ZBTB20 Regulates Prolactin Expression and Lactotrope Function in Adult Mice

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Supplemental Table 1. Sequence of the primers used for genotyping

Primer	Sequence (5' to 3')	PCR product
J_F	CTGTGGATGCCACCTCTGATG	2905bp
J_R	ACAGTGGGAGTGGCACCTT	
5J_F	GGCTGATGGTAGAATTCTAGAG	1891 bp
5J_R	GGCTTGCAGGTACAGGAGGTAG	
3J_R	TACAGTATGGTTTGTCCTCAATCAAGACC	1865 bp
P3	GCATCGATACCGTCGACCTC	
P1	GCTAATTGACTTGTTGGACCCTAC	P1/P2:621 bp
P2	GTATCGATACCGTCGACCTC	P3/P2:499 bp

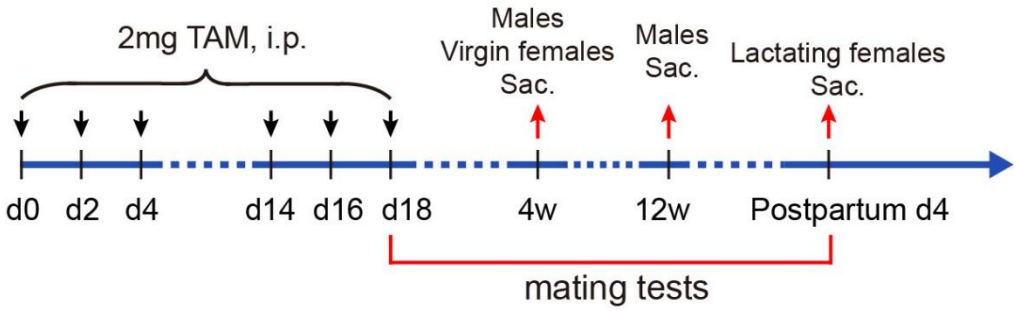
Supplemental Table 2. Primer sequence for RT-PCR analysis

Gene	Orientation	Sequence (5'-3')	PCR product
CreER	Forward	ACAGATGCCAGGACATCAGGAA	237 bp
	Reverse	CAGCCACACCAGACACAGAGA	
Prl	Forward	CTCACTACATCCATACCCTG	242 bp
	Reverse	TTGAATACCACCTACTCCA	
Gh	Forward	CTTCTCGCTGCTGCTCATC	213 bp
	Reverse	TTGGCGTCAAACTTGTCATA	
Tshβ	Forward	GTAGTGGGTGGAGAAGAGT	180 bp
	Reverse	CAGATGGTGGTGTTGATG	
Zbtb20	Forward	GCAGCCGGCAGCCCCTTCTTC	410 bp
	Reverse	CGCTCGCCGCTGCCATTCTG	
Pou1f1	Forward	TCCCCAGAAATCCGAGAACT	350 bp
	Reverse	TGCGAGGAAGGCTTGCTGTGC	
Drd2	Forward	ACCTGTCCTGGTACGATGATG	105 bp
	Reverse	GCATGGCATAGTAGTTGTAGTGG	
Erα	Forward	GCGGCATACGGAAAGACC	430 bp
	Reverse	ATGGAGCGCCAGACGAGAC	
Actb	Forward	CCCTAAGGCCAACCGTGAAAAGAT	431 bp
	Reverse	ACCGCTCGTTGCCAATAGTGATGA	

