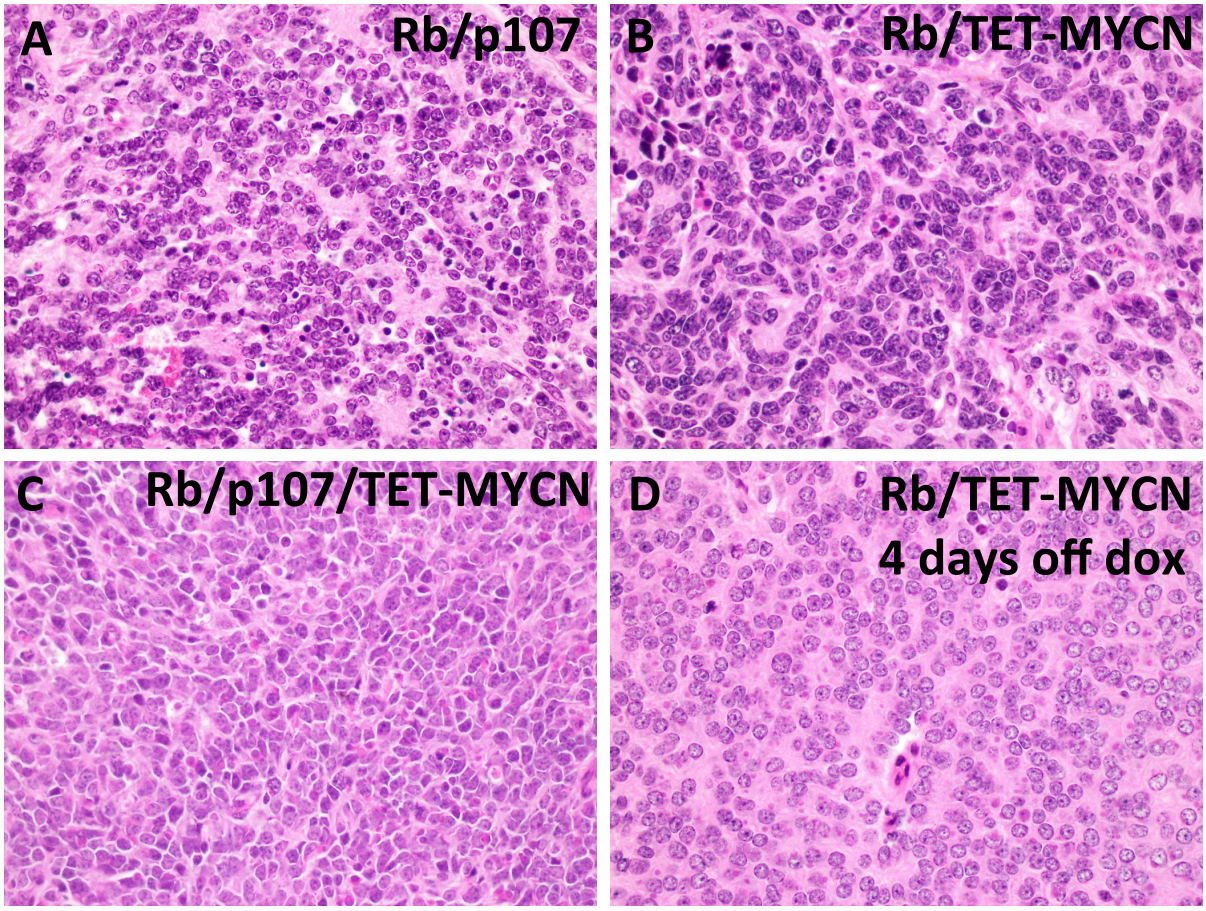
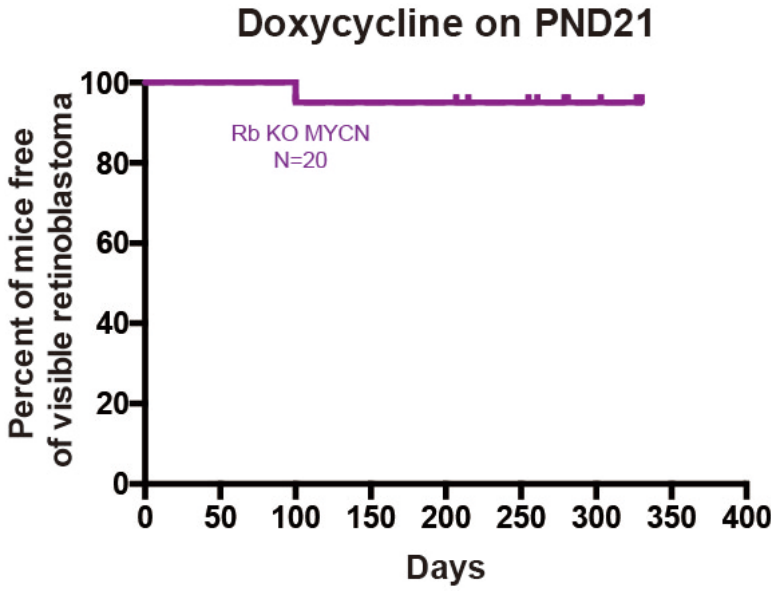
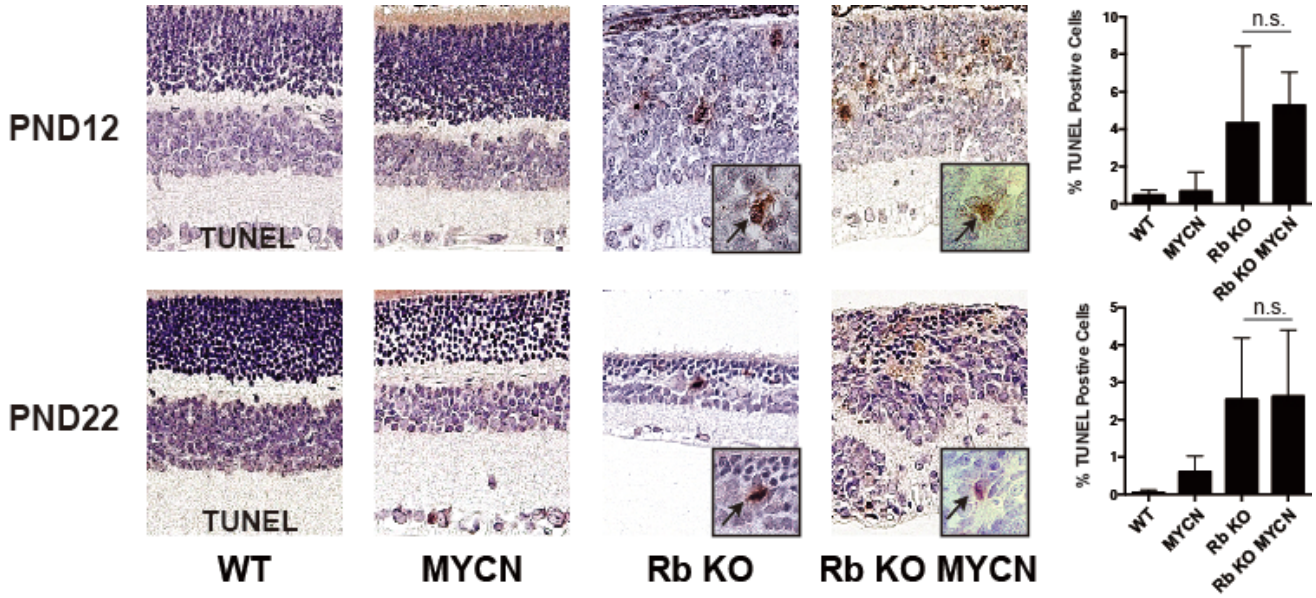


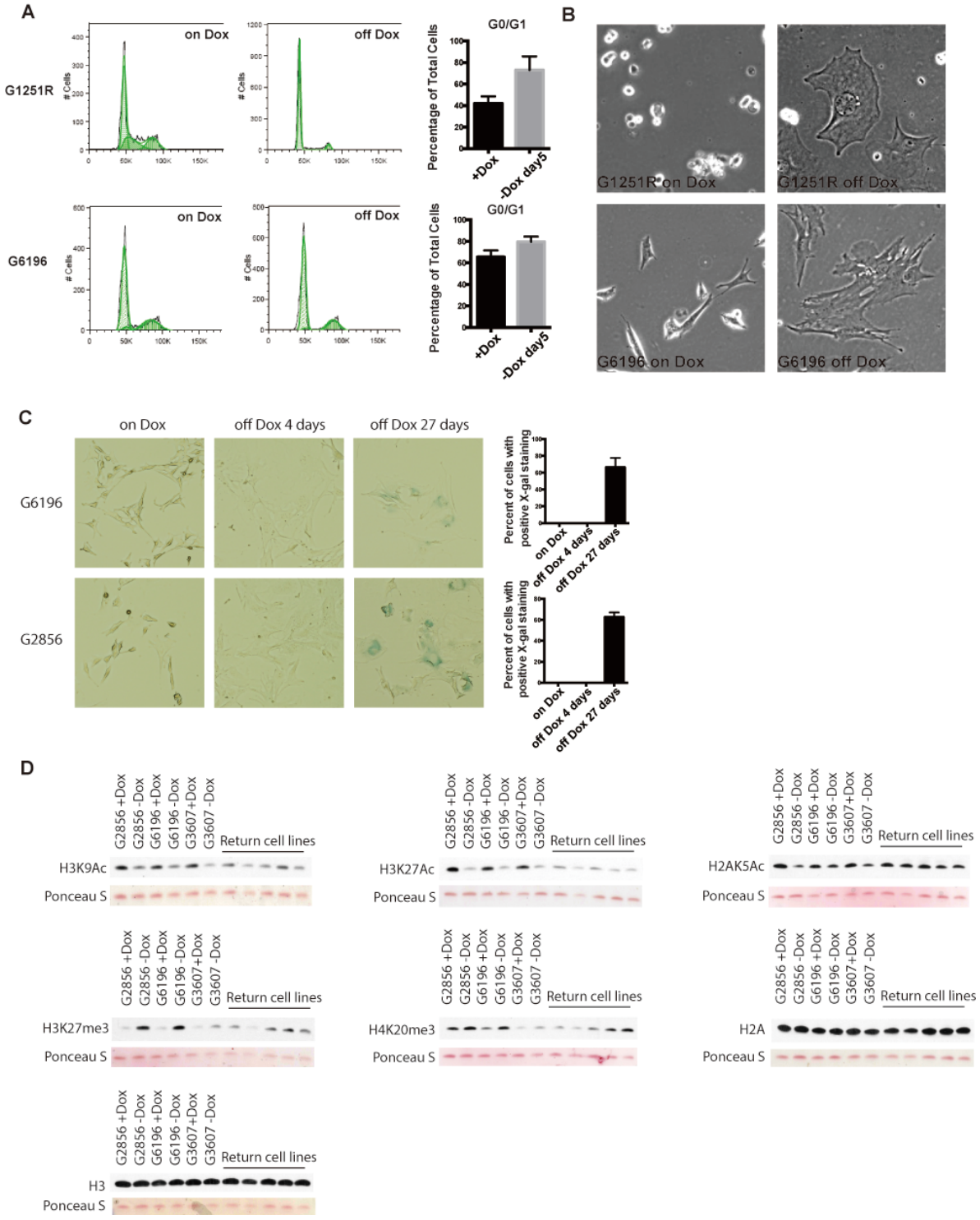
Supplemental Figure S1. ***Increased anaplasia in MYCN-overexpressing mouse retinoblastomas*** **A)** Loss of both *Rb* and *p107* results in the growth of small round blue cell tumors in the eye, morphologically consistent with retinoblastoma. **B)** The combination of *Rb* deletion and overexpression of *MYCN* results in intraocular tumors with somewhat larger and more pleomorphic nuclei. **C)** Anaplasia was even more pronounced in tumors arising in *Rb/p107/TET-MYCN* animals. **D)** 4 days after doxycycline removal, reduction in MYCN expression in *Rb/TET-MYCN* tumors results in tumor cells with more round and regular nuclear morphology, with almost no mitotic figures present. All photographs were taken at original 400X magnification and data are representative of 4 retinoblastomas examined per condition.



