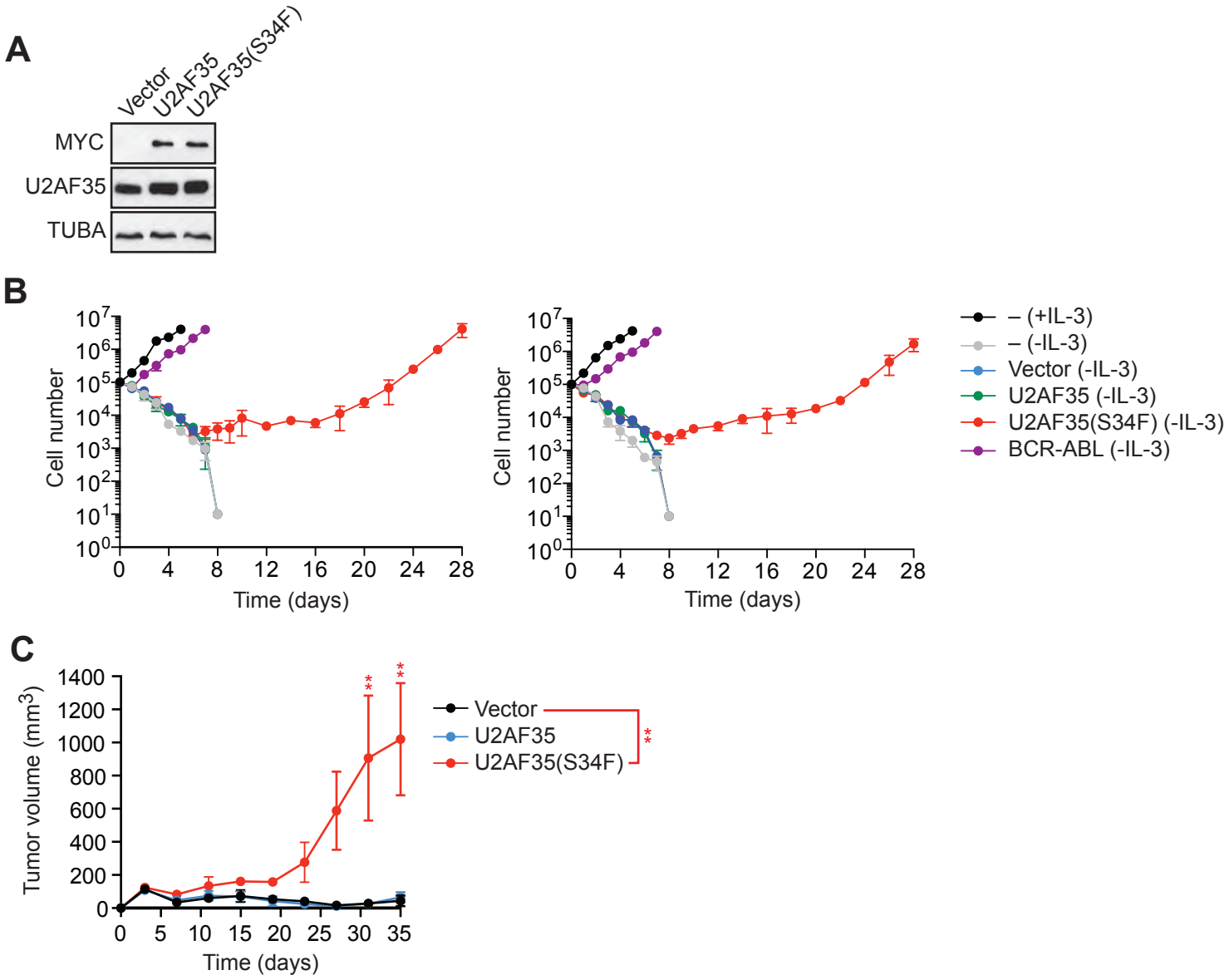
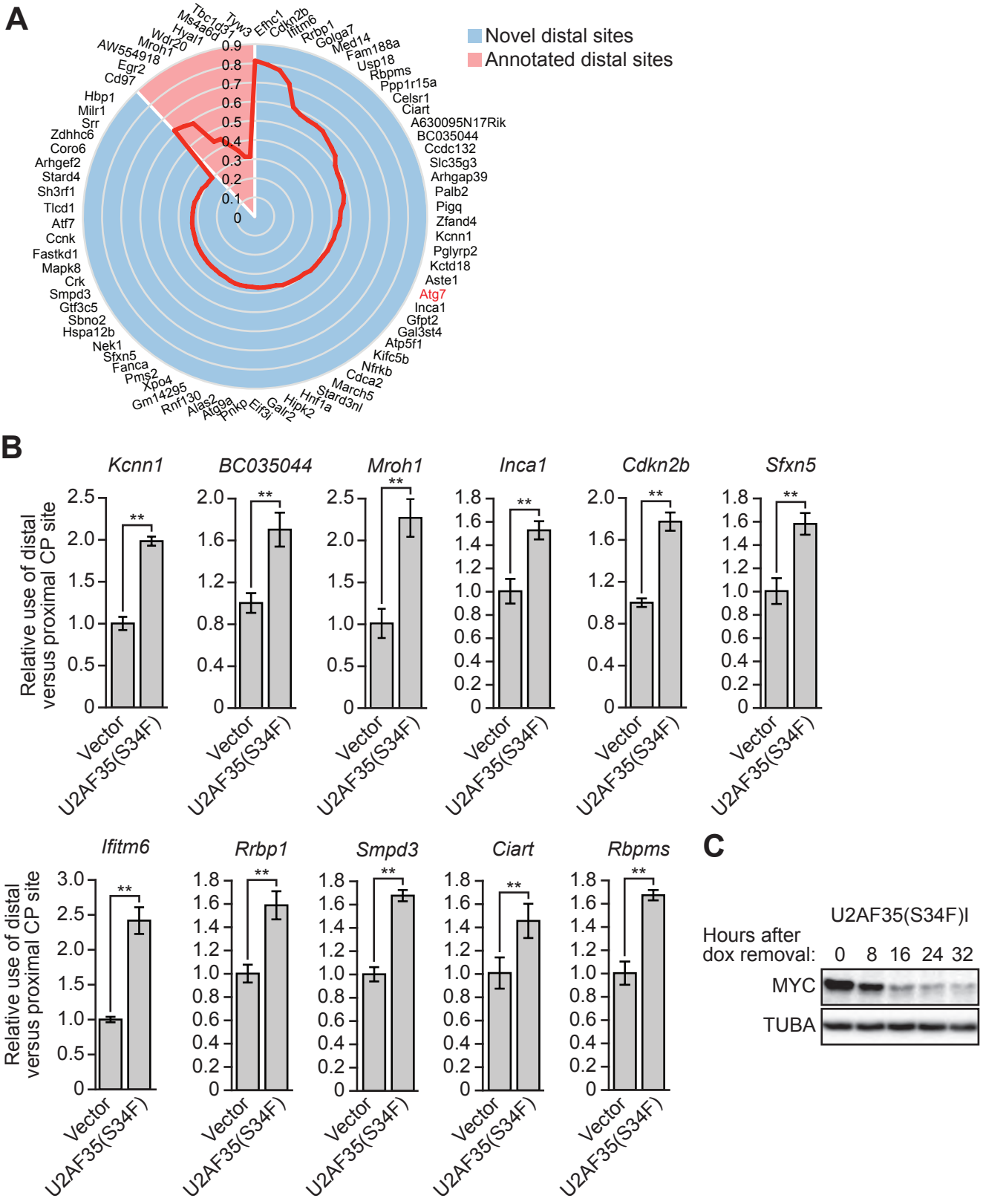
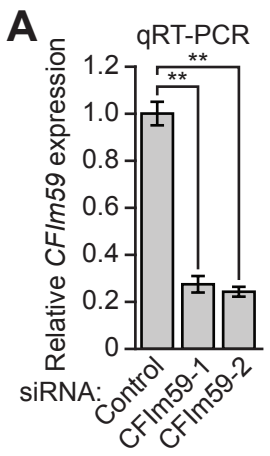
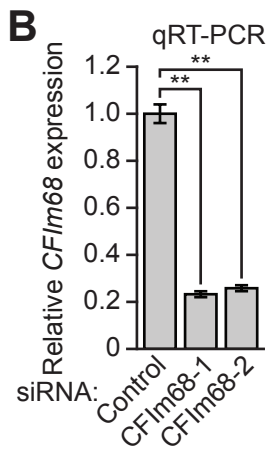
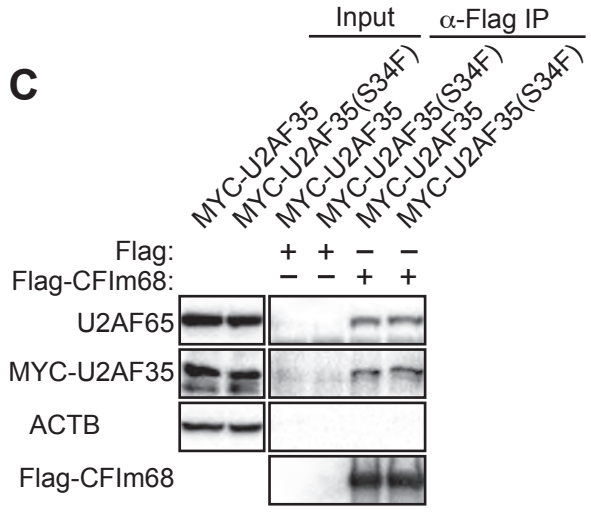
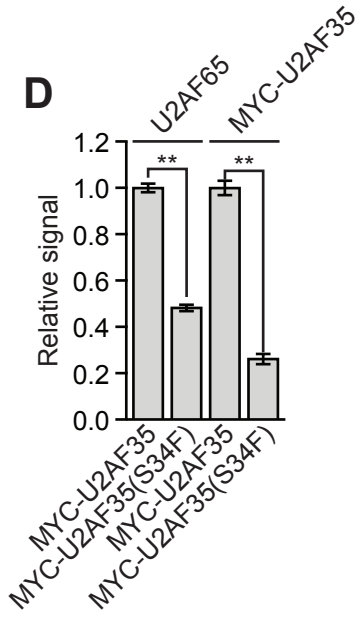

A
Vector
U2AF35
U2AF35(S34F)
MYC
U2AF35
TUBA
B
Cell number
Time (days)
– (+IL-3)
– (-IL-3)
Vector (-IL-3)
U2AF35 (-IL-3)
U2AF35(S34F) (-IL-3)
BCR-ABL (-IL-3)
C
Tumor volume (mm3)
Time (days)
Vector
U2AF35
U2AF35(S34F)
**

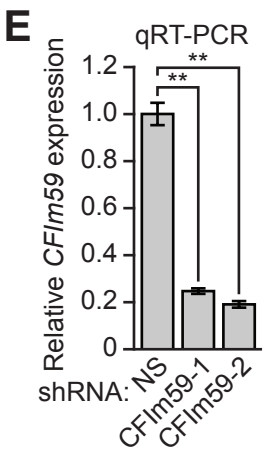

A
Novel distal sites
Annotated distal sites
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Tbc1d31
Ms4a6d
Hyal1
Wdr20
Mroh1
AW554918
Egr2
Cd97
Hbp1
Milr1
Srr
Zdhhc6
Coro6
Arhgef2
Stard4
Sh3rf1
Tlcd1
Atf7
Ccnk
Fastkd1
Mapk8
Crk
Smpd3
Gtf3c5
Sbno2
Hspa12b
Nek1
Sfxn5
Fanca
Pms2
Xpo4
Gm14295
Rnf130
Alas2
Atg9a
Pnkp
Eif3l
Galr2
Hipk2
Hnf1a
Stard3nl
March5
Cdca2
Nfrkb
Kifc5b
Atp5f1
Gal3st4
Gfpt2
Inca1
Atg7
Aste1
Kctd18
Pglyrp2
Kcnn1
Zfand4
Pigq
Palb2
Arhgap39
Slc35g3
Ccdc132
BC035044
A630095N17Rik
Ciart
Celsr1
Ppp1r15a
Rbpms
Usp18
Fam188a
Med14
Golga7
Rrbp1
Ifitm6
Cdkn2b
Eftc1
Tyw3
B
Kcnn1
BC035044
Mroh1
Inca1
Cdkn2b
Sfxn5
Ifitm6
Rrbp1
Smpd3
Ciart
Rbpms
Relative use of distal versus proximal CP site
**
Vector
U2AF35(S34F)
C
U2AF35(S34F)I
Hours after dox removal:
0
8
16
24
32
MYC
TUBA

