

Supplemental Figures:

Fig. S1: Immunohistochemistry of ErbB2 (A, C), Cyclin D1 (E, G), and pAkt (I, K) in mammary glands of MMTV-neu MMTV-Cre *Jak2^{fl/fl}* mice (C, D, G, H, K, L) and MMTV-neu *Jak2^{fl/fl}* control females (A, B, E, F, I, J) at day 11.5 of pregnancy. Panels B, D, F, H, J, and L show serial sections that were stained without the corresponding primary antibodies. All slides were counterstained with hematoxylin (bar, 50 μ m). Arrows and asterisks in panels A, E, and I indicate epithelial cells within developing alveoli and mammary ducts, respectively.

Fig. S2: The reproductive state modulates the onset of Her2/neu-associated mammary cancer in wildtype controls but not females lacking Jak2. **A.** Kaplan-Meier survival curve (left panel) and mean age of onset of palpable tumors (right panel) in ErbB2/Neu-expressing females that lack Jak2 in alveolar progenitors of primiparous (i.e. single pregnancy; white circles, n=18) and multiparous (black circles, n=9) MMTV-neu WAP-Cre *Jak2^{fl/fl}* females; p-value, t-test. **B.** Kaplan-Meier survival curve (left panel) and mean age of onset of palpable tumors (right panel) in primiparous (white squares, n=9) and multiparous (black squares, n=9) MMTV-neu *Jak2^{fl/fl}* control females; p-value, t-test.

Fig. S3: Histological analysis of Her2/neu-expressing mammary cancer cells lacking Jak2 and their Jak2-expressing isogenic controls. **A.** Hematoxylin/Eosin (H&E) staining (upper panels) and immunohistochemistry against Ki67 as cellular marker for proliferation (lower panels; bar, 200 μ m); arrows indicate mitotic figures. **B.** Quantitative analysis of Ki67-positive cells. **C.** Quantitative analysis of cell density (mean and \pm S.D) within a defined

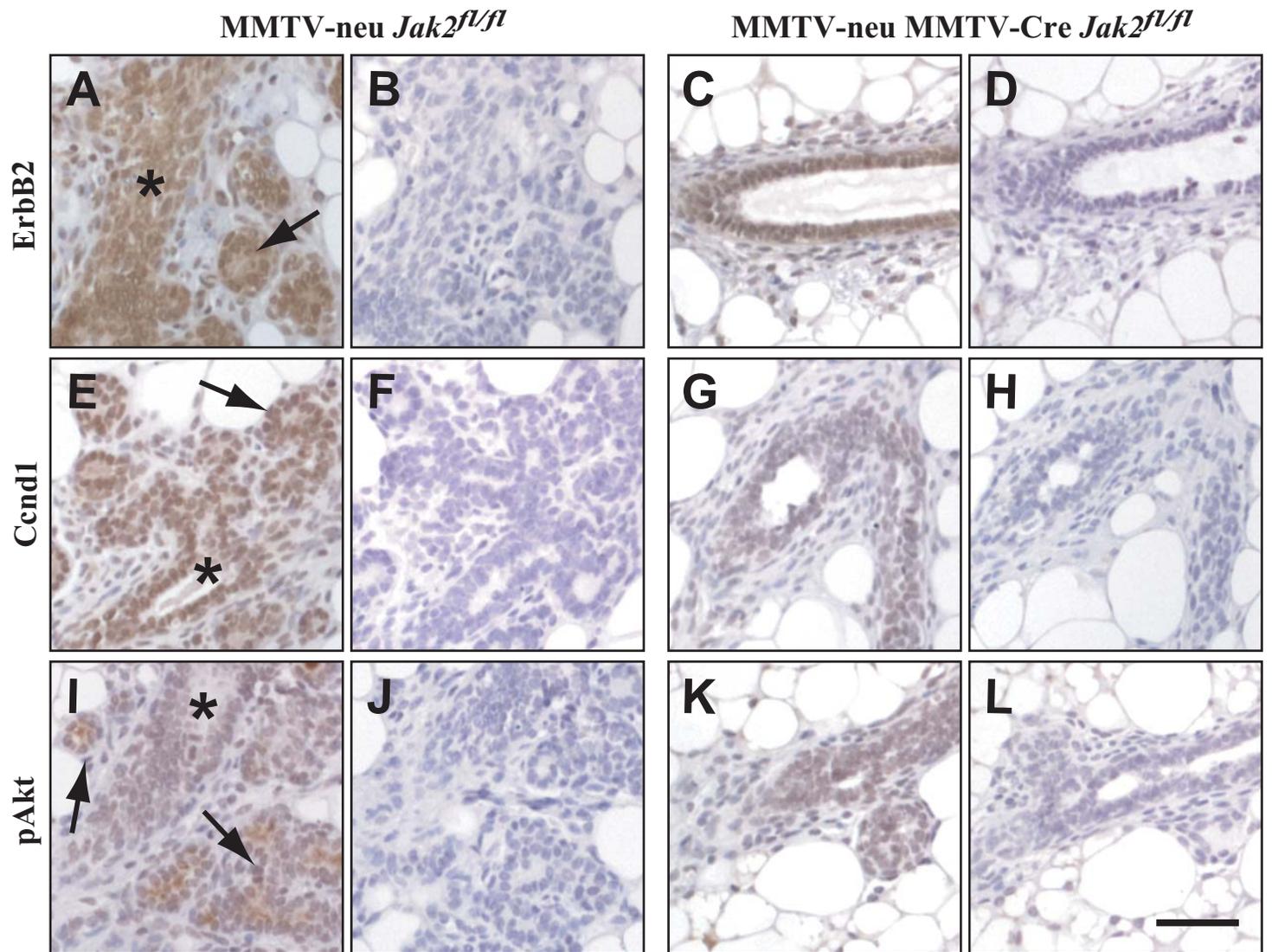
area. More than one thousand cells were counted in several randomly selected areas of histological sections; p-value, t-test.

