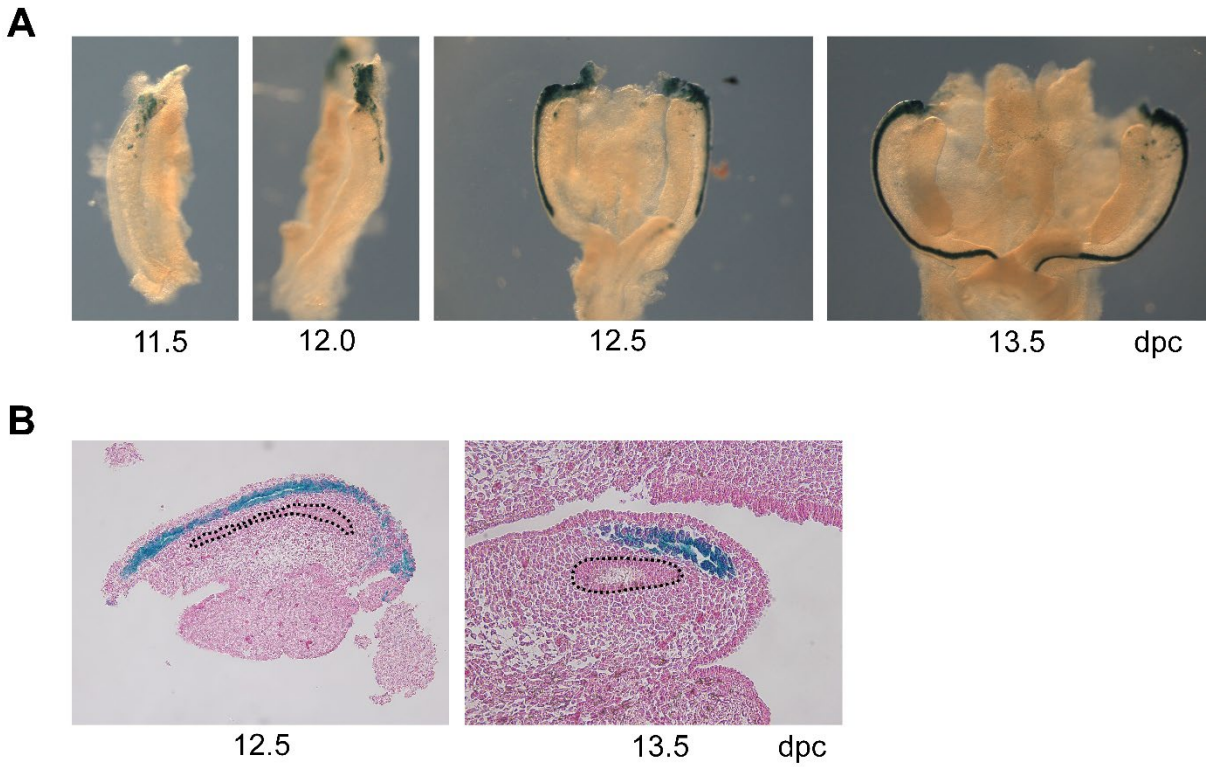
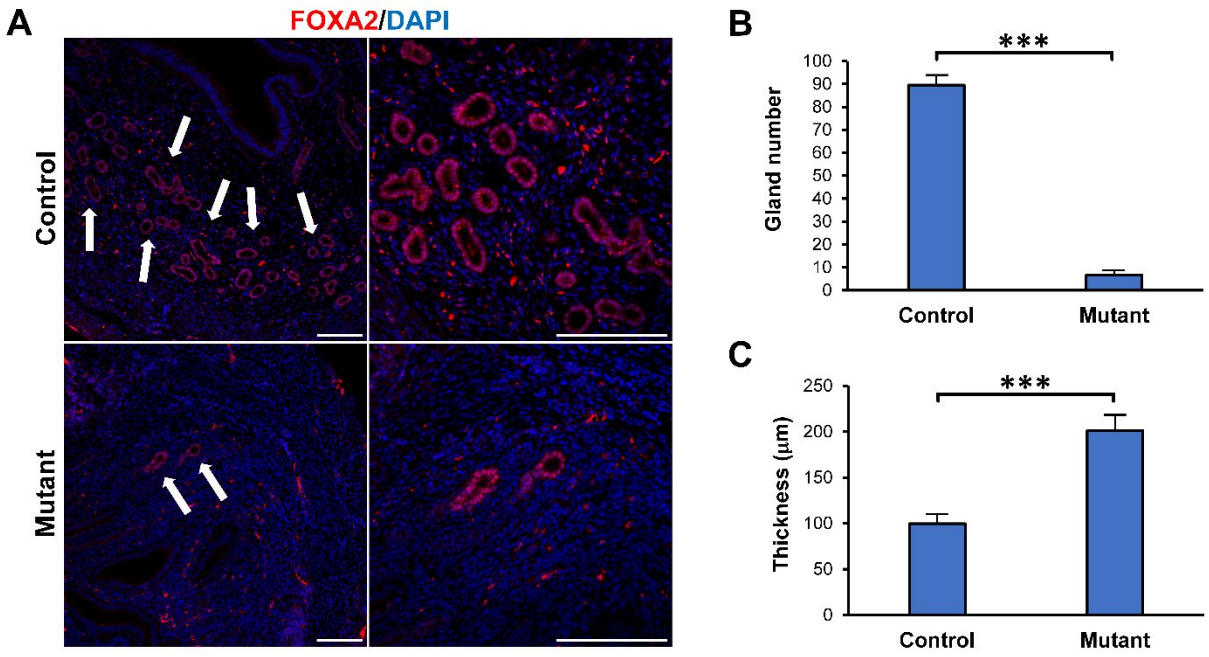
Supplementary Figures



