Supplemental Materials

Supplementary Figure 1.

(A) RNAi targets designed against the 3 and 5'UTR of Shot, as well as RNAi designed against an exon within Shot, effectively deplete Shot. Non-treated (NT) and Control RNAi also shown. Immunoblot for tubulin is provided to demonstrate protein loads. (B) Immunoprecipitates from S2 cell lysates transiently expressing GFP CTD-fragments probed with anti-GFP (Top) and anti-EB1 (middle). Inputs are shown probed with the same antibodies (bottom). (C) RNAi depletion of DHC verified by immunoblot using tubulin levels to normalize protein loads.

Supplementary Figure 2.

S2 cells transfected with Shot A-EGFP (top), the GAS2 domain of Shot tagged with EGFP (middle), and ShotΔGAS2-EGFP (bottom) arranged with increasing fluorescent intensity from left to right. At low expression levels Shot A-EGFP has two distinct distributions: in the cell interior it appears comet-like and at the cell periphery it appears MAP-like. At high expression levels, Shot A-EGFP fully decorates microtubules. The GAS2 domain localizes along the length of microtubules regardless of expression level, while ShotΔGAS2-EGFP does not localize along of the length of microtubules even at higher fluorescent intensity levels.

Supplemental table 1. Primers used.

Supplemental Videos.

All time-lapsed images were taken at 3 second time intervals and are played at a rate of 7 frames per second.

Video 1. Shot demonstrates two modes of dynamics in S2 cells.

An S2 cell transiently expressing mRFP-EB1 (left panel, red in merge) and Shot A-EGFP (middle panel, green in merge). In the interior of the cell Shot's dynamics are more similar to EB1 while at the cell periphery Shot binds along the lattice of microtubules.

Video 2. The GAS2 domain of Shot does not plus end track.

An S2 cell transiently expressing mRFP-EB1 (left panel, red in merge) and an EGFP-fusion of the GAS2 domain of Shot (middle panel, green in merge). EGFP-GAS2 does not plus in track, rather it localizes along the length of microtubules.

Video 3. Deletion of the GAS2 abolishes Shot's lattice binding.

An S2 cell transiently expressing mRFP-EB1 (right panel, red in merge) and ShotΔGAS2-EGFP (middle panel, green in merge). The GAS2 domain of Shot is required for Shot's lattice binding, ShotΔGAS2-EGFP tracks along the plus end of microtubules and does not bind to the lattice.

Video 4. Shot maintains lattice association in the absence of EB1.

An S2 cell transiently expressing mCherry-tubulin (left panel, red in merge) and ShotA-EGFP (middle panel, green in merge). RNAi depletion of EB1 eliminates Shot's plus end dynamics but not its lattice binding. .

Video 5. The Shot C-terminal domain (CTD) is sufficient for targeting to plus ends.

Shown, two different S2 cells expressing CTD-WT-EGFP, however, the cell of the left has not been treated with RNAi while the cell on the right has been treated with RNAi targeted against EB1 for sevens days prior to transient transfection.

Video 6. ShotΔC-TagRFP does not plus-end track but does bind weakly to the MT lattice.

Shown, S2 cells co-expressing EGFP-Tubulin (left, red in merge) and ShotΔC-TagRFP (middle, green in merge).

Video 7. CTD- β and γ-EGFP fusions plus end track, CTD-α-EGFP does not.

