Summer Forum 2021

Examining Patterns of Rostral Migration in Celsr1 and Wnt5a Double Mutants

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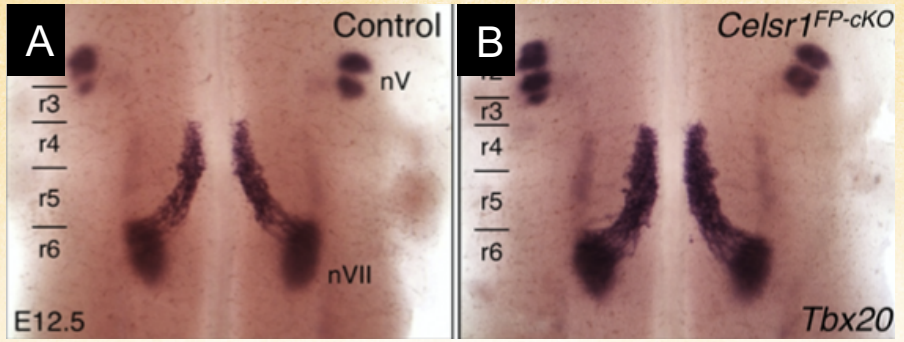
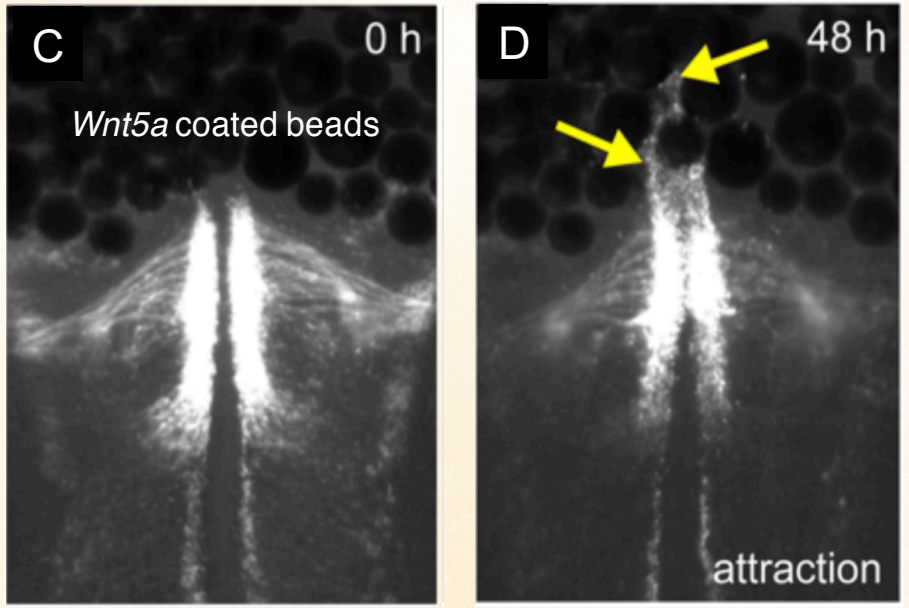
Experimental Basis of the Current Model

- (A-B) While investigating Celsr1's role in the Wnt/PCP pathway, it was found that Celsr1 acted to influence the direction of migration
- (C-D) Previous experiments have proven that *Wnt5a* coated beads can elicit rostral migration, supporting the hypothesis that *Wnt5a* acts as a chemoattractant