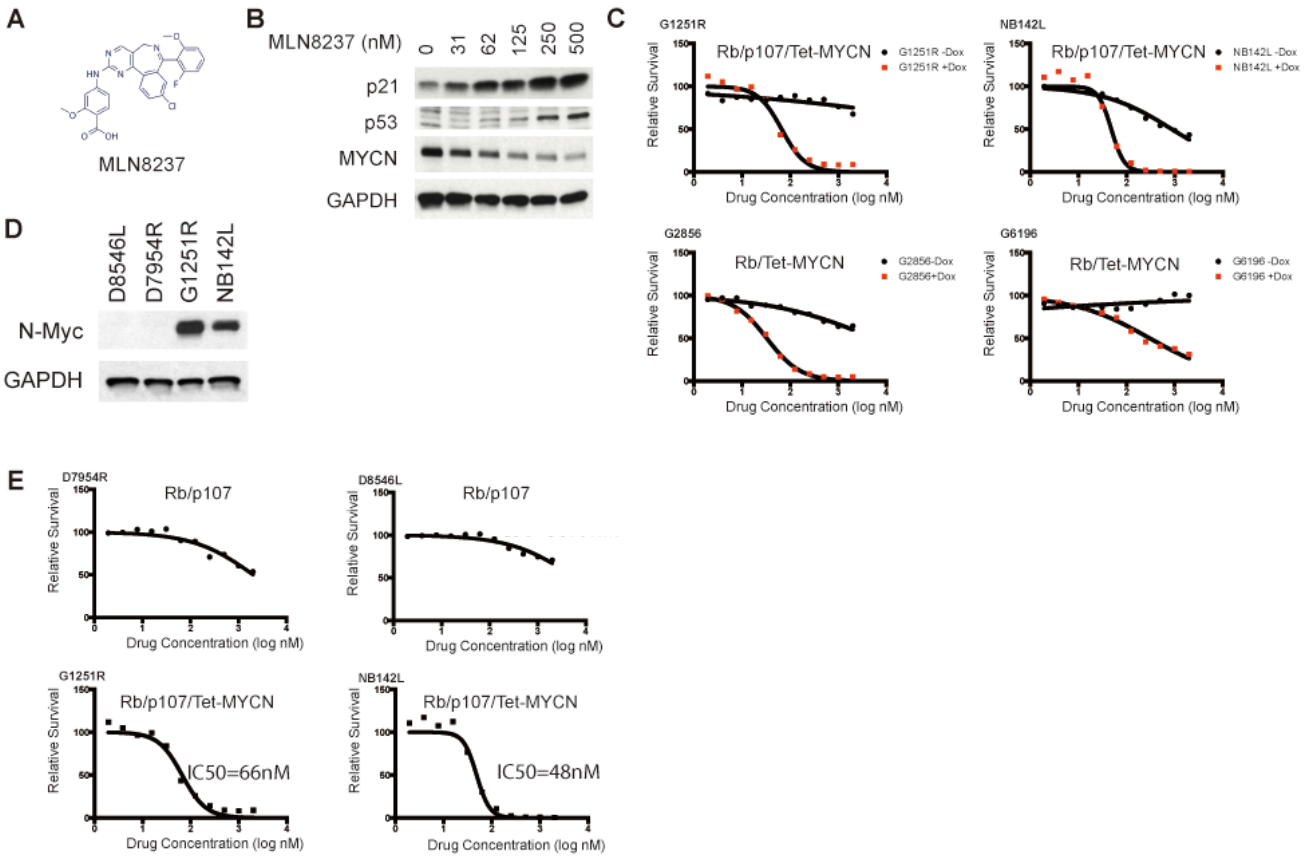
Supplementary Figure S2. ***Retinoblastomas in Rb/TET-MYCN mice when DOX is first given at PND21.*** Kaplan-Meier curve showing time to first appearance of retinoblastoma in the anterior chamber of the eye. *Rb/TET-MYCN-TRE* mice were fed doxycycline containing food beginning on PND21. N=20



