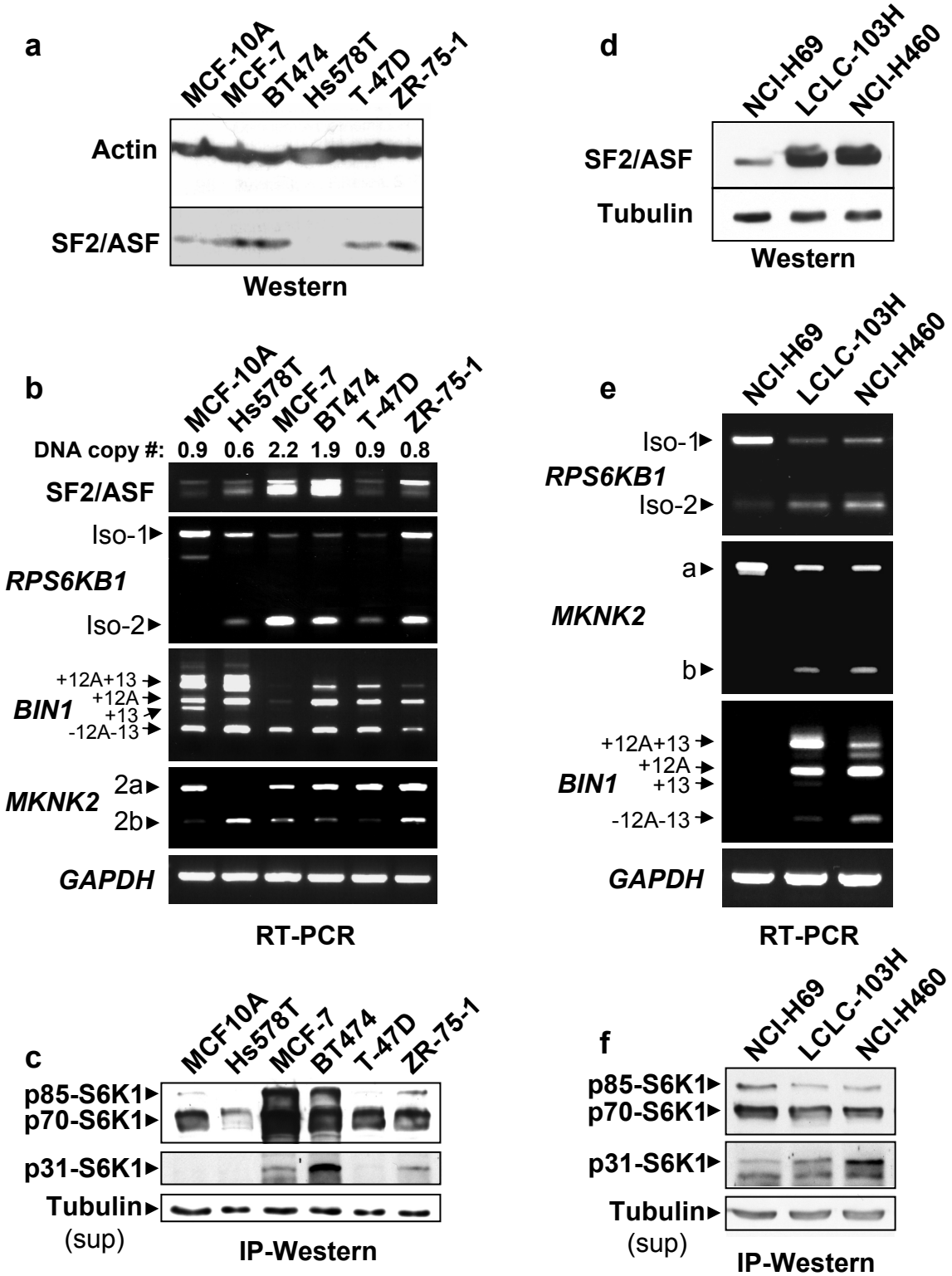
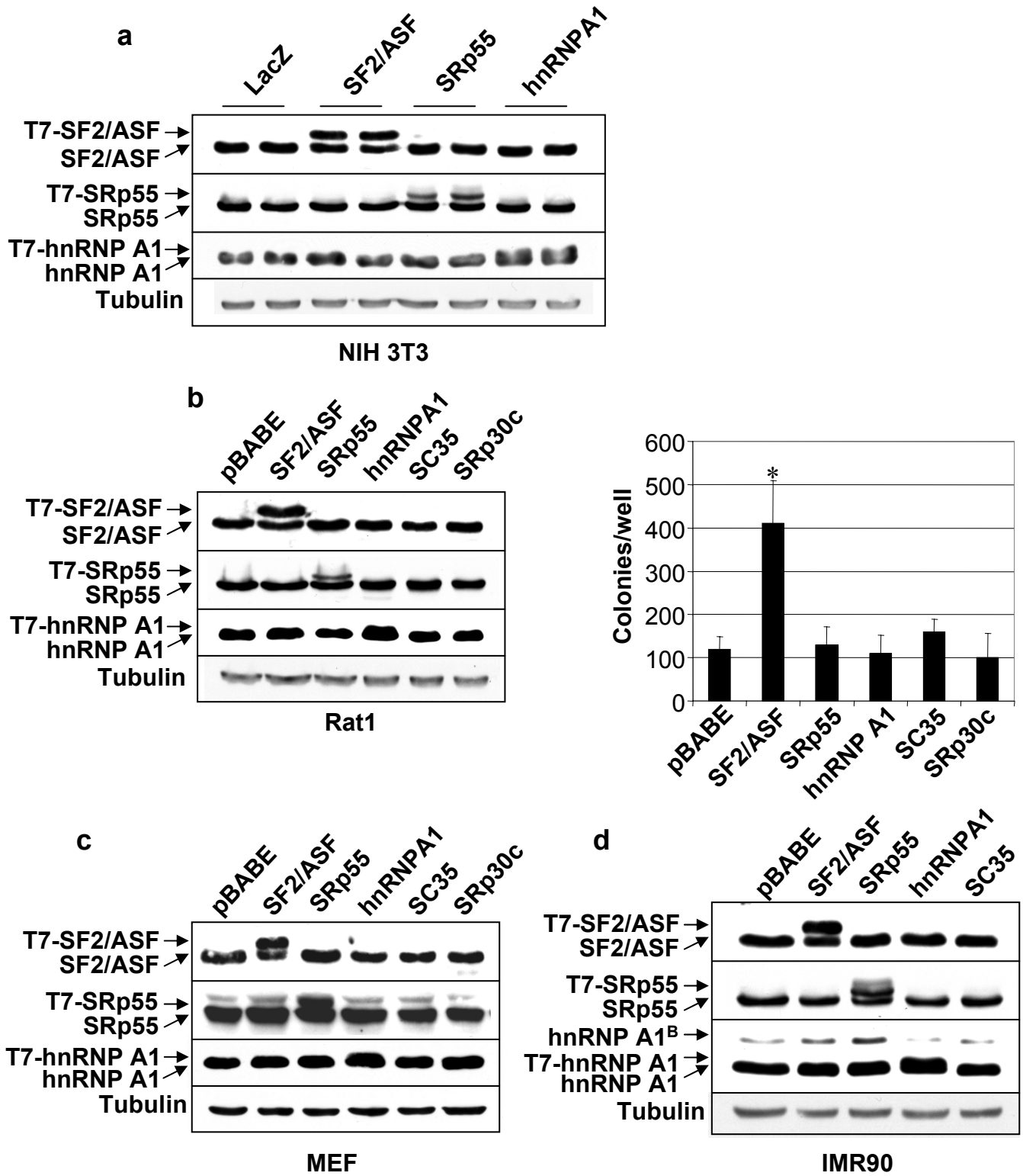


Supplementary Figure 1. Amplification and overexpression of SF2/ASF in cancer cell lines, and correlation with alternative splicing of its targets. (**a**) Western blot analysis of SF2/ASF levels in lysates of an immortal breast cell line (MCF10A) or five breast-cancer cell lines. (**b**) The mRNA levels of SF2/ASF, *GAPDH*, and the isoforms of *RPS6KB1*, *MKNK2*, and *BIN1* were detected by RT-PCR as in **Fig. 4**, in RNA samples from the indicated breast cell lines. MCF7, BT474, and ZR751 are known to have 17q23 amplification[8]. The DNA copy number of *SFRS1* (SF2/ASF) (measured by TaqMan and normalized to β-actin) is shown above the RT-PCR panel. (**c**) IP-Western analysis of S6K1 isoforms in the breast cell lines, carried out as in **Fig. 7e**. (**d**) Western blot analysis of SF2/ASF levels in three lung-cancer cell lines. (**e**) The levels of *GAPDH*, and the isoforms of *RPS6KB1*, *MKNK2*, and *BIN1* in the lung-cancer cell lines were detected by RT-PCR. (**f**) IP-Western analysis of S6K1 isoforms in the lung-cancer cell lines.



