Table S1. Clinical information about CML patient samples used in this study

Sample number	Sample type	Primary diagnosis	Point in treatment	Phase
1	PBMC	CML	Pre-treatment	Chronic phase
2	PBMC	CML	Pre-treatment	Chronic phase
3	PBMC	CML	Active treatment	Chronic phase

PBMC, peripheral blood mononuclear cell

Table S2. Oligo ID numbers for shRNAs obtained from Open Biosystems

Gene symbol (species)	**Oligo ID**
Atf5 (mouse)	TRCN0000075555
ATF5 (human)	TRCN0000017638
Foxo1	TRCN0000054882
Foxo3A	TRCN0000071616
Foxo4	TRCN0000071562

Table 3S. Primer sequences for qRT-PCR

Gene symbol	Forward primer (5' → 3')	Reverse primer (5' → 3')
Akt1	CTTCTATGGTGCGGAGATTGTG	CCCGGTACACCACGTTCTTC
Atf5	GGGTCATTTTAGCTCTGTGAGAGAA	ATTTGTGCCCATAACCCCTAGA
Atg3	CATCACTTACGACAAATATTACCAGACA	CTGCCGTTGCTCATCATAGC
Atg4a	TCACACTGGCCTCCCTTTG	GCCCCTGTGGTTGTCACTTC
Atg4b	GGGCCTGCTCTGCTCATG	GGCAGTCTGACTCCAAGCATT
Atg4c	CTAATGGAACACTACATCAACTGCAA	CAATGGGCTCAGGGAGAAAA
Atg4d	GGGCTCCAGGGCACTCA	GGACCAGTGACCCAATGCA
Atg5	ACTGCTTCGCTGAGACACACA	GCTTCGGCTGCATTGCAT
Atg7	CAGCCAGCAAGCGAAAGC	TCTCATGACAACAAAGGTATCAAACC
Atf9a	GAGATCATAGACTTCTTCCGCAACT	GAACGTCCATCTGAGCAAAGG
Atg9b	CCAGCTGCAGGAAATTTGATG	AAATAGGAATGGAGCTCAGAAGATG
Atg10	ATAGGCGATGGCTGGGAAT	TTTGCACATGTAGCCATCAGAAC
Atg12	GGTTCTTGCGCCTCATCCT	GGTGTCCTGAGCGCTGAGA
Atg16l1	CAGCCGAGGGTTCTCTTTATGT	TGTTTTGAAAGAACCTTCTCCACTT
Atg16l2	GACAGGTGTTCAGGGCAGATG	GGGCTGAACACAGCTTTGGT
Bad	CAGGGAGAAGAGCTGACGTACA	CCCTCCGTGGCTATTGCA
Bak1	CCGGGAATGCCTACGAACT	CCAGCTGATGCCACTCTTAAATAG
Bax	AGGATGCGTCCACCAAGAAG	CCATATTGCTGTCCAGTTCATCTC
Bbc3	ACGACCTCAACGCGCAGTA	AGGAGTCCCATGAAGAGATTGTACA
Bcl2	AAGGGCTTCACACCCAAATCT	TTCTACGTCTGCTTGGCTTTGA
Bcl2a1a	TCAAGTAACACTACGGACCAGAGAGA	AAATGCCAAGTGCTGATAACCA
Bcl2l1	GAGCCATTGAGTGAGGTGCTTT	TCCCAAGCAGCCTGAATTTC
Bcl2l2	GCTCTCCAAAGCCCAAAGG	GTGCCCACTTGCGCTCTT
Bcl2l11	GCCGGTGAGATACCCTAAGATG	ATGGGTTTGATGGGTATTTTCTTAGA
Bcl2l12	CCCGCCTTGTGGAACTCTT	ACGGGCTGGAGCAATTTG
Becn1	GGACAAGCTCAAGAAAACCAATG	TGTCCGCTGTGCCAGATGT
Bid	CAAGCCCGATGGTGACACT	TTCAGGCCAAGGTCTTTCCA
Bik	TCATACAGGTTTTCTGGGTTTTTG	CTTAGCCAGTAATGCTCCTGAGGTA
Bmf	CGGCTTCATACGCAACAACA	GGGCGAGGTTTTGAAGGAA
Bnip3	CCCACGAACCCCACTTTG	GACAGCAAGGCGAGAATCCT
Bnip3l	GCAGGCTTCGTTTTAAGTTTATTGA	TGCCGCTTTCCTCTTGACTTAT
Cdkn2a	CTCAACTACGGTGCAGATTCGA	CACCGGGCGGGAGAAG
Cdkn1b	TCTTCGGCCCGGTCAAT	CATATCCCGGCAGTGCTTCT
Ctsb	TCCATGCCCCAGTAATCCA	GGCCAGTTCAGTGGTCCATT
Ctss	GCTGTGGAGGCGGCTACAT	CTGCCTCTATGCCCCCATT
Cxcr4	TCGGCAATGGATTGGTGAT	CCGTCATGCTCCTTAGCTTCTT
Dapk1	TGGTGGCCAAACACAACGT	CACTGGGCTAGGGAGGAAATC
Eif2ak3	ACCGTGGACGGCAATGAC	AGAAGCTTCGGACGGACAAA
Eif4g1	GGGCAAAGCTACTTCTTTATTGCT	CCTTTTTGGGACCCATGCT
Esr1	AGCCTTGTCTCTTCCCTGATGTC	TGTGACTGCCCTTGATCAATCT

