Phenotype-based screening rediscovered benzopyran-embedded microtubule inhibitors as anti-neuroinflammatory agents by modulating the tubulin-p65 interaction

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Neuroinflammation is one of the critical processes implicated in central nervous system (CNS) diseases. Therefore, alleviating neuroinflammation has been highlighted as a therapeutic strategy for treating CNS disorders.

However, the complexity of neuroinflammatory processes and poor drug transport to the brain are considerable hurdles to the efficient control of neuroinflammation using small-molecule therapeutics. Thus, there is a significant demand for new chemical entities (NCEs) targeting neuroinflammation.

- Herein, we rediscovered benzopyran-embedded tubulin inhibitor 1 as an anti-neuroinflammatory agent via phenotype-based screening.
- A competitive photoaffinity labeling study revealed that compound 1 binds to tubulin at the colchicine-binding site.
- Structure–activity relationship analysis of 1's analogs identified SB26019 as a lead compound with enhanced anti-neuroinflammatory efficacy.
- Mechanistic studies revealed that upregulation of the tubulin monomer was critical for the anti-neuroinflammatory activity of SB26019.
- We serendipitously found that the tubulin monomer recruits p65, inhibiting its translocation from the cytosol to the nucleus and blocking NF-κB-mediated inflammatory pathways.
- Further in vivo validation using a neuroinflammation mouse model demonstrated that SB26019 suppressed microglial activation by downregulating Iba-1 and proinflammatory cytokines.
- Intraperitoneal administration of SB26019 showed its therapeutic potential as an NCE for successful anti-neuroinflammatory regulation.

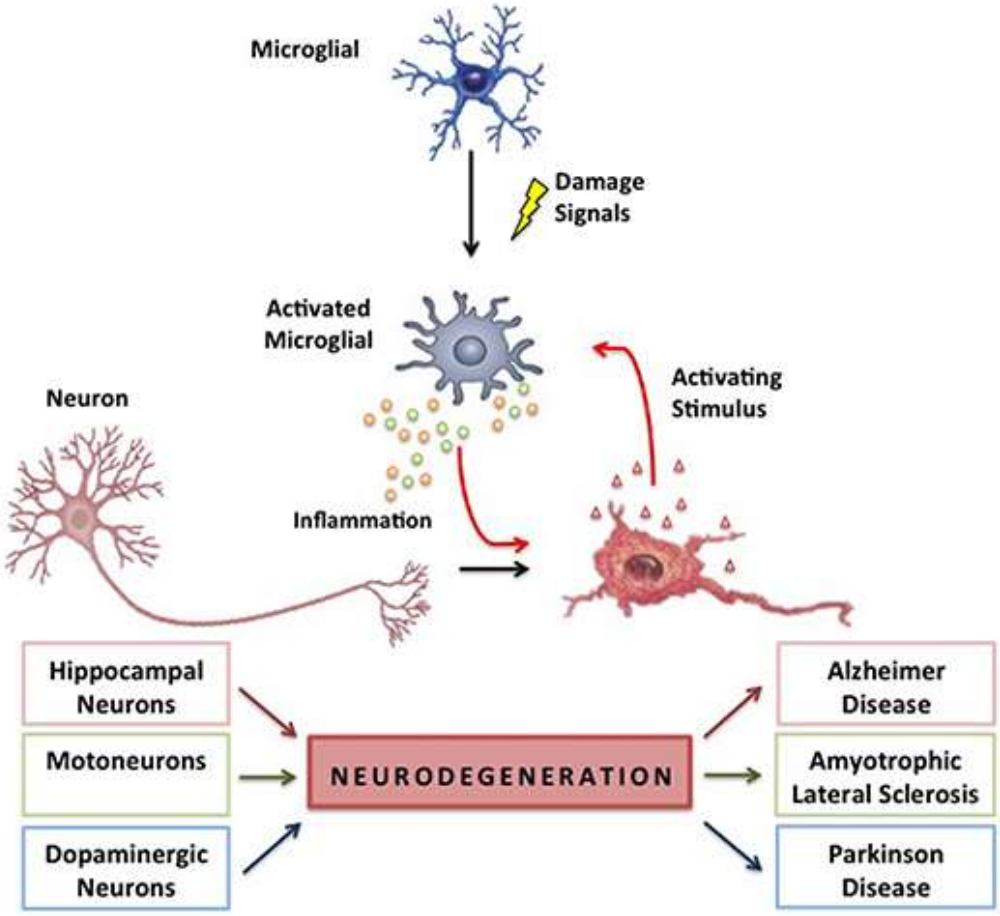
Along with the recent growing demands on tubulin modulators for treating various inflammatory diseases, our results suggest that colchicine-binding site-specific modulation of tubulins can be a potential strategy for preventing neuroinflammation and treating CNS diseases.

神経炎症は、中枢神経系疾患に関与する重要なプロセスの一つであり、その緩和は中枢神経系疾患の治療戦略として注目されている。しかし、神経炎症過程の複雑さや脳への薬物輸送の難しさが、低分子治療薬を用いて神経炎症を効率的に制御する上での大きな障害となっている。このため、神経炎症を標的とした新規化学物質（NCEs）の開発が求められている。

- ここでは、表現型に基づくスクリーニングにより、ベンゾピラン包接型チューブリン阻害剤1を抗神経炎症剤として再発見した。
- 競合的光親和性標識法により、化合物1がコルヒチン結合部位でチューブリンに結合することを明らかにした。
- 1のアナログの構造活性相関解析により、SB26019が抗神経炎効果を増強するリード化合物であることが判明した。
- そして、SB26019の抗神経炎作用には、チューブリン単量体のアップレギュレーションが重要であることが明らかになった。
- また、偶然にも、チューブリン単量体がp65をリクルートし、細胞質から核への移行を阻害し、NF-κBを介した炎症経路をブロックすることを発見した。
- さらに、神経炎症マウスモデルを用いたin vivo検証では、SB26019がIba-1と炎症性サイトカインをダウンレギュレートすることにより、ミクログリアの活性化を抑制することが示された。
- SB26019の腹腔内投与は、抗神経炎症制御を成功させるためのNCEとしての治療の可能性を示した。

近年、様々な炎症性疾患の治療においてチューブリン調節物質に対する需要が高まっており、今回の結果は、コルヒチン結合部位特異的なチューブリン調節が神経炎症の予防および中枢神経系疾患の治療のための戦略として可能であることを示唆している。

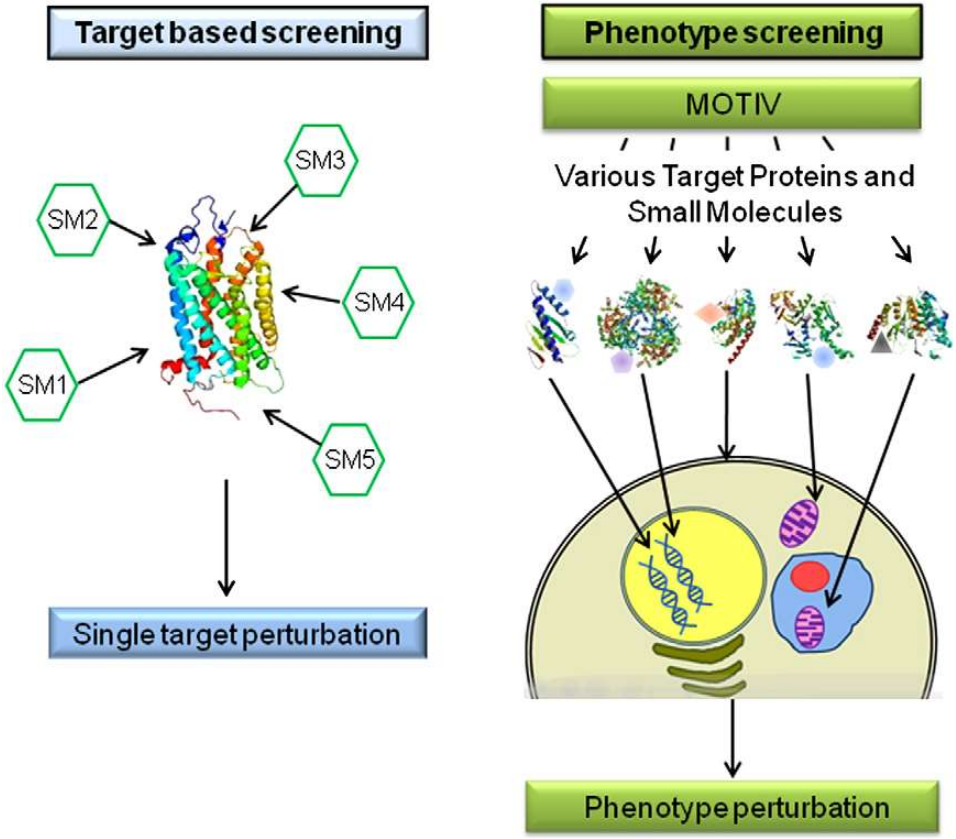
Neuroinflammation and neurodegenerative disorders



