

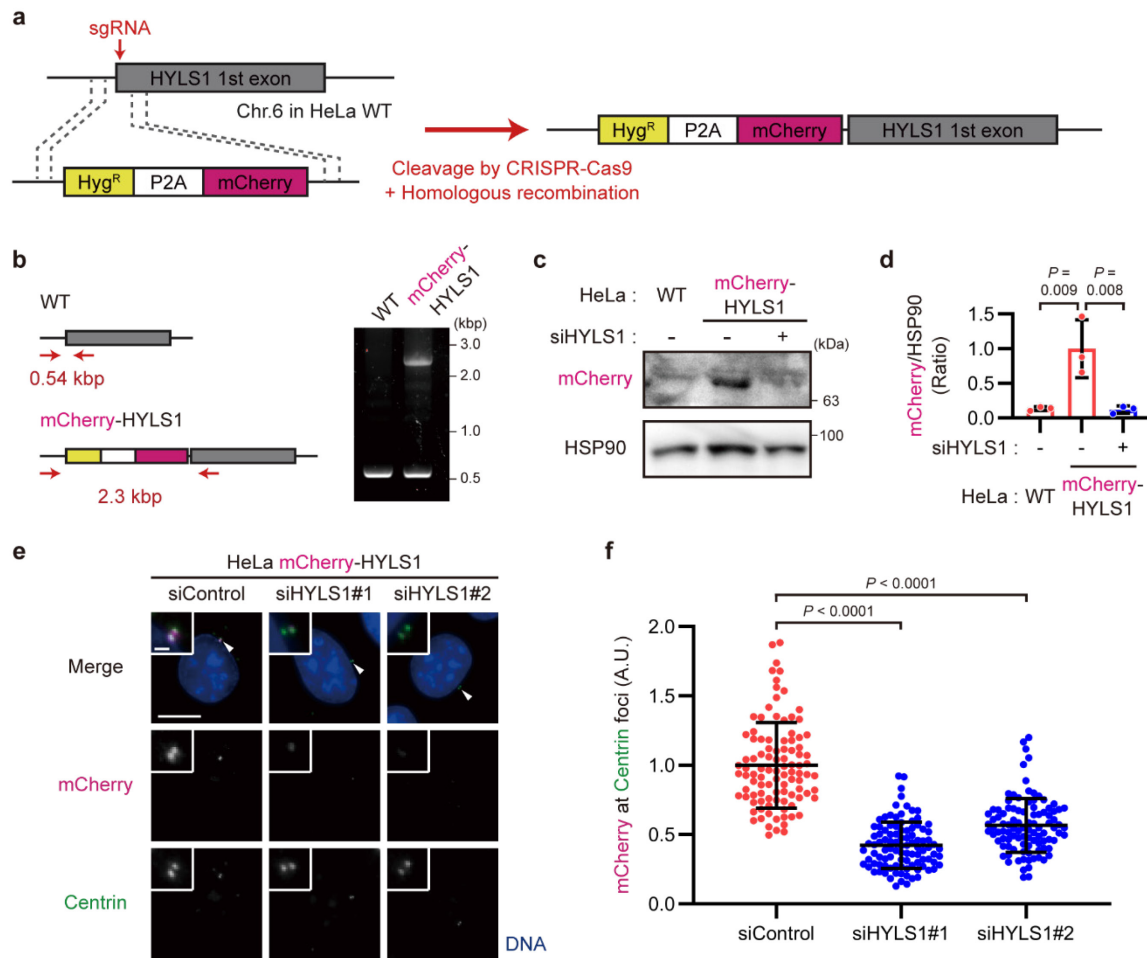
## **Supplementary Information**

### **Molecular basis promoting centriole triplet microtubule assembly**

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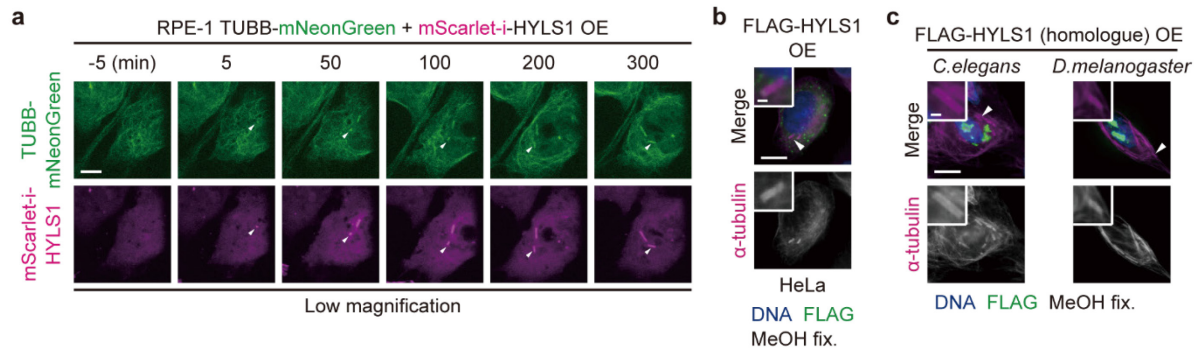
## Supplementary Fig. 1



## Supplementary Fig. 1 Generation of HeLa mCherry-HYLS1 knock-in cells.

**a**, Schematic of CRISPR-mediated knock-in method to generate HeLa mCherry-HYLS1 knock-in cells. Chr.: chromosome, Hyg<sup>R</sup>: hygromycin resistance gene. **b**, Genomic PCR and agarose gel electrophoresis of HeLa wildtype (WT) and HeLa mCherry-HYLS1 cells using the indicated primers. **c**, Immunoblotting images of lysates from HeLa WT and HeLa mCherry-HYLS1 cells treated with siControl or siHYLS1. **d**, Quantification of the intensity of mCherry normalized to that of HSP90 in **c**.  $n = 3$  independent experiments. **e**, IF images of HeLa mCherry-HYLS1 cells treated with siControl or siHYLS1. Scale bars: 10  $\mu\text{m}$  and 1  $\mu\text{m}$ . Arrowheads: magnified areas. **f**, Quantification of the intensity of mCherry at Centrin foci (centrioles) in **e**. A.U.: arbitrary unit.  $n = 100$  cells. Data are represented as mean  $\pm$  s.d..  $P$  values were calculated by one-way ANOVA with Dunnett's multiple comparisons test (**d**) or Dunn's multiple comparisons test (**f**). Source data are provided as a Source Data file.

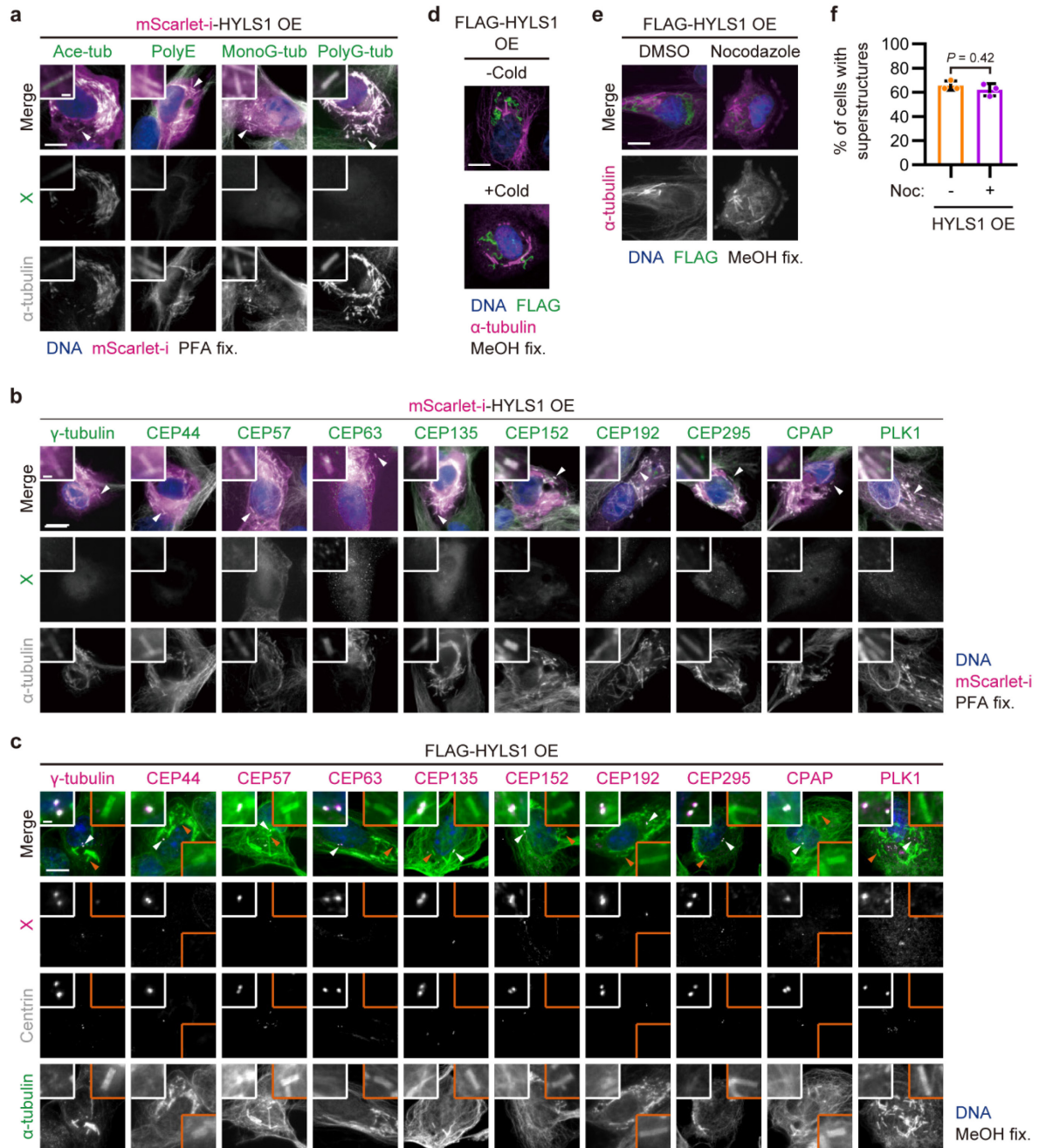
## Supplementary Fig. 2



### Supplementary Fig. 2 Overexpression of the ciliopathy protein HYLS1 leads to assembly of tubulin-based superstructures.

**a**, Supplementary time-lapse images related to Fig. 1h. Scale bar: 10  $\mu$ m. Arrowheads: magnified areas in Fig. 1h. **b**, IF images of HeLa cells transfected with pCMV-FLAG-HYLS1. Scale bars: 10  $\mu$ m and 1  $\mu$ m. Arrowhead: magnified area. **c**, IF images of RPE-1 cells transfected with pCMV-FLAG-HYLS1 homologues of *C. elegans* or *D. melanogaster*. Scale bars: 10  $\mu$ m and 1  $\mu$ m. Arrowheads: magnified areas.

