Supplementary Figure Legends

Figure 1-Supplement 1: Biochemical purification of Alp14 constructs used in this study

A) A schematic description of all the Alp14 constructs used in this study Two sets of constructs with different C-terminal tags: mNeon Green (mNG) tags for bulk studies, and SNAPf tag for single molecule studies (SANPf).
B) Structural locations of mutant residues for constructs used in this study. TOG1M, TOG2M mutations inactivate the intra-HEAT repeat loops residues from $\alpha\beta$-tubulin binding in TOG1 and TOG2 domains, respectively. TOG12M combines both TOG1M and TOG2M mutations. INT1 8-residue mutations destabilize two interfaces stabilizing the inter-subunit contact in the TOG square. INT2 7-residue mutations destabilize the interfaces the intra-subunit TOG1-TOG2 interfaces. INT12 combines both INT1 and INT2 mutations. INT1,INT2 and INT12 mutants don't interfere with $\alpha\beta$-tubulin binding as described in Nithianantham et al 2018.

C) Above, Size exclusion chromatography trace for Alp14-SNAPf. Below, SDS-PAGE for fractions from SEC.
D) Single SDS-PAGE bands showing each of the 13 constructs used in this study. mNG notes the C-terminally tagged mNeonGreen constructs, SNAPf notes the C-terminally tagged SNAP constructs

Figure 1-Supplement 2: TIRF microscopy studies of MT polymerase activity for Alp14-mNG and its mutants at dynamic MT plus ends

A) Scheme for TIRF assay utilized in this study. MT seeds are anchored on glass surfaces via Neutravidin-Biotin interface and then dynamic MTs are polymerized in the presence of Alp14-mNG at their plus ends
B) Raw image fields for TIRF microscopy experiments revealing all Alp14-mNG track MT plus ends, except TOG12M, which does not localize to MT plus ends *in vitro.*
C) Dynamic MT polymerization parameters as described in Table S1.

Figure 2-Supplement 1: Approach to measure MT plus end Residence ratio and examples for TOG1M, TOG2M, and TOG12M tracking at MT plus ends

A) Left, 640 nm emission channel showing the kymograph for MT. The yellow transparent line depicts measured pixel intensities. Note measurement extends beyond the MT intensity at catastrophe to depict the boundary of the event. Bottom, left, Intensity trace for MT lattice intensity and the drop at catastrophe. This data is used to determine the duration of MT polymerization event.
Right, 488 nm emission channel shows a kymograph for Alp14-mNG localization at the MT plus end. The yellow transparent line depicts measured pixel intensities. Note the measurement extends beyond catastrophe to determine background value. Bottom, right intensity trace

for Alp14-mNG showing the intensity value throughout polymerization and drop off to background level at catastrophe. This data is used to determine the duration of Alp14 tracking during MT polymerization event.

B) Five additional examples for wt-Alp14-mNG residence at MT plus ends. Top, showing actual dual channel kymograph. Below intensity profiles for Alp14-NG and MT lattice to mark catastrophe event.
C) Five additional examples for TOG1M MT plus-end tracking as shown in B
D) Five additional examples for TOG2M MT plus-end tracking as shown in B

Figure 2-Supplement 2: Single molecule TIRF tracking event examples used to determine the dwell time for wt-Alp14, TOG1M and TOG2M at MT plus ends

A) Schematic description of single molecule TIRF experiment using Alp14-SNAP consisting of 5 nM Alp14-SNAP Atto-488 and 195 nM Alp14-SNAP unlabeled. In which few Atto-488 Alp14- SNAP molecules bind MT plus-ends during MT polymerization events.
B) Photobleaching curve measuring the duration for single Alp14-SNAP-Atto-488 molecules using the same intensity conditions utilized in these studies revealing the length time that a single fluorophore retains fluorescence. The duration is longer than 200 sec, which is 80% longer than the longest Alp14-tracking events.

C-E) show a variety of event short medium and long depicting the range of wt-Alp14, TOG1M, TOG2M localization at MT plus ends

Figure 3-Supplement 1: Analysis of Alp14 mutants in fission yeast

A) Left, Dilution Growth series for *S. pombe* strains at 20,25,30,36 °C (aligned vertically) reveal only defects with growth in the TOG12M mutant. Middle, Growth of *S. pombe* strains in the presence of MT depolymerization drug, MBC, at 20,25,30,36 °C (aligned vertically) reveal growth defects in with TOG12M cells in all conditions and TOG2M cells in 25 °C. Right, Phloxine plates show dead cells (dark pink) in assays shown in left panels.
B) Raw images of *S. pombe* cells expressing mCherry-Atb2 and Alp14-GFP. Lower left, Montage of cells showing two individual MTs used for MT residence ratios revealing the dissociation of Alp14 signal from MT plus-end during polymerization events. These residence ratio measurements are described in Figure 3C and Figure 6C.

Figure 3-Supplement 2: Approach to study Alp14 tracking at MT plus-ends in fission yeast cells.

A) Top panel, mage of fission yeast cell with mCherry-Atb2 (α-tubulin; red) and Alp14-GFP (green). Lower panel, In interphase fission yeast cells, MTs are generally organized into bundles containing 2-4 MTs of mixed polarity. In this schematic, we show one bundle in the context of the cell and nucleus. Typically, in each bundle, there are “lead MTs” (arrows) whose plus ends face the cell tips; these grow towards the cell tip, and then undergo catastrophe after they contact the cell tip. Alp14 is located on growing MT plus ends, as

well as MTOCs such as the spindle pole body on the medial nucleus (large Alp14 dot on nucleus). In our analyses, we analyzed primarily the plus ends of the “lead MTs” that grow out towards the cell tips. We analyzed MTs, which were marked with mCherry-tubulin, and Alp14-GFP by examination of movies and kymographs (see Methods).

B) Kymographs of dynamic interphase MTs in yeast cells. Left, merge dynamic MTs (red) with Alp14-GFP (green) along their ends of the TOG1M mutant strain.
C) A "Find Edges" filtered kymograph s in B generated using ImageJ plugin (see materials and methods) which reveals a sharper image of the GFP intensity in the kymograph in B
D) Frame images of dynamic MT polymerization in fission yeast cells revealing the formation, dimming and re-appearance of alp14 signal in the Alp14-TOG1M mutant. The frames are arrayed left to right from top row to the bottom row.

Figure 3-Supplement 3: Additional dynamic parameters and example kymographs for TOG1M, TOG2M and TOG12M mutants studied in yeast cells.

A) Dynamic MT lengths measured for individual MTs in wt, TOG1M, TOG2M and TOG12M strains
B) MT shrinkage rates for dynamic MTs in wt, TOG1M, TOG2M and TOG12M strains.
C) The proportion of MTs undergoing catastrophe prior to reaching the cell cortex (inset image) in wt, TOG1M, TOG2M and TOG12M strains.

