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Three-dimensional reconstruction of regular and damaged podocytes by FIB-SEM

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Nail-patella syndrome (NPS) is a rare autosomal-dominant hereditary disorder with an incidence of ~1:50,000. The prognosis of NPS patients is determined by chronic kidney failure. In 1998 the first mutations in *LMX1B*, a gene encoding the LIM-homeodomain transcription factor 1 beta, were reported to be responsible for NPS [1-3]. Ultrastructural analyses of affected kidneys revealed pronounced alterations of the glomerular filtration barrier. This barrier consists of endothelial cells, the glomerular basement membrane and podocytes. Podocytes form a tight meshwork of primary and secondary processes on the urinary side of the glomerular filtration barrier. Beside a thickened glomerular basement membrane NPS patients regularly show an effacement of foot processes accompanied by the loss of slit diaphragm [4,5]. This prompted us to develop and characterize inducible podocyte-specific *Lmx1b* knock-out mice in order to analyze the role of LMX1B in maintaining the structural differentiation status of podocytes.

3D reconstructions of the glomerular filtration barrier were performed on induced and noninduced 3 month old *Lmx1b* knock-out mice. Mice were fixed by perfusion through the distal abdominal aorta with 4% formaldehyde, 1x PBS. Subsequently, kidney slices were fixed overnight in 2% glutaraldehyde, 1x PBS. The specimens were incubated with cacodylate-buffered 1% OsO_4 for 2 hours at room temperature and then embedded in Epon. The specimens were initially screened by conventional TEM (Zeiss 902), before they were analyzed by focused ion beam scanning electron microscopy (FIB-SEM). Data were collected using a Zeiss Auriga FIB-SEM. Subsequent digital analysis, 3D reconstruction and manual segmentation was performed with the software package Amira ®.

Our data allow a comprehensive view onto ultrastructural changes at the glomerular filtration barrier in kidneys of *Lmx1b* knock-out mice. The 3D reconstruction emphasizes the essential role of Lmx1b for the maintenance of appropriately structured podocytes. Already one week after the inactivation of *Lmx1b* in adult mice, the highly ordered structure of podocytes is markedly disturbed. The foot processes disappear thus leading to increased leakage of the glomerular filter and the loss of protein into the urine. Further 3D analyses will resolve more details of the podocyte processes at higher resolution using TEM tomography.

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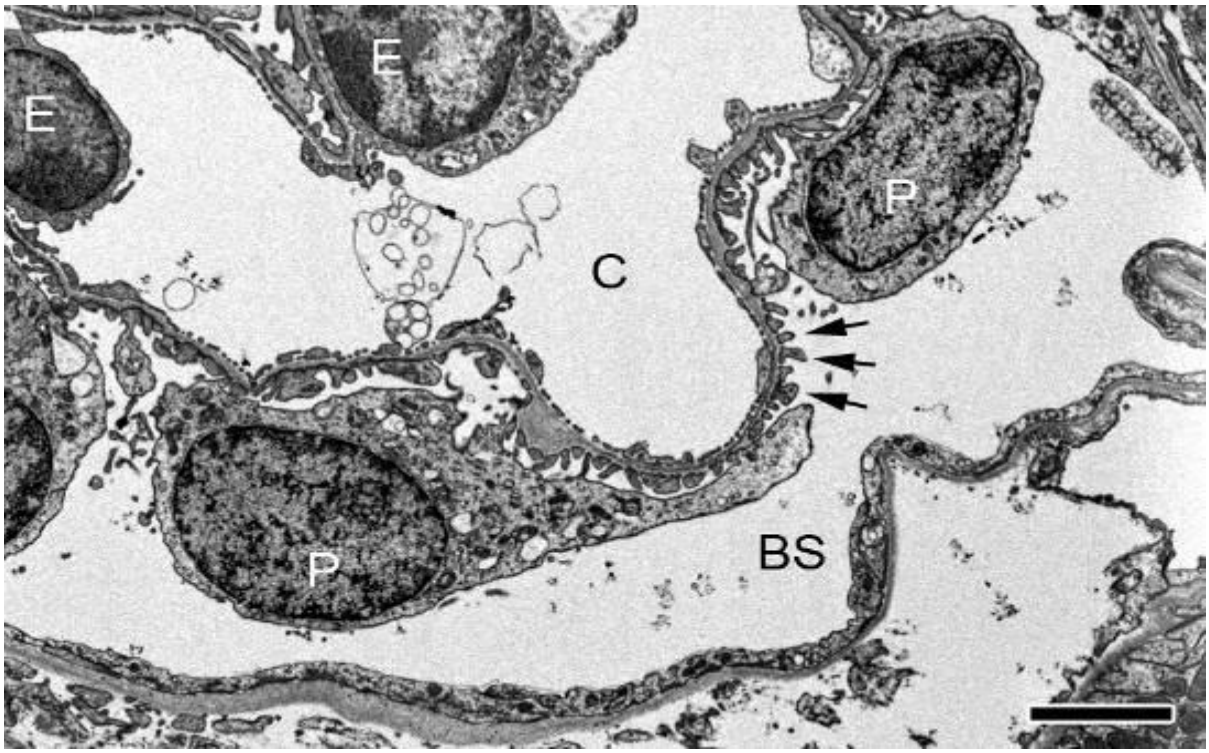

