

# **Super-resolution imaging reveals dynamic reticular cytoophidia**

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## **SUPPLEMENTAL INFORMATION**

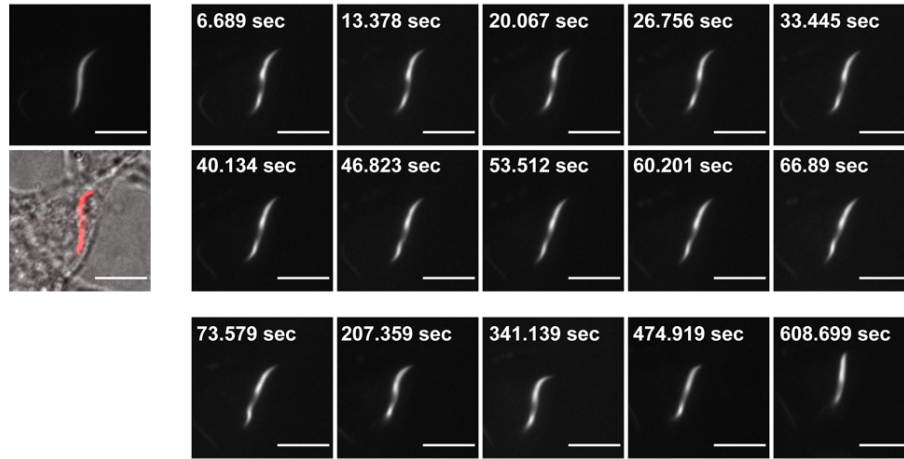
**SUPPLEMENTAL FIGURES S1 - S3**

**SUPPLEMENTAL TABLES S1 AND S2**

## SUPPLEMENTAL FIGURES S1 - S3 AND FIGURE LEGENDS

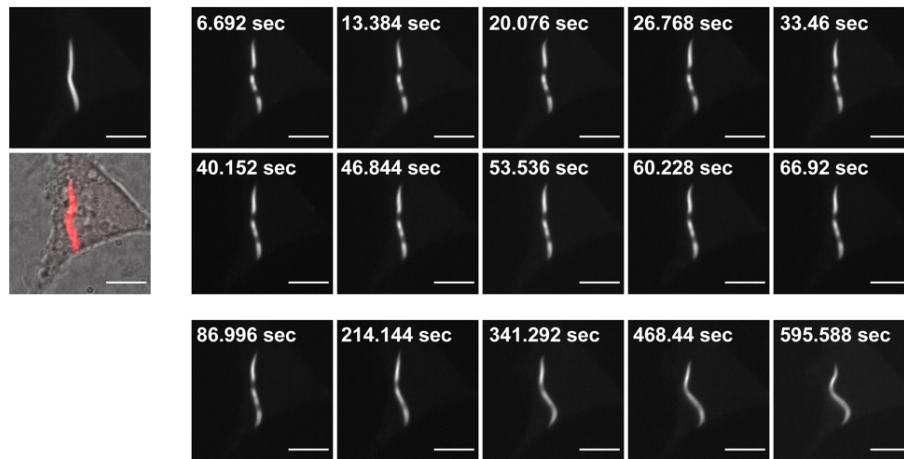
**A**

hCTPS1-mCherry DON+ 100 $\mu$ g/mL 8hours



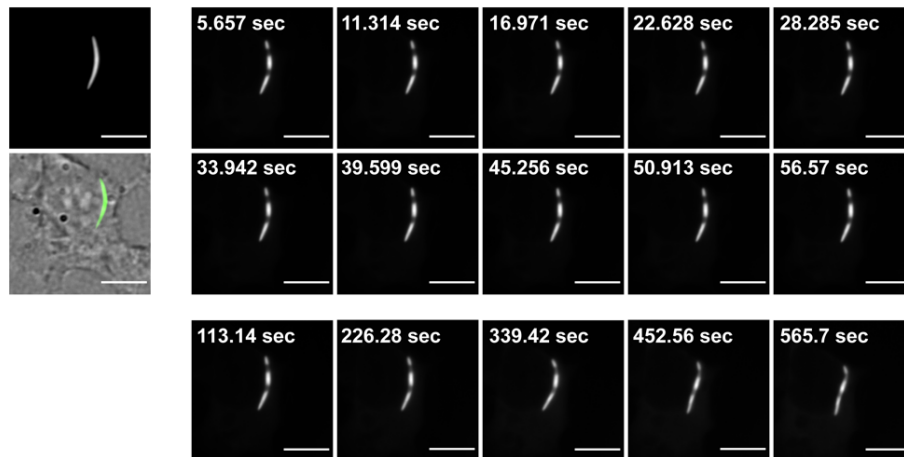
**B**

hCTPS1-mCherry DON+ 20 $\mu$ g/mL 25hours