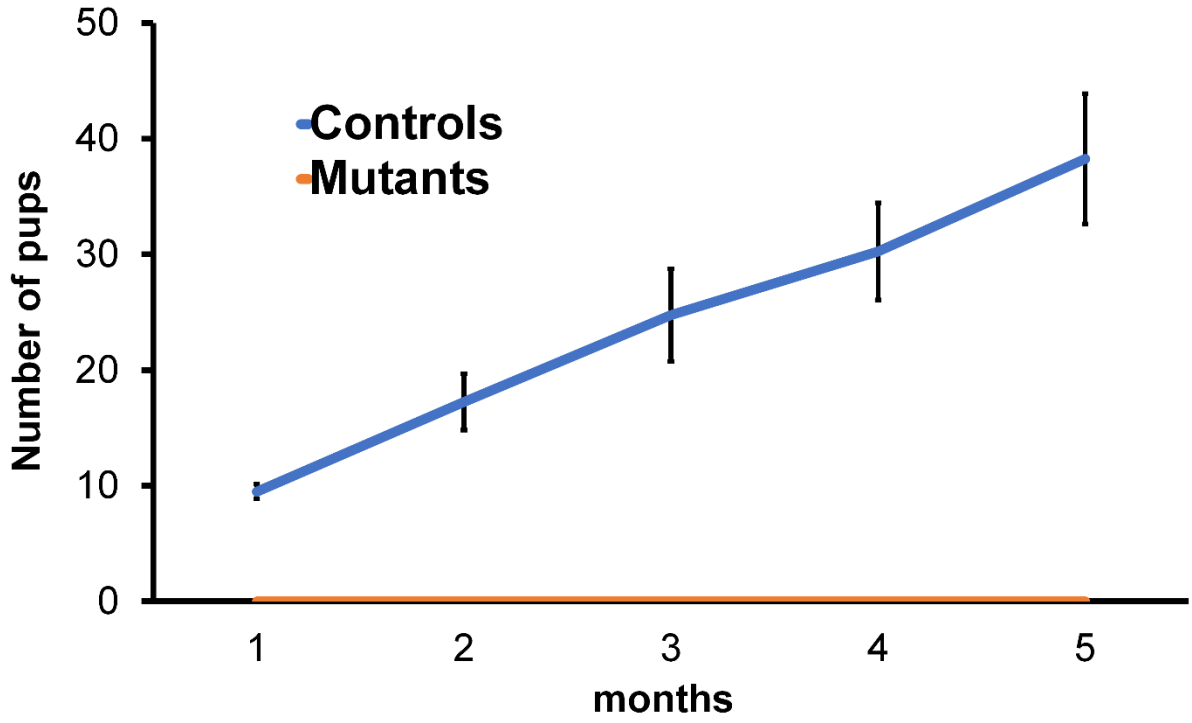
Suppl Figure 1. Characterization of Wnt7a-Cre expression.

