

## Supplementary Information

**Title: Wnt5a is a crucial regulator of neurogenesis during cerebellum development**

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**Fig. S1**

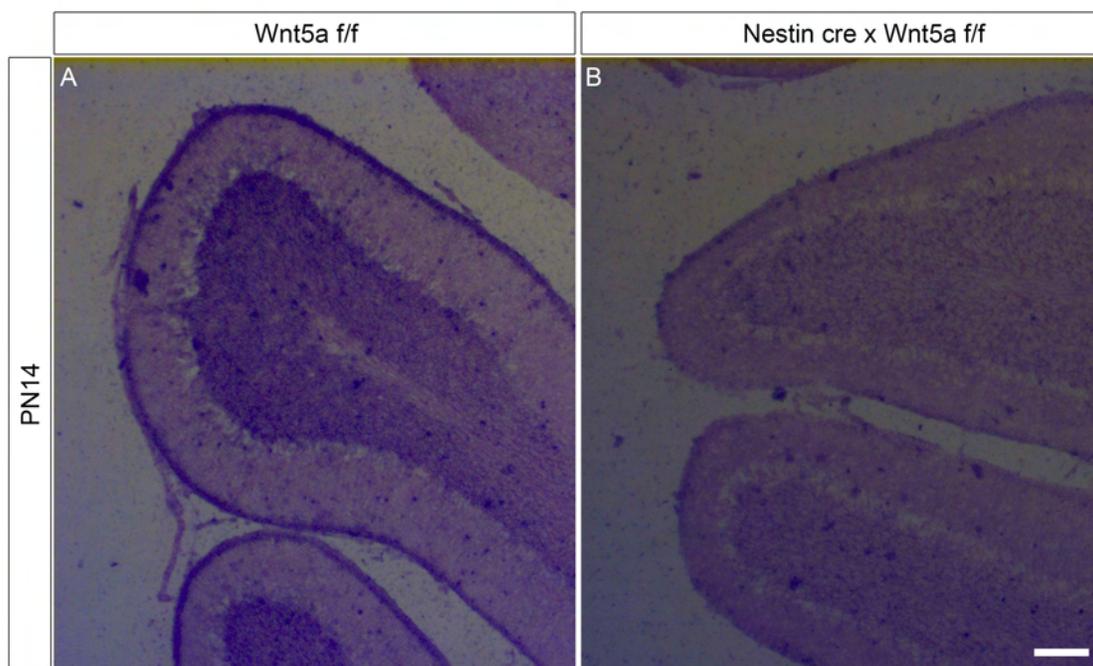


Fig. S2

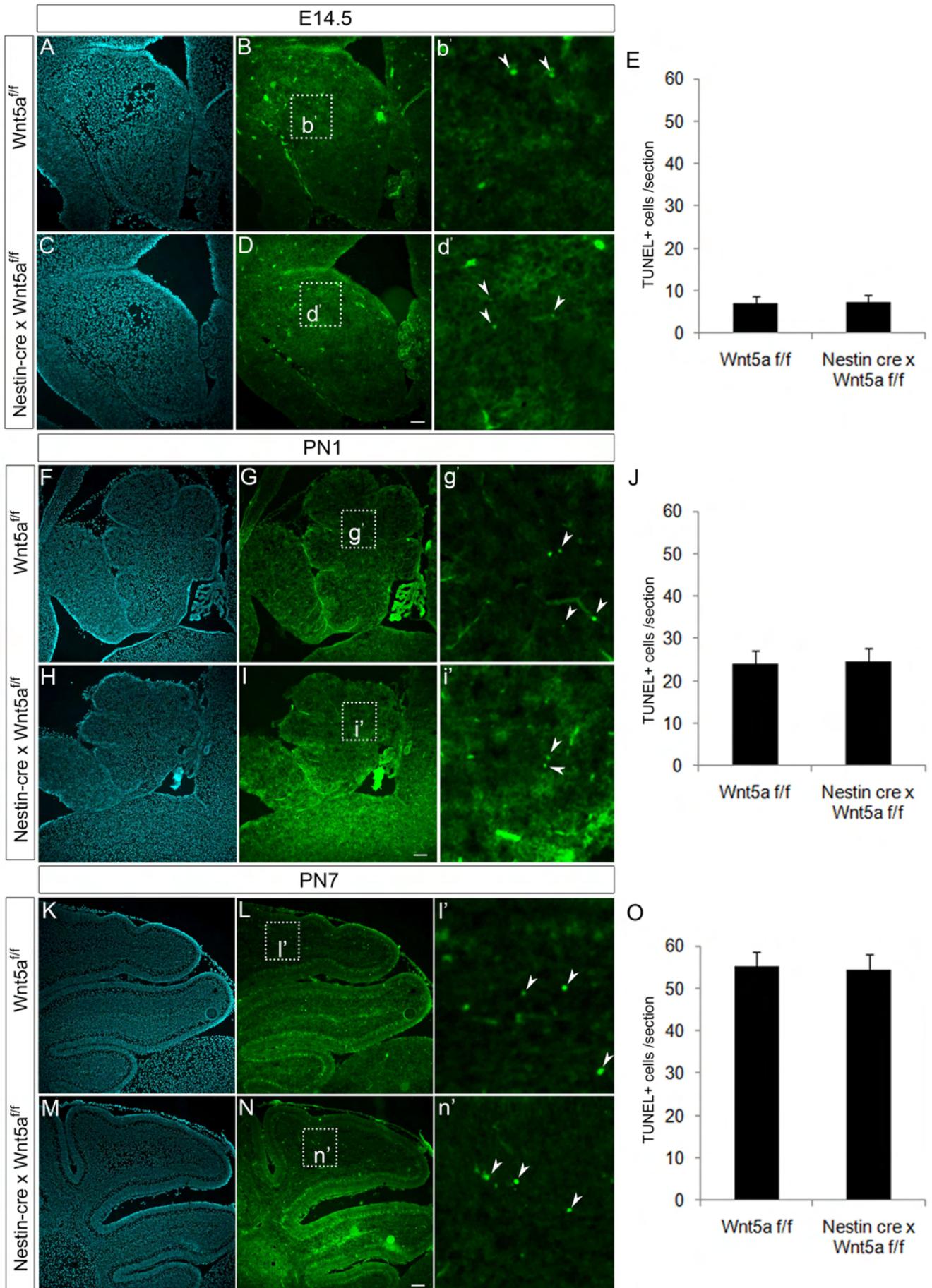


