Supplementary Information

Repurposing protein degradation for optogenetic modulation of protein activities

Payel Mondal[†], Vishnu V. Krishnamurthy[†], Savanna R. Sharum[†], Neeka Haack[†], Huiwen Zhou[†], Jennifer Cheng[†,⸸], Jing Yang[#], Kai Zhang[†,‡,§,¶,*]

[†]*Department of Biochemistry,* [‡]*Center for Biophysics and Quantitative Biology,* [§]*Neuroscience Program, and* [¶]*Beckman Institute, University of Illinois at Urbana-Champaign, 600 South Mathews Avenue, Urbana, Illinois 61801, United States.*

[#]*Department of Comparative Biosciences, University of Illinois at Urbana-Champaign, 2001 S Lincoln Ave, Urbana, Illinois 61802, United States.*

[⸸] *Current address: Feinberg School of Medicine, Northwestern University, Ward 9-250, 303 E Chicago Ave, Chicago, Illinois 60611, United States.*

Corresponding Author

* Kai Zhang, kaizkaiz@illinois.edu

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Supplementary Methods

Reagents

Phusion DNA polymerase master mix (M0530L) was purchased from NEB. In-Fusion HD Cloning Plus kit was from Takara (638909). DreamTaq PCR Master Mix (2×) (K1081), Turbofect (R0532) and Pierce Protease and Phosphatase Inhibitor Mini Tablets (A32959), F12K (21127-022) medium, and horse serum (26050088) were from Thermo Fisher Scientific. Fetal bovine serum (F1051), RIPA Lysis Buffer, 10× (20-188) was from Millipore Sigma. PBS (21-040-CV), DMEM (10-017-CV), Penicillin-streptomycin solution (30-002-CI), Trypsin EDTA (0.25% Trypsin, 0.1% EDTA) 1× (25-053-CI) was from Corning. Precast protein gels (456-1024) and ECL substrate (170-5060) were from Bio-Rad. Antibodies used in this work are Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) (Cell Signaling Technology, 9101S, 1:1000), p44/42 MAPK (Erk1/2) (Cell Signaling Technology, #9102S, 1:1000), HA-Tag (C29F4) (Cell Signaling Technology, #3724S, 1:1000), GAPDH (14C10) (Cell Signaling Technology, #2118S, 1:1000) and Anti-rabbit IgG, HRP-linked Antibody (Cell Signaling Technology, 7074S, 1:2000).

Plasmid construction

Constitutive active MEK (S218D, S222D) was constructed by site-directed mutagenesis. Evolved LOV (eLOV) was amplified from a synthetic gBlock from IDT based on a sequence from the previous work[24]. The sequence of LEXY was amplified from NLS-mCherry-LEXY (Addgene, catalog #72655). The full sequence of GLIMPSe was shown in Figure S8.

Cell culture and transfection

HEK293T cells were cultured in DMEM supplemented with 10% fetal bovine serum and penicillin-streptomycin. PC12 cells were cultured in F12K medium supplemented with 15% horse serum and 2.5% FBS. All cell cultures were maintained in a standard incubator at 37 ºC with 5% CO_2. HEK293T and PC12 cells were seeded in 12 well plates, and cells were transfected once they reached 60% - 80% confluency. Transfection was performed using Turbofect transfection reagent following the vendor’s instruction. For light-induced stabilization of MKP3 experiments, 100 ng CA MEK-EGFP, 200 ng EGFP-p2A-MKP3-eLOVtevS-deg and 700 ng of NLS-mCherry-TEV-LEXY plasmid were cotransfected in PC12 cells. For light-induced CA MEK stabilization experiments 50 ng of EGFP-p2A-CA MEK-eLOVtevS-deg and 950 ng of NLS-mCherry-TEV-LEXY plasmid was cotransfected in PC12 cells. After 3 hours of transfection, transfection medium was replaced with growth medium (F12K + 15% horse serum +2.5% FBS). After overnight recovery, the cell culture was exchanged to a low-serum medium (1.5% horse serum + 0.025% FBS) for another 24 hours to reduce the base-level ERK activity.

