

Supplement S3

Supplementary data on extra-cardiac phenotypes: kidney, liver, spleen, and testis

In addition to heart, we confirmed that shRNA was delivered to other major visceral organs, including the kidney, liver, testis, and spleen (**Figure 1**). Throughout our studies, no significant change in kidney volume was observed (**Movie S13**). While no polycystic kidney phenotype was observed in *F11R* and *PGRMC2* mice (**Figure 2**), mild isolated renal cysts including liver cysts were observed (**Figure 3**). A decrease in vascularization was apparent in the testis from the *PGRMC2* and *F11R* mice, as indicated by the lack of surface vasculature. Testicular vascularization in *F11R* or *PGRMC2* mice was significantly decreased by $15\pm 3\%$, compared to control mice. Neither cysts nor abnormal spermatogenesis was observed in the testes. Fibrosis was detected in the kidney, liver, testis and spleen (**Figure 4**). Significant fibrosis was observed around the red pulps of the spleen in *PGRMC2* knockdown mice. The trabecular structures and periarteriolar lymphoid sheaths of the spleen were comparable between the *F11R* or *PGRMC2* and scrambled control mice.

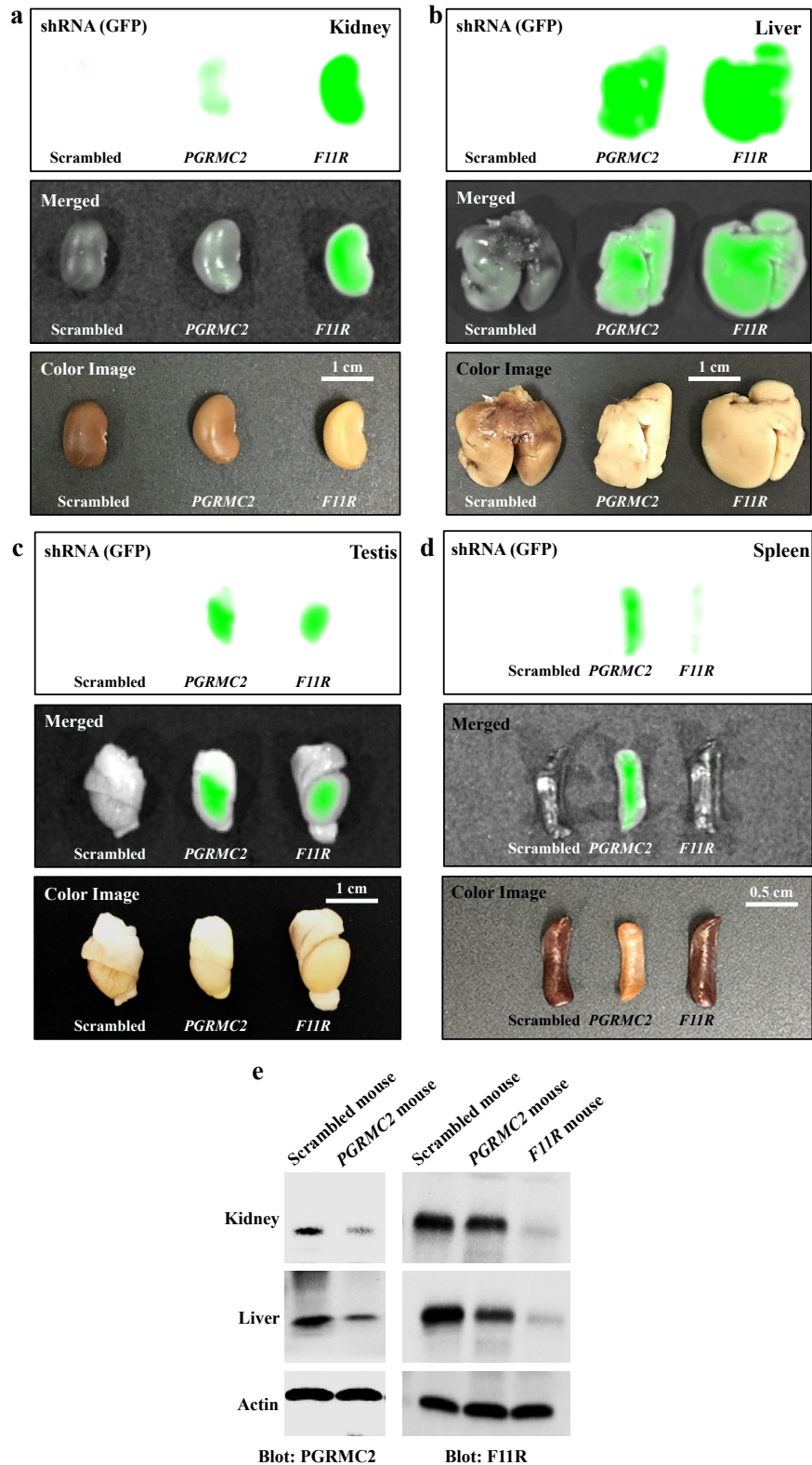


Figure 1. Confirmation of *PGRMC2* and *F11R*-targeted lentivirus carrying GFP marker in mice.

