Klotho-derived peptide 1 inhibits cellular senescence in the fibrotic kidney by restoring Klotho expression via posttranscriptional regulation

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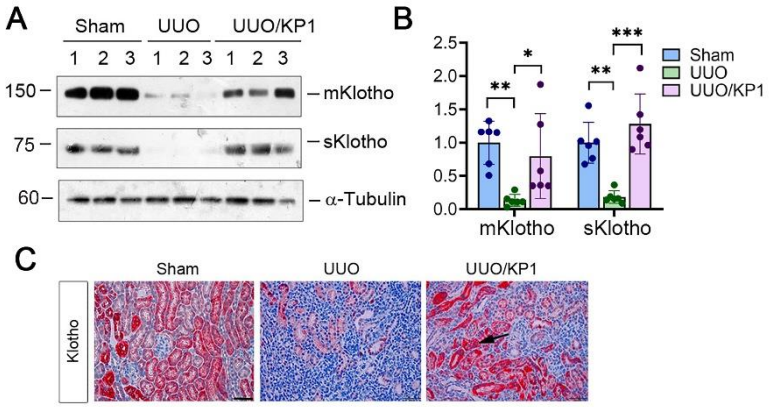

Figure S1. KP1 restores Klotho expression in the kidney of UUO mice. (**A**) Representative Western blot analyses showed renal expression of mKlotho, sKlotho proteins in UUO mice. (**B**) Quantitative data of mKlotho and sKlotho protein levels. (**C**) Representative immunostaining of Klotho in UUO mice. Arrows indicate positive staining. Scale bar, 50 μm. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