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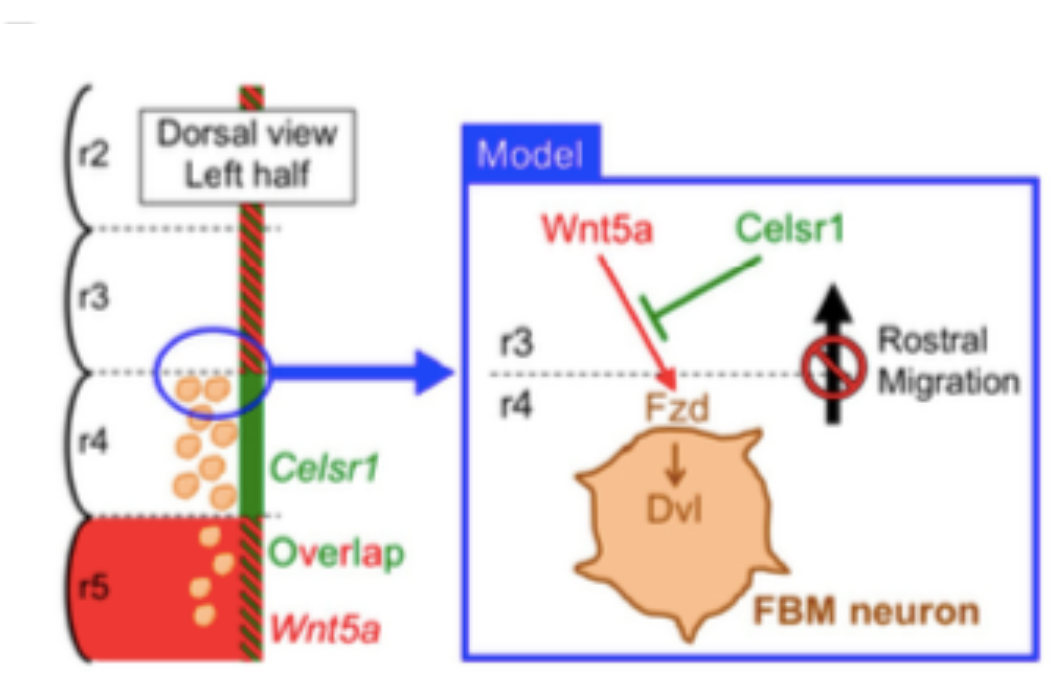
Current Model

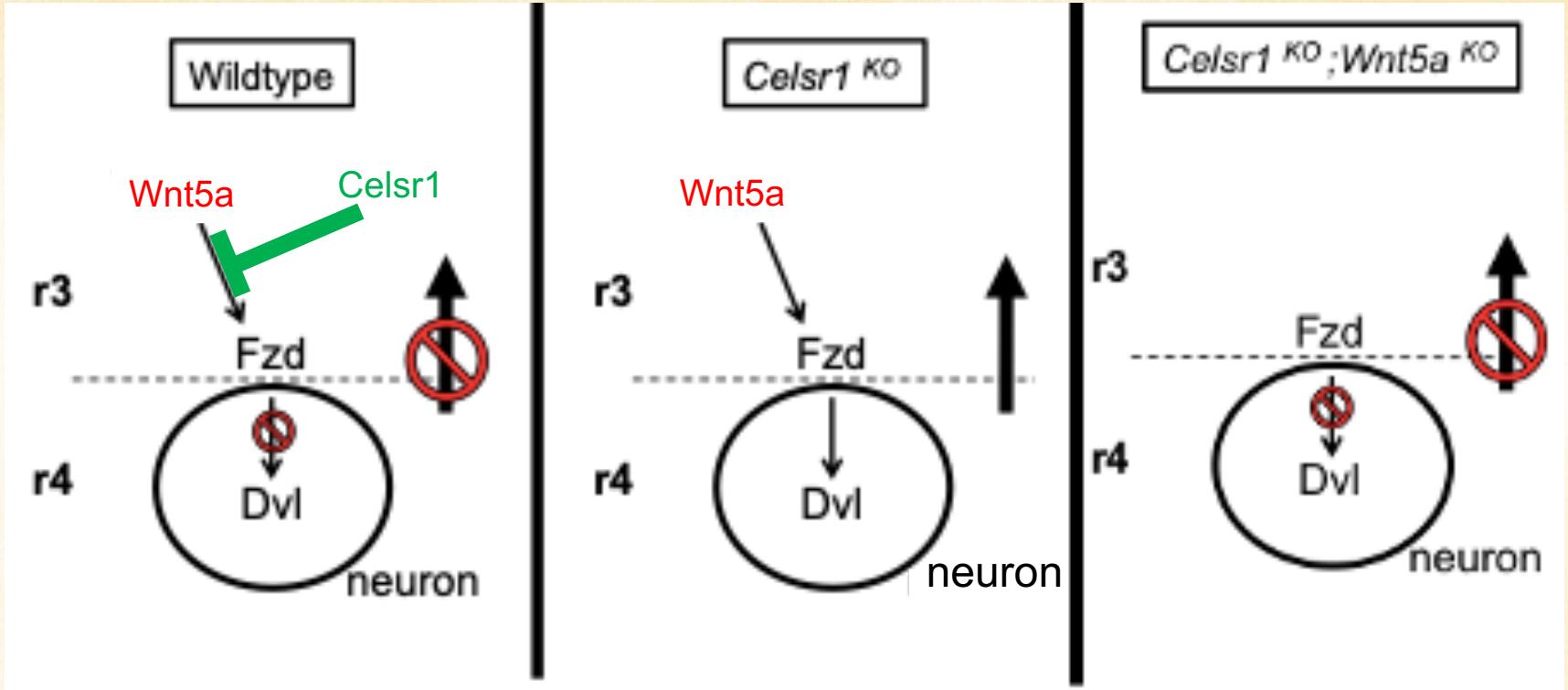
Roles of Celsr1 and Wnt5a in Suppressing Rostral Migration



