Microglia reactivity entails microtubule remodeling from acentrosomal to centrosomal arrays

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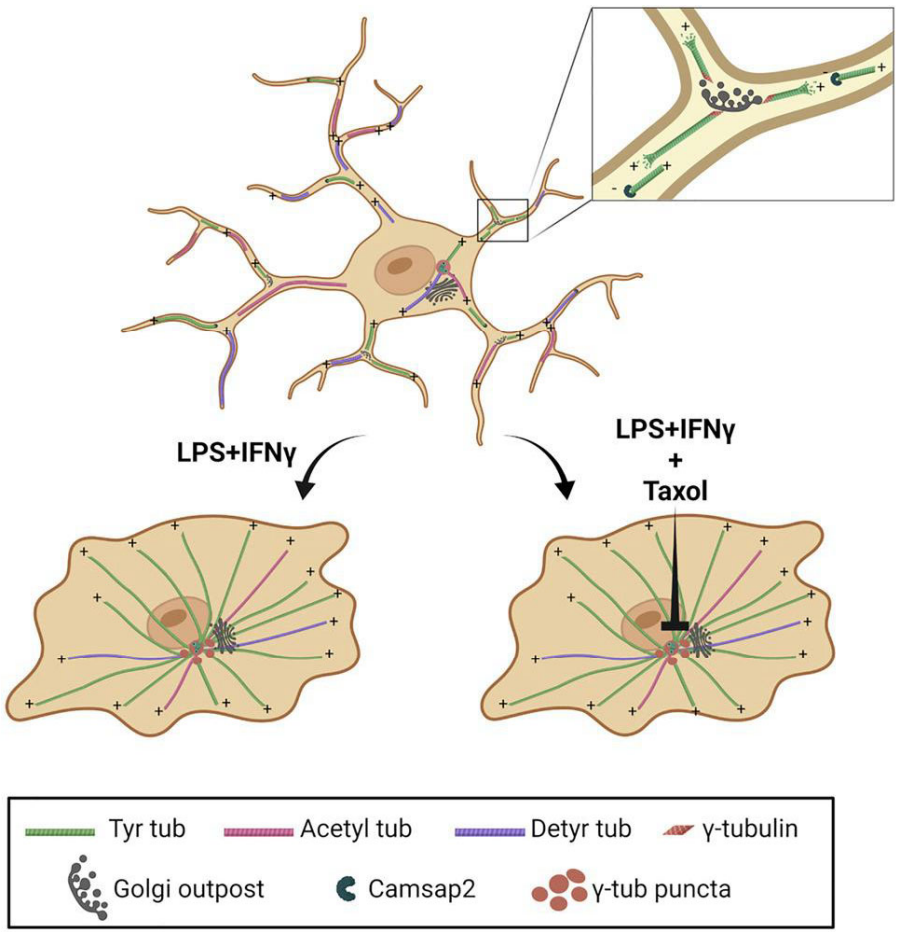
Maria Rosito, Caterina Sanchini, Giorgio Gosti, Manuela Moreno, Simone De Panfilis, Maria Giubettini, Doriana Debellis, Federico Catalano, Giovanna Peruzzi, Roberto Marotta, Alessia Indrieri, Elvira De Leonibus, Maria Egle De Stefano, Davide Ragozzino, Giancarlo Ruocco, Silvia Di Angelantonio, and Francesca Bartolini

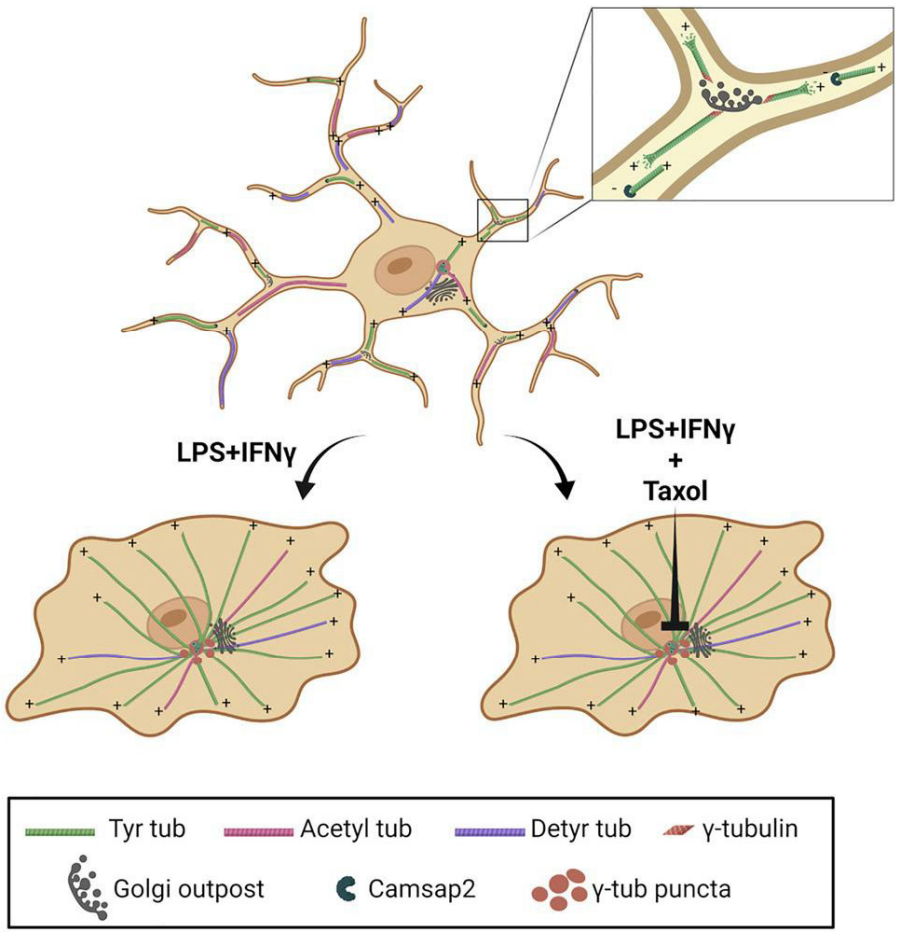
Presenter: Haruka Kanamaru



Microglia reactivity entails a large-scale remodeling of cellular geometry, but the behavior of the microtubule cytoskeleton during these changes remains unexplored.

- Here we show that activated microglia provide an example of microtubule reorganization from a non-centrosomal array of parallel and stable microtubules to a radial array of more dynamic microtubules.
- While in the homeostatic state, microglia nucleate microtubules at Golgi outposts, and activating signaling induces recruitment of nucleating material nearby the centrosome, a process inhibited by microtubule stabilization.
- Our results demonstrate that a hallmark of microglia reactivity is a striking remodeling of the microtubule cytoskeleton and suggest that while pericentrosomal microtubule nucleation may serve as a distinct marker of microglia activation.

Inhibition of microtubule dynamics may provide a different strategy to reduce microglia reactivity in inflammatory disease.



ミクログリアの活性化には、細胞形状の大規模なリモデリングが伴うが、その際の微小管細胞骨格の挙動は未解明である。

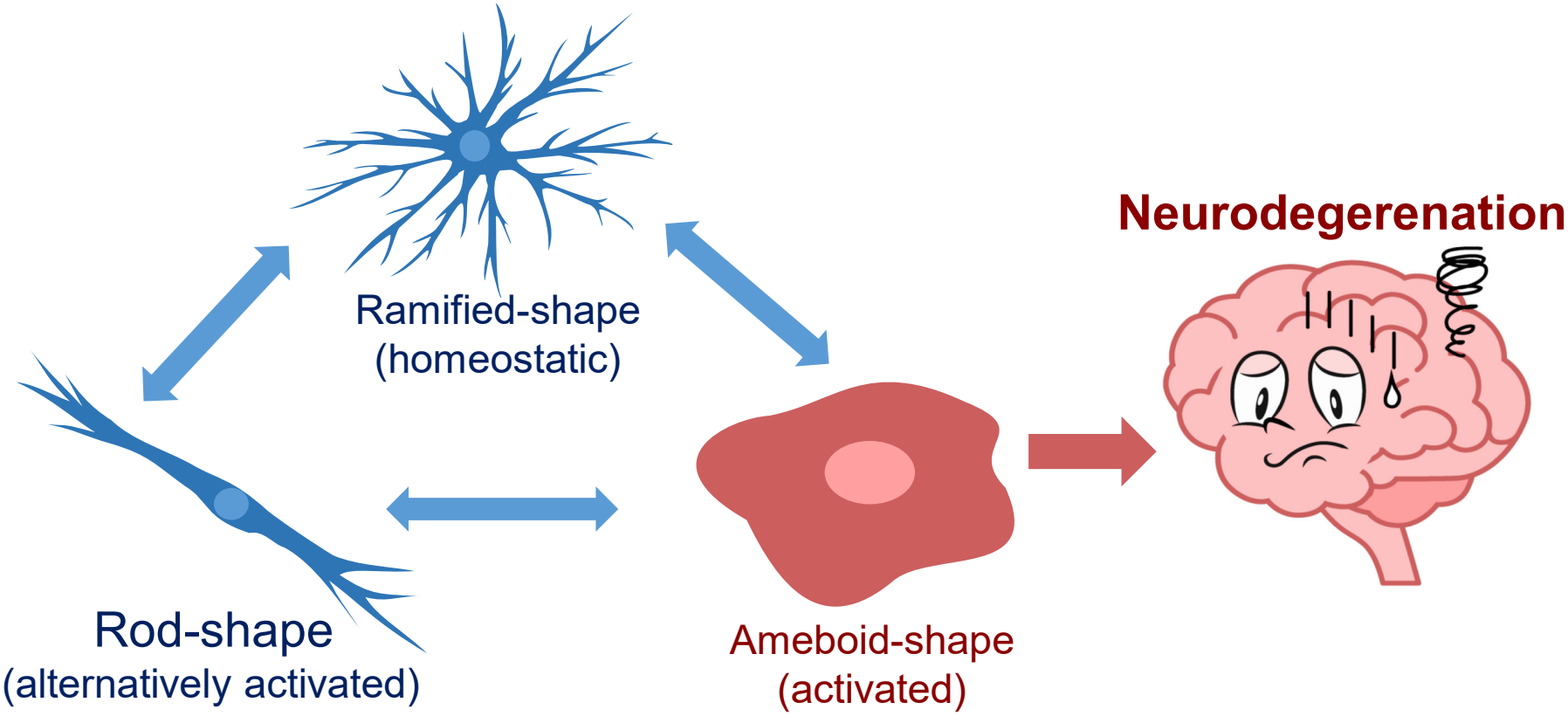
ここでは、活性化したミクログリアが、平行で安定な微小管のセントロソーム非依存的な配置から、より動的な微小管の放射状配置への微小管の再編成の一例となることを示す。

恒常状態では、ミクログリアはゴルジ体のアウトポストで微小管の核形成を行い、活性化シグナルは、微小管の安定化によって抑制されるセントロソーム付近の核形成物質のリクルートを誘導する。

この結果は、ミクログリアの反応性の特徴が微小管細胞骨格の顕著なリモデリングであることを示し、セントロソーム周辺の微小管核形成がミクログリア活性化の明確なマーカーとして機能する可能性を示唆する。

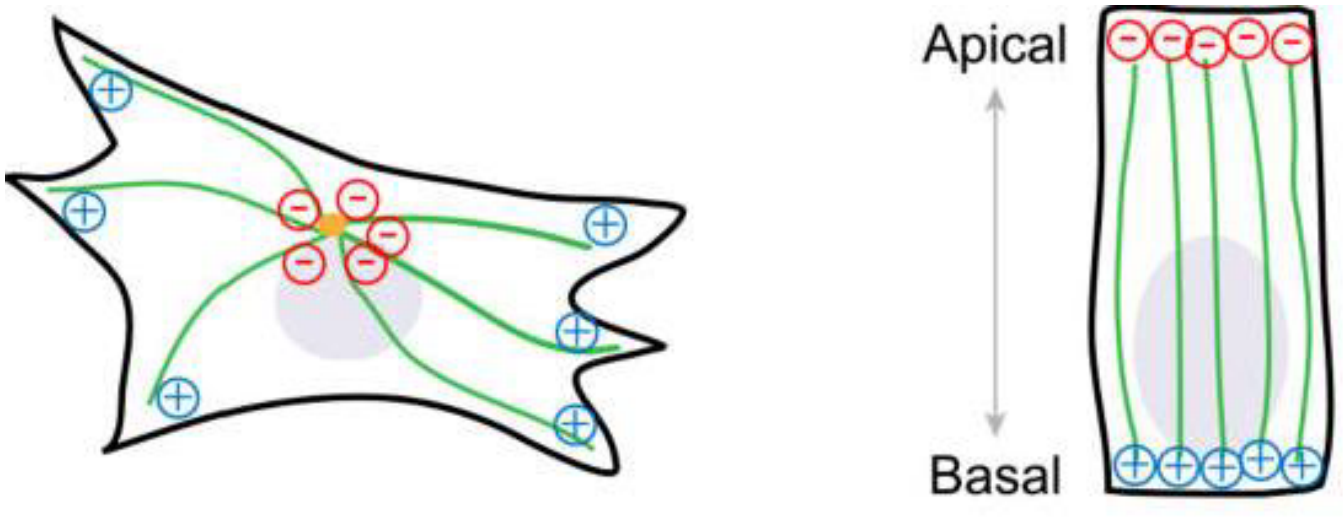
微小管ダイナミクスを阻害することで、炎症性疾患におけるミクログリアの反応性を低下させる別の戦略を提供することができるかもしれない。

