

Figure S1

Fig. S1. Id4 expression is highest in the mammary glands of pubertal mice. (A) Southern blot analysis to identify positive offspring carrying the wild-type and floxed *Id4* allele. Arrows indicate the wild-type and floxed alleles. (B) FACS analysis of GFP reporter expression in MaSC/basal ($CD29^{hi}CD24^{+}$) cells at 4, 6, 8 and 12 weeks-of-age ($n = 3$ mice \pm s.e.m.). (C) FACS analysis of GFP reporter expression in luminal progenitor ($CD29^{lo}CD24^{+}CD49b^{+}$) cells at 4, 6, 8 and 12 weeks-of-age ($n = 3$ mice \pm s.e.m.). Representative image of Id4 immunostaining of freshly sorted $CD29^{lo}CD24^{+}CD49b^{+}$ cells. The arrow indicates a positive cell. Scale bar, 25 μ m. (D) Western blot showing Id4 expression in virgin mice at 4, 6 and 8 weeks-of-age. Actin provided the protein loading control. (E) Quantitation of Id4 immunohistochemical data at various time-points in virgin (wk), pregnant (dP), lactating (L) and involuting (I) mice. The percentage of Id4-positive ducts is shown as a proportion of total ducts per section (virgin) or per field of view (pregnancy, lactation, involution) ($n = 3$ mice \pm s.e.m.). (F) Immunostaining of sections from 6 week-old virgin $Id4^{f/+}$ and $Id4^{K14\Delta/\Delta}$ mice for Id4. Scale bar, 25 μ m. (G) Whole-mount of representative mammary glands from 4 week-old virgin $Id4^{f/+}$, $K14cre$, $Id4^{K14\Delta/+}$ and $Id4^{K14\Delta/\Delta}$ mice ($n = 3$ mice \pm s.d.). (H) Extent of fat pad filling in 8 week-old $Id4^{f/+}$, $MMTVcre$, $Id4^{MMTV\Delta/+}$ and $Id4^{MMTV\Delta/\Delta}$ mammary glands, quantified as a percentage of total fat pad ($n = 3$ mice \pm s.d.).

