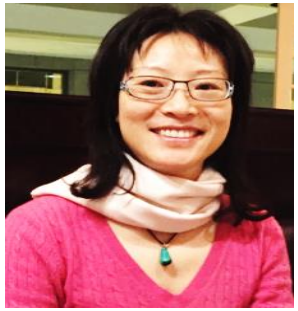
嗜神经病毒与神经环路示踪

罗敏华

病毒学国家重点实验室
中国科学院武汉病毒研究所

简介



罗敏华

中科院武汉病毒所 研究员

主要研究对象：

嗜神经疱疹病毒

HCMV/MCMV

HSV-1/2, VZV

长期从事医学病毒学基础及应用研究

主要成就：**1.**建立和完善**CMV**等致神经损伤模型；**2.** 研发顺向神经环路示踪工具，填补空白，转化；**3.** 疫苗研发，与国药集团合作研发神经减毒疫苗。

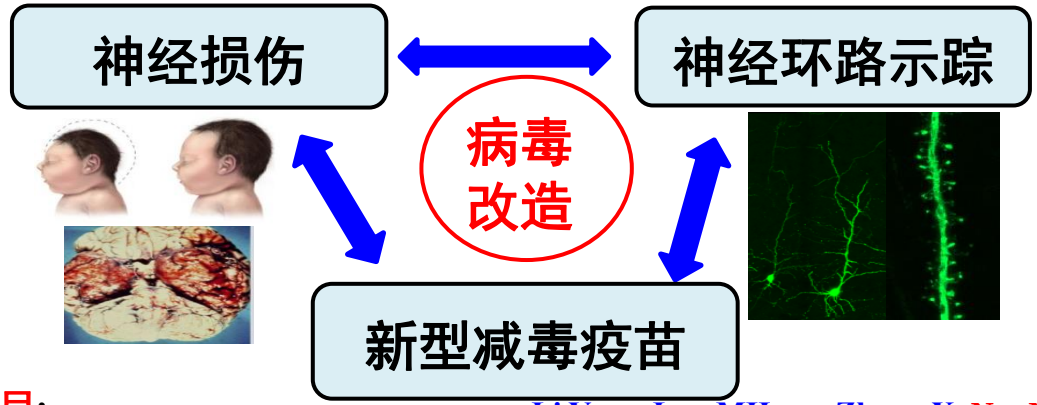
发表研究论文60余篇， Nat. Neurosci, Neuron, Nat. Comms, Cell Host & Microbe、PLoS Pathog、J Virol等。

曾获湖南省自然科学一等奖，教育部科学技术二等奖

研究方向和平台

Liu XJ, …, Luo MH*. *PLoS Pathog*. 2017
Jiang HF, …, Luo MH*. *J Virol*. 2017
Yang B,… Luo MH*. *J Virol*. 2018
Wu C…, Luo MH*. *J Virol* 2018
Jiang X…, Luo MH* Frontiers. 2018
Wen L… Luo MH*. Protein and Cell 2020

Zeng WB, …, Luo MH*. *Mol Neurodegener.* 2017
Zhao F …, Luo MH*. Neurosci Bull. 2017
Yang H…. Luo MH*. Neurosci Bull. 2020
Xu X…Luo MH..Neuron 2020
Dong X…., Luo MH. *J Virol* 2020
Sun J…Luo MH*..Wang P Nat. Comms 2020



近期主要科研项目:

卓越中心-神经环路示踪技术先导 (XDBS010302)
国家自然科学基金国际合作重点 (81620108021)
国家自然科学基金重大科研仪器研制项目 (81427801)
国家科技部973（2015CBR55600）
美国NIHRF1 (1RF1MH120020-01) (2019-2022)
国药/中生/祈健 新型减毒疫苗 （2020-2025）

Li Y, …, Luo MH, …, Zhang X. Nat Neurosci. 2017
Zhu H, …, Luo MH, …, Lu Y. Nat Commun. *2017*
Wang YY, …, luo MH, …Wang XM. Exp Neurol. 2017
Yu K, …, Luo MH, …, Li B. Nat Neurosci 2018
Fu Y …, Luo MH….Shu H. Cell Host & Microbe 2017
Huang ZF …, Luo MH….Wang YY. Cell Host & Microbe 2018
Ye L… Luo MH..Zhong B. PLoS Pathog. 2019
Sun T… Luo MH…Zhang Z. PNAS 2019

Wuhan Institute of Virolog, CAS

Sate Key Laboratory of Virology

Luo Lab

National Biosafty Laboratory (P3/P4)

Center for Biosafety Mega-Science

(生物安全大科学中心）

大纲

◆ 脑计划与病毒工具的需求

◆ 病毒的特性与病毒工具

AAV； RV； HSV-1(H129)

◆ 病毒工具与生物安全

Brain Projects - worldwide

Neurotechnologies —Brain connectome— How brain works

US BRAIN Initiative
(Brain Research through Advancing Innovative Neurotechnologies)

European Human Brain Project
(Neuroscience, computing and brain-related medicine)

Brain Connectome

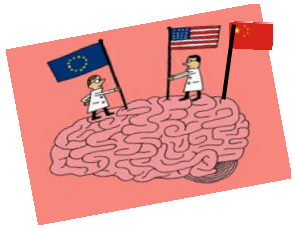
中国脑计划：一体两翼

Flow with the flow

Hope not be drown

神经环路与脑联结和脑疾病

- **脑联结**
  - 脑区内局域神经回路（Local)
  - 脑区间长程投射回路（Projection)



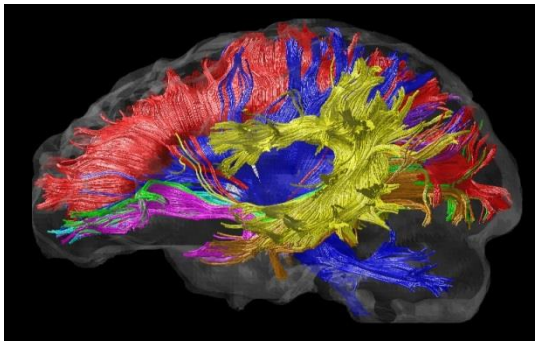
- **脑疾病**
  - 脑疾病：神经回路的结构和功能变化

关键问题：神经回路的解析

关键技术：神经回路的标记与标记工具

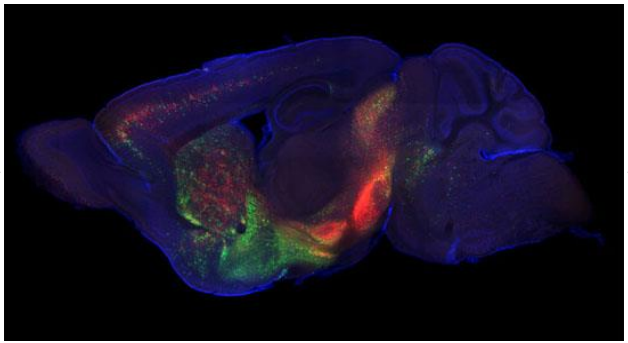
Brain connectome at different scales

Macroscale



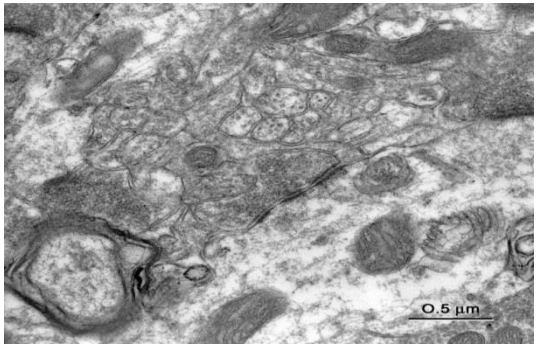
Diffusion MRI

Mesoscale



Neuron-neuron, circuits

Microscale

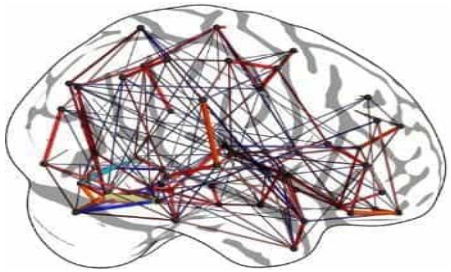


Electron microscope

Transsynaptic tracers are required

Nature 2014, 508(7495):207-14.

Neural Circuit



Neural circuit Connectivity: **The key to understand the principles of motor, sensory, behavior and cognitive systems.**

Circuit pathway: **input pathway and output pathway**

Questions： **How to trace the neural circuits?** **Innovative tools/technology**

大纲

◆ 脑计划与病毒工具的需求

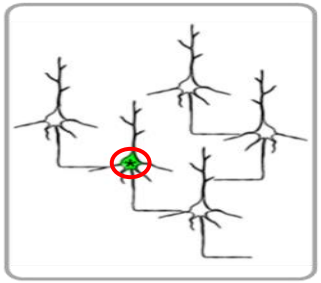
◆ 病毒的特性与病毒工具

AAV； RV； HSV-1(H129)

◆ 病毒工具与生物安全

Viral Tracers

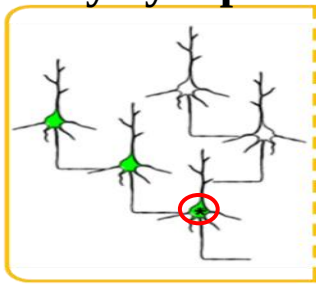
Non-transneuronal



AAV, CAV

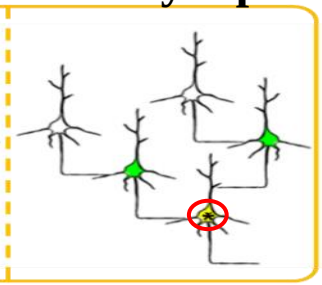
Retrograde

Poly-synaptic



PRV

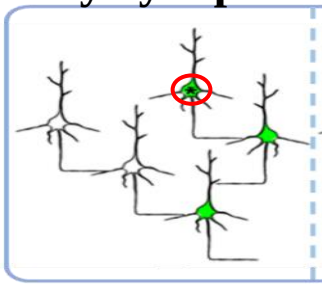
Mono-synaptic



RV, PRV

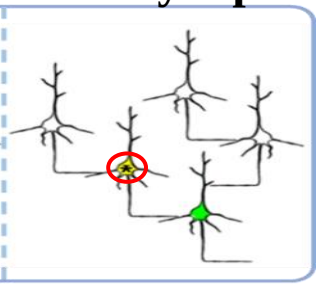
Anterograde

Poly-synaptic

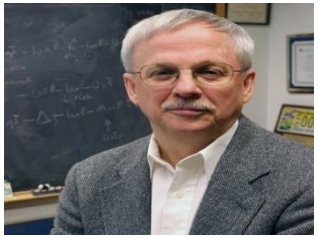


HSV-1

Mono-synaptic



HSV-1 dTK



Lynn Enquist

Edward Callaway

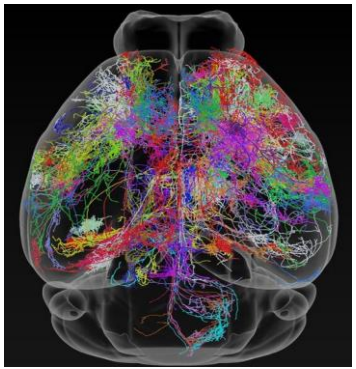
David Anderson

Pomeranz L E , *et al.* The J Neurosci, 2017

Wall N R, *et al.* PNAS, 2010
Wickersham I R , *et al.* Nature Methods, 2007

Lo L, *et al.* Neuron. 2011
Nassi JJ, *et al.* Front Neuroanat. 2015
Zingg B, *et al.* Neuron. 2017
Zeng WB, *et al.* Mol Neurodegener. 2017

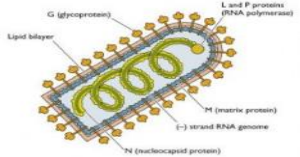
Question: Why Virus and Viral Tools



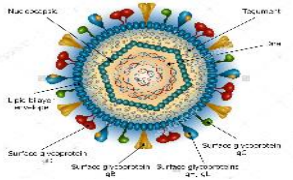
The advantages of virus:

- **Virus replication: amplify the signal**
- **Virus transmission: transneuronal tracing**
- **Neurotropism: modify and specific neuron type tracing**

AAV



RV

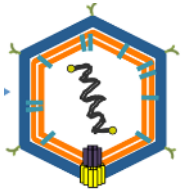


HSV

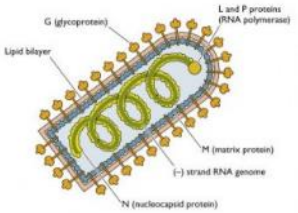
病毒的生物学特性

病毒： 严格活细胞内寄生的最小微生物，电镜下可见

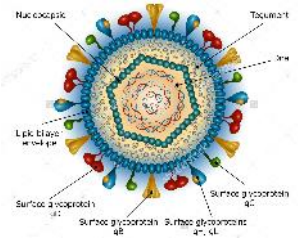
结构： 简单



AAV



RV



HSV/PRV

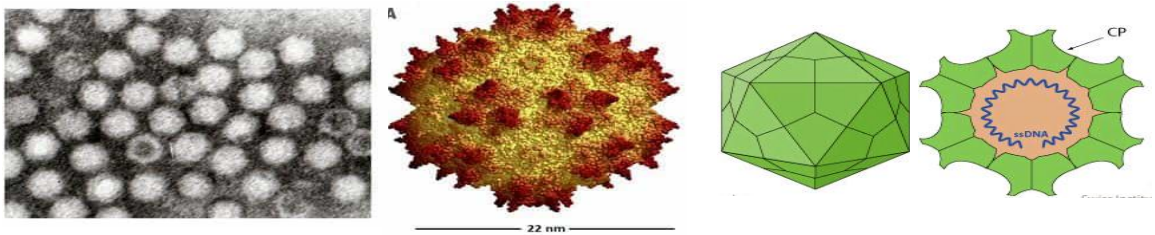
分类： 1）根据基因组分RNA、DNA病毒：

AAV/PRV/HSV-DNA病毒；RV-RNA病毒

2) 有无包膜分包膜病毒、无包膜病毒

AAV-无包膜V；PRV/HSV/RV-包膜V

腺相关病毒
Adeno-associated virus (AAV)



分类

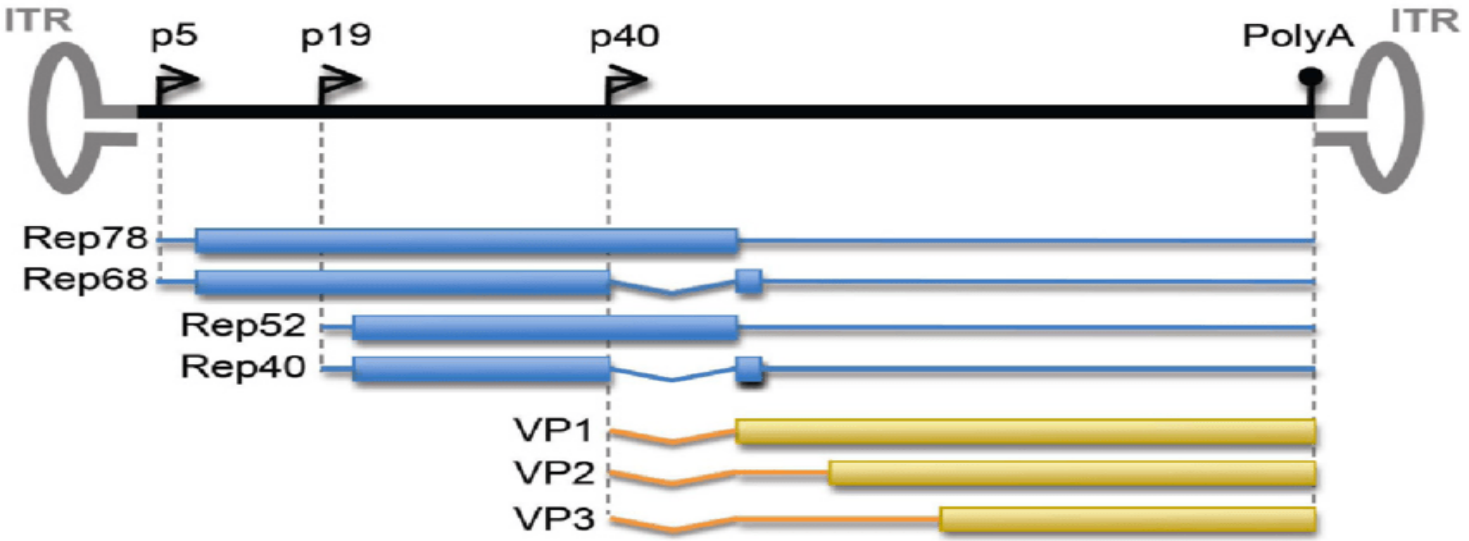
- **Parvoviridae family (细小病毒科)：** 25 nm diameter (small), non-enveloped
- **Genome:** ssDNA, genome size of 4.7 kb
- **Serotype（血清型）:** multiple distinct serotypes, capsid variants
- **Toxicity:** no known disease association， promising vector for gene delivery

腺相关病毒（AAV）

生物学特性：

- ssDNA virus，4.7kb
- Depends on helper virus for their replication and gene expression, defective virus
- There are 2 ORFs: 4 Rep proteins, 3 cap proteins, Flanked by ITR
- Very stable: temperature, acid

AAV基因组



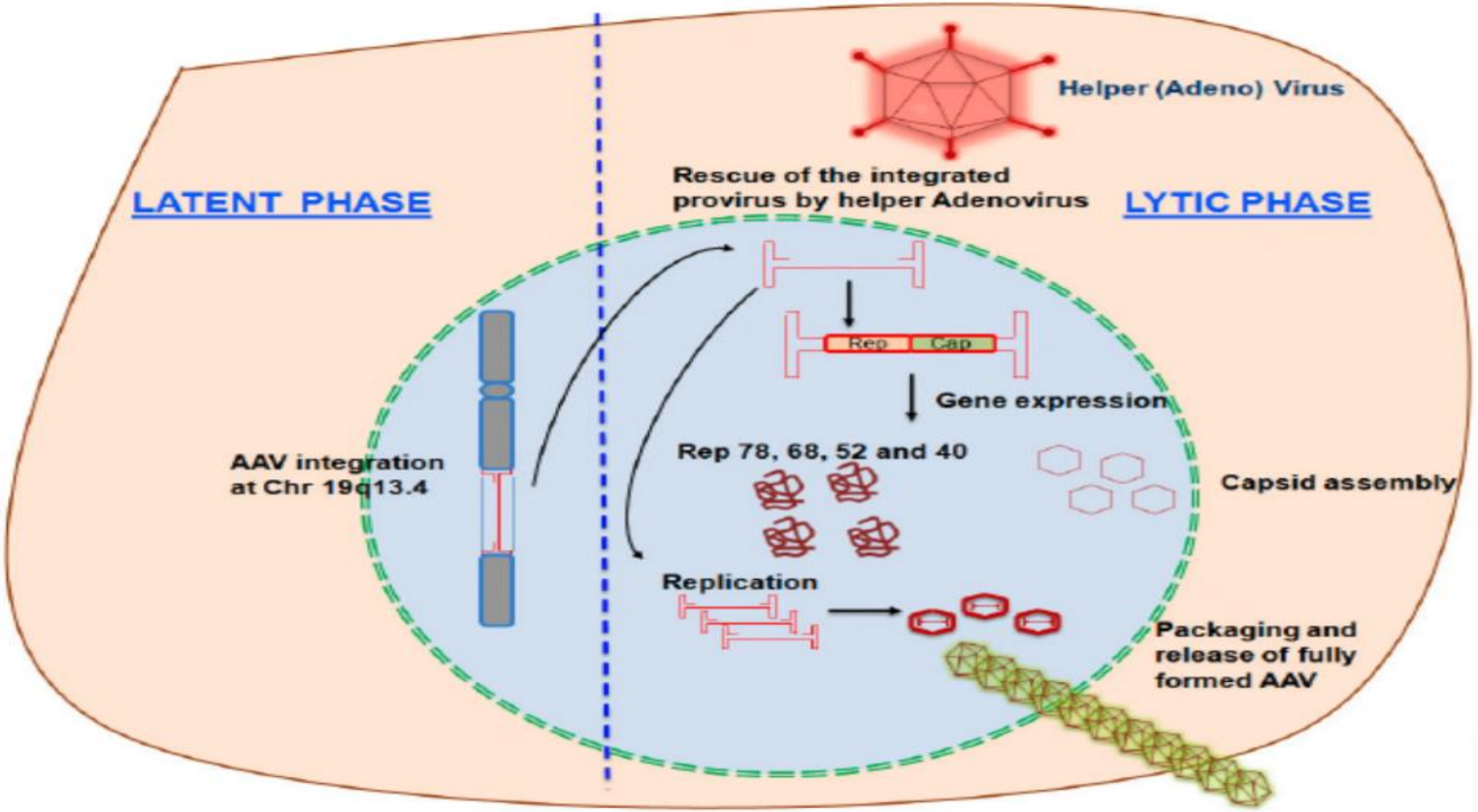
主要基因的功能：

Rep78/68: involved in genome replication

Rep52/40: involved in genome packaging

VP1/2/3: capsid proteins

AAV生命周期


Helper (Adeno) Virus
LATENT PHASE
Rescue of the integrated provirus by helper Adenovirus
LYTIC PHASE
Rep
Cap
Gene expression
AAV integration at Chr 19q13.4
Rep 78, 68, 52 and 40
Capsid assembly
Replication
Packaging and release of fully formed AAV

