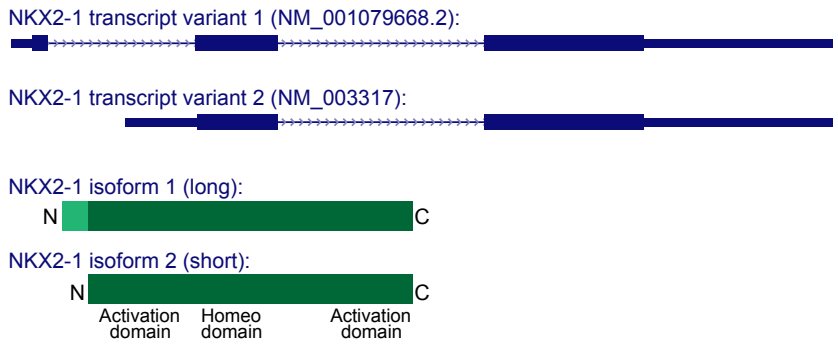
Figure A

A


NKX2-1 transcript variant 1 (NM_001079668.2):
NKX2-1 transcript variant 2 (NM_003317):
NKX2-1 isoform 1 (long):
N
C
NKX2-1 isoform 2 (short):
N
C
Activation domain
Homeo domain
Activation domain

B

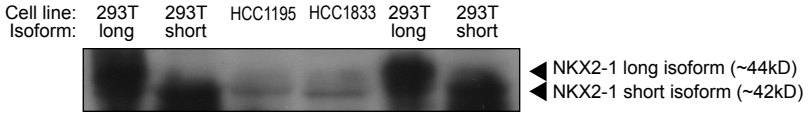

Cell line:
Isoform:
293T long
293T short
HCC1195
HCC1833
293T long
293T short
NKX2-1 long isoform (~44kD)
NKX2-1 short isoform (~42kD)

