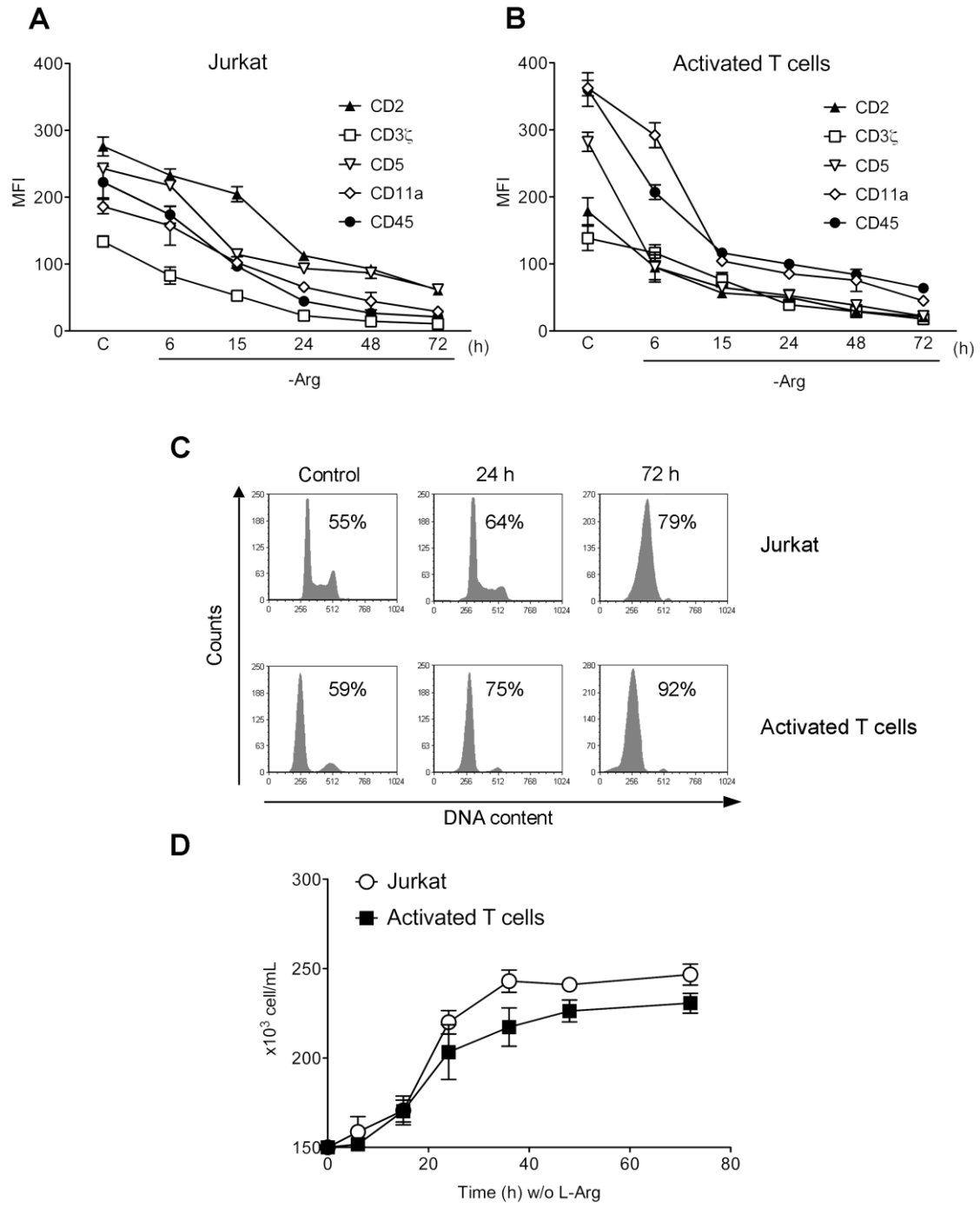

A
Jurkat
MFI
400
300
200
100
0
CD2
CD3ζ
CD5
CD11a
CD45
C
6
15
24
48
72
(h)
-Arg
B
Activated T cells
MFI
400
300
200
100
0
CD2
CD3ζ
CD5
CD11a
CD45
C
6
15
24
48
72
(h)
-Arg
C
Control
24 h
72 h
55%
64%
79%
59%
75%
92%
Jurkat
Activated T cells
Counts
DNA content
D
Jurkat
Activated T cells
x10³ cell/mL
300
250
200
150
0
20
40
60
80
Time (h) w/o L-Arg