Inelia Morales, Gonzalo A. Farías, Nicole Cortes and Ricardo B. Maccioni (2016)

- Activation of microglia induces the secretion of neurotoxic cytokines and chemokines resulting in neuronal dysfunction
- Many drugs regulating inflammation failed to treat neuroinflammation due to the complexity of neuroinflammatory processes and the lack of available knowledge

Phenotype-based drug screening



- Target-based drug discovery has been leading approach for the past few decades
- Pharmaceutical industry has suffered from a significant decrease in new drug with new mode of action
- Phenotype-based drug screening enables to discover bioactive molecules that restore abnormal disease- relevant phenotypes without knowing their exact modes of action
- Phenotype-based screening could facilitate the unbiased discovery of effective bioactive molecules for regulating neuroinflammatory

Yoon Sun Cho, H. Kwon (2012)

① Phenotype-based screening for an anti-neuroinflammatory agent and identification of its binding site

② A structure-activity relationship study generates SB26019 as a potent anti-neuroinflammatory agent

③ SB26019 regulates NF-κB activation by inducing monomeric α-tubulin formation

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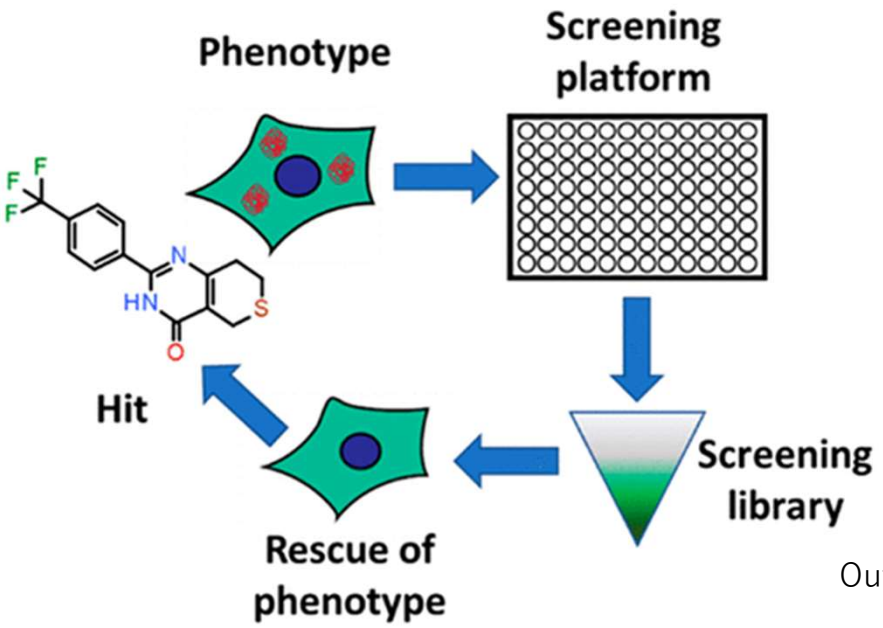
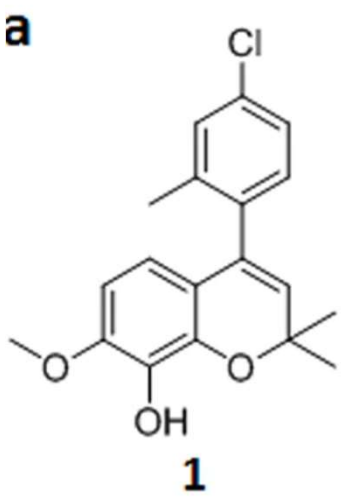
①Phenotype-based screening for an anti-neuroinflammatory agent and identification of its binding site

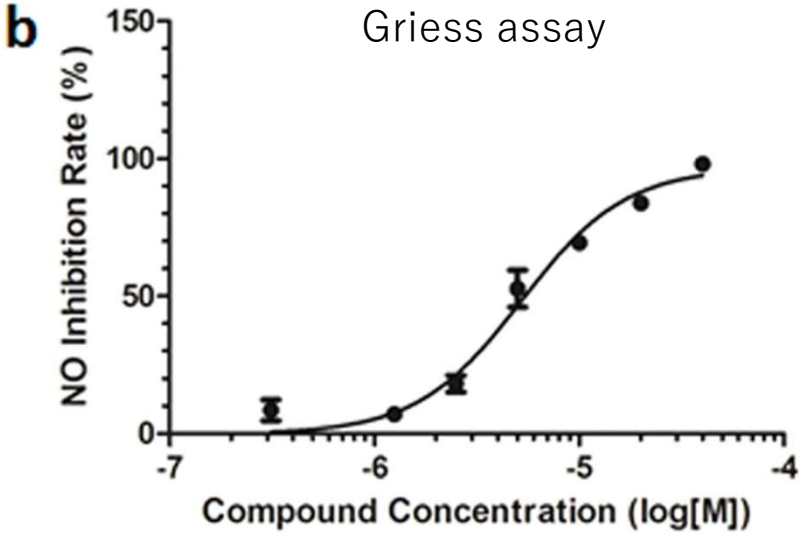


Phenotype-based screening



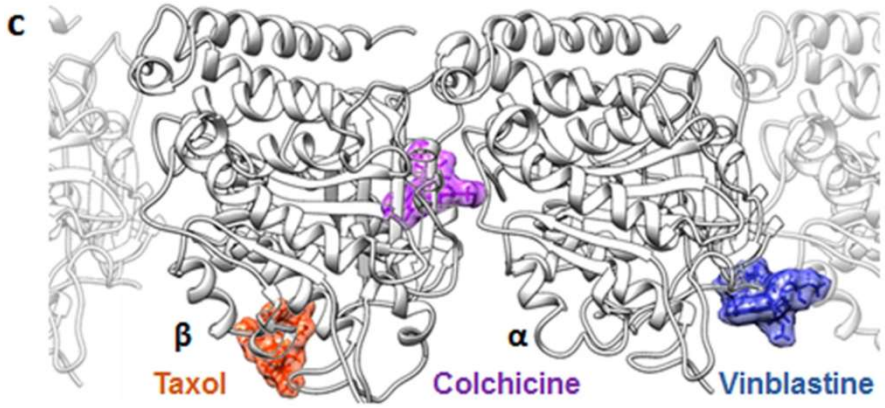
Hit compound **1**

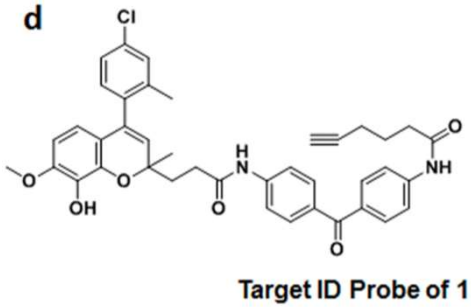
Out of 6000 candidate drug like molecules



