Supplemental Information: Optogenetic control of organelle transport using a photocaged chemical inducer of dimerization
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Supplemental Results

Figure S1

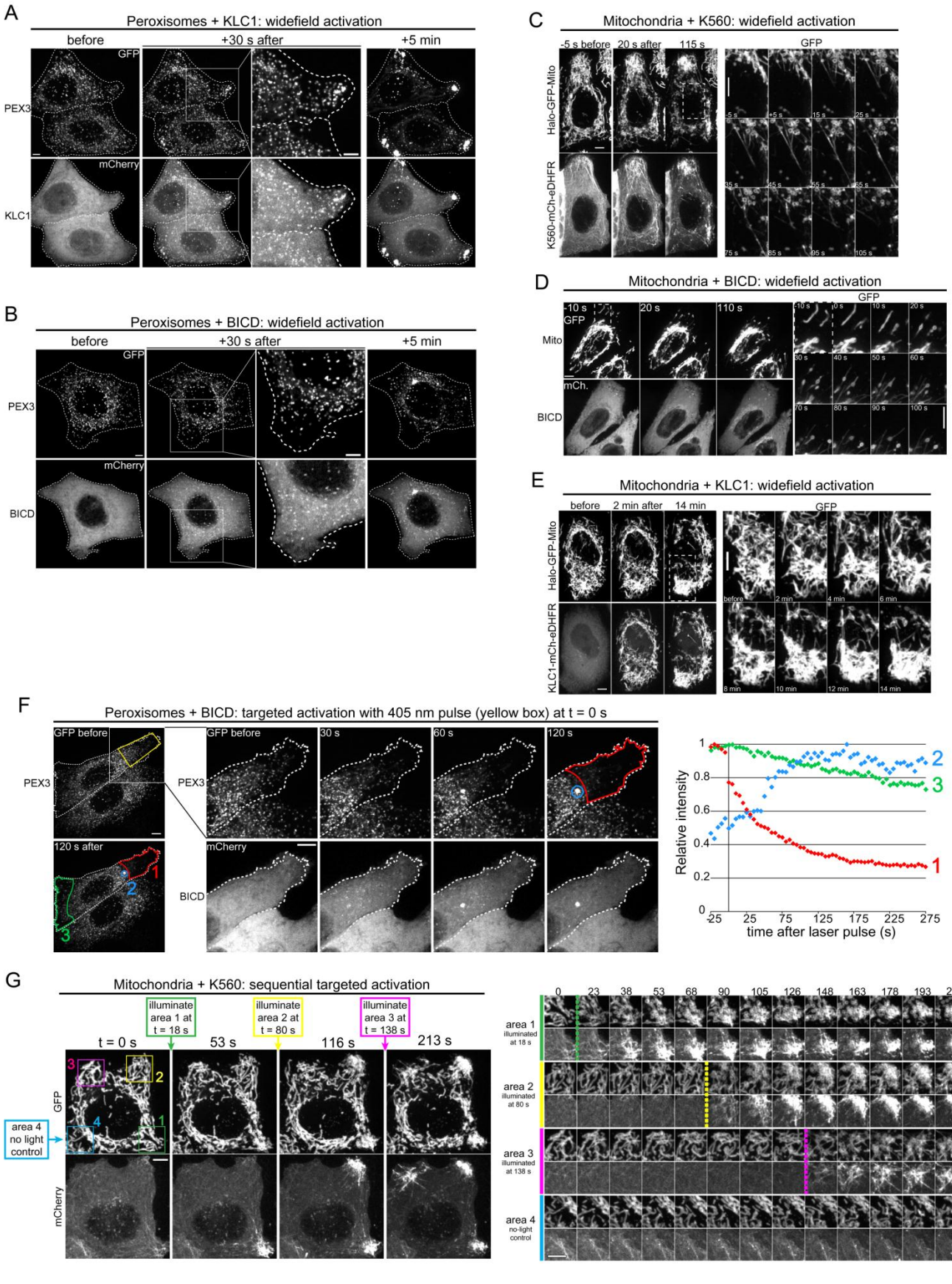

Figure S1: Additional examples of light-induced motor recruitment in HeLa cells.