Supplementary Figure 2. Analysis of transduced splicing factor levels in mouse, rat, and human cell lines. Stable cell lines were selected after infection with the indicated retroviruses. Cells were lysed in SDS, and Western blots were carried out using monoclonal antibodies to SF2/ASF, SRp55, hnRNP A1, and tubulin. The T7-tagged proteins migrate slightly above the endogenous ones. Ectopic expression of SF2/ASF reproducibly results in slight downregulation of endogenous SF2/ASF. (**a**) NIH 3T3 immortal mouse fibroblasts. (**b**) Rat1 immortal rat fibroblasts. Soft-agar colonies were counted as in **Fig. 2b**. Means ± s.d. are shown. * $P < 5.6E\text{-}7$ (**c**) Mouse embryonic fibroblasts. (**d**) Human primary lung fibroblasts. hnRNP $A1^B$ is an alternatively spliced isoform of hnRNP A1

a

b

```
1- MRRRRRRDGFYLAPDFRHREAEDMAGVFDIDLDQPEDAGSEDELEEGGQLNESMDHGGVGPYELG  65
2- MRRRRRRDGFYLAPDFRHREAEDMAGVFDIDLDQPEDAGSEDELEEGGQLNESMDHGGVGPYELG  65

                                        I                    II
1- MEHCEKFEISETSVNRGPEKIRPECFELLRVLGKGGYGKVFQVRKVTGANTGKIFAMKVLKKAMI  130
2- MEHCEKFEISETSVNRGPEKIRPECFELLRVLGKGGYGKVFQVRKVTGANTGKIFAMKVLKKAMI  130

          III          IV                V
1- VRNAKDTAHTKAERNILEEVKHPFIVDLIYAFQTGGKLYLILEYLSGGELFMQLEREGIFMEDTA  195
2- VRNAKDTAHTKAERNILEEVKHPFIVDLIYAFQTGGKLYLILEYLSGGELFMQLEREGIFMEDTA  195

     VIA          VIB                VII          VIII
1- CFYLAEISMALGHLHQKGIIYRDLKPENIMLNHQGHVKLTDFGLCKESIHDGTVTHTFCGTIEYM  260
2- WPWVDRSSLQNFLELTFQFPRCSLREVITLNIHLWIDFIARMGSALLFAFSNTEAAFKSLRDEVP  260

               IX                X
1- APEILMRSGHNRAVDWWSLGALMYDMLTGAPPFTGENRKKTIDKILKCKLNLPPYLTQEARDLLK  325
2- LFGGGSLSPVEAVVLLAVKLPQSSRTHPPSWSNLQDCNIVTQPVLQCFVLFESRQG          316

          XI
1- KLLKRNAASRLGAGPGDAGEVQAHPFFRHINWEELLARKVEPPFKPLLQSEEDVSQFDSKFTRQT  390

1- PVDSPDDSTLSESANQVFLGFTYVAPSVLESVKEKFSFEPKIRSPRRFIGSPRTPVSPVKFSPGD  455