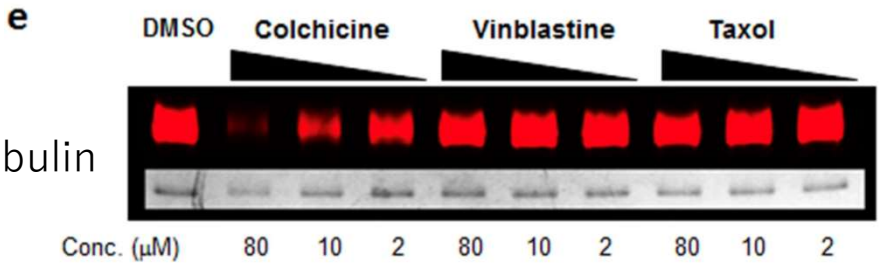
→Hit compound 1 inhibited LPS-mediated inflammation in cultured BV-2 microglial cells

①Phenotype-based screening for an anti-neuroinflammatory agent and identification of its binding site

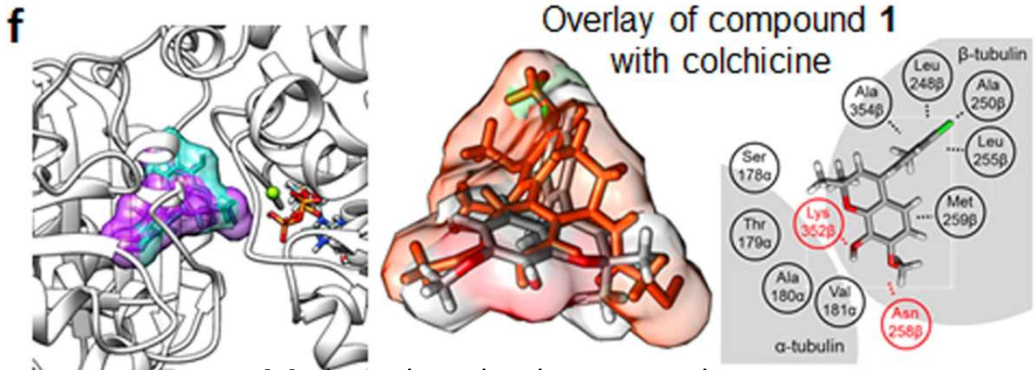




Cy5-azide (far-red fluorescent probe)



Photoaffinity-based competition assay

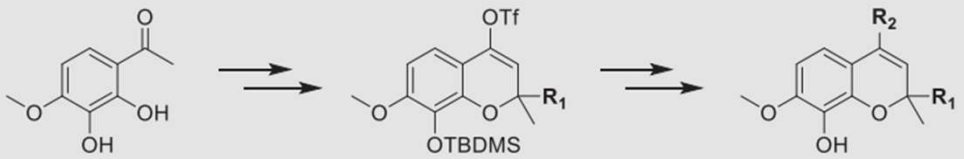


Molecular docking analysis

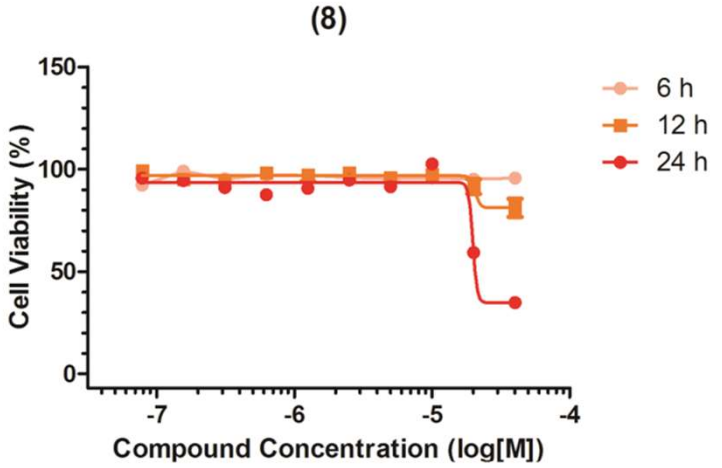
→Hit compound 1 and colchicine share the same binding site

②A structure-activity relationship study generates SB26019 as a potent anti-neuroinflammatory agent

Table 1. Structure–activity relationship (SAR) study by modifications at the R_1 and R_2 positions of compound **1**.



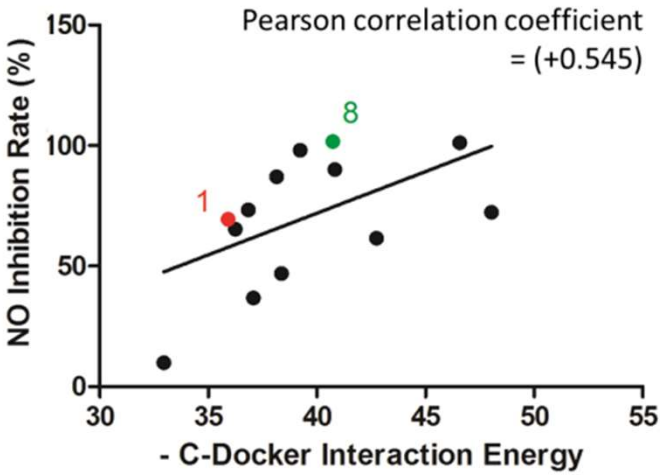
Cpd.	R_1	R_2	IC_{50} of Griess Assay	% NO inh. at 10 μM	% Viab. at 10 μM	- C-Docker Interaction energy
1	Methyl	4-Chloro-2-methylphenyl	5.22	69.4	102.4	35.91
2	Methyl	4-Fluoro-2-methylphenyl	5.94	65.3	105.3	36.24
3	Methyl	4-Fluorophenyl	22.28	9.9	88.0	32.96
4	Methyl	2-Methylphenyl	7.19	73.3	106.1	36.85
5	Methyl	2,5-Dimethylphenyl	9.89	46.9	99.7	38.37
6	Methyl	2-Methoxyphenyl	5.09	87.1	113.8	38.15
7	Methyl	Quinolin-8-yl	5.01	98.0	101.5	39.23
8 (SB26019)	Methyl	Dibenzofuran-4-yl	1.13	101.7	108.0	40.73
9	Methyl	Dibenzothiophen-4-yl	1.00	90.0	103.6	40.82
10	Methyl	Thianthren-1-yl	13.12	36.8	102.1	37.07
11	Methyl	1,1-Biphenyl-3-yl	6.71	61.6	106.1	42.74
12	Ethyl propionate	4-Chloro-2-methylphenyl	7.45	72.3	105.6	48.03
13	Ethyl propionate	Dibenzofuran-4-yl	1.79	101.2	111.4	46.58
Nocodazole			13.44	54.9	93.8	
Colchicine			4.20	51.8	113.8	