Fig.S3

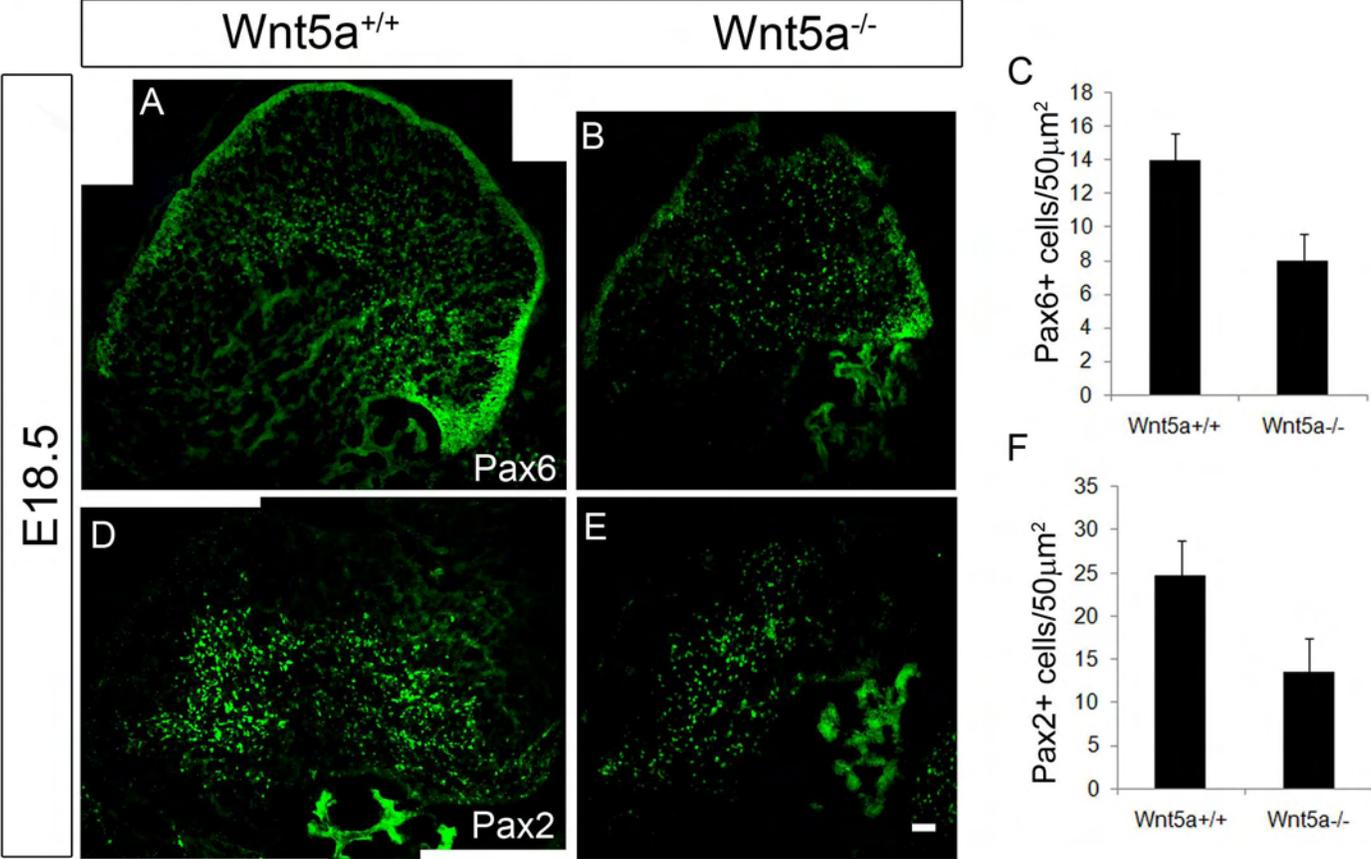


Fig.S4

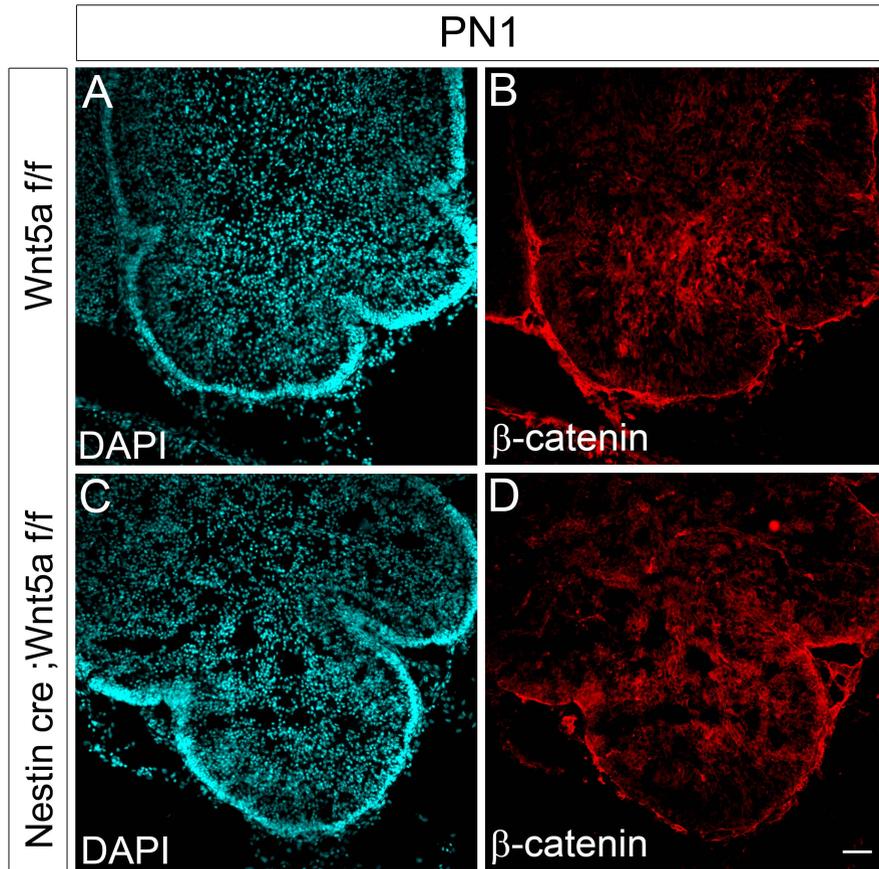
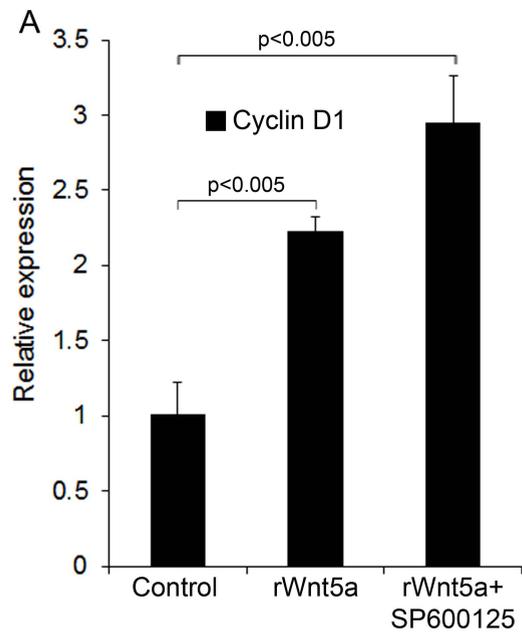


