Local control of intracellular microtubule dynamics by EB1 photodissociation

Jeffrey van Haren1, Rabab A. Charafeddine1, Andreas Ettinger1,3, Hui Wang2, Klaus M. Hahn2 and Torsten Wittmann1,3*

End-binding proteins (EBs) are adaptors that recruit functionally diverse microtubule plus-end-tracking proteins (+TIPs) to growing microtubule plus ends. To test with high spatial and temporal accuracy how, when and where +TIP complexes contribute to dynamic cell biology, we developed a photo-inactivated EB1 variant (π-EB1) by inserting a blue-light-sensitive protein-protein interaction module between the microtubule-binding and +TIP-binding domains of EB1. π-EB1 replaces endogenous EB1 function in the absence of blue light. By contrast, blue-light-mediated π-EB1 photodissociation results in rapid +TIP complex disassembly, and acutely and reversibly attenuates microtubule growth independent of microtubule end association of the microtubule polymerase CKAP5 (also known as ch-TG and XMAP215). Local π-EB1 photodissociation allows subcellular control of microtubule dynamics at the second and micrometre scale, and elicits aversive turning of migrating cancer cells. Importantly, light-mediated domain splitting can serve as a template to optically control other intracellular protein activities.

Microtubules (MTs) self-organize to form polarized arrays of dynamic intracellular tracks, enabling directional transport, chromosome segregation and cell polarity. MT network remodelling through stochastic switching of dynamic MT plus ends between phases of growth and shortening and selective stabilization is critical for all MT functions in cells. Despite extensive biochemical insights from in vitro experiments and computational models, how MT dynamics are locally controlled inside cells is incompletely understood. In addition, how local control of MT dynamics contributes to complex cell and tissue morphology remains unclear, which represents a gap in our understanding of physiological MT function. Interactions of growing MT plus ends with other intracellular components are mediated by a class of proteins referred to as microtubule plus-end-tracking proteins (+TIPs)1–4. Association of most, if not all, +TIPs with growing MT ends requires end-binding proteins (EBs), in particular, EB1 (encoded by MAPRE1) and EB3 (encoded by MAPRE3) in mammalian cells. Although EBs associate with all growing MT ends, the composition of the EB-recruited +TIP complex is tightly controlled in cells in space and time5–9. In addition, different +TIP complexes can have antagonistic effects. EBs can recruit enzymes to growing MT ends, promoting either MT polymerization10 or depolymerization11, and how these opposing activities are balanced remains unknown. Here, we developed a strategy to inactivate the +TIP adaptor EB1 by light, a stimulus that can be controlled with high spatial and temporal accuracy and is compatible with high-resolution microscopy of cell dynamics. We demonstrate that a photo-inactivated EB1 variant (π-EB1) allows accurate control of intracellular MT dynamics and function, allowing new experimental paradigms and serving as proof of principle of a new optogenetic protein inactivation design.

Results

Design of a photo-inactivated EB1 variant. EB1 consists of two functional domains. The amino-terminal CH domain recognizes growing MT ends12,13, whereas the carboxy-terminal EBH domain recruits +TIPs13. We predicted that controlling their connection using light would disrupt EB1-mediated +TIP interactions with growing MT ends, without interfering with EB1 binding to MTs. Unlike most other optogenetic reagents in which light induces protein–protein interactions14, the protein A Z-domain-derived affibody, Zdk1 (ref.15), functions the opposite way and binds to the oat phototropin 1 LOV2 domain with high affinity in the dark, but dissociates in blue light (Fig. 1a). To develop a light-sensitive EB1 variant, we inserted the LOV2–Zdk1 module into the intrinsically disordered linker between the N-terminal and C-terminal EB1 domains. Because efficient EB1 plus-end tracking requires dimerization16,17, we further inserted a GCN4 leucine zipper between the CH and LOV2 domains to retain dimerization and plus-end tracking of the N-terminal half by itself. We refer to these photo-inactivated π-EB1 constructs as EB1N-LOV2 and Zdk1-EB1C (Fig. 1b). Affinity-purified glutathione S-transferase (GST)-tagged EB1N-LOV2 bound poly-histidine (6xHis)-tagged Zdk1-EB1C and precipitated both SxIP motif (CLIP-associated protein 2 (CLASP2)) and CAP-Gly motif (p15002nd)-TIPs from cell lysates. This indicates that the two π-EB1 halves interact and that Zdk1-EB1C is functional in binding known classes of +TIPs (Supplementary Fig. 1a). To directly visualize π-EB1 dynamics in cells and because tagging EB1 at either the N terminus or the C terminus interferes with EB1 function18, we inserted an enhanced green fluorescent protein (eGFP) tag N-terminal to the Zdk1 peptide. To initially test the π-EB1 light response, the LOV2 domain was photoactivated by turning on the 488 nm acquisition channel, which represents saturating blue light exposure. eGFP-Zdk1-EB1C was recruited to MT ends by non-tagged EB1N-LOV2, and dissociated from MT ends in response to blue light with a half-life (t1/2) of <200 ms (Fig. 1c,d), which is close to the diffusion-limited turnover time of EB1 molecules on MT ends19,20. Zdk1-EB1C remained enriched near centrosomes that bind SxIP-motif +TIPs independent of growing MT ends21. Because Zdk1 binding requires the LOV2 C terminus, we further inserted mCherry between EB1N and LOV2 to verify that the π-EB1 N-terminal half remained on growing MT ends (Fig. 1e,f).

1Department of Cell and Tissue Biology, University of California, San Francisco, CA, USA. 2University of North Carolina, Chapel Hill, NC, USA. 3Institute of Epigenetics and Stem Cells, Helmholtz Center Munich, München, Germany. *e-mail: torsten.wittmann@ucsf.edu

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.

NATURE CELL BIOLOGY | VOL 20 | MARCH 2018 | 252-261 | www.nature.com/naturecellbiology
EB1 activity was largely replaced by π-EB1. We chose H1299 non-small-cell lung cancer cells because they express almost no EB3 (ref. 22) (Supplementary Fig 1c). We first made H1299 cell lines with both π-EB1 halves at similar and near endogenous levels (46 ± 17% EB1N-LOV2; 29 ± 11% eGFP-Zdk1-EB1C) by depletion of EB1. However, EB1 depletion is known to affect both EB1 halves equally. Thus, we generated cell lines with only endogenous levels of one EB1 half. These data demonstrate that both halves of π-EB1 are recruited to MT ends efficiently in the dark, and that blue light induces rapid π-EB1 photodissociation.

