Supplemental data

Figure S1. The *IKBKE* promoter sequence including exon 1 and intron 1. Putative STAT3 and NF-κB binding sites are boxed. Transcriptional start site and boundary of exon-1, intron-1 and exon-2 are indicated. Translation initiation site, ATG, was labeled in red. The primers used for preparing different deletion mutants of IKBKE promoter are underlined.

Figure S2. Inhibition of STAT3 reduces IKBKE expression. H157 and OVCAR-3 cells were transfected with STAT3-shRNA (A) or treated with STAT3 inhibitor JSI-124 (B) and then subjected to Western blot and RT-PCR analyses.

Figure S3. Knockdown of STAT3 decreases STAT3 binding to *IKBKE* promoter. H292 cells expressing elevated pSTAT3 and IKBKE were transfected with STAT3-shRNA and then subjected to ChIP assay.

Figure S4. Knockdown of STAT3 reduces wild-type but not the mutant *IKBKE* promoter activity. H292 cells were transfected with indicated shRNA as well as WT- and STAT3-binding site mutation-IKBKE-Luc and β-galactosidase. After incubation for 36 hours, luciferase activity was measured and normalized to β-galactosidase.

Figure S5. Positive correlation of IKBKE expression with pSTAT3. Immunohistochemistry was carried out with antibodies against IKBKE and pSTAT3-Y705 in paraffin sections from primary NSCLCs.

Figure S6. Nicotine and NNK induce IKBKE. H1299 cells were treated with nicotine (A) and NNK (B) for indicated times and then subjected to Western blot analysis.

Figure S7. NNK induces IKBKE promoter activity via STAT3. A549 cells were transfected with IKBKE-Luc together with/without shRNA-STAT3 or treated with/without STAT3 inhibitor. Following exposure to NNK (+ = 0.5 μM and ++ = 1.0 μM) for 12 hours,

luciferase reporter assay was performed.

Figure S8. IKBKE mediates nicotine-induced chemoresistance. H661 cells were transfected with IKBKE-shRNA and then treated with/without nicotine together with/without gemcitabine and cisplatin for 36 hours. Caspase 3/7 activity was assayed.

Figure S9. Inhibition of NF-κB moderately reduces nicotine-stimulated IKBKE expression and does not compromise STAT3-induced IKBKE. H1299 cells were treated or transfected with indicated agents (A) or plasmids (B) and then were immunoblotted with indicated antibodies.

