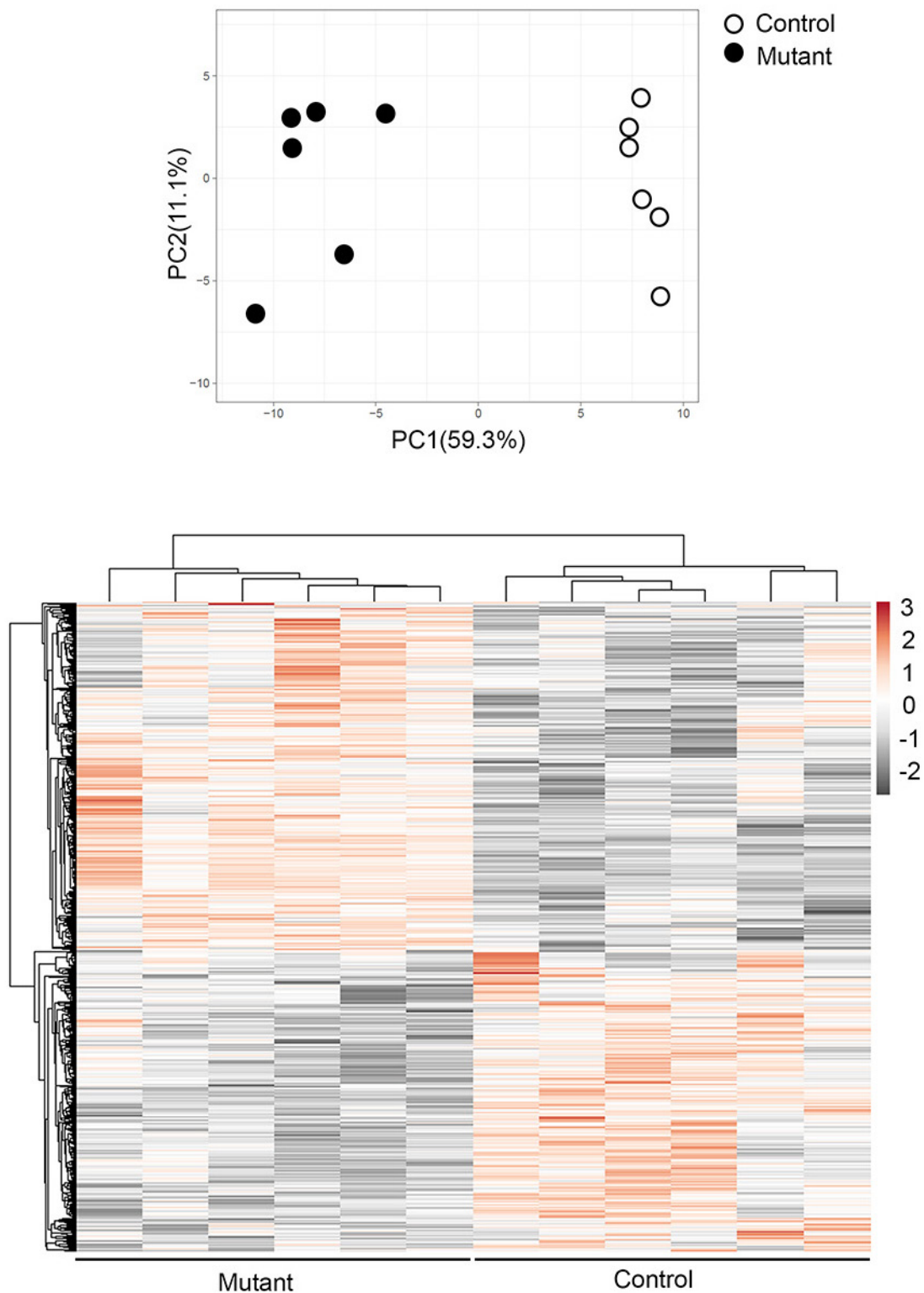
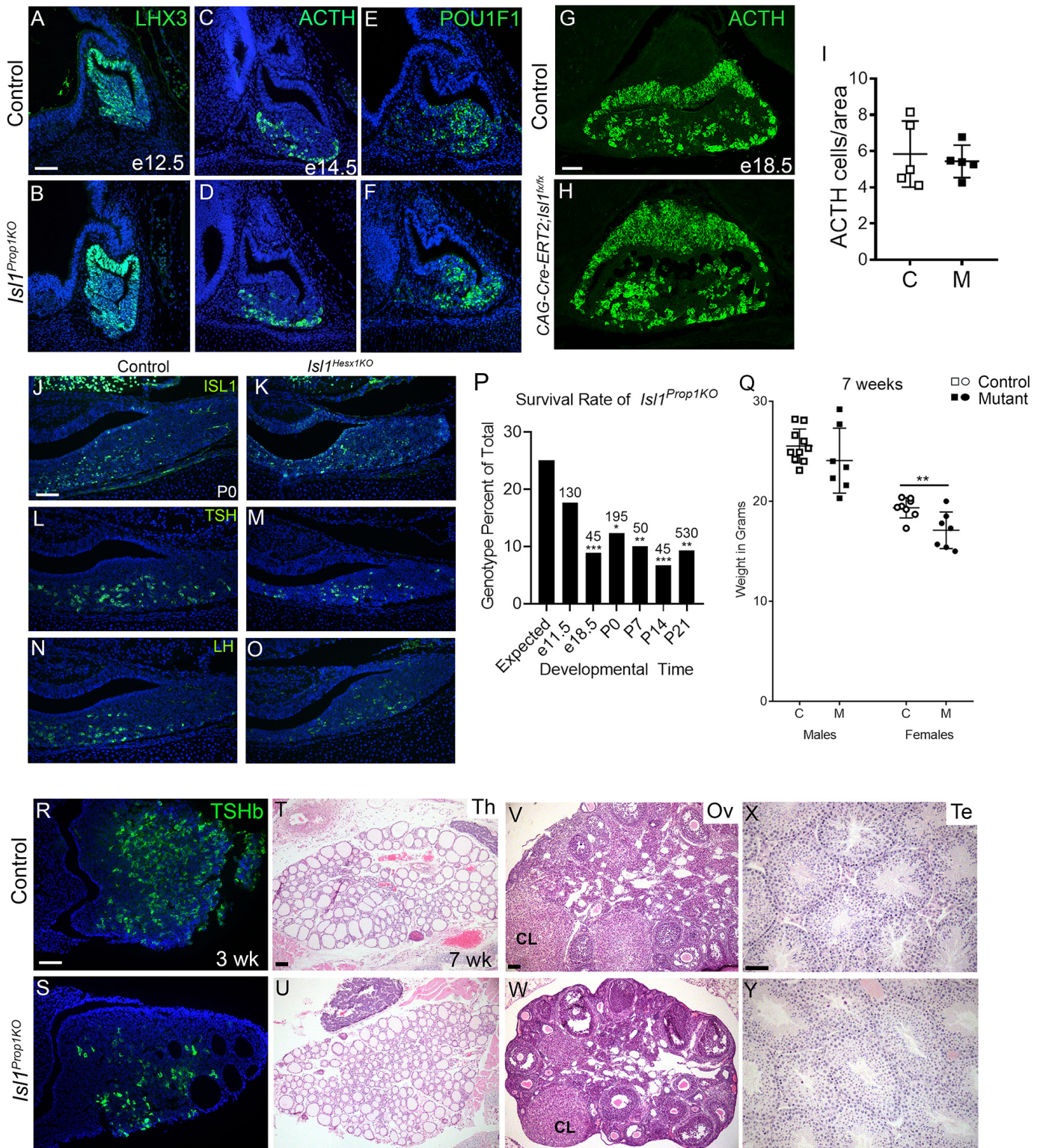