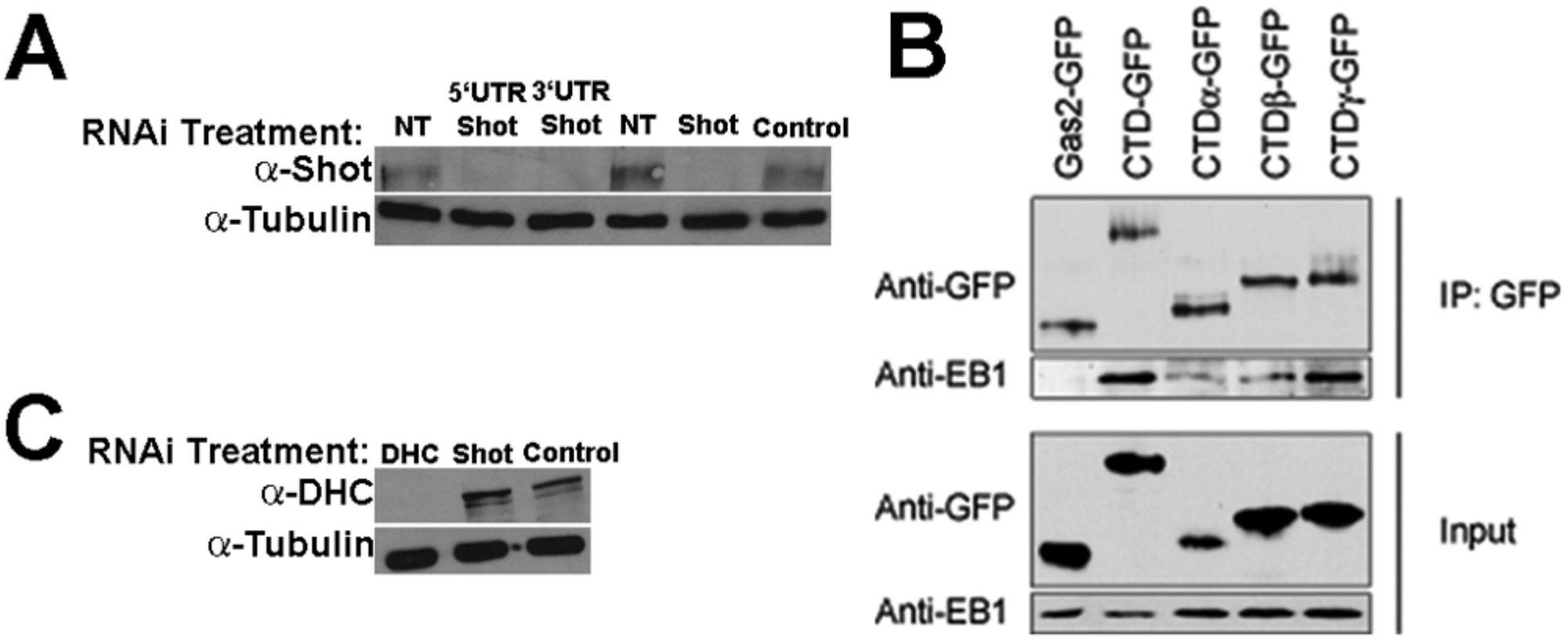
Shown S2 cells expressing either (left) CTD-α-EGFP, (middle) CTD-β-EGFP, or (right) CTD-Y-EGFP. Both CTD-β-EGFP and CTD-Y-EGFP demonstrate plus-end tracking while CTD-α-EGFP does not.

