# 記憶と海馬神経細胞における DNA 切断

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https://moonbeam.sakura.ne.jp/gene/download

# DNA 修復と神経系

- 神経細胞のように、DNA 複製のない細胞における DNA 修復は未解明な部分が多い。
  https://www.biorxiv.org/content/10.1101/2024.06.25.600517v2
- 神経活動に伴う酸素ラジカルが DNA を切断する？？？

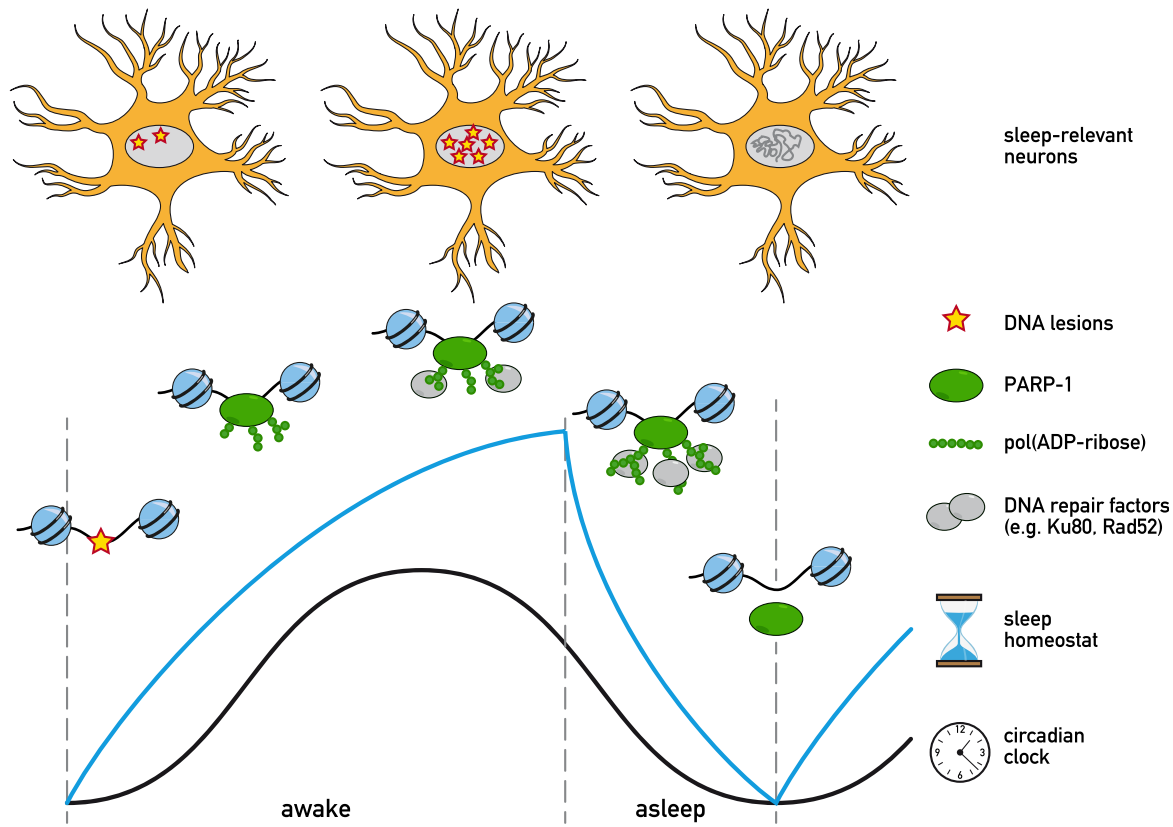
- DNA の変化に伴う細胞機能の低下／老化／細胞死が予想される。

# DNA 修復と神経系

- ***Atm*** (毛細血管拡張性運動失調症), ***Xpa*** (色素性乾皮症), ***Ercc8/Ercc6*** (Cockayne 症候群), ***Xrcc1/Xrcc4, Lig4, Prkdc/Ku70/Ku80*** (重症複合免疫不全症), ***Tdp1*** (小脳性運動失調)
  - これらのノックアウトマウスは脳に表現型が現れる
- 夜間の PARP1 蓄積と睡眠

# PARP と睡眠

- 覚醒中に DNA 損傷が蓄積
- 徐々に PARP-1 が集積し、眠気を誘発
- 睡眠中に PARP-1 依存的に DNA 修復
- 以上をゼブラフィッシュ (一部マウス) で示した




**Parp1 promotes sleep, which enhances DNA repair in neurons**

Molecular Cell 81: 4958–4959 (2021)

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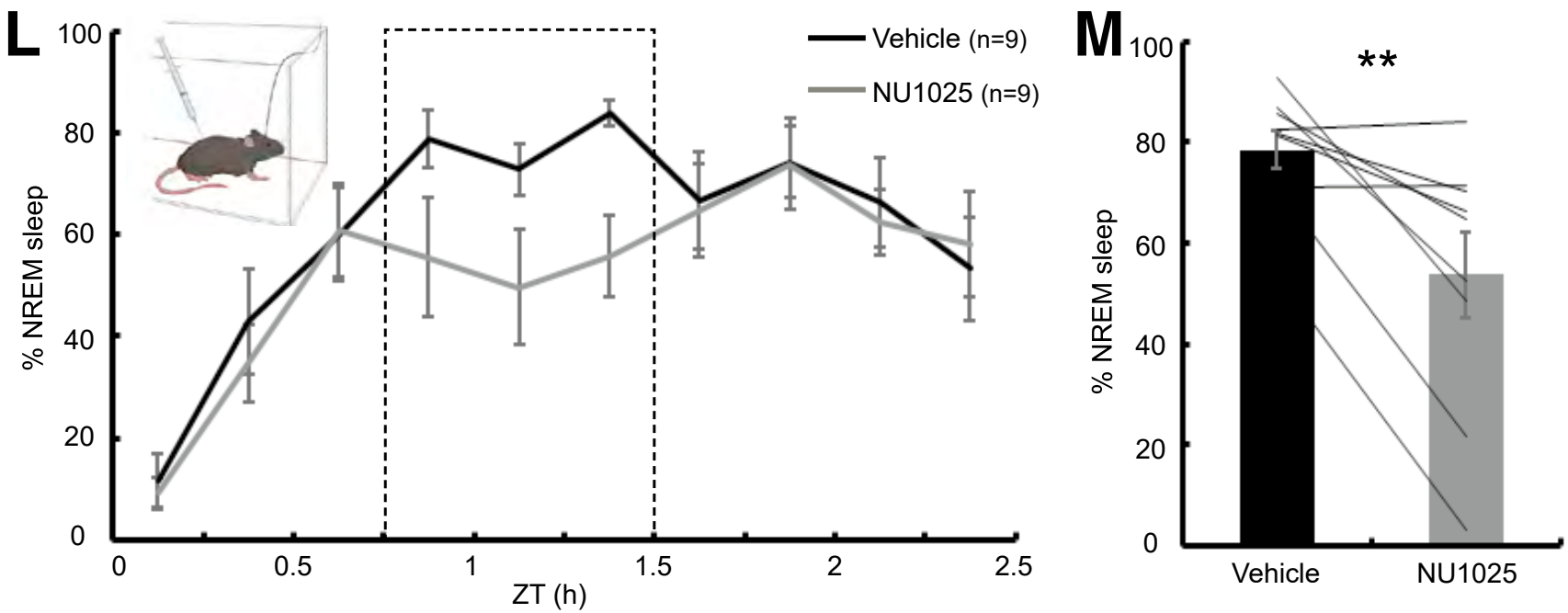
Molecular Cell 81: 4979–4993 (2021)

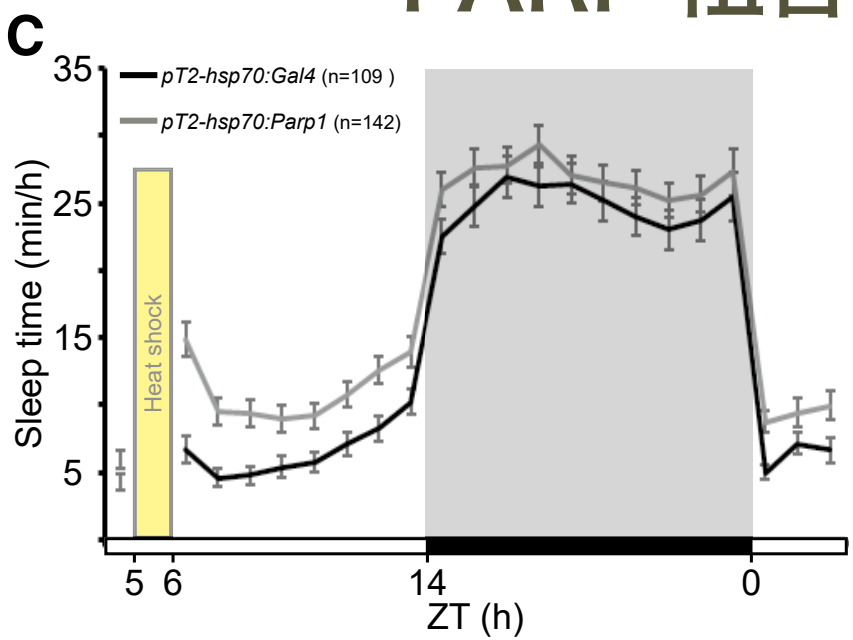
# マウスで、 PARP 阻害剤は NREM 睡眠を減少させる

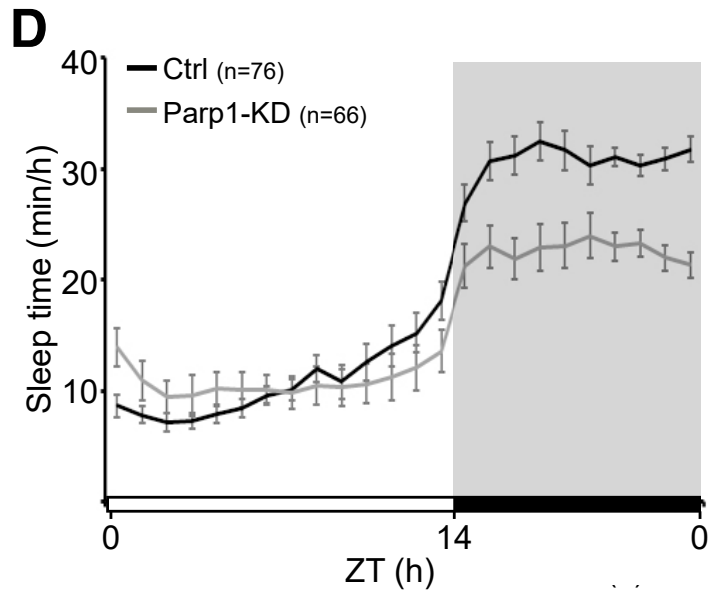
脳波を記録

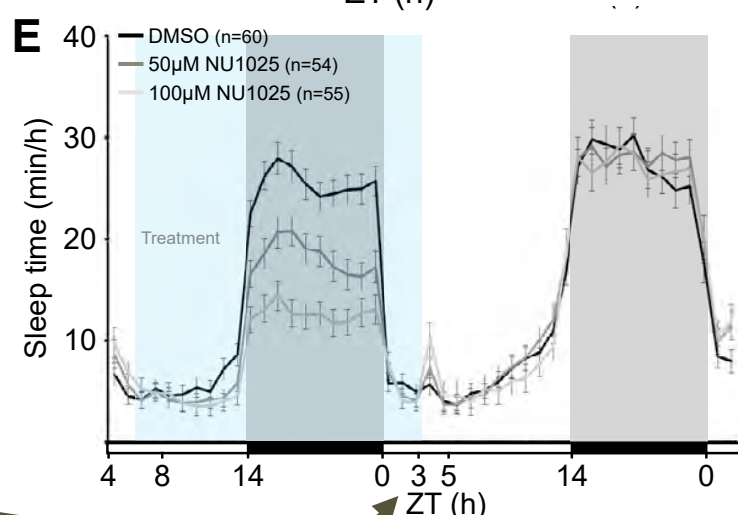



Molecular Cell 81: 4979–4993 (2021)

# PARP1過剰発現ですぐ眠くなる
# knockdown で寝なくなる
# PARP 阻害剤でも寝なくなる



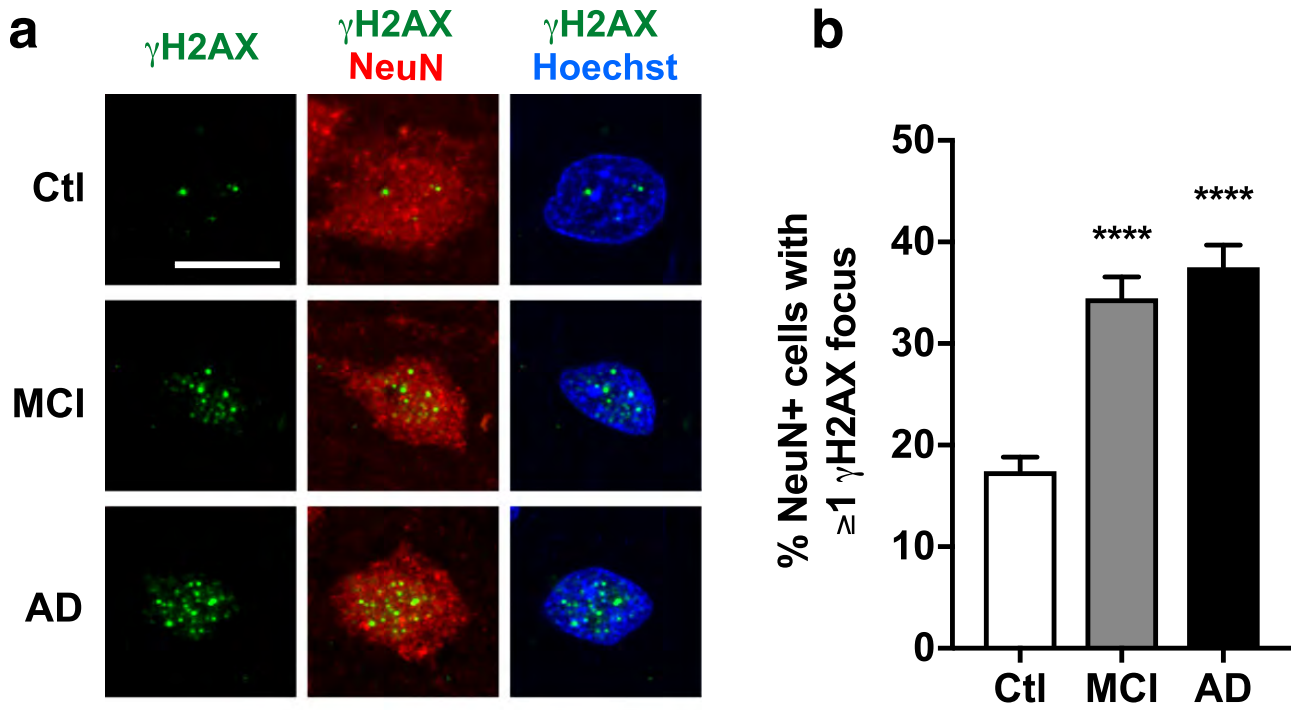




この時 (ZT3)、PARP 阻害剤群で
γH2AX foci が増えている！！
DNA 損傷ではなく
PARP 活性が眠りをさそっている！！

Molecular Cell 81: 4979–4993 (2021)

# アルツハイマー病死後脳における DNA 切断の増加

- アルツハイマー病や軽度認知障害の neuron で γH2AX foci（DNA 二重鎖切断のマーカー）が増加。astrocyte でも！




Shanbhag et al. Acta Neuropathologica Communications (2019) 7:77
https://doi.org/10.1186/s40478-019-0723-5

# Toll-like receptors (TLRs)

- もともとショウジョウバエの胚発生における背腹軸の決定に関わる分子として発見、後に抗菌反応に関与することが示された。
- 脊椎動物にも存在し、自然免疫を司る。
- ヒトでは10種類。

| | リガンド | 局在 |
|---|---|---|
| TLR1/TLR2 | lipopeptides | 表面 |
| TLR2 | peptidoglycan | 表面 |
| TLR3 | 二本鎖RNA | 細胞内小胞 |
| TLR4 | LPS | 表面 |
| TLR5 | flagellin | 表面 |
| TLR2/TLR6 | lipopeptides | 表面 |
| TLR7 | 一本鎖RNA | 細胞内小胞 |
| TLR8 | 一本鎖RNA | 細胞内小胞 |
| TLR9 | CpG DNA | 細胞内小胞 |

**ヒトの細胞には無い物質か、本来の場所以外にある物質**

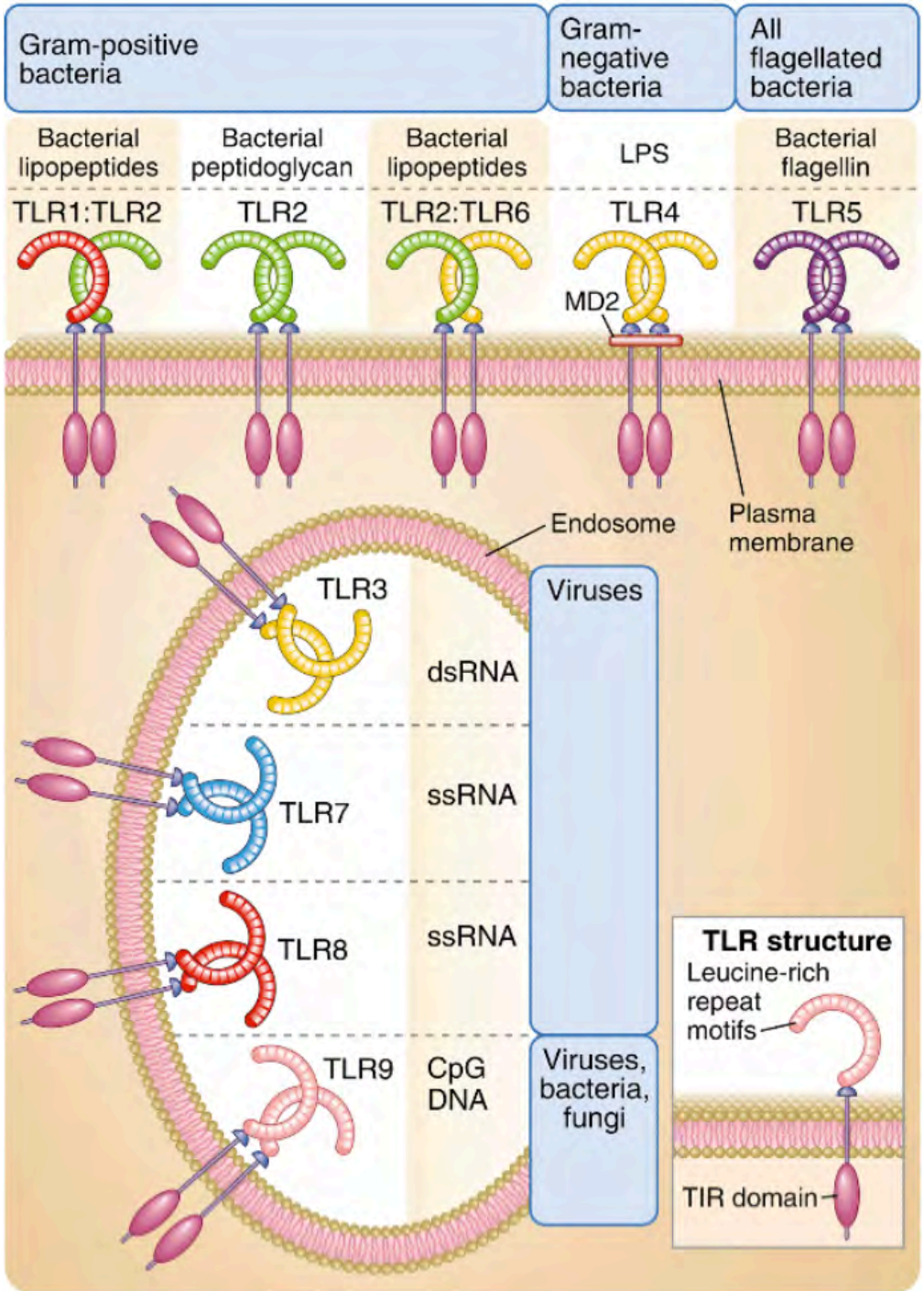


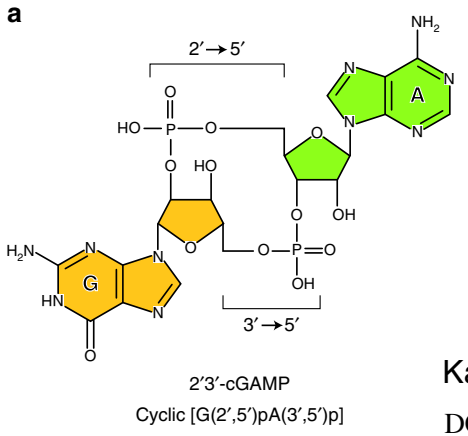

Figure 4.2: Structure, location, and specificities of mammalian Toll-like receptors.

