Supporting Information

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SI Methods

Animals and Cell Lines. Generation of Pact-deficient mice was described previously (1). All Pact$^{-/-}$ mice used in this study were those backcrossed into the C57BL/6 background for a minimum of 10 generations. All experiments were approved by the CCF Institutional Animal Care and Ethics Committee. Rat GH3 cells were obtained from American Type Culture Collection (ATCC) and LβT2 cells were a kind gift of John Nilson, Washington State University.

Whole-Mount Mammary Gland Analysis. Mammary glands were prepared as described (2).

Hormone Measurements. Serum FSH and LH levels were measured by the standard protocols of the Reproductive Endocrine Laboratory (Colorado State, CO). Serum progesterone levels were measured by RIA using a commercial direct ^{125}I Progesterone kit (Pantex), which was previously validated for use with mouse serum (3).

Histology and Immunohistochemistry. Tissues were fixed by immersion in Histochoice fixative (Sigma) overnight, dehydrated through a graded-ethanol series, and embedded in paraffin. Five-micrometer sections were cut and stained with hematoxylin and eosin (H&E). Postnatal-day 1 heads were placed in 2-methylbutane cooled in a dry ice bath and stored at -80 °C before cryosectioning. For Ki67 immunostaining, cryosections were fixed in 4% paraformaldehyde and antigen retrieval performed by boiling for 10 min in 10 mM sodium citrate, pH 6.0. Goat anti-mouse Ki67 (Santa Cruz Biotechnology) and FITC-conjugated donkey anti-goat IgG were used to detect Ki67 protein. For identification of pituitary cell lineages, the following antibodies were obtained from the National Hormone and Peptide Program (NHPP), National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and Dr. Parlow: rabbit anti-mouse GH (1:200); rabbit anti-human ACTH (1:200); rabbit anti-rat PRL (1:200); rabbit anti-mouse TSH (1:1,000), rabbit anti-mouse FSH (1:200) and guinea pig anti-rat LH (1:200). For immunofluorescence, pituitary sections with deparaffinized, rehydrated, blocked in PBS with BSA and incubated with primary antibody overnight at 4 °C.

Superovulation. Immature female mice (25 days) were primed with pregnant mare serum gonadotrophin (PMSG, 5 IU/100 μL, ip; Sigma), and given human chorionic gonadotrophin (hCG,100 μL, ip; Ayerst APL) 48 h later. The Wt or Pact$^{-/-}$ mice were then mated to proven breeder Wt males and monitored for vaginal plugs the next morning. Oocytes from oviducts were collected into M2 medium and counted.

Primer Sets for Quantitative RT-PCR. Real-time quantitative PCR was performed using the following primer sets (4): Growth hormone 5′TGAGAAACTGAAGGACCTGGAAGAG3′; 5′GTTGGCGTCAAACTTGTCATAGG3′; 18S rRNA 5′ATTGACGGAAGGGCACCACCAG3′, 5′CAAATCGCTCCACCAACTAAGAACG3′; and PACT 5′TCCTCAGTCTCCCGAACAC3′, 5′CTAAGTCGCTCCAAGCCTAC3′ (located within the coding region of PACT domain 3) (5).

Pituitary Extract Preparation/Western Blotting. Pituitary extracts were made by homogenizing the tissue on ice in buffer containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 1% Nonidet P-40, 0.2 mM PMSF, 100 U/mL aprotinin, and 20% glycerol. Rabbit polyclonal antibody against PACT was raised against full-length recombinant PACT by the Hybridoma Core Facility at the Cleveland Clinic Foundation (6). The cell extract was used in western blot analysis with anti-PACT polyclonal antibody, anti-β-actin monoclonal antibody (Sigma), or anti-Ki67 antibody (Santa Cruz Biotechnology).

Pituitary Extract Preparation/Caspase 3/7 Assay. Pituitaries from Pact$^{+/+}$ or Pact$^{-/-}$ mice were isolated and flash frozen in liquid nitrogen. Pituitary extracts were made by homogenizing the tissue on ice in buffer containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 1 mM EDTA, 1 mM DTT, 1% Nonidet P-40, 0.2 mM PMSF, 100 U/mL aprotinin, and 20% glycerol. Caspase 3/7 activity was measured directly on these extracts as described in Materials and Methods.

Statistical Analysis. Student's *t* test was used for statistical analysis to compare the means between Pact$^{+/+}$ and Pact$^{-/-}$ mice.

