SUPPLEMENTARY FIGURES

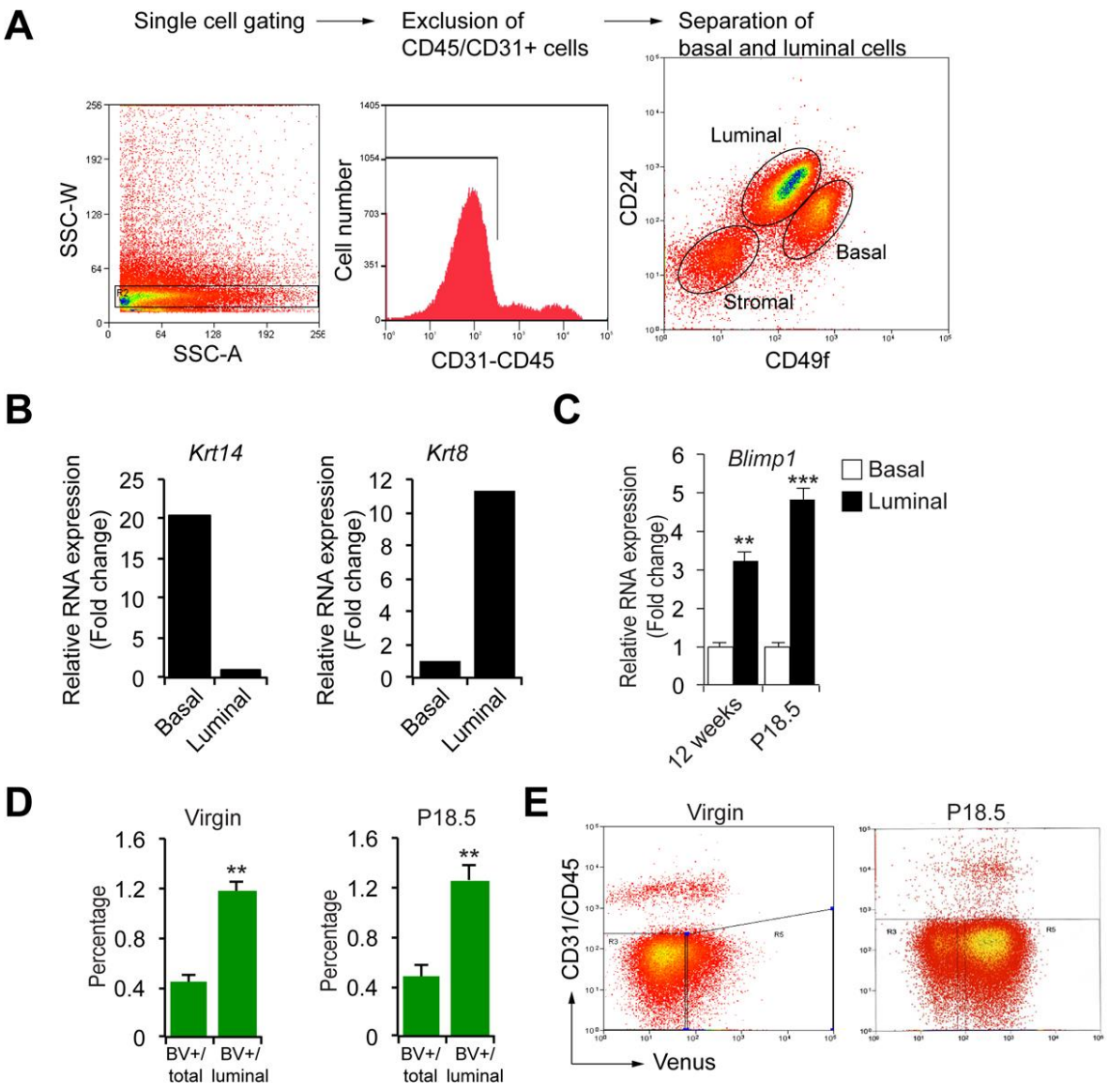

Fig. S1. Characterization of Blimp1-expressing cells using flow cytometry. (A) Gating cascade: samples were gated on forward and side scatter (FSC/SSC) to exclude debris, and single cells were isolated using time-of-flight analysis on both FSC and SSC. $CD45^+$ leukocytes and $CD31^+$ endothelial cells were removed and total epithelial cells gated on a CD24/CD49f plot. Epithelial subpopulations were isolated from the total epithelial cell

