Identification of Genes Required for Nuclear Exclusion of Prospero During Neural Stem Cell Self-Renewal

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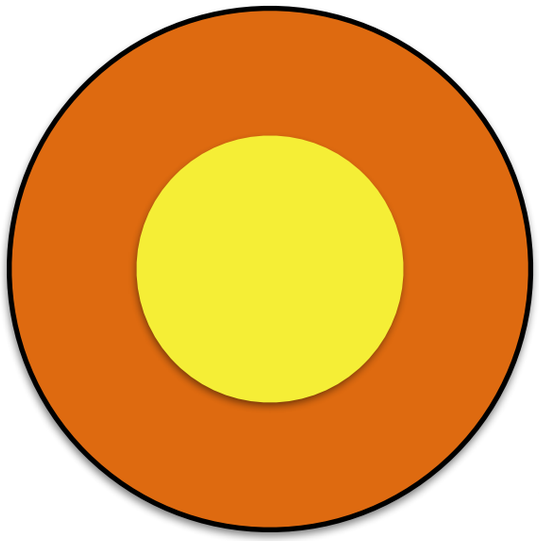
Principal Investigator: Dr. Chris Doe

Stem cells and neurogenesis - “the birth of neurons”

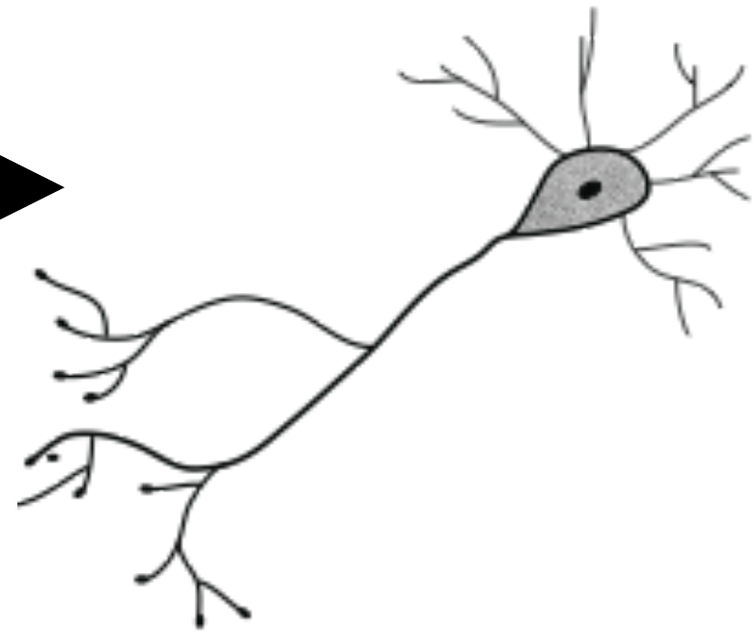
Neural Stem Cell
(NSC)



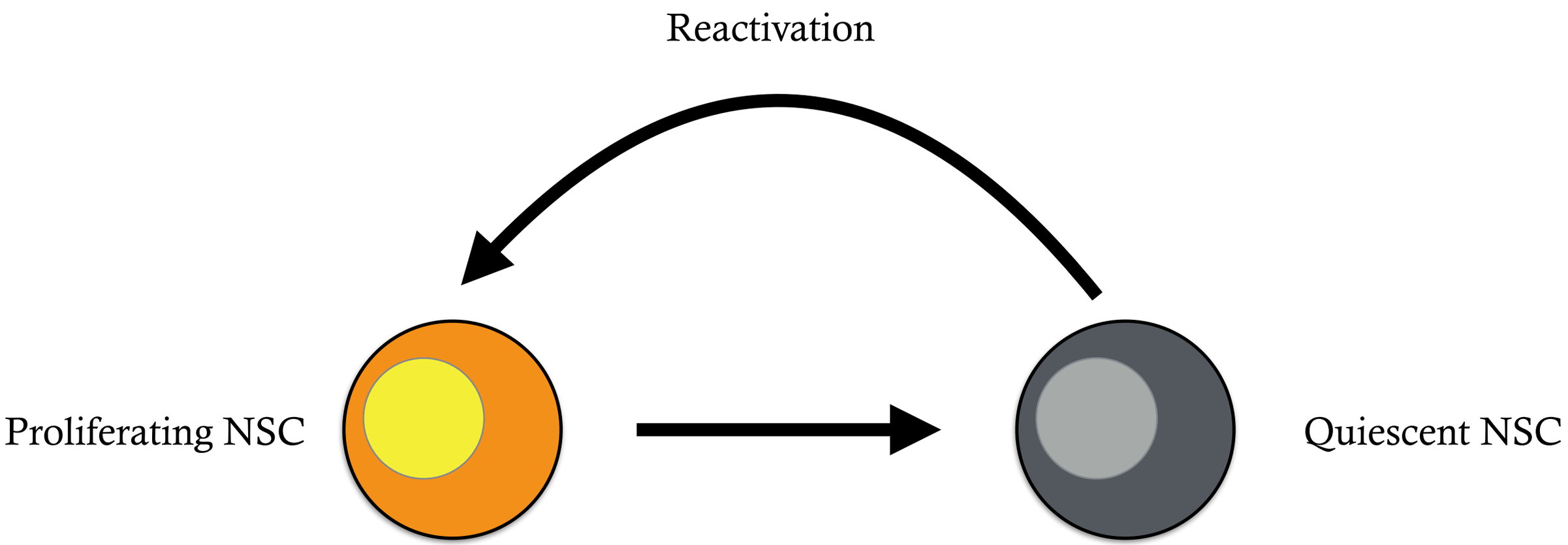
Asymmetric Division

Self-renewal

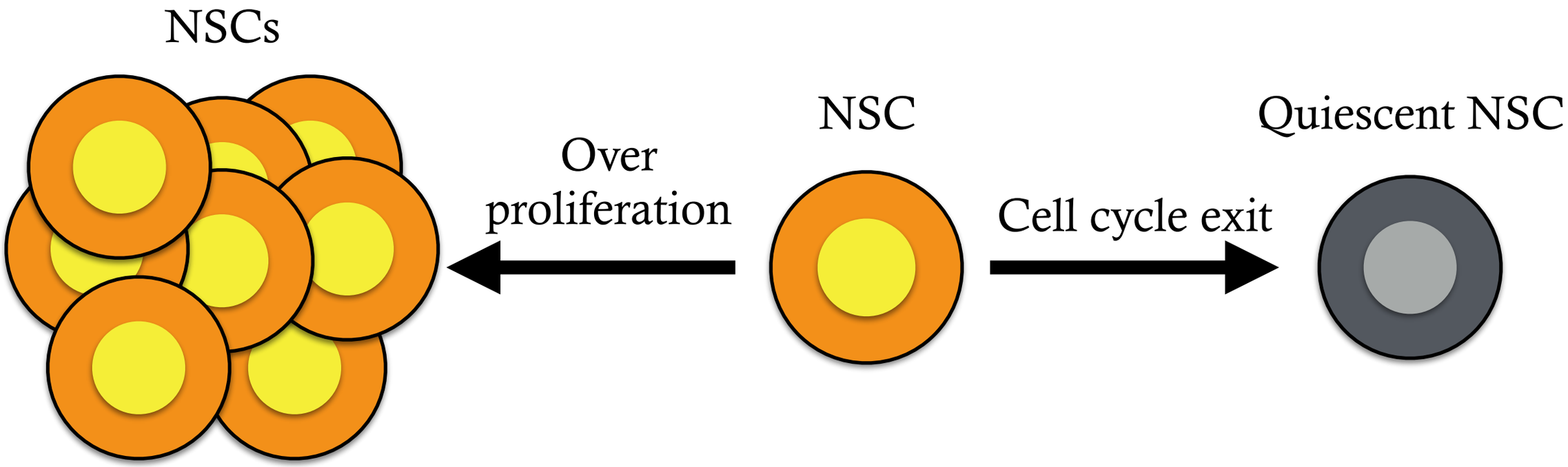
Differentiated Cell
(Neuron)



NSCs can persist in a reversible state of quiescence



Neural development requires a balance between neural stem cell self-renewal and quiescence



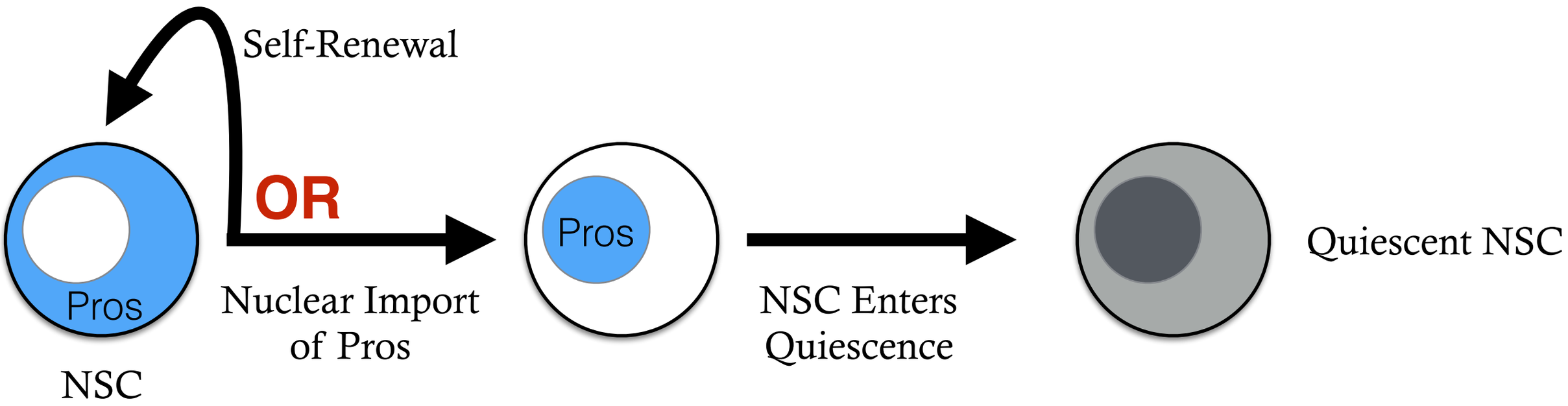
Drosophila neural stem cells (neuroblasts) are a well-established genetic modeling system

Human Genetics

Drosophila Genetics

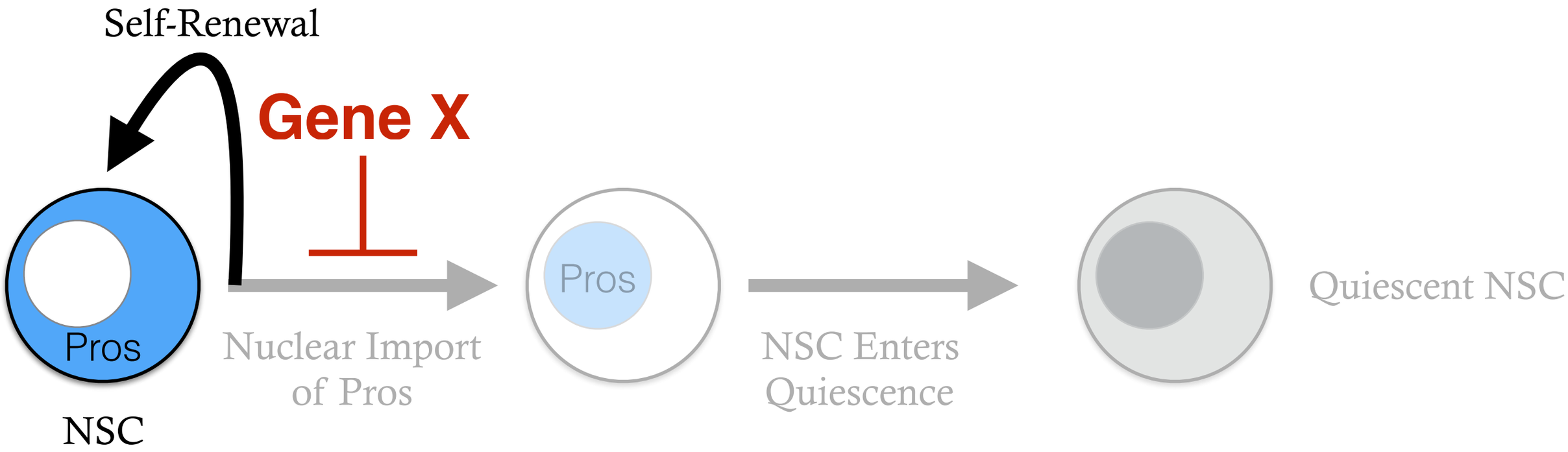
- Highly conserved mechanisms
- Well known model
- Quick life cycle
- Genetically manipulable

Pros is *necessary* and *sufficient* to induce quiescence



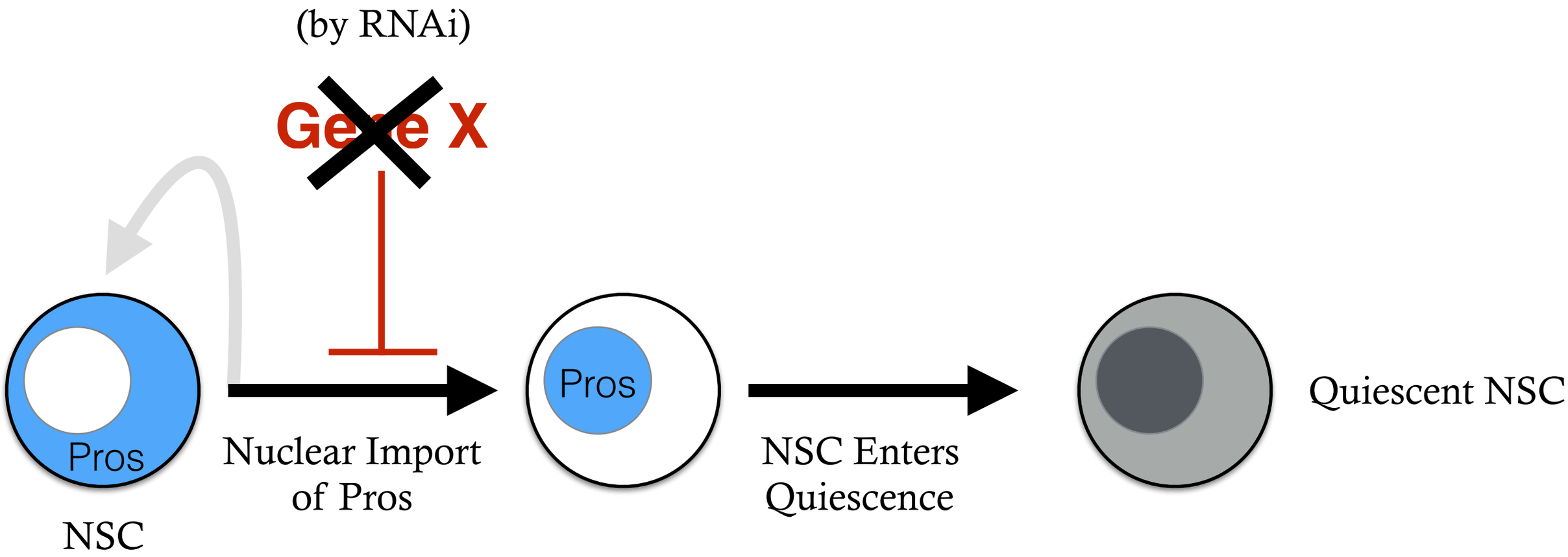
Pros = Prospero (Conserved atypical homeodomain transcription factor)

Pros has to be excluded from entering the nucleus to allow self-renewal



What gene(s) regulate the transient importation of Pros into the nucleus?