Western Blot

After transfection, recovery, and starvation, cells were harvested and lysed with a mixture of RIPA buffer and protease/phosphatase inhibitor cocktail. Lysates were centrifuged, and supernatants were mixed with NuPAGE™ LDS Sample Buffer and β-Mercaptoethanol. Samples were subjected to SDS-PAGE on a precast gel, followed by overnight transfer to PVDF membrane. Two different blots were run using the same cell lysate to probe the phospho-ERK and pan ERK, respectively.

Construction of a programmable LED device

For both long-term and short-term light illumination, the LED array was constructed by assembling a 6-by-4 blue LED array with 24 blue LEDs (B4304H96, Linrose Electronics) on a breadboard. LED intensity can be continuously tuned through a tunable voltage and a current-limiting resistor.

The breadboard was hosted in an aluminum box, and a light diffuser film was positioned above the LED array to make the light intensity homogeneous in the defined area. The light intensity at the cell culture plate was measured by a power meter (PM100D, S121C, Thorlabs).

Statistical analysis

The p-values were determined by performing two-tailed, unpaired t-test, or one-way ANOVA test using the GraphPad Prism software.

Supplementary Figure 1

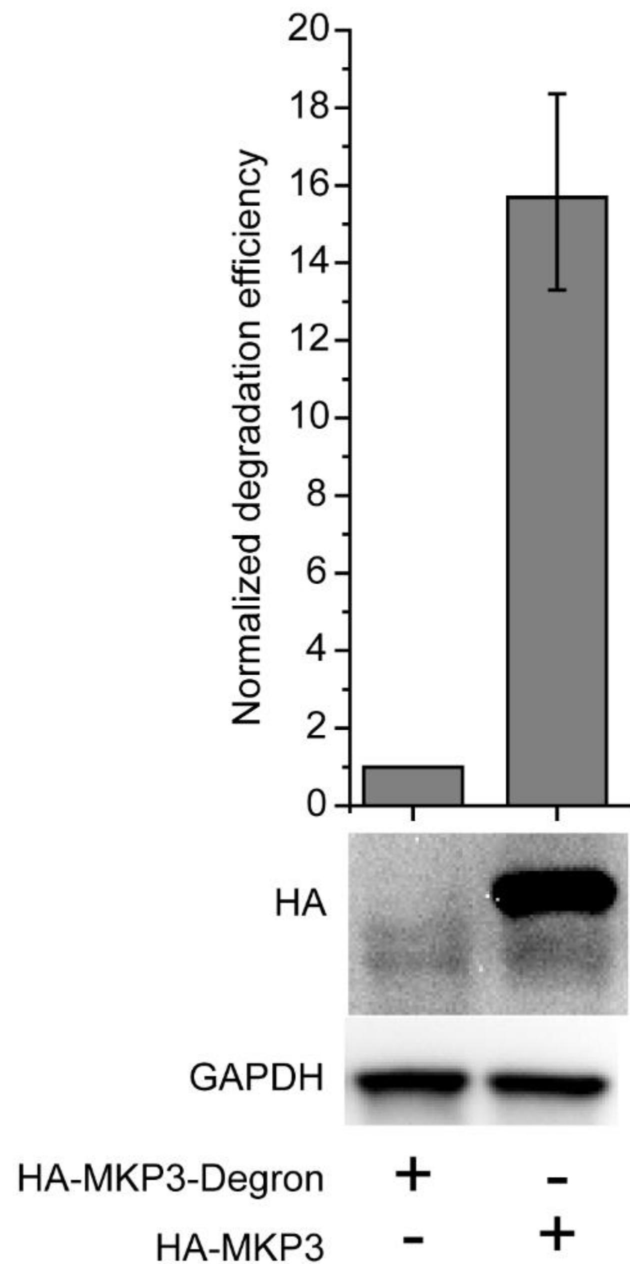

Figure S1. Degron functions normally with the fusion protein HA-MKP3. In PC12 cells, the fusion of degrons at the C-terminus of HA-MKP3 resulted in a 15.7-fold reduction of protein level compared to HA-MKP3 assayed by Western blot analysis with anti-HA antibody. Values were presented by mean ± SD (n=2).

