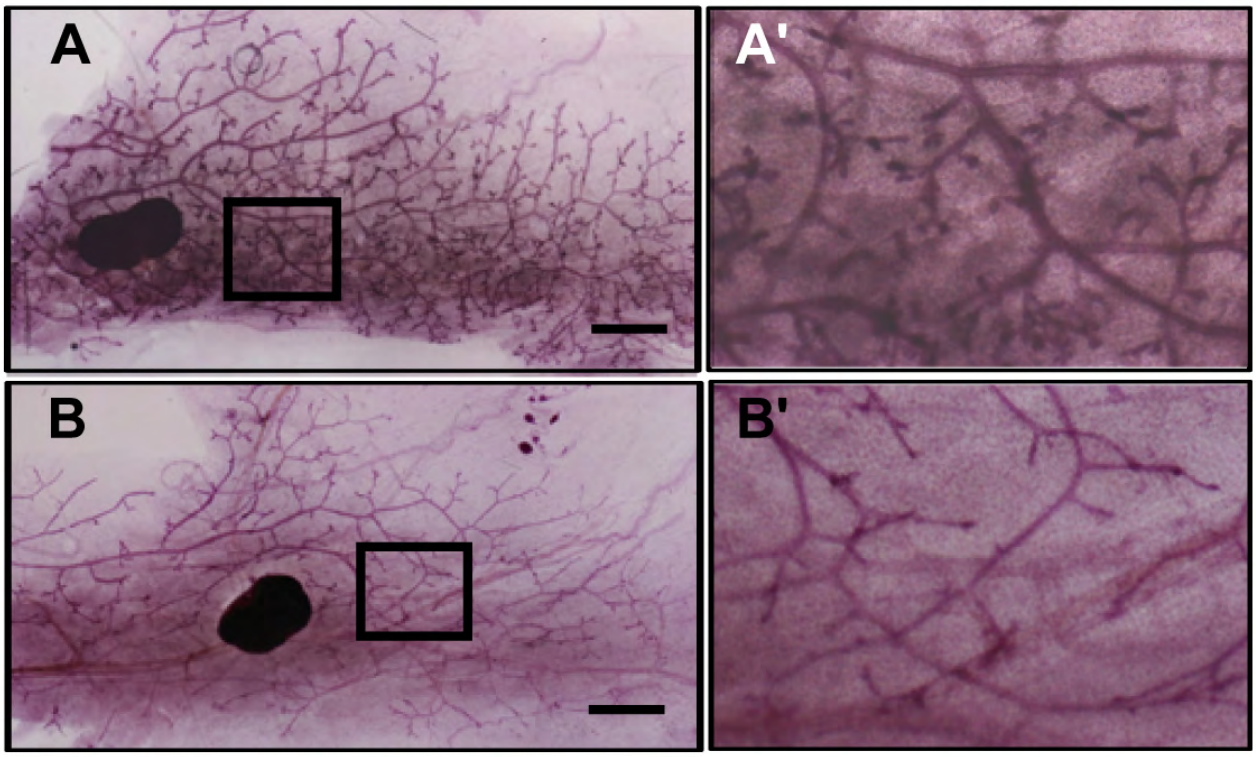

Fig. S1. Ductal network in 5-month-old RIP140 KO mice. (**A-B′**) Mammary gland whole-mounts from representative RIP140 heterozygous (RIP140 Het) (A) and RIP140 KO (B) mice. RIP140 KO mice show a sparse ductal tree compared with the extensive ductal network seen in the RIP140 Het. A′ and B′ show magnified images of the boxed areas in A and B, respectively (*n*=2). Scale bars: 1 mm.

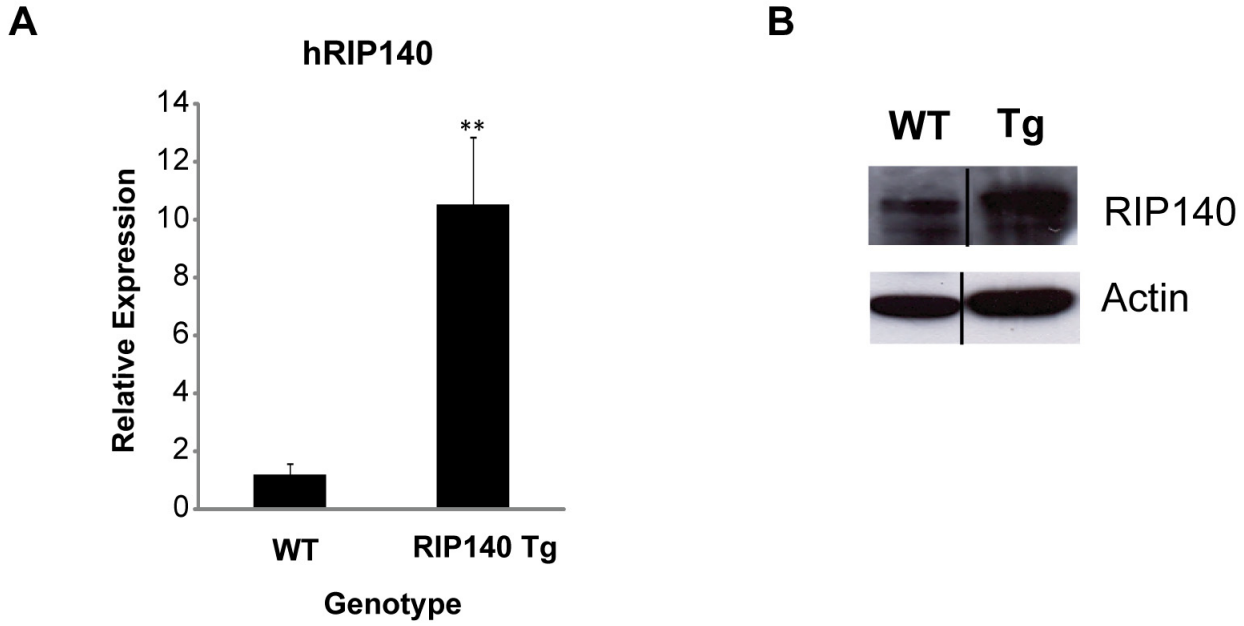

Fig. S2. Analysis of RIP140 over expressing transgenic mammary glands. (**A**) Q-PCR analysis shows over expressed human RIP140 (hRIP140) in the mammary glands from RIP140 transgenic mice. (**B**) Western blots using RIP140 (1:1000; 6D7, in-house monoclonal) and β-actin (1:5000; Abcam, Cambridge, MA) primary antibodies, show increased RIP140 expression in the mammary glands of the RIP140 transgenic mice (*n*=3). Error bars represent s.e.m. ***P*<0.005.