Panels A and B relate to Figure 1B, panel F relates to Figure 1E. HeLa cells expressing PEX3-GFP-Halo or Halo-GFP-Mito and BICD-mCherry-eDHFR, KLC1-mCherry-eDHFR or K560-mCherry-eDHFR as indicated were incubated with 10 μM cTMP-Htag prior to imaging. (A,B) Expanded version of Figure 1B, showing mCherry images. KLC1 (A) and BICD (B) were recruited to peroxisomes using a 500 ms widefield pulse of 387 nm light. mCherry images show both mCherry-eDHFR motor effector proteins diffuse in the cytosol prior to photoactivation, localized to peroxisomes 30 seconds after activation (whole cell image and inset) and accumulated with peroxisomes 5 minutes after photoactivation. (C-E) K560 (C), BICD (D) or KLC1 (E) were recruited to mitochondria with a 500 ms widefield pulse of 387 nm light. Images on left show whole cell in GFP and mCherry, insets on right show area in dashed box in GFP. (F) BICD was recruited to peroxisomes in a defined region (yellow box) with a ~100 ms pulse of 405 nm light in an experiment analogous to that shown in Figure 1C for KLC1. Images on the left show whole cell (with dashed outline) in GFP, insets show area in white square in GFP and mCherry. Solid colored lines mark regions (1-3) for quantitation. Graph shows average GFP intensity in regions 1-3 before and after 405 nm pulse at t = 0, normalized for each region to the maximum value observed. Results from these experiments are quantified in Figure 1E. (G) K560 was sequentially recruited to mitochondria in regions 1-3 (green, yellow and magenta boxes) using ~100 ms pulses of 405 nm light at the timepoints indicated. Images on left show the whole cell in GFP and mCherry, insets on right show the three photoactivated regions as well as an unilluminated control region (4, cyan) in GFP and mCherry. Heavy dashed lines indicate intervals when photoactivation pulses in the respective regions were applied. All scale bars 5 μm.