Supplementary Figure 2

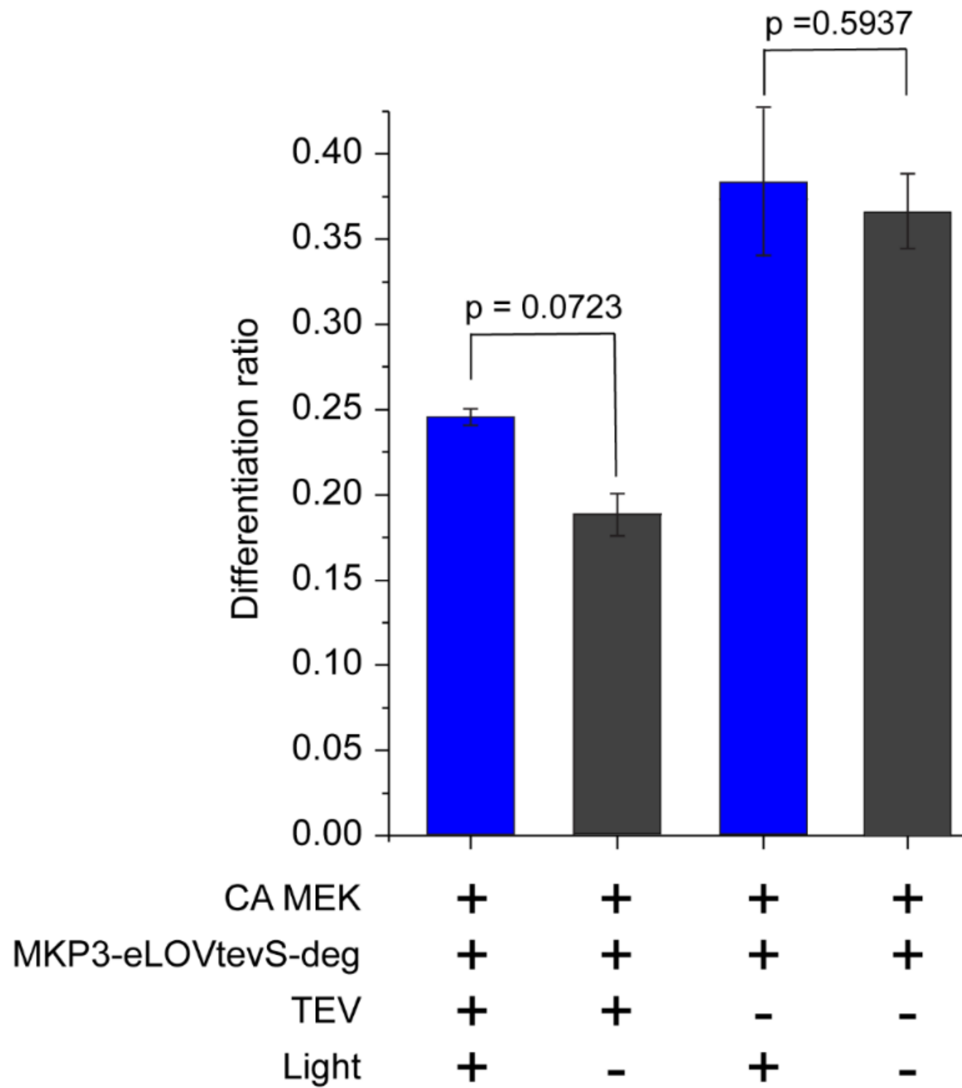

Figure S2. Absence of LEXY shows increased stabilization of MKP3 and reduced PC12 differentiation ratio in the dark. Differentiation ratio calculated for PC12 cells transfected with CA MEK-EGFP, EGFP-p2A-MKP3-eLOVtevS-3X degron, and TEV-mRuby2. Cells were illuminated with 0.5 mW/cm^2 blue light or kept in the dark for 45 h before imaging. Values represent the mean ± SD of two biological replicates (n = 2) with more than 150 cells counted per replicate. Differentiation ratio= (# of transfected + differentiated cells) / # of transfected cells.

