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## Septin-microtubule association requires a MAP-like motif unique to Sept9 isoform 1 embedded into septin octamers

Mira Kuzmić, Gerard Castro Linares, Jindřiška Leischner Fialová, Francois Iv, Danièle Salaün, Alex Llewellyn, Maxime Gomes, Mayssa Belhabib, Yuxiang Liu, Keisuke Asano, et al.

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1 **Septin-microtubule association requires a MAP-like motif unique to Sept9 isoform 1**  
2 **embedded into septin octamers**

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47 **Abstract**

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Septins, a family of GTP-binding proteins assembling into higher order structures, interface with the membrane, actin filaments and microtubules, which positions them as important regulators of cytoarchitecture. Septin 9 (Sept9), which is frequently overexpressed in tumors and mutated in hereditary neuralgic amyotrophy (HNA), mediates the binding of septins to microtubules, but the molecular determinants of this interaction remained uncertain. We demonstrate that a short MAP-like motif unique to Sept9 isoform 1 (Sept9\_i1) drives septin octamer-microtubule interaction in cells and *in vitro* reconstitutions. Septin-microtubule association requires polymerizable septin octamers harboring Sept9\_i1. Although outside of the MAP-like motif, HNA mutations abrogates this association, identifying a putative regulatory domain. Removal of this domain from Sept9\_i1 sequesters septins on microtubules, promotes microtubule stability and alters actomyosin fiber distribution and tension. Thus, we identify key molecular determinants and potential regulatory roles of septin-microtubule interaction, paving the way to deciphering the mechanisms underlying septin associated pathologies.

## 93 Introduction

94  
95 Microtubules and actin filaments are cytoskeletal polymers involved in key cellular  
96 functions. Microtubules assemble from tubulin dimers to form hollow tubes that have the ability  
97 to switch between growing and shortening phases, a process called dynamic instability.  
98 Microtubule diversity arises, from tubulin isotypes, from post-translational modifications,  
99 including acetylation and glutamylation, often associated with stable sub-populations of  
100 microtubules, and from associations with specific microtubule-associated proteins (MAPs) that  
101 modulate microtubule dynamics (Janke & Magiera, 2020). Microtubules are critically involved  
102 in the segregation of chromosomes during cell division and the directed transport of various  
103 intracellular cargoes (Carlton, Jones et al., 2020). Actin monomers assemble into double helical  
104 strands that in cells are often cross-linked into bundles and networks (Maninova, Caslavsky et  
105 al., 2017). In particular, the presence of associated myosins confers contractility to these actin  
106 fiber assemblies, that is essential during both cell division and migration. Such bundled  
107 actomyosin fibers form stress fibers that are qualified as peripheral and ventral when running  
108 near the cortex and the cell base, respectively. Stress fibers connect focal adhesions and the  
109 perinuclear actin cap linked to the top of the nucleus controlling its position and shape (Sneider,  
110 Hah et al., 2019). Several biological processes depend on the coordinated regulation of the actin  
111 and microtubule cytoskeletons, mediated by a diversity of molecular crosstalk between the two  
112 cytoskeletons. Direct and indirect contacts are involved, for instances, in cytokinetic furrow  
113 positioning, cell migration steering or maturation of neuronal dendritic spines (Dogterom &  
114 Koenderink, 2019).

115 Septins are GTP-binding protein that form heteropolymeric complexes associating with  
116 membranes, actin filaments and a subset of microtubules (Woods & Gladfelter, 2020). Like yeast  
117 and *Drosophila* septins, human septin hexameric and octameric protomers can polymerize into  
118 filaments (Bertin, McMurray et al., 2012, DeRose, Kelley et al., 2020, Iv, Martins et al., 2021,  
119 Ong, Wloka et al., 2014, Soroor, Kim et al., 2021, Szuba, Bano et al., 2020, Valadares, d' Muniz  
120 Pereira et al., 2017). Septins are thought to function as diffusion barriers for protein  
121 compartmentalization or as scaffolds for protein-protein interactions during cells division and in  
122 an increasing number of processes during interphase (Fung, Dai et al., 2014, Marquardt, Chen et  
123 al., 2019, Mostowy & Cossart, 2012). During cytokinesis, septins are recruited to the sub-  
124 membrane cortex by anillin (Oegema, Savoian et al., 2000, Renshaw, Liu et al., 2014) and co-  
125 localize with the constricting actomyosin ring, prior to the specification and the maintenance of  
126 the ingression furrow position by microtubules (Straight & Field, 2000, Verma & Maresca,  
127 2019). Septins appear also to control abscission (Addi, Bai et al., 2018, Estey, Di Ciano-Oliveira  
128 et al., 2010, Kim, Froese et al., 2011, Kinoshita, Kumar et al., 1997, Renshaw et al., 2014, Surka,  
129 Tsang et al., 2002) that achieves cell division. It is still unclear how human septins interface with  
130 microtubules and the acto-myosin networks. Septins form a collar structure at the neck of  
131 dendritic spines, nanotubule-like protrusions and micro-tentacles to guide microtubules (Nolke,  
132 Schwan et al., 2016, Ostevold, Melendez et al., 2017, Tada, Simonetta et al., 2007, Yadav, Osés-  
133 Prieto et al., 2017). In adherent cells, septins concentrate on ventral stress fibers, and  
134 occasionally on perinuclear microtubules, depending on the cell type. Despite studies showing  
135 that septins regulate the traffic of motor proteins, like kinesin, the binding of MAPs and the  
136 guiding of microtubules in polarizing epithelia (Spiliotis, 2010), the functional consequences of  
137 septin-microtubule interaction are still largely unknown.

138 The 13 human septin genes encode four protein sequence homology-groups named after  
139 Sept2, Sept6, Sept7 and Sept3; septins from each group interact with each other in a specific  
140 order to form hexamers and octamers (Kim et al., 2011, Sellin, Stenmark et al., 2012, Sirajuddin,  
141 Farkasovsky et al., 2007). Septin octamers differ from hexamers by the addition of septins from  
142 the Sept3 group, with their arrangement recently determined as Sept2-Sept6-Sept7-Sept3-Sept3-  
143 Sept7-Sept6-Sept2 (Mendonca, Macedo et al., 2019, Soroor et al., 2021). Septins include a  
144 conserved GTP-binding domain (G domain) flanked by variable N- and C-termini. Septins  
145 assemble by the G domain on one side forming the G:G interface and by the N- and C- termini  
146 forming the NC:NC interface on the opposite side. This results in  
147 ( ${}_{\text{NC}}\text{Sept2}_{\text{G:G}}\text{Sept6}_{\text{NC:NC}}\text{Sept7}_{\text{G:G}}\text{Sept7}_{\text{NC:NC}}\text{Sept6}_{\text{G:G}}\text{Sept2}_{\text{NC}}$ ) hexamers and  
148 ( ${}_{\text{NC}}\text{Sept2}_{\text{G:G}}\text{Sept6}_{\text{NC:NC}}\text{Sept7}_{\text{G:G}}\text{Sept3}_{\text{NC:NC}}\text{Sept3}_{\text{G:G}}\text{Sept7}_{\text{NC:NC}}\text{Sept6}_{\text{G:G}}\text{Sept2}_{\text{NC}}$ ) octamers that can  
149 polymerize via a universal  $\text{Sept2}_{\text{NC:NC}}\text{Sept2}$  interface, that is labile in presence of high salt  
150 concentrations. In the Sept3 group, Sept9 has been the most studied because it is ubiquitously  
151 expressed, and often overexpressed in tumors (Connolly, Abdesselam et al., 2011). Interestingly  
152 Sept9 is mutated in a large number of hereditary neuralgic amyotrophy (HNA) patients (Collie,  
153 Landsverk et al., 2010, Hannibal, Ruzzo et al., 2009, Kuhlenbaumer, Hannibal et al., 2005).  
154 Alternative splicing resulting in alternative translation start sites gives rise to five Sept9 isoforms  
155 (Sept9\_i1 to\_i5) differing by the length and/or sequence of their N-terminus and by their  
156 functions (Connolly, Yang et al., 2011, McIlhatton, Burrows et al., 2001, Verdier-Pinard, Salaun  
157 et al., 2017). Sept9\_i1, \_i2 and \_i3 long isoforms share a common structurally disordered N-  
158 terminal domain (common N-ter) that was proposed, based on *in vitro* studies, to mediate  
159 binding to microtubules (Bai, Bowen et al., 2013). However, several studies indicate that only  
160 the isoform 1 of Sept9 (Sept9\_i1) drives the association of septins with microtubules (Nagata,  
161 Kawajiri et al., 2003, Sellin et al., 2012, Surka et al., 2002, Verdier-Pinard et al., 2017). In order  
162 to understand the contribution of septin-microtubule interaction to cell physiology and disease, it  
163 is critical to identify and characterize precisely the molecular determinants of this interaction.

164  
165 In the present study, we show that septin association with microtubules requires a MAP-  
166 like motif specific of Sept9\_i1 and the integration of Sept9\_i1 within polymerized septin  
167 octamers. *In vitro* reconstitution allowed us to demonstrate a direct and specific interaction of  
168 Sept9\_i1-harboring septin octamers with microtubules, which slows down depolymerization of  
169 microtubules. Based on this molecular characterization, we designed specific genetic variants,  
170 including HNA-like mutations, which modulate Sept9\_i1-microtubule interaction and we show  
171 that relocalizing septins to microtubules disturbs stress fiber distribution and tension.

## 172 173 **Results**

174  
175 **Different Sept9 isoform expression profiles are associated with distinct**  
176 **octamers/hexamers ratios and cytoskeletal localization.** We have analyzed septin expression  
177 profiles in commonly used cell models, namely U2OS osteosarcoma, HeLa cervical carcinoma  
178 cells, RPE1 retinal epithelial cells and SKBr3 breast carcinoma cells (Fig. 1a). Sept7 being  
179 unique in its group, it is essential for the formation of hexamers and octamers (Supplementary  
180 Fig. 1d). The four cell lines examined expressed very similar levels of Sept7. In the Sept2 group,  
181 Sept2 was expressed in all cell lines and Sept5 was only detected in U2OS cells. Expression of  
182 septins from the Sept6 group was more variable, except for Sept8 that was expressed at similar  
183 levels in all cell lines. U2OS and HeLa cells expressed similar levels of total Sept9. However,

184 U2OS cells expressed mostly isoform 3, whereas HeLa cells expressed mostly isoform 1. RPE1  
185 and SKBr3 cells expressed higher levels of Sept9 with similar levels of Sept9\_i1 and Sept9\_i2,  
186 and no or very low levels of Sept9\_i3.

187 To assess how Sept9 expression profiles affected its incorporation in septin hexamers and  
188 octamers, we extracted cells in conditions that preserved oligomers and performed native gel  
189 electrophoresis and analysis by Western blotting (Fig. 1b and Supplementary Fig. 1). Based on  
190 the detection of the essential Sept7 and pan-expressed Sept8, U2OS and HeLa cells contained  
191 more hexamers than octamers, as opposed to RPE1 and SKBr3 cells. This result is consistent  
192 with higher Sept9 expression levels in RPE1 and SKBr3 leading to higher levels of octamers. In  
193 addition, our results indicate that long Sept9 isoforms incorporate indifferently in octamers. We  
194 did not detect smaller septin assemblies containing Sept9 monomers or dimers, suggesting that,  
195 at endogenous levels, the entire pool of Sept9 is stably incorporated into octamers.

196 Next, we examined the localization of Sept9 in these cell lines either in interphase or in  
197 late cytokinesis (Fig. 1c, d). We observed that Sept9 was associated with actin fibers in all  
198 interphase cells, especially on ventral stress fibers (Fig. 1c, d). Interestingly, consistent with  
199 previous observation (Verdier-Pinard et al., 2017), Sept9 co-localized with a subpopulation of  
200 acetylated microtubules in HeLa and SKBr3 cells; in cytokinetic cells, Sept9 concentrated  
201 cortically at the ingression furrow and on the thick bundles of acetylated microtubules contained  
202 in the intercellular bridge. In U2OS cells, which express primarily Sept9\_i3, septins associated  
203 with the actin cytoskeleton but did not co-localize with microtubules. These results indicate that  
204 the association of septins with microtubules is dependent on the presence of octamers harboring  
205 Sept9\_i1. It is less clear why in RPE1 cells, despite Sept9\_i1 levels comparable to SKBr3 cells,  
206 there was no association of Sept9 with microtubules, except in primary cilia (Fig. 1e).

207  
208 **Sept9\_i1 interacts with microtubules via a specific MAP-like motif.** The fact that  
209 only Sept9\_i1, but not the closely related i2 and i3 isoforms, is required for septins to bind  
210 microtubules identifies the sequence formed by the first specific 25 amino acid residues of the  
211 Sept9\_i1 protein, as specific to this isoform and as key for its interaction with microtubules  
212 (Verdier-Pinard et al., 2017). This sequence being well conserved in vertebrates (Supplementary  
213 Fig. 2), it is also indicative of an important function. Searching for similar sequences in proteins  
214 related to the microtubule cytoskeleton by using the NCBI Blastp tool, we found the Arabidopsis  
215 thaliana AIR9 protein, a plant MAP involved in cytokinesis (Buschmann, Chan et al., 2006). The  
216 sequence of AIR9 similar to the Sept9\_i1 specific sequence was located in the AIR9-microtubule  
217 binding domain (AIR9 1-234) (Buschmann et al., 2006). Alignment of these two sequences  
218 reveals a block of similar sequence that we named AIR-9-like (Fig. 2a). Another sequence,  
219 located upstream in the AIR9 N-terminus (aa 46-78), presents a lower similarity with the  
220 Sept9\_i1 specific sequence (Fig. 2a). Strikingly, the AIR9-like block had similarities with  
221 canonical repeats found in the microtubule binding domains (MBDs) of the structural human  
222 MAPs, tau, MAP2 and MAP4, which are known to interact directly with the surface of  
223 microtubules (Fig. 2a). An upstream smaller block in the Sept9\_i1 specific sequence showed  
224 similarities with the MAP4 R2 repeat. Thus, Sept9\_i1 displays a putative MBD, similar to the  
225 MBD repeats of several MAPs and constituted by a MAP4 R2-like sequence followed by an  
226 AIR9-like sequence.

227 To unequivocally demonstrate that this motif is actually the MBD of Sept9\_i1, we  
228 examined the localization of Sept9\_i1 mutants (C-terminally tagged with GFP), presenting  
229 deletions or point mutations in the first 25 amino acid residues (Fig. 2b), in U2OS cells (Fig. 2c,

230 d and Supplementary Fig. 3a-c). U2OS cells were chosen because they express very low levels  
231 of Sept9\_i1 (Fig. 1a), and show no Sept9-microtubule co-localization (Fig. 1c, d). In addition,  
232 U2OS cells were amenable to simultaneous siRNA-mediated knockdown (KD) of Sept9 and  
233 expression of exogenous Sept9\_i1-GFP constructs at near-endogenous Sept9 levels  
234 (Supplementary Fig. 3a). Wild type Sept9\_i1 co-localized with actin in all cells and with  
235 microtubules in more than 60% of the cells. Sept9\_i3 and the  $\Delta$ 1-25 Sept9\_i1 mutant also  
236 associated with actin fibers, but never localized to microtubules (Fig. 2c, d, f and Supplementary  
237 Fig. 3b, d). This result is consistent with our previous observations of Sept9\_i3 and Sept9\_i1  $\Delta$ 1-  
238 25 vs Sept9\_i1 localization in SKBr3 cells (using N-terminally tagged constructs) (Verdier-  
239 Pinard et al., 2017). Deletion of the AIR9-like portion ( $\Delta$ 10-25 mutant) or of the MAP4 R2-like  
240 portion ( $\Delta$ 1-7 mutant) totally or largely abrogated Sept9 binding to microtubules, respectively  
241 (Fig. 2c and Supplementary Fig. 3b). Next, we introduced point mutations in the AIR9-like  
242 portion (Fig. 2b). Independent mutations of two sets of contiguous serine residues (S12A/S13A  
243 and S22A/S23A), conserved in the AIR9 MBD, did not significantly alter the co-localization of  
244 Sept9 with microtubules (Fig. 2c, d and Supplementary Fig. 3b, c). Importantly, mutations of the  
245 two arginine residues (R10A/R15A), conserved in all vertebrates (Supplementary Fig. 2) and  
246 present in the AIR9 MBD, completely abrogated the binding of Sept9 to microtubules (Fig. 2c, d  
247 and Supplementary Fig. 3b, c). Collectively, our data show that the Sept9\_i1 specific N-terminal  
248 sequence is an MBD analogous to the ones found in human MAPs and the plant AIR9 MAP. Of  
249 note, in contrast to MAP MBDs, the Sept9\_i1 specific sequence is not tandem-repeated and lacks  
250 upstream conserved residues (Fig. 2a).

251 A previous report proposed, based on *in vitro* studies using recombinant polypeptides,  
252 that Sept9\_i1<sub>61-113</sub> within the N-terminal region common to all Sept9 long isoforms (i1, i2 and i3)  
253 was the main contributor to microtubule binding (Bai et al., 2013). To evaluate a potential  
254 contribution of Sept9 N-terminal region common to all long isoforms (common N-ter), we  
255 generated a chimeric Sept9 deleted of the entire common N-ter by fusing the isoform 1 specific  
256 sequence to Sept9\_i5, the shortest Sept9 isoform, and named it Sept9\_i1-i5 (Fig. 2e). Whereas  
257 Sept9\_i5 localized strictly to actin fibers (Fig. 2f, h and Supplementary Fig. 3e), its fusion to the  
258 Sept9\_i1 specific motif was sufficient to localize septins to microtubules in all interphase cells.  
259 Surprisingly, this was accompanied by the complete loss of Sept9 co-localization with actin  
260 fibers (Fig. 2f, h). Consistent with these findings, expression of Sept9\_i1-i5 during cytokinesis  
261 increased strongly the percentage of cells showing Sept9 association with microtubule bundles at  
262 the intercellular bridge (Supplementary Fig. 3d).

