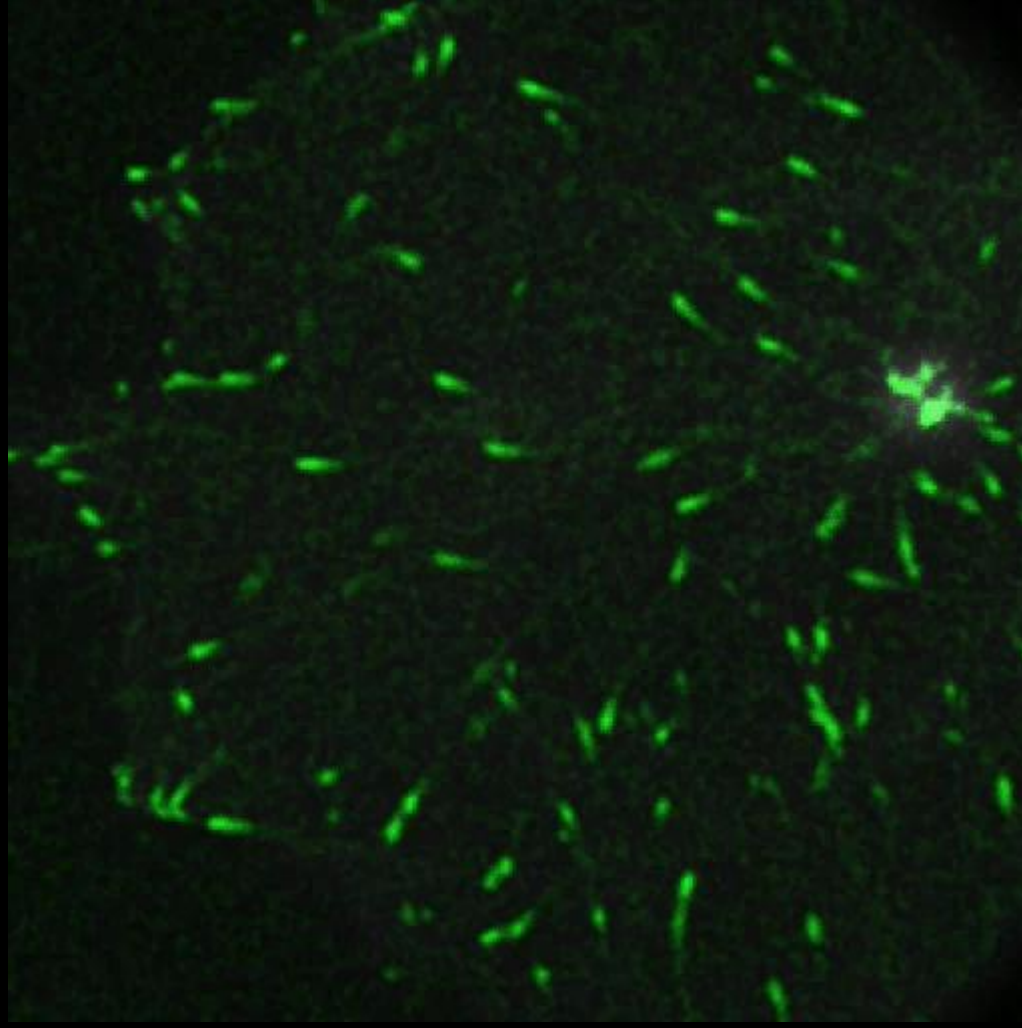
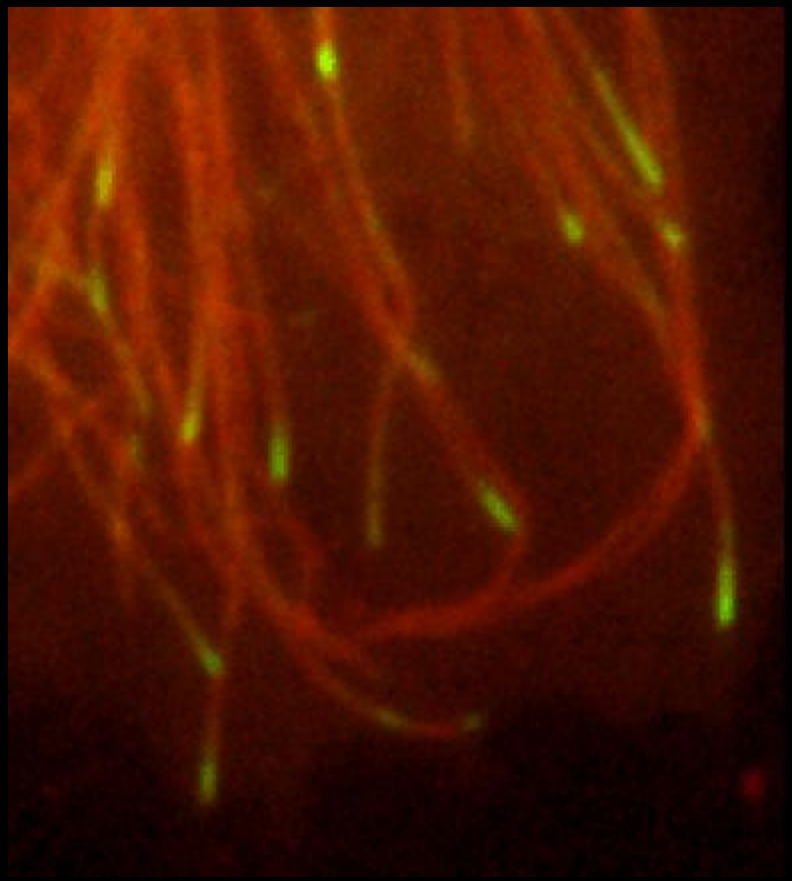
Anna Akhmanova
Cell Biology
Utrecht University
The Netherlands



A zoo at the tip: a dynamic protein network controls the fate of microtubule ends

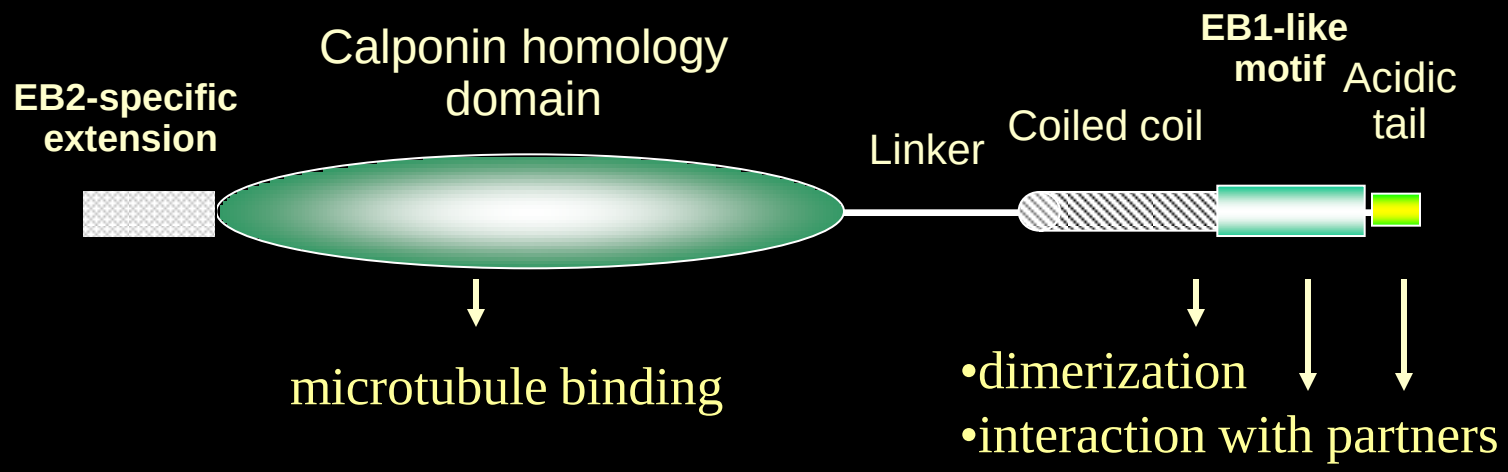
Plus end-tracking proteins accumulate at the growing microtubule ends

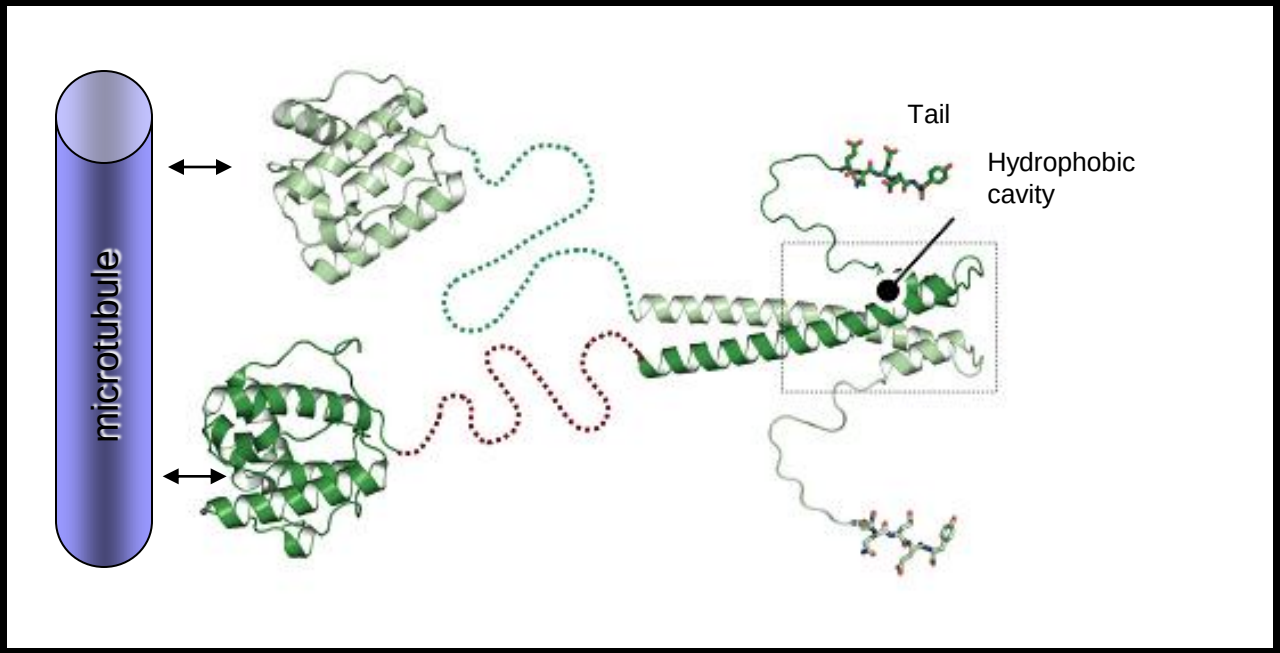


GFP-EB3 and mCherry-α-tubulin
in a MRC5 human lung fibroblast

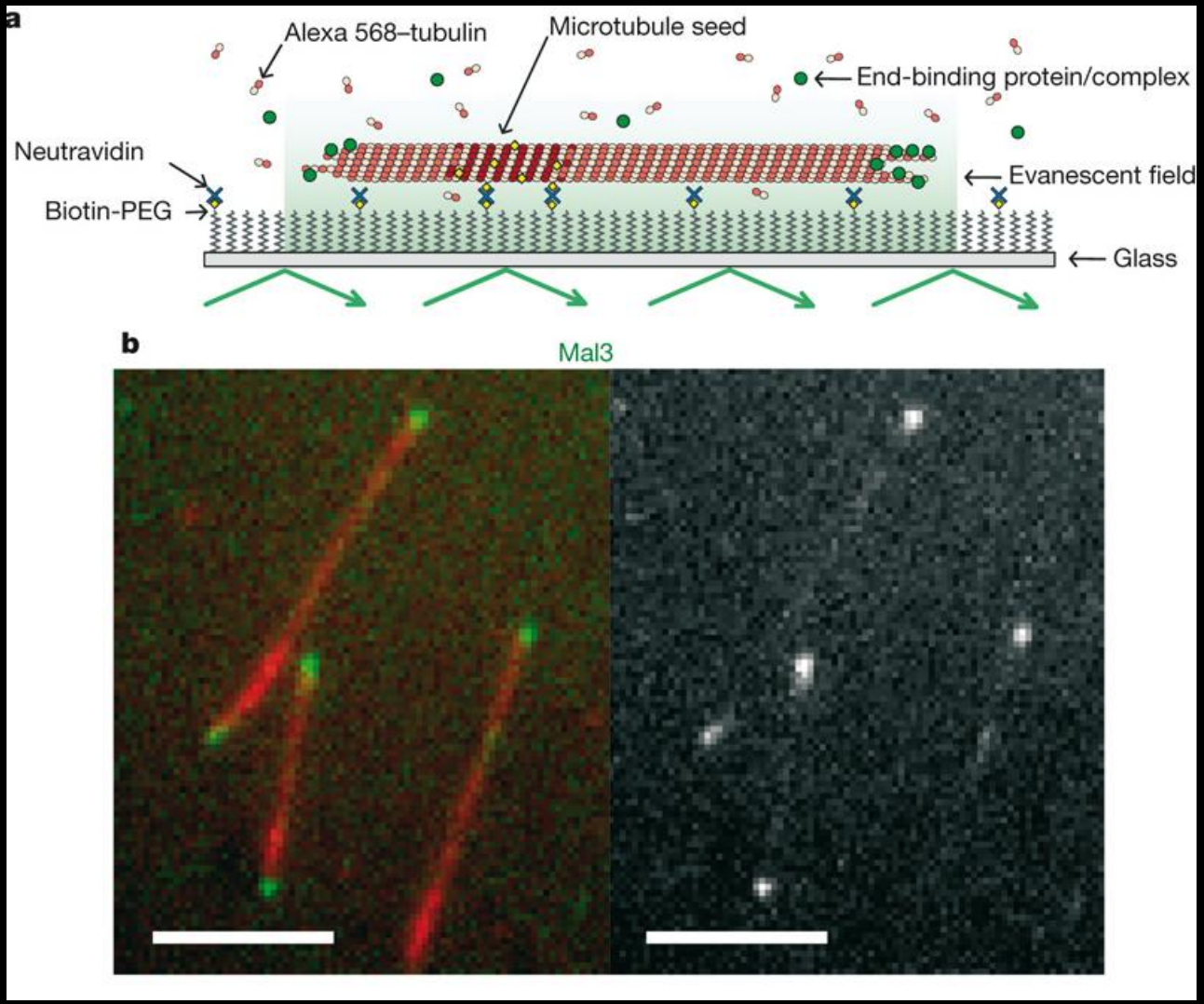
0.5s/frame

EB1 family members: EB1, EB2 (RP1) and EB3 (EBF3)





