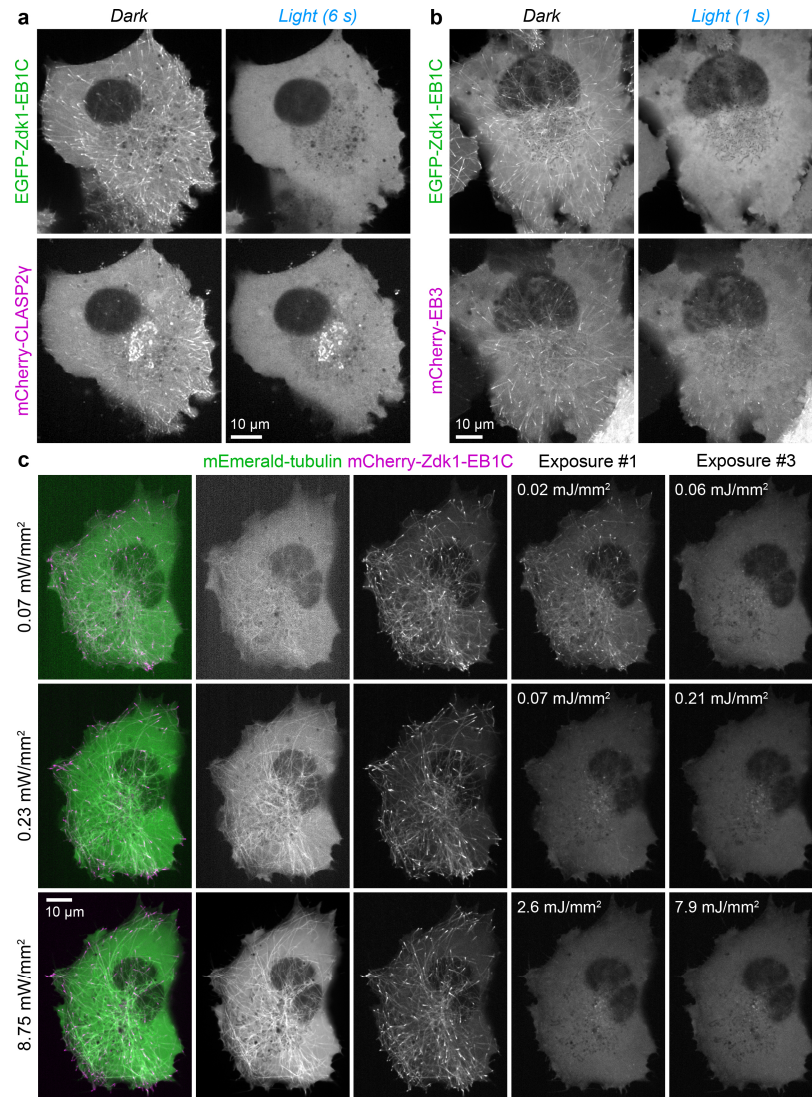


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Local control of intracellular microtubule dynamics by EB1 photodissociation