Acute and reversible +TIP complex disruption by π-EB1 photodissociation. To analyse the functional consequences of π-EB1 photodissociation, we generated cell lines in which endogenous EB1 activity was largely replaced by π-EB1. We chose H1299 non-small-cell lung cancer cells because they express almost no EB3 (ref. 23) (Supplementary Fig 1c). We first made H1299 cell lines that stably expressed π-EB1 constructs. Even though both π-EB1 halves were expressed from different plasmids, selecting clonal lines that show normal MT plus-end association of eGFP-Zdk1-EB1C, which requires untagged EB1N-LOV2 to be present, resulted in H1299 cell lines with both π-EB1 halves at similar and near endogenous levels (46 ± 17% EB1N-LOV2; 29 ± 11% eGFP-Zdk1-EB1C).
compared with endogenous EB1; \( n = 4 \). Next, endogenous EB1 was depleted in \( \pi \)-EB1-expressing cells by lentivirus-mediated short hairpin RNA (shRNA) by -90 ± 2% \( (n = 3) \) (Fig. 1g). These \( \pi \)-EB1/EB1 shRNA cells used in subsequent experiments were viable with no obvious phenotype, indicating that \( \pi \)-EB1 expression rescues loss of endogenous EB1 function.

We then asked how \( \pi \)-EB1 photodissociation affected the dynamics of mCherry-tagged +TIPs at growing MT ends. All +TIPs that were tested localized to MT plus ends in non-illuminated cells, but disappeared rapidly from MT ends during blue light exposure, confirming that MT end association of mitotic centromere-associated kinesin (MCAK; also known as KIF2C), CLASP2 and SLAIN motif-containing protein 2 (SLAIN2) critically depends on interactions with the C-terminal domain of EB1 (Fig. 2a). In addition, +TIP dissociation was reversible over multiple cycles of light exposure (Fig. 2b and Supplementary Video 1), indicating that \( \pi \)-EB1 photodissociation rapidly and reversibly disrupts the MT plus-end +TIP complex. +TIPs only dissociated from growing MT ends and not from other binding sites; for example, CLASP2 remained at the Golgi apparatus \( ^{25} \) (Supplementary Fig. 2a), demonstrating that +TIP dissociation from MT ends is specific to the light-sensitive \( \pi \)-EB1 dissociation. EB3-mCherry also partially dissociated from MT ends upon \( \pi \)-EB1 photodissociation, which probably reflects heterodimerization with \( \pi \)-EB1 and supports a dominant effect of \( \pi \)-EB1 photodissociation even in the presence of small amounts of endogenous EB1s \( ^{23,24} \) (Supplementary Fig. 2b).

Subcellular spatial control of \( \pi \)-EB1 photodissociation. Because EB1 diffuses freely through the cytoplasm \( ^{26} \), spatial control requires rapid re-association of photodissociated \( \pi \)-EB1 molecules in the absence of blue light to prevent diffusion throughout the cell. Compared with wild-type (WT) LOV2, \( \pi \)-EB1 containing the LOV2 (I427V) variant with faster dark-state recovery \( ^{15,25} \) re-associated on growing MT ends approximately twice as fast (Fig. 2c). To minimize blue-light-induced phototoxicity, we determined the minimum dosage of blue light exposure required for sustained \( \pi \)-EB1 photodissociation. To achieve a range of radiant exposures, we used pulse-width modulation of a 470-nm LED \( (0.25-20\text{-ms pulse lengths}) \) at a fixed irradiance and a pulse frequency of 1 Hz between camera exposures (Fig. 2d). Compared with WT LOV2, the faster LOV2 domain required approximately three-times more blue light to reach the same steady-state \( \pi \)-EB1 photodissociation. Thus, faster LOV2 dark recovery comes at a cost of increased dosage of blue light exposure. To test how this blue light exposure related to typical fluorescence microscopy light levels \( ^{26} \), we compared \( \pi \)-EB1 photodissociation with images of mEmerald-tagged tubulin at identical exposure settings (Supplementary Fig. 2c), indicating that \( \pi \)-EB1 photodissociation-associated photodamage is probably small.

Next, we used a digital micromirror device mask to test whether LOV2 kinetics are sufficient for localized \( \pi \)-EB1 photodissociation. With the faster LOV2 variant, 20-ns 470-nm light pulses of <1 mJ mm \(^{-2} \) every second achieved intracellular gradients with 95% difference in \( \pi \)-EB1 photodissociation over a width of ~20 μm (Fig. 2e,f), and reversibly switched \( \pi \)-EB1 photodissociation between different intracellular regions (Fig. 2g and Supplementary Video 2). The photodissociation boundary was similar to the edge steepness of the illumination pattern (Fig. 2f), which was much shallower than expected from the theoretical resolution limit. Thus, boundary steepness was mostly determined by the sharpness of the illumination pattern edge, which is a potential technical limit, and to a lesser extent by diffusion of photodissociated molecules out of the blue-light-exposed region. Because WT LOV2 achieved a boundary that was almost as steep at reduced blue light exposure, we continued to use \( \pi \)-EB1/EB1 shRNA cells expressing WT LOV2 \( \pi \)-EB1 and in which endogenous EB1 was stably depleted by shRNA.

Acute and local inhibition of MT growth and organization by \( \pi \)-EB1 photodissociation. Many EB1-recruited +TIPs influence MT polymerization dynamics \( ^{1} \), but it is not understood how potentially antagonistic +TIP activities are integrated and spatially controlled inside cells. Although previous EB1/3 short interfering RNA depletion studies demonstrated MT dynamics defects \( ^{12,27} \), such experiments are confounded by indirect and adaptive mechanisms in genetically depleted cells and cannot directly address protein actions in real time. Thus, we tested how \( \pi \)-EB1 photodissociation acutely altered MT dynamics and organization. As expected, the MT-binding half of \( \pi \)-EB1 remained on growing MT ends following \( \pi \)-EB1 photodissociation (Fig. 1e). However, after a few seconds, EB1N-mCherry-LOV2 comet length decreased (Supplementary Fig. 4d,e), which is indicative of a decreased MT growth rate \( ^{24} \). Indeed, computational tracking of EB1N-mCherry-LOV2 revealed an overall attenuation of MT growth and a reduction in the number of growing MT ends within 30 s of blue light exposure (Fig. 3a,b and Supplementary Video 3), and the shift in the frame-to-frame growth rate distribution indicated that \( \pi \)-EB1 photodissociation predominantly affected fast-growing MT ends (Fig. 3c). Importantly, these changes were reversible, and MT dynamics recovered within minutes of terminating blue light exposure (Fig. 3b). Moreover, the fast kinetics of \( \pi \)-EB1 photodissociation allowed locally restricted inhibition of MT growth (Fig. 3d and Supplementary Video 4). Kymograph analysis indicated that polymerization of individual MT ends did not slow gradually. Instead, MTs abruptly switched to a slower growth rate or underwent a catastrophe following \( \pi \)-EB1 photodissociation (Fig. 3e). However, this response was not always immediate and resulted in a delay of the MT population growth rate with \( t_{c} = \approx 5 \text{ s} \) (Fig. 3f), which is at least an order of magnitude slower than \( \pi \)-EB1 photodissociation itself (Fig. 1d). Together, these data demonstrate that the +TIP-recruiting activity of EB1 is acutely required and sufficient to sustain fast and persistent MT growth in interphase cells.

