2022年11月28日

Synaptic pruning of murine adult-born neurons by microglia depends on phosphatidylserine

Chihiro Kurematsu, Masato Sawada, Masaki Ohmuraya, Motoki Tanaka, Kazuya Kuboyama, Takashi Ogino, Mami Matsumoto, Hisashi Oishi, Hiroyuki Inada, Yuri Ishido, Yukina Sakakibara, Huy Bang Nguyen, Truc Quynh Thai, Shinichi Kohsaka, Nobuhiko Ohno, Maki K. Yamada, Masato Asai, Masahiro Sokabe, Junichi Nabekura, Kenichi Asano, Masato Tanaka, and Kazunobu Sawamoto

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医学群医学類

プレスリリース

大人の神経細胞を接続する「シナプス」の数を調節するしくみ：名市大医学部生らが発見―脳疾患の治療法開発への新たな期待―

公立大学法人名古屋市立大学
Public University Corporation
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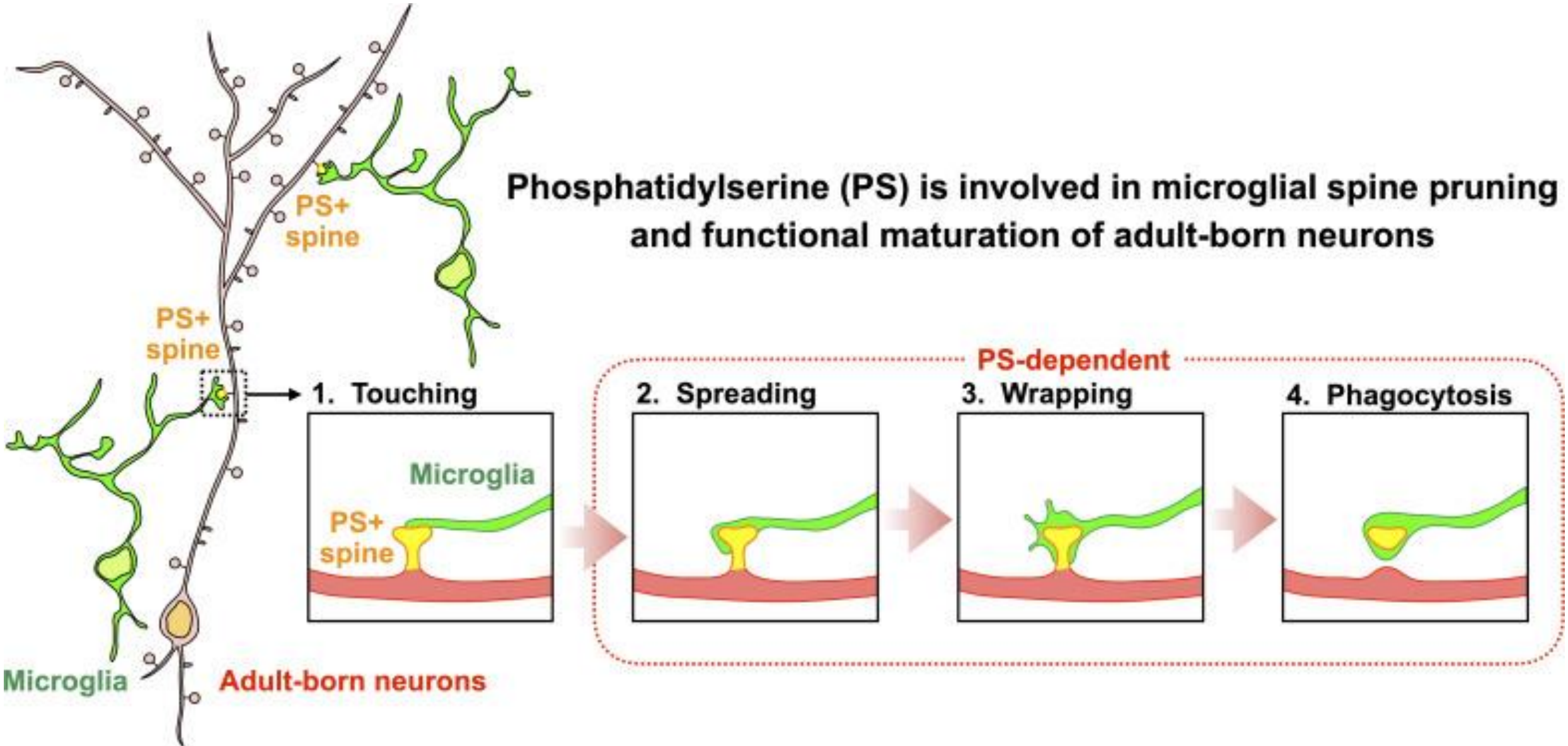
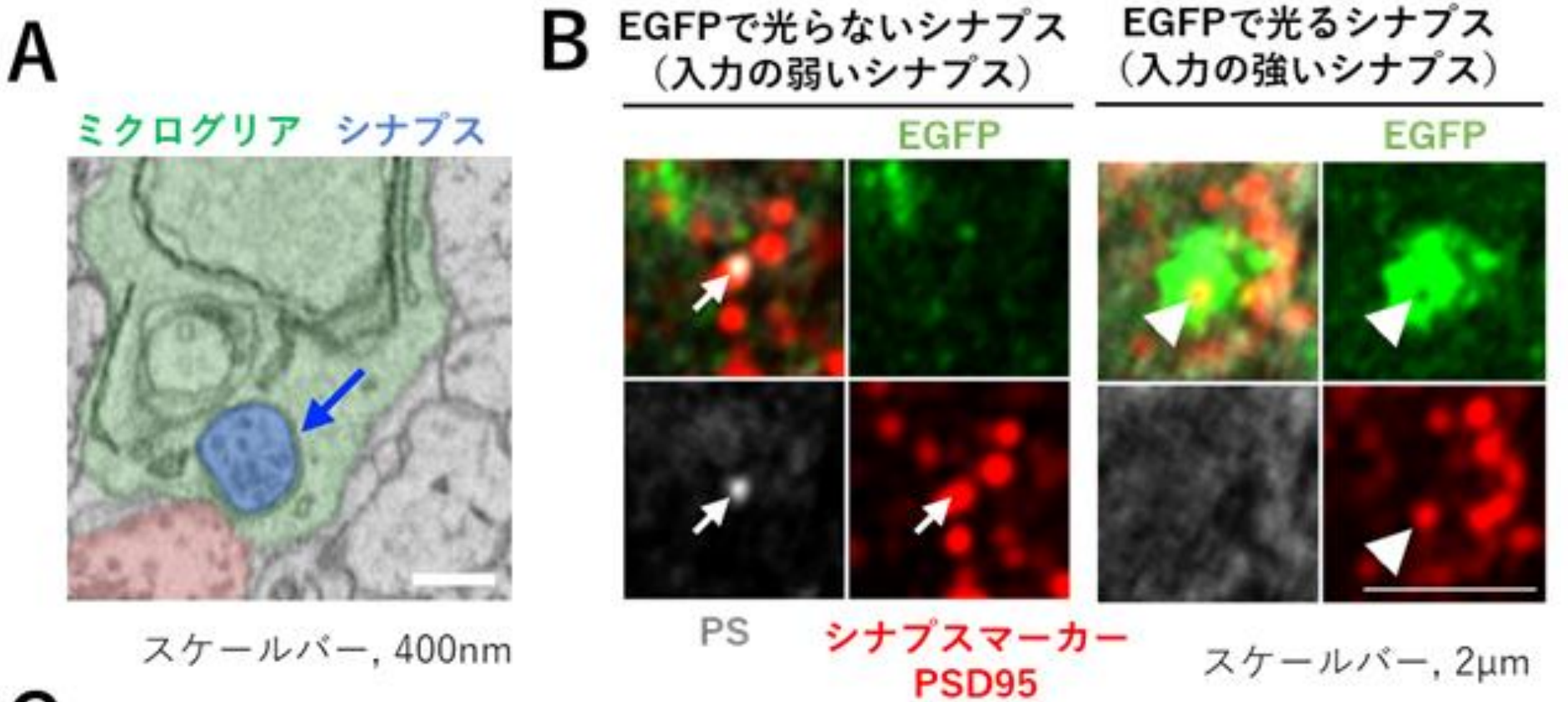
- New neurons, continuously added in the adult olfactory bulb (OB) and hippocampus, are involved in information processing in neural circuits.
- Here, we show that synaptic pruning of adult-born neurons by microglia depends on phosphatidylserine (PS), whose exposure on dendritic spines is inversely correlated with their input activity.
- To study the role of PS in spine pruning by microglia in vivo, we developed an inducible transgenic mouse line, in which the exposed PS is masked by a dominant-negative form of milk fat globule-EGF-factor 8 (MFG-E8), MFG-E8D89E.
- In this transgenic mouse, the spine pruning of adult-born neurons by microglia is impaired in the OB and hippocampus.
- Furthermore, the electrophysiological properties of these adult-born neurons are altered in MFG-E8D89E mice.
- These data suggest that PS is involved in the microglial spine pruning and the functional maturation of adult-born neurons.
- The MFG-E8D89E–based genetic approach shown in this study has broad applications for understanding the biology of PS-mediated phagocytosis in vivo.


Phosphatidylserine (PS) is involved in microglial spine pruning and functional maturation of adult-born neurons
PS+ spine
PS+ spine
1. Touching
Microglia
PS+ spine
PS-dependent
2. Spreading
3. Wrapping
4. Phagocytosis
Microglia
Adult-born neurons


A
ミクログリア　シナプス
スケールバー, 400nm
B
EGFPで光らないシナプス
（入力の弱いシナプス）
EGFPで光るシナプス
（入力の強いシナプス）
EGFP
EGFP
PS
シナプスマーカー
PSD95
スケールバー, 2μm
C
コントロールマウス
D89Eマウス
振幅
間隔
5 pA
1 s

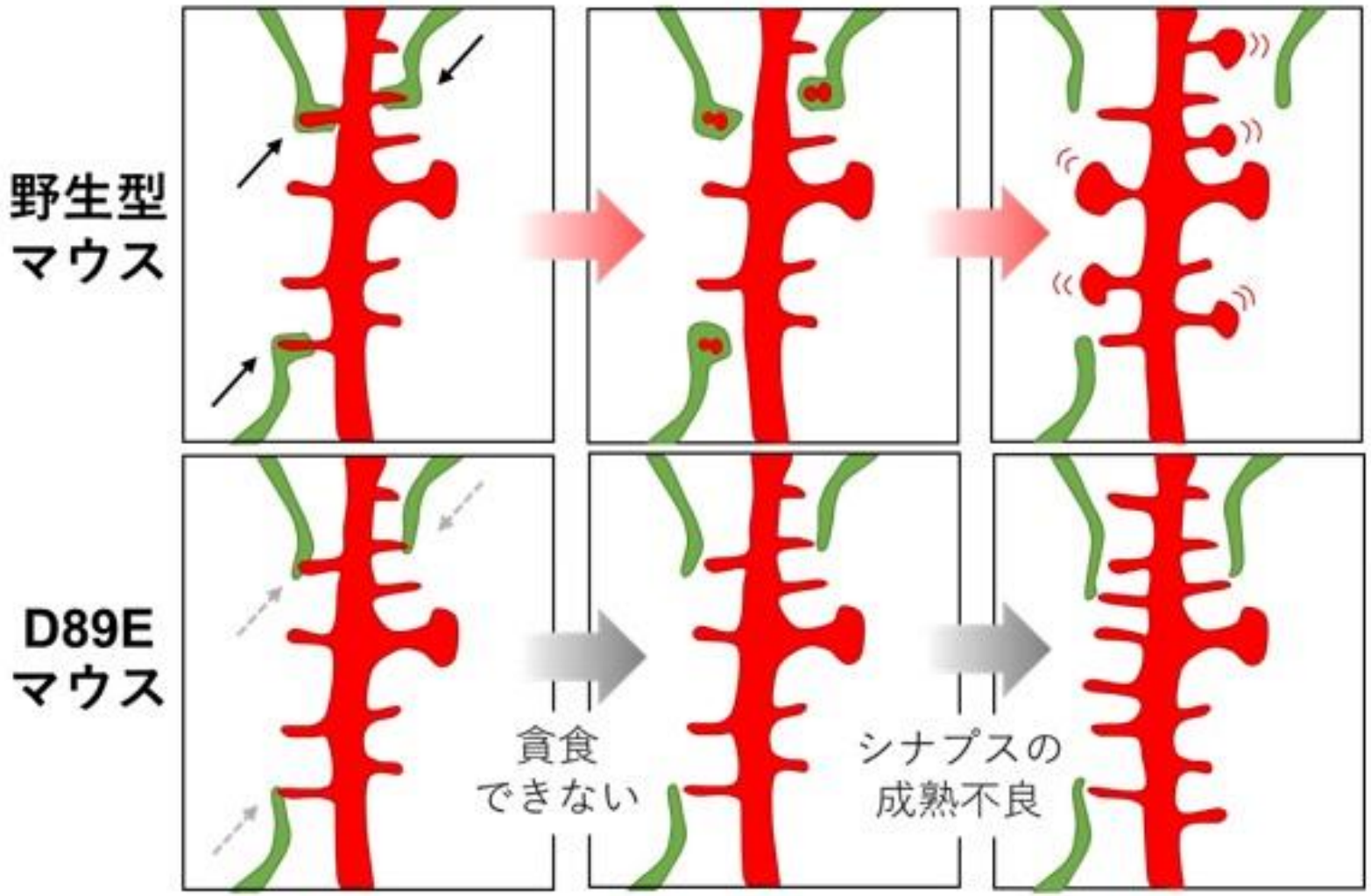
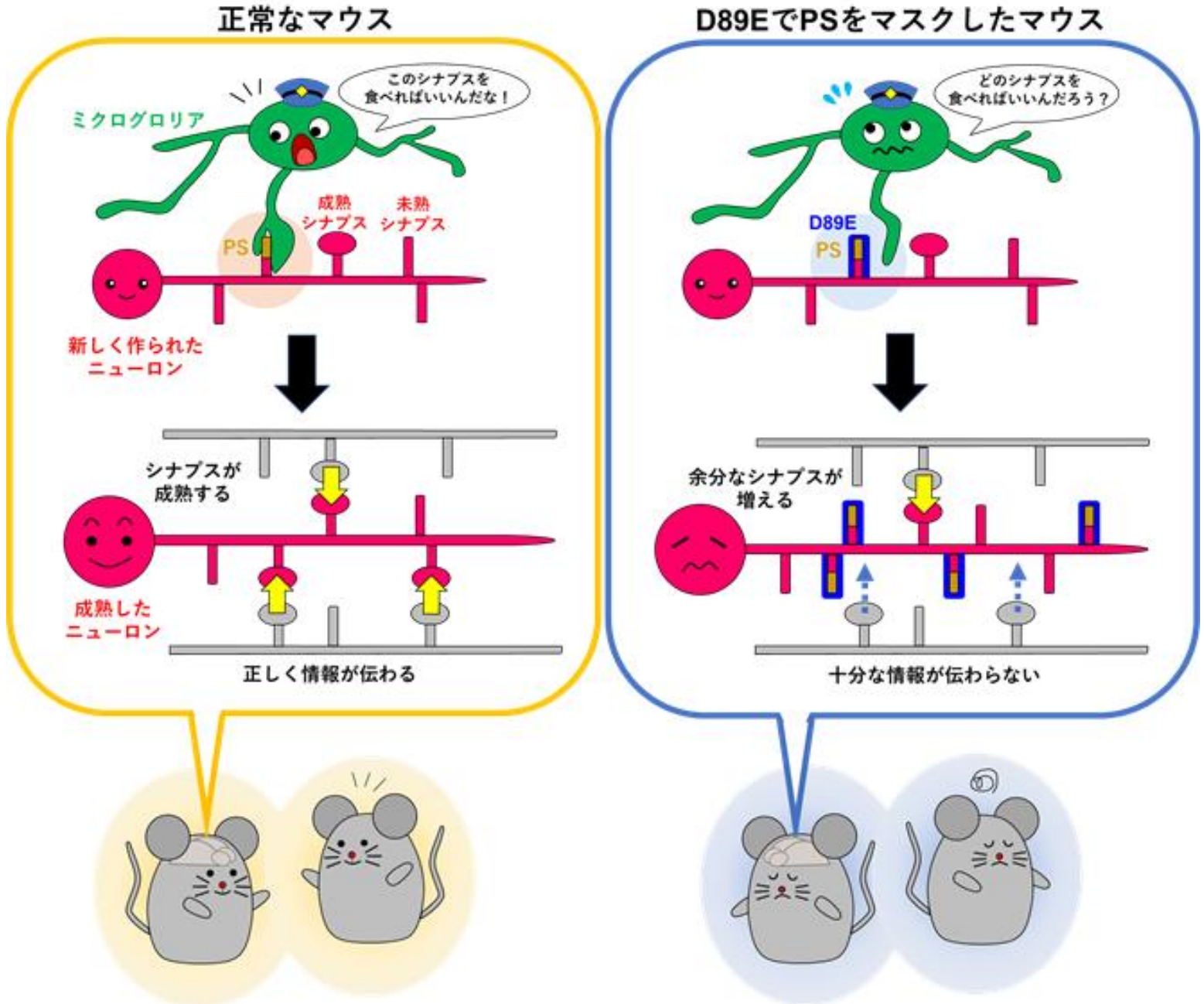
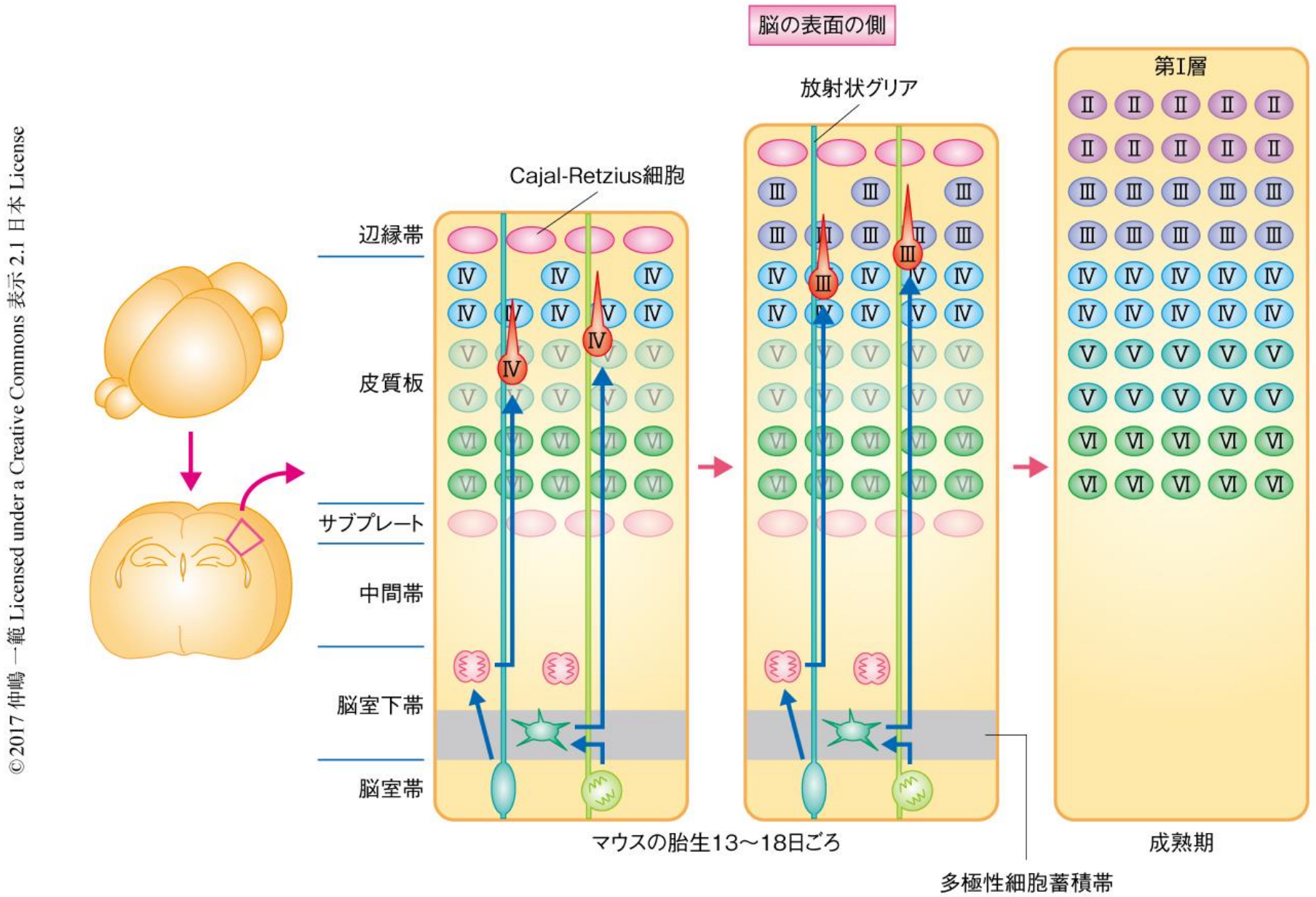
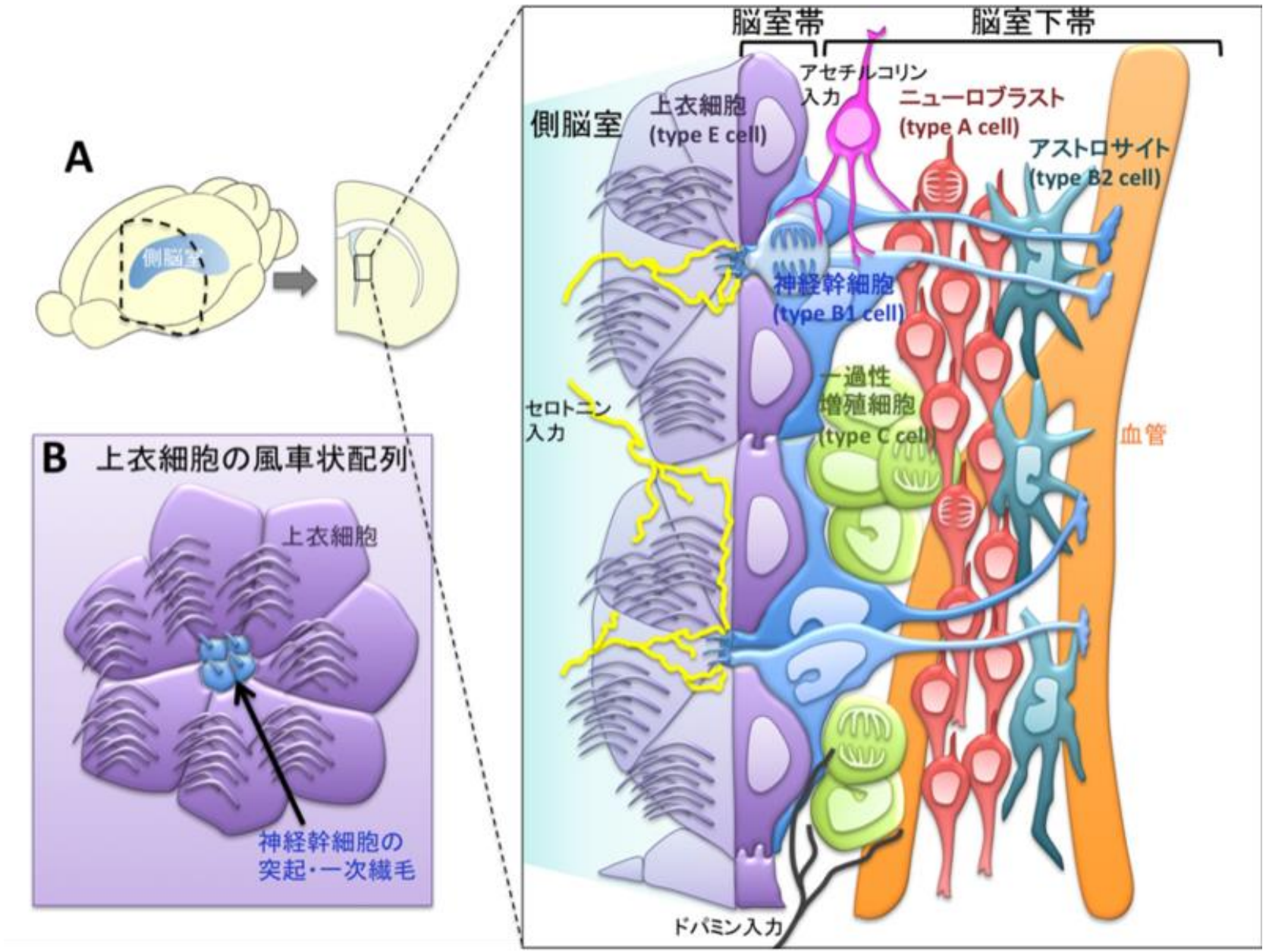
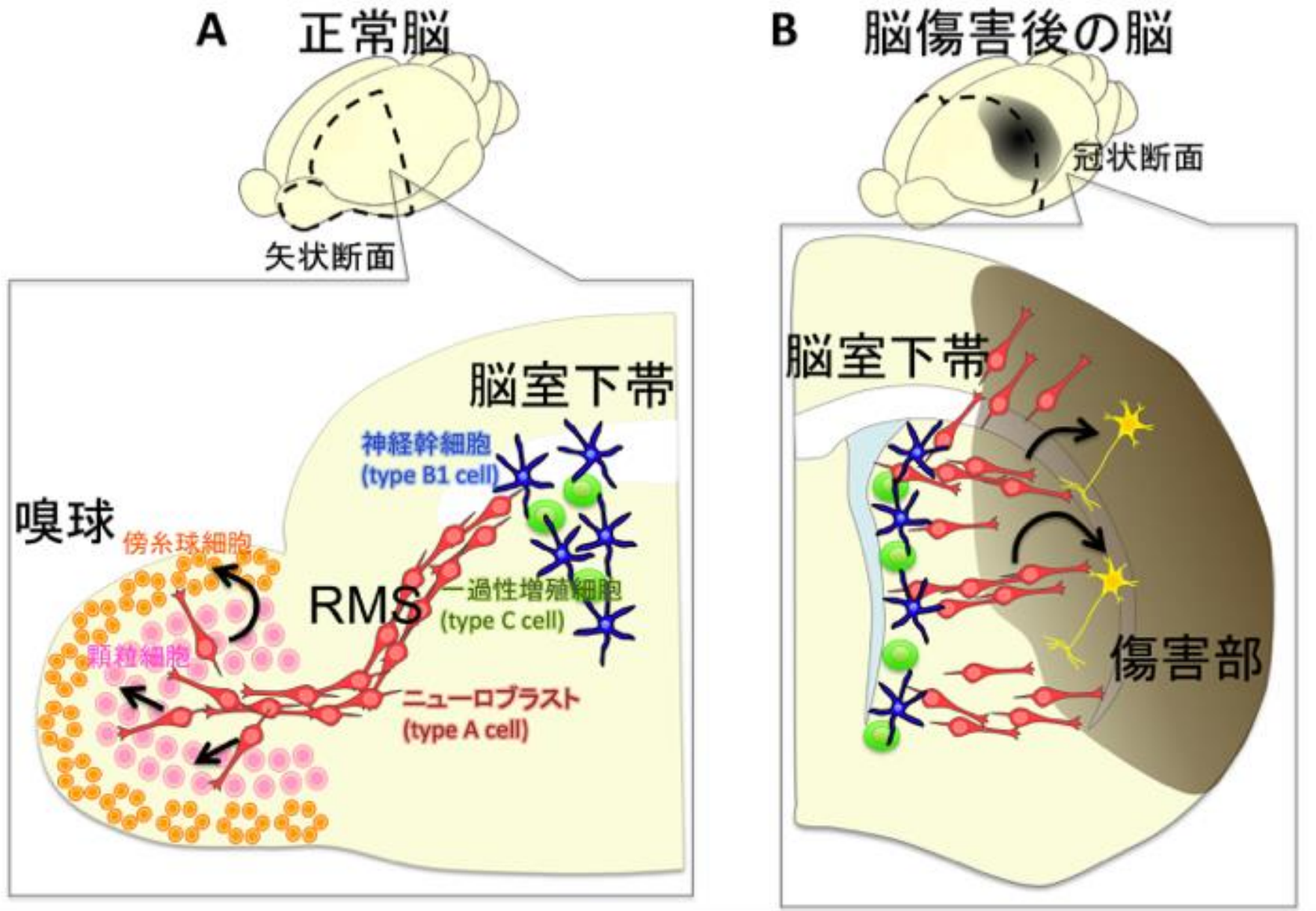