RNAi screen was used to suppress genes active in NBs

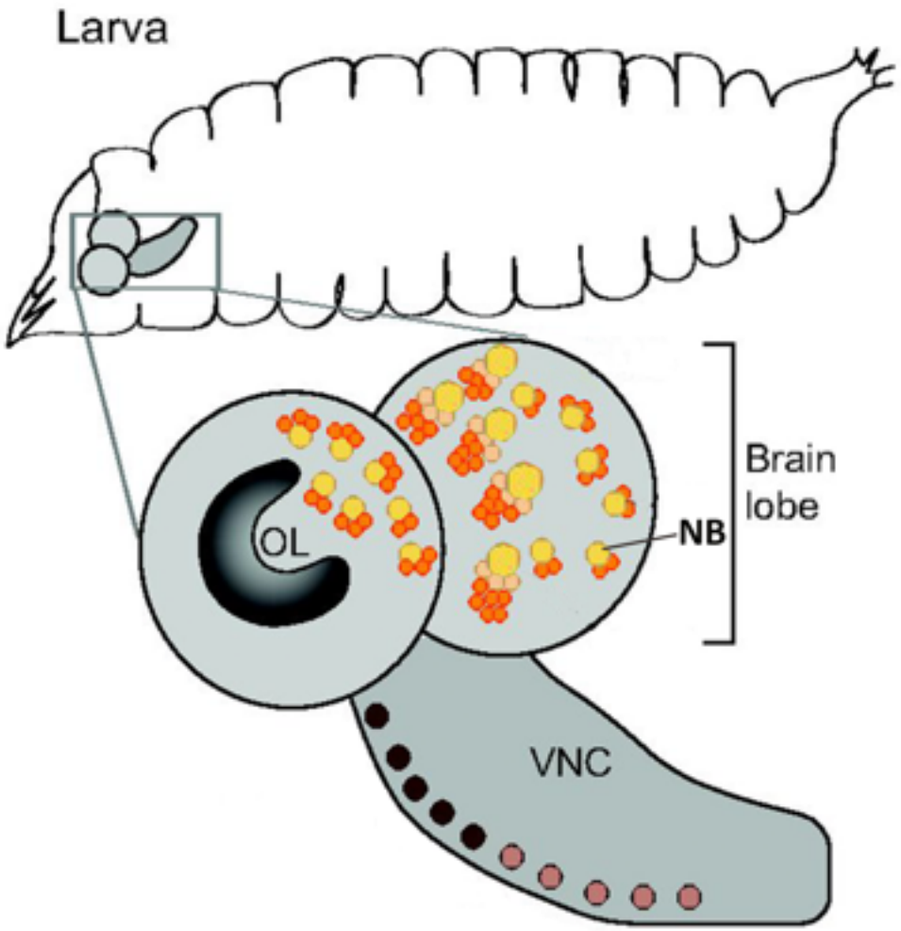


Suppression of a gene that aids in the exclusion of nuclear Prospero would arrest the cell cycle of nuclear stem cells

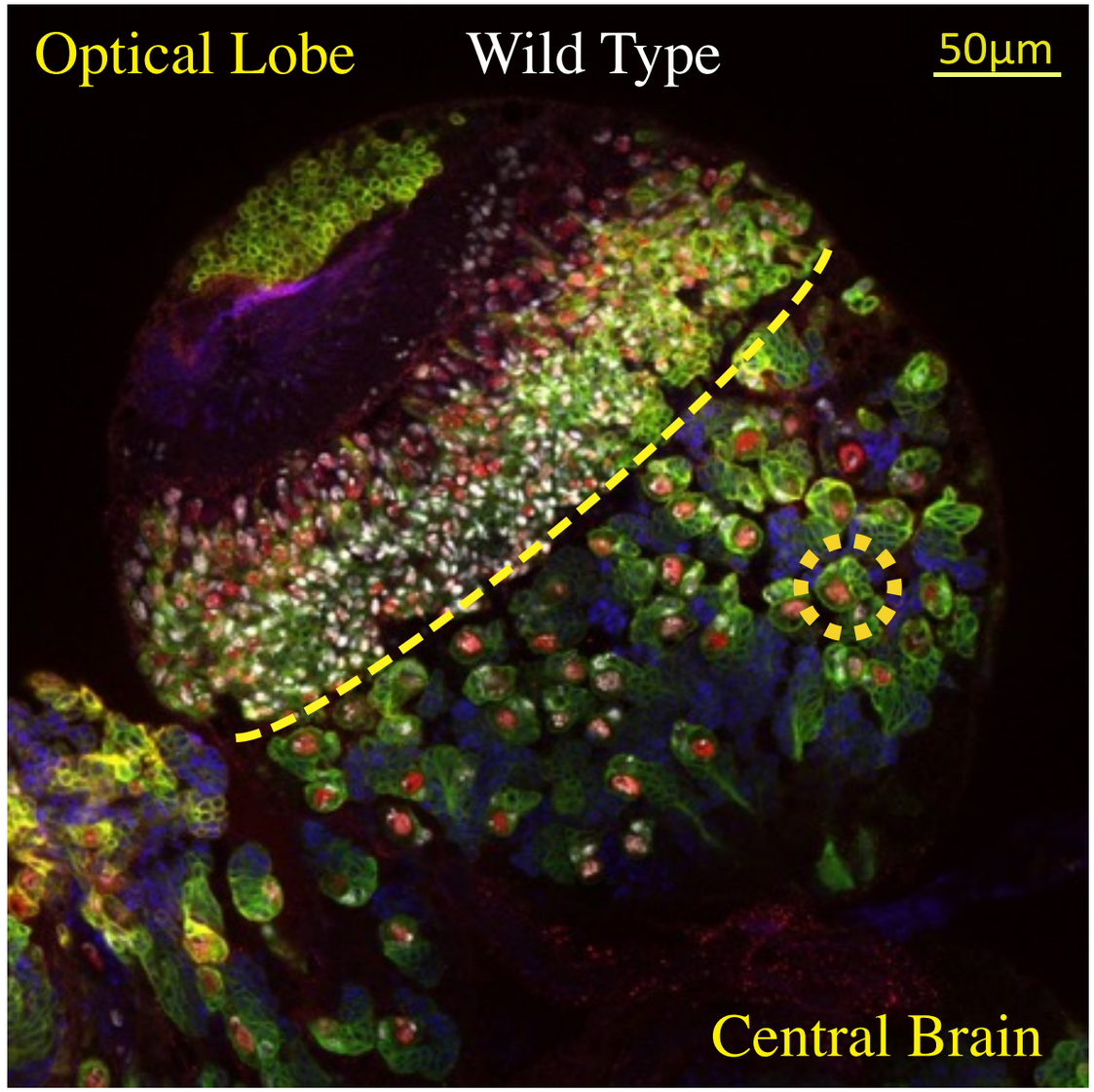
Proliferating and quiescent neuroblasts express unique molecular markers

Molecular Markers		Neuroblast (NB) State	
	Function	**Proliferative**	**Quiescent**
Deadpan (Dpn)	NB proliferation and specification	+	+
Worniu (Wor)	NB delamination, proliferation	+	-
EdU	Nucleoside analog, measures S-phase synthesis	+	-
Prospero (Pros)	Cell-cycle arrest, promotes quiescence and differentiation	Cytoplasmic	Nuclear

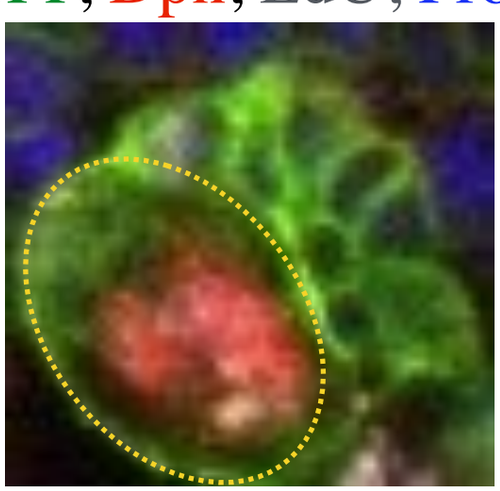
Confocal microscopy was used for visualization



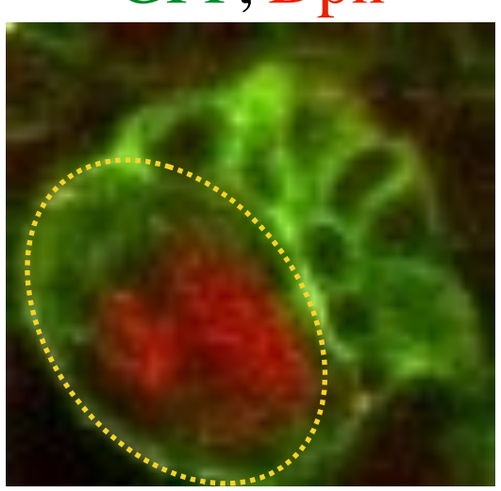
Adapted from Development 139, 4297-4310 (2012)



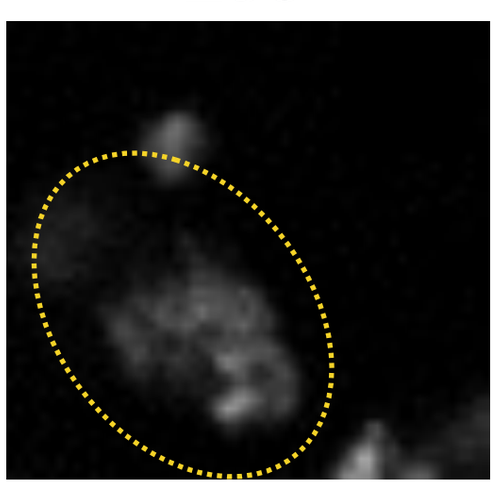
GFP, Dpn, EdU, Pros



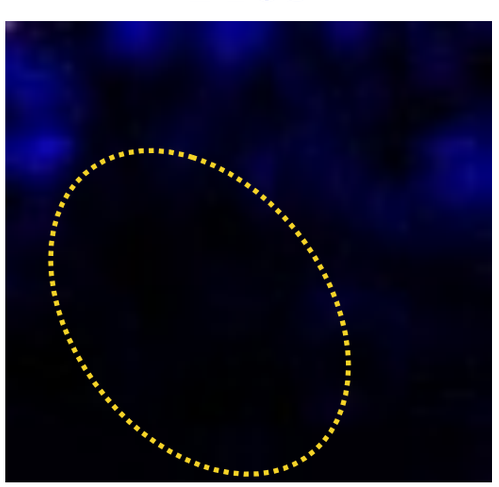
GFP, Dpn



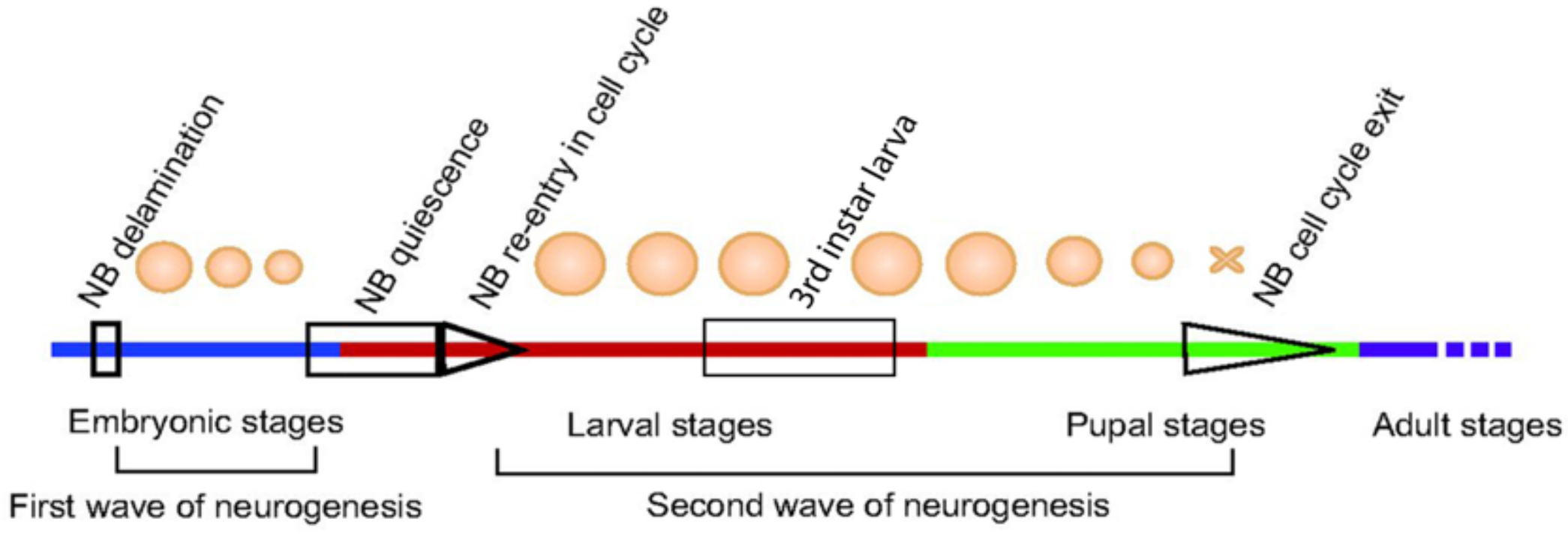
EdU



Pros



Dissections were performed on 3rd instar larva



Adapted from Development 139, 4297-4310 (2012)

3rd Instar Larva

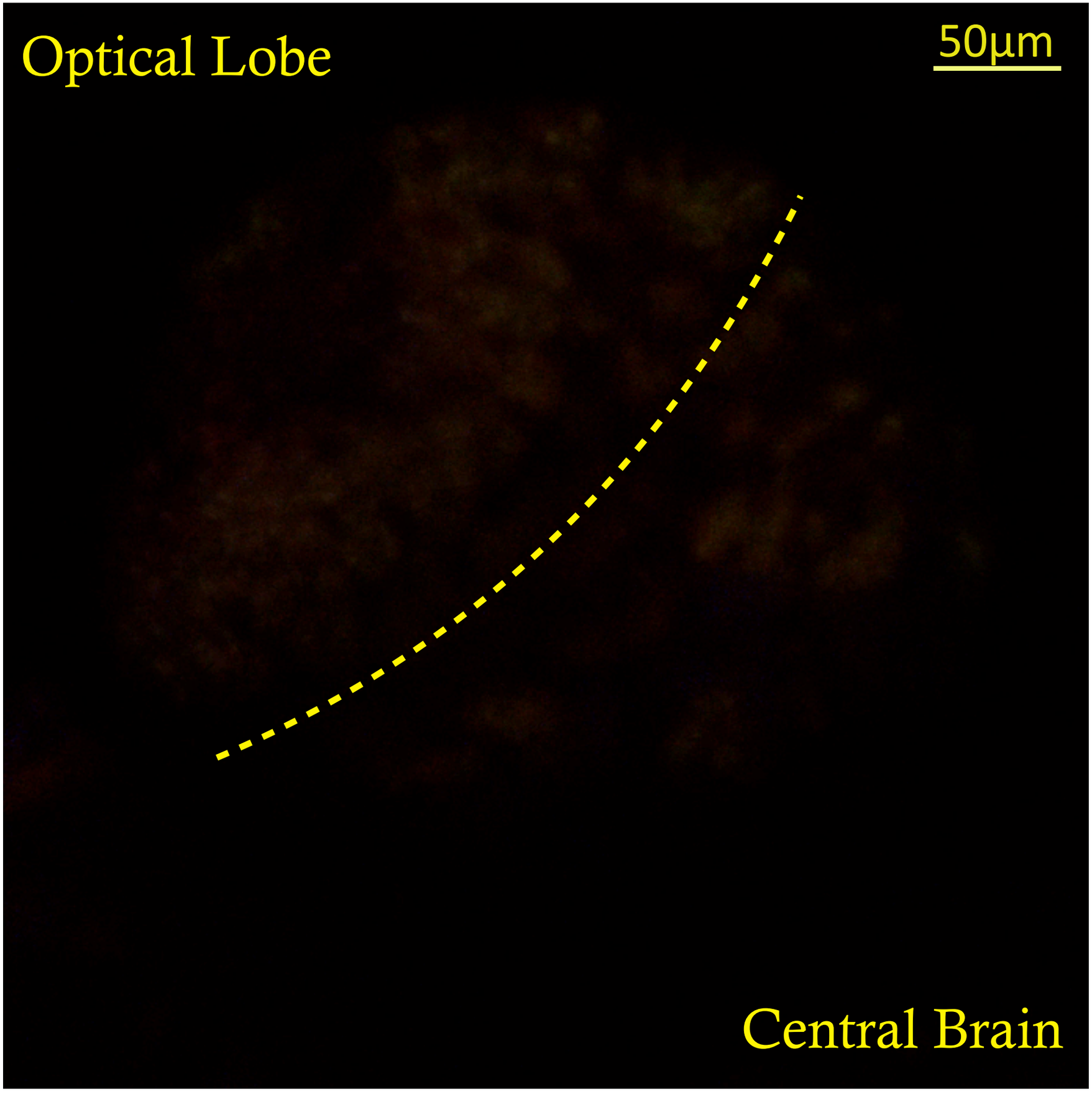
Stage begins about 3 days (72 hours) after fertilization

