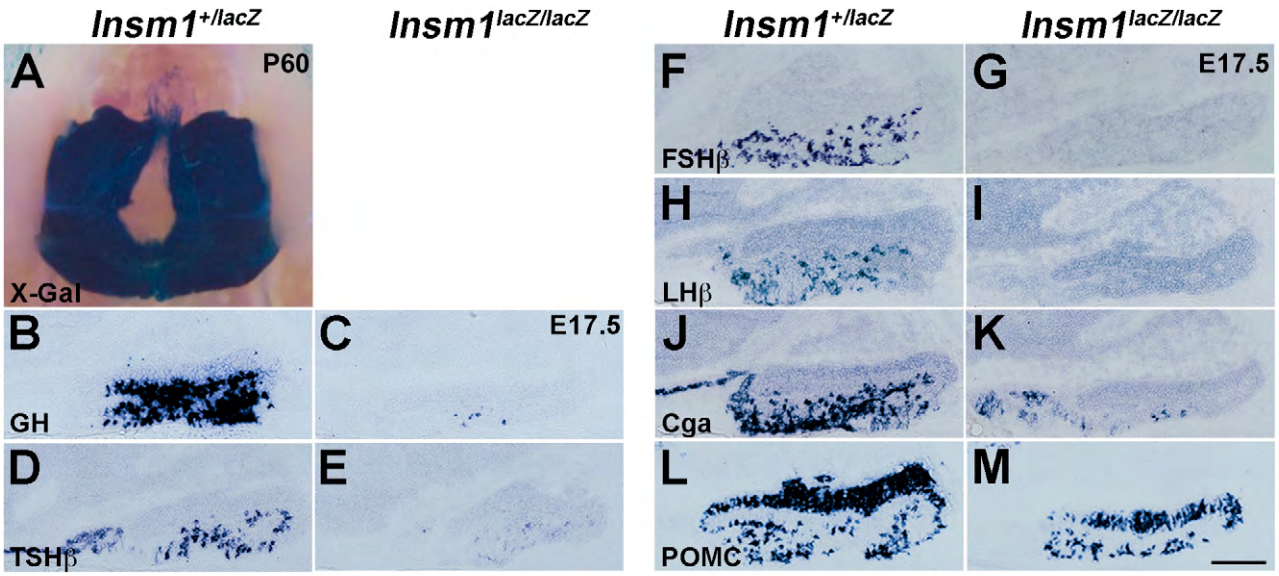

Fig. S1. Insm1 expression in the adult pituitary gland and reduced transcription of genes encoding pituitary hormones. Whole mount X-Gal staining of a P60 *Insm1*$^{+/lacZ}$ pituitary gland demonstrates transcriptional activity of the *Insm1* locus in the adult (A). Transcript levels of hormone coding genes were assessed by *in situ* hybridization in control and mutant (*Insm1*$^{lacZ/lacZ}$) mice at E17.5 (B-M). GH (B,C), TSHb (D,E), FSHb (F,G) and LHb (H,I) transcripts were abundant in control pituitaries and absent in pituitaries of *Insm1*$^{lacZ/lacZ}$ mice, and Cga (J,K) and POMC (L,M) transcripts were present at reduced levels in mutants. Scale bar**:** 100 µm.

