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Supplemental Information

Long Noncoding RNA SBF2-AS1 Is Critical for Tumorigenesis of Early-Stage Lung Adenocarcinoma

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Supplementary Materials

Supplementary Table1. Primers and siRNA sequences

Supplementary Table2. Differentially expressed genes after knockdown of SBF2-AS1

Supplementary Table3. Function enrichment analyses of differentially expressed genes after knockdown of SBF2-AS1

Supplementary Table4. miRNA binding sites within SBF2-AS1 sequence predicted by miRanda algorithm and CLIP-seq

Supplementary Table5. Cell cycle-related genes that were downregulated after knockdown of SBF2-AS1

Supplementary Table6. Co-expression between 19 cell cycle-related genes and SBF2-AS1 among cancers

Supplementary File 1. Constructed plasmids vectors used in this study.

The following plasmids were generated by inserting the indicated sequences in the Pezx-FR02 dual- Luciferase reporter vector.

E2F1-WT, wild type E2F1 3'UTR

CAGGGCTTGGAGGGACCAGGGTTTCCAGAGATGCTCACCTTGTCTCTGCA
GCCCTGGAGCCCCCTGTCCCTGGCCGTCCTCCCAGCCTGTTTGGAAACATT
TAATTTATACCCCTCTCCTCTGTCTCCAGAAGCTTCTAGCTCTGGGGTCTGG
CTACCGCTAGGAGGCTGAGCAAGCCAGGAAGGGAAGGAGTCTGTGTGGTG
TGTATGTGCATGCAGCCTACACCCACACGTGTGTACCGGGGGTGAATGTGT
GTGAGCATGTGTGTGTGCATGTACCGGGGAATGAAGGTGAACATACACCTC
TGTGTGTGCACTGCAGACACGCCCCAGTGTGTCCACATGTGTGTGCATGAG
TCCATGTGTGCGCGTGGGGGGGCTCTAACTGCACTTTCGGCCCTTTTGCTC
TGGGGGTCCCACAAGGCCCAGGGCAGTGCCTGCTCCCAGAATCTGGTGCT
CTGACCAGGCCAGGTGGGGAGGCTTTGGCTGGCTGGGCGTGTAGGACGGT
GAGAGCACTTCTGTCTTAAAGGTTTTTTCTGATTGAAGCTTTAATGGAGCGT
TATTTATTTATCGAGGCCTCTTTGGTGAGCCTGGGGAATCAGCAAAGGGGA
GGAGGGGTGTGGGGTTGATACCCCAACTCCCTCTACCCTTGAGCAAGGGC
AGGGGTCCCTGAGCTGTTCTTCTGCCCCATACTGAAGGAACTGAGGCCTGG
GTGATTTATTTATTGGGAAAGTGAGGGAGGGAGACAGACTGACTGACAGC
CATGGGTGGTCAGATGGTGGGGTGGGCCCTCTCCAGGGGGCCAGTTCAGG
GCCCCAGCTGCCCCCCAGGATGGATATGAGATGGGAGAGGTGAGTGGGGG
ACCTTCACTGATGTGGGCAGGAGGGGTGGTGAAGGCCTCCCCCAGCCCAG
ACCCTGTGGTCCCTCCTGCAGTGTCTGAAGCGCCTGCCTCCCCACTGCTCT
GCCCCACCCTCCAATCTGCACTTTGATTTGCTTCCTAACAGCTCTGTTCCCT
CCTGCTTTGGTTTTAATAAATATTTTGATGACGTT

E2F1-MUT1, binding site of miR-338-3p was deletion-mutated

CAGGGCTTGGAGGGACCAGGGTTTCCAGAGATGCTCACCTTGTCTCTGCA
GCCCTGGAGCCCCCTGTCCCTGGCCGTCCTCCCAGCCTGTTTGGAAACATT
TAATTTATACCCCTCTCCTCTGTCTCCAGAAGCTTCTAGCTCTGGGGTCTGG
CTACCGCTAGGAGGCTGAGCAAGCCAGGAAGGGAAGGAGTCTGTGTGGTG
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GAGAGCACTTCTGTCTTAAAGGTTTTTTCTGATTGAAGCTTTAATGGAGCGT
TATTTATTTATCGAGGCCTCTTTGGTGAGCCTGGGGAATCAGCAAAGGGGA
GGAGGGGTGTGGGGTTGATACCCCAACTCCCTCTACCCTTGAGCAAGGGC

