGCGGCAGTGGAGGGTCTGGTGGCAGCGGACATACTCTTTATGCCCCGGTGGATATGACATTATGGGA
TATCTGATTGAGATCATGAACCGGCCAAACCCGAGGTGGAAGTGGGCCCGTGGATACATCCTGCGCCTTGATTCTTTGT
GACCTGAAACAGAAAGACACCCCGATAGTTTACGCGAGTGAAAGCCTTCCTCTACATGACAGGTTACAGCAACGCAGAGGTG
CTGGGCCGAATTGCCGGTTTCTGCAAAGCCTGACGGCATGGTGAAGCCCAAGAGCACCCGGAAGTACGTGGATAGTAAC
ACAATCAATACTATGCGCAAGGCAATCGACAGGAATGCCGAGGTGCAGGTTGAAGTAGTCAATTTTAAAAAGAATGGACAG
CGATTTGTAAATTTCTGACTATGATACCTGTTAGGGACGAAACAGGCGAGTATCGATACTCTATGGGATTCCAGTGCGA
ACAGAAAGGGGTTCTGGGGGAGCGGCGGGGATCCGAAGATGCCAAAACATTAGAAGGGCCAGCGCCATTCTACCCA
CTCGAAGACGGGACCGCCGCGAGCAGCTGCACAAAGCCATGAAGCGCTACGCCCTGGTGCCCGGCACCATCGCCTTTACC
GACGCACATATCGAGGTGGACATTACCTACGCCGAGTACTTCGAGATGAGCGTTCCGGCTGGCAGAAGCTATGAAGCGCTAT
GGGCTGAATACAAACCATCGGATCGTGGTGTGCAGCGAGAATAGCTTGAGTTCTTCATGCCCGTGTGGGTGCCCTGTTT
ATCGGTGTGGCTGTGGCCCCAGCTAACGACATCTACAACGAGCGCGAGCTGCTGAACAGCATGGGCATCAGCCAGCCCACC
GTCGTATTGCTGAGCAAGAAAGGGCTGCAAAAGATCCTCAACGTGCAAAAGAAGCTACCGATCATACAAAGATCATCATC
ATGGATAGCAAGACCGACTACCAGGGCTTCAAAGCATGTACACCTTCGTGACTTCCCATTGGCCACCCGGCTTCAACGAG
TACGACTTCGTGCCGAGAGCTTCGACCGGGACAAACCATCGCCCTGATCATGAACAGTAGTGGCAGTACCGGATTGCC
AAGGGCGTAGCCCTACCGACCGCACCGCTTGTGTCCGATTGAGTCATGCCCGGACCCCATCTTCGGCAACCAGATCATC
CCCGACACCGCTATCCTCAGCGTGGTGCCATTTACCACGGCTTCGGCATGTTACCACGCTGGGCTACTTGATCTGCGGC
TTTCGGGTGCTGCTCATGTACCGCTTCGAGGAGGAGCTATTCTTGCGCAGCTTGCAAGACTATAAGATTCAATCTGCCCTG
CTGGTGCCCACTATTTAGCTTCTCGCTAAGAGCACTCTCATCGACAAGTACGACCTAAGCAACTTGACAGAGATCGCC
AGCGGCGGGGCGCCGCTCAGCAAGGAGGTAGGTGAGGCCGTGGCCAAACGCTTCCACCTACCAGGCATCCGCCAGGGCTAC
GGCCTGACAGAAACAACAGCGCCATTCTGATACCCCCGAAGGGGACGACAAGCCTGGCGCAGTAGGCAAGGTGGTGCC
TTCTTCGAGGCTAAGGTGGTGGACTTGGACACCGGTAAGACACTGGGTGTGAACCAGCGCGGCGAGCTGTGCGTCCGTGGC
CCCATGATCATGCTCGAGTCTAGAGGGCCCTTCGAAGGTAAGCCTATCCCTAACCTCTCCTCGGTCTCGATTCTACGCGT

ACCGGTCATCATCACCATCACCATTGA

C

pcDNA3.1/V5-His A_Cfluc-VVD

>Amino acid sequence

MGSGPMIMSGYVNNPEATNALIDKDGWLHSGDIAYWDEDEHFFIVDRLKSLIKYKGYQVAPAELESILLQHPNIFDAGVAG
LPDDDAGELPAAVVLEHGKTMTEKEIVDYVASQVTTAKKLRGGVVFVDEVPKGLTGKLDARKIREILIKAKKGGKIAVLE
GGSGSGSGSGHTLYAPGGYDIMGYLIQIMNRPNPQVELGPVDTSCALILCDLKQKDTPIVYASEAFLYMTGYSNAEVLGRN
CRFLQSPDGMVKPKSTRKYVDSNTINTMRKAIDRNAEVQVEVNFKNQGRFVNFLTMIPVRDETGEYRYSMGFQCETEGG
SGSGSGSGSSRGPFEKGKIPNPLLGLDSTRTGHHHHH*

>DNA sequence

ATGGGATCCGGCCCCATGATCATGAGCGGCTACGTTAACAACCCCGAGGCTACAAACGCTCTCATCGACAAGGACGGCTGG
CTGCACAGCGGCGACATCGCCTACTGGGACGAGGACGAGCACTTCTTCATCGTGGACCGGCTGAAGAGCCTGATCAAATAC
AAGGGCTACCAGGTAGCCCCAGCCGAAGTGGAGAGCATCCTGCTGCAACACCCCAACATCTTCGACGCCGGGGTCGCCGGC
CTGCCCGACGACGATGCCGGCGAGCTGCCCGCCGCACTCGTCTGCTGGAACACGGTAAACCATGACCGAGAAGGAGATC
GTGGACTATGTGGCCAGCCAGGTTACAACCGCCAAGAAGCTGCGCGGTGGTGTTGTGTTCTGGACGAGGTGCCTAAAGGA
CTGACCGGCAAGTTGGACGCCCGCAAGATCCGCGAGATTCTCATTAAAGCCAAGAAGGGCGGCAAGATCGCCGTGCTCGAG
GGCGGCAGTGGAGGGTCTGGTGGCAGCGGA**CATACTCTTTATGCCCGCGGTGGATATGACATTATGGGATATCTGATTCAG**
ATCATGAACCGGCCAAACCCGCAAGTGGAACTGGGCCCCGTGGATACATCCTGCGCCTTGATTCTTTGTGACCTGAAACAG
AAAGACACCCCGATAGTTTACGCGAGTGAAGCCTTCTCTACATGACAGGTTACAGCAACGCAGAGGTGCTGGGCCGGAAT
TGCCGGTTTCTGCAAAGCCCTGACGGCATGGTGAAGCCCAAGAGCACCCGGAAGTACGTGGATAGTAACACAATCAATACT
ATGCGCAAGGCAATCGACAGGAATGCCGAGGTGCAGGTTGAAGTAGTCAATTTTAAAAAGAATGGACAGCGATTTGTTAAT
TTCTGACTATGATACCTGTTAGGGACGAAACAGGCGAGTATCGATACTCTATGGGATTCCAGTGCGAAACAGAAAGGGGGT
TCTGGGGGGAGCGGGGGGGATCCTCTAGAGGGCCCTTCGAAGGTAAGCCTATCCCTAACCTCTCCTCGGTCTCGATTCT
ACGCGTACCGGTCATCATCACCATCACCATTGA

d

pcDNA3.1/V5-His A_VVD-Cfluc

>Amino acid sequence

MGLEGGSGGSGSGHTLYAPGGYDIMGYLIQIMNRPNPQVELGPVDTSCALILCDLKQKDTPIVYASEAFLYMTGYSNAEV
LGRNCRFLQSPDGMVKPKSTRKYVDSNTINTMRKAIDRNAEVQVEVNFKKNGQRFVNFLTMIPVRDETGEYRYSMGFQCE
TEGGSGGSGGSGGPMIMSGYVNNPEATNALIDKDWLHSGDIAYWDEDEHFFIVDRLKSLIKYKGQVAPAELESILLQHP
NIFDAGVAGLPDDDAGELPAVVVLEHGKTMTEKEIVDYVASQVTTAKKLGGVVVFVDEVPKGLTGKLDARKIREILIKAK
KGGKIAVLESRGPFEGKPIPNLLGLDSTRTGHHHHHH*

>DNA sequence

ATGGGACTCGAGGGCGGCAGTGGAGGGTCTGGTGGCAGCGGACATACTCTTTATGCCCCCGGTGGATATGACATTATGGGA
TATCTGATTCAGATCATGAACCGGCCAAACCCGCAGGTGGAAGTGGCCCCCGTGGATACATCCTGCGCCTTGATTCTTTGT
GACCTGAAACAGAAAGACACCCCGATAGTTTACGCGAGTGAAGCCTTCCTCTACATGACAGGTTACAGCAACGCAGAGGTG
CTGGGCCGGAATTGCCGGTTTCTGCAAAGCCCTGACGGCATGGTGAAGCCCAAGAGCACCCGGAAGTACGTGGATAGTAAC
ACAATCAATACTATGCGCAAGGCAATCGACAGGAATGCCGAGGTGCAGGTTGAAGTAGTCAATTTTAAAAAGAATGGACAG
CGATTTGTTAATTTCTGACTATGATACCTGTTAGGGACGAAACAGGCGAGTATCGATACTCTATGGGATTCCAGTGCGAA
ACAGAAAGGGGGTTCTGGGGGGAGCGGGGGGGATCCGGCCCCATGATCATGAGCGGCTACGTTAACAACCCCGAGGCTACA
AACGCTCTCATCGACAAGGACGGCTGGCTGCACAGCGGCGACATCGCCTACTGGGACGAGGACGAGCACTTCTTCATCGTG
GACCGGCTGAAGAGCCTGATCAAATACAAGGGCTACCAGGTAGCCCCAGCCGAAGTGGAGAGCATCCTGCTGCAACACCCC
AACATCTTCGACGCCGGGGTGC CGGGCCTGCCGACGACGATGCCGGCGAGCTGCCGCGCGAGTCGTCGTGCTGGAACAC
GGTAAAACCATGACCGAGAAGGAGATCGTGGACTATGTGGCCAGCCAGGTTACAACCGCCAAGAAGCTGCGCGGTGGTGT
GTGTTGCTGGACGAGGTGCCTAAAGGACTGACCGGCAAGTTGGACGCCCGCAAGATCCGCGAGATTCTCATTAAAGGCCAAG
AAGGGCGGCAAGATCGCCGTGCTCGAGTCTAGAGGGCCCTTCGAAGGTAAGCCTATCCCTAACCCCTCTCCTCGGTCTCGAT
TCTACGCGTACCGGTCATCATCACCATCACCATTGA

