Cancer Research

Supplementary Data

Runx2 is a novel regulator of mammary epithelial cell fate in development and breast cancer

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Supplementary Figure 1

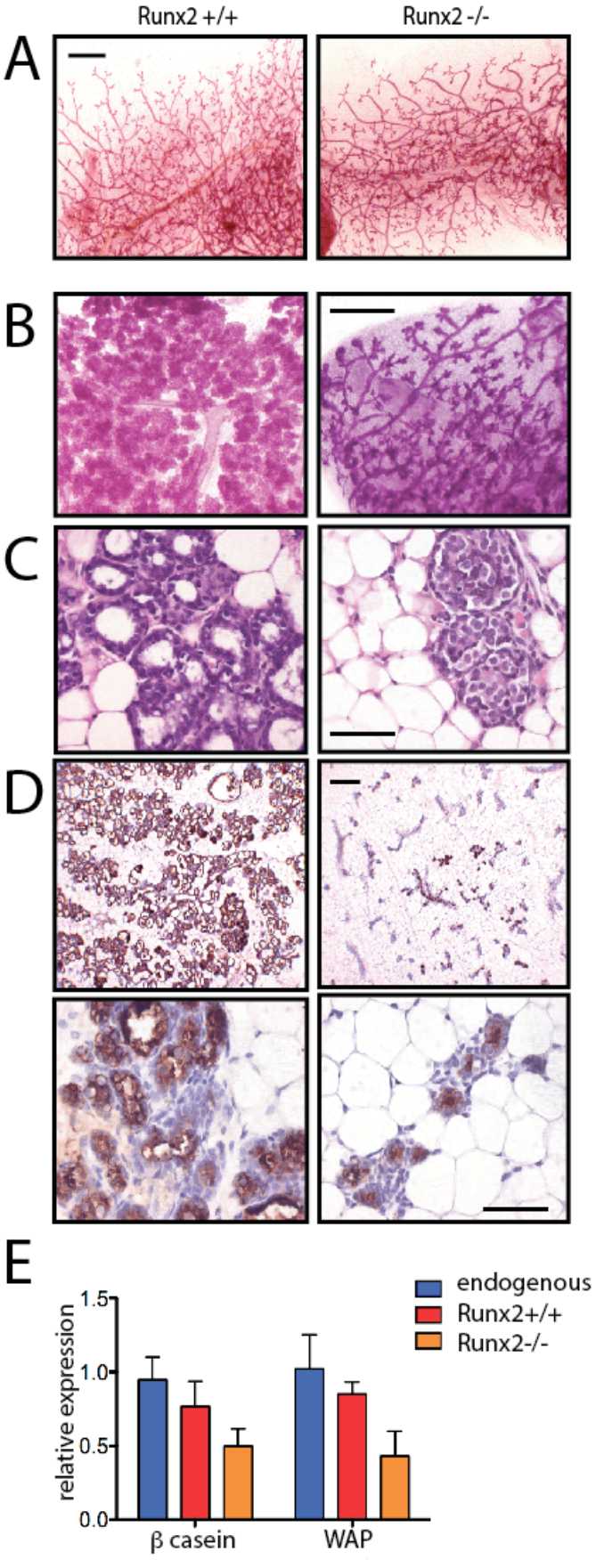

Figure S1. Failed lobuloalveolar development in Runx2$^{-/-}$ mammary glands is retained at the first day postpartum.

A) Whole mounts of transplanted Runx2$^{+/+}$ and Runx2$^{-/-}$ mammary glands isolated from Rag1$^{-/-}$ recipients pregnancy day 7 (Scale bar 1 mm).

B) Whole mounts of transplanted Runx2$^{+/+}$ and Runx2$^{-/-}$ mammary glands isolated from Rag1$^{-/-}$ recipients first day postpartum (Scale bar 1 mm).

C) H&E stained section of glands shown in B (Scale bar 12.5 μm).

D) Immunohistochemical analysis of β casein expression in Runx2$^{+/+}$ and Runx2$^{-/-}$ mammary glands at on the first day postpartum. Low and high magnification representative images are shown (Scale bar 25 μm).

E) qPCR analysis of WAP and β casein expression at pregnancy day 18.5.

