MicroRNAs (miRNAs) are emerging as effective therapeutic agents. When testing whether miR-145-5p could alleviate kidney injury, we unexpectedly found that extracellular vesicles loaded with miR-145-5p induced proteinuria and podocyte foot process effacement in normal control mice. To explore the mechanism of miR-145-5p’s toxicity to podocytes, we hypothesized that miR-145-5p could enter podocytes and inhibit genes essential for podocytes. We demonstrated that systematically administered miRNA can enter podocytes. Next, we predicted 611 podocyte essential genes based on single-cell RNA sequencing (RNA-seq) and found that 32 of them are predicted to be targeted by miR-145-5p. Functional annotation of the 32 podocyte essential genes revealed small GTPase-mediated signal transduction as the top pathway. We experimentally validated that miR-145-5p targeted Arhgap24 and Srgap1, the essential regulators of the Rho family of small GTPases, increased the activity of Rac1 and Cdc42, and reduced RhoA activity, accompanied by cellular injury, in podocytes. These results explain how miR-145-5p has deleterious effect on podocytes. Most importantly, our study provides a novel approach to identify genes essential for podocytes and then systematically administer miRNA-based drugs.

miR-145-5p dysregulation has been shown to be involved in kidney injury by mediating the effect of MALAT117 and MEG318 on kidney. Recently, we investigated whether systemically administered miR-145-5p could mitigate kidney injury in mouse models, and, unexpectedly, we found that treatment of normal control mice with extracellular vesicles (EVs) loaded with miR-145-5p resulted in albuminuria and podocyte foot process effacement. In the present study, we studied how the systemically administered miR-145-5p caused podocyte injury. We speculated that the circulating miR-145-5p in EVs can enter podocytes and inhibit the expression of target genes that are essential for podocytes, leading to podocyte injury and glomerular filtration barrier disruption. To prove this hypothesis, it is required to determine the essential genes of podocytes and then compare them with miR-145-5p’s predicted targets in order to identify which podocyte essential genes are targeted by miR-145-5p.

Recently, we designed an approach to identify genes essential for mouse podocytes and other cell types.19 The rationale of the approach is that individual cells of the same type exhibit tremendous difference in gene expression, with correlation coefficients of 0.1–0.5, as shown by single-cell RNA sequencing (RNA-seq) studies,20–22 indicating that most genes are differentially expressed among individual cells of the same type, and the genes differentially expressed are
dispensable, whereas those expressed commonly in all individual cells would be essential for the cell type. By this criterion, we identified candidate podocyte essential genes based on our mouse podocyte single-cell RNA-seq data and experimentally validated their essentiality for podocytes, demonstrating the feasibility of the approach.19 The availability of podocyte essential genes made it possible to test whether miR-145-5p could target podocyte essential genes and induce podocyte injury.

We then compared the predicted miR-145-5p targets with the 611 podocyte essential genes and identified 32 miR-145-5p targets. Gene ontology (GO) analysis of the predicted 32 podocyte essential genes revealed small GTPases as the top biological process affected by miR-145-5p. Optimal activity of small GTPases is known to be crucial for podocyte survival, structure, and function.23–25 We have proved that miR-145-5p alters small GTPase activity and induces podocyte injury. Our interpretation of the mechanism underlying miR-145-5p toxicity on podocytes provides a novel approach for understanding the mechanism underlying the side effects of miRNA-based drugs, as well as for prediction of toxicity of a miRNA drug.

RESULTS
Systemically administered miR-145-5p induced podocyte injury in mice
miR-145-5p has been reported to be dysregulated and involved in kidney injuries.17,18 To determine whether supplement of miR-145-5p could alleviate kidney injury in mouse models, we purified the EVs from miR-145-5p transfected Jurkat or HEK293 cells and obtained the miR-145-5p-enriched EVs (Figure S1). We first tested the miR-145-5p EV samples for toxicity or side effects on healthy control mice by administering miR-145-5p EVs intravenously every other day for a total of 3 times. Unexpectedly, we found that miR-145-5p EVs induced albuminuria in the mice (Figure S2). To further confirm the albuminuria-inducing effect of miR-145-5p, we repeated the experiment with miR-145-5p EV injection once a day for a total of 6 days. To support that the toxicity of miR-145-5p EVs was miR-145-5p specific, we included a group of mice that were simultaneously injected with miR-145-5p inhibitor using TransIT-EE Delivery Solution, expecting an alleviated albuminuria in the mice. As shown in Figure 1, miR-145-5p EVs induced albuminuria and podocyte foot process effacement, and this effect was abolished by miR-145-5p inhibitor.