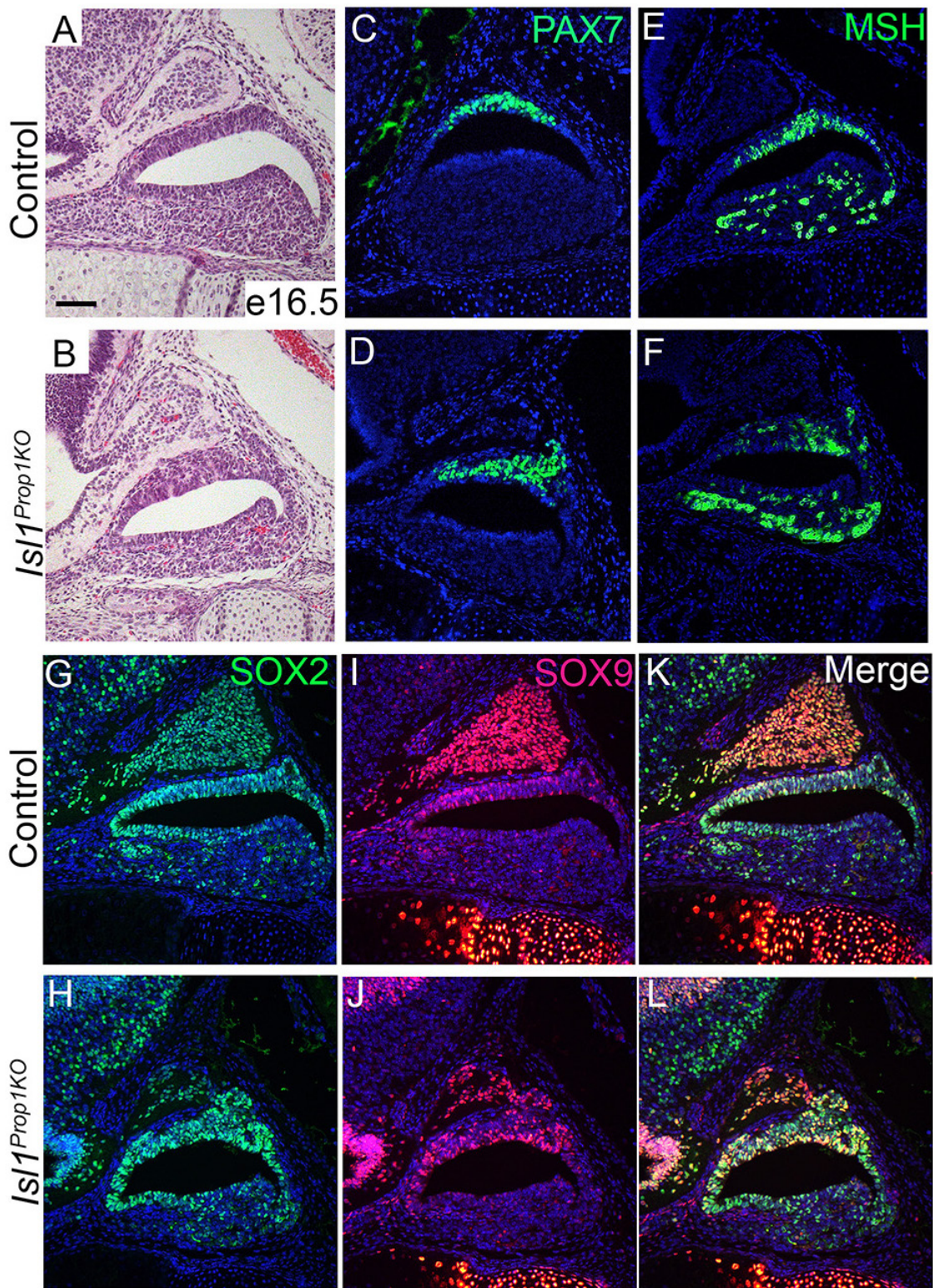
Supplemental Figure 1. Prop1cre effectively deletes *Isl1fx* in the pituitary gland. (A) PCR on DNA from P0 pituitary glands amplifies an approximately 1600bp fragment representing the *Isl1fx* and *Isl1* wild type alleles plus a 478bp fragment representing the *Isl1null* allele demonstrates the loss of the *Isl1 wt/fx* allele in the *Prop1cre;Isl1fx/-* mutants. (B-E) Prominent ISL1 protein expression is detected in control mice at e16.5 and P3. A significant reduction in ISL1 expression is observed at e16.5 progressing to a complete loss at P3 in the *Isl1^{Prop1KO}* mice. (F, G) ISL1 protein is reduced in both *Isl1^{Prop1KO}* (F) and *Isl1^{Hesx1KO}* (G) mutants at e11.5. (H, I) YFP immunostaining was not detected in control pituitary glands at P0 (H) but was prominent in all cells of *Isl1^{Prop1KO};R26R^{EYFP/+}* mutants (I) including cysts. (J) Higher magnification of boxed region in I. The scale bar in panel B and F represents 50 μ m and is applicable to panels B, D, F, G. The scale bar in panel C represents 50 μ m and is applicable to panels C, E, H, I.



