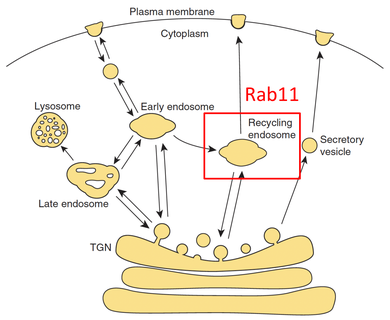
Purkinje cell development, survival, and adaptive motor behavior in mice require the redundant function of the small GTPase Rab11a and Rab11b

Jack DeLucia[1], Edward Martinez[1], Haniya Naveed[1], Michael W. Shiflett[2], Tracy S. Tran[1]

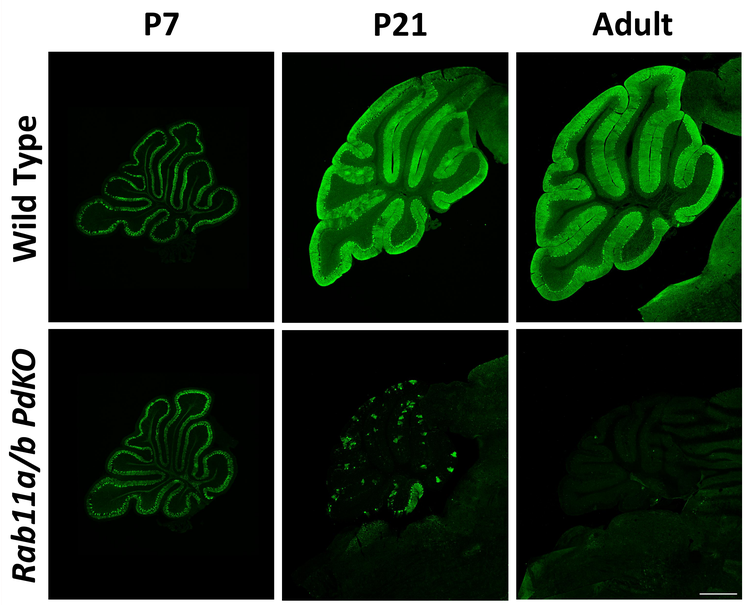
[1]Department of Biological Sciences, Rutgers University, Newark, NJ 07102; [2]Department of Psychology, Rutgers University, Newark, NJ 07102

Introduction

Cerebellar Purkinje neurons form the core of cerebellar circuitry, both as the sole outputs of the cerebellar cortex and the chief organizers of cerebellar development. One of the critical functions necessary in development is the trafficking of signaling proteins between membrane compartments. Rab11, a member of the Rab GTPase family, resides within the recycling endosome and is responsible for trafficking signaling proteins to and from the plasma membrane in addition to its many other cellular functions. Mutations in the *RAB11A* and *RAB11B* genes produce severe neurodevelopmental disorders in humans, including ataxia and cerebellar hypoplasia. However, the role Rab11 plays in the development and function of cerebellar Purkinje neurons is unknown. Here, we show that loss of both Rab11a/b in Purkinje neurons (*Rab11a/b PdKO*), causes a dramatic reduction in whole cerebellar area and the molecular layer and internal granule cell layer of the cerebellar cortex. *Rab11a/b PdKO* animals show a severe loss of Purkinje neurons by P21 which is near total by adulthood, in addition to a significant loss of climbing fiber synaptic input to the cerebellum. Lastly, *PdKO* animals trend toward ataxia and impaired locomotion, demonstrating that Rab11a/b are required for normal cerebellar development and the behavioral output of the animal.

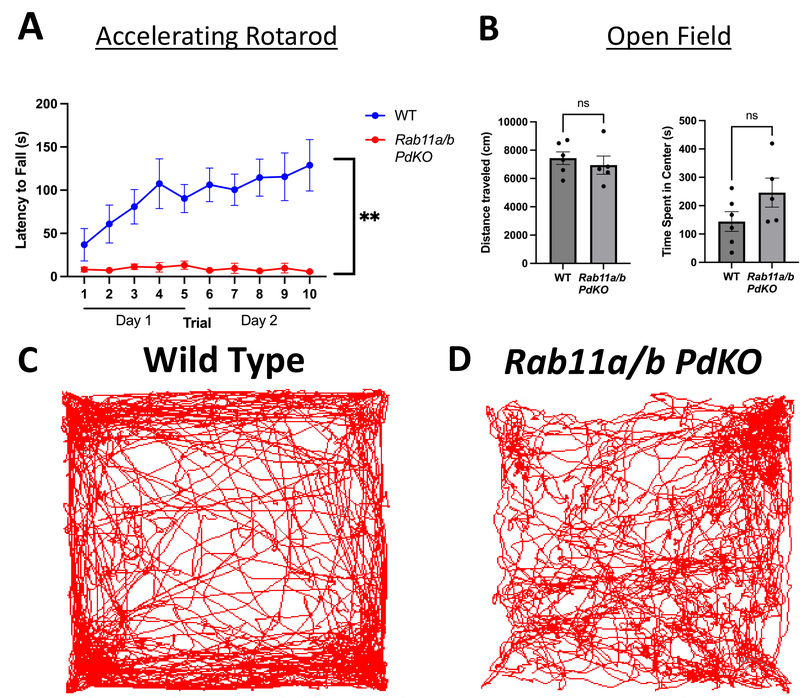


Severe loss of Purkinje cells in *PdKO* cerebellum by P21, near total by adulthood



