Supplementary Figure 1

A

5 weeks of age

Ccn6^{wt/wt} *Ccn6^{fl/fl}*

B

C

8 weeks of age

Ccn6^{wt/wt} *Ccn6^{fl/fl}*

D

Supplementary Figure 2

A 16 week old

15 mm   10 mm   5 mm

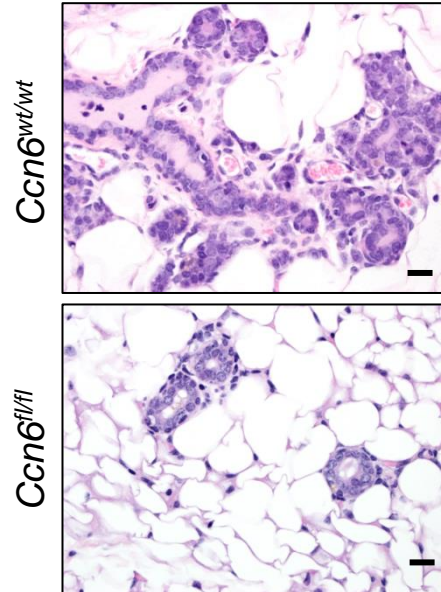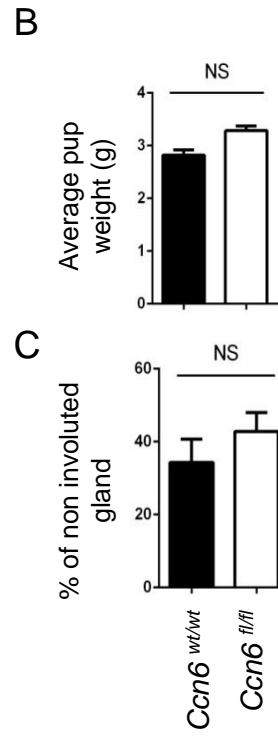Distance from the lymph node
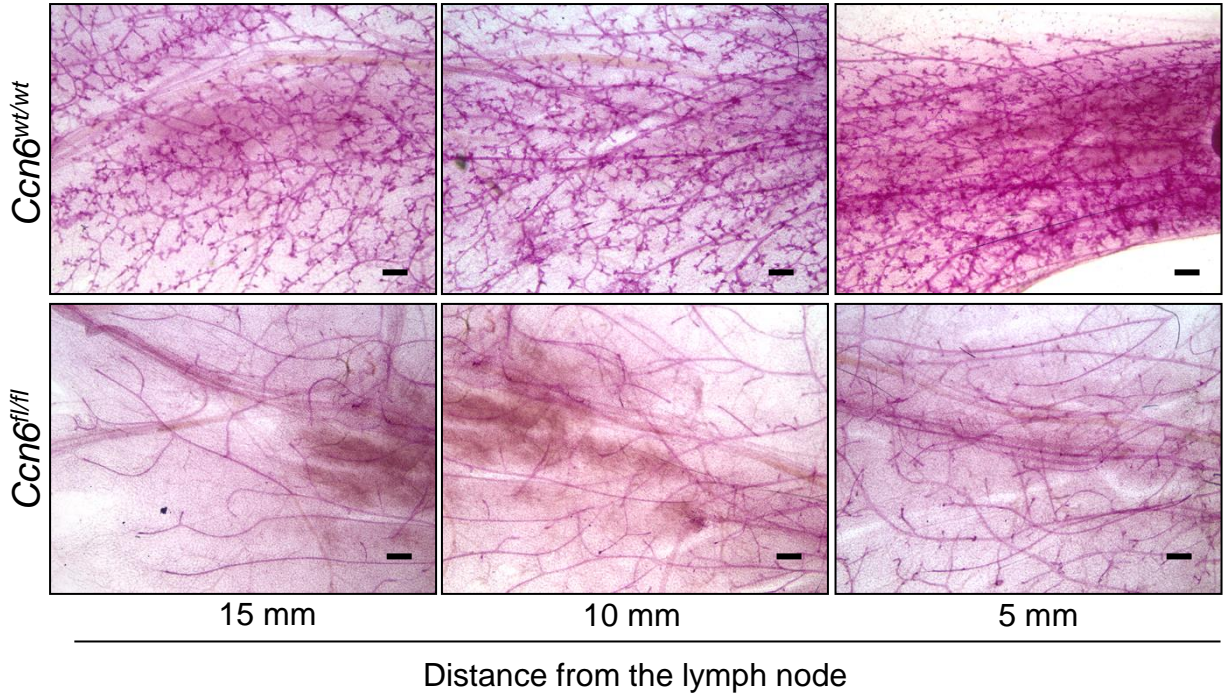
B 16 week old whole mounts
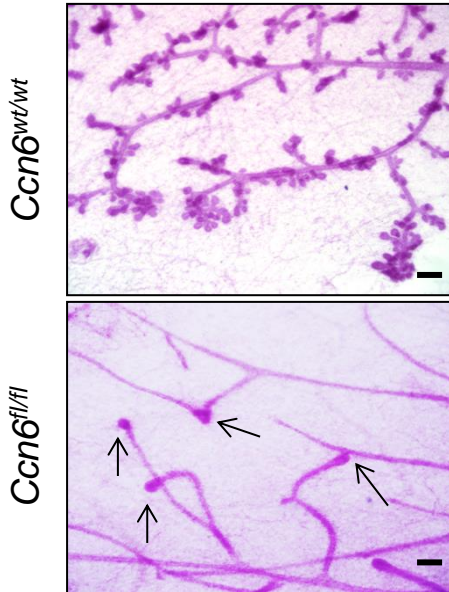
C 16 week old histology