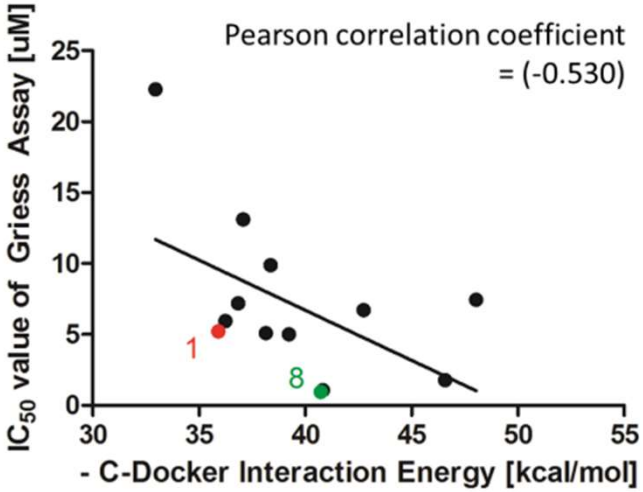


Cell viability curve for compound **8**

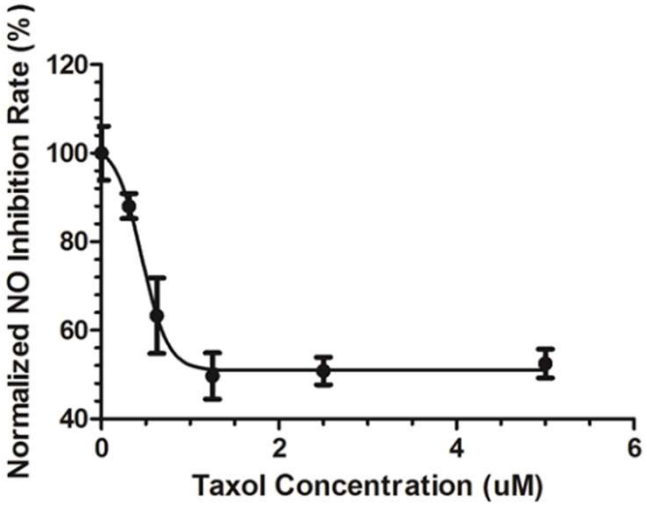
→SB26019 indicated high affinity to tubulin without considerable cytotoxicity

②A structure-activity relationship study generates SB26019 as a potent anti-neuroinflammatory agent





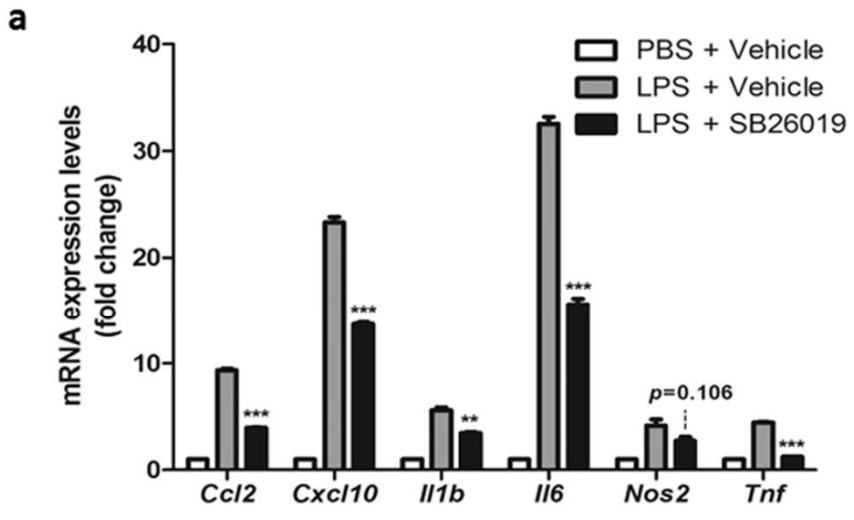
Correlation between anti-inflammatory effect and absolute C-Docker Interaction Energy.

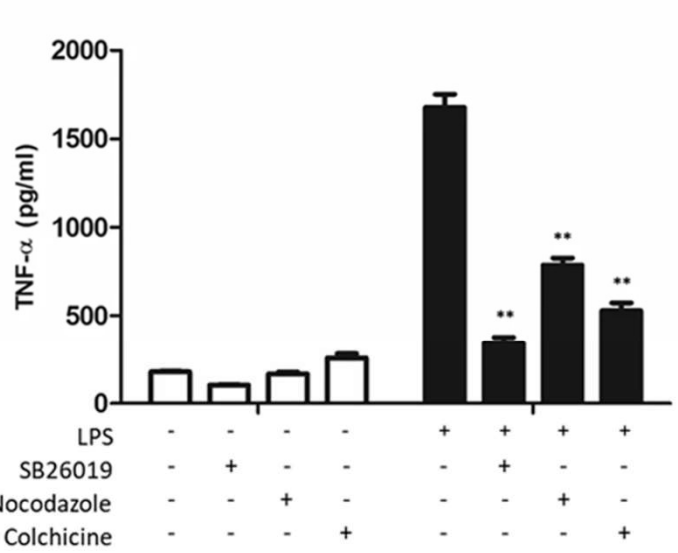


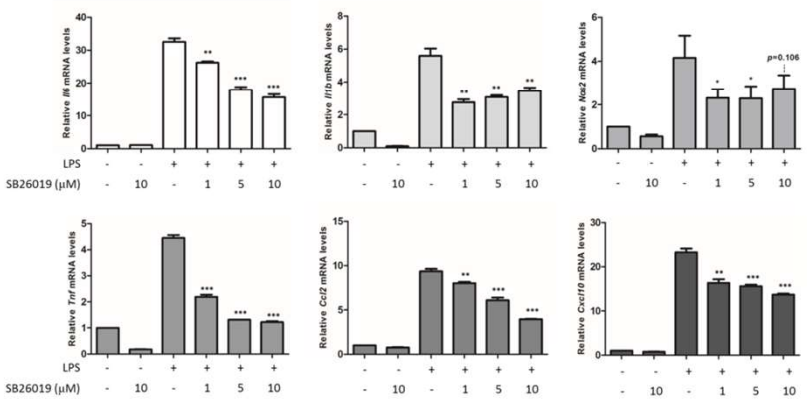
Anti-inflammatory effects of SB26019 (5 μM) were compensated by tubulin stabilizer (taxol) in a dose-dependent manner (n=6).
Data are presented as the mean ± SD.

→Tubulin polymerization inhibition triggered the anti-neuroinflammatory effect of SB26019

③ SB26019 regulates NF-κB activation by inducing monomeric α-tubulin formation

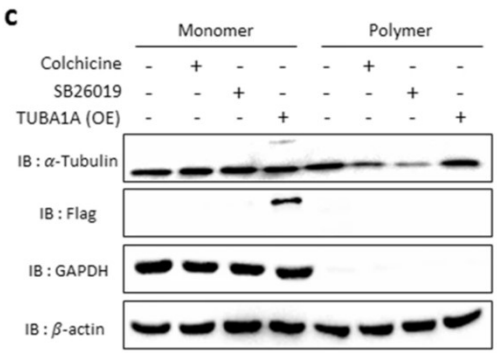


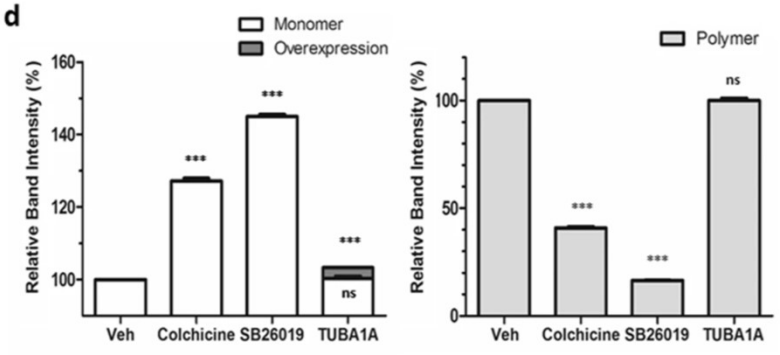


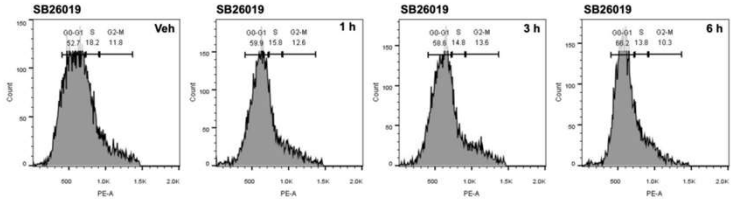


→SB26019 and other drugs which share colchicine-domain binders reduced expression of NF-κB-mediated cytokines and chemokines