Fadd	CCACACTTGGAGCCCAATAAA	TTATCACACCCAGCCAAAGGT
Fas	TTCTCCTGGCTGTGAACACTGT	CACGGCTCAAGGGTTCCAT
Foxo1	TTCAGCAGCAACCCCTGTTT	AGACAACTGCCCATGATTCACA
Foxo3a	TGGTGCTAAGCAGGCCTCAT	CCCCTTTCCTCAGTGATCCTT
Foxo4	CGGCTGTCGCCAATGAG	CCGAGGCTGGCATTTCCT
Hdac1	GTGGCTACACCATCCGGAAT	GGCCACCGCTGTTTCGTA
Hrk	TTTTTCCTGGTTGGATTTGCA	TGCTTTCTAGCAGACCCTCTGAT
Hsp90aa1	AGCAAACATGGAGAGAATCATGAA	CTGCCATGTAACCCATTGTTGA
Hspa8	CCATTACCCGGGCTCGAT	CCAGTGTGCCACGGAACA
Htt	GGGCAGGAAGCCGTCAT	GCGACTCGAAAGCCTTCATC
Ifna2	GGACAGGCAGGACTTTGGATT	GCCTTCTGGATCTGCTGGTTA
Mcl1	CTGGCTTTTGCGGGTGTT	TTGGTGGCTGGAGCTTTAAGA
mTOR (mouse)	CCAACCAGCCAATCATTCG	GCTTGGATGTGATGACTTGCA
mTOR (human)	GGATGTTCCAACGCAAGTTGA	GCAGAGGTTTTCATGGGATGTC
Pmaip1	TTTCCCACCGTAAAGCGACTA	CATCACTTCTGGTAGGCATCAAGA
Tgfb1	CCAGCCGCGGGACTCT	TTCCGTTTCACCAGCTCCAT
Tnf	CACCGTCAGCCGATTTGC	TTGACGGCAGAGAGGAGGTT
Trp53	TGGACCCTGGCACCTACAAT	GGAAAGTAGGCCCTGGAGGAT
Trp73	CAAGTCTAAAACGCCGAGCAT	CTGCACTCCCACGGCTATG
Pten	CAGAGCAAGCTCAGTGTGGGTAT	ACTCAAAGGGTGACCAAATTCTCTA
Rab24	TTGATGCATTTCCTTGCTGTGT	ACGTGGCCCCAGGAACA
Rb1	GTCTGCCAACACCCACAAAA	GATGTCCCAAATGATTCACCAATT
Sqstm1	GGAGGCACCCCGAAACA	GCCCGTTGCAACCATCAC
Tsc1	CTCCTGCCTCTGCTCACACA	CTGGTTTGCCAGGGAGTCAA
Tsc2	CACCTACAGCCGGTCATCCT	GCAGCAAGCTCCTCCAAATG
Uvrag	GAACGAGAGACTCCAGTACAAAACC	CCGGGCTGTGTCAATGCT
Zbtb24	TGGGCAGTGAGGAACATGTC	CCGGGAGGTGGAGAATGG

