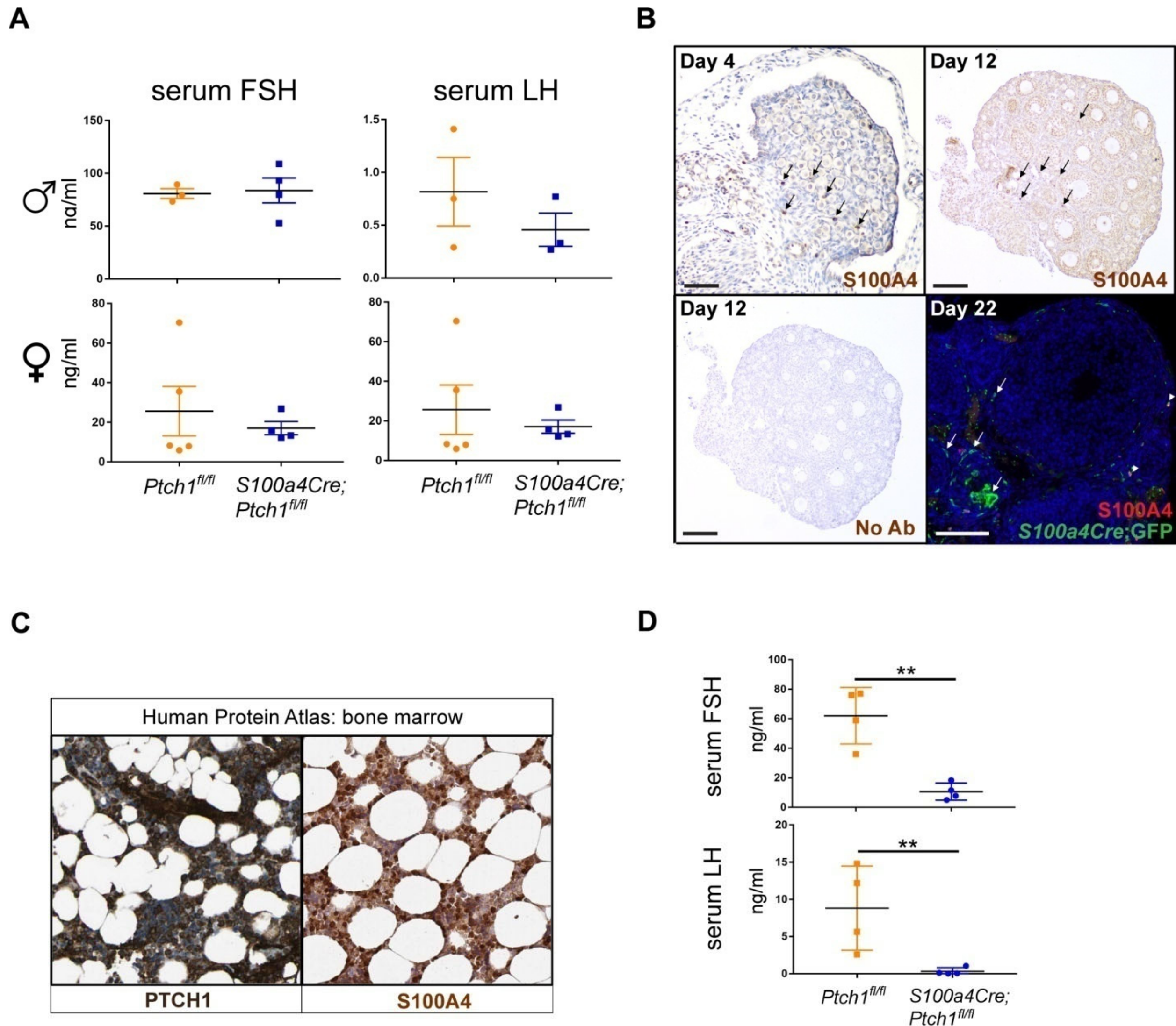
Supplemental Figure 1.



