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Supplemental information

A nonautophagic role of ATG5 in regulating cell growth by targeting c-Myc for proteasome-mediated degradation

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Supplementary Figure Legends

Figure S1. Serum starvation enhances autophagy activity, Related to Figure 1.

A, 293T cells were cultured under normal conditions or serum starvation conditions as indicated. Cell extracts were prepared and subjected to Western blot analyses with antibodies as indicated.

B, 293T cells transfected with a GFP-tagged LC3 construct were cultured under normal conditions or serum starvation conditions as indicated. Cells were then harvested, and fluorescence microscopy analyses were performed. The average numbers of LC3 puncta per cell were calculated. The bars and error bars indicate the mean±s.d. values; n=3 independent replicates.

C, 293T cells transfected with a DsRed2-GFP double-labeled LC3 reporter construct were cultured under normal conditions or serum starvation conditions as indicated. Cells were then harvested, and fluorescence microscopy analyses were performed.

Figure S2. The effects of loss of ATG5 on cell growth under serum starvation conditions, Related to Figure 1.

HeLa cells were transfected with a control or an ATG5-specific siRNA and cultured under serum starvation conditions for 6 h. Cells were then treated with or without 1 μM Baf A1 as indicated and were further cultured for different durations as indicated. The relative cell growth ability was examined by a cell counting kit-8 (CCK-8) assay. The bars and error bars indicate the mean±s.d. values; n=3 independent replicates. Two-tailed unpaired Student's t test was performed. *: $P<0.05$, ***: $P<0.001$.

Figure S3. Determination of overexpression and RNAi efficiencies of ATG5 by Western blot analyses, Related to Figure 1.

293T cells transfected with a control siRNA, either of two ATG5-specific siRNAs, or a Flag-tagged ATG5 construct were cultured under normal conditions or serum starvation conditions as indicated. Cell extracts were prepared and subjected to Western blot analyses with antibodies as indicated.

Figure S4. ATG5 shows similar effects on cell migration under both normal culture conditions and serum starvation conditions, Related to Figure 1.

A, HeLa cells were transfected with a control siRNA, either of two ATG5-specific siRNAs, or a Flag-tagged ATG5 as indicated. Transwell assays were then performed with or without Baf A1 in the upper Transwell chamber as indicated.

B, Quantification of the Transwell assay results. The bars and error bars indicate the mean ±s.d. values; n=3 independent replicates.

Figure S5. The effect of ATG5 on ATG12, Related to Figure 2.

HeLa cells were transfected with an ATG5-specific siRNA or a Flag-tagged ATG5 construct and were further cultured under normal conditions or serum starvation conditions for 48 h. Western blot analyses were then performed.

Figure S6. ATG5 regulates c-Myc protein degradation through the 26S proteasome pathway, Related to Figure 3.

293T cells were transfected with a Flag control or a Flag-tagged ATG5 construct for 48 h. Cells were then treated with or without 1 μM Baf A1 (bafilomycin A1, an autophagy inhibitor) and multiple 26S proteasome inhibitors (including 25 μM MG132, 1 μM Bortezomib, or 1 μM Carfilzomib) as indicated for another 10 h. Cell extracts were prepared, and Western blot analyses were performed.

Supplementary Tables

Table S1. The sequences of the primers used for plasmid construction, Related to STAR Methods.

Plasmid Name	Primer Sequences
GST-ATG5 (Homo)	Forward: 5’-CGGACTAGTATGACAGATGACAAAGATGTG-3’ Reverse: 5’-CGCGGATCCTCAATCTGTTGGCTGTGGGAT-3’
His-c-Myc (Homo)	Forward: 5’-CGCCATATGATGCCCCTCAACGTTAGCTTC-3’ Reverse: 5’-CCGGAATTCCGCGCACAAGAGTTCCGTAG-3’
HA-FBW7 (Homo)	Forward: 5’-CCGGAATTCCGATGTGTGTCCCGAGAAGC-3’ Reverse: 5’-GGAAGATCTTCACTTCATGTCCACATCAAA-3’
His-Fbw7 (Homo)	Forward:

