Supplementary Figure 1. Light-responsiveness of chimeric TrkB receptors conferred by the PHR motif. (a) Schematic showing the molecular architecture of PHR- and CIBN-fused Trk receptors and activation process of them. (b) Immunoblot analysis of endogenous ERK activity in the absence or presence of a 30-s illumination using a blue LED array (470 nm, 5.5 μW) in PC12 cells transfected with chimeric TrkBs. ERK activity was examined 5 min after illumination. α-actin was included as a protein loading control. (c) Time course of changes in R-GECO1 fluorescence before and after delivery of three 0.5-s pulses of blue-light (488 nm, 6.5 μW) in HeLa cells expressing the indicated constructs. Chimeric TrkB localization is shown on the left. Note that TrkB-PHR\textsuperscript{D387A}-mCit expression evoked Ca\textsuperscript{2+} transients (inset) when coupled to BDNF stimulation (50 ng ml\textsuperscript{-1}). Scale bars, 20 μm.
Supplementary Figure 2. Activation of Akt and Ca^{2+} signalings by photostimulation of optoTrks. Time-lapse images showing (a) plasma membrane recruitment of mCherry-PH\textsubscript{AKT1} and (b) increase in R-GECO1 fluorescence intensity in response to blue-light stimulation (three 0.5-s pulses, 6.5 μW) of optoTrk-cotransfected HeLa cells. Graphs shown in (a) denote representative fluorescence intensity profiles across a transverse line in indicated cells before (grey) and after (blue) exposure to light. Scale bars, 20 μm.
Supplementary Figure 3. Differential ERK dynamics induced by light and BDNF stimulation. (a) Immunoblot analysis of endogenous ERK activity in the absence (-) or presence of a 30-s illumination using a blue LED array (470 nm, 5.5 μW) or of 50 ng ml⁻¹ BDNF in HeLa cells transfected with optoTrkB. ERK activity was examined at indicated times after stimulation. (b) Quantitative time-course changes in normalized phospho-ERK levels induced by BDNF incubation (red) or a single light stimulation (blue). Phospho-ERK levels normalized to total ERK levels at each time points were calculated and the normalized phospho-ERK levels over time, relative to baseline (0 min), were plotted.
**Supplementary Figure 4. Comparison of optoTrkB performance with wildtype TrkB activity.** (a) Representative time-lapse images showing a nuclear translocation of ERK-mCh after BDNF (100 ng ml⁻¹) or light (three 0.5-s pulses, 6.5 μW, once (“1 pulse”) or every 5 min (“repetitive”)) stimulation in HeLa cells co-expressing TrkB-mCit (“WT TrkB”) or optoTrkB, respectively. Scale bar, 20 μm. (b) The graph shows quantified translocation of ERK-mCh by calculating the ratio of nuclear to cytosolic ERK-mCh (ERK_{nuc}/ERK_{cyt}) normalized to baseline (at -1 min). Black arrow indicates stimulation. Error bars, ± SEM (n = 8). Note that repetitive exposure of optoTrkB ("opto") to blue-light pulses sustains normalized ERK_{nuc}/ERK_{cyt}, similarly as BDNF-WT TrkB activation ("WT"). (c) Immunoblot analysis of endogenous ERK activity in optoTrkB- or TrkB-mCit-transfected HeLa cells stimulated with a single 30-s illumination (470 nm, 5.5 μW) or BDNF (100 ng ml⁻¹), respectively. ERK activity was examined at indicated times after stimulation. (d) Quantitative time-course changes in normalized phospho-ERK levels induced by BDNF incubation (red) or a single light stimulation (blue).
Supplementary Figure 5. Inhibition of light-mediated activation of canonical TrkB signalling pathways by Trk receptor inhibitor. (a) HeLa cells transfected with optoTrkB were left unstimulated (- Light) or illuminated using a blue LED array for 30 s (+ Light) without (-) or with a 30-min pre-incubation with 100 nM K252a or 50 nM PD0325901 (PD03). (b) Translocation of mCherry-PH$_{\text{AKT1}}$ to the plasma membrane by photostimulation of optoTrkB-cotransfected HeLa cells was completely blocked in the presence of 100 nM K252a or 50 μM LY294002 (LY29). Scale bars, 20 μm.
Supplementary Figure 6. Relationship between interstimulus interval and reversibility efficacy. HeLa cells cotransfected with optoTrkB and R-GECO1 were stimulated with three 0.5-s pulses of blue-light (6.5 µW) twice with varying interstimulus intervals (2, 3, 4, 5, and 10 min). (a) Time series of ratio images showing changes in R-GECO1 fluorescence, including baseline (Before) and peak (1st and 2nd peak) levels, for each interstimulus interval are displayed. Scale bars, 20 µm. (b) Normalized changes in R-GECO1 fluorescence over time in cells shown in (a) were plotted.
Supplementary Figure 7. Reversible modulation of endogenous ERK activity using light. HeLa cells transfected with optoTrkB were left unexposed (-L) or exposed to a 60-s illumination once (1st stim.) or twice consecutively with a 20-min interval (1st and 2nd stim.) using a blue LED array. Phospho-ERK levels were examined 5 and 20 min after the first and second stimulation (blue arrows) and quantified by normalization to total ERK. Each bar on the graph below shows the relative level of normalized phospho-ERK.