We then tested whether the observed MT growth inhibition was sufficient to reorganize the intracellular MT network. Although difficult to quantify precisely, the density of the peripheral MT network dropped rapidly during blue light exposure, and recovered within minutes after terminating blue light exposure (Fig. 4a). A decrease in the amount of MT polymer was further supported by a robust increase in cytoplasmic tubulin-mCherry fluorescence, which reached a new equilibrium in <1 min \( (t_{c} = \approx 12 \text{ s}) \) as individual MTs depolymerized in response to \( \pi \)-EB1 photodissociation (Fig. 4c and Supplementary Video 5). Interestingly, two distinct MT populations responded differently. Many radial MTs underwent catastrophes and depolymerized within seconds of \( \pi \)-EB1 photodissociation (Fig. 4d), which is consistent with the observed delay in growth rate attenuation (Fig. 3f). By contrast, a population of more-curved MTs appeared more resistant to \( \pi \)-EB1 photodissociation, indicating stabilization by mechanisms that no longer require a growth-promoting +TIP complex. To focus on the population of ‘pioneer’ MTs that grow towards the leading edge of migrating cells \( ^{29} \), we expressed constitutively active Rac1(Q61L) in \( \pi \)-EB1 cells, which induces isotropic leading-edge F-actin polymerization, and exposes fast MT growth that counter-balances retrograde F-actin flow \( ^{10} \). Local \( \pi \)-EB1 photodissociation resulted in a dramatic retrac- tion of these MTs from Rac1(Q61L)-induced lamellipodia (Fig. 4e and Supplementary Video 6), indicating that EB1-recruited +TIP complexes maintain fast ‘pioneer’ MT growth near a migrating cell’s leading edge. Importantly, by using patterned blue light exposure, intracellular asymmetry of MT network organization could be generated rapidly and maintained for extended periods of time.

EB1 is not required to recruit the MT polymerase CKAP5 to MT ends. Because CKAP5 accelerates MT growth in vitro \( ^{21,23} \) and can bind to the EB-dependent +TIP SLAIN2 \( ^{30} \), we hypothesized...
that loss of CKAP5 from MT ends in response to $\pi$-EB1 photodissociation may cause the observed MT growth inhibition. Consistent with previous reports, $\text{mKate2-tagged CKAP5 localized to dynamic dots at distal MT ends}^{5,9}$. However, even in control cells, only a subset of EB1 comets had associated dots of CKAP5-mKate2 (Fig. 5g), and CKAP5-mKate2 was often completely absent from elongated EB1 comets on fast-growing MT ends (Fig. 5b). In addition, quantitative comparison revealed no clear correlation between the amount of EB1 and CKAP5 associated with the same MT plus ends (Fig. 5c). We then tested whether $\pi$-EB1 photodissociation altered intracellular CKAP5 dynamics: even after 30 s of blue light exposure, when persistent MT growth has ceased, the relative brightness of CKAP5 dots did not significantly decrease (Fig. 5d–f and Supplementary Video 7).
Fig. 3 | Attenuation of MT growth by \( \pi \)-EB1 photodissociation. a, EB1N-mCherry-LOV2-labelled MT plus ends before blue light exposure, after 30 s during blue light exposure and after 3-min recovery in the dark in \( \pi \)-EB1/EB1 shRNA cells. Insets show maximum intensity projections over 20-s time windows of the indicated regions at higher magnification. b, Quantification of MT polymerization dynamics by tracking EB1N-mCherry-LOV2-labelled MT ends. Growth rates are frame-to-frame measurements from images acquired at 0.5-s intervals. \( n = 11 \) cells. Box plots show median, first and third quartile, with whiskers extending to observations within 1.5 times the interquartile range, and all individual data points. Statistical analysis by Tukey-Kramer HSD test. c, Comparison of the frame-to-frame MT growth rate distribution in the dark and during blue light exposure, demonstrating a specific loss of fast growth events as a result of \( \pi \)-EB1 photodissociation. Shown are the mean distributions from the cells in b (lines) and the 95% confidence intervals (shaded areas). d, Local inhibition of MT growth by patterned blue light exposure in a \( \pi \)-EB1/EB1 shRNA cell expressing EB1N-mCherry-LOV2. Shown is a maximum intensity projection over 20 s. Only the top half above the dashed line was exposed to blue light pulses between image acquisitions. Insets show the indicated regions at higher magnification. This experiment was replicated more than five times with similar results. e, Representative kymographs illustrating the sudden response of rapidly growing MT ends to \( \pi \)-EB1 photodissociation. f, Analysis of the intracellular MT population growth rate response as a function of time after \( \pi \)-EB1 photodissociation. Shown are all measurements from \( n = 5 \) cells. The purple solid line is an exponential fit of the mean during blue light exposure. The purple shaded area is the 95% confidence interval of the fit. In a and d, sequential time points in maximum intensity projections are shown in alternating green and purple to better visualize MT growth tracks. Source data are shown in Supplementary Table 3.
Because we were concerned about incomplete and variable EB1 depletion by shRNA, we next deleted the genes encoding EB1 and EB3 (EB1/3–/–) in H1299 cells by CRISPR–Cas9 genome editing (Supplementary Fig. 3a). This eliminated both EB1 and potentially compensatory EB3 expression (Supplementary Fig. 3b,c) and EB-dependent +TIP recruitment (Supplementary Fig. 3d). Nevertheless, even in these genetically deleted EB1/3−/− cells, CKAP5-mKate2 still associated with growing MT ends (Supplementary Fig. 3e and Supplementary Video 8). In addition, MT growth was inhibited in EB1/3−/− cells and indistinguishable from what we observed in blue-light-exposed π-EB1-expressing EB1 shRNA cells (Fig. 5g).