e

pcDNA3.1/V5-His A_pMag-mCherry

>Amino acid sequence

MGLEGGSGGSGSGHTLYAPGGYDIMGYLRQIRNRPNPQVELGPVDTSCALILCDLKQKDTPIVYASEAFLYMTGYSNAEV
LGRNCRFLQSPDGMVKPKSTRKYVDSNTINTMRKAIDRNAEVQVEVNFKKNGQRFVNFLTMIPVRDETGEYRYSMGFQCE
TEGSMVSKGEEDNMAI I KEFMRFKVMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAWDILSPQFMYGSKAY
VKHPADIPDYLKLSFPEGFKWERVMNFEDEGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEASSERMPYE
DGALKGEIKQRLKLDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTI VEQYERAEGRHSTGGMDELYKLES
RGPFEKGKIPNPLLGLDSTRTGHHHHHH*

>DNA sequence

ATGGGACTCGAGGGCGGCAGTGGAGGGTCTGGTGGCAGCGGACATACTCTTTATGCCCCCGGTGGATATGACATTATGGGA
TATCTGAGGCAGATCAGGAACCGGCCAAACCCGAGGTGGAAGTGGGCCCGGTGGATACATCCTGCGCCTTGATTCTTTGT
GACCTGAAACAGAAAGACACCCGATAGTTTACGCGAGTGAAGCCTTCCTCTACATGACAGGTTACAGCAACGCAGAGGTG
CTGGGCCCGAATTGCCGTTTCTGCAAAGCCCTGACGGCATGGTGAAGCCCAAGAGCACCCGGAAGTACGTGGATAGTAAC
ACAATCAATACTATGCGCAAGGCAATCGACAGGAATGCCGAGGTGCAGGTTGAAGTAGTCAATTTTAAAAAGAATGGACAG
CGATTTGTTAATTTCTGACTATGATACCTGTTAGGGACGAAACAGGCGAGTATCGATACTCTATGGGATTCCAGTGCGAA
ACAGAAAGGATCCATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATG
GAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCGGCCCTACGAGGGCACCCAGACCGCCAAG
CTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTAC
GTGAAGCACCCCGCCGACATCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAAGTTC
GAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGC
ACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCGAG
GACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACC
TACAAGGCCAAGAAGCCCGTGAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCACAACGAGGAC
TACACCATCGTGGAACAGTACGAACGCGCCGAGGGCGGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGCTCGAGTCT
AGAGGGCCCTTCGAAGGTAAGCCTATCCCTAACCCCTCTCCTCGGTCTCGATTCTACGCGTACCGGTCATCATCACCATCAC
CATTGA

f

pcDNA3.1/V5-His A_pMag(2x)-mCherry

>Amino acid sequence

MGLEGGSGGSGSGHTLYAPGGYDIMGYLRQIRNRPNPQVELGPVDTSCALILCDLKQKDTPIVYASEAFLYMTGYSNAEV
LGRNCRFLQSPDGMVKPKSTRKYVDSNTINTMRKAIDRNAEVQVEVNFKKNGQRFVNFLTMIPVRDETGEYRYSMGFQCE
TEGSGGSGGSGSGHTLYAPGGYDIMGYLRQIRNRPNPQVELGPVDTSCALILCDLKQKDTPIVYASEAFLYMTGYSNAEV
LGRNCRFLQSPDGMVKPKSTRKYVDSNTINTMRKAIDRNAEVQVEVNFKKNGQRFVNFLTMIPVRDETGEYRYSMGFQCE
TEGSMVSKGEEDNMAIKEFMRFKVMHEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPAWDILSPQFMYGSKAY
VKHPADIPDYLKLSFPEGFKWERVMNFEDEGGVVTVTQDSSLQDGEFIYKVKLRGTNFPDGPVMQKKTMGWEASSERMPYE
DGALKGEIKQRLKLDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIQEYERAEGRHSTGGMDELYKLES
RGPFEKGKIPNPLLGLDSTRTGHHHHHH*

>DNA sequence

ATGGGACTCGAGGGCGGCAGTGGAGGGTCTGGTGGCAGCGGACATACTCTTTATGCCCCCGGTGGATATGACATTATGGGA
TATCTGAGGCAGATCAGGAACCGGCCAAACCCGCAGGTGGAAGTGGGCCCGGTGGATACATCCTGCGCCTTGATTCTTTGT
GACCTGAAACAGAAAGACACCCCGATAGTTTACGCGAGTGAAGCCTTCCTCTACATGACAGGTTACAGCAACGCAGAGGTG
CTGGGCCCGAATTGCCGTTTCTGCAAAGCCCTGACGGCATGGTGAAGCCCAAGAGCACCCGGAAGTACGTGGATAGTAAC
ACAATCAATACTATGCGCAAGGCAATCGACAGGAATGCCGAGGTGCAGGTTGAAGTAGTCAATTTTAAAAAGAATGGACAG
CGATTTGTAAATTTCTGACTATGATACCTGTTAGGGACGAAACAGGCGAGTATCGATACTCTATGGGATTCCAGTGCGAA
ACAGAAAGGATCTGGCGGCAGTGGAGGGTCTGGTGGCAGCGGACATACTCTTTATGCCCCCGGTGGATATGACATTATGGGA
TATCTGAGGCAGATCAGGAACCGGCCAAACCCGCAGGTGGAAGTGGGCCCGGTGGATACATCCTGCGCCTTGATTCTTTGT
GACCTGAAACAGAAAGACACCCCGATAGTTTACGCGAGTGAAGCCTTCCTCTACATGACAGGTTACAGCAACGCAGAGGTG
CTGGGCCCGAATTGCCGTTTCTGCAAAGCCCTGACGGCATGGTGAAGCCCAAGAGCACCCGGAAGTACGTGGATAGTAAC
ACAATCAATACTATGCGCAAGGCAATCGACAGGAATGCCGAGGTGCAGGTTGAAGTAGTCAATTTTAAAAAGAATGGACAG
CGATTTGTAAATTTCTGACTATGATACCTGTTAGGGACGAAACAGGCGAGTATCGATACTCTATGGGATTCCAGTGCGAA
ACAGAAAGGATCCATGGTGAAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATG
GAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCGCCCTACGAGGGCACCCAGACCGCCAAG
CTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTAC
GTGAAGCACCCCGCGACATCCCGACTACTTGAAGCTGTCTTCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACCTC
GAGGACGGCGGCGTGGTACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGC
ACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCGAG
GACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACC
TACAAGGCCAAGAAGCCCGTGAGCTGCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCACAACGAGGAC
TACACCATCGTGGAACAGTACGAACGCGCCGAGGGCGGCCACTCCACGGCGGCATGGACGAGCTGTACAAGCTCGAGTCT
AGAGGGCCCTTCGAAGGTAAGCCTATCCCTAACCTCTCCTCGGTCTCGATTCTACGCGTACCGGTATCATCACCATCAC

CATTGA

pcDNA3.1/V5-His A_pMag(3x)-mCherry

>Amino acid sequence

MGLEGGSGGSGSGHTLYAPGGYDIMGYLRQIRNRPNPQVELGPVDTSCALILCDLKQKDTPIVYASEAFLYMTGYSNAEV
 LGRNCRFLQSPDGMVKKPKSTRKYVDSNTINTMRKAIDRNAEVQVEVNFKKNGQRFVNFLTMIPVRDETGEYRYSMGFQCE
 TEGSGGSGGSGSGHTLYAPGGYDIMGYLRQIRNRPNPQVELGPVDTSCALILCDLKQKDTPIVYASEAFLYMTGYSNAEV
 LGRNCRFLQSPDGMVKKPKSTRKYVDSNTINTMRKAIDRNAEVQVEVNFKKNGQRFVNFLTMIPVRDETGEYRYSMGFQCE
 TEGSGGSGGSGSGHTLYAPGGYDIMGYLRQIRNRPNPQVELGPVDTSCALILCDLKQKDTPIVYASEAFLYMTGYSNAEV
 LGRNCRFLQSPDGMVKKPKSTRKYVDSNTINTMRKAIDRNAEVQVEVNFKKNGQRFVNFLTMIPVRDETGEYRYSMGFQCE
 TEGSMVSKGEEDNMAIKEFMRFKVMHEGVSNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPAWDILSPQFMYGSKAY
 VKHPADIPDYLKLSFPEGFKWERVMNFEDEGGVVTVDSSLDQGEFIYKVKLRGTNFPDGPVMQKKTMGWEASSERMPYE
 DGALKGEIKQRLKLDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIQEYERAEGRHSTGGMDELYKLES
 RGPFEKPIPNPLLGLDSTRTHHHHHH*

