Fig.1


atggcaggacagcccggccacatgcccca**tggagggagttccaacaacctct**gccacaccctggggcctgtgcatcctcctga
KB-001
cccacagaggcatcccaacacgctgtcttttcgctgctcgctggcggacttccagatcgaaaagaagataggccgaggacagt
tcagcgaggtgtacaaggccacctgcctgctggacaggaagacagtggctctgaagaaggtgcagatctttgagatgatgg
acgccaa**ggcgaggcaggactgtgtcaagg**agatcggcctcttgaagcaactgaaccacccaaatatcatcaagtatttgga
KB-002
ctcgtttatcgaagacaacgagctgaacattgtgctggagttggctgacgcaggggacctctcgcagatgatcaagtacttta
agaagcagaagcggctcatccc**ggagaggacagtatggaagtactt**tgtgcagctgtgcagc**gccgtggagcacatgcattc**
Stealth
KB-003
acgccgggtgatgcaccgagacatcaagcctgccaacgtgttcatcacagccacgggcgtcgtgaagctcggtgaccttggtc
tgggccgcttcttcagctctgagaccaccgcagcccactccctagtggggacgccctactacatgtcaccggagaggatccatg
agaacggctacaacttcaagtccgacatctggtccctgggctgtctgctgtacgagatggcagccctccagagccccttctatg
gagataagatgaatctcttctccctgtgccagaagatcgagcagtgtgactacccccactccccggggagcactactccgag
KB-004
aagttacgagaactggtcagcatgtgcatctgccctgaccccc**accagagacctgacatcggatac**gtgcaccaggtggcca
KB-005
agcagatgcacatctggatgtccagcacctga

Fig.2

(A)



(B)