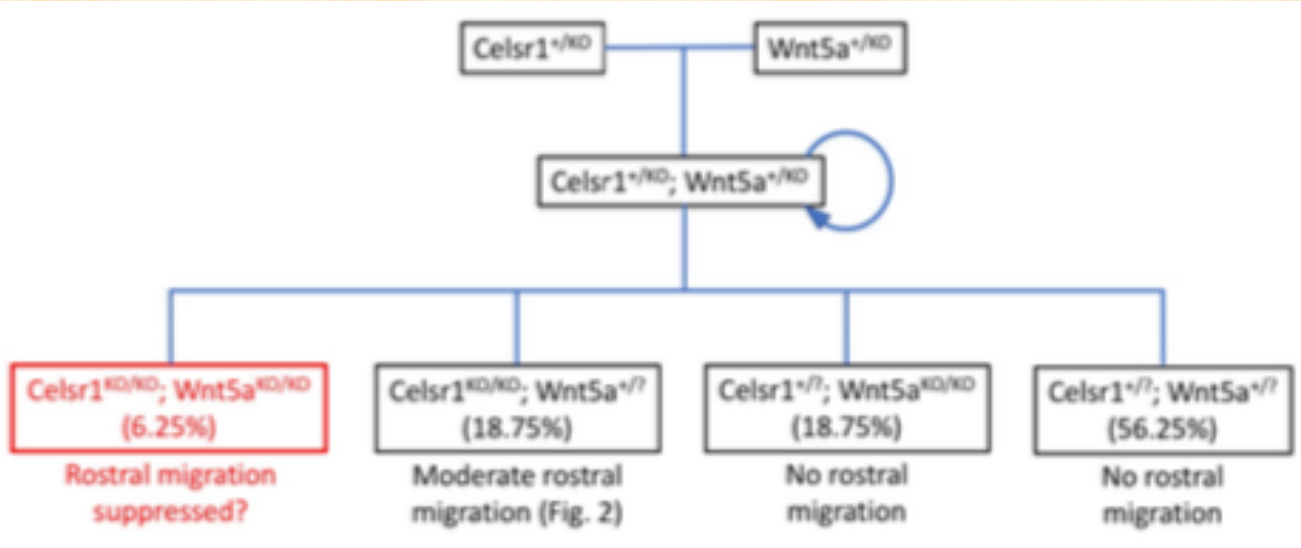
The current model proposes that *Celsr1* prevents rostral migration into r3. As shown in the diagram on the left, it is expressed in the floorplate rather than within neurons.

