Supplementary data

Supplementary Figures and Legends

Figure S1

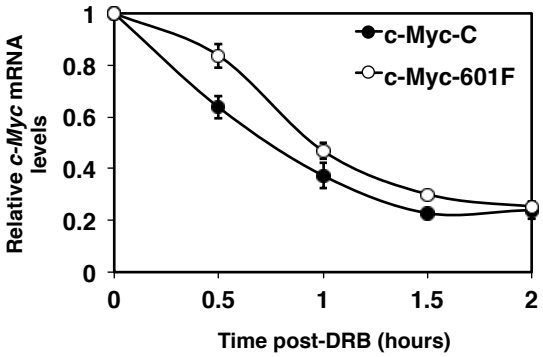

Figure S1. *c-Myc-C* and *c-Myc-601* mRNA degradation time course. *(A)* Cells were subjected to DRB treatment (100μM) and samples were harvested at the indicated time points. RNA was analyzed by RT-qPCR as described in Figure 2E. Time point prior DRB treatment was set to 1.

Figure S2

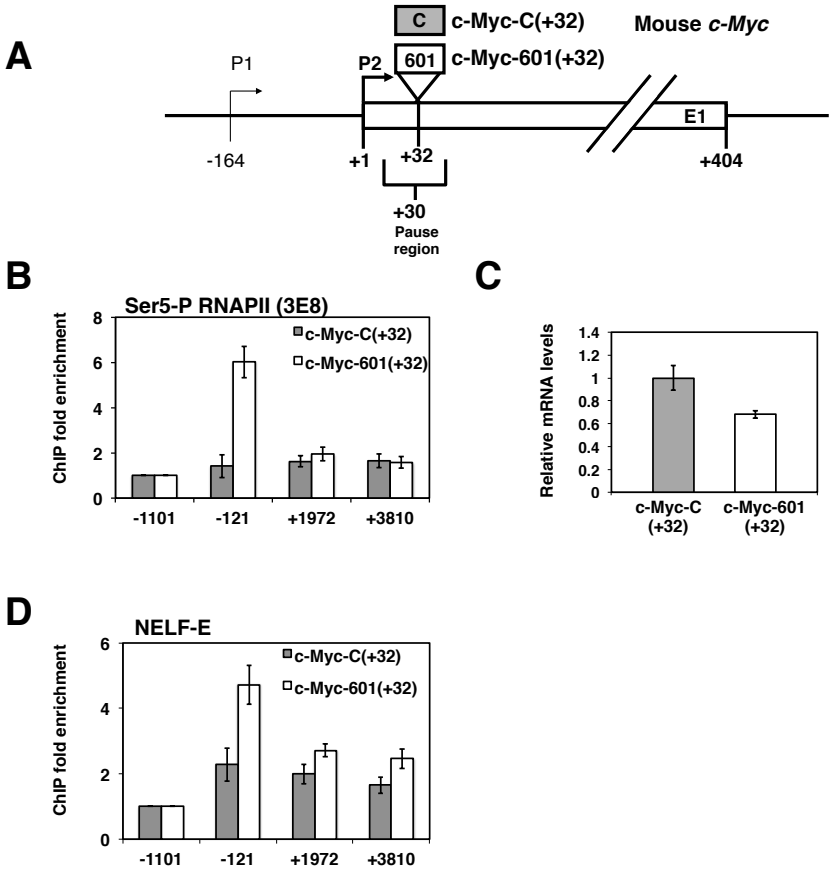

Figure S2. Increased promoter-proximal pausing under a strong +1 nucleosome positioning at position +32. *(A)* Schematic representation of promoter and exon 1 of the mouse *c-Myc* gene showing the position in which either the 601 sequence (601) or the control sequence (C) have been inserted. Distances (pb) of pause region and insertions from the transcription start site (TSS) are indicated below the gene. *(B)* RNAPII ChIP analysis of *c-Myc-601(+32)* and *c-Myc-C(+32)* genes. Ser5 phosphorylated form of RPB1 was immunoprecipitated using 3E8 antibody. ChIP values were normalized to the values from -1101 amplicon. *(C) c-Myc* mRNA levels measured by RT-qPCR using the primers c-Myc-1972-up and c-Myc-1972-low. Values were normalized to *GAPDH* amplification. *c-Myc-C* signal was set to 1. *(D)* NELF-E ChIP assay

in *c-Myc-601(+32)* and *c-Myc-C(+32)* genes as described in Figure 2B. Primer sequences are listed in Supplementary Table S1. Average and standard deviation from 2 independent experiments are displayed.

