

## **Table of Contents for Supplemental Data**

1. Supplemental Figure 1. Re-analysis of RNA-seq data using Hisat2.
2. Supplemental Figure 2. (A, B, C, D) Full scans of Western blots and band density analysis.
3. Supplemental Figure 3. (A, B) Immunohistochemical staining for c-MYC and Grhl2 in kidney samples from 10 week old control and TCKD mice.
4. Supplemental Figure S4. Real-time quantitative PCR validation of differentially expressed genes identified by RNA-seq.
5. Supplemental Table 1. Primer list for genotyping
6. Supplemental Table 2. Primary antibody list
7. Supplemental Table 3. Secondary antibody list
8. Supplemental Table 4. RT-PCR primer list

## Supplemental Data

### **Supplemental Figure 1. Re-analysis of RNA-seq data using Hisat2.**

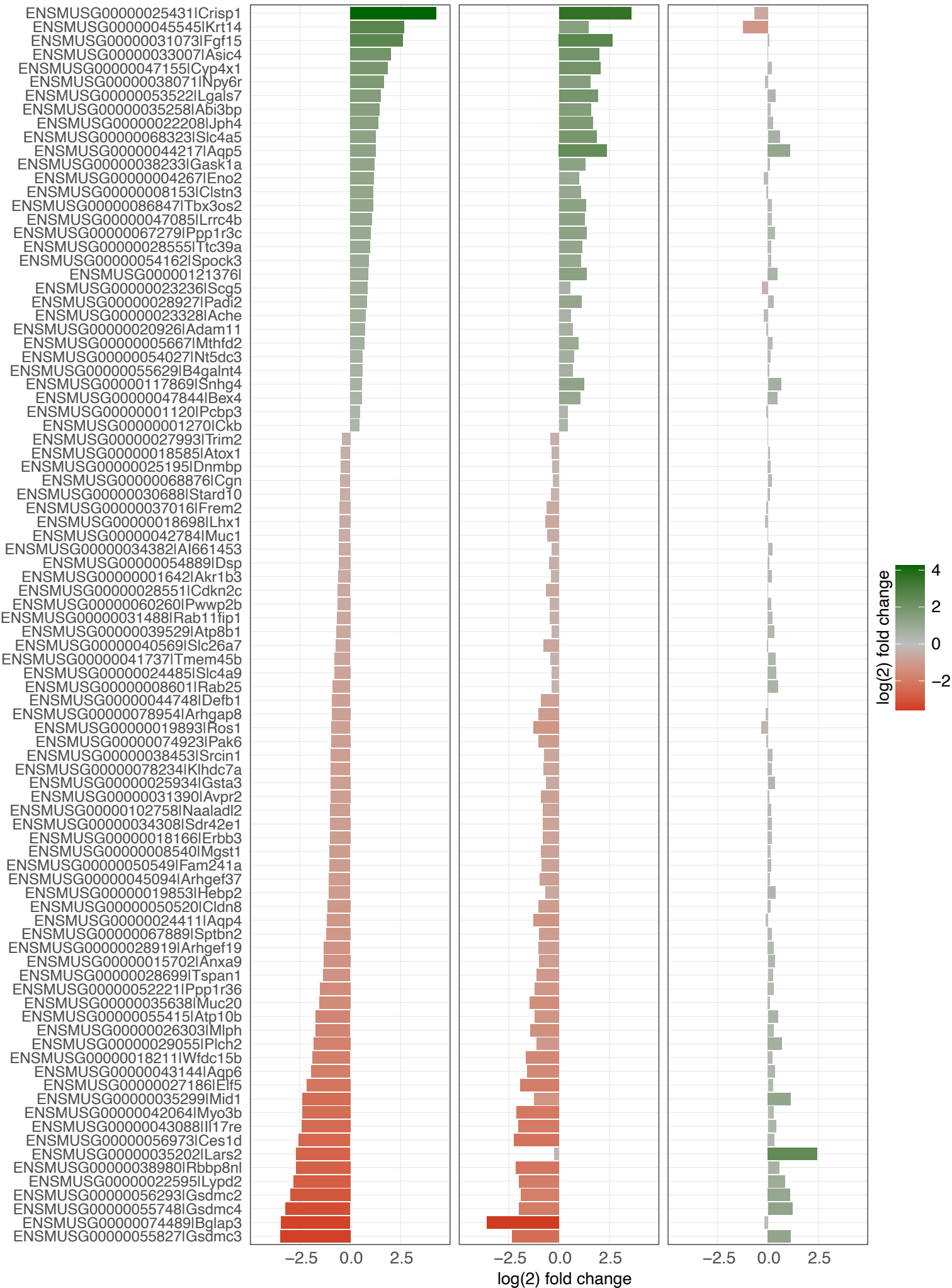
The re-analysis of RNA-seq data using Hisat2 was conducted on lysates of whole kidneys from control (n=5), TCKD (n=3), and TCKD-Grhl2KO (n=4) mice at P1.

Genes upregulated in TCKD-Grhl2KO kidneys compared to TCKD kidneys are labeled in green, while downregulated genes are labeled in red. Bars represent log2 fold changes (FDR<0.01 and p values<0.0001).

