Construction of a viral T2A-peptide based knock-in mouse model for enhanced Cre recombinase activity and fluorescent labeling of podocytes

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Podocyte injury is a key event in glomerular disease leading to proteinuria and opening the path toward glomerular scarring. As a consequence, glomerular research strives to discover molecular mechanisms and signaling pathways affecting podocyte health. The hNphs2.Cre mouse model has been a valuable tool to manipulate podocyte-specific genes and to label podocytes for lineage tracing and purification. Here we designed a novel podocyte-specific tricistronic Cre mouse model combining codon improved Cre expression and fluorescent cell labeling with mTomato under the control of the endogenous *Nphs2* promoter using viral T2A-peptides. Independent expression of endogenous podocin, codon improved Cre, and mTomato was confirmed by immunofluorescence, fluorescent activated cell sorting and protein analyses. Nphs2$^{pod.T2A.ciCre.T2A.mTomato/wild\text{-}type}$ mice developed normally and did not show any signs of glomerular disease or off-target effects under basal conditions and in states of disease. Nphs2$^{pod.T2A.ciCre.T2A.mTomato/wild\text{-}type}$-mediated gene recombination was superior to conventional hNphs2.Cre mice–mediated gene recombination. Last, we compared Cre efficiency in a disease model by mating Nphs2$^{pod.T2A.ciCre.T2A.mTomato/wild\text{-}type}$ and hNphs2.Cre mice to Phb2$^{fl/fl}$ mice. The podocyte-specific Phb2 knockout by Nphs2$^{pod.T2A.ciCre.T2A.mTomato/wild\text{-}type}$ mice resulted in an aggravated glomerular injury as compared to a podocyte-specific *Phb2* gene deletion triggered by hNphs2.Cre. Thus, we generated the first tricistronic podocyte mouse model combining enhanced Cre recombinase efficiency and fluorescent labeling in podocytes without the need for additional matings with conventional reporter mouse lines.

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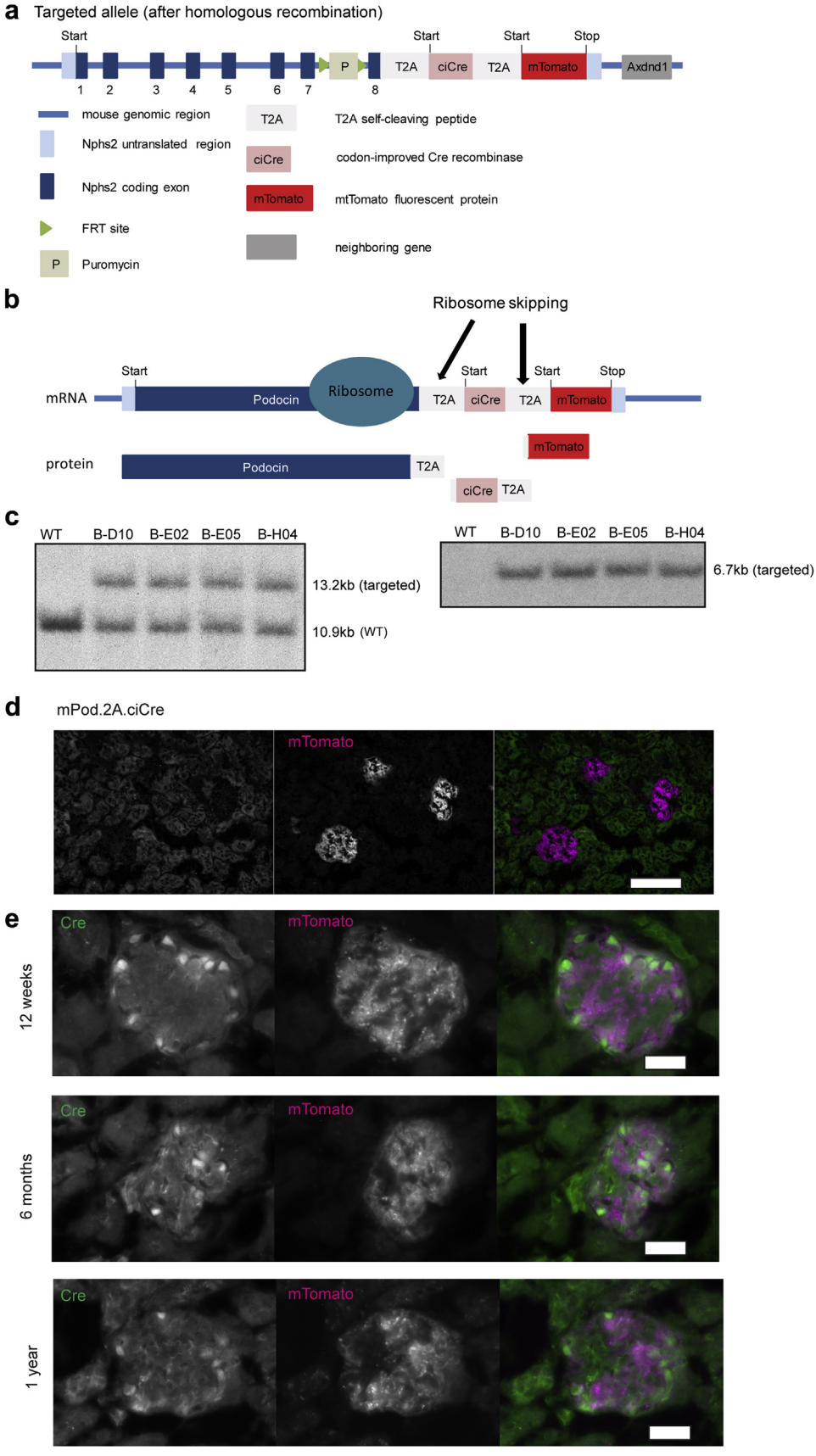
The kidney filtration barrier consists of fenestrated endothelial cells, the glomerular basement membrane, and highly specialized epithelial cells, the podocytes.[1] Podocyte injury and the development of albuminuria are hallmarks of glomerular disease and are considered to be the common fate-determining path.[2–4] The hNphs2.Cre (hPod.Cre) mouse has been the backbone of podocyte research despite certain limitations.[5] Due to the random integration of the hPod.Cre transgene and the prokaryotic nucleotide sequence, Cre expression is limited and might be affected by gene silencing.

