Supplementary Materials

Supplementary Figures

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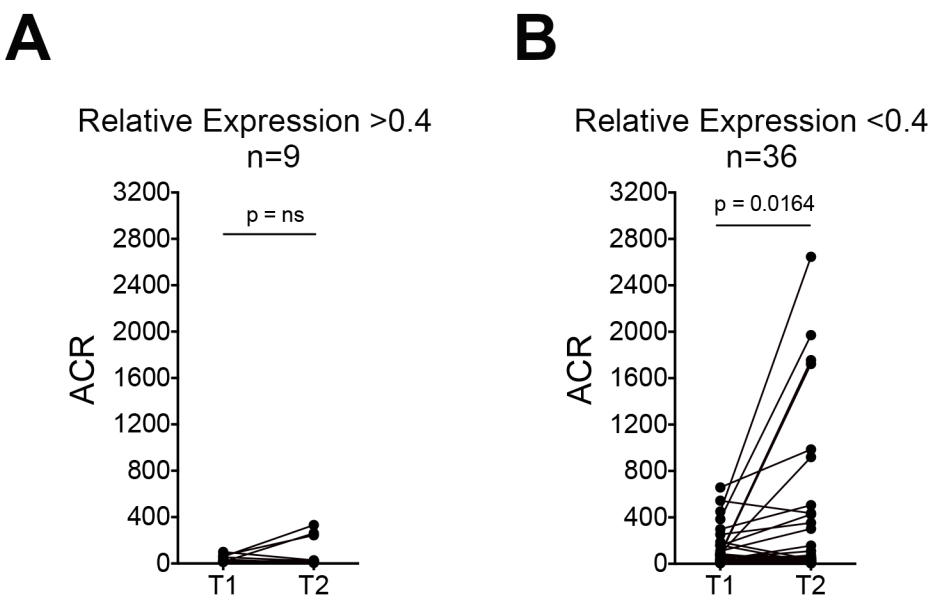

A
B
Relative Expression >0.4
n=9
p = ns
ACR
3200
2800
2400
2000
1600
1200
800
400
0
T1
T2
Relative Expression <0.4
n=36
p = 0.0164
ACR
3200
2800
2400
2000
1600
1200
800
400
0
T1
T2

Figure S1

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.

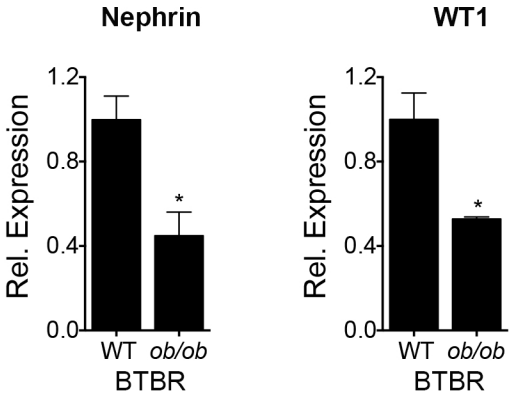


Figure S2

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). *, $P<0.05$.

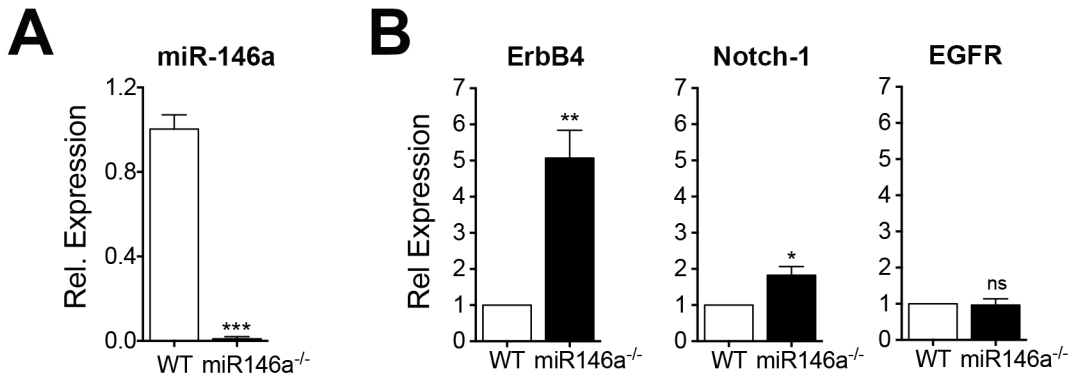

A
miR-146a
Rel. Expression
1.2
0.8
0.4
0.0
***
WT
miR146a-/-
B
ErbB4
Notch-1
EGFR
Rel Expression
7
6
5
4
3
2
1
0
**
*
ns
WT
miR146a-/-

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). **, $P<0.01$, *, $P<0.05$.