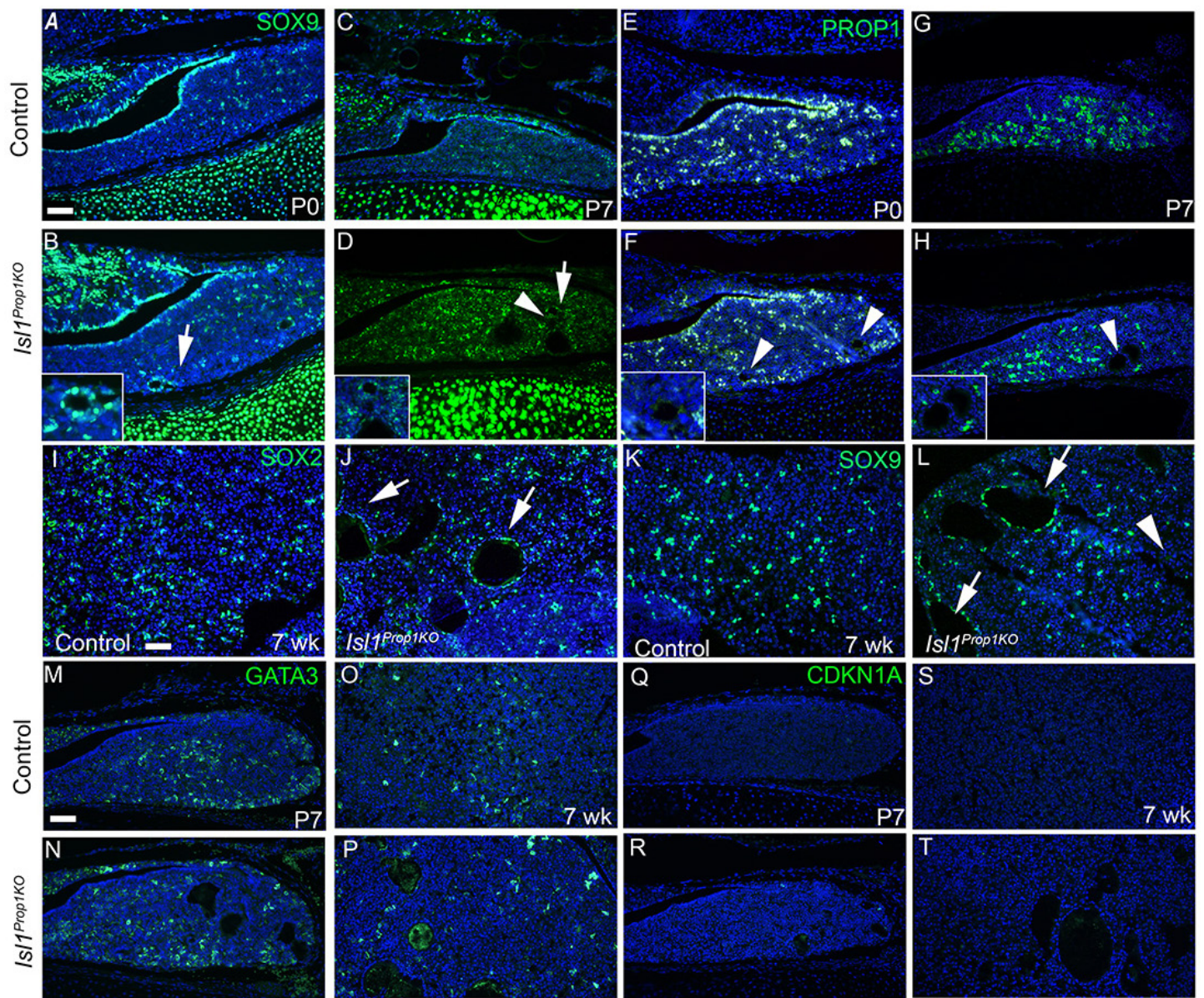
Supplemental Figure 2. Principal Component Analysis and heat map clustering of differentially expressed genes. (A) PCA was performed using ClustVis SVD with imputation and preprocessing $\ln(x+1)$ transformation. Principal components 1 and 2 explain 59.3% and 11.1% of the variance, respectively. (B) Heat map clustering of differentially expressed genes from 6 mutant and 6 control samples. Red indicates genes with elevated expression and grey indicates genes with reduced expression.



