Genetic deficiency or pharmacological inhibition of miR-33 protects from kidney fibrosis

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Running Title: miR-33 in renal fibrosis.

SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Renal SREBP2/miR-33 expression is not altered in folic acid and UUO-induce kidney fibrosis.

(**A-D**) *miR-33* (**A** and **C**) and *Srebp2* (**B** and **D**) expression in non-treated (NT) or folic acid (FA) treated mice (**A** and **B**) or in mice that underwent UUO surgery (**C** and **D**) (n=6). Statistical significance was

determined using unpaired two-sided Student's t-test. Data represent the mean ± S.E.M. of relative expression levels normalized to NT mice or CT kidney.

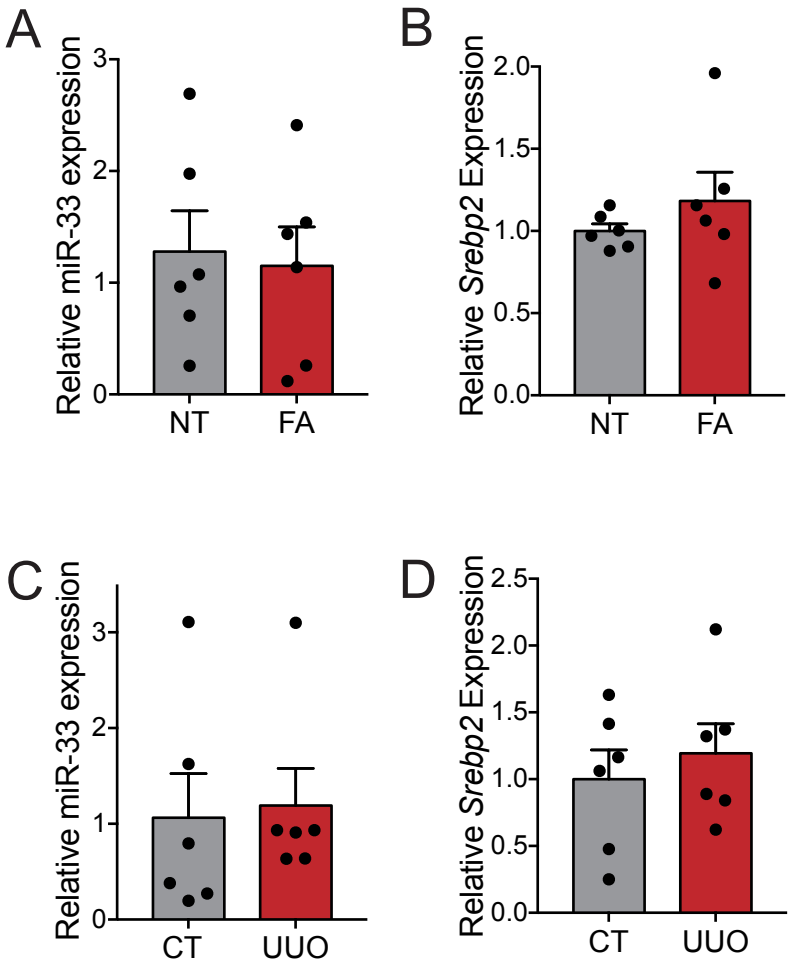
Supplemental Figure 2. miR-33 is expressed in the kidney and renal TECs. (**A**) miR-33 expression in several human tissues. Data obtained from the human miRNA tissue atlas project (https://ccb-web.cs.uni-saarland.de/tissueatlas/. Red arrow indicates miR-33 expression in the kidney. (**B**) Functional annotation of mammalian genome (FANTOM5) analysis of miR-33 expression analysis in 400 human primary cells (fanton.grs.riken.jp). Red squares highlight the expression of miR-33 in renal TECs. (**C**) qRT-PCR analysis of miR-33 expression in mouse primary renal TECs. CT value for the analysis is indicated in red. Statistical significance was determined using unpaired two-sided Student's t-test.

Supplemental Figure 3. miR-33 expression is not affected by TGFB treatment in renal TEC. qRT-PCR analysis of miR-33 expression in the HKC-8 human renal TEC line treated with TGFb for 24 and 48 h. Statistical significance was determined using non-parametric two-tailed Mann-Whitney U test.

