Supporting materials

Supporting table: PCR primers.

	Genes	Forward (from 5' to 3')	Reverse (from 5' to 3')	NCBI reference seq.
Guinea pig	*Ar*	CTACTACAACTTTCCGCTACCC	CAGAGTCATCCCTGCTTCATAG	XM_063245991.1
	Esr1	AGCGACAAGGGAAGCATAAC	CTGATCGTGGAGGGTCAAATC	XM_003468423.3
	Actb	AGAGGTATCCTGACCCTGAAA	AGCACTGTGTTGGCATAGAG	NM_001172909.1
	Egf	CACAGCCATCCTTTCCATCT	TACCCGCCAGGACAATAAATC	XM_013150606.3
	Klk1	CGTGCTACAAGGCATCATCT	CTCGTGGTACTCTGTCACTTTG	XM_003465406.4
	Klk2	GGTTCTGTGCCTTGTCCTATTC	CTGGGAATGCTTCTCACACTC	XM_063252406.1
	Ccnd1	TCGATTCCAACGCACTCTTATC	CCTCCCTTCAACACTTCCTAAA	XM_003468321.5
	Ctnnb1	CTGACCTGTAACCCTCCTTTAG	GCAAGGCTAGGGTCTGATAAA	XM_063260962.1
	Wisp2	CTGTAAACTCATTAGGAGGCTATGT	AATTCAGCCACTCAGTTCTCTC	XM_063248285.1
	Wnt4	CTAAGGCCTAGGGCTGAATAAA	GGATGTCACAATGGCAAGAAG	XM_063235714.1
	Gapdh	ACAGTGACAGCCATTCTTCC	AGCCGAACTCATTGTCATACC	XM_063237559
Mouse	*Ar*	CATGCACAAGTCTCGGATGTA	GAAGGAGAAGCCCAGGAAATAG	NM_013476.4
	Esr1	CTGGCTGGAGATTCTGATGATT	CGTAGCAAAGGGAGAAAGAGAG	NM_001302531.2
	Actb	GAGGTATCCTGACCCTGAAGTA	GCTCGAAGTCTAGAGCAACATAG	NM_007393.5
	Egf	ACTGGTGTGACACCAAGAGGTC	CCACAGGTGATCCTCAAACACG	NM_010113.4
	Klk1b3	GGATGGAGGCAAAGACACTTGC	TGGTGTAGACGCCTGGCATATC	NM_008693.2
	Klk1b21	GGAGTCCTGTTGAACCCCAACT	TGACCAATCGGTGCTGAGCAGA	NM_010642.3
	Ccnd1	GCAGAAGGAGATTGTGCCATCC	AGGAAGCGGTCCAGGTAGTTCA	NM_007631.3
	Ctnnb1	GTTCGCCTTCATTATGGACTGCC	ATAGCACCCTGTTCCCGCAAAG	NM_007614.3
	Wisp2	TGTGTGACCAGGCAGTGATGCA	CAGGCTGTGCTCCAGTTTGGAC	NM_016873.2
	Wnt4	GAGAACTGGAGAAGTGTGGCTG	CTGTGAGAAGGCTACGCCATAG	NM_009523.2
	Gapdh	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTCAG	NM_008084.4

Ar, Esr1 and *Actb* primers are reverse transcription PCR (RT-PCR) primers, and the products span introns. All other primers are quantitative PCR (qPCR) primers. Guinea pig qPCR primer sequences were designed using PrimerQuest tool from Integrated DNA Technologies, Inc. (IDT, Coralville, Iowa, USA). Mouse qPCR primer sequences were obtained from OriGene Technologies, Inc, (Rockville, MD, USA).

