

Supplemental Information

Therapeutic miR-21 Silencing Ameliorates Diabetic

Kidney Disease in Mice

Malte Kölling, Tamas Kaucsar, Celina Schauerte, Anika Hübner, Angela Dettling, Joon-Keun Park, Martin Busch, Xaver Wulff, Matthias Meier, Kristian Scherf, Nóra Bukosza, Gábor Szénási, Mária Godó, Amit Sharma, Michael Heuser, Peter Hamar, Claudia Bang, Hermann Haller, Thomas Thum, and Johan M. Lorenzen

Figure S1

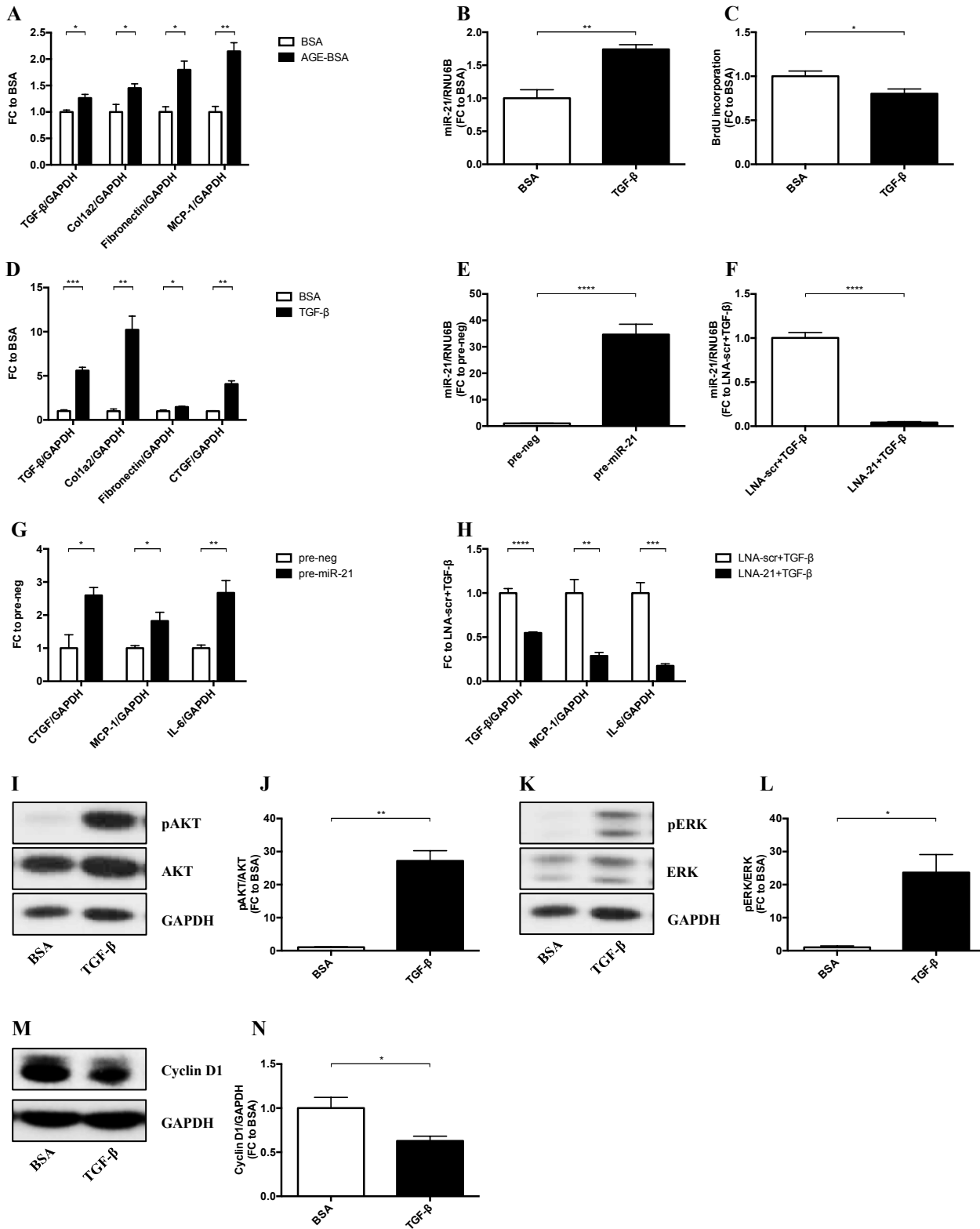


Figure S2

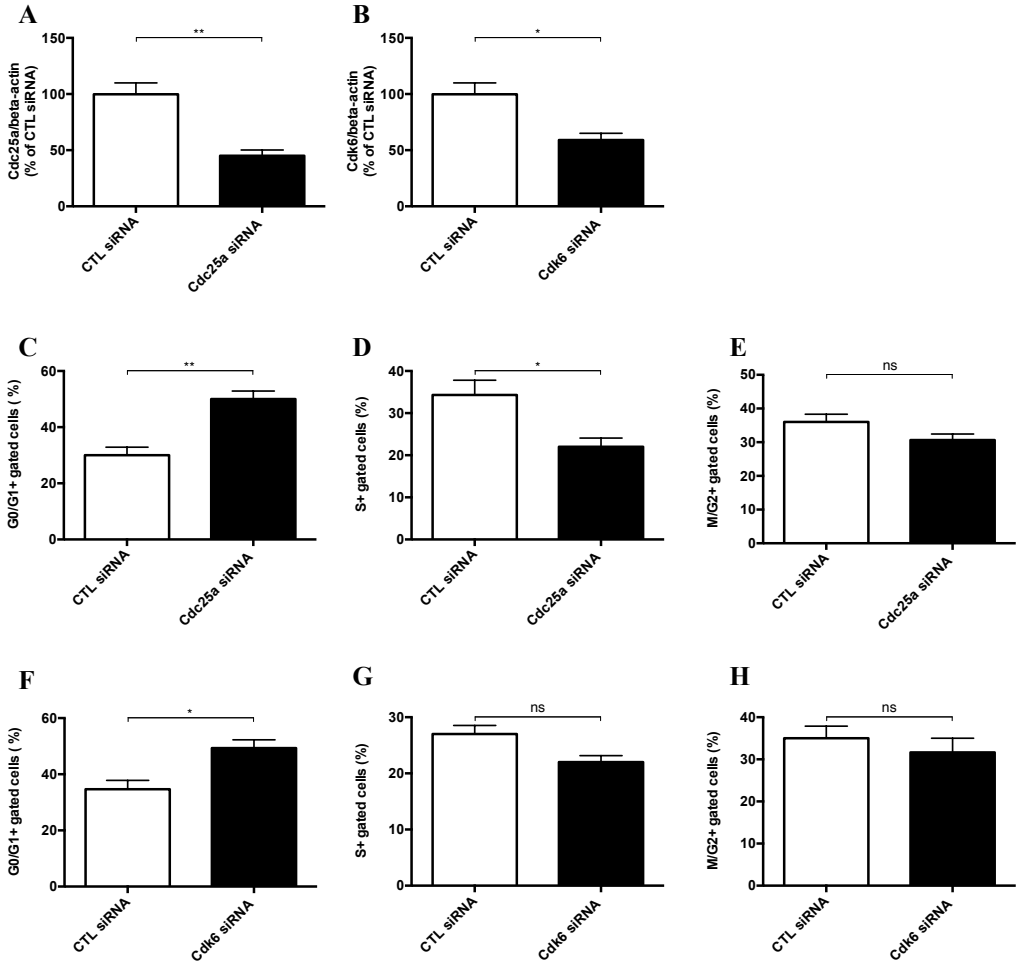


Figure S3

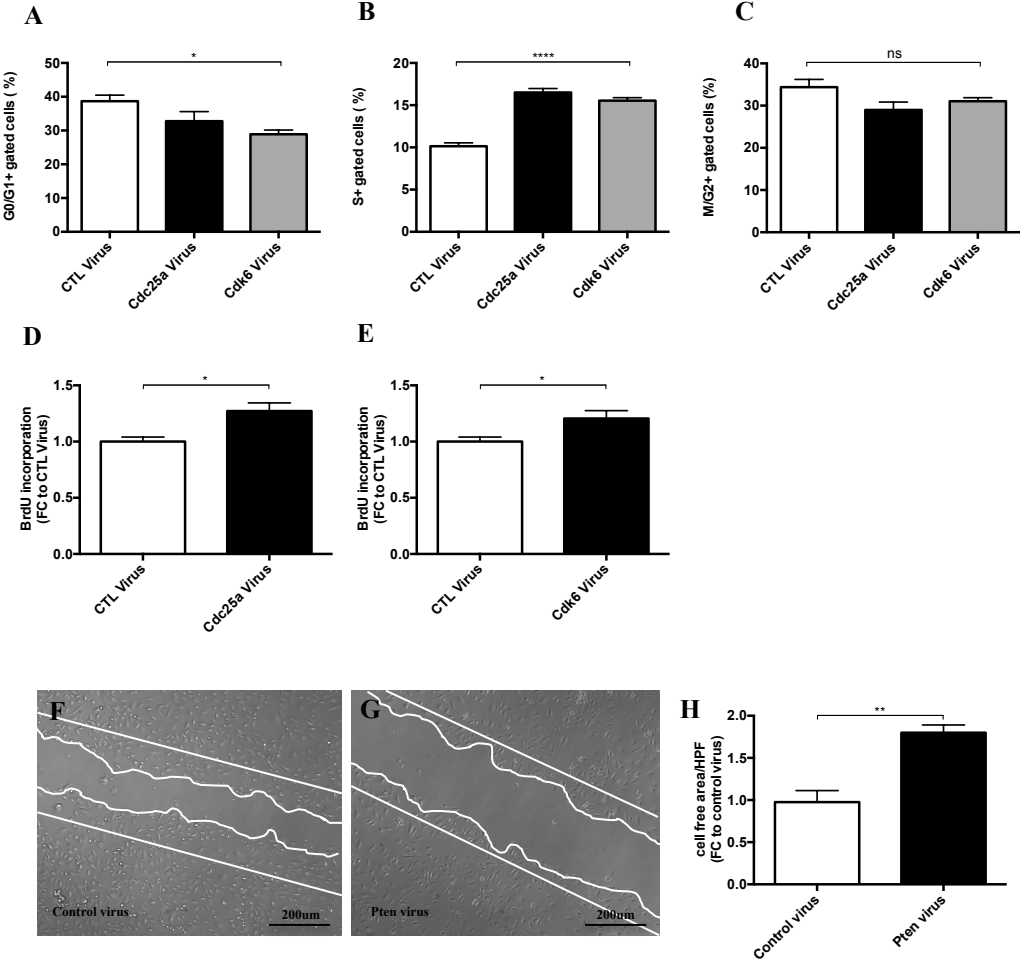


Figure S4

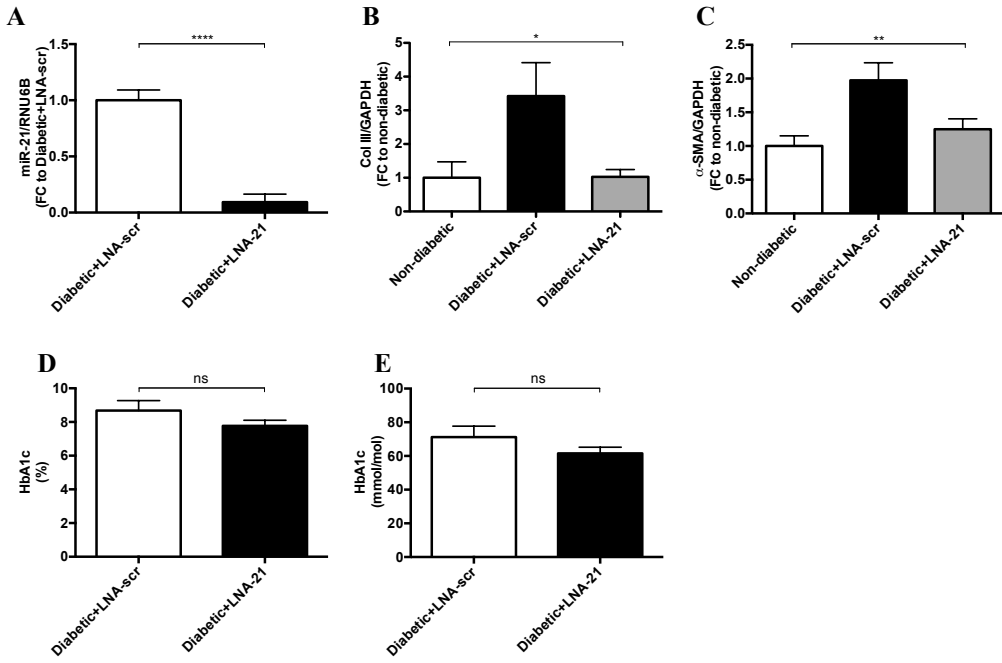
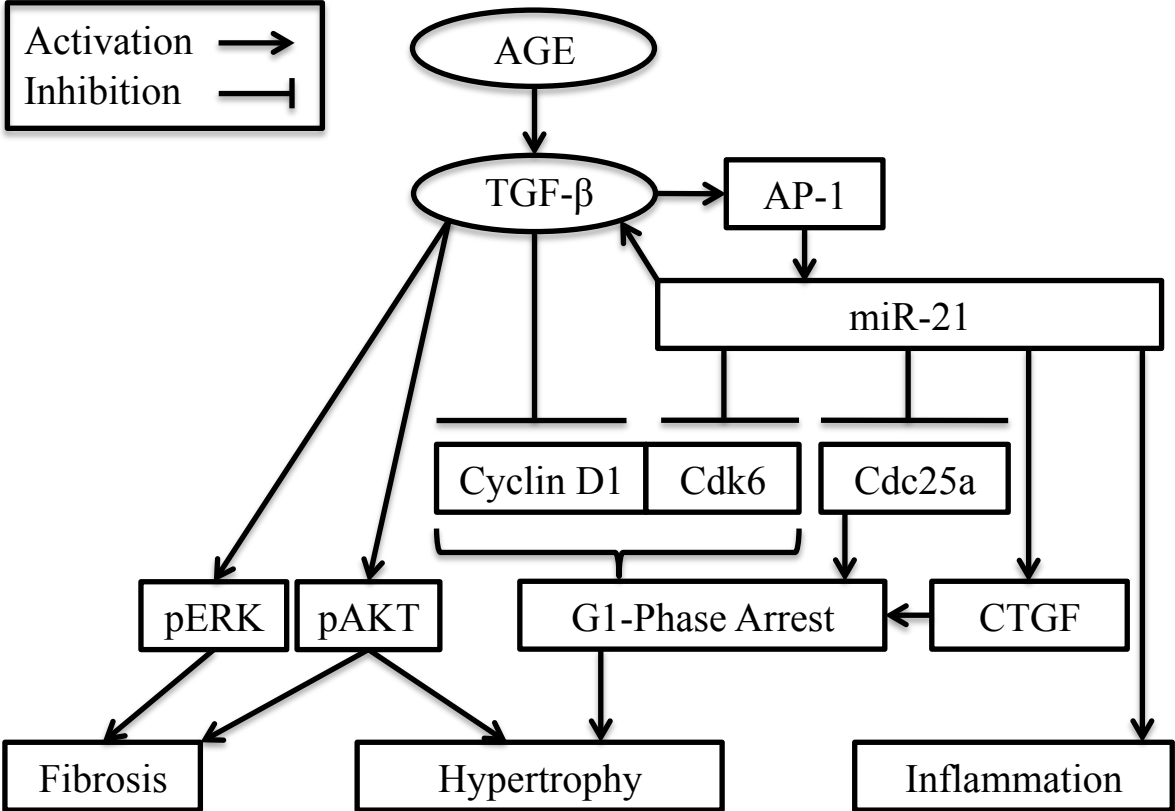


Figure S5



Supplemental Figure Legends

Figure S1: MiR-21 promotes inflammatory and pro-fibrotic processes in mesangial cells. qPCR quantifications of TGF- β , Colla2-, Fibronectin- and MCP-1 gene expression after treating mesangial cells with advanced glycation end products (A) (n=4). qPCR quantification of miR-21 (B) (n=4). BrdU incorporation in mesangial cells after TGF- β treatment (C) (n=6). qPCR quantifications of TGF- β , Colla2, Fibronectin and CTGF (n=3) after TGF β treatment (D). Efficacy of pre-miR-21/pre-neg transfection (E) (n=5) and LNA-21/LNA-scr (n=4) transfection with additional TGF- β treatment (F) by qPCR quantification of miR-21. qPCR quantification of CTGF, MCP-1 and Il-6 after overexpression of miR-21 (G) (n=3). qPCR quantification of TGF- β , MCP-1 and Il-6 after TGF- β treatment upon miR-21 silencing (H) (n=3). Western Blot analysis of AKT phosphorylation (I-J), ERK phosphorylation (K-L), and protein expression of Cyclin D1 (M-N) after TGF- β treatment (n=3). *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

