Supporting Information

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SI Materials and Methods

Plasmids. The cDNAs of PhyB (1–908 aa), PIF6 (1–100 aa), and PIF3 (1–100 aa), which were derived from *Arabidopsis thaliana*, were synthesized with codon optimization for humans by GenScript. According to a previous report (18), the cDNAs of PhyB, linker, mCherry, and the C terminus of H-Ras (HRasCT), a plasma membrane localization signal, were inserted into the pCAGGS vector to generate pCAGGS-PhyB-mCherry-HrasCT. The Tyr-276 of PhyB was substituted to His by two-step overlap PCR, generating pCAGGS-PhyB-Y276H-mCherry-HRasCT. The cDNA of PhyB (1–621 aa) was amplified by PCR to obtain pCAGGS-PhyB621-mCherry-HRasCT. The cDNAs of PIF3 or PIF6 and mEGFP were inserted into the pCAGGS vector to construct pCAGGS-PIF3-mEGFP or pCAGGS-PIF6-mEGFP. The cDNAs of PIF3 and full-length human CRaf were fused and subcloned into the pCX4puro vector (46) to produce pCX4puro-PIF3-CRaf. The cDNAs of PIF3, human Tiam1 (952–1,532 aa), and mEGFP were fused and subcloned into the pCX4bsr vector to generate pCX4bsr-Tiam1-PIF3-mEGFP. pPBbsr-EKAREV-NLS, a *piggyBac* transposon donor vector (47), has been described previously (37). The cDNAs of human *HO1*, *Fd* (Fd-1 also known as adrenodoxin), and *Fnr* were amplified from a cDNA pool obtained from mRNA of HeLa cells and subcloned into a pCAGGS vector, generating pCAGGS-MTS-human HO1 (hHO1)-mCFP, pCAGGS-MTS-human Fd (hFd)-mVenus, and pCAGGS-MTS-human Fnr (hFnr)-tagBFP.

pSpCas9(BB)-2A-Puro (pX459) and lentiCRISPR v2 were gifts from Feng Zhang (Addgene plasmids 62988 and 52961, respectively) (48). pX459-BVRA was generated by annealing oligo inserts, followed by subcloning into the pX459 vector. The single-guide RNA (sgRNA) targets the following sequence of the human *BVRA* gene: 5′-CCGCAAGTCCCTCATCCGCACGG-3′. The PAM sequence is underlined. As a control, lentiCRISPRv2-RLuc, which targeted *Renilla Luciferase*, was generated according to the above-described protocol. The sgRNA targets the following sequence of the *Renilla Luciferase* gene: 5′-AGGTGTACGACCCCGAGCAGAGG-3′. The shRNA-targeting human BVRA construct, pSUPER-sh-hBVRA (#1) and pSUPER-sh-hBVRA (#2), was generated by using pSUPER.retro.puro vector (OligoEngine). The 22-nucleotide sequences used to target the human *BVRA* gene were 5′-CATATACTG-ATCTTCCTTTCGC-3′ (#1) and 5′-GATC-TTTCAGAAATATGTTCTT-3′ (#2). To generate pPHFF-sh-hBVRA (#1) and pPHFF-sh-hBVRA (#2), the H1 promoter and sh-hBVRA sequence were amplified by PCR and subcloned into pPHFF by Gibson assembly (49). pPHFF-sh-mBVRA, which expressed PHFF and shRNA targeting to mouse BVRA, was generated as in pPHFF-sh-hBVRA. The 21-nucleotide sequences used to target the m*BVRA* gene were 5′-GCCAAATGTAGGAGT-CAATAA-3′.

Cells and Reagents. HeLa cells were purchased from the Human Science Research Resources Bank and maintained in DMEM (Wako) supplemented with 10% FBS (Sigma-Aldrich). HEK-293 cells were maintained in minimum essential medium (MEM) (Sigma-Aldrich) supplemented with 10% FBS. HEK-293T cells were obtained from Invitrogen as Lenti-X 293 cells and maintained in DMEM supplemented with 10% FBS. mESCs and MEFs were a kind gift of Toshihiko Fujimori, National Institute for Basic Biology, Japan. The mESCs were maintained in DMEM supplemented with 10% FBS, 1% GlutaMAX (Thermo Fisher Scientific), 1% nonessential amino acids (Thermo Fisher Scientific), 1% sodium pyruvate (Thermo Fisher Scientific), 1% penicillin streptomycin (Nacalai Tesque), 1,000 units per mL ESGRO Leukemia Inhibitory Factor (Merck), 4×10^{-4}% 2-mercaptoethanol (Nacalai Tesque), 1 μM PD0325901 (Tocris Bioscience), and 3 μM CHIR99021 (Cayman Chemical Company). The MEFs were maintained in DMEM supplemented with 10% FBS.

PCB was purchased from Santa Cruz Biotechnology, dissolved in DMSO (final concentration: 5 mM), and stored at −30 °C. Biliverdin was purchased from Sigma-Aldrich, dissolved in DMSO (final concentration: 25 mM), and stored at −30 °C. Bilirubin was purchased from Wako, dissolved in DMSO, and diluted in PBS. Anti-mCherry rabbit polyclonal antibody was obtained from Abcam. The IRDye800CW-conjugated anti-mouse Ig secondary antibodies were purchased from LI-COR. 5-ALA hydrochloride and FeSO4·7H2O [iron(ii) sulfate] was purchased from Sigma-Aldrich and dissolved in H_2O. We treated cells with 250 μM 5-ALA and 40 μM iron(ii) sulfate for 2 d.

Establishment of Stable Cell Lines. HeLa cells were transfected with lentiCRISPRv2-RLuc or pX459-BVRA by 293fectin (Invitrogen). Two days after transfection, the cells were selected by treatment with 1.0 μg/mL puromycin for 48 h, followed by single-cell cloning to establish control HeLa cells or *BVRA* KO HeLa cells. HeLa cells stably expressing EKAREV-NLS were generated as previously reported (37).

For retroviral production, HEK-293T cells were cotransfected with each pCX4 vector or pSUPER vector (50), pGP, and pCMV-VSV-G-RSV-Rev, a gift from Dr. Miyoshi (RIKEN BioResource Center, Ibaraki, Japan), by using polyethyleneimine "Max" MW 40,000 (Polyscience Inc.). For lentiviral production, HEK-293T cells were cotransfected with pCSIIbleo or pCSIIbleo-PHFF vector, psPAX2, a gift from Didier Trono (Addgene plasmid 12260), and pCMV-VSV-G-RSV-Rev. Virus-containing media were collected at 48 h after transfection, filtered, and applied to target cells with 10 μg/mL polybrene (Nacalai Tesque). Two days after infection, the infected cells were selected with the following antibiotics: 10 μg/mL blasticidin S (InvivoGen), 200 μg/mL hygromycin (Wako), 800 μg/mL G418 (InvivoGen), and 1.0 μg/mL puromycin (InvivoGen). The infected mESCs were selected with the following antibiotics: 1.0 μg/mL puromycin and 10 μg/mL zeocin (InvivoGen).

