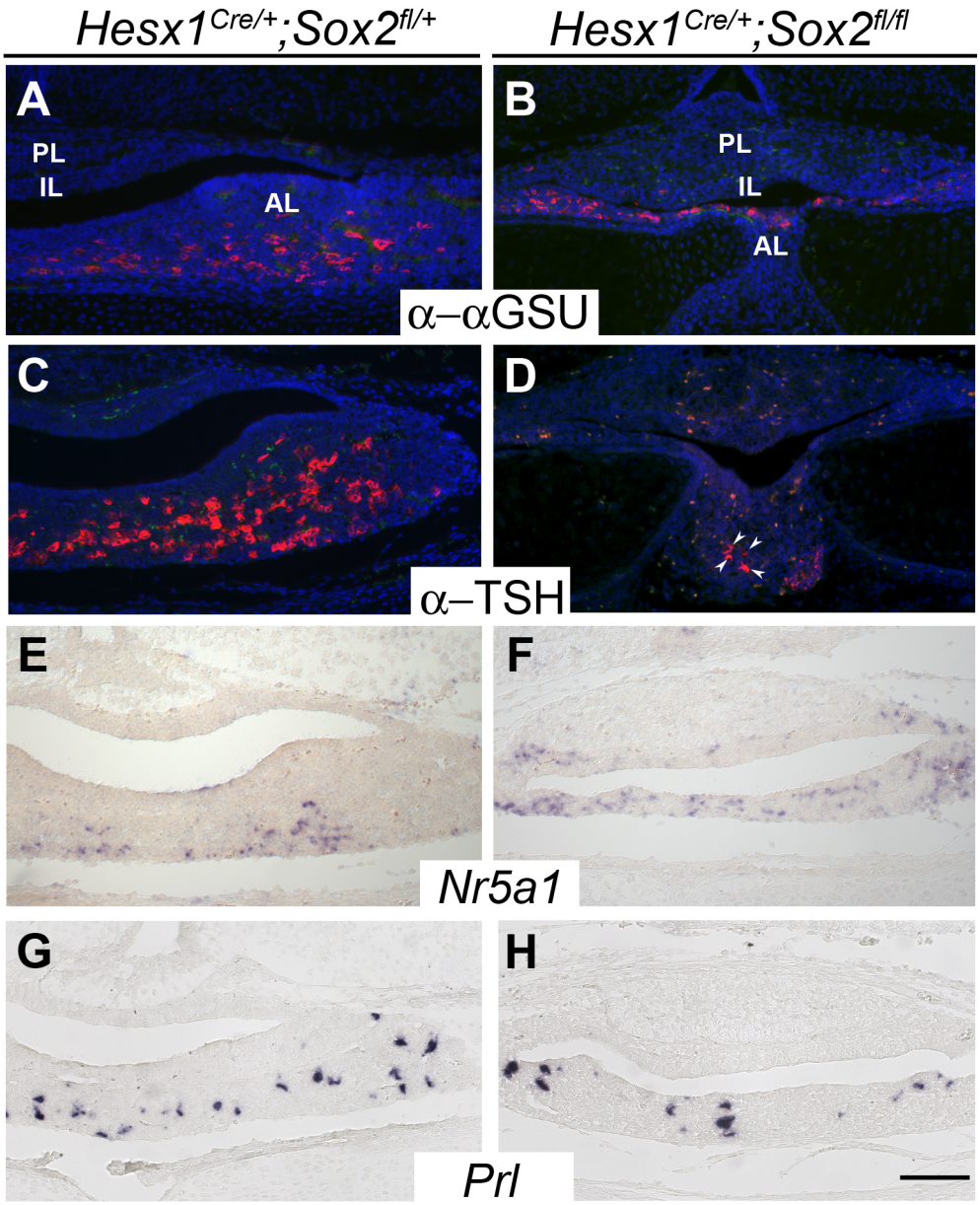
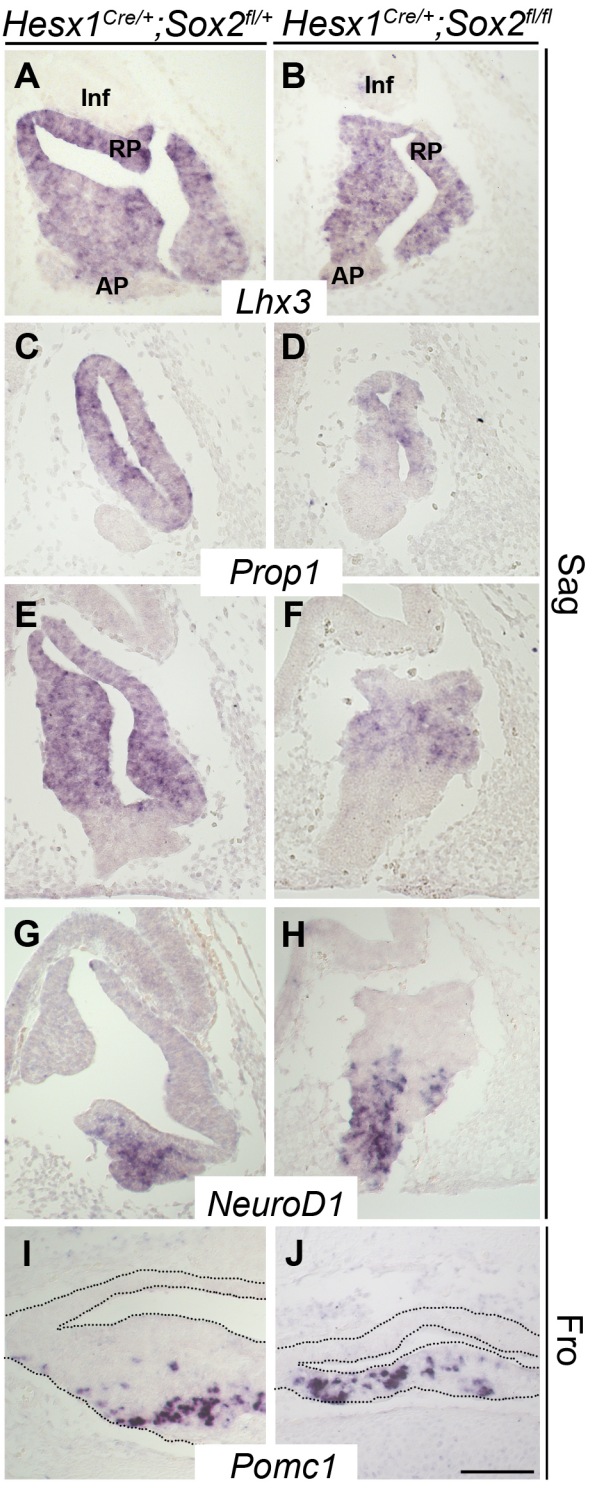