NBs regrow to their original size after each cell division

NB populations have distinct anatomical locations

Constant number of NBs

NBs undergo continuous proliferation


Optical Lobe
50µm
Central Brain

RNAi-based functional screen identified candidate genes for regulating NSC self-renewal/quiescence

Functional genomics identifies neural stem cell sub-type expression profiles and genes regulating neuroblast homeostasis

Travis D. Carney, Michael R. Miller, Kristin J. Robinson, Omer A. Bayraktar, Jessica A. Osterhout, Chris Q. Doe *

Institute of Molecular Biology, Institute of Neuroscience, Howard Hughes Medical Institute, University of Oregon, Eugene, OR 97403, USA

Analyzed 6 genotypes that generate ectopic NBs

Transcriptional profiling

Cluster Analysis

1045 genes expressed in all NBs

Enhance relevance

681 genes have mammalian orthologes

Score lethality

RNAi constructs of 179 genes caused lethality

Assess number of NBs

71 genes did not alter the number of neuroblasts

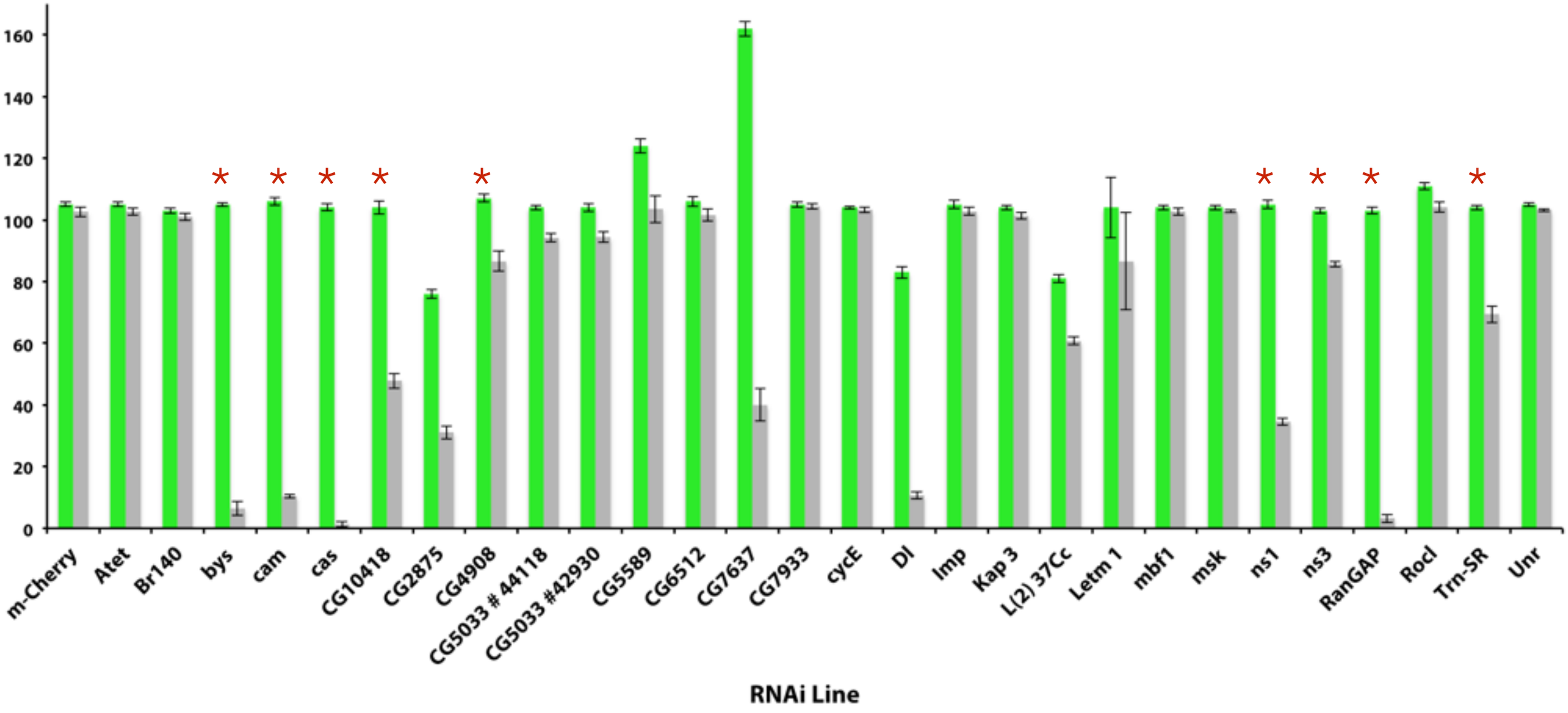
NB survival

Quiescence

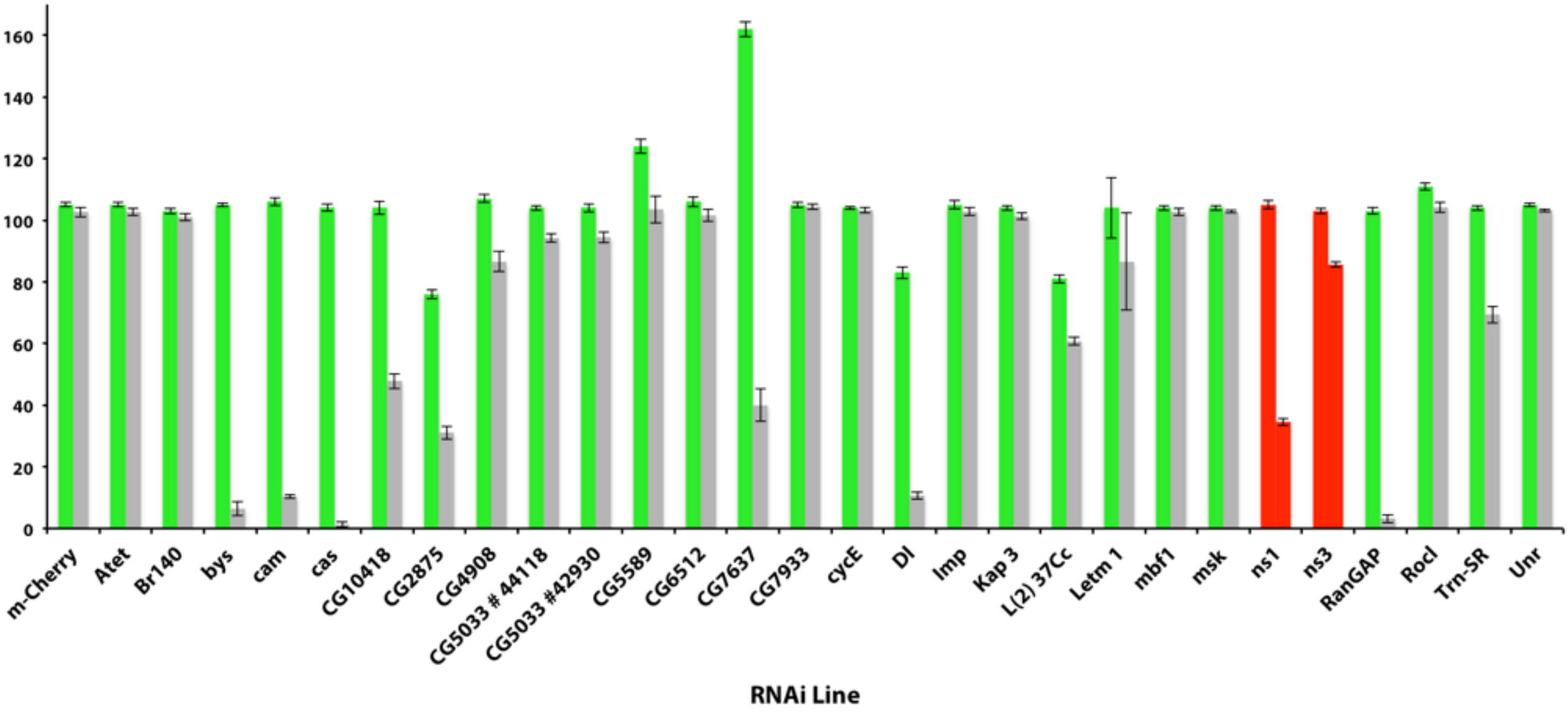
Self-renewal

Asymmetric Cell-Division

RNAi screen identified 9 genes as being required for neuroblast self-renewal



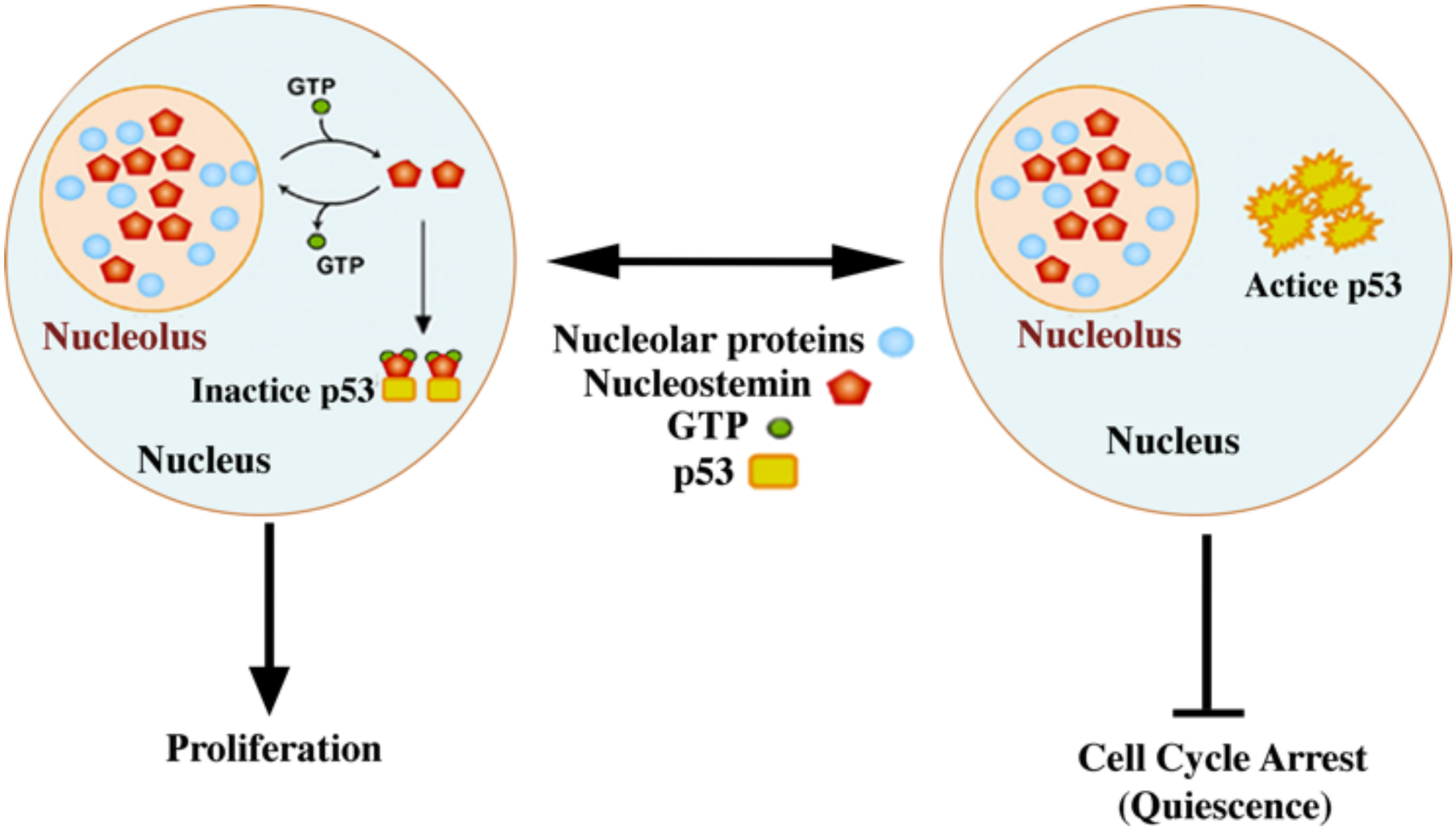
RNAi screen identified 10 genes as being required for neuroblast self-renewal



***Of these, there was a particular interest in the nucleostemin family**

Nucleostemin Family

- Nucleostemin is enriched in mammalian neural stem cells and multiple types of cancer cells.
- Knocking down nucleostemin inhibits cancer cells proliferation
- Nucleostemin controls stem cell activities via regulating p53
- NS3 is a conserved GTP binding protein
- It is unknown if NS3 regulates neural stem cell proliferation in any manner