Table S4. Primer sequences for ChIP analysis of the *mTOR* and *ATF5* promoters

***mTOR* promoter region (bp)**	**Forward primer (5' → 3')**	**Reverse primer (5' → 3')**
163 – 265	GTGTGGAGGGGACTTGTAGTAGC	AAGGAGGCGAAGGACTATTCC
490 – 567	GTAGTTCGGTGATCATCTTATCCTG	TGTTTTTTTTTATTCTCACTGTCCC
908 – 1003	TTATATATTGGAGCAGGTTCTCTGG	TGGTGGTGGAGGTTTAGAGGG
1560 – 1666	CTACATAGCAAGTTTTAGGCCATCC	GGTGTTCCTCTAAGTTGGCTGTTC
2124 – 2227	AGGCTGGGCAAGGAGGTG	TTTCTTAGGGCCTCCTATTTGTG
2597 - 2693	CTGGAATTACAAGCATTCACCC	GGGTTGGGATGTAGCTCAGATAG
***ATF5* promoter region (bp)**		
-3562 – -3797	TGGTACAGAAGCATGAGGCCA	AGGGACAGGAGTTGGAGGTGT
-3165 – -3269	GATGAAGACTCACCCTTCCCT	TACTTGTCTTCCCAGTGTGAG
-2447 – -2562	TTCCAAGCAGCTCCACCCTTG	CTCTCTTCCTAACCCTCACCC
-1587 – -1683	CCCATTCCAAGGGGAATTATC	CTGGTGTCCATCCCTGGTACT
-880 – -1106	CCCAATCCATGTGGTGCTTGG	GGGATGATGCGTGAACCCTGT
-434 – -599	AGCAGTGGGTAAAGGCTGCCA	AGGCCACGAAGTCCTAACCAC
834 – 1055	GGATCTGTGTGTGTGACACTG	TCCTCCCTCAACACAGAAACC

Figure S1. An ATF5 doublet is evident on 15% but not 10% SDS-polyacrylamide gels. Cell lysates prepared from 32D/BCR-ABL or K562 cells were resolved by either 10% or 15% SDS-PAGE. In an immunoblot ATF5 is a doublet, which is evident on 15% but not 10% SDS-PAGE gels. The variable appearance of the doublet in this manuscript is a reflection of the fact that some of the experiments were run on 10% gels and others on 15% gels. As indicated, the size of the major ATF5 band is ~35 kDa.

Figure S2. siRNA-mediated knockdown efficiency of ATF5 in 32D and 32D/BCR-ABL cells. Immunoblot analysis of ATF5 levels in 32D or 32D/BCR-ABL treated with a luciferase or ATF5 siRNA. β-actin (ACTB) was monitored as a loading control.

Figure S3. Representative FACS plots analyzing the effect of ATF5 knockdown. 32D or 32D/BCR-ABL cells were treated with a luciferase or ATF5 siRNA and monitored for cell death by annexin V-PE/7-AAD staining.

Figure S4. ATF5 does not regulate autophagy in 32D cells. 32D cells treated with either a NS or ATF5 shRNA were monitored for expression of LC3B by immunoblot analysis. ACTB was monitored as a loading control. The level of LC3B-II induction was quantified by measuring the intensity of the LC3B-II signal in the immunoblots and normalizing to ACTB levels.

Figure S5. Knockdown of ATF5 in BCR-ABL-transformed cells induces autophagy

Quantification of the results presented in Figure 2B. Error bars represent SD. Single asterisks indicate $p<0.05$.

Figure S6. Schematic diagram of the autophagic process. Autophagy is a dynamic process that involves the formation of autophagosomes and their subsequent fusion with lysosomes to form autolysosomes. The schematic diagram indicates the pH as well as the presence or absence of LC3B-II and p62 in each of these three compartments. N/A, not applicable. Following fusion of the autophagosome and lysosome, the contents of the autolysosome are degraded by lysosomal enzymes, which indicates the completion of autophagy.

Figure S7. Imatinib induces autophagy in K562 cells. K562 cells stably expressing EGFP-LC3B or, as a control, EGFP were treated with DMSO or imatinib (10 μM for 24 h) and then visualized under an inverted fluorescence microscope. Representative images are shown (left panel). Quantification of the results for the EGFP-LC3B expressing cells (right panel). Error bars represent SD. Single asterisks indicate $p<0.05$.

Figure S8. Imatinib treatment induces autophagy in 32D/BCR-ABL cells

Immunoblot analysis monitoring LC3B levels in 32D/BCR-ABL cells treated in the absence or presence of imatinib (5 μM for 24 h). ACTB was monitored as a loading control. The level of LC3B-II induction was quantified by measuring the intensity of the LC3B-II signal in the immunoblots and normalizing to ACTB levels.