Supplemental Figure 4. Gating strategy used for the analysis of pHLIP association to renal TECs. Representative dot plot FACS analysis of pHLIP associated to TECs treated with saline (left panel), pHLIP-A546-5K (middle panel) and pHLIP-A546 (right panel). LTL (lotus tetragonolobus lectin)-FITC labelling was used for the identification of renal TECs.

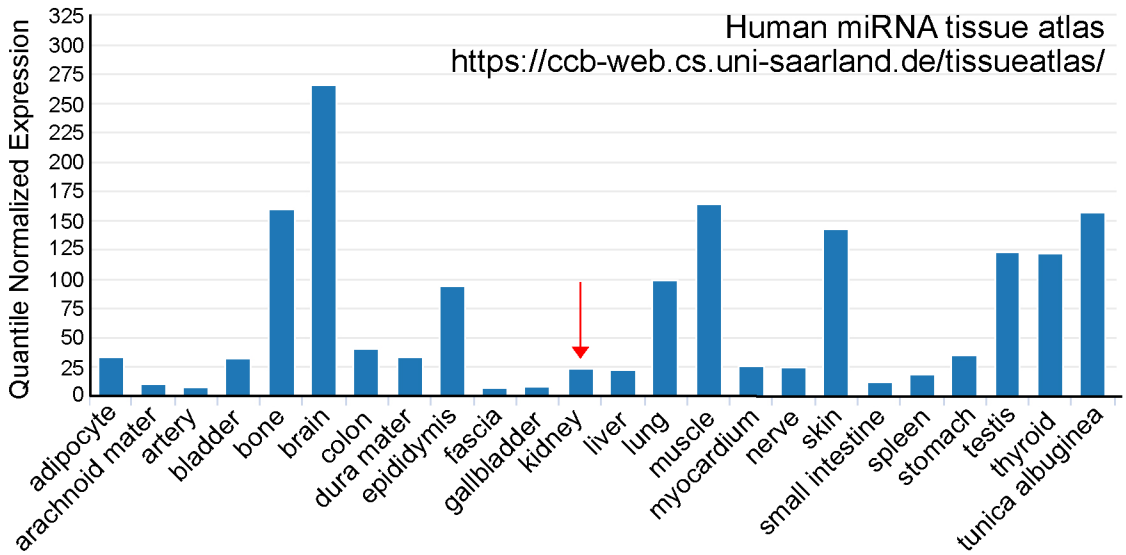
Supplemental Figure 5. miR-33 silencing does not influence renal AMPK and PGC1a expression. Representative WB analysis of PGC1a, AMPK, p-AMPK and vinculin in kidneys of mice injected with Src^{pHLIP} or anti-miR-33^{pHLIP} treated or not with folic acid.