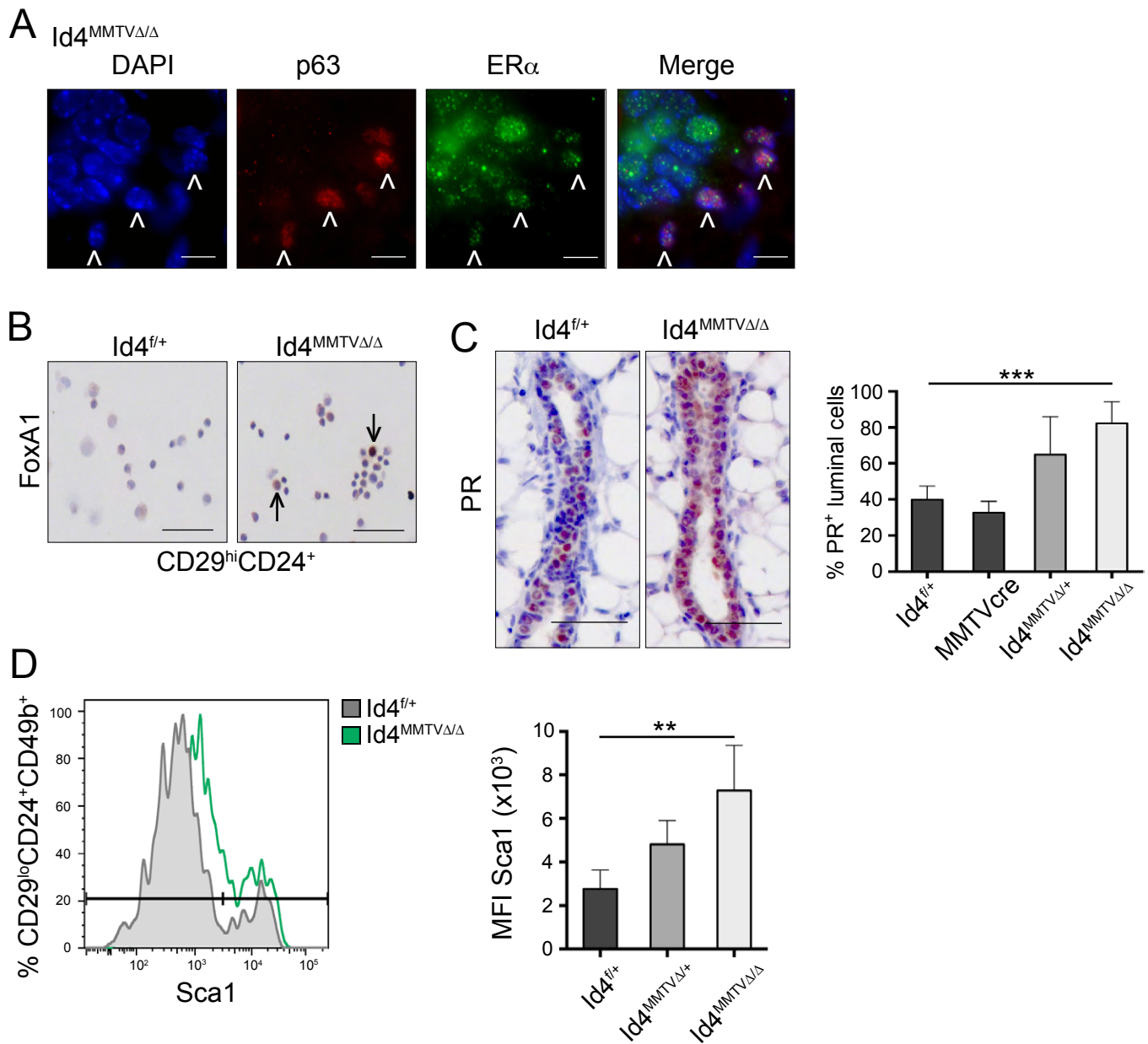


Fig. S2. *Id4*-deficiency affects the luminal population. (A) Co-staining of p63, ER α and DAPI on 6 week-old $Id4^{MMTV\Delta/\Delta}$ mammary sections. Arrows indicate basal cells expressing both p63 and ER α in a representative duct. (B) FoxA1 immunostaining on sorted, cyto-spun MaSC/basal cells (CD29^{hi}CD24⁺) from 6 week-old $Id4^{f/+}$ and $Id4^{MMTV\Delta/\Delta}$ mice (n = 4 mice). Scale bar, 25 μ m. (C) Immunostaining for Progesterone Receptor (PR) expression on sections from 6 week-old $Id4^{f/+}$ and $Id4^{MMTV\Delta/\Delta}$ mice. Bar graph indicates percent PR-positive ductal cells depicted as a proportion of total ductal cells for $Id4^{f/+}$, MMTVcre, $Id4^{MMTV\Delta/+}$ and $Id4^{MMTV\Delta/\Delta}$ mice (n = 7 mice \pm s.d.). (D) FACS analysis of Sca1 expression in the luminal progenitor population (CD29^{lo}CD24⁺CD49b⁺) of 6 week-old $Id4^{f/+}$ and $Id4^{MMTV\Delta/\Delta}$ mice. Bar graph indicates the mean fluorescence intensity (MFI) of Sca1 in the luminal population of 6 week-old $Id4^{f/+}$, $Id4^{MMTV\Delta/+}$ and $Id4^{MMTV\Delta/\Delta}$ mice (n = 4 mice \pm s.d.).

