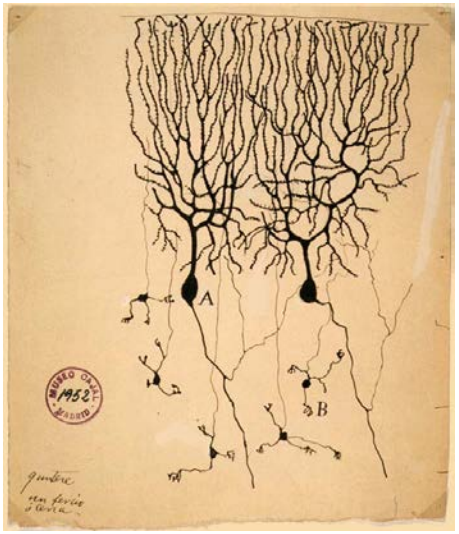
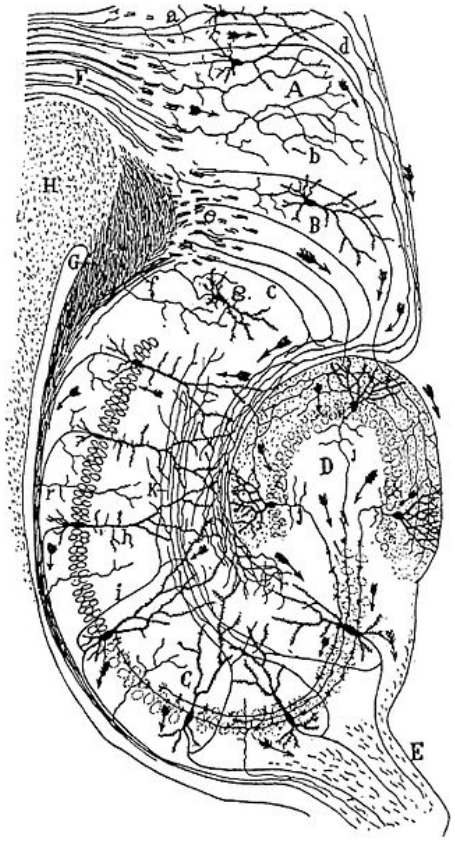
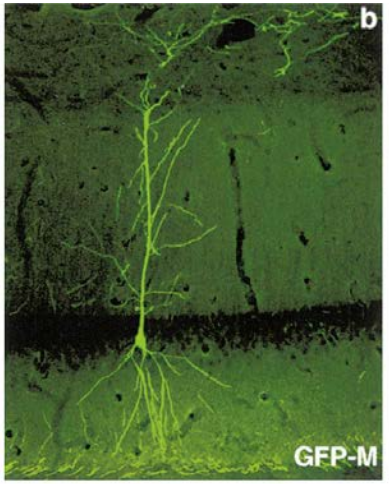
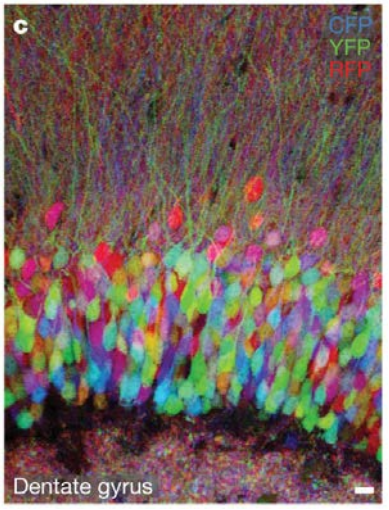
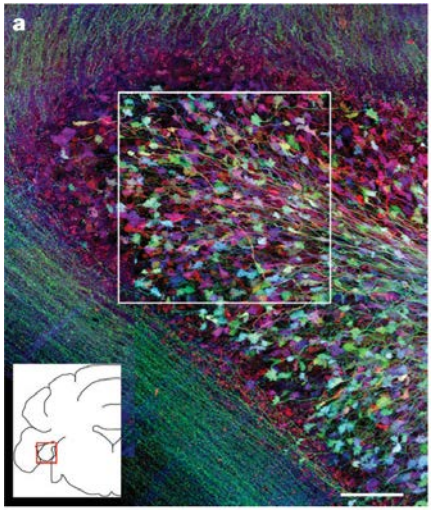
Introduction to Genetic Tools in Neurobiology & NS201B Overview











Eric J. Huang, Professor of Pathology

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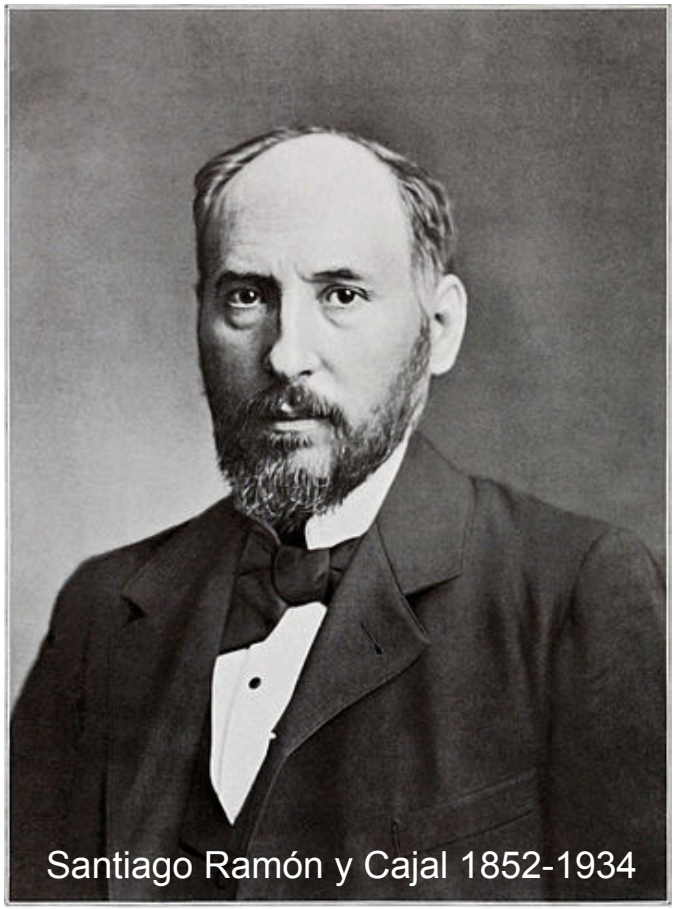
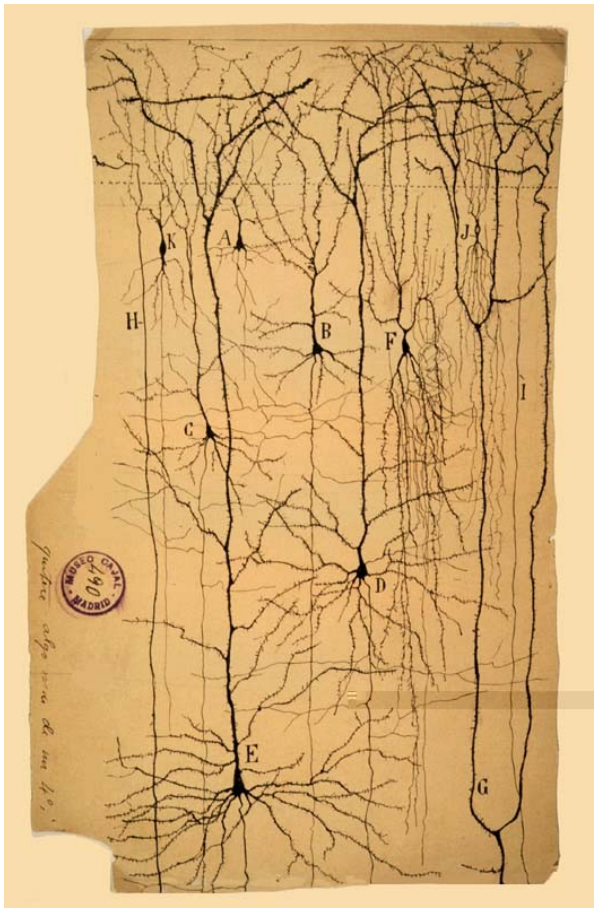
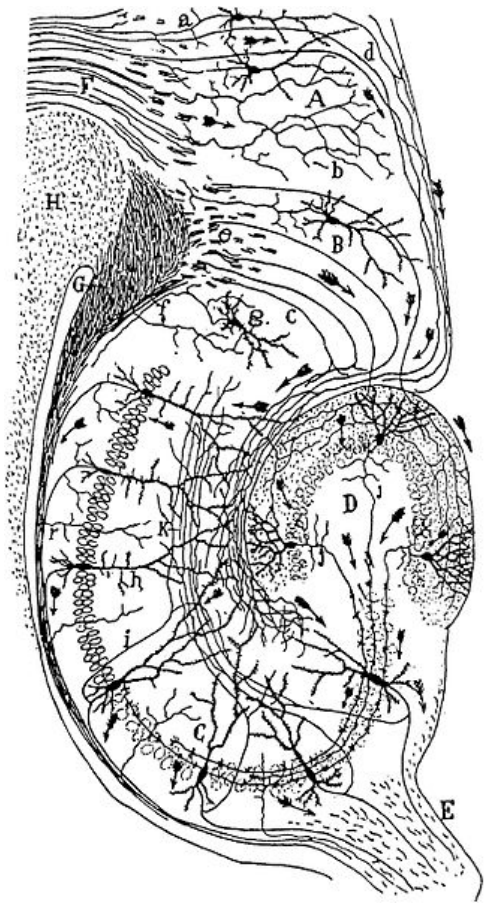
The Neuron Doctrine

The fundamental unit in the nervous system



Santiago Ramón y Cajal 1852-1934





1906 Nobel Prize in Physiology or Medicine (shared with Camillo Golgi)

The Polarity Hypothesis

Directional flows in dendrites, soma and axons

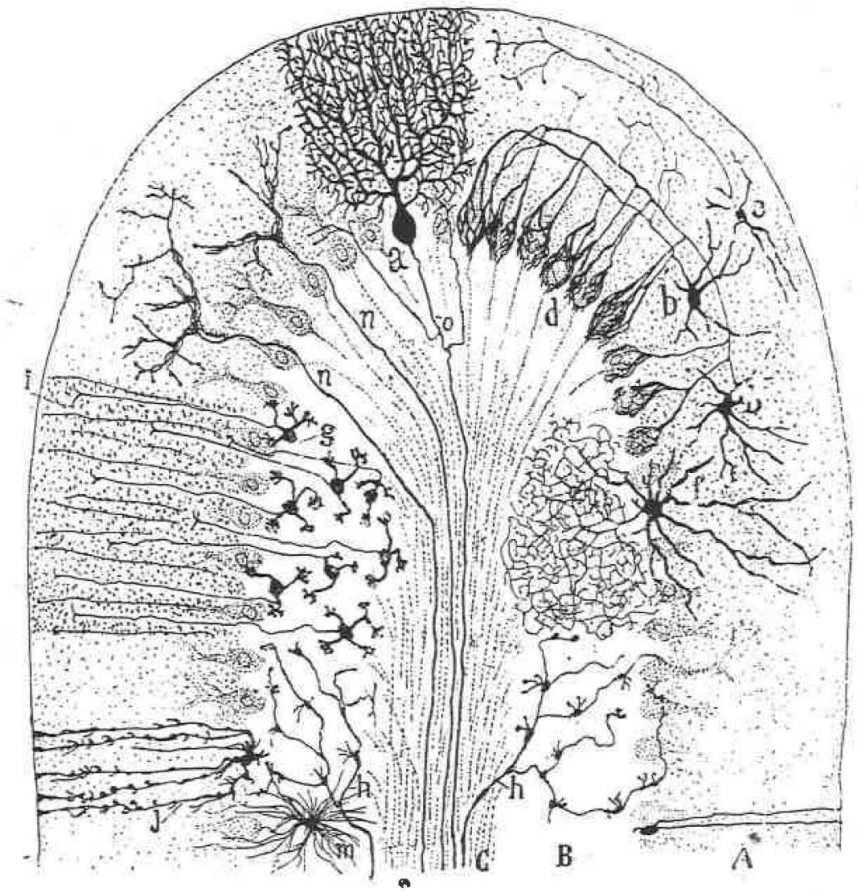


FIG. 28. SEMIDIAGRAMMATIC TRANSVERSE SECTION OF A CEREBELLAR CONVOLUTION OF A MAMMAL. *A*, molecular layer; *B*, granular layer; *C*, layer of white matter; *a*, Purkinje cell with its dendrites spread out in the plane of section; *b*, small stellate cells of the molecular layer; *d*, descending terminal arborizations embracing the cells of Purkinje; *e*, superficial stellate cell; *f*, large stellate cell of the granule layer; *g*, granules with their ascending axons bifurcating at *i*; *h*, mossy fibres; *j*, tufted neuroglia cell; *n*, climbing fibres; *m*, neuroglia cell of the granule layer.

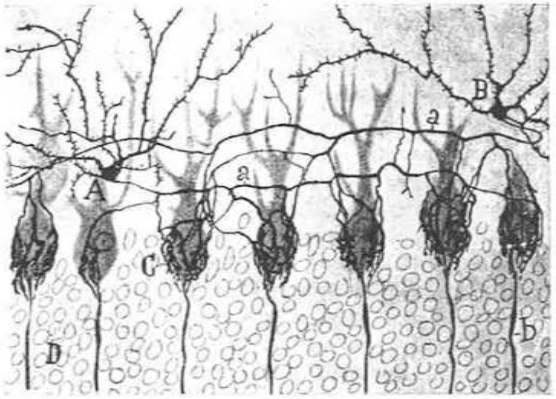


FIG. 26. TRANSVERSE SECTION OF A CEREBELLAR LAMELLA. Semidiagrammatic. *A* and *B*, stellate cells of the molecular layer (basket cells), of which the axon (*a*) produces terminal nests about the cells of Purkinje (*C*): *b*, axon of the Purkinje cell.

