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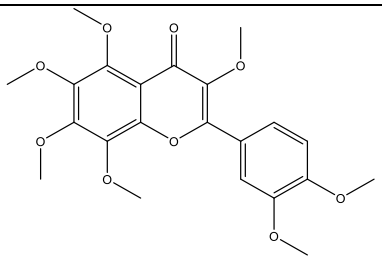
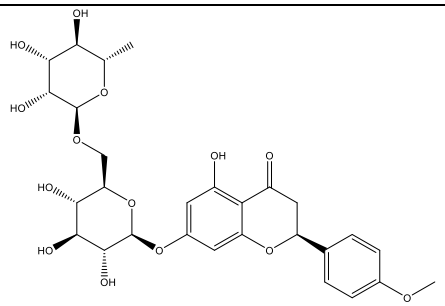
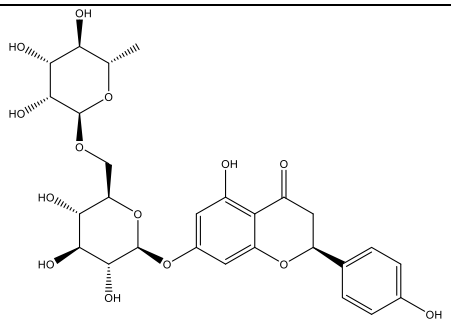
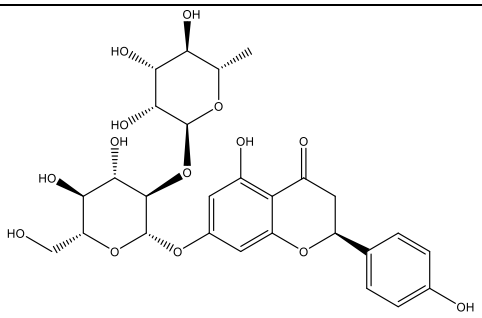
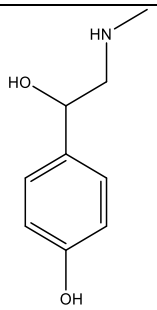
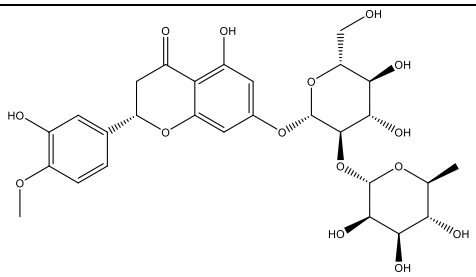
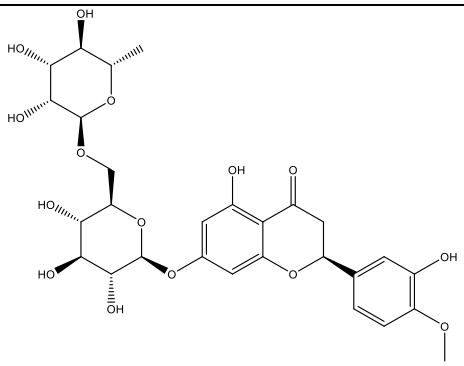
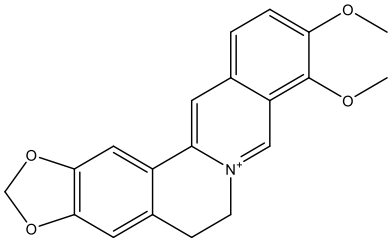
Table S1 Clinical information of the subjects.

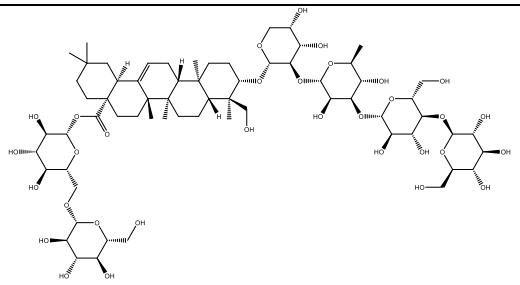
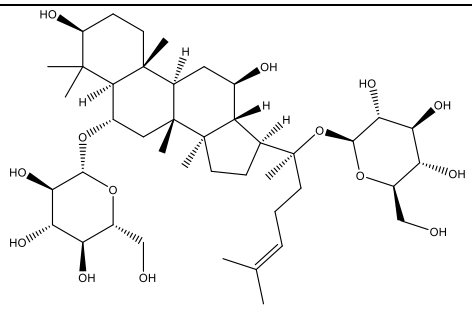
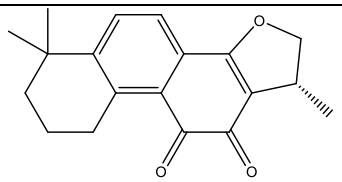
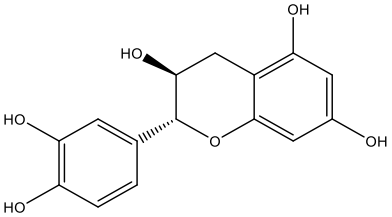
Characteristics	**Renal Fibrosis (*n* = 5)**	**Non-Renal Fibrosis (*n* = 4)**	***p* value**
Age (years)	41.6±12.7	23.25±10.3	0.05272
Male (n, %)	3 (20%)	1 (25%)	0.35582
SCr (μmol/L)	212.96±60.03	54.5±12.71	0.001368
BUN (mmol/L)	9.38±3.92	3.65±0.98	0.025749
eGFR (mL/(min*1.73m^2))	30.79±9.26	129.34±17.21	3.71E-05
Comorbidities/Medical history			
Hypertension	3 (60%)	0	
Diabetes mellitus	0	0	
COPD or asthma	1 (25%)	0	
Coronary disease	0	0	
Morbid obesity	0	0	
Neurologic	1 (25%)	0	
Neoplasms (extra-renal)	0	0	

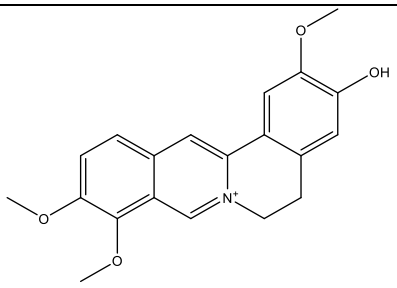
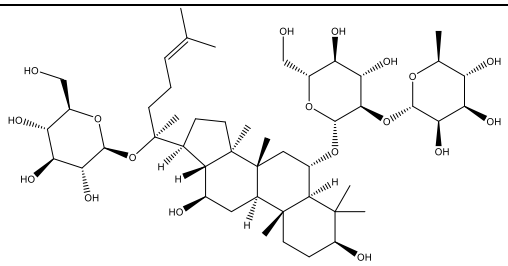
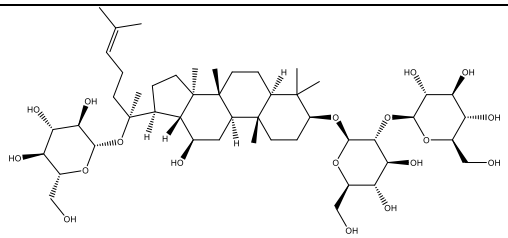
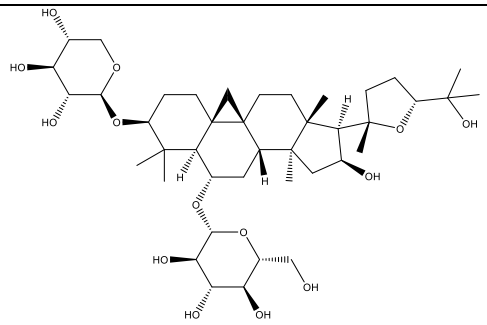
Unpaired two-tailed *t*-test. SCr: serum creatinine; BUN: Blood urea nitrogen. eGFR: Estimated glomerular filtration rate; COPD: chronic obstructive pulmonary disease.

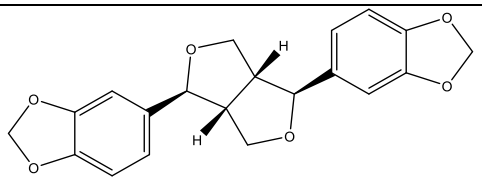
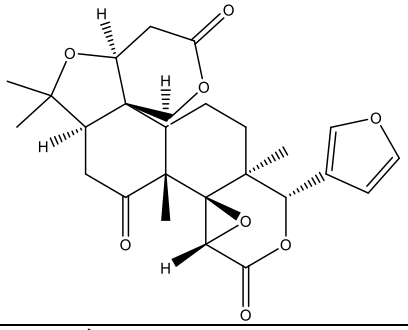
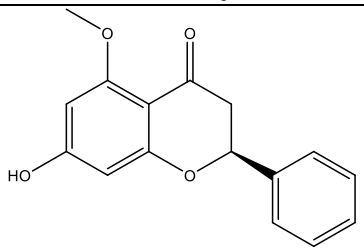
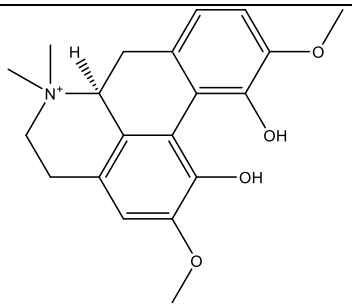
Table S2 The inhibitory rate of 67 compounds on NEU4 enzyme activity.

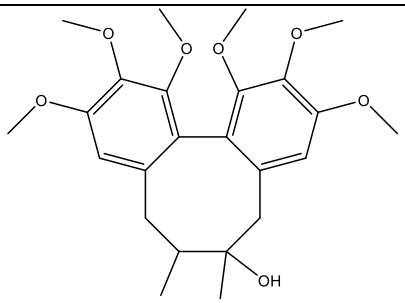
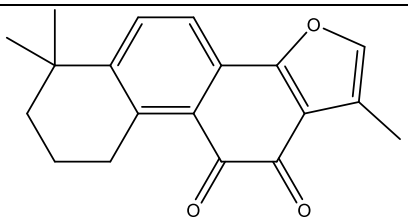
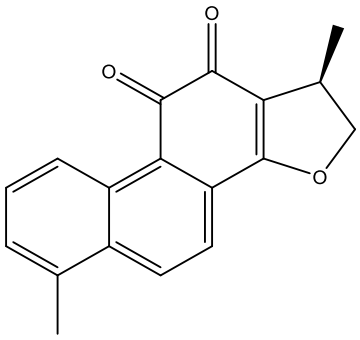
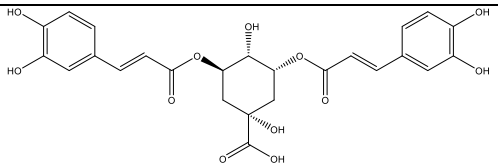
ID	Natural compound	Inhibition ratio±SD	Soureces	Structure
1	3,5,6,7,8,3′,4′-Heptamethoxyflavone	44.6%±0.016	Biopurify Phytochemicals	
2	Didymin	10.9%±0.01	Biopurify Phytochemicals	
3	Narirutin	14.7%±0.023	Biopurify Phytochemicals	
4	Naringin	16.9%+0.03	Must bio-technology	

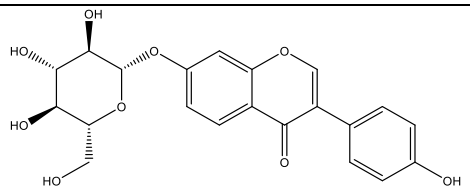
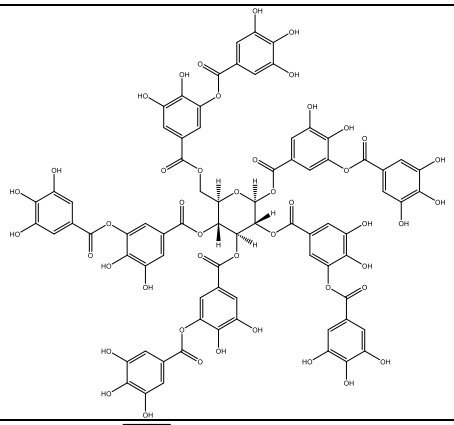
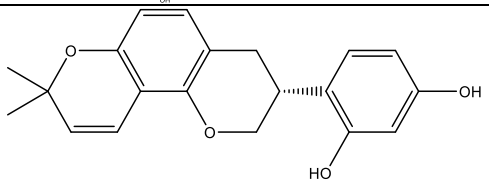
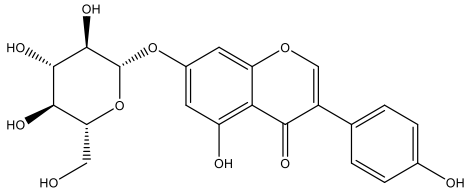
5	Synephrine	22.5%±0.074	Must bio-technology	
6	Neohesperidin	27.5%±0.009	Prepared in our laboratory	
7	Hesperidin	25.7%±0.013	Must bio-technology	
8	Berberine	15.6%±0.024	Must bio-technology	

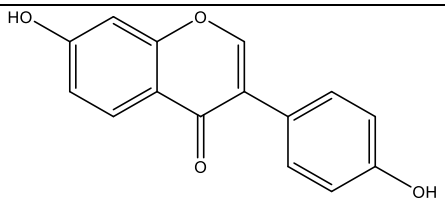
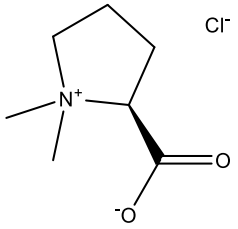
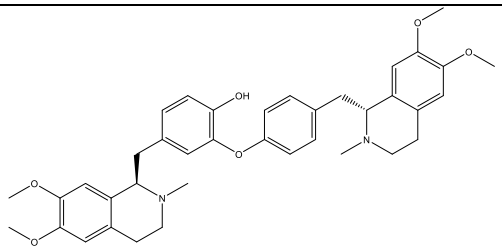
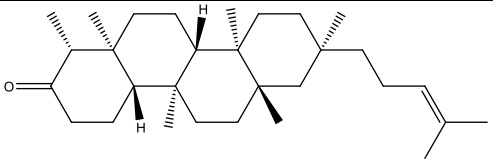
9	Macranthoidin B	6.4%±0.033	Must bio-technology	
10	Ginsenoside Rg1	2.1%±0.014	Must bio-technology	
11	Cryptotanshinone	6.4%±0.032	Must bio-technology	
12	Catechin	16.2%±0.008	Must bio-technology	

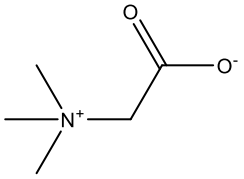
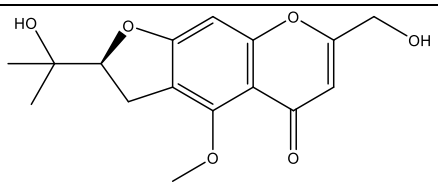
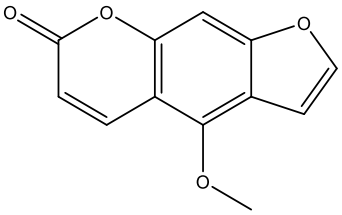
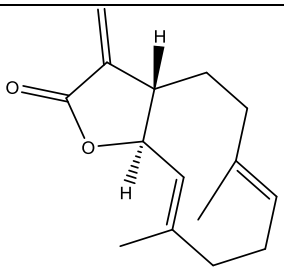
13	Jatrorrhizine	15.6%±0.04	Must bio-technology	
14	Ginsenoside Re	7.6%±0.012	Must bio-technology	
15	Ginsenoside Rd	11.3%±0.009	Must bio-technology	
16	Astragaloside A	10.5%±0.02	Must bio-technology	

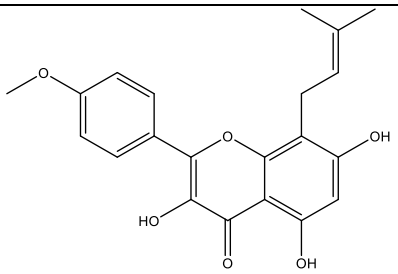
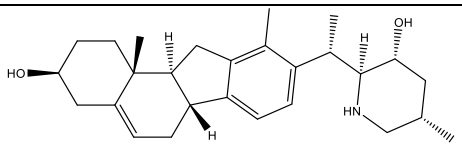
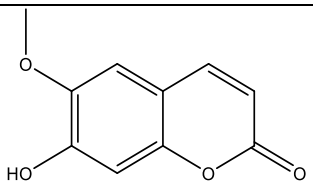
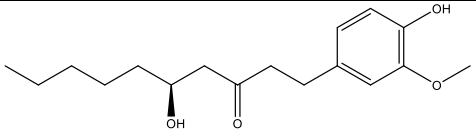
17	Sesamin	13.9%±0.018	Must bio-technology	
18	Limonin	10.6%±30.011	Must bio-technology	
19	Alpinetin	14.1%±0.04	Must bio-technology	
20	Magnoflorine	-182.4%±0.198	Must bio-technology	

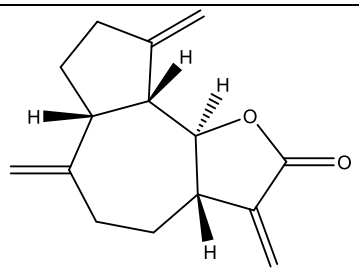
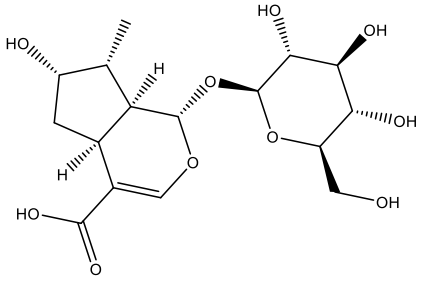
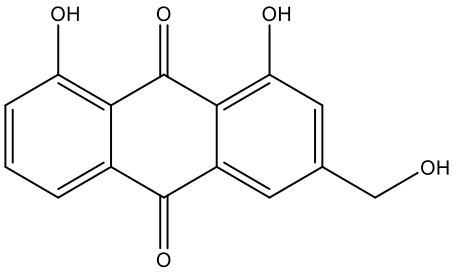
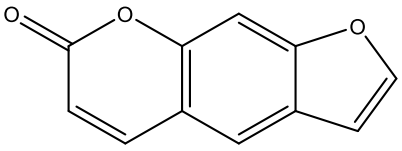
21	Schisandrin	7.4%±0.013	Must bio-technology	
22	Tanshinone ⅡA	3.3%±0.013	Must bio-technology	
23	Dihydrotanshinone I	17.5%±0.02	Must bio-technology	
24	Isochlorogenic acid A	39.5%±0.103	Must bio-technology	