>DNA sequence

ATGGGACTCGAGGGCGGCAGTGGAGGGTCTGGTGGCAGCGGACATACTCTTTATGCCCCCGGTGGATATGACATTATGGGA
 TATCTGAGGCAGATCAGGAACCGGCCAAACCCGAGGTGGAAGTGGGCCCGGTGGATACATCCTGCGCCTTGATTCTTTGT
 GACCTGAAACAGAAAGACACCCGATAGTTTACGCGAGTGAAGCCTTCCTCTACATGACAGGTTACAGCAACGCAGAGGTG
 CTGGGCCGAATTGCCGTTTCTGCAAGCCCTGACGGCATGGTGAAGCCCAAGAGCACCCGGAAGTACGTGGATAGTAAC
 ACAATCAATACTATGCGCAAGGCAATCGACAGGAATGCCGAGGTGCAGGTTGAAGTAGTCAATTTTAAAAAGAATGGACAG
 CGATTTGTTAATTTCTGACTATGATACCTGTTAGGGACGAAACAGGCGAGTATCGATACTCTATGGGATTCCAGTGCAGAA
 ACAGAAAGGATCTGGCGGCAGTGGAGGGTCTGGTGGCAGCGGACATACTCTTTATGCCCCCGGTGGATATGACATTATGGGA
 TATCTGAGGCAGATCAGGAACCGGCCAAACCCGAGGTGGAAGTGGGCCCGGTGGATACATCCTGCGCCTTGATTCTTTGT
 GACCTGAAACAGAAAGACACCCGATAGTTTACGCGAGTGAAGCCTTCCTCTACATGACAGGTTACAGCAACGCAGAGGTG
 CTGGGCCGAATTGCCGTTTCTGCAAGCCCTGACGGCATGGTGAAGCCCAAGAGCACCCGGAAGTACGTGGATAGTAAC
 ACAATCAATACTATGCGCAAGGCAATCGACAGGAATGCCGAGGTGCAGGTTGAAGTAGTCAATTTTAAAAAGAATGGACAG
 CGATTTGTTAATTTCTGACTATGATACCTGTTAGGGACGAAACAGGCGAGTATCGATACTCTATGGGATTCCAGTGCAGAA
 ACAGAAAGGATCTGGCGGCAGTGGAGGGTCTGGTGGCAGCGGACATACTCTTTATGCCCCCGGTGGATATGACATTATGGGA
 TATCTGAGGCAGATCAGGAACCGGCCAAACCCGAGGTGGAAGTGGGCCCGGTGGATACATCCTGCGCCTTGATTCTTTGT
 GACCTGAAACAGAAAGACACCCGATAGTTTACGCGAGTGAAGCCTTCCTCTACATGACAGGTTACAGCAACGCAGAGGTG
 CTGGGCCGAATTGCCGTTTCTGCAAGCCCTGACGGCATGGTGAAGCCCAAGAGCACCCGGAAGTACGTGGATAGTAAC
 ACAATCAATACTATGCGCAAGGCAATCGACAGGAATGCCGAGGTGCAGGTTGAAGTAGTCAATTTTAAAAAGAATGGACAG
 CGATTTGTTAATTTCTGACTATGATACCTGTTAGGGACGAAACAGGCGAGTATCGATACTCTATGGGATTCCAGTGCAGAA
 ACAGAAAGGATCCATGGTGAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATG
 GAGGGCTCCGTGAACGGCCACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCTACGAGGGCACCCAGACCGCCAAG

CTGAAGGTGACCAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTAC
GTGAAGCACC CGCGGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAAGTTC
GAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGC
ACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCGAG
GACGGCGCCCTGAAGGGCGAGATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACC
TACAAGGCCAAGAAGCCCGTGACGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACGAGGAC
TACACCATCGTGGAACAGTACGAACGCGCGGAGGGCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGCTCGAGTCT
AGAGGGCCCTTCGAAGGTAAGCCTATCCCTAACCTCTCCTCGGTCTCGATTCTACGCGTACCGGTCATCATCACCATCAC
CATTGA

j

pcDNA3.1/V5-His A_nMagHigh1-mKikGR-CAAX

>Amino acid sequence

MGLEGGSGGSGSGHTLYAPGGYDIMGYLDQIGNRPNPQVELGPVDTSCALILCDLKQKDTPIVYASEAFLYMTGYSNAEV
LGRNCRFLQSPDGMVKPKSTRKYVDSNTINTIRKAIDRNAEVQVEVNFKNQGRFVNFLTIIIPVRDETGEYRYSMGFQCE
TEGGSGGSGGSGMSVITSEMKIELRMEGSVNGHKFVITGKGSGRPYEGTQTVDLTVIEGGPLPFAFDILTTFHYGNRVF
VEYPEEIVDYFKQSFPEGYSWERSMSFEDGGICLATNNITMKKDGSNFVNEIRFDGTNFPANGPVMQRKTVEWEPSTEKM
YVRDGVLKGDIEALLLEGGGHYRCDFRTTYKAKKVQLPDYHYVDHQMEITSHDKDYNKVKAYEHAKAYSGTYRGAKYEF
EAGGSGGQGCMLPCVVM*

>DNA sequence

ATGGGACTCGAGGGCGGCAGTGAGGGTCTGGTGGCAGCGGACATACTCTTTATGCCCCGGTGGATATGACATTATGGGA
TATCTGGACCAGATCGGCAACCGGCCAAACCCGAGGTGGAAGTGGGCCCGGTGGATACATCCTGCGCCTTGATTCTTTGT
GACCTGAAACAGAAAGACACCCGATAGTTTACGCGAGTGAAGCCTTCCTCTACATGACAGGTTACAGCAACGCAGAGGTG
CTGGGCCGAATTGCCGTTTCTGCAAAGCCCTGACGGCATGGTGAAGCCCAAGAGCACCCGGAAGTACGTGGATAGTAAC
ACAATCAATACTATCCGCAAGGCAATCGACAGGAATGCCGAGGTGCAGGTTGAAGTAGTCAATTTTAAAAAGAATGGACAG
CGATTTGTAAATTTCTGACTATCATACCTGTTAGGGACGAAACAGGCGAGTATCGATACTCTATGGGATTCCAGTGC
AACAAGAGGGGGTTCTGGGGGGAGCGGCGGGGGATCCATGGTGAGCGTGATCACCAGCGAGATGAAGATCGAGCTGAGGATG
GAGGGCTCCGTGAACGGCCACAAGTTCGTGATCACCAGGCAAGGGCAGCGGCCGGCCCTACGAGGGCAGCCAGACCGTGGAC
CTGACCGTGATCGAGGGCGGCCCTGCCCTTCGCCTTCGACATCCTGACCACCGCCTTCCACTACGGCAACAGGGTGTTT
GTGGAGTACCCCGAGGAGATCGTGGACTACTTCAAGCAGAGCTTCCCCGAGGGCTACAGCTGGGAGAGGAGCATGAGCTTC
GAGGACGGCGGCATCTGCCTGGCCACCAACAACATCACCATGAAGAAGGACGGCAGCAACACCTTCGTGAACGAGATCAGG
TTCGACGGCAGCAACTTCCCCGCCAACGGCCCCGTGATGCAGAGGAAGACCGTGGAGTGGGAGCCCAGCACCGAGAAGATG
TACGTGAGGGACGGCGTGCTGAAGGGCGACATCGAGATGGCCCTGCTGCTGGAGGGCGGCGGCCACTACAGGTGCGACTTC
AGGACCACCTACAAGGCCAAGAAGGTGGTGCAGCTGCCCGACTACCACTACGTGGACCACCAGATGGAGATCACCAGCCAC
GACAAGGACTACAACAAGGTGAAGGCCTACGAGCACGCCAAGGCCTACAGCGGCACCTACAGGGGCGCCAAGTACGAGTTC
GAGGCCGGCGGAAGTGGAGGCCAAGGTTGTATGGGTCTTCCTTGTTGTTATGTAA

k

pcDNA3.1_pMagFast2 (3x) –iRFP

>Amino acid sequence

MHTLYAPGGYDIMGYLRQIRNRPNPQVELGPVDTSCALVLCDLKQKDTPVVYASEAFLYMTGYSNAEVLGRNCRFLQSPDG
MVKPKSTRKYVDSNTINTMRKAIDRNAEVQVEVVNFKNQGRFVNFLTMIPVRDETGEYRYSMGFQCETEGSGGSGGSGGS
GHTLYAPGGYDIMGYLRQIRNRPNPQVELGPVDTSCALVLCDLKQKDTPVVYASEAFLYMTGYSNAEVLGRNCRFLQSPDG
MVKPKSTRKYVDSNTINTMRKAIDRNAEVQVEVVNFKNQGRFVNFLTMIPVRDETGEYRYSMGFQCETEGSGGSGGSGGS
GHTLYAPGGYDIMGYLRQIRNRPNPQVELGPVDTSCALVLCDLKQKDTPVVYASEAFLYMTGYSNAEVLGRNCRFLQSPDG
MVKPKSTRKYVDSNTINTMRKAIDRNAEVQVEVVNFKNQGRFVNFLTMIPVRDETGEYRYSMGFQCETEGSGGSGGSGGS
GGTGGSGGSGGSGMAEGSVARQPDLLTCDEPIHIPGAIQPHGLLLALAADMTIVAGSDNLPETGLAIGALIGRSAADV
F DSETHNRLTIALAEPGAAGVAPITVGFTMRKDAGFIGSWHRHDQLIFLELEPPQRDVAEPQAFFRRTNSAIRRLQAAETLE
SACAAAAQEVRKITGFDRVMIYRFASDFSGEVIAEDRCAEVESKGLHYPASTVPAQARRLYTINPVRIIPDINYRPVPVT
PDLNPVTGRPIDLSFAILRSVSPVHLEFMRNIGMHGTMSISILRGERLWGLIVCHHRTPYYVDLGRQACELVAQVLAWQI
GVME*