	5’-TGCTCTAGAATGTGTGTCCCGAGAAGCGGT-3’ Reverse: 5’-CCCAAGCTTCTTCATGTCCACATCAAAGTC-3’

Table S2. The sequences of the siRNAs, Related to STAR Methods.

siRNA Name	Sequences
siATG5-#486	5’-GACGUUGGUAACUGACAAATT-3’ 5’-UUUGUCAGUUACCAACGUCTT-3’
siATG5-#938	5’-GACCUUUCAUUCAGAAGCUTT-3’ 5’-AGCUUCUGAAUGAAAGGUCTT-3’
sic-Myc-#630	5’-GCCGUAUUUCUACUGCGACTT-3’ 5’-GUCGCAGUAGAAAUACGGCTT-3’
sic-Myc-#1740	5’-CCAAGGUAGUUAUCCUUAATT-3’ 5’-UUAAGGAUAACUACCUUGGTT-3’
sic-Myc#1939	5’-CACCUAUGAACUUGUUUCATT-3’ 5’-UGAAACAAGUUCAUAGGUGTT-3’
siFBW7	5’-GCACUCUAUGUGCUUUCAUTT-3’ 5’-AUGAAAGCACAUAGAGUGCTT-3’

Table S3. The sequences of the primers used for RT-PCR analyses, Related to STAR Methods.

Gene Name	Primer Sequences
GAPDH (Homo)	Forward: 5’-GACAGTCAGCCGCATCTTCT-3’ Reverse: 5’-AAATGAGCCCCAGCCTTCTC-3’
ODC1 (Homo)	Forward: 5’-CTGGGCGCTCTGAGATTGTC-3’ Reverse: 5’-CAAGGGTCTTCACGATGGCT-3’
GADD45 (Homo)	Forward:

	5’-AAGGATGGATAAGGTGGGGGA-3’ Reverse: 5’-TGATGTCGTTCTCGCAGCAA-3’
γ-GCSH (Homo)	Forward: 5’-GAGAACATGAAGGTAGCAC-3’ Reverse: 5’-CTTGCTTGTAGTCAGGATG-3’
C/EBPα (Homo)	Forward: 5’-GAGGGGAGAATTCTTGGGGC-3’ Reverse: 5’-GGAAGGAGGCAGGAAACCTC-3’
c-Myc (Homo)	Forward: 5’-GGGCGCTTTGCACTGGA-3’ Reverse: 5’-GCAGAGTAGCCTCCCCGC-3’
ATG5 (Homo)	Forward: 5’-ATGACAGATGACAAAGAT-3’ Reverse: 5’-CAGTGGTGTGCCTTCATA-3’