Importantly, both in EB1 shRNA cells and in EB1/3−/− cells, only expression of both π-EB1 halves (Supplementary Fig. 4a,b) rescued MT growth rate and comet length in a light-dependent manner (Fig. 5h, Supplementary Video 9 and Supplementary Fig. 4c–e), but expression of the N-terminal MT-binding half alone, EB1N-mCherry-LOV2, did not (Fig. 5g). CKAP5-mKate2 associated with a population of growing MT ends in EB1/3−/− π-EB1 rescue cells both in the dark and during blue light exposure (Supplementary Fig. 4f). Together, these data demonstrate that CKAP5 MT plus-end association is independent of EB1-mediated +TIP recruitment, and therefore cannot explain MT growth inhibition by π-EB1 photodissociation.
Local $\pi$-EB1 photodissociation induces rapid cell turning. Persistent MT growth towards the leading edge has long been speculated to maintain directional cell migration $^{34-36}$. However, owing to the lack of experimental tools to manipulate intracellular MT dynamics with high spatial and temporal accuracy, the central question to what extent MT network polarity is a cause or consequence of directional migration has not been adequately addressed. Thus, we used our CRISPR EB1/3-/- $\pi$-EB1 rescue cells to ask how acute interference with EB-dependent MT plus-end interactions influenced directional cell migration. We focused on randomly migrating single cells in the absence of other chemical or haptotactic cues. As expected, WT H1299 cells did not react to local blue light.

Fig. 5 | EB1-independent MT plus-end localization of the MT polymerase CKAP5. a, TIRF microscopy of a control H1299 cell expressing EB1N-eGFP-LOV2 and CKAP5-mKate2. CKAP5 forms dots at growing MT ends that are unlike the typical comet-shaped EB1 distribution. Note that wavelengths were acquired sequentially, resulting in a small temporal shift between channels. Insets show indicated regions at higher magnification. b, Time-lapse sequence showing variable and transient CKAP5 accumulation on MT ends. Arrowheads highlight representative MT ends. Elapsed time is shown in seconds. c, Scatter plot of the relative accumulation of CKAP5 and EB1 on the same MT ends showing only a very weak correlation. The dashed line indicates the ratio of 1 at which no MT end accumulation of CKAP5-mKate2 can be detected. The solid line is a linear fit. $n=252$ MT ends from 5 cells. d, TIRF microscopy of CKAP5-mKate2 in a $\pi$-EB1/EB1 shRNA cell 15 s before (left panel) and during (right panel) $\pi$-EB1 photodissociation, showing qualitatively indistinguishable CKAP5 distribution. Insets show projections over 20-s time windows of the indicated regions, revealing linear tracks of CKAP5 dots. e, Time-lapse sequence of CKAP5-mKate2 dynamics before and during blue light exposure in a $\pi$-EB1/EB1 shRNA cell. Arrowheads highlight the continued linear movement of a MT-end-associated CKAP5 dot. Elapsed time is shown in seconds. f, CKAP5 enrichment on MT ends in the indicated time intervals before and during blue light exposure, indicating no change in response to $\pi$-EB1 photodissociation. 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 all individual data points. $n=8$ cells. g, EB1N-mCherry-LOV2-labelled MT plus ends in control H1299 cells and in cells in which both EB1 and EB3 expression were deleted by CRISPR–Cas9 genome editing, showing attenuation of MT growth in EB1/3-/- cells. h, EB1N-mCherry-LOV2-labelled MT plus ends in an EB1/3-/- $\pi$-EB1 rescue cell before and during blue light exposure, demonstrating light-induced MT growth attenuation very similar to what we observe in $\pi$-EB1/EB1 shRNA cells. In g and h, insets show maximum intensity projections over 20-s time windows of the indicated regions. Sequential time points in these projections are shown in alternating green and purple to better visualize MT growth tracks. Experiments were replicated more than five times with similar results. Source data are shown in Supplementary Table 3.
Fig. 6 | Aversive cell turning in response to local π-EB1 photodissociation. a, Time-lapse sequence of a control H1299 cell expressing EB3-mCherry showing no response to local blue light exposure. b, Two examples of EB1/3<sup>−/−</sup> π-EB1 rescue cells that turn away from local blue light exposure in the front half of the cell. Cells also express mCherry-Zdk1-EB1C to show local π-EB1 photodissociation. Blue areas are images of the patterns of blue light exposure reflected off the coverslip. Yellow lines show the cell centroid trajectory. 0 min indicates the time when the respective blue light pattern was switched on. c, Analysis of cell migration direction changes over 30-min time intervals expressed as the angle between smoothed centroid positions 15 min before and 15 min after the indicated time points. A significant non-random change in migration direction only occurred in response to local blue light exposure in EB1/3<sup>−/−</sup> π-EB1 rescue cells, which consistently turned away from the pattern. n = 12 cells (control); n = 13 cells (EB1/3<sup>−/−</sup> π-EB1). Dashed line indicates no direction change. Box plots show median, first and third quartile, with whiskers extending to observations within 1.5 times the interquartile range, and all individual data points. Statistical analysis by Tukey–Kramer HSD test. d, Time-lapse sequence of a migrating EB1/3<sup>−/−</sup> π-EB1 rescue cell trapped inside a virtual blue light box for >8 h. The yellow line marks the centroid trajectory. This experiment was replicated more than three times with similar results.
exposure (Fig. 6a). By contrast, EB1/3−/− πEB1 rescue cells consistently turned away from blue-light-exposed regions that were placed in the front half of a migrating cell (Fig. 6b). Although H1299 cells often switched direction and random protrusion dynamics contributed substantially to centroid trajectory directional noise, in a quantitative comparison of multiple cells, the only significant direction change occurred as a response to local blue light exposure in EB1/3−/− πEB1 rescue cells (Fig. 6c). We then tested whether this aversive turning response to local πEB1 photodissociation was sufficient to confine a migrating cell within a predefined region. Indeed, even though these cells are highly motile, EB1/3−/− πEB1 rescue cells were unable to escape from a blue light box and reversed direction every time the cell touched the virtual blue light barrier for an observation period of >8 h (Fig. 6d and Supplementary Video 10).

Thus, EB1-mediated interactions of +TIP complexes with growing MT ends are necessary to maintain leading-edge identity and directionality during cell migration.

Discussion

To locally dissect complex and dynamic cell biology, new methods are needed to rapidly inactivate specific protein activities inside cells. Here, we use the +TIP-recruiting activity of EB1 as a proof of principle to photo-inactivate specific protein functions by inserting a light-sensitive LOV2–Zdk1 protein–protein interaction module between functional domains. Unlike genetic methods that are orders of magnitude too slow to dissect highly dynamic MT and +TIP functions and offer no spatial control, we demonstrate acute, reversible πEB1 photodissociation and control of intracellular MT dynamics at second and micrometre accuracy inside live cells. In contrast to genetic methods to silence or knockout genes, this approach allows the replacement of a protein of interest with a photo-inactivated π-variant that can be fully characterized for normal function in the absence of blue light. Thus, acute and local photo-inactivation can be used to directly observe and analyse a cellular response, rather than inferring protein function from a cell population in which a gene of interest has been removed and in which cells may have adapted to the loss of function through poorly controlled compensatory mechanisms. In the case of +TIP or other complex protein networks, biochemical competition may further complicate mechanistic interpretation of depletion or knockout phenotypes. To our knowledge, this light-induced reversible domain splitting is the only optogenetic method to allow acute, reversible and specific loss-of-function experiments. Most other optogenetic tools rely on gain of function, in which proteins are activated, relocalized or aggregated by light. There are only a few methods of light-induced protein inactivation that are either slow and irreversible, such as protein dephosphorylation by light-induced proteolysis or genome editing, or require large a priori knowledge of target protein structure.

