



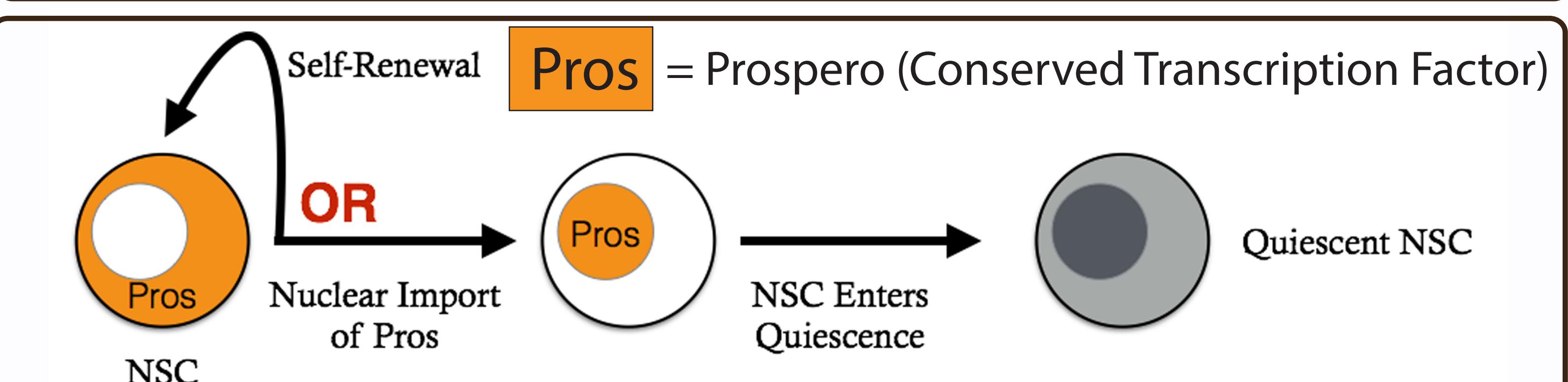
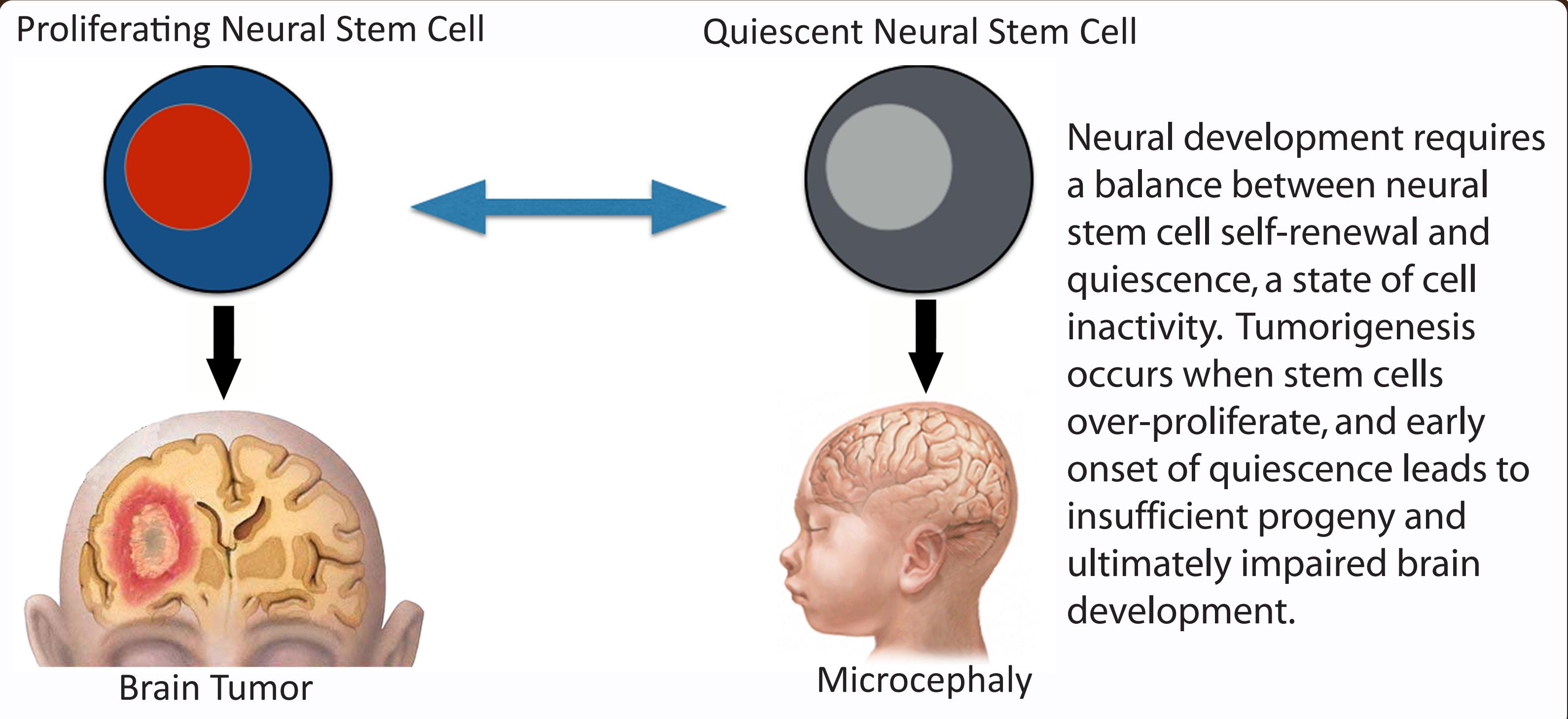
Identification of Genes Required for Nuclear Exclusion of Prospero During Neural Stem Cell Self-Renewal

