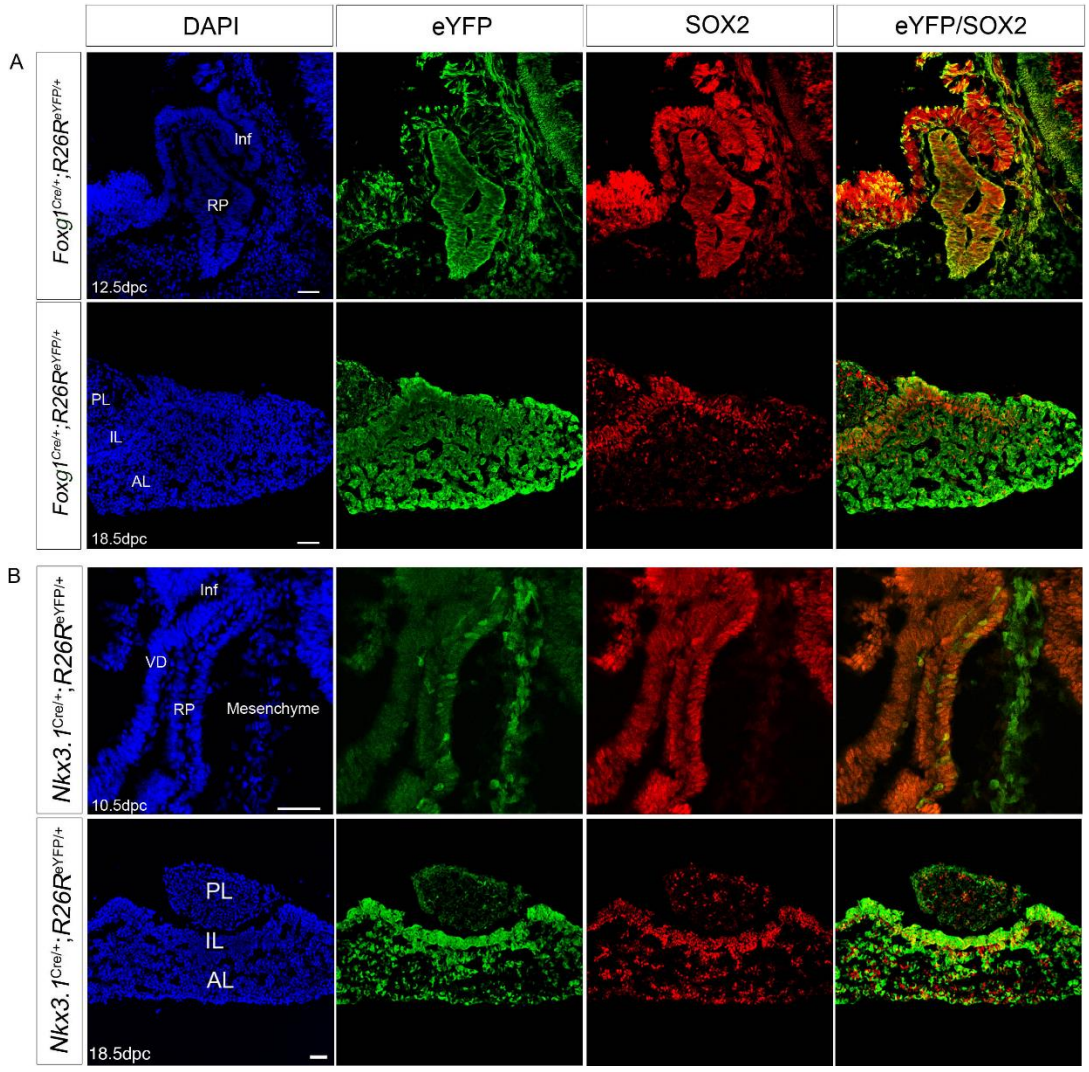

Fig. S1. Cre drivers lineage tracing analysis in embryonic pituitaries.