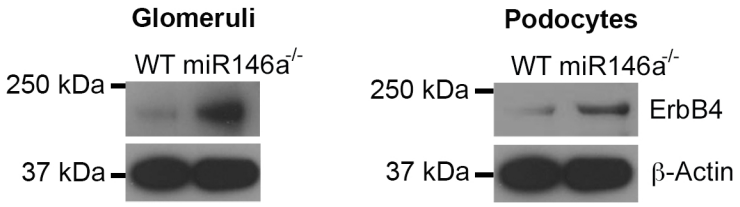
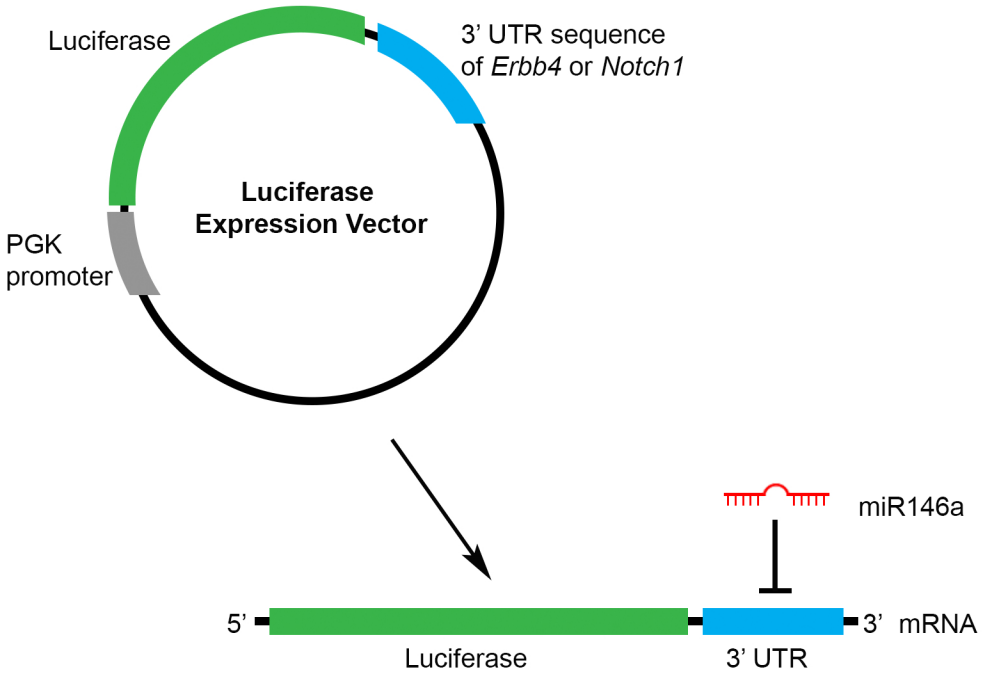

Glomeruli
WT miR146a-/-
250 kDa
37 kDa
Podocytes
WT miR146a-/-
250 kDa
ErbB4
37 kDa
β-Actin

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.

A



B

Inserted 3'-UTR Sequence

Wild type Erbb4 3' UTR: 5'-AAGAAATGTCCACATAACTTCGTGGTAGATTCCAGTTCTTGTGTACGAGCCTGCCCTAGT
Mutated Erbb4 3' UTR: 5'-AAGAAATGTCCACATAACTTGCTAAAAGATCCCAATTCTTATGTACGAGCCTGCCCTAGT
Wild type Notch1 3' UTR: 5'-GGAAAAACATATCTGTTCCAAGAAAATAAACTAGTTCTCAGAGCCTTGATTTTCCTGG
Mutated Notch1 3' UTR: 5'-GGAAAAACATATCTGTTCGATCTACTTACACTGGATCTCAGAGCCTTGATTTTCCTGG

Figure S5

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.

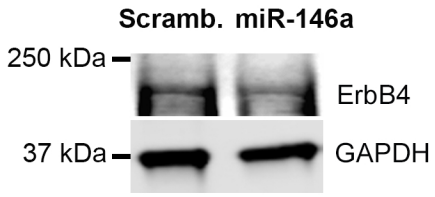


Figure S6

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.