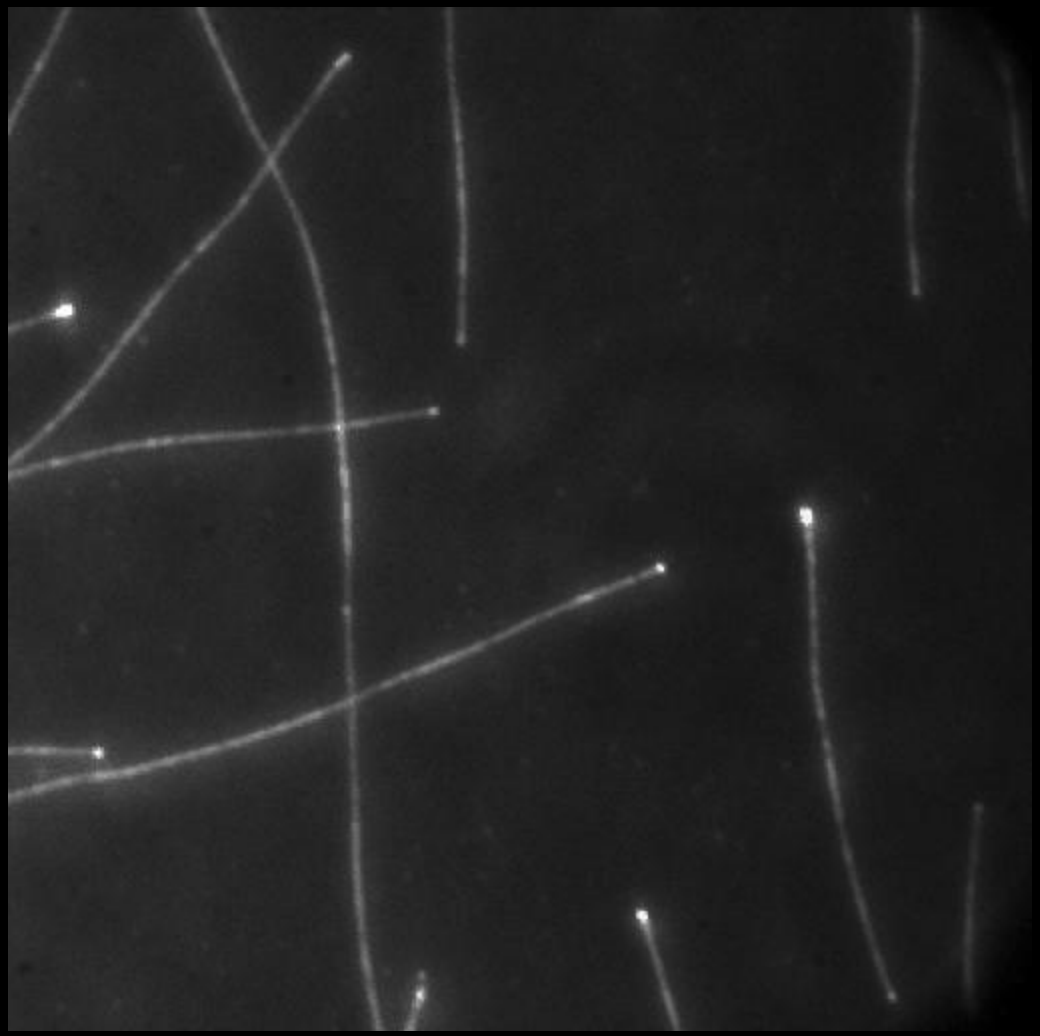
Plus end tracking in vitro



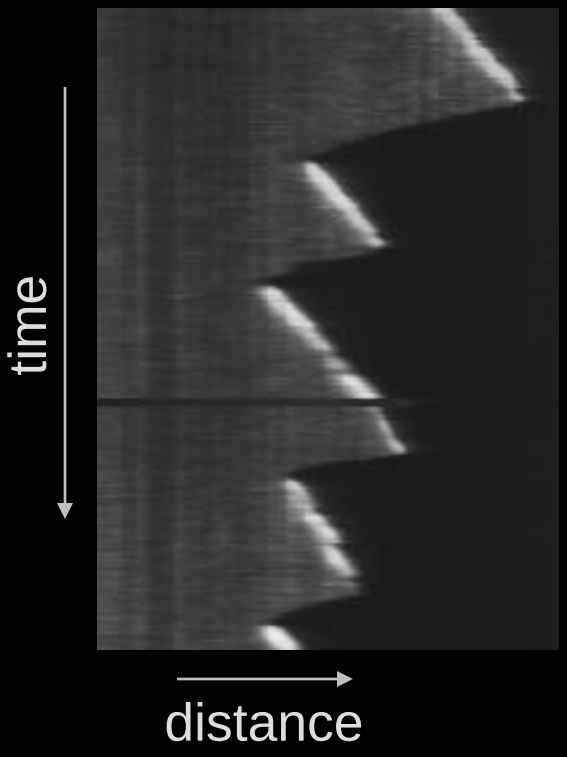
Bieling et al., Nature 2007 (Surrey, Dogterom, Brunner labs)