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GGTCAGATGGTGGGGTGGGCCCTCTCCAGGGGGCCAGTTCAGGGCCCCAG
CTGCCCCCCAGGATGGATATGAGATGGGAGAGGTGAGTGGGGGACCTTCA
CTGATGTGGGCAGGAGGGGTGGTGAAGGCCTCCCCCAGCCCAGACCCTGT
GGTCCCTCCTGCAGTGTCTGAAGCGCCTGCCTCCCCACTGCTCTGCCCCAC
CCTCCAATCTGCACTTTGATTTGCTTCCTAACAGCTCTGTTCCCTCCTGCTT
TGGTTTTAATAAATATTTTGATGACGTT

E2F1-MUT2, binding sites of miR-362-3p were deletion-mutated
CAGGGCTTGGAGGGACCAGGGTTTCCAGAGATGCTCACCTTGTCTCTGCA
GCCCTGGAGCCCCCTGTCCCTGGCCGTCCTCCCAGCCTGTTTGGAAACATT
TAATTTATACCCCTCTCCTCTGTCTCCAGAAGCTTCTAGCTCTGGGGTCTGG
CTACCGCTAGGAGGCTGAGCAAGCCAGGAAGGGAAGGAGTCTGTGTGTGT
GCATGCAGCCTACACCCACACCCGGGGGTGAATGTGTGTGAGCATGTGTGT
GTGCATGTACCGGGGAATGAAGGTGAACATACACCTCTGTGTGTGCACTGC
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GGCCCAGGGCAGTGCCTGCTCCCAGAATCTGGTGCTCTGACCAGGCCAGG
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CTTAAAGGTTTTTTCTGATTGAAGCTTTAATGGAGCGTTATTTATTTATCGAG
GCCTCTTTGGTGAGCCTGGGGAATCAGCAAAGGGGAGGAGGGGTGTGGG
GTTGATACCCCAACTCCCTCTACCCTTGAGCAAGGGCAGGGGTCCCTGAGC
TGTTCTTCTGCCCCATACTGAAGGAACTGAGGCCTGGGTGATTTATTTATTG
GGAAAGTGAGGGAGGGAGACAGACTGACTGACAGCCATGGGTGGTCAGA
TGGTGGGGTGGGCCCTCTCCAGGGGGCCAGTTCAGGGCCCCAGCTGCCCC
CCAGGATGGATATGAGATGGGAGAGGTGAGTGGGGGACCTTCACTGATGT
GGGCAGGAGGGGTGGTGAAGGCCTCCCCCAGCCCAGACCCTGTGGTCCCT
CCTGCAGTGTCTGAAGCGCCTGCCTCCCCACTGCTCTGCCCCACCCTCCAA
TCTGCACTTTGATTTGCTTCCTAACAGCTCTGTTCCCTCCTGCTTTGGTTTTA
ATAAATATTTTGATGACGTT

The following plasmids were generated by inserting the indicated sequences between the BamHI site and XhoI sites of pcDNA3.1(+) (Life Technologies).

SBF2-AS1 WT, wild SBF2-AS1 full length sequence
CAGGTTCCAGCCCCGACCCGGGCGCGCGGGGCCGACTAGGGTCGGGTCCA
GTGTGCGGTGGTCGCTCCGCTCCGGGCCGCTCCGCTCTGGGCGTCAGGGC
GCGGGGAGCTGCCCCGGGGTTCTGTCCACCGGGGAGGAAAGCCACGAGC
ACTGAGCGCCTCCTGAGAGCCAGCCCTGACGTGAACTCATTTTATCTGCCA