Fig. S4: The PRL-induced activation of Stat5 and expression of Cyclin D1 in Her2/neu-associated mammary cancer cells lacking Jak2 (*Jak2^{-/-}*) and their Jak2-expressing isogenic controls (*Jak2^{fl/fl}*) in an orthotopic transplant model. **A.** Immunohistochemistry against the phosphorylated form of Stat5. Slides were counterstained with hematoxylin (bar, 200 μ m). **B.** Western blot analysis to assess the expression of Cyclin D1 in individual mammary tumors that lack Jak2 (right panel) and their controls (left panel). β -Actin (ActB) served as loading control.

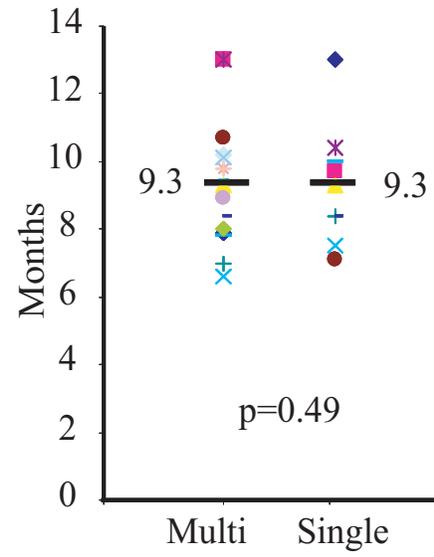
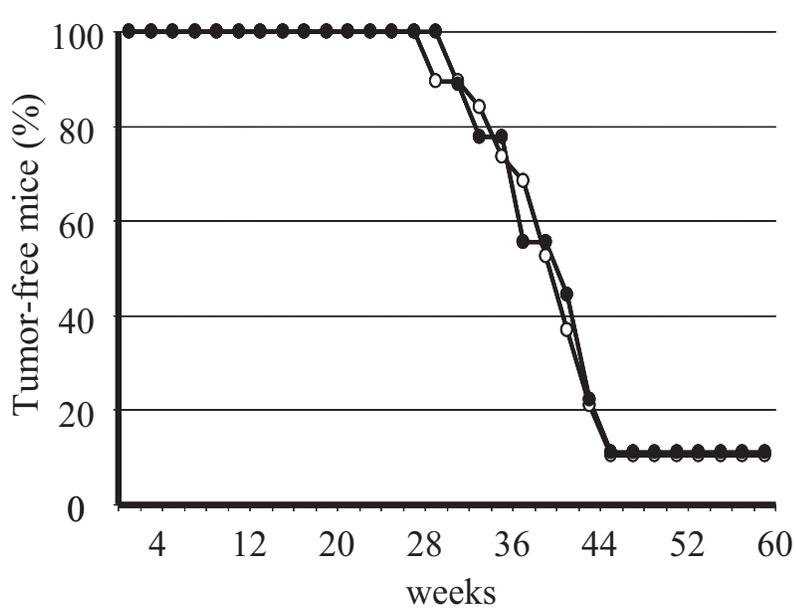
Fig. S5: Expression of Cyclin D1 in individual clones of Her2-associated mammary cancer cells expressing the Janus kinase 2 (*Jak2^{fl/fl}*) and their derived subclones lacking Jak2 (*Jak2^{-/-}*). **A.** PCR assay to verify the Cre-mediated deletion of *Jak2* from individual subclones. The parental Jak2-expressing clones (T1a/b T2a/b) were generated from two primary mammary cancers (T1, T2) of different females. **B.** Western blot analysis to assess the expression of Jak2 and Cyclin D1 in individual clones lacking Jak2 and their Jak2-expressing parental cells. β -Actin (ActB) served as loading control.