Figure S1

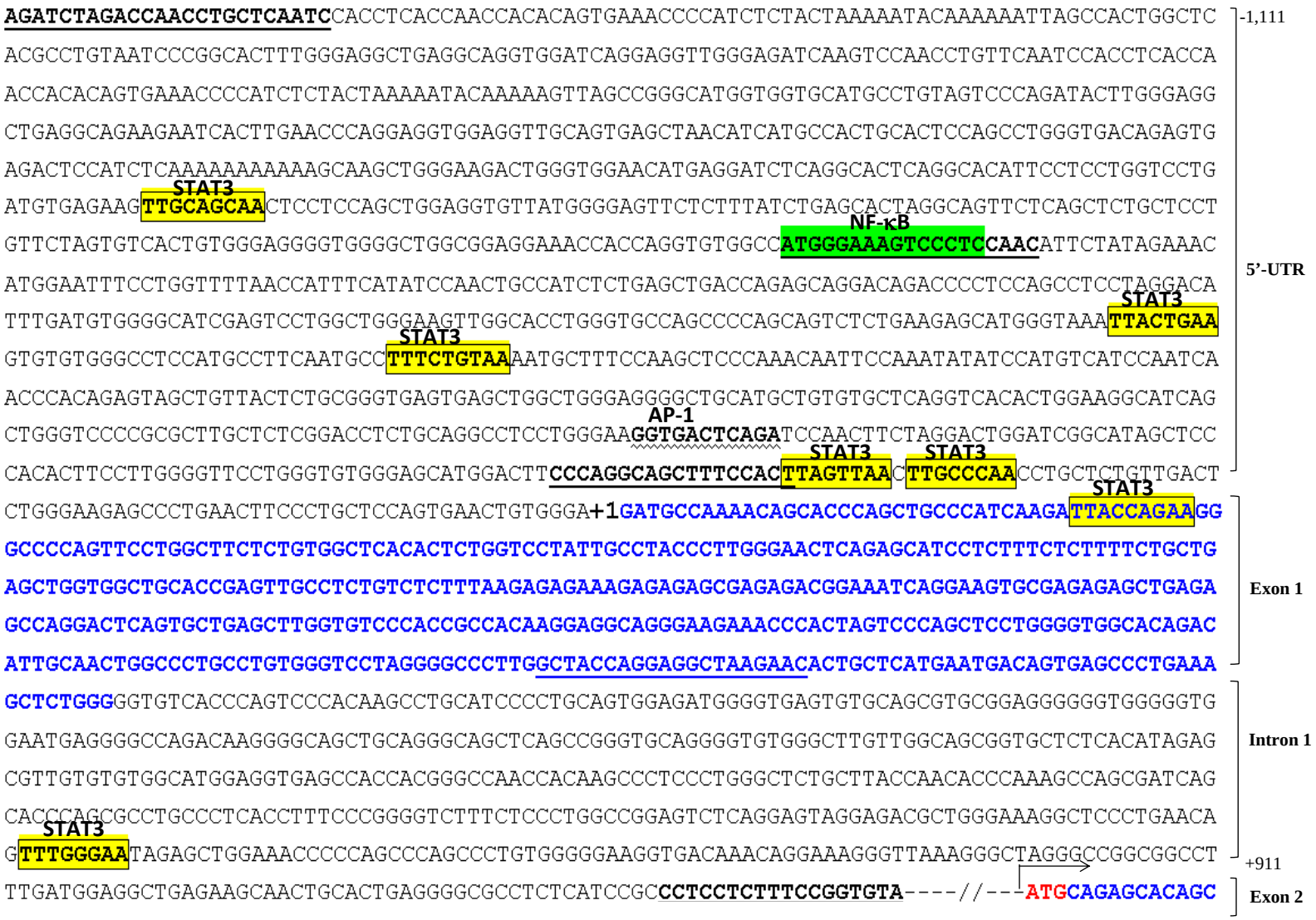

AGATCTAGACCAACCTGCTCAATC
-1,111
STAT3
TTGCAGCAA
NF-κB
ATGGGAAAGTCCCTCCAAC
STAT3
TTACTGAA
STAT3
TTTCTGTAA
AP-1
GGTGACTCAGA
STAT3
TTAGTTAA
STAT3
TTGCCCAA
STAT3
TTACCAGAA
5'-UTR
Exon 1
Intron 1
STAT3
TTTGGGAA
+911
Exon 2
CCTCCTCTTTCCGGTGTA
ATG
CAGAGCACAGC
CATTACCTGTGG