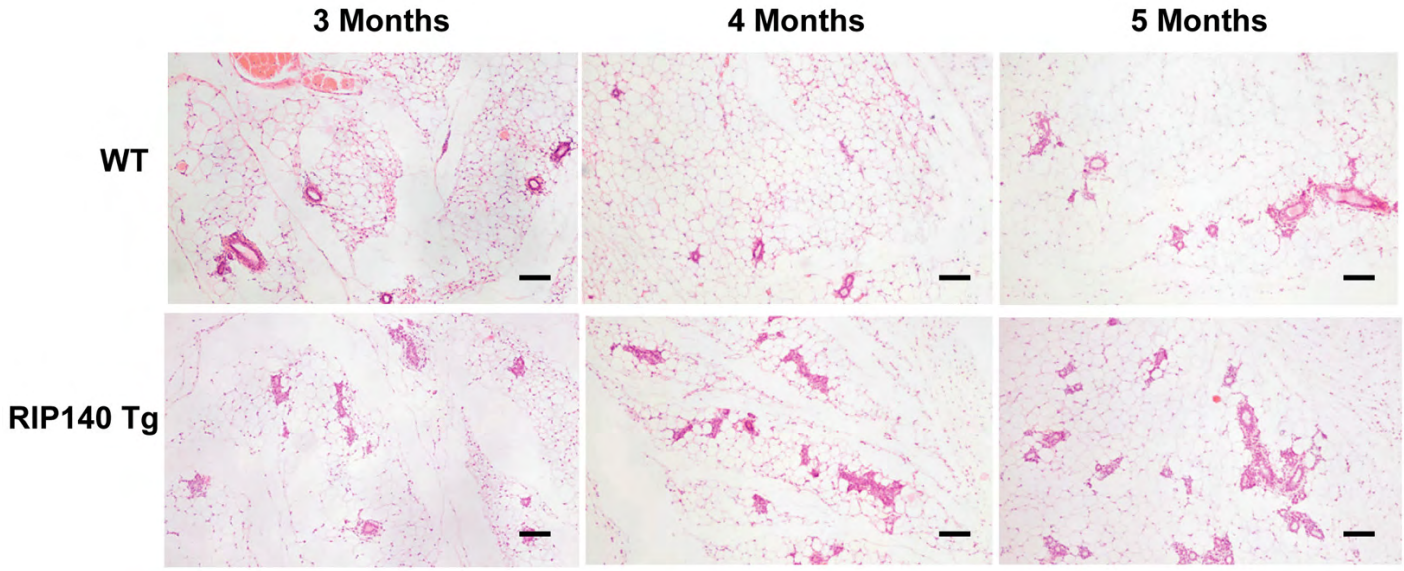

Fig. S3. Mammary gland histology. H&E-stained cross-sections through the mammary glands of 3-, 4- and 5-month-old WT and RIP140 Tg mice showing increased hyper budding in RIP140 Tg mice (*n*=3). Scale bars: 200 μm.

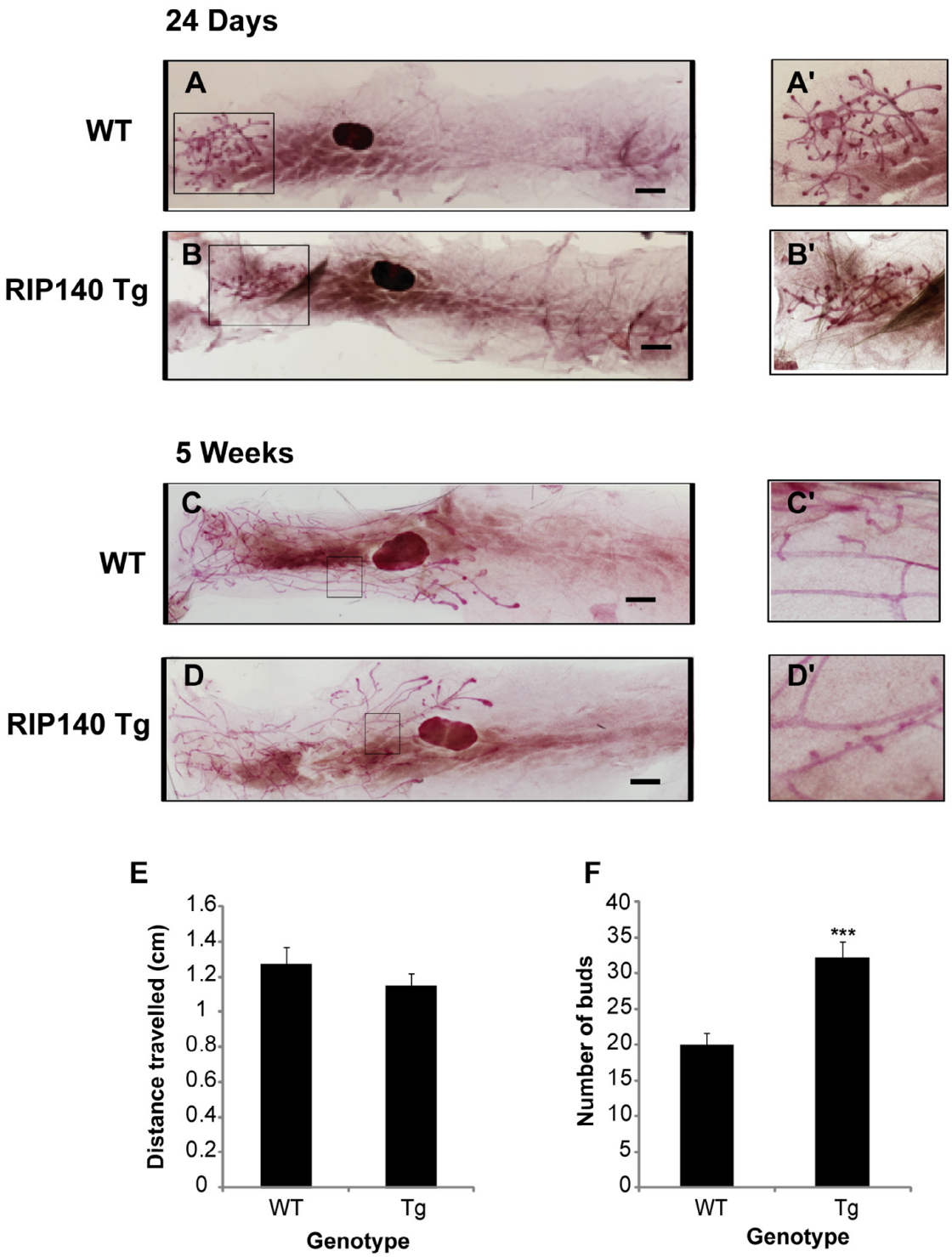

Fig. S4. RIP140 Tg mammary gland development. (**A-D′**) Whole-mounts of 24-day-old WT (A) and RIP140 Tg (B), and 5-week-old WT (C) and RIP140 Tg (D) mice. A′, B′, C′ and D′ are magnified images of the boxed areas in A, B, C and D, respectively. (**E**) Distance travelled by the ducal network from the root of the epithelim to the tip of the terminal end buds in 5-week-old WT and RIP140 Tg mice. (**F**) Quantification of budding points in 5-week-old WT and RIP140 Tg mice (n=5).***P<0.0001. Error bars represent s.e.m. Scale bars: 1 mm.