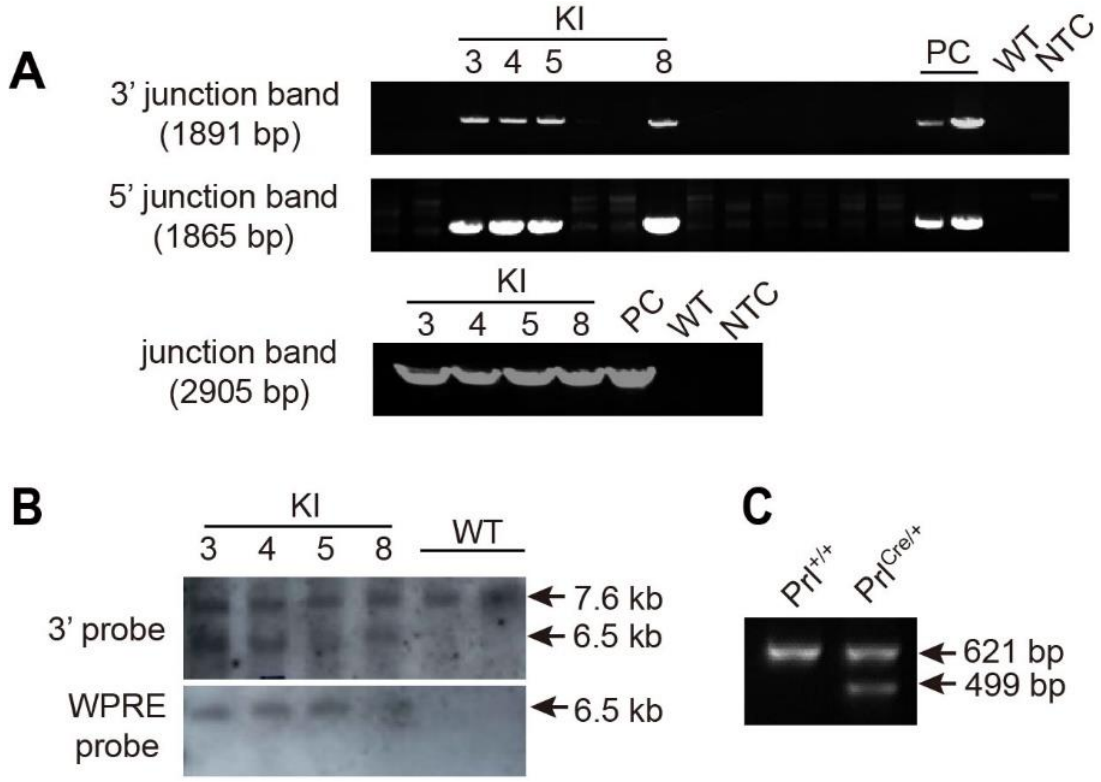
Supplemental Table 3. Reproductive performance of female mice.

Genotype	Treatment	Pregnancy rate (%)	Intervals from mating to first litter (days)	Litter size (n)
Prl$^{+/+}$	No	100 (9/9)	25.78 ± 1.92	6 ± 0.81
Prl$^{Cre/+}$	No	100 (9/9)	23.78 ± 0.98	6 ± 0.55
Control	Tamoxifen	42.9 (6/14)	77.67 ±7.55	3.33 ± 0.5
ZB20-cKO	Tamoxifen	47.1 (8/17)	89.63 ± 9.75	2.50 ± 0.5

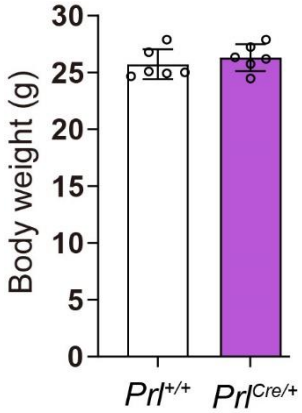
Data are expressed as mean ± SEM. No significant difference was observed between *Prl*$^{Cre/+}$ and *Prl*$^{+/+}$, nor between ZB20-cKO and Control.