Acute πEB1 photodissociation allowed us to obtain new insights into the role of EB1 and the associated +TIP complex in the physiological control of MT dynamics and function. Although we only tested a subset of SxIP-motif +TIPs, recruitment of all known +TIPs depends on interactions with the EB1 C terminus41. Thus, we think that πEB1 photodissociation results in a broad disruption of +TIP association with MT ends. In vitro, EB1 stimulates tubulin GTP hydrolysis and increases the catastrophe frequency12,42,43, but only moderately increases the MT growth rate at high EB1 concentrations. By contrast, we find that in cells, EB1-mediated +TIP complexes maintain fast and persistent MT growth. Thus, the EB1 intrinsic MT-stabilizing activity is outweighed by the growth-promoting activity of the EB1-recruited +TIP complex. Because the N-terminal half of πEB1 remains on growing MT ends after photodissociation, structural changes induced by EB1 binding are insufficient to maintain persistent MT growth. In contrast to previous reports15, at physiological expression levels, the N-terminal MT-binding half of EB1 by itself had no noticeable effect on intracellular MT dynamics.

The growth-promoting activity of the EB1-mediated +TIP complex also cannot easily be explained by interactions with the canonical MT polymerase CLASP. CLASP association with the most distal tips of MT plus ends does not correlate well with EB1 localization and does not depend on EB1 either in the πEB1 photodissociation experiments or in cells in which we removed EB1 and EB3 by genome editing. Although this is consistent with EB1-independent CLASP localization to MT ends in vitro15, this was surprising as CLASP recruitment to growing MT ends in cells was previously attributed to EB1-mediated interactions with the +TIP SLAIN2 (refs 7,8), which rapidly disappears from growing MT ends upon πEB1 photodissociation. In any case, our results show that, in the absence of an EB1-recruited +TIP complex, CKAP5 by itself cannot maintain physiological MT growth, and additional mechanisms that control CKAP5 activity could be involved. Strikingly, individual MTs respond to πEB1 photodissociation stochastically and often continue to grow for some time before switching abruptly from fast to slow growth, indicating a sudden state change of the growing MT ends after +TIP complex dissociation. Thus, alternatively, the +TIP complex may stabilize the protective GTP-like MT end cap10 in cells independent of CKAP5.

As an example to demonstrate the utility of πEB1 in interrogating dynamic cell biology, we asked whether local +TIP dissociation is sufficient to influence migration of cancer cells in which we replaced EB1 and EB3 with πEB1. We find that the response of migrating H1299 non-small-cell lung cancer cells to local πEB1 photodissociation is remarkably robust: cells invariably turned away from the area of blue light exposure within minutes and were unable to cross a virtual blue light boundary. Thus, even in this highly transformed metastatic cancer cell line, +TIP-mediated MT interactions in the front of the cell are essential to define and maintain directional polarity. This is consistent with our previous finding that cell–matrix-adhesion-associated MT tracks support localized secretion of matrix metalloproteinase activity, which supports MT involvement in invasive cancer cell migration and emerging ideas that MT-directed cancer chemotherapy targets cell functions other than mitosis.

In summary, regardless of the precise mechanism by which EB1-recruited +TIP complexes maintain physiological MT growth rates, our data demonstrate local and acute modulation of intracellular MT dynamics with spatial and temporal accuracy that surpasses recent photopharmacological approaches7, and together with selective optogenetic recruitment of specific proteins to MT ends61 will allow precise dissection of +TIP functions in complex cell biology. Finally, insertion of a light-sensitive protein–protein interaction module between functional domains is a powerful strategy applicable to a large fraction of the proteome, enabling new spatially and temporally resolved experimental questions that cannot be addressed by other genetic or pharmacological means.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41556-017-0028-5.

Received: 10 January 2017; Accepted: 13 December 2017; Published online: 29 January 2018

References

1. Ohi, R. & Zanic, M. Ahead of the curve: new insights into microtubule dynamics. F1000Res. 5, 314 (2016).
2. Akhmanova, A. & Steinmetz, M. O. Control of microtubule organization and dynamics: two ends in the limelight. Nat. Rev. Mol. Cell. Biol. 16, 711–726 (2015).
3. Kumar, P. & Wittmann, T. +TIPs: SxIPping along microtubule ends. Trends Cell Biol. 22, 418–428 (2012).
4. Kumar, P. et al. GSK3beta phosphorylation modulates CLASP–microtubule association and lamella microtubule attachment. J. Cell Biol. 184, 895–908 (2009).

© 2018 Macmillan Publishers Limited, part of Springer Nature. All rights reserved.
30. Wittmann, T., Bokoch, G. M. & Waterman-Storer, C. M. Regulation of cell migration by dynamic TIP complexes to regulate neurite extension. *Curr. Biol.*, 24, 1778–1785 (2014).

31. Wang, X. et al. Skin stem cells orchestrate directional migration by regulating microtubule–AxeT connections through GSK3beta. *Cell*, 144, 341–352 (2011).

32. Montenegro, G. S. et al. In vitro reconstitution of the functional interplay between MCAK and EB3 at microtubule plus ends. *Curr. Biol.*, 20, 1717–1722 (2010).

33. Maurer, S. P. et al. EBs recognize a nucleotide-dependent structural cap at growing microtubule ends. *Cell*, 149, 371–382 (2012).

34. Zhang, R., Alushin, G. M., Brown, A. & Nogales, E. Mechanistic origin of microtubule dynamic instability and its modulation by EB proteins. *Cell*, 162, 849–859 (2015).

35. Honnappa, S. et al. An EB1-binding motif acts as a microtubule tip localization signal. *Cell*, 138, 366–376 (2009).

36. Repina, N. A. et al. At light speed: advances in optogenetic systems for regulating cell signaling and behavior. *Annu. Rev. Chem. Biomol. Eng.*, 8, 13–39 (2017).

37. Wang, H. et al. LOVTRAP: an optogenetic system for photoinduced protein dissociation. *Nat. Methods*, 13, 755–758 (2016).

38. Slep, K. C. & Vale, R. D. Structural basis of microtubule plus end tracking by EB1. *Nat. Struct. Mol. Biol.*, 16, 792–797 (2009).

39. Komarova, Y. et al. Mammalian end binding proteins control persistent microtubule growth. *J. Cell Biol.*, 184, 691–706 (2009).