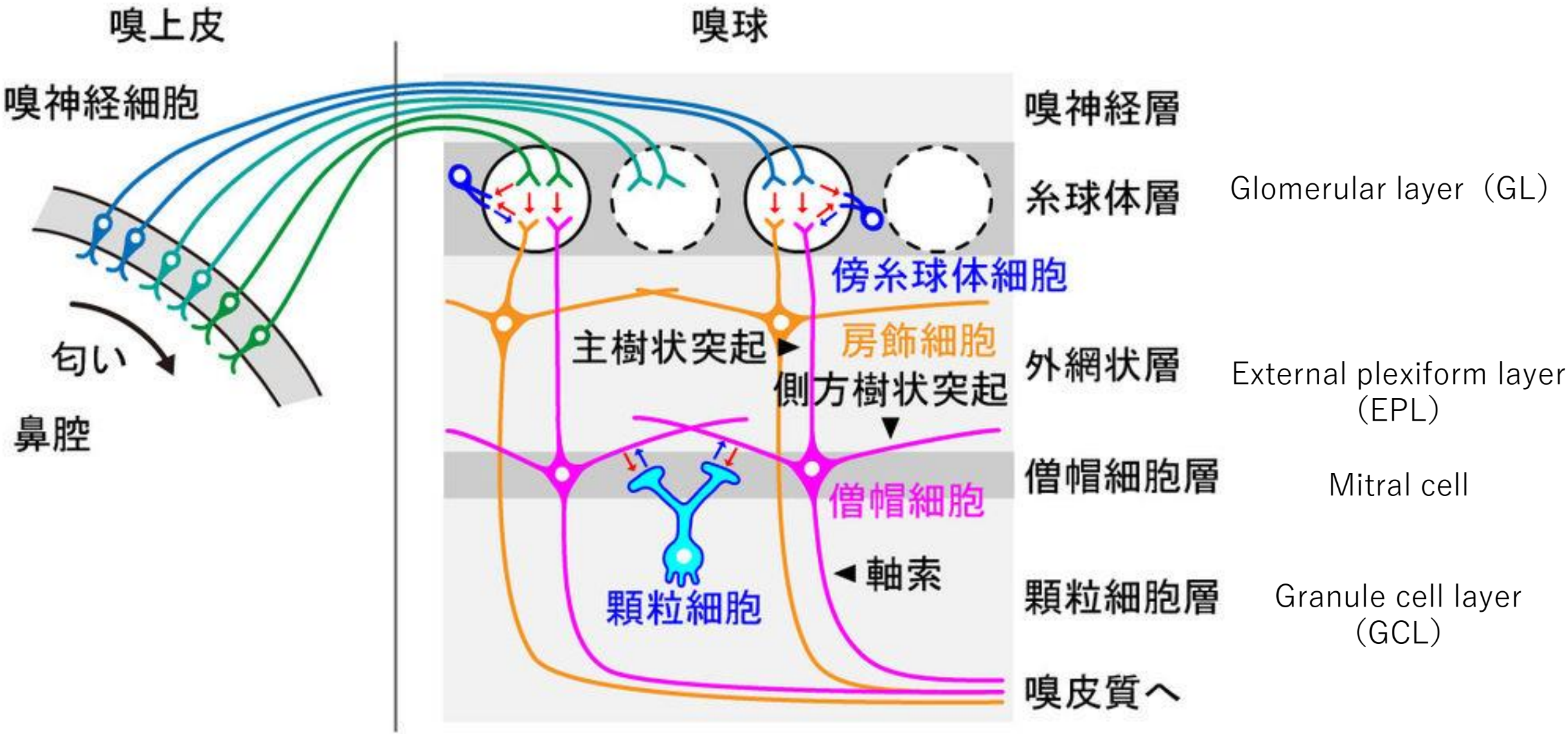
図 2：ミクログリアによる PS 依存的な成体新生ニューロンのシナプス貪食の意義

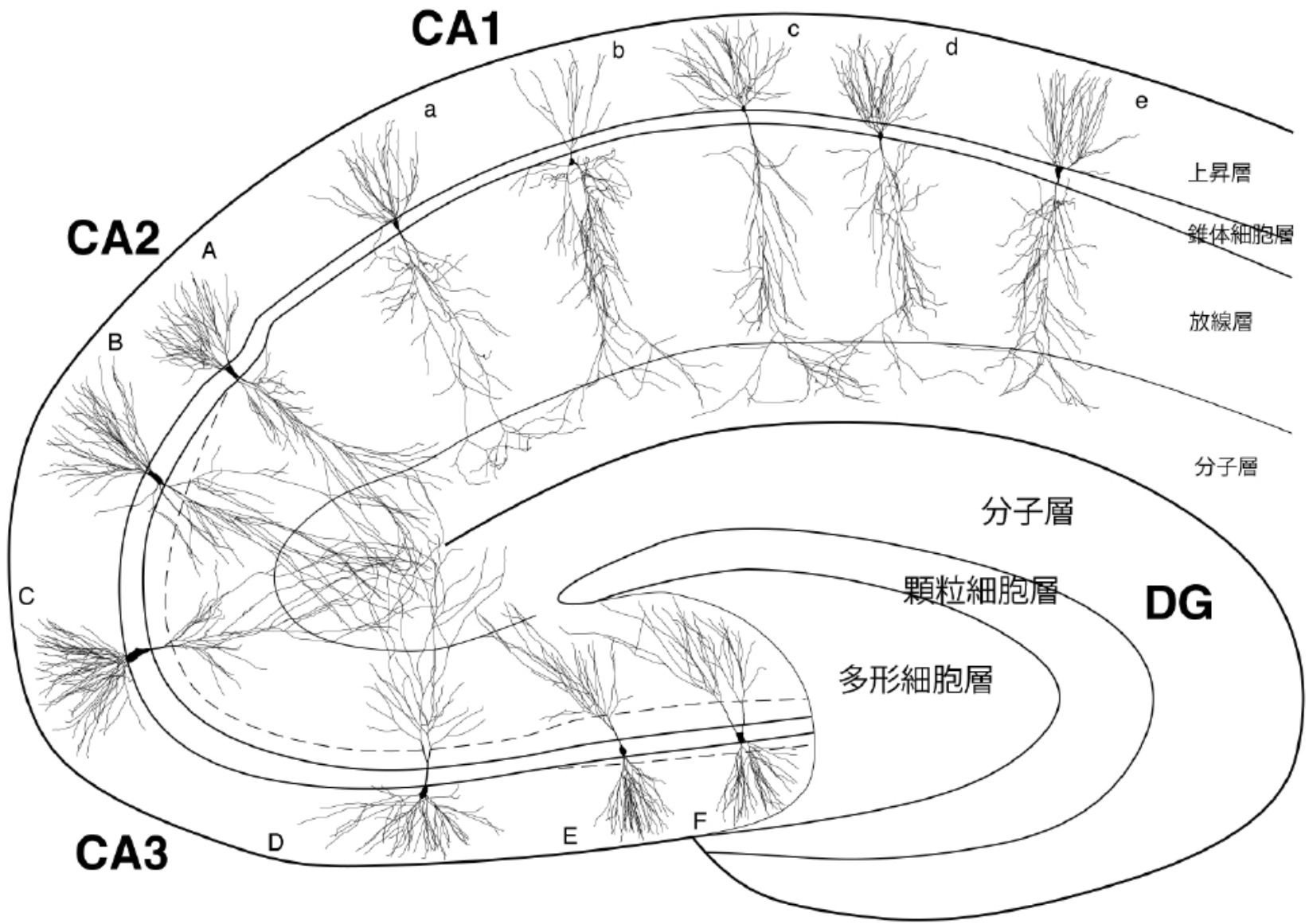

正常なマウス
このシナプスを
食べればいいんだな！
ミクログロリア
成熟
シナプス
未熟
シナプス
PS
新しく作られた
ニューロン
シナプスが
成熟する
成熟した
ニューロン
正しく情報が伝わる
D89EでPSをマスクしたマウス
どのシナプスを
食べればいいんだろう？
D89E
PS
余分なシナプスが
増える
十分な情報が伝わらない

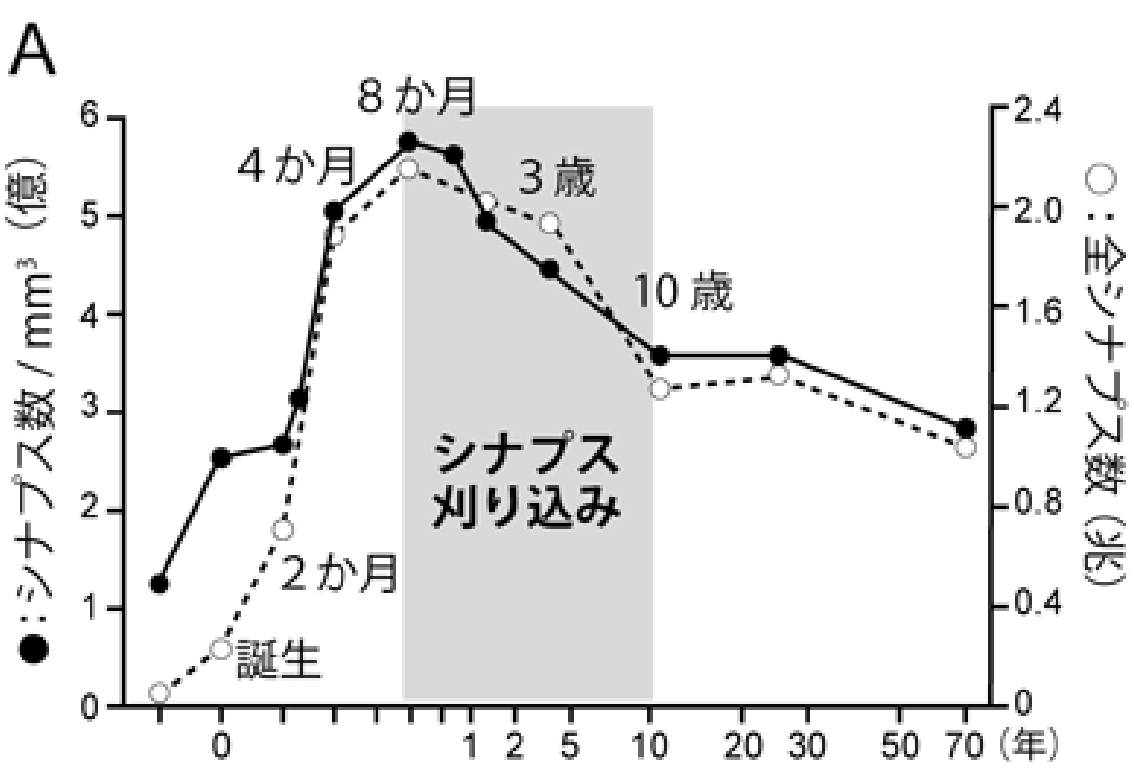


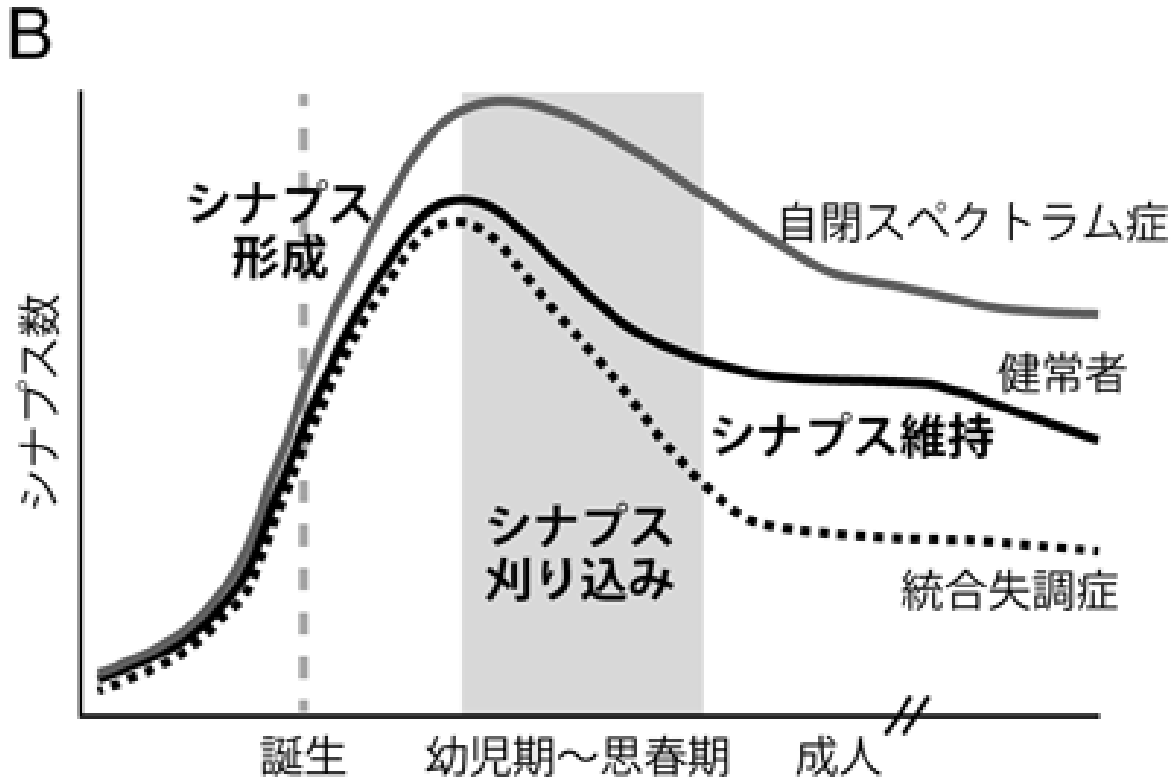

A
側脳室
B 上衣細胞の風車状配列
上衣細胞
神経幹細胞の
突起・一次繊毛
脳室帯
脳室下帯
側脳室
上衣細胞
(type E cell)
アセチルコリン
入力
ニューロブラスト
(type A cell)
アストロサイト
(type B2 cell)
神経幹細胞
(type B1 cell)
一過性
増殖細胞
(type C cell)
セロトニン
入力
血管
ドパミン入力

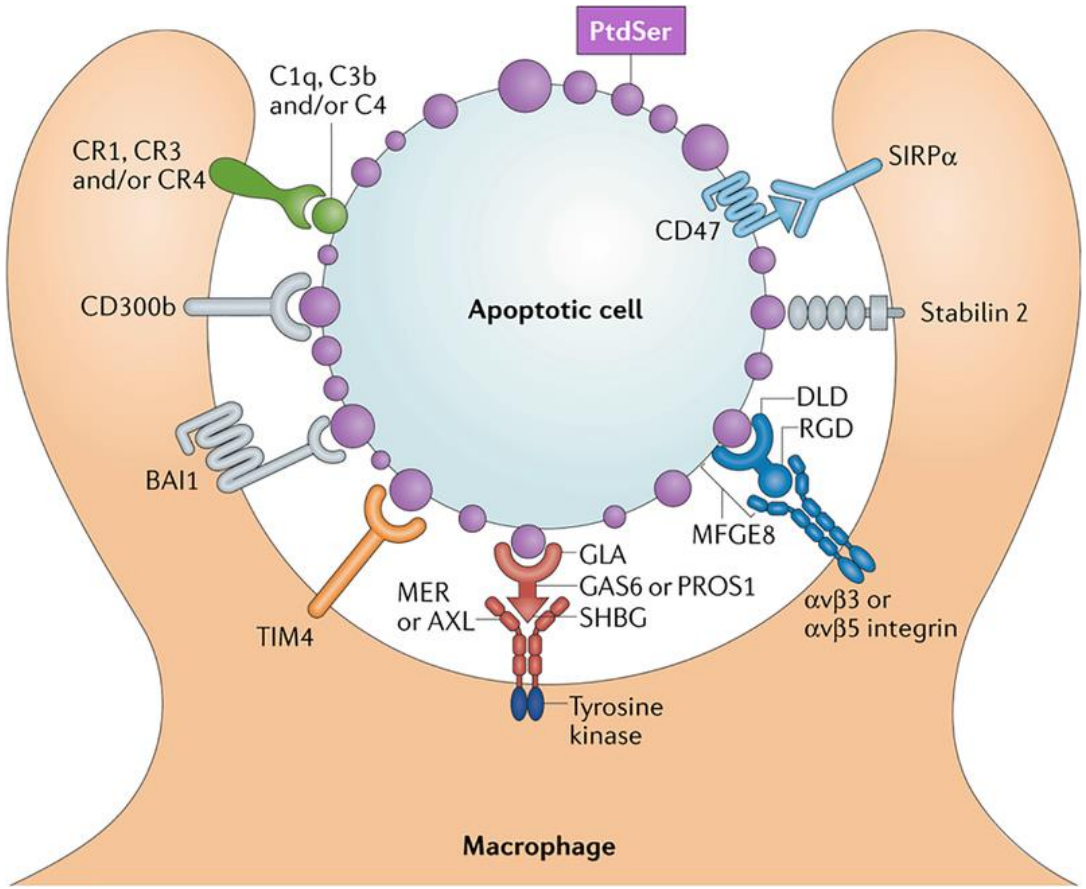

A 正常脳
矢状断面
脳室下帯
神経幹細胞
(type B1 cell)
嗅球
傍糸球細胞
RMS
一過性増殖細胞
(type C cell)
顆粒細胞
ニューロブラスト
(type A cell)
B 脳傷害後の脳
冠状断面
脳室下帯
傷害部

