Introduction

Keratin 7 (K7) is a type II member of the keratin superfamily and despite its widespread expression in different types of simple and transitional epithelia, its functional role in vivo remains elusive, in part due to the lack of any appropriate mouse models or any human diseases that are associated with KRT7 gene mutations. Using conventional gene targeting in mouse embryonic stem cells, we report here the generation and characterisation of the first K7 knockout mouse. Loss of K7 led to increased proliferation of the bladder urothelium although this was not associated with hyperplasia. K18, a presumptive type I assembly partner for K7, showed reduced expression in the bladder whereas K20, a marker of the terminally differentiated superficial urothelial cells was transcriptionally up-regulated. No other epithelia were seen to be adversely affected by the loss of K7 and western blot and immunofluorescence microscopy analysis revealed that the expression of K8, K18, K19 and K20 were not altered in the absence of K7, with the exception of the kidney where there was reduced K18 expression.

Abstract

Keratin 7 (K7) is a Type II member of the keratin superfamily and despite its widespread expression in different types of simple and transitional epithelia, its functional role in vivo remains elusive, in part due to the lack of any appropriate mouse models or any human diseases that are associated with KRT7 gene mutations. Using conventional gene targeting in mouse embryonic stem cells, we report here the generation and characterisation of the first K7 knockout mouse. Loss of K7 led to increased proliferation of the bladder urothelium although this was not associated with hyperplasia. K18, a presumptive type I assembly partner for K7, showed reduced expression in the bladder whereas K20, a marker of the terminally differentiated superficial urothelial cells was transcriptionally up-regulated. No other epithelia were seen to be adversely affected by the loss of K7 and western blot and immunofluorescence microscopy analysis revealed that the expression of K8, K18, K19 and K20 were not altered in the absence of K7, with the exception of the kidney where there was reduced K18 expression.

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Generation and Characterisation of Keratin 7 (K7) Knockout Mice

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placenta caused by the lack of an intact keratin cytoskeleton [13]. Therefore in the placenta at least, simple keratins provide an essential structural role in maintaining the integrity of the trophoblast layer, much akin to the role played by the epidermally-expressed keratins which give structural support to the skin and its appendages.

In an attempt to understand better K7 function in vivo, as well as to increase the overall number of keratin knockout mice that are available for study, we used our previous experience with the mouse Krt7 gene [2] to introduce a null mutation into mouse embryonic stem cells by gene targeting. By generating K7 deficient mice, the consequences of the absence of K7 on the development and differentiation of simple epithelia can be studied, the outcome of which might be useful in discovering hitherto unknown human disorders associated with KRT7 gene mutations.

Materials and Methods

Construction of the Krt7 Gene Targeting Vector

The mouse Krt7 gene was isolated from a PAC 12986/SsEcoTaq genomic DNA library, subcloned into pUC18 and completely sequenced [2]. To facilitate the construction of the K7 knockout vector, a 2063 bp PCR product which comprised the short arm of Krt7 homology was amplified from the original pUC18 clone and cloned into pCR2.1 (Invitrogen). The amplification primers for the short arm of homology incorporated HindIII and SacII sites to select the formation of targeted ES cell clones. HindIII and SacII double-digestion of the short arm of homology in pCR2.1 produced a ~2 kb fragment which was subcloned into the HindIII and SacII sites of the targeting vector pNTKV-1906 (Clontech) to generate the construct pNTKV-1906 K7. pNTKV-1906 K7 was then digested with EcoRI and a 4148 bp EcoRI/MfeI restriction fragment (the long arm of homology) was subcloned into this site using blunt-ending cloning to generate the complete Krt7 knockout vector (Figure 1). The targeting vector was linearised with NotI prior to electroporation into E14 mouse embryonic stem cells.