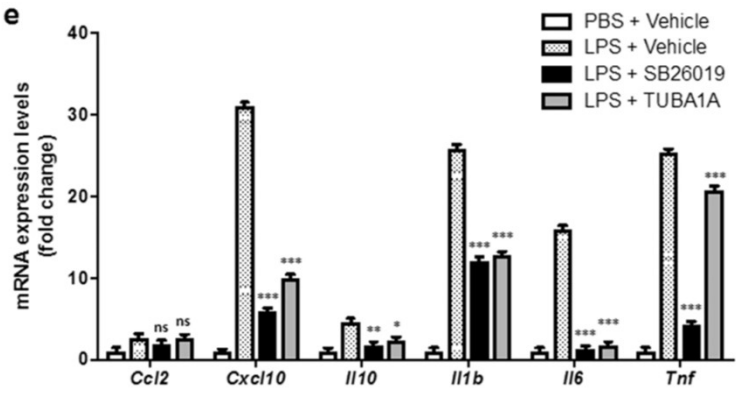
③ SB26019 regulates NF-κB activation by inducing monomeric α-tubulin formation

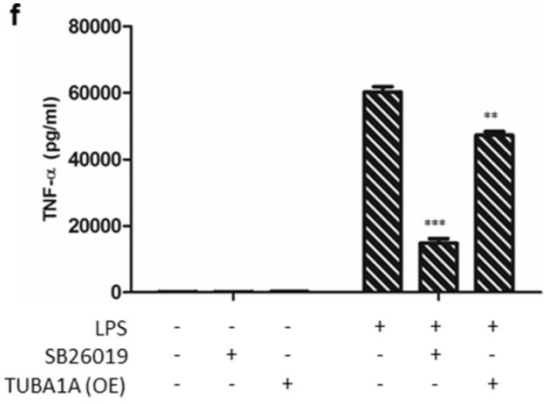






Supplementary Fig. 13. 20 μM of SB26019 treatment didn't show any mitotic arrest within 6 h confirmed by flow cytometry in J774A.1 murine macrophage cell.

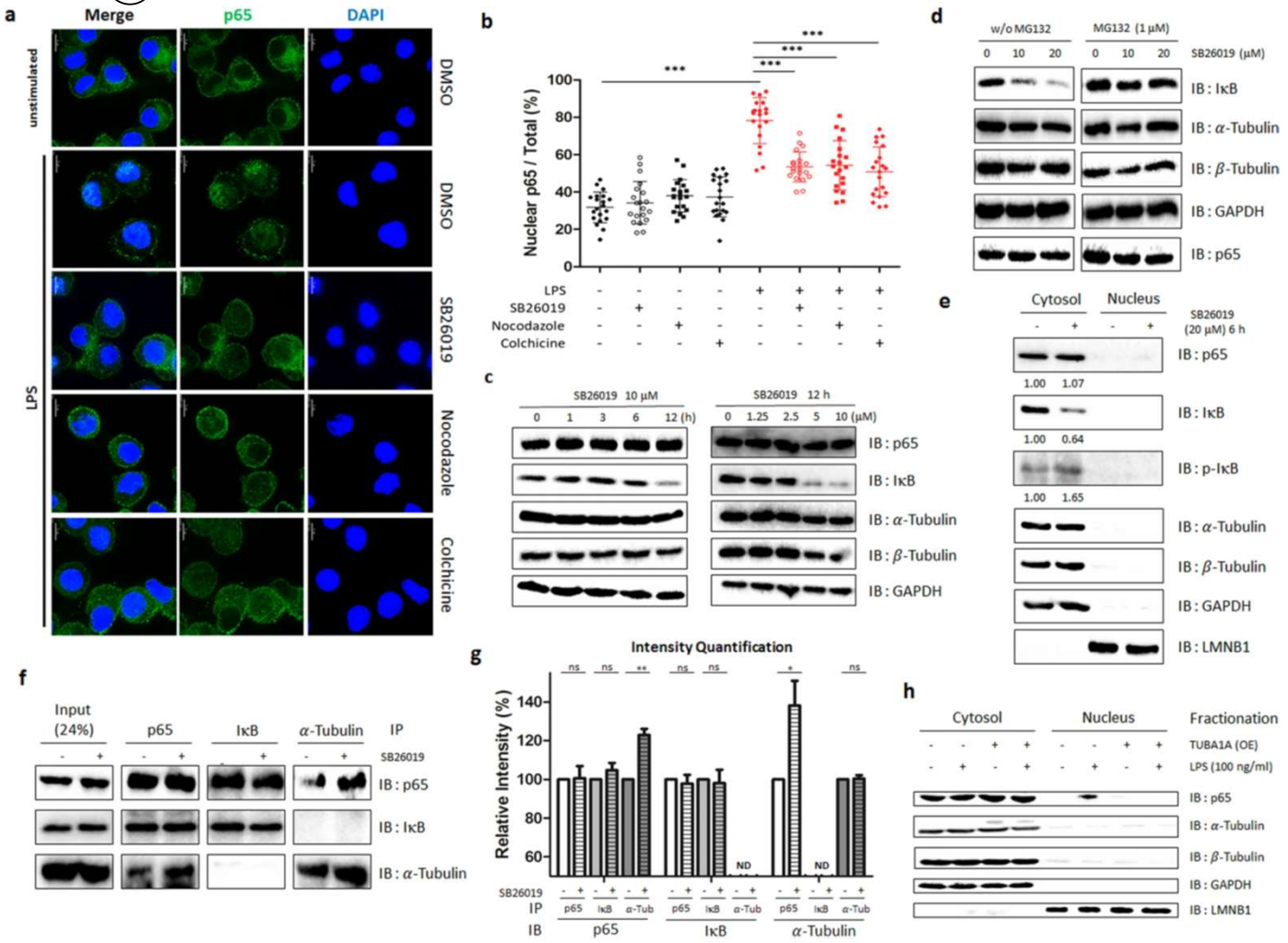


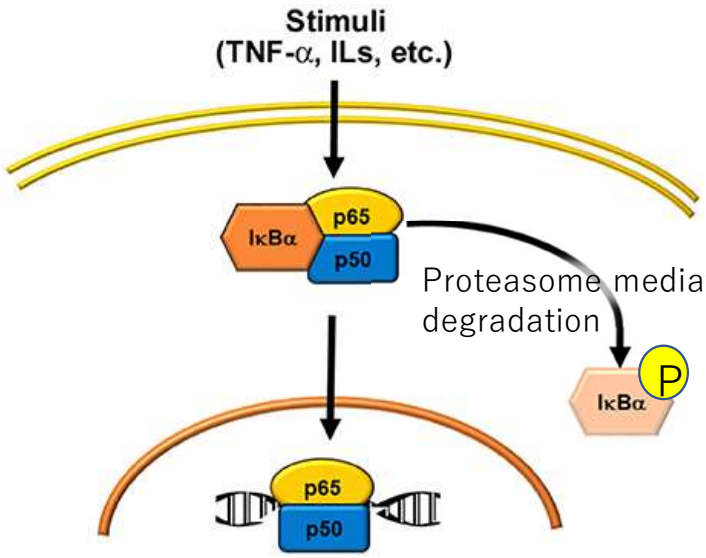


Transfection host :
RAW264.7 murine macrophage cells

→SB26019 treatment induces transition from tubulin polymers to monomers and α-tubulin monomers downregulate the expression of NF-κB-related genes and TNF-α level

