ONLINE METHODS

Plasmid and strain construction. S. Koide (University of Chicago) provided plasmids encoding ePDZ variants. B. Glick (University of Chicago) provided plasmids encoding PMA1 and DsRedMax. F. Cross (Rockefeller University) provided a plasmid encoding the Gal4-rMR construct. All ARS/CEN plasmids used in this study were from the pGREG series[31]. All integrating plasmids were of the YIplac series[32]. The *MET25*, *TEF*, and *ADH* promoters were from the PCR Toolbox plasmids[33]. All other yeast CDS were obtained by PCR from the Yeast Genomic Tiling Collection (Open Biosystems) or from genomic DNA.

DNA manipulations were simulated with a pre-release version of the SnapGene software (GSL Biotech). Plasmids were generated using a combination of conventional ligation, InFusion cloning (Clontech) and recombination in yeast[31]. Yeast were transformed using LiAc/SS-DNA/PEG[34]. All plasmids and strains were verified by colony PCR or DNA sequencing.

We constructed the background strain YLS1254 by integrating a Gal4-rMR expression cassette[35] into W303 *MAT***a** so as to delete the endogenous *TRP1* coding sequence using a *URA3* marker, which was itself subsequently deleted[36]. We then integrated a C-terminal mCherry tag at the endogenous *ABP1* coding sequence using a *HIS3MX* marker[37].

Affinity, caging and switching. We use the term "intrinsic affinity" to denote the intrinsic affinity of binding between a photoactivated, helix-undocked LOVpep and free

ePDZ (**Supplementary Fig. 1**). We use the term "caging" for the diminishment of LOVpep–ePDZ binding in the dark state. Quantitatively this is the ratio of the dark-state dissociation constant to the intrinsic dissociation constant. We use the term "switching" to refer to the ratio of the dark- and lit-state dissociation constants. Because it is possible for a highly stabilized helix to remain substantially docked to the LOV core even in the lit state, caging and switching may have different values. However, caging is always numerically greater than or equal to switching. We use the term "overall affinity" to refer to the observed affinity of the reaction scheme depicted in **Fig. 1a**.

Plasma membrane recruitment assay. We used a plasma-membrane recruitment assay in living yeast to assess the lit- and dark-state binding between ePDZ and the LOV–peptide fusions. We fused GFP–AsLOV2–peptide constructs to the C-terminus of the integral plasma membrane protein Mid2[22]. We expressed the P_{TEF}–Mid2–GFP–LOVpep constructs from ARS/CEN plasmids maintained with a LEU2 or KanMX marker. Generally, GFP fluorescence was cleanly and evenly localized to the plasma membrane and accumulation in endocytic compartments was minimal. Sometimes GFP fluorescence was also localized to the vacuole or nuclear periphery, but this was always less intense than the plasma membrane signal and did not interfere with image thresholding

We co-expressed the LOV–peptide constructs with mCherry-tagged ePDZ (**Fig. 1b**). To ensure that binding affinity was in a sensitive range for the assay, we used moderate- and high-affinity ePDZ variants (ePDZb and ePDZb1, respectively, having a 10-fold difference in affinity for model peptides)[20]. We expressed P_{TEF} –ePDZ–mCherry or P_{TEF}

–PDZ–mCherry constructs from a plasmid integrated at the *URA3* locus. This combination of ARS/CEN and integrating plasmids provided the most consistent expression levels as judged by GFP and mCherry fluorescence levels. Diploid JK9-3d strains harboring both plasmids were constructed by mating singly transformed haploids and selecting on SD –LEU –URA.

We grew cells in liquid culture (YPD + G418), then pelleted and resuspended in minimal media. We plated 3 μL on a 2 × 2 × 0.1 cm, 1.2% agar pad made with the same media. We then placed a #1.5 coverslip over the pad and sealed the edges with petroleum jelly[38]. We imaged the cells on an Axiovert 200M microscope (Zeiss) equipped with a spinning disk confocal (CSU10, Yokogawa) and an EMCCD camera (Cascade 512B, Photometrics) using a 63 ×, 1.4 NA objective. The microscope was controlled using MetaMorph (Molecular Devices). We placed a 550 nm long pass filter (Edmund Optics) in the transmitted light path to avoid photoexciting the LOV domain when using phase contrast.