Note that some TLRs are expressed on the cell surface and others in endosomes. TLRs may form homodimers or heterodimers. dsRNA, Double-stranded RNA; LPS, lipopolysaccharide; ssRNA, single-stranded RNA; TIR, Toll IL-1 receptor; TLR, Toll-like receptor.

Cellular and Molecular Immunology 10th ed.

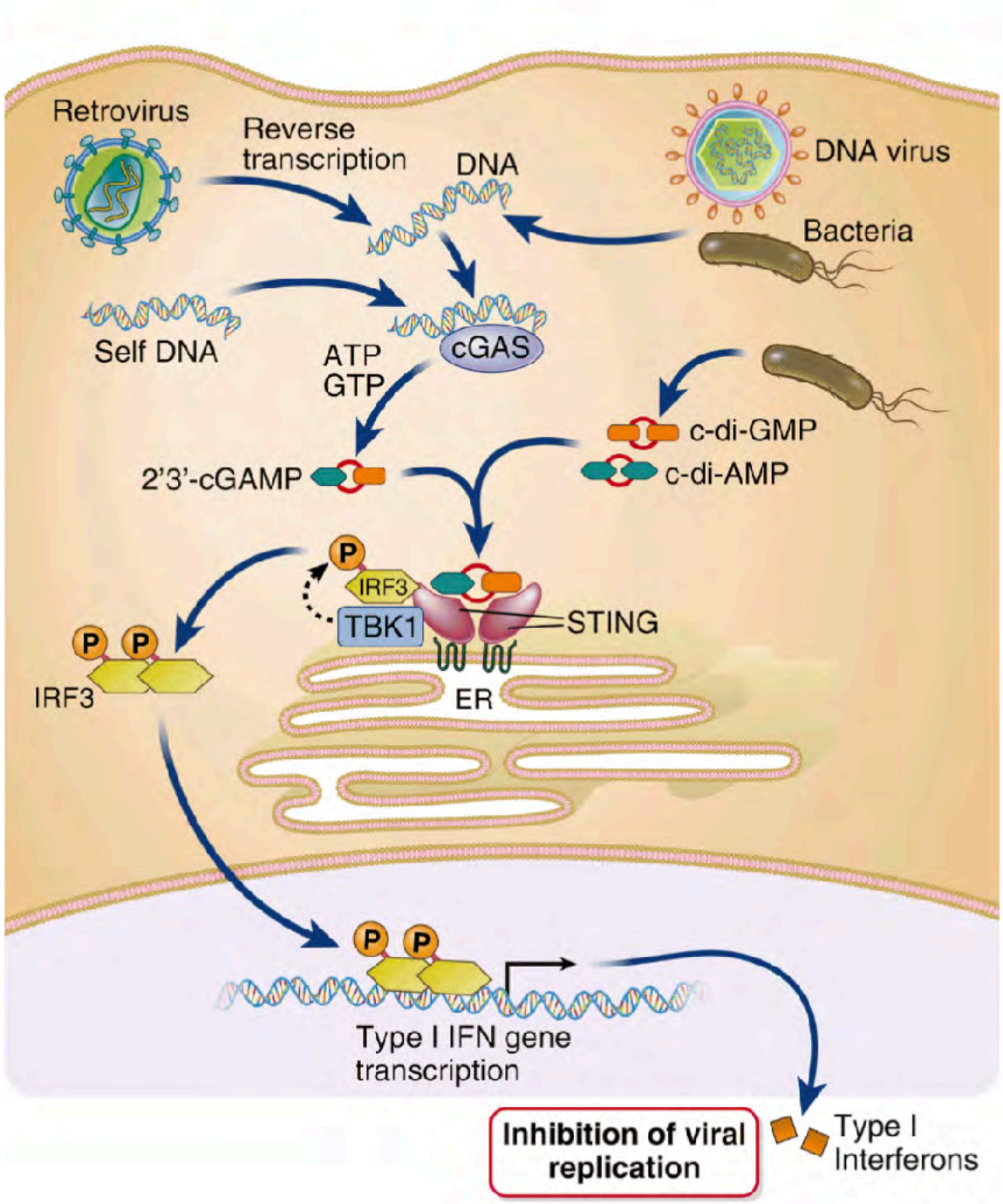
# cGAS-STING 経路

- ウイルス由来 DNA に反応
- 自己 DNA に反応 (自己炎症性疾患)
- cGAS が感知して、cGAMP を生成
- cGAMP を結合した STING が小胞体からゴルジ体へ移動、 インターフェロン経路をオンに




2'3' cyclic GMP-AMP (cGAMP)

Kato et al., Nature Communications 9: 4424 (2018)
DOI: 10.1038/s41467-018-06922-7




# Figure 4.5: The STING cytosolic DNA sensing pathway.

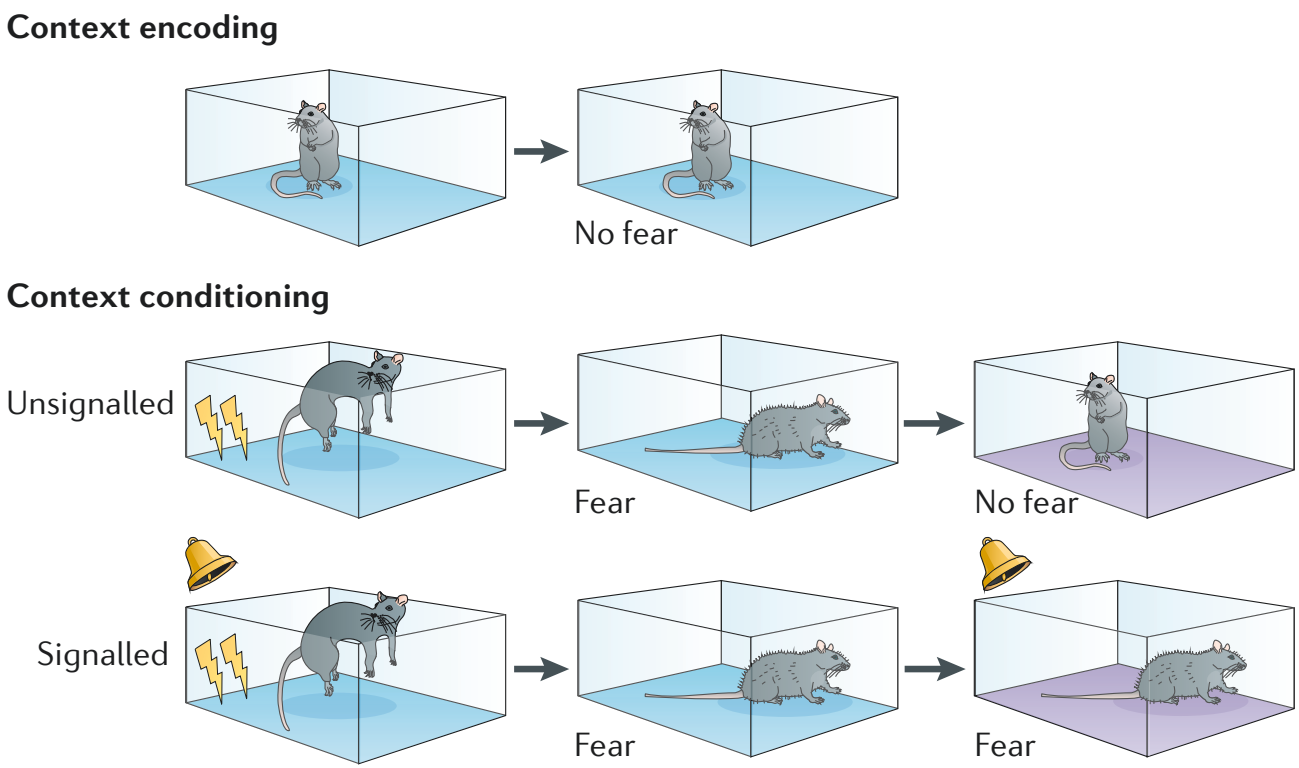
Cytoplasmic microbial DNA and self DNA that accumulates in the cytosol activate the enzyme cGAS, which catalyzes the synthesis of cyclic GMP-AMP (cGAMP) from ATP and GTP. cGAMP binds to STING in the endoplasmic reticulum membrane, causing STING to translocate to the Golgi (not shown), and then STING recruits and activates the kinase TBK1, which phosphorylates IRF3. Phospo-IRF3 moves to the nucleus, where it induces type I IFN gene expression. Self DNA may be produced as a result of genomic or mitochondrial damage or from turnover of DNA. The bacterial second messenger molecules cyclic di-GMP (c-di-GMP) and cyclic di-AMP (c-di-AMP) are directly sensed by STING. ATP, Adenosine triphosphate; cGAS, cyclic GMP-AMP synthase; ER, endoplasmic reticulum; GTP, guanosine triphosphate; IFN, interferon; IRF3, interferon response factor 3.

# cGAS-STING 経路

- がん免疫（STINGアゴニストを抗がん剤へ？）
- 老化における炎症 (transposons？)
- ALS など神経変性疾患 (transposons？)

# contextual fear conditioning (CFC)

- context （場所）やシグナル（音）の組合せによって、警戒すべき状況（電気ショック）を記憶させる実験。




Nat. Rev. Neurosci. 14: 418 (2013)

Article

# Formation of memory assemblies through the DNA-sensing TLR9 pathway

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https://www.nature.com/articles/s41586-024-07220-7

Jovasevic et al., Nature 628: 150 (2024)

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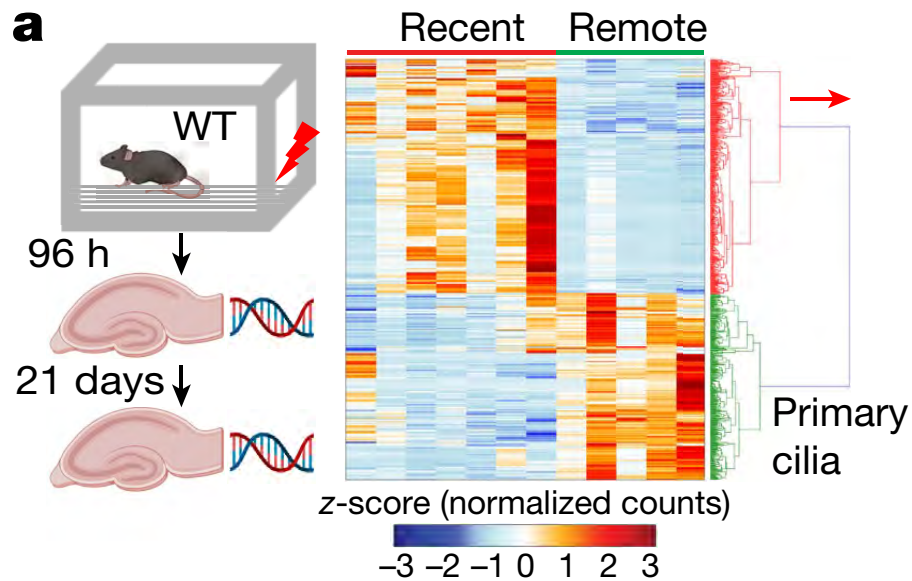
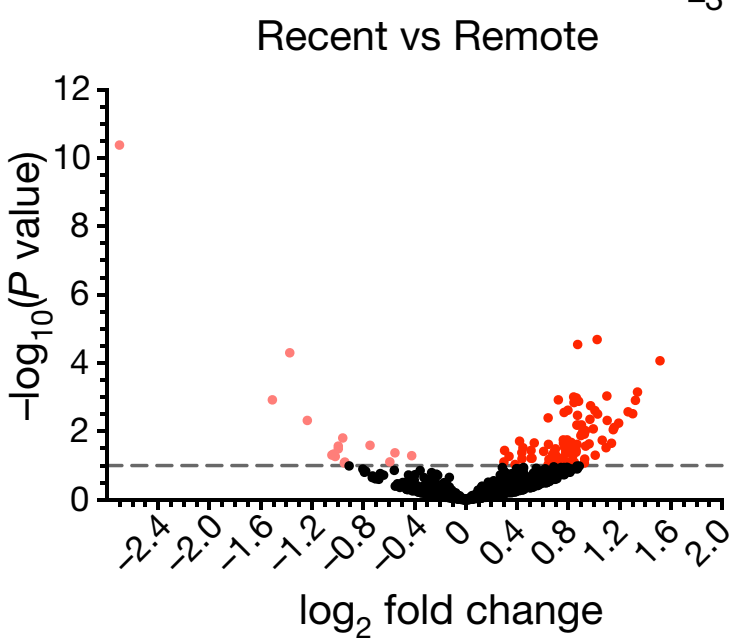
https://einsteinmed.edu/faculty/16551/jelena-radulovic

# CFC 96h 後、海馬 *Tlr9* 発現上昇



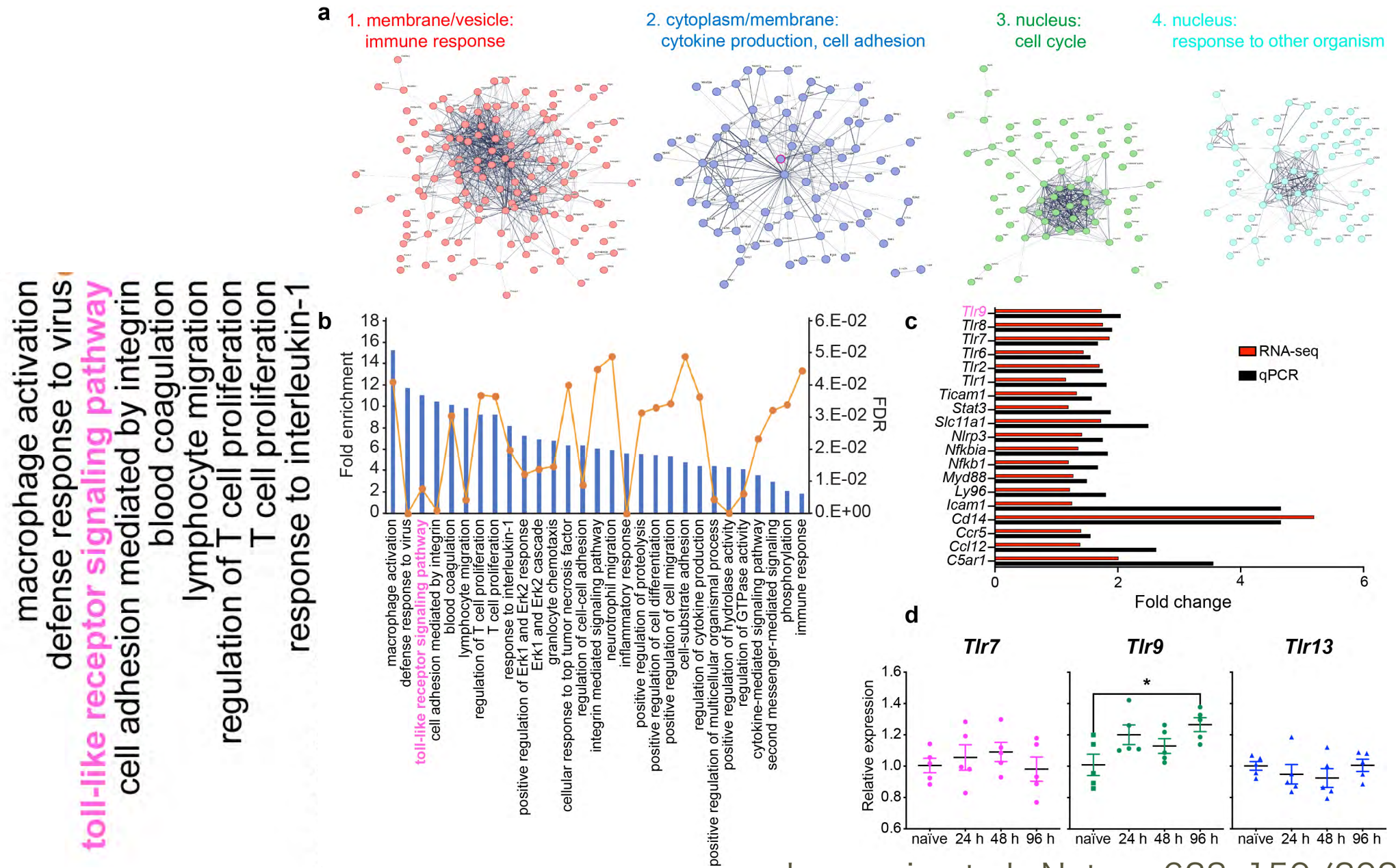




| Gene | Fold change | *P* | *P* adj |
|---|---|---|---|
| *Tlr7* | 1.86 | 0.0002 | 0.013 |
| *Tlr9* | 1.73 | 0.0001 | 0.002 |
| *Tlr13* | 1.71 | 0.0015 | 0.040 |
| *Irf7* | 2.06 | 0.0003 | 0.017 |
| *Irf8* | 1.81 | 0.0001 | 0.007 |
| *Irf5* | 1.70 | 0.0002 | 0.002 |
| *Irf9* | 1.53 | 0.0015 | 0.041 |
| *Ifnar1* | 1.19 | 0.0014 | 0.039 |