TCKD-Grhl2KO vs. TCKD

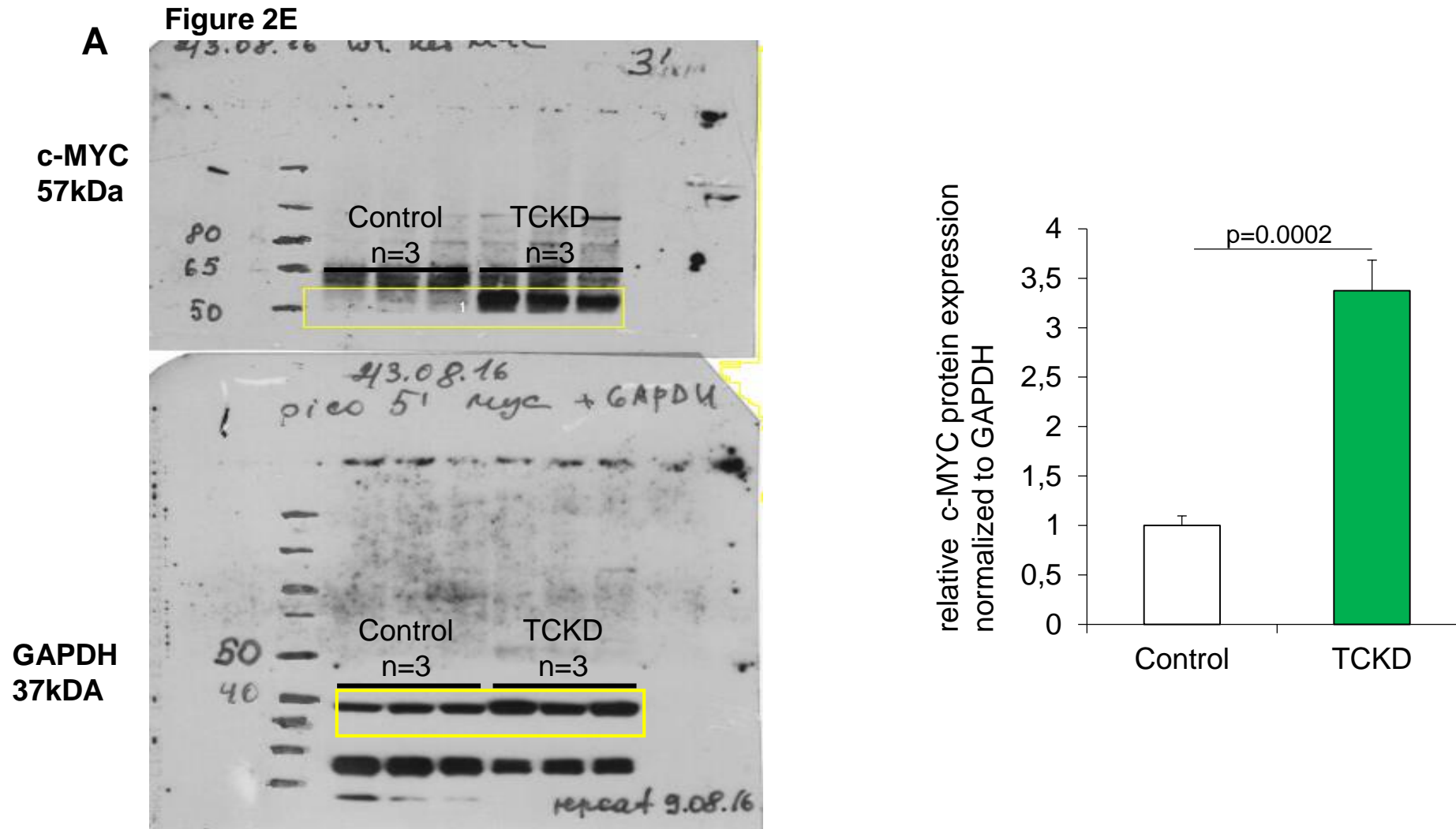
TCKD-Grhl2KO vs. Ctrl

TCKD vs. Ctrl



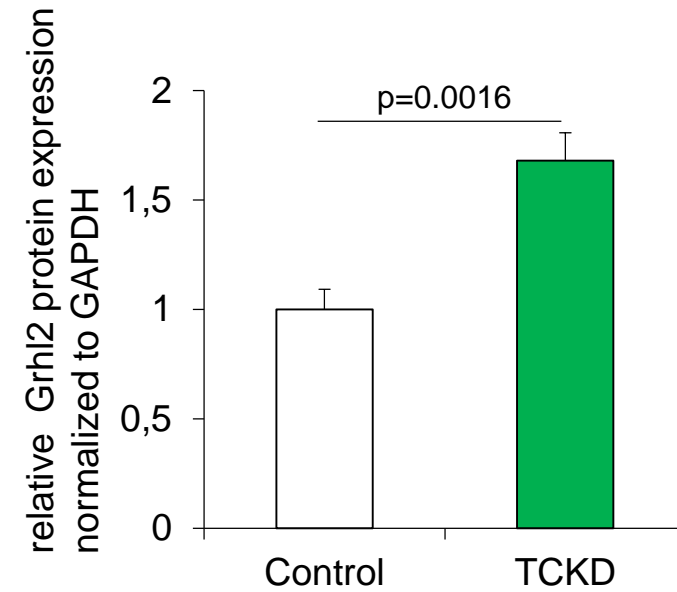
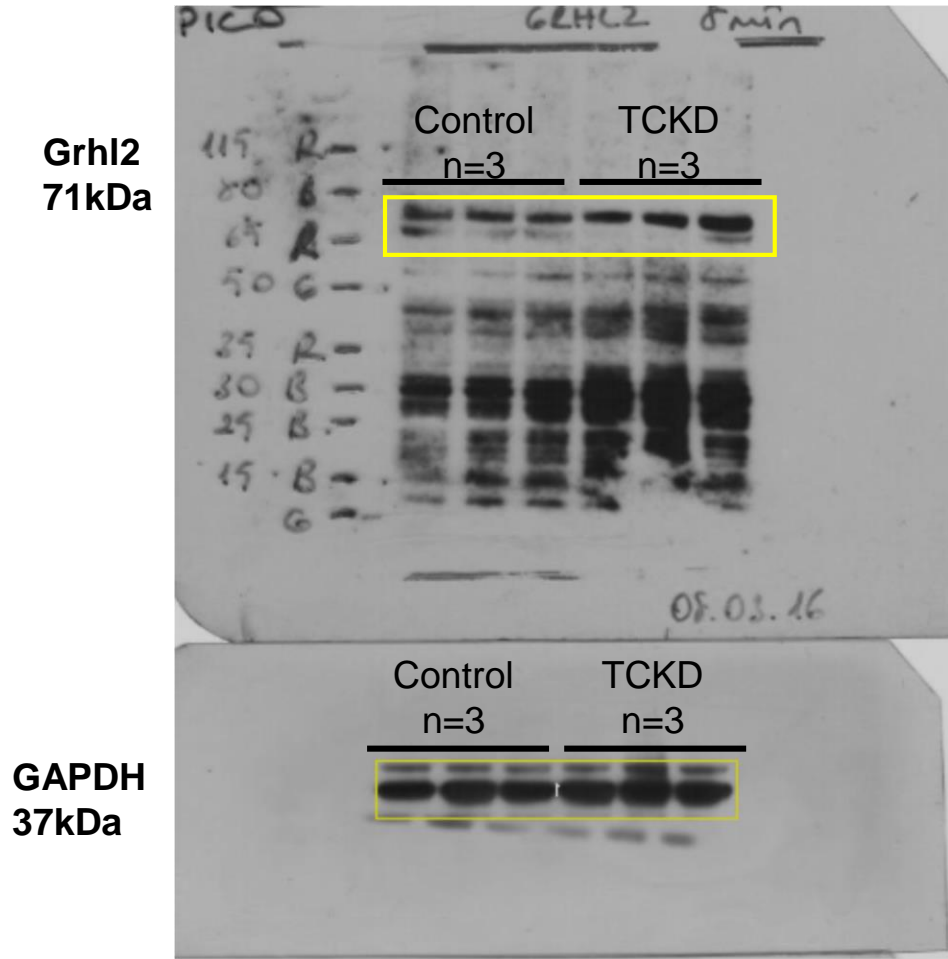
**Supplemental Figure 2. Full scans and band density analysis of Western blots corresponding to Figure 2E (A), Figure 3F (B), Figure 5B (C), and Figure 5D (D).**