Figure S1

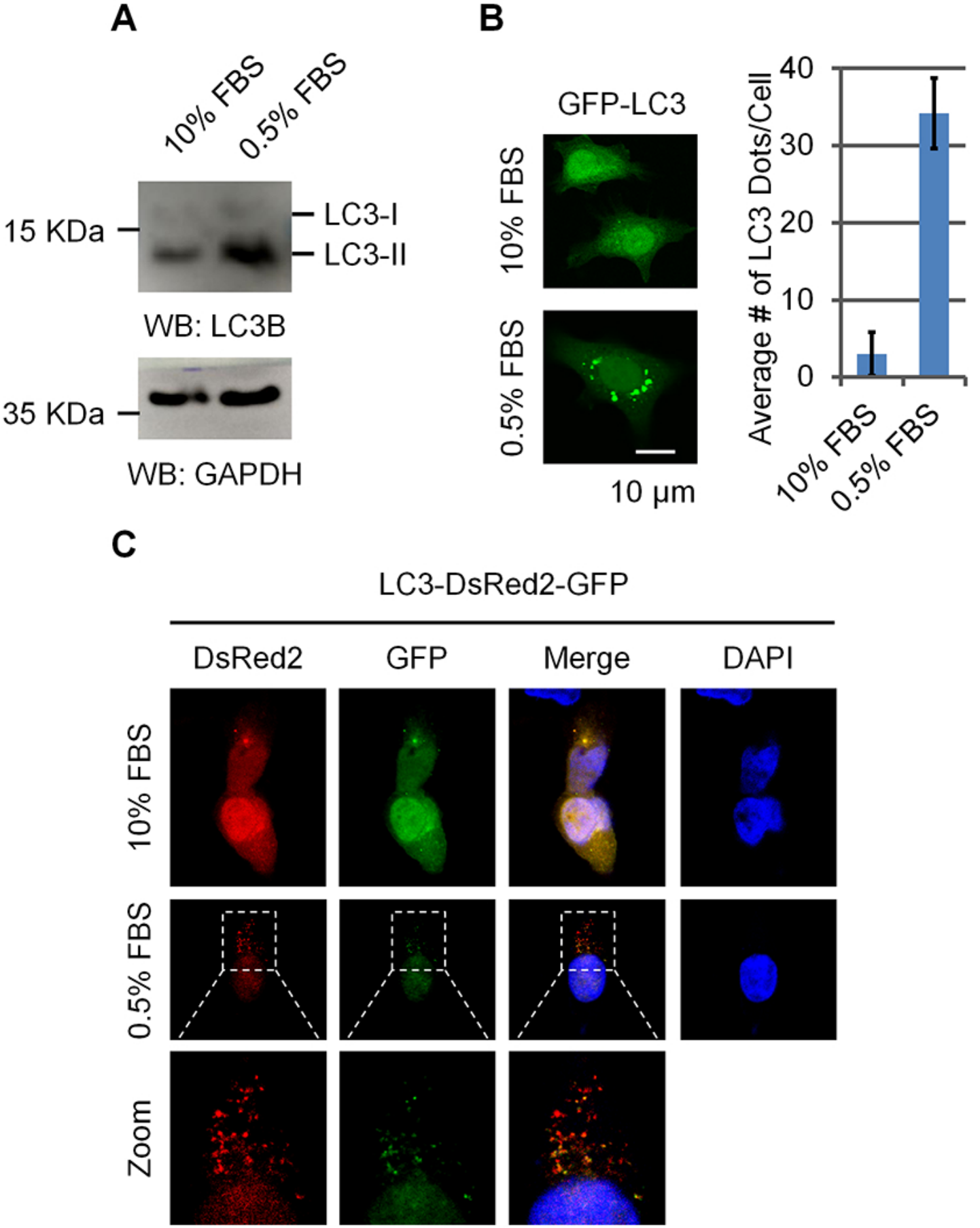

A
10% FBS
0.5% FBS
15 KDa
LC3-I
LC3-II
WB: LC3B
35 KDa
WB: GAPDH
B
GFP-LC3
10% FBS
0.5% FBS
10 μm
Average # of LC3 Dots/Cell
40
30
20
10
0
10% FBS
0.5% FBS
C
LC3-DsRed2-GFP
DsRed2
GFP
Merge
DAPI
10% FBS
0.5% FBS
Zoom