AAV天然血清型

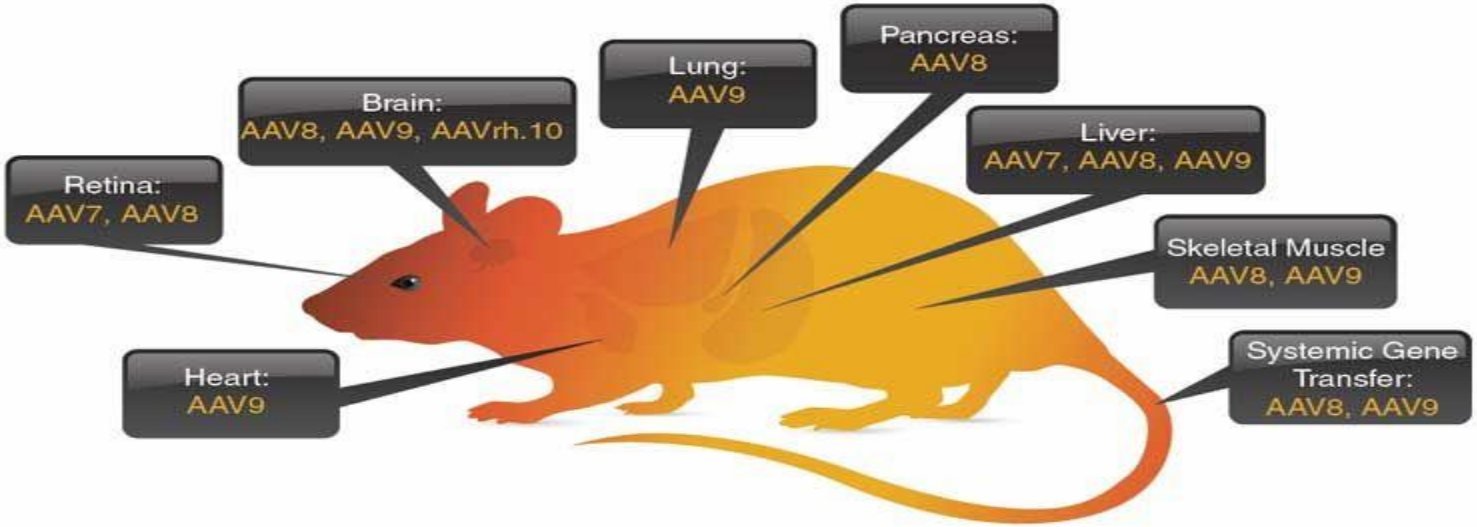
	AAV1	AAV2	AAV3	AAV4	AAV5	AAV6	AAV7	AAV8	AAV9
Primary receptor	N-linked sialic acid	HSPG	HSPG	O-linked sialic acid	N-linked sialic acid	N-linked sialic acid; HSPG	unknown	unknown	N-linked galactose
Secondary receptor	unknown	FGFR1, HGFR, integrins, CD9, LamR	FGFR1, HGFR, LamR	unknown	PDGFR	EGFR	unknown	LamR	LamR

HSPG = Heparan Sulfate ProteoGlycans
FGFR1 = Fibroblast Growth Factor Receptor 1
HGFR = Hepatocyte Growth Factor Receptor 1

PDGFRB = Platelet-derived Growth Factor Receptor beta
EGFR = Epidermal Growth Factor Receptor
LamR = Laminin Receptor

区别：衣壳蛋白/嗜性，ITR序列及复制相关蛋白/复制能力

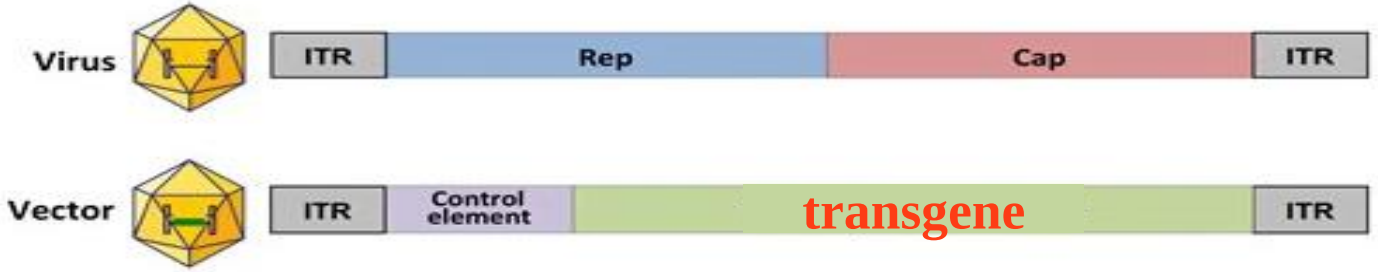
不同血清型的组织嗜性



病毒的组织嗜性与受体的组织分布密切相关

异源表面受体——异源配体：**ENVA/TVA, HER2?**

AAV作为工具的优势



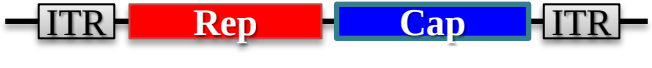
- **AAV is effective in transducing many mammalian cell types**
- defective in replication, low toxicity
- long-term transgene expression in animal models
- various serotypes for different tissues
- A major practical advantage： AAV can be handled in biosafety level 1 (BSL1) facilities.

This makes AAV the ideal viral vector system for many studies.

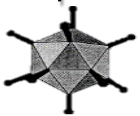
AAV载体

◆ 自然感染

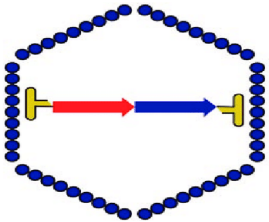
AAV genome



\+



Adenovirus



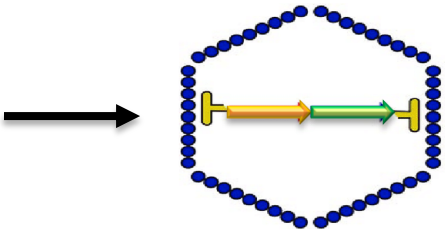
◆ 改造后制备

AAV vector

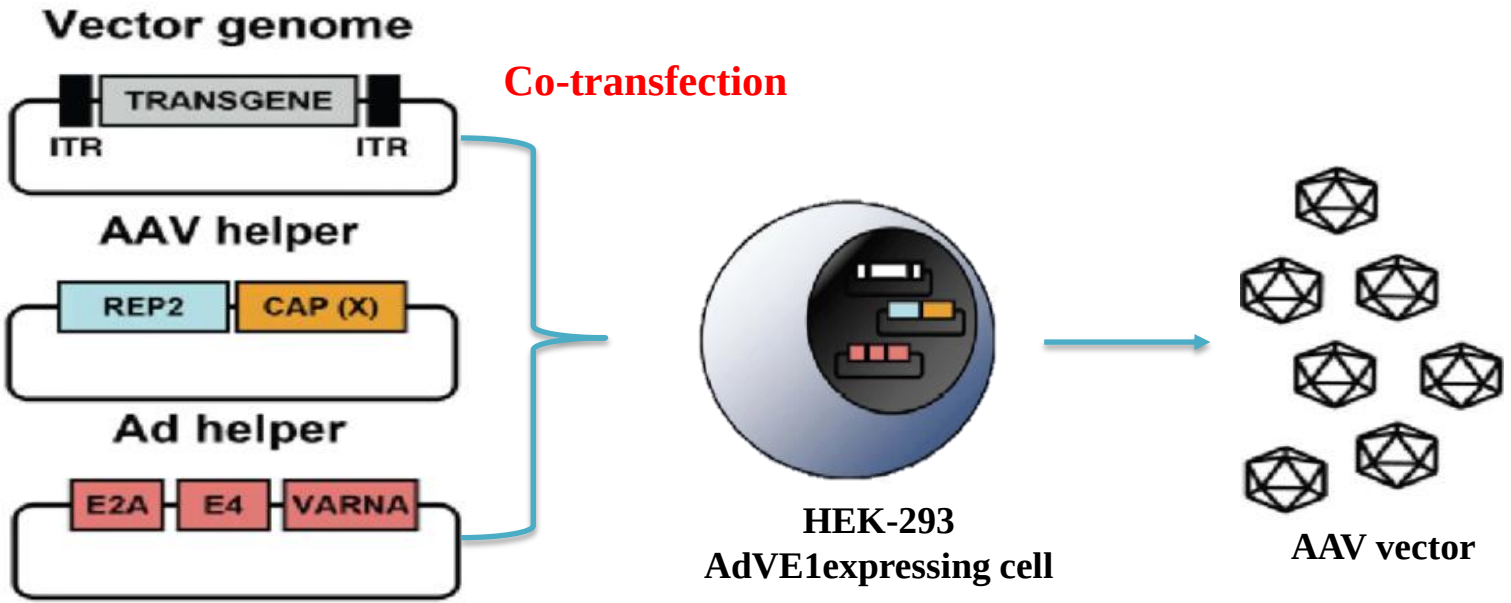
\+

Rep/Cap plasmid

Adv helper plasmid

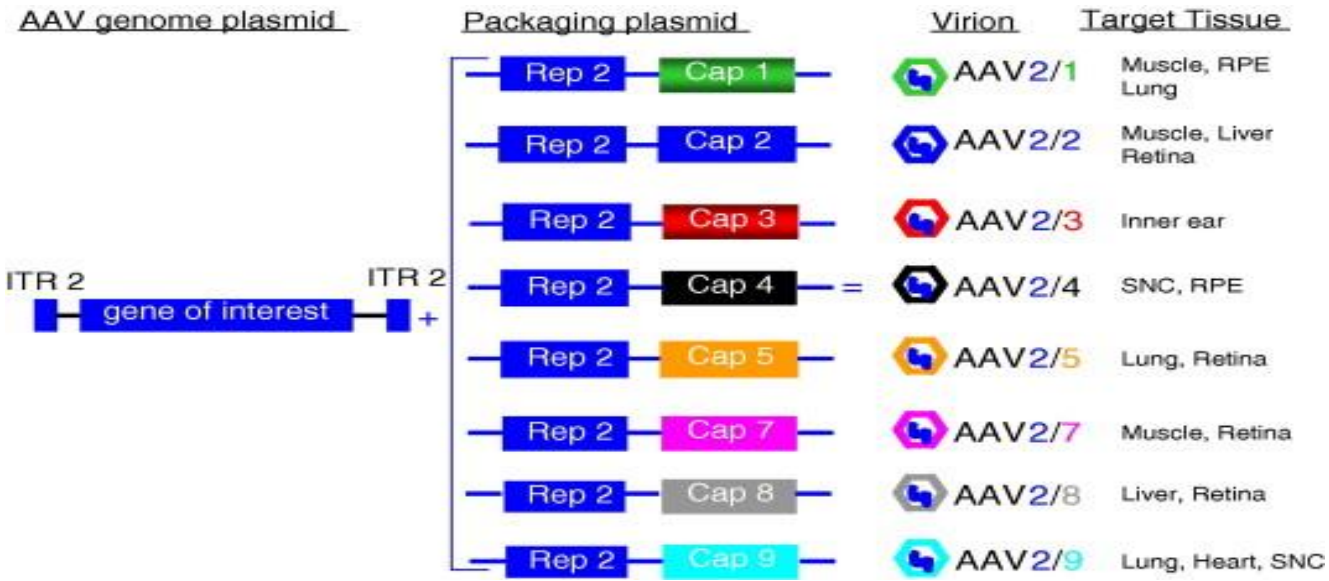


AAV载体的生产过程



ITR和目的外源基因
ITR cap基因，rep基因
AdV含辅助载体（E1，E2a），AAV复制

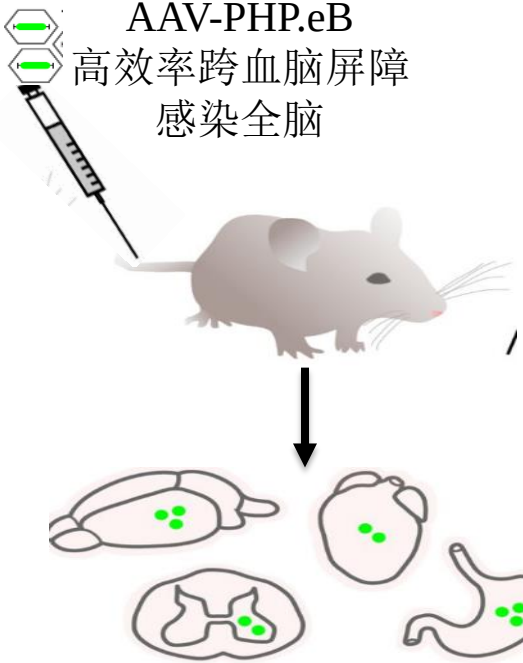
重组AAV载体的血清型和靶向性

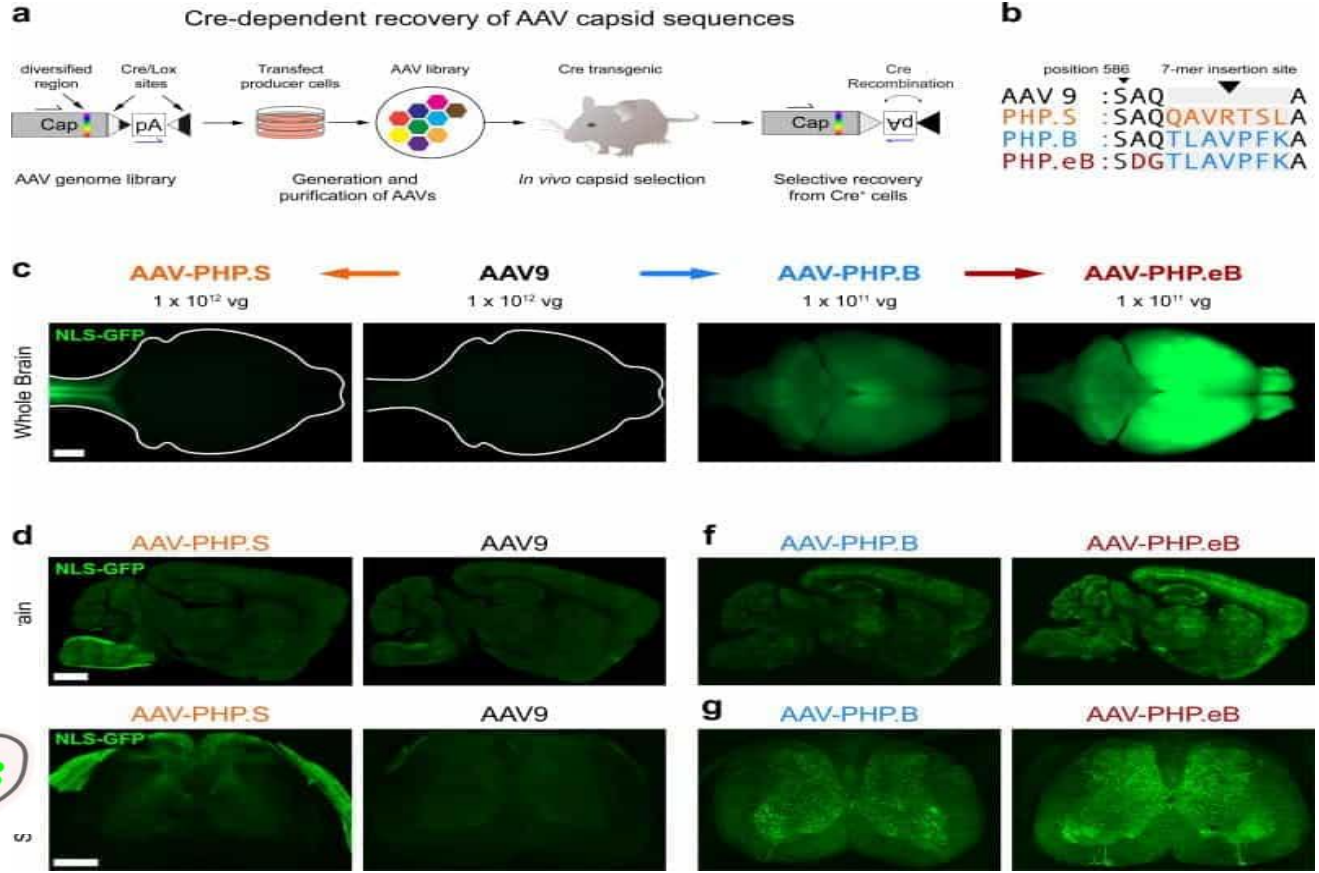


1. AAV载体都使用AAV2 的ITR，并利用Rep2进行复制
2. 选择不同Cap以获得不同血清型，产生不同嗜性

人工筛选获得 新的AAV血清型: AAV-PHP.eB

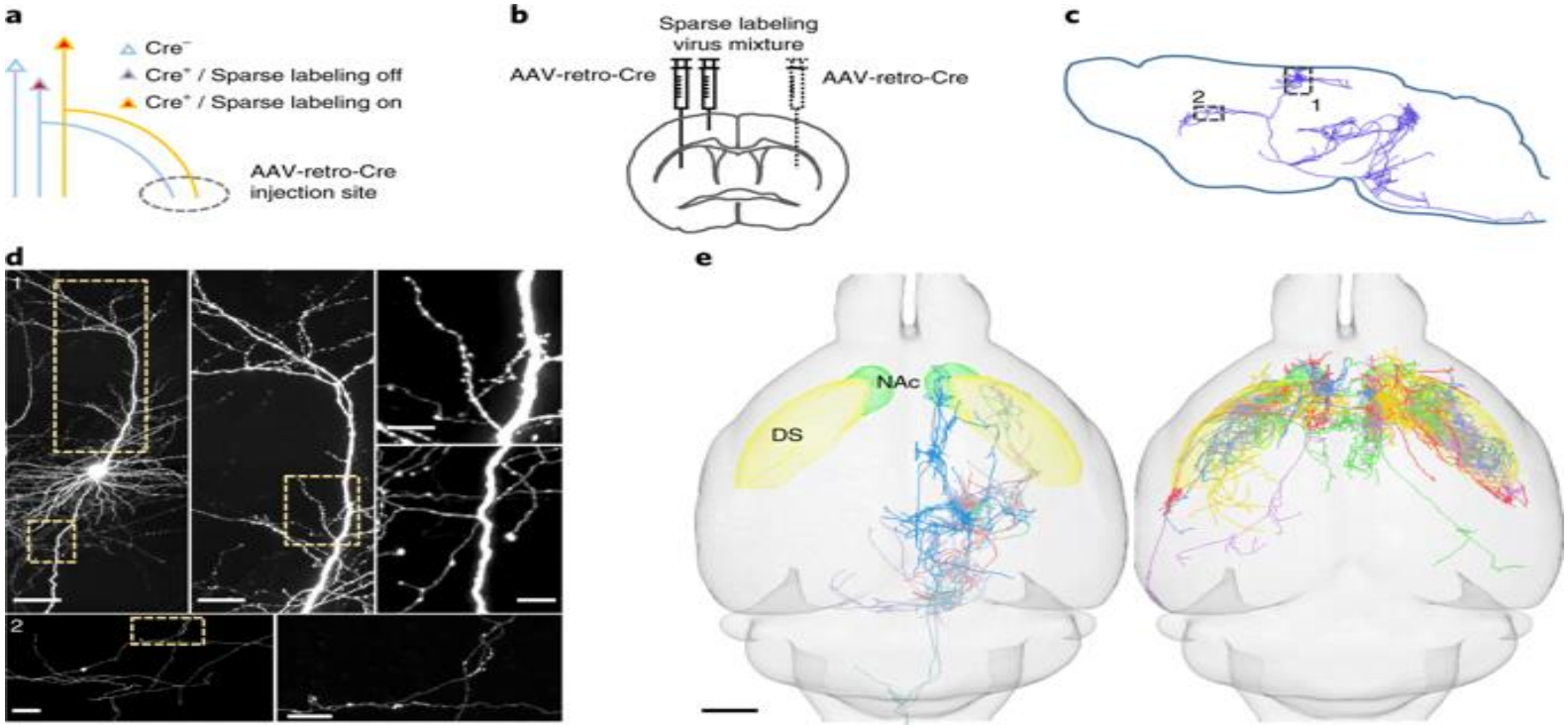
AAV-PHP.eB
高效率跨血脑屏障
感染全脑





Nat Neurosci. 2017 Aug;20(8):1172-1179

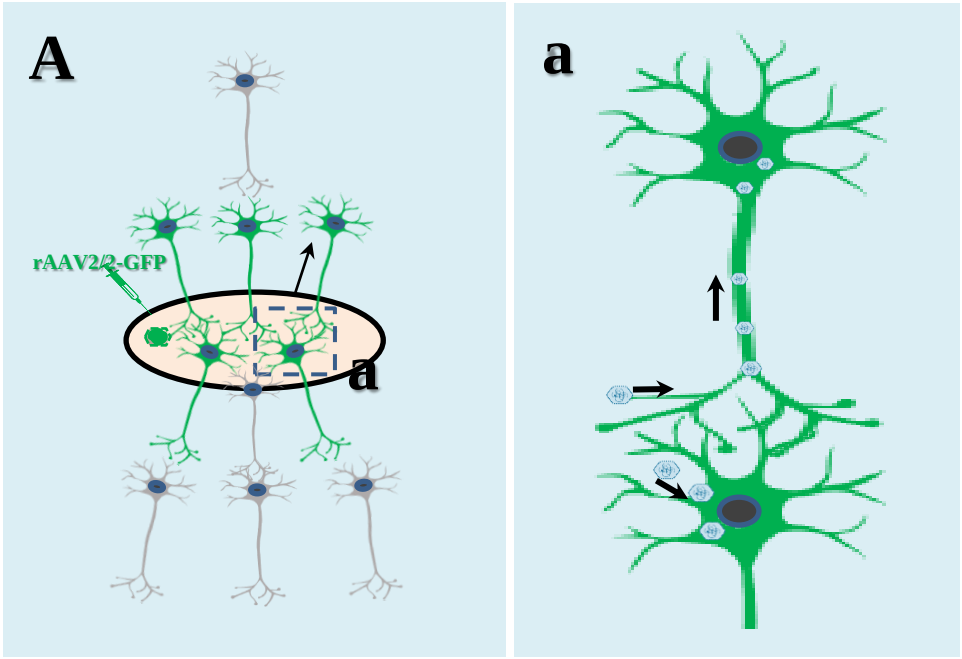
人工筛选获得 新的AAV血清型：
AAV-Retro-Cre：Sparse Labeling