EB family members autonomously track growing microtubule ends

GFP-EB3

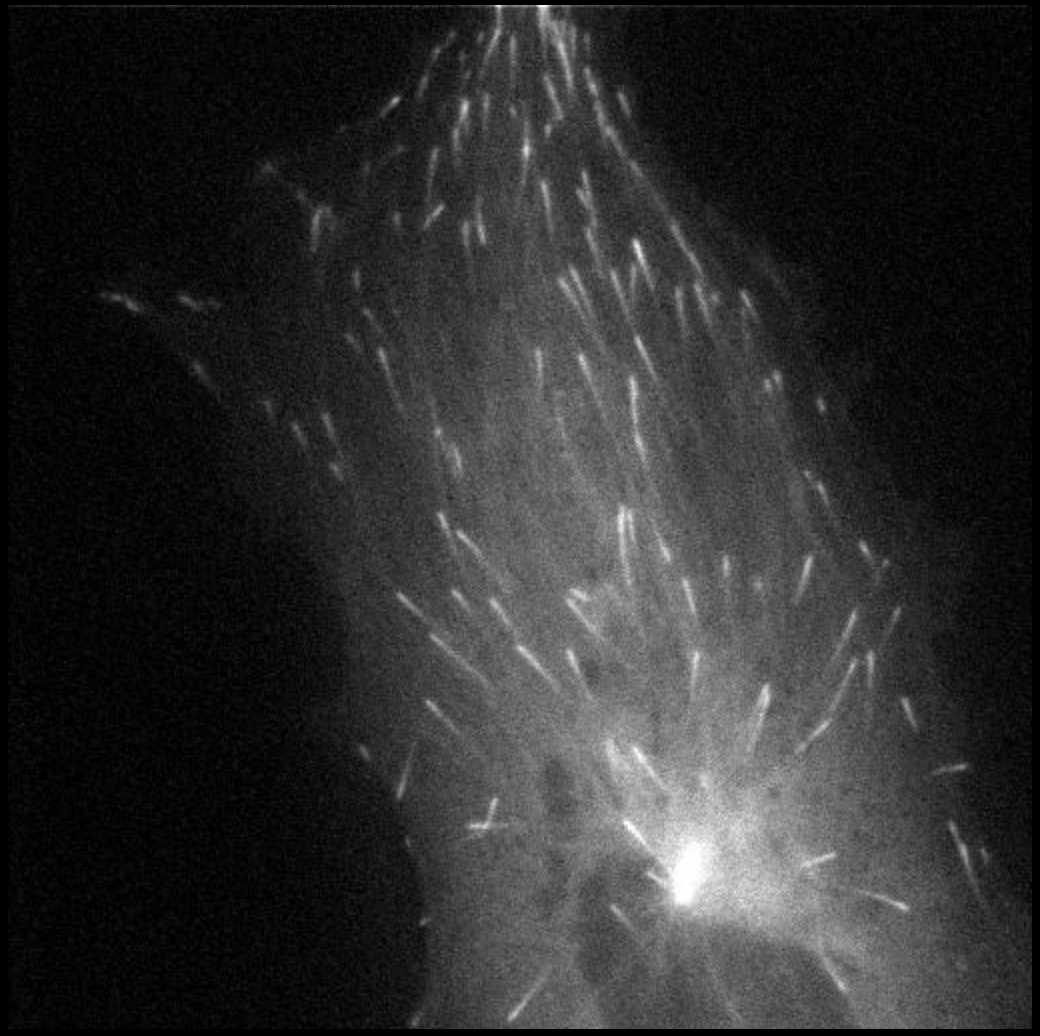


kymograph



Komarova et al., J Cell Biol 2009

Persistent growth of microtubules the role of EB proteins

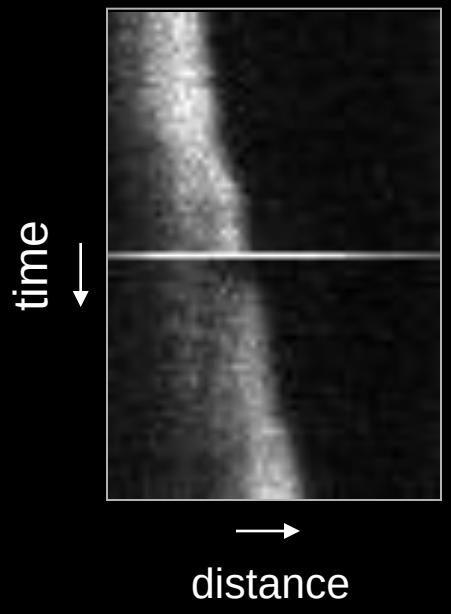


EB3-GFP in a CAR goldfish fibroblast

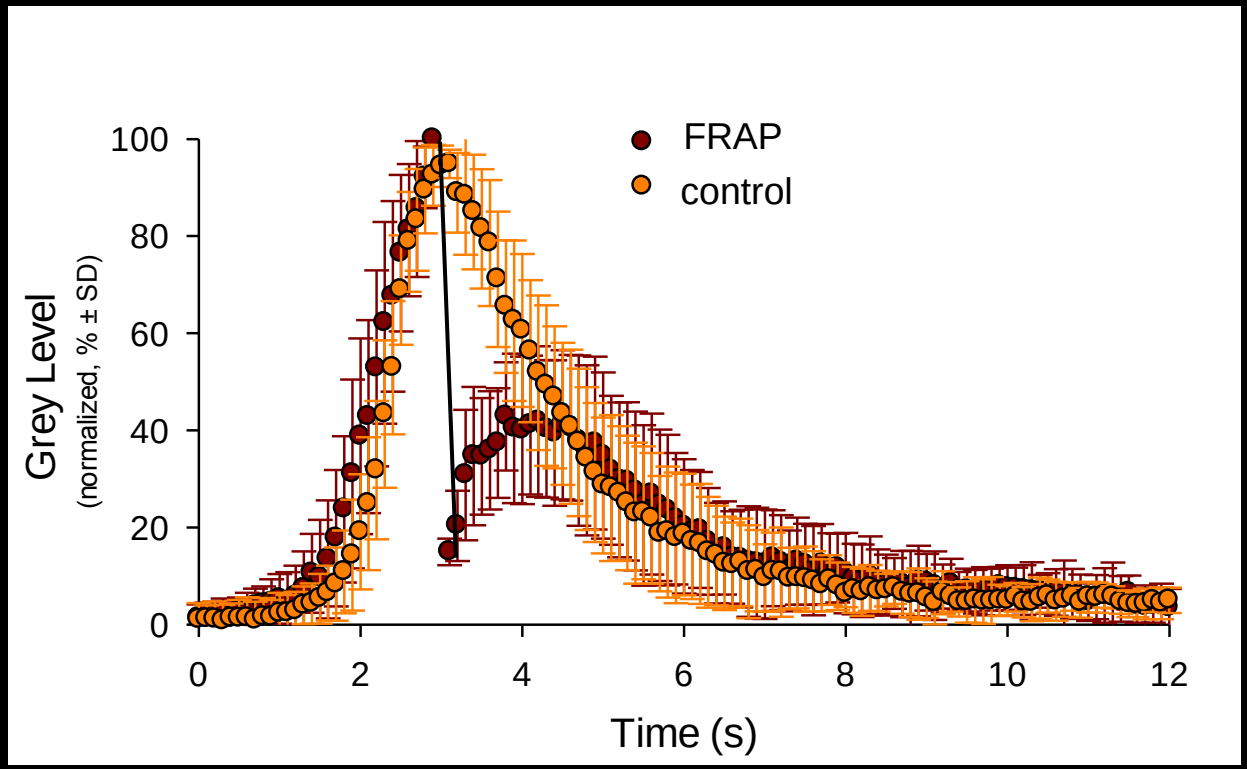
movie: Ilya Grigoriev

FRAP-based analysis of protein exchange on microtubule ends

30 fps



EB3-GFP residence time 0.3-0.4 s

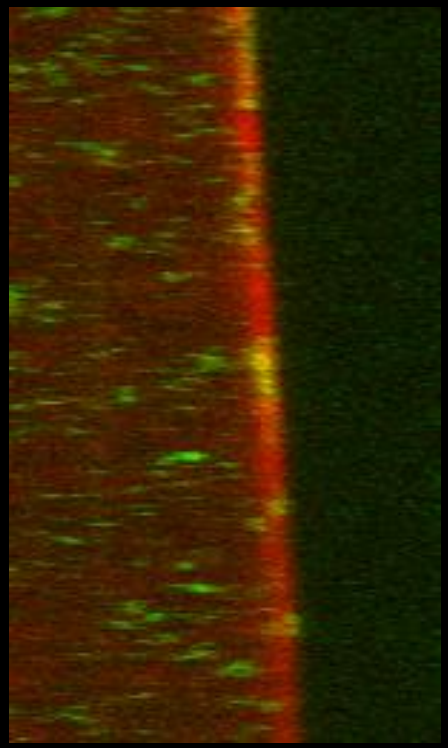


Ilya Grigoriev

Single molecule analysis of EB3 interaction with microtubules

GFP-EB3

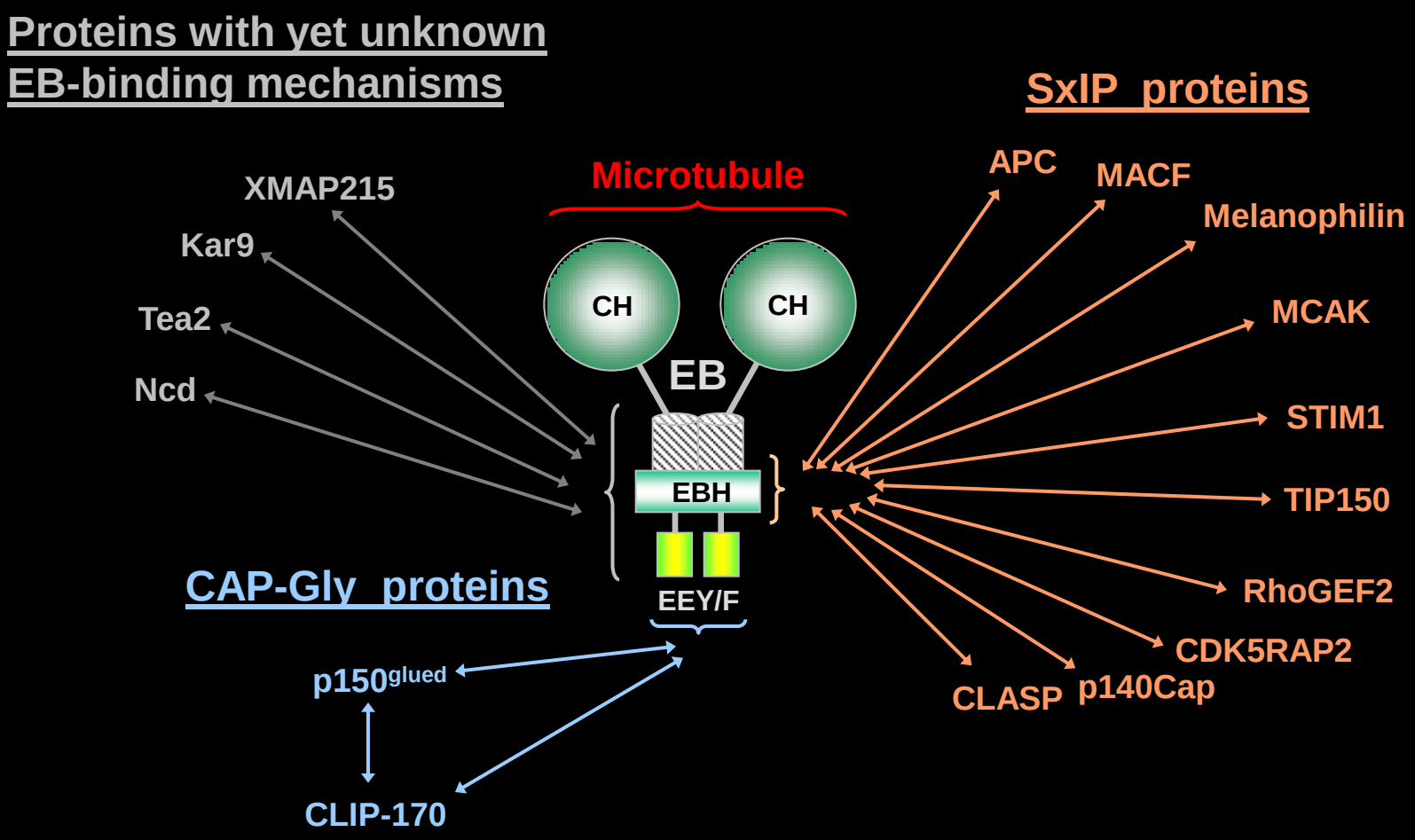
mCherry-EB3



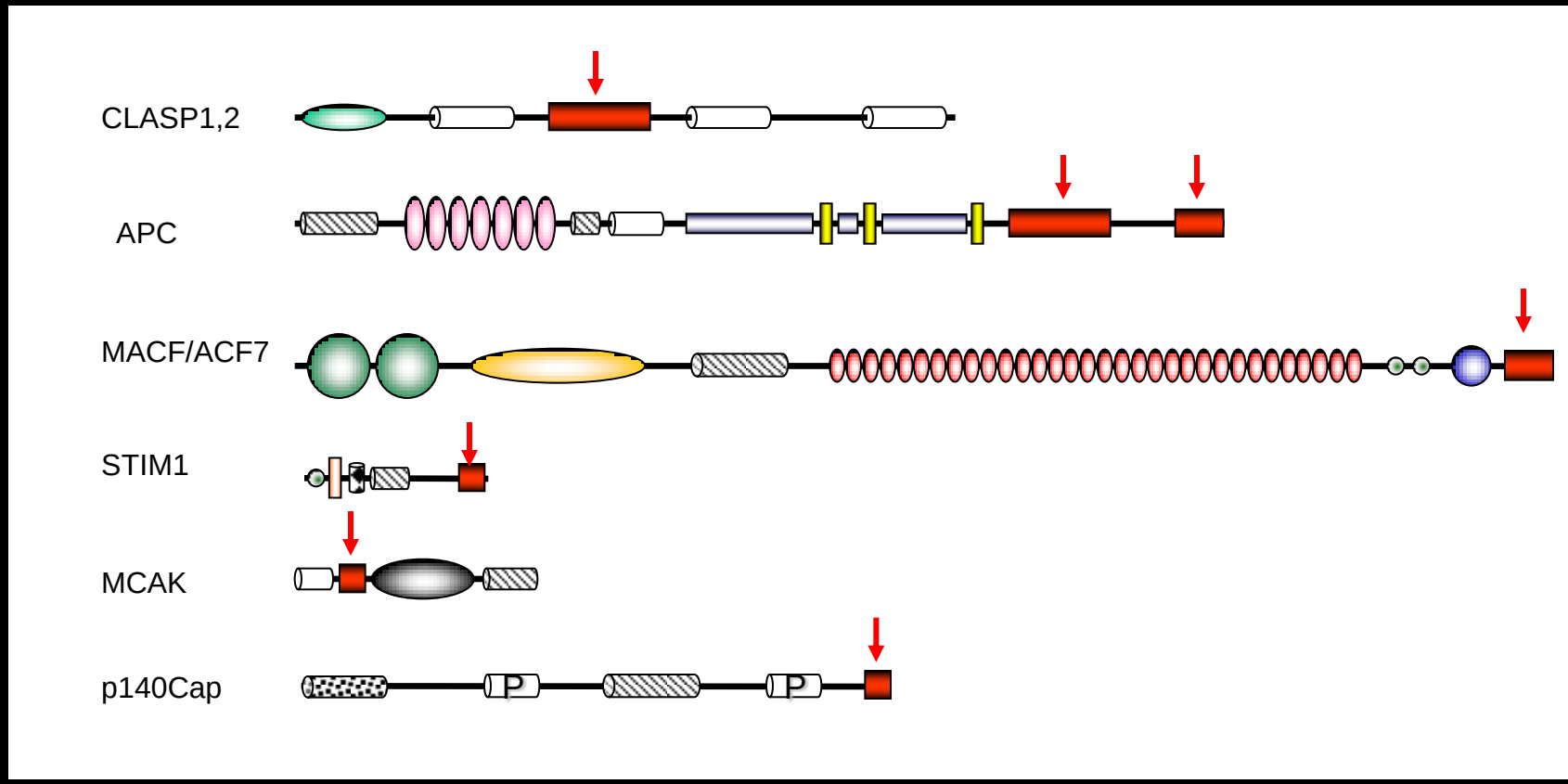
Association Rate (n per 1 µm per 1 nM per 1 s)		Dwell Time (s)	
Lattice	**Tip**	**Lattice**	**Tip**
2.3 ± 0.9	**6.5 ± 0.5**	**0.16 ± 0.03**	**0.34 ± 0.04**

Kris Leslie, Lukas Kapitein

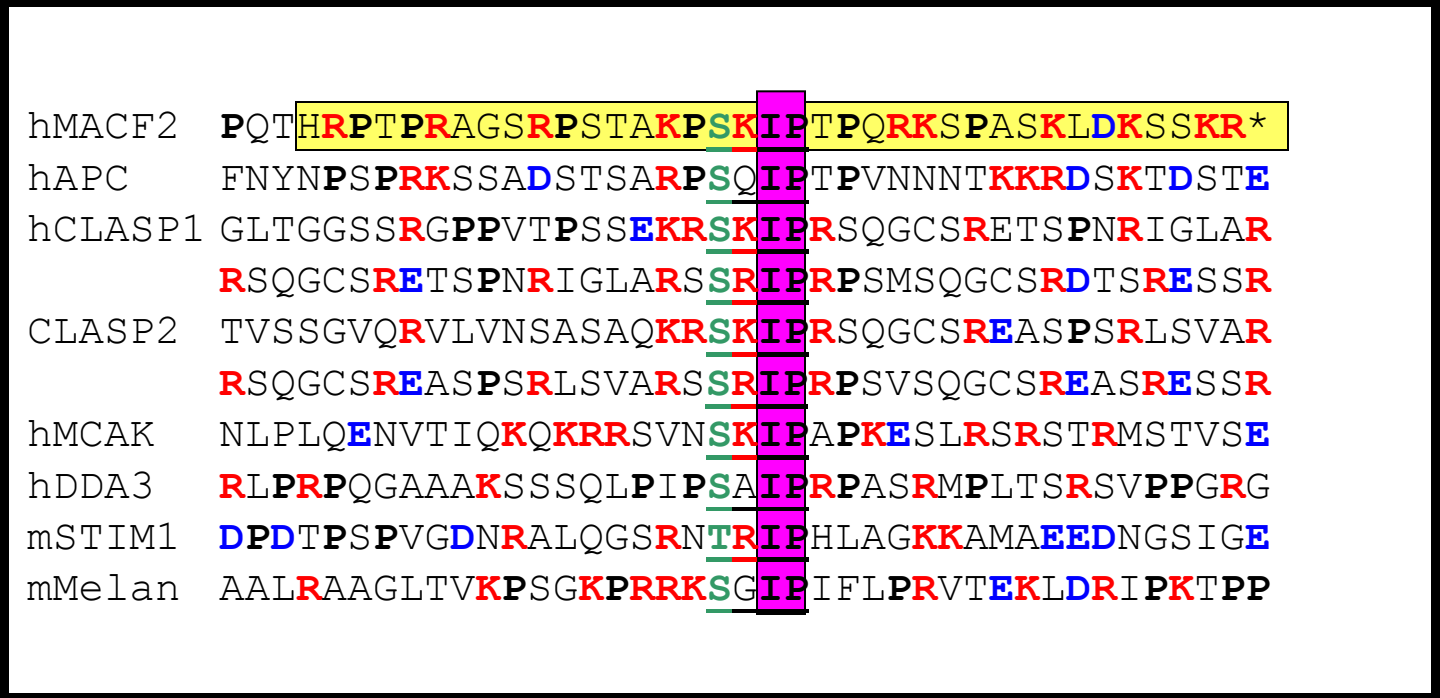
EBs – the “core “ of +TIP network



+TIPs with basic/serine-rich regions



SxIP motif is a common feature of +TIPs with basic/serine rich regions



Honnappa et al.,Cell 2009

Dimerization stimulates EB1-binding and plus end tracking of IP motif peptides

hMACF2-18

GSRPSTAKPSKIPTPQRKSPASK

hMACF2-12

AKPSKIPTPQRK

monomer

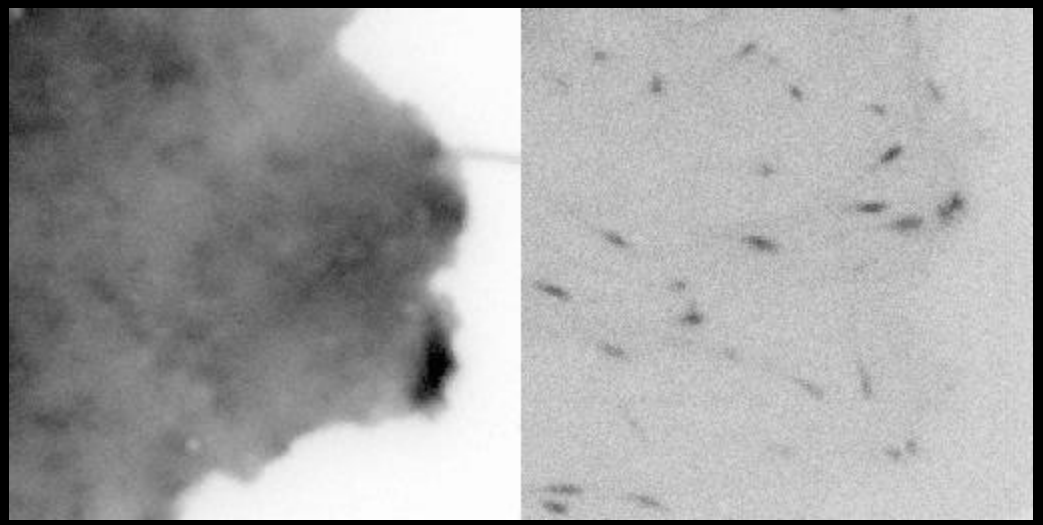
GFP MACF-18

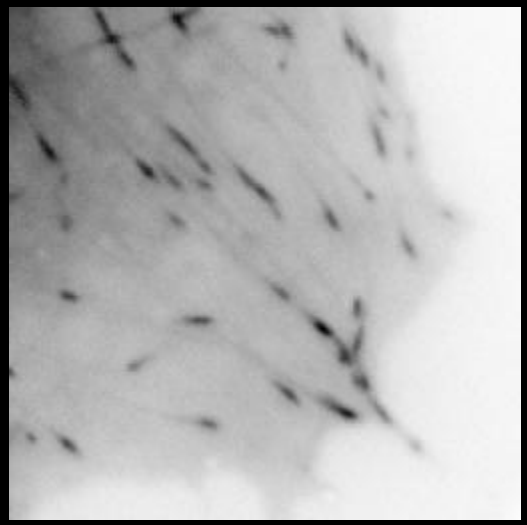
dimer

GFP GCN4 MACF-18

dimer

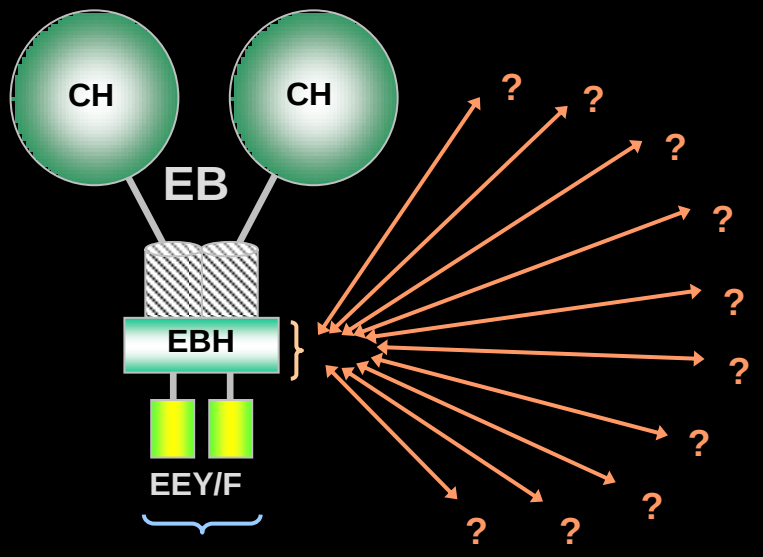
GFP GCN4 MACF-12





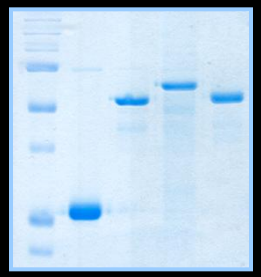
Honnappa et al.,Cell 2009

Searching for new SxIP +TIPs



Bioinformatics approaches

GST-EB pull downs + Mass Spec



Bioinformatics prediction of SxIP motifs

		-5 SxIP-9AA +5	
APC	2796	DSTSARPSQIPTPVNNNTK	2814
APC2	2075	RTTSESPSRLPVRAPAARP	2093
CLASP1	711	TPSSEKRSKIPRSQGCSRE	729
CLASP1	734	RIGLARSSRIPRPSMSQGC	752
CLASP2	487	SASAQKRSKIPRSQGCSRE	505
CLASP2	510	RLSVARSSRIPRPSVSQGC	528
TIP150	916	SVTAPRRSLLPAPKSTSTP	934
Dystonin	7543	RPSTAKPSKIPTPQRKSPA	7561
MACF1	5402	RRGLNKPSKIPTMSKKTTT	5420
MCAK	91	QKRRSVNSKIPAPKESLRS	109
STIM1	635	ALQASRNTRIPHLAGKKAV	653
p140Cap	1031	EKPSASRTSIPVLTSFGAR	1049
FGFR1OP	165	TSAQTTPSKIPRYKGQGKK	183
Melanophilin	503	SGKPRRKSNLPIFLPRVAG	521
DDA3	274	SSQLPIPSAIPRPASRMPL	292
CDK5RAP2	929	TNREAKKSRLPILIKPSRS	947
		x_1x_2 x_3 $x_4x_5x_6$	

x_1-x_2-S/T-x_3-I/L-P-x_4-x_5-x_6 9AA
x_1-x_4: at least one of them is R or K
x_1-x_6: none of them are D or E