④ SB26019-induced α-tubulin monomer inhibits p65 translocation

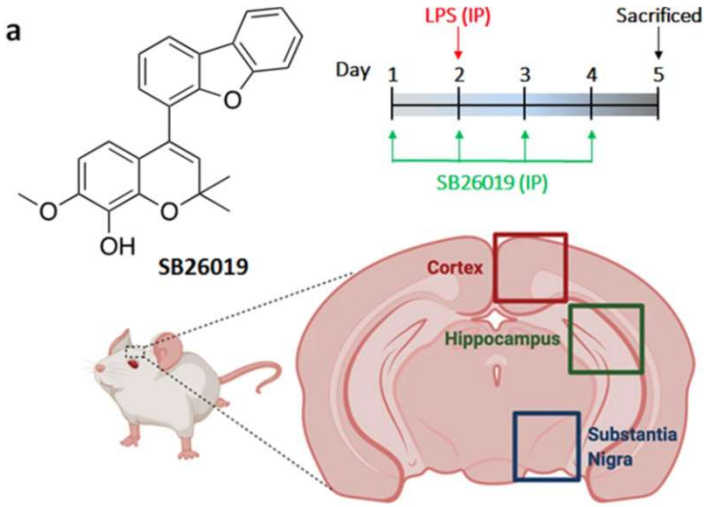


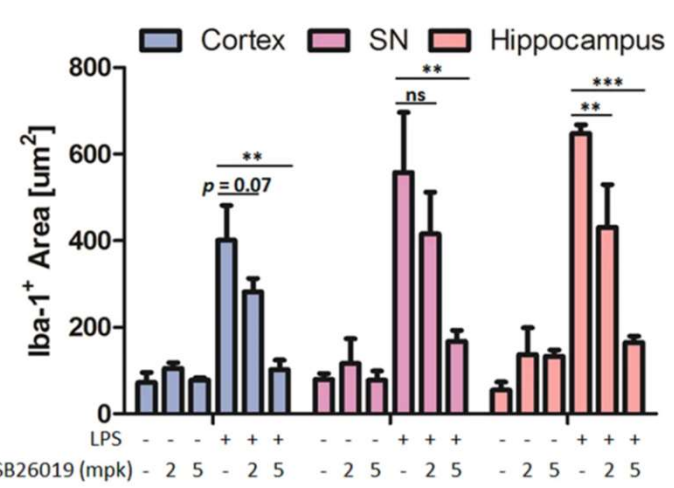


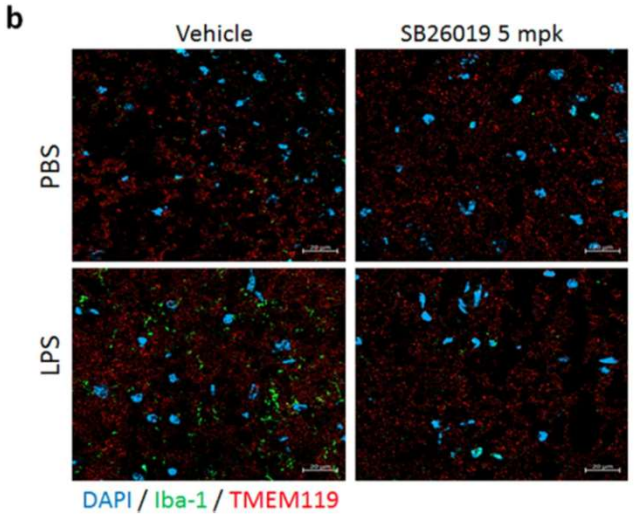
Giuliani C, Bucci I and Napolitano G (2018)

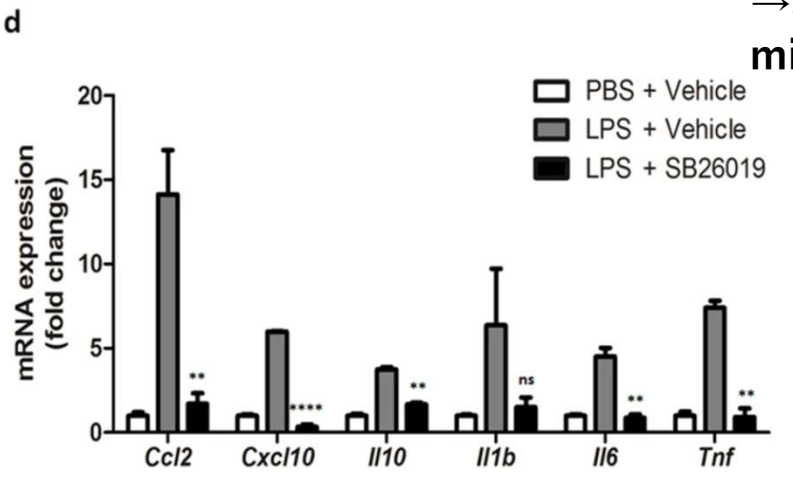
→ α-tubulin monomers bind to p65 and inhibit its nuclear translocation

⑤ SB26019 ameliorates neuroinflammation in vivo









→SB26019 suppresses LPS-mediated microglial activation in vivo

① Phenotype-based screening for an anti-neuroinflammatory agent and identification of its binding site