Supplementary Figure 2

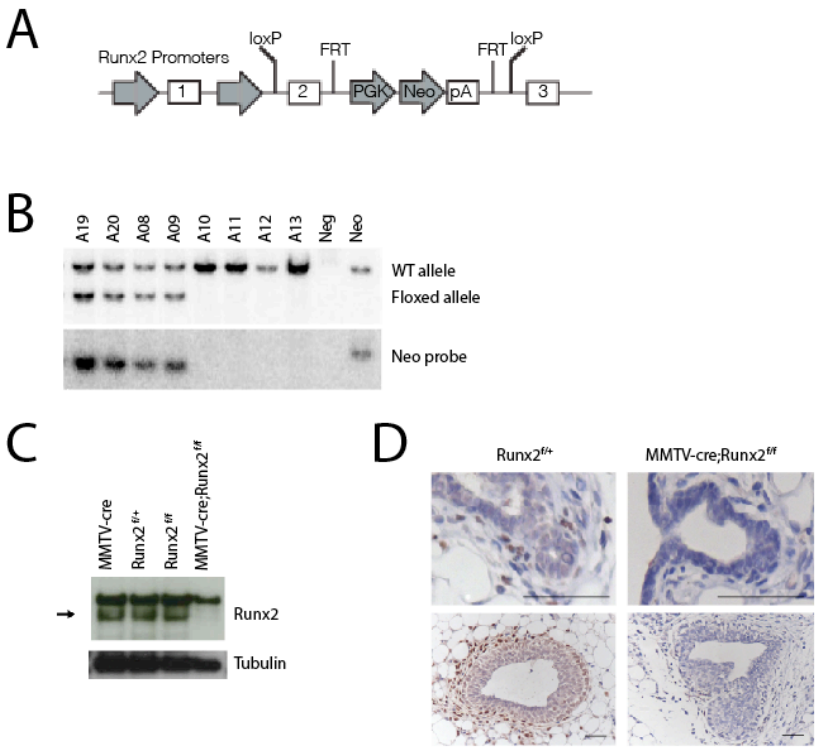

Figure S2. Generation and validation of Runx2$^{f/f}$;MMTV-cre mice.

A) Schematic of floxed allele construct, generated by OzGene. LoxP sites flank exon 2, present in all isoforms of Runx2.

B) Southern Blot of Runx2 floxed allele. Positive clones identified using 5' probe in Runx2 allele and 3' probe in neomycin cassette.

C) Western Blot of littermate whole ground mammary glands. Runx2 band is indicated (55 kDa), tubulin is used as a loading control.

D) Immunohistochemistry of Runx2 in ducts (top) and terminal end buds (below) of Runx2$^{f/+}$ (left) and MMTV-cre;Runx2$^{f/f}$ (right) in 8 week virgin sections. Runx2 is not expressed in the mammary epithelium of Runx2$^{f/f}$; MMTVcre mice (Scale bar: 0.2 μm).