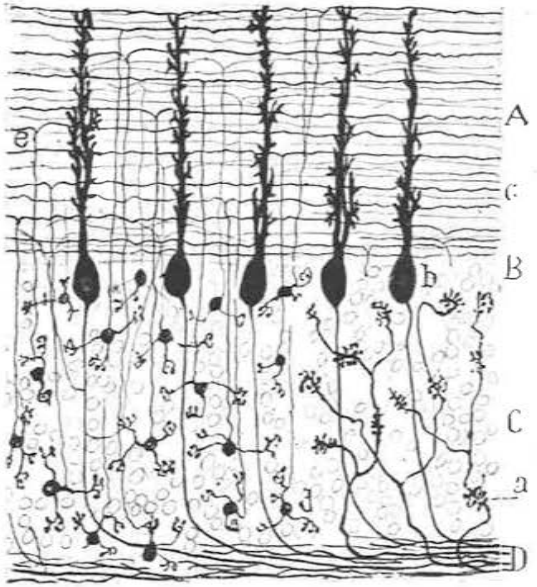


FIG. 27. LONGITUDINAL SECTION OF A CEREBELLAR CONVOLUTION. *A*, molecular layer; *B*, layer of Purkinje cells; *C*, granular layer; *D*, white matter; *a*, tuft of a mossy fibre; *b*, body of a Purkinje cell; *c*, parallel fibres; *d*, granule cell with its ascending axon; *e*, division of this axon. (Semidiagrammatic.)

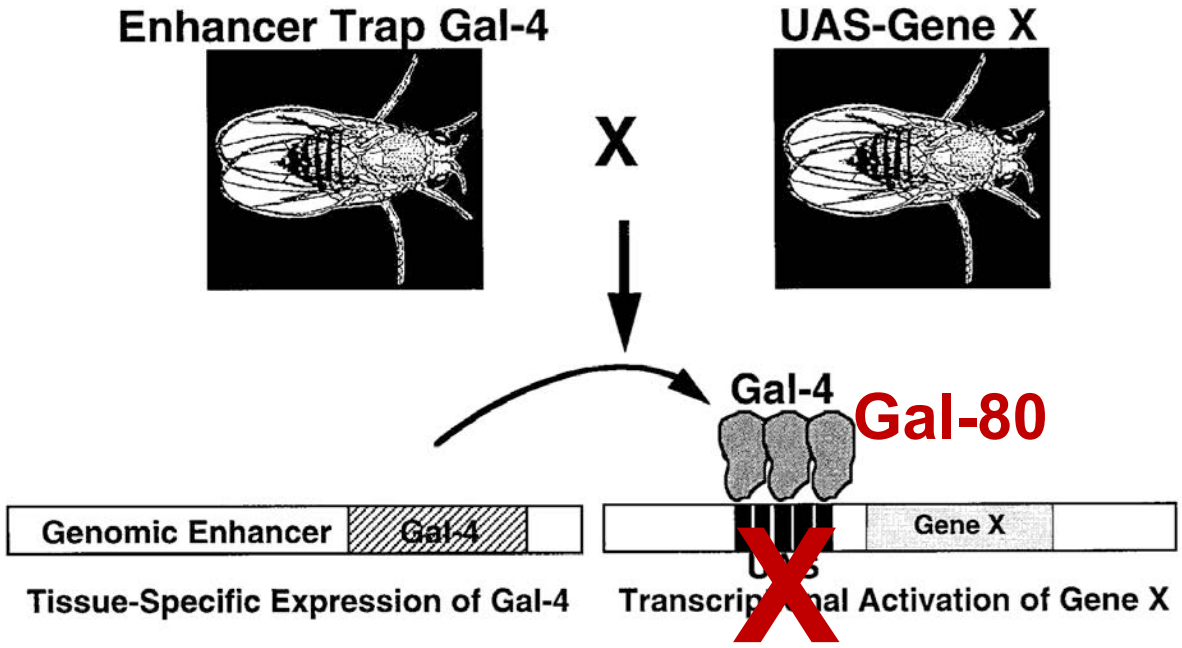
Introduction to Genetic Tools in Neurobiology

- **Genetic approaches to investigate the complexity of the nervous system**
  - **Lineage tracing of neuronal origin**
  - **Cell biology of neuron**
  - **Neural circuits and connectivity**
- **Model organisms**
  - ***Caenorhabditis elegans***
  - ***Drosophila melanogaster***
  - ***Mus musculus***
  - ***Rattus rattus***
- **Technical approaches**
  - **Transgenic**
  - **Conditional gene targeting**
- **Functional outcomes**
  - **Loss of function vs. Gain of function**
  - **Cell type-specific vs. circuit-dependence**

Take Home Message:
Genetic tools allow temporal and spatial controls to study gene and circuit functions in neurobiology

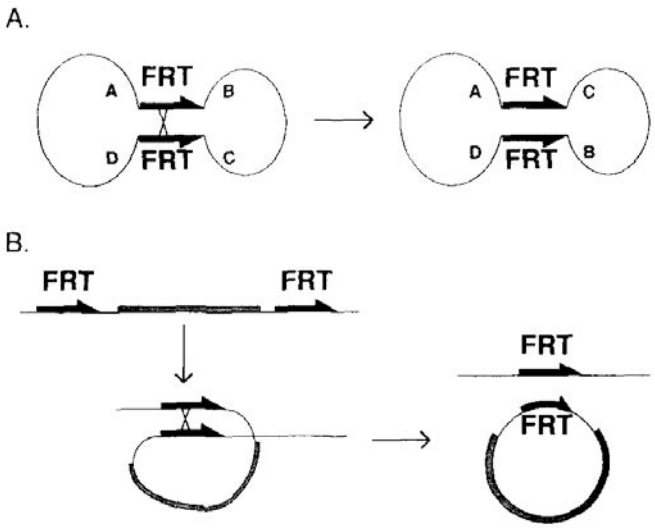
Genetic tools in neurobiology research in *Drosophila*

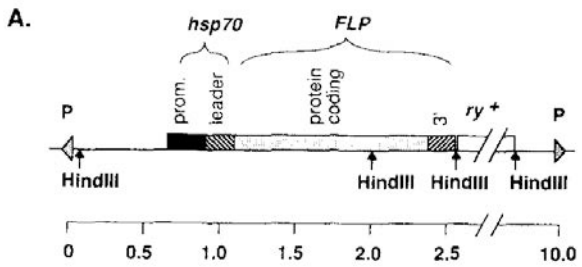
- The "GAL4-UAS" technique – misexpression of gene
- The "FLP/FRT" technique – germline mosaics
- The MARCM system – single cells & clonal analyses



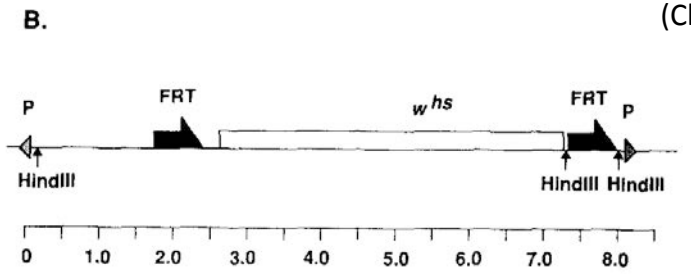
The "FLP/FRT" technique

- **FLP: a recombinase encoded by the yeast 2μm plasmid**
- **FRT: FLP recombination target present in the 2μm plasmid as 599bp inverted repeats**





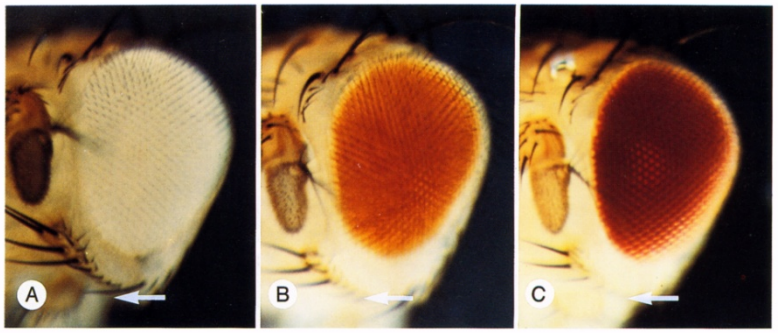
(Chromosome 2)



(Chromosome 3)

(Golic and Linquist, Cell 1989)

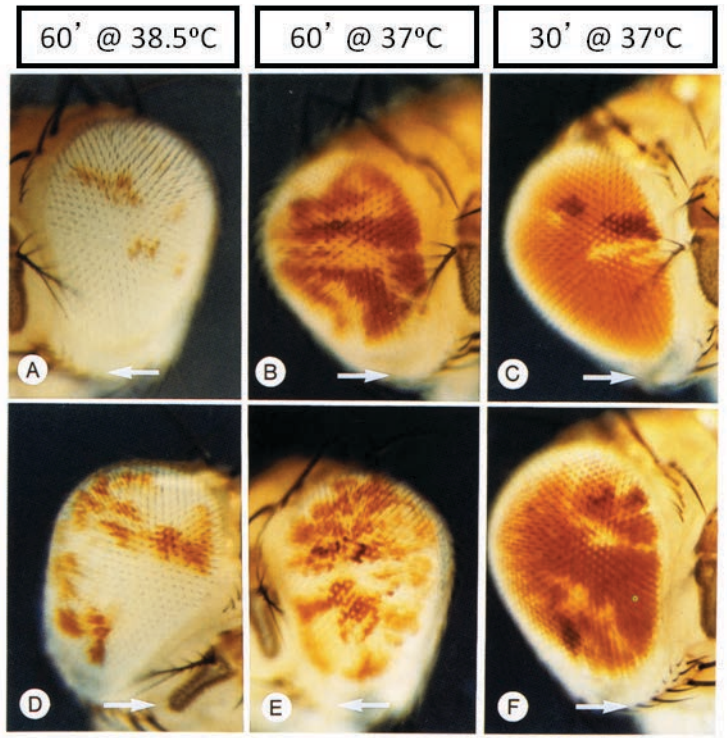
The "FLP/FRT" technique



w /w
loss of eye color; 'white' eye

w / hsp70-W+
One copy of eye color transgene

hsp70-W+/ *hsp70*-W+
two copies of eye color transgene



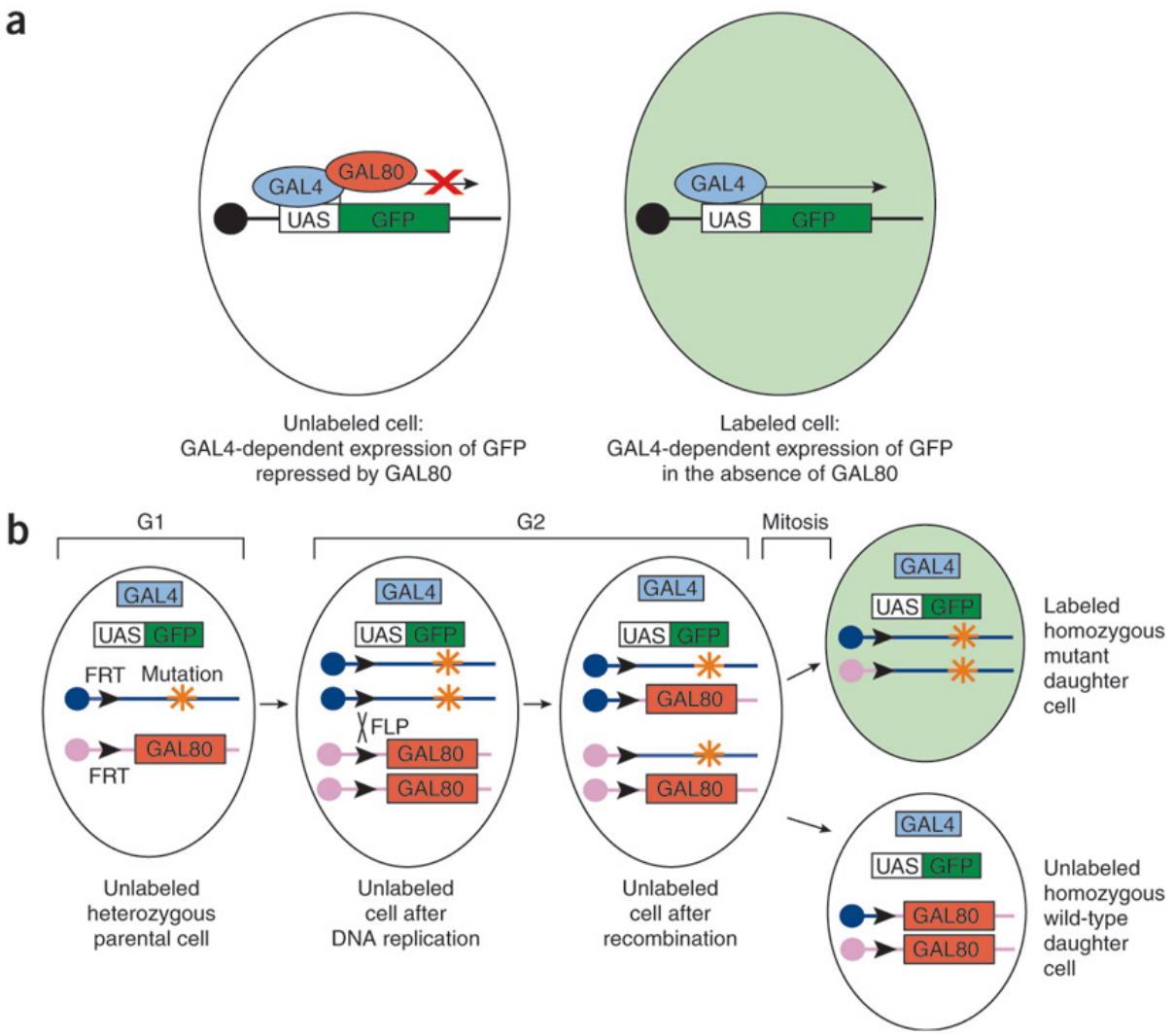
(Golic and Linquist, Cell 1989)

