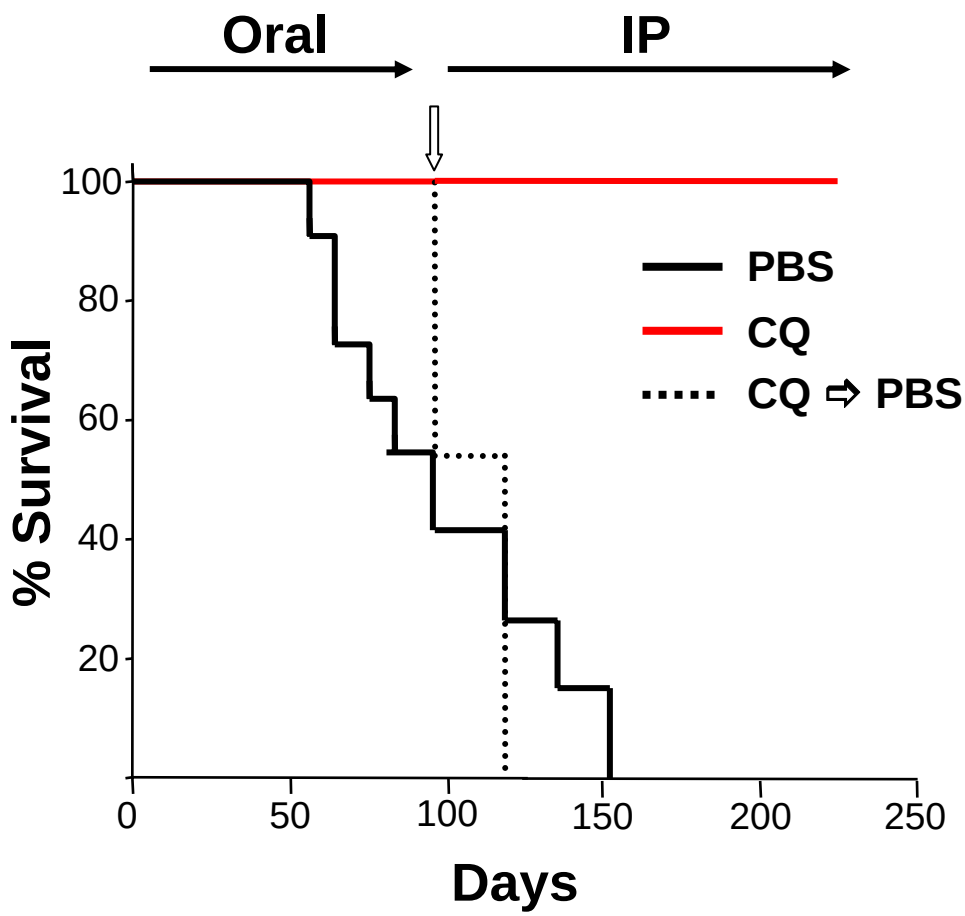
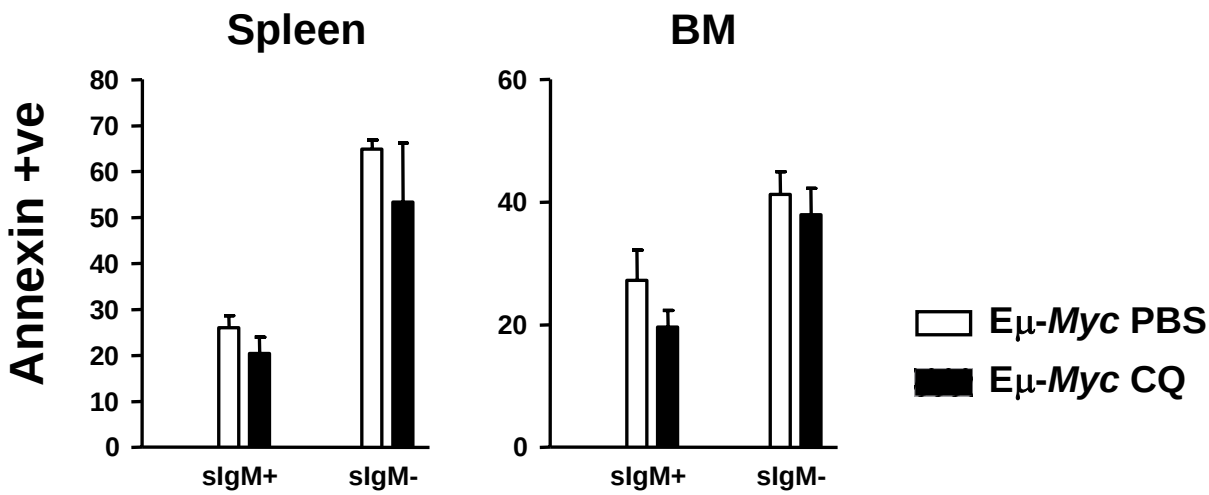
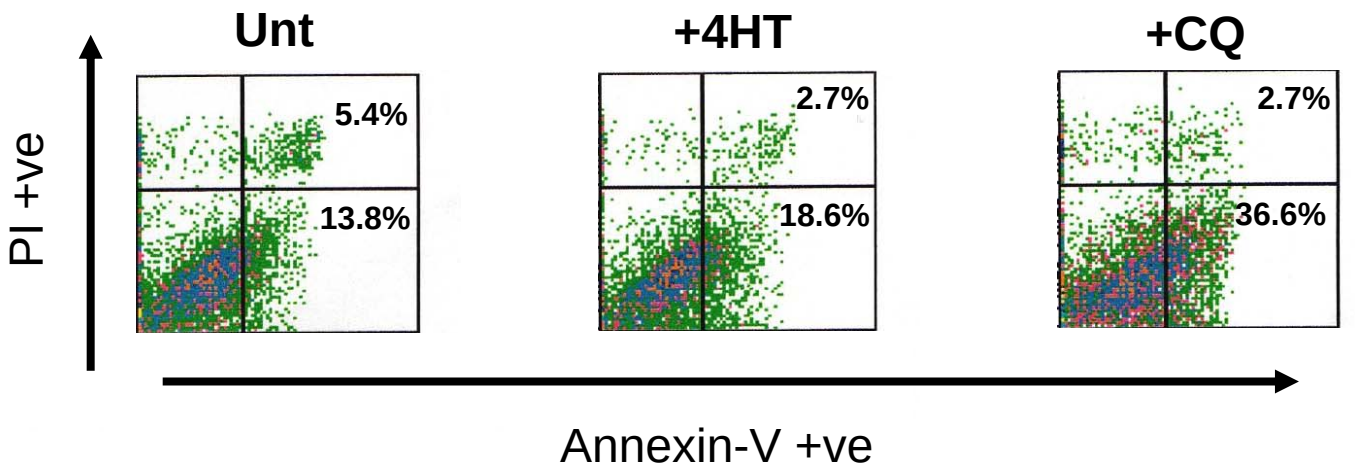
Supplemental Figure S1