We assayed recruitment of ePDZ–mCherry to the plasma membrane in the dark and immediately after photoexcitation with a 473 nm laser. To quantify the plasma membrane association of ePDZ–mCherry, we measured the ratio of plasma membrane and cytoplasmic fluorescence, averaged over a population of cells ($\langle R_{obs} \rangle$, below & **Supplementary Fig. 3**). We used an ImageJ macro to quantify recruitment with minimal user intervention. Because the algorithm works best with individual cells or mother-daughter pairs that are well-separated from other cells, we searched for fields of well-

separated cells using Nomarski illumination. We then took a 500 ms image of mCherry fluorescence, and a 125 ms image of GFP fluorescence. We used an additional 1 s pulse (473 nm) to ensure the LOV domain was fully photoexcited (see below for more on light requirements). For basic recruitment assays, we took a single 500 ms image of mCherry fluorecence after a 1–10 s delay to allow recruitment to reach the maximum level. For kinetic assays, we acquired a time lapse of 500 ms images.

Quantification included the following steps: First, a stack registration plugin (StackReg[39]) corrected for stage drift. This was especially important for long time lapse imaging in kinetic assays. Second, a thresholding method automatically defined regions of interest (ROIs) for the plasma membrane, cytoplasm, and background based on the GFP image (**Supplementary Fig. 3a**). There was no user intervention in defining the ROIs, but cells that were not thresholded accurately (*e.g.* because of nearby dust particles) were discarded. Third, for each frame of a given cell the average, background-subtracted intensities were measured in the plasma membrane, cytoplasm ROIs. The ratio of the plasma membrane and cytoplasm intensities (R_{obs}) were also calculated. Notably, the background autofluorescence of the media and the cellular mCherry fluorescence have different photobleaching properties. Because R_{obs} is a ratio of two background-subtracted values it is somewhat sensitive to this difference, and this sensitivity is especially apparent when photoexciting over multiple cycles. However, we have not attempted to correct for this phenomenon in any assays. We collated the data and calculated the mean, denoted $<R_{obs}>$, and standard error for populations of cells using Excel (Microsoft), and plotted the data using Igor Pro (Wavemetrics).

Interpretation of $\langle R_{obs} \rangle$. Empirically, $\langle R_{obs} \rangle$ ranges from ~ 0.35 to ~ 2.5. We estimated the lower value by globally evaluating multiple data sets. We found that $\langle R_{obs} \rangle$ is never less than ~ 0.30, and all data approach a value of ~ 0.35 as the expected affinity decreases. We confirmed the assignment of ~ 0.35 as 100% cytoplasmic fluorescence by inspecting a subset of cells with individual R_{obs} of 0.34–0.36. mCherry fluorescence was strongly cytoplasmic in these cells (**Supplementary Fig. 3b**). In Fig. 2 and Supplementary Fig. 2b, we indicate that $\langle R_{obs} \rangle = 0.35$ is estimated to be 100% cytoplasmic. In Supplementary Fig. 6, the lowest observed $\langle R_{obs} \rangle$ is ~ 0.30, and we adjusted the 100% cytoplasmic estimate to this lower value. While not ideal, this adjustment is needed due to a small amount of systematic variation in $\langle R_{obs} \rangle$ seen across experiments (data not shown).

We designed our thresholding algorithm to analyze large and variable populations of cells quickly and with minimal user intervention. In choosing an automated thresholding algorithm we favored robustness and a high signal-to-noise ratio. However, this robustness comes at the expense of capturing the true extremes of plasma membrane and cytoplasmic fluorescence, and the method tends to compress the numerical range of $\langle R_{obs} \rangle$. Furthermore, a given $\langle R_{obs} \rangle$ value should not be interpreted as representing a clearly defined ratio of bound and unbound molecules. For example, $\langle R_{obs} \rangle = 1$ should not be taken to mean that 50% of the molecules are plasma membrane-bound, and 50% are cytoplasmic.

Global illumination during live-cell microscopy. We used the same 473 nm laser as for GFP imaging. The light intensity measured at the back of the objective was 750 $\mu J \cdot s^{-1}$. Using the conservative assumption that all of this light was evenly distributed across the area imaged by the camera (1.23×10^{-4} cm^2), the irradiance was 6.1 $J \cdot cm^{-2} \cdot s^{-1}$. We generally used 1.125 seconds total blue light photoexcitation (6.9 $J \cdot cm^{-2}$) for ePDZ–mCherry recruitment assays. For comparison, a recent study examining the effects of phototoxicity in budding yeast[40] used 4 s blue light pulses of 4.9 $J \cdot cm^{-2}$ every 20 s for GFP image acquisition (*i.e.*, in addition to constant illumination used as the experimental source of phototoxicity). Imaging illumination itself was well below the apparent threshold for a detectable stress response in their experiments[40].