**C**

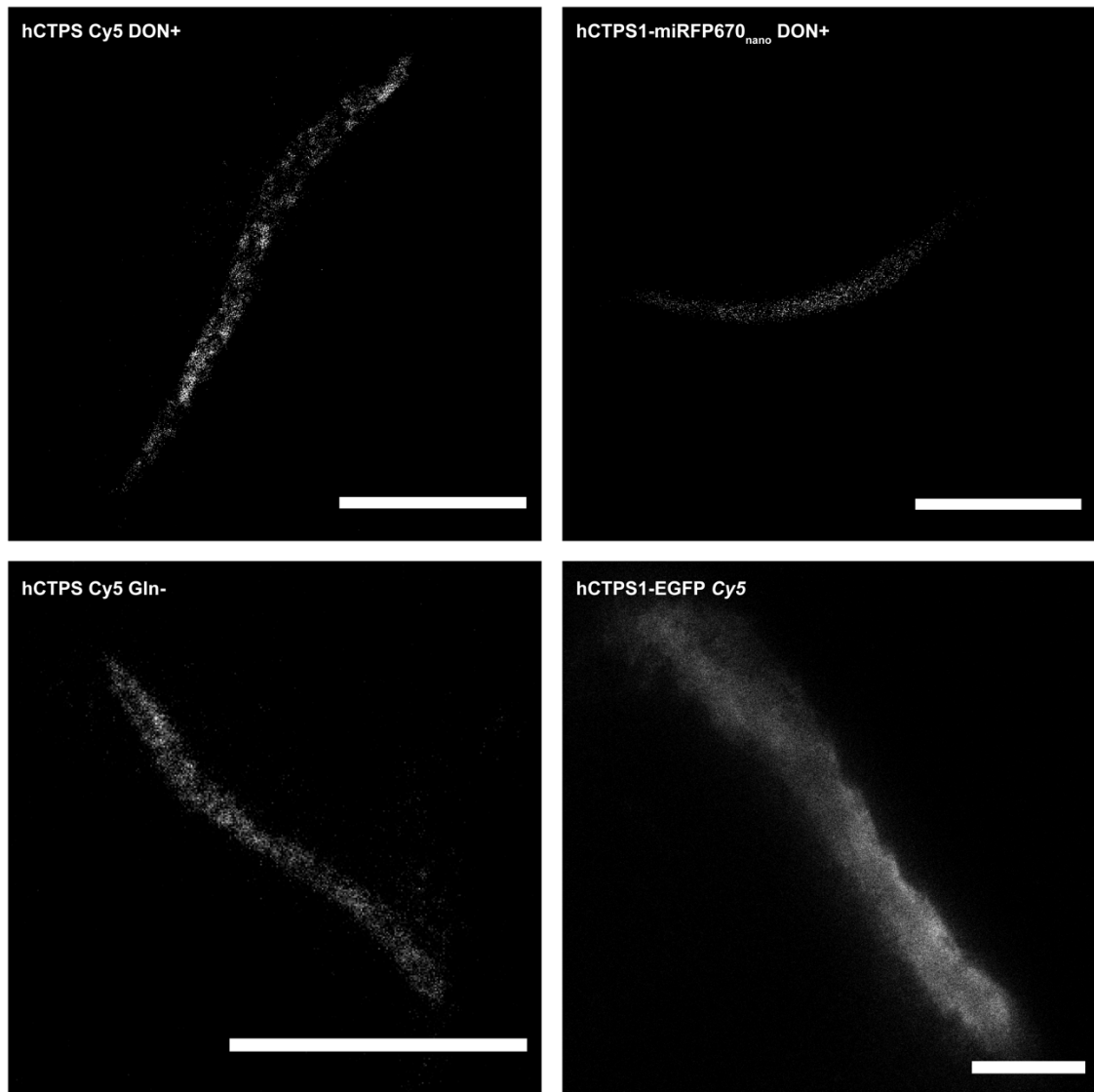
hCTPS1-EGFP



**Figure S1. FRAP on cytoophidia.**

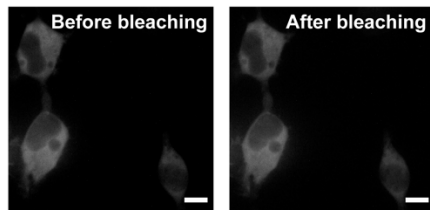
(A) Living cell images of FRAP on hCTPS-mCherry cytoophidia induced by 100  $\mu\text{g/mL}$  DON for 8 hours. (B) Living cell images of FRAP on hCTPS-mCherry cytoophidia induced by 20  $\mu\text{g/mL}$  DON for 25 hours. (C) Living cell images of FRAP on hCTPS1-EGFP Cytoophidium-like condensates. Scale bars, 10  $\mu\text{m}$  (A-C).