Figure 5-Supplement 1: Approach to measure MT plus end Residence ratio and examples for INT1, INT2 and INT12 tracking at MT plus ends

A) Left, 640 nm emission channel showing the kymograph for MT. The yellow transparent line depicts measured pixel intensities. Note measurement extends beyond the MT intensity at catastrophe to depict the boundary of the event. Bottom, left, Intensity trace for MT lattice intensity and the drop at catastrophe. This data is used to determine the duration of MT polymerization event. Right, 488 nm emission channel shows a kymograph for Alp14-mNG localization at the MT plus end. The yellow transparent line depicts measured pixel intensities. Note the measurement extends beyond catastrophe to determine background value. Bottom, right intensity trace for Alp14-mNG showing the intensity value throughout polymerization and drop off to background level at catastrophe. This data is used to determine the duration of Alp14 tracking during MT polymerization event.
B) Five examples for INT1-mNG residence at MT plus ends . Top, showing actual dual channel kymograph. Below intensity profiles for Alp14-NG and MT lattice to mark catastrophe event.
C) Five additional kymograph examples for INT2 as shown in B
D) Five additional kymograph examples for INT12 as shown in B

Figure 5-Supplement 2: Single molecule TIRF tracking event examples used to determine the dwell time for INT1, INT2, INT12 at MT plus ends

A) Schematic description of single molecule TIRF experiment using Alp14-SNAP consisting of 5 nM Alp14-SNAP Atto-488 and 195 nM Alp14-SNAP unlabeled. In which few Atto-488 Alp14- SNAP molecules bind MT plus-ends during MT polymerization events.

B-E) show a variety of event short medium and long depicting the range of dwell time events in INT1, INT2 and INT12 localization at MT plus ends

Figure 6-Supplement 1: Additional dynamic parameters and kymograph examples for INT1, INT2 and INT12 mutants studied in yeast cells.

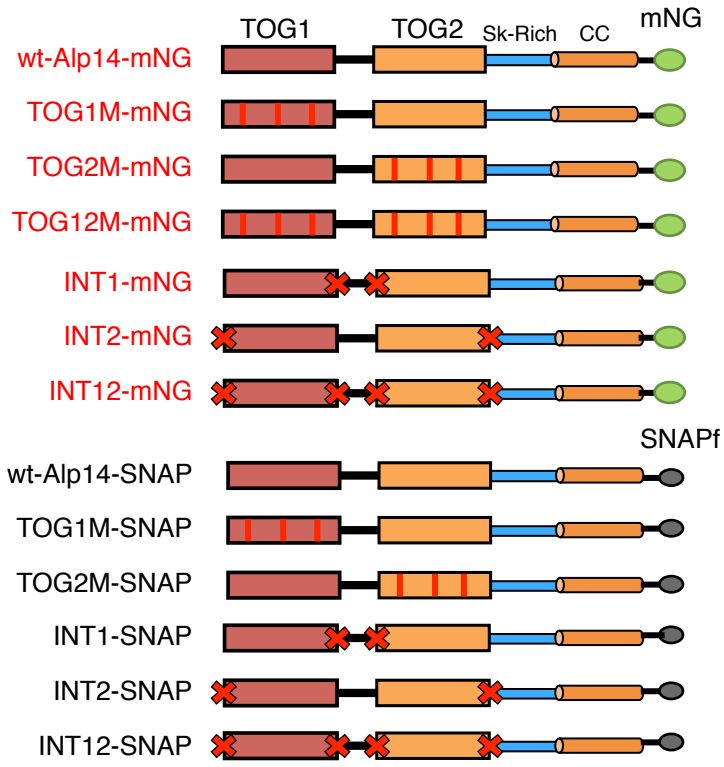
A) Dynamic MT lengths measured for individual MTs in wt, INT1, INT2, and INT12 strains

B) MT shrinkage rates for dynamic MTs in wt, INT1, INT2, and IN12 strains.

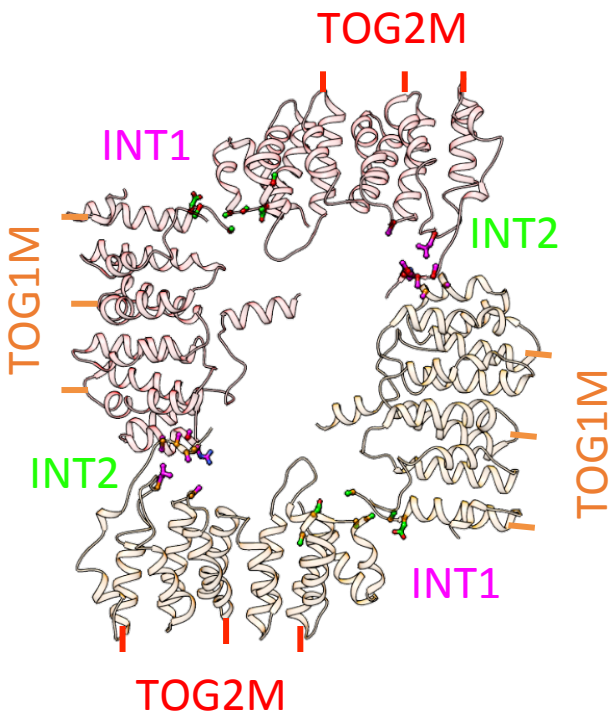
C) The proportion of MTs undergoing catastrophe prior to reaching the cell cortex (inset image) in wt, INT1,INT2, INT12 strains.

