**a**

```
                    ┌─────────────────────┐
                    │  GOCAR enrollees    │
                    │      n=589          │
                    └─────────┬───────────┘
              ┌───────────────┴─────────────────┐
              ▼                                  ▼
   ┌─────────────────────┐          ┌─────────────────────┐
   │  Non-microarray     │          │  Microarray Cohort  │
   │      n=440          │          │      n=159          │
   └─────────┬───────────┘          └─────────┬───────────┘
             ▼                                ▼
   ┌─────────────────────┐          ┌─────────────────────┐
   │     CADI-12         │          │     eGFR-12         │
   │      n=102          │          │      n=147          │
   └─────────────────────┘          └─────────┬───────────┘
                                              ▼
                                    ┌─────────────────────┐
                                    │     CADI-12         │
                                    │     *n=101          │
                                    └─────────┬───────────┘
                                              ▼
                                    ┌─────────────────────┐
                                    │  CADI-3 &CADI-12    │
                                    │      n=85           │
                                    └─────────────────────┘

   ┌─────────────────────┐          ┌─────────────────────┐
   │  SNP genotyping     │          │  SNP genotyping     │
   │  Donors=381         │          │  Donors=136         │
   │  Recipients=395     │          │  Recipients=145     │
   └─────────────────────┘          └─────────────────────┘
```
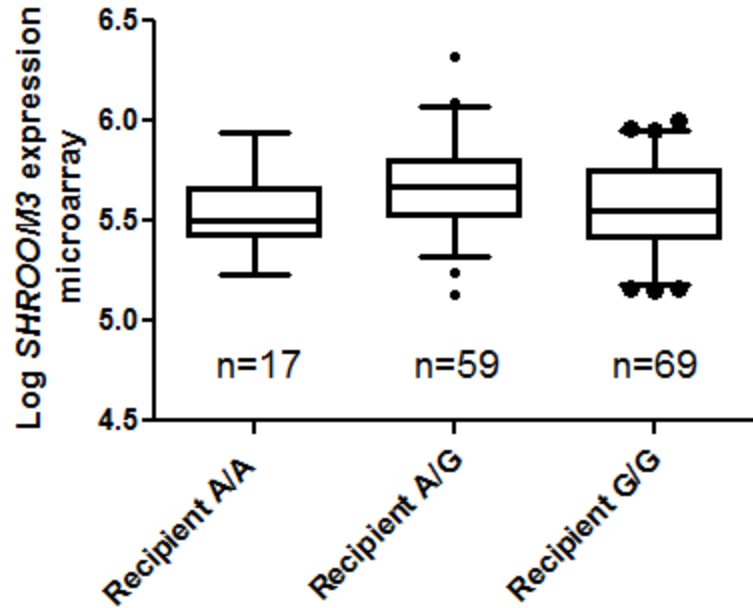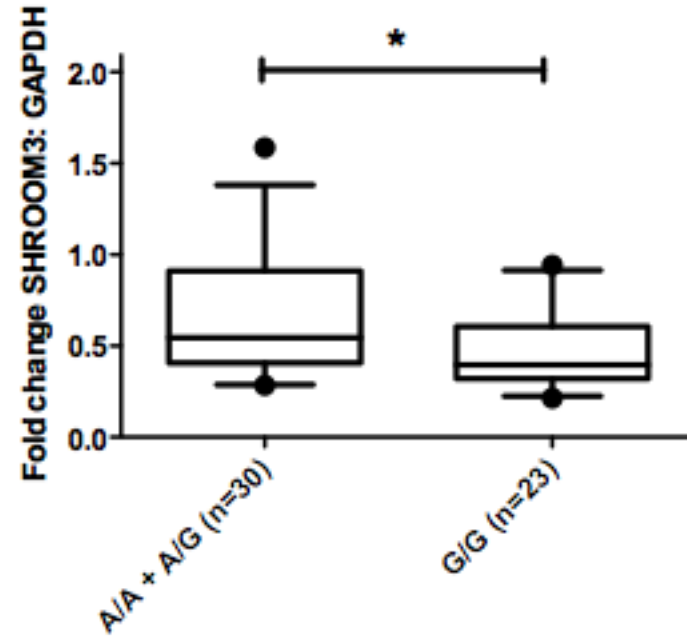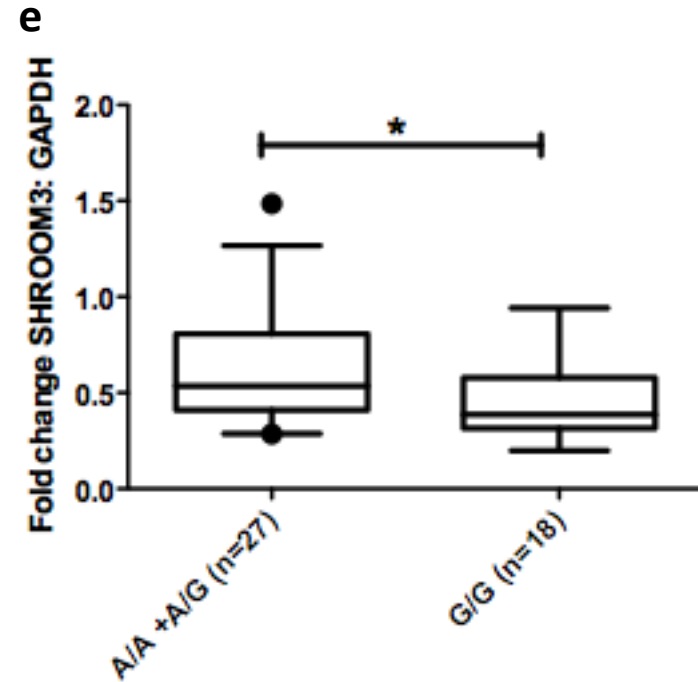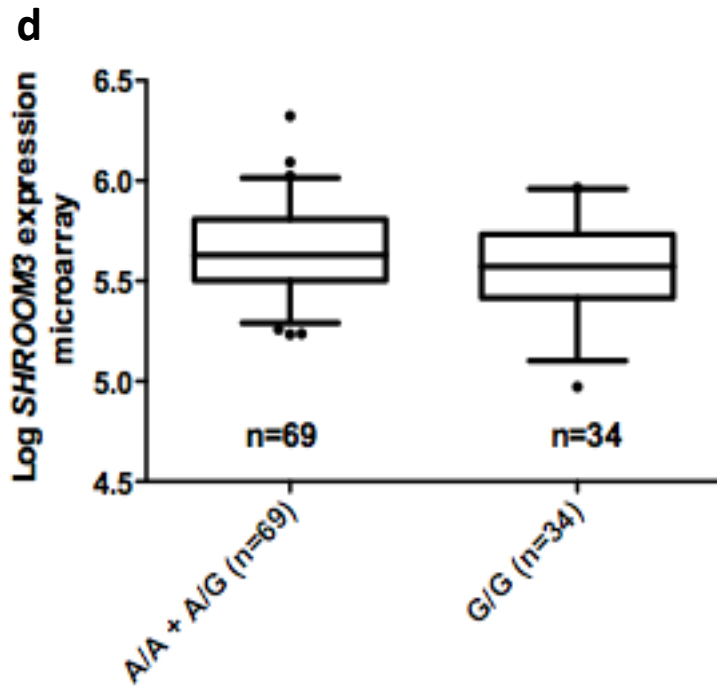
**Suppl Fig 1a.**
Of 589 GoCAR enrollees, 159 patients had 3-month allograft biopsy RNA extracted for microarray based upon date of enrollment into the study and consent for 3-month biopsy . Among these 159 patients, at 12-months post transplant, 147 had eGFR-12, 101 had CADI-12, 85 had CADI-3 and CADI-12 reported. Reasons for not having a 12-month biopsy included graft loss (n=8), death (n=1), lost-to-follow up (n=9), contraindication for or inability to obtain a renal allograft biopsy (n=40). In the non-microarray cohort, 102/439 have CADI-12 scored till Jan 2013. [*80/101 with CADI-12 were Caucasian-donor allografts].
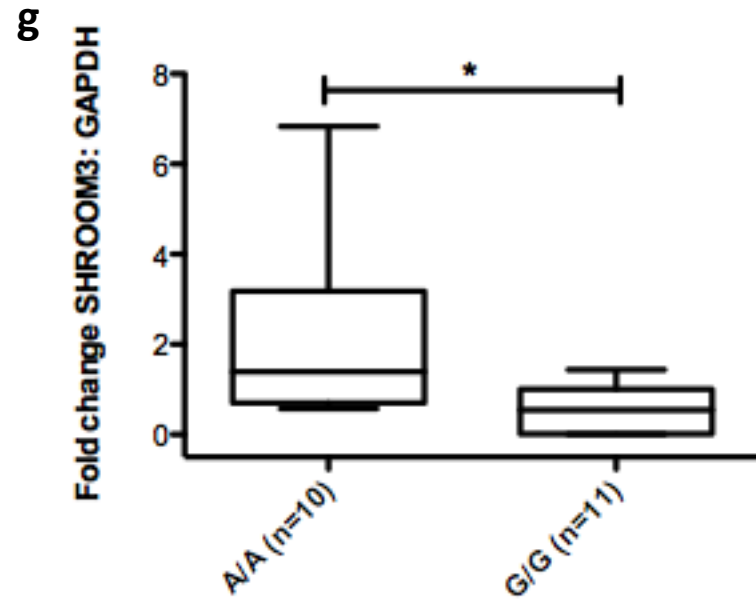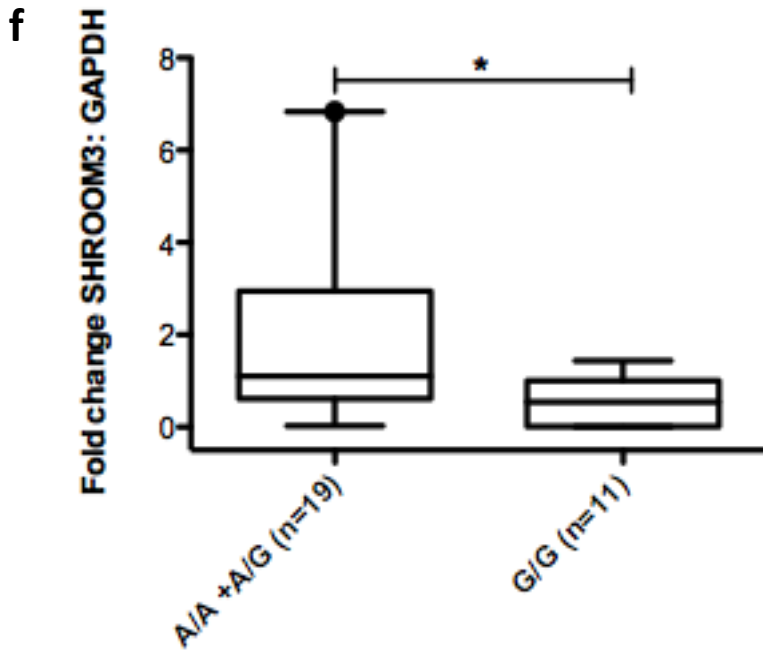
**Suppl Fig 1b-c:(b)** Box plots compare SHROOM3-3M (microarray) to corresponding recipient SHROOM3 SNP genotype; and **(c)** compares SHROOM3-expression (normalized to GAPDH) at 3-months with corresponding SHROOM3 allele-type; (whiskers: $5^{th}$-$95^{th}$ percentile; Line at Median; ANOVA p>0.05). An allograft with intermediate SHROOM3-expression was arbitrarily selected as control sample (Fold Change = 1)

