

Supplementary information

Impaired NEPHRIN localization in kidney organoids derived from nephrotic patient iPS cells

Tomoko Ohmori¹, Shankhajit De¹, Shunsuke Tanigawa¹, Koichiro Miike¹, Mazharul Islam¹, Minami Soga², Takumi Era², Shinichi Shiona³, Koichi Nakanishi^{4,5}, Hitoshi Nakazato⁶, and Ryuichi Nishinakamura^{1,*}

¹Department of Kidney Development, Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto 860-0811, Japan

²Department of Cell Modulation, Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto 860-0811, Japan

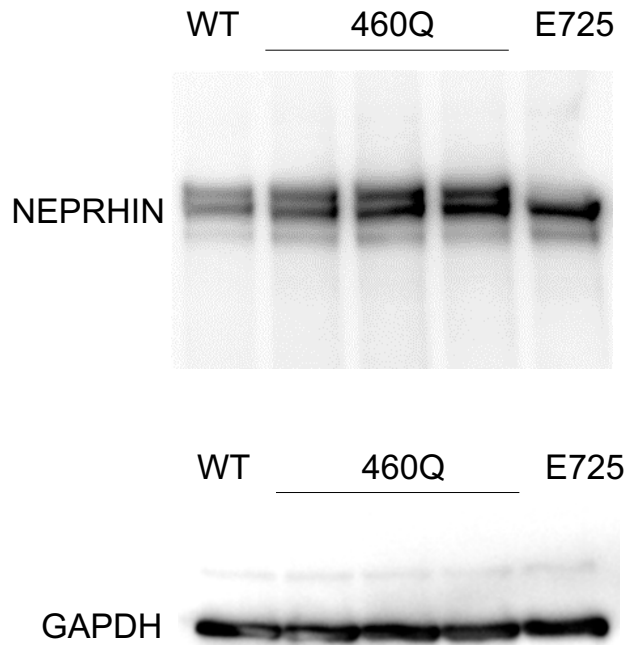
³Department of Pediatrics, Oita Prefectural Hospital, Oita 870-0855, Japan

⁴Department of Child Health and Welfare (Pediatrics), Graduate School of Medicine, University of the Ryukyus, Okinawa 903-0215, Japan

⁵Genetic Consulting Unit, University of the Ryukyus Hospital, Okinawa 903-0215, Japan

⁶Department of Pediatrics, Faculty of Life Sciences, Kumamoto University, Kumamoto 860-8556, Japan