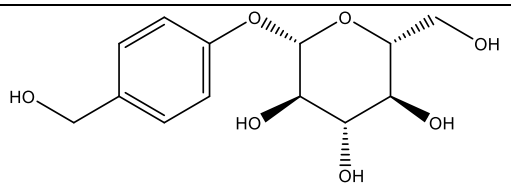
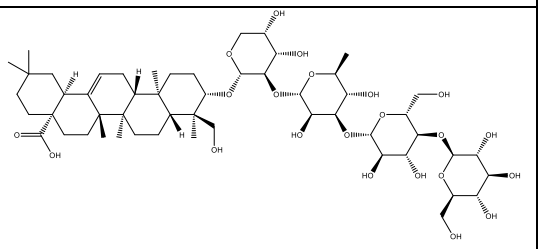
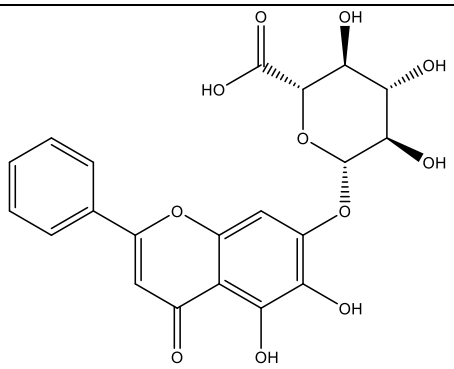
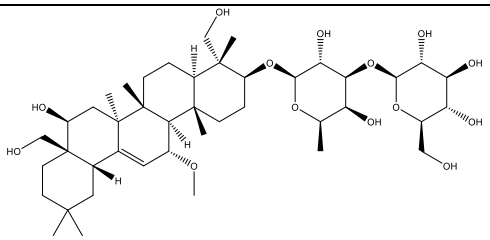
25	Daidzin	43.9%±0.036	Must bio-technology	
26	Tannic acid	-25.0%±0.03	Must bio-technology	
27	Glabridin	10.2%±0.038	Must bio-technology	
28	Genistin	19.3%±0.04	Must bio-technology	

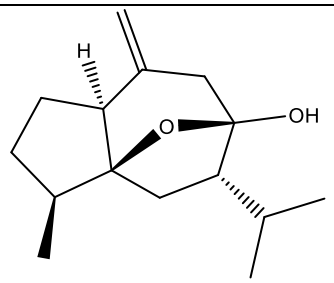
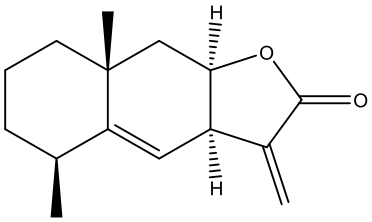
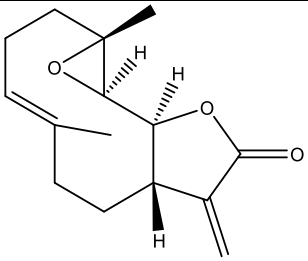
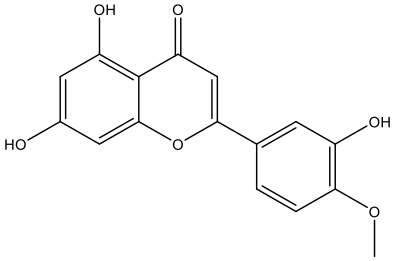
29	Daidzein	34.6%±0.053	Must bio-technology	
30	Stachydrine hydrochloride	4.9%±0.047	Must bio-technology	
31	Dauricine	29.9%±0.088	Must bio-technology	
32	Shionone	1.7%±0.059	Must bio-technology	

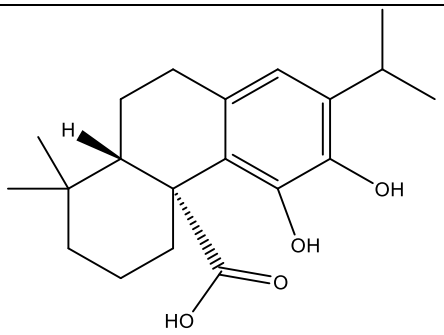
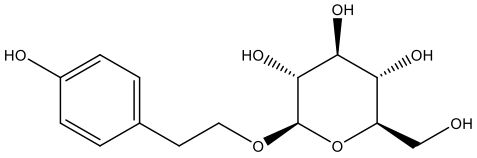
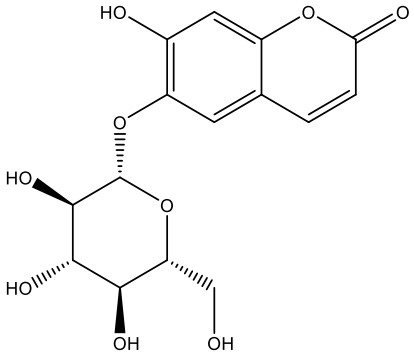
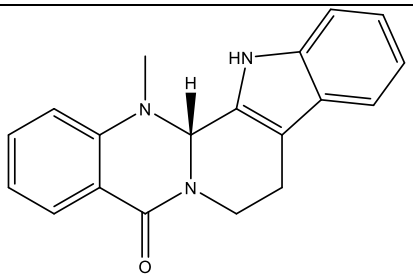
33	Betaine	2.8%±0.059	Must bio-technology	
34	Cimifugin	-24.9%±0.026	Must bio-technology	
35	Bergapten	23.3%±0.024	Must bio-technology	
36	Costunolide	15.2%±0.014	Must bio-technology	

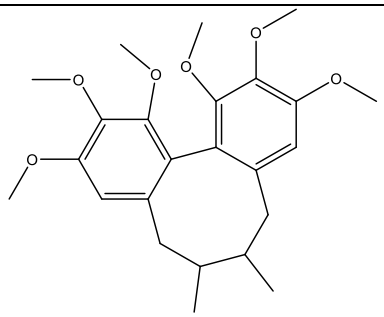
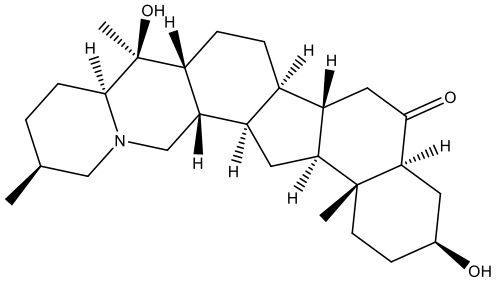
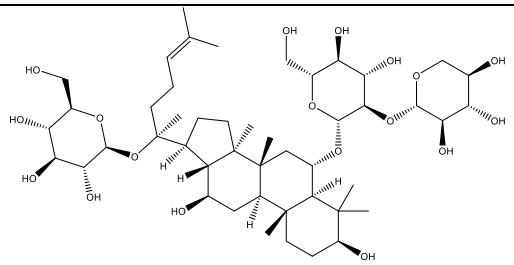
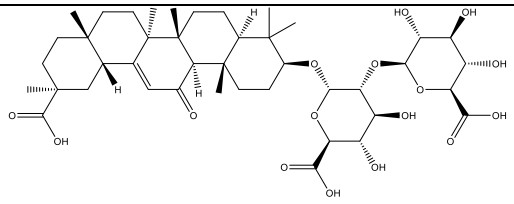
37	Icaritin	19.6%±0.022	Must bio-technology	
38	Veratramine	19.2%±0.011	Must bio-technology	
39	Scopoletin	-891.1%±0.242	Must bio-technology	
40	6-Gingerol	9.9%±0.032	Must bio-technology	

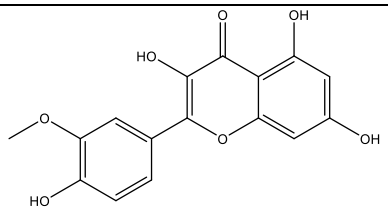
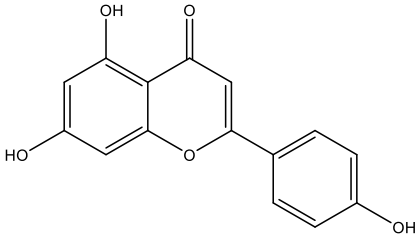
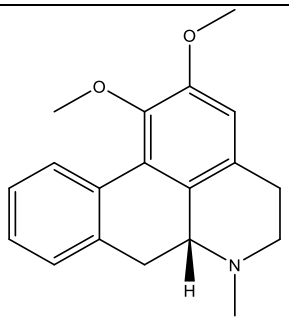
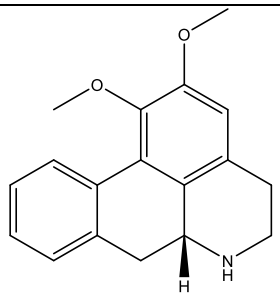
41	Dehydrocostus lactone	17.9%±0.019	Must bio-technology	
42	Loganic acid	-5.2%±0.012	Must bio-technology	
43	Aloe emodin	25.9%±0.208	Must bio-technology	
44	Psoralen	29.8%±0.012	Must bio-technology	

45	Gastrodin	3.6%±0.023	Must bio-technology	
46	Macranthoside B	10.9%±0.033	Must bio-technology	
47	Baicalin	32.1%±0.033	Must bio-technology	
48	Saikosaponin	3.9%±0.045	Must bio-technology	

49	Curcumol	6.9%±0.004	Must bio-technology	
50	Alantolactone	12.4%±0.021	Must bio-technology	
51	Parthenolide	15.1%±0.014	Must bio-technology	
52	Diosmetin	17.5%±0.025	Must bio-technology	

53	Carnosic acid	35.2%±0.004	Must bio-technology	
54	Salidroside	1.1%±0.011	Must bio-technology	
55	Esculin	-1054.69%±12.193	Must bio-technology	
56	Evodiamine	-18.3%±0.087	Must bio-technology	

57	Deoxyschizandrin	1.4%±0.039	Must bio-technology	
58	Sipeimine	1%±0.01	Must bio-technology	
59	Notoginsenoside R1	-1.5%±0.008	Must bio-technology	
60	Glycyrrhizic acid	6.8%±0.019	Must bio-technology	

61	Isorhamnetin	22%±0.022	Must bio-technology	
62	Apigenin	27.8%±0.010	Must bio-technology	
63	Nuciferine	14.1%±0.018	Must bio-technology	
64	N-Nornuciferine	7.4%±0.042	Must bio-technology	

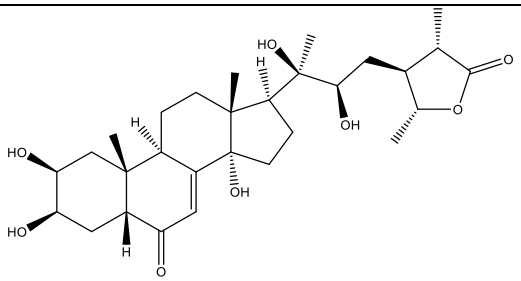
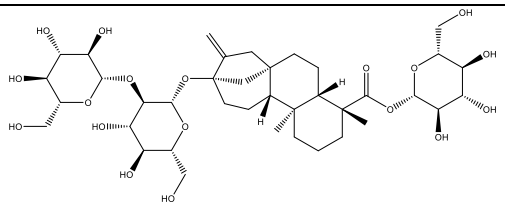
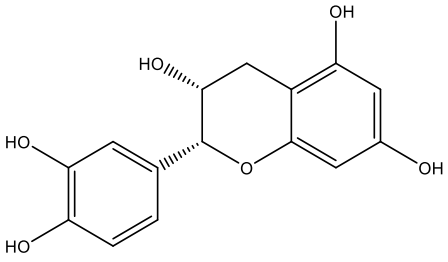
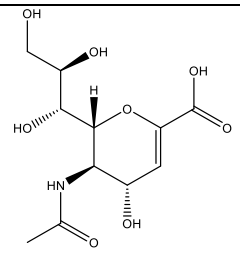
65	Cyasterone	6.3%±0.035	Must bio-technology	
66	Stevioside	7.4%±0.014	Must bio-technology	
67	Epicatechin	28.1%±0.027	Must bio-technology	
68	2-deoxy-2,3-didehydro-N-acetylneuraminic acid	53.3%±0.017	TCI	

Table S3 Nucleotide sequences of gene-specific primers used for quantitative real-time reverse transcription PCR.

Gene name	Primer	Sequence of primers (5′ to 3′)
Human *α-SMA*	Forward	GCTGCCCAGAGACCCTGTT
	Reversed	TTTCATGGATGCCAGCAGACT
Human *VIMENTIN*	Forward	CAGAGAGAG GAAGCCGAAAG
	Reversed	ATGCTGTTCCTGAATCTGGG
Human *N-CADHERIN*	Forward	AAGAGAGACTGGGTCATCC
	Reversed	TGAGATGGGGTTGATAATG
Human *E-CADHERIN*	Forward	TAACAGGAACACAGGAGTCATCA
	Reversed	GTGGTGGGATTGAAGATCGG
Human *FN1*	Forward	CCACAGTGGAGTATGTGGTTAG
	Reversed	CAGTCCTTTAGGGCGATCAAT
Human *COL1A1*	Forward	TGTGCCACTCTGACTGGAAG
	Reversed	CGCCATACTCGAACTGGAATC
Human *COL3A1*	Forward	CGCCCTCCTAATGGTCAAGG
	Reversed	TTCTGAGGACCAGTAGGGCA
Human *COL4A1*	Forward	TGTTGACGGCTTACCTGGAGAC
	Reversed	GGTAGACCAACTCCAGGCTCTC
Human *CCL2*	Forward	CAGGTCCCTGTCATGCTTCT
	Reversed	GTCAGCACAGACCTCTCTCT
Human *PAI1*	Forward	GCACCACAGACGCGATCTT
	Reversed	ACCTCTGAAAAGTCCACTTGC
Human *TGF-β*	Forward	CTAATGGTGGAAACCCACAACG
	Reversed	TATCGCCAGGAATTGTTGCTG
Human *ARG1*	Forward	TCAGAGCATGAGCGCCAAGT
	Reversed	CCTCGTGGCTGTCCCTTTGA
Human *NEU4*	Forward	GGCCACGGGATGACAGTTG
	Reversed	CAGGCGGATACCCATGTGTAG
Human *SNAI1*	Forward	TCGGAAGCCTAACTACAGCG
	Reversed	CAGATGAGCATTGGCAGCGA
Human *KIM-1*	Forward	CCCACGTCACCTATCGGAAG

	Reversed	GTGCTCAACACGGCAACAAT
Human *TNFα*	Forward	CCTCTCTCTAATCAGCCCTCTG
	Reversed	GAGGACCTGGGAGTAGATGAG
Human *IL6*	Forward	ACTCACCTCTTCAGAACGAATTG
	Reversed	CCATCTTTGGAAGGTTCAGGTTG
Human *IL1β*	Forward	AGCTACGAATCTCCGACCAC
	Reversed	CGTTATCCCATGTGTCGAAGAA
Human *IL10*	Forward	ACCTGCCTAACATGCTTCGAG
	Reversed	GGCATCACCTCCTCCAGGTA
Human *P21*	Forward	TCTTGTACCCTTGTGCCTCG
	Reversed	ATCTGTCATGCTGGTCTGCC
Human *P16*	Forward	TGAAGCTCCCAGAATGCCAG
	Reversed	GCTGCCCTGGTAGGTTTTCT
Human *P53*	Forward	ACTTCAGGGGTGCCACATTC
	Reversed	CGACCCTGTCCCTCACCTCC
Human *IL8*	Forward	ACTCCAAACCTTTCCACCCC
	Reversed	CCTCTGCACCCAGTTTTCCT
Human *KI67*	Forward	GGAAGCTGGACGCAGAAGAT
	Reversed	CAGCACCATTTGCCAGTTCC
Human *γH2AX*	Forward	AGAAGACGCGAATCATCCCC
	Reversed	TGGTCTTCTTGGGCAGCAG
Human *GAPDH*	Forward	GGAGCGAGATCCCTCCAAAAT
	Reversed	GGCTGTTGTCATACTTCTCATGG
Human *β-actin*	Forward	GATCATTGCTCCTCCTGAGC
	Reversed	ACTCCTGCTTGCTGATCCAC
Mouse *α-Sma*	Forward	GGACTTTGAAAATGAGATGG
	Reversed	TGATGCTGTTATAGGTGGTT
Mouse *Vimentin*	Forward	CAGAGAGAG GAAGCCGAAAG
	Reversed	ATGCTGTTCCTGAATCTGGG
Mouse *N-cadherin*	Forward	AAGAGAGACTGGGTCATCC
	Reversed	TGAGATGGGGTTGATAATG
Mouse *Fibronectin*	Forward	CCACAGTGGAGTATGTGGTTAG

	Reversed	CAGTCCTTTAGGGCGATCAAT
Mouse *Kim-1*	Forward	CTATGTTGGCATCTGCATCG
	Reversed	AAGGCAACCACGCTTAGAGA
Mouse *Neu4*	Forward	GAACAGCGACTTAGCCCTGATG
	Reversed	TAGACCTGTGCTCCTCCAGTAC
Mouse *Snai1*	Forward	CTCCAAACCCACTCGGATGT
	Reversed	AGCCAGACTCTTGGTGCTTG
Mouse *Snai2*	Forward	GCCTCCAAGAAGCCCAACTA
	Reversed	GCCGACGATGTCCATACAGT
Mouse *E-cadherin*	Forward	TAACAGGAACACAGGAGTCATCA
	Reversed	GTGGTGGGATTGAAGATCGG
Mouse *Col1a1*	Forward	TTGGAGAGAGCATGACCG
	Reversed	TACGCTGTTCTTGCAGTG
Mouse *Col3a1*	Forward	GTCTGGTGGCTTTTCACCCT
	Reversed	AGTTCGGGGTGGCAGAATTT
Mouse *Col4a1*	Forward	AACAACGTCTGCAACTTCGC
	Reversed	CTTCACAAACCGCACACCTG
Mouse *Ccl2*	Forward	CAGGTCCCTGTCATGCTTCT
	Reversed	GTCAGCACAGACCTCTCTCT
Mouse *Tgf-β*	Forward	GACCGCAACAACGCCATCTA
	Reversed	GGCGTATCAGTGGGGGTCAG
Mouse *Mmp2*	Forward	ACCTGAACACTTTCTATGGCTG
	Reversed	CTTCCGCATGGTCTCGATG
Mouse *Mmp7*	Forward	TAGGCGGAGATGCTCACTTT
	Reversed	TTCTGAATGCCTGCAATGTC
Mouse *Mmp9*	Forward	CTGGACAGCCAGACACTAAAG
	Reversed	CTCGCGGCAAGTCTTCAGAG
Mouse *Mmp13*	Forward	CTGGTCTTCTGGCACACGCT
	Reversed	GCAGCGCTCAGTCTCTTCAC
Mouse *Fsp1*	Forward	TGAGCAACTTGGACAGCAACA
	Reversed	TTCCGGGGTTCCTTATCTGGG
Mouse *Timp1*	Forward	GCAACTCGGACCTGGTCATAA