Figure S9. Viability of K562 cells following treatment with a low dose of imatinib. K562 cells were treated with 5 μM imatinib for 48 h. Cell viability was monitored by an MTT assay. Error bars represent SD. Double asterisks indicate $p>0.05$.

Figure S10. Ectopic expression of ATF5 in 32D cells does not antagonize autophagy induced by IL-3 withdrawal. Immunoblot analysis of LC3B levels in 32D cells stably expressing FLAG or FLAG-ATF5 in the absence or presence of IL-3 and the apoptosis inhibitor zVAD (100 μM for 24 h). The results show that ectopic ATF5 expression does not inhibit IL-3-withdrawal induced autophagy in 32D cells in the presence or absence of zVAD. The level of LC3B-II induction was quantified by measuring the intensity of the LC3B-II signal in the immunoblots and normalizing to ACTB levels.

Figure S11. Knockdown of FOXO1 or FOXO3A does not prevent the loss of *ATF5* expression following PI3K/AKT inhibition. (A) Knockdown efficiency of FOXO1, FOXO3A and FOXO4 in 32D/BCR-ABL cells. 32D/BCR-ABL cells were transduced with viruses expressing either a non-silencing (NS), FOXO1, FOXO3A or FOXO4 shRNA, and target gene expression was monitored by qRT-PCR. (B) 32D/BCR-ABL cells expressing a NS, FOXO1 or FOXO3A shRNA were treated with 20 μM LY294002 for 18 h, and *Atf5* expression was monitored by qRT-PCR. Error bars represent SD. Single asterisks indicate $p<0.05$; double asterisks indicate $p>0.05$.

Figure S12. siRNA-mediated knockdown of ATF5 decreases expression of *mTOR*. qRT-PCR analysis monitoring expression of *mTOR* in 32D/BCR-ABL cells following

treatment with an ATF5 siRNA or, as a control, a luciferase siRNA. Error bars represent SD. Single asterisks indicate $p<0.05$.

Figure S13. Ethidium bromide-stained gels from ChIP experiments monitoring ATF5 binding to the *mTOR* promoter. DNA was immunoprecipitated in the presence or absence of an ATF5 antibody. Input levels represent one-hundredth the levels loaded in the IP lanes. The results show that ATF5 binds to the *mTOR* promoter region ~1500-2200 bp upstream from the transcription start-site.

Figure S14. Imatinib regulates expression of *ATF5* and *mTOR* in the absence of apoptosis induction. qRT-PCR analysis monitoring levels of *ATF5* and *mTOR* in K562 cells treated in the absence or presence of imatinib (10 μM) and zVAD (100 μM) for 24 h. Error bars represent SD. Single asterisks indicate $p<0.05$.

Figure S15. Analysis of FOXO4 binding to the *Atf5* promoter following treatment with imatinib or LY294002. 32D/BCR-ABL cells were treated with DMSO, imatinib (5 μM for 24 h) or LY294002 (20 μM for 24 h) and binding of FOXO4 to the *Atf5* promoter analyzed in a ChIP assay using a series of primer-pairs located in various regions of the *Atf5* promoter (see schematic at the bottom). Red lines indicate the position of putative FOXO-binding sites (predicted by TRANSFAC analysis); their positions and sequences are also indicated. Primer-pairs that encompass the predicted FOXO binding sites are indicated with a red asterisk. TSS, transcription start site. Error bars represent SD.

Figure S1

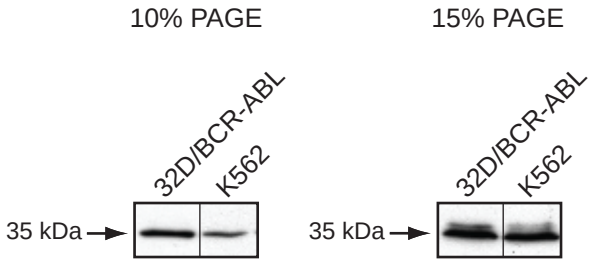
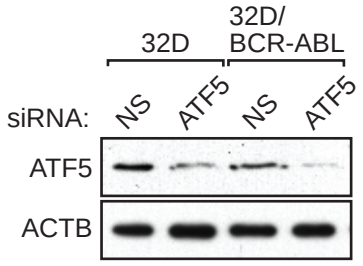


