Supplementary Information

Embigin is involved in the regulation of early mouse kidney development

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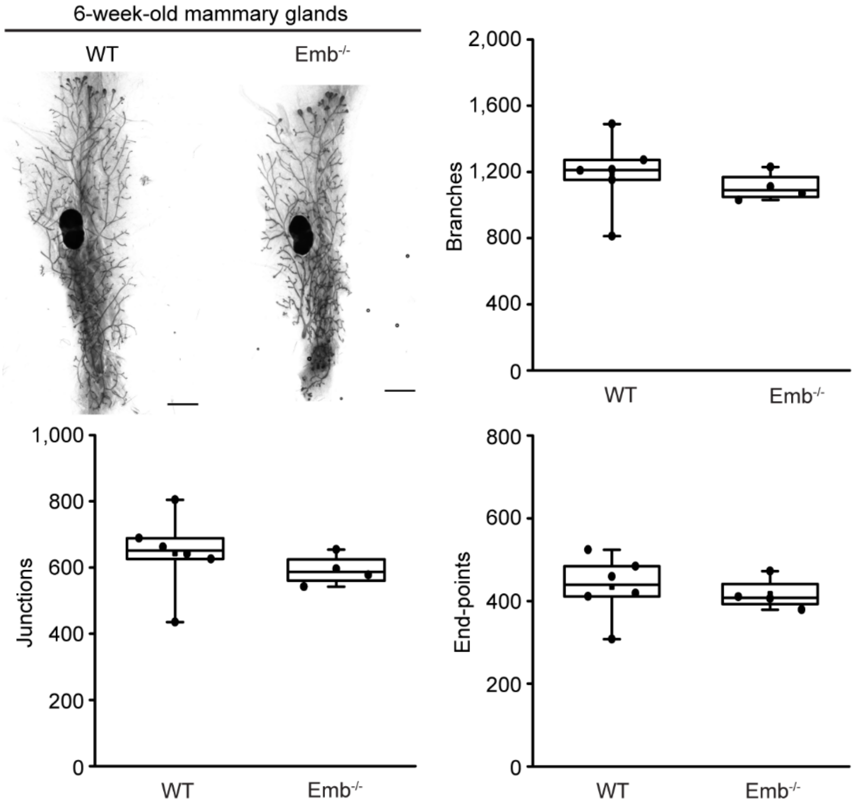