CGACCCAGAAGGAGTCTACTGCTAAGATTTCAGCATGTCCTGTGGCTGAG
TTAATCAGAGTTATGACAGGAAGGTACCGGGCACACCATCGCAATGCTCC
ATCAATGCTAGTATGTTGTGTTCTTTCCTTCATATCAAGTCAACTCAAGCT
TGCTCTACTTACCTGGTGTACACAGTCTAAGAACTGTAAGAAGACTGGAG
CAAAACCACTCCCCTGACAGTTGAGGGTCAAGCTGCTCCTCTGACTGAAT
TTGTGACCAAAAGAGAGCCACTCTTTTTCAACCAACATCTGGAAGCCTTC
AAGTGTCCTATAAAAGGGATCACTGAGTAACTGAACCAGGGATGTCACCT
AGGGCATAAGCAGGATGGATTGTCATTAATTTTAGTTCTGAAAAAGGCCT
ATTACTAAGATAAAAGCACTTCCTTCTGATGATAGCTAATTCACAAATTTA
CCTGGACAGCAAATTTGTTCACTAACCATTCCAGGATGGCCAATAAAATT
AATTTTGTAAACTTGCCAGTAAAAACTAAAGCTCAAATTCATTTTGGCTAC
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GACAGCAACCTTTTCCTAAGTTAATCAGAGGCCAGAGGATCAGTTAAAAT
CAATTCTACTTTTTGGCAGATGCAGATTAATGTCTTAATGTAGTCTGAACT
CACACTTTTCACTCTGGAGTGAGTTAAGGCGGGGCTTATAGAGAGCCAGG
GGATGGACTGACAAAACCCAGGAAGGATGGTCCACGCCTCACACTGCTGG
TCAAGGGCTCCATCCGCAAGCCTGCATGGTACATCTGTGGATGGGAGAGC
TTGATGCCCGGAGCCAGAGCTCCAGGCTGCGTAGGGGCTAAGAGCTCATG
GTACGGAGTTTTCTGATGGATACCAGGTGCTGTTTAACACAGGACTTTCTC
TGTCCATAAACACATTAGTCATTATACAAAAGAGTCATCTGAAGAATTTA
AAAATGACCAAATGGAAATATAAATATAAAATATCAAAAAATATGTCTAA
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GAGGAACCATTCTCAAGTACTTCTGATACAACCACAGCCAGCTGCATTAT
CCTGTGAAGCTAAGAGACAGGAAGGAGAACACAATTAGACCAAGCGCTT
TCTGAGTGCAGAAGTCAGAGAGAGTTGCTTTCAACCCTGGATAATCAAAC
GACTTAGGGATAAAGCGCTTGCACAAAAGAACCACATAATGTTTTAAATA
CCTAAAGTTCTTTCAAATCAAGATTAAACAATGACACTTTTGACTGGATGC
AATGACTCATGCCCATAATCCCAGCACTTTGGGAGGCCGAGCCGGGCAGG
TCATTTGAGGTTCGGAGTTCGAGACCAGCCTGGGCAACATAAGCAAGACC
CTGTCTCGAAAAATAAAAATAAAAAAGATACTCCTATTTGTCTATACTGT
GTACTAAATGAGAAAAAAACTTTCAATATATTTGTGCTAAGTTTTTAAAGC
CACATCAATTGGCTCAAAGTCCTTAATAAACAATTAAGGTATGAGAATAG
TATATAGATTTTAAAACCAGATGTATAACATTGTAATTTTAAAAAATATTT
TAATGATCATCAGACATAATTTGCAATTTGAATCCAACTCTGAACAAGAA
CAAAAATGAAAAAACCCATCTCTTAAACAGAGCTAGGCTACTTTAAACAC
AGGAATAAGGCAAATGCCCTACTCAGTAATGAGAAGCTCCTCATGTAACT
GTTTCTACAACATAGCTATCACCAGGCCTGAATTCTTTTGGGCTCAGAATT
TGTTCTAAGAAATGGCTATTATTAAAAAGTCAAAAAATAACAGATGCTGG
CGAGCCTGTGGAGAAAAGGGAATGTATATACATTTCTGGTGGGAATGTAA
ATTAGTTCAGCCATTGTAGAAAGTAGTTTGGTGATTTCTCAAATAACTTAA
AACAGAATTACCATTTGACCTAGCAATCCCATTACTGAGTATATACCCAA
AGGAATATAAATTGTTCTACCATAAAGTCACATGCACACACATGTTCAGT
GCTGTACTATTCACAATAGCAAAGACATGGAGTCAACCTATATGCCCATC
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ACACAGCCATAAAAAGAATGAGCTTATGTCCTTTGCAGTAACACAGCTGA
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AAGTGGGAGCTAAACATTGAGTACACATGGACACAAAGAAAGGAATAGA
CGCCTGGGGCGTATTTGAGGTTGGAGGTGAGAGGAGGGTGAGGATAGAA
AAACTACCTATCAGGTACTATGCTTATTACCTGGGTGATGAAATAATCTGT
ACACCAAACTCCCAAGATATGCAATGTACCTATACAACAAACCTACAT