***S. pombe* Strain Construction and Cell Culture.** All fission yeast strains used in this study, with their genotypes and origins, are listed in the Table S1. They are made from WT strains, 972 (h−), or TN366 (h+ ade6-M216 leu1-32), which are a kind gift of Jun-ichi Nakayama, National Institute for Basic Biology, Okazaki, Japan, by transformation and crossing. Growth medium, sporulation medium, and other techniques for fission yeast were based on the protocol as described previously (51) unless otherwise noted.

To generate PhyB-expressing fission yeast cells, a construction cassette was inserted at the gene-free region of chromosome II (adjacent to the zfs1+ gene locus: Z1; see primer lists described below). The construction cassette contains genes of PhyB-Y276H fused with mCherry and expressed under the constitutively active adh1 promoter. The terminator sequence of adh1 was set downstream of the PhyB stop codon. The selection marker (kan^R, hyg^R, nat^R, or bsd^R) was placed in the end of the cassette. The construction cassettes were created by the sewing PCR by KOD FX Neo (KFX-201; TOYOBO). PCR products were EtOH-precipitated and transformed into cells according to the high-efficiency transformation method (52). Transformed cells were placed on the nonselective

YEA plate and incubated at 32 °C for 20 h, followed by the replica to selection YEA plates containing drugs [100 μg/mL G418 (23986–84; Invitrogen), 50 μg/mL Hygromicene B (085–06153; Wako), 100 μg/mL ClonNAT (GOLDBIO.com, *N*-500-1), and 10 μg/mL Blasticidin S (03759–71; Invitrogen)]. The PHFF gene and PcyA and HO1 genes were also introduced by the same method as PhyB. They were inserted at the gene-free region adjacent to the zfs1+ (Z1; chromosome II) or hgh1+ (C1, C2; chromosome I) gene loci (see also the primer lists described below). The genes of PhyB-Y276H, PcyA, and HO1 are human-codon optimized. Strains having more than one insertions were made by crossing. Different mating type strains were mixed and suspended into Leucine solution and spot on the SPA plate. One day after incubation at 25 °C, spore formation was checked by microscope and random spore was done. Asci were incubated with 2% glusurase (NEE154001EA; PerkinElmer) for 2 h at room temperature and then spread onto selection plates.

The following primers were used for generating fission yeast strains in this study: C1F1, AGAGATTCGCGAAGTTTG; C1 F2 connect to Padh, TGTTGTAGGGCATGCtgtctgcataacccattt; C1 R2 connect from Ttef, ACGCCGCCATCCAGTgcagagcacaccaaagaa; C1 R1, tggattaatgatatgatg; C2 F1, tgtgttaactcacttaag; C2 F2 connect to Padh, TGTTGTAGGGCATGCactgagcgacgaaccaag; C2 R2 connect from Ttef, ACGCCGCCATCCAGTccttgctctgatcgtttg; C2 R1, agcgatactacactgtgg; Z1 F1, acactacagtgttgaaatg; Z1 F2 connect to Padh, TGTTGTAGGGCATGCtgaatggaagaacaatta; Z1 R2 connect from Ttef, ACGCCGCCATCCAGTttggagagtattggcgat; Z1 R1, ccaaaattcaatcatcac.

Fluorescence Imaging with Confocal Microscope. HeLa cells and HEK-293 cells were plated on poly–L-lysine (PLL)-coated 35-mm glass-base dishes coated with collagen type I. The plasmids expressing PhyB:PIF-mEGFP:PHFF were transfected by 293fectin at a ratio of 50:1:50. After 48 h, the medium was replaced with FluoroBrite D-MEM (Thermo Fisher Scientific) supplemented 1% GlutaMAX (Thermo Fisher Scientific) and 0.1% BSA. mESC cells were plated on PLL-coated 35-mm glass-base dishes coated with 0.1% Gelatin. MEF cells were plated on PLL-coated 35-mm glass-base dishes. The plasmids expressing PhyB:PIF-mEGFP:PHFF were transfected by Lipofectamine 3000 (Thermo Fisher Scientific) at a ratio of 10:1:10. After 72 h, the medium was replaced with FluoroBrite D-MEM supplemented with 1% GlutaMAX and 0.1% BSA. The fission yeast cells were cultured with liquid YEA media at 32 °C to 5.0×10^6 cells per mL. The cells were washed and suspended with liquid MM media. For the addition of exogenous PCB to fission yeast, PCB solution was added to 1.0×10^6 cells per mL cell culture, followed by a 6-h shake at 32 °C for PCB to permeate into the cells. Cell suspension was mounted on the slide glass (MAS-01; Matsunami) and covered by cover glass (18 × 18 mm No.1; Matsunami).

For confocal fluorescence imaging, cells were imaged with an IX81 inverted microscope (Olympus) equipped with an FV1000 confocal imaging system (Olympus) or a TCS SP5 microscope (Leica Microsystems). In the Olympus FV1000 confocal imaging system, an oil immersion objective lens (UPLANSAPO 60 × O/N.A. 1.35; Olympus) was used. The excitation laser and fluorescence filter settings were as follows: excitation laser, 488 nm (mEGFP), 559 nm (mCherry), and 635 nm (PCB fluorescence); excitation dichroic mirror, DM 405/488/559 dichroic mirror, 500–545 nm (mEGFP), 570–625 nm (mCherry), and 655–755 nm (PCB fluorescence). LEDs for illumination with red (625 nm) and far-red (735 nm) light were purchased from Optocode and controlled manually. In the Leica TSC SP5 confocal imaging system, an oil immersion objective lens (HCX PL APO 63/×1.4–0.6 oil; Leica Microsystems) was used. The excitation laser and fluorescence filter settings were as follows: excitation laser, 488 nm (mEGFP) and 633 nm (PCB fluorescence); excitation dichroic mirror, TD 488/543/633 dichroic mirror; detector, HyD 520–590 nm (mEGFP) and HyD 670–720 nm (PCB fluorescence). LEDs for illumination with red (625 nm) and far-red (735 nm) light were controlled manually.

Fluorescence Imaging with Epifluorescence Microscope. HeLa cells expressing EKAREV-NLS were plated on PLL-coated 35-mm glass-base dishes and transfected with plasmids (PhyB:PIF:PHFF = 1:1:1) by 293fectin. Two days after transfection, the cells were starved for 3 h with FluoroBrite D-MEM supplemented with 1% GlutaMAX and 0.1% BSA. Imaging was performed with an inverted microscope IX81 (Olympus) equipped with an oil immersion objective lens (UPlanSApo 60×/1.35; Olympus), a Retiga 4000R cooled CCD camera (Photometrics), a Spectra-X light engine (Lumencor Inc.), an IX2-ZDC laser-based autofocusing system (Olympus), a MAC5000 controller for filter wheels and XY stage (Ludl Electronic Products), and an incubation chamber (Tokai Hit). The microscope was controlled by MetaMorph software (Molecular Devices). LEDs for the illumination with red (625 nm) and far-red (735 nm) light were controlled manually. The images were analyzed by MetaMorph software. FRET/CFP ratio images are shown in the intensity-modulated display mode, in which eight colors from red to blue are used to represent the FRET/CFP ratio, with the intensity of each color indicating the mean intensity of FRET and CFP.