Mosaic Analysis with a Repressible Cell Marker (MARCM)

- **Positively marks a small population of wild type or mutant cells**
- **Generate homozygous mutant cells from heterozygous precursors via mitotic recombination**
- **MARCM-ready flies: GAL4-UAS, GAL80 and FLP/FRT**
- **Applications:**
  - **Lineage analysis**
  - **Investigating gene function in single or small populations of cells**
  - **Neuronal circuit tracing**
  - **Growth cone signaling**
  - **Axon pruning**

(Lee T, Luo L, Neuron, 1999)

Mosaic Analysis with a Repressible Cell Marker (MARCM)

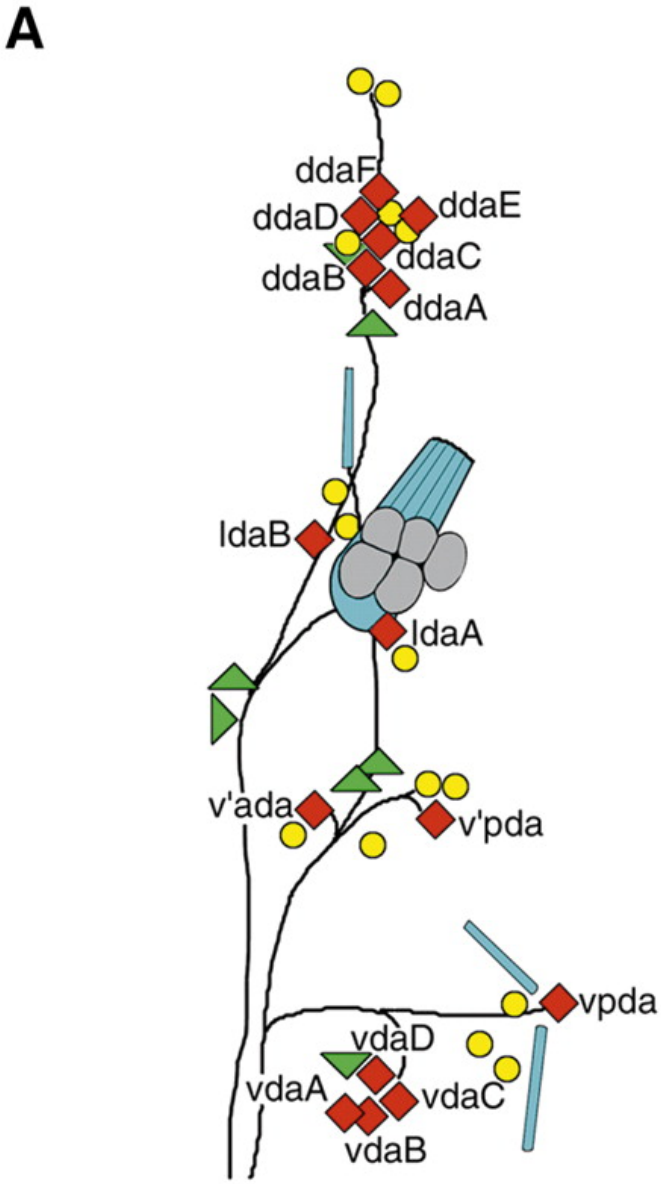


Example: MARCM used to analyze morphology

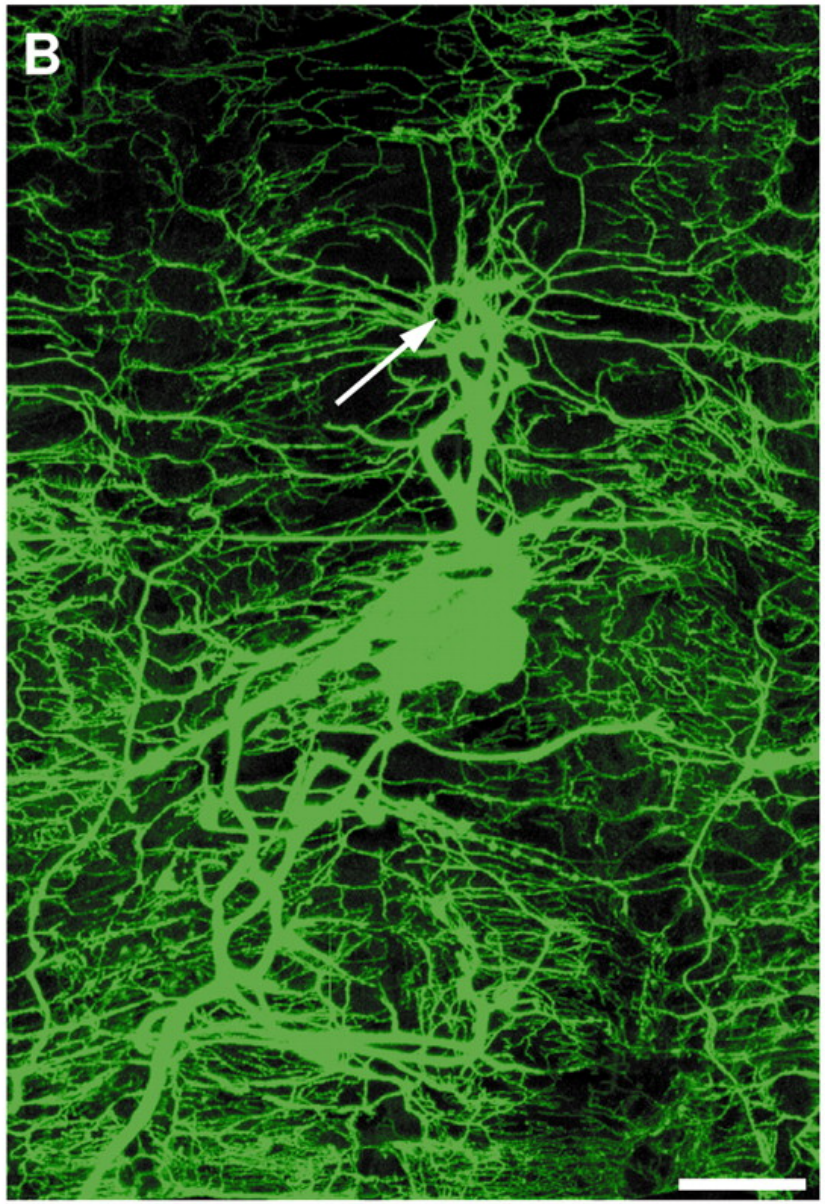
- Assessment of cell autonomous gene function
  - Single cells labeled
  - Early lethal genes can be analyzed in specific cell types at later stages of development

- Cut (ct) gene
  - Transcription factor (homeobox gene)
  - Mutants are embryonic lethal
  - Expressed in Drosophila Peripheral Nervous System (PNS) sensory neurons

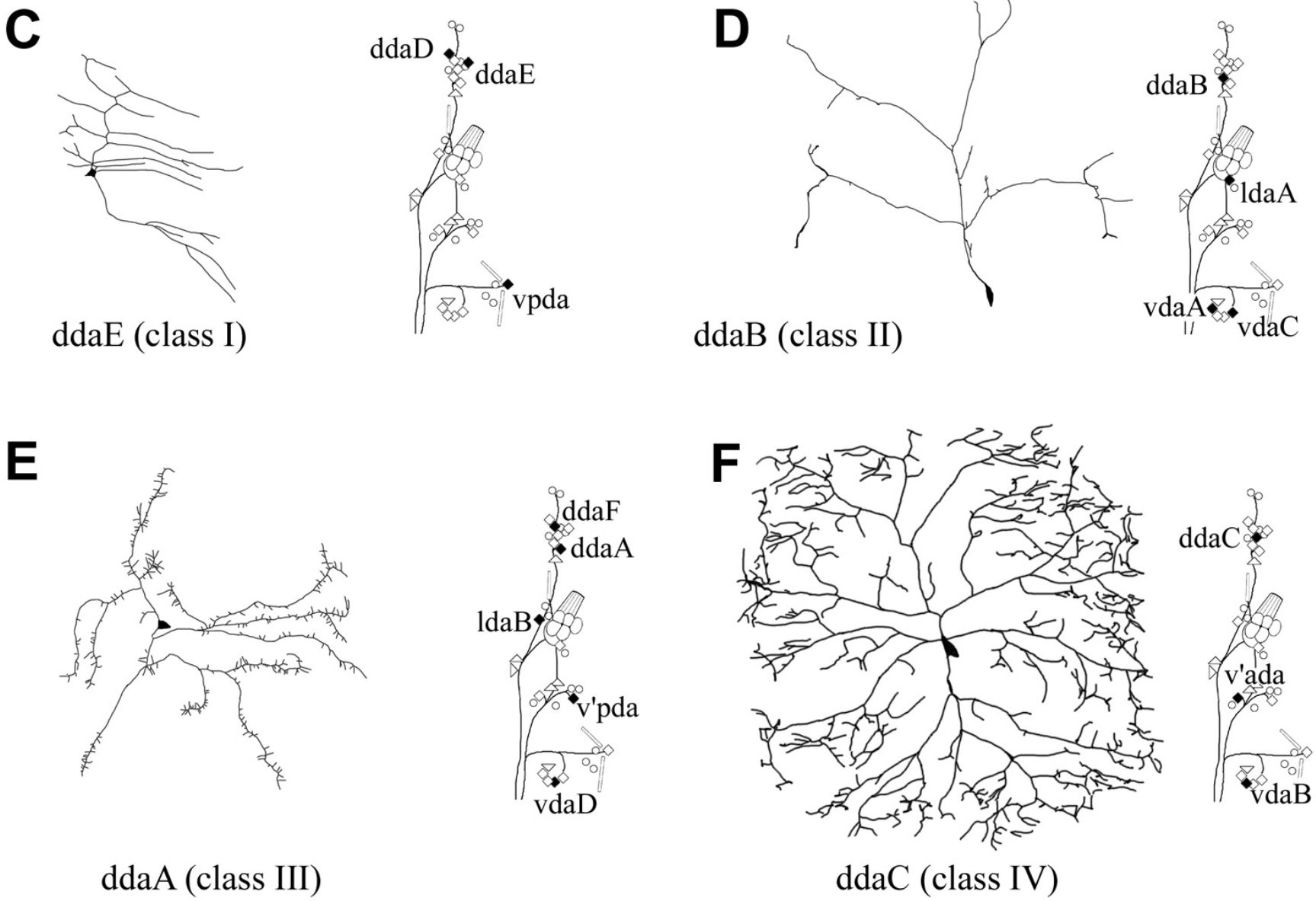
Drosophila da neuron clusters
(from one segment)