263 Our results demonstrate that the Sept9\_i1 MBD is required and sufficient to target Sept9  
264 to microtubules, whereas the N-terminal region common to all long isoforms is dispensable.  
265 Interestingly the Sept9\_i1-i5 is much more efficient than Sept9\_1 for targeting septins to  
266 microtubules (Fig. 2f, h and Supplementary Fig. 3d), hinting that the common N-ter might have a  
267 negative regulatory function. Sept9 is mutated in hereditary neuralgic amyotrophy (HNA)  
268 patients. HNA-associated mutations are located in the Sept9 common N-ter (Kuhlenbaumer et  
269 al., 2005), but their impact on Sept9 function remains to be elucidated. We introduced separately  
270 the two most frequently described HNA mutations (R106W or S111F) in Sept9\_i1 constructs  
271 and evaluated their impact on Sept9\_i1 localization. Both Sept9\_i1 R106W and S111F  
272 maintained co-localization with ventral stress fibers (Fig. 2g, h). However, both mutations  
273 suppressed the association with microtubules (Fig. 2g, h). This result confirms that the common  
274 N-ter, while not required for Sept9 microtubule binding per se, is an important regulatory domain  
275 and provides novel insights into the molecular etiology of HNA.

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**Sept9\_i1 must be included in a polymerized septin octamer to associate with microtubules.** In contrast to typical MBDs in structural MAPs, the Sept9\_i1 specific sequence does not have tandem repetitions. We hypothesized that the dimerization of Sept9\_i1 in octamers via the NC interface brings two Sept9\_i1 specific sequences in close proximity, to form a MBD mimicking tandem arrangements of repeats. To test this hypothesis and the oligomeric environment required for microtubule binding, we introduced point mutations into Sept9\_i1 that should disturb the NC interface to generate tetramers, or the G interface to generate non-incorporated Sept9\_i1 (Sirajuddin et al., 2007) (Fig. 3a). We then expressed these constructs in U2OS cells in place of endogenous Sept9 (KD via siRNA). Importantly, re-expression of wild type Sept9\_i1-GFP restored close to endogenous levels of octamers and hexamers (Fig. 3a). As expected, most of the Sept9\_i1 G interface mutant (Sept9\_i1 G<sub>mut</sub>) did not incorporate in octamers (Fig. 3a). Instead, it migrated with an apparent molecular weight of 138 kDa, corresponding most likely to monomeric Sept9\_i1, whose gel motility is retarded by the long and disordered N-terminus (Sellin et al., 2012) (Supplementary Fig. 1a). Based on previous studies (Bertin, McMurray et al., 2010, Kim et al., 2011, Sirajuddin et al., 2007), we generated two Sept9\_i1 NC interface mutants with mutations in the  $\alpha 0$  helix either in the basic residue stretch (Sept9\_i1 NC<sub>mut1</sub>) or upstream of this stretch in key hydrophobic residues (Sept9\_i1 NC<sub>mut2</sub>). Sept9\_i1 NC<sub>mut1</sub> incorporated in tetramers and Sept9\_i1 NC<sub>mut2</sub> incorporated partly in tetramers, but mostly migrated like the monomeric protein (Fig. 3a), indicating that mutations in Sept9\_i1 NC<sub>mut2</sub> might affect both NC and G interfaces. Importantly, we observed no association of Sept9\_i1 G or NC mutants with microtubules, but rather a diffuse cytosolic distribution (Fig. 3b). These results show that neither septin tetramers nor Sept9\_i1 monomers bind to microtubules, and that the octameric septin context, which brings together two Sept9 units via their NC interface, is mandatory for Sept9\_i1 association with microtubules.

Next, we evaluated if septin octamers harboring Sept9\_i1 bind microtubules by themselves or if they need to assemble into longer polymers via Sept2:Sept2 NC interfaces. Mutation of the Sept2 NC interface (Kim, Froese et al., 2012, Sirajuddin et al., 2007) (Sept2 NC<sub>mut</sub>) should produce a mixture of isolated octamers and hexamers in cells (Fig. 3c). To evaluate its impact on Sept9\_i1 localization, we knocked down both Sept9 and Sept2, and re-expressed Sept9\_i1 (tagged with GFP to monitor Sept9 localization), together with wild type or Sept2 NC<sub>mut</sub> (tagged with mApple to identify transfected cells). Using native gel separation, we verified that wild type Sept2 and Sept2 NC<sub>mut</sub> generated the same relative expression levels of octamers and hexamers (Fig. 3c). However, when Sept2 NC<sub>mut</sub> was expressed Sept9\_i1 (and Sept2) no longer co-localized with microtubules (Fig. 3d). Thus, the association of Sept9\_i1 with microtubules requires its incorporation in polymerizable octamers.

**Sept9\_i1 associates with stable microtubules.** Because of the sequence similarities between Sept9\_i1 and MAP4 MBDs, the two proteins might use the same tubulin interdimer binding pocket at the surface of microtubules (Kellogg, Hejab et al., 2018, Shigematsu, Imasaki et al., 2018). At endogenous Sept9\_i1 levels in SKBr3 cells, we observed that MAP4 associated with the entire microtubule cytoskeleton, whereas Sept7 was concentrated on acetylated bundled microtubules (Fig. 4a). In U2OS cells transfected with Sept9\_i1, the same differential distribution between MAP4 and Sept9\_i1 was observed. When the Sept9\_i1-i5 chimera, which showed a stronger affinity for microtubules, was expressed, MAP4 was absent on many



321 microtubule bundles (Fig. 4b). These observations suggest that the binding sites of Sept9\_i1  
322 MBD and MAP repeats partially overlap on the microtubule lattice.

323 As we repeatedly observed the preference of Sept9\_i1 for acetylated microtubules (Fig.  
324 1d, e, Fig. 2d, h, Fig. 3b, d, Fig. 4a and Supplementary Fig. 3c, g), we wondered whether  
325 microtubule acetylation was a determinant of septin binding. We first examined whether the  
326 unexpected lack of Sept9\_i1 association with microtubules in RPE1 cells (Fig. 1c, d) was  
327 associated with a deficit of microtubule acetylation. Tubulin acetylation levels in RPE1 cell line  
328 were indeed low, especially compared to those in another retina epithelial cell line (ARPE19),  
329 which expressed similar levels of Sept9\_i1 (Fig. 5a) but showed frequent septin association with  
330 microtubules (Fig. 5b, supplementary Fig. 3f). Other posttranslational modifications (PTMs) of  
331 tubulins, such as polyglutamylation, that could affect Sept9\_i1 binding (Froidevaux-Klipfel,  
332 Targa et al., 2015, Spiliotis, Hunt et al., 2008) were equally low in these cell lines (Fig. 5a).  
333 Moreover, treating RPE1 cells with paclitaxel, a drug that induces acetylation and bundling of  
334 microtubules in cells, induced the association of septins with microtubules (Fig. 5c). Of note, the  
335 paclitaxel-driven re-localization of septins to microtubules was dependent on the expression of  
336 Sept9\_i1 (Fig. 4c and Fig. 5c). Thus, Sept9\_i1 association with microtubules appears to be  
337 correlated with tubulin acetylation. To determine if paclitaxel-induced tubulin acetylation was  
338 responsible for re-localization of septins on microtubules,  $\alpha$ TAT1, the major tubulin acetyl  
339 transferase (Janke & Montagnac, 2017), was knocked down to prevent tubulin acetylation, prior  
340 to paclitaxel treatment (Fig. 5d). This experiment clearly showed that re-localization of septins  
341 on paclitaxel-bundled microtubules was independent of tubulin acetylation levels (Fig. 5d).

342 Finally, we evaluated whether Sept9\_i1, like MAPs, contributes to microtubule  
343 stabilization in the presence of nocodazole, a microtubule-depolymerizing agent. We observed  
344 that, whereas Sept9\_i1 moderately stabilized microtubules, Sept9\_i1-i5 strongly promoted the  
345 resistance of microtubules against nocodazole-induced depolymerization (Fig. 4c, d and  
346 Supplementary Fig. 3g). Of note, depolymerization of microtubules allowed Sept9\_i1-i5 to re-  
347 localize to actin fibers in nearly 60% of the cells (Fig. 4c).

348 Collectively, these data establish that septin octamers harboring Sept9\_i1 associate with a  
349 population of stable bundled microtubules in cells, independently of their acetylation levels, and  
350 contribute to microtubule stabilization.

351

352 **Recombinant septin octamers harboring Sept9\_i1 interact directly and specifically**  
353 **with microtubules and slow down their depolymerization.** In order to assess if recombinant  
354 octamers harboring (Oct\_9i1) are able to bind directly to microtubules, we purified recombinant  
355 septin octamers (Sept2-Sept6-Sept7-Sept9\_i1-Sept9\_i1-Sept7-Sept6-Sept2 and Sept2-Sept6-  
356 Sept7-Sept9\_i3-Sept9\_i3-Sept7-Sept6-Sept2)(Iv et al., 2021) (Supplementary Fig. 1b) and  
357 reconstituted dynamic microtubules made from purified tubulin  $\alpha/\beta$ -heterodimers in the presence  
358 of each type of octamer. Total internal reflection fluorescence (TIRF) microscopy allowed  
359 visualizing both dynamic microtubules incorporating rhodamine-tagged tubulin and septin  
360 oligomers incorporating GFP-tagged Sept2 (Fig. 6a). Microtubules were polymerized from a 10  
361  $\mu$ M tubulin heterodimer solution and in the presence of 10 to 300 nM of octamers, i.e. at  
362 concentrations comparable to the tubulin  $\alpha/\beta$ -heterodimer ( $\sim$ 10  $\mu$ M) and septin complex ( $\sim$ 400  
363 nM) concentrations measured in Hela cells (Hein, Hubner et al., 2015).

364 We clearly observed Oct\_9i1 binding to microtubules at the lowest concentration (10 nM,  
365 Fig. 6b, c). Oct\_9i1 diffused randomly on the lattice of dynamic microtubules (Fig. 6c and  
366 Supplementary Movie 1), and were also on the stable microtubule seeds. On kymographs,

367 diffusion on microtubules during the growing phases was clearly apparent, with a reduced  
368 presence on the microtubule seeds (Fig. 6c). Of note, upon microtubule depolymerization,  
369 Oct\_9i1 accumulated and remained on seeds, then diffused again on the microtubule upon its  
370 regrowth (Fig. 6c). At 10 nM concentration, there were no detectable recombinant octamers  
371 harboring Sept9\_i3 (Oct\_9i3) on dynamic or stable seed microtubules. Decoration of  
372 microtubules increased with increasing concentrations of Oct\_9i1, until saturation at 300 nM  
373 (Fig. 6b, c and Supplementary Movie 1). In comparison, Oct\_9i3 only showed significant  
374 binding at the highest concentration (300 nM), which was restricted to the stable microtubule  
375 seeds (Fig. 6b, c and Supplementary Movie 2). Altogether these results indicate that octamers  
376 harboring Sept9\_i1 bind specifically and directly dynamic individual microtubules.

377 We then analyzed how the addition of Oct\_9i1 or Oct\_9i3, at 10 to 300 nM, affected  
378 parameters of microtubule dynamic instability (Fig. 7a, b). Both types of octamer were similarly  
379 and moderately affecting microtubule growth rate at intermediate concentrations. Strikingly,  
380 Oct\_9i1 induced a strong and dose-dependent decrease of the microtubule shortening rate, while  
381 Oct\_9i3 had no effect, even when used at the highest concentration. The catastrophe time  
382 frequency was not consistently altered by the addition of either types of octamer. Intriguingly,  
383 we observed that, solely in the presence of 200 nM or 300 nM of Oct\_9i1, about 10% of  
384 depolymerizing microtubules had an unusual curved tubulin-containing structure at their plus-  
385 end that was decorated by septins (Fig. 7c and Supplementary Movie 3). These structures could  
386 be made of few protofilaments whose shortening is slowed down by the association with  
387 Oct\_9i1.

388

### 389 **Re-localization of octamers from stress fibers to microtubules alters stress fiber** 390 **organization and function.**

391 Our observations indicate that the population Sept9\_i1 octamers associate with both  
392 microtubules and actin fibers and can shuttle between them. This was for instance illustrated by  
393 the observation that the Sept9\_i1-i5 chimera co-localized with microtubules only (Fig. 2f, h and  
394 Fig. 4b, c), but was able to co-localize with actin fibers upon nocodazole-induced  
395 depolymerization of microtubules (Fig. 4c). Thus, beyond its direct contribution to microtubule  
396 stability and dynamics (this study) and microtubule-linked processes, e.g. cell resistance to  
397 paclitaxel (Targa, Klipfel et al., 2019), the relative distribution of Sept9\_i1 on microtubules vs  
398 actin fibers might indirectly affect actomyosin fibers distribution and cellular events dependent  
399 on their contractility. Previous studies showed that Sept9 associates with perinuclear actin fibers,  
400 especially ventral stress fibers whose organization depends on Sept9 (Farrugia, Rodriguez et al.,  
401 2020, Verdier-Pinard et al., 2017) (Supplementary Fig. 5a). We similarly observed that in U2OS  
402 (Fig. 8a) and RPE1 cells (Supplementary Fig 5d), the knockdown of Sept9 strongly decreased  
403 the percentage of cells with sub-nuclear stress fibers. To test the hypothesis that septin octamer  
404 association with microtubules impacts actin fiber organization and function, we generated U2OS  
405 cell lines stably expressing Sept9\_i1 variants (Supplementary Fig. 4).

406 Expression of Sept9\_i1-i5, only associating with microtubules, was concomitant with the  
407 strong reduction in the percentage of cells having sub-nuclear stress fibers (Fig. 8b), while  
408 expression of Sept9 constructs with no or reduced microtubule association (Sept9\_i1  $\Delta$ 1-25,  
409 Sept9\_i1 R10A/R15A and Sept9\_i3) preserved sub-nuclear stress fibers (Fig.8b). Likewise,  
410 paclitaxel-induced re-localization of Sept9\_i1 octamers from stress fibers to microtubule bundles  
411 in RPE1 cells induced the loss of sub-nuclear stress fibers (Fig. 5c), whereas paclitaxel-treatment  
412 of U2OS expressing Sept9\_i3 as the major isoform did not result in any loss of sub-nuclear stress

413 fibers (Fig. 5c). Thus, the sequestration of septin octamers on microtubules is dependent on the  
414 MBD of Sept9\_i1 and induces a loss of sub-nuclear stress fibers. These results suggest that the  
415 precise relative distribution of septins on microtubules vs on actin fibers is critical for an intact  
416 actin cytoskeleton.

417 As a functional assay for testing the interplay between Sept9\_i1-microtubule association  
418 and stress fiber function, we plated U2OS cells KD for Sept9 or expressing different Sept9\_i1  
419 variants on adhesive micropatterns. Indeed, when plated on adhesive micropatterns, cells form  
420 actin stress fibers across adhesives edges, and the convexity of the non-adhesive cell edges  
421 provides a readout for stress fiber tension (Thery, Pepin et al., 2006). Partial or total localization  
422 of septins on microtubules vs stress fibers in U2OS cells stably expressing Sept9\_i1 or Sept9\_i1-  
423 i5, respectively, promoted the concavity of non-adhesive cell edges, reflective of the relaxation  
424 of peripheral stress fibers compared to cells stably expressing Sept9\_i1  $\Delta$ 1-25, that is solely  
425 located on actin fibers (Fig. 8c). The knockdown of Sept9 in U2OS cells, leading to the loss of  
426 septin octamers, induced a similar detectable relaxation of peripheral stress fiber (Fig. 8d).

427  
428 Altogether, these results suggest that the relative abundance of octamers vs hexamers  
429 and/or the relative proportion of septin octamers able to associate to microtubules contribute  
430 critically to the maintenance of the actomyosin organization and tension. Our study indicates that  
431 the propensity of octamers harboring Sept9\_i1 to associate with microtubule depends on  
432 Sept9\_i1 expression levels, the state of its N-terminal regulatory domain, and the extent of  
433 microtubule bundling.

## 434 435 **Discussion**

436  
437 Septins associate in a dynamic way with different major constituents of the cell, i.e.  
438 membranes, actin fibers and microtubules (Woods & Gladfelter, 2020). Septin octamers and  
439 hexamers co-associate to form septin filaments (Bertin et al., 2012, Iv et al., 2021, Ong et al.,  
440 2014, Soroor et al., 2021, Szuba et al., 2020, Valadares et al., 2017), whose properties may  
441 depend in part on the relative abundance of incorporated octamers and hexamers. In addition, the  
442 expression profile of different septin isoforms, including those of Sept9, may direct both  
443 localization and function of septin octamers. It is thus critical to define precisely the molecular  
444 basis of the different subcellular localizations of septin complexes, in order to develop tools  
445 differentially altering their distribution. This will help us understand how the different septin  
446 pools respectively contribute to cell physiology and pathology.