The kidney (a), liver (b), testis (c), and spleen (d) were confirmed for virus infection, as evidenced by the lentivirus-GFP marker, immediately after the mice were sacrificed. (e) Expressions of *PGRMC2* and *F11R* in only kidney and liver organs were analyzed.

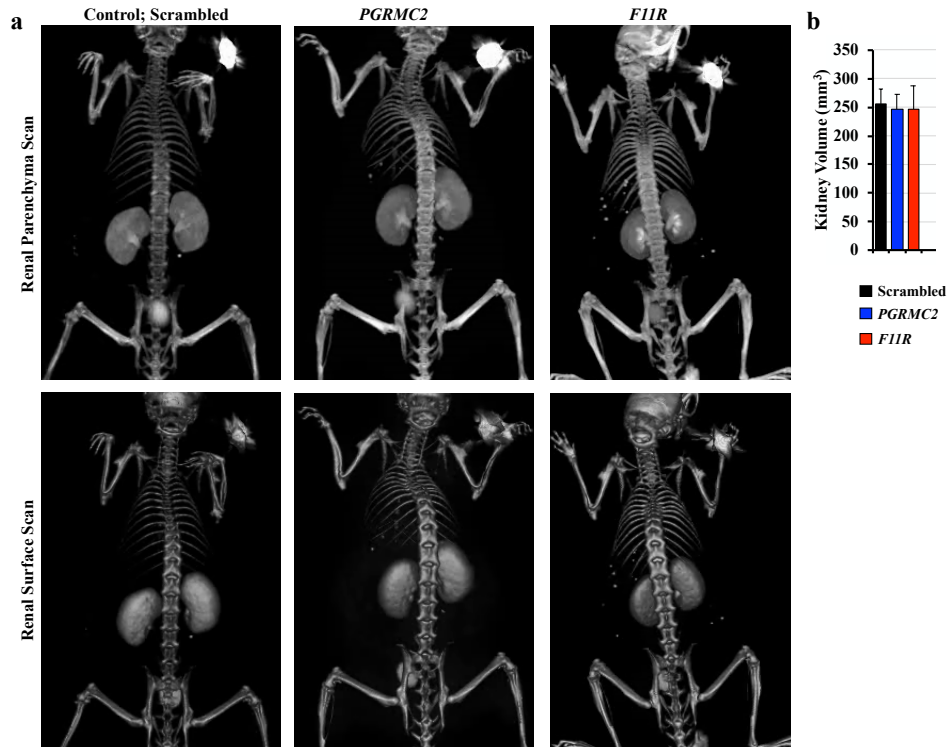


Figure 2. No significant change in kidney volume from *PGRMC2* and *F11R* mice.

a, The kidney-specific contrast agent OptiPrep was intravenously injected into the tail of the mice. Mice were imaged with the IVIS Spectrum CT imaging system. A computed tomography scan of the pair of kidneys was performed in 9-week-old live mice. The renal parenchyma scan (top panel) and renal surface scan (lower panel) were used to evaluate the volumes of the cyst and kidney, respectively (**Movie S13**). **b**, Total kidney volume shows that due to the small kidney cysts, there was no significant difference in renal volume between knockdown and scrambled control mice. N=3 for each group.