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3 **Supplemental Figure 1. Serum gonadotropin levels and S100A4 expression in the mouse**
4 **ovary and human bone marrow tissue.** (**A**) Serum FSH and LH levels in wild-type controls and
5 homozygous *Ptch1* mutants at 5 weeks of age. No statistically significant difference was detected
6 by t test. (**B**) Representative images of S100A4 IHC in wild-type mice (Days 4 and 12), as well
7 asS100A4 and GFP IF in the *S100a4-Cre;mTmG* reporter control mice (day 22). Scale bars: 100
8 µm and 200 µm. (**C**) Representative images of PTCH1 and S100A4 IHC in human bone marrow
9 tissue (images are from Human Protein Atlas). (**D**) Serum FSH and LH levels in wild-type controls
10 and homozygous *Ptch1* mutants after ovariectomy (OVX) at 8 weeks of age. ***P*<0.01; *t* test.

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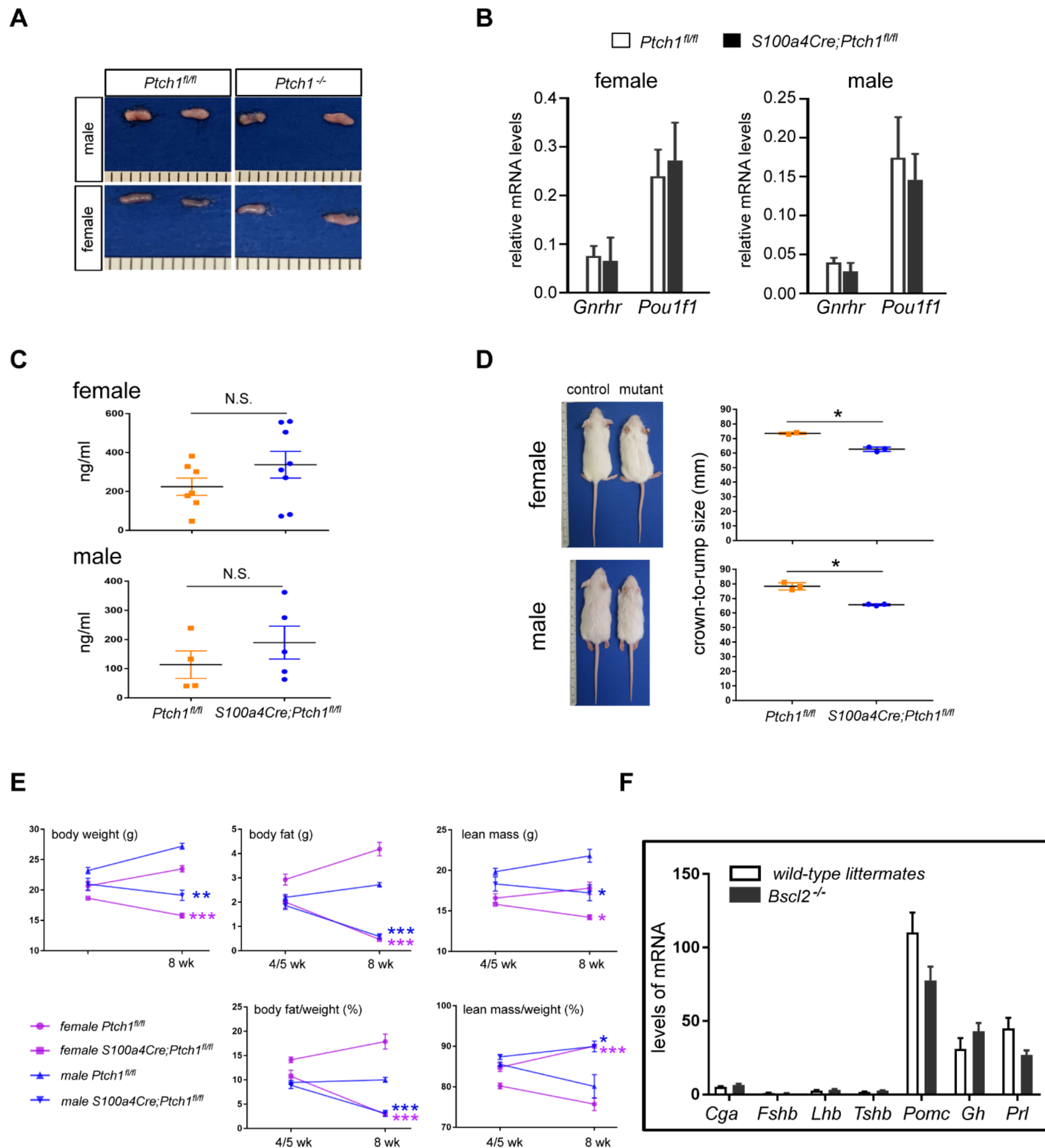
Supplemental. Figure 2.