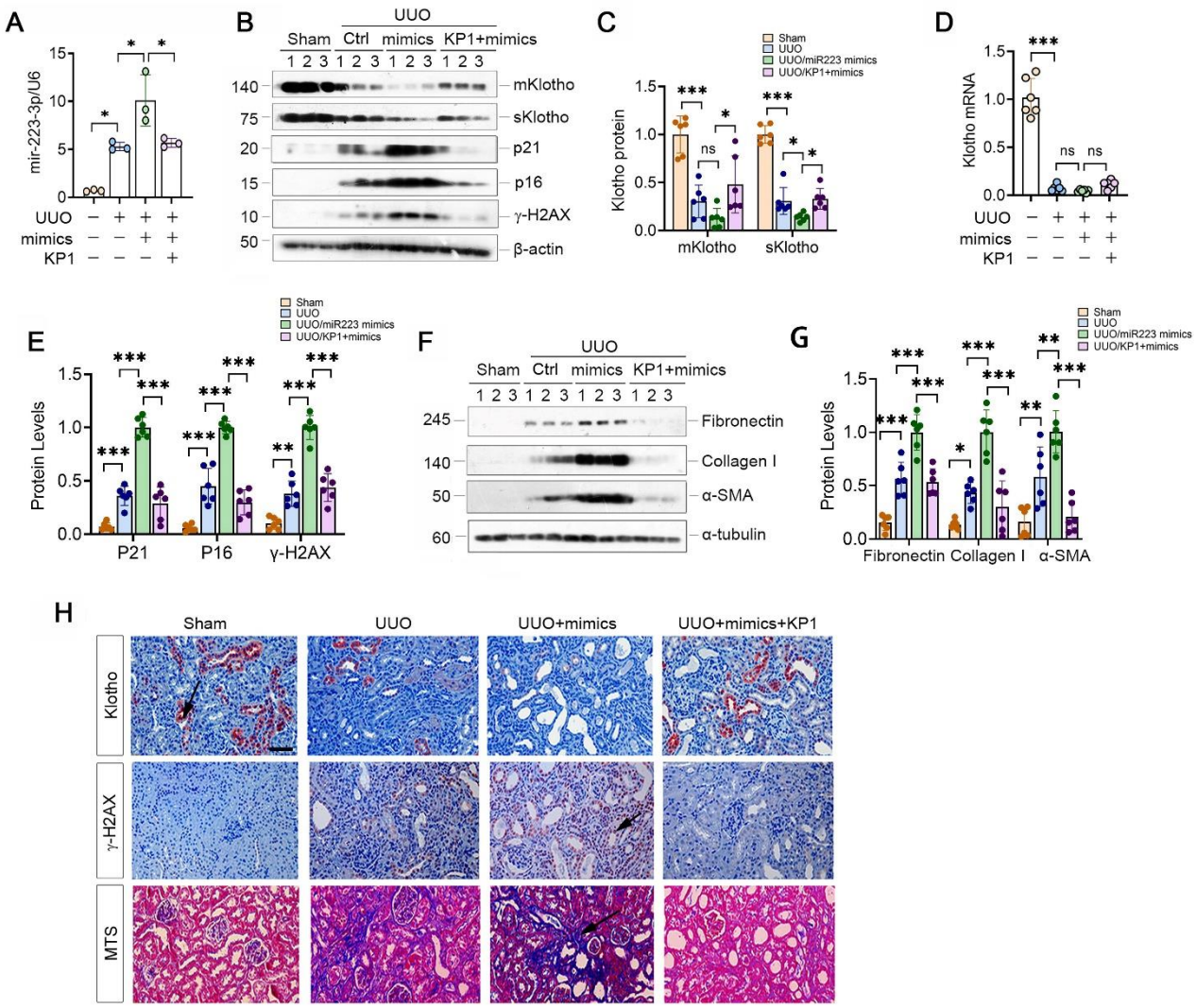

Figure S2. KP1 upregulates Klotho expression and ameliorates nephropathy by repressing miR-223-3p in UUO mice. (**A**) qRT-PCR analysis of miR-223-3p levels in different groups as indicated. (**B**) Representative Western blot analyses showed renal expression of mKlotho, sKlotho, p21, p16 and γ-H2AX proteins in UUO mice. (**C**) Quantitative data of mKlotho and sKlotho protein levels. (**D**) qRT-PCR analysis showed Klotho mRNA expression in different groups as indicated. (**E**) Quantitative data of p21, p16 and γ-H2AX proteins. (**F**, **G**) Representative Western blot (**F**) and quantitative data (**G**) showed renal expression of fibronectin, collagen I and α-SMA proteins in different groups as indicated. (**H**) Representative immunostaining of Klotho and γ-H2AX, and MTS for collagens in UUO mice. Arrows indicate positive staining. Scale bar, 50 μm. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, n=6. ns, no statistical difference.