# Supplemental Figure 2. (A) Full scans of Western blots and band density analysis for Figure 2E.



# Supplemental Figure 2. (B) Full scans of Western blots and band density analysis for Figure 3F.

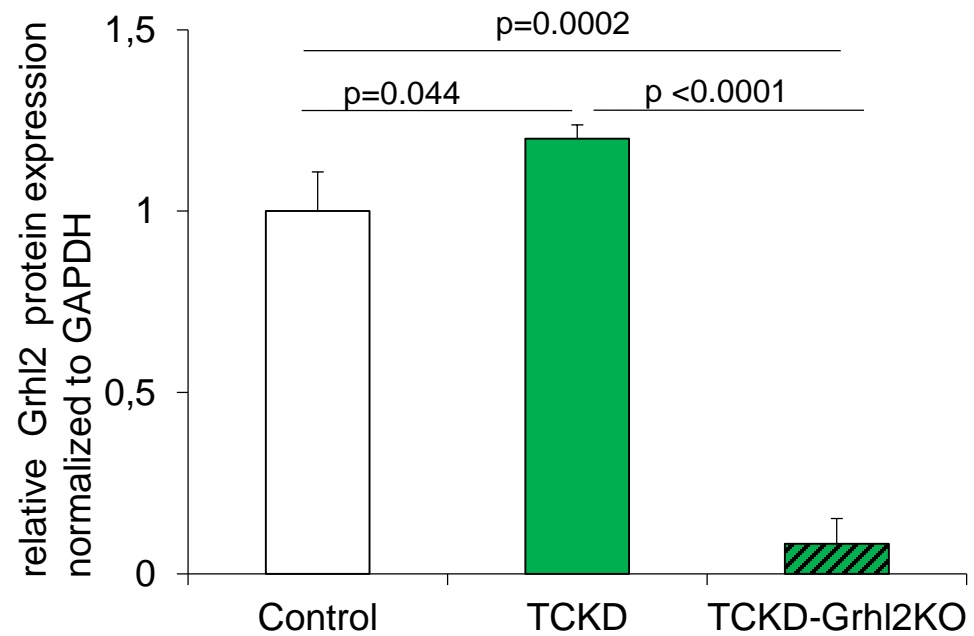
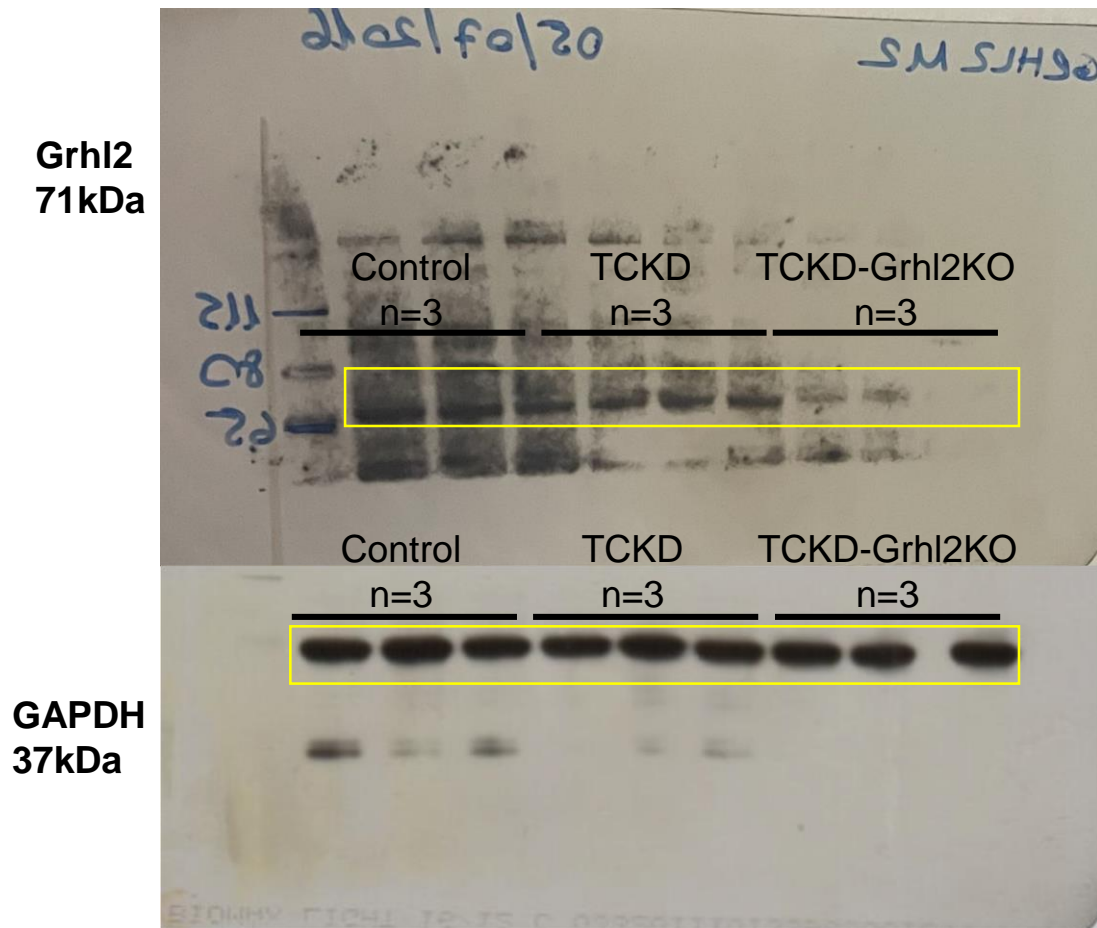
**B** Figure 3F