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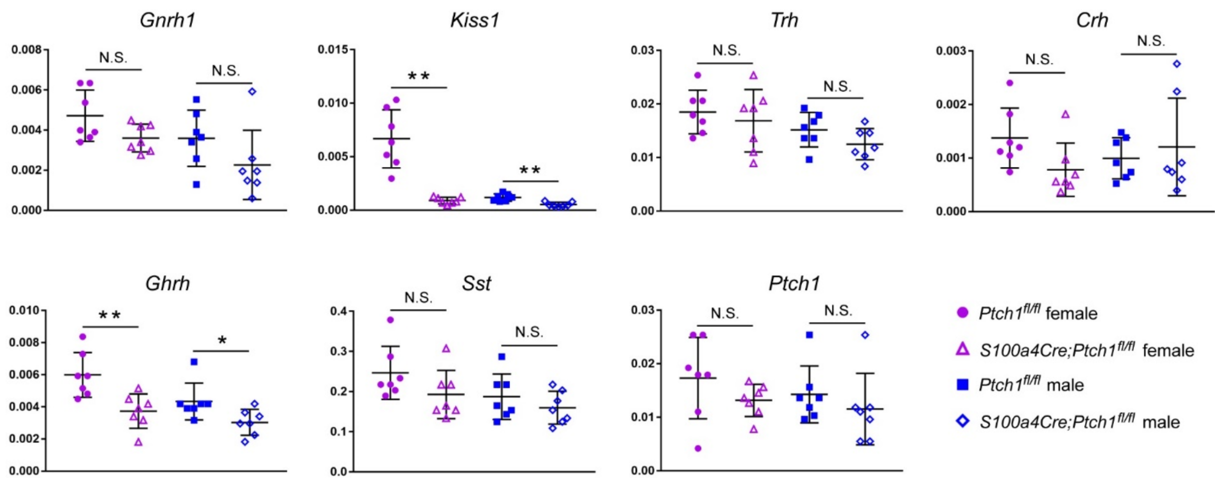
15 **Supplemental Figure 2. Pituitary differentiation and reduced adiposity in *S100a4-*
16 ***Cre;Ptch1$^{fl/fl}$* mutant mice are unlikely the major cause of the severely abnormal pituitary**
17 **phenotype in these mice. (A)** Representative images of pituitary morphology at 4.5 weeks of
18 age. Scale is in units of millimeters. (**B**) Relative mRNA levels of *Gnrhr* and *Pou1f1* in whole
19 pituitary tissues of wild-type controls and homozygous *Ptch1* mutants at 8 weeks of age (n≥ 5).

20 Total RNA was assayed by qPCR and the concentration of each transcript was normalized to that
21 of a housekeeping gene *Rpl19*. Data are represented as mean ± SD. No statistically significant
22 differences were detected between samples. *P<0.05; t test. (**C**) Serum concentration of
23 corticosterone in control and homozygous *Ptch1* mutant male and female mice at 8 weeks of age
24 (n=5~8). *N.S.*: no statistically significant difference; P<0.05, t test. (D) Body size comparison
25 between wild-type controls and homozygous *Ptch1* mutant mice at 8 weeks of age (n=3). *P<0.05;
26 t test. (**E**) Body composition of wild-type control and homozygous *Ptch1* mutant mice at 8 weeks
27 of age assessed by magnetic resonance imaging (n=3). **P<0.01; *P<0.05; ***P<0.001; t test. (**F**)
28 Relative mRNA levels of pituitary endocrine function genes in whole pituitary tissues of male wild-
29 type controls and homozygous *Bscl2* knockout mice (*Bscl2*$^{-/-}$) at 10 weeks of age (n=7). Total
30 RNA was assayed by qPCR and the concentration of each transcript was normalized to that of a
31 housekeeping gene *Rpl19*. Data are represented as mean ± SD. No statistically significant
32 differences were detected between samples. *P<0.05; t test.