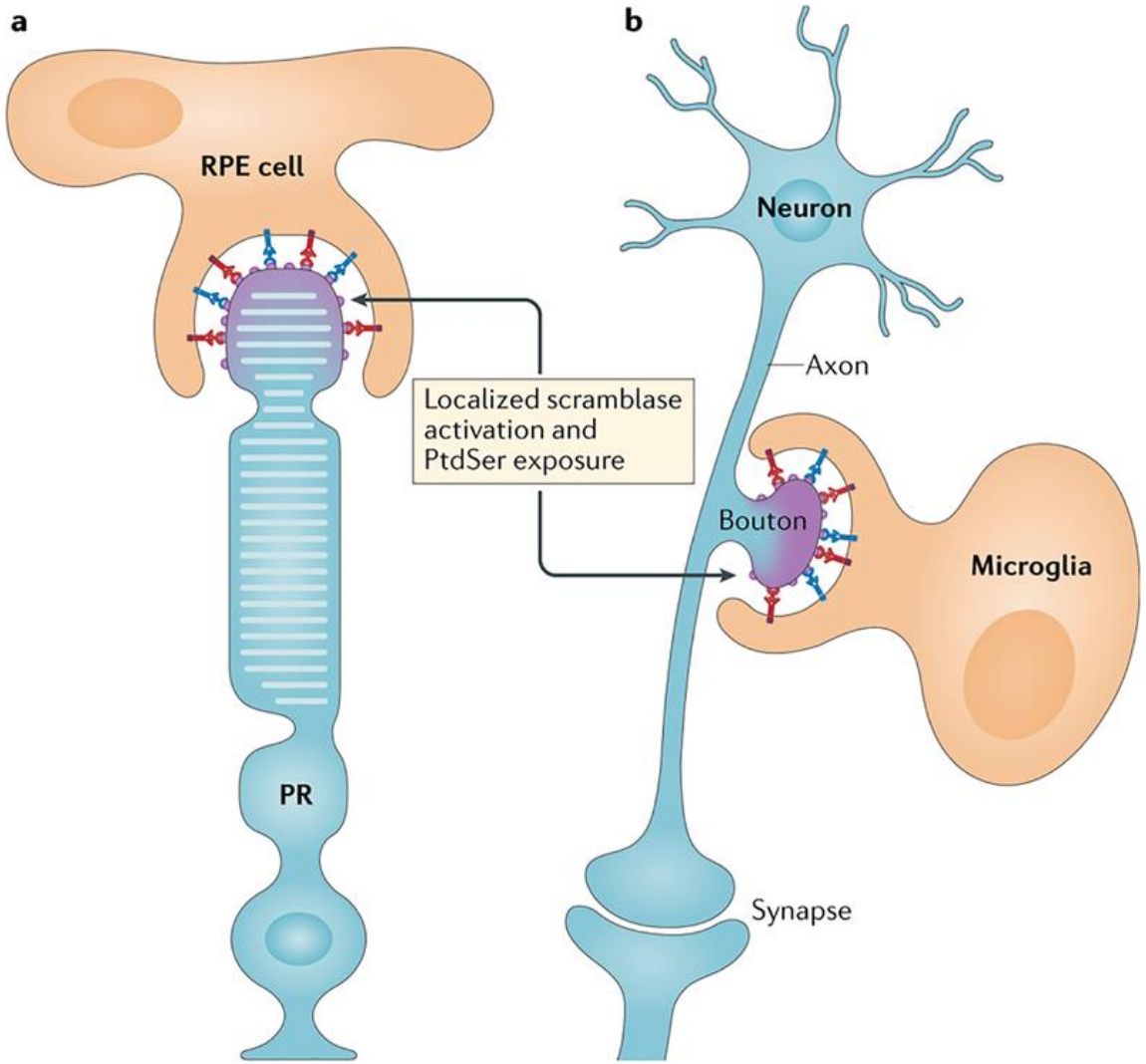

嗅上皮
嗅球
嗅神経細胞
匂い
鼻腔
嗅神経層
糸球体層
傍糸球体細胞
房飾細胞
主樹状突起
外網状層
側方樹状突起
僧帽細胞層
僧帽細胞
軸索
顆粒細胞
顆粒細胞層
嗅皮質へ
Glomerular layer（GL）
External plexiform layer (EPL)
Mitral cell
Granule cell layer (GCL)


CA1
a
b
c
d
e
上昇層
錐体細胞層
放線層
分子層
CA2
A
B
C
分子層
顆粒細胞層
DG
多形細胞層
CA3
D
E
F


A
8か月
4か月
3歳
10歳
シナプス
刈り込み
2か月
誕生
●：シナプス数 / mm³（億）
○：シナプス数（兆）
0
1
2
5
10
20
30
50
70（年）


B
シナプス
形成
自閉スペクトラム症
健常者
シナプス維持
シナプス
刈り込み
統合失調症
シナプス数
誕生
幼児期～思春期
成人

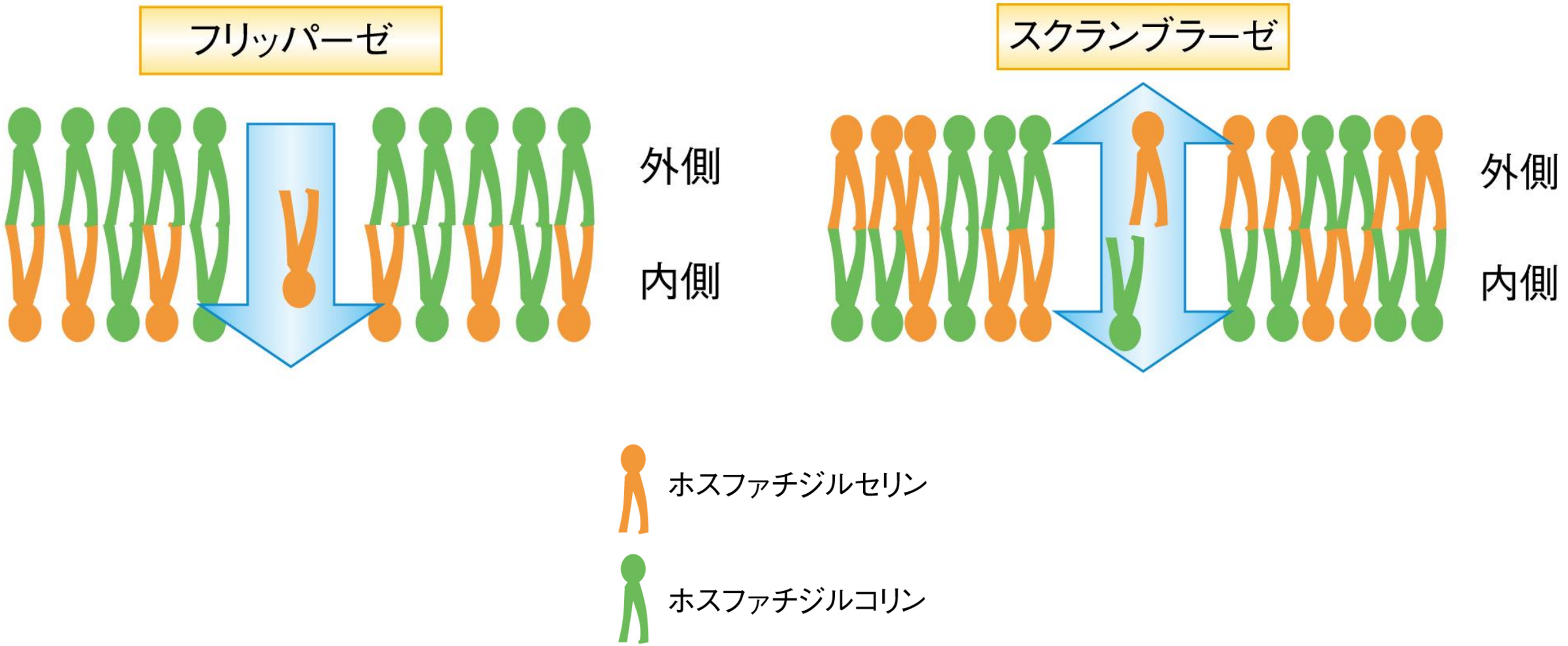

PtdSer
C1q, C3b
and/or C4
CR1, CR3
and/or CR4
CD300b
BAI1
TIM4
MER
or AXL
GLA
GAS6 or PROS1
SHBG
Tyrosine
kinase
MFGE8
DLD
RGD
αvβ3 or
αvβ5 integrin
CD47
SIRPα
Stabilin 2
Apoptotic cell
Macrophage

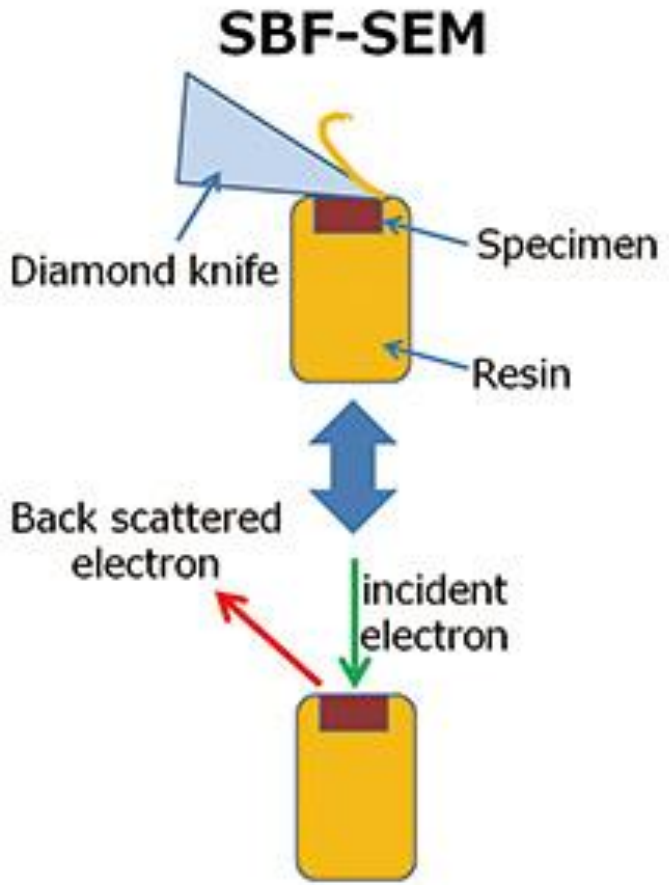

a
b
RPE cell
PR
Neuron
Axon
Localized scramblase
activation and
PtdSer exposure
Bouton
Microglia
Synapse

milk fat globule-EGF-factor 8

MFG-E8：milk fat globule-EGF-factor 8．アポトーシス細胞上のPSとαvβ3インテグリンを架橋するタンパク質．αvβ3インテグリンを発現する貪食細胞がPSを発現するアポトーシス細胞を貪食する際に機能する．本稿では，この変異タンパク質（D89E MFG-E8）で，PSには結合するものの，インテグリンには結合しないものをCD300aとPSの結合を阻害するために用いた．

実験医学増刊 Vol.34 No.7

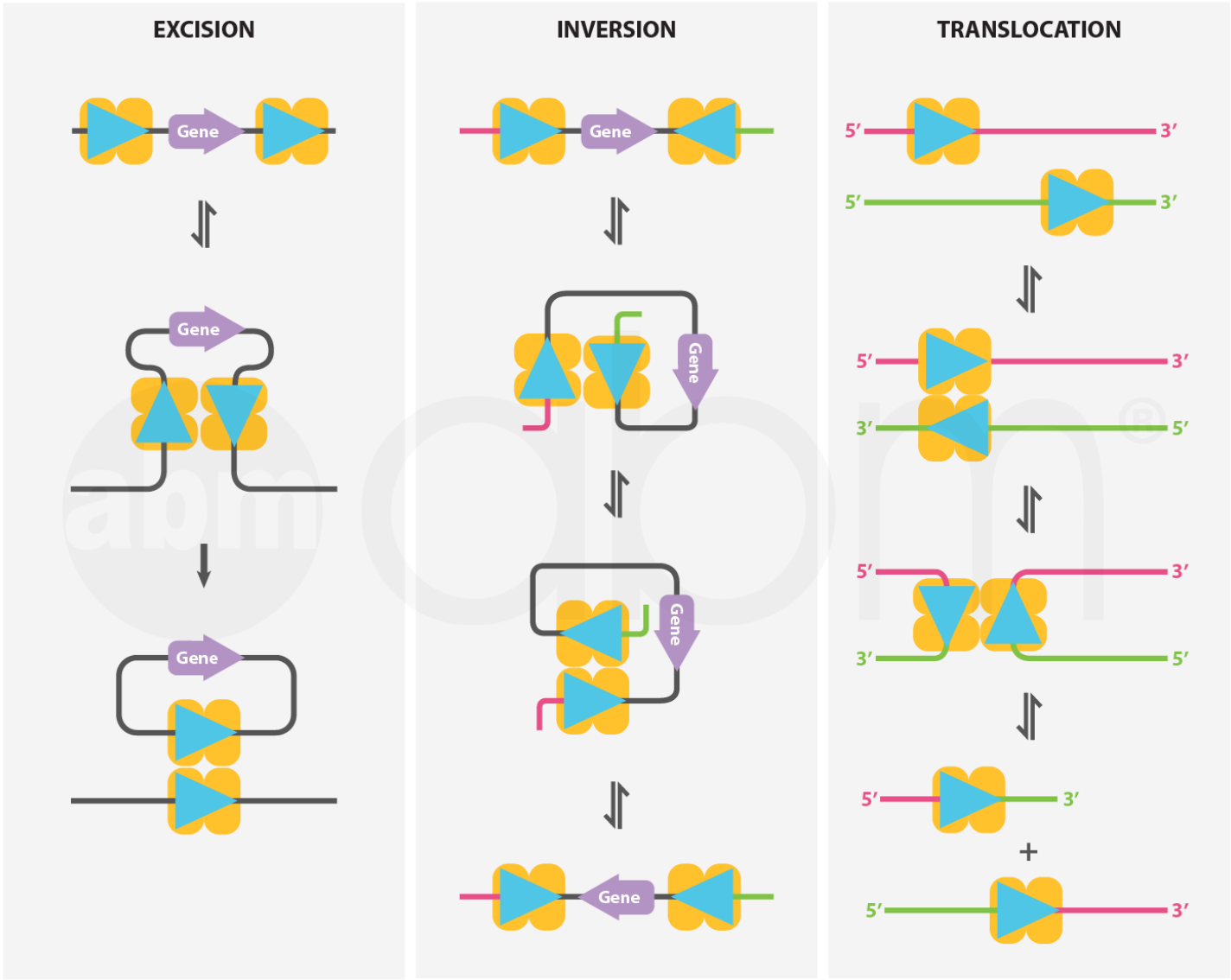

フリッパーゼ
外側
内側
スクランブラーゼ
外側
内側
ホスファチジルセリン
ホスファチジルコリン

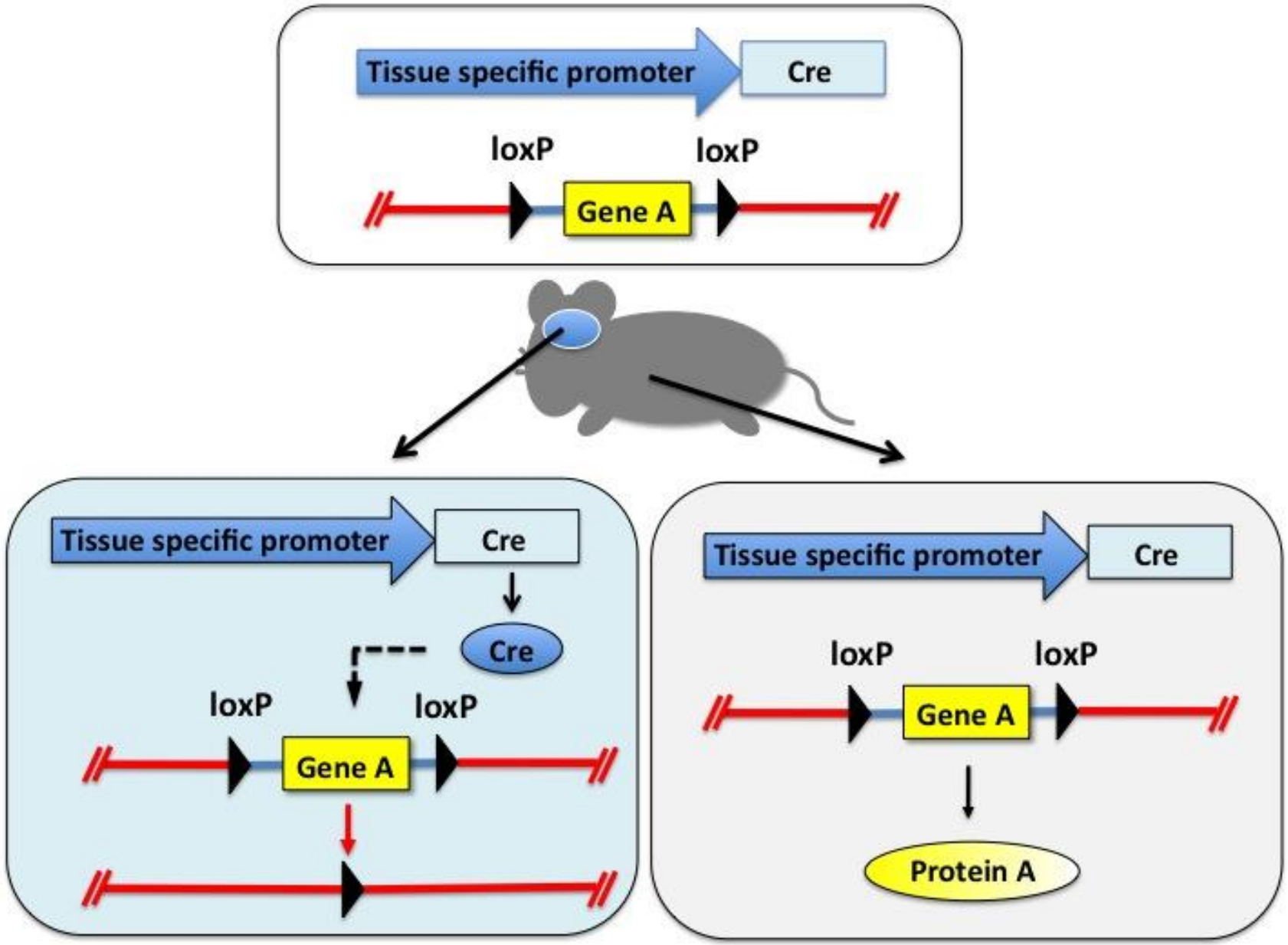


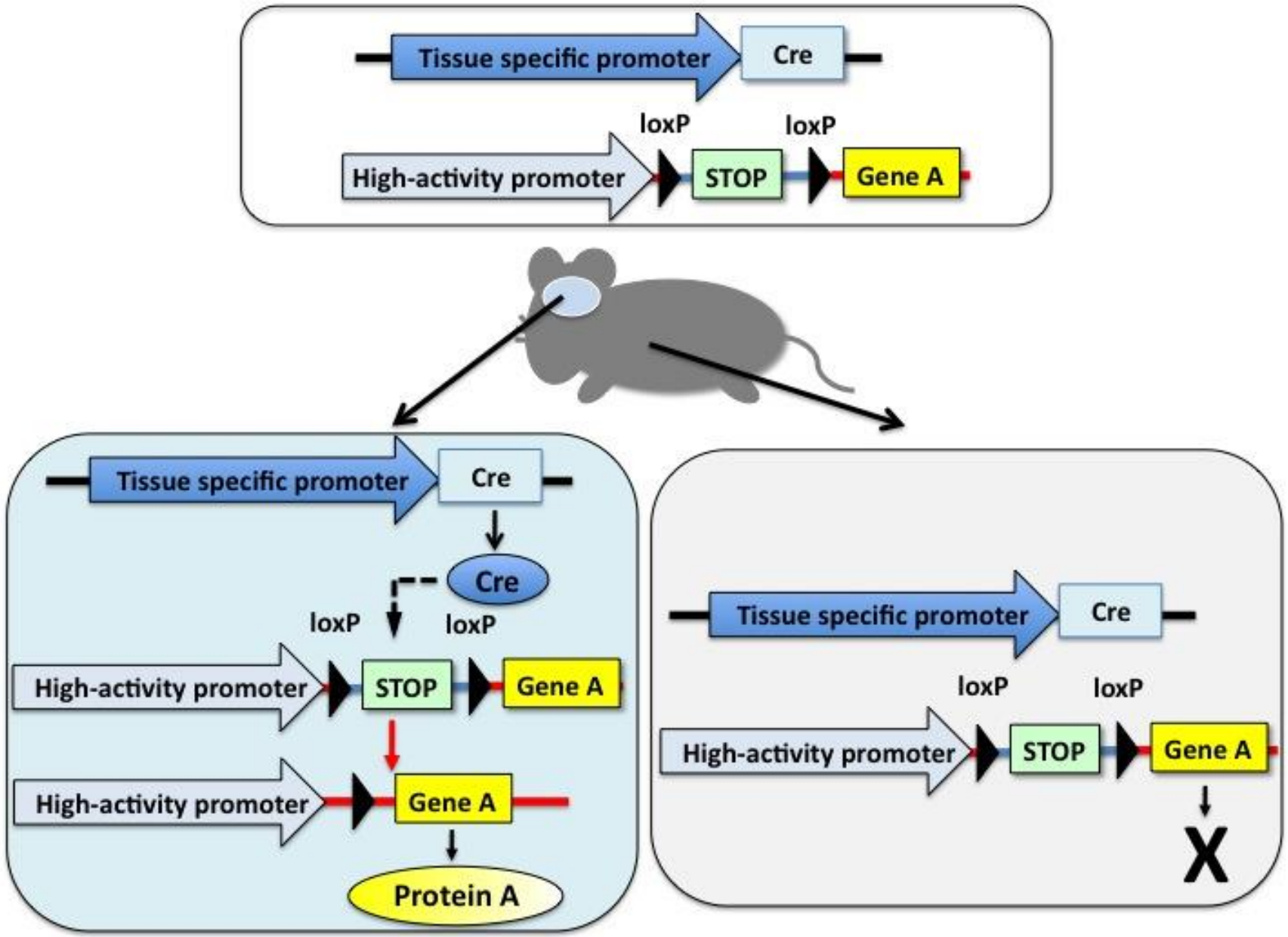
Serial block-face scanning electron microscopy