miRNA loaded in extracellular vesicles can reach podocytes after being systemically administered
To determine whether EVs and the enclosed miRNA can be delivered to podocytes after being administered systemically, we first performed live fluorescence imaging of the distribution of EVs labeled by DiR (a near-infrared dye) in mice after injection via tail vein. The fluorescence signal was predominantly present in liver (Figures 2A and 2B); meanwhile, abundant fluorescence was also observed in spleen and kidney, but not muscle (Figure 2B), which was in accordance with former publications.26,27

We next examined whether miRNA mimic labeled with Cy5 in the EVs was successfully delivered to podocytes and found that Cy5-miR-145-5p was clearly present in all glomerular cells, including podocytes that were located at the periphery of each glomerular tuft (Figure 2C). This observation was in accordance with results of in situ hybridization of an artificial miRNA mimic (miR#) (Figure 2D). No miR# was detected in mice injected with PBS, while in the mice injected with miR# EVs, all glomerular cells, including podocytes, were positive for miR# staining. These results were consistent with studies showing that intravenously administered EVs were readily delivered to extensive kidney tissues, including glomerular podocytes.28,29

Systemically administered miR-145-5p agomir entered podocytes and caused injury
We next tested the toxicity of miR-145-5p in the form of agomir, which is commonly used for miR-based drug delivery via systemic administration.30 We first verified that Cy5-agomir injected via tail vein was able to reach podocytes (Figure S3A). Then, miR-145-5p and scramble agomirs were injected every other day for a total of 6 times, and...
proteinuria developed in the miR-145-5p agomir-treated mice but not the scramble-treated mice (Figure S3B). EM examination consistently showed podocyte foot process effacement in the miR-145-5p- but not scramble-treated mice (Figure S3C). Additionally, we injected miR-145-5p agomir locally to mouse kidney, and 3 days after injection, marked foot process effacement was observed in the kidney injected with miR-145-5p agomir but not scramble (Figure S3D).

miR-145-5p induced injury in cultured podocytes
We tested whether miR-145-5p could cause injury in immortalized podocyte cell line in culture, which is a commonly used model for podocyte research. We added miR-145-5p EVs, which were labeled with PKH67, to podocytes in culture and observed fluorescence in the cells after 12 h (Figure 3A). We then quantified the reduction of podocyte F-actin stress fibers, because loss of F-actin stress fibers is characteristic of podocyte injury. The result showed that F-actin stress fibers were greatly reduced in the miR-145-5p EV-treated podocytes (Figure 3B). We also used miR-145-5p mimic to transfect the podocytes and observed a similar reduction of F-actin stress fibers in the cells (Figure 3C). The consistent toxic effect of miR-145-5p on the podocytes in culture with those in mice suggests that the podocyte cell line is a good model for exploring how miR-145-5p induces podocyte injury.

Identification of 611 podocyte essential genes by single-cell RNA-seq
We speculated that miR-145-5p is toxic to podocytes because it is not normally expressed in podocytes, and exogenous miR-145-5p can effectively target genes essential for podocytes, as shown in the schematic diagram (Figure 4). We previously performed single-cell RNA-seq of mouse podocytes and found enormous heterogeneity of gene expression among individual podocytes. By using the concept that genes commonly expressed in all individual podocytes are likely podocyte essential genes, we predicted and validated 335 podocyte essential genes when expression cutoff was set as >0.5 Reads Per Kilobase per Million mapped reads (RPKM). According to the estimation that there are approximately 2,000 genes that are essential for a cell type, we lowered the expression cutoff to >0.1 RPKM for the present study, resulting in 611 genes that are predicted to be podocyte essential genes (Table S1). GO analysis of the predicted 611 essential genes revealed housekeeping and other processes known to be essential for podocytes (e.g., actin cytoskeleton organization [Figure S4]), supporting the reliability of our prediction approach.