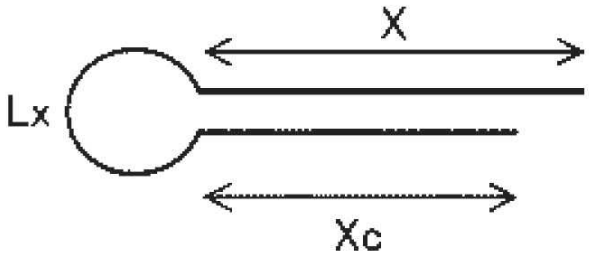


Fig.3

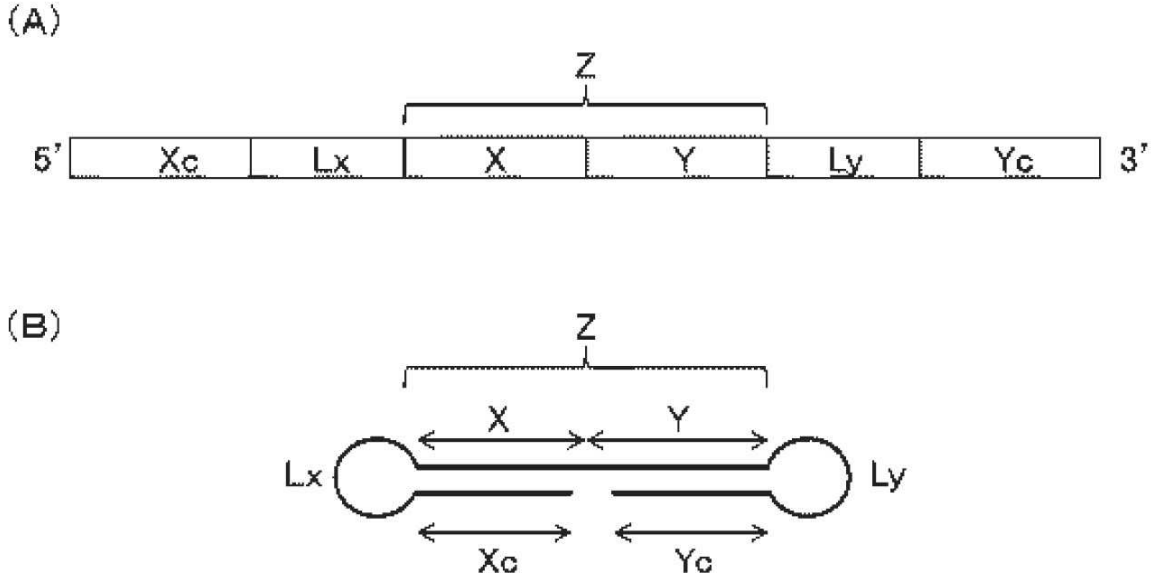


Fig.4

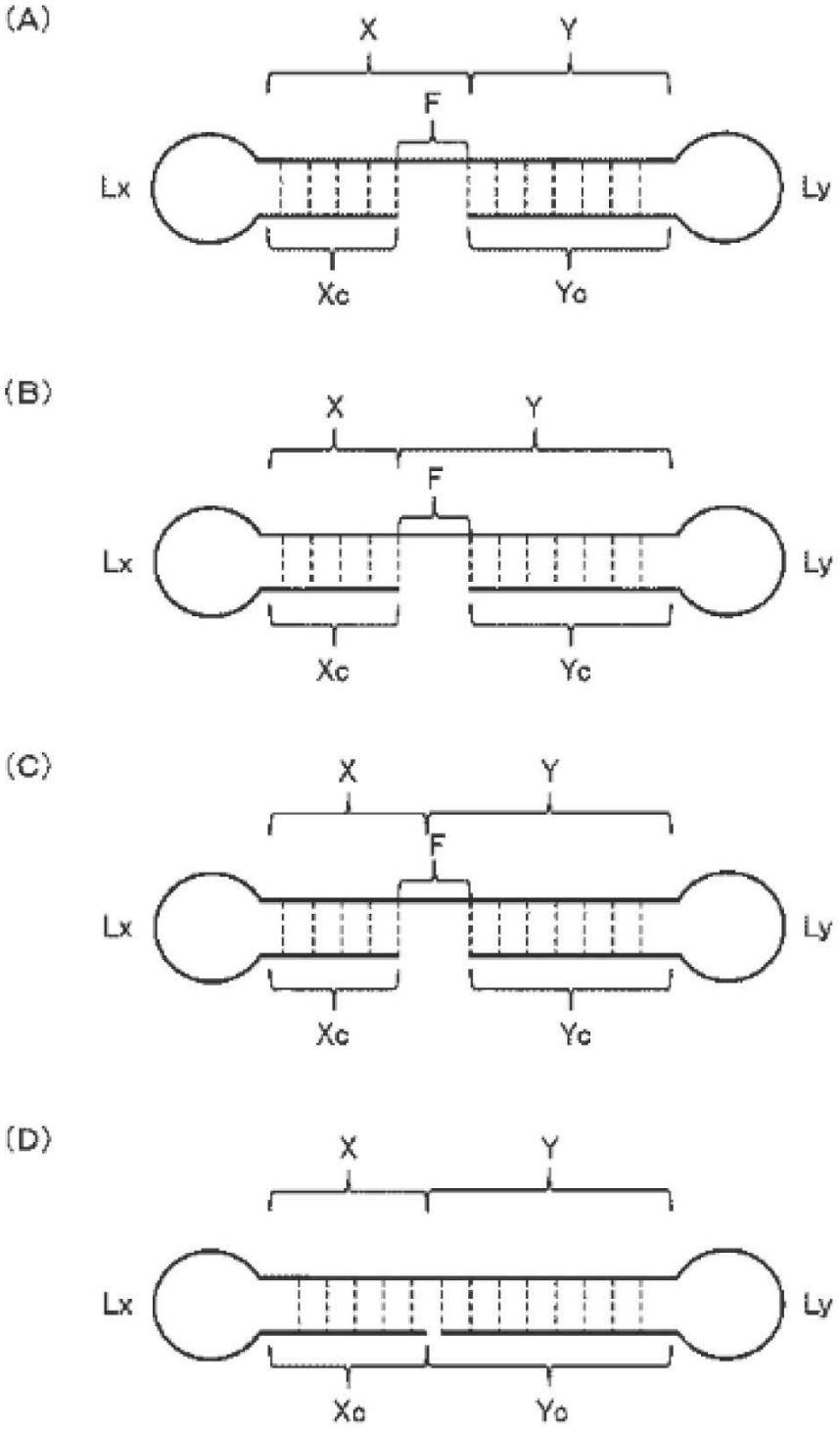


Fig.5

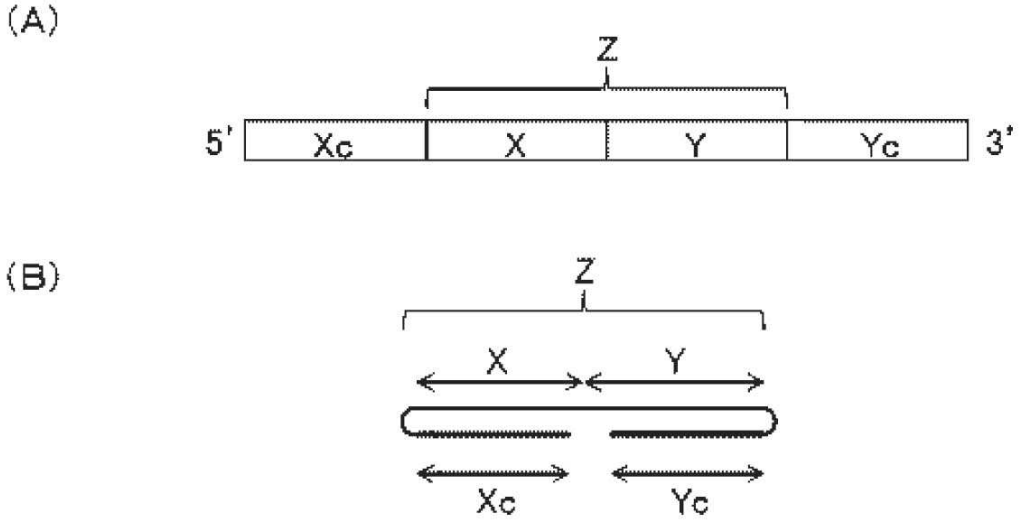


Fig.6

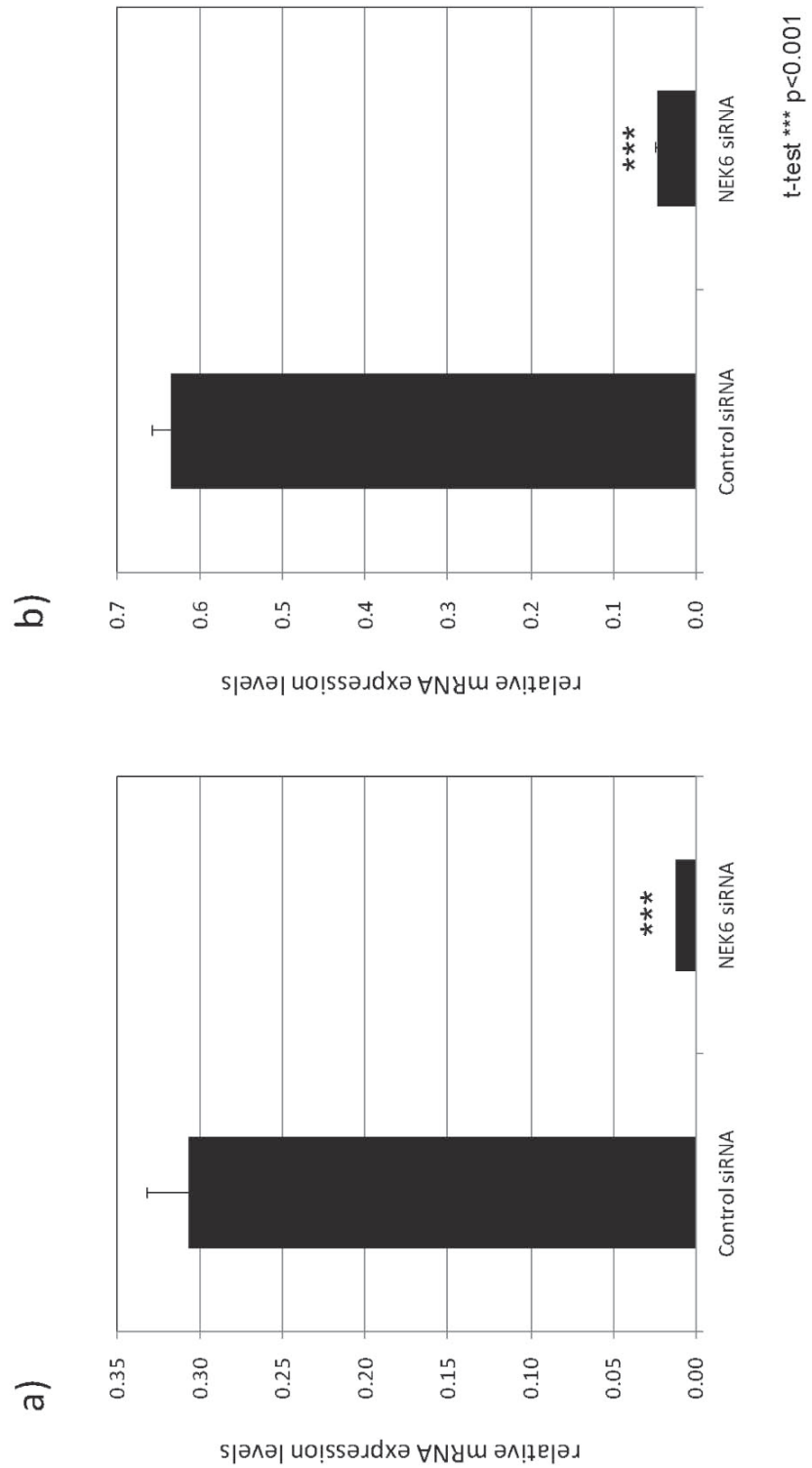

a)
relative mRNA expression levels
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
***
Control siRNA
NEK6 siRNA
b)
relative mRNA expression levels
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
***
Control siRNA
NEK6 siRNA
t-test *** p<0.001