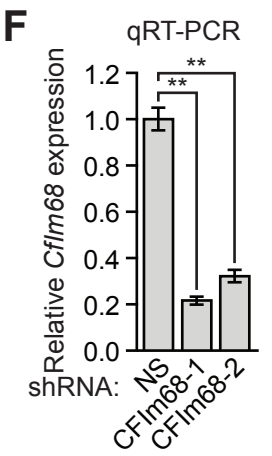

A
qRT-PCR
Relative CFIm59 expression
siRNA: Control CFIm59-1 CFIm59-2

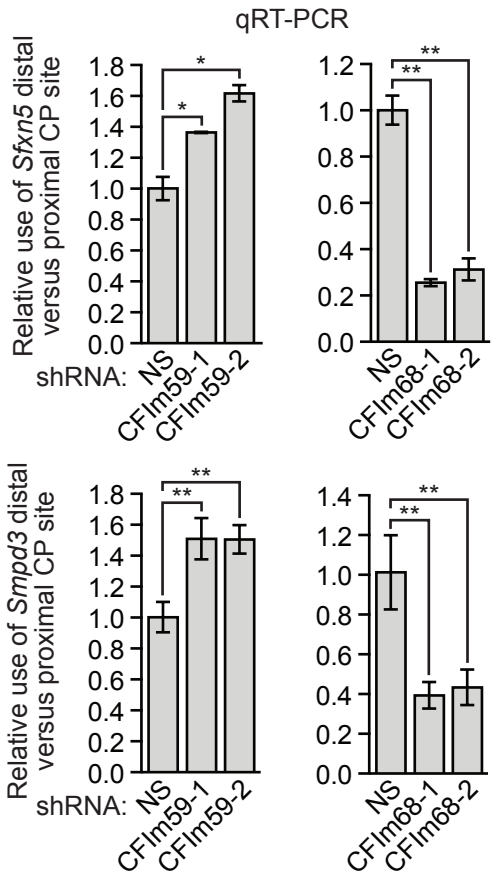

B
qRT-PCR
Relative CFIm68 expression
siRNA: Control CFIm68-1 CFIm68-2

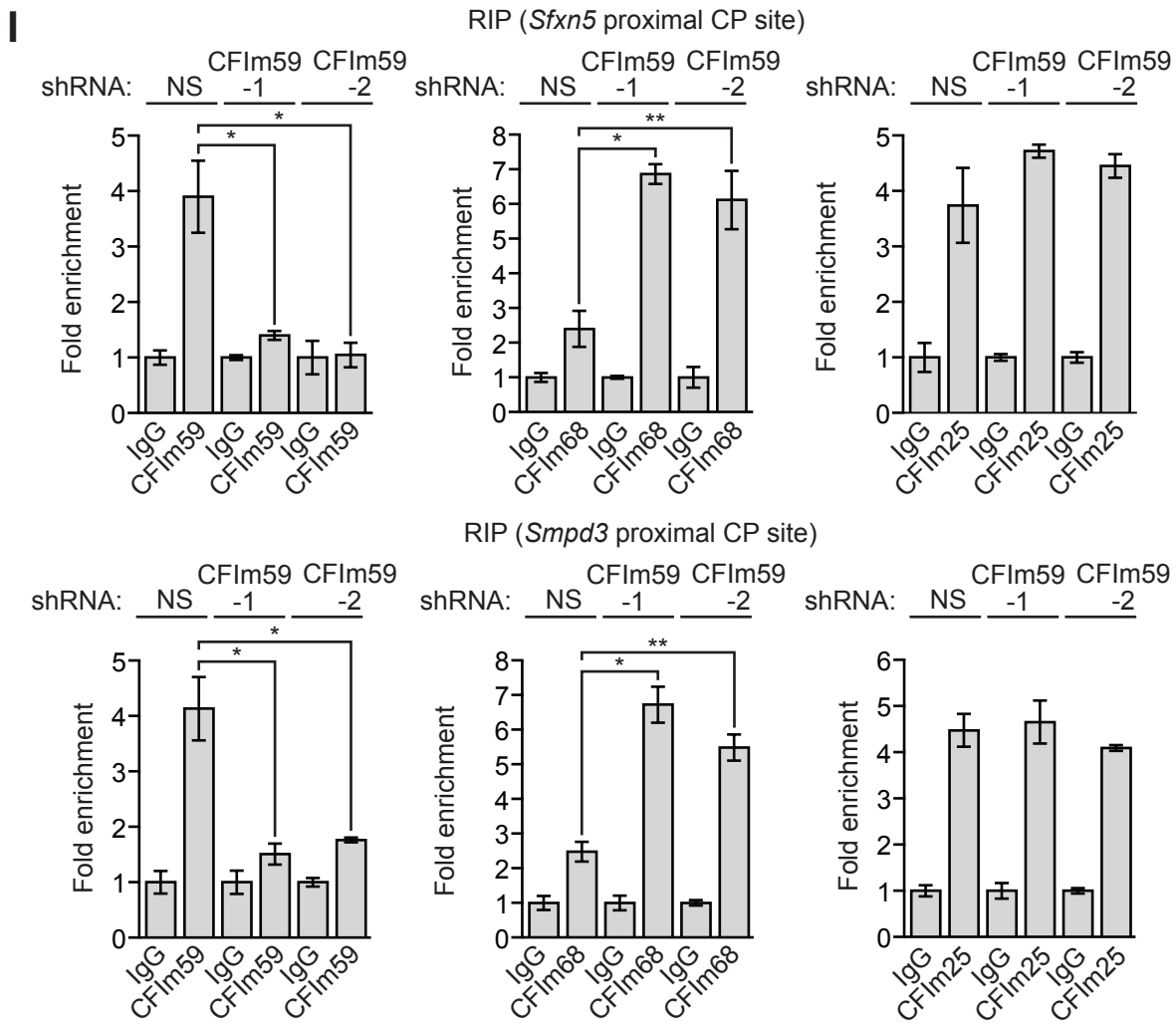

C
Input
α-Flag IP
MYC-U2AF35
MYC-U2AF35(S34F)
Flag:
Flag-CFIm68:
U2AF65
MYC-U2AF35
ACTB
Flag-CFIm68

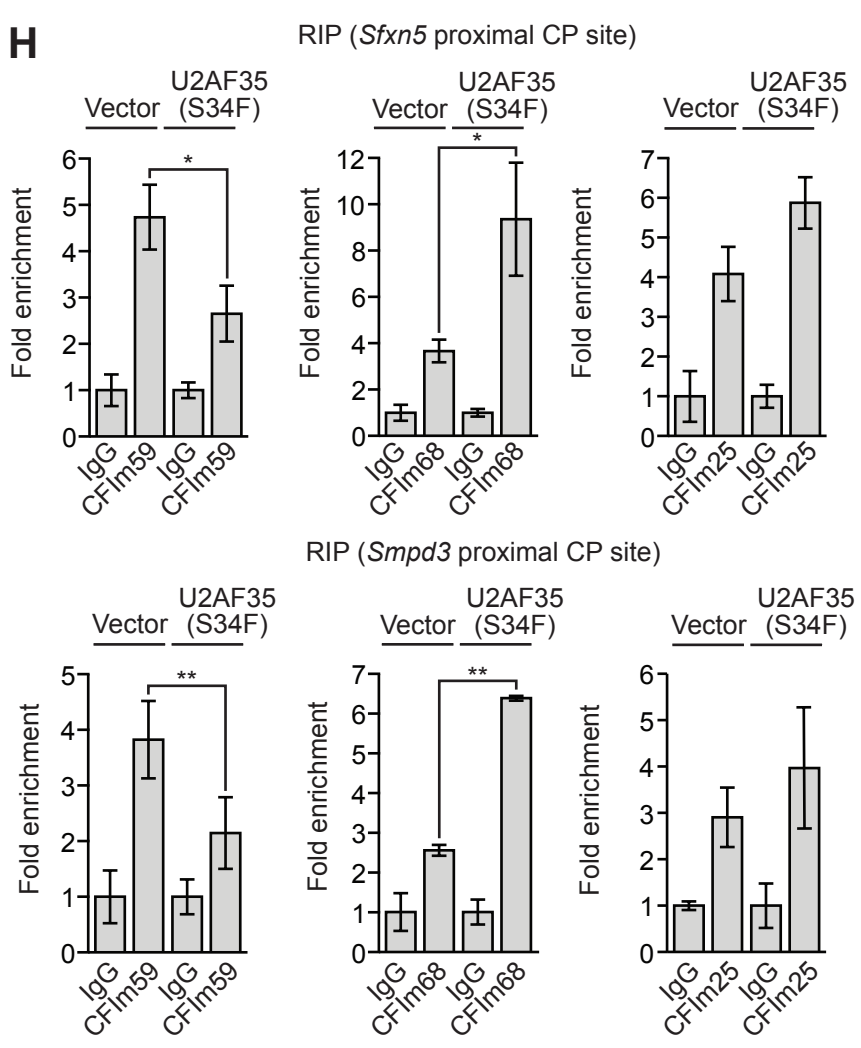

D
U2AF65
MYC-U2AF35
Relative signal
MYC-U2AF35
MYC-U2AF35(S34F)
MYC-U2AF35
MYC-U2AF35(S34F)