稀疏标记单个神经元全脑投射

Nature Methods volume 15, pages1033–1036 (2018)

Principles for retrograde labeling with rAAV2/2



◆ **Mutant sites**

LADQDYTKTA

N587 and R588 of the AAV2 VP1 capsid gene

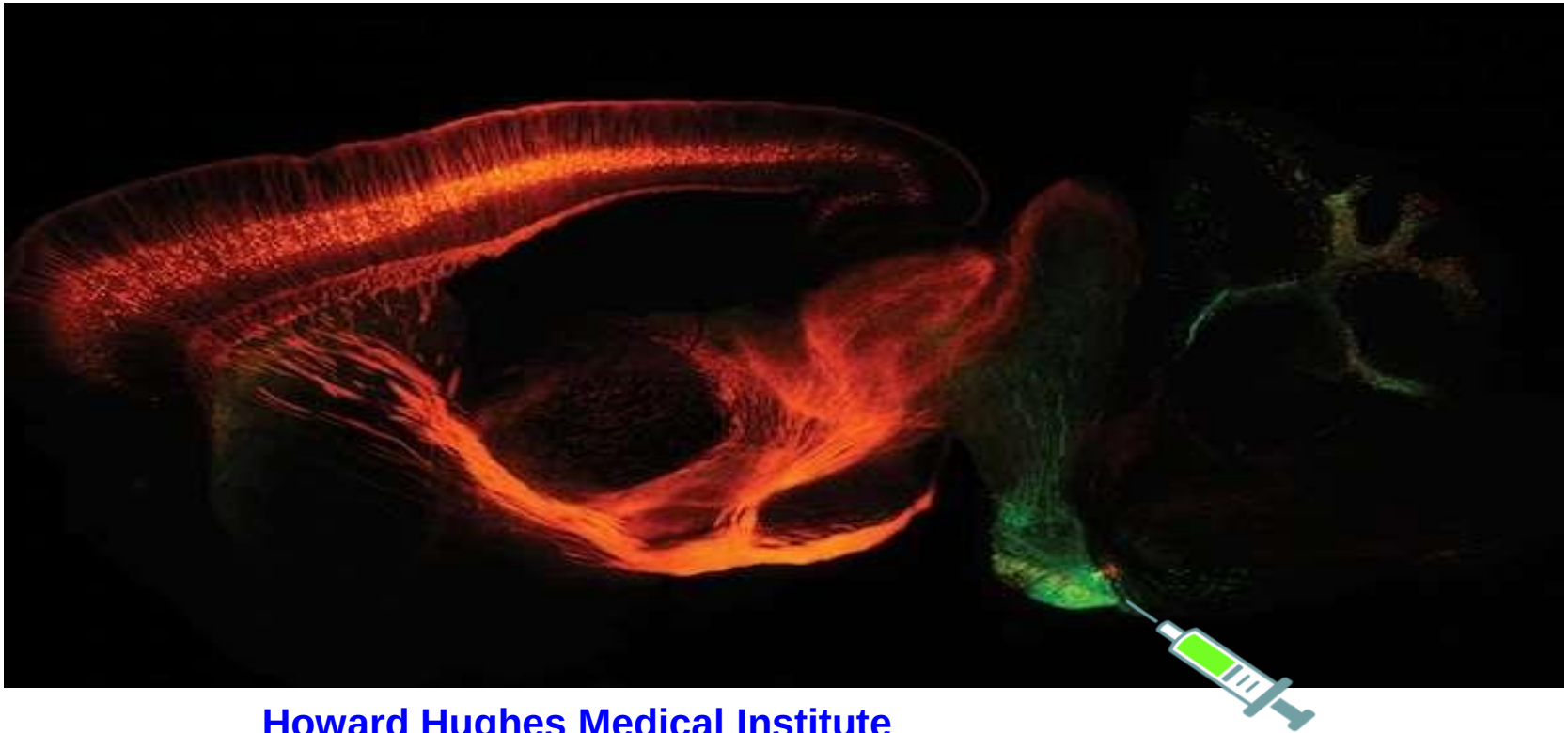
◆ **The mutants displayed the strongest retrograde transport**

◆ **The rAAV2/2 have two ways to entry neuron.**

- rAAV2/2 can directly entry the soma of neuron
- rAAV2/2 can entry the axon terminal of neurons and retro-spread to the soma

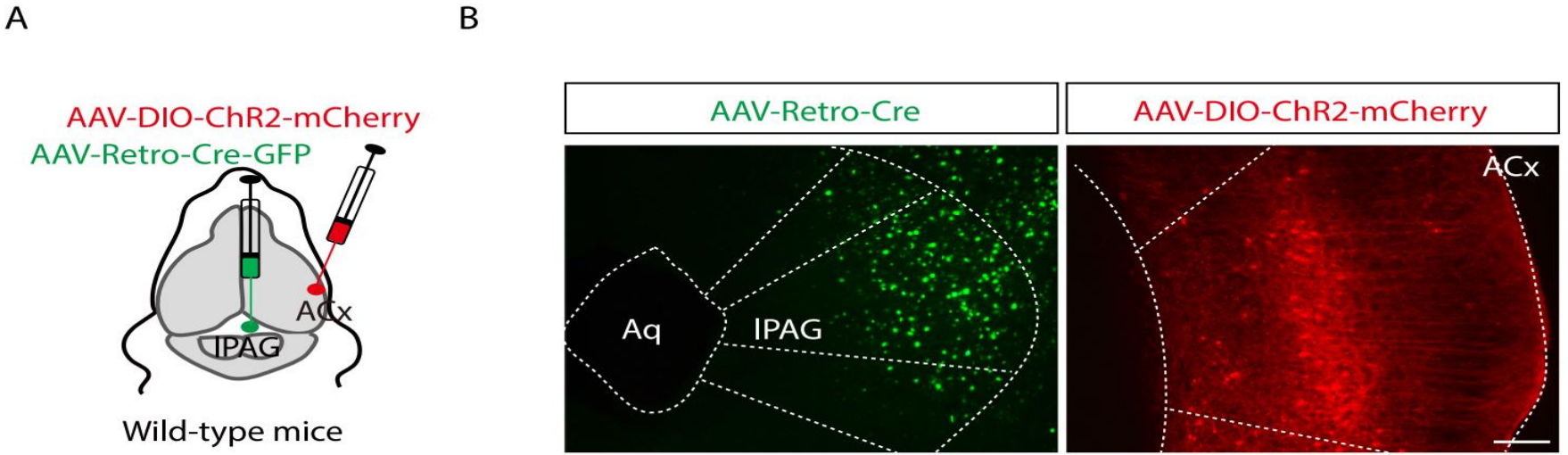
Tervo et al., *et al*. Neuron, 2016, 92, 1–11.

AAV-Retro(red)/AAV(green)



Howard Hughes Medical Institute

The application of rAAV2/2



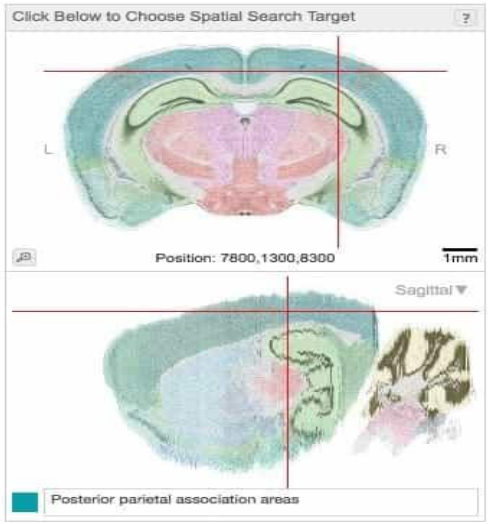
The GluACx--GlulPAG pathway. (A) Schematic showing a combinational viral strategy for mapping collateral pathways. (B) Typical images of the lPAG expressing AAV-Retro-Cre-GFP (left panel) and the ACx expressing AAV-DIOChR2-mCherry (right panel) in wild-type mice. Scale bar, 100 μm.

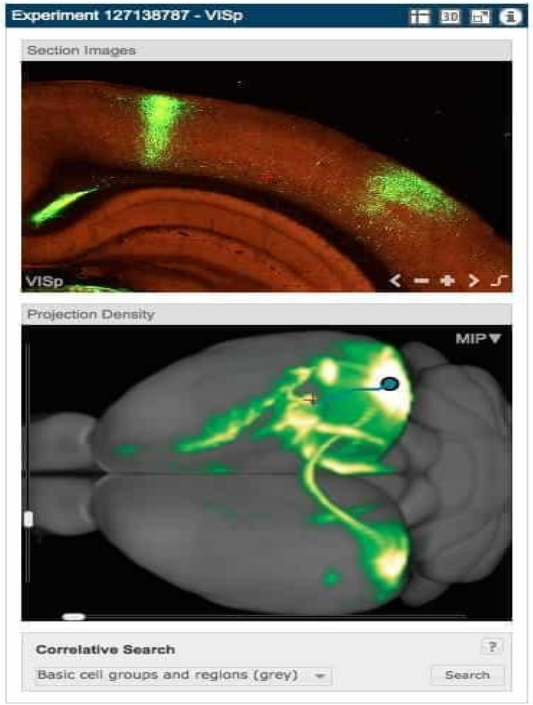
Wang H, *et al*. PLoS Biology, 2019, 17(8):e3000417.

不同血清型的组织嗜性

Serotype	Tissue tropism
AAV1	Smooth muscle, CNS, lung, retina, inner ear, pancreas, heart, liver
AAV2	Smooth muscle, CNS, liver, kidney, retina, inner ear
AAV3	Smooth muscle, liver, lung
AAV4	CNS, retina, lung, kidney
AAV5	Smooth muscle, CNS, lung, retina
AAV6	Smooth muscle, heart, lung, adipose, liver
AAV6.2	Lung, liver, inner ear
AAV7	Smooth muscle, retina, CNS, liver
AAV8	Smooth muscle, CNS, retina, inner ear, liver, pancreas, heart, kidney, adipose
AAV9	Smooth muscle, lung, liver, heart, pancreas, CNS, retina, inner ear, testes, kidney
AAVrh10	Smooth muscle, lung, liver, heart, pancreas, CNS, retina, kidney
AAV-DJ	Liver, heart, kidney, spleen
AAV-DJ/8	Liver, brain, spleen
AAV-PHP.eB	CNS
AAV-PHP.S	PNS
AAV2-retro	Spinal nerves
AAV2-QuadYF	Endothelial cell
AAV2.7m8	Retina, inner ear

AAV:Mouse Brain Map

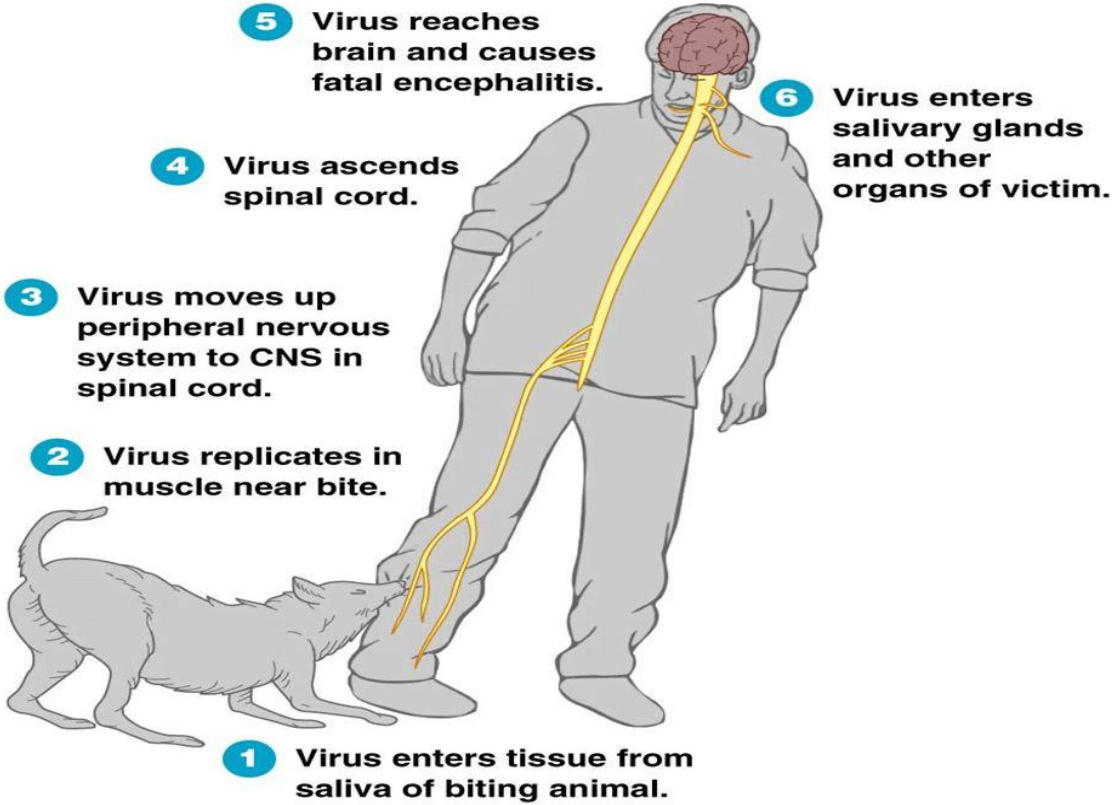




https://mouse.brain-map.org/

Mouse brain map – Allen institute

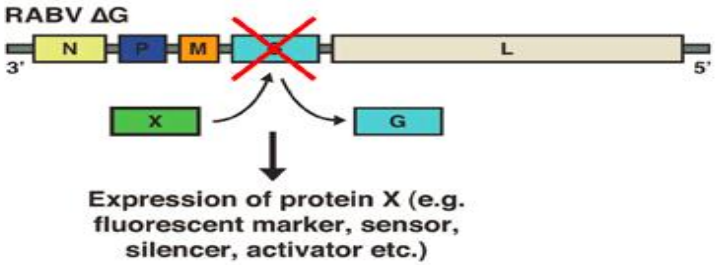
Rabiesvirus （RV）-天然逆向传播

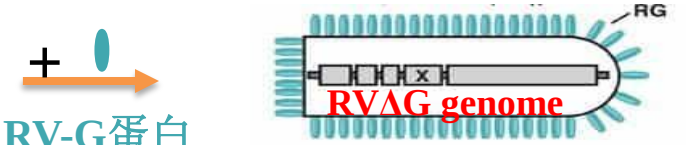


RV改造 - RVΔG

RV

RVΔG

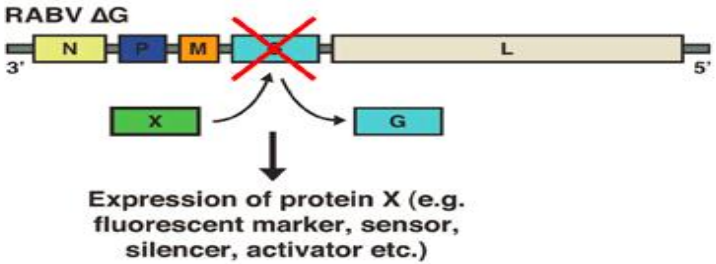




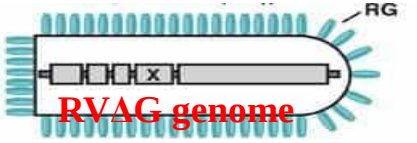
RV-G蛋白
其他表面蛋白？

RVΔG pseudotyping

RVΔG

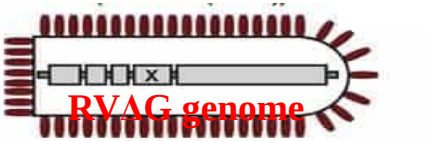


\+ RV-G蛋白



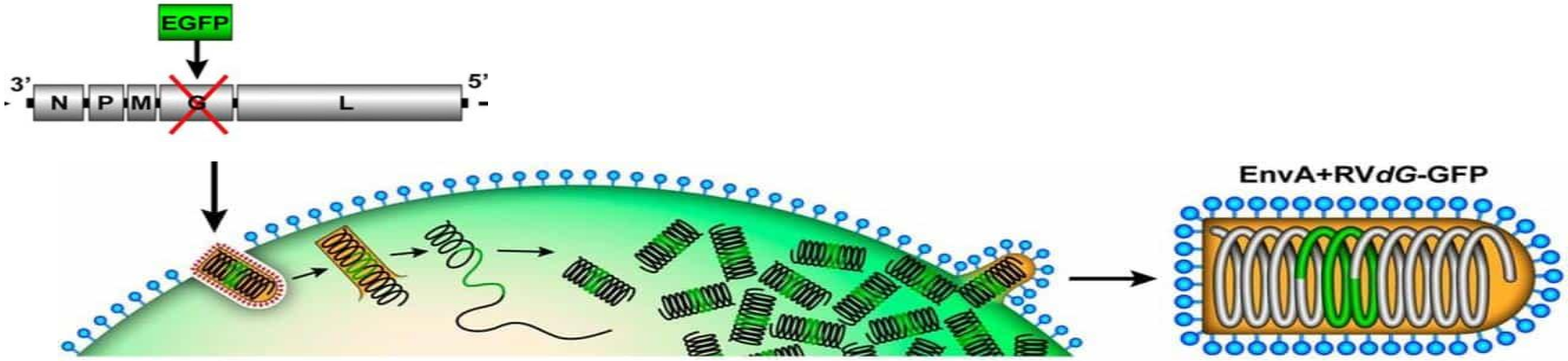
RVΔG

\+ 其它G蛋白 EnvA



用其他兼容的G蛋白替代RV-G包被RVΔG

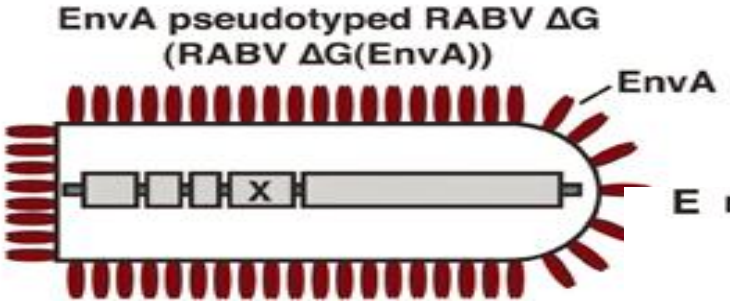
生产pseudotyped RVΔG



Cells expressing substituting envelope protein：**EnvA**

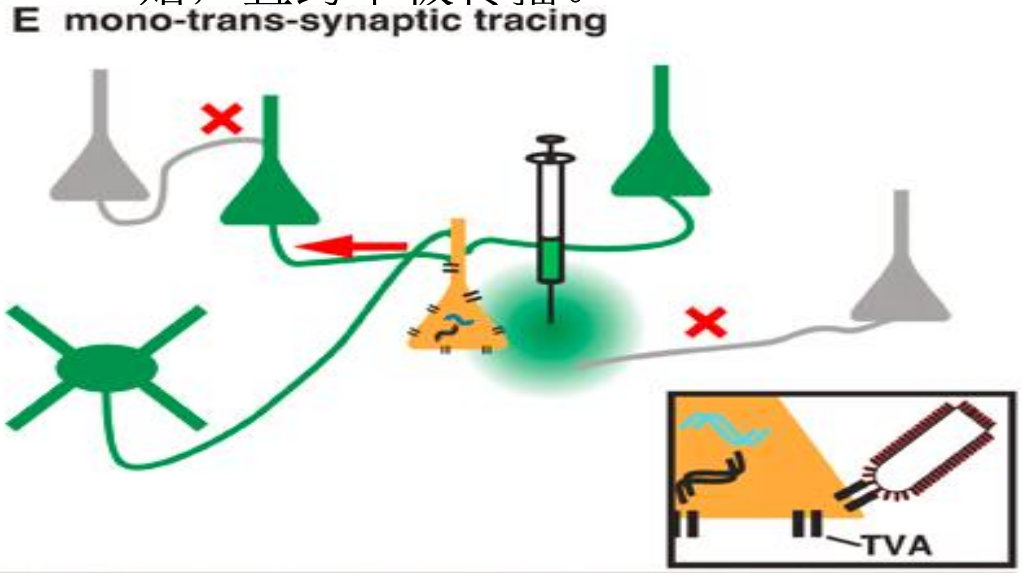
EnvA：avian sarcoma and leukosis virus 包膜（Envelope）糖蛋白，它的特异性受体为TVA，仅表达于鸟类细胞。ENV宝贝的病毒可选择性感染表面据TVA受体的细胞。