### Supplementary Fig. 3

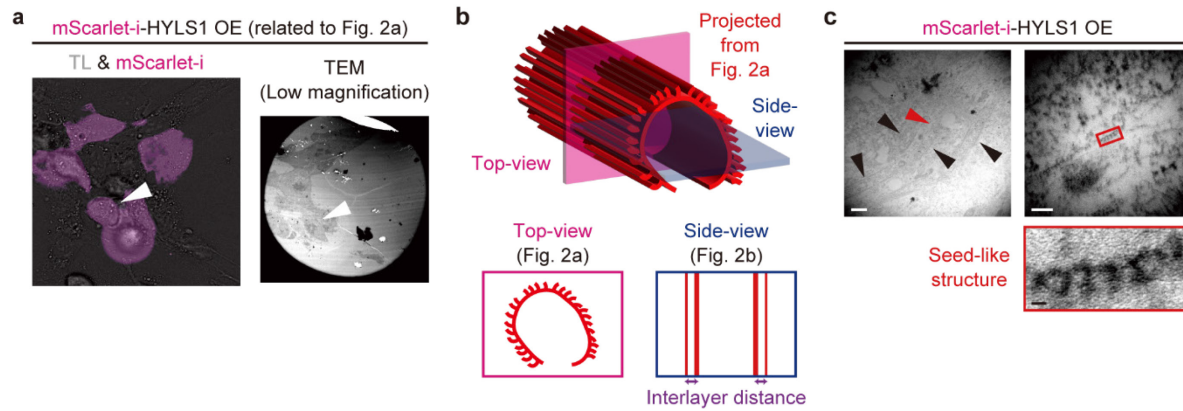


**Supplementary Fig. 3 Overexpression of HYLS1 leads to assembly of tubulin-based superstructures with high stability similar to that of centrioles.**

**a, b**, IF images of RPE-1 cells transfected with pCMV-mScarlet-i-HYLS1 and fixed with PFA. Scale bars: 10  $\mu$ m and 1  $\mu$ m. Arrowheads: magnified areas. Ace-tub: acetylated tubulin, PolyE: polyglutamylation modification, MonoG-tub: monoglycylated tubulin, PolyG-tub: polyglycylated

tubule. **c**, IF images of RPE-1 cells transfected with pCMV-mScarlet-i-HYLS1 and fixed with MeOH. Scale bars: 10  $\mu\text{m}$  and 1  $\mu\text{m}$ . Arrowheads: magnified areas including centrioles (white) or superstructures (orange). **d**, Supplementary IF images related to Fig. 1k. Scale bar: 10  $\mu\text{m}$ . **e**, IF images of RPE-1 cells transfected with pCMV-FLAG-HYLS1 and then treated with DMSO or nocodazole for 2 hours before fixation. Scale bar: 10  $\mu\text{m}$ . **f**, Quantification of frequency of interphase cells with the tubulin-based superstructures in **e**.  $n = 3$  independent experiments, 30 cells each. Data are represented as mean  $\pm$  s.d..  $P$  value was calculated by two-tailed unpaired Student's  $t$ -test (**f**). Source data are provided as a Source Data file.

## Supplementary Fig. 4

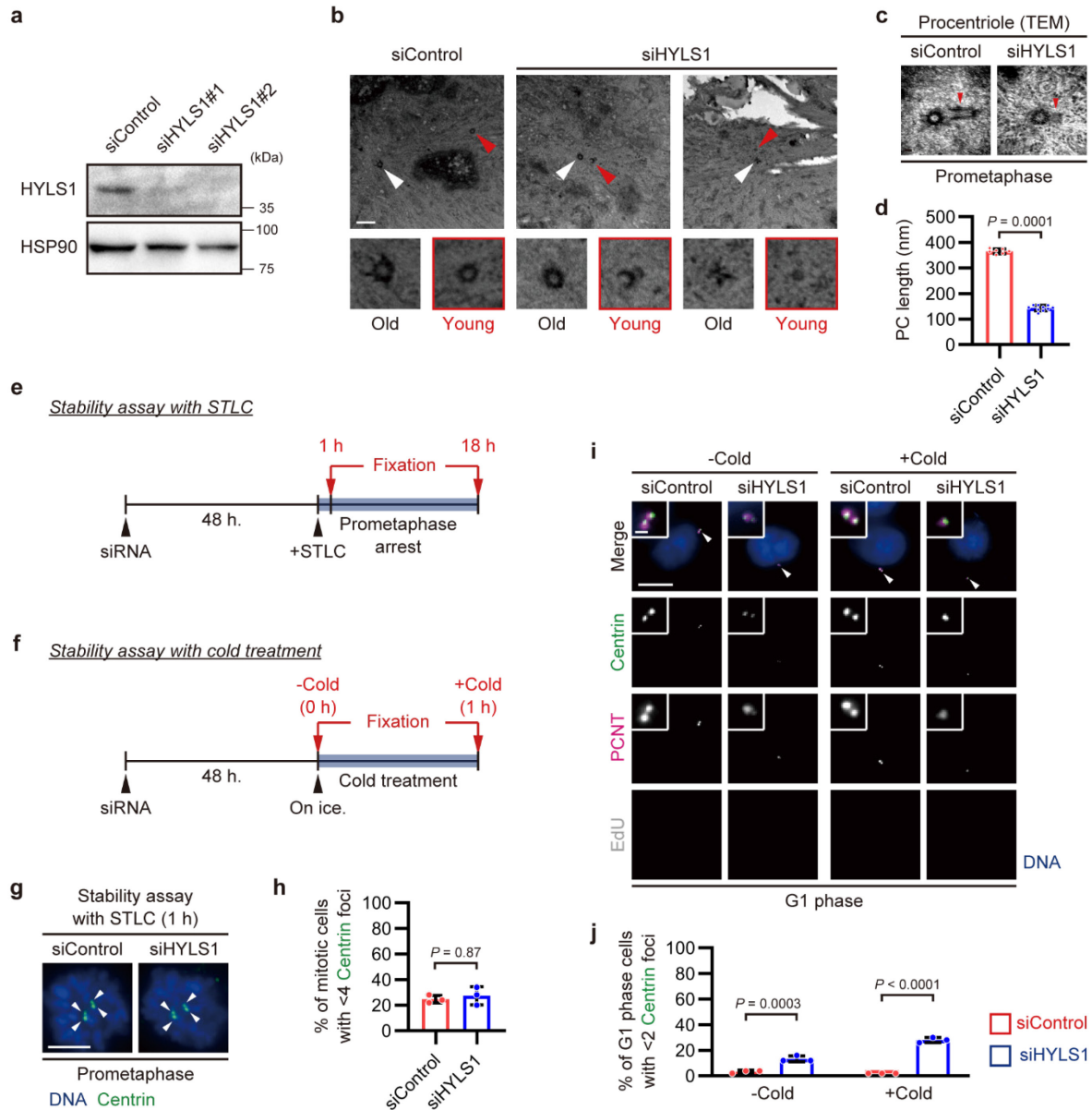


### Supplementary Fig. 4 Overexpression of HYLS1 leads to assembly of tubulin-based superstructures with incomplete microtubule chains.

**a**, Supplementary CLEM images related to Fig. 2a. Fluorescence image of pre-fixed cells and TEM image at low magnification of them. Arrowheads: observed cell in Fig. 2a. TL: transmitted light.

**b**, Schematic showing assumed geometry of the superstructure and its TEM images. **c**, TEM images of seed-like structures in RPE-1 cells transfected with pCMV-*mScarlet-i*-HYLS1. Scale bars: 500 nm, 100 nm, and 10 nm. Red arrowhead: observed seed-like structure, black arrowheads: superstructures.

## Supplementary Fig. 5

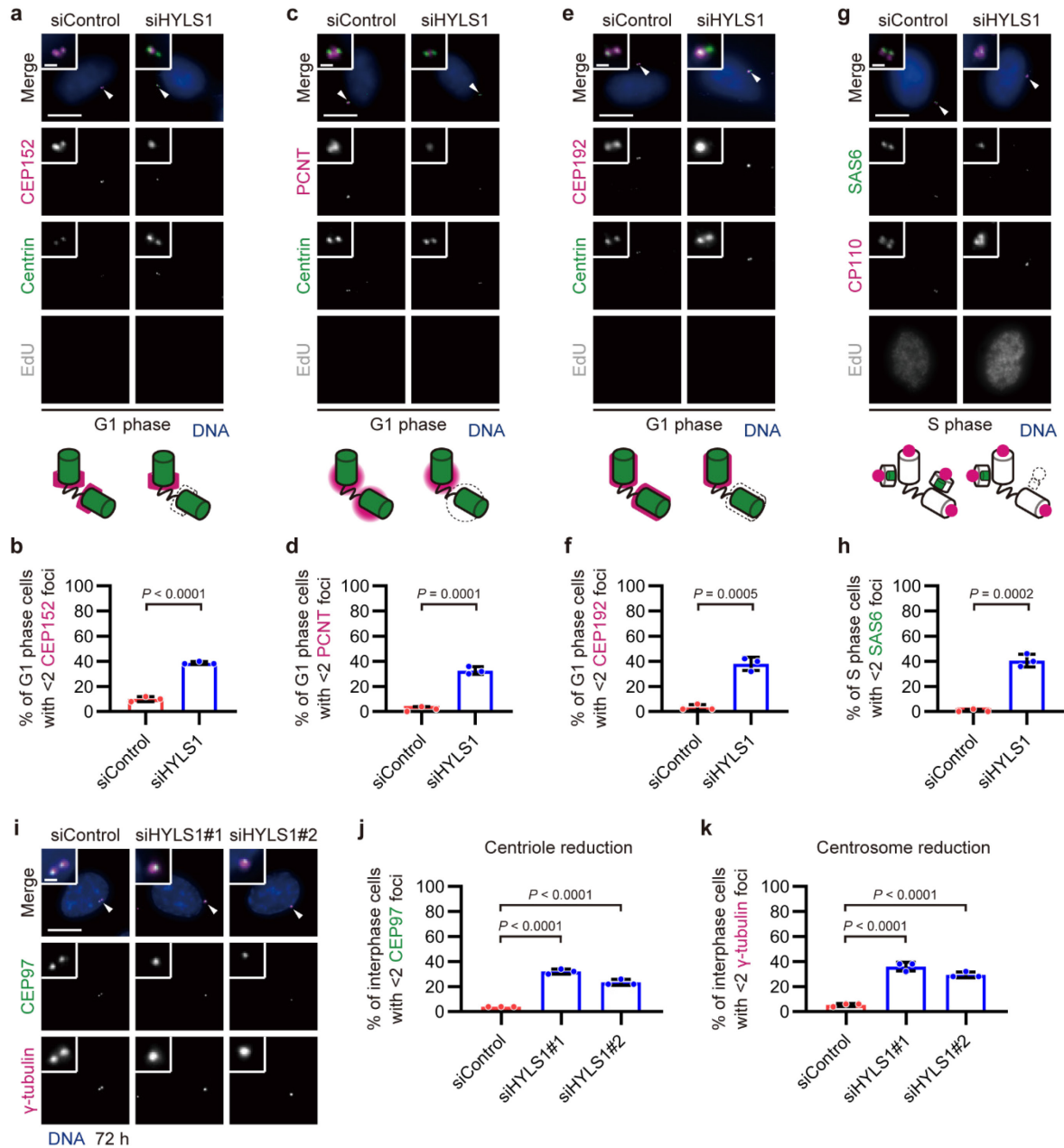


## Supplementary Fig. 5 HYLS1 provides the structural integrity of centriole triplet microtubules.

**a**, Immunoblotting images of lysates from RPE-1 cells treated with siControl or siHYLS1. **b**, Supplementary TEM images at low magnification related to Fig. 2d. The younger centrioles were distinguished by the absence of appendages. Scale bar: 1  $\mu$ m. White arrowheads: older centrioles, red arrowheads: younger centrioles. **c**, TEM images of procentrioles in prometaphase RPE-1 cells transfected with siControl or siHYLS1. To induce prometaphase arrest, the cells were treated with