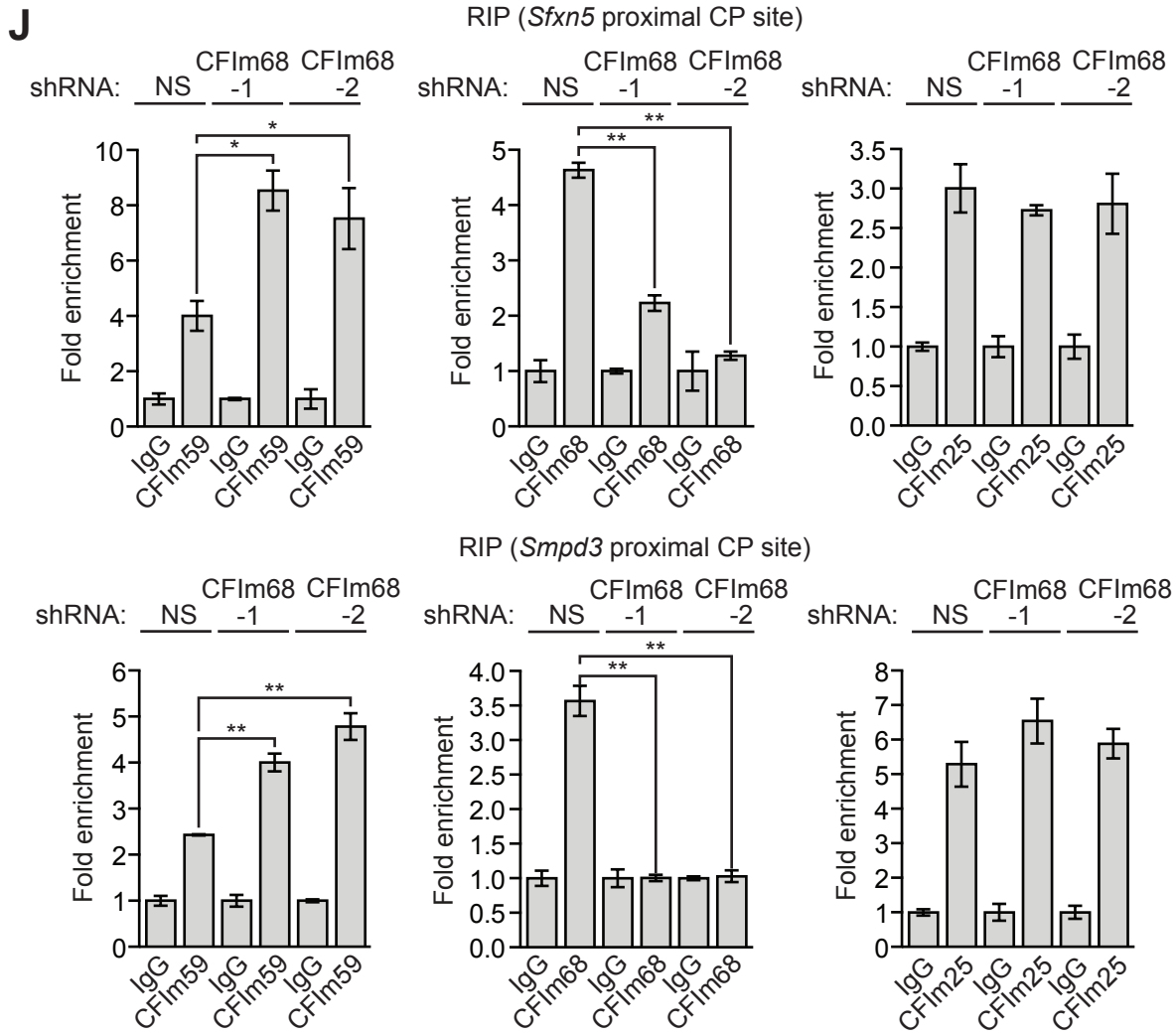

E
qRT-PCR
Relative CFIm59 expression
shRNA: NS CFIm59-1 CFIm59-2

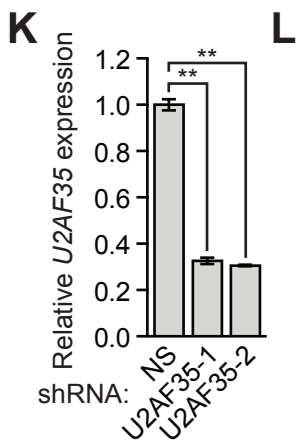

F
qRT-PCR
Relative Cfim68 expression
shRNA: NS CFIm68-1 CFIm68-2

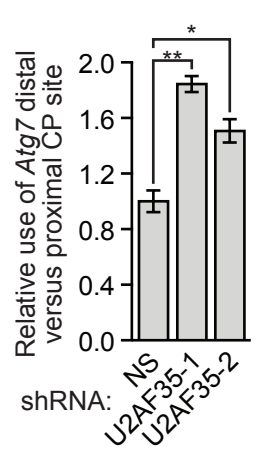

G
qRT-PCR
Relative use of Sfxn5 distal versus proximal CP site
shRNA: NS CFIm59-1 CFIm59-2
shRNA: NS CFIm68-1 CFIm68-2
Relative use of Smpd3 distal versus proximal CP site
shRNA: NS CFIm59-1 CFIm59-2
shRNA: NS CFIm68-1 CFIm68-2

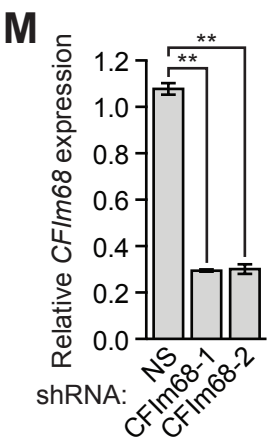

I
RIP (Sfxn5 proximal CP site)
shRNA: NS CFIm59 -1 CFIm59 -2
Fold enrichment
IgG CFIm59
IgG CFIm68
IgG CFIm25
RIP (Smpd3 proximal CP site)
shRNA: NS CFIm59 -1 CFIm59 -2
Fold enrichment
IgG CFIm59
IgG CFIm68
IgG CFIm25


H
RIP (Sfxn5 proximal CP site)
Vector U2AF35 (S34F)
Fold enrichment
IgG CFIm59
IgG CFIm68
IgG CFIm25
RIP (Smpd3 proximal CP site)
Vector U2AF35 (S34F)
Fold enrichment
IgG CFIm59
IgG CFIm68
IgG CFIm25


J
RIP (Sfxn5 proximal CP site)
shRNA: NS CFIm68 -1 CFIm68 -2
Fold enrichment
IgG CFIm59
IgG CFIm68
IgG CFIm25
RIP (Smpd3 proximal CP site)
shRNA: NS CFIm68 -1 CFIm68 -2
Fold enrichment
IgG CFIm59
IgG CFIm68
IgG CFIm25


K
Relative U2AF35 expression
shRNA: NS U2AF35-1 U2AF35-2


L
Relative use of Atg7 distal versus proximal CP site
shRNA: NS U2AF35-1 U2AF35-2


M
Relative CFIm68 expression
shRNA: NS CFIm68-1 CFIm68-2

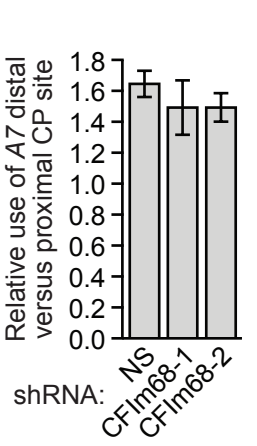
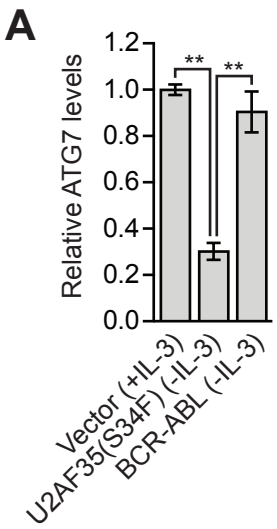
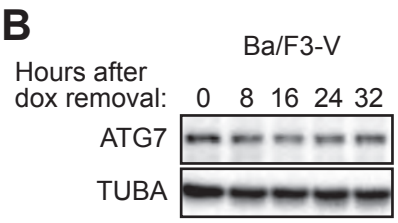
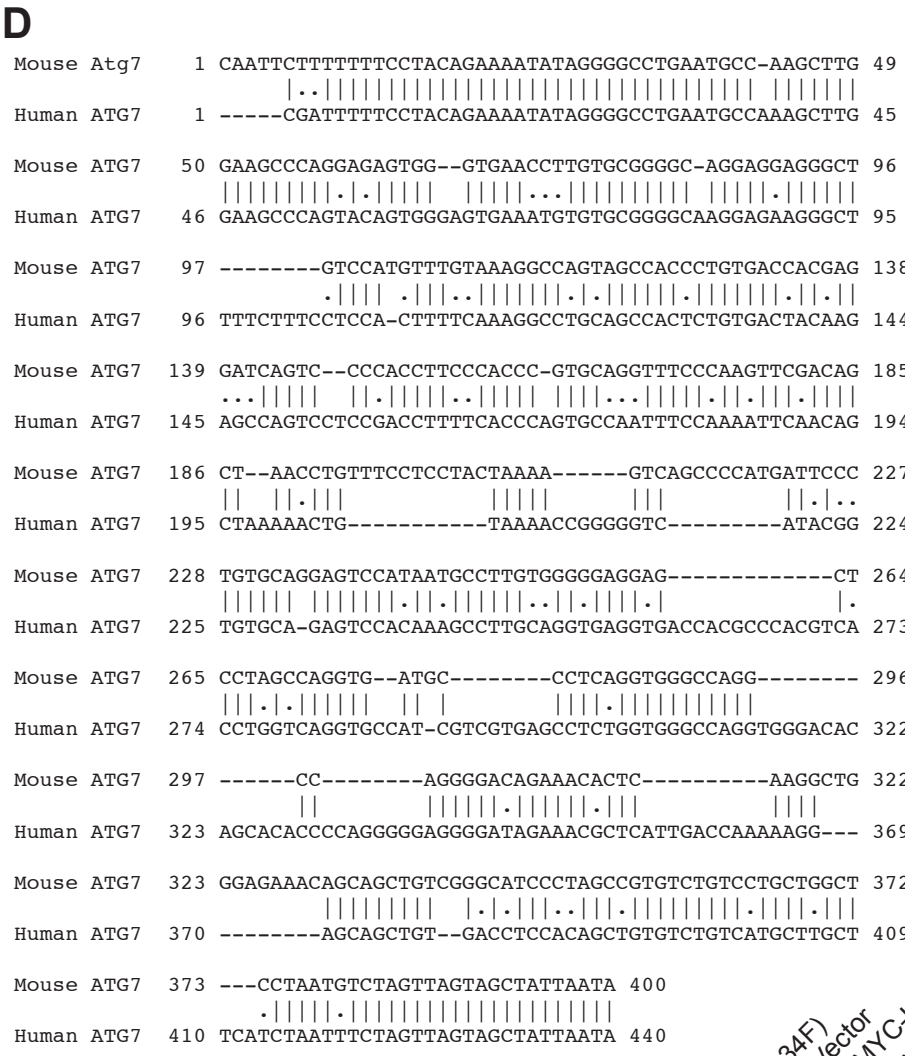
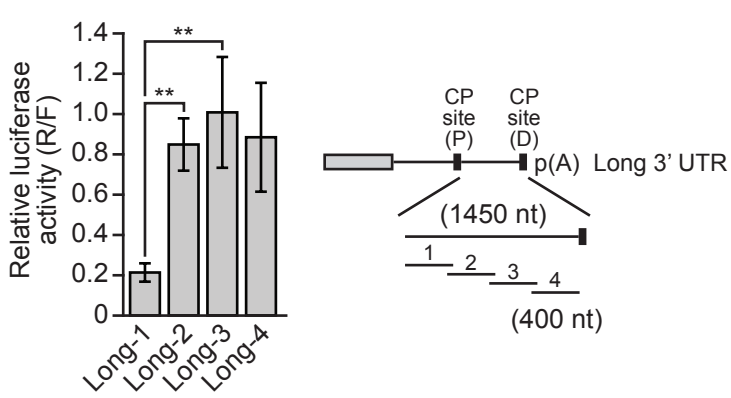

N
Relative use of A7 distal versus proximal CP site
shRNA: NS CFIm68-1 CFIm68-2

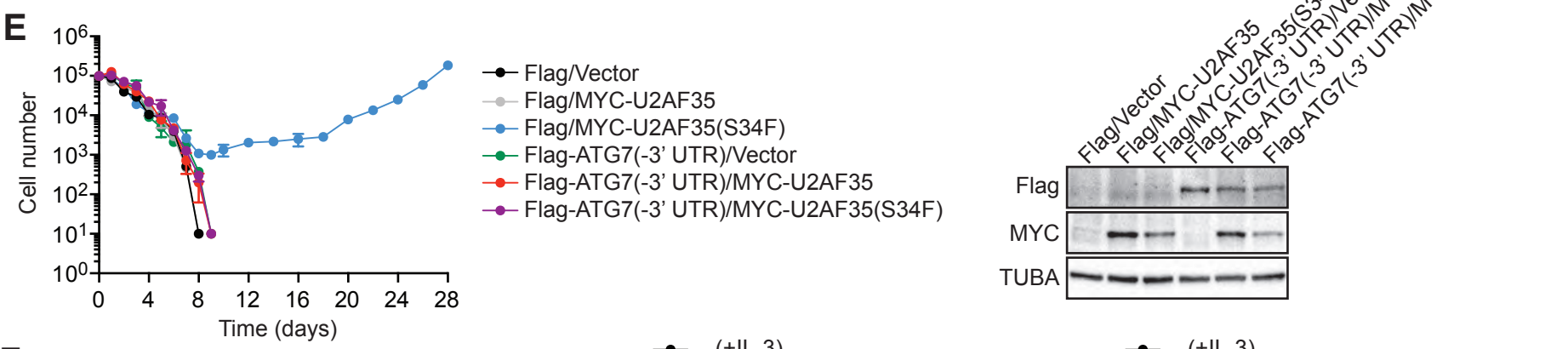
Figure S4

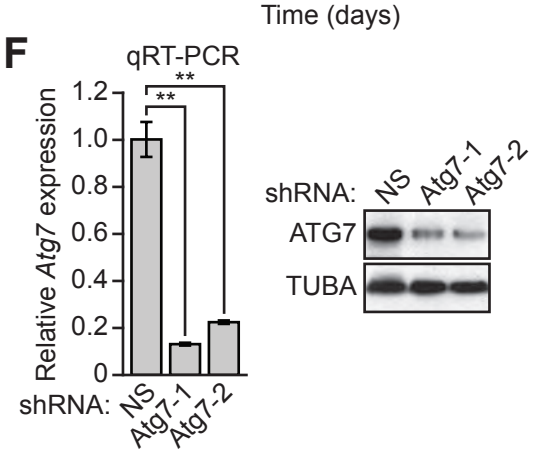

A
Relative ATG7 levels
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
**
Vector (+IL-3)
U2AF35(S34F) (-IL-3)
BCR-ABL (-IL-3)