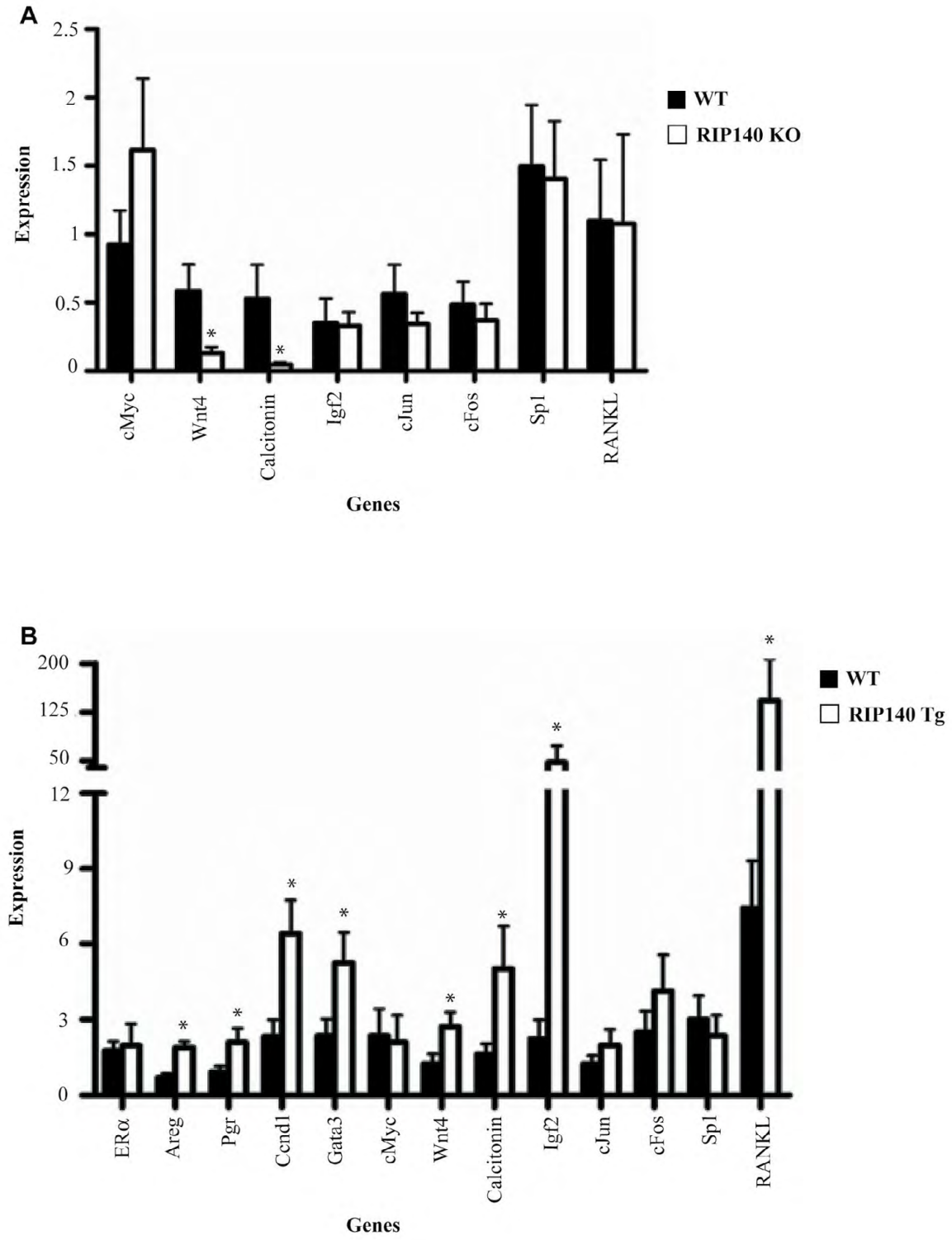

Fig. S5. Mammary gland gene expression. Quantitative RT-PCR analysis of gene expression in mammary glands of WT/RIP140 KO (**A**) and WT/RIP140 Tg (**B**) mice (n=4). *P<0.05. Error bars represent s.e.m.

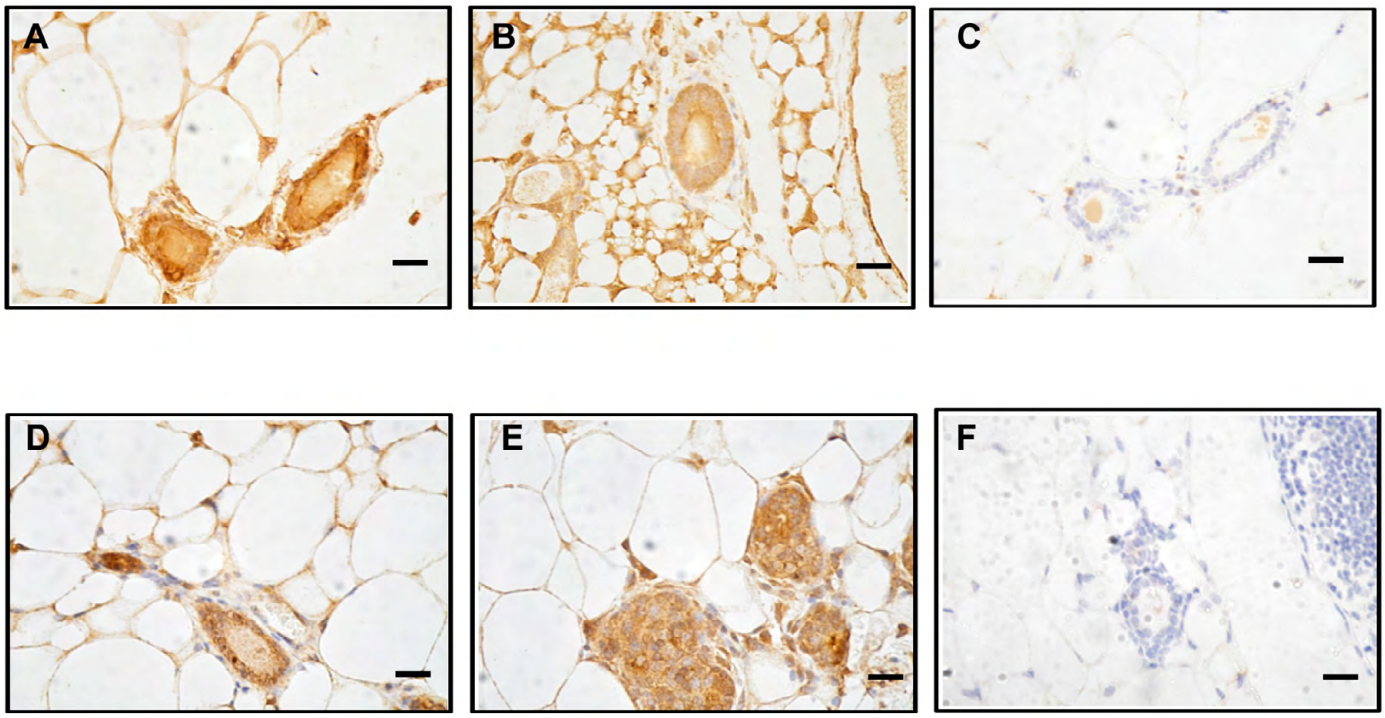

Fig. S6. Immunocytochemical analysis of RANKL expression in adult (virgin) mice. (**A-C**) RANKL staining (brown) in WT (C57BL/6) (A) and RIP140 KO (B) and the no primary antibody control (C). (**D-F**) RANKL staining in WT (FVB/N) (D) and RIP140 Tg (E) and the no primary antibody control (F). (*n*=3). Scale bars: 50 μm.