Supplementary Figure 3

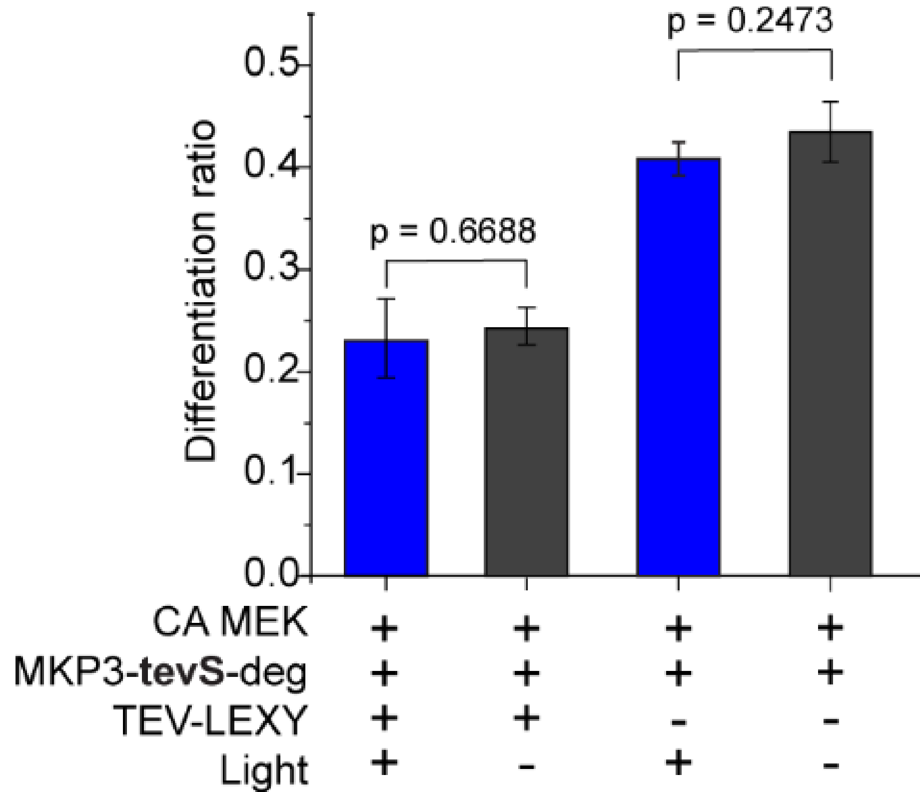

Figure S3. Absence of eLOV in MKP3-tevS-deg abolishes light-dark contrast for MKP3-reduced PC12 cell differentiation. Differentiation ratio calculated for PC12 cells transfected with CA MEK-EGFP, mRuby2-p2A-MKP3-tevS-3X degron, and NLS-mCherry-TEV-LEXY. Cells were illuminated with 0.5 mW/cm^2 blue light or kept in the dark for 45 h before imaging. Values represent the mean ± SD of two biological replicates (n = 2) with more than 150 cells counted per replicate. Differentiation ratio= (# of transfected + differentiated cells) / # of transfected cells.