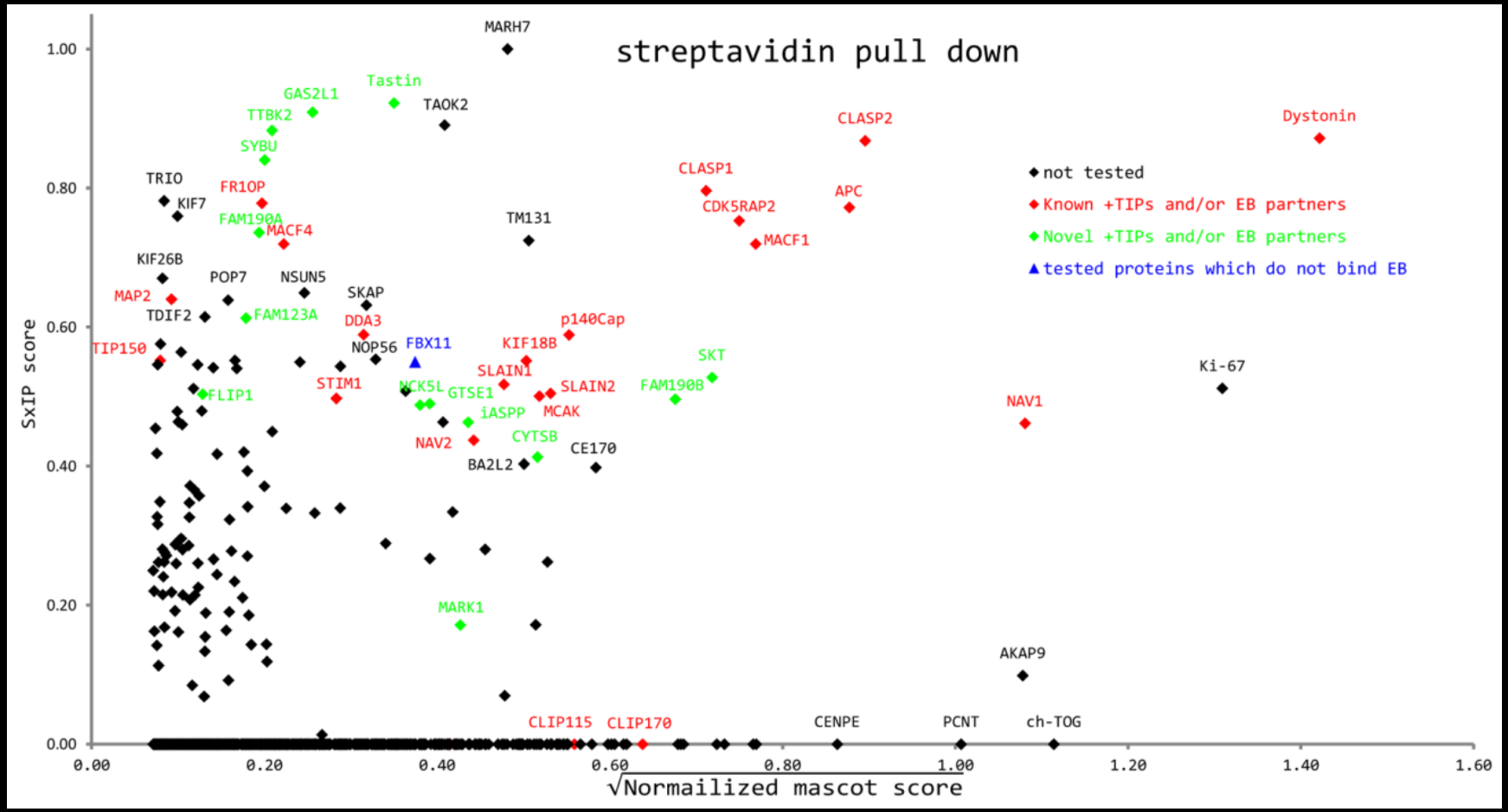
Folding and Conservation →

833 proteins
(~4%)

Jiang et al., Curr Biol 2012

Grischa Toedt, Toby Gibson

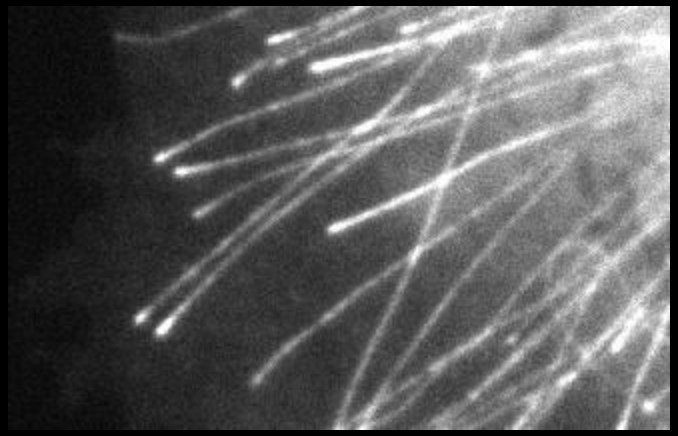
Comparison between EB pull down and bioinformatics prediction



Jiang et al., Curr Biol 2012

Back-tracking +TIPs:

DDA3 (proline/serine-rich coiled-coil 1)



Regulated by p53/p73

(Lo et al., Oncogene 1999; (Hsieh et al., Oncogene 2002)

Binds to EB3

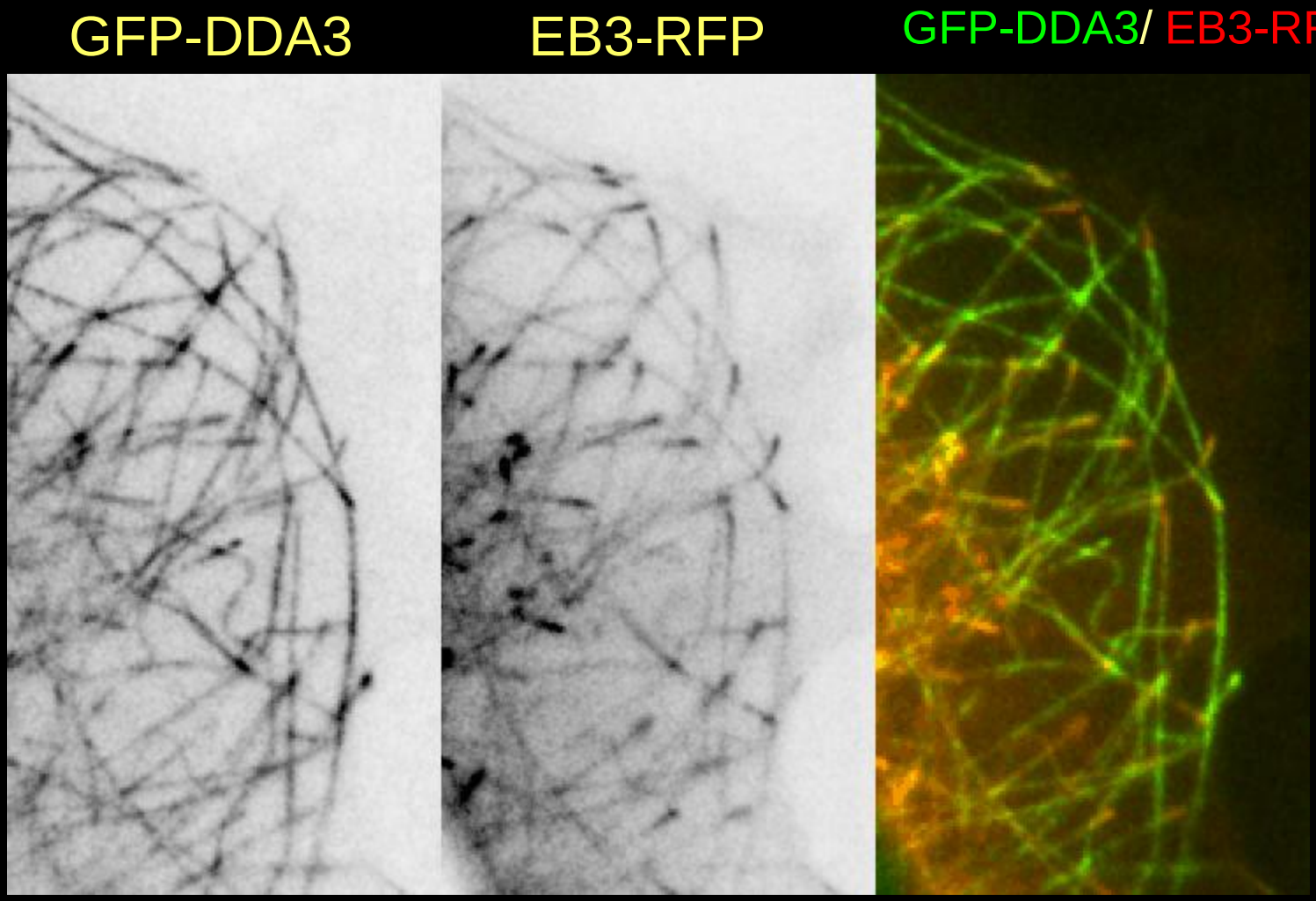
(Hsieh et al., Oncogene 2007)

Targets kinesin-13 KIF2A (MT depolymerase) to the spindle

(Jang et al., JCB 2008)

Jiang et al., Curr Biol 2012

Back-tracking +TIPs: DDA3



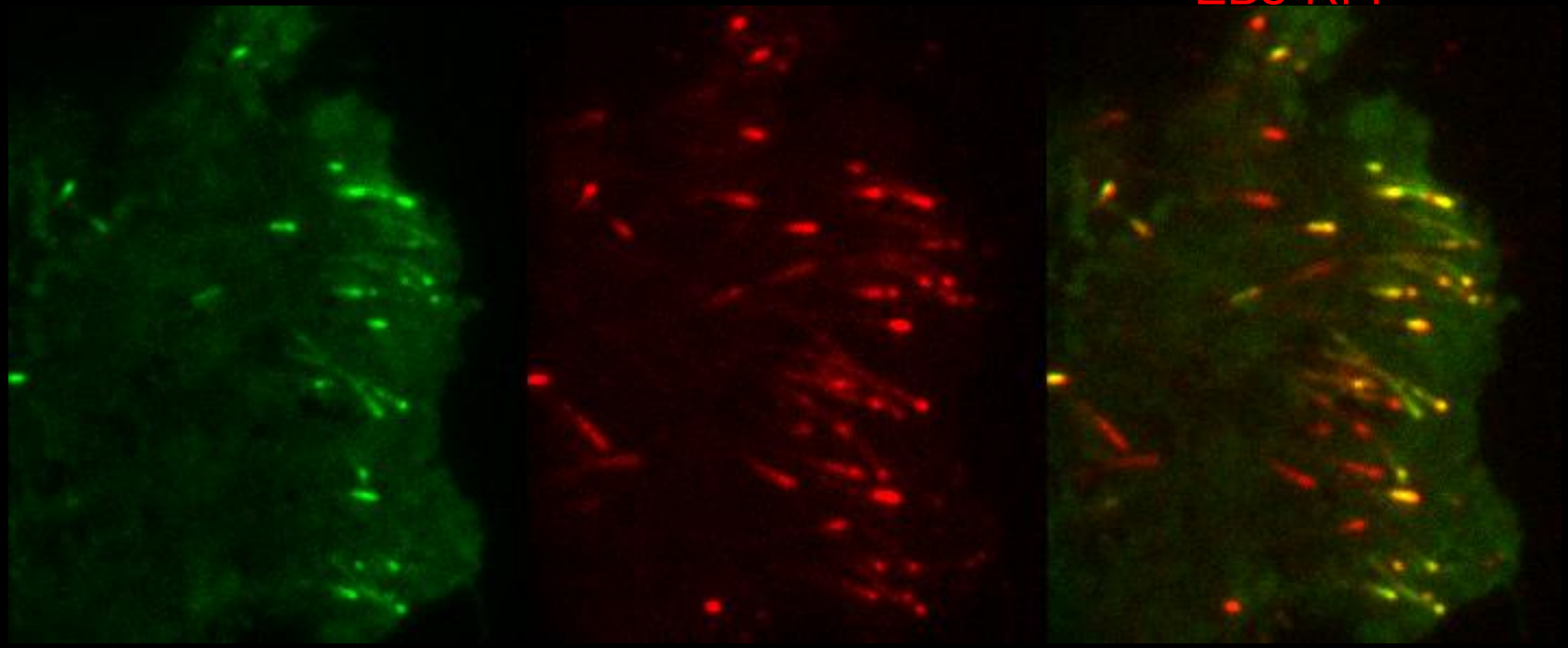
Jiang et al., Curr Biol 2012

Plasma membrane binding: FAM123A/AMER2 (homologue of WTX)

GFP-FAM123A

EB3-RFP

GFP- FAM123A / EB3-RFP

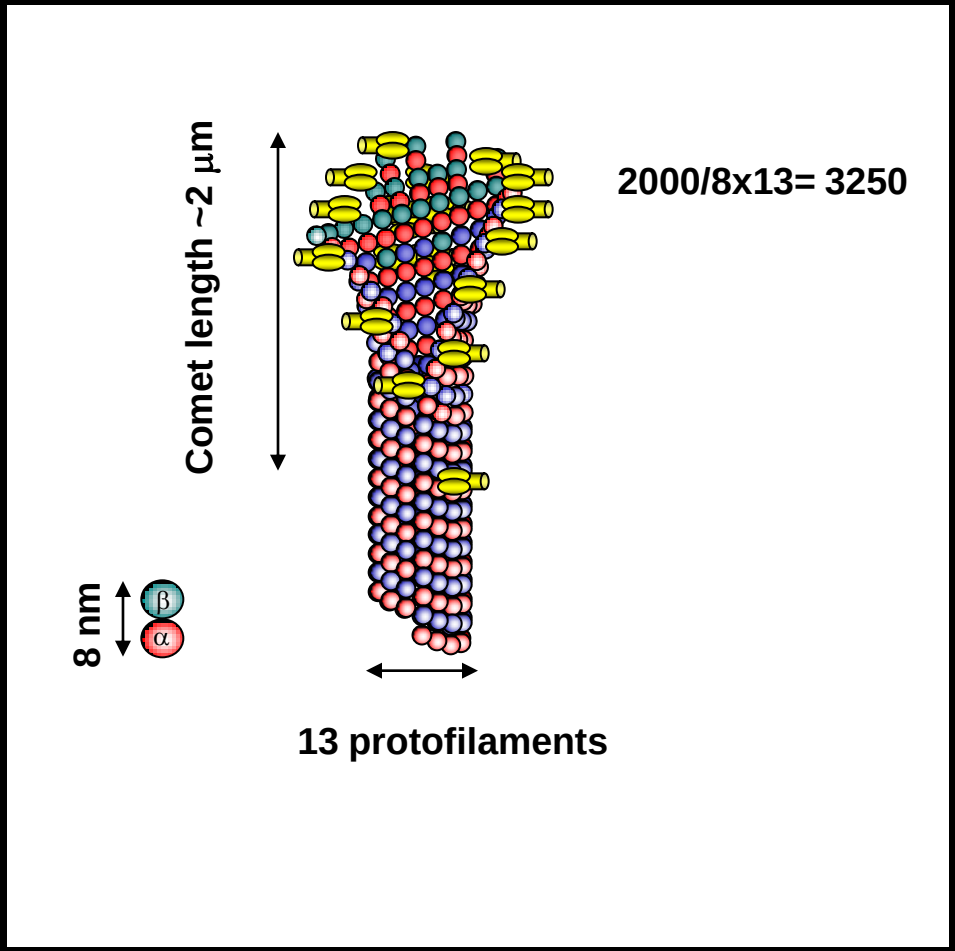


WTX=AMER1 (Wilms Tumor gene on the X chromosome)
Regulates Wnt signaling, binds to APC,
Binds to PIP2 on the plasma membrane

Jiang et al., Curr Biol 2012

How many different proteins can bind to one microtubule tip at the same time?