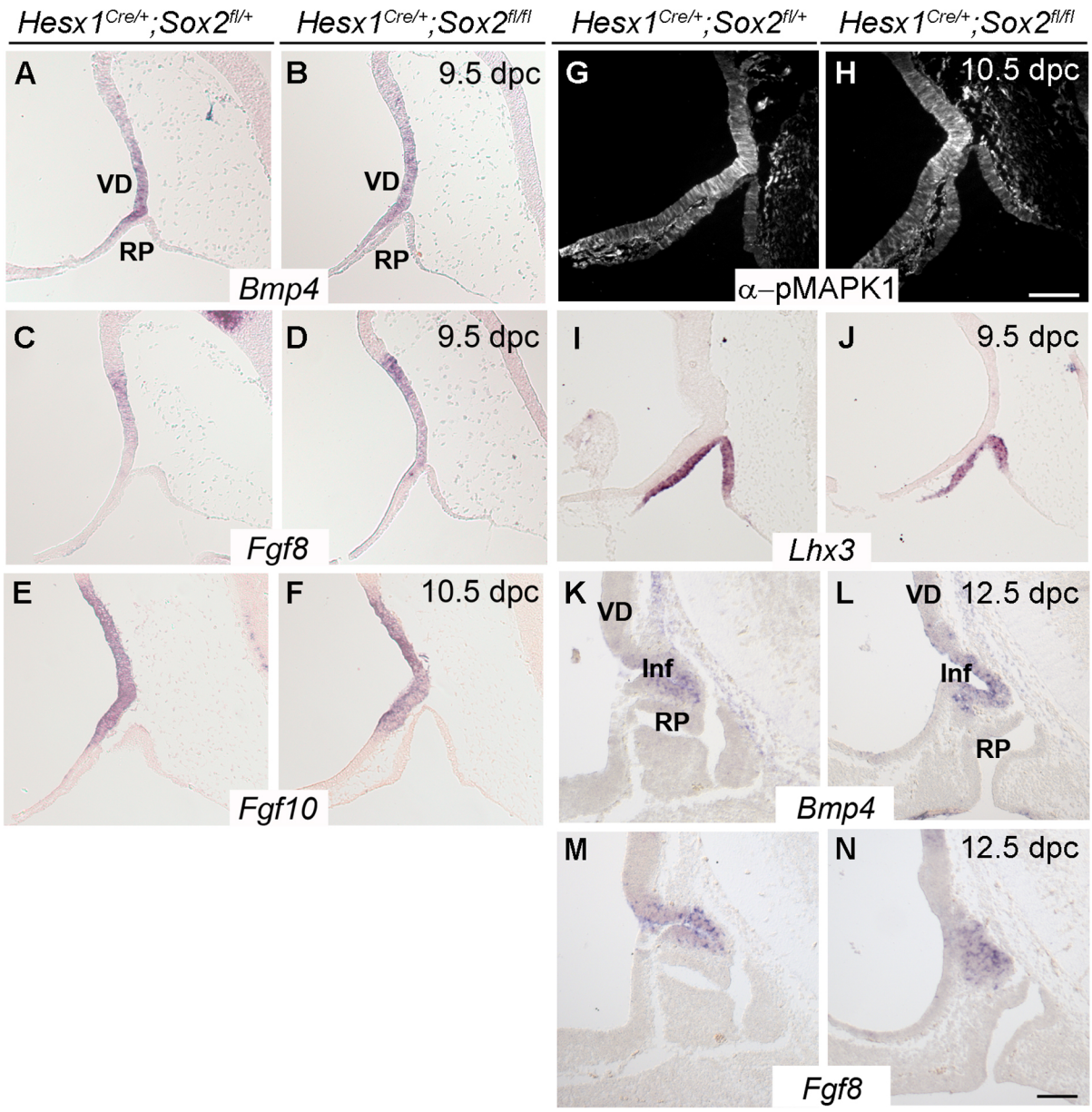


