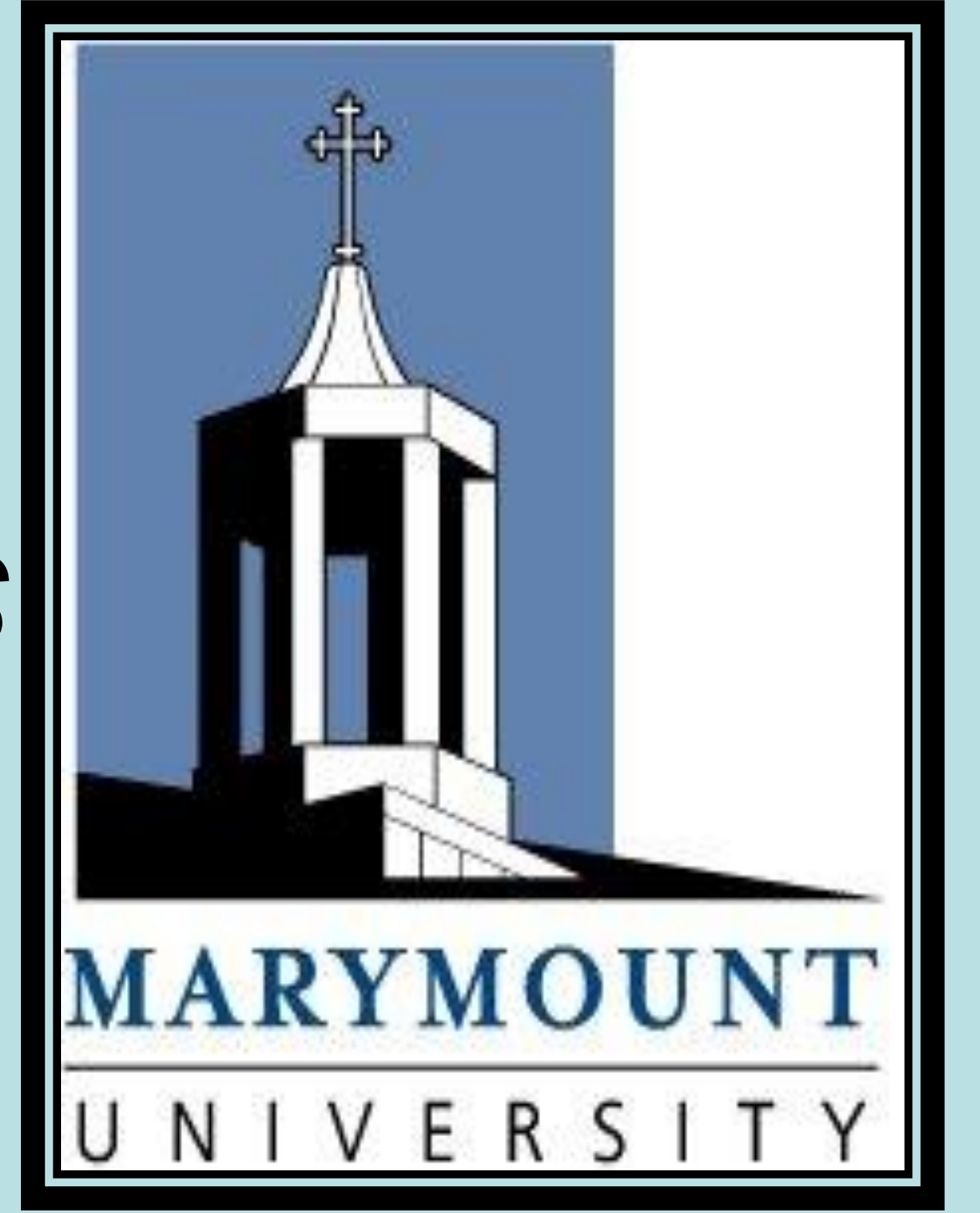