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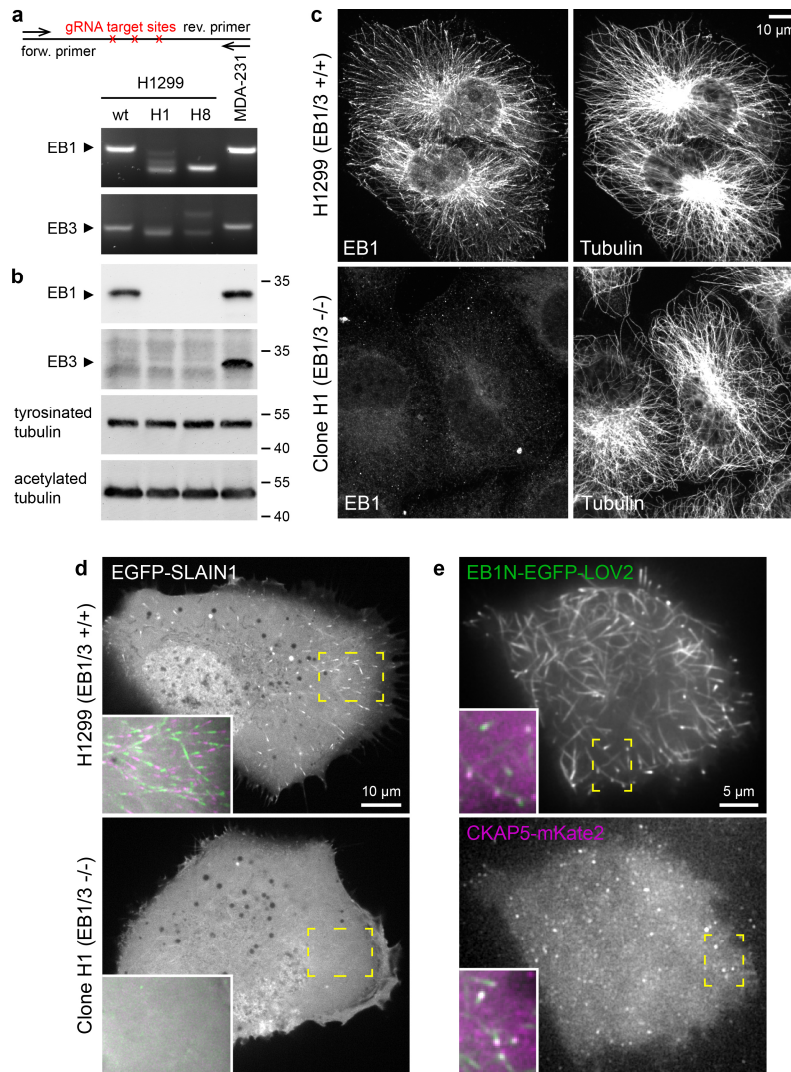
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Supplementary Figure 2

Additional characterization of π -EB1 photo-dissociation.

(a) π -EB1 photo-dissociation results in dissociation of transiently transfected mCherry-CLASP2 γ from growing MT ends. mCherry-CLASP2 γ remains at the Golgi apparatus to which it binds independent of MTs, indicating specific disruption of MT interactions only. **(b)** π -EB1 photo-dissociation partially dissociates transiently transfected mCherry-EB3 from growing MT ends. This is likely due to EB1/3 heterodimerization and indicates that π -EB1 may act as a dominant negative even in the background of small amounts of remaining endogenous EBs. In both **a** and **b** the dark panel is the first exposure at 488 nm. **(c)** To qualitatively compare blue light intensity (i.e. irradiance in mW mm⁻²) and light dose (i.e. radiant exposure in mJ mm⁻²) requirements for π -EB1 photo-dissociation to fluorescent protein imaging by spinning disk confocal microscopy, the same H1299 cell expressing π -EB1 (mCherry-Zdk1-EB1C) and tubulin-mEmerald was recorded 3 times at increasing 488 nm laser power levels (300 ms exposures in the 488 nm channel). π -EB1 was allowed to recover for a few minutes between the recordings. Substantially less light is required for sustained π -EB1 photo-dissociation compared with high signal-to-noise imaging of tubulin-mEmerald. All experiments were replicated at least 3 times with similar results.