Growing Microtubule Tip



Are all growing microtubule tips

in the same cell the same?

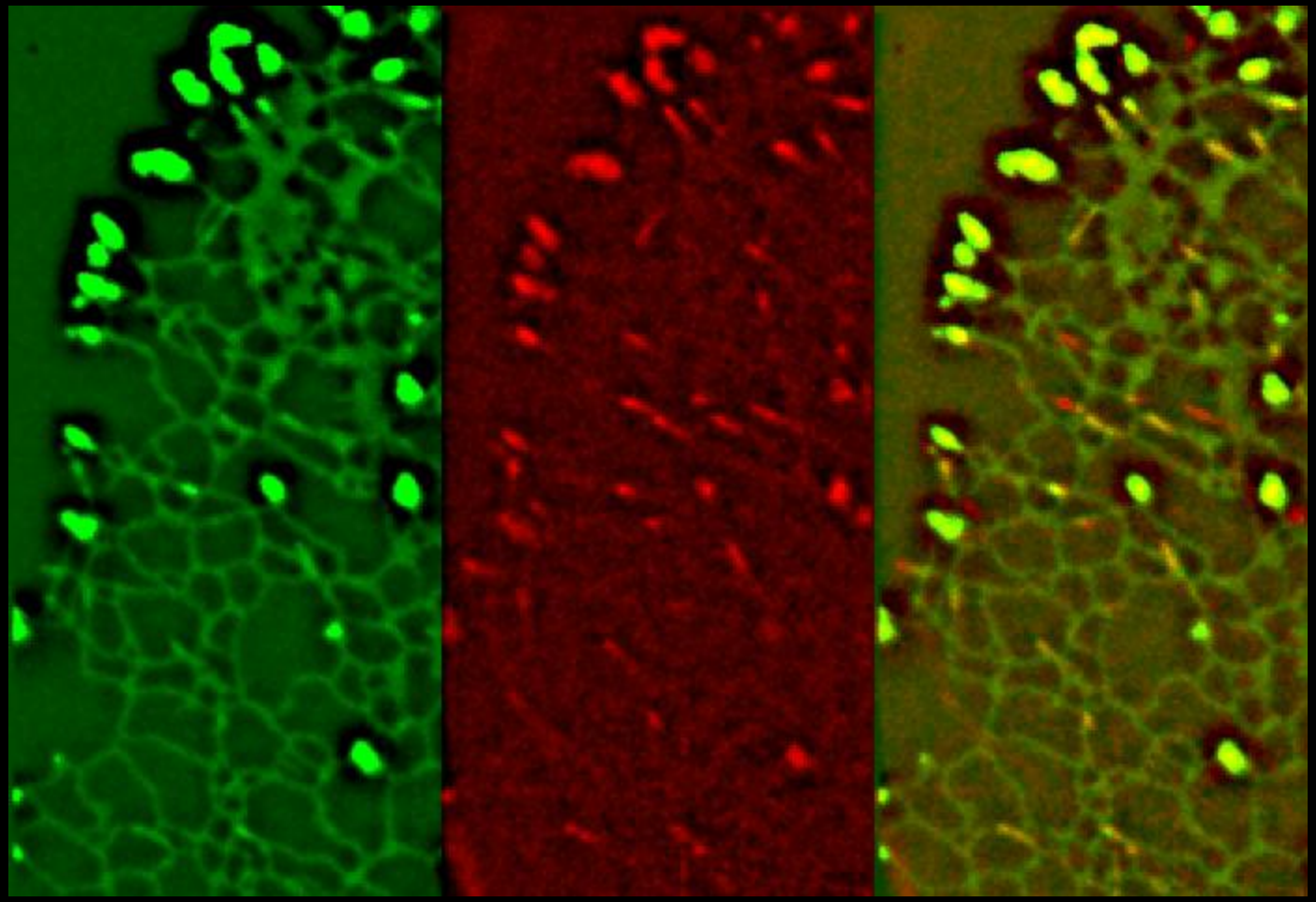
Stromal Interaction Molecule: STIM

- STIM1 is a transmembrane protein, which localizes predominantly to the ER

- STIM 1 is as a sensor of ER luminal Ca^{2+} and is essential for store-operated Ca^{2+} entry

- Upon Ca^{2+} release from the ER, STIM1 locally activates Ca^{2+} channels in the plasma membrane

GFP-STIM1 colocalizes with plus end marker EB3



GFP-STIM1 and EB3-mRFP
in a MRC5 human lung fibroblast

1 s/ frame

Grigoriev et al.,Curr. Biol 2008

Tip Attachment Complex-mediated ER protrusion

GFP-STIM1 and mCherry-tubulin

in a MRC5 human lung fibroblast

1 s/frame

Grigoriev et al.,Curr. Biol 2008

What do +TIPs do?

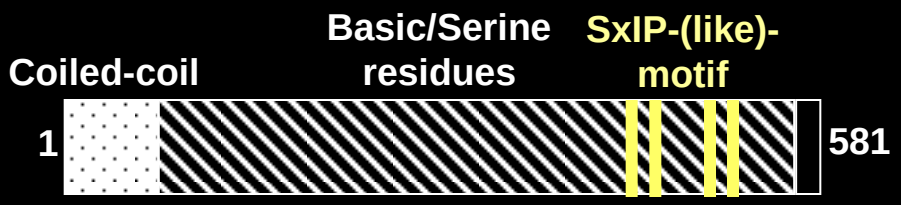
Regulation of microtubule dynamics

SLAINs

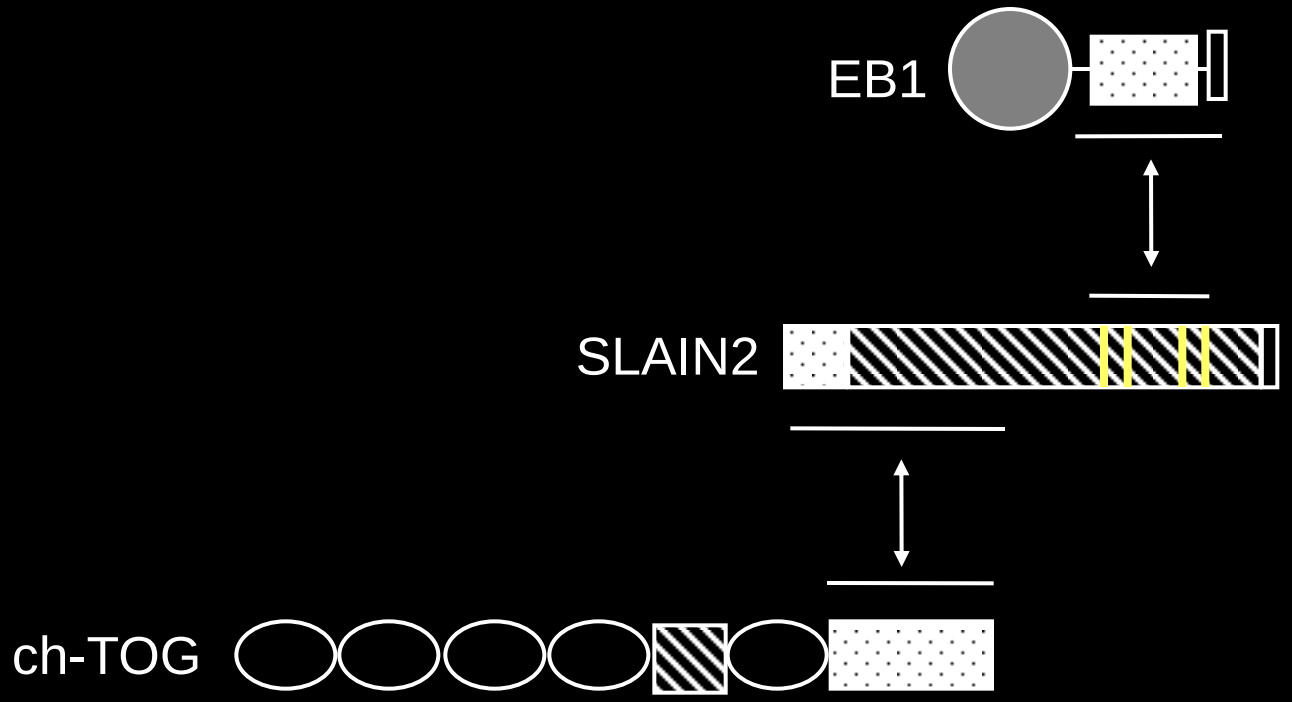
- SLAIN1 and SLAIN2 are present in all vertebrates
- SLAIN1 was identified as a novel embryonic stem cell gene
  Hirst, CE et al., Dev Biol. 2006 May 1;293(1):90-103
- SLAINs were named after amino acid stretch in SLAIN1:

```
HsSL1 528-ALPRPSLAINGSN
HsSL2 466-GLPRPSAPSAGGI
```

- Protein structure:

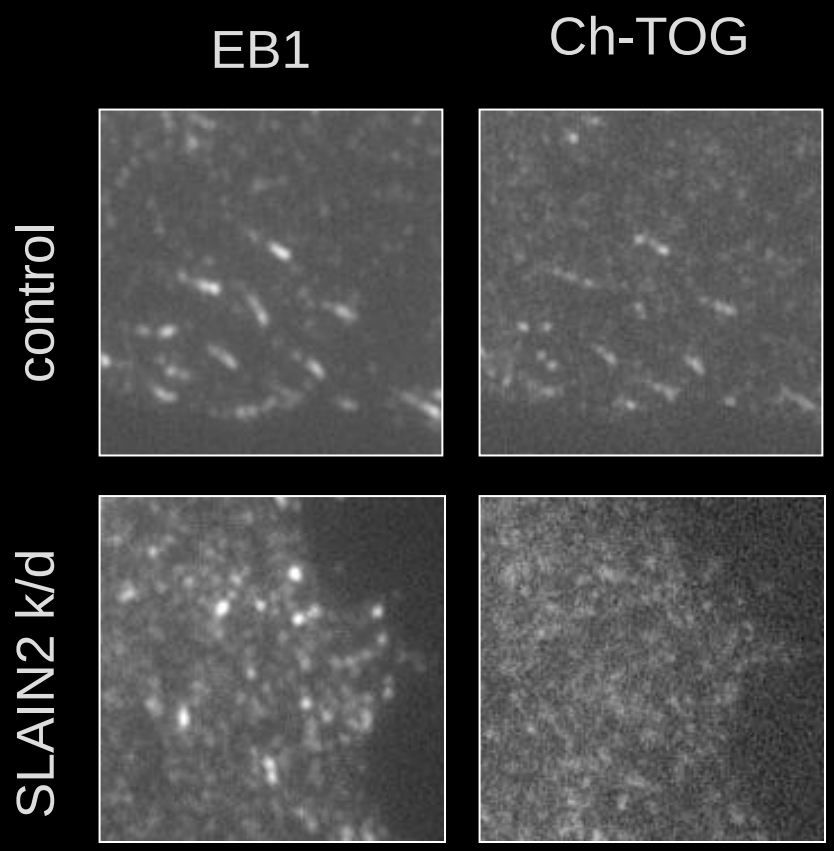


SLAINs link EB1 to the microtubule polymerase ch-TOG



van der Vaart et al, JCB 2011

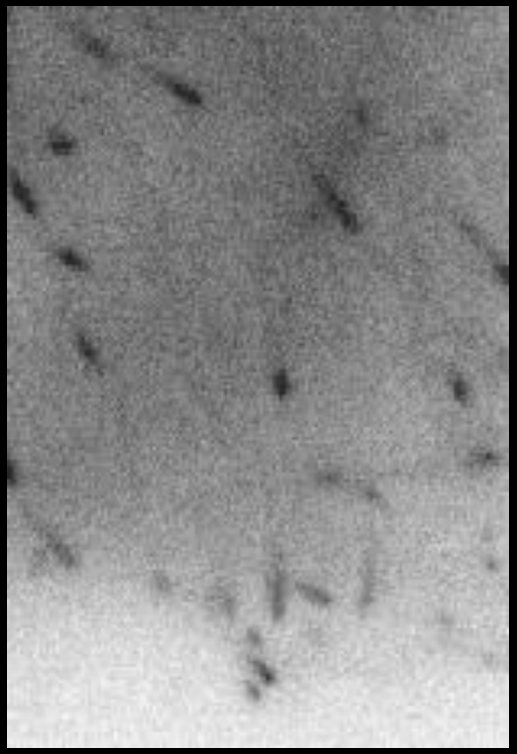
SLAIN2 depletion causes loss of ch-TOG from microtubule tips