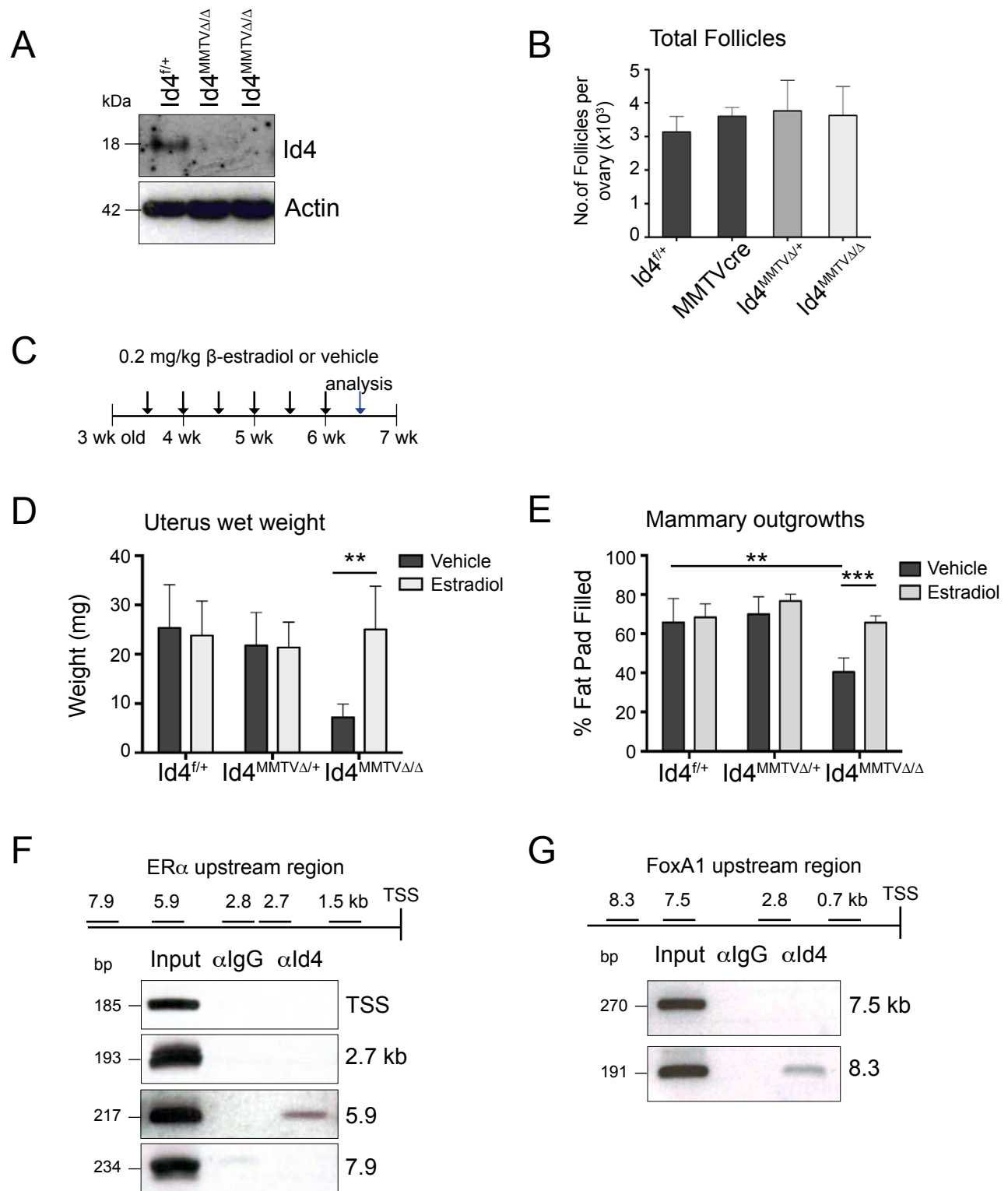


Fig. S3. Exogenous β -estradiol rescues ductal elongation defects in *Id4*-deficient mammary glands. (A) Western blot analysis of *Id4* expression in whole ground ovaries from 6 week-old *Id4*^{f/+} and *Id4*^{MMTV $\Delta\Delta$} mice. Actin provides the loading control. (B) Analysis of the total number of follicles per ovary in 6 week-old *Id4*^{f/+}, MMTVcre, *Id4*^{MMTV Δ /+} and *Id4*^{MMTV $\Delta\Delta$} mice ($n = 3$ mice \pm s.e.m.). (C) Schematic diagram showing days of treatment with β -estradiol: 0.2 mg/kg β -estradiol or vehicle were injected twice per week over three weeks. Mammary glands and ovaries were collected at 6.5 weeks of age for analysis. (D) Uterus wet weight in *Id4*^{f/+}, *Id4*^{MMTV Δ /+} and *Id4*^{MMTV $\Delta\Delta$} mice in the vehicle and estradiol-treated arms at proestrus and estrus ($n = 4$ mice \pm s.d.). (E) Fat pad filling by the mammary ductal tree for *Id4*^{f/+}, *Id4*^{MMTV Δ /+} and *Id4*^{MMTV $\Delta\Delta$} mice in the vehicle and estradiol-treated arms ($n = 4$ mice \pm s.d.). (F) ChIP analysis of *Id4* binding on the *ER α* promoter region in CommaD β geo cells. Input and immuno-precipitations with IgG or *Id4* antibody on promoter regions 2.7, 5.9 and 7.9 kb upstream of the TSS ($n = 2$). (G) ChIP analysis of *Id4* binding on the *FoxA1* promoter region in CommaD β geo cells. Input and immuno-precipitations with IgG or *Id4* antibody on promoter regions 7.5 and 8.3 kb upstream of TSS ($n = 2$).