Fig.1

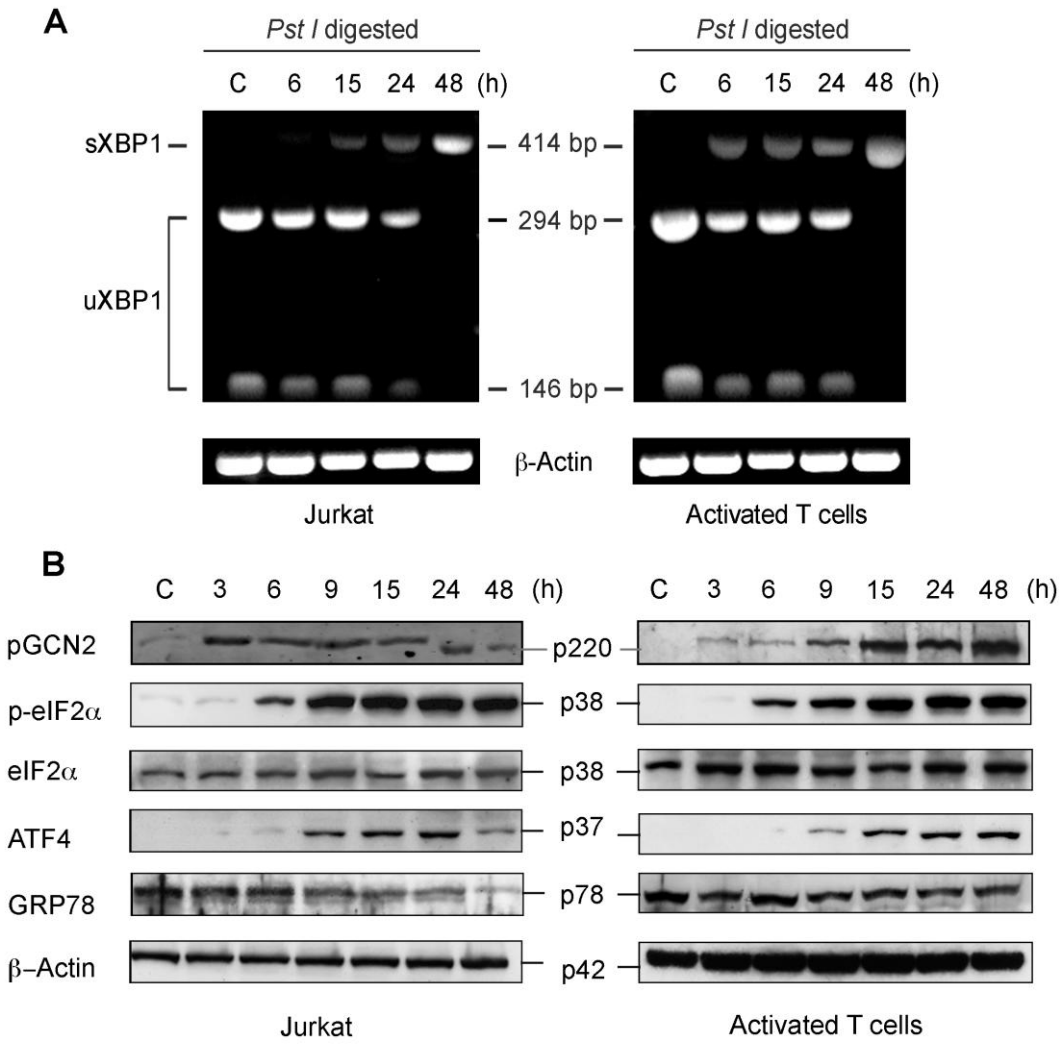

A
Pst I digested
C 6 15 24 48 (h)
sXBP1
uXBP1
414 bp
294 bp
146 bp
β-Actin
Jurkat
Pst I digested
C 6 15 24 48 (h)
Activated T cells
B
C 3 6 9 15 24 48 (h)
pGCN2
p-eIF2α
eIF2α
ATF4
GRP78
β–Actin
p220
p38
p38
p37
p78
p42
Jurkat
C 3 6 9 15 24 48 (h)
Activated T cells

Fig. 2

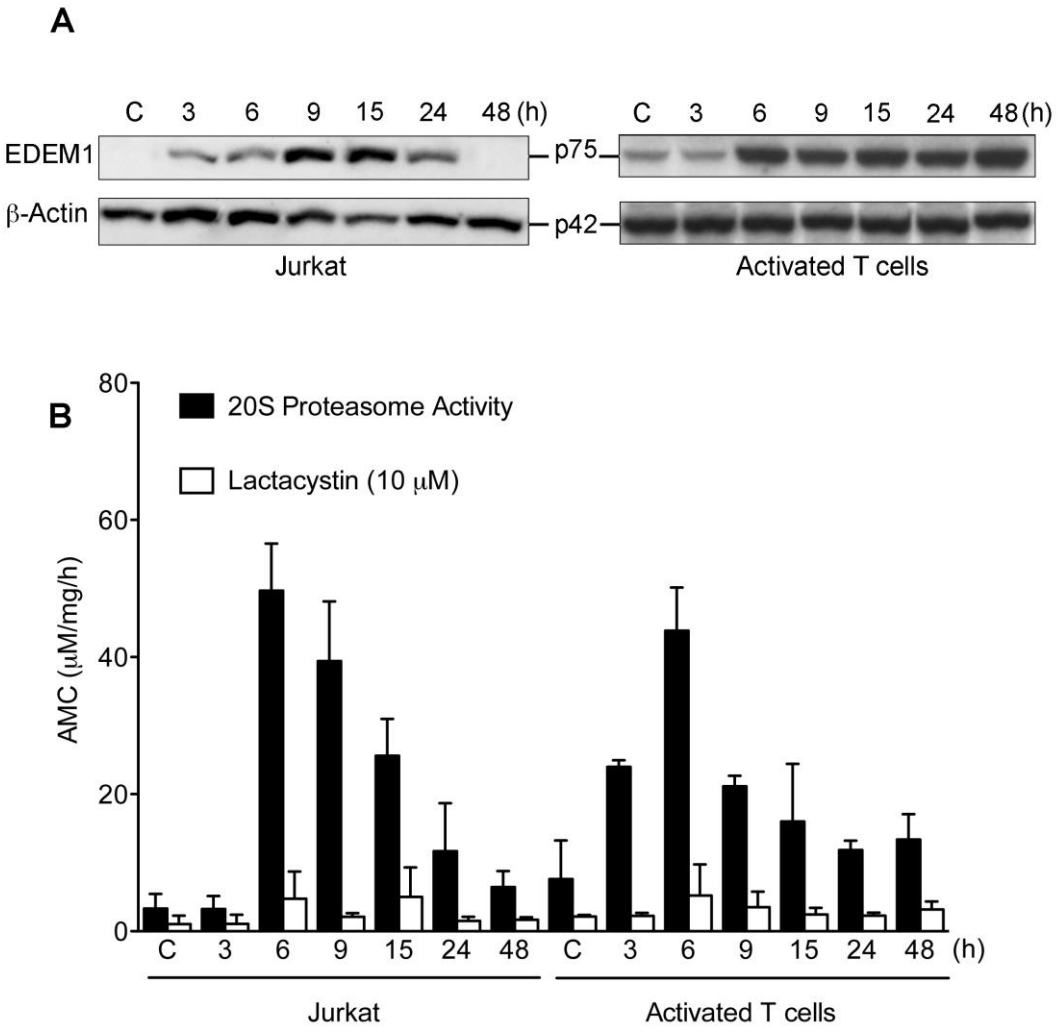

A
C 3 6 9 15 24 48 (h)
C 3 6 9 15 24 48 (h)
EDEM1
β-Actin
p75
p42
Jurkat
Activated T cells
B
20S Proteasome Activity
Lactacystin (10 μM)
AMC (μM/mg/h)
80
60
40
20
0
C 3 6 9 15 24 48
C 3 6 9 15 24 48 (h)
Jurkat
Activated T cells