	Reversed	CGGCCCGTGATGAGAAACT
Mouse *Tnfα*	Forward	TCTCATGCACCACCATCAAGGACT
	Reversed	ACCACTCTCCCTTTGCAGAATCA
Mouse *Il6*	Forward	ATCCAGTTGCCTTCTTGGGACTGA
	Reversed	TAAGCCTCCGACTTGTGAAGTGGT
Mouse *Il1β*	Forward	GCAACTGTTCCTGAACTCAACT
	Reversed	ATCTTTTGGGGTCCGTCAACT
Mouse *Yap*	Forward	CCAGACGACTTCCTCAACAGTG
	Reversed	GCATCTCCTTCCAGTGTGCCAA
Mouse *Negr1*	Forward	GCCTTCGAGTGGTACAAAGGA
	Reversed	CTGTACTTGGAGGGTTGAGGG
Mouse *Ankrd1*	Forward	ATAAACGGACGGCACTCCAC
	Reversed	CATCTGCGTTTCCTCCACGA
Mouse *Ctgf*	Forward	GCCTACCGACTGGAAGACAC
	Reversed	GTAACTCGGGTGGAGATGCC
Mouse *Cyr61*	Forward	ATGACCTCCTCGGACTCGAT
	Reversed	GGGTTGAAAAGAACTCGCGG
Mouse *P21*	Forward	TTGTCGCTGTCTTGCACTCT
	Reversed	TTTCGGCCCTGAGATGTTCC
Mouse *P16*	Forward	GAACTCTTTCGGTCGTACCCC
	Reversed	TAGTGGGGTCCTCGCAGTT
Mouse *P53*	Forward	TGGAGGAGTCACAGTCGGAT
	Reversed	CGTCCATGCAGTGAGGTGAT
Mouse *Il8*	Forward	TGGGTGAAGGCTACTGTTGG
	Reversed	AGCTTCATTGCCGGTGGAAA
Mouse *GAPDH*	Forward	TGTGTCCGTCGTGGATCTGA
	Reversed	CCTGCTTCACCACCTTCTTGAT
Mouse *β-actin*	Forward	GCTCTGGCTCCTAGCACC
	Reversed	CCACTATCCACACAGAGTACTTG

14

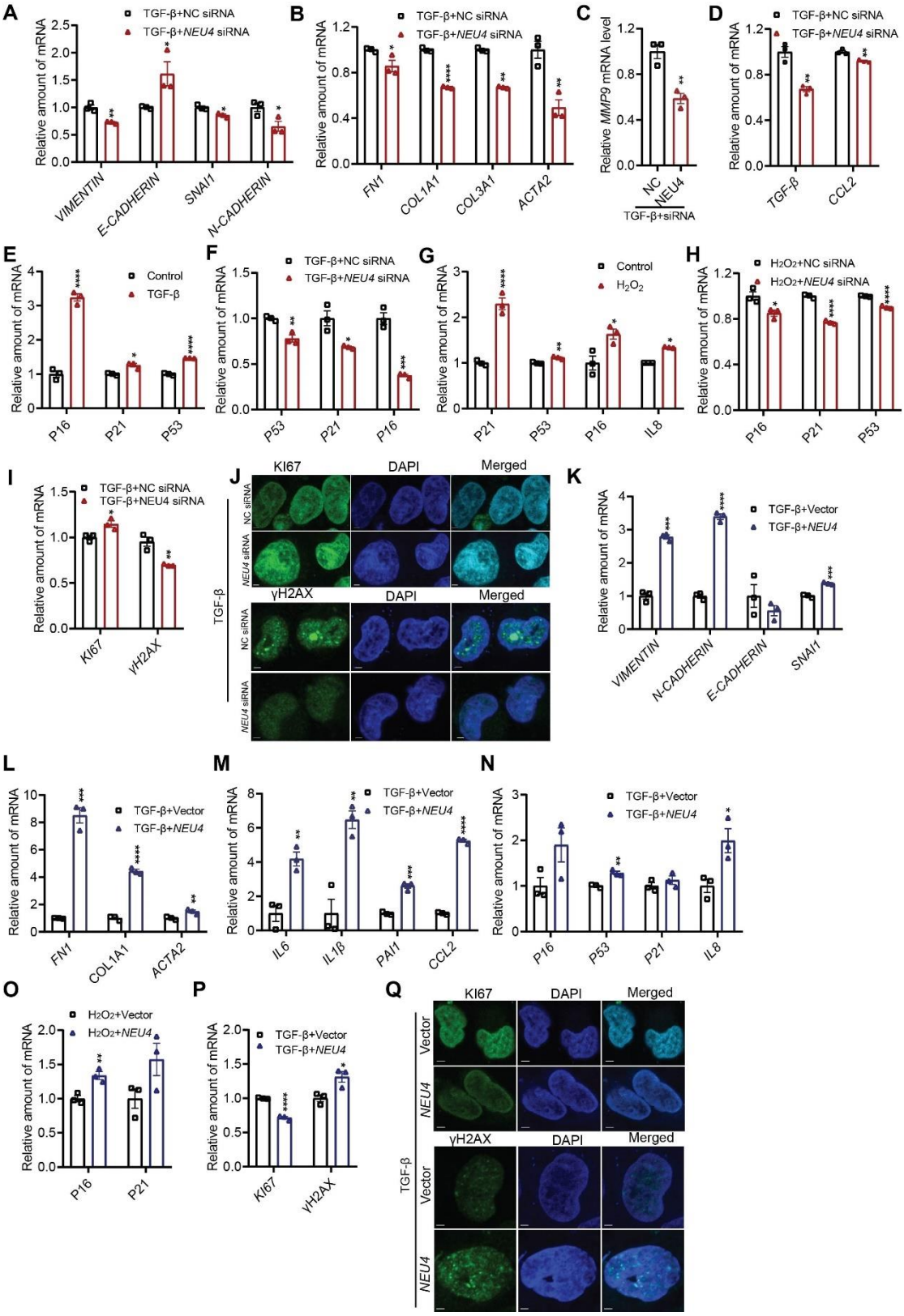


Figure S1. NEU4 promoted epithelial-mesenchymal transition (EMT) and cellular senescence in TGF-β-induced HK-2 cells. (**A-F, I**) Relative EMT associated gene (**A**), ECM associated gene (**B**), *MMP9* (**C**), chemokine associated (**D**), senescence-

associated gene (**E, F**) and DNA damage marker gene (I) mRNA level in HK-2 cells. *n* = 3 samples. (**G, H**) Relative senescence-associated gene mRNA level in HK-2 cells. *n* = 3 samples. (**J, Q**) Immunofluorescent staining represents KI67 or γH2AX in HK-2 cells. Scale bar, 10 μm, *n* = 3 samples. (**K**-**P**) Relative EMT associated gene (**K**), ECM associated gene (**L**), inflammation associated gene (**M**), senescence-associated gene (**N**, **O**) and DNA damage marker gene (**P**) mRNA level in HK-2 cell. *n* = 3 samples. (**A**-**D, F, I, J**) HK-2 cells treatment with TGF-β 24 h after transfection with *NEU4* siRNA. (**E, G**) HK-2 cells treatment with TGF-β 24 h or H_2O_2 6 h. (**K**-**Q**) HK-2 cells treatment with TGF-β 24 h after transfection with *NEU4*-overexpression plasmids. (**H**, **O**) HK-2 cells treatment with H_2O_2 6 h after transfection with *NEU4* siRNA (**H**) or *NEU4*-overexpression plasmids (**O**). Data are presented as mean ± SEM. Comparisons between two groups were analyzed by using a two-tailed Student′s *t* test. **p*<0.05, ***p*<0.01, ****p*<0.001, *****p*<0.0001 versus the NC siRNA or Vector group.

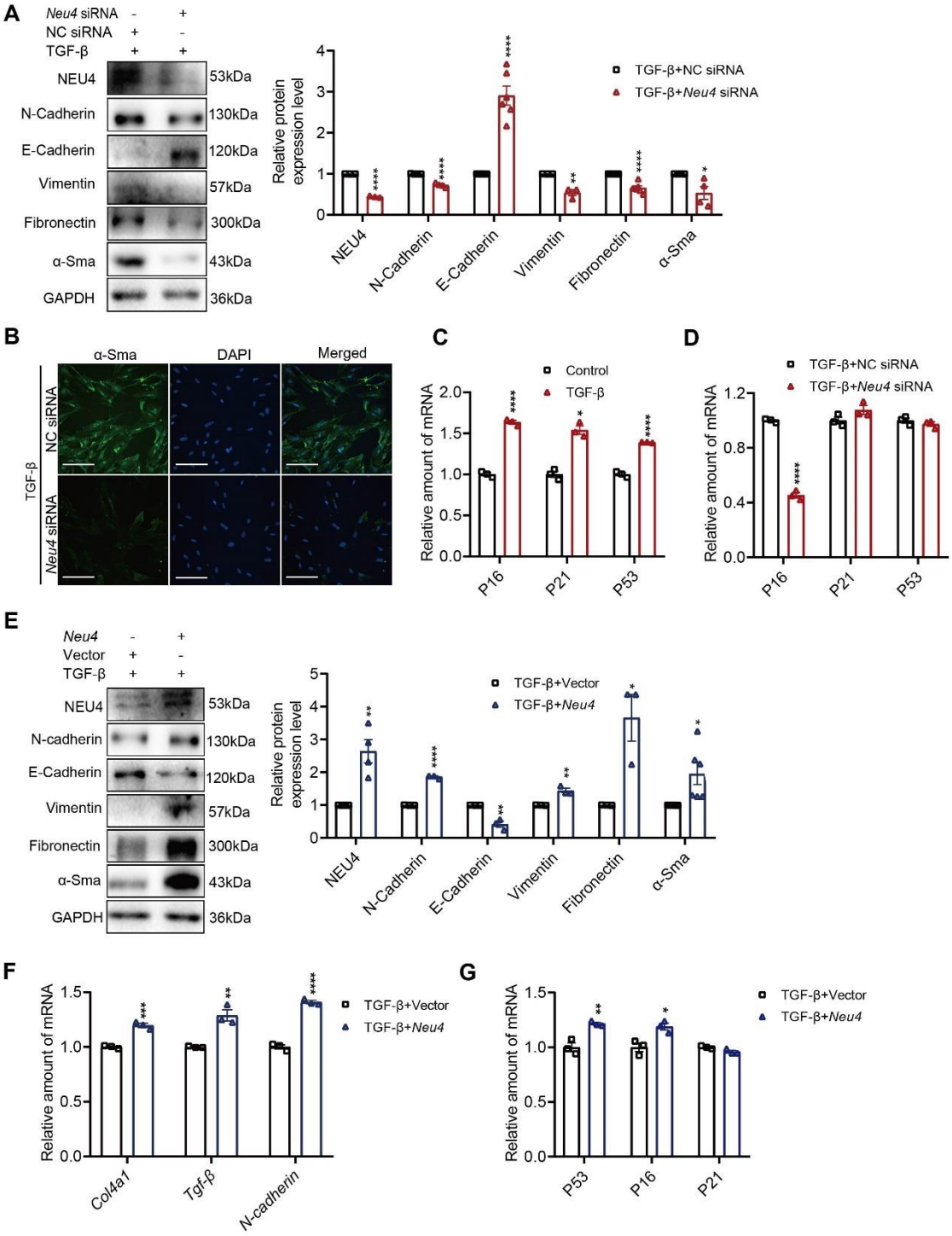


Figure S2. NEU4 promoted EMT and cell senescence in TGF-β-induced PTECs. (**A**) Western blot (left panel) and quantification (right panel) of the protein expression of NEU4, N-Cadherin, E-Cadherin, Vimentin, Fibronectin and α-Sma in PTECs. GAPDH served as loading control, *n* = 3-6 samples. (**B**) Immunofluorescent staining represents α-Sma in PTECs. Scale bar, 100 μm, *n* = 3 samples. (**C**, **D**) Relative senescence associated gene mRNA level in PTECs, *n* = 3 samples. (**E**) Western blot

(left panel) and quantification (right panel) of the protein expression of NEU4, N-Cadherin, E-Cadherin, Vimentin, Fibronectin and α-Sma in PTECs. GAPDH served as loading control, n = 3-6 samples. (**F, G**) Relative EMT associated gene, ECM associated gene (**F**) and senescence associated gene (**G**) mRNA level in PTECs, n = 3 samples. (**C**) PTECs treatment with TGF-β 24 h. (**A-B, D**) PTECs treatment with TGF-β 24 h after transfection with *Neu4* siRNA. (**E-G**) PTECs treatment with TGF-β 24 h after transfection with *Neu4*-overexpression plasmids. Error bars represent mean ± SEM. Comparisons between two groups were analyzed by using a two-tailed Student′s t test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 versus the NC siRNA or Vector group.

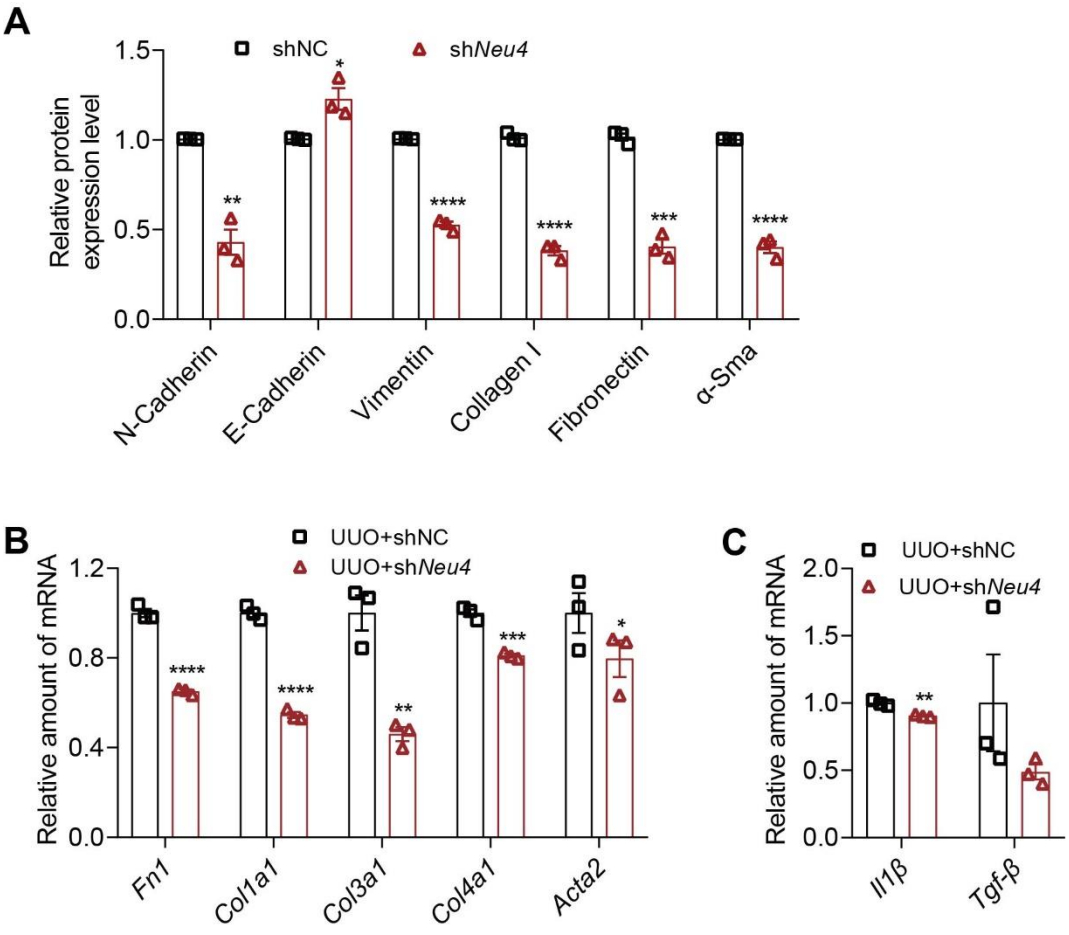


Figure S3. ***Neu4*** **knockdown alleviated UUO-induced EMT, ECM and inflammation in mice.** (**A**) Quantification of the expression of N-Cadherin, E-Cadherin, Vimentin, Collagen I, Fibronectin and α-Sma as shown in Figure 3E. GAPDH served as loading control. n = 3-5 mice. (**B**, **C**) Relative mRNA level of ECM associated genes (**B**) and inflammation associated genes (**C**) were determined by RT-qPCR, n = 3 mice. Error bars represent mean ± SEM. Comparisons between two groups were analyzed by using a two-tailed Student′s t test. *p<0.05, ****p<0.0001 versus the shNC.