Generation of K7 Knockout Mice

107 E14 (129P) embryonic stem cells were electroporated with 35 µg of linearised targeting vector and seeded onto mitomycin-C treated embryonic fibroblast feeder cells. Transfected ES cells underwent double-selection with the neomycin analogue G418 (Gibco), at a concentration of 200 µg/ml and with gancyclovir (2 mM). ES clones were screened by Southern blot analysis using DNA probes external to both the 5′ and 3′ ends of the Krt7 homology arms. Microinjection of ES cell clones and generation of chimeric mice were performed as described previously [14]. Chimeric male mice were mated with C57BL/6 female mice and germline transmission of the targeted Krt7 allele was confirmed by PCR analysis of the resulting offspring. For continued maintenance of the line, K7 knockout mice were backcrossed onto the C57B6 background. Genotyping of K7 knockout mice was performed using the following primers: forward primer 5′ CTA CGT GGC TCA GTA TAG G 3′; reverse primer 1 5′ AAG AAC CGT GGC ACT GAG 3′ and reverse primer 2 5′ GAA TAT CAT GGT GGA AAA TGG C 3′ to generate PCR products of 743 bp (wildtype allele) and 593 bp (knockout allele). PCR conditions were as follows: 1 cycle at 94°C for 3 minutes followed by 40 cycles of 94°C (30 sec); 58°C (30 sec); 72°C (1 minute) and a final extension cycle of 72°C for 5 minutes.

Southern Blotting

DNA from either targeted ES cells or mouse tail tips was digested overnight with appropriate restriction enzymes and then separated on 1% (w/v) agarose gels. DNA gels were transferred to Hybond N+ membrane (GE Healthcare) overnight. DNA probes for Southern blotting were non-radioactively labelled using fluorescein (GE Healthcare). Probes were hybridised overnight at 60°C then washed in 1×SSC/0.1% (w/v) SDS followed by 0.5×SSC/0.1% (w/v) SDS. The bound probes were visualised using an anti-fluorescein antibody conjugated to alkaline phosphatase followed by chemiluminescent detection.

Northern Blotting

mRNA was purified from mouse tissues using the QuickPrep Micro mRNA purification kit (GE Healthcare) according to the manufacturer’s instructions. mRNA samples were separated on 1.2% (w/v) formaldehyde-agarose gels with MOPS running buffer and transferred overnight onto Hybond N+ membrane. Blots were incubated overnight at 68°C with an in vitro transcribed digoxigenin-labelled K7 RNA probe corresponding to exons 6–9 of the murine K7 cDNA. Following probe hybridisation, blots were washed twice in 2×SSC/0.1% (w/v) SDS at room temperature (5 minutes per wash) followed by 2 washes in 0.2×SSC/0.1% (w/v) SDS at 68°C (15 minutes per wash). The bound probe was detected using a sheep anti-digoxigenin antibody (Fab fragments) conjugated to alkaline phosphatase (Roche) followed by chemiluminescent detection.

Quantitative RT-PCR

Tissues were mechanically disrupted using the Qiagen Tissue-Lyser LT. Total RNA was extracted using the Qiagen RNeasy extraction kit according to the kit protocol. In-column treatment of the RNA with DNase was performed to remove genomic DNA contamination. 2 µg of RNA was reverse transcribed into cDNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems). 0.5 µl of cDNA was amplified in a 20 ul reaction using pre-designed Taqman® Gene Expression assays for Krt7 (Mm00466676_m1), Krt8 (Mm0429043_g1), Krt18 (Mm01601704_g1), Krt19 (Mm00492909_m1) and Krt20 (Mm00508106_m1) and ran on a 7900HT Fast Real-Time PCR system (Applied Biosystems) following the manufacturer’s recommended protocol. A Taqman® probe for mouse GAPDH was used as the endogenous control. Relative quantification (RQ) using the Comparative Ct method was determined using the RQ Manager 1.2.1 software (Applied Biosystems).

Immunofluorescence Microscopy

Mouse tissues (a minimum of 3 mice per genotype) were embedded in OCT (Agar Scientific) and immediately frozen in liquid nitrogen. 10 µm sections were fixed in methanol-acetone (−20°C) and blocked with 10% (v/v) normal goat serum (Sigma) in PBS buffer containing 0.1% (w/v) BSA. Primary and secondary antibodies were diluted in PBS buffer containing 0.1% (w/v) BSA and incubated for 1 hour at room temperature. Sections were extensively washed between antibody incubations with PBS. Primary antibodies were detected using appropriate Alexafluor-488 or Alexafluor-594 conjugated-secondary antibodies (Molecular Probes). Following antibody labelling, sections were mounted under glass coverslips with Hydromount containing 2.5% (v/v) DABCO (Sigma).