**Suppl Fig 1d-e: Donor rs17319721 genotype is associated with increased SHROOM3-3M by RTPCR in Caucasian-donor allografts.**

Box plots compare SHROOM3-expression (normalized to GAPDH) at 3-months with corresponding SHROOM3 allele-type in Caucasian-donor allografts by **(f)** microarray [p=0.05] and **(g)** by RTPCR. (whiskers: 5th-95th percentile; Line at Median; *Mann-Whitney p<0.05). An allograft with intermediate SHROOM3-expression was arbitrarily selected as control sample (Fold Change = 1)
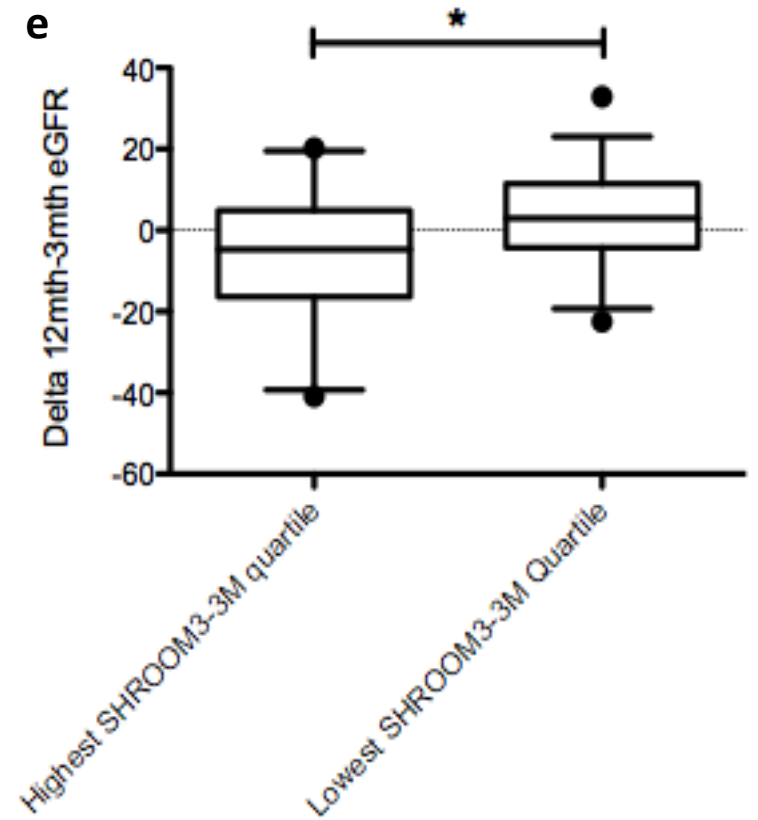
**Suppl Fig 1f-g: Donor rs17319721 genotype is associated with increased SHROOM3-0M by RTPCR.**

Box plots compare SHROOM3-expression (normalized to GAPDH) at **(d)** baseline with corresponding SHROOM3 allele-type; while **(e)** compares SHROOM3-expression at baseline between Donor A/A and G/G genotypes (whiskers: 5th-95th percentile; Line at Median; *Mann-Whitney p<0.05). An allograft with intermediate baseline SHROOM3-expression was arbitrarily selected as control sample (Fold Change = 1)