Fig.7

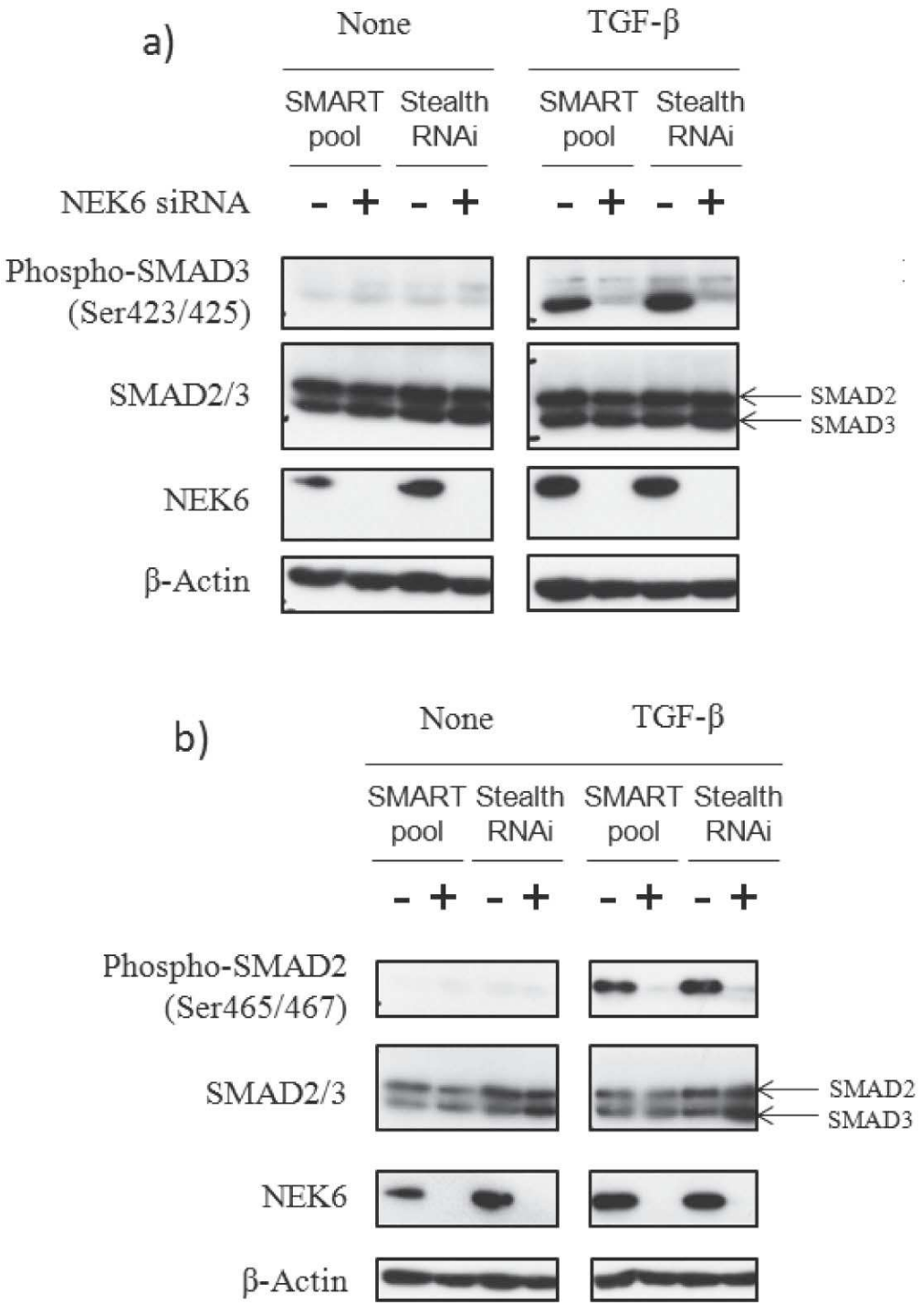
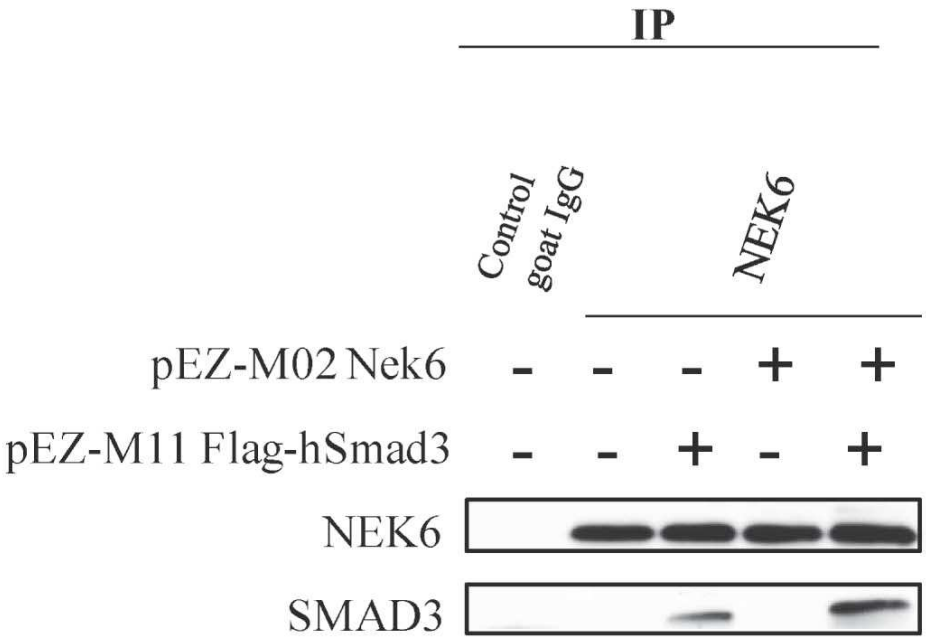

a)
None
TGF-β
SMART pool
Stealth RNAi
SMART pool
Stealth RNAi
NEK6 siRNA
- + - + - + - +
Phospho-SMAD3 (Ser423/425)
SMAD2/3
SMAD2
SMAD3
NEK6
β-Actin
b)
None
TGF-β
SMART pool
Stealth RNAi
SMART pool
Stealth RNAi
- + - + - + - +
Phospho-SMAD2 (Ser465/467)
SMAD2/3
SMAD2
SMAD3
NEK6
β-Actin

Fig.8

a)


IP
Control goat IgG
NEK6
pEZ-M02 Nek6 - - - + +
pEZ-M11 Flag-hSmad3 - - + - +
NEK6
SMAD3

b)

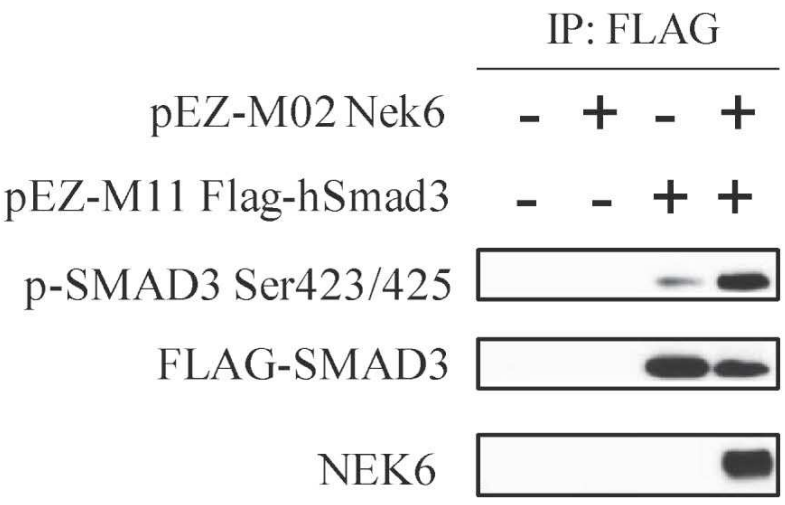

IP: FLAG
pEZ-M02 Nek6 - + - +
pEZ-M11 Flag-hSmad3 - - + +
p-SMAD3 Ser423/425
FLAG-SMAD3
NEK6

Fig.9

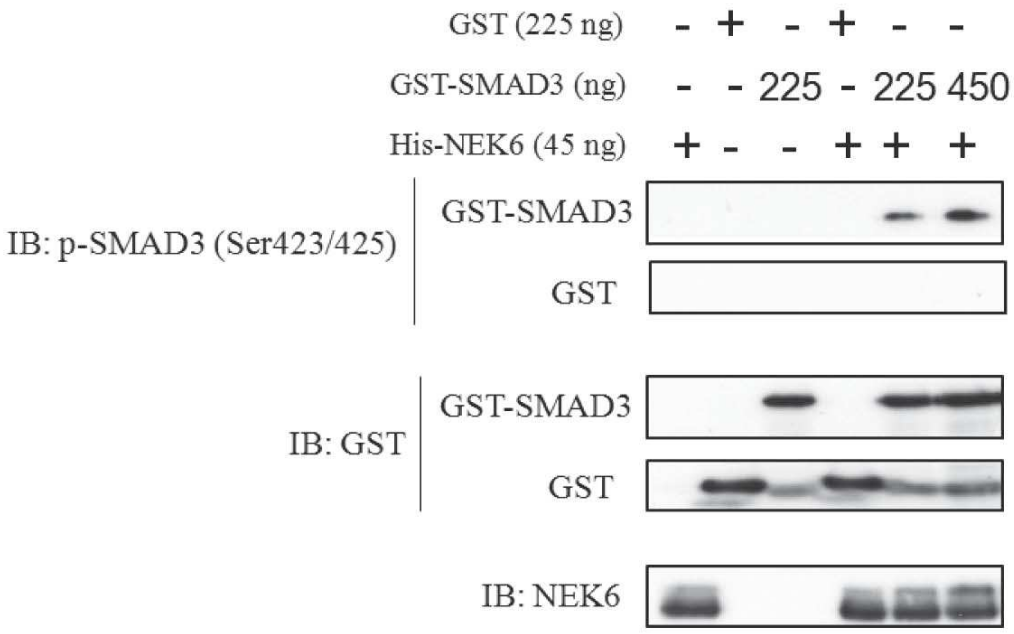
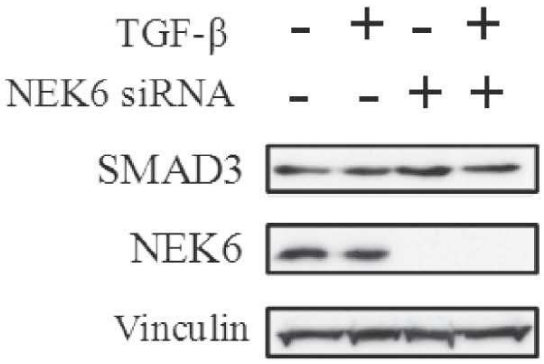
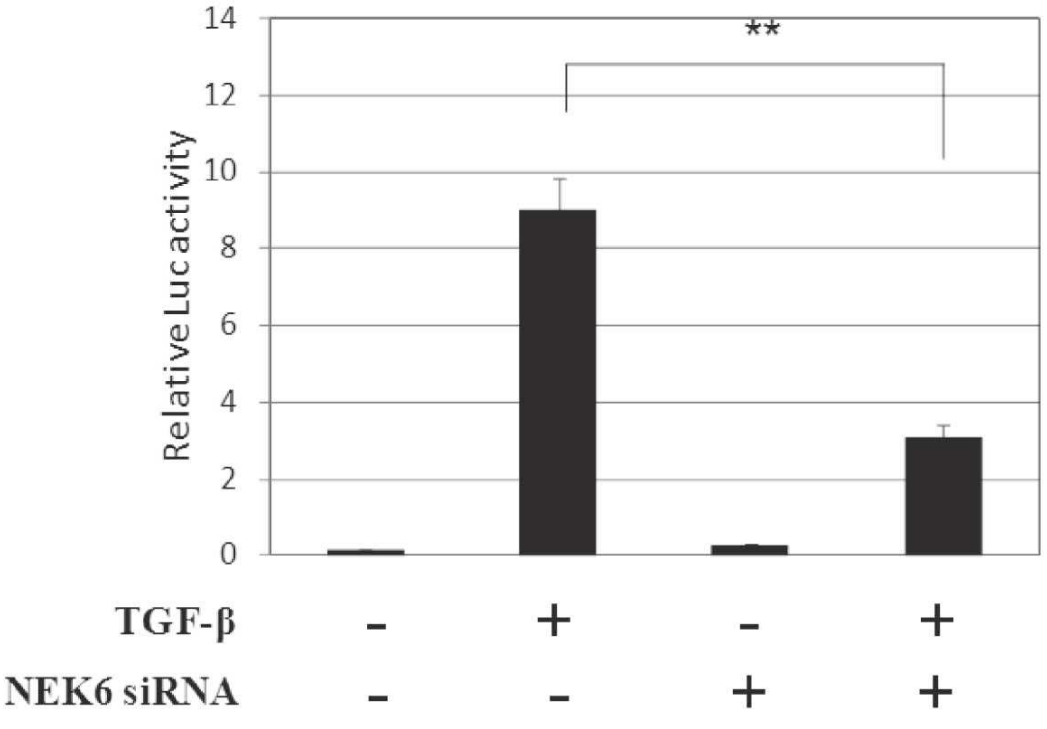