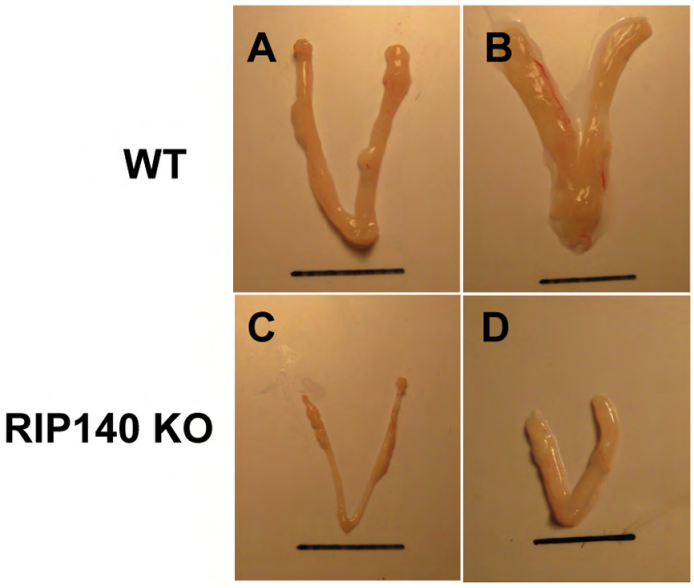

Fig. S7. Efficacy of estradiol treatment in vivo. (**A-D**) Uteri from WT (A,B) and RIP140 KO (C,D) mice treated with 21-day slow release oestradiol (B,D) or placebo (A,C) pellets. Mice treated with oestradiol pellets showed heavier uteri with water retention (*n*=3).

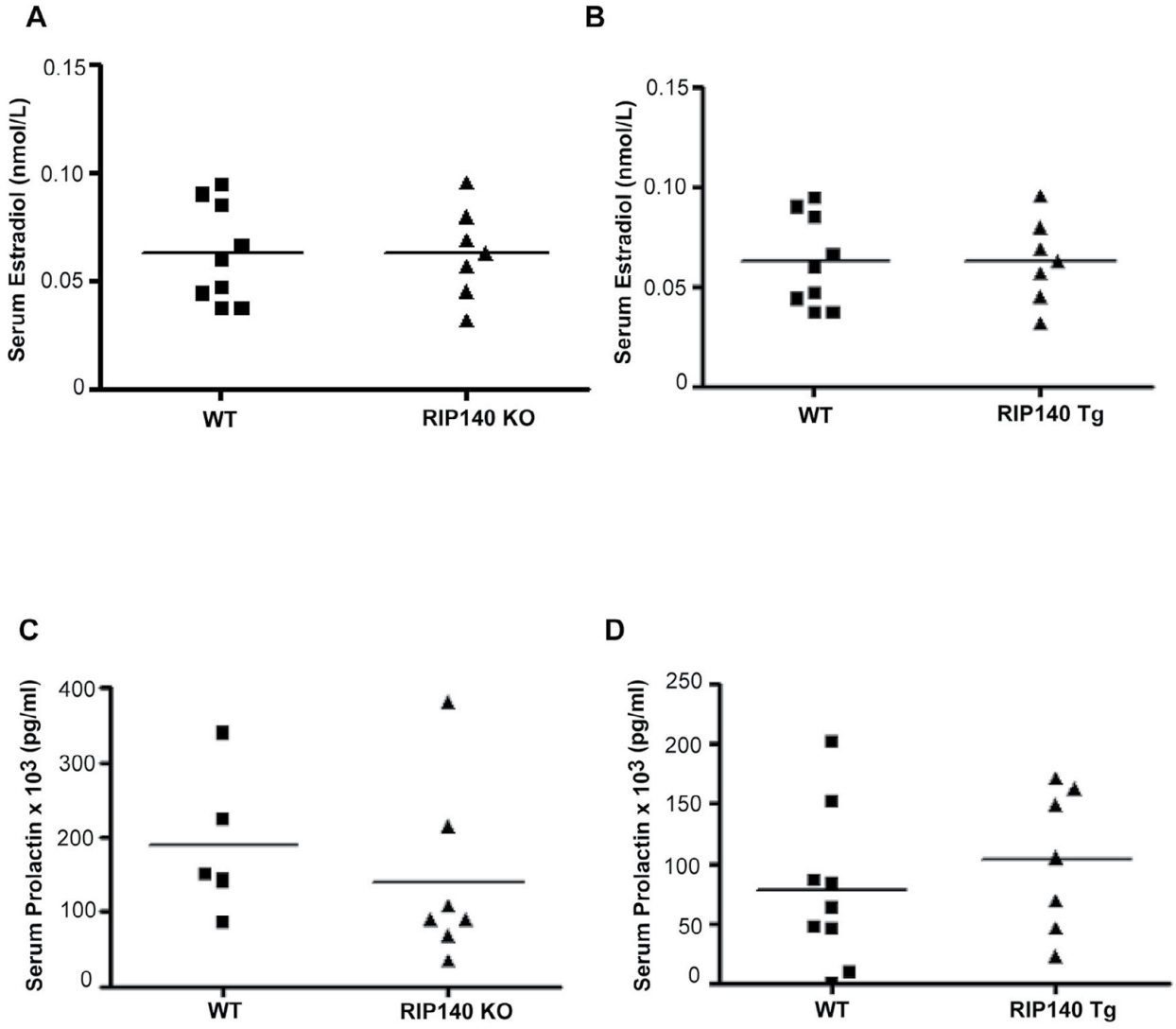

Fig. S8. Hormone measurements. (**A-D**) Serum estradiol (A,B) and prolactin (C,D) concentration in individual 8- to 12-week-old virgin WT/RIP140 KO (C57BL/6) (A,C) and WT/RIP140 Tg (FVB/N) (B,D) mice. Horizontal line represents the mean value.

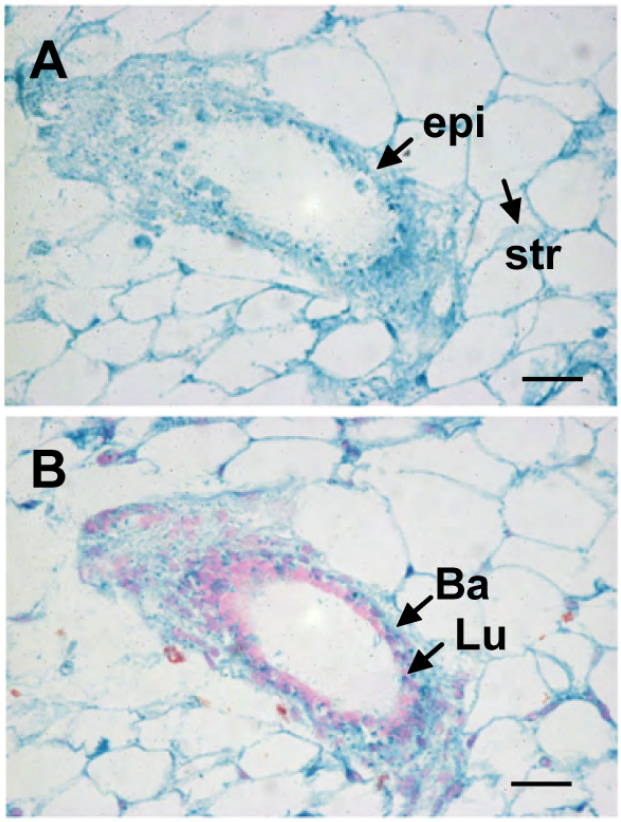

Fig. S9. Mammary gland sections from RIP140 KO mice stained for β-galactosidase activity. (**A**,**B**) Blue staining depicts RIP140 expression both in the epithelium (epi) and stroma (str) (A). Staining with nuclear fast red (a counterstain) helps highlight RIP140 expression in both the luminal (Lu) and the basal (Ba) layers of the epithelium (B). Scale bars: 50 μm.