Supplementary Figure 3

A

| | Ccn6^wt/wt | Ccn6^fl/fl |

6.5 dP

18.5 dP

1 dL

4 dI

B

NS

Average pup weight (g)
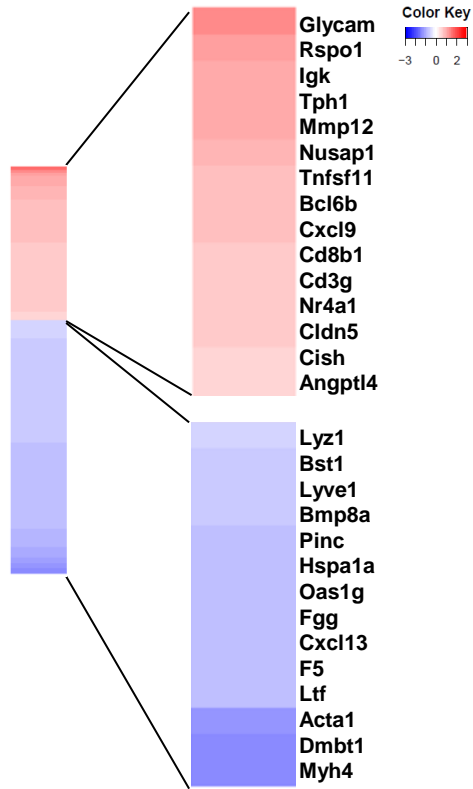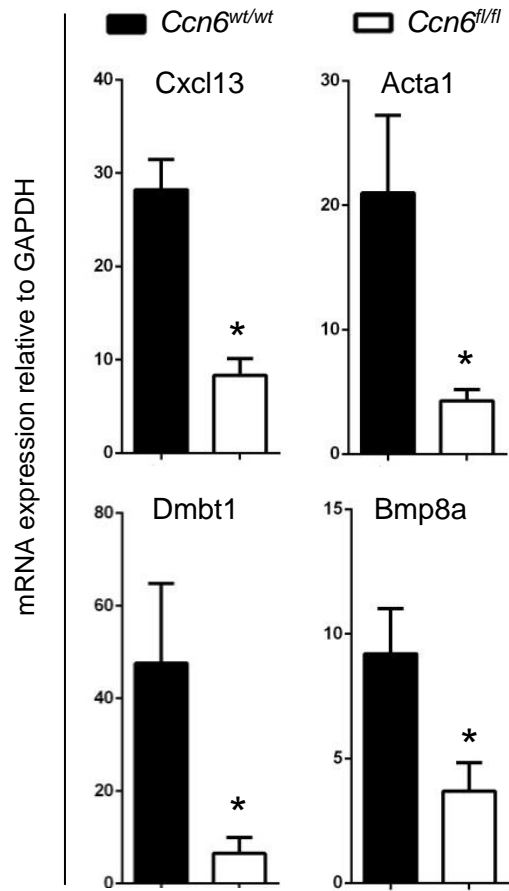
C