Microglial Morphology and Association with Diseases



- Microglia change their morphology responding to various stimulation
- Overactivation is associated with neurodegeneration

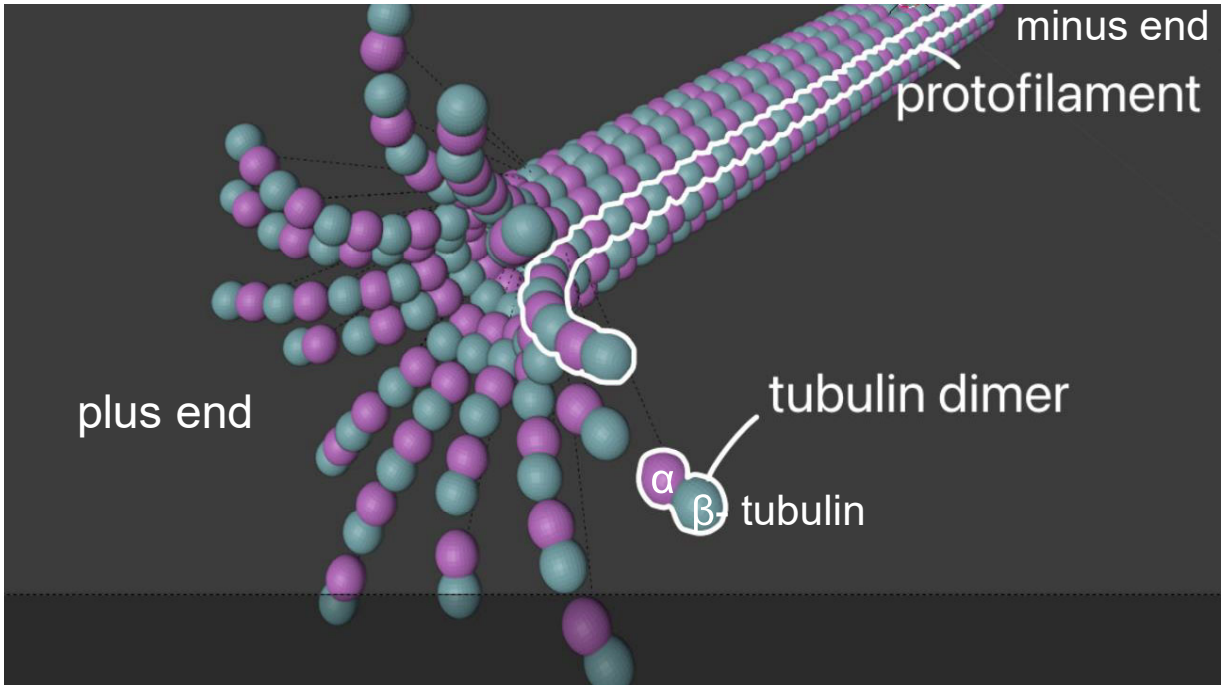
Change in cellular polarity and cytoskeleton

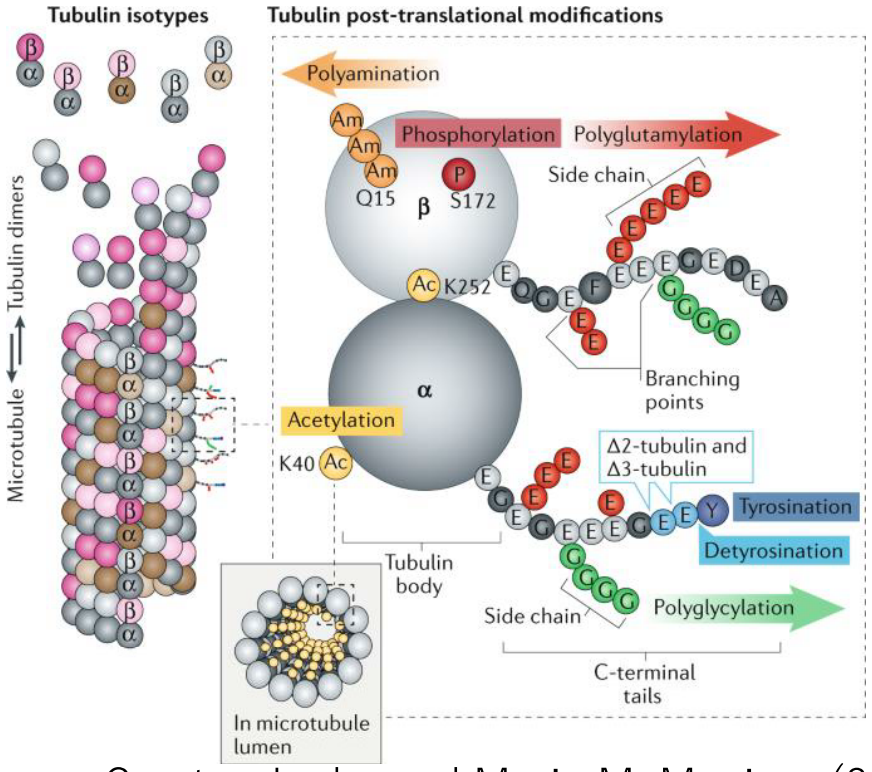


M. Toya, M. Takeichi (2016)

- In eukaryotes, changes in cellular symmetry are associated with cytoskeletal rearrangement
- The role of microtubules in breaking cellular polarity during transition from ramified to ameboid are largely unknown.

The Organization of Microtubules in Cells

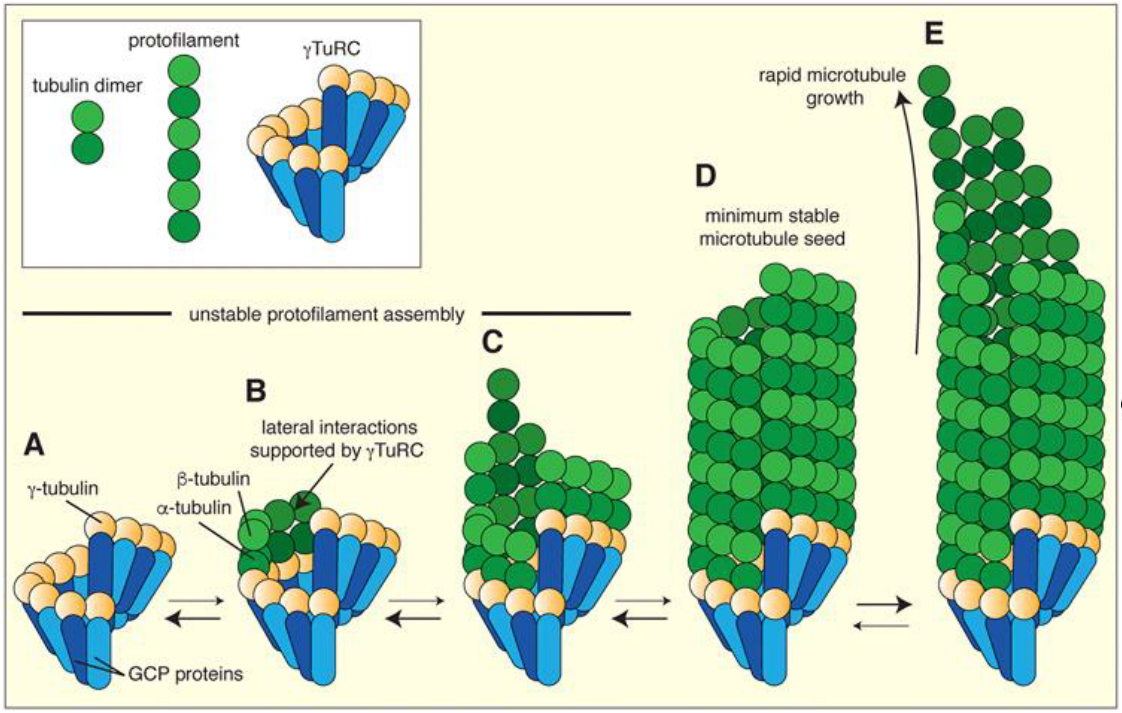




Carsten Janke and Maria M. Magiera (2020)

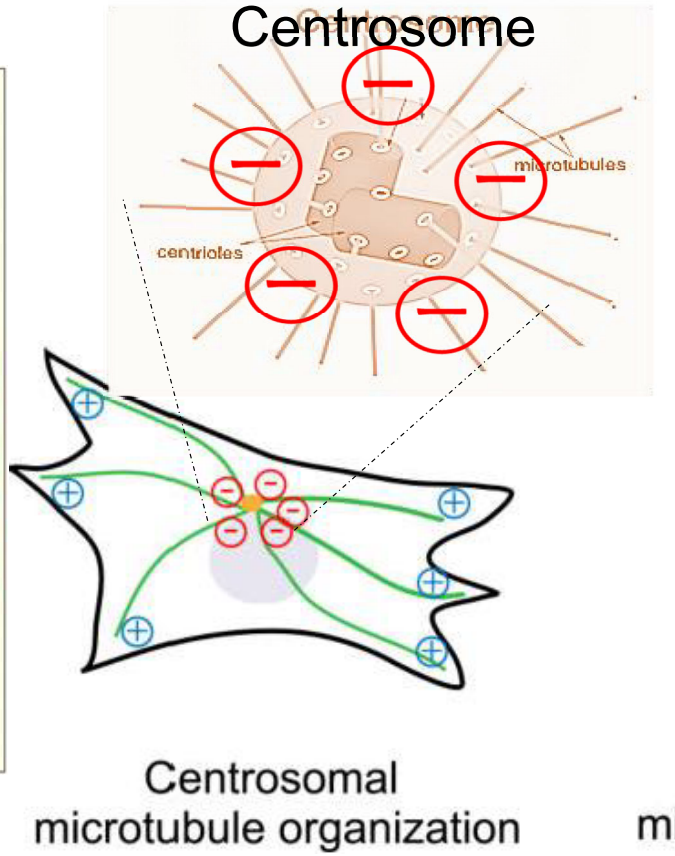
- Microtubules are polarized polymers composed of α- and β-tubulin dimers.
- Plus end : fast-growing Minus end : slow-growing
- α- and β-tubulins receive post-translational modifications which controls microtubule dynamics and organelle transport

Centrosomal and Acentrosomal MTOC

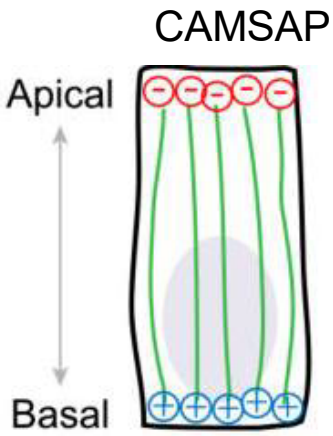


Microtubule Nucleation

Corinne A. Tovey and Paul T. Conduit (2018)