Figure S3

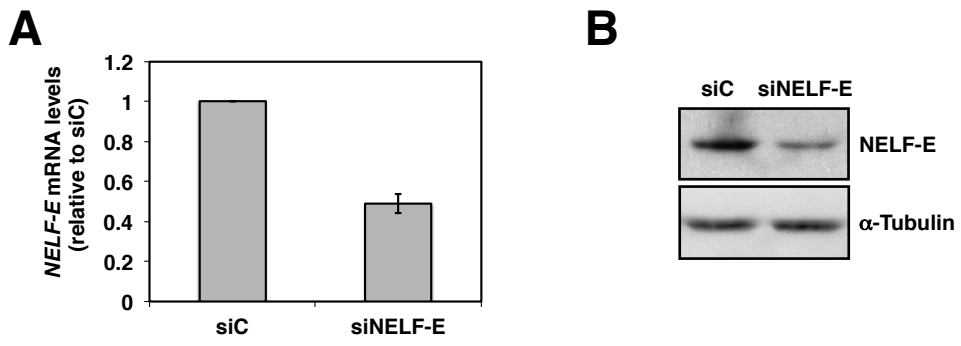

Figure S3. *NELF-E* downregulation. *(A and B) NELF-E* mRNA levels and NELF-E protein levels after *NELF-E* siRNA or control siRNA treatment measured by RT-qPCR and Western blot, respectively. Cells were harvested for RNA or protein extraction 72 hours after siRNA transfection. *GAPDH* amplification was used as RNA loading control and α-tubulin was used as a protein loading control. *NELF-E* mRNA values were normalized to the control siRNA. Primers and siRNAs used are listed in Supplementary Table S1. Average and standard deviation from 3 independent experiments are displayed.

Figure S4

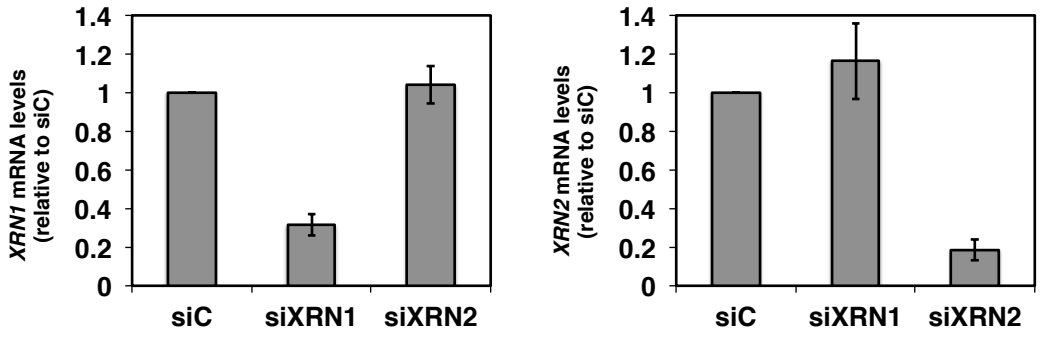

Figure S4. *XRN1* and *XRN2* downregulation. *XRN1* (left panel) and *XRN2* (right panel) mRNA levels three days after the indicated siRNA treatment. RNA extraction was carried out and levels of mRNA was determined by RT-qPCR. Control siRNA data were set to 1. Primers and siRNAs used are listed in Supplementary Table S1. Average and standard deviation from 3 independent experiments are displayed.

Figure S5

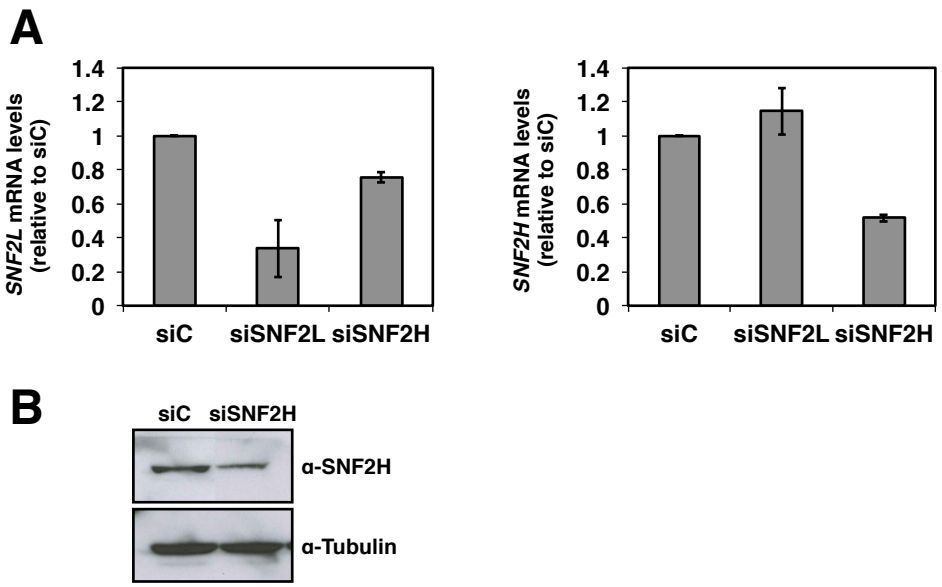

Figure S5. *SNF2L* and *SNF2H* downregulation. *(A) SNF2L* (left panel) and *SNF2H* (right panel) mRNA levels 72 hours after the indicated siRNA treatment. RNA extraction was performed and mRNA levels were determined by RT-qPCR. Primers and siRNA used are listed in Supplementary Table S1. Average and standard deviation from 3 independent experiments are displayed. *(B)* SNF2H protein levels after 72 hours of SNF2H or control siRNA treatment measured by Western blot.