**A**



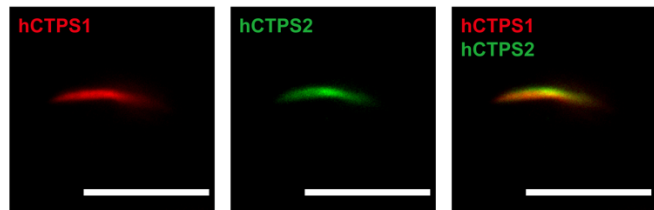
**B**

hCTPS1<sup>H355A</sup>-mCherry DON+

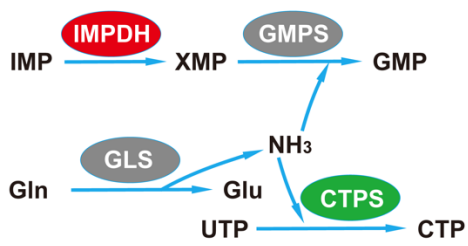


**C**

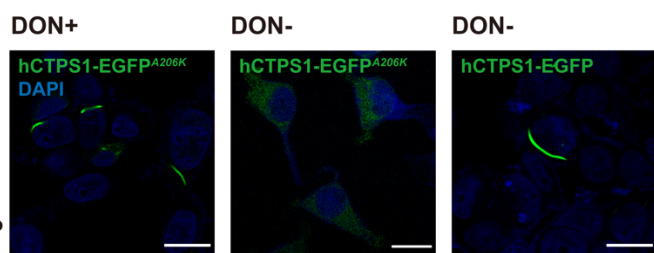
DON+



**D**

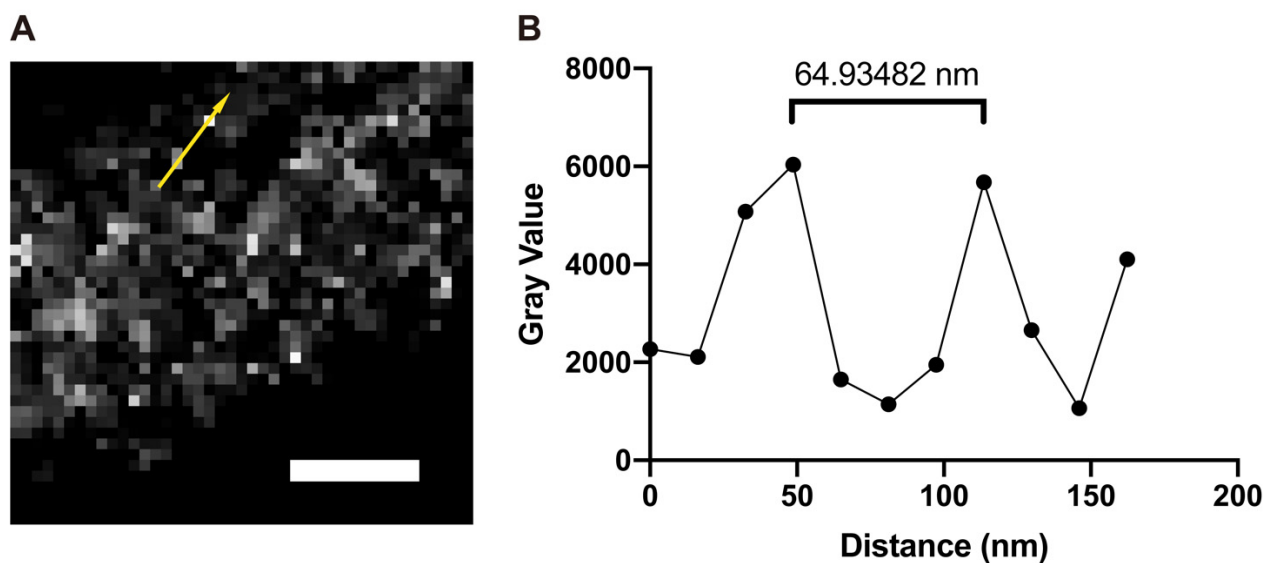


**E**



## Figure S2. Cytoophidia in super-resolution.

(A) STED images before deconvolution of 1) hCTPS cytoophidia induced by DON in 293T cells, 2) hCTPS1 cytoophidia with mRFP670nano tags induced by DON in living 293T cells, 3) hCTPS cytoophidia induced by glutamine deprivation in SW480 cells and 4) hCTPS1-EGFP cytoophidium-like condensates in 293T cells. (B) There was no difference in hCTPS1 signal before and after bleaching. (C) hCTPS1 and hCTPS2 were colocalized under DON treated in the form of cytoophidia. For DON treatment, DON (PBS solution) was added into fresh DMEM media 8 hours before living cell imaging. Images are not analyzed by SIM algorithm. (D) IMPDH and CTPS are both parts of  $\text{NH}_3$  metabolic pathways. (E) hCTPS-EGFPA206K can form cytoophidia with DON treatment. Without DON, hCTPS-EGFPA206K cannot form cytoophidia. hCTPS1-EGFP can form cytoophidium-like condensates. For DON treatment, 20  $\mu\text{g/mL}$  DON (PBS solution) was added into fresh DMEM media 8 hours before fixation. Scale bars, 3  $\mu\text{m}$  (A), 10  $\mu\text{m}$  (B, C) and 20  $\mu\text{m}$  (E).



**Figure S3. Resolution of STED microscopy.**

(A) STED image zoomed in from figure 3A, hCTPS1-miRFP670nano cytoophidia induced by DON in live 293T cells, for resolution measurement along the arrow line. (B) By measuring intensity profiles along the lines marked in the image, the approximate resolution is 64.93482nm. Scale bars, 200 nm.