# Supplemental Figure 2. (C) Full scans of Western blots and band density analysis for Figure 5B.

## C

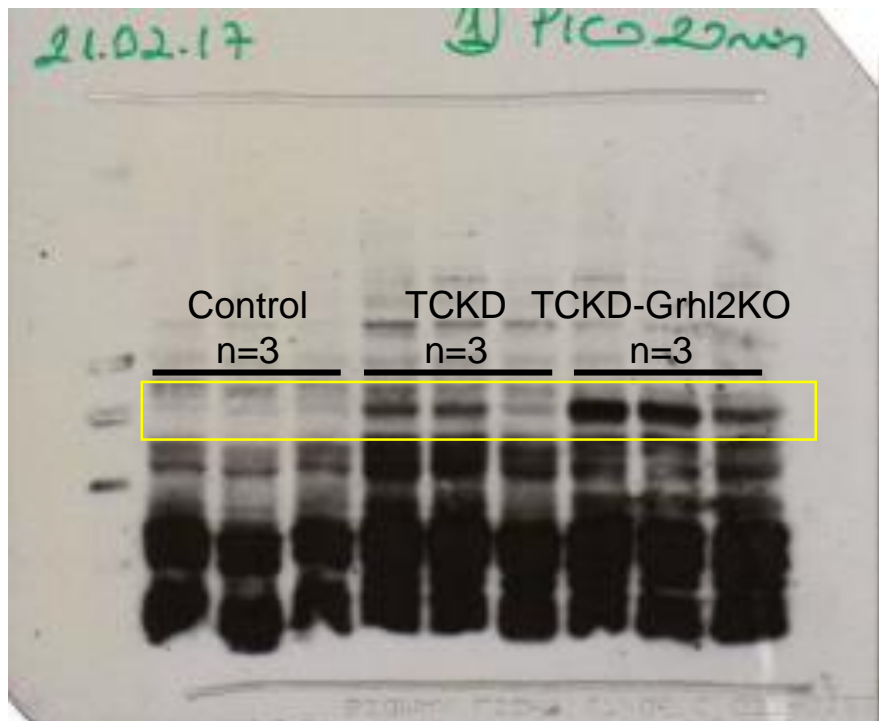
### Figure 5B



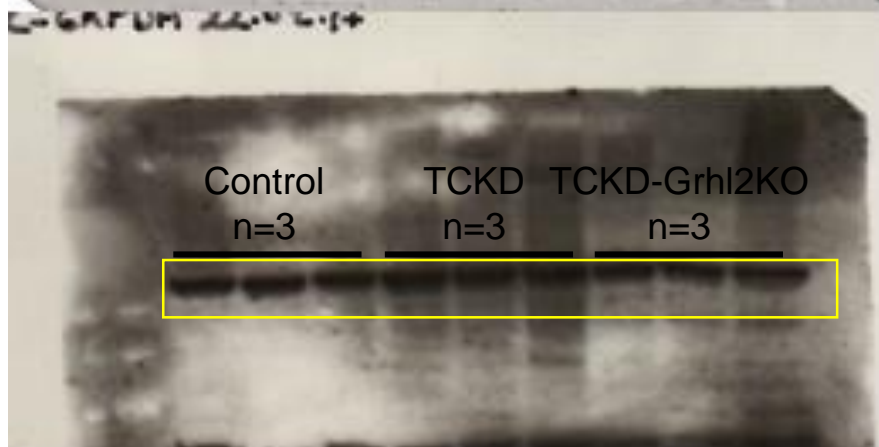
# Supplemental Figure 2. (D) Full scans of Western blots and band density analysis for Figure 5D.