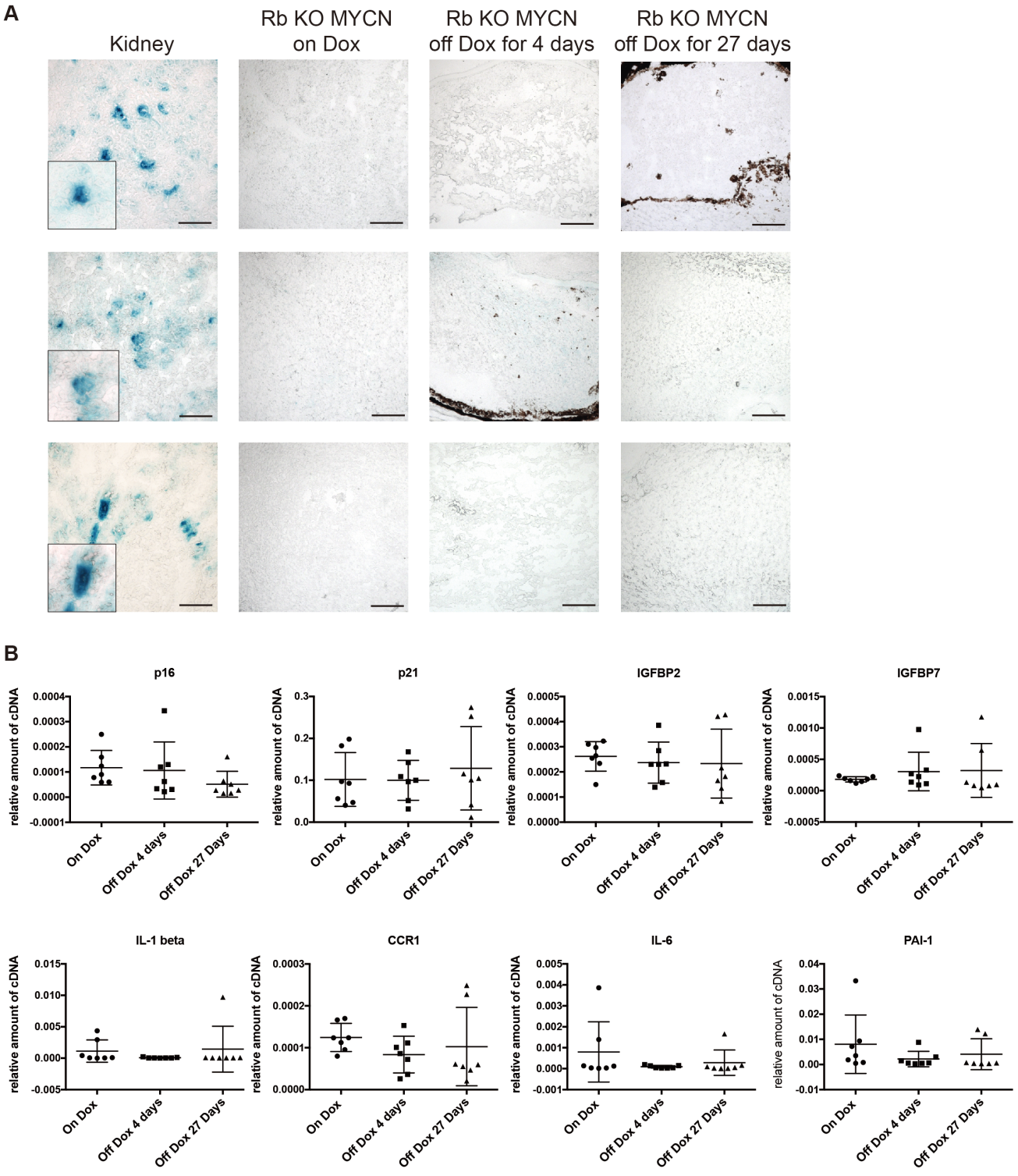
Supplementary Figure S3. ***Histology and quantification of TUNEL analysis showing levels of apoptosis at PND12 and PND22.*** *WT*: n=5; *MYCN*: n=7; *RbKO*: n=4; *RbKO/ MYCN*: n=5 independent retinas. Error bars in figure indicate the standard deviation. n.s. not significant, Student's t-test.