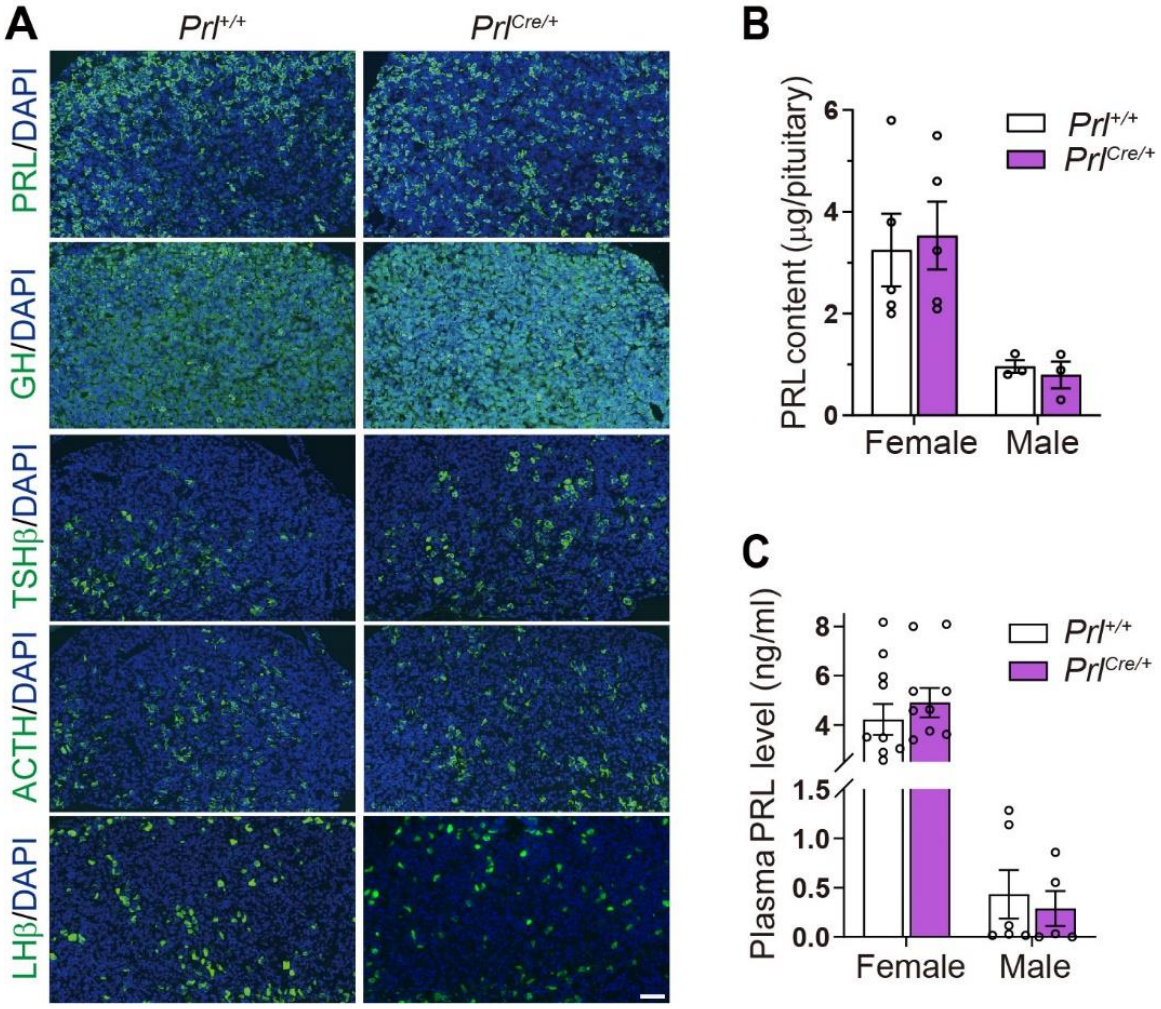
Supplemental Fig. 1. Protocol for tamoxifen treatments and samples collection. The time point when the adult mice were initially administered tamoxifen injection was designated as d0. Totally, two rounds of tamoxifen induction were carried out. Mice were sacrificed at 4 weeks or 12 weeks after initial induction for male mice and virgin female mice. After tamoxifen induction, most females were mated with male breeders until being pregnancy, and the lactating females were sacrificed at 4 days after pup delivery. See the Materials and Methods section for the details. TAM, tamoxifen.