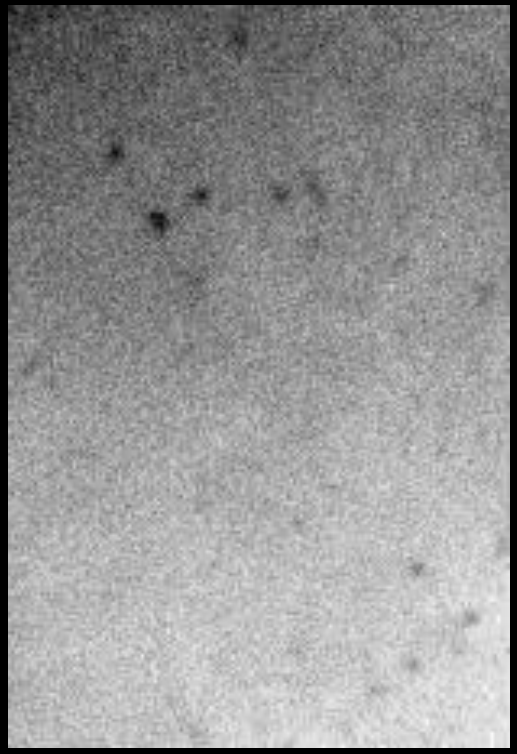
van der Vaart et al, JCB 2011

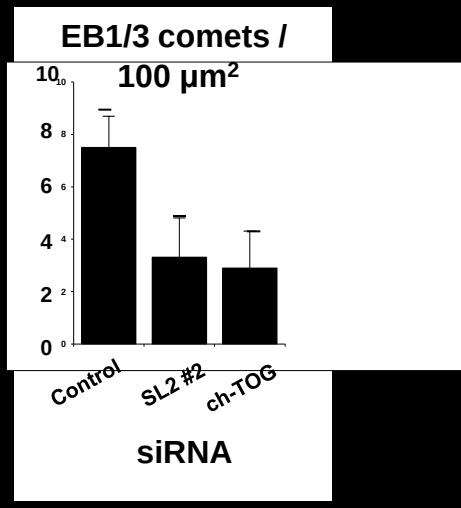
SLAIN2 knockdown in HeLa cells

Control siRNA



SLAIN2 siRNA

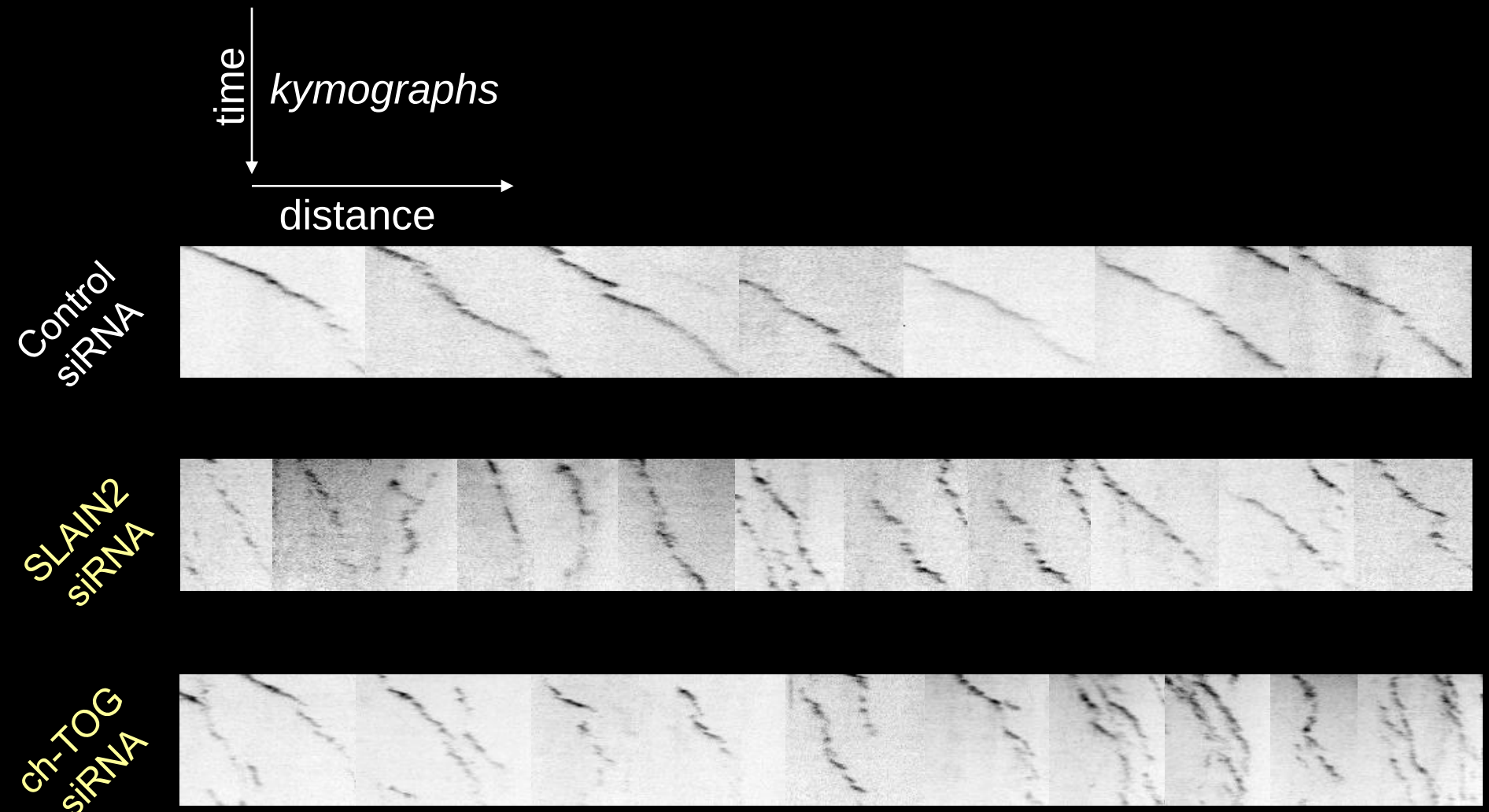




Stable EB3-GFP HeLa cell line

van der Vaart et al, JCB 2011

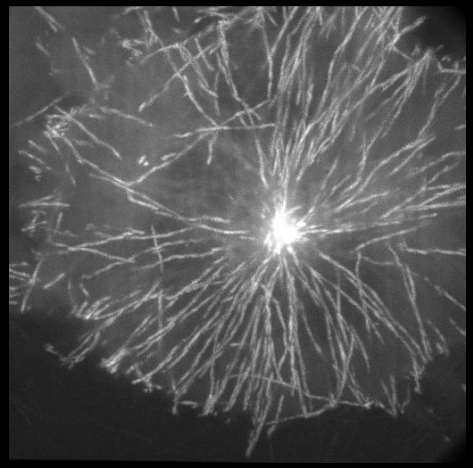
Knockdown of SLAIN2 and ch-TOG gives a similar MT dynamics phenotype



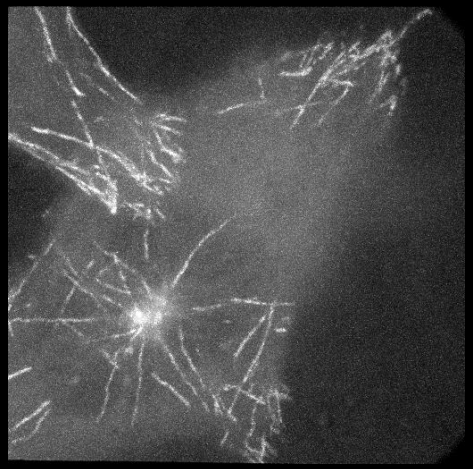
van der Vaart et al, JCB 2011

SLAIN2 and ch-TOG depletion increases catastrophe frequency in CHO cells

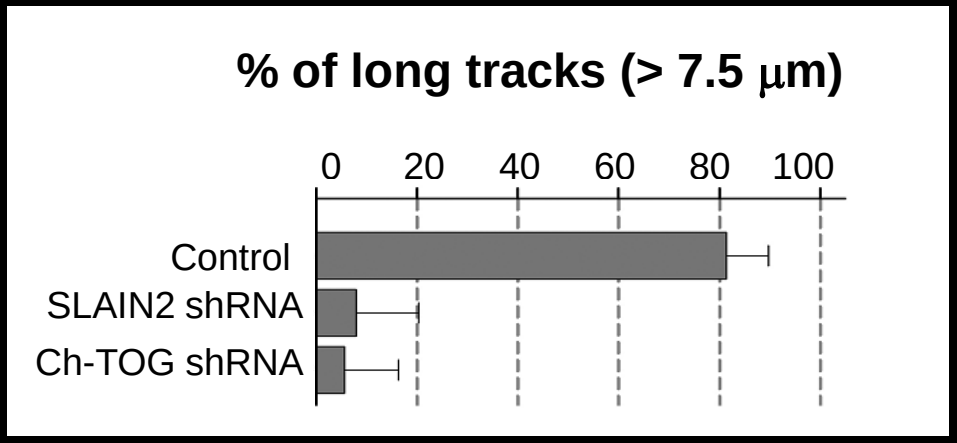
Control shRNA



SLAIN2 shRNA



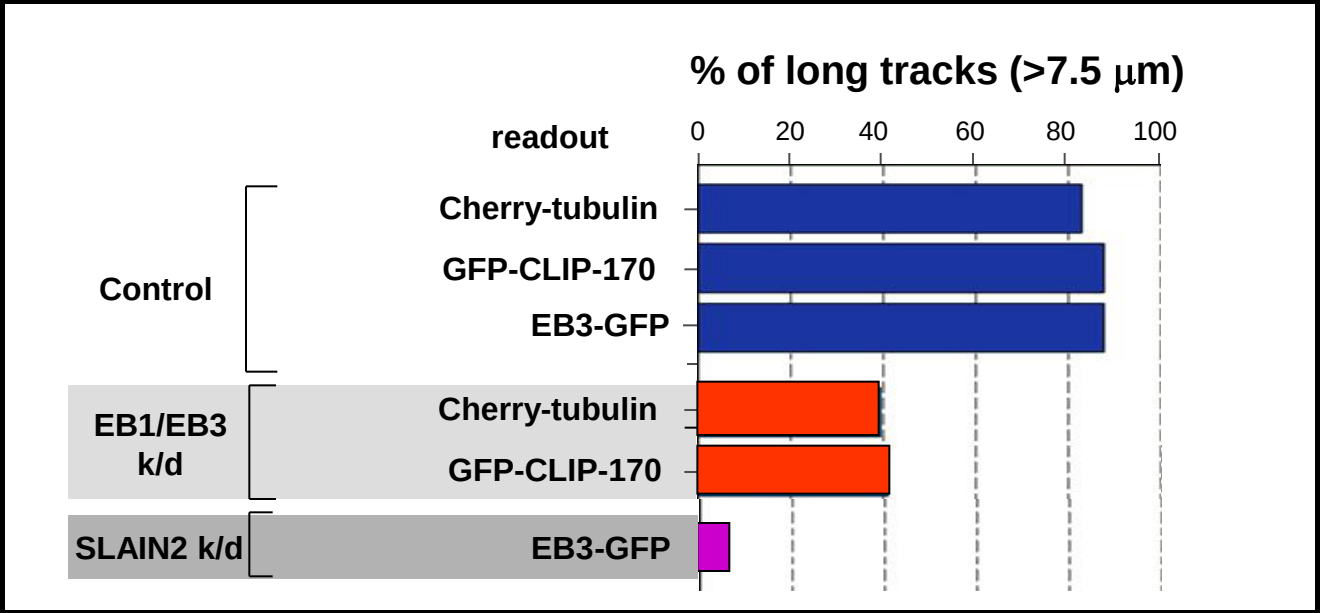
EB3-GFP,
Projection analysis



van der Vaart et al, JCB 2011

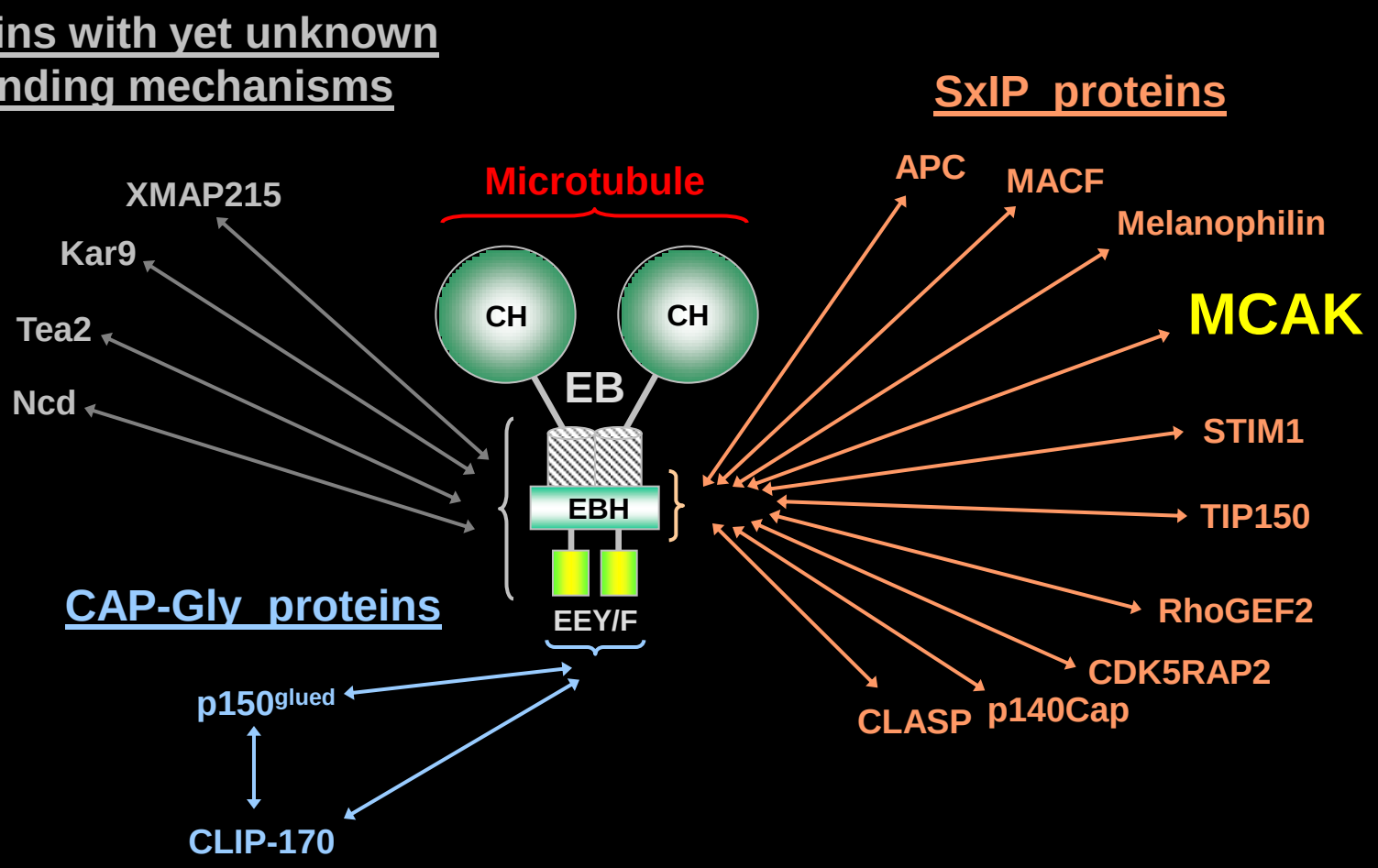
SLAINs are part of a complex protein network that promotes microtubule growth

