Supplementary Material for

Myc inhibition impairs autophagosome formation

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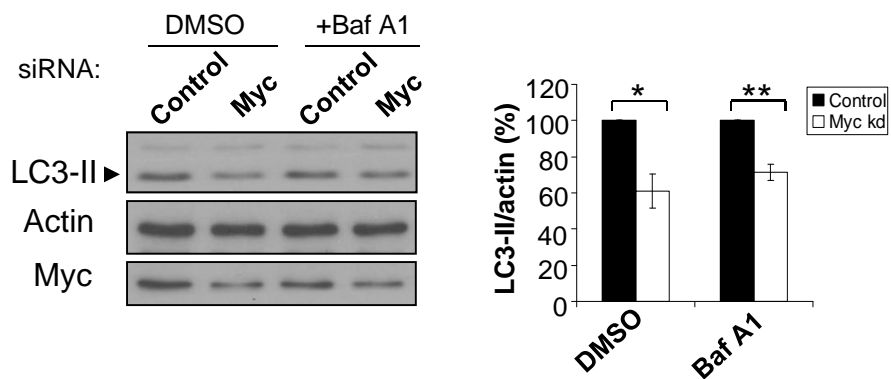*To whom correspondence should be addressed. Email: dcr1000@cam.ac.uk

This file includes:

1.   Supplementary Figures (Fig. S1-S6)
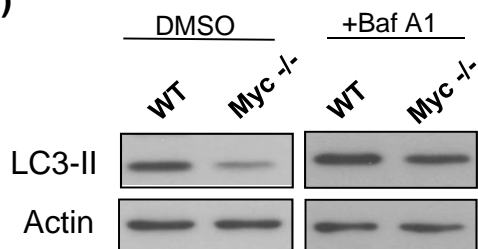2.   Supplementary Figure Legends

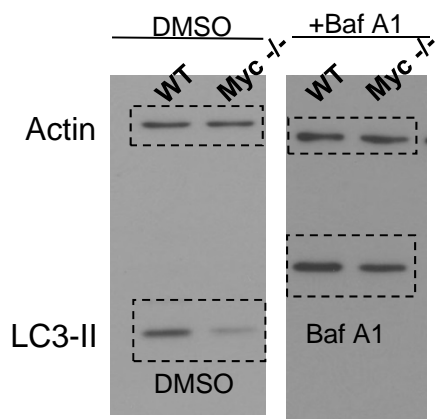# 1. SUPPLEMENTARY FIGURES

FIGURE S1

A

siRNA:

DMSO — Control, Myc

+Baf A1 — Control, Myc

LC3-II ▶
Actin
Myc

Bar chart: LC3-II/actin (%), y-axis 0–120

■ Control □ Myc kd

x-axis: DMSO, Baf A1

* (DMSO), ** (Baf A1)

B (i)

DMSO — WT, Myc -/-

+Baf A1 — WT, Myc -/-

LC3-II
Actin

(ii) Whole blots for Figure S1B:

DMSO — WT, Myc -/-

+Baf A1 — WT, Myc -/-

Actin

LC3-II

DMSO

Baf A1

C

Myc-I :   -   +   -   +

LC3-II
Actin
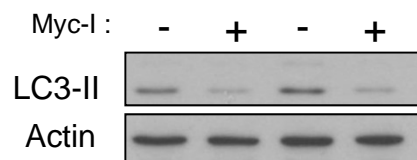
FIGURE S2

FIGURE S3

FIGURE S4
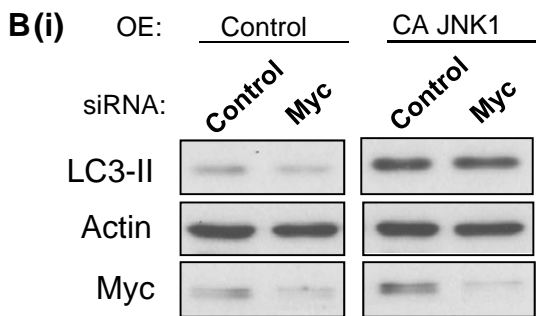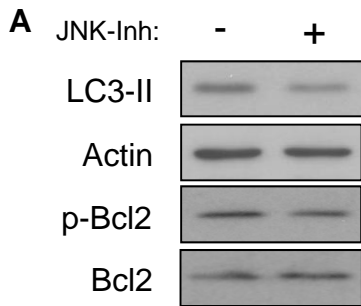
A JNK-Inh: **−**   **+**

LC3-II

Actin

p-Bcl2

Bcl2

B(i)   OE: Control   CA JNK1

siRNA: Control   Myc   Control   Myc

LC3-II

Actin

Myc

(ii) Whole blots for Figure S4B:

Control   CA JNK1   Control   CA JNK1

Myc siRNA: **− +**   **− +**   **− +**   **− +**

Actin

LC3-II

Longer exposure   Shorter exposure

C

FIGURE S5

A

siRNA: Control Myc

p-p38

p38

siRNA: Control Myc

p-ERK1/2

ERK1/2

B

H2O2(250uM):	-	-	+	+
Control siRNA:	+	-	+	-
Myc siRNA:	-	+	-	+

LC3-II

Actin

FIGURE S6

Whole blots for Figure 5D:

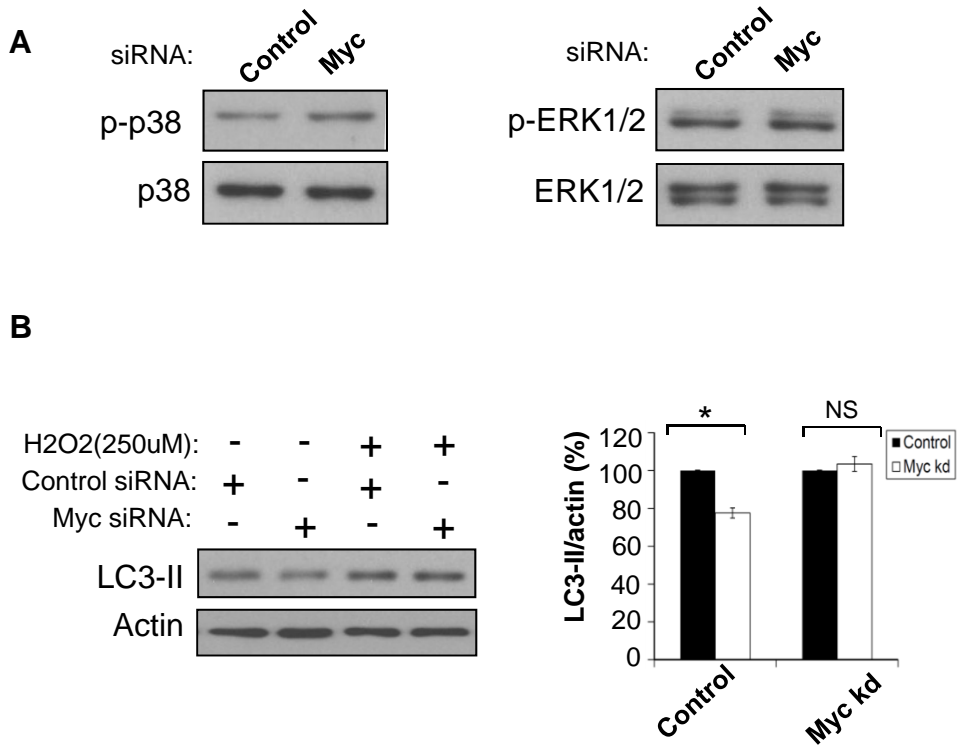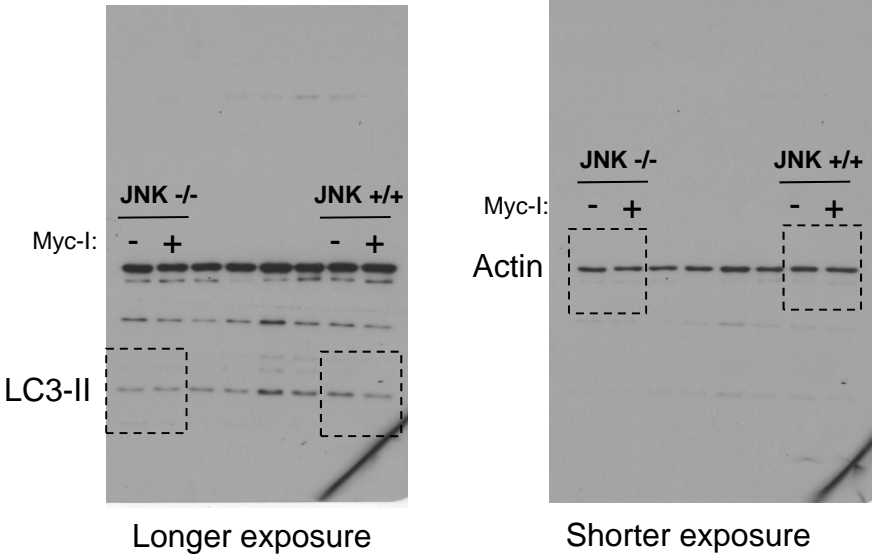Longer exposure   Shorter exposure
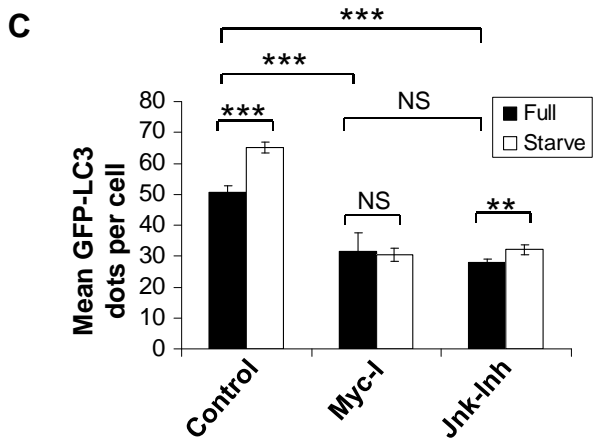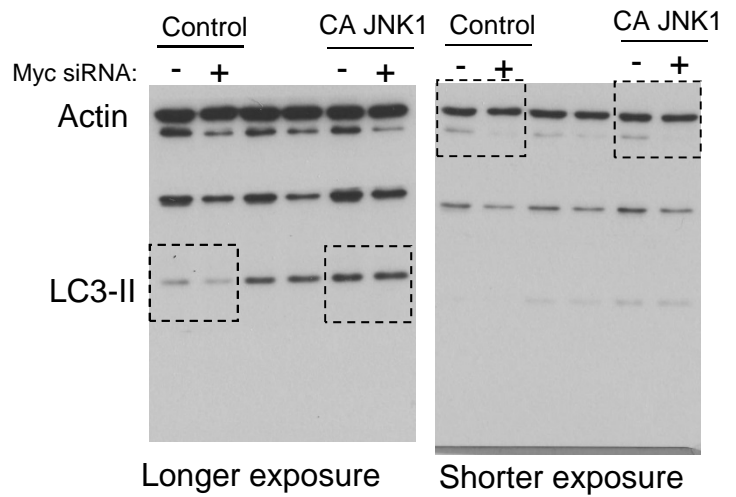
2. LEGENDS FOR SUPPLEMENTARY FIGURES