Fig. S6: Biochemical and cytological analysis of mammary tumors derived from orthotopically transplanted mammary cancer cells that express Jak2 in a doxycycline (Dox)-regulated manner (tet-off system, i.e. Dox administration results in repression of the Jak2 target gene). **A.** IP/western blot analysis to assess the downregulated expression of exogenous, myc-

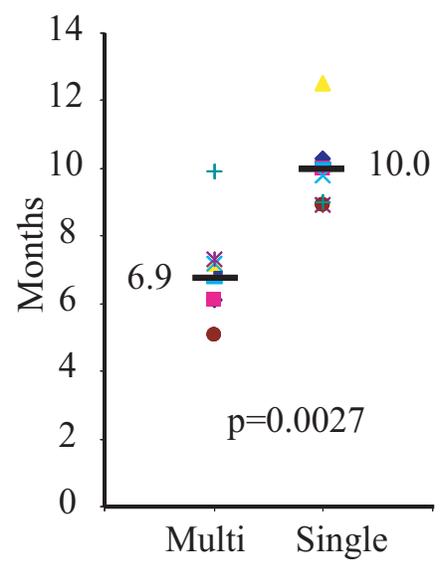
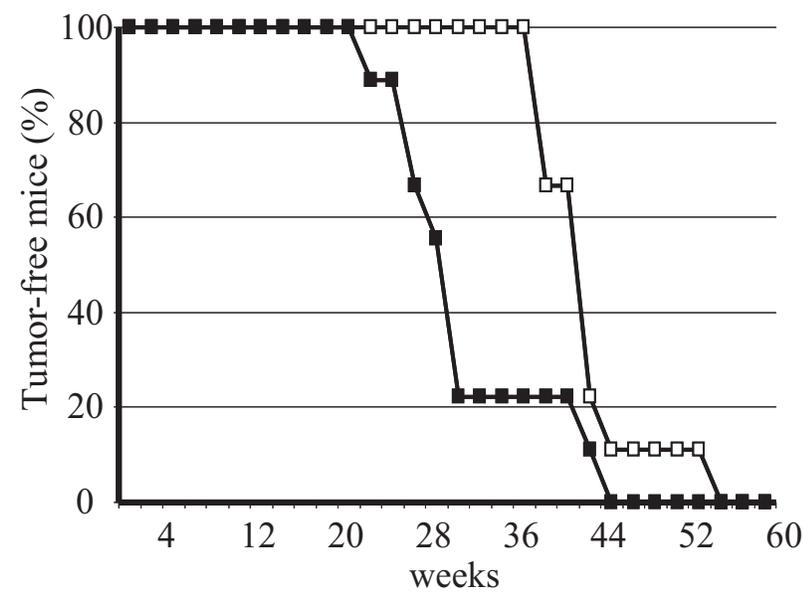
tagged Jak2 in individual mammary tumors from Dox-treated recipient females and their untreated controls. Note that some Jak2 protein was still present in tumors from Dox-treated animals despite the absence of myc-tagged exogenous Jak2. The residual expression of Jak2 in these tumors may originate from blood vessels and stromal cells of the wildtype hosts (see PCR results in Fig. 5B). **B.** Quantitative analysis of the cell density (left panel; mean and \pm S.D) as well as Ki67-positive cells (right panel) within a defined area of histological sections from tumors derived from Dox-treated recipients and their untreated controls. More than one thousand cells were counted in several randomly selected areas of histological sections. **C.** Western blot analysis to assess the expression of Cyclin D1 in individual mammary tumors that lack Jak2 (Dox) and their controls. β -Actin (ActB) served as loading control.