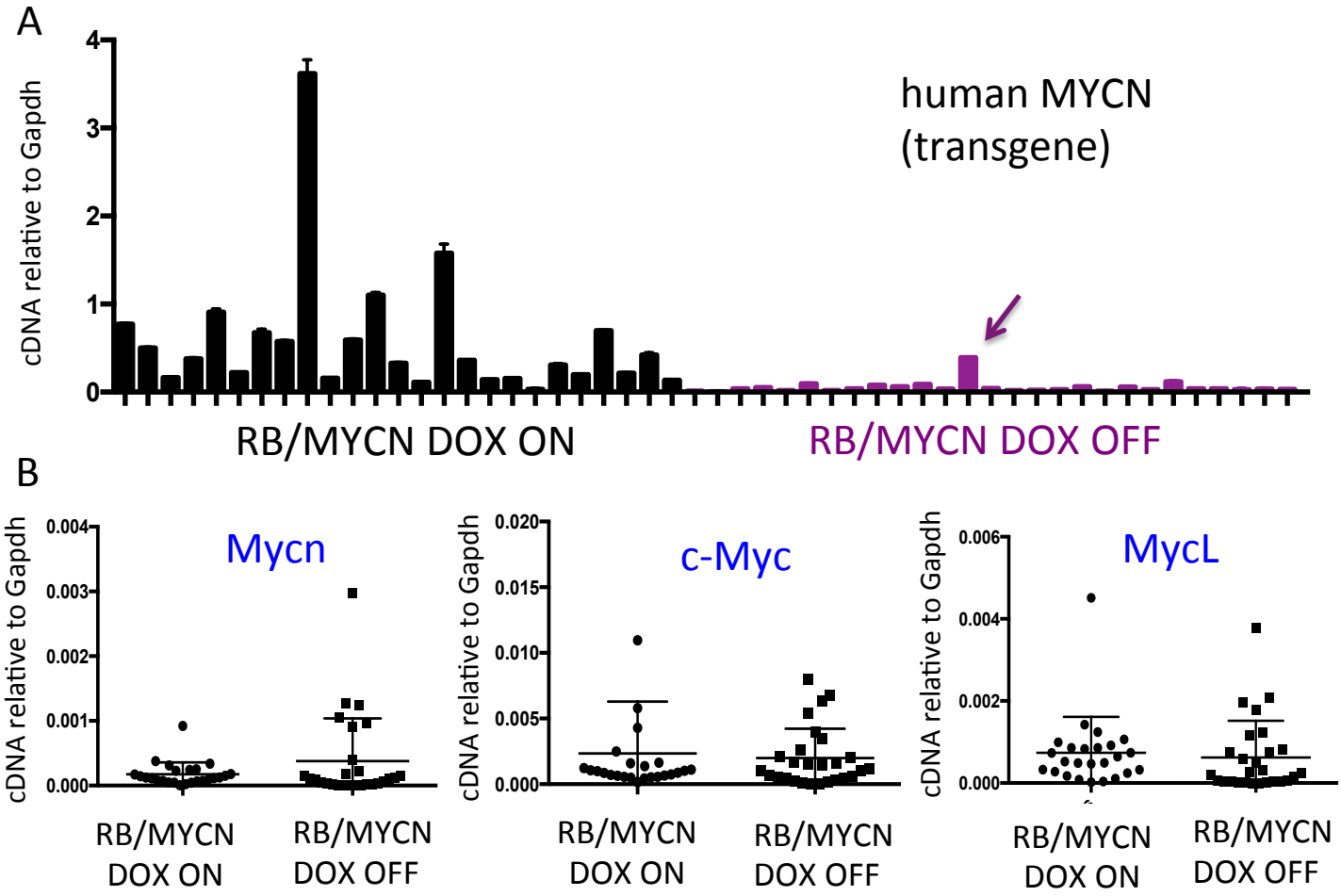
Supplementary Figure S4. ***MYCN inhibition represses retinoblastoma cell growth.*** **A)** *Rb/TET-MYCN* retinoblastoma cells were cultured under doxycycline containing or withdrawal conditions for five days, fixed, stained with propidium iodide, and then analyzed using flow cytometry. Error bars indicate the standard deviation, pooling data from 3 independent experiments with duplicate samples per experiment **B)** Cell morphology of *Rb/TET-MYCN* cells under doxycycline containing or withdrawal conditions. Pictures were taken at seven days post doxycycline withdrawal. **C)** SA-beta gal senescence staining of *Rb/TET-MYCN* inducible cells. Quantification showing average of three experiments is shown to right **D)** Expansion of data from Figure 4C. Western blots showing histone modifications in *Rb/TET-MYCN* retinoblastoma cell lines ON DOX, with DOX removal. Also included for comparison are 5 cell lines derived from retinoblastomas that returned OFF DOX. Ponceau S staining showing loading conditions for each tested histone marker. Note that this figure includes the same western blots as shown in Figure 4C.