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NICHD R25 Summer Research Program

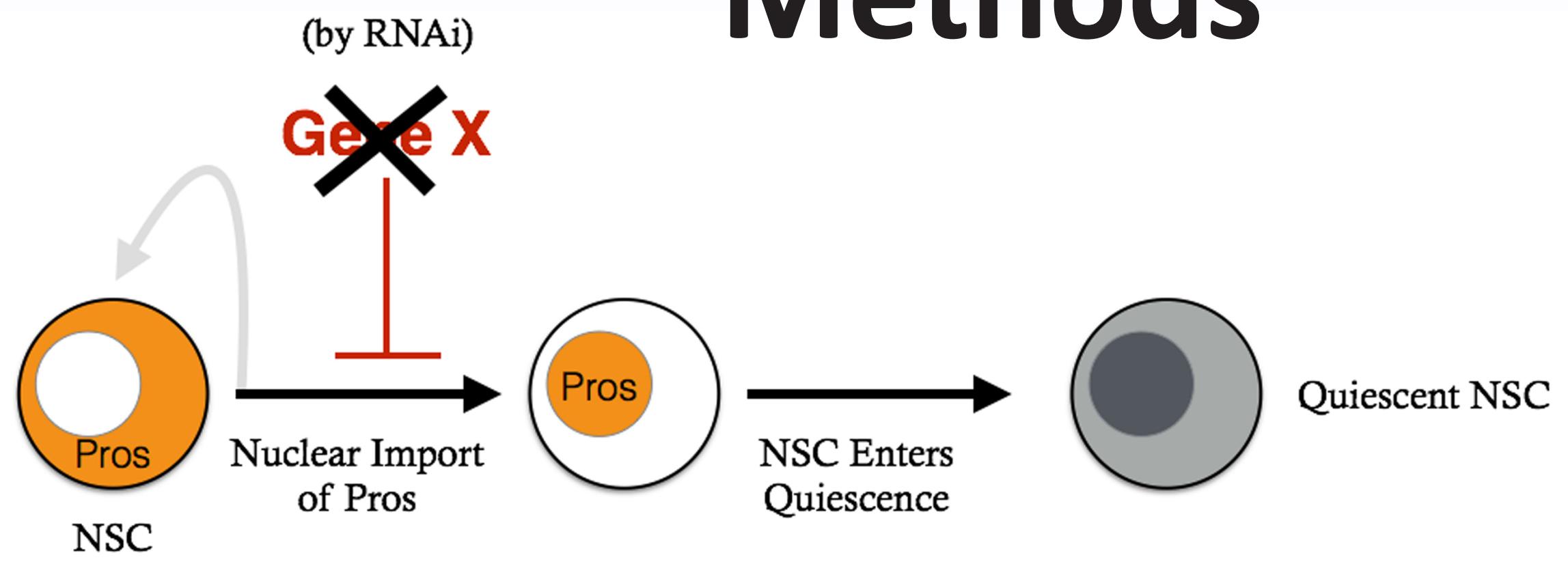
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Introduction