Figure S2

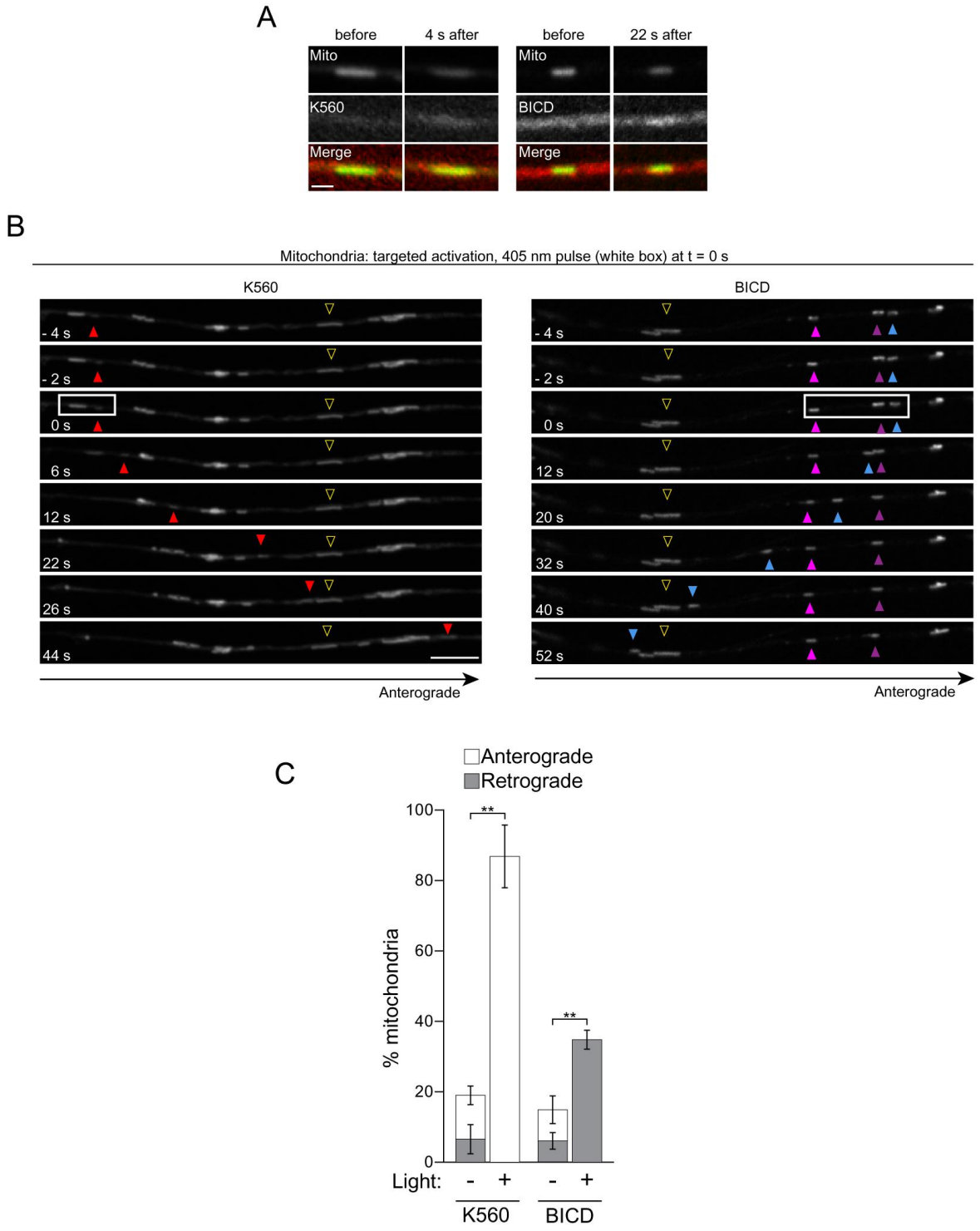

Figure S2: Recruitment of motors to mitochondria in neurons.

(A) Representative images of recruitment of motor proteins or adaptors (K560 or BICD) to mitochondria before and after photoactivation. Scale bar, 500 nm. (B) Time series of mitochondria movement before and after photoactivation. White box indicates the photoactivated region. Filled arrowheads mark positions of photoactivated mitochondria; open yellow arrowheads mark unilluminated mitochondria. Scale bar, 5 µm. Note that unlike peroxisomes, not all mitochondria become motile within 5 min after photoactivation. (C) Quantitation of the percent of mitochondria exhibiting anterograde or retrograde movement with or without photoactivation in K560 and BICD recruitment experiments in neurons (Mean ± SEM, n=10 neurons from 3 independent experiments). **$p<0.05$ Student's t-test. This figure is not related to Figure 1.

Supplemental Experimental Procedures

Plasmids

All plasmids in this study are derived from pEM705, which contains a CAG promoter for constitutive expression, obtained from E. V. Makeyev [S1]. Halo-GFP-Mito is previously described [S2], and includes the C-terminal 47 amino acids of the the *Listeria monocytogenes* ActA gene, which confer mitochondrial outer membrane targeting. PEX3-GFP-Halo includes the N-terminal 42 amino acids of the human Pex3 gene, which confer peroxisome targeting [S3]. The mCherry-eDHFR constructs in this study were derived from a previously described mCherry-eDHFR plasmid [S2], augmented with motor and motor effector domains described in [S3]. BICD-mCherry-eDHFR includes residues 1-572 of mouse BICD2, KLC1-mCherry-eDHFR includes residues 1-175 of rat Kinesin-1 light chain, and K560-mCherry-eDHFR includes residues 1-560 of human Kinesin-1 heavy chain.