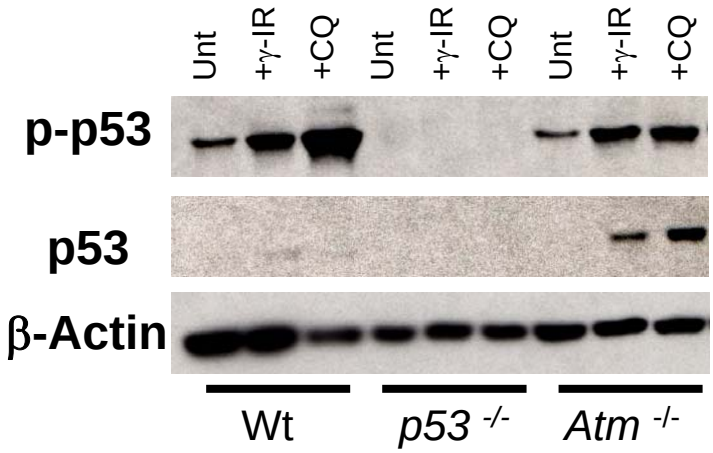
Supplemental Figure S2

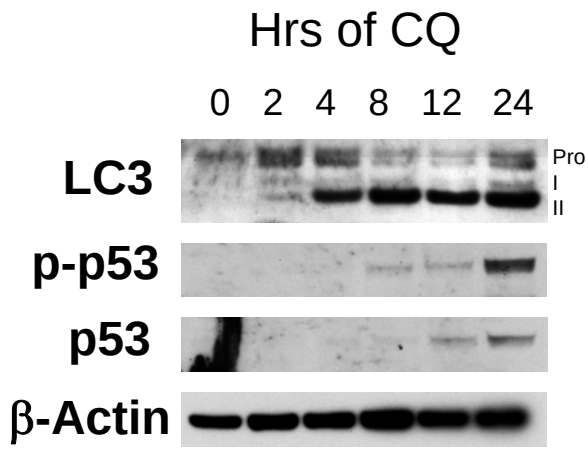


Supplemental Figure S3

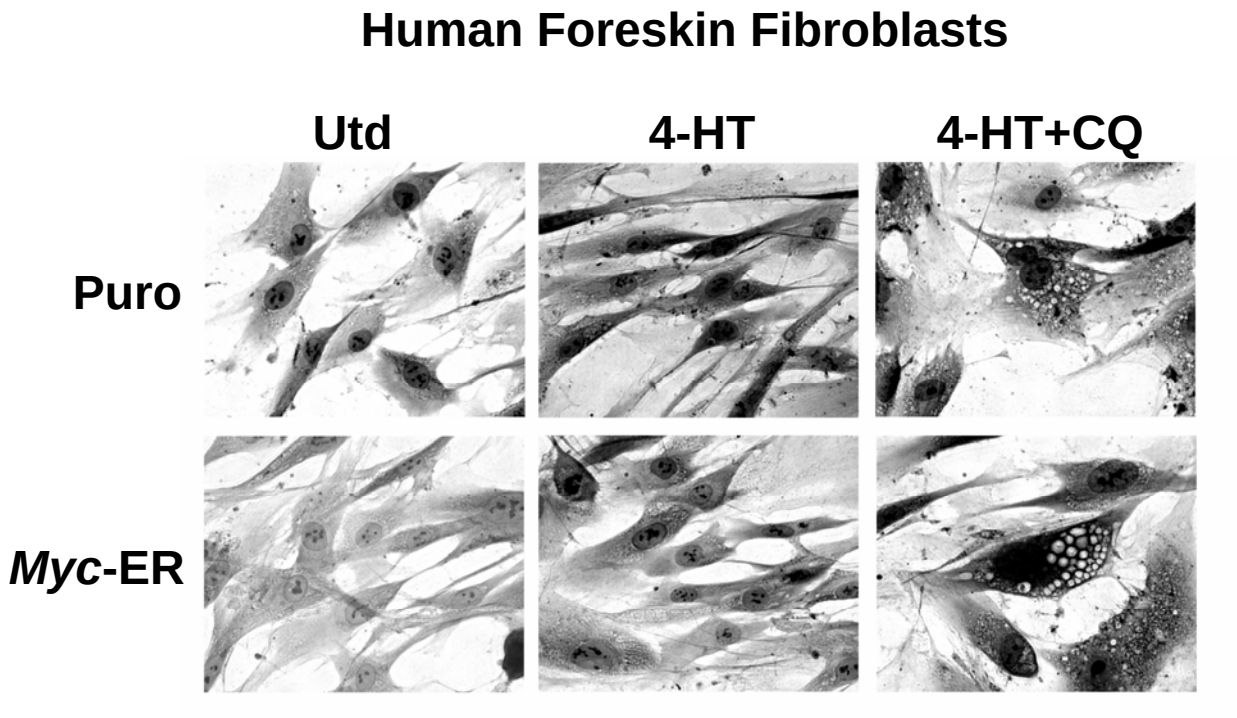


Supplemental Figure S4



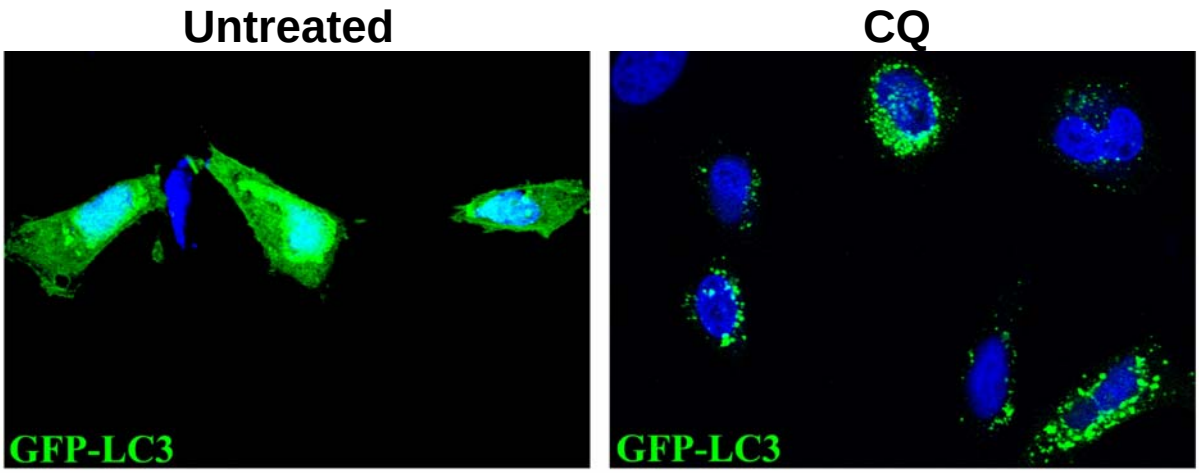


Supplemental Figure S5



Supplemental Figure S6

A



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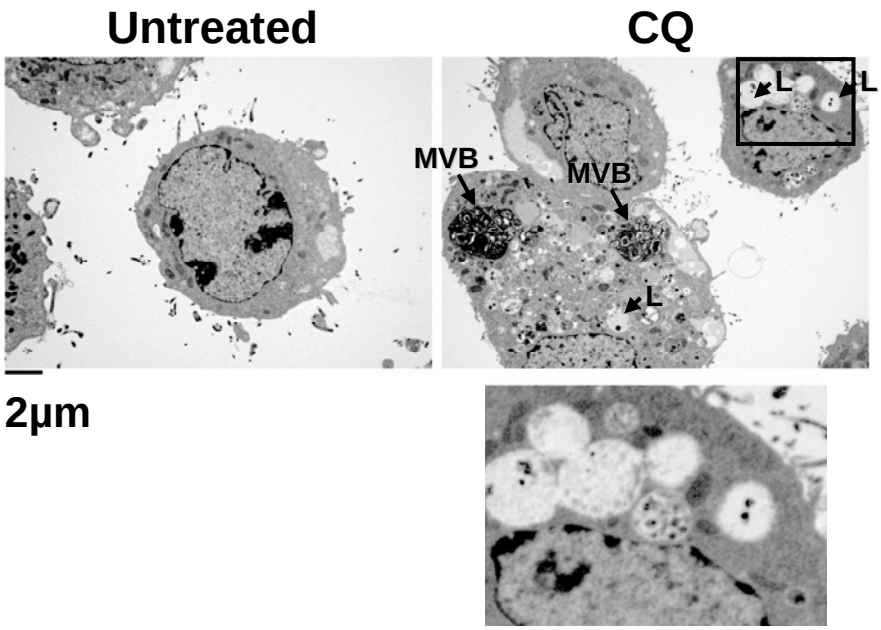

Supplementary Material

Cancer chemoprevention by disrupting lysosomal function and inducing p53-dependent cell death

Kirsteen H. Maclean,[1*] Frank C. Dorsey,[2*]
John L. Cleveland,[2#] and Michael B. Kastan[1#]

[1]St. Jude Children's Research Hospital, Memphis, Tennessee, USA.
.[2] The Scripps Research Institute-Florida, Jupiter, Florida, USA.

#Correspondence: michael.kastan@stjude.org; jcleve@scripps.edu

* Both of these authors have equal contribution

Supplementary online materials:

Supplementary Video V2

Supplemental Figure S1

Oral/I.P. administration of chloroquine impairs Myc-induced lymphoma development**.