STLC 3 hours before fixation. Scale bar: 100 nm. Arrowheads: procentrioles. **d**, Quantification of the length of procentrioles in **c**.  $n = 7$  (siControl) or 10 (siHYLS1) cells. **e**, Time-course of centriole stability assay with STLC. **f**, Time-course of centriole stability assay with cold treatment. **g**, Supplementary IF images related to Fig. 2e. Scale bar: 5  $\mu\text{m}$ . Arrowheads: Centrin foci (centrioles). **h**, Supplementary quantification of frequency of mitotic cells with  $<4$  Centrin foci related to Fig. 2f. Groups treated with STLC for 1 hour are shown.  $n = 3$  independent experiments, 50 cells each. **i**, IF images of RPE-1 cells subjected to centriole stability assay with cold treatment. Before incubation on ice, the cells were transfected with siControl or siHYLS1. Scale bars: 10  $\mu\text{m}$  and 1  $\mu\text{m}$ . Arrowheads: magnified areas. **j**, Quantification of frequency of G1 phase cells with  $<2$  Centrin foci in **i**.  $n = 3$  independent experiments, 50 cells each. Data are represented as mean  $\pm$  s.d..  $P$  values were calculated by Mann–Whitney  $U$  test (**d**) or one-way ANOVA with Tukey’s multiple comparisons test (**h**, **j**). Source data are provided as a Source Data file.

## Supplementary Fig. 6

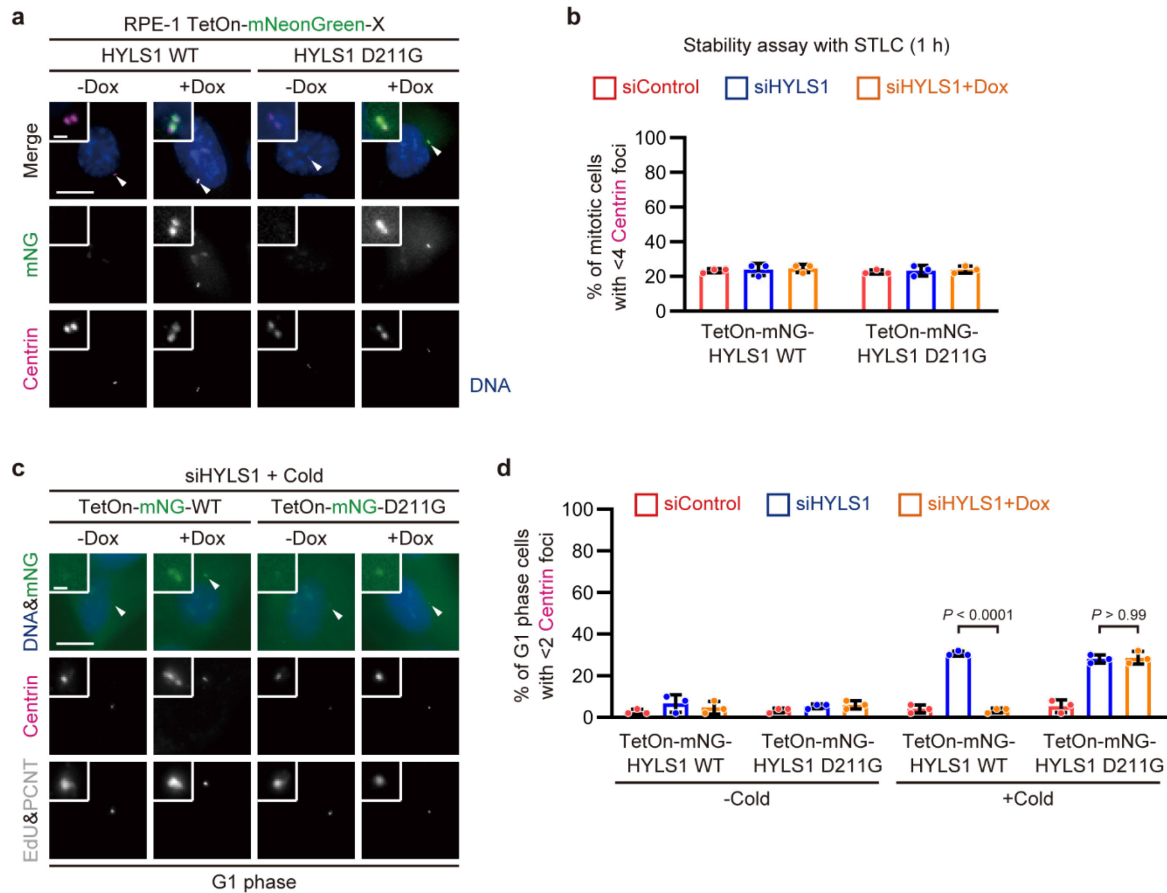


## Supplementary Fig. 6 HYLS1 ensures the ability of centrioles to organize centrosomes.

**a**, IF images of G1 phase RPE-1 cells transfected with siControl or siHYLS1. Scale bars: 10  $\mu$ m and 1  $\mu$ m. Arrowheads: magnified areas. **b**, Quantification of frequency of G1 phase cells with <2 CEP152 foci in **a**.  $n = 3$  independent experiments, 50 cells each. **c**, IF images of G1 phase RPE-1 cells transfected with siControl or siHYLS1. Scale bars: 10  $\mu$ m and 1  $\mu$ m. Arrowheads: magnified areas. **d**, Quantification of frequency of G1 phase cells with <2 PCNT foci in **c**.  $n = 3$  independent

experiments, 50 cells each. **e**, IF images of G1 phase RPE-1 cells transfected with siControl or siHYLS1. Scale bars: 10  $\mu\text{m}$  and 1  $\mu\text{m}$ . Arrowheads: magnified areas. **f**, Quantification of frequency of G1 phase cells with  $<2$  CEP192 foci in **e**.  $n = 3$  independent experiments, 50 cells each. **g**, IF images of S phase RPE-1 cells transfected with siControl or siHYLS1. Scale bars: 10  $\mu\text{m}$  and 1  $\mu\text{m}$ . Arrowheads: magnified areas. **h**, Quantification of frequency of S phase cells with  $<2$  SAS6 foci in **g**.  $n = 3$  independent experiments, 50 cells each. **i**, IF images of RPE-1 cells transfected with siControl or siHYLS1 for 72 hours. Scale bars: 10  $\mu\text{m}$  and 1  $\mu\text{m}$ . Arrowheads: magnified areas. **j**, Quantification of frequency of interphase cells with  $<2$  CEP97 foci in **i**.  $n = 3$  independent experiments, 50 cells each. **k**, Quantification of frequency of interphase cells with  $<2$   $\gamma$ -tubulin foci in **i**.  $n = 3$  independent experiments, 50 cells each. Data are represented as mean  $\pm$  s.d..  $P$  values were calculated by two-tailed unpaired Student's  $t$ -test (**b**, **d**, **f**, **h**) or one-way ANOVA with Dunnett's multiple comparisons test (**j**, **k**). Source data are provided as a Source Data file.