Table S1. List of primers for genotyping, qRT-PCR analysis and ChIP-PCR

Name	Sequence 5' to 3'	Product Length
MMTVcre Genotyping	CATCACTCGTTGCATCGACC CTGATCTGAGCTCTGAGTG	Transgene: 280 bp
K14cre Genotyping	CGATGCAACGCGTGATGAGGTTC GCACGTTACCGGCATCAAC	Transgene: 350 bp
Id4 floxed Genotyping	GAGCAGCTCTCCGGTCGATTTCTG GGCTGCCGAGCCACCCAGGCTGTGG	WT: 269 bp Floxed: 320 bp
GAPDH qPCR	TGACATCAAGAAGGTGGTGAAGC AAGGTGGAAGAGTGGGAGTTGCTG	117 bp
ERalpha qPCR	CTGTCCGGCTGCGCAAGTGTT CATCTCTCTGACGCTTGTGCT	101 bp
FoxA1 qPCR	GCTGGCTCCAGGATGTTAGGGAC GCTGACAGGGACAGAGGAGTAGG	108 bp
FSH-R qPCR	ACCGCTTGAAAAAGCTCCCT GTTCAAGAGGTTTGCCGCCTC	114 bp
FoxL2 qPCR	AGCCGGCTTTTGTTCATGATGG TACTGGTAGATGCCGGACAGA	250 bp
Cyp11 qPCR	ACATGGCCAAGATGGTACAGTTG ACGAAGCACCAGGTCATTCAC	119 bp
StAR qPCR	CGGGTGGATGGGTCAAGTTC CCAAGCGAAACACCTTGCC	230 bp
Cyp17 qPCR	TGACCAGTATGTAGGCTTCAGTCG TCCTTCGGGATGGCAAACCTC	171 bp
HSD3 β qPCR	TGGACAAAGTATTCCGACCAGA GGCACACTTGCTTGAACACAG	250 bp
Cyp19 qPCR	TGGAGAACAATTCGCCCTTTC TGGTTTGATGAGGAGAGCTTGC	273 bp
TSS ER α ChIP	CCAGTCTGAAATGCAGAG GGCTCAGCAGTTCTTG	185 bp
2.7 kb upstream ER α ChIP	GAGAGGATGTGTGTGCTG GAAGCCAGCCTGATCTAC	193 bp
5.9 kb upstream ER α ChIP	CCCTCACAGAGATATCT CACAAAGGAAGTAAAAGG	217 bp
7.9 kb upstream ER α ChIP	CGCTTGCTTCCTAGCCA GATCTCAGTCTTCTAGC	234 bp
7.5 kb upstream FoxA1 ChIP	CAACCTTCAGTCTCTCTC CTATTCAGGAATGACG	270 bp
8.3 kb upstream FoxA1 ChIP	GCCCTCTTCTCTTGCAGGG GGGAAGACAGCTGTGCTC	191 bp

Table S2. List of antibodies for western blot, FACS analysis and immunohistochemistry

Antigen	Clone	Conjugate	Species	Supplier
Id4	82-12	Unconjugated	Rabbit	Biocheck
p63	4E5	Unconjugated	Mouse	AbCam
Keratin-8	-	Unconjugated	Mouse	Progen
ER α	MC-20	Unconjugated	Rabbit	Santa Cruz
PR	C-19	Unconjugated	Rabbit	Santa Cruz
FOXA1	-	Unconjugated	Rabbit	Abcam
β -Actin	AC-15	Unconjugated	Mouse	Sigma
Mouse IgG	-	HRP	Sheep	GE Healthcare
Rabbit IgG	-	HRP	Goat	GE Healthcare
Rabbit IgG	-	Biotin	Goat	Vector Laboratories
Mouse IgG	-	Biotin	Horse	Vector Laboratories
Rat IgG	-	Biotin	Goat	Vector Laboratories
Rabbit IgG	-	Alexa-647	Donkey	Life Technologies
Mouse IgG	-	Alexa-555	Donkey	Life Technologies
CD31	MEC 13.3	APC	Rat	BD Pharmingen
CD45	30-F11	APC	Rat	BD Pharmingen
TER-119	TER-119	APC	Rat	BD Pharmingen
CD24	M1/69	Pacific Blue	Rat	BioLegend
CD29	HM β 1-1	APCcy7	Hamster	BioLegend
CD49b	HMa2	Biotin	Hamster	eBioscience
CD61	HM β 3-1	APC	Hamster	Life Technologies
Sca1(Ly-6A/E)	E13-161.7	PE	Rat	BD Pharmingen
Streptavidin	-	Qdot-655	-	Life Technologies
Streptavidin	-	APCcy7	-	BD Pharmingen