(A) LacZ staining of *Wnt7a-Cre mice crossed with* ROSA26-LacZ mice showing Wnt7a-directed expression of Cre started at 11.5 dpc at the tip of the MD and progressed posteriorly during MD elongation. (B) Sections of LacZ-stained samples showed Wnt7a-Cre expression in the MD (blue) but not the WD (dashed lines). Counterstaining was performed with Nuclear Fast Red.



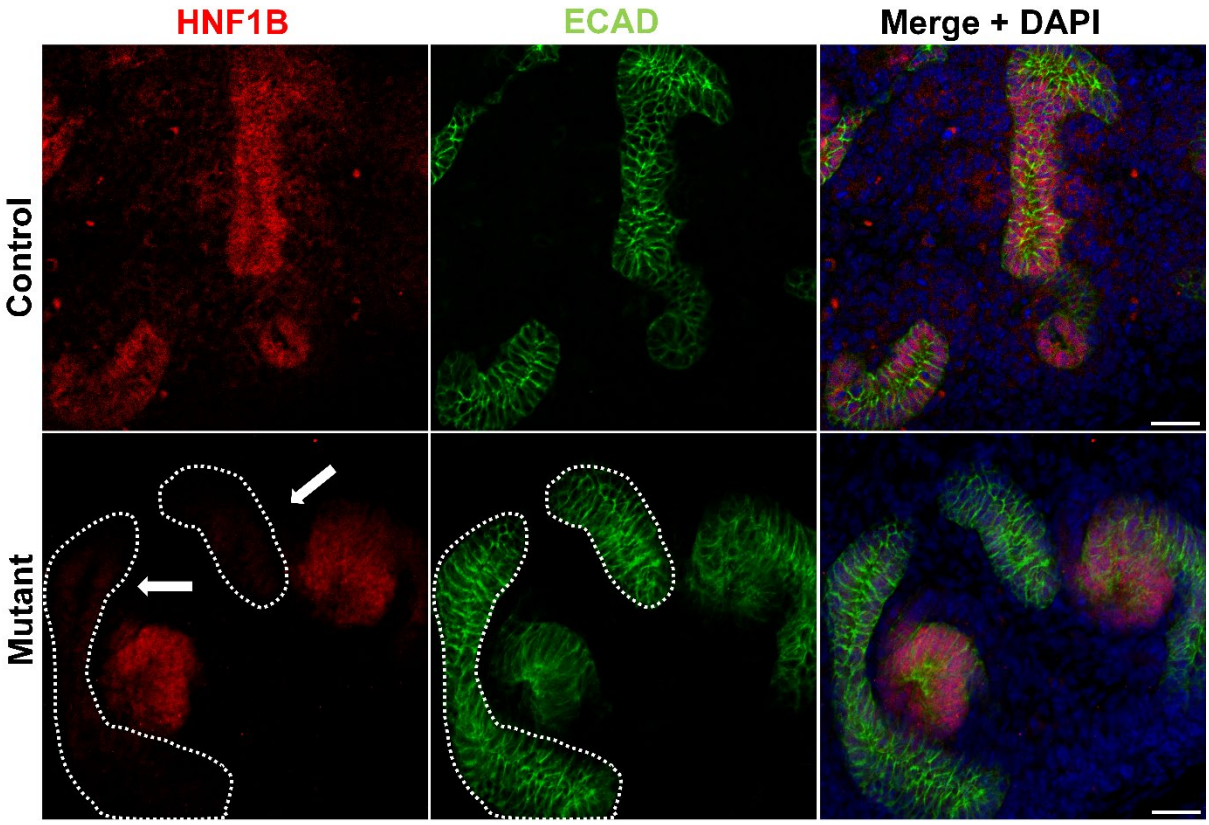
Suppl Figure 2. Impaired development of uterine glands in Hnf1b-mutant uteri.