A) *Foxg1*^{Cre} lineage tracing analysis. Immunofluorescence for eYFP and SOX2, at 12.5 and 18.5dpc in *Foxg1*^{Cre}*;R26R*^{eYFP} embryos. eYFP is observed in all cells of RP at 12.5dpc and in some cells in the VD (these are not present on a pure 129 background, data not shown). At the end of gestation the pituitary is essentially ubiquitously eYFP positive.

B) *Nkx3.1*^{Cre} lineage tracing analysis. Immunofluorescence for eYFP and SOX2 at 10.5dpc and 18.5dpc in *Nkx3.1*^{Cre}*;R26R*^{eYFP} embryos. eYFP is initially observed in a small number of cells in RP at 10.5dpc. By 18.5dpc nearly all cells in IL are eYFP positive. In the anterior lobe, eYFP positive cells also make a significant contribution.

10.5dpc – 12.5dpc sections are orientated sagittally, 18.5dpc are orientated coronally. Scale bar = 50μm.

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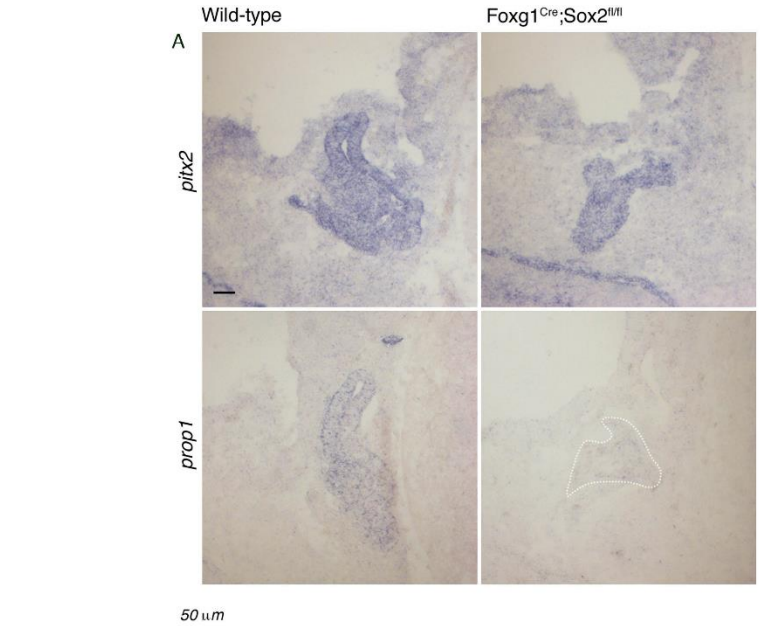