D Figure 5D

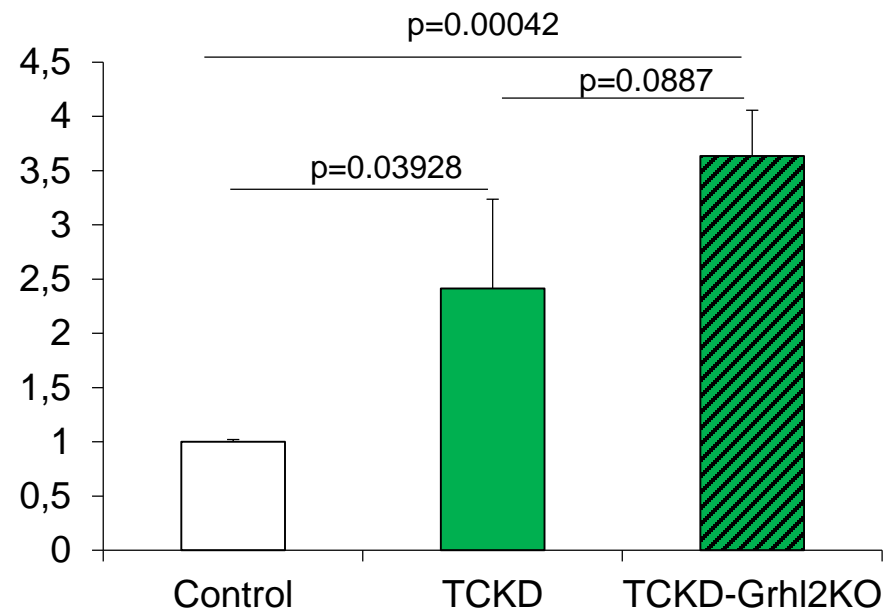
c-MYC  
57kDa



GAPDH  
37kDa



relative c-MYC protein expression  
normalized to GAPDH

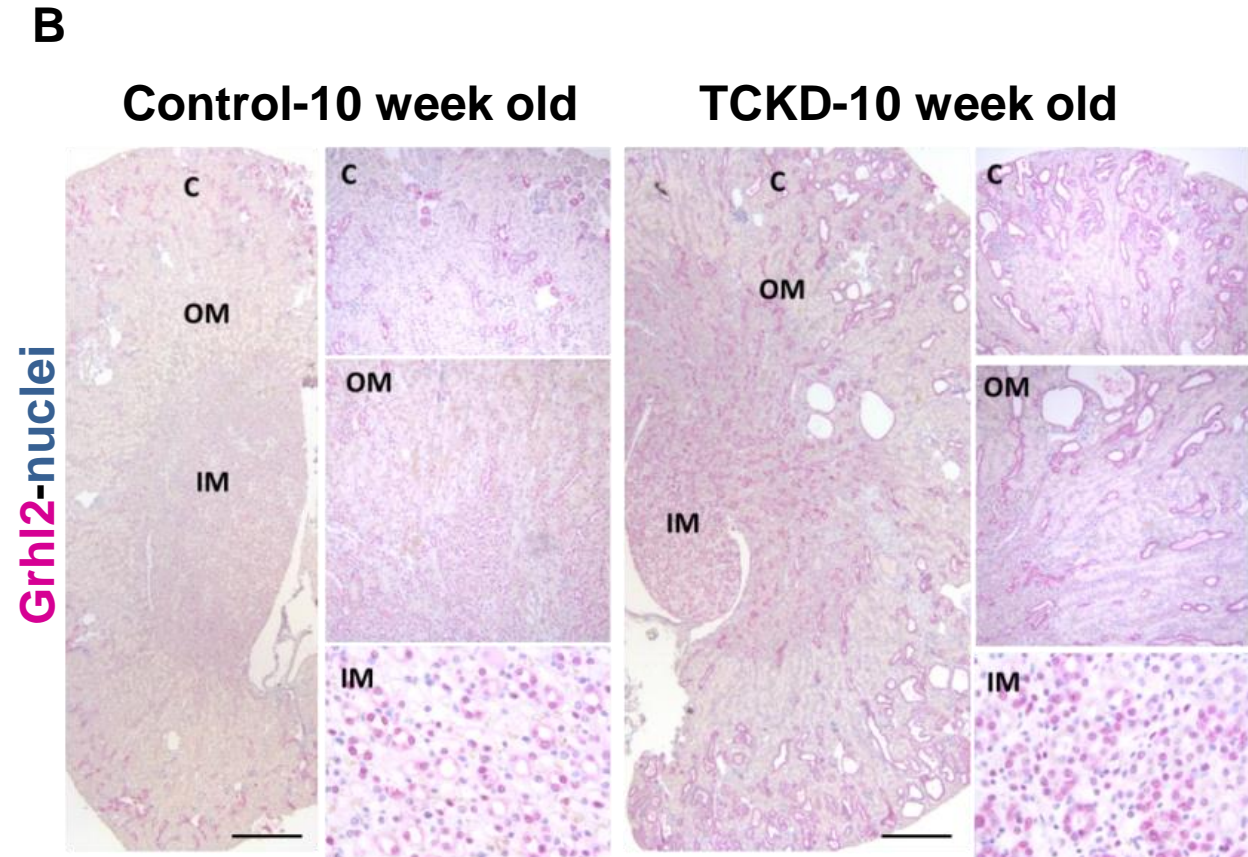
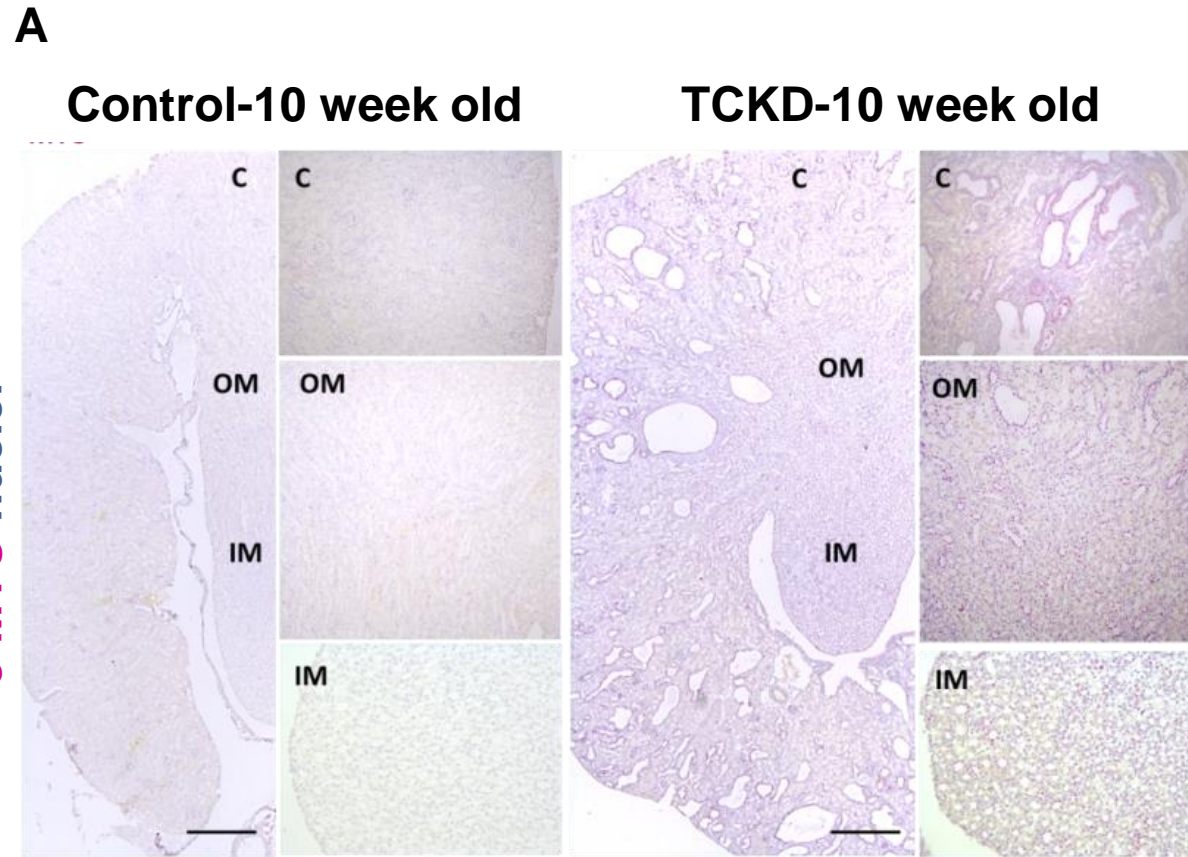




**Supplemental Figure 3. (A, B) Immunohistochemical staining for c-MYC and Grhl2 in kidney samples from 10 week old control and TCKD mice.**

The immunohistochemical staining for c-MYC (A) and Grhl2 (B) was performed on kidneys from control and TCKD. No MYC-positive cells were observed in the kidneys of control mice. Images were captured from the cortex, outer medulla, and inner medulla.