33

34 **Supplemental Figure 3.**



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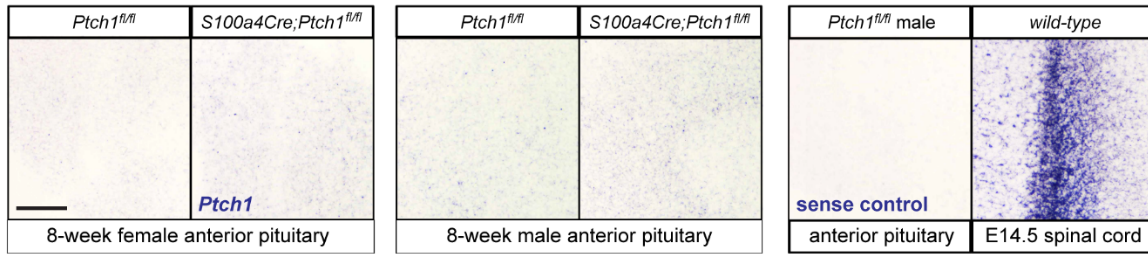
37 **Supplemental Figure 3. Hypothalamic functions of *S100a4-Cre;Ptch1$^{fl/fl}$* mutant mice are**
38 **impaired but cannot fully account for the pituitary defects in these mice.** Relative mRNA
39 levels of genes associated with hypothalamic functions and *Ptch1* in hypothalamic tissues of
40 wild-type controls and homozygous *S100a4-Cre;Ptch1$^{fl/fl}$* mutant mice at 8 weeks of age (n=7).
41 Total RNA was assayed by qPCR and the concentration of each transcript was normalized to
42 that of a housekeeping gene *Rpl19*. Data are represented as mean ± SD. N.S.: no statistically
43 significant differences were detected between samples. **P*<0.05; ***P*<0.01 *t* test.

Supplemental Figure 4.

A



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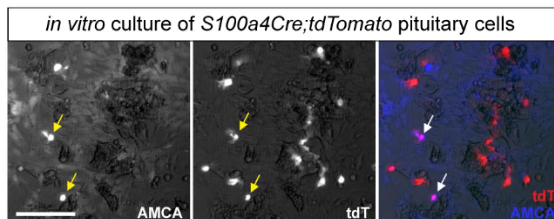

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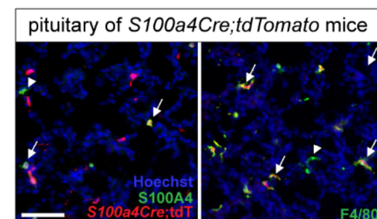

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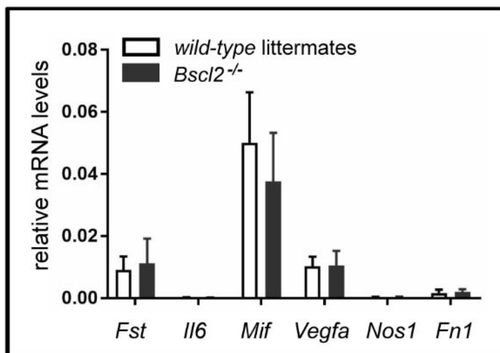
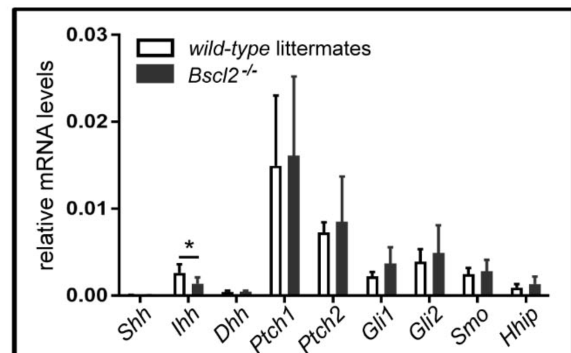


E



Supplemental Figure 4. Pituitary hematopoietic cells express *S100a4-Cre* and are likely the cause of the abnormal pituitary endocrine function in *S100a4-Cre;Ptch1*$^{fl/fl}$ mutant mice. (**A**) Representative images of *in situ* hybridization for *Ptch1* in the anterior pituitary of wild-type control and *Ptch1* mutant mice at 8 weeks of age. Sense probes hybridized on anterior pituitary of wild-type control male mice were used as negative control, and antisense probes hybridized on whole mouse embryo (embryonic day 14.5) sections was used as positive control. Scale bar: 100 µm. (**B**) Representative images of IF staining of S100A4, tdTomato (tdT; expression driven by *S100a4-Cre*), FOXL2 (a gonadotrope and thyrotrope marker), and F4/80 a macrophage and monocyte marker, on frozen tissue sections of anterior pituitaries from *S100a4-Cre;tdTomato* reporter mice. Arrows: cells positive for both green and red staining; arrowheads: cells that are only positive for green signal. Scale bar: 50 µm. (**C**) Representative images of AMCA labeling of *in vitro* cultured pituitary cells from *S100a4-Cre;tdTomato* reporter mice. Arrows: cells positive for both AMCA and tdTomato. (**D**) Relative mRNA levels of genes associated with pituitary