Control and BVRA KO HeLa cells were plated on PLL-coated 35-mm glass-base dishes and transfected with plasmids encoding UnaG-mCherry by 293fectin. Three hours after transfection, the medium was replaced with FluoroBrite D-MEM supplemented with 1% GlutaMAX and 0.1% BSA. One day after transfection, cells were imaged with the aforementioned epifluorescence microscope.

Zinc Blotting and Immunoblotting. Cells were lysed in 1× SDS sample buffer. After sonication, the samples were separated by 7.5% SDS-polyacrylamide gel electrophoresis (Nagaiki precast gels; Oriental Instruments, Ltd.). The gel was incubated in buffer containing 150 mM zinc acetate and 150 mM Tris·HCl (pH 6.8) for 3 h. PCB fluorescence was detected using an Odyssey infrared scanner (LICOR) with 680 nm excitation light. For immunoblotting, proteins were separated by SDS/PAGE and transferred to polyvinylidene difluoride membranes (Millipore). After blocking with 2.5% skim milk (Morinaga) in TBS-T for 1 h, the membranes were incubated with primary antibodies diluted in blocking buffer, followed by secondary antibodies diluted in blocking buffer. Proteins were detected by an Odyssey infrared scanner (LICOR).

Cell Growth Assay. HeLa cells or mESCs were plated 1.0×10^4 cells per well in each well of a six-well plate. The cell number was counted every day by Coutness (Thermo Fisher Scientific). The experimental growth curves were fitted with the following equation:

$$N(t) = A_0 \exp(kt)$$

$N(t)$ and k indicate cell number at time t and growth rate (per day). A_0 is the initial cell number at the time = 0—namely, 1.0×10^4.

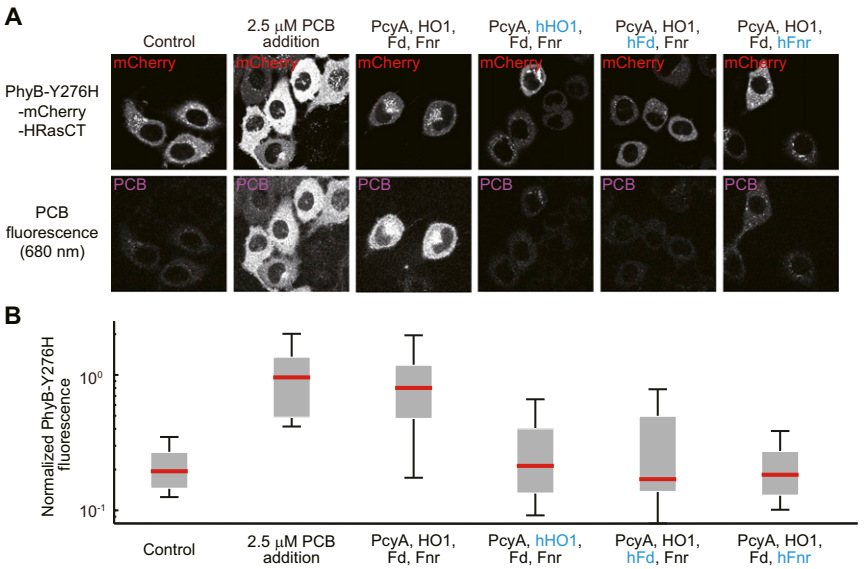


Fig. S1. Human HO1, Fd, or Fnr was unable to substitute for HO1, Fd, or Fnr in PCB synthesis. (*A*) Fluorescence images of PhyB-Y276H-mCherry-HRas C terminus (HRasCT) (*Upper*) and PCB-bound PhyB-Y276H (*Lower*) are shown for the indicated condition in HeLa cells. PCB fluorescence was diminished by replacing HO1, Fd, or Fnr with hHO1, hFd, or hFnr. (Magnification: 60×.) (*B*) Quantification of PCB synthesis. PCB-bound PhyB-Y276H fluorescence was divided by mCherry fluorescence, followed by normalization to the average PCB-bound PhyB-Y276H/mCherry value of 2.5 μM PCB-treated cells. The box extends from the first to the third quartile, with the whiskers denoting 1.5 times the interquartile range. Red crosses are outliers. $n = 28$.

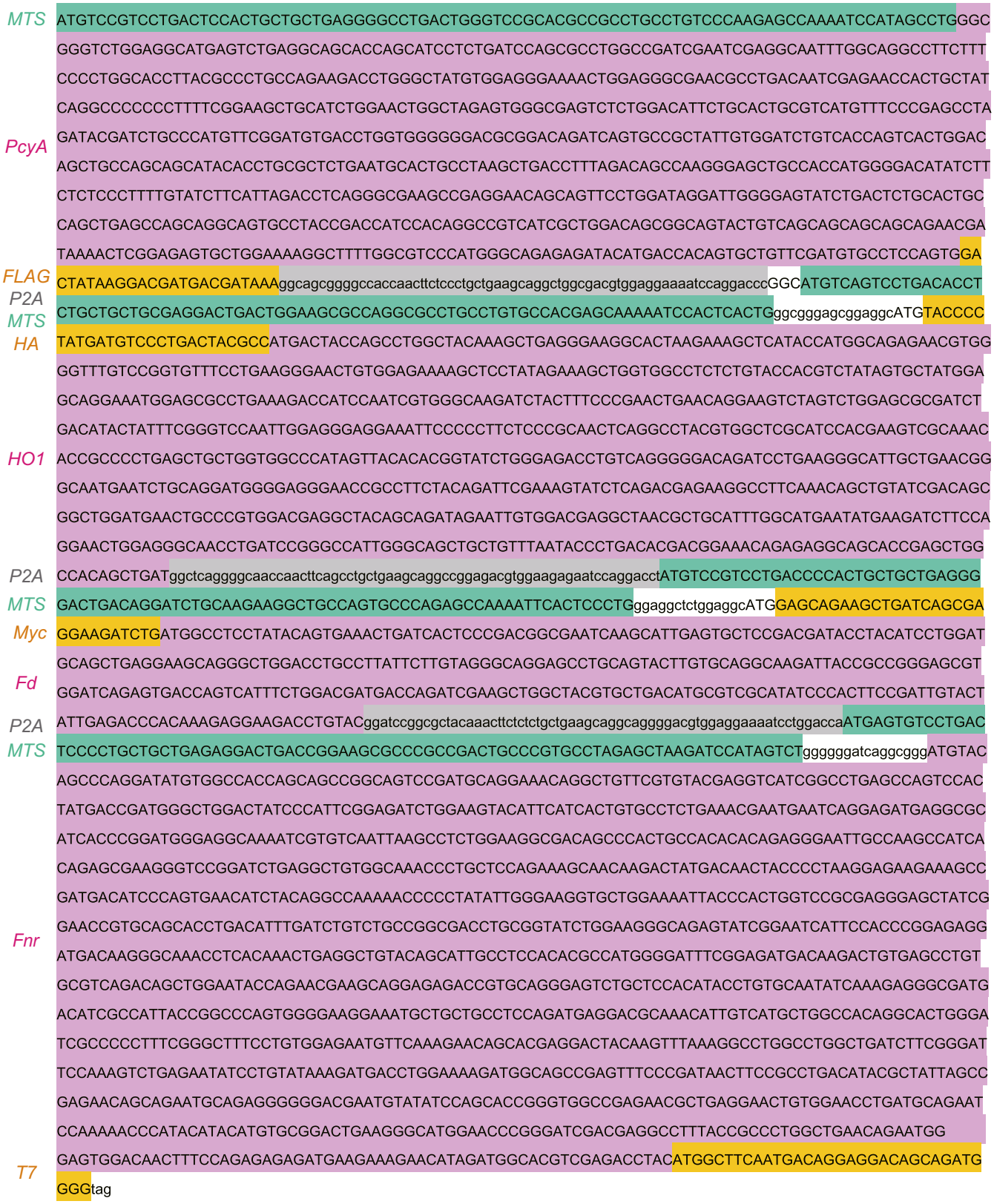


Fig. S2. DNA sequence of PHFF. DNA sequence of PHFF is shown with the indication of individual genes on the left.

