

Single genetic trigger model of CKD-associated cardiac remodeling in podocyte-specific *MafB* knockout mice

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Abstract

Chronic kidney disease (CKD) causes systemic complications that contribute to renal-cardiac disease (RCD), but experimental models that reproducibly link defined renal injury to cardiac remodeling and CKD-associated complications remain limited. Here, we examined whether podocyte-specific *MafB* knockout mice, in which glomerular injury is genetically induced, can serve as a single-gene-driven model for studying renal-cardiac interactions. Mice (*Mus musculus*) with a C57BL/6 background were used in this study. *MafB*-deficient mice developed progressive glomerulosclerosis and tubulointerstitial injury, followed by cardiac hypertrophy and fibrosis. These cardiac changes were accompanied by CKD-associated systemic abnormalities, including hypertension, renal anemia, and elevated serum fibroblast growth factor 23 levels. Echocardiographic and hemodynamic analyses supported the development of cardiac remodeling during CKD progression. Pharmacological intervention with angiotensin receptor–neprilysin inhibition partially improved disease-related parameters, including blood pressure, cardiac hypertrophy, renal function, and the urinary albumin levels. These findings suggest that podocyte-specific *MafB* knockout mice provide a genetically defined experimental setting in which CKD, cardiac remodeling, hypertension, renal anemia, and mineral metabolism abnormalities develop in sequence. This model may complement conventional multi-hit RCD models and may be useful for investigating kidney–heart interactions and evaluating therapeutic strategies for cardiovascular complications secondary to renal injury.

Keywords Chronic kidney disease · Renal-cardiac disease · Animal study

Introduction

Glomerular and tubular injuries contribute to chronic kidney disease (CKD), but podocyte dysfunction is an important initiating event. Clinically, glomerular injury presents as albuminuria, and leaked proteins damage tubular epithelial cells, causing secondary tubular atrophy and interstitial fibrosis. CKD progression is accompanied by systemic complications, including hypertension, renal anemia, and mineral metabolism abnormalities such as elevated fibroblast growth factor 23 (FGF23) levels. These systemic abnormalities may contribute to

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cardiac hypertrophy, cardiac fibrosis, and other forms of cardiac remodeling. Cardiac remodeling contributes to renal-cardiac disease (RCD), and nearly half of patients with advanced CKD die from cardiovascular events, making the mechanisms underlying RCD a major clinical and experimental challenge^{1–4}.

Animal models are essential for understanding kidney–heart interactions. In conventional RCD models, renal injury alone often fails to induce sufficient cardiac remodeling, so multiple factors are frequently combined, such as surgical or chemical induction of renal injury together with high-fat diet, a high-phosphate diet, diabetes, or hypertension-inducing interventions^{5–10}. These multi-hit models make it difficult to dissect the individual contributions of each intervention to renal lesions, cardiac lesions, hypertension, anemia, and mineral metabolism abnormalities. In addition, strain-dependent variability underscores the need for models in which renal pathology, CKD-associated systemic complications, and cardiac pathology develop in a defined and interpretable manner¹¹.

To address these limitations, a simple, reproducible, genetically defined “single-factor RCD model” is needed. Such a model should meet three criteria: (1) CKD onset is genetically driven, not induced by surgery or nephrotoxic agents; (2) disease arises from a single-gene mutation, minimizing direct effects on other organs; and (3) glomerular injury is reproducibly induced. Additionally, such a model should allow evaluation of RCD-associated systemic abnormalities, including hypertension, renal anemia, mineral metabolism abnormalities, and cardiac remodeling during CKD progression. Although some existing models partially meet these criteria, models that stably recapitulate the sequence from renal injury to systemic complications and cardiac lesions remain limited.

The *Col4a3* global knockout mouse has been used as one of the “single-cause RCD models” that develop renal injury due to a single-gene defect. The progression rate and reproducibility of renal and cardiac lesions vary greatly depending on the genetic background. In 129Sv mice, renal function is maintained until approximately 4 weeks of age, after which it rapidly declines, accompanied by proteinuria and elevated FGF23 levels. Because end-stage renal failure progresses rapidly, most mice die around 15 weeks of age, making stable observation of cardiac lesions difficult¹². By contrast, C57BL/6J (B6) *Col4a3*-deficient mice exhibit slower renal disease progression, with end-stage renal failure developing at 20–30 weeks, allowing for long-term observation. This gradual progression has led to reports that male B6 mice show increased blood pressure, left ventricular wall thickening, and cardiac hypertrophy compared with the 129Sv strain¹³. However, other studies found no significant changes in blood pressure in C57BL/6-background mice, and female mice exhibited slightly more pronounced systolic and diastolic dysfunction than males, although the severity remained lower than that observed in 129x1/SvJ mice¹⁴. Furthermore, 129x1/SvJ mice do not develop cardiac hypertrophy or fibrosis, indicating an absence of overt cardiac disease. These discrepancies among reports suggest that, at least under certain experimental conditions, the reproducibility of the reno-cardiac interaction is limited. Therefore, the development of a new, simpler, and more reproducible “single-cause RCD model mouse” that can stably recapitulate CKD-associated systemic complications and cardiac lesions secondary to renal injury is highly anticipated.

From this perspective, podocyte-specific knockout of *MafB*, which is essential for podocyte differentiation and maintenance, induces severe progressive glomerulosclerosis and is therefore a promising candidate for a single-factor RCD model¹⁵. Because kidney injury in *MafB*-deficient mice originates exclusively in podocytes, systemic abnormalities and secondary cardiac remodeling can be analyzed under more controlled conditions in this model than in multi-hit or nonspecific genetic models. Furthermore, this approach allows for a clearer evaluation of organ-to-organ crosstalk and the relationship between glomerular injury and systemic complications. In this model, hypertension, renal anemia, elevated FGF23 levels, and cardiac remodeling can potentially be evaluated within the same disease sequence following podocyte injury.

Here, we used podocyte-specific *MafB* knockout mice to investigate the development of RCD under a single-gene-driven CKD background. We aimed to determine whether genetically induced glomerular injury is followed by CKD progression, CKD-associated systemic complications, including hypertension, renal anemia, and elevated FGF23 levels, and cardiac remodeling. We also sought to evaluate whether *MafB*-deficient mice can serve as a reproducible and mechanistically defined model for studying renal-cardiac interactions and assessing therapeutic interventions.

Results

Evaluation of the kidney in *MafB*^{flx/flx}; *NPHS2*-CreER^{T2} (*MafB* CKO) mice

The *MafB* podocyte-specific knockout mice used in this study have previously been reported to exhibit severe glomerulosclerosis¹⁵. However, sex differences in this phenotype have not been described. Therefore, we first examined whether this mouse model reproduced renal injury under the experimental conditions of the present study and whether phenotypic differences were present between males and females. Tamoxifen-treated *MafB*^{flx/flx} littermates were used as controls. After induction of *MafB* deletion by TAM administration, time-dependent changes in the renal phenotype were evaluated at two time points: 18 weeks post-TAM administration (T18w) and 23–28 weeks post-TAM administration (T23–28w). These groups were further stratified by sex, resulting in four subgroups for renal assessment.