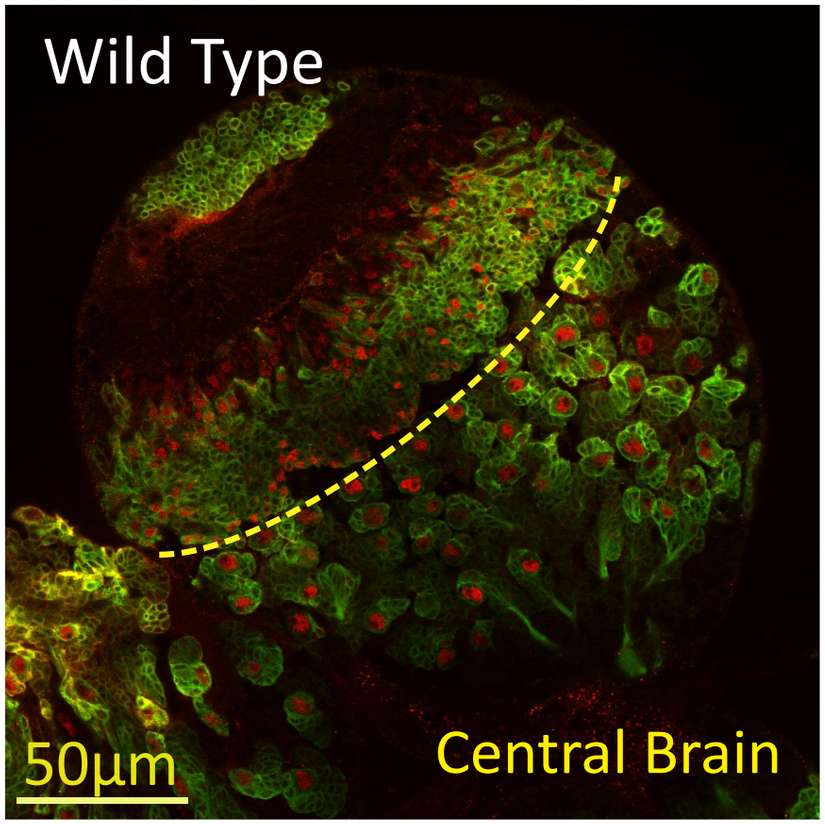


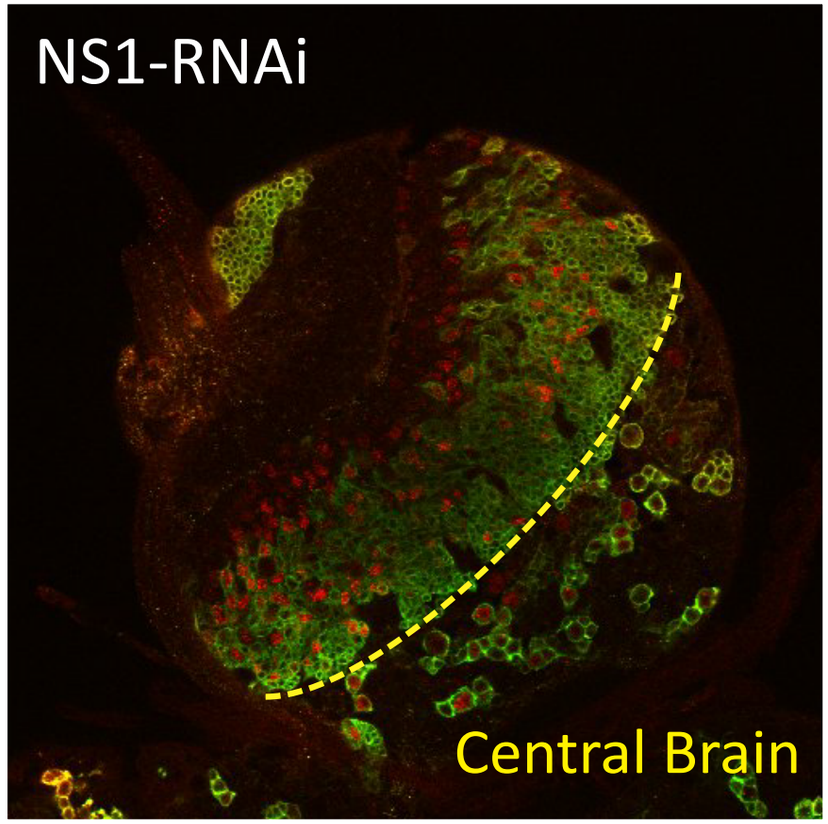
Adapted from *Nature Medicine* **9**, 24 - 25 (2003)

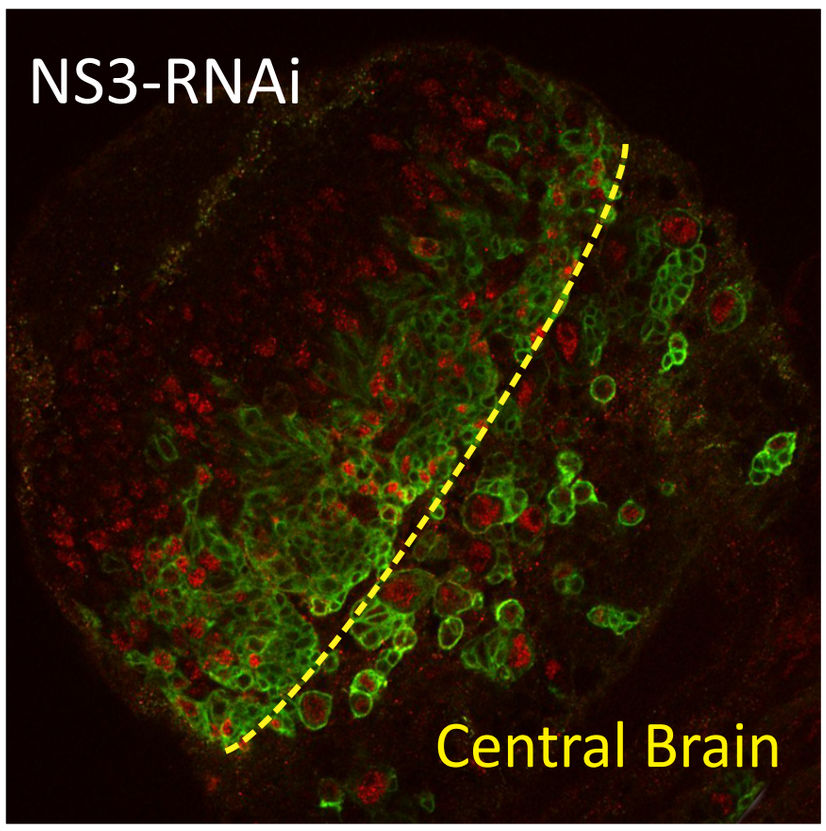
NS1-RNAi and NS3-RNAi do not decrease the total number of neuroblasts