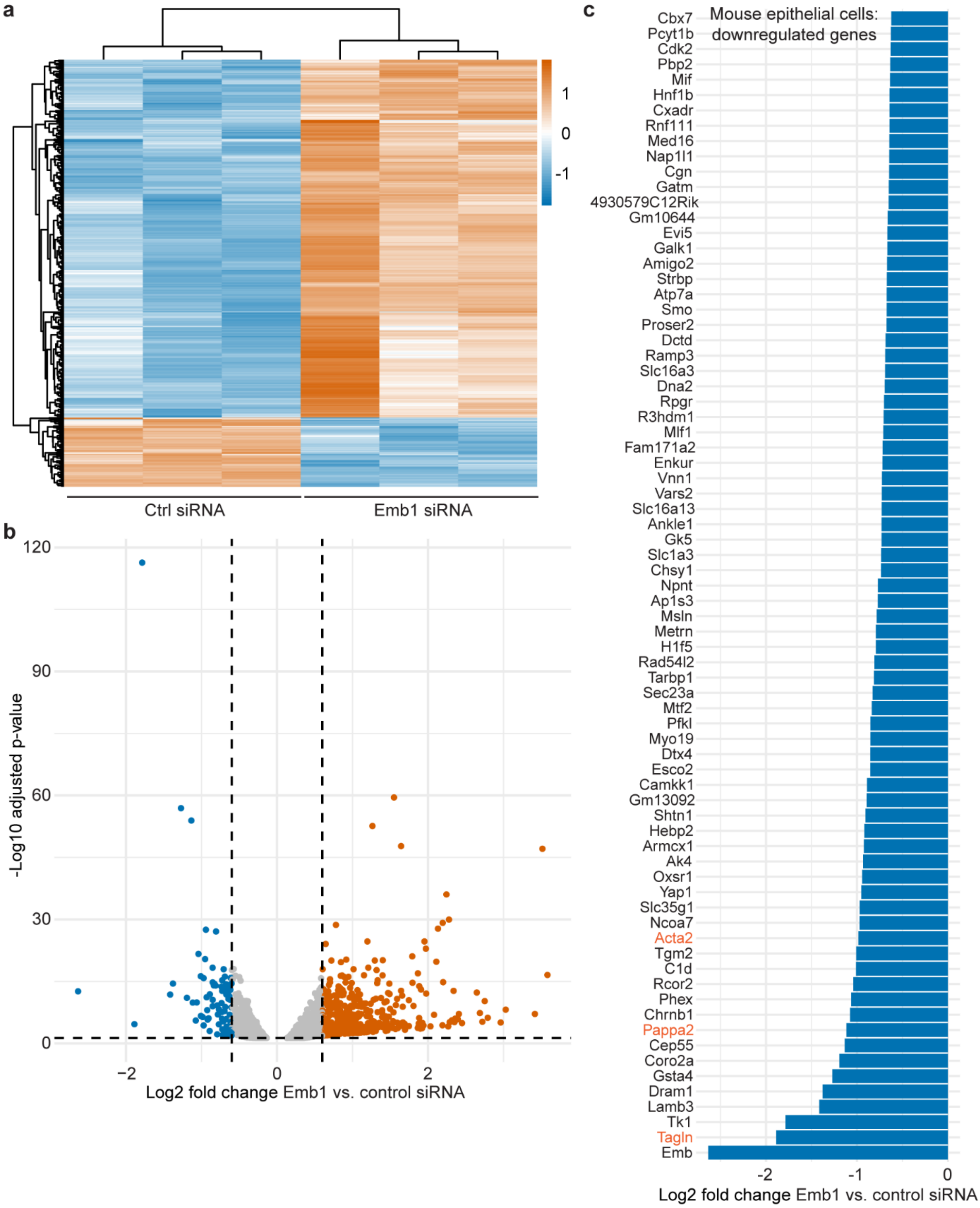
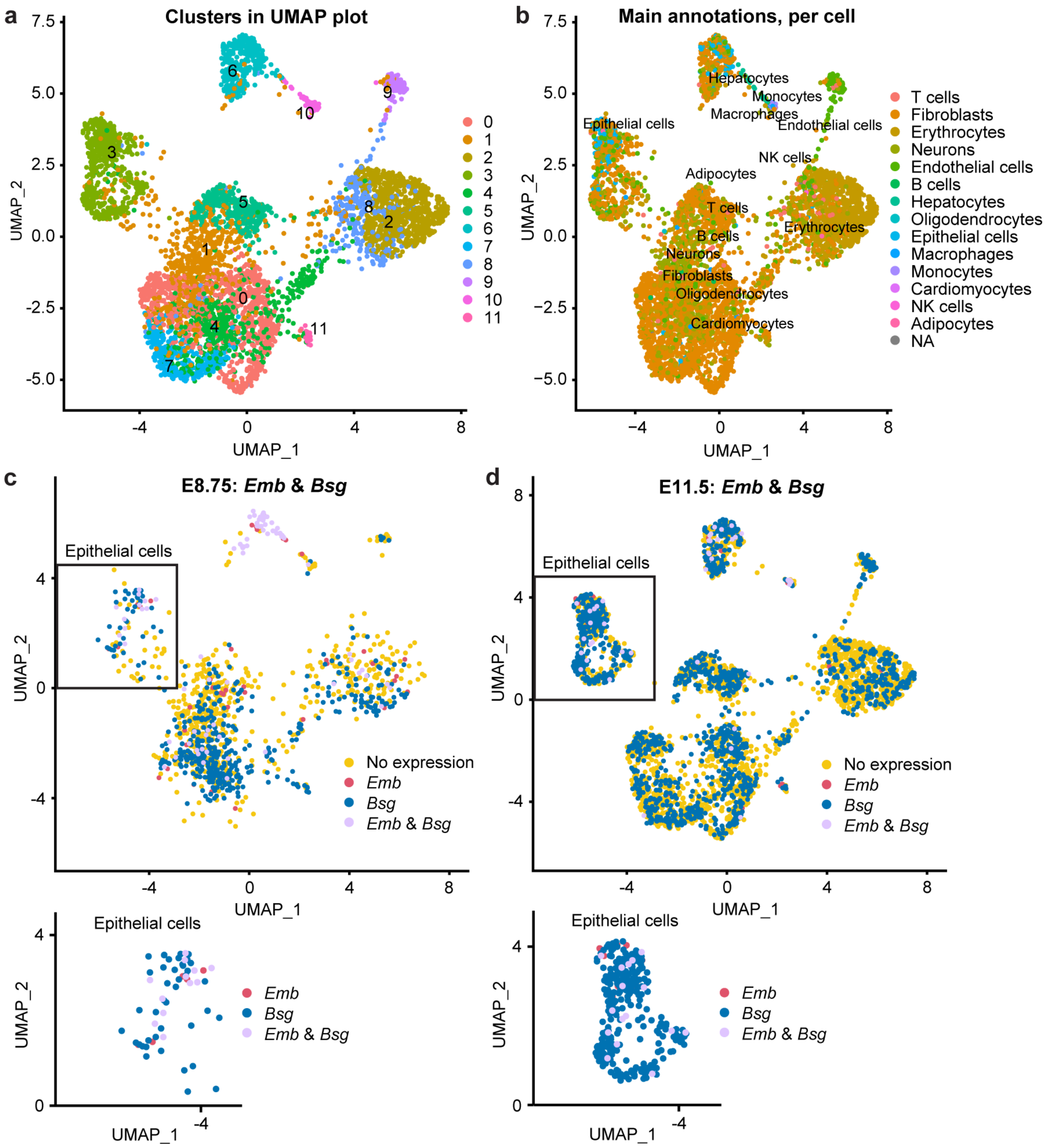