>DNA sequence

ATGCACACTCTTTACGCCCTGGAGGATACGACATTATGGGATATTTGCGGCAGATTAGGAACCGCCAAACCCTCAGGTC
GAACTGGGGCCTGTGGACACGTCATGTGCCCTGGTCTGTGCGATCTGAAGCAAAGGACACTCCGGTGGTCTACGCCTCG
GAAGCCTTCTTGATATGACCGGATACAGCAATGCAGAGGTGCTCGGCAGGAAGTGCAGATTCCTGCAGTCCCCGACGGG
ATGGTGAAACCAAAGTCGACTCGCAAATATGTGGAAGTCAACACCATGCGGAAGGCCATCGACCGGAACGCC
GAGGTCCAGGTGGAGGTGGTCAACTTTAAGAAGAACGGCCAGCGGTTCTGAACTTTCTGACCATGATTCCGGTCCGGGAT
GAAACCGGAGAGTACAGATACTCCATGGGATTCCAGTGCAGAAACCGAAGGTTCCGGCGGGCTCGGGCGGATCAGGGGGAAGC
GGACACACACTCTACGCCCGGGAGGCTACGATATCATGGGCTACCTGAGGCAGATCCGGAACAGACCGAACCCCAAGTG
GAACTCGGCCCCGTGGACACTTCTTGCGCTCTGGTGTGTGCGACCTGAAGCAGAAGGATACCCCGTGGTGTACGCATCC
GAAGCGTTCCTCTACATGACCGGTACTCGAACGCGGAGGTGCTGGGTCGCAACTGCCGTTCTGCAGAGCCCCGACGGA
ATGGTCAAGCCCAAGTCCACTCGGAAATACGTGGATTCCAACACCATCAATACTATGAGGAAGGCTATCGATAGGAACGCG
GAAGTCCAAGTGGAGGTGGTGAAGTTCAAGAAGATGGCCAGAGATTCTCAATTTCTTACCATGATCCCTGTGCGCGAT
GAGACTGGAGAGTACCGATACTCAATGGGCTTCCAGTGTGAAACCGAGGGAAGCGGCGGTTCCGGGGGGTCCGGAGGTTCC
GGACATACCCTCTACGCGCCGGGGGTTATGACATCATGGGTTACCTCAGACAGATCAGAAACCGGCCGAACCCACAAGTG
GAGCTGGGACCCGTGACACCTCTGCGCCCTCGTGTGTGTGACCTTAAGCAGAAGGACACCCCTGTGGTGTACGCCTCC
GAAGCATTCTGTACATGACCGGTACTCGAACGCGGAAGTGTGGGACGGAAGTCCGCTTCTGCAATCCCCGGATGGA
ATGGTGAAGCCTAAGTCAACCCGCAAATACGTGGACTCCAACACTATCAACACCATGCGCAAGGCCATTGACCGCAATGCT
GAGGTGCAAGTGAAGTGGTGAAGTTCAAGAAGAACGGACAGCGCTTCTCAACTTCTGACTATGATTCCCGTGGGGAC
GAAACCGGCGAATACCGGTACAGCATGGGGTTTCAGTGCAGACTGAGGGCTCCGGCGGCAGCGGTGGCTCCGGAGGCAGC
GGAGGTACCGGCGGCAGTGGAGGGTCTGGTGGCAGCGGAATGGCCGAAGGGAGTGTGCCAGACAGCCTGACCTGCTGACT

TGCGATGACGAACCAATCCACATTCCCGGCGCTATCCAGCCCCACGGACTGCTGCTGGCTCTGGCCGCTGACATGACTATC
GTGGCAGGCTCTGATAACCTGCCAGAGCTGACCGGGCTGGCTATCGGAGCACTGATTGGCCGGTCCGCAGCCGACGTGTTCC
GATTCTGAGACTCACAATAGACTGACCATCGCCCTGGCTGAACCTGGAGCCGCGTGGGAGCCCCAATTACCGTCGGCTTC
ACAATGCGAAAGGACGCTGGCTTTATCGGGTCCTGGCACCGCCATGATCAGCTGATTTTTCTGGAGCTGGAACCACCTCAG
AGAGACGTGGCAGAGCCACAGGCTTTCTTTCGGAGAACAAACAGCGCCATCCGGCGGCTCCAGGCCGCTGAGACTCTGGAA
TCCGCTTGCGCAGCCGCTGCACAGGAAGTGAGGAAGATCACCGGGTTCGATCGCGTCATGATCTACCGTTTCGCCTCAGAC
TTTAGCGGAGAAGTGATCGCAGAAGATCGCTGTGCCGAGGTGAAAAGTAACTGGGCCTGCATTACCCAGCCTCAACCGTG
CCTGCACAGGCACGACGGCTGTATACAATCAACCCAGTCCGGATCATTCCCGACATTAATTACAGACCAGTGCCCGTCACA
CCTGACCTGAACCCAGTGACTGGGAGGCCCATCGACCTGAGCTTCGCCATTCTGCGCAGCGTGTCCCCTGTCCACCTGGAG
TTTATGAGGAATATCGGAATGCATGGCACCATGTCTATCAGTATTCTGAGAGGCGAAAGGCTGTGGGGGCTGATCGTGTGC
CACCATCGGACACCTTACTATGTGGACCTGGACGGCAGGCAGGCTTGTGAACTGGTCGCTCAGGTGCTGGCTTGGCAGATT
GGCGTGATGGAAGAGTAA

I

pcDNA3.1_nMagHigh1-EGFP-CAAX

>Amino acid sequence

MGHTLYAPGGYDIMGYLDQIGNRPNPQVELGPVDTSCALILCDLKQKDTPIVYASEAFLYMTGYSNAEVLGRNCRFLQSPD
GMVKPKSTRKYVDSNTINTIRKAIDRNAEVQVEVNFKNQGRFVNFLTIIIPVRDETGEYRYSMGFQCETE
GSGSGSGSGSG
GSGSGSMVSKGEELFTGVVPILEVELDGDVNGHKFSVSGEGEGDATYGKLTCLKFICTTGKLPVPWPTLVTTLT
YGVQCFSRY
PDHMKQHDFFSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYN
SHNVYIMAD
KQKNGIKVNFKIRHNIEDGVSQVLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMLLEFVTAAG
ITLGMDEL
YKGKKKKKKSKTKCVIM*

>DNA sequence

ATGGGACACACTCTTTACGCCCTGGAGGATACGACATTATGGGATATTTGGATCAGATTGGAACGCCCCAAACCTCAG
GTCGAACTGGGGCCTGTGGACACGTCATGTGCCCTGATCCTGTGCGATCTGAAGCAAAGGACACTCCGATCGTCTACGCC
TCGGAAGCCTTCTTGTATATGACCGGATACAGCAATGCAGAGGTGCTCGGCAGGAAGTGCAGATTCTGCAGTCCCCGAC
GGGATGGTGAAACCAAAGTCGACTCGAAATATGTGGACTCGAACACGATCAACACCATCCGGAAGGCCATCGACCGGAAC
GCCGAGGTCCAGGTGGAGGTGGTCAACTTTAAGAAGAACGCCAGCGGTTCTGTAACCTTCTGACCATCATTCCGGTCCGG
GATGAAACCGGAGAGTACAGATACTCCATGGGATTCCAGTGCGAAACCGAAGCGGCTCCGGAGGCAGCGGCGGTTCTGGA
GGTTCCGGTGGCGGCTCCATGGTGTGAAGGGAGAGGAGCTTTTCACCGGCGTGGTGCCGATTCTTGTGGAAGTGGACGGC
GACGTGAACGGACATAAGTTCAGCGTGTGAGGAGAAGGAGAGGGGACGCGACTTACGGAAGCTGACCCTGAAGTTCATC
TGTACCACCGGGAAGCTCCCGGTGCCCTGGCCGACCCTCGTGAAGTCTGACCTACGGAGTGCAGTGCTTCTCCCGCTAC
CCGGACCATATGAAGCAGCAGGATTTCTTCAAGAGCGCTATGCCCGAGGGTTACGTGCAAGAACGGACCATCTTCTTCAAG
GACGACGGAACTACAAGACCAGAGCCGAAGTGAAATTCGAAGGAGACACCCTCGTCAATCGCATCGAGCTGAAGGGAATC
GACTTTAAGGAAGATGGGAATATCCTGGGTGACAAGCTGGAGTACAAGTACAAGTCCACAATGTGTACATCATGGCGGAT
AAGCAAAGAAGCGGATTAAGGTCAACTTCAAGATCCGGCATAACATAGAAGATGGCTCCGTGCAAGTGGCGGACCACTAC
CAGCAGAACACCCCCATTGGGGACGGTCCGGTGCTGCTCCCTGATAACCACTACCTGTCAACCCAAAGCGCACTGTCCAAG
GACCCCAACGAGAAGAGGGACCATGGTGTGCTGGAGTTCGTCACTGCCGCTGGCATCACTCTCGGAATGGACGAGCTG
TACAAGGGGAAGAAAAAGAAGAAGTCCAAGACAAATGCGTGATTATGTAG