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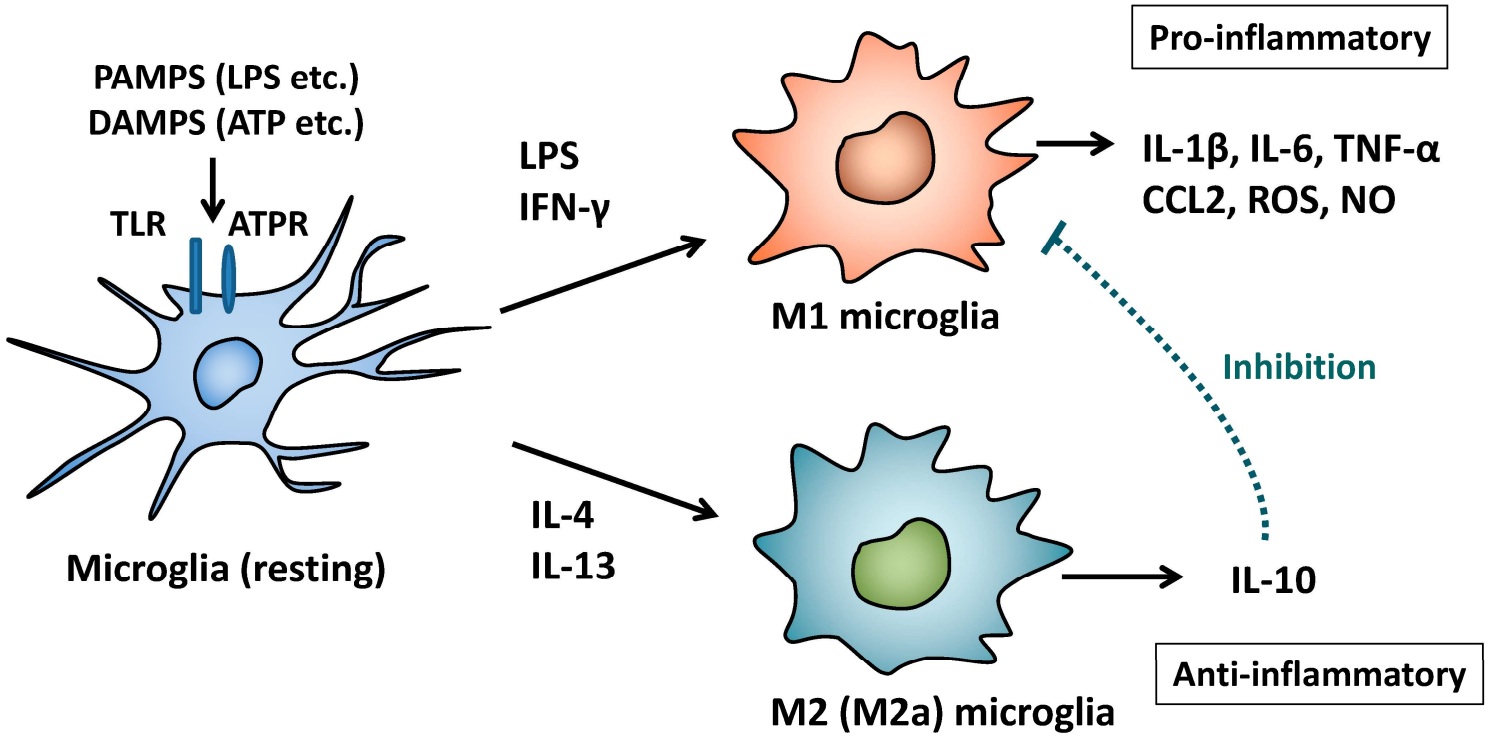
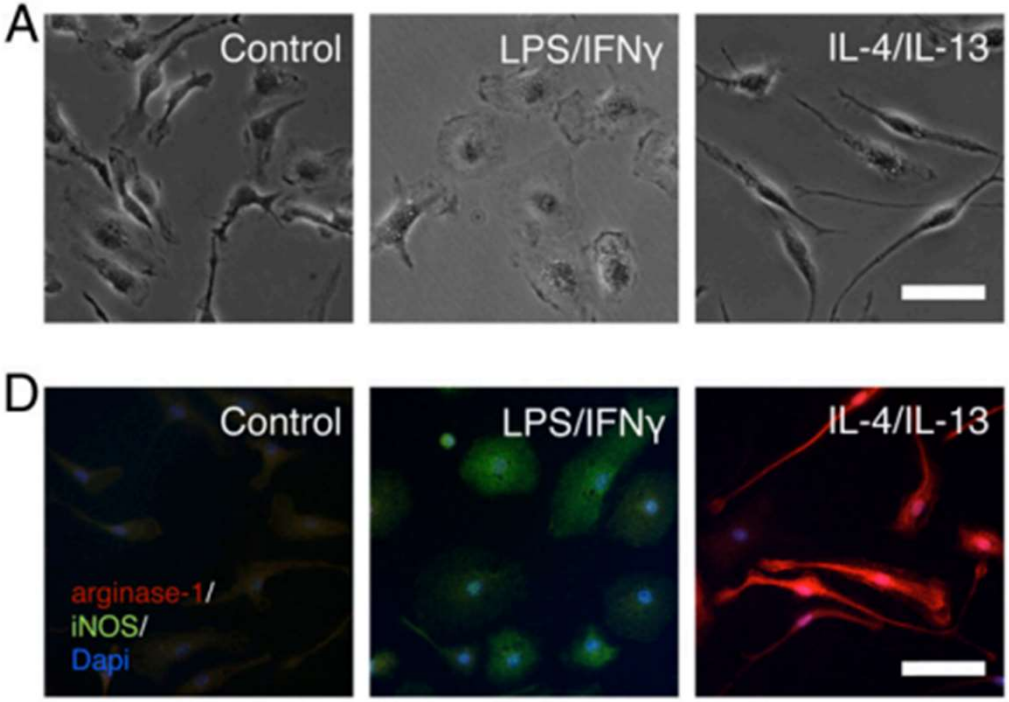
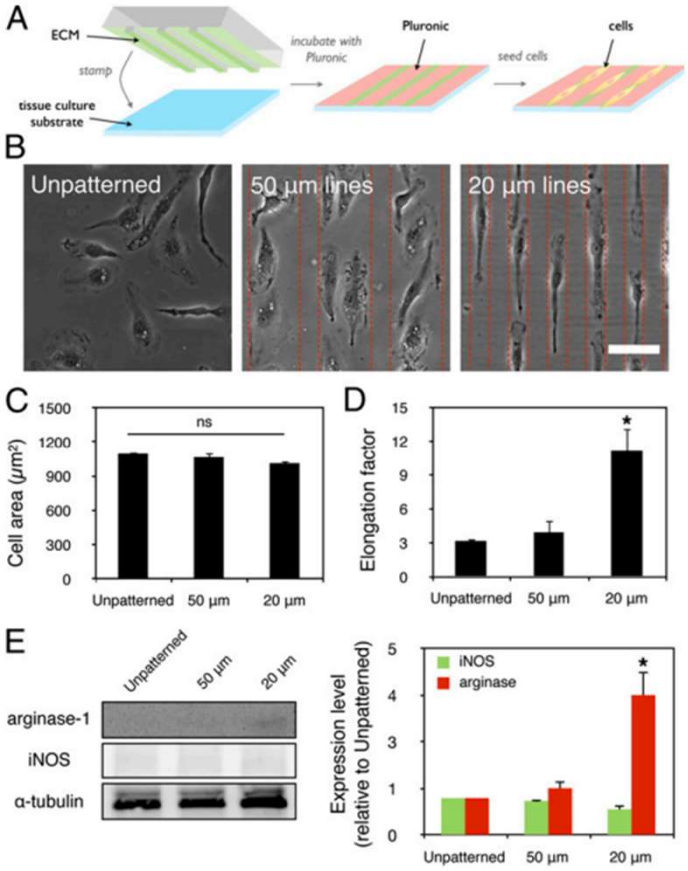
- Their findings demonstrated that colchicine-binding site-specific modulation of tubulin could be a potential strategy for treating neurodegenerative diseases
- Unwanted side effects due to cytotoxicity when treated in a long term could be a significant limitation for clinical application
- Combination of lower dosage of tubulin modulator with other anti-neuroinflammatory agents having different mode of action may be desirable
- Discovering various types of tubulin modulators is essential for the efficient control of neuroinflammation to overcome the complexity of neuroinflammatory processes and the poor drug transport to brains due to the blood– brain barrier



Yutaka Nakagawa, Kenji Chiba(2014)

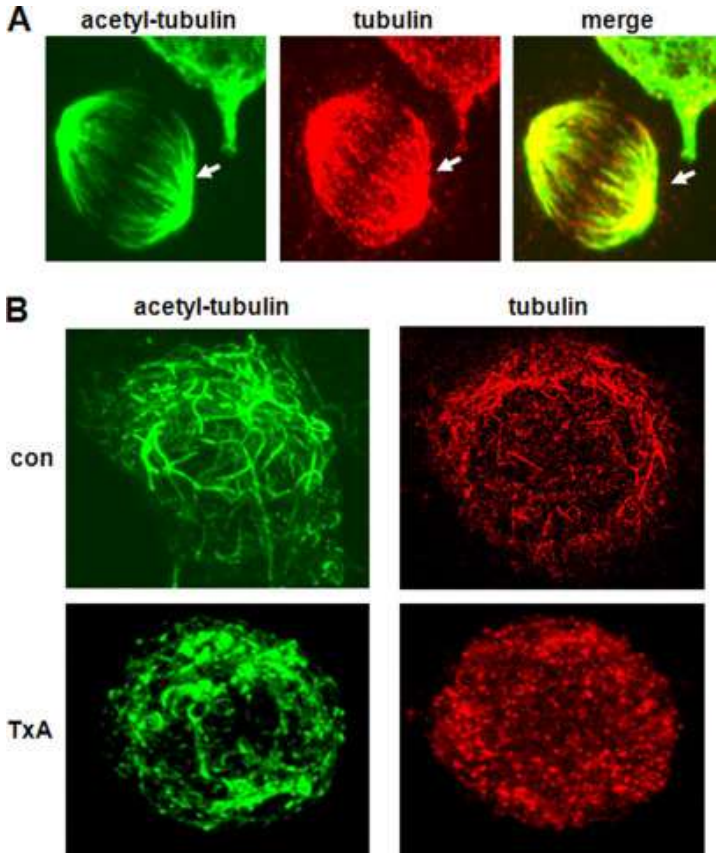
Modulation of macrophage phenotype by cell shape