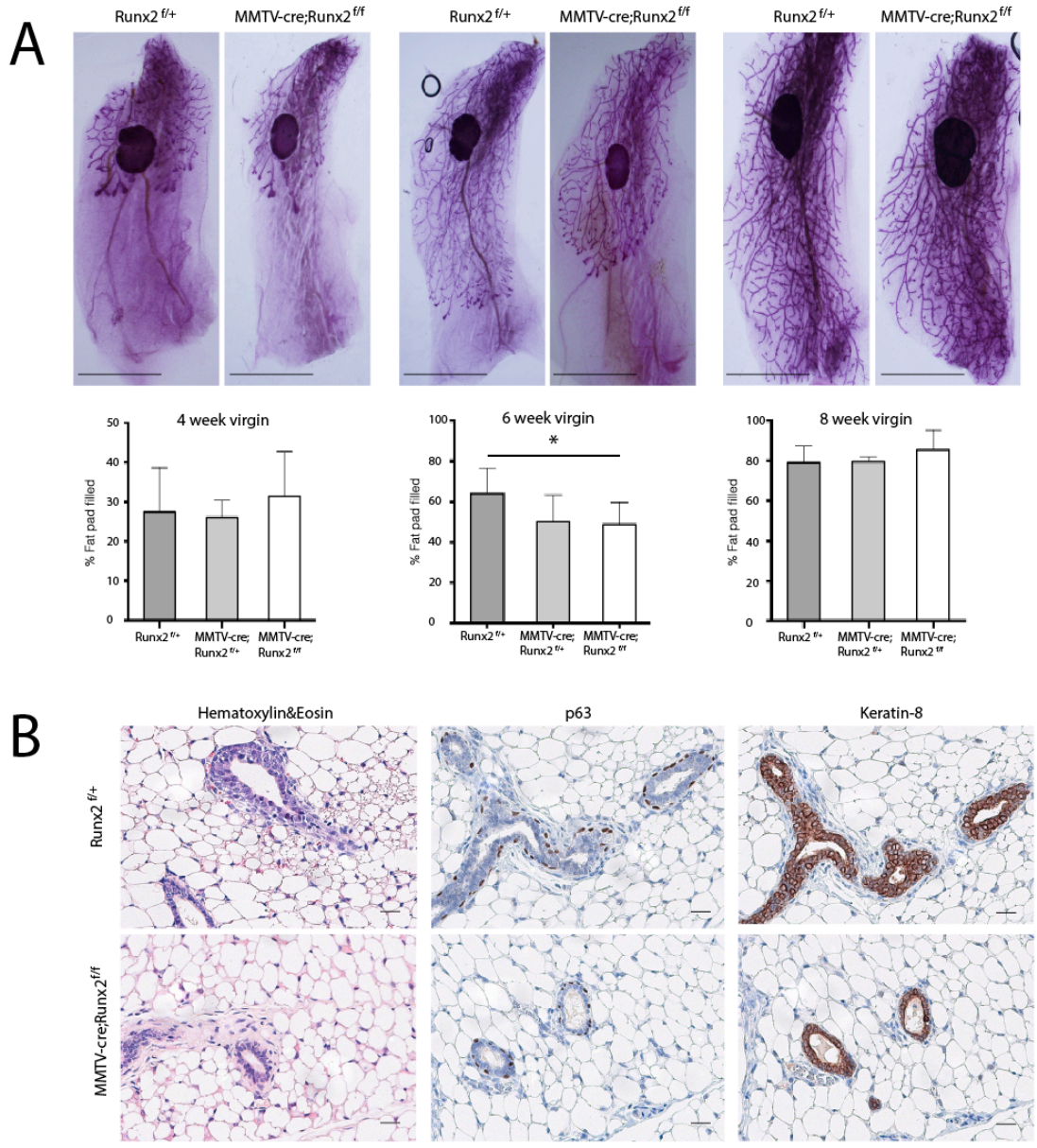

Figure S3. Runx2$^{f/f}$;MMTV-cre mice exhibit slightly delayed ductal elongation during puberty.

A) Virgin inguinal mammary glands stained with carmines to visualise the ductal tree. The area of ductal tree present in the total area of the gland was graphically quantified (Image J software), comparing Runx2$^{f/+}$ mice, MMTV-cre;Runx2$^{f/+}$ and MMTV-cre;Runx2$^{f/f}$ (representative images above. Scale bar 5 mm).

B) Immunohistochemistry of Runx2$^{f/+}$ and MMTV-cre;Runx2$^{f/f}$ mice. Sections were stained with H&E, p63 (myoepithelial) and Keratin-8 (luminal) to investigate ductal structure.