Supplementary Figure 1
Gonadotrophs and lactotrophs are present in the hypoplastic pituitary of *Hesx1*$^{Cre/+}$;*Sox2*$^{fl/fl}$ pituitaries at late gestation. Immunofluorescence with specific antibodies against alpha-glycoprotein subunit (αGSU) and thyroid-stimulating hormone (TSH), and in situ hybridization against *Nr5a1* and prolactin (*Prl*) on transverse histological sections on *Hesx1*$^{Cre/+}$;*Sox2*$^{fl/+}$ control and *Hesx1*$^{Cre/+}$;*Sox2*$^{fl/fl}$ mutant embryos at 18.5 dpc. (**A** and **B**) αGSU^{+ve} cells, marking prospective thyrotrophs and gonadotrophs, are abundant in the pituitary gland of both genotypes. (**C** and **D**) The severe reduction of TSH^{+ve} cells (a specific marker of thyrotrophs) in the mutant pituitary implies that most of the αGSU^{+ve} cells detected in B may correspond with gonadotrophs. The green signal in A-D is due to blood cells. (**E** and **F**) In situ hybridization with *Nr5a1* antisense riboprobes demonstrates the presence of differentiating gonadotrophs in the control and mutant pituitary. (**G** and **H**) Fully differentiated lactotrophs, expressing *Prl* mRNA, are detected in the mutant and control pituitaries. PL, posterior lobe; IL, intermediate lobe; AL, anterior lobe. Scale bar: 100 μm.