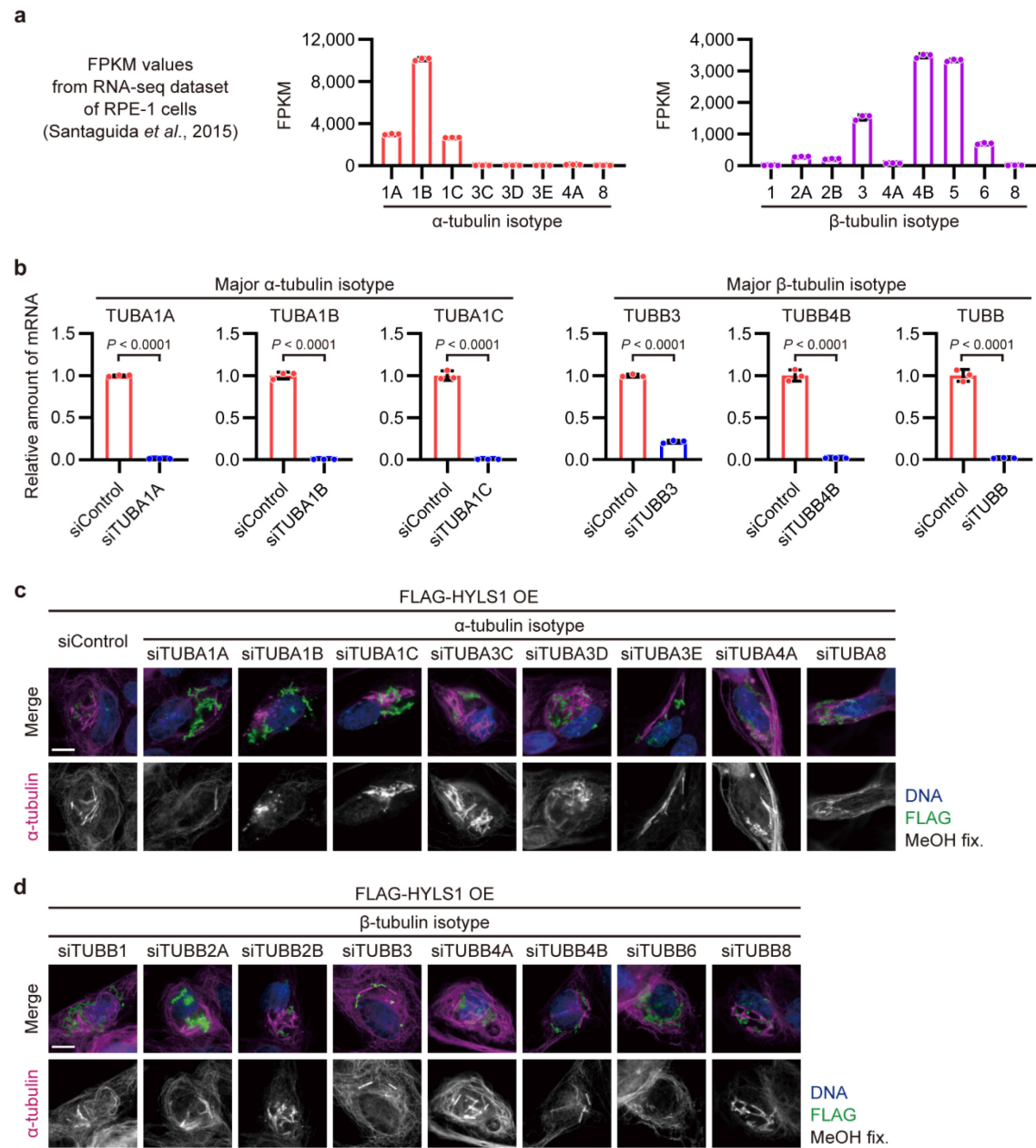
## Supplementary Fig. 7



### Supplementary Fig. 7 The ciliopathy-related D211G mutant of HYLS1 fails to stabilize centrioles.

**a**, IF images of RPE-1 TetOn-mNeonGreen-X cells (X = HYLS1 WT or D211G) in presence and absence of doxycycline. Scale bars: 10  $\mu$ m and 1  $\mu$ m. Arrowheads: magnified areas. **b**, Supplementary quantification of frequency of mitotic cells with <4 Centrin foci related to Fig. 3h. Groups treated with STLC for 1 hour are shown.  $n = 3$  independent experiments, 50 cells each. **c**, Representative IF images of RPE-1 TetOn-mNeonGreen-X cells (X = HYLS1 WT or D211G) subjected to centriole stability assay with cold treatment. Before incubation on ice, the cells were transfected with siControl or siHYLS1 and were treated with doxycycline. Scale bars: 10  $\mu$ m and 1  $\mu$ m. Arrowheads: magnified areas. **d**, Quantification of frequency of G1 phase cells with <2 Centrin foci in **c**.  $n = 3$  independent experiments, 50 cells each. Data are represented as mean  $\pm$  s.d..  $P$  values were calculated by one-way ANOVA with Tukey's multiple comparisons test (**d**). Source data are provided as a Source Data file.

**Supplementary Fig. 8**

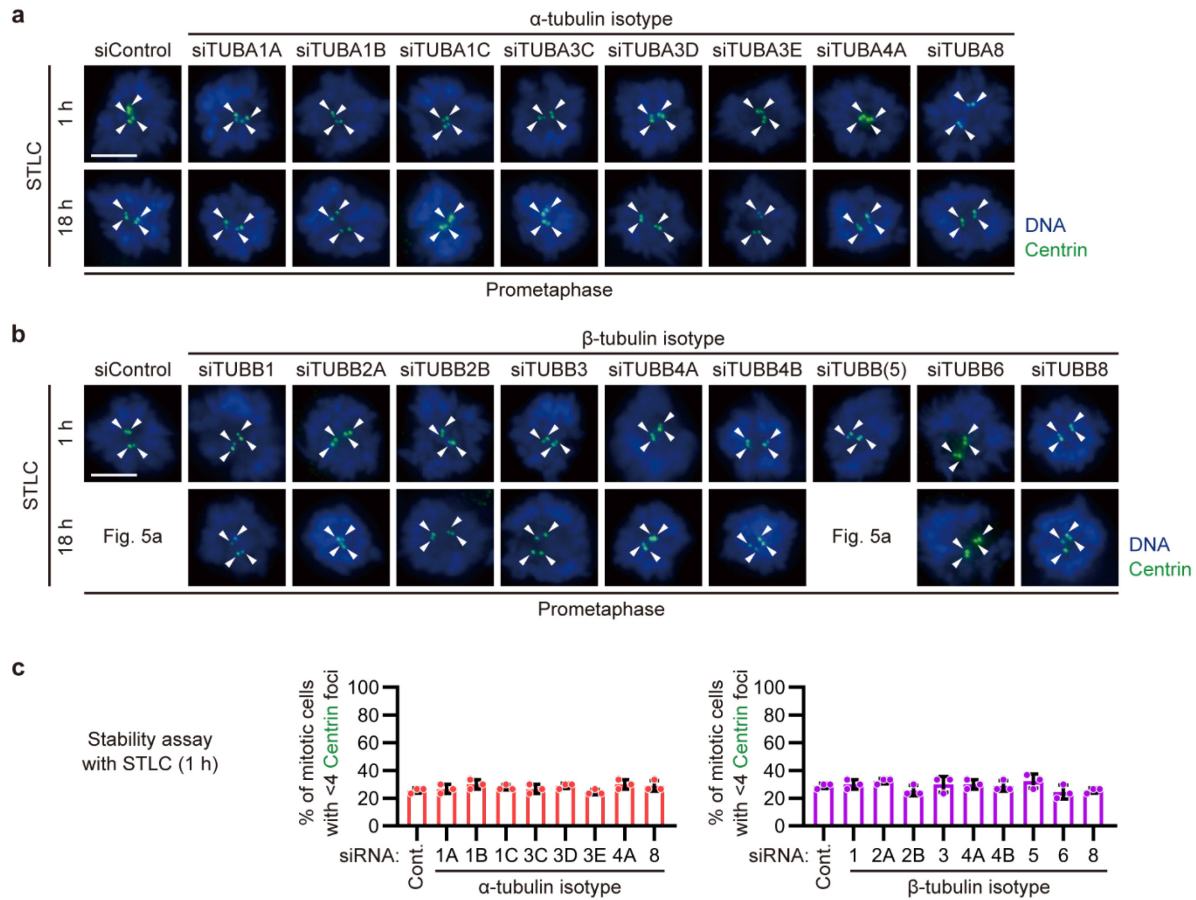


**Supplementary Fig. 8 The  $\beta$ -tubulin isotype TUBB is specifically required for the HYLS1-dependent assembly of tubulin-based superstructures.**

**a**, FPKM values for human tubulin isotypes from an RNA-seq dataset of RPE-1 cells in a previous study (GSE60570).  $n = 3$  biological replicates. Data for untreated samples were used. **b**, Quantitative PCR analyses of major tubulin isotypes in RPE-1 cells treated with siControl or siRNAs targeting each isotype.  $n = 3$  biological replicates. **c**, **d**, Supplementary IF images related

to Fig. 4e. Scale bars: 10  $\mu\text{m}$ . Data are represented as mean  $\pm$  s.d.. *P* values were calculated by two-tailed unpaired Student's *t*-test (**b**). Source data are provided as a Source Data file.

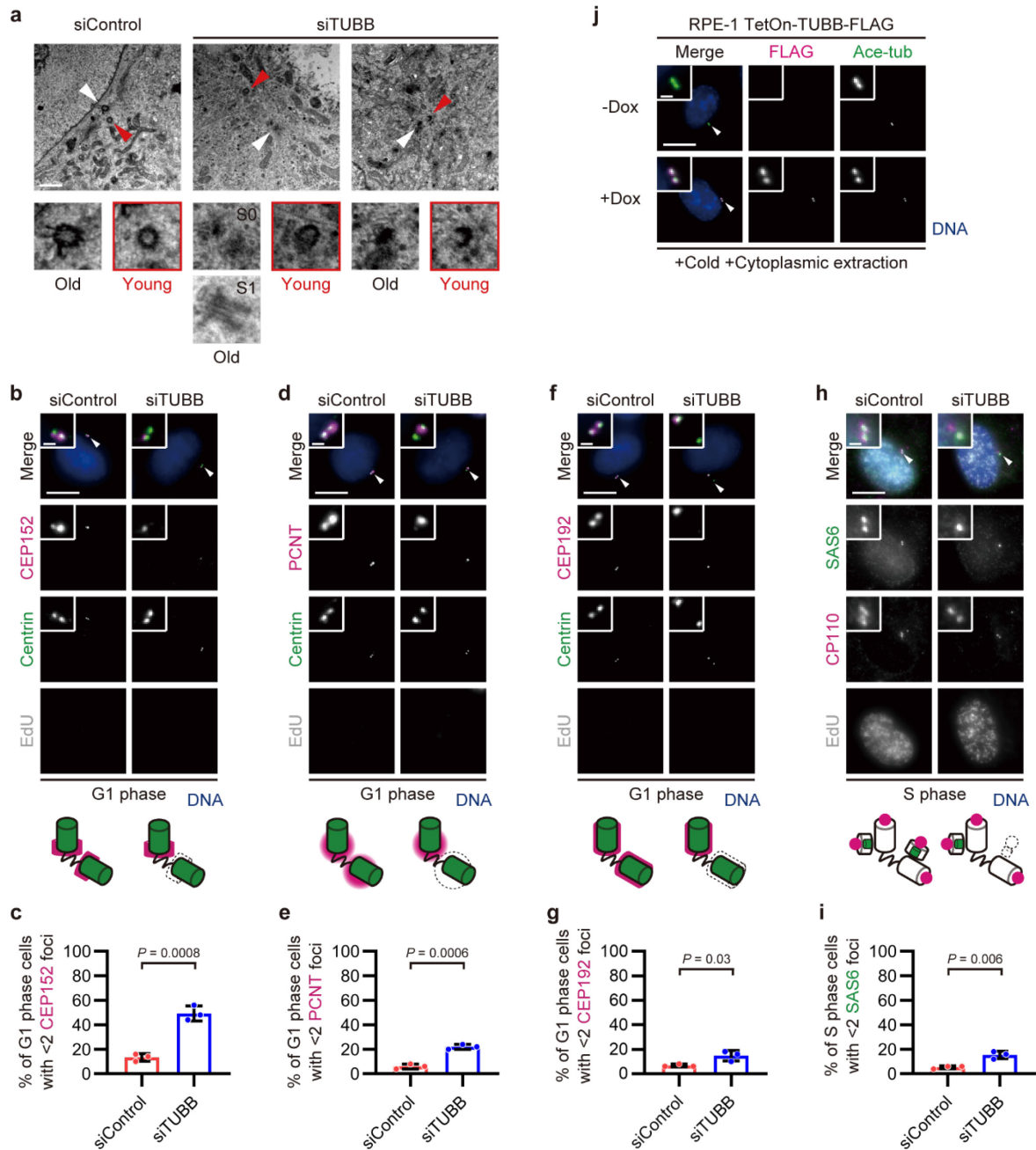
## Supplementary Fig. 9



### Supplementary Fig. 9 TUBB is specifically required for the assembly of stable centrioles.

**a, b**, Supplementary IF images related to Fig. 5a. Scale bars: 5  $\mu$ m. Arrowheads: Centrin foci (centrioles). **c**, Supplementary quantification of frequency of mitotic cells with <4 Centrin foci related to Fig. 5b. Groups treated with STLC for 1 hour are shown. Cont.: control.  $n = 3$  independent experiments, 30 cells each. Data are represented as mean  $\pm$  s.d.. Source data are provided as a Source Data file.