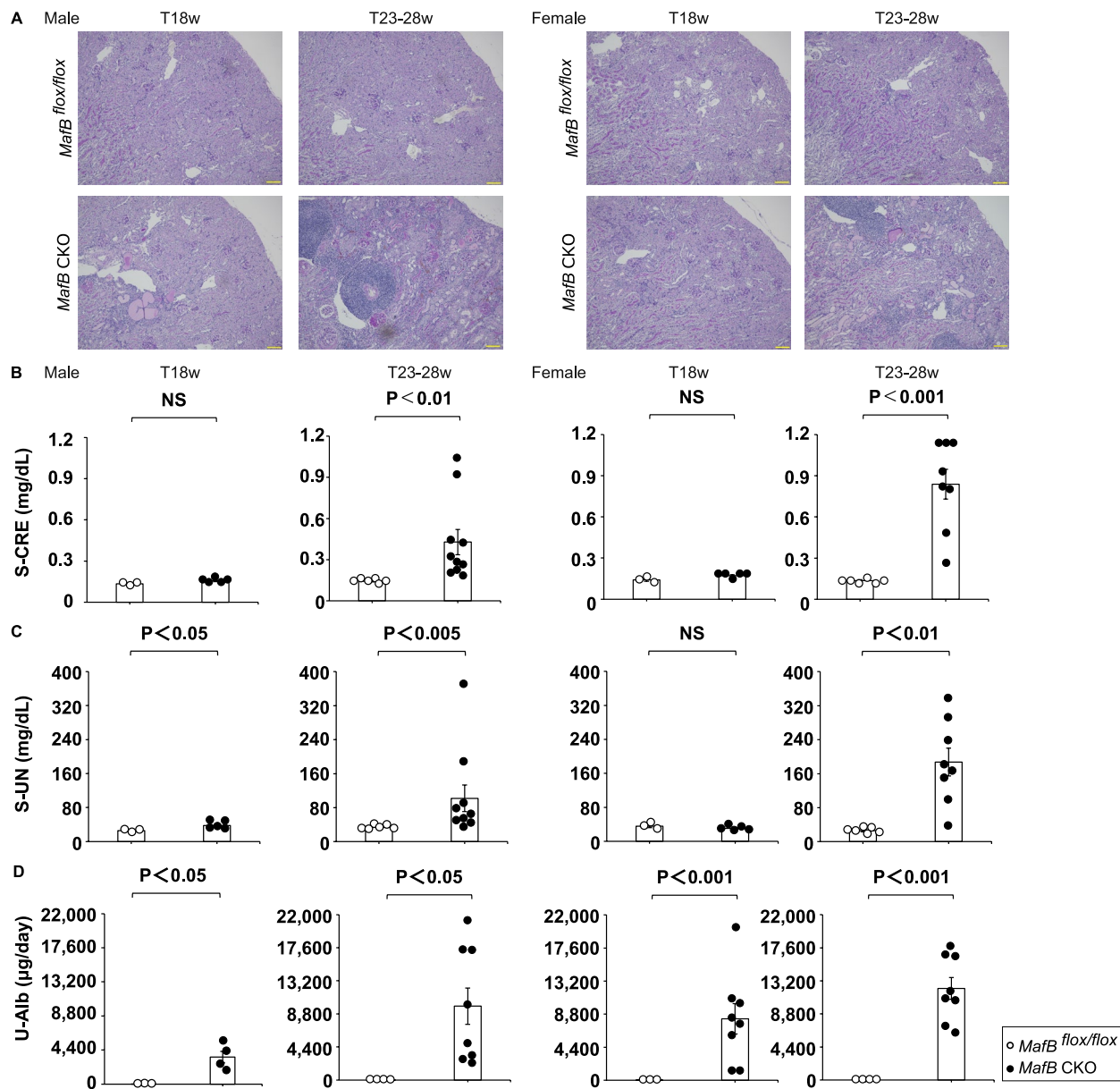
First, survival was analyzed in TAM-administered *MafB* CKO mice of both sexes. Deaths began to occur approximately 20 weeks after TAM administration, and most mice died by 38 weeks post-TAM. The mean survival time differed significantly from that of the control group (*MafB*^{flx/flx} mice), whereas no sex difference was observed within the experimental group (Supplementary Fig. S1A). Moreover, 9 of 50 mice survived for more than 1 year beyond this 38-week period, including 4 males and 5 females. The mortality rate of non-TAM-administered control *MafB*^{flx/flx}; *NPHS2*-CreER^{T2} mice was 0% (n = 20); however, the possibility that increased Cre activity in certain cell types affected survival cannot be excluded.

Next, PAS staining was performed for histological assessment of the kidney. Renal injury was observed in both sexes at T18w, and its progression was confirmed at T23–28w (Fig. 1A). To quantitatively evaluate renal function, serum creatinine (S-CRE), serum urea nitrogen (S-UN), and urinary albumin (U-Alb) levels were measured. At T18w, although the increase in S-UN in males did not reach statistical significance, the other parameters were markedly elevated in both sexes (Fig. 1B–D). Furthermore, at T23–28w, all three parameters were elevated regardless of sex.

Development of cardiac hypertrophy and fibrosis in *MafB* CKO mice

Because severe and progressive renal injury was observed in both male and female *MafB* CKO mice, we next examined the cardiac phenotype to investigate how renal disease in these mice affects systemic pathology. First, body weight, heart weight, and left ventricular anterior wall mass (LVAW mass) were evaluated in male and female mice at T18w and T23–28w. Body weight did not differ significantly between control and *MafB* CKO mice at T18w, but was significantly reduced in *MafB* CKO mice at T23–28w (Supplementary Fig. S2A). In contrast, heart weight was already significantly increased in *MafB* CKO mice at T18w and was increased further at T23–28w (Fig. 2A). The body weight of mice used for LVAW mass analysis showed a similar pattern to that of mice used for heart weight measurement, with no significant difference at T18w and a significant reduction in *MafB* CKO mice at T23–28w (Supplementary Fig. S2B). Nevertheless, LVAW mass assessed by echocardiography began to increase at T18w and showed evidence of significant cardiac hypertrophy by T23–28w (Fig. 2B). However, no significant changes in ejection fraction (EF) were observed in any group (Fig. 2C). These findings suggest that *MafB* CKO mice develop cardiac hypertrophy with preserved ejection fraction.

To evaluate cardiac fibrosis histologically, Masson's trichrome (MT) staining and gene expression analyses of the extracellular matrix components *Col4a3* and Fibronectin were performed. The longitudinal sections of the hearts at T23–28w showed clear evidence of fibrosis in both male and female *MafB* CKO mice (Fig. 2D). Moreover, both genes were significantly upregulated in male and female mice at T18w and T23–28w (Fig. 2E, F). Finally, serum analyses showed elevated levels of intact FGF23 (S-iFGF23), a factor known to increase with CKD and contribute to left ventricular hypertrophy, in both male and female mice at T18w and T23–28w (Fig. 2G). Collectively, these results demonstrated that *MafB* CKO mice exhibit concurrent severe kidney injury, cardiac hypertrophy, cardiac fibrosis, and elevated circulating FGF23.



Hypertension and renal anemia in *MafB* CKO mice

Furthermore, to assess the systemic effects of renal disease in *MafB* CKO mice, we evaluated hypertension and renal anemia, which are clinically relevant features associated with RCD. First, we assessed systolic blood pressure (SBP) and BNP expression, a cardiac hormone secreted in response to increased cardiac load and pressure. Compared with the control mice, SBP was significantly elevated in male and female *MafB* CKO mice at T18w, with further progression at T23–28w (Fig. 3A). Similarly, BNP expression increased significantly in both sexes at T18w and T23–28w (Fig. 3B).