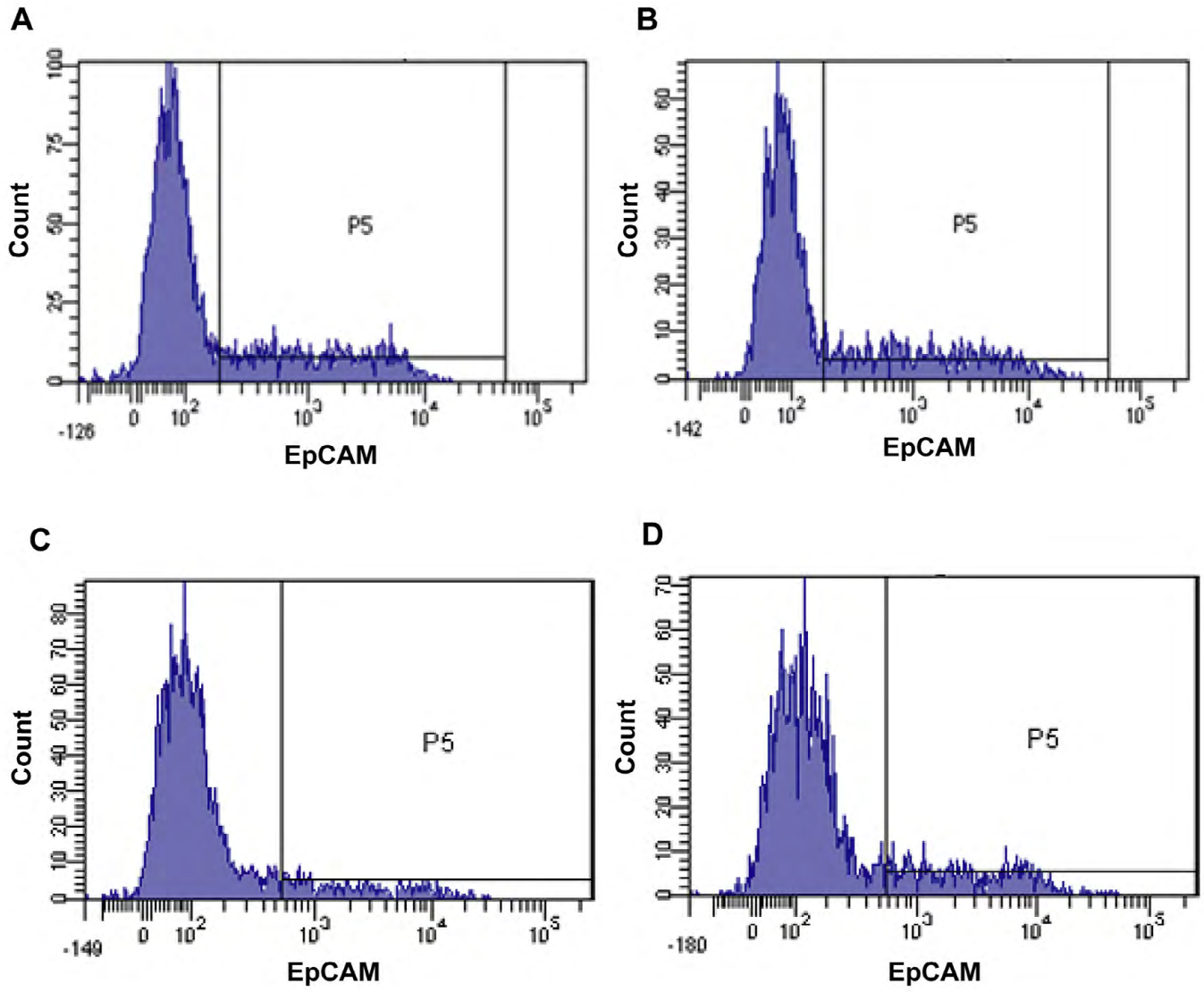

Fig. S10. Purification of epithelial (EpCAM$^+$) cells from single cell suspensions of mouse mammary cells. Histograms show EpCAM-expressing cells (P5) in total mammary cell preparations isolated from WT (C57BL/6) (**A**), RIP140 KO (**B**), WT (FVB/N) (**C**) and RIP140 Tg (**D**) mice. These purified epithelial cells were sorted and injected as donor cells in the tissue recombinant experiments.