Identification of podocyte essential genes targeted by the miR-145-5p
To determine how many of the podocyte essential genes can be targeted by miR-145-5p, we searched Targetscan and retrieved the top 500 predicted target genes of miR-145-5p. Comparison of miR-145-5p’s target genes with the predicted 611 podocyte essential genes revealed 32 podocyte essential genes that may be targeted by miR-145-5p (Table 1).

miR-145-5p is predicted to target Rho family of small GTPases in podocytes
To explore the mechanism of miR-145-5p toxicity on podocytes, we performed GO functional annotation of the 32 podocyte essential genes that are miR-145-5p potential targets (Table 1) and found small GTPase-mediated signal transduction was at the top of the functions (Figure 5; Table S2). The members of Rho family of small GTPases

Figure 2. Tissue distribution of injected EVs and the miRNA in a mouse
(A) Fluorescence imaging of the mouse injected with DiR-labeled EVs through tail vein. The image was taken 30 min after the injection, showing predominant fluorescence signal in liver as well as other tissues. (B) Fluorescence imaging of individual organs from the same mouse, showing that EVs were accumulated in liver, kidney, and spleen but not muscles. (C) Confocal imaging of Cy5-miR-145-5p in an isolated glomerulus 48 h after the Cy5-miR-145-5p-loaded EVs were injected via tail vein to a Nps2-cre/eGFP transgenic mouse whose podocytes express eGFP. Arrows: Cy5-miR-145-5p-loaded EVs accumulated in podocytes. G, glomerulus; T, tubule. Scale bar: 20 μm. (D) In situ hybridization of artificial miR# in kidney after the miR#-containing EVs were injected via tail vein to a mouse. It was clearly shown that miR# was delivered to all glomerular cells, including podocytes that are localized at periphery of each glomerular tuft. Arrows: miR#-loaded EVs accumulated in podocytes. G, glomerulus; T, tubule. Scale bar: 20 μm.
(RhoA, Rac1, and Cdc42) have been best described and shown to be crucial for many cellular processes in podocytes. Aberrant activity of RhoA, Rac1, and Cdc42 has been shown to cause podocyte injury.23,24,35

miR-145-5p inhibited Arhgap24 and Srgap1, altering the activity of Rho family of small GTPases in podocytes

miR-145-5p was predicted to target three GTPase-activating proteins, Arhgap24, Arhgap28, and Srgap1, which are associated with the small GTPase pathway (Table 2). Since loss of Arhgap24 is known to cause podocyte injury,35 we examined whether Arhgap24 is truly a miR-145-5p target and contributes to injurious effect of miR-145-5p on podocytes. We found that Arhgap24 is expressed specifically in podocytes in glomeruli (Figure S5). Luciferase reporter assay and immunoblotting showed that miR-145-5p significantly repressed Arhgap24 expression in podocytes in vitro (Figures 6A–6C). In mice, miR-145-5p EV administration resulted in significant reduction of Arhgap24 in podocytes, as shown by immunohistochemistry of the kidney (Figure 6D). Both in vitro and in vivo, miR-145-5p inhibitor was capable of abolishing the effect of miR-145-5p EVs on Arhgap24 expression (Figures 6C and 6D), indicating that it was miR-145-5p in the EVs that inhibited Arhgap24 expression in the podocytes. These results together supported that Arhgap24 is a direct target of miR-145-5p in podocytes. Furthermore, Arhgap24 is known to downregulate the activity of Rac1 and Cdc42,35 and we consistently found that miR-145-5p-transfected podocytes, in which Arhgap24 was downregulated, had increased activity of both Rac1 and Cdc42 (Figure 6E). We also tested whether miR-145-5p could downregulate Srgap1 in podocytes. As shown in Figure S6, miR-145-5p induced a marked decrease of Srgap1 protein in cultured podocytes, as well as the podocytes in mice. Srgap1 was reported to activate RhoA and inactivate Rac1.36 Consistently, we found that miR-145-5p decreased RhoA activity (Figure 6E), likely through its inhibition of Srgap1. We also tested glomerular Rac1/Cdc42 total protein levels in mice and found that miR-145-5p did not change the total protein levels (Figure S7).

miR-145-5p altered podocyte behaviors and induced injuries that involve Rho family of small GTPases