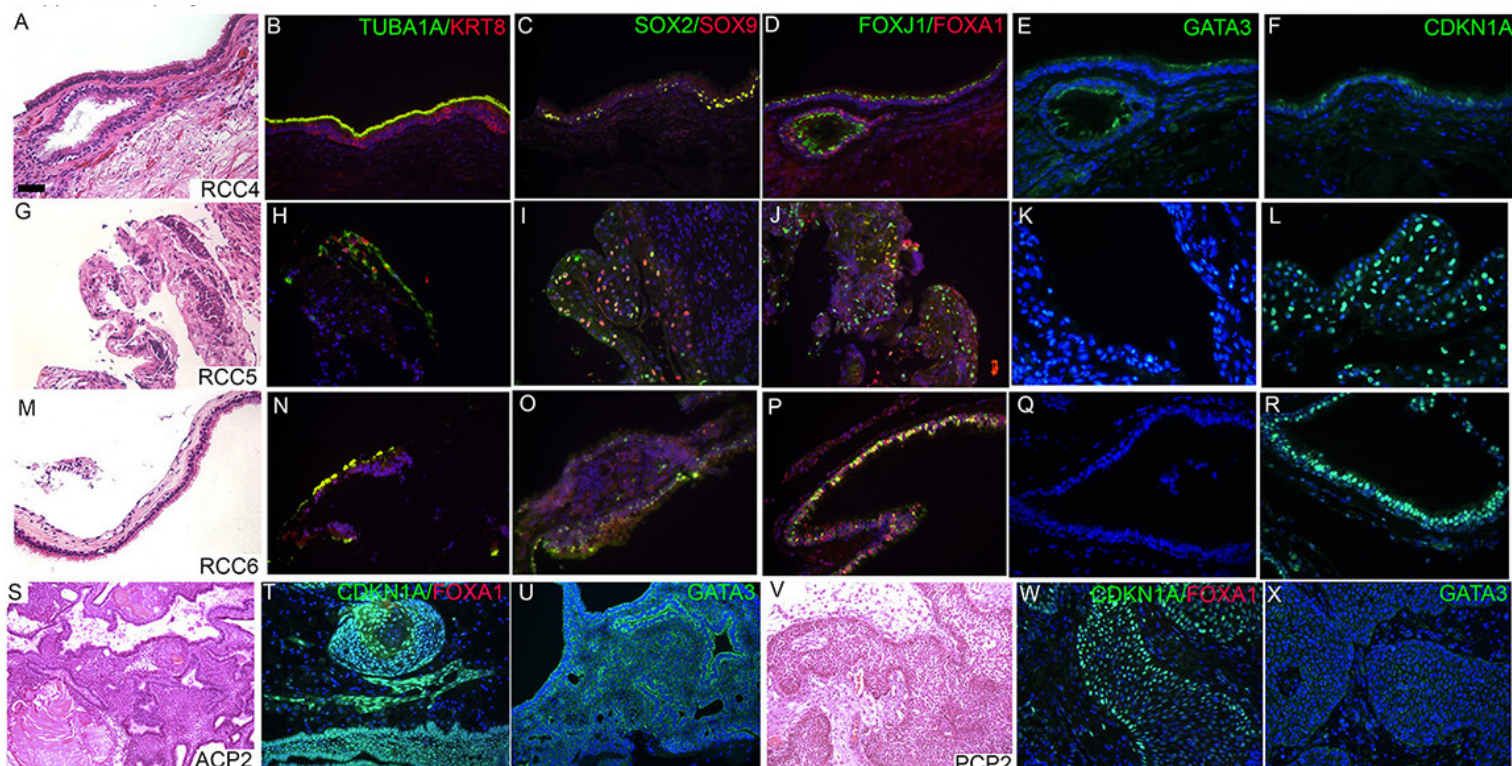
Supplemental Figure 3. Transient decrease in thyrotrope and gonadotrope specification does not impact adult thyroid gland, ovary, or testis. (A-F) Protein expression of LHX3, ACTH, and POU1F1 were comparable between control and *Is1^{Prop1KO}* embryos. (G-I) Inducible deletion of *Is1* using the *CAGCre-ERT2* did not alter specification of the corticotrope cell population. (J-O) Deletion of *Is1^{flx}* using the *Hexx1-cre* resulted in variable deletion of ISL1 and reduced levels of TSH and LH. (P) *Is1^{Prop1KO}* mice have reduced viability beginning in late embryonic stages. Total number of mice analyzed for each time point is listed and Chi Square analysis was used to determine the significance of distribution away from the expected Mendelian ratios. (Q) Weights of individual mice, male and female, are graphed for Control (C) and *Is1^{Prop1KO}* mutants (M) at 7 weeks of age. The significance of weight variance was evaluated using the Student T test with one tail distribution and 2 sample unequal variance, and the female weights were significantly different. (R,S) TSHb protein expression is reduced in *Is1^{Prop1KO}* mutants compared to controls at weaning. (T-Y) The transient decrease in thyrotrope and gonadotrope specification does not impact thyroid gland, ovary, or testis development. In addition, female mice are fertile. The scale bars represent 50 μ m and can be applied to adjacent panels.



Supplemental Figure 4. Cells of the developing intermediate lobe are properly specified in *Is1^{Prop1KO}* mutants at e16.5. (A, B) H&E staining of sagittal sections highlights the dysmorphism of the developing intermediate lobe in the *Is1^{Prop1KO}* mice compared to Controls. (C, D) PAX7 and (E, F) MSH were used as markers of intermediate lobe and are properly specified in *Is1^{Prop1KO}* mutants. (G-L) Co-im-munostaining of SOX2 and SOX9 was used to analyze the pituitary stem cells, demonstrating no changes in *Is1^{Prop1KO}* mice compared to controls. The scale bar in panel A represents 50µm and is applicable to panels A-L.



Supplemental Figure 5. Developing and mature cysts express a subset of progenitor cell markers. (A-D) SOX9 is transiently expressed in the cells lining the walls of forming cysts in *Isl1^{Prop1KO}* mutants at P0, however expression within the cyst wall remains only in a small subset of cells at P7. (E-H) PROP1 expression is not detected in the cells lining the cysts of *Isl1^{Prop1KO}* mutants at P0 or P7. (I-L) SOX2 and SOX9 expression is abundant in the cells lining the cyst walls in *Isl1^{Prop1KO}* mutants at 7 wks. (M-P) GATA3 is not expressed in the maturing *Isl1^{Prop1KO}* cysts at P7 or 7 wks. (Q-T) CDKN1A is not expressed in the maturing *Isl1^{Prop1KO}* cysts at P7 or 7 wks. Scale bars represent 50 μ m and apply to adjacent panels.



Supplemental Figure 6. Additional Human Rathke's Cleft Cysts confirm FOXA1 expression.

(A-R) Classification of human Rathke's cleft cyst samples was confirmed by staining with H&E to detect characteristic RCC morphology (A, G, M) and co-staining with antibodies for cytokeratin8 (KRT8) and acetylated tubulin (TUBA1A) (B, H, N) Co-staining with antibodies for progenitor markers SOX2 and SOX9 detected many positive cells (C, I, O). Co-staining with antibodies FOXJ1 and FOXA1 detected positive cells lining the cysts (D, J, P). Immunostaining for GATA3 was negative in all additional samples (E, K, Q). CDKN1A (F, L, R) detected positive cells lining the cysts in some, but not all additional samples. **(S-X)** Surgical samples from craniopharyngiomas of both the adamantinomatous (ACP) (S-U) and papillary (PCP) (V-X) subtypes were stained with H&E, co-stained for CDKN1A and FOXA1, and immunostained for GATA3. Samples were positive for CDKN1A and negative for both FOXA1 and GATA3. Magnification: scale bar in panel A corresponds to 50 μ m.

Supplemental Table 1: Stem Cell Markers unchanged in *Is/1^{Prop1KO}*

Stem Cell Marker	Fold Change
Cadherin1, <i>Cdh1</i>	1.5x
Cyclin-dependent kinase inhibitor 2a, <i>Cdkn2a</i>	1.2x
SRY-box 2, <i>Sox2</i>	1.2x
S100 protein, beta peptide, <i>S100b</i>	1.2x
Paired related homeobox 2, <i>Prrx2</i>	1.1x
Lymphocyte antigen 6 complex locus A, <i>Ly6a</i>	1.1x
Beta catenin, <i>Ctnnb1</i>	1.0x
Paired like homeodomain factor 1, <i>Prop1</i>	1.0x
Caspase 3, <i>Casp3</i>	1.0x
SRY-box 9, <i>Sox9</i>	1.0x
Coxsackie virus and adenovirus receptor, <i>Cxadr</i>	1.0x
Glial cell line derived neurotrophic factor family receptor alpha 1, <i>Gfra1</i>	0.9x
Paired related homeobox 1, <i>Prrx1</i>	0.9x
Glial cell line derived neurotrophic factor family receptor alpha 2, <i>Gfra2</i>	0.9x
Neuronatin, <i>Nnat</i>	0.9x
Glial cell line derived neurotrophic factor family receptor alpha 4, <i>Gfra4</i>	0.9x
Nestin, <i>Nes</i>	0.9x
LIM homeobox protein 3, <i>Lhx3</i>	0.9x
Ret proto-oncogene, <i>Ret</i>	0.8x