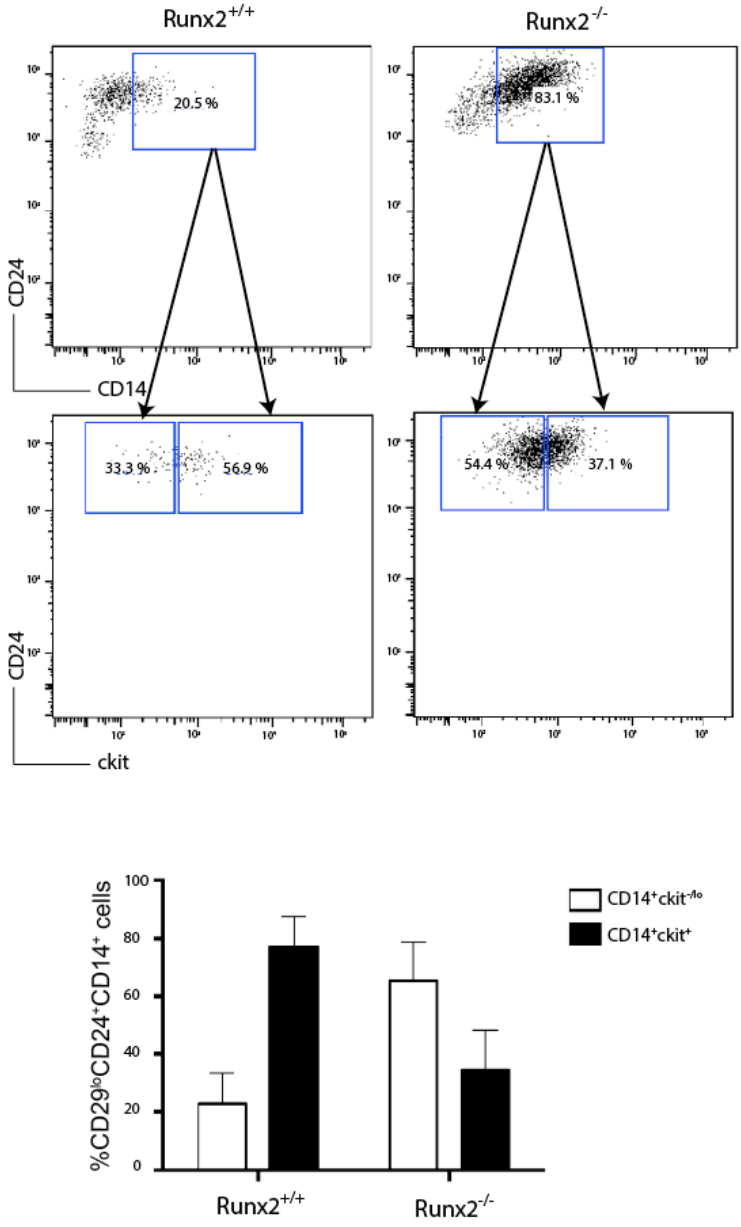

Figure S4. Runx2 regulates luminal progenitor populations in Runx2$^{-/-}$ mammary glands.

Flow cytometry analysis of pregnancy day 18.5 Runx2$^{+/+}$ and Runx2$^{-/-}$ luminal epithelial cells (CD29^{lo}CD24^{+}). Luminal cells were analysed further on the basis of CD14 expression and then ckit expression. Bar graph indicates proportion of CD14^{+}ckit$^{-/lo}$ (left arrow) and CD14^{+}ckit^{+} (right arrow) in Runx2$^{+/+}$ and Runx2$^{-/-}$ transplanted mammary glands. Representative FACS plots are shown. CD14^{+}ckit^{+} p=0.0374, CD14^{+}ckit$^{-/lo}$ p=0.0286; unpaired students t-test.

Supplementary Figure 5

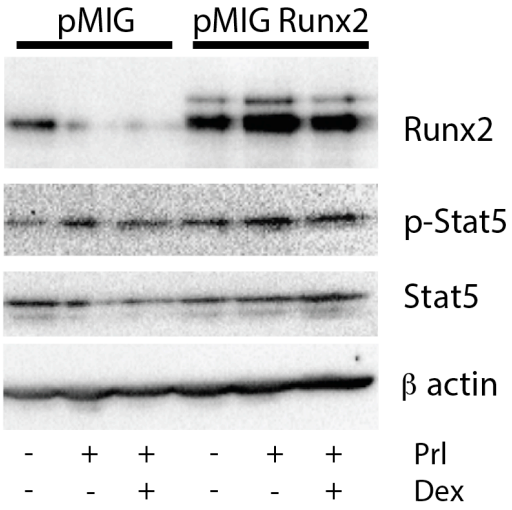

Figure S5. Forced Runx2 expression does not perturb prolactin induced Stat5 activation in HC11 cells.

Control HC11 cells (pMIG) or Runx2 stably over-expressing HC11 cells (pMIG Runx2) were serum staved in media with 5 µg/ml Insulin for 24 hours, before a 10 min induction with 1µM dexamethasone and/or 5 ug/ml prolactin as indicated. Cells were lysed on ice using prewarmed 2% SDS/50mM Tris pH 7.2 lysis buffer and protein lysates subjected to western analysis.