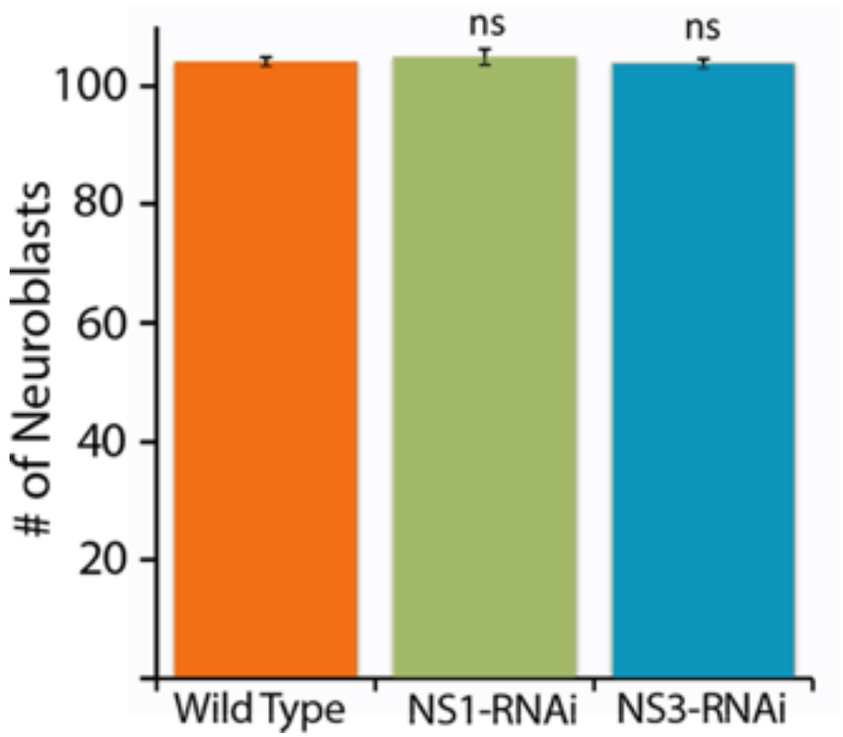
GFP

Dpn



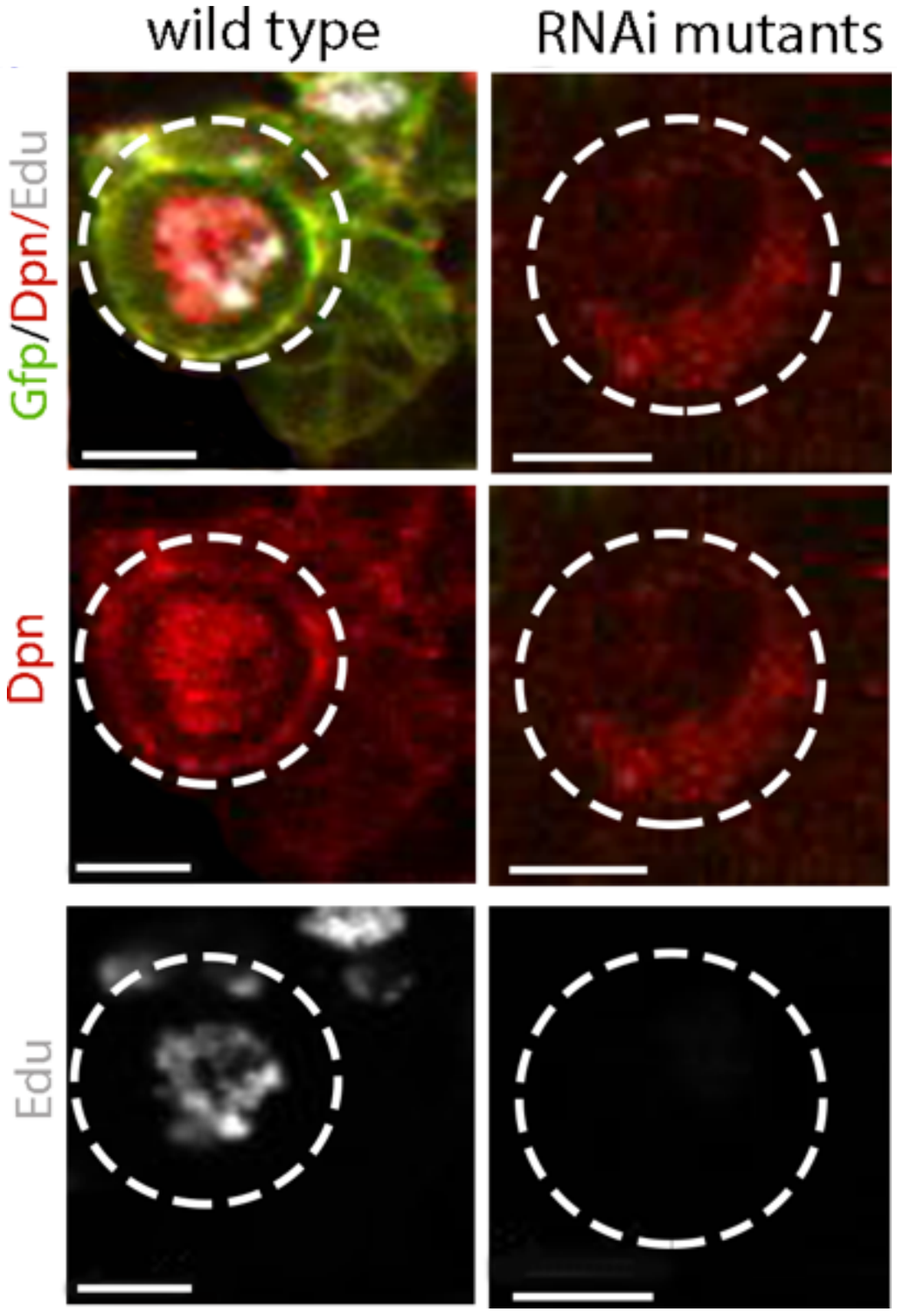


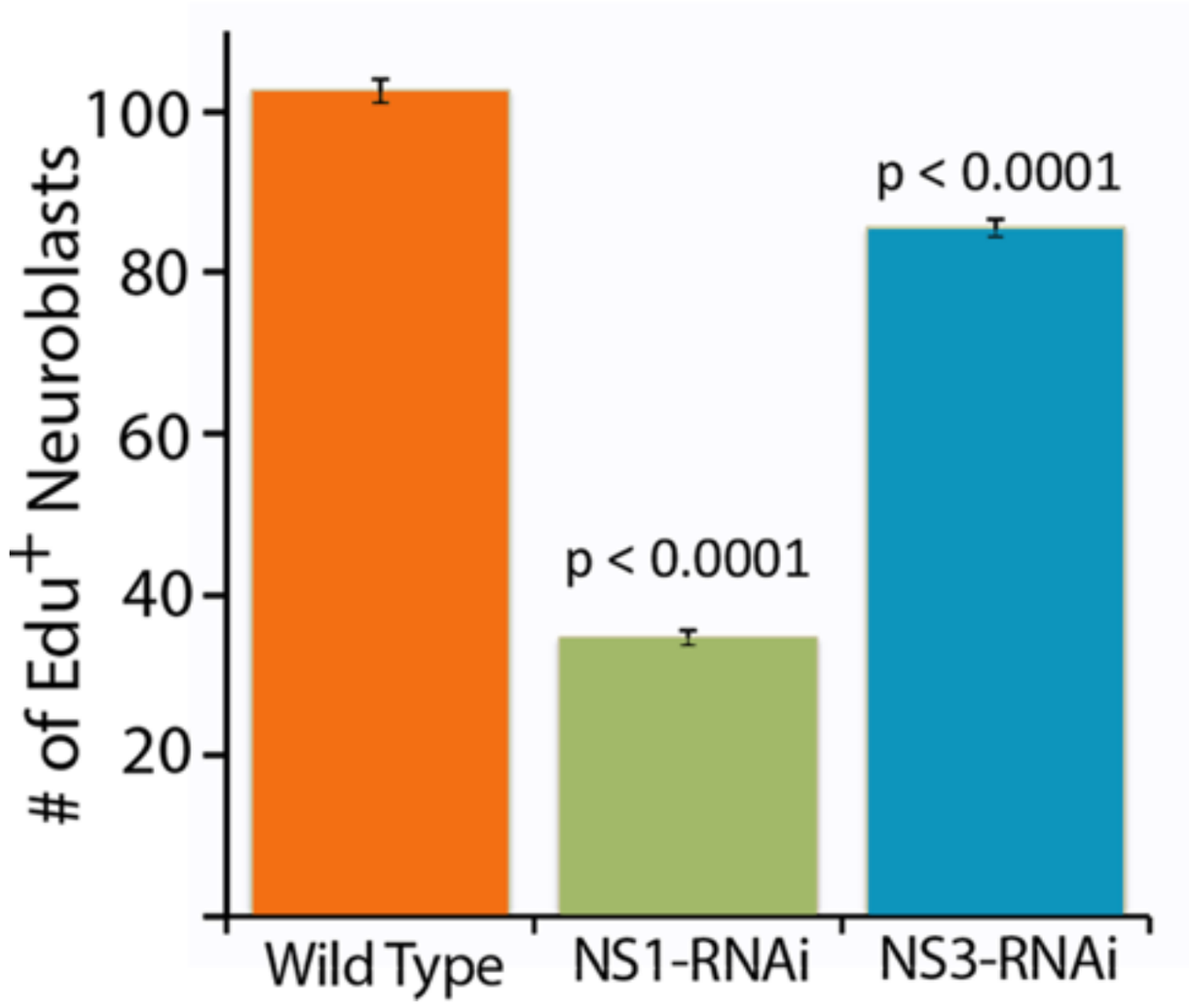




n=5, Genotype: wor-gal4, uas-mcd8:gfp

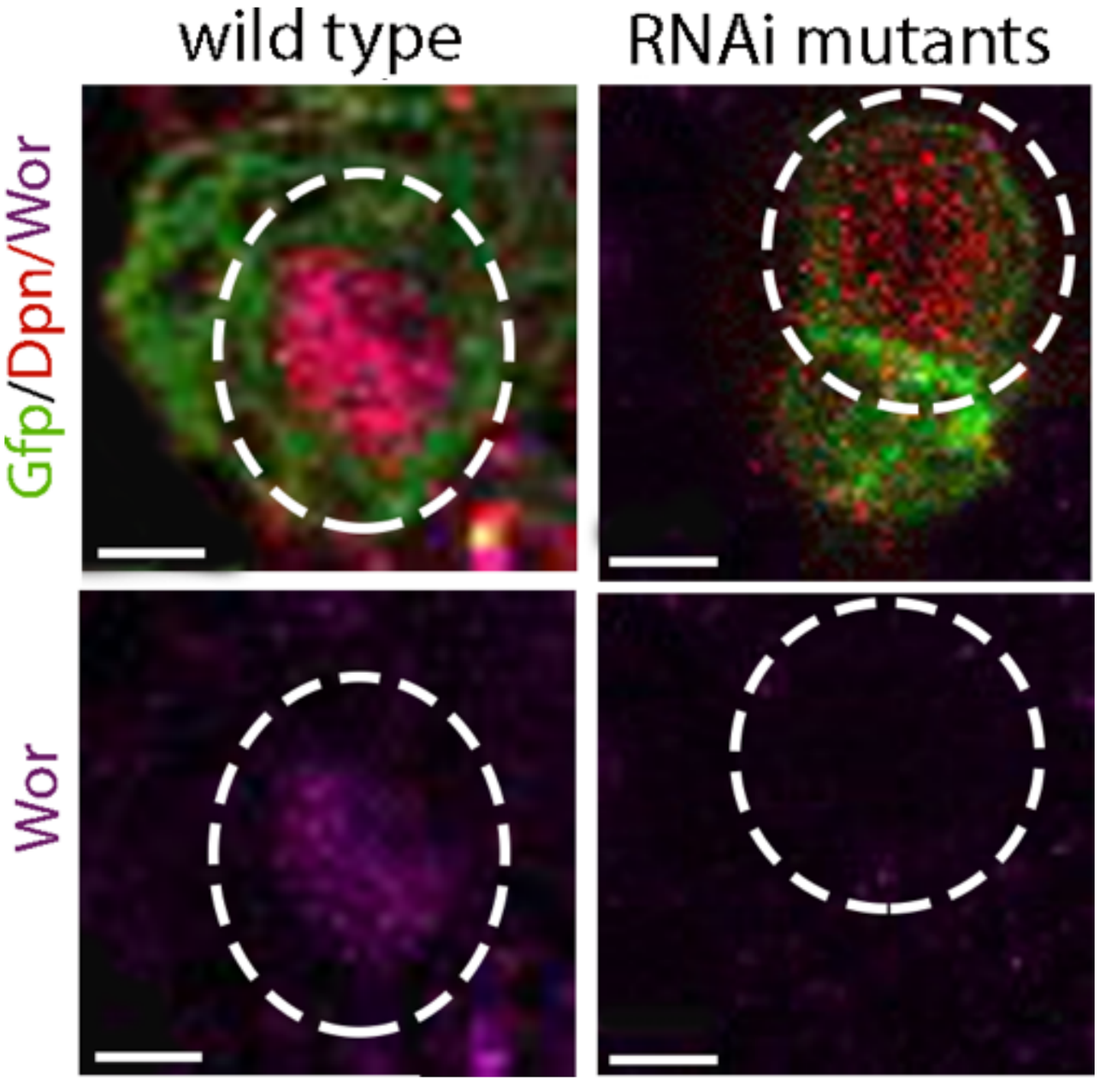
NS1-RNAi and NS3-RNAi decrease the total number of neuroblasts in the cell cycle

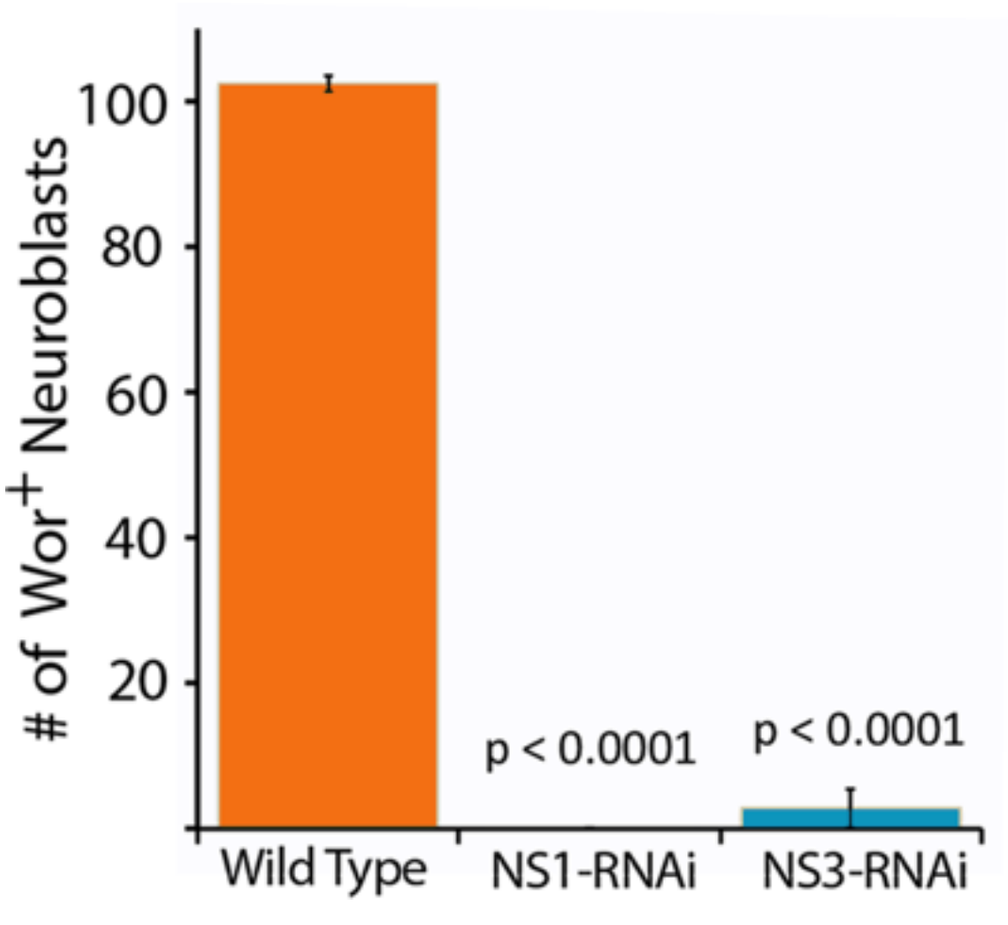




n=5, Genotype: wor-gal4, uas-mcd8:gfp

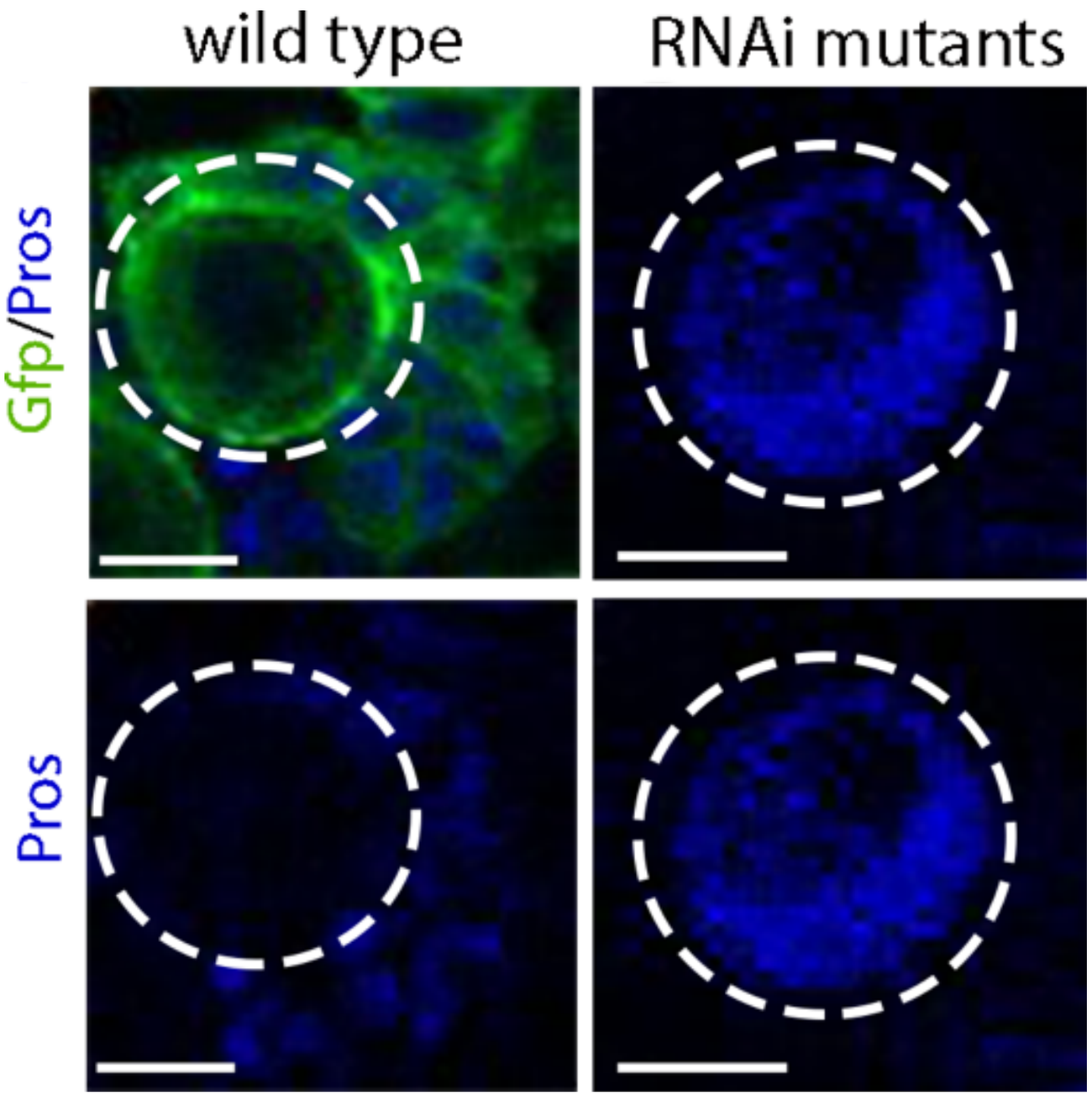
NS1-RNAi and NS3-RNAi cause neuroblast entry into quiescence

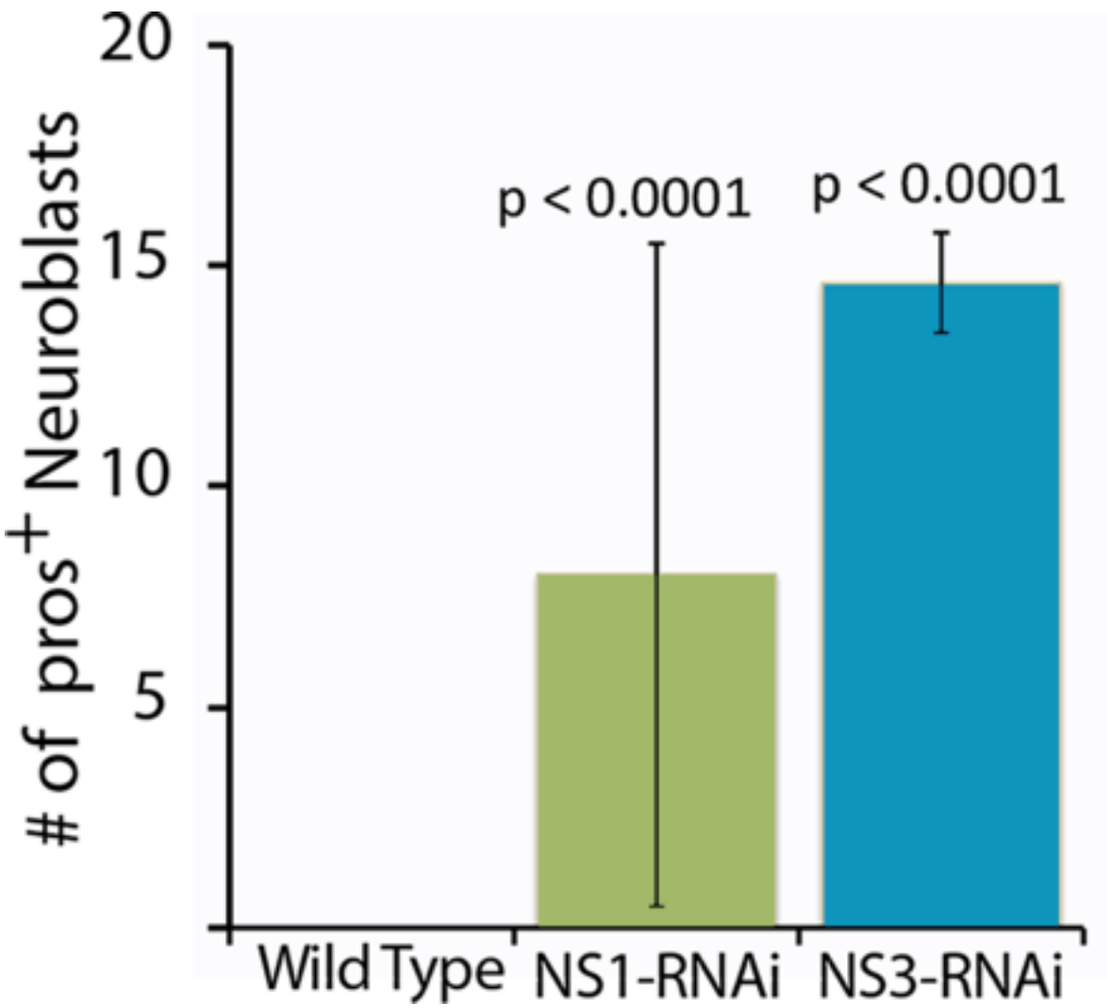




n=5, genotype: wor-gal4, uas-mcd8:gfp

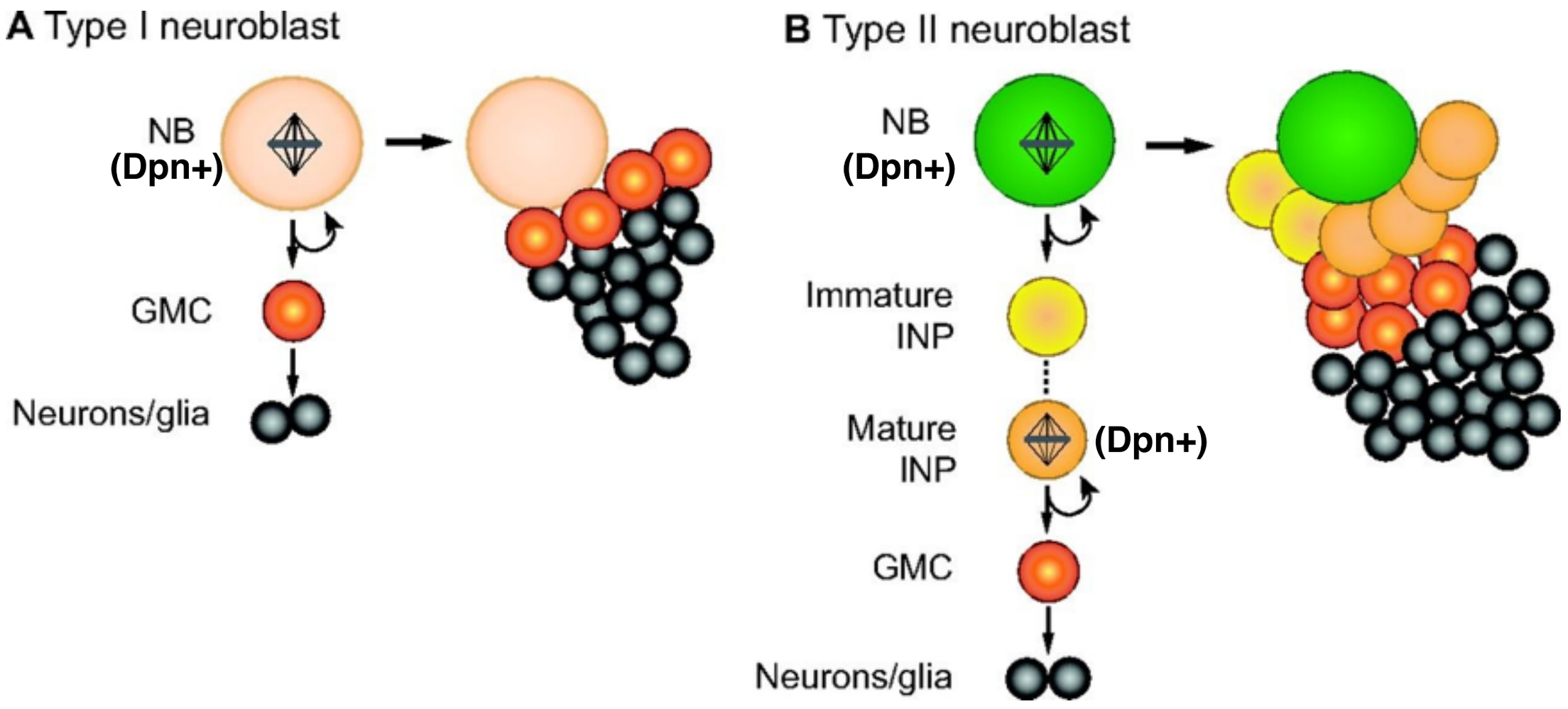
NS1-RNAi and NS3-RNAi induce nuclear localization of Pros





n=5, Genotype: wor-gal4, uas-mcd8:gfp

Type I neuroblasts vs Type II neuroblasts



Development 139, 4297-4310 (2012)