Centrosomal microtubule organization



Non-centrosomal microtubule organization

M. Toya, M. Takeichi (2016)

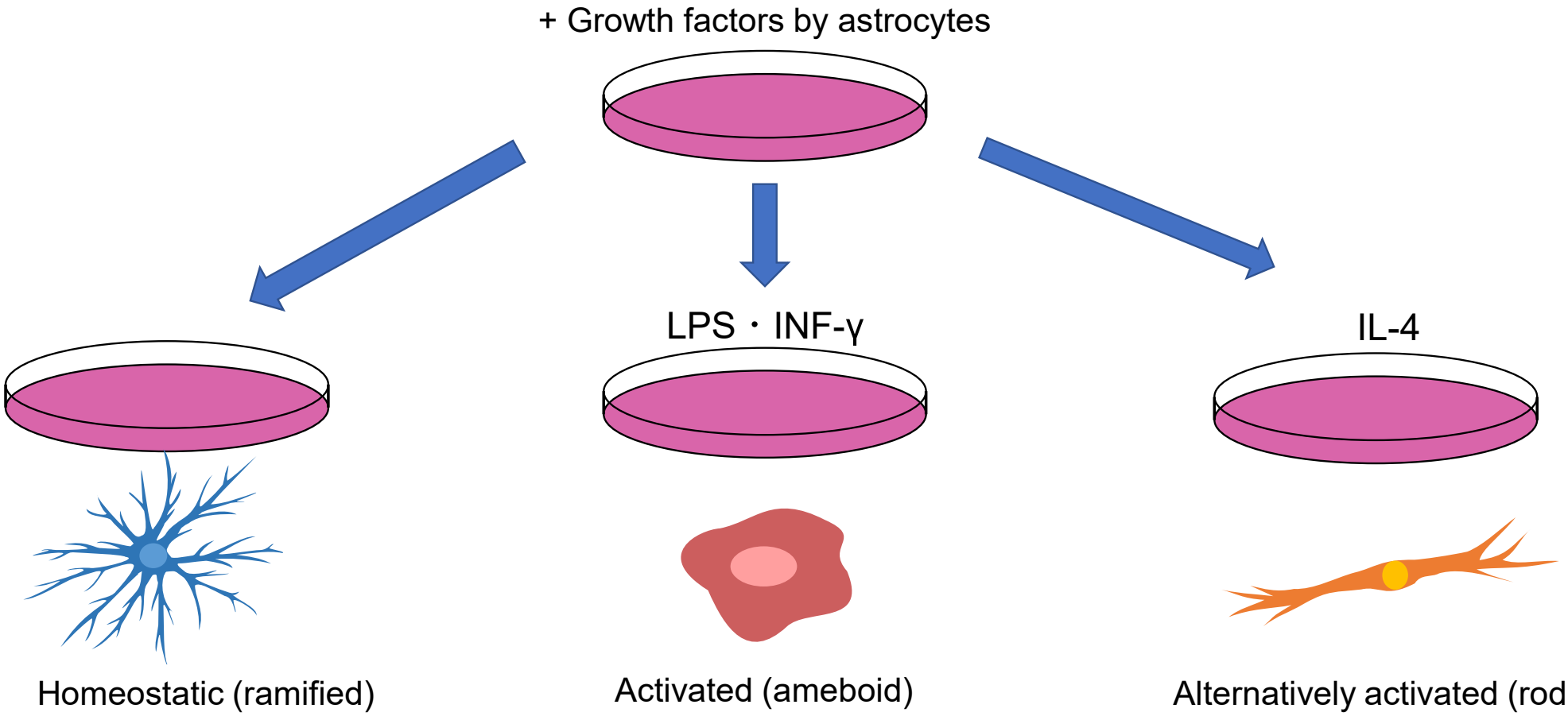
① Homeostatic, activated, and alternatively activated primary microglia differ in MT distribution, stability, and dynamic behavior

② Homeostatic, activated, and alternatively activated microglia differ in MT orientation

③ Homeostatic microglia nucleate non-centrosomal MTs from Golgi outposts

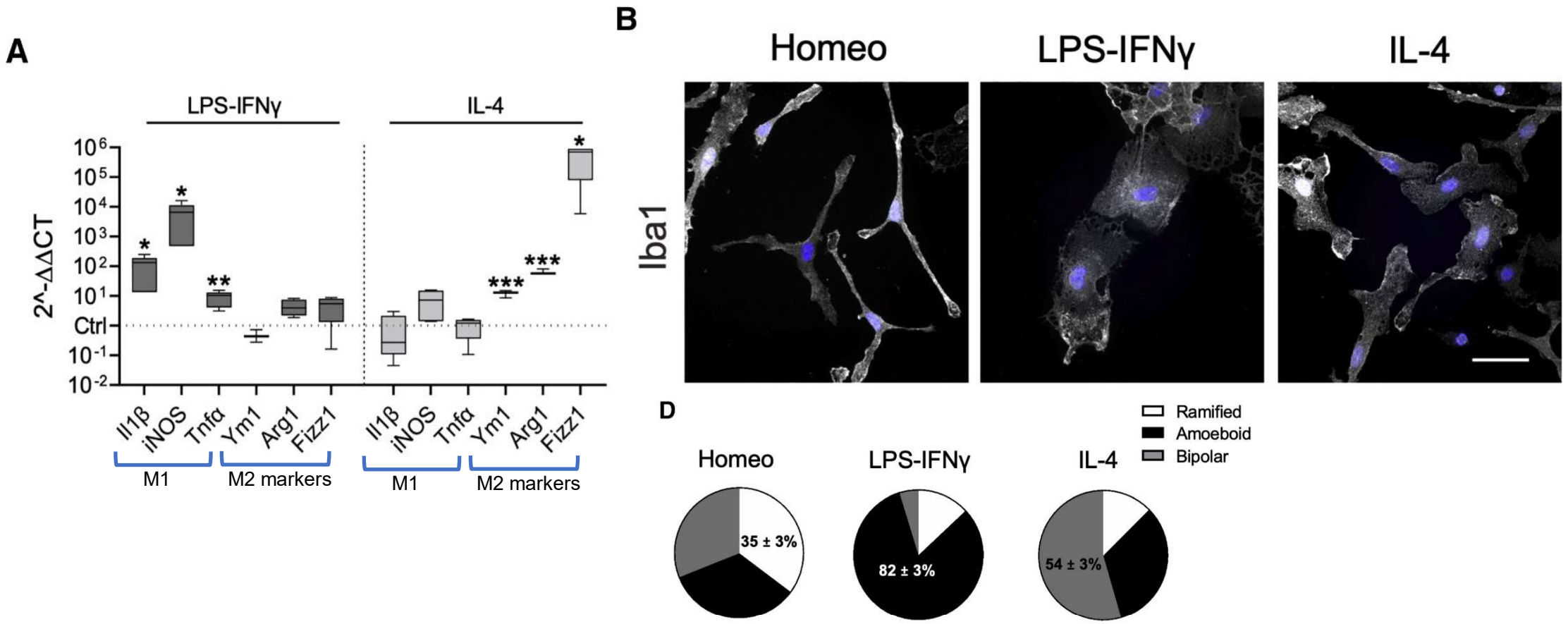
④ Pericentrosomal redistribution of microtubule-nucleating material is a hallmark of activated microglia

① Homeostatic, activated, and alternatively activated primary microglia differ in MT distribution, stability, and dynamic behavior



① Figure 1

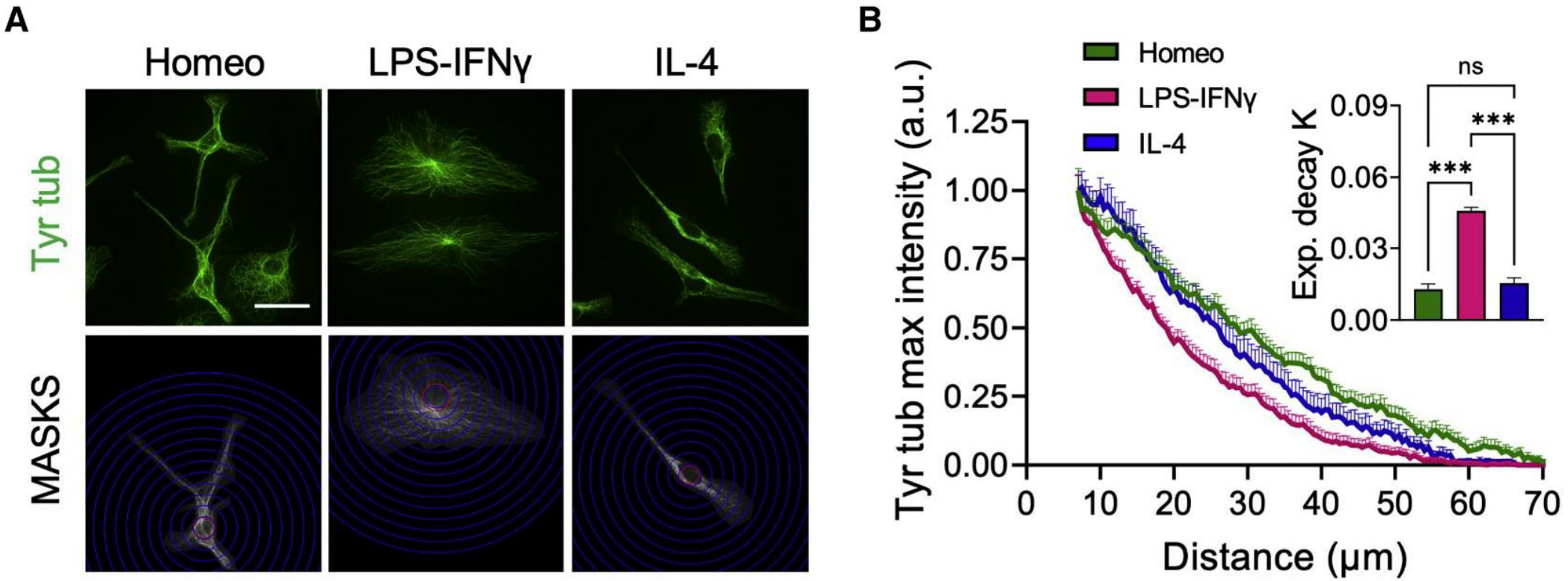
Q: What are characteristics of each microglia they cultured?



Homeostatic, activated and alternatively activated microglia exhibited each characteristic as previously reported.

① Figure 2

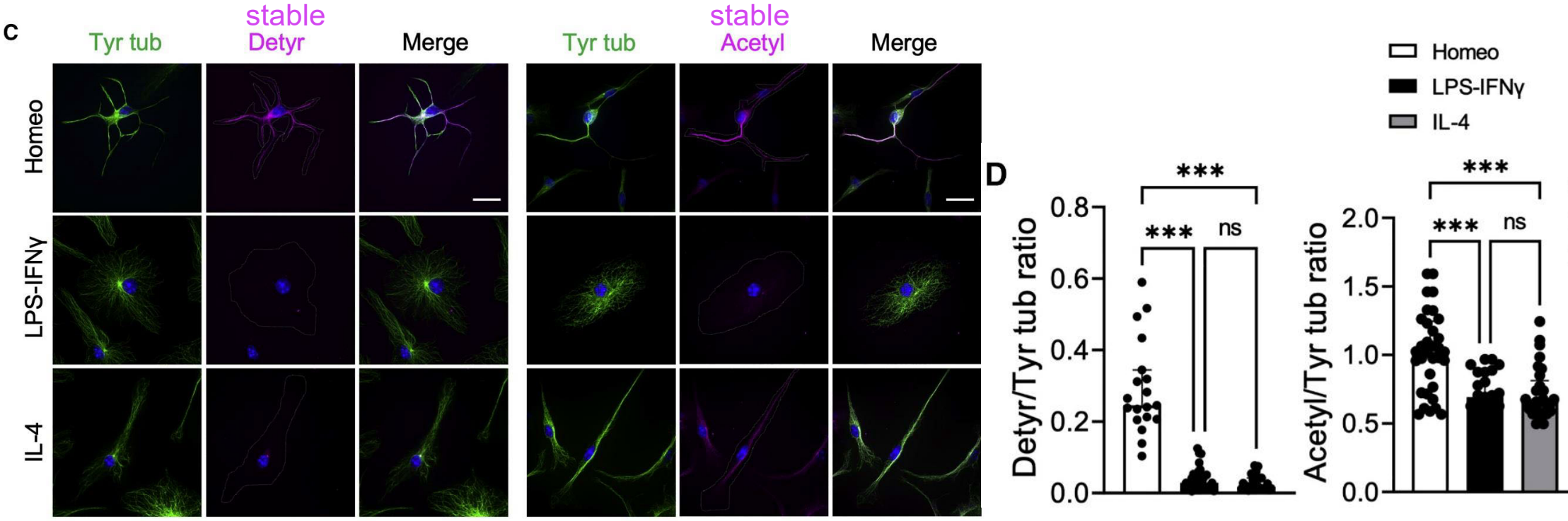
Q: What do the MT arrays of each cell look like?



Activated microglia exhibit faster decay pf Tyr tub signal.