SBF2-AS1-MUT1, **binding sites of miR-338-3p were deletion-mutated**
CAGGTTCCAGCCCCGACCCGGGCGCGCGGGGCCGACTAGGGTCGGGTCCA
GTGTGCGGTGGTCGCTCCGCTCCGGGCCGCTCCGCTCTGGGCGTCAGGGC
GCGGGGAGCTGCCCCGGGGTTCTGTCCACCGGGGAGGAAAGCCACGAGC
ACTGAGCGCCTCCTGAGAGCCAGCCCTGACGTGAACTCATTTTATCTGCCA
CGACCCAGAAGGAGTCTACTGCTAAGATTTCAGCATGTCCTGTGGCTGAG
TTAATCAGAGTTATGACAGGAAGGTACCGGGCACACCATCGCAATGCTCC
ATCAATGCTAGTATGTTGTGTTCTTTCCTTCATATCAAGTCAACTCAAGCT
TGCTCTACTTACCTGGTGTACACAGTCTAAGAACTGTAAGAAGACTGGAG
CAAAACCACTCCCCTGACAGTTGAGGGTCAAGCTGCTCCTCTGACTGAAT
TTGTGACCAAAAGAGAGCCACTCTTTTTCAACCAACATCTGGAAGCCTTC
AAGTGTCCTATAAAAGGGATCACTGAGTAACTGAACCAGGGATGTCACCT
AGGGCATAAGCAGGATGGATTGTCATTAATTTTAGTTCTGAAAAAGGCCT
ATTACTAAGATAAAAGCACTTCCTTCTGATGATAGCTAATTCACAAATTTA
CCTGGACAGCAAATTTGTTCACTAACCATTCCAGGATGGCCAATAAAATT
AATTTTGTAAACTTGCCAGTAAAAACTAAAGCTCAAATTCATTTTGGCTAC
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CAATTCTACTTTTTGGCAGATGCAGATTAATGTCTTAATGTAGTCTGAACT
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GGCTCCATCCGCAAGCCTGCATGGTACATCTGTGGATGGGAGAGCTTGAT
GCCCGGAGCCAGAGCTCCAGGCTGCGTAGGGGCTAAGAGCTCATGGTACG
GAGTTTTCTGATGGATACCAGGTGCTGTTTAACACAGGACTTTCTCTGTCC
ATAAACACATTAGTCATTATACAAAAGAGTCATCTGAAGAATTTAAAAAT
GACCAAATGGAAATATAAATATAAAATATCAAAAAATATGTCTAATAGTT
TACTTGTGCAGTGATGTCCCAGCCTTCCTCCAAACAGACCAAAACTGAGG
AACCATTCTCAAGTACTTCTGATACAACCACAGCCAGCTGCATTATCCTGT
GAAGCTAAGAGACAGGAAGGAGAACACAATTAGACCAAGCGCTTTCTGA
GTGCAGAAGTCAGAGAGAGTTGCTTTCAACCCTGGATAATCAAACGACTT
AGGGATAAAGCGCTTGCACAAAAGAACCACATAATGTTTTAAATACCTAA
AGTTCTTTCAAATCAAGATTAAACAATGACACTTTTGACTGGATGCAATG
ACTCATGCCCATAATCCCAGCACTTTGGGAGGCCGAGCCGGGCAGGTCAT
TTGAGGTTCGGAGTTCGAGACCAGCCTGGGCAACATAAGCAAGACCCTGT
CTCGAAAAATAAAAATAAAAAAGATACTCCTATTTGTCTATACTGTGTAC
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TCTAAGAAATGGCTATTATTAAAAAGTCAAAAAATAACACGAGCCTGTGG
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AGGTACTATGCTTATTACCTGGGTGATGAAATAATCTGTACACCAAACTCC
CAAGATATGCAATGTACCTATACAACAAACCTACAT

SBF2-AS1-MUT2, **binding site of miR-362-3p was deletion-mutated**
CAGGTTCCAGCCCCGACCCGGGCGCGCGGGGCCGACTAGGGTCGGGTCCA
GTGTGCGGTGGTCGCTCCGCTCCGGGCCGCTCCGCTCTGGGCGTCAGGGC
GCGGGGAGCTGCCCCGGGGTTCTGTCCACCGGGGAGGAAAGCCACGAGC
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TTAATCAGAGTTATGACAGGAAGGTACCGGGCACACCATCGCAATGCTCC
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CCTATCAGGTACTATGCTTATTACCTGGGTGATGAAATAATCTGTACACCA
AACTCCCAAGATATGCAATGTACCTATACAACAAACCTACAT

SBF2-AS1-MUT, **binding sites of miR-338-3p and miR-362-3p were deletion-mutated**

CAGGTTCCAGCCCCGACCCGGGCGCGCGGGGCCGACTAGGGTCGGGTCCA
GTGTGCGGTGGTCGCTCCGCTCCGGGCCGCTCCGCTCTGGGCGTCAGGGC
GCGGGGAGCTGCCCCGGGGTTCTGTCCACCGGGGAGGAAAGCCACGAGC
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TCTACTTACCTGGTGTACACAGTCTAAGAACTGTAAGAAGACTGGAGCAAA
ACCACTCCCCTGACAGTTGAGGGTCAAGCTGCTCCTCTGACTGAATTTGTG
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TCCTATAAAAGGGATCACTGAGTAACTGAACCAGGGATGTCACCTAGGGCA
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CTTGCCAGTAAAAACTAAAGCTCAAATTCATTTTGGCTACAAGTTTACTTTC
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TTCCTAAGTTAATCAGAGGCCAGAGGATCAGTTAAAATCAATTCTACTTTTT
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TGACACTTTTGACTGGATGCAATGACTCATGCCCATAATCCCAGCACTTTGG
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CCATTGTAGAAAGTAGTTTGGTGATTTCTCAAATAACTTAAAACAGAATTAC
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ACACATGGACACAAAGAAAGGAATAGACGCCTGGGGCGTATTTGAGGTTG
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ATTACCTGGGTGATGAAATAATCTGTACACCAAACTCCCAAGATATGCAATG
TACCTATACAACAAACCTACAT