EnvA pseudotyped RVΔG

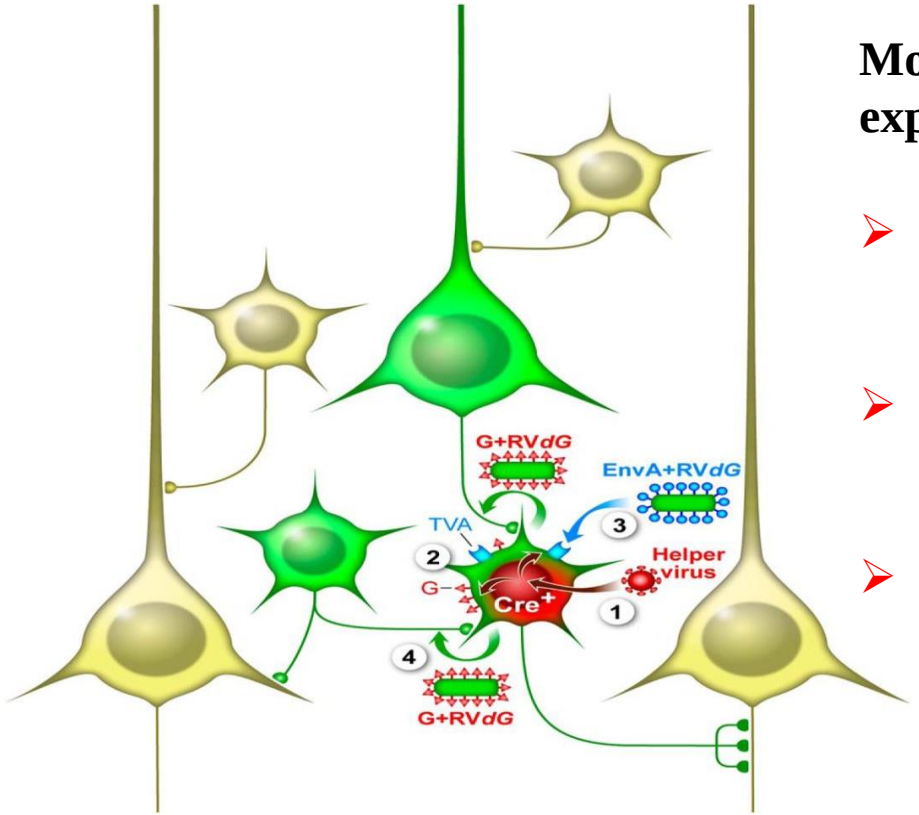


糖蛋白EnvA特异性识别受体TVA

RV的跨神经元传播与膜蛋白G有关，用EnvA替换G蛋白可使改造后的RV在表达其受体TVA的细胞特异性起始，且跨单极传播。



Monosynaptic tracing of RV



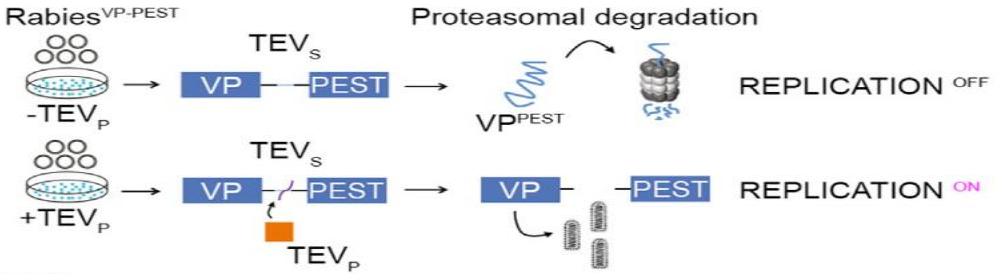
Monosynaptic rabies tracing of inputs to Cre-expressing starter cells.

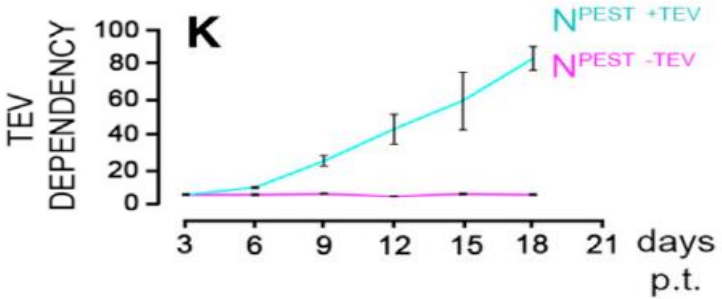
- **Helper virus** （**AAV**）: expresses TVA, G, and RFP in a Cre-dependent manner in **starter cell**s.
- **infect** with EnvA + RV*dG* （*GFP*） is injected，infects the TVA+ starter cells.
- **The result** is that starter cells are marked with both RFP and GFP; while input cells express only GFP. Other neurons that do not express Cre and are not directly presynaptic to starter cells remain unlabeled (light yellow).

Journal of Neuroscience. 2015; 35(24):8979-8985

RV减毒

- 在病毒蛋白上融合蛋白酶体识别域PEST及TEV蛋白识别的切割序列TEVs，能控制病毒蛋白的量。





控制RV的N蛋白，能控制RV的复制

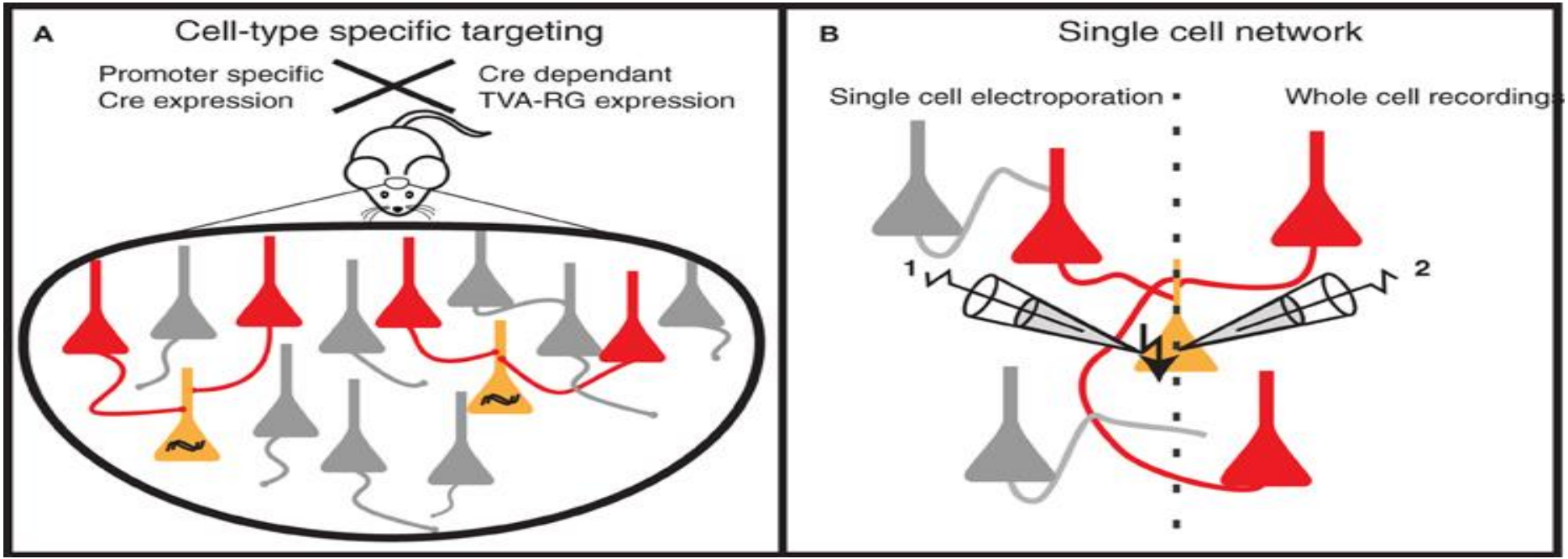
控制RV的复制，能降低RV的细胞毒性

(Ernesto Ciabatti et al., 2017)

RV tracer：map the connectome

比如利用Cre-Lox系统
标记特定类型神经元

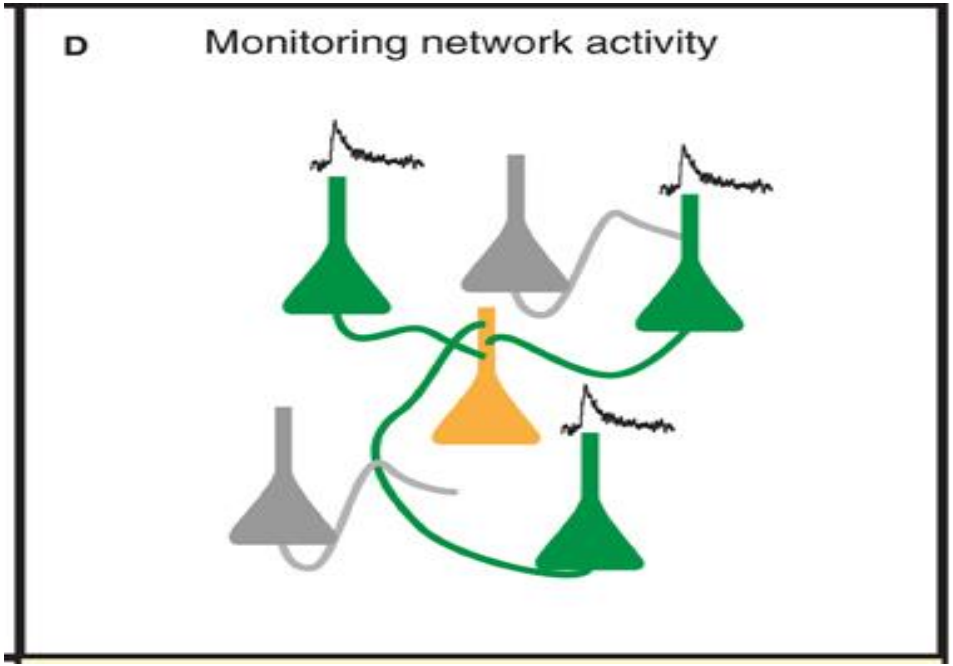
病毒感染/质粒DNA
转染单个神经元



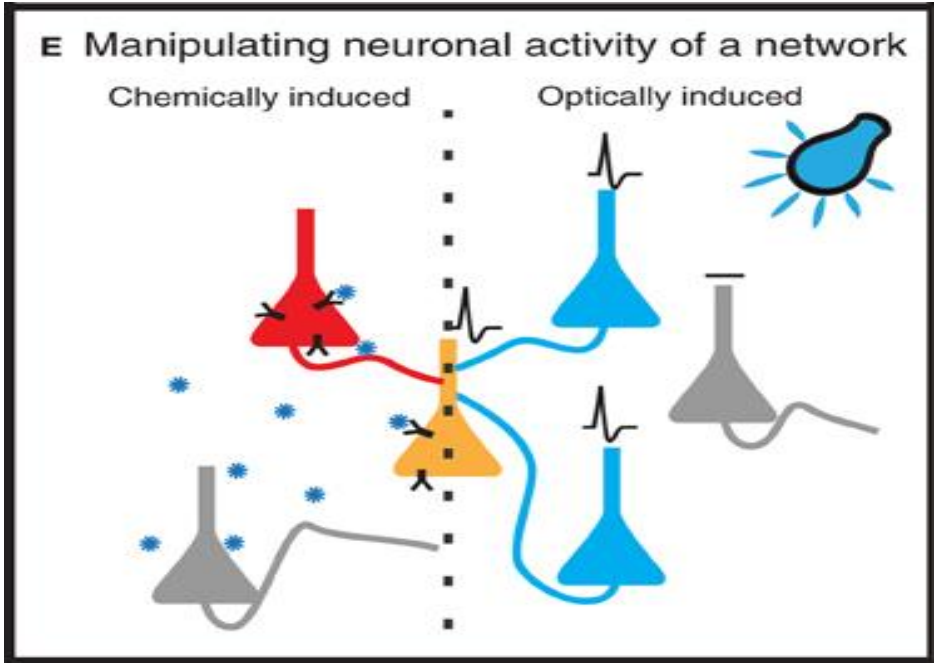
Front. Neural Circuits, 2013.7:1

Application of RV tracer

表达钙调蛋白，特定神经元激活状态

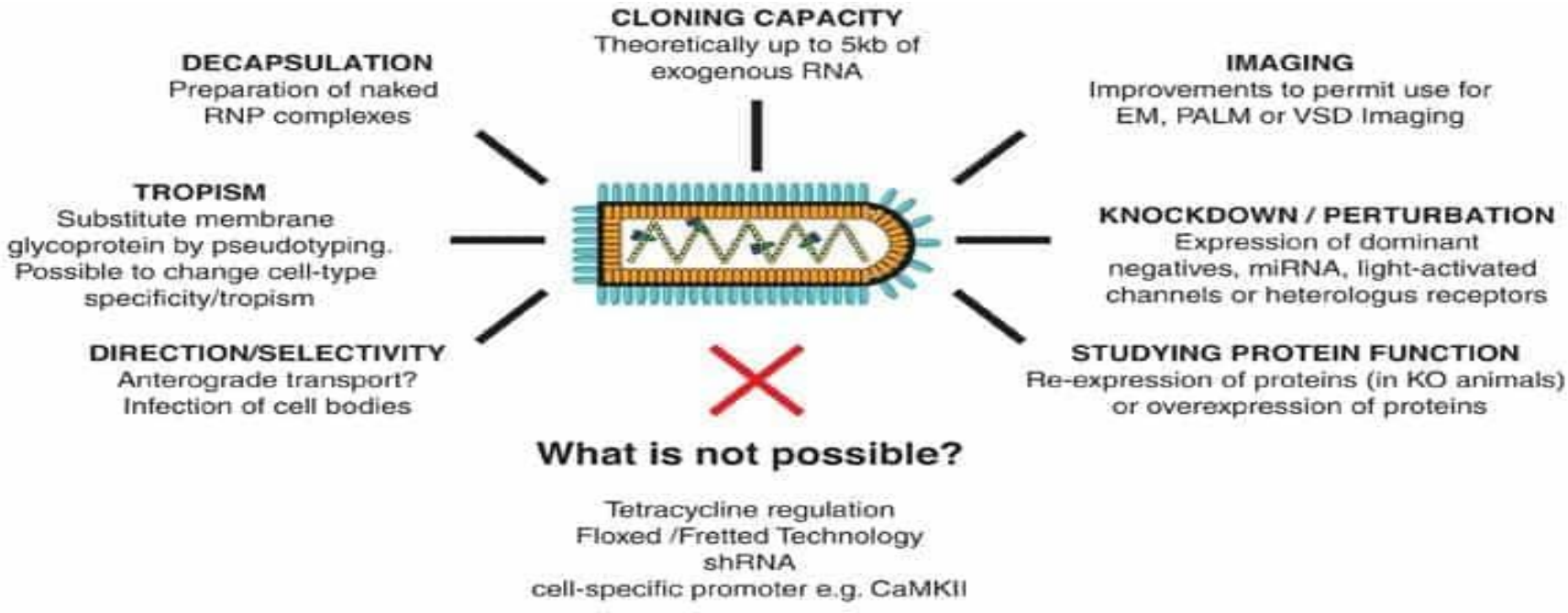


表达功能元件，用于光遗传学研究



Front. Neural Circuits, 2013.7:1

RVdG示踪与限制


CLONING CAPACITY
Theoretically up to 5kb of exogenous RNA
DECAPSULATION
Preparation of naked RNP complexes
IMAGING
Improvements to permit use for EM, PALM or VSD Imaging
TROPISM
Substitute membrane glycoprotein by pseudotyping. Possible to change cell-type specificity/tropism
KNOCKDOWN / PERTURBATION
Expression of dominant negatives, miRNA, light-activated channels or heterologus receptors
DIRECTION/SELECTIVITY
Anterograde transport? Infection of cell bodies
STUDYING PROTEIN FUNCTION
Re-expression of proteins (in KO animals) or overexpression of proteins
What is not possible?
Tetracycline regulation
Floxed /Fretted Technology
shRNA
cell-specific promoter e.g. CaMKII

RV示踪特点

优点：

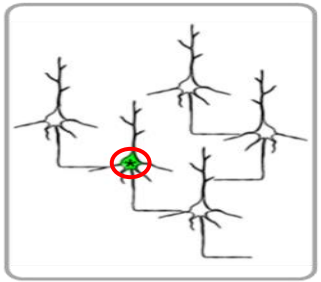
- 基因组小，结构清楚，易改造；
- 严格逆向传播，方向确定；
- 标记效率高，信号强；
- 毒性比较低；
- 发展较成熟，应用广泛；
- 与ENV/TVA系统结合，特异更强；
- …

缺点：

- 承载外源基因能力有限；
- 可感染人，如发生回复突变或重组，具有一定危险性；
- …

Viral Tracers

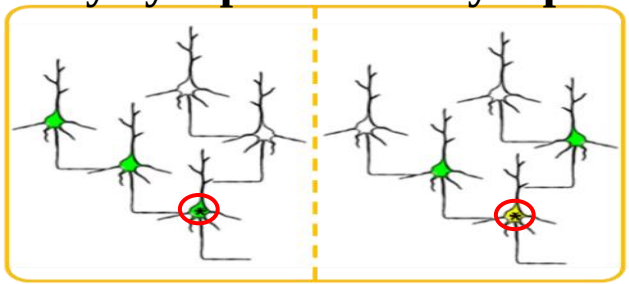
Non-transneuronal



AAV, CAV

Retrograde

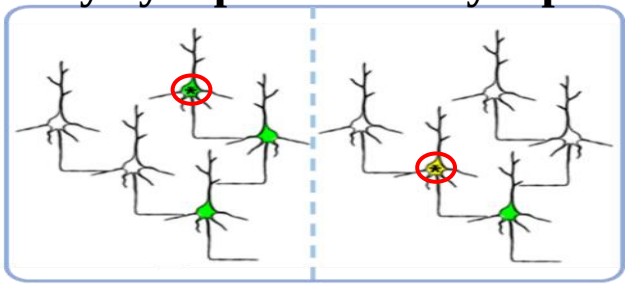
Poly-synaptic **Mono-synaptic**



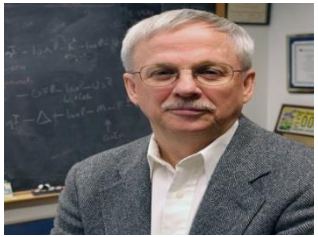
PRV **RV**, PRV

Anterograde

Poly-synaptic **Mono-synaptic**



HSV-1 **HSV-1 dTK**



Lynn Enquist

Pomeranz L E , *et al.* The J Neurosci, 2017

Edward Callaway

Wall N R, *et al.* PNAS, 2010

Wickersham I R , *et al.* Nature Methods, 2007

David Anderson

Lo L, *et al.* Neuron. 2011

Nassi JJ, *et al.* Front Neuroanat. 2015

Zingg B, *et al.* Neuron. 2017

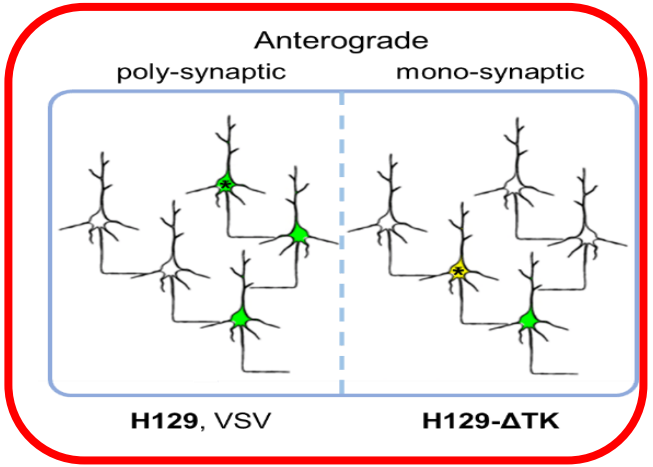
Zeng WB, *et al.* Mol Neurodegener. 2017