Figure S2



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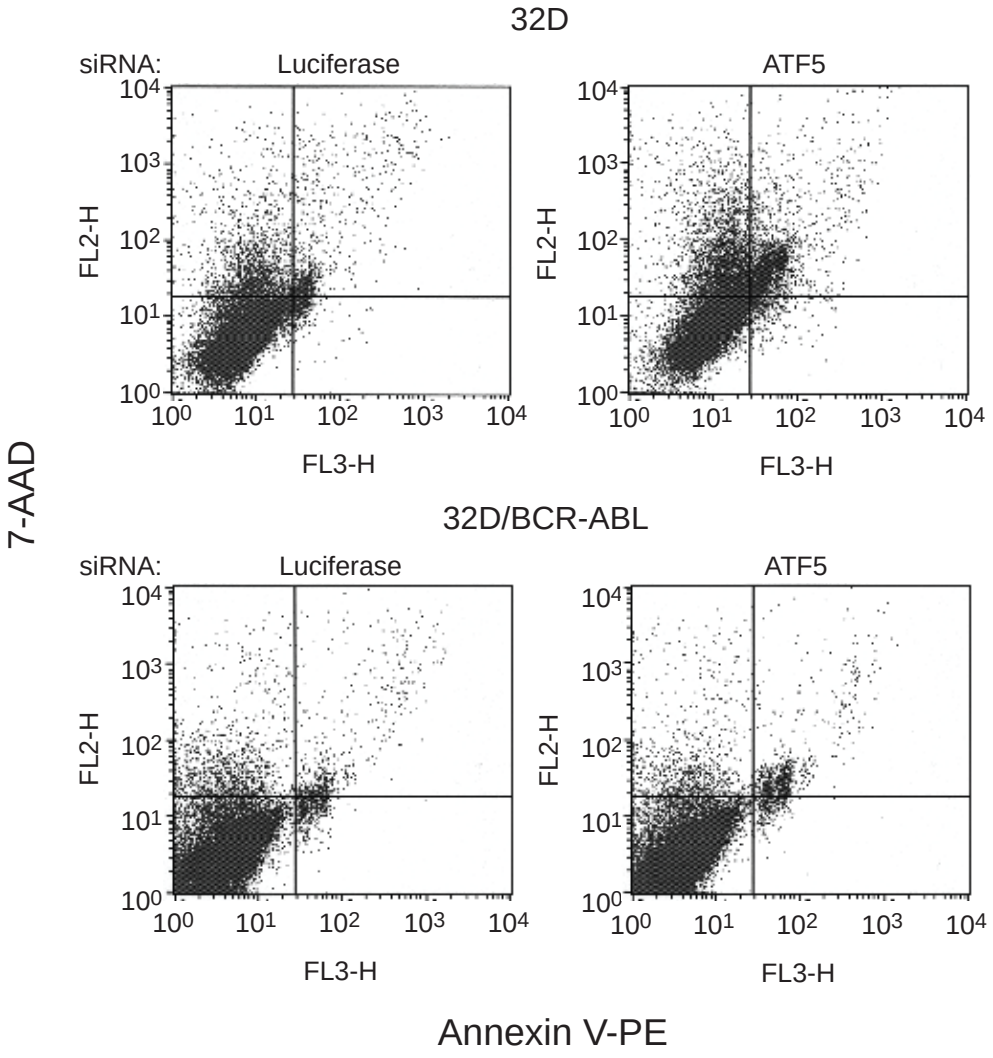
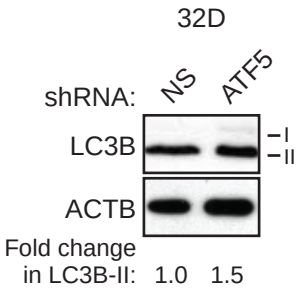

32D
siRNA:
Luciferase
ATF5
FL2-H
FL3-H
7-AAD
32D/BCR-ABL
siRNA:
Luciferase
ATF5
FL2-H
FL3-H
Annexin V-PE

Figure S4



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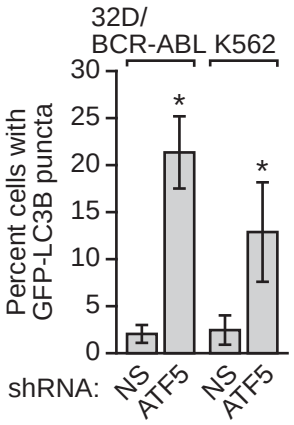