組み込み式ミクロトームによる表層切削
＋
SEMによる試料の断面観察

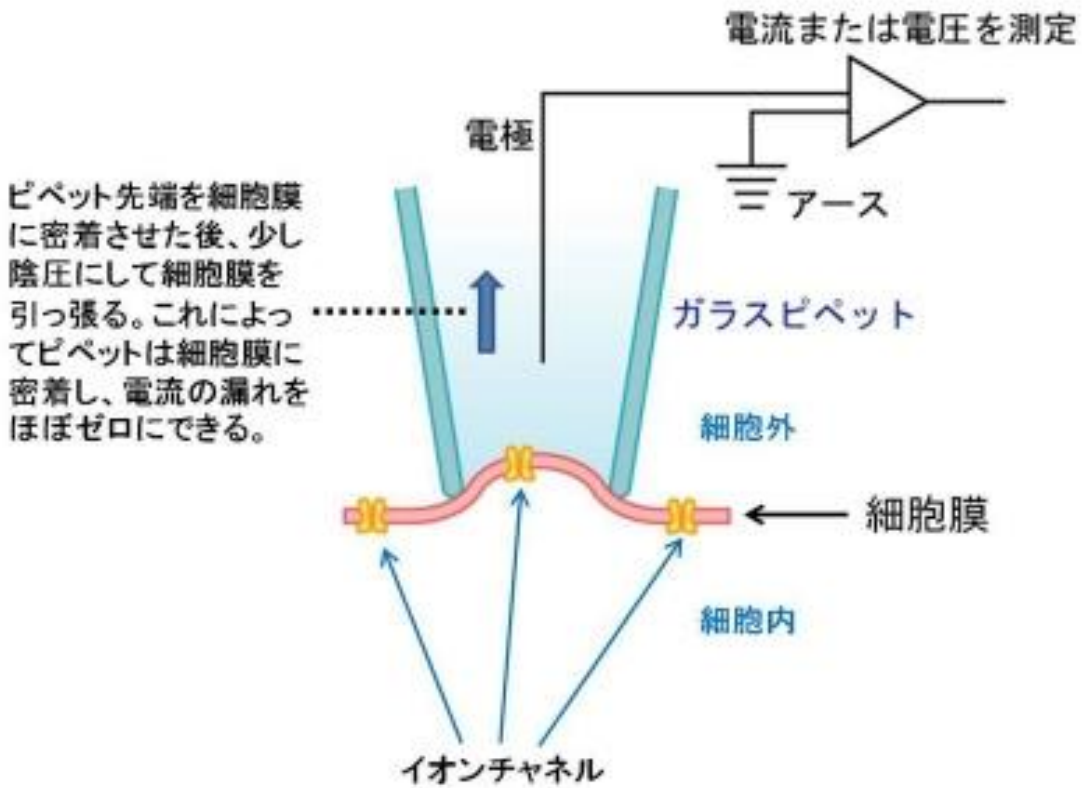
交互に反復
↓
数百mm^2以上に及ぶ比較的広範囲の領域から
透過型電子顕微鏡による連続切片観察に類似した
画像を、数nm程度の解像度で迅速に取得できる

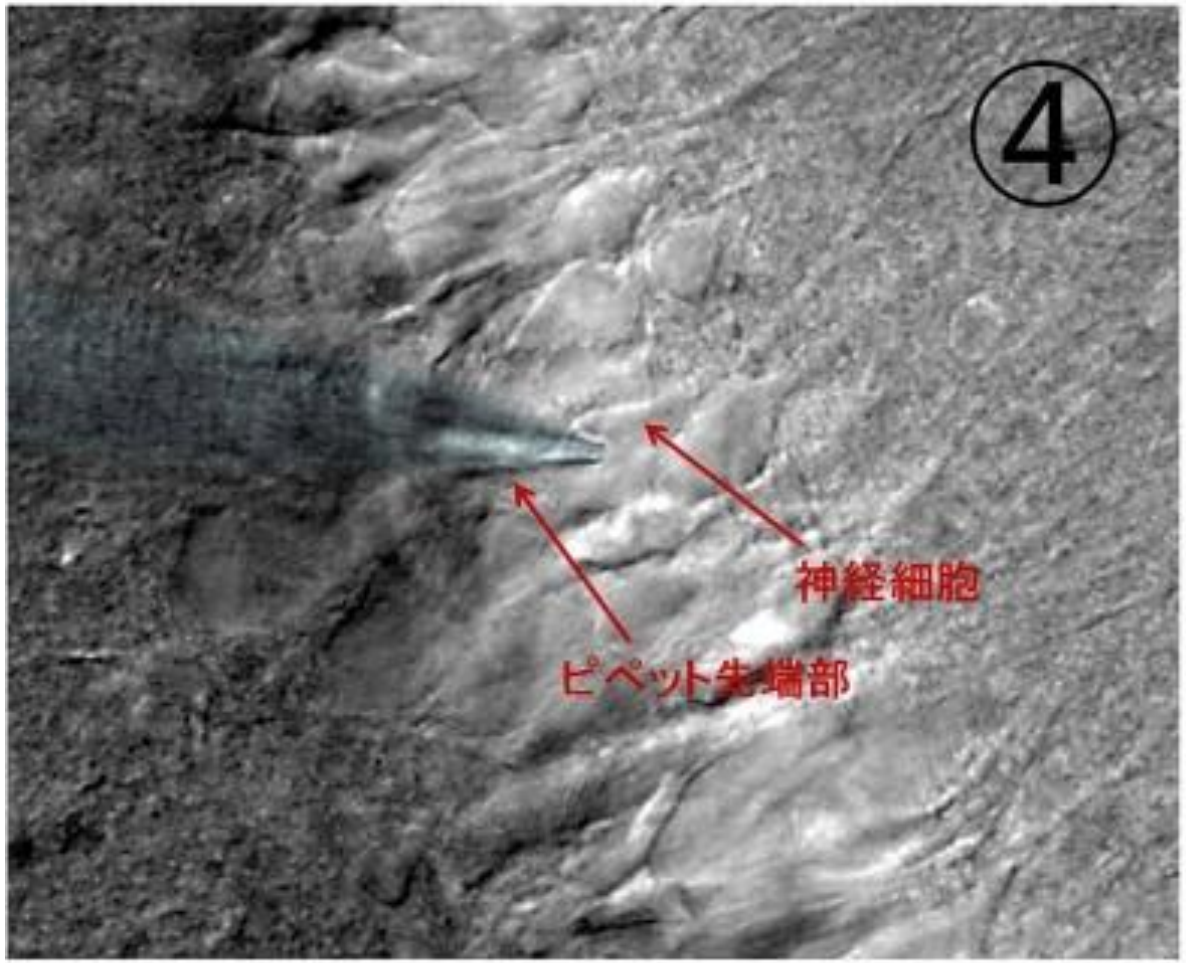

EXCISION
INVERSION
TRANSLOCATION
Gene
5′
3′
= Lox
= Cre


Tissue specific promoter
Cre
loxP
loxP
Gene A
Tissue specific promoter
Cre
Cre
loxP
loxP
Gene A
Tissue specific promoter
Cre
loxP
loxP
Gene A
Protein A


Tissue specific promoter
Cre
loxP
loxP
High-activity promoter
STOP
Gene A
Tissue specific promoter
Cre
Cre
loxP
loxP
High-activity promoter
STOP
Gene A
High-activity promoter
Gene A
Protein A
Tissue specific promoter
Cre
loxP
loxP
High-activity promoter
STOP
Gene A
X

パッチクランプ法 ③





Kathrin Bonni, Dimitrios Psyrakis & Sodikdjon A. Kodirov (2008)
From wikipedia

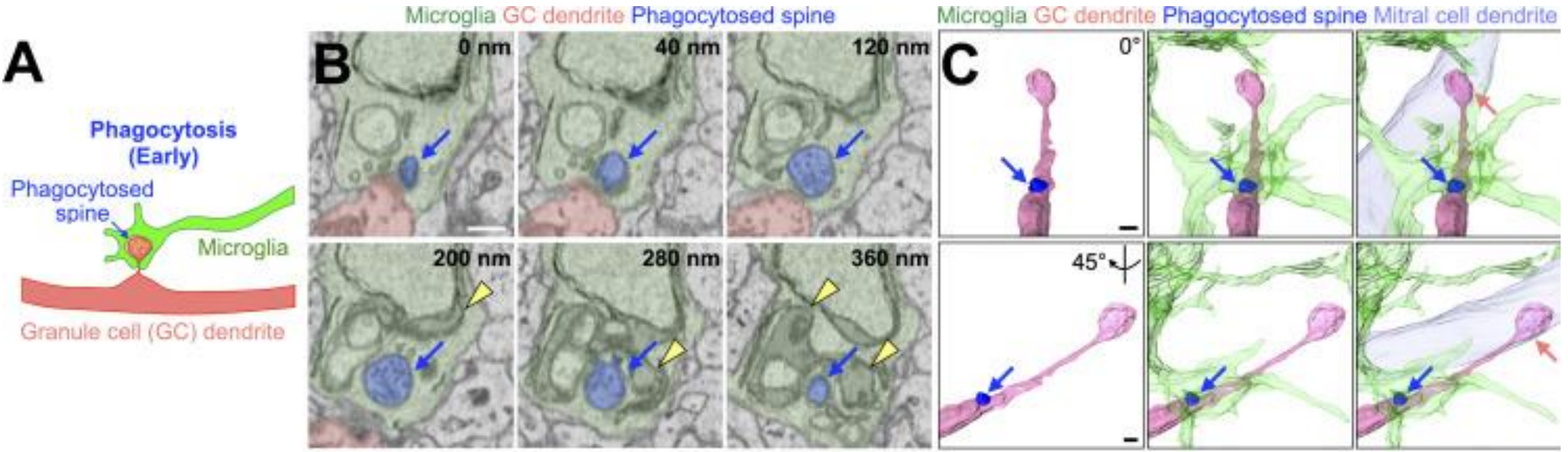
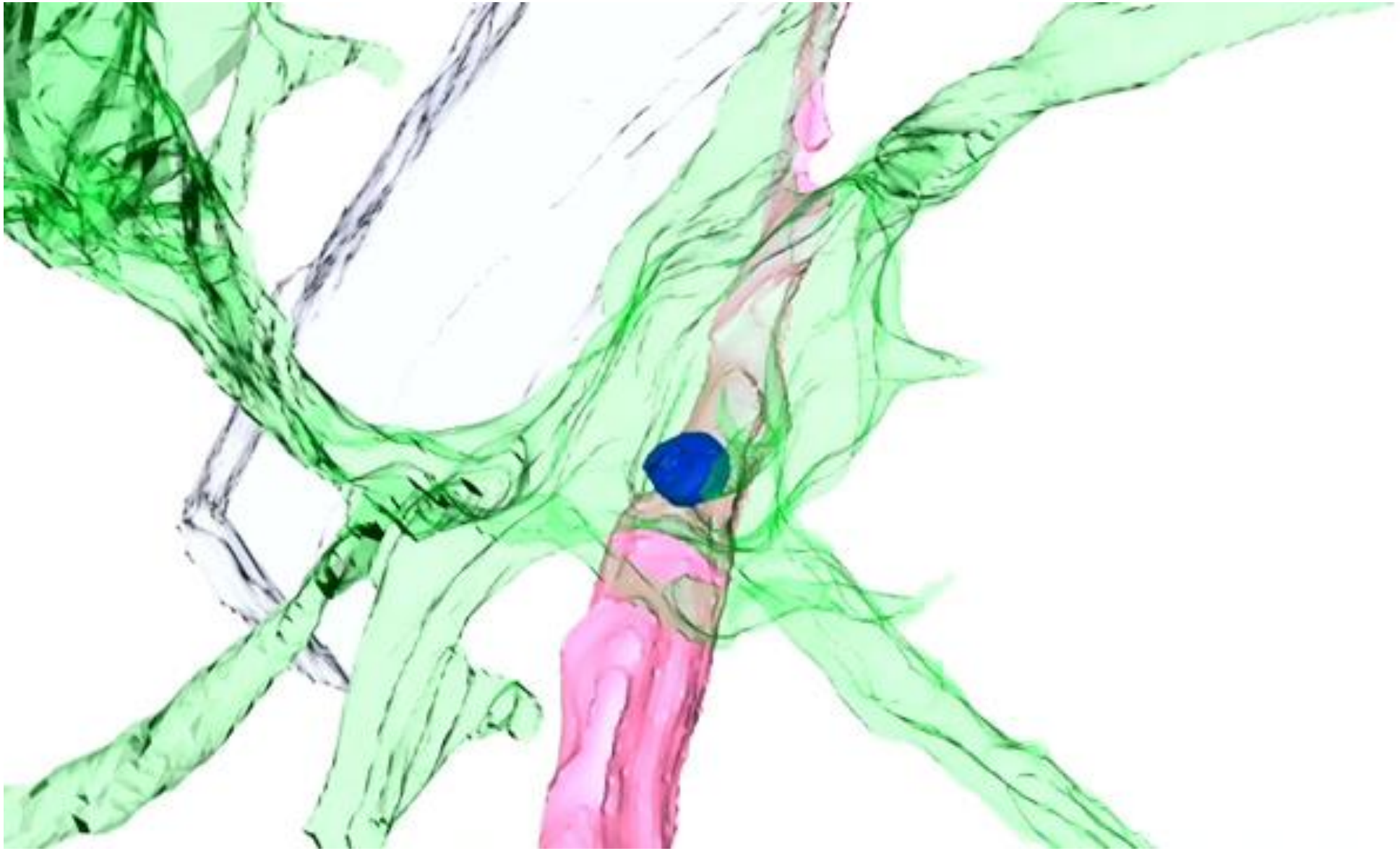
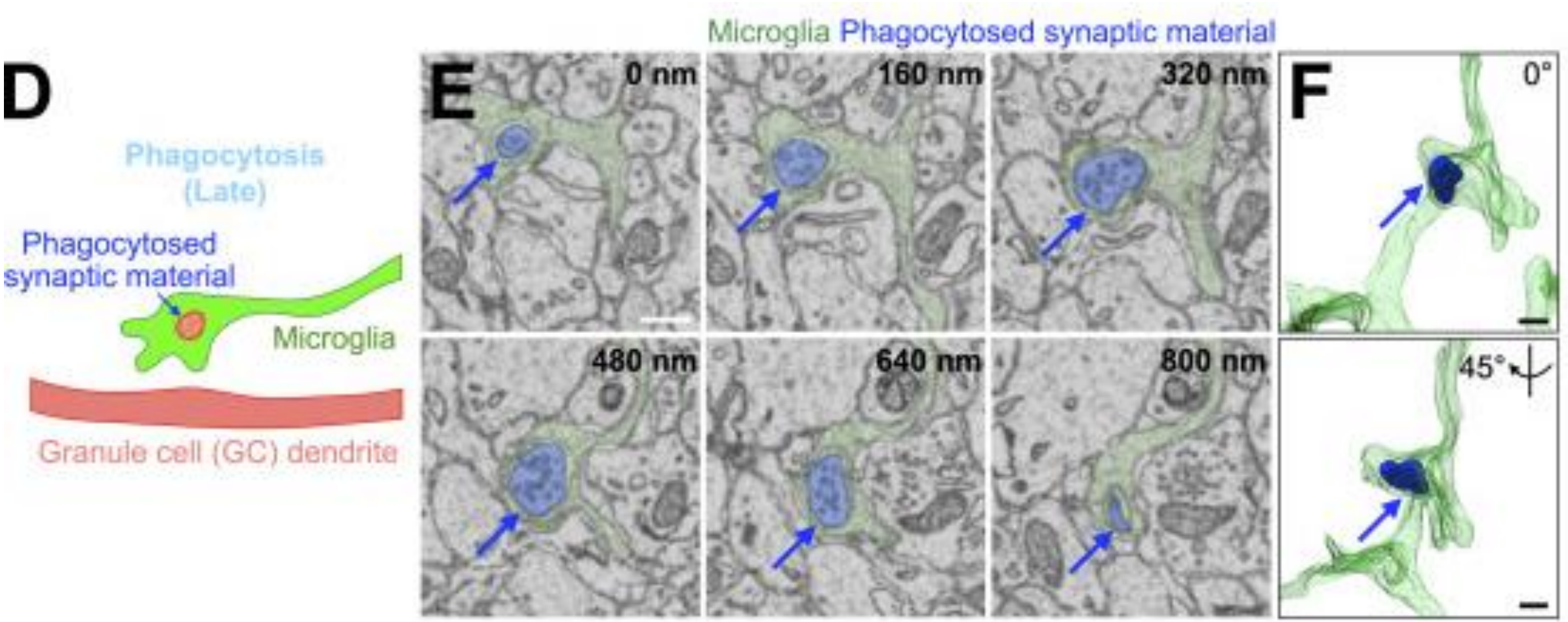


Figure 1. Microglia phagocytose a subset of granule cell spines in the adult OB.



Video 1.



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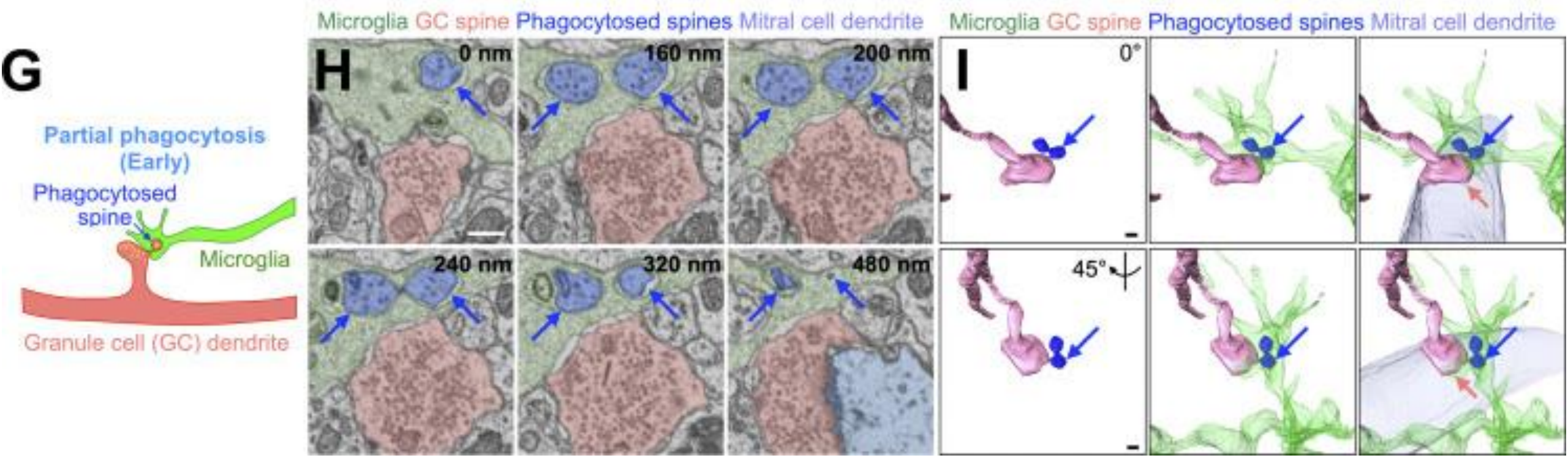