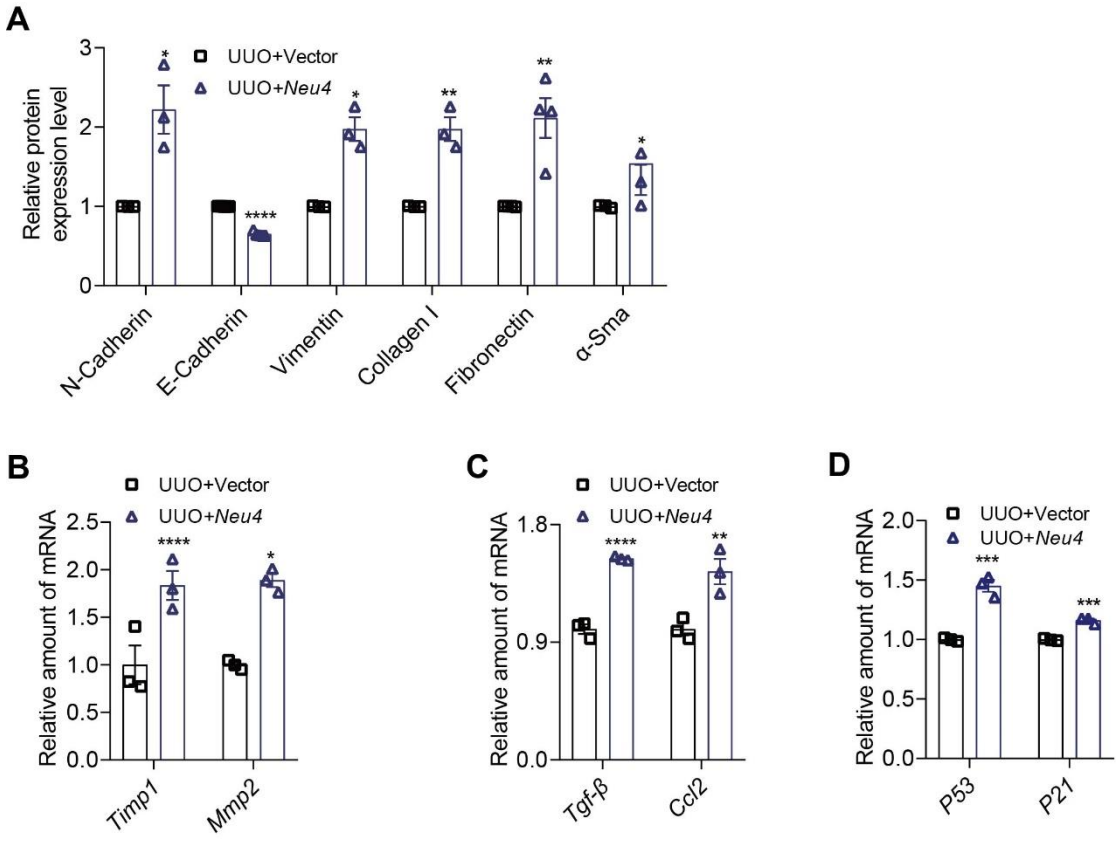
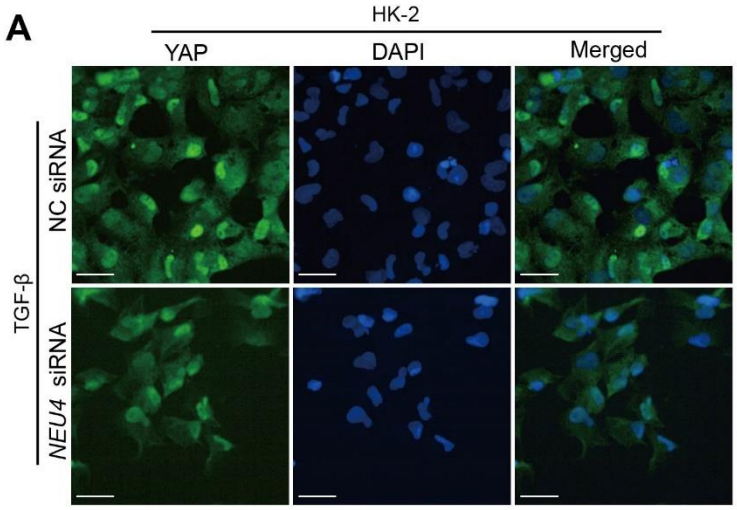
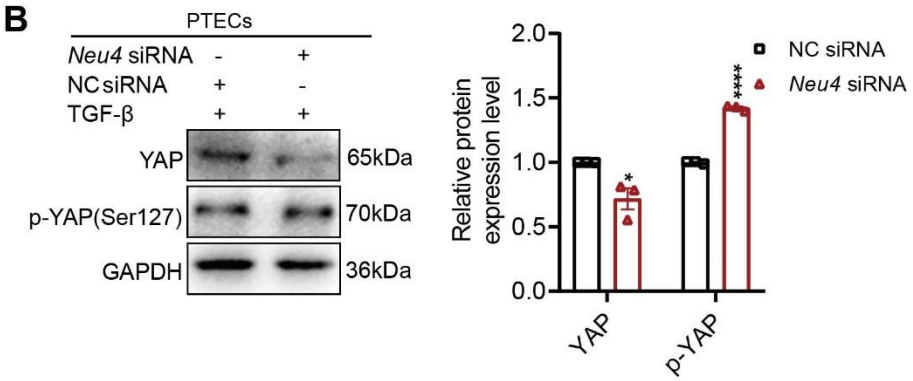
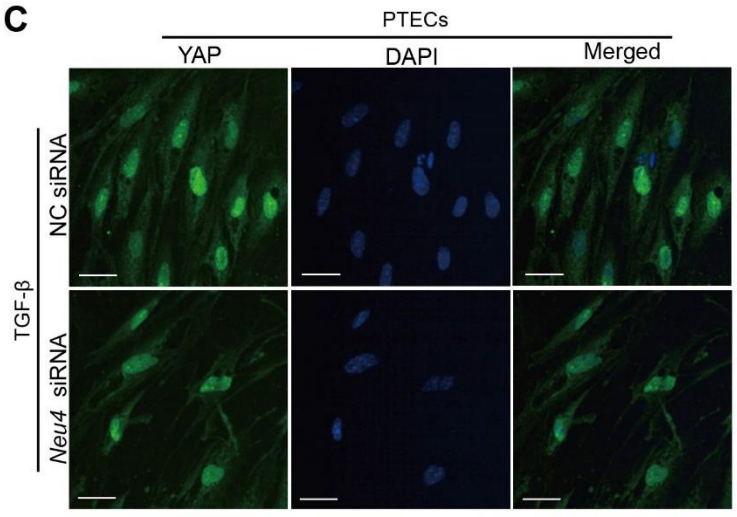


Figure S4. *Neu4* overexpression aggravated UUO-induced EMT and cellular senescence in mice. (**A**) Quantification of the protein expression of α-Sma, N-Cadherin, E-Cadherin, Vimentin, Fibronectin and Collagen I in kidneys as shown in Figure 4E. GAPDH served as loading control, n = 3-5 mice. (**B-D**) Relative extracellular matrix associated gene (**B**), chemokine associated (**C**) and senescence associated gene (**D**) mRNA level in left kidney. n = 3 mice. Error bars represent mean ± SEM. Comparisons between two groups were analyzed by using a two-tailed Student′s t test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 versus the Vector group.


A
HK-2
YAP
DAPI
Merged
TGF-β
NC siRNA
NEU4 siRNA


B
PTECs
Neu4 siRNA - +
NC siRNA + -
TGF-β + +
YAP 65kDa
p-YAP(Ser127) 70kDa
GAPDH 36kDa
Relative protein expression level
2.0
1.5
1.0
0.5
0.0
NC siRNA
Neu4 siRNA
*
****
YAP
p-YAP


C
PTECs
YAP
DAPI
Merged
TGF-β
NC siRNA
Neu4 siRNA

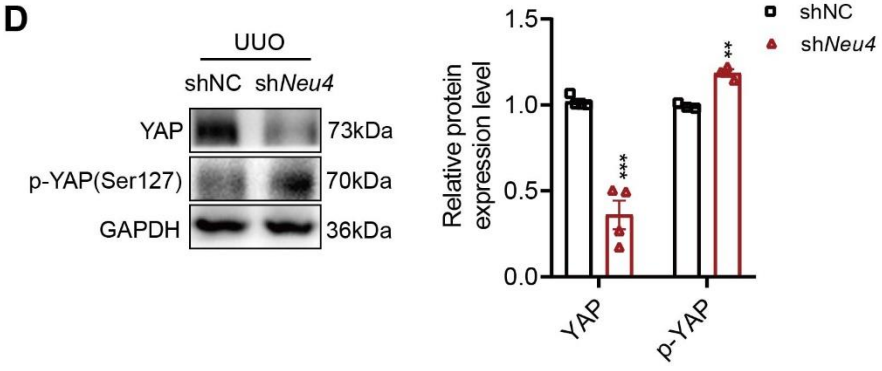

D
UUO
shNC shNeu4
YAP 73kDa
p-YAP(Ser127) 70kDa
GAPDH 36kDa
Relative protein expression level
1.5
1.0
0.5
0.0
shNC
shNeu4
***
**
YAP
p-YAP

Figure S5. NEU4 knockdown inhibits activation of Yes-associated protein (YAP). Related to Figure 6. (**A**) Immunofluorescent staining represents YAP in HK-2 cells treatment with TGF-β 24 h after transfection with *Neu4* siRNA. Scale bar, 100 μm, n = 3 samples. (**B**) Western blot (left panel) and quantification (right panel) of the protein expression of YAP and phosphorylation of YAP in PTECs. GAPDH served as loading control, n = 3 samples. (**C**) Immunofluorescent staining represents YAP in PTECs. Scale bar, 100 μm, n = 3 samples. (**D**) Western blot (left panel) and quantification (right panel) of the protein expression of YAP and phosphorylation of YAP in left kidneys from UUO mice with sh*Neu4*. GAPDH served as loading control, n = 3-4 mice. (**B** and **C**) PTECs treatment with TGF-β 24 h after transfection with *Neu4* siRNA. Error bars represent mean ± SEM. Comparisons between two groups were analyzed by using a two-tailed Student′s t test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 versus the NC siRNA or shNC group.

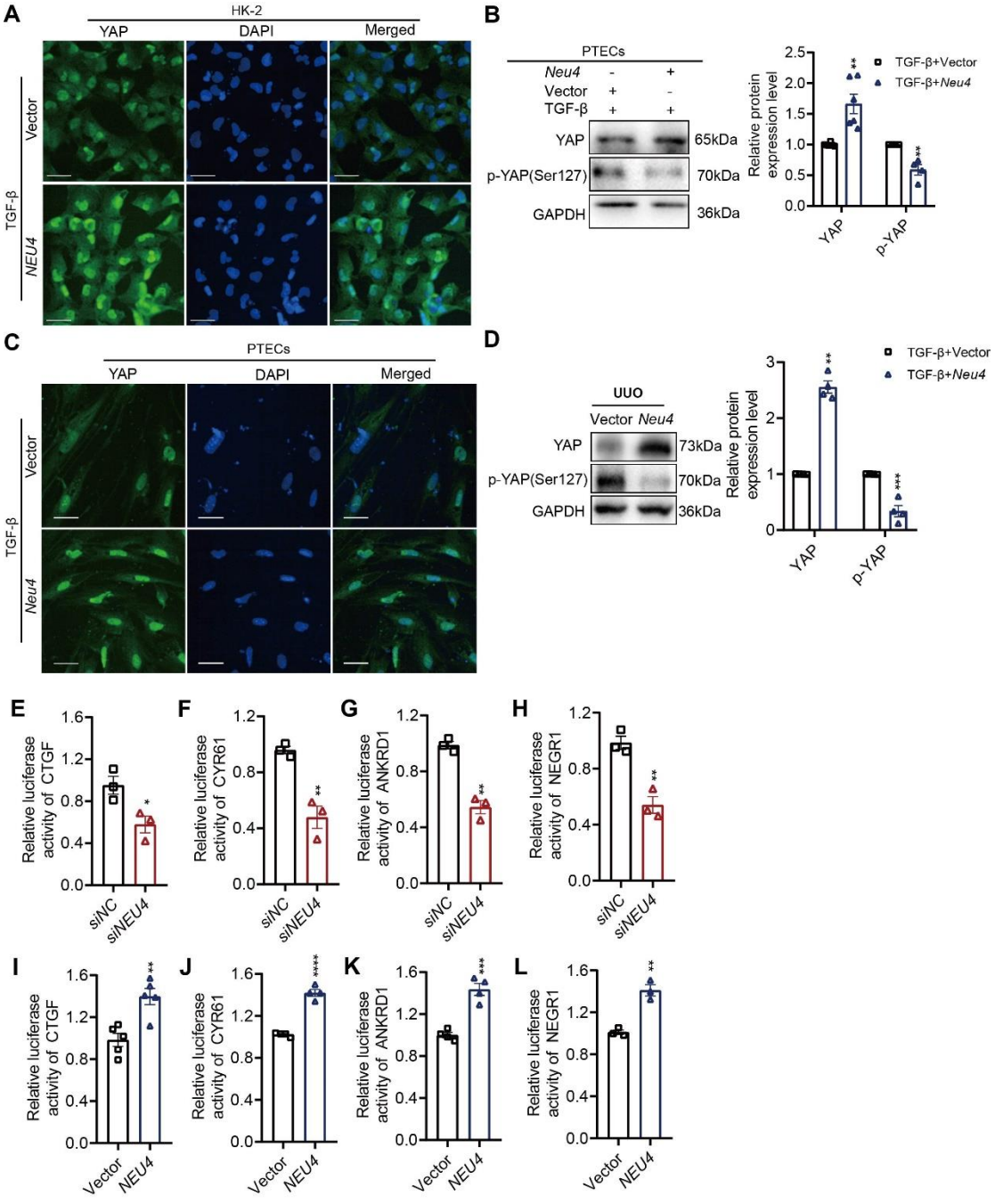


Figure S6. NEU4 overexpression promotes activation of YAP. (**A**) Immunofluorescent staining represents YAP in HK-2 cells treatment with TGF-β 24 h after transfection with *NEU4*-overexpression plasmids. Scale bar, 100 μm, n = 3 samples. (**B**) Western blot (left panel) and quantification (right panel) of YAP and phosphorylation of YAP in PTECs, GAPDH served as loading control, n = 4-6 samples. (**C**) Immunofluorescent staining represents YAP in PTECs. Scale bar, 100 μm, n = 3 samples. (**D**) Western blot (left panel) and quantification (right panel) of the protein

expression of YAP and phosphorylation of YAP in left kidneys from UUO mice with *Neu4*-overexpression plasmids. GAPDH served as loading control, $n = 4$ mice. (**B** and **D**) PTECs treatment with TGF-β 24 h after transfection with *Neu4*-overexpression plasmids. (**E-L**) 293T cells were co-transfected with *YAP* and *Luc-CTGF* or *Luc-CYR61* or *Luc-ANKRD1* or *Luc-NEGR1* and si*NEU4* (E-H) or *NEU4* plasmid (I-L) for 48 h. Luciferase activity was determined using the luciferase reporter system. $n = 3$-5 samples. Error bars represent mean ± SEM. Comparisons between two groups were analyzed by using a two-tailed Student′s *t* test. *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$ versus the Vector group.

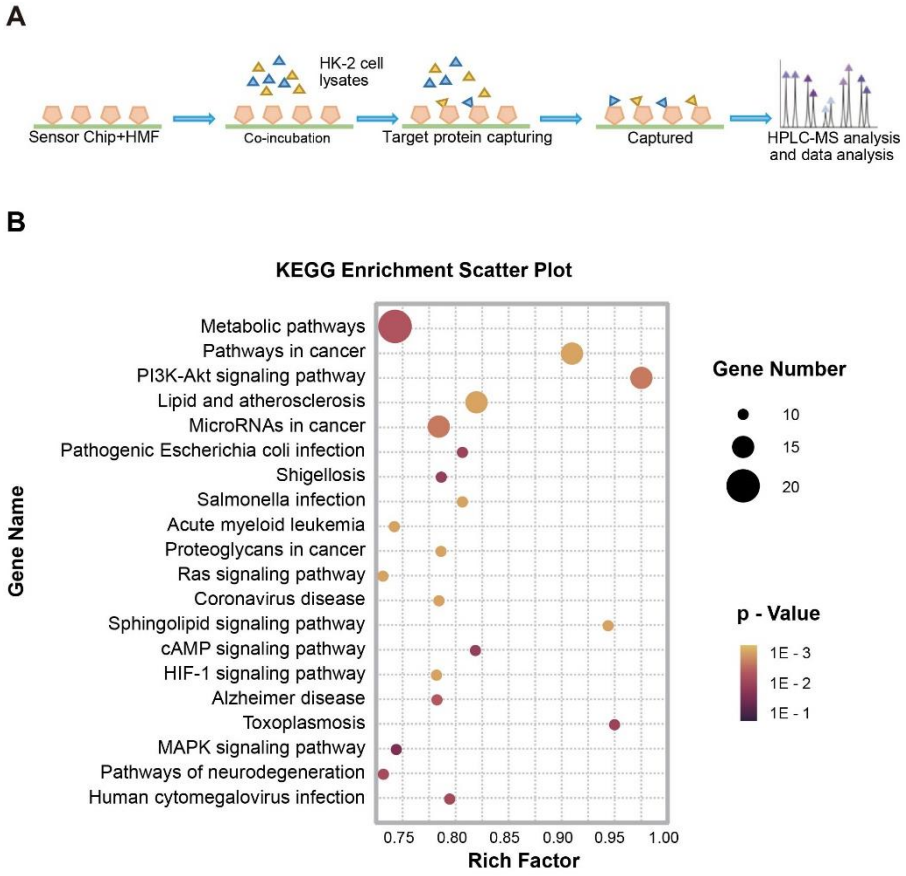


Figure S7. NEU4 served as a cellular target of anti-fibrosis small-molecule HMF. (**A**) Schematic representation of the HMF molecular capture protocol. (**B**) Genomes (KEGG) pathway analysis of the identified changed proteins in HK-2 cells 24 h after treatment with TGF-β.

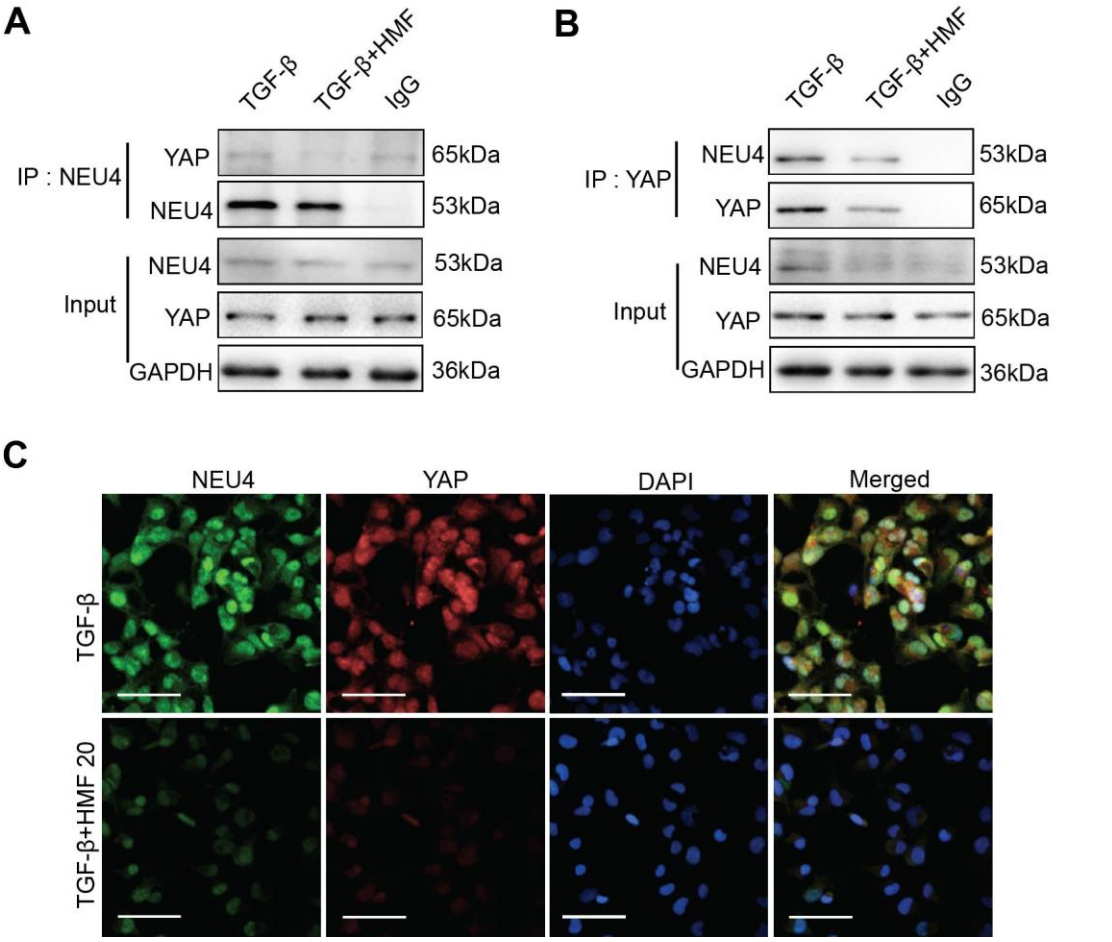


Figure S8. HMF inhibited the interaction between NEU4 and YAP. (**A** and **B**) Western blotting of CoIP of NEU4 and YAP in HK-2 cells treated with TGF-β and in the presence of DMSO or HMF. (**C**) Colocalization of NEU4 and YAP was analyzed by immunofluorescence in HK-2 cells stimulated with TGF-β and in the presence of DMSO or HMF 24 h. Scale bar, 100 μm, n = 3 samples.