A yet unmet challenge in podocyte research lies in the lack of *in vitro* models that mimic the podocyte's *in vivo* environment. Isolated primary podocytes are considered to be the best approximation. However, culturing primary podocytes is technically demanding. For efficient purification of primary podocytes, additional breedings with a reporter line are required. These are time-consuming and cost intensive. We set out to generate a podocyte-specific Cre recombinase mouse line that allows for improved Cre expression and simultaneous fluorescent labeling in podocytes. Here, we describe a tricistronic mouse model combining expression of a codon-improved Cre recombinase[6] with expression of a membrane-targeted tandem dimer tdTomato (mTomato) under the control of the endogenous *Nphs2* promoter.[7]

The widely used Cre recombinase originates from the bacteriophage P1.[6] Shimshek *et al.*[6] introduced a codon-improved nucleotide sequence (ciCre) adapted to eukaryotic species by minimizing the CpG (5′—C—phosphate—G—3′) content to reduce the chances of epigenetic gene silencing and altering the stop codon leading to increased Cre expression. To link ciCre expression to a fluorescent reporter and to ascertain exclusive expression in podocytes, we used the


a
Targeted allele (after homologous recombination)
Start
Start
Start
Stop
P
T2A
ciCre
T2A
mTomato
Axdnd1
1 2 3 4 5 6 7 8
mouse genomic region
Nphs2 untranslated region
Nphs2 coding exon
FRT site
P Puromycin
T2A
T2A self-cleaving peptide
ciCre
codon-improved Cre recombinase
mTomato
mtTomato fluorescent protein
neighboring gene
b
Ribosome skipping
Start
Start
Start
Stop
mRNA
Podocin
Ribosome
T2A
ciCre
T2A
mTomato
mTomato
protein
Podocin
T2A
ciCre T2A
c
WT
B-D10
B-E02
B-E05
B-H04
13.2kb (targeted)
10.9kb (WT)
WT
B-D10
B-E02
B-E05
B-H04
6.7kb (targeted)
d
mPod.2A.ciCre
mTomato
e
12 weeks
Cre
mTomato
6 months
Cre
mTomato
1 year
Cre
mTomato