HeLa cell culture and transfection

Hela cells (obtained from E. V. Makeyev, Nanyang Technological University) were cultured in growth medium (DME with 10% FBS and penicillin-streptomycin) at 37 °C in a humidified atmosphere with 5% CO_2. Peroxisome recruitment experiments in HeLa cells were performed by transiently cotransfecting plasmids expressing PEX3-GFP-Halo and either BICD-mCherry-eDHFR or KLC1-mCherry-eDHFR. Mitochondrial recruitment experiments in HeLa cells were performed by transiently transfecting BICD-mCherry-eDHFR, KLC1-mCherry-eDHFR or K560-mCherry-eDHFR into a stable cell line constitutively expressing Halo-GFP-Mito. The Halo-GFP-Mito stable cell line was created using the Recombinase Mediated Cassette Exchange technique described by Makeyev and coworkers [S1]. For single-plasmid transient transfections, cells at ~60% confluency in a single well of a 6-well plate were transfected with 1 μg of plasmid using 3 uL of Fugene 6 (Promega). Double-plasmid cotransfections were performed similarly, but with 1 μg of each plasmid and 6 μL of Fugene 6. Transient transfections were performed 40 hours prior to experiment.

Neuronal cell culture and transfection

Rat hippocampal neurons obtained from the Neuron Culture Service Center at the University of Pennsylvania were dissected from the hippocampus of rat embryos at embryonic day 18-20 as previously described [S4]. Cells were plated at a density of 1,00,000 cells/ml on glass coverslips coated with 0.5 mg/ml poly-L-lysine in 2 ml Neurobasal medium (Gibco) supplemented with 2% B27 (Invitrogen), 1% GlutaMax (Gibco) and cultured at 37°C in a 5% CO_2 incubator.

Neuronal experiments were performed after either 8 or 9 DIV (days in vitro) with DNA plasmids transfected on 7 or 8 DIV respectively. Halo-GFP-mito and PEX3-GFP-Halo were co-transfected with either K560-mcherry-eDHFR or BICD-mcherry-eDHFR using Lipofectamine 2000 reagent (Invitrogen). Cells were imaged 12-18 hours post transfection.

Dimerizer treatment

cTMP-Htag was dissolved in DMSO at 10 mM and stored in amber plastic microcentrifuge tubes at -80 °C, then diluted in medium to a final working concentration of 10 μM. Care was taken to minimize incidental exposure of cTMP-Htag or treated cells to

light prior to experiment. We found that working quickly in low levels of normal room lighting did not cause any detectable premature uncaging. The low levels of white light necessary for differential interference contrast microscopy also did not cause any detectable cTMP-Htag uncaging. Cells were incubated with 10 μM cTMP-Htag for 5-60 minutes in culture medium, then washed with culture medium for 5-15 minutes prior to experiment. In our hands, 5 minute incubations were as effective as 60 minute incubations.

Image acquisition and photoactivation

For live imaging, HeLa cells were plated on 22 x 22 mm glass coverslips (no. 1.5; Fisher Scientific) coated with poly-D-lysine (Sigma-Aldrich). Coverslips were mounted in magnetic chambers (Chamlide CM-S22-1, LCI). During imaging, cells were maintained in L-15 medium without phenol red (Invitrogen) supplemented with 10% FBS and penicillin/streptomycin. Temperature was maintained at ~35 °C using an environmental chamber (Incubator BL; PeCon GmbH).