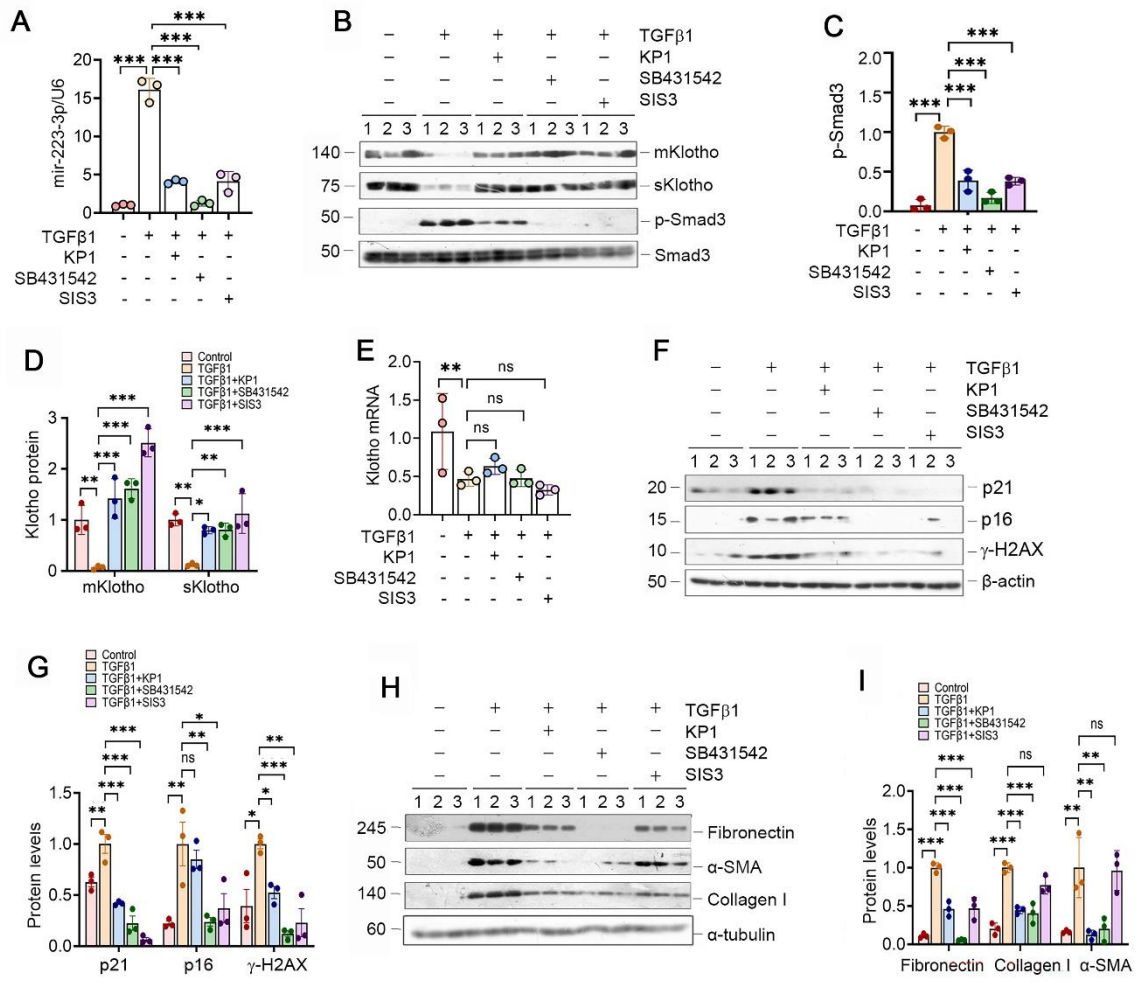

Figure S3. KP1 restores endogenous Klotho expression through inhibiting TGF-β/Smad3/miR-223-3p axis. (**A**) qRT-PCR analysis of miR-223-3p levels in TGF-β1-stimulated HK-2 cells in the absence or presence of KP1, SB431542 and SIS3, respectively. (**B**-**D**) Representative Western blot (**B**) and quantitative data (**C** and **D**) showed renal expression mKlotho, sKlotho and p-Smad3 proteins in TGF-β1-stimulated HK-2 cells in the absence or presence of KP1, SB431542 and SIS3. (**E**) qRT-PCR analysis of Klotho mRNA levels in different groups as indicated. (**F**, **G**) Representative Western blot (**F**) and quantitative data (**G**) showed the expression of p21, p16 and γ-H2AX proteins in TGF-β1-stimulated HK-2 cells in the absence or presence of KP1, SB431542 and SIS3. (**H**, **I**) Representative Western blot (**H**) and quantitative data (**I**) showed the expression of fibronectin, collagen I and α-SMA proteins in TGF-β1-stimulated HK-2 cells in the absence or presence of KP1, SB431542 and SIS3. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

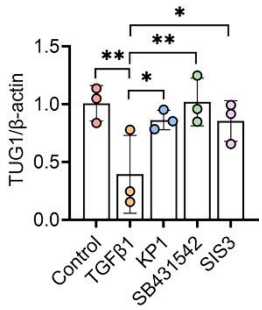

Figure S4. KP1 restores lncRNA-TUG1 expression in HK-2 cells after treatment with TGF-β1. HK-2 cells were treated with TGF-β1 in the absence or presence of KP1, SB431542 or SIS3. LncRNA-TUG1 levels were assessed by qRT-PCR. $^{*}P < 0.05$, $^{**}P < 0.01$.