Immunohistochemistry

Mouse tissues (a minimum of 3 mice per genotype) were immediately fixed in 10% (v/v) Gurr neutral buffered formalin (pH 7.4) for 48 hours before dehydration and embedding in paraffin wax. 5 µm sections were stained with Mayer’s
Figure 1. *Krt7* gene targeting strategy. A. Schematic diagram of the mouse *Krt7* gene and upstream sequences, only the proximal part of the gene encompassing exons 1 to 3 is shown. Filled black boxes denote exons 1, 2, 3. The long and short homology arms of the targeting vector are indicated by filled black rectangles. Restriction enzyme sites are as indicated and the open black boxes denote the locations of the DNA probes that were used for southern blotting at the 5' and 3' ends of recombination in targeted ES cells. B. Genotyping of K7 knockout mice, the wildtype allele is 743 bp, the targeted allele is 593 bp. C. RT-PCR analysis of cDNA from the bladder (lanes 1–3), lung (lanes 4–6), colon (lanes 7–9) and kidney (lanes 10–12) amplified with primers to full-length K7 (1.5 kb) and GAPDH (509 bp). Lanes 1, 4, 7 and 10 are from wildtype mice; lanes 2, 4, 6 and 8 are from heterozygote K7 knockout mice; lanes 3, 6, 9 and 12 are from homozygous K7 knockout mice. M = DNA size standards. D. Northern blot of bladder RNA from wildtype (+/+) heterozygous (+/-) and homozygous (−) mice detected with RNA probes to K7 and GAPDH. The position of the 18S RNA.
haematoxylin and eosin. Tissue sections were examined by a clinical pathologist experienced in the histological analysis of mouse tissues. For antibody staining, high-temperature antigen retrieval was performed overnight by incubating sections with 0.01 M citrate buffer. Endogenous peroxidase was quenched by 1% v/v H2O2 in PBS for 30 minutes. Sections were blocked with either goat or rabbit serum (10% v/v in PBS) depending on the host species of the secondary antibody. Primary antibodies were incubated for 1 hour at room temperature on the host species of the secondary antibody. Primary and secondary antibodies were diluted in 1% (w/v) BSA in TBS buffer containing 0.06% (v/v) Tween-20. Primary and secondary antibodies were diluted in 1% (v/v) BSA in TBS buffer containing 0.06% (v/v) Tween-20. Primary antibodies were detected using goat anti-rabbit, goat anti-mouse or rabbit anti-rat immunoglobulins conjugated to horseradish peroxidase (DAKO). The antigen-antibody complex was then visualised chemilluminescently using luminol (Fluka) as substrate.

Ethical Considerations
All work involving animals was approved by the University of Dundee ethical review committee and all scientific procedures were performed under project licence authority (to WHIM and EBL) from the Home Office in accordance with the Animals (Scientific Procedures) Act 1986.

Results
To generate K7 knockout mice, we replaced exon 1 of the Krt7 gene and ~270 bp of the proximal promoter with a neomycin resistance cassette (Figure 1A) in order to prevent transcripts originating from this locus. Homozygous K7 knockout mice, on either the original 129P2/C57Bl6 mixed genetic background or those on the inbred C57Bl6 background, were born from heterozygous intercrosses at the expected Mendelian frequency of 1:2:1 indicating that the absence of K7 did not affect embryonic development. Homozygous K7 knockout mice were phenotypically indistinguishable from heterozygous and wildtype littermates and both male and female homozygotes were fertile and reproduced normally.

In the mouse, K7 is primarily expressed in various ductal and glandular epithelia and is highly expressed in transitional epithelia such as the bladder urothelium [2]. Semi-quantitative RT-PCR analysis of cDNA prepared from the bladder, lung, colon and kidney confirmed the absence of Krt7 transcripts in homozygous K7 knockout tissues (Figure 1C). Northern blotting of mRNA prepared from the bladder using the mouse K7 cDNA (exons 1–6) as a probe showed the absence of any K7 mRNA transcripts in homozygous mouse tissues whereas in heterozygous mice there was approximately half the amount of Krt7 mRNA as compared to wildtype mice (Figure 1D). Western blotting of cytoskeletal-enriched extracts prepared from the bladder, lung and colon of homozygous K7 knockout mice showed no K7 protein (Figure 1E).