population. (B) qRT-PCR analysis of *Krt14* and *Krt18* expression in basal ($CD31^-/CD45^-$ / $CD24^+/CD49f^{high}$) and luminal ($CD31^-/CD45^-/CD24^+/CD49f^{low}$) cell populations isolated from 12-week virgin control mammary glands. Data are presented as mean ± s.e.m values from n=2 independent experiments. (C) qRT-PCR analysis of Blimp1 mRNA in basal *versus* luminal mammary epithelial cells isolated from 12-week virgins and P18.5 mice. qRT-PCR values were normalized to *Hprt* expression. Data are presented as mean ± s.e.m values from n=2 independent experiments, and confirm that Blimp1 is restricted to luminal cells. (D) Histograms showing the percentages of BV^+ luminal cell relative to gated total or luminal cells isolated from 12-week virgin or P18.5 mammary glands. Data are presented as mean ± s.e.m values from n=2 independent experiments. Error bars, s.e.m. ** $p < 0.01$. (E) Representative dot plots showing an enrichment of BV^+ cells within the luminal population from 12-week virgin and P18.5 mammary glands.

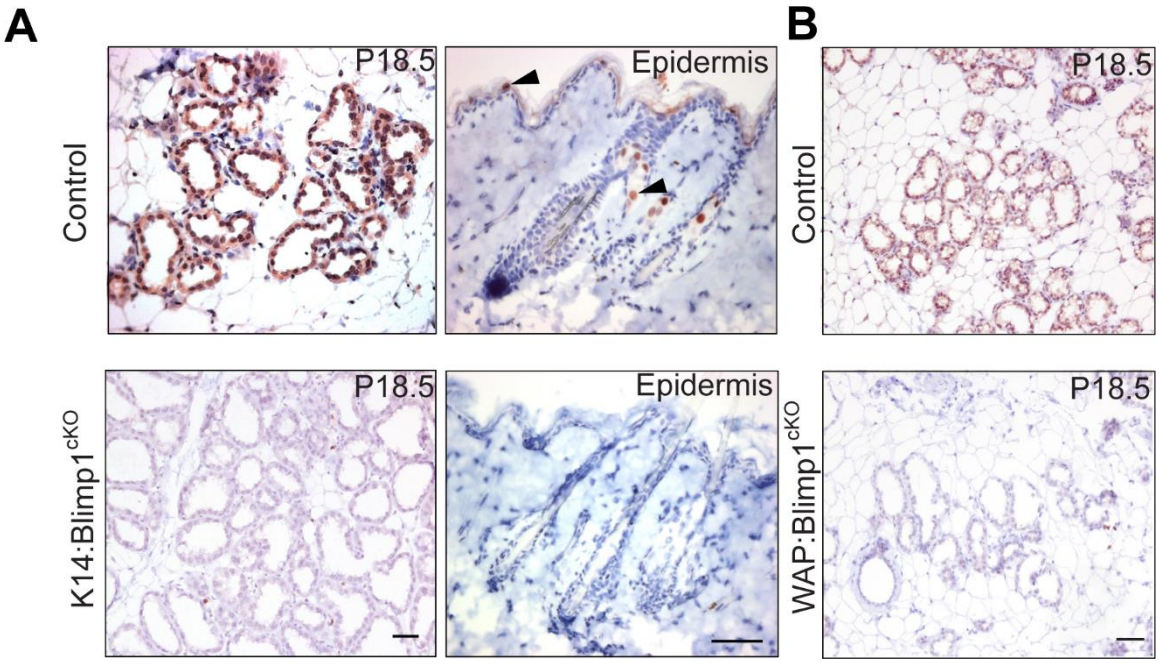

Fig. S2. Efficacy of Cre-mediated of Blimp1 deletion from mammary gland epithelia. (A and B) Immunostaining of mammary glands (P18.5) and epidermis from K14:Blimp1^{cKO} and WAP:Blimp1^{cKO} and littermate control mice (representative of n=3 mice of each genotype). Blimp1 protein was not detected in mammary epithelia of either K14:Blimp1^{cKO} or WAP:Blimp1^{cKO} tissue. Images representative of n=3 mice of each genotype. Arrowheads indicate Blimp1^{+} cells in the hair follicle (sebocytes) and in the granular layer of the epidermis. Scale bars, 50µm.