顺向跨突触的示踪病毒工具

AAV：效率差 Zingg B, *et al*. Neuron. 2017

水泡口炎病毒（VSV）有问题 Beier KT, PNAS, 2011

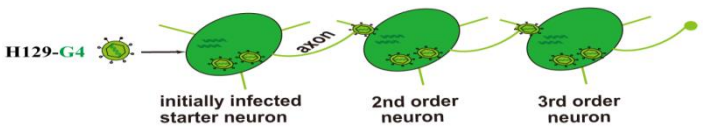
单纯疱疹病毒（HSV）129株（H129）

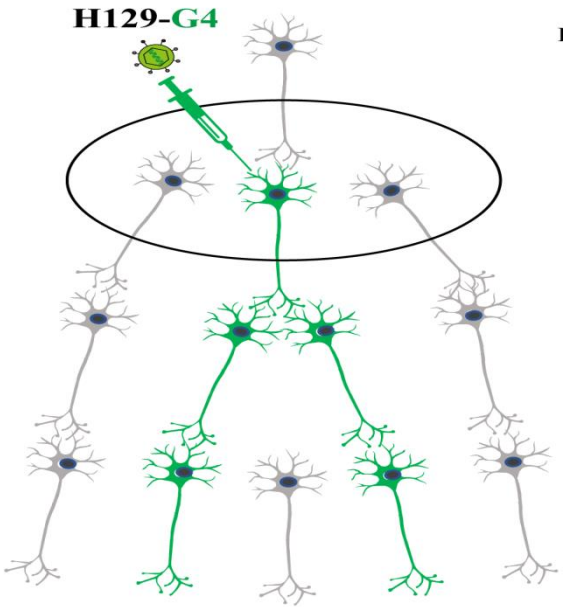


Lo L, *et al*. Neuron. 2011
Zingg B, *et al*. Neuron. 2017
Zeng WB, *et al*. Mol Neurodegener. 2017

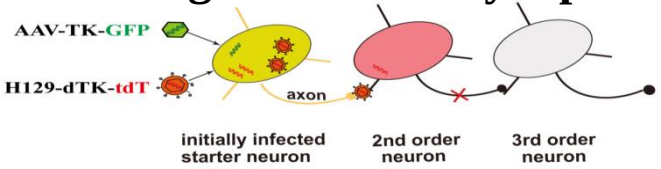
顺向病毒工具示踪神经环路原理

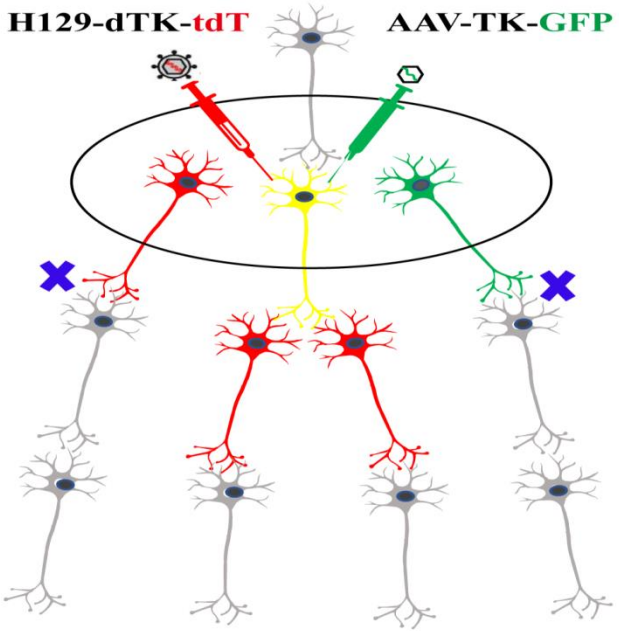
◆ **Anterograde Poly-synaptic**



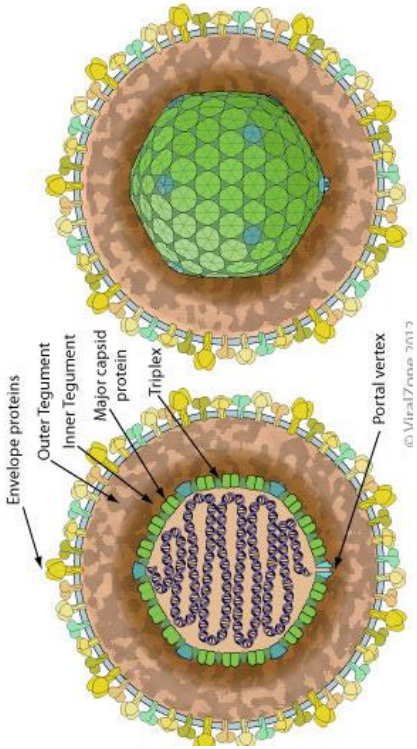


◆ **Anterograde Mono-synaptic**





HSV-1的结构

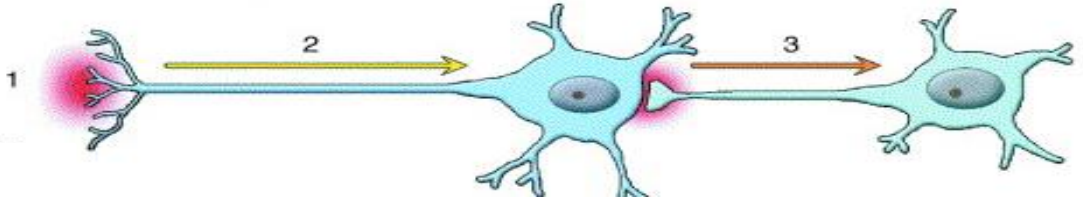


疱疹病毒颗粒准球形，直径120-200nm：

- 包膜/envelope：含病毒糖蛋白的脂质双层膜
- 皮膜/tegument：包膜与衣壳间、不定形，主要为蛋白，厚度不等
- 核衣壳/neuleocapsid：核衣壳100－125nm，正20面体，病毒基因组+衣壳
- 基因组/ viral genome：**dsDNA, 152kb**, 线性

HSV-1的传播方向与顺向示踪

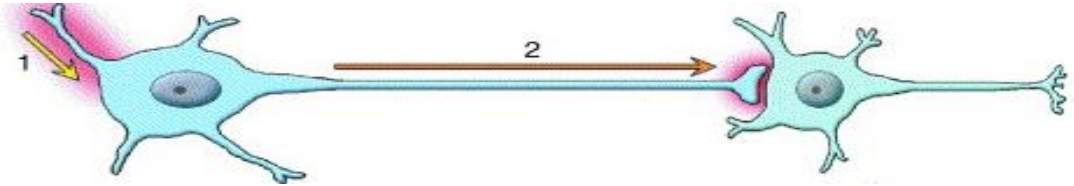
RV
逆行工具



Retrograde trans-synaptic tracing for input network

Nassi, J.J, 2015 *Front Neuroanat* doi: 10.3389/fnana.2015.00080

HSV-H129



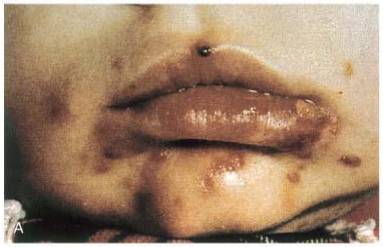
Anterograde trans-synaptic tracing for **output network**