NS

% of non involuted gland
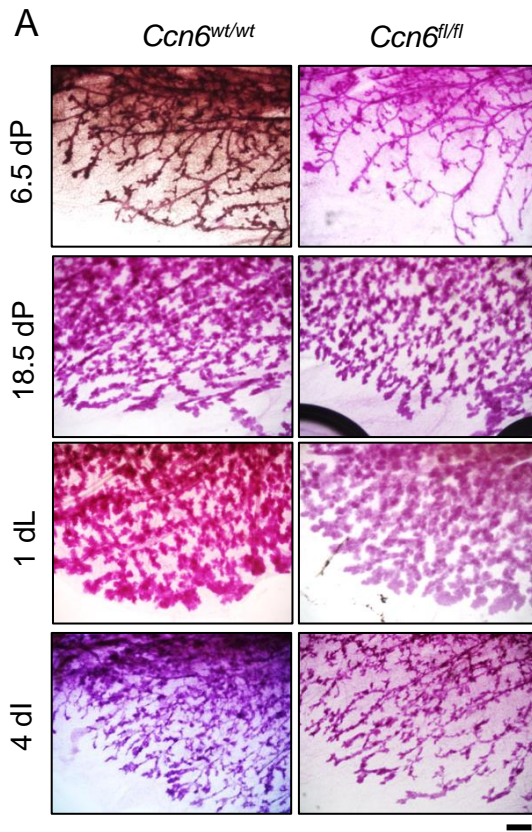
Ccn6^wt/wt   Ccn6^fl/fl

Supplementary Figure 4

A

4 month old virgin mice mammary glands
Ccn6 $^{fl/fl}$ vs. *Ccn6* $^{wt/wt}$

Color Key
-3 0 2

Glycam
Rspo1
Igk
Tph1
Mmp12
Nusap1
Tnfsf11
Bcl6b
Cxcl9
Cd8b1
Cd3g
Nr4a1
Cldn5
Cish
Angptl4