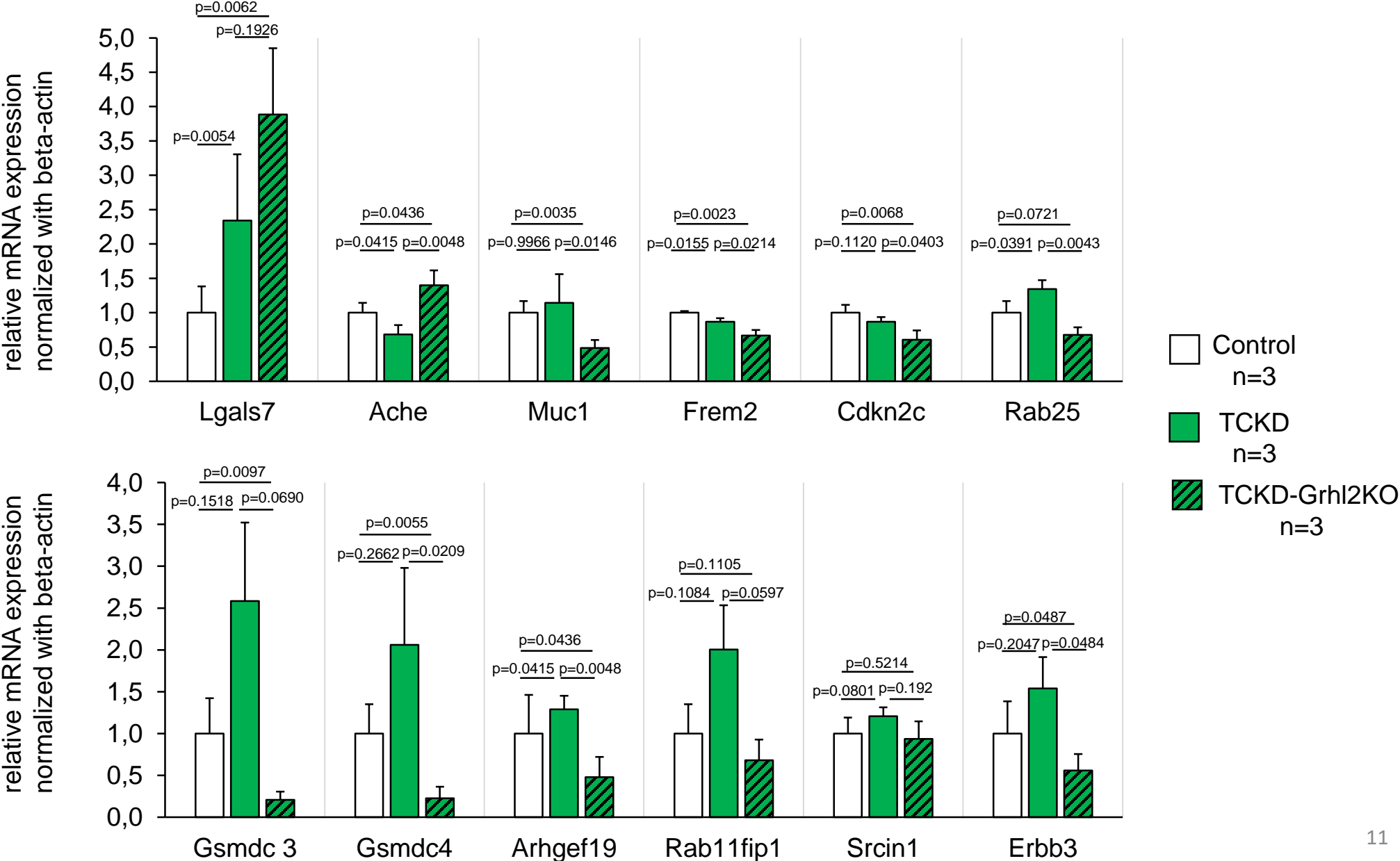
**Supplemental Figure 3. (A,B) Immunohistochemical staining for c-MYC and Grhl2 in kidney samples from 10 week old control and TCKD mice.**



**C: Cortex, OM: Outer Medulla, IM: Inner Medulla**

**Supplemental Figure 4. Real-time quantitative PCR validation of differentially expressed genes identified by RNA-Seq.** Real-time quantitative PCR analysis for the expression profiles of 12 randomly selected differentially expressed genes between the kidneys of control, TCKD, and TCKD-Grhl2KO mice (n=3 for each) at P1. Beta-actin mRNA was used as an internal control. Data are shown as mean  $\pm$  SD, P values calculated with t-test.

**Supplemental Figure 4. Real-time quantitative PCR validation of differentially expressed genes identified by RNA-seq.**



## Supplementary Table S1. Primer list for genotyping

Gene	Forward primer sequence 5' to 3'	Reverse primer sequence 5' to 3'	PCR Product Length
Rosa26	GGAGTGTTG CAATACCTTTCTGGGAGTTC	TGTCCCTCCAATTTTACACCTGTTCAATTC	250 bp
MYC	CCAAGAGGGTCAAGTTGG A	GCAATATGGTGGAAAATAAC	500 bp
HoxB7-Cre	AGCGCGATCACATGGTCCTG	ACGATCCTGAGACTTCCACACT	214 bp
Grhl2 flox/flox	CCAACCTTCCCTTTCCATTC	AGAGGACTTGAAGGTCCGGAG	672bp (flox) /564bp(wt)