Figure S2

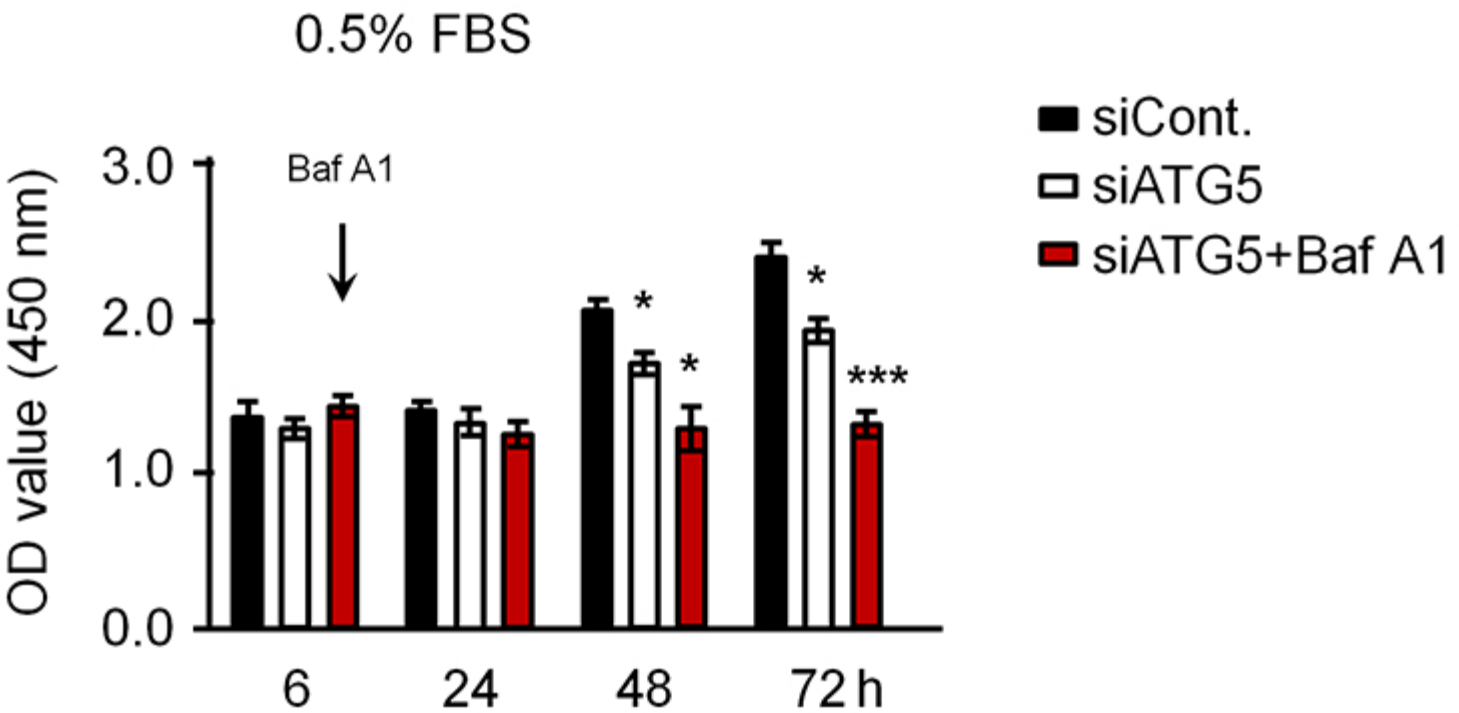

0.5% FBS
Baf A1
OD value (450 nm)
3.0
2.0
1.0
0.0
6
24
48
72 h
*
*
*
***
siCont.
siATG5
siATG5+Baf A1