GST (225 ng) - + - + - -
GST-SMAD3 (ng) - - 225 - 225 450
His-NEK6 (45 ng) + - - + + +
IB: p-SMAD3 (Ser423/425)
GST-SMAD3
GST
IB: GST
GST-SMAD3
GST
IB: NEK6

Fig.10

a)



b)



t-test ** p<0.01

Fig.11

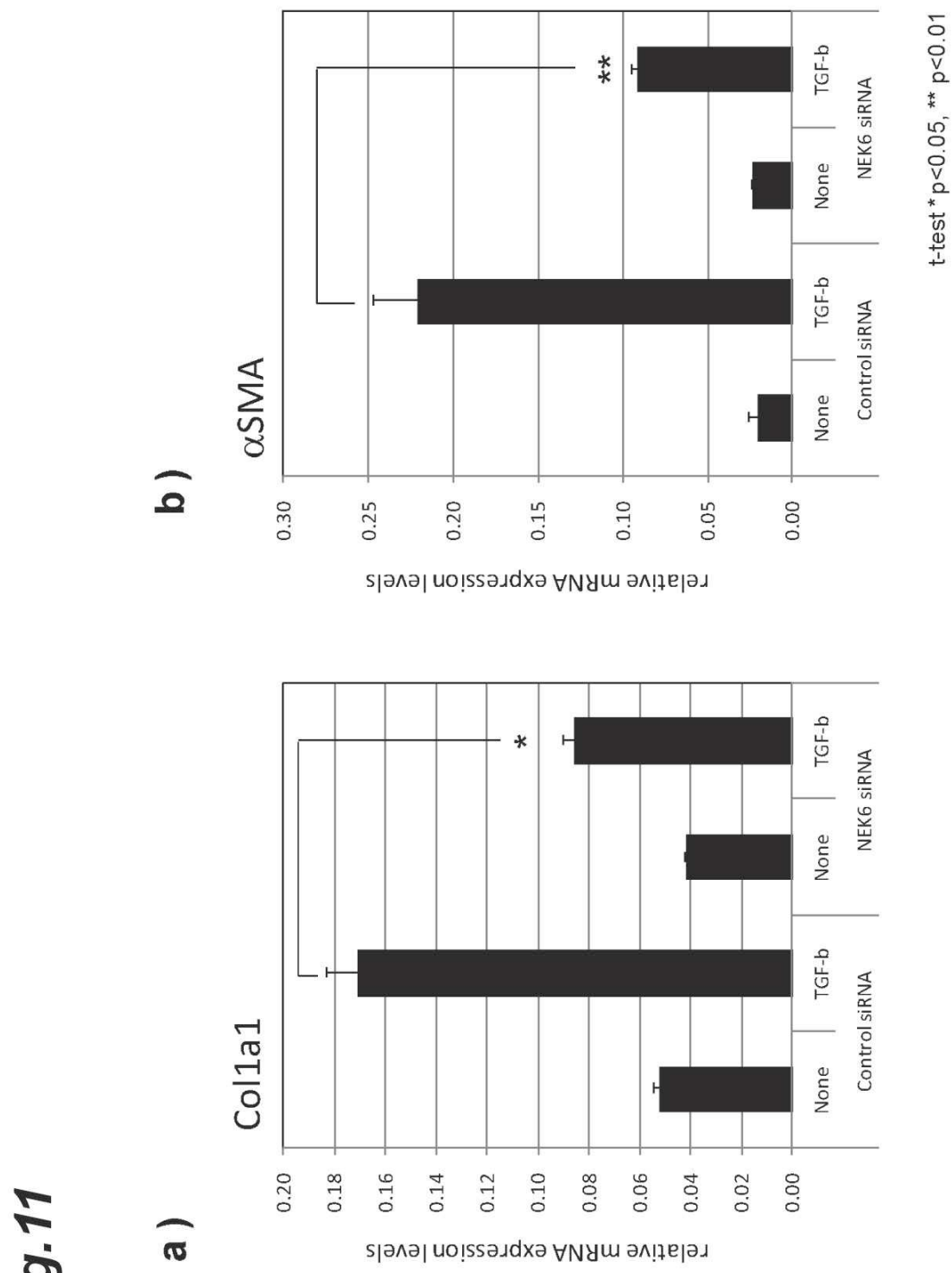

a)
Col1a1
relative mRNA expression levels
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
*
None
TGF-b
None
TGF-b
Control siRNA
NEK6 siRNA
b)
αSMA
relative mRNA expression levels
0.30
0.25
0.20
0.15
0.10
0.05
0.00
**
None
TGF-b
None
TGF-b
Control siRNA
NEK6 siRNA
t-test * p<0.05, ** p<0.01