32D/
BCR-ABL
K562
30
25
20
15
10
5
0
Percent cells with
GFP-LC3B puncta
*
*
shRNA:
NS
ATF5
NS
ATF5

Figure S6

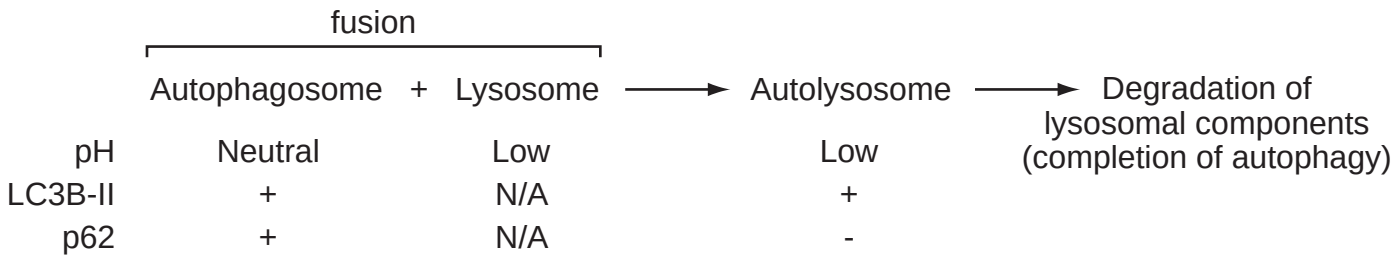

fusion
Autophagosome
+
Lysosome
Autolysosome
Degradation of lysosomal components (completion of autophagy)
pH
Neutral
Low
Low
LC3B-II
+
N/A
+
p62
+
N/A
-

Figure S7

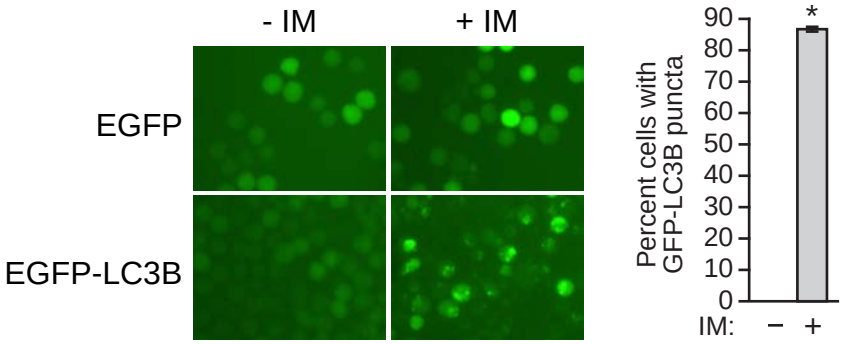

- IM
+ IM
EGFP
EGFP-LC3B
Percent cells with GFP-LC3B puncta
90
80
70
60
50
40
30
20
10
0
*
IM:
−
+

Figure S8

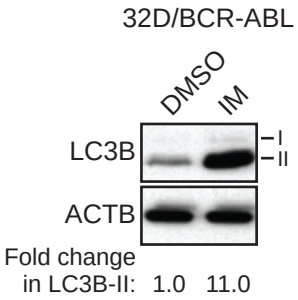


Figure S9

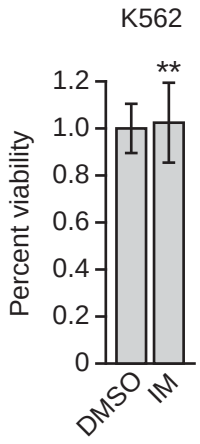


Figure S10

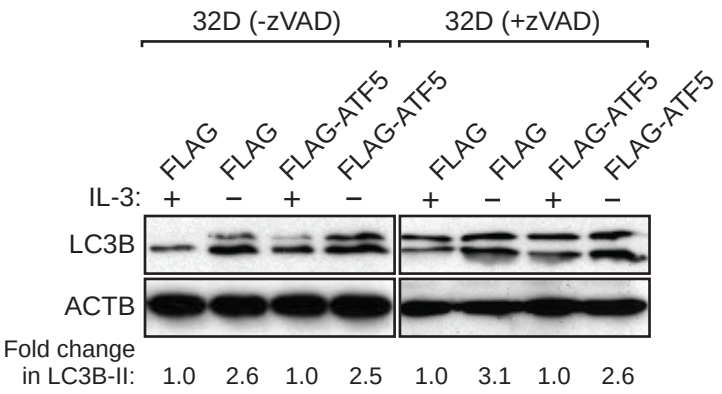

32D (-zVAD)
32D (+zVAD)
FLAG
FLAG
FLAG-ATF5
FLAG-ATF5
FLAG
FLAG
FLAG-ATF5
FLAG-ATF5
IL-3: + − + − + − + −
LC3B
ACTB
Fold change in LC3B-II: 1.0 2.6 1.0 2.5 1.0 3.1 1.0 2.6