Type II neuroblasts express low levels of Pros compared to Type I neuroblasts

Bayraktar *et al. Neural Development* 2010, **5**:26
http://www.neuraldevelopment.com/content/5/1/26

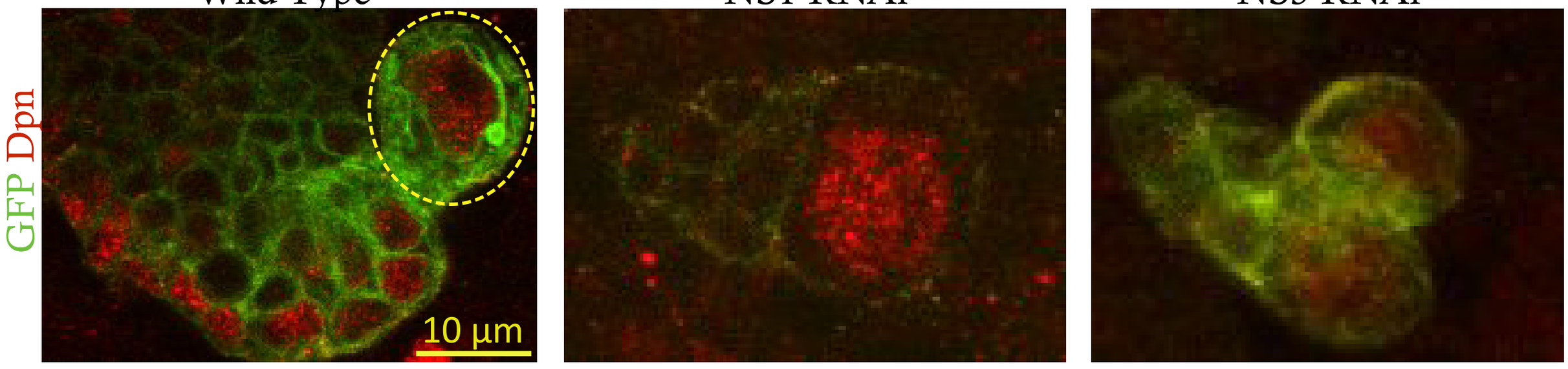
RESEARCH ARTICLE Open Access

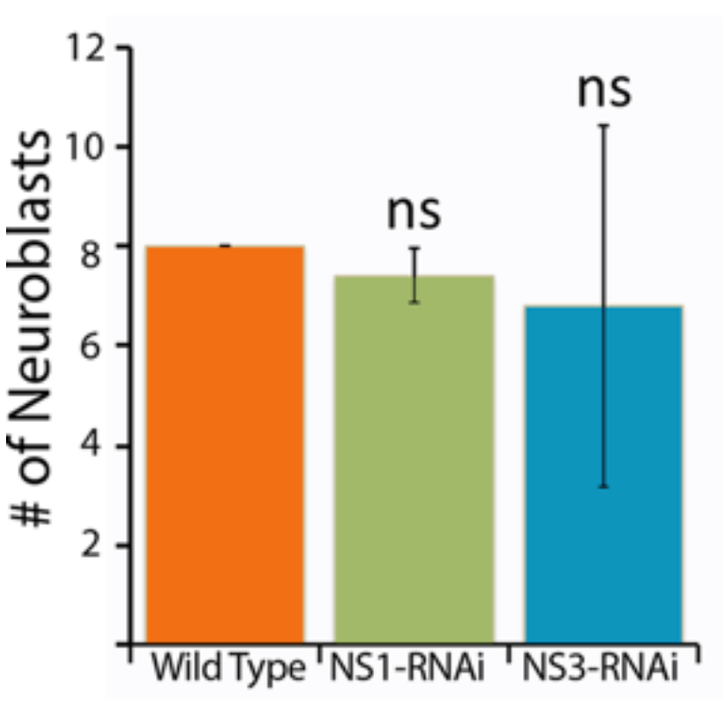
Drosophila type II neuroblast lineages keep Prospero levels low to generate large clones that contribute to the adult brain central complex

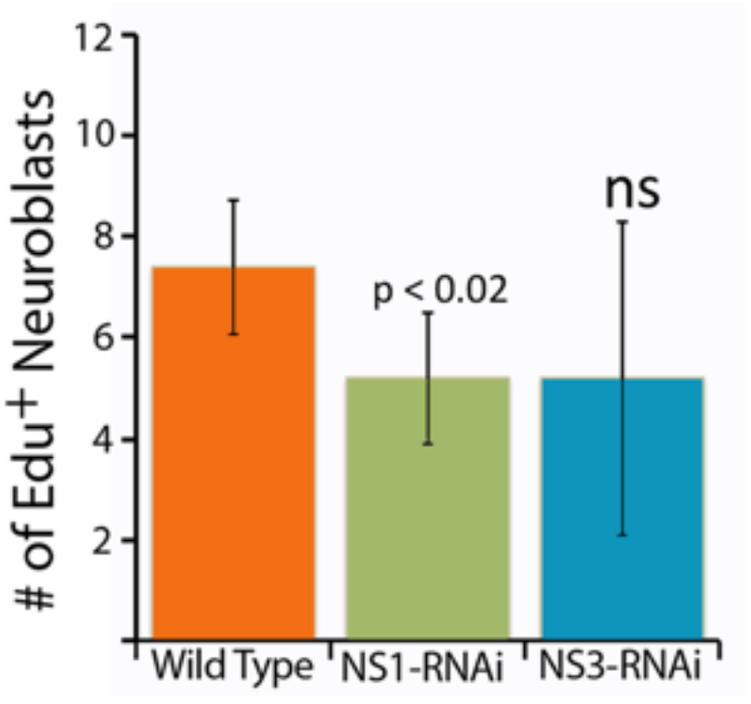
Omer Ali Bayraktar[1], Jason Q Boone[2], Michael L Drummond[1], Chris Q Doe[1,2*]

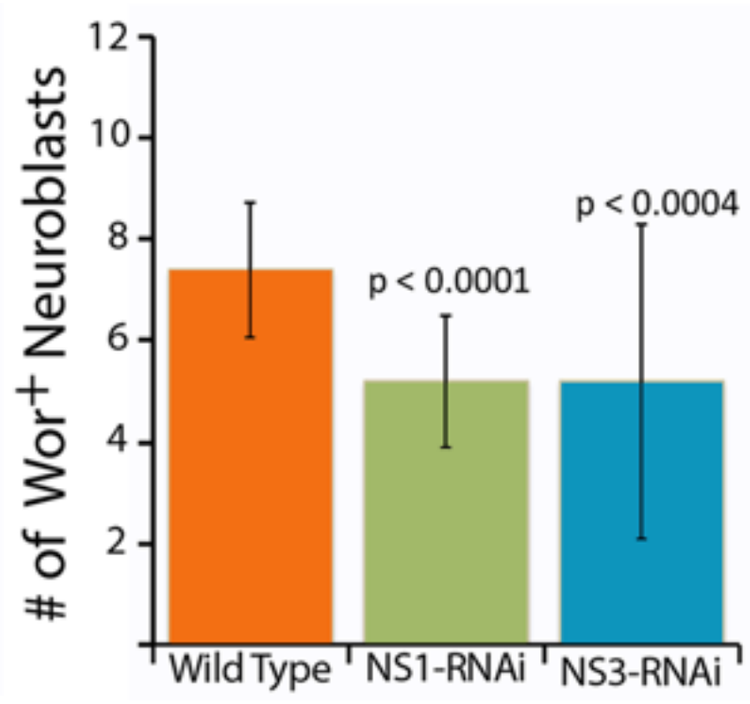
Do NS1 and NS3 regulate type II neuroblast quiescence as well?

Similar results are found in type II neuroblasts





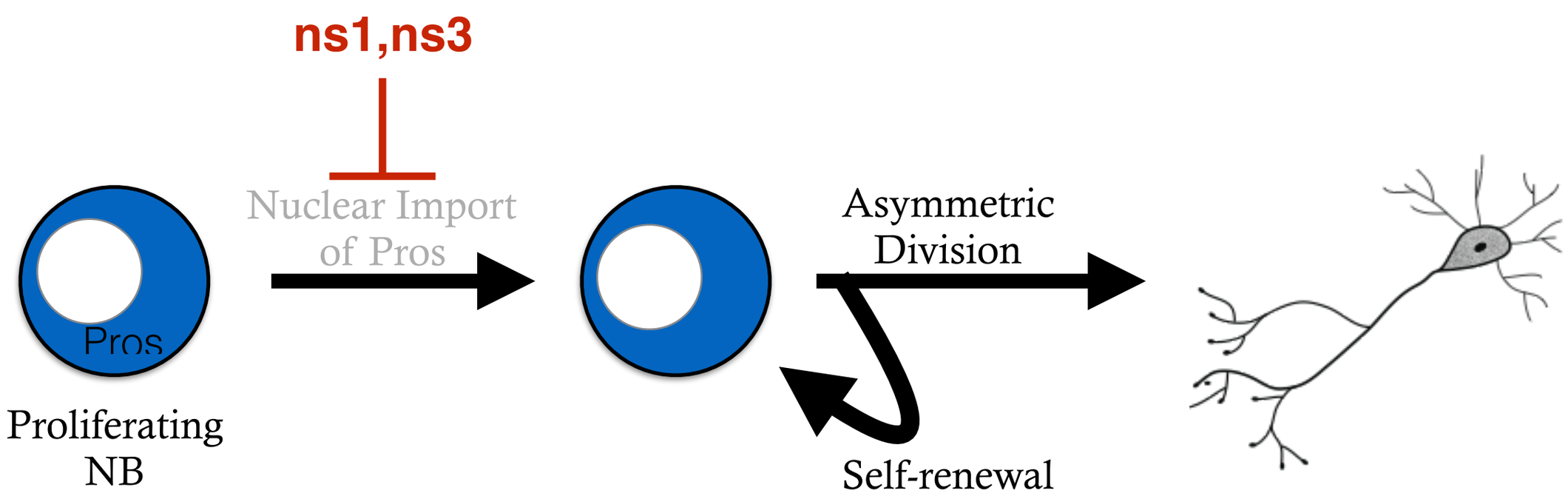




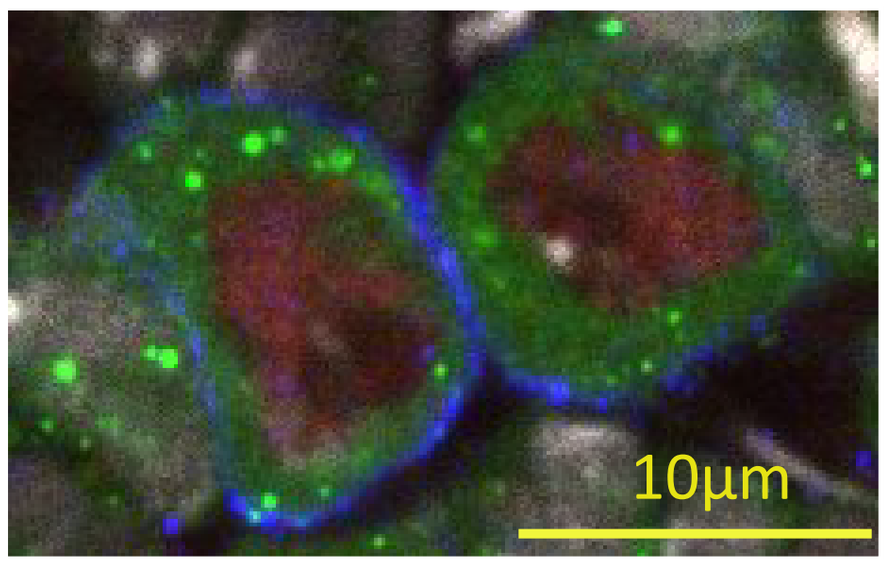
n=5, Genotype: wor-gal4, ase-gal80, uas-mcd8:gfp

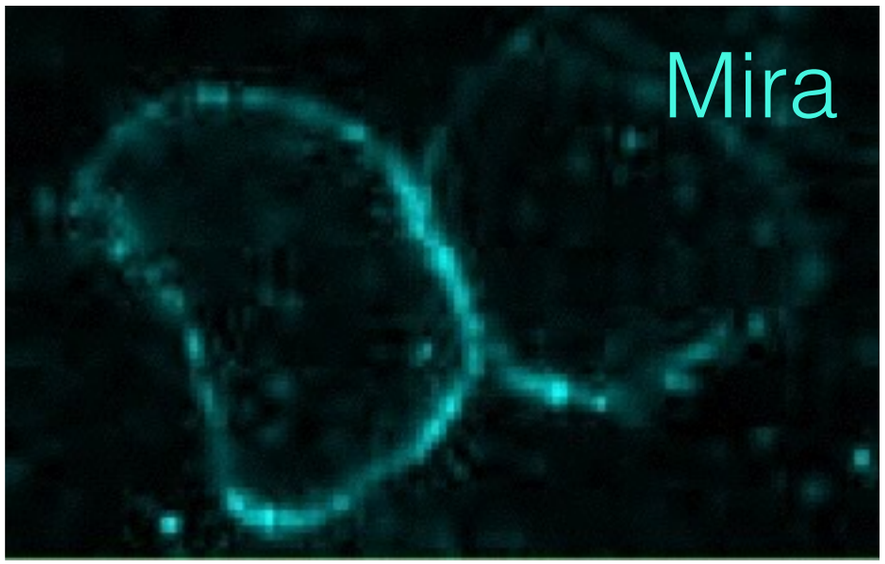
NS1 and NS3 promote neuroblast proliferation