Figure S1: Screening early stage-specific lncRNAs. 5 outliers were excluded from hierarchical clustering of cancer tissue samples (A). 12 significant co-expression gene modules across all 508 sampling sets were detected with WGCNAs (B). Soft thresholding power to achieve scale-free topology ($\beta = 2$) (C). The correlation within gene sets of module greenyellow (D). The heatmap of all 3250 differentially expressed genes in GSE19804 (E).

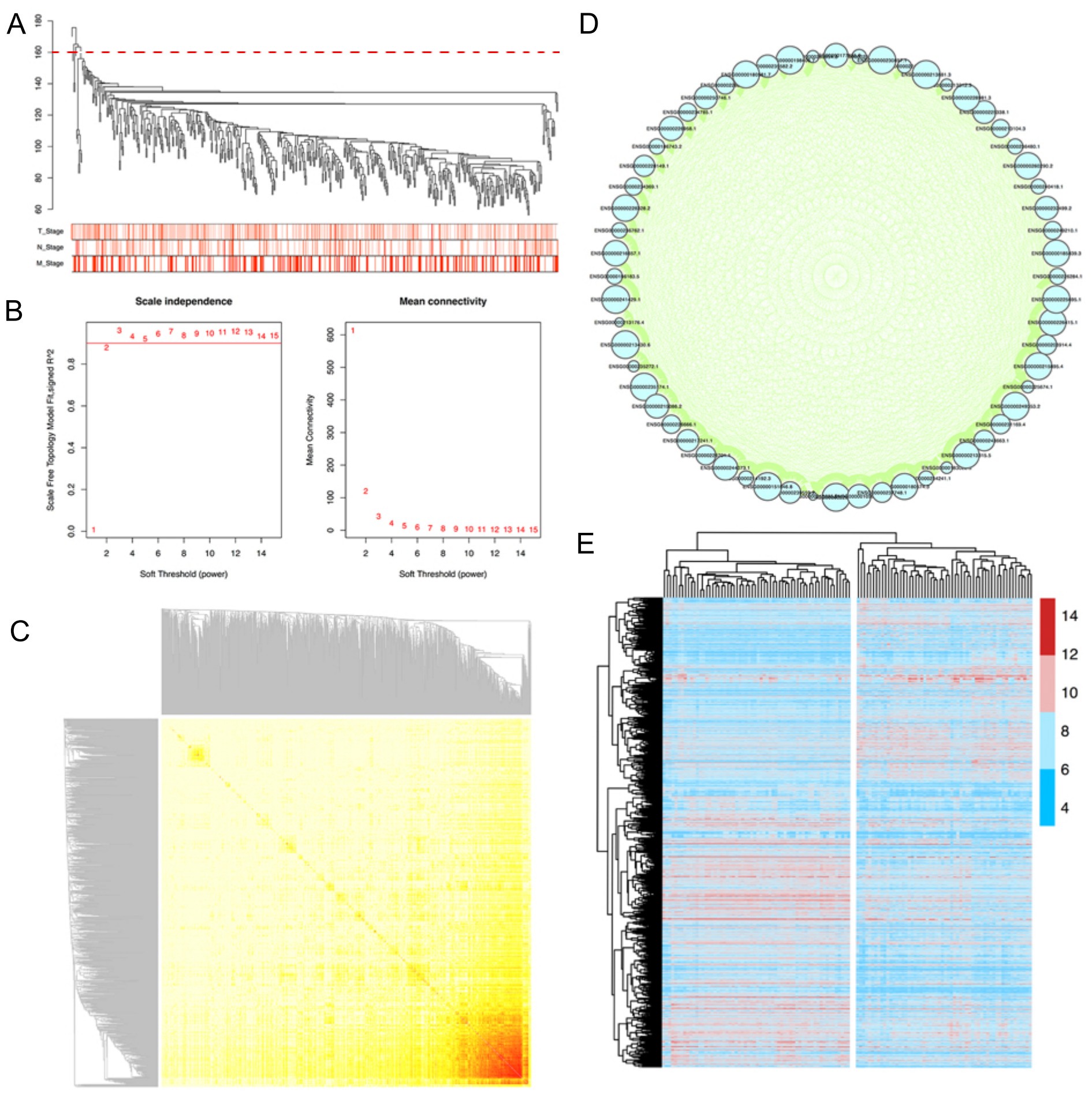

A
T_Stage
N_Stage
M_Stage
B
Scale independence
Mean connectivity
Scale Free Topology Model Fit,signed R^2
Mean Connectivity
Soft Threshold (power)
Soft Threshold (power)
C
D
E
14
12
10
8
6
4