In addition to podocyte cytoskeletal injury induced by miR-145-5p as shown in Figure 3, which involves aberrant activity of Rho family of small GTPases, other podocyte cellular processes that involve the small GTPases include cell spreading, adhesion, migration, and apoptosis.23,24,34,37 Since miR-145-5p was capable of altering the Rho GTPase activities in podocytes (Figure 6), we tested miR-145-5p’s effect on spreading, adhesion, migration, and apoptosis of podocytes. Real-time cell analysis (RTCA) spreading assay showed that cell index of miR-145-5p-transfected podocytes was significantly lower than that of scramble-transfected cells (Figure 7A), indicating that miR-145-5p compromised podocyte spreading. In adhesion assay,
cell index of the miR-145-5p-treated podocytes was lower than the scramble-transfected podocytes (Figure 7B); consistently, the level of vinculin, a component of focal adhesion, decreased in the miR-145-5p-treated podocytes (Figure 7C). In contrast, miR-145-5p appeared not to affect podocyte migration, as shown by both RTCA and wound-healing assays (Figures S8A and S8B). miR-145-5p-treated podocytes exhibited increased apoptosis, as shown by nuclear condensation (Figure S8C) and annexin V–fluorescein isothiocyanate/propidium iodide (PI) double staining (Figure 7D). miR-145-5p transfection also downregulated CD2AP and synaptopodin (Figure 7E), which are sensitive markers of podocyte injury (https://www.nephroseq.org/) and known to be regulated by Rho GTPases.24,38

### Table 1. Predicted targets of miR-145-5p among the 611 podocyte essential genes

| Symbol | Entrez gene name                                      | Average expression in single podocyte (RPKM) | Cumulative weighted context++ score |
|--------|-------------------------------------------------------|---------------------------------------------|-----------------------------------|
| ACTB   | actin, beta                                           | 13,889.86                                   | −0.43                             |
| GNB1   | guanine nucleotide-binding protein (G protein), beta polypeptide 1 | 2,439.721                                   | −0.15                             |
| ST13   | suppression of tumorigenicity 13                      | 2,106.819                                   | −0.04                             |
| DYSPL2 | dihydropyrimidinase-like 2                           | 1,419.238                                   | −0.05                             |
| EIF4A2 | eukaryotic translation initiation factor 4A2          | 1,082.761                                   | −0.14                             |
| Podxl  | podocalyxin-like                                      | 1,050.493                                   | −0.18                             |
| SET    | SET nuclear proto-oncogene                           | 951.0317                                    | −0.11                             |
| QKI    | QKI, KH domain containing, RNA binding               | 776.3372                                    | −0.12                             |
| TMOD3  | tropomodulin 3 (ubiquitous)                          | 531.727                                     | −0.24                             |
| SKP1   | S-phase kinase-associated protein 1                  | 503.8126                                    | −0.08                             |
| ARHGAP24 | Rho GTPase-activating protein 24                    | 452.6906                                    | −0.23                             |
| MPP5   | membrane protein, palmitoylated 5 (MAGUK p55 subfamily member 5) | 270.9088                                   | −0.35                             |
| NRAS   | neuroblastoma RAS viral (v-ras) oncogene homolog     | 249.8165                                    | −0.15                             |
| ACSL4  | acyl-CoA synthetase long-chain family member 4       | 246.0129                                    | −0.09                             |
| EPB41LS | erythrocye membrane protein band 4.1 like 5         | 214.3441                                    | −0.24                             |
| ARHGAP28 | Rho GTPase-activating protein 28                    | 199.0145                                    | −0.07                             |
| SRGAP1 | SLIT-ROBO Rho GTPase-activating protein 1            | 172.8949                                    | −0.31                             |
| CORO2B | coronin, actin-binding protein, 2B                   | 172.8764                                    | −0.19                             |
| DENND5B | DENN/MADD domain containing 5B                  | 152.5768                                    | −0.12                             |
| SPOP   | speckle-type POZ protein                             | 146.9724                                    | −0.43                             |
| OGT    | O-linked N-acetylglucosamine (GlcNAc) transferase    | 137.9184                                    | −0.05                             |
| PCE1   | phospholipase C, epsilon 1                           | 128.7123                                    | −0.29                             |
| MAGI2  | membrane-associated guanylate kinase, WW and PDZ domain containing 2 | 120.5446                                   | −0.33                             |
| PDCD4  | programmed cell death 4 (neoplastic transformation inhibitor) | 109.5968                                   | −0.18                             |
| NFIA   | nuclear factor 1A                                    | 97.51652                                    | −0.1                              |
| Dst    | dystonin                                              | 97.29717                                    | −0.33                             |
| NFE2L1 | nuclear factor, erythroid 2-like 1                   | 56.95308                                    | −0.17                             |
| UBN2   | ubiquinol 2                                          | 44.89126                                    | −0.1                              |
| PURA   | purine-rich element binding protein A                 | 35.38958                                    | −0.12                             |
| SIK2   | salt-inducible kinase 2                              | 32.43537                                    | −0.12                             |
| ERMP1  | endoplasmic reticulum metalloproteinase 1            | 12.50254                                    | −0.1                              |
| HELLS  | helicase, lymphoid-specific                          | 1.301073                                    | −0.2                              |