Fig. 3

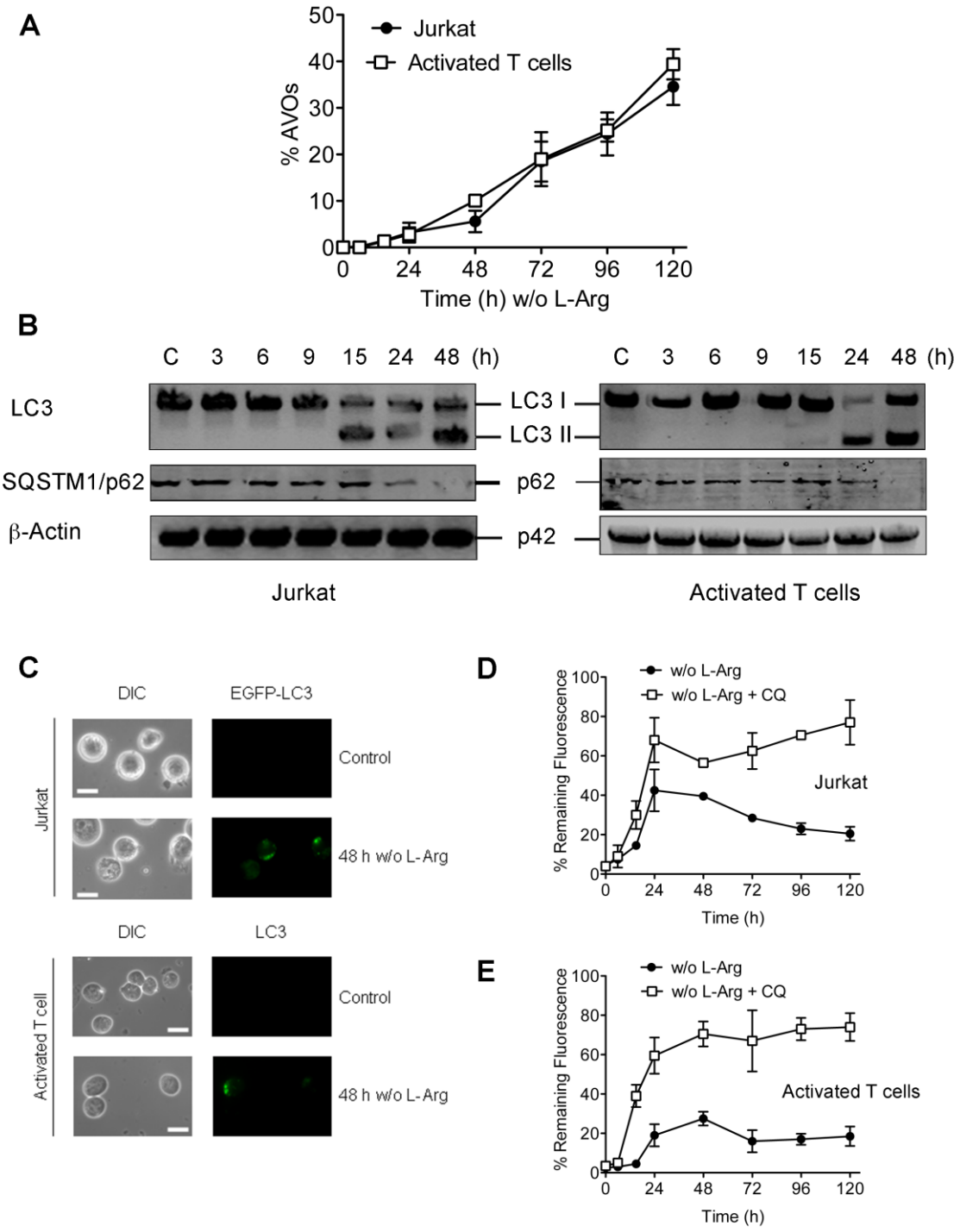

A
Jurkat
Activated T cells
% AVOs
Time (h) w/o L-Arg
B
C 3 6 9 15 24 48 (h)
LC3
LC3 I
LC3 II
SQSTM1/p62
p62
β-Actin
p42
Jurkat
Activated T cells
C
DIC
EGFP-LC3
Control
48 h w/o L-Arg
Jurkat
DIC
LC3
Control
48 h w/o L-Arg
Activated T cell
D
w/o L-Arg
w/o L-Arg + CQ
% Remaining Fluorescence
Jurkat
Time (h)
E
w/o L-Arg
w/o L-Arg + CQ
% Remaining Fluorescence
Activated T cells
Time (h)

Fig. 4

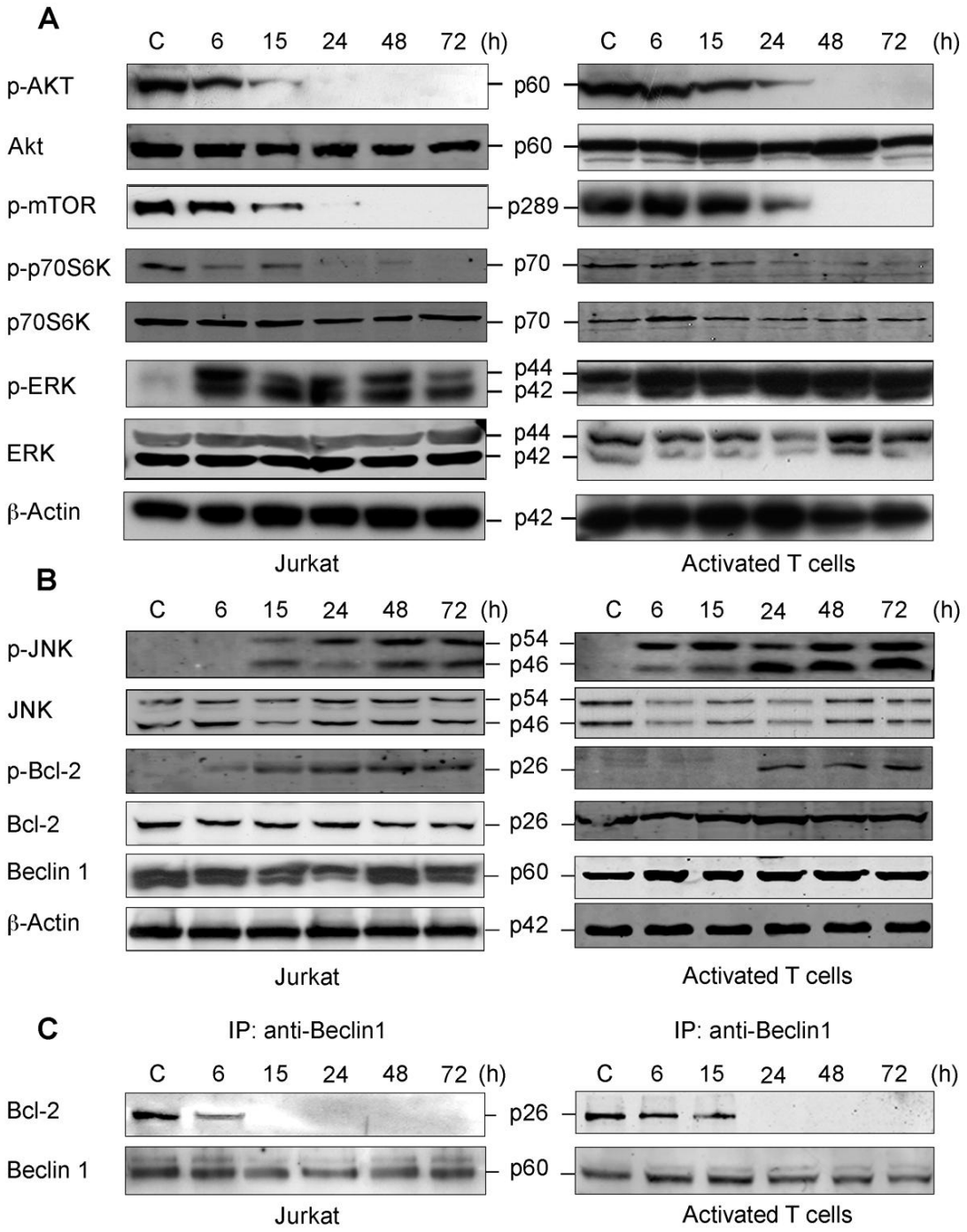

A
C 6 15 24 48 72 (h)
C 6 15 24 48 72 (h)
p-AKT
p60
Akt
p60
p-mTOR
p289
p-p70S6K
p70
p70S6K
p70
p-ERK
p44
p42
ERK
p44
p42
β-Actin
p42
Jurkat
Activated T cells
B
C 6 15 24 48 72 (h)
C 6 15 24 48 72 (h)
p-JNK
p54
p46
JNK
p54
p46
p-Bcl-2
p26
Bcl-2
p26
Beclin 1
p60
β-Actin
p42
Jurkat
Activated T cells
C
IP: anti-Beclin1
IP: anti-Beclin1
C 6 15 24 48 72 (h)
C 6 15 24 48 72 (h)
Bcl-2
p26
Beclin 1
p60
Jurkat
Activated T cells