EB1+EB3 depletion increases catastrophe frequency, but not as dramatically as SLAIN depletion

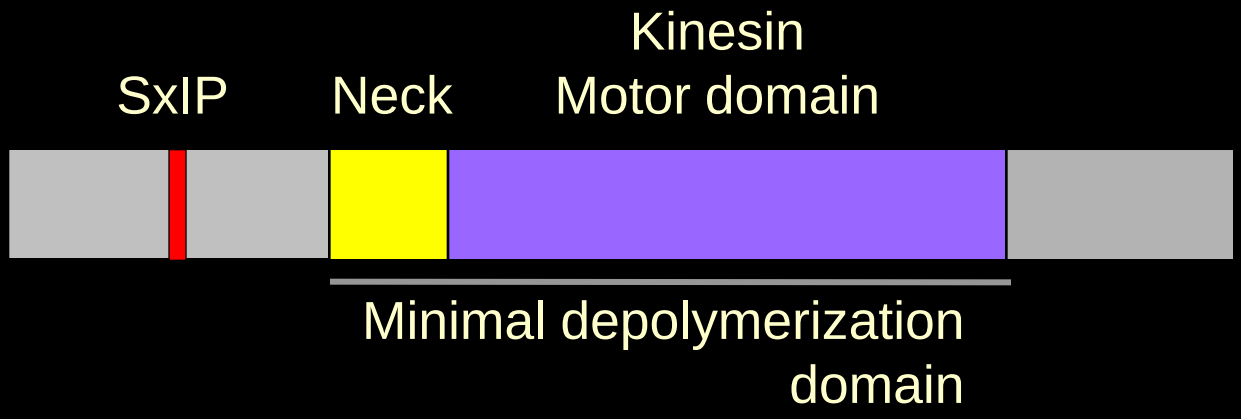


Ilya Grigoriev

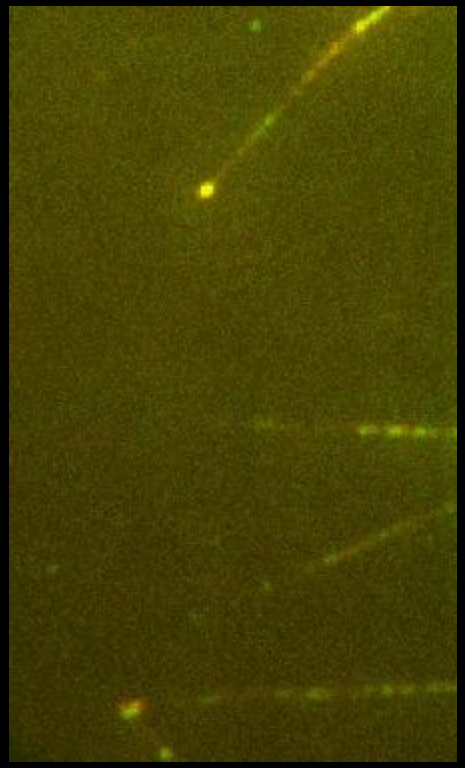
EBs bind not only to MT stabilizers but also to destabilizers



MCAK is a microtubule depolymerase

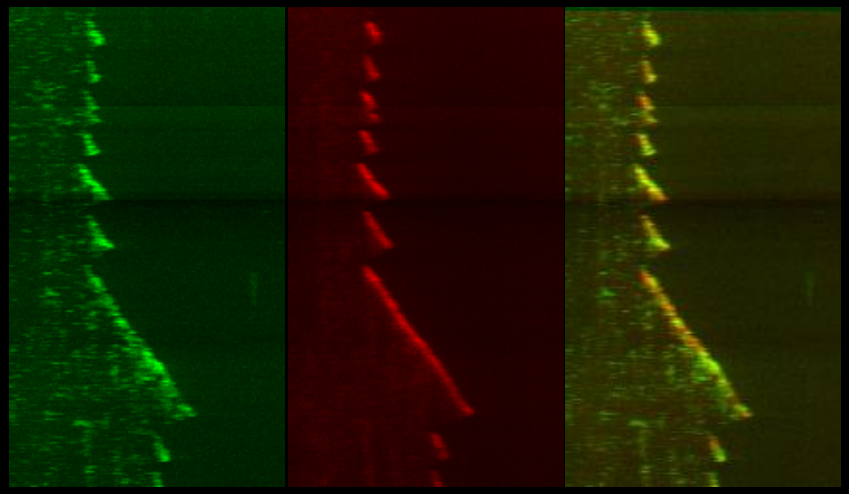


GFP-MCAK + mCherry-EB3 (+ATP)



GFP-MCAK 6 nM

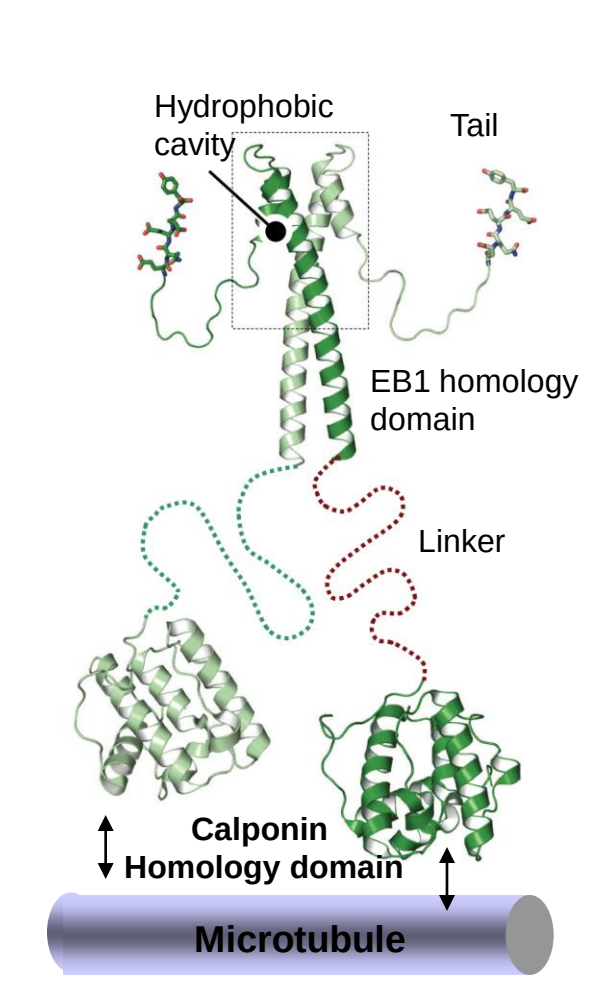
mCherry-EB3 300 nM



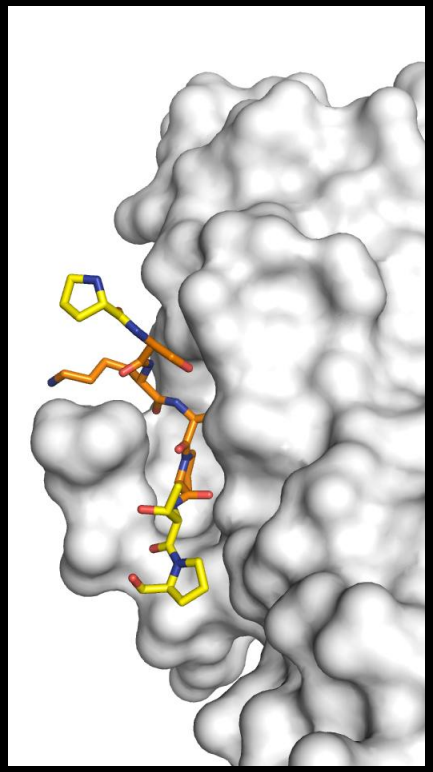
Montenegro Gouveia et al., Curr Biol 2010

GFP-MCAK is a gift of L.Wordeman

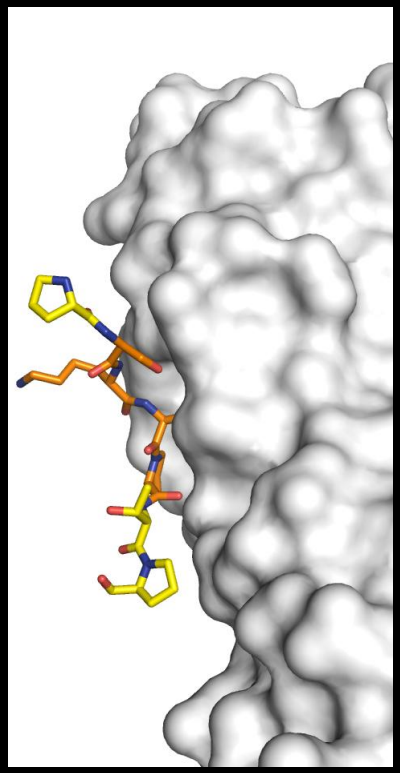
The proximal part of EB1 tail participates in binding to SxIP peptides



EB1c
-SxIP peptide



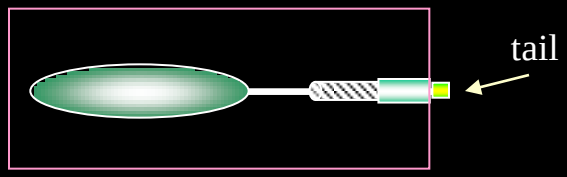
EB1c-Δ tail
-SxIP peptide



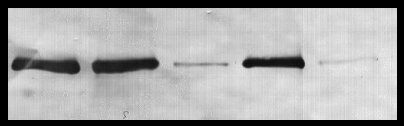
Michel Steinmetz

EB3 lacking the tail does not bind to MCAK and does not target it to the tips

EB-Δtail

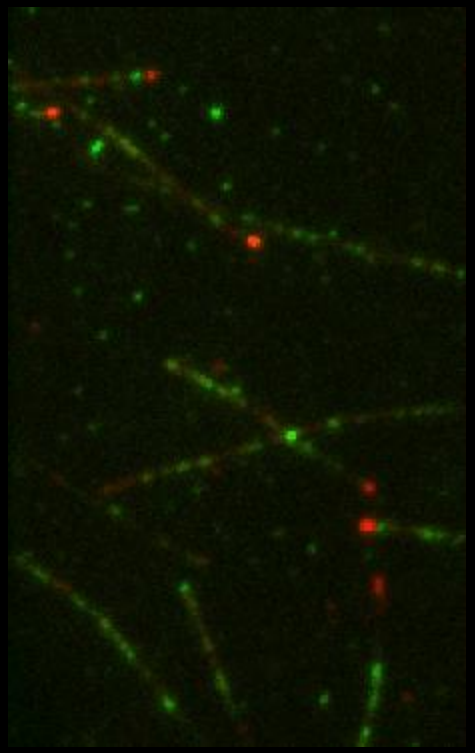


GST pull down



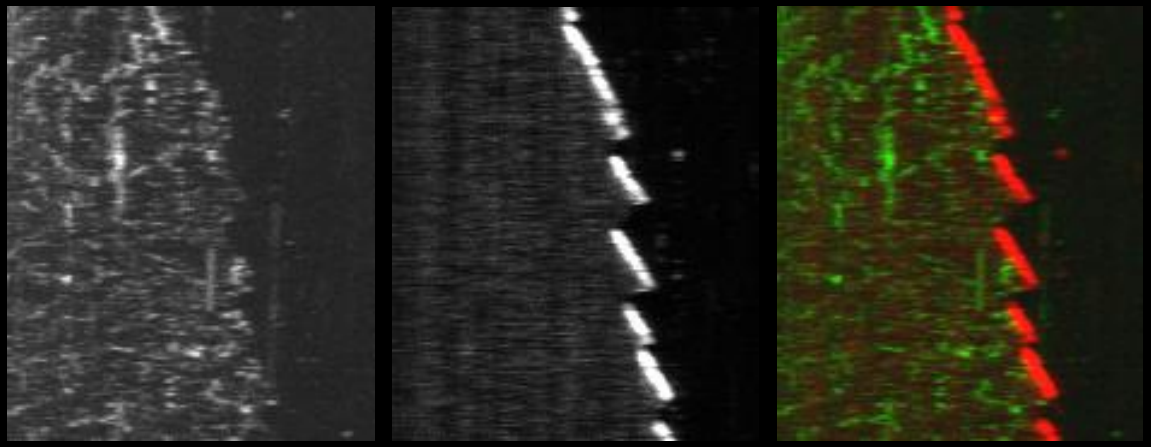
GFP-MCAK
mCherry-EB3 Δtail

ATP



GFP-MCAK
12 nM

mCherry-EB3
Δtail (75 nM)



Montenegro Gouveia et al., Curr Biol 2010

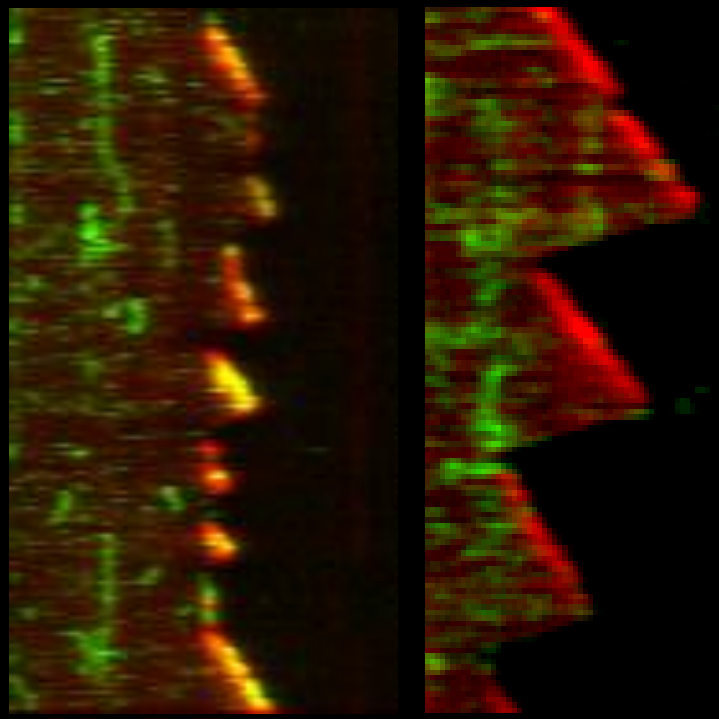
Targeting to microtubule tips by EB3 promotes MCAK-mediated catastrophe induction