1- FWGRGASASTANPQTPVEYPMETSGIEQMDVTVSGEASAPLPIRQPNSGPYKKQAFPMISKRPEH  520

1- LRMNL                                                              525
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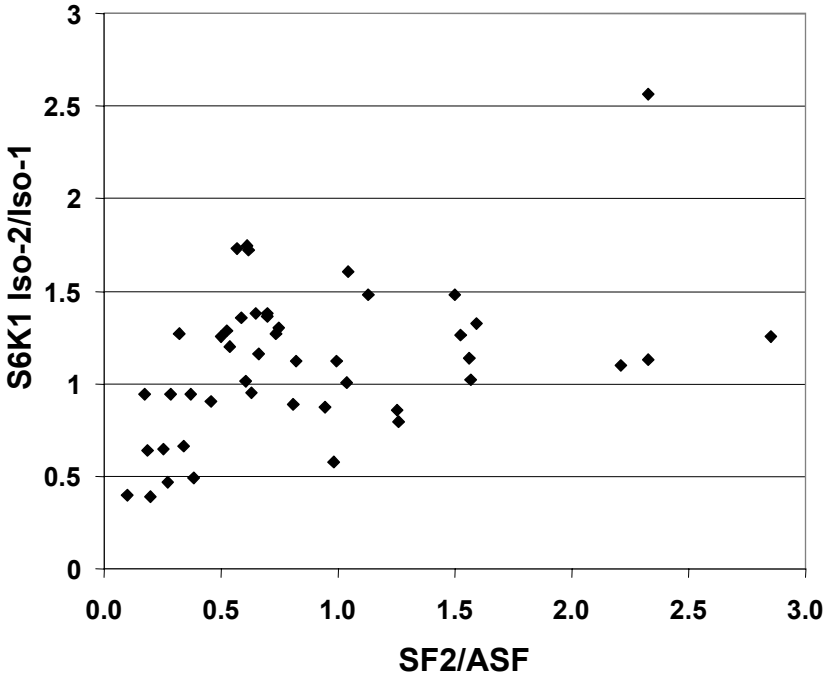
Supplementary Fig. 3 Alternative splicing of mouse *Rps6kb1* and primary structure of the two S6K1 isoforms. (**a**) The mouse *Rps6kb1* gene (middle) is alternatively spliced to generate two

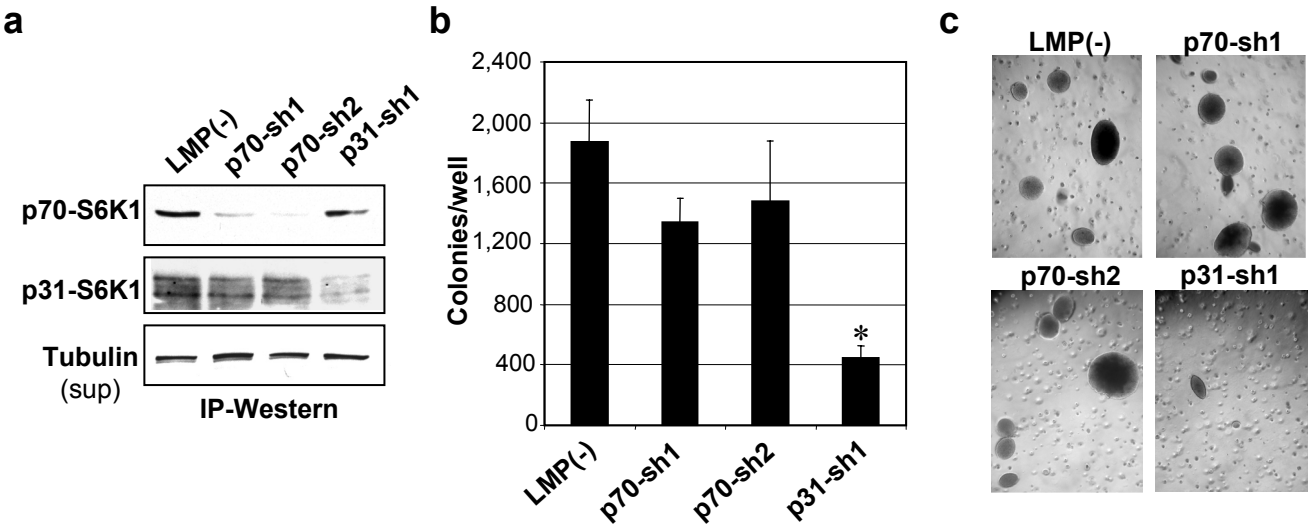
isoforms: a long, 15-exon isoform-1 (top) that encodes the 525- and 502-residue "p85" and "p70" S6K1 polypeptides through alternate translation start sites in exon 1; and a short, 10-exon isoform-2 (bottom) that incorporates three alternative cassette exons after exon 6 plus a longer 3' non-coding exon generated by alternative cleavage/polyadenylation within intron 7, and encodes a 316-residue protein (designated "p31" based on its apparent mobility, see **Fig. 5a**). Constitutive exons are shown as open boxes, and alternative exons or exon extensions are shown as black boxes. Introns are shown as horizontal lines, and splicing events are indicated by angled lines. (**b**) Alignment of S6K1 isoform-1 and isoform-2 protein sequences. The segments encoded by the 15 exons of isoform-1, and nine coding exons of isoform-2, are indicated by alternating black and blue lettering. Residues overlapping exon junctions are shown in red. The 12 conserved kinase subdomains present in isoform-1 are indicated by arrows and Roman numerals. Subdomains I-IV comprise the ATP-binding site. A catalytic aspartic acid (D218) in subdomain VIB is highlighted in magenta. An alternate translational start site (M24) that gives rise to p70-S6K1 is highlighted in green. T389 (T412 in p85), the main site of activation through phosphorylation by mTOR, is highlighted in cyan. The TOR signaling (TOS) motif is underlined.