Supplmental Figure 1



Supplemental Figure 2

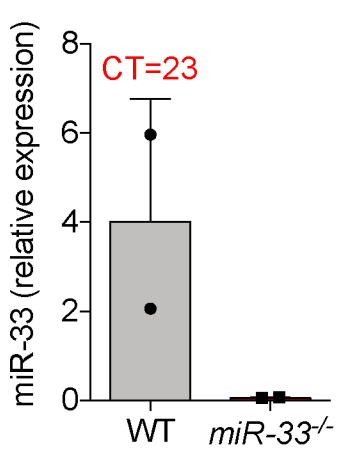
A



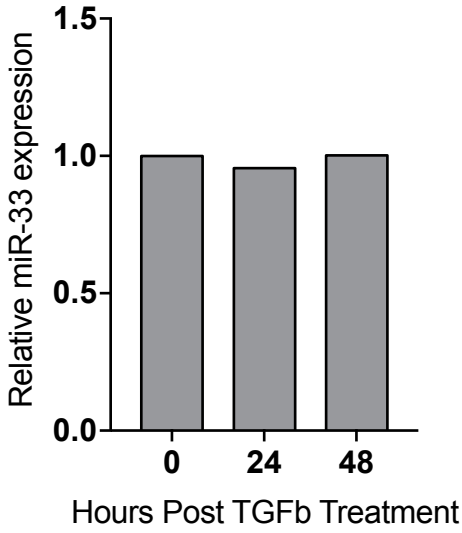
B

fanton.grs.riken.jp

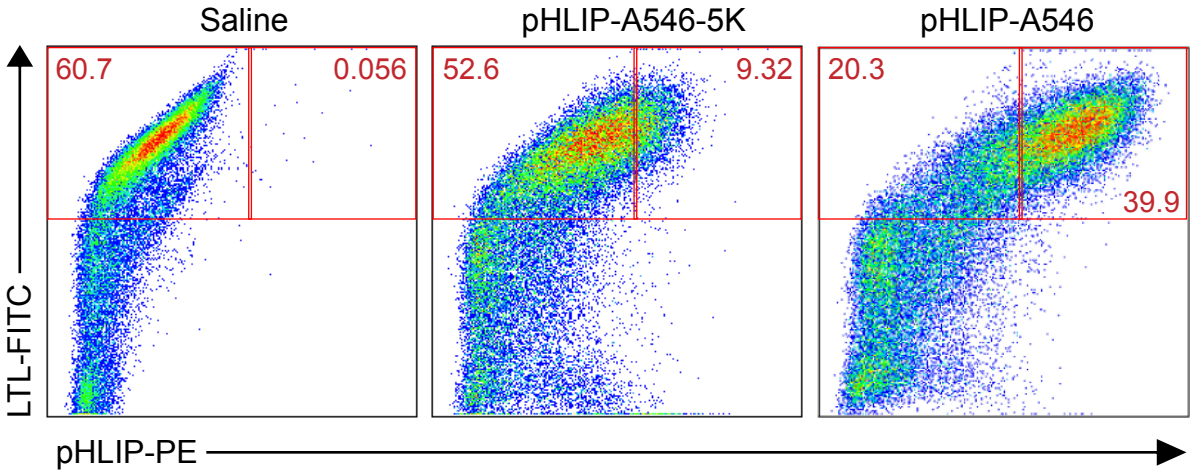
C



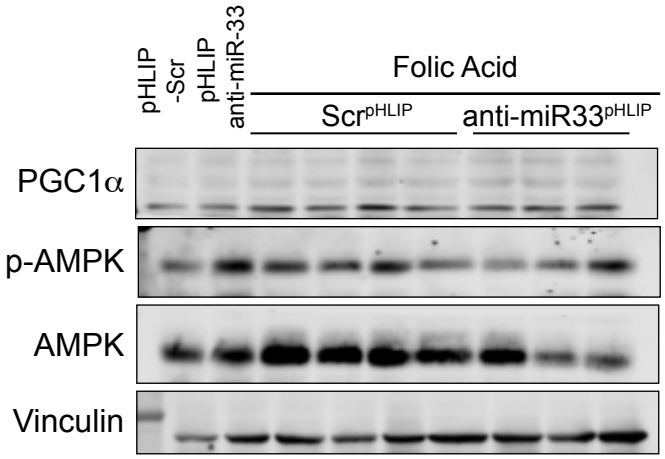
Supplemental Figure 3



Supplmental Figure 4



Supplemental Figure 5



Genotyping Primers	
miR-33 Knockout	FW: AGCCTGCTGGGCTCTCTGAGAC
	RE: AGCCGCTACCATGACATTCCAG
qPCR Primers	
αSMA	FW: CTGACAGAGGCACCACTGAA
	RE: CATCTCCAGAGTCCAGCACA
FN1	FW: ACCGACAGTGGTGTGGTCTA
	RE: CACCATAAGTCTGGGTCACG
COL1A1	FW: CTGCTGGCAAAGATGGAGA
	RE: ACCAGGAAGACCCTGGAATC
CPT1A	FW: TTGATCAAGAAGTGCCGGACGAGT
	RE: GTCCATCATGGCCAGCACAAAGTT
CROT	FW: ACTGAGAGTGAAGGGCATTGTCCA
	RE: AATGCCGCTATACTGGGTCCAACA
HADHβ	FW: CACTTTCGGGTTTGTTGCATCGGA
	RE: GCTGTGGTCATGGCTTGGTTTGAA
IL-6	FW: AGTTGCCTTCTTGGGACTGA
	RE: TCCACGATTTCCCAGAGAAC
IL-1β	FW: CCAAAATACCTGTGGCCTTGG
	RE: GCTTGTGCTCTGCTTGTGAG
18s	FW: TTCCGATAACGAACGAGACTCT
	RE: TGGCTGAACGCCACTTGTC
CD68	FW: CCAATTCAGGGTGGAAGAAA
	RE: CTCGGGCTCTGATGTAGGTC
TNFα	FW: CCCTCACACTCAGATCATCTTCT
	RE: GCTACGACGTGGCTACAG

Supplementary Table 1