(A) Immunofluorescence of the glandular marker FOXA2 showing fewer uterine glands in 4M Hnf1b-mutant uteri compared to controls. Scale bar = 100 µm. (B) Quantification of uterine glands in 4M control and Hnf1b-mutant uteri (n = 15). (C) Thickness of the outer longitudinal myometrium in 4M control and Hnf1b-mutant uteri (n = 9). Data are mean ± s.e.m. *** = $p < 0.001$.



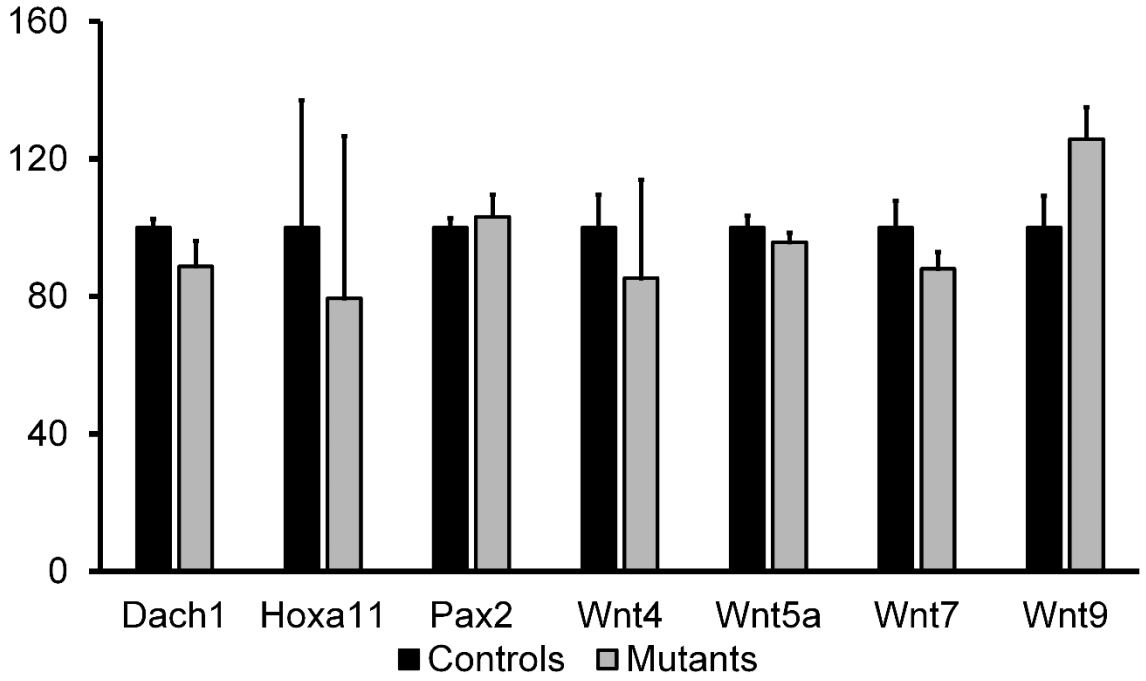
Suppl Figure 3. Hnf1b-mutant mice fail to remain pregnant.

Progeny analysis showed that Hnf1b mutant mice failed to remain pregnant and deliver pups.