Spearman *r* = 0.2978

95% CI = 0.2364 to 0.3545

Supplementary Figure 4. Correlation between SF2/ASF expression and S6K1 isoform ratios in 50 lung tumors. SF2/ASF and S6K1 isoform-1 and isoform-2 mRNA levels in 50 lung tumors were determined using RT-qPCR and an actin mRNA probe as a reference. The scatter plot shows the relationship between SF2/ASF mRNA levels and the ratio of S6K1 isoform-2/isoform-1 mRNA evels. The correlation coefficient *r* was calculated by non-parametric Spearman rank correlation, and a bootstrap technique was used to determine a 95% confidence interval (CI).



Supplementary Figure 5. Knockdown of isoform-2 of S6K1 reverses NCI-H460 cell transformation. (**a**) NCI-H460 cells were transduced with retroviruses encoding shRNAs against S6K1 isoform-1 (p70-sh1, p70-sh2) or isoform-2 (p31-sh1), or with the empty vector, LMP(-). S6K1 isoforms were immunoprecipitated from lysates using antibody against the N-terminus, and detected by Western blotting with the same antibody; IP-Western was used because of the low levels of p31-S6K1 in epithelial cells. (**b**) Cells described in **a** were seeded in soft agar and the colony number was measured 14 days later. Means ± s.d. are shown; * P = 6 × 10-8. (**c**) Representative fields from soft-agar plates with cells described in **b**.

Supplementary Table 1: Alternative splicing changes induced by overexpression (↑) or knockdown (↓) of specific splicing factors

Gene[1]	Isoforms	SF2/ASF ↑	SF2/ASF↓[2]	SRp55 ↑	SC35 ↑	SRp30c ↑	hnRNP A1 ↑
ADD1 exon 15	2	Inclusion (h)	Skipping	NE (h)	Inclusion (h)	NE (h)	NE (h)
BCL2L1 exon 2	2	NE (h)	NE	NE (h)	NE (h)	NE (h)	NE (h)
BIN1 exon 10	1	NE (m, h)	ND	NE (m, h)	ND	ND	ND
BIN1 exon 12A	4	Inclusion (m, r, h)	Skipping	NE (m, r, h)	Inclusion (weakly) (m, r, h)	Skipping (m, r, h)	Skipping (m, r, h)
BRCA1 exons 9,10	3	NE (h)	Strong skipping	NE (h)	NE (h)	NE (h)	NE (h)
CASP2 exon 9	2	Inclusion (weak) (m, h)	ND	NE (m, h)	Inclusion (weak) (m, h)	NE (m, h)	Inclusion (weak) (m, h)
CASP9 exons 3 - 6	2	NE (m, h)	NE	NE (m, h)	NE (h)	NE (h)	NE (h)
CCNE1 exon7	2	NE (m)	ND	NE (m)	ND	ND	ND
CHEK2 exon 3	2	NE (h)	NE	NE (h)	NE (h)	NE (h)	NE (h)
CHEK2 exon 6	2	NE (h)	NE	NE (h)	NE (h)	NE (h)	NE (h)
HNRNPA2B1 exon 3	2	Inclusion (h)	Skipping	Inclusion (weak) (h)	NE (h)	NE (h)	NE (h)
MGEA6 exon 19	2	Skipping (h)	Inclusion	NE (h)	Skipping (h)	NE (h)	NE (h)