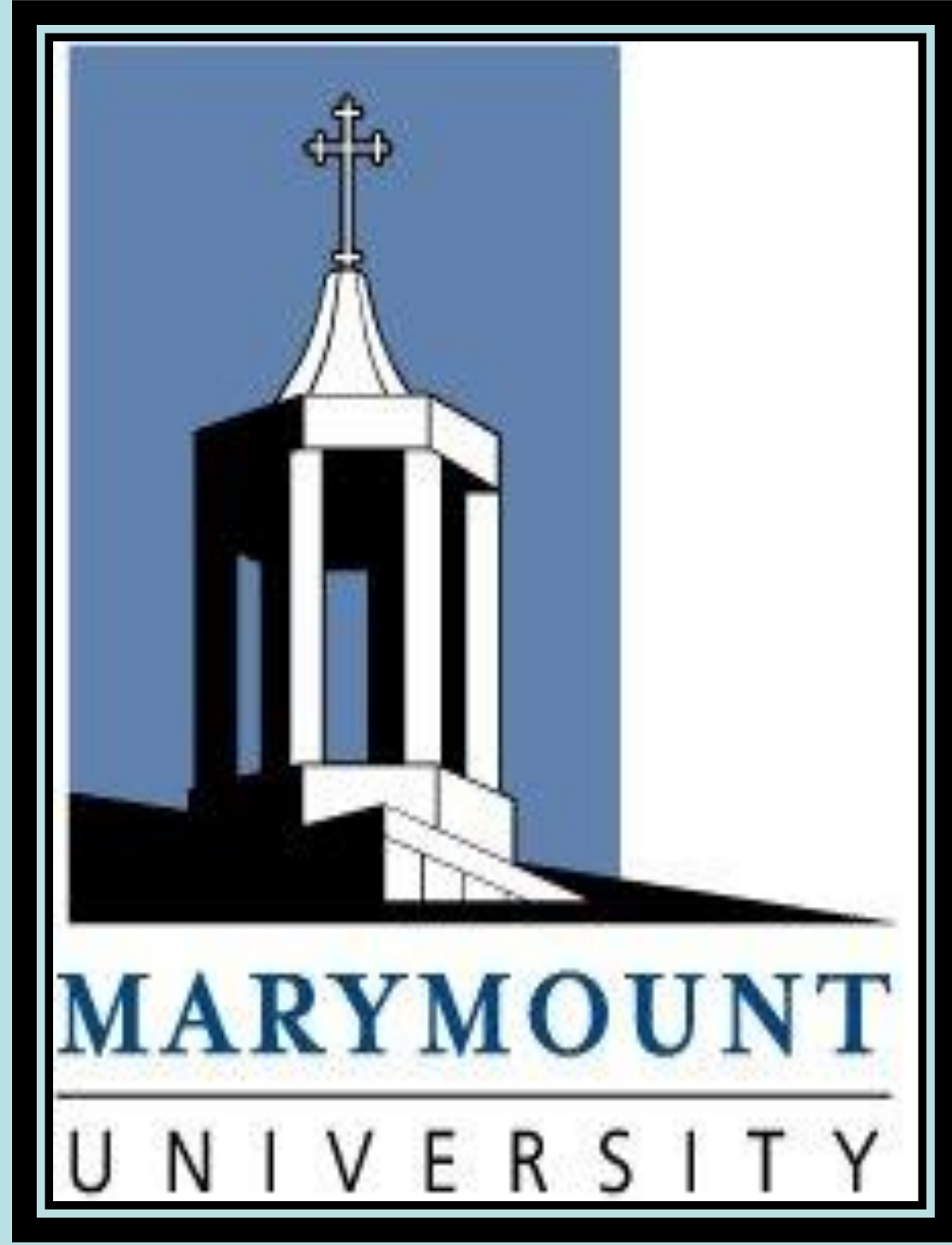