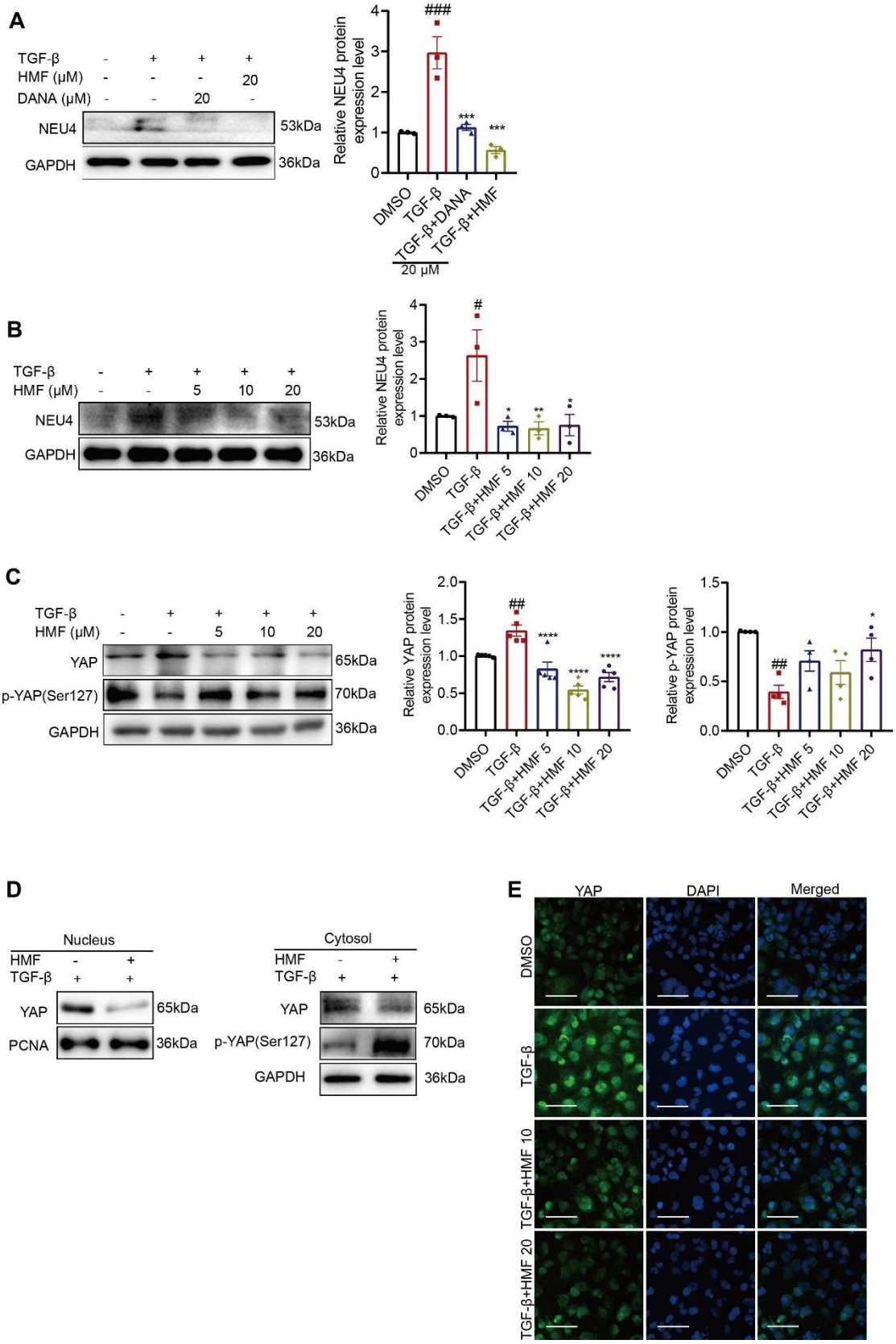


Figure S9. HMF inhibited NEU4 and YAP expression. (**A**) Western blot (left panel) and quantification (right panel) of the protein expression of NEU4 in HK-2 cells 24 h

after treatment with TGF-β and in the presence of DANA or HMF. GAPDH served as loading control, n = 3 samples. (**B** and **C**) Western blot (left panel) and quantification (right panel) of the protein expression of NEU4 (**B**), YAP and phosphorylation of YAP (**C**) in HK-2 cells 24 h after treatment with TGF-β and in the presence of DMSO or HMF. GAPDH served as loading control, n = 3-5 samples. (**D**) Western blot of the protein expression of YAP in nuclear and cytosol, phosphorylation of YAP in cytosol in HK-2 cells 24 h after treatment with TGF-β and in the presence of DMSO or HMF. (**E**) Immunofluorescent staining represents YAP expressions in HK-2 cells treatment with TGF-β and in the presence of DMSO or HMF 24 h. Scale bar, 100 μm, n = 3 samples. Error bars represent mean ± SEM. Comparisons those among three or more groups by using one-way analysis of variance (ANOVA) followed by Dunnett′s post hoc tests. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 versus the TGF-β group, $^{\#}p$<0.05, $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001 versus the DMSO group.

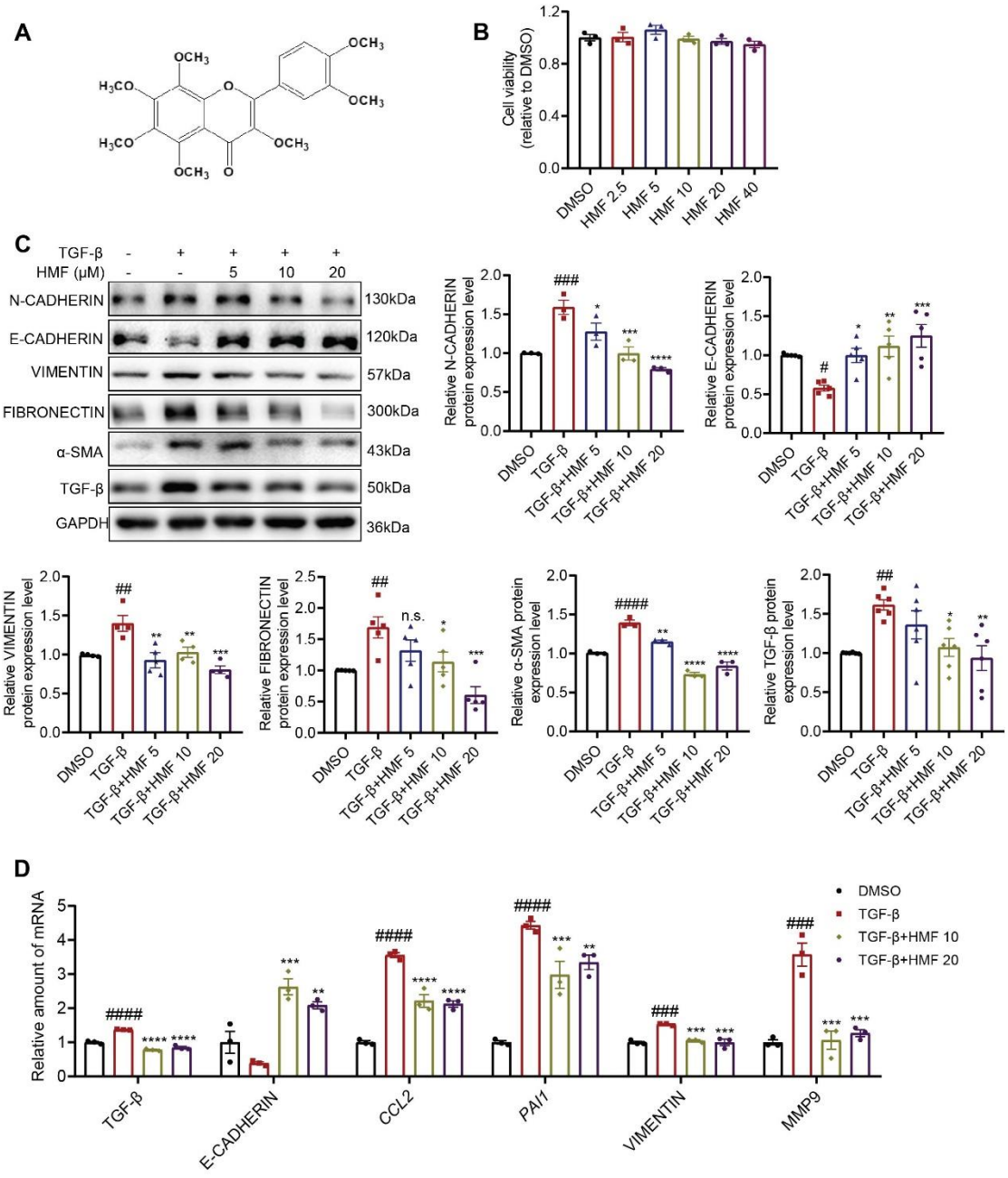
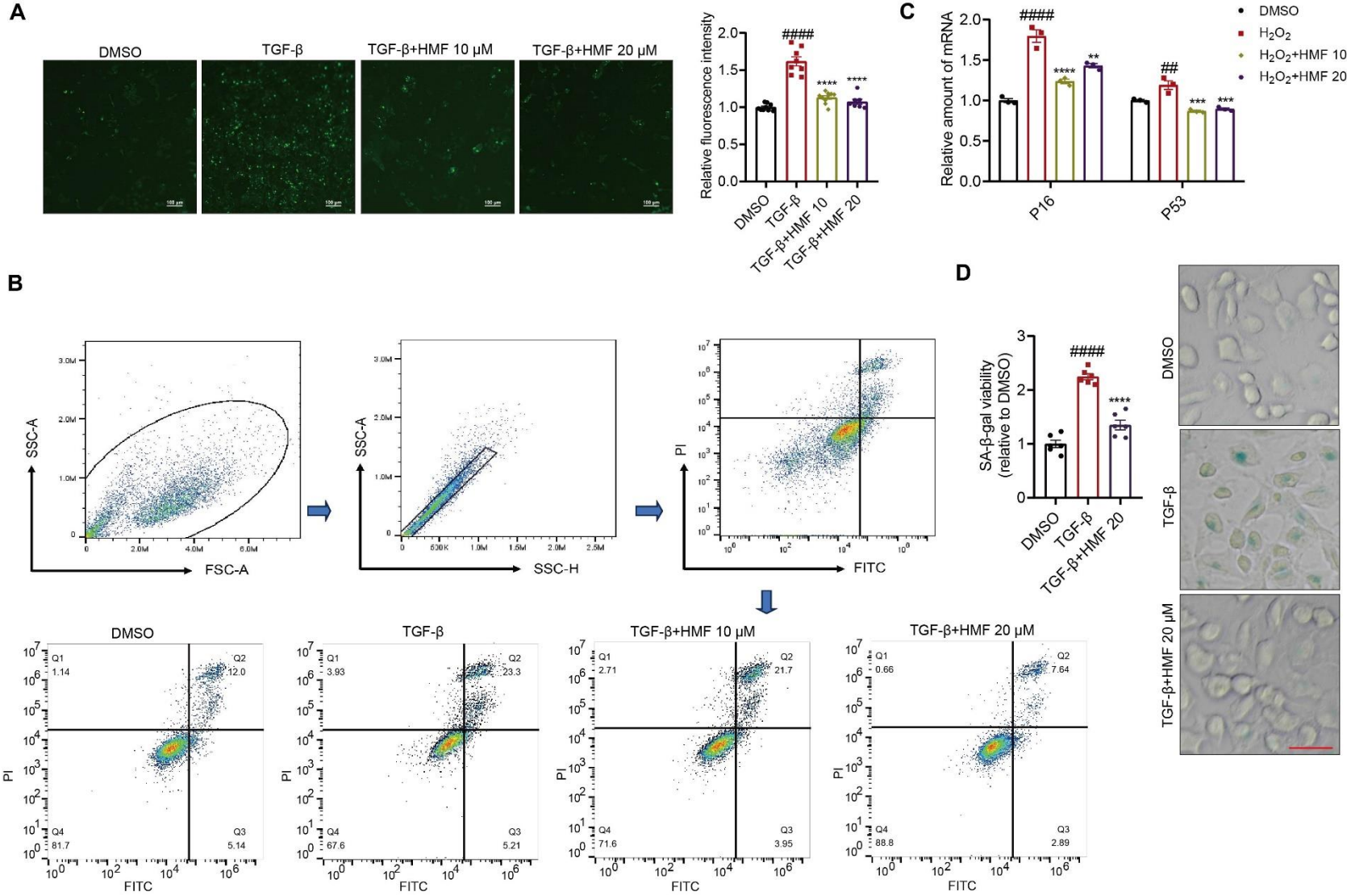


Figure S10. HMF inhibited TGF-β-induced EMT, and the production of proinflammatory cytokines in HK-2. (**A**) The structure of HMF. (**B**) Cell viability after treatment with increasing concentrations of HMF in HK-2 cells for 24 h. *n* = 3 biologically independent samples. (**C**) Western blot (left panel) and quantification (right panel) of the protein expression of N-CADHERIN, E-CADHERIN, VIMENTIN, FIBRONECTIN, α-SMA and TGF-β in HK-2 cells. GAPDH served as loading control. *n* = 3-6 biologically independent samples. (**D**) Relative EMT associated gene and extracellular matrix associated gene mRNA level in HK-2 cells. *n* = 3 biologically

independent samples. (**C** and **D**) HK-2 cells treated with TGF-β and in the presence of DMSO or HMF for 24 h. Error bars represent mean ± SEM. Comparisons those among three or more groups by using one-way analysis of variance (ANOVA) followed by Dunnett′s post hoc tests. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 versus the TGF-β group, $^{\#}p$<0.05, $^{\#\#}p$<0.001, $^{\#\#\#}p$<0.001, $^{\#\#\#\#}p$<0.0001 versus the DMSO group.


A
DMSO
TGF-β
TGF-β+HMF 10 μM
TGF-β+HMF 20 μM
Relative fluorescence intensity
DMSO
TGF-β
TGF-β+HMF 10
TGF-β+HMF 20
C
Relative amount of mRNA
DMSO
H2O2
H2O2+HMF 10
H2O2+HMF 20
P16
P53
B
SSC-A
FSC-A
SSC-H
PI
FITC
DMSO
TGF-β
TGF-β+HMF 10 μM
TGF-β+HMF 20 μM
Q1 1.14
Q2 12.0
Q4 81.7
Q3 5.14
Q1 3.93
Q2 23.3
Q4 67.6
Q3 5.21
Q1 2.71
Q2 21.7
Q4 71.6
Q3 3.95
Q1 0.66
Q2 7.64
Q4 88.8
Q3 2.89
D
SA-β-gal viability (relative to DMSO)
DMSO
TGF-β
TGF-β+HMF 20
TGF-β+HMF 20 μM

142

Figure S11. HMF ameliorated TGF-β-induced apoptosis and cellular senescence in HK-2. (**A**) Measurement (left panel) and the quantification (right panel) of apoptosis by TUNEL staining in HK-2 cells. Scale bar, 100 μm. n = 10 samples. (**B**) Measurement of apoptosis by flow cytometric analysis in HK-2 cells. (**C**) Relative senescence associated gene mRNA level in HK-2 cells after treatment with H_2O_2 in the presence of DMSO or HMF, n = 3 biologically independent samples. (**D**) Measurement (right panel) and the quantification (left panel) of SA-β-gal activity by SA-β-gal staining in HK-2 cells. Scale bar, 100 μm. n = 6 samples. (A, B and D) HK-2 cells treated with TGF-β and in the presence of DMSO or HMF for 24 h. Error bars represent mean ± SEM. Comparisons those among three or more groups by using one-way analysis of variance (ANOVA) followed by Dunnett′s post hoc tests. $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$ versus the TGF-β or H_2O_2 group, $^{\#\#}p<0.01$, $^{\#\#\#\#}p<0.0001$ versus the DMSO group.