Fig. 5

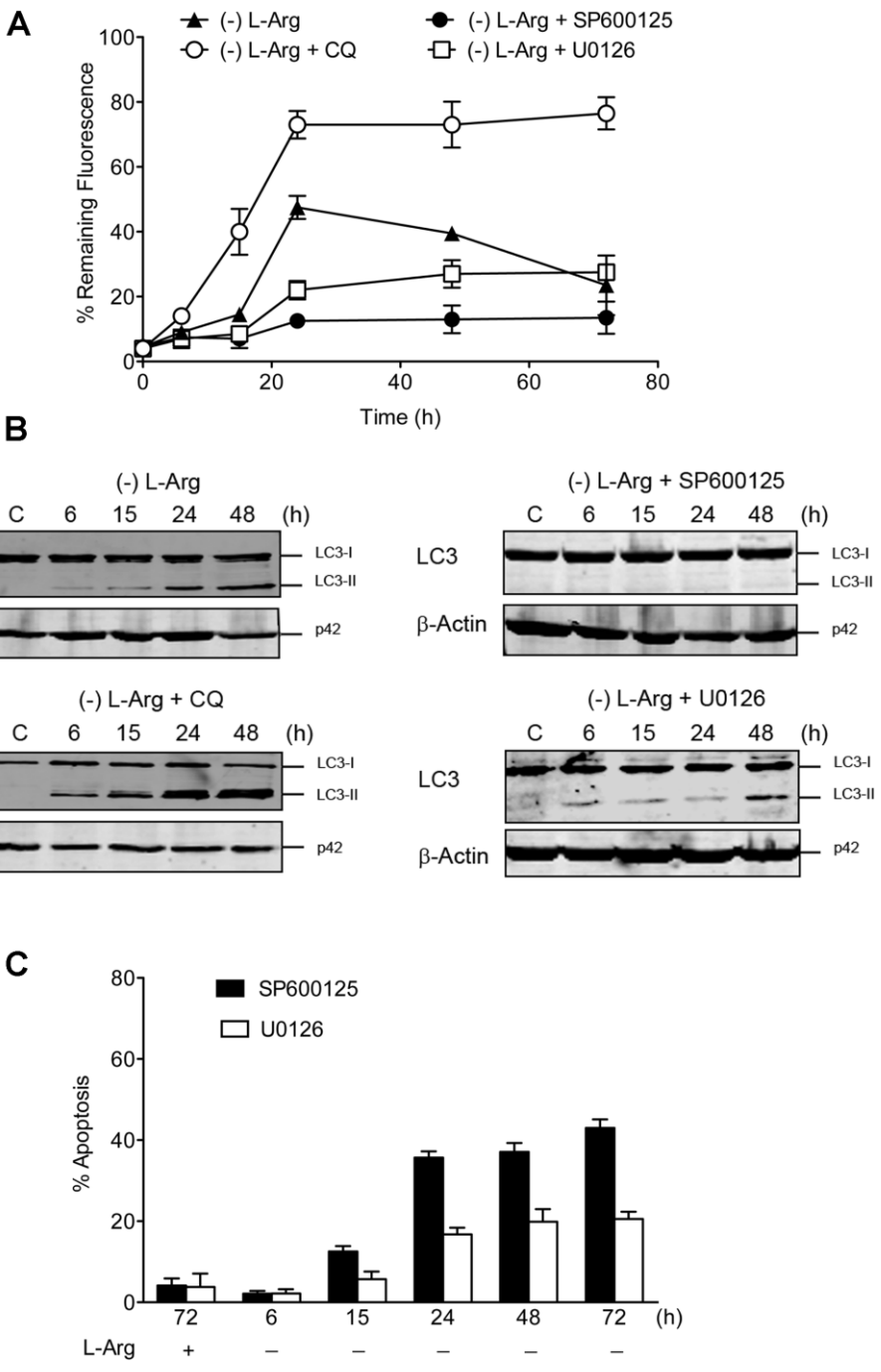

A
(-) L-Arg
(-) L-Arg + CQ
(-) L-Arg + SP600125
(-) L-Arg + U0126
% Remaining Fluorescence
Time (h)
B
(-) L-Arg
(-) L-Arg + SP600125
(-) L-Arg + CQ
(-) L-Arg + U0126
C 6 15 24 48 (h)
LC3
LC3-I
LC3-II
β-Actin
p42
C
SP600125
U0126
% Apoptosis
72 6 15 24 48 72 (h)
L-Arg + – – – – –