Figure B

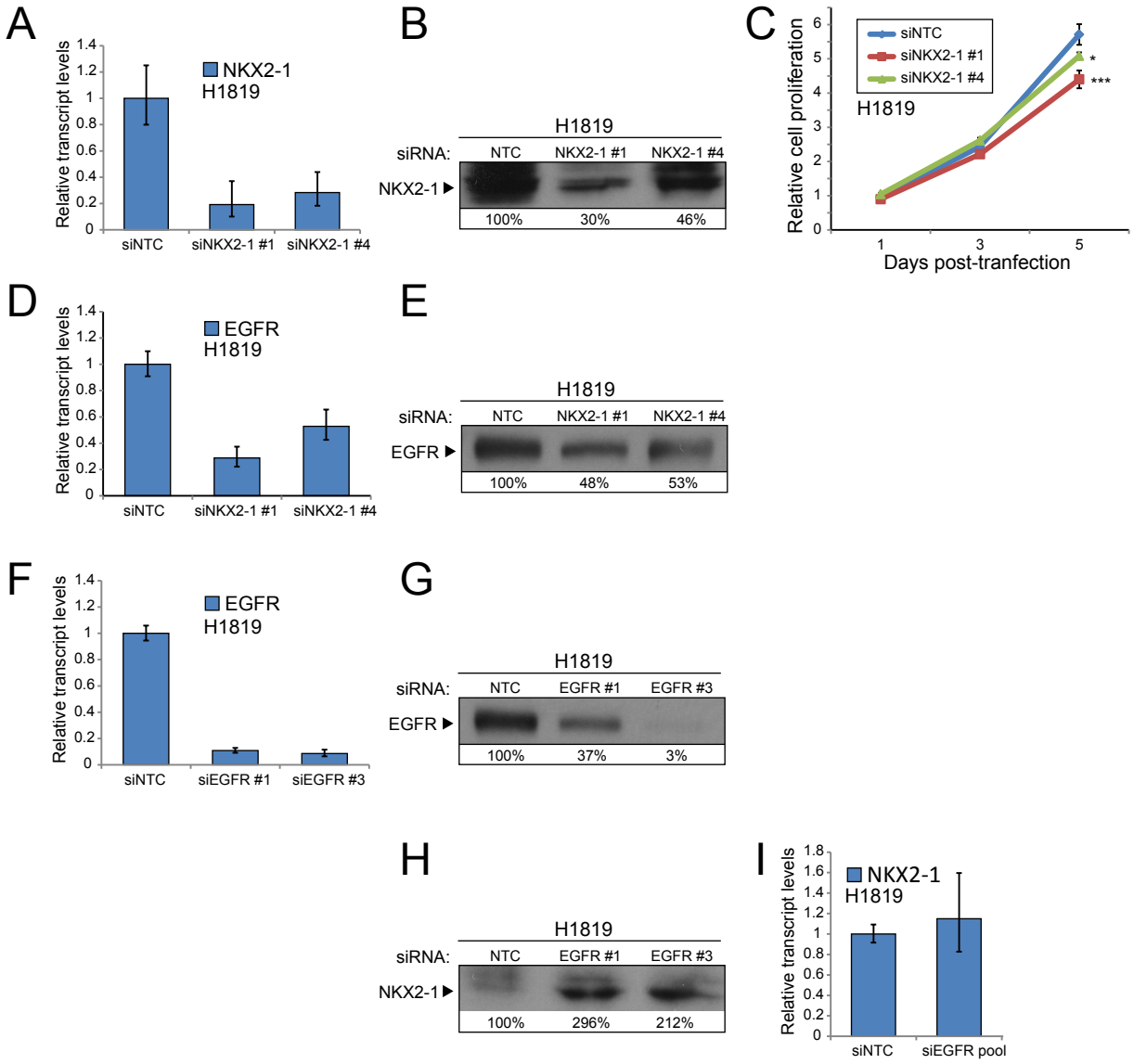
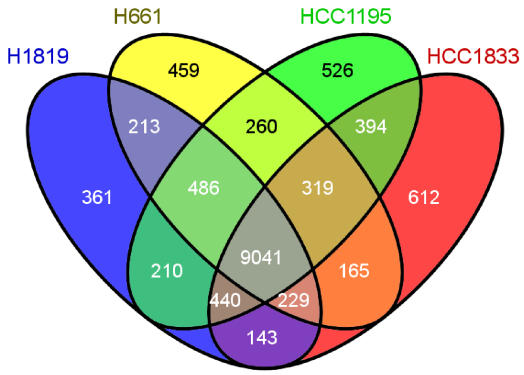
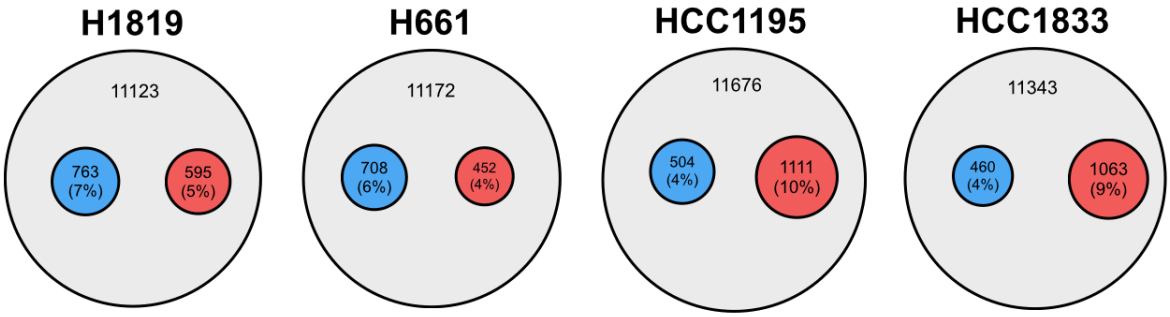

A
Relative transcript levels
NKX2-1
H1819
siNTC
siNKX2-1 #1
siNKX2-1 #4
B
H1819
siRNA:
NTC
NKX2-1 #1
NKX2-1 #4
NKX2-1
100%
30%
46%
C
Relative cell proliferation
siNTC
siNKX2-1 #1
siNKX2-1 #4
H1819
*
***
Days post-tranfection
D
Relative transcript levels
EGFR
H1819
siNTC
siNKX2-1 #1
siNKX2-1 #4
E
H1819
siRNA:
NTC
NKX2-1 #1
NKX2-1 #4
EGFR
100%
48%
53%
F
Relative transcript levels
EGFR
H1819
siNTC
siEGFR #1
siEGFR #3
G
H1819
siRNA:
NTC
EGFR #1
EGFR #3
EGFR
100%
37%
3%
H
H1819
siRNA:
NTC
EGFR #1
EGFR #3
NKX2-1
100%
296%
212%
I
Relative transcript levels
NKX2-1
H1819
siNTC
siEGFR pool

Figure C

A


H661
HCC1195
H1819
HCC1833
459
526
213
260
394
361
486
319
612
9041
210
165
440
229
143

B


H1819
11123
763 (7%)
595 (5%)
H661
11172
708 (6%)
452 (4%)
HCC1195
11676
504 (4%)
1111 (10%)
HCC1833
11343
460 (4%)
1063 (9%)