Figure S2

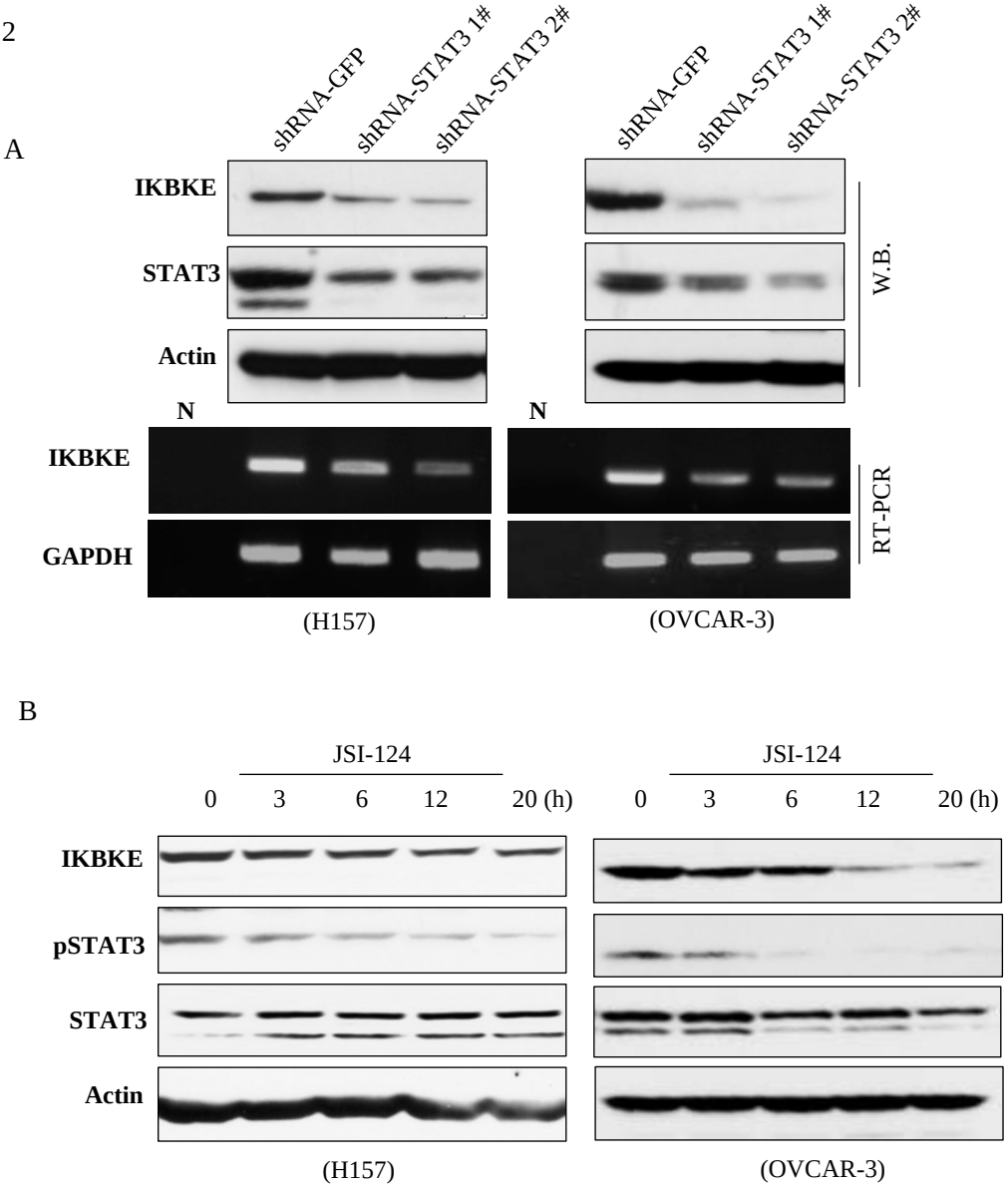

A
shRNA-GFP
shRNA-STAT3 1#
shRNA-STAT3 2#
shRNA-GFP
shRNA-STAT3 1#
shRNA-STAT3 2#
IKBKE
STAT3
Actin
W.B.
N
N
IKBKE
GAPDH
RT-PCR
(H157)
(OVCAR-3)
B
JSI-124
JSI-124
0 3 6 12 20 (h)
0 3 6 12 20 (h)
IKBKE
pSTAT3
STAT3
Actin
(H157)
(OVCAR-3)