Fig. 6

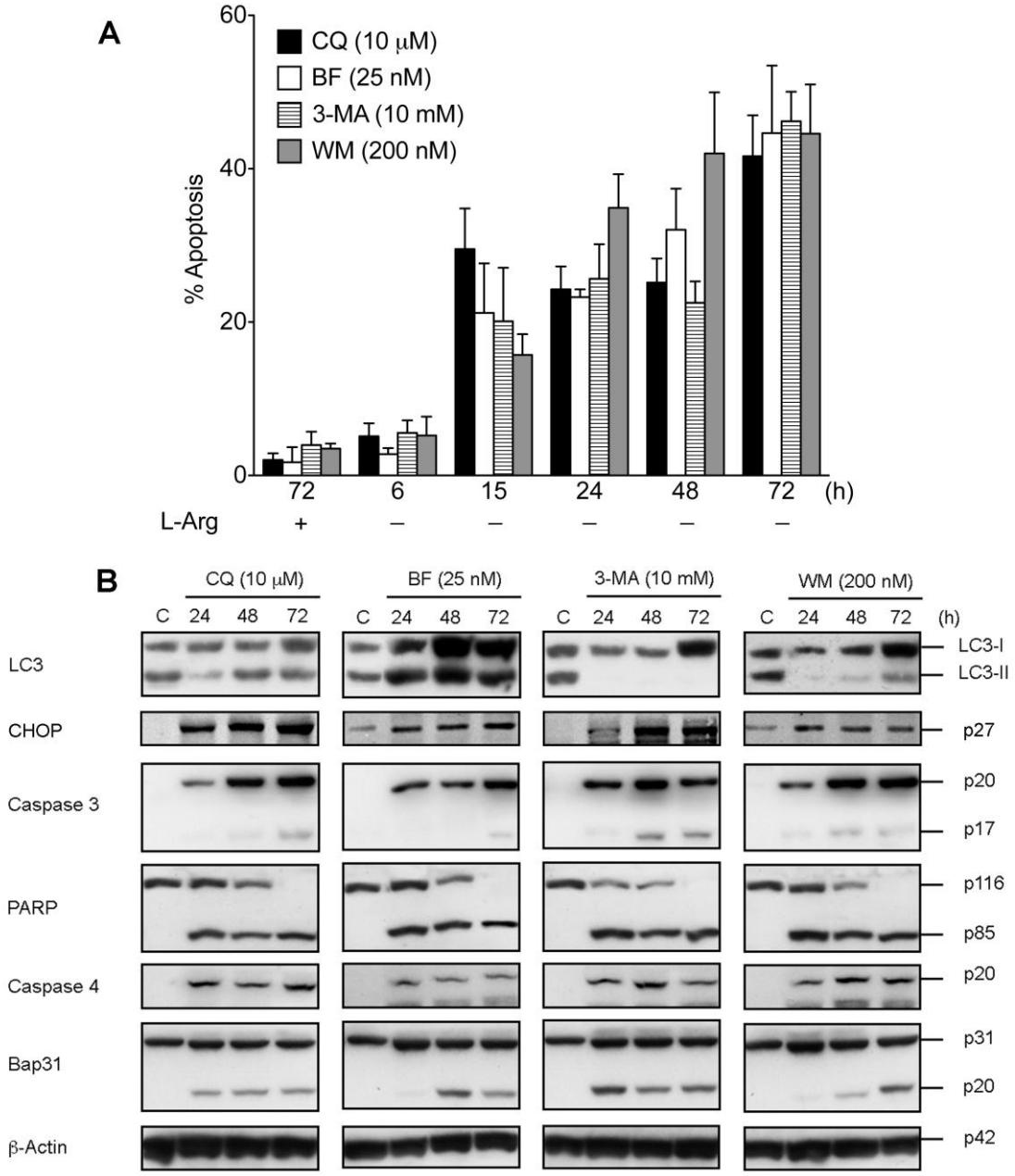

A
CQ (10 μM)
BF (25 nM)
3-MA (10 mM)
WM (200 nM)
% Apoptosis
60
40
20
0
72
6
15
24
48
72
(h)
L-Arg
+
–
–
–
–
–
B
CQ (10 μM)
BF (25 nM)
3-MA (10 mM)
WM (200 nM)
C
24
48
72
(h)
LC3
LC3-I
LC3-II
CHOP
p27
Caspase 3
p20
p17
PARP
p116
p85
Caspase 4
p20
Bap31
p31
p20
β-Actin
p42

Fig. 7

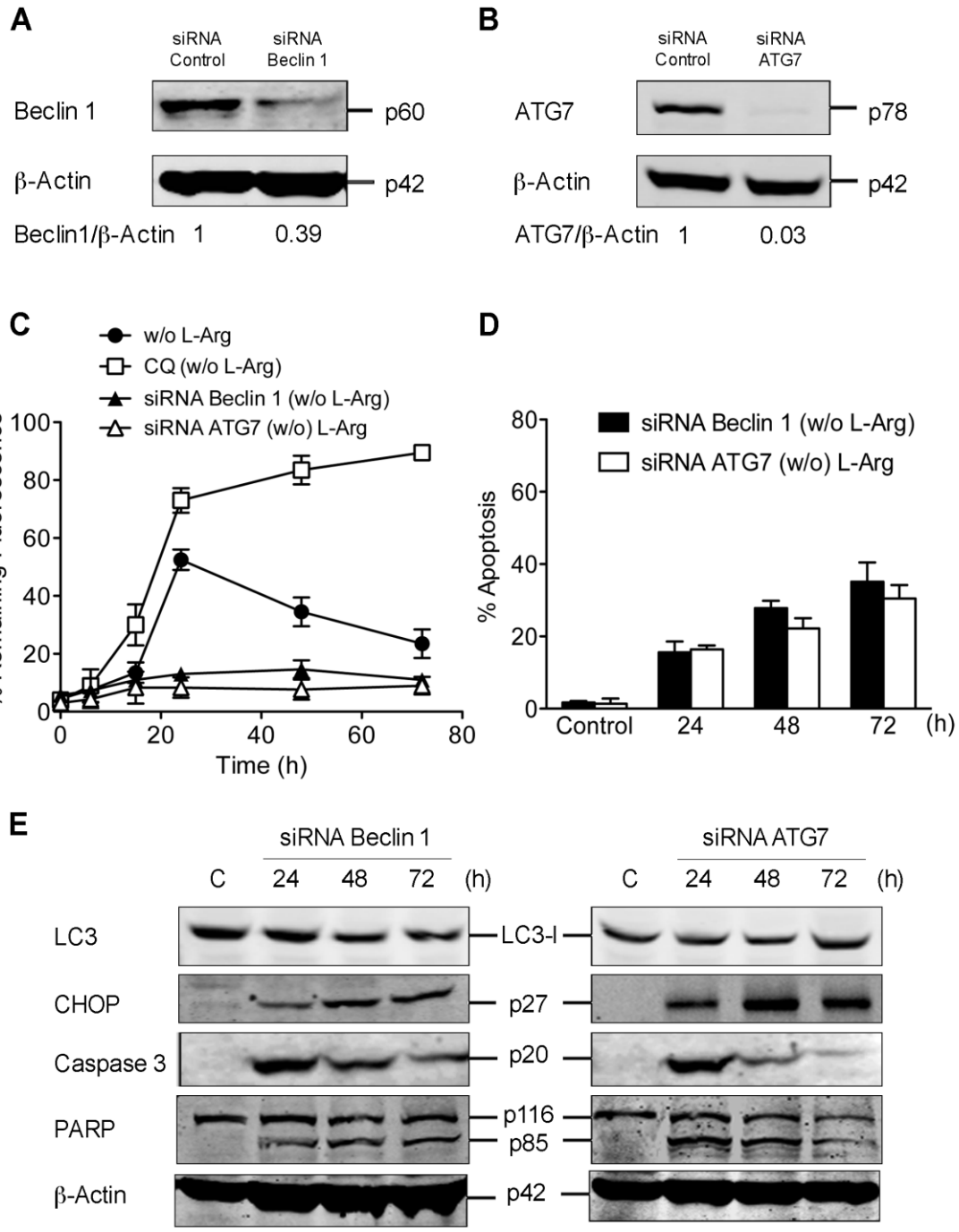

A
siRNA Control
siRNA Beclin 1
Beclin 1
p60
β-Actin
p42
Beclin1/β-Actin 1 0.39
B
siRNA Control
siRNA ATG7
ATG7
p78
β-Actin
p42
ATG7/β-Actin 1 0.03
C
w/o L-Arg
CQ (w/o L-Arg)
siRNA Beclin 1 (w/o L-Arg)
siRNA ATG7 (w/o) L-Arg
% Remaining Fluorescence
Time (h)
D
siRNA Beclin 1 (w/o L-Arg)
siRNA ATG7 (w/o) L-Arg
% Apoptosis
Control
24
48
72
(h)
E
siRNA Beclin 1
siRNA ATG7
C
24
48
72
(h)
LC3
LC3-I
CHOP
p27
Caspase 3
p20
PARP
p116
p85
β-Actin
p42