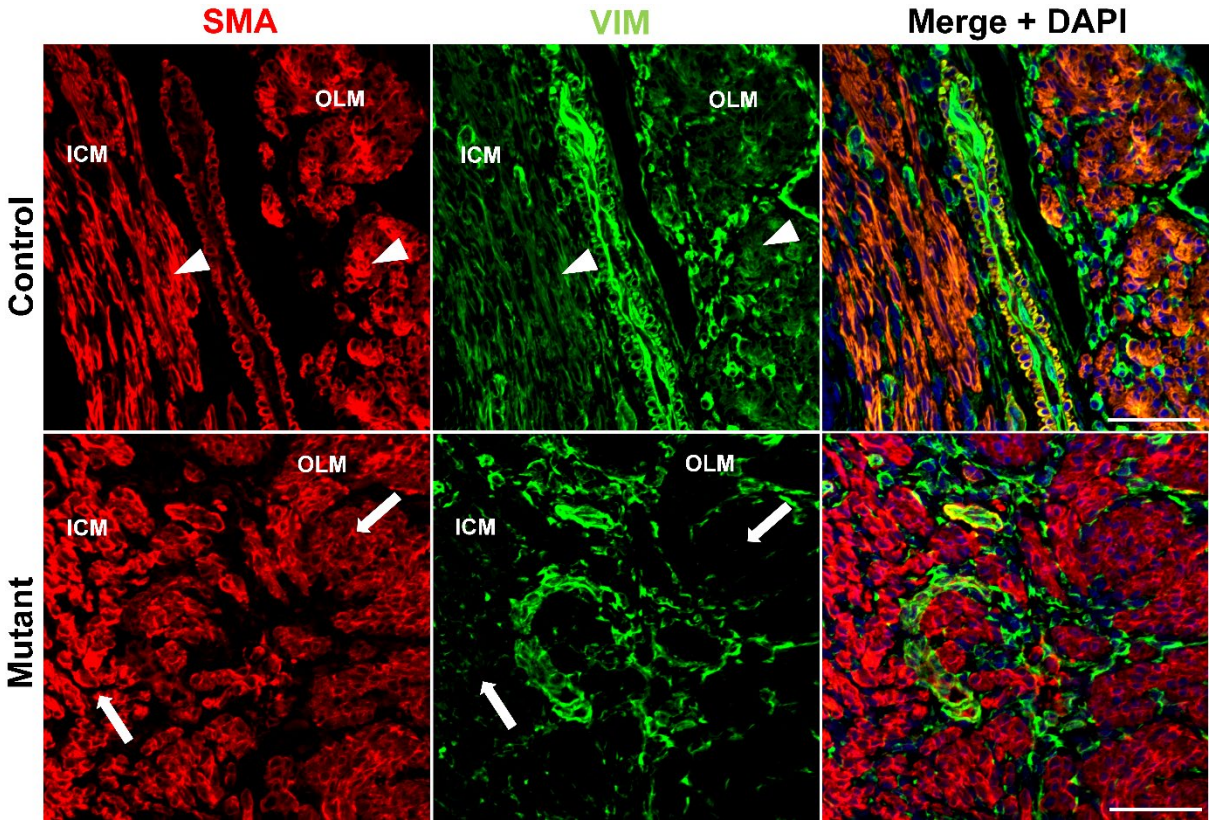
Suppl Figure 4. Decreased expression of HNF1B in kidneys of mutant mice.

Immunofluorescence staining of HNF1B and ECAD in kidney sections at 13.5 dpc. In mutant mice, HNF1B showed reduced expression in the epithelium of branches of the developing kidney (dashed lines, arrows). Scale bar = 25 μm.