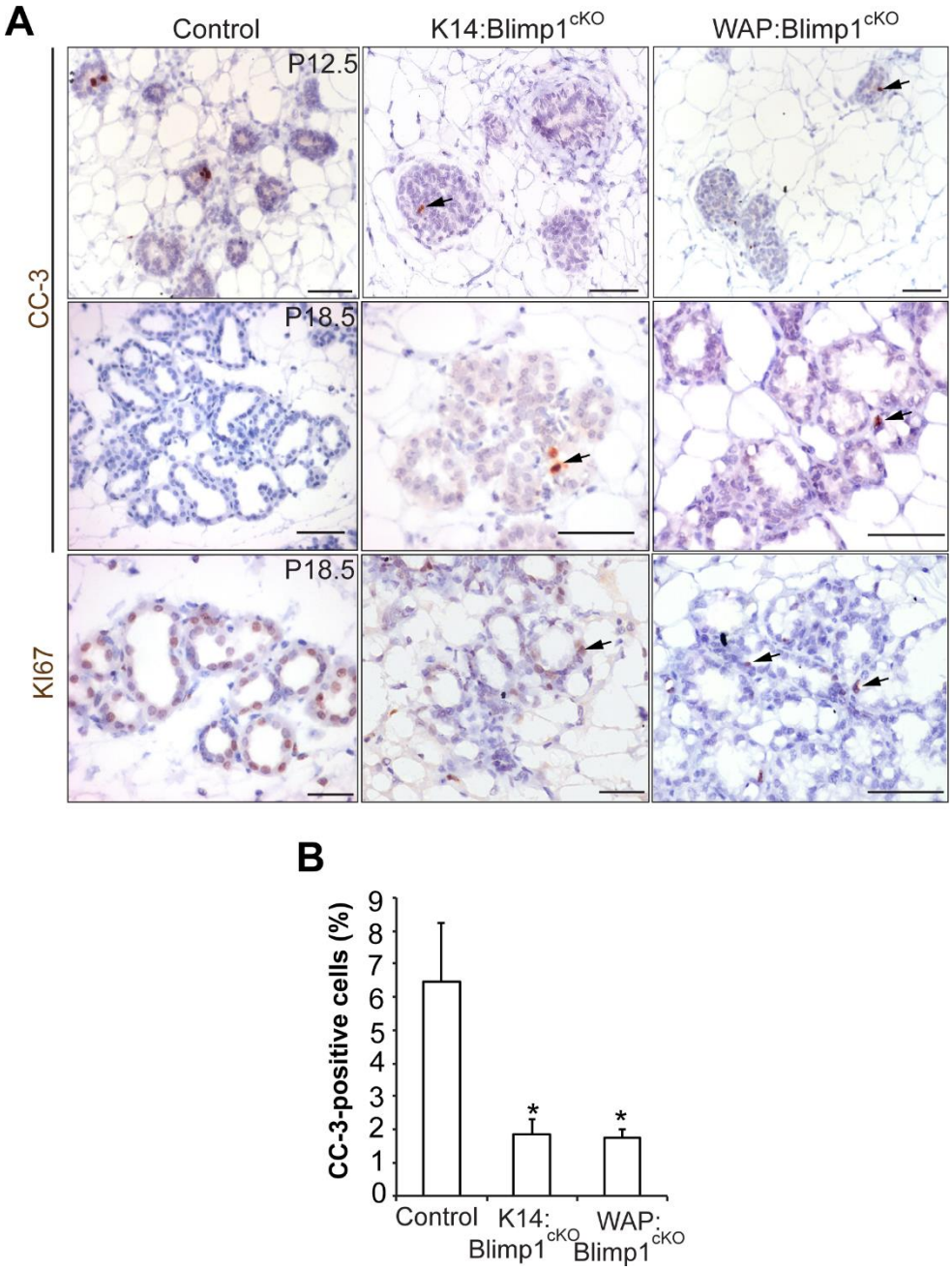

Fig. S3. Loss of Blimp1 alters lumen formation during pregnancy. (A and B) Immunostaining of Blimp1 mutant mammary glands and littermate controls for CC-3 and KI67 at P12.5 and P18.5 (representative images from n=3 mice of each genotype). Immunostaining shows a reduction in the number of CC-3^{+} cells (arrow) at P12.5 mutant mammary glands. Data are presented as mean ± s.e.m values from n=3 mice of each genotype. Error bars represent s.e.m values. * p <0.05. CC-3^{+} cells (arrow) were detected in mutant mammary epithelia at P18.5, while markedly reduced levels of KI67^{+} cells (arrow) were observed in mutant alveoli (representative images from n=3 mice of each genotype). All scale bars, 50µm.