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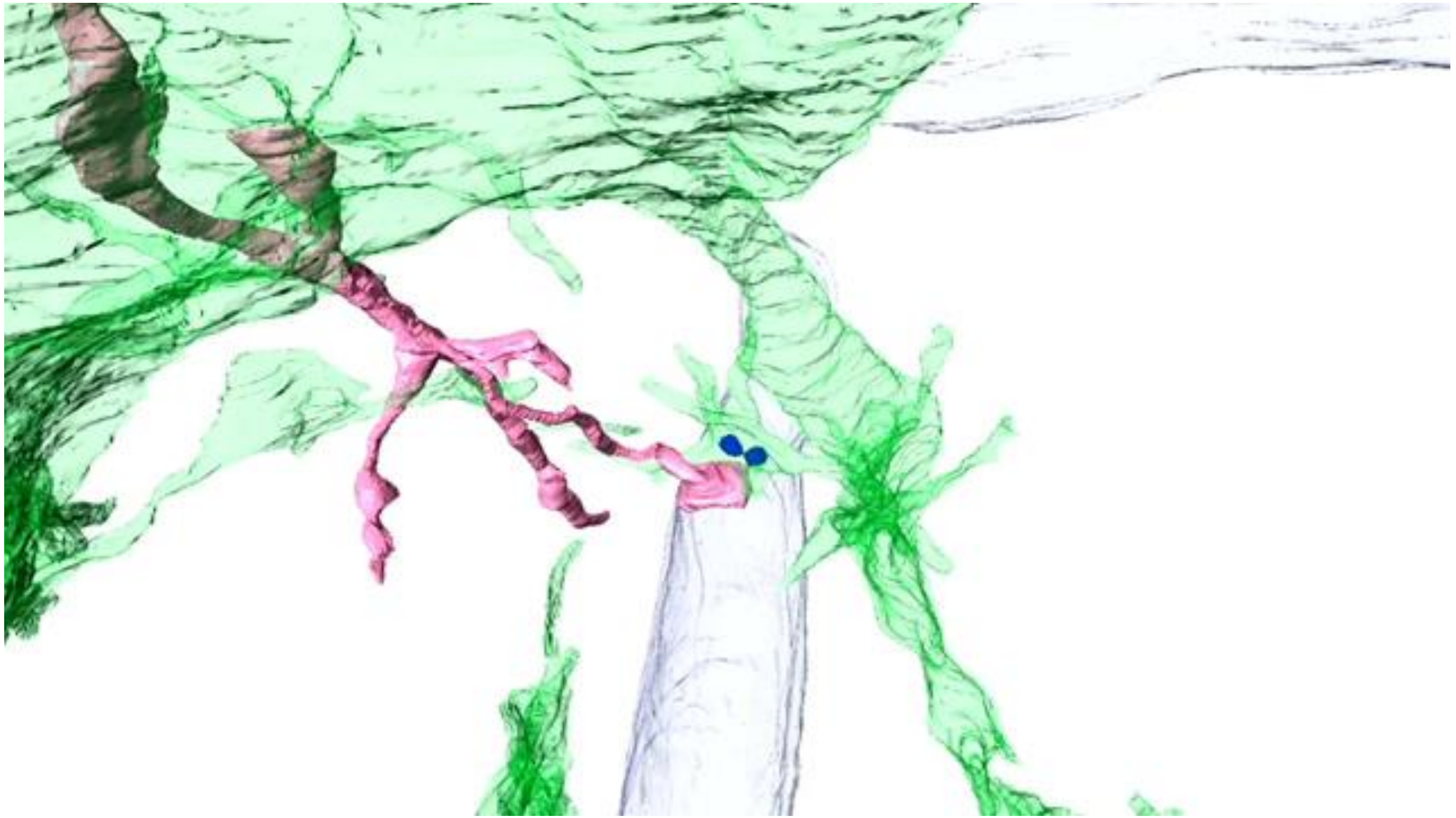
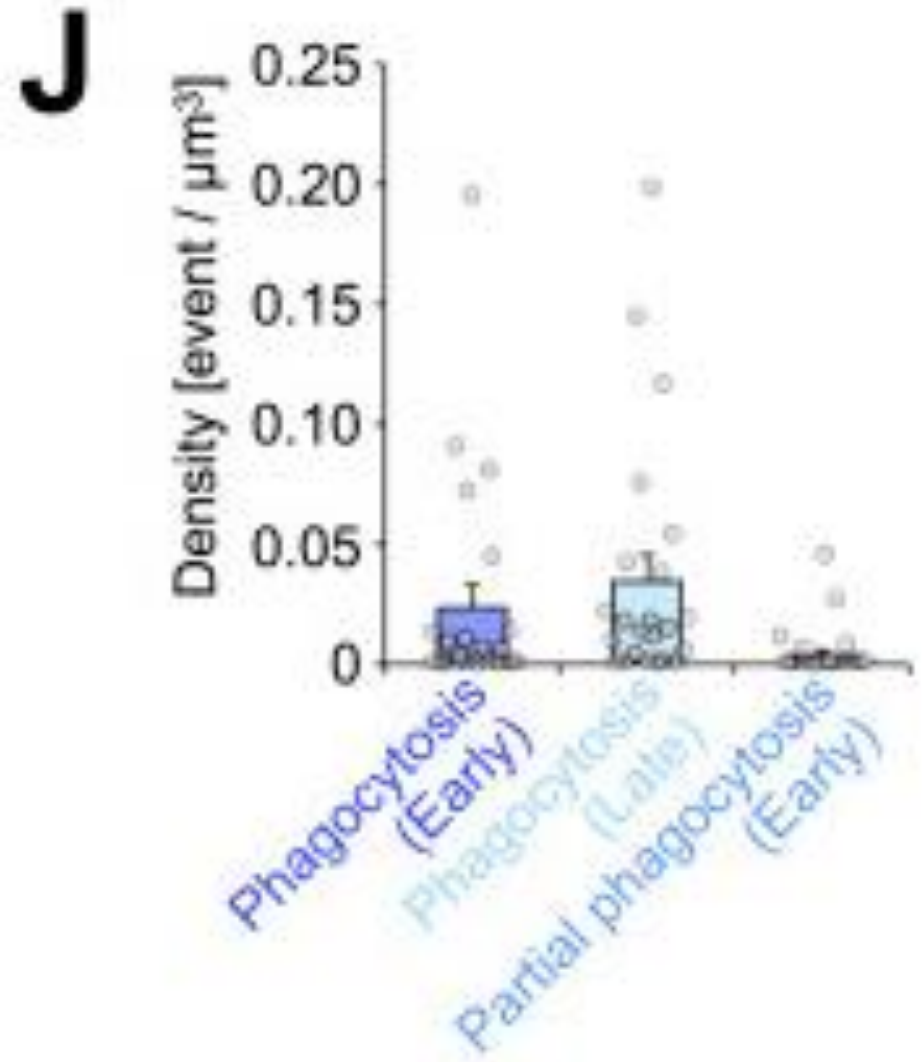


Video 2.



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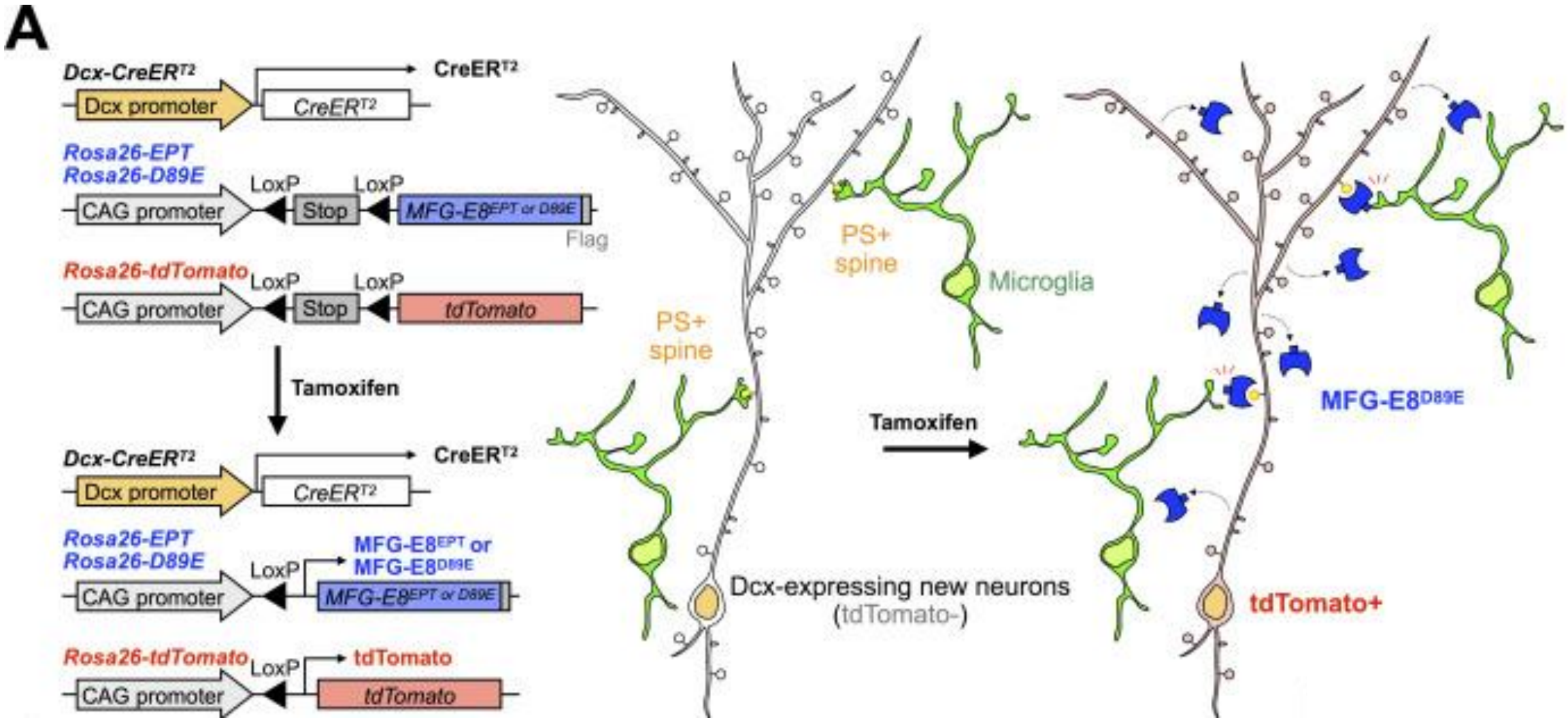
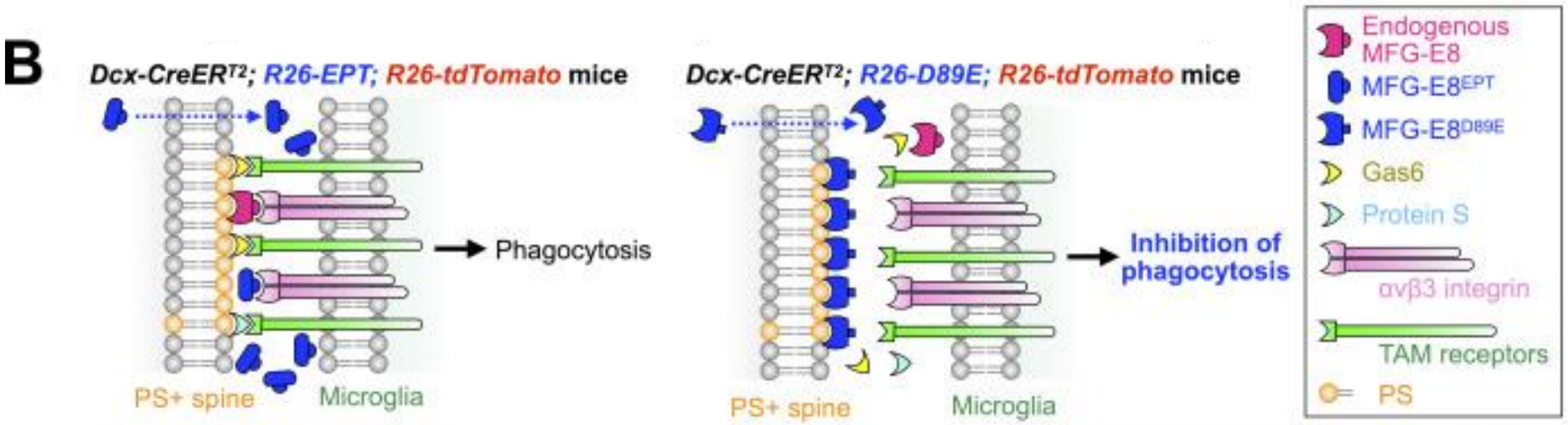


Figure 2. MFG-E8D89E masks PS exposed at spines in vivo.



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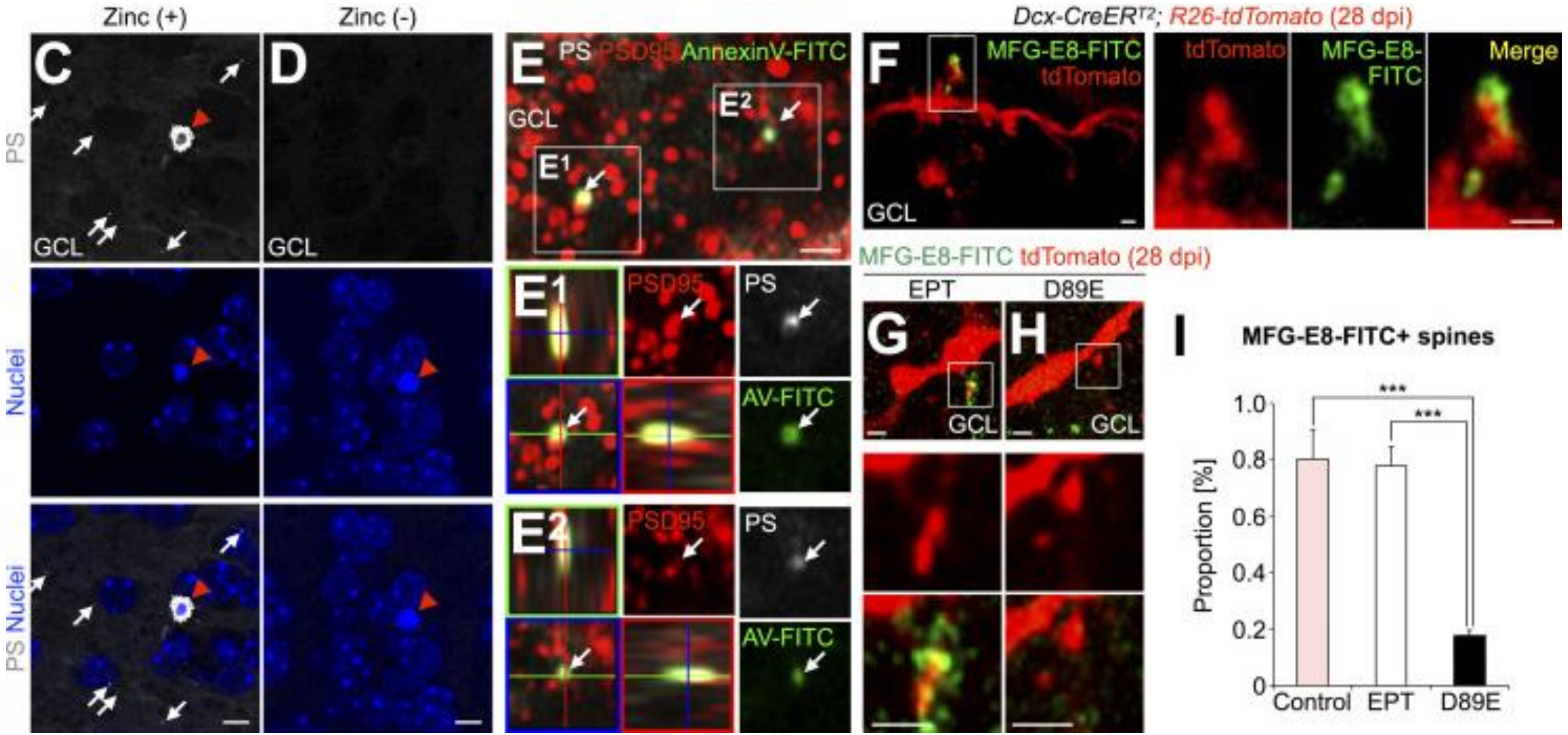

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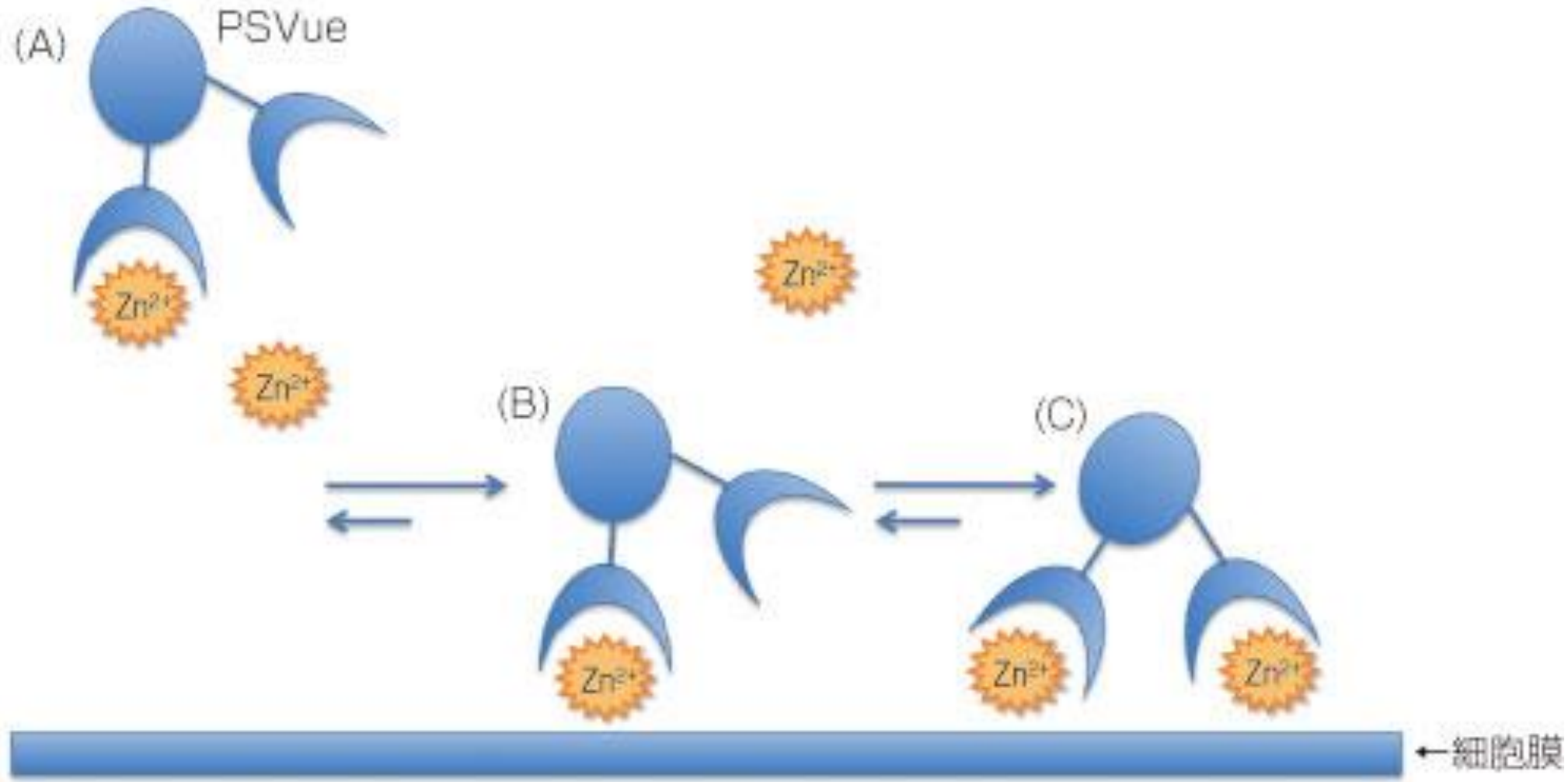

(A)
PSVue
Zn^{2+}
Zn^{2+}
Zn^{2+}
(B)
Zn^{2+}
(C)
Zn^{2+}
Zn^{2+}
←細胞膜

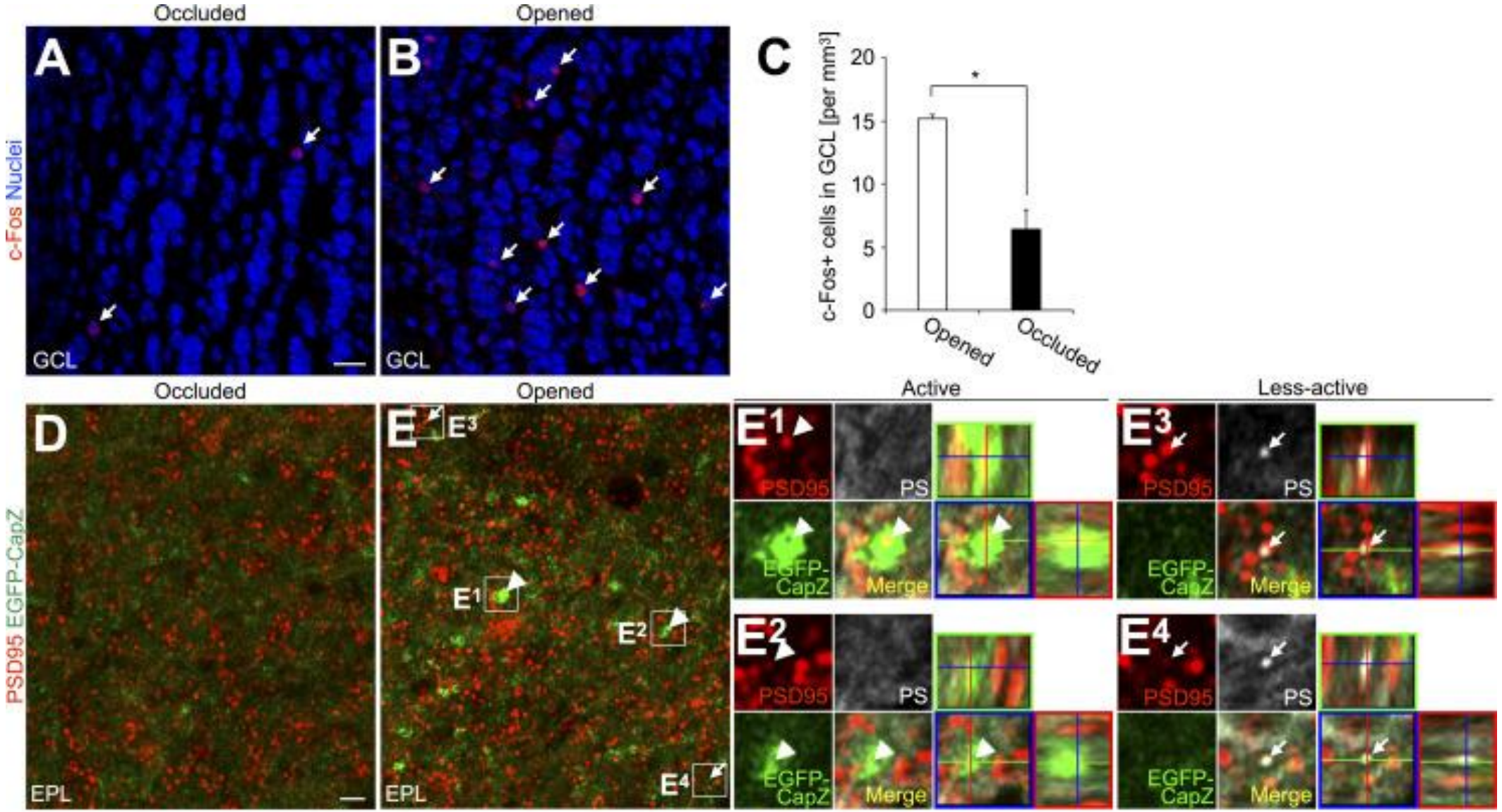
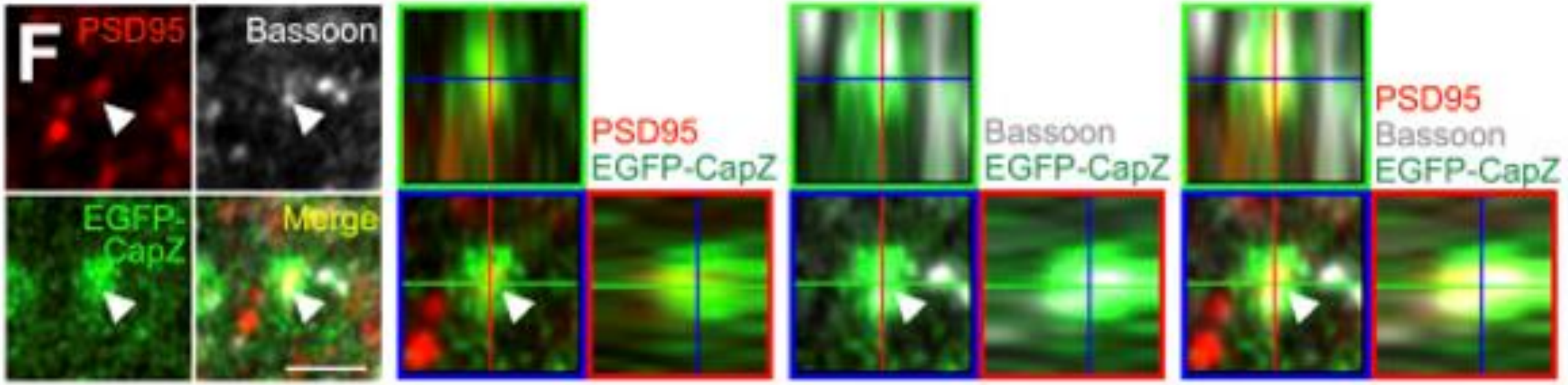


Figure 3. PS exposure on less-active spines, and its suppression by olfactory inputs in the adult OB.