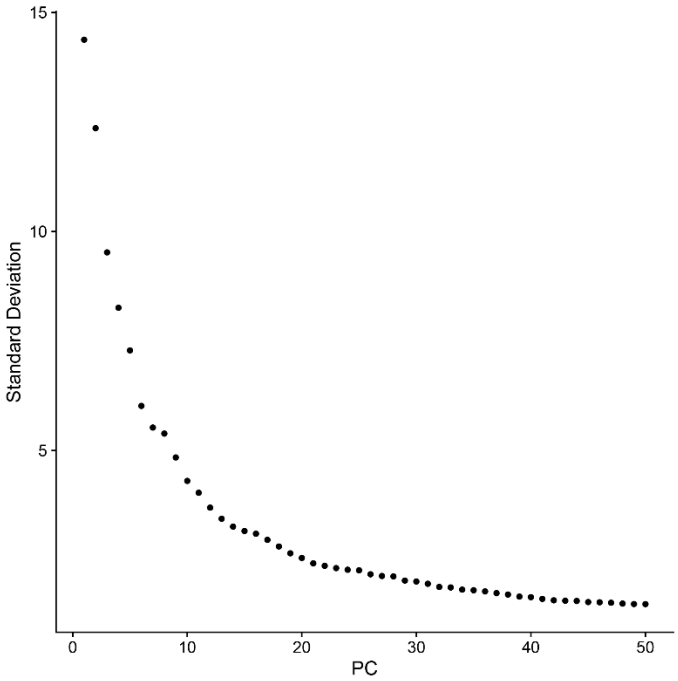
Suppl Figure 5. Gene expression of markers of MD development in 13.5 dpc samples.

Expression of several factors known to play an important role in MD development was not significantly different between controls and Hnf1b mutant samples.



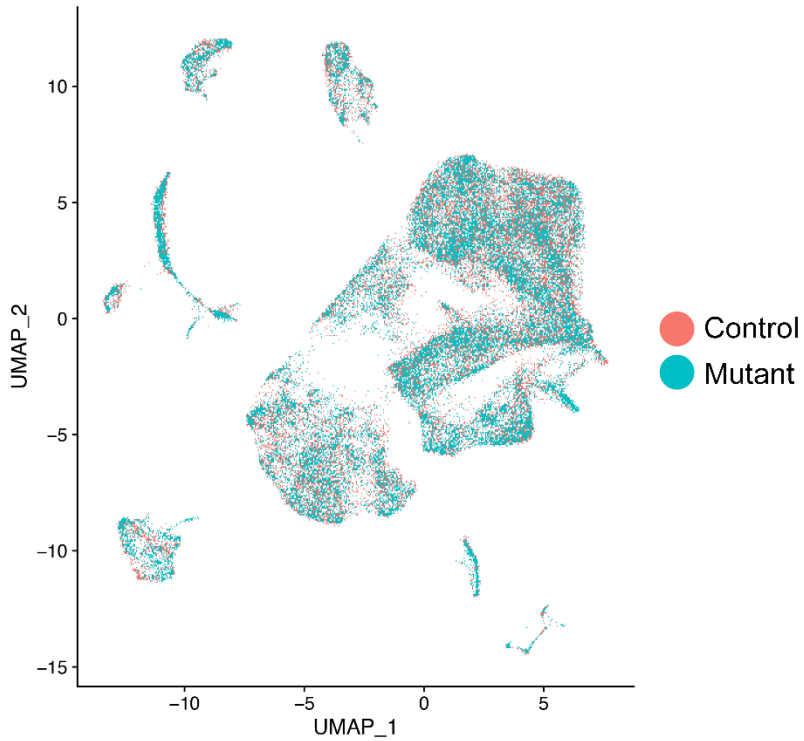
Suppl Figure 6. Dysregulated expression of vimentin in the mutant myometrium.

Vimentin is expressed and colocalizes with SMA (arrowheads) in the inner circular (ICM) and outer longitudinal myometrium (OLM) of the control uterus. In the Hnf1b mutant uterus, vimentin was downregulated in both ICM and OLM (arrows). Scale bar = 50 μm.



Suppl Figure 7. Elbow plot of principal components.

Elbow plot of the principal components (PC) ranked based on the percentage of variance explained after Principal Component Analysis.



Suppl Figure 8. Uniform manifold approximation and projection of single cell analysis.

Uniform manifold approximation and projection (UMAP) plot shows data integration from control and Hnf1b mutants.