Figure S2. SBF2-AS1promotes H1299 cell proliferation. Cell cycle was arrested at G1 phase in H1299 cells upon SBF2-AS1 knockdown (A). Expression of Cyclin D1 and P21 after ectopic expression and silence of SBF2-AS1 in H1299 cells (B). CCK8 assay (C), EdU (D), and colony formation assay (E) in H1299 cells after ectopic and silence of SBF2-AS1

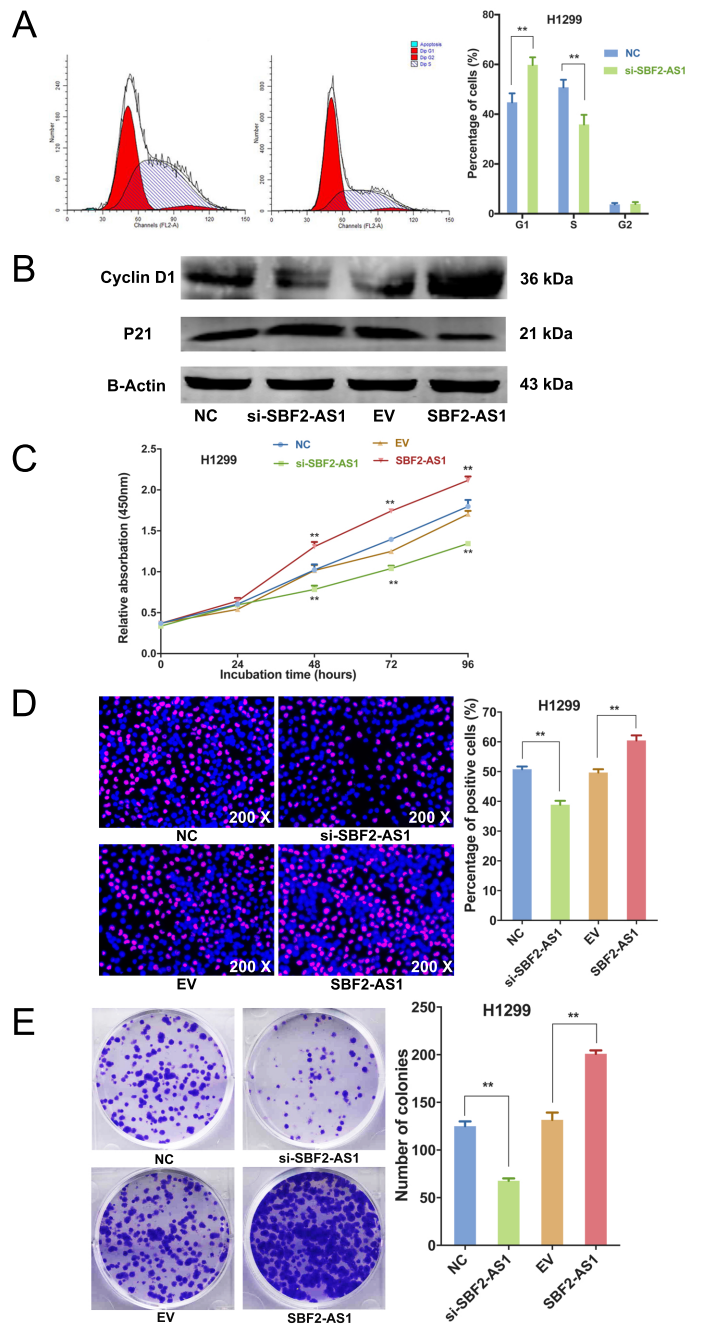

A
H1299
Percentage of cells (%)
NC
si-SBF2-AS1
G1
S
G2
B
Cyclin D1
P21
B-Actin
36 kDa
21 kDa
43 kDa
NC
si-SBF2-AS1
EV
SBF2-AS1
C
H1299
Relative absorbation (450nm)
Incubation time (hours)
D
200 X
Percentage of positive cells (%)
E
Number of colonies

Figure S3. Colony formation (A) and EdU (B) assay in H1299 cells. CCK8 assay in A549 (C) and H1299 (D) cells.