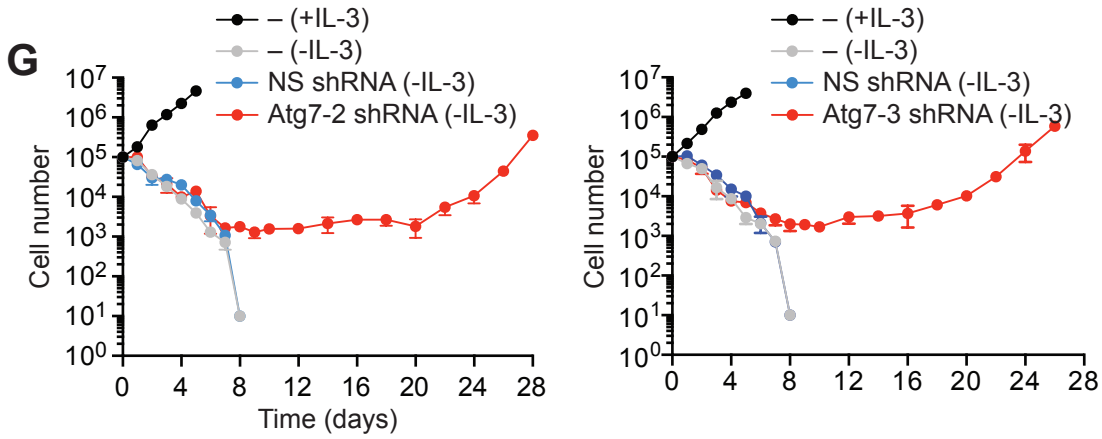

B
Ba/F3-V
Hours after dox removal:
0 8 16 24 32
ATG7
TUBA

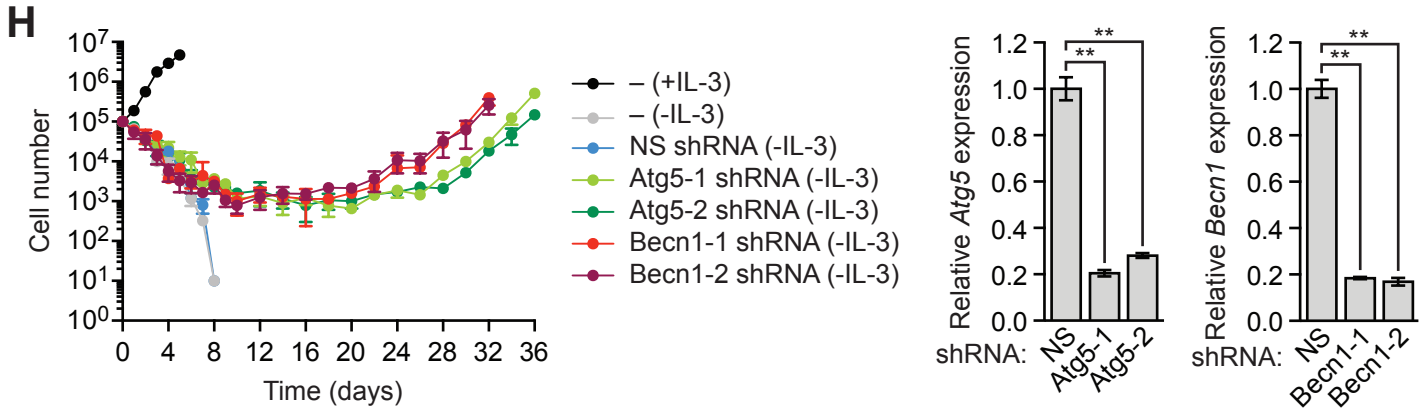

D
Mouse Atg7 1 CAATTCTTTTTTTCCTACAGAAAATATAGGGGCCTGAATGCC-AAGCTTG 49
Human ATG7 1 -----CGATTTTTCCTACAGAAAATATAGGGGCCTGAATGCCAAAGCTTG 45
Mouse ATG7 50 GAAGCCCAGGAGAGTGG--GTGAACCTTGTGCGGGGC-AGGAGGAGGGCT 96
Human ATG7 46 GAAGCCCAGTACAGTGGGAGTGAAATGTGTGCGGGGCAAGGAGAAGGGCT 95
Mouse ATG7 97 --------GTCCATGTTTGTAAAGGCCAGTAGCCACCCTGTGACCACGAG 138
Human ATG7 96 TTTCTTTCCTCCA-CTTTTCAAAGGCCTGCAGCCACTCTGTGACTACAAG 144
Mouse ATG7 139 GATCAGTC--CCCACCTTCCCCACCC-GTGCAGGTTTCCCAAGTTCGACAG 185
Human ATG7 145 AGCCAGTCCTCCGACCTTTTCACCCAGTGCCAATTTCCAAAATTCAACAG 194
Mouse ATG7 186 CT--AACCTGTTTCCTCCTACTAAAA------GTCAGCCCCATGATTCCC 227
Human ATG7 195 CTAAAAACTG----------TAAAACCGGGGGTC---------ATACGG 224
Mouse ATG7 228 TGTGCAGGAGTCCATAATGCCTTGTGGGGGAGGAG----------CT 264
Human ATG7 225 TGTGCA-GAGTCCACAAAGCCTTGCAGGTGAGGTGACCACGCCCACGTCA 273
Mouse ATG7 265 CCTAGCCAGGTG--ATGC--------CCTCAGGTGGGCCAGG-------- 296
Human ATG7 274 CCTGGTCAGGTGCCAT-CGTCGTGAGCCTCTGGTGGGCCAGGTGGGACAC 322
Mouse ATG7 297 ------CC--------AGGGGACAGAAACACTC----------AAGGCTG 322
Human ATG7 323 AGCACACCCCAGGGGGAGGGGATAGAAACGCTCATTGACCAAAAAGG--- 369
Mouse ATG7 323 GGAGAAACAGCAGCTGTCGGGCATCCCTAGCCGTGTCTGTCCTGCTGGCT 372
Human ATG7 370 --------AGCAGCTGT--GACCTCCACAGCTGTGTCTGTCATGCTTGCT 409
Mouse ATG7 373 ---CCTAATGTCTAGTTAGTAGCTATTAATA 400
Human ATG7 410 TCATCTAATTTCTAGTTAGTAGCTATTAATA 440

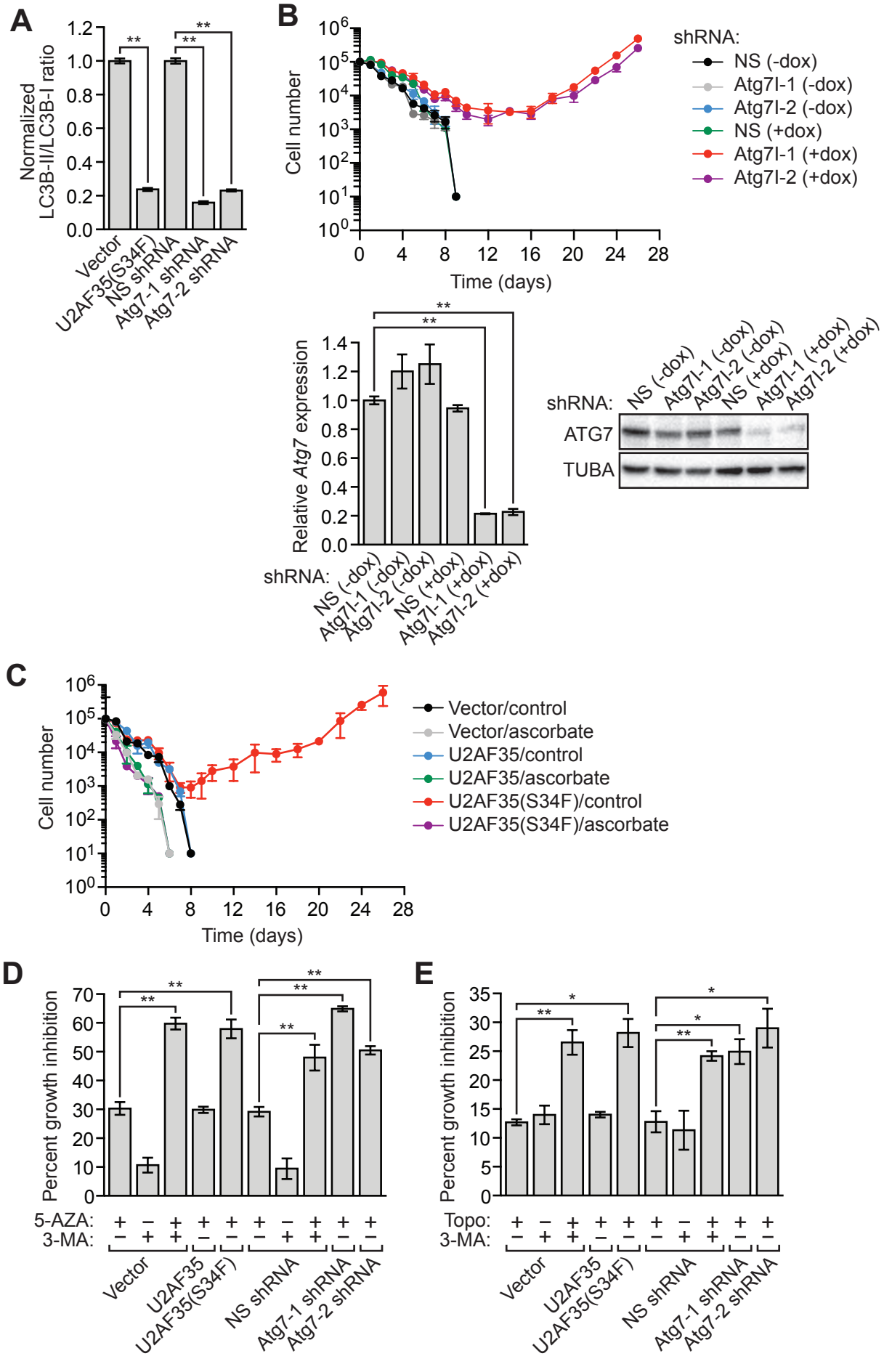

C
Relative luciferase activity (R/F)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
**
**
Long-1
Long-2
Long-3
Long-4
CP site (P)
CP site (D)
p(A)
Long 3' UTR
(1450 nt)
1
2
3
4
(400 nt)


E
Cell number
Time (days)
Flag/Vector
Flag/MYC-U2AF35
Flag/MYC-U2AF35(S34F)
Flag-ATG7(-3' UTR)/Vector
Flag-ATG7(-3' UTR)/MYC-U2AF35
Flag-ATG7(-3' UTR)/MYC-U2AF35(S34F)
Flag
MYC
TUBA


F
qRT-PCR
Relative Atg7 expression
shRNA: NS Atg7-1 Atg7-2
ATG7
TUBA


G
– (+IL-3)
– (-IL-3)
NS shRNA (-IL-3)
Atg7-2 shRNA (-IL-3)
Atg7-3 shRNA (-IL-3)
Cell number
Time (days)