ヒトには TLR13 はありません

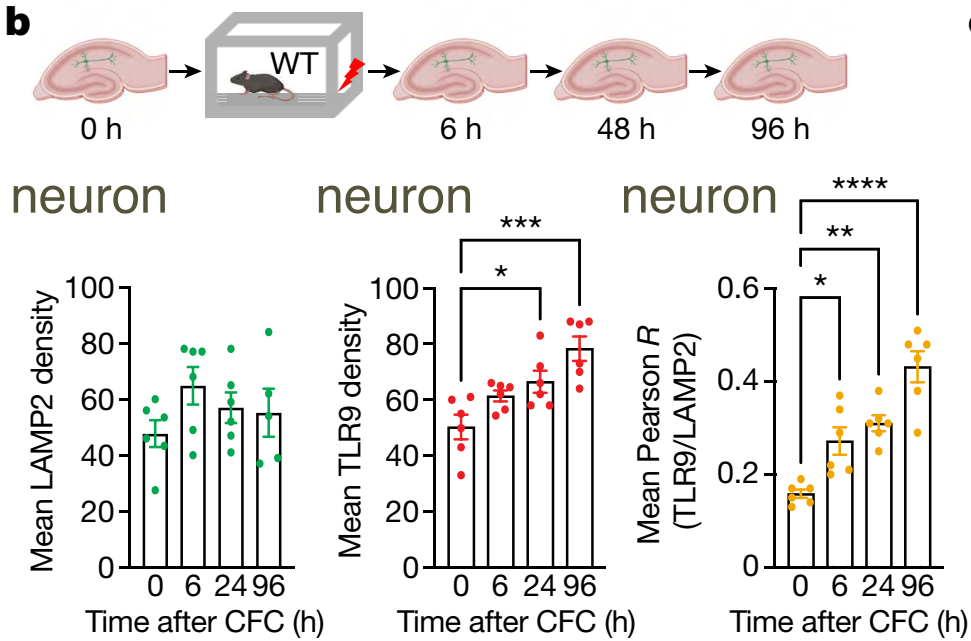
Jovasevic et al., Nature 628: 150 (2024)

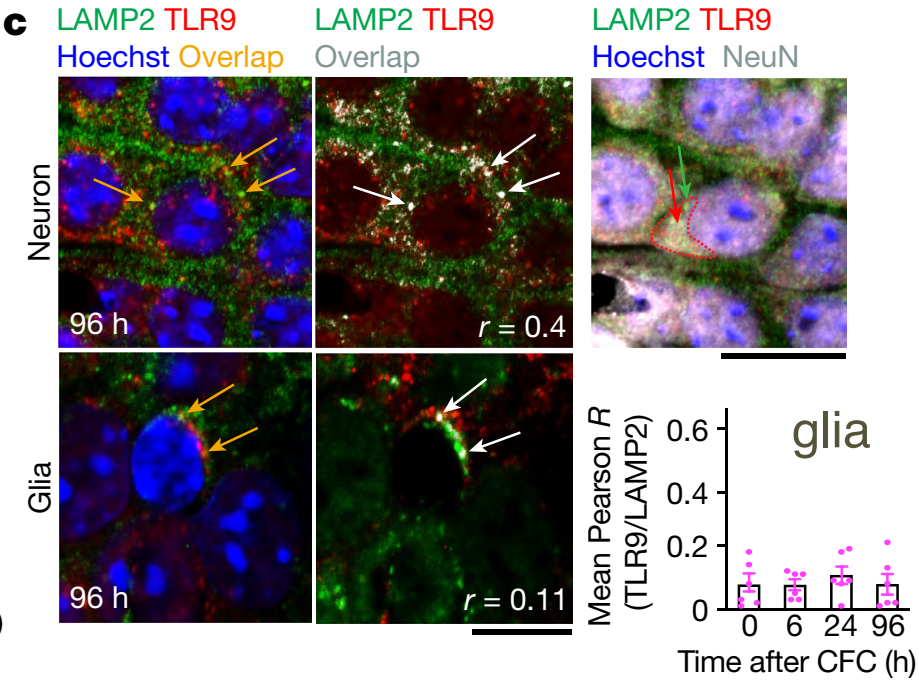
# 記憶形成中、炎症／サイトカイン分泌／細胞周期・DNA修復関連遺伝子が発現上昇

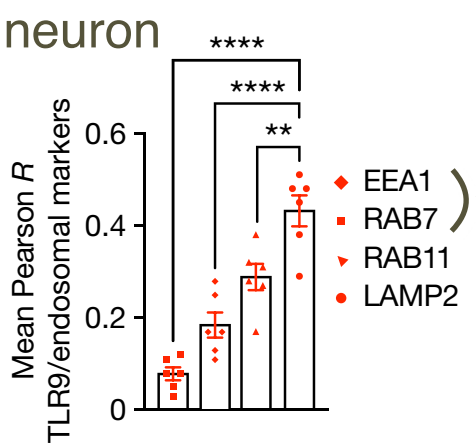



Jovasevic et al., Nature 628: 150 (2024)

# TLR9 の後期エンドソーム局在は神経細胞で認められ、グリアでは認められない




early endosome marker

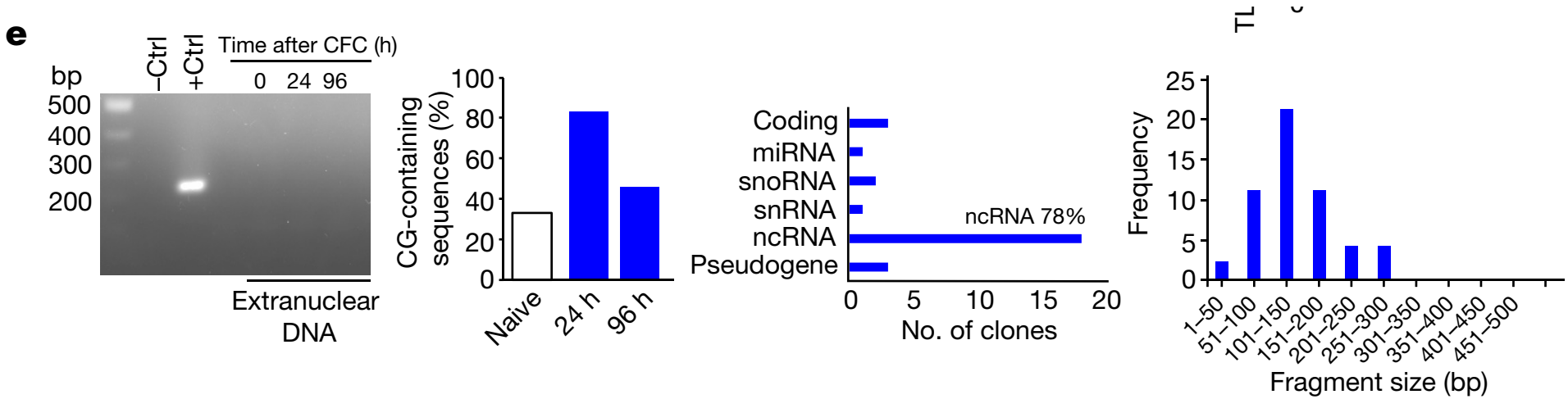
recycling endosome marker

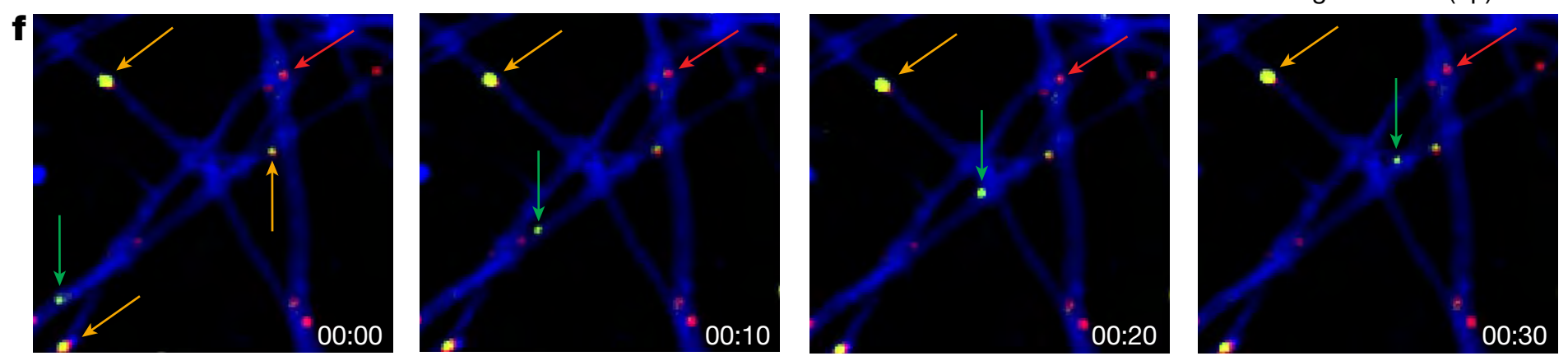
late endosome & lysosome marker

DNA recognition & NF-κB activation

Jovasevic et al., Nature 628: 150 (2024)

# 細胞質に non-coding DNA 遺伝子由来 DNA が CFC 24h後に増加




hippocampal neuron (P1)

NMDA, 10 min

Mitochondrial dsDNA
MitoTracker
PicoGreen

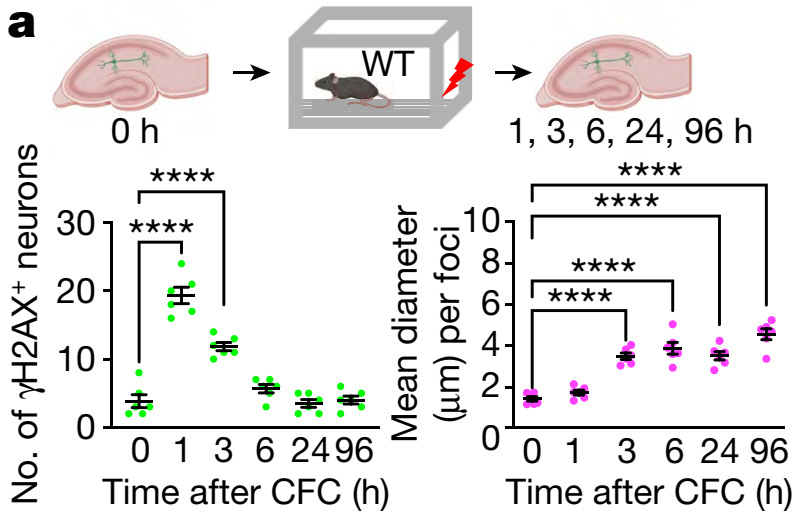
Mitochondria
MitoTracker

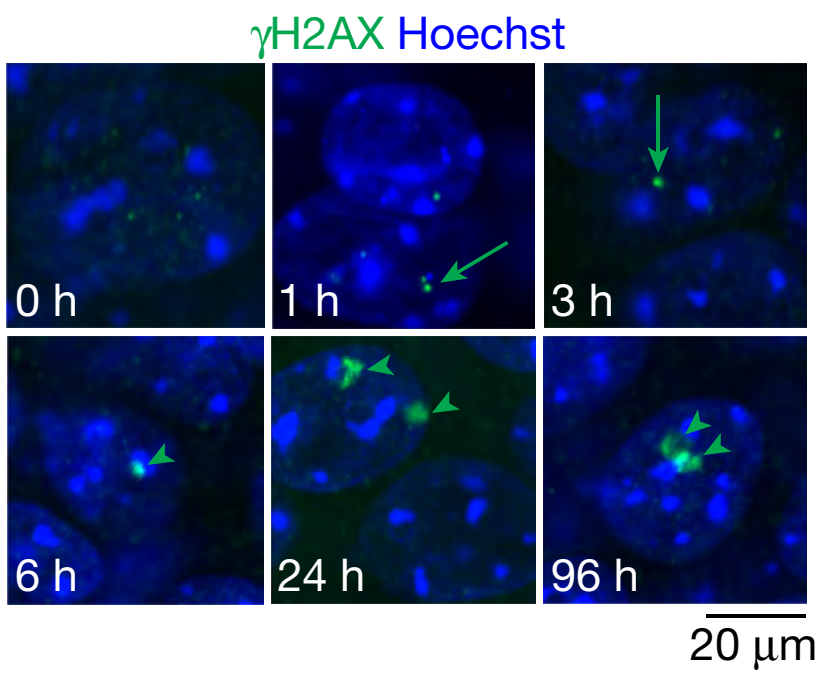
Extranuclear dsDNA
PicoGreen

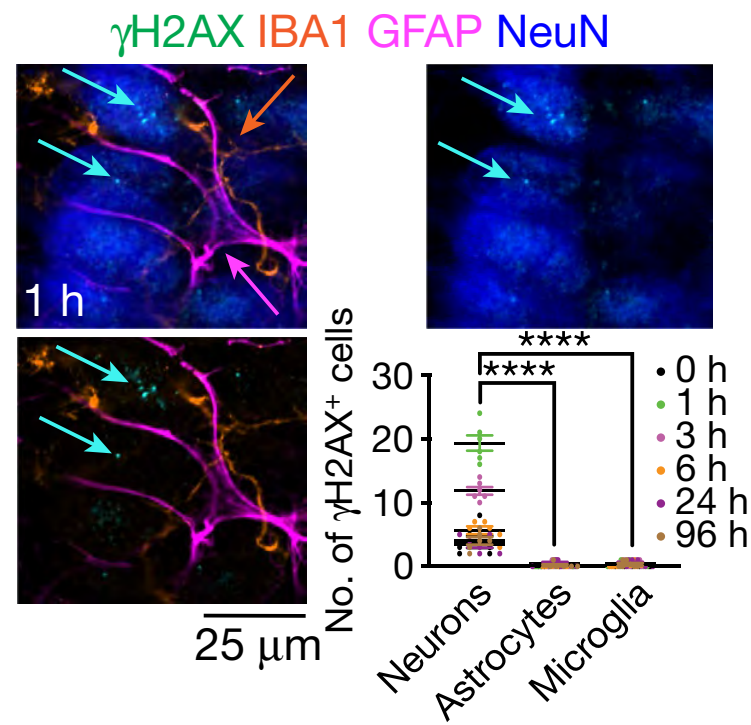
cell membrane
CellMAsk

Jovasevic et al., Nature 628: 150 (2024)

# CA1 neuron で γH2AX foci が CFC 1 h 後に増加

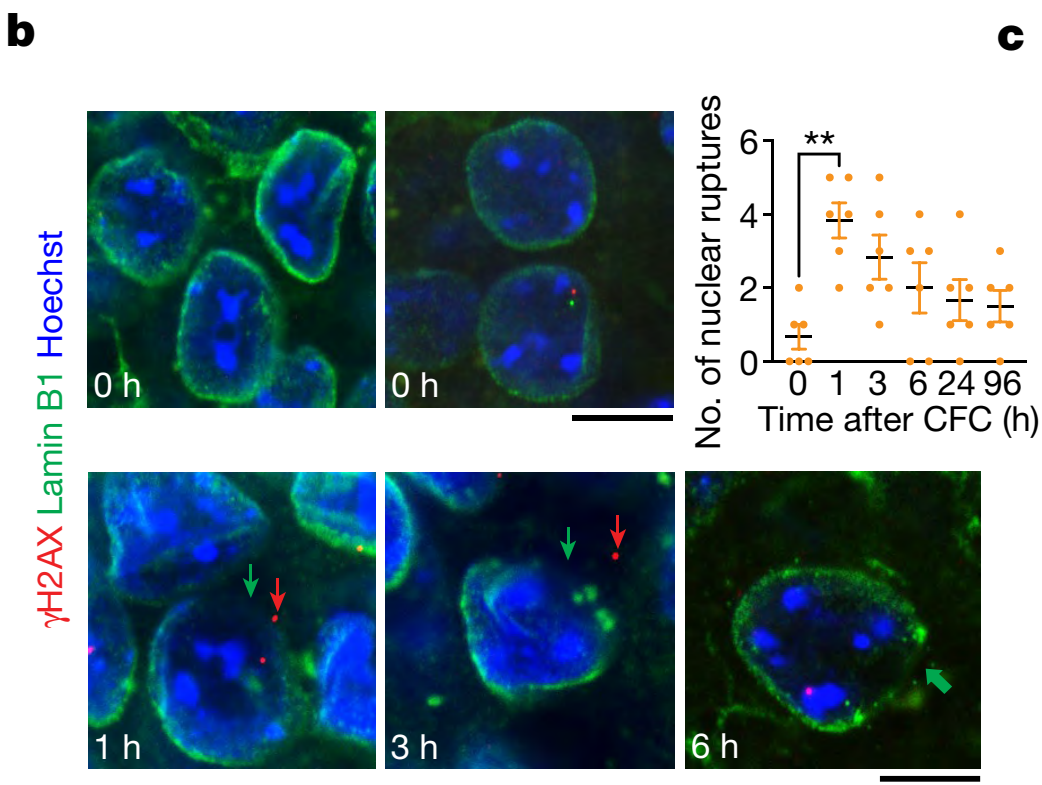


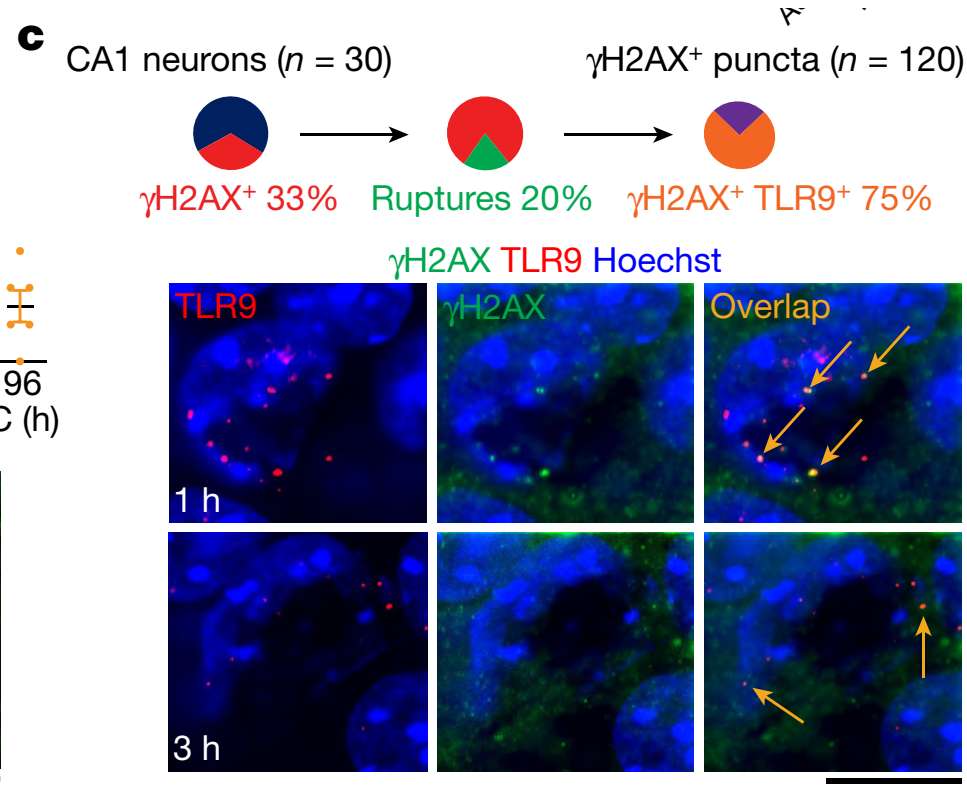




Jovasevic et al., Nature 628: 150 (2024)