447 Earlier studies have shown that Sept9\_1 has a preferential affinity for microtubules in  
448 cells (Nagata et al., 2003, Sellin et al., 2012, Surka et al., 2002, Verdier-Pinard et al., 2017)  
449 indicating that the first 25 aa residues defining the sequence unique to this long isoform was  
450 responsible for specific binding to microtubules. In the present study, we identify a conserved  
451 MAP-like MBD in Sept9\_i1, including two distinct modules and key conserved residues whose  
452 mutation abrogates microtubule binding. We and others have identified imperfect repeats  
453 containing basic amino acid residues in the common long N-terminal sequence of Sept9\_i1, \_i2  
454 and \_i3 (Bai et al., 2013, Verdier-Pinard et al., 2017). These repeats were proposed to constitute  
455 the MBD of Sept9 via electrostatic interactions with the acidic C-termini of  $\beta$ -tubulin exposed at  
456 the surface of microtubules (Bai et al., 2013), in line with the accepted mode of interaction of  
457 conventional MAPs at the time (Serrano, Montejo de Garcini et al., 1985). However, recent  
458 advances in the determination of the structure of MAP tandem repeats bound to microtubules by

459 cryo-EM contradicted this model (Kellogg et al., 2018, Shigematsu et al., 2018). These studies  
460 revealed that a sequence in Tau and MAP4 repeats anchors MAPs to a binding pocket at the  
461 interface between consecutive tubulin heterodimers in protofilaments, independently of tubulin  
462 acidic C-termini. We actually found numerous similarities between the MAPs repeated  
463 sequences and the AIR9-like module of Sept9\_i1 MBD, that are not present in the repeats shared  
464 with the other Sept9 long isoforms. Most importantly, our data show that, contrary to the  
465 Sept9\_i1 MBD, the N-terminal region common to all long isoforms is not required for the  
466 association of Sept9\_i1 harboring octamers with microtubules in cells. Deletion of this region  
467 actually favored septin association with microtubules at the expense of actin fibers. These  
468 findings led us to speculate that the common N-terminal domain is not directly involved in the  
469 interaction of Sept9\_i1 with the microtubule lattice, but negatively regulates it. The imperfect  
470 repeats of the common N-terminal domain were predicted to form short  $\beta$ -sheets whereas the rest  
471 of the domain is disordered (Verdier-Pinard et al., 2017). One of the imperfect repeats of the  
472 common N-terminal region is the locus of mutations detected in HNA patients (Collie et al.,  
473 2010, Hannibal et al., 2009, Kuhlenbaumer et al., 2005). To this date the mechanisms whereby  
474 Sept9 mutations contribute to this disease are not known. Remarkably, when we expressed  
475 Sept9\_i1 harboring HNA-like mutations, septin octamers were no longer able to associate with  
476 microtubules, while still associating with actin fibers. This finding suggests that HNA-associated  
477 mutations alter the putative “Sept9\_i1 MBD regulatory region” providing clues for the  
478 determination of the molecular mechanisms underlying HNA.

479 Importantly, we demonstrated that Sept9\_i1 localizes septins on microtubules, only if it is  
480 included in polymerizable septin octamers. At endogenous expression levels, in the diverse  
481 cellular models we studied, we did not observe the presence of a significant pool of monomeric  
482 Sept9 (Fig. 1b), which suggests that microtubule-associated Sept9 is wholly incorporated in  
483 octamers in these cells. Conversely, the large excess of Sept9\_i1 monomers in cells expressing  
484 the Sept9\_i1 G interface mutant was not associated with microtubules (Fig. 3a, b). Thus, *in vitro*  
485 studies with isolated recombinant Sept9\_i1 suggesting that Sept9\_i1 can binds and bundle  
486 microtubules by itself (Bai et al., 2013, Karasmanis, Phan et al., 2018, Nakos, Rosenberg et al.,  
487 2019b) must be interpreted with caution.

488 In previous studies, recombinant Sept9\_i3 or the common N-ter fragments were shown to  
489 associate with microtubules, assembled from purified tubulin (Bai et al., 2013, Nakos et al.,  
490 2019b). The discrepancy is most likely linked to the fact that in these *in vitro* experiments, Sept9  
491 was used outside of its natural octameric context, often at high concentrations (up to 10-30  $\mu$ M  
492 in pelleting assays) and using paclitaxel or GMPCPP stabilized microtubules in very low salt  
493 buffers, which likely favored electrostatic interactions. Using recombinant octamers harboring  
494 Sept9\_i1 and Sept9\_i3 (Iv et al., 2021), we observed that only those harboring Sept9\_i1  
495 associated with dynamics microtubules at low nanomolar concentrations, *in vitro*. Our *in vitro*  
496 reconstitution experiments results are the first one to recapitulate the specific binding of Sept9\_i1  
497 octamers to microtubules observed in cells here and by others (Targa et al., 2019, Verdier-Pinard  
498 et al., 2017). Of note, although recombinant Sept2-Sept6-Sept7-Sept7-Sept6-Sept2 hexamers  
499 were found to interact with microtubules *in vitro* (Nakos, Radler et al., 2019a), this is not  
500 consistent with the absence of co-localization of septin hexamers with microtubules, when Sept9  
501 was knocked down in cells (Verdier-Pinard et al., 2017) (Fig 8a and Supplementary Fig. 1d).

502 Septins associate with a sub-population of bundled microtubules (Bai et al., 2013,  
503 Bowen, Hwang et al., 2011, Nagata et al., 2003). We confirm that Sept9\_i1 co-localize with  
504 acetylated, bundled microtubules (much more selectively than structural MAPs, such as MAP4).

505 As we also show that microtubule acetylation is not a requirement, Sept9\_i1 binding appears  
506 rather to rely on microtubule bundling in cells. Intriguingly, microtubule bundling is not required  
507 *in vitro*. This paradox will require other studies, including structural ones, to further characterize  
508 the molecular bases of the association of septins with individual and bundled microtubules.

509 Our data show that octamers harboring Sept9\_i1 slowed down microtubule  
510 depolymerization *in vitro* and stabilized microtubules in cells treated with nocodazole. It will be  
511 important to determine how these molecular events contribute to microtubule-dependent cellular  
512 processes such as spatiotemporal sorting of vesicles and organelles (Karasmanis et al., 2018,  
513 Spiliotis et al., 2008), formation of protrusive structures (Ghossoub, Hu et al., 2013, Hu, Bai et  
514 al., 2012, Nolke et al., 2016, Ostevold et al., 2017, Tada et al., 2007, Yadav et al., 2017) or cell  
515 survival against cytotoxic doses of paclitaxel (Targa et al., 2019).

516 Association of septins with microtubules is likely to be regulated by cell- and tissue-  
517 specific contexts. Indeed, septin association with microtubules is clearly correlated to the specific  
518 expression of the Sept9\_i1 isoform and is sensitive to the bundling state of microtubules. Septin-  
519 microtubule interaction might also be modulated by the presence of competing MAPs or PTMs  
520 targeting the Sept9\_i1 MBD and/or its putative regulatory domain. Importantly, we show that  
521 deficient Sept9\_i1 binding to microtubules is associated with a diseased state, as two distinct  
522 HNA-like point mutations prevented Sept9 binding to microtubules. We are still far from  
523 understanding the underlying mechanism leading to HNA, which might be related to  
524 dysregulated microtubule function, but might be related also to an excessive association of  
525 septins with actin fibers and perturbation of actomyosin functions. Thus, our work not only  
526 establishes a new molecular basis for studying the physiological relevance of the association of  
527 septin with microtubules, but also open new avenues in the difficult search for the causative  
528 events leading to this rare neuropathy.

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551 **Materials and methods**

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553 **Human Cell lines.**

554 U2OS (ATCC), SKBR-3 (ATCC), HeLa (a kind gift from Dr Patrice Dubreuil, CRCM,  
555 Marseille, France), RPE-1 and ARPE19 (kind gifts from Dr Michael Sebbagh, CRCM,  
556 Marseille) cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Gibco)  
557 supplemented with 4 mM GlutaMAX™, 10 % fetal bovine serum (FBS) and 1% sodium  
558 pyruvate (supplemented DMEM), except ARPE19 cells that were cultivated in DMEM/F12 (1:1  
559 mixture of DMEM and Ham's F12, Gibco) supplemented as for DMEM. All cell lines were  
560 maintained in the presence of 5% CO<sub>2</sub> humidified atmosphere at 37°C. RPE-1 cells were also  
561 grown on coverslips coated with collagen (Corning, Cat#354236) until they reached confluency  
562 and ciliogenesis was induced by growing cells in DMEM containing only 0.5% of fetal bovine  
563 serum for 24 hours.

564

565 **Plasmids coding for fluorescent septin constructs and transfection**

566 To drive expression of the constructs in mammalian cells, we used the immediate early enhancer  
567 and promoter of human cytomegalovirus (CMV promoter, 508 base pairs). Human Sept9\_i1  
568 cDNA was a gift from C. Montagna (Albert Einstein College of Medicine, USA). Human  
569 Sept9\_i3 cDNA was a gift from W. Trimble (University of Toronto, Canada). A synthetic human  
570 Sept2 coding sequence (Eurofins Genomics, Germany) was generated using the codon usage of  
571 mouse Sept2 except for the five codons that differ between the two species, for which we used  
572 codons encoding the human residues. C-terminal green and red fluorescent protein fusions were  
573 generated using monomeric (V206K) superfolder GFP (msfGFP) (Costantini, Fossati et al.,  
574 2012, Cranfill, Sell et al., 2016, Pedelacq, Cabantous et al., 2006, Zacharias, Violin et al., 2002)  
575 and monomeric Apple (mApple) (Cranfill et al., 2016, Shaner, Lin et al., 2008), respectively. All  
576 constructs were generated with two-insert seamless cloning (In-Fusion HD Cloning Plus Kit  
577 from Takara Bio, Cat# 638910) using NheI/BamHI linearized plasmid backbones (Addgene  
578 plasmid #54759) and the oligonucleotide primer sequences (listed in Supplementary Table 1).  
579 Primers for seamless cloning were Cloning Oligo (<60 bp) or EXTREmer (>60 bp) synthesis and  
580 purification quality from Eurofins Genomics, Germany. Restriction enzymes were FastDigest  
581 enzymes from Thermo Scientific. All plasmids were verified by sequencing (Eurofins Genomics,  
582 Germany) after each cloning step, including the midpreps used for plasmid production.  
583 Constructs were transiently transfected by nucleofection in U2OS cells (Amaxa nucleofector  
584 program U2OS X-001, Lonza) as described previously (Verdier-Pinard et al., 2017).

585

586 **siRNAs and transfection**

587 The following 19-mer duplex siRNAs were purchased from LifeTechnologies: siRNA control  
588 (siCtrl) targeting the E.Coli  $\beta$  galactosidase (LacZ) (5'-GCGGCUGCCGGAUUUACC-3'),  
589 Sept9 (5'-GGAUCUGAUUGAGGAUAAA-3') targeting the 3'UTR of all human Sept9 mRNA  
590 variants, Sept7 (5'-CGACUACAUUGAUAGUAAA-3') targeting the human mRNA coding  
591 region,  $\alpha$ TAT1 (5'-CGCACCAACUGGCAAUUGA-3'), whose design was based on validated  
592 siRNA  $\alpha$ TAT1#2 from Shida et al (Shida, Cueva et al., 2010). Transfection of siRNA was  
593 performed as described previously (Verdier-Pinard et al., 2017) either with Lipofectamine  
594 RNAiMAX (Invitrogen, Cat# 13778075) or Amaxa cell line nucleofection kit V (Lonza,  
595 Cat#VCA-1003).

596

597 **Drug treatments**

598 For some experiments, cells were treated for two hours with 2  $\mu$ M paclitaxel (Sigma-Aldrich,  
599 Cat#T7191) or 10  $\mu$ M nocodazole (Sigma-Aldrich, Cat#M1404) in DMSO or with DMSO alone  
600 (Sigma-Aldrich, Cat#D8418).

601

602 **Antibodies**

603 Labeling of septins on Western blots (WB) and/or on immunocytochemistry coverslip (ICC) was  
604 achieved with the following antibodies: rabbit polyclonals against human Sept2 (Sigma-Aldrich,  
605 Cat#HPA018481, WB), against human Sept9 (Sigma-Aldrich, Cat#HPA042564 and  
606 HPA050627, WB and ICC; Proteintech, Cat#10769-1-AP, WB), against Septin 7 (IBL,  
607 cat#18991, WB and ICC), against Sept11 (Sigma-Aldrich, Cat#SAB2102111, WB), against  
608 human Sept5, against Sept8 (kind gifts from Barbara Zieger, University of Freiburg, Germany,  
609 WB), against Sept6 (a kind gift from Makoto Kinoshita (Nagoya University, Japan, WB), against  
610 Sept10 (Sigma, Cat#HPA047860, WB); rat monoclonals against human Sept9\_i1 (clone 4D2A5,  
611 WB) or human Sept9\_i3 (clone 1A6C2, WB), and against human Sept7 (clone 10A7, WB and  
612 ICC) were produced as described previously (Verdier-Pinard et al., 2017) (WB). Other  
613 antibodies were against  $\alpha$ -tubulin: mouse monoclonal (Sigma-Aldrich, DM1A Cat#05-829, WB  
614 and ICC), rat monoclonal (Invitrogen, YL1/2 Cat#MA1-80017, ICC), mouse monoclonal anti-  
615 acetylated K40 (Santa Cruz Biotechnology, 6-11B1 Cat#sc-23950, WB and ICC), against  
616 tubulin: mouse monoclonal anti-glutamylated tubulin (Adipogen, GT-335 Cat#AG-20B-0020-  
617 C100 and polyE Cat#AG-25B-0030-C050, WB). Labeling of actin was performed with  
618 phalloidin-Atto 390 (Sigma-Aldrich, Cat#50556, ICC) or phalloidin-TRITC (Sigma-Aldrich,  
619 Cat#P1951, ICC). Secondary antibodies conjugated with HRP were used for WB (Dako  
620 Agilent). For ICC application, and for WB application when indicated in figures, the following  
621 fluorophores conjugated to secondary antibodies were used: DYLIGHT405, AlexaFluor405,  
622 AlexaFluor488 and AlexaFluor594 (Jackson, Immunoresearch) and AlexaFluor 647 (Invitrogen).

623

624 **U2OS cell lines stably co-expressing mCherry-H2B and Sept9-GFP constructs**

625 U2OS and HeLa cell lines stably expressing mCherry-H2B and a Sept9\_X-GFP construct were  
626 generated by co-transfecting the plasmid pBabeD hygro mCherry-Histone H2B (a kind gift from  
627 Christophe Lachaud, CRCM, Marseille, France) with hygromycin B resistance and one of the  
628 plasmids encoding for different Sept9 isoforms and mutants-GFP constructs, with geneticin  
629 resistance. U2OS cells were nucleo-transfected (Amaxa nucleofector program U2OS X-001,  
630 Lonza) with Amaxa kit V. Three days after transfection, co-expressing cells were selected with  
631 hygromycin B (Invitrogen, Cat#10687-010) and geneticin (Gibco, Cat#10131-027) at 0.5 mg/mL  
632 each. GFP and mCherry fluorescence positive cells were sorted by flow cytometry. Sorted cell  
633 populations were cultured in supplemented DMEM in the presence of 0.5 mg/mL of antibiotics.  
634 After three passages, cell line stocks were stored in liquid nitrogen.

635

636 **Micropatterning of U2OS cells**

637  $6 \times 10^3$  U2OS cells KD for Sept7 or Sept9 or U2OS cells stably co-expressing mCherry-H2B and  
638 Sept9-GFP constructs were seeded in each well formed by the assembly of a 35 mm collagen  
639 coated coverslip with H-shaped medium size adhesive micropatterns (Cytoo, Cat#10-008-00-18)  
640 and a magnetic four-well chamber (Cytoo, Cat#30-011) following manufacturer instructions.  
641 Coverslips were fixed with 4% formaldehyde and processed for ICC as previously described

642 (Verdier-Pinard et al., 2017). Distance measurements were performed using the Graphics line  
643 tool in Zen blue software as described in more details in Fig. 8c legend.

#### 644 **SDS-PAGE and Western blotting**

645 Total protein extraction from cells in culture by scraping the cells off in NP-40 lysis buffer,  
646 separation of proteins by SDS-PAGE using NuPAGE 4-12 % gradient Bis-Tris gels (Invitrogen,  
647 Cat#NP0322BOX) and MOPS SDS running buffer (Invitrogen, Cat#NP0001-02) and Western  
648 blotting of separated proteins on nitrocellulose were performed as described previously (Verdier-  
649 Pinard et al., 2017).

650

#### 651 **Separation of septin complexes by native gel electrophoresis and detection**

652 Native cellular protein extraction was carried out as published by Sellin et al. (Sellin, Stenmark  
653 et al., 2014). Briefly, proteins from cells at 80% confluence in one 100 mm petri dish were  
654 extracted in 40  $\mu$ l of native lysis buffer (80 mM PIPES pH 6.9, 2 mM  $MgCl_2$ , 4 mM EGTA,  
655 0.2% saponin and protease inhibitor cocktail (Roche, Cat#04693159001); the extract was  
656 incubated for 10 min on ice and centrifuged and the supernatant was supplemented with 0.45 M  
657 sodium chloride, incubated on ice, centrifuged, concentrated and exchanged twice with  
658 phosphate buffer (pH 7.5, 0.45 M NaCl, 1 mM EGTA and protease inhibitors) on an Amicon 30  
659 kDa cut off concentrator (Merck, Cat# UFC 503096). Protein concentration was determined  
660 using a BCA protein assay (Thermo Scientific, Cat#23223 and 23224), glycerol was added at 1:1  
661 (vol:vol), and extracts stored at  $-20^{\circ}C$  until use. Native PAGE was performed on 4-16%  
662 NativePAGE Novex Bis-Tris polyacrylamide gels (Thermo Scientific, Cat#BN1002BOX)  
663 following instructions from the manufacturer. Briefly, native protein extracts were mixed with 4x  
664 sample buffer (Thermo Scientific, Cat#BN2003) and water (final sample buffer 1x, and about  
665 150 mM NaCl). Eight  $\mu$ g of proteins were loaded per lane. Unstained native protein molecular  
666 weight standards were used (Thermo Scientific, Cat#LC0725) and stained with Coomassie blue  
667 during electrophoresis. Protein complexes were transferred overnight on a PVDF membrane  
668 under 20 V constant voltage. The membrane was partially destained with 25% MeOH and 10%  
669 acetic acid, positions of native protein molecular weight standards were marked, and the  
670 membrane was placed briefly in MeOH for complete destaining. The membrane was processed  
671 for Western blot detection following the protocol used for nitrocellulose membranes described  
672 previously (Verdier-Pinard et al., 2017).

673

#### 674 **Immunocytochemistry**

675 Multiplex immunocytochemistry was carried out on cells cultured on collagen coated coverslips  
676 followed by 4% formaldehyde fixation as described previously (Verdier-Pinard et al., 2017).  
677 Images were acquired on a Zeiss structured light ApoTome microscope equipped with a 63x/1.4  
678 Plan Apochromat objective and an AxioCam MRc5 camera using AxioVision software or a Zeiss  
679 LSM880 META confocal microscope equipped with a 63x/1.46 Plan Apochromat objective and  
680 a GaAsP detector using Zen software.

