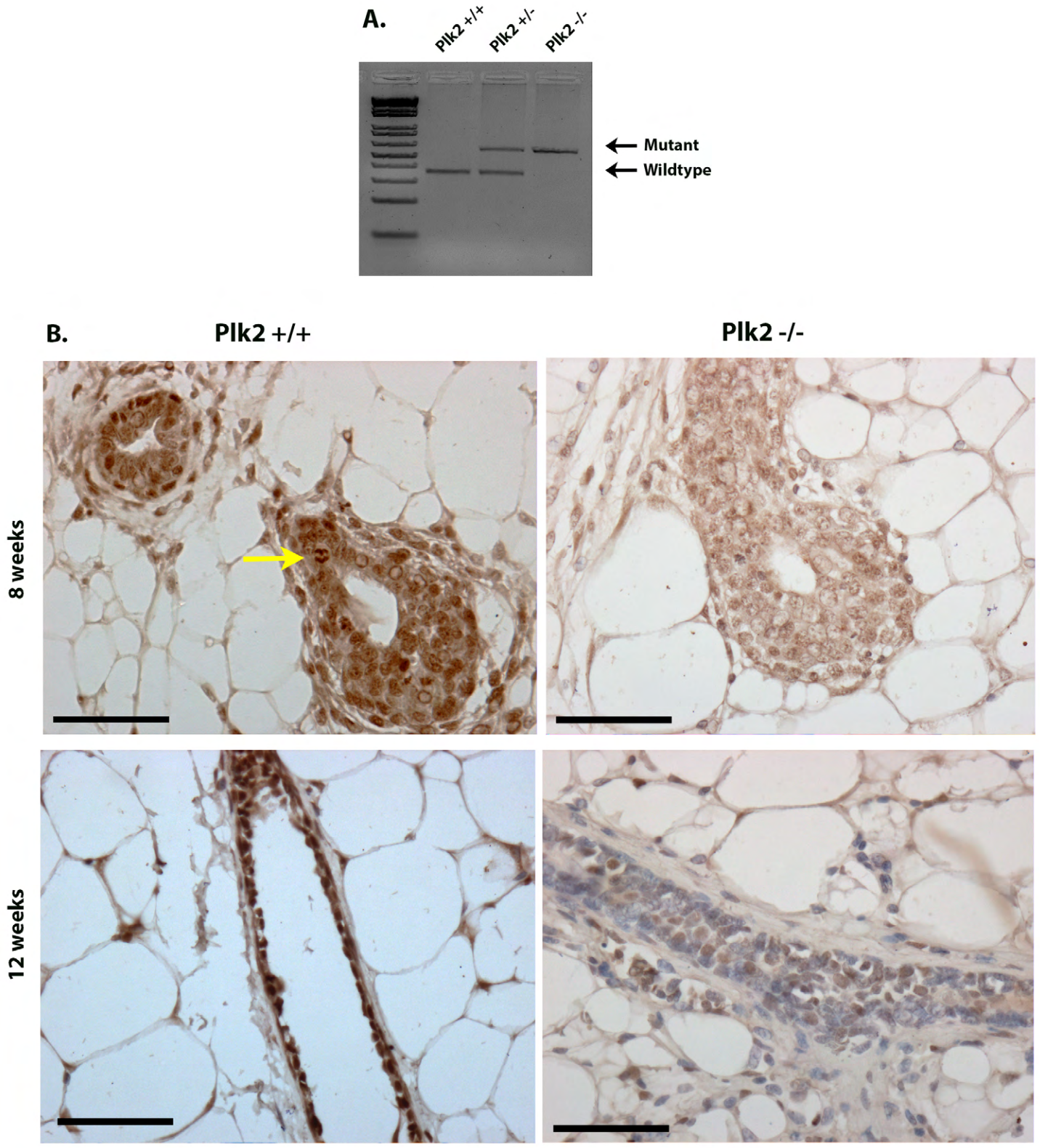
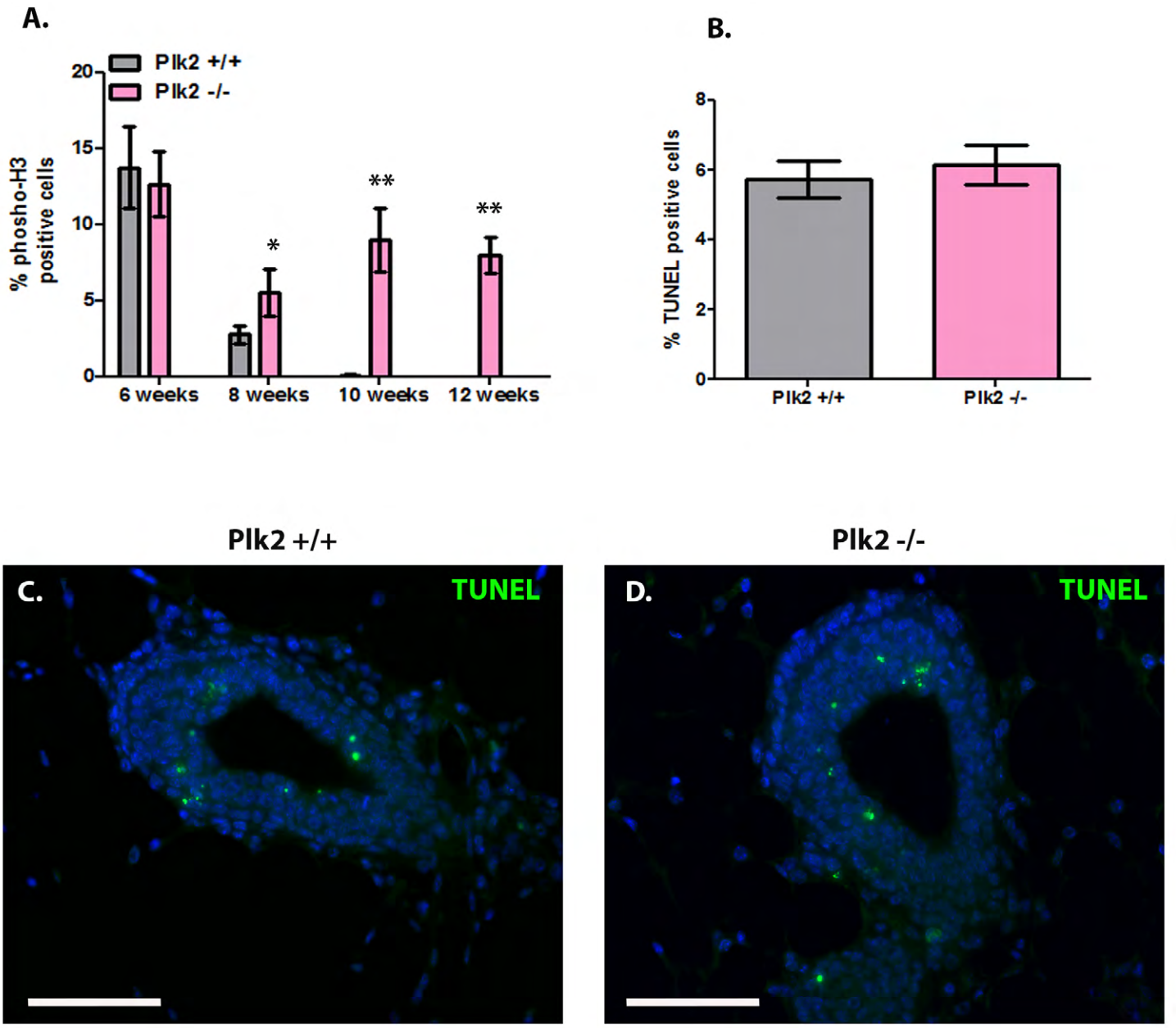
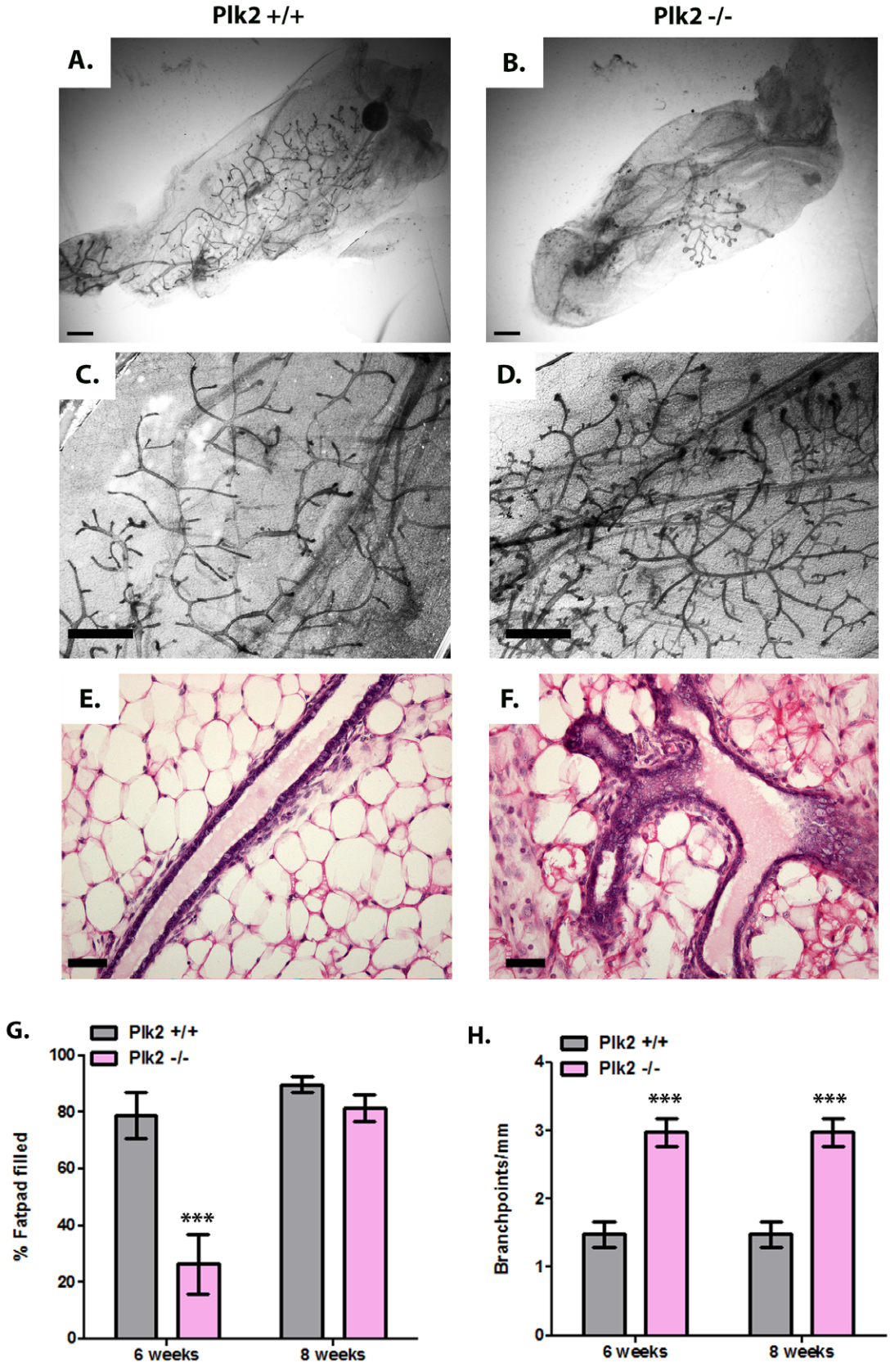


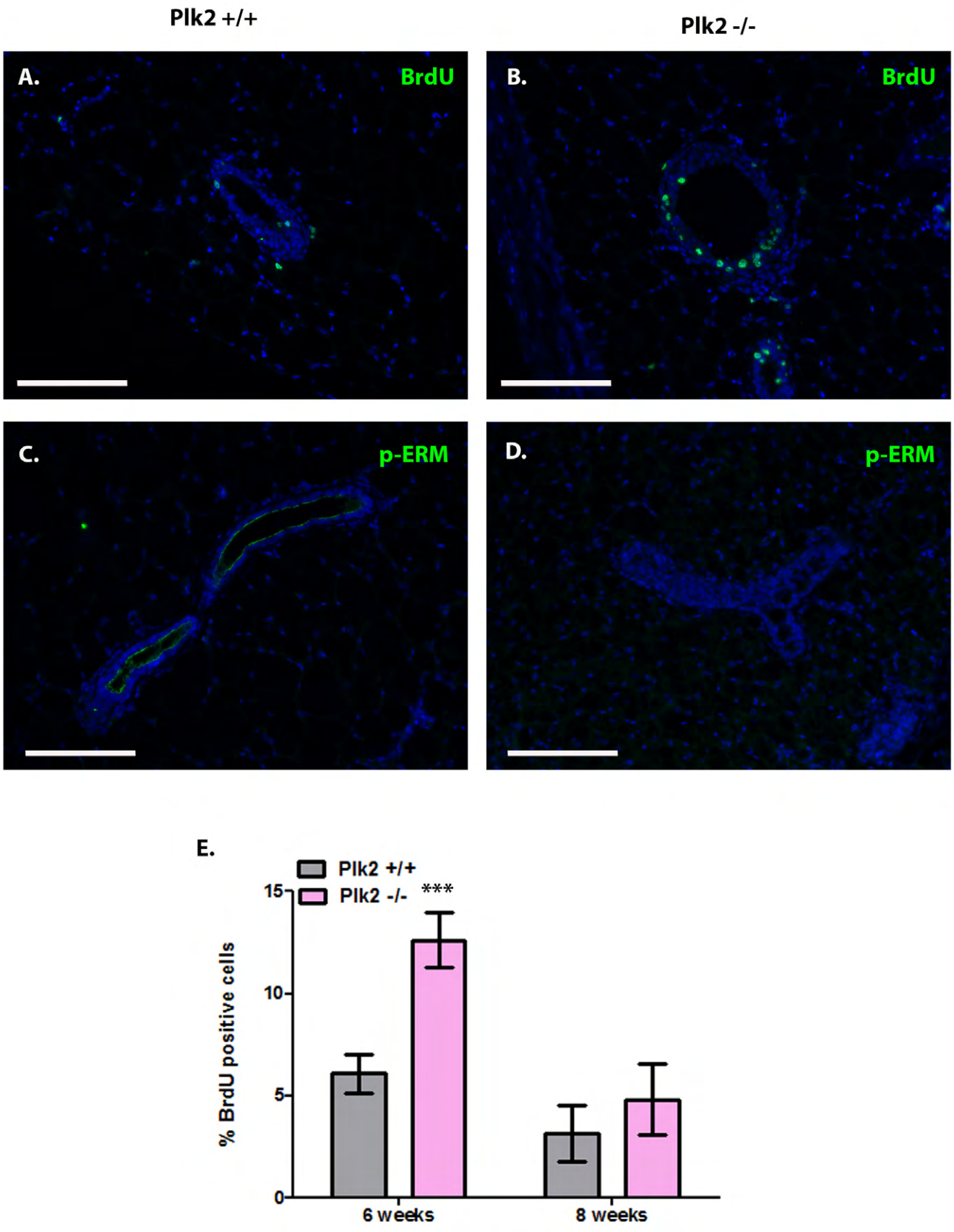
Supplemental Figure 1. Plk2 is expressed in both the luminal and myoepithelial compartments but is more pronounced in mitotic cells. (A) Image of an electrophoresis gel denoting the three genotypes observed using a germline knockout mouse model of Plk2. Two bands of distinct size were observed for the wildtype and mutant allele. **(B)** Immunohistochemistry performed using a Plk2 antibody on mammary glands from 8 and 12 week old mice. Plk2 