① Figure 2

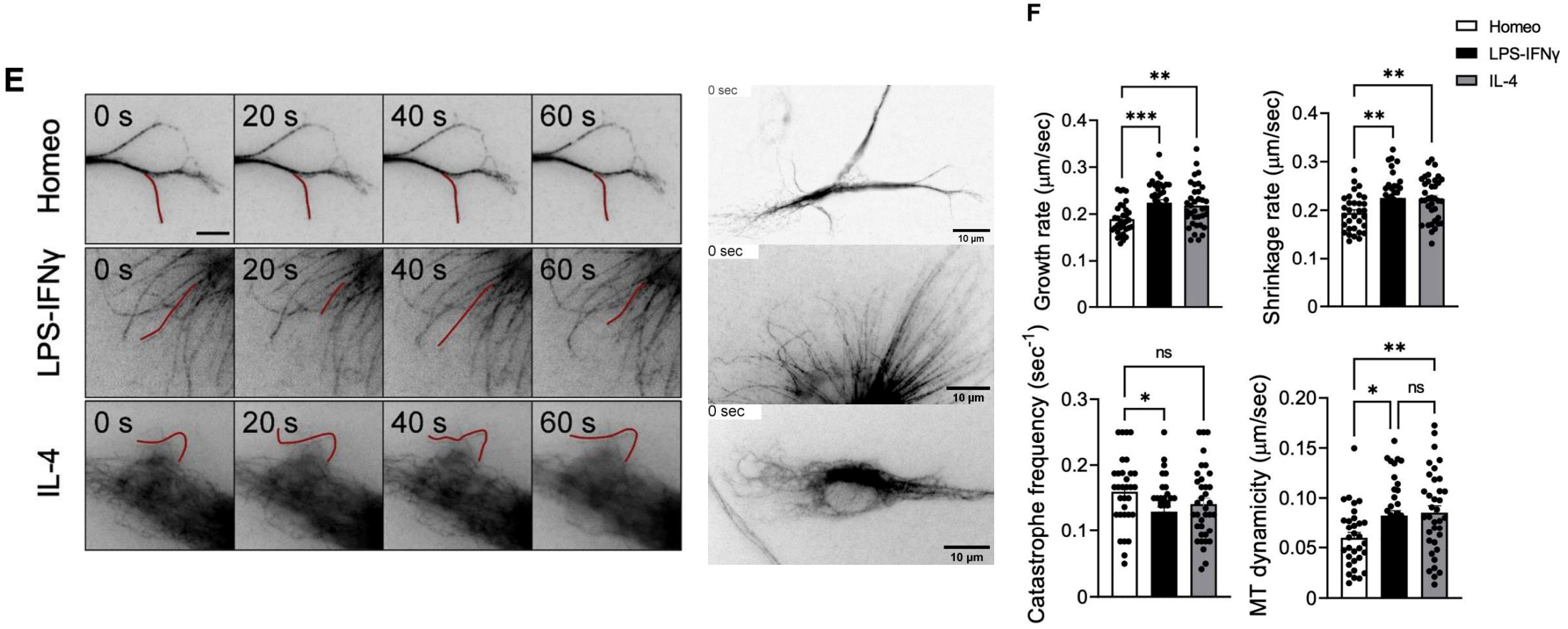
Q: What are the difference in the MT stability in each cell?



Homeostatic microglia display more stable MTs.

① Figure 2

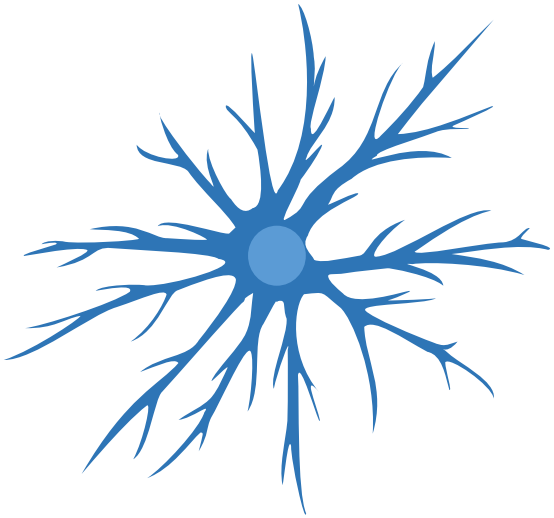
Q: What are the difference in the MT dynamics in each cell?



Activated and alternatively activated microglia have more MT dynamicity compared with homeostatic microglia.

Short Summary ①

Homeostatic, activated, and alternatively activated primary microglia differ in MT distribution, stability, and dynamic behavior



Homeostatic (ramified)

MT stability

>

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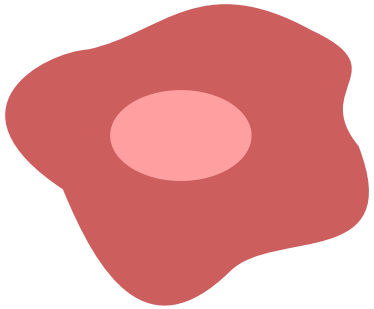
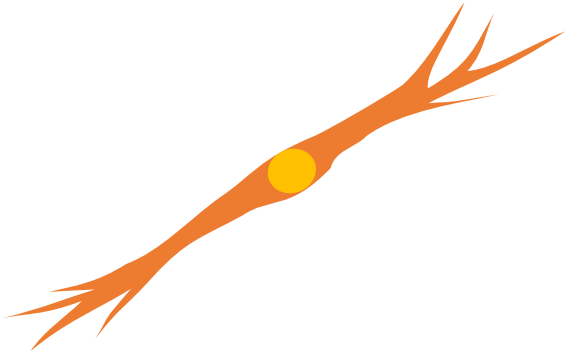
MT dynamics

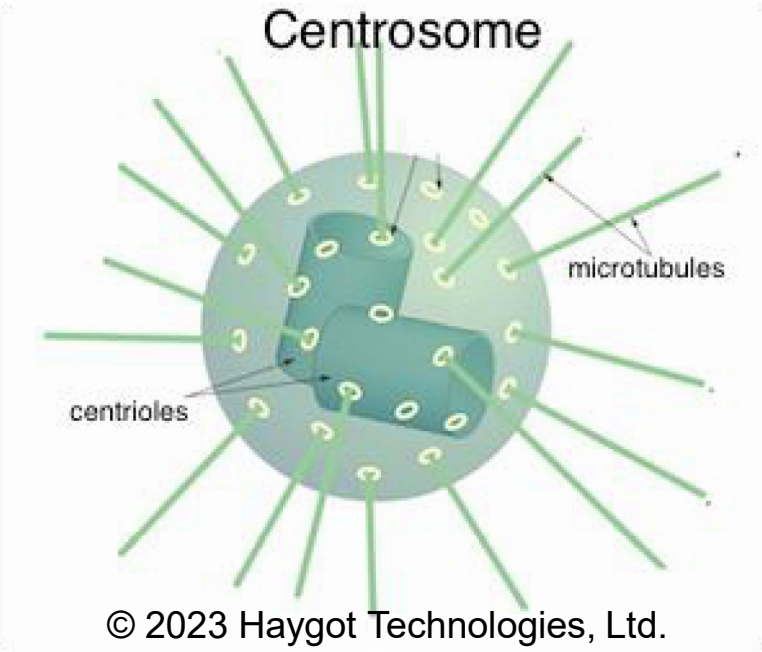


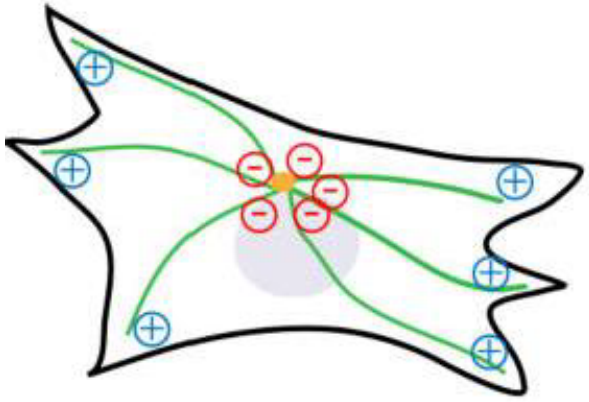
Activated (ameboid)



Alternatively activated (rod)

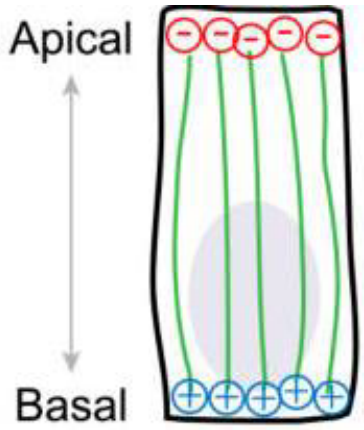
② Homeostatic, activated, and alternatively activated microglia differ in MT orientation





Centrosomal microtubule organization

CAMSAP



Non-centrosomal microtubule organization

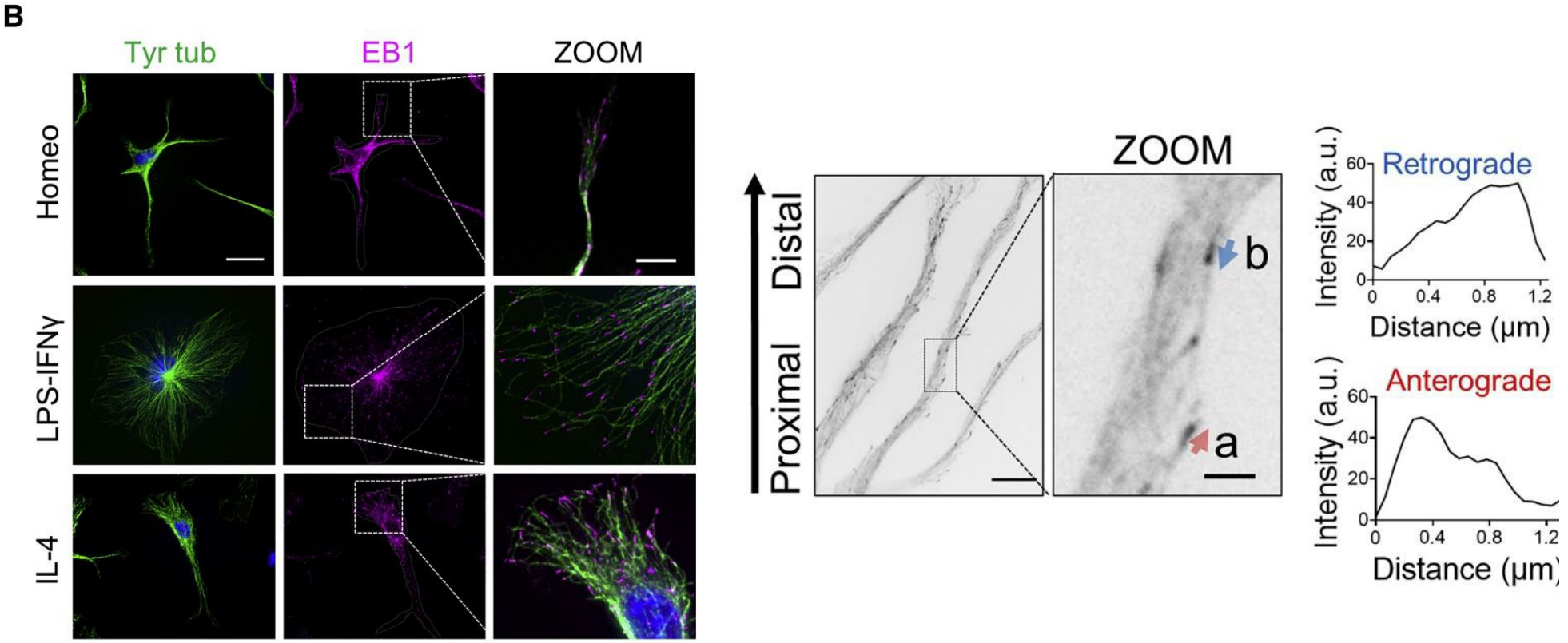
M. Toya, M. Takeichi (2016)

EB : microtubule plus-end end binding proteins

CAMSAP : regulate the stability and formation of non-centrosomal MT minus-end

② Figure 3

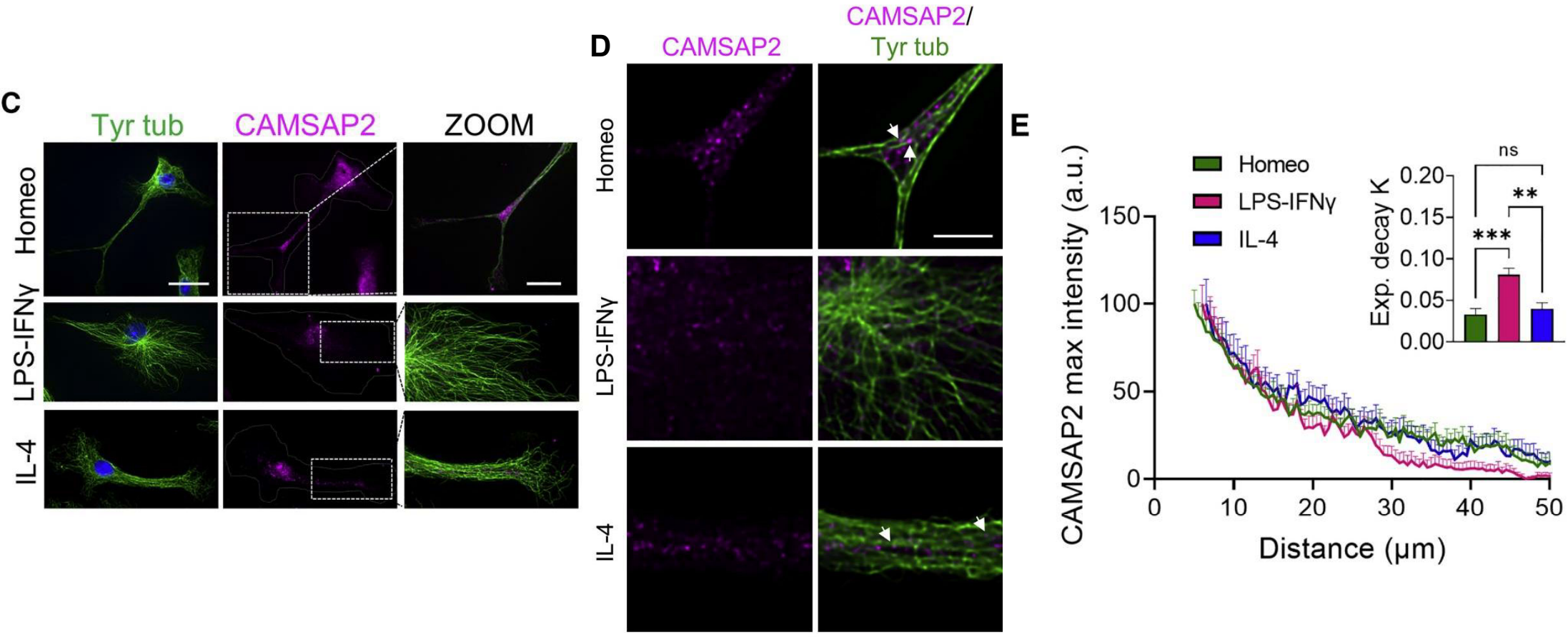
Q: What are the difference in the distribution of actively growing MT plus-end in each cell?