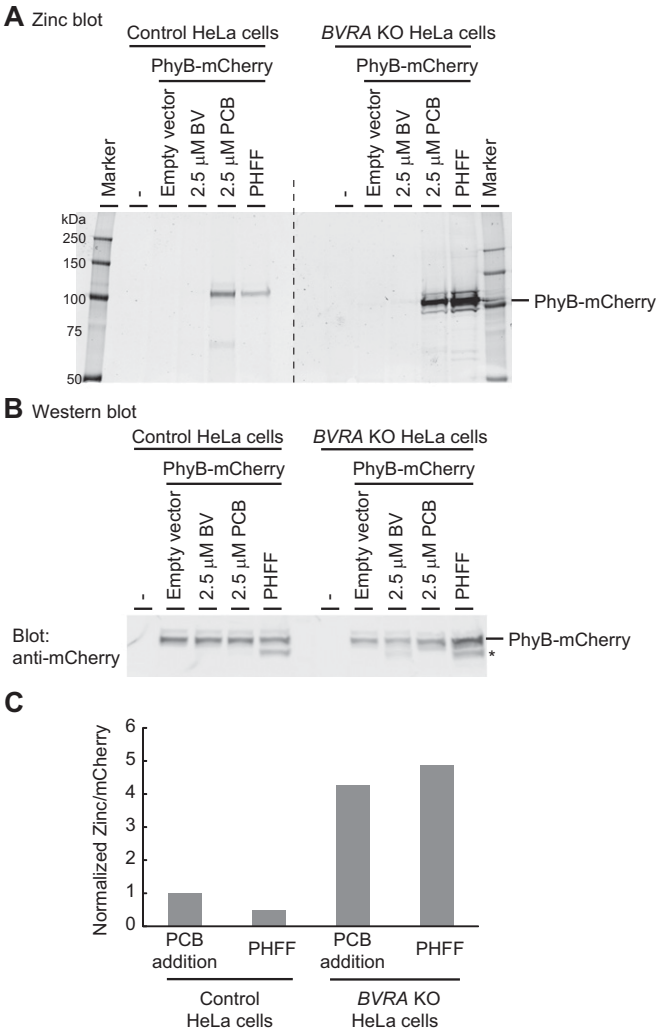


Fig. S3. Zinc blot analysis of PCB-bound PhyB. (*A*–*C*) HeLa cells expressing PhyB-mCherry-HRasCT and/or PHFF were treated under the following conditions: BV, 2.5 μM biliverdin for 30 min; PCB, 2.5 μM purified PCB for 30 min. Left half and right half samples were derived from control HeLa and *BVRA* KO HeLa cells, respectively. Cell lysates were separated by SDS/PAGE, followed by detection of PCB fluorescence bound to zinc (*A*) and mCherry with anti-mCherry antibody (*B*) by using an Odyssey infra-red imaging system. An asterisk shows the extra bands produced for unknown reasons. Signals of PhyB-mCherry-HRasCT in Zinc blot (*A*) were divided by those in Western blotting (*B*) and then normalized by dividing by the value in control HeLa cells treated with 2.5 μM PCB (*C*).