A

Constructs used in the *in vitro* studies



B

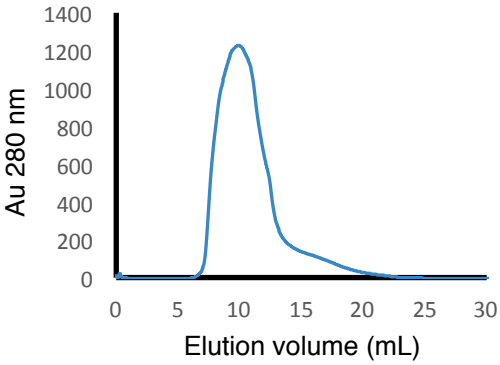


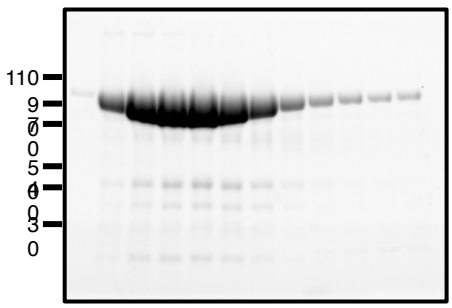
TOG12M = TOG1M+TOG2M

INT12 = INT1+INT2

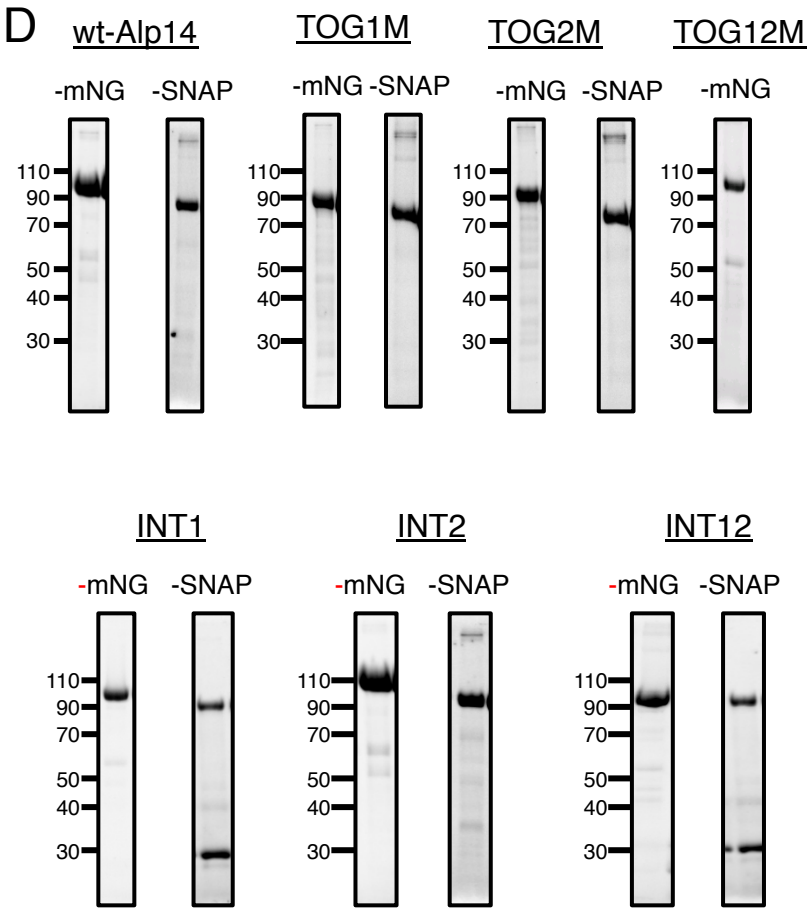
C

SEC purified wt-Alp14-SNAP

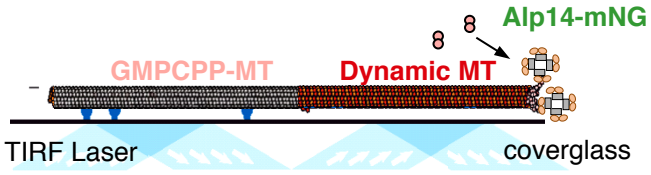




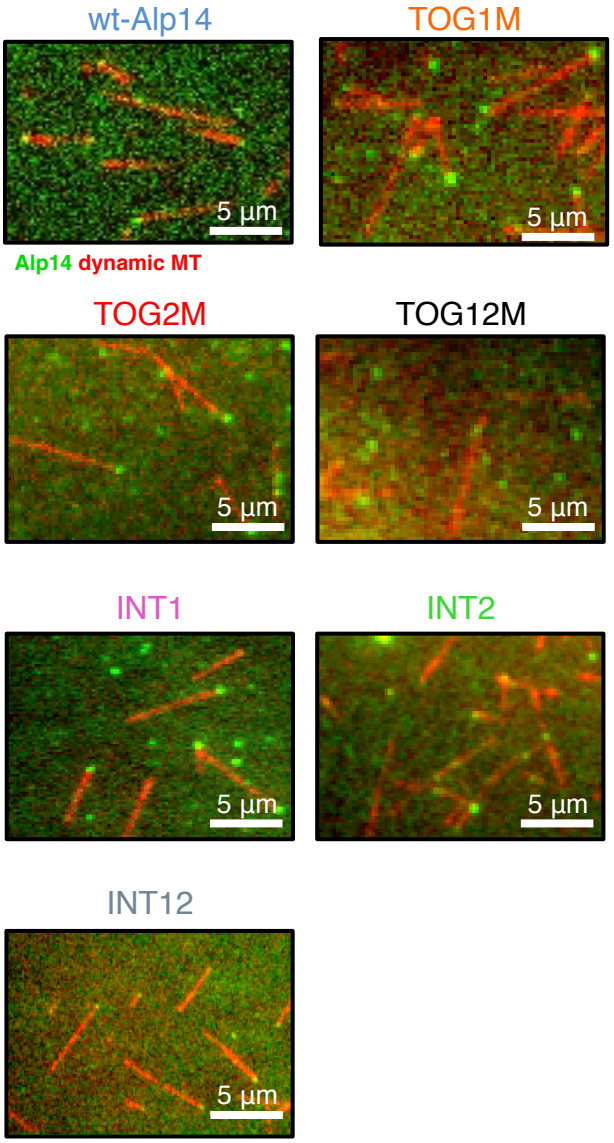
D



A Reconstituting Alp14 MT polymerase