We assessed whether lower levels of illumination could elicit ePDZ–mCherry recruitment. We clearly detected recruitment after a 0.063 s pulse with a 10% transmission filter in the excitation path (0.038 $J \cdot cm^{-2}$, **Supplementary Fig. 10a**). This is considerably less power than would be used for routine GFP imaging.

Spot illumination. We used a galvanometer-steerable 440 nm dye laser (Micropoint, Photonics Instruments) to locally photoexcite Mid2-localized LOVpep. We controlled the illumination intensity using an adjustable internal attenuator plate and an external 1.0 OD absorptive neutral density filter (Thor Labs) placed in the beam path.

We did not measure the Micropoint laser intensity directly. However, with the attenuator plate set to ~ 30% transmission, three pulses of the laser were just sufficient to ablate the reflective coating on the calibration slide provided with the instrument. We used this setting as the reference power for experiments. Five pulses at the reference power was sufficient to slightly bleach Mid2-GFP (**Supplementary Fig. 10b**). After five pulses at 10–11% of the reference power (attenuated with either the attenuator plate or the external filter), Mid2–GFP bleaching was nearly undetectable. For spot photoexcitation experiments we used five pulses at ~1% of the reference power (*i.e.*, with the attenuator set at 3% and the external filter in place). Five pulses at this power was more effective than a single higher power pulse for spot recruitment.

Spot photoexcitation kinetics. We manually defined ROIs corresponding to the recruited spot, cytoplasm, and background, and measured the average pixel intensities for these regions over all timepoints. We fit background-subtracted spot intensities to one- and two-exponential functions using IgorPro (Wavemetrics). For the spot only experiments, a two-exponential function did not offer any improvement over a one-exponential function. For the spot + global experiments both data sets were better approximated by a two-exponential function (not shown). Nevertheless, we have provided the rate constant for the one-exponential fit for wild-type cycling LOVpep (**Supplementary Table 2**).

HeLa culture and transfection. We grew Hela cells in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10%FBS, 100 U penicillin and 0.1 mg/ml

streptomycin at 37C in 5% CO2. We transfected cells using Lipofectomine 2000 (Invitrogen) according to standard protocols. We grew cells overnight on glass coverslips and transfected with 0.5–1 μg of plasmid DNA the next day. The following day, we transferred the cells to phenol-free DMEM, laid the coverslips directly on a microscope slide and sealed the edges with petroleum jelly.

For HeLa experiments we used LOVpep with either the K–6R, T–2S mutations (Lyn and Tom70 global) or the T406A, T407A and I532A mutations (Tom70 spot). The choice of the first allele was arbitrary, and we have no reason to expect that any of the mutations are optimal for mammalian cells. Indeed, we fount the more highly caged T406A, T407A, I532A variant superior in the Tom70 spot recruitment experiment. We performed global and spot recruitment assays essentially as described for yeast.

Blue LED illumination. Blue AlGaInP LEDs (http://theledlight.com, 20° viewing angle, 8,000 millicandela, 468-nm λmax at 3.4 V) were arranged into 6 × 8 arrays by pressing into an empty pipet tip rack and soldered together in parallel. The entire array was powered with a 3.3 V, 1.2 A power supply (Phihong PSA05R-033).

The unfiltered light intensity from the LED arrays was ~ 0.005 $J \cdot cm^{-2} \cdot s^{-1}$. A considerably higher intensity (> 0.037 $J \cdot cm^{-2} \cdot s^{-1}$) is required to elicit nuclear shuttling of the transcription factor Msn2, an indicator of environmental stress in budding yeast[40]. For MAPK activation and polarity disruption experiments, we attenuated the light intensity with either colored plastic notebook dividers (Avery) or transparency sheets laser printed

with a uniform gray tone. In either case, we determined the transmission at 465 nm spectroscopically. In these experiments, 10% of the raw LED intensity was sufficient to elicit a strong biological response.