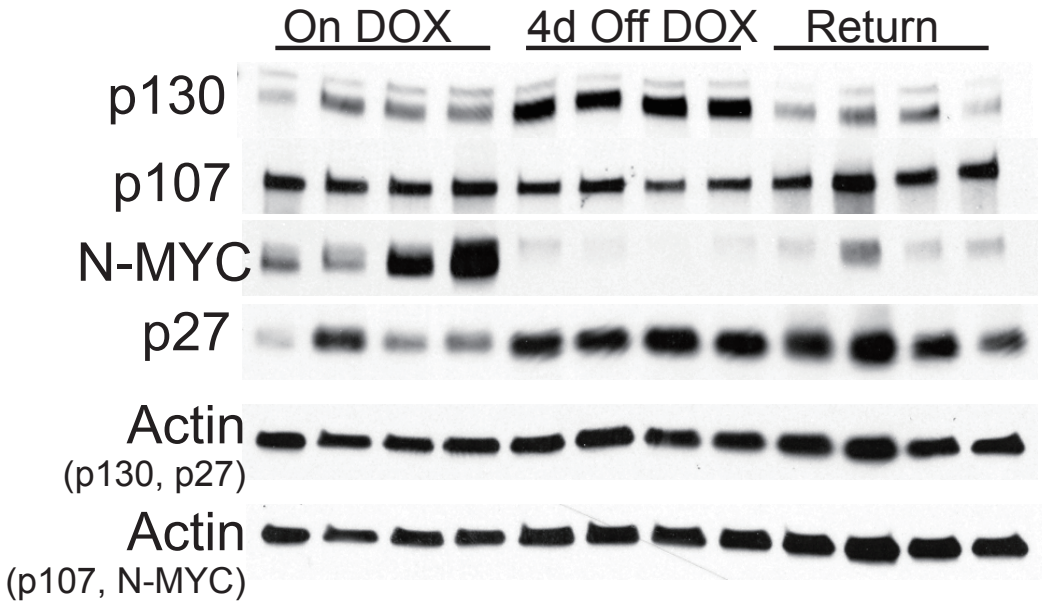
Supplementary Figure S5. ***MYCN overexpressing retinoblastoma cells are sensitive to Aurora A inhibitor MLN8237*** **A)** Chemical structure of MLN8237 **B)** G2856 *Rb/TET-MYCN* cells treated with different doses of MLN8237 for 24 hrs and collected for western blot analysis showing MYCN downregulation and p53 pathway increases. **C)** MLN8237 treatment in two *Rb/p107/TET-MYCN* and two *Rb/TET-MYCN* cells in presence or absence of DOX. Cells were treated with MLN8237 for 72 hrs. CellTiter-Glo was used for quantification of cell viability. Each curve shows the nonlinear regression fit for the average of three independent experiments with triplicate wells. **D)** Western blot showing N-MYC protein levels in cell lines from *Rb/p107* retinoblastomas that arose without MYCN overexpression compared to *Rb/p107/TET-MYCN* cell lines **E)** MLN8237 treatment in two *Rb/p107* and two *Rb/p107/TET-MYCN* cell lines. Cells were treated with MLN8237 for 72 hrs and CellTiter-Glo used for quantification of viability. Each curve shows the nonlinear regression fit for the average of three independent experiments with triplicate wells.



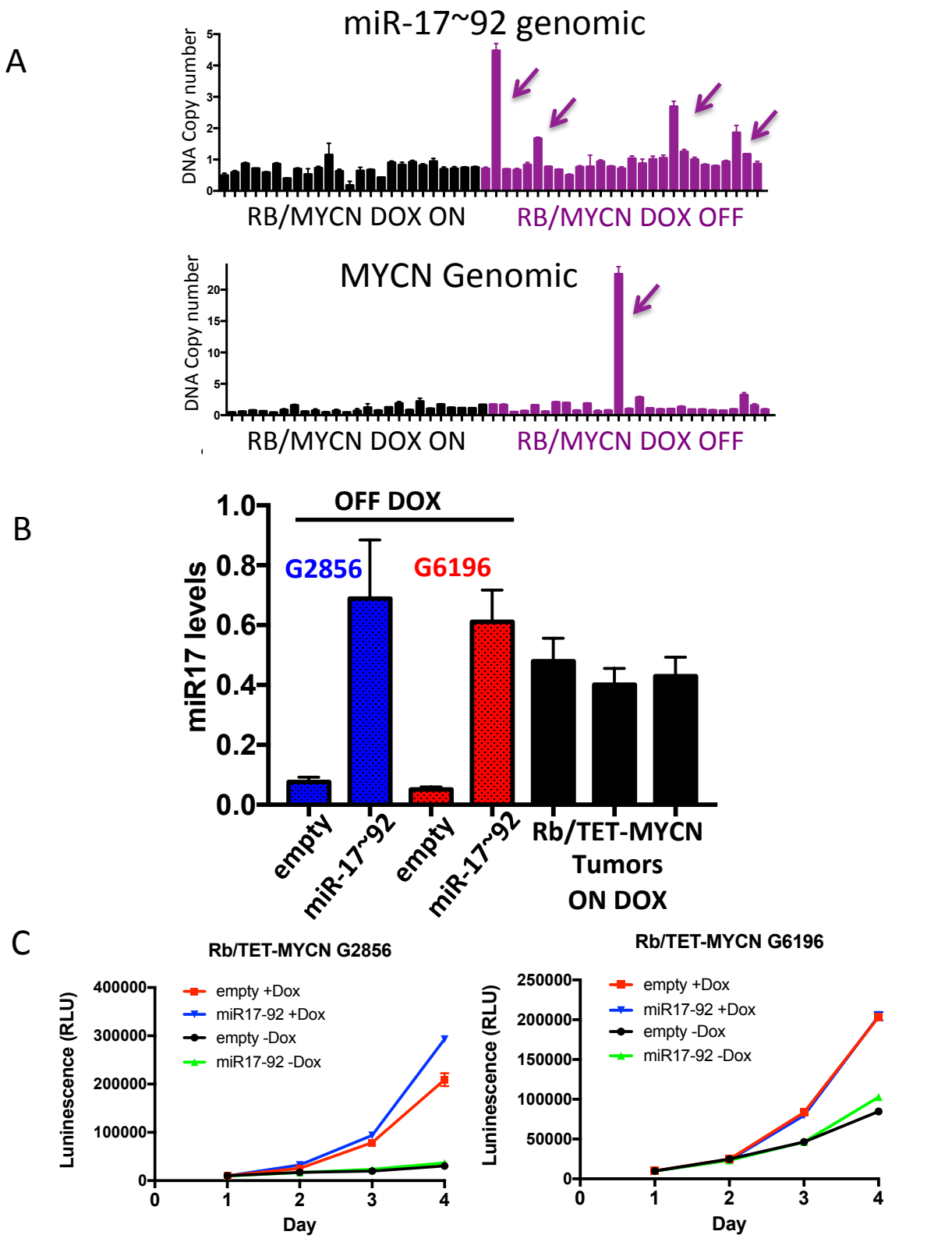
Supplemental Figure S6. ***No evidence of senescence in retinoblastomas following MYCN suppression in vivo*** **A)** Senescence associated beta galactosidase (SA-betaGal). Adult kidneys from 3 aging mice (18-22 months of age) were used as a positive control. Three retinoblastoma bearing eyes ON DOX or at 4 and 27 days after DOX removal are shown. Scale bars: 100 microns. **B)** Real time PCR for a panel of senescence associated markers in *Rb/TET-MYCN* tumors ON DOX, or OFF DOX for either 4 or 27 days. N=7 tumors per group, average and s.d. are indicated.