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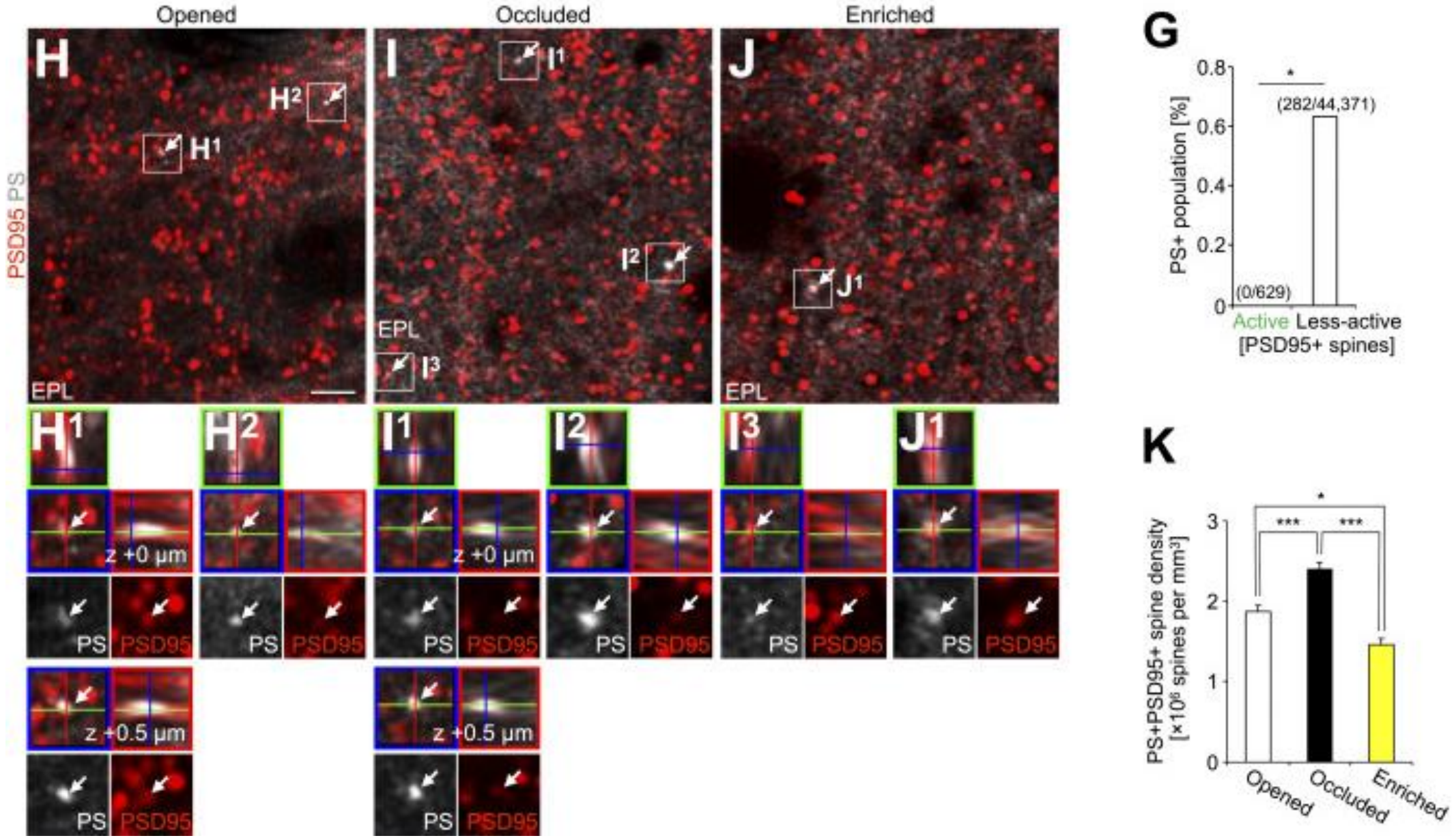

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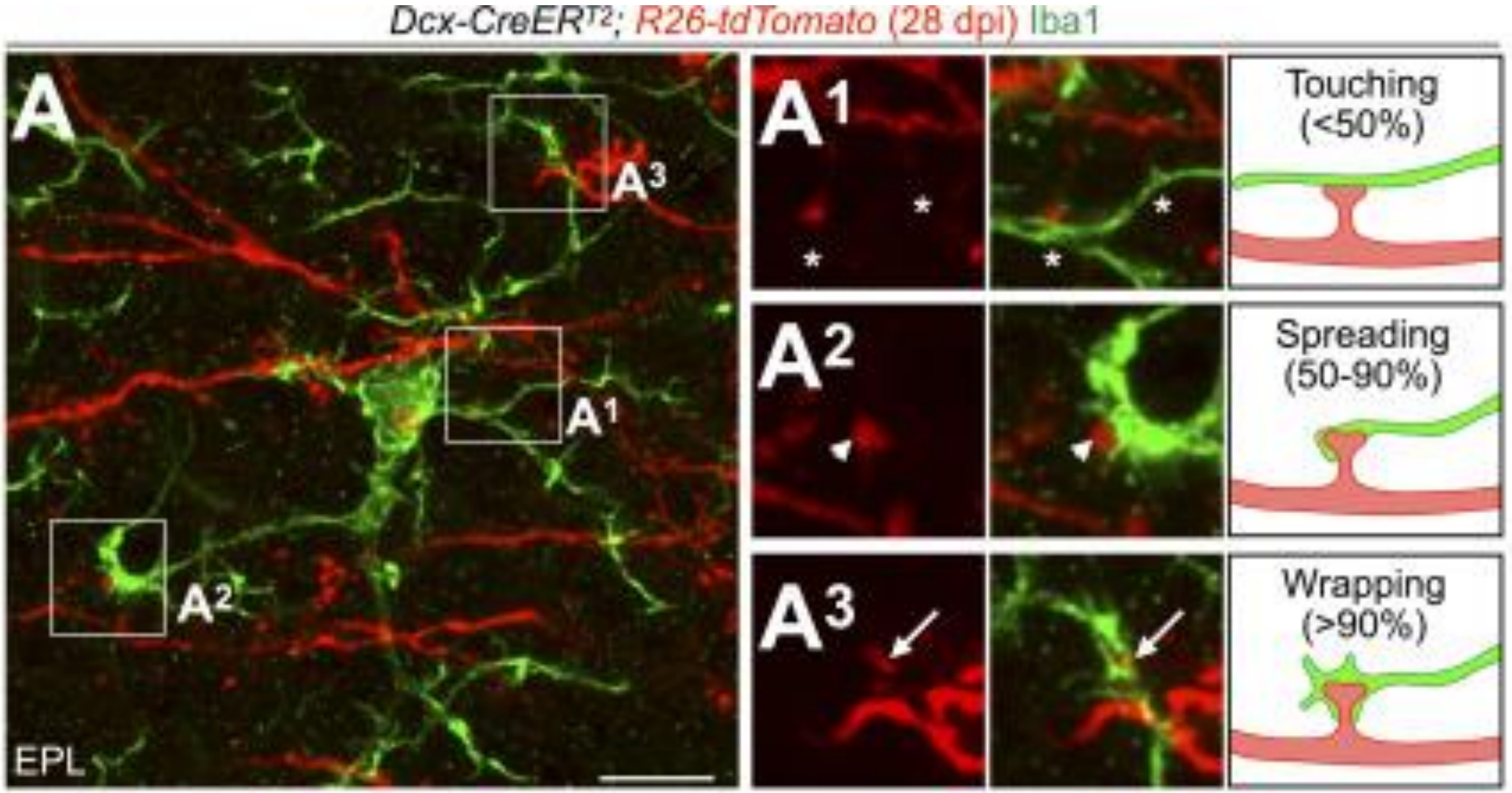
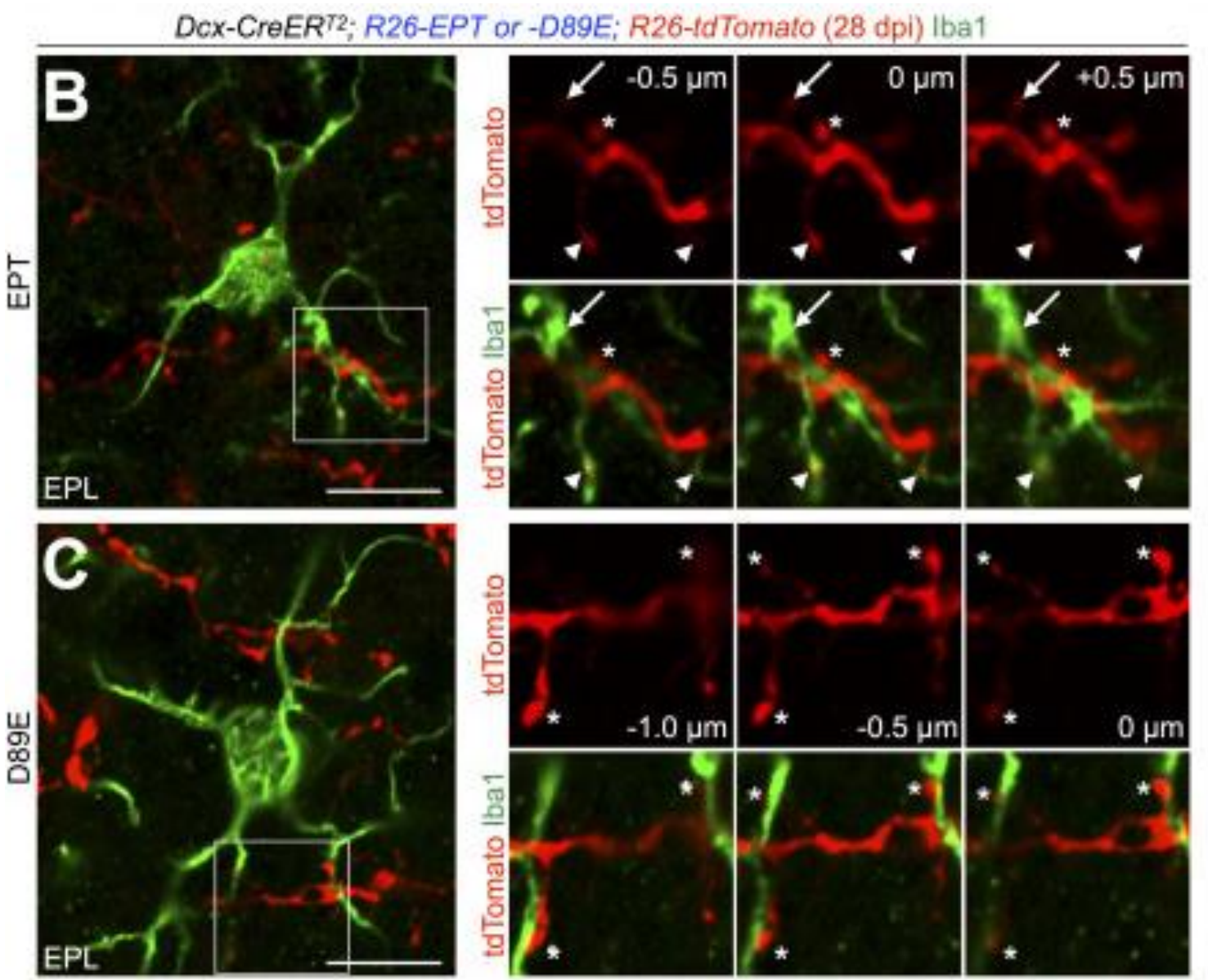


Figure 4. PS is involved in microglial phagocytosis of spines of adult-born neurons in the OB.



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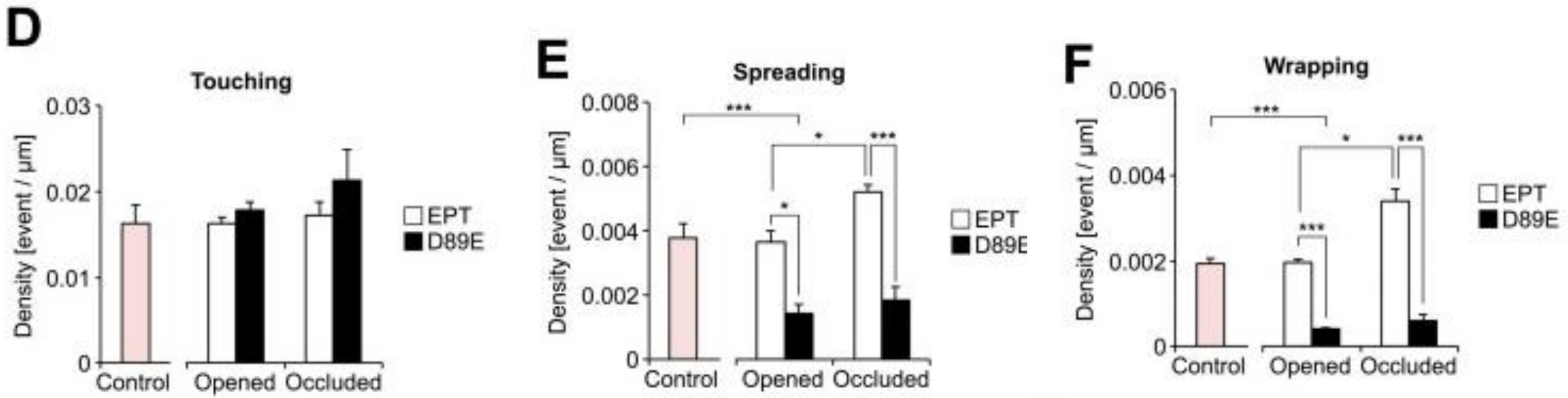

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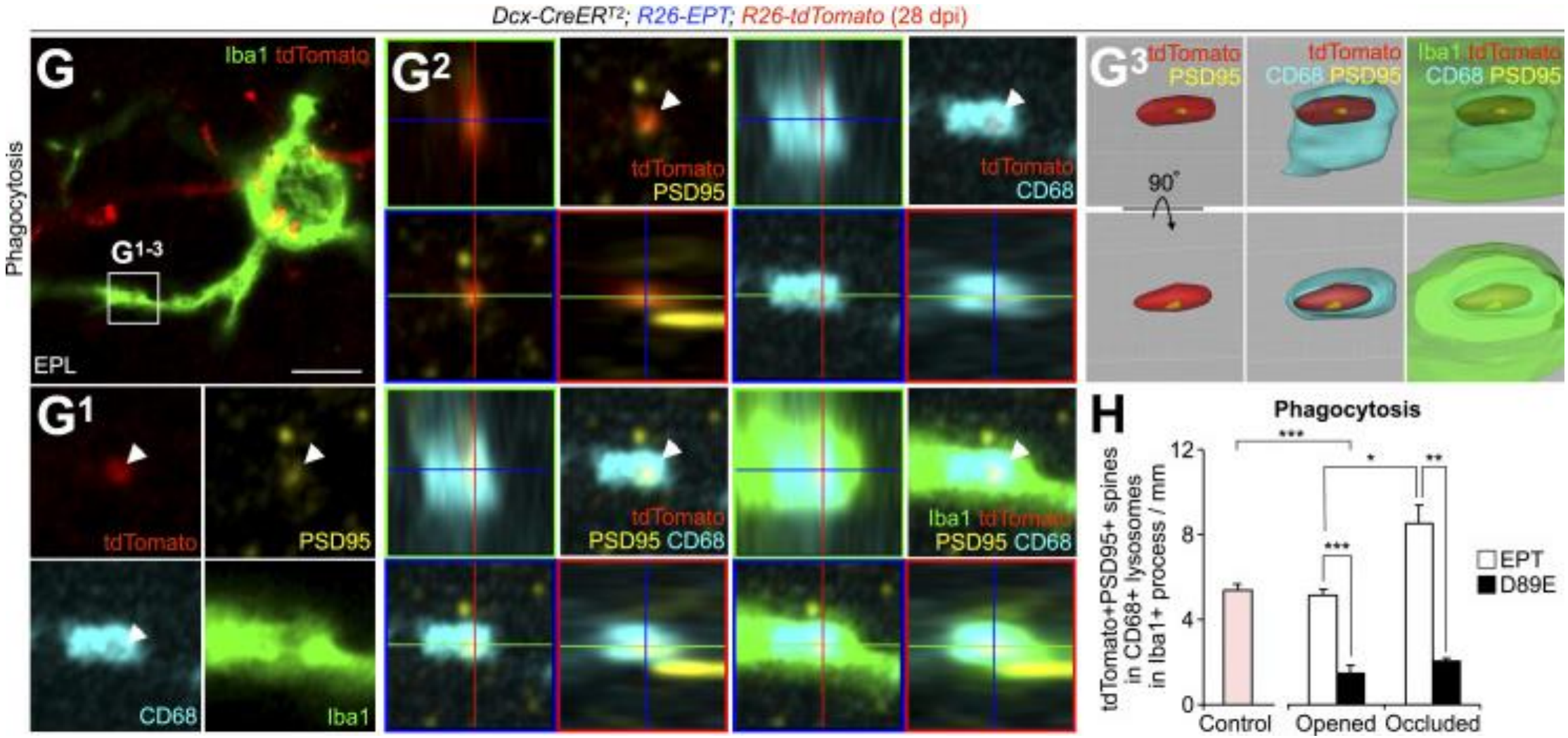
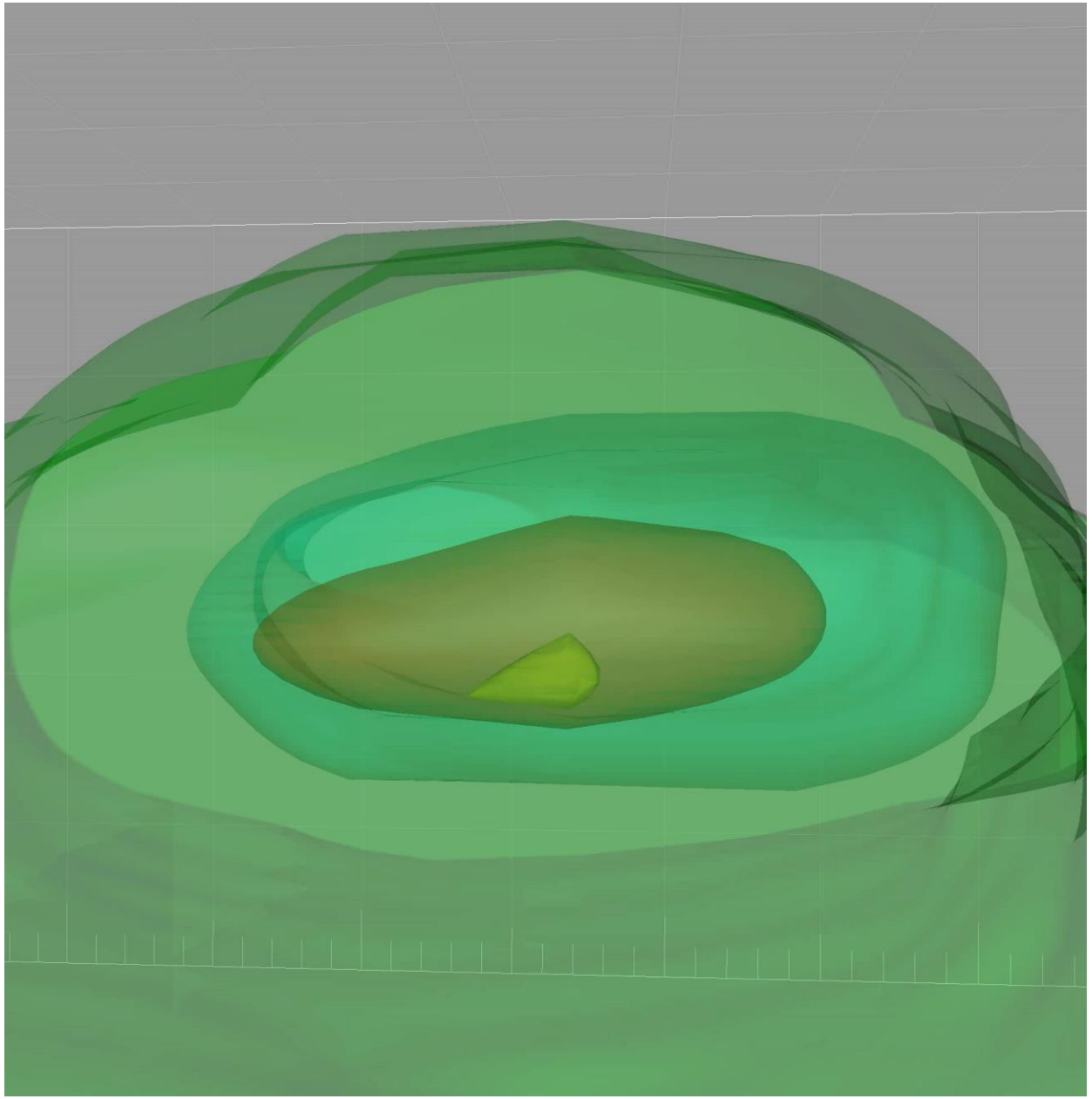


Figure 4. PS is involved in microglial phagocytosis of spines of adult-born neurons in the OB.