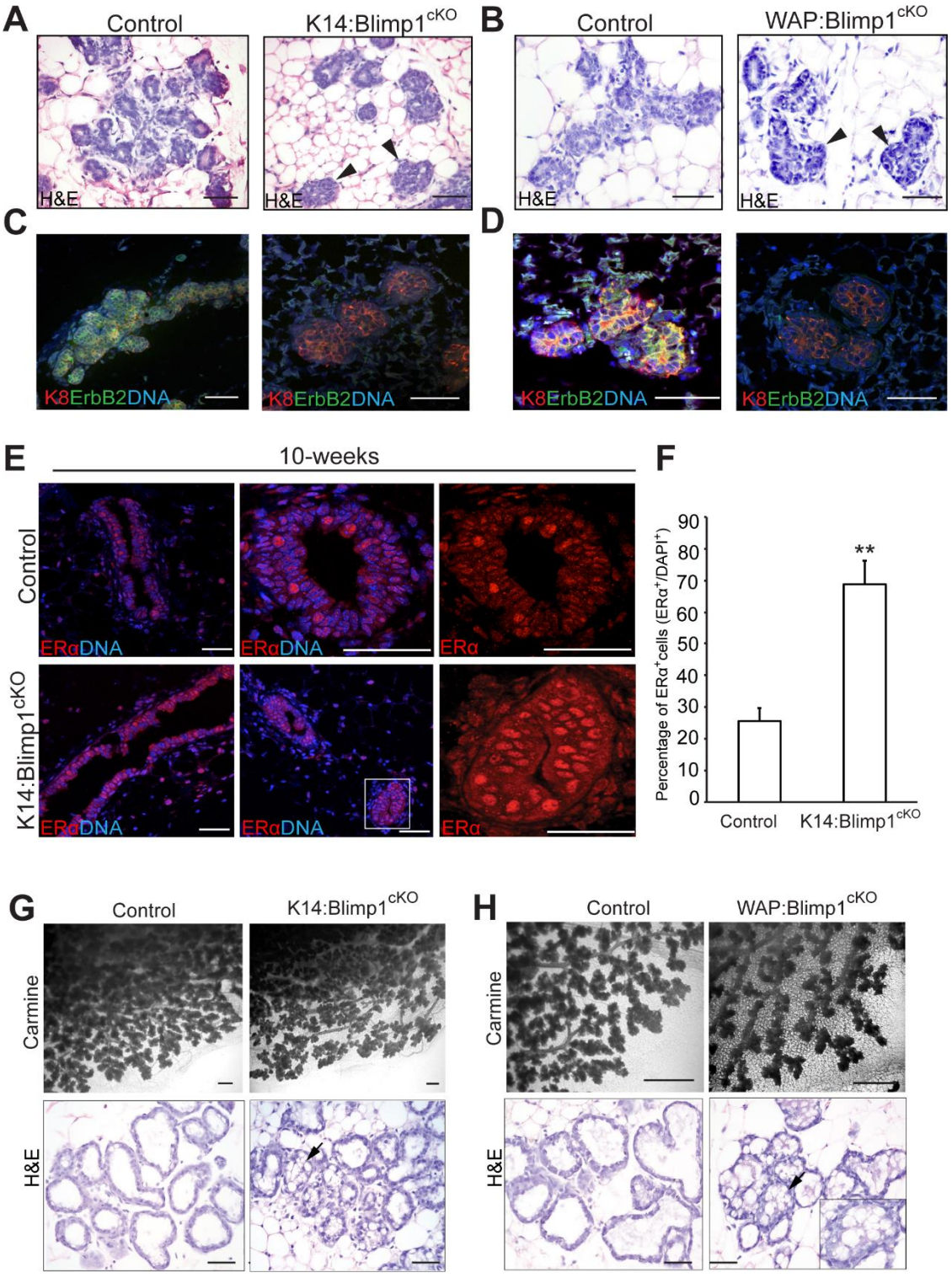

Figure S4. Loss of Blimp1 severely impacts lobuloalveolar development. (A and B) Histological sections of mammary glands at P12.5 from K14:Blimp1^{cKO}, WAP:Blimp1^{cKO} and littermate control mice. Arrowheads indicate collapsed alveoli in mutant mammary

epithelia (representative images from n=3 mice of each genotype). (C and D) Immunofluorescence staining for K8 (red) and ErbB2 (green). (E) Increased number of $ER\alpha^{+}$ cells observed in 10-week-old mutant mammary epithelia (images representative of n=3 mice of each genotype). (F) Histograms showing the percentages of $ER\alpha^{+}$ at 10 weeks of age in control and mutant mammary epithelia's. Data are presented as mean ± s.e.m values from n=3 independent experiments. (G and H) Whole mount carmine staining and histological sections from P18.5 K14:Blimp1^{cKO}, WAP:Blimp1^{cKO} and control littermate mammary glands (representative images from n=3 mice of each genotype). Arrows indicate disorganized and vacuolated alveoli in Blimp1 mutant mammary glands. ** $p < 0.01$. All scale bars, 50 µm.