Figure S11

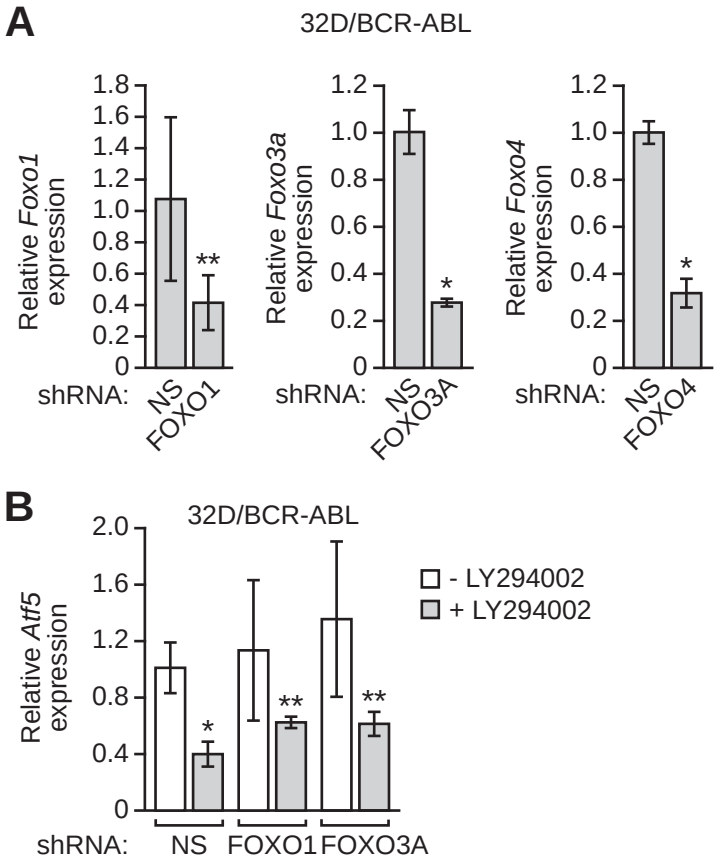

A
32D/BCR-ABL
Relative Foxo1 expression
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
**
shRNA:
NS
FOXO1
Relative Foxo3a expression
1.2
1.0
0.8
0.6
0.4
0.2
0
*
shRNA:
NS
FOXO3A
Relative Foxo4 expression
1.2
1.0
0.8
0.6
0.4
0.2
0
*
shRNA:
NS
FOXO4
B
32D/BCR-ABL
Relative Atf5 expression
2.0
1.6
1.2
0.8
0.4
0
*
**
**
- LY294002
+ LY294002
shRNA:
NS
FOXO1
FOXO3A

Figure S12

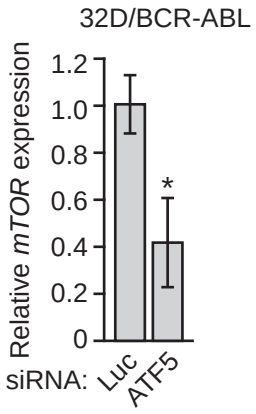
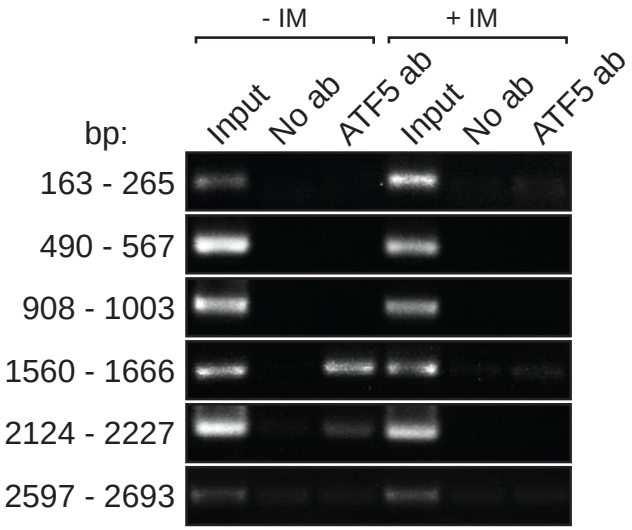