## SUPPLEMENTAL TABLES S1 AND S2

**Table S1. Over-expression proteins with fluorescence tags used in this study.**

Exogenous proteins with tags	Figure
hCTPS1-mCherry	Figure 1A, B; Figure 2C; Figure 4A, C; Figure S1A, B; Figure S2B.
hCTPS1-EGFP <sup>A206K</sup>	Figure 1H, Figure 3C, D.
hCTPS1-EGFP	Figure 1H; Figure S1C.
hCTPS1 <sup>H355A</sup> -EGFP	Figure 1H.
hCTPS1-miRFP670nano	Figure 3A, Figure S2A.
hCTPS2-miRFP670nano	Figure S2C.

**Table S2. List of oligonucleotides used in this study.**

Primer name	Sequence (5'-3')	Target	Source
FYF-14F	ACGCGTTAAGTCGACAATCAAC CTC	pLV-hCTPS1	This paper
FYF-14R	GTCATGATTTATTGATGGAACT TCAGTTCGGTG	pLV-hCTPS1	This paper
FYF18-mCherry-F	TAAATCATGACCCACCGGTCAT GGTGAGCAAGGGCGAGG	mCherry	This paper
FYF-13R	TGATTGTCGACTTAACGCGTTTA CTTGTACAGCTCGTCCATGCCG	mCherry	This paper
FYF69-gfpK206A-F	GCCCTGAGCAAAGACCCCAAC GAGAAGC	EGFP <sup>K206A</sup>	This paper
FYF70-gfpK206A-R	GTCTTTGCTCAGGGCGGACTG GGTGCTCAGGTAGTGG	EGFP <sup>K206A</sup>	This paper
FYF31F	GCAGAAGCTTGGCAGAAGCTC TGTAGTGC	hCTPS1 <sup>H355A</sup>	This paper
FYF32-R	CTGCCAAGCTTCTGCGTAGCGC ACGGGCTCTTC	hCTPS1 <sup>H355A</sup>	This paper
FYF25-miRFPnano-FS	TAAATCATGACCCACCGGTCAT GGCAAACCTGGACAAGATGCT	miRFP670nano	This paper



	GAA		
FYF28- miRFPnano-RS	TTGTCGACTTAACGCGTTTAGC TCTGCTGGATGGCGATGC	miRFP670n ano	This paper
FYF50-S2-temp- R	CAGGATGTACTTCATGGTGGCA GCGCTCTAGAACCG	miRFP670n ano-pLV	This paper
FYF51-S2-temp- F	GAGTTGGAAATAAGCCCACCGG TCATGGCAAACCTGG	miRFP670n ano-pLV	This paper
FYF43-hCTPS2- F	ATGAAGTACATCCTGGTCACGG GTGG	hCTPS2	This paper
FYF44-hCTPS2- R	GCTTATTTCCAACTCAGCTATCC TTGGCTCT	hCTPS2	This paper