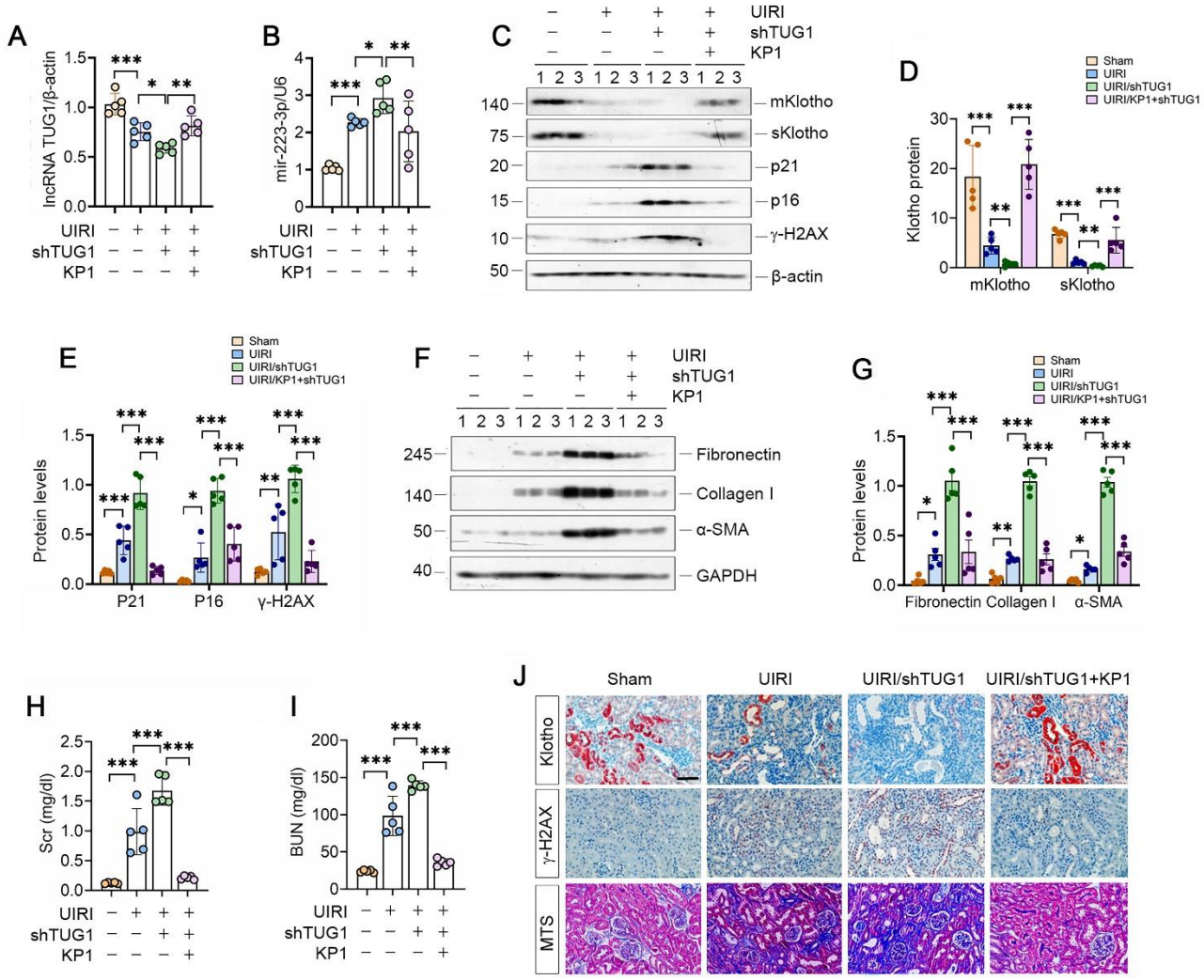

Figure S5. KP1 ameliorates kidney injury by restoring lncRNA-TUG1 and Klotho expression in the fibrotic kidney of UIRI mice. (**A**, **B**) qRT-PCR analysis of lncRNA-TUG1 (**A**) and miR-223-3p

(**B**) levels in UIRI mice injected with TUG1-shRNA in the absence or presence of KP1. (**C-E)** Representative Western blot (**C**) and quantitative data (**D**, **E**) showed renal expression of mKlotho and sKlotho, p21, p16 and γ-H2AX proteins in different groups as indicated. (**F**, **G**) Representative Western blot (**F**) and quantitative data (**G**) showed renal expression of fibronectin, collagen I and α-SMA proteins in different groups as indicated. (**H**, **I**) Serum creatinine (Scr) and blood urea nitrogen (BUN) levels in different groups as indicated. (**J**) Representative micrographs showed immunostaining for Klotho and γ-H2AX, and MTS for collagens in different groups as indicated. Scale bar, 50 μm. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, n=5.