Supplementary Figure 4

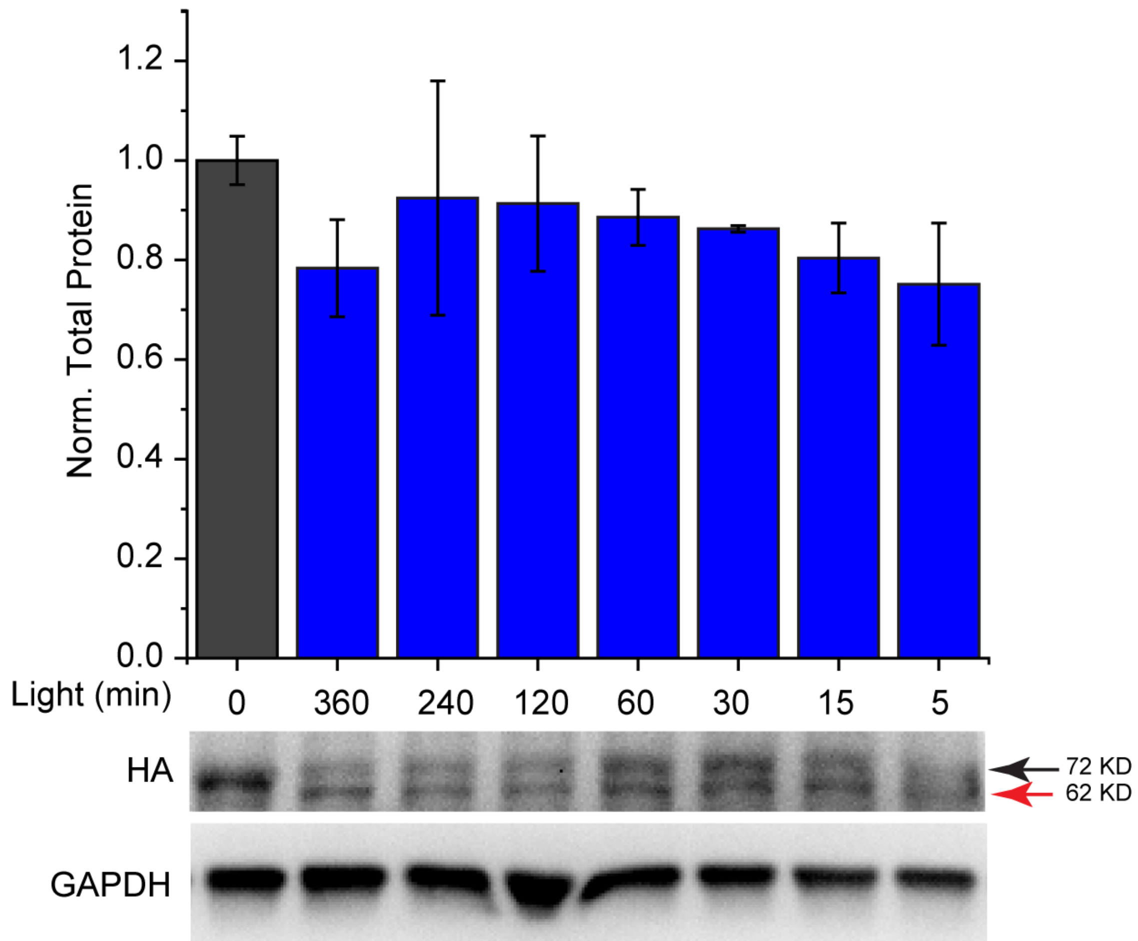

Figure S4. The total amount of proteins remains consistent between the light-stimulated sample and the dark control upon inhibition of new protein synthesis with cycloheximide. PC12 cells transfected with CA MEK-EGFP, EGFP-p2A-HA-MKP3-eLOVtevS-3X degron, and NLS-mCherry-TEV-LEXY were treated with 100 ng/μL cycloheximide, a translation inhibitor, 24 h after transfection. Cell were incubated in cycloheximide for 18 h followed by light exposure for different time interval. The total amount of HA-tagged protein remained consistent across different conditions as new protein synthesis was blocked.