Neural stem cells in *Drosophila* (neuroblasts) are a well-established genetic model system capable of identifying conserved signaling pathways that regulate stem cell activities. Prospero, a conserved transcription factor, is expressed in neuroblasts, but must be excluded from the nucleus in order for neuroblast self-renewal. Transient import of Prospero into the nucleus of a neuroblast results in termination of the cell cycle and induces neuroblast quiescence.

Methods



In order for neural stem cell self-renewal to occur, there must be a gene that prevents Pros from entering the nucleus. If a gene aids in the exclusion of nuclear Pros, the suppression of that gene would lead to the nuclear import of Prospero and thus arrest the cell cycle of neuroblasts. To find this gene(s), the RNAi approach was used to selectively suppress neuroblast genes, and then antibody staining was used to determine the cellular localization of Pros along side with EdU incorporation to assay neuroblast activity.

	Proliferating Neural Stem Cell	Quiescent Neural Stem Cell
Dpn	+	+
EdU	+	-
Wor	+	-
Pros	Cytoplasmic	Nuclear

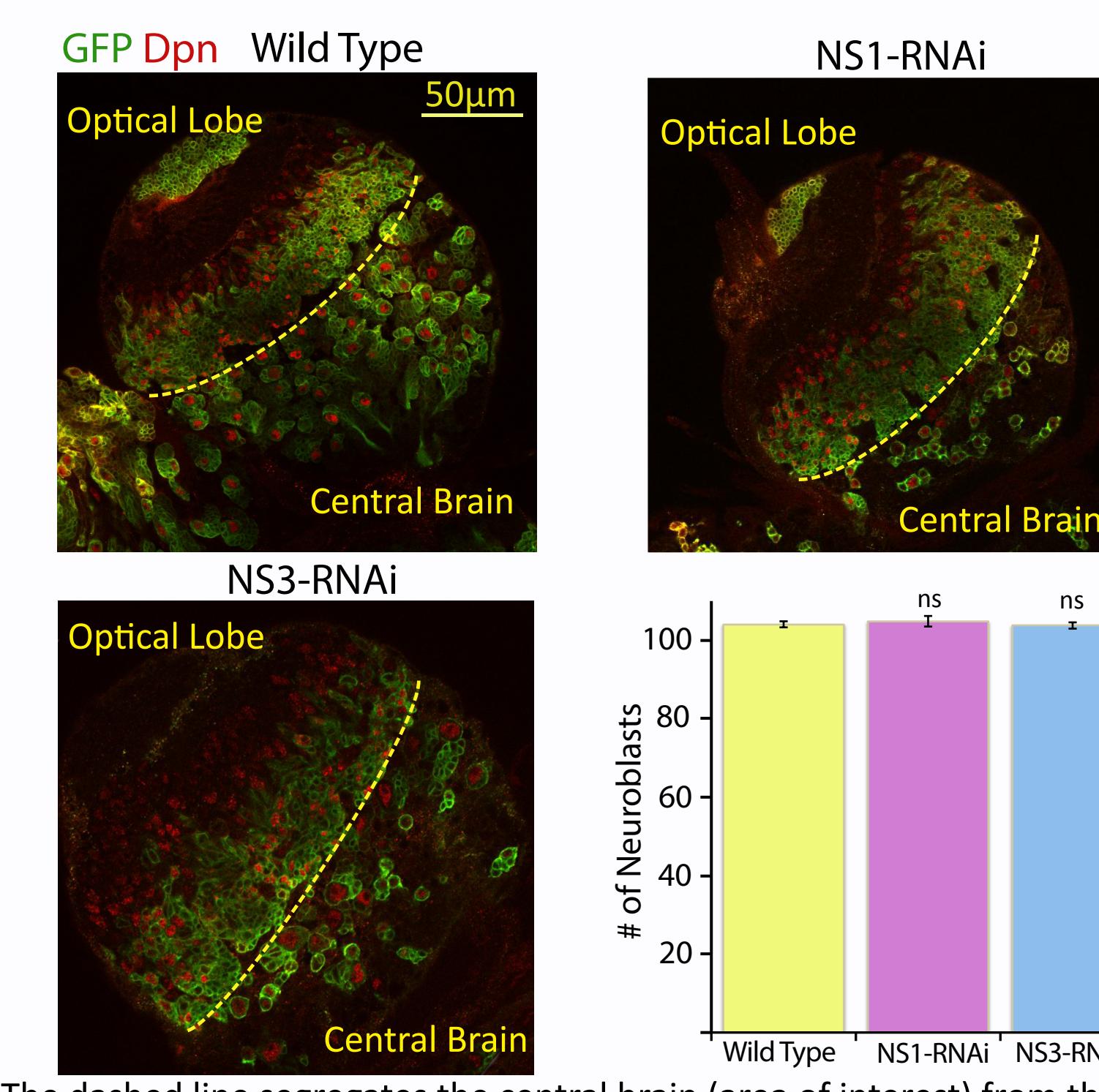
These markers were used to access whether a neuroblast was proliferating or in a state of quiescence.

Dpn (Deadpan): basic helix-loop-helix transcription factor
Wor (Worni): zinc finger transcription factor
Pros (Prospero): a typical homeodomain transcription factor