Figure S3

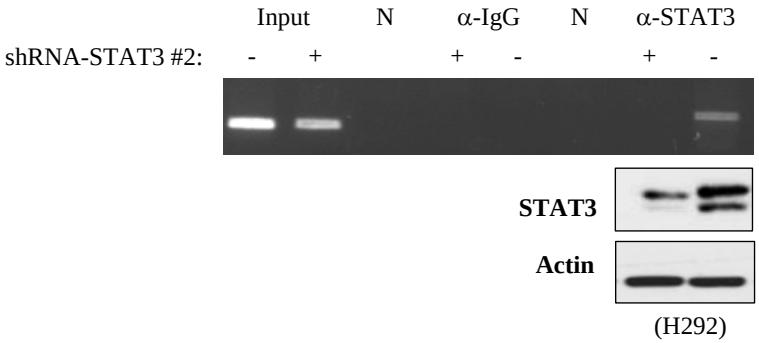
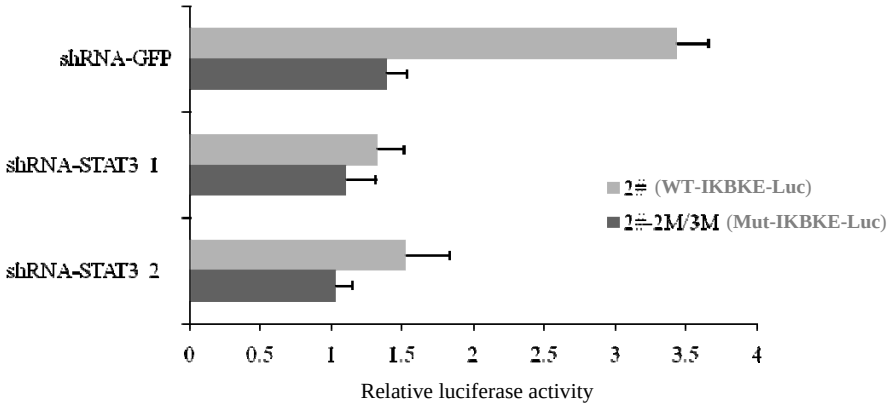


Figure S4



(H292)