C

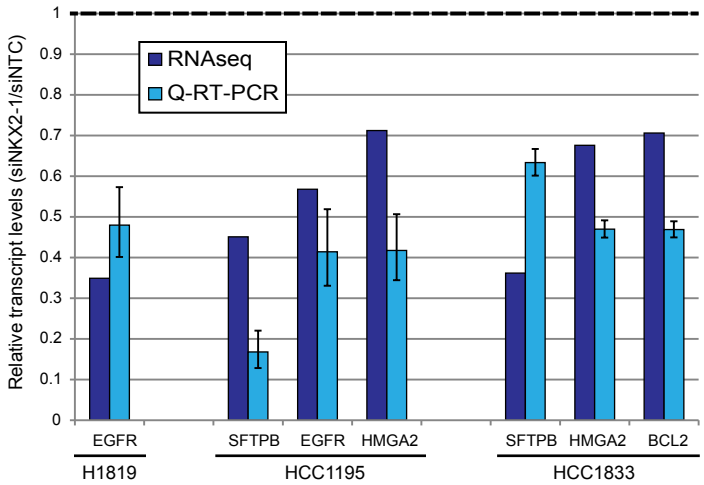
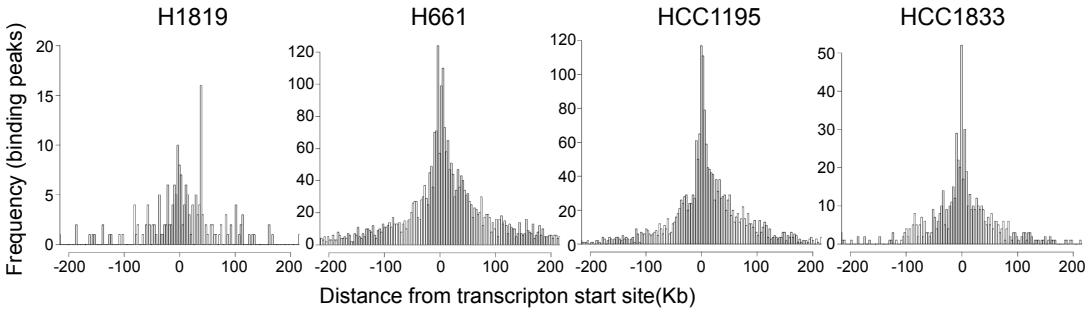

Relative transcript levels (siNKX2-1/siNTC)
RNAseq
Q-RT-PCR
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
EGFR
SFTPB
EGFR
HMGA2
SFTPB
HMGA2
BCL2
H1819
HCC1195
HCC1833

Figure D

A



B

① 5′CATAATAACATATTATGCACTTGAAATTTGCTAAGAGAGTAGATCTTAGCTATTCTCACCACATAACAACAACATAAGTGGCTAGGTGAGGTGATATGCTA
GTATTATTGTATAATACGTGAACTTTAAACGATTCTCTCATCTAGAATCGATAAGAGTGGTGTATTGTTGTTGTATTCACCGATCCACTCCACTATACGAT 5′

② 5′TAGATGTCGCCTTCTCAAGTGGCCTTCTCTGACAGTTGAAAAGTCACGACGCCTCTTCATCCTCCCTCTCCCCTACTCATTTTCTCTCATTTCTCTGAAGT
ATCTACAGCGGAAGAGTTCACCGGAAGAGACTGTCAACTTTTCAGTGCTGCGGAGAAGTAGGAGGGAGAGGGGATGAGTAAAAGAGAGTAAAGAGACTTCA 5′

③ 5′ACACCAAAGGCATTCAAAAGTGCACTCAGAAAGGTCAAGAATTAACAGTAGGGCTGAAGTATCTAGCATAGGGAACTATAAACCTGCCCTGACAGAATGCT
TGTGGTTTCCCTAAGTTTTCACGTGAGTCTTTCCAGTTCTTAATTGTCATCCCGACTTCATAGATCGTATCCCTTGATATTTGGACGGGACTGTCTTACGA 5′

④ 5′CAAGCTCTTCACATGCCAGAGCCTTCCAGTGCCCTTGAGACCCCTCATTCCAAAGATCTCTTCATAGTTTTTCTCCTTCACAGAGCCAAGAGGGAATTCCC
GTTCGAGAAGTGTACGGTCTCGGAAGGTCACGGGAACTCTGGGGAGTAAGGTTTCTAGAGAAGTATCAAAAAGAGGAAGTGTCTCGGTTCTCCCTTAAGGG 5′

⑤ 5′GTTTGGTTTACGACACTCACAGGAGAGACCTCATCCAAAGCCAATGTCATCCTTATGTAATTCTAACCAGCACAATTGTTAGATTTCTCAGCCACAAATAG
CAAACCAAATGCTGTGAGTGTCCTCTCTGGAGTAGGTTTCGGTTACAGTAGGAATACATTAAGATTGGTCGTGTTAACAATCTAAAGAGTCGGTGTTTATC 5′