**a**



**b**



**c**
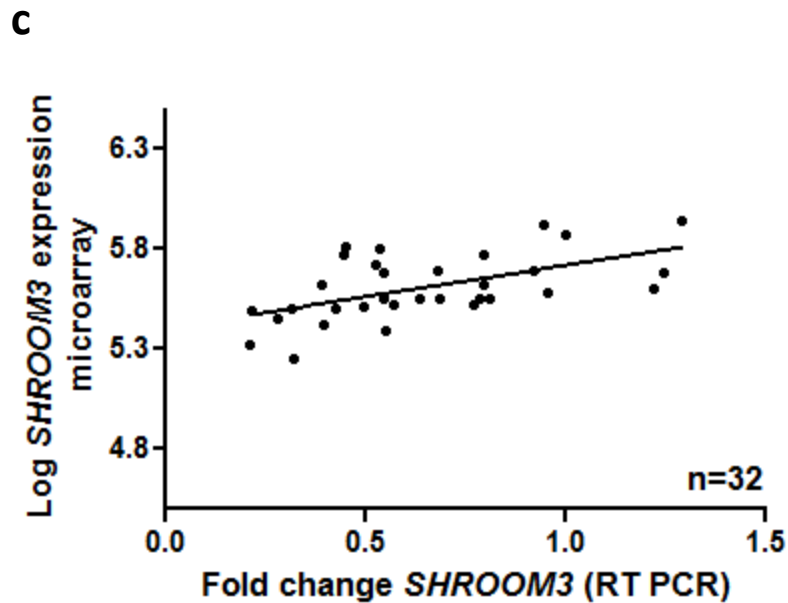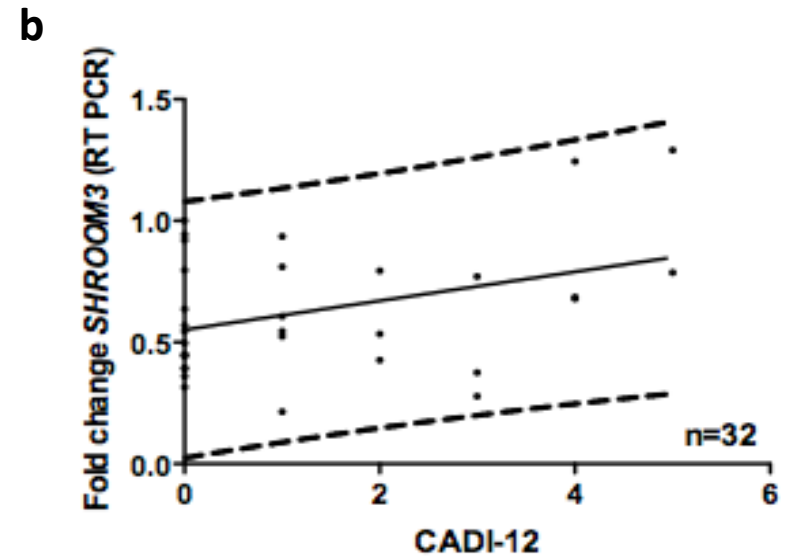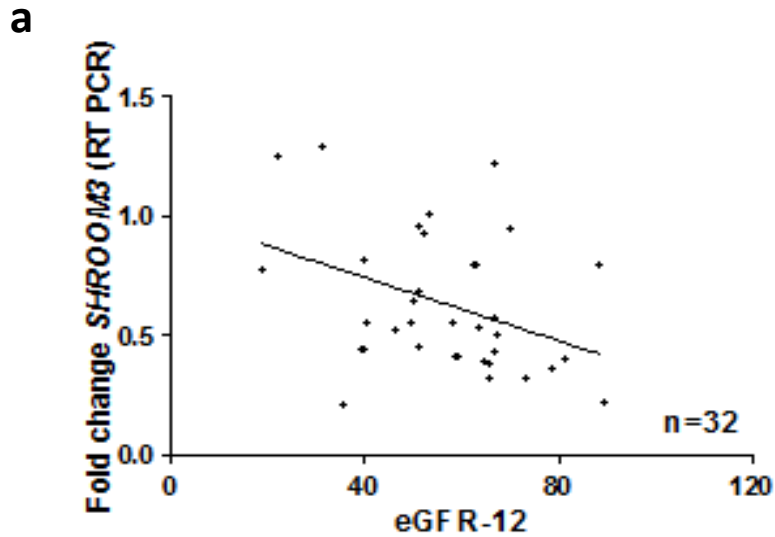


**Suppl Fig 2a-c: RT-PCR validation of SHROOM3-3M and outcomes**

a: Correlation of Fold change *SHROOM3* expression (normalized to GAPDH by RT-PCR) at 3 months and eGFR creatinine at 12 months(r=0.32; p=0.04). b: Correlation of Fold change *SHROOM3* expression (RT-PCR) at 3 months and CADI-12 months (r = 0.42; p=0.01) [Dotted lines represent 95% CI for Fold-change SHROOM3]. c: Correlation between SHROOM3-3M and Fold change *SHROOM3* at 3 months (by RT PCR) (r = 0.56; p<0.001) An allograft with intermediate SHROOM3-expression was arbitrarily selected as control sample (Fold Change = 1) in each analysis.

**d** Log *SHROOM3* expression microarray vs CADI-3; n=135
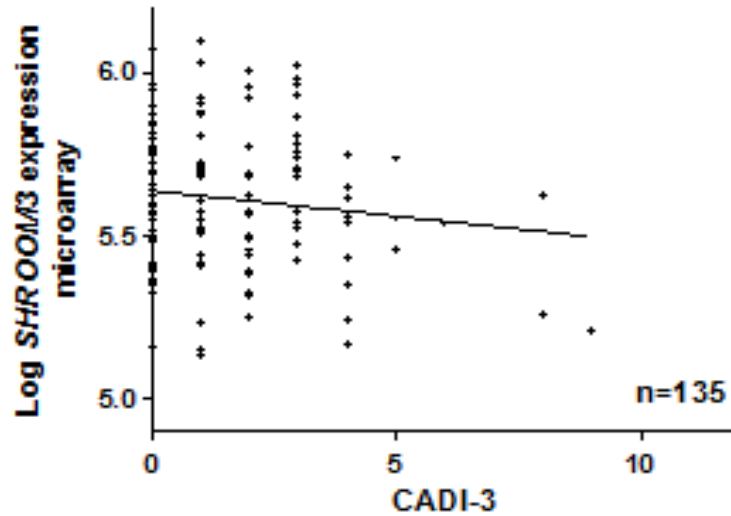
**e** Delta 12mth-3mth eGFR; Highest SHROOM3-3M quartile vs Lowest SHROOM3-3M Quartile; *