Fig. 8

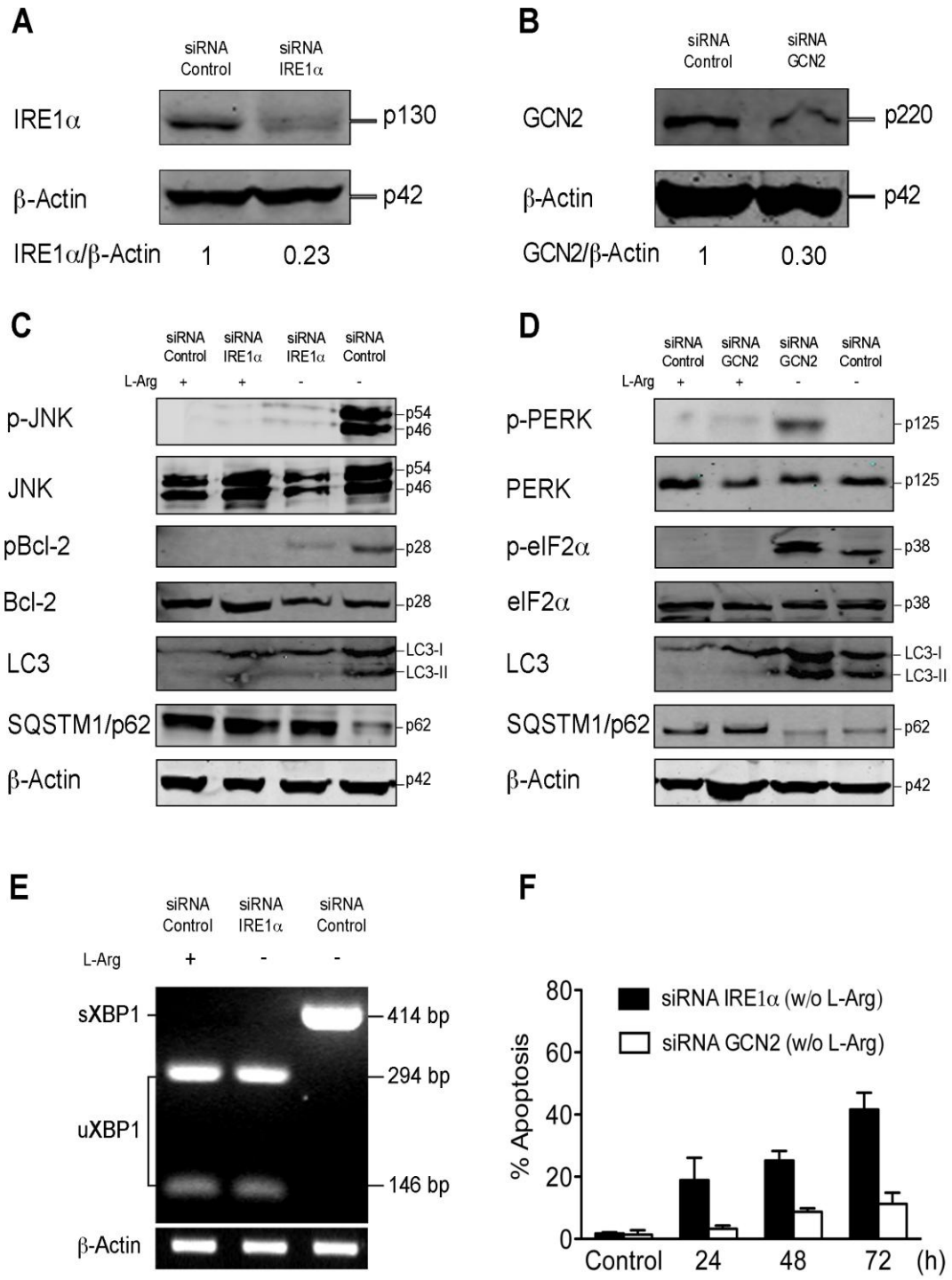

A
siRNA Control
siRNA IRE1α
IRE1α
p130
β-Actin
p42
IRE1α/β-Actin 1 0.23
B
siRNA Control
siRNA GCN2
GCN2
p220
β-Actin
p42
GCN2/β-Actin 1 0.30
C
siRNA Control
siRNA IRE1α
siRNA IRE1α
siRNA Control
L-Arg + + - -
p-JNK
p54
p46
JNK
p54
p46
pBcl-2
p28
Bcl-2
p28
LC3
LC3-I
LC3-II
SQSTM1/p62
p62
β-Actin
p42
D
siRNA Control
siRNA GCN2
siRNA GCN2
siRNA Control
L-Arg + + - -
p-PERK
p125
PERK
p125
p-eIF2α
p38
eIF2α
p38
LC3
LC3-I
LC3-II
SQSTM1/p62
p62
β-Actin
p42
E
siRNA Control
siRNA IRE1α
siRNA Control
L-Arg + - -
sXBP1
414 bp
294 bp
uXBP1
146 bp
β-Actin
F
siRNA IRE1α (w/o L-Arg)
siRNA GCN2 (w/o L-Arg)
% Apoptosis
80
60
40
20
0
Control
24
48
72
(h)