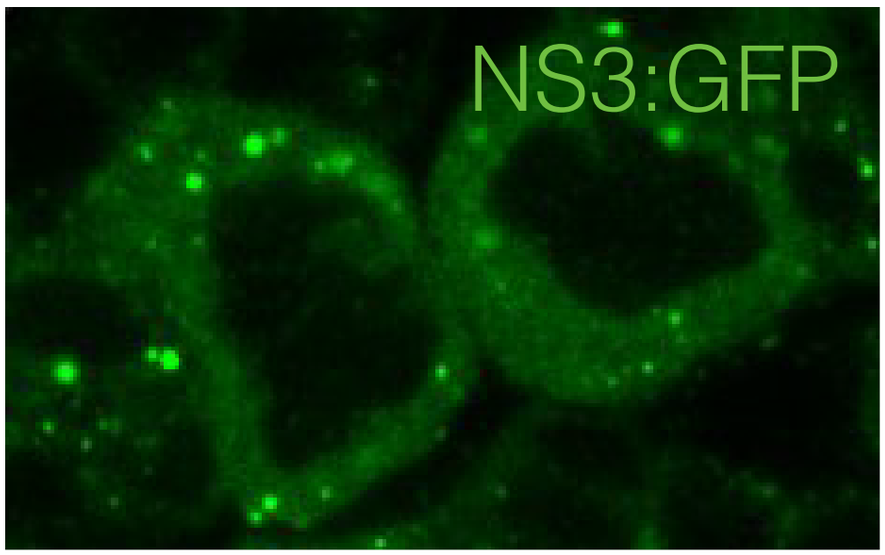
	Wild Type	NS1-RNAi	NS3-RNAi
Deadpan (Dpn)	+	+	+
EdU	+	-	-
Worniu (Wor)	+	-	-
Nuclear Pros	-	+	+
Neuroblast State	Proliferating	Quiescent	Quiescent

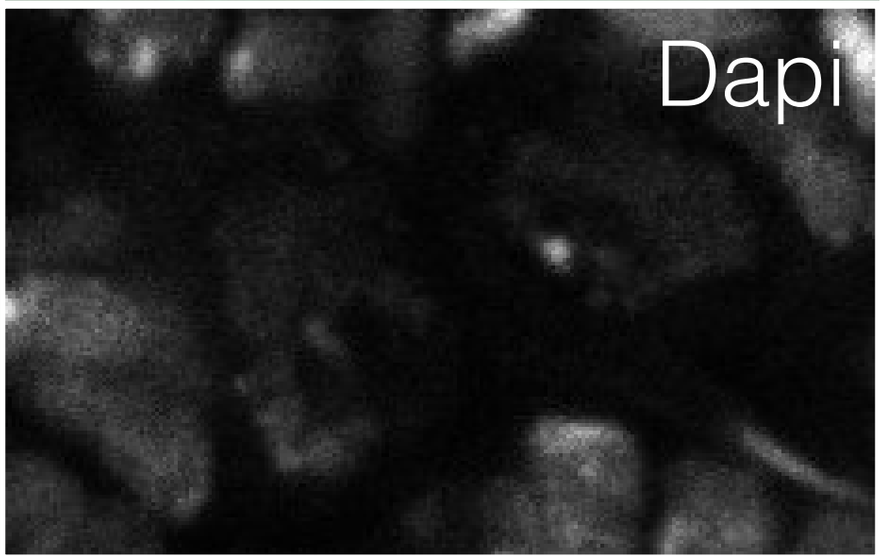


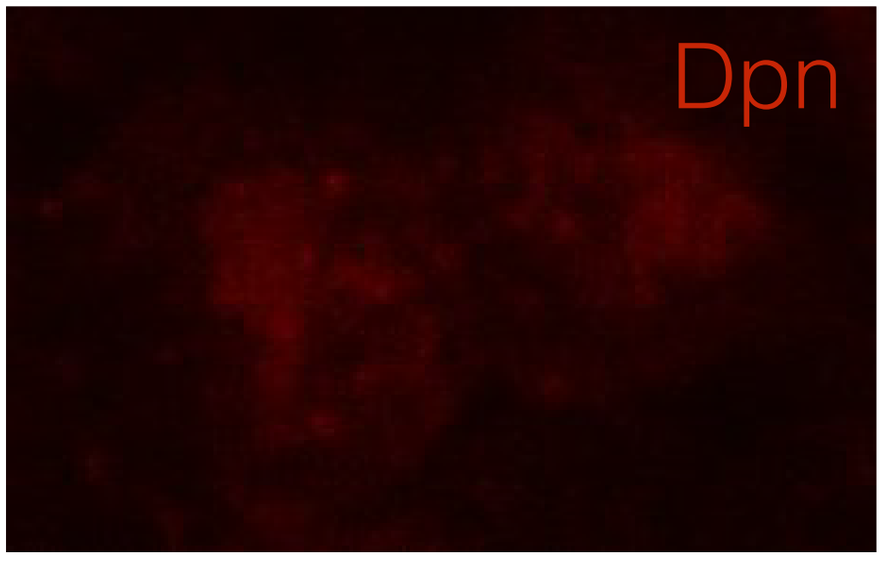
NS3 is located in the cytoplasm

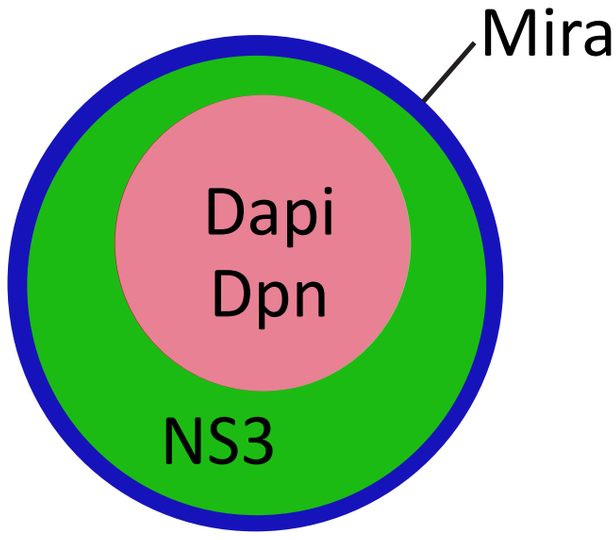












Genotype: wor-gal4, uas-ns3:gfp

Nucleostemins could regulate the cellular localization of Prospero in several ways

1) NS directly regulates expression levels of Prospero
    - Transcriptional control
    - Translational control
    - Post-translational control
2) NS acts as part of the Ran pathway
3) NS sequesters Prospero in the cytoplasm

Nucleostemins could regulate the cellular localization of Prospero in several ways

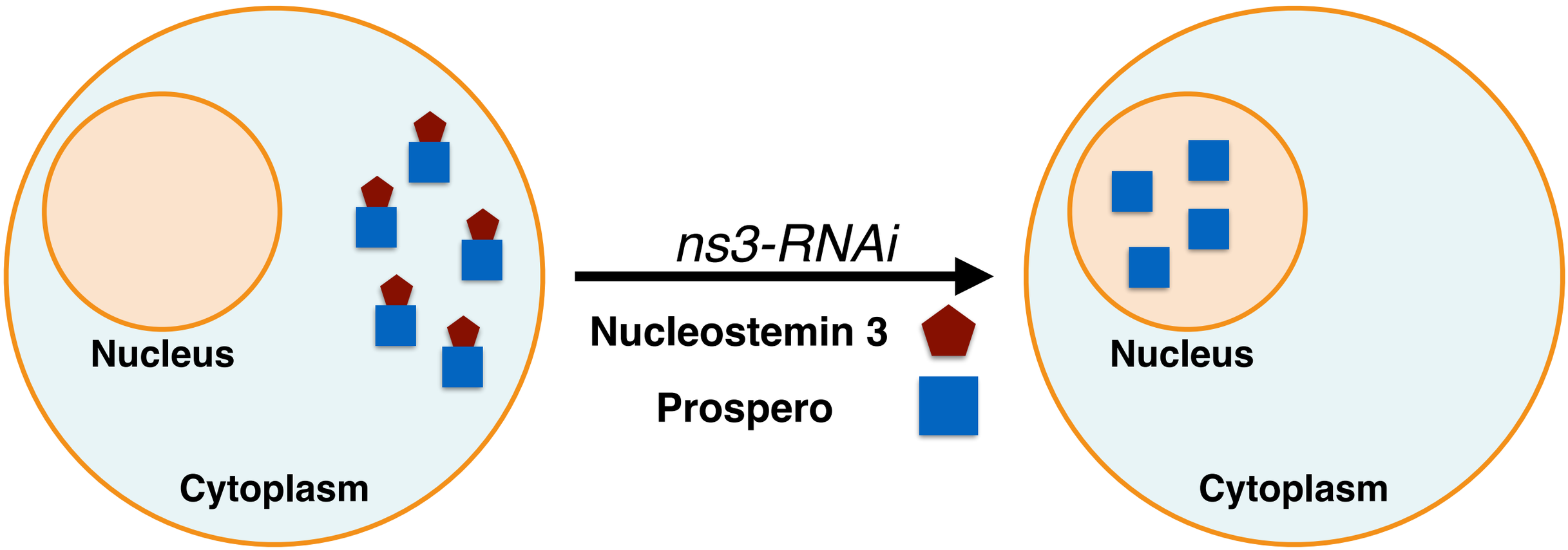
1) NS directly regulates expression levels of Prospero

- Transcriptional control
- Translational control
- Post-translational control

2) NS acts as part of the Ran pathway

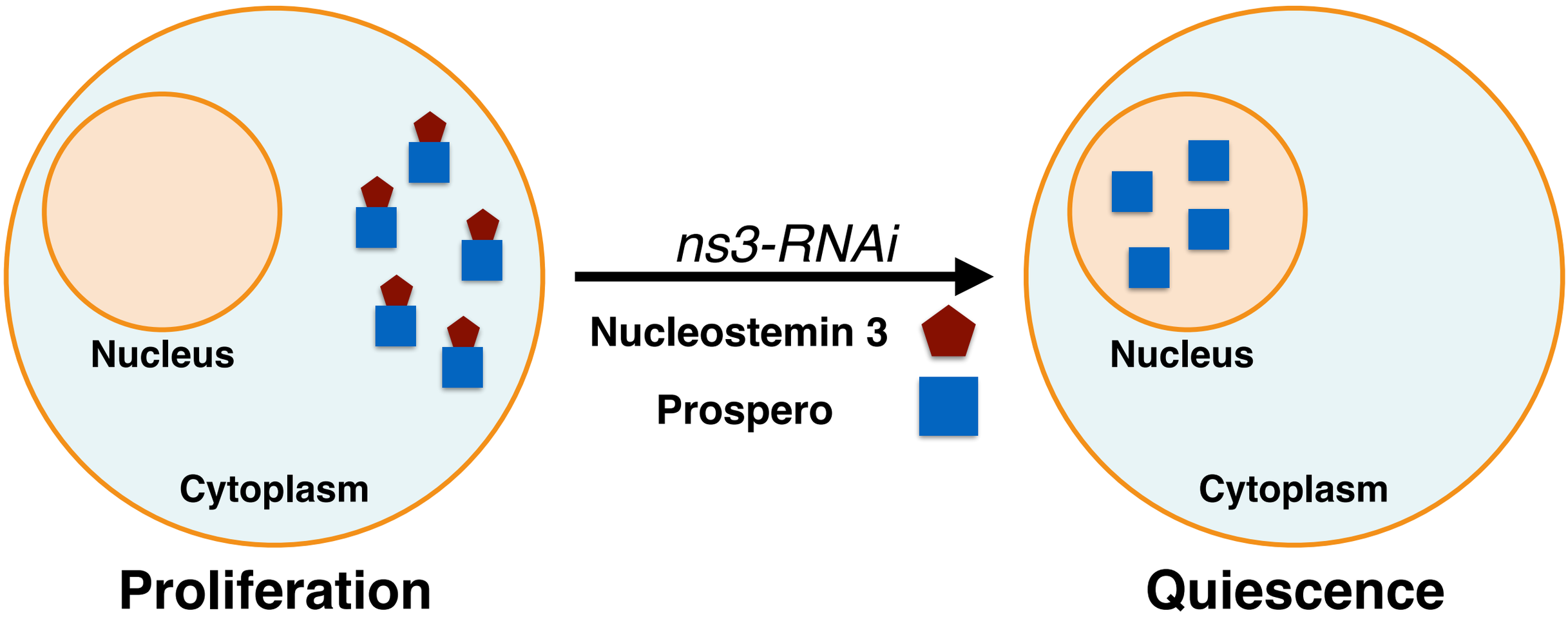
3) NS sequesters Prospero in the cytoplasm

Hypothesis: NS3 directly binds and sequesters Prospero in the cytoplasm

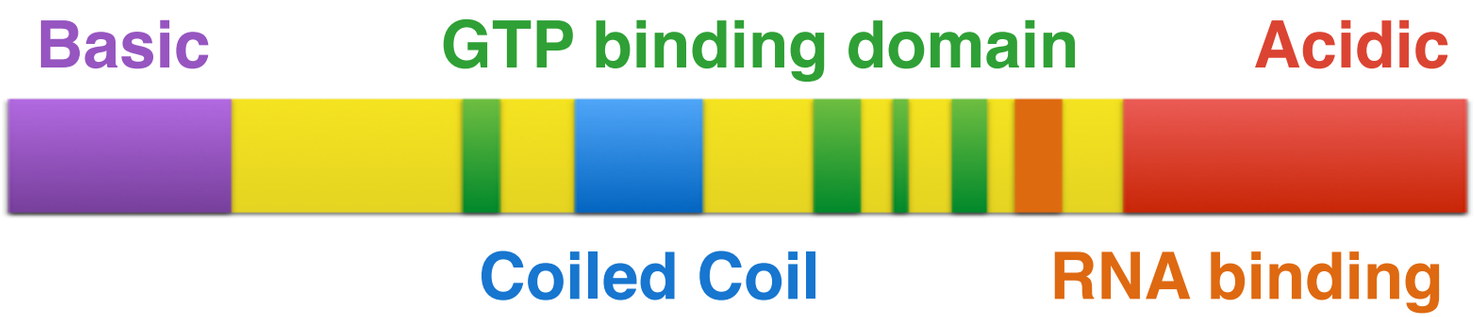


Proliferation **Quiescence**

Hypothesis: NS3 directly binds and sequesters Prospero in the cytoplasm



If this is true, which domain binds Prospero?