Supplemental Figure S7. ***Expression of Myc family members in Rb/MYCN retinoblastomas on DOX and tumors that recurred OFF DOX.*** **A)** Human MYCN transgene expression relative to Gapdh as assessed using real time PCR. Arrow points to tumor with reactivation of human MYCN transgene expresson **B)** Murine Mycn, c-Myc and MycL expression levels. Data are normalized to GAPDH. N=25 tumors On Dox, n=27 tumors Off Dox.



Supplemental Figure S8. ***Expression of p107, p130 and p27 in retinoblastomas that return OFF DOX*** Western blot analysis of *Rb/TET-MYCN tumors* ON DOX, 4 days post DOX removal (OFF DOX) and tumors that returned OFF DOX (Return). N=4 tumors per condition. Beta actin is used as a loading control. Data are compiled from two separate blots with actin results from both blots shown.



Supplemental Figure S9. ***miR-17~92 amplification in MYCN independent tumors*****. A)** Real time PCR showing *MYCN* and *miR-17~92* copy number comparing *RB/TET-MYCN* tumors ON DOX and DOX-independent return tumors. ON DOX: n=25, OFF DOX: n=27. Data were normalized to *ANT1* genomic control and expressed relative to tail DNA. **B)** Taqman PCR analysis of miR-17 microRNA expression in two *Rb/TET-MYCN* cell lines transduced with MSCV-I *miR-17~92* or MSCV-empty. Levels of miR-17 upon DOX removal are similar to *Rb/TET-MYCN* tumors maintained On DOX. **C)** Cell Titer-Glo assay showing that overexpression of *miR-17~92* does not rescue the proliferation arrest that occurs upon DOX removal.