Celsr1 acts to block *Wnt5a* from inducing rostral migration. Previous experiments have demonstrated that *Celsr1*$^{KO/KO}$ mice exhibit rostral migration, specifically at the r3/r4 boundary.

Rescue of Migration Defect in Double Mutants

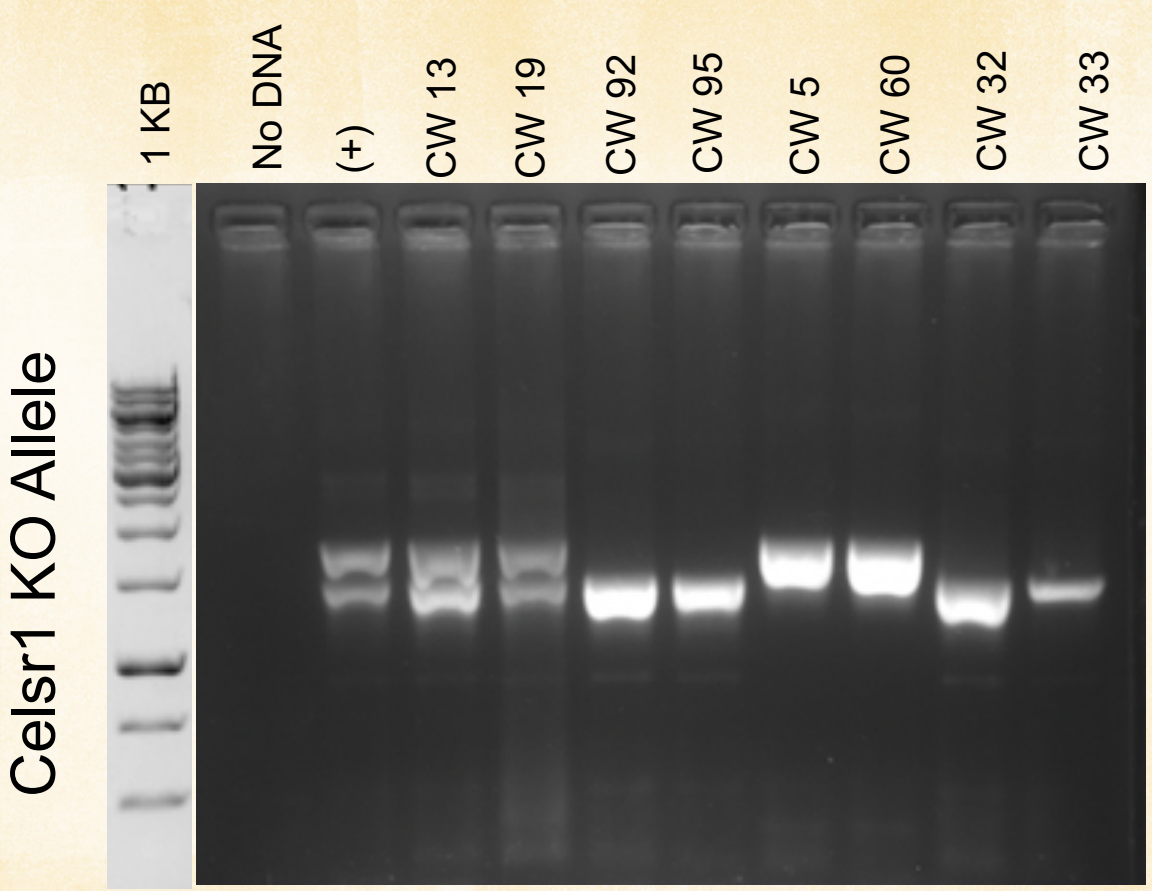