Supplementary Figure S1

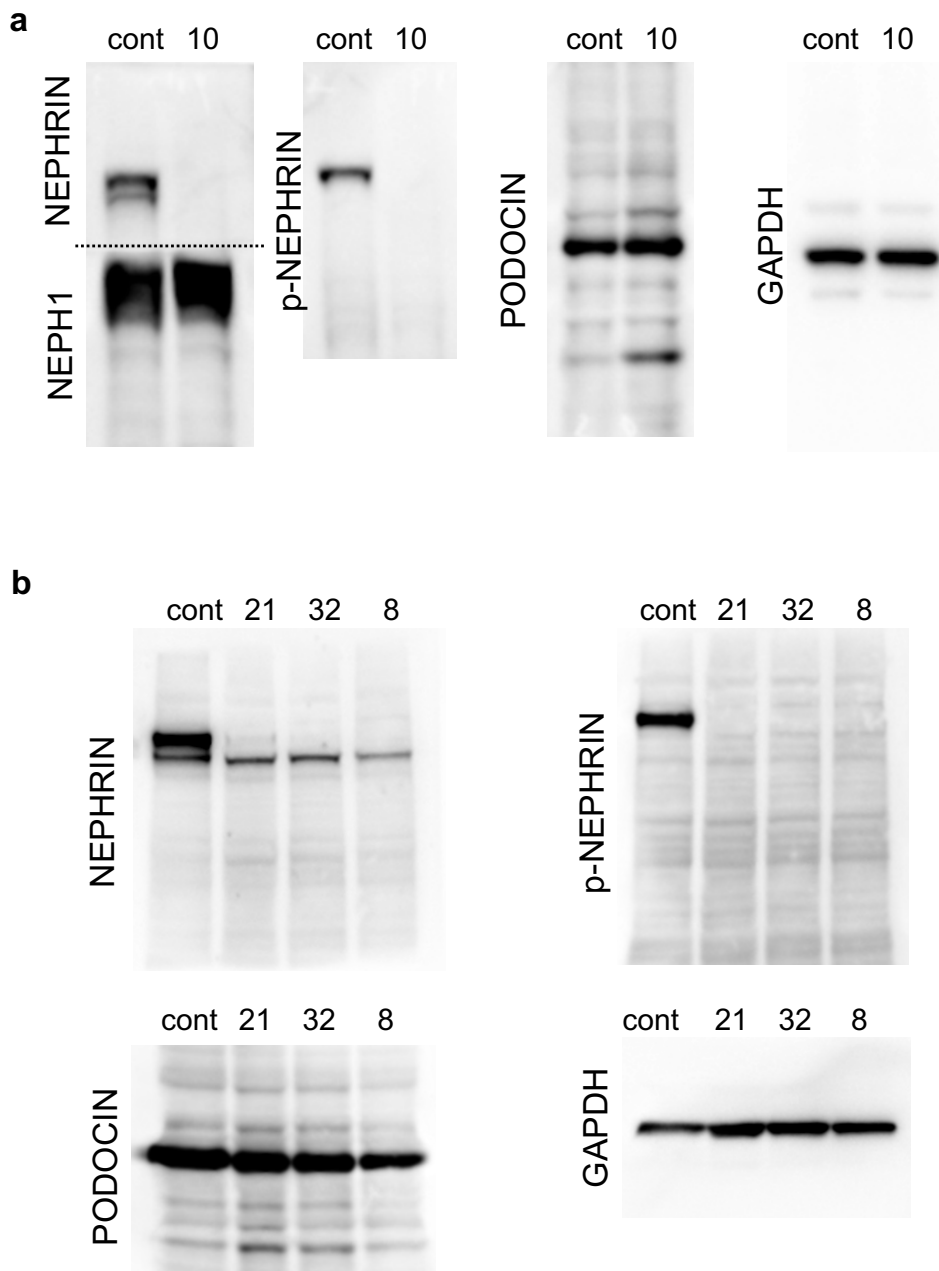


Supplementary Figure S1

The full-length western blots used for Figure 2

Western blotting analysis of HEK293 cells overexpressing wild-type (WT), R460Q mutant (Patient 3), and E725D mutant (Patient 0) NEPHRIN. GAPDH is used as a loading control.