In homozygous mice, there was an appreciable reduction in the amount of K7 protein as compared to cytoskeletal extracts prepared from wildtype tissues. Immunofluorescence microscopy of tissue cryosections using a polyclonal antibody raised against the C-terminus of murine K7 confirmed the absence of K7 protein in homozygous K7 knockout mice (Figure 2 and results not shown). In heterozygotes, K7 staining was comparable to that observed in wildtype tissues (results not shown). Overall, these series of experiments demonstrated that our inactivation of the Krt7 gene using gene targeting had been successful.

Based on our earlier study of K7 expression in the mouse [2], we undertook a comprehensive histological analysis of tissues from 6–8 week old homozygous K7 knockout mice (Table S1). We could detect no gross histological differences between the tissues and organs of homozygous K7 knockout mice as compared to wildtype littermates (Figure 3 and results not shown). Furthermore, the genetic background did not appear to influence these results since we could detect no histological differences between the tissues of homozygous K7 knockout mice that were from the original 129P2/C57Bl6 mixed genetic background and those that were maintained on a C57Bl6 background (results not shown). We also considered the possibility that a phenotype could be late-onset, as has been shown for K18 knockout mice [11], therefore we performed the same histological analysis in a small number (n = 3) of homozygous K7 knockout mice from the original 129P2/C57Bl6 mixed genetic background (results not shown).
urothelial cells, was similar to wildtype mice indicating normal with antibodies to uroplakin 3a, a differentiation marker of overt histological evidence of hyperplasia (Figure 4H). Immuno-bladder urothelium of homozygous K7 knockout mice showed no gous K7 knockout mice, around 2.5% of urothelial cells were Ki- mouse urothelium of around 0.4% [16]. In contrast, in homozy-

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around 0.5% in wildtype and heterozygous K7 knockout mouse bladder (Figure 4G) which is in close agreement with an earlier study by Farsund who reported a proliferation index in the normal mouse urothelium of around 0.4% [16]. In contrast, in homozygo-
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histochemical staining of tissue sections with antibodies to the cell proliferation marker Ki-67 revealed normal epithelial cell proliferation in homozygous K7 knockout mouse tissues (results not shown) with the exception of the urinary bladder where there appeared to be more Ki-67 stained nuclei (Figure 4F). Counting of Ki-67 positive cell nuclei in the transitional epithelium of the bladder, the urothelium, revealed a proliferation index of around 0.5% in wildtype and heterozygous K7 knockout mouse bladder (Figure 4G) which is in close agreement with an earlier study by Farsund who reported a proliferation index in the normal mouse urothelium of around 0.4% [16]. In contrast, in homozygo-
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K18, K19 and K20 are all potential in vivo assembly partners for K7 in the bladder, meaning that their expression could be affected by the loss of K7, whereas K8 represents the remaining simple type II keratin in this tissue. Western blotting (Figure 5A) and immunofluorescence microscopy (Figure S2) showed reduced K18 expression in the bladder of homozygous K7 knockout mice although gene expression analysis showed this was not due to reduced Krt18 mRNA (Figure 5B). K8 and K19 were not affected by the loss of K7 (Figure 5) but there was a significant increase in K20 mRNA expression in homozygous K7 knockout mice (Figure 5B). However a concomitant increase in K20 protein was not detected by western blotting (Figure 5A) or by immunofluorescence microscopy (Figure S2).