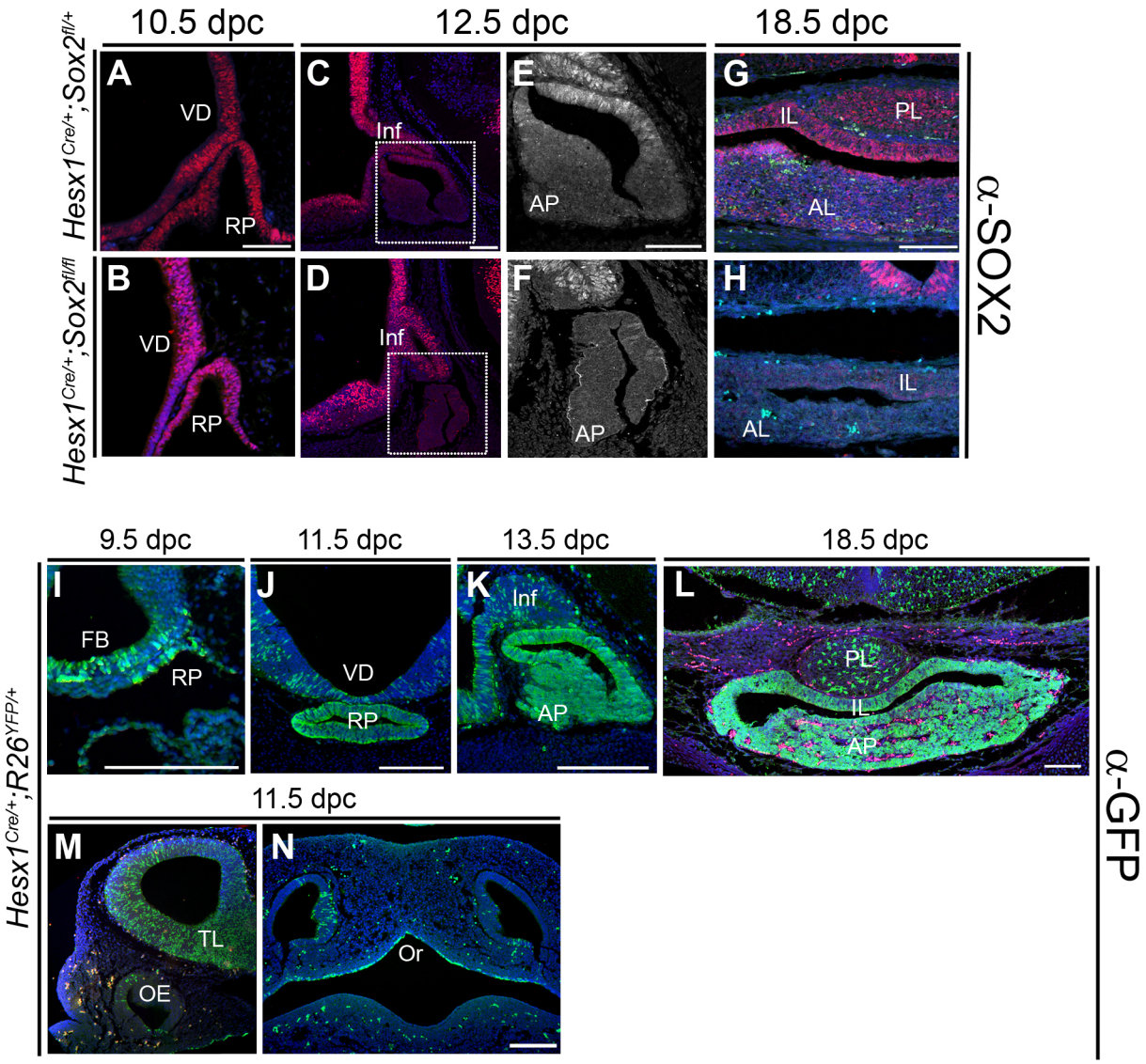
Supplementary Figure 2

Reduced *Prop1* expression in the developing pituitary of *Hesx1*$^{Cre/+}$;*Sox2*$^{fl/fl}$ embryos. *In situ* hybridization with antisense riboprobes against *Lhx3*, *Prop1*, *NeuroD1* and *Pomc1* on sagittal (A-H) and frontal (I,J) histological sections in *Hesx1*$^{Cre/+}$;*Sox2*$^{fl/+}$ control and *Hesx1*$^{Cre/+}$;*Sox2*$^{fl/fl}$ mutant embryos at 12.5 dpc. (**A** and **B**) *Lhx3* is expressed in the mutant pituitary, but a reduction in its expression domain is noted, corresponding to the smaller size of the mutant (B) relative to the control (A) pituitary. (**C**-**F**) *Prop1* expression domain is reduced in the mutant (D,F) compared with the control pituitary (C,E). (**G**-**J**) *NeuroD1* and *Pomc1* expression domains are comparable to the control pituitaries. Scale bar: 100 µm.