60 microenvironment, and of genes within the HH signaling pathway (**E**) in whole pituitary tissues of
61 male wild-type controls and homozygous *Bscl2* knockout mice ($Bscl2^{-/-}$) at 10 weeks of age (n=7).
62 Total RNA was assayed by qPCR and the concentration of each transcript was normalized to that
63 of a housekeeping gene *Rpl19*. Data are represented as mean ± SD. No statistically significant
64 differences were detected between samples except for *Ihh*. *P<0.05; *t* test.

65

66 **Supplemental Table 1. Real-time PCR primer set sequences (mouse).**

	Primer Sequences (5'-3')	
Gene Symbol	**Forward**	**Reverse**
Cyp19a1	TGTTGTGGACTTGGTCATGC	CAAAGCCAAAAGGCTGAAAG
Fshr	CAGGTCAACATACCGCTTGA	TCCCCAGGCTGAGTCATATC
Amh	ACCCTTCAACCAAGCAGAGA	AAGCGAGTGAGGGTCTCTAGG
Ar	GGACCATGTTTTACCCATCG	TCGTTTCTGCTGGCACATAG
Cyp17a1	GTCGCCTTTGCGGATAGTAGT	TGAGTTGGCTTCCTGACATATCA
Cyp11a1	GTACTTGGGCTTTGGCTGGG	CAGGTCCTGCTTGAGAGGCT
Lhcgr	CTGAAAACTCTGCCCTCCAG	AATCGTAATCCCAGCCACTG
Sfrp4	AGAAGGTCCATACAGTGGGAAG	GTTACTGCGACTGGTGCGA
Insl3	TACTGATGCTCCTGGCTCTG	TGTCTCTGCTCTAGCCACTGC
Lhb	AGTTCTGCCCAGTCTGCATC	GACCCCCACAGTCAGAGCTA
Fshb	GAAGAGTGCCGTTTCTGCAT	GTGCTGTCGCTGTCACACTT
Cga	CAAGCTAGGAGCCCCCATCTA	CACTCTGGCATTTCCCATTACT
Tshb	TCAACACCACCATCTGTGCT	TCTGACAGCCTCGTGTATGC
Pomc	GAAGATGCCGAGATTCTGCT	TTTTCAGTCAGGGGCTGTTC
Gh	CTGGCTGCTGACACCTACAA	AAGCGAAGCAATTCCATGTC
Prl	TGGGATCTACTTTGTTTGGTCAC	ATGGGCAATTTGGCACCTCA
Shh	AAAGCTGACCCCTTTAGCCTA	TTCGGAGTTTCTTGTGATCTTCC
Ihh	CTCTTGCCTACAAGCAGTTCA	CCGTGTTCTCCTCGTCCTT
Dhh	CTTGGCACTCTTGGCACTATC	GACCCCCTTGTTACCCTCC
Ptch1	TGTGGTCATCCTGATTGCAT	AAGAGGACAGGCAGCAGAAC
Ptch2	CTCCGCACCTCATATCCTAGC	TCCCAGGAAGAGCACTTTGC
Gli1	TCAATCCAATGACTCCACCA	TCTCTGGCTGCTCCATAACC
Gli2	CCCTGCACTGGAGAAGAAAG	TCTCATGTCAATCGGCAAAG
Smo	CTACGGAGCCACCACCAC	TCCACTCGGTCATTCTCACA
Hhip	AGCTGCTCAGTGGAGGAGAG	CAGGGAGCACATTGGATCTC
Fst	TGCTGCTACTCTGCCAGTTC	GTGCTGCAACACTCTTCCTTG

Il6	GATGGATGCTACCAAACTGGA	GGAAATTGGGGTAGGAAGGA
Mif	GCCAGAGGGGTTTCTGTCG	GTTCGTGCCGCTAAAAGTCA
Vegfa	CCCACGACAGAAGGAGAGCAGAAGT	CATCAGCGGCACACAGGACGG
Nos1	CTGGTGAAGGAACGGGTCAG	CCGATCATTGACGGCGAGAAT
Fn1	TTCAAGTGTGATCCCCATGAAG	CAGGTCTACGGCAGTTGTCA

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70 **Supplemental Table 2.** Primary antibody information and immunostaining conditions.