For the evaluation of renal anemia, hemoglobin (Hb) and hematocrit (HCT) levels, primary markers of renal anemia, were examined. Hb levels were significantly reduced in both sexes at T18w and T23–28w (Fig. 3C). Moreover, HCT levels showed no change at T18w but decreased significantly at T23–28w in males and females (Fig. 3D). Moreover, serum iron levels (S-Fe; Fig. 3E), unsaturated iron-binding capacity (UIBC; Fig. 3F), and

Fig. 1 Sex-based assessment of kidney injury in *MafB* podocyte-specific conditional knockout (CKO) mice. **(A)** Kidney histology assessed using periodic acid-Schiff (PAS) staining in *MafB* CKO mice at 18 weeks (T18w) and 23–28 weeks (T23–28w) following tamoxifen (TAM)-induced *MafB* deletion ($n = 7$ for each group). Male and female *MafB* CKO mice displayed evidence of kidney injury at T18w, with progressive glomerular damage observed at T23–28w. Extent of damage was comparable between sexes at both time points. **(B)** Serum creatinine (S-CRE) levels were significantly elevated at T18w ($n = 4$ and $n = 8$ in the control and CKO groups, respectively, in both sexes) and T23–28w in CKO mice (male: $n = 6$ and $n = 10$ in control and CKO groups, respectively; female: $n = 6$ and $n = 8$ in control and CKO groups, respectively), reflecting impaired kidney function. **(C)** Serum urea nitrogen (S-UN) levels were significantly increased in males and females at T18w ($n = 4$ and $n = 8$ in control and CKO groups, respectively), while male and female *MafB* CKO mice exhibited elevated S-UN levels at T23–28w (male: $n = 6$ and $n = 9$ in control and CKO groups, respectively; female: $n = 6$ and $n = 8$ in control and CKO groups, respectively), suggesting gradual progression of kidney dysfunction. **(D)** Urinary albumin (U-Alb) levels were significantly elevated in male and female CKO mice at T18w (male: $n = 3$ and $n = 4$ in control and CKO groups, respectively; female: $n = 3$ and $n = 8$ in the control and CKO groups, respectively), indicating glomerular injury and albumin leakage into the urine. This increase persisted at T23–28w ($n = 4$ and $n = 8$ in control and CKO groups, respectively), confirming ongoing kidney damage in both sexes. Scale bars = 100 μm for PAS-stained images. Data in Figs. 1B–D is presented as mean and standard error of the mean (SEM). To assess whether differences between *MafB*^{fl_{ox}/fl_{ox}} (control) and *MafB* CKO mice were statistically significant, a minimum of three biological replicates were analyzed using Welch's *t*-test; a *P* value of < 0.05 was considered statistically significant. NS, not significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. White circles: control (*MafB*^{fl_{ox}/fl_{ox}}) groups, and black circles: *MafB* CKO groups.

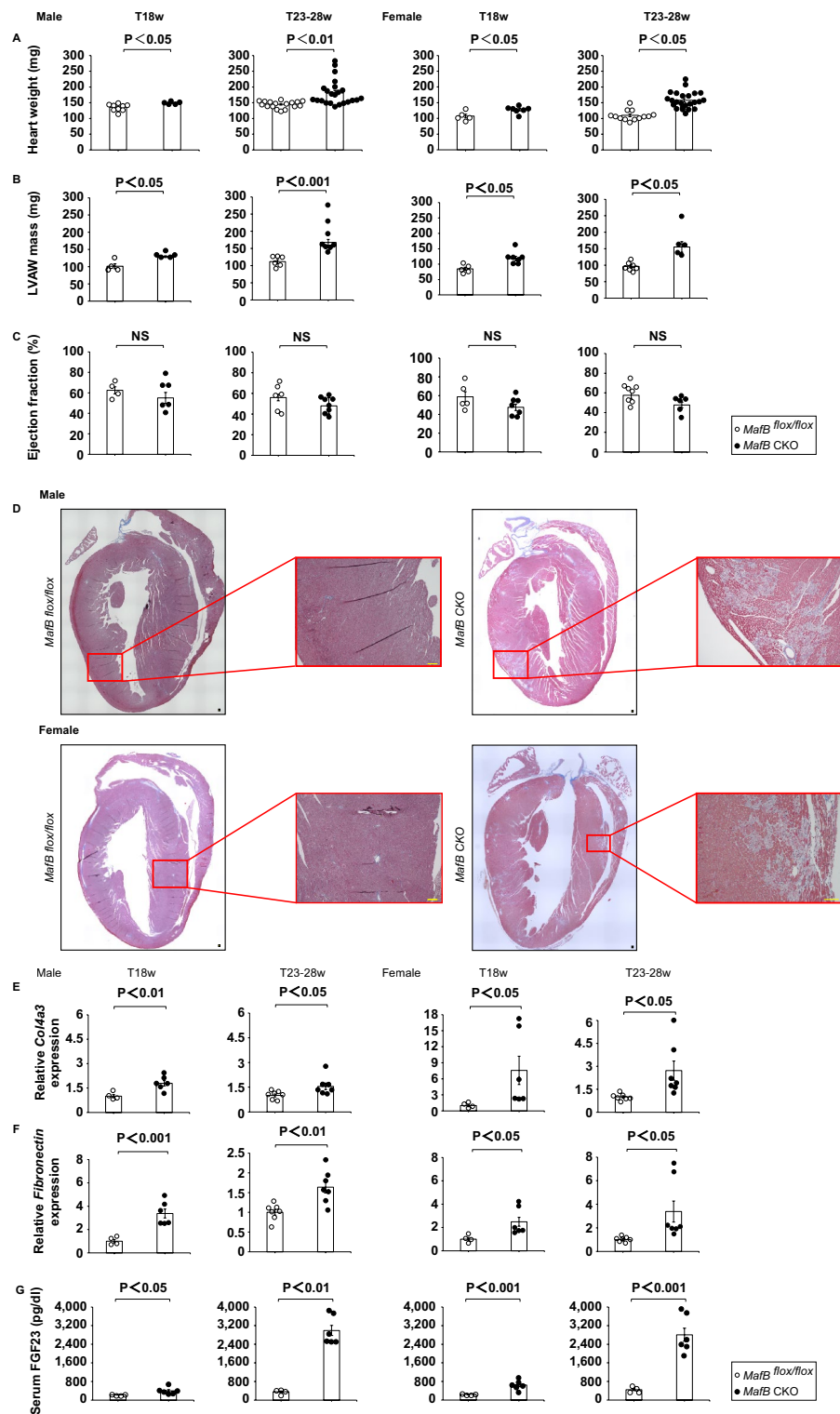
total iron-binding capacity (TIBC; Fig. 3G) displayed high variability at T18w but were significantly reduced at T23–28w, indicating progressive iron deficiency in both sexes. Taken together, these results confirmed that *MafB* CKO mice exhibit systemic abnormalities, including hypertension and renal anemia, in addition to cardiac remodeling.

ARNI treatment improves blood pressure and left ventricular wall thickness in *MafB* CKO mice

To evaluate the utility of *MafB* CKO mice in pharmacological studies, we examined the effects of an angiotensin receptor–neprilysin inhibitor (ARNI), a therapeutic agent for cardiovascular disease. Male and female *MafB* CKO mice at 17 weeks after tamoxifen administration (T17w) that exhibited mild to moderate hypertension [systolic blood pressure (SBP), 120–130 mmHg] were randomly assigned to the ARNI-treated or solvent control group ($n = 7$ per group; 4 males and 3 females). ARNI or solvent was administered five times per week for 6 weeks, and SBP was measured at the start of treatment (A0w) and at 1 week (A1w), 4 weeks (A4w), and 6 weeks (A6w) after treatment initiation.

First, the change in SBP relative to the A0w value was calculated for each mouse, and its time course is shown in Fig. 4A. Although the change in SBP tended to increase from A0w to A6w in both groups, the increase in SBP at A6w was significantly attenuated in the ARNI-treated group compared with that in the solvent control group (Fig. 4B). Next, the time course of the actual SBP values at each measurement point is shown in Fig. 4C. Although the actual SBP values increased over time in both groups, this increase tended to be attenuated in the ARNI-treated group. Among these measurements, comparison of the actual SBP values at A6w showed that SBP was significantly lower in the ARNI-treated group than in the solvent control group (Fig. 4D). These results indicate that ARNI treatment suppressed the progressive increase in SBP in *MafB* CKO mice.