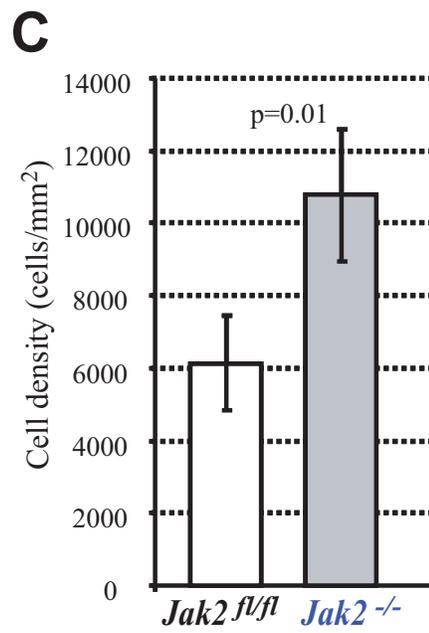
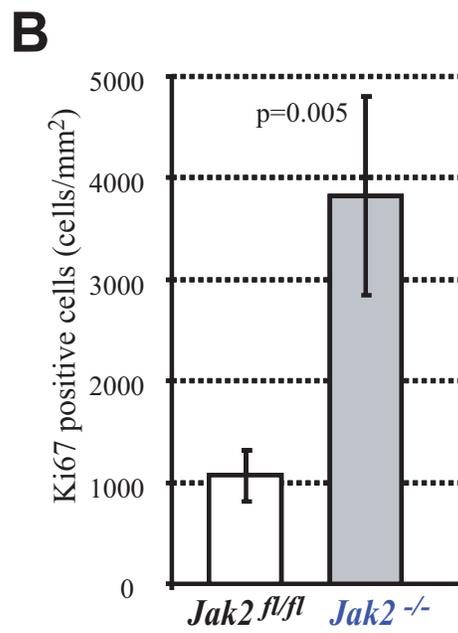
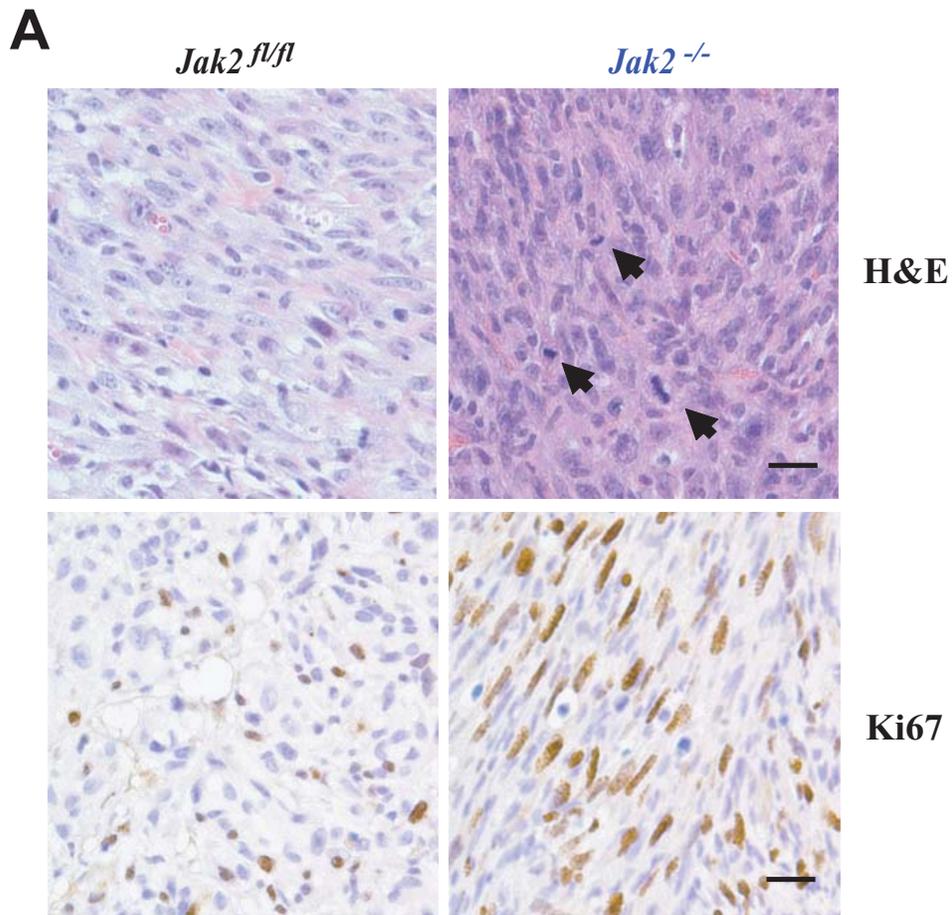