40. Skube, S. B., Chaverrri, J. M. & Goodson, H. V. Effect of GFP tags on the localization of EB1 and EB1 fragments in vivo. *Cytoskeleton (Hoboken)*, 67, 1–12 (2010).

41. Dragestein, K. A. et al. Dynamic behavior of GFP-CLIP-170 reveals fast microtubule turnover on microtubule plus ends. *J. Cell Biol.*, 180, 729–737 (2008).

42. Sestapun, D. et al. Estimating the microtubule GTP cap size in vivo. *Curr. Biol.*, 22, 1681–1687 (2012).

43. Yan, X., Habedank, R. & Nigg, E. A. A complex of two centrosomal proteins, CAP350 and FOP, cooperates with EB1 in microtubule anchoring. *Mol. Biol. Cell.*, 17, 634–644 (2006).

44. Wang, T. et al. Translating mRNAs strongly correlate to proteins in a multivariate manner and their translation ratios are phenotype specific. *Nucleic Acids Res.*, 41, 4743–4754 (2013).

45. Miller, P. M. et al. Golgi-derived CLASP-dependent microtubule control Golgi organization and polarized trafficking in motile cells. *Nat. Cell. Biol.*, 11, 1069–1080 (2009).

46. De Groot, C. O. et al. Molecular insights into mammalian end-binding protein heterodimerization. *J. Biol. Chem.*, 285, 5802–5814 (2010).

47. Christie, J. M. et al. Steric interactions stabilize the signaling state of the LOV2 domain of phototropin 1. *Biochemistry*, 46, 9310–9319 (2007).

48. Ettinger, A. & Wittmann, T. Fluorescence live cell imaging. *Methods Cell Biol.*, 123, 77–94 (2014).

49. Gierke, S. & Wittmann, T. EB1-recruited microtubule +TIP complexes coordinate protrusion dynamics during 3D epithelial remodeling. *Curr. Biol.*, 22, 753–762 (2012).

50. Bieling, P. et al. CLIP-170 tracks growing microtubule ends by dynamically recognizing composite EB1/tubulin-binding sites. *J. Cell Biol.*, 183, 1223–1233 (2008).

51. Kaverina, I. & Straube, A. Regulation of cell migration by dynamic microtubules. *Semin. Cell. Dev. Biol.*, 22, 968–974 (2011).

52. Wittmann, T., Bokoch, G. M. & Waterman-Storer, C. M. Regulation of leading edge microtubule and actin dynamics downstream of Rac1. *J. Cell Biol.*, 161, 845–851 (2003).
Methods
DNA constructs and molecular cloning. mCherry-EB3 was from the Michael Davidson Plasmid Collection (Addgene plasmid 55037). mCherry-CLASP2y was cloned by replacing the AgeI/KpnI restriction fragment of peGFP-CLASP2y with the corresponding fragment of pmCherry-C1. Similarly, mCherry-MCAK was cloned by replacing the AgeI/BglII fragment of mMICAL-MCAK (Addgene plasmid 54161, from the Michael Davidson Plasmid Collection) with the corresponding fragment of pmCherry-C1. mCherry-α-tubulin was obtained from R. Tsien (Addgene plasmid 49149). PCR-based cloning strategies for other constructs are summarized below, and all primers used are listed in Supplementary Table 2.

(1) EB1N-LZ-LOV2 was constructed as follows: the coding regions corresponding to amino acids 1-185 of an EB1 shRNA-resistant variant7 and a GCN4 leucine zipper (generated by oligonucleotide assembly PCR) were first amplified, and then connected by overlap extension PCR, and the resulting product ligated into the NheI/Xhol-digested backbone of peGFP-C1. The LOV2 coding sequence was amplified from the pTriEx-mCherry-Zdk1 (Addgene plasmid 20227) and L606/607 A mutations that were reported to stabilize docking of the Jx–helix6 as well as a GSGS linker sequence were introduced by PCR. This modified LOV2 domain including the linker was amplified and inserted into the SacII/BamHI sites of the EB1N-LZ plasmid.

(2) EB1N-mCherry-LOV2 was cloned by inserting PCR-amplified mCherry coding sequences into Xhol/SacII sites between LZ and LOV2 in EB1N-LZ-LOV2.

(3) EB1N-eGFP-LOV2 was cloned by inserting the coding sequence for the PCR-amplified GCN4 leucine zipper into the SacII site of EB1N-eGFP-LOV2 by Gibson assembly between eGFP and LOV2.

(4) mCherry-Δ1 was cloned in the following way: PCR-amplified mCherry-Zdk1-linker (from pTriEx-mCherry-Zdk1, Addgene plasmid 81057) and the EB1 C-terminal fragment corresponding to amino acids 186–268 were connected by overlap extension PCR. The resulting PCR product was inserted into the NheI/Xhol sites of peGFP-C1. eGFP-Zdk1-EB1C was generated by excising eGFP from peGFP-C1 by restriction digestion with NheI/BstXI and ligation into NheI/BstXI-digested mCherry-Zdk1-EB1C, thereby replacing mCherry with eGFP.

(5) GST-EB1N-LZ-LOV2 was cloned by inserting PCR-amplified EB1N-LZ-LOV2 into the BamHI/Xmal sites of pGEX-4T-2 (GE Healthcare Life Sciences).

(6) The GST–Zdk1-EB1C was cloned by inserting PCR-amplified Zdk1-EB1C into NheI/Xhol sites of pET28a.

(7) The GST–LOV2 was cloned by inserting the PCR-amplified LOV2 coding sequence including a GSGS linker sequence into BamHI/Xmal sites of pGEX-4T-2.

(8) mCherry-SLAIN2 was cloned by inserting the PCR-amplified SLAIN2 coding sequence into the Xhel/EcoRI sites of pBso-mCherry-C1.

(9) CAK5-mKate2 was cloned by inserting the PCR-amplified mKate2 coding sequence into the BamHI/NotI sites of chTOG-GFP (Addgene 29480, from L. Wodarzan).

Cell culture and generation of π-EB1 cell lines. H1299 human non-small-cell lung cancer cells were cultured in RPMI1640 supplemented with 10% FBS, penicillin/streptomycin and non-essential amino acids at 37 °C, 5% CO2 in a humidified atmosphere. H1299–EB1 cell lines were cultured in RPMI1640 supplemented with 10% FBS, penicillin/streptomycin and non-essential amino acids at 37 °C, 5% CO2 in a humidified atmosphere. H1299 human non-small-cell lung cancer cells were cultured in RPMI1640 supplemented with 10% FBS, penicillin/streptomycin and non-essential amino acids at 37 °C, 5% CO2 in a humidified atmosphere. H1299 cells and THO1(6,9.3); TPOX(8); and vW A(16,17,18). The profile obtained was identical with both untagged EB1N-LOV2 and eGFP-Zdk1-EB1C plasmids and selected.