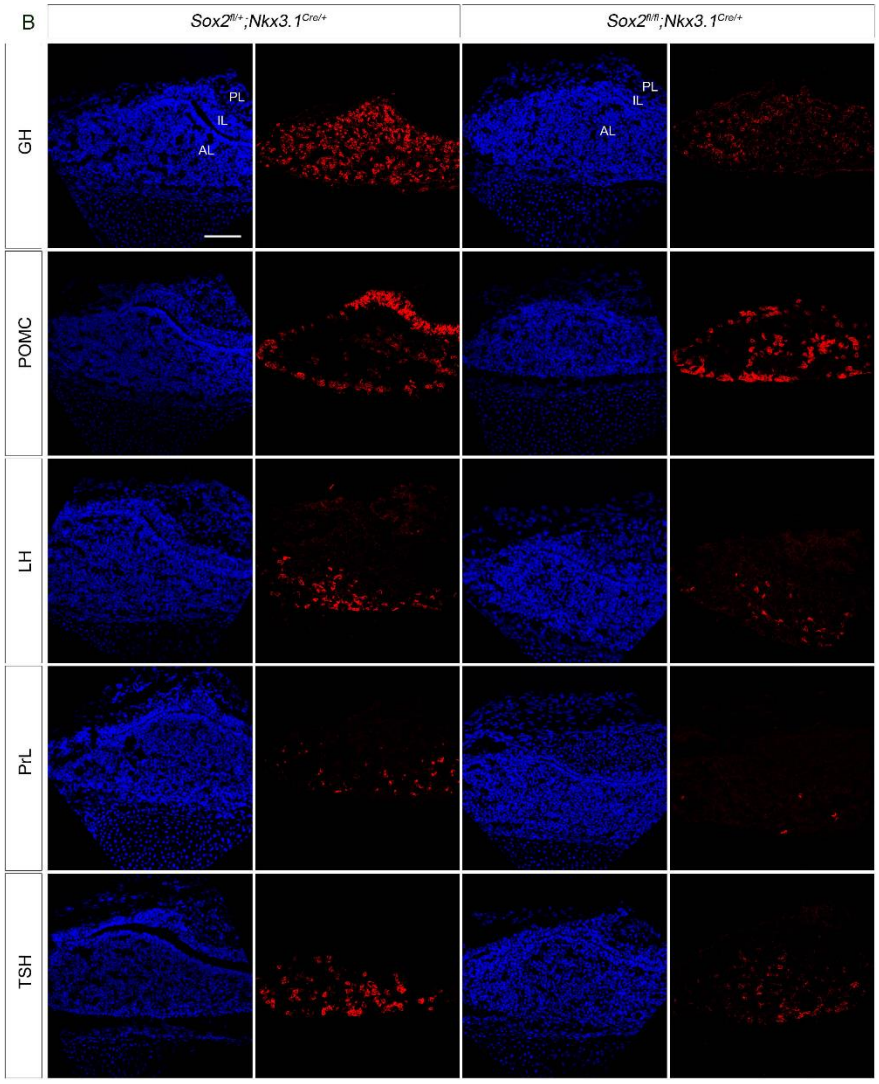

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Fig. S2. Analysis of *Pitx2* and *Prop1* transcripts and reduction in pituitary hormones in *Sox2*$^{fl/fl}$;*Nkx3.1*$^{Cre/+}$ embryos.

A) *In situ* hybridization for *Pitx2* and *Prop1* at 12.5dpc. *Pitx2* expression is seemingly unaffected by the loss of *Sox2* while *Prop1* expression is clearly downregulated in *Sox2* $^{fl/fl}$; *Foxg1*$^{Cre/+}$ mutants.

B) Immunofluorescence for GH, POMC, LH, PrL and TSH at 18.5dpc. The number of hormone positive cells is generally reduced in *Sox2*$^{fl/fl}$;*Nkx3.1*$^{Cre/+}$ pituitaries compared to *Sox2*$^{fl/+}$;*Nkx3.1*$^{Cre/+}$ ones, with AL corticotrophs appearing less affected than the other endocrine cell types.

Scale bar = 50 μm for A and 100μm for B.