EB1 decorated MT ends to a lesser extent in homeostatic microglia.
Homeostatic and alternatively activated microglia had some retrograde comets while activated cells do not

② Figure 3

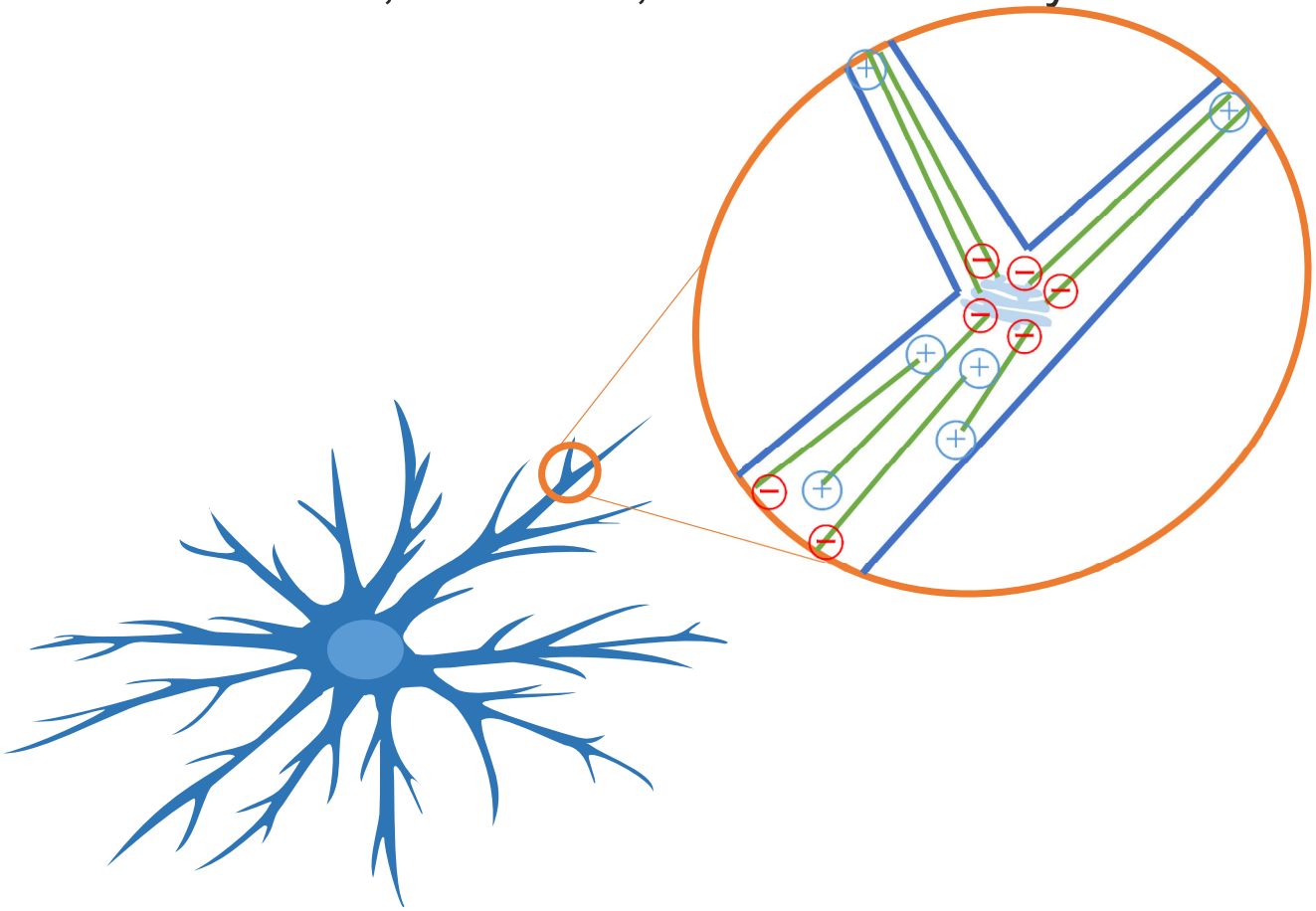
Q:What are the distribution of MT minus-end like?

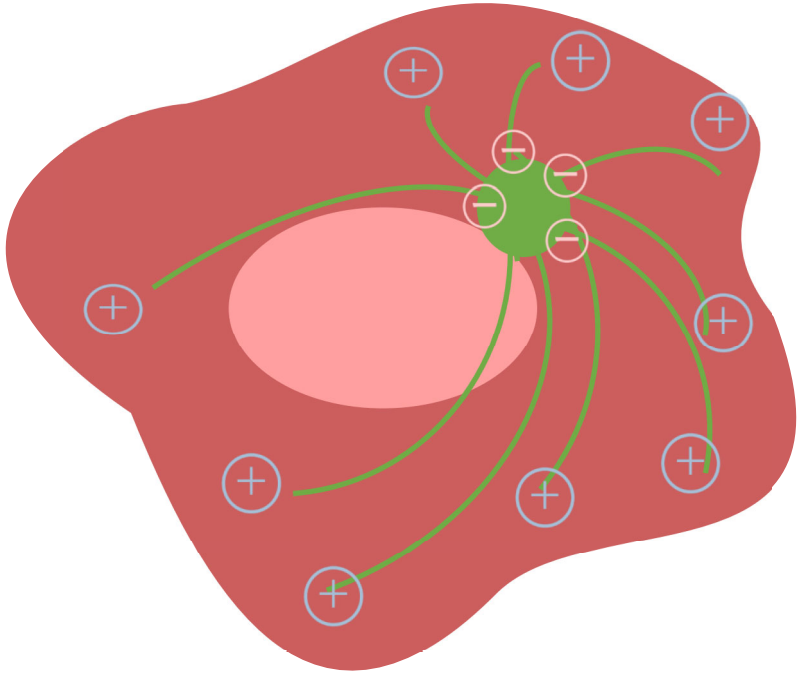


While CAMSAP2 in homeostatic and alternatively activated microglia distributed to isolated clustered puncta along ramifications, CAMSAP2 in activated microglia are in perinuclear region

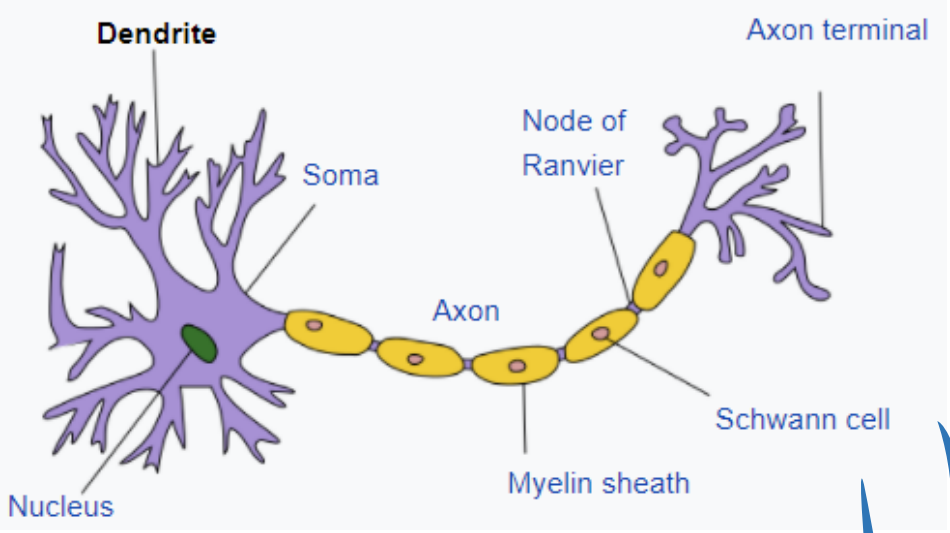
Short Summary ②

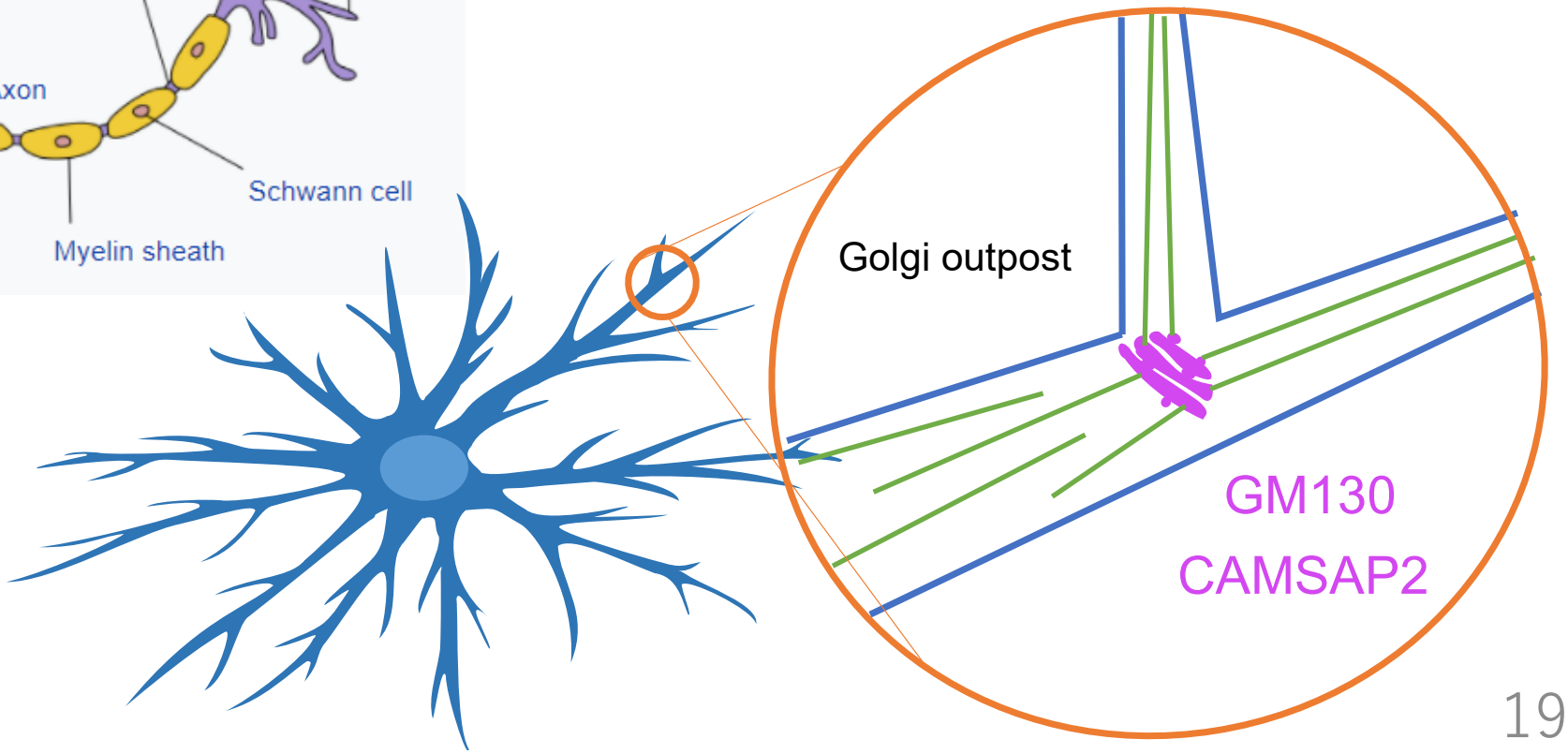
Homeostatic, activated, and alternatively activated microglia differ in MT orientation