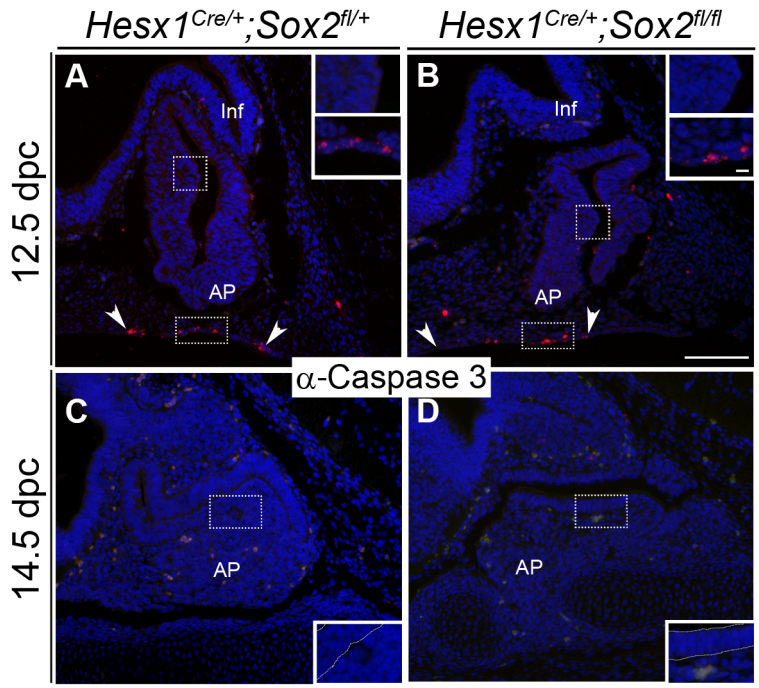
Supplementary Figure 3

Induction of Rathke's pouch is not affected in *Hesx1*$^{Cre/+}$;*Sox2*$^{fl/fl}$ mutant embryos. *In situ* hybridization (A-F,I-N) and immunostaining (G,H) on sagittal histological sections of *Hesx1*$^{Cre/+}$;*Sox2*$^{fl/+}$ control and *Hesx1*$^{Cre/+}$;*Sox2*$^{fl/fl}$ mutant embryos during early developmental stages. (**A**-**F**) Expression of *Bmp4*, *Fgf8* and *Fgf10* in the ventral diencephalon (VD) is comparable between genotypes. (**G** and **H**) pMAPK1 immunofluorescence staining in the VD and Rathke's pouch (RP) is similar in the control and mutant pituitary. (**I** and **J**) *Lhx3* expression domain within the developing RP is comparable between genotypes. (**K**-**N**) Expression of *Bmp4* and *Fgf8* in the infundibulum (Inf) of the mutant and control pituitary is analogous, but there is an obvious hypoplasia of the developing RP. Scale bars: 100 µm.