Figure S5

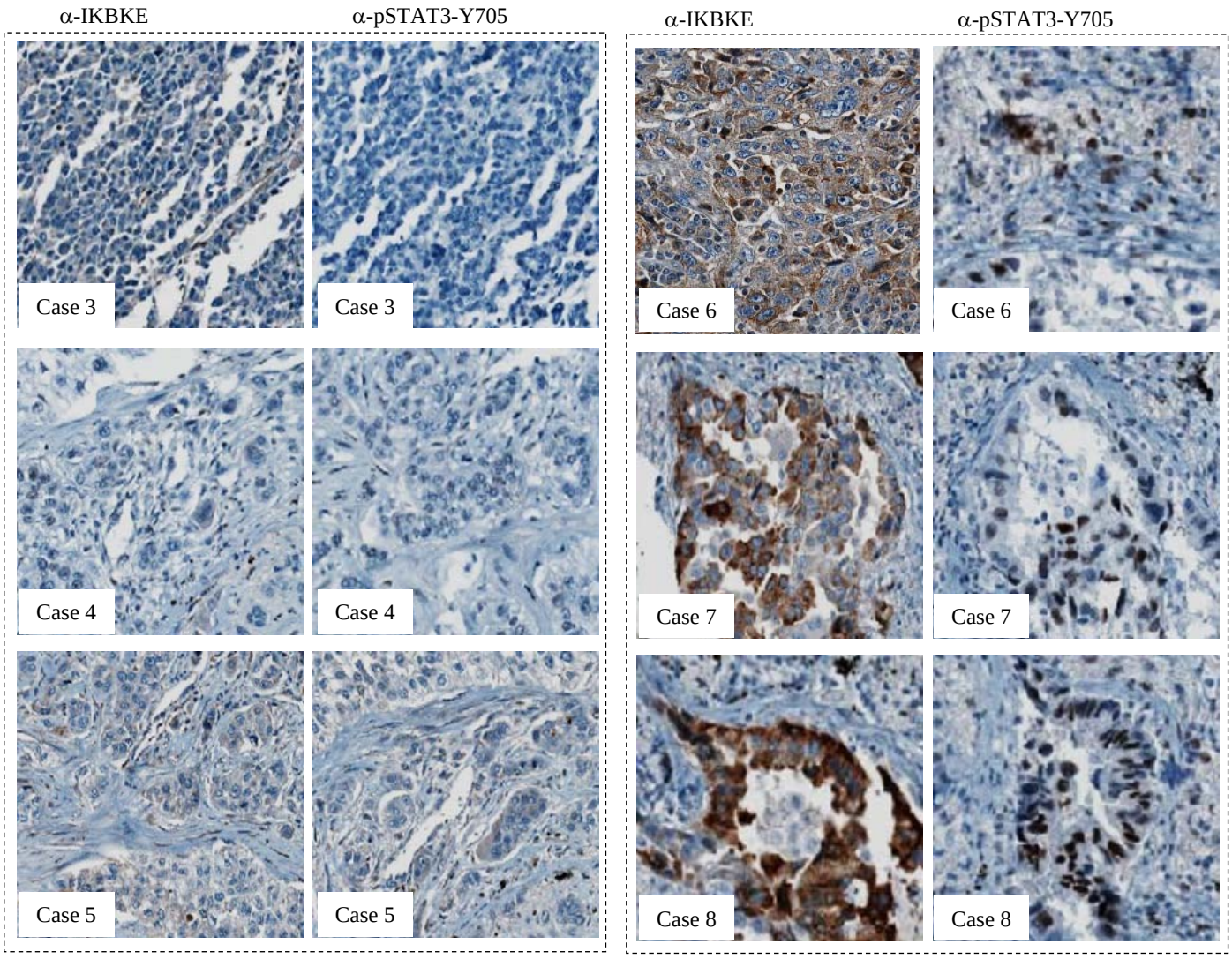
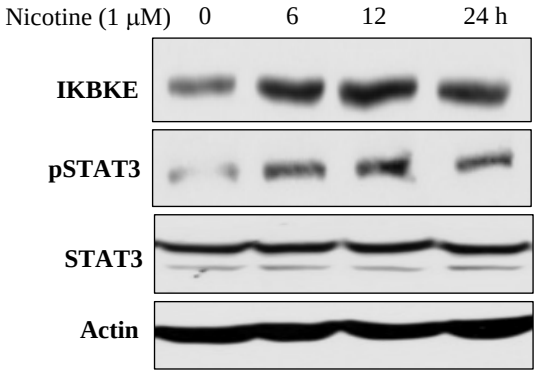

α-IKBKE
α-pSTAT3-Y705
α-IKBKE
α-pSTAT3-Y705
Case 3
Case 3
Case 6
Case 6
Case 4
Case 4
Case 7
Case 7
Case 5
Case 5
Case 8
Case 8

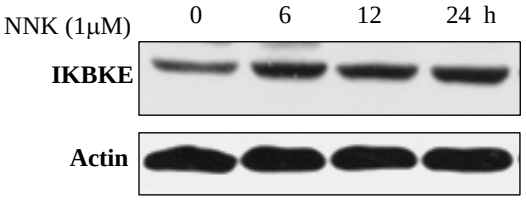
Figure S6

A



(H1299)

B



(H1299)