B Raw images of MT Alp14



C

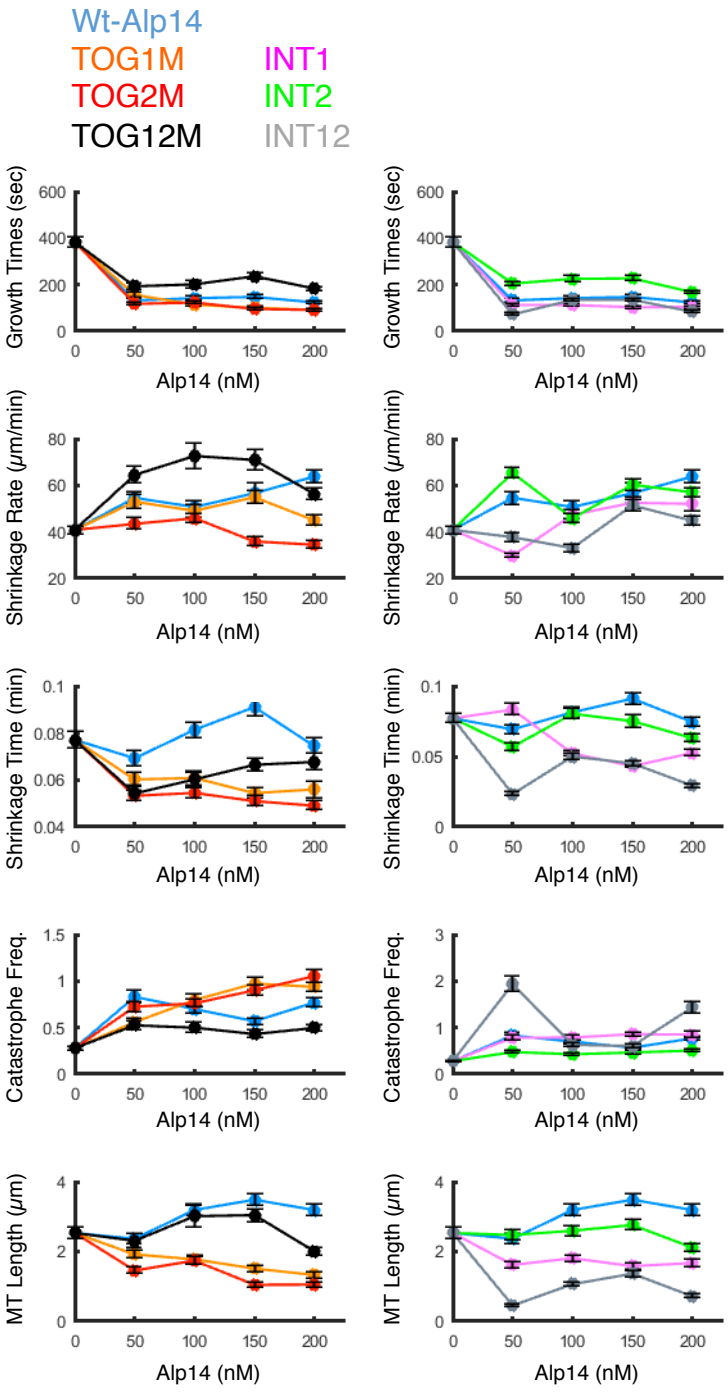


Fig2 −S1

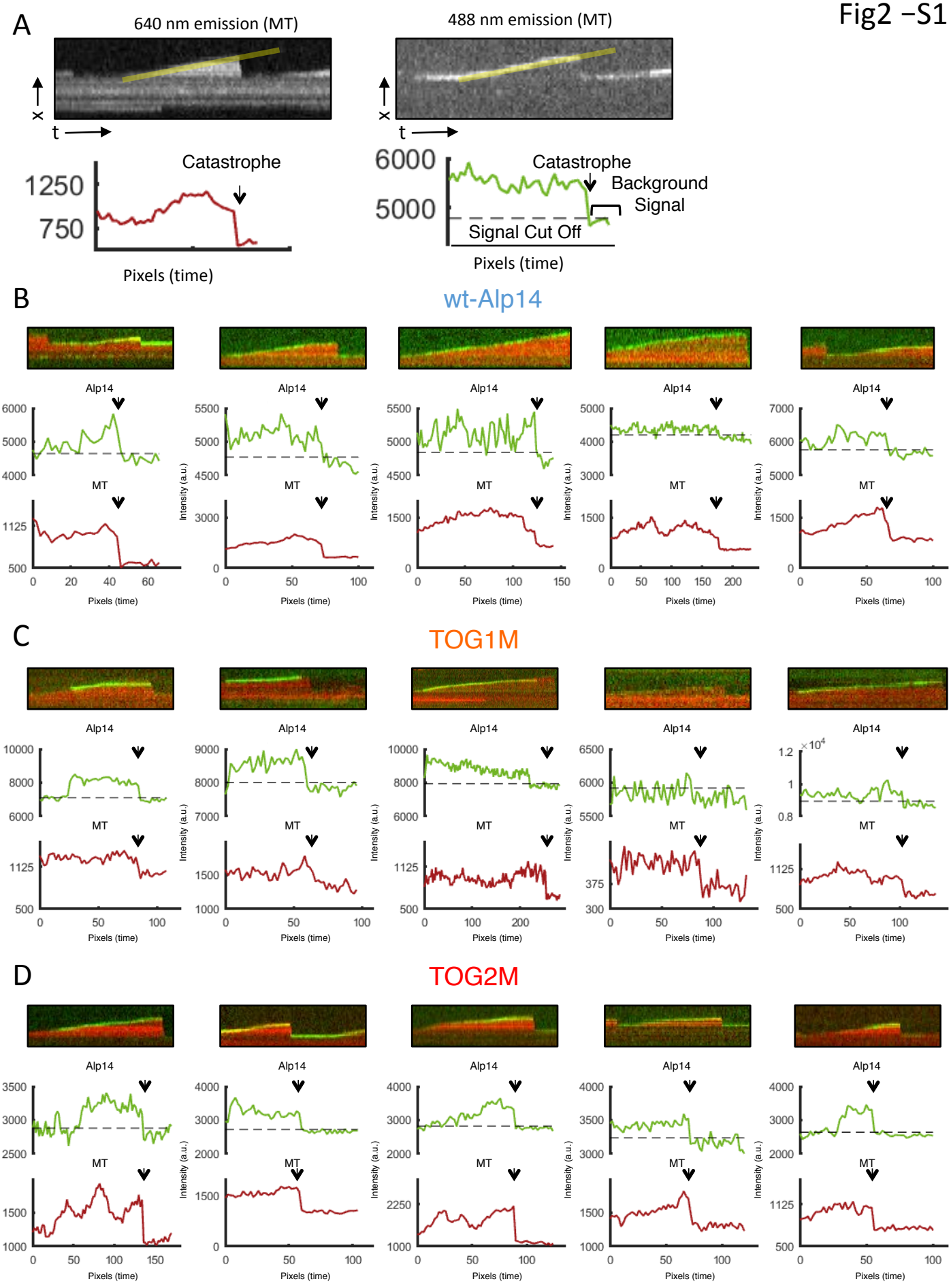
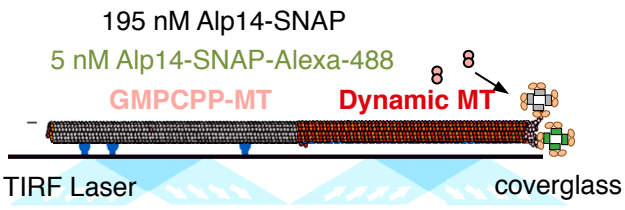
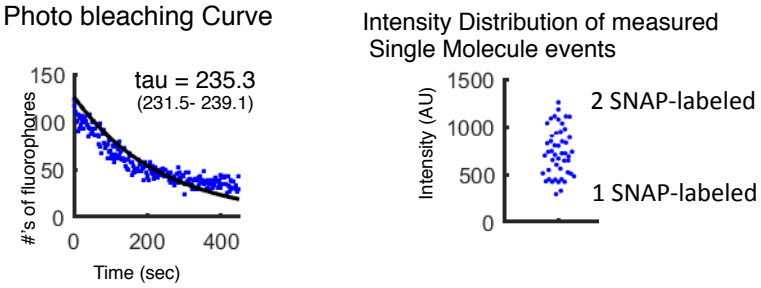

A
640 nm emission (MT)
488 nm emission (MT)
x
t
Catastrophe
1250
750
Pixels (time)
6000
5000
Background
Signal
Signal Cut Off
B
wt-Alp14
C
TOG1M
D
TOG2M
Alp14
MT
Intensity (a.u.)
Pixels (time)