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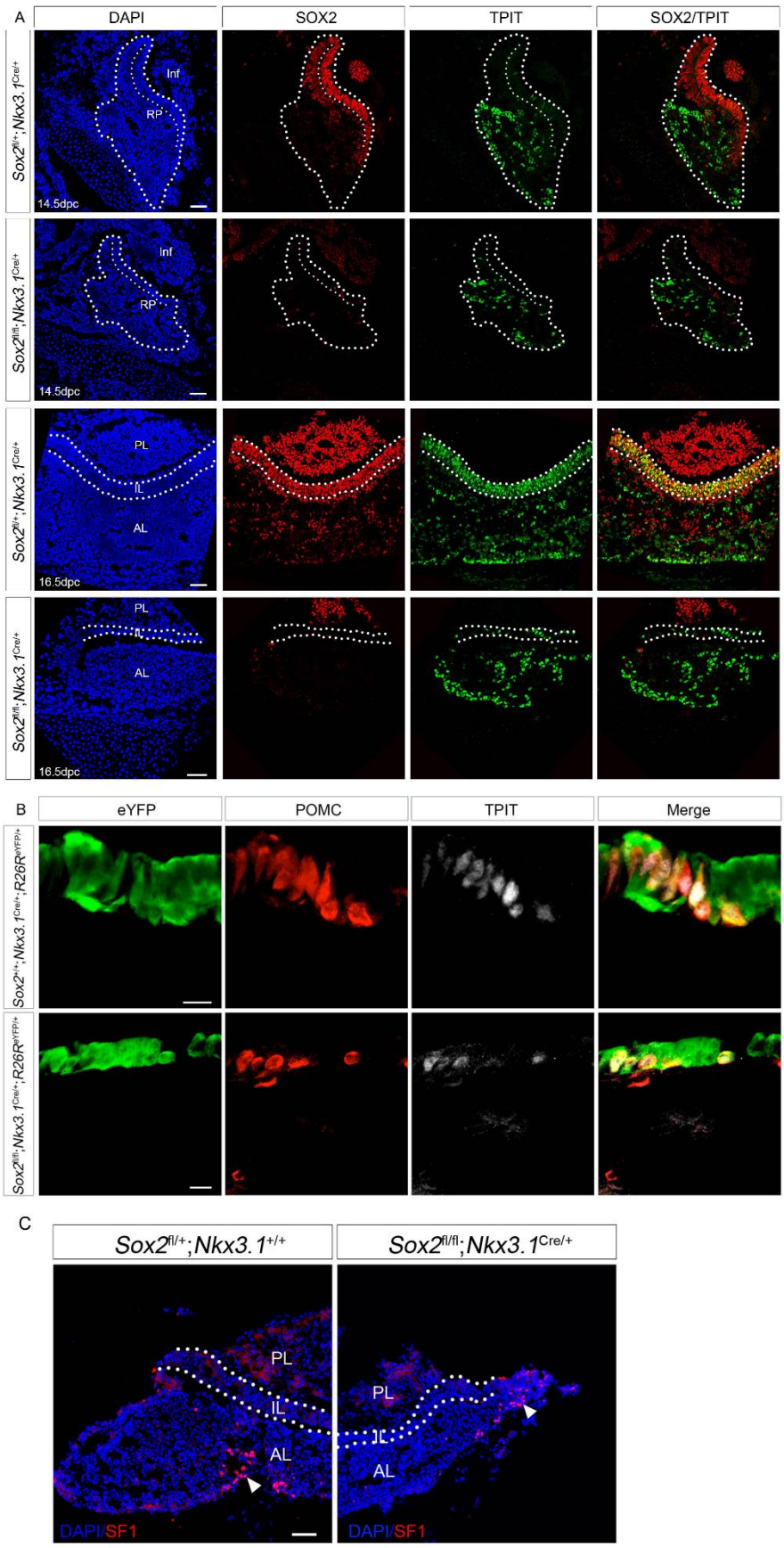

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Fig. S3. Analysis of Tpit and SF1 expression in *Sox2$^{fl/fl}$;Nkx3.1$^{Cre/+}$* embryos.

A) Immunofluorescence for SOX2 and TPIT at 14.5dpc and 16.5dpc in Nkx3.1^{Cre} mutants. At 14.5dpc, TPIT is expressed in AL corticotrophs. It is later upregulated in IL melanotrophs, by 16.5dpc, as observed in *Sox2$^{fl/+}$;Nkx3.1$^{Cre/+}$* control embryos. Its expression is dramatically reduced in the IL of *Sox2$^{fl/fl}$;Nkx3.1$^{Cre/+}$* embryos.

B) Immunofluorescence for eYFP, POMC and TPIT at 16.5dpc. TPIT is expressed in all eYFP, POMC positive cells in control *Nkx3.1$^{Cre/+}$; R26R$^{eYFP/+}$* embryos. This is also true in *Sox2 $^{fl/fl}$; Nkx3.1$^{Cre/+}$; R26R$^{eYFP/+}$* embryos where TPIT positives cells are also POMC positive.

C) Immunofluorescence for SF1 at 18.5dpc in Nkx3.1^{Cre} mutants. SF1 is expressed in AL gonadotrophs in both *Sox2$^{fl/+}$;Nkx3.1$^{Cre/+}$* and *Sox2$^{fl/fl}$;Nkx3.1$^{Cre/+}$* embryos (arrows). There is no ectopic expression in IL of *Sox2$^{fl/fl}$;Nkx3.1$^{Cre/+}$* embryos.