Figure E

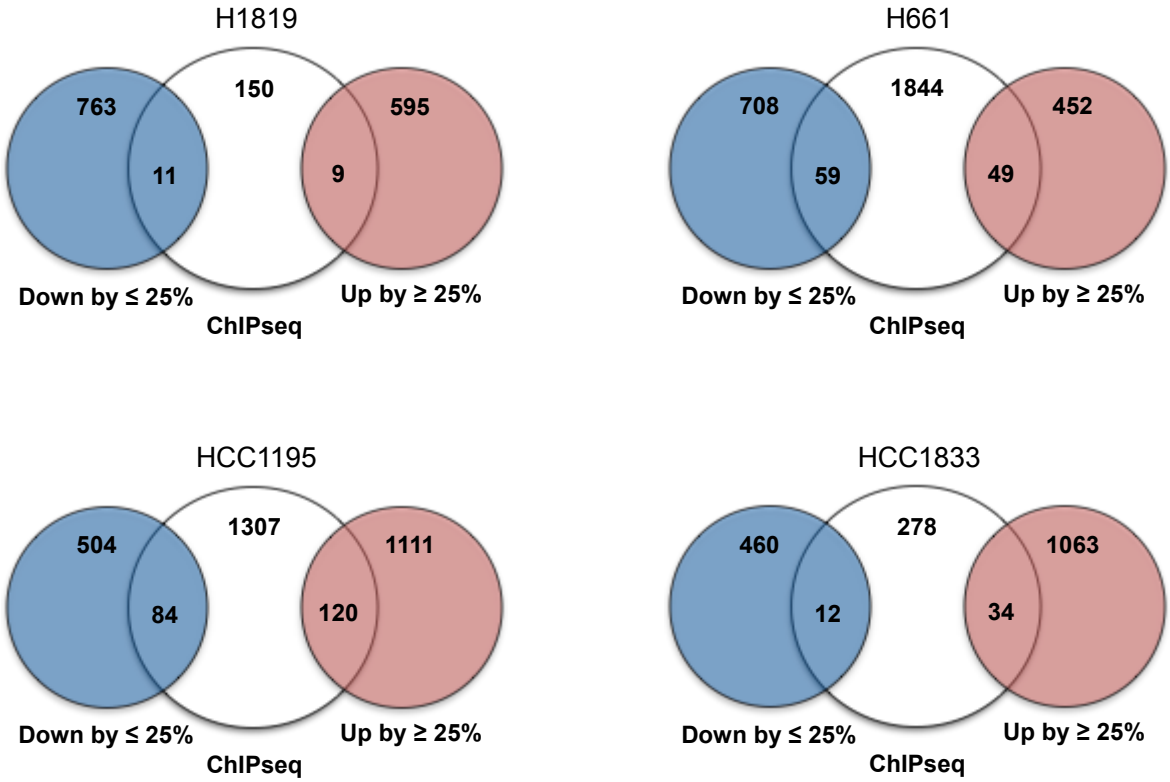
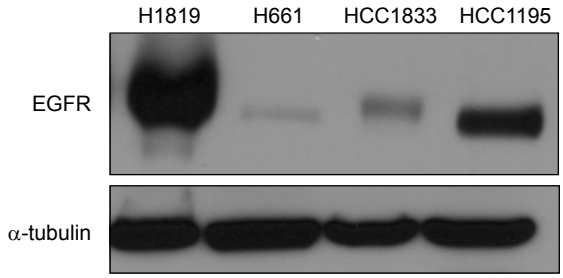
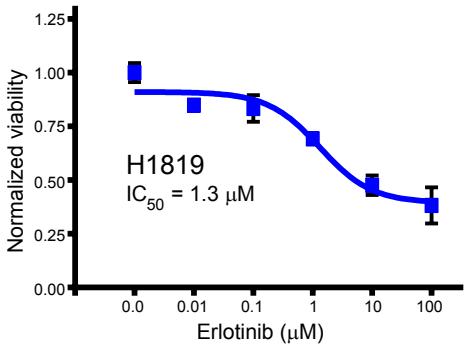

H1819
763
150
595
11
9
Down by ≤ 25%
Up by ≥ 25%
ChIPseq
H661
708
1844
452
59
49
Down by ≤ 25%
Up by ≥ 25%
ChIPseq
HCC1195
504
1307
1111
84
120
Down by ≤ 25%
Up by ≥ 25%
ChIPseq
HCC1833
460
278
1063
12
34
Down by ≤ 25%
Up by ≥ 25%
ChIPseq

Figure F

A


H1819
H661
HCC1833
HCC1195
EGFR
α-tubulin

B


Normalized viability
1.25
1.00
0.75
0.50
0.25
0.00
H1819
IC50 = 1.3 μM
0.0
0.01
0.1
1
10
100
Erlotinib (μM)