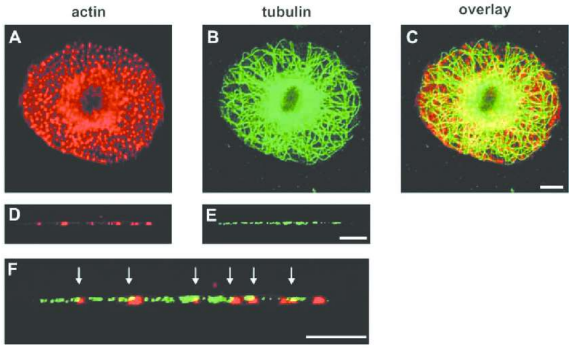


Frances Y. McWhortera et al. (2013)

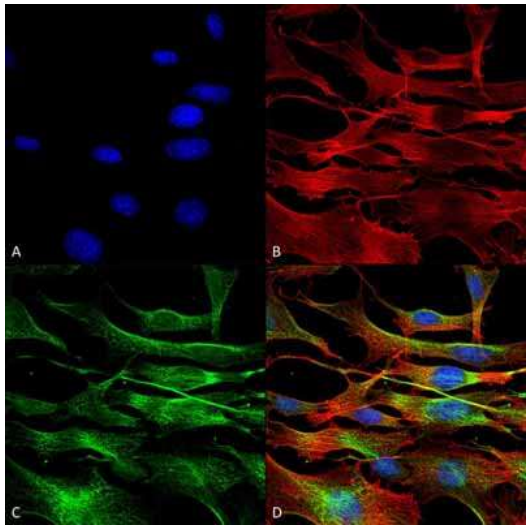
Clostridium difficile toxin A deacetylate α-tubulin and induce tubulin monomer dissociation

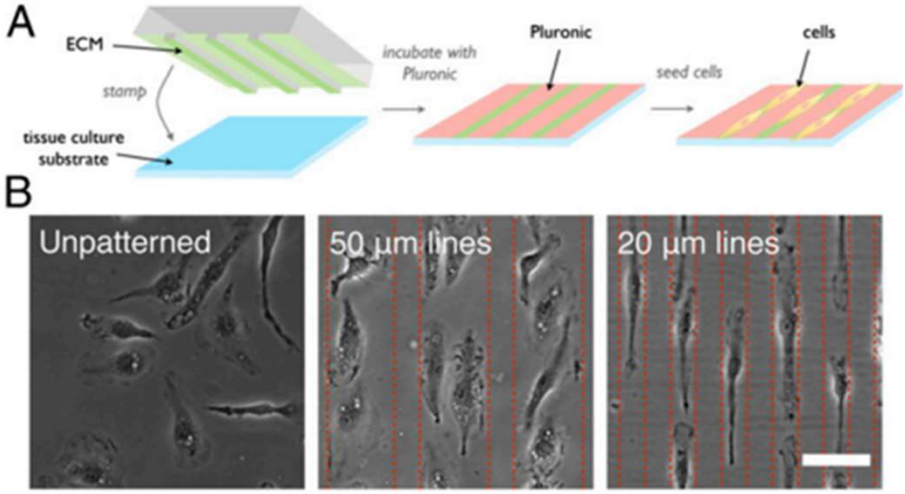


Hyo Jung Nam, Jin Ku Kang et al. (2010)

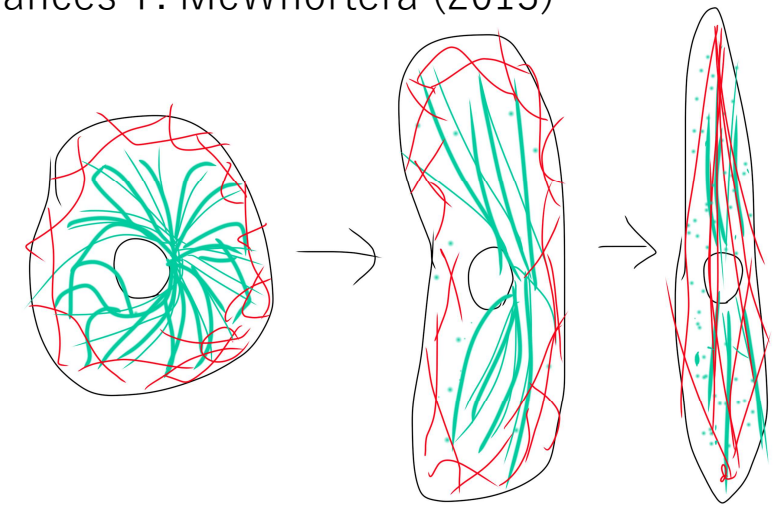


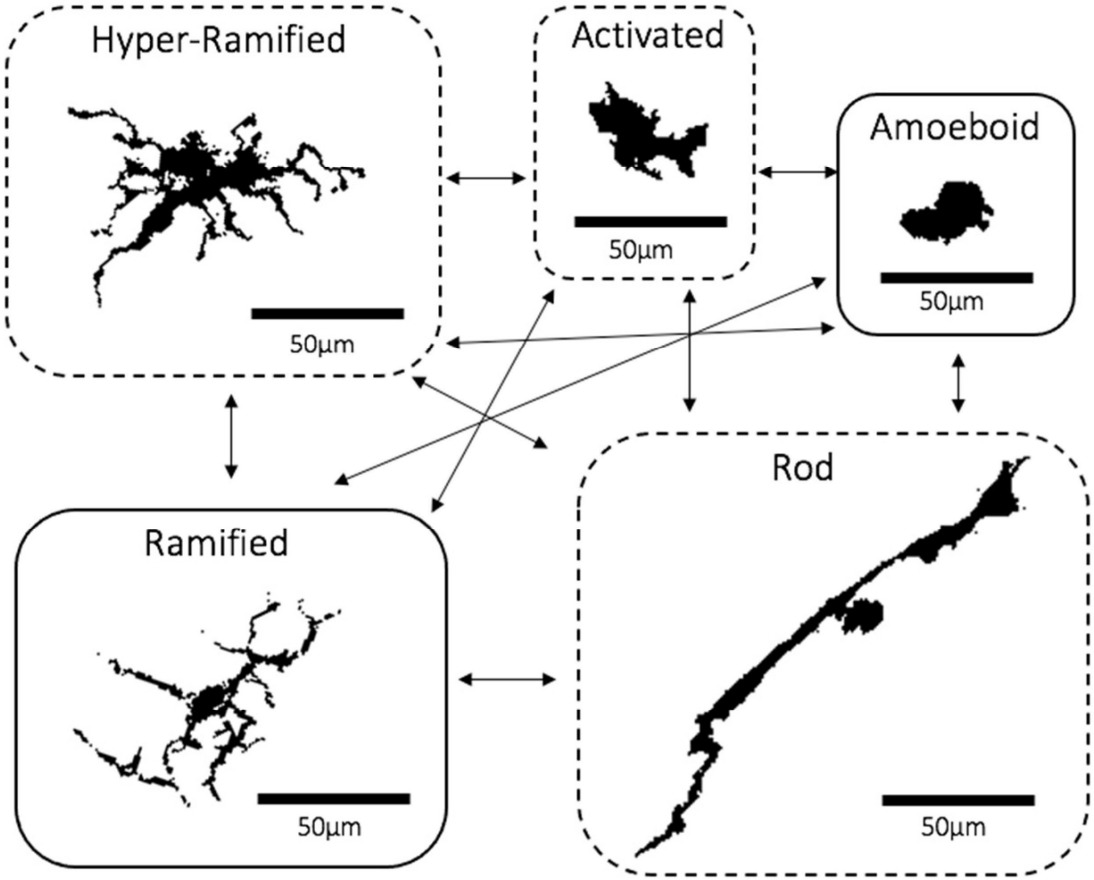
Stefan Linder (2001)





Frances Y. McWhortera (2013)





Soyoung Choi et al. (2021)