Video 3.

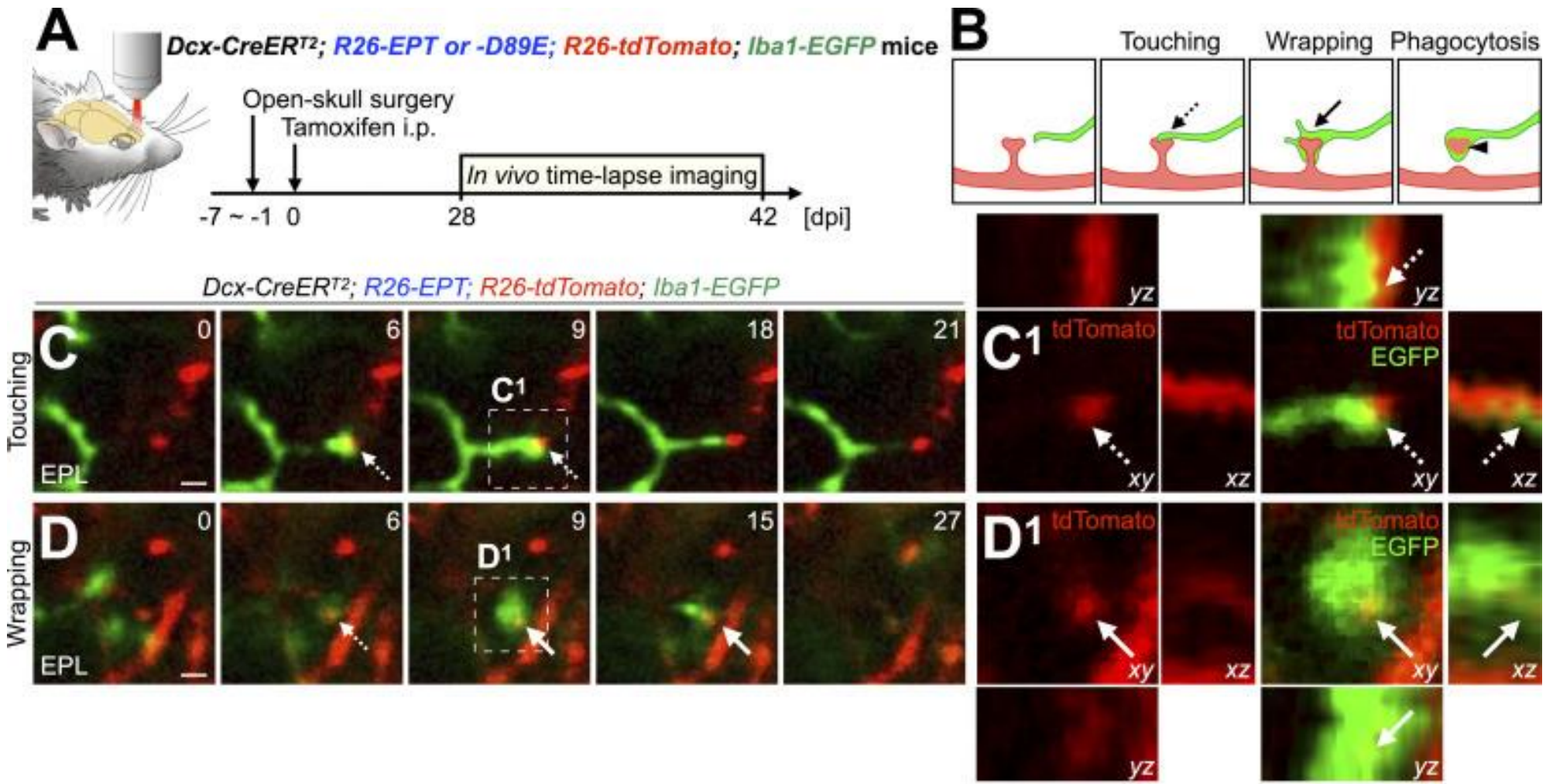
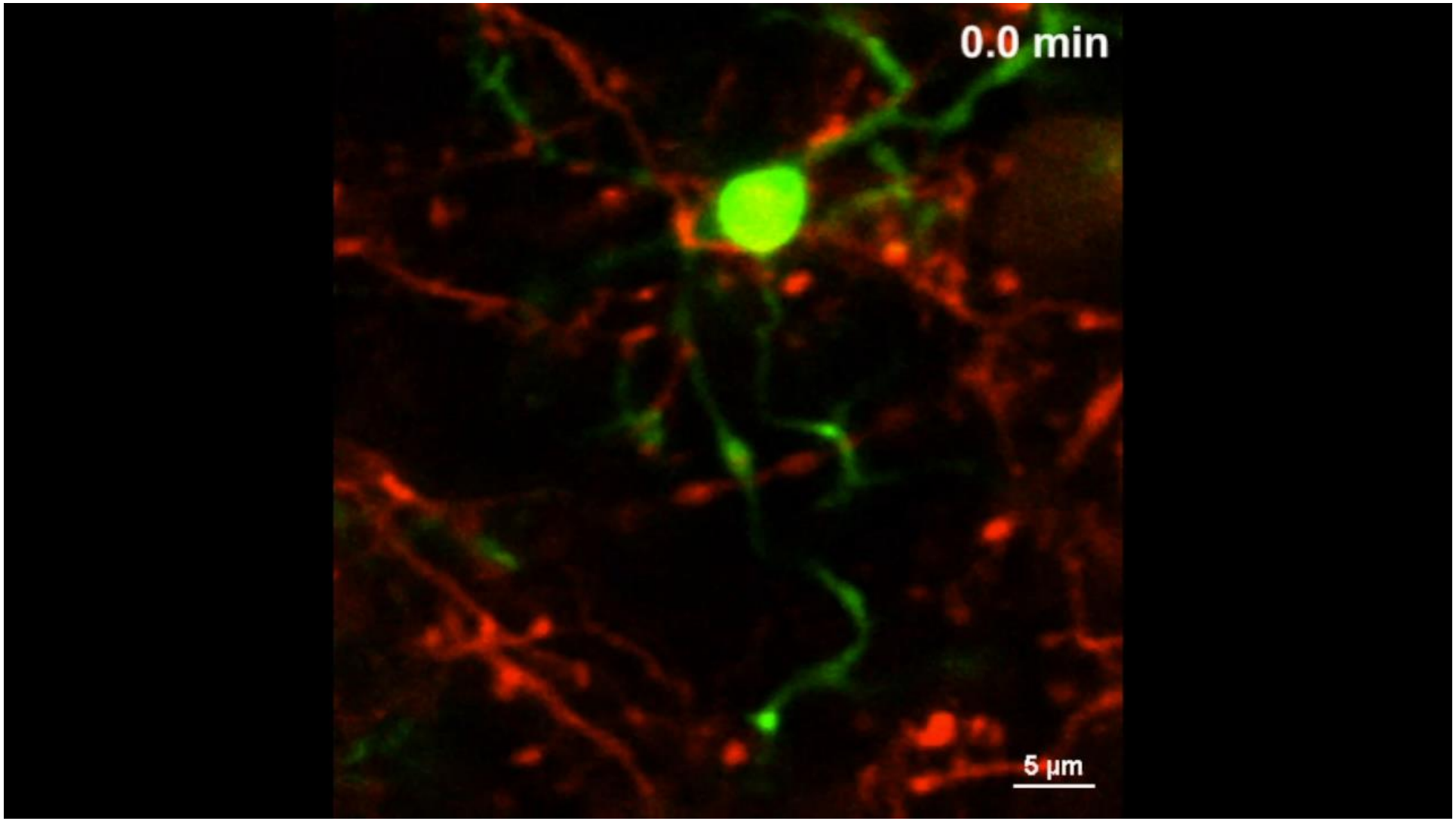
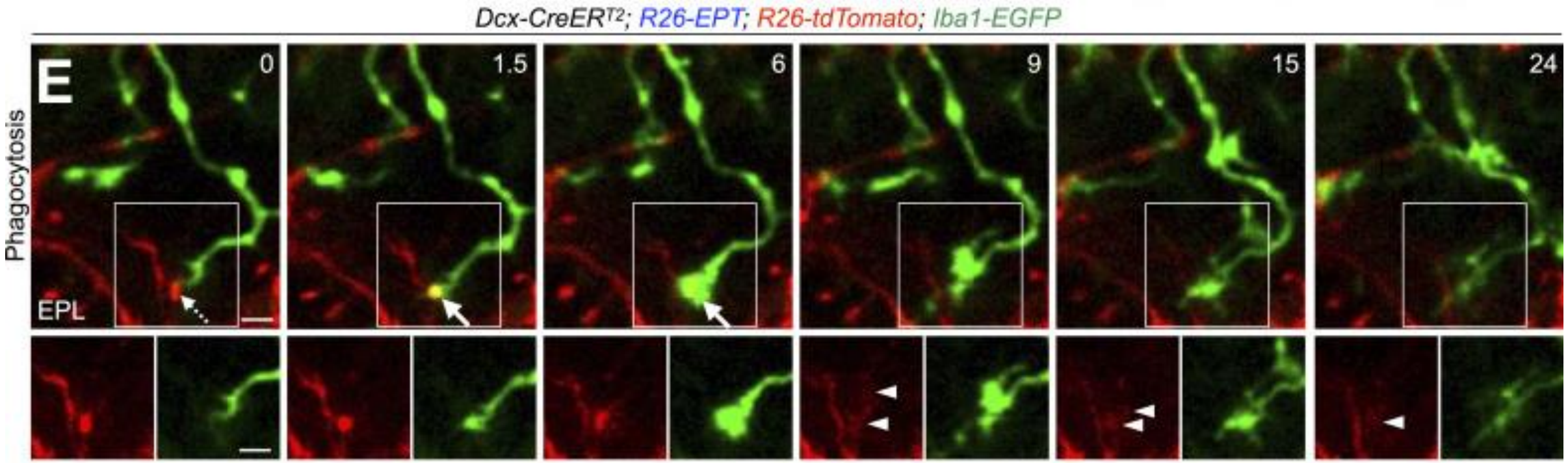


Figure 5.
PS is involved in the microglial membrane extension along targeted spines to proceed phagocytosis in adult-born neurons in the OB.



Video 4.



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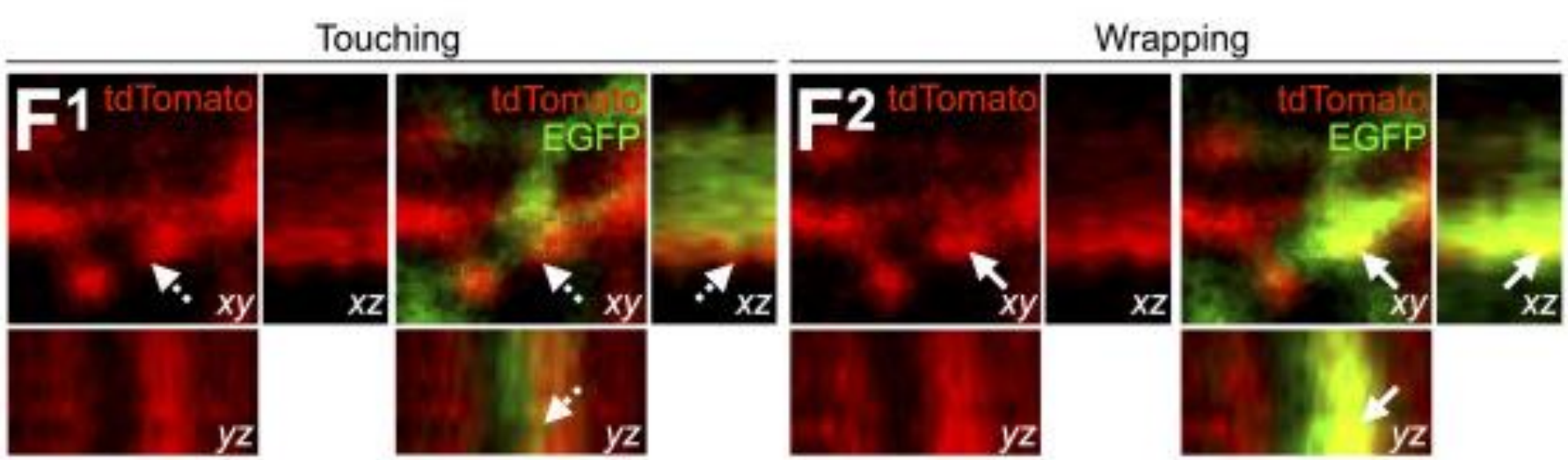

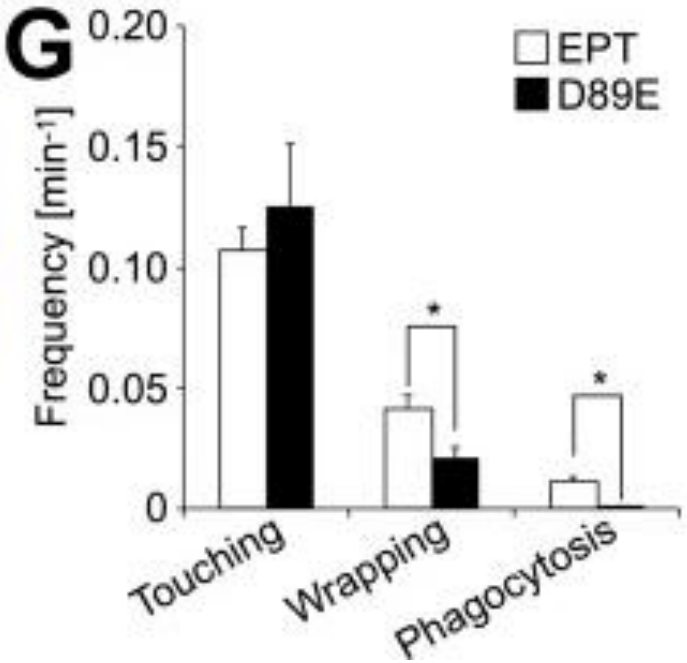

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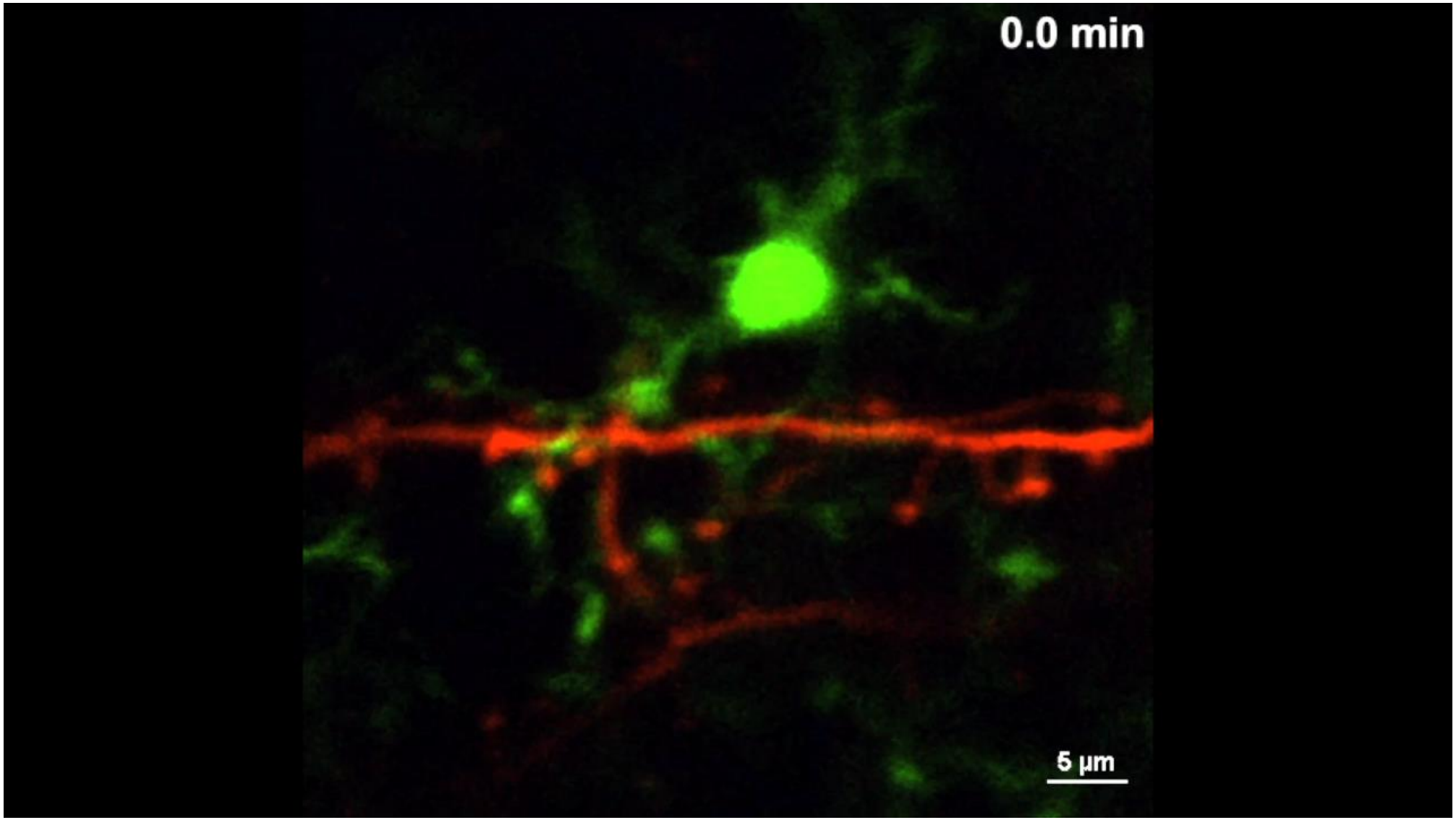
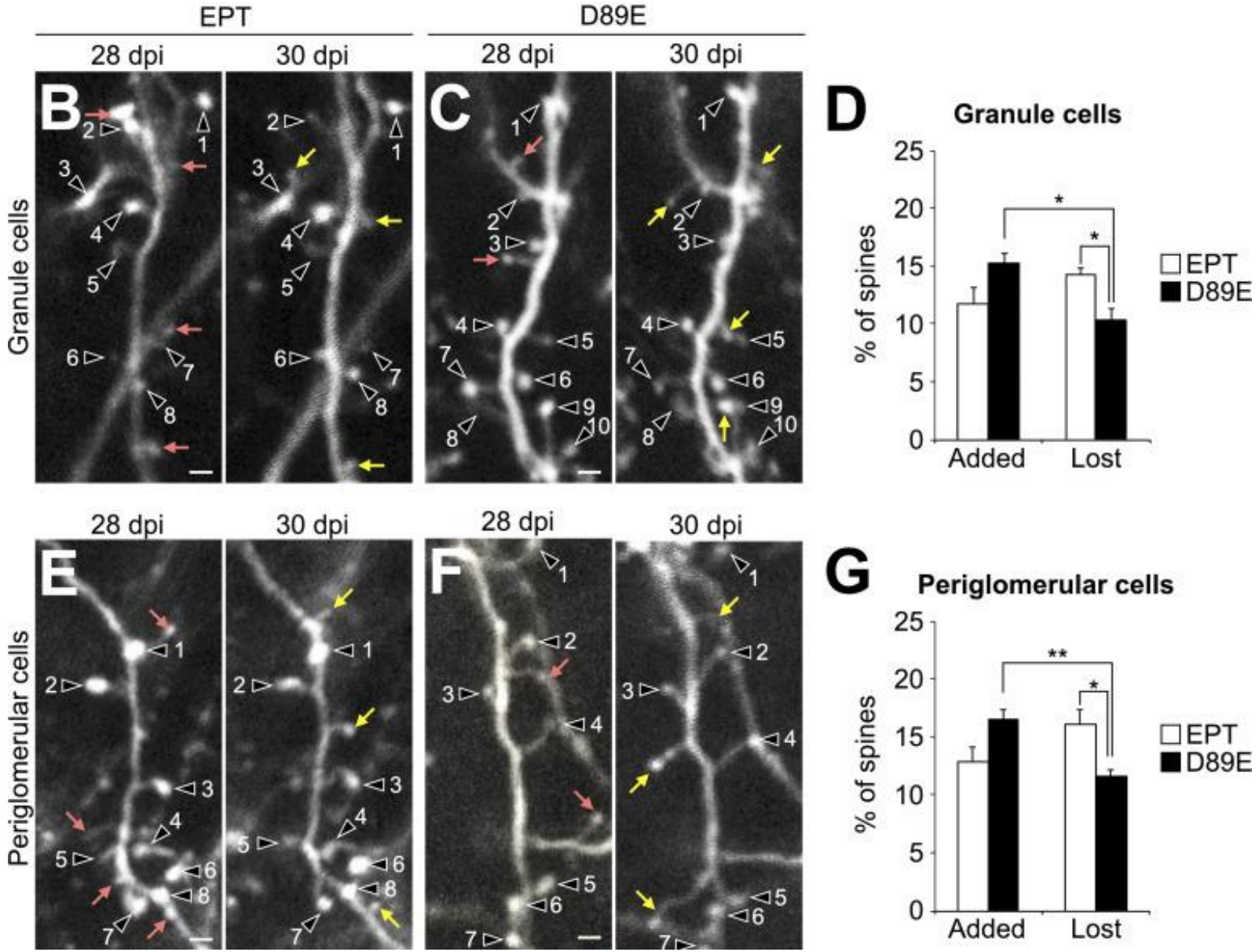


Video 5.

Figure 6. PS is involved in the spine pruning of adult-born neurons in the OB.