## Supplementary Fig. 10

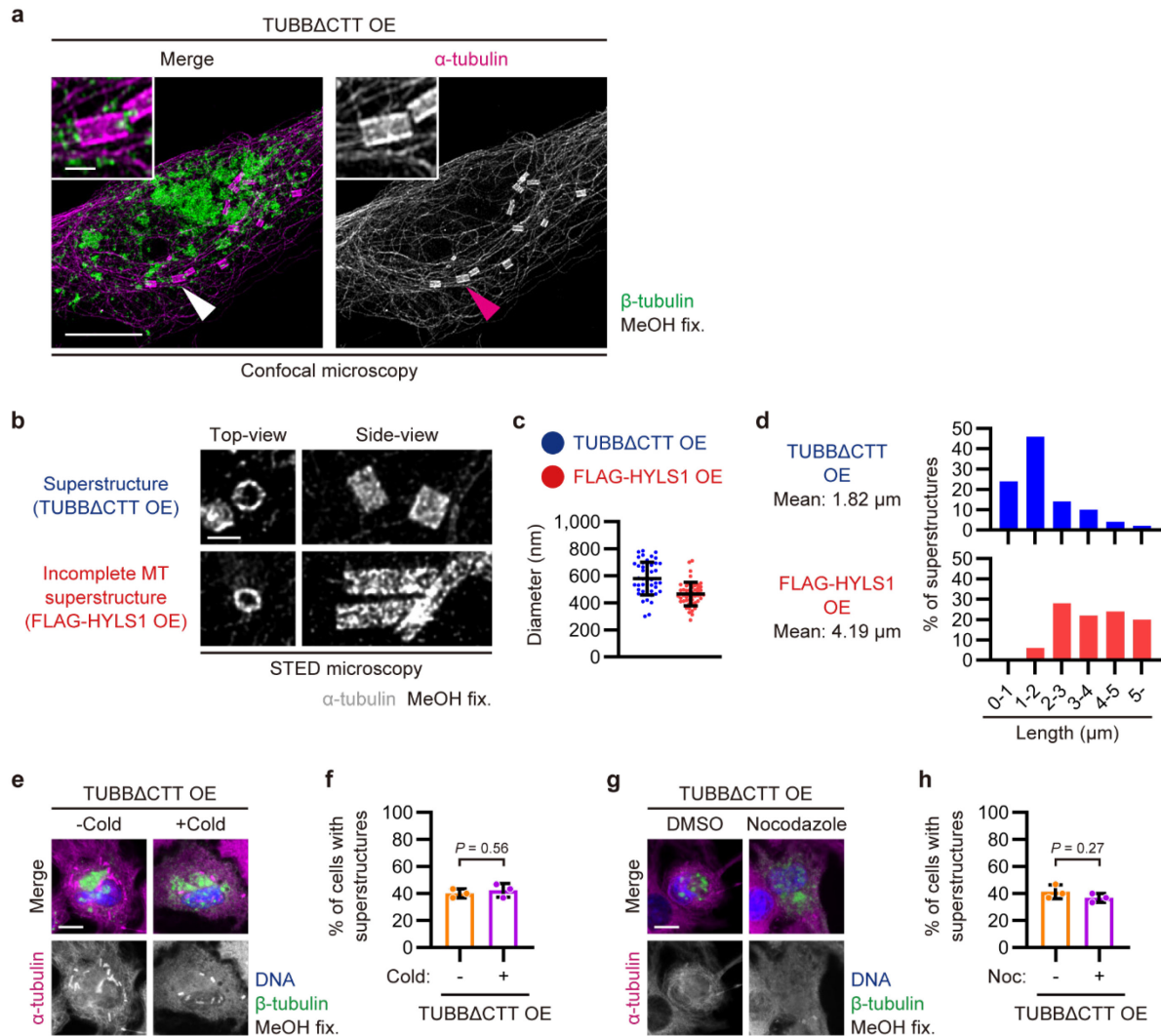


## Supplementary Fig. 10 TUBB provides the structural integrity of centriole triplet microtubules.

**a**, Supplementary TEM images at low magnification related to Fig. 5c. The younger centrioles were distinguished by the absence of appendages. Scale bar: 1  $\mu$ m. White arrowheads: older centrioles, red arrowheads: younger centrioles. S0: serial#0 (the same serial as the low

magnification image above), S1: serial#1 (adjacent serial). **b**, IF images of G1 phase RPE-1 cells transfected with siControl or siTUBB. Scale bars: 10  $\mu\text{m}$  and 1  $\mu\text{m}$ . Arrowheads: magnified areas. **c**, Quantification of frequency of G1 phase cells with  $<2$  CEP152 foci in **b**.  $n = 3$  independent experiments, 50 cells each. **d**, IF images of G1 phase RPE-1 cells transfected with siControl or siTUBB. Scale bars: 10  $\mu\text{m}$  and 1  $\mu\text{m}$ . Arrowheads: magnified areas. **e**, Quantification of frequency of G1 phase cells with  $<2$  PCNT foci in **d**.  $n = 3$  independent experiments, 50 cells each. **f**, IF images of G1 phase RPE-1 cells transfected with siControl or siTUBB. Scale bars: 10  $\mu\text{m}$  and 1  $\mu\text{m}$ . Arrowheads: magnified areas. **g**, Quantification of frequency of G1 phase cells with  $<2$  CEP192 foci in **f**.  $n = 3$  independent experiments, 50 cells each. **h**, IF images of S phase RPE-1 cells transfected with siControl or siTUBB. Scale bars: 10  $\mu\text{m}$  and 1  $\mu\text{m}$ . Arrowheads: magnified areas. **i**, Quantification of frequency of S phase cells with  $<2$  SAS6 foci in **h**.  $n = 3$  independent experiments, 50 cells each. **j**, IF images of RPE-1 TetOn-TUBB-FLAG cells in presence and absence of doxycycline. The cells were subjected to cold treatment and cytoplasmic extraction to depolymerize cytoplasmic microtubules. Scale bars: 10  $\mu\text{m}$  and 1  $\mu\text{m}$ . Arrowheads: magnified areas. Ace-tub: acetylated tubulin. Data are represented as mean  $\pm$  s.d.  $P$  values were calculated by two-tailed unpaired Student's  $t$ -test (**c**, **e**, **g**, **i**). Source data are provided as a Source Data file.

## Supplementary Fig. 11

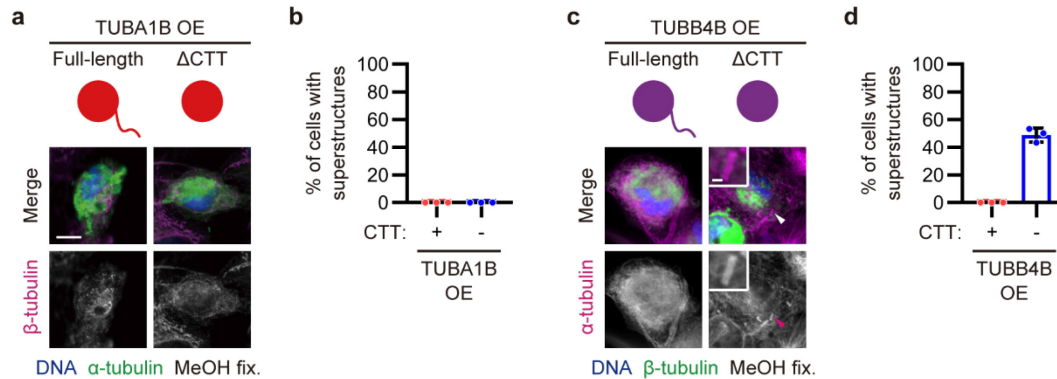


## Supplementary Fig. 11 The unstructured C-terminal tail of TUBB prevents the assembly of incomplete microtubules.

**a**, Confocal microscopy images of RPE-1 cells transfected with pCMV-TUBB $\Delta$ CTT. Scale bars: 10  $\mu$ m and 1  $\mu$ m. Arrowheads: magnified area. **b**, STED microscopy images of the tubulin-based superstructures in RPE-1 cells transfected with pCMV-TUBB $\Delta$ CTT or pCMV-FLAG-HYLS1. Scale bar: 1  $\mu$ m. **c**, Quantification of diameter of the tubulin-based superstructures in **b**.  $n = 50$  superstructures. **d**, Quantification of length of the tubulin-based superstructures in **b**.  $n = 50$  superstructures. **e**, IF images of RPE-1 cells transfected with pCMV-TUBB $\Delta$ CTT and then incubated on ice for 1 hour before fixation. Scale bar: 10  $\mu$ m. **f**, Quantification of frequency of interphase cells with the tubulin-based superstructures in **e**.  $n = 3$  independent experiments, 30

cells each. **g**, IF images of RPE-1 cells transfected with pCMV-TUBB $\Delta$ CTT and then treated with DMSO or nocodazole for 2 hours before fixation. Scale bar: 10  $\mu$ m. **h**, Quantification of frequency of interphase cells with the tubulin-based superstructures in **g**.  $n = 3$  independent experiments, 30 cells each. Data are represented as mean  $\pm$  s.d..  $P$  values were calculated by two-tailed unpaired Student's  $t$ -test (**f**, **h**). Source data are provided as a Source Data file.