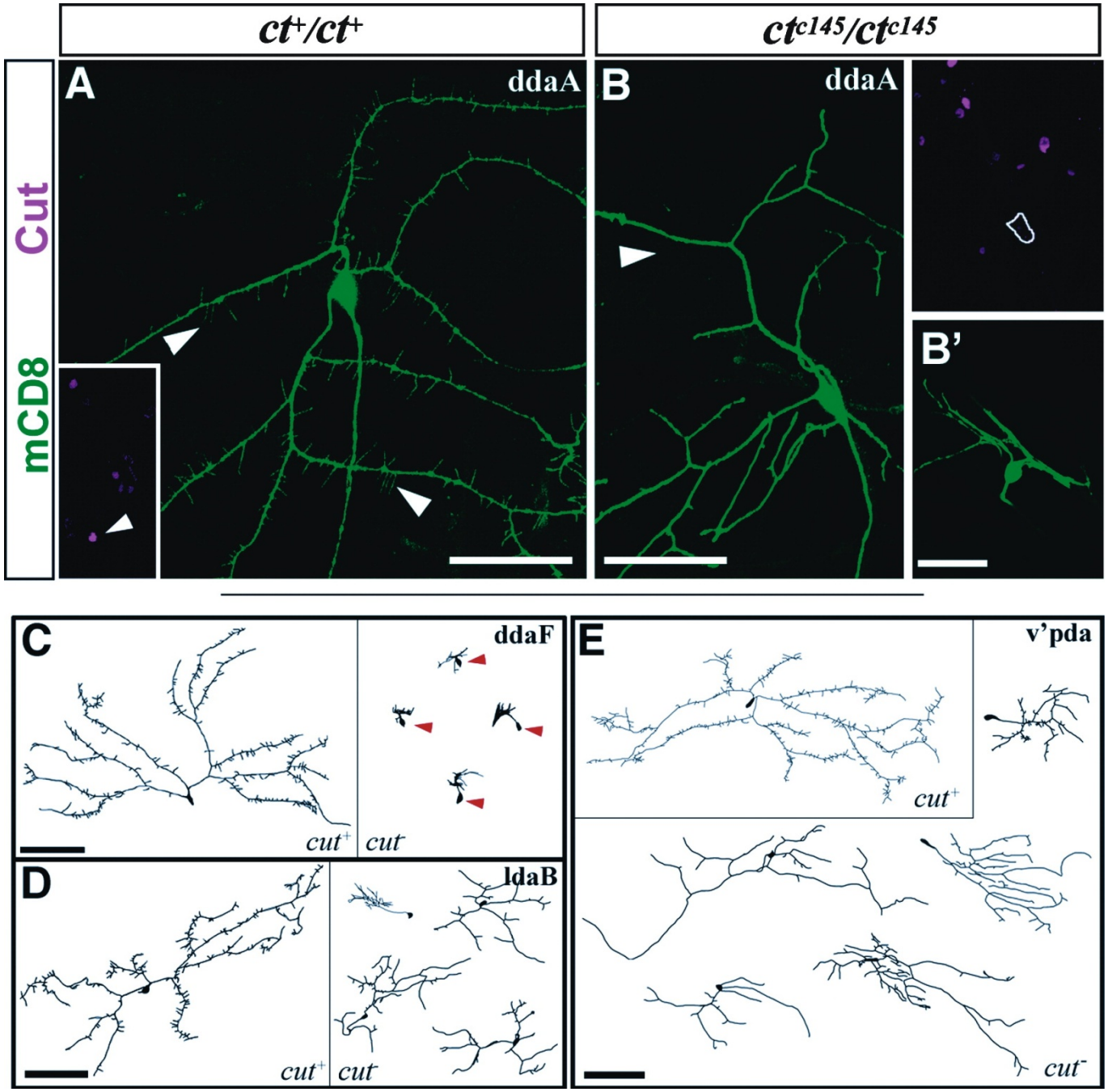
Gal4$^{109(2)80}$, UAS-mCD8-GFP (all da neurons)