Lo, L, 2011, *Neuron*: 938-950

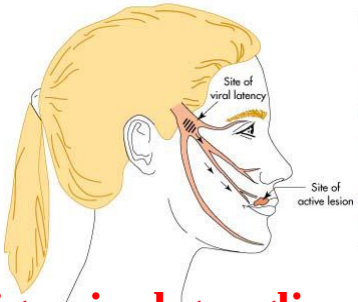
问题：H129能否改造成好用的踪顺行工具？

Zhao, et al. J Med Virol 2016，专利 201511029238.8；201510287275.2

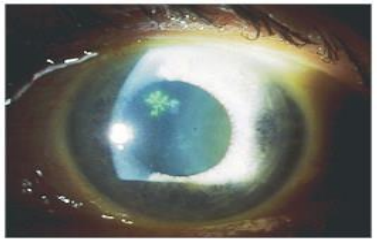
HSV-1 与疾病



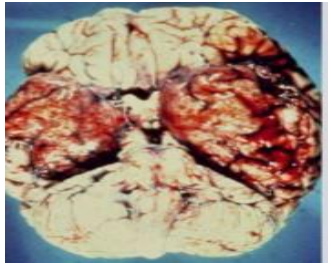
Cold sores



Trigeminal ganglia



Herpes Keratitis

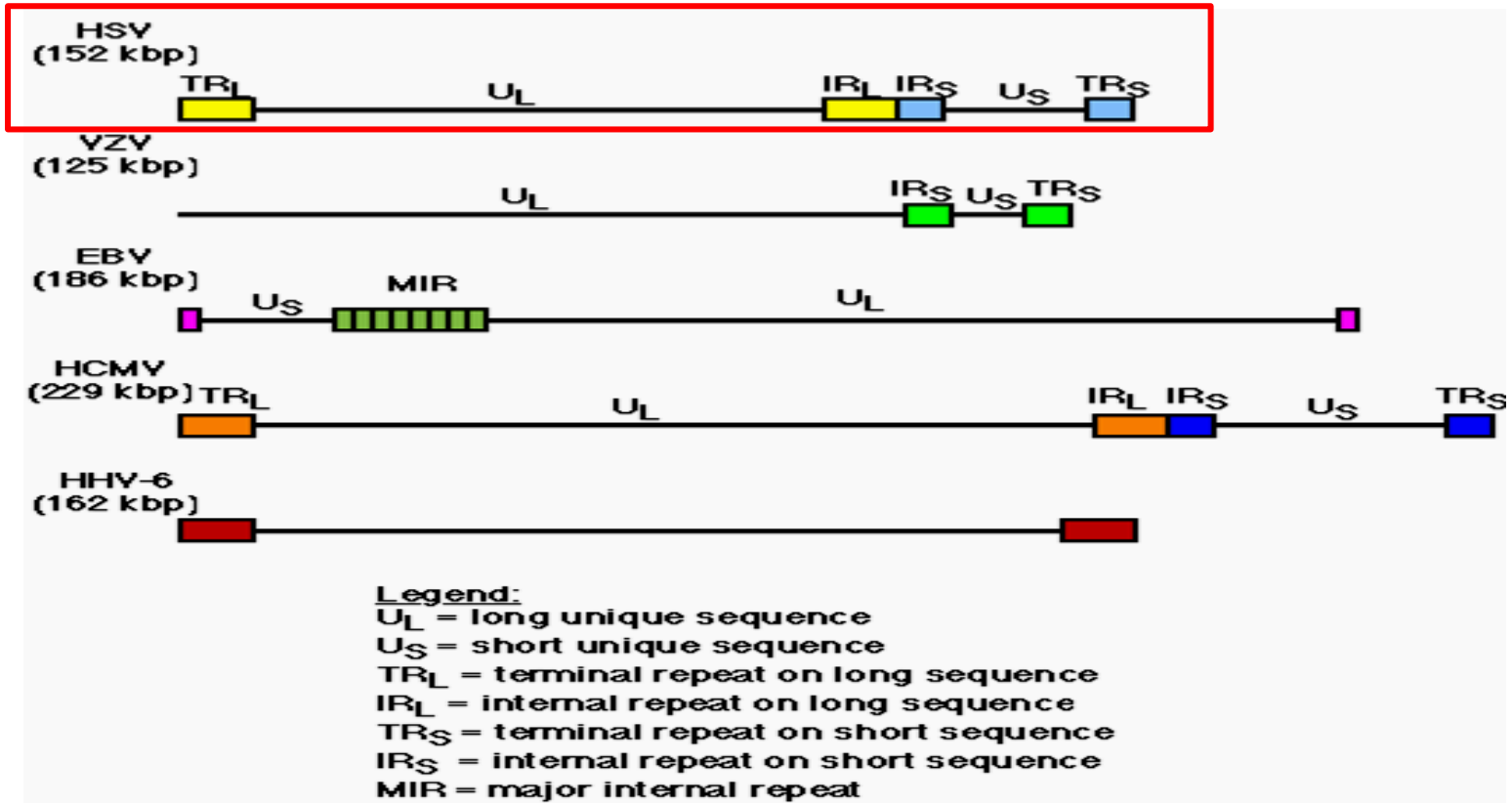


Encephalitis

Herpes Keratitis: Leading cause of corneal blindness

Herpes Brain infection: Encephalitis, AD, epilepsia

疱疹病毒基因组：大



Fiels Virology 2014

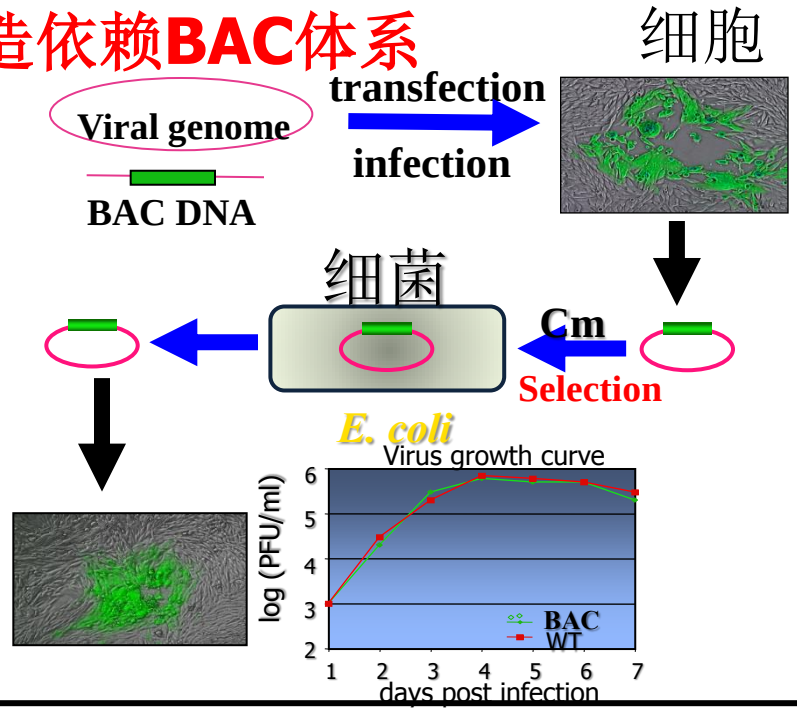
病毒的遗传改造平台—BAC

疱疹病毒基因组大，改造依赖**BAC**体系

1. 病毒基因组整合入BAC

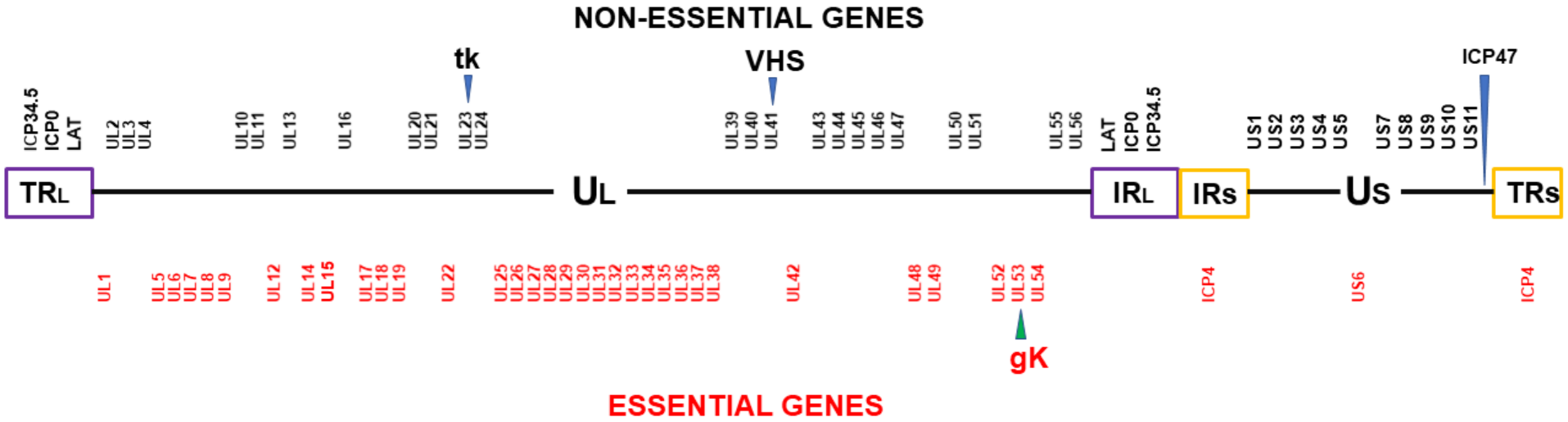
2. HSV-BA C筛选鉴定

3. 拯救鉴定HSV



获得了系列病毒突变体和专利； 致病机制
新的神经示踪工具； 新型神经减毒疫苗

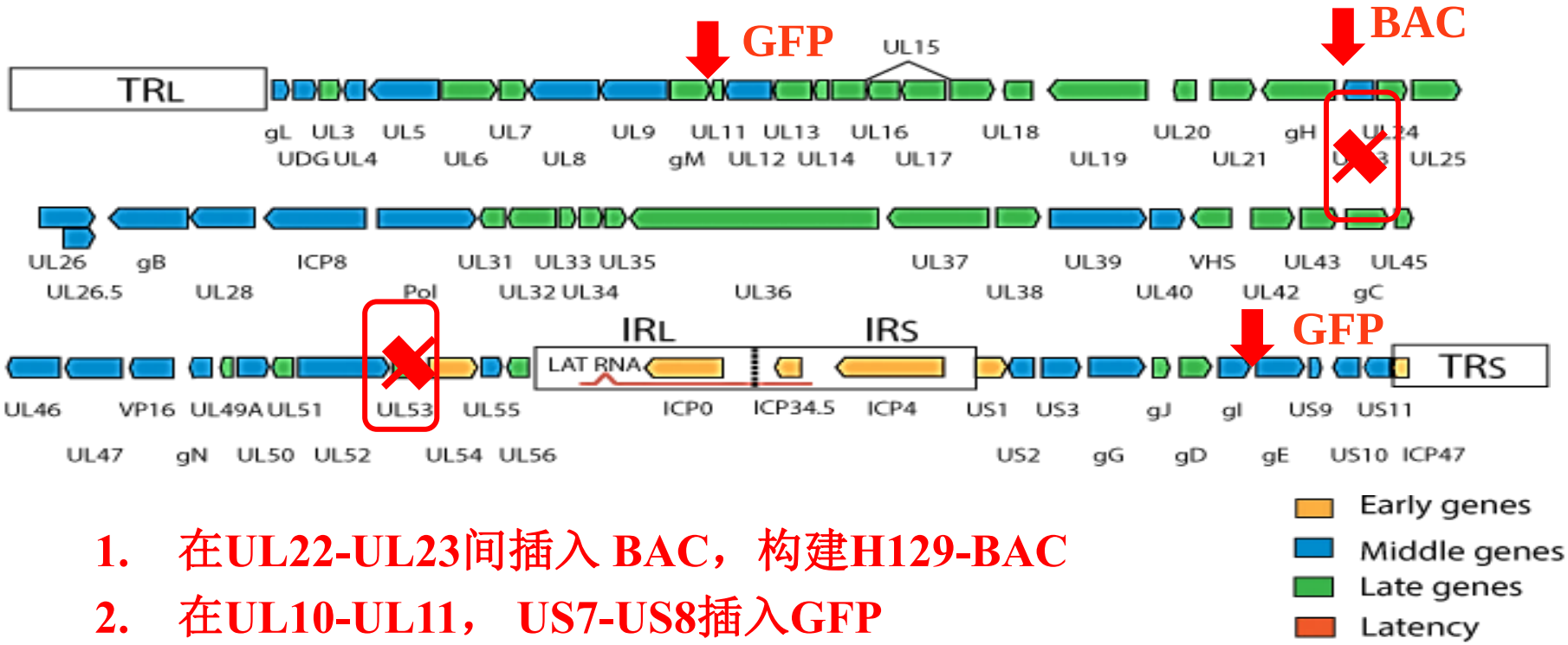
HSV-1基因组结构



VHS: virion host shut off protein

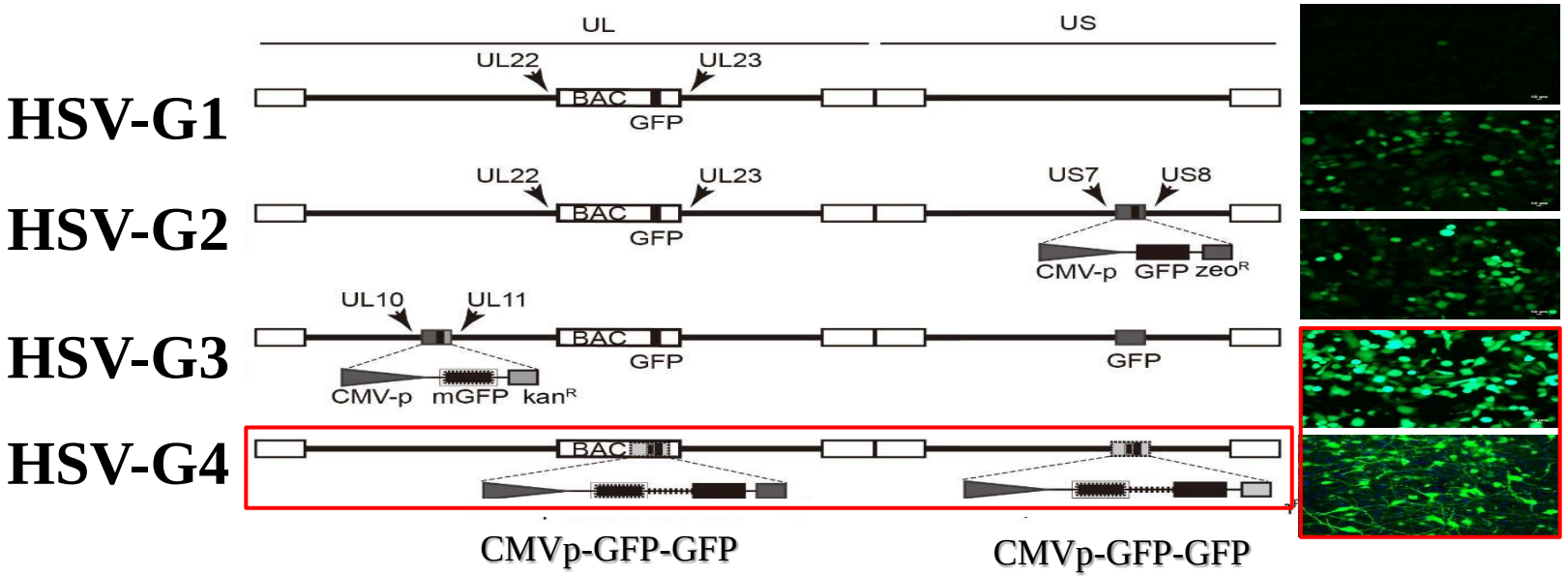
HSV-1有36个必需基因，有37个非必需基因

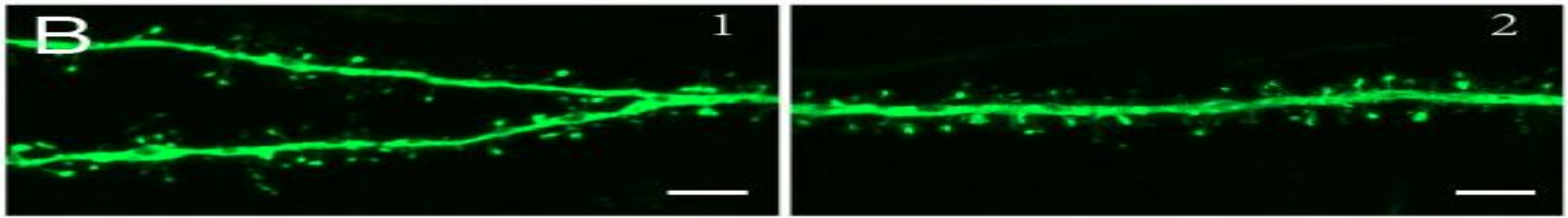
H129基因组结构图与改造



1. 在UL22-UL23间插入 BAC，构建H129-BAC
2. 在UL10-UL11， US7-US8插入GFP
3. 从H129基因组中敲除 UL23(TK)、UL53（gK）基因，构建顺向跨单级工具H129-dTK，H129-dgK

改造HSV-1 H129

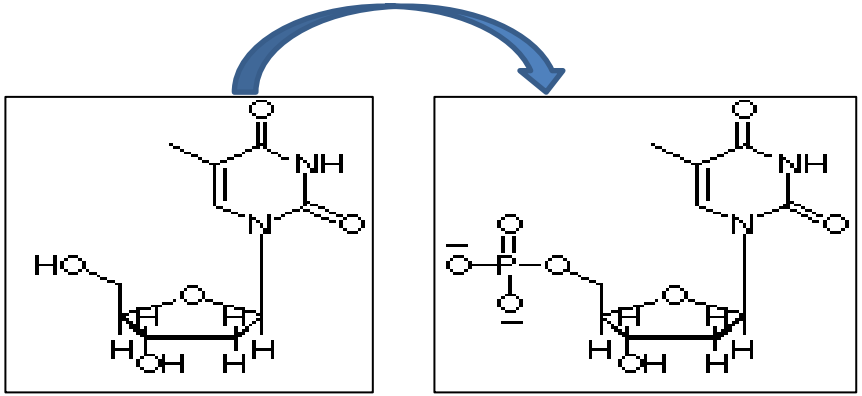




Bright, efficient, showing details of labeled neurons-spikes

Zeng WB, *et al*. Mol Neurodegener. 2017

Role of TK



脱氧胸苷
(Thd)

一磷酸-脱氧胸苷
(TMP)

TK (Thymidine kinase)

↓

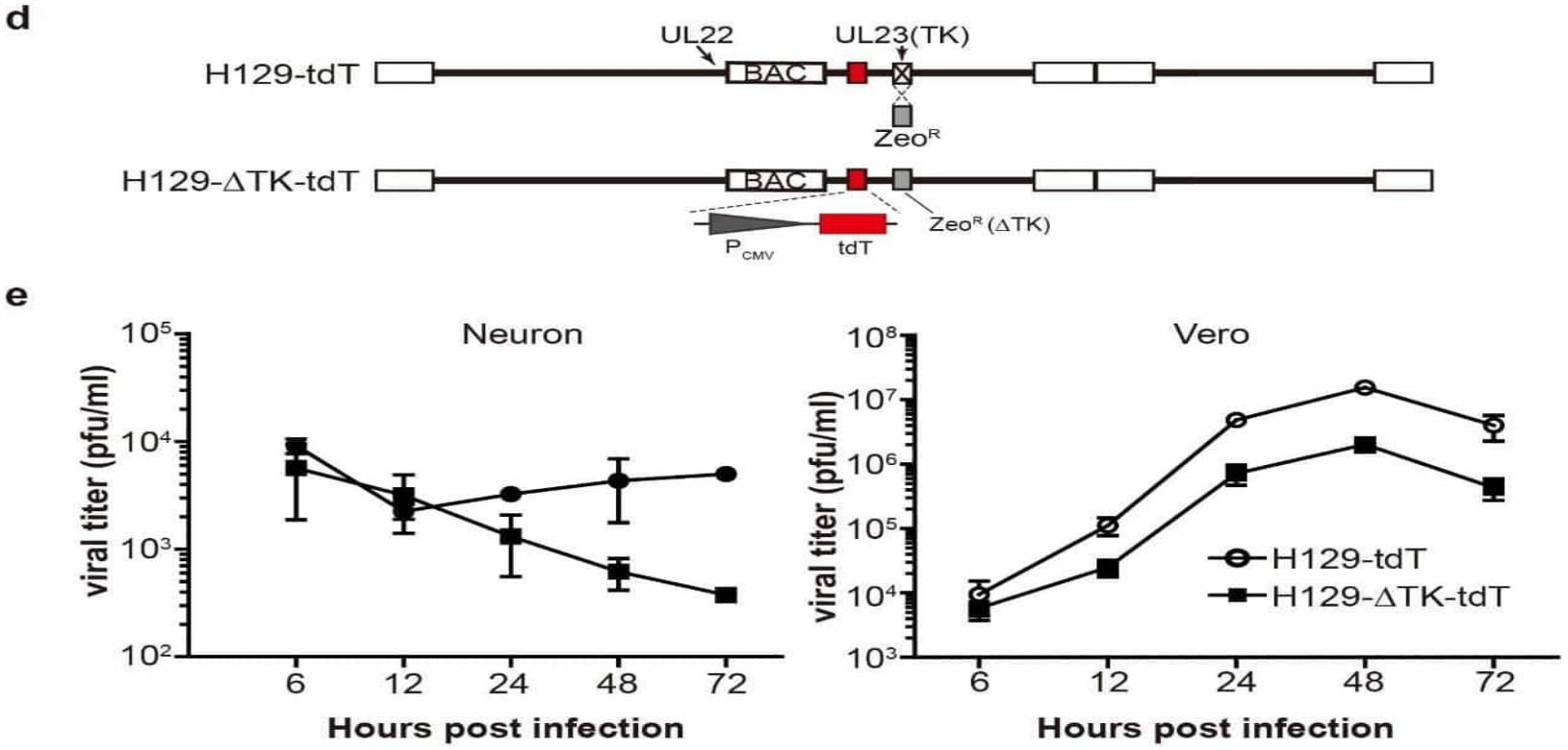
essential for TTP synthesis and viral genome replication

↓

TK deficient H129 can't replicate in neurons

TK催化Thd合成TMP, 合成TTP的关键一步, DNA复制所必须

H129-dTK


d
UL22
UL23(TK)
H129-tdT
BAC
Zeo^R
H129-ΔTK-tdT
BAC
Zeo^R (ΔTK)
P_CMV
tdT
e
Neuron
viral titer (pfu/ml)
10^5
10^4
10^3
10^2
6
12
24
48
72
Hours post infection
Vero
viral titer (pfu/ml)
10^8
10^7
10^6
10^5
10^4
10^3
H129-tdT
H129-ΔTK-tdT
6
12
24
48
72
Hours post infection

已开发的顺向示踪工具与特点

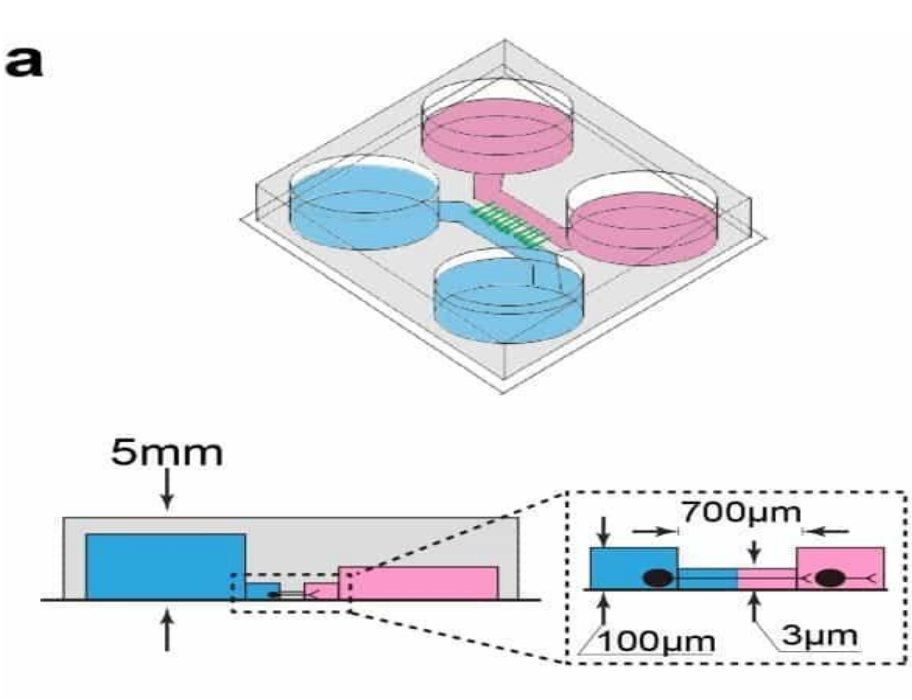
顺向跨多级工具	顺向跨单级工具
H129-G4，绿色，高亮	**H129-dTK-tdT，红色，需染色** **H129-dTK-T2, 红色，不需染色，染效果佳**
H129-R4，红色，高亮	H129-dTK-G4，绿色，需染色
H129-LSL-TK-G4，起始特异，高亮	**H129-dgK-G4，绿色，高亮，效果好**
H129-G2-FLH2, 绿色，MRI联用	**H129-dgK-G4-d56，减毒，高亮，测试中**
H129-G3-ChR2，高亮，功能耦合	**病毒粒子轴突运输和跨突触**
H129-R4-GCaMP，高亮，功能耦合	H129-vp26-mCh，核衣壳带红色荧光
减毒	H129-gD-GFP，囊膜带绿色荧光
H129-41G4，高亮，减毒，测试中	**H129-G/R，病毒颗粒红绿双标**
H129-56G4，高亮，减毒，测试中	**H129-G/R-dX，多种红绿双标缺陷病毒**

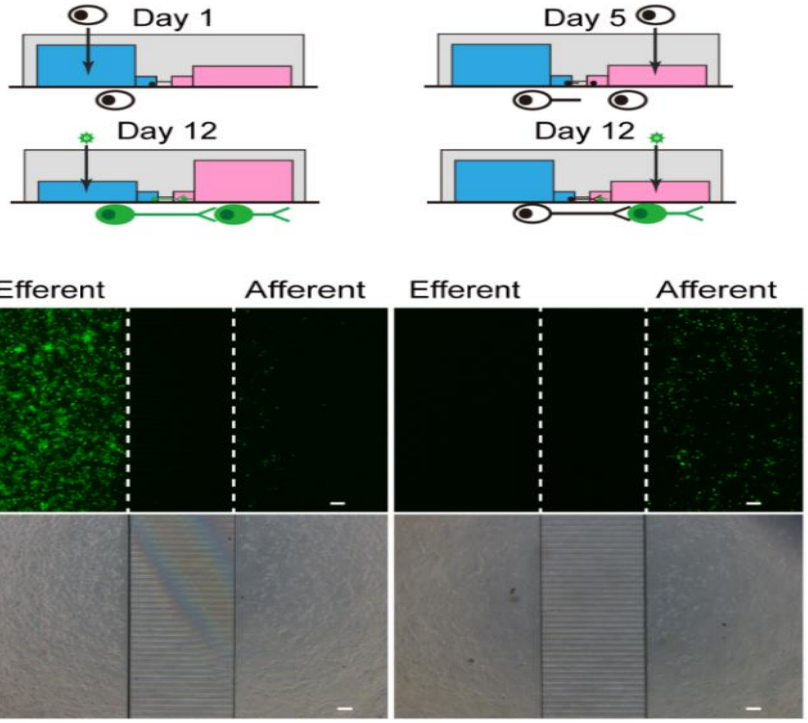
可标记，条件优化中

示踪效果好

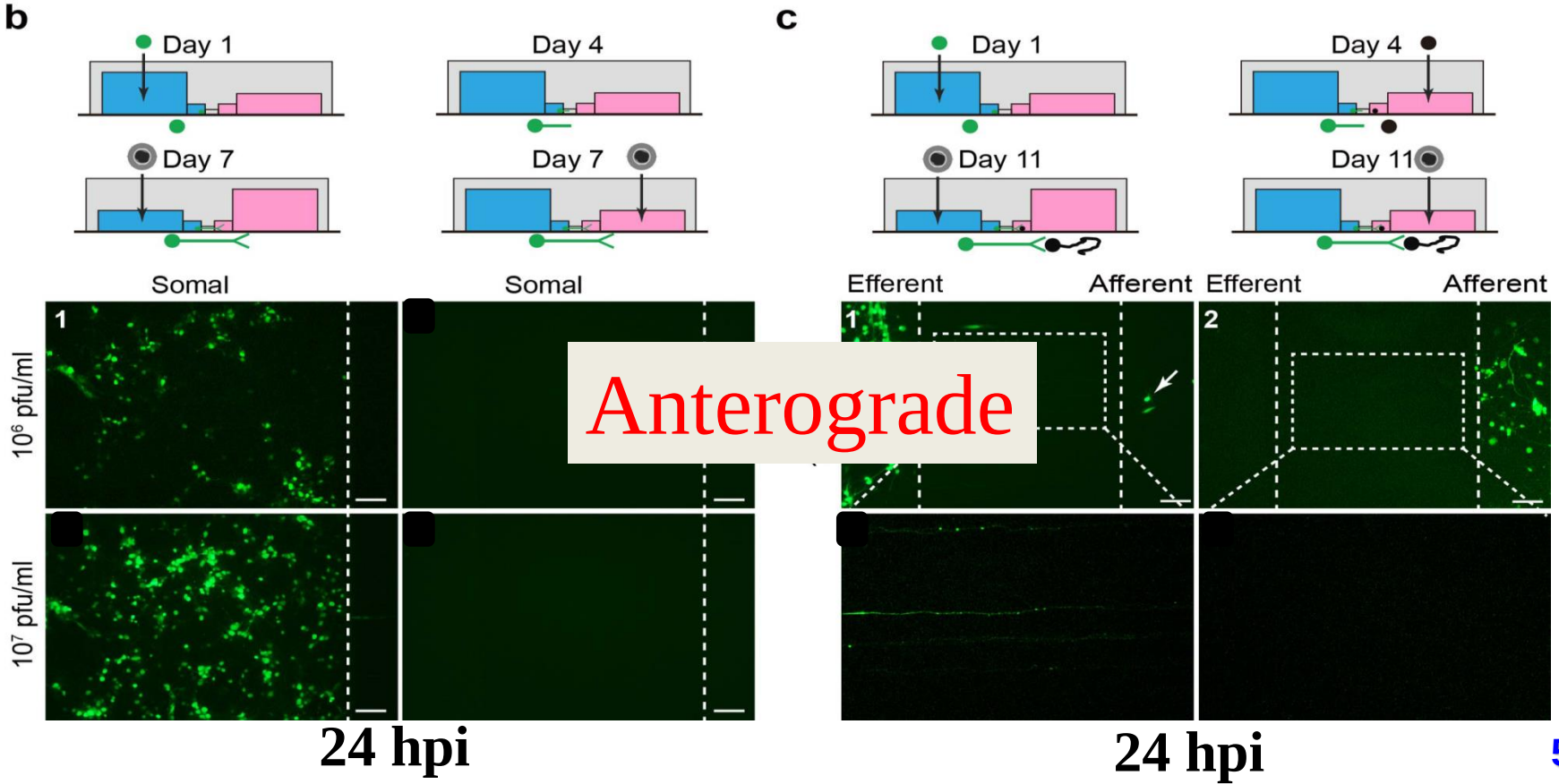
Zeng WB, *et al*. Mol Neurodegener. 2017