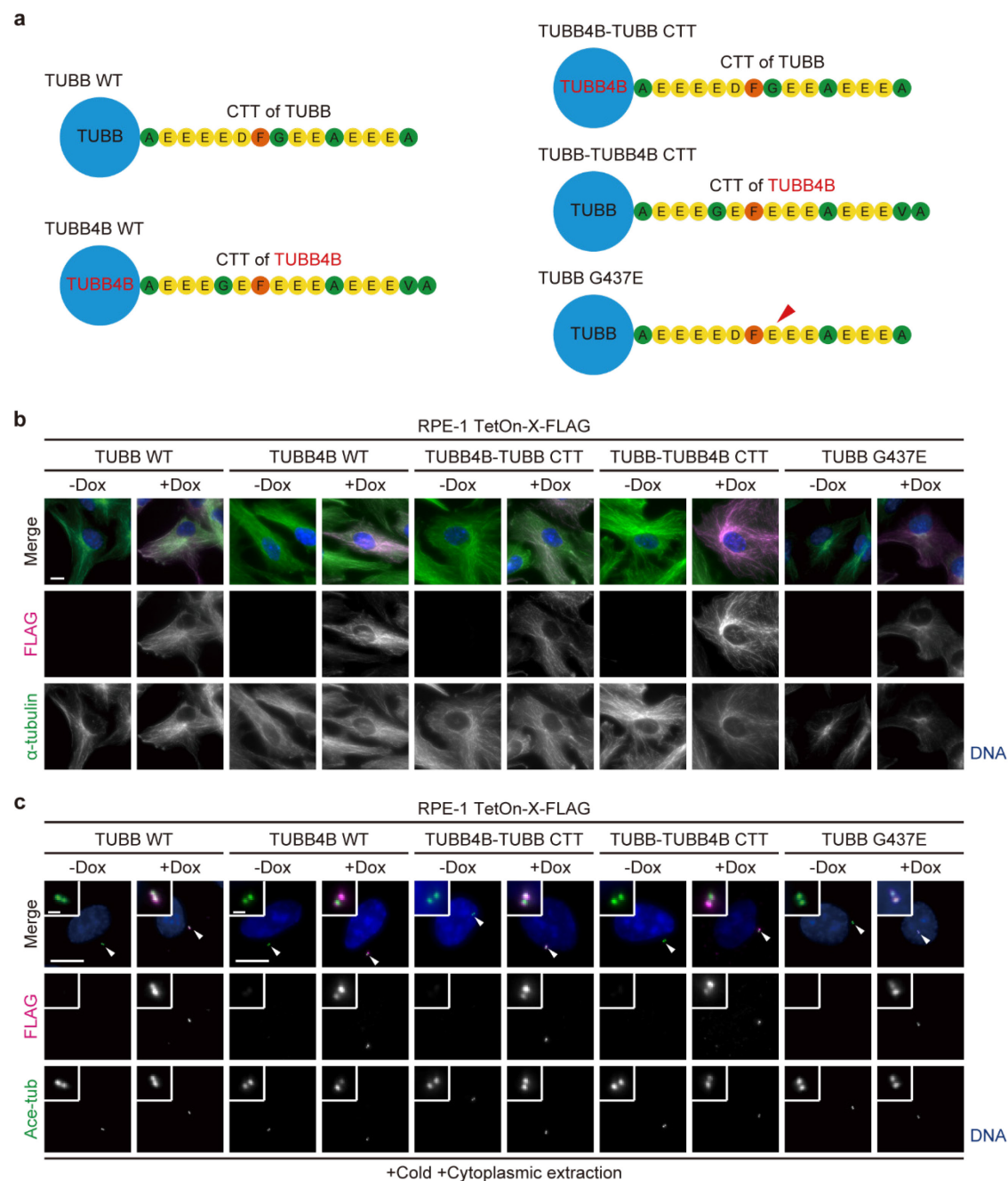
## Supplementary Fig. 12



## Supplementary Fig. 12 TUBB4B $\Delta$ CTT, but not TUBA1B $\Delta$ CTT, can also induce the assembly of incomplete microtubules.

**a**, IF images of RPE-1 cells transfected with pCMV-TUBA1B with or without the C-terminal tail (CTT). Scale bar: 10  $\mu$ m. **b**, Quantification of frequency of interphase cells with tubulin-based superstructures similar to incomplete MT superstructures in **a**.  $n = 3$  independent experiments, 30 cells each. **c**, IF images of RPE-1 cells transfected with pCMV-TUBB4B with or without the CTT. Scale bars: 10  $\mu$ m and 1  $\mu$ m. Arrowheads: magnified area. **d**, Quantification of frequency of interphase cells with tubulin-based superstructures similar to incomplete MT superstructures in **c**.  $n = 3$  independent experiments, 30 cells each. Data are represented as mean  $\pm$  s.d.. Source data are provided as a Source Data file.

Supplementary Fig. 13

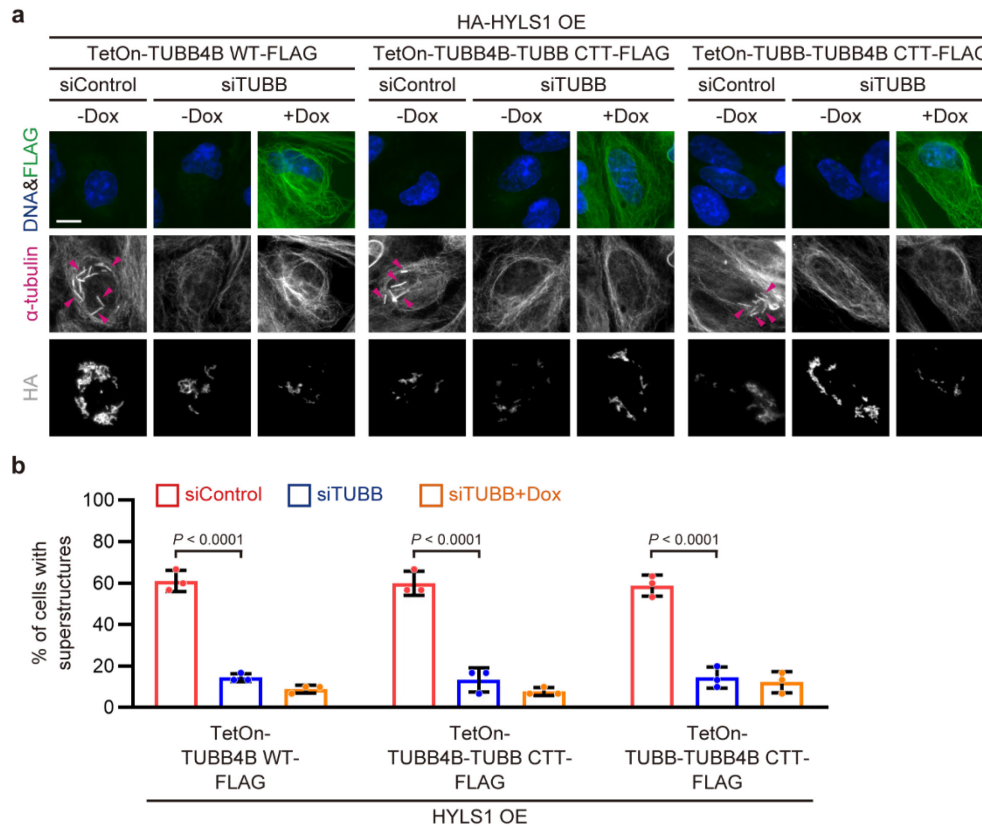


Supplementary Fig. 13 Generation of RPE-1 TetOn-X-FLAG cells.

**a**, Schematic of TUBB WT, TUBB4B WT, TUBB4B-TUBB CTT, TUBB-TUBB4B CTT, and TUBB G437E. CTT: C-terminal tail. **b**, IF images of RPE-1 TetOn-X-FLAG cells (X = TUBB WT, TUBB4B WT, TUBB4B-TUBB CTT, TUBB-TUBB4B CTT, or TUBB G437E) in presence and absence of doxycycline. Scale bar: 10  $\mu$ m. **c**, IF images of RPE-1 TetOn-X-FLAG cells (X =

TUBB WT, TUBB4B WT, TUBB4B-TUBB CTT, TUBB-TUBB4B CTT, or TUBB G437E) in presence and absence of doxycycline. The cells were subjected to cold treatment and cytoplasmic extraction to depolymerize cytoplasmic microtubules. Scale bars: 10  $\mu$ m and 1  $\mu$ m. Arrowheads: magnified areas. Ace-tub: acetylated tubulin.

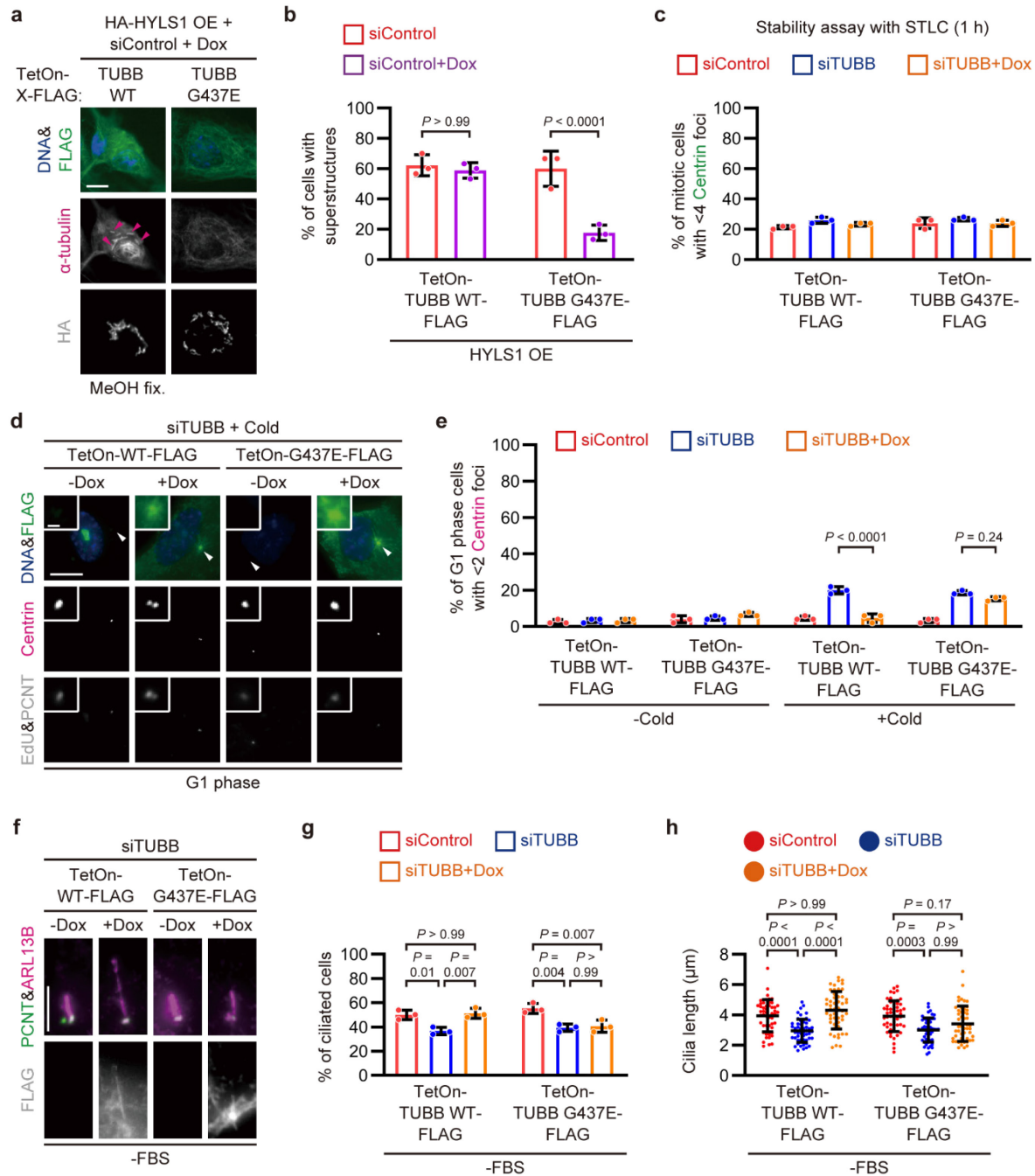
## Supplementary Fig. 14



**Supplementary Fig. 14 The TUBB-specific CTT, as well as the body of TUBB, is required for the HYLS1-dependent assembly of incomplete microtubules.**

**a**, IF images of RPE-1 TetOn-X-FLAG cells (X = TUBB4B, TUBB4B-TUBB CTT, or TUBB-TUBB4B CTT) transfected with pCMV-HA-HYLS1 and siControl or siTUBB, and treated with doxycycline. Scale bar: 10  $\mu$ m. Arrowheads: incomplete MT superstructures. **b**, Quantification of frequency of interphase cells with incomplete MT superstructures in **a**.  $n = 3$  independent experiments, 30 cells each. Data are represented as mean  $\pm$  s.d..  $P$  values were calculated by one-way ANOVA with Tukey's multiple comparisons test (**b**). Source data are provided as a Source Data file.