**Endogenously expressed miRNAs are predicted to target few podocyte essential genes**

Since the miR-145-5p is predicted to target 32 podocyte essential genes (Table 1), among which genes in the small GTPase-mediated...
pathway are highly enriched, we wondered how many podocyte essential genes would be targeted by the miRNAs endogenously expressed in podocytes. We sequenced miRNA contents of podocytes (Figure 8). We sorted out the 10 most abundant miRNA families, which together accounted for 90.1% of total miRNA content in podocytes (Table 2). Comparisons of their targets with the predicted 611 podocyte essential genes (Table S1) showed that they could target 2 to 16 podocyte essential genes (10.2 on average) (Table 2), which are much fewer than that of miR-145-5p (32 genes) (Table 1). Moreover, there was not any significant enrichment of genes for any specific molecular and cellular processes among the predicted target genes according to GO analysis (data not shown). From the sequencing result as shown in Figure S8, we confirmed that miR-145-5p is indeed not expressed in podocytes.

**DISCUSSION**

In the present study, we found miR-145-5p induced podocyte injury both in vitro and in vivo. We speculated that miR-145-5p could induce podocyte injury because it might effectively target podocyte essential genes, and the targeted genes are enriched in important biological processes in podocytes.

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**Table 2. Top 10 most abundant miR families in podocytes and their targets of podocyte essential genes**

| miRs | let-7 | miR-26-5p | miR-10a | miR-486-5p | miR-22-3p | miR-27-3p | miR-30-5p | miR-125-5p | miR-99-5p | Total |
|------|-------|-----------|---------|-----------|-----------|-----------|-----------|-----------|-----------|-------|
| RPKM | 83,700 | 37,646    | 9,938   | 8,870     | 8,185     | 5,951     | 5,352     | 3,802     | 1,883     | 171,016|
| % total | 44.1 | 19.8 | 5.2 | 4.7 | 4.3 | 3.1 | 2.8 | 3.0 | 2.0 | 90.1 |
| Gene no. | 12 | 16 | 9 | 4 | 13 | 14 | 16 | 11 | 2 | average: 10.2 |

These miRNAs target a total of 72 podocyte essential genes, and GO functional annotation of the 72 genes did not yield any significant enrichment of terms, suggesting the endogenous miRNAs of podocytes do not affect podocyte homeostasis.
Proving the speculation requires identification of podocyte essential genes. Genome-wide identification of genes essential for a cell type was impossible until our recent study. We recently performed single-cell RNA-seq on mouse podocytes, and, based on the notion that genes that are expressed in every cell of the given cell type would be indispensable for the cell type, we identified genes essential for mouse podocytes.19 This work made our approach to predict a miRNA's toxicity to podocytes possible. However, we reported only 335 podocyte essential genes in the work due to the use of the stringent cutoff of 0.5 RPKM for defining expression of a gene in a podocyte.31–33 Since the typical number of genes that are required for survival and function of a cell type is about 2,000 (9%–10% of entire genes in the genome),31–33 which is far beyond 335, we thus lowered the expression cutoff to 0.1 RPKM, resulting in 611 genes that were considered commonly expressed in all single podocytes and used as the predicted podocyte essential genes for the present study.

