Supplementary Figure Legends

**Figure S1. Pax6 α-enhancer Cre activity**
A) LacZ staining of control PND21 mouse expressing Pax6 α-enhancer Cre transgene and containing the Gtrosa26tm1Sor (R26R) reporter (Soriano, 1999). Whole mount stained retina is shown to the left, and 4 micron section to the right, showing location of widespread Cre-mediated recombination in the retina periphery. Cre is expressed by E10.5 in mid-far-peripheral retina. In addition to the widespread Cre expression in peripheral progenitors, some scattered LacZ positive cells can be found in the inner nuclear layer and ganglion cell layer of central retina reflecting Cre expression at later stages in retinal development.
B) LacZ staining of Rb/p130 DKO retina at PND31 showing early lesion (arrow) adjacent to hypocellular, degenerative region of LacZ positive cells. Note that the early lesions are present at the extreme periphery of the LacZ-positive region and that multiple lesions appear to be present.

**Figure S2. Rb/p107 DKO retinoblastoma tumor characteristics**
A) Late stage retinoblastoma from Rb/p107 DKO animal at 8 months. Scale bar = 500 microns. Inset, high magnification, scale bar = 60 microns.
B) Immunohistochemical analysis of Rb/p107 DKO lymph node metastases. Tumors are widely positive for syntaxin, exhibit scattered and variable positivity for caretinin and calbindin. Some GFAP positive cells can also be found. Scale bar is 200 microns for low power, and 60 microns for high power inset.

**Figure S3. Late Proliferation and early tumorigenesis occurs at far periphery of retina in Rb/p107 DKO s**
A) H+E of peripheral early retinoblastoma at PND60. Cell loss and disorganization is found in distal retina, where Cre is expressed in progenitor cells by E10.5. At the extreme periphery, early lesions resembling retinoblastomas were found in 4 of 14 eyes. Scale bar = 200 microns.
B) At PND31, cell proliferation was found in Rb/p107 DKO eyes without histological evidence of retinoblastoma. Left, H+E. Optic nerve (O.N.) is indicated, as well as the central retina, where Cre is not expressed at early progenitor stages and normal retina histology is seen. Right, BrdU analysis of the same retina focusing on the mid-far-peripheral region. This region was separated into three bins, 400microns each, with Bin 1 extending from the most distal region of the retina towards central retina. Bin1 and Bin2 are well within the Rb deleted region based on the complete lack of photoreceptor nuclei, and consistent with the examination of α-enhancer Cre-activity in LacZ stained sections from R26R reporter mice. In some sections Bin3 extended into the transition region and should be considered mosaic for Rb deletion. No BrdU positive cells were found in central retina. Note that most proliferation is concentrated at the extreme periphery (black arrows) but occasional BrdU-positive cells are found outside of this region (blue arrows). Shown are two eyes at this stage. Scale bar for H+E is 500 microns, BrdU 200 microns.
C) Quantification of BrdU data from B (n=13 eyes). *p<0.01 comparing Bin 1 to either Bin2 or Bin3 (Student’s T-test). PND31 samples with clear histological evidence
of early retinoblastoma were excluded from quantitative analysis but such early tumors also exhibited far-peripheral localization.

**Figure S4. Quantitative real-time RT-PCR analysis of N-myc expression in murine Rb/p130 and Rb/p107 DKO primary and metastatic retinoblastoma.** Data are expressed relative to the E18 wild-type retina value. The expression levels in normal adult retina is also shown. Each sample was normalized to the levels of actin. Error bars represent standard deviation. Samples with genomic N-myc amplification are indicated (*).

**Figure S5. Quantitative real-time RT-PCR analysis of C-myc and L-myc expression in murine Rb/p130 and Rb/p107 DKO primary and metastatic retinoblastoma.** Data are expressed relative to the E18 wild-type retina value. The expression levels in normal adult retina is also shown. The order of samples graphed is the same as shown for Supplemental Figure 4. Each sample was normalized to the levels of actin. Error bars represent standard deviation.

**Reference for supplemental figure legends**