Supporting figures

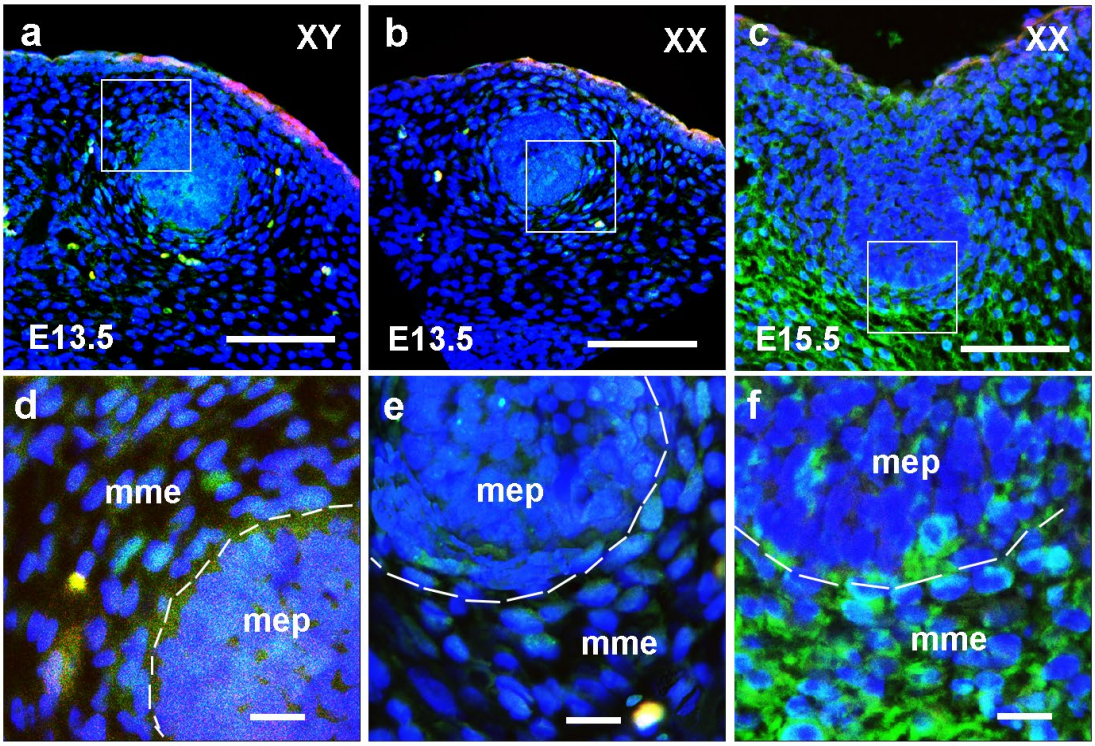

Figure S1. **ERα protein localization in developing mammary glands (MGs) of embryonic mice.**

(*a-c*) ERα immunofluorescence in MGs of E13.5 male (*a*), female (*b*) and E15.5 (female) mice. ERα signal is green, and Dapi is blue. Red color is non-specific staining. (*d-f*) are amplified images from square-marked regions in (*a-c*), respectively. Stages are indicated at bottom left of each image, and sex is indicated by chromosome symbols XY (male) and XX (female) at top right of each image in all supporting figures. Broken white lines in (*d-f*) indicate boundary of mammary epithelium and mesenchyme. *mep*, mammary epithelium; *mme*, mammary mesenchyme. Scale bars: *a-c*, 20μm; *d-f*, 5μm.

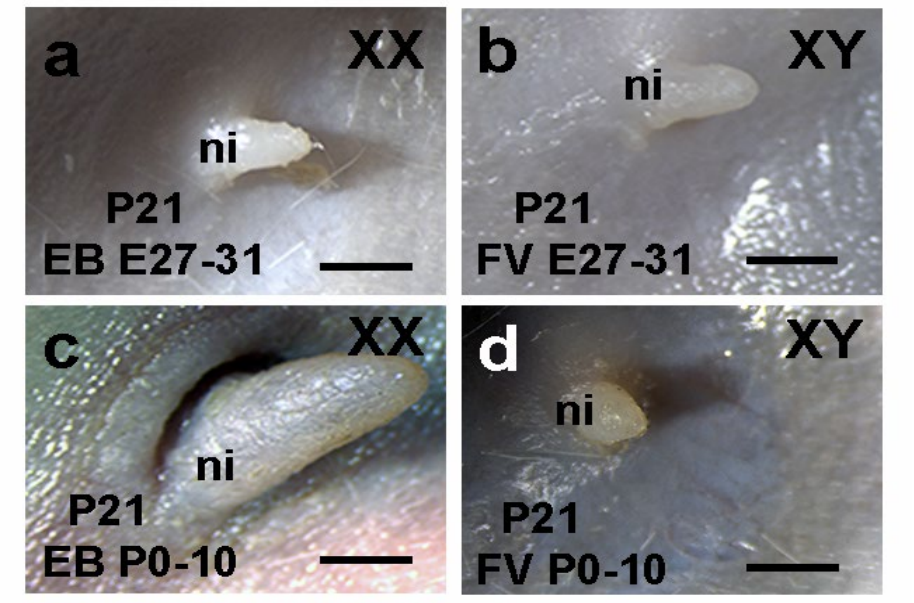

Figure S2. **The effect of estrogen signaling on nipple development in guinea pigs.**

Images show nipples of prenatally estradiol benzoate (EB)-treated female (A), prenatally fulvestrant (FV)-treated male (B), neonatally EB-treated female (C) and neonatally FV-treated male (D) P21 guinea pigs. Note that neonatal, but bot prenatal EB treatment increased nipple size. *ni*, nipple. Scale bars: 2mm.

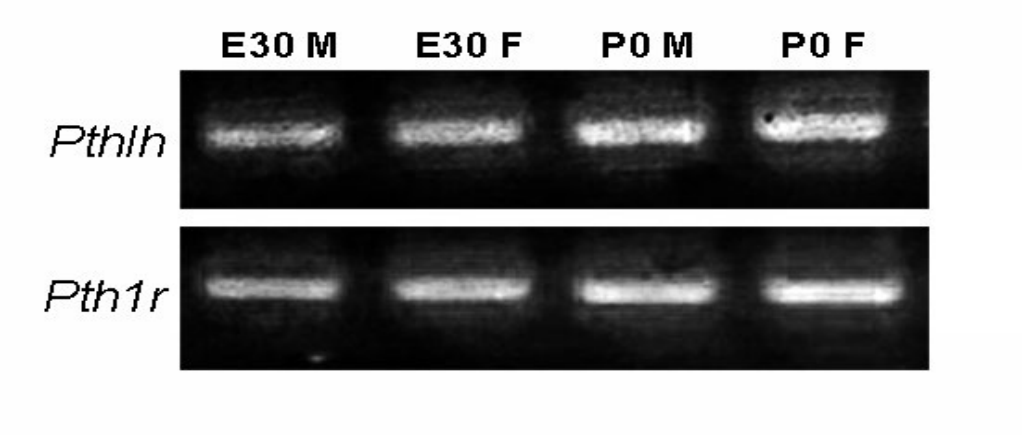

Figure S3. ***Pthlh* and *Pth1r* expression were detected in developing MGs of E30 and newborn (P0) guinea pigs of both sexes.**
cDNA fragments of *Pthlh* and its receptor gene *Pth1r* were detected using RT-PCR followed by agarose gel electrophoresis and ethidium bromide staining. n=3. *M*, male; *F*, female.