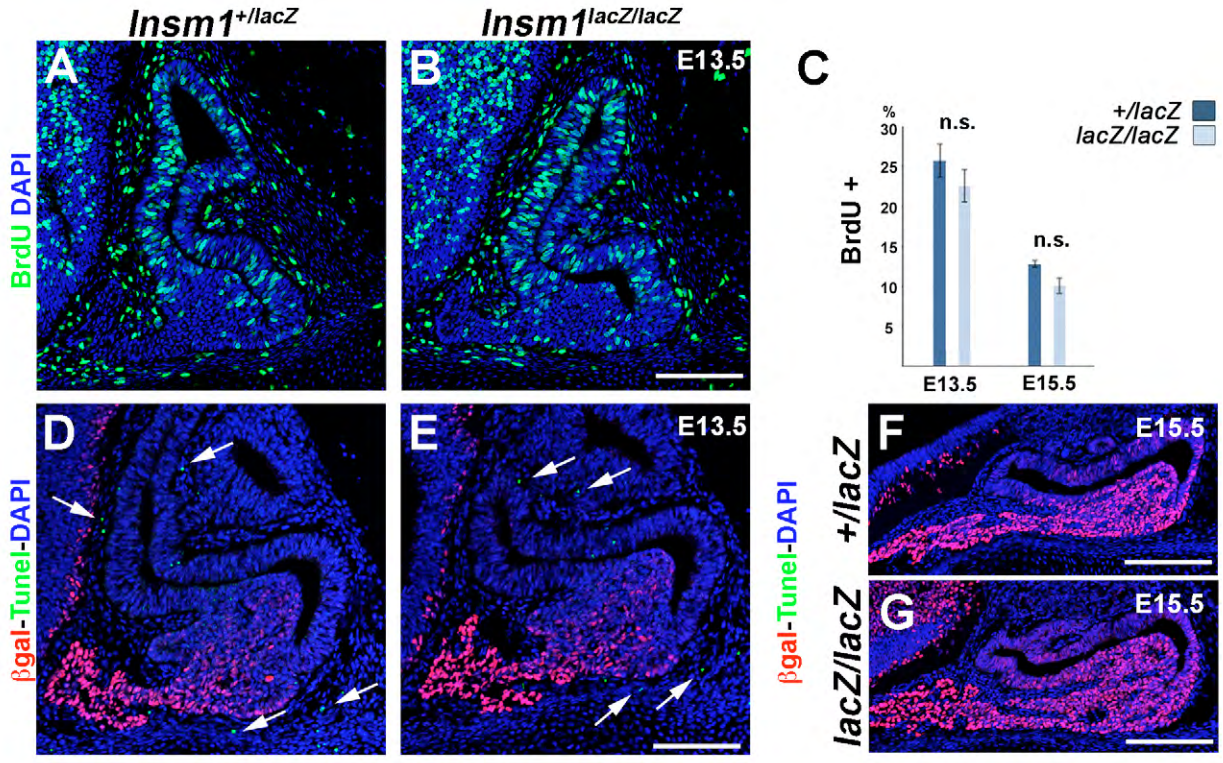

Fig. S2. The *Insm1* mutation does not promote proliferation or cell death. Proliferation was assessed at E13.5 and E15.5 by BrdU incorporation (A-C) in control (*Insm1*$^{+/lacZ}$) and mutant (*Insm1*$^{lacZ/lacZ}$) mice. Nuclei were counterstained with DAPI, and the percentage of BrdU+ cells was determined (C). Apoptosis was assessed by Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay at E13.5 (D,E) and E15.5 (F,G). In control and mutant mice at E13.5 or E15.5, a few apoptotic cells (arrows) were observed in parenchymal and other tissues adjacent to the pituitary gland, but not within the gland (D-G). Scale bars**:** 100 µm.