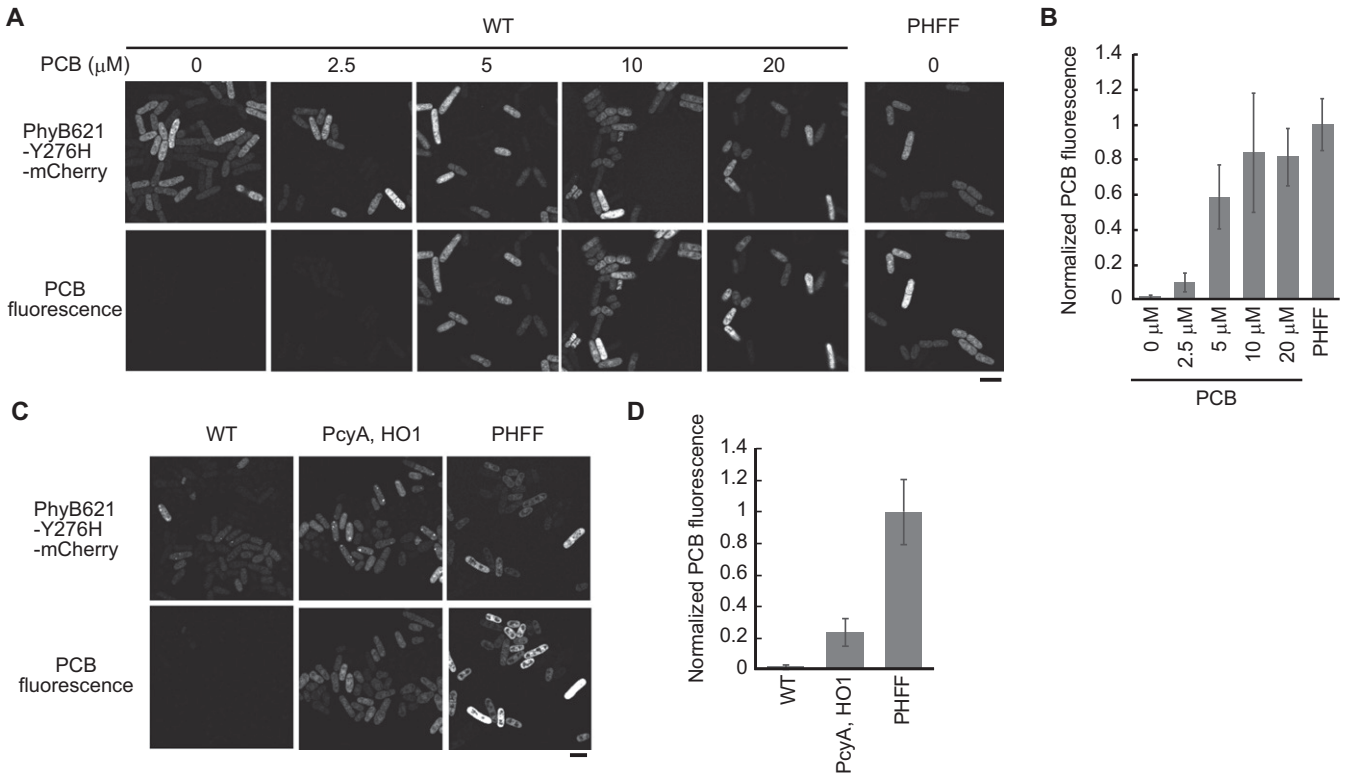


Fig. S4. PCB synthesis in fission yeasts. (*A*) Representative images of PhyB-Y27H-mCherry fluorescence (*Upper*) and PCB fluorescence (*Lower*) in WT cells treated with the indicated concentration of PCB (*Left*) and PHFF-introduced cells (*Right*). (Scale bar, 10 μm.) (*B*) Quantification of PCB synthesis. PCB fluorescence in *A* was divided by mCherry fluorescence and normalized to the average PCB/mCherry value in PHFF-introduced cells as 1.0. The normalized PCB/mCherry is shown in the bar graph with the SD. $n = 211$ (0 μM), 117 (2.5 μM), 126 (5 μM), 174 (10 μM), 97 (20 μM), and 112 (PHFF) cells. (*C*) Representative images of PhyB-Y27H-mCherry fluorescence (*Upper*) and PCB fluorescence (*Lower*) in WT cells (*Left*), PcyA and HO1-expressing cells (*Middle*), and PHFF-introduced cells (*Right*). (Scale bar, 10 μm.) (*D*) The PCB synthesis was quantified as in *B*. $n = 50$ for all conditions.

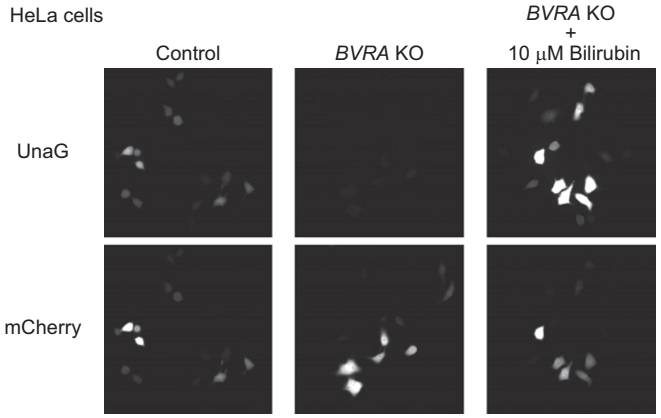


Fig. S5. Detection of Bilirubin by UnaG fluorescence in control and BVRA KO HeLa cells. Control and *BVRA* KO HeLa cells expressing UnaG and mCherry, which was a transfection marker, were treated with or without 10 μM bilirubin for 30 min. The cells were imaged with an epifluorescence microscope. UnaG fluorescence was clearly diminished by *BVRA* KO cells, showing the predominant role of BVRA in bilirubin synthesis. (Magnification: 40×.)

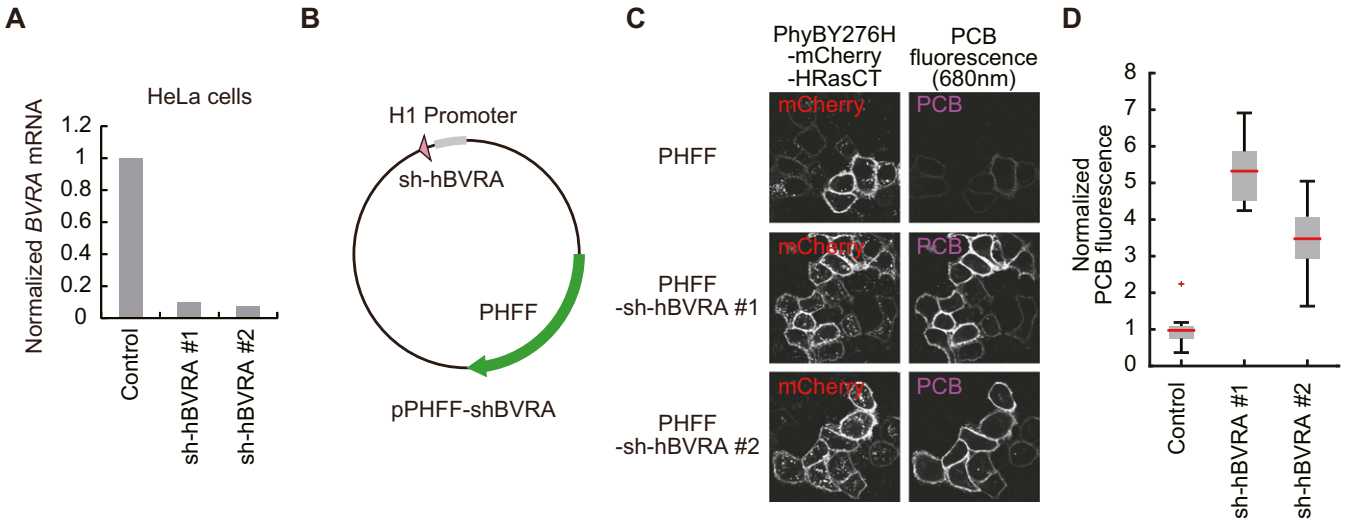


Fig. S6. Increase in PCB synthesis by the depletion of BVRA with shRNA. (*A*) mRNA levels of *BVRA* in HeLa cells transfected with control, human *BVRA* shRNA-expressing (#1), and shRNA (#2)-expressing vectors were measured by quantitative PCR. (*B*) Schematic representation of pPHFF-sh-hBVRA. (*C*) Fluorescence images of mCherry (*Left*) and PCB (*Right*) in control, BVRA shRNA #1, and #2 HeLa cells expressing PhyBY276H-mCherry-HrasCT and PHFF. (Magnification: 60×.) (*D*) The box plot represents the normalized PCB fluorescence in *C*. $n = 12$. The box plot was prepared as in Fig. 3*C*.