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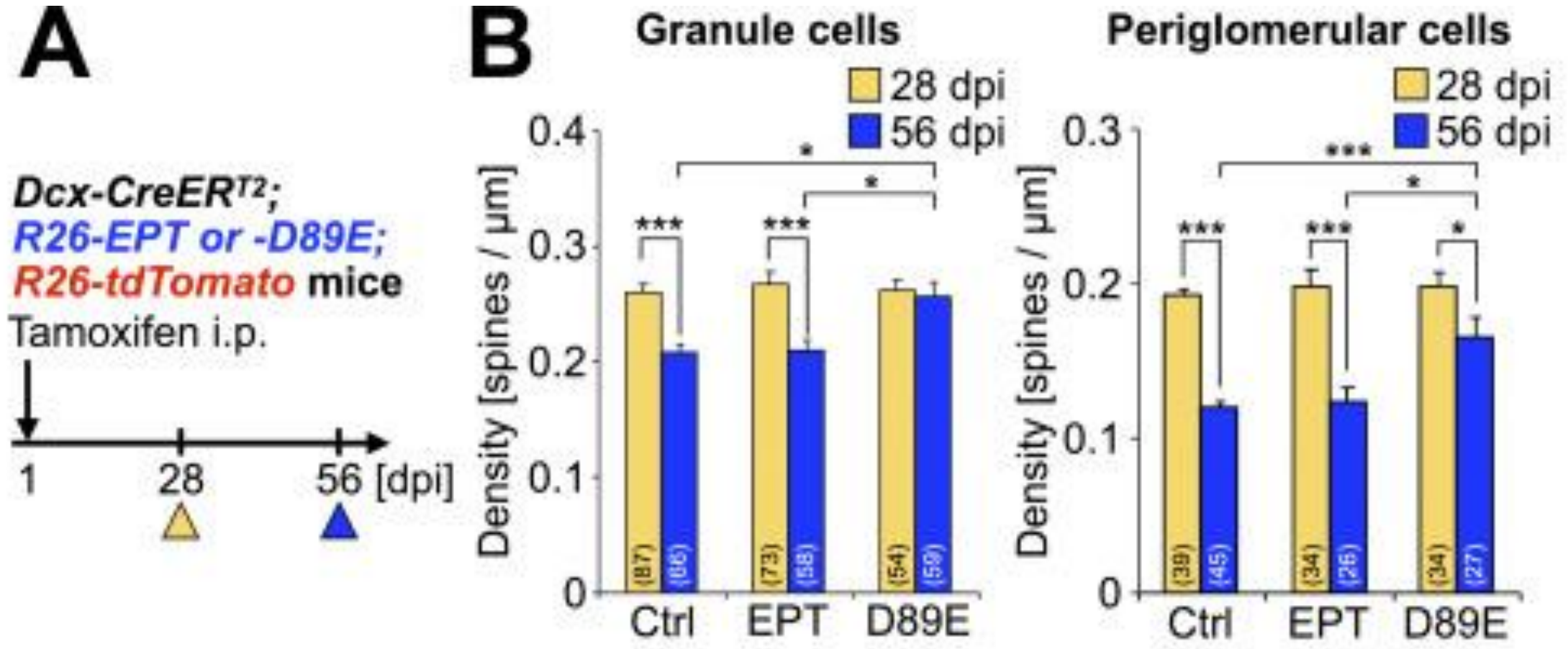
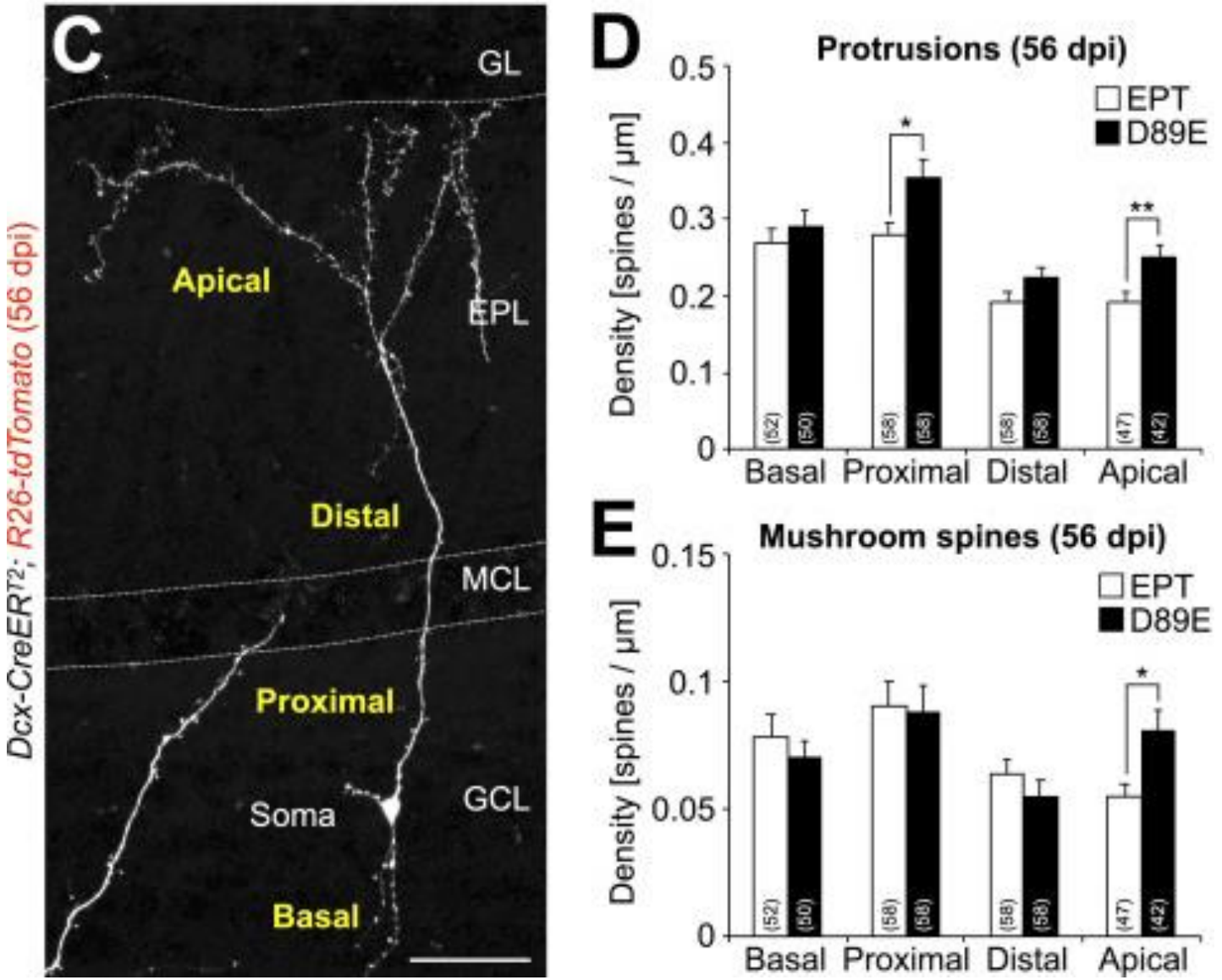


Figure 7.
Inhibition of PS-dependent spine pruning by MFG-E8D89E increases the synaptic density in adult-born mature neurons in the OB.



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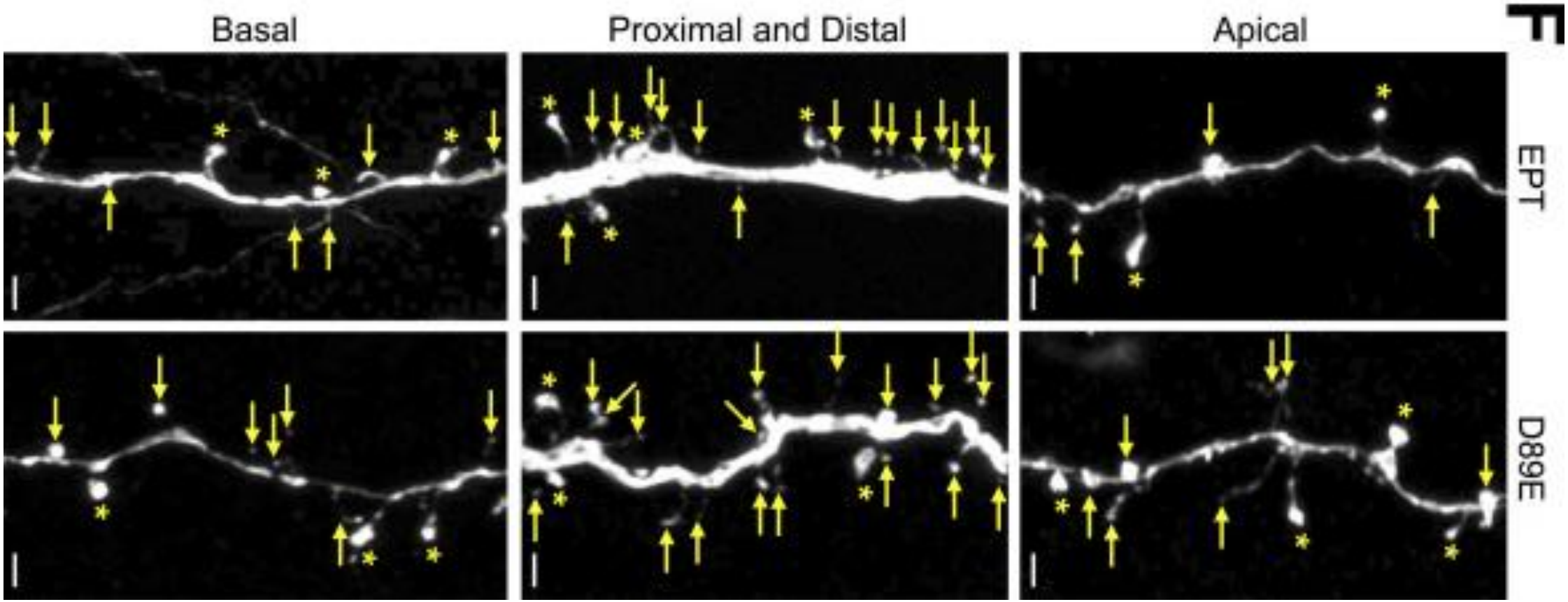

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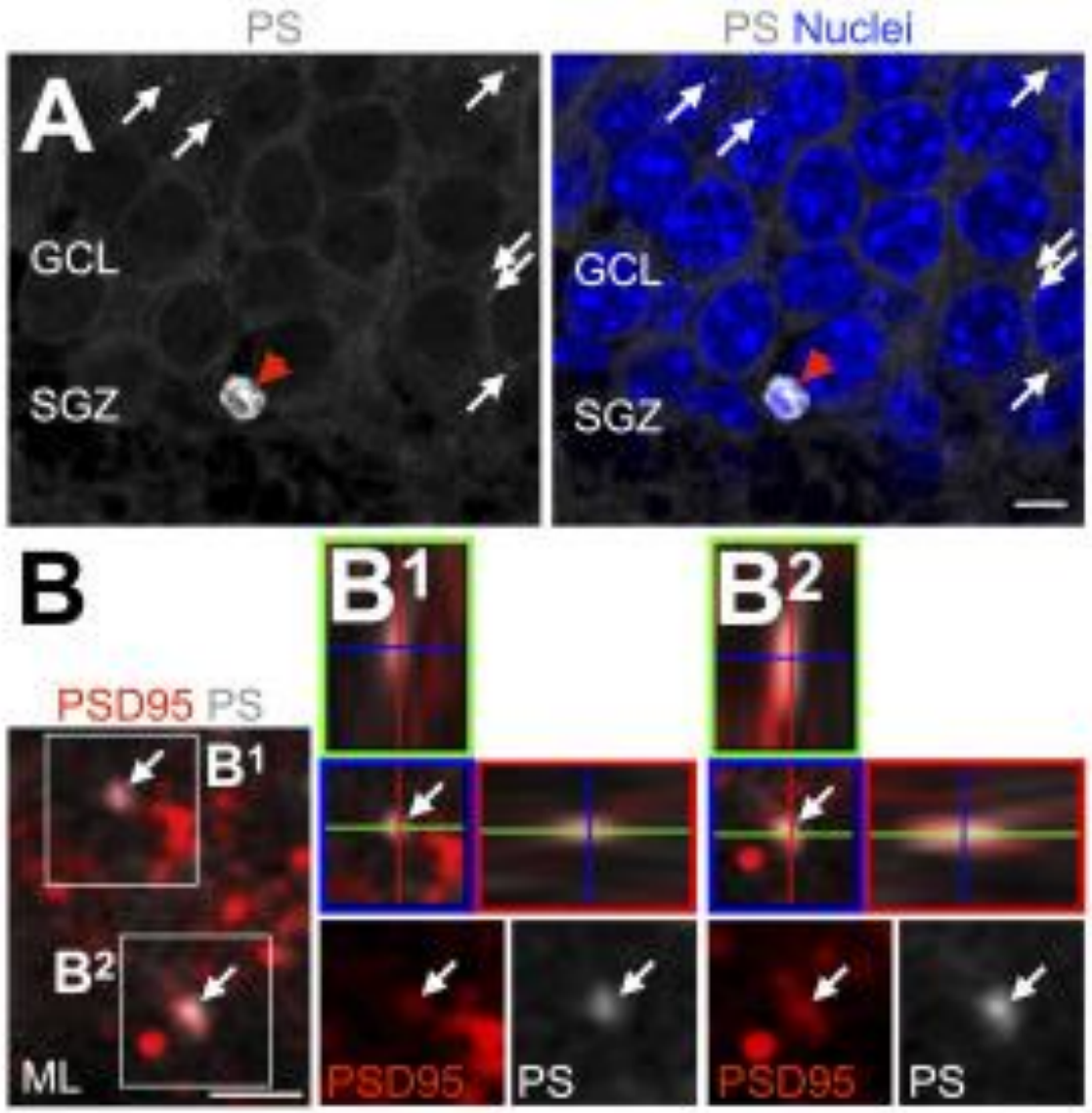
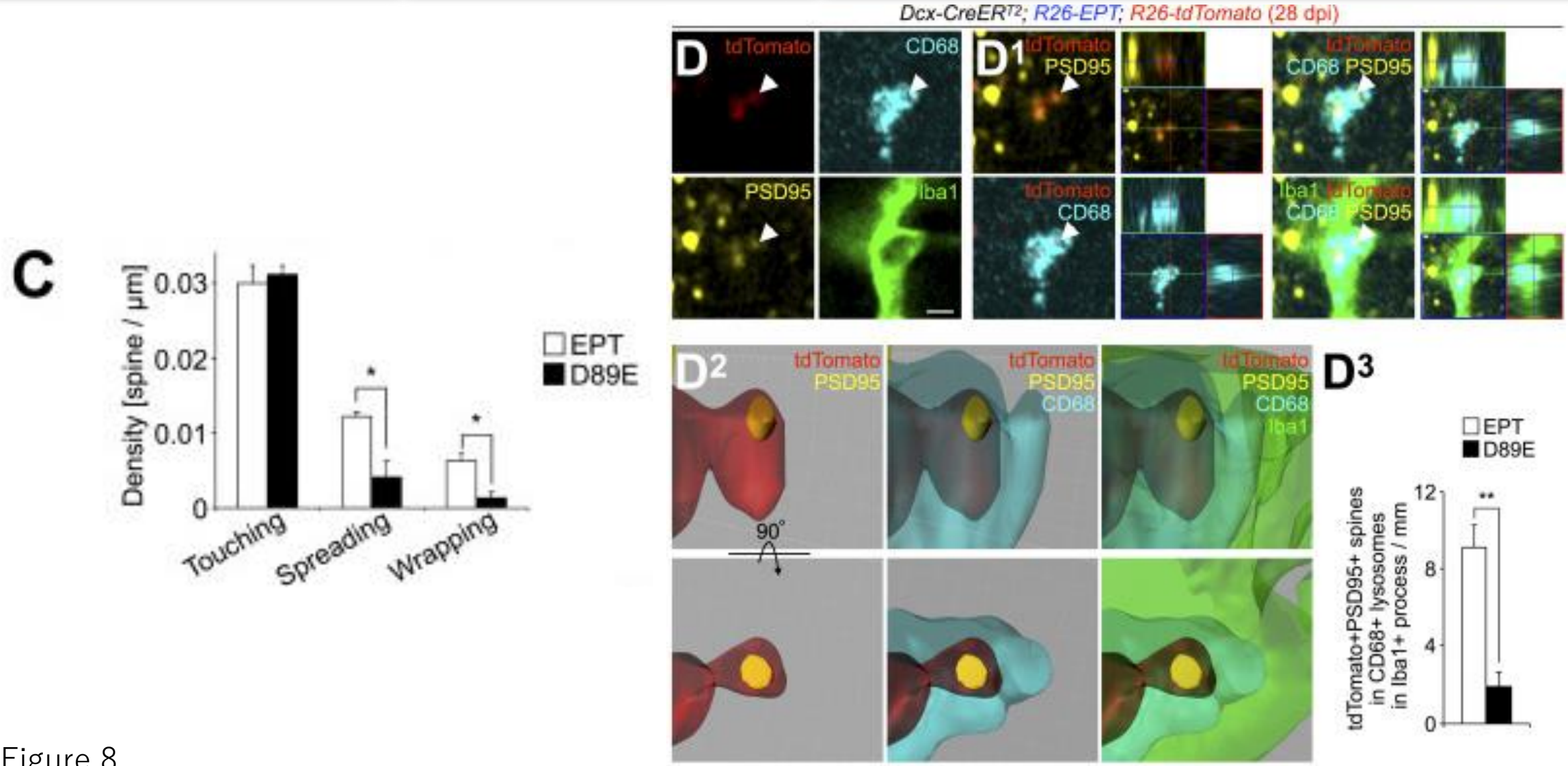


Figure 8.
Inhibition of PS-dependent spine pruning by MFG-E8D89E disrupts the synaptic maturation of adult-born neurons in the hippocampal DG.



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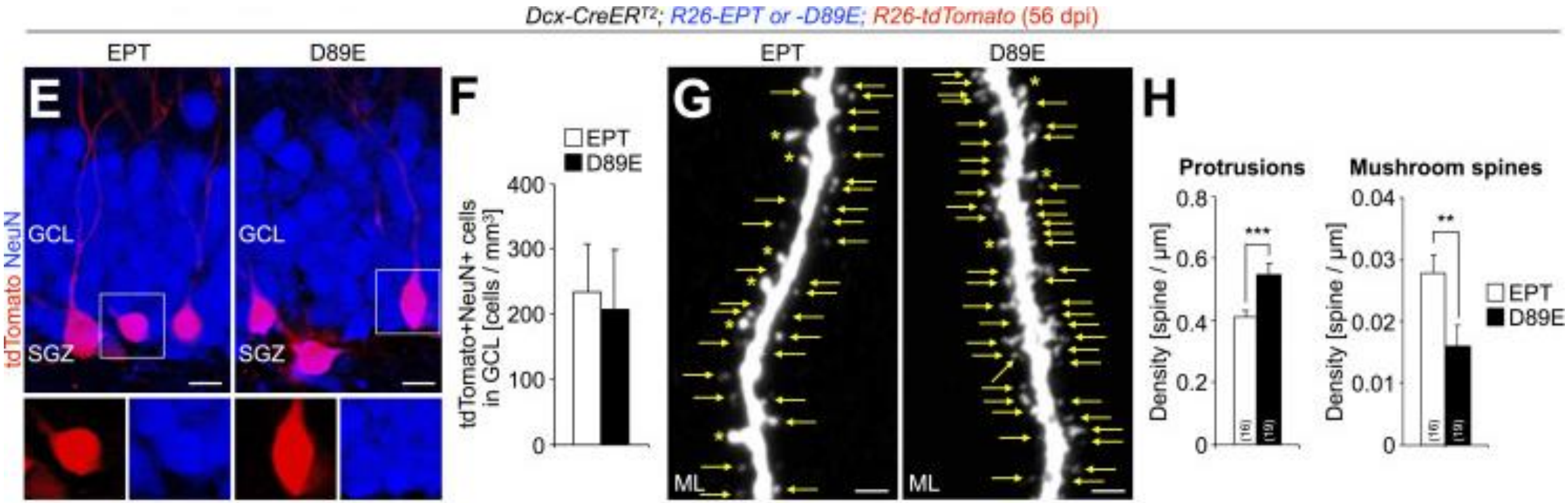
Inhibition of PS-dependent spine pruning by MFG-E8D89E disrupts the synaptic maturation of adult-born neurons in the hippocampal DG.

Video 6.



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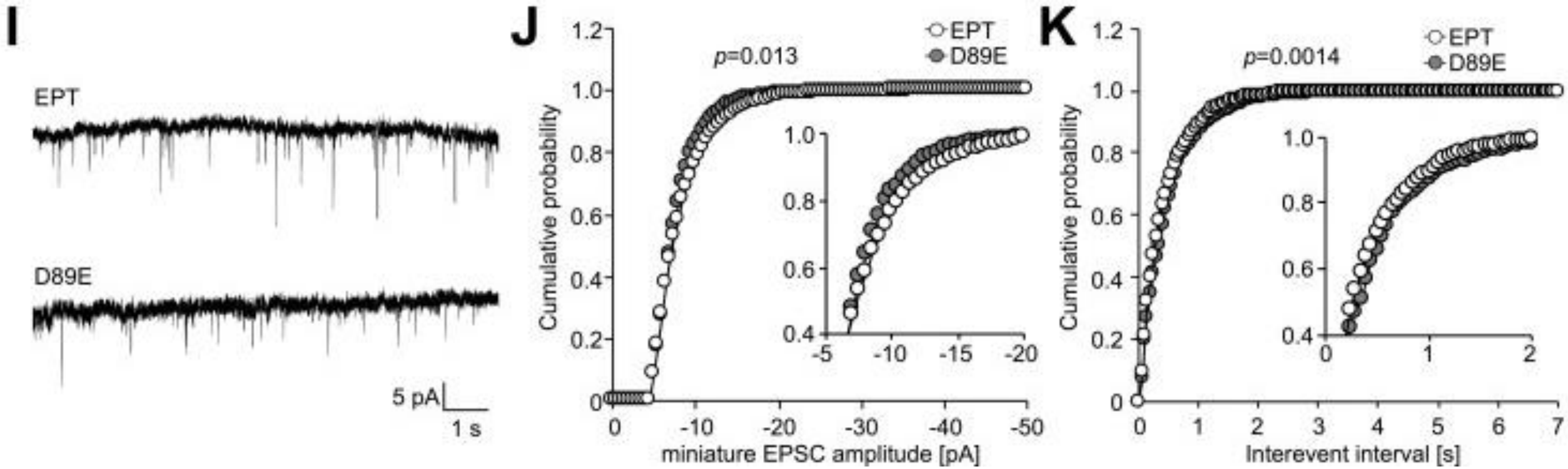

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Inhibition of PS-dependent spine pruning by MFG-E8D89E disrupts the synaptic maturation of adult-born neurons in the hippocampal DG.

◆PS as a synaptic eat-me signal

◆Roles of PS-dependent phagocytosis by microglia in spine pruning of developing adult-born neurons

◆R26-MFG-E8D89E mice: a novel tool to inhibit PS-dependent phagocytosis

◆Microglial phagocytosis of synapses and neurodevelopmental disorders