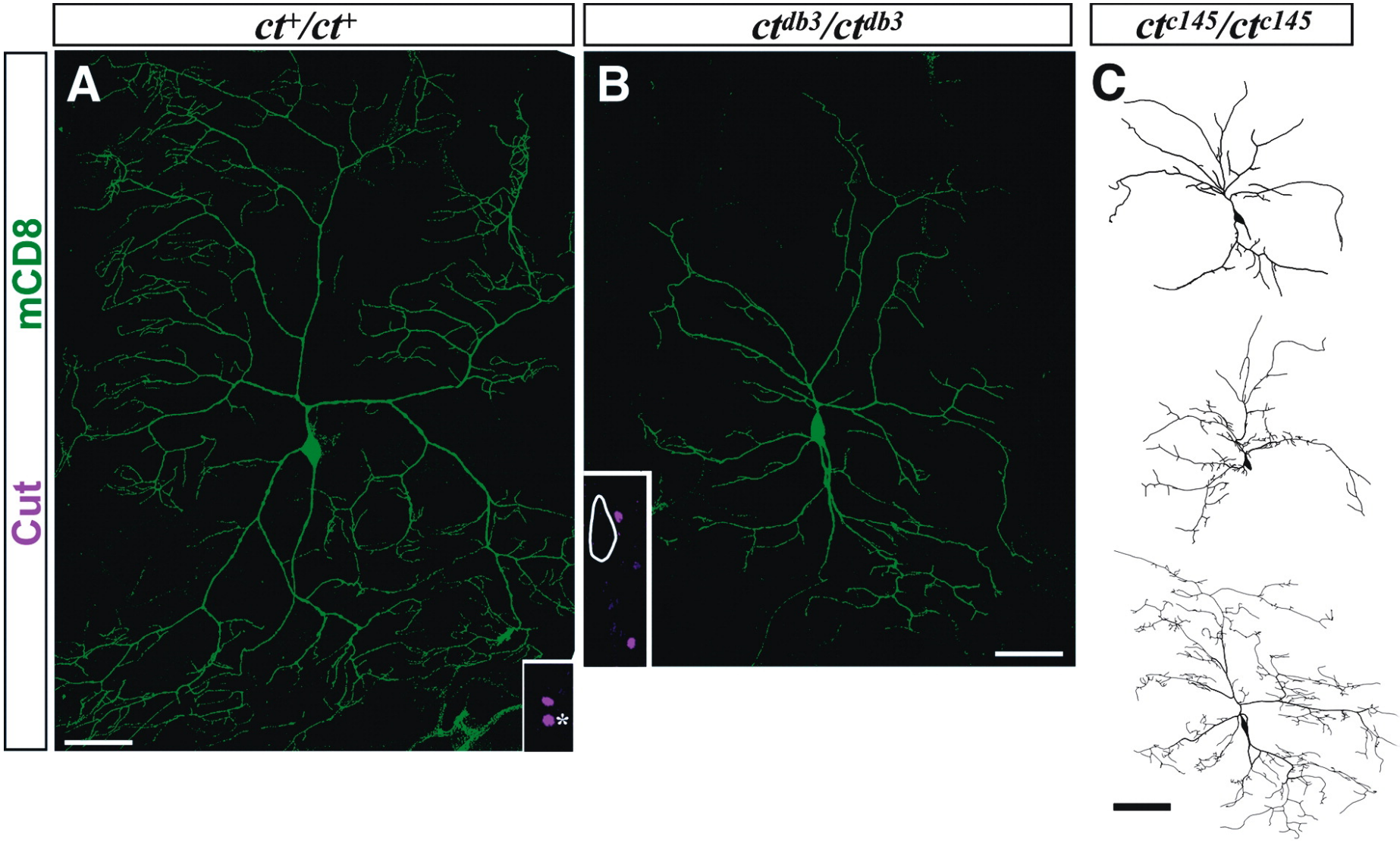


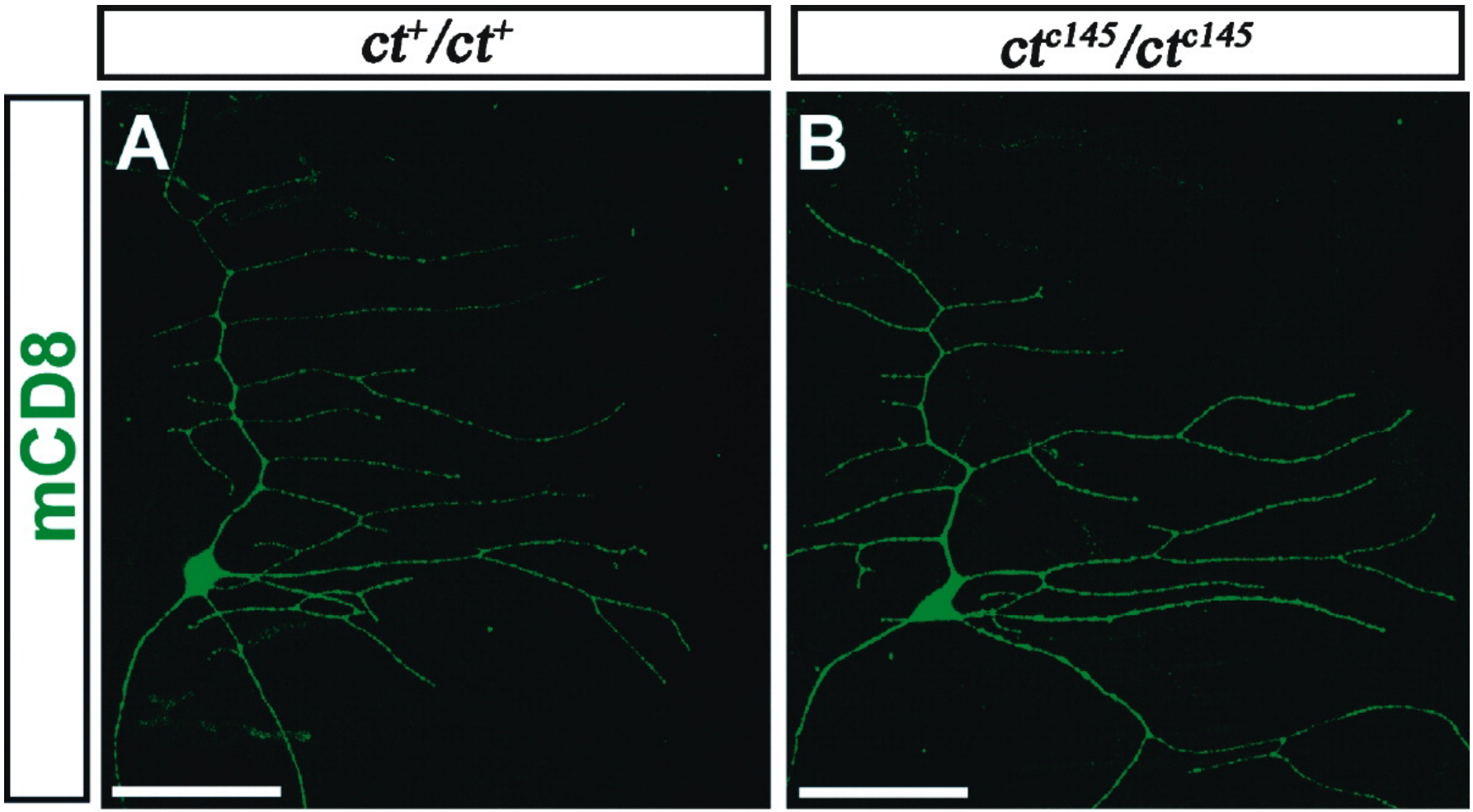
Grueber W B et al. Development 2002

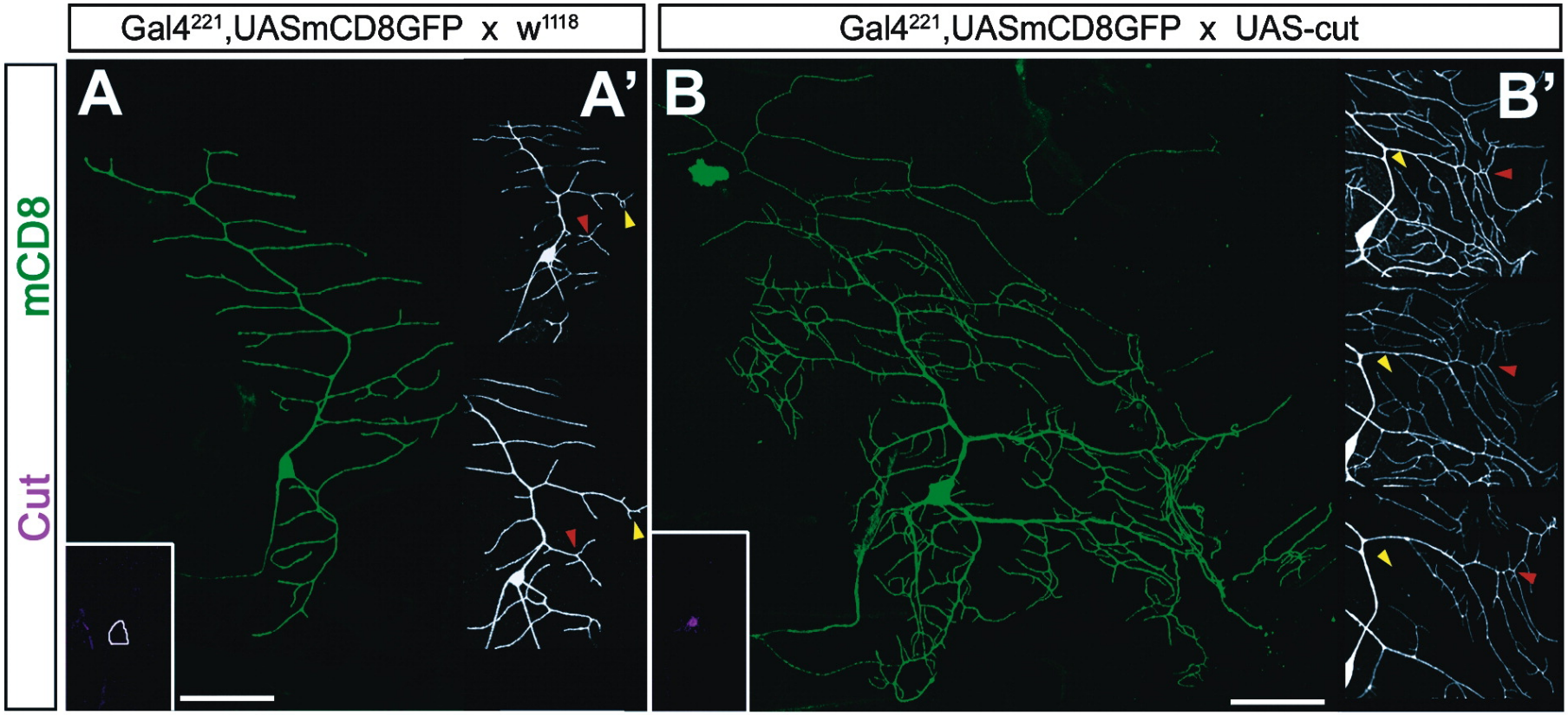


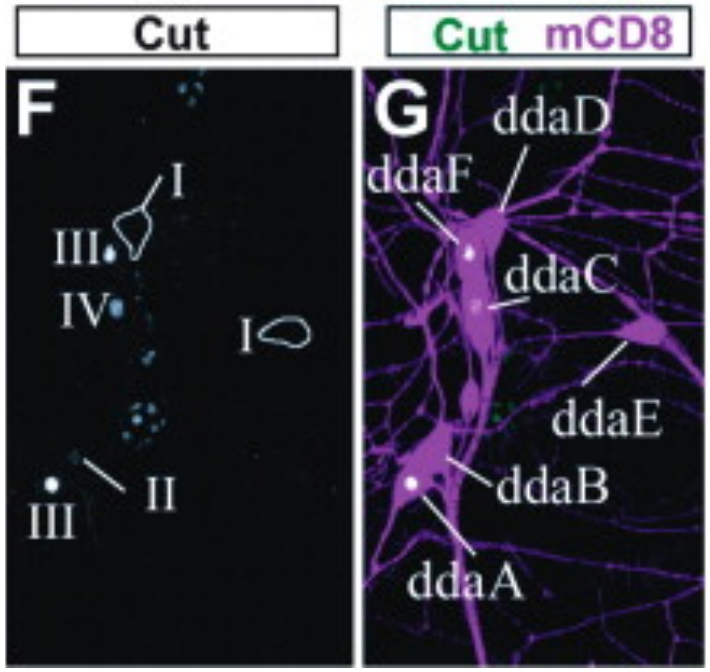
Grueber W B et al. Cell, 2003

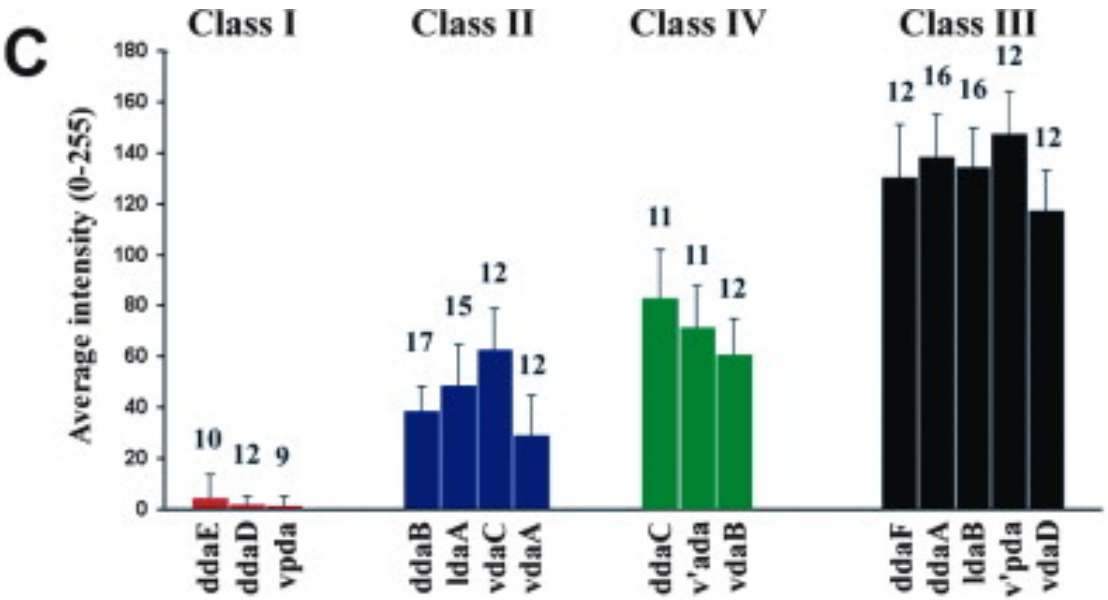

ct+/ct+
ctc145/ctc145
A
ddaA
B
ddaA
B'
Cut
mCD8
C
ddaF
cut+
cut-
D
ldaB
cut+
cut-
E
v'pda
cut+
cut-


ct+/ct+
ctdb3/ctdb3
ctc145/ctc145
A
B
C
mCD8
Cut
*


ct^{+}/ct^{+}
ct^{c145}/ct^{c145}
mCD8
A
B


Gal4221,UASmCD8GFP x w1118
Gal4221,UASmCD8GFP x UAS-cut
A
A'
B
B'
mCD8
Cut





H

	Class I	Class II	Class IV	Class III
Cut Immunoreactivity	Undetectable	Low	Medium	High
Dendrite morphology	Simple	Simple	Complex space-filling	Complex "spiked"

Limitations of MARCM

- **Timing of GAL80 elimination:** due to protein perdurance, MARCM can only be used to reliably label single cells 24-48 hours after the induction of mitotic recombination
- **Maternal GAL80 contribution:** limits the efficacy of the MARCM system in studying early embryo development

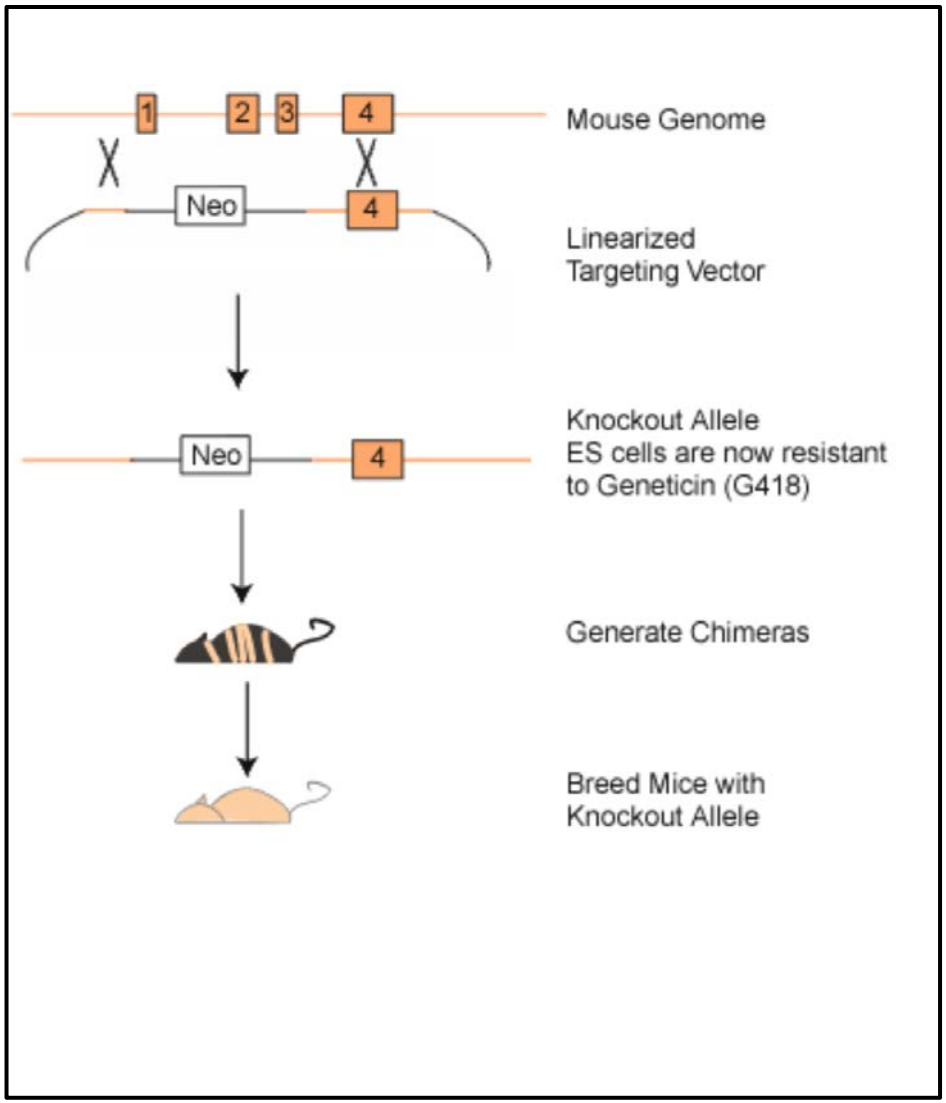
- **Gene of interest:** the high abundance and perdurance of protein may interfere with interpretations

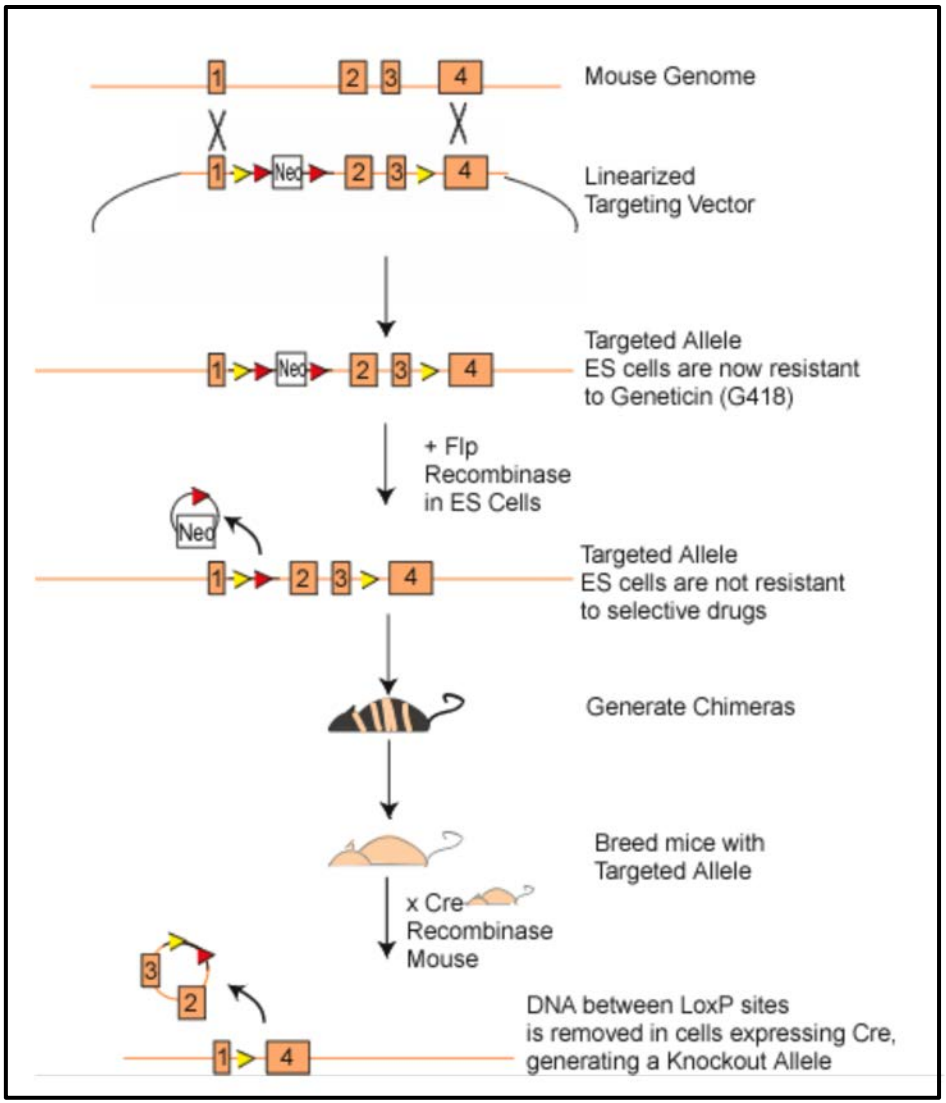
- **Some mutant cells may not be labeled by Gal4-UAS:** Gal4 is only expressed in subset of cells, but hs-FLP/FRT recombination is ubiquitous

Genetic tools for neuronal functions and neural circuits in mouse brain

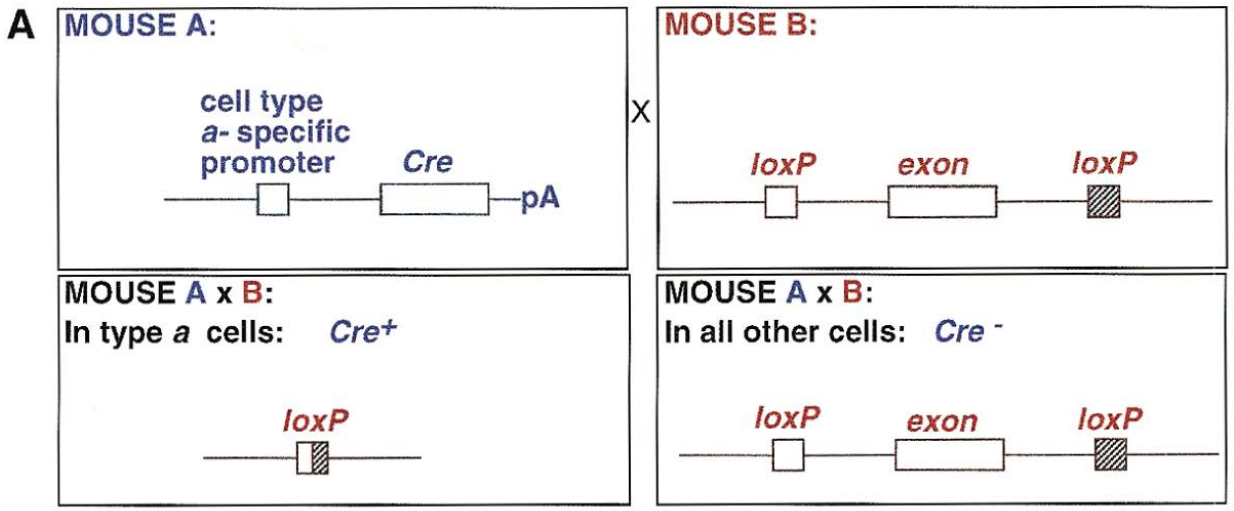
- Transgenic targeting of specific neuron type
  - *cis*-regulatory elements as drivers
  - Bacterial artificial chromosome (BAC) transgenics
  - Gene targeting ("knock-in") w/ or w/o internal ribosomal entry site (IRES)
  - Enhancer trap – random insertion of target genes in the genome under the control of a minimal promoter
- Binary expression strategy – Cre-loxP, FLP-FRT & Tet-on/Tet-off systems
  - Cre drivers
  - Cre reporter lines
- GFP reporter and its variants