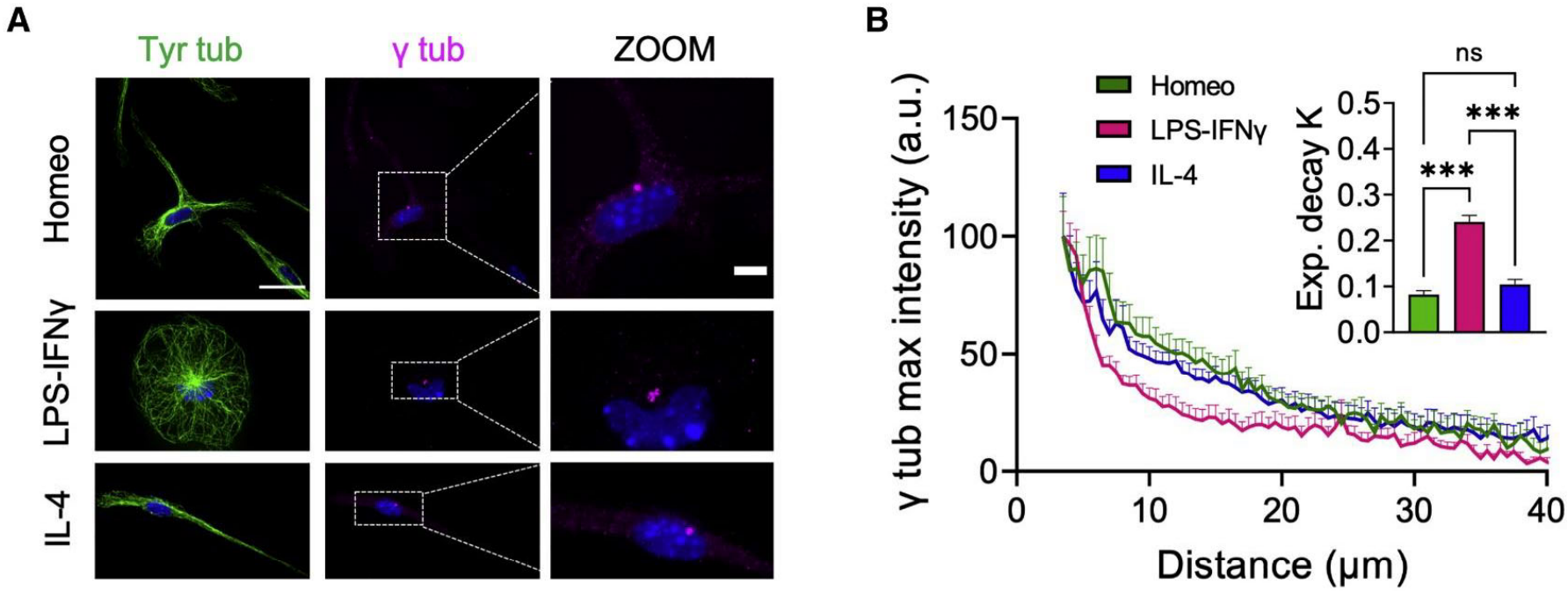
③ Homeostatic microglia nucleate non-centrosomal MTs from Golgi outposts





③ Figure 4

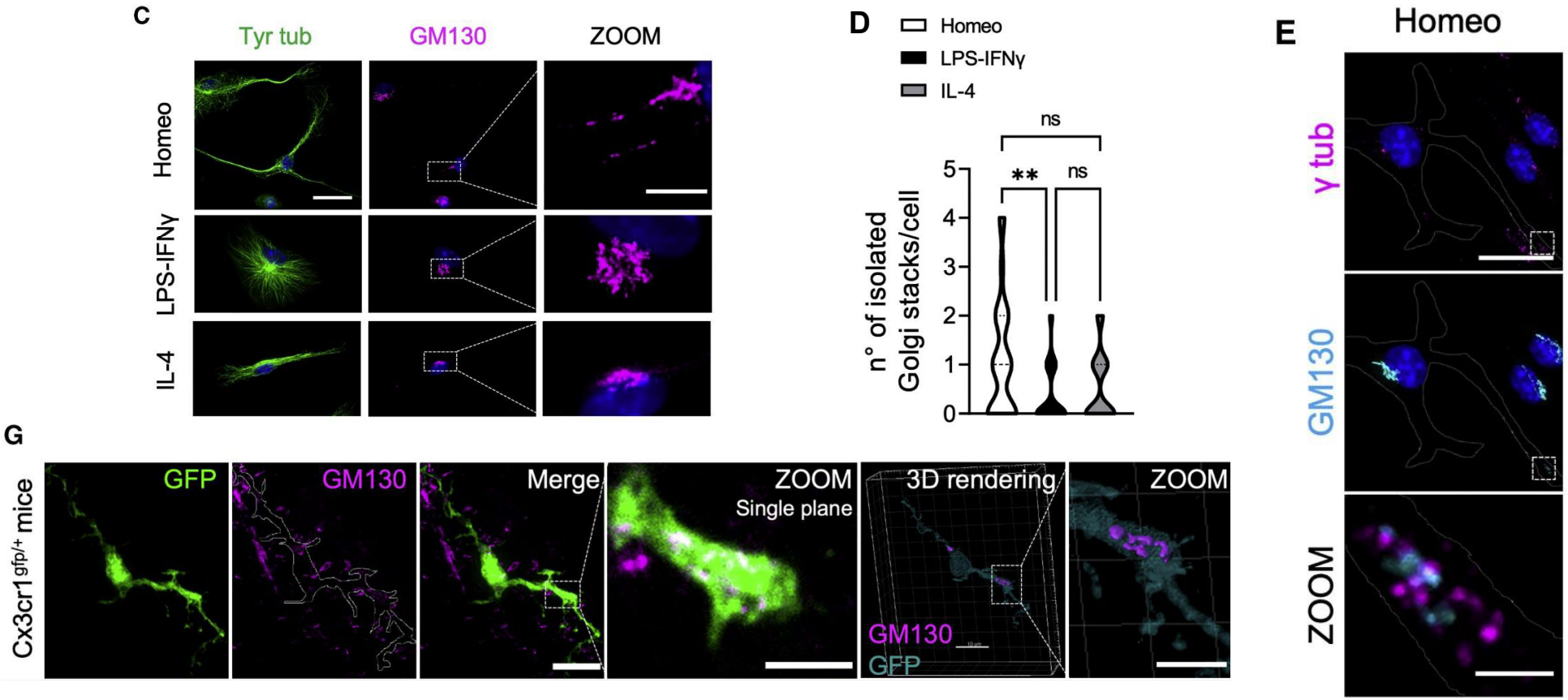
Q: Where do the MT nucleating sites distribute in each cell?



Compared with homeostatic and alternatively activated microglia, γ-tubulin accumulated in perinuclear region in activated cells.

③ Figure 4

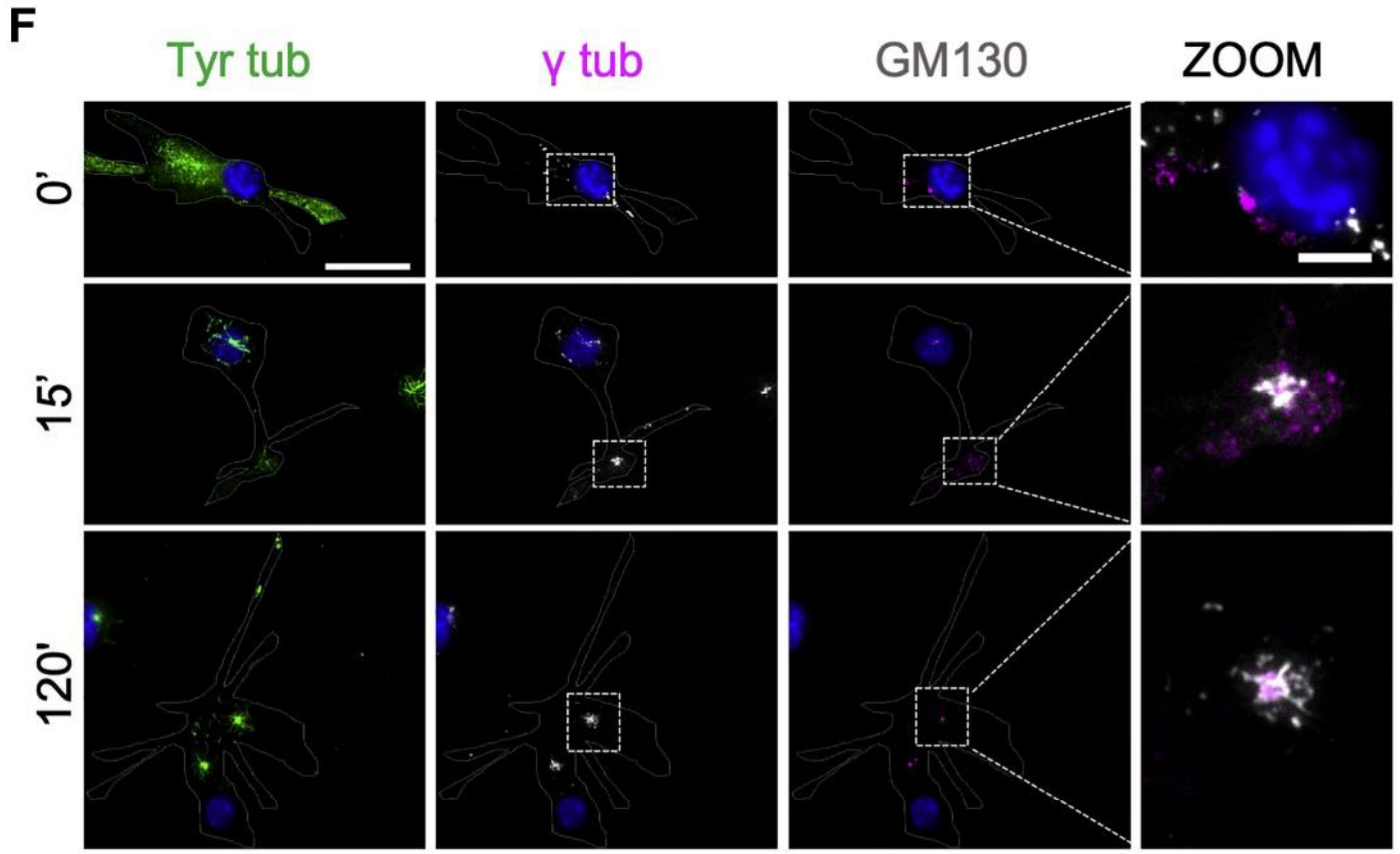
Q: What are the difference in distributions of Golgi outposts in each cell?



Homeostatic microglia had increased signal of Golgi outpost for MT nucleation.

③ Figure 4

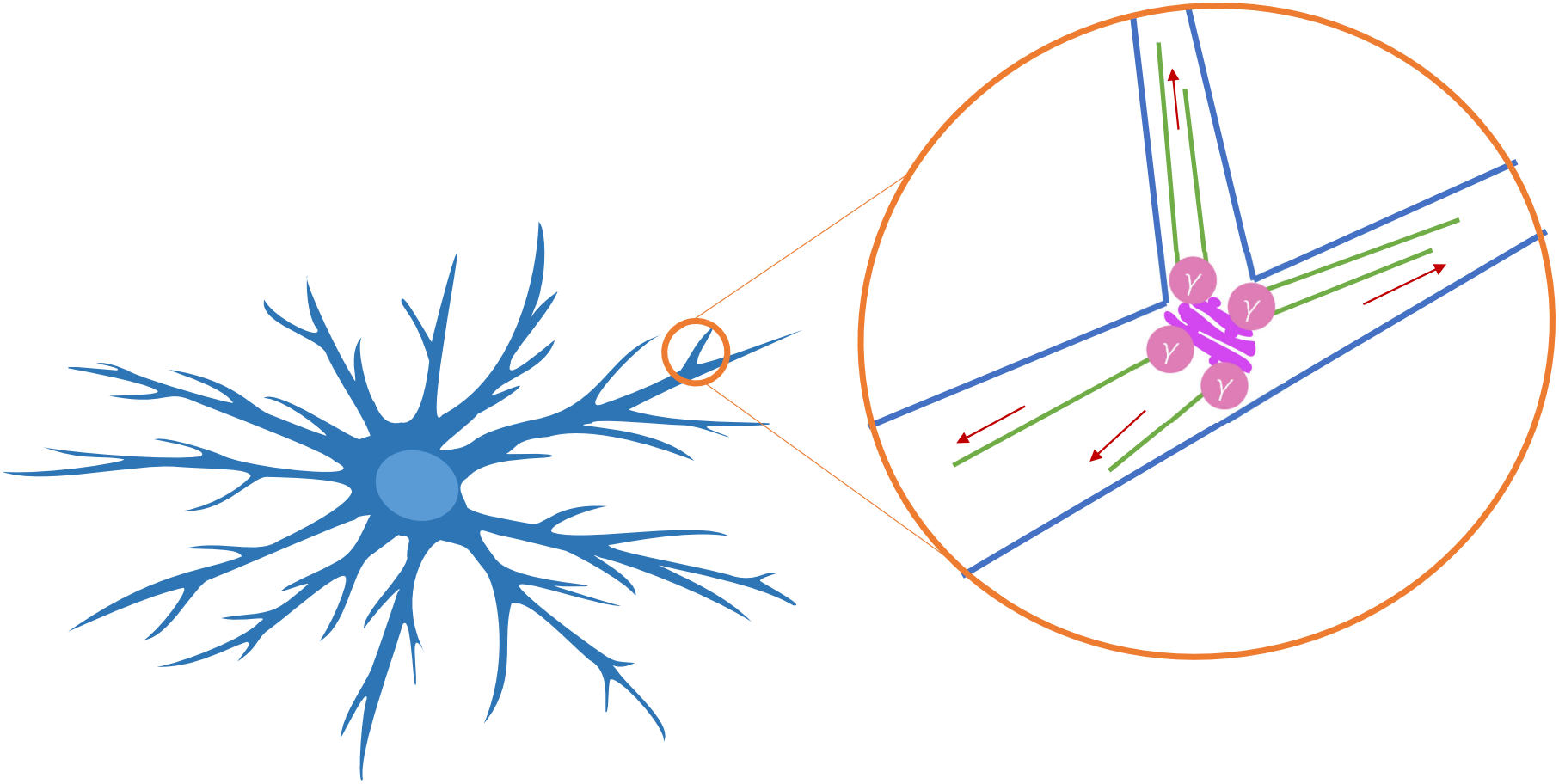
Q: Can Golgi outposts function as MTOCs in homeostatic microglia?