# CFC 1h 後 neuron nuclear envelope が破裂
# 3h 後、γH2AX が細胞質小胞体で TLR9 と共局在




γH2AX: perinuclear > envelope rapture > ER




cytosolic DNA meets with TLR9 at ER

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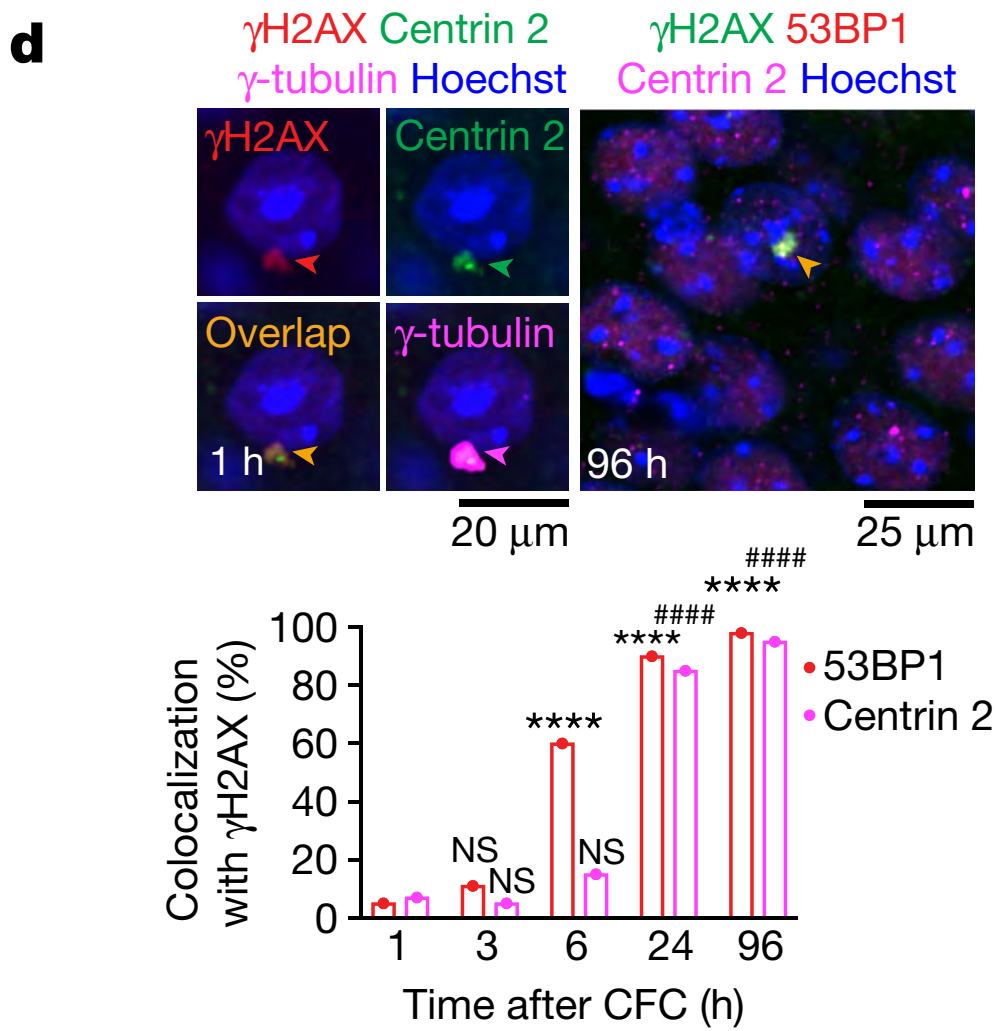
# その後、γH2AX は中心体に移動し、大きくぼやけた姿を呈し、53BP1と共局在する

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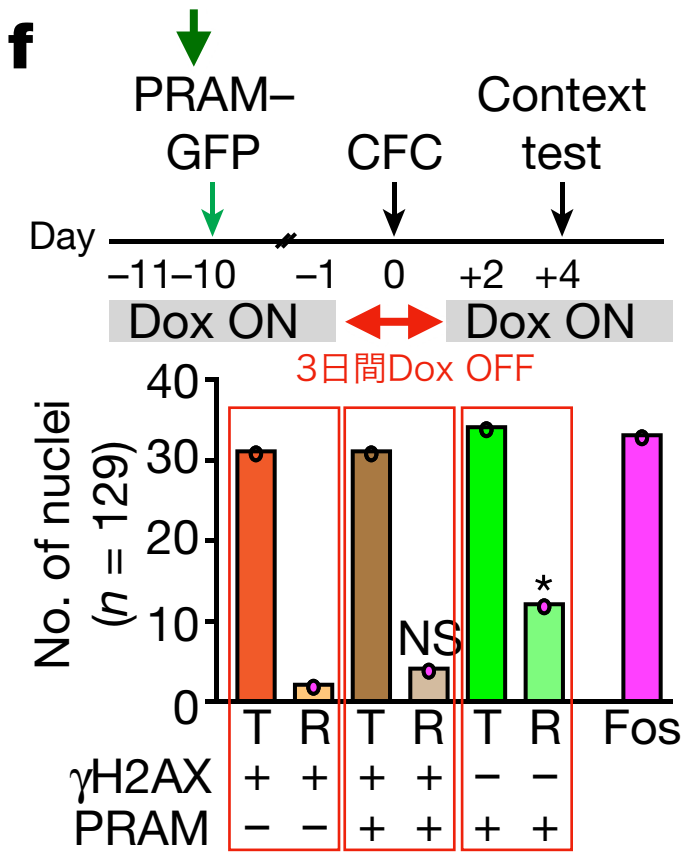
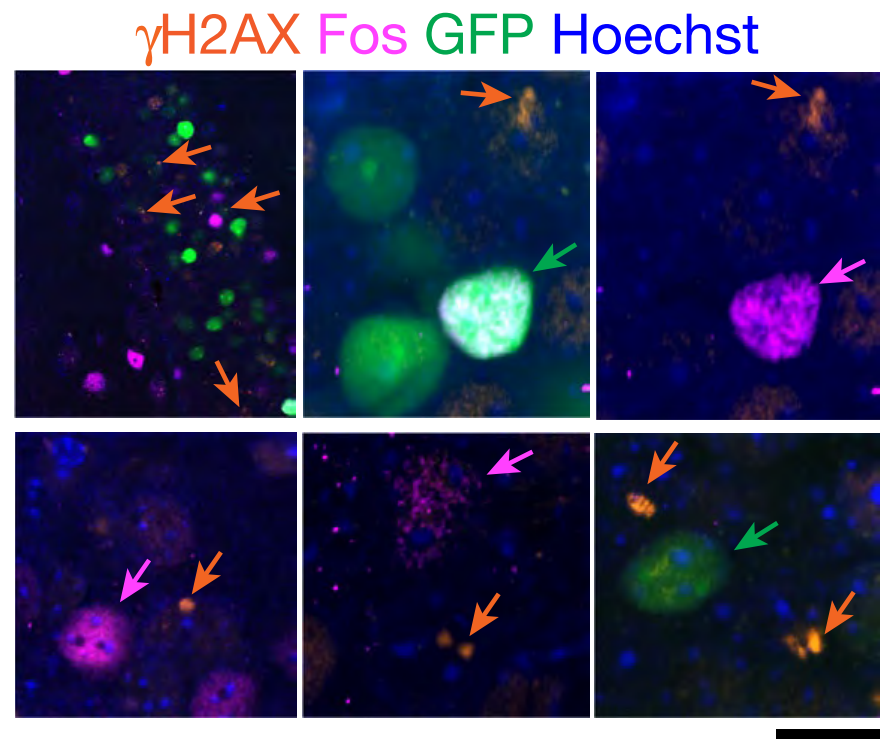
# CFC と memory activation 後、
# Fos が制御する遺伝子(immediate early gene: IEG)発現細胞と
# γH2AX－炎症シグナル発現細胞は異なる神経細胞

tet + Fos minimal promoter

Dox-OFF で転写 ON にする d2tTA

【Fos が活性化し、Dox-OFF の時に GFP が発現】




**T**: CFC training 1hr後、安楽死

**R**: memory reactivation (Context test) 1hr 後、安楽死

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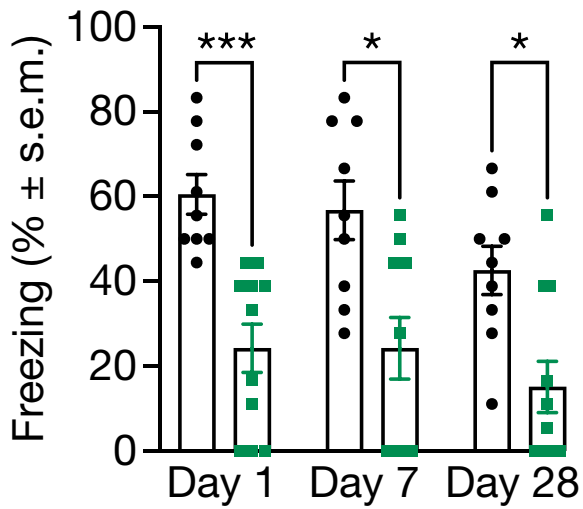
# 神経細胞特異的 *Tlr9* knockout により context memory が減弱する

**a**

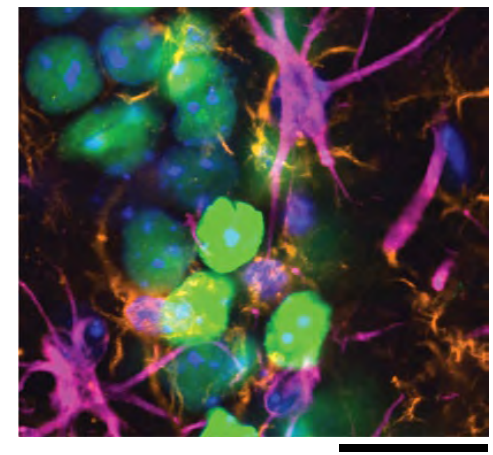


- AAV9-Syn-GFP
- AAV9-Syn-Cre

Syn: synapsin promoter




IBA1 GFP
GFAP Hoechst



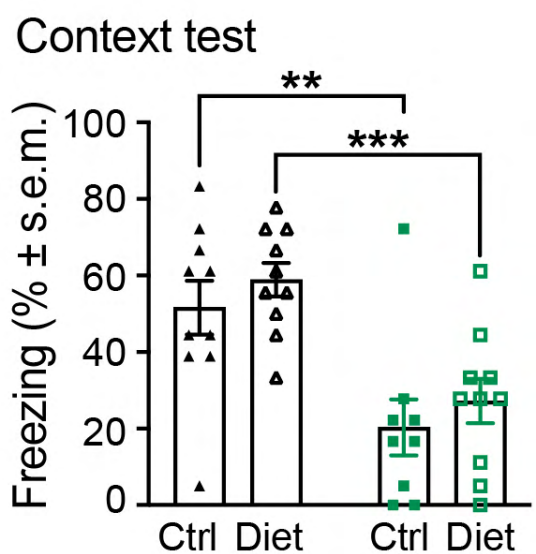
Cre-GFP expression only in neurons

**d**



- AAV9-Syn-Cre
- AAV9-Syn-Cre + microglia depletion diet

CSF1R inhibitor

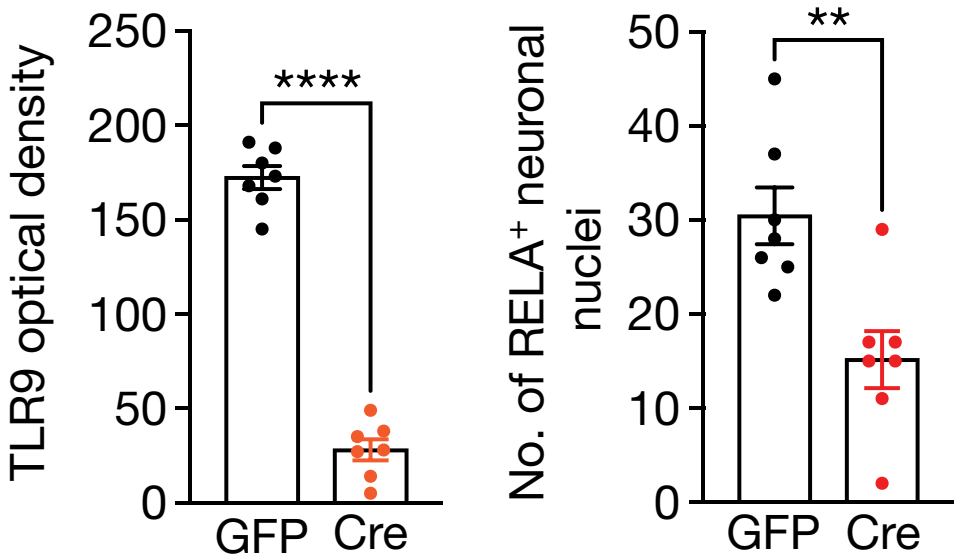


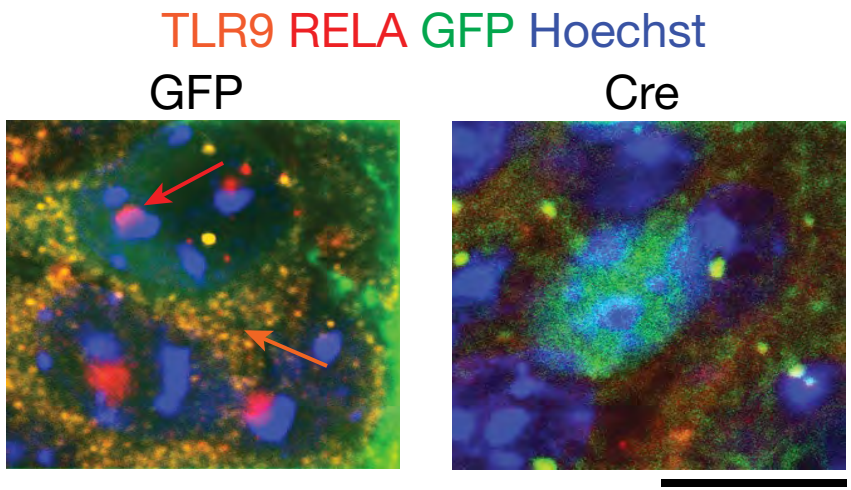

AAV9-Syn-*Tlr9* shRNA -> WT
でも context memory 減弱

microglia depletion は影響なし

Jovasevic et al., Nature 628: 150 (2024)

# *Tlr9* knockout を TLR9 と RELA タンパクが減少により確認

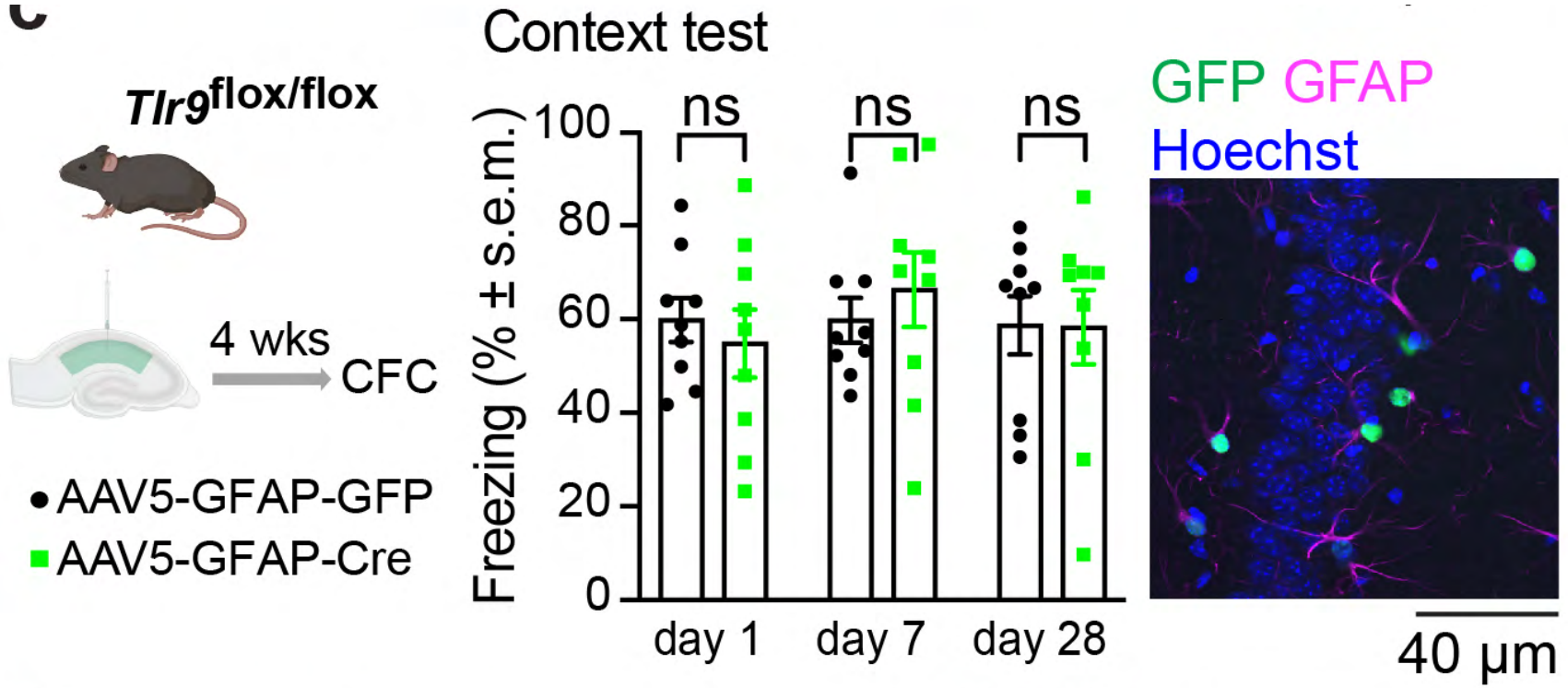



RELA: NF-κB を構成する転写活性化サブユニット

Jovasevic et al., Nature 628: 150 (2024)