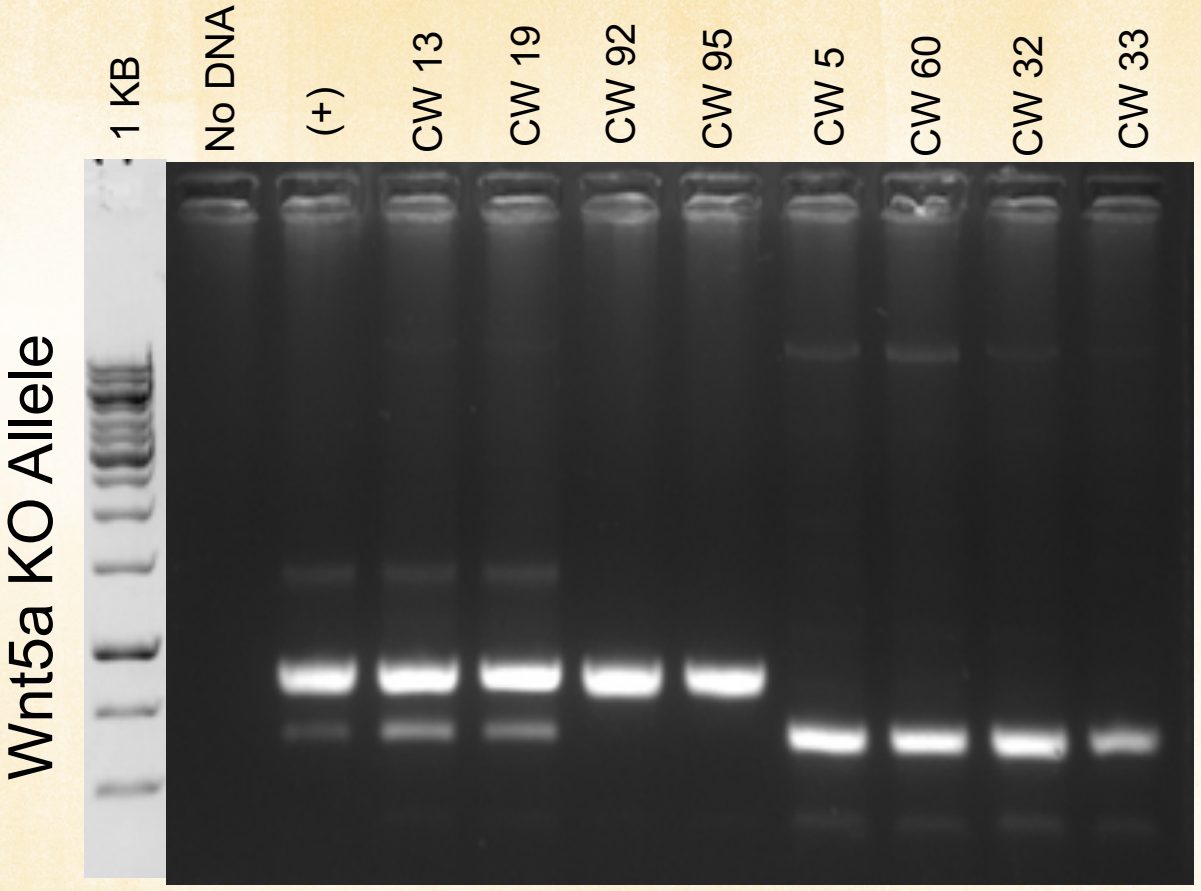
Experimental Crosses to Generate Double Mutant Embryos



Celsr1 and *Wnt5a* single heterozygous lines were crossed to generate double heterozygotes *Celsr1*$^{+/KO}$ *Wnt5a*$^{+/KO}$ mice. Mice were mated to each other in a two-factor cross to produce double mutant embryos.