Supplemental Table 2: Gene Ontology Terms Associated With Elevated Gene Expression in *Isl1*^{Prop1KO}

Database ID	Category	#Genes/Total	Fold Change	P Value
GO: 0005576	Extracellular Region	92/351	2.0	1.52E-10
GO: 0006811	Ion Transport	47/366	2.5	2.71E-08
GO: 0005886	Plasma Membrane	121/351	1.5	1.31E-06
GO:0030855	Epithelial Cell Differentiation	15/366	4.5	5.40E-06
GO:0022803	Passive Transmembrane Transporter Activity	27/363	2.7	7.81E-06
GO:0015267	Channel Activity	27/363	2.7	7.81E-06
GO: 0022838	Substrate Specific Channel Activity	26/363	2.6	1.80E-05
GO: 0030594	Neurotransmitter Receptor Activity	12/363	5.0	2.31E-05
GO: 0006812	Cation Transport	32/366	2.3	2.36E-05
GO: 0030001	Metal Ion Transport	29/366	2.4	2.36E-05
PIRSF001630	Serpin	10/253	6.1	3.06E-05
PIRSF002282	Cytoskeletal Keratin	10/253	6.1	3.06E-05
GO: 0004857	Enzyme Inhibitor Activity	20/363	3.0	3.78E-05
GO: 0060429	Epithelium Development	21/366	2.9	4.50E-05
GO: 0042573	Retinoic Acid Metabolic Process	6/366	13.1	6.45E-05
GO: 0030414	Peptidase Inhibitor Activity	16/363	3.3	9.45E-05
GO: 0016324	Apical Plasma Membrane	12/351	4.3	9.85E-05
IPR000215	Protease Inhibitor I4, Serpin	10/493	5.2	1.17E-04
IPR016044	Filament	10/493	5.1	1.29E-04
GO: 0046873	Metal Ion Transmembrane Transporter	21/363	2.7	1.37E-04
GO: 0034702	Ion Channel Complex	15/351	3.4	1.40E-04
GO: 0042445	Hormone Metabolic Process	11/366	4.5	1.46E-04
GO: 0003700	Transcription Factor Activity	40/363	1.9	1.58E-04
IPR001664	Intermediate Filament Protein	10/493	5.0	1.60E-04
IPR001314	Peptidase S1A, Chymotrypsin	14/493	3.5	1.75E-04
GO: 0006833	Water Transport	5/366	15.5	2.14E-04
GO: 0042044	Fluid Transport	5/366	15.5	2.14E-04
GO: 0005216	Ion Channel Activity	23/363	2.4	2.33E-04
GO: 0022836	Gated Channel Activity	20/363	2.6	2.85E-04
GO: 0048568	Embryonic Organ Development	18/366	2.8	2.81E-04
GO: 0004867	Serine-Type Endopeptidase Inhibitor Activity	12/363	3.8	2.98E-04
GO:0005261	Cation Channel Activity	18/363	2.7	4.59E-04
IPR001254	Peptidase S1 and S6, Chymotrypsin/Hap	14/493	3.2	4.92E-04
GO: 0008188	Neuropeptide Receptor Activity	7/363	6.7	5.18E-04
GO: 0006776	Vitamin A Metabolic Process	6/366	8.6	5.62E-04
GO: 0043565	Sequence Specific DNA Binding	30/363	2.0	6.28E-04
GO: 0010817	Regulation of Hormone Level	12/366	3.5	6.65E-04
GO: 0008236	Serine-Type Peptidase Activity	16/363	2.8	7.10E-04
GO: 0016101	Diterpenoid Metabolic Process	6/366	8.0	8.03E-04
GO: 0001523	Retinoid Metabolic Process	6/366	8.0	8.03E-04
IPR001846	von Willebrand Factor, Type D	5/493	11.3	8.12E-04
GO: 0048732	Gland development	15/366	2.8	8.90E-04
GO: 0006721	Terpenoid Metabolic Process	6/366	7.7	9.50E-04
IPR001766	Transcription factor, forkhead	7/493	6.0	9.80E-04
GO: 0044421	Extracellular Region Part	38/351	1.7	9.90E-04
IPR016054	Ly-6 Antigen/uPA Receptor-Like	6/493	1.7	1.09E-03
GO: 0043068	Positive Regulation of Programmed Cell Death	17/366	2.5	1.20E-03
GO: 0015672	Monovalent Inorganic Cation Transport	19/366	2.4	1.43E-03
GO:0005929	Cilium	12/351	3.2	1.40E-03
GO: 0042277	Peptide Binding	13/363	3.0	1.44E-03
GO: 0045177	Apical Part of Cell	12/351	3.1	1.52E-03
GO: 0004866	Endopeptidase Inhibitor Activity	13/363	3.0	1.52E-03
GO: 0031420	Alkali Metal Ion Binding	15/363	2.7	1.55E-03
GO: 0030216	Keratinocyte Differentiation	7/366	5.4	1.71E-03
IPR002957	Keratin, type I	6/493	6.8	1.70E-03
GO: 0007605	Sensory Perception of Sound	9/366	4.0	1.85E-03
GO: 0006775	Fat Soluble Vitamin Metabolic Process	6/366	6.6	1.90E-03
GO: 0004252	Serine-type Endopeptidase Activity	14/363	2.7	2.04E-03
GO: 0048878	Chemical Homeostasis	21/366	2.1	2.08E-03
IPR006208	Cystine Knot	4/493	14.4	2.19E-03
GO: 0044447	Axoneme Part	4/351	14.3	2.25E-03
IPR006788	Myelin-Associated Oligodendrocytic Basic Protein	3/493	36.0	2.25E-03
GO: 0009913	Epidermal Cell Differentiation	7/366	5.1	2.30E-03