Supplementary Figure 5

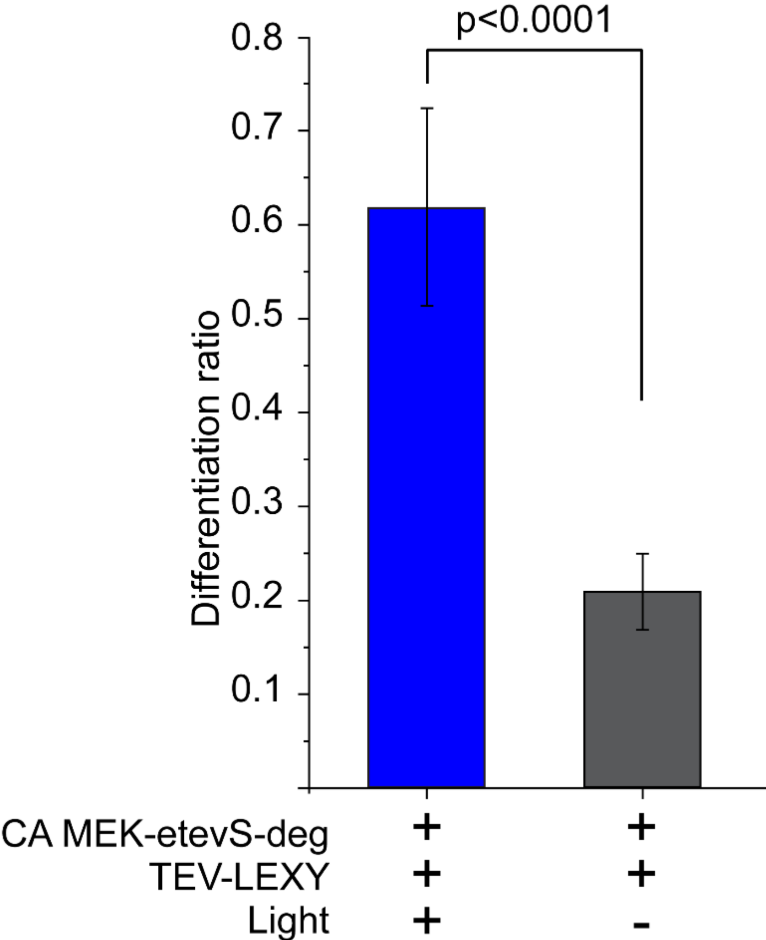

Figure S5. Light illumination for 30 min in PC12 cells transfected with GLIMPSe-CA MEK is sufficient to induce significant neurite outgrowth. CA-MEK rescued by 30 min light treatment shows a differentiation ratio of 0.6 compared with 0.2 for the dark control. The bar graph is presented with mean ± SD averaged over three biological replicates (n = 3) with more than 200 cells counted per replicate.