is ubiquitously expressed in both the luminal and myoepithelial compartments of the mammary gland but is more pronounced in mitotic cells at 8 weeks of age indicated by yellow arrow. Scale bar 40μ.



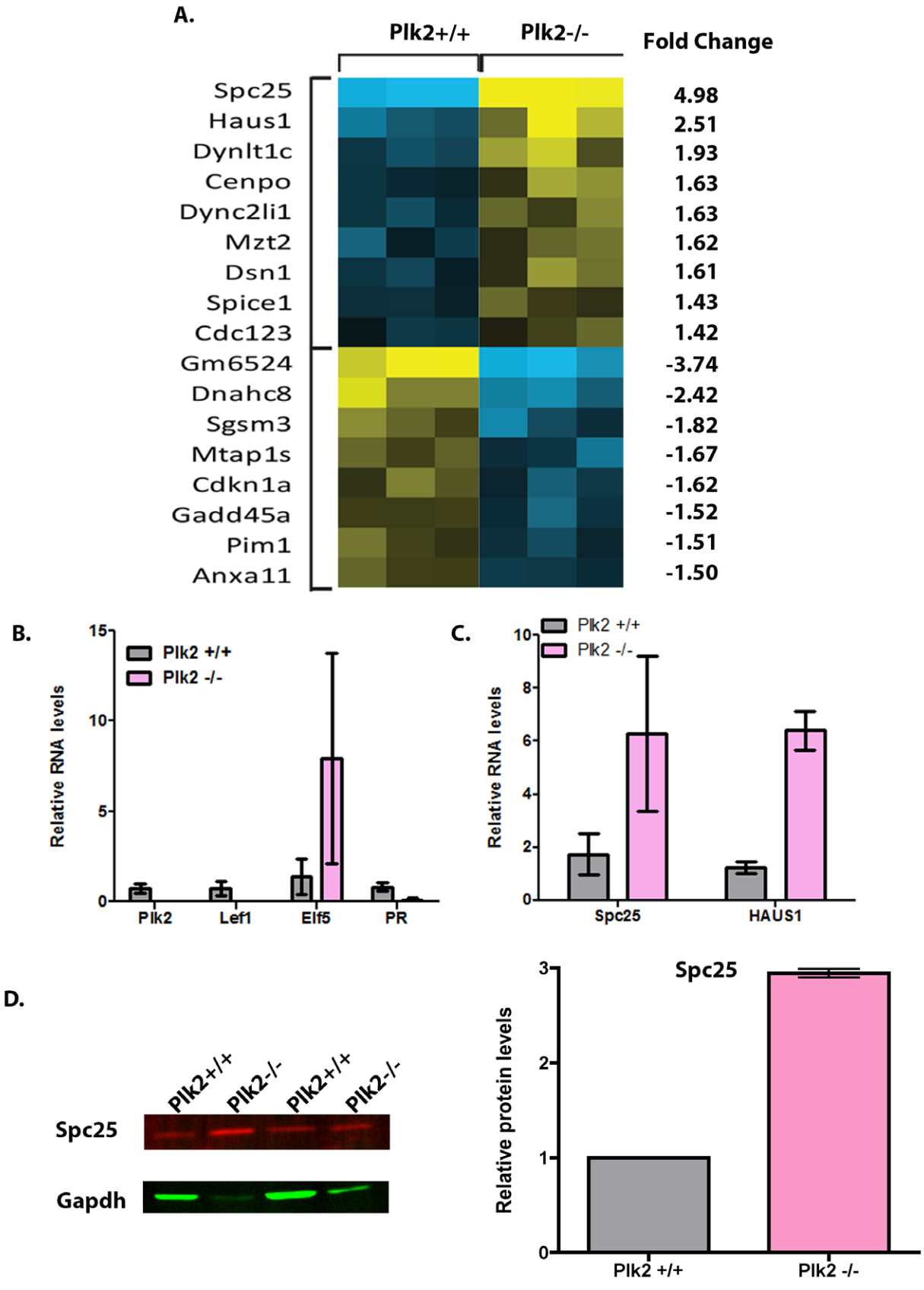
Supplemental Figure 2. Increase in proliferation and no changes in cell death in the absence of Plk2. (A) Proliferation was measured using an additional marker for proliferation p-H3 that stains mitotic cells. A significant increase in proliferation in mammary glands observed in the absence of Plk2 at 8, 10, 12 weeks of age. No observable changes in cell