# astrocyte 特異的 *Tlr9* knockout では context memory が減弱しない

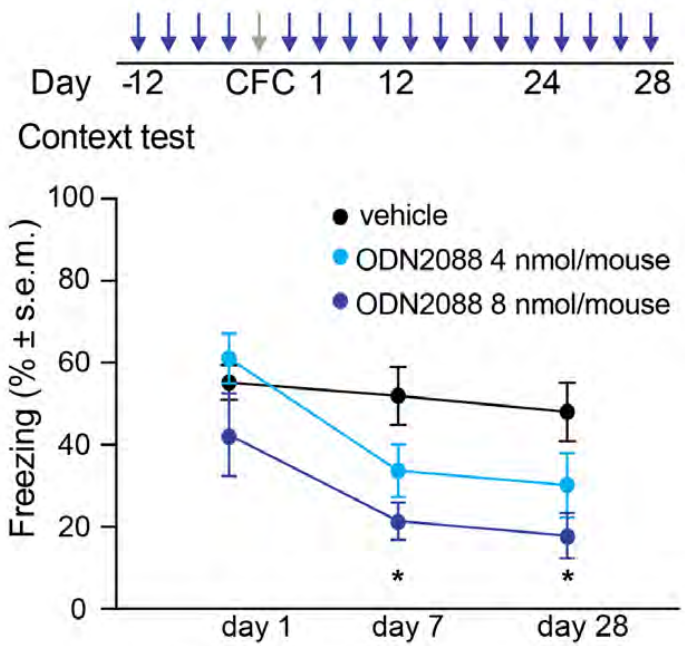


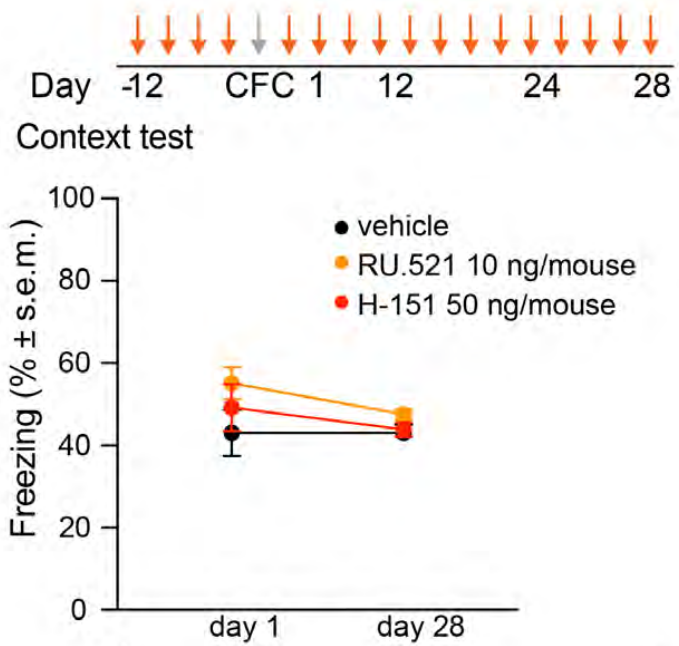

GFAP promoter

Cre-GFP expression only in astrocytes

Jovasevic et al., Nature 628: 150 (2024)

# TLR9 / cGAS-STING 阻害薬 では context memory が減弱する / しない


カニューレで海馬に投与

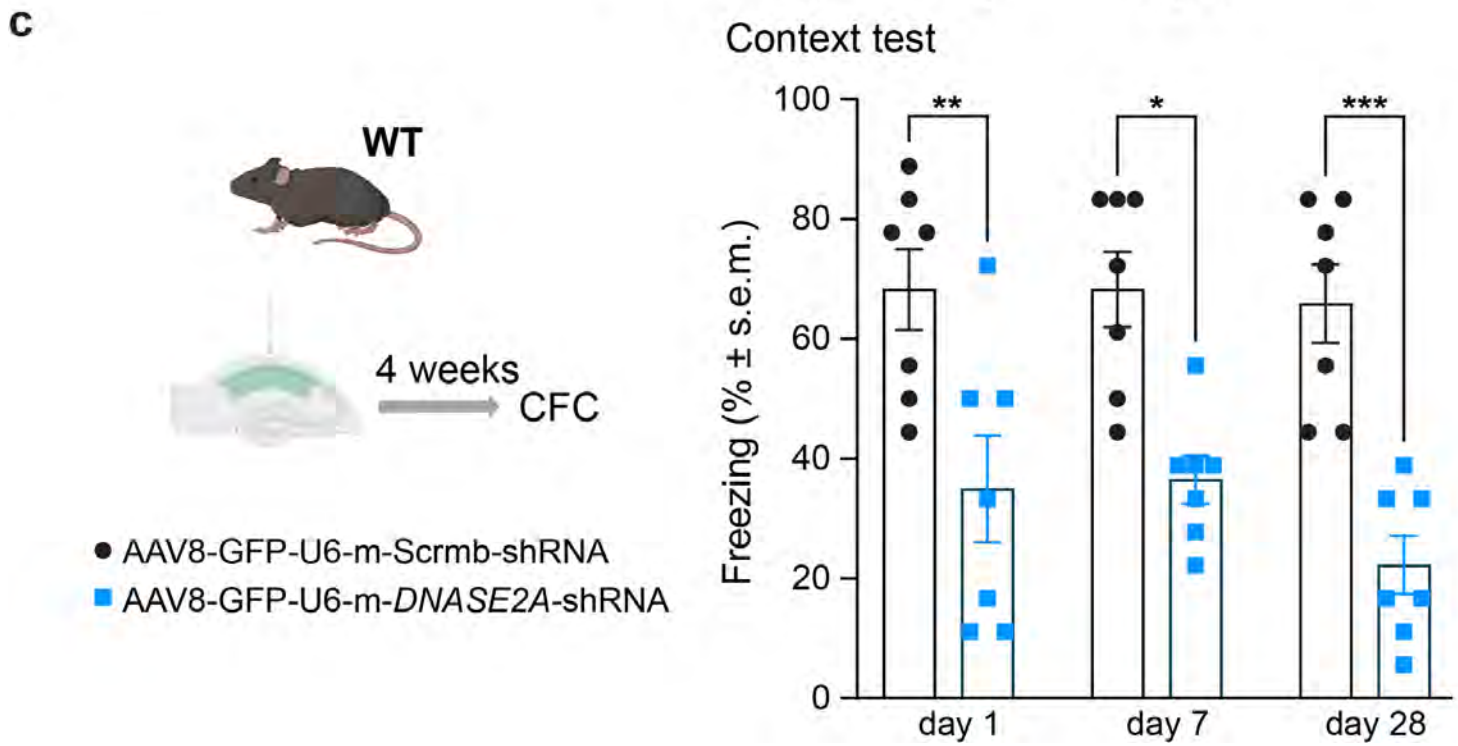
ODN2088:
TLR9 antagonist

RU-521, H-151:
cGAS-STING antagonist

*Sting1* KO は memory 正常

Jovasevic et al., Nature 628: 150 (2024)

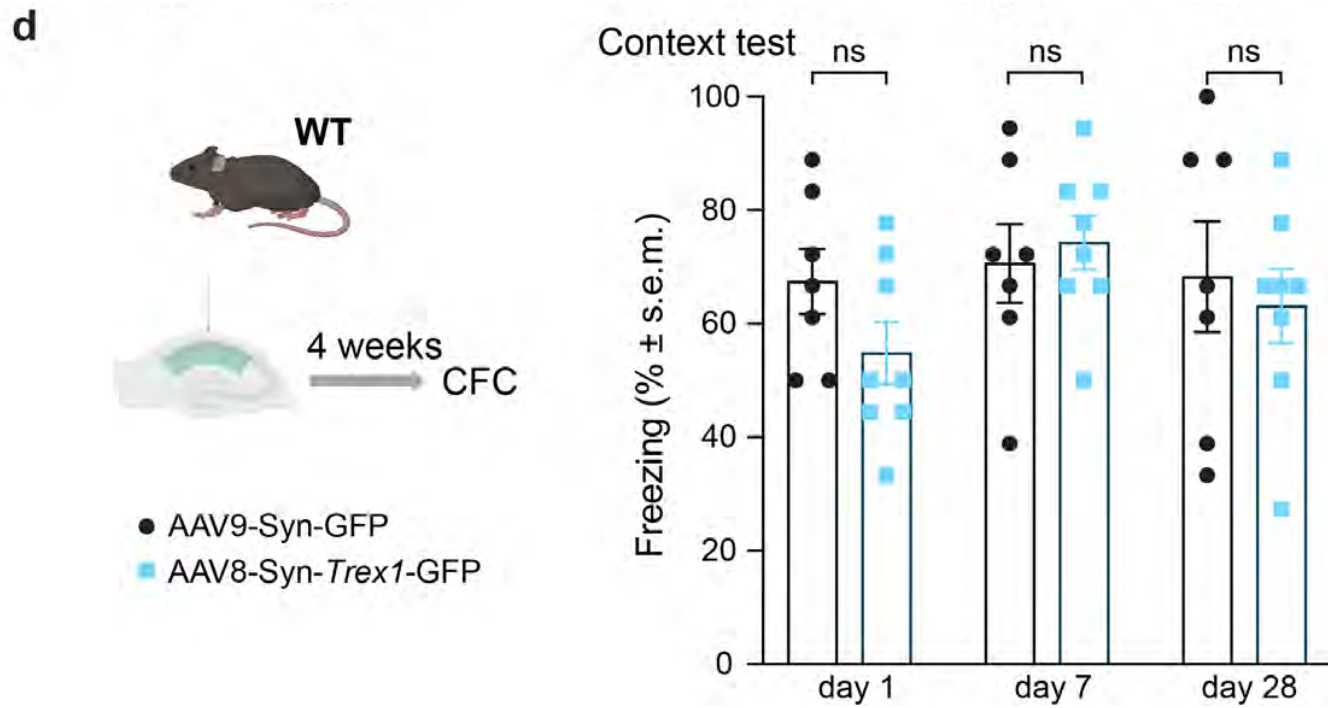
# *Dnase2* knockdown で context memory が減弱する




Dnase2 は 二本鎖 DNA を分解し、TLR9 に結合できる短い DNA 断片を生成するのに必要な酵素

Jovasevic et al., Nature 628: 150 (2024)

# *Trex1* 過剰発現 で context memory が減弱しない




TREX1 は 細胞質の DNA 分解酵素で
cGAS-STING 経路を主に抑制する

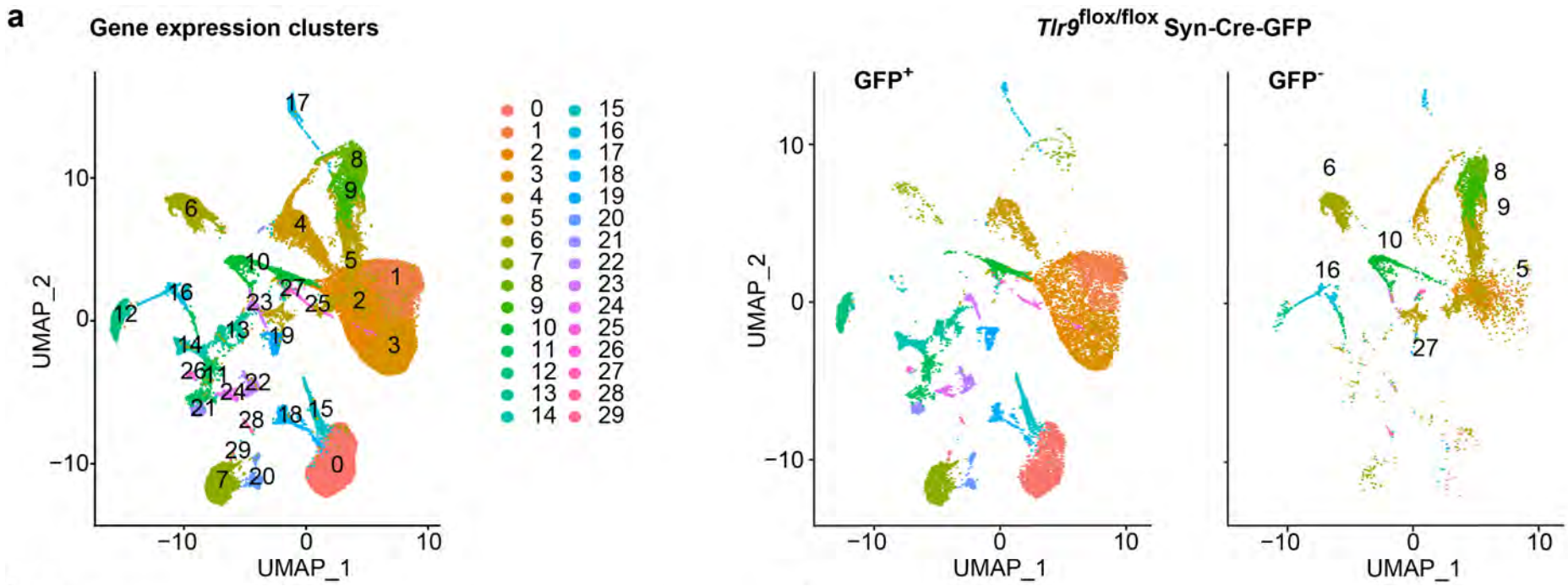
Jovasevic et al., Nature 628: 150 (2024)

# single-nucleus RNA-sequencing (snRNA-seq)

Syn-Cre > *Tlr9*$^{fl/fl}$ , no CFC
[no sorting]

Syn-Cre > *Tlr9*$^{fl/fl}$ , no CFC
[GFP(+) sorted]

Syn-Cre > *Tlr9*$^{fl/fl}$ , no CFC
[GFP(–) sorted]



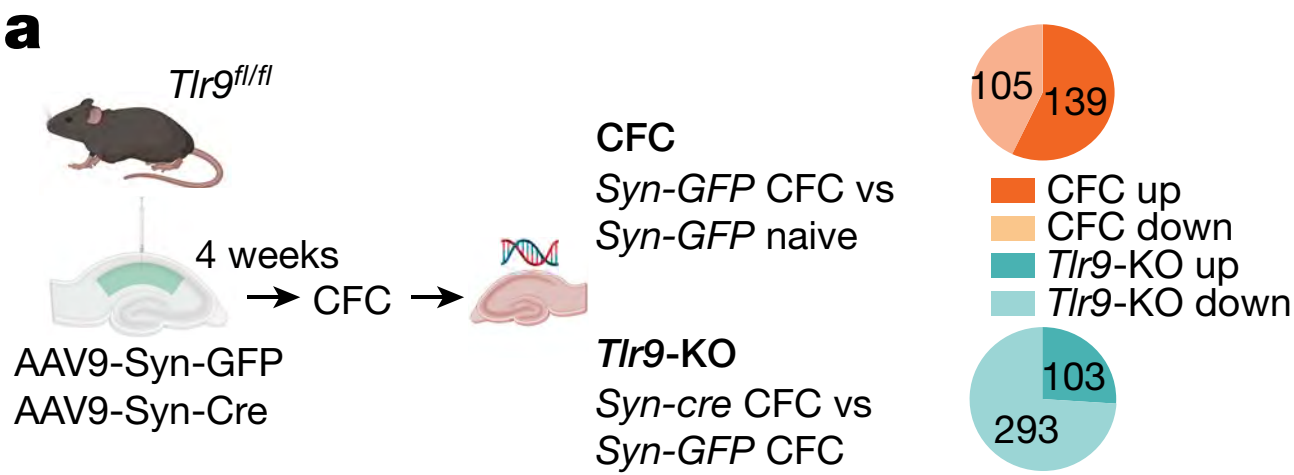

neuron

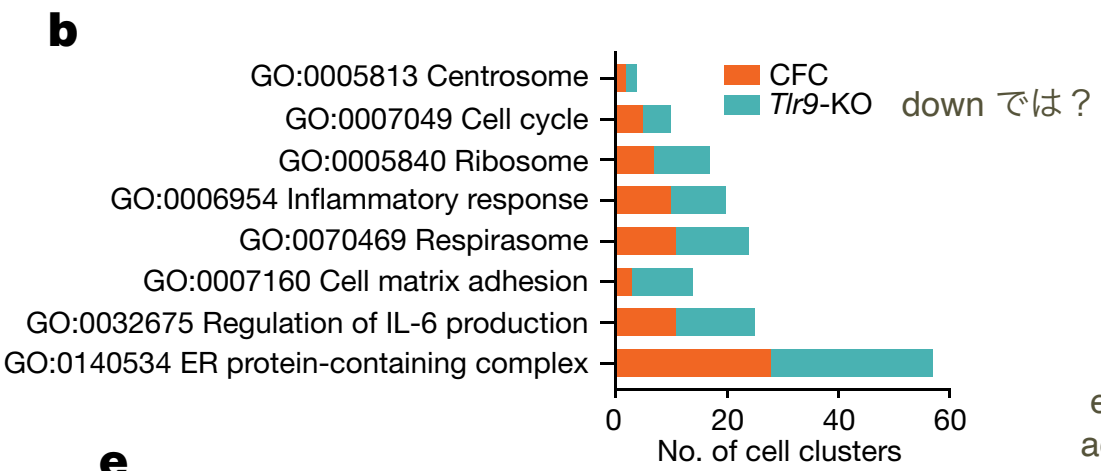
non-neuron

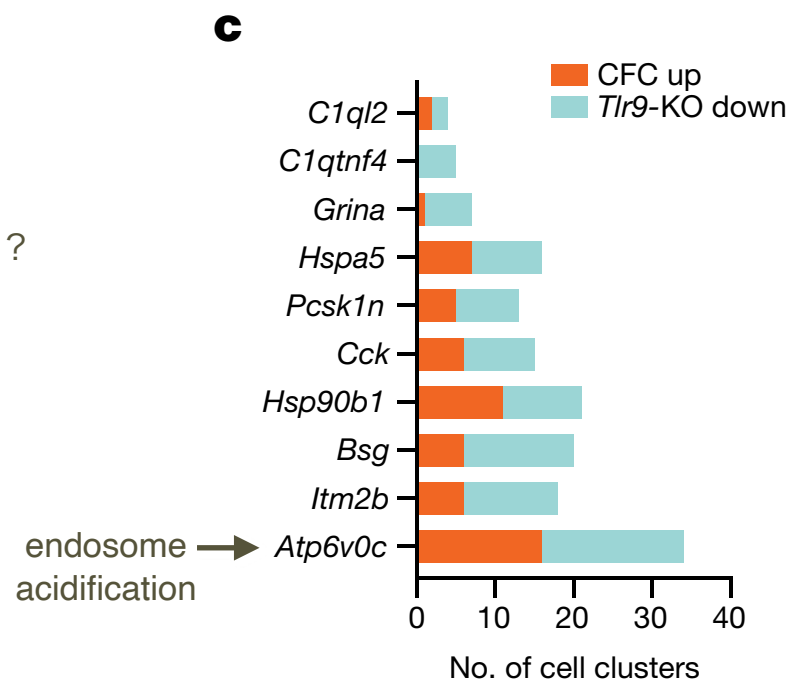
Jovasevic et al., Nature 628: 150 (2024)