Figure S7

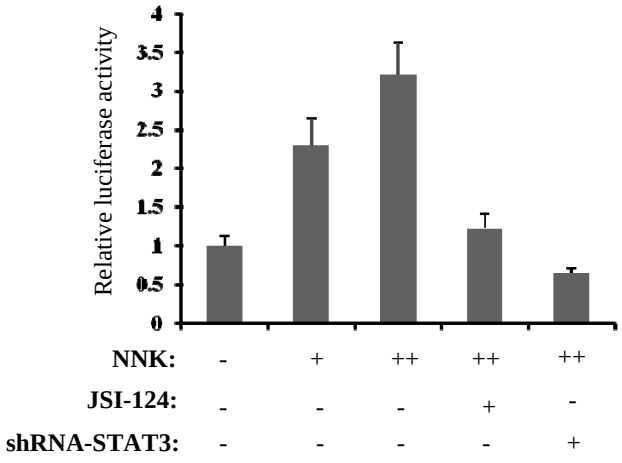
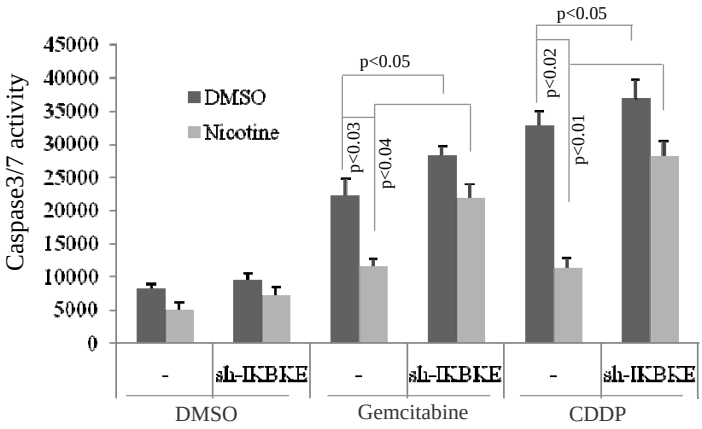
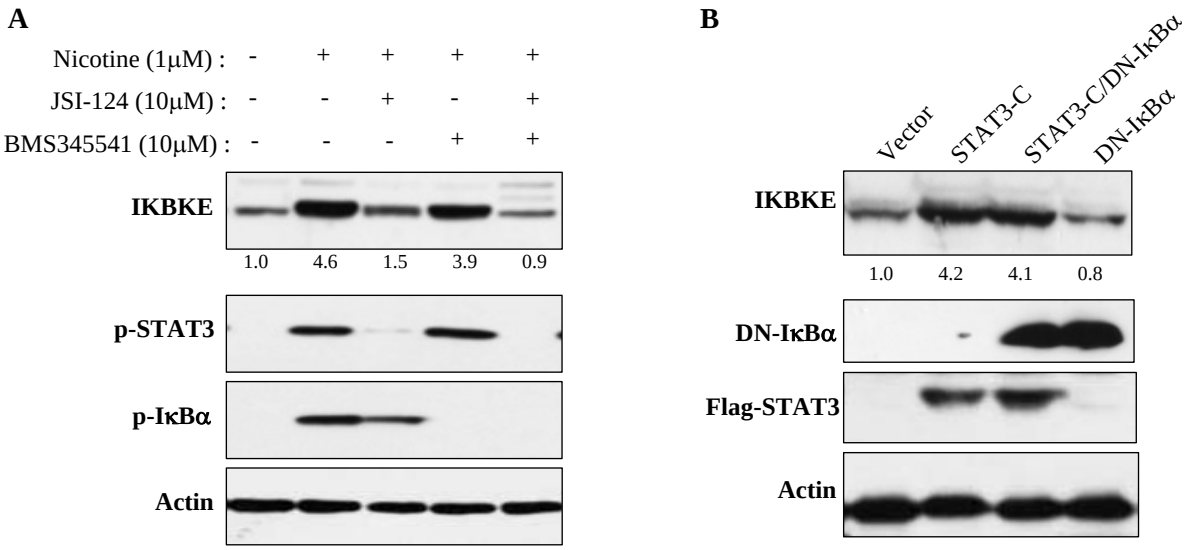

Relative luciferase activity
4
3.5
3
2.5
2
1.5
1
0.5
0
NNK: - + ++ ++ ++
JSI-124: - - - + -
shRNA-STAT3: - - - - +

Figure S8



H661

Figure S9


A
Nicotine (1μM) : - + + + +
JSI-124 (10μM) : - - + - +
BMS345541 (10μM) : - - - + +
IKBKE
1.0 4.6 1.5 3.9 0.9
p-STAT3
p-IκBα
Actin
B
Vector
STAT3-C
STAT3-C/DN-IκBα
DN-IκBα
IKBKE
1.0 4.2 4.1 0.8
DN-IκBα
Flag-STAT3
Actin