**Suppl Fig2d-e: (d)** Correlation between SHROOM3-3M and simultaneous CADI (3-month CADI score) (r=-0.1273, P=0.14)

**(e)** Box plots show Delta-eGFR between allografts in the highest (n=23) versus the lowest (n=31) quartiles of SHROOM3-3M. (whiskers: 5th-95th percentile; Line at Median; *Mann-Whitney p<0.05)
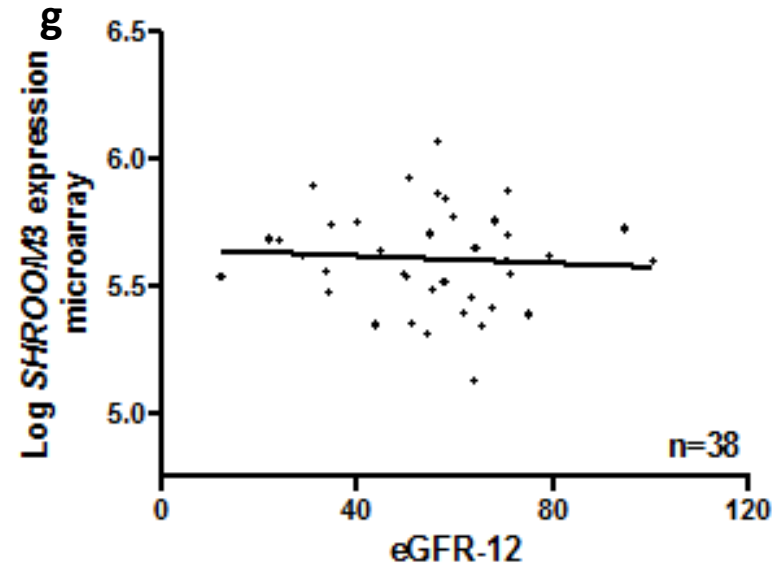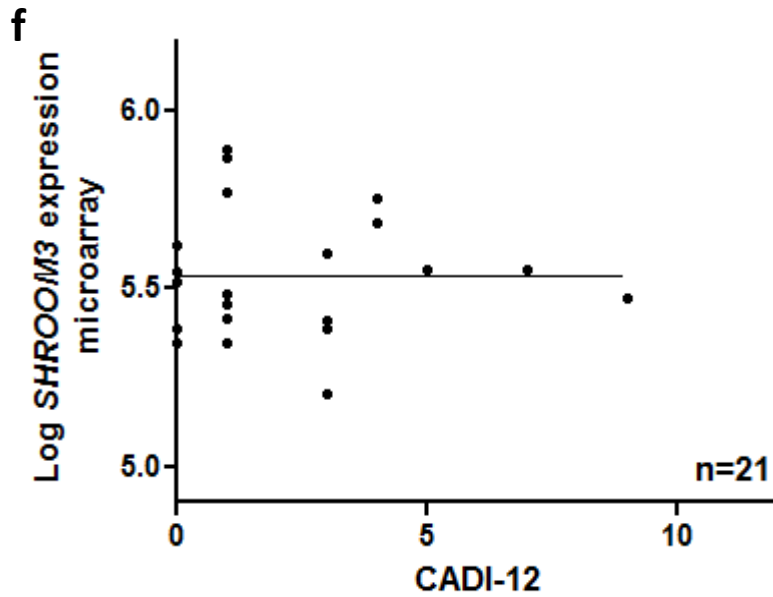
**Suppl Fig 2f-g:**
e: Correlation lines  between SHROOM3-3M and eGFR-12 and, f: CADI-12 in non-Caucasian
allografts is shown [p=ns]
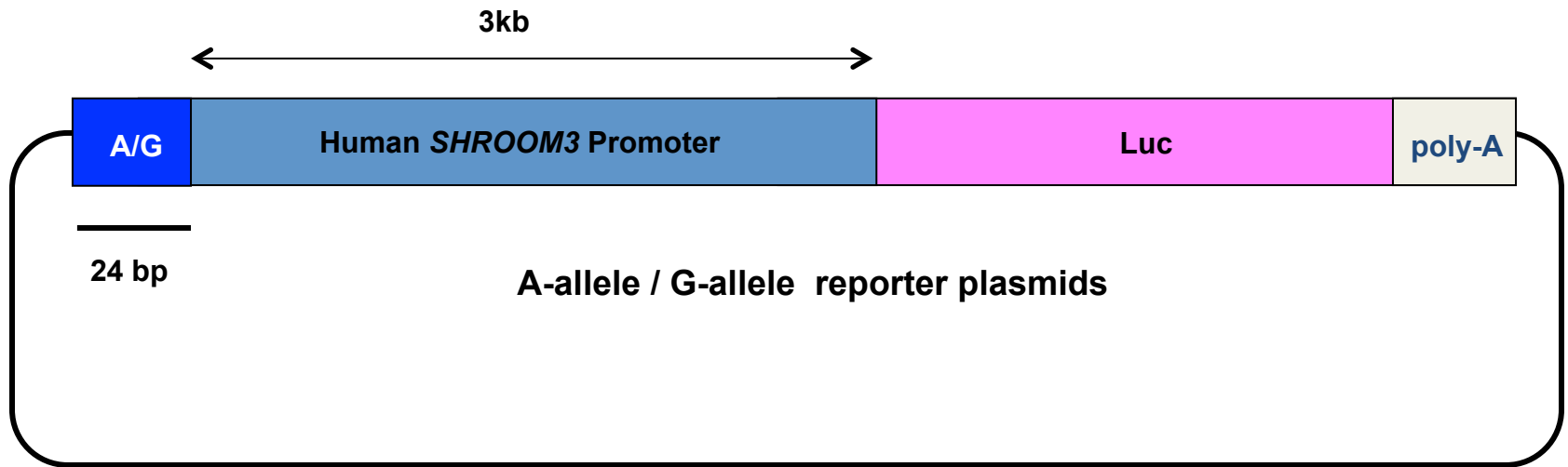
**Suppl Fig 3: Luciferase reporter plasmids: Promoter-Enhancer construct design**
Promoter fragments spanning -3000 bases 5' of the translation start site of *SHROOM3* were PCR amplified using primer sets that optionally included 24-bp sequences of the intron-1 of *SHROOM3* including the rs17319721 site containing either of the two alleles (A or G) (labelled A/G in figure). The PCR products were then cloned into luciferase reporter vector-pGL3 Basic (E-1751, Promega, WI, USA) using Restriction endonuclease (Kpn I and Hind III) sites (Suppl Table 3b).
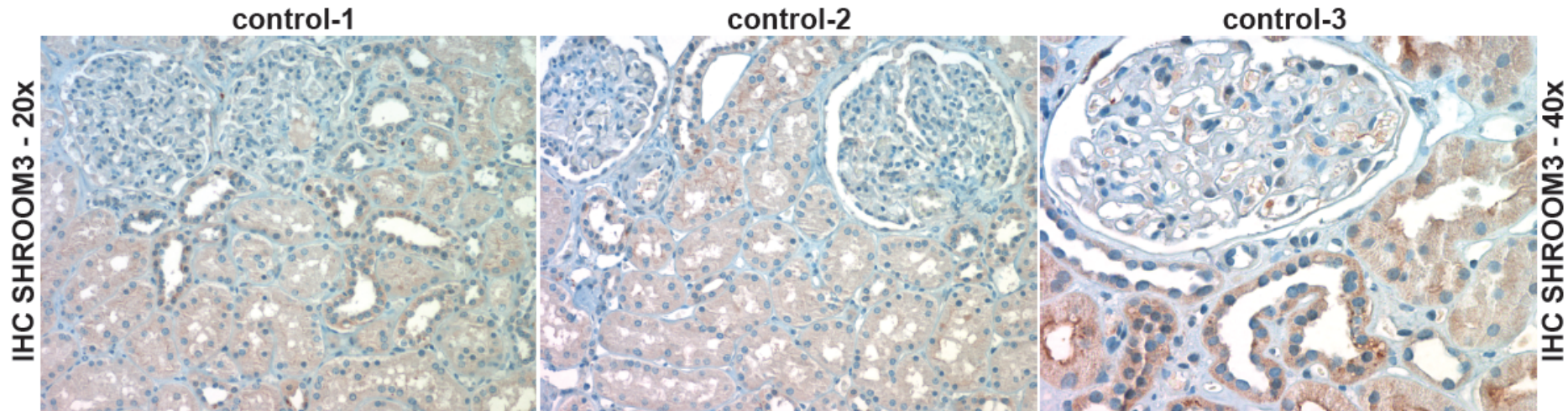
**Supplemental Fig 4: Tubular localization of SHROOM3 in kidney sections**
Paraffinized sections from healthy donor control kidneys were deparaffinized and treated with citrate buffer (pH-6). Immunohistochemistry was performed using rabbit polyclonal anti-SHROOM3 antibody with biotinylated goat anti-rabbit IgG followed by Streptavidin HRP. 20x- and 40x-images are displayed with hematoxylin counterstain.