Figure S2: Knockdown efficacy of Cdc25a- (A) and Cdk6 (B) siRNA (n=4). Quantification of mesangial cells detected in G0/G1-phase, S-phase and M/G2-phase after liposomal transfection of siRNA silencing either Cdc25a (C-E) or Cdk6 (F-H) compared to control siRNA, respectively (n=3). *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

Figure S3: Quantification of mesangial cells detected in G0/G1-phase, S-phase and M/G2-phase after adenoviral overexpression of Cdc25a and Cdk6 compared to Beta-Gal control virus, respectively (A-C) (n=4). BrdU incorporation in mesangial cells after adenoviral overexpression of Cdc25a and Cdk6 compared to Beta-Gal control virus, respectively (D-E) (n=4). Scratch Migration for 8h in podocytes with viral overexpression of Pten compared to viral overexpression of control (F-G); quantification of the cell free area after 8h migration (H) (n=4). *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

Figure S4: Therapeutic efficacy of miR-21 inhibition by LNA-21 in kidneys of diabetic mice (A). qPCR quantification of ColIII (B) and α -SMA (C) in kidneys of non-diabetic mice and both diabetic mice treated with LNA-scr and LNA-21. Glycated hemoglobin count (HbA1c) in % (D) and mmol/mol (E) in diabetic mice treated with LNA-21 and LNA-scrambled oligonucleotides. *P<0.05; **P<0.01; ***P<0.001; ****P<0.0001.

Figure S5: Summary of the main findings in MC. Hypertrophy, inflammation and fibrogenesis in MCs: Advanced glycation end products (AGE) upon diabetes induction result in an increase in TGF- β , thereby leading to an AP-1 dependent increase of miR-21, which in turn potentiates TGF- β . MiR-21 represses Cdc25a and Cdk6 leading to reduced G1/S phase transition. Protein production in G1-phase results in MC hypertrophy. TGF- β degrades Cyclin D1, further disturbing cell cycle progression. MiR-21 mediated CTGF induction further promotes G1-phase arrest. AKT and ERK signaling are activated by TGF- β . AKT signaling endorses MC hypertrophy. Both activated AKT and ERK signaling are involved in fibrogenesis. Enhanced levels of miR-21 provoke inflammation.

Table S1*Primer pairs used for gene expression analysis*

Gene symbol	Gene name	Primer pairs
Tgfb	transforming growth factor beta [<i>Mus musculus</i>]	Fwd: CAACAATTCCTGGCGTTACCTTGG Rev: GAAAGCCCTGTATTCCGTCTCCTT
Ctgf	connective tissue growth factor [<i>Mus musculus</i>]	Fwd: CCTAGCTGCCTACCGACTG Rev: TTAGAACAGGCGCTCCACTC
Col1a2	collagen, type I, alpha 2 [<i>Mus musculus</i>]	Fwd: CAGAACATCACCTACCACTGCAA Rev: TTCAACATCGTTGGAACCCTG
Fn1	fibronectin 1 [<i>Mus musculus</i>]	Fwd: CAGACCTACCCAGGCACAAC Rev: CAGCGACCCGTAGAGGTTTT
Acta2	actin, alpha 2, smooth muscle, aorta [<i>Mus musculus</i>]	Fwd: ACTACTGCCGAGCGTGAGAT Rev: AAGGTAGACAGCGAAGCCAG
Ccl2 (MCP-1)	chemokine (C-C motif) ligand 2 (Monocyte chemoattractant protein-1) [<i>Mus musculus</i>]	Fwd: GGCTCAGCCAGATGCAGTTA Rev: ACTACAGCTTCTTTGGGACA
Il6	interleukin 6 [<i>Mus musculus</i>]	Fwd: GAGAAAAGAGTTGTGCAATG Rev: ATTTTCAATAGGCAAATTC