- EB1N-Δ1-LOV2 cells were then transfected with mCherry, GFP-Δ1-LOV2 and eGFP-Zdk1-EB1C, and the EB1 C-terminal fragment corresponding to amino acids 186–268 were connected by overlap extension PCR. The resulting PCR product was inserted into the NheI/Xhol sites of peGFP-C1. eGFP-Zdk1-EB1C was generated by excising eGFP from peGFP-C1 by restriction digestion with NheI/BstXI and ligation into NheI/BstXI-digested mCherry-Zdk1-EB1C, thereby replacing mCherry with eGFP.

- mCherry-SLAIN2 was cloned by inserting the PCR-amplified SLAIN2 coding sequence into the Xhel/EcoRI sites of pBso-mCherry-C1.

- CAK5-mKate2 was cloned by inserting the PCR-amplified mKate2 coding sequence into the BamHI/NotI sites of chTOG-GFP (Addgene 29480, from L. Wodarzan).

- GST–LOV2 was cloned by inserting the PCR-amplified LOV2 coding sequence including a GSGS linker sequence into BamHI/Xmal sites of pGEX-4T-2.

- The GST–Zdk1-EB1C was cloned by inserting PCR-amplified Zdk1-EB1C into NheI/Xhol sites of pET28a.

- The GST–LOV2 was cloned by inserting the PCR-amplified LOV2 coding sequence including a GSGS linker sequence into BamHI/Xmal sites of pGEX-4T-2.

- mCherry-SLAIN2 was cloned by inserting the PCR-amplified SLAIN2 coding sequence into the Xhel/EcoRI sites of pBso-mCherry-C1.

- CAK5-mKate2 was cloned by inserting the PCR-amplified mKate2 coding sequence into the BamHI/NotI sites of chTOG-GFP (Addgene 29480, from L. Wodarzan).

Cell culture and generation of π-EB1 cell lines. H1299 human non-small-cell lung cancer cells were cultured in RPMI1640 supplemented with 10% FBS, penicillin/streptomycin and non-essential amino acids at 37 °C, 5% CO2 in a humidified atmosphere. H1299 cells and THO1(6,9.3); TPOX(8); and vW A(16,17,18). The profile obtained was identical with both untagged EB1N-LOV2 and eGFP-Zdk1-EB1C plasmids and selected.

- EB1N-Δ1-LOV2 cells were then transfected with mCherry, GFP-Δ1-LOV2 and eGFP-Zdk1-EB1C, and the EB1 C-terminal fragment corresponding to amino acids 186–268 were connected by overlap extension PCR. The resulting PCR product was inserted into the NheI/Xhol sites of peGFP-C1. eGFP-Zdk1-EB1C was generated by excising eGFP from peGFP-C1 by restriction digestion with NheI/BstXI and ligation into NheI/BstXI-digested mCherry-Zdk1-EB1C, thereby replacing mCherry with eGFP.

- mCherry-SLAIN2 was cloned by inserting the PCR-amplified SLAIN2 coding sequence into the Xhel/EcoRI sites of pBso-mCherry-C1.

- CAK5-mKate2 was cloned by inserting the PCR-amplified mKate2 coding sequence into the BamHI/NotI sites of chTOG-GFP (Addgene 29480, from L. Wodarzan).

- GST–LOV2 was cloned by inserting the PCR-amplified LOV2 coding sequence including a GSGS linker sequence into BamHI/Xmal sites of pGEX-4T-2.

- The GST–Zdk1-EB1C was cloned by inserting PCR-amplified Zdk1-EB1C into NheI/Xhol sites of pET28a.

- The GST–LOV2 was cloned by inserting the PCR-amplified LOV2 coding sequence including a GSGS linker sequence into BamHI/Xmal sites of pGEX-4T-2.

- mCherry-SLAIN2 was cloned by inserting the PCR-amplified SLAIN2 coding sequence into the Xhel/EcoRI sites of pBso-mCherry-C1.

- CAK5-mKate2 was cloned by inserting the PCR-amplified mKate2 coding sequence into the BamHI/NotI sites of chTOG-GFP (Addgene 29480, from L. Wodarzan).

Microscopy, photoactivation and image analysis. Fluorescent protein dynamics were imaged by spinning disk or total internal reflection fluorescence (TIRF) microscopy on a customized microscope setup essentially as described previously43,44, except that the system was upgraded with a next-generation scanning charge-coupled device (CCD) camera (C4742-90, Hamamatsu) with 4.5 µm pixels that allowed optimal spatial sampling using a x60 NA 1.49 objective (CFI APO TIRF; Nikon). Global π-EB1 photodissociation was achieved by turning on the 488-nm excitation channel. An irradiance of ~250 mW per cm2 was sufficient to photodissociate the LOV2 domain, although simultaneous imaging of eGFP-tagged proteins required higher light intensity. Irradiance at the specimen plane was measured using a X-Cite XR2100 light power meter (EXFO Photonic Solutions).

To achieve subcellular π-EB1 photodissociation, a digital micromirror device (Polygon 400, MicroTec) equipped with a 470 nm LED was mounted on a Nikon Ti auxiliary camera port equipped with a beamsplitter, such that 20% of the light path was diverted for photoactivation and 80% was used for spinning disk confocal microscopy. This also allowed for direct imaging of the reflected illumination pattern to accurately focus and align the Polygon 400, as well as simultaneous imaging and π-EB1 photodissociation. To eliminate scattered photoactivation light
in the imaging channel, the Polygon 400 was operated such that short 10–20-ms pulses of blue light were triggered to occur between image acquisitions. For fast time-lapse experiments (at or above 1 frame per second), the Polygon 400 was directly triggered by the camera using a short delay of a few hundred milliseconds after camera exposure. For slower time-lapse experiments, the camera trigger was used to start a pre-programmed pulse sequence between exposures using an ASI MS2000 Sequencer module.

Image analysis was performed in NIS Elements 4.3 or Fiji. Fluorescence intensities were measured in small regions with a diameter of 3–5 pixels on MT ends that were moved in time with MT growth, and nearby cytoplasm as local background. MT end/cytoplasm ratios were calculated as previously described. A ratio of one indicates no measurable difference between the MT end and the local cytoplasm. MT plus-end tracking of mCherry-EB1N was done using u-track version 2.1.3 (refs. 18,19). Comet detection parameters were adjusted to decrease the number of false-positive detections as follows: 'High-pass Gaussian standard deviation': 6; Watershed segmentation 'Minimum threshold': between 6 and 8; and other parameters remained at default settings. Similarly, in the 'Tracking' step, the 'Minimum length of track segments' was set to 4. To not bias average MT growth rates toward shorter tracks, frame-to-frame MT growth rates were extracted from the 'tracksFinal' structure using a custom MatLab script, and frame-to-frame displacements of <0.5 pixels were excluded. Tubulin-mCherry-labelled MTs ends were tracked using the Fiji MtrackJ plugin.