death, **(B-D)** measured by using a TUNEL assay on mammary glands isolated from 8 week old mice. * $p<0.01$, ** $p<0.001$. Scale bar 40µ.



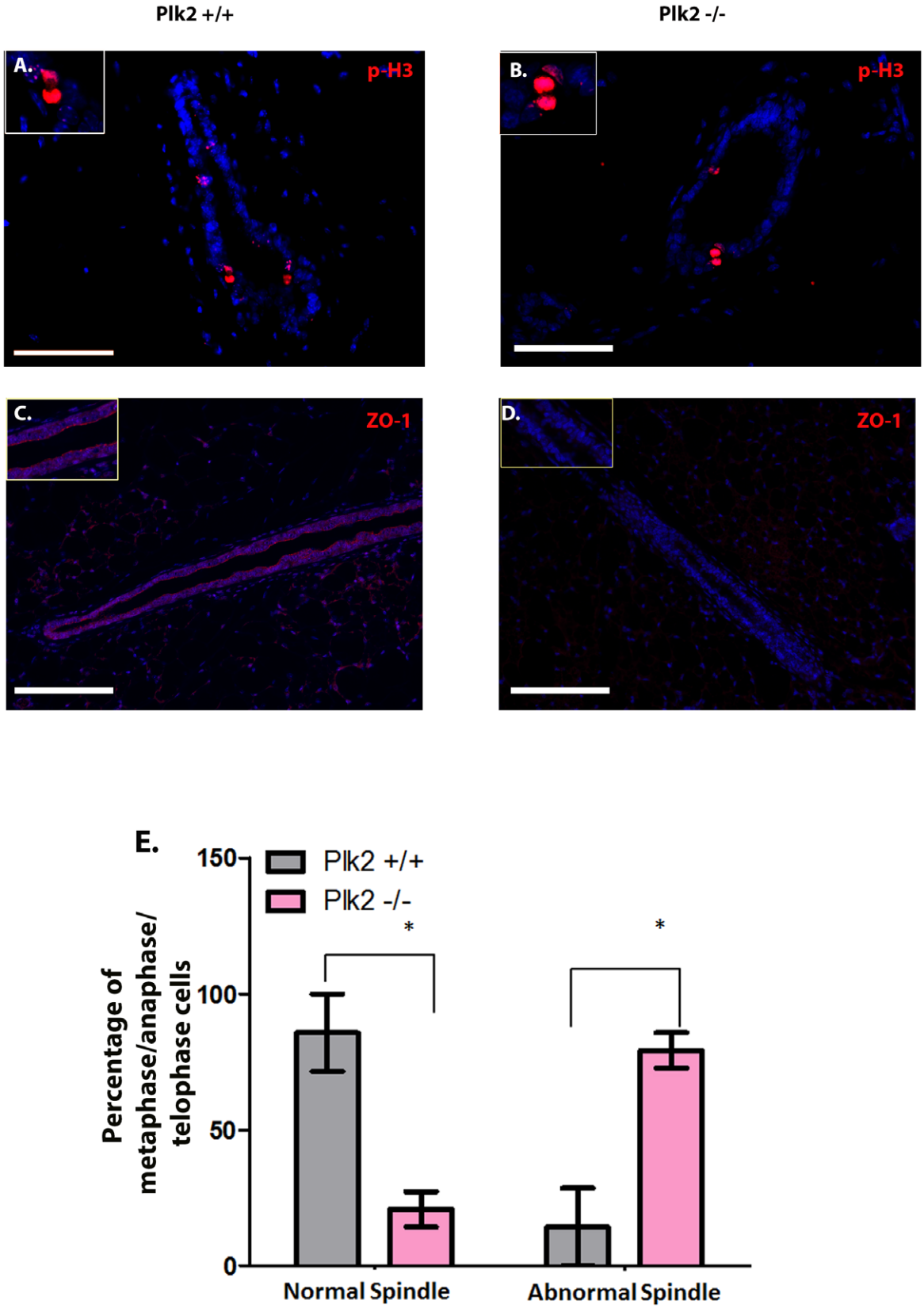
Supplemental Figure 3. Plk2 loss in the epithelium leads to a delay in ductal elongation and an increase in branching. (A,B,G) Carmine stained whole-mount glands of transplants collected 6 weeks post-transplantation reveals a delay in ductal elongation. Ductal development is completed 8 weeks post-transplantation **(G).** Whole- mount analysis **(C,D)** and H&E staining **(E,F)** denote an increase in branching in Plk2 null glands as