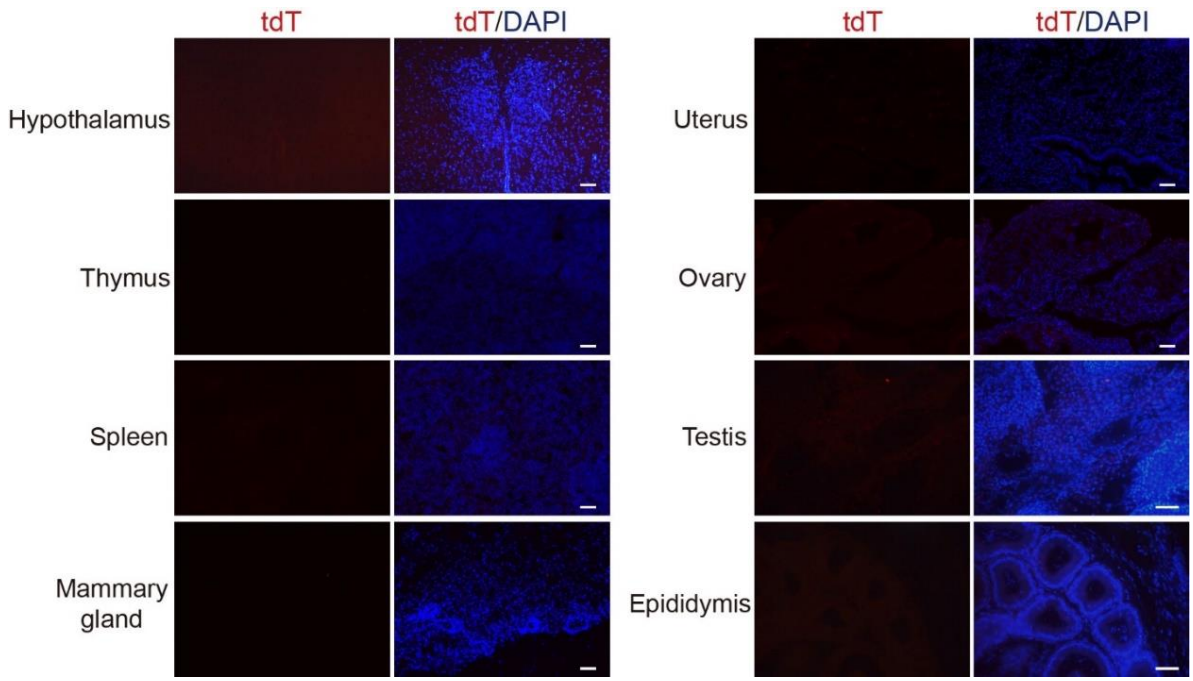
Supplemental Fig. 2. Genotyping of *Prl-CreER* knock-in mice. (A) Tail junction PCR of mouse tail genomic DNA in F1 heterozygous mice using 3 pairs of primers. F1 mice KI-3, -4, -5 and -8 had positive Cre insertion. WT, wild-type; KI, knock-in; PC, positive control; NTC, no-template control. **(B)** Southern blot of genomic DNA in F1 heterozygous mice. The 7.6 kb wild-type band and 6.5 kb knock-in band were identified using 3'-probe, and only knock-in allele generated a 6.5 kb band using WPRE probe. **(C)** Mouse genotyping was performed using tail DNA from offspring mice by PCR with three primers (P1-P3). The wild-type allele produced a 621 bp band, and knock-in allele generated a 499 bp band.



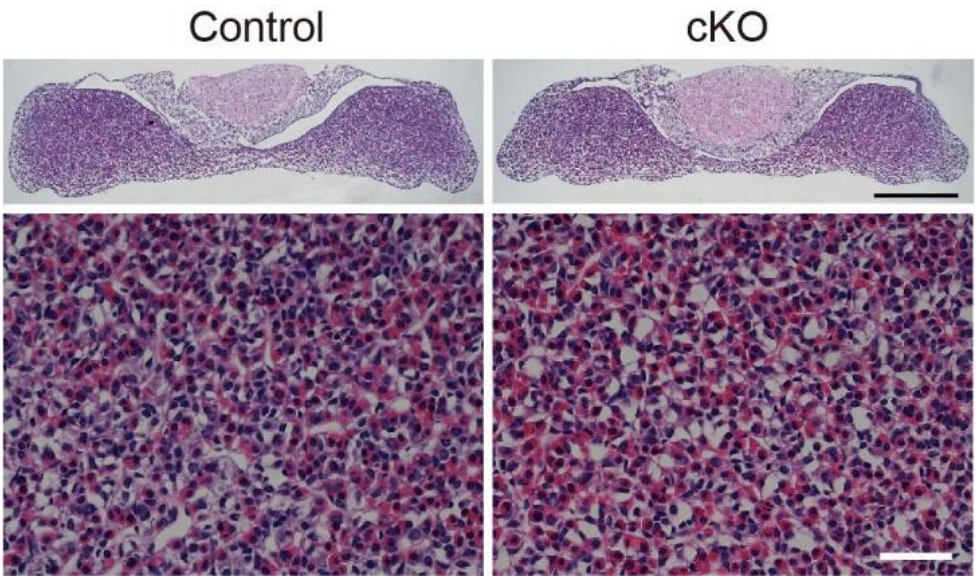
Supplemental Fig. 3. Female *Prl-CreER* hemizygotes exhibit normal body weight. Body weight of *Prl*$^{Cre/+}$ females and their wild-type littermates were measured at age of 2-3 months. n=6. Values represent mean ± SEM.