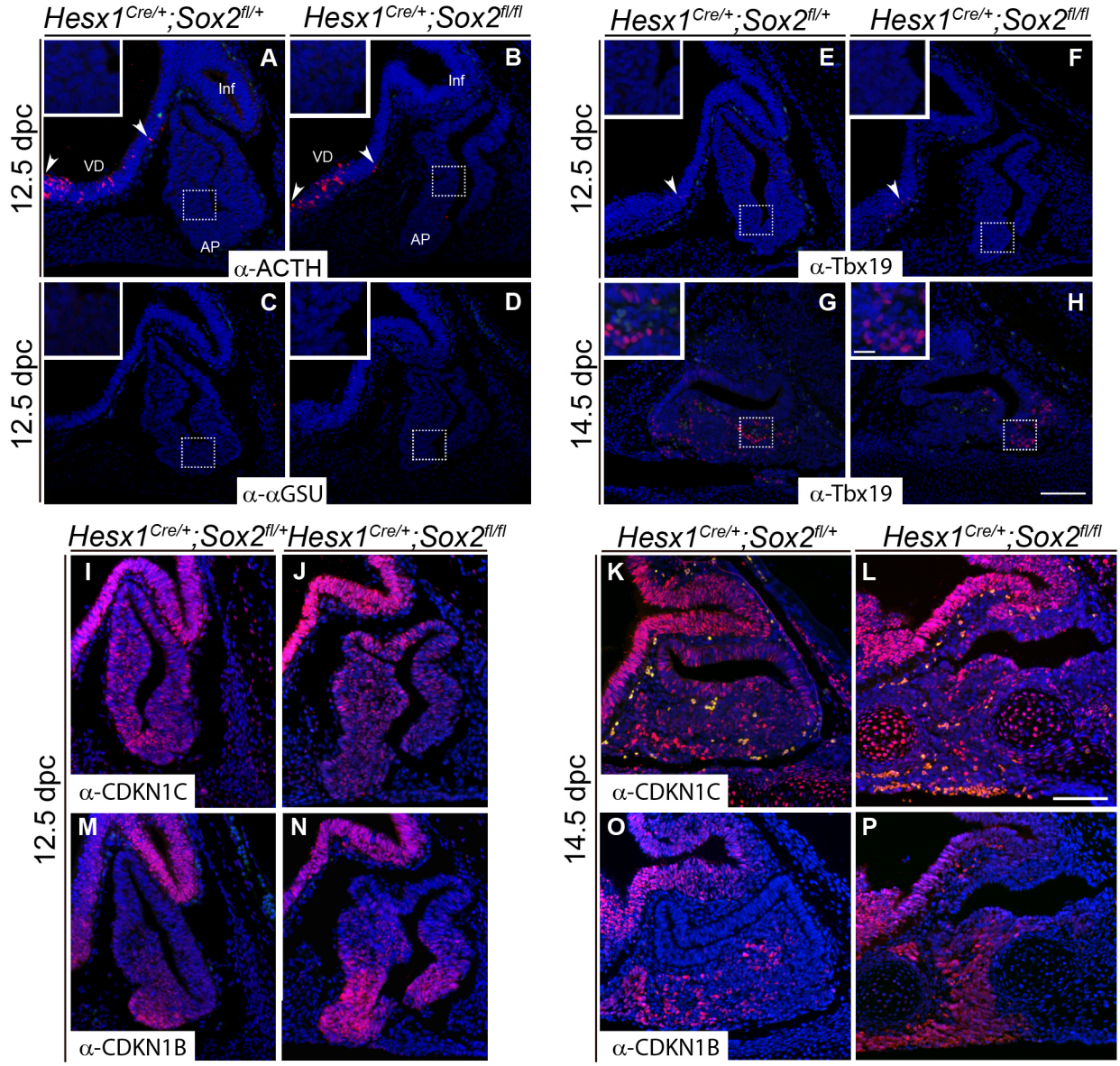


Supplementary Figure 4

Analysis of *Sox2* excision in *Hesx1*$^{Cre/+}$*;Sox2*$^{fl/fl}$ mutant embryos. SOX2 (A-H) and GFP (I-N) immunofluorescence staining on sagittal (A-F,I,K) or transverse (G,H,J,L-N) histological sections of *Hesx1*$^{Cre/+}$*;Sox2*$^{fl/+}$ control and *Hesx1*$^{Cre/+}$*;Sox2*$^{fl/fl}$ mutant embryos. (**A** and **B**) SOX2 is detectable in the ventral diencephalon (VD) and developing Rathke's pouch (RP) in both genotypes at 10.5 dpc. (**C**-**F**) At 12.5 dpc, SOX2 is detected in the infundibulum and neuroepithelium of control and mutant embryos, but expression in the periluminal epithelium is observed only in the control embryo. Note that SOX2 expression is not detectable in the developing AP at this stage. (**G** and **H**) SOX2 expression is detected in the pituitary gland in the control (G), but not in the mutant (H) pituitary at 18.5 dpc. The green signal in G and F is due to blood cells. (**I**) Cre-mediated recombination is detected in the forebrain (FB) and Rathke's pouch (RP) of *Hesx1*$^{Cre/+}$*;R26*$^{YFP/+}$ embryos at 9.5 dpc. (**J**) At 11.5 dpc, most of the cells in the developing RP have recombined and express YFP, but the pattern of excision in the VD is mosaic. (**K** and **L**) YFP expression is evident throughout the entire developing pituitary and only sparse cells express YFP in the infundibulum (Inf) or posterior lobe (PL). (**M** and **N**) YFP expression is also detected in the telencephalon (TL), olfactory epithelium (OE) and oral ectoderm (Or). YFP expression has been revealed using an anti-GFP antibody. Scale bars: 100 µm.