To adjust for differences in body size between the solvent control and ARNI-treated groups ($n = 7$ per group, consisting of 4 males and 3 females), body weight was first measured (Supplementary Fig. S3A), and heart weight (HW) and left ventricular anterior wall (LVAW) mass were then normalized to body weight (BW). Although no significant change in HW/BW was observed between the groups (Fig. 4E), LVAW mass/BW was significantly reduced in the ARNI-treated group (Fig. 4F). Regarding renal function assessed using serum samples from both groups, S-UN was significantly reduced in the ARNI-treated group (Fig. 4G), whereas no change was observed in S-CRE (Fig. 4H). Furthermore, quantitative analyses of glomerulosclerosis and tubulointerstitial injury scores in PAS-stained and MT-stained kidney sections from the solvent control and ARNI-treated groups showed no significant differences. Nevertheless, BP and left ventricular wall mass were significantly ameliorated (Fig. 4I–L).



Taken together, renal injury in *MafB* CKO mice was accompanied by CKD-associated systemic changes, including cardiac hypertrophy, cardiac fibrosis, hypertension, and renal anemia. In addition, ARNI treatment improved SBP, LV mass/BW, and S-UN.

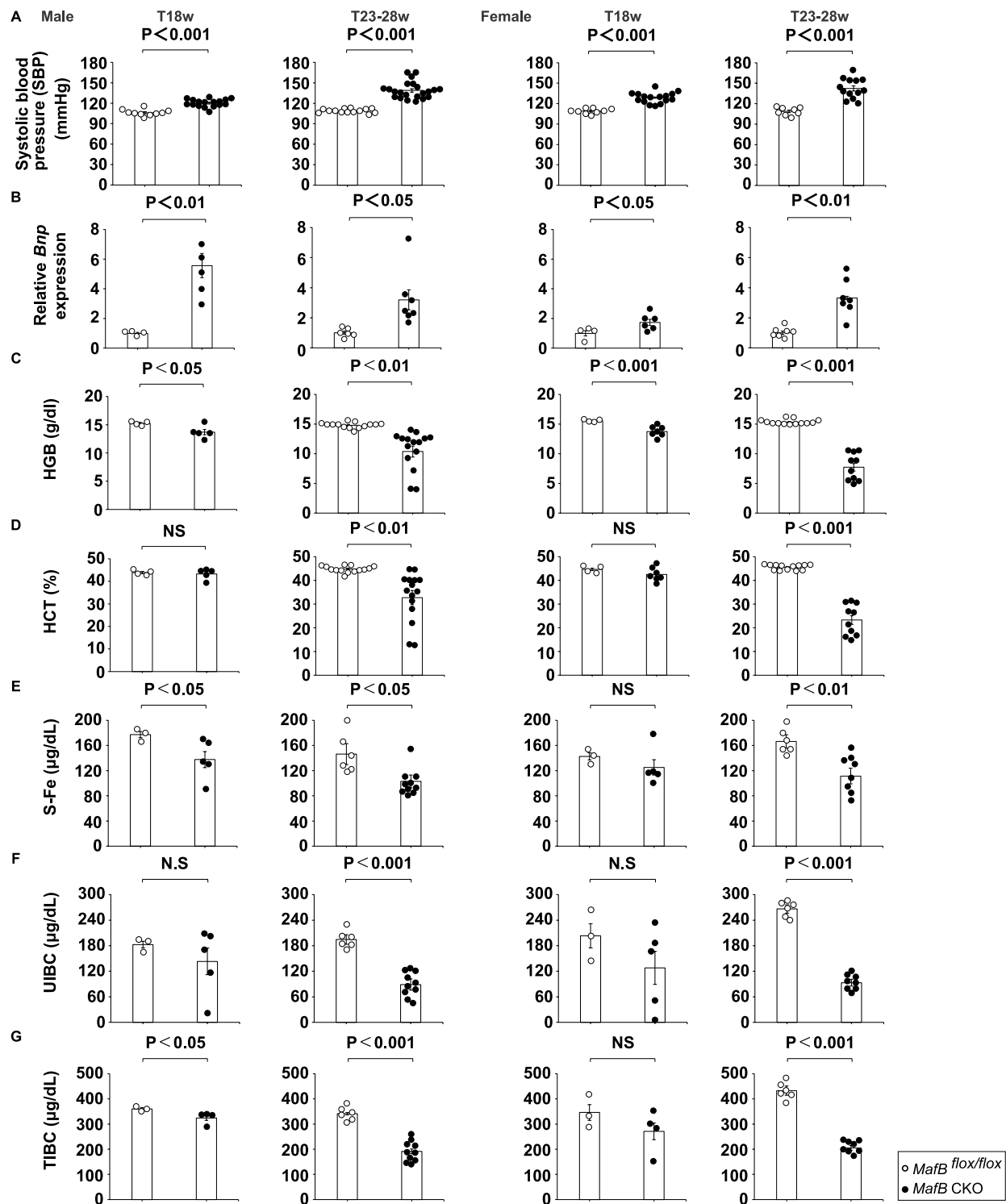
Fig. 2 Development of cardiac hypertrophy and fibrosis in *MafB* conditional knockout (CKO) mice. **(A)** Heart weight was significantly increased in male and female *MafB* CKO mice at T18w and was further increased at T23–28w compared with the corresponding control mice, indicating progressive cardiac hypertrophy (control and CKO groups, respectively: male, T18w, $n = 9$ and $n = 5$; T23–28w, $n = 17$ and $n = 23$; female, T18w, $n = 5$ and $n = 7$; T23–28w, $n = 13$ and $n = 24$). **(B)** Left ventricular anterior wall mass (LVAW mass), assessed by echocardiography, began to increase in *MafB* CKO mice at T18w and was significantly increased at T23–28w, indicating progressive left ventricular hypertrophy (control and CKO groups, respectively: male, T18w, $n = 4$ and $n = 5$; T23–28w, $n = 6$ and $n = 8$; female, T18w, $n = 5$ and $n = 7$; T23–28w, $n = 7$ and $n = 5$). **(C)** No significant changes in ejection fraction (EF) were observed in *MafB* CKO mice at either T18w or T23–28w, suggesting that cardiac systolic function was preserved despite the progression of cardiac hypertrophy (control and CKO groups, respectively: male, T18w, $n = 4$ and $n = 6$; T23–28w, $n = 6$ and $n = 8$; female, T18w, $n = 5$ and $n = 7$; T23–28w, $n = 7$ and $n = 6$). **(D)** Representative Masson's trichrome (MT)-stained longitudinal heart sections from male and female mice at T23–28w. Clear cardiac fibrosis was observed in *MafB* CKO mice of both sexes (male: $n = 6$ and $n = 6$ in the control and CKO groups, respectively; female: $n = 6$ and $n = 8$ in the control and CKO groups, respectively). **(E, F)** Gene expression analyses of the extracellular matrix components collagen type IV alpha 3 chain (*Col4a3*) and Fibronectin showed significant upregulation in male and female *MafB* CKO mice at T18w and T23–28w, indicating the progression of cardiac fibrosis (control and CKO groups, respectively: male, T18w, $n = 4$ and $n = 6$; T23–28w, $n = 7$ and $n = 7$; female, T18w, $n = 4$ and $n = 6$; T23–28w, $n = 7$ and $n = 7$). **(G)** Serum intact fibroblast growth factor 23 (S-iFGF23) levels were significantly elevated in male and female *MafB* CKO mice at T18w and T23–28w ($n = 4$ and $n = 6$ in the control and CKO groups, respectively, for each sex and time point). Scale bars = 100 μm for MT-stained images. Data in Figs. 2A–C and E–G are presented as the mean and standard error of the mean (SEM). To assess whether differences between *MafB*^{flox/flox} (control) and *MafB* CKO mice were statistically significant, a minimum of three biological replicates were analyzed using Welch's *t*-test, and a *P* value of < 0.05 was considered statistically significant. NS, not significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. White circles: control (*MafB*^{flox/flox}) groups, and black circles: *MafB* CKO groups.