Figure S1

(A) HEK293 cells subjected to control or Myc siRNA knockdown for 72 h were treated with 400 nM bafilomycin A1 (Baf A1) or DMSO during the last 4 h. Cells were lysed and assessed for LC3-II levels by Western blotting. Graph shows quantification of LC3-II/actin ratio. The values represent the mean ± SEM of the percentage of LC3-II/actin from three independent experiments. *P<0.05; **P<0.005 by two-tailed Student's t-test as described in methods.

(B) (i) Myc+/+ (WT) or Myc-/- MEFs treated with 400 nM Baf A1 or DMSO for 4 h were assessed for LC3-II levels by Western blotting. **(ii)** Whole blots for (i).

(C) HEK293 cells treated with 60 μM Myc inhibitor (Myc-I) for 8 h were assessed for LC3-II levels by Western blotting.

Figure S2

Myc+/+ (WT) or Myc-/- rat fibroblasts were probed for p62. Graph shows quantification of p62/actin ratio. The values represent the mean ± SEM of the percentage of p62/actin from three independent experiments. *P<0.05.

Figure S3

HeLa cells treated with 30 μM of another Myc inhibitor (10074-G5) for 16 h were assessed for phospho-Bcl2 and LC3-II levels by Western blotting. The values represent the mean ± SEM of the percentage of LC3-II/actin from three independent experiments. **P<0.005.

Figure S4

(A) HeLa cells treated with 20 μM JNK inhibitor VIII (JNK-Inh) for 3 h were assessed for phospho-Bcl2 and LC3-II levels by Western Blotting.

(B) (i) HeLa cells subjected to control or Myc siRNA knockdown for 72 h were transfected with pcDNA3.1 empty vector, or constitutive active (CA) JNK1 overexpression constructs for the last 24 h. Cell lysates were assessed for LC3-II levels by Western blotting. **(ii)** Whole blots for (i).

(C) HeLa cells stably expressing GFP-LC3 treated with either 60 μM Myc-I for 16 h or 20 μM JNK-Inh for 3 h were cultured under full medium or 3 h HBSS starvation

condition before being fixed for quantification of LC3 dots. The number of LC3 dots was quantified by automated counting (see methods). At least 300 cells were counted per sample. Graph shows quantification of the mean of GFP-LC3 dots per cell. The values represent the mean ± SEM of the mean of GFP-LC3 dots per cell whereby ≥300 cells per sample were analysed in a study representative of three independent experiments. ***$P < 0.0005$; **$P < 0.005$, by one-way ANOVA, with Bonferrroni post hoc test. ***$P < 0.0005$ for control vs. treatment pair (with either Myc-I or JNK-Inh) in each full medium condition and starvation condition.

Figure S5

(A) HeLa cells subjected to control or Myc siRNA knockdown for 72 h were assessed for phospho- or total p38 (left panel) or phospho- or total ERK1/2 (right panel) by Western blotting.

(B) HeLa cells subjected to control or Myc siRNA knockdown for 72 h were treated with 250 µM H2O2 for the last 24 h. Cell lysates were assessed for LC3-II levels by Western Blotting. Graph shows quantification of LC3-II/actin ratio. The values represent the mean ± SEM of the percentage of LC3-II/actin from three independent experiments. *$P < 0.05$; NS, not significant.

Figure S6

Whole blots for Figure 5D.