H
Cell number
Time (days)
– (+IL-3)
– (-IL-3)
NS shRNA (-IL-3)
Atg5-1 shRNA (-IL-3)
Atg5-2 shRNA (-IL-3)
Becn1-1 shRNA (-IL-3)
Becn1-2 shRNA (-IL-3)
Relative Atg5 expression
Relative Becn1 expression
shRNA: NS Atg5-1 Atg5-2
shRNA: NS Becn1-1 Becn1-2


A
Normalized LC3B-II/LC3B-I ratio
Vector
U2AF35(S34F)
NS shRNA
Atg7-1 shRNA
Atg7-2 shRNA
B
Cell number
Time (days)
shRNA:
NS (-dox)
Atg7l-1 (-dox)
Atg7l-2 (-dox)
NS (+dox)
Atg7l-1 (+dox)
Atg7l-2 (+dox)
Relative Atg7 expression
ATG7
TUBA
C
Cell number
Time (days)
Vector/control
Vector/ascorbate
U2AF35/control
U2AF35/ascorbate
U2AF35(S34F)/control
U2AF35(S34F)/ascorbate
D
Percent growth inhibition
5-AZA:
3-MA:
E
Percent growth inhibition
Topo:
3-MA:

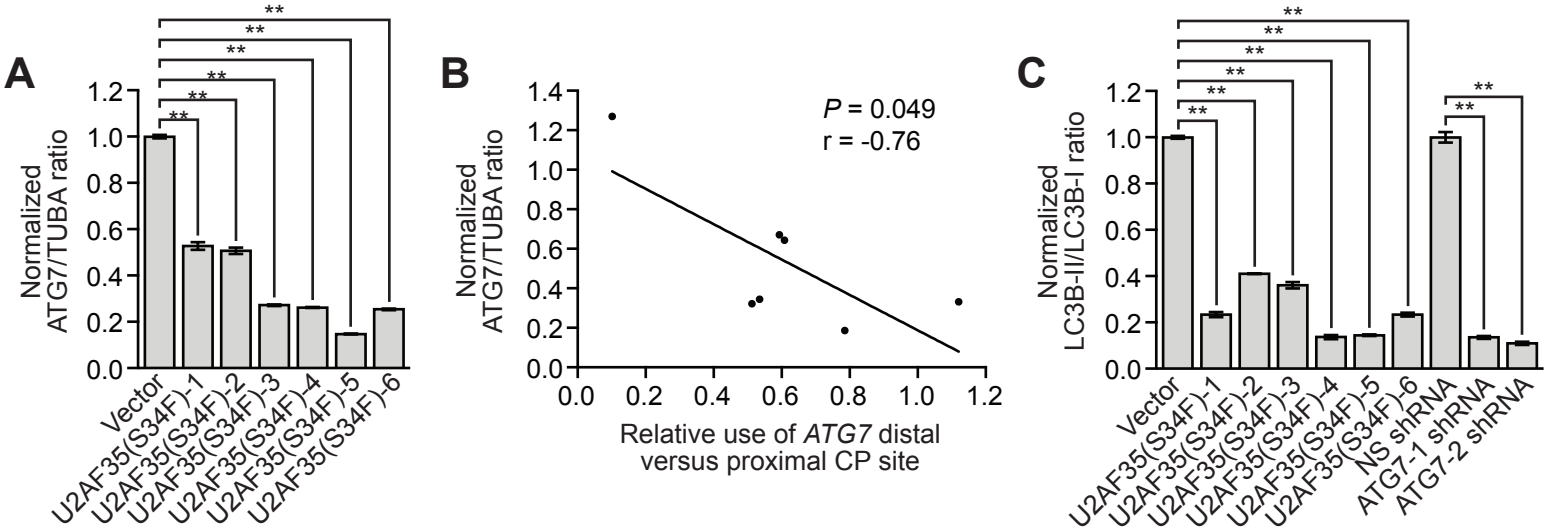
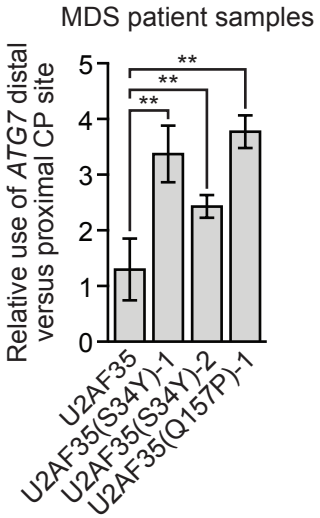

A
Normalized ATG7/TUBA ratio
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
Vector
U2AF35(S34F)-1
U2AF35(S34F)-2
U2AF35(S34F)-3
U2AF35(S34F)-4
U2AF35(S34F)-5
U2AF35(S34F)-6
B
Normalized ATG7/TUBA ratio
P = 0.049
r = -0.76
Relative use of ATG7 distal versus proximal CP site
C
Normalized LC3B-II/LC3B-I ratio
NS shRNA
ATG7-1 shRNA
ATG7-2 shRNA

Figure S7


MDS patient samples
Relative use of ATG7 distal versus proximal CP site
5
4
3
2
1
0
**
**
**
U2AF35
U2AF35(S34Y)–1
U2AF35(S34Y)–2
U2AF35(Q157P)–1

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Additional Experiments Related to Figure 1

(A) Immunoblot monitoring MYC-U2AF35 and U2AF35 levels in Ba/F3-V, Ba/F3-U2AF35 and Ba/F3-U2AF35(S34F) cells cultured in the presence of IL-3. α-tubulin (TUBA) was monitored as a loading control.

(B) Independent biological replicates of the experiment shown in Figure 1A. Proliferation of parental Ba/F3 cells cultured in the presence (+) or absence (-) of IL-3, and Ba/F3-V, Ba/F3-U2AF35, Ba/F3-U2AF35(S34F) or Ba/F3-BCR-ABL cells cultured in the absence of IL-3.

(C) Tumor formation in mice (n=5 mice per group) injected with Ba/F3-V, Ba/F3-U2AF35, or Ba/F3-U2AF35(S34F) cells that had not been pre-selected for IL-3-independent proliferation in culture. The results confirm that U2AF35(S34F) can directly transform cells without pre-selection for IL-3-independent growth. Error bars indicate SD. **P*<0.05; ***P*<0.01.

Figure S2. Identification and Validation of Pre-mRNAs Identified by the Modified DaPars Algorithm (Related to Figure 2)

(A) Radar plot showing the pre-mRNAs identified by the modified DaPars algorithm. The red line represents ΔPDUI (change in percentage distal polyadenylation site usage index between Ba/F3 and Ba/F3-U2AF35(S34F) cells). The modified DaPars algorithm identified novel distal sites (blue) as well as previously annotated sites (in the UCSC Genome Browser; red).

(B) qRT-PCR analysis monitoring the relative use of the distal versus proximal CP site of several candidate pre-mRNAs obtained using the DaPars algorithm in Ba/F3-U2AF35 or Ba/F3-U2AF35(S34F) cells. Error bars indicate SD. **P*<0.05; ***P*<0.01.

(C) Immunoblot analysis showing loss of MYC-U2AF35(S34F) expression in Ba/F3-U2AF35(S34F)I cells following doxycycline (dox) withdrawal.

Figure S3. Target Gene Expression Analysis (Related to Figure 3)

(A and B) Knockdown efficiencies of two unrelated siRNAs directed against CFIm59 (A) or CFIm68 (B) in Ba/F3 cells. The results were normalized to expression obtained with the control siRNA, which was set to 1.

(C) Co-immunoprecipitation analysis. FLAG-tagged CFIm68-bound beads were added to extracts from Ba/F3 cells expressing MYC-U2AF35 or MYC-U2AF35(S34F) in the presence of RNase. The beads were collected and washed, and the bound proteins were analyzed by immunoblotting for U2AF65, MYC-U2AF35 or FLAG-CFIm68. β-actin (ACTB) was monitored as a control. The results show that CFIm68 comparably interacts with U2AF35 and U2AF35(S34F).

(D) Quantification of the co-immunoprecipitation experiment shown in Figure 3B. The signal for U2AF65 or MYC-U2AF35 was quantified relative to that obtained for Flag-CFIm59. The level of U2AF65 or MYC-U2AF35 in the immunoprecipitate from cells MYC-U2AF35 was set to 1.

(E and F) Knockdown efficiencies of two unrelated shRNAs directed against CFIm59 (E) or CFIm68 (F) in Ba/F3 cells. The results were normalized to expression obtained with the non-silencing (NS) shRNA, which was set to 1.

(G) qRT-PCR monitoring relative use of the *Sfxn5* (top) or *Smpd3* (bottom) CP site in Ba/F3 cells expressing a NS, CFIm59 or CFIm68 shRNA.

(H-J) RIP assay monitoring binding of CFIm59, CFIm68 or CFIm25 to the *Sfxn5 or Smpd3* proximal CP site in Ba/F3-V or Ba/F3-U2AF35(S34F) cells (H), in Ba/F3 cells expressing a NS

or CFIm59 shRNA (I), or in Ba/F3 cells expressing a NS or CFIm68 shRNA (J). Binding was normalized to IgG, which was set to 1.
(K) Knockdown efficiencies of two unrelated shRNAs directed against U2AF35 in Ba/F3 cells.
(L) qRT-PCR monitoring relative use of the *Atg7* CP site in Ba/F3 cells expressing a NS or U2AF35 shRNA.
(M) Knockdown efficiencies of two unrelated shRNAs directed against CFIm68 in Ba/F3-U2AF35(S34F) cells.
(N) qRT-PCR monitoring relative use of the *Atg7* CP site in Ba/F3-U2AF35(S34F) cells expressing a NS or CFIm68 shRNA. Error bars indicate SD. **P*<0.05; ***P*<0.01.

Figure S4. Additional Experiments and Sequence Alignments Related to Figure 4
(A) Quantification of the levels of ATG7 protein in Ba/F3-V, Ba/F3-U2AF35(S34F) and Ba/F3-BCR-ABL cells. ATG7 protein levels were analyzed by immunoblotting in six independently-derived Ba/F3-U2AF35(S34F)-transformed cell lines, three independently-derived Ba/F3 cell lines transformed by BCR-ABL and three comparable untransformed control cell lines all run in parallel on the same gel, and the results were quantified relative to levels of α-tubulin (TUBA). This quantitative analysis, which includes the original blot shown in Figure 4A, demonstrates that expression of U2AF35(S34F) results in a statistically significant reduction of ATG7 protein levels.
(B) Immunoblot analysis monitoring ATG7 levels in Ba/F3-V cells following doxycycline withdrawal. The results show that doxycycline itself does not affect ATG7 protein levels.
(C) Luciferase reporter assay. Four fragments (400-nucleotides in length and tiled with a 50 nt overlap) of the 3' end of the long 3' UTR of *Atg7* were tested in a luciferase reporter assay. The results show that the repressive element is located in nt 1-350 of the 3' UTR.
(D) Multi-sequence alignment of the 3' UTR region of the mouse *Atg7* and human *ATG7* genes. The overall identity between the two sequences is 63.4%.
(E) (Left) Proliferation of Ba/F3 cells stably expressing a Flag-tagged *Atg7* derivative lacking the 3' UTR [ATG7(-3'UTR)] or, as a control, Flag alone and infected with a retrovirus expressing empty expression vector, U2AF35 or U2AF35(S34F). The results demonstrate that expression of *Atg7* lacking the 3' UTR blocks the ability of U2AF35(S34F) to transform cells. (Right) Immunoblot analysis monitoring levels of Flag-ATG7(-3'UTR) and MYC.
(F) qRT-PCR (left) and immunoblot (right) analysis monitoring *ATG7* knockdown efficiency in Ba/F3 cells using two unrelated shRNAs. The qRT-PCR results were normalized to that obtained with a control NS shRNA, which was set to 1.
(G) Independent biological replicates of the experiment shown in Figure 4E. Proliferation of parental Ba/F3, Ba/F3-NSshRNA or Ba/F3-Atg7-2shRNA cells.
(H) (Left) Proliferation of parental Ba/F3 cells cultured in the presence (+) or absence (-) of IL-3, and Ba/F3-NSshRNA, Ba/F3-Atg5shRNA, or Ba/F3-Becn1shRNA cells cultured in the absence of IL-3. (Middle and right) qRT-PCR analysis monitoring knockdown efficiencies of *Atg5* and *Becn1* shRNAs. These results show that depletion of other essential autophagy factors also transform Ba/F3 cells, consistent with our conclusion that transformation by U2AF35(S34F) is due to promotion of defective autophagy and not some putative non-autophagic function of ATG7. Error bars indicate SD. **P*<0.05; ***P*<0.01.