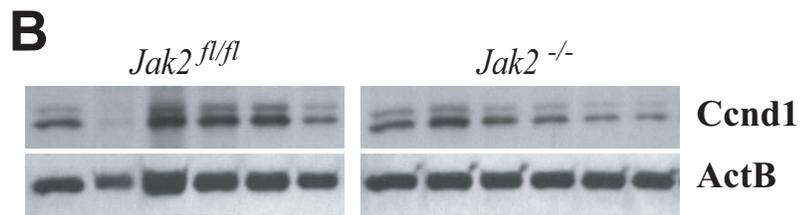
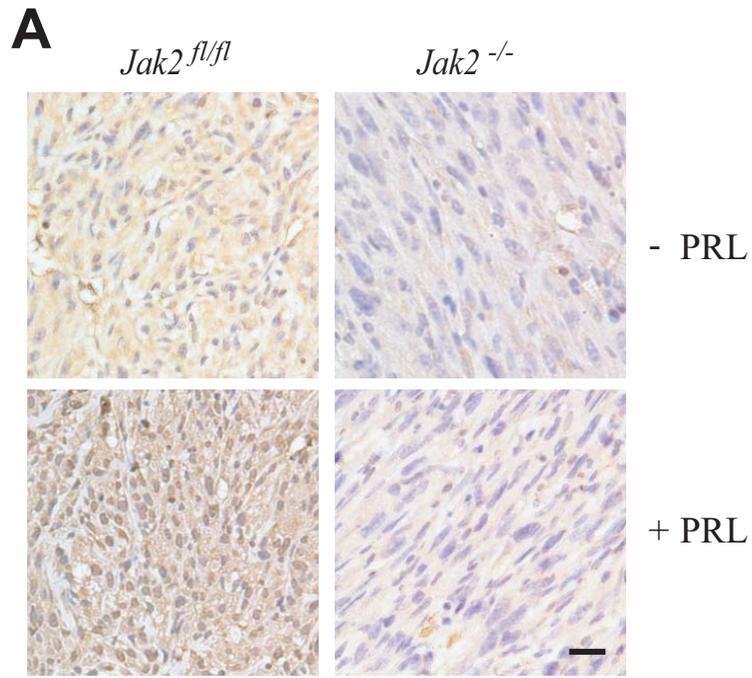
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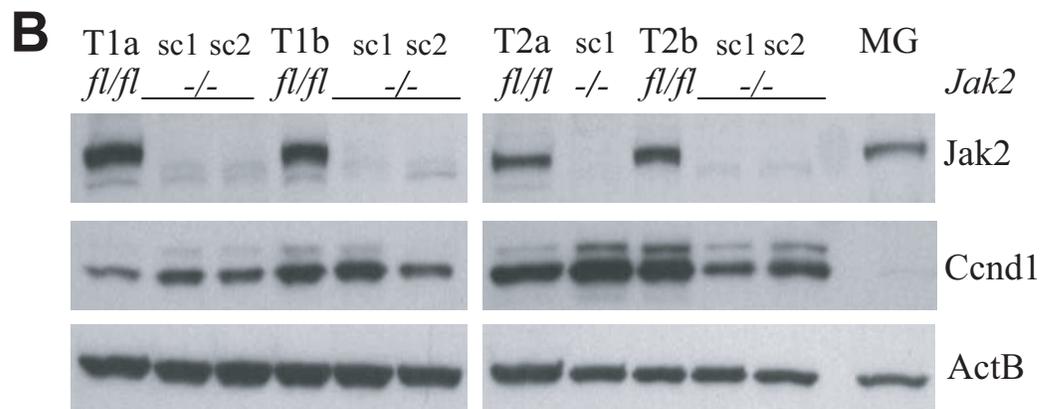
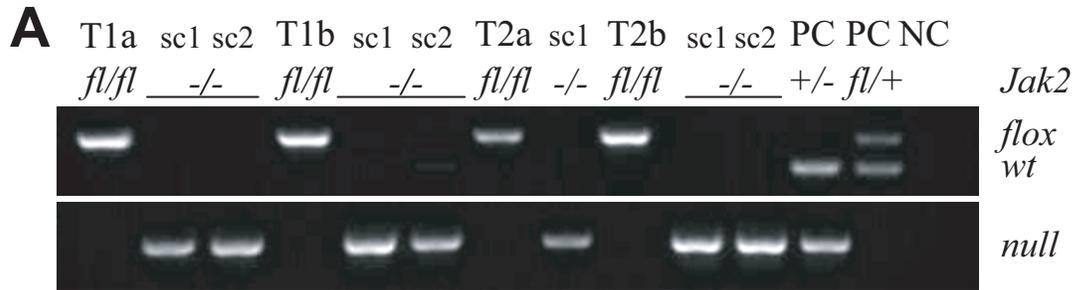


B









Supplemental Fig. S6