# 発現量が変動する遺伝子

# by single-nucleus RNA-sequencing (snRNA-seq)



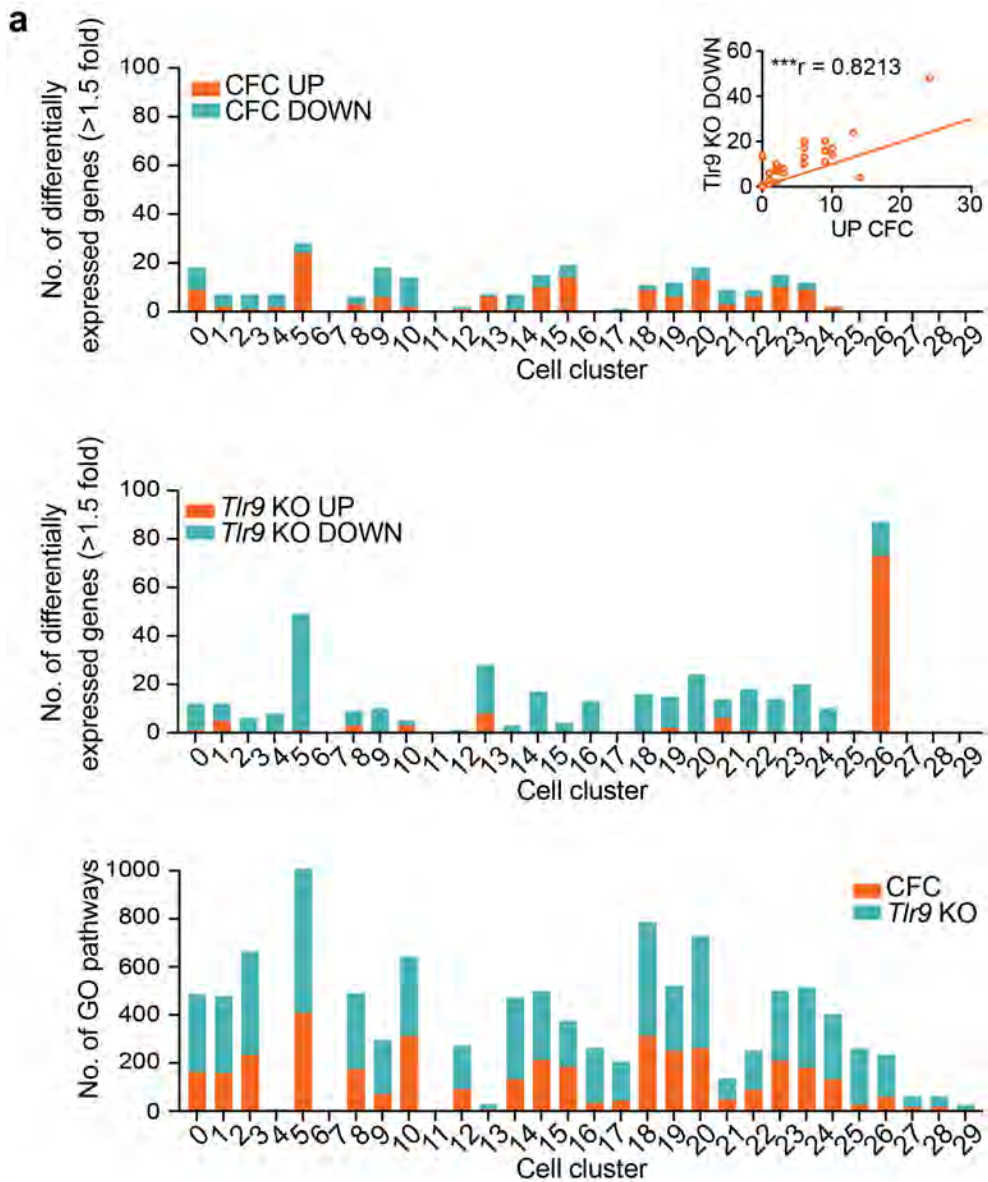




Jovasevic et al., Nature 628: 150 (2024)

# 発現量が変動する遺伝子

# by single-nucleus RNA-sequencing (snRNA-seq)

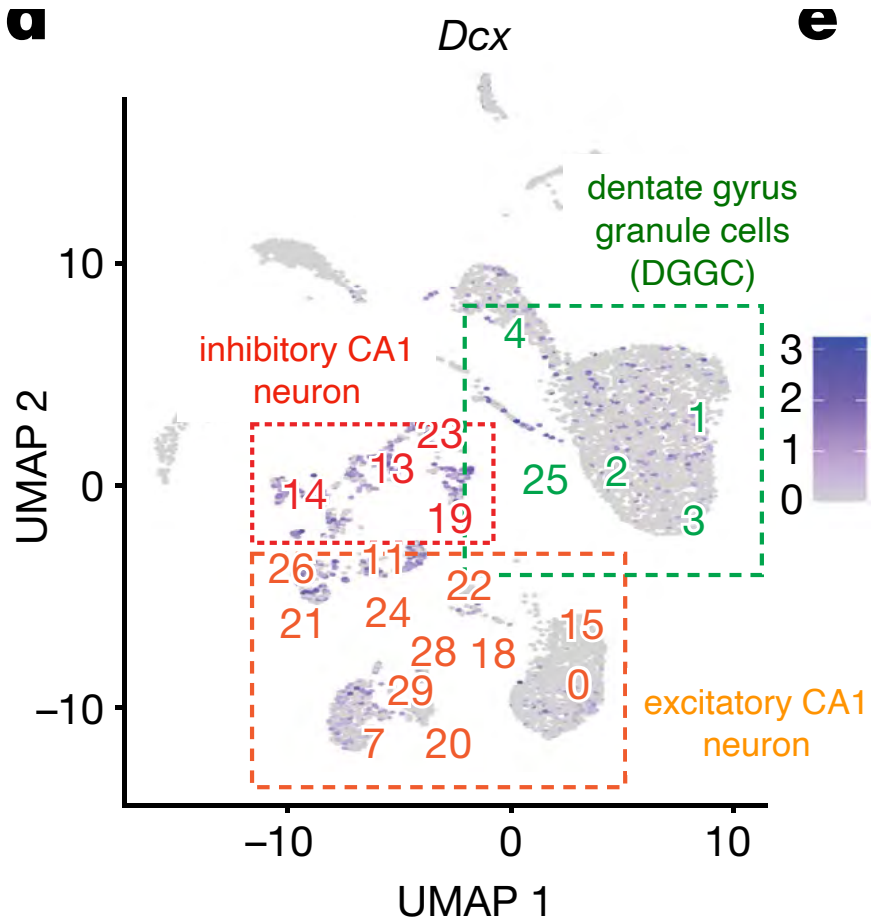


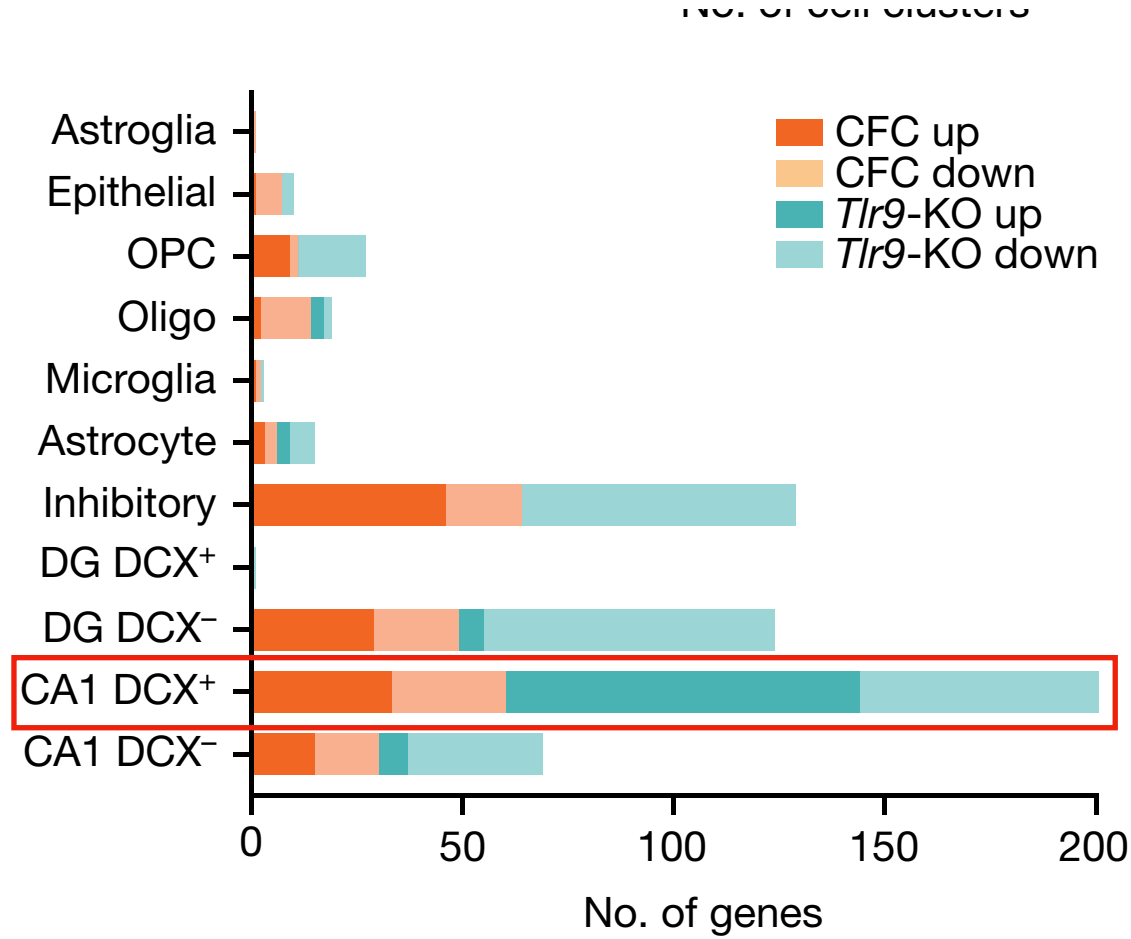

Jovasevic et al., Nature 628: 150 (2024)

# 発現量が変動する遺伝子

# by single-nucleus RNA-sequencing (snRNA-seq)

Doublecortin: 微小管関連タンパク




Jovasevic et al., Nature 628: 150 (2024)

# conserved neuronal marker expression in each cluster

excitatory neurons (0-4, 7, 11, 15, 18, 20-22, 24-26, 28, 29)

inhibitory neurons (13, 14, 19, 23)

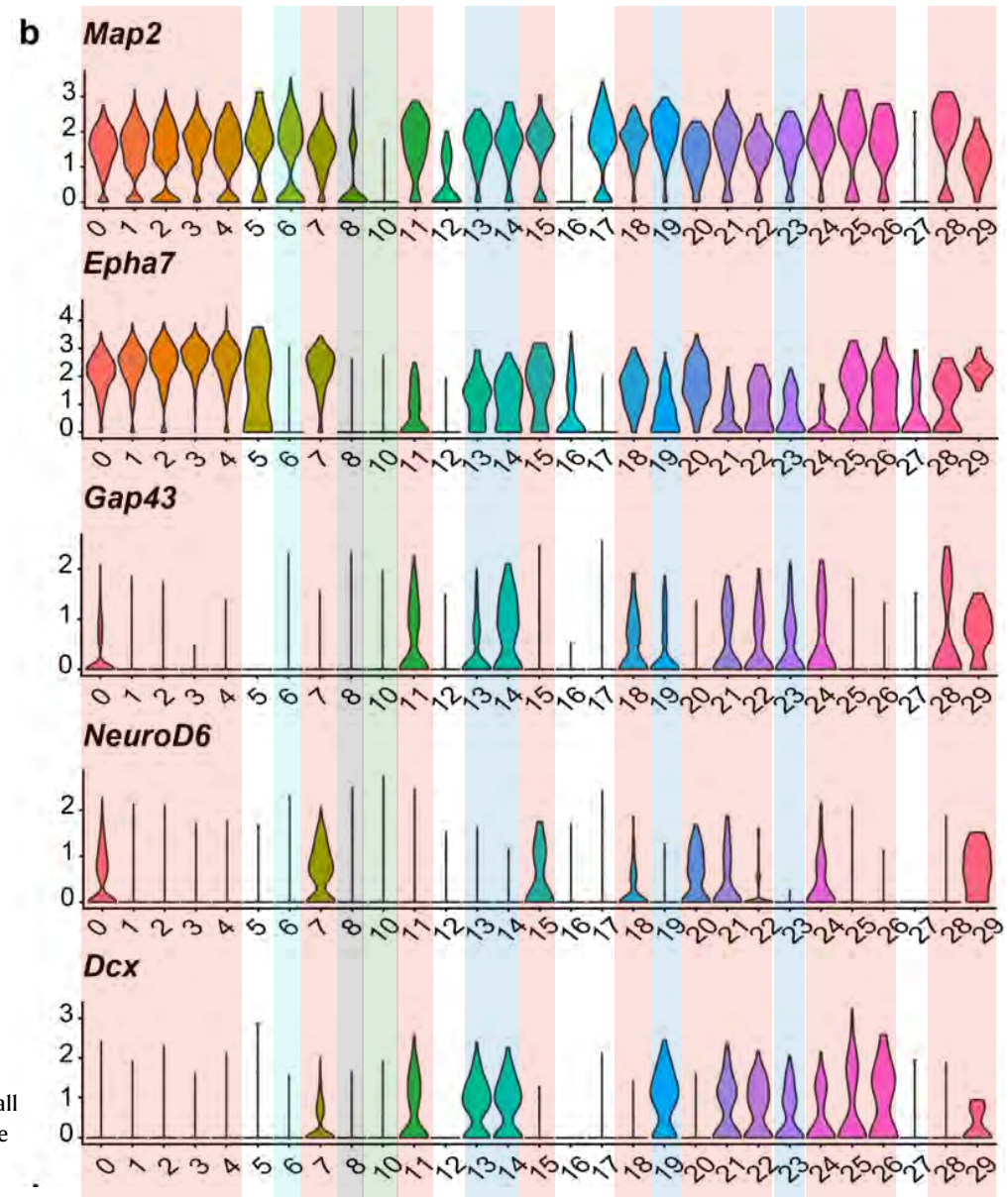
microglia (10)

oligodendrocytes (8,9)

astrocytes (6)

興奮性ニューロンのマーカー

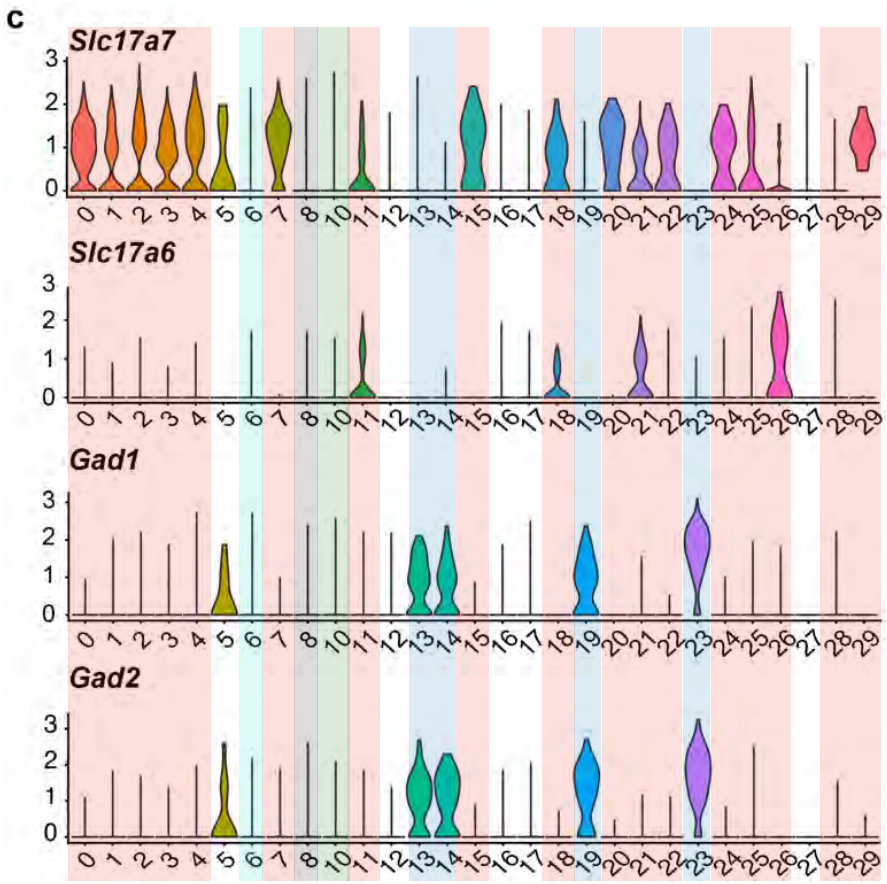
Doublecortin: immature DGGC marker
予想以上に広範な発現

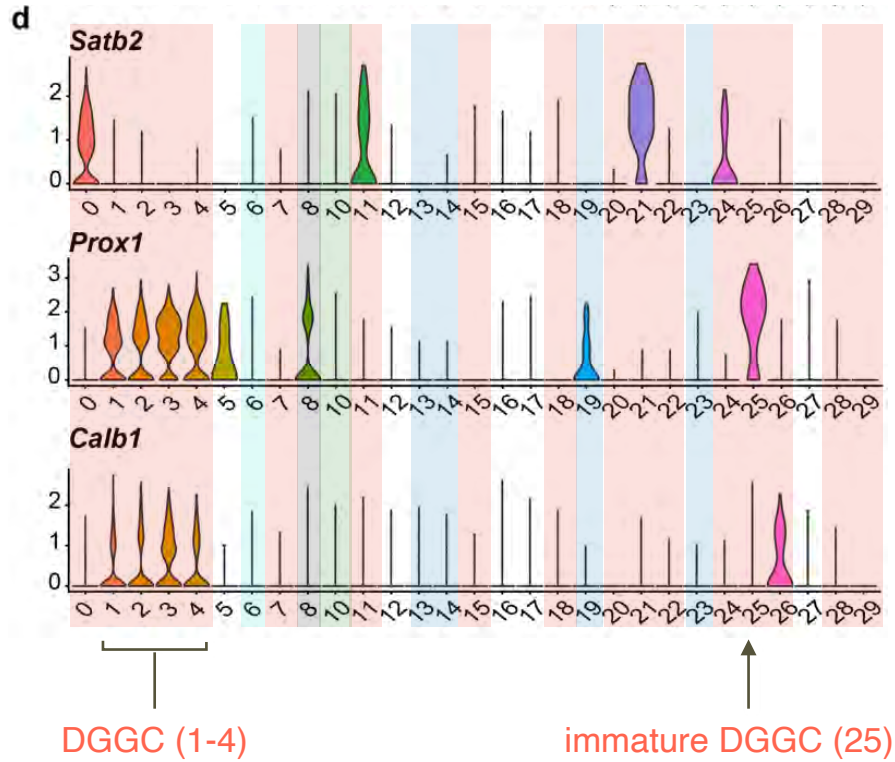



down-regulation of functional pathways (bottom). **b** Neuronal phenotyping with conserved markers revealing presence of Dcx in several excitatory and all inhibitory neuronal clusters in addition to immature DGGC (cluster 25). **c** The