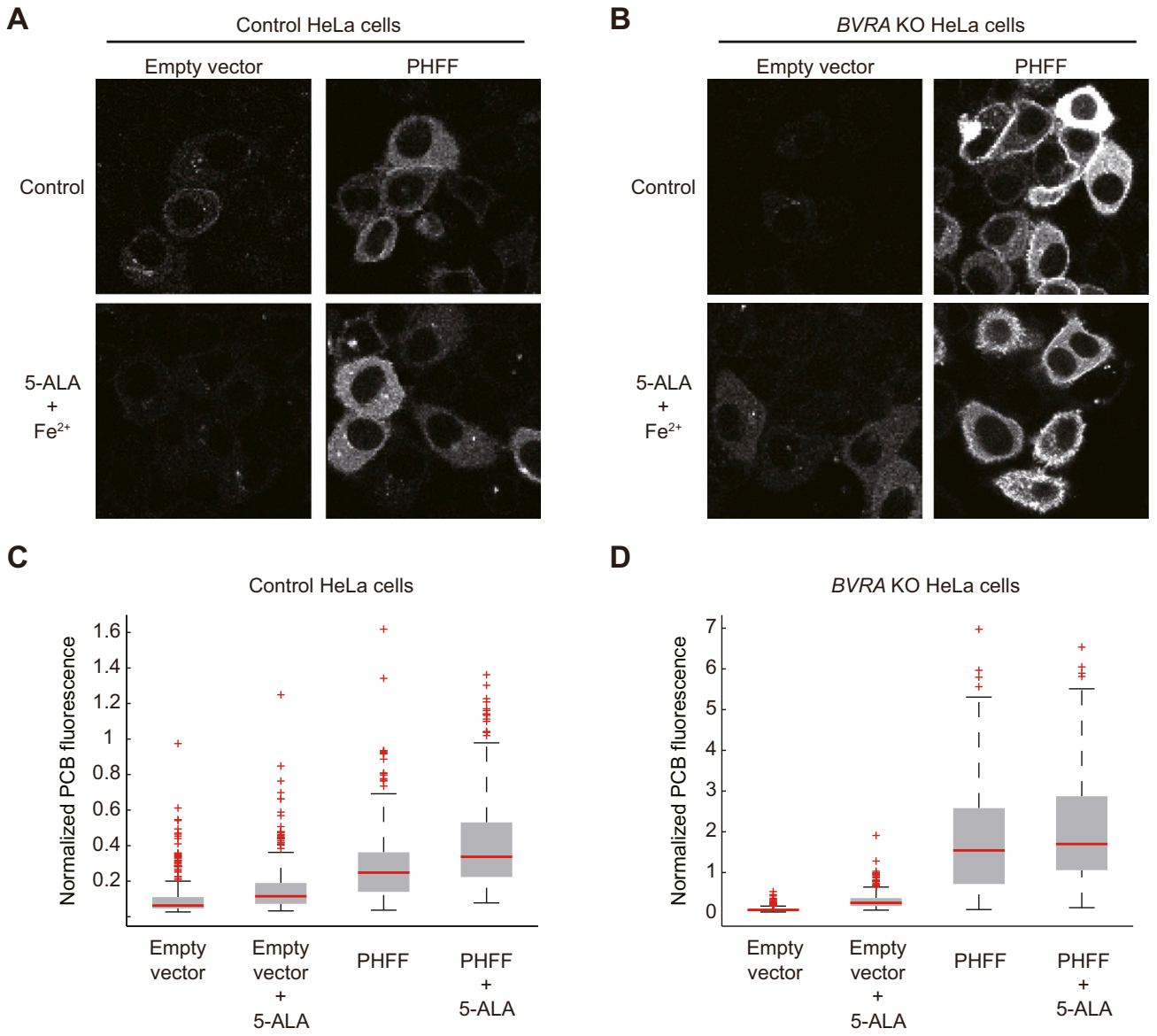


Fig. S7. The effect of 5-ALA and iron(ii) sulfate on PCB synthesis. (*A* and *B*) Fluorescence images of PCB-bound PhyB-Y276H are shown for the indicated condition in control HeLa cells (*A*) and *BVRA KO* HeLa cells (*B*). (*C* and *D*) Quantification of PCB synthesis in control HeLa cells (*C*) and *BVRA* KO HeLa cells (*D*). PCB-bound PhyB-Y276H fluorescence was divided by mCherry fluorescence. The box extends from the first to the third quartile, with the whiskers denoting 1.5 times the interquartile range. Red crosses are outliers. $n > 200$ in all experimental conditions. (Magnification: *A* and *B*, 60×.)

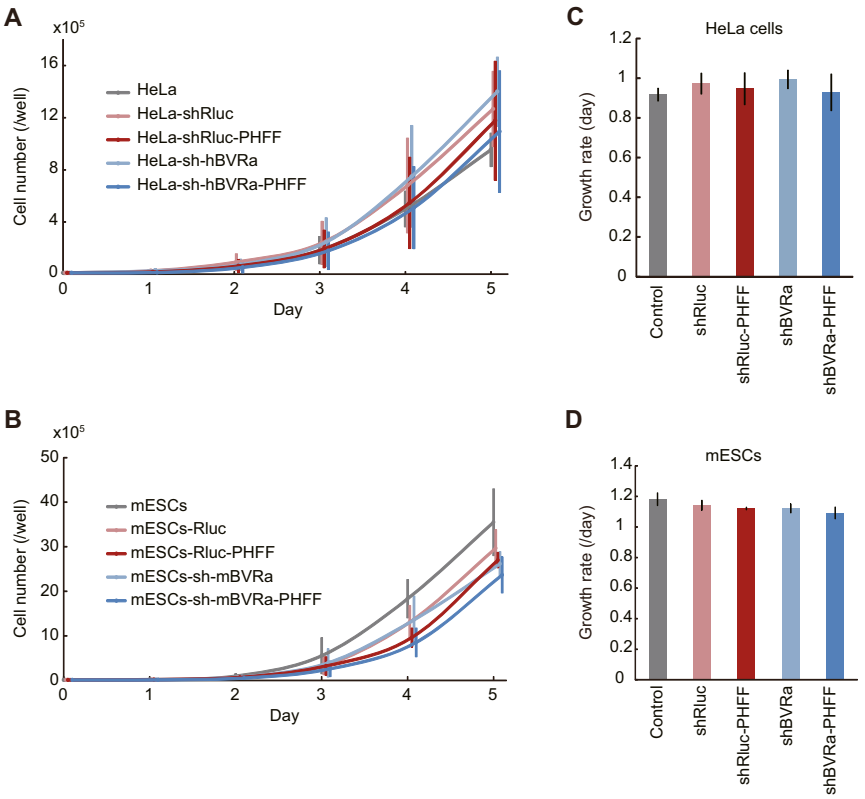


Fig. S8. Cell growth rates with or without PCB synthesis in HeLa cells and mESCs. (*A* and *B*) The averages of cell numbers in HeLa cells (*A*) and mESCs (*B*) are plotted as a function of time (day) after seeding with the SD ($n = 3$). Gray line, parental cells; light red, shRNA against *RLuc* gene (shRuc) for control and Bleomycin resistance gene-introduced cells; dark red, shRluc and PHFF-introduced cells; light blue, shRNA against BVRA gene (shBVRA) and Bleomycin resistance gene-introduced cells; dark blue, shBVRA and PHFF-introduced cells. The averages of cell numbers are plotted as a function of time with the SD ($n = 3$). (*C* and *D*) The growth rates in HeLa cells (*C*) and mESCs (*D*) were quantified by curve fitting with the data in *A* and *B*, respectively. The averaged growth rates (/day) are represented in the bar graphs with the SD ($n = 3$). The *P* values were 0.61 (*C*) and 0.07 (*D*), which were obtained by one-way ANOVA.