MKNK1 exon 2	2	NE (m)	ND	NE (m)	NE (m)	NE (m)	NE (m)
MKNK2 exon 13	2	Inclusion (h)	Skipping (h)	Inclusion (h)	NE (h)	NE (h)	NE (h)
B-MYB exon 8(9A)	2	NE (h)	Skipping	Skipping (h)	Skipping (h)	NE (h)	Inclusion (h)
C-MYB exon 9A	2	Inclusion (h)	Inclusion	Skipping and increased expression (h)	Skipping and increased expression (h)	NE (h)	Increased expression (h)
S6K1 Iso-1 and Iso-2	2	Increased Iso-2 Reduced Iso-1 (m, r, h)	Increased Iso-1 Reduced Iso-2	NE (m, r, h)	Increased Iso-2 Reduced Iso-1 (m, r, h)	NE (m, r, h)	NE (m, r, h)
TEAD1 exon 5	2	Inclusion (m, h)	Skipping	NE (m, h)	Inclusion (m, h)	NE (m, h)	Skipping (m, h)
TFDP2 exon6	3	Inclusion (weak) (m)	ND	NE (m)	ND	ND	ND
TP73 exon 2	2	NE (h)	ND	Skipping (h)	ND	ND	ND
TSC2 exon 3	2	Inclusion (m) Increase of both isoforms (h)	NE	Increase of both isoforms (h)	Increase of both isoforms (h)	ND	Increase of both isoforms (h)
TSC2 exon 25	2	NE (h)	NE	NE (h)	NE (h)	NE (h)	NE (h)
VHL exon 2	2	NE (h)	NE	NE (h)	NE (h)	NE (h)	NE (h)

[1] Grey shading indicates genes that showed changes in alternative splicing
[2] RNA interference done in HeLa cells only
NE: No effect; ND: Not determined
h: human (IMR90 cells); m: mouse (MEF cells); r: rat (Rat1 cells)

For the following additional genes, only a single isoform was detected in the stable mouse and human cell lines: *CASP8* (exon 9), *FAS* (exon 6), *CD44* (exon v5), *FRAP1* (mTOR)(intron 32), *HER-2* (intron 8), *HER-2* (exon 16), *MAX* (exons 1-5), and *HIF1A* (exon 11).

Supplementary Table 2: Primers, probes, and shRNA templates

Q-PCR probes

Probe	Location
S1F	exon 1 of SF2/ASF
S2F	exon 4 of SF2/ASF
RA1	3' UTR of hnRNPA1
RA2	exon 4 of hnRNPA1
RA3	exon 6 of SRp55
RA4	exon 6 of SRp55 (within 3' UTR)
S6KA	exon 6c of S6K1 isoform-2
S6KB	exon 15 of S6K1 isoform-1

S1F QF: CTTGGTGGGAAGGCCTGTT
S1F QR: AGATGCGGCAATCGTTGTTC
S1F QP: (6-FAM)-CGAGTCCGCGCTTTTCGTCACC-(TAMRA)
S2F QF: CAGATCAATAATGGAGGCAATGG
S2F QR: AATCATTTTTAACCCCTGCCTTT
S2F QP: (6-FAM)-CAATACTGCCAATTTCATCTGTGACA-(TAMRA)
RA1 QF: GGAAAGTGTAAAGCATTCCAACAA
RA1 QR: TCAGCGTCACGATCAGACTGT
RA1 QP: (6-FAM)-TTTTGCACCCCATGCTGTTGATTGCTAA-(TAMRA)
RA2 QF: AGAACATCACCTAAGAGATTATTTTGAACA
RA2 QR: CAAAGGCAAAGCCCCTTTTC
RA2 QP: (6-FAM)-TGCCACTGCCTCGGTCAGTCATG-(TAMRA)
RA3 QF: CGATCCCCTAAAGAAAATGGAA
RA3 QR: GGCCTTTGAGGGTGGAACA