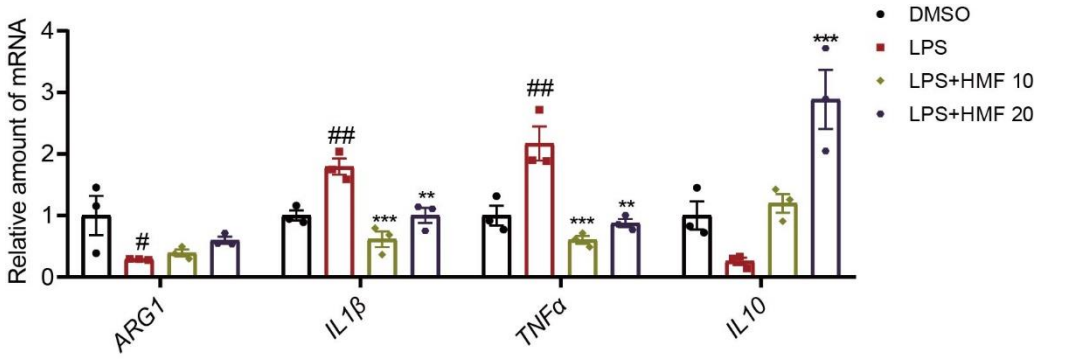
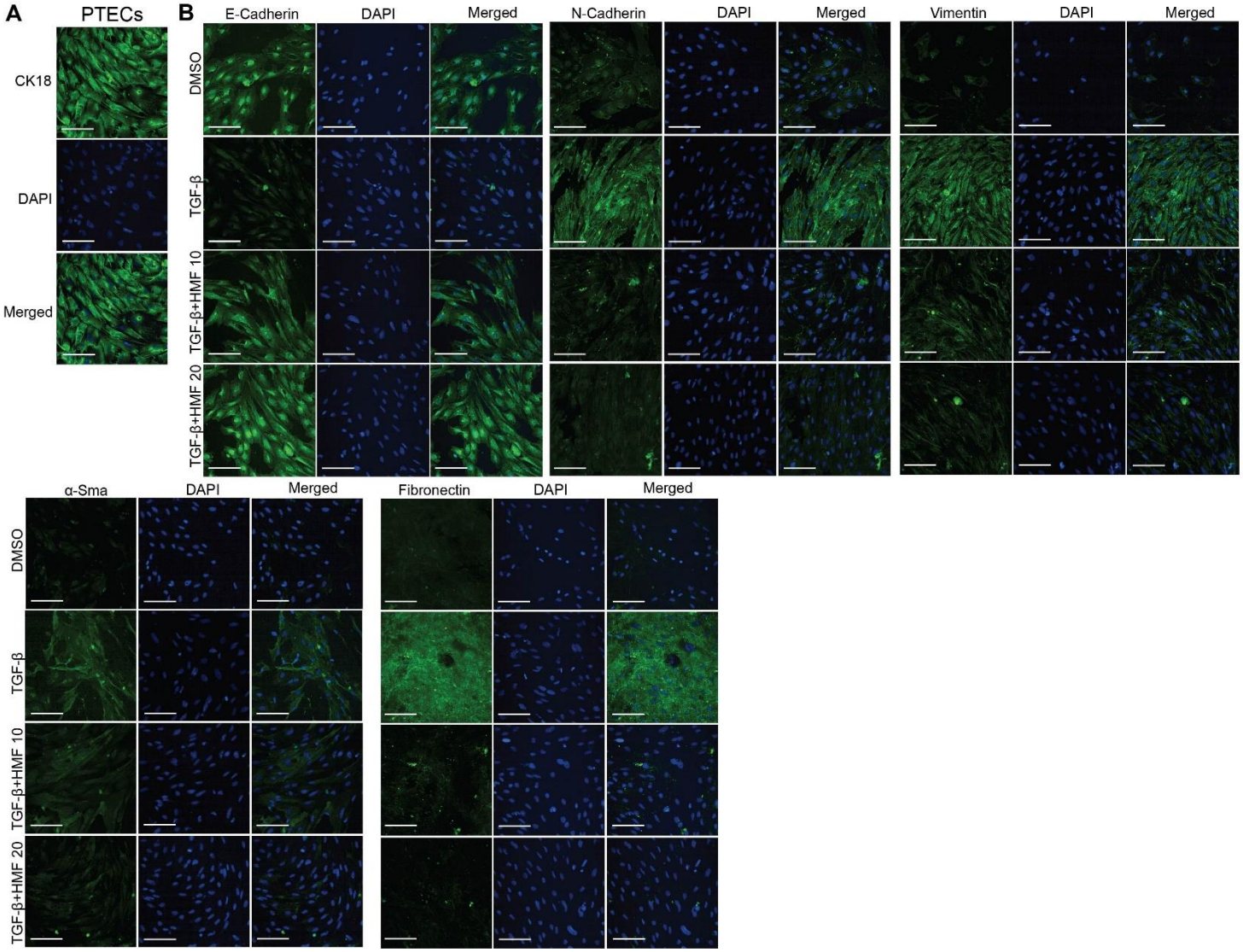


Figure S12. HMF inhibited LPS-induced inflammation in HK-2. Relative inflammation associated gene mRNA level in HK-2 cells 24 h after treatment with LPS in the presence of DMSO or HMF, n = 3 biologically independent samples. Error bars represent mean ± SEM. Comparisons those among three or more groups by using one-way analysis of variance (ANOVA) followed by Dunnett′s post hoc tests. $^{**}p<0.01$, $^{***}p<0.001$ versus the LPS group, $^{\#}p<0.05$, $^{\#\#}p<0.01$ versus the DMSO group.


A
PTECs
CK18
DAPI
Merged
B
E-Cadherin
DAPI
Merged
N-Cadherin
DAPI
Merged
Vimentin
DAPI
Merged
DMSO
TGF-β
TGF-β+HMF 10
TGF-β+HMF 20
α-Sma
DAPI
Merged
Fibronectin
DAPI
Merged
DMSO
TGF-β
TGF-β+HMF 10
TGF-β+HMF 20

164

Figure S13. HMF inhibited TGF-β-induced EMT in PTECs. (**A**) Immunofluorescent staining represents CK18 expressions in PTECs. Scale bar, 100 μm, n = 3 samples. (**B**) Immunofluorescent staining represents N-Cadherin, E-Cadherin, Vimentin, α-Sma and Fibronectin expressions in PTECs 24 h after treatment with TGF-β and in the presence of DMSO or HMF. Scale bar, 100 μm, n = 3 samples.

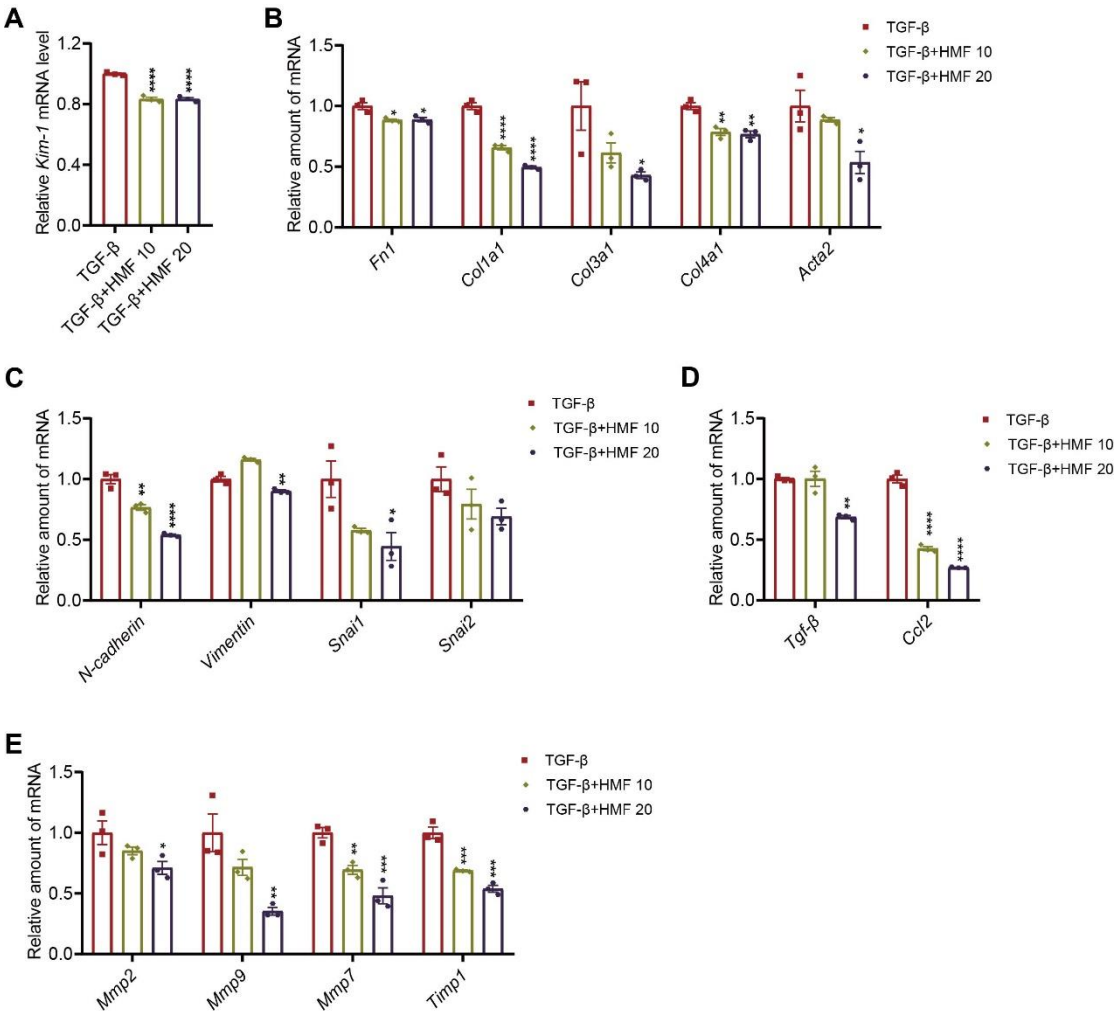


Figure S14. HMF inhibited TGF-β-induced EMT, and the production of proinflammatory cytokines in PTECs. (**A**-**E**) PTECs treated with TGF-β and in the presence of DMSO or HMF for 24 h. (**A**) *Kim-1* mRNA level in PTECs, n = 3 biologically independent samples. (**B**-**E**) Relative ECM associated gene (**B**), EMT associated gene (**C**), chemokine associated gene (**D**), and extracellular matrix associated gene (**E**) mRNA level in PTECs, n = 3 biologically independent samples. Error bars represent mean ± SEM. Comparisons those among three or more groups by using one-way analysis of variance (ANOVA) followed by Dunnett′s post hoc tests. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 versus the TGF-β group.

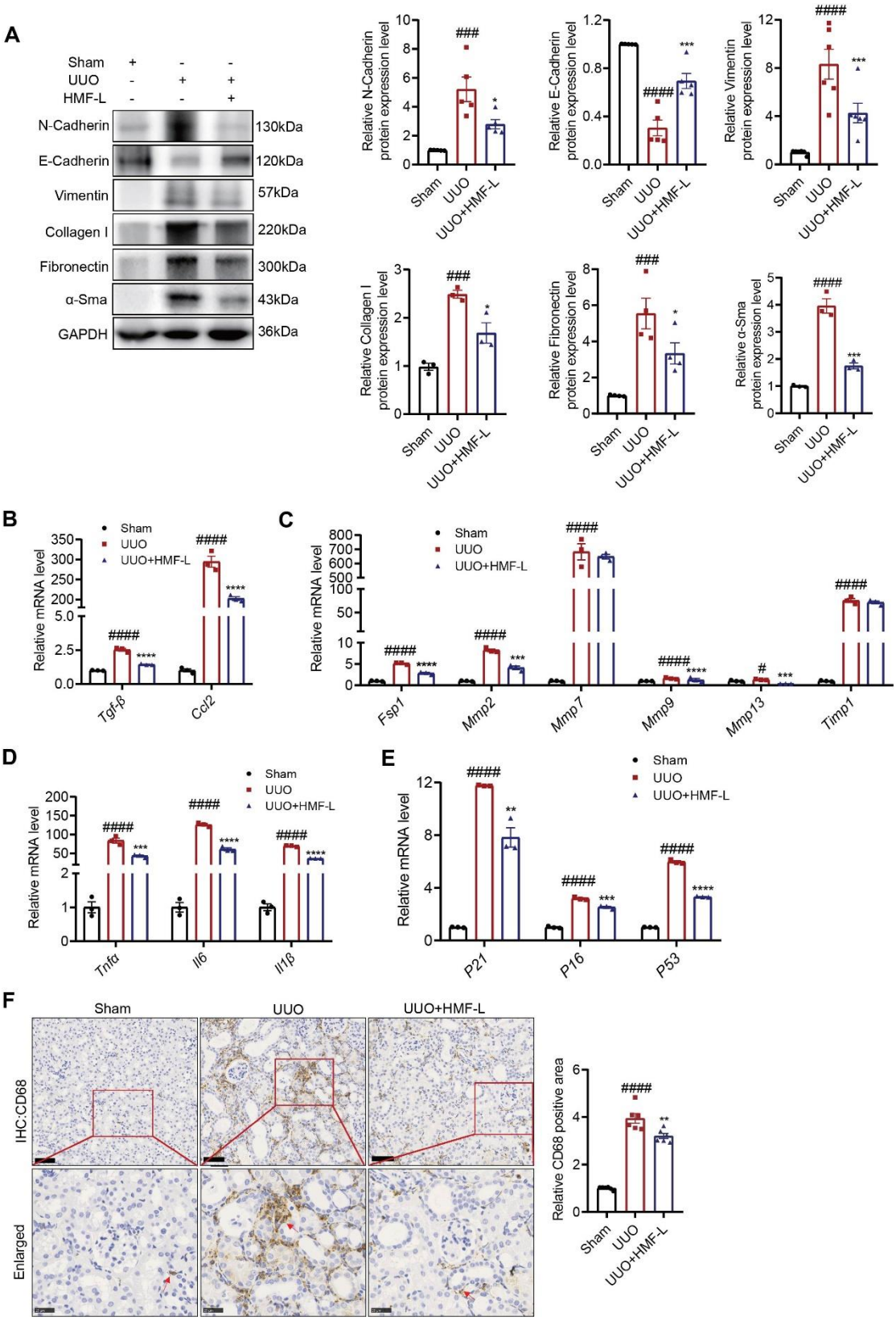


Figure S15. HMF improved UUO-induced renal fibrosis in mice. Vehicle, or HMF (50 or 100 mg/kg/day) was administrated to UUO mice by gastric irrigation once daily

for 10 days. UUO, unilateral ureteral obstruction. (**A**) Western blot (left panel) and quantification (right panel) of the protein expression of N-Cadherin, E-Cadherin, Vimentin, Collagen I, Fibronectin and α-Sma in left kidneys, GAPDH served as loading control, n = 3-6 mice. (**B**-**E**) Relative mRNA level of chemokine-associated genes (**B**), extracellular matrix-associated genes (**C**), inflammation associated genes (**D**) and senescence-associated genes (**E**) were determined by RT-qPCR from left kidneys. n = 3 biologically independent samples. (**F**) Immunohistochemistry staining analysis (left panel) and quantification (right panel) of CD68 expression in left kidney tissues, n = 6 mice. Scale bar, 50 μm. Error bars represent mean ± SEM. Comparisons those among three or more groups by using one-way analysis of variance (ANOVA) followed by Dunnett′s post hoc tests. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 versus the UUO group, $^{\#\#\#}p$<0.001, $^{\#\#\#\#}p$<0.0001 versus the Sham group.

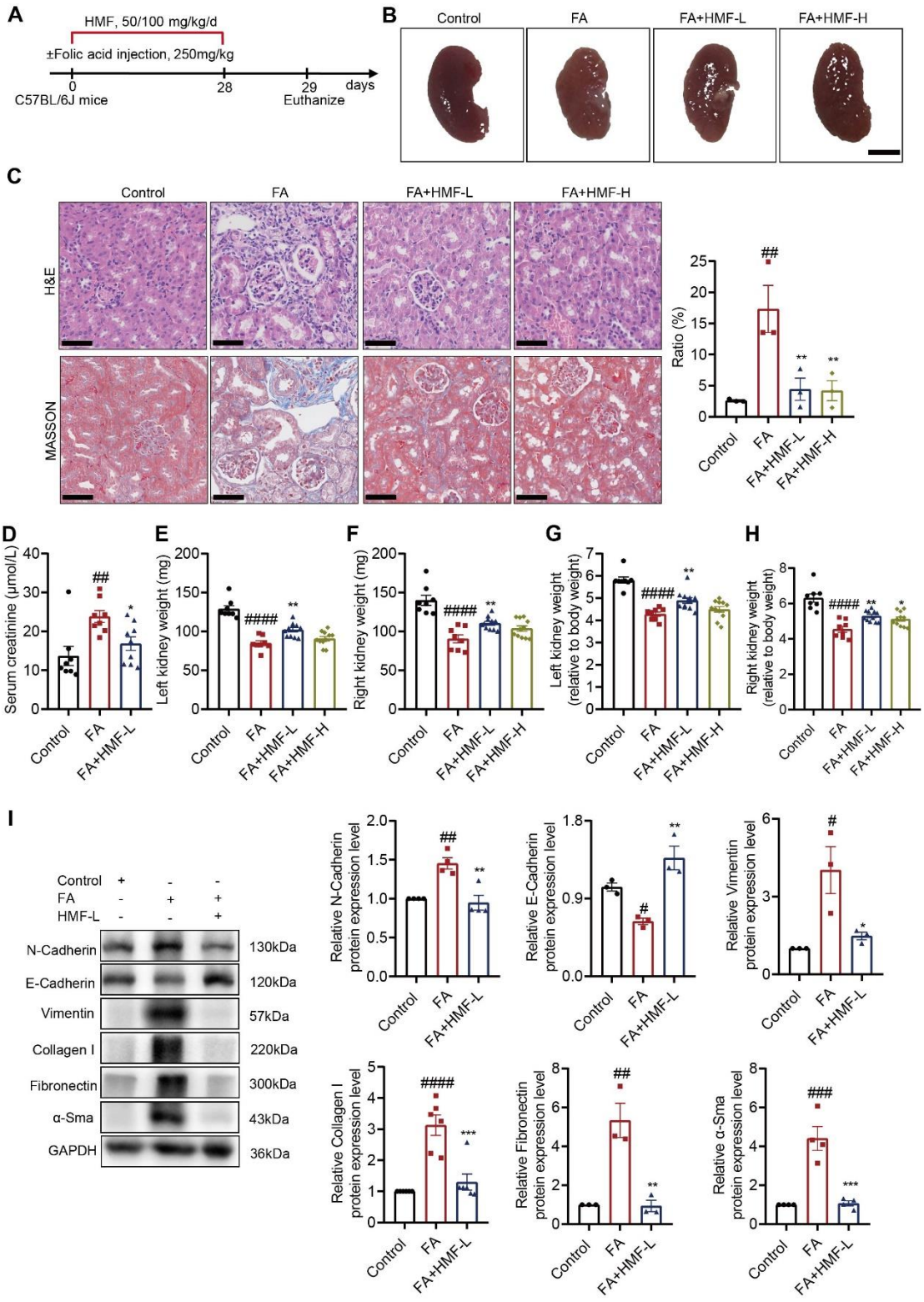


Figure S16. HMF ameliorated FA-induced renal fibrosis in mice. The mice were intraperitoneally injected with folic acid (250 mg/kg). Vehicle, or HMF (50 or 100 mg/kg/day) was administrated to folic acid mice by gastric irrigation once daily for 28 days. (**A**) Scheme of the experimental approach. (**B**) Representative picture of left

kidneys of mice with different treatments. (**C**) Representative photomicrographs of the H&E staining and Masson's trichrome staining from left kidneys, renal interstitial fibrosis scores based on Masson's trichrome staining (right panel), n = 3 mice. Scale bar, 50 μm. (**D**) Creatinine in serum, n = 8-11 mice. (**E** and **F**) The left (**E**) and right (**F**) renal weight. (**G** and **H**) The ratio of left (**G**) and right (**H**) renal weight to body weight (BW), n = 8-11 mice. (**I**) Western blot (left panel) and quantification (right panel) of the protein expression of N-Cadherin, E-Cadherin, Vimentin, Collagen I, Fibronectin and α-Sma in left kidneys, GAPDH served as loading control, n = 3-6 mice. Error bars represent mean ± SEM. Comparisons those among three or more groups by using one-way analysis of variance (ANOVA) followed by Dunnett′s post hoc tests. *p<0.05, **p<0.01, ***p<0.001 versus the FA group, $^{\#}p$<0.05, $^{\#\#}p$<0.01, $^{\#\#\#}p$<0.001, $^{\#\#\#\#}p$<0.0001 versus the Control group.