**a**

Fold change *SHROOM3* (RT PCR)

Control Vector

SHROOM3 Vector

**b**

ANTI-V5 — 250 KD

β-ACTIN — 45 KD

Control Vector

SHROOM3 Vector
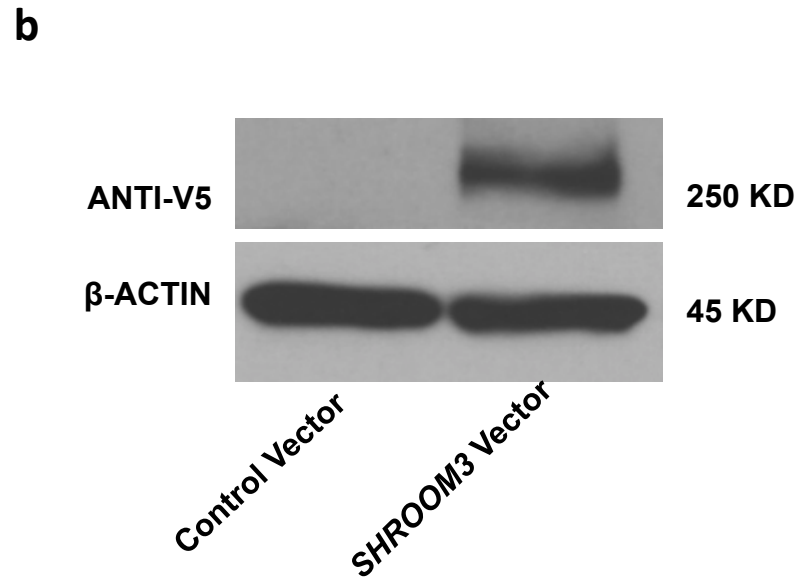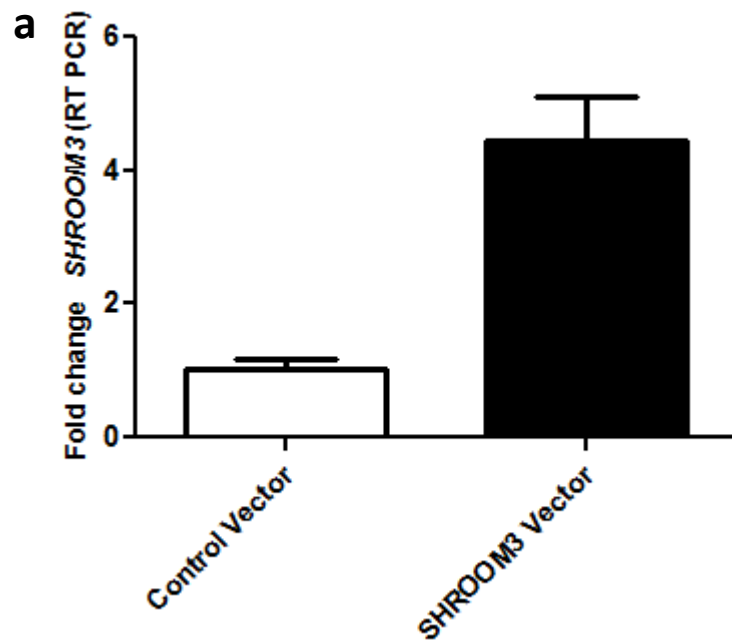
**Suppl Figure 5a-b: *SHROOM3* overepression by PC-*SHROOM3* was confirmed by R TPCR and Western blot.**

*SHROOM3* overexpression with PC-*SHROOM3* transfection was achieved by electroporation in PRCEC. (5a) Bar graph compares fold change *SHROOM3* mRNA by RT-PCR in Control-transfected PRCEC to PC-*SHROOM3* transfected cells after 36-hours of transfection (normalized to *HGAPDH;* after DNAse digestion). (5b) Figure displays Western blots of SHROOM3 (Anti-V5 tag antibody) and β-ACTIN from PRCEC lysates 48-hours after transfection [error bars = mean±SEM]
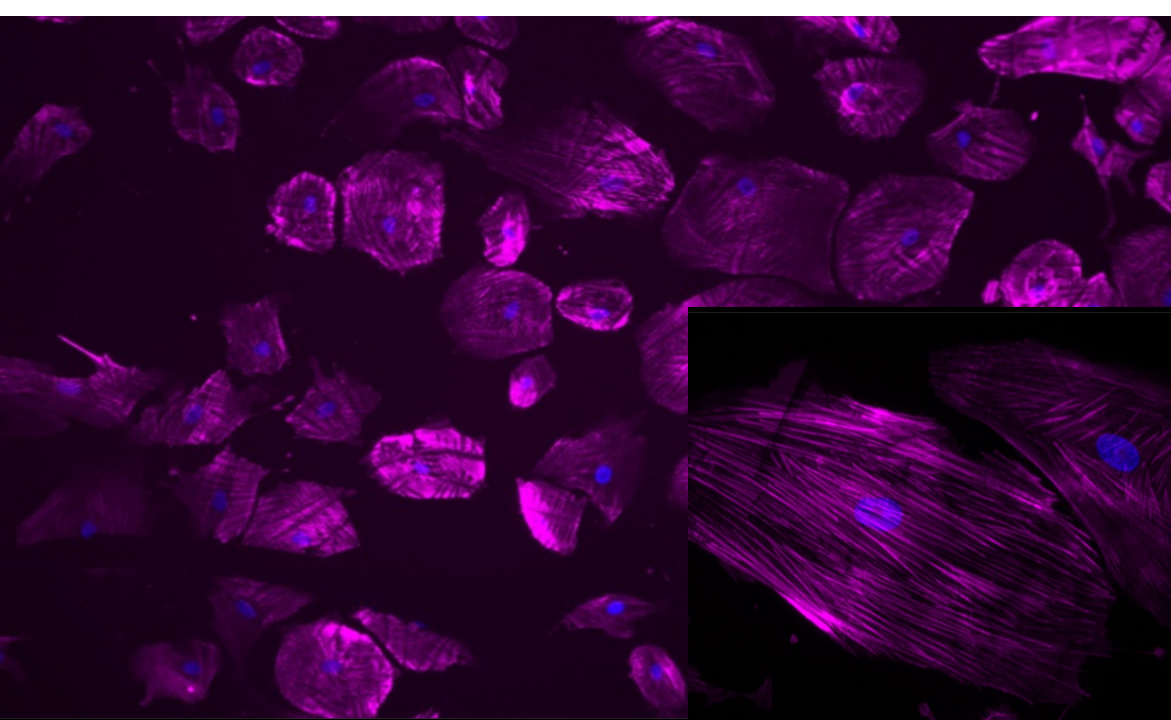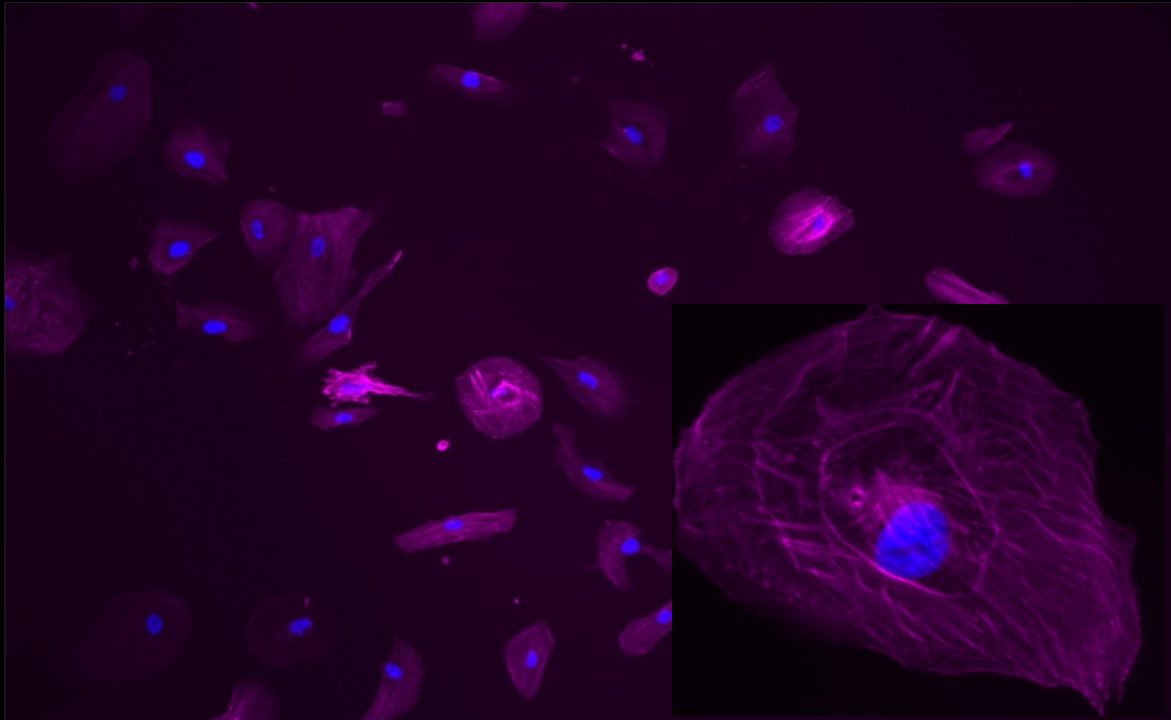
**Suppl Figure 5c:** *SHROOM3* **overexpression causes changes in cytoskeletal organization of PRCEC at 36-hours:** Upper and lower Panels: F-Actin staining in PRCEC: *SHROOM3*-transfected cells (lower panel and inlay) and Control-transfected cells (upper panel and inlay) grown on collagen-coated coverslips for 36-hours, formaldehyde-Triton X fixed, and stained with Phalloidin (Alexa-Fluor 647 Phalloidin, Invitrogen) are depicted (inlay- 40x magnification)