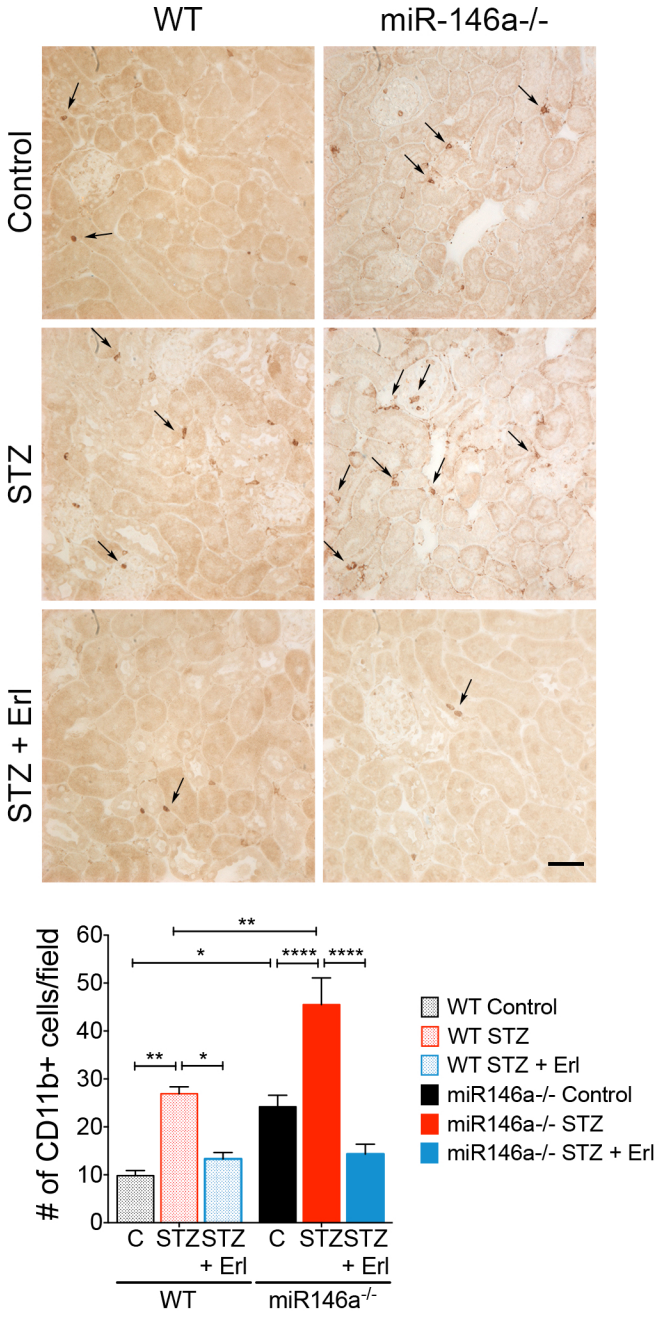

WT
miR-146a-/-
Control
STZ
STZ + Erl
# of CD11b+ cells/field
60
50
40
30
20
10
0
**
*
****
****
**
*
C
STZ
STZ + Erl
C
STZ
STZ + Erl
WT
miR146a-/-
WT Control
WT STZ
WT STZ + Erl
miR146a-/- Control
miR146a-/- STZ
miR146a-/- STZ + Erl

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). *, $P<0.05$; **, $P<0.01$.

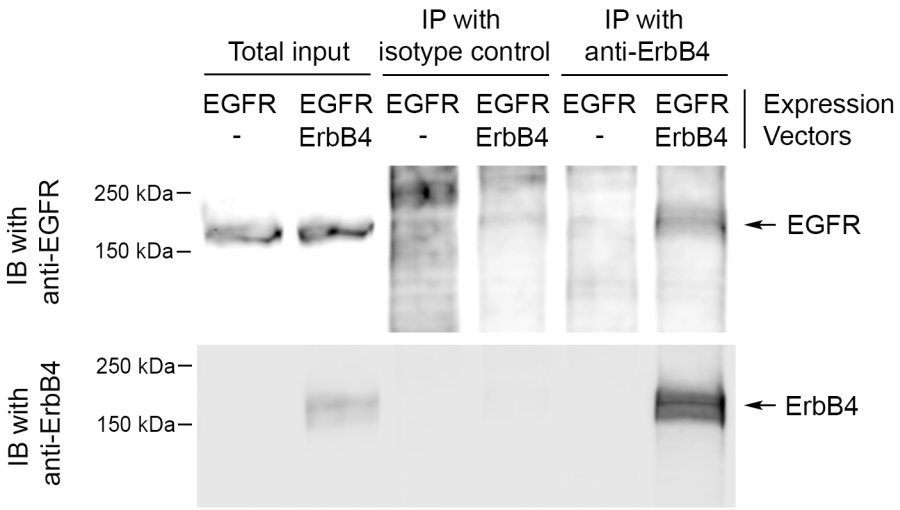

Total input
IP with isotype control
IP with anti-ErbB4
EGFR -
EGFR ErbB4
EGFR -
EGFR ErbB4
EGFR -
EGFR ErbB4
Expression Vectors
IB with anti-EGFR
250 kDa
150 kDa
EGFR
IB with anti-ErbB4
250 kDa
150 kDa
ErbB4

Figure S8

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.