Figure S3

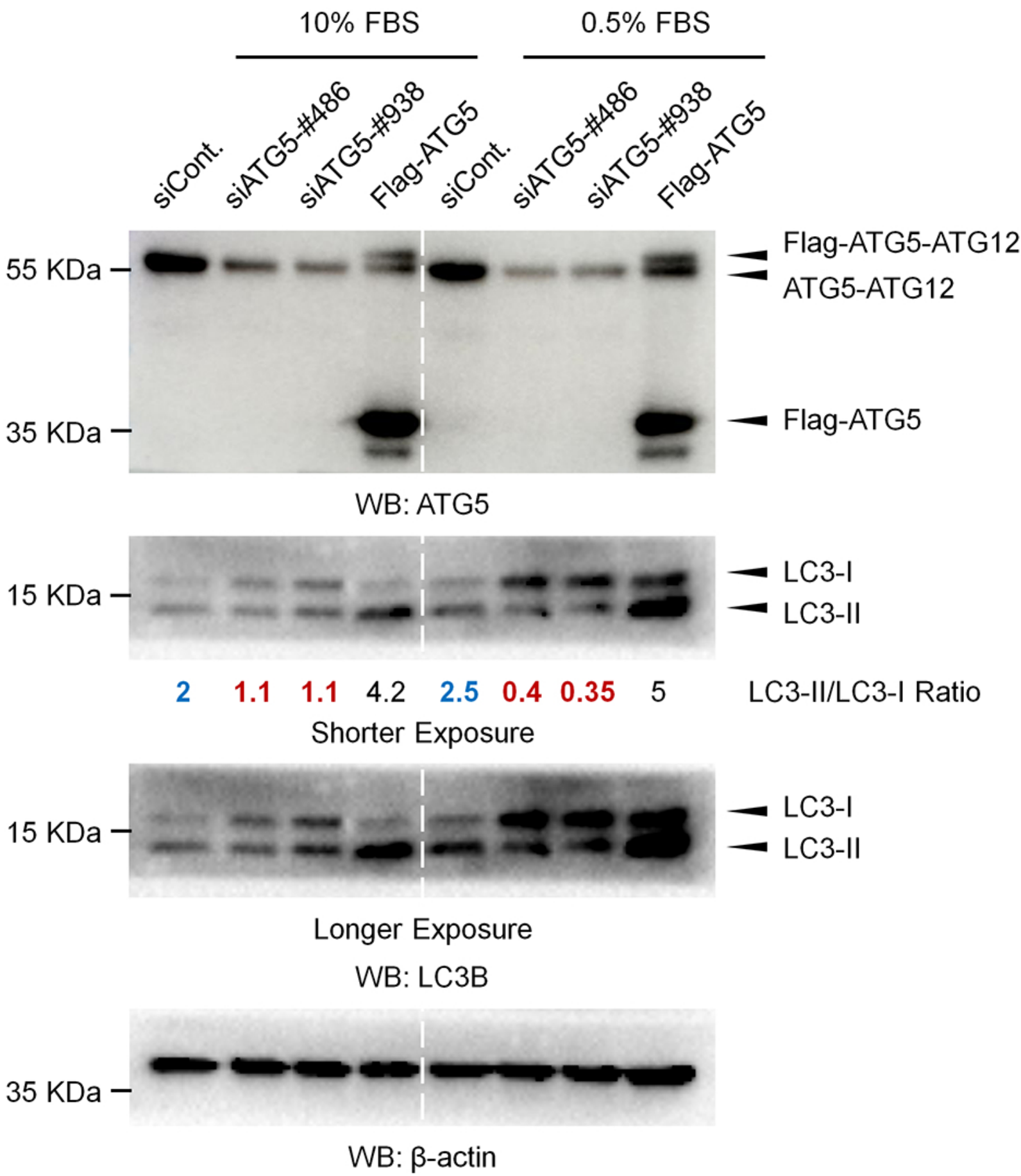
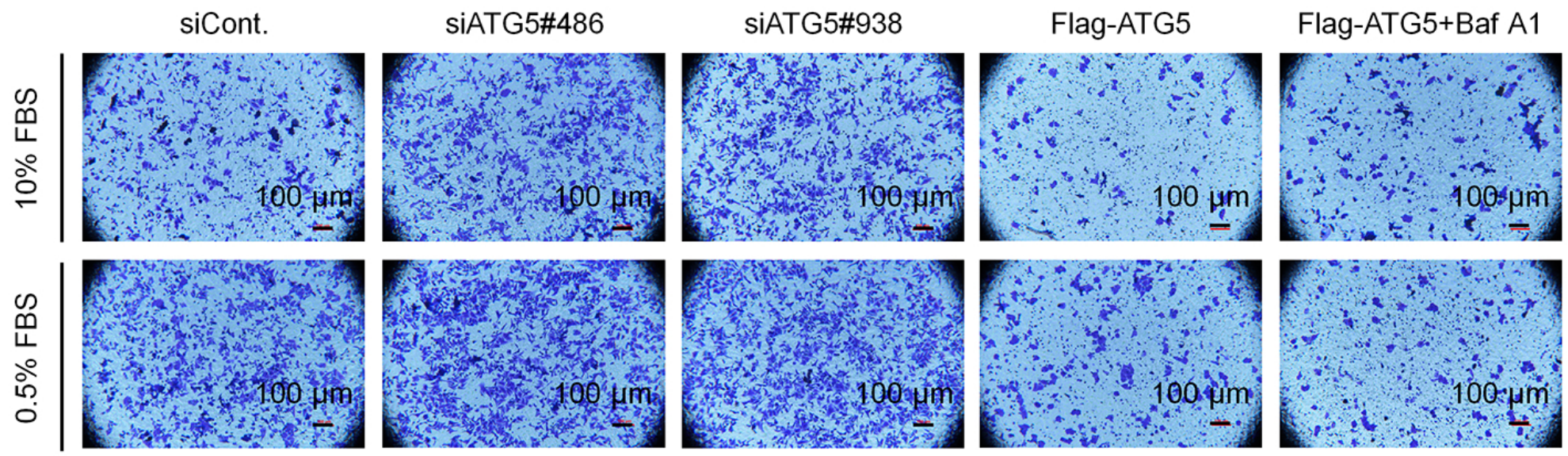