Table S1. The sources of antibodies used in this study

Antibodies	Company	Catalogue number	Location
Primary antibodies			
Anti-Fibronectin	Sigma-Aldrich	F3648	St. Louis, MO
Anti-α-SMA	Sigma-Aldrich	A2547	St. Louis, MO
Anti-α-SMA	Abcam	AB7817	Cambridge, MA
Anti-COL1A1	Boster Biological Technology	BA0325	Wuhan, China
Anti-mKlotho	R&D Systems	AF1819	Minneapolis, MN
Anti-CDKN1A/p21CIP1	ABclonal	A2691	Cambridge, MA
Anti-p16 INK4A	Santa Cruz Biotechnology	Sc-1661	Dallas, TX
Anti-Histone H2AX	ABclonal	A11463	Cambridge, MA
Anti-gamma-H2AX (phospho)	Abcam	Ab26350	Cambridge, MA
Anti-Phospho-Smad3	ABclonal	AP0727	Cambridge, MA
Anti-Smad2/3	Cell Signaling Technology	8685S	Danvers, MA
Anti-TGFβ RII	Santa Cruz Biotechnology	Sc-17791	Dallas, TX
Anti-GAPDH	Ray Antibody	RM2002	Peachtree Corners, GA
Anti-β-actin	Ray Antibody	RM2001	Peachtree Corners, GA
Anti-α-tubulin	Ray Antibody	RM2007	Peachtree Corners, GA
Secondary antibodies			
Peroxidase-conjugated AffiniPure Goat Anti-Mouse IgG (H+L)	Jackson Immuno-Research Laboratories.	115-035-003	West Grove, PA
Peroxidase-conjugated AffiniPure Rabbit Anti-Goat IgG (H+L)	Jackson Immuno-Research Laboratories.	305-035-003	West Grove, PA
Peroxidase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L)	Jackson Immuno-Research Laboratories.	111-035-003	West Grove, PA
Biotin-SP-conjugated AffiniPure Donkey Anti-Goat IgG (H+L)	Jackson Immuno-Research Laboratories.	705-065-147	West Grove, PA
Biotin-SP-conjugated AffiniPure Donkey Anti-Rabbit IgG (H+L)	Jackson Immuno-Research Laboratories.	711-065-152	West Grove, PA
Biotin-SP-conjugated AffiniPure Donkey Anti-Mouse IgG (H+L)	Jackson Immuno-Research Laboratories.	715-065-150	West Grove, PA

Table S2. The nucleotide sequences of the primers used for qPCR

Gene	Primer Sequences 5' to 3'	
	Forward	**Reverse**
mmu-Klotho	AAAGTAGACGGGGTTGTAGCC	CGGTAGAAGTGCAGAACCGT
hsa-Klotho	GTGCGTCCATCTGGGATACG	TGTCGCGGAAGACGTTGTT
has-miR-223-3p	UGUCAGUUUGUCAAAUACCCCA	
mmu-miR-223-3p	UGUCAGUUUGUCAAAUACCCCA	
hsa-TUG1	TAGCAGTTCCCCAATCCTTG	CACAAATTCCCATCATTCCC
mmu-TUG1	GAGACACGACTCACCAAGCA	GAAGGTCATTGGCAGGTCCA