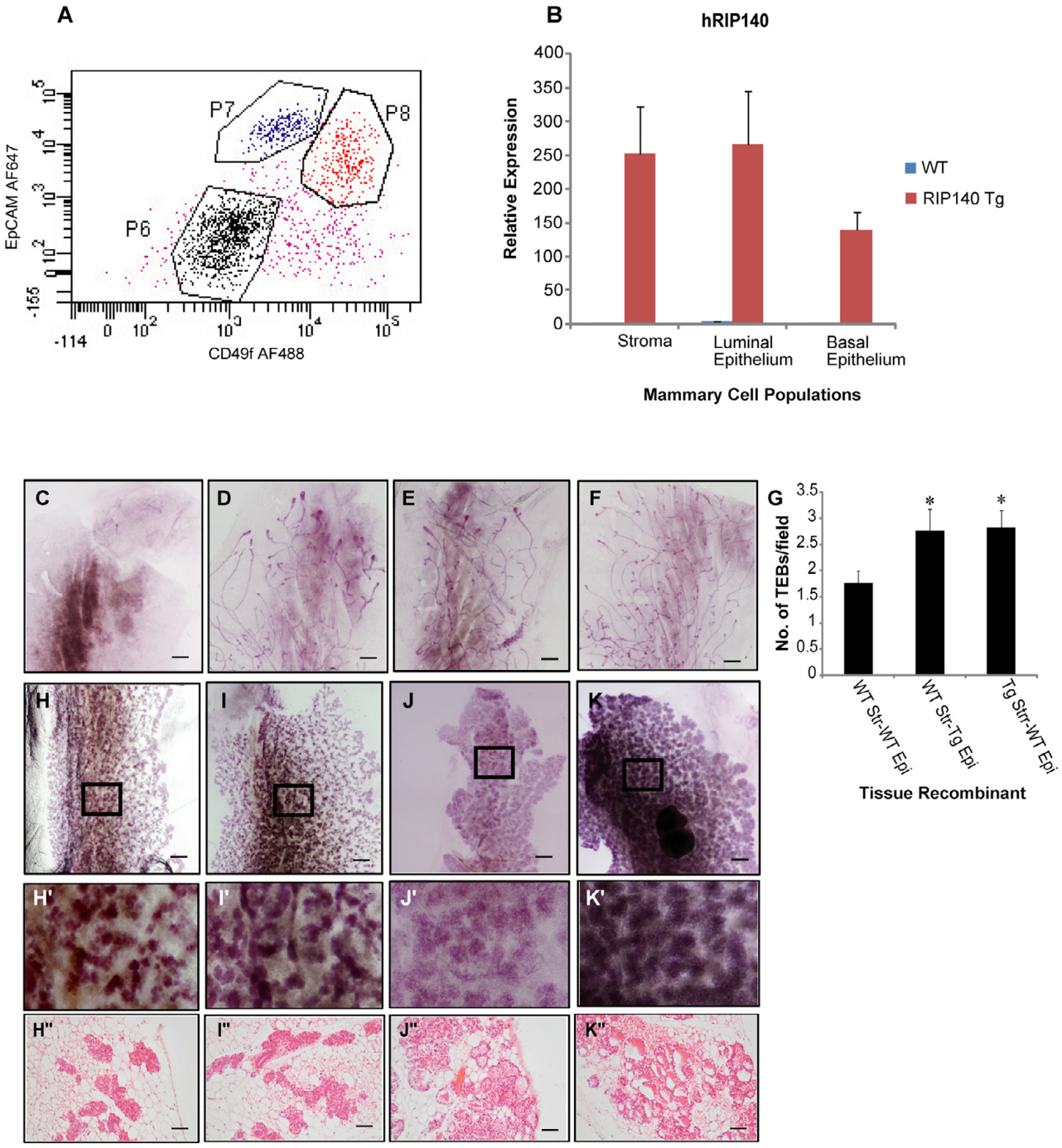

Fig. S11. Tissue recombination experiments. (**A**) Sorting strategy for mammary cell populations for gene expression analysis. Cell population in black (P6) represents stromal, in blue (P7) luminal and in red (P8) basal cells. (**B**) Q-PCR for RIP140 over expression (hRIP140) in mammary cell populations (n=4). (**C-F**) Tissue recombinants for pubertal growth in the mammary gland. (C) Representative image of a fat pad cleared of endogenous epithelium but not injected with donor epithelial cells. (D,E) Fat pads cleared of endogenous epithelium from 3-week-old WT mice, which were either injected with epithelium from WT (D) or RIP140 Tg (E) donors. (F) Three-week-old RIP140 Tg mice injected with epithelium from WT donors after removal of endogenous epithelium. Images represent recipient fat pads after 6 weeks of donor epithelium injections. (**G**) Quantification of number of TEBs in D-F. (**H-J**) Tissue recombinants at day 15 of pregnancy. (H,I) Fat pads from WT mice injected with epithelium from WT (H) or RIP140 Tg (I) donor mice. (J) Fat pad from RIP140 Tg mice injected with epithelium from WT donor. (**K**) Representative whole-mount of an intact RIP140 Tg mouse at day 15 of pregnancy (n=3). *P<0.05. (**H′**,**I′**,**J′**,**K′**) Magnified images of boxed area in from H,I,J,K, respectively. (**H″**,**I″**,**J″**,**K″**) Cross-sections through H,I,J,K, respectively. Error bars represent s.e.m. Scale bars: 1 mm in C-F,H-K; 100 μm in H″,I″,J″,K″.

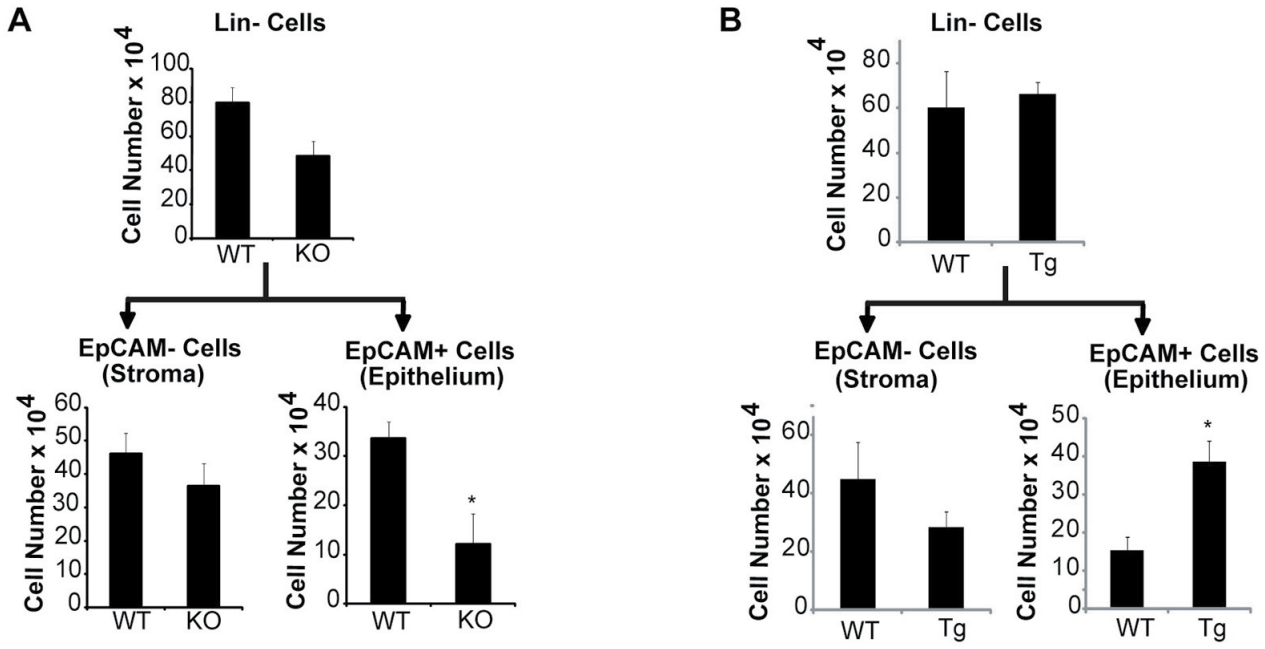

Fig. S12. Mammary gland cell populations. Histograms representing number of cells in different cell populations obtained from single cell suspensions of WT/RIP140 KO (**A**) and WT/RIP140 Tg (**B**) mammary glands.**P*<0.05. Error bars represent s.e.m.