GFP-MCAK
6 nM

mCherry-EB3
75nM

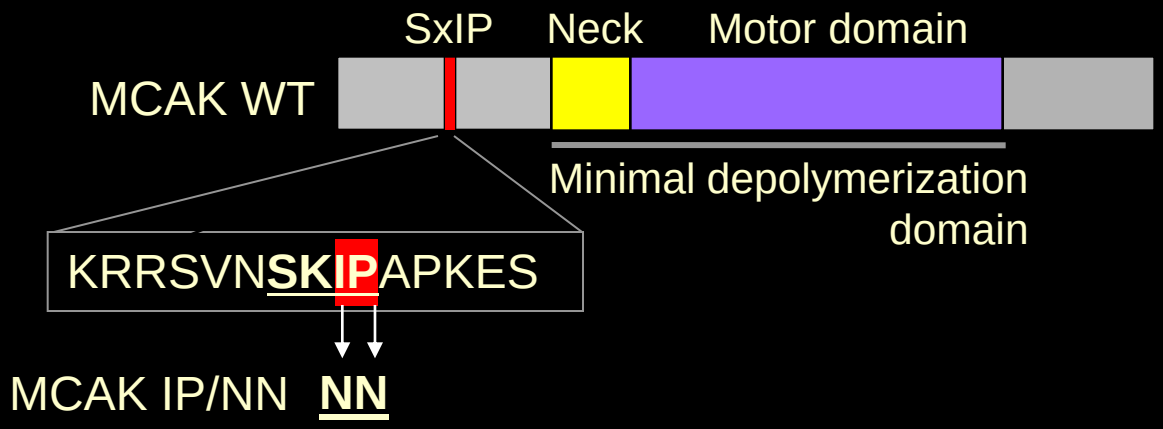
GFP-MCAK
24 nM

mCherry-EB3ΔT
75 nM

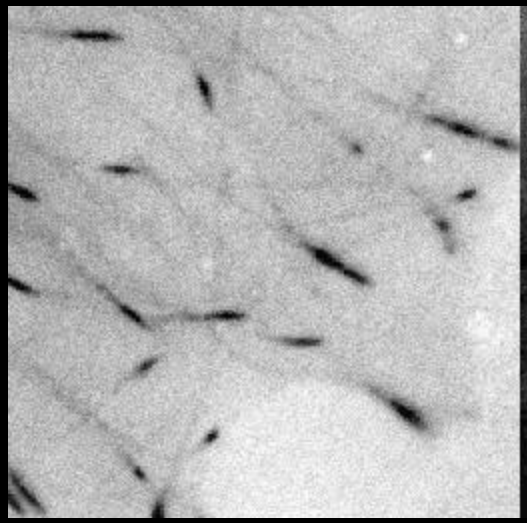


Montenegro Gouveia et al., Curr Biol 2010

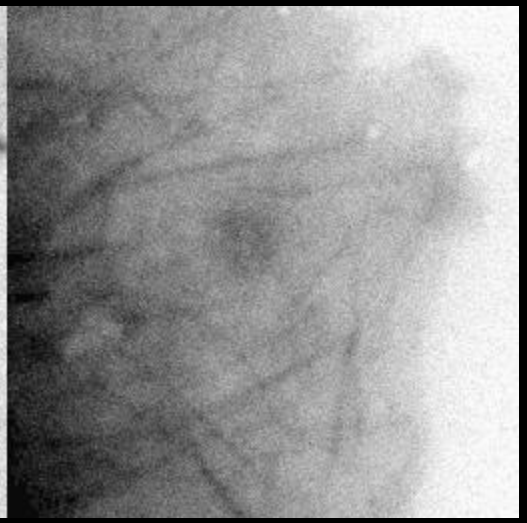
IP->NN mutation in MCAK abolishes plus end tracking in cells

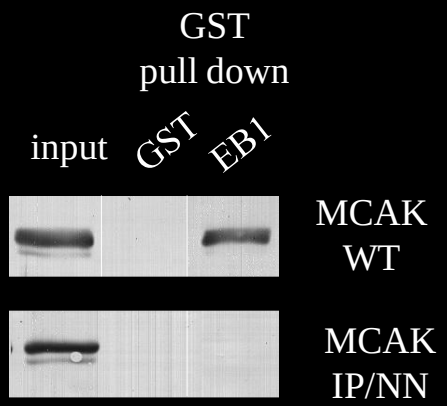


MCAK WT



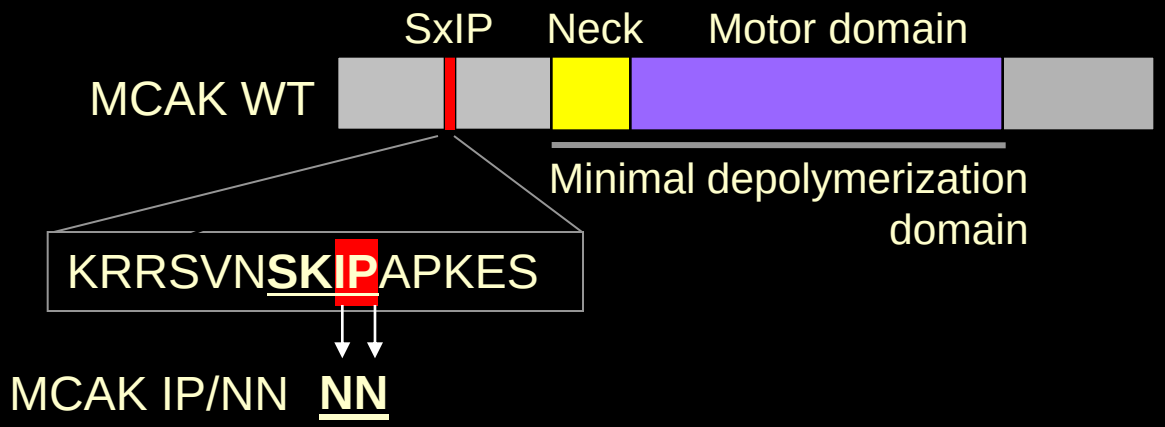
MCAK IP/NN

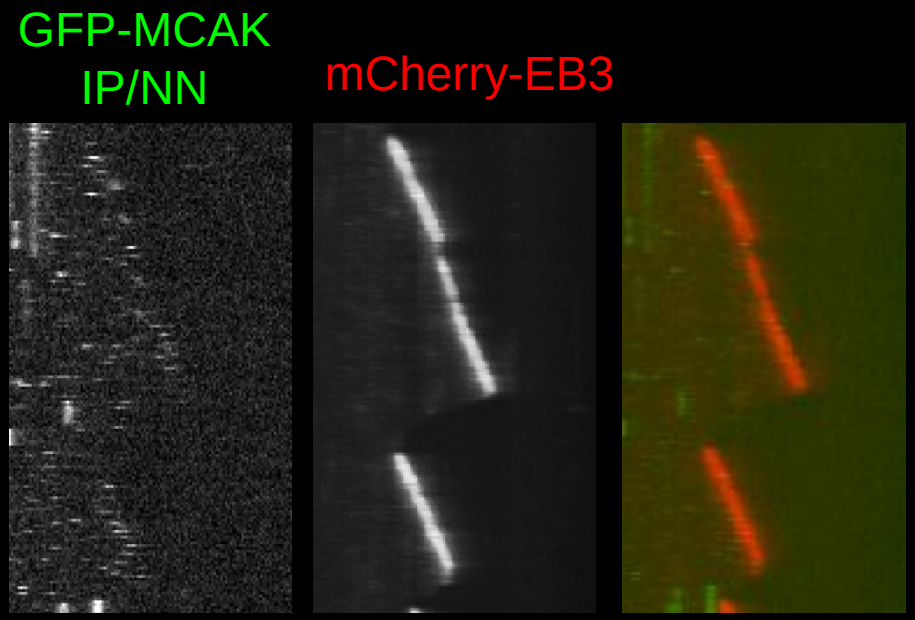




Honnappa et al., Cell 2009

IP->NN mutation in MCAK abolishes plus end tracking in vitro

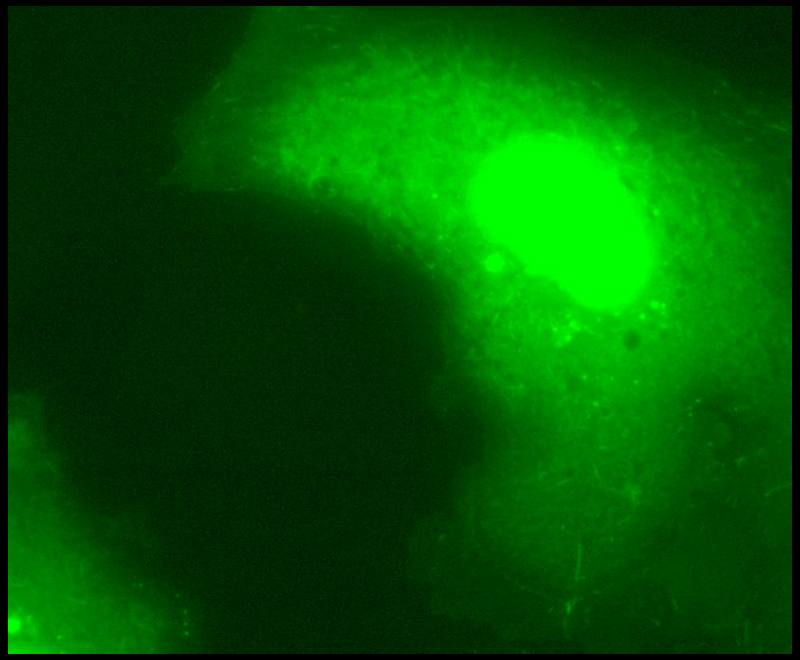




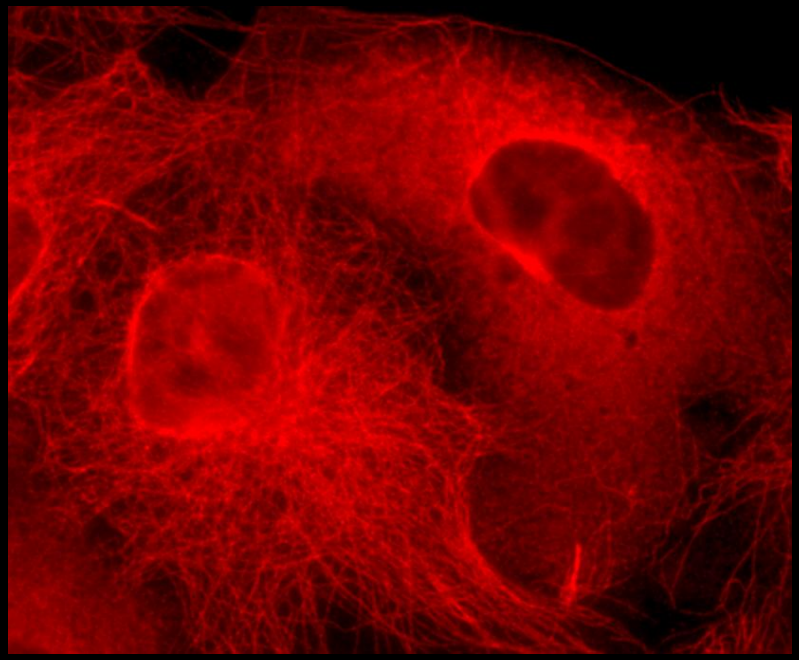
Montenegro Gouveia et al., Curr Biol 2010

MCAK is a microtubule depolymerase

GFP-MCAK

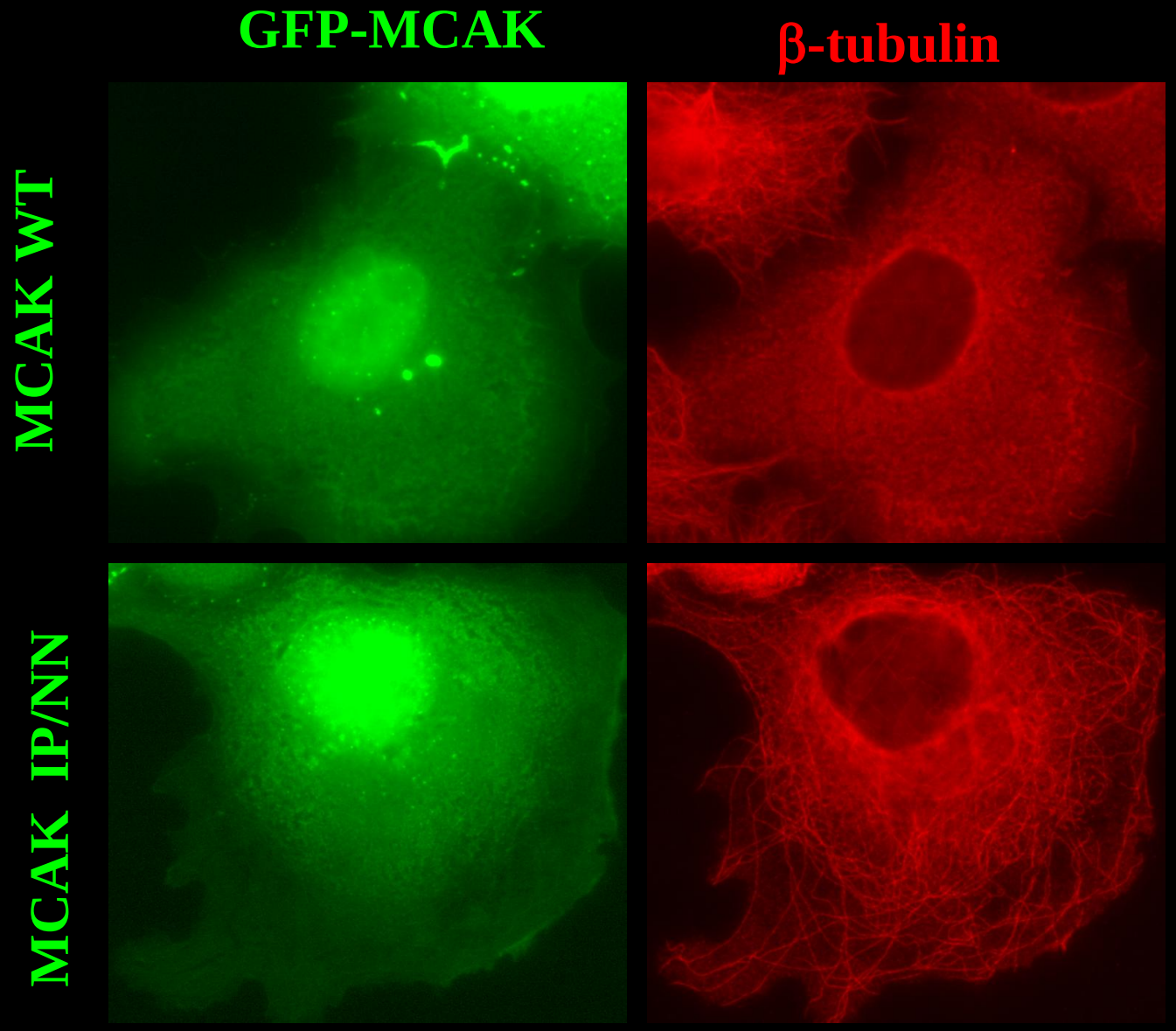


β-tubulin



Does plus end targeting enhance or inhibit depolymerization activity?

MCAK IP->NN mutant depolymerizes microtubules less efficiently



EB-dependent targeting of MCAK to microtubule tips makes it a more potent catastrophe inducer

A complex and flexible network of microtubule tip-interacting proteins and their partners controls the dynamics of individual microtubule ends

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