Fig2-S2

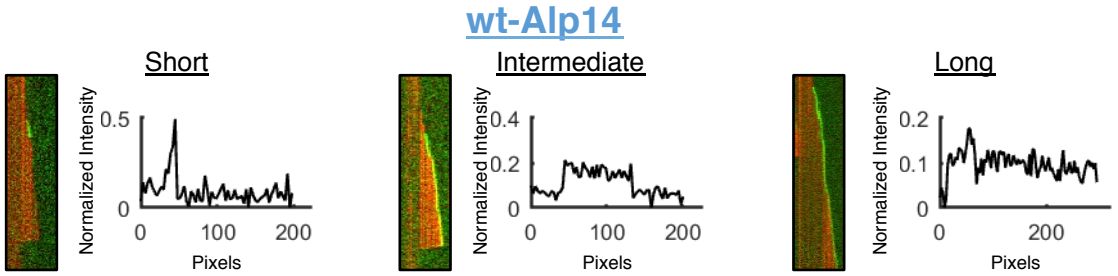
A



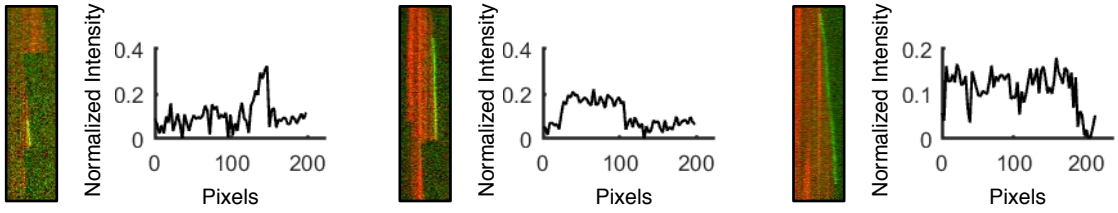
B



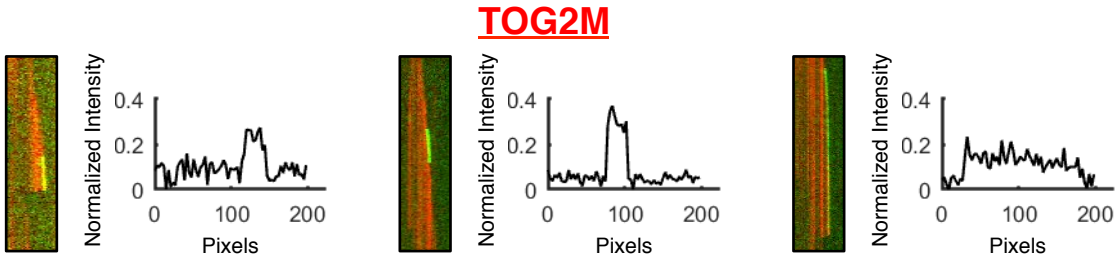
C

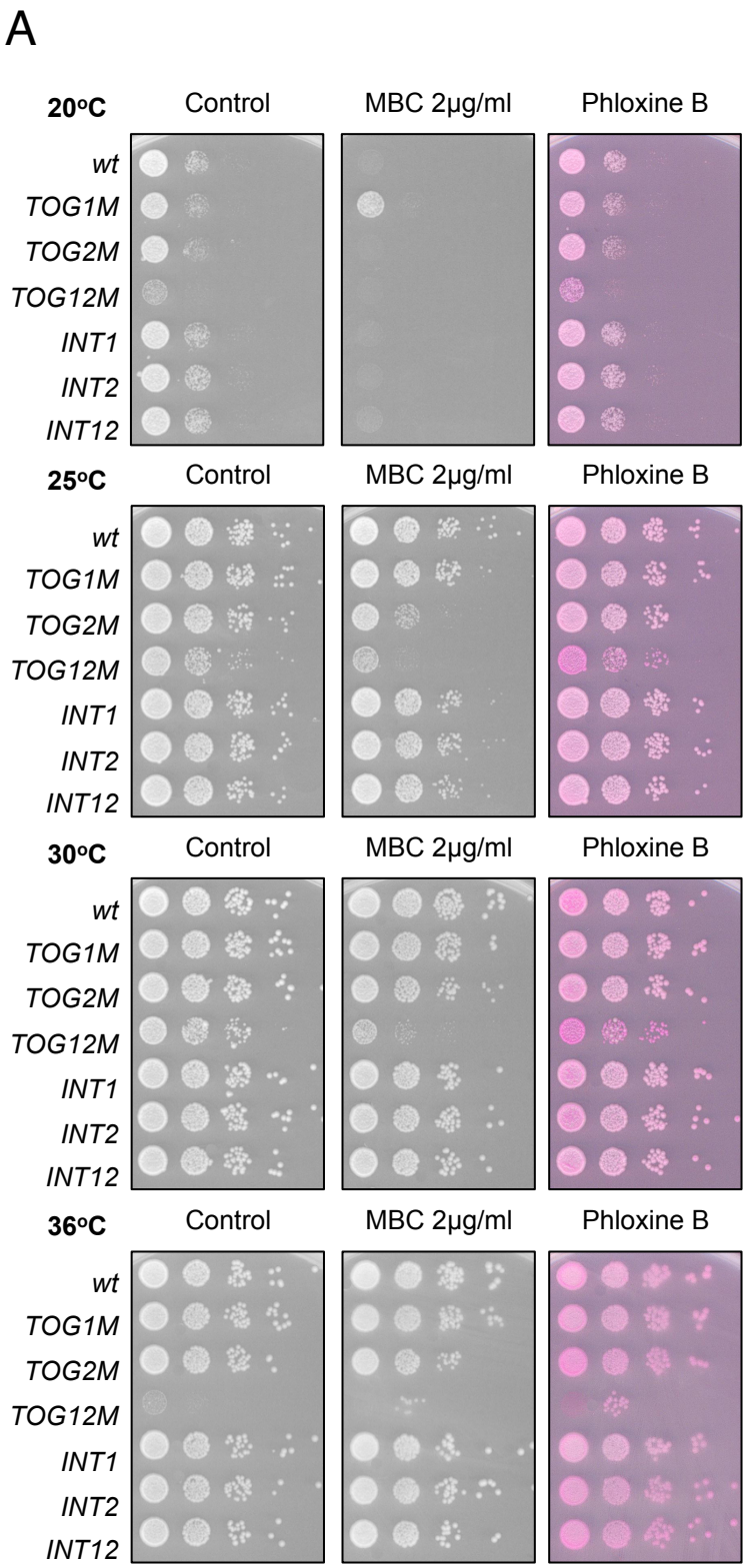


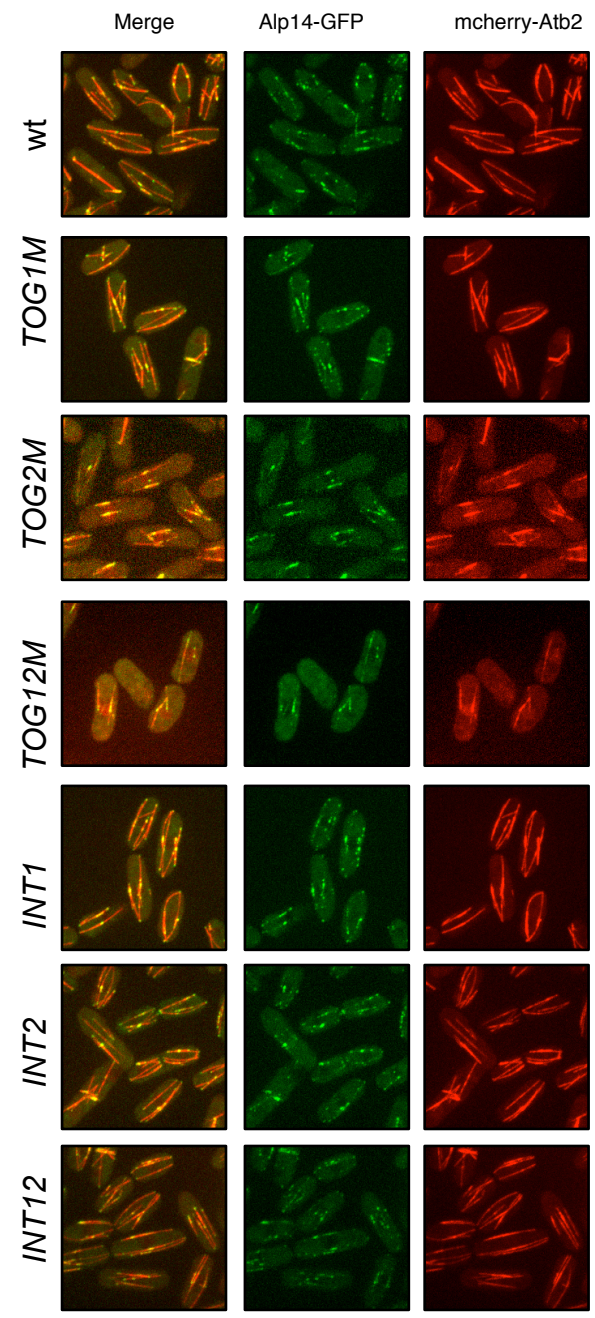
D



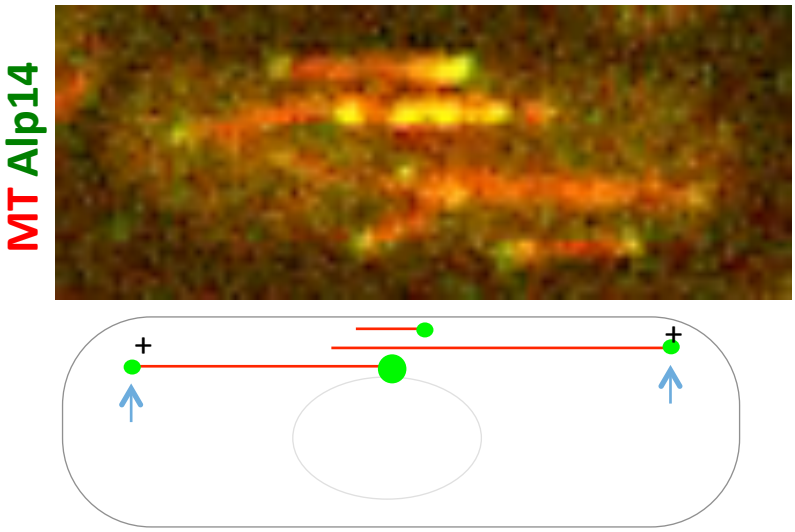
E



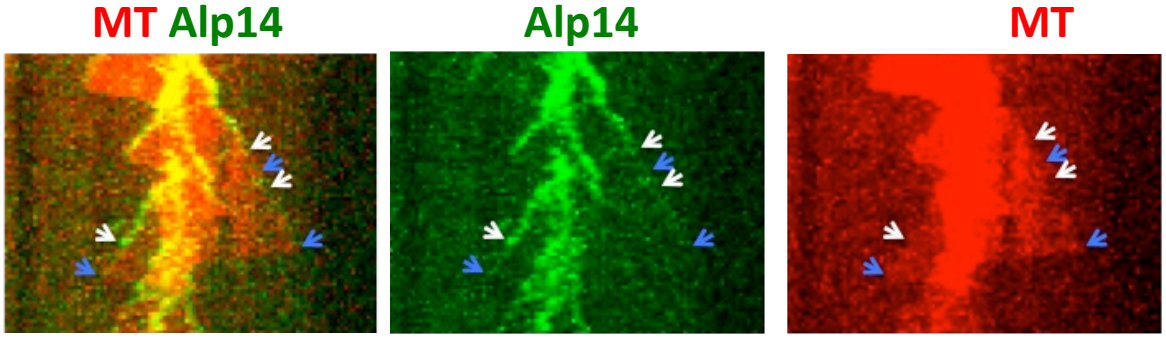

A
20°C
Control
MBC 2μg/ml
Phloxine B
wt
TOG1M
TOG2M
TOG12M
INT1
INT2
INT12
25°C
Control
MBC 2μg/ml
Phloxine B
wt
TOG1M
TOG2M
TOG12M
INT1
INT2
INT12
30°C
Control
MBC 2μg/ml
Phloxine B
wt
TOG1M
TOG2M
TOG12M
INT1
INT2
INT12
36°C
Control
MBC 2μg/ml
Phloxine B
wt
TOG1M
TOG2M
TOG12M
INT1
INT2
INT12


