SUPPLEMENTARY INFORMATION

Podocin-GFP zebrafish allows *in vivo* visualization and functional analysis of glomerular podocytes

Bing He et al.

FULL METHODS

Zebrafish husbandry

The zebrafish wild-type AB and TL strains were maintained, mated and raised as described (8) at the Karolinska institutet zebrafish core facility. Embryos were kept in E3 water with or without 0.003% of PTU (1-Phenyl-2-thiourea) to inhibit pigmentation.

Generation transgenic zebrafish

Transgenic zebrafish were generated using the *Tol2* transposon system (4). The zebrafish *podocin* promoter sequence was amplified from AB strain genomic DNA. The 2.5-kb amplicon was first cloned into the pCRII-TOPO vector (Invitrogen) followed by sequencing. The cloned fragment was then transferred into the pT2KXIG plasmid (A gift from K. Kawakami, National Institute of Genetics, Shizuoka, Japan) (**Figure 1A**). The injected G_0 embryos were raised and outcrossed with TL zebrafish to identify germline founders. All experiments were approved by the Stockholm North Ethical Committee for animal research.

Embryo microinjection

The pT2KXIG-based construct, together with the *Tol2* transposase mRNA, were co-injected into the yolk of zebrafish embryos at the late one-cell or early two-cell stage as

described (3). We analyzed GFP expression in embryos at ages between 1-6 dpf using a stereomicroscope equipped for epifluorescence.

Scanning electron microscopy

Tg(podocin:GFP) embryos at 6 dpf were briefly fixed by immersion in 2,5 % glutaraldehyde in 0.1M PBS (pH 7.4). The glomerulus, localized by GFP fluorescence under fluorescence microscopy, was exposed by dissection followed by further fixation at 4°C. The specimens were briefly rinsed in distilled water and placed in 70% ethanol for 10 min, 95% ethanol for 10 min, absolute ethanol for 15 min at room temperature and in pure acetone for 10 min and then were transferred to Tetramethylsilane (Merck, Germany) for 10 min and air-dried (1). After drying, the specimens were mounted on an aluminum stub and coated with Carbon (Balzer, SCD 005, Liechtenstein). The specimens were analyzed in an Ultra 55 (Carl Zeiss, Oberkochen, Germany) field emission SEM at 3 kV.

In situ hybridization, histological analysis and crb2b knockdown

Whole-mount in situ hybridization was performed as described (7). Histological analysis was carried out as described (5). Knockdown of Crb2b using translation-blocking morpholinos was done as previously described (2).

qPCR

The qPCR was performed on cDNA using SYBR Green PCR Master Mix (Applied Biosystems) on the ABI PRISM 7300 System. The cDNA was reverse-transcribed from total RNA extracted from single 4-dpf whole embryos. The relative expression quantification was analyzed using the comparative threshold (Ct) method (6).

Primer sequences

All primers used in the present study are listed in Table S1.

Table S1 . Zebrafish primer sequences used

Gene	Primer sequence
Podocin 5′	5′-GGTGATTCTATGCTCTTTGCGCTTTGT
	5′-TTTCTCTATCTCCGCAGGAAGCATCGT
GFP probe	5′-GACGTAAACGGCCACAAGTT
	5′-TTCTCGTTGGGGTCTTTGCT
Podocin probe	5′-GTCTGGAATGCTAGCGAAGG
	5′-GTCTGGAATGCTAGCGAAGG
β-actin qPCR	5′-CGAGCAGGAGATGGGAACC
	5′-CAACGGAAACGCTCATTGC
GFP qPCR	5′-ACCACTACCTGAGCACCCAGTC
	5′-GTCCATGCCGAGAGTGATCC
Podocin qPCR	5′-CGAGAGATACTGGCCCATCA
	5′-CCACTTTAATACCCCACCTG

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