**a**

Transgene 1: *TGM*  Col1a1 — SAdpA (stop) — TRE — CFP — shRNAmir — PGK — Hygro^R

Transgene 2: *ROSAm26-rtTA* — ROSAm26 — rtTA₃

**b**

WT  Control  Test

Rosa-m2RTTA - 350 bp

WT/WT  WT/Col  Col/Col

COLTGM - 295 bp
WT - 220 bp

**c**

Doxycycline feed (200 mg/gm) + water (0.2 mg/ml)
OR
Regular feed (No DOX)

4-6 week male mice — 21 days — Right UUO — 10 days — Sacrifice
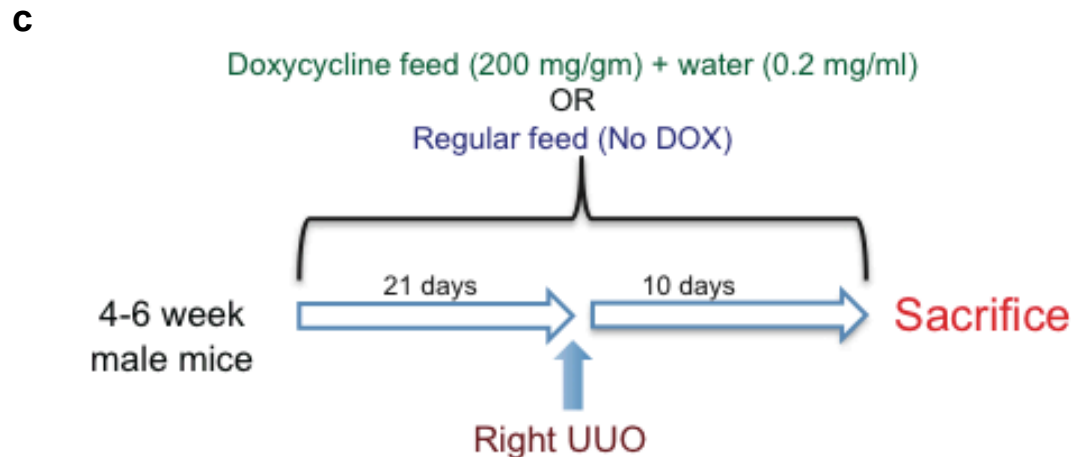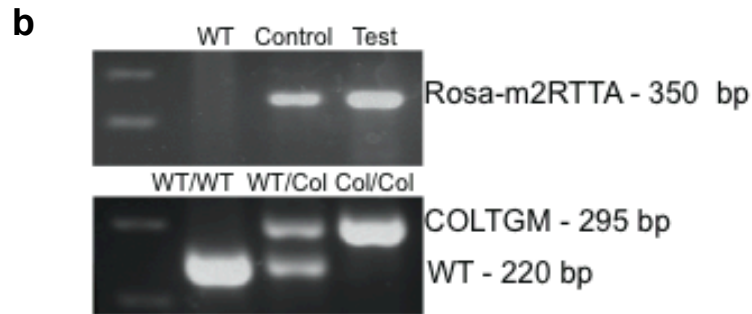
**Suppl Fig 6 a-c**: **Murine model of inducible *Shroom3* knockdown**

**a:** Doxycycline (DOX**)** inducible shRNA-mediated *Shroom3* knockdown mice (C57B6/129B background)  were generated in collaboration with Mirimus, inc. NY.

**b:** Representative genotyping results for ROSA-m26RTTA(upper) and COLTGM (lower) are depicted. Double-transgenic ROSA-rtTA-*Shroom3*-RNAi mice were selected for experiments. In these, shRNAmir-mediated knockdown was driven by the universal promoter, ROSAm26 and inducible by DOX-feeding.