Lyz1
Bst1
Lyve1
Bmp8a
Pinc
Hspa1a
Oas1g
Fgg
Cxcl13
F5
Ltf
Acta1
Dmbt1
Myh4

B

■ *Ccn6* $^{wt/wt}$ □ *Ccn6* $^{fl/fl}$

mRNA expression relative to GAPDH

Cxcl13   Acta1

Dmbt1   Bmp8a

C

Ccn6 $^{wt/wt}$ *Ccn6* $^{fl/fl}$

Dmbt1

■ *Ccn6* $^{wt/wt}$
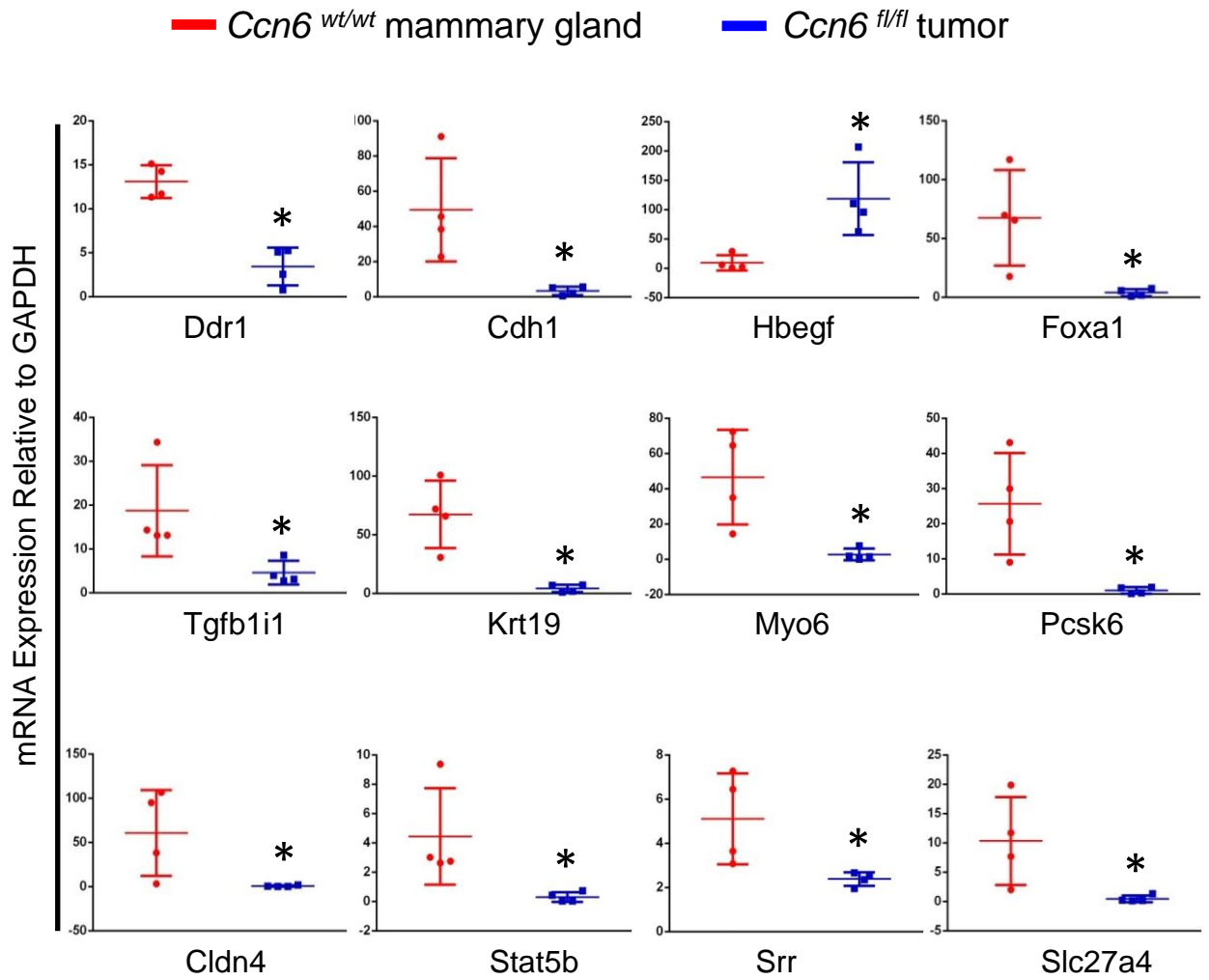□ *Ccn6* $^{fl/fl}$

**
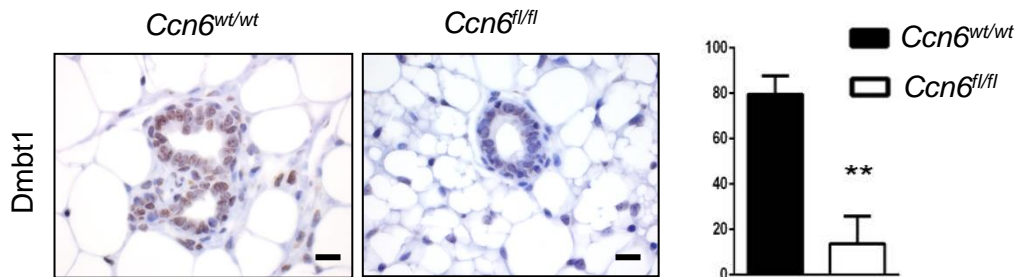
Supplementary Figure 5

Supplementary Figure Legends.