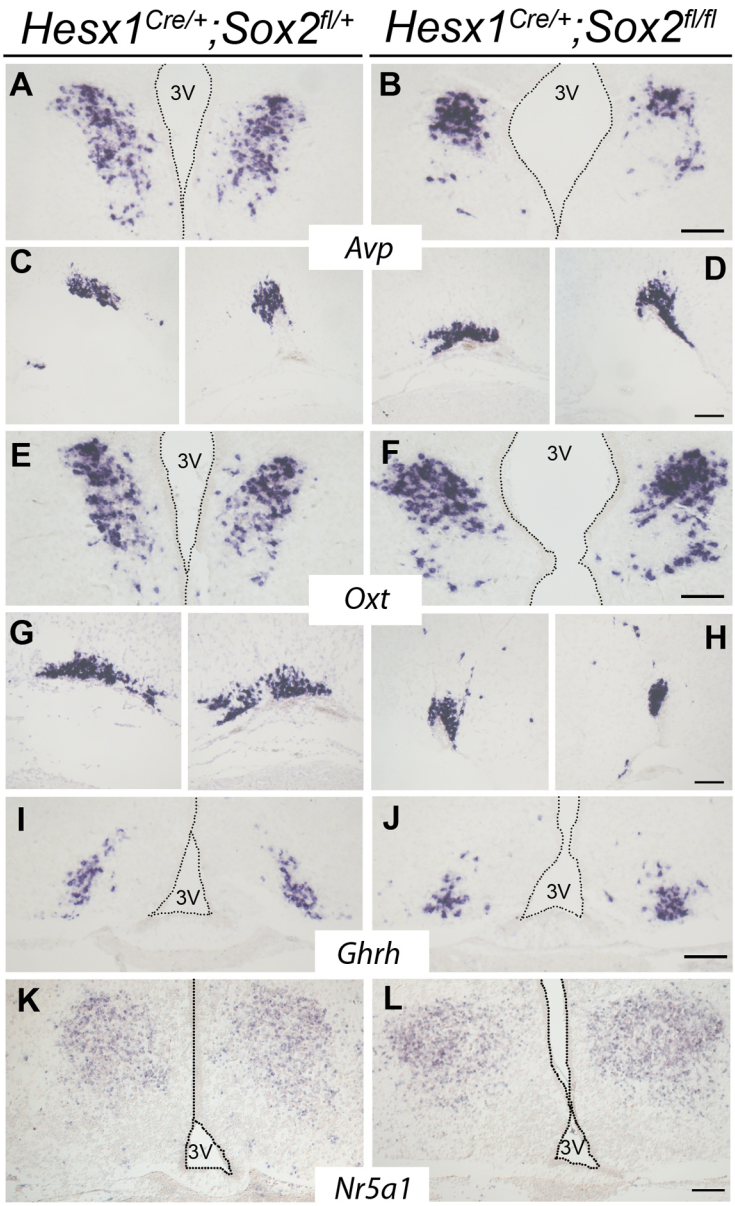


Supplementary Figure 5
Increased apoptosis does not contribute to the hypoplastic phenotype observed in *Hesx1*$^{Cre/+}$;*Sox2*$^{fl/fl}$ pituitaries. (**A-D**) At 12.5 dpc, cell death, as detected by cleaved Caspase 3 immunofluorescence staining, is observed in the region of the oral ectoderm adjacent to the ventral region of the developing pituitary gland at comparable levels in both genotypes. Cell death is absent in the developing anterior pituitary (AP) and infundibulum (Inf) at both stages examined. Scale bars: 100 μm; 10 μm in the insets.



Supplementary Figure 6

Premature cell differentiation is not detectable in the developing pituitary of *Hesx1*$^{Cre/+}$;*Sox2*$^{fl/fl}$ embryos. Immunofluorescence with specific antibodies against adrenocorticotrophic hormone (ACTH), alpha glycoprotein subunit (αGSU), T-box pituitary transcription factor (TBX19), CDKN1C and CDKN1B on sagittal histological sections of *Hesx1*$^{Cre/+}$;*Sox2*$^{fl/+}$ control and *Hesx1*$^{Cre/+}$;*Sox2*$^{fl/fl}$ mutant embryos. (**A-H**) There is no premature expression of any of the markers analysed in the mutant compared with control pituitaries. Expression of ACTH and TBX19 in neuroepithelial cells in the ventral diencephalon (VD) is indicated with arrowheads in A, B, E and F. (**I-L**) There are no overall significant differences in the expression of CDKN1C between genotypes. (**M-P**) Expression of CDKN1B appears more extensive in the mutant compared with the control pituitaries at all stages analysed. Scale bars: 100 μm; 20 μm in the insets.