Figure S5. Additional Experiments Related to Figure 5

(A) Quantification of the LC3B-II/LC3B-I ratio in Ba/F3-V, Ba/F3-U2AF35(S34F), Ba/F3-NSshRNA or Ba/F3-Atg7shRNA cells. The results were normalized to those obtained in Ba/F3-V or Ba/F3-NSshRNA cells, which was set to 1.

(B) (Top) Proliferation of Ba/F3 cells expressing a NS shRNA or a doxycycline-inducible *Atg7* shRNA and cultured in the absence (-) or presence (+) of doxycycline (dox). The results show that transient depletion of *Atg7* promotes transformation. (Bottom) qRT-PCR analysis monitoring *Atg7* mRNA levels (left) or immunoblot analysis monitoring ATG7 protein levels (right) in Ba/F3 cells expressing a NS shRNA or a doxycycline-inducible *Atg7* shRNA and cultured in the absence (-) or presence (+) of doxycycline.

(C) Proliferation of Ba/F3-V, Ba/F3-U2AF35 or Ba/F3-U2AF35(S34F) cells treated with water (control) or the anti-oxidant ascorbate. Briefly, 1x105 cells were plated in the absence of IL-3 and in the presence of ascorbate (250 μM final concentration). The results show that ascorbate suppresses the ability of U2AF35(S34F) to promote transformation, demonstrating that the increase in ROS production is critical for U2AF35(S34F)-mediated transformation.

(D and E) Proliferation of Ba/F3-V, Ba/F3-U2AF35, Ba/F3-U2AF35(S34F), Ba/F3-NSshRNA or Ba/F3-Atg7shRNA cells treated with 5-azacytine (5-aza) (D) or topotecan (topo) (E) or, as a positive control, the autophagy inhibitor 3-methyladenine (3-MA), or both. The results show that Ba/F3-U2AF35(S34F) and Ba/F3-Atg7shRNA cells are more sensitive to 5-azacytidine and topotecan compared to their control, non-transformed counterparts. Error bars indicate SD. **P*<0.05; ***P*<0.01.

Figure S6. Additional Experiments Related to Figure 6

(A) Quantification of ATG7 protein levels in SA cells expressing vector or U2AF35(S34F). The results were normalized to those obtained in vector control cells, which was set to 1.

(B) Pearson correlation analysis showing significant negative correlation ($P = 0.49$, $r = -0.76$) between the levels of *Atg7* distal CP site usage (Figure 6D) and the levels of ATG7 protein in the different SA/U2AF35(S34F)-expressing clones (Figures 6E and S6A).

(C) Quantification of the LC3B-II/LC3B-I ratio in SA-V, SA-U2AF35(S34F), SA-NSshRNA or SA-ATG7shRNA cells. The results were normalized to those obtained in Ba/F3-V or Ba/F3-NSshRNA cells, which was set to 1. Error bars indicate SD. **P*<0.05; ***P*<0.01.

Figure S7. Increased Selection of the ATG7 Distal CP site in MDS Patient Samples Harboring Other U2AF35 Mutants (Related to Figure 7)

qRT-PCR monitoring *ATG7* pre-mRNA CP site use in MDS patient samples containing U2AF35(S34Y) or U2AF35(Q157P) relative to wild-type U2AF35, which was set to 1. The average value for patient samples containing wild-type U2AF35 is the same as that shown in Figure 7A. Error bars indicate SD. **P*<0.05; ***P*<0.01.

Table S1. List of Genes, Identified by RNA-Seq Analysis, Whose Splicing is Significantly Altered upon Expression of U2AF35(S34F) (Related to Figure 2)

See attached Excel table.

Table S2. List of Pre-mRNAs Identified by the Modified DaPars Algorithm (Related to Figure 2)

See attached Excel table.

Table S3. List of MDS Patient Samples and Characteristics (Related to Figure 7)

Sample	Diagnosis *	U2AF35 mutation	Age at sample collection (years)	Sex	Karyotype	Survival status
U2AF35-1	MDS	–	69	Male	46,XY[20]	Deceased
U2AF35-2	MDS	–	64	Male	46,XY,t(2;11)(p21;q23)[20]	Deceased
U2AF35-3	MDS	–	78	Male	46,XY[20]	Deceased
U2AF35-4	MDS	–	77	Male	46,XY[30]	Deceased
U2AF35-5	AML	–	71	Male		Deceased
U2AF35-6	MDS	–	69	Female	46,XX,del(20)(q11.2q13.1)	Deceased
U2AF35-7	AML	–		Female	46,XX[20]	Deceased
U2AF35-8	MDS	–	71	Male	44,XY,del(5)(q11.2q33),-7,add(13)(q34),-17[7]/43,sl,-13,-14,-22,+mar1,+mar2[4]/42,sdl1,-18[2]/42,sl,-14,-18,-20,-20,-22,+mar4,+mar5,+mar6[5],41,sdl2,-18[2]	Deceased
U2AF35-9	MDS	–	56	Male	46,XY,del(7)(q31)[5]/46,XY[15]	Deceased
U2AF35-10	MDS	–	71	Male	44 46,XY,del(4)(q27q35)[2],del(5)(q13q33)[18],-7[18],add(14)(p11.2)[4],del(17)(p11.2)[18],-18[2],add(19)(p13.3)[4],-21[4],i(21)(q10)[14],+mar[11][cp18]/46,XY[2]	Deceased
U2AF35-11	CMML	–	72	Female	47,XX,+8[13]/46,XX[7]	Deceased
U2AF35-12	AML	–	70	Male	46,XY[20]	Deceased
U2AF35-13	MDS	–	77	Male	46,XY,del(5)(q22q34),del(12)(q15q22)[2]/46,idem,-17,+mar[2]/45,idem,add(11)(p15),-17,+mar[4]/45,idem,add(11)(p15),-17[12]	Deceased
U2AF35-14	CMML	–	71	Male	46,XY[20]	Deceased
U2AF35(S34F)-1	MDS	S34F	75	Female	46,XX[20]	Deceased
U2AF35(S34F)-2	MDS	S34F	58	Male	46,XY[20]	Deceased
U2AF35(S34F)-3	MDS	S34F	64	Male	46,XY,add(5)(q22)[14]/46,XY[6]	Deceased
U2AF35(S34F)-4	MDS	S34F	87	Male	47,XY,+8[17]/46,XY[3]	Deceased
U2AF35(S34F)-5	MDS	S34F	68	Male	46,XY[20]	Alive
U2AF35(S34F)-6	MDS	S34F	73	Male	47,XY,+8[11]/45,X,-Y[4]/46,XY[5]	Deceased
U2AF35(S34F)-7	MDS	S34F	79	Male	46,XY,del(20)(q11.2q13.1)[5]/46,idem,dup(9)(p13p22)[10]/46,idem,add(3)(q26.2)[4]/46,XY[5]	Deceased
U2AF35(S34F)-8	MDS	S34F	83	Male	46,XY[24]	Deceased
U2AF35(S34F)-9	MDS	S34F	72	Male	47,XY,+Y,del(5)(q15q33)[12]/47,idem,del(	Deceased

					17)(p13.1)[8]	
U2AF35(S34F)-10	MDS	S34F	90	Male	46,XY[20]	Deceased
U2AF35(S34F)-11	MDS	S34F	70	Male	46,XY[20]	Alive
U2AF35(S34Y)-1	MDS	S34Y	74	Male	47,XY,+8[7]/46,XY[13]	Alive
U2AF35(S34Y)-2	MDS	S34Y	65	Male	46,XY[20]	Deceased
U2AF35(Q157P)-1	MDS	Q157P	73	Male	46,XY[20]	Alive

*MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia

Table S4. List of Samples Obtained from the TCGA LAML Dataset, and their U2AF35(S34F) Mutational Status (S34F, U2AF35(S34F); WT, wild-type) (Related to Figure 7)

See attached Excel table.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Cell Lines and Culture

Ba/F3 cells were grown in RPMI-1640 medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS) (Atlanta Biologicals) and 5 ng/ml murine IL-3 (PeproTech). SA cells (provided by Scott Randell, University of North Carolina School of Medicine) were grown in BEGM medium (Lonza).

To generate Ba/F3 and SA cell lines stably expressing either MYC-U2AF35 or MYC-U2AF35(S34F), human U2AF35 cDNA was PCR amplified from pET15b-U2AF35 (Shen et al., 2010) using forward (5'-ATACTCGAGGATCCGCCATGGAGCAGAAGCTGATCTCCGAGGAGGACCTGAACGCGGAGTATCTGGCCTCCAT-3') and reverse (5'-ATAGAATTCTCAGAATCGCCCAGATCTTTCA-3') primers (where underlining indicates the MYC epitope taq sequence). The PCR product was then digested with XhoI and EcoRI and cloned into the retroviral vector pMSCV-IRES-Blasticidin, which was generated by inserting IRES (PCR amplified from pMXs-IRES-GFP [Cell Biolabs] and digested with EcoR1 and NcoI) and the blasticidin-resistance gene (sub-cloned from pcDNA6/myc-HisA [Invitrogen] using NcoI and ClaI, and modified for codon optimization) into pMSCV-puro (Clonetech), to generate pMSCV-MYC-U2AF35-IRES-Blasticidin. The U2AF35(S34F) mutant was generated by site-directed mutagenesis of pMSCV-MYC-U2AF35-IRES-Blasticidin using forward (5'-TGTCGTCATGGAGACAGGTGCTTTCGGTTGCACAATAAACCGACG-3') and reverse (5'-CGTCGGTTTATTGTGCAACCGAAAGCACCTGTCTCCATGACGACA-3') primers (where underlining indicates the mutated residue) to create pMSCV-MYC-U2AF35(S34F)-IRES-Blasticidin. The retroviral vectors were then stably transduced into Ba/F3 or SA cells. Ba/F3-BCR-ABL cells were established using the previously described pMSCV-BCR-ABL-IRES-GFP retroviral clone (Li et al., 1999).

To construct a tetracycline-inducible lentiviral vector, pMSCV-MYC-U2AF35-IRES-Blasticidin or pMSCV-MYC-U2AF35(S34F)-IRES-Blasticidin was treated with BamHI-Klenow-EcoRI, and the resulting fragment was cloned into pTRIPZ (GE Dharmacon) that had been digested with AgeI-Klenow-EcoRI.

To construct a Ba/F3 cell line stably expressing Flag-ATG7(-3' UTR), mouse *Atg7* cDNA (MGC clone 5368226) was PCR amplified using forward (5'-ATACTCGAGGATCCGCCATGGACTACAAAGACGATGACGACAAGGGGGACCCTGGACTGG-3') and reverse (5'-ATAGAATTCTCAGACAGTCTCCTCGTCACTC-3') primers (where underlining indicates the Flag epitope taq sequence). The PCR product was then digested with XhoI and EcoRI and cloned into the retroviral vector pMSCV-puro (Clonetech) to generate pMSCV-puro-Flag-mATG7. To generate cell lines stably co-expressing Flag-ATG7 and U2AF35 or U2AF35(S34F), Ba/F3 cells were infected with retroviruses expressing pMSCV-puro-Flag or pMSCV-puro-Flag-mATG7, followed by retroviral infection with pMSCV-IRES-Blasticidin, pMSCV-MYC-U2AF35-IRES-Blasticidin or pMSCV-MYC-U2AF35(S34F)-IRES-Blasticidin.