Fig.S5



**Table S1: List of primers used for Quantitative RT-PCR analysis**

<b>Gene Name</b>	<b>Primer Sequence</b>	<b>Ann. Temp (°C)</b>	<b>Product Size (bp)</b>
<b>β-actin</b>	F- AGACTTCGAGCAGGAGATG R- CTTGATCTTCATGGTGCTAGG	56	322
<b>Hes-1</b>	F- TCAACACGACACCGGACAAAC R- TTCATGCACTCGCTGAAGCC	56	295
<b>Hes-5</b>	F- CAAGGAGAAAAACCGACTGCG R- GGTAAGCAGCTTCATCTGCG	56	314
<b>Sox2</b>	F- CCAAGACGCTCATGAAGAAG R- TGGTCATGGAGTTGTACTGC	60	288
<b>Cyclin D1</b>	F- GGAAGTCTTCTTCTGGTGAACAAG R- TGGAGGGTGGGTTGGAATG	58	185

## Supplementary Figure Legend

**Figure S1: Wnt5a expression is lost in Nestin-Cre conditional knockout mutant cerebella.** (A-B) *In situ* hybridization analysis using Wnt5a specific DIG labelled probes indicates loss of Wnt5a expression in Wnt5a cKO (B) as compared to Wt-type controls (A) at PN14 stage. Scale Bar = 50  $\mu$ m.

**Figure S2: Analysis of cell death in control and Wnt5a cKO using TUNEL assay.** (A-E, F-J&K-O) TUNEL assay revealed no significant difference in number of TUNEL<sup>+</sup> cells at E14.5, PN1 and PN7 respectively. (b'&d') Magnified view of selected area in B and C. (g'&i') Magnified view of selected area in G and I. (l'&n') Magnified view of selected area in L and N. Arrowheads indicate the TUNEL<sup>+</sup> cells at each stage. Data expressed as Mean  $\pm$  SD,  $n=3$ . Scale. Bar = 50  $\mu$ m.

**Figure S3: Wnt5a<sup>-/-</sup> null mutants exhibit severe reduction in GABAergic interneuron and granule neuron progenitors.** (A-B) Immunohistochemical analysis using Pax6 antibody showed significant reduction in granule neuron progenitors in Wnt5a<sup>-/-</sup> null mutants at E18.5 compared to Wnt5a<sup>+/+</sup> controls (C) Quantitation of Pax6<sup>+</sup> cells in Wnt5a<sup>+/+</sup> control and Wnt5a<sup>-/-</sup> mutants. (D-E) Immunostaining of Pax2 at E18.5 stage in Wnt5a<sup>+/+</sup> and Wnt5a<sup>-/-</sup> sagittal cerebellar sections. (F) Quantitative analysis indicates significant reduction of Pax2<sup>+</sup> cells in Wnt5a<sup>-/-</sup> mutants as compared to Wnt5a<sup>+/+</sup> control. Data expressed as Mean  $\pm$  SD,  $n=3$ . Scale. Bar = 50  $\mu$ m.

**Figure S4: Wnt5a cKo animals do not exhibit any difference in  $\beta$ -catenin expression.** (A-D) Immunohistochemical analysis using  $\beta$ -catenin antibody did not show any significant difference in expression of  $\beta$ -catenin between PN1 wnt5a<sup>fl/fl</sup> and Nestin-cre;Wnt5a<sup>fl/fl</sup>. Scale. Bar = 50  $\mu$ m.

**Figure S5: Wnt5a does not activate non-canonical JNK signalling during cerebellar development.** (A) Treatment of CGN cultures with rWnt5a and JNK inhibitor SP600125 did not show any down regulation in expression of Cyclin D1 excluding the involvement of non-canonical Wnt5a mediated JNK activation. Data expressed as Mean  $\pm$  SD,  $n=3$ .

**Table S1:** List of primers used