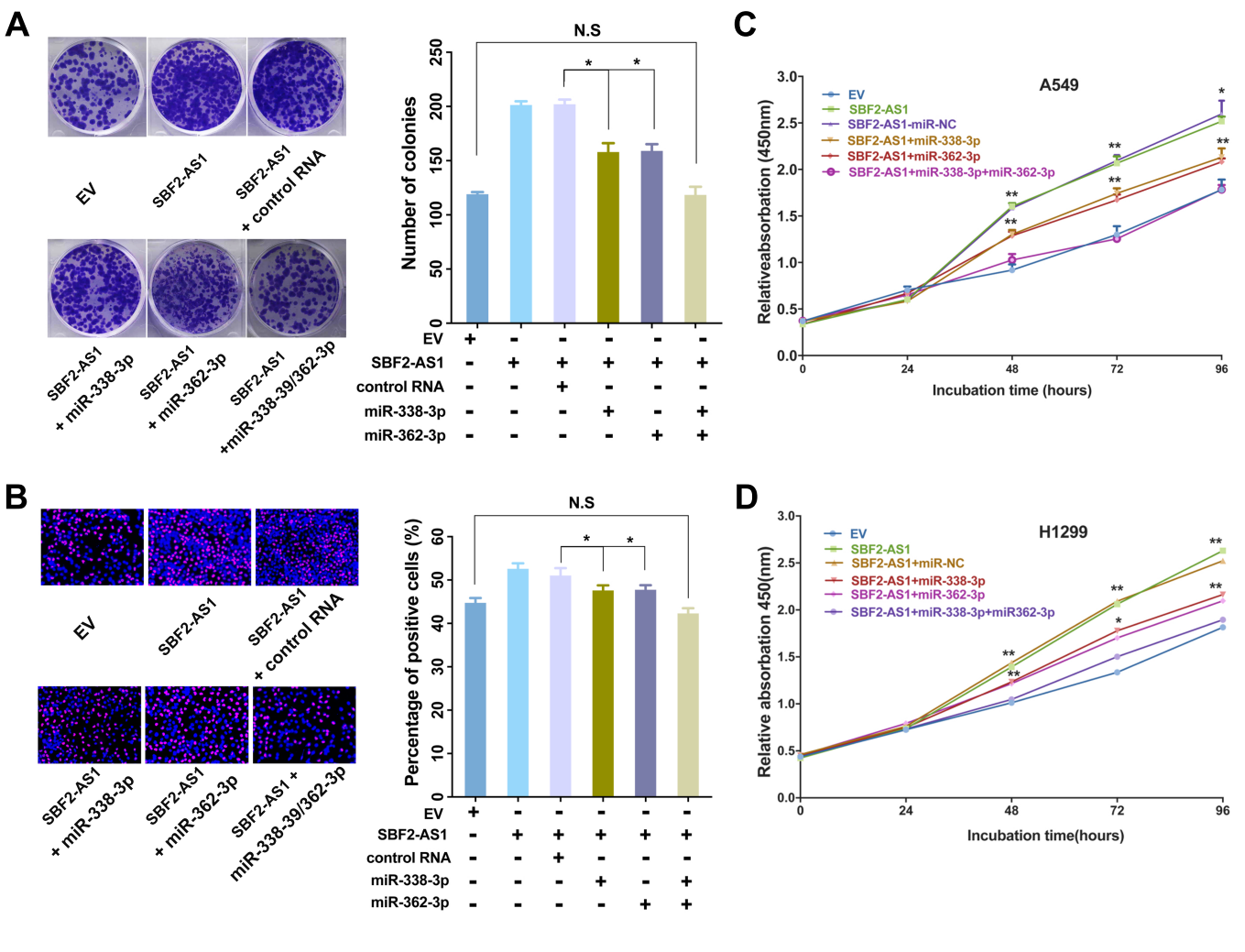
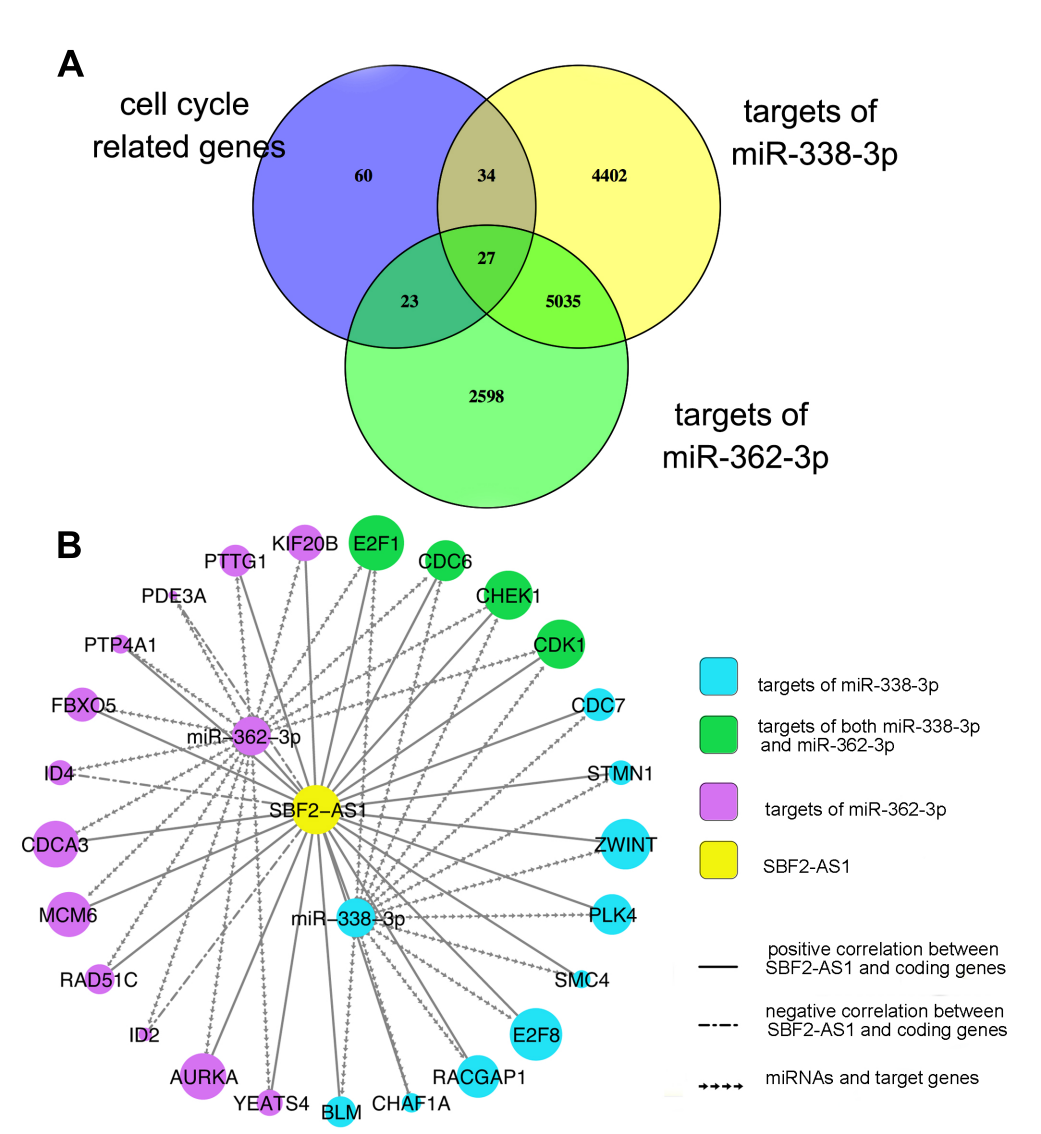