Jovasevic et al., Nature 628: 150 (2024)

# conserved neuronal marker expression in each cluster




excitatory neurons (0-4, 7, 11, 15, 18, 20-22, 24-26, 28, 29)

inhibitory neurons (13, 14, 19, 23)

microglia (10)

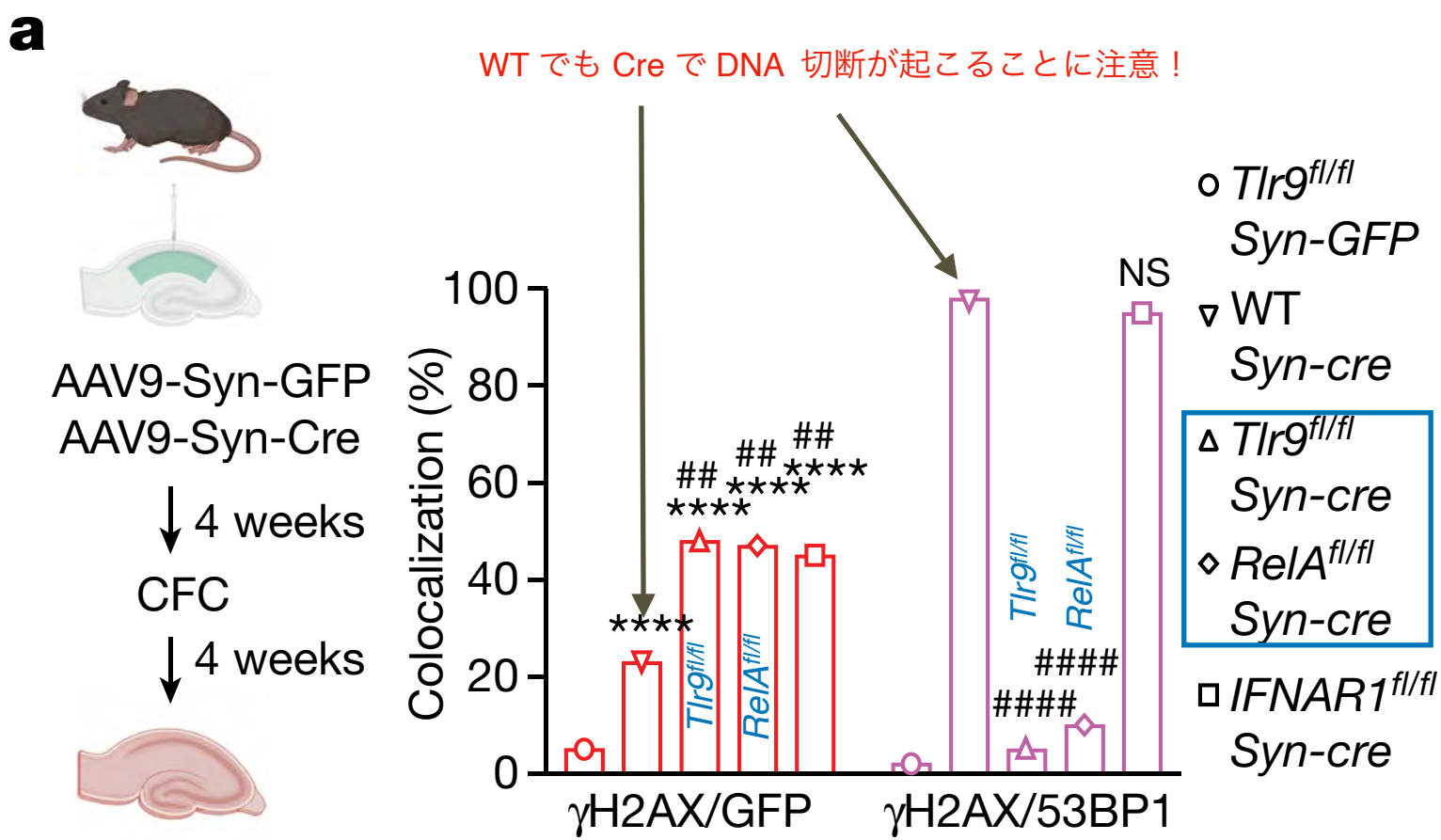
oligodendrocytes (8,9)

astrocytes (6)

inhibitory neuronal clusters in addition to immature DGGC (cluster 25). **c** The expression of excitatory and inhibitory neuron markers as well as **d** CA and DGGC markers were consistent with the clustering method and identified clusters 0, 7, 11, 15, 18, 20, 21, 22, 24, 26, 28, and 29 as excitatory CA neurons, clusters 1-4 as DGGC, cluster 25 as immature DGGC, and clusters 13, 14, 19, and 23 as inhibitory neurons.

Jovasevic et al., Nature 628: 150 (2024)

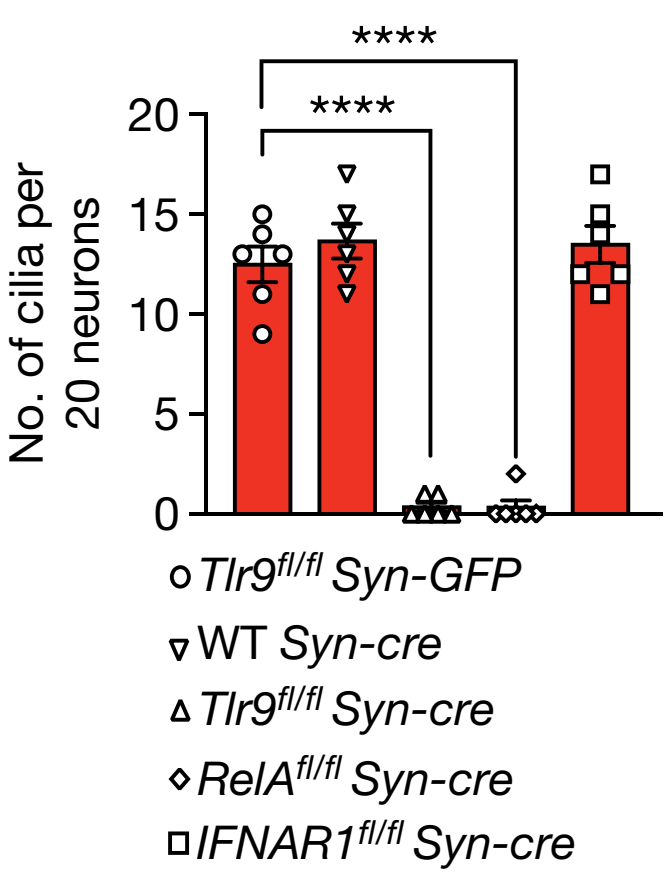
# *Tlr9* KO または *RelA* KO で γH2AX と 53BP1 との共局在（中心体）が減弱する

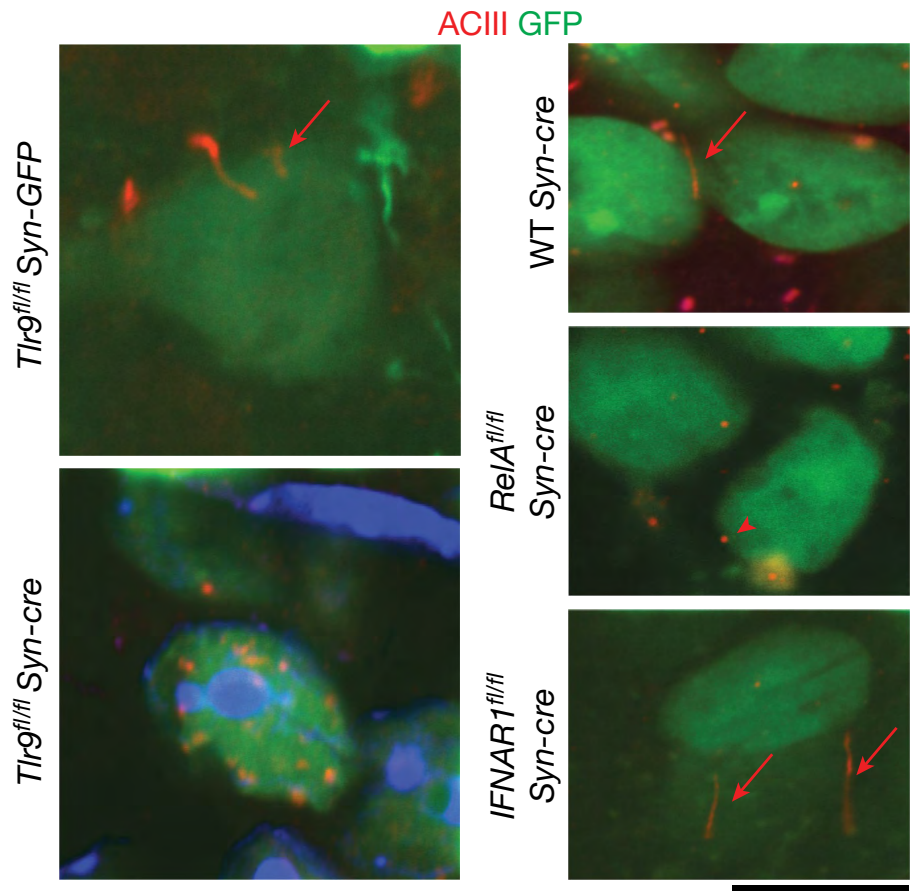



**NF-κB シグナルが DNA 修復反応ー＞繊毛形成に必要**

Jovasevic et al., Nature 628: 150 (2024)

# *Tlr9* KO または *RelA* KO で neuronal cillia が減少する

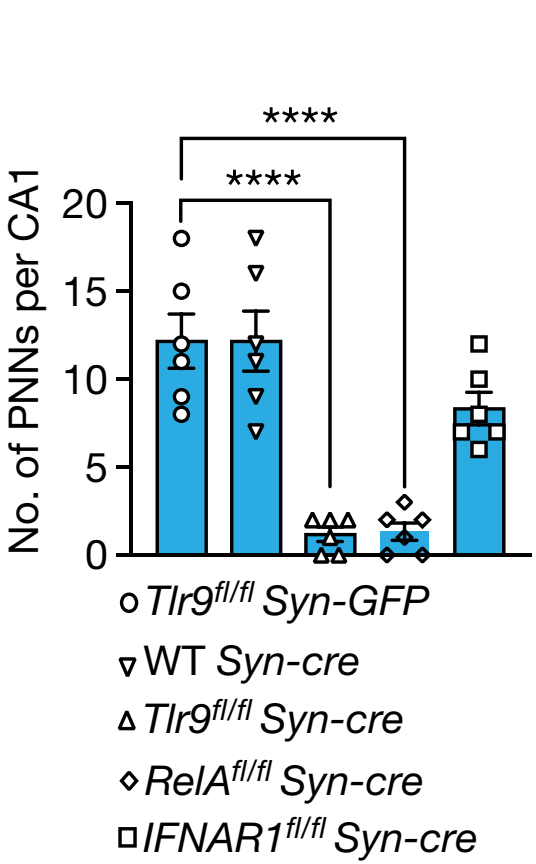


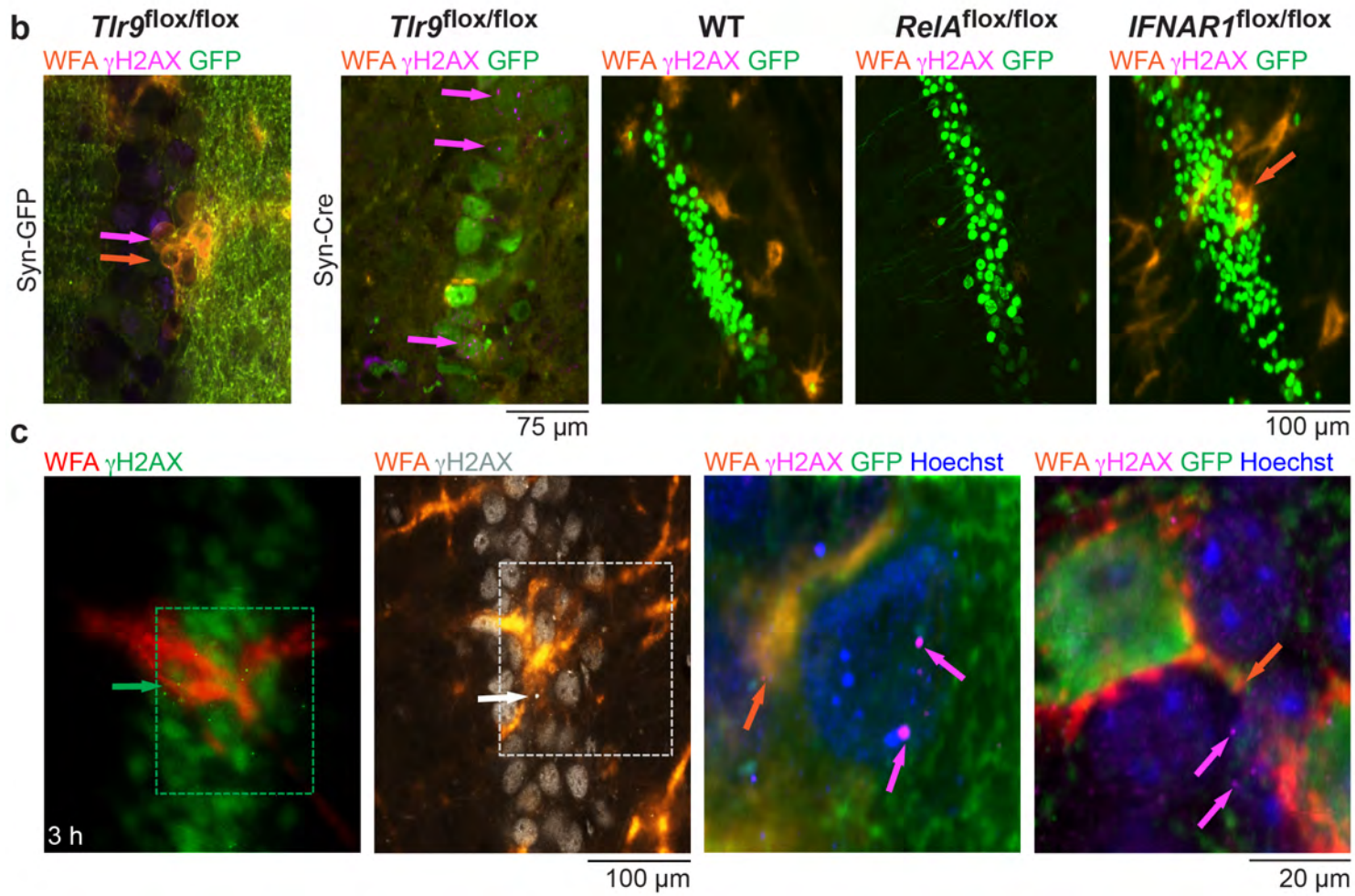




adenyl cyclase III (ACIII) は神経細胞の一次繊毛に局在

Jovasevic et al., Nature 628: 150 (2024)

# *Tlr9* KO または *RelA* KO で perineuronal net (PNN) が減弱する




perineuronal net: 神経細胞の細胞体と樹状突起を覆う細胞外マトリックス。神経可塑性やシナプスの安定化に関与。含まれる糖鎖 N-アセチルガラクトサミンが Wisteria floribunda lectin (WFA) と結合する。

Jovasevic et al., Nature 628: 150 (2024)

# まとめ

- 記憶形成(CFCに関わる)に核膜破裂、 DNA の流出と TLR9 -> NF-κB (RELA) 活性化が必要
- DNA切断＆修復を行う細胞は immediate early gene (IEG, Fos) 発現 を示す細胞とは別。
- NF-κB 活性化の下流で繊毛 & PNN 形成が起こる
- 今後解明されるべきは：
  - 中心体/γH2AX/53BP と繊毛形成の関係
  - DNA 切断メカニズム
  - 記憶できる回数は有限？
  - ODN2088 は記憶定着を阻害？PTSD の予防薬？

# 本研究のその後

- 19の論文で引用 (PMID: 38712188, 38632491, 38920491, 38849394, 39000135, 39063016, 39063148, 39123407, 39095921, 39315362, 39457754, 39354711, 39483923, 39518906, 39468666, 39364746, 39300271, 39511426, 38979269, )

- そのうちの一つ、

Li *et al. Translational Neurodegeneration* (2024) 13:39
https://doi.org/10.1186/s40035-024-00427-8

Translational
Neurodegeneration

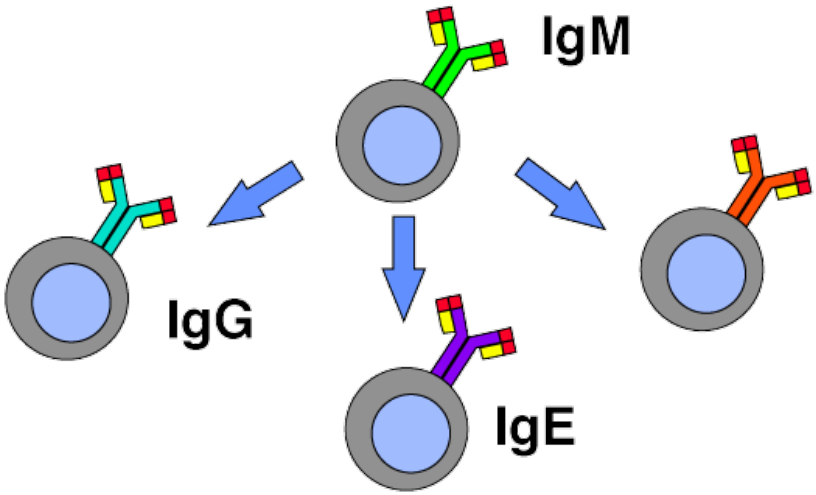
**RESEARCH** **Open Access**

## Neuronal double-stranded DNA accumulation induced by DNase II deficiency drives tau phosphorylation and neurodegeneration