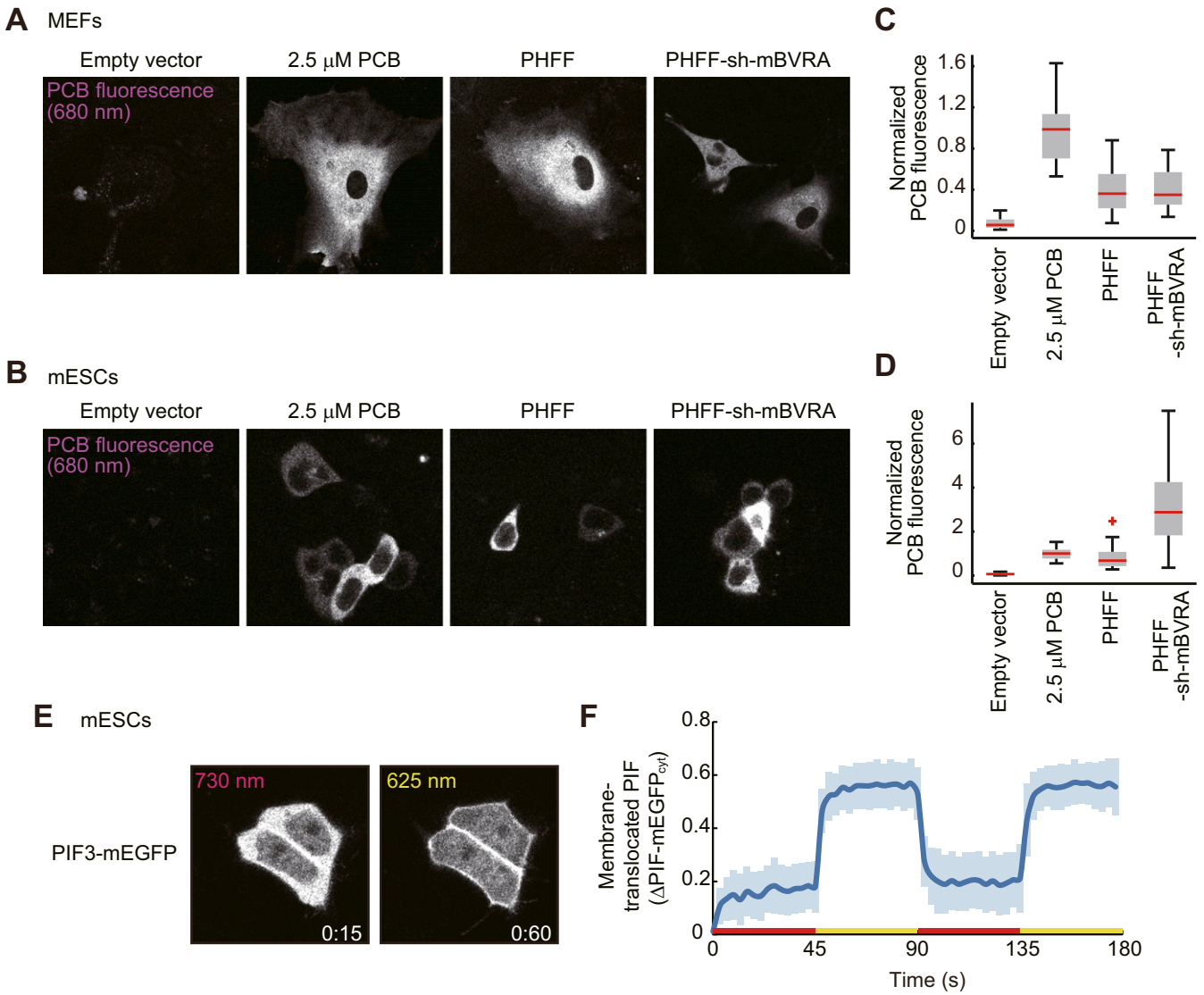
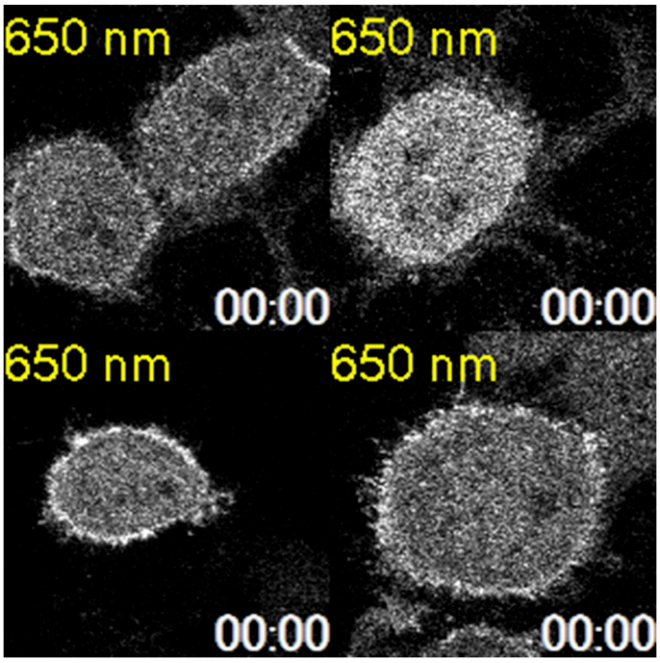


Fig. S9. PCB synthesis and PhyB–PIF LID system in mESCs and MEFs. (*A* and *B*) Fluorescence images of PCB-bound PhyB-Y276H are shown for the indicated condition in MEFs (*A*) and mESCs (*B*). PHFF-sh-mBVRA is a coexpression vector for PHFF and shRNA targeting to mBVRA as shown in Fig. S6. (*C* and *D*) Quantification of PCB synthesis in MEFs (*C*) and mESCs (*D*). PCB fluorescence that bound to PhyB-Y276H-mVenus was divided by mVenus fluorescence, followed by normalization to the average PCB-bound PhyB-Y276H/mVenus value of 2.5 μM PCB-treated cells. $n = 12$ for MEFs and $n = 32$ for mESCs. (*E*) PhyB–PIF LID system in mESCs. PIF3-mEGFP fluorescence images are shown in mESCs expressing PhyB-mCherry-HrasCT, PIF3-mEGFP, and PHFF-sh-mBVRA. (*F*) Quantification of PhyB–PIF LID in mESCs. Membrane-recruited PIF-mEGFP was quantified, and the average values (bold lines) are plotted with SD ($n = 10$) as in Fig. 2*D*. (Magnification: *A*, *B*, and *E*, 60×.)