32D/BCR-ABL
Relative mTOR expression
1.2
1.0
0.8
0.6
0.4
0.2
0
*
siRNA:
Luc
ATF5

Figure S13


- IM
+ IM
Input
No ab
ATF5 ab
Input
No ab
ATF5 ab
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908 - 1003
1560 - 1666
2124 - 2227
2597 - 2693

Figure S14

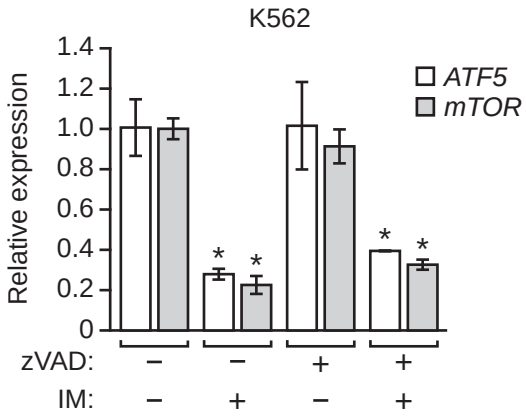
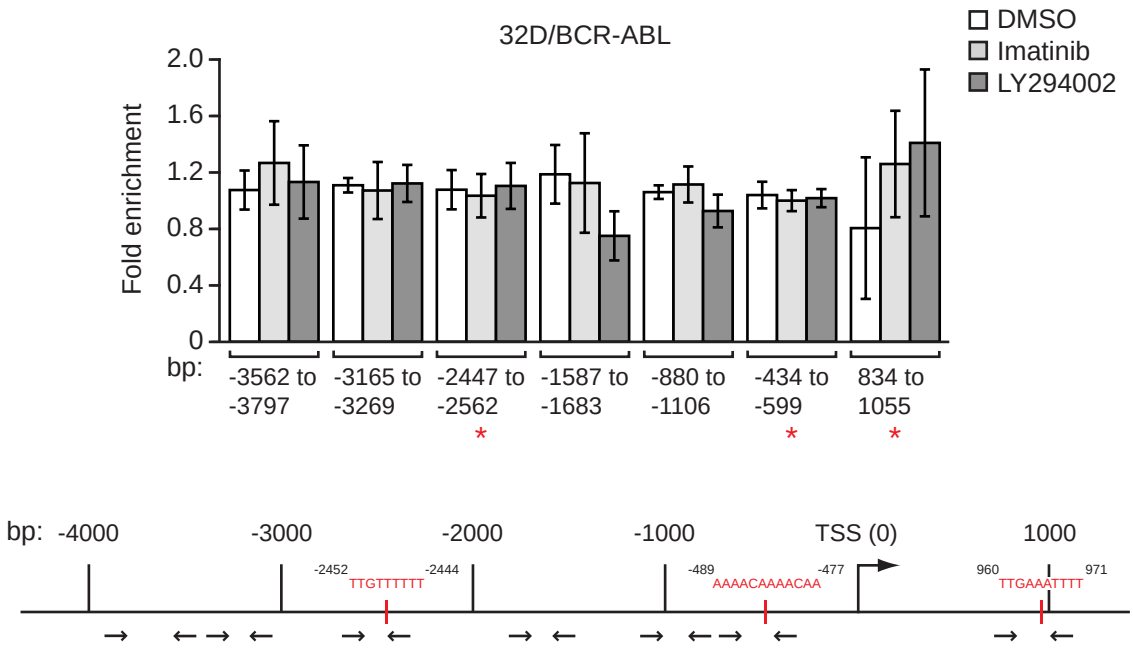

K562
Relative expression
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
ATF5
mTOR
*
*
*
*
zVAD:
IM:
–
–
+
+
–
+
–
+

Figure S15


32D/BCR-ABL
DMSO
Imatinib
LY294002
Fold enrichment
2.0
1.6
1.2
0.8
0.4
0
bp:
-3562 to -3797
-3165 to -3269
-2447 to -2562
-1587 to -1683
-880 to -1106
-434 to -599
834 to 1055
*
*
*
bp: -4000
-3000
-2000
-1000
TSS (0)
1000
-2452
TTGTTTTTT
-2444
-489
AAAACAAAACAA
-477
960
TTGAAATTTT
971