To construct a tetracycline-inducible lentiviral vector expressing *Atg7* shRNA, a pGIPZ-based *Atg7* shRNA clone (V2LMM_218931 or V2LMM_220588) was digested with XhoI and MluI, and the resulting fragment was cloned into pTRIPZ (GE Dharmacon) to generate pTRIPZ-Atg7shRNA. For proliferation assays, Ba/F3 cells expressing pTRIPZ-Atg7shRNA were cultured in 2 μg/ml doxycycline for 30 days to induce shRNA expression, and then doxycycline and IL-3 were removed to test for IL-3 independent growth.

Tumor Formation Assays

All animal protocols were approved by the Institution Animal Care and Use Committee at UMMS (A-2247). For the assays shown in Figure 1C, 4G and 6C, Ba/F3 or SA cells (6×10^6) expressing either vector, U2AF35(S34F), NS shRNA or Atg7/ATG7 shRNA were suspended in 100 µl Matrigel (BD Biosciences) and PBS mix (1:1) and injected subcutaneously into the right flank of 5–6 week old athymic BALB/c (nu/nu) male mice (Taconic Farms). Tumor dimensions were measured every 4 days for ~3 weeks and tumor volume was calculated using the formula $\pi/6 \times (\text{length}) \times (\text{width})^2$. For the assay using non-transformed cells shown in Figure S1C, 1×10^7 non-transformed Ba/F3 cells were suspended in BD Matrigel Matrix High Concentration (BD Biosciences) prior to injection into mice.

RNA-Seq

Total RNA from parental Ba/F3 or Ba/F3-U2AF35(S34F) cells was isolated using TriPure Isolation Reagent (Roche), and mRNA purification, cDNA synthesis, and amplification were carried out according to the TrueSeq RNA sample preparation guide (Illumina). Libraries were sequenced as 100-bp paired end using Illumina HiSeq 2000. All reads were mapped to the mouse genome (mm10) using TopHat, followed by running Cufflinks to assemble and quantify the transcriptome (Trapnell et al., 2012). Alternative splicing events were first identified using Cuffdiff2 and further analyzed as follows. Gene-level normalized isoform expression (NI) is defined as the ratio of the isoform level to the gene level [isoform FPKM / gene FPKM]. Relative difference of NI (RDni), a differential alternative splicing event measurement, was calculated as [NI for Ba/F3-U2AF35(S34F) / NI for Ba/F3]. Absolute difference of NI (ADni) was calculated as [NI for Ba/F3-U2AF35(S34F) − NI for Ba/F3] to indicate the magnitude of the alternative splicing. Isoforms with RDni >1.2 or <1/1.2, and ADni >0.1, and gene level expression greater than median FPKM in both conditions (Table S1) were selected for further validation. Alternative CP site use was evaluated using Identification of novel alternative polyadenylation sites (InPAS, version 0.0.9; http://www.bioconductor.org/packages/release/bioc/html/InPAS.html), the details of which will be published separately.

qRT-PCR

Total RNA was isolated using TriPure Isolation Reagent (Roche) and treated with Turbo DNA-free kit (Ambion). Reverse transcription was performed using SuperScript II Reverse Transcriptase (Invitrogen) and oligodT primers followed by qRT-PCR using Fast SYBR Green Master Mix (Applied Biosystems) by StepOne Plus system (Applied Biosystems). Gene-specific primers are listed below. The expression level of each gene was normalized to that of *GAPDH*.

Gene	**Forward primer sequence (5'->3')**	**Reverse primer sequence (5'->3')**
Atg7	AGCAGTGATGACCGCATGAA	TCAGCAGCTTGGGTCTCTTG
m*Atg7*_proximal CP	GCTGCTGAGATCTGGGACAT	GAGATGTGGAGATCAGGACCAG
m*Atg7*_distal CP	TCTGTACATTCTTTACGCACAG C	CCACTCTCCTGGGCTTCCAA
h*ATG7*_proximal CP	GCTGCTGAGATCTGGGACAT	CAGAGGGGGGAATCCCA
h*ATG7*_distal CP	GGGCATCGTCTTTCCTGCTA	TGGCTACTTTGGGAGAAGCG
Atg5	TTCATCCAGAAGCTGTTCCG	ATTGGCTCTATCCCGTGAATC
Becn1	GTACCGACTTGTTCCCTATGG	ACACAGTCCAGAAAAGCTACC

*BC035044*_proximal CP	CACGAACCTGGGAGACTTGA	CAAGAACCAGCGGTGTCCAA
*BC035044*_distal CP	CCAGGCATGGCATGTGACTA	ATCCACAGGCACAAGGTAGC
*Cdkn2b*_proximal CP	CCTTTCAGGACGCGGTGTAA	AAGGTACTGACTGCACCCAC
*Cdkn2b*_distal CP	GTTACCTTGGGCTACTCCCC	CCCCAGGGCACCCATAATTT
CFIm59	AGAAGCAGTAGCACGGAACC	CACCAGGAGAAGCTGCCTAC
CFIm68	TCGAGTCCAAGTCCTATGGGT	ATGGCGACGACTCTTTTCCC
*Ciart*_proximal CP	CCACCAACTCCACTGTCTCC	GGACAGGCGGACATAGGATG
*Ciart*_distal CP	CTCGCTGAGCAACTGACAAGA	CCCACAGGTTTTGTACCATCC
Gapdh	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG
*Ifitm6*_proximal CP	CAGGGACCGGAAGATGGT	GAAGATGAGGGAGATGACCAG
*Ifitm6*_distal CP	TCCAGGGATAGAGGACCAGC	CCGAAGGCCACCATAGAGTT
*Inca1*_proximal CP	TAGGCCAGCAGGGGACTTAT	CATCGCCTGGGGATCGTAAA
*Inca1*_distal CP	GGAAGGGGAATGAAAAGGCG	GGCAAAGGAGAGTGCTTGAG
*Kcnn1*_proximal CP	AAACATTTGGGCGTGGAGGA	CCGGTGATGCACAGACCTTA
*Kcnn1*_distal CP	CCTCGGTCAGAGGACACATT	GTCCCTAGACACCCGGACTT
*Mroh1*_proximal CP	GCTGGGCAGCCATAGAGTAG	TGGCAGGTGACTGTGGTAAC
*Mroh1*_distal CP	TTGCCTGTGCTTTCCTGACT	TGGGAAGCTCCCTGATTTCG
*Rbpms*_proximal CP	TTGCTGAAGTCCAAGGGACG	TGACAGACCGAAGCCTGGTA
*Rbpms*_distal CP	GTGTGGCGATCACCTCAACT	AGCAGCGCAGAAGGAATGAT
*Rrbp1*_proximal CP	AGGCCACATGGGACACAAAT	ACCCAATTTTATGCCCCTACAGT
*Rrbp1*_distal CP	ATTGTCAGACCAAAGCAGGC	CAACCATCAGGGCCATACTGA
*Sfxn5*_proximal CP	ACCTCCGTCAGTAGGGAGAC	GGCCCAGAGATGCACTTGAT
*Sfxn5*_distal CP	AAATGGCCACCGAACCAAGA	CTTAGCATGGACCTGCCTCC
*Smpd3*_proxima CP	AGTACGAGGACCGGGTTTCT	GGCATGCGAGACAACATTGG
*Smpd3*_distal CP	GTCCCTCCCGGATTGTTTCA	AAGTGCAGATTCCCAGTCGG
U2AF35	ATGAACGTCTGCGACAACCT	CTCTGCATCCTCCTCACGTC

Northern Blot Analysis

Poly(A) RNA was extracted from Ba/F3-V or Ba/F3-U2AF35(S34F) cells using PolyATtract mRNA Isolation System IV (Promega) and analyzed by northern blot analysis as previously described (Green and Sambrook, 2012). Briefly, 5 µg of poly(A) RNA was separated on a 1% formaldehyde agarose gel and soaked in 75 mM NaOH. The fractionated RNA was transferred onto Zeta-Probe GT Membranes (Bio-Rad) and crosslinked by UV irradiation. The blots were hybridized with a ^{32}P-labeled probe specific for mouse *Atg7* short 3' UTR ($2x10^8$ cpm/µg; the DNA used to prepare the probe was amplified from BAC clone RP24-316O8 using forward [5'-ACCTCCCTGGTCCTGATCTC-3'] and reverse [5'-GCTGGACACACCCATCCTAC-3'] primers) or *Gapdh* ($1x10^6$ cpm/µg; the DNA used to prepare the probe was amplified using forward [5'-TCCATGACAACTTTGGCATTGTGG-3'] and reverse [5'-GTTGCTGTTGAAGTCGCAGGAGAC-3'] primers) in ExpressHyb Hybridization Solution (Clontech) for 3 h at 68°C. Membranes were washed twice in 1x SSC-0.1% sodium dodecyl sulfate (SDS) for 10 min at room temperature and 0.1x SSC-0.1% SDS for 10 min at 68°C, and exposed for 24-72 h.] Signals were visualized by phosphorimager using a Fujifilm FLA-7000 imaging system and quantified using Image Gauge v4.22 software.

RNA Interference

For siRNA-mediated knockdown of *CFIm59* or *CFIm68*, Ba/F3 cells were transfected with 100 nM of either the *CFIm59* or *CFIm68* siRNA (see below) or control siRNA (Sigma-Aldrich). For siRNA-mediated knockdown of U2AF35, Ba/F3 cells were transfected with 200 nM of U2AF35 siRNA (see below) and 36 h later transfected again.

siRNA	Sense (5'→3')	Antisense (5'→3')
CFIm59-1	GCAUAGAGGCCAAGUCAUAUU	UAUGACUUGGCCUCUAUGCUU
CFIm59-2	GAAGCGAAACAGAGCAAUUUU	AAUUGCUCUGUUUCGCUUCUU
*CFIm68-1**	CAUAGUAGAUCACGGGAAAUU	UUUCCCGUGAUCUACUAUGUU
	GGAAAUGGAUACUGCAAGAUU	UCUUGCAGUAUCCAUUUCCUU
	GCAGAGAACGAGAGAGACAUU	UGUCUCUCUCGUUCUCUGCUU
*CFIm68-2**	CCAAACGUUGUGUACACUUTT	AAGUGUACACAACGUUUGGTT
	GCCCUUGUUGGUGUUGGAUTT	AUCCAACACCAACAAGGGCTT
	GCAGUUCCUGAGUCAAUUUTT	AAAUUGACUCAGGAACUGCTT
U2AF35-1	AGCCGTGATCGACTTGAAUTT	ATTCAAGTCGATCACGGCTTdT
U2AF35-2	CUUGAAUAACCGUUGGUUUAAUG dGdA	UCCAUUAAACCAACGGUUAUUCAAGUC
Control	UAACUGUAUAAUCGACUAGTT	CUAGUCGAUUAUACAGUUAGA

* Consists of a pool of three duplexes.