Suppl Table 1. Differentially expressed genes associated with uterine anomalies in the epithelial cell cluster. Excel file.

Suppl Table 2. Differentially expressed genes associated with uterine anomalies in the stromal cell cluster. Excel file.

Suppl Table 3. Differentially expressed genes associated with uterine anomalies in the proliferating cell cluster. Excel file.

Suppl Table 4. Differentially expressed genes with apico-basal and baso-lateral polarity in the epithelial cell cluster.

Cellular Component	p-value	No. Genes	Genes
Apical plasma membrane	5.50E-09	52	Anxa1, Anxa4, Aqp1, Aqp5, Atp6v0a4, Atp6v1a, Atp6ap2, Atp1b1, Birc5, Bst2, Cd55, Cd81, Cdh2, Cldn1, Cldn25, Crb3, Ctsb, Ctsl, Dpp4, Emp2, Enpp3, Epcam, Ezr, Fat1, Fzd3, Gnas, Hax1, Igfbp2, Igsf5, Itpk1, Jag1, Kcnk1, Lmo7, Lrp2, Muc20, Pard6g, Pdpn, Prom1, Prom2, S100g, Slc6a6, Slc7a5, Slc9a3r1, Slc12a2, Slc15a2, Slc16a3, Slc34a2, Slc39a4, Stc1, Tgfbr1, Tjp3, Tnik
Basolateral plasma membrane	2.30E-04	29	Anxa1, Aqp1, Atp1b1,Bsg, Cadm1, Car2, Cd81, Cdh2, Cldn1, Cldn7, Ddr1, Epcam, Ezr, Hpgd, Itga6, Lepr, Muc20, Orai1, Pdpn, Prom2, S100g, Slc6a6, Slc7a5, Slc12a2,Slc13a5, Slc16a3, Slc40a1, Slc51a, Tgfbr1
Lateral plasma membrane	5.30E-03	11	Anxa1, Atp1b1, Bsg, Cldn1, Cldn3, Cldn7, Epcam, Fzd3, Lepr, Slc12a2, Slc16a3
Basal plasma membrane	3.40E-02	8	Aqp1, Aqp5, Cd81, Itga6, Itgb4, Muc20, Slc7a5, Slc12a2

Underlined genes have been reported in HNF1B ChIP-Seq experiments (Aboudehen K et al, 2016).

Suppl Table 5. List of primers for real-time PCR

Hnf1b-Fw	CTATAGCTCCAACCAGACGC
Hnf1b-Rev	AGTGACCTCATTGTTTCCCG
Wnt9-Fw	GGGACAACCTCAAGTACAGC
Wnt9-Rev	TTCCACTCCAGCCTTTATCAC
Dach1-Fw	ACTTTCTCTAACTGGGCATGG
Dach1-Rev	AGCTCTGGCATTGTCTATGG
Dach2-Fw	CTCCGACCCTTAATCCACTTC
Dach2-Rev	ATTCATCTGATTCATTGCCATGG
Hoxa11-Fw	AGGAGAAGGAGCGACGG
Hoxa11-Rev	GGTATTTGGTATAAGGGCAGCG
Wnt7a-Fw	ACGAGTGTCAGTTTCAGTTCC
Wnt7a-Rev	AATCGCATAGGTGAAGGCAG
Lhx1-Fw	CCAGTGCTGTGAATGTAAATGC
Lhx1-Rev	GAACCAGATCGCTTGGAGAG
Pax2-Fw	GATCCTACTCCATCAACGGG
Pax2-Rev	GACCAGATGTAAACCTCCACC
Wnt4-Fw	CTGGACTCCCTCCCTGTCTTT
Wnt4-Rev	CATGCCCTTGTCACTGCAA
Wnt5A-Fw	AATGAAGCAGGCCGTAGGA
Wnt5A-Rev	AGCCAGCACGTCTTGAGG
TBP-Fw	ACGGACAACTGCGTTGATTTT
TBP-Rev	ACTTAGCTGGGAAGCCCAAC