Growth arrest assay. For all signaling assays, we used a modified Mid2 construct, Mid2(SS/TM), in which the extracellular serine-and-threonine rich and cytoplasmic domains were deleted. To assay growth arrest on solid media, we made 1:10 serial dilutions of cells (grown in liquid culture or resuspended from plates) in water. We spotted the dilutions onto YP + 2% Dextrose or 2% Galactose, with G418 to maintain CEN plasmids. We grew the plates at room temperature, either foil wrapped for dark plates, or under an LED array with filters for 10% transmission for lit plates. We wrapped the edges of the plates with parafilm to prevent drying, and kept the plates with the growth surface facing down. For lit plates, we positioned fans to blow across the plates to dissipate heating from the LED array, and placed the plates on a foil surface to reflect transmitted light back onto the growth surface.

Assay for light-dependent mating pathway activation. We grew overnight cultures (YLS2067 background with plasmids as indicated) in 5 mL YP + 2% galactose + G418. If the overnight cultures were above OD_{600} = 0.8, then we diluted the cultures with the same media to OD_{600} = 0.2 and grew them for an additional 2 hours. We diluted the log-phase cultures to OD_{600} = 0.1 to 0.2 and aliquoted 100 μL into standard clear 96-well microtiter plates. For alpha factor stimulation, we added 5 μL media + 20X alpha factor.

We incubated the cultures at room temperature with shaking for 4 hours. Dark plates were foil wrapped, and lit plates were under an LED array with filters for 10% transmission.

For microscopy, we spun down 50–100 µL and resuspended in 5–10 µL SC. We spotted 2 µL onto 10 mm × 10 mm × 1 mm thick agarose pad made with SC (4 pads per slide) and sealed the edges with vaseline[38]. We imaged with a 40 × objective (Zeiss).

For flow cytometry, we pelleted the cells and resuspended in phosphate buffered saline. We collected DsRedMax[28] fluorescence intensities on a BD Biosciences LSR II flow cytometer using a 561 nm excitation laser and a 610 nm ± 20 nm emission filter, and analyzed the data using FlowJo (Tree Star).

Assay for light-dependent polarity disruption. We grew overnight cultures (YLS1254 background with plasmids as indicated) in 5 mL YP + 2% galactose. We aliquoted 100 µL into standard clear 96-well microtiter plates and incubated at room temperature with shaking for 5 hours. Dark plates were foil wrapped, and lit plates were under an LED array with filters for 10% transmission.

For microscopy, we spun down 50–100 uL and resuspended in 5–10 uL SC. We spotted 2 µL onto 10 mm × 10 mm × 1 mm thick agarose pad made with SC (4 pads per slide), placed a coverslip on the pad and sealed the edges with vaseline. We imaged with a 40 × objective.

Assay for light-dependent polarity specification. We grew overnight cultures (YLS2446) in SC –HIS –LEU –MET –URA +2X ADE with 20 μM deoxycorticosterone (DOC^{35}) in foil-wrapped tubes. We spun down 1–1.5 mL of the overnight culture and resuspended in 20 μL of the same media with 10 μM DOC and 10 μg / mL αF, and incubated in the dark for 30 min. We spotted 2 μL onto 10 mm × 10 mm × 1 mm thick agarose pad made with the same media, including DOC and αF, placed a coverslip on the pad and sealed the edges with vaseline.

We imaged the cells on the same microscope used for recruitment assays. We used a 550-nm long pass filter (Edmund Optics) in the transmitted light path to avoid photoexciting the LOV domain when using phase contrast. Once per minute minute we took a 1 s confocal image of mCherry fluorescence and a 100 ms confocal phase contrast image, and photoexcited the cells using the Micropoint laser. We used the same photoexcitation duration and intensity as for spot recruitment. For "– Photoexcitation", the experiment was performed identically, except with the laser switched off.

A MetaMorph journal recorded the laser targets directly into a stack of phase contrast images. Using ImageJ, we made composites of the phase contrast and mCherry images, and measured the angle between the laser target and the incipient polarized growth. We binned the measured angles using Excel, and plotted the results using Igor Pro. We also performed a two-sample Kolmogorov–Smirnov test using Igor Pro.