681

682 **Co-localization of septins with microtubules and with stress fibers.** Individual cells were  
683 examined by ICC for co-localization of septins with total  $\alpha$ -tubulin, acetylated K40  $\alpha$ -tubulin and  
684 F-actin, from top to bottom. Cells were counted as positive for co-localization of septins with  
685 microtubules or with F-actin when septin fluorescence signal was co-aligned with microtubules  
686 and/or acetylated microtubules or with ventral stress fibers, respectively. The percentage of cells



687 positive for both septin-microtubule and septin-stress fibers co-localization, only positive for  
688 septin-stress fibers co-localization and only positive for septin-microtubule co-localization were  
689 designated in graphs as Microtubule+Actin, Actin, and Microtubule, respectively. The extent of  
690 the co-localizations in each cell was not considered.

691

### 692 **Quantification of acetylated microtubules in cells**

693 The fluorescence surface corresponding to acetylated microtubules in individual cells was  
694 determined using the ImageJ 1.48v software (Schneider, Rasband et al., 2012): the fluorescence  
695 image of acetylated microtubules was converted in 8-bits, adjusted manually with the threshold  
696 function for black and white levels to maximally remove black pixels unrelated to acetylated  
697 microtubules, the actin image was synchronized with the acetylated microtubule image  
698 (Synchronize Windows tool) and used to delimit the ROI corresponding to the entire cell surface  
699 with the freehand selection tool, and finally the image was analysed using the measure function to  
700 obtain the percentage of surface area represented by the remaining black pixels corresponding to  
701 acetylated microtubule.

702

### 703 **Production and purification of recombinant human septin complexes**

704 Human septin hexameric and octameric complexes with either Sept9\_i1 or Sept9\_i3 were  
705 produced, purified and analyzed as detailed in Iv et al (Iv et al., 2021). No difference in apparent  
706 MW was observed between recombinant septin octamers or hexamers and those extracted from  
707 cells after native gel separation and WB (Supplementary Fig. 1b, c).

708

### 709 **Septin/microtubule reconstitution and TIRF imaging**

710 GMPCPP-stabilized microtubule seeds serving as a nucleation site for dynamic microtubules  
711 were prepared using an established double-cycle method (Gell, Bormuth et al., 2010). Briefly, a  
712 ~22  $\mu\text{M}$  mixture of tubulin (~11  $\mu\text{M}$  tubulin dimers) in MRB80 (80 mM PIPES pH 6.8, 4 mM  
713  $\text{MgCl}_2$ , 1 mM EGTA), composed of 75% unmodified tubulin dimers (Cytoskeleton, Inc.,  
714 Ct#T240), 15% rhodamine-labeled tubulin dimers (Cytoskeleton, Inc., Cat#TL590M) and 10%  
715 biotin-modified tubulin dimers (Cytoskeleton, Inc., Cat#T333P), was spun down using an  
716 Airfuge® Air-driven ultracentrifuge (Beckman Coulter Inc., Brea, California, USA) for 5  
717 minutes at 30 psi with a cold rotor. Then, the mixture was complemented with 1 mM GMPCPP  
718 from a 10 mM stock solution, thus diluting the tubulin to 20  $\mu\text{M}$ . This mixture was incubated at  
719 37°C for 30 minutes to polymerize tubulin, and immediately airfuged for 5 minutes at 30 psi  
720 with the rotor at room temperature to pellet GMPCPP-stabilized microtubules. Afterwards, the  
721 supernatant was discarded and the pellet was resuspended in warm MRB80 to a final tubulin  
722 concentration of 20  $\mu\text{M}$ , considering an 80% recovery of the tubulin. The mixture was incubated  
723 on ice for 20 minutes to depolymerize the microtubules. Subsequently, it was complemented  
724 with 1 mM GMPCPP, incubated at 37°C for 30 minutes to repolymerize the microtubules, and  
725 airfuged for 5 minutes at 30 psi with a warm rotor. The pellet, containing GMPCPP-stabilized  
726 microtubule seeds, was resuspended in warm MRB80 supplemented with 10% glycerol, snap-  
727 frozen, and kept at -80°C until use.

728 Nr. 1 Menzel coverslips (Thermo Fisher scientific, product number 11961988) and glass slides  
729 (Thermo Fisher scientific, product number 11879022) were cleaned in base piranha solution (5%  
730 hydrogen peroxide, 5% ammonium hydroxide) at 70°C for 10 minutes, washed extensively by  
731 rinsing with Milli-Q water and stored in Milli-Q water for up to 5 days. Just before use, a  
732 coverslip and a glass slide were blow dried with a stream of  $\text{N}_2$  gas. Flow channels were

733 prepared by placing parallel 2x20 mm parafilm strips spaced by 2-3 mm between the glass slide  
734 and the coverslip. The parafilm was melted by placing the chambers on a hotplate at 120°C.  
735 After cooling down, the chambers were passivated by incubation with 0.2 mg/mL Poly(L-  
736 lysine)-graft-biotinylated PEG (SuSoS, product number CHF560.00) in MRB80, 0.2 mg/mL  
737 neutravidin in MRB80, 0.5mg/mL  $\kappa$ -casein in MRB80, and 1 wt% Pluronic F-127 in MRB80, in  
738 that order and without intervening washing steps. The channels were washed with three channel  
739 volumes (~30  $\mu$ L) of MRB80 at the end of the passivation process. Then the channels were  
740 incubated for 10 minutes with an aliquot of GMPCPP-stabilized seeds that was quickly thawed at  
741 37°C, allowing the microtubule seeds to bind to the surface via biotin-neutravidin interactions.  
742 Microtubule polymerization was induced by immediately flushing into the channel 20  $\mu$ M  
743 tubulin (3% rhodamine-labeled tubulin) in a 5:1 volume ratio of MRB80:septin buffer  
744 complemented with 0.5 mg/mL  $\kappa$ -casein to prevent unspecific interactions, 0.1% methylcellulose  
745 as a crowding agent, 1 mM ATP, 1 mM GTP and an oxygen scavenging system composed of 50  
746 mM glucose, 200  $\mu$ g/ml catalase, 400  $\mu$ g/ml glucose-oxidase, and 4 mM DTT. A mixture of 90%  
747 unlabelled and 10% msGFP-labeled septin hetero-hexamers or hetero-octamers (containing  
748 either SEPT9\_i1 or Sept9\_i3) were added to the previous mix at different concentrations in the  
749 range of 10 to 300 nM.

750 The samples were immediately imaged using a Nikon Ti2-E microscope complemented with a  
751 Gataca iLAS2 azimuthal TIRF illumination system heated to 30°C using an Okolab incubator  
752 system. The sample was illuminated with 488-nm and 561-nm lasers (Gataca laser combiner  
753 iLAS2) to visualize the septin and the tubulin signals, respectively. To achieve fast imaging, the  
754 fluorescence signal was split with a Cairn Research Optosplit II ByPass containing a Chroma ZT  
755 543 rdc dichroic mirror and filtered with either a 525/50 or a 600/50 chroma bandpass filter. The  
756 images were recorded with a Andor iXon Ultra 897 EM-CCD camera using an exposure time of  
757 75 ms, for 10-20 minutes with a frame rate of 1 frame/second.

758 Microtubule dynamics were analysed by kymograph analysis (Bieling, Laan et al., 2007).  
759 Kymographs were built with the reslice tool in FIJI (Schneider et al., 2012) on a manually drawn  
760 line that went from the beginning of the microtubule seed to the tip of the microtubule in its  
761 maximum length. In the kymographs, we can observe the positions of the microtubule tips over  
762 time. The analysis was only done on the plus ends of the microtubules, which can be  
763 distinguished from the minus ends via the longer final length and higher growth velocity of the  
764 former. Growth and shortening rates were obtained as the slopes of manually fitted straight lines  
765 on the growing or shortening contour phases, determined by manual inspection, of the  
766 microtubule plus ends. The catastrophe rate was calculated as the inverse of the time that a  
767 microtubule spends growing.

## 768 **Statistical analysis**

770 Unless indicated otherwise, statistical analysis was performed using GraphPad Prism software  
771 and unpaired two-tailed t-test with Welch correction for cell related data or using the function  
772 compare\_means from the package ggpubr from R and two-tailed t-test with Benjamini &  
773 Hochberg p-value correction (<https://rpkgs.datanovia.com/ggpubr/index.html>) for the *in vitro*  
774 data related to microtubule dynamics.

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779 **References**

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1050 supervision, writing – original draft, writing – review & editing.  
1051  
1052  
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1054 **Figure legends**

1055

1056 **Fig 1. Sept9 expression, incorporation in octamers and localization in human cell lines. a**  
1057 Western blots of total protein extracts from U2OS, HeLa, RPE1 and SKBr3 cells probed with  
1058 antibodies against septins and tubulin. **b** Native extracts from the same cell lines were analyzed  
1059 by Western blotting for their content in both septin hexamers and octamers using antibodies  
1060 against constitutively required Sept7 and ubiquitously expressed Sept8; septin octamers were  
1061 also specifically labeled with a pan Sept9 antibody. Relative content in septin octamers and  
1062 hexamers in each cell line based on three independent Sept7 WB is presented below the blots  
1063 (mean  $\pm$  SEM). **c** and **d** Cellular localization by immunocytochemistry of Sept9 in the same cell  
1064 lines, during interphase and cytokinesis; in **c**, the percentages of cells displaying both co-  
1065 localization of Sept9 with microtubules and co-localization with stress fibers (Microtubule +  
1066 Actin) or only stress fibers (Actin) were determined from three independent experiments based  
1067 on a total of 90 cells (30 cells per experiment). Unpaired, two-tailed t-test with Welch's  
1068 correction, \*\*  $p < 0.01$ , \*\*\*  $p < 0.0005$ , Microtubule and Actin localization vs in SKBr3; in **d**,  
1069 white scale bars correspond to 5  $\mu\text{m}$ , insets are two-fold zoomed regions framed by a white  
1070 square in the corresponding original images. **e** RPE1 cells were starved for 24 h and  
1071 immunofluorescence was performed as in **c** and **d**; white scale bars correspond to 5  $\mu\text{m}$ , a three-  
1072 fold zoom of a region framed in white containing a primary cilium is presented below the  
1073 original images.

1074

1075 **Fig. 2. Identification of the microtubule binding domain in Sept9\_i1. a** Top part: human  
1076 Sept9\_i1 specific amino-acid N-terminal sequence (1-24) was aligned with the homologous plant  
1077 *A. thaliana* AIR9 sequences (173-205 and 46-78) contained in the AIR-9 microtubule binding  
1078 domain (MBD) (Bushman et al. 2006); bottom part: Sept9\_i1 and AIR9 sequences in top part  
1079 were aligned with sequences of tandem repeats involved in the binding of human structural  
1080 MAPs (Tau, MAP4 and MAP2) to microtubules (ex: Tau 4R isoform positions of repeats are  
1081 R1<sub>242-273</sub>, R2<sub>274-304</sub>, R3<sub>305-335</sub> and R4<sub>336-367</sub>). Alignments were adjusted by introducing gaps and  
1082 similar aa residues were manually coded, orange: common to AIR9 and MAPs; yellow: common  
1083 to Sept9\_i1, AIR9 and MAPs; light green: common to Sept9\_i1 and MAPs; dark green: common  
1084 to MAPs; dark blue: common to Sept9\_i1 and AIR9; light blue: common to AIR9 repeats.  
1085 Important serine residues and hydrophobic residues of MAPs involved in MAP repeat-  
1086 microtubule lattice interactions are framed in red and marked with a red asterisk, respectively.  
1087 Horizontal blue line and red line indicate, respectively, the position of Tau R2 repeat and Tau  
1088 R1/MAP4 R1 repeat modeled based on the cryo-EM structural determination of MAP repeated  
1089 sequences bound on microtubules *in vitro* (<sup>1</sup>Kellogg et al., 2018, <sup>2</sup>Shigematsu et al., 2018). **b**  
1090 Schematic representation of the Sept9\_i1 mutants in the predicted MBD tagged with GFP at their  
1091 C-terminus. **c**, **d** Co-localization of each Sept9 construct transfected in U2OS cells with  
1092 microtubules or stress fibers during interphase was analyzed as described in Fig. 1 legend.  
1093 Unpaired, two-tailed t-test with Welch's correction, \*\*  $p < 0.01$ , Microtubule and Actin  
1094 localization vs Sept9\_i1 wt; white scale bars 5  $\mu\text{m}$ , insets: three-fold zooms. **e** Schematic  
1095 representation of Sept9 isoforms (Sept9\_i1, \_i5 and \_i3), of the chimeric construct fusing the  
1096 Sept9\_i1 MBD to the N-terminus of Sept9\_i5 (Sept9\_i1-i5) and of Sept9\_i1 HNA mutants, all  
1097 tagged with GFP at their C-terminus. **f** Co-localization of Sept9\_i5 or \_i1-i5 or \_i3, and **g** of  
1098 Sept9 HNA point mutants with microtubules or stress fibers in U2OS transfected cells during  
1099 interphase. Unpaired, two-tailed t-test with Welch's correction, \*\*  $p < 0.01$ . **h** Images showing

1100 co-localization of Sept9 in U2OS cells transfected with constructs described in e; white scale  
1101 bars 10  $\mu\text{m}$ , insets: 1.4-fold zooms.

1102  
1103 **Fig. 3. Dependence of septin association with microtubules on the incorporation of Sept9\_i1**  
1104 **in polymerized septin octamers.** **a** Top, predicted septin complexes when Sept9\_i1 wt, or  
1105 Sept9\_i1 mutated on its G ( $G_{\text{mut}}$ , blue dots) or NC interface ( $NC_{\text{mut}}$ , yellow dot) is expressed in  
1106 cells; bottom, Western blots of native gels resolving septin complexes present in U2OS cells  
1107 expressing indicated siRNA and Sept9\_i1 wt or Sept9\_i1 interface mutants. **b** GFP-fluorescence  
1108 based co-localization of Sept9\_i1 wt or interface mutants with actin and microtubules; Sept9\_i1  
1109 wt co-localized both with microtubule and actin fibers as expected, but non-octameric septin  
1110 complexes containing an interface mutant Sept9\_i1 did not. White scale bar 10  $\mu\text{m}$ , insets: two-  
1111 fold zooms of frames region. **c** Top, predicted septin complexes when Sept2 wt or Sept2 mutated  
1112 on its NC interface ( $NC_{\text{mut}}$ , yellow dots) is co-expressed with Sept9\_i1 in U2OS cells; bottom,  
1113 Western blots of native gels resolving septin complexes present in U2OS expressing indicated  
1114 siRNAs and septin constructs. **d** mApple and GFP-based co-localization of Sept2 and Sept9\_i1,  
1115 respectively, with actin and microtubules; expression of Sept2 NC mutant prevented normal co-  
1116 localization of octamers harboring Sept9\_i1 with acetylated microtubules and actin fibers. Insets:  
1117 individual fluorescence channel images of framed regions.

1118  
1119 **Fig. 4. Sept9\_i1 selective binding on, and stabilization of, microtubule bundles.** **a**  
1120 Differential co-localization of endogenous Sept7 vs MAP4 with microtubule cytoskeleton in  
1121 SKBr3 cells. **b** Differential co-localization between endogenous MAP4 and transfected  
1122 Sept9\_i1-GFP or Sept9\_i1-i5-GFP with microtubule cytoskeleton in U2OS cells. White scale  
1123 bars 5  $\mu\text{m}$  in merge images. **c** Analysis of the percent of U2OS cells KD for Sept9 and  
1124 expressing the indicated Sept9-GFP constructs, displaying either microtubule or actin or mixed  
1125 cytoskeleton co-localization with GFP during interphase, and treated either with DMSO vehicle  
1126 alone (D) or 2  $\mu\text{M}$  paclitaxel (P) or 10  $\mu\text{M}$  nocodazole (N) for 2 hrs. Results are from the  
1127 analysis on a total of 90 cells from three independent experiments based (30 cells per  
1128 experiment). Unpaired, two-tailed t-test with Welch's correction, \*\*  $p < 0.01$ , microtubule and  
1129 actin localization vs DMSO. **d** Quantification of nocodazole-resistant acetylated microtubules  
1130 (treatment as in c) in U2OS cells KD for Sept9 and expressing the indicated Sept9-GFP  
1131 constructs. Results are from the analysis of a total of 150 cells from three independent  
1132 experiments (50 cells per experiments). Unpaired, two-tailed t-test with Welch's correction, \*  
1133  $p < 0.05$ , \*\*\*  $p < 0.0005$ .

1134  
1135 **Fig. 5. Dependence of Sept9\_i1 binding to microtubules on their bundling but not on their**  
1136 **acetylation or polyglutamylation.** **a** Left: Western blots of total protein extracts from indicated  
1137 cell lines, probed for Sept9\_i1,  $\alpha$ -tubulin acetylation on K40, glutamylation (one or two  
1138 glutamate residues) and polyglutamylation of tubulin; as a positive control for these tubulin  
1139 posttranslational modifications, an equivalent amount of purified porcine brain tubulin (Brain  
1140 tub) was included in the last lane. Right: quantification of tubulin acetylation levels in cell lines  
1141 based on acetylated  $\alpha$ -tubulin and total  $\alpha$ -tubulin Western blots. Results are from three  
1142 independent determinations. Unpaired, two-tailed t-test with Welch's correction, \*  $p < 0.05$ , \*\*  $p <$   
1143  $0.01$ , tubulin acetylation levels vs ARPE19. **b** Left: Co-localization by immunocytochemistry of  
1144 Sept7 with microtubules and/or actin fibers in RPE1 and ARPE19 cells during interphase; white  
1145 scale bars 10  $\mu\text{m}$ , insets: two-fold zooms of framed regions. Right: quantification of this co-

1146 localization from three independent experiments on a total of 90 cells (30 cells per experiments).  
1147 **c** Left: Co-localization by immunocytochemistry of Sept7 with microtubules bundles induced by  
1148 paclitaxel treatment (PTX) and/or with actin fibers in RPE1 and ARPE19 cells; white scale bar  
1149 10  $\mu\text{m}$ , insets: two-fold zooms of framed regions. Right: Western blots as described in **a** for total  
1150 protein extracts of cells that were treated with vehicle alone (DMSO) or PTX. **d** Left: Co-  
1151 localization by immunocytochemistry of Sept7 with microtubules bundles induced by paclitaxel  
1152 treatment (PTX) in RPE1 cells either transfected with a control siRNA (siCtrl) or a siRNA  
1153 against the  $\alpha$ -tubulin acetyl transferase 1 ( $\alpha$ TAT1); white scale bar 10  $\mu\text{m}$ . Right: Western blot of  
1154 total protein extracts from PTX treated RPE1 cells as described in the left part, showing down-  
1155 regulation of  $\alpha$ -tubulin acetylation upon  $\alpha$ TAT1 KD.