Fig.12

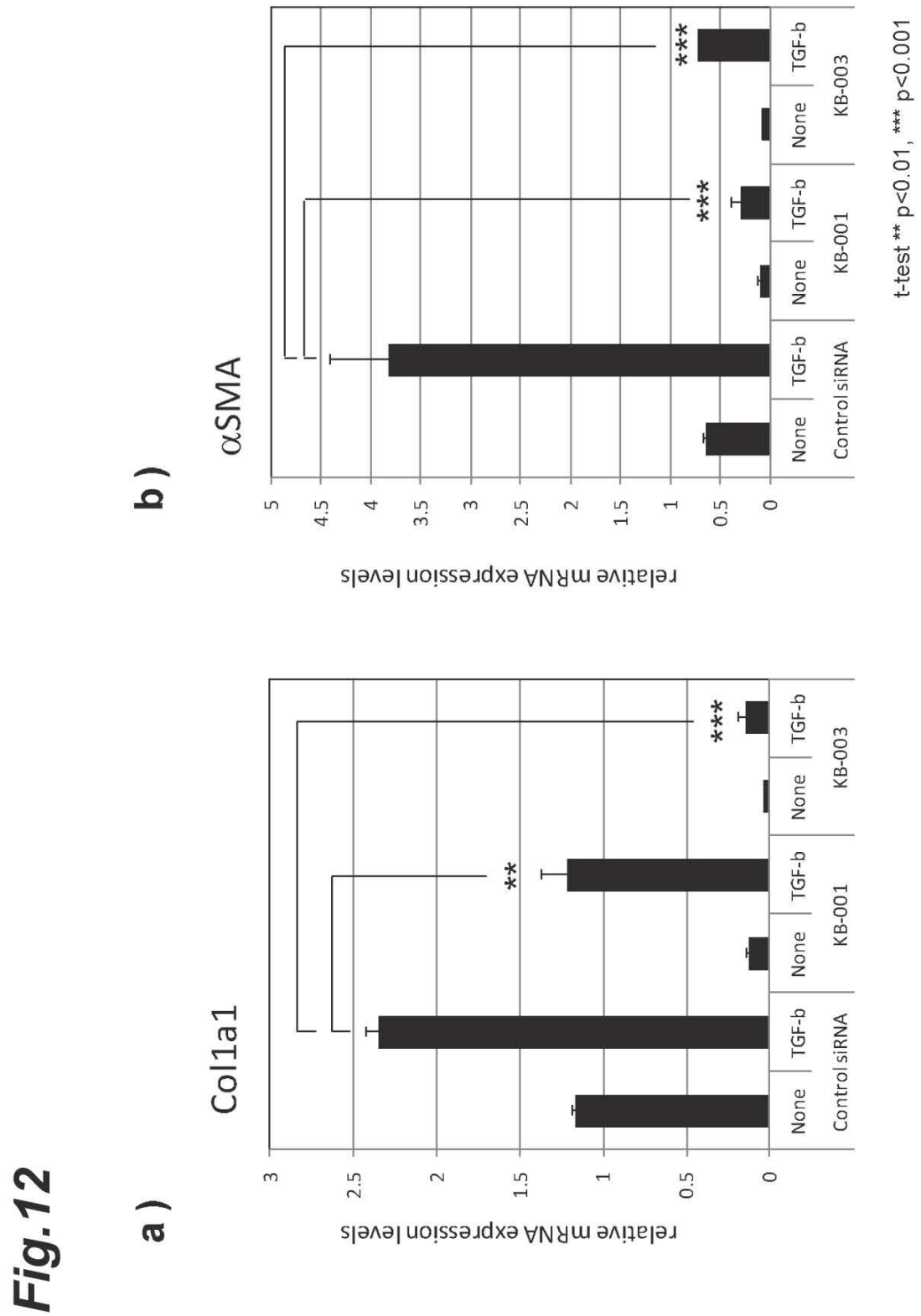

a)
Col1a1
relative mRNA expression levels
3
2.5
2
1.5
1
0.5
0
**
***
None
TGF-b
None
TGF-b
None
TGF-b
Control siRNA
KB-001
KB-003
b)
αSMA
relative mRNA expression levels
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
***
***
None
TGF-b
None
TGF-b
None
TGF-b
Control siRNA
KB-001
KB-003
t-test ** p<0.01, *** p<0.001

Fig.13

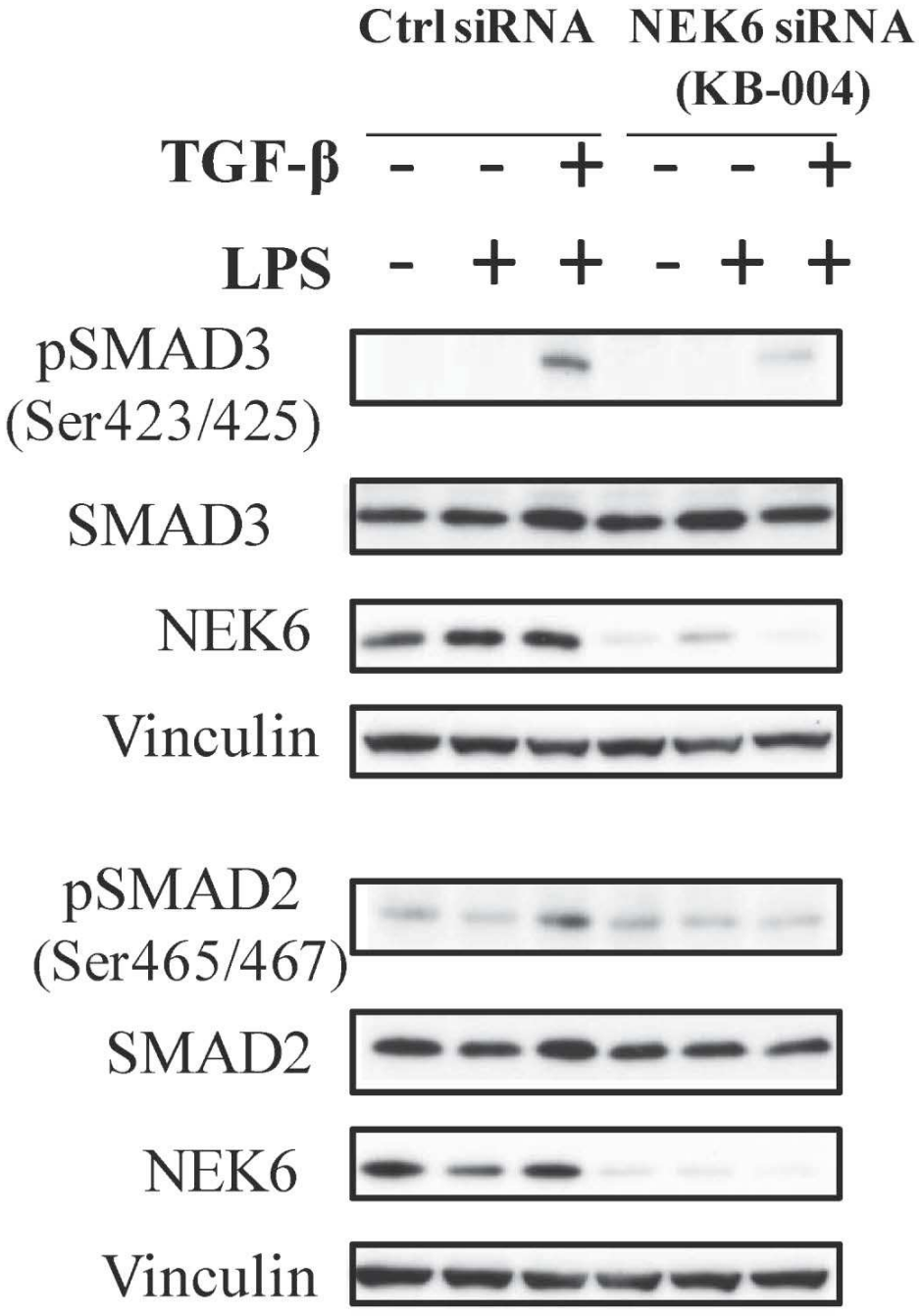

Ctrl siRNA
NEK6 siRNA
(KB-004)
TGF-β - - + - - +
LPS - + + - + +
pSMAD3
(Ser423/425)
SMAD3
NEK6
Vinculin
pSMAD2
(Ser465/467)
SMAD2
NEK6
Vinculin