**c:** Male mice were DOX-fed for 3 weeks, and subjected to Unilateral Ureteric obstruction surgery (UUO) at 6-8 weeks age to stimulate kidney fibrosis. Non DOX-fed littermates with UUO were used as controls. After 10-days kidney tissues were collected for histology, immunostaining for fibrosis markers, RNA isolation for RTPCR, protein extraction for WB, and IHC.
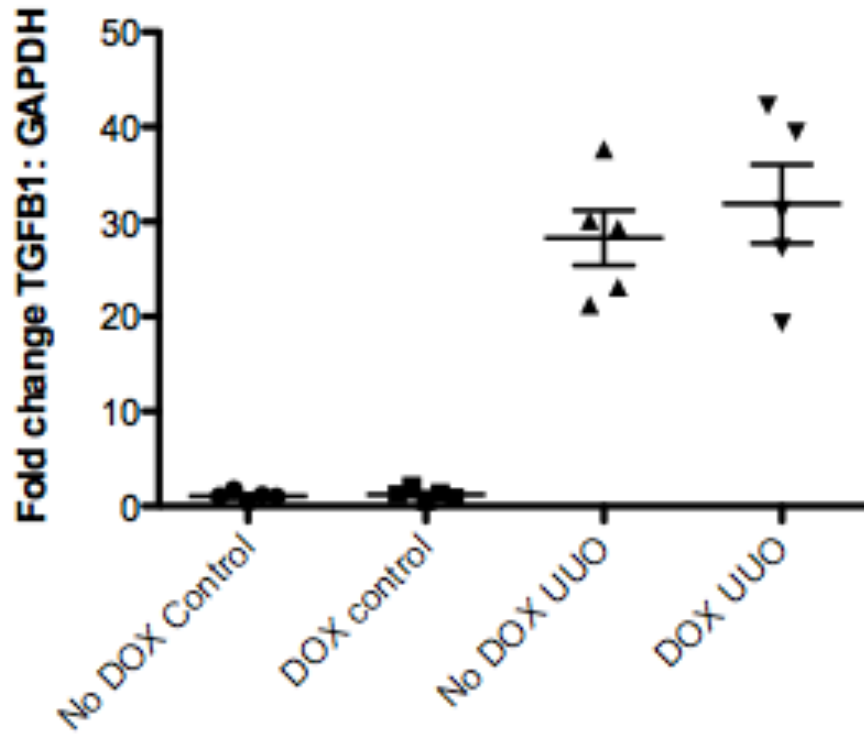
**Suppl Fig 6d:** Graph compares TGFβ1 mRNA levels (expressed as Fold change normalized to GAPDH) between control- and UUO- kidneys of DOX-fed and Non-DOX-fed Rosa-RTTA/Shroom3 RNAi animals (n=5 pairs)[Line/Whiskers represent Mean/SEM in each group]
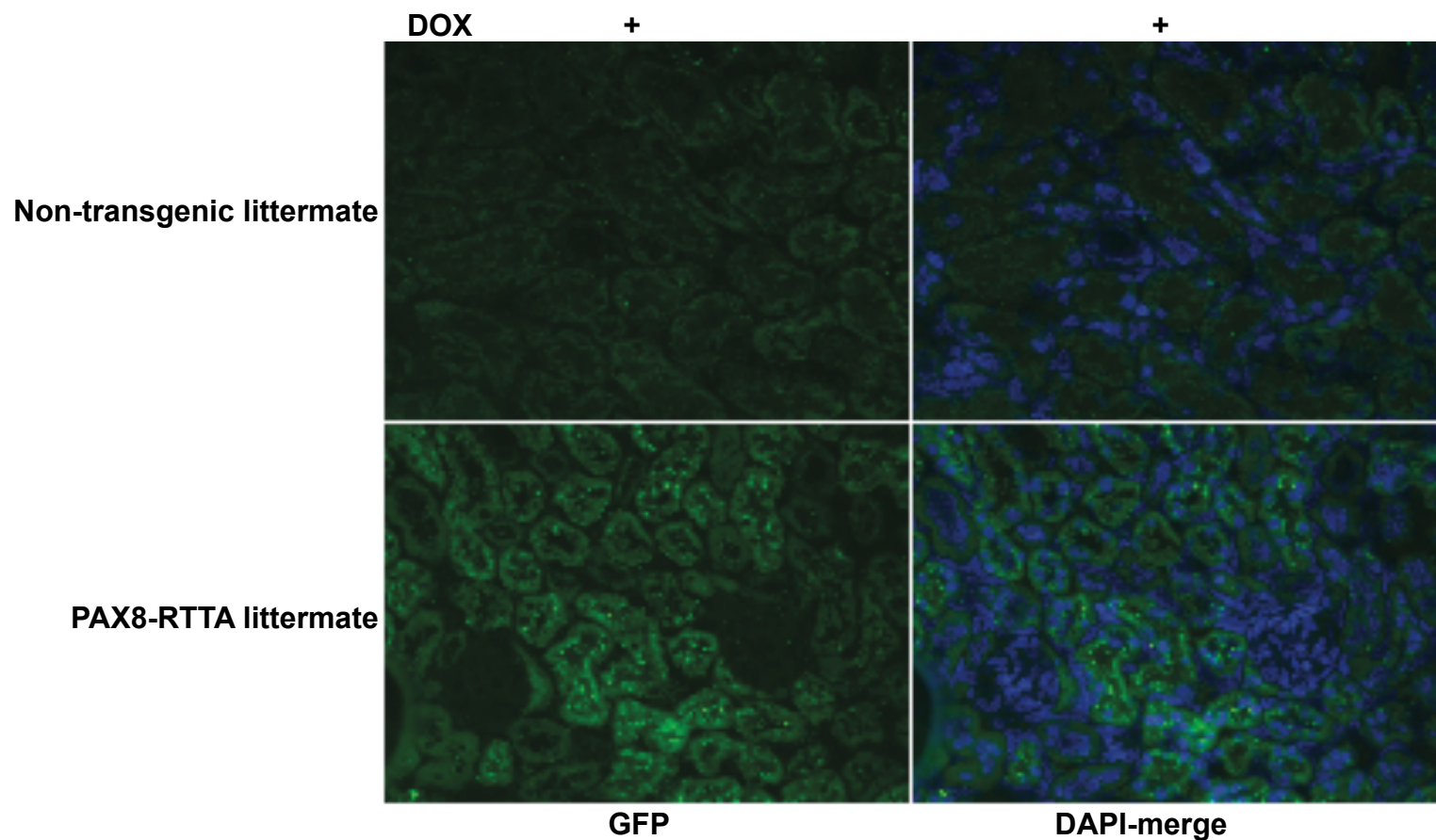
**Suppl Fig 7:** Control kidneys of PAX8-RTTA mice show GFP production exclusively in the tubular cells (sparing the glomerulus) with 3-weeks of DOX feeding in PAX8-RTTA transgenic animals (lower figures), but not in non-transgenic littermates.

Supplementary Table 1: Multivariate analysis with SHROOM3-3M as predictor CADI-12 ≥ 2 as outcome measure.

| Covariates | All Donors | | Caucasian Donors | | Deceased donors | | Caucasian DDs | |
|---|---|---|---|---|---|---|---|---|
| | OR | p-value | OR | p-value | OR | p-value | OR | p-value |
| SHROOM3-3M* | 1.269 | 0.0351 | 1.344 | 0.0235 | 2.040 | 0.0036 | 2.124 | 0.0062 |
| Recipient Age | 1.002 | 0.9127 | 1.005 | 0.8165 | 1.029 | 0.3450 | 1.034 | 0.3496 |
| Recipient Gender | 3.760 | 0.0110 | 4.979 | 0.0127 | 16.834 | 0.0065 | 12.095 | 0.0218 |
| Recipient Race | 0.603 | 0.2874 | 0.449 | 0.2377 | 0.343 | 0.2070 | 0.413 | 0.4444 |
| Acute Rejection | 2.714 | 0.0338 | 3.043 | 0.0494 | 12.407 | 0.0072 | 20.313 | 0.0077 |
| Cold-Ischemia time | NA | NA | NA | NA | 1.048 | 0.5100 | 1.163 | 0.1168 |

*OR for SHROOM3-3M corresponds to a 0.1 unit increase in allograft Log-SHROOM3 expression

Supplementary Table 2: Multivariate analysis with SHROOM3-3M as predictor and eGFR-12 as outcome measure

| Covariate | All Donors | | Caucasian Donors | | Deceased donors | | Caucasian DDs | |
|---|---|---|---|---|---|---|---|---|
| | Estimate | p-value | Estimate | p-value | Estimate | p-value | Estimate | p-value |
| SHROOM3-3M* | -1.59 | 0.0227 | -1.94 | 0.0159 | -2.00 | 0.0531 | -2.30 | 0.0315 |
| Recipient Age | -0.21 | 0.0729 | -0.28 | 0.0398 | -0.38 | 0.0326 | -0.48 | 0.0132 |
| Recipient Gender | 1.58 | 0.6425 | 1.28 | 0.7684 | 0.79 | 0.8806 | 1.76 | 0.7562 |
| Recipient Race | -10.72 | 0.0044 | -9.68 | 0.0666 | -15.69 | 0.0047 | -9.84 | 0.1311 |
| Acute Rejection | -7.62 | 0.0233 | -10.53 | 0.0114 | -9.56 | 0.0786 | -11.82 | 0.0578 |
| Cold-Ischemia | NA | NA | NA | NA | 0.05 | 0.8931 | -0.36 | 0.3884 |

* Estimate for SHROOM3-3M corresponds to a 0.1 unit increase in allograft Log-SHROOM3 expression

Supplementary table 3: Presence of one or two copies of the risk-allele (A) at rs1739721 is associated with significantly greater risk of CADI-12≥2.