Supplementary Figure S2

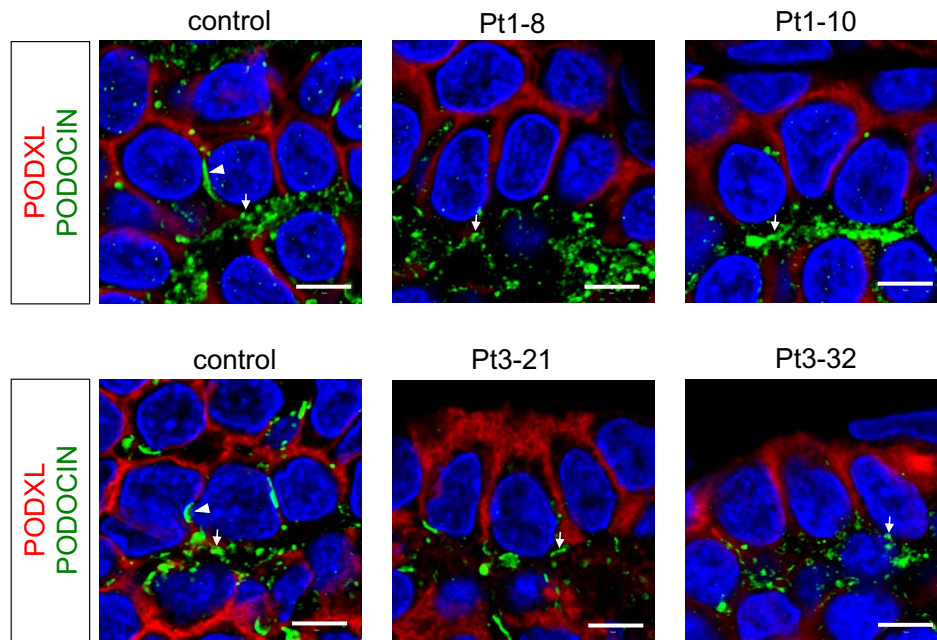


Supplementary Figure S2

The full-length western blots used for Figure 6

Western blot analyses of NEPHRIN, phosphorylated NEPHRIN (p-NEPHRIN), and PODOCIN in kidney organoids derived from Patient 1 (**a**) and 3 (**b**). The clone numbers are indicated at the top. GAPDH is used as a loading control. cont: control clones.

Supplementary Figure S3



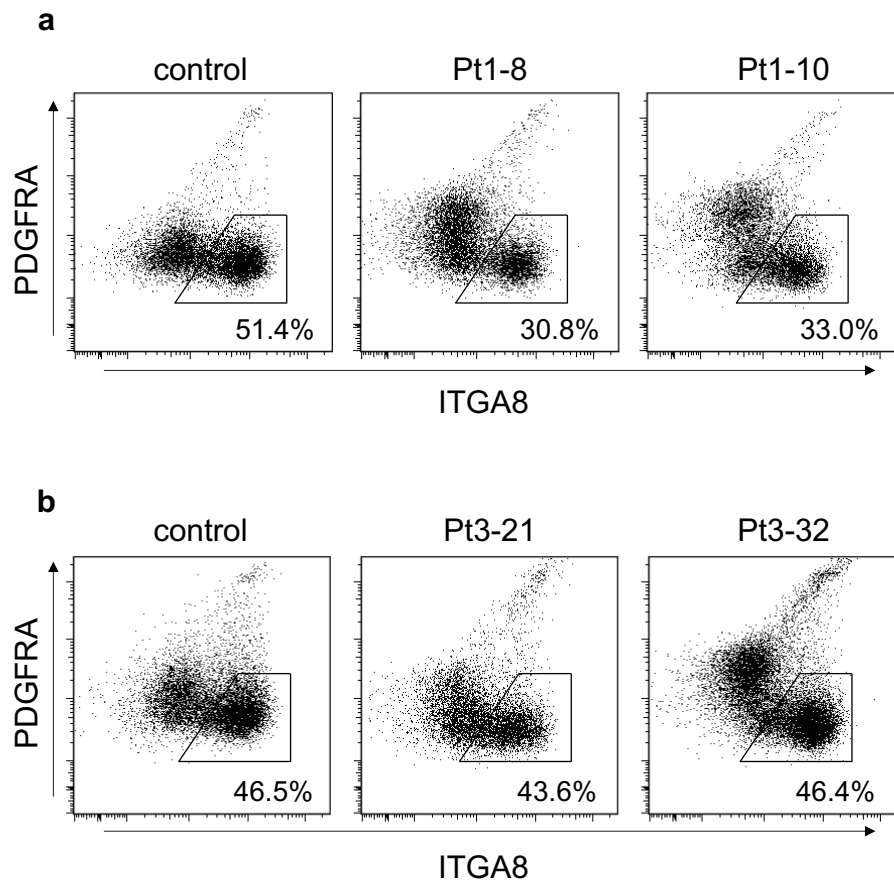
Supplementary Figure S3

Localization of PODOCIN is impaired in patient-derived podocytes

Section immunostaining of kidney organoids was performed with anti-PODOCIN and anti-PODXL antibodies (upper row: Patient 1; lower row: Patient 3). PODOCIN is undetectable in the lateral domains of the podocytes derived from both patients.

Arrowheads: lateral SD precursors; arrows: basal PODOCIN+ domains. Scale bars: 5 μ m.

Supplementary Figure S4



Supplementary Figure S4

Induction of nephron progenitors from iPSCs

Induction was performed for the control clone (201B7), two Patient 1-derived clones (Pt1-8, Pt1-10), and two Patient 3-derived clones (Pt3-21, Pt3-32), and the formation of ITGA8⁺/PDGFRA⁻ nephron progenitors was analyzed by flow cytometry on day 13. Representative data are shown.