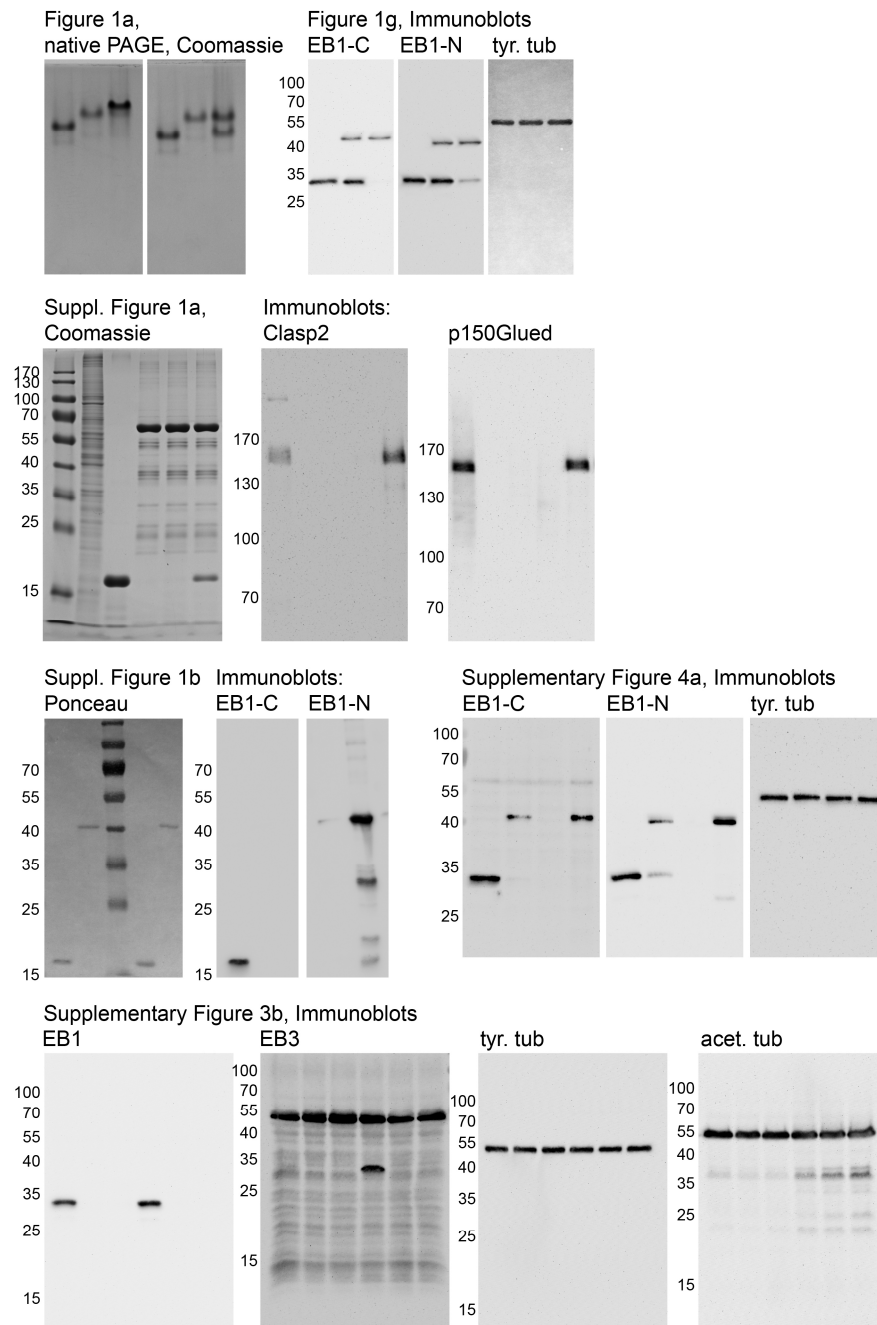
Supplementary Figure 3

Characterization of EB1/3 -/- CRISPR/Cas9 knockout cells.

(a) Knockout strategy using a combination of three guide RNAs targeting exon 1 of the EB1 and EB3 encoding genes (*MAPRE1* and *MAPRE3*). Gels show PCR products of genomic DNA across exon1 from control H1299, two H1299 EB1/3 knockout clones (clones H1 and H8), and control MDA-MB-231 cells. Shifts in PCR product size result from deletions/insertions from non-homologous end joining of Cas9-induced double strand breaks, indicating successful genome editing of all *MAPRE1* and *MAPRE3* alleles in both knockout clones. **(b)** Immunoblot for EB1 and EB3 from lysates of the same cells as indicated above showing absence of both EB1 and EB3 protein from both knockout lines. MDA-MB-231 cells were included as additional controls because H1299 cells express very little if any EB3. Molecular size markers in kDa. **(c)** Immunofluorescence of control H1299 and EB1/3 -/- knockout cells confirms that EB1 is absent in EB1/3 -/- cells. Although EB1/3 -/- cells still had an extensive MT network, compared with controls the MT network in EB1/3 -/- cells was less radial and appeared less dense. **(d)** Control and EB1/3 -/- H1299 cells transiently transfected with EGFP-SLAIN1 showing that EGFP-SLAIN1 is completely absent from growing MT ends in EB1/3 -/- cells. Insets show maximum intensity projections over 20 s time windows of the indicated regions at higher magnification. **(e)** TIRF microscopy of EB1N-GFP-LOV2 and CKAP5-mKate2 in an EB1/3 -/- cell. EB1N-GFP-LOV2 marks MT ends but does not rescue the EB1 +TIP-recruiting activity. Even though these cells do not have functional EB1 and do not recruit SLAIN1/2 or other +TIPs to growing MT ends, CKAP5 still associates with MT ends. Insets show two different regions illustrating CKAP5-mKate2 dots on MT ends. Unprocessed original blots in Supplementary Fig. 5. All experiments were replicated at least 3 times with similar results.