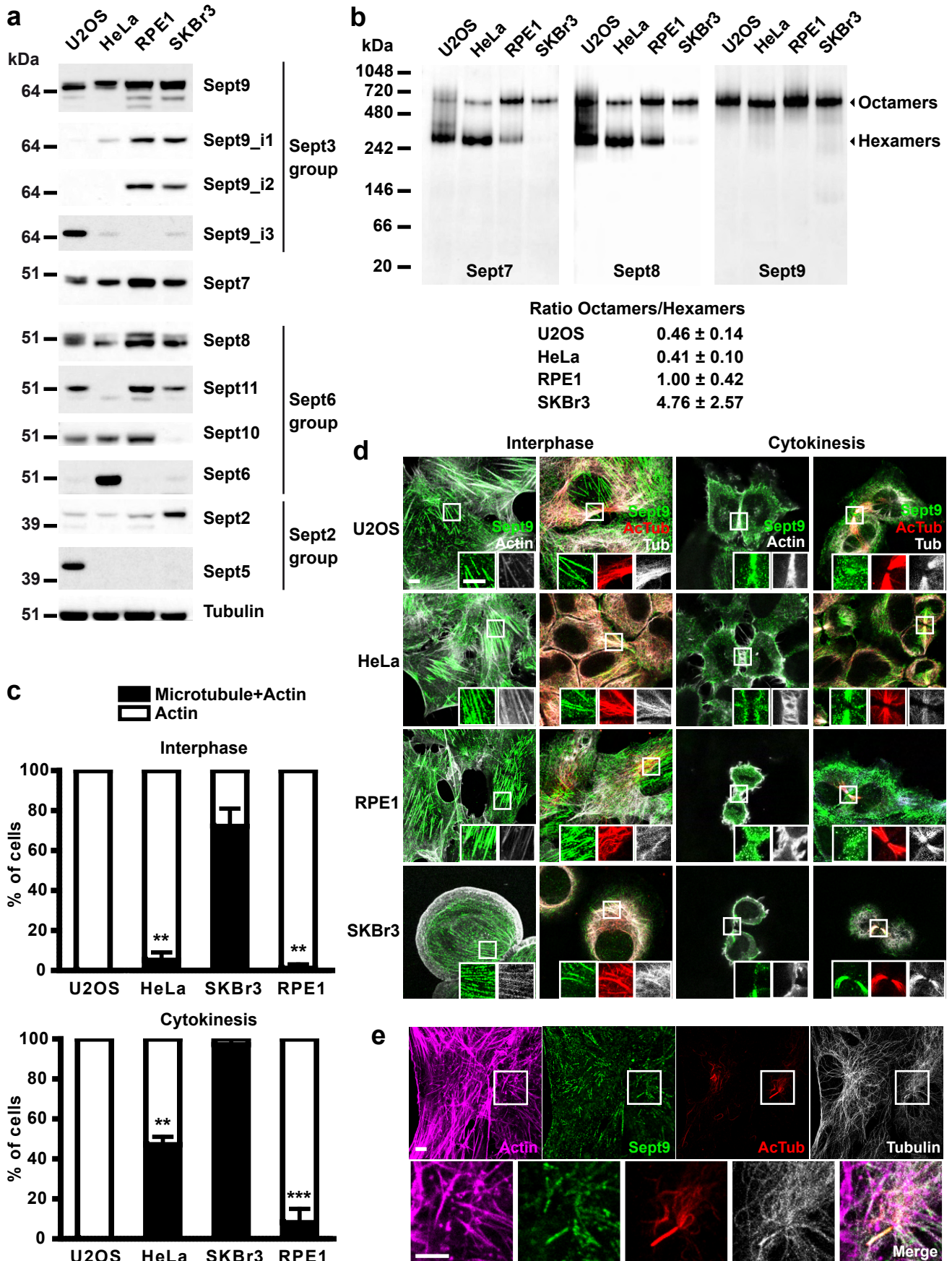
1156  
1157 **Fig. 6. Recombinant septin octamers containing Sept9\_i1 specifically bind microtubules *in***  
1158 ***vitro*.** **a** Schematic representation of the *in vitro* assay. Biotinylated GMPCPP-stabilized  
1159 microtubule seeds were immobilized on a streptavidin coated passivated glass coverslip, and free  
1160 tubulin heterodimers (10  $\mu\text{M}$ ) along with septin octamers (nM concentrations as indicated  
1161 containing 10% msfGFP-labeled septins) were added. The seeds were labelled with a higher  
1162 percentage (30%) of rhodamine-tubulin heterodimers than the free tubulin added (3-6%) to be  
1163 able to distinguish the seed from the dynamic microtubules while using the same fluorophore. **b**  
1164 Septin octamers harboring either Sept9\_i1 or \_i3 subunits (Oct\_9i1 or Oct\_9i3) were introduced  
1165 in the chamber at the indicated concentrations and their binding observed on dynamic  
1166 microtubules (examples of sporadic binding indicated by white arrow heads) and stable  
1167 microtubule seeds (examples indicated by yellow arrow heads). White scale bar 5  $\mu\text{m}$ . **c**  
1168 Examples (#1 and #2) of representative kymographs of microtubules observed as in **b** in the  
1169 presence of the minimal (10 nM) or maximal (300 nM) septin octamer concentrations. False  
1170 colors for rhodamine (Tub) and GFP (Sept2) fluorescence signals were used to optimize visually  
1171 their merged image (third image from the left of each condition). Horizontal white scale bar 5  
1172  $\mu\text{m}$ ; vertical white scale bar 50 sec.

1173  
1174 **Fig. 7. Recombinant septin octamers containing Sept9\_i1 reduce the shortening rate of**  
1175 **dynamic microtubules *in vitro*.** **a** Quantitation of microtubule dynamic parameters (growth rate,  
1176 shortening rate, and catastrophe time frequency) at increasing septin octamer concentrations.  
1177 Results are presented as box and whiskers plots superposed with individual data points including  
1178 outliers. The number of microtubules used (n) and mean value are indicated under each plot.  
1179 (grey: no septin octamers, pink: octamers with Sept9\_i1, light blue: octamers with Sept9\_i3).  
1180 Two-tailed t-test with Benjamini & Hochberg p-value correction with R, \* p<0.05, \*\* p<0.01,  
1181 \*\*\* p<0.001, \*\*\*\* p<0.0001. Right inset graphs show median values of each parameter in the  
1182 absence of octamers (black dots) and direct comparison in presence of either octamers with  
1183 Sept9\_i1 (red dots) or octamers with Sept9\_i3 (blue dots) and corresponding interquartile ranges.  
1184 **b** Representative kymographs (rhodamine channel for tubulin) representing the effect of  
1185 recombinant septin octamers on microtubule dynamics. Such kymographs were used to calculate  
1186 the parameters values presented in **a** from individual microtubules. First kymograph on the left is  
1187 in the absence of septin octamers. Horizontal white scale bar 5  $\mu\text{m}$ ; vertical white scale bar 50  
1188 sec. **c** Snapshots at indicated times (min:sec) of a microtubule and associated septin octamers  
1189 harboring Sept9\_i1 (200 nM, Supplementary Movie 3). Yellow arrow heads point at a curved  
1190 structure on a depolymerizing microtubule (+) end. White scale bar 5  $\mu\text{m}$ .

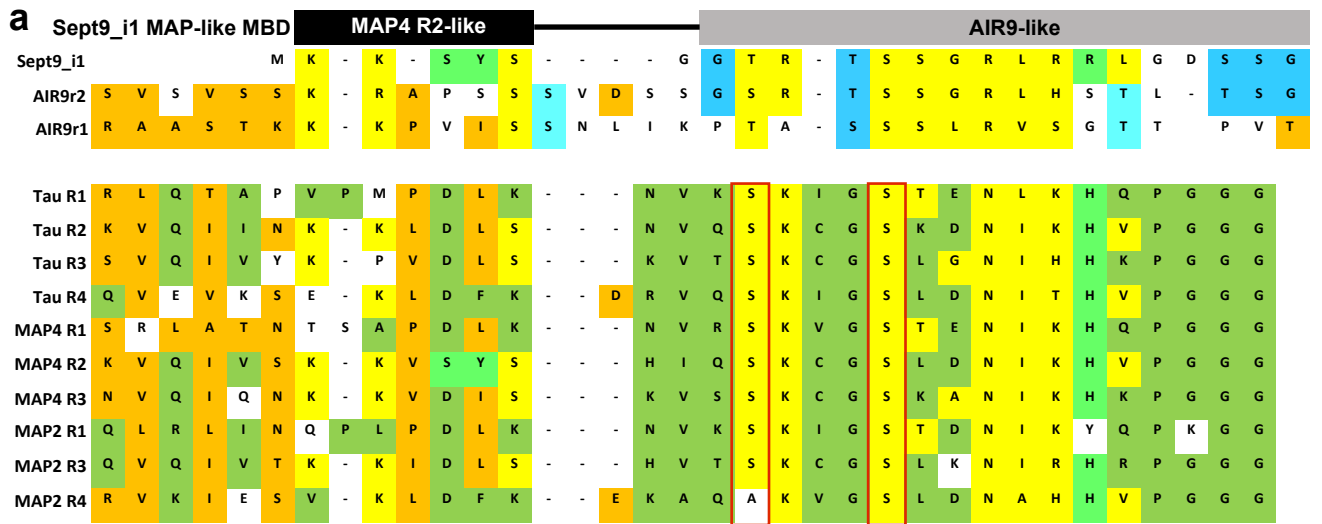
1191

1192 **Fig. 8. Impact on actin cytoskeleton and cell shape of septin octamer sequestration by**  
1193 **microtubules via Sept9\_i1 MBD. a** Analysis of the presence of sub-nuclear actin fibers in  
1194 U2OS transfected by either siCtrl or siSept9. Results are from three independent experiments on  
1195 a total of 90 cells per condition (30 cells per experiments). Unpaired, two-tailed t-test with  
1196 Welch's correction, \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.0005$ , % of cells vs siCtrl. Right: Co-  
1197 localization of Sept7 with sub-nuclear actin stress fibers. White scale bar 10  $\mu\text{m}$ , insets: two-fold  
1198 zooms of frames region. **b** Analysis of the presence of sub-nuclear actin fibers in U2OS derived  
1199 cell lines stably expressing each one of the indicated C-terminally GFP tagged Sept9\_i1  
1200 constructs and the histone mCherry-H2B fusion. Results are from a single determination on 100  
1201 cell images per cell lines. **c** Analysis of shape changes on derived U2OS cell lines described in **b**  
1202 and adhering to medium size H micropatterns. d1: average maximum width of 35.170  $\mu\text{m}$   
1203 observed from measurement on four empty H micropatterns; d2: observed width of single  
1204 adherent cells at mid-maximal height of the H micropatterns. Representative images of merged  
1205 fluorescence channels of the three U2OS derived cell lines selected for analyses. Note that as for  
1206 the rest of the figure, these cell lines (U2OS.Sept9\_i1-GFP.mCherry-H2B, U2OS.Sept9\_i1-i5-  
1207 GFP.mCherry-H2B and U2OS.Sept9\_i1 $\Delta$ 1-25-GFP.mCherry-H2B) are indicated simply by the  
1208 originally transfected Sept9-GFP construct names. White scale bar is 10  $\mu\text{m}$ . Right:  
1209 measurements of the difference between d1 and d2 in cells from selected lines showing a higher  
1210 value when septins bind to microtubules, due to a reduction of the cell width at mid-maximal  
1211 height. Results are from two independent experiments on a total of 120 cells (60 cells per  
1212 experiment). Unpaired, two-tailed t-test with Welch's correction, \*\*\*  $p < 0.0005$ , d1-d2 vs  
1213 Sept9\_i1 $\Delta$ 1-25. Red lines represent mean values  $\pm$  SEM. **d** Measurements of d1-d2, as described  
1214 in c, in U2OS cells transfected either by either siCtrl or siSept9 as described in c showing that  
1215 Sept9 KD reduced cell width at mid-maximal height. Results are from three independent  
1216 experiments on a total of 120 cells (40 cells per experiment). Unpaired, two-tailed t-test with  
1217 Welch's correction, \*\*\*  $p < 0.0005$ , d1-d2 vs siCtrl. Red lines represent mean values  $\pm$  SEM.

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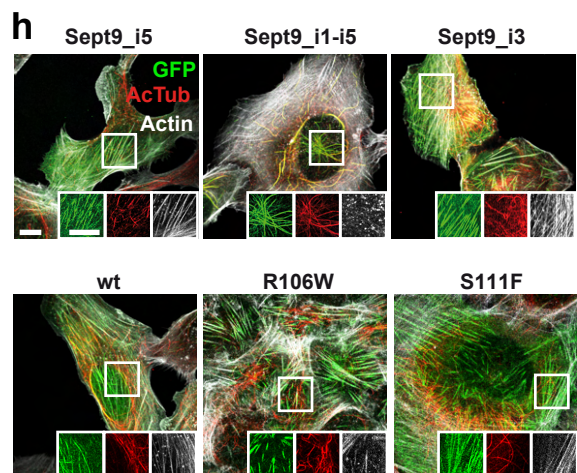
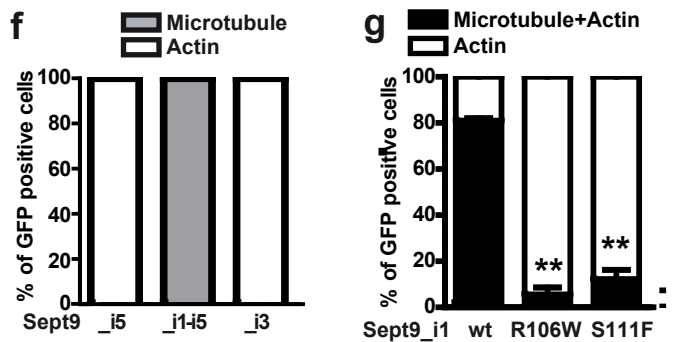
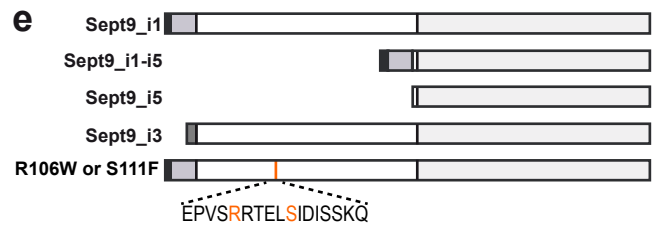
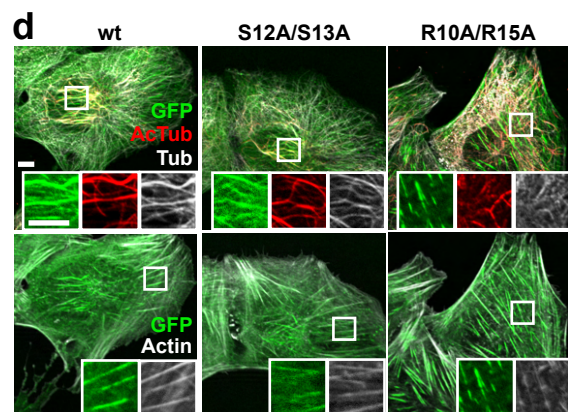
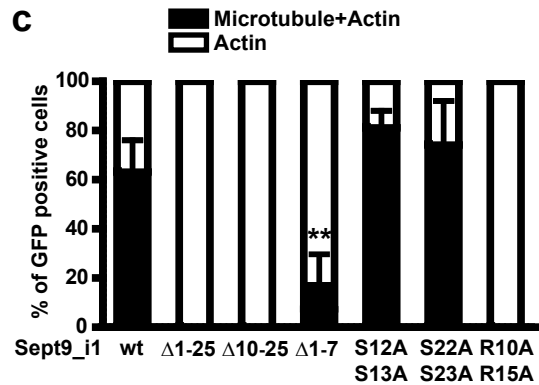
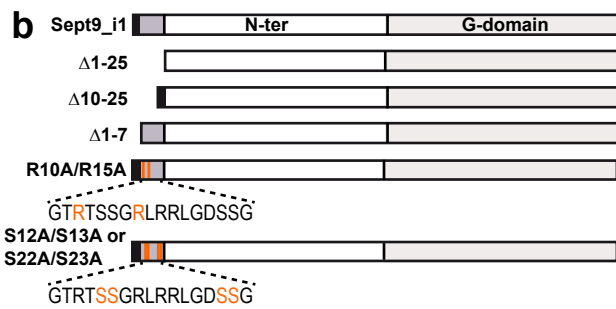