Completion of our characterisation of K7 knockout mice, we extended our analysis of simple epithelial keratin expression to other tissues using double-label immunofluorescence microscopy using the K7 polyclonal antibody in combination with monoclonal antibodies to K8, K18, K19 and K20 and the results are summarised in Table 1. We confirmed some of the results using western blotting (Figure S3) although this method was not applicable to every tissue due to the restricted expression of K7 to certain cell types such as the ducts of the liver and pancreas. Based on these analyses we could not detect any changes in K8 expression in homozygous K7 knockout mouse tissues as compared to tissues from wildtype mice (Table 1 and Figure S3). Immunofluorescence staining suggested reduced K18 expression within the collecting tubules of the kidney of K7 knockout mice (Figure S4) whereas other tissues showed no difference in K18 expression (Table 1). Despite extensive co-expression with K7 in wildtype tissues, there was no difference in K19 expression in the tissues of homozygous K7 knockout mice as compared to wildtype tissues (Table 1 and Figure S5). K20 is even more restricted in its pattern of expression as compared to K8 or K19, and in wildtype tissues where it is expressed along with K7, it did not appear to co-localise with K7 except for in the bladder where there was co-localisation of K7 with K20 at the apical cell membranes of superficial urothelial cells (Figure S2). However, in the absence of K7, K20 still remained localised at the apical cell membranes of urothelial cells (Figure S2). In other K7 knockout tissues, K20 expression was unchanged (Table 1).

**Discussion**

In this paper we have described the generation and character-

isation of K7 knockout mice, one of the remaining so-called “simple” epithelial keratin genes to be knocked out in the mouse using conventional gene targeting. Although the absence of K7 protein was not associated with any pathological phenotype this is not an unexpected result given that K18 knockout mice, which also show secondary loss of K7, only developed a mild late-onset phenotype that was restricted to hepatocytes with no other associated pathology [11]. Compensation for the loss of K7 by other type II keratins, in particular K8 whose expression closely overlaps with K7 [1], provides the most likely reason for the absence of any overt phenotype in K7 knockout mice but overcoming this problem of functional redundancy, through the generation of a K7/K8 double knockout mouse for example, would be difficult since both genes are closely associated within the keratin gene cluster on mouse chromosome 15 and based on previous studies embryos lacking both K7 and K8 are likely to be non-viable anyway [12,13].

Despite the absence of any pathology associated with the loss of K7, homozygous K7 knockout mice showed increased prolifera-

tion of the bladder urothelium.

Hyperproliferation is a feature of several keratin mouse knockouts and includes those which affect internal epithelial such as K8 [10] and K4 [17] as well as certain epidermally-expressed keratins such as K10 [18]. However, unlike these keratin knockout mice where hyperproliferation was associated with hyperplasia, there was no apparent urothelial hyperplasia in the bladder of K7 knockout mice. Unlike other types of epithelia such as the colon and epidermis, urothelial cells are characterised by their low proliferation index and longevity [16,19]. Therefore the five-fold increase in urothelial cell proliferation that we observed in K7 knockout mice, although similar to the four-fold increase in keratinocyte proliferation observed in the hyperplastic epidermis of K10 knockout mice [18], may simply have been insufficient to produce a phenotype in this particular type of epithelium. It is not clear how the absence of K7 led to stimulation of the normally quiescent urothelium since histologically there was no obvious disruption to the urothelium such as loss of the superficial umbrella cell layer, nor any evidence of apoptotic urothelial cells. Moreover, there was no evidence of any inflammatory cellular infiltrate present within the urothelium or the underlying bladder mucosa and the expression of the differentiation-associated plaque protein uroplakin 3a, which is important for limiting transcellular permeability across the urothelium [20], was also normal in K7

![Figure 2. Loss of K7 expression in K7 knockout mouse tissues.](image-url)
K7 Knockout Mice
knockout mice suggesting that the urothelial barrier was intact. Further study is therefore required in order to understand how the loss of K7 leads to changes in urothelial cell proliferation.

Our analysis of the fate of remaining simple keratins in K7 knockout mice suggests that K7 is required in part for the stabilisation of K18 in vivo. Unlike K18 knockout mice which showed complete loss of K7 [11], in K7 knockout mice K18 protein levels were only reduced but this appears to be a tissue-dependent effect since it was only noted in the bladder and in the kidney. In contrast to K18, the tail-less type I keratin K19, despite extensive co-expression with K7 in all of the tissues we examined, did not appear to be affected by the loss of K7 suggesting that K19 must be stabilised through pairing with another type II keratin, most likely K8. Although we only found minimal overlap between

Figure 3. Histological analysis of K7 knockout tissues. Haematoxylin and eosin stained formalin-fixed tissue sections from wildtype (A, C, E, G) and homozygous K7 knockout mice (B, D, F, H). Images show the cortical collecting tubules of kidney (A, B), bile ducts in liver (C, D), pancreatic ducts (E, F) and columnar epithelium of uterus (G, H). Scale bars = 50 μm. doi:10.1371/journal.pone.0064404.g003