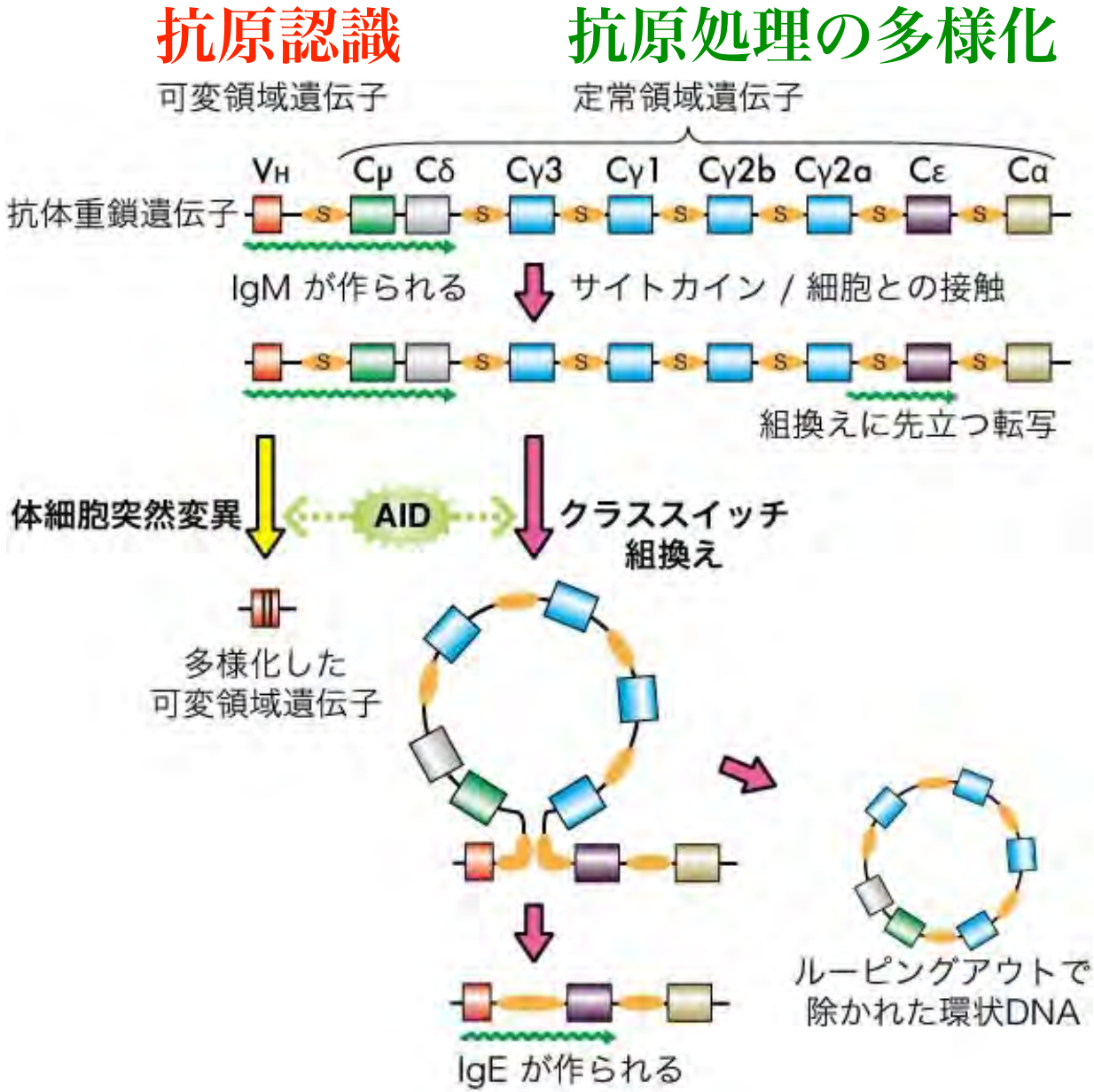
Ling-Jie Li[1,2†], Xiao-Ying Sun[1†], Ya-Ru Huang[1], Shuai Lu[1], Yu-Ming Xu[3], Jing Yang[3], Xi-Xiu Xie[1], Jie Zhu[1,2], Xiao-Yun Niu[1,4], Dan Wang[5], Shi-Yu Liang[1,2], Xiao-Yu Du[1,2], Sheng-Jie Hou[1,2], Xiao-Lin Yu[1*] and Rui-Tian Liu[1*]

AD 患者の神経細胞で DNase II 減少。
DNase II 減少は CDK5, CaMKII, PP2A により tau リン酸化を促進。
マウスで DNase II KD は神経細胞喪失、神経炎症、認知障害を誘導し、
DNase II 強制発現はこれらを改善した。

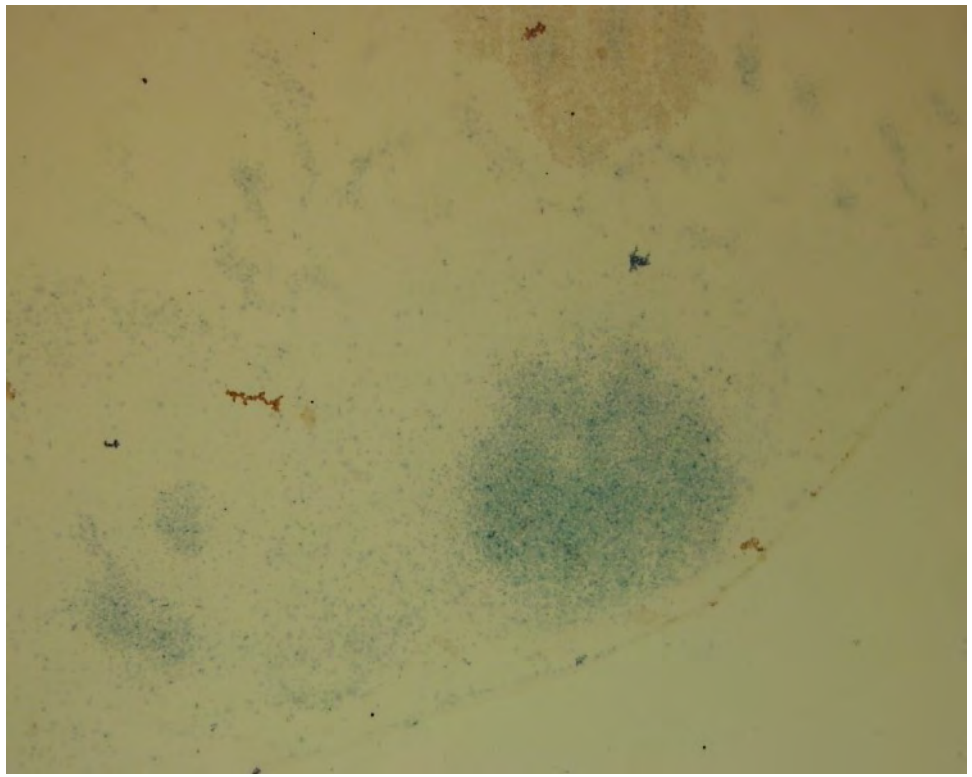
# 抗体のクラススイッチ



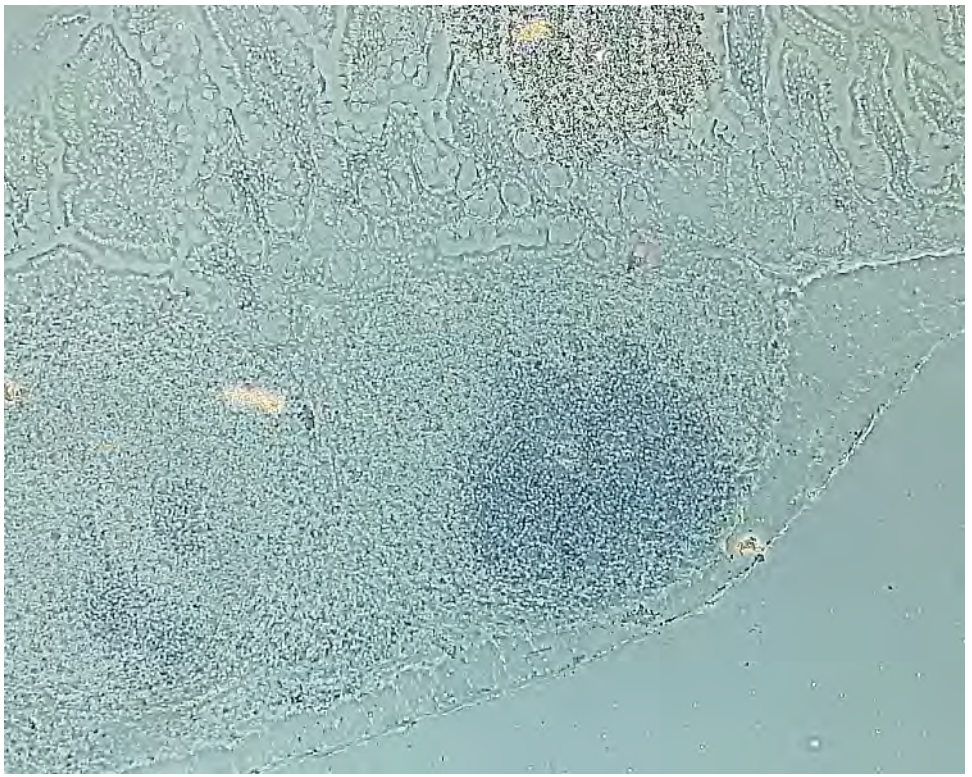

本庶　佑



# AID expression history in the Peyer's patch by lineage tracing (LacZ)



LacZ



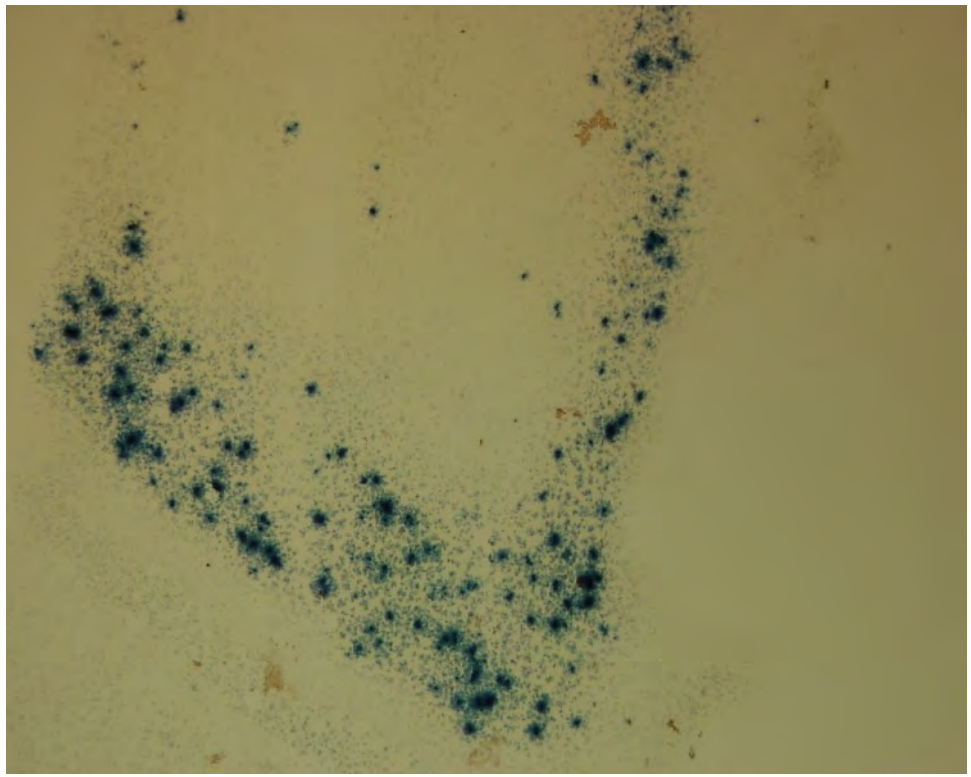
LacZ + phase contrast

木下　未発表データ

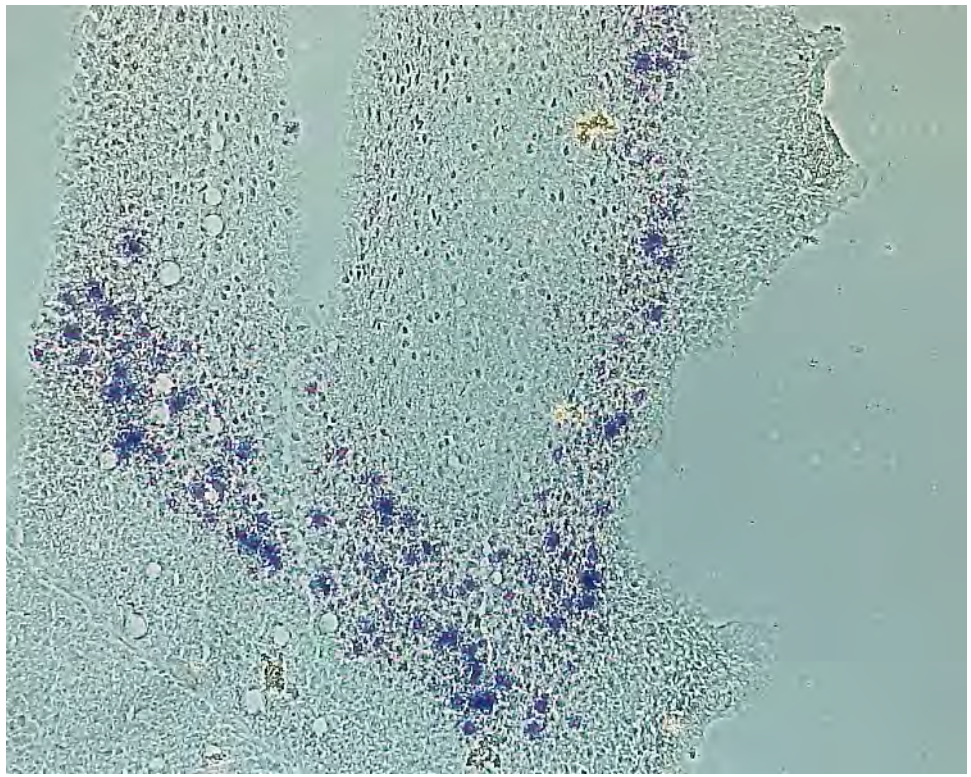
Aicda-cre + R26R-LacZ, female, 25 weeks

x 100

# AID expression history in the brain by lineage tracing (LacZ)



LacZ



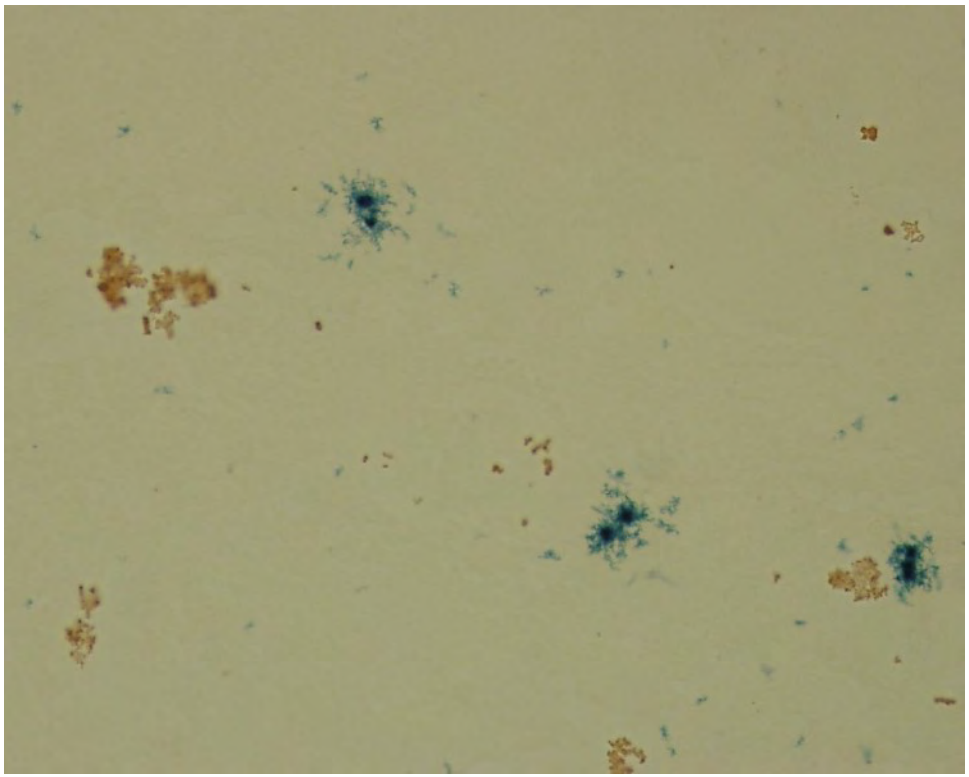
LacZ + phase contrast

木下　未発表データ

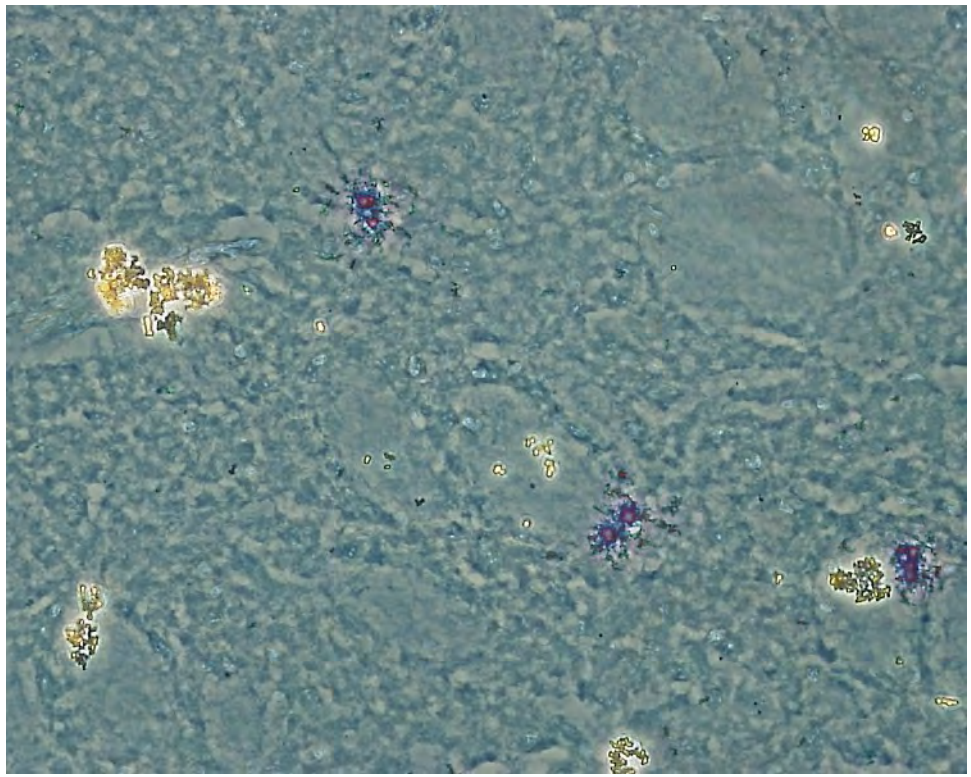
Aicda-cre + R26R-LacZ, male, 63 weeks

x 100

# AID expression in the brain by lineage tracing (LacZ)



LacZ



LacZ + phase contrast

木下　未発表データ

Aicda-cre + R26R-LacZ, female, 25 weeks

x 400