B
Merge
Alp14-GFP
mcherry-Atb2
wt
TOG1M
TOG2M
TOG12M
INT1
INT2
INT12

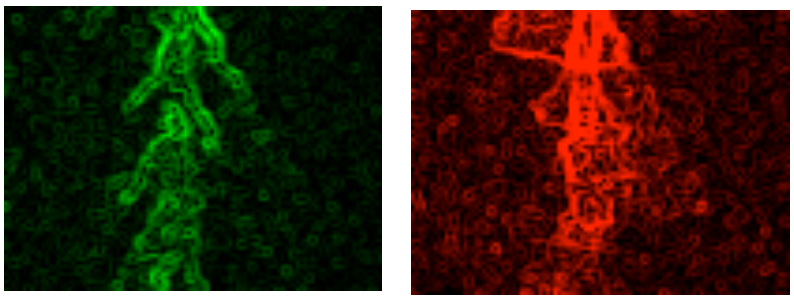
A



B



C



D

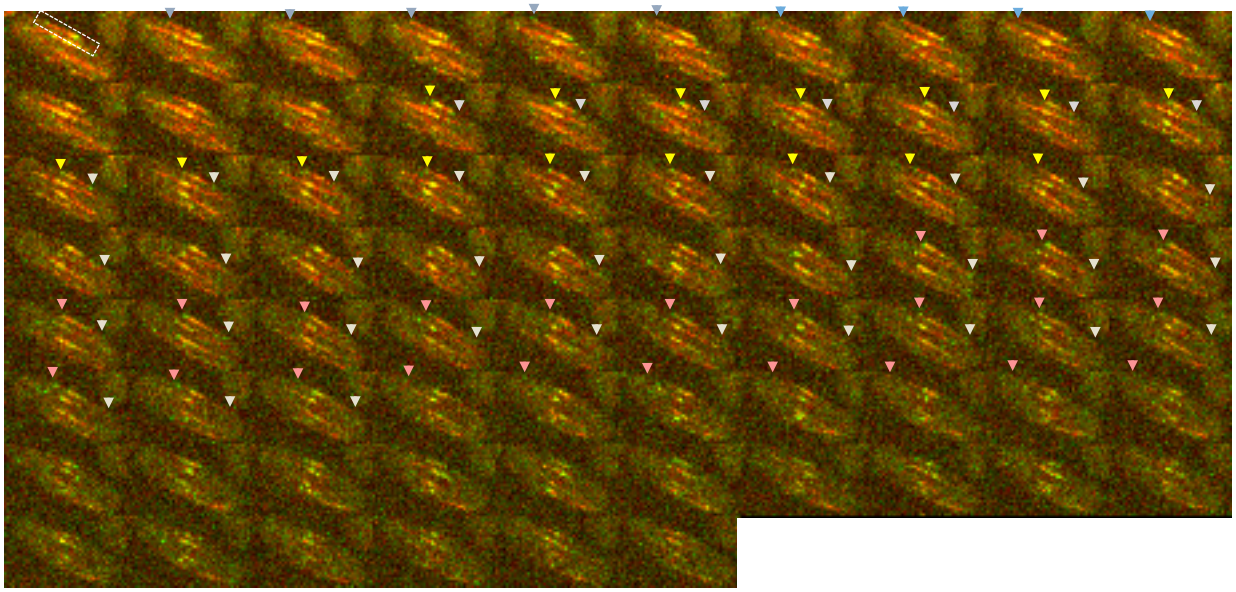
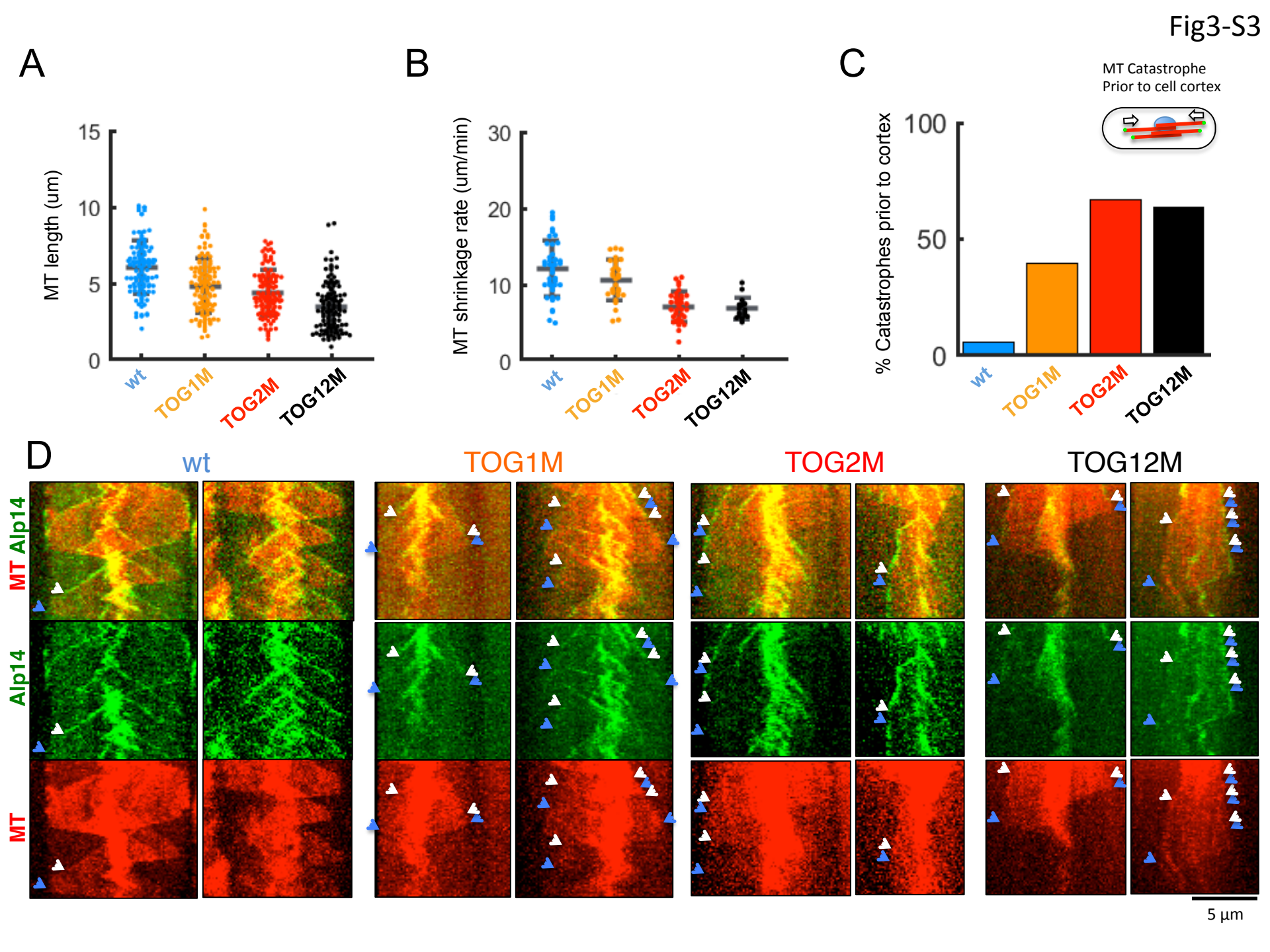
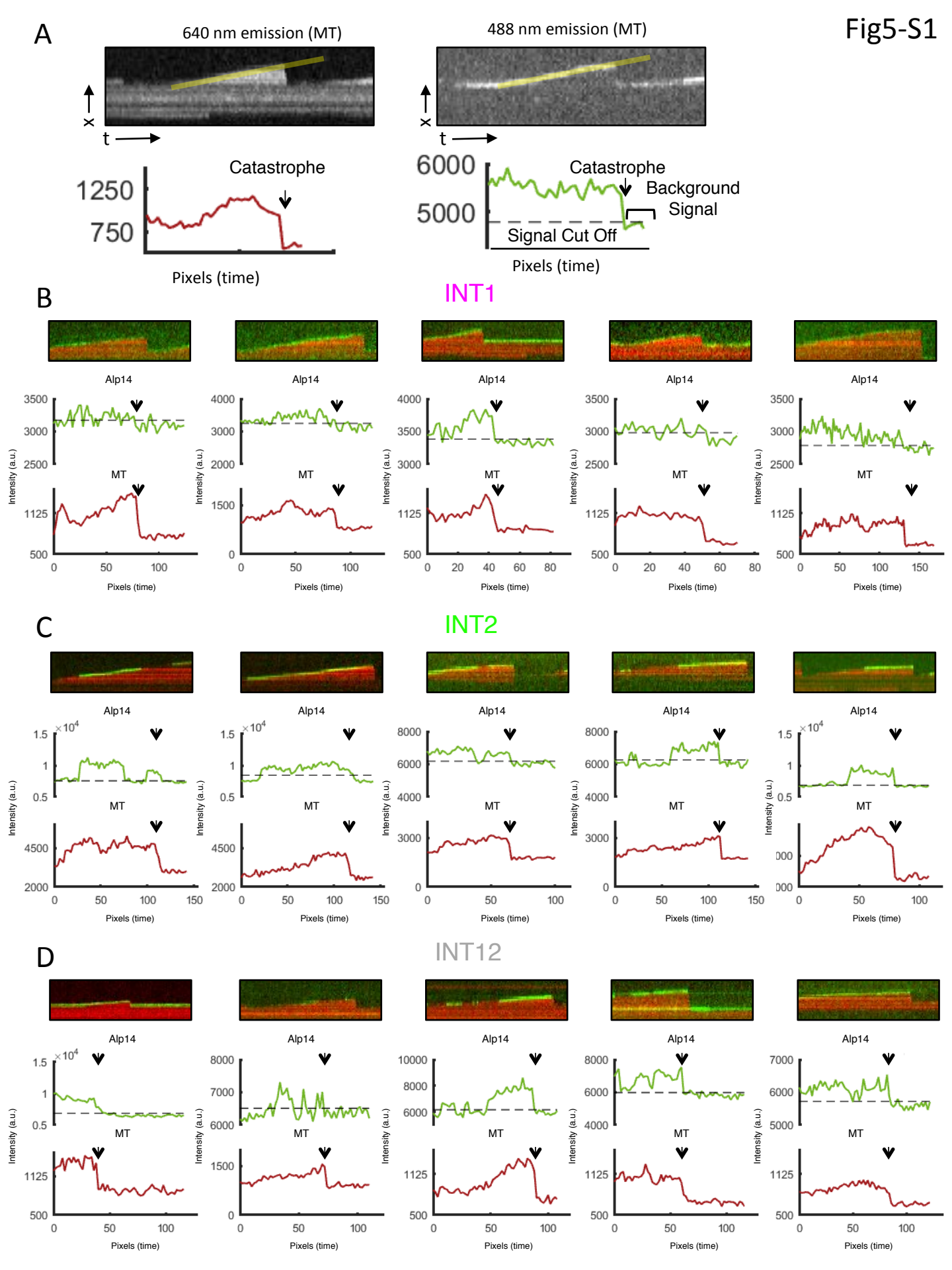
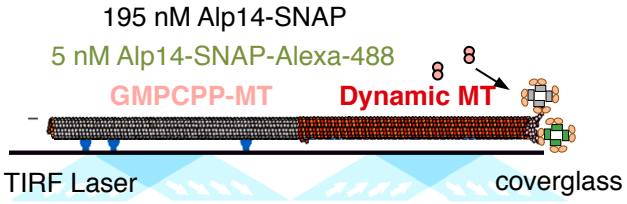


Fig3-S3