Table S1: List of Antibodies

Antibody	Supplier	Cat No	Host Species	Dilution	Application
anti-Cleaved-Caspase-3	Cell Signaling	9664	Rabbit polyclonal	1 in 100	IHC
anti-β-casein	Santa Cruz Biotechnology	sc-30042	Rabbit polyclonal	1 in 100	IF
anti-Blimp-1	Santa Cruz Biotechnology	sc-130917	Rat monoclonal	1 in 500	IHC/IF/ICC
anti-BrdU	Becton Dickinson	347580	mouse monoclonal	1 in 100	IF
anti-CD24-PerCP-Cy5.5 clone M1/69	BD Pharmingen	562360	Rat monoclonal	1 in 200	FACS
anti-CD31-APC clone MEC13.3	BD Pharmingen	551262	Rat monoclonal	1 in 200	FACS
anti-CD45-APC clone 30-F11	BD Pharmingen	559864	Rat monoclonal	1 in 200	FACS
anti-CD49f-PE clone GoH3	BD Pharmingen	561894	Rat monoclonal	1 in 50	FACS
anti-Elf5	Santa Cruz Biotechnology	sc-9645	Goat polyclonal	1 in 100	IF
anti-ErbB2	Abcam	ab2428	Rabbit polyclonal	1 in 100	IF
anti-ERα	Santa Cruz Biotechnology	sc-542	Rabbit polyclonal	1 in 200	IHC/IF
anti-GFP-A488-conjugate	Invitrogen/MP	A-31851	Rabbit polyclonal	1 in 400	IF
anti-GFP	Abcam	Ab13970	chicken polyclonal	1 in 1000	IF
anti-Keratin 14	Cambridge Bioscience	PRB-155P	Rabbit Polyclonal	1 in 2000	IF
anti-Keratins 8	Progen Biotechnik	GP11	Guinea-Pig polyclonal	1 in 50	IF
anti-Ki67	Abcam	ab15580	Rabbit polyclonal	1 in 100	IF
anti-p63	Abcam	ab735	Mouse monoclonal	1 in 40	IF
Phalloidin-Alexa Fluor 633	Invitrogen/MP	A22284		1 in 100	IF/ICC
anti-pStat5 (Tyr694)	Cell Signaling	9314	Rabbit polyclonal	1 in 100	IHC
anti-WAP	Santa Cruz Biotechnology	sc-25526	Rabbit polyclonal	1 in 100	IF
GM130	BD Pharmingen	610822	Mouse monoclonal	1 in 200	IF
Par3	Millipore	07-330	Rabbit polyclonal	1 in 200	IF
Podocalyxin	R&D systems	MAB1556	Rat polyclonal	1 in 200	IF

Table S2: List of Primers

Gene	Protein	Epithelial compartment	Primer sequence
Prdm1	Blimp1	Luminal	Forward: GGCTCCACTACCCTTATCCTG; Reverse: TCCTTTTGGAGGGATTGGAGTC
Venus	Yellow fluorescent protein (YFP)		Forward: CGACTCTAGATCATAATCAGCC; Reverse: TAAGATACATTGATGAGTTTGGAC
Elf5	E74-like factor 5 (ELF5), luminal progenitors		Forward: CCAACGCATCCTTCTGTGAC; Reverse: AGGCAGGGTAGTAGTCTTCA
Esr1	Estrogen receptor 1 (ER1), mature luminal cells		Forward: CTGGACAGGAATCAAGGTAAA; Reverse: GAGGCACACAAACTCTTCTC
WAP	Whey acidic protein (Milk protein)		Forward: AACATTGGTGTTCCGAAAGC; Reverse: GGTCGCTGGAGCATTCTATC
Csn2	Beta casein (Milk protein)		Forward: TGCAGGCAGAGGATGTGCTCCAGGC; Reverse: GGCCTGGGGCTGTGACTGGATGCT
Krt8/18	Cytokerain 8/18		Forward: CGAGGCACTCAAGGAAGAAC-; Reverse: AATCTGGGCTTCCAGACCTT
Pgr	*Progestrone Receptor*		Forward: CCACCTGTACTGCTTGAATAC, Reverse: CAACTGGGCAGCAATAACTTC
Krt14	Cytokerain 14	Basal	Forward: GCTCTTGTGGTATCGGTGGT; Reverse: GAGGAGAAGCGAGAGGAGGT
Hprt	Hypoxanthine-guanine phosphoribosyltransferase (Hprt)		Forward: GCTGGTGAAAAGGACCTCT; Reverse: CACAGGACTAGAACACCTGC