Kuzmic et al. Figure 1

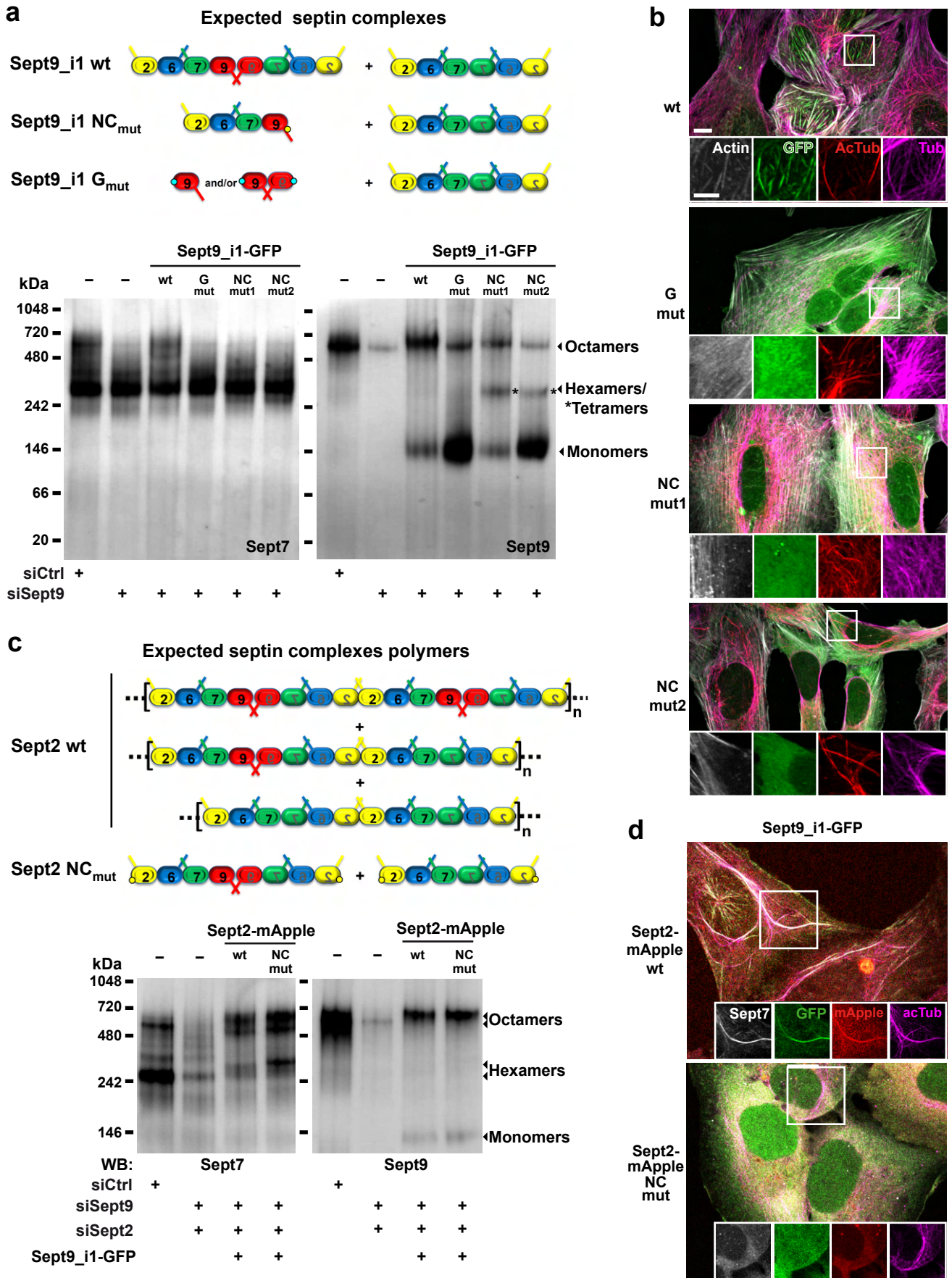


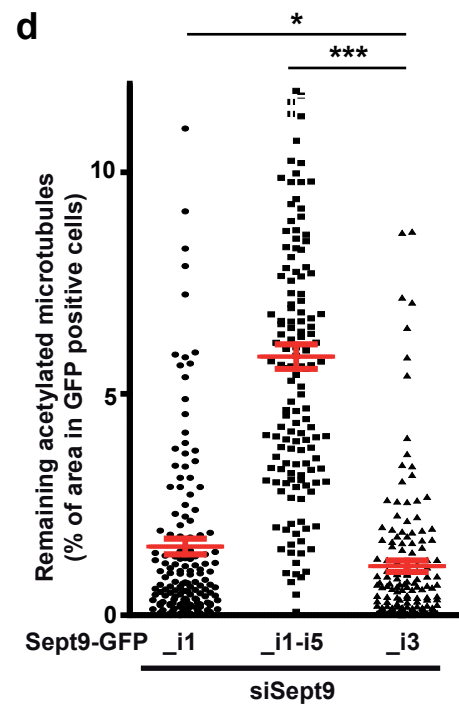
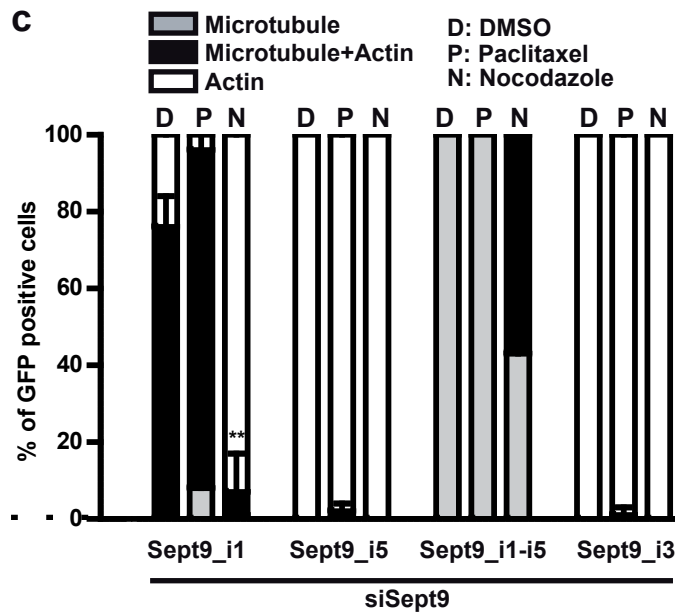
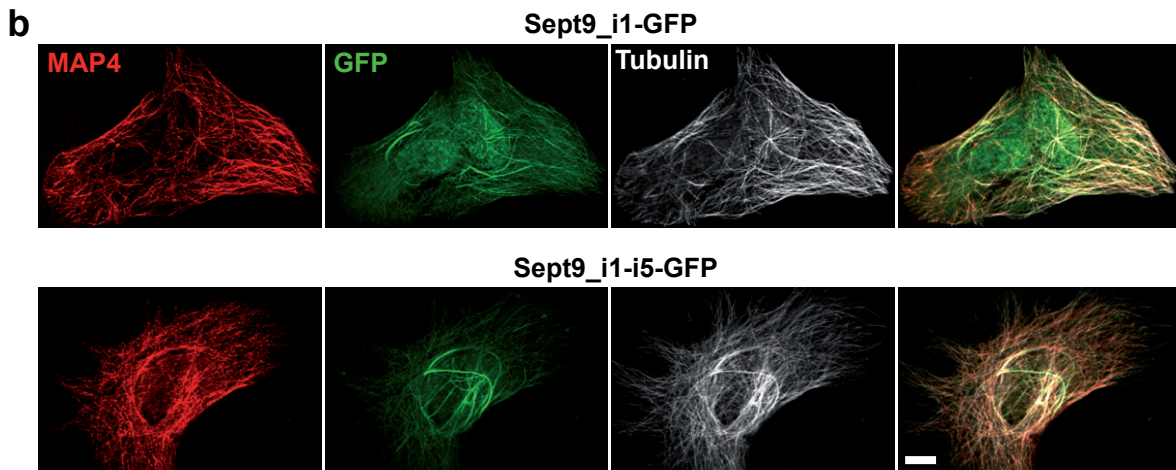
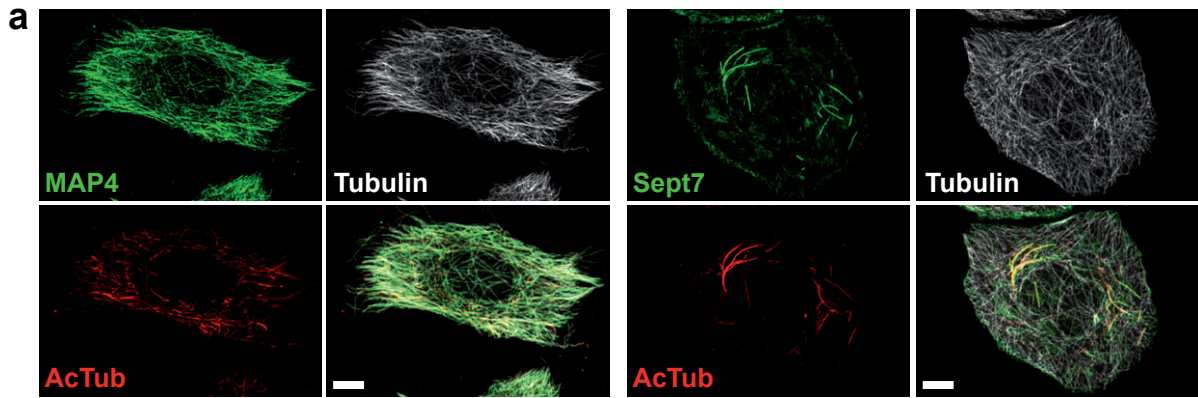
modeled Tau R2<sup>1</sup>

modeled Tau R1<sup>1</sup> or MAP4 R1<sup>2</sup>

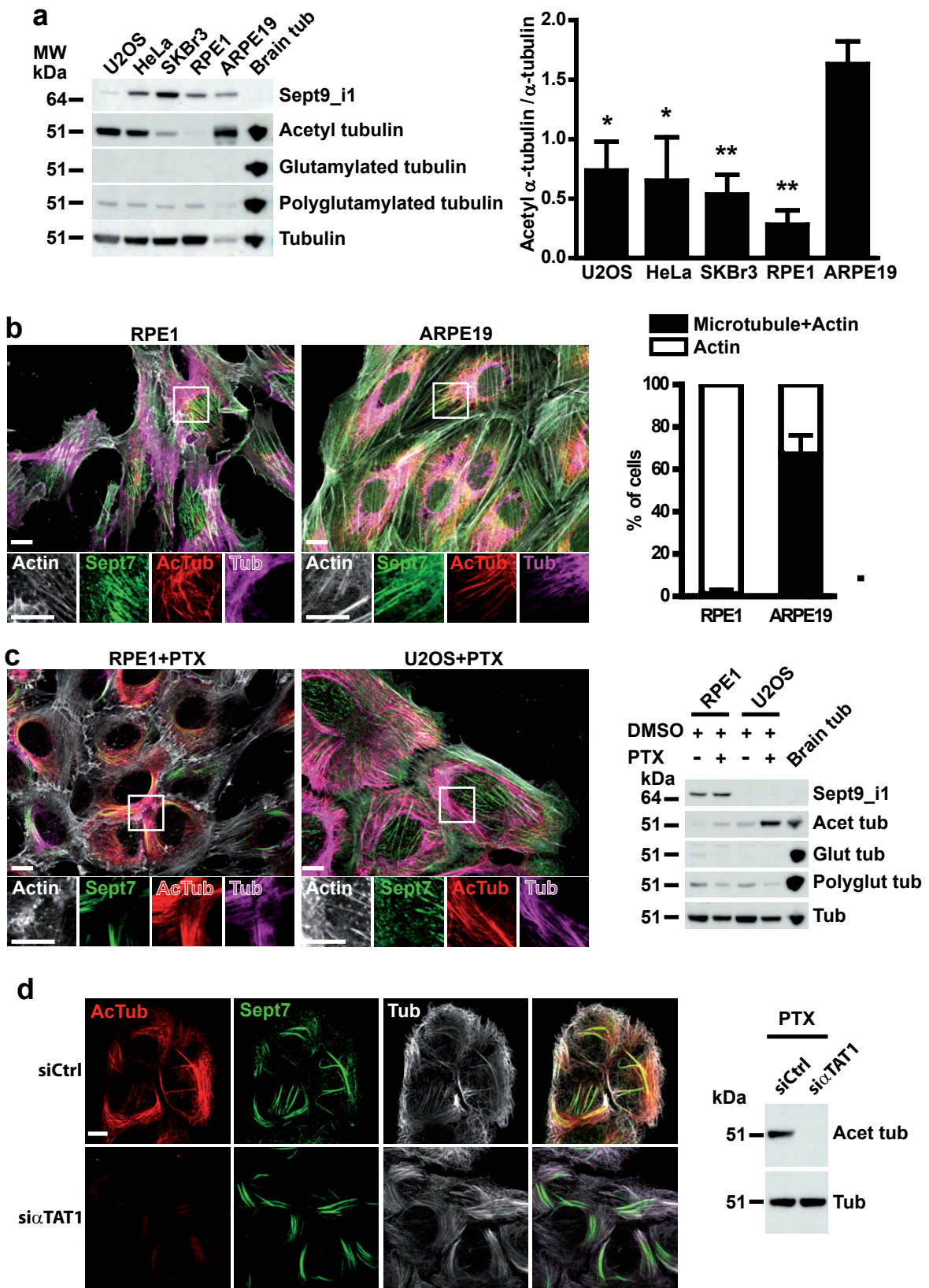


Kuzmic et al. Figure 2

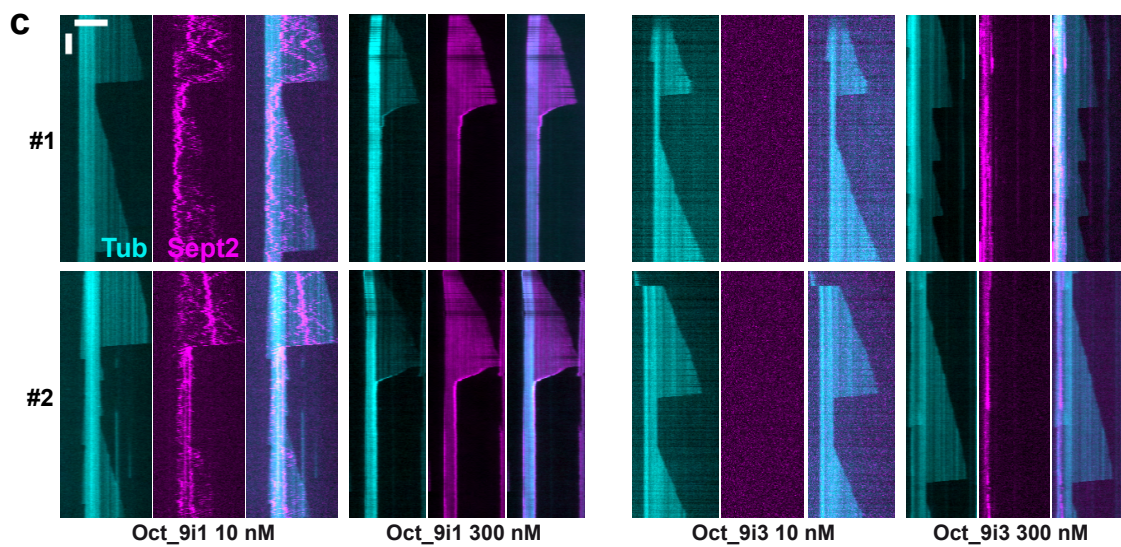
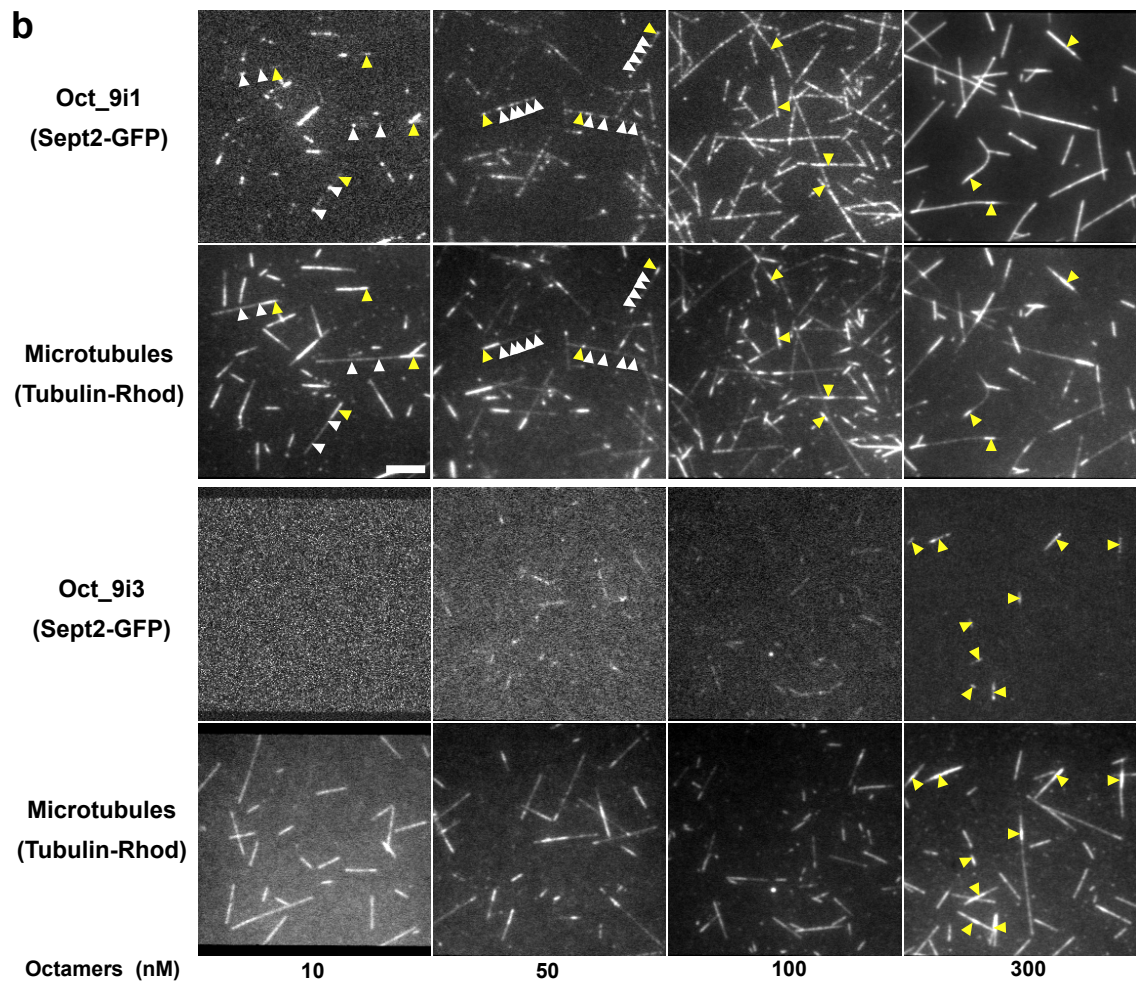
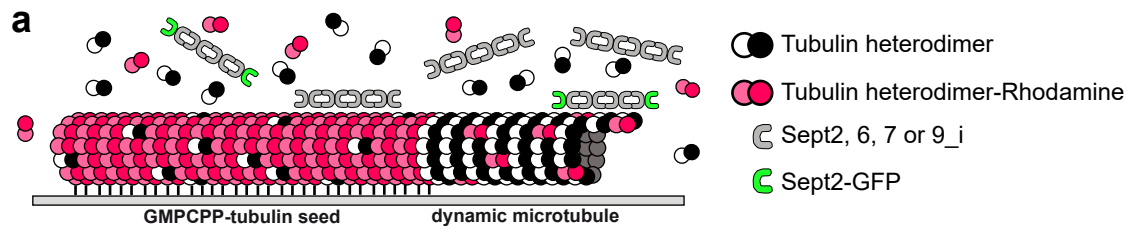