Figure 4. Loss of K7 is associated with hyperproliferation but not hyperplasia of the bladder urothelium. Immunohistochemistry of bladder sections from wildtype (A, D), heterozygous (B, E) and homozygous K7 knockout mice (C, F) stained with a rabbit polyclonal antibody to K7 (A, B, C) and mouse monoclonal antibody MM1 to the cell proliferation marker Ki-67 (D, E, F). Arrowheads and insets in panels D and E indicate Ki-67 positive nuclei in wildtype (D) and heterozygous K7 knockout (E) bladder. More Ki-67 positive cell nuclei can be seen in the bladder of homozygous K7 knockout mice (arrowheads in F). Scale bars = 50 μm. G. Graph showing the percentage of Ki-67 positive urothelial cells in wildtype, heterozygous and homozygous K7 knockout mice (5 bladders per genotype). For each bladder, 10 random images were collected and an average of 1480 (SD ±300) urothelial cell nuclei were counted. Standard errors (SE) are indicated by the capped lines. * indicates a p value of less than 0.05 (WT p = 0.01; HET p = 0.007). H. H&E stained sections of the bladder urothelium of wildtype, heterozygous and homozygous K7 knockout mice. Scale bar = 25 μm. doi:10.1371/journal.pone.0064404.g004
Figure 5. Simple keratin expression in the bladder of K7 knockout mice. A. Coomassie blue stained SDS-PAGE gel and western blots of cytoskeletal-enriched extracts prepared from the bladder, lung and colon of wildtype (+/+), heterozygous (+/-) and homozygous (-/-) K7 knockout mice probed with antibodies to K8, K18, K19 and K20. A monoclonal antibody to β-actin was used to monitor protein loading and was co-incubated with the polyclonal K7 antibody on the same blot. M denotes molecular weight standards, sizes in kDa are as indicated. B. Quantitative RT-PCR of
the expression pattern of K20 and K7 in the tissues that we examined, both proteins are strongly expressed in the bladder urothelium although they only co-localise at the apical surface of the terminally differentiated superficial cells where they contribute to a trajectorial keratin network that underlies the plasma membrane [21]. The upregulation of Krt20 gene expression that we observed in the bladder of K7 knockout mice might be interpreted as an attempt to compensate for the loss of K7 protein from the sub-apical cytoskeleton in these superficial cells. Although we measured a significant increase in Krt20 mRNA expression in the bladder of K7 knockout mice, we could not detect any concomitant increase in the amount of K20 protein. This disparity could simply be due to differences in the sensitivity of the gene expression assay versus western blotting which is only semi-quantitative. It is also possible that since we blotted cytoskeletal extracts which contain filamentous ie. assembled keratin, rather than total protein extracts which would have included any soluble keratin that was not incorporated into filaments, any additional K20 protein may not have been detected using this approach.

Overall our characterisation of K7 knockout mice indicates that K7 is largely dispensable for the development, differentiation and maintenance of those simple epithelia in which it is normally expressed. However, the absence of K7 does appear to affect the normal homeostasis of the bladder urothelium as shown by the increase in urothelial cell proliferation. The urothelium acts as a highly effective barrier by preventing the leakage of urine into the underlying bladder mucosa and is physiologically important in terms of preventing urinary tract infections as well as being clinically important in terms of its susceptibility to carcinoma. Further functional studies using K7 knockout mice may be useful towards understanding the role of K7 within this specialised epithelium in greater detail.

**Table 1. Immunofluorescence analysis of simple keratin expression in K7 knockout mice.**

| Tissue              | K7 expression | K8   | K18  | K19  | K20  |
|---------------------|---------------|------|------|------|------|
| Bladder Urothelium  | =             | =    | reduced* | =   | =    |
| Liver Bile ducts    | =             | =    | =    | ne.  | =    |
| Colon Basal cells in crypts, goblet cells | = | = | = | = |
| Kidney Collecting tubules & ducts | = | reduced | = | ne. |
| Lung Alveolar & bronchial epithelium | = | = | = | ne. |
| Pancreas Ductal epithelial cells | = | = | = | ne. |
| Duodenum Brunner’s gland & specific cells in crypt | = | = | = |
| Stomach Squamo-columnar cells | = | = | = | = |

* = intensity of staining and localization similar to wildtype tissue.
*confirmation by western blotting.
ne. no protein expression.
*glandular cell staining.