Microfluidic device and transmit direction of H129 *in vitro*

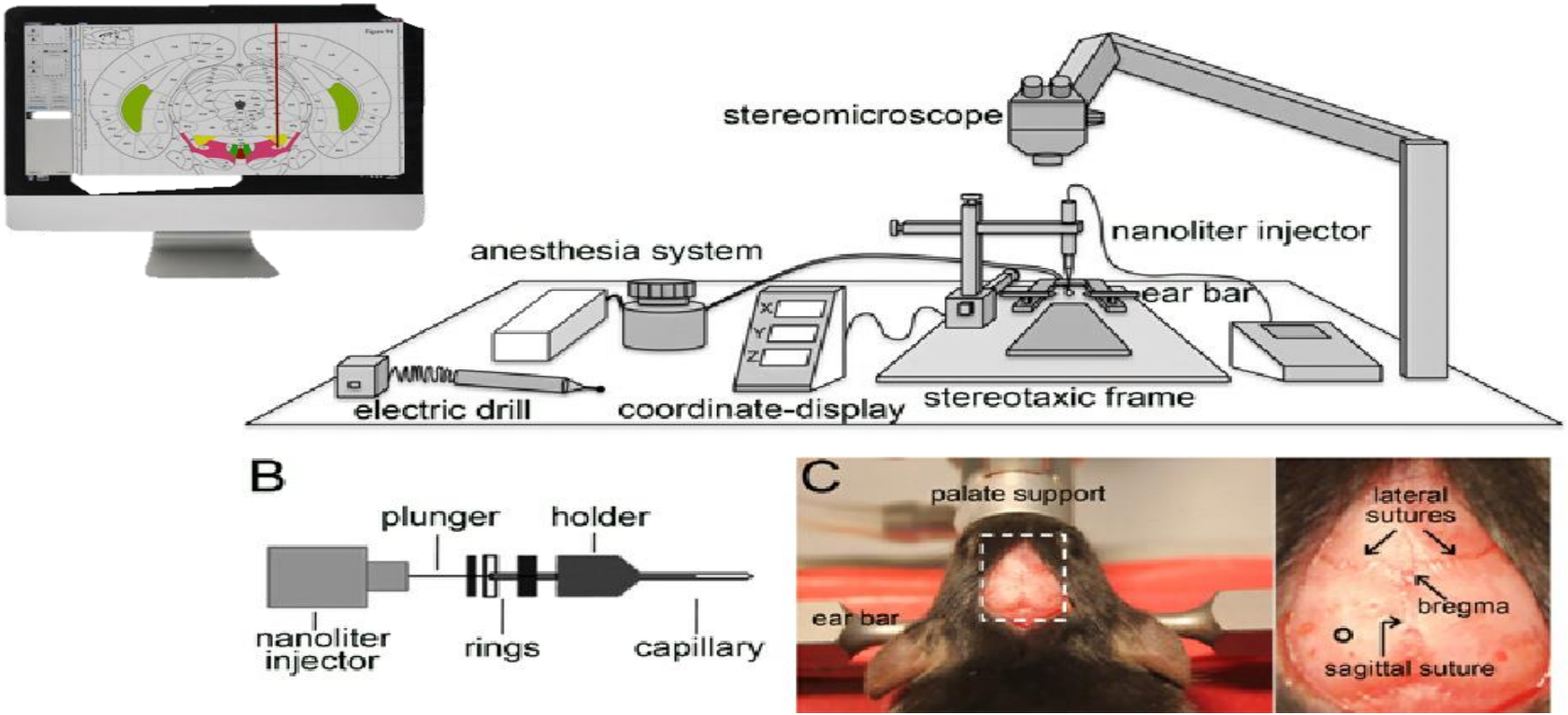




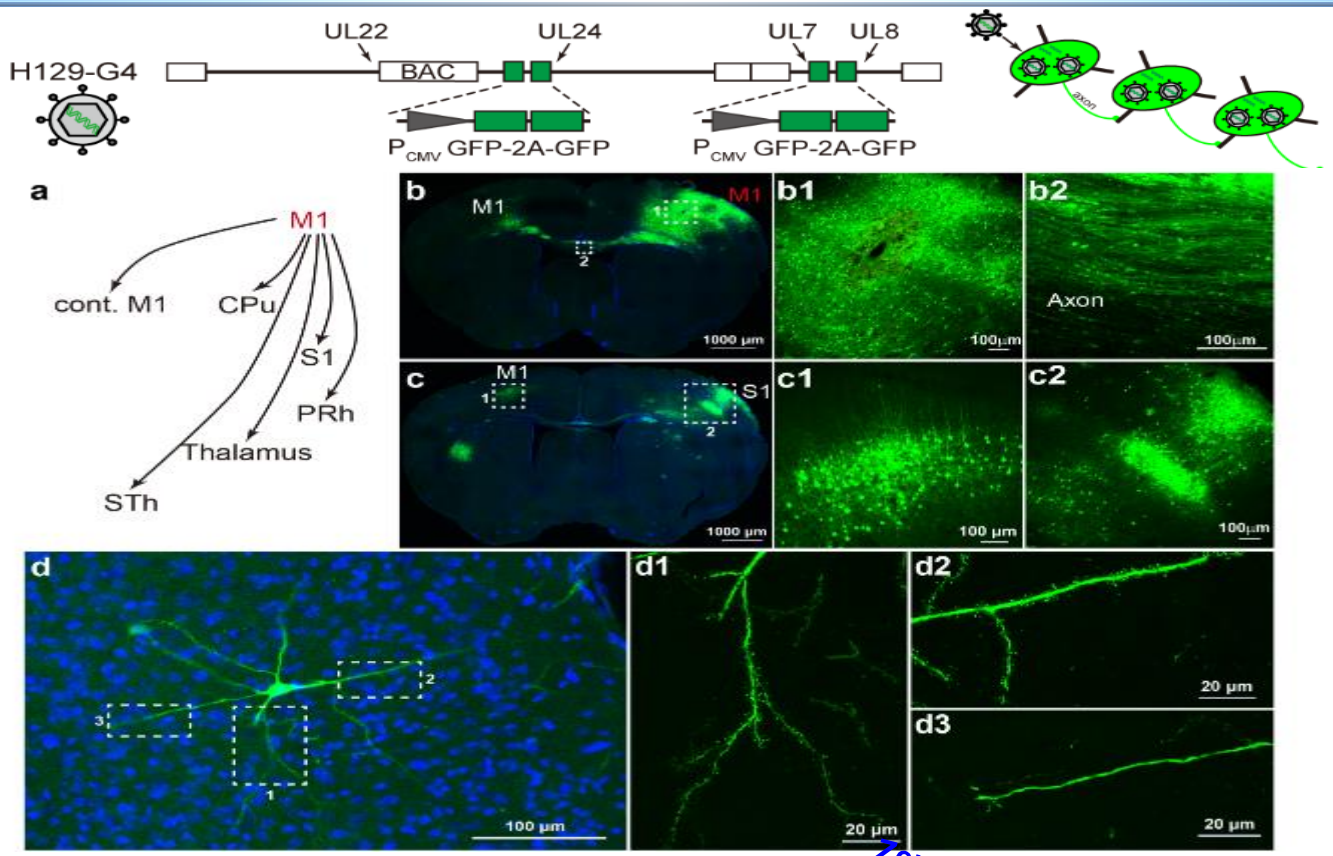
H129-G4 transmission direction *in vitro*,



Brain stereotaxic injection



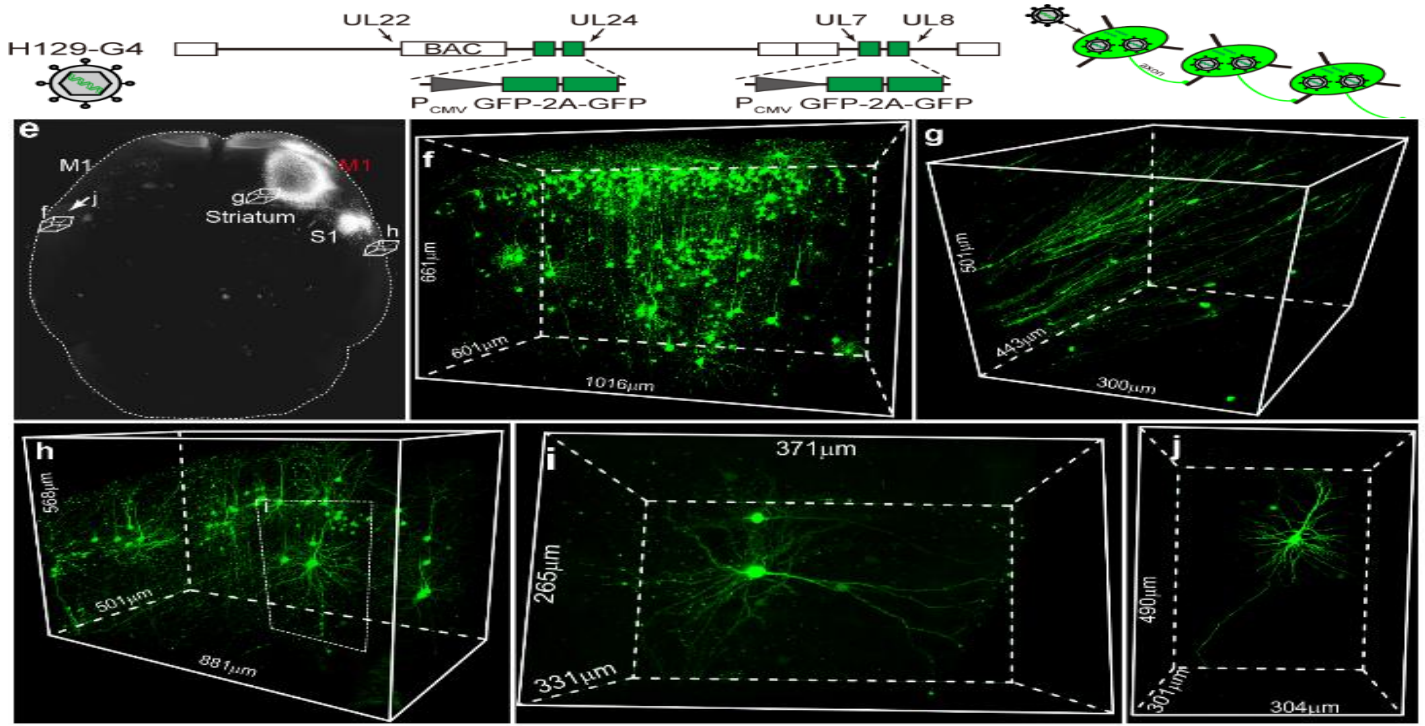
Anterograde Polysynaptic Tracing with H129-G4



High labeling efficiency and intensity: details of neurons

Zeng Wei et al. Mol Neurod

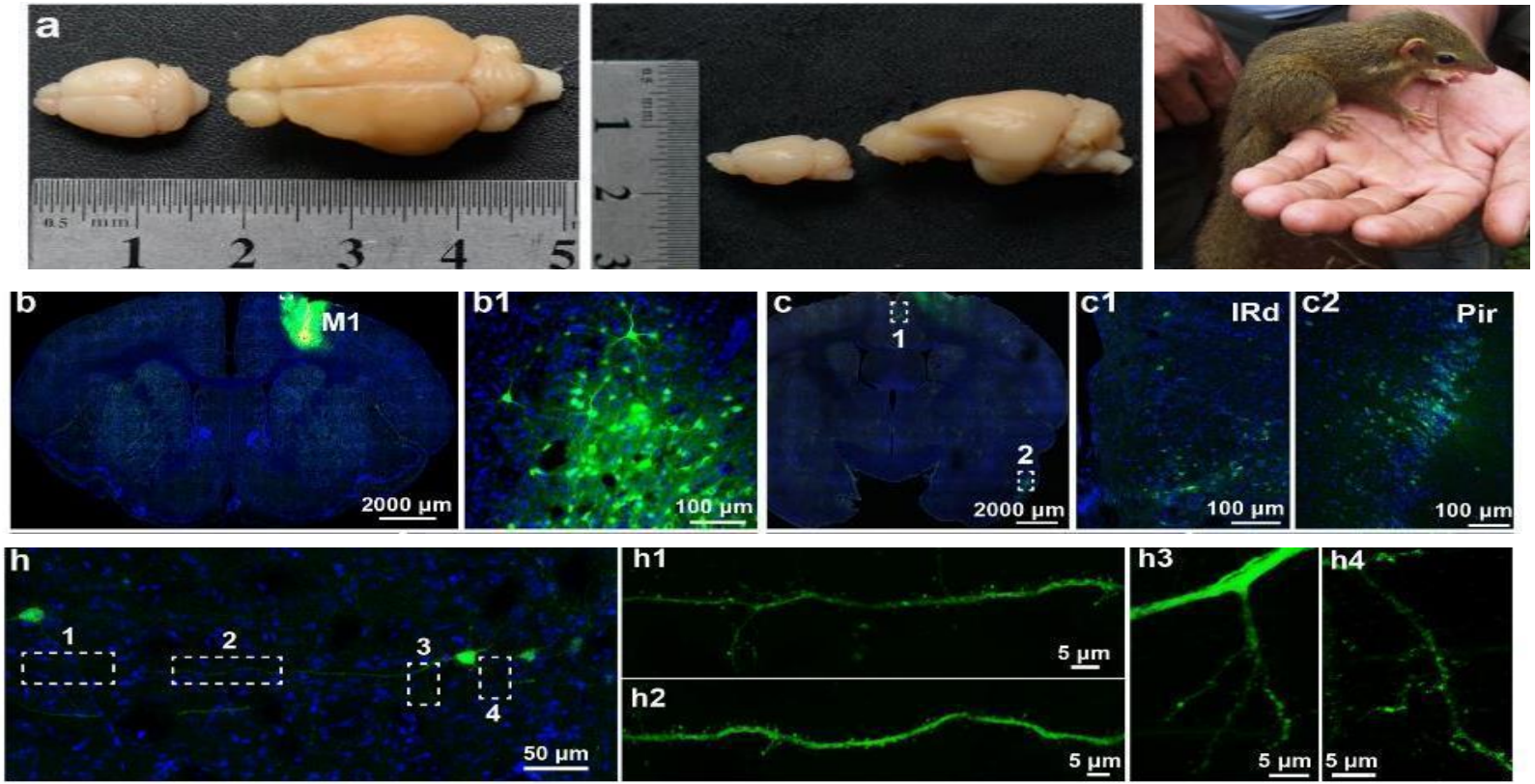
Polysynaptic Tracing with H129-G4 and imaging by fMOST



High intensity H129-G4 labeling is compatible with fMOST whole brain high resolution imaging

fMOST: fluorescence Micro-Optical Sectioning Tomography Zeng WB, *et al.* Mol Neurodegener. 2017

Tree Shrew Neural Circuitry Tracing with H129-G4

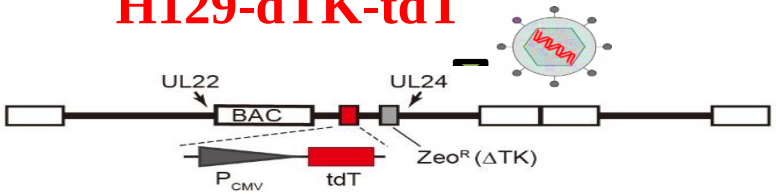


Tree shrew

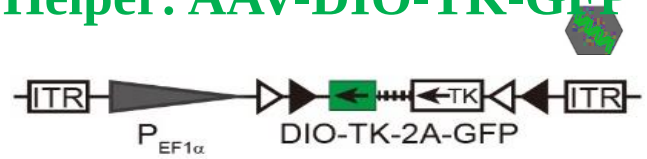
Zeng WB, *et al*. Mol Neurodegener. 2017

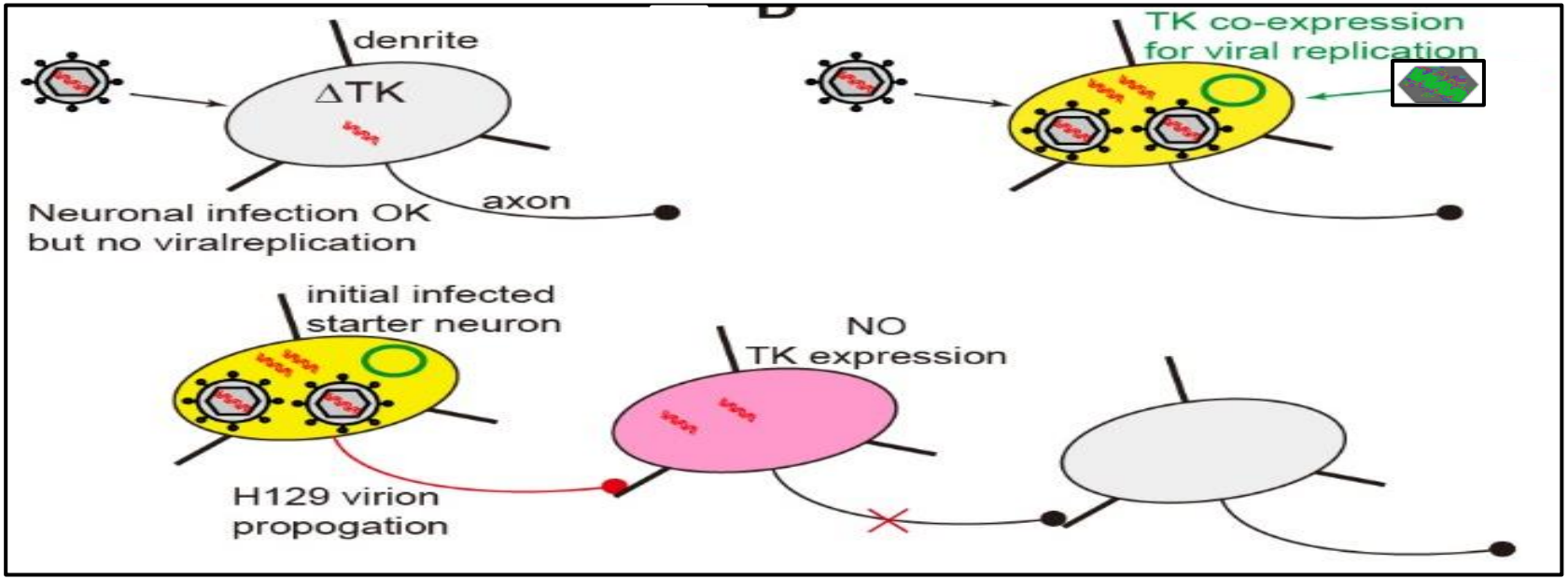
H129-dTK-tdT：anterograde mono-synaptic tracer
顺向跨单级工具的0到1突破