Protein	Antibody	Type	Company	Dilution	Conditions
GFP	11122	Rabbit polyclonal	Invitrogen	1:1000	2 hours, room temperature
GFP	JL8	Mouse polyclonal	Clontech	1:250	Overnight, 4 C°
GFP	Ab290	Rabbit polyclonal	Abcam	1:200	Overnight, 4 C°
S100A4	07-2277	Rabbit polyclonal	Millipore	1:250	Overnight, 4 C°
Vimentin	5741	Rabbit monoclonal	Cell Signaling Technology	1:100	Overnight, 4 C°
ACTA2	Ab18147	Mouse monoclonal	Abcam	1:500	Overnight, 4 C°
CD45	550539	Rat monoclonal	BD Biosciences	1:20	1 hour, room temperature
CYP17A1	N/A	Rabbit polyclonal	Gift from Dr. Alan J. Conley	1:200	2 hours, room temperature
RFP (tdTomato)	600-401-379	Rabbit polyclonal	Rockland	1: 1000	Overnight, 4 C°
F4/80	Ab6640	Rat monoclonal	Abcam	1:500	Overnight, 4 C°

71

72 **Supplemental Methods**

73 *Real-time Q-PCR analysis of gene expression.* Total mRNA was extracted from whole ovaries,
74 testes, and pituitaries using a RNeasy Mini kit (QIAGEN). Total RNA extraction, reverse
75 transcription, and qPCR were performed using previously described procedures (65). Sequences
76 of primers used are listed in Supplementary Table 1. Relative levels of mRNA were analyzed
77 using the Rotor-Gene 6.0 software and normalized to the levels of endogenous ribosomal protein
78 L19 (*Rpl19*). The number of replicates and statistical analysis used for each data set is denoted
79 in their respective figure legends.

80 *Histology and immunostaining.* Ovaries and pituitaries were fixed in 4% paraformaldehyde at 4°C
81 for 4 hours. Testes were fixed in Bouin's (Ricca Chemical Company, Catalog 1120-32) solution
82 at room temperature for 5 hours. Tissues were paraffin-embedded and sectioned at 5 μm
83 thickness for staining. Primary antibodies and their incubation conditions are listed in
84 Supplementary Table 2. For Xgal staining, testes were fixed in 4% paraformaldehyde and lacZ
85 staining was performed as previously described followed by counter staining with nuclear fast red.
86 Micrographs were taken with a Zeiss Leica Axioskop 2 Plus with an AxioCamMRm FX camera.
87 Tissues from at least four animals per sex and genotype were examined and representative
88 images are presented in the result section.

89 *Flow Cytometry.* Freshly isolated ovaries and pituitaries from *S100a4-Cre;mTmG* reporter mice
90 were enzymatically dissociated into single cell suspension at 37°C for one hour in DMEM/F12
91 medium (Gibco) containing 0.1% collagenase/dispase (Roche, 10 269 638 001), 1mg/ml trypsin
92 (Gibco) , and 25 μg/ml DNase I (Sigma Aldrich, D4527). The tissues and the dissociation medium
93 were pipetted five times with 1 ml tip at 10-minute intervals, centrifuged for 5 minutes at 500 g at
94 the end of the dissociation, and ran five times through a 26-gauge syringe. Dispersed cells were
95 further incubated in the dissociation medium at 37°C for 10 minutes, centrifuged again, and ran

96 through a 40 μm cell strainer. Dispersed cells were span down at 3000 rpm for 5 minutes, re-
97 suspended in FcR blocking solution (Miltenyi Biotec, 130-092-575), and incubated on ice for 10
98 minutes. Fluoro-conjugated anti-mouse CD45 (Tonbo Biosciences, 75-0451-U025) were added
99 to the cells at 1:100 dilution and incubated on ice for 25 minutes. Dispersed cells without any
100 endogenous fluorescence and cells with single fluorescent signal were used for gating. The
101 percentage of $CD45^+$ cells among GFP positive cells were assessed for ovaries and pituitaries
102 from four mice and graphed using FlowJo Software (FlowJo, LLC).