Schematic diagrams of conventional and conditional knockout in mice



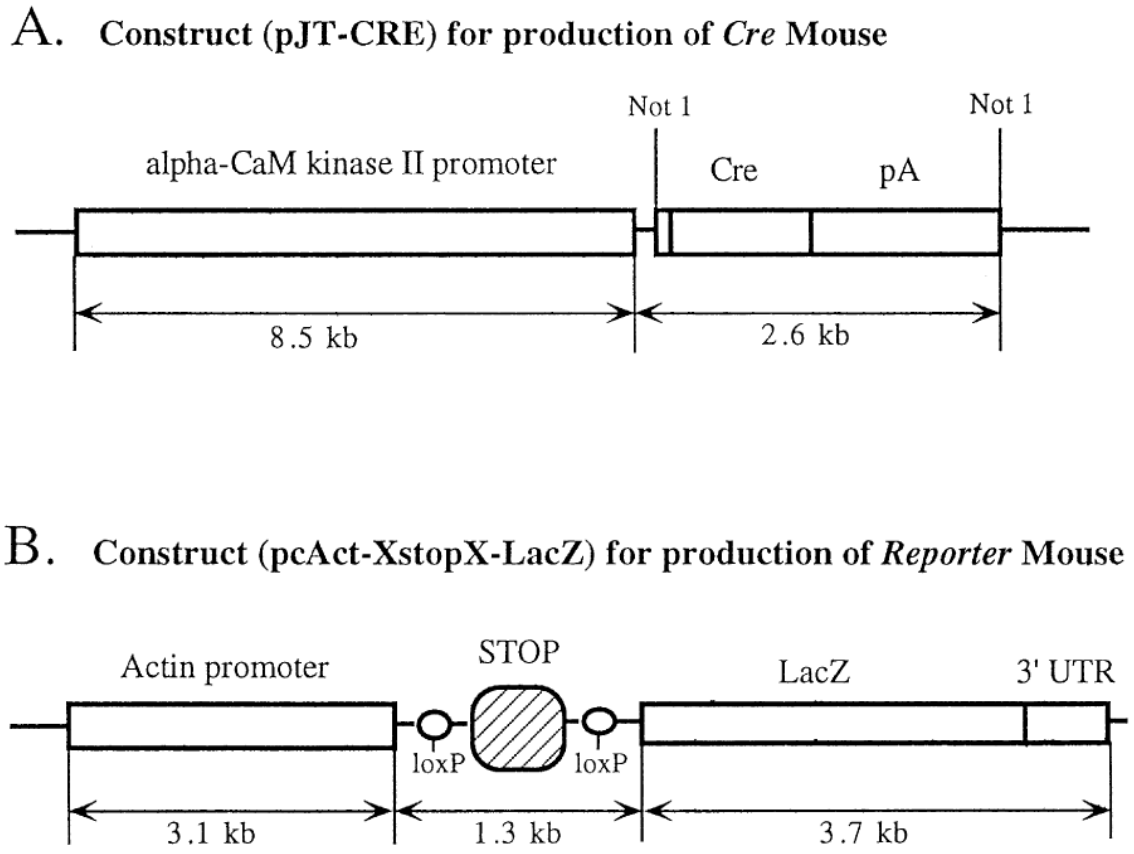


Strategy for cell type-specific gene knockout

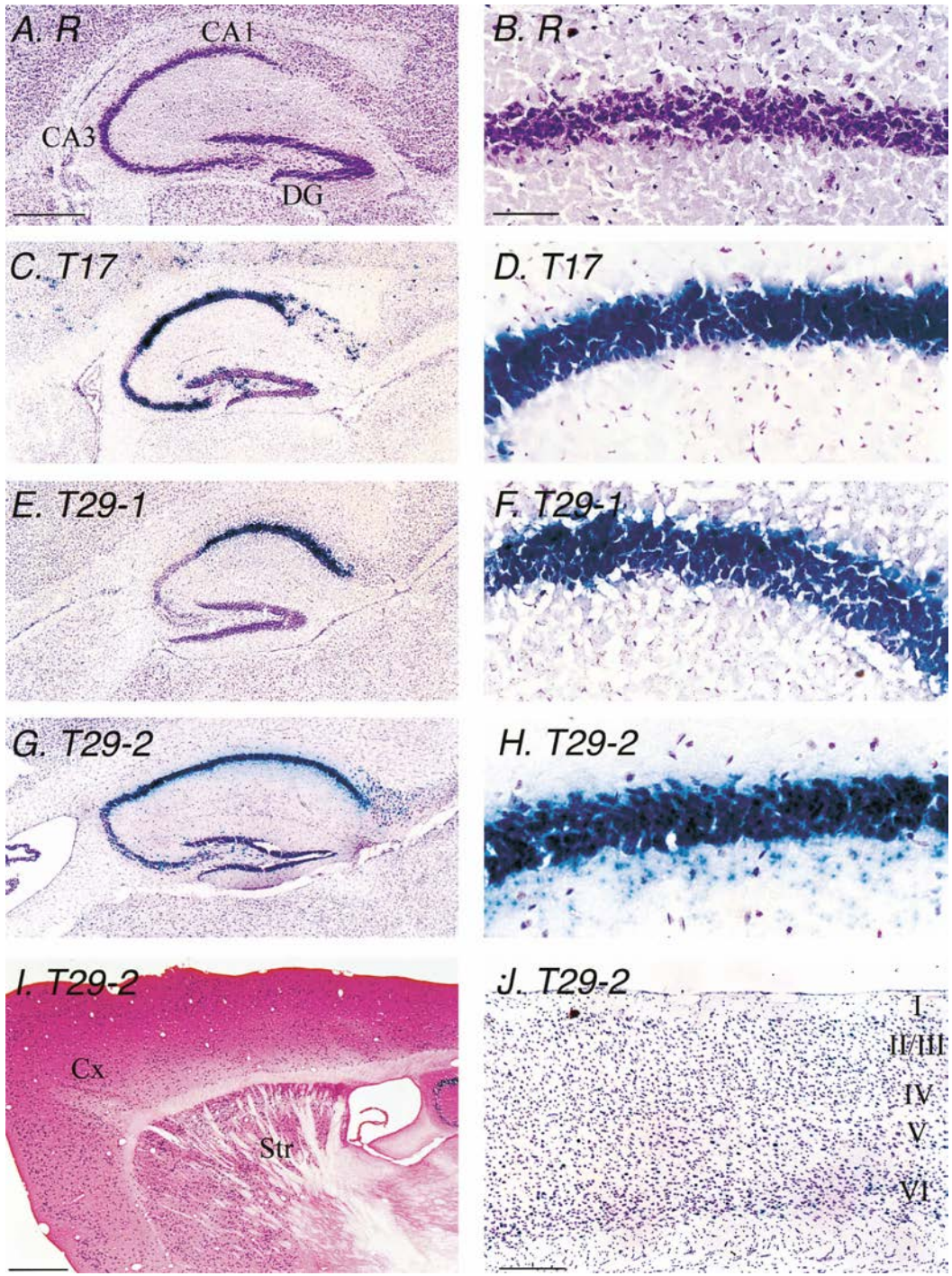


(Tsien et al., Cell 1996a)

Strategy for cell type-specific gene knockout

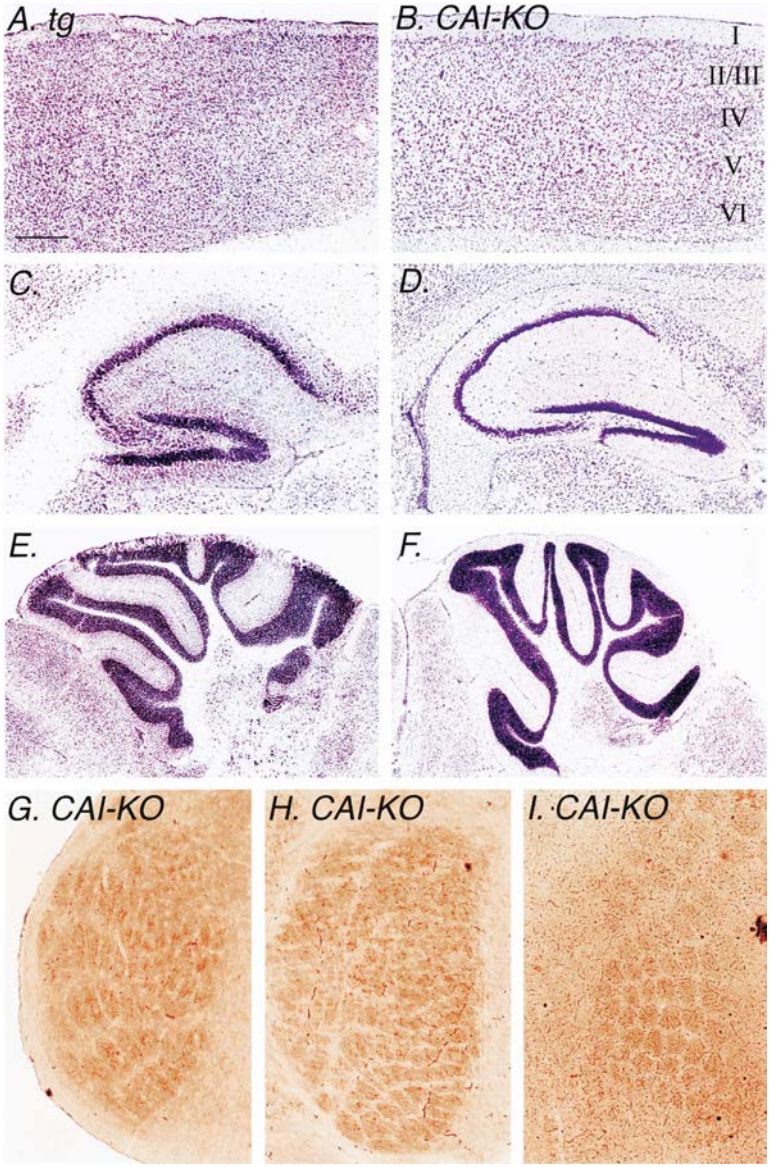


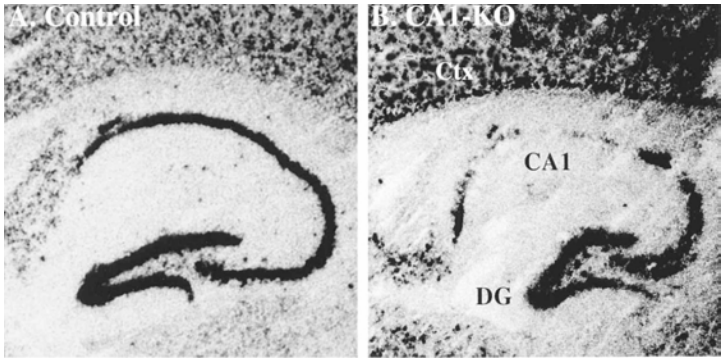
(Tsien et al., Cell 1996a)

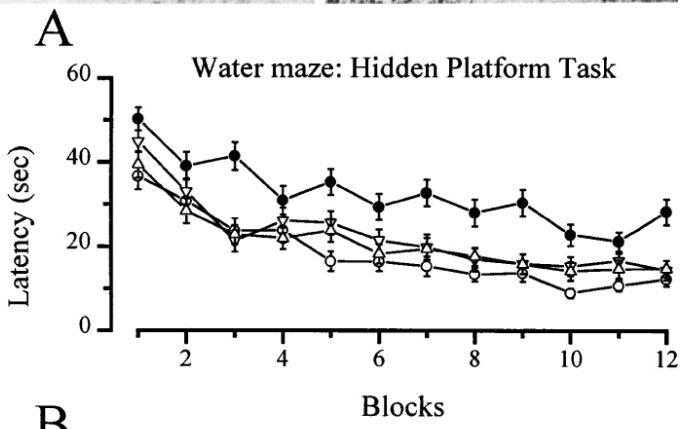


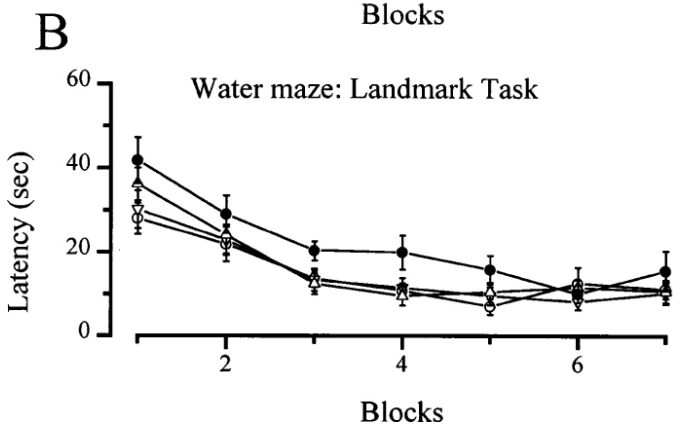
(Tsien et al., Cell 1996a)