m

pcDNA3.1_iSH2-pMag (3x)-iRFP

>Amino acid sequence

MVSKYQQDQVVKEDNIEAVGKKLHEYNTQFQEKSRDYRLYEDYTRTSQEIQMKRTAIEAFNETIKIFEEQCQTQERYSK
YIEKFKREGNETEIQRIIMHNYEKLKSRISEIVDSRRRLEEDLKKQAAEYREIDKRMNSIKPDLIQLRKTRDQYLMWL
TQKV
VRQKKLNEWLGNENTEDQYSLVEDDEDLPHHDEKTGSGGSGGSGGSGGSGGSGHTLYAPGGYDIMGYLRQIRNRPNPQVEL
GPVDTSCALILCDLKQKDTPIVYASEAFLYMTGYSNAEVLGRNCRFLQSPDGMVKKPKSTRKYVDSNTINTMRKAIDRNAEV
QVEVVNFKKNGQRFVNFLTMIPVRDETEYRYSMGFQCETEGSGGSGGSGGSGHTLYAPGGYDIMGYLRQIRNRPNPQVEL
GPVDTSCALILCDLKQKDTPIVYASEAFLYMTGYSNAEVLGRNCRFLQSPDGMVKKPKSTRKYVDSNTINTMRKAIDRNAEV
QVEVVNFKKNGQRFVNFLTMIPVRDETEYRYSMGFQCETEGSGGSGGSGGSGHTLYAPGGYDIMGYLRQIRNRPNPQVEL
GPVDTSCALILCDLKQKDTPIVYASEAFLYMTGYSNAEVLGRNCRFLQSPDGMVKKPKSTRKYVDSNTINTMRKAIDRNAEV
QVEVVNFKKNGQRFVNFLTMIPVRDETEYRYSMGFQCETEGSGGSGGSGGSGGTGGSGGSGGSGMAEGSVARQPDLLTCD
DEPIHIPGAIQPHGLLLALAADMTIVAGSDNLPETGLAIGALIGRSAADVDFSETHNRLTIALAEPGAAGVAPITVGFTM
RKDAGFIGSWHRHDQLIFLELEPPQRDVAEPQAFFRRTNSAIRRLQAAETLESACAAAQEVRKITGFDRVMIYRFASDFS
GEVIAEDRCAEVESKLGHPASTVPAQARRLYTINPVRIIPDINRYPVPTPDLPNVTGRPIDLSFAILRSVSPVHLEFM
RNIGMHGTMSISILRGERLWGLIVCHHRTPYYVDLDGRQACELVAQVLAWQIGVME*

>DNA sequence

ATGGTGTCCAAATACCAACAGGATCAAGTTGTCAAAGAAGATAATATTGAAGCTGTAGGGAAAAAATTACATGAATATAAC
ACTCAATTTCAAGAAAAAGTCGGGAATATGATAGATTATATGAAGACTATACCCGAACCTCCAGGAAATCCAAATGAAA
AGAACAGCTATTGAAGCATTTAATGAAACCATAAAAAATATTTGAAGAACAGTGCCAAACCCAGGAGCGGTACAGCAAAGAA
TACATAGAAAAGTTCAAACGTGAAGGCAACGAGACGGAAATCCAAAGGATTATGCATAATTATGAAAAGTTAAAGTCTCGA
ATCAGTGAAATTGTGACAGCAGAAGGAGATTGGAAGAGGACTTGAAGAAGCAGGCGGCTGAGTATCGTGAGATTGACAAA
CGCATGAACAGCATTAAACCAGACCTCATTGAGCTAAGAAAGACGAGAGACCAATACTTGATGTGGTTGACTCAGAAAGGT
GTTCCGCAGAGAAATTAACGAGTGGCTGGGCAATGAAAACACTGAAGACCAATATTCGCTGGTAGAAGATGATGAGGAT
TTGCCCCACCACGATGAGAAGACTGGATCCGGCGGCTCCGGAGGCAGCGCGGTTCTGGAGGTTCCGGTGGCGGCTCCAC
ACTCTTTACGCCCTGGAGGATACGACATTATGGGATATTTGCGGCAGATTAGGAACGCCCAAACCTCAGGTGGAAGT
GGGCTGTGGACACGTATGTGCCCTGATCCTGTGCGATCTGAAGCAAAGGACACTCCGATCGTCTACGCCTCGGAAGCC
TTCTTGATATGACCGGATACAGCAATGCAGAGGTGCTCGGCAGGAAGTGCAGATTCTGCAGTCCCCGACGGGATGGTG
AAACCAAAGTCGACTCGAAATATGTGGACTCGAACACGATCAACACCATGCGGAAGGCCATCGACCGGAACGCCGAGGTC
CAGGTGGAGGTGGTCAACTTTAAGAAGAACGGCCAGCGGTTCTGAACTTTCTGACCATGATTCCGGTCCGGGATGAAACC
GGAGAGTACAGATACTCCATGGGATTCCAGTGCGAAACCGAAGGTTCCGGCGGCTCGGGCGGATCAGGGGAAGCGGACAC
ACACTCTACGCCCGGGAGGCTACGATATCATGGGCTACCTGAGGCAGATCCGGAACAGACCGAACCCCAAGTGGAAGTC
GGCCCCGTGGACACTTCTTGCGCTCTGATCCTGTGCGACCTGAAGCAGAAGGATACCCCATCGTGTACGCATCCGAAGCG
TTCTCTACATGACCGGGTACTCGAACCGGAGGTGCTGGGTGCAACTGCCGTTCTCTGCAGAGCCCCGACGGAATGGTC

AAGCCCAAGTCCACTCGGAAATACGTGGATTCCAACACCATCAATACTATGAGGAAGGCTATCGATAGGAACGCGGAAGTC
CAAGTGGAGGTGGTGAACCTCAAGAAGAATGGCCAGAGATTGTCATTTTCCTTACCATGATCCCTGTGCGCGATGAGACT
GGAGAGTACCGATACTCAATGGGCTTCCAGTGTGAAACCGAGGGAAGCGGCGGTCCGGGGGGTTCGGGAGGTTCGGACAT
ACCCTCTACGCGCCGGGGGGTTATGACATCATGGGTTACCTCAGACAGATCAGAAACCGGCCGAACCCACAAGTGGAGCTG
GGACCCGTGACACCTCCTGCGCCCTCATCCTGTGTGACCTTAAGCAGAAGGACACCCCTATCGTGTACGCCTCCGAAGCA
TTCCTGTACATGACCGGGTACTCGAACGCCGAAGTGCTGGGACGGAAGTCCGCTTCCTGCAATCCCGGATGGAATGGTG
AAGCCTAAGTCAACCCGAAATACGTGGACTCCAACACTATCAACACCATGCGCAAGGCCATTGACCGCAATGCTGAGGTG
CAAGTGGAAAGTGGTGAACCTCAAGAAGAACGGACAGCGCTTCGTCAACTTCCTGACTATGATTCCCGTGCGGGACGAAACC
GGCGAATACCGGTACAGCATGGGGTTTCAAGTGCAGACTGAGGGCTCCGGCGGCAGCGGTGGCTCCGGAGGCAGCGGAGGT
ACCGGCGGCAGTGGAGGGTCTGGTGGCAGCGGAATGGCCGAAGGGAGTGTGCCAGACAGCCTGACCTGCTGACTTGCGAT
GACGAACCAATCCACATTCGCGGCGCTATCCAGCCCCACGGACTGCTGCTGGCTCTGGCCGTGACATGACTATCGTGGCA
GGCTCTGATAACCTGCCAGAGCTGACCGGGCTGGCTATCGGAGCACTGATTGGCCGGTCCGCAGCCGACGTGTTGATTCT
GAGACTCACAATAGACTGACCATCGCCCTGGCTGAACCTGGAGCCGCGTGGGAGCCCCAATTACCGTCCGGCTTCACAATG
CGAAAGGACGCTGGCTTTATCGGGTCCTGGCACC GCCATGATCAGCTGATTTTTCTGGAGCTGGAACCACCTCAGAGAGAC
GTGGCAGAGCCACAGGCTTTCTTTCGGAGAACAACAGCGCCATCCGGCGGCTCCAGGCCGTGAGACTCTGGAATCCGCT
TGCGCAGCCGCTGCACAGGAAGTGAGGAAGATCACCGGGTTCGATCGCGTCATGATCTACCGGTTGCCTCAGACTTTAGC
GGAGAAGTGATCGCAGAAGATCGCTGTGCCGAGGTGAAAAGTAACTGGGCCTGCATTACCCAGCCTCAACCGTGCCTGCA
CAGGCACGACGGCTGTATACAATCAACCCAGTCCGGATCATTCCCGACATTAATTACAGACCAGTGCCCGTCACACCTGAC
CTGAACCCAGTGACTGGGAGGCCCATCGACCTGAGCTTCGCCATTCTGCGCAGCGTGTCCCCTGTCCACCTGGAGTTTATG
AGGAATATCGGAATGCATGGCACCATGTCTATCAGTATTCTGAGAGGCGAAAGGCTGTGGGGGCTGATCGTGTGCCACCAT
CGGACACCTTACTATGTGGACCTGGACGGCAGGCAGGCTTGTAAGTGGTCCGTCAGGTGCTGGCTTGGCAGATTGGCGTG
ATGGAAGAGTAA

n

pcDNA3.1_iSH2-pMagFast2 (3x)-iRFP

>Amino acid sequence

MVSKYQQDQVVKEDNIEAVGKKLHEYNTQFQEKSREYDRLYEDYTRTSQEIQMKRTAIEAFNETIKIFEEQCQTQERYSK
YIEKFKREGNETEIQRIIMHNYEKLKSRISEIVDSRRRLEEDLKKQAAEYREIDKRMNSIKPDLIQLRKTRDQYLMWLTKG
VRQKKLNEWLGNENTEDQYSLVEDDEDLPHHDEKTGSGGSGGSGGSGGSGGSGHTLYAPGGYDIMGYLRQIRNRPNPQVEL
GPVDTSCALVLCDLKQKDTPVVYASEAFLYMTGYSNAEVLGRNCRFLQSPDGMVKKPKSTRKYVDSNTINTMRKAIDRNAEV
QVEVVNFKKNGQRFVNFLTMIPVRDETGEYRYSMGFQCETEGSGGSGGSGGSGHTLYAPGGYDIMGYLRQIRNRPNPQVEL
GPVDTSCALVLCDLKQKDTPVVYASEAFLYMTGYSNAEVLGRNCRFLQSPDGMVKKPKSTRKYVDSNTINTMRKAIDRNAEV
QVEVVNFKKNGQRFVNFLTMIPVRDETGEYRYSMGFQCETEGSGGSGGSGGSGHTLYAPGGYDIMGYLRQIRNRPNPQVEL
GPVDTSCALVLCDLKQKDTPVVYASEAFLYMTGYSNAEVLGRNCRFLQSPDGMVKKPKSTRKYVDSNTINTMRKAIDRNAEV
QVEVVNFKKNGQRFVNFLTMIPVRDETGEYRYSMGFQCETEGSGGSGGSGGSGGTGGSGGSGGSGMAEGSVARQPDLLTCD
DEPIHIPGAIQPHGLLLALAADMTIVAGSDNLPETGLAIGALIGRSAADVFDSETHNRLTIALAEPGAAVGAPITVGFTM
RKDAGFIGSWHRHDQLIFLELEPPQRDVAEPQAFFRRTNSAIRRLQAAETLESACAAAQEVRKITGFDRVMIYRFASDFS
GEVIAEDRCAEVESKLGHPASTVPAQARRLYTINPVRIIPDINRYPVPVTPDLNPVTGRPIDLSFAILRSVSPVHLEFM
RNIGMHGTMSISILRGERLWGLIVCHHRTPYVVDLGRQACELVAQVLAWQIGVMEE*