Fig.14

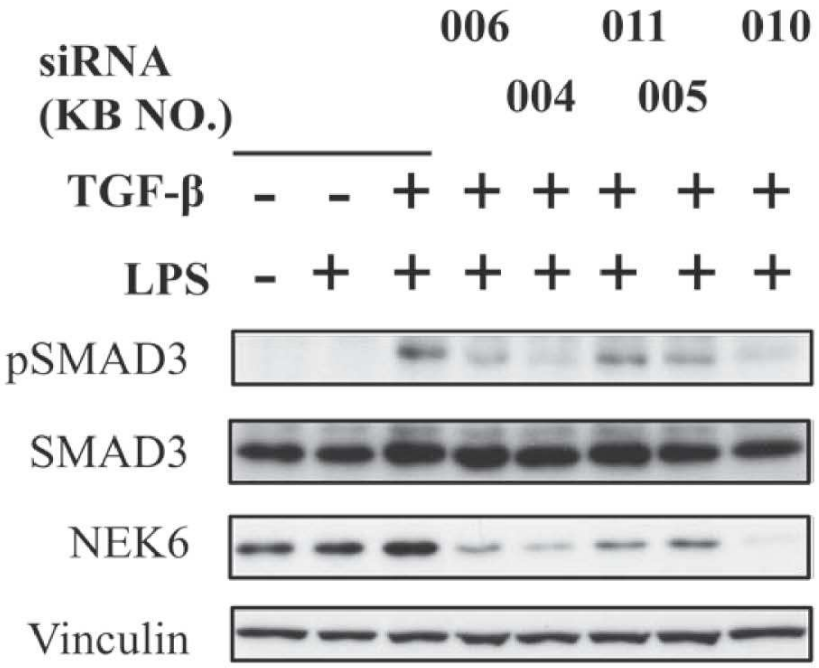
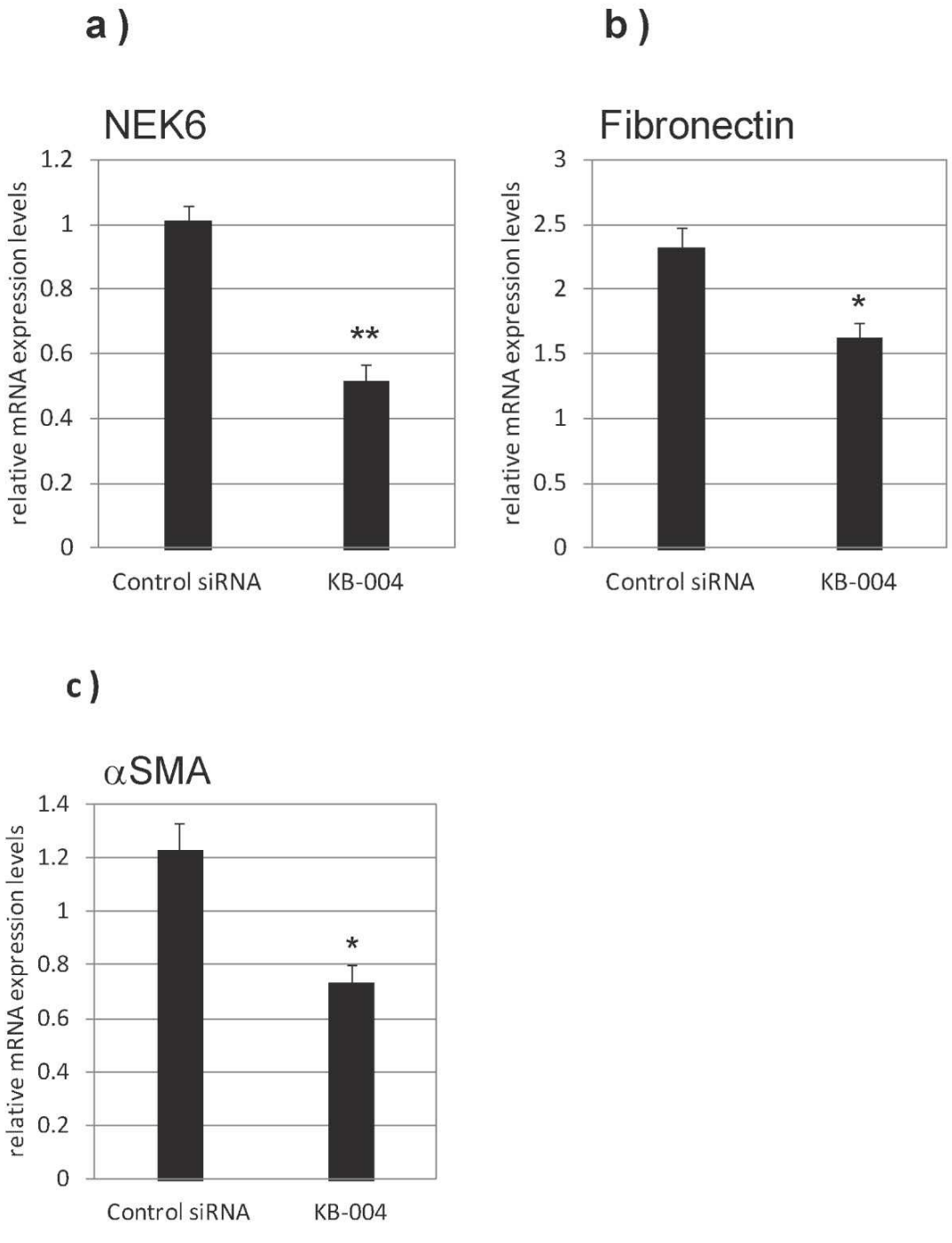

siRNA
(KB NO.)
006
004
011
005
010
TGF-β - - + + + + + +
LPS - + + + + + + +
pSMAD3
SMAD3
NEK6
Vinculin

Fig.15



t-test * p<0.05, ** p<0.01

Fig.16

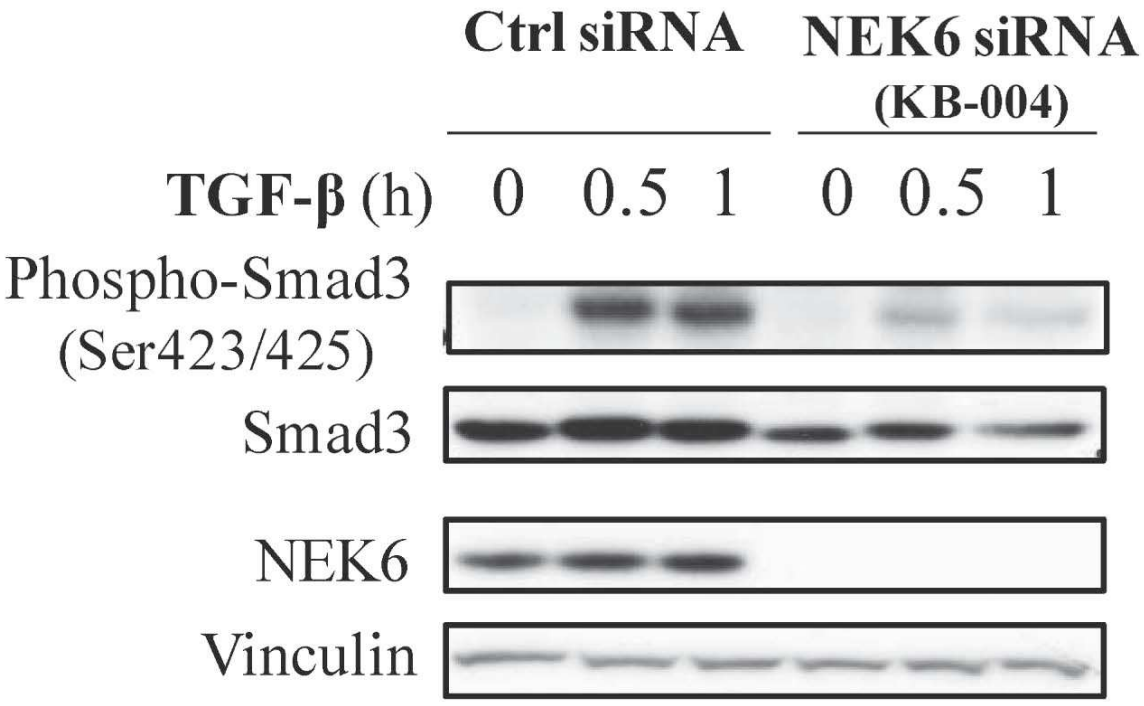

Ctrl siRNA
NEK6 siRNA
(KB-004)
TGF-β (h)
0
0.5
1
0
0.5
1
Phospho-Smad3
(Ser423/425)
Smad3
NEK6
Vinculin