## Supplementary Fig. 15



**Supplementary Fig. 15 HYLS1 regulates the C-terminal tail of TUBB to promote the assembly of incomplete microtubules and stable centrioles.**

**a**, Supplementary IF images related to Fig. 6f. Arrowheads: incomplete MT superstructures. Scale bar: 10  $\mu$ m. **b**, Supplementary quantification of frequency of interphase cells with incomplete MT superstructures related to Fig. 6g.  $n = 3$  independent experiments, 30 cells each. **c**, Supplementary

quantification of frequency of mitotic cells with  $<4$  Centrin foci related to Fig. 6i. Groups treated with STLC for 1 hour are shown.  $n = 3$  independent experiments, 50 cells each. **d**, Representative IF images of RPE-1 TetOn-X-FLAG cells ( $X = \text{TUBB WT or G437E}$ ) subjected to centriole stability assay with cold treatment. Before incubation on ice, the cells were transfected with siControl or siTUBB and were treated with doxycycline. Scale bars:  $10\ \mu\text{m}$  and  $1\ \mu\text{m}$ . Arrowheads: magnified areas. **e**, Quantification of frequency of G1 phase cells with  $<2$  Centrin foci in **d**.  $n = 3$  independent experiments, 50 cells each. **f**, Representative IF images of serum-starved RPE-1 TetOn-X-FLAG cells ( $X = \text{TUBB WT or G437E}$ ). The cells were transfected with siControl or siTUBB and were treated with doxycycline, followed by serum starvation for 48 hours. Scale bar:  $5\ \mu\text{m}$ . **g**, Quantification of frequency of interphase cells with primary cilia in **f**.  $n = 3$  independent experiments, 50 cells each. **h**, Quantification of the length of primary cilia in **f**.  $n = 50$  cells pooled from 3 independent experiments. Data are represented as mean  $\pm$  s.d..  $P$  values were calculated by one-way ANOVA with Tukey's multiple comparisons test (**b**, **e**, **g**) or Dunn's multiple comparisons test (**h**). Source data are provided as a Source Data file.

**Supplementary Table 1 Plasmids generated in this study.**

| Plasmid                                        | Vector                     | Insertion                           | Primer sequence for PCR (5'-3') |                                                                        |
|------------------------------------------------|----------------------------|-------------------------------------|---------------------------------|------------------------------------------------------------------------|
|                                                |                            |                                     |                                 |                                                                        |
| pCMV5-FLAG-HYLS1                               | pCMV5-FLAG                 | HYLS1                               | F                               | TTCTCTGCAGGTCGACATGGAAGAAGCTTCTACCTGA                                  |
|                                                |                            |                                     | R                               | CCGGGATCAGGATCCTTAAGAAGGAGAAAGAGGGA                                    |
| pCMV5-HA-HYLS1                                 | pCMV5-HA                   | HYLS1                               | F                               | TTCTCTGCAGGTCGACATGGAAGAAGCTTCTACCTGA                                  |
|                                                |                            |                                     | R                               | CCGGGATCAGGATCCTTAAGAAGGAGAAAGAGGGA                                    |
| pCMV5-FLAG-HYLS1<br>( <i>C. elegans</i> )      | pCMV5-FLAG                 | HYLS1<br>( <i>C. elegans</i> )      | F                               | TTCTCTGCAGGTCGACATGGCCAATTTTACTACAG                                    |
|                                                |                            |                                     | R                               | CCGGGATCAGGATCCTCAGTCAATATAAGGTCGTG                                    |
| pCMV5-FLAG-HYLS1<br>( <i>D. melanogaster</i> ) | pCMV5-FLAG                 | HYLS1<br>( <i>D. melanogaster</i> ) | F                               | TTCTCTGCAGGTCGACATGTCCCATCTGCCGCTCG                                    |
|                                                |                            |                                     | R                               | CCGGGATCAGGATCCTCACTTCTTGAGTCCCATG                                     |
| pCMV5-mScarlet-i-HYLS1                         | pCMV5-FLAG-HYLS1           | mScarlet-i                          | F                               | GAATTGGCCGCCACCATGGTGAGCAAGGGCGAG                                      |
|                                                |                            |                                     | R                               | TAGAAGTTCTTCCATTGCACCAGCTCCTGCACC                                      |
| pCMV5-FLAG-HYLS1<br>(1-100 a.a.)               | pCMV5-FLAG                 | HYLS1<br>(1-100 a.a.)               | F                               | TTCTCTGCAGGTCGACATGGAAGAAGCTTCTACCTGA                                  |
|                                                |                            |                                     | R                               | CCGGGATCAGGATCCTTAGCGCAGCACCTTTCTCTT                                   |
| pCMV5-FLAG-HYLS1<br>(1-150 a.a.)               | pCMV5-FLAG                 | HYLS1<br>(1-150 a.a.)               | F                               | TTCTCTGCAGGTCGACATGGAAGAAGCTTCTACCTGA                                  |
|                                                |                            |                                     | R                               | CCGGGATCAGGATCCTTAAACATCAAATGAAGATTC                                   |
| pCMV5-FLAG-HYLS1<br>(1-200 a.a.)               | pCMV5-FLAG                 | HYLS1<br>(1-200 a.a.)               | F                               | TTCTCTGCAGGTCGACATGGAAGAAGCTTCTACCTGA                                  |
|                                                |                            |                                     | R                               | CCGGGATCAGGATCCTTACAGCTTTGGGAGAATAAAG                                  |
| pCMV5-FLAG-HYLS1<br>(101-200 a.a.)             | pCMV5-FLAG                 | HYLS1<br>(101-200 a.a.)             | F                               | TTCTCTGCAGGTCGACAGAAAGCCAGATGGGGAAG                                    |
|                                                |                            |                                     | R                               | CCGGGATCAGGATCCTTACAGCTTTGGGAGATAAAG                                   |
| pCMV5-FLAG-HYLS1<br>(101-299 a.a.)             | pCMV5-FLAG                 | HYLS1<br>(101-299 a.a.)             | F                               | TTCTCTGCAGGTCGACAGAAAGCCAGATGGGGAAG                                    |
|                                                |                            |                                     | R                               | CCGGGATCAGGATCCTTAAAGAAGGAGAAAGAGGGA                                   |
| pCMV5-FLAG-HYLS1<br>(151-299 a.a.)             | pCMV5-FLAG                 | HYLS1<br>(151-299 a.a.)             | F                               | TTCTCTGCAGGTCGACTCACAAAAATTAACCTAC                                     |
|                                                |                            |                                     | R                               | CCGGGATCAGGATCCTTAAAGAAGGAGAAAGAGGGA                                   |
| pCMV5-FLAG-HYLS1<br>(201-299 a.a.)             | pCMV5-FLAG                 | HYLS1<br>(201-299 a.a.)             | F                               | TTCTCTGCAGGTCGACGACCAGTTAAGCCGAAACC                                    |
|                                                |                            |                                     | R                               | CCGGGATCAGGATCCTTAAAGAAGGAGAAAGAGGGA                                   |
| pCMV5-FLAG-HYLS1<br>D211G                      | pCMV5-FLAG-HYLS1           |                                     | F                               | AAGACAGGCCGGGTAGCCCCGGTATTTTG                                          |
|                                                |                            |                                     | R                               | TACCCGGCCTGTCTTGCCCCGGTTTCGG                                           |
| pCMV5-FLAG-TUBB                                | pCMV5-FLAG                 | TUBB                                | F                               | TTCTCTGCAGGTCGACATGAGGGAAATCGTGCAC                                     |
|                                                |                            |                                     | R                               | CCGGGATCAGGATCCTTTAGGCCTCCTCTTCGGC                                     |
| pCMV5-TUBB                                     | pCMV5                      | TUBB                                | F                               | GAATTGGCCGCCACCATGAGGGAAATCGTGCAC                                      |
|                                                |                            |                                     | R                               | CCGGGATCAGGATCCTTAGGCCTCCTCTTCGGCCTC                                   |
| pCMV5-TUBB<br>(1-429 a.a.)                     | pCMV5                      | TUBB<br>(1-429 a.a.)                | F                               | GAATTGGCCGCCACCATGAGGGAAATCGTGCAC                                      |
|                                                |                            |                                     | R                               | CCGGGATCAGGATCCTTAGGTGGCATCCTGGTACTG                                   |
| pCMV5-TUBA1B                                   | pCMV5                      | TUBA1B                              | F                               | GAATTGGCCGCCACCATGCGTGAGTGCATCTCC                                      |
|                                                |                            |                                     | R                               | CCGGGATCAGGATCCTTAGTATTCCTCTCCTTC                                      |
| pCMV5-TUBA1B<br>(1-439 a.a.)                   | pCMV5                      | TUBA1B<br>(1-439 a.a.)              | F                               | GAATTGGCCGCCACCATGCGTGAGTGCATCTCC                                      |
|                                                |                            |                                     | R                               | CCGGGATCAGGATCCTTAATCCACACCAACCTCCTC                                   |
| pCMV5-TUBB4B                                   | pCMV5                      | TUBB4B                              | F                               | GAATTGGCCGCCACCATGAGGGAAATCGTGCAC                                      |
|                                                |                            |                                     | R                               | CCGGGATCAGGATCCTTAGGCCACCTCCTCCTCAGC                                   |
| pCMV5-TUBB4B<br>(1-429 a.a.)                   | pCMV5                      | TUBB4B<br>(1-429 a.a.)              | F                               | GAATTGGCCGCCACCATGAGGGAAATCGTGCAC                                      |
|                                                |                            |                                     | R                               | CCGGGATCAGGATCCTTATGTGGCATCCTGGTACTG                                   |
| pRetroX-TRE3G-mNG-HYLS1                        | pRetroX-TRE3G-mNeonGreen   | HYLS1                               | F                               | TGGTGCAGGCGGCCGCATGGAAGAAGCTTCTACCTG                                   |
|                                                |                            |                                     | R                               | CTACCCGGTAGAATCTTAAGAAGGAGAAAGAGGG                                     |
| pRetroX-TRE3G-mNG-HYLS1 RR                     | pRetroX-TRE3G-mNG-HYLS1    |                                     | F                               | GACCGACGAATCCATCATCAGTGAATCAGAATCTG                                    |
|                                                |                            |                                     | R                               | GATGGATTCTGTCGGTCACTAATACTTCCCCATCTG                                   |
| pRetroX-TRE3G-mNG-HYLS1 D211G RR               | pRetroX-TRE3G-mNG-HYLS1 RR |                                     | F                               | AAGACAGGCCGGGTAGCCCCGGTATTTTG                                          |
|                                                |                            |                                     | R                               | TACCCGGCCTGTCTTGCCCCGGTTTCGG                                           |
| pRetroX-TRE3G-TUBB-FLAG                        | pRetroX-TRE3G-FLAG         | TUBB                                | F                               | ATCCATCGATGCCACCATGAGGGAAATCGTGCACAT                                   |
|                                                |                            |                                     | R                               | TTCCGGCCCTTACTGTGTCATCGTCCTTGATGTCGGCCTCTCTTCGGCCTCCT                  |
| pRetroX-TRE3G-TUBB RR-FLAG                     | pRetroX-TRE3G-TUBB-FLAG    |                                     | F                               | GTAATAATTCGGGAGGAGTACCCTGATCGCATCATGAA                                 |
|                                                |                            |                                     | R                               | TACTCCTCCCGAATTTTACTGATAAGGAGAGTGCCCAT                                 |
| pRetroX-TRE3G-TUBB G437E RR-FLAG               | pRetroX-TRE3G-TUBB RR-FLAG |                                     | F                               | GATTTTCGAGGAGGAGGCCGAAGAGGAG                                           |
|                                                |                            |                                     | R                               | CTCCTCCTCGAAATCCTCCTCCTCTTC                                            |
| pRetroX-TRE3G-TUBB4B-FLAG                      | pRetroX-TRE3G-FLAG         | TUBB4B                              | F                               | CTTATACTTGGATCCATCGATGCCACCATGAGGGAAA                                  |
|                                                |                            |                                     | R                               | TACCCGGTAGAATTCGGGCCCTTACTTGTGTCATCGTCGTCCTTGATGTCGGCCACCTCCTCCTCAGCCT |