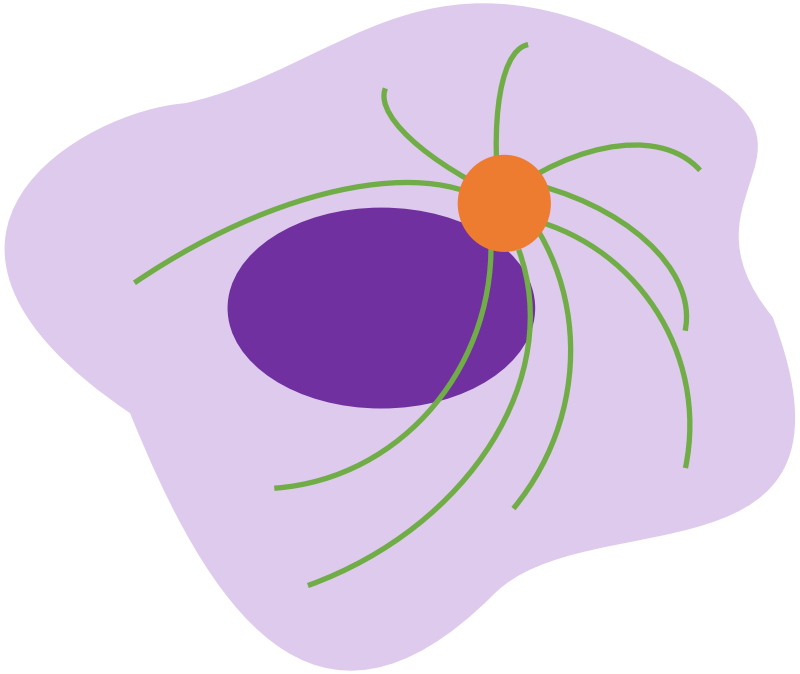
Golgi outposts in homeostatic microglia can act as sites for acentrosomal MT nucleation.
γ-tubulin-mediated non-centrosomal nucleation is essential for establishing an asymmetric MT array.

Short Summary ③

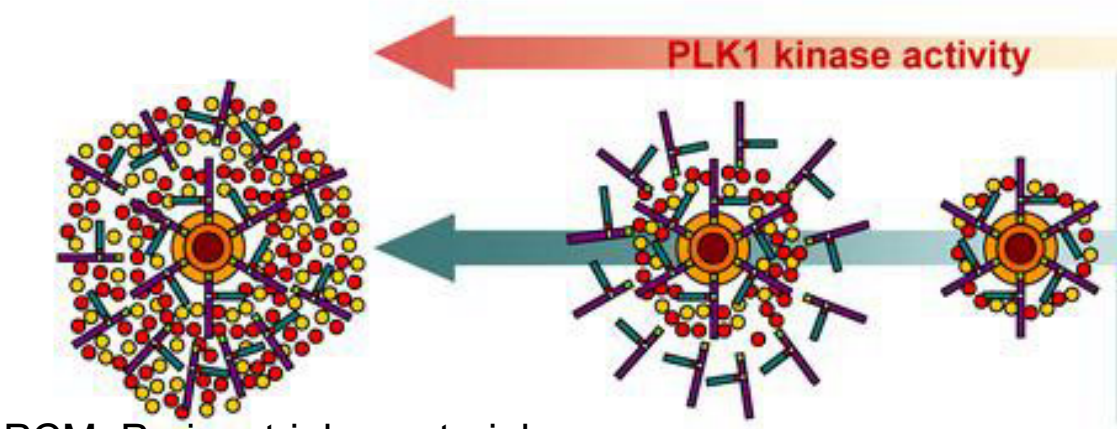
Homeostatic microglia nucleate non-centrosomal MTs from Golgi outposts



④ Pericentrosomal redistribution of microtubule-nucleating material is a hallmark of activated microglia



Activated Macrophage



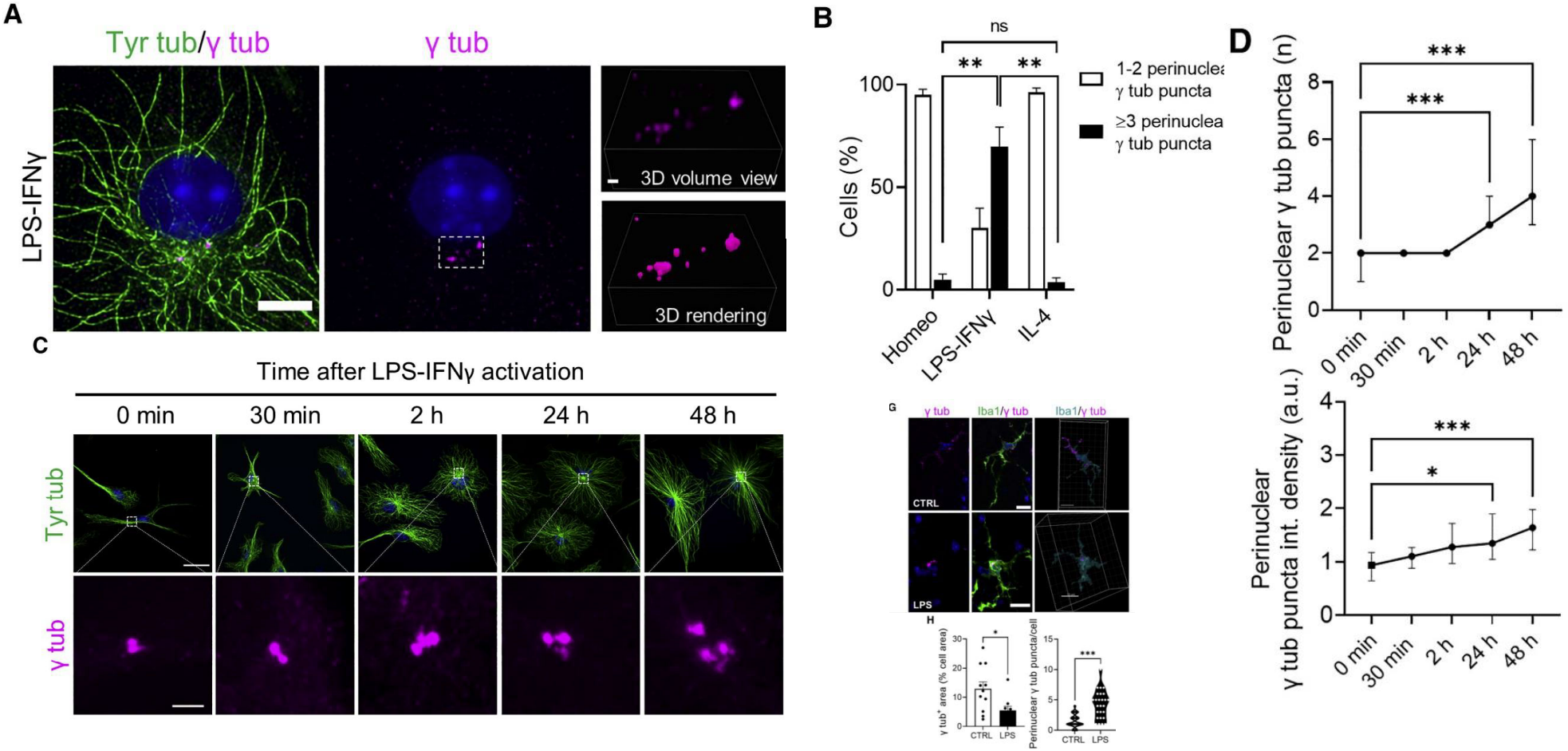
PCM: Pericentriolar materials

Centrosome Maturation

Seongjae Kim & Kunsoo Rhee (2014)

④ Figure 5

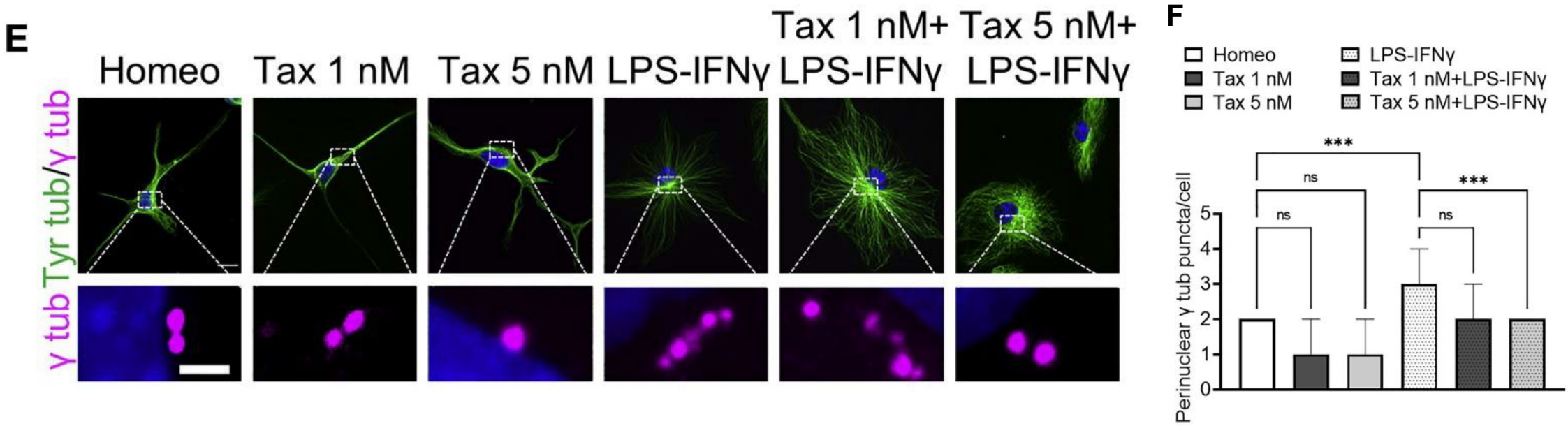
Q: Was the recruitment of γ-tubulin to a region near the centrosome also a characteristic of activated microglia?



The clustering of γ-tubulin to peri-centrosomal region seems a characteristic of activated cells.

④ Figure 5

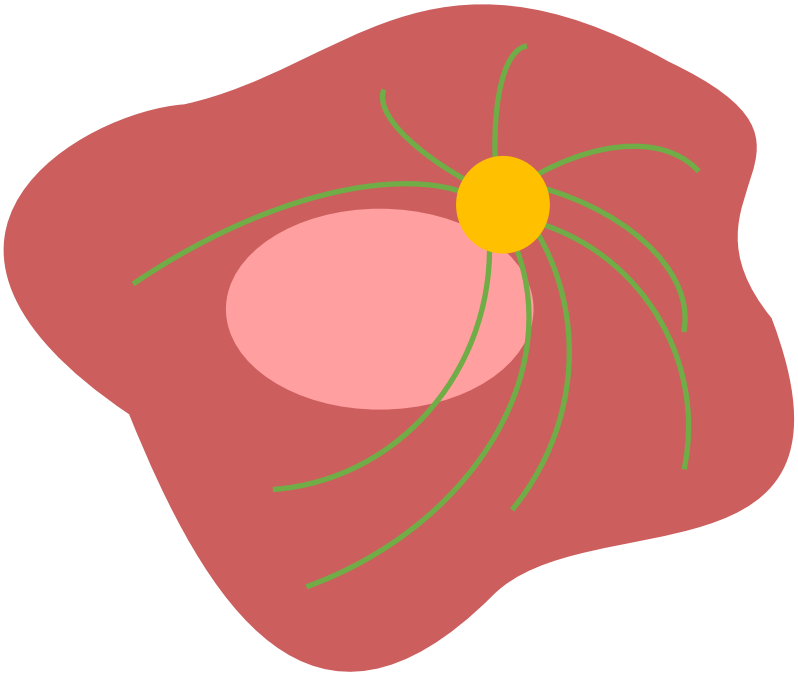
Q: How does a microtubule stabilizer affect the reactivity of microglia?