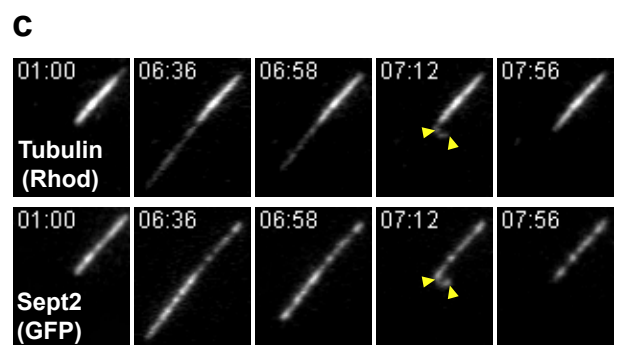
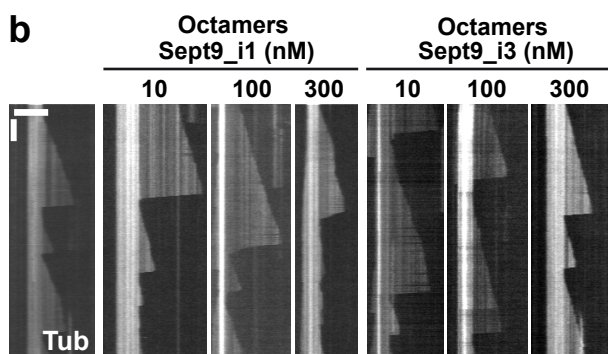
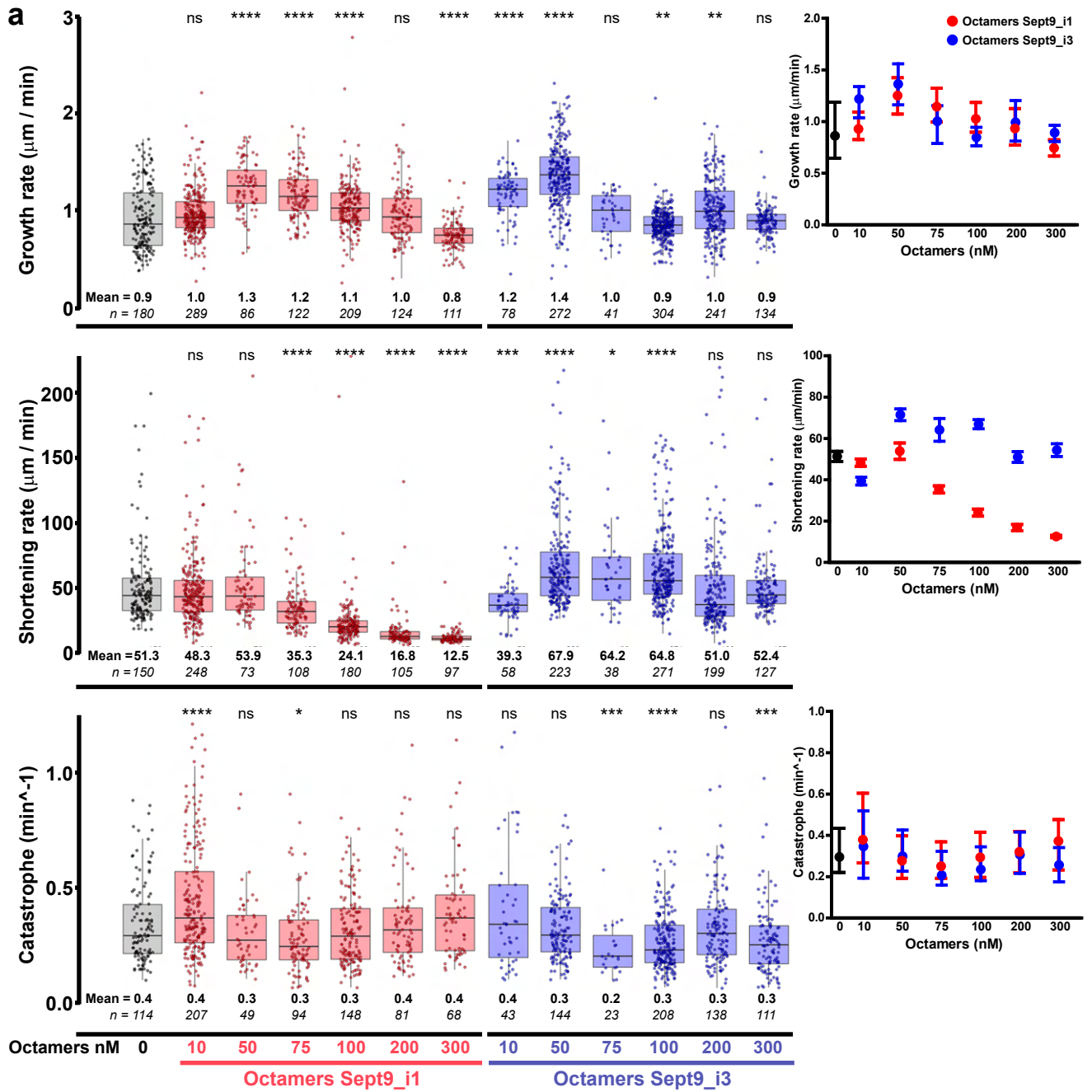


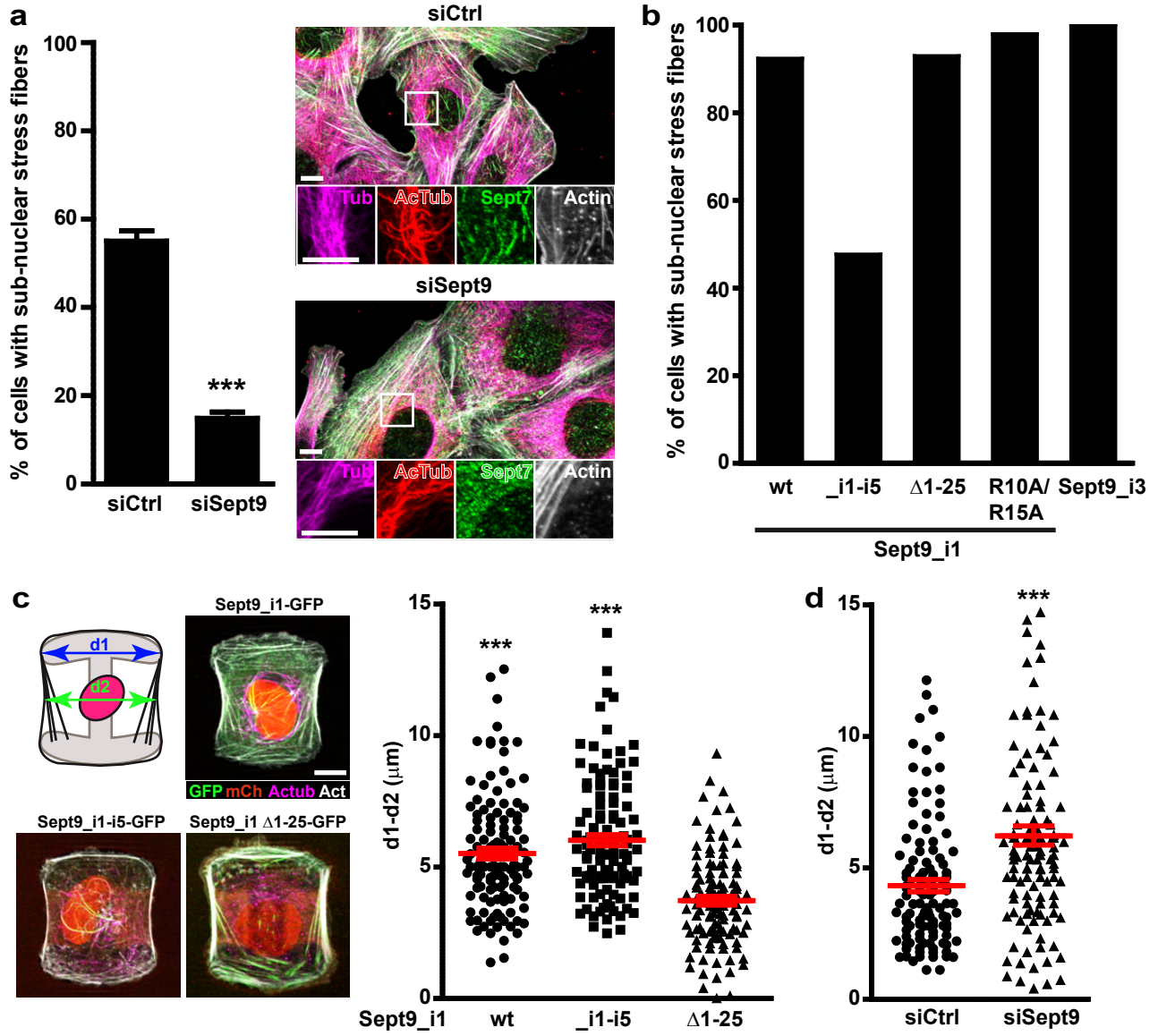




Kuzmic et al. Figure 5







Kuzmic et al. Figure 8

1238 **Supplementary Figure legends**

1239 **Supplementary Fig. 1. Separation of septin complexes on native blue gels and detection by**  
1240 **Western blot. a** Relation between migration distances of native septin complexes from the top  
1241 of the 4-16% acrylamide gradient gels and those of native MW markers; each data point  
1242 represents the average of two or three independent determinations. Right: table presenting  
1243 observed apparent MW and theoretical MW of the different complexes. **b** Recombinant septin  
1244 hexamers and octamers were run on gradient native gels as in **a** and were detected by Coomassie  
1245 blue staining; the asterisk indicates the migration position of putative septin monomers. **c** Native  
1246 protein extracts from Hela cells were run on native gels and blotted on a PVDF membrane;  
1247 septin hexamers and octamers were detected by WB. **d** U2OS cells were KD for Sept7 or Sept9  
1248 and native extracts were analyzed as in **c**.

1249 **Supplementary Fig. 2. Protein sequence conservation of the Sept9\_i1 MBD. a** Alignment of  
1250 Sept9\_i1 sequences grouped by phylogenetically closely related organisms (birds and reptiles  
1251 highlighted in green) and mammals (quadrupeds in blue, rodents in purple, and primates in  
1252 turquoise). Amino acid residues are highlighted based on Clustal X color coding. A conservation  
1253 score histogram for each position is presented below the alignments of sequences; under each  
1254 column is indicated the numerical index reflecting the conservation of physico-chemical  
1255 properties of amino acid residues (asterisk : 100% identical residues, plus sign: alternative  
1256 residues with conserved properties). Columns exhibit a color shading coding from the maximal  
1257 conservation index (11=asterisk) in pure yellow to the minimal conservation index (2) in dark  
1258 brown. Sept9\_i1 1-25 sequences were recovered using the PSI-BLAST tool and the human  
1259 sequence as the seed, imported in Jalview, aligned with Mafft (redundant and truncated  
1260 sequences were eliminated), and grouped by phylogenic proximity by BLOSUM52; the two  
1261 highly conserved arginine residues R10 and R15 mutated in this study are indicated at the top of  
1262 alignments. **b** Consensus logo of the Sept9\_i1 MBD was generated using Jalview.

1263 **Supplementary Fig. 3. Cellular localization of Sept9 by immunocytochemistry. a**  
1264 Fluorescent detection of Sept9 and tubulin on Western blots of total protein extracts from U2OS  
1265 cells transiently transfected by a control siRNA (siCtrl) or co-transfected with an siRNA against  
1266 the 3'UTR region of Sept9 mRNA (siSept9) and with respective constructs described in Fig 2b.  
1267 Numbers at the bottom of each lane indicate the levels of expression of Sept9 constructs relative  
1268 to those of endogenously expressed Sept9 in first lane as quantified on this WB. **b** Percentages of  
1269 U2OS cells transfected with the indicated Sept9-GFP constructs displaying co-localization of  
1270 Sept9 with microtubules and actin fibers or only actin fibers during cytokinesis. Percentages  
1271 were determined from three independent experiments based on a total of 90 cells (30 cells per  
1272 experiment). Unpaired, two-tailed t-test with Welch's correction, \*  $p < 0.05$ , microtubule and  
1273 actin localization vs Sept9\_i1 wt. **c** U2OS cells transfected with the indicated Sept9-GFP  
1274 constructs in cytokinesis. Insets are two-fold zoomed regions framed by a white square in the  
1275 corresponding original images. **d** Percentages of U2OS cells transfected with the indicated  
1276 Sept9-GFP constructs displaying co-localization of Sept9 with microtubules and actin fibers or  
1277 only actin fibers during cytokinesis. Percentages were determined from three independent  
1278 experiments based on a total of 90 cells (30 cells per experiment). Unpaired, two-tailed t-test

1279 with Welch's correction, \*\*  $p < 0.01$ , microtubule and actin localization vs Sept9\_i5. **e** U2OS  
1280 cells expressing Sept9\_i5-GFP. Insets are 2.5-fold zoomed regions framed by a white square in  
1281 the corresponding original images. **f** Comparison of percentages of cells displaying co-  
1282 localization of Sept9 with microtubules and actin fibers or only actin fibers during cytokinesis in  
1283 RPE1 and ARPE19 cell lines. Percentages were determined from three independent experiments  
1284 based on a total of 90 cells (30 cells per experiment). Unpaired, two-tailed t-test with Welch's  
1285 correction. **g** U2OS cells transfected with the indicated Sept9-GFP constructs and treated either  
1286 by vehicle (DMSO) or 10  $\mu\text{M}$  nocodazole for two hours.

1287 **Supplementary Fig. 4. Characterization of U2OS cell lines stably co-expressing Sept9-GFP**  
1288 **and mCherry-H2B constructs.** **a** Expression of Sept9-GFP constructs (Sept9\_X-GFP) and total  
1289 endogenous Sept9 (top Western blot) and expression of Sept9\_i3-GFP constructs and  
1290 endogenous Sept9\_i3 (bottom Western blot) in U2OS cell lines stably co-expressing mCherry-  
1291 H2B and Sept9-GFP constructs (U2OS.Sept9\_X-GFP.mCherry-H2B abbreviated here as  
1292 U2OS.Sept9\_X-GFP) and in the non-transfected parental U2OS cell line. **b** Percentages of cells  
1293 displaying co-localization of Sept9\_X-GFP with microtubules and actin fibers in  
1294 U2OS.mCherry-H2B.Sept9\_X-GFP cell lines. Results are from one determination in 30 cells  
1295 from each cell line. **c** Images of Sept9\_X-GFP constructs localization in U2OS.Sept9\_X-  
1296 GFP.mCherry-H2B cell lines, mCherry-H2B and acetylated tubulin fluorescence signals are  
1297 shown in false blue and red colors, respectively.

1298 **Supplementary Fig. 5. Phenotyping cells based on the presence of sub-nuclear actin fibers.**  
1299 **a** Top and bottom view of actin fibers (phalloidin-Atto 390), Sept9\_i1 (GFP), and nucleus  
1300 (mCherry-H2B) in a U2OS.Sept9\_i1-GFP.mCherry-H2B cell. White scale bar 10  $\mu\text{m}$ . **b** Effects  
1301 of KD of Sept7 or Sept9 in RPE1 cells on the relative expression of Sept7 and Sept9 and **c** on the  
1302 presence of hexamers and octamers. **d** presence of sub-nuclear actin fibers in RPE1 cells KD for  
1303 Sept9, i.e. in the absence of octamers. In **b**, Western blot signals of Sept7 or Sept9 expression  
1304 were first normalized to the respective Western blot signals of  $\alpha$ -tubulin and then further  
1305 normalized to the ratio obtained from cells transfected with Ctrl siRNA; results are from three  
1306 independent experiments. Paired, two-tailed t-test with Welch's correction, \*\*  $p < 0.01$ , \*\*\*  
1307  $p < 0.0005$ . In **c**, Western blots of native extracts resolved on native 4-16% acrylamide native gels.  
1308 In **d**, results are from three independent experiments on a total of 90 cells (30 cells per  
1309 experiment). Unpaired, two-tailed t-test with Welch's correction, \*\*  $p < 0.01$ , % of cells vs siCtrl.

1310 **Supplementary Table 1. Oligonucleotide primer sequences used for the generation of the**  
1311 **plasmids used in this study.** Oligonucleotides marked with an asterisk are shared among several  
1312 constructs, but are listed only once.

1313 **Supplementary Movie 1. Dynamics of septin octamers harboring Sept9\_i1 (Oct\_9i1)**  
1314 **binding with microtubules *in vitro*.** Series of videos corresponding to each of the 10 to 300 nM  
1315 of Oct\_9i1 that were used. White scale bar 5  $\mu\text{m}$ .

