Supplemental data

Reversible tagging of parietal cells with nuclear eGFP

In order to allow fluorescent double stainings, we sought to label specifically PECs with the nuclear fusion protein histone-eGFP (suppl. fig. 1A). For this purpose, PEC-rtTA transgenic mice were crossbred to (tet-O)$_7$-histone-eGFP transgenic mice[1]. Upon administration of dox, the nuclei of PECs were specifically loaded with histone-eGFP, as verified by co-staining with the PEC marker claudin-1 (suppl. fig. 1B, white arrowhead). Transitional cells, located at the vascular stalk[2], were also labeled (suppl. fig. 1B, arrow). Similar to the efficiency of somatic recombination[2], about 66% of the PECs were labeled by this method (suppl. fig. 1B,C, black arrowhead). No cell within the glomerular tuft was aberrantly labeled (suppl. fig. 1C).

CD44 is specifically expressed on PECs

To verify if CD44 is a specific marker for PECs also in a model for FSGS, the Thy1.1 transgenic mouse was crossed to the doubly transgenic PEC-rtTA/(tet-O)$_7$-histone-eGFP mouse (suppl. fig. 1A).

After reversible labeling of PECs with histone-eGFP, we first employed the accelerated Thy1.1 model. The accelerated Thy1.1 model has the advantage that the mice can be analyzed already 7 days after induction, so that the reversible labeling of PECs is still preserved. As depicted on suppl. fig. 1D-D'', PECs with eGFP labeled nuclei expressed CD44 de novo. As shown on suppl. fig. 1E, a labeled PEC has migrated onto the glomerular tuft (arrow) co-expressing CD44 de novo as marker for activation. When comparing the relatively low eGFP fluorescence intensity with

other labeled PECs of the same glomerulus, it is suggestive that this activated PEC has already undergone cellular divisions.

Legends

Suppl. figure 1

Tracking parietal cells with the reversible tag eGFP-histone in the accelerated Thy1.1 mouse model for FSGS

A. Schematic of inducible reversible tagging of PECs in the murine Thy1.1 model. Upon administration of doxycycline (Dox), histone-eGFP is expressed within the nuclei of PECs. **B.** In control transgenic PEC-rtTA/(tet-O)7 histone-eGFP mice (negative for the Thy1.1 transgene), PECs (white arrowhead) as well as transitional cells (arrow) were efficiently labeled with nuclear eGFP-histone. Due to mosaic expression, not all PECs were labeled (black arrowhead). No CD44 expression was detected in these mice (not shown). **C.** No evidence for direct labeling of podocytes was observed when co-staining for the podocyte marker protein synaptopodin. **D, E.** The Thy1.1 transgene was crossed into the experimental mice (panel A). These mice developed collapsing FSGS after injection of the Thy1.1 antiserum within 8 – 14 days. **D.** CD44 positive FSGS lesions contained predominantly eGFP positive cells in injected triple transgenic Thy1.1/PEC-rtTA/(tet-O)7 histone-eGFP mice (white arrowheads). Significant differences in eGFP labeling intensities suggest, that a subpopulation of the PECs have undergone cellular divisions (black arrowhead), while others have not (arrow). **E.** A CD44 positive cells within the glomerular tuft originated from PECs, as shown by nuclear labeling with eGFP (arrow).

References

1. Tumbar, T, G Guasch, V Greco, C Blanpain, WE Lowry, M Rendl, and E Fuchs: Defining the epithelial stem cell niche in skin. *Science* 303: 359-63, 2004.

2. Appel, D, DB Kershaw, B Smeets, G Yuan, A Fuss, B Frye, M Elger, W Kriz, J Floege, and MJ Moeller: Recruitment of podocytes from glomerular parietal epithelial cells. *J Am Soc Nephrol* 20: 333-43, 2009.

Suppl. figure 1