Discussion

Previous studies have focused primarily on kidney disease in *MafB*-deficient mice¹⁵. This study revealed that these mice naturally develop CKD-associated cardiac hypertrophy and fibrosis, establishing them as a novel and highly reproducible RCD model. Nearly 100% of mice showed increased heart weight and left ventricular wall thickness from T18w, with significant cardiac hypertrophy at T23–28w, accompanied by hypertension and renal anemia. Extensive cardiac fibrosis was also observed, indicating progressive cardiac remodeling. These findings identify *MafB* CKO mice as a highly reproducible model for RCD associated with kidney disease.

One of the important advantages of this mouse model is that the phenotypes observed as systemic consequences of renal injury are obtained with high consistency. In conventional animal models of RCD, renal injury is induced by surgical reduction of renal function^{16,17}, unilateral ureteral obstruction, ischemia–reperfusion injury⁵, 5/6 nephrectomy¹⁸, or adenine-induced nephropathy^{9,19}. Cardiac remodeling has been reported to occur without additional interventions in some of these models, including unilateral ureteral obstruction²⁰ and adenine-induced nephropathy⁹. However, surgically or chemically induced renal injury models may show variability in phenotype, mortality, and the extent of cardiac involvement, and cardiac remodeling may not always be induced sufficiently or reproducibly¹¹. Therefore, multi-hit approaches combining renal injury with additional factors, such as a high-fat diet⁹, DOCA–salt loading¹⁰, a high-phosphate diet¹⁸, or angiotensin II administration²¹, have also been widely used to enhance renal dysfunction and cardiac disease^{5–10}. Diabetic nephropathy models, including *db/db* mice and streptozotocin-induced type 1 diabetic mice, are also widely used; however, in these models, renal injury alone may not always sufficiently reproduce cardiovascular lesions, and additional risk factors are sometimes introduced^{21,22}. Because multiple factors act simultaneously in such multi-hit models, it is difficult to clearly distinguish the extent to which each intervention contributes to renal lesions, cardiac lesions, hypertension, anemia, and mineral metabolism abnormalities. Moreover, strain-dependent differences in phenotype have also been reported. Thus, models in which renal pathology, CKD-associated systemic complications, and cardiac pathology progress in a clearly defined and interpretable manner are needed¹¹.

Several models that spontaneously develop renal injury due to a single-gene mutation have been reported, one of which is the global *Col4a3* knockout mouse. In this mouse, abnormalities of the glomerular basement membrane led to progressive renal injury and CKD with albuminuria, and it is therefore widely used as a genetic kidney



disease model resembling Alport syndrome^{12–14}. However, phenotypic discrepancies among reports suggest that, at least under certain experimental conditions, the reproducibility of reno-cardiac interactions is limited. Another model is the *Ksp*-Cre-mediated *Fgfr1* conditional knockout mouse²³. In this mouse, a kidney-derived single-gene model primarily targeting distal tubules was generated, and the mice were reported to exhibit hypertension, left ventricular hypertrophy, and myocardial fibrosis. However, *Ksp*-Cre activity is also observed in collecting ducts and in a subset of proximal tubules, and this model primarily represents a tubular-origin disease model rather

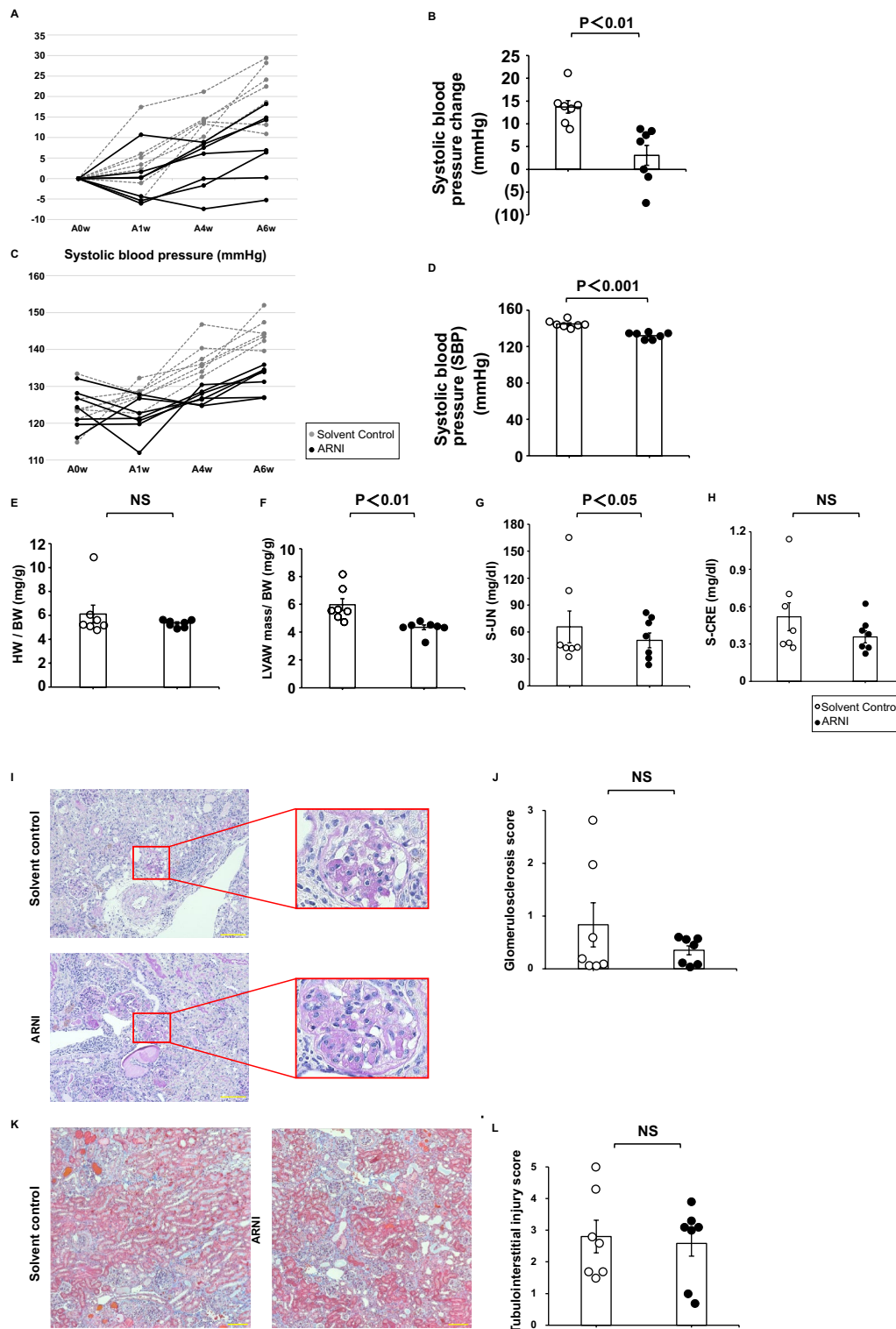
Fig. 3 Hypertension and renal anemia in *MafB* conditional knockout (CKO) mice. **(A)** Systolic blood pressure (SBP) was significantly elevated in *MafB* CKO mice at T18w, with further progression of hypertension observed at T23–28w. Male and female mice exhibited similar trends in BP elevation over time (control and CKO groups, respectively: male, T18w, $n = 10$ and $n = 17$; T23–28w, $n = 14$ and $n = 22$; female, T18w, $n = 9$ and $n = 16$; T23–28w, $n = 8$ and $n = 13$). **(B)** B-type natriuretic peptide (BNP) expression was significantly elevated in male and female *MafB* CKO mice at T18w and T23–28w, reflecting increased cardiac stress associated with hypertension and cardiac hypertrophy (control and CKO groups, respectively: male, T18w, $n = 3$ and $n = 5$; T23–28w, $n = 6$ and $n = 7$; female, T18w, $n = 4$ and $n = 6$; T23–28w, $n = 7$ and $n = 7$). **(C)** Hemoglobin (Hb) levels were significantly reduced in male and female *MafB* CKO mice at T18w and T23–28w (control and CKO groups, respectively: male, T18w, $n = 4$ and $n = 5$; T23–28w, $n = 14$ and $n = 15$; female, T18w, $n = 4$ and $n = 7$; T23–28w, $n = 13$ and $n = 10$). **(D)** Hematocrit (HCT) levels remained unchanged at T18w but significantly decreased at T23–28w in both sexes (control and CKO groups, respectively: male, T18w, $n = 4$ and $n = 5$; T23–28w, $n = 14$ and $n = 15$; female, T18w, $n = 4$ and $n = 7$; T23–28w, $n = 13$ and $n = 10$). **(E)** Serum iron (S-Fe) levels varied at T18w but showed a significant reduction at T23–28w, suggesting worsening renal anemia in *MafB* CKO mice as the disease progressed (control and CKO groups, respectively: male, T18w, $n = 3$ and $n = 5$; T23–28w, $n = 6$ and $n = 10$; female, T18w, $n = 3$ and $n = 5$; T23–28w, $n = 6$ and $n = 8$). **(F)** Unsaturated iron-binding capacity (UIBC) was significantly reduced at T23–28w in male and female *MafB* CKO mice (control and CKO groups, respectively: male, T18w, $n = 3$ and $n = 5$; T23–28w, $n = 6$ and $n = 10$; female, T18w, $n = 3$ and $n = 5$; T23–28w, $n = 6$ and $n = 8$). **(G)** Total iron-binding capacity (TIBC) levels also showed a significant reduction at T23–28w in both sexes, consistent with the progression of renal anemia in *MafB* CKO mice (control and CKO groups, respectively: male, T18w, $n = 3$ and $n = 4$; T23–28w, $n = 6$ and $n = 10$; female, T18w, $n = 3$ and $n = 4$; T23–28w, $n = 6$ and $n = 8$). All data are presented as the mean and standard error of the mean (SEM). To assess whether differences between *MafB*^{flx/flx} (control) and *MafB* CKO mice were statistically significant, a minimum of three biological replicates were analyzed using Welch's *t*-test, and a *P* value of < 0.05 was considered statistically significant. NS, not significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. White circles: control (*MafB*^{flx/flx}) groups, and black circles: *MafB* CKO groups.