Supplementary Figure 8. Effects of illumination intensity on the light-induced neurite outgrowth. PC12 cells transfected with optoTrkB were serum-starved for 24 h and exposed to a 5-s light stimulation with various intensities (0, 1.7, 2.8, 6.5, and 13.5 μW) every 5 min for another 24 h using a blue-LED array. (a) Representative fluorescence and DIC images of paraformaldehyde-fixed PC12 cells. Scale bars, 50 μm. (b-d) Quantitative data showing the effect of illumination intensity on the presence of neurites (b), the total neurite length (c), and the length of the longest neurite (d). Cells presenting neurites were defined as those with at least one neurite more than one cell-body diameter in length. At least 50 cells were counted in each experiment and the data were pooled from three independent experiments. Data are means ± SEM. *p<0.05 (One way ANOVA).
Supplementary Figure 9. Functional expression of optoTrkB in hippocampal neurons. (a) A confocal image of a hippocampal neuron (DIV 7) cotransfected with optoTrkB and mCh-PHAKT1. Illuminated region was marked by a white box. Inset denotes optoTrkB expression. Scale bar, 100 μm. (b) Time-lapse images of mCh-PHAKT1 taken before (-1 min) and after (0, 1, 2, 3 min) blue-light stimulation (five 0.5-s pulses, 60 μW). (c) The graph shows a fluorescence intensity profile across a transverse line shown in (b) before (gray) and after (blue) exposure to light. (d) Images showing optoTrkB expression before and 10 min after illumination. Note that lamellipodia (blue arrows)- and filopodia (yellow arrows)-like structures were present around the illuminated soma of the neuron.
Supplementary Figure 10. Live-cell imaging of BDNF-induced filopodia formation in hippocampal neurons. (a) A hippocampal neuron (DIV 6) transfected with TrkB-mCit and mCh-LifeAct. Scale bar, 50 μm. (b) Time-lapse mCh-LifeAct images of a region outlined with white dashed lines in (a) before and after BDNF (100 ng ml⁻¹) addition. White arrows indicate filopodia. Scale bar, 20 μm. (c) Quantification of the number of filopodia. The number of filopodia in neuritic processes per 10 μm was calculated at each time points and normalized to baseline (at -30 min). Error bars, ± SEM (n = 6). The black bar in the graph indicates stimulation period.
Supplementary Figure 11. Filopodia formation induced by BDNF or light stimulation in hippocampal neurons. (a-b) Representative images of (a) optoTrkBexpressing hippocampal neurons (DIV 6) unstimulated (-light) or photostimulated using a blue-LED array (25 μW) either repetitively (10-s on/20-s off, rep.) or continuously (cont.) for 30 min, and (b) TrkB-mCit-expressing neurons untreated or treated with 100 ng ml⁻¹ BDNF for 30 min. OptoTrkB- or TrkB-mCit-expressing, phalloidin-stained neurons were imaged and analyzed. Bottom panels in (a) and (b) showed enlarged images of the regions outlined with white dashed lines. Scale bars, 20 μm. (c) The number of filopodia in neuritic processes per 10 μm were calculated. The results of three independent experiments were compiled. Data are presented as means ± SEM. ***p<0.0005 (paired t-test).
Supplementary Figure 12. Effects of optoTrkB activation on neurite outgrowth in hippocampal neurons. Freshly dissociated hippocampal neurons were transfected with optoTrkB, and at DIV 1, neurons were either non-exposed (control) or exposed to BDNF (100 ng ml\(^{-1}\)) or light (25 μW) stimulation for 48 h. (a) Representative images of optoTrkB-transfected hippocampal neurons exposed to different stimulation conditions at DIV 3. Scale bar, 50 μm. (b, c) The total neurite length and the number of branch points were quantified. Data are presented as means ± SEM. *p<0.05, **p<0.005, ***p<0.0005 (paired t-test).
Supplementary Figure 13. Loss of Ca\textsuperscript{2+} elevation after light-activation of mutant optoTrkB. HeLa cells transfected with mutant optoTrkB (TrkB\textsuperscript{Y816F}-PHR-mCit) and Ca\textsuperscript{2+}, PI3K, or ERK signal sensors were exposed to blue-light stimulation (three 0.5-s pulses, 6.5 μW). Note that PI3K and ERK signaling was activated whereas no Ca\textsuperscript{2+} elevation was detected. Scale bars, 20 μm.
**Supplementary Figure 14. Effects of optoTrkB expression level on the background ERK activity using light.** Non-transfected (Cell) and transfected PC12 cells with indicated constructs (TrkB-mCit, CMV-optoTrkB, PGK-optoTrkB) were kept in the dark or exposed to blue-light illumination using a blue-LED array (25 μW) for 1 min. ERK activity was examined 5 min after illumination. Note that PGK promoter-driven optoTrkB expression is much lower than CMV promoter-driven expression, below the detection level, but still phospho-ERK level was increased after light stimulation.
Supplementary Figure 15. Amino acid and nucleotide sequences of codon-optimized PHR.
Supplementary Figure 16. The LED array devices for illuminating live cells. Two different types of the customized LED arrays made of (a) 96- or (b) 24-blue LEDs were used for light stimulation. (a) The 96-LED array was modulated by an electronic switch which could control each column (8-LEDs in a row) independently. This inverted LED array was used to illuminate cells in a 96-well plate in the incubator for a long time. (b) The 24-LED array was used for stimulating cells in a 6-well plate by light for a short time. Most of immunoblot analysis were done by the upright 24-LED array controlled by a switch.
Supplementary Figure 17. Uncropped gel scans of immunoblots.