1. Rowe TM, et al. (2006) A role of the double-stranded RNA-binding protein PACT in mouse ear development and hearing. *Proc Natl Acad Sci USA* 103:5823–5828.
2. Landis MD, Seachrist DD, Abdul-Karim FW, Keri RA (2006) Sustained trophism of the mammary gland is sufficient to accelerate and synchronize development of ErbB2/Neu-induced tumors. *Oncogene* 25:3325–3334.
3. Mann RJ, Keri RA, Nilson JH (1999) Transgenic mice with chronically elevated luteinizing hormone are infertile due to anovulation, defects in uterine receptivity, and midgestation pregnancy failure. *Endocrinology* 140:2592–2601.
4. Rosenberg LA, Schluchter MD, Parlow AF, Drumm ML (2006) Mouse as a model of growth retardation in cystic fibrosis. *Pediatr Res* 59:191–195.
5. Rowe TM, Sen GC (2001) Organizations and promoter analyses of the human and the mouse genes for PACT, the protein-activator of the interferon-induced protein kinase, PKR. *Gene* 273:215–225.
6. Marques JT, White CL, Peters GA, Williams BR, Sen GC (2008) The role of PACT in mediating gene induction, PKR activation and apoptosis in response to diverse stimuli. *J Interferon Cytokine Res* 28:469–476.

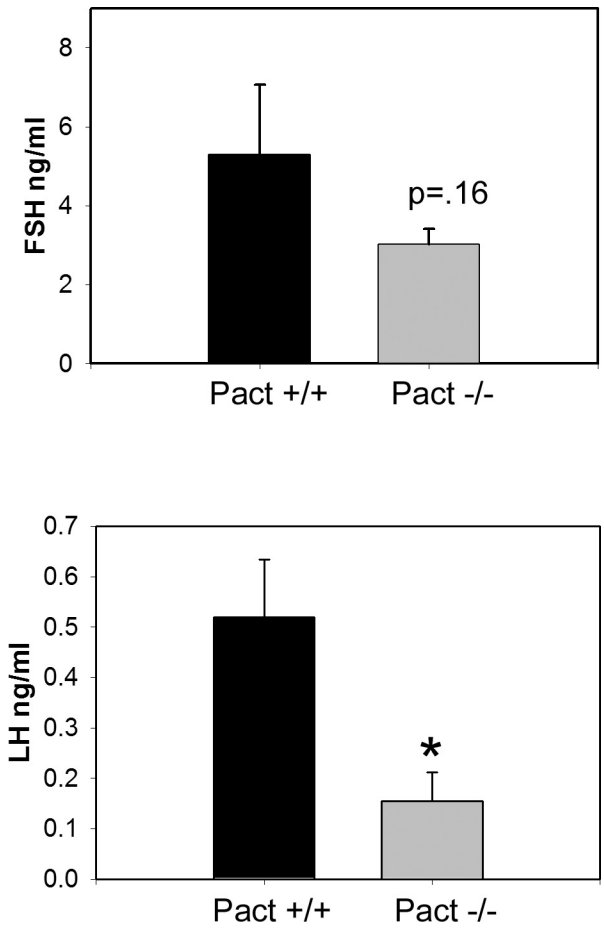

Fig. S1. Low FSH and LH levels in Pact$^{-/-}$ mice. (*A*) Serum FSH levels in female Pact$^{+/+}$ and Pact$^{-/-}$ mice, measured by RIA. $P = 0.16$, when comparing Pact$^{+/+}$ vs. Pact$^{-/-}$ mice; $n = 8$–11 mice per each group. (*B*) Serum LH levels in female Pact$^{+/+}$ and Pact$^{-/-}$ mice, measured by RIA. $n = 7$–8 mice per each group. * = $P <$ 0.05, when comparing Pact$^{+/+}$ vs. Pact$^{-/-}$ mice.