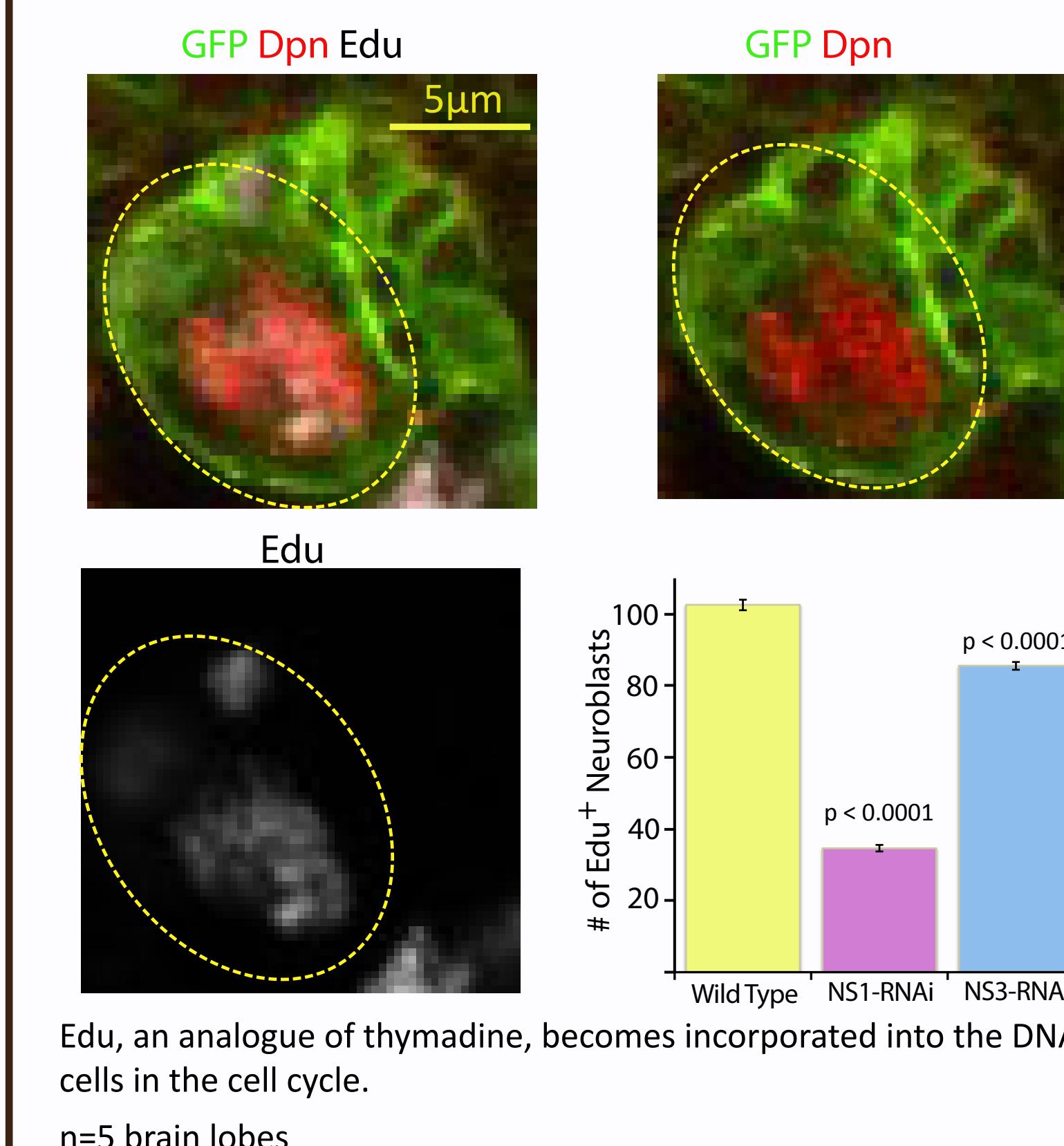
Results

Upon screening 20 different genes, nucleostemin 1 (NS1) and nucleostemin 3 (NS3) had the most profound impact on the neuroblast activities without altering the total number of neuroblasts. NS1 has been previously characterized as aiding in large ribosomal subunit biogenesis, cell growth and midgut precursor cell maintenance¹ while NS3 regulates body size². Both NS1 and NS3 encode nucleostemin GTPase and the mammalian orthologs are enriched in the neural stem cells.