We estimate the uncertainty in laser targeting to be 0.6–0.8 μm, and the corresponding angular uncertainty to be ~ 15° for a 5 μm yeast cell. This uncertainty limits the precision with which we can measure the angle between photoexcitation and polarized growth. Furthermore, this uncertainty is compounded by human error in updating targets in real time. Thus it is likely that the laser narrowly missed some cells during some photoexcitation cycles, although we do not know to what extent a near miss by the laser would photoexcite LOVpep.

We also note a slight tendency of polarization towards the mock photoexcitation target (**Fig. 3c**). To facilitate interpretation of the data, we avoid placing the laser target at points of cell-cell contact. This may have the unintended effect of biasing target placement towards the default polarization cue or away from regions of higher pheromone degradation.

TULIPs plasmid system

We have deposited a set of plasmids for the TULIPs system, along with maps and sequences, in the Addgene database (www.addgene.org, **Supplementary Table 3**). The plasmids allow cloning of protein coding sequences with GFP–LOVpep, cpPDZ, ePDZb, and ePDZb1 as tags. We have provided integrating versions, based on the YIplac series of plasmids, and centromeric versions, based on the pGREG series of plasmids.

Our cloning scheme is based on *in vitro* recombination cloning such as the InFusion system (Clontech), or the method of Gibson *et al*.[41]. Cloning by recombination in yeast

can also be used with the centromeric plasmids[31]. See **Supplementary Table 5** for primer details.

Supplementary File	Title

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Supplementary Figure 1	Definition of caging and switching.
Supplementary Figure 2	Register series of LOV–peptide fusions.
Supplementary Figure 3	Thresholding of cells for the PM recruitment assay.
Supplementary Figure 4	Reversible light-triggered recruitment of ePDZb by LOVpep.
Supplementary Figure 5	ePDZ–mCherry localization in HeLa cells in the absence of LOVpep.
Supplementary Figure 6	Lit- and dark-state $\langle R_{obs} \rangle$ using LOVpep mutations in the peptide epitope.
Supplementary Figure 7	Growth arrest assay for ePDZ fusions.
Supplementary Figure 8	Light induced growth arrest by MAPK activation.
Supplementary Figure 9	PFUS1–DsRed reporter activation for ePDZb–Ste5ΔN and Ste11–ePDZb constructs measured using flow cytometry.
Supplementary Figure 10	Light induced growth arrest by polarity disruption.
Supplementary Figure 11	Photoexcitation requirements.
Supplementary Table 1	Fitted values of k_{obs} and k_{phot}.
Supplementary Table 2	Fitted parameters for dissociation from spot recruitment.
Supplementary Table 3	Yeast plasmids used in this study.
Supplementary Table 4	Strains used in this study.
Supplementary Table 5	Suggested primers for TULIPs plasmids.
Supplementary Video 1	Repeated photoexcitation, mitochondrial recruitment of ePDZ–mCherry and dark-state recovery in HeLa cell expressing Tom70–GFP–LOVpep (K–6R, T–2S) and ePDZb1–mCherry. mCherry fluorescence images taken every 5 s.
Supplementary Video 2	Photoexcitation, PM recruitment of ePDZ–mCherry and dark-state recovery in cells expressing Mid2–GFP–LOVpep (T406A, T407A). Left, ePDZb–mCherry. Right, ePDZb1–mCherry. Photoexcitation occurs between the first and second frames. mCherry fluorescence images taken every 5 s.
Supplementary Video 3	Spot photoexcitation, PM recruitment of ePDZb1–mCherry and dark-state recovery in cells expressing Mid2–GFP–LOVpep (V416I, T406A, T407A) and ePDZb1–mCherry. Left, spot recruitment of ePDZb1–mCherry only. Right, spot recruitment of ePDZb1–mCherry followed by global recruitment and cytoplasmic depletion. mCherry fluorescence images 1–17 taken every 2 s, 18–30 every 10 s, and 31–42 every 60 s.
Supplementary Video 4	Light-directed polarized growth in budding yeast. Cells are exposed to mating pheromone to induce cell cycle arrest, then stimulated with spot photoexcitation to recruit Cdc24–ePDZb1 to PM-tethered LOVpep. Red, actin labeled using Abp1–mCherry. Blue, phase contrast image processed using an edge detection filter. Frames taken every 1 min. Spot photoexcitation occurs at the white crosshairs, only in the frames where they are visible.
Supplementary Note 1	Protein design considerations.
Supplementary Note 2	Suggested workflow for using the TULIPs system.