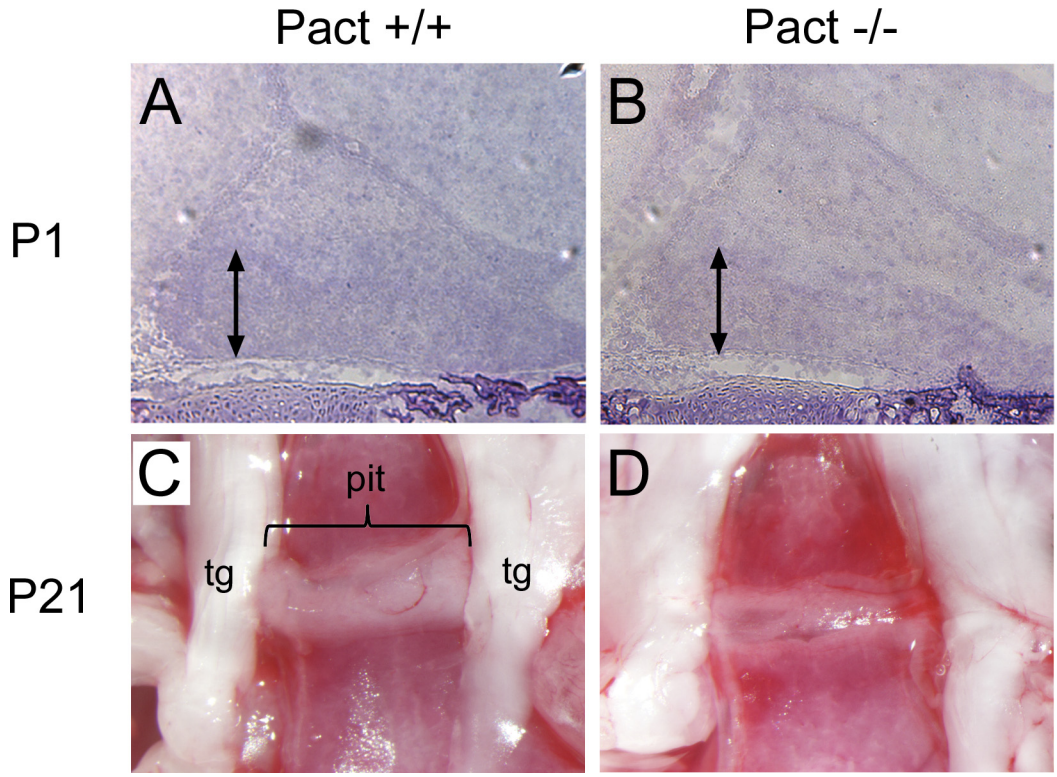

Fig. S2. Size of anterior pituitary lobe in Pact$^{-/-}$ mice is normal at birth. H&E staining of head sections from postnatal day 1 (P1) Pact$^{+/+}$ (*A*) and Pact$^{-/-}$ (*B*) littermates. Arrows indicate the location of the anterior pituitary lobe, showing similar size in Pact$^{+/+}$ and Pact$^{-/-}$. Light micrographs showing P21 pituitaries from (*C*) Pact$^{+/+}$ or (*D*) Pact$^{-/-}$ littermates. Hypoplasia is apparent in Pact$^{-/-}$ mice by day P21. pit, pituitary; tg, trigeminal nerve.

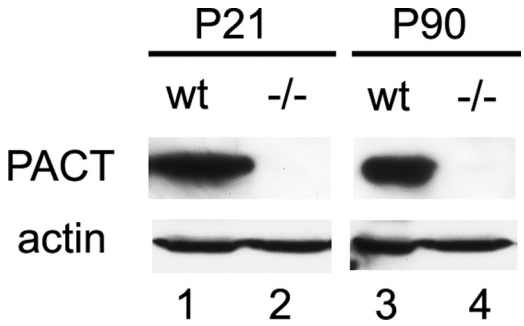

Fig. S3. PACT protein is expressed in pituitary of postnatal day 21 and day 90 Wt mice. Western blotting using anti-PACT or anti-actin antibodies was done on pooled pituitary extracts. Pact +/+, $n = 3$; Pact −/−, $n = 3$. Western blotting for mouse actin serves as a protein loading control.