Supplemental Table 4. Antibody Conditions

Experiment	Citric Acid Antigen Retrieval	CH ₃ OH: H ₂ O ₂	Block	Primary Antibody	Secondary Antibody and Detection	Biotin Block	Second Primary Antibody	Second Secondary Antibody and Detection	Reference
ACTH	No	No	NGD	1:1000, National Hormone and Peptide Program	Anti-rabbit Biotin, Strep Cy2	No			
ACTH	No	No	NGD	1:1000, AFP-156102789, National Hormone and Peptide Program	Anti-rabbit Alexa Fluor 488 (Invitrogen)	No			
Caspase 3	10 min	Yes	TNB	1:50, Cell Signaling Technology 9661	Anti-rabbit Biotin, Strep Cy2	No			
CCND1	10 min	Yes	M.O.M., TNB	1:200, Santa Cruz, sc-8396, A-12	Anti-Mouse Biotin, Perkin Elmer TSA-FITC or Biotium TSA-CF488	No			
CDKN1A	10 min	Yes	M.O.M., TNB	1:100, BD Pharmingen, 556431, SXM30	Anti-Mouse Biotin, Perkin Elmer TSA-FITC or Biotium TSA-CF488	No			
CDKN1A/FOXA1	10 min	Yes	M.O.M., TNB	1:100, BD Pharmingen, 556431, SXM30, anti-CDKN1A	Anti-Mouse Biotin, Perkin Elmer Biotium TSA-CF488	Yes	1:100, ThermoFisher PA5-18168, anti-FOXA1	Anti-goat Biotin, Biotium TSA-CF543	
CGA	No	No	NGD	1:1000, National Hormone and Peptide Program	Anti-rabbit Biotin, Strep Cy2	No			
FOXA1	10 min embryo, 15 min postnatal	Yes	TNB	1:100, ThermoFisher PA5-18168	Anti-goat Biotin, Perkin Elmer TSA-FITC or Biotium TSA-CF488	No			
FOXJ1	10 min embryo, 15 min postnatal	Yes	M.O.M., TNB	1:100, ThermoFisher 14-9965-80	Anti-mouse Biotin, Perkin Elmer TSA-FITC	No			
FOXJ1/FOXA1	10 min	Yes	M.O.M., TNB	1:100, ThermoFisher 14-9965-80, Anti-FOXJ1	Anti-mouse Biotin, Perkin Elmer TSA-FITC	Yes	1:100, ThermoFisher PA5-18168, Anti-FOXA1	Anti-goat Biotin, Perkin Elmer TSA-TRITC	
FOXL2	10 min	Yes	TNB	1:100, Novus Bio NB100-1277	Anti-goat Biotin, Perkin Elmer TSA-FITC	No			
FSHb	No	No	NGD	1:250, National Hormone and Peptide Program	Anti-rabbit AlexaFluor 488 (Invitrogen)	No			
GATA3	10 min embryo, 15 min postnatal	Yes	M.O.M., TNB	1:100, BD Biosciences 558686	Anti-mouse Biotin, Biotium TSA-CF488	No			
GH	No	No	NGD	1:600, National Hormone and Peptide Program	Anti-human Biotin, Strep Cy2	No			
ISL1	10 min embryo, 15 min postnatal	Yes	M.O.M., TNB	1:100, Developmental Studies Hybridoma Bank (DSHB) 40.2D6, Anti-ISL1	Anti-mouse Biotin, Perkin Elmer TSA-FITC	No			
ISL1/FOXA1	10 min	Yes	M.O.M., TNB	1:100, DSHB, 40.2D6, Anti-ISL1	Anti-mouse Biotin, Perkin Elmer TSA-FITC	Yes	1:100, ThermoFisher PA5-18168, Anti-FOXA1	Anti-goat Biotin, Perkin Elmer TSA-TRITC	
ISL1/PROP1	15 min	Yes	M.O.M., TNB	1:100, Dr. Aimee Ryan, Anti-PROP1	Anti-guinea pig biotin, Perkin Elmer TSA-FITC	Yes	1:100, DSHB 40.2D6, Anti-ISL1	Anti-mouse Biotin, Perkin Elmer TSA-TRITC	7
ISL1/SOX2	15 min	Yes	M.O.M., TNB	1:100, DSHB, 40.2D6, Anti-ISL1	Anti-mouse Biotin, Perkin Elmer TSA-FITC	Yes	1:250, Neuromics GT15098, Anti-SOX2	Anti-goat Biotin, Perkin Elmer TSA-TRITC	
KRT8	10 min	Yes	TNB	1:100, Abcam ab5940	Anti-rabbit Biotin, Perkin Elmer TSA-FITC	No			
LHb	No	No	NGD	1:1000, National Hormone and Peptide Program	Anti-guinea pig Biotin, Strep Cy2	No			
LHX3	10 min	Yes	M.O.M., TNB	1:100 DSHB, 67.4E12	Anti-mouse Biotin, Perkin Elmer TSA-FITC or Biotium TSA-CF488	No			
MSH	No	No	NGD	1:100, MilliporeSigma AB5087	Anti-sheep Biotin, StrepCy2	No			
NR5A1	Yes	Yes	TNB	1:500, Dr. Gary Hammer	Anti-rabbit Biotin, Perkin Elmer TSA-FITC	No			8
PAX7	10 min	Yes	M.O.M., TNB	1:100, DSHB	Anti-mouse Biotin, Perkin Elmer TSA-FITC	No			
PITX1	10 min	Yes	TNB	1:250, Dr. Jacques Drouin	Anti-rabbit Biotin, Perkin Elmer TSA-FITC	No			9
POU1F1	10 min	Yes	NGD	1:100, Dr. Simon Rhodes	Anti-rabbit Biotin, Strep Cy2	No			10
PROP1	10 min embryo, 15 min postnatal	Yes	TNB	1:100, Dr. Aimee Ryan	Anti-guinea pig biotin, Perkin Elmer TSA-FITC	No			7
SHH	10 min	Yes	M.O.M., TNB	1:100 DSHB, 5E1	Anti-mouse Biotin, Biotium TSA-CF488	No			
SOX2	10 min embryo, 15 min postnatal	Yes	TNB	1:100, Neuromics GT15098	Anti-goat Biotin, Perkin Elmer TSA-FITC	No			
SOX2/SOX9	10 min	Yes	TNB	1:250, Neuromics GT15098, Anti-SOX2	Anti-goat Biotin, Perkin Elmer TSA-FITC	Yes	1:50, Millipore AB5535, Anti-SOX9	Anti-rabbit Biotin, Perkin Elmer TSA-TRITC	
SOX9	10 min embryo, 15 min postnatal	Yes	TNB	1:100, Abcam ab185966	Anti-rabbit Biotin, Perkin Elmer TSA-FITC	No			
TSHb	No	No	NGS	1:1000, National Hormone and Peptide Program	Anti-guinea pig AlexaFluor 555 (Invitrogen) or anti-guinea pig TRITC (Invitrogen)	No			
TSHb	No	No	NGD	1:1000, National Hormone and Peptide Program	Anti-rabbit Biotin, Strep Cy2	No			
TUBA1A	10 min	Yes	M.O.M., TNB	1:200, Sigma T6793	Anti-mouse Biotin, Perkin Elmer TSA-FITC	No			
TUBA1A/KRT8	10 min	Yes	M.O.M., TNB	1:100, Sigma T6793, Anti-TUBA1A	Anti-mouse Biotin, Perkin Elmer TSA-FITC	Yes	1:100, Abcam ab5940, Anti-KRT8	Anti-rabbit Biotin, Perkin Elmer TSA-TRITC	
YFP	10 min	Yes	TNB	1:100, Abcam ab6556	Anti-rabbit Biotin, Perkin Elmer TSA-FITC or Biotium TSA-CF488	No			