Colocalization of CHL1 and SLIT1 During Embryonic Development of Mouse Brain

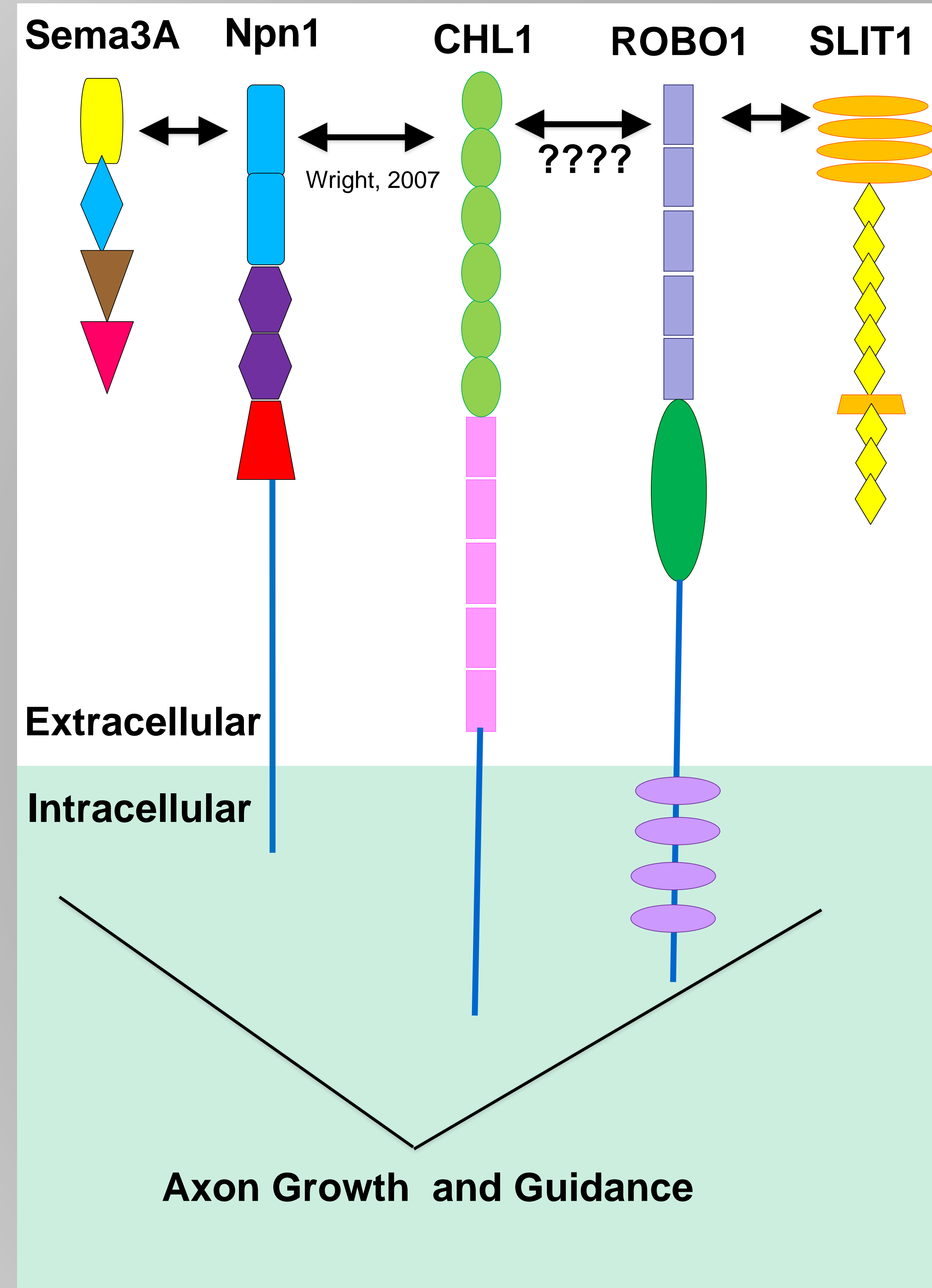
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Program Number: P2343



Abstract

Axons are an integral part of a neuron responsible for transmitting electrical signals within the brain. It is important to understand the mechanics of axon growth, including how growth cones function and which proteins are involved in axon migration and how they co-exist with other proteins. This hierarchy of information is essential to combat certain types of cancers and neurological disorders such as Alzheimer's disease. We currently know of several families of proteins that play major roles in regulating axon guidance. Two such families are neural cell adhesion molecules (NCAMs) of the immunoglobulin superfamily (Maness and Schachner, 2007) and the family of SLIT proteins (Andrews, *et al.*, 2007). Close Homolog of L1 (CHL1), a NCAM, is part of the mammalian L1 family, which is known to play a role in axon growth and migration of developing neurons (Wright, *et al.*, 2007). Roundabout1 (ROBO1), also a member of the immunoglobulin super family, plays a role in axon guidance in the developing forebrain by serving as a receptor for the guidance cue, SLIT1 (Andrews, *et al.*, 2007). Our objective in this study is to investigate a potential interaction between CHL1 and SLIT1. We hypothesize that these two proteins function together to regulate SLIT-mediated axon guidance in developing neurons. The first step in our investigation involved the use of co-immunofluorescence staining to visualize CHL1 and SLIT1 colocalization. We demonstrate here that CHL1 and SLIT1 colocalize in the intermediate zone of the cerebral cortex during axonal targeting (E16). We also demonstrate that CHL1 and ROBO1, the receptor for SLIT1, are coordinately expressed during this same period but do not colocalize. This data suggests an interaction between CHL1 and SLIT1 and a cooperation between CHL1 and ROBO1 that may help control axon guidance to regulate proper neuronal topographic mapping.



Methods

E16 mouse brain sections were purchased from Zymagen. To label CHL1, sections were incubated with goat anti-mouse CHL1 antibody (R&D Systems) in 1% goat serum for one hour at room temperature. To observe SLIT1, anti-SLIT1 rabbit antibody (abcam) in goat serum was incubated on the slides overnight at 4°C. To visualize ROBO1, anti-ROBO1 rabbit polyclonal antibody (abcam) was added to the slides for one hour at room temperature. Secondary antibodies, TRITC-conjugated donkey anti-goat (CHL1) or FITC-conjugated donkey anti-rabbit (SLIT1 or ROBO1) (JacksonImmuno), was added to the slides for thirty minutes. The slides were mounted with Vectashield prior to viewing with an EVOS digital fluorescent microscope.