Fig. 9

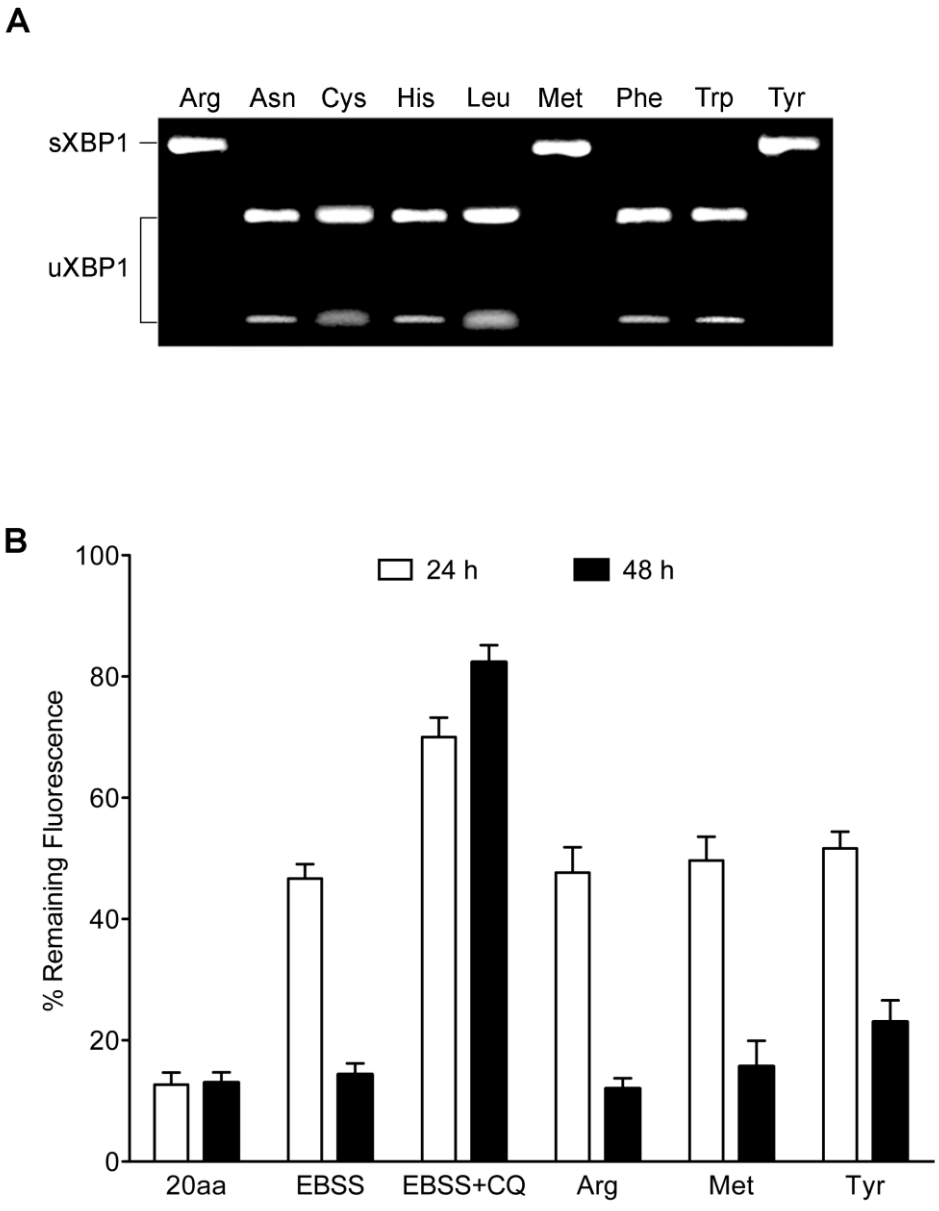

A
Arg
Asn
Cys
His
Leu
Met
Phe
Trp
Tyr
sXBP1
uXBP1
B
24 h
48 h
% Remaining Fluorescence
100
80
60
40
20
0
20aa
EBSS
EBSS+CQ
Arg
Met
Tyr

Fig. 10

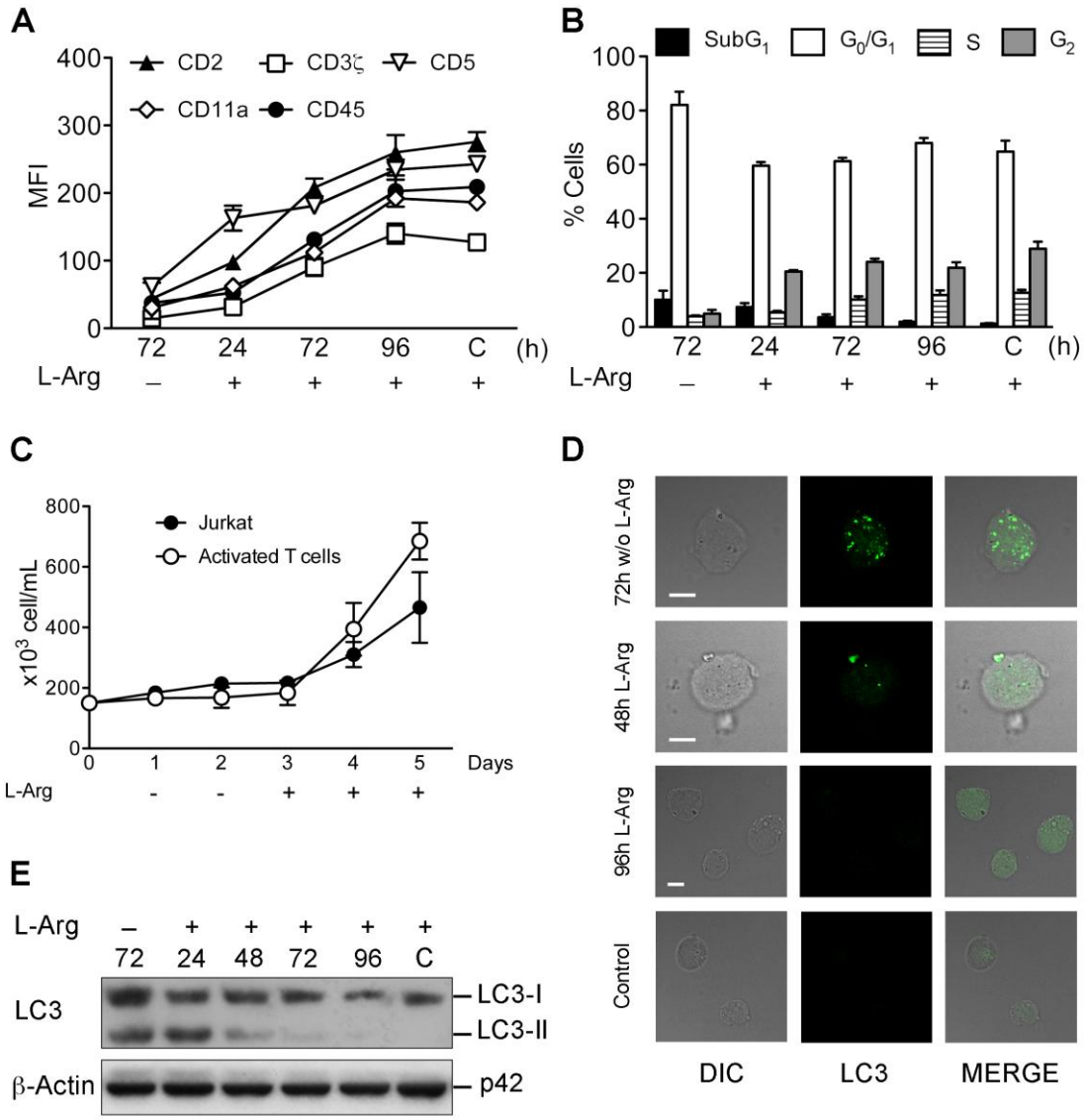
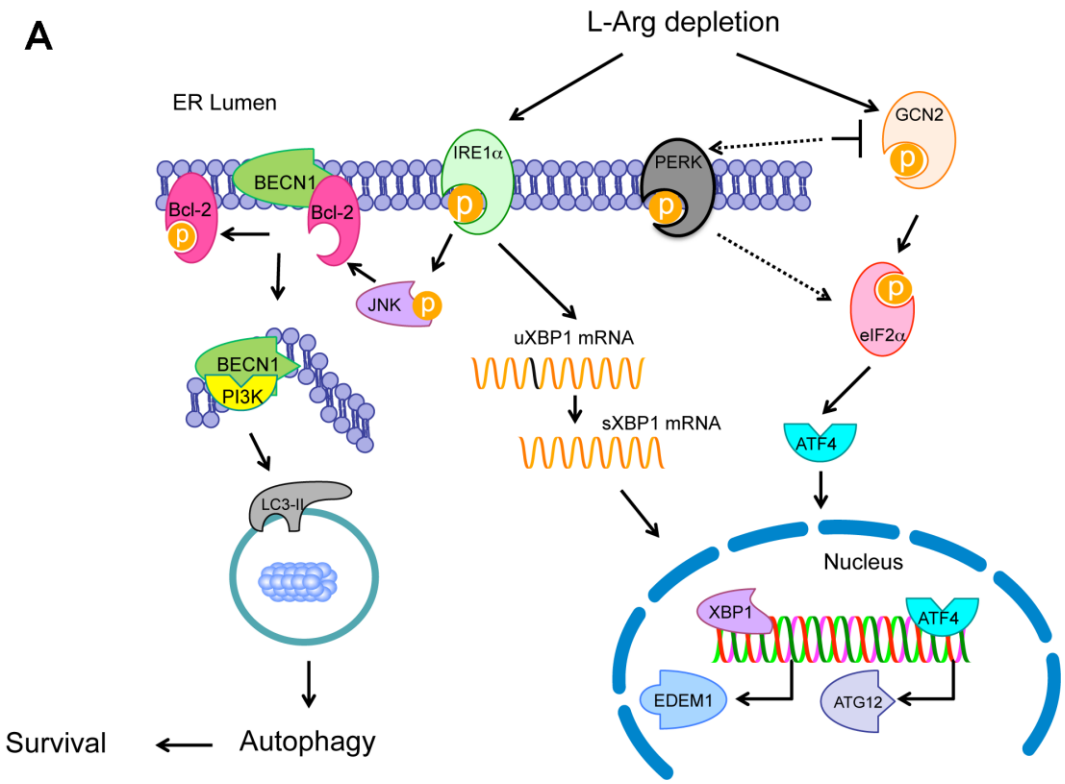