To analyse the cell migration response to π-EB1 photodissociation, cells were recorded at 1 frame per minute for at least 30 min to select cells that were actively migrating and to determine the pre-exposure direction of migration. Regions of blue light exposure were manually drawn, roughly targeting the front or side of the migrating cell, and blue light patterns were pulsed at a frequency of 1 Hz. The cell outline was determined by fluorescent signal threshold, and the centroid position was calculated in NIS Elements for each time point. To reduce positional noise of the centroid position due to transient changes in cell shape and protrusive activity, the centroid position was smoothed using a nine-frame running average. The angle between 15-min linear segments along this centroid trajectory and centred on the time of π-EB1 photodissociation (that is, 0 min) was then calculated by linear algebra. Turning away from the light-exposed region was defined as a positive angle.

Statistics and reproducibility. Statistical analysis was done with the Analyse-It plugin for Microsoft Excel. Significance of multiple comparisons was calculated using the Tukey–Kramer honest significant difference (HSD) test after confirming normal distribution of the data by Shapiro–Wilks testing. Least square curve fitting was performed using the Solver plugin in Microsoft Excel, and 95% confidence intervals of the fit were calculated as described. Figures were assembled in Adobe Illustrator CS5, and videos were made using Apple QuickTime Pro. Box-and-whisker plots show median, first and third quartile, observations within 1.5 times the interquartile range, and all individual data points. All experiments showing representative gel images or immunoblots (Fig. 1a,g and Supplementary Figs. 1a,b,3a,b and 4a), or representative microscopy data (Figs. 2b,c,e and 6d and Supplementary Figs. 1c,2c,e and 4b,c,f) were repeated at least three times with similar results.

Life Sciences Reporting Summary. Further information on experimental design is available in the Life Sciences Reporting Summary.

Code availability. The u-track particle tracking package for MATLAB is available from the Danuser Lab at http://www.utsouthwestern.edu/labs/danuser/software/.

Data availability. Statistical source data for Figs. 1d,f,2a–d,3b,c,f,4b,d,5c,f and 6c and Supplementary Fig. 4e are included in Supplementary Table 3. All other data supporting the findings of this study are available from the corresponding author on reasonable request.

References
49. Ahkmanova, A. et al. Clasps are CLIP-115 and -170 associating proteins involved in the regional regulation of microtubule dynamics in motile fibroblasts. Cell 104, 923–935 (2001).
50. Wu, Y. I. et al. A genetically encoded photoactivatable Rac controls the motility of living cells. Nature 461, 104–108 (2009).
51. Strickland, D. et al. TULIPx: tunable, light-controlled interacting protein tags for cell biology. Nat. Methods 9, 379–384 (2012).
52. Ran, F. A. et al. Genome engineering using the CRISPR–Cas9 system. Nat. Protoc. 8, 2281–2308 (2013).
53. van Haren, J. & Wittmann, T. Generation of cell lines with light-controlled microtubule dynamics. Protoc. Exch., https://doi.org/10.1038/protx.2017.155 (2018).
54. Stehbens, S., Pemble, H., Murrow, L. & Wittmann, T. Imaging intracellular protein dynamics by spinning disk confocal microscopy. Methods Enzymol. 504, 293–313 (2012).
55. Schindelin, J. et al. Fiji: an open-source platform for biological-image analysis. Nat. Methods 9, 676–682 (2012).
56. Ettinger, A., van Haren, J., Ribeiro, S. A. & Wittmann, T. Doublecortin is excluded from growing microtubule ends and recognizes the GDP-microtubule lattice. Curr. Biol. 26, 1549–1555 (2016).
57. Matov, A. et al. Analysis of microtubule dynamic instability using a plus-end growth marker. Nat. Methods 7, 761–768 (2010).
58. Jaqaman, K. et al. Robust single-particle tracking in live-cell time-lapse sequences. Nat. Methods 5, 695–702 (2008).
59. Meijering, E., Dzyubachyk, O. & Smal, I. Methods for cell and particle tracking. Methods Enzymol. 504, 183–200 (2012).
60. Stehbens, S. J. & Wittmann, T. Analysis of focal adhesion turnover: a quantitative live-cell imaging example. Methods Cell Biol. 123, 335–346 (2014).
61. Brown, A. M. A step-by-step guide to non-linear regression analysis of experimental data using a Microsoft Excel spreadsheet. Comput. Methods Prog. Biomed. 65, 191–200 (2001).
Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

1. **Sample size**
   
   Describe how sample size was determined.
   
   No statistical method was used to determine sample size. Sample size was chosen based on previous experience and standards in the field.

2. **Data exclusions**
   
   Describe any data exclusions.
   
   No data were excluded. Cells imaged and analyzed were chosen based on fluorescent protein expression levels.

3. **Replication**
   
   Describe whether the experimental findings were reliably reproduced.
   
   All experiments were repeated at least three times with similar outcome.

4. **Randomization**
   
   Describe how samples/organisms/participants were allocated into experimental groups.
   
   No randomization of experimental groups was performed.

5. **Blinding**
   
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   
   Investigators were not blinded during data collection and analysis.

6. **Statistical parameters**
   
   For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

   - The exact sample size ($n$) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
   - A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
   - A statement indicating how many times each experiment was replicated
   - The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
   - A description of any assumptions or corrections, such as an adjustment for multiple comparisons
   - The test results (e.g. $P$ values) given as exact values whenever possible and with confidence intervals noted
   - A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
   - Clearly defined error bars

   See the web collection on statistics for biologists for further resources and guidance.
Software

Describe the software used to analyze the data in this study.

NIS Elements 4.3
Image J/Fiji (latest updated version available at the time)
Microsoft Excel 2010 with Analyse-It plug-in (Standard Edition 3.90.7)
Matlab R2014b
u-track 2.1.3 (particle tracking Matlab code from Danuser lab; details in methods)

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

No unique / restricted materials were used. Relevant plasmid reagents will be made available on Addgene.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

All antibody information including dilutions used for immunoblotting and immunofluorescence, and how specific antibodies were validated is included in Supplementary Table 1.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

NCI-H1299 (ATCC)

b. Describe the method of cell line authentication used.

STR profiling

c. Report whether the cell lines were tested for mycoplasma contamination.

Cells regularly tested negative for mycoplasma.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No cell lines used in this study were found in the database of commonly misidentified cell lines that is maintained by ICLAC and NCBI Biosample.

Animals and human research participants

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

No animals were used in this study.

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

This study did not involve human research participants.