A
EV
SBF2-AS1
SBF2-AS1 + control RNA
SBF2-AS1 + miR-338-3p
SBF2-AS1 + miR-362-3p
SBF2-AS1 +miR-338-39/362-3p
N.S
Number of colonies
EV
SBF2-AS1
control RNA
miR-338-3p
miR-362-3p
B
EV
SBF2-AS1
SBF2-AS1 + control RNA
SBF2-AS1 + miR-338-3p
SBF2-AS1 + miR-362-3p
SBF2-AS1 + miR-338-39/362-3p
N.S
Percentage of positive cells (%)
EV
SBF2-AS1
control RNA
miR-338-3p
miR-362-3p
C
A549
EV
SBF2-AS1
SBF2-AS1-miR-NC
SBF2-AS1+miR-338-3p
SBF2-AS1+miR-362-3p
SBF2-AS1+miR-338-3p+miR-362-3p
Relativeabsorbation (450nm)
Incubation time (hours)
D
H1299
EV
SBF2-AS1
SBF2-AS1+miR-NC
SBF2-AS1+miR-338-3p
SBF2-AS1+miR-362-3p
SBF2-AS1+miR-338-3p+miR362-3p
Relative absorbation 450(nm)
Incubation time(hours)

Figure S4. Venn plot of candidate target genes (A). ceRNA network driven by SBF2-AS1 (B).


A
cell cycle related genes
targets of miR-338-3p
targets of miR-362-3p
60
34
4402
27
23
5035
2598
B
KIF20B
E2F1
PTTG1
CDC6
PDE3A
CHEK1
PTP4A1
CDK1
FBXO5
CDC7
miR-362-3p
ID4
STMN1
SBF2-AS1
CDCA3
ZWINT
MCM6
miR-338-3p
PLK4
RAD51C
SMC4
ID2
E2F8
AURKA
RACGAP1
YEATS4
BLM
CHAF1A
targets of miR-338-3p
targets of both miR-338-3p and miR-362-3p
targets of miR-362-3p
SBF2-AS1
positive correlation between SBF2-AS1 and coding genes
negative correlation between SBF2-AS1 and coding genes
miRNAs and target genes