For HeLa cell experiments, images were acquired with a spinning disk confocal microscope (DM4000; Leica) with a 100x 1.4 NA objective, an XY Piezo-Z stage (Applied Scientific Instrumentation), a spinning disk (Yokogawa), an electron multiplier charge-coupled device camera (ImageEM; Hamamatsu Photonics), and a laser merge module equipped with 488- and 593-nm lasers (LMM5; Spectral Applied Research) controlled by MetaMorph software (Molecular Devices). Images in Figure 1 B and Figure S1 are maximum-intensity projections of 5 confocal Z-sections, 1 μm spacing. Images in Figure 1C, F, G and Figure S2 are single confocal sections.

For whole-cell UV exposure experiments in Figure 1B and Figure S1 A-E, light from a mercury arc lamp (Osram HXP R 120W/45c Vis) was filtered through a 387/11 nm bandpass filter (Semrock part #FF01-387/11 as a component in a DAPI filter cube) and focused through the objective. 5 x 100 ms exposures were used for widefield UV activation. Targeted laser experiments in Figure 1C and Figure S1 panels F,G employed an iLas2 illuminator system (Roper Scientific), equipped with a 405 nm laser (CrystaLaser LC model # DL405-050-O, output of 27 mW after fiber coupling) operated at 10% intensity, controlled using the iLas2 software module within Metamorph. Defined areas (2-10 μm^2) were rasterized 2 times over ~100 ms.

Neurons were imaged in low-fluorescence nutrient media (Hibernate E, Brain Bits) supplemented with 2% B27 and 1% GlutaMax. For neuron experiments, all images were acquired on a spinning-disk confocal UltraView VOX (Perkin Elmer) with a 405 nm Ultraview Photokinesis (Perkin Elmer) unit on an inverted Nikon Ti microscope with apochromat 100X 1.49 NA oil-immersion objective and a C9100-50 EMCCD camera (Hamamatsu) controlled by Volocity software (Perkin Elmer). The Photokinesis module at 25% laser power (0.6 W/cm^2) for 25 cycles was used for localized photoactivation. Only neurons expressing both GFP and mCherry (co-transfected) were imaged and the axons were selected based on morphologic criteria as previously described [S5]. Sequential dual colored images (GFP and mCherry) were acquired for 20 s at 2 s per frame prior to photoactivation and for 5 min at 2 s per frame post photoactivation. There was no evidence of cellular phototoxicity with the photoactivation conditions described here, and we note that these doses of light are less intense than those required for standard FRAP experiments.

Image Processing

All image processing and analysis was performed using ImageJ [S6]. For quantification of peroxisome density vs time in Figures 1D and S1F, average GFP intensity in the indicated regions was measured at each timepoint (cells were imaged every 5 seconds). Background signal was estimated as average intensity in large areas outside the cells and subtracted. To normalize between different regions, the background-subtracted measurements for each region at each timepoint were divided by the maximum intensity observed for each region over the course of the experiment. For the endpoint analysis in Figure 1E, 10 cells were analyzed for KLC1 recruitment and 11 cells were analyzed for BICD recruitment. Two regions were defined for each cell: 1 peripheral region contained in the area targeted by the activating 405 nm laser pulse, and one unilluminated peripheral region (on the other side of the cell). Average GFP intensity within these regions was measured before photoactivation ("start") and at the final timepoint (275 seconds after photoactivation, "end"). Measured values were corrected for background signal and observational photobleaching. The final calculated values are the ratio of end intensity/start intensity for each region.

For analysis of peroxisome (Figure 1H) and mitochondria (Figure S2C) transport in neurons , photoactivated and non-photoactivated organelles within an axon were classified as exhibiting anterograde movement, retrograde movement or no movement. Organelles were considered motile if they moved greater than 5 um distance in a given direction within a time window of 5 min.

Supplemental References

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S3. Kapitein, L. C., Schlager, M. a, van der Zwan, W. a, Wulf, P. S., Keijzer, N., and Hoogenraad, C. C. (2010). Probing intracellular motor protein activity using an inducible cargo trafficking assay. Biophys. J. *99*, 2143–52.

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S6. Schneider, C. a, Rasband, W. S., and Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nat. Methods *9*, 671–675.