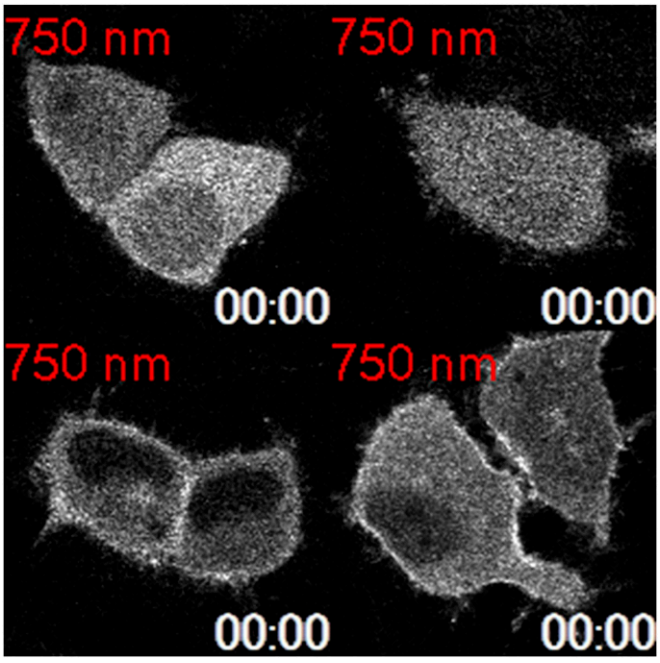
Table S1. Fission yeast strain list

Strain	Genotype	Origin	Figure
YG051	h− c1::Padh1-MTS-PcyA-FLAG<<kan	972 tf	
YG073	h− c1::Padh1-PHFF<<kan	972 tf	
YG075	h− c1::Padh1-MTS-PcyA-FLAG<<kan c2::Padh1-MTS-HA-HO1<<bsd	YG051 tf	
YG103	h− ade6-M216 leu1-32 z1::Padh1-PhyB621-Y276H-mCherry<<hyg	TN366 tf	Fig. S3 *A* and *B*
YG084	h+ ade6-M216 leu1-32 z1::Padh1-PhyB621-Y276H-mCherry<<bsd	TN366 tf	Fig. S3 *A* and *B*
YG090	h− leu1? z1::Padh1-PhyB621-Y276H-mCherry<<bsd c1::Padh1-PHFF<<kan	YG73 × YG84	Fig. S3 *C* and *D*
YG102	h− z1::Padh1-PhyB621-Y276H-mCherry<<hyg	972 tf	Fig. S3 *C* and *D*
YG107	h? leu? c1::Padh1-MTS-PcyA-FLAG<<kan c2::Padh1-MTS-HA-HO1<<bsd	YG75 × YG103	Fig. S3 *C* and *D*
YG107	z1::Padh1-PhyB621-Y276H-mCherry<<hyg	YG75 × YG103	Fig. S3 *C* and *D*
YG090	h− leu1? z1::Padh1-PhyB621-Y276H-mCherry<<bsd c1::Padh1-PHFF<<kan	YG73 × YG84	



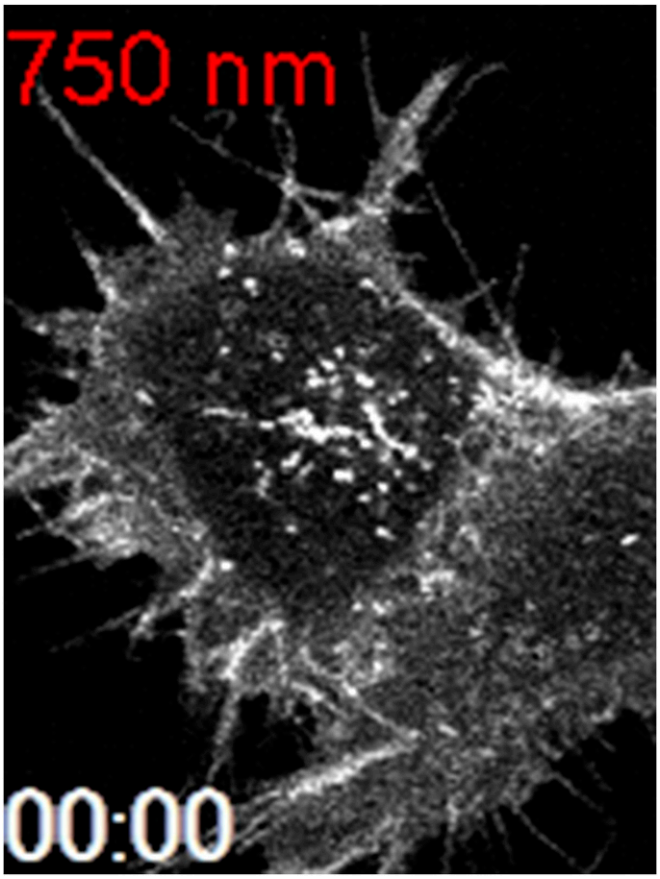
Movie S1. Recruitment and dissociation of PIF-mEGFP in HeLa cells expressing PHFF. HeLa cells expressing the following proteins were imaged with red and far-red illumination: (*Upper Left*) PHFF, PhyB-mCherry-HRasCT, and PIF3-mEGFP; (*Upper Right*) PHFF, PhyB621-mCherry-HRasCT, and PIF3-mEGFP; (*Lower Left*) PHFF, PhyB-mCherry-HRasCT, and PIF6-mEGFP; (*Lower Right*) PHFF, PhyB621-mCherry-HRasCT, and PIF6-mEGFP. Timestamp shows time in minutes:seconds.

Movie S1



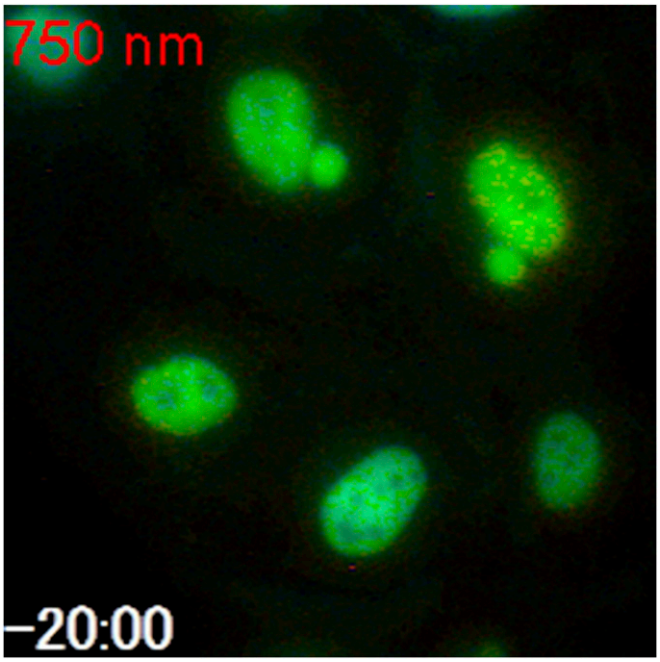
Movie S2. Recruitment and dissociation of PIF-mEGFP in BVRA KO HeLa cells expressing PHFF. BVRA KO HeLa cells expressing the following proteins were imaged with red and far-red illumination: (*Upper Left*) PHFF, PhyB-mCherry-HRasCT, and PIF3-mEGFP; (*Upper Right*) PHFF, PhyB621-mCherry-HRasCT, and PIF3-mEGFP; (*Lower Left*) PHFF, PhyB-mCherry-HRasCT, and PIF6-mEGFP; (*Lower Right*) PHFF, PhyB621-mCherry-HRasCT, and PIF6-mEGFP. Timestamp shows time in minutes:seconds.

Movie S2



Movie S3. Regulation of lamellipodial formation by the membrane recruitment of Tiam1 in BVRA KD HEK-293 cells. BVRA KD HEK-293 cells stably expressing PhyB-mCherry-HRasCT and Tiam1-PIF3-mEGFP were transfected with pPHFF. Two days after transfection, the cells were imaged with confocal microscopy. Timestamp shows time in minutes:seconds.

Movie S3



Movie S4. Repeated ERK activation by membrane recruitment of CRaf in BVRA KO HeLa 293 cells. BVRA KO HeLa cells stably expressing EKAREV-NLS were transfected with pPHFF, pCAGGS-PhyB-mCherry-HRas, and pCX4puro-PIF3-CRaf. Two days after transfection, the cells were imaged with epifluorescence microscopy. Timestamp shows time in minutes:seconds after first red light illumination.

Movie S4