Supplementary Figure 6

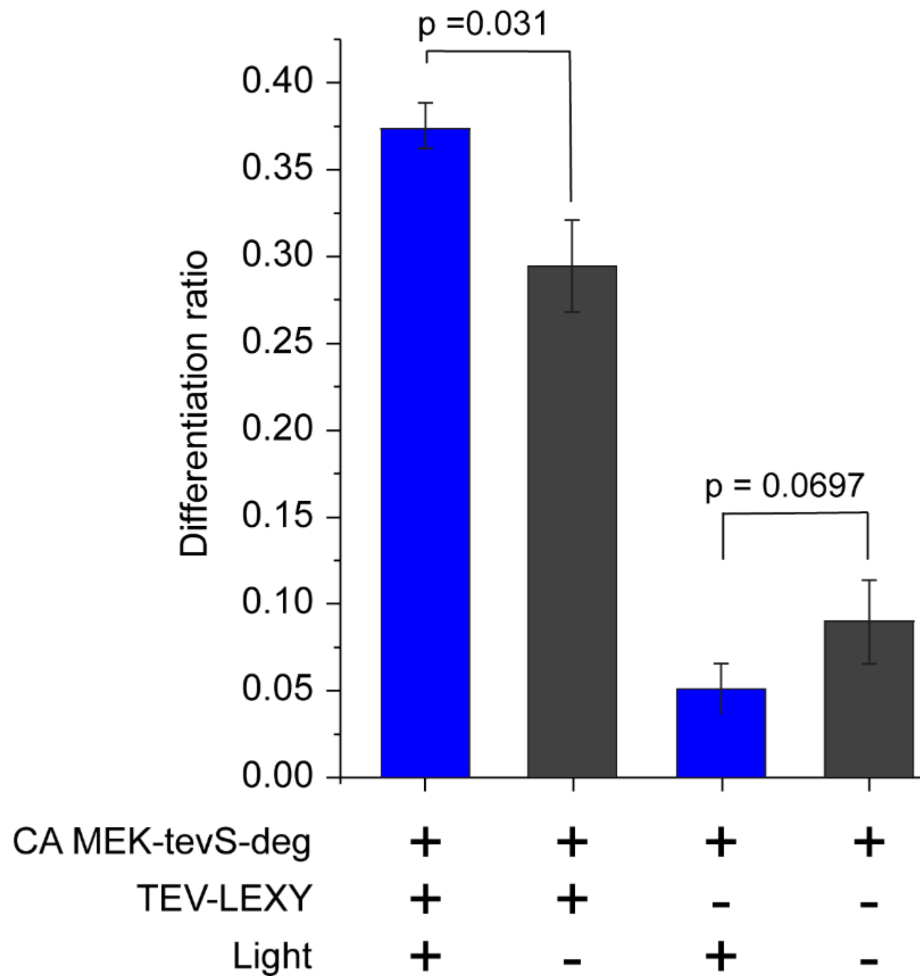

Figure S6. Absence of eLOV in CA MEK-tevS-deg abolishes light-dark contrast for PC12 cell differentiation. Differentiation ratio calculated for PC12 cells transfected with mRuby2-p2A-CA MEK-tevS-3X degron and NLS-mCherry-TEV-LEXY. Cells were illuminated with 0.5 mW/cm^2 blue light or kept in the dark for 45 h before imaging. Values represent the mean ± SD of three biological replicates (n = 3) with more than100 cells counted per replicate. Differentiation ratio= (# of transfected + differentiated cells) / # of transfected cells

Supplementary Figure 7

Figure S7. Long-term light exposure does not cause cell death. (a) PC12 cells were exposed under blue light for 45-h (0.5 mW/cm^2) followed by co-staining with Calcein AM (staining live cells). Less than 1% dead cells were observed in both light and dark conditions. Values represent the mean ± SD of three biological replicates (n = 3) with more than 500 cells counted per replicate. (b) Representative fluorescent and phase-contrast images of PC12 cells in the same field of view under light and dark treatment. Scale bar: 50 μm.

Supplementary Figure 8

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 61 CCAGATAATCCCATTATATTCGTTTCCGATAGTTTCTTGCAGTTGACAGAATATAGCCGT
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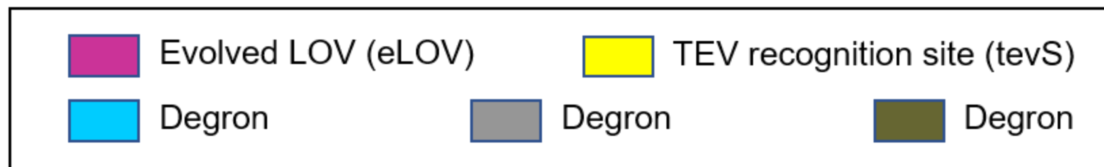

Figure S8. DNA and amino acid sequences of GLIMPSe. The domain of evolved LOV domain, tevS, and three codon-optimized degron are marked in distinct colors. The protein of target (e.g., MKP3 and CA MEK) were fused at the N-terminus of GLIMPSe.