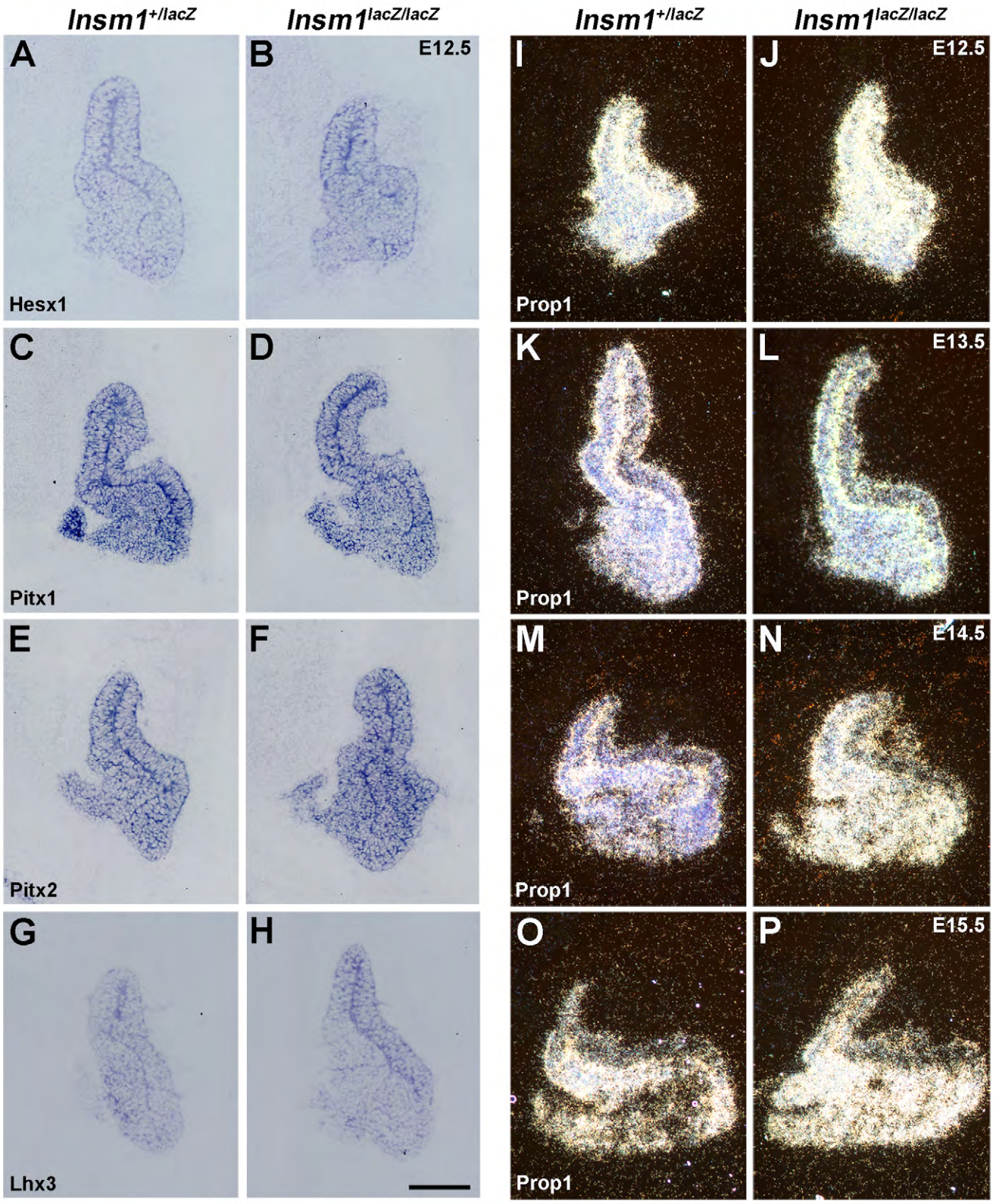

Fig. S3. Pituitary endocrine cells are specified correctly in *Insm1* mutant mice. The expression of transcription factors expressed during specification of pituitary endocrine cells was assessed by *in situ* hybridization. The transcripts of Hesx1 (A,B), Pitx1 (C,D), Pitx2 (E,F), Lhx3 (G,H), and Prop1 (I-P) were detected at comparable levels in control (*Insm1*$^{+/lacZ}$) and mutant (*Insm1*$^{lacZ/lacZ}$) mice at E12 or E13.5. At E14.5 and E15.5, expression of Prop1 was mildly enhanced in the anterior lobe. Scale bar: 100 μm.

