Supplementary Materials

Supplementary Figures

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Figure S1

A
Relative Expression >0.4
n=9

\[ p = \text{ns} \]

B
Relative Expression <0.4
n=36

\[ p = 0.0164 \]
Figure S1. Reduction in glomerular miR-146a levels correlate with progression to higher albuminuria in diabetic patients. A-B. Two graphs showing change in urinary albumin to creatinine ratio (ACR) in diabetic patients (n=45) over 5±1 years ($T_1$ = initial ACR measurement, at the time of kidney biopsy, $T_2$ = second ACR measurement 5±1 years later), where patients with relative glomerular miR-146a levels of >0.4 show slower increase in ACR over this time period and patients with relative miR-146a levels of <0.4 show progression to a higher level of ACR over the same period. Each dot represents an individual patient. Significance of difference between the two sets was determined using student’s t-test.
Figure S2
Figure S2. Expression level of podocyte markers Nephrin and WT1 are reduced in the kidney sections of diabetic mice. Bar graphs showing relative expression levels of Nephrin and WT1 in the kidney sections from 12 wk old BTBR WT and BTBR ob/ob mice, as measured by qRT-PCR. Their expression was normalized with Gapdh mRNA expression. Data shown are mean ± SEM (n=3). *, P<0.05.
Figure S3
Figure S3. Expression level of miR-146a and its targets in primary podocytes. A. A bar graph showing relative expression level of miR-146a in the kidney sections from 10-12 wk old WT and miR-146a−/− mice, as measured by qRT-PCR. Their expression was normalized using miR-361 miRNA expression. Data shown are mean ± SEM (n=3). *, P<0.05. B. Bar graphs showing relative expression level of miR-146a targets ErbB4 and Notch-1 in the primary podocytes isolated from the glomeruli of 10-12 wk old WT and miR-146a−/− mice, as measured by qRT-PCR. Their expression was normalized using GAPDH mRNA expression. Expression level of EGFR, not a miR-146a target, was not changed. Data shown are mean ± SEM (n=3). **, P<0.01, *, P<0.05.
Figure S4
Figure S4. WB analysis of ErbB4 expression level in primary podocytes and murine kidney sections. ErbB4 levels are basally increased in miR-146a−/− samples. Immunoblot analysis of total ErbB4 protein in the lysates from isolated glomeruli of WT or miR-146a−/− (−/−) animals (left blot) or lysates of cultured primary podocytes from isolated glomeruli of WT and miR-146a−/− (−/−) (right blot). β-actin was used as the loading control. Relative position of the molecular weight markers is shown on the left.
**Figure S5**

**A**

Luciferase Expression Vector

3' UTR sequence of Erbb4 or Notch1

miR146a

**B**

Inserted 3'-UTR Sequence

Wild type Erbb4 3' UTR: 5'-AAGAAATGTCCACATAACTTGTGGTAGATCCAGTCTTGTGTAAGGCCTGCCCTAGT

Mutated Erbb4 3' UTR: 5'-AAGAAATGTCCACATAACTTGCTAAAGATCCACCTAATG TGTAAGGCCTGCCCTAGT

Wild type Notch1 3' UTR: 5'-GGAAAAACATATCTGTTCGGAAGAAAATAAAAACCTAGGTTCTCAGAGCCTTTGATTTTCTCTG

Mutated Notch1 3' UTR: 5'-GGAAAAACATATCTGTTCGATCTACTTACACTGGGATCTCAGAGCCTTTGATTTTCTCTG
**Figure S5. Schematic of the luciferase reporter assay.** A. Schematic graph of luciferase reporter assay. Luciferase expression plasmid contains 3’-UTR sequence of our interest at the 3’-end of luciferase gene. Transfection of the luciferase expression plasmid into HEK293T cells produces the luciferase mRNA with 3’-UTR of our interest. In the absence of a specific miR-146a sequence to suppress the 3’-UTR, the luciferase protein product is produced, which can be detected using a luciferase assay. However, in the presence of a co-transfected miR-146a construct, and if this construct recognizes the inserted 3’-UTR sequence, the translation of luciferase mRNA is suppressed and the expression and activity of luciferase is reduced, which can be measured using a luciferase assay system. B. Sequence of the wide type and mutated 3’-UTRs of ErbB4 and Notch1 that were inserted in the luciferase vector are listed.
Figure S6
Figure S6. Ectopic expression of miR-146a results in reduced ErbB4 expression in murine podocytes. Immunoblot analysis of total ErbB4 protein in the lysates from cultured WT podocytes transduced with viral particles containing plasmids that express either miR-146a or a scrambled sequence. GAPDH was used as the loading control. Relative position of the molecular weight markers is shown on the left.
Figure S7
Figure S7. STZ treatment increases renal leukocyte infiltration that is suppressed by erlotinib. Diabetic milieu increases inflammation and tissue infiltration of leukocytes. Representative immunohistochemical images of anti-CD11b antibody stained murine kidney sections from WT (left panels) and miR-146a−/− (right panels) mice treated with vehicle alone (Control), with STZ and vehicle (STZ) or with STZ and erlotinib (STZ + Erl) at the end of the experiment (16 weeks post-STZ) and show increased leukocyte infiltration in the STZ-treated tissues, which is reduced in the STZ and erlotinib group. Images were taken at 40X magnification. Scale bar, 50 µm. A bar graph below shows quantification of CD11b positive cells in four independent, randomly selected fields observed at 10X magnification. Data shown are mean ± SEM (n=3-5/grp). *, P<0.05; **, P<0.01.
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**Figure S8**
miR-146 in podocytes and diabetic injury

Figure S8. ErbB4 and EGFR heterodimerize in cells. A. HEK293T cells were transfected with the plasmids expressing either EGFR or human ErbB4 and EGFR (as indicated) and cultured for 2 days. Subsequently, the lysates were immunoprecipitated with antibody against ErbB4 or an isotype control according to literature protocols. The immunoprecipitated extracts were immunoblotted (IB) for EGFR or ErbB4 using specific antibodies to study co-immunoprecipitation of ErbB4 with EGFR. Total cell lysates were used as a control to confirm expression of each protein. Relative position of the molecular weight markers is shown on the left.