| Plasmid                               | Vector                        | Insertion              | Primer sequence for PCR (5'-3') |                                     |
|---------------------------------------|-------------------------------|------------------------|---------------------------------|-------------------------------------|
| pRetroX-TRE3G-TUBB4B-TUBB CTT-FLAG    | pRetroX-TRE3G-TUBB CTT-FLAG   | TUBB4B<br>(1-429 a.a.) | F                               | TCCATCGATGCCACCATGAGGGAAATCGTGCAC   |
|                                       |                               |                        | R                               | CTCCTCCTCTTCTGCTGTGGCATCCTGGTACTG   |
| pRetroX-TRE3G-TUBB-TUBB4B CTT-FLAG    | pRetroX-TRE3G-TUBB4B CTT-FLAG | TUBB<br>(1-429 a.a.)   | F                               | TCCATCGATGCCACCATGAGGGAAATCGTGCAC   |
|                                       |                               |                        | R                               | GCCCTCCTCCTCGGCGGTGGCATCCTGGTACTG   |
| pQCXIZ-TUBB-mNG                       | pQCXIZ-mNeonGreen             | TUBB                   | F                               | CGCGGCCGCGCCACCATGAGGGAAATCGTGCAC   |
|                                       |                               |                        | R                               | ACCTGCACCAGCTCCGGCCTCCTCTTCGGCCTC   |
| pBluescript-HYLS1 N ter.              | pBluescript                   | HYLS1 N ter.           | F                               | GGTATCGATAAGCTTAGACAGTAGAAAGCAGGAGT |
|                                       |                               |                        | R                               | CGCTCTAGAACTAGTCAGAGACTGTTTCTGAAGGG |
| pBluescript-HygR-mCherry-HYLS1 N ter. | pBluescript-HYLS1 N ter.      | HygR-mCherry           | F                               | ATGAAGCCTGAACTCACC GC               |
|                                       |                               |                        | R                               | GGCGCCTGCACCGGATCCCT                |
| pX330-hSpCas9-sgHYLS1                 | pX330-hSpCas9                 | sgHYLS1                | F                               | CACCGCTGCCAACATTCTTCTTC             |
|                                       |                               |                        | R                               | AAACGAAGAACGAATGTTGGCAGC            |

**Supplementary Table 2 siRNAs used in this study.**

| <b>siRNA</b> | <b>Supplier</b>          | <b>Cat#</b> | <b>Sense sequence (5'-3')</b> |
|--------------|--------------------------|-------------|-------------------------------|
| siHYLS1#1    | Thermo Fisher Scientific | s47731      | GUAACAGAUGAGUCGAUUATT         |
| siHYLS1#2    | Thermo Fisher Scientific | s47732      | GCCCGGUAUUUUGAGUACATT         |
| siTUBA1A#1   | Thermo Fisher Scientific | s15400      | GGUUAUAGGUCAAAUUGUTT          |
| siTUBA1A#2   | Thermo Fisher Scientific | s15401      | GUUGUGGUCUGAUCAGUUATT         |
| siTUBA1B#1   | Thermo Fisher Scientific | s20288      | CAUCAUAUCUCAAAGUAAATT         |
| siTUBA1B#2   | Thermo Fisher Scientific | s20289      | AAAGCUUUCUGGUUAGAUUTT         |
| siTUBA1C#1   | Thermo Fisher Scientific | s195488     | ACCUGUGUGCUGUACUUUUTT         |
| siTUBA1C#2   | Thermo Fisher Scientific | s224963     | GUCUUGGAACUGUCUUUUTT          |
| siTUBA3C#1   | Thermo Fisher Scientific | s194842     | CGGUCAUCUCAGCCGAGAATT         |
| siTUBA3C#2   | Thermo Fisher Scientific | s194843     | AGGAGAUCGUCGACCUGGUTT         |
| siTUBA3D     | Thermo Fisher Scientific | s195595     | GCCUGGACCAUAAGUUCGATT         |
| siTUBA3E#1   | Thermo Fisher Scientific | s41356      | CAAGCGCACUAUCCAGUUUTT         |
| siTUBA3E#2   | Thermo Fisher Scientific | s195587     | GCACUAUCCAGUUUGUGGATT         |
| siTUBA4A#1   | Thermo Fisher Scientific | s14497      | GCCGCAACCUAGACAUCGATT         |
| siTUBA4A#2   | Thermo Fisher Scientific | s14498      | CUAUGCCCGUGGUCACUAUTT         |
| siTUBA8#1    | Thermo Fisher Scientific | s28671      | GGAUGUGAAUGUCGCUAUUTT         |
| siTUBA8#2    | Thermo Fisher Scientific | s28672      | GGGCCGUCAUGAUAGAUCUTT         |
| siTUBB1#1    | Thermo Fisher Scientific | s37479      | CAGUCUUGGUGGACCUAGATT         |
| siTUBB1#2    | Thermo Fisher Scientific | s37480      | CAUUCGAUCUAGCAAAUATT          |
| siTUBB2A#1   | Thermo Fisher Scientific | s14500      | GUAUUGUCUGUGAAGGUATT          |
| siTUBB2A#2   | Thermo Fisher Scientific | s14501      | UCCUCUAAAAAUACAGUATT          |
| siTUBB2B#1   | Thermo Fisher Scientific | s51294      | CAACCUGAGUUUUAAAAUATT         |
| siTUBB2B#2   | Thermo Fisher Scientific | s223916     | GGUAAAAGCUCAAAUGAAUTT         |
| siTUBB3#1    | Thermo Fisher Scientific | s20296      | GCAAGGUGCGUGAGGAGUATT         |
| siTUBB3#2    | Thermo Fisher Scientific | s20297      | AAUCUAUUGCUGUCAGAUATT         |
| siTUBB4A#1   | Thermo Fisher Scientific | s20299      | ACUUCUCUUUGAUCUCUGATT         |
| siTUBB4A#2   | Thermo Fisher Scientific | s20300      | GGAGGUUAUCAGUGACGAATT         |
| siTUBB4B#1   | Thermo Fisher Scientific | s20301      | AGACCUACUGCAUUGAUAATT         |
| siTUBB4B#2   | Thermo Fisher Scientific | s20302      | ACAACUUCGUUUUCGGUCATT         |
| siTUBB#1     | Thermo Fisher Scientific | s284        | GCAAGAUCCGAGAAGAAUATT         |
| siTUBB#2     | Thermo Fisher Scientific | s283        | GCACUCUCCUUAUCAGCAATT         |
| siTUBB6#1    | Thermo Fisher Scientific | s39180      | GCAAGAUCCGUGAGGAGUUTT         |
| siTUBB6#2    | Thermo Fisher Scientific | s39182      | GGAACAAAGACUAAAAACATT         |
| siTUBB8#1    | Thermo Fisher Scientific | s51282      | ACAACUUCAUCUUCGGUCATT         |
| siTUBB8#2    | Thermo Fisher Scientific | s196882     | AACUCUCCUUUUCUAGGUATT         |
| siControl    | Thermo Fisher Scientific | 4390843     |                               |

**Supplementary Table 3 Primers used in genomic PCR.**

| Figure                | Target | Sequence (5'-3') |                      |
|-----------------------|--------|------------------|----------------------|
|                       |        |                  |                      |
| Supplementary Fig. 1b | HYLS1  | F                | GACCTGAAGGTGGCAAGACA |
|                       |        | R                | TTTCGGAGTCTTTGGGAGGC |

**Supplementary Table 4 Primers used in real-time PCR.**

| Figure                | Target | Sequence (5'-3') |                        |
|-----------------------|--------|------------------|------------------------|
| Supplementary Fig. 8b | TUBA1A | F                | CTTCGTCTCCGCCATCAG     |
|                       |        | R                | TTGCCAATCTGGACACCA     |
|                       | TUBA1B | F                | CCCTAGCCACTATGCGTGA    |
|                       |        | R                | CGTGTTCCAGGCAGTAGAGC   |
|                       | TUBA1C | F                | CCCCTTCAAGTTCTAGTCATGC |
|                       |        | R                | GCATTGCCAATCTGGACAC    |
|                       | TUBB3  | F                | GCAACTACGTGGGCGACT     |
|                       |        | R                | CGAGGCACGTACTTGTGAGA   |
|                       | TUBB4B | F                | CTGCTGCTGTTTGTCTACTTCC |
|                       |        | R                | CTGATCACCTCCCCAAAACCTG |
|                       | TUBB   | F                | ATACCTTGAGGCGAGCAAAA   |
|                       |        | R                | TCACTGATCACCTCCCAGAAC  |
|                       | GAPDH  | F                | TCCACTGGCGTCTTCACC     |
|                       |        | R                | GGCAGAGATGATGACCCTTTT  |