To construct Ba/F3 cell lines stably expressing an *Atg7* shRNA, a lentiviral plasmid containing mouse *Atg7* shRNA (Open Biosystems/GE Dharmacon; see clone IDs below) was transfected into HEK293T cells using Effectene (QIAGEN) according to the manufacturer's instructions. Viral supernatants were collected 48 h later and used to infect Ba/F3 cells followed by selection using 1 μg/ml puromycin.

shRNA	Clone ID
Atg7-1	TRCN0000092164
Atg7-2	TRCN0000092166
Atg7-3	V2LMM_194162

To construct Ba/F3 cell lines stably expressing a *CFIm59* or *CFIm68* shRNA, the cloning vector pLKO.1-TRC (Addgene) was digested with AgeI and EcoRI, and ligated to annealed oligos listed below. Constructs were transfected into HEK293T cells and viral supernatants collected as described above.

shRNA	Forward sequence (5' → 3')*	Reverse sequence (5' → 3')
CFIm59-1	CCGGTGGCATAGAGGCCAAGTCATACTCG AGTATGACTTGGCCTCTATGCCATTTTTG	AATTCAAAAATGGCATAGAGGCCAAGTCA TACTCGAGTATGACTTGGCCTCTATGCCA
CFIm59-2	CCGGATGAAGCGAAACAGAGCAATTCTC GAGAATTGCTCTGTTTCGCTTCATTTTTTG	AATTCAAAAAATGAAGCGAAACAGAGCA ATT CTCGAGAATTGCTCTGTTTCGCTTCAT
CFIm68-1	CCGGACCATAGUAGAUCACGGGAAACTC GAGTTTCCCGTGATCTACTATGGTTTTTTG	AATTCAAAAAACCATAGUAGAUCACGGG AAACTCGAGTTTCCCGTGATCTACTATGG T
CFIm68-2	CCGGAGGGAAAUGGAUACUGCAAGACTC GAGTCTTGCAGTATCCATTTCCCTTTTTTG	AATTCAAAAAAGGGAAAUGGAUACUGCA AGACTCGAGTCTTGCAGTATCCATTTCCCT

* Underlining indicates siRNA sequences

Co-immunoprecipitation Assays

To express and purify Flag-CFIm59, CFIm59 cDNA (MGC clone 4506179) was PCR amplified using forward (5'-CGGGGTACCTCAGAAGGAGTGGATTTGATTGATA-3') and reverse (5'-ATTTGCGGCCGCTCAGTGGTGCCGGTCCC-3') primers (where underlining indicates KpnI and NotI sites, respectively), and cloned into pcDNA3.1-3xFlag (Ryu et al., 2013). Similarly, to express and purify Flag-CFIm68, CFIm68 cDNA (MGC clone 6813785) was PCR amplified using forward (5'-CGGGGTACCGCGGACGGTGTGGACC-3') and reverse (5'-

ATTTGCGGCCGCCTAACGATGACGGTATTCTCGCT-3') primers. Each construct was transfected into HEK293T cells, and 56 h later cells were harvested and lysed with high salt lysis buffer (20 mM Tri-HCl (pH 8.0), 300 mM NaCl, 0.5% NP-40, 1 mM EDTA, protease inhibitors) and briefly sonicated. The extract was cleared by centrifugation at 15,000g for 15 min and then incubated with anti-FLAG M2 affinity gel (Sigma-Aldrich), for 2 at 4°C. Beads were then washed three times with high salt lysis buffer, treated with 10 μg/ml RNase A for 30 min at room temperature, and washed again. Flag-CFIm59/68-bound beads were incubated with Ba/F3-U2AF35 or Ba/F3-U2AF35(S34F) nuclear extracts prepared as previously described (Allemand et al., 2005) except after 5 h incubation at 4°C, beads were washed three times and treated with 100 μg/ml RNase A for 30 min at room temperature and washed again. Protein elution was performed by adding Laemmli buffer to the beads Proteins were analyzed by immunoblotting with the following antibodies: U2AF65 (Santa Cruz Biotechnology, MC3), MYC (Roche, 11-667-149-001), β-actin (Sigma, AC74), FLAG M2 (Sigma, F1804).

RNA Immunoprecipitation Assay

RNA immunoprecipitation was performed as described (Ruepp et al., 2010) with minor modifications as described in the Supplemental Experimental Procedures. Briefly, 2×10^7 cells were fixed in 1% formaldehyde for 10 min at room temperature and lysed in FA lysis buffer [50 mM HEPES-KOH (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% sodium deoxycholate, protease inhibitors] containing RNasin (Promega; 160 U/ml buffer). Nucleic acids were fragmented by extensive sonication using a Bioruptor sonicator (Diagenode). The extract was treated with RNase-free DNase (Promega) for 10 min at 37°C and then cleared by centrifugation at 15,000 g for 15 min. Immunoprecipitation was performed by adding the relevant antibody-conjugated Dynabeads® Protein G (Life Technologies) to the DNase-treated extract and incubating at 4°C overnight. The following antibodies were used: CFIm25 (Santa Cruz, sc-81109), CFIm59 (Bethyl Laboratories, A301-360A) and CFIm68 (Abcam, ab175237) were used for RNA immunoprecipitation and mouse IgG (Abcam, ab18413) or rabbit IgG (Abcam, ab46540) was used as a negative control. 1% of the DNase-treated extract was taken for an input fraction. All subsequent precipitate washes, final elution, proteinase K treatment and reverse cross-linking steps were performed as described (Ruepp et al., 2010). The precipitate was dissolved in 11 μl nuclease-free water and reverse transcription was performed using SuperScript III Reverse Transcriptase (Invitrogen) at 50°C followed by qPCR using primers listed below. The qRT-PCR results were analyzed using the comparative Ct method (Pfaffl, 2001). The qRT-PCR levels of the IP samples were normalized against the input of the same samples and calibrated to an IgG control followed by normalization to an irrelevant region in the 18S rRNA. Fold enrichment was calculated by setting the IgG control IP sample to a value of 1.

Gene	**Forward primer sequence (5' → 3')**	**Reverse primer sequence (5' → 3')**
m*Atg7*_proximal CP	CACCTACGACAAAAAAGATCAACT	AGACCATCATTACGCTGTGC
m*Sfxn5*_proximal CP	GATTTCTGCCATGCTCCAAT	TGTCACAGCAAATCCAAAGG
m*Smpd3*_proximal CP	GGGACACTAGGGGTTTTATATGG	TTCTAGGGAAGAATTGTCTGGA
18S rRNA	TTGACGGAAGGGCACCACCAG	GCACCACCACCCACGGAATCG

Immunoblot Analysis

Cells were collected with ice-cold NETN Buffer [20 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.5% NP-40, 1 mM EDTA) in the presence of a protease inhibitor cocktail (Roche). Cell extracts were prepared by lysis in Laemmli buffer [62.5 mM Tris-HCl (pH 6.8), 5% 2-mercaptoethanol,

10% glycerol, 2% SDS, 0.002% bromophenol blue]. Protein (30-60 μg) was fractionated by SDS-PAGE and transferred to a PVDF membrane, which was then incubated with the following primary antibodies at appropriate dilutions in 1% BSA TBS-T buffer at 4°C overnight: U2AF65 (Santa Cruz Biotechnology, MC3), MYC (Roche, 11-667-149-001), β-actin (Sigma, AC74), FLAG M2 (Sigma, F1804), ATG7 (ProSci, 3615), CFIm59 (Bethyl Laboratories, A301-359A), CFIm68 (Abcam, ab175237), LC3B (Cell Signaling Technology, #3868), p62 (Santa Cruz Biotechnology, sc-28359), U2AF35 (Proteintech, 10334-1-AP) and α-tubulin (Sigma, B5-1-2). Immunoblots were visualized by a ChemiDoc™ MP System (Bio-Rad).

Luciferase Reporter Assay

To construct the reporter plasmids, different regions of the mouse *Atg7* 3'UTR cDNA were PCR amplified from a BAC clone (BACPAC Resources Center, clone RP24-316O8) using primers listed below. The PCR products were digested with XhoI and NotI and cloned into the multiple cloning region downstream of the *Renilla* luciferase stop codon in the vector psiCHECK-2 (Promega), which also contains a second reporter gene, firefly luciferase. Individual constructs (0.4 μg) were transfected into HEK293T cells using Effectene (QIAGEN). Luciferase activity was measured 48–60 h later using the Dual-Luciferase Reporter Assay System (Promega). Transfection efficiency was normalized by firefly luciferase activity.

Gene	Forward primer sequence (5'->3')	Reverse primer sequence (5'->3')
ATG7_Short 3'UTR	CCCTCGAGAGCAAGCAACCACAGCTCAG	ATTTGCGGCCGCTTCAGAACACTTTTTTTATATAGGTAATTTTAAAG
ATG7_Long 3'UTR	CCCTCGAGCAATTCTTTTTTTCCTACAGAAAATATAGG	ATTTGCGGCCGCGGTGGAAGAGCACTTCCGT
Long-1	CCCTCGAGCAATTCTTTTTTTCCTACAGAAAATATAGG	ATTTGCGGCCGCTATTAATAGCTACTAACTAGACATTAGGAGCC
Long-2	CCCTCGAGAGCCGTGTCTGTCCTGCTG	ATTTGCGGCCGCTGTCTGGCCTCTTGTCAGC

Cyto-ID Fluorescence Spectrophotometric Assay

Cells were treated with 10 μM PP242 (Sigma-Aldrich), or DMSO as a negative control, for 4 h and then washed once with pre-warmed PBS supplemented with 5% FBS. Cells ($6x10^4$) were then divided: one half was used for the measurement of Cyto-ID fluorescence, and the other half was used for an MTS assay. Cyto-ID (Enzo Life Sciences) staining was performed according to the manufacturer's instructions with minor modifications (see Supplemental Experimental Procedures). In brief, cells were mixed with Cyto-ID staining solution and incubated for 30 min at 37°C in the dark, then washed twice with PBS (without FBS) to remove the free dyes. Cells were then plated in a black round-bottom 96-well plate and incubated for 10 min at room temperature. Cyto-ID fluorescence was read using a SpectraMax M5 microplate reader (Molecular Devices; excitation 480 nm, emission 530 nm). To minimize the difference of cell density among each sample, we determined the relative number of live cells using a Cell-Titer 96 Aqueous One solution cell proliferation assay (MTS) (Promega). Cells in 100 μl culture media were plated ($1x10^4$ cells per well) in a 96-well plate. MTS (10 μl) was added to each well and incubated for 1 h at 37°C. The absorbance of MTS (490 nm) was measured using a SpectraMax M5 microplate reader. These readings were used to normalize the Cyto-ID fluorescence of each corresponding sample.

Correlation Determination

To test whether there was a significant correlation between relative *Atg7* distal CP site usage and ATG7 protein level, Pearson correlation analysis was performed using log2-transformed data in R, a system for statistical computation and graphics (Ihaka and Gentleman, 1996).

MDS Patient Samples

The U2AF35 mutation status was determined by PCR amplifying a ~400 bp fragment containing the C101 nucleotide using forward (5'-AGTCGATCACCTGCCTCACT-3') and reverse (5'-GGTGCTTAATACCACGGAAAA-3') primers, followed by Sanger sequencing of the amplicon using the reverse primer. To detect lengthening of the human *ATG7* 3'UTR in MDS patient samples, 3'-end qRT-PCR was performed as previously described (Jenal et al., 2012) with minor modifications. Briefly, total RNA was extracted from bone marrow samples using TRIzol reagent (Life Technologies) and 3 µg was heat fragmented in Ambion fragmentation buffer for 5 min at 70°C. cDNA was synthesized using a specific P7-oligodT primer (5'-CAAGCAGAAGACGGCATACGAGATTTTTTTTTTTTTTTTTTTTTTTTVN-3') to amplify the 3' end. Then target regions were amplified using gene specific forward and P7 reverse primers listed below. To determine if there was a significant difference in *ATG7* CP site use between normal and U2AF35(S34F)-containing samples, the average values for each group were determined.

Gene	Forward primer sequence (5'->3')	Reverse primer sequence (5'->3')
P7-h*ATG7* proximal	CTCGGGATTCAAGATACCACC	
P7-h*ATG7* distal	CCCGCACTACTTGTGAGTAAAGTG	
P7-*GAPDH* distal	CCGCACCTTGTCATGTACC	
P7		CAAGCAGAAGACGGCATACGAGA

Cytotoxicity Assays

Ba/F3 cells were plated in a 96-well plate (2,500 cells per well) in the presence of 1 µM etoposide (Sigma-Aldrich), 1 mM 3-methyladenine (Sigma-Aldrich), or both. For Figure S5D and E, cells were treated with 50 nM topotecan hydrochloride hydrate (Sigma-Aldrich) or 2 µM 5-azacytidine (Sigma-Aldrich). SA cells were plated (4,000 cells per well) in the presence of 5 µM etoposide, 5 mM 3-methyladenine (Sigma-Aldrich), or both. For the radiosensitivity test, 2 h following drug treatment, Ba/F3 cells were irradiated with x-rays (4 Gy) at a dose rate of 220 cGy/min using a Faxitron RX-650 radiation source (Faxitron Bioptics). Cytotoxic effects were measured using an MTS assay. Briefly, 24 h after treatment, MTS (20 µl) was added to each well and incubated for 3 h at 37°C. The percentage of growth inhibition was calculated as follows: growth inhibition (%) = (1-A of experiment well/A of negative control well)×100% (where A is absorbance, and the negative control is non-treated cells).

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