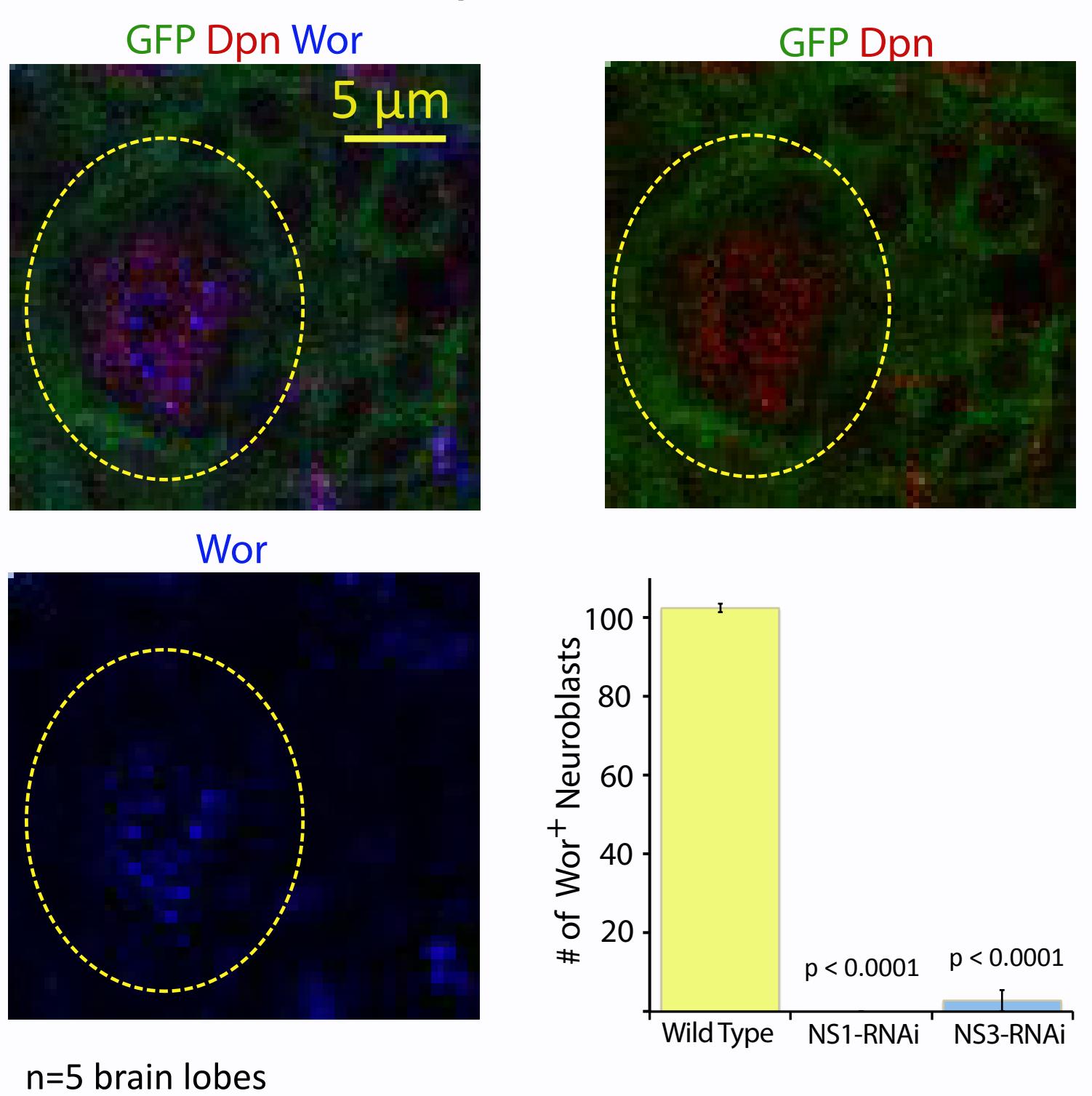
NS1-RNAi and NS3-RNAi do not decrease the total number of neuroblasts