Scale bar = 50μm for A and C and 10μm for B.

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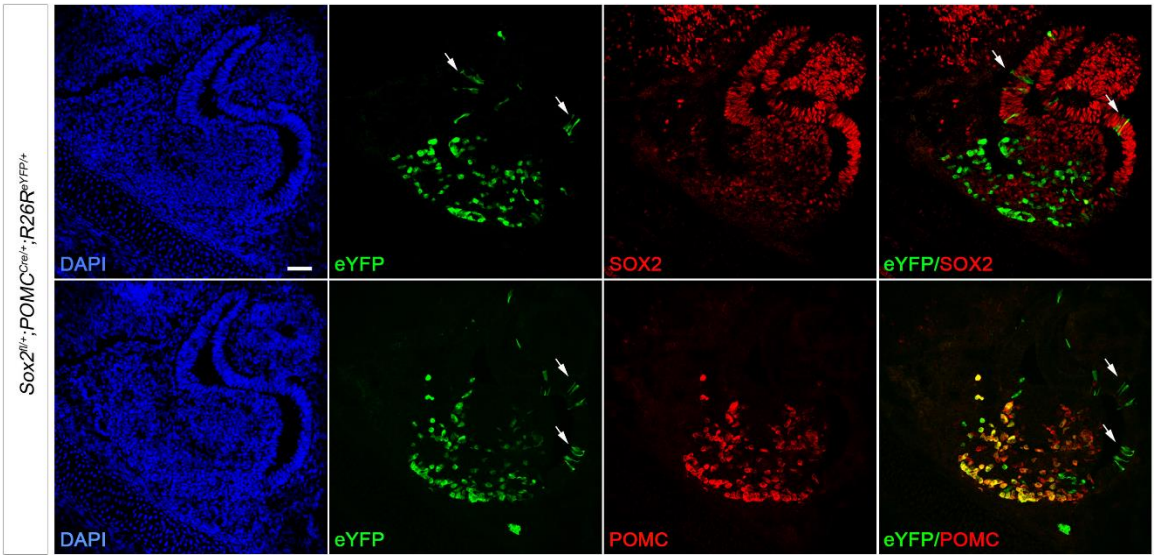

Fig. S4. Pomc-Cre lineage tracing analysis at 14.5dpc.

Immunofluorescence for eYFP, SOX2 and POMC in *Sox2$^{fl/+}$;Pomc-Cre;R26R^{eYFP}* embryos. eYFP is expressed in the developing AP, always co-localising with endogenous POMC in about half of the corticotrophs, as described (Langlais et al., 2013). In addition, we observe some minor ectopic activity in the SOX2 positive cells lining the cleft, both ventrally and dorsally where the future IL will develop (arrows). These eYFP positive cells do not express POMC and therefore likely represent ectopic activity of the promoter. Scale bar = 50 μm.

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Table S1

List of antibodies used.

	Host specie	Dilution	Source	Catalogue Number
BrdU	Rat	1:100	Abcam	Ab6326
E-cadherin	Rat	1:1000	Sigma	U3254
GFP	Rat	1:1000	Nacalai-Tesque	GF090R
GR	Rabbit	1:100	Santa Cruz Biotechnology	Sc-1004
LHX3	Rabbit	1:500	Abcam	ab14555
PAX7	Mouse	1:50	DSHB	AB_528428
Pituitary hormones	Rabbit	1:500	NHPP	
SF1	Rabbit	1:300	Cell Signalling Solution	12800
SIX6	Rabbit	1:500	Sigma	HPA001403
SOX2	Goat	1:500	ISL	GT15098
TBX19	Rabbit	1:1000	Gift from J. Drouin, IRCM, Montreal	

The secondary antibodies used at a dilution of 1 in 500 were donkey anti-goat, rabbit, rat and goat anti-mouse IgG (H+L) Alexa 488, 568 and 647 (Molecular Probes).

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