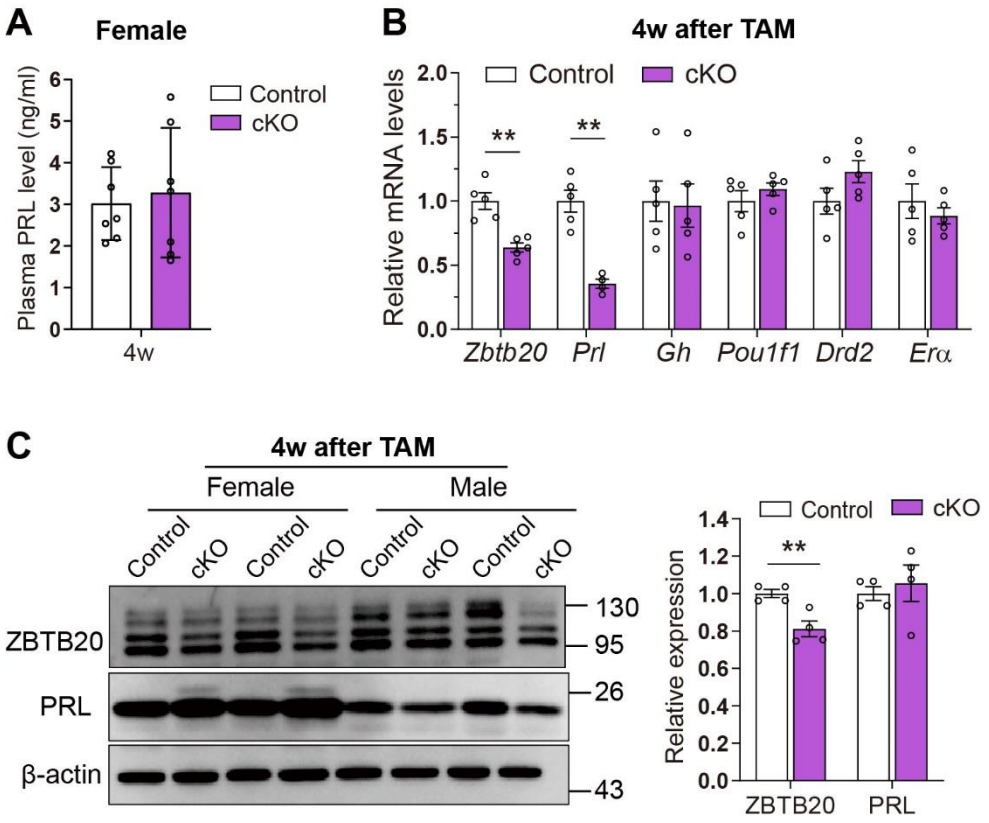
Supplemental Fig. 4. The pituitary from *Prl-CreER* hemizygote has normal cell composition and PRL production. (A) All five types of endocrine cell types in anterior pituitary were detected by immunohistochemical staining using anti-PRL, -GH, -TSHβ, -ACTH and -LHβ antibodies in adult wild-type and hemizygous *Prl-CreER* mice. Scale bar, 50 μm. **(B-C)** PRL content in pituitary extracts **(B)** and plasma PRL level **(C)** were measured by ELISA in adult wild-type and hemizygous mice. n=5 females and 3 males in (B); n=10 females and 6 males in (C). Values represent mean ± SEM.



Supplemental Fig. 5. Absence of Cre recombinase activity in extra-pituitary tissues from Prl-CreER mice. The tdTomato fluorescence signal (red) was undetectable in indicated tissues of adult *Prl*$^{CreER/+}$; *Rosa*$^{tdT/+}$ mice at 4 weeks after tamoxifen induction. Cell nuclei were stained with DAPI (blue). Scale bar, 50 μm.



Supplemental Fig. 6. Adult ZB20-cKO mice exhibit normal morphology and structure of pituitary. Hematoxylin and eosin (H&E) staining for the sections of pituitaries from adult wild-type and ZB20-cKO mice at 4 weeks after tamoxifen induction. cKO, ZB20-cKO. Scale bar, 200 μm for the upper panel and 20 μm for the lower panel.



Supplemental Fig. 7. Adult ZB20-cKO mice exhibit normal PRL protein levels in the pituitary and plasma at 4 weeks after tamoxifen induction. (A) Plasma PRL levels in ZB20-cKO and control females at 4 weeks after tamoxifen induction. n=7. **(B)** Relative mRNA levels of the indicated genes in control and ZB20-cKO pituitaries were detected by real-time RT-PCR and normalized to *Actb* at 4 weeks after tamoxifen induction. n=4~5. **(C)** PRL and ZBTB20 protein levels in both female and male pituitaries were detected by Western blot at 4 weeks after tamoxifen induction. Relative protein levels were analyzed by gray scanning and normalized to β-actin. n=4. Values represent mean ± SEM. $^{**}P < 0.01$ (Student's *t*-test). cKO, ZB20-cKO.