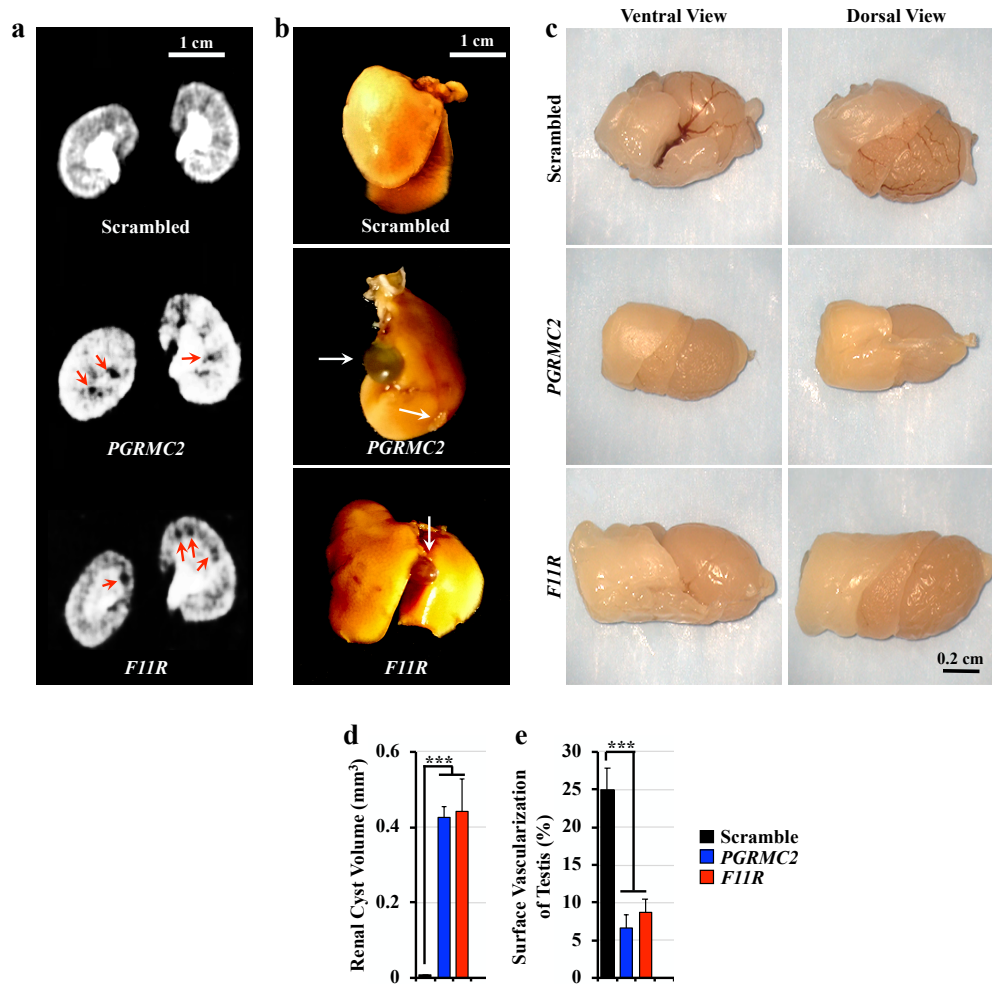


Figure 3. *PGRMC2* and *F11R* knockdown induce kidney cyst, liver cyst, and testicular devascularization.

a, A computed tomography scan was done on a pair of kidneys from a live 8-week-old mouse. Red arrows indicate mild isolated cysts. **b**, Isolated livers were immediately imaged after the mice were sacrificed; white arrows indicate isolated fluid-filled cysts. **c**, Representative images show the low vascular density around the testes in *PGRMC2* and *F11R* knockdown mice compared with scrambled control mice. Kidney cyst volume (**d**) and percentage of surface vascularization area in testis (**e**) were quantitated. N=3 mice in each scrambled control, *PGRMC2* and *F11R* knockdown groups. ***, $p < 0.001$ compared with the corresponding scrambled control group.