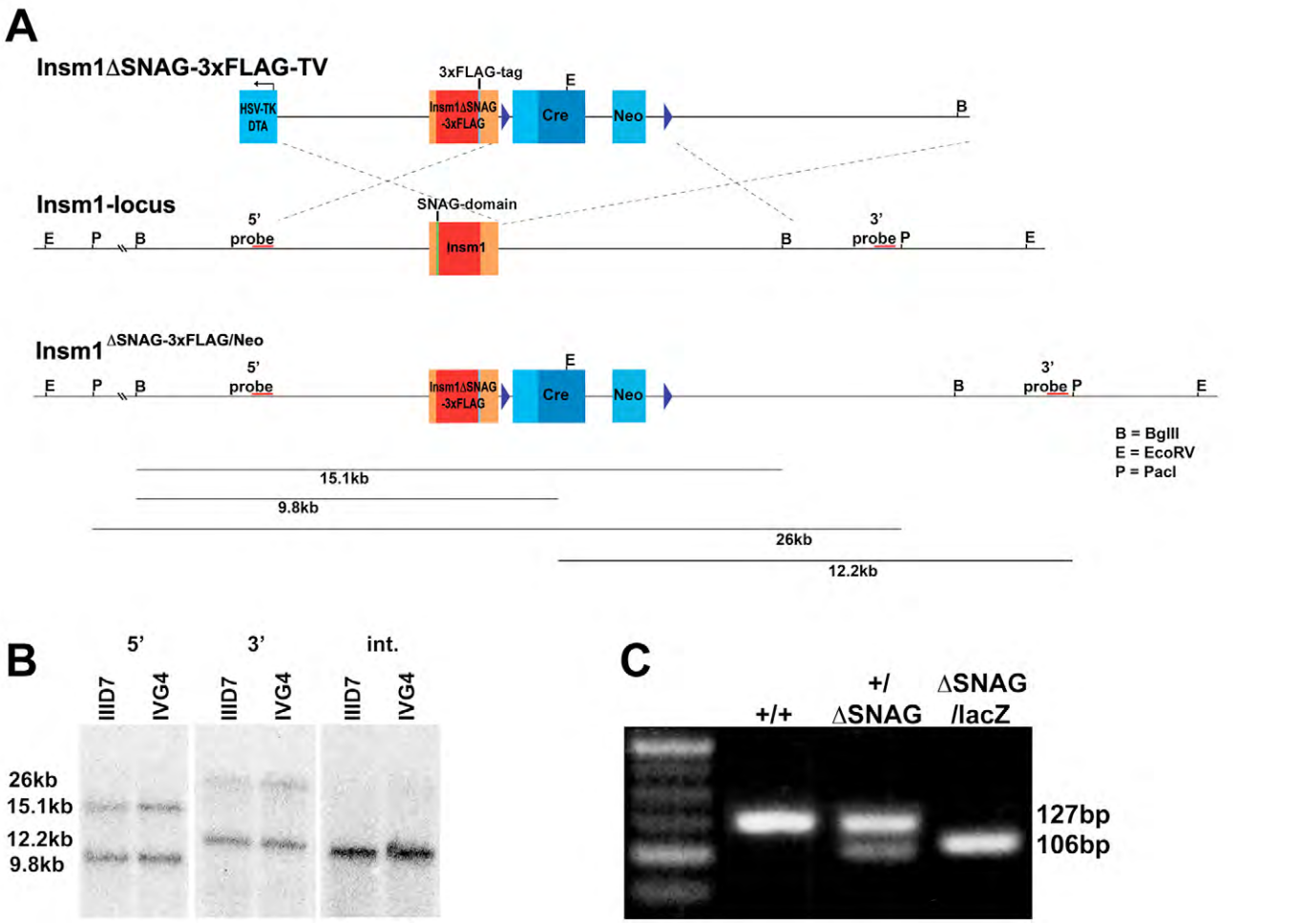

Fig. S4. Generation of *Insm1*$^{\Delta SNAG}$ mutant mice. Schematic representation of the targeting vector, the wild-type *Insm1* locus, and the mutated *Insm1*$^{\Delta SNAG}$ allele (A). Coding (red) and non-coding (orange) sequences, the SNAG domain (green), the N-terminal 3xFLAG-tag (grey), DTA, the self-excision neomycin cassette, loxP (arrowhead), Southern blot probes (red bars) and *Bgl*II, EcoRV and PacI restriction sites are depicted. Black lines indicate the predicted fragment sizes obtained after digestion of genomic DNA. G418 resistant clones were analyzed by Southern blotting (B). Hybridization with a probe located 5′ of the targeting vector detected 15.1 kb and 9.8 kb DNA fragments corresponding to *Insm1* wildtype and *Insm1*$^{\Delta SNAG}$ alleles, respectively; the DNA from ES cells was digested with *Bgl*II/EcoRV. Hybridization with a probe located 3′ of the targeting vector detected 26 kb and 12.2 kb fragments of *Insm1* wildtype and *Insm1*$^{\Delta SNAG}$ alleles; the DNA was digested by PacI/EcoRV. A probe containing sequences present in the targeting vector was used to ensure the absence of random integration events. (C) *Insm1* wildtype and *Insm1*$^{\Delta SNAG}$ alleles are distinguishable by PCR. PCR amplification of *Insm1* wildtype and *Insm1*$^{\Delta SNAG}$ alleles produce a 127 bp and 106 bp fragment, respectively.