compared to wildtype glands indicating that the hyperbranching is due to loss of Plk2 in the epithelium. **(F)** Quantitative analysis indicates a statistically significant increase in branchpoints per millimeter of duct in Plk2 null glands present at 6 and 8 weeks post-transplantation. Scale bar 10mm **(A-D).** Scale bar 40μ **(E,F)**. *** $p<0.0001$



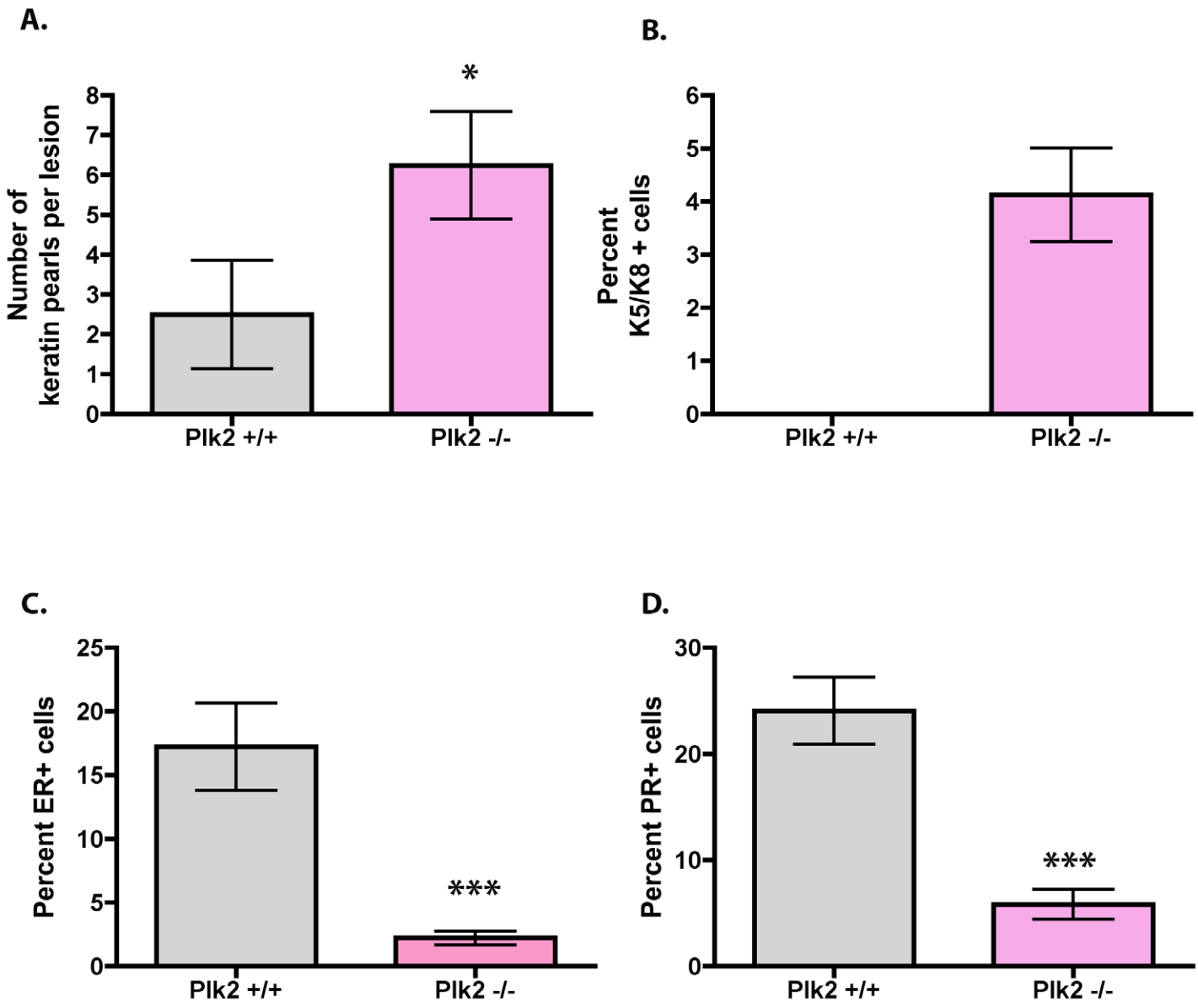
Supplemental Figure 4. Increase in proliferation and loss of apical polarization is a result of Plk2 loss in epithelial cells. Plk2 +/+ and Plk2 -/- mammary gland outgrowths were stained for BrdU incorporation as a