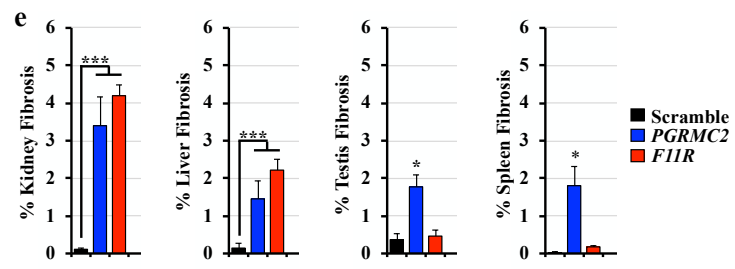
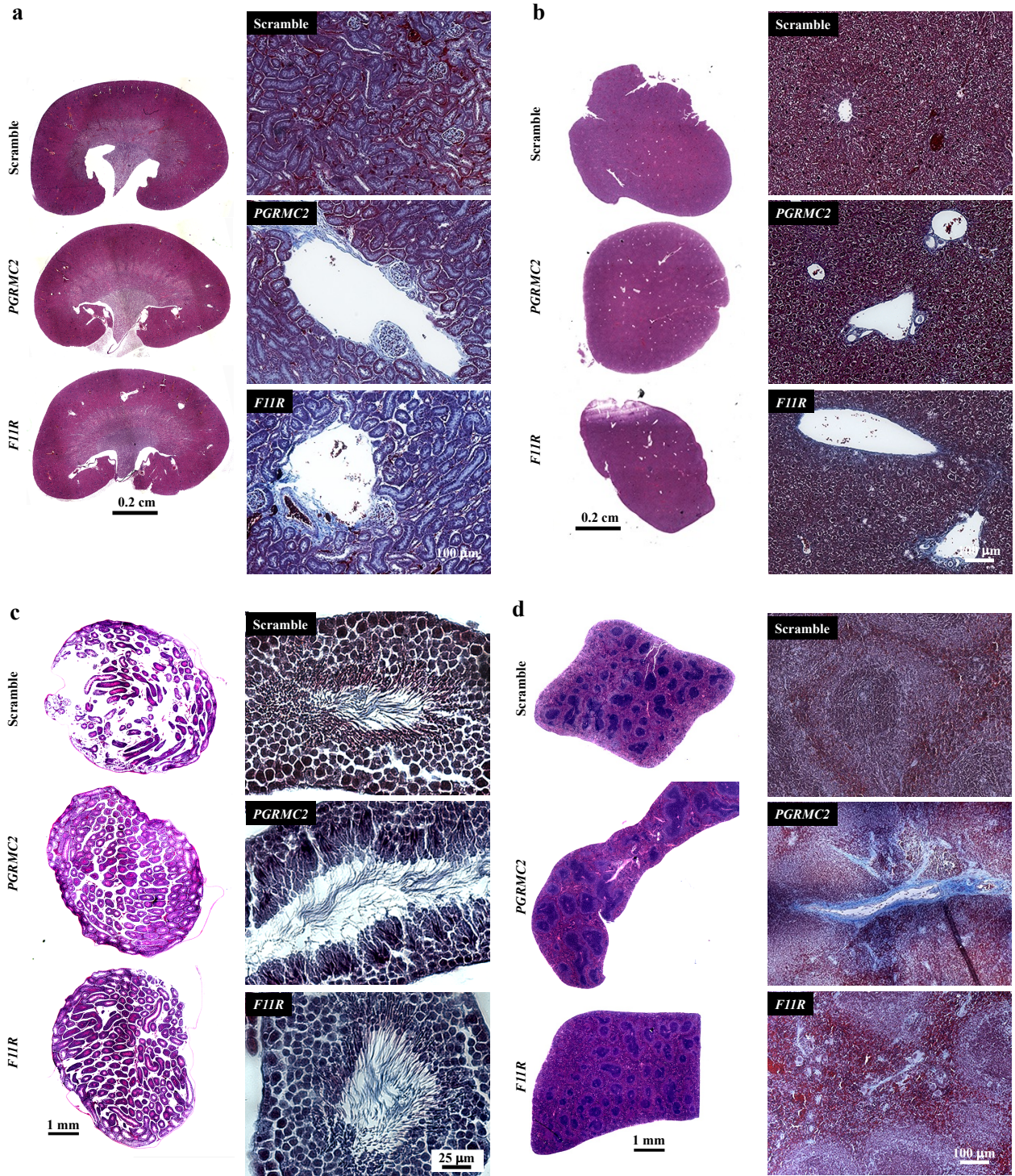


Figure 4. *PGRMC2* and *F11R* knockdown result in kidney, liver, testis and spleen fibrosis.

Hematoxylin and Eosin (H&E; left panels) and Masson's Trichrome (right panels) staining were performed for structural morphology analyses. **a**, Representative H&E staining shows isolated cysts around the cortex region of the kidneys from *PGRMC2* and *F11R* knockdown mice. Masson's Trichrome staining shows severe fibrosis around the cortex region. **b**, Representative H&E staining shows isolated cysts around the livers from the *PGRMC2* and *F11R* knockdown mice. Representative images of Masson's Trichrome staining shows fibrosis around the cyst area. **c**, Representative images show H&E staining of the testes and Masson's Trichrome staining shows fibrosis with potential testicular tubular ectasia with a decreased spermatogenesis in *PGRMC2* knockdown mice. **d**, Representative images of the H&E staining of the spleen and Masson's Trichrome staining show fibrosis in the spleens from *PGRMC2* knockdown mice. **e**, Percent of fibrosis in kidney, liver, testis and spleen were quantified. N=3 for each group. *, p<0.05; ***, p<0.001 compared with the corresponding scrambled control group.