>DNA sequence

ATGGTGTCCAAATACCAACAGGATCAAGTTGTCAAAGAAGATAATATTGAAGCTGTAGGGAAAAAATTACATGAATATAAC
ACTCAATTTCAAGAAAAAGTCGGGAATATGATAGATTATATGAAGACTATACCCGAACCTCCCAGGAAATCCAAATGAAA
AGAACAGCTATTGAAGCATTTAATGAAACCATAAAAAATATTTGAAGAACAGTGCCAAACCCAGGAGCGGTACAGCAAAGAA
TACATAGAAAAGTTCAAACGTGAAGGCAACGAGACGGAAATCCAAAGGATTATGCATAATTATGAAAAGTTAAAGTCTCGA
ATCAGTGAAATTGTCGACAGCAGAAGGAGATTGGAAGAGGACTTGAAGAAGCAGGCGGCTGAGTATCGTGAGATTGACAAA
CGCATGAACAGCATTAAACCAGACCTCATTGAGCTAAGAAAGACGAGAGACCAATACTTGATGTGGTTGACTCAGAAAGGT
GTTCCGCAGAGAAATTAACGAGTGGCTGGGCAATGAAAACACTGAAGACCAATATTCGCTGGTAGAAGATGATGAGGAT
TTGCCCCACCACGATGAGAAGACTGGATCCGGCGGCTCCGGAGGCAGCGCGGTTCTGGAGGTTCCGGTGGCGGCTCCAC
ACTCTTTACGCCCTGGAGGATACGACATTATGGGATATTTGCGGCAGATTAGGAACGCCCAAACCTCAGGTGGAAGT
GGGCCTGTGGACACGTCATGTGCCCTGGTCTGTGCGATCTGAAGCAAAGGACACTCCGGTGGTCTACGCCTCGGAAGCC
TTCTTGATATGACCGGATACAGCAATGCAGAGGTGCTCGGCAGGAAGTGCAGATTCTGCAGTCCCCGACGGGATGGTG
AAACCAAAGTCGACTCGCAAATATGTGGACTCGAACACGATCAACACCATGCGGAAGGCCATCGACCGGAACGCCGAGGTC
CAGGTGGAGGTGGTCAACTTTAAGAAGAACGCCAGCGGTTCTGTAACCTTCTGACCATGATTCCGGTCCGGGATGAAACC
GGAGAGTACAGATACTCCATGGGATTCAGTGCGAAACCGAAGGTTCCGGCGGCTCGGGCGGATCAGGGGAAGCGGACAC
ACACTCTACGCCCGGGAGGCTACGATATCATGGGCTACCTGAGGCAGATCCGGAACAGACCGAACCCCAAGTGGAAGTC
GGCCCCGTGGACACTTCTTGCGCTCTGGTGTGTGCGACCTGAAGCAGAAGGATACCCCGTGGTGTACGCATCCGAAGCG
TTCTCTACATGACCGGGTACTCGAACCGGAGGTGCTGGGTGCGAACTGCCGTTCTCTGCAGAGCCCCGACGGAATGGTC

AAGCCCAAGTCCACTCGGAAATACGTGGATTCCAACACCATCAATACTATGAGGAAGGCTATCGATAGGAACGCGGAAGTC
CAAGTGGAGGTGGTGAACCTCAAGAAGAATGGCCAGAGATTCTCAATTTCTTACCATGATCCCTGTGCGCGATGAGACT
GGAGAGTACCGATACTCAATGGGCTTCCAGTGTGAAACCGAGGGAAGCGGCGGTCCGGGGGGTTCGGGAGGTTCGGACAT
ACCCTCTACGCGCCGGGGGGTTATGACATCATGGGTTACCTCAGACAGATCAGAAACCGGCCGAACCCACAAGTGGAGCTG
GGACCCGTGACACCTCCTGCGCCCTCGTGTGTGACCTTAAGCAGAAGGACACCCCTGTGGTGTACGCCTCCGAAGCA
TTCCTGTACATGACCGGGTACTCGAACGCCGAAGTGTGGGACGGAAGTCCGCTTCCTGCAATCCCGGATGGAATGGTG
AAGCCTAAGTCAACCCGAAATACGTGGACTCCAACACTATCAACACCATGCGCAAGGCCATTGACCGCAATGCTGAGGTG
CAAGTGGAAAGTGGTGAACCTCAAGAAGAACGGACAGCGCTTCGTCAACTTCCTGACTATGATTCCCGTGGGGACGAAACC
GGCGAATACCGGTACAGCATGGGGTTTCAAGTGGGAGCTGAGGGCTCCGGCGGCAGCGGTGGCTCCGGAGGCAGCGGAGGT
ACCGGCGGCAGTGGAGGGTCTGGTGGCAGCGGAATGGCCGAAGGGAGTGTGCCAGACAGCCTGACCTGCTGACTTGCGAT
GACGAACCAATCCACATTCGCGGCGCTATCCAGCCCCACGGACTGCTGCTGGCTCTGGCCGTGACATGACTATCGTGGCA
GGCTCTGATAACCTGCCAGAGCTGACCGGGCTGGCTATCGGAGCACTGATTGGCCGGTCCGCAGCCGACGTGTTGATTCT
GAGACTCACAATAGACTGACCATCGCCCTGGCTGAACCTGGAGCCGCGTGGGAGCCCCAATTACCGTGGGCTTCACAATG
CGAAAGGACGCTGGCTTTATCGGGTCCTGGCACC GCCATGATCAGCTGATTTTTCTGGAGCTGGAACCACCTCAGAGAGAC
GTGGCAGAGCCACAGGCTTTCTTTCGGAGAACAACAGCGCCATCCGGCGGCTCCAGGCCGTGAGACTCTGGAATCCGCT
TGCGCAGCCGCTGCACAGGAAGTGAGGAAGATCACCGGGTTCGATCGCGTCATGATCTACCGGTTGCCTCAGACTTTAGC
GGAGAAGTGATCGCAGAAGATCGCTGTGCCGAGGTGAAAAGTAACTGGGCCTGCATTACCCAGCCTCAACCGTGCCTGCA
CAGGCACGACGGCTGTATACAATCAACCCAGTCCGGATCATTCCCGACATTAATTACAGACCAGTGCCCGTCACACCTGAC
CTGAACCCAGTGACTGGGAGGCCCATCGACCTGAGCTTCGCCATTCTGCGCAGCGTGTCCCCTGTCCACCTGGAGTTTATG
AGGAATATCGGAATGCATGGCACCATGTCTATCAGTATTCTGAGAGGCGAAAGGCTGTGGGGGCTGATCGTGTGCCACCAT
CGGACACCTTACTATGTGGACCTGGACGGCAGGCAGGCTTGTGAACTGGTCCGCTCAGGTGCTGGCTTGGCAGATTGGCGTG
ATGGAAGAGTAA

O

pcDNA3.1_Tiam1-pMagFast2 (3x)-iRFP

>Amino acid sequence

MRQLSDADKLRKVICELLETERTYVKDLNCLMERYLKPLQKETFLTQDELDFLGNLTEMVEFQVEFLKTLEDGVRLVPDL
EKLEKVDQFKKVLFSLGGSFLLYADRFKLYSAFCASHTKVPKVLVKAKTDTAFKAFLDAQNPRQQHSSTLESYLKPIQRV
LKYPLLLRELFALTDAESEEHYHLDVAIKTMNKVASHINEMQKIH EEF GAVFDQLIAEQTGEKKEVADLSMGDLLHTSVI
WLNPPASLGKWKKEPELAAFVFKTAVVLVYKDGSKQKKLVGSHRLSIYEEWDPFRFRHMIPTALQVRALPSADAEANAV
CEIVHKSESEGRPERVFHLCSSPESRKDFLKS VHSILRDKHRRQLLKTEGSGSGSGSGSGSGSGSGSHTLYAPGGYDIM
GYLRQIRNRPNPQVELGPVDTSCALVLCDLKQKDTPVVYASEAFLYMTGYSNAEVLGRNCRFLQSPDGMVKPKSTRKYVDS
NTINTMRKAIDRNAEVQVEVVNFKNQGQRFVNFLTMIPVRDETGEYRYSMGFQCETEGSGSGSGSGSGSHTLYAPGGYDIM
GYLRQIRNRPNPQVELGPVDTSCALVLCDLKQKDTPVVYASEAFLYMTGYSNAEVLGRNCRFLQSPDGMVKPKSTRKYVDS
NTINTMRKAIDRNAEVQVEVVNFKNQGQRFVNFLTMIPVRDETGEYRYSMGFQCETEGSGSGSGSGSGSHTLYAPGGYDIM
GYLRQIRNRPNPQVELGPVDTSCALVLCDLKQKDTPVVYASEAFLYMTGYSNAEVLGRNCRFLQSPDGMVKPKSTRKYVDS
NTINTMRKAIDRNAEVQVEVVNFKNQGQRFVNFLTMIPVRDETGEYRYSMGFQCETEGSGSGSGSGSGSGTGGSGSGSGSG
MAEGSVARQPDLLTCDDEPIHIPGAIQPHGLLLALAADMTIVAGSDNLP E LTGLAIGALIGRSAADVDFDSETHNRLTIALA
EPGAAVGAPITVGFTMRKDAGFIGSWHRHDQLIFLELEPPQRDVAEPQAFFRRTNSAIRRLQAAETLESACAAAAQEVRI
TGFDRVMIYRFASDFSGEVIAEDRCAEVESKLG LHY PASTVPAQARRLYTINPVRIIPDINYPVPVTPDLNPVTGRPIDL
SFAILRSVSPVHLEFMRNIGMHGTMSISILRGERLWGLIVCHHRTPIYYVDLDGRQACELVAQVLAWQIGVMEE*