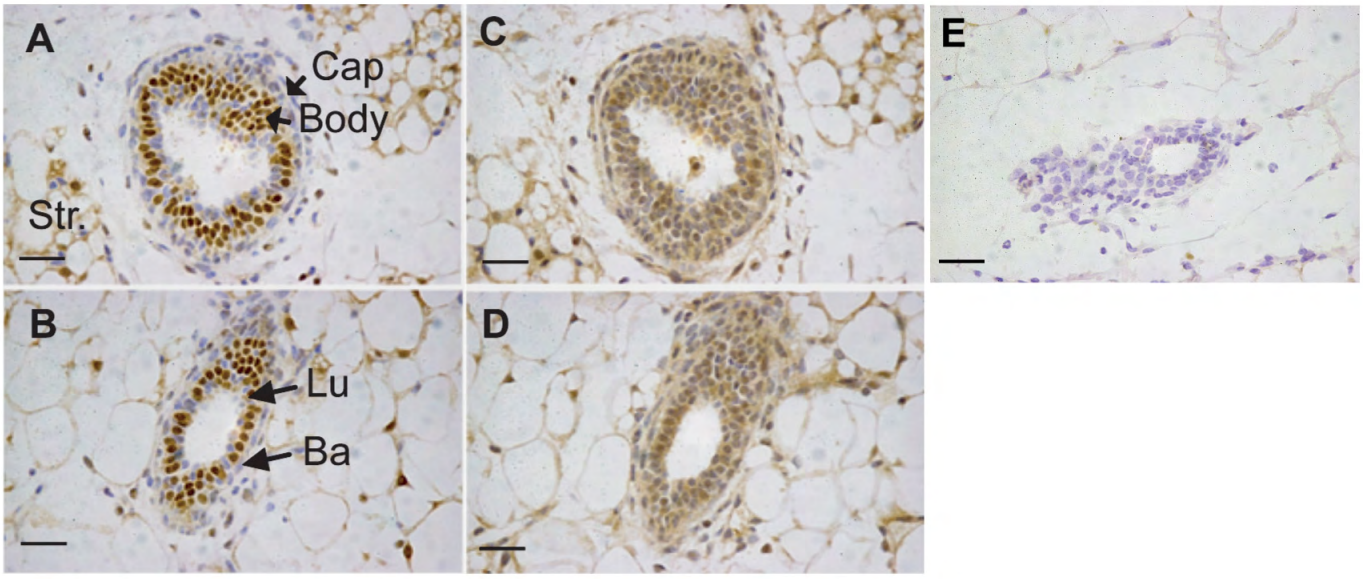

Fig. S13. ERα and RIP140 co-localization analysis. (**A-D**) Immunocytochemical expression (brown) of ERα (A,B) and RIP140 (C,D) in mammary gland sections showing expression in the terminal end buds (A,C), epithelial ducts (B,D) and stroma (Str). Sections are counterstained with Haematoxylin to highlight nuclei. In the TEBs, ERα expression is absent in the cap cells, which are positive for RIP140. In the mature ducts, RIP140 is expressed both in the luminal (Lu) and basal (Ba) layers. (**E**) RIP140 KO mammary gland section stained with RIP140 antibody. Scale bars: 50 μm.

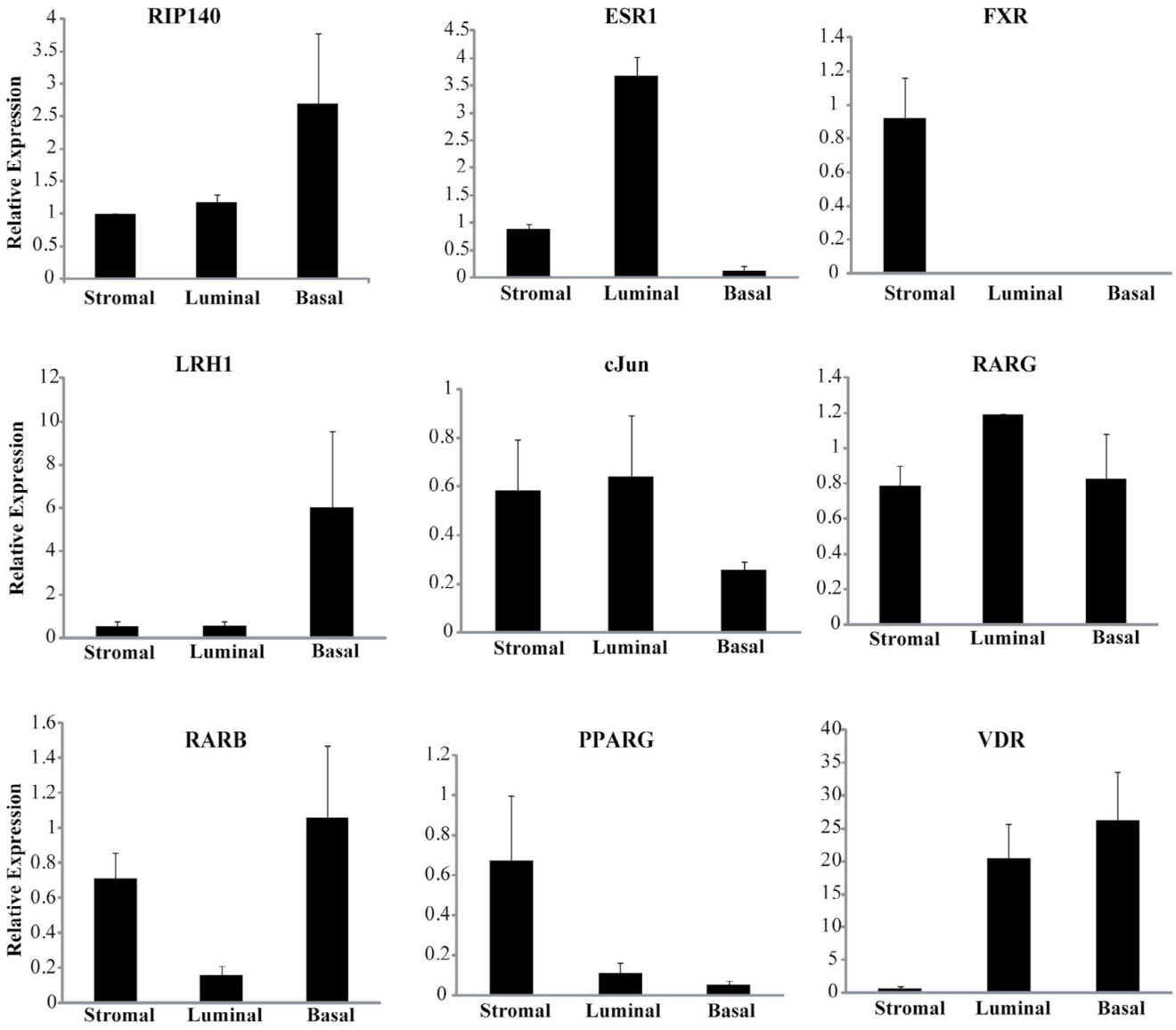

Fig. S14. Q-PCR analysis of gene expression for RIP140 and several nuclear receptors and transcription factors in sorted mammary cell populations from adult virgin WT mice. Error bars represent s.e.m.

Table S1. Sequences for Q-PCR and ChIP primers.