10% FBS
0.5% FBS
siCont.
siATG5-#486
siATG5-#938
Flag-ATG5
siCont.
siATG5-#486
siATG5-#938
Flag-ATG5
55 KDa
35 KDa
Flag-ATG5-ATG12
ATG5-ATG12
Flag-ATG5
WB: ATG5
15 KDa
LC3-I
LC3-II
2 1.1 1.1 4.2 2.5 0.4 0.35 5
LC3-II/LC3-I Ratio
Shorter Exposure
15 KDa
LC3-I
LC3-II
Longer Exposure
WB: LC3B
35 KDa
WB: β-actin

Figure S4

A


siCont.
siATG5#486
siATG5#938
Flag-ATG5
Flag-ATG5+Baf A1
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0.5% FBS
100 μm

B

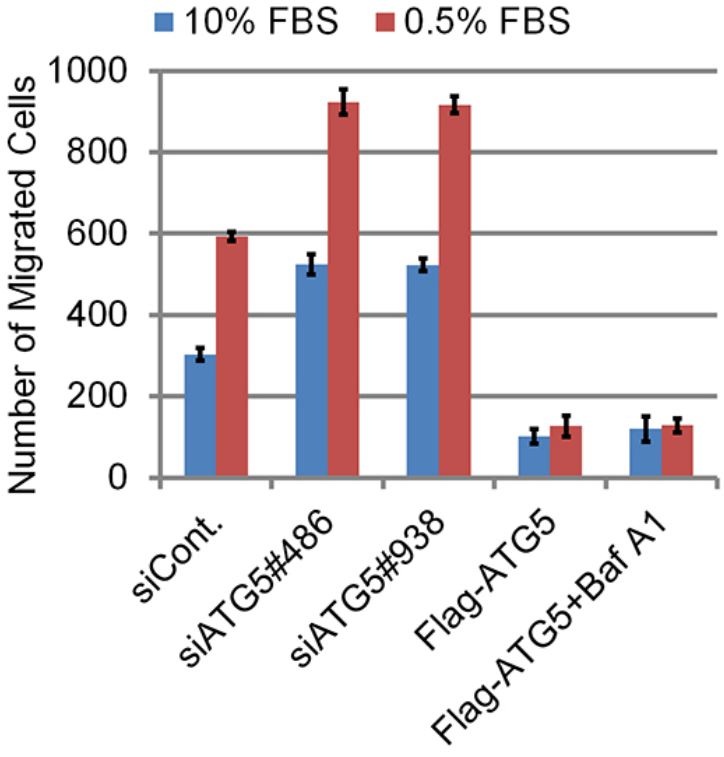

10% FBS
0.5% FBS
Number of Migrated Cells
1000
800
600
400
200
0
siCont.
siATG5#486
siATG5#938
Flag-ATG5
Flag-ATG5+Baf A1