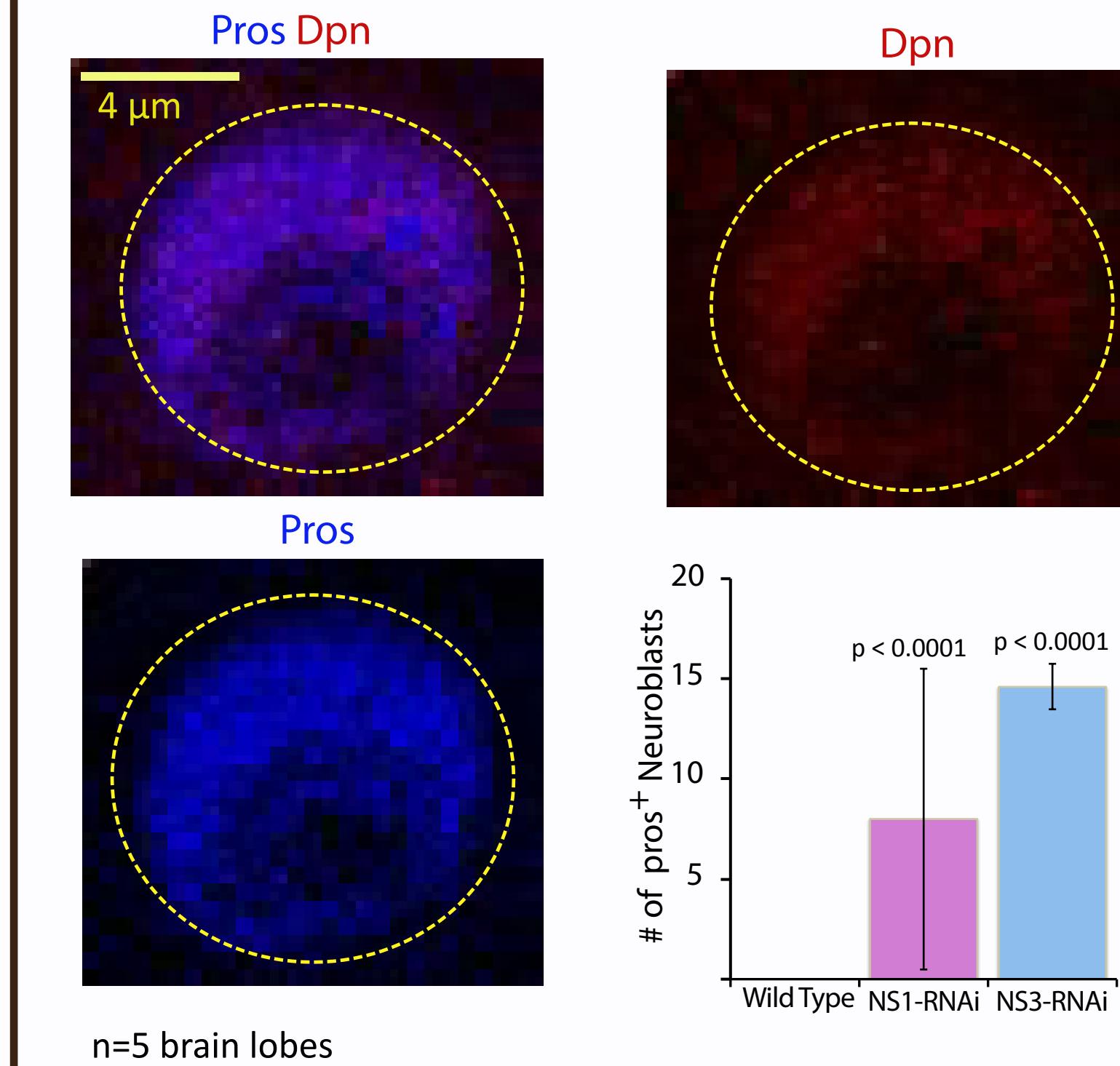
NS1-RNAi and NS3-RNAi decrease the total number of neuroblasts in the cell cycle