(a) Analysis of EB1 and π -EB1 (EB1N-LOV2 and EGFP-Zdk1-EB1C) expression in control H1299, π -EB1 / EB1 shRNA, EB1/3 $-/-$, and EB1/3 $-/-$ π -EB1 rescue cells. Immunoblots were probed with antibodies specific to either the C- or N-terminus of EB1 and anti- α -tubulin as loading control. Note that the EB1/3 $-/-$ π -EB1 rescue cells express slightly more π -EB1, compared with the shRNA line, and have no residual wild-type EB1. Molecular size markers in kDa. **(b)** π -EB1 photo-dissociation in a field of EB1/3 $-/-$ π -EB1 rescue cells demonstrating the homogeneity of the cell line and of the blue light response (immediate dissociation of EGFP-Zdk1-EB1C from growing MT ends). **(c)** Kymograph analysis of MT growth in EB1N-mCherry-LOV2 expressing EB1/3 $-/-$ π -EB1 rescue cells before and during blue light exposure showing MT growth attenuation similar to what we observed in the π -EB1 / EB1 shRNA cells. **(d)** Representative EB1N-mCherry-LOV2 comet profiles in a π -EB1 / EB1 shRNA cell before and after 15 s during blue light exposure showing shortening of the comet as expected as a result of the reduced MT growth rate. **(e)** Analysis of EB1N-mCherry-LOV2 comet half-length calculated from exponentially modified Gaussian fits of fluorescence intensity profiles in control H1299, π -EB1 / EB1 shRNA, EB1/3 $-/-$, and EB1/3 $-/-$ π -EB1 rescue cells, in the dark and exposed to blue light as indicated. $n = 35$ comet profiles (from 5 cells). Statistical analysis by Tukey-Kramer HSD test. Box plots show median, first and third quartile, with whiskers extending to observations within 1.5 times the interquartile range, and outliers. Notches indicate 95% confidence interval. Note that EB1N-mCherry-LOV2 comet length and thus likely MT growth dynamics are indistinguishable in control H1299 cells compared with EB1/3 $-/-$ π -EB1 rescue cells in

the dark (i.e. complete rescue), and in EB1/3 $-/-$ compared with EB1/3 $-/-$ π -EB1 rescue cells in blue light (i.e. complete knockout phenotype), respectively. **(f)** Time-lapse sequence of CKAP5-mKate2 dynamics before and during blue light exposure in an EB1/3 $-/-$ π -EB1 rescue cell. Top row shows individual time points, bottom row shows maximum intensity projections (MIP) over the indicated time intervals. The second column shows an overlay of EGFP-Zdk1-EB1C (green) and CKAP5-mKate2 (purple), and $t = 0$ s marks the time when blue light was turned on. Note that linear tracks of CKAP5-mKate2 dots persist long after π -EB1 photo-dissociation indicating continued association with MT ends. Unprocessed original blots in Supplementary Fig. 5. All experiments were replicated at least 3 times with similar results.



Supplementary Figure 5

Original immunoblot images.

Uncropped images of all gels and blots as indicated. Molecular size markers in kDa, except Fig. 1a is a native gel without size markers. The ~50kDa band recognized by the EB3 antibody is non-specific.

Supplementary Table 1. List of antibodies used in this study.

Supplementary Table 2. List of PCR primer and gRNA sequences used in this study.

Supplementary Table 3. Statistics source data.

Video 1. Light-mediated SLAIN2 dissociation from MT ends in a π -EB1 / EB1 shRNA cell.

Time-lapse sequence of five consecutive cycles of π -EB1 photo-dissociation showing immediate and reversible release of both the EGFP-tagged π -EB1 C-terminal half and mCherry-SLAIN2 from growing MT ends when 488 nm blue light is turned on. Images were acquired every 2 seconds with a 3 min dark recovery phase between cycles. Scale bar: 10 μ m.