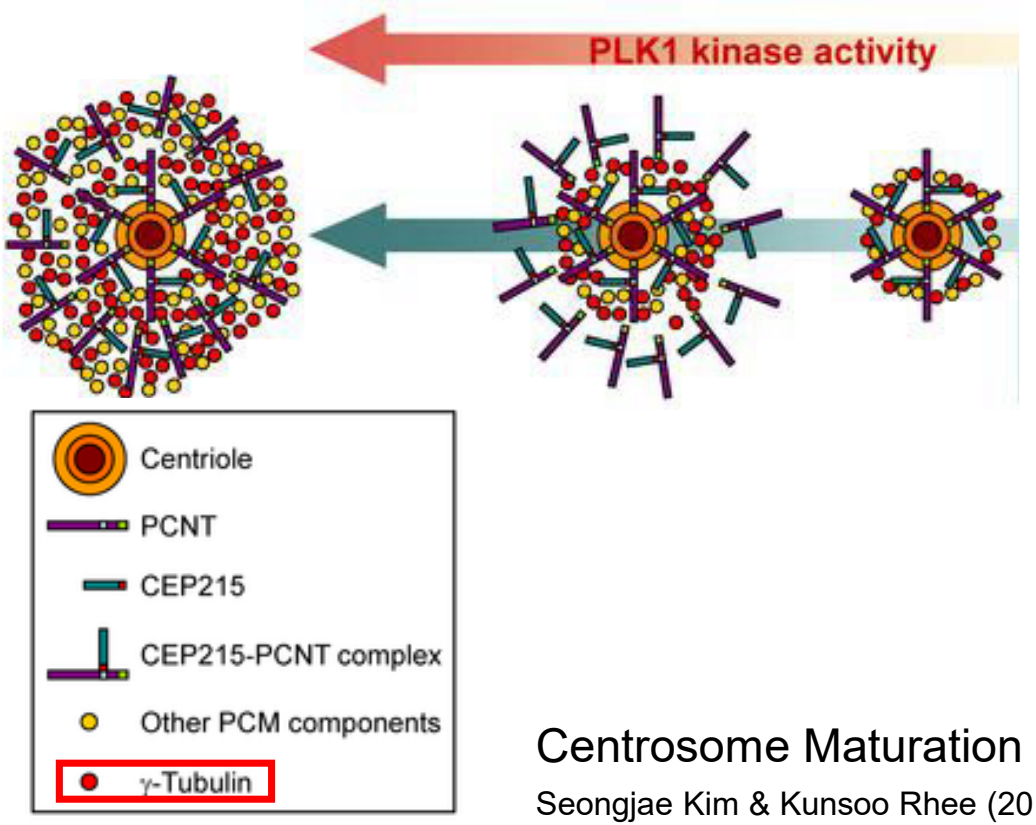
Taxol treatment seems reduce the reactivity of microglia.

Short Summary ④

Pericentrosomal redistribution of microtubule-nucleating material is a hallmark of activated microglia



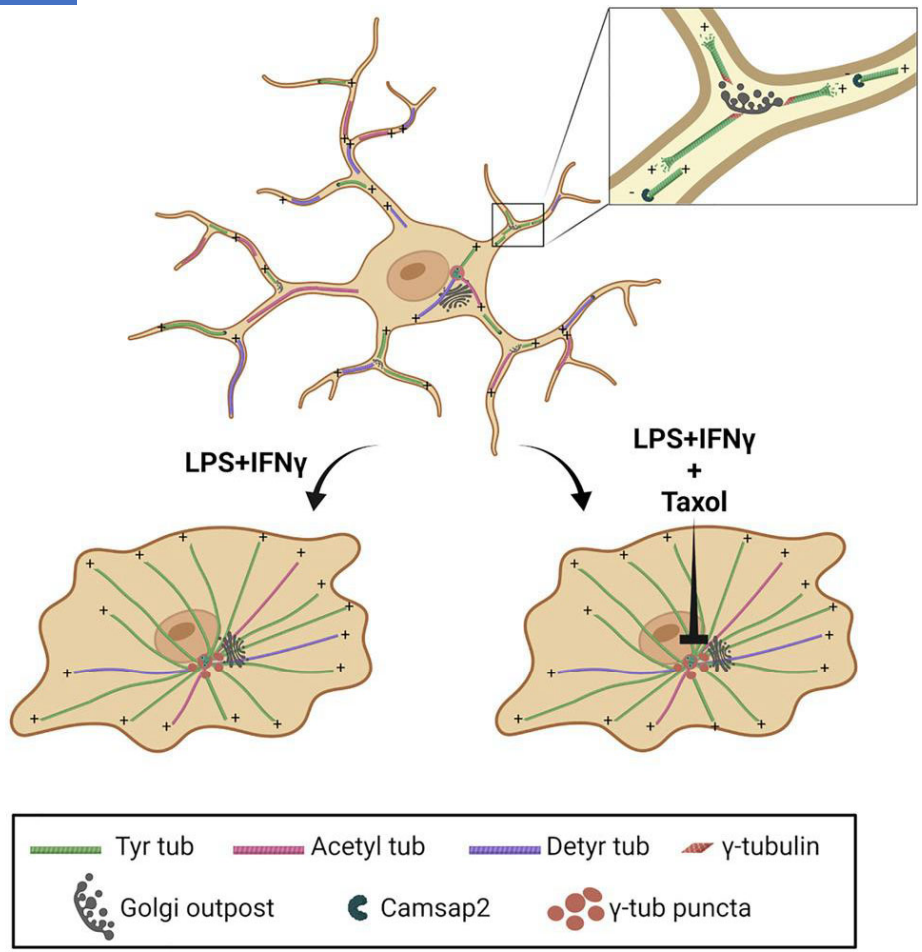
Activated Microglia



Centrosome Maturation

Seongjae Kim & Kunsoo Rhee (2014)

Summary



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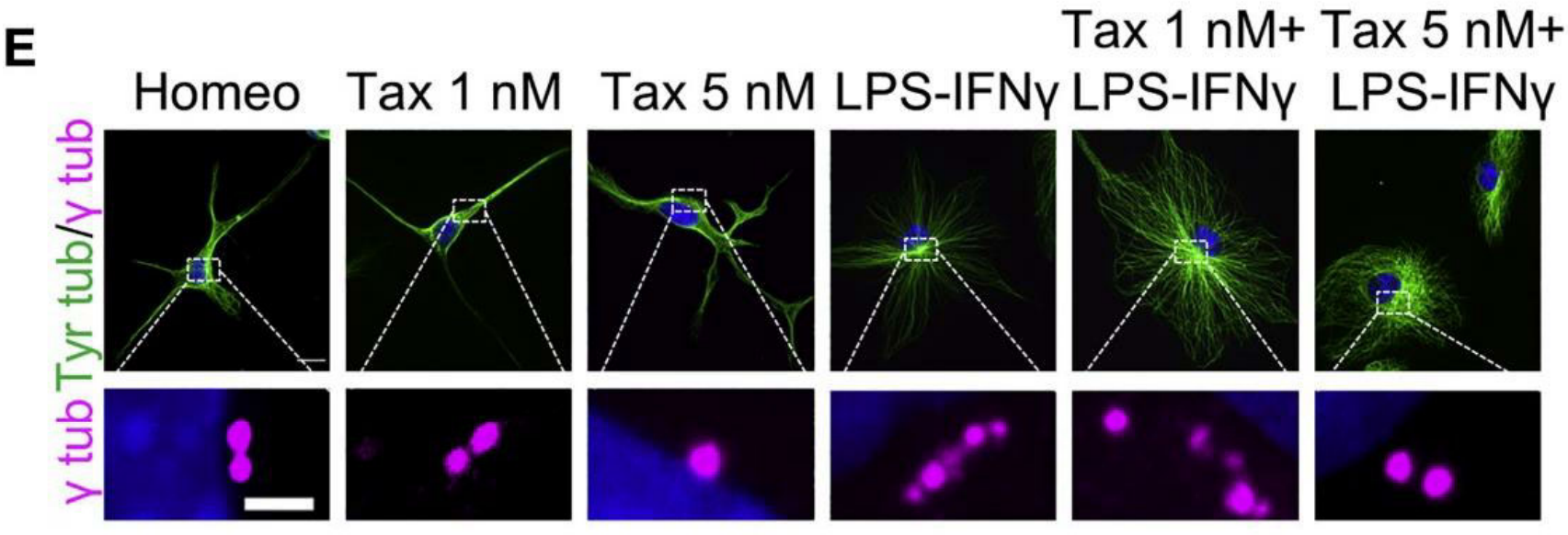
④ Pericentrosomal redistribution of microtubule-nucleating material is a hallmark of activated microglia

- Unique Microtubule Reorganization: Transition from Non-Centrosomal to Radial Array in Microglia
- Role of Golgi Outpost MT Nucleation in Supporting Microglia Patrolling Behavior
- Tubulin Remodeling: Enabling Microglia Reactivity in vitro and in vivo
- Targeting Pericentrosomal Matrix Maturation to Limit Tissue Damage in Neurodegenerative Diseases
- Investigating the Role of Spinal Microglial MT Dysfunction in Peripheral Neuropathy

They did not prove the anti-inflammatory effect of taxol treatment



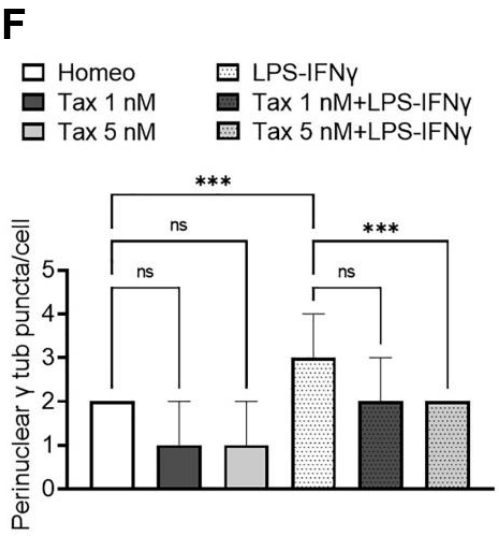


Figure 5

Taxol inhibits PCM maturation

They did not prove the anti-inflammatory effect of taxol treatment

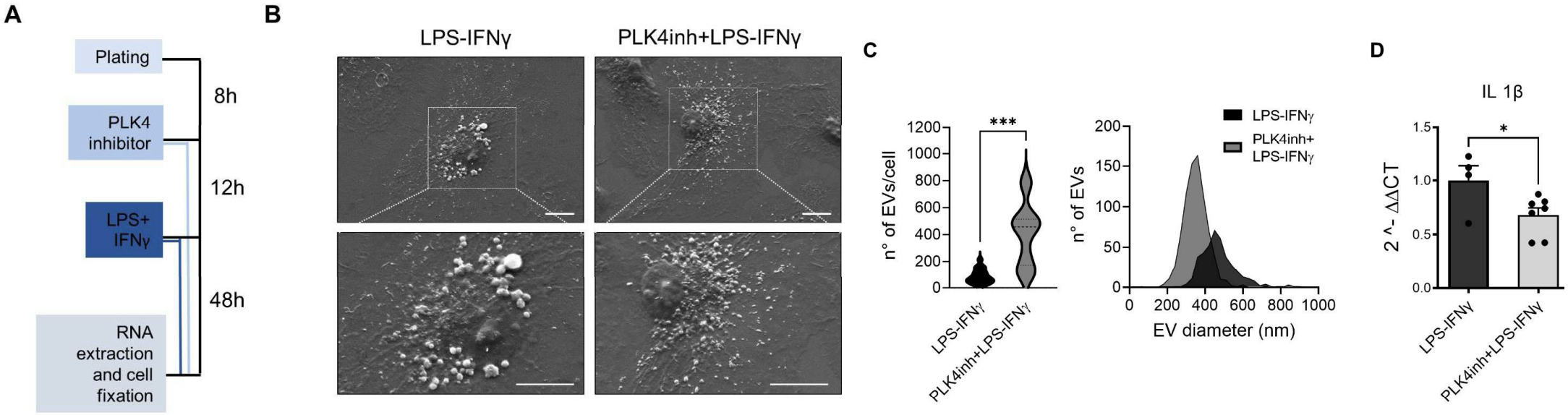


Figure S4

PLK4: inhibits centrosome maturation

PLK4 treatment reduces the number of EV and IL-1β expression levels.

They did not prove the anti-inflammatory effect of taxol treatment

Fig. 3　タンパクの量を測る

- ameboid型とrod型で極端に発現が違うキネシン、変化ないキネシンでwestern blotting
（mRNA量とタンパク量がだいたい比例するっていうことを示しておく）

Fig. 4 キネシンの局在、動態

- Fig.3で調べたキネシンで局在調べる。抗体染色

Fig. 5　とあるキネシンまたは他の微小管関連遺伝子がameboid⇄rodの変化に重要

- 形態変化に関わってそうな遺伝子を選んでiRNAでノックダウン、もしくは過剰発現したときにameboid⇄rodの変化に違いが見られるか調べる。もしアメボイド型への変化が阻害されたら、免疫マーカー遺伝子の発現量を調べてみる

It may be necessary to investigate expression levels of immuno-marker genes in microglia upon the treatment, to make the thesis more convincing