RA3 QP: (6-FAM)-ATCAAGGAGCCAGTCCCGTTCCAATT-(TAMRA)
RA4 QF: ATGAACATGCCGTAGTGCCTTT
RA4 QR: GGTGAACAAATCGGGAGGAA
RA4 QP: (6-FAM)-TGGCCAGTTTGAGTCCTGCCTACTTTGA-(TAMRA)
S6A QF: CTGTGAAACTGCCTCAGTCCTCTA
S6A QR: AAAGCACAAGGCACTGCAATACT
S6A QP: CACCCTCTCCATCCTGGAGTAATCTGCA
S6B QF: GAGCAGATGGATGTGACAATGAG
S6B QR: GAGATCATGGGAAAAGCTTGTTTT
S6B QP: ATACGACAGCCGAACTCTGGGCCAT
β-actin forward primer: GCAAAGACCTGTACGCCAACA;
β-actin reverse primer: TGCATCCTGTCGGCAATG
β-actin probe: (6-FAM)-TGGCGGCACCACCATGTACC-(TAMRA)

RT-PCR primers

1. Human and mouse *CASP2* (exon 9)

Human: 5'hICH-1(exon 8): AACTGCCCAAGCCTACAGAA
3'hICH-1 (exon 10): GCGTGGTTCTTTCCATCTTGTTGGTCA
Mouse: 5'mICH-1 (exon 8): ATGCTAACTGTCCAAGTCTACAGAAC

3'mICH-1 (exon 10): GTCTCATCTTCATCAACTCCTCTTTGCC

2. Human *FAS* (exon 6)

FasS1: ATGCTGTGGATCTGGGCTGTC
FASAS: TGT CTT CAG CAA TTC TCG GGA TG

3. Human *TP73* (exon2)

p73(exon2)For: GGACGGACGCCGATGCC
p73(exon2)Rev: GGTCCATGGTGCTGCTCAGC

4. Mouse *TFDP2* (exon 6)

DP2-e2-For: GCTGAAGAGAGAGCCC
DP2-e8-Rev: ATCATAAACTCTTCGTCTAATG

5. Mouse *BIN1* (exon 10)

m*BIN1* mid-For: AAGCCCAGAAGGTGTTCGAG
m*BIN1* mid-Rev: TGGCTGAGATGGGGACTT

6. Mouse *BIN1* (exon 11-15)

m*BIN1* CT-For: CTGAGATCAGAGTGAACCATG
m*BIN1* CT-Rev: CACCCGCTCTGTAAAATTC

7. Mouse and human *BIN1* exon 11-14

m/H*BIN1*e11-For: CCTCCAGATGGCTCCCCTGC
m/H*BIN1*e14-Rev: CCCGGGGGCAGGTCCAAGCG

8. Mouse, human, and rat *RPS6KB*

S6K-P1-For: GAGGAGAACTATTTATGCAGTTAG
S6K-P2-Rev: GGGGCACTTCATCCCTAAGG
S6K-P4-Rev: GAACGCCGAGATGTTGCTAGG

9. Mouse *MKNK1* (exon 1-5)

MNK1-e1-For: AGGTGGGGGTGCTCGCGGCCG
MNK1-e5-Rev: CCACGATGGGAAGGGGCTCAC

10. Human MKNK2 (exon 13a and 13b)

MKNK2 e11 for: CCAAGTCCTGCAGCACCCCTG
MKNK2 e13a rev: GATGGGAGGGTCAGGCGTGGTC
MKNK2 e13b rev: GAGGAGGAAGTGACTGTCCCAC

11. Mouse and human *TEAD1* (exon 4-8)

TEF-1-e3,4-For: AGACGAAGGCAAAATGTATGG
TEF-1-e9,8-Rev: CGTAGGCTCAAACCCTGGAAT

12. Human *BRCA1* (exon 10)

hBRCA1ex9-For: TAATAAGGCAACTTATTGCAG
hBRCA1ex11-RevS: CTTCTCAGTGGTGTTCAAATC

13. Human *CCNE1* (exon 7)

hCyclinE-For: ATCATGCCGAGGGAGCGCCAGGGA
hCyclinE-ET-Rev: CCAGGACACAGAGATCCAACAGCTTC

14. Human *CASP8* (exon 9)

Caspase-8-For: TCTGTGCCCAAATCAACAAG
Caspase-8-Rev: GCCACCAGCTAAAAACATTCC

15. Human *CASP9* (exons 3-6)

Caspase 9-For: GCTCTTCCTTTGTTCATCTCC
Caspase 9-Rev: CATCTGGCTCGGGGTTACTGC

16. Human and mouse *TSC2* (exon 3)

h/mTSC2 e2-For: GGCCTCAACAATCGCATCCG