Genotyping Protocol to Identify Celsr1 and Wnt5a Double Mutants

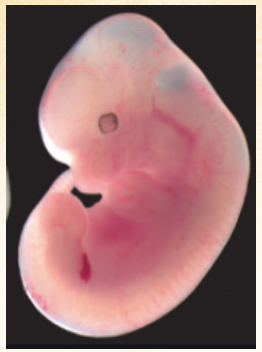




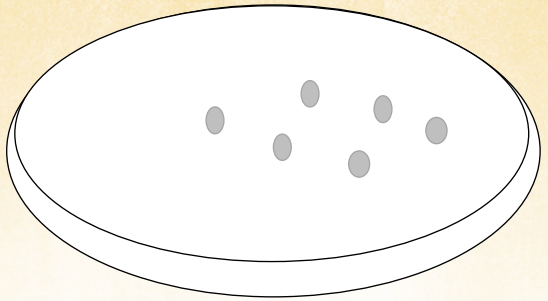
In Situ Hybridization

Technique Used to Examine Neuron Migration Phenotypes

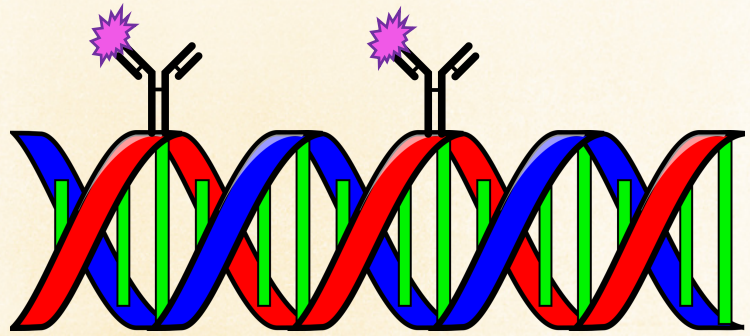
Mate *Celsr1*$^{+/KO}$, *Wnt5a*$^{+/KO}$ mice