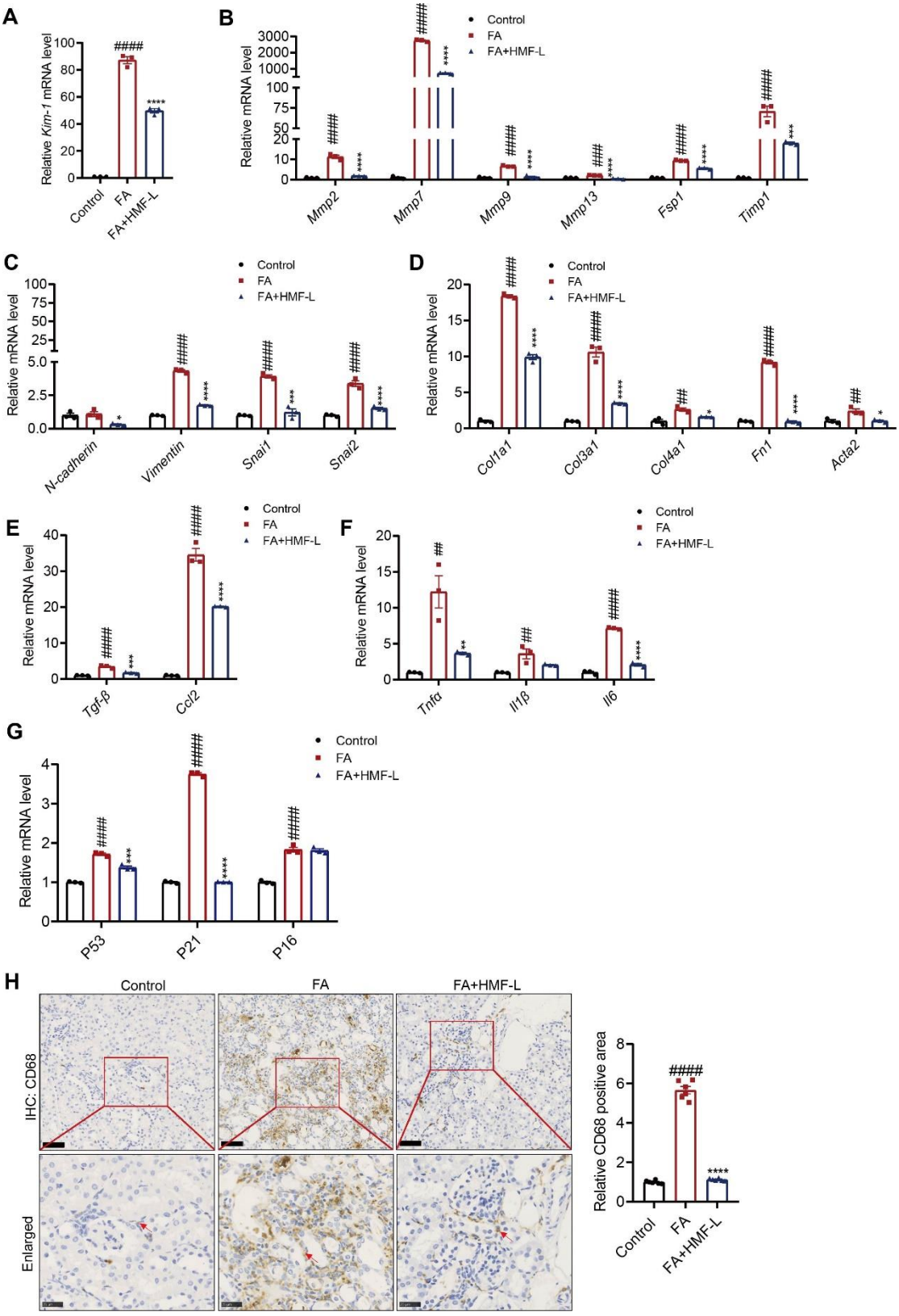
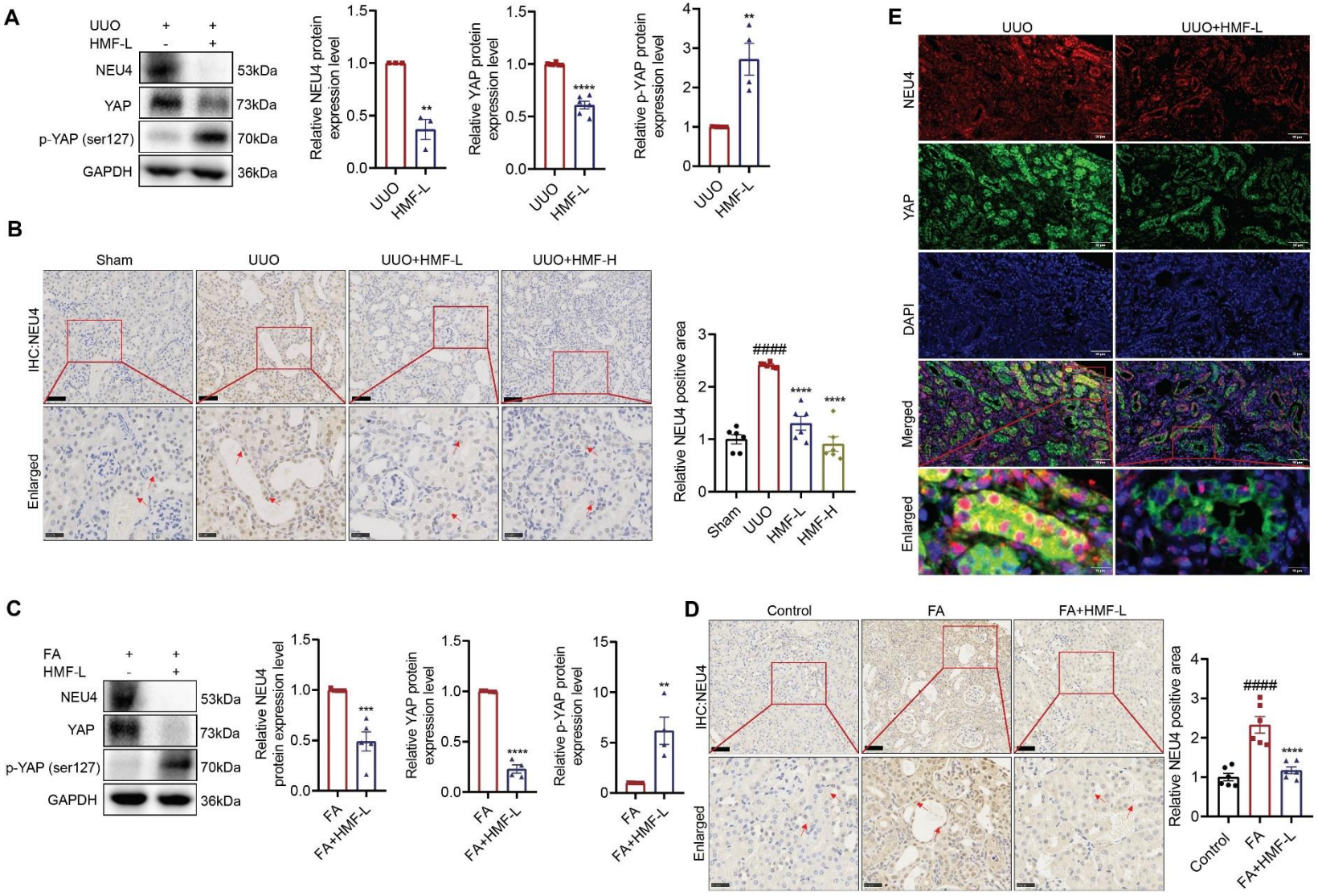


Figure S17. HMF inhibited FA-induced EMT, the production of proinflammatory cytokines, and macrophage infiltration in mice. The mice were intraperitoneally

injected with folic acid (250 mg/kg). Vehicle, or HMF (50 or 100 mg/kg/day) was administrated to folic acid mice by gastric irrigation once daily for 28 days. (**A**) *Kim-1* mRNA level in left kidneys, $n = 3$ mice. (**B-G**) Relative extracellular matrix associated gene (**B**), EMT associated gene (**C**), ECM associated gene (**D**), chemokine associated gene (**E**), inflammation associated gene (**F**) and senescence-associated genes (**G**) mRNA level in left kidneys, $n = 3$ mice. (**H**) Immunohistochemistry staining analysis (left panel) and quantification (right panel) of CD68 expression in kidney tissues, $n = 6$ mice. Scale bar, 50 μm. Error bars represent mean ± SEM. Comparisons those among three or more groups by using one-way analysis of variance (ANOVA) followed by Dunnett′s post hoc tests. *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$ versus the FA group, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$, $^{\#\#\#\#}p<0.0001$ versus the Control group.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HMF-L
NEU4
YAP
p-YAP (ser127)
GAPDH
53kDa
73kDa
70kDa
36kDa
Relative NEU4 protein expression level
Relative YAP protein expression level
Relative p-YAP protein expression level
UUO
HMF-L
B
Sham
UUO
UUO+HMF-L
UUO+HMF-H
IHC:NEU4
Enlarged
Relative NEU4 positive area
C
FA
HMF-L
NEU4
YAP
p-YAP (ser127)
GAPDH
53kDa
73kDa
70kDa
36kDa
Relative NEU4 protein expression level
Relative YAP protein expression level
Relative p-YAP protein expression level
FA
FA+HMF-L
D
Control
FA
FA+HMF-L
IHC:NEU4
Enlarged
Relative NEU4 positive area
E
UUO
UUO+HMF-L
NEU4
YAP
DAPI
Merged
Enlarged

228

Figure S18. HMF inhibited the interaction between NEU4 with YAP, and activation of YAP in UUO or folic acid-induced mice. (**A**) Western blot (left panel) and quantification (right panel) of the protein expression of NEU4, YAP and phosphorylation of YAP in left kidneys. GAPDH served as loading control, n = 3-5 mice. (**B**) Immunohistochemistry staining analysis (left panel) and quantification (right panel) of NEU4 in left kidney tissues. n = 6 mice. Scale bar, 50 μm. (**C**) Western blot (left panel) and quantification (right panel) of the protein expression of NEU4, YAP and phosphorylation of YAP in kidneys. GAPDH served as loading control, n = 3-5 mice. (**D**) Immunohistochemistry staining analysis (left panel) and quantification (right panel) of NEU4 in kidney tissues. n = 6 mice. Scale bar, 50 μm. (**E**) Colocalization of NEU4 and YAP was analyzed by immunofluorescence in left kidneys. Scale bar, 50 μm. n = 6 mice. (**A**, **B** and **E**) Vehicle, or HMF (50 or 100 mg/kg/day) was administrated to UUO mice by gastric irrigation once daily for 10 days. (**C** and **D**) The mice were intraperitoneally injected with folic acid (250 mg/kg). Vehicle, or HMF (50 or 100 mg/kg/day) was administrated to folic acid mice by gastric irrigation once daily for 28 days. Error bars represent mean ± SEM. Comparisons between two groups were analyzed by using a two-tailed Student′s t test. Comparisons those among three or more groups by using one-way analysis of variance (ANOVA) followed by Dunnett′s post hoc tests. $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$ versus the UUO or FA group, $^{\#\#\#\#}p<0.0001$ versus the Sham or Control group.

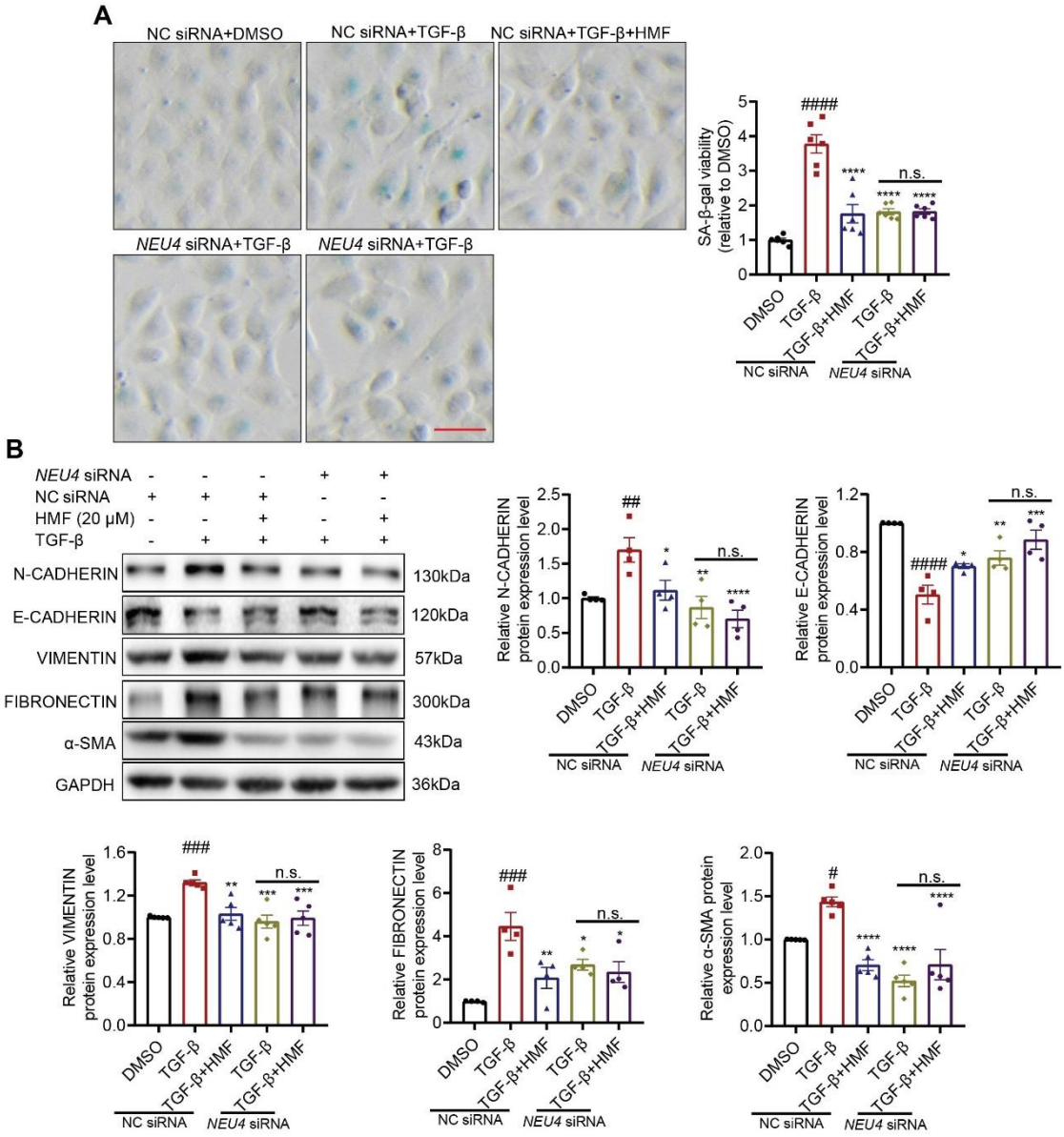

Figure S19. NEU4 knockdown abolished the anti-fibrotic effect of HMF in HK-2. HK-2 cells treatment with TGF-β and in the presence of DMSO or HMF 24 h after transfection with *NEU4* siRNA. (**A**) Measurement (left panel) and quantification (right panel) of SA-β-gal activity by SA-β-gal staining in HK-2 cells, n = 6 biologically independent samples. Scale bar, 100 μm. (**B**) Western blot (left panel) and quantification (right panel and bottom panel) of the protein expression of N-CADHERIN, E-CADHERIN, VIMENTIN, FIBRONECTIN and α-SMA in HK-2 cells. GAPDH served as loading control. n = 4-5 biologically independent samples. Error bars represent mean ± SEM. Comparisons those among three or more groups by using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc tests. *p<0.05, **p<0.01,

260 ***p<0.001, ****p<0.0001 versus the NC siRNA+TGF-β group, $^{\#}p$<0.05, $^{\#\#}p$<0.01,
261 $^{\#\#\#}p$<0.001, $^{\#\#\#\#}p$<0.0001 versus the NC siRNA+DMSO group. n.s.: no significance.
262

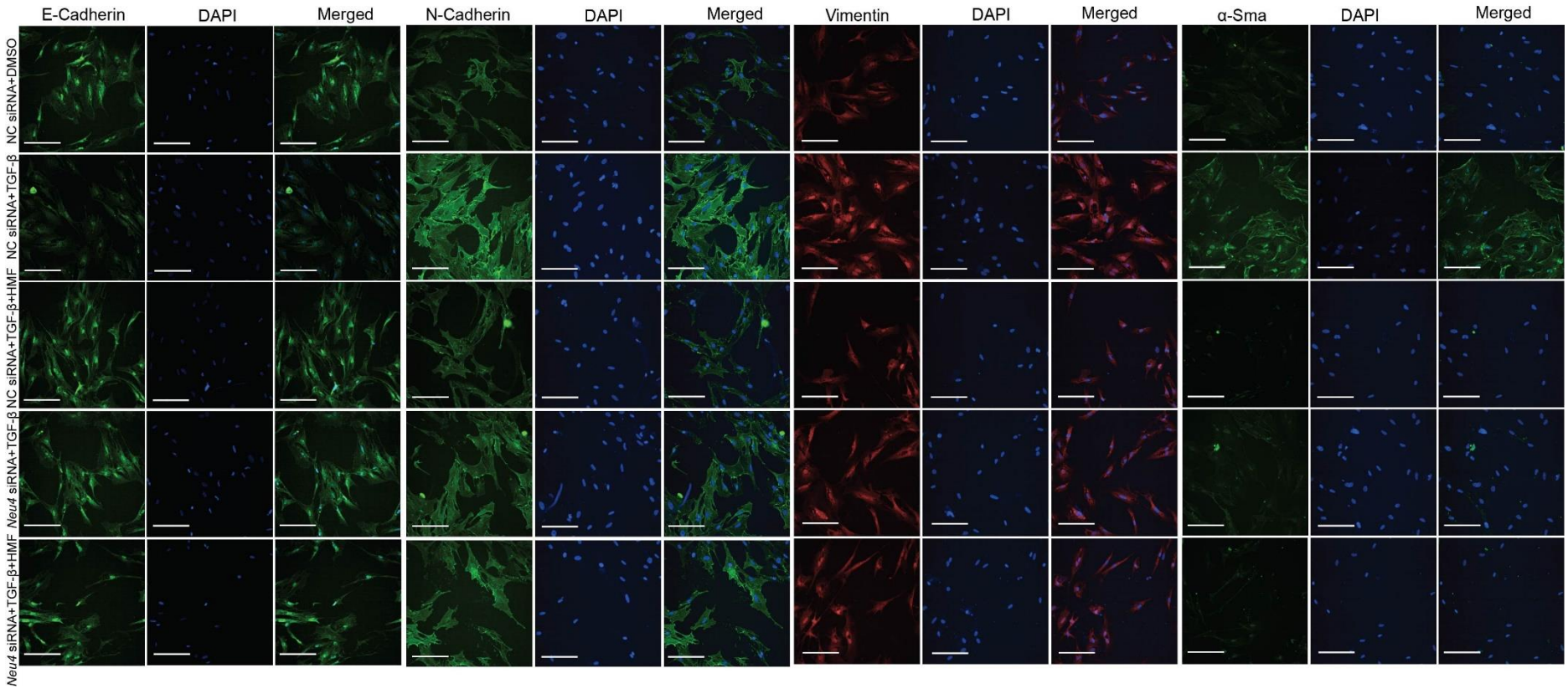

Figure S20. Knockdown of NEU4 abolished the anti-fibrotic effect of HMF in PTECs. Immunofluorescent staining represents N-Cadherin, E-Cadherin, Vimentin and α-Sma expressions in PTECs treatment with TGF-β and in the presence of DMSO or HMF 24 h after transfection with *NEU4* siRNA. $n = 3$ biologically independent samples. Scale bar, 100 μm.