Activity dependent modification of CA1 synapse, mediated by NMDA receptor, is required for spatial learning



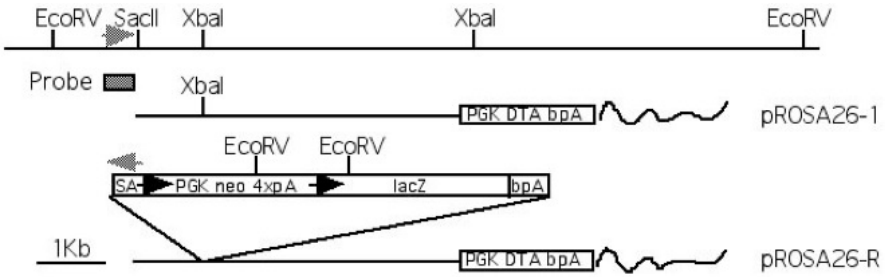


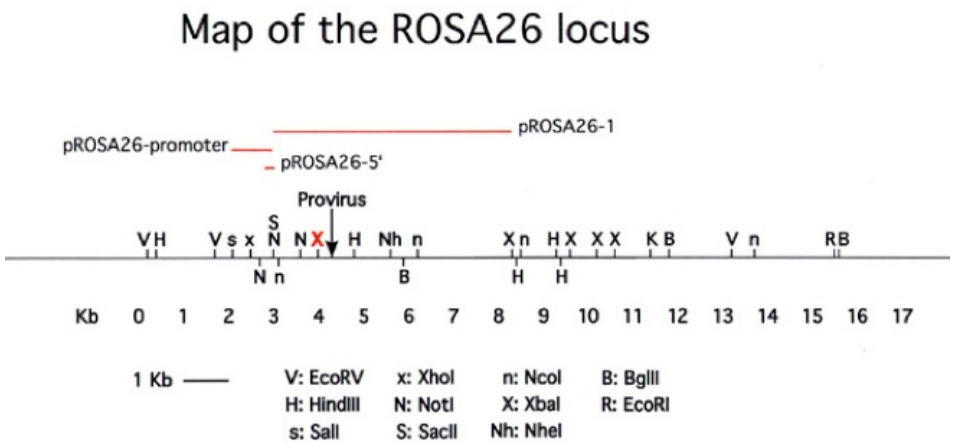


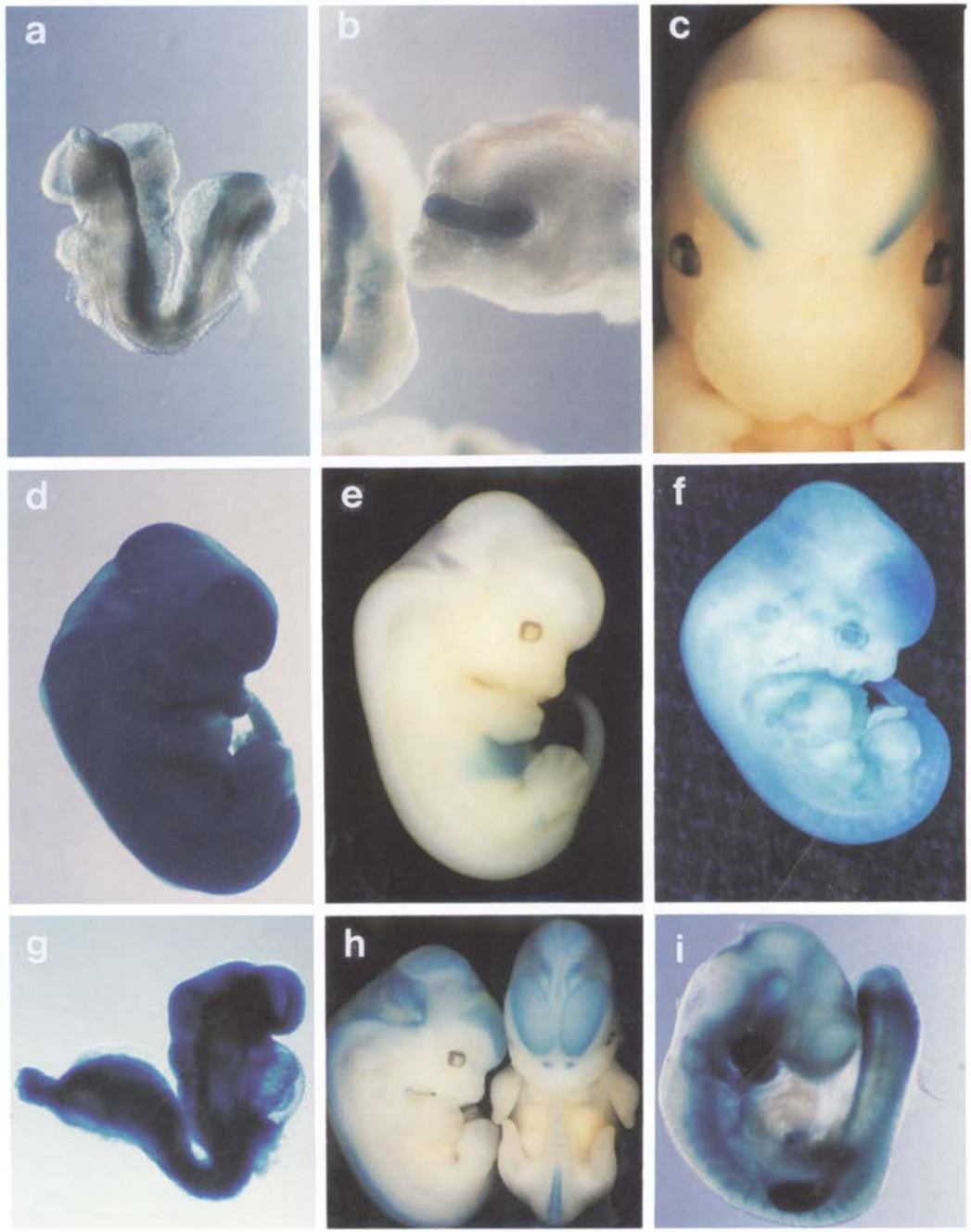


ROSA26 & its variants

- The gold standard in reporting Cre activity
- A “promoter trap” – similar to the concept of “enhancer trap” in Drosophila
- The ROSAβgeo26 (GtROSA26) line was initially derived from pools of ES cells infected with the retroviral gene trap vector Gen-ROSAβgeo at low multiplicity of infection
- βgeo encodes a bifunctional lacZ/neomycin phosphotransferase gene and the ROSA26 strain is one of several strains that exhibits broad lac Z staining.



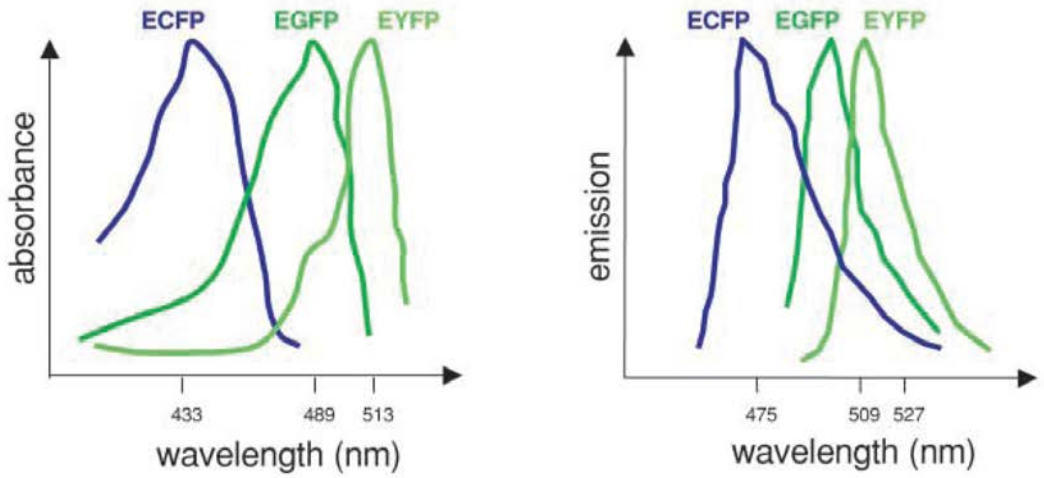




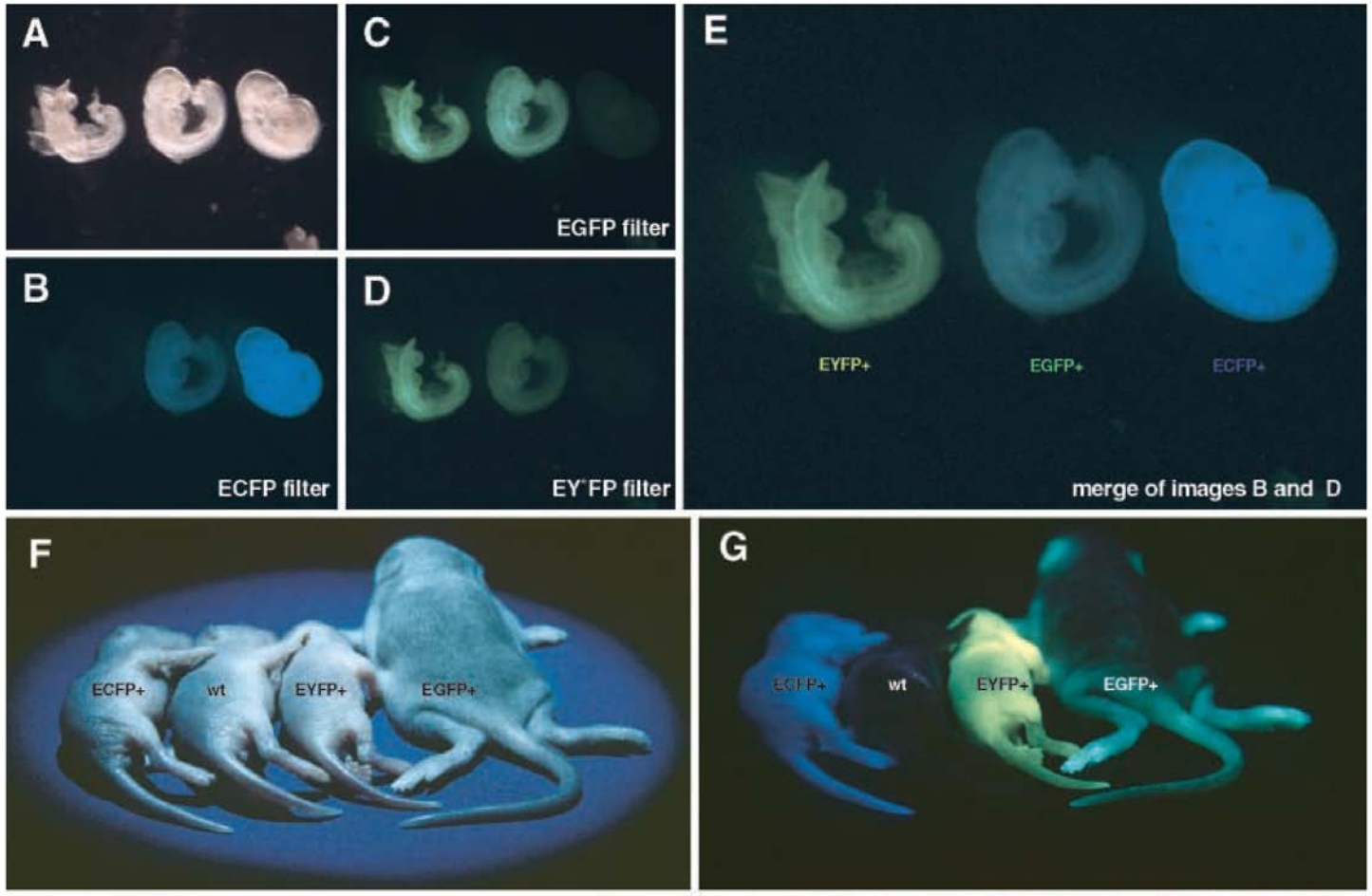
(Friedrich and Soriano, 1991)