A
CD2
CD3ζ
CD5
CD11a
CD45
MFI
400
300
200
100
0
72
24
72
96
C
(h)
L-Arg
–
+
+
+
+
B
SubG1
G0/G1
S
G2
% Cells
100
80
60
40
20
0
72
24
72
96
C
(h)
L-Arg
–
+
+
+
+
C
Jurkat
Activated T cells
x10³ cell/mL
800
600
400
200
0
0
1
2
3
4
5
Days
L-Arg
-
-
+
+
+
D
72h w/o L-Arg
48h L-Arg
96h L-Arg
Control
DIC
LC3
MERGE
E
L-Arg
–
+
+
+
+
+
72
24
48
72
96
C
LC3
LC3-I
LC3-II
β-Actin
p42

Fig. 11


A
L-Arg depletion
ER Lumen
GCN2
IRE1α
PERK
BECN1
Bcl-2
Bcl-2
JNK
uXBP1 mRNA
eIF2α
BECN1
PI3K
sXBP1 mRNA
ATF4
LC3-II
Nucleus
XBP1
ATF4
EDEM1
ATG12
Survival
Autophagy

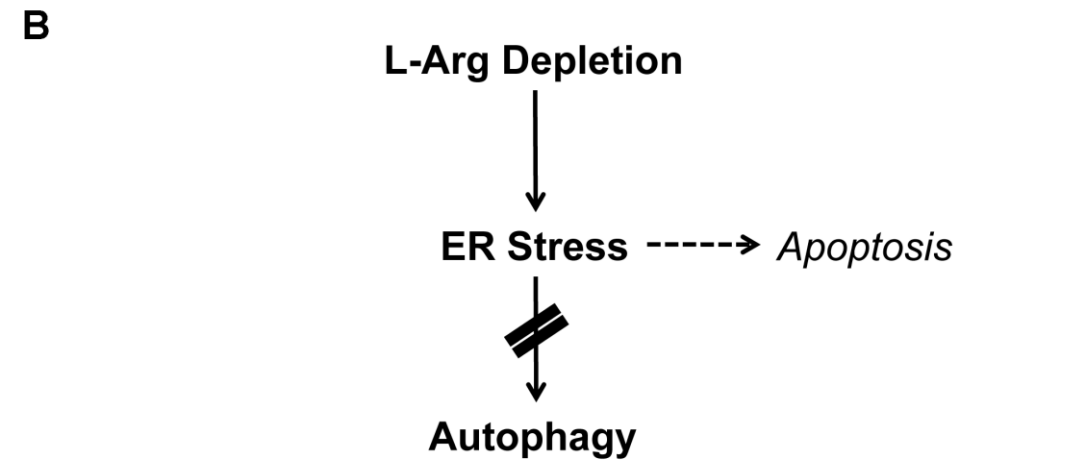
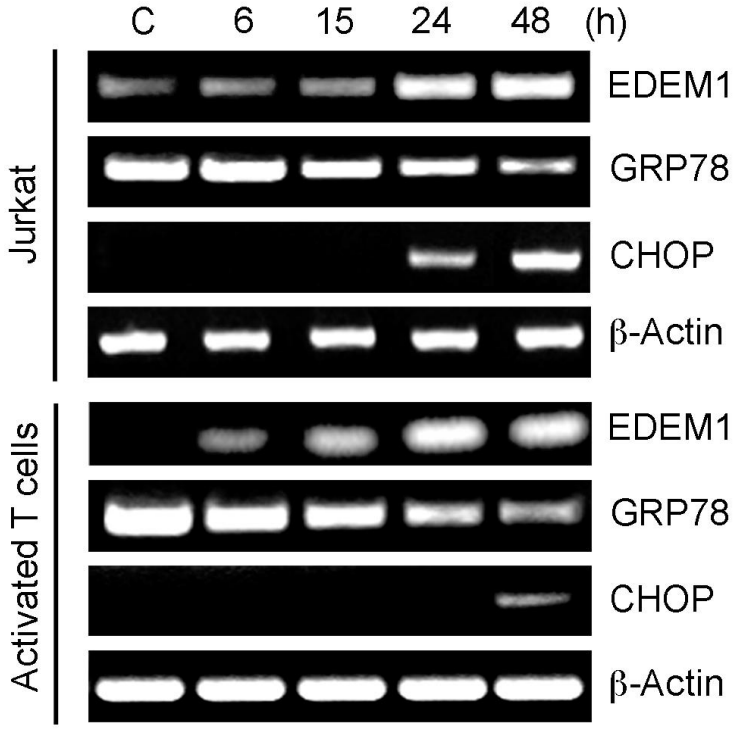

B
L-Arg Depletion
ER Stress
Apoptosis
Autophagy

Fig. 12



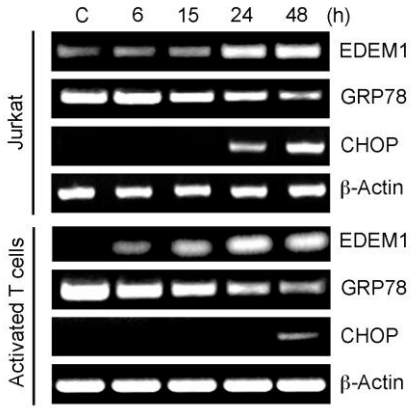


Figure S1. Semiquantitative RT-PCR of ER stress markers in Jurkat cells and peripheral blood mitogen-activated T cells following L-Arg depletion. Jurkat and peripheral blood mitogen-activated T cells were incubated with (*C*) or without L-Arg for the indicated times, and then total RNA was isolated and subjected to semiquantitative RT-PCR using specific primers for the following UPR genes: *EDEM1* (ER degradation-enhancing alpha-mannosidase-like 1), 300 bp; *GRP78* (78 kDa glucose-regulated protein), 306 bp; *CHOP* (C/EBP homologous protein), 357 bp. *β-Actin* was used as a loading control. Data shown are representative of 3 experiments performed.