marker for proliferation and with p-ERM an apical polarization marker. Loss of Plk2 in the epithelium resulted in a significant increase in proliferation and loss of apical polarization 6 weeks post-transplantation **(A-E).** Scale bar 40µ. *** $p<0.0001$



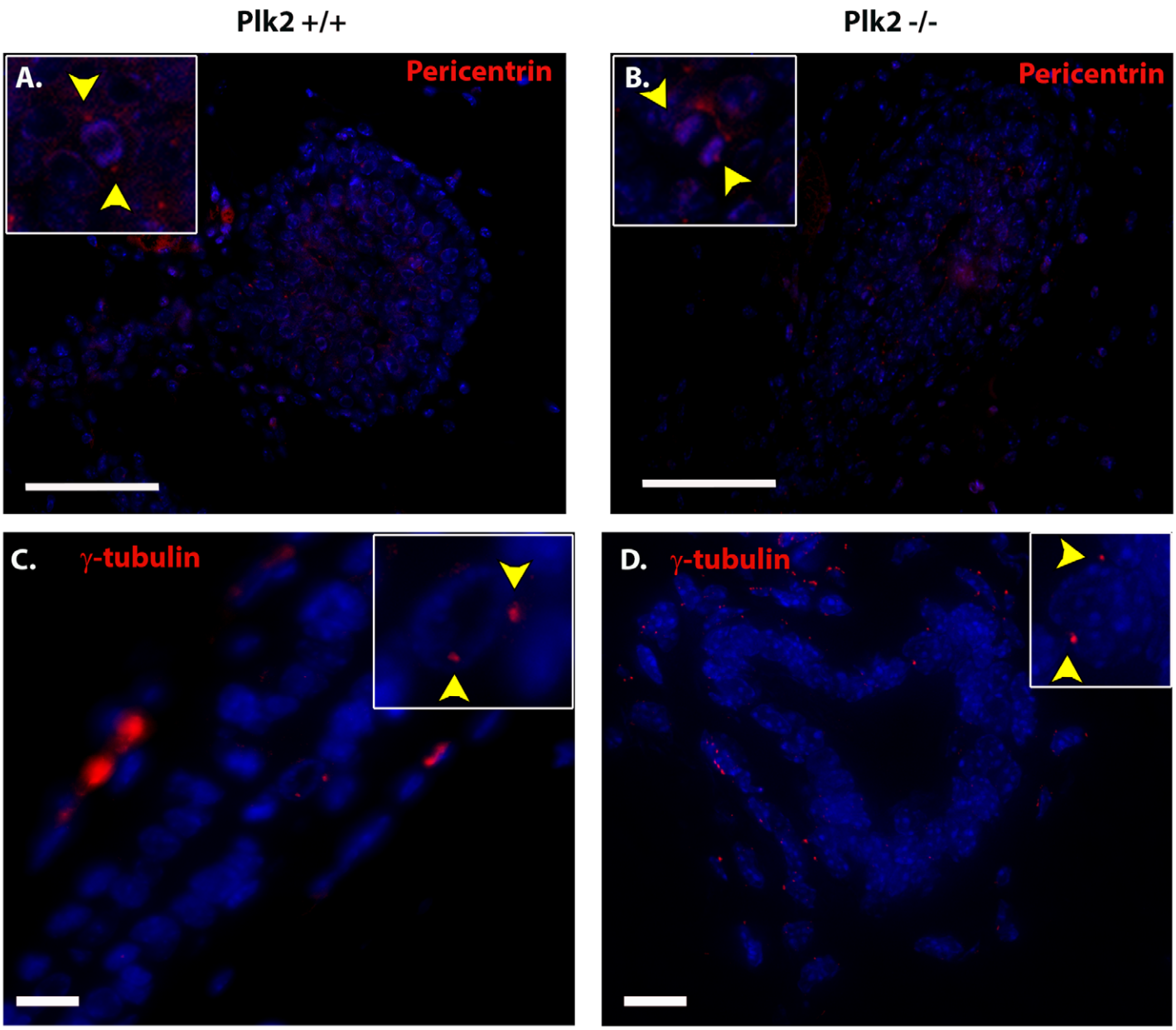
Supplemental Figure 5. Alterations in cell cycle genes upon Plk2 loss. (A) Heat map depicting gene expression alterations in genes involved in cell cycle regulation. Genes involved in establishing the mitotic spindle were found to have an increase in gene expression upon Plk2 loss. **(B,C)** QPCR showing