Figure 1. Kidney section from a noninduced *Lmx1b* knock-out mouse analyzed by FIB-SEM. The glomerular filtration barrier is properly formed between the capillary space (C) and the Bowman space (BS). The filter is composed of endothelial cells (E), the glomerular basement membrane, and podocytes (P) branching into characteristic foot processes (arrows). Bar, 2 µm

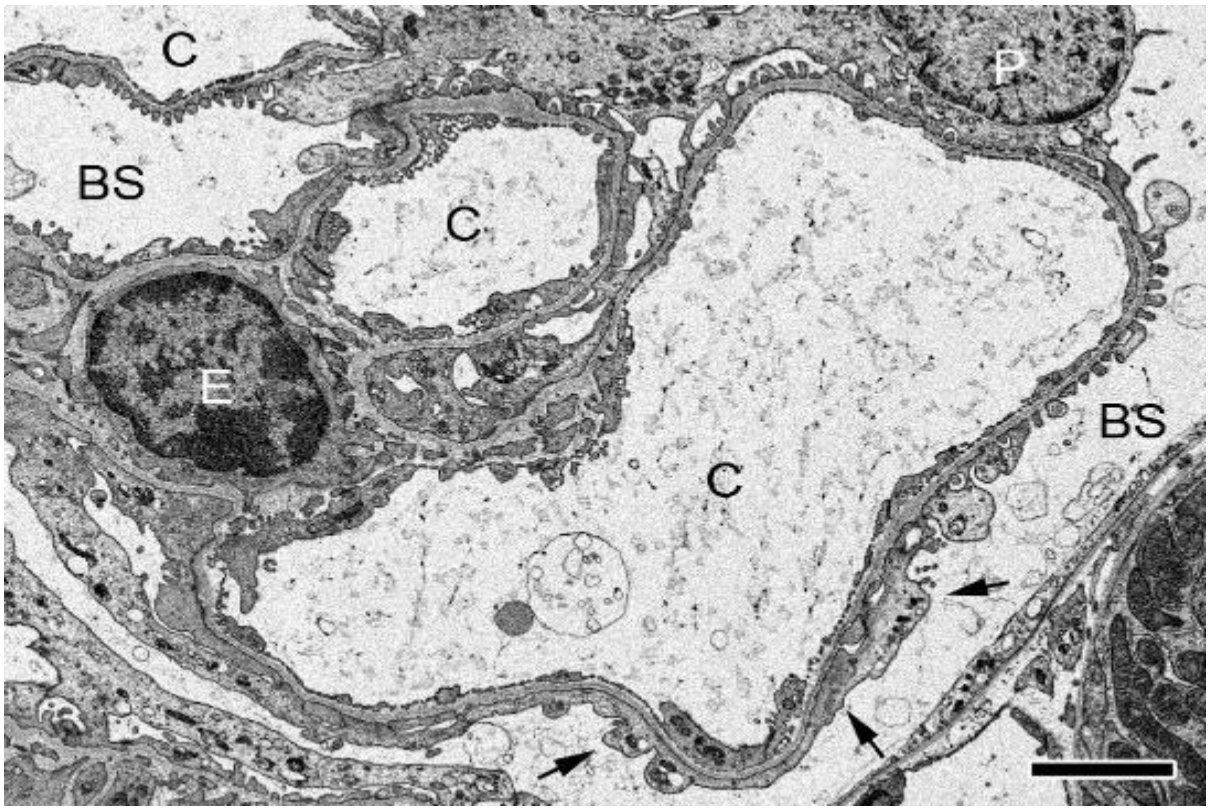

Figure 2. Kidney section from an induced *Lmx1b* knock-out mouse analyzed by FIB-SEM. The organization of the glomerular filter is disturbed; effacement of podocyte foot processes is evident (arrows). Bar, 2 µm