Supplementary Figure 7

Hypothalamic development is not overtly affected in the *Hesx1*$^{Cre/+}$*;Sox2*$^{fl/fl}$ mutant embryos. In situ hybridization with arginine vasopressin (*Avp*), oxytocin (*Oxt*), growth hormone releasing hormone (*Ghrh*) and *Nr5a1* on transverse histological sections of *Hesx1*$^{Cre/+}$*;Sox2*$^{fl/+}$ control and *Hesx1*$^{Cre/+}$*;Sox2*$^{fl/fl}$ mutant embryos at 18.5 dpc. (**A-H**) *Avp*$^{+ve}$ and *Oxt*$^{+ve}$ neurons are detected at comparable levels in the paraventricular (A,B,E,F) and supraoptic (C,D,G,H) nuclei in control and mutant embryos. (**I-L**) *Ghrh*$^{+ve}$ (I,J) and *Nr5a1*$^{+ve}$ (K,L) neurons are detected in the arcuate and ventro-medial hypothalamic nuclei, respectively, at similar levels. 3V, 3rd ventricle. Scale bars: 100 µm.

Supplementary Table 1

Genotypes obtained from *Hesx1*$^{Cre/+}$;*Sox2*$^{fl/+}$ x *Sox2*$^{fl/+}$ intercrosses.

Stage	Genotypes (% expected)[a]						Total
	Sox2$^{+/+}$; *Hesx1*$^{+/+}$ (12.5%)	*Sox2*$^{+/+}$; *Hesx1*$^{Cre/+}$ (12.5%)	*Sox2*$^{fl/+}$; *Hesx1*$^{+/+}$ (25%)	*Sox2*$^{fl/+}$; *Hesx1*$^{Cre/+}$ (25%)	*Sox2*$^{fl/fl}$; *Hesx1*$^{+/+}$ (12.5%)	*Sox2*$^{fl/fl}$; *Hesx1*$^{Cre/+}$ (12.5%)	
10.5 dpc	5	6	8	3	8	4	34
12.5 dpc	1	2	2	1	2	1	9
13.5 dpc	2	4	4	3	3	4	20
15.5 dpc	2	3	8	6	4	6	29
17.5 dpc	6	1	3	5	2	1	18
18.5 dpc	1	2	8	7	3	5	26
Embryos† (% observed)	17 (12.5%)	18 (13%)	33 (24%)	25 (18%)	22 (16%)	21 (15.5%)	136
Pups* (% observed)	19 (21.5%)	15 (17%)	16 (18%)	26 (29.5%)	12 (13.5%)	0	88

[a] Derived from expected Mendelian ratios.

*Chi-square test showed a significant deviation from the expected 12.5% ratio (P= 0.010).

†Chi-square test showed no significant deviation from the expected Mendelian ratio.

Supplementary Table 2

Genotypes obtained from *Hesx1$^{Cre/+}$;Sox2$^{fl/+}$ x Sox2$^{fl/fl}$* intercrosses.

Stage	Genotypes (% expected)[a]				Total
	Hesx1$^{+/+}$;Sox2$^{fl/+}$ (25%)	*Hesx1$^{+/+}$;Sox2$^{fl/fl}$* (25%)	*Hesx1$^{Cre/+}$;Sox2$^{fl/+}$* (25%)	*Hesx1$^{Cre/+}$;Sox2$^{fl/fl}$* (25%)	
9.5 dpc	4	3	10	9	26
10.5 dpc	5	6	6	4	21
11.5 dpc	1	2	2	1	6
12.5 dpc	9	8	15	9	41
13.5 dpc	2	1	3	2	8
14.5 dpc	5	8	16	6	35
18.5 dpc	7	7	6	8	28
Embryos† (% observed)	33 (20%)	35 (21%)	58 (35%)	39 (24%)	165
Pups* (% observed)	26 (54%)	7 (14.5%)	15 (31%)	0	48

[a] Derived from expected Mendelian ratios.

*Chi-square test showed a significant deviation from the expected 25% ratio ($P < 0.001$).

†Chi-square test showed no significant deviation from the expected Mendelian ratio.

Supplementary Table 3

Pituitary function tests performed in Patients 1 and 2 carrying *SOX2* heterozygous mutations

	Patient 1 (Ref. range)	Patient 2 (Ref. range)
Free T4 (pmol/L)	14.3 (10.8-19.0)	17.8 (10.2-20.6)
TSH (mU/L)	1.6 (< 6.0)	0.9 (< 4.0)
IGF-1 (ng/ml)	289 (237-996)	97 (116-358)
IGFBP-3 (mg/L)	6.67 (3.5-10.0)	3.33 (3.4-7.8)
Peak GH to glucagon stimulation (μg/L)	28.1 (> 6.7)	38.8 (> 6.7)
09.00h Cortisol (nmol/L)	516	483
Prolactin (mU/L)	69 (76-551)	211 (76-551)
Oestradiol (pmol/L)	<44	<44