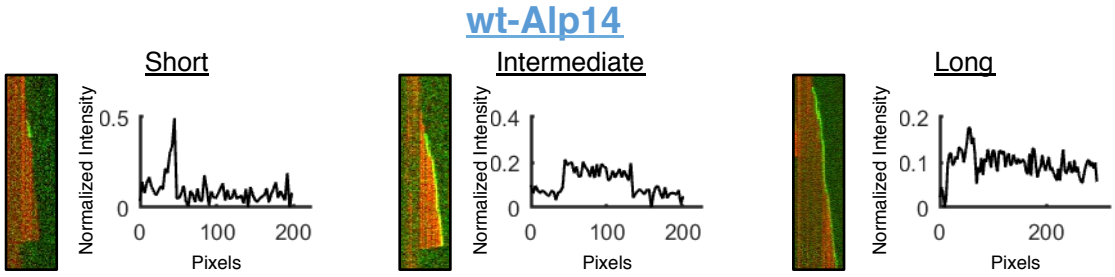

A
MT length (um)
15
10
5
0
wt
TOG1M
TOG2M
TOG12M
B
MT shrinkage rate (um/min)
30
20
10
0
wt
TOG1M
TOG2M
TOG12M
C
MT Catastrophe
Prior to cell cortex
% Catastrophes prior to cortex
100
50
0
wt
TOG1M
TOG2M
TOG12M
D
wt
TOG1M
TOG2M
TOG12M
MT Alp14
Alp14
MT
5 µm