H129-dTK-tdT

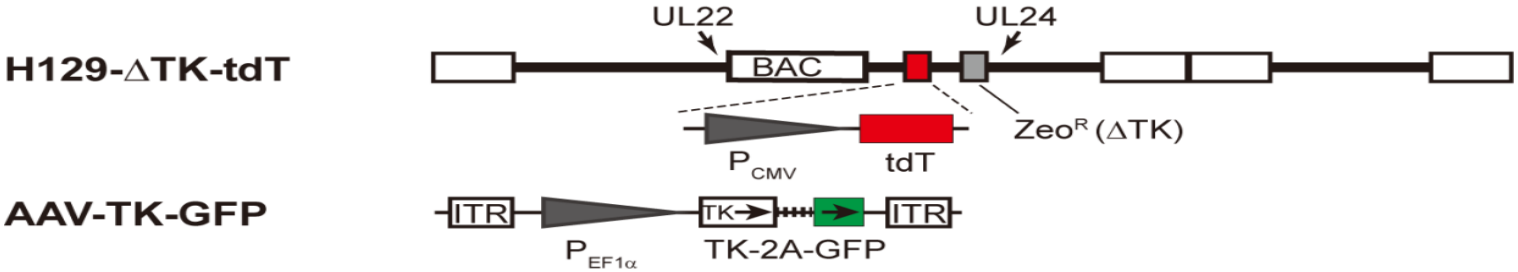


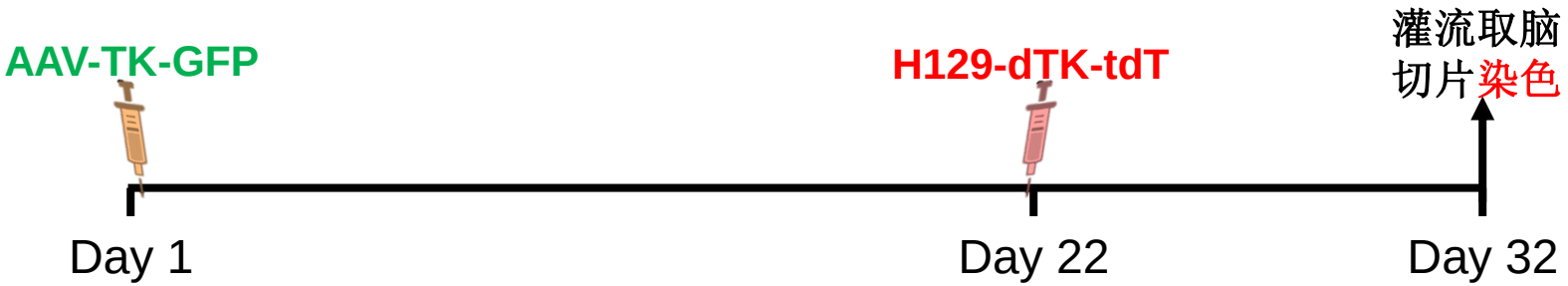
Helper: AAV-DIO-TK-GFP



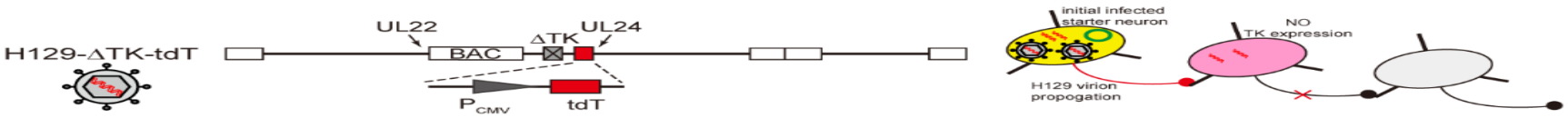


H129-dTK顺向跨单级示踪

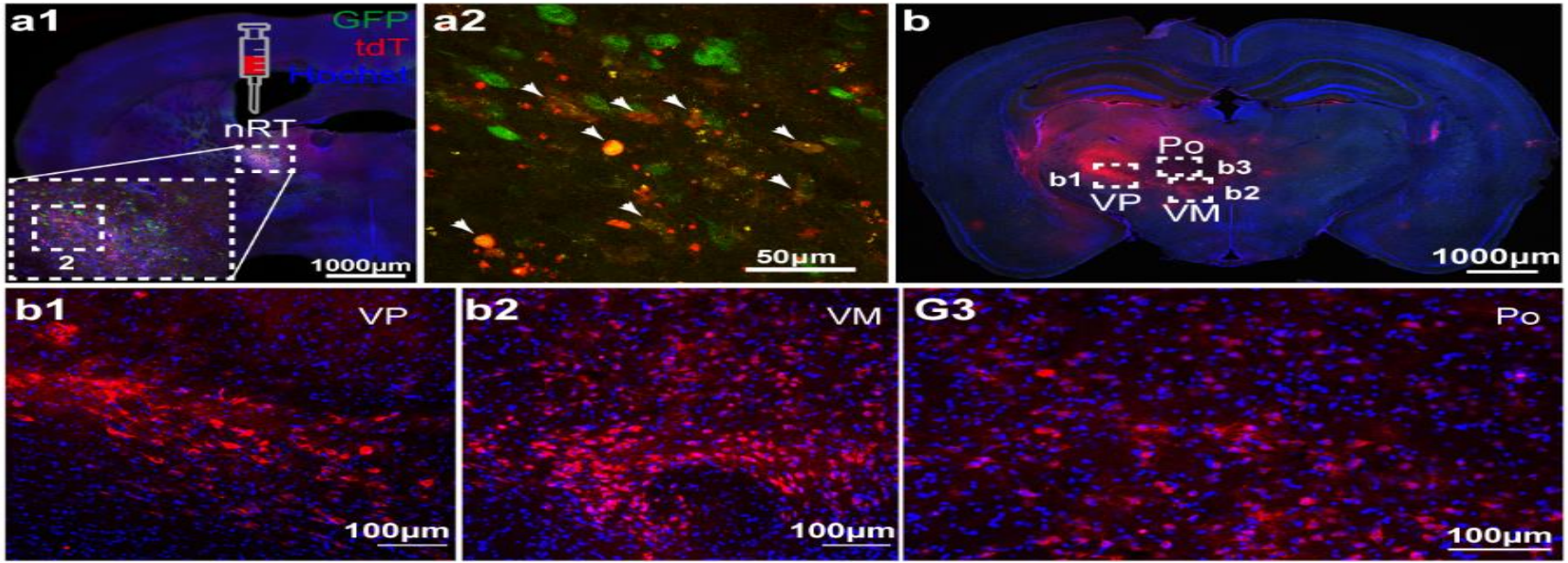




H129-dTK-dTd: direct projectom tracing



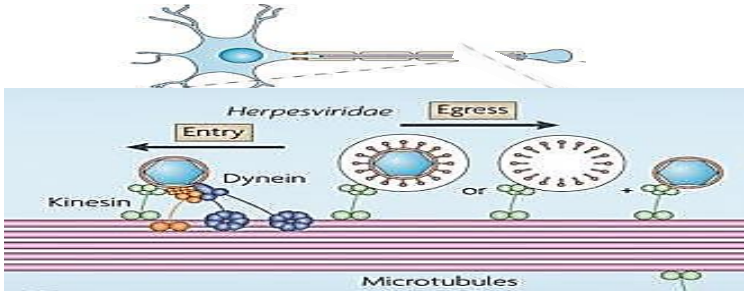
nRT-PV projectome

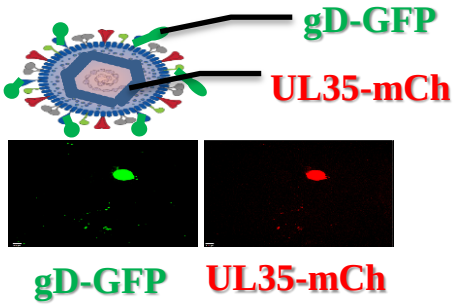


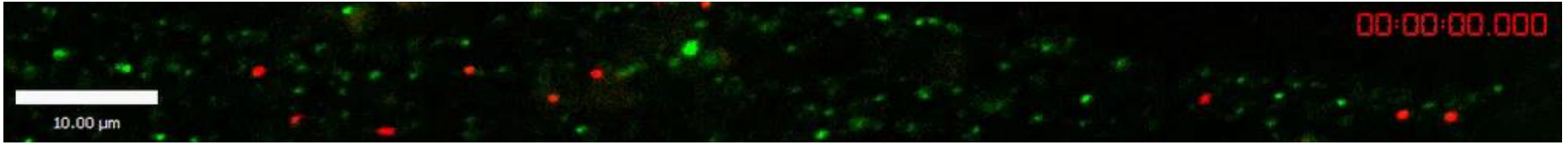
The first anterograde mono-transneuronal tracer

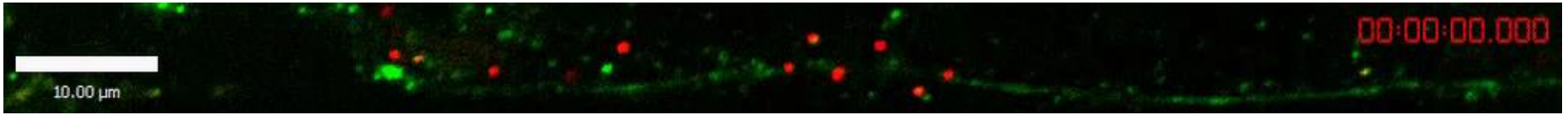
Zeng WB, *et al*. Mol Neurodegener. 2017

Axonal Transport of H129

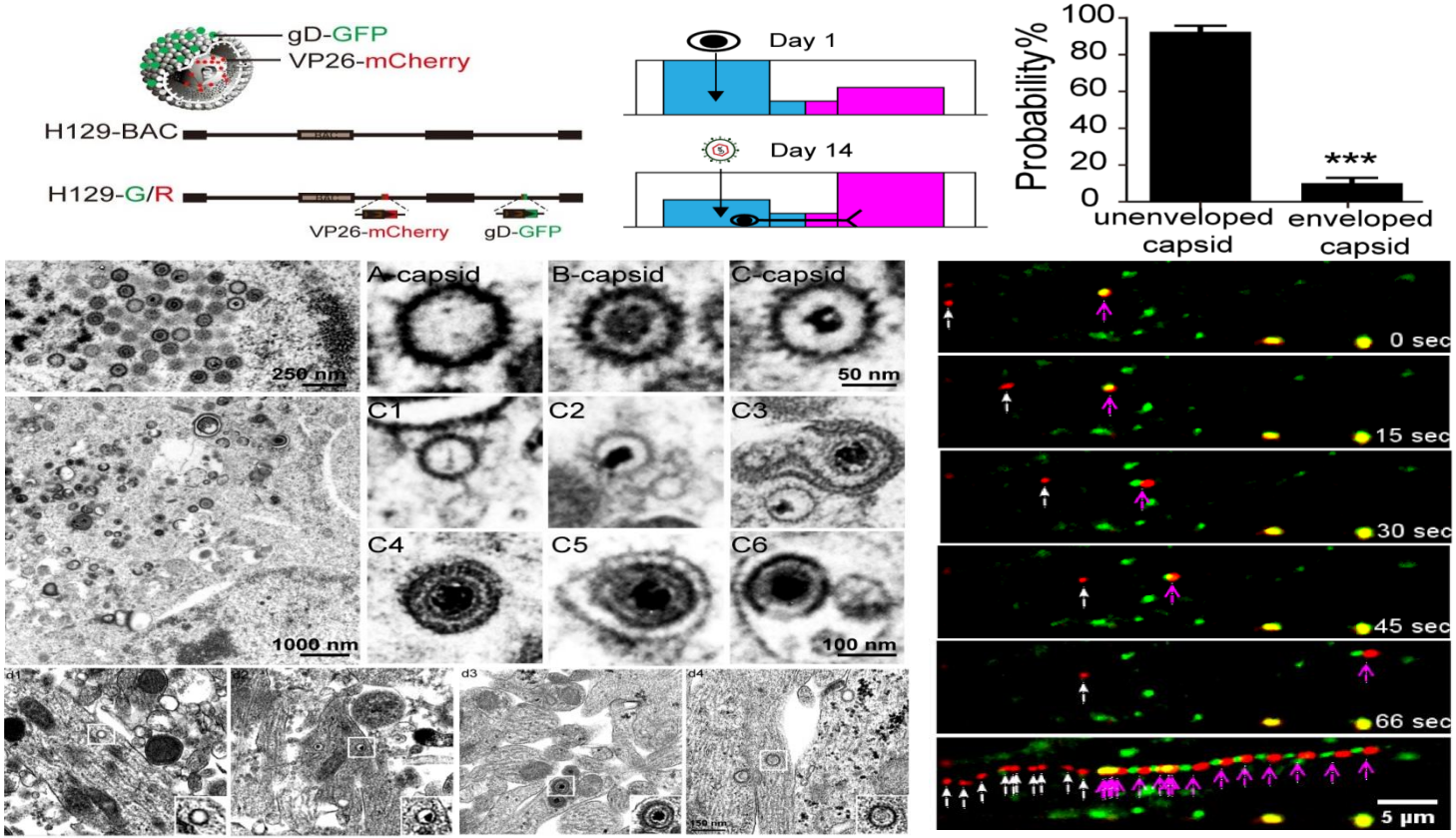








Axonal Transportation of H129 Particles: Primarily as Capsids in Cortical Neuron Axons



CA1-EC connectivities with H129 tracers

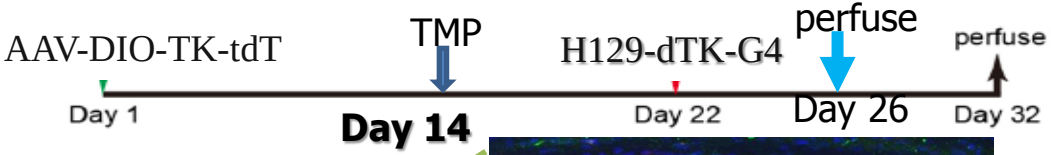
EC ⟹ CA1

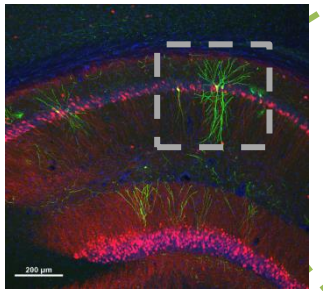
A distinct entorhinal cortex to hippocampal CA1 direct circuit for olfactory associative learning. **Nat Neurosci. 2017; 20:559-570**.

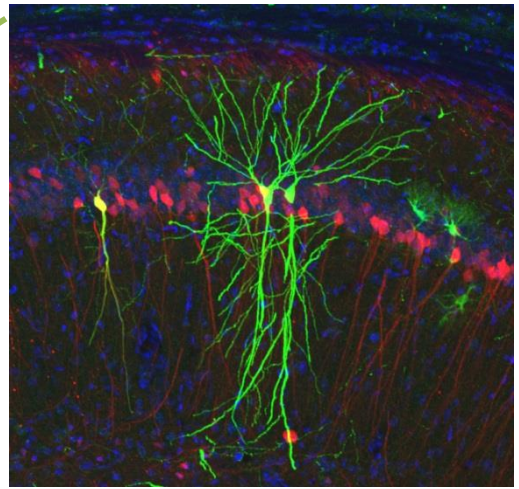
Collaborations

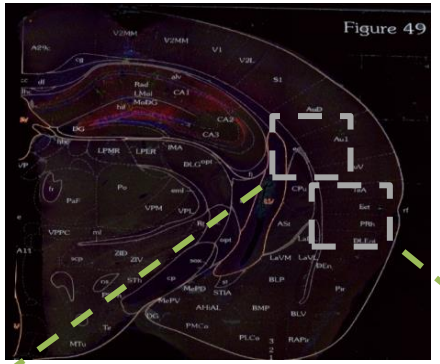
EC ⟸ CA1

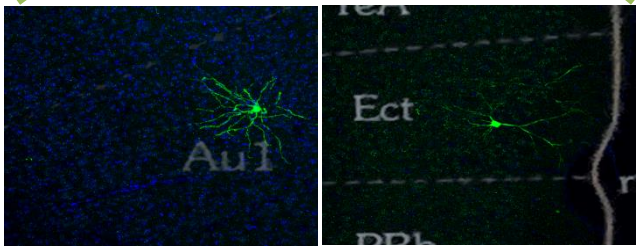
submitted











Starter cells in dCA1

Target cells in AuD and Ect

病毒与生物安全

- **AAV：** 生物安全1级

- **Rabies virus:** 弱毒株，生物安全2级；
  街毒株，生物安全3级

- **HSV-1 H129:** 生物安全2级，有效药物

大纲

◆ 脑计划与病毒工具的需求

◆ 病毒的特性与病毒工具

AAV； RV； HSV-1(H129)

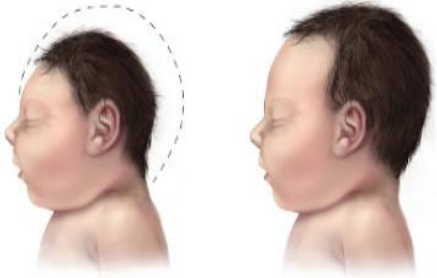
◆ 病毒工具与生物安全

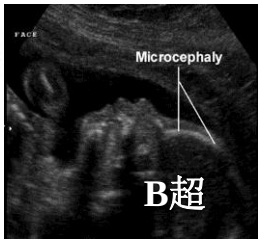
先天性HCMV感染与胎脑发育异常

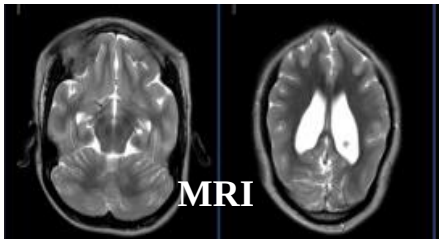
1. 天性HCMV感染是出生缺陷的最常见病因

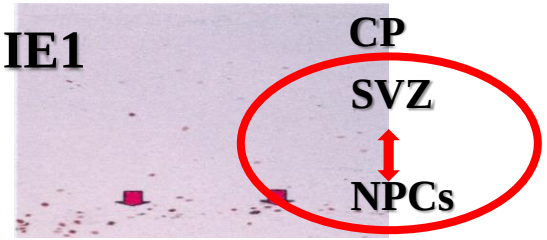
2. 我国先天性HCMV感染率为2%，加上人口基数大，受累人数多（10倍于唐氏综合征）

3. 先天性HCMV感染主要引起神经发育畸形，如小脑畸形、神经性耳聋等









胎脑为靶器官

Boppana[#]... Britt[*]. N Engl J Med. 2011: 2111-8; Kimberlin[#]… Britt[*]. N Engl J Med. 2015: 933-43

HCMV Projects at WIV since 2009

2009 2010 2013 2014 2015 2017 2018 2020

Mouse model, better tracers and vaccine

NPC : IE1/STAT3/SOX2 *J Virol*, Jiang
WDR5 J Virol, Yang
NPC and mouse: *Front Microbiol*, Jiang

NPC : Hes1/IE1/E3 ligase, *PLoS Pathog*, Liu
VZV-7D, *J Virol*, Jiang
Neural tracers: *Mol Neurodegener*, Zeng

NPC: Notch1/Jag1, *J Virol , Li; miR21/CDC25, J Virol , Fu*

HCMV Latent cell model with high genome copies (T98G), *J Virol*, ***Spotlight,*** Duan

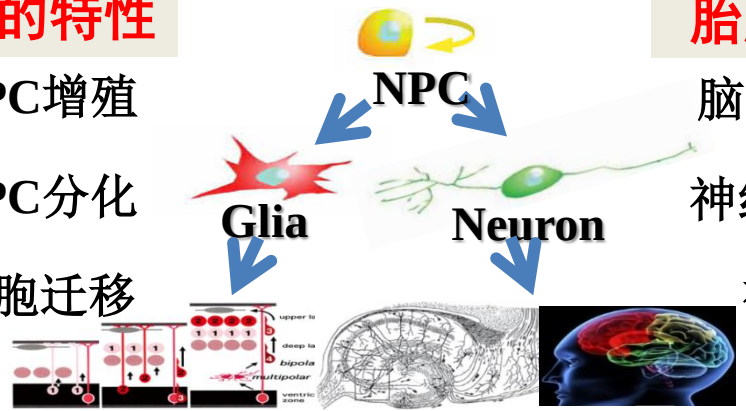
NPC Bank， HCMV acute NPC model, *J Virol*, *Pan*

NPC differentiation, *J Virol*

HCMV致脑发育畸形细胞模型：NPCs

NPC的特性

1. NPC增殖
2. NPC分化
3. 细胞迁移



胎脑发育

脑细胞数量

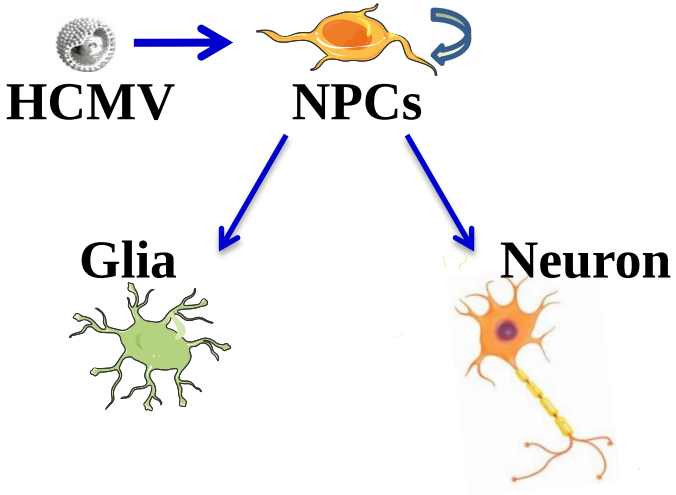
神经元/胶质

神经环路

NPC是胎脑发育的基石，对CMV感染最敏感

- 建成了初具规模的NPC细胞库，持续研究的资源优势
- 建立了CMV感染的NPC细胞模型，最接近临床的细胞模型

HCMV 感染NPC的细胞模型



HCMV infection cell model

Luo MH *et al.* J Virol 2007
Luo MH *et al.* J Virol 2008
Luo MH *et al.* J Virol 2010
Pan X *et al.* J Virol 2013

HCMV and NPC cell fate

Luo MH *et al.* J Virol 2007
Duan YL *et al.* J Virol 2014 (亮点)
Li XJ *et al.* J Virol 2015
Fu YR *et al.* J Virol 2015
Jiang HF *et al.* J Virol 2017
Liu XJ *et al.* PLoS Pathog 2017
Wu CC *et al.* J Virol 2018
Yang B *et al.* J Virol 2018
Wen L, *et al* Proteins & Cells 2020

Major Collaboration works

Li Y, …, Luo MH, …, Zhang X. Nat Neurosci. 2017
Zhu H, …, Luo MH, …, Lu Y. Nat Commun. *2017*
Wang YY, …, luo MH, …Wang XM. Exp Neurol. 2017
Yu K, …, Luo MH, …, Li B. Nat Neurosci 2018
Fu Y …, Luo MH….Shu H. Cell Host & Microbe 2017
Huang ZF …, Luo MH….Wang YY. Cell Host & Microbe 2018
Sun T….Luo MH…Zhang Z. PNAS 2019

Acknowledgement

Lab members:

Fei Zhao, Wenbo Zeng
Shuang Cheng, Xuan Jiang
Jinyan Sun, Xiao Dong
Haifei Jiang, Xijuan Liu
Bo Yang, Le Wen
Hong Yang, Yuepeng Zhou
Yige Song, Xianzhang Wang
Jing Zhou, Shengnan Huang
Haibin Qin, Mengjie Mei

Major Funding:

Strategic Priority Research Program (XDB32030000)
Key project of NSFC for international collaboration (81620108021)
Key project of NSFC for imaging devices (81427801)
Key Basic Porject from MOST 973（2015CBR55600）
NIHRF1 (1RF1MH120020-01) (2019-2022)

Collaborators:

Lee Fortunato (ID, USA)
Michael McVoy (VA, USA)
Michael Nevels (St Andrews, UK)
William J. Britt (AL, USA)
Simon Rayner (Oslo, Norway)
Xiangmin Xu (CA, USA)
Greg Horwitz (WA, USA)