GFP reporter and its variants

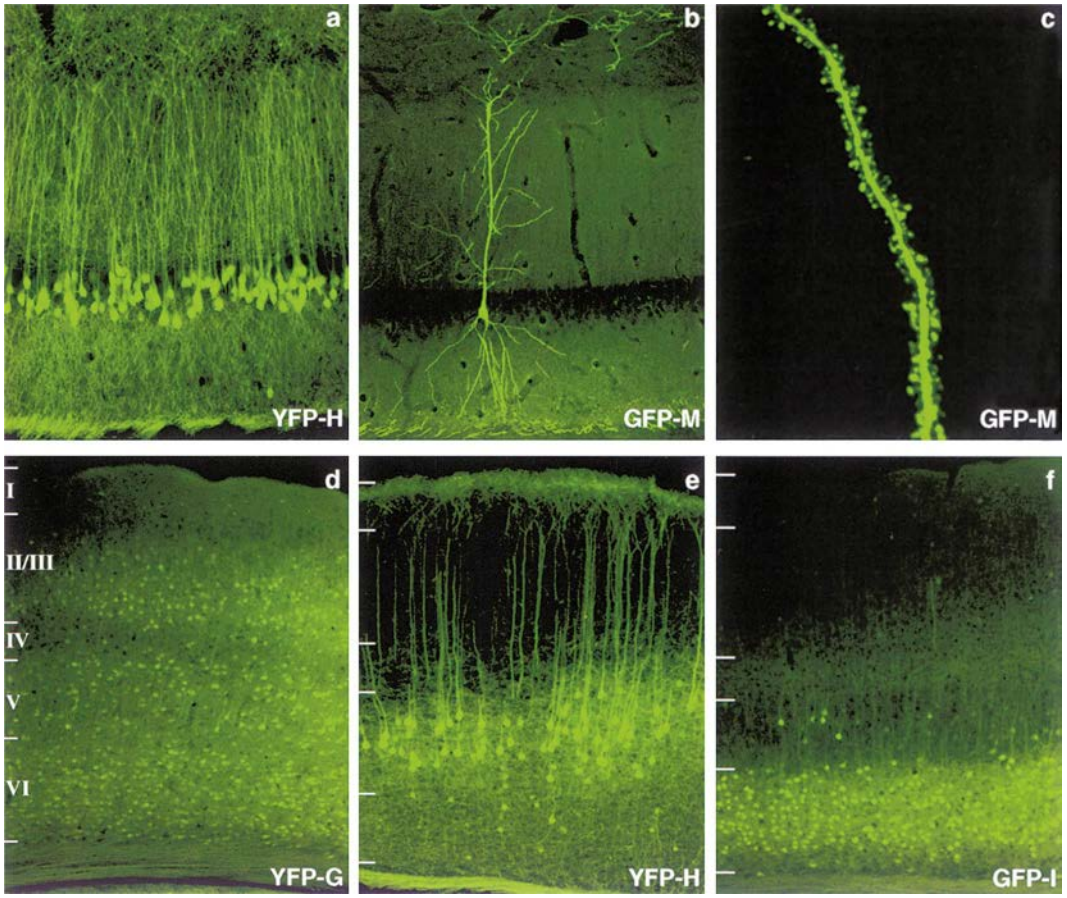
wtGFP	Phe^{64}-Ser-Tyr-Gly-Val-Gln^{69}...Ser^{72}...Tyr^{145}...Thr^{203}	Chalfie et al. 1994
ECFP	Phe64Leu, Ser65Thr, Tyr66Trp, Asn146Ile, Met153Thr, Val163Ala blue shifted humanized codon usage	Heim and Tsien 1996
EGFP	Phe64Leu, Ser65Thr red shifted humanized codon usage	Cormack et al. 1996
EYFP	Ser65Gly, Val68Leu, Ser72Ala, Thr203Tyr red shifted humanized codon usage	Ormo et al. 1996



GFP reporter and its variants



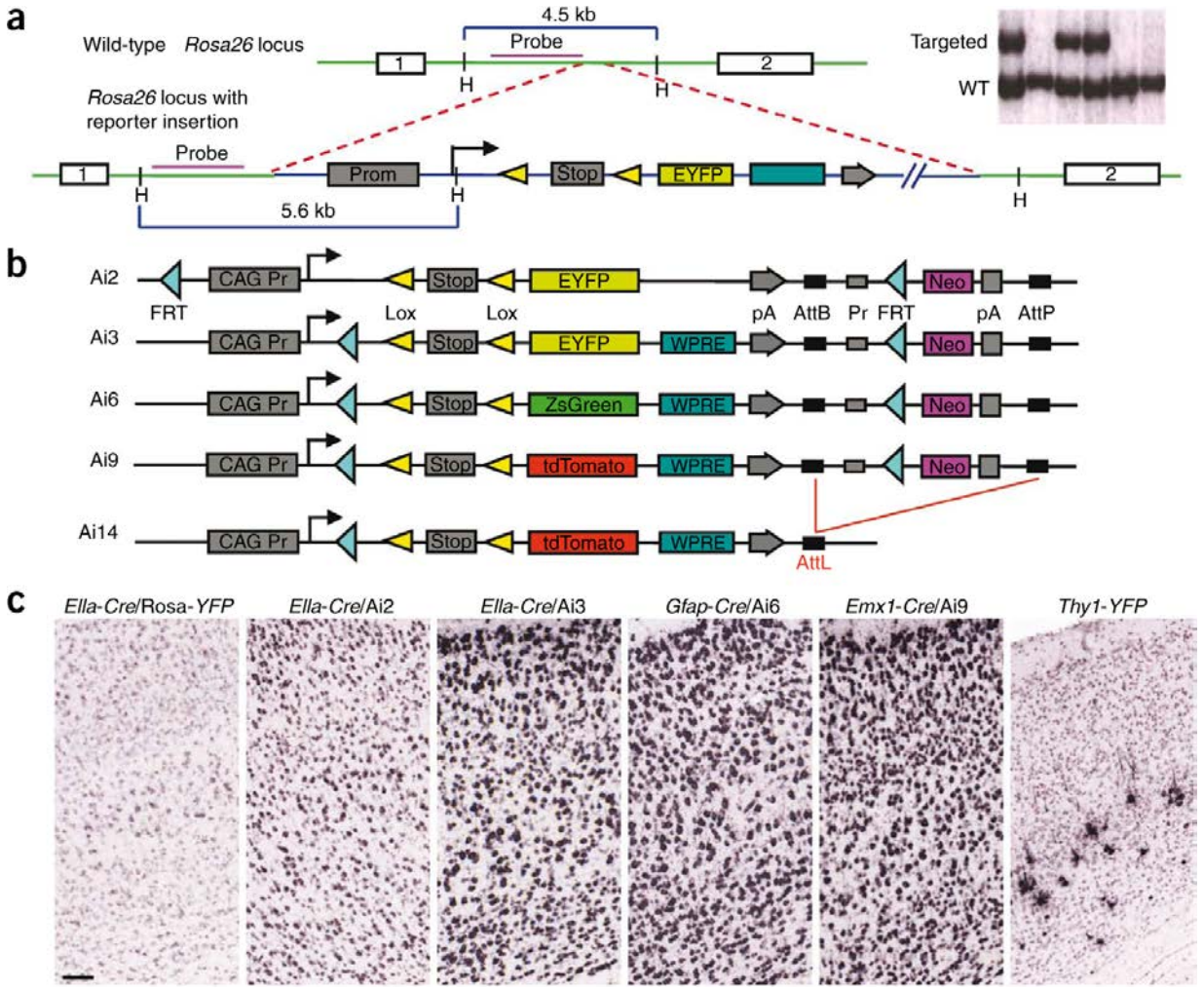
Variable expression patterns of XFP in the hippocampus and cerebral cortex



(Feng et al., Neuron 28: 41-51, 2000)

- Thy1 – a member of the Ig superfamily expressed by projection neurons in many parts of the nervous system
- Characteristics of thy1-XFP transgenic mice
  - Label axons and dendrites even though not fused to peptides designed to facilitate transport
  - Stable expression of XFP up to 9 months with no discernible effect on synaptic structure
  - Remarkable variability in patterns of XFP expression in mice generated from the same construct
  - Each line exhibit unique, heritable pattern of expression
  - Double transgenic lines can be achieved

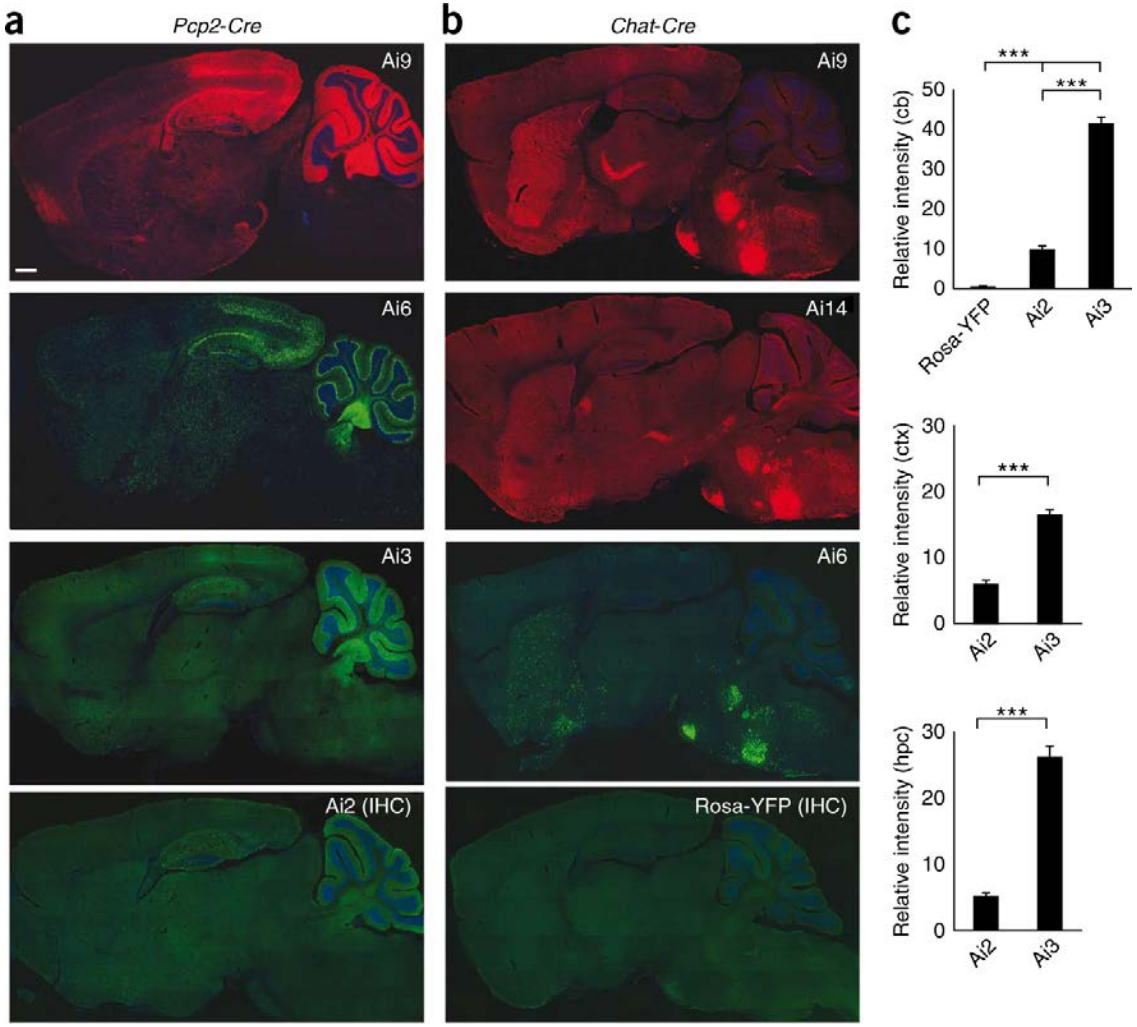
Cre reporter lines



WPRE: woodchuck hepatitis virus posttranscriptional regulatory element (to enhance RNA stability)

(Madisen et al., Nature Neurosci 13: 133-140, 2010)

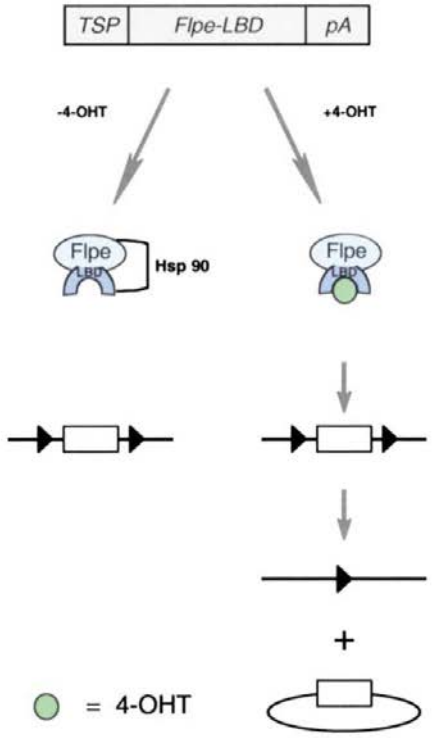
Enhanced fluorescent labeling in the new Cre reporter lines



(Madisen et al., Nature Neurosci 13: 133-140, 2010)

Mechanisms of temporal control of site-specific recombination

A Post - translational control



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Reviews

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2016-2017 NS201B Overview

- Signaling I: Notch signaling & lateral inhibition (Cheyette)
- Signaling II: Shh & Wnt (Cheyette)
- Neurocircuit development (Stryker)
- Signaling III: Neurotrophic factors (Huang)
- Growth cone dynamics (Weiner)
- Dendritogenesis (YN Jan)
- Synapse formation I (Ullian)
- Problem set I (2nd students)
- Synapse formation II (Ullian)
- Synapse homeostasis (Davis)
- Cortical development I (Rubenstein)
- Cortical development II (Pleasure)
- Cortical development III (Alvarez-Buylla)

NS201B 2016-2017

- Neurodevelopment & behavior (Manoli)
- In vitro models & neuropsychiatric diseases (Willsey)
- Paper discussion – Cortical development (Pleasure)
- Neurogenetics I (Sanders)
- Neurogenetics II (Ptacek)
- Myelination/Oligodendroglia/Schwann cells (Chan)
- Astrocytes (Molosky)
- Problem set II (2nd year students)
- Glia paper discussion (Molosky)
- Final Exam (1/18/17 – 1/20/17)
- ***Topics to be covered by minicourses:*** "Neurobiology of disease" (2016), "Glia biology" (2016), "Neuroinflammation" (2016), "Addiction" (2017), "Thalamocortical circuit" (2017), "Basal ganglia" (2017), etc