103 *Body composition analysis (MRI):* The whole body weight and the weight of lean mass and
104 adipose tissue were quantified in *S100a4Cre;Ptch1$^{fl/fl}$* mutant mice and their littermate controls at
105 4/5 (4 for females and 5 for males) weeks and 8 weeks of age using the EchoMRI (Echo Medical
106 system, Houston, TX), which is an Nuclear Magnetic Resonance system (NMR-MRI-based
107 technology) that measures whole body fat mass (1), lean tissue mass, free fluid, and total body
108 water in live animals up to 100 grams (2), without the need for anesthesia, in 69 seconds per
109 mouse.

110 *Immunogold labeling and optimal cell structure imaging of pituitary.* For immunogold labeling,
111 fixed tissues were stained with uranyl acetate (2% w/v in0.1M acetate buffer), dehydrated through
112 increasing concentration of methanol (70–100%) at 20 °C and embedded in LR Gold acrylic resin
113 (London Resin Company Ltd, Reading, UK). Pituitary sections were incubated for 2 hours at room
114 temperature with GFP primary antibody (Invitrogen) followed by a 1-hour incubation with a 15 nm
115 gold-conjugated donkey anti-rabbit secondary antibody (1:60; British Biocell International, Cardiff,
116 UK). Specificity of antibody labeling was confirmed in negative control sections in which the
117 primary antibody was replaced with non-immune serum and no labeling was observed in these
118 sections. Finally, sections were counterstained with lead citrate and uranyl acetate. For optimal
119 cell structure imaging, fixed tissue were stained with osmium tetroxide (1% in 0.1M phosphate
120 buffer) for 1 hour, and uranyl acetate (2% w/v in distilled water) for 1 hour. Stained sections were

121 examined on a JOEL 1010 transmission electron microscope (JOEL USA Inc., Peabody, MA,
122 USA) fitted with a Orius digital camera (Gatan, USA). Sections from three animals per group were
123 examined.

124 *In situ hybridization (ISH)* was performed on 25 µm thick sections cut from fresh-frozen pituitaries
125 collected from *S100a4Cre;Ptch1$^{fl/fl}$* mutant mice and their wild-type littermate controls at 8 weeks
126 of age. Sections from embryonic day 14.5 mice were used as positive controls. Digoxigenin (DIG)-
127 labeled antisense probes against *Ptch1* mRNA and the corresponding sense control probes were
128 synthesized from reverse-transcribed mouse cDNA as templates using RNA labeling kits (Roche).
129 The primers used for generating *Ptch1* probes were published in Allen Brain Atlas (Forward:
130 AAGCCCATCGACATTAGTCAGT; Reverse: ATAAGAGGACAGGCAGCAGAAC). The ISH
131 procedure was performed by the RNA *In Situ* Hybridization Core at Baylor College of Medicine
132 using an automated robotic platform as previously described (3).

133 *Labeling of folliculo-stellate cells with β-Ala-Lys-N$^{\varepsilon}$-AMCA (AMCA).* AMCA is a UV-excitable
134 dipeptide used for labeling and imaging of FS cells (4-6). Pituitaries from *S100a4-Cre;tdTomato*
135 reporter mice were removed immediately after euthanasia of the mice and washed in warm
136 Hank's balanced salt solution (HBSS) before they were enzymatically digested and dispersed into
137 single cells suspensions using protocol from a previous study (7). Dispersed cells were re-
138 suspended and plated into 24-well cell culture plate (Thermo Fisher Scientific, 144530) coated
139 with poly-D-Lysine (Sigma, P6407) at a density of 1x 10^5 in M-199 medium (GIBCO,31100-035)
140 supplemented with 10% FBS, 1% antibiotic-antimyotic (Wisent 450-115-EL) and 50 µg/ml
141 gentamicin (Wisent 450-135-XL). After overnight culture, cells were washed with warm HBSS and
142 incubated with 40 µM AMCA (Biotrend, BP0352) for 3 hours (37 °C; 5% CO_2). As a control, cells
143 were also incubated with 40 µM AMCA but at 4 °C for 3 hours. Cells were then washed three
144 times with cold HBSS and imaged for florescent signals using Zeiss AxioPlan2 microscope.

145 **Reference for Supplementary Material**

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