Fig.17

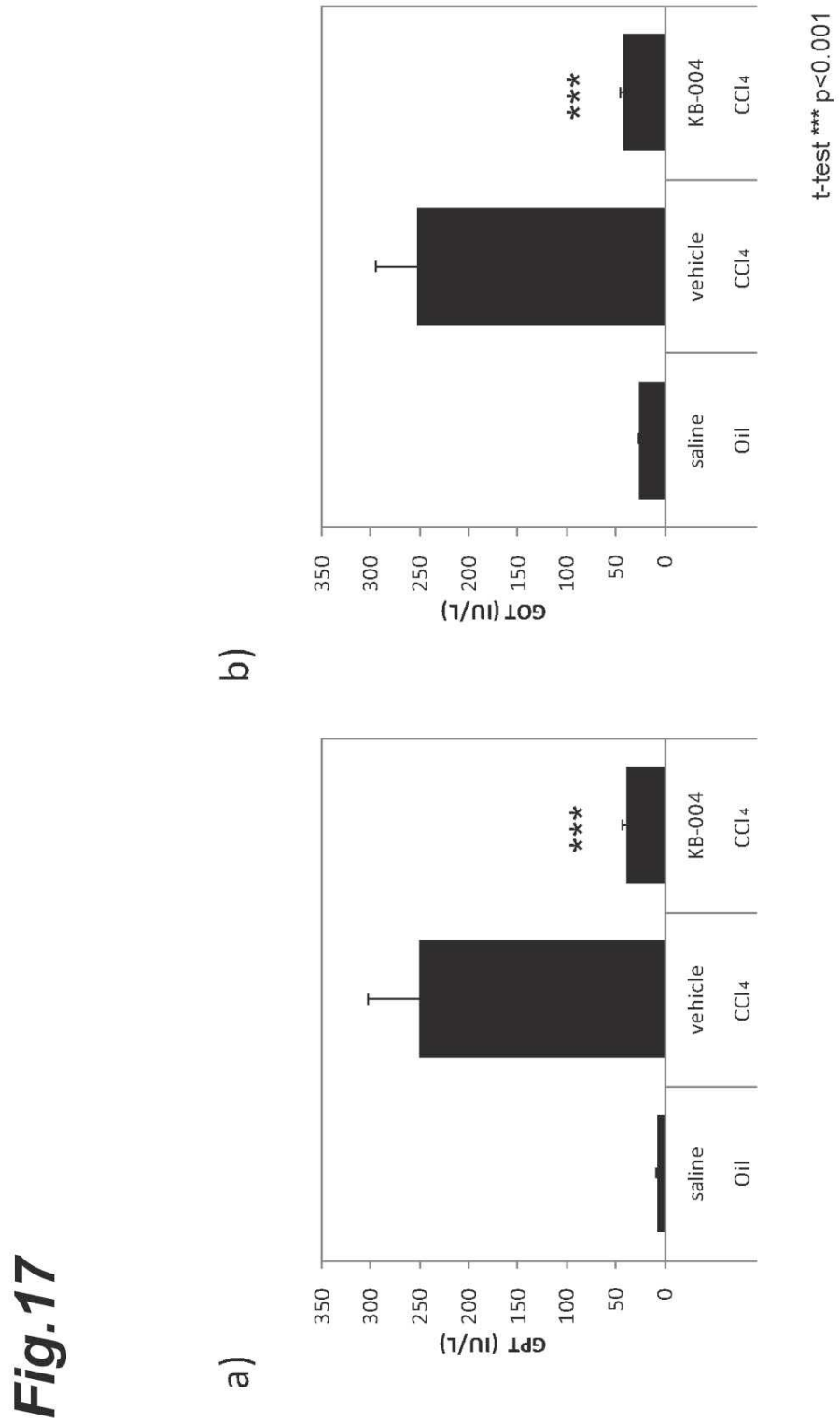
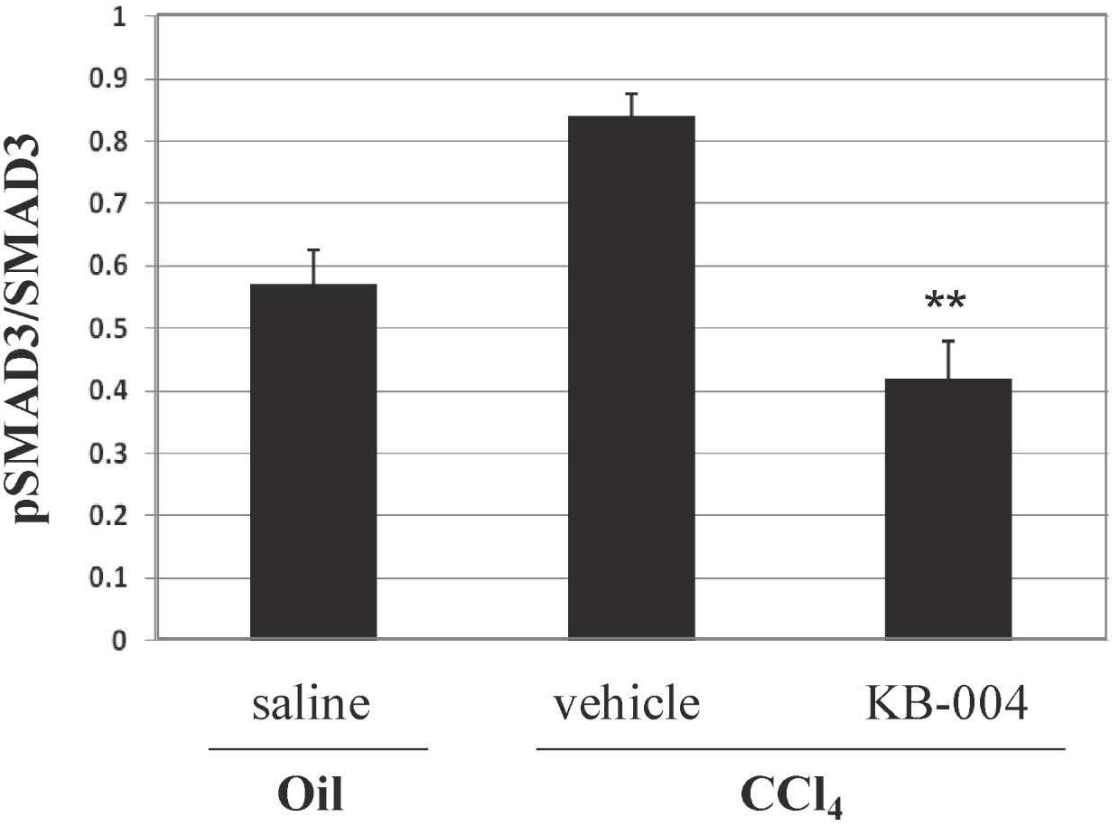

a)
GPT (IU/L)
350
300
250
200
150
100
50
0
***
saline
Oil
vehicle
CCl4
KB-004
CCl4
b)
GOT (IU/L)
350
300
250
200
150
100
50
0
***
saline
Oil
vehicle
CCl4
KB-004
CCl4
t-test *** p<0.001