A
640 nm emission (MT)
488 nm emission (MT)
Fig5-S1
x
t
Catastrophe
1250
750
Pixels (time)
6000
5000
Background
Signal
Signal Cut Off
B
INT1
C
INT2
D
INT12
Alp14
MT
Intensity (a.u.)
Pixels (time)

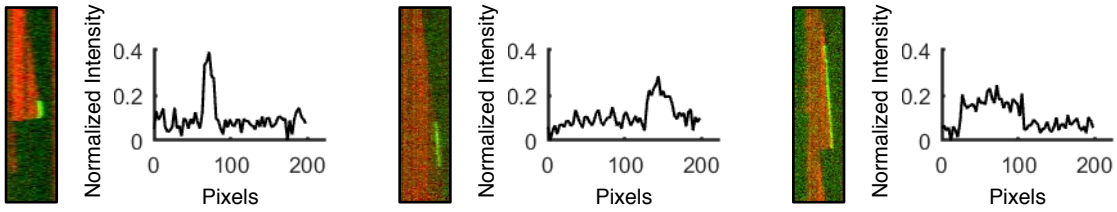
A


195 nM Alp14-SNAP
5 nM Alp14-SNAP-Alexa-488
GMPCPP-MT
Dynamic MT
TIRF Laser
coverglass

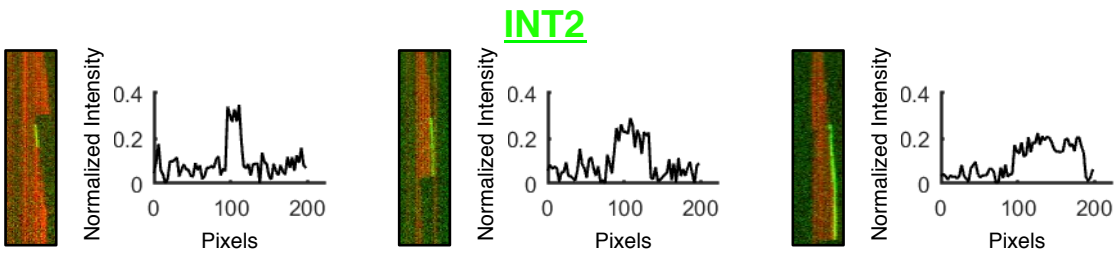
B


wt-Alp14
Short
Intermediate
Long
Normalized Intensity
Pixels

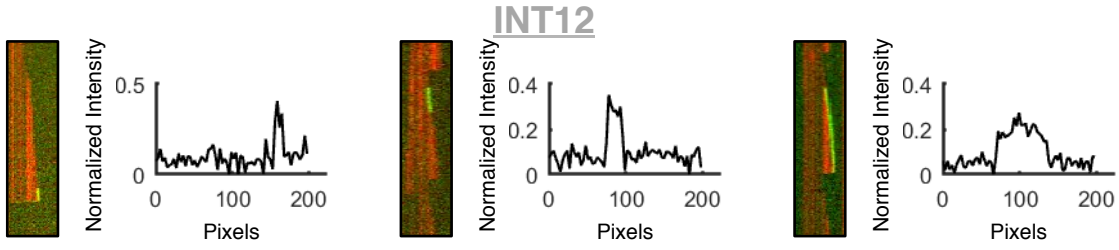
C

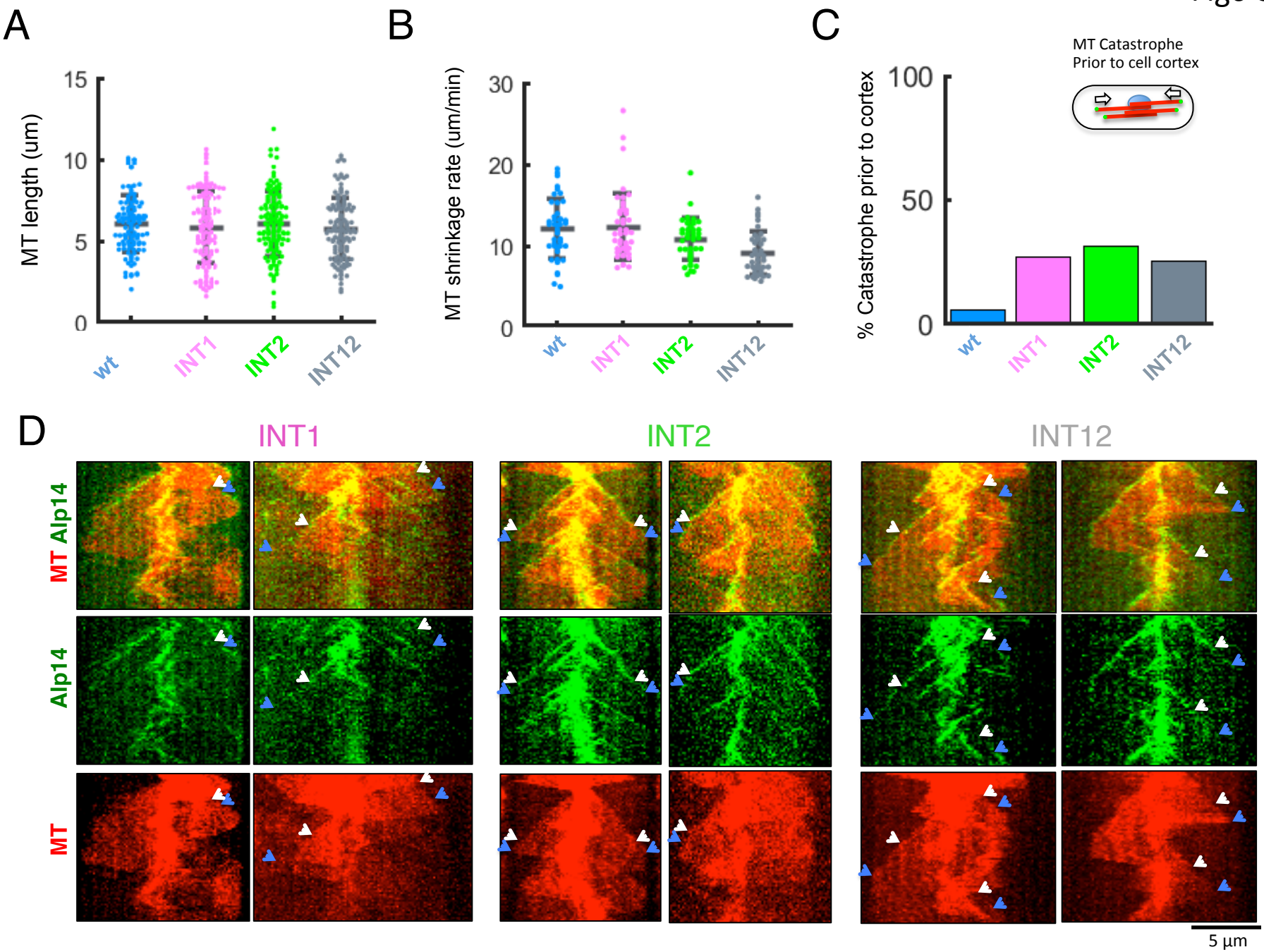

INT1
Normalized Intensity
Pixels

D


INT2
Normalized Intensity
Pixels

E


INT12
Normalized Intensity
Pixels


A
MT length (um)
15
10
5
0
wt
INT1
INT2
INT12
B
MT shrinkage rate (um/min)
30
20
10
0
wt
INT1
INT2
INT12
C
MT Catastrophe
Prior to cell cortex
% Catastrophe prior to cortex
100
50
0
wt
INT1
INT2
INT12
D
INT1
INT2
INT12
MT Alp14
Alp14
MT
5 µm