Supplementary Figure 1. Mammary epithelial cell specific *Ccn6* knockout leads to reduced numbers of TEBs and mammary gland complexity in pre-pubertal and pubertal mice. A. Mammary whole mounts from 5 week old control (*Ccn6$^{wt/wt}$*) and CCN6 knockout (*Ccn6$^{fl/fl}$*) mice. **B.** Quantification of the number of terminal end buds and bifurcated terminal end buds in 5 week old mammary CCN6 knockout (*Ccn6$^{fl/fl}$*) and control glands (*Ccn6$^{wt/wt}$*). * p<0.02. **C**. TOP, Mammary whole mounts from 8 week old control (*Ccn6$^{wt/wt}$*) and CCN6 knockout (*Ccn6$^{fl/fl}$*) mice (top). Arrows point to bifurcating terminal end buds. BOTTOM, H&E sections of re-imbedded mammary whole mounts stained with H&E. **D.** Quantification of the number of secondary and tertiary branch points in 8 week old CCN6 knockout (*Ccn6$^{fl/fl}$*) and control glands (*Ccn6$^{wt/wt}$*) . *p<0.05. All quantification was performed blinded to genotype by two independent counters with results reported as average +/- SEM. N=at least 3 mice per genotype. Two-tailed Student's *t*-test was used for all statistical analyses. (Scale bars, 500 μm.)

Supplementary Figure 2. Adult virgin *Ccn6* knockout mice exhibit a hypobranching mammary phenotype. A. Mammary whole mounts from 16 week old female virgin control (*Ccn6$^{wt/wt}$*) and CCN6 knockout (*Ccn6$^{fl/fl}$*) mice at 5 mm, 10 mm, and 15 mm away from the mammary lymph node. (Scale bars, 500 μm.) **B.** A close up view of mammary whole mounts. Arrows point to residual terminal end buds. (Scale bars, 250 μm.) **C.** H&E stained sections of mammary glands of 16 week old female virgin mice. Note the hypoplastic appearance of CCN6 KO mice glands compared to controls. (Scale bars, 50 μm.)

Supplementary Figure 3. *Ccn6* deletion does not affect lactational hyperplasia, or post-lactational involution. A. Mammary whole mounts from control and knockout mice throughout pregnancy. From top to bottom: 6.5 days pregnant (6.5 dP), 18.5 days pregnant (18.5 dP), 1

day of lactation (1 dL), and 4 days involution (4 dI). **B.** Average ± SEM pup weight (g) for $Ccn6^{fl/fl}$;*MMTV-Cre* and $Ccn6^{wt/wt}$;*MMTV-Cre* litters. **C.** Quantification of the percentage of the mammary gland that had regressed to an approximately pre-pregnant state in 4 dI dams. Quantification was performed blinded to genotype by two independent counters with results reported as average ± SEM. N=at least 3 mice per genotype. A two-tailed Student's *t*-test was used for all statistical analyses. (Scale bars, 500 µm.)

Supplementary Figure 4.   Transcriptional expression profiles of pubertal virgin *Ccn6* knockout glands and controls. A. Heatmap showing genes up or down regulated in virgin female pubertal knockout mice homogenates compared to controls. mRNA was isolated from 8 week old mammary glands from both groups and analyzed using an Affymetrix Plus Kit. **B.** Quantitative RT-PCR validation of selected genes from A. showing mRNA expression relative to *gapdh.* **C.** Immunohistochemical staining for DMBT1 in 8 week old control and Ccn6 knockdout glands. Data were quantified using image J. All quantification was performed blinded to genotype by two independent counters with results reported as average ± SEM, N= at least 3 mice per genotype. Two-tailed Student's t-test was used for statistical analyses, with *p<0.01. (Scale bars, 20 µm.)

Supplementary Figure 5. Further validation of significantly deregulated transcripts in $Ccn6^{fl/fl}$;*MMTV-Cre* mice tumors compared to $Ccn6^{wt/wt}$;*MMTV-Cre* mammary glands. Quantitative RT-PCR results for significantly deregulated genes in triplicate samples of at least three mice in each experimental group. * p<0.05.