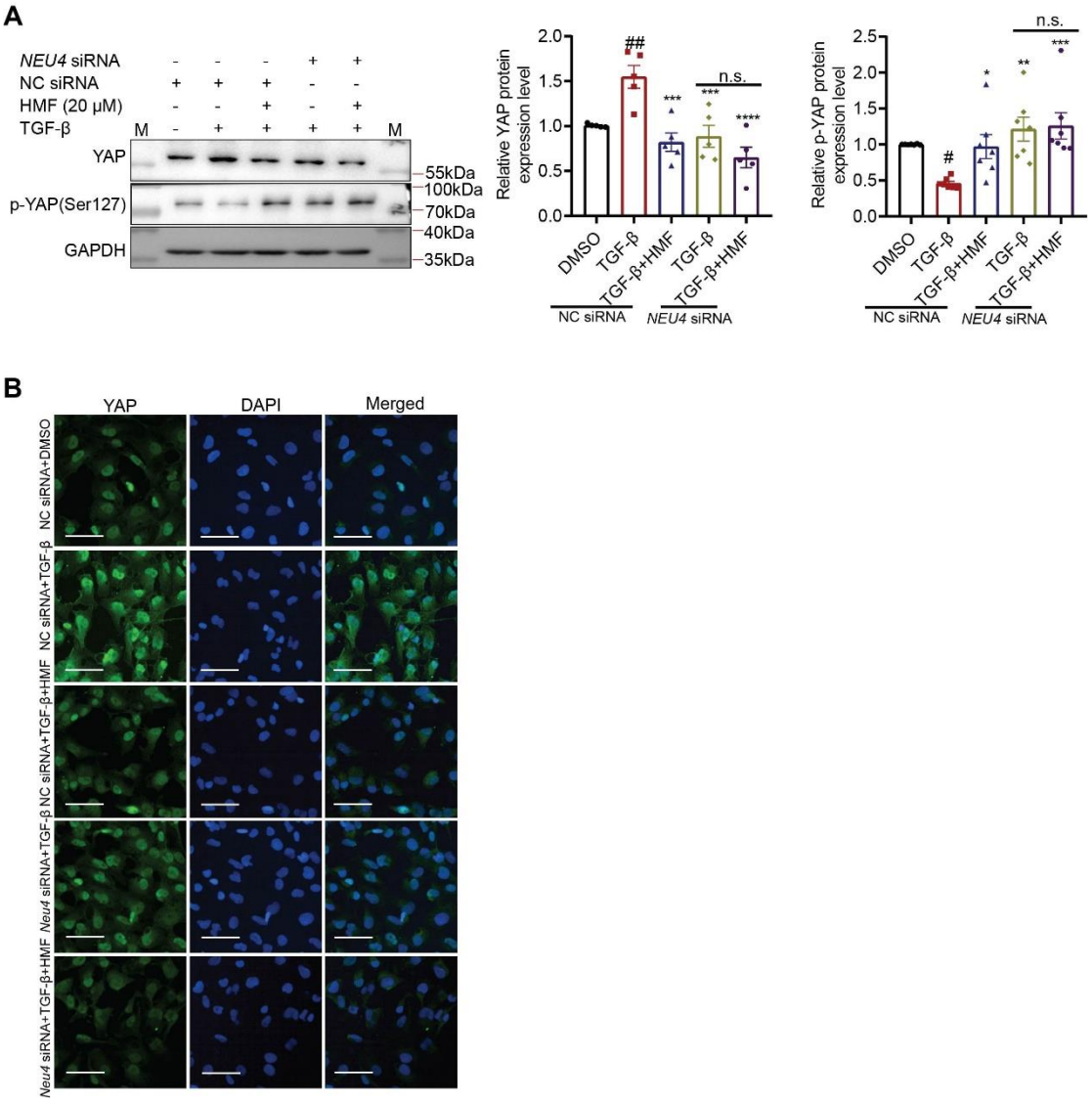

Figure S21. NEU4 knockdown abolished the downregulation of YAP by HMF. HK-2 cells treatment with TGF-β and in the presence of DMSO or HMF 24 h after transfection with *NEU4* siRNA. (**A**) Western blot (left panel) and quantification (right panel) of the protein expression of YAP and phosphorylation of YAP in HK-2 cells. GAPDH served as loading control. n = 5-7 biologically independent samples. M, Marker. (**B**) Immunofluorescent staining represents YAP expressions in HK-2 cells treatment with TGF-β and in the presence of DMSO or HMF 24 h after transfection with *NEU4* siRNA. Scale bar, 100 μm, $n = 3$ samples. Error bars represent mean ± SEM. Comparisons those among three or more groups by using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc tests. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 versus the NC siRNA+TGF-β group, $^{\#}p$<0.05, $^{\#\#}p$<0.001 versus the NC siRNA+DMSO group. n.s.: no significance.

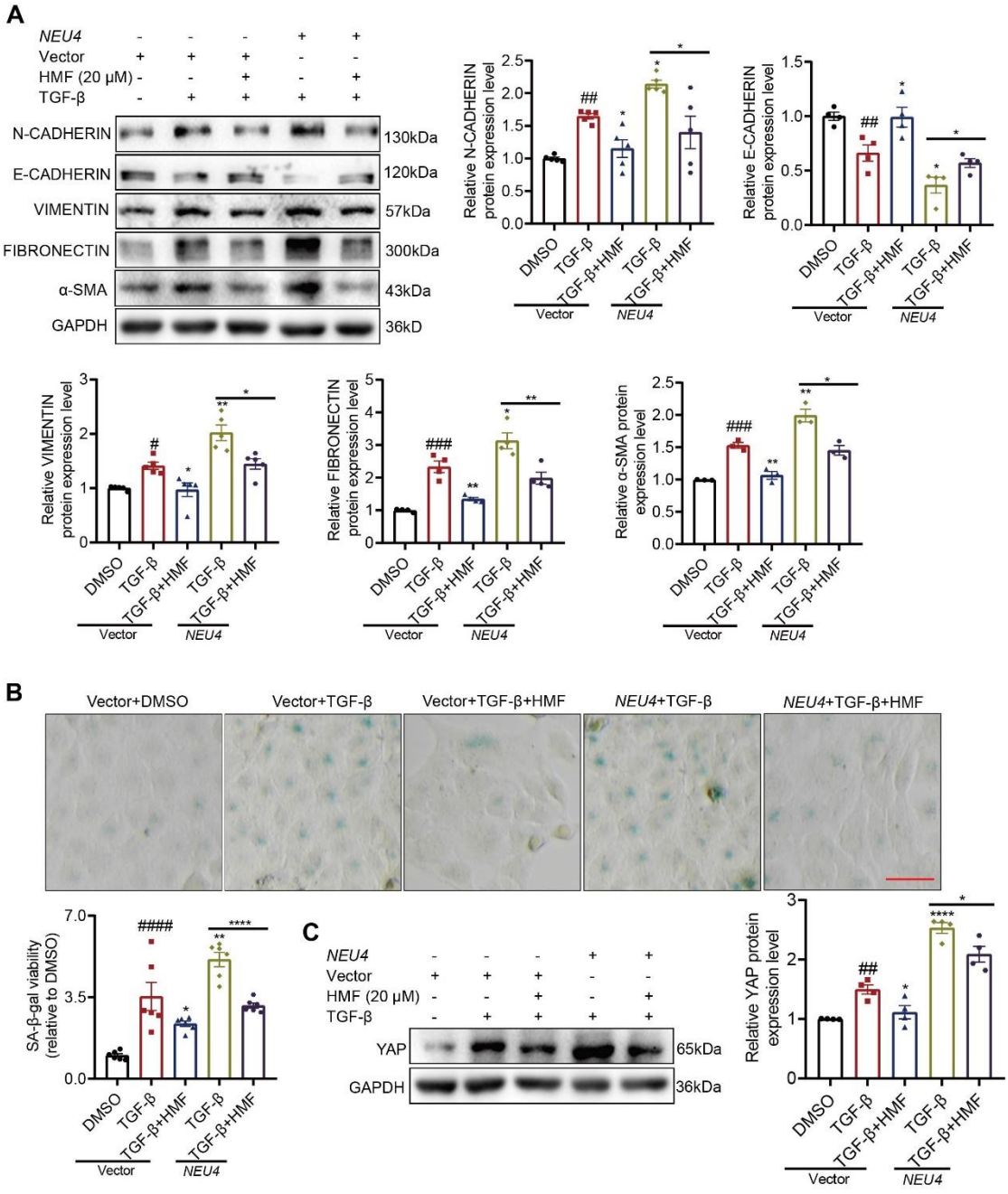

Figure S22. HMF ameliorated TGF-β-induced EMT and cellular senescence is dependent on NEU4 in HK-2. HK-2 cells treated with TGF-β and in the presence of DMSO or HMF 24 h after transfection with *NEU4*-overexpression plasmids. (**A**) Western blot (left panel) and quantification (right panel and bottom panel) of the protein expression of N-CADHERIN, E-CADHERIN, VIMENTIN, FIBRONECTIN and α-SMA in HK-2 cells. GAPDH served as loading control. *n* = 3-6 biologically independent samples. (**B**) Measurement (top panel) and the quantification (bottom panel) of SA-β-gal activity by SA-β-gal staining in HK-2 cells. *n* = 6 biologically

independent samples. Scale bar, 100 μm. (**C**) Western blot (left panel) and quantification (right panel) of the protein expression of YAP in HK-2 cells, GAPDH served as loading control, $n = 4$ biologically independent samples. Error bars represent mean ± SEM. Comparisons those among three or more groups by using one-way analysis of variance (ANOVA) followed by Dunnett′s post hoc tests. *$p<0.05$, **$p<0.01$, ****$p<0.0001$ versus the Vector+TGF-β or *NEU4*+TGF-β group, $^{\#}p<0.05$, $^{\#\#}p<0.001$, $^{\#\#\#}p<0.01$, $^{\#\#\#\#}p<0.0001$ versus the Vector+DMSO group.

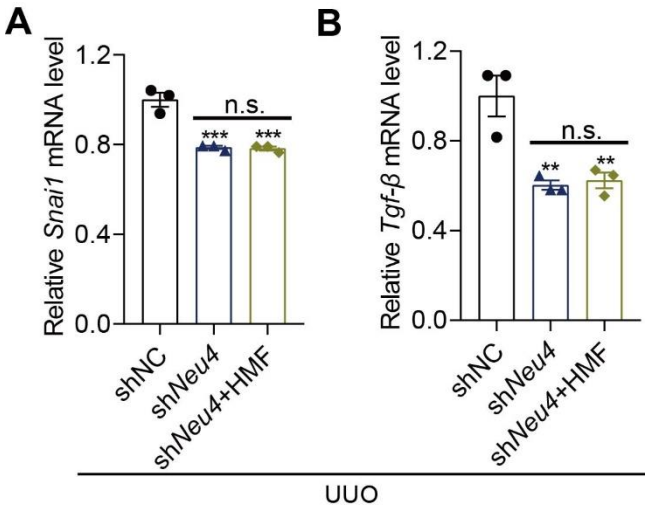

Figure S23. *Neu4* knockdown relieved *Snai1* and *Tgf-β* inhibition effect of HMF in UUO model. Six-week-old male C57BL/6J mice were injected with shNC or sh*Neu4* adenoviruses. Five weeks after injection, the mice were subjected to UUO surgery, then vehicle or HMF (50 mg/kg/day) was administrated to mice by gastric irrigation once daily for 10 days. (**A** and **B**) Relative EMT associated gene mRNA level in left kidneys, $n = 3$ mice. Error bars represent mean ± SEM. Comparisons those among three or more groups by using one-way analysis of variance (ANOVA) followed by Dunnett′s post hoc tests. $^{**}p<0.01$, $^{***}p<0.001$ versus the shNC group. n.s.: no significance.

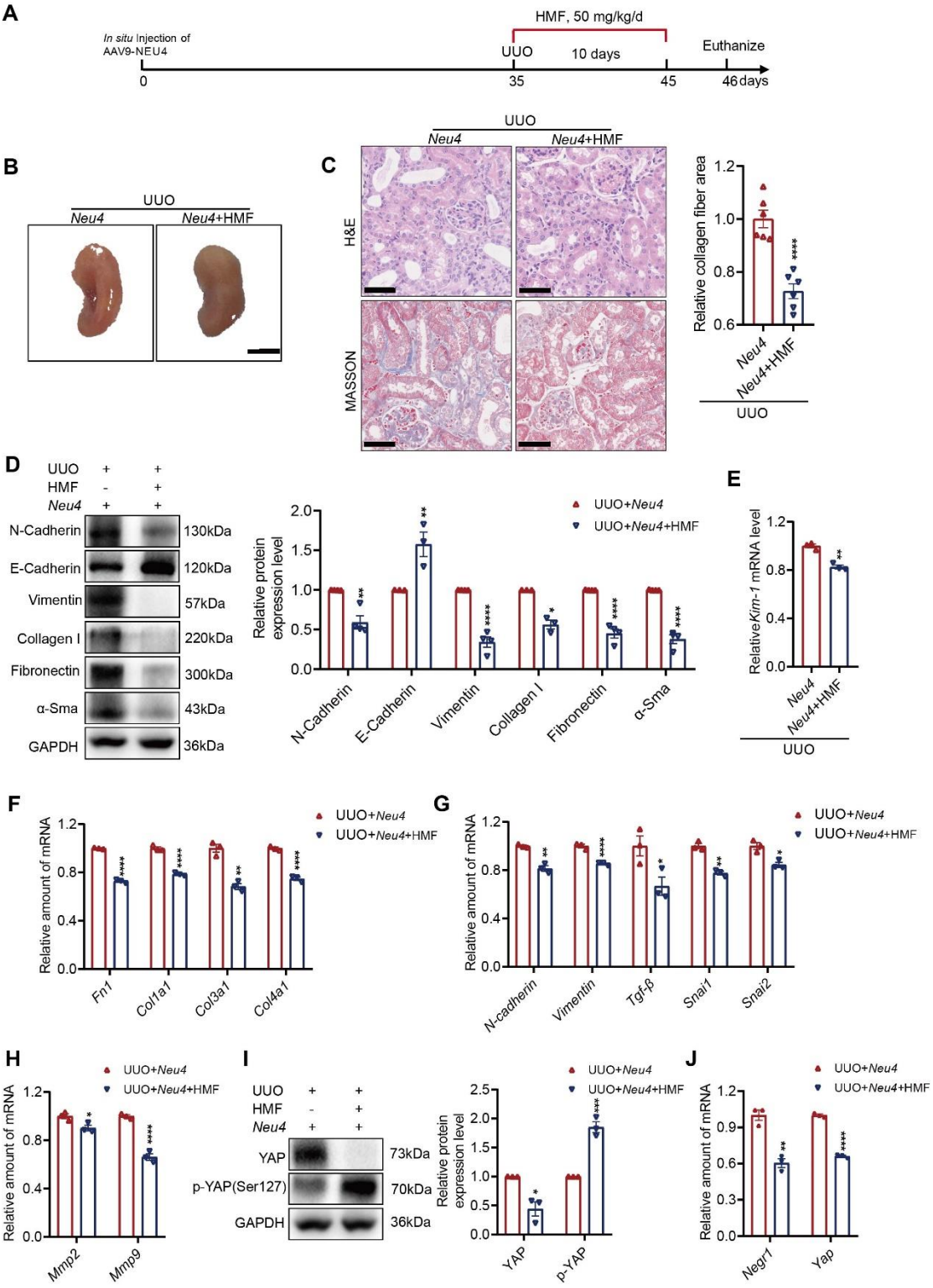

Figure S24. Relief of renal fibrosis by HMF was dependent on NEU4. Mice was *in situ* injected with AAV9 encoding GFP-*Neu4*. Five weeks after injection, the mice were subjected to UUO surgery, then vehicle or HMF (50 mg/kg/day) was administrated to mice by gastric irrigation once daily for 10 days. (**A**) The schematic of experimental

design. (**B**) Representative picture of left kidneys of UUO mice. Scale bar, 100 μm, *n* = 3 mice. (**C**) Representative photomicrographs of the H&E staining and Masson's trichrome staining from left kidneys of UUO mice (left panel), and renal interstitial fibrosis scores based on Masson's trichrome staining (right panel). *n* = 6 mice. H&E staining, scale bar, 50 μm. Masson's trichrome staining, scale bar, 100 μm. (**D**) Western blot (left panel) and quantification (right panel) of the protein expression of N-Cadherin, E-Cadherin, Vimentin, Collagen I, Fibronectin and α-Sma in kidneys, GAPDH served as loading control. *n* = 3-4 mice. (**E**) *Kim-1* gene mRNA level in left kidneys. *n* = 3 mice. (**F**-**H**) Relative ECM associated gene (**F**), EMT associated gene (**G**), extracellular matrix associated gene (**H**) mRNA level in left kidneys. *n* = 3 mice. (**I**) Western blot (left panel) and quantification (right panel) of the protein expression of YAP and phosphorylation of YAP in kidneys. GAPDH served as loading control, *n* = 3 mice. (**J**) Relative mRNA abundance of *Yap* target genes in left kidney tissue, *n* = 3 mice. Error bars represent mean ± SEM. Comparisons between two groups were analyzed by using a two-tailed Student′s *t* test. **p*<0.05, ***p*<0.01, ****p*<0.001, *****p*<0.0001 versus the *Neu4* group.