Supplementary Fig. 1. Embigin deficiency does not affect the mammary gland branching of young adult mice. Representative images of the mammary glands of 6-week-old mice. The mammary glands were collected at the diestrus stage of the estrous cycle and stained with carmine alum. The number of branches, junctions, and terminal end-points of 6-week-old mice mammary glands was analysed with ImageJ/Fiji software (n = 6 for WT and n = 4 for Emb$^{-/-}$). Data are represented as a Spear style box plot. A square shows the mean value. Scale bars: 2000 μm.

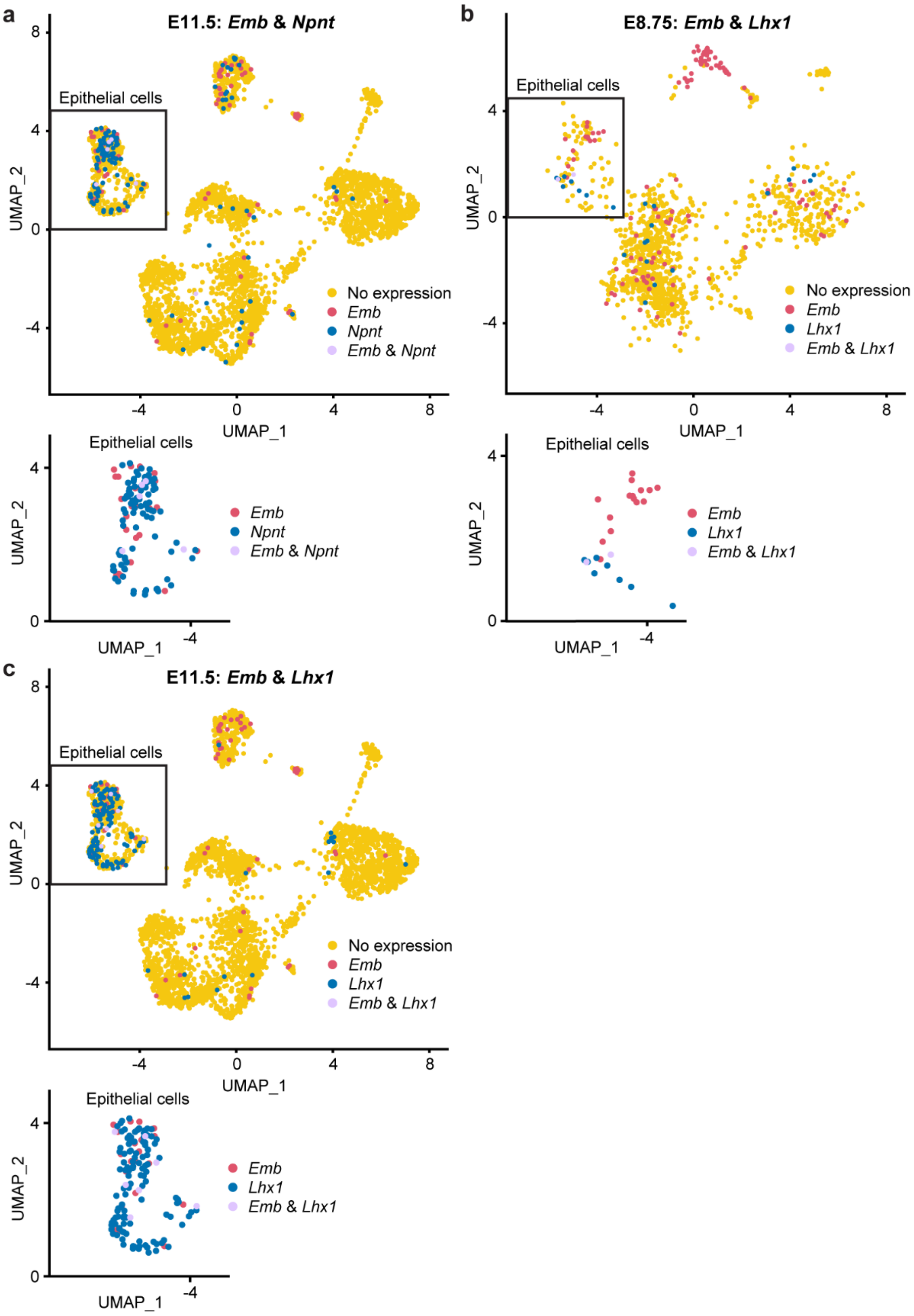


Supplementary Fig. 2. Knockdown of embigin in mouse epithelial cells leads to differential expression of several genes involved in kidney development. (**a**) Heatmap of differentially expressed genes (log2FC > 0.6 or < -0.6 and adjusted p-value < 0.05) in mouse epithelial cells treated with Emb1 siRNA (n = 3) and negative control siRNA (n = 3) based on RNA-seq analysis. (**b**) Volcano plot of the differentially expressed genes (log2FC > 0.6 or < -0.6 and adjusted p-value

< 0.05) in mouse epithelial cells treated with Emb1 siRNA (n = 3) vs. negative control siRNA (n = 3) based on RNA-seq analysis. (**c**) Bar plot of the 75 most downregulated genes (log2FC > 0.6 or < -0.6 and adjusted p-value < 0.05) based on RNA-seq analysis of Emb1 siRNA- and negative control siRNA-treated mouse epithelial cells. The three genes also downregulated in E13.5 $\mathrm{Emb}^{-/-}$ kidneys are highlighted: *Pappa2*, *Acta2*, and *Tagln*.