Table S3. The nucleotide sequences of Oligo DNA/RNA used in this study

RNA	Sequences 5'to 3'
hsa-miRNA inhibitor N.C.	CAGUACUUUUGUGUAGUACAA
hsa-miR-223-3p inhibitor	UGGGGUAUUUGACAAACUGACA
hsa-miRNA mimics N.C.	sense: UUCUCCGAACGUGUCACGUTT
	antisense: ACGUGACACGUUCGGAGAATT
hsa-miR-223-3p mimics	sense: UGUCAGUUUGUCAAAUACCCCA
	antisense: GGGUAUUUGACAAACUGACAUU
siRNA N.C.	sense: UUCUCCGAACGUGUCACGUTT
	antisense: ACGUGACACGUUCGGAGAATT
siTUG1-homo-1144	sense: GCUACAACUAUCUUCCUUUTT
	antisense: AAAGGAAGAUAGUUGUAGCTT
siTUG1-homo-222	sense: GAGCAGGCUAUCAGAAUAATT
	antisense: UUAUUCUGAUAGCCUGCUCTT
siTUG1-homo-3739	sense: GCCUCUAUUCCUGUAUGUATT
	antisense: UACAUACAGGAAUAGAGGCTT
siRNA-TUG1-4134	sense: GGACAAACUUAUCUCUCAUTT
	antisense: AUGAGAGAUAAGUUUGUCCTT
mmu-miRNA-mimics-NC	sense: AATTCGTTCTCCGAACGTGTCACGTGTTTTGG
	CCACTGACTGACACGTGACATTCGGAGAAA
	antisense: CCGGTTTCTCCGAATGTCACGTGTCAGTCAGT
	GGCCAAAACACGTGACACGTTCGGAGAACG
mmu-miR-223-3p mimics	sense: TGCTGTGTCAGTTTGTCAAATACCCCAGTTTTGG
	CCACTGACTGACTGGGGTATGACAAACTGACA
	antisense: CCTGTGTCAGTTTGTCATACCCCAGTCAGTCAGT
	GGCCAAAACTGGGGTATTTGACAAACTGACAC
mmu-miR-223-3p antagomir	UGGGGUAUUUGACAAACUGACA (3'cholesterol modification)
mmu-shRNA-NC	sense: CACCGTTCTCCGAACGTGTCACGTTTCAA
	GAGAACGTGACACGTTCGGAGAACTTTTTTG
	antisense: GATCCAAAAAAGTTCTCCGAACGTGT
	CACGTTCTCTTGAAACGTGACACGTTCGGAGAAC
mmu-shRNA-TUG1-2335	sense: CACCGCATATTGTCAACCTGTTTGCTTCAAGA
	GAGCAAACAGGTTGACAATATGCTTTTTTG
	antisense: GATCCAAAAAAGCATATTGTCAACCTGTTTGCT
	CTCTTGAAGCAAACAGGTTGACAATATGC
mmu-shRNA-TUG1-500	sense: CACCGCACTGTCACTGGGAACTTGATTCAAG
	AGATCAAGTTCCCAGTGACAGTGCTTTTTTG
	antisense: GATCCAAAAAAGCACTGTCACTGGGAACTTGA
	TCTCTTGAATCAAGTTCCCAGTGACAGTGC
mmu-shRNA-TUG1-3525	sense: CACCGCTACTGCAATTAGACTAACTTTCAAG
	AGAAGTTAGTCTAATTGCAGTAGCTTTTTTG
	antisense: GATCCAAAAAAGCTACTGCAATTAGACTAAC

		TTCTCTTGAAAGTTAGTCTAATTGCAGTAGC
mmu-shRNA-TUG1-277	sense:	CACCGGAGGTTCATAAAGTACATGCTTCAA
		GAGAGCATGTACTTTATGAACCTCCTTTTTTG
	antisense:	GATCCAAAAAGGAGGTTCATAAAGTACATG
		CTCTCTTGAAGCATGTACTTTATGAACCTCC

Table S4. The plasmid vectors of Oligo DNA/RNA and control vectors used in this study

Oligo DNA/RNA	Plasmid vectors	Control vectors
hsa-miR-223-3p inhibitor	microRNA inhibitors	hsa-miRNA inhibitor N.C.
hsa-miR-223-3p mimics	Double-stranded microRNA mimics	hsa-miRNA mimics N.C.
mmu-miR-223-3p antagomir	miR-DownTM antagomir	mmu-miRNA antagomir N.C.
mmu-miR-223-3p mimics	CMV/EGFP/miR/Blasticidin	mmu-miRNA-mimics-NC
siTUG1-homo-1144	Double-stranded siRNA	siRNA N.C.
siTUG1-homo-222	Double-stranded siRNA	siRNA N.C.
siTUG1-homo-3739	Double-stranded siRNA	siRNA N.C.
siTUG1-homo-4134	Double-stranded siRNA	siRNA N.C.
mmu-shRNA-TUG1-2335	pGPU6/GFP/Neo	pGPU6/GFP/Neo shNC
mmu-shRNA-TUG1-500	pGPU6/GFP/Neo	pGPU6/GFP/Neo shNC
mmu-shRNA-TUG1-3525	pGPU6/GFP/Neo	pGPU6/GFP/Neo shNC
mmu-shRNA-TUG1-277	pGPU6/GFP/Neo	pGPU6/GFP/Neo shNC
pmirGLO-h-KL-miR223-wt	pmirGLO Vector	pmirGLO-h-miR223-mut
pmirGLO-h-NR152868.2-miR223-wt	pmirGLO Vector	pmirGLO-h-NR152868.2-miR223-mut