than a mouse model in which CKD is initiated by glomerular injury. Furthermore, an important limitation of these mouse models is that they are not temporally inducible knockout models. Therefore, the development of a simpler, more reproducible, and temporally controllable “single-cause RCD mouse model” that can stably recapitulate CKD-associated systemic complications and cardiac lesions secondary to renal injury is needed.

A characteristic feature of the *MafB* CKO mice used in the present study is that the timing of gene deletion can be controlled using the CreER^{T2}/TAM system, allowing podocyte-specific gene deletion to be induced in adult mice. Previous studies have reported that *MafB* expression changes in association with the differentiation and fate of cardiac macrophages²⁴. In contrast, *NPHS2*–CreER^{T2} expression was confirmed to be absent at the RNA level in the heart, and although protein-level data were not shown, no cardiac protein expression was reported²⁵. Therefore, the direct impact of *MafB* deletion on the heart in this model is expected to be limited. Thus, this inducible model enables experimental control of CKD onset while minimizing the influence of gene deletion during development.

In this regard, a recent study reported another inducible, podocyte-specific mouse model in which overexpression of a constitutively active *NFATc1* mutant in adult mice induced nephrotic syndrome that subsequently progressed to CKD and pathological cardiac remodeling²⁶. This mutation is an experimentally engineered gain-of-function variant designed to remain constitutively localized in the nucleus. However, human pathogenic *NFATc1* variants that have been functionally characterized to date have predominantly exhibited loss-of-function properties, including impaired nuclear translocation and reduced transcriptional transactivation activity²⁷. Furthermore, the effects of pharmacological interventions were not evaluated in that study using the *NFATc1* mutant model.

In contrast, the *MafB* CKO mouse used in the present study is a loss-of-function model in which endogenous *MafB*, which is required for podocyte differentiation and homeostasis, is deleted in adulthood. Importantly, pathogenic *MAFB* variants have been reported in patients with FSGS, proteinuria, and progressive renal dysfunction²⁸. Reduced *MAFB* expression has also been observed in podocytes from patients with FSGS. These findings support the genetic and clinical relevance of *MAFB* to human glomerular disease. Moreover, within the same disease course, this model exhibited multiple CKD-associated systemic complications, including hypertension and renal anemia, in addition to cardiac hypertrophy, cardiac fibrosis, elevated FGF23 levels, and hyperphosphatemia. Collectively, these findings highlight the distinctive strengths of the *MafB* CKO mouse as a model for comprehensively evaluating the systemic and cardiac complications secondary to progressive renal injury.



However, this study has some limitations. Although the incidence of renal disease in this model was very high, not all mice exhibited an identical phenotype. In the present study, renal disease developed in more than 80% of the mice, whereas some mice did not show apparent renal disease. Additionally, serum and urinary biochemical parameters and anemia-related parameters showed a certain degree of variability. Nevertheless, survival analysis showed that almost all mice died by 30–35 weeks after TAM administration, and the mean survival time

Fig. 4 Angiotensin receptor–neprilysin inhibitor (ARNI) treatment improves blood pressure and left ventricular wall thickness in *MafB* conditional knockout (CKO) mice. **(A)** Time course of changes in systolic blood pressure (SBP) relative to value at the start of treatment (A0w) in ARNI-treated and solvent control groups. SBP was measured at A0w and at 1 week (A1w), 4 weeks (A4w), and 6 weeks (A6w) after treatment initiation. Each line represents longitudinal changes in an individual mouse. **(B)** Comparison between the groups of change in SBP from A0w to A6w. **(C)** Time course of the actual SBP values in ARNI-treated and solvent control groups. SBP was measured at A0w, A1w, A4w, and A6w. Each line represents longitudinal changes in an individual mouse. **(D)** Comparison between groups of the actual SBP values at A6w. **(E)** Heart weight/body weight ratio (HW/BW) showed no significant differences between the ARNI-treated and solvent control groups. **(F)** Anterior wall mass/body weight (AW mass/BW) was significantly reduced in ARNI-treated group compared with solvent control group at A6w. **(G)** Serum creatinine (S-CRE) levels did not differ significantly between solvent control and ARNI-treated groups. **(H)** Serum urea nitrogen (S-UN) levels were significantly reduced in ARNI-treated group. **(I)** Representative PAS-stained kidney sections from solvent control and ARNI-treated *MafB* CKO mice, shown at low and higher magnifications. **(J)** Quantitative analysis of glomerulosclerosis (PAS) in both groups. **(K)** Representative MT-stained kidney sections and **(L)** quantitative analysis of tubulointerstitial injury (MT) scores from the solvent control and ARNI-treated groups. All experiments included $n = 7$ per group, comprising 4 males and 3 females. Scale bars = 100 μm for PAS- and MT-stained images. Data in Figs. A–H, J and L are presented as mean and standard error of the mean (SEM). To assess whether differences between the solvent control and ARNI-treated groups were statistically significant, a minimum of three biological replicates were analyzed using Welch's *t*-test, and a *P* value of < 0.05 was considered statistically significant. NS, not significant; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. White circles: solvent control group, and black circles: ARNI-treated group.

converged within a defined range (Supplementary Fig. S1A). These results confirmed that the survival pattern in this model was consistent. Therefore, although the incidence of renal disease in this model was not 100%, disease progression and survival patterns were consistent among mice that developed renal injury, suggesting that this model has a certain degree of reproducibility for evaluating systemic changes associated with renal injury.