Q-PCR primer name	Primer sequences (5′-3′)
ERα	**F:**CCTCCCGCCTTCTACAGGT **R:**CACACGGCACAGTAGCGAG
Areg	**F:** TCCGGCTATATTATAGATGATTCAGTCA **R:**TCTCCTTCTGTCTTGTTTTTCTTGG
Pgr	**F:**GGTGGAGGTCGTACAAGCAT **R:**GGATTTGCCACATGGTAAGG
Ccnd1	**F:**CAGAAGTGCGAAGAGGAGGTC **R:**TCATCTTAGAGGCCACGAACAT
Gata3	**F:**AGTCGAGGCCCAAGGCACGA **R:**GCTGCCGACAGCCTTCGCTT
RIP140	**F:**CCCCAGTACCAACAGGACTACC **R:**TGAACGTGGCGGAATTTTGT
Stat5a	**F:**CGCCAGATGCAAGTGTTGTAT **R:**TCCTGGGGATTATCCAAGTCAAT
Lifr	**F:**TACGTCGGCAGACTCGATATT **R:**TGGGCGTATCTCTCTCTCCTT
Cav1	**F:**GCGACCCCAAGCATCTCAA **R:**ATGCCGTCGAAACTGTGTGT
Rpl7	**F:** AGCGAGGCTACGGCAAAA **R:**GAGACCGAGCAATCAAGGAATT
hRIP140	**F:**GAGAAACCAGCCCAAAATGA **R:**CTGGGTCTCTGCTCTTCCAC
cmyc	**F:**TCTCCATCCTATGTTGCGGTC **R:**TCCAAGTAACTCGGTCATCATCT
Wnt4	**F:**AGGAGTGCCAATACCAGTTCC **R:**TGTGAGAAGGCTACGCCATA
Calcitonin	**F:**GCCTTTGAGGTCAATCTTGGAA **R:**GCTTCAACCCCAATTGAAGTTT
Igf2	**F:**GTGCGGAGGGGAGCTTGTTGAC **R:**GTGGGCGTCTTTGGGTGGTA
c Jun	**F:** CGGACCGTTCTATGACTGCAA **R:**GGAGGAACGAGGCGTTGA
c Fos	**F:**CCTGCCCCTTCTCAACGAC **R:**GCTCCACGTTGCTGATGCT
sp1	**F:**GCCTCAGCTGCCTCCATTC **R:**CCTGGCATGGAGGAGAGTTG
RANKL	**F:**TGTACTTTCGAGCGCAGATG **R:**CCCACAATGTGTTGCAGTTC
FXR	**F**:CACGAAGATCAGATTGCTTTGC **R:**CTCCGCCGAACGAAGAAA
LRH1	**F**:TGCATGCCAAAAGAGCCTAAG **R:**TCCTCCTCTTCTCCTTCCAGTCT
RARG	**F**:TGACAGCGAGACTGGGCTACT **R:**ACCTTCTCGGGCTCTTCCA
RARB	**F**:ACCCAGCAAGCCTCACATGT **R:**AATTACACGTTCGGCACCTTTC
PPARG	**F**:CCCCTGCTCCAGGAGATCTAC **R:**TGCAATCAATAGAAGGAACACGTT
VDR	**F**:ACAGGACGCTAAGCTGGTTGA **R:**GGCGGCAGCGGATGT

ChIP Primer Name	Primer sequences (5′-3′)
rip140 prom	**F:**GTCTCCCTTTCTCCCGTTTC **R:**TGCACTCCTCAGGTCTCCTT
areg Prom	**F:**CCGGTGGAACCAATGAGAACT **R:**TGAGCCTAAGACCAGCAGCAA
areg Enh	**F:**ACAGTGACCCAGACGCTTTT **R:**ACACATGTCCCACAAGGTCA
pgr Prom	**F:**GGGTGGGGCTGGCATGCTTC **R:**CCGCCCAAAGCCCCTCCCTA
pgr Enh	**F:**GTTGACAGCATTCCAAAGCA **R:**TTCGCCATGAGTCGTAAGAA
ccnd1 Prom	**F:**CACCCCAGTGCGCCAGGATG **R:**TCCGAGTACGCCACGAGGCA
gata3 Prom	**F:**GGGAAGTTGCGGCTGGGTCC **R:**TTCGCCTAGCTCGGGGTGCT
gata3 Enh	**F:**GAGATGGGAGAGGACACAGC **R:**CAGTTGGAGCTGTCTGGTGA
stat5a	**F:**GTTCAAATGGCCAGGAAAAA **R:**GGTCACACTGACCTGGTCCT
ccdc6	**F:**GTCACCTGTCCCTCTGGGTA **R:**CTGTGGTTCAAGCGAAAACA
myc enh	**F:**GCTTAGCCATTCCTGCAGTC **R:**GCCAAAGGTCATGGTGTTCT
cued6	**F:**TGCTTCTGCCAGTTCTACCC **R:**GCTCTCTCTGCATGGCTTTC
lifr	**F:**GAAGCCAGGAGGTCACTGAG **R:**ATAGCAGTTTCCAGCCCAGA
greb1	**F:**CTCTGGCTGCCTAGGTGACT **R:**GAAGATCCACCGCAAACTGT
tprg	**F:**CTCGAGATGTTGATGCCAGA **R:**CACTTCCACCCACAACTCCT
mef2a	**F:**CTGGCTCTCCTCCAACACTC **R:**TCAGGGACTCTGCTCCTGAT
cav1	**F:**CCCTGGGCTAACAGAACTCA **R:**CAGCTCGAGTCCAGGATTTC
vegfa	**F:**AGACACTTCAGGGAGCAGGA **R:**TAAAGGGAGGTTTGGCTGTG
b Globin	**F:**CCTGCCCTCTCTATCCTGTG **R:**GCAAATGTGTTGCCAAAAAG

F, forward primer; R, reverse primer

Table S2. List of 458 genes for which ERα binding could be detected within 20 kb of gene promoters.

Entrez ID	Gene symbol	Gene description	ER– progenitor	ER+ progenitor	Differentiated luminal	Basal	Stromal
2103	**ESRRB**	**Estrogen-related receptor beta**	5.325514728	6.45856139	6.606453279	5.365730097	5.25737677
2104	**ESRRG**	**Estrogen-related receptor gamma**	5.254721211	5.353738986	5.66897559	5.18125953	5.263388265
6095	**RORA**	**RAR-related orphan receptor A**	6.90625365	6.242223271	6.074482833	7.154547644	6.861127074
7421	**VDR**	**Vitamin D (1,25-dihydroxyvitamin D3) receptor**	6.443922757	5.753070844	5.661924723	6.18300242	5.136685212
6097	**RORC**	**RAR-related orphan receptor C**	6.104929531	5.465568827	5.719564933	5.391660104	5.095232079
5468	**PPARG**	**Peroxisome proliferetor-activated receptor gamma**	5.319266772	5.401434715	5.670253622	5.512429536	6.429797525
5915	**RARB**	**Retenoic acid receptor, beta**	5.032788316	5.147905268	5.145224064	5.179409455	5.29418781
5916	**RARG**	**Retenoic acid receptor, gamma**	5.45835644	5.270686782	5.650083338	5.348901578	5.514703504
3725	**JUN**	**Jun oncogene**	7.801161199	7.271524941	7.241651942	7.463239551	6.995269918
2494	**NR5A2 (LRH1)**	**Nuclear receptor subfamily 5, group A, member 2**	5.558124566	6.156559978	5.531169896	5.746186332	5.452759444
2099	**ESR1**	**Estrogen receptor 1**	5.640602984	6.888940536	6.867860909	5.408619722	5.713373377
9971	**NR1H4 (FXR)**	**Nuclear receptor subfamily 1, group H,member 4**	5.101617011	5.127743796	5.122012353	5.146607576	5.575165887

Table S3. Data mined from a microarray of gene expression signatures from mouse mammary cell populations, showing differential expression of nuclear receptors and transcription factors, which RIP140 could potentially co-regulate. Centroids of gene expression for each cell subpopulation were built from the union set of top differentially expressed genes between each pair of cell subtypes. To identify differentially expressed genes, genes were first filtered according to variability and then the limma R package was used to rank them according to differential expression using B-statistics. The False Discovery Rate (FDR) was estimated using the q-value R package. In order to avoid skewing the number of centroid genes to specific cell types, top 250 up- and top 250 downregulated genes were selected in each cell-type comparison. All of these passed FDR corrected *P*-values <0.05. Data taken from Shehata et al. (Shehata et al., 2012).