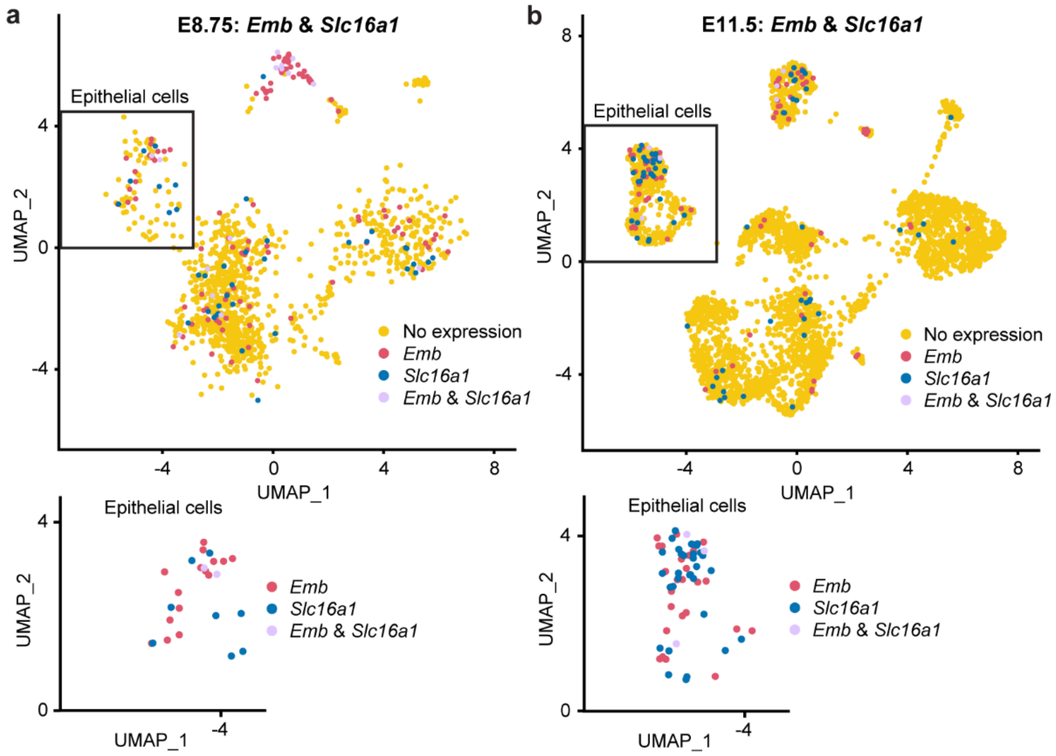


Supplementary Fig. 3. Reanalysis of mouse embryonic single-cell RNA sequencing data reveals the presence of embigin in the kidney primordium. Embigin is also co-expressed with basigin during early kidney development. (**a-b**) Single-cell RNA sequencing (scRNA-seq) data[31] obtained from the mouse Pax2-expressing renal cells at E8.75 and the caudal urogenital system cells (containing the caudal nephric duct, the UB, the surrounding mesenchyme, and the cloaca) at E11.5 was reanalysed. The data were normalised, integrated, and clustered using the Seurat Bioconductor package implemented in Chipster. Annotation was done in R using SingleR Bioconductor package and Mouse RNA-seq data as CellDex reference. Seurat R package was used to visualize gene expression in the UMAP plots. (**c-d**) We analysed embigin (gene *Emb*) and basigin (gene *Bsg*) RNA expression during early kidney development from scRNA-seq data[31] obtained from the mouse Pax2-expressing renal cells at E8.75 and the caudal urogenital system cells at E11.5. Smaller images are magnifications from the epithelial cell-type cluster area where the co-expression is observed.



Supplementary Fig. 4. Embigin is partially co-expressed with nephronectin and Lim1 in early renal epithelial-type cells. We reanalysed scRNA-seq data[31] obtained from the caudal urogenital system cells (containing the caudal nephric duct, the UB, the surrounding mesenchyme, and the cloaca) at E11.5 for embigin (gene *Emb*) and nephronectin (gene *Npnt*) RNA expression (**a**). Additionally, embigin and Lim1 (gene *Lhx1*) RNA expression were analysed from the data obtained from the mouse Pax2-expressing renal cells at E8.75 (**b**) and the caudal urogenital system cells at

E11.5 (**c**). Smaller images are magnifications from the epithelial cell-type cluster area where partial co-expression is observed.