Supplementary Table

Table S1. Primer and siRNA sequences.

Primer	Sequence
c-Myc 601/ACTB (-38)	Up: CCCCTCCTGCCTCCTGAA
	Low: CCTCTGTCTCTCGCTGGAATTAC
c-Myc 601/ACTB (+23)	Up: TGACTCGCTGTAGTAATTCCAGC
	Low: TCCGCTCACTCCCTCTGTC
c-Myc 601/ACTB (+314)	Up: AAACTTTGCCCATTGCAGC
	Low: AGGCAGAGAACACTGTCCCC
c-Myc 601/ACTB (+446)	Up: CGTCTTGAATGTAGCGGCC
	Low: TCCCTTCCCCTTTCCTCTG
c-Myc ACTB (+51)	Up: ATTCCAGCGAGAGACAGAGG
	Low: GGATGCCACAGAATTCCAA
c-Myc ACTB (+58)	Up: AGCGAGAGACAGAGGGAGTG
	Low: TGGAGTTGAAGGTAGTTTCGTG
c-Myc ACTB (+115)	Up: AGTAATTCCAGCGAGAGACAGAG
	Low: CTGCAATGGGCAAAGTTTC
c-Myc ACTB (+132)	Up: AGTGTGACGTGGACATCCGC
	Low: GGTACATGGTGGTGCCGC
c-Myc-601 (+51)	Up: AGCGAGAGACAGAGGGAGTG
	Low: AAGCGGTGCTAGAGCTGTCT
c-Myc-601 (+101)	Up: AGACAGCTCTAGCACCGCTTA
	Low: TATCTGACACGTGCCTGGAG
c-Myc-601 (+129)	Up: CTGGAGAATCCCGGTGCC
	Low: CGTACGTGCGTTTAAGCGG
c-Myc-601 (+203)	Up: CTCCAGGCACGTGTCAGATA
	Low: CCGCACTAGTGATTGGGAAT
c-Myc (+1)	Up: TGACTCGCTGTAGTAATTCCAGC
	Low: TCCGCTCACTCCCTCTGTC
c-Myc (+481)	Up: CCATTCCTGTGCTTTTGACACTT
	Low: CCCCTTTCCTCTGTCATCTTGAC
c-Myc (+1972)	Up: GATGCCCCTCAACGTGAAC
	Low: AAATAGGGCTGTACGGAGTCG
c-Myc (+3810)	Up: AGATGAGGAAGAAATTGATGTGG
	Low: TCGGGATGGAGATGAGCC
c-Myc (-1101)	Up: TGTATGGGGTGTAGACCGGC
	Low: ACTCCAGCACCTCCGGTTC
NELF-E	Up: GACATGACACCCACCCTTCT
	Low: TGACGAAGGCACAGTTTCTG
XRN1	Up: GCCCTGACTGGGATTAGTGT
	Low: GCCCTGACTGGGATTAGTGT
XRN2	Up: TCCTTCGGCTTAATGTTCTTC
	Low: AGATGTGAAACTCGTATTAGG
SNF2L	Up: CCTCCAAAACAGCCAAATGT
	Low: TTTGAGCCAGAGCTGGATTT
SNF2H	Up: GAACTGCCTTAGCCACCTG

	Low: ACCCATGAAGGGAGGAAACT
GAPDH	Up: GAGTCAACGGATTTGGTCGT
	Low: AATGAAGGGGTCATTGATGG
28S	Up: TACCCACCCGACCCGTCTTG
	Low: CTGGAGAGGCCTCGGGATCC

siRNA	**Sequence (reference)**
NELF-E	GGCAUUGCUGGCUCUGAAGUU (1)
XRN1	UGAUGAUGUUCACUUUAGA (2)
XRN2	AAGAGUACAGAUGAUCAUGUU (3)
SNF2L	UAACAUAGCUCGAGAGGUA (4)
SNF2H	GGAAUGGUAUACUCGGAUA (4)
Luciferase	CGUACGCGGAAUACUUCGA

Supplementary References

1. Aiyar, S.E., Sun, J.L., Blair, A.L., Moskaluk, C.A., Lu, Y.Z., Ye, Q.N., Yamaguchi, Y., Mukherjee, A., Ren, D.M., Handa, H. *et al.* (2004) Attenuation of estrogen receptor alpha-mediated transcription through estrogen-stimulated recruitment of a negative elongation factor. *Genes Dev*, **18**, 2134-2146.
2. Stoecklin, G., Mayo, T. and Anderson, P. (2006) ARE-mRNA degradation requires the 5'-3' decay pathway. *EMBO Rep*, **7**, 72-77.
3. West, S., Gromak, N. and Proudfoot, N.J. (2004) Human 5' --> 3' exonuclease Xrn2 promotes transcription termination at co-transcriptional cleavage sites. *Nature*, **432**, 522-525.
4. Eckey, M., Kuphal, S., Straub, T., Rummele, P., Kremmer, E., Bosserhoff, A.K. and Becker, P.B. (2012) Nucleosome remodeler SNF2L suppresses cell proliferation and migration and attenuates Wnt signaling. *Mol Cell Biol*, **32**, 2359-2371.