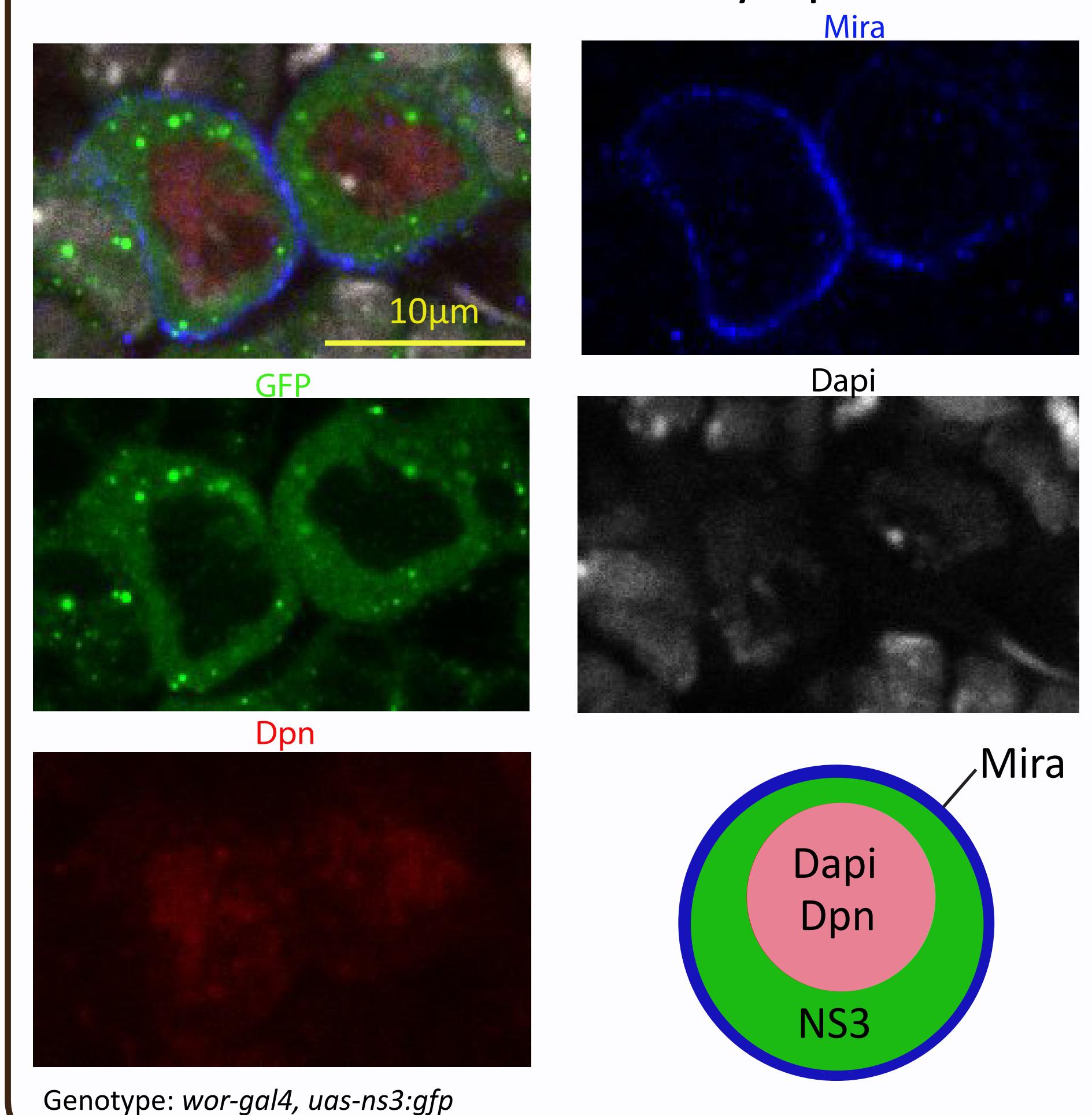
NS1-RNAi and NS3-RNAi increase the total number of quiescent neuroblasts



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



NS3 is located in the cytoplasm



Conclusions

NS1 and NS3 have a clear role in aiding in the nuclear exclusion of Pros during stem cell self-renewal. When the genes are suppressed via RNAi, Pros enters the nucleus and the neuroblasts become quiescent.

Future Directions

- 1) Perform *in situ* hybridization of NS1 and NS3 to determine if they are expressed in neuroblasts only or ubiquitously in the CNS.
- 2) Determine cellular localization of NS1.
- 3) Determine if Pros is the direct downstream of NS1 and NS3.
- 4) Determine if NS1 and NS3 are sufficient to promote neuroblast self-renewal.
- 5) Determine if type II neuroblast quiescence is Pros dependent.

References

- ¹ Rosby et al. (2009) Molecular Biology of the Cell 20, 4424-4434
² Kaplan et al. (2008) Genes & Development 22, 1877-1893

Acknowledgments

Doe Lab

