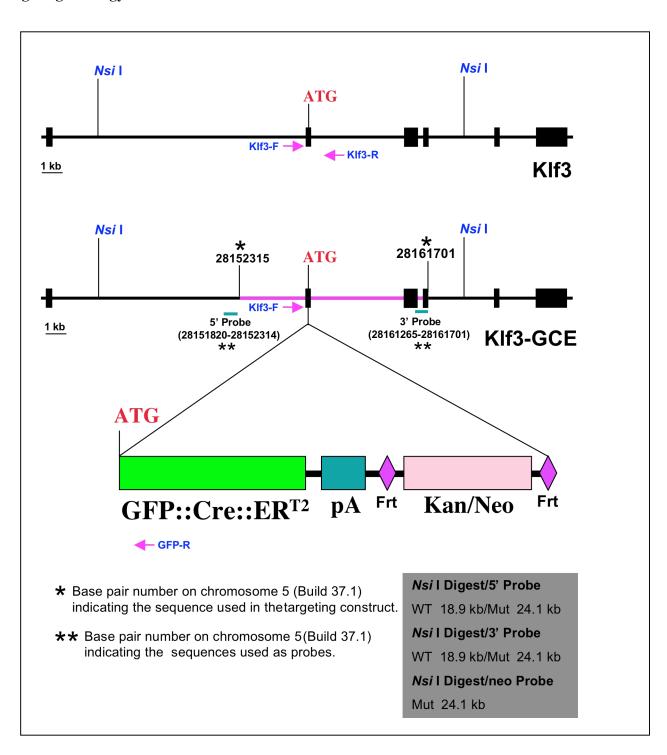
Klf3

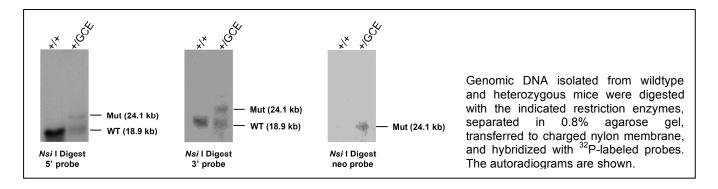
A. Rationale

Klf3 expression is restricted to the developing collecting ducts. A Klf3 knock-in approach was employed by the GUDMAP consortium to investigate the role of this population of cells in more detail.

B. Targeting Strategy



C. Southern Blot Analysis of the Targeted Allele in Mice



D. PCR Genotyping

a. Primers

Klf3-F: 5' cagttagtcctgcccgggag 3' Klf3-R: 5' agatcggctacggttgacc 3' GFP-R: 5' gtccagctcgaccaggatgg 3'

b. Expected Band Sizes

Klf3-F + Klf3-R: 437 bp Klf3-F + RFP-R: 276 bp

E. Relevant Sequences

a. Genomic clone used for targeting construct

agaggagagggaatgtcaggcaagtcttgctttgtcctcagcaaacagaactcttggctctagttagcctccacct ccattcattgcctcccttaggaagtagttgtaccgggagcctcacagactgtaagagctgtatcatttctagaagtcgcct aaatgattctccaggcttctaatatacttctacagttacatgctgacagaaacctgacccttggtttgataatctgcag tcaaaagtagaagaggggagggctcccgaaggcacatcacttttcaatgaaaattcggtttgactagcccttccccacaaa ataacccaqttqtccccattatqqqaaattatttqqqqqaaaactqqtttttcctatattqttqqttttttaaaqcacctc attgcctaattcagattgagagtgtttagctaaacagaactaagatgtaaagaaacaaccacattcgaggctttctcattt ctgcaatgcctacataaagtggttagtaagattcctcagcttggttaaaacccacaaggttttaaaagaccccaaggctggc qtacaacqccaatcttctqccaqcqtcttaaqqctccaaaqcctqttttcqtqcqccacctactqqttctctttaqaattq ctgctagggtggtaagtaaaagacttgcgtctttggtttcagaatcccttttgaaaaaatgaaggagatgtggtagtatgca cctqqcaatccaacacttqqaaqqcaqqtaqaqctctaqqtcaqccqaqqctatataqqtqaqactctqttttaaaaqaaqa aaaacqaqctttaaaaaaaqqataacqataactqcaqaqcactqtccctqqataacaqcqactqcaaaccaaqtttaaaqa tcccaqttcttacaqqtcttaqcttacqtqttaqttacqtqqaaqqcacaqtqqtaqttctcttctcattctatcctqatt ttgtacccctcttccctggttcctcaccagaaaccataaagcccaaagcaatggccagggtagttggtacagaagaattgc aaacqtttaactataatqaacaacttaaccatcatcacacaqqcatqcaaaqctqctqctcccaactcttqqtqaqcctaq gacggccttgaacttgaggcatcctcttgcatcggcatcagctttatcctgagtgccgagatctaagcatacacagcctca tgggatagatctgaggtgttgttaggaagctgctaaactgtctgaccttatcagtgggagaagaacacctgtttgctagct qtqtaqttqctcaattaqqaccaqttcttaqatqccaaaacacaqqttttcttcttcctaataaqatcacccaqttt tcgtttgtttgtttgtttgtttgtttgctgtcattttgtcttttgcgaggatacctaagagggtaatggcaccatggagtt qaqcaqcctqqtqtcacattqtatttqcaqattattatacatcctaqqqtctaqqacaattaqttqtqaaqtcaqqqcctt gtgttggtgggtagaatttacatgttgccatgaaaattaaaagaggttccaatgacatgctgaaggacaagagccacctgg caccacattcqqqcttatqtatqcqtatqtcttaactqctaqaacacaqqtatqqataqqcqacqtqqqcaccqcattctc