To explore how miR-145-5p is toxic to podocytes, we performed GO functional annotation of the 32 genes and found that miR-145-5p may preferentially target small GTPase-mediated signal transduction (Figure 3; Table S2). The GTPase pathway has been extensively studied in podocytes and shown to be crucial for many cellular processes in podocytes.23,24,34,37 We therefore focused on this pathway. There are three GTPase-activating proteins, Arhgap24, Arhgap28, and Srgap1 among predicted podocyte essential genes that are possible miR-145-5p targets. Since miRNAs act on a pathway often through inhibiting multiple components in the pathway, it is possible that inhibition of each of the three GTPase-activating proteins and other proteins contributed to the injurious effect of miR-145-5p on podocytes. We tested Arhgap24 and Srgap1, which were known to regulate the members of the Rho family of small GTPases (RhoA, Rac1, and Cdc42) in podocytes; Arhgap24 mutations can cause focal segmental glomerulosclerosis (FSGS),35 and Srgap1 is important for podocyte foot process maintenance.36 The results confirmed that miR-145-5p reduced the expression of Arhgap24 and Srgap1 and induced activity alteration of RhoA, Rac1, and Cdc42. In addition to Arhgap24, Srgap1, and other GTPase-activating proteins, other genes involving small GTPase-mediated pathways (Table 2) may also mediate miR-145-5p-induced podocyte injury (e.g., PLCE1, which mutations have been shown to cause podocyte injury and kidney diseases).39 Besides the pathway of small GTPase-mediated signal transduction, the
other highly ranked predicted pathways enriched with miR-145-5p-targeted podocyte essential genes include those associated with cytoskeleton, cell junction, and cell protrusions (actomyosin structure organization, cell junction assembly, and axon guidance). These pathways and cellular processes have also been reported to be important for podocyte structure and function. Among the genes, Epb41l5 was reported to regulate actomyosin contractility and focal adhesion formation to maintain the kidney filtration barrier.40 In addition, the deletion of Podxl in podocytes was found to result in nephrotic syndrome and FSGS in mice.41 Thus, miR-145-5p toxicity to podocytes may involve its target genes in other important pathways in podocytes at the same time. Based on these studies, we suggest that toxicity of a miRNA is determined by whether the miRNA could effectively alter a pathway or process that is essential for the cells and that targeting effectiveness of the miRNA is determined by whether the miRNA could simultaneously target multiple components in the pathway. Accordingly, prediction of toxicity of a miRNA drug could be implemented by examining whether the miRNA could target many essential genes of the cell type and whether any pathways essential for the cell type are enriched in the functional annotation of the targeted essential genes of the cell type.

To support the validity of our approach that is based on podocyte essential genes and the enrichment of miR-145-5p targets in a critical function or process in podocytes, it would be helpful to learn how many podocyte essential genes are targeted by the miRNAs endogenously expressed in podocytes. As the endogenous miRNAs are not toxic to podocytes, they are expected to target very few podocyte essential genes. We then examined the 10 most abundant miRNA families (accounting for 90.1% of total miRNA content in podocytes) and found that they could target only 10.2 podocyte essential genes on average (Table 2). Importantly, there was not any significant enrichment of genes in a function or process according to GO analysis. These results support that our approach to predict miRNA toxicity is valid.

The applications of our method to investigate miRNA toxicity can be extended. First of all, it can be applied to any other cell types as long as they have been subjected to single-cell RNA-seq and their essential genes are thus available. At present, most cell types have undergone single-cell RNA-seq, and their essential genes can be determined by using our method.19 We expect that there will be databases of essential genes of all cell types that are derived from single-cell RNA-seq very soon. Based on these databases, a bioinformatics tool will be generated, which allows toxicity screening for all miRNAs in all cell types in the body, thereby facilitating the understanding of side effects of miRNA drugs, as well as the development of miRNA-based drugs, by skipping the miRNAs that are predicted to inhibit genes essential for a cell type and thus be toxic.
The idea of the approach can be adapted for extended uses. First, the approach can be used to associate diseases with the elevated levels of miRNAs in circulation and further explore roles of the miRNAs in the pathogenesis of the diseases. Second, the idea of the approach also applies to toxicity studies of miRNA inhibitor-based drugs. In this scenario, prediction and validation of upregulation of target genes of the miRNA can be conducted to identify the responsible genes whose upregulation results in injury of a cell type. Similarly, if an anti-miRNA drug is already known to be toxic to a cell type, it is feasible to identify the responsible genes in the cell type using the same approach as miRNA-based drugs.