Video 2. Spatiotemporal control of π -EB1 photo-dissociation.

Time-lapse sequence of mCherry-Zdk1-EB1C showing local and reversible π -EB1 photo-dissociation by alternating patterns of blue light exposure. The boundary sharpness in this video illustrates what we can currently achieve. Images were acquired at one frame per second, and playback is accelerated 15x. Scale bar: 10 μ m.

Video 3. Reversible inhibition of MT growth by π -EB1 photo-dissociation.

Time-lapse sequence of EB1N-mCherry-LOV2 before, during and after blue light exposure showing reversible inhibition of MT growth as a result of π -EB1 photo-dissociation. Images were acquired at two frames per second, and playback is accelerated 7.5x. Scale bar: 10 μ m.

Video 4. Local control of MT growth.

Time-lapse sequence of EB1N-mCherry-LOV2 showing local inhibition of MT growth. Only the top half of the cell is exposed to 470 nm blue light during the 40-79 second time window illustrating near immediate MT growth inhibition. Images were acquired at one frame per second, and playback is accelerated 15x. Scale bar: 10 μ m.

Video 5. Light-induced MT depolymerization by π -EB1 photo-dissociation.

Time-lapse sequence of tubulin-mCherry before and during blue light exposure illustrating rapid depolymerization of a population of microtubules in response to π -EB1 photo-dissociation. Left panel shows the EGFP-tagged C-terminal half of π -EB1 dissociating from MT ends when 488 nm exposure is turned on. Images were acquired every 2.5 seconds and playback is accelerated 30x. Scale bar: 10 μ m.

Video 6. Light-induced MT reorganization by π -EB1 photo-dissociation.

Time-lapse sequence of tubulin-mCherry in a Rac1(Q61L)-expressing π -EB1 cell. Only approximately the right third of the cell is exposed to blue light resulting in retraction of the MT network from this part of the cell. Images were acquired every 30 seconds and playback is accelerated 450x. Scale bar: 10 μ m.

Video 7. CKAP5 association with MT ends is independent of π -EB1 photo-dissociation.

Time-lapse sequence of CKAP5-mKate2 dynamics by TIRF microscopy before and during π -EB1 photo-dissociation. Dynamic CKAP5-mKate2 dots remain during blue light exposure indicating that CKAP5 association with the most distal tip of growing MTs does not depend on functional EB1. Top panel shows

the EGFP-tagged C-terminal half of π -EB1 dissociating from MT ends when 488 nm exposure is turned on. Images were acquired at 0.6 frames per second, and playback is accelerated 25x. Scale bar: 10 μ m.

Video 8. CKAP5 dynamics in EB1/3 -/- cells.

Time-lapse sequence of CKAP5-mKate2 dynamics by TIRF microscopy in a control and a CRISPR/Cas9 EB1/3 knockout cell. Dynamic CKAP5-mKate2 dots are still visible in the EB1/3 -/- cells further indicating that CKAP5 association with growing MT ends is independent of EBs. Images were acquired at two frames per second, and playback is accelerated 7.5x. Scale bar: 10 μ m.

Video 9. Light-induced MT growth inhibition in π -EB1-expressing EB1/3 -/- cells.

Time-lapse sequence of EB1N-mCherry-LOV2 before and during blue light exposure (30 s after blue light was switched on) in a CRISPR/Cas9 EB1/3 knockout cell stably expressing π -EB1 (EB1N-LOV2 and EGFP-Zdk1-EB1C) showing inhibition of MT growth as a result of π -EB1 photo-dissociation. Images were acquired at two frames per second, and playback is accelerated 7.5x. Scale bar: 10 μ m.

Video 10. π -EB1-expressing EB1/3 -/- cell trapped in a virtual blue light box.

Time-lapse sequence of EB1/3 -/- π -EB1 rescue cell migration confined by a surrounding blue light pattern. Over the approximately 8.5 hour duration of the experiment, the cell is unable to cross the blue light barrier. Images were acquired every 2 minutes, and playback is accelerated 1800x. Scale bar: 10 μ m.