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Klf3-F

Klf3-R

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b. The final construct (excluding plasmid backbone and the negative selection marker)

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Klf3-F

Intron/exon junction

G ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCACA

Start of GFP

GFP-R

AGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGC
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End of GFP Start of Cre

End of Cre Start of ER

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End

TTGATGA AGATCTGAGCTCCCTGGCGGAATTCGGATCTTATTAAAGCAGAACTTGTTTATTGCAGCTTATAATGGTTACA

of ER Start of SV40 polyA

End of SV40 polyA

Frt

Start of Kan/Neo

ggcgcttttcccaaggcagtctggagcatgcgctttagcagccccgctgggcacttggcgctacacaagtggcctctggcc tegeacacattecacatecaeeggtaggegeeaaeeggeteegttetttggtggeeeettegegeeaeettetaeteetee cctagtcaggaagttccccccccgcccgcagctcgcgtcgtgcaggacgtgacaaatggaagtagcacgtctcactagtct cgtgcagatggacagcaccgctgagcaatggaagcgggtaggcctttggggcagcggccaatagcagctttgctccttcgc gtcctccggaggcccggcattctgcacgcttcaaaagcgcacgtctgccgcgctgttctcctcttcctcatctccgggcct ttcgacctgcagcctgttgacaattaatcatcggcatagtatatcggcatagtataatacgacaaggtgaggaactaaacc atgggatcggccattgaacaagatggattgcacgcaggttctccggccgcttgggtggagaggctattcggctatgactgg gcacaacagacaatcggctgctctgatgccgccgtgttccggctgtcagcgcaggggcgcccggttctttttgtcaagacc gctgtgctcgacgttgtcactgaagcgggaagggactggctgctattgggcgaagtgccgggggcaggatctcctgtcatct caccttgctcctgccgagaaagtatccatcatggctgatgcaatgcggcggctgcatacgcttgatccggctacctgccca ttcgaccaccaagcgaaacatcgcatcgagcgagcacgtactcggatggaagccggtcttgtcgatcaggatgatctggac gaagagcatcaggggctcgccagccgaactgttcgccaggctcaaggcgcgcatgcccgacggcgatgatctcgtcgtg acccatggcgatgcctgcttgccgaatatcatggtggaaaatggccgcttttcttggattcatcgactgtggccggctgggt gtggcggaccgctatcaggacatagcgttggctacccgtgatattgctgaagagcttggcggcgaatgggctgaccgcttc ctcgtgctttacggtatcgccgctcccgattcgcagcgcatcgccttctatcgccttcttgacgagttcttctgaggggat tccttgaccctggaaggtgccactcccactgtcctttcctaataaaatgaggaaattgcatcgcattgtctgagtaggtgt cattctattctggggggtggggtggggcaggacagcaagggggaggattgggaagacaatagcaggcatgctggggatgcg gtgggetetatggettetgaggeggaaagaaceagetggggetegaetagagettgeggaaceette **gaagtteetatte**

End of Kan/Neo Frt tctagaaagtataggaacttc ATCAGTCAGGTAC CTCATGTTTGATCCAGTCCCTGTCAAGCAGGAGGCCATGGACCCT

Frt Klf3 continues

 ${\tt GTCTCGGTGgtaagctgccccaaacggatcaactcttaactgataacatcgtgctctgtgttttcctaaagtgcttcc}$

Exon/intron junction

 $\tt Tgcttgacaggtggtgtttctcaaagcccccagtagtaaacgaggtgtcactatccccacacccctagacagggtcaaccg$

Klf3-R

```
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c. 5' probe

d. 3' probe