Supplementary Fig. 5. Embigin is partially co-expressed with MCT1 in early renal epithelial-type cells. We reanalysed scRNA-seq data[31] obtained from the mouse Pax2-expressing renal cells at E8.75 (**a**) and the caudal urogenital system cells (containing the caudal nephric duct, the UB, the surrounding mesenchyme, and the cloaca) at E11.5 (**b**) for embigin (gene *Emb*) and MCT1 (gene *Slc16a1*) RNA expression. Smaller images are magnifications from the epithelial cell-type cluster area where partial co-expression is observed.

Supplementary Table 1. RNA sequencing analysis of mouse Emb$^{-/-}$ kidneys at E13.5 identified several downregulated genes associated with kidney development.

Gene	Long name	log2FC
Nphs1	Nephrosis 1, nephrin	-6.2
R3hdml	R3H domain containing-like	-5.2
Sim2	Single-minded family bhlh transcription factor 2	-2.3
Irx2	Iroquois homeobox 2	-2.0
Irx1	Iroquois homeobox 1	-1.9
Hnf4a	Hepatic nuclear factor 4, alpha	-1.9
Ptpro	Protein tyrosine phosphatase receptor type O	-1.5
Slc22a6	Solute carrier family 22 member 6	-1.4
Folr1	Folate receptor alpha	-1.2
Slc39a5	Solute carrier family 39 member 5	-1.1
Ren1	Renin 1 structural	-1.1
Nkx6-1	NK6 homeobox 1	-1.1
Pappa2	Pregnancy-associated plasma protein A2, pappalysin 2	-1.0
Acta2	Actin alpha 2, smooth muscle, aorta	-1.0
Clic5	Chloride intracellular channel 5	-1.0
Aqp1	Aquaporin 1	-0.9
Cbln4	Cerebellin 4 precursor protein	-0.9
Cldn1	Claudin 1	-0.9
Sall3	Spalt like transcription factor 3	-0.8
Foxa1	Forkhead box A1	-0.8
Hes5	Hes family bhlh transcription factor 5	-0.8
Tagln	Transgelin	-0.7
Hmgcs2	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 2	-0.7
Cacna2d2	Calcium channel, voltage-dependent, alpha 2/delta subunit 2	-0.7
Lama3	Laminin, alpha 3	-0.7
Osr2	Odd-skipped related 2	-0.7
Enpep	Glutamyl aminopeptidase	-0.7
Irx5	Iroquois homeobox 5	-0.7
Nog	Noggin	-0.7
Reln	Reelin	-0.7
Erbb4	Erb-b2 receptor tyrosine kinase 4	-0.6

Supplementary Table 2. RNA sequencing analysis of embigin-silenced mouse epithelial cells identified several downregulated genes involved in kidney development.

Gene	Long name	log2FC
Tagln	Transgelin	-1.9
Pappa2	Pregnancy-associated plasma protein A2, pappalysin 2	-1.1
Chrnb1	Cholinergic receptor nicotinic beta 1 subunit	-1.1
Tgm2	Transglutaminase 2, C polypeptide	-1.0
Acta2	Actin alpha 2, smooth muscle, aorta	-1.0
Yap1	Yes-associated protein 1	-1.0
Mtf2	Metal response element binding transcription factor 2	-0.8
Npnt	Nephronectin	-0.8
Chsy1	Chondroitin sulfate synthase 1	-0.7
Enkur	Enkurin, TRPC channel interacting protein	-0.7
Smo	Smoothened, frizzled class receptor	-0.7
Atp7a	ATPase, Cu++ transporting, alpha polypeptide	-0.7
Gatm	Glycine amidinotransferase (L-arginine:glycine amidinotransferase)	-0.7
Rnf111	Ring finger 111	-0.6
Cxadr	Coxsackie virus and adenovirus receptor	-0.6
Hnf1b	HNF1 homeobox B	-0.6
Wnt7b	Wingless-type MMTV integration site family, member 7B	-0.6