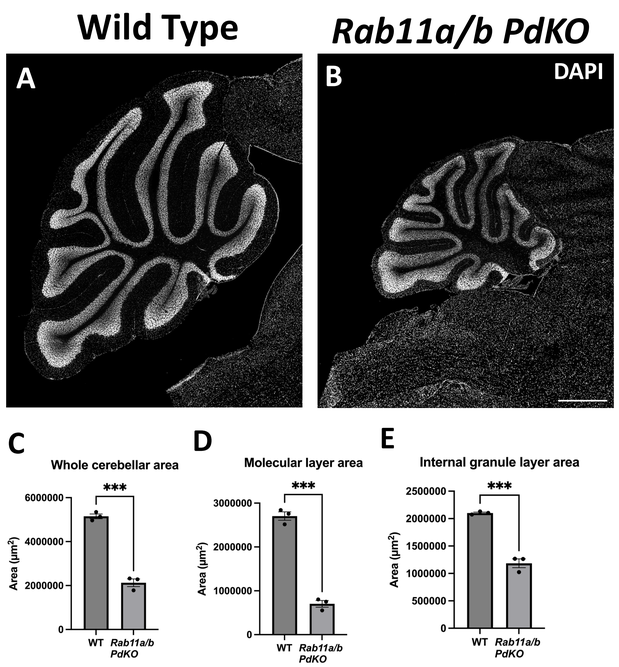
A-D. Para-midsagittal sections of P7 (**A, D**), P21 (**B, E**), and adult (**C, F**) cerebella stained with anti-calbindin taken from WT (**A-C),** and *Rab11a/b PdKO* (**D-F**) mice. Scalebar = 500 µm in D for A-D.

PdKO trend toward ataxia, reduced locomotion



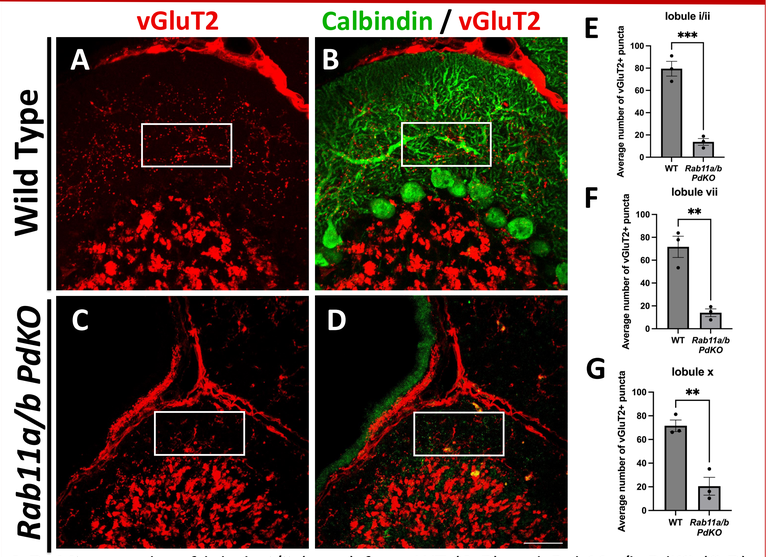
A. Quantification of latency to fall from accelerating rotarod test. **B.** Quantification of the time spent in the center of the arena and the total distance traveled from the open field test. **C.** Representative tracings of a mouse center-point in the open field test from WT and *Rab11a/b PdKO* animals. Data are means ± SEM from n = 5-6 animals per genotype, $^{**}P < 0.01$ by unpaired t-test. Welch's correction used for rotarod analysis in A.

PdKO cerebellum has reduced whole area, molecular layer area, and internal granule layer area



A-B. DAPI-stained sagittal cerebellar sections of wild type (A) and *Rab11a/b PdKO* (B) cerebella at adulthood.
C. Quantification of the whole cerebellar area.
D. Quantification of the molecular layer area.
E. Quantification of the internal granule layer area. Data are means ± SEM from n = 3 animals per genotype. $^{***}P < 0.001$ by unpaired t-test. Scalebar in B = 500 µm for A and B.

Reduced climbing fiber synapse number in *PdKO* cerebellum



A-D. Micrographs of lobule i/ii (apex) from WT (**A-B**) and *Rab11a/b PdKO* (**C-D**) cerebella stained with anti-vGluT2 (**A, C**) and anti-vGluT2 + anti-calbindin (**B, D**). **E-G.** Quantification of average vGluT2+ puncta from fixed ROI in lobule i/ii (**E**), lobule vii (**F**), and lobule X (**G**). Data are means ± SEM from n = 3 animals per genotype $^{***}P < 0.001$, $^{**}P < 0.01$ by unpaired t-test. Scalebar in D = 25 µm for A-D. White frame in A-D indicates region of quantification.

Summary and Conclusions

- Rab11a and Rab11b (Rab11a/b) are required for normal size development of the cerebellum as *PdKO* animals show a dramatic reduction in their whole cerebellar area, molecular layer area, and internal granule cell area compared to WT controls.
- Rab11a/b are required for the survival and maintenance of Purkinje neuron numbers as *PdKO* cerebella display widespread loss of Purkinje neurons during development, which is severe by P21 and near total loss by adulthood.
- Rab11a/b are required for the proper synaptic input from the brainstem (inferior olives) as *PdKO* cerebella show a dramatic loss of climbing fiber inputs as measured by the number of vGluT2+ presynaptic puncta in anterior, central, and posterior / nodular lobules.
- *Rab11a/b PdKO* animals display impaired motor coordination (ataxia) and learning and trend toward increased time in the center of the open field.

Acknowledgments

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