Supplemental Materials

Applewhite et al.
Supplemental Figure Legends

Supplemental Figure 1. Perfusion of rapamycin reduced the length of Shot’s lattice association.
S2 cells expressing FKBP-Shot-FRB-EGFP before (A) and after perfusion of 250 nM rapamycin (A’) or before (B) and after perfusion of DMSO (B’). Shown at higher magnification (white boxes) is Shot at the plus-end and along the lattice of microtubules. (C) Quantification of Shot’s length along the microtubule lattice taken from time-lapsed movies of the S2 cells pictured in A and B. Error bars denote SD of the mean marked by the dotted line. Approximately 200 comets length were measured for each condition. Asterisk denotes a statistically significant reduction in Shot’s length along microtubule lattice of the cell treated with 250 nM rapamycin as compared to DMSO control (p-value < 0.0001, Student’s t-test). Scale bars are 10 μm in low magnification images and 2 μm in high magnification images.

Supplemental Figure 2. Zw3 does not regulate Shot’s interaction with microtubules.
(A and B) Localization of endogenous Shot following co-expression of constitutively active mCherry-Zw3 (A) or control (B). Shown at lower magnification in A is mCherry-Zw3-CA. (C) Western blot of Control RNAi, Zw3 RNAi, or cells treated with Zw3 RNAi followed by transient expression of mCherry-Zw3-CA. RNAi depletion of Zw3 leads to an accumulation of β-catenin, while in control treated cells, the levels of β-catenin remain low. Transient expression of mCherry-Zw3-CA can facilitates the destruction of β-catenin. α-Tubulin is loading control. (D - H) Localization of endogenous Shot (green), endogenous EB1 (red) and graphical representations of line scans plotting the distribution of Shot (green) and EB1 (red) at the cell periphery following Control RNAi (D and E), treatment with 20mM LiCl (F and G) and Zw3 RNAi (H and I). For each graph, the normalized fluorescent intensity is plotted against the distance from the microtubule plus-end for 10-15 different microtubules found in the cell periphery (approximately 1-5 μm from the cell edge). Error bars represent SE, scale bar 10 μm. Images were acquired by TIRF microscopy.

Supplemental Figure 3. Shot’s dynamics are not affected by expression of constitutively active Zw3 (S9A).
S2 cells transfected with Shot A-EGFP (A) or co-transfected with Shot A-EGFP and mCherry-Zw3-CA (B). (C and D) S2 cells co-expressing Shot’s CTD along with mCherryZw3-CA. The black arrowhead in C indicates a cell lacking the mCherry-Zw3-CA construct. Shown in the upper right hand corner is an image of mCherry-Zw3-CA (B-D). Scale bar 10 μm in high magnification images and 2 μm in low magnification images. All images taken from time-lapsed TIRF-microscopy movies.

Supplemental Figure 4. Mander’s Coefficient vs. Fluorescent Intensity/μm².
A graphical representation of the the fluorescent intensity per cell area compared the Mander’s coefficient for cells co-expressing either Shot A-EGFP, FKBP-Shot-FRB-EGFP (treated with DMSO or 500 nM rapamycin), ShotΔRod-EGFP, ShotΔEFhand-
EGFP, ShotΔGAS2-EGFP, or ShotGAS2-EGFP and mCherry-Tubulin (from the cells quantified in Figure 3). We performed a Pearson product-moment correlation analysis to determine the dependence of the Mander’s coefficient on cell fluorescent intensity. Shown are the correlation coefficient (R value), the variance (r² value) and the P-value (two-tailed). We observed a positive correlation between the Mander’s coefficient and the fluorescent intensity per cell area in cells expressing Shot A-EGFP, FKBP-Shot-FRB-EGFP (treated with DMSO), and ShotGAS2-EGFP (denoted by an asterisk), suggesting that the Mander’s coefficient and thus the degree of co-localization between the EGFP-tagged Shot construct and mCherry-Tubulin increased with increase fluorescent intensity. For all other conditions, there was no correlation, suggesting that the degree of co-localization was not dependent on the cell’s overall fluorescent intensity. Analysis was performed on approximately 30-40 different cells per condition.

Supplemental Movies.
All time-lapsed movies were acquired at 3 sec time intervals and are presented at 7 frames per second.

Supplemental Movie 1. Bimolecular Fluorescence Complementation Assay.
S2 cells transfected with EB1-VN and EB1-VC (left), NH₂-(VN) and COOH-terminal (VC) halves of Venus alone (middle) and VN-Shot-VC in the cis orientation (right). EB1 is an obligate dimer and is a positive control for the reconstitution of fluorescence, and untagged VN and VC should not fluoresce. We observed fluorescence at the tips of microtubules in cells expressing VN-Shot-VC indicating that Shot’s NH₂- and COOH-termini are close enough for reconstitution of the Venus fluorescent protein. Images were acquired by Hawk-VT multi-point array scanning confocal microscope (Visitech International, Sunderland, UK) for 2 min.

Supplemental Movie 2. The central rod domain is required for the interaction between Shot’s ABD and EF-hand-GAS2 domain.
S2 cells transfected with VN-Shot-VC (left), VN-ShotΔRod-VC (middle), and ShotΔRod-EGFP (right). Without the central rod domain Shot’s NH₂- and COOH are unable to interact. Images were acquired by Hawk-VT multi-point array scanning confocal microscope (Visitech International, Sunderland, UK) for 2 min.

Supplemental Movie 3. EF-hand motifs contribute to Shot’s regulation.
S2 cells transfected with VC-ShotΔEF-hand-VN (left), VC-ShotEF-handMut-VN (middle), and VC-Shot-VN (right). Without the EF-hand motifs, Shot is trapped in an “open” conformation and is unable to reconstitute fluorescence, however, a mutant version of Shot where key calcium coordinating residues in the EF-hand motif were mutated (D5080A, D5082A, N5084A) reconstituted fluorescence at the tips of microtubules similar to VC-Shot-VN. Images were acquired by Hawk-VT multi-point array scanning confocal microscope (Visitech International, Sunderland, UK) for 45 sec.
Supplemental Movie 4. Rapamycin induced folding targets Shot to the microtubule plus end.
S2 cells co-transfected with FKBP-Shot-FRB-EGFP and EB1-mRFP following perfusion with 500 nM rapamycin (left) and DMSO (right). Perfusion of rapamycin induces dimerization and targets Shot to the tips of microtubules while DMSO treated cells appeared wild-type. Images were acquired on laser TIRF system (Nikon, Tokyo, Japan) for 3 min.

Supplemental Movie 5. Live-Cell imaging of rapamycin perfusion. S2 cells expressing FKBP-Shot-FRB-EGFP perfused with either DMSO (left) or 500 nM rapamycin (right). Images were acquired on laser TIRF system (Nikon, Tokyo, Japan) for 3 min.