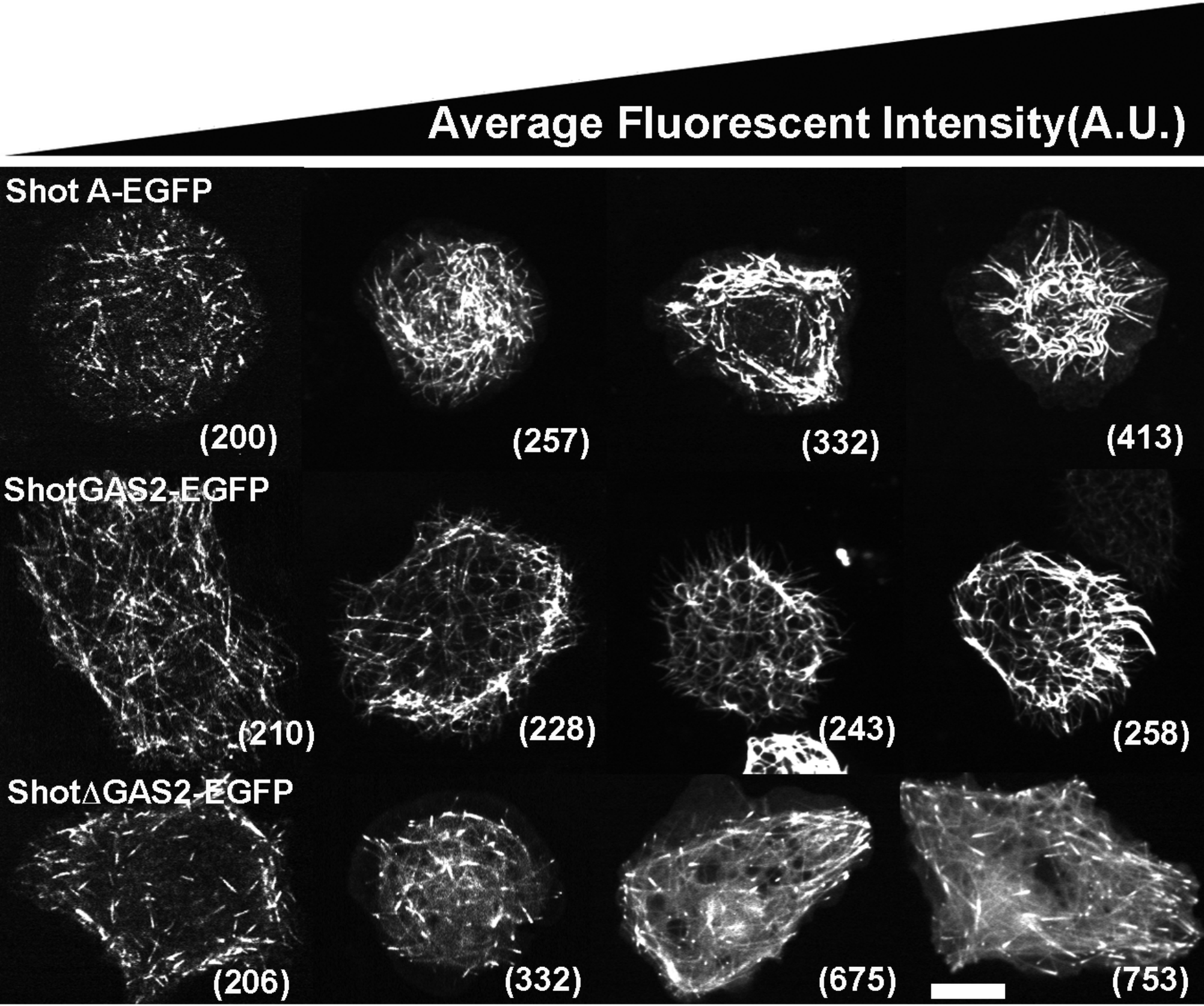
Video 8. Deletion of SxIP motif in the CTD ablates plus end tracking.

Shown S2 cells expressing (left) CTD-βΔSxIP1-EGFP and (right) CTD-δΔSxIP2. Deletion of SxIP motifs ablates plus-end tracking.

Video 9. Depletion of Shot leads to exaggerated lateral "fishtailing" movements of microtubules.

S2 cells expressing EGFP-tubulin (left and middle) and mCherry-tubulin (right). Left, a control cell demonstrating normal microtubule dynamics. Middle, an S2 cell demonstrating the exaggerated 'fish-tailing' we observed following RNAi depletion. Right, microtubule dynamics following double RNAi depletion of DHC and KHC.


A
5'UTR 3'UTR
RNAi Treatment: NT Shot Shot NT Shot Control
α-Shot
α-Tubulin
B
Gas2-GFP
CTD-GFP
CTDα-GFP
CTDβ-GFP
CTDγ-GFP
Anti-GFP
Anti-EB1
IP: GFP
Anti-GFP
Anti-EB1
Input
C
RNAi Treatment: DHC Shot Control
α-DHC
α-Tubulin


Average Fluorescent Intensity(A.U.)
Shot A-EGFP
(200)
(257)
(332)
(413)
ShotGAS2-EGFP
(210)
(228)
(243)
(258)
ShotΔGAS2-EGFP
(206)
(332)
(675)
(753)