h/mTSC2 e4-Rev: CCAACATCCATCCACTGCAGG

17. Human *TSC2* (exon 25)

hTSC2 e24-For: GGCCTGCGGTCCAATGTCCTC
hTSC2 e26-Rev: CTCTTCGGGACAGCCGTGAAG

18. Human *C-MYB* (exon 9A)
c-Myb e8-For: CCACACATGCAGCTACCCCG
c-Myb e10-Rev: CACAGTCTGGTCTCTATGAAATGG

19. Human *B-MYB* (exon 9A)
B-myb e7-For: GGAAGTCTTCTGACCAACTGGC
B-myb e9-Rev: GCAGCATGTTTCTGGTGCAGGGG

20. Human *HNRNPA2B1* (exon 2)
HnRNPA2B1 e1-For: GCGGCAGTAGCAGCAGCGCC
HnRNPA2B1 e3-Rev:CTTACGGAACTGTTCCTTTTCTC

21. Human *MGEA6* (exon 19)
MGEA6 e-18-For: CTGAAACAGAGCTTAAATTTGAAC
MGEA6 e-20-Rev: CTGGCGGAGGAAACATCATCC

22. Human *VHL* (exon 2)
VHL e-1-For: AACTTCGACGGCGAGCCGCAG
VHL e-3-Rev: CTGTGTCAGCCGCTCCAGGTC

23. Human *BCL2L1* (exon 2)
Bcl-Xfor: GAGGCAGGCGACGAGTTTGAA
Bcl-Xrev: TGGTAGGTGAGATGGGAGGGT

24. Human *FRAP1* (intron 32)
FRAP1-e32-For: ACTGCATCAGGACCTCTTCTCC
FRAP1-e34-Rev: GGTCTAGACCACCCTCTCTCTGACG

25. Human *HER2* (intron 8)
HER2-E8-For: CAACTACCTTTCTACGGACGTG
HER2-in8-Rev: GGAGGTGGGGGTGGGTGGGG
HER2-E9-Rev: AGCTCTCCGGCAGAAATGCCAGG

26. Human *HER2* (exons 15-17)
HER2-E15-For: CTGCGTGGCCCGCTGCCCCAG
HER2-E17-Rev: GTTTCCTGCAGCAGTCTCCGC

27. Mouse *MAX* (exons 1-5)
dMAX-For: ATGAGCGATAACGATGACATC
dMAX-Rev: GCCCCGCATGGTTAGC

28. *CD44* (exon v5)
CD44-For: CTATTGTCAACCGTGATGGTAC
CD44-Rev: GCCAGGAGAGATGCCAAGATG

29. *HIF1A* (exon)11
hHIF-1a-e10-For: CCCCAGATTCAGGATCAGACA
hHIF-1a-e13-Rev: CCATCATGTTCCATTTTTCGC

Cloning primers for *RPS6KB1*:
T7s6k For: GGGGAAAGATCTACCAGCCACCATGGCATCGATG
ACAGGTGGCCAACAGATGGGTATGAGGCGACGAAGGAGGCGGGAC
T7s6k Rev-1: GGGGAAGAATTCACTATGTAATGACATTGACTCTCTG
T7s6k Rev-2: GGGGAAGAATTCAAGGGCTGAATCAACCCTGTCTGG

SF2/ASF shRNA templates:

SF2-sh***:**
TGCTGTTGACAGTGAGCGAGGGCATCTACGTGGGTAACTTTAGTGAAGCCAC
AGATGTAAAGTTACCCACGTAGATGCGGCTGCCTACTGCCTCGGA

SF2-sh1:
TGCTGTTGACAGTGAGCGCTGGCAGTATTGACCTTATACTTAGTGAAGCCACA
GATGTAAGTATAAGGTCAATACTGCCAATGCCTACTGCCTCGGA

SF2-sh2:
TGCTGTTGACAGTGAGCGCGGGCTAAAGTGTTGAATTGCATAGTGAAGCCAC
AGATGTATGCAATTCAACACTTTAGCCCATGCCTACTGCCTCGGA

S6K shRNA templates:
S6K1 p70-sh1:
TGCTGTTGACAGTGAGCGCGGCATGGAACATTGTGAGAAATAGTGAAGCCAC
AGATGTATTTCTCACAATGTTCCATGCCATGCCTACTGCCTCGGA

S6K1 p70-sh2:
TGCTGTTGACAGTGAGCGCTGGAACATTGTGAGAAATTTGTAGTGAAGCCAC
AGATGTACAAATTTCTCACAATGTTCCATTGCCTACTGCCTCGGA

S6K1 p31-sh1:
TGCTGTTGACAGTGAGCGACCGGAGAACATCATGCTTAATTAGTGAAGCCAC
AGATGTAATTAAGCATGATGTTCTCCGGCTGCCTACTGCCTCGGA