**MATERIALS AND METHODS**

**Cell culture and transfection, EV isolation, and labeling**

Conditional immortalized human podocytes (kind gift from M. Saleem, University of Bristol, Bristol, UK) were cultured as described and are detailed in the supplemental methods. HEK293 cells were cultured in DMEM (high glucose) (Gibco-BRL, Gaithersburg, MD, USA) with 10% FBS and 1% penicillin-streptomycin. Jurkat cells were cultured in RPMI 1640 (Gibco-BRL, Gaithersburg, MD, USA) with 10% FBS and 1% penicillin-streptomycin. For transient transfection, Lipofectamine RNAiMAX (Life, China) was used following the manufacturer’s instructions. EVs were collected from cell culture medium through a series of differential centrifugation as described. The purity of EVs was assessed by electron microscopy and immunoblotting. To trace EVs in vitro and in vivo, EVs were labeled with PKH67 (Sigma) and DiR (Invitrogen) as previously described. Detailed methods are in the supplemental methods.

**Animals and treatment**

All following animal protocols and procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Jinling Hospital (2019JLHGKJDWLS-141). Eight-week-old male BALB/c or NPSH2-Cre/eGFP mice were injected with EVs or miRNA agomir through the tail vein. miR-145-5p inhibitor was delivered simultaneously with EVs using TransIT-EE Delivery Solution. Kidney samples were collected after mice were euthanized. For transmission electron microscopy and quantification of podocyte foot process effacement, renal cortex was minced, fixed in 2.5% glutaraldehyde, and post-fixed in phosphate-buffered 1% osmium tetroxide. Ultrathin sections (50 nm) were stained and examined by Hitachi 7500 transmission electron microscope (Hitachi, Tokyo, Japan). Three glomeruli per mouse were evaluated with five images for each glomerulus. Resulting images were analyzed by Gatan 2.0 software. Podocyte foot process width quantification was adapted from a previous report. Mean foot process width (FPW) was calculated by the equation FPW = π/4 × (ΣGBMlength/Σfoot process). Detailed methods are in supplemental methods.

**miR# in situ hybridization**

miR# was an artificial mutation of mmu-miR-21: mmu-miR-21 (5’-uag cuu auc aga cug augu uga-3’), mutant mmu-miR-21 (miR#) (5’-uag cuu auc aga cug caca aua-3’). Locked nucleic acid (LNA) probes were purchased from Exiqon (Copenhågen, Denmark). Mouse kidneys were fixed in 10% formaldehyde and incubated with 18% sucrose/PBS overnight at 4°C. Kidney sections (15 mm) were incubated with LNA miRNA probes labeled with digoxigenin at 55°C overnight. After wash, kidney sections were incubated with anti-digoxigenin antibody conjugated with alkaline phosphatase at 25°C.
for 3 h. Nitro-Blue-Tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate (NBT/BCIP) (Roche) was used for color development with substrates.

**Luciferase reporter assay**
The 3’ UTR of ARHGAP24 was obtained by PCR using human genomic DNA and inserted downstream of the pGL3-promoter (Promega, Madison, WI, USA). Luciferase assays were done using the Dual-Luciferase Report Assay System as described in the supplemental methods.

**Flow cytometric analysis of apoptosis via annexin V staining**
Podocyte apoptosis was measured by fluorescein isothiocyanate (FITC)-conjugated annexin V/PI apoptosis kit (Multisciences, China) as detailed in the supplemental methods.

**Podocyte adhesion and wound-healing assay**
Real-time adhesion and migration assays were performed using the xCELLigence system (ACEA Biosciences, China) in E-plate 16 and CIM plate16, respectively, according to the manufacturer’s instructions, and are detailed in the supplemental methods.

**Quantitation of the actin cytoskeleton, immunohistochemical staining, immunofluorescence staining of podocytes, western blotting, and GTPase activity assay**
See supplemental methods.

**Statistical analyses**
The data are presented as the mean ± SD. Differences between two groups were analyzed using the t test incorporated in Prism6 software (GraphPad Software, La Jolla, CA, USA). p < 0.05 was considered statistically significant. Differences among four groups were compared using the ANOVA method, and post hoc analyses were analyzed using the Bonferroni correction or Dunnett T correction. Differences in albuminuria between the groups at time points were analyzed by the ANOVA method, and post hoc analyses were analyzed using the let-7 microRNA family. Cell 120, 665–667.

**DECLARATION OF INTERESTS**
The authors declare no competing interests.

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