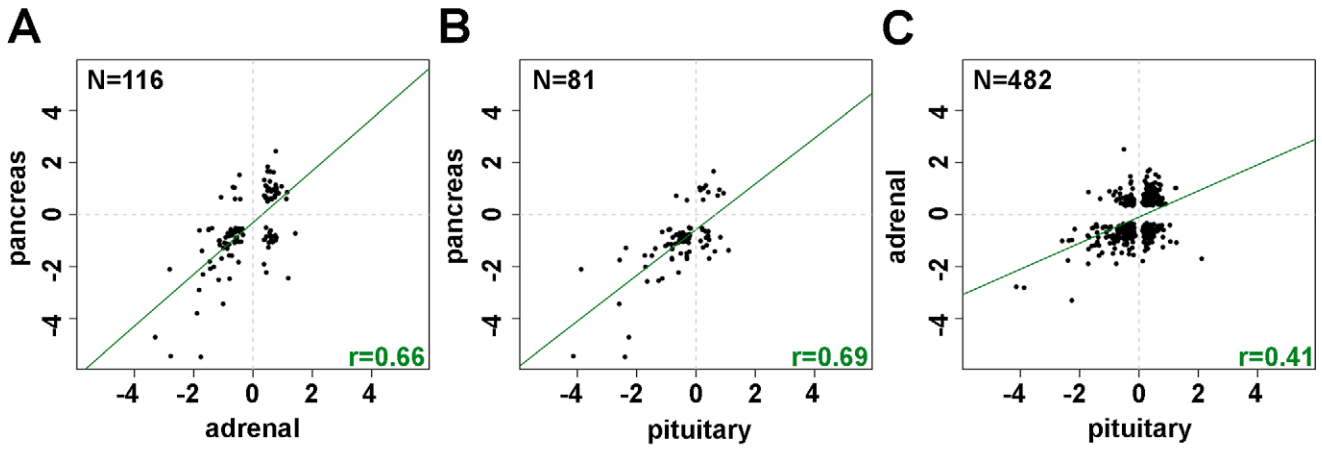

Fig. S5. Comparison of gene expression changes in three different endocrine cell types of Insm1 mutant mice. Cross comparison of expression changes (log2 values are given) in three different (adrenergic, pancreatic and pituitary) endocrine organs of *Insm1* mutant versus control mice. Green: linear regression line and Pearson's correlation coefficients. Each scatter plot represents genes that are deregulated in both organs (FC more than 1.7). (A) Comparison of deregulated genes in the adrenal gland and pancreas (116 genes); (B) Comparison of deregulated genes in the pituitary and pancreas (81 genes); (C) Comparison of deregulated genes in the pituitary and adrenal gland (482 genes).