validation of microarrays using established mammary gland genes as well as cell cycle specific genes. **(D)** Plk2 -/- MECs display a 3 fold increase in protein levels.



Supplemental Figure 6. Plk2 regulates the orientation of the mitotic spindle and apical polarization. Immunofluorescence for phosphorylated histone H3 highlight the dividing cells in mitosis. An altered spindle orientation was observed in the absence of Plk2 **(A,B)**. Mitotic cells are dividing parallel to the basement membrane in wildtype glands and perpendicular to the basement membrane in Plk2 null glands. Quantitation of this phenomenon reveals 90% of wildtype cells with normal spindle orientation and nearly 90% of Plk2 null cells with an abnormal spindle orientation **(E)**. Immunofluorescent staining for ZO-1 a marker for apical polarization on Plk2 +/+ and Plk2 -/- mammary glands highlights the loss of apical polarization in the absence of Plk2. Scale bar 40μ. * $p<0.01$



Supplemental Figure 7. Plk2 loss leads to less differentiated lesions and a significant decrease in ER and PR staining. Quantitation for keratinization was performed on Plk2 +/+ and Plk2 -/- multiparous mammary glands using H&E staining and immunofluorescent staining for K5 and K8. Plk2 -/- mammary glands portrayed an increase in keratin pearls per lesion and an increase of positive cells for K5 and K8 **(A,B)**. Quantitation of ER and PR staining reveals a significant decrease in these steroid receptors in multiparous Plk2 -/- mammary glands **(C,D)**. * $p<0.01$, *** $p<0.0001$



Supplemental Figure 8. Loss of Plk2 does not disrupt centriole duplication. (A-B) Plk2-/- epithelial cells have no detectable alterations in duplicating centrioles indicated by pericentrin that stains the percentriolar material and γ-tubulin a marker for centrioles. Scale bar 40μ.