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**Supporting Information**

**Figure S1** Immunohistochemistry of wildtype (A) and homozygous K7 knockout (B) bladder sections stained with antibodies to the urothelial cell differentiation marker uroplakin 3a. Notice the intense staining of the intermediate and superficial urothelial cells layers in both samples. m indicates the bladder muscular; * indicates the lumen of the bladder. Scale bars = 50 μm.

**Figure S2** K18 and K20 expression in the bladder of K7 knockout mice. Double label immunofluorescence microscopy of wildtype (A-C) and homozygous K7 knockout (D-F) bladder cryosections stained with antibodies to K7 (A, D) and K18 (B, E). Merged images (C, F) show both proteins co-localised at the apical cell membrane of superficial urothelial cells in wildtype mice (arrowheads, C). In homozygous K7 knockout mice, K18 expression appears to be reduced (E) but remains restricted to the superficial cell layer in the absence of K7 (E and F). Wildtype (G-I) and homozygous K7 knockout mice (J-L) bladder cryosections double-labelled with antibodies to K7 (G, J) and K20 (H, K). Merged images are shown in I and L. In the bladder of wildtype mice, K20 is also restricted to the superficial urothelial cells (H) and merged images of G and H shows colocalisation with K7 at the apical cell membrane (arrowheads, I). In homozygous K7 knockout mice, K20 expression (K) appeared similar to wildtype mice (merged image L). Cryosections were counterstained with DAPI. * indicates the lumen of the bladder and m denotes the position of the underlying bladder mucosa. Scale bars = 50 μm.

**Figure S3** Western blots of simple keratin expression in the colon and lung of K7 knockout mice. A. Coomassie Blue stained SDS-PAGE gel and B. western blots of cytoskeletal extracts of the colon and lung of wildtype (+/-), heterozygous (+/-) and homozygous (-/-) K7 knockout mice probed with antibodies to K8, K18, K19 and K20. K20 expression was not detected in cytoskeletal extracts from the lung (not shown). M denotes molecular weight standards, sizes in kDa as indicated.

**Figure S4** K18 expression in the kidney of homozygous K7 knockout mice. Double-label immunofluorescence microscopy of kidney cryosections from wildtype (A, C, E) and homozygous K7 knockout mice (B, D, F) stained with a rabbit polyclonal antibody to K7 (A, B) and mouse monoclonal antibody Ks18.04 to K18 (C, D). Merged images of A and C and B and D are shown in panels E and F respectively. In wildtype kidney, both K7 and K18 co-localise and show strong membranous staining of ductal epithelial cells (arrowheads, E). In homozygous K7 knockout mice, the intensity of K18 staining is overall weaker (D) than wildtype kidney (C) although some membranous staining can still be detected (arrowhead, F). Cell nuclei are counterstained with DAPI. Scale bar = 50 μm.

**Figure S5** K7 and K19 expression in the liver of K7 knockout mice. Double-label immunofluorescence microscopy
of liver cryosections from wildtype (A, C, E) and homozygous K7 knockout mice (B, D, F) stained with a rabbit polyclonal antibody to K7 (A, B) and rat monoclonal antibody Troma III to K19 (C, D). Merged images of A and C and B and D are shown in panels E and F respectively. In wildtype mice, K7 and K19 colocalise and specifically stain the bile duct epithelium (E). In the liver of homozygous K7 knockout mice, K19 staining is not altered by the absence of K7 (D, F). Cell nuclei are counterstained with DAPI. Scale bar = 50 μm.

Table S1 List of K7 KO tissues examined by H&E staining.

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Conceived and designed the experiments: AS FJDS EBL WHIM. Performed the experiments: AS FJDS DPL L. Campbell KMD SFM L. Corden L, Christie. Analyzed the data: AS FJDS DPL L. Christie SF. Wrote the paper: AS.