1316 **Supplementary Movie 2. Dynamics of septin octamers harboring Sept9\_i3 (Oct\_9i3)**  
1317 **binding with microtubules *in vitro*.** Series of videos corresponding to each of the 10 to 300 nM  
1318 of Oct\_9i3 that were used. White scale bar 5  $\mu\text{m}$ .

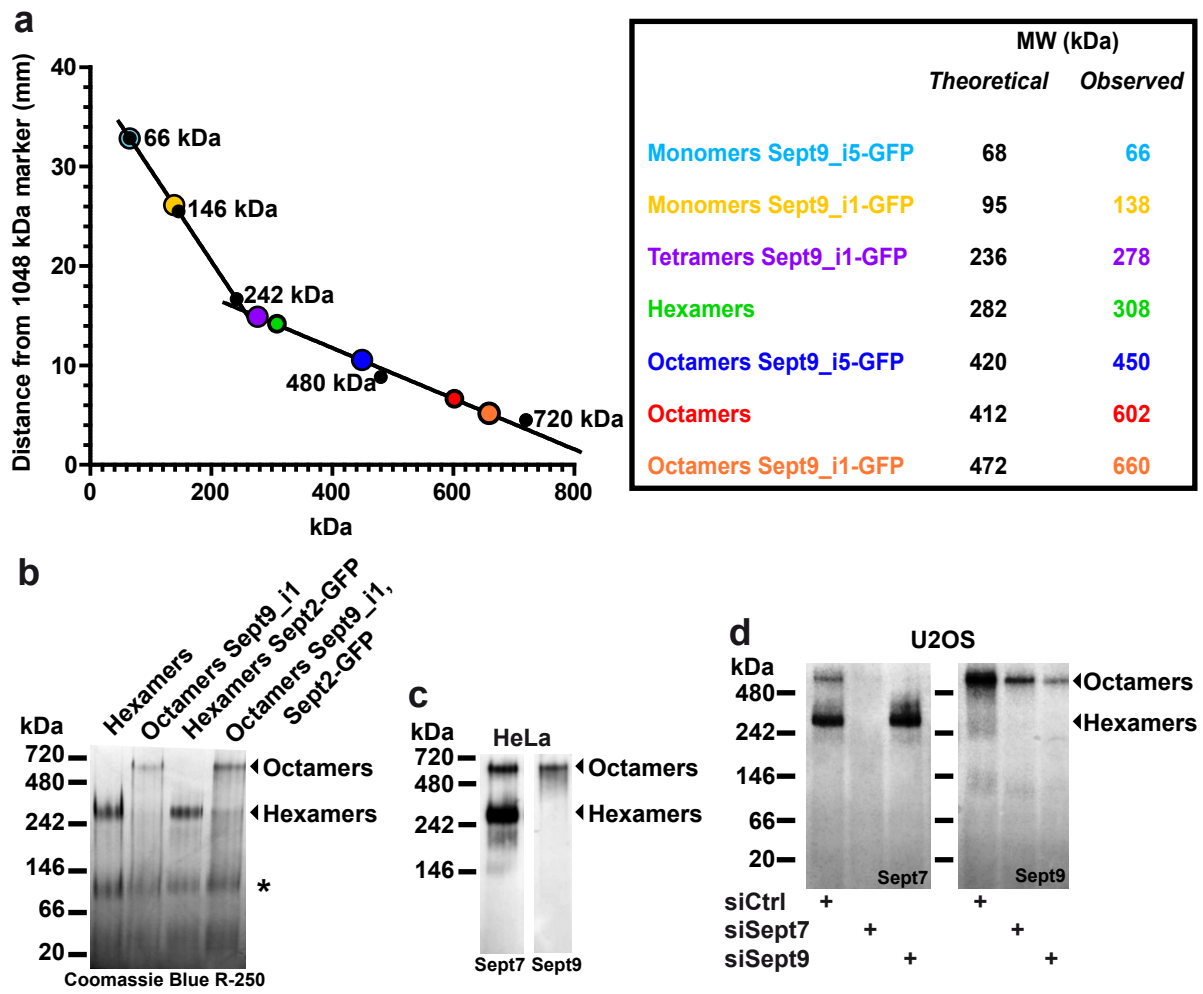
1319 **Supplementary Movie 3. Depolymerizing microtubules (examples 1 and 2) in presence of**  
1320 **200 nM of septin octamers harboring Sept9\_i1 (Oct\_9i1) *in vitro*.** Series of videos  
1321 corresponding to each example. White scale bar 5  $\mu$ m.

1322

promoter	in-house plasmid name	plasmid	primer name	primer sequence (5' to 3')	primer length	
CMV			Sept9_i1-msfGFP	NheISept9_i1FOR	CGTCAGATCCGCTAGCATGAAGAAGTCTTACTCAGGAGGCAC	42
			Sept9_i3-msfGFP	NheISept9_i3FOR	CGTCAGATCCGCTAGCATGGAGAGGGACCGGATCTC	36
				Sept9sfGFPREV	TTGGACACCATCTCTGGGGCTTCTGGCTCC	30
				Sept9sfGFPFOR	AGAGATGGTGTCCAAGGGCGAGGAGC	26
				BamHIsfGFPREV	TAGATCCGGTGGATCCTTACTGTACAGCTCATCCATGCC	40
CMV		Sept2-mApple	NheIhSept2FOR	CGTCAGATCCGCTAGCATGTCTAAGCAACAACCAACTCAGT	41	
			hSept2mAppleREV	CTCGCCCTTGCTCACCACATGGTGGCCGAGAGC	33	
			mAppleFOR	GTGAGCAAGGGCGAGGAG	18	
			BamHImAppleREV	TAGATCCGGTGGATCCTCACTGTACAGCTCGTCCATGCC	40	
			NdeICMVpromoterFOR	ATCAAGTGTATCATATGCCAAGTACGCCCCCTATTGACGTCAATGACG	48	
CMV	pl-14	Sept2 F20D V27D-mApple	Sept2FDREV	GATTGGGAAGATTTGCATCTCCAACATAGCCAGGAGTTTCTGG	43	
			Sept2FDFOR	CAAATCTTCCAATCAAGATCACCGAAAATCAGTGAAGAAGGGGTTTCG	48	
			Sept9IDREV	TGCTCAGGATGGAGTCATCCCCACGTAGCCGAAGTCC	39	
CMV	pl-6	Sept9_i1-1281D M288D-msfGFP (Sept9_i1 NCmut2)	Sept9MDFOR	CTCCATCTGGAGCAGGATCGCCGGAAGCCATGAAGC	38	
			a0h_Sept9_REV	CGCCGCGCATCTGCTCCAGGATGGAGTCAATCC	35	
CMV	α0h-4	Sept9_i1 R289A R290A K291A K294A-msfGFP (Sept9_i1 NCmut1)	a0h_Sept9_FOR	CAGATGGCCGCGCGCCATGGCGCAGGGCTTCGAGTTCAACATCATG	48	
			Sept9i1W520AREV	AACTTCGATGGTACCCGCTTGGTCTTCTCCCAAGGATCC	41	
CMV	pl-12	Sept9_i1 W520A H530D-msfGFP (Sept9_i1 Gmut)	Sept9i1H530DFOR	GGTACCATCGAAGTTGAAAACACCACAGATTGTGAGTTGCCTACCTGCGG	51	
			NheISept9dNFOR	CGTCAGATCCGCTAGCATGGCCTTGAAAAGATCTTTTGAGGTCG	44	
CMV	is-7	Sept9_i5-msfGFP	NheISept9i5FOR	CGTCAGATCCGCTAGCATGGCCGACACCCCCAG	33	
CMV	is-9	Sept9_i1-i5-msfGFP	NheI9i1_Sept9i5FOR	CGTCAGATCCGCTAGCATGAAGAAGTCTTACTCAGGAGGCACGCGACCTCCAGTGCCCGGCTCCGGAGGCTTGGTGACTCCAGTGGCCAGCCGACACCCCCAGAGATG	110	
CMV	mt-1	Sept9_i1 Δ10-25-msfGFP	NheISept9i1D10FOR	CGTCAGATCCGCTAGCATGAAGAAGTCTTACTCAGGAGGCACGCGACCTCCAGTGCCCGCTCCGGAGGCTTGGTGACTCC	73	
CMV	mt-2	Sept9_i1 R10A R15A-msfGFP	NheISept9i1R10R15FOR	CGTCAGATCCGCTAGCATGAAGAAGTCTTACTCAGGAGGCACGCGACCTCCAGTGCCCGCTCCGGAGG	82	
CMV	mt-5	Sept9_i1 S12A S13A-msfGFP	NheISept9i1S12S13FOR	CGTCAGATCCGCTAGCATGAAGAAGTCTTACTCAGGAGGCACGCGACCGCCGCGCCGGCTCCGGAGG	70	
CMV	mt-6	Sept9_i1 S22A S23A-msfGFP	NheISept9i1S22S23FOR	CGTCAGATCCGCTAGCATGAAGAAGTCTTACTCAGGAGGCACGCGACCTCCAGTGCCCGGCTCCGGAGGCTTGGTGACTCCAGTGGCCAGCCGACCTTGAAGAAGTCTTTGAGG	113	
CMV	mt-20	Sept9_i1 ΔN7-msfGFP (Sept9_i1 Δ1-7)	NheISept9i1DN7FOR	CGTCAGATCCGCTAGCATGGGCACGCGGACCTCCAG	36	
CMV	Hna-1	Sept9_i1 R106W-msfGFP	Sept9RWREV	CAGTGCCAGGACACCGCTCGGCC	26	
			Sept9RWFOR	TGCTTGGCGCACTGAGCTGTCCATTGAC	29	
CMV	Hna-2	Sept9_i1 S111F-msfGFP	Sept9SFREV	GATGTCAATGAACAGCTCAGTGCGCCGG	28	
			Sept9SFFOR	CTGTTCAATGACATCTCGTCCAAGCAGGTG	30	

Supplementary Table 1. Oligonucleotide primer sequences used for the generation of the plasmids used in this study.



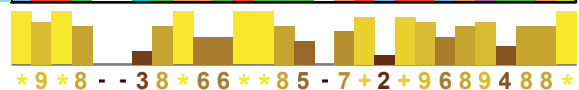


Kuzmic et al. Supplementary Figure 1

**a**

		R10	R15
XP_034645779.1 [ <i>Trachemys scripta elegans</i> ]	MKK	S-VHSGIARTSS	-GR LRR LGDPTSP
XP_030390263.1 [ <i>Gopherus evgoodei</i> ]	MKK	S-VHSGISRTSS	-GR LRR LGDPTSP
XP_024062065.1 [ <i>Terrapene carolina triunguis</i> ]	MKK	S-VHSGITRTSS	-GR LRR LGDPTSP
XP_014457381.1 [ <i>Alligator mississippiensis</i> ]	MRK	S-SYSGARTTSS	-GK LRK AGDPTSP
XP_019398237.1 [ <i>Crocodylus porosus</i> ]	MRK	S-SYSGARTTSS	-GR LRK AGDPTSP
XP_015269831.1 [ <i>Gekko japonicus</i> ]	MRK	T-SYSGISRTSS	-GR LRR LGNPTSP
XP_020641631.1 [ <i>Pogona vitticeps</i> ]	MRK	T-SYSGISRTSS	-GR LRR LGDPTSP
XP_028572882.1 [ <i>Podarcis muralis</i> ]	MRK	T-SNTGISRTSS	-GR LRR FGDPTSP
XP_026557860.1 [ <i>Pseudonaja textilis</i> ]	MKK	TSSYTGT SRTSS	G-VR LRR FGDPTSP
XP_026523333.1 [ <i>Notechis scutatus</i> ]	MKK	TSSYTGT SRTSS	-GR LRR FGDPTSP
XP_008102562.1 [ <i>Anolis carolinensis</i> ]	MKK	T-SYTGISRTSS	-GR LRR LGDPTSP
XP_021270206.1 [ <i>Numida meleagris</i> ]	MRK	S-MYSGITRTSS	-GR LRR LSDQ TSP
XP_015735503.1 [ <i>Coturnix japonica</i> ]	MRK	S-TYSGITRTSS	-GR LRR LSDQ TSP
XP_013034321.1 [ <i>Anser cygnoides domesticus</i> ]	MRK	S-SYSGITRTSS	-GR LRR LSDQ TSP
XP_026716736.1 [ <i>Athene cucularia</i> ]	MRK	S-SYSGISRTSS	-GR LRR LSDQ TSP
XP_030900675.2 [ <i>Melopsittacus undulatus</i> ]	MKK	S-SYSGISRTSS	-GR PRR LSDQ TSP
XP_027741897.1 [ <i>Melopsittacus undulatus</i> ]	MKK	S-SYSGISRTSS	-GR LGR LSDQ TSP
XP_029870538.1 [ <i>Aquila chrysaetos chrysaetos</i> ]	MKK	S-SYSGISRTSS	-GR LRR LSDQ TSP
XP_014740523.1 [ <i>Sturnus vulgaris</i> ]	MKK	S-SYSGITRTSS	-GR LRR SEQ TSP
XP_025959670.1 [ <i>Dromaius novaehollandiae</i> ]	MKK	S-SYSGITRTSS	-GR LQR LSDQ TSP
XP_026654321.1 [ <i>Zonotrichia albicollis</i> ]	MKK	S-SYSGITRTSS	-GR LRR LSDQ TSP
XP_023387505.1 [ <i>Pteropus vampyrus</i> ]	MKK	S--YSGVTRTSS	-DR LRK LGDPAGP
XP_025323914.1 [ <i>Canis lupus dingo</i> ]	MKK	S--SSGVTRTSS	-NRLRK LGDPTGP
XP_016047785.1 [ <i>Erinaceus europaeus</i> ]	MKK	S--SSGV SRTSS	-SRLRR LGDPSGP
XP_027705848.1 [ <i>Vombatus ursinus</i> ]	MKK	S-SGSGVMRTS	I-SR-RRF GDTSGP
XP_020821661.1 [ <i>Phascolarctos cinereus</i> ]	MKK	S-SGSGVMRTS	I-SR-RRL GDTSGP
XP_017506039.1 [ <i>Manis javanica</i> ]	MKK	S--SSGVTRTSS	-SRLRR LGDL SGP
XP_025715323.1 [ <i>Callorhinus ursinus</i> ]	MKK	S--YSGVTRTSS	-NRLRK LGDT TGP
XP_012415706.1 [ <i>Odobenus rosmarus divergens</i> ]	MKK	S--YSGVTRTSS	-NRLRK LGDATGP
XP_026339835.1 [ <i>Ursus arctos horribilis</i> ]	MKK	S--YSGVTRTSS	-NRLRK LGEP TGP
XP_023099861.1 [ <i>Felis catus</i> ]	MKK	S--YSGVTRTSS	-NRLRK LGDPTGP
XP_032726397.1 [ <i>Lontra canadensis</i> ]	MKK	S--YSGVTRTSS	-NRLRK LGDPSGP
XP_007185825.1 [ <i>Balaenoptera acutorostrata scammonii</i> ]	MRK	S--YSGVARTSS	-GRLRK LGDPTGP
XP_026936614.1 [ <i>Lagenorhynchus obliquidens</i> ]	MRK	S--YSGVMRTS	G-GRLRK LGDPTGP
XP_024616632.1 [ <i>Neophocaena asiaeorientalis asiaeorientalis</i> ]	MRK	S--YSGVTRTSS	G-GRLRK LGDPTGP
XP_010949017.1 [ <i>Camelus bactrianus</i> ]	MKK	S--YSGVARTSS	-GRLRK LGDPTGP
KAF6458727.1 [ <i>Rousettus aegyptiacus</i> ]	MKK	S--YSGVTRTSS	-GRLRK LGDPTGP
KAF6299646.1 [ <i>Rhinolophus ferrumequinum</i> ]	MKK	S--YTG VTRTSS	-GRLRK LGDPTGP
KAF6417104.1 [ <i>Molossus molossus</i> ]	MKK	S--YSGVTRTSS	-GRLRK LGDSTGP
XP_019499882.1 [ <i>Hipposideros armiger</i> ]	MKK	S--YTG VTRTSS	-GRLRK LGDT TGP
XP_017919201.1 [ <i>Capra hircus</i> ]	MKK	S--YTG VTRTSS	-GRLRK LGDSTGP
XP_004860849.1 [ <i>Heterocephalus glaber</i> ]	MKK	S--YSGVTRTAS	-GRLRR LGDPTGP
XP_023507708.1 [ <i>Heterocephalus glaber</i> ]	MKK	S--YSGVTRTSS	-GRLRR LGDL TGP
XP_015864047.1 [ <i>Peromyscus maniculatus bairdii</i> ]	MKK	T--NSGVARTSS	-GRLRR LADPTGP
XP_021496060.1 [ <i>Meriones unguiculatus</i> ]	MKK	S--YSGVGR TSS	SGRLRR LADPTGP
XP_031210187.1 [ <i>Mastomys coucha</i> ]	MKK	S--YSGVTRTSS	-GRLRR LADPTGP
XP_012968864.1 [ <i>Mesocricetus auratus</i> ]	MKK	S--YSGVTRTSS	-GRLRR LADSTGP
VTJ77022.1 [ <i>Marmota monax</i> ]	MKK	S--YSGVTRTSS	-GRLRR LGDPTGP
XP_010340668.1:1-25 [ <i>Saimiri boliviensis boliviensis</i> ]	MKK	S--YSGGTRTAS	-GRLRR LGDPSGP
XP_030779009.1 [ <i>Rhinopithecus roxellana</i> ]	MKK	S--YSGGTRTSS	-GRLRR LGDPSGP
XP_032097420.1 [ <i>Sapajus apella</i> ]	MKK	S--YSGGTRTSS	-GRLRR LGDASGP
Sept9_i1 [ <i>Homo sapiens</i> ]	MKK	S--YSGGTRTSS	-GRLRR LGDSSGP

Conservation



**b**

Consensus sequence