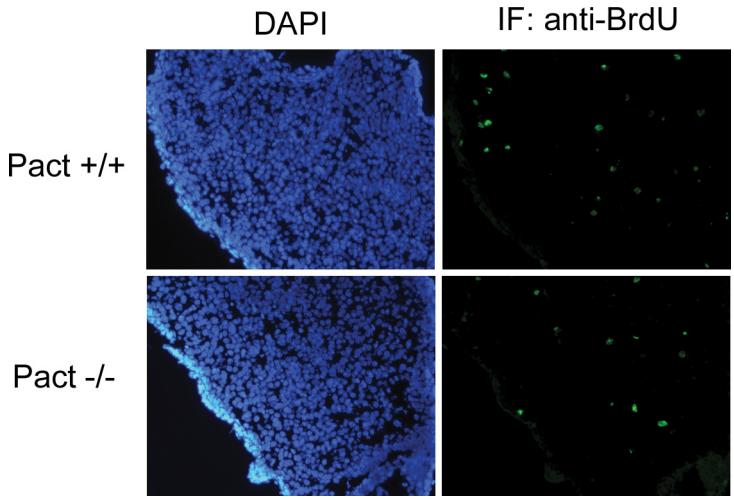

Fig. S4. BrdU incorporation in the anterior pituitary lobe as a measure of proliferation in P21 Pact$^{+/+}$ and Pact$^{-/-}$ mice. (*Left*) DAPI. (*Right*) BrdU-labeled proliferating cells revealed by green immunofluorescence.

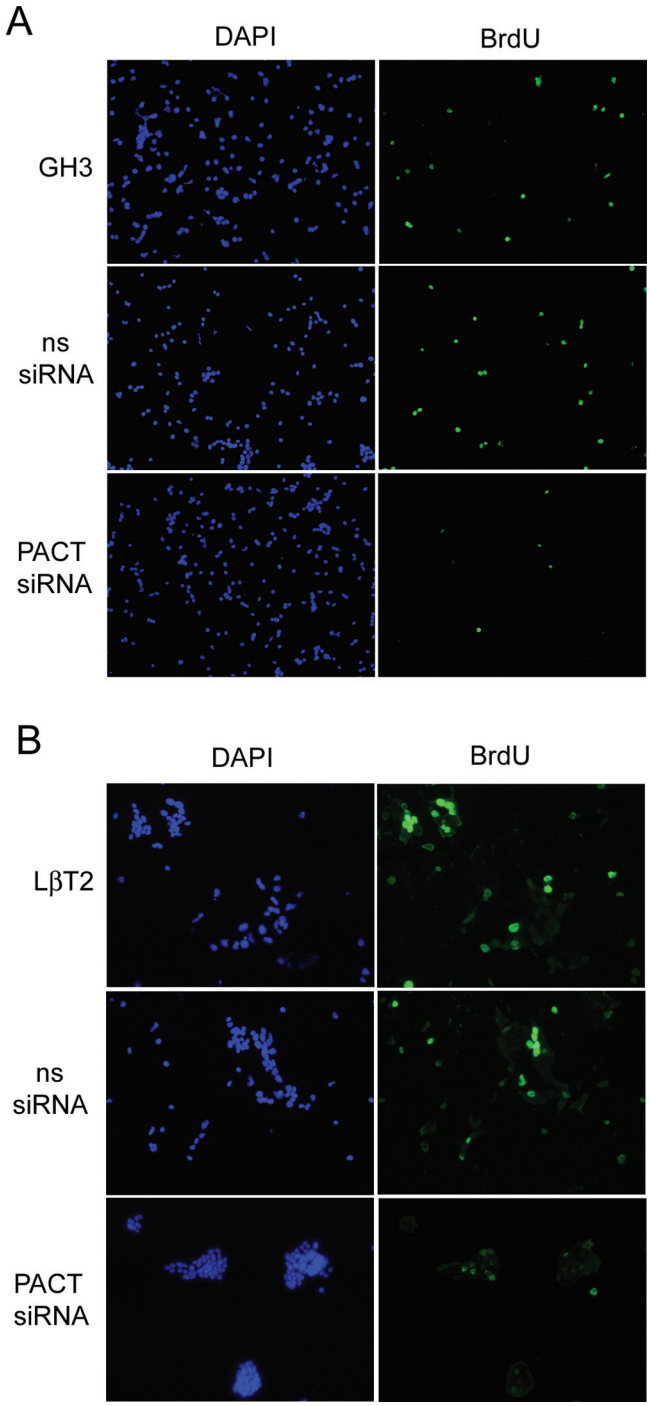

Fig. S5. Knockdown of PACT with siRNA in somatolactotrophs and in gonadotroph cell lines decreases BrdU incorporation. After 72 h of adding siRNA to PACT to actively dividing cells, BrdU was added for 1 h and proliferation measured by immunostaining for the amount of BrdU incorporation. (*A*) Reduced BrdU incorporation after PACT knockdown in GH3 somatolactotrophs (*Left*) DAPI staining, showing all nuclei. (*Right*) BrdU-labeled proliferating cells revealed by green immunofluorescence. (*B*) Reduced BrdU incorporation after PACT knockdown in LβT2 gonadotrophs. Legends for panels are the same as in (*A*).