>DNA sequence

ATGCGACAGCTGTCTGGATGCGGATAAGCTGCGCAAGGTGATCTGTGAAGTCTGCTGGAGACTGAACGCACCTATGTGAAAGAC
TTAAACTGCCTCATGGAGAGATACCTGAAGCCCCTTCAGAAGGAGACCTTTCTCACCCAGGATGAGCTTGATGTACTTTTT
GGAAATTTAACCGAAATGGTGGAGTTTCAAGTCGAGTTCCTTAAGACTCTAGAAGATGGAGTAAGACTGGTCCCTGACTTG
GAAAAGCTGGAGAAGTTGACCAGTTCAGAAAGTGCTCTTCTCTCTGGGGGATCCTTCCTGTACTACGCGGACCGCTTC
AAGCTCTACAGTGCCTTCTGTGCGAGCCACACAAAAGTCCCCAAGGTCTGGTGAAAGCCAAGACGGACACAGCCTTCAAG
GCGTTCCTCGATGCCAGAACCCGAGGCAGCAGCACTCATCCACGCTGGAGTCTTACCTCATCAAGCCCATCCAGAGGGTC
CTCAAGTACCCGCTTCTGCTCAGGGAGCTGTTTGCGCTGACCGATGCGGAGAGCGAGGAACACTACCACCTGGACGTGGCC
ATCAAGACTATGAACAAGGTGCGCAGTCACATCAATGAGATGCAGAAGATCCACGAAGAGTTTGGTGCTGTGTTTGACCAG
CTGATTGCTGAGCAGACGGGAGAGAAGAAAGAGGTTGCAGATCTGAGCATGGGTGACCTGCTCTTGACACCAGCGTCATC
TGGCTGAACCCACCCGCCTCACTGGGAAAGTGGA AAAAGGAGCCAGAATTAGCAGCCTTCGTCTTCAAACGGCCGTGGTC
CTTGATATAAAGACGGTCCAAGCAGAAGAAGAACTTGTTGGCTCTCACAGGCTGTCAATCTACGAGGAGTGGGACCCT
TTCCGGTTTCGCCACATGATCCCTACCGAAGCTTTGCAGGTCGAGCTCTGCCAGTGCAGATGCAGAGGCAAATGCGGTA
TGCGAAATTGTCCACGTGAAATCAGAGTCAGAAGGGAGGCCCGAGCGGGTTTTCCACCTCTGCTGCAGTTCGCCAGAGAGC
AGAAAGGACTTTCTGAAGTCTGTGCATTGATCCTGCGAGATAAACACAGAAGACAGCTCCTCAAACGGAAAGGATCCGGC
GGCTCCGGAGGCAGCGGGCGTTCTGGAGGTTCCGGTGGCGGCTCCACACTCTTTACGCCCTGGAGGATACGACATTATG

GGATATTTGCGGCAGATTAGGAACCGCCAAACCTCAGGTGGAAGTGGGGCCTGTGGACACGTATGTGCCCTGGTCTG
TGCGATCTGAAGCAAAGGACACTCCGGTGGTCTACGCCTCGGAAGCCTTCTTGTATATGACCGGATACAGCAATGCAGAG
GTGCTCGGCAGGAAGTGCAGATTCTGCAGTCCCCGACGGGATGGTGAACCAAAGTCGACTCGCAAATATGTGGACTCG
AACACGATCAACACCATGCGGAAGGCCATCGACCGGAACGCCGAGGTCCAGGTGGAGGTGGTCAACTTTAAGAAGAACGGC
CAGCGGTTCTGTAACCTTCTGACCATGATTCCGGTCCGGGATGAAACCGGAGAGTACAGATACTCCATGGGATTCCAGTGC
GAAACCGAAGGTTCCGGCGGCTCGGGCGGATCAGGGGGAAGCGGACACACACTCTACGCCCCGGGAGGCTACGATATCATG
GGCTACCTGAGGCAGATCCGGAACAGACCGAACCCCAAGTGGAAGTGGGCCCCGTGGACACTTCTTGCGCTCTGGTCTG
TGCGACCTGAAGCAGAAGGATACCCCGTGGTGTACGCATCCGAAGCGTTCTCTACATGACCGGGTACTCGAACGCGGAG
GTGCTGGGTGCAACTGCCGTTCTGCAGAGCCCCGACGGAATGGTCAAGCCCAAGTCCACTCGGAAATACGTGGATTCC
AACACCATCAATACTATGAGGAAGGCTATCGATAGGAACGCGGAAGTCCAAGTGGAGGTGGTGAAGTTCAAGAAGAATGGC
CAGAGATTCTGTAATTTCTTACCATGATCCCTGTGCGCGATGAGACTGGAGAGTACCGATACTCAATGGGCTTCCAGTGT
GAAACCGAGGGAAGCGGCGGTTCCGGGGGTGCGGAGGTTCCGGACATACCCTCTACGCGCCGGGGGTTATGACATCATG
GGTTACCTCAGACAGATCAGAAACCGGCCGAACCCACAAGTGAGCTGGGACCCGTGACACCTCTGCGCCCTCTGCTGTG
TGTGACCTTAAGCAGAAGGACACCCCTGTGGTGTACGCCTCCGAAGCATTCTGTACATGACCGGGTACTCGAACGCGGAA
GTGCTGGGACGGAAGTGCCGTTCTGCAATCCCGGATGGAATGGTGAAGCCTAAGTCAACCCGCAAATACGTGGACTCC
AACACTATCAACACCATGCGCAAGGCCATTGACCGCAATGCTGAGGTGCAAGTGAAGTGGTGAAGTTCAAGAAGAACGGA
CAGCGCTTCTGTAACCTCTGACTATGATTCCCGTGGGGGACGAAACCGGCGAATACCGGTACAGCATGGGGTTTCAGTGC
GAGACTGAGGGCTCCGGCGGCAGCGGTGGCTCCGGAGGCAGCGGAGGTACCGGCGGCAGTGGAGGGTCTGGTGGCAGCGGA
ATGGCCGAAGGGAGTGTGCGCAGACAGCCTGACCTGCTGACTTGCGATGACGAACCAATCCACATTCGCGGCGCTATCCAG
CCCCACGGACTGCTGCTGGCTCTGGCCGCTGACATGACTATCGTGGCAGGCTCTGATAACCTGCCAGAGCTGACCGGGCTG
GCTATCGGAGCACTGATTGGCCGGTCCGACGCCGACGTGTTGATTCTGAGACTCACAATAGACTGACCATCGCCCTGGCT
GAACCTGGAGCCGCCGTGGGAGCCCCAATTACCGTCCGCTTCAATGCGAAAGGACGCTGGCTTTATCGGGTCTGGCAC
CGCCATGATCAGCTGATTTTTCTGGAGCTGGAACACCTCAGAGAGACGTGGCAGAGCCACAGGCTTTCTTTGCGAGAACA
AACAGCGCCATCCGGCGGCTCCAGGCCGCTGAGACTCTGGAATCCGCTTGCGCAGCCGCTGCACAGGAAGTGAGGAAGATC
ACCGGGTTCGATCGCGTCATGATCTACCGGTTGCGCTCAGACTTTAGCGGAGAAGTGATCGCAGAAGATCGCTGTGCCGAG
GTCGAAAGTAACTGGGCCTGCATTACCCAGCCTCAACCGTGCCTGCACAGGCACGACGGCTGTATACAATCAACCCAGTC
CGGATCATTCCCGACATTAATTACAGACCAGTGCCCGTACACCTGACCTGAACCCAGTGACTGGGAGGCCCATCGACCTG
AGCTTCGCCATTCTGCGCAGCGTGTCCCCTGTCCACCTGGAGTTTATGAGGAATATCGGAATGCATGGCACCATGTCTATC
AGTATTCTGAGAGGCGAAAGGCTGTGGGGGCTGATCGTGTGCCACCATCGGACACCTTACTATGTGGACCTGGACGGCAGG
CAGGCTTGTGAAGTGGTCGCTCAGGTGCTGGCTTGGCAGATTGGCGTGATGGAAGAGTAA

p

pcDNA3.1_AktPH-mCherry

>Amino acid sequence

MSDVAIVKEGWLHKRGEYIKTWRPRYFLLKNDGTFIGYKERPDVDQREAPLNNFSVAQCQLMKTERPRPNTFII RCLQWT
TVIERTFHVETPEEREETTAIQTVADGLKKQEEEEEMDFRSGSPSDNSGAEEMEVS LAKPKHRVTMNEFEYLKLLGKGTFG
KVEFGSGGGGSGGGGSGGRSMVSKGEEDNMAI I KEFMRFKVHMEG SVNGHEFE I EGEGGRPYEGTQTAKLKVTKGGPLP
FAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVM
QKKTMGWEASSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTI VEQYERA
EGRHSTGGMDELYK*