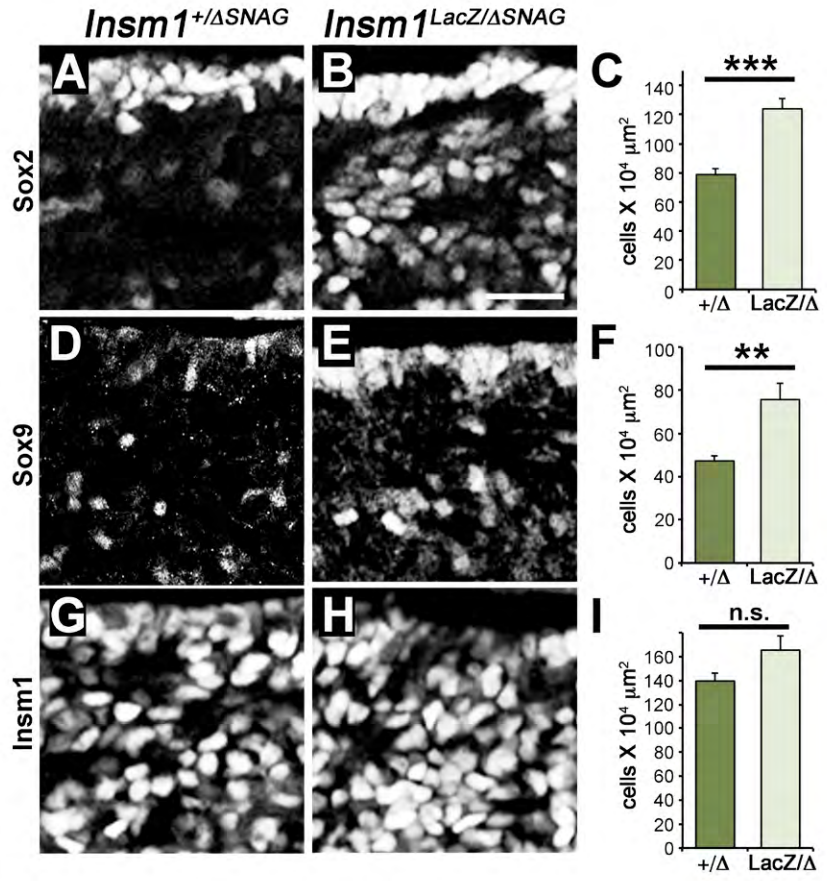

Fig. S6. Mutation of *Insm1* results in an increased number of cells that retain progenitor characteristics. Immunohistochemical analysis of endocrine progenitor cells using antibodies directed against Sox2 (A,B), Sox9 (D,E) and Insm1 (G,H) in the pituitary of Insm1 control (*Insm1*$^{+/\Delta SNAG}$) and mutant (*Insm1*$^{lacZ/\Delta SNAG}$) mice at E17.5. Quantification of numbers of Sox2+ (C), Sox9+ (F) and Insm1+ cells (I) in the anterior lobe of the pituitary; whereas the numbers of Sox2+ and Sox9+ cells were increased, the number of Insm1+ cells was not significantly changed. Scale bars: 25 µm. $^{**}P<0.01$

Table S1. Primers used for qRT-PCR

Name	Primer Sequence 5' to 3'
Myl1-fw	CACATCATGTCTGTCTAAACGG
Myl1-rv	CTGGTGTTGACAGTTAGCCAT
Actc1-fw	AGGCCCATCCATTGTCCA
Actc1-rv	CAAGAAGCACAATACGGTCA
Msc-fw	GCTTTGTGGAACTTCCGCTT
Msc-rv	AGGGCAAACCACACTTGTCT
Chga-fw	ACACTTCTGCAGGGCAGC
Chga-rv	AGTTATTGCAGTTGTGCCCC
Chgb-fw	ATTCACCCACAGGCAGAAAG
Chgb-rv	ACAAGTCACGCTAGTCACATGG
Pcsk1-fw	CCATGCTGCGACTCCT
Pcsk1-rv	TGATTGTTTTGAAAGTGCATT
Pcsk2-fw	ACCTTTGGCATCAGTATTAACACC
Pcsk2-rv	CATCAGACTCAGGGGCATCA
Scg3-fw	TGTCTCGGCATGCTAGACAC
Scg3-rv	GACGTGGGTTTATTTCCGTG
Notch2-fw	GCTATAAGTGCCTCTGCGAT
Notch2-rv	AGGCACACTCATCTATATTCACC
Dll1-fw	GATACACACAGCAAACGTGACACC
Dll1-rv	TTCCATCTTACACCTCAGTCGCTA
Hes1-fw	CAGACATTCTGGAAATGACTGTGAA
Hes1-rv	CGCGGTATTTCCCCAACAC
Hes5-fw	GCTCCGCTCGCTAATCGCCTCCAG
Hes5-rv	GTCCCGACGCATCTTCTCCACCAC
Hey1-fw	GCCGACGAGACCGAATCAATAACA
Hey1-rv	TCCCGAAACCCCAAACTCCGATAG