Regarding variability in the measured parameters, indices of renal dysfunction, hypertension, anemia, and structural cardiac changes showed clearly abnormal values compared with those in the control group in most mice. Thus, although interindividual variability was present, the overall ranges of these measurements remained clearly within pathological ranges.

Regarding the assessment of cardiac hypertrophy, heart weight and left ventricular anterior wall mass were presented as absolute values when comparing non-diseased mice with *MafB* CKO mice with CKD, taking into consideration the body weight loss associated with CKD progression. As a result, heart weight and left ventricular anterior wall mass were already increased at a time point when body weight did not significantly differ between the groups, and these indices of cardiac hypertrophy remained significantly increased even after body weight had significantly decreased. These findings suggest that cardiac hypertrophy in *MafB* CKO mice is likely accompanied by actual increases in heart weight and left ventricular wall thickness.

In contrast, in the comparison between the ARNI-treated and solvent control groups, these parameters were normalized to body weight to adjust for interindividual differences in body size, including differences between male and female mice. Although normalization to tibial length is a useful method that is less affected by CKD-associated changes in body weight, this comparison was performed between groups with the same CKD background caused by *MafB* CKO. Furthermore, because heart weight and LVAW mass are weight- or mass-based indices, we considered normalization to body weight to be more dimensionally interpretable and therefore used this method. As a result, although heart weight/body weight did not change, left ventricular wall mass/body weight was significantly reduced. In addition, improvements in blood pressure and S-UN were observed.

This study also did not fully elucidate the molecular mechanisms underlying the development of cardiac lesions, hypertension, renal anemia, and other complications associated with renal injury. Several pathways, including FGF23 signaling, RAAS activation, inflammatory signaling, and oxidative stress, have been reported to contribute to cardiovascular pathology in CKD, and these factors may also be involved in this model. However, the present study mainly focused on the time-dependent evaluation of phenotypes and did not include detailed analyses of these molecular mechanisms. However, the *MafB* CKO mice established in this study enabled control of the timing of renal injury onset and analysis of disease progression over time. This characteristic may be

useful for future studies aimed at elucidating the mechanisms underlying systemic complications secondary to renal injury.

Lastly, although ARNI treatment improved BP and left ventricular wall mass in this study, its therapeutic effects were limited. The sample size was small, and the treatment was evaluated under a single administration condition. Therefore, further studies using larger sample sizes and different doses and treatment durations will be required to evaluate the therapeutic effects of ARNI in greater detail. Furthermore, in addition to clinically established therapies such as SGLT2 inhibitors, future studies evaluating interventions targeting FGF23–FGFR4 signaling, including genetic suppression or antibody-mediated inhibition of FGFR4, would be valuable²⁹. Nevertheless, because pharmacological intervention produced detectable changes in BP and left ventricular wall mass in this study, this model may be applicable to pharmacological studies targeting systemic changes associated with renal injury. In particular, the ability to evaluate cardiac structural changes and BP changes originating from renal injury suggests that this model may be useful as an experimental system for assessing the effects of therapeutic interventions.

Taken together, podocyte-specific *MafB* deletion induced glomerular injury with high reproducibility in *MafB* CKO mice, accompanied by systemic changes such as cardiac hypertrophy, cardiac fibrosis, hypertension, and renal anemia. These findings suggest that this model may allow time-course evaluation of renal injury and its systemic consequences starting from a single genetic alteration. Moreover, because the timing of gene deletion can be controlled in this model, it has the advantage of enabling stepwise analysis of renal disease progression and associated systemic changes. Furthermore, the improvement in BP and left ventricular wall mass after ARNI treatment suggests that this model may also be applicable to pharmacological studies evaluating systemic changes associated with renal injury. These characteristics indicate that *MafB* CKO mice may serve as a useful experimental model for analyzing systemic pathologies secondary to renal injury and for exploring new therapeutic strategies.

Methods

Mice

The mice used in this study were *Mus musculus* with a C57BL/6 background and were purchased from SLC (Hamamatsu, Japan). These mice were used to generate *MafB*^{flox/flox} mice and podocyte-specific inducible *MafB* knockout mice (*MafB*^{flox/flox}; *NPHS2*-CreER^{T2}, hereafter referred to as *MafB* CKO mice). The *NPHS2*-CreER^{T2} mice were initially obtained from Professors Hideki Yokoi (currently affiliated with Kumamoto University) and Masato Kasahara (currently affiliated with Nara Medical University) during their tenures at Kyoto University. The use of these mice was facilitated as part of a collaborative study, and Professors Yokoi and Kasahara are authors of this manuscript. The *MafB*^{flox/flox} mouse line is not commercially available and is currently available only through scientific collaboration. Male and female mice aged 26–36 weeks were included, with body weights of approximately 20–30 g. To generate the genetically modified mice, 7–10 backcrosses were performed. The tamoxifen-treated *MafB*^{flox/flox} littermates without *NPHS2*-CreER^{T2} were used as controls.

Podocyte-specific inducible Cre recombinase transgenic mice activated by tamoxifen administration were used to generate *MafB* CKO mice²⁵. The mice had free access to commercial chow (Oriental Yeast, Tokyo, Japan) and filtered water.

Mice with the expected genotype that received tamoxifen according to the experimental protocol were included in the analyses. Mice or individual samples were excluded from the relevant analyses only when genotyping failed, tamoxifen administration was incomplete, or reliable data could not be obtained because of technical problems during sample collection or measurement. *MafB* CKO mice that survived beyond 36 weeks after tamoxifen administration were continuously monitored because *NPHS2*-CreER^{T2}-mediated recombination is not complete in every podocyte, a small proportion of mice may undergo incomplete *MafB* deletion (26). We therefore

considered that all of these mice survived for more than one year after tamoxifen administration were most likely represent animals with incomplete recombination and excluded them from the endpoint analyses. All of these mice survived for more than one year after tamoxifen administration and were included in the survival analysis but excluded from the endpoint analyses of renal disease progression and renal–cardiac phenotypes. Given the reported incomplete recombination efficiency of the *NPHS2*-CreER^{T2} line, these rare long-term survivors were considered likely to represent animals in which *MafB* deletion had been incompletely induced.

In accordance with the animal experiment protocol approved by the University of Tsukuba, a humane endpoint was defined as a body weight loss of $\geq 10\%$ within one week in mice exhibiting renal failure and cardiac hypertrophy. Mice were euthanized by cervical dislocation under deep isoflurane anesthesia promptly upon reaching this endpoint. Because the timing at which individual mice reached the humane endpoint varied according to disease progression, endpoint analyses were performed between 23 and 28 weeks after tamoxifen administration (T23–T28).

Conditional deletion of the floxed *MafB* allele

To activate the Cre recombination system, both 8-week-old *MafB* CKO mice and their control *MafB*^{flox/flox} littermates were injected intraperitoneally with tamoxifen (TAM) at 75 mg/kg once daily for 5 consecutive days. TAM (Sigma-Aldrich, Tokyo, Japan) was first dissolved in ethanol and then mixed with filter-sterilized corn oil as described previously³⁰. The time after TAM administration was defined as weeks post-TAM administration.

Histopathological analysis of the kidney and heart and evaluation of glomerulosclerosis and tubulointerstitial injury scores

Kidneys were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned at a thickness of 2 μm . Before fixation, the heart was perfused with phosphate-buffered saline to remove intravascular blood by allowing the heart to beat. Subsequently, the heart was immersed in 50 mM KCl to induce diastolic arrest and ensure uniform fixation. After carefully removing residual moisture, the heart weight was measured, and fixation was performed using the same procedure as for the kidney.

