Legends to Supplemental Figures

Supplemental Figure 1. Retinal expression of Fz8 and Fz5 revealed by beta-gal **reporter**, alkaline phosphatase and in situ hybridization. A. Weak expression of Fz8 in neural retina of E13.5 embryo by X-gal staining. Strong expression appears in optic disc (asterisk) and proliferating marginal zone (PMZ). **B-C**, Fz5 is expressed in optic fissure (OF) and neural retina (NR) observed at E12.5 by in situ hybridization. Left panels are Fz5 heterozygous, right panels are Fz5 mutant (Fz5^{CKOAP/lacZ} allele in Sox2-Cre background). The in situ hybridization showed the spatial distribution Fz5 transcripts in retina (left panels) and a high efficiency of Cre excision driven by Sox2 promoter: no detectable Fz5 transcripts were found in the mutant retina (right panels). Antisense probes against Fz5 mRNA were used in both panel B and C. D-E, Fz5 is expressed in adult Müller and Amacrine cells by X-gal and X-phos staining. E, Fz5 is expressed in adult Müller cells (arrow) revealed by X-gal staining. F: A conditional knock-in alkaline phosphatase marker (Fz5^{CKOAP/+)} in Rosa26-CreER background was used to monitor Fz5 expression in adult retina controlled by 4-hydroxytamoxifen injections. Arrowheads point to sparsely labeled Müller and Amacrine cells.

Supplemental Figure 2. Axon sprouting in 6-month old *Fz5^{-/-}* mutant retina. A, NFL stained RGC and HCs axons (green). Note the enhanced OPL and IPL staining and diminished RGC axon bundles in mutant retina (A, C, right panels). B, Brn3a-labeled RGC cells (red) show attenuated staining in mutant retina compared to the wild type. The bright red fluorescence in IPL, OPL and GCL is nonspecific labeling of blood vessels for using mouse primary Brn3a antibody. C, Merged images from A, B and

DAPI.

Supplemental Figure 3. Increased BrdU-labeled cells in mutant retina 16 hr after BrdU injection. A, Merged images from Ki67 (green) and BrdU (red) labeled cells 16 hr after BrdU injection at late E13. More BrdU-positive cells were founded in the mutant inner neural blast layer (INBL) (Right panel, dashed closed lines) and outer neural blast layer (ONBL) than the wild type (Left panel). BrdU signal in ONBL was subjected to ImageJ software detection, while in the INBL was counted cell by cell. Total 9 sections from 3 retinas of 3 independent animals were used for BrdU analysis. Students' t-test obtained P values are less than 0.05 in both INBL and ONBL.

Supplemental Figure 4. IHC detection of p27Kip1 in E13.5 retina and western blot detection of p27Kip and Ccnd1. A, IHC of p27Kip1 shows more apical cells staining in the mutant retina and less INBL staining (mutant shown in right panel). **B**, Western Blot showing slight reduction of p27Kip1 but unchanged Ccnd1 in the mutant retina. w, wild type; m, mutant.

Supplemental Figure 5. Laminin staining of the retinal neuroblasts. A,

laminin staining (green) is reduced in the mutant retina. Note the laminin disposition at E13.5 inversely correlated with neurogenic wave, which spreads from the central to peripheral area. **B**, DAPI staining shows nuclear structures of the same sections from A.



Fz5 ^{CKOAP/+} ; Rosa26-CreER





Fz5^{+/-}

Fz5^{-/-}





Fz5^{+/-};Fz8^{+/-}

R









Fz5^{+/-};Fz8^{+/-}

Fz5^{-/-}; Fz8 +/-