Collect E11.5 embryos



RNA probe for in situ

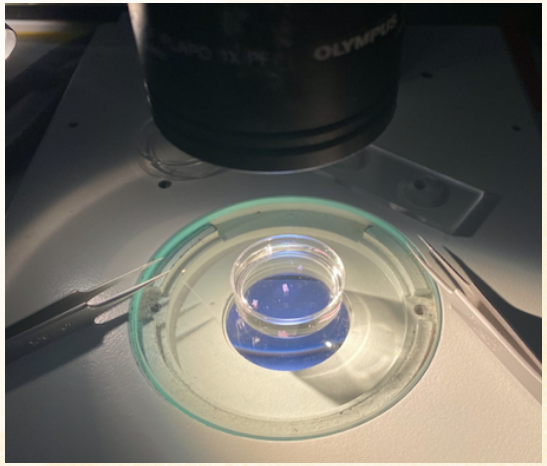


Antibody with fluorescent marker

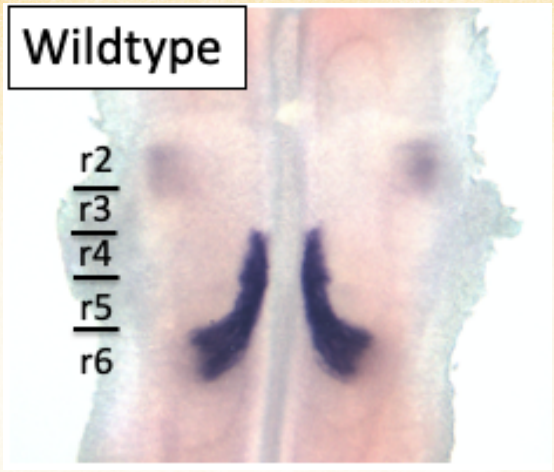
Blue-Target mRNA

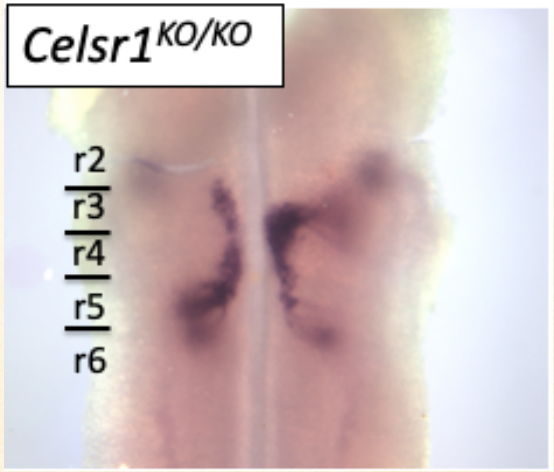
Red-Probe mRNA

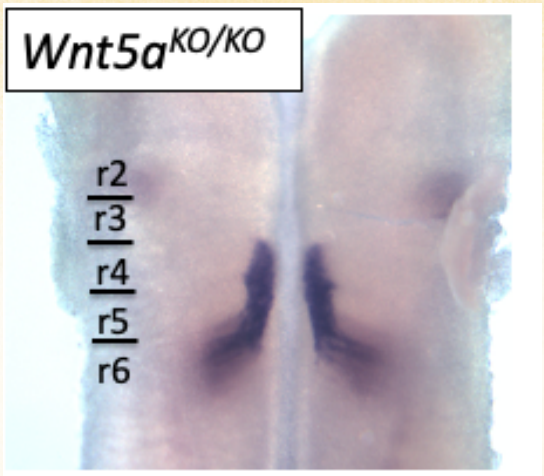
Dissection and Mounting



Rostral Migration of Neurons is Not Seen in Double Mutants



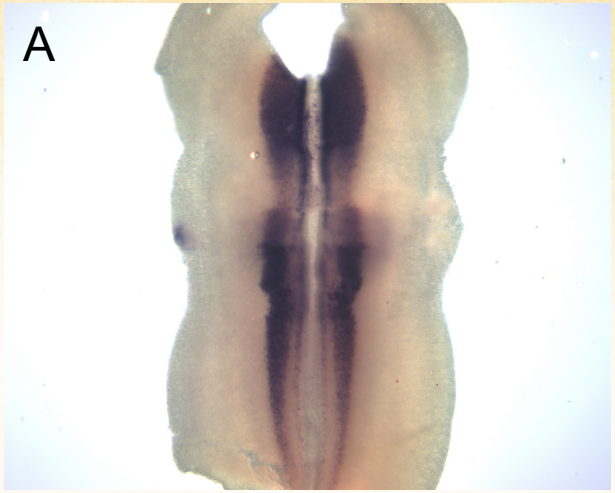


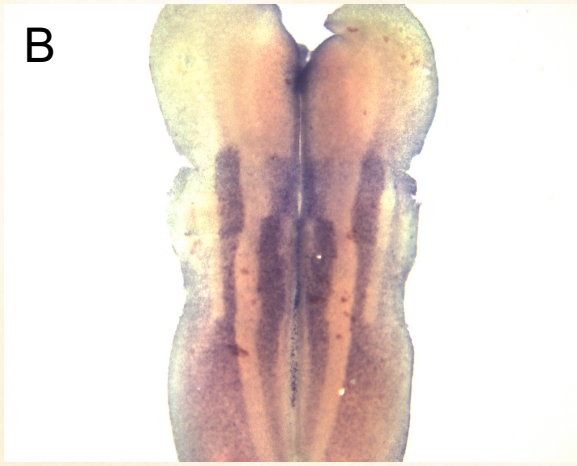


Examining Markers to Ensure Hindbrain Tissues Are Unaffected in Double Mutants

The double mutant embryos generated exhibit an open neural tube phenotype, which could potentially affect the ability of FBM neurons to migrate rostrally. To ensure the hindbrain phenotypes were not affected, the lab will examine the expression patterns of genes expressed in specific hindbrain segments, also known as rhombomeres, in double mutant and control embryos using in situ hybridization

Figure A shows an in situ of the gene GATA3 and figure B is an in situ example of MASH1





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