Figure S5

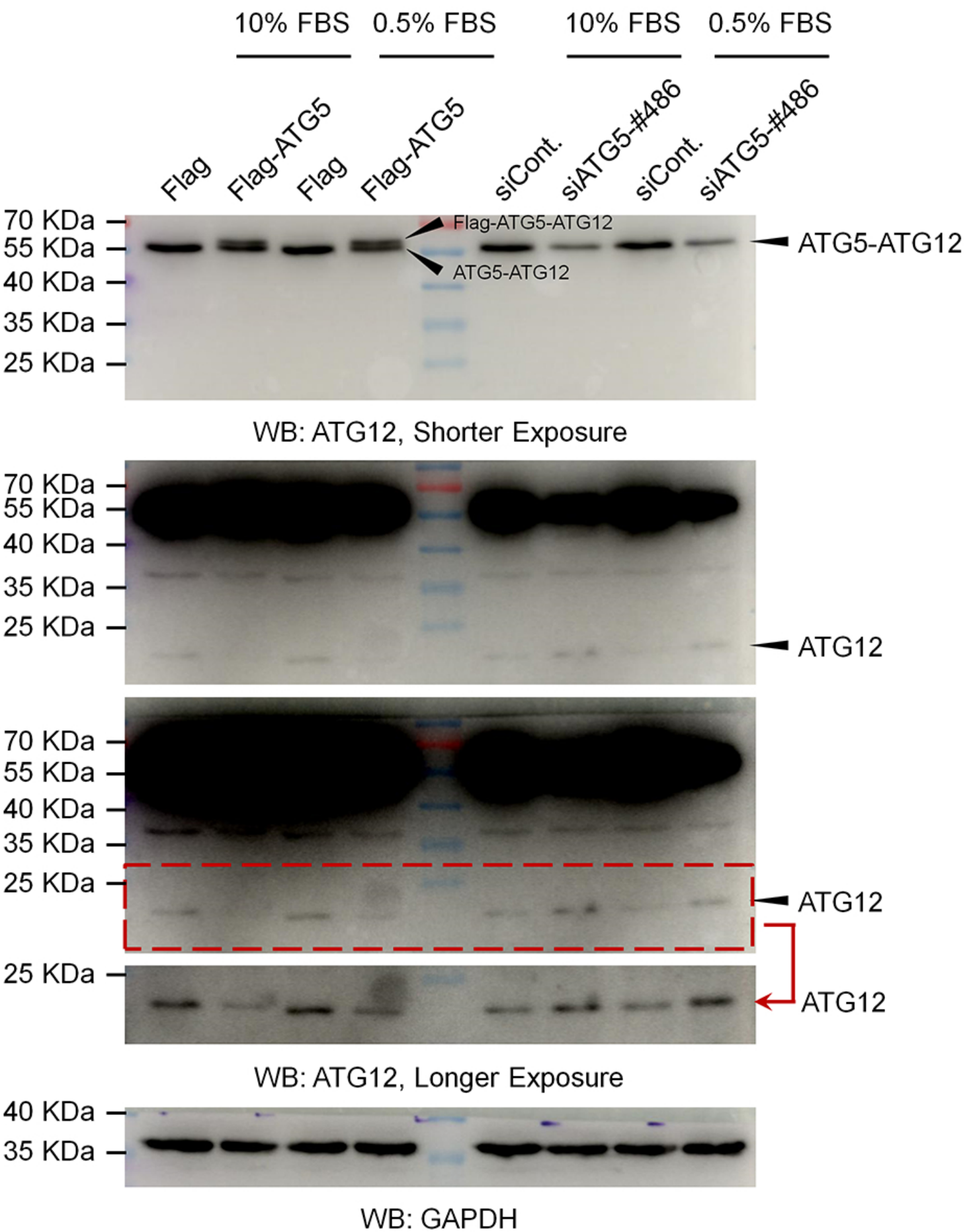
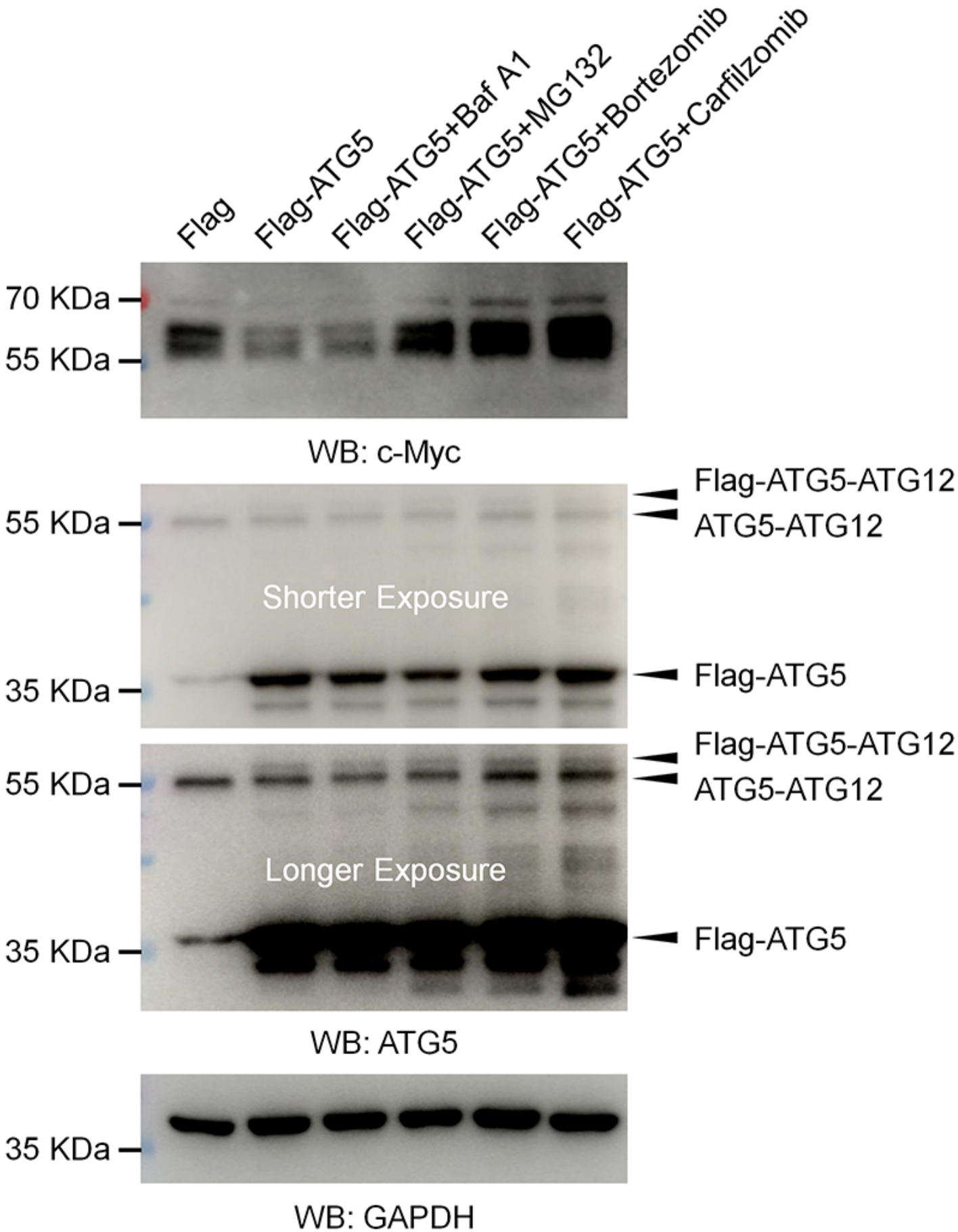

10% FBS
0.5% FBS
10% FBS
0.5% FBS
Flag
Flag-ATG5
Flag
Flag-ATG5
siCont.
siATG5-#486
siCont.
siATG5-#486
70 KDa
55 KDa
40 KDa
35 KDa
25 KDa
Flag-ATG5-ATG12
ATG5-ATG12
ATG5-ATG12
WB: ATG12, Shorter Exposure
70 KDa
55 KDa
40 KDa
35 KDa
25 KDa
ATG12
70 KDa
55 KDa
40 KDa
35 KDa
25 KDa
ATG12
25 KDa
ATG12
WB: ATG12, Longer Exposure
40 KDa
35 KDa
WB: GAPDH

Figure S6


Flag
Flag-ATG5
Flag-ATG5+Baf A1
Flag-ATG5+MG132
Flag-ATG5+Bortezomib
Flag-ATG5+Carfilzomib
70 KDa
55 KDa
WB: c-Myc
55 KDa
Shorter Exposure
35 KDa
Flag-ATG5-ATG12
ATG5-ATG12
Flag-ATG5
55 KDa
Longer Exposure
35 KDa
Flag-ATG5-ATG12
ATG5-ATG12
Flag-ATG5
WB: ATG5
35 KDa
WB: GAPDH