| Allografts with CADI_12 scored | Total n | Donor Genotype- A/A and A/G | | Donor Genotype- G/G | | OR-CADI-12≥2 (95% CI) (A/A+A/G Vs G/G) | p-value |
|---|---|---|---|---|---|---|---|
| | | CADI-12<2 n | CADI-12≥2 n | CADI-12<2 n | CADI-12≥2 n | | |
| All Donors | 203 | 63 | 67 | 47 | 26 | 1.98 (1.10-3.59) | 0.028* |
| All LDs[#] | 106 | 34 | 40 | 27 | 7 | 4.54 (1.76-11.72) | 0.001* |
| All DDs** | 97 | 29 | 29 | 20 | 19 | 1.05 (0.47-2.45) | 0.9 |
| Caucasian-Donors | 165 | 53 | 57 | 34 | 21 | 1.74 (0.89-3.37) | 0.09 |
| Caucasian-Donors LDs | 61 | 29 | 32 | 4 | 16 | 5.41 (1.56-18.39) | 0.004* |
| Caucasian-Donor DDs | 94 | 24 | 25 | 18 | 17 | 1.10 (0.46-2.62) | 0.89 |
| Non-Caucasian-Donors | 38 | 10 | 10 | 13 | 5 | 2.6 (0.67-10.07) | 0.16 |
| Non-Caucasian-Donor LDs | 25 | 5 | 6 | 11 | 3 | 4.4 (0.77-25.16) | 0.08 |
| Non-Caucasian-Donor DDS | 13 | 5 | 4 | 2 | 2 | 0.80 (0.07-8.48) | 1.0 |

#LDs- Live donors, **DDs-Deceased donors, *P<0.05 Chi-square

Supplementary Table 4: Prediction of CADI-12≥2 and Delta CADI≥2 with logistic models including clinical predictors with/without SHROOM3-3M

| OUTCOME- CADI-12≥2 | AUC | |
|---|---|---|
| | With Shroom3 | Without Shroom3 |
| **All Donors** | | |
| Covariates: Recipient Age, Recipient Gender, Recipient White, Acute rejection before 3 months | 0.73 | 0.70 |
| **Caucasian Donors** | | |
| Covariates: Recipient Age, Recipient Gender, Recipient White, Acute rejection before 3 months | 0.77 | 0.73 |
| **OUTCOME – CADI-progressors** | | |
| **All Donors** | | |
| Covariates: Recipient Age, Recipient Gender, Recipient White, Acute rejection before 3 months | 0.77 | 0.72 |
| **Caucasian Donors** | | |
| Covariates: Recipient Age, Recipient Gender, Recipient White, Acute rejection before 3 months | 0.81 | 0.74 |

Supplementary Table 5: Genes significantly upregulated and co-expressed in 3-month biopsies of patients in highest quartile of SHROOM3-3M when compared to lowest quartile of SHROOM3-3M.

| Gene Symbol | Probe ID | Gene name | Log2 Ratio | P-value | *Adjusted P-value |
|---|---|---|---|---|---|
| TCF7L2 | 3264621 | T-cell factor 7 like-2 | 0.73 | 1.37E-10 | 1.30E-09 |
| COL4A1 | 3525313 | Collagen-IV | 0.44 | 1.04E-06 | 6.61E-06 |
| CTNNB1 | 2618940 | β-catenin | 0.43 | 0.001 | 0.007 |
| VIM | 3236958 | Vimentin | 0.29 | 0.003 | 0.014 |
| FGF1 | 2879166 | Fibroblast growth factor-2 | 0.39 | 0.008 | 0.026 |
| CTGF | 2974330 | Connective tissue growth factor | 0.22 | 0.014 | 0.039 |

* P-values are adjusted for multiple testing

Supplementary Table 6: Primer sequences used in Luciferase promoter-enchancer cloning protocols and Probe sequences used for EMSA.

| Constructs | Forward (5'—3') | Reverse (5'-3') |
|---|---|---|
| Wild-type (promoter only) | TTATA<u>GGTAC</u>C*CTTGAGACAATAGAGTTGCC* | TTA<u>AGCTT</u>C*CATGCCAAACACATGATCCCTC* |
| A-allele enhancer sequence (24 bp) | TTT<u>GGTACC</u>G**AGTAGCAGGGCAAAAACAA--AAGC***CCTTGAGACAATAGAGTTGCC* | TTA<u>AGCTT</u>C*CATGCCAAACACATGATCCCTC* |
| G-allele enhancer sequence (24 bp) | TTT<u>GGTACC</u>G**AGTAGCAGGGCAAAAACAA--AGGC***CCTTGAGACAATAGAGTTGCC* | TTA<u>AGCTT</u>C*CATGCCAAACACATGATCCCTC* |
| **EMSA Probes** | **Sense (5'—3')** | **Antisense(5'—3')** |
| HSHM3EMSA-**A**-PN2 F & R | GGTAGCAGGGCAAAAACAA**A**GCAGCCATTGATCT | GGAGATCAATGGCTGC**T**TTGTTTTTGCCCTGCTA |
| HSHM3EMSA-**G**-PN2 F & R | GGTAGCAGGGCAAAAACAA**G**GCAGCCATTGATCT | GGAGATCAATGGCTGC**C**TTGTTTTTGCCCTGCTA |

**Legend:** KpnI was introduced in all forward primer and Hind III in the Reverse ones (underlined). Human promoter sequences in initiation and SNPs sequences in bold. SNP-position is in Red.

Highlighted in blue – Exogenous (non-intronic) sequence for P$^{32}$Cytidine labelling

Highlighted in yellow – Consensus binding site for TCF7L2 (A or G in bold lettering)

Supplementary Table 7: Multivariate analysis with SHROOM3-3M as predictor CADI-12 ≥ 2 as outcome measure, adding CTGF-levels as a covariate.

| Covariates | All Donors | | Caucasian Donors | | Deceased donors | | Caucasian DDs | |
|---|---|---|---|---|---|---|---|---|
| | OR | p-value | OR | p-value | OR | p-value | OR | p-value |
| *SHROOM3-3M** | 1.248 | 0.0561 | 1.337 | 0.0327 | 2.072 | 0.0045 | 2.022 | 0.0140 |
| Recipient Age | 1.002 | 0.8894 | 1.005 | 0.8280 | 1.031 | 0.3402 | 1.021 | 0.5829 |
| Recipient Gender (Female) | 3.692 | 0.0126 | 4.854 | 0.0168 | 17.752 | 0.0075 | 9.505 | 0.0410 |
| Recipient Race | 0.562 | 0.2342 | 0.440 | 0.2345 | 0.344 | 0.2084 | 0.464 | 0.5200 |
| Acute Rejection (<3 months) | 2.619 | 0.0411 | 3.035 | 0.0499 | 12.688 | 0.0073 | 20.691 | 0.0073 |
| Cold-Ischemia time (hours) | NA | NA | NA | NA | 1.042 | 0.5825 | 1.216 | 0.0979 |
| CTGF | 1.039 | 0.3555 | 1.008 | 0.8690 | 0.984 | 0.8342 | 1.074 | 0.4487 |

*OR for SHROOM3-3M corresponds to a 0.1 unit increase in allograft Log-*SHROOM3* expression

Supplementary Table 8: Primer sequences used in RT-PCR

| Gene name | Forward primer (5' ➜ 3') | Reverse primer (5'➜ 3') |
|-----------|--------------------------|--------------------------|
| HGAPDH | TGTTGCCATCAATGACCCCTT | CTCCACGACGTACTCAGCG |
| SHROOM3 | CCCTCTCGGGGCGTCTAGCC | GCCCAGCACTACTCGCTCCC |
| COL1A1 | GATGGTGAAGATGGTCCCAC | GGCCAAGTCCAACTCCTTTT |
| TCF7L2 | GCCTCTTATCACGTACAGCAAT | GCCAGGCGATAGTGGGTAAT |