>DNA sequence

ATGAGCGACGTGGCTATTGTGAAGGAGGGTTGGCTGCACAAACGAGGGGAGTACATCAAGACCTGGCGGCCACGCTACTTC
CTCCTCAAGAATGATGGCACCTTCATTGGCTACAAGGAGCGGCCGAGGATGTGGACCAACGTGAGGCTCCCCCAACAAC
TTCTCTGTGGCGCAGTGCCAGCTGATGAAGACGGAGCGGCCCGGCCAACACCTTCATCATCCGCTGCCTGCAGTGGACC
ACTGTCAATCGAACGCACCTTCATGTGGAGACTCCTGAGGAGCGGGAGGAGTGGACAACCGCCATCCAGACTGTGGCTGAC
GGCCTCAAGAAGCAGGAGGAGGAGGAGATGGA CTTCGGTCTGGGCTCAGGAGTGAACACTCAGGGGCTGAAGAGATGGAG
GTGTCCCTGGCCAAGCCCAAGCACC GCGTGACCATGAACGAGTTTGAGTACCTGAAGCTGCTGGGCAAGGGCACTTTCTGGC
AAGGTGGAATTCGGAGGGTCTGGAGGGGGTGGCTCAGGTGGCGGCGGAAGTGGCGGAAGATCTATGGTGAGCAAGGGCGAG
GAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTTCGAG
ATCGAGGGCGAGGGCGAGGGCGGCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGCCCCCTGCCC
TTGCGCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCGGACATCCCCGACTAC
TTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAG
GACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCGACGGCCCCGTAATG
CAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGGCCCTGAAGGGCGAGATCAAGCAG
AGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTGACGCTGCCC
GGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCACAACGAGGACTACACCATCGTGGAACAGTACGAACGCGCC
GAGGGCCGCCACTCCACCGCGGCATGGACGAGCTGTACAAGTAA

q

pcDNA3.1_Lifeact-mCherry

>Amino acid sequence

MGVADLIKKFESISKEEGDPPVATMVSKEEDNMAIIKEFMRFKVHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGG
PLPFAWDILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDGGVVTVTQDSSLQDGEFIYKVKLRGTNFPSDG
PVMQKKTMGWEASSERMYPEDGALKGEIKQRLKLDGGHYDAEVKTTYKAKKPVQLPGAYNVNIKLDITSHNEDYTIIVEQY
ERAEGRHSTGGMDELYK*

>DNA sequence

ATGGGCGTGGCCGACCTGATCAAGAAGTTCGAGAGCATCAGCAAGGAGGAGGGCGACCCACCTGTGGCCACCATGGTGAGC
AAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCAC
GAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGGC
CCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATC
CCCGACTACTTGAAGCTGTCCTTCCCGAGGGCTTCAAGTGGGAGCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACC
GTGACCCAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGC
CCCGTAATGCAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCGAGGACGGCGCCCTGAAGGGCGAG
ATCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGCCAAGAAGCCCGTG
CAGCTGCCCCGGCGCTACAACGTCAACATCAAGTTGGACATCACCTCCACAACGAGGACTACACCATCGTGGAACAGTAC
GAACGCGCGGAGGGCCGCCACTCCACCGCGGCATGGACGAGCTGTACAAGTAA

Supplementary Figure 10. List of DNA sequences and amino acid sequences of constructs used in this study. Dark green; Nfluc and Cfluc, Orange; VVD, Red; pMag and pMagFast2, Magenta; mCherry, Blue; nMagHigh1, Light green, mKikGR and EGFP, Gray; CAAX, Dark red; iRFP, Light blue; iSH2 domain and Tiam1 domain, Purple; AktPH, Brown; Lifeact, Black; Start codon, Stop codon, linkers and histidine tag.

Supplementary Table

Supplementary Table 1 List of mutation primers used in this study

Number	Mutation	Primer sequence 5' → 3'	
#1	I47E	CCCGGTGGATATGACGAGATGGGATATCTGATT	Supplementary Figure 3a,b
#2	M48E	GGTGGATATGACATTGAGGGATATCTGATTCAG	
#3	G49E	GGATATGACATTATGGAGATATCTGATTCAGATC	
#4	Y50E	TATGACATTATGGGAGAGCTGATTCAGATCATG	
#5	L51E	GACATTATGGGATATGAGATTCAGATCATGAAC	
#6	I52E	ATTATGGGATATCTGGAGCAGATCATGAACCGG	
#7	Q53E	ATGGGATATCTGATTGAGATCATGAACCGGCCA	
#8	I54E	GGATATCTGATTCAGGAGATGAACCGGCCAAAC	
#9	M55E	TATCTGATTCAGATCGAGAACCGGCCAAACCCG	
#10	N56E	CTGATTCAGATCATGGAGCGGCCAAACCCGCAG	
#11	I47R	CCCGGTGGATATGACAGGATGGGATATCTGATT	Figure 1e,f Supplementary Figure 3b,d,f
#12	M48R	GGTGGATATGACATTAGGGGATATCTGATTCAG	
#13	Y50R	TATGACATTATGGGAAGGCTGATTCAGATCATG	
#14	L51R	GACATTATGGGATATAGGATTCAGATCATGAAC	
#15	I52R	ATTATGGGATATCTGAGGCAGATCATGAACCGG	
#16	I54R	GGATATCTGATTCAGAGGATGAACCGGCCAAAC	
#17	M55R	TATCTGATTCAGATCAGGAACCGGCCAAACCCG	
#18	M55K	TATCTGATTCAGATCAAGAACCGGCCAAACCCG	Supplementary Figure 3d
#19	M55D	TATCTGATTCAGATCGACAACCGGCCAAACCCG	
#20	I52K	ATTATGGGATATCTGAAGCAGATCATGAACCGG	Figure 1e,f Supplementary Figure 3f
#21	I52D	ATTATGGGATATCTGGACCAGATCATGAACCGG	
#22	I52R/M55K	TATCTGAGGCAGATCAAGAACCGGCCAAACCCG	Figure 1e,f Supplementary Figure 3h,i,j,l
#23	I52R/M55R	TATCTGAGGCAGATCAGGAACCGGCCAAACCCG	
#24	I52R/M55D	TATCTGAGGCAGATCGACAACCGGCCAAACCCG	
#25	I52R/M55E	TATCTGAGGCAGATCGAGAACCGGCCAAACCCG	
#26	I52D/M55K	TATCTGAGCCAGATCAAGAACCGGCCAAACCCG	
#27	I52D/M55R	TATCTGAGCCAGATCAGGAACCGGCCAAACCCG	
#28	I52D/M55D	TATCTGAGCCAGATCGACAACCGGCCAAACCCG	
#29	I52D/M55E	TATCTGAGCCAGATCGAGAACCGGCCAAACCCG	

#30	I52D/M55H	TATCTGGACCAGATCCACAACCGGCCAAACCCG	Figure 1e,f Supplementary Figure 3l,m
#31	I52D/M55N	TATCTGGACCAGATCAACAACCGGCCAAACCCG	
#32	I52D/M55Q	TATCTGGACCAGATCCAGAACCGGCCAAACCCG	
#33	I52D/M55S	TATCTGGACCAGATCAGCAACCGGCCAAACCCG	
#34	I52D/M55T	TATCTGGACCAGATCACCAACCGGCCAAACCCG	
#35	I52D/M55Y	TATCTGGACCAGATCTACAACCGGCCAAACCCG	
#36	I52D/M55F	TATCTGGACCAGATCTTCAACCGGCCAAACCCG	
#37	I52D/M55W	TATCTGGACCAGATCTGGAACCGGCCAAACCCG	
#38	I52D/M55A	TATCTGGACCAGATCGCCAACCGGCCAAACCCG	
#39	I52D/M55V	TATCTGGACCAGATCGTGAACCGGCCAAACCCG	
#40	I52D/M55L	TATCTGGACCAGATCCTGAACCGGCCAAACCCG	
#41	I52D/M55I	TATCTGGACCAGATCATCAACCGGCCAAACCCG	
#42	I52D/M55P	TATCTGGACCAGATCCCCAACCGGCCAAACCCG	
#43	I52D/M55C	TATCTGGACCAGATCTGCAACCGGCCAAACCCG	
#44	I52D/M55G	TATCTGGACCAGATCGGCAACCGGCCAAACCCG	
#45	C71S	CCCGTGGATACATCCAGCGCCTTGATTCTTTGT	Figure 1f Supplementary Figure 3a
#46	I74V	ACATCCTGCGCCTTGGTGCTTTGTGACCTGAAA	Figure 2 Supplementary Figure 5
#47	I85V	CAGAAAGACACCCCGGTGGTTTACGCGAGTGAA	
#48	M135I	AACACAATCAATACTATCCGCAAGGCAATCGAC	
#49	M165I	GTTAATTTCTGACTATCATACCTGTTAGGGAC	

Mutation-related codons are shown in red.

Supplementary Movies

Supplementary Movie 1. Blue light-dependent translocation of pMagFast2(3x)-iRFP to the plasma membrane (PM) and its fast dissociation when the blue light is turned off. nMagHigh1 is tethered to the PM of the NIH3T3 cell. Imaging was conducted at 37 °C with a 63× oil objective on the stage of a LSM 710 confocal laser scanning microscope. Fluorescence images of iRFP were taken using a HeNe laser (633 nm). Scale bar, 10 μm.

Supplementary Movie 2. Blue light-dependent morphological changes in the PM of a COS-7 cell expressing iSH2-pMag(3x)-iRFP and nMagHigh1-EGFP-CAAX. Lifeact-mCherry is expressed to observe actin polymerization in the cell. Imaging was conducted at room temperature with a 63× oil objective on the stage of a LSM 710 confocal laser scanning microscope. Fluorescence images of mCherry were taken using a HeNe laser (543 nm). Scale bar, 10 μm.