For the assessment of glomerulosclerosis, periodic acid–Schiff (PAS)-stained sections were evaluated according to previously described methods³¹. Briefly, all glomeruli in a longitudinal kidney section were scored on a five-point scale (0–4) based on the percentage of the glomerular tuft area affected by sclerosis: 0, no lesion; 1, $>0\%$ to $<25\%$; 2, 25% to $<50\%$; 3, 50% to $<75\%$; and 4, $\geq 75\%$. For each mouse, the glomerulosclerosis score was calculated as the average of all glomerular scores.

For the assessment of tubulointerstitial injury, Masson trichrome (MT)-stained sections were analyzed according to previously described methods³². The entire cortical region of each longitudinal kidney section was first surveyed at low magnification. Ten nonoverlapping cortical fields were then sampled systematically at approximately equal intervals throughout the cortex at $\times 40$ magnification. When arteries were present within a field, the vessel wall and the immediately surrounding perivascular connective tissue were excluded from the tubulointerstitial area evaluated. Tubular atrophy, tubular dilatation, protein casts, and interstitial fibrosis were semiquantitatively scored on the basis of the percentage of the affected area as follows: 0, 0%; 1, 1–10%; 2, 11–25%; 3, 26–50%; 4, 51–75%; and 5, 76–100%. For each mouse, the tubulointerstitial injury score was determined as the average score from the 10 fields.

Cardiac fibrosis was evaluated using MT-stained heart sections. Hematoxylin and eosin (HE), PAS, and MT staining were performed using standard procedures. An observer blinded to the experimental groups performed all morphological analyses.

Echocardiography

The mice were anesthetized with isoflurane in oxygen at approximately 3% and 1.5–2.5% for induction and maintenance, respectively. The mice were placed on a platform in the supine position, and electrocardiography was used to continuously monitor heart rate; the isoflurane dosage was adjusted to maintain a heart rate of 300–450 beats/min. Transthoracic echocardiography was performed using a high-frequency ultrasound system with a 40-MHz linear probe (Vevo 2100, VisualSonics Inc., Toronto, Canada). The images were analyzed offline in a blinded manner. From the systolic and diastolic phases, left ventricular anterior wall mass/body weight (LVAW mass/BW) and ejection fraction were calculated.

Blood pressure

Tail artery blood pressure (BP) in mice was measured noninvasively using a Softron device (Softron, Brussels, Belgium). Before measurement, mice were placed calmly in a holder, and their tails were warmed at approximately 37 °C for 5–10 minutes to promote blood flow. Subsequently, an appropriate cuff was attached to the tail, and multiple measurements were obtained in automatic mode. For each mouse, at least 10 stable readings were collected, and their average was recorded as the systolic BP (SBP). Stable readings were defined as measurements obtained without movement detected by the device.

Urine, serum, and hematological analyses

The concentrations of urinary and serum components were measured following standard protocols. Mice were housed in metabolic cages for 24 hours, and urine samples were collected from each mouse. The urine samples were placed on ice during collection and then stored at –30 °C. Urinary components were evaluated by Oriental Yeast Corporation (Shiga, Japan).

Serum samples were stored at approximately 25 °C for approximately 15 minutes, followed by 2–4 hours on ice, centrifuged at 3,000 rpm for 10 minutes at 15 °C, and frozen at –30 °C. Serum components were evaluated by Oriental Yeast Corporation. Serum intact FGF23 levels were examined using an enzyme-linked immunosorbent assay kit (TCY4000 [11A735]; Kainos, Belfast, Ireland). Additionally, hematologic parameters were determined using a Celltac MEK-6558K automated analyzer (Nihon Kohden, Tokyo, Japan).

Quantitative polymerase chain reaction (qPCR)

Hearts were excised from each mouse, finely minced with scissors, and immediately snap-frozen in liquid nitrogen. For RNA extraction, each frozen heart was placed in a metal cone crushing tube (ST-0350F-0, Yasui Kikai Co., Ltd., Osaka, Japan) together with a metal cone (MC-0316; Yasui Kikai Co., Ltd.). While maintaining the frozen state, 1 mL of ISOGEN reagent (311-02501; Nippon Gene, Tokyo, Japan) was added, and the tissues were pulverized using a Multi Beads Shocker (Yasui Kikai Co., Ltd.). Total RNA was extracted according to the manufacturer's instructions.

Complementary DNA was synthesized from total RNA using the QuantiTect Reverse Transcription Kit (205313; Qiagen, Hilden, Germany). qPCR was performed on the Thermal Cycler Dice Real-Time System (TaKaRa, Shiga, Japan) using THUNDERBIRD SYBR qPCR Mix (QPS-201; Toyobo, Osaka, Japan). Primer sequences for collagen type IV alpha 3 chain (*Col4a3*), fibronectin, B-type natriuretic peptide (BNP), and hypoxanthine-guanine phosphoribosyl transferase (*Hprt*) are listed in Supplementary Table S1. Expression levels of target genes were normalized to *Hprt*.

Evaluation of the effects of sacubitril/valsartan (LCZ696) on BP and cardiac parameters in *MafB* CKO mice

Sacubitril/valsartan (LCZ696), an angiotensin receptor–neprilysin inhibitor, was used in the drug administration experiments in *MafB* CKO mice. LCZ696 was dissolved in corn oil and administered by oral gavage at a dose of 33 mg/kg per administration, whereas corn oil alone was administered to the solvent control group. The dose was selected with reference to previous animal studies evaluating LCZ696 at doses of approximately 20–60 mg/kg, including studies using 50–60 mg/kg, while taking into consideration the longer treatment duration used in the present study^{33–37}. Both groups received the respective treatment five times per week for 6 weeks.

The treatment and solvent control groups each consisted of seven randomly selected mice at 17 weeks post-TAM administration (T17w), with moderately elevated BP of approximately 120–130 mmHg. The start of LCZ696 treatment was defined as A0w. BP changes and actual measurements were compared between the two groups at A0w, A1w, A4w, and A6w. Additionally, at A6w, the left ventricular wall was measured using echocardiography, and heart weights were measured. Renal function and urinary albumin were also measured at A6w.

Statistical analysis

Data are presented as the mean $\pm$ standard error of the mean (SEM) unless otherwise indicated. Statistical analyses were performed using appropriate tests according to the number of groups and data distribution. Comparisons between two groups were performed using the Student's *t*-test or Mann–Whitney *U* test, as appropriate. Comparisons among multiple groups were performed using one-way analysis of variance followed by post hoc tests or the Kruskal–Wallis test followed by post hoc comparisons, as appropriate. A *P* value of <0.05 was considered statistically significant.

Ethics statements

All experiments were performed in accordance with the guidelines of the Care and Use of Laboratory Animal Resource Center at the University of Tsukuba and were approved by the Institutional Review Board of the University of Tsukuba (Approval number 24-122).

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Author contributions Satoru Takahashi supervised the study; Mitsunori Fujino designed experiments; Mitsunori Fujino, Shun Ishibashi, Walaa Basha, and Masami Ojima performed experiments; and Mitsunori Fujino, Shun Ishibashi, Walaa Basha, Kazuya Murata, Hideki Yokoi, Masato Kasahara, Masami Ojima, and Satoru Takahashi edited and revised the manuscript. All the authors interpreted the experimental results and approved the final version of the manuscript.

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Data availability The data supporting the findings of this study are available on Figshare under a private link. The datasets generated during the current study are available in Figshare (<https://doi.org/10.6084/m9.figshare.28657262>). Access to the data is currently restricted because this manuscript is prior to submission.

Declarations

Competing interests The authors declare that they have no conflicts of interest.

Ethics All experiments were performed according to the guidelines of the Care and Use of Laboratory Animal Resource Center at the University of Tsukuba and were approved by the Institutional Review Board of the University of Tsukuba (Approval number for animal experiments: 24-122).

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