Molecular cloning was used to produce NS3 deletion constructs

NS3

PCR amplification

Remove a single domain

PCR Product/ annealed oligos

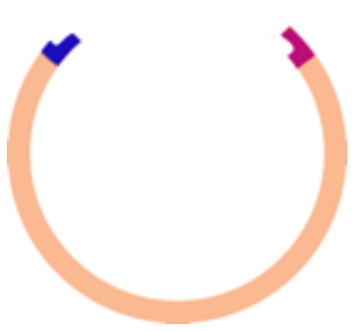
+

E. coli Vector

Digestion

Ligation

+



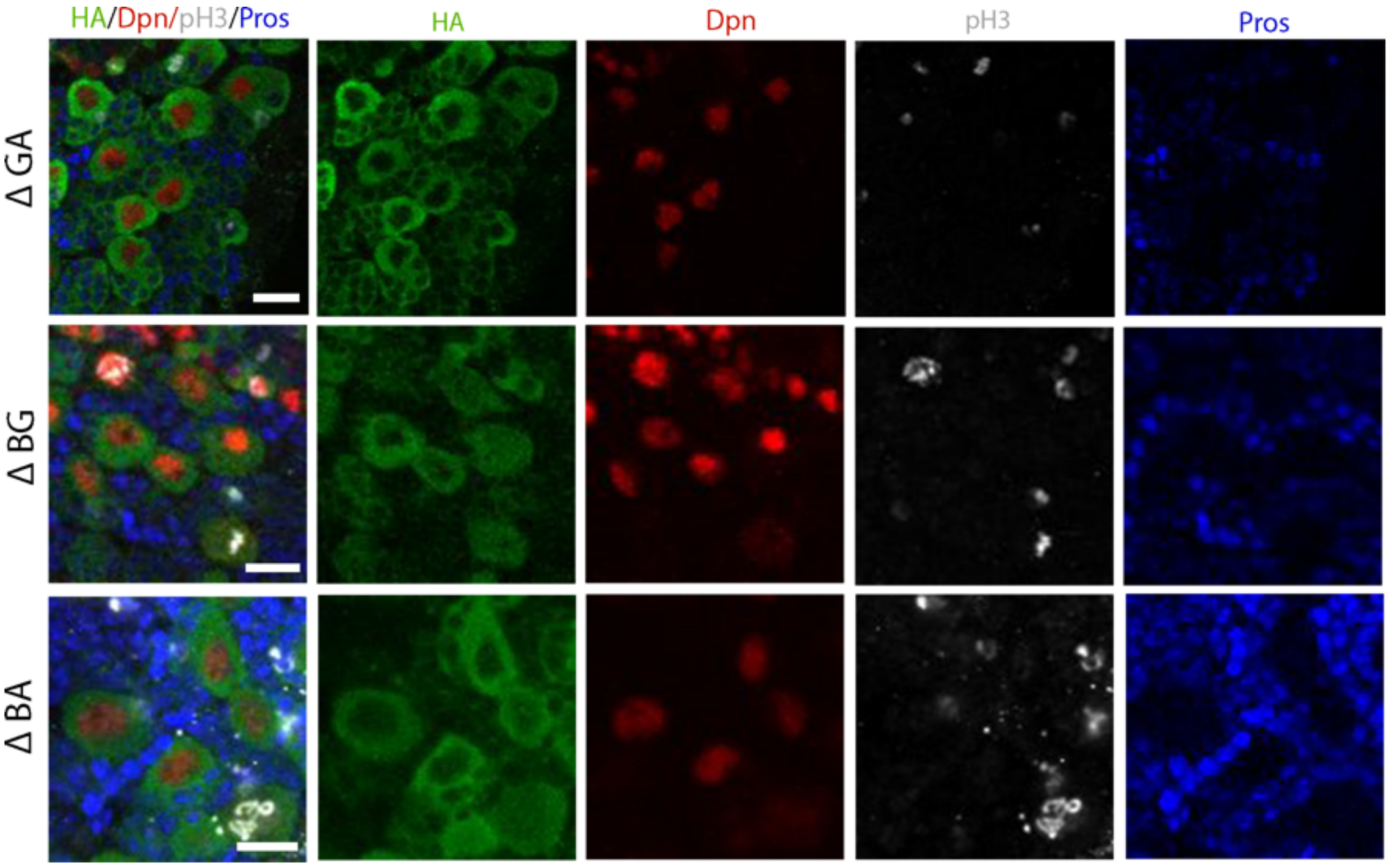
Injection into *Drosophila*

ΔAcidic Domain

ΔBasic Domain

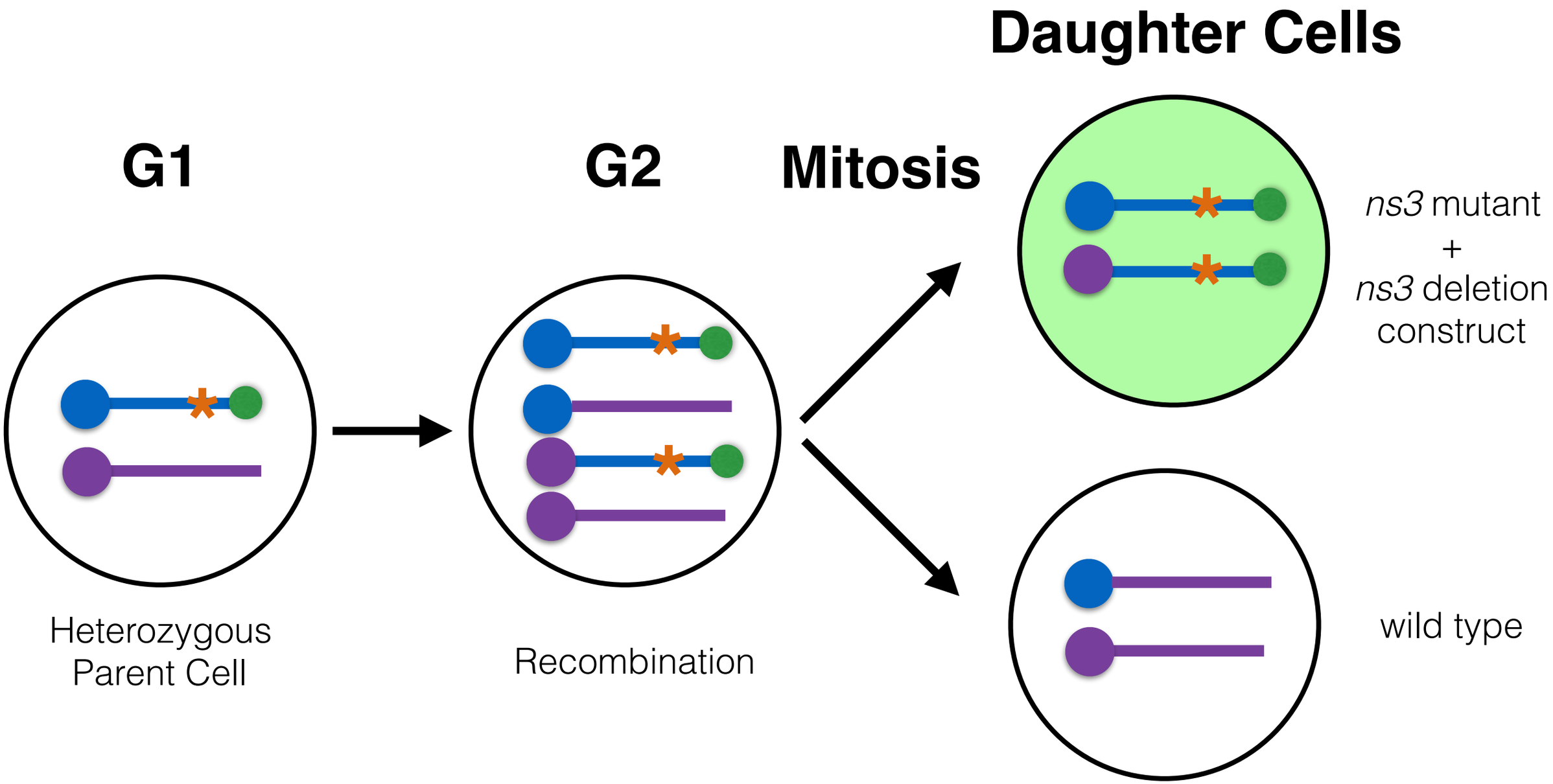
ΔGTP, coiled-coil

ns3 mutant constructs



MARCM will be used to determine if any NS3 deletion construct can rescue the *ns3* mutant phenotype

MARCM = Mosaic Analysis with Repressible Marker



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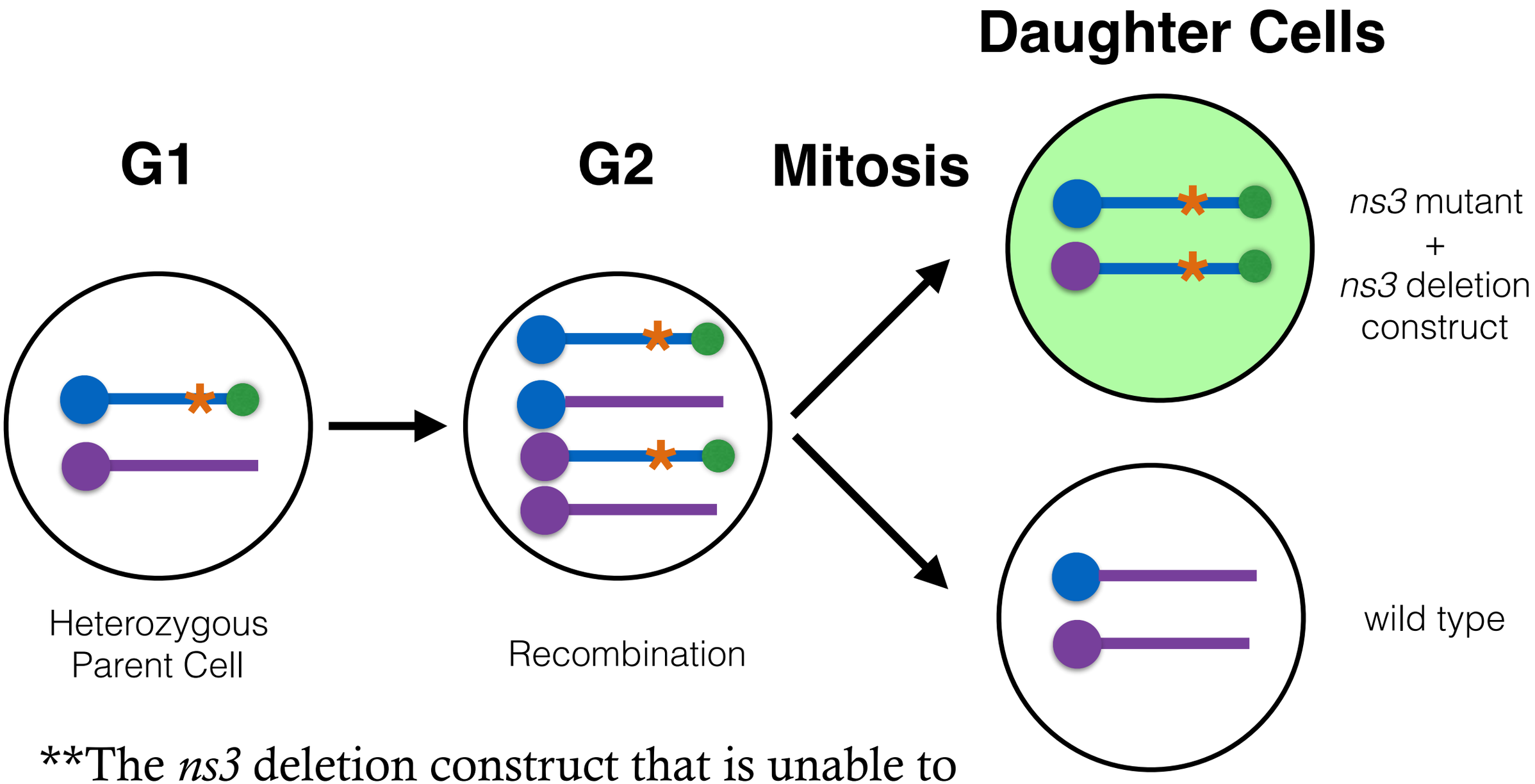

**The *ns3* deletion construct that is unable to rescue the *ns3* mutant phenotype must contain the Prospero binding domain

Conclusions

When NS1 and NS3 are suppressed via RNAi:

- There is a decrease in proliferating neuroblasts
- Neuroblasts cease to express neural progenitor markers
- Prospero is imported into the nucleus of neuroblasts

*** NS1 and NS3 are required to promote neural stem cell proliferation in *Drosophila***

Significance

Nucleostemin is a highly conserved GTP-binding protein

- Findings here may propose a novel function for how mammalian systems regulate the fine balance between proliferation and quiescence

Future Directions

- Complete MARCM analysis
- Continue investigating the possible relationship between NS and the Ran-dependent nuclear transport system

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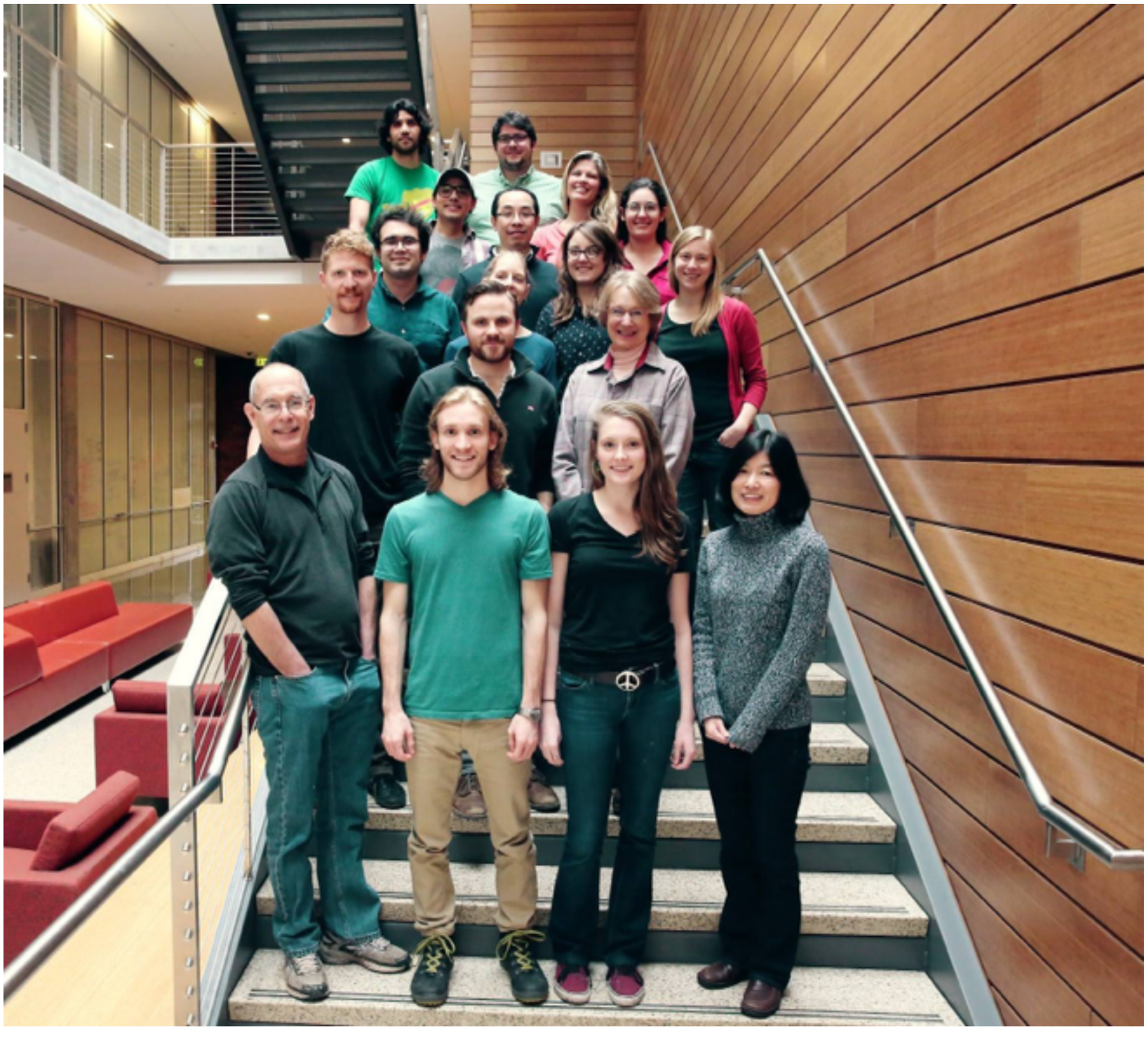

HHMI

Questions?