Fig.18



t-test** p<0.01

Fig.19

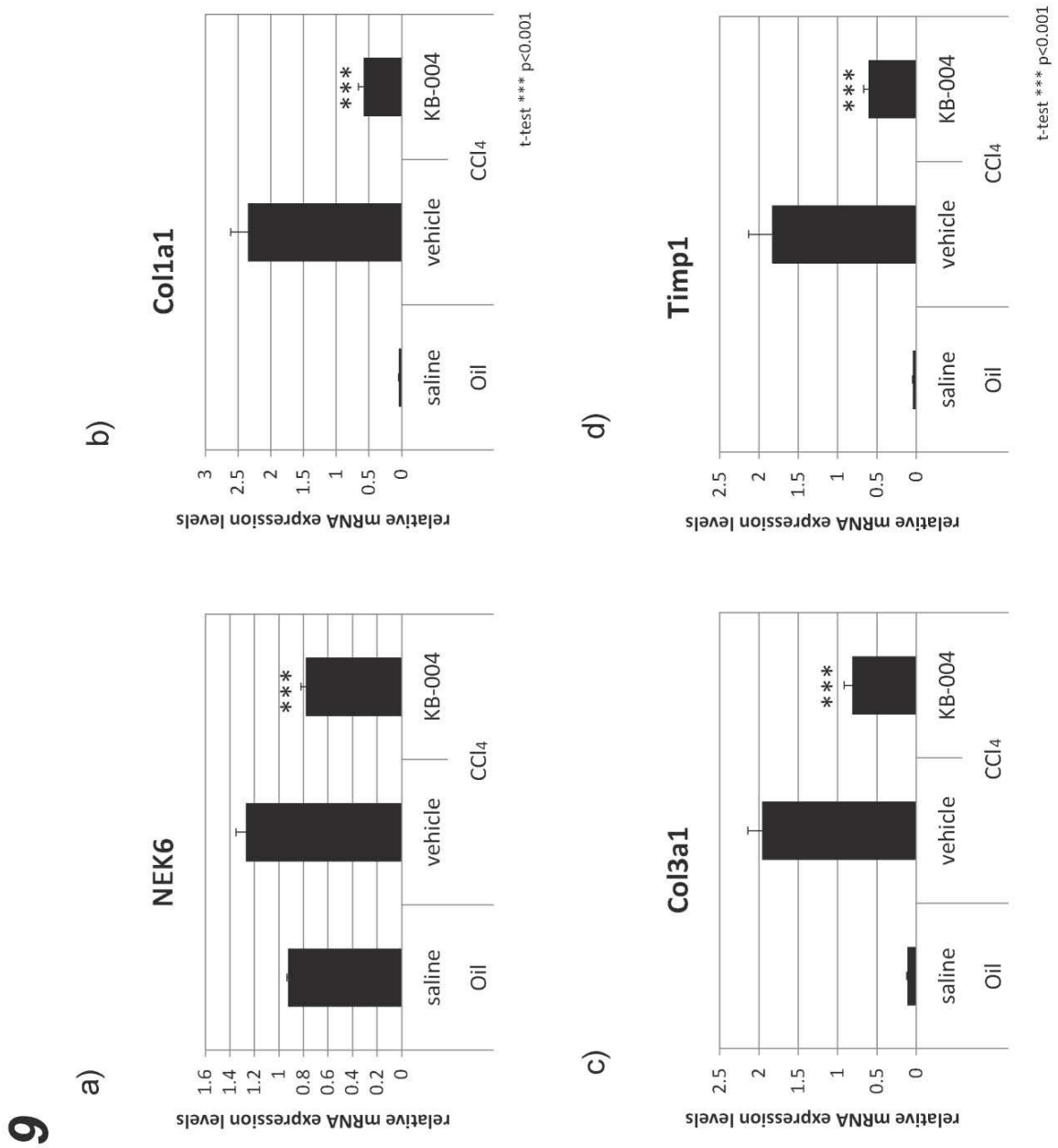

a)
NEK6
relative mRNA expression levels
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
***
saline
vehicle
KB-004
Oil
CCl4
b)
Col1a1
relative mRNA expression levels
3
2.5
2
1.5
1
0.5
0
***
saline
vehicle
KB-004
Oil
CCl4
t-test *** p<0.001
c)
Col3a1
relative mRNA expression levels
2.5
2
1.5
1
0.5
0
***
saline
vehicle
KB-004
Oil
CCl4
d)
Timp1
relative mRNA expression levels
2.5
2
1.5
1
0.5
0
***
saline
vehicle
KB-004
Oil
CCl4
t-test *** p<0.001

Fig.20

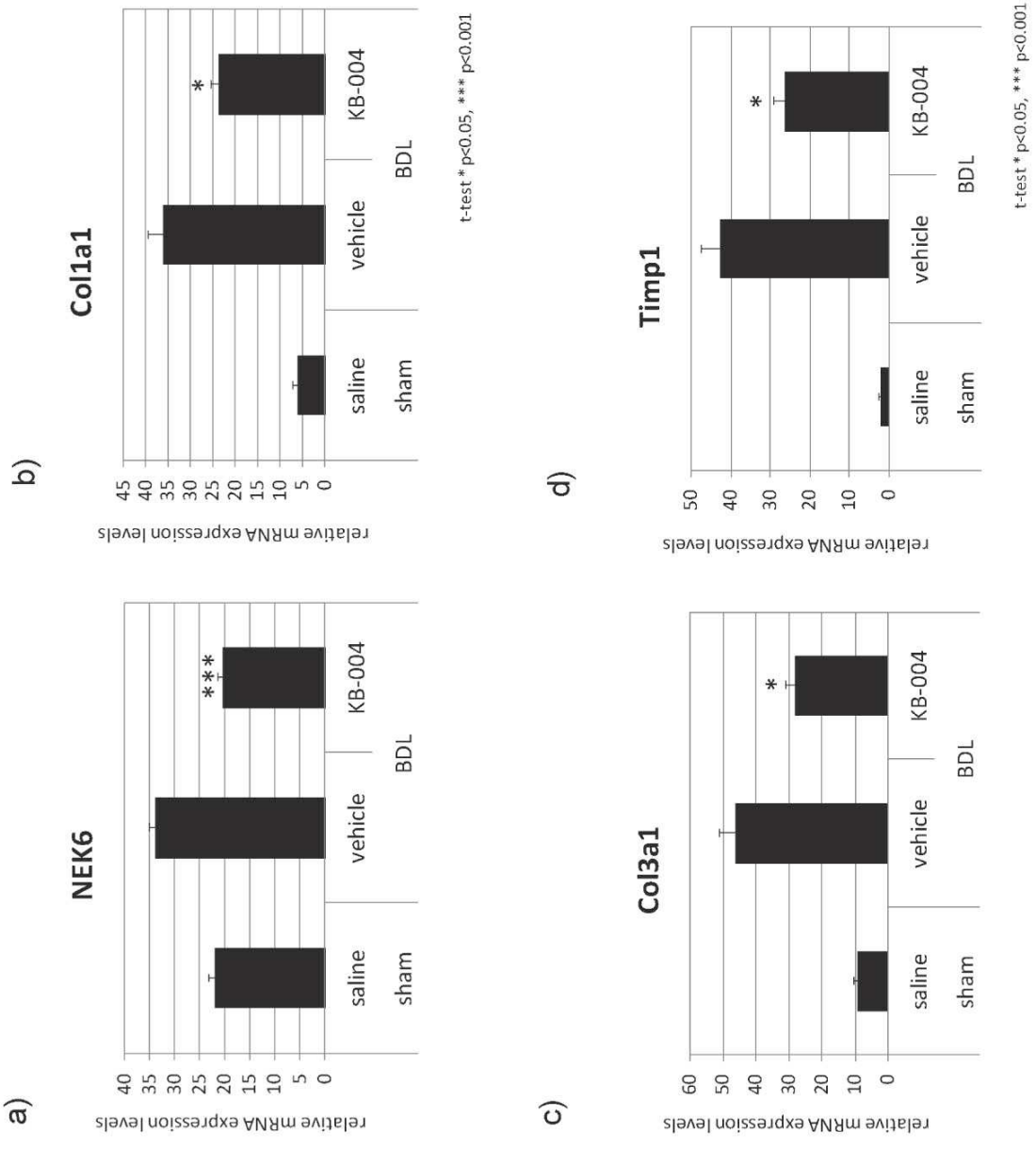
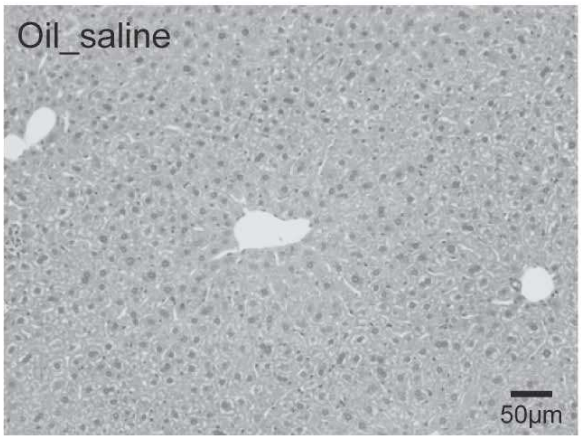
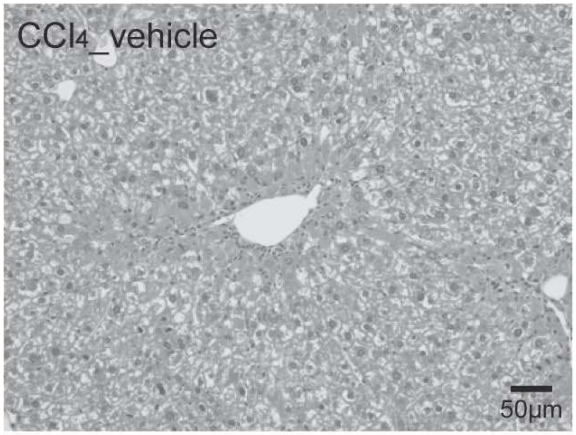

a)
NEK6
relative mRNA expression levels
saline
sham
vehicle
BDL
KB-004
***
b)
Col1a1
relative mRNA expression levels
saline
sham
vehicle
BDL
KB-004
*
t-test * p<0.05, *** p<0.001
c)
Col3a1
relative mRNA expression levels
saline
sham
vehicle
BDL
KB-004
*
d)
Timp1
relative mRNA expression levels
saline
sham
vehicle
BDL
KB-004
*
t-test * p<0.05, *** p<0.001

Fig.21

(a)



(b)



(c)