Results

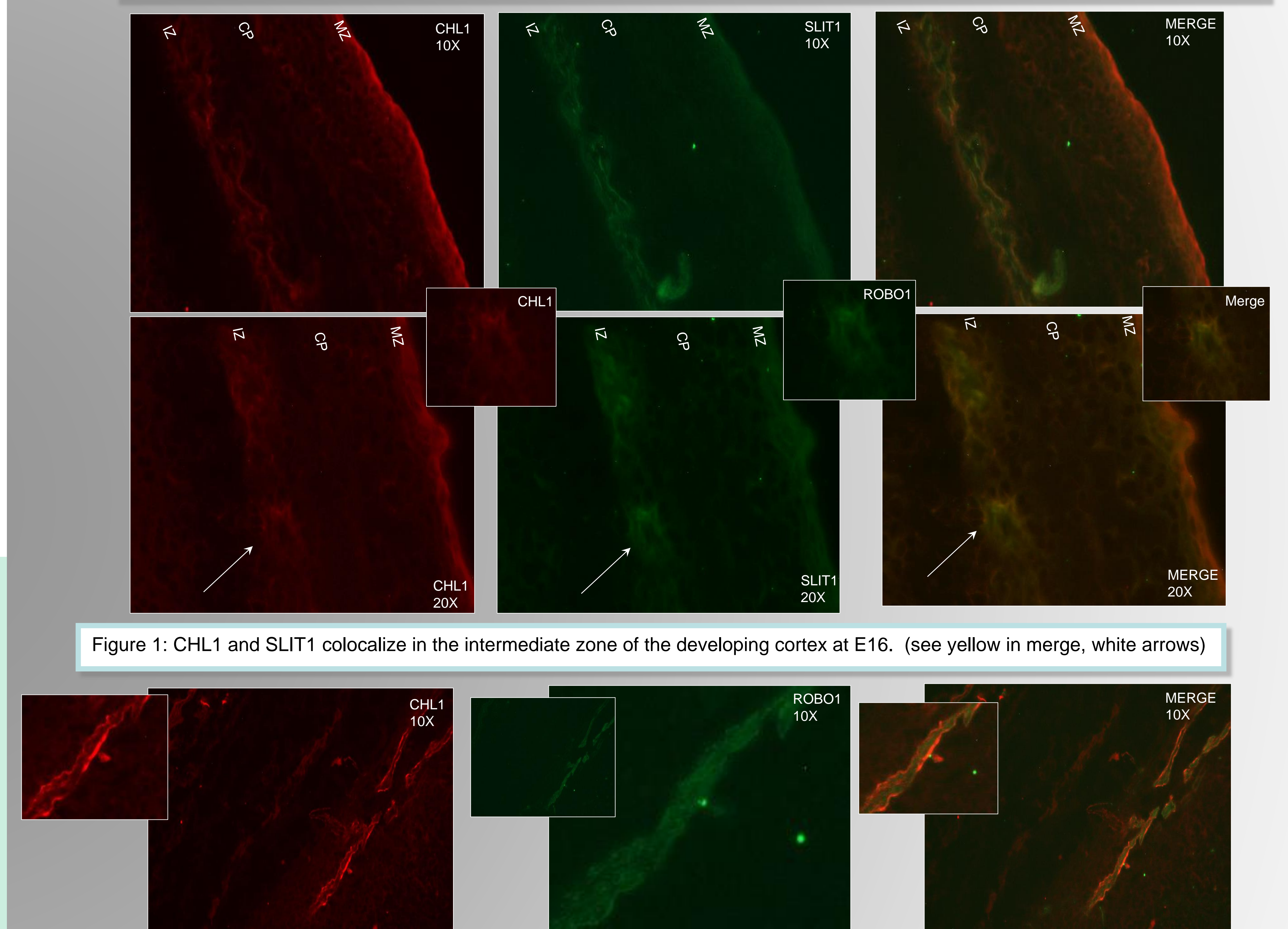


Figure 1: CHL1 and SLIT1 colocalize in the intermediate zone of the developing cortex at E16. (see yellow in merge, white arrows)

Figure 2: CHL1 and ROBO1 are expressed in a coordinating pattern but do not colocalize at E16.

Conclusion

Our results demonstrate a potential interaction between CHL1 and SLIT1 during the time of axonal targeting. Colocalization was observed in the mouse brain at E16 using co-immunofluorescence. A coordinating expression pattern of CHL1 and ROBO1 was also observed during this same time frame. These results suggest that CHL1 and ROBO1 may work together to mediate SLIT1 regulated axonal guidance. Future co-immunoprecipitation studies are planned to demonstrate definitive binding of CHL1 and SLIT1.

References

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Acknowledgements

We would like to thank Marymount University's DISCOVER program for financial support.