*NGD= Normal Goat Diluent

*M.O.M= Mouse on Mouse Blocking Kit, Vector Labs

*TNB= 0.1M Tris HCl, pH 7.5; 0.15M NaCl, 0.5% Blocking Reagent

Supplemental Methods

Mouse sources and genotyping. *Isl1*^{tm2Gan} mice were a generous gift from Dr. Lin Gan, Dept. of Neuroscience and Regenerative Medicine, Medical College of Georgia, Augusta University, Augusta, GA (1). These mice, referred to here as *Isl1*^{fl}, have loxP sites flanking exon 2. The *Isl1*^{+/-} mice were generated by breeding the *Isl1*^{fl} to B6.C-Tg(CMV-cre)^{1Cgn/J} mice (stock number 006054; The Jackson Laboratory) (2). Tg(*Prop1-cre*)^{432Sac}, referred to here as *Prop1-cre*, were generated at University of Michigan (3). The cre reporter strain, B6.129X1-Gt(ROSA)26Sor^{tm1(EYFP)Cos/J}, referred to as R26R-EYFP, were purchased from the Jackson Laboratory, Stock no. 006148, and genotyped according to the Jackson Laboratory recommended primers and conditions (4). The tamoxifen inducible cre strain, B6.Cg-Tg(CAG-cre;Esr1*)5Amc/J stock 004682 (5), *CAG-Cre-ERT2* was purchased from the Jackson Laboratory. The B6-*Hesx1-cre* strain was generously donated by Dr. Juan Pedro Martinez-Barbera, Developmental Biology and Cancer, Birth Defect Research Centre, GOS Institute of Child Health, University of College London, London, UK (6).

Isl1 and *Prop1-cre* mice were genotyped as previously described (2, 3). *Hesx1-cre* mice were genotyped with oligos specific to the cre recombinase: forward 5'-GCATAACCAGTGAAACAGCATTGCTG-3' and reverse 5'-GGACATGTTCAGGGATCGCCAGGCG-3' using the following conditions: 94°C for 3 min followed by 33 cycles of 94°C 30 sec, 60°C 45 sec, 72°C 90 sec then ending with a final extension of 72°C for 10 min. *CAG-Cre-ERT2* mice were genotyped as described (5), using the following conditions: 94°C for 5 min followed by 35 cycles of 94°C 30 sec, 55°C 30 sec, 72°C 30 sec and a final extension of 72°C for 5 min.

Tissue Preparation. Staged embryos were established using timed pregnancy breeding with the observance of the copulation plug documented at E0.5. Embryos were collected and fixed in 4% buffered formaldehyde with time of fixation depending on the age of the embryo. E11.5 and

E12.5 were fixed for 45 mins and E13.5 thru E16.5 were fixed for 1 hr. After fixation the embryos were washed in 1xPBS then dehydrated through a series of ethanol solutions to 70%. For analysis of neonatal pituitary glands, P0, P3, and P7 heads were dissected and the skin, lower jaw, and skull removed to aid in the penetration of the fixative solutions. P0 and P3 heads were fixed for 4 hours and P7 heads were fixed overnight at 4°C. After fixation, the heads were fixed and treated with 10% EDTA 24-36 hrs to soften the bone and aid in sectioning. Heads were dehydrated through a series of ethanol solutions to 70%. Dissected pituitaries from 3 wk – 1 yr old mice were fixed overnight in 4% buffered formaldehyde, rinsed in 1xPBS, then treated for 24 hrs in 10% EDTA. Pituitaries were then dehydrated through a series of ethanol solutions to 70%. All tissues were processed in the Miles Scientific Tissue-Tek VP Model #20 embedding machine and the Shandon Histocentre 2 Model #64000012 embedding station. Embedded tissues were sectioned to 6µm thickness on an American Optical Microtome and mounted onto SuperFrost Plus microscope slides (Fisher Scientific). Sections from a minimum of 3 animals per genotype per time point were processed by Hematoxylin and Eosin staining or used for immunohistochemistry. Relative Rathke's pouch size was determined by expressing the average Rathke's pouch area as a percentage of the average head area. Areas were calculated with Image J software from five hematoxylin and eosin stained sections per embryo in 7 controls and 10 *Isl1*^{Prop1KO} samples.

For the analysis of *CAG-Cre-ERT2; Isl1*^{fl/fl} conditional mutant embryos, pregnant females were injected with tamoxifen at e12.5 and embryos were extracted at e18.5. *CAG-Cre-ERT2; Isl1*^{fl/fl} embryos were compared to their *Isl1*^{fl/fl} siblings, which served as negative controls. Embryo heads were fixed in 4% paraformaldehyde for 5 hrs and cryoprotected in 10% sucrose/PBS overnight at 4°C. The following day, heads were embedded in 10% gelatin (Sigma-Aldrich) in 10% sucrose/PBS solution, frozen in isopentane at -60°C and stored at -80°C

until use. Gelatin-embedded heads were sliced in 20µm cryosections using a cryostat (Microm 505n).