## Supplementary Table S2. Primary antibody list

Antigen	Species	Company	Serial Number	Applications/ Dilutions
GRHL2	rabbit polyclonal	Sigma-Aldrich	HPA004820	IHC 1:100, IF 1:100
GRHL2	rabbit polyclonal	Abcam	ab136682	WB 1:500
MYC	rabbit polyclonal	Biolegend	626801	IHC 1:200, IF 1:100, WB 1:500
VATPaseB1/2 (H180)	rabbit polyclonal	Santa Cruz Biotechnology	SC-20943	IF 1:100
VATPaseB1/2 (H5)	mouse monoclonal	Santa Cruz Biotechnology	SC-166848	IF 1:75
AQP2	goat polyclonal	Santa Cruz Biotechnology	SC-9882	IF 1:200
Ki-67	rat monoclonal	Biolegend	652402	IF 1:80
Phospho-Histone H3 (Ser10) (6G3)	mouse monoclonal	Cell Signaling Technology	9706	IF 1:100
E-cadherin	mouse monoclonal	BD Transduction lab	610181	IF 1:500
Vimentin	rabbit polyclonal	Abcam	ab92547	IF 1:200
Acetylated $\alpha$ -tubulin	mouse monoclonal	Sigma-Aldrich	T6793	IF 1:200
ZO-1 (Tjp1)	rabbit polyclonal	Thermo Fisher Scientific	61-7300	IF: 1:200
GAPDH (V-18)	goat polyclonal	Santa Cruz Biotechnology	SC-20357	WB 1:1000

## Supplementary Table S3. Secondary antibody list

Antibody	Species	Company	Serial Number	Applications/ Dilutions
<b>Fluorophore conjugated</b>				
anti-goat Alexa 488	donkey polyclonal	Jackson ImmunoResearch	705-545-147	IF 1:200
anti-mouse Alexa 488	donkey polyclonal	Jackson ImmunoResearch	715-545-150	IF 1:200
anti-goat Alexa 647	donkey polyclonal	Jackson ImmunoResearch	705-605-147	IF 1:200
anti-rat CY5	donkey polyclonal	Jackson ImmunoResearch	712-175-153	IF 1:200
anti-rabbit CY3	donkey polyclonal	Jackson ImmunoResearch	711-165-152	IF 1:200
anti-mouse CY3	donkey polyclonal	Jackson ImmunoResearch	715-165-151	IF 1:200
anti-rat CY3	donkey polyclonal	Jackson ImmunoResearch	715-165-153	IF 1:200
<b>Enzyme conjugated</b>				
anti-rabbit Alkaline Phosphatase	donkey polyclonal	JacksonImmunoResearch	711-056-152	IHC 1:500
anti-goat Alkaline Phosphatase	donkey polyclonal	JacksonImmunoResearch	705-056-147	IHC 1:500
anti-rabbit Horseradish Peroxidase	donkey polyclonal	GE Healthcare	NA934	WB 1:1000
anti-goat Horseradish Peroxidase	donkey polyclonal	Abcam	ab97110	WB 1:2000

IHC: Immunohistochemistry, IF: Immunofluorescence, WB: Western Blot.

## Supplementary Table S4. RT-PCR primer list

Gene	Forward primer sequence 5' to 3'	Reverse primer sequence 5' to 3'
Grhl2	GCTCTTGTCTGCCATCTCG	CGGGACAGTCTTCAGAACCT
MYC	CTCTCAACGACAGCAGCCCG	AGGTGATCCAGACTCTGACC
Lgal7	AAGAACAAGGCAAATGGGGC	CGGTGGTGGAAAGTGGAGATA
Ache	TTTCCTTCGTGCCTGTGGTA	GTCACCTTGCTTGGGGTACAC
Muc1	GGCTCCTTTCTTCCTGCTG	CTGCTGGTGTCTGAGATGA
Frem2	CCTCCTTGTCTTCTGCTGGA	GCCTGTCCCTTTGAGTGAAG
Cdkn2c	GATTTGGGAGAAGTGCCTG	CCAGCAAAGCCTGTACAGTG
Rab25	TTCTCCACCCGCACTGTAAT	TAGGTCTGGTGTCTGGTCAG
Gsmdc3	GGCCTTTGTGAATAGTCTGCC	GGGCTCCAAGAGATCCAGAA
Gsmdc4	TTGCAAGAGGAGGTGTAGGG	AGGCCTCTGTCACCCACATAC
Arhgef19	ATACCCATATCGCCTCCACC	CATGTGCTGAGAACTCCACG
Rab11fip1	CGTTGCTCGGTCTCGATAAG	GGGTTTGGACTTCAGGGTGT
Srcin1	AGAGCAAGTACCCTCAGCAC	CACACAGGGTCCACGGTC
ErbB3	GCTCCGGTTCACCTCAGCTTA	CTGGAACCCTCACGATGTCC
Pkd1	CATTAGAAGAAGGCGTGTTCG	GCACCTGATGTTCTTTGCC
Pkd2	ACTACTACACTCGGACACTGTC	CCTTCCAGTACAGCCCATCC
Tsc1	AGCCATCTTCAAGGAGTCCC	GGATGTGCAATACCGGCTGA
Hnf1 $\beta$	TGACACTCCTCCATCCTCA	ATCATTGTCAGCCCTCCA
$\beta$ -actin	GGCTGTATTCCCCTCCATCG	GGCCTCGTCACCCACATAG