Immunohistochemistry. Prior to immunohistochemistry paraffin was removed from tissue sections using xylene and sections were rehydrated using a series of ethanol solutions and 1 x PBS. Single and double immunohistochemistry was performed as previously described (7). Specific antibody information, dilutions, and detection methods are listed in Supplemental Table 4. The ISL1 antibody, 40.2D6, was deposited to the Developmental Studies Hybridoma Bank (DSHB) by Jessell, T.M./Brenner-Morton, S.). The PROP1 antibody was a generous gift from Dr. Aimee K. Ryan, Department of Human Genetics, McGill University, Montreal, QC (7). The LHX3 antibody, 67.4E12, was deposited to the DSHB by Jessell, T.M./Brenner-Morton, S.). The NR5A1 antibody was a generous gift from Dr. Gary Hammer, Cell and Developmental Biology, University of Michigan (8). The PAX7 antibody was deposited to the DSHB by Kawakami, A. (DSHB Hybridoma Product PAX7). The PITX1 antibody was a generous gift from Dr. Jacques Drouin, Laboratoire de Genetique Moleculaire, Institut de recherches cliniques de Montreal, Montreal, QC (9). This POU1F1 antibody was a kind gift from Dr. Simon Rhodes, University of North Florida, Jacksonville, FL (10). The SHH antibody, 5E1, was deposited to the DSHB by Jessell, T.M./Brenner-Morton, S.). The number of LH positive and TSH positive cells per unit area was determined using the Image J software and counting the number of immunopositive cells per unit area in an average of 4 slides per sample across 5 control and 4 *Isl1^{Prop1KO}* neonates. The area of the cysts was excluded from the overall pituitary area in *Isl1^{Prop1KO}* samples. The number of FSH and TSH cells in *CAG-Cre-ERT2; Isl1^{fl/fl}* pituitaries was determined by counting immunopositive cells per unit area using FIJI (Image J) software in an average of 6 slides per sample across 5 control and 5 mutant embryos.

Alcian Blue/PAS staining. Prior to Alcian Blue/PAS staining, paraffin was removed from tissue sections using Xylene and sections were rehydrated to 95% ethanol, then rinsed in distilled water. Sections were then incubated in 3% acetic acid for 3 mins then incubated for 15 mins in Alcian Blue, pH 2.5. Sections were then rinsed in running tap water for 2 mins, followed by a 5-min incubation in 0.5% periodic acid. Sections were again rinsed for 2 mins under gently running tap water then incubated for 10 mins in Schiff's Reagent. Sections were then washed for 5 mins in lukewarm tap water, dehydrated to 100% ethanol, cleared with xylene and mounted with Permount Mounting Media.

In Situ Hybridization. The *Gata2* cDNA clone K7B20006K16 was from an E14.5 mouse pituitary cDNA library (11). The *Gata2* plasmid was linearized with *XhoI* and transcribed with T3 polymerase to generate the antisense probe and linearized with *SfiI* and transcribed with T7 to generate the sense control probe. The probes were diluted 1:100 in hybridization solution and the ISH protocol was performed as previously described (12).

RNAseq. P0 pituitaries were harvested from 6 control mice and 6 *Isl1^{Prop1KO}* mice. RNA was prepared using the RNA Micro Kit (Ambion). Libraries were prepared from the RNA using the TruSeq RNA Library Prep Kit v2 (Illumina) by the University of Michigan Sequencing Core. The quality of the library preps was confirmed using the Bioanalyzer 2200 (Agilent, Santa Clara, CA). 50 bp single-end sequencing was performed on Illumina HiSeq 4000, each sample was barcoded and pooled and the samples were run across two lanes. Sequencing reads were analyzed by the University of Michigan Bioinformatics Core. The quality of the raw reads data for each sample was confirmed using FastQC (version 0.10.1) to identify features of the data that may indicate quality problems (e.g. low quality scores, over-represented sequences, inappropriate GC content, etc.). The reads were trimmed based on these quality scores. The Tuxedo Suite software package was utilized for alignment, differential expression analysis, and

post-analysis diagnostics (13-15). Briefly, reads were aligned to the combined reference mRNA and lncRNA total transcriptome (UCSC mm10) (<http://genome.ucsc.edu/>) using TopHat (version 2.0.9) and Bowtie (version 2.1.0.). Default parameter settings were used for alignment, with the exception of: “--b2-very-sensitive” telling the software to spend extra time searching for valid alignments. FastQC was used for a second round of quality control (post-alignment), to ensure that only high-quality data would be input to expression quantitation and differential expression analysis. Cufflinks/CuffDiff (version 2.1.1) was used for expression quantitation, normalization, and differential expression analysis, using UCSC mm10.fa as the reference genome sequence. For this analysis, the parameter settings: “--multi-read-correct” were used to adjust expression calculations for reads that map in more than one locus, as well as “--compatible-hits-norm” and “--upper-quartile -norm” for normalization of expression values. Diagnostic plots were generated using the CummeRbund package. We used locally developed scripts to format and annotate the differential expression data output from CuffDiff. Briefly, we identified genes and transcripts as being differentially expressed based on three criteria: test status = “OK”, FDR \leq 0.05 and fold change $\geq \pm 1.5$. We annotated genes and isoforms with NCBI Entrez GeneIDs and text descriptions. We further annotated differentially expressed genes with Gene Ontology (GO) (<http://www.geneontology.org/>) terms using NCBI annotation. We used Gene Sequence Enrichment Analysis software (GSEA_4.0.3) for enrichment analysis of the set of differentially expressed genes to identify significantly enriched functional categories. Principal Component Analysis was performed using ClustVis (16). Data from the RNAseq has been deposited in NCBI’s Gene Expression Omnibus (17) and is accessible through the Geo accession number GSE149019.

CAG-Cre-ERT2; Isl1^{fl/fl} tamoxifen injection. Tamoxifen (T56648, Sigma-Aldrich) was dissolved in sesame oil (S3547, Sigma-Aldrich) at 10 mg/ml. A single dose of tamoxifen (75 μ g per gram of body weight) was administered to the mother by intraperitoneal injection at e12.5.

Serum T4 analysis. Blood was collected from 7 control male mice and 7 *Isl1^{Prop1KO}* mutant male mice at 3 wks of age. Blood was allowed to coagulate at room temperature for 20 mins and serum was isolated by centrifugation at 1000rpm for 30 mins. Free T4 levels were analyzed using 25ul of serum with the Total T4 EIA Kit (MP Biomedical).

Statistics. Significance of normalized Rathke's pouch size, LH, TSH, and FSH cells/area, and T4 serum analysis were determined using the Student's T-Test, one tail distribution, 2 sample unequal variance.

Study approval. All mouse studies were approved by the University of Michigan IACUC (PRO00008714) or by the FCEN-University of Buenos Aires CICUAL (protocol no. 68). Mice were housed in an AALAC approved animal facility at University of Michigan and at the Bioterio Central of FCEN-UBA.

Informed consent for collection of human pituitary specimens was approved by the ethical committee of Kagoshima University (reference no. 180135). Anonymized specimens were sent to University of Michigan for immunostaining, which was classified as exempt by the IRB-MED.

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