** Beginning at weaning (4 weeks), wild type and Eμ-*Myc* mice were given 15mg chloroquine/liter in their drinking water, or they were provided with normal drinking water (*n* = 8 for each group). Due to signs of muscle wasting at day 100 of treatment (evident in both wild type non-transgenic and Eμ-*Myc* littermates, data not shown) mice were shifted to 3.5 mg/kg chloroquine (in PBS) I.P. every five days, or they received PBS alone. Median survival time was 90 days for mice treated with water/PBS alone versus >400 days for mice treated with this regimen of chloroquine. By contrast, when Eμ-*Myc* mice on this regimen were removed from chloroquine, they rapidly succumbed to lymphoma (dotted line, *n* = 4).

Supplemental Figure S2

Effect of chloroquine on apoptosis in vivo. Beginning at 4 weeks, Eμ-*Myc* transgenics were injected with 3.5 mg/kg chloroquine (CQ, in PBS) I.P. every five days, or they were injected with PBS alone. At 7 weeks mice were sacrificed and $B220^{+}$ B cells derived from both the spleen and bone marrow and analyzed for apoptosis using annexin-V.

Supplemental Figure S3

Chloroquine induces apoptosis in MEFs. Early passage (p2) wild type MEFs expressing the MSCV-*Myc*-ER^{TAM}-IRES-puro retrovirus were treated for 24 h with 4-HT to activate

Myc-ER^{TAM} and left untreated or were treated with 50μM chloroquine (CQ) for 24h. The percentage of apoptotic cell death was determined by staining cells with Annexin-V. Results shown are representative of three independent experiments.

Supplemental Figure S4

Chloroquine induces p53 in primary MEFs. *Top Panel*: Early passage (p2) wild type, *p53*-, and *Atm*-deficient, MEFs were treated with 50μM chloroquine (CQ) for 24h. Effects on p53 were assessed by western blot, γ-IR treated MEFs were used as a positive control for p53 induction. *Bottom Panel*: Early passage wild type MEFs were treated with chloroquine (CQ, 50 μM) and at the indicated intervals cell extracts were prepared and assessed for modifications of LC3, p53, phosphor-Ser^{15}-p53, and β-actin by western blot analyses. Note that LC3 modification greatly precedes increases in the steady state levels of p53 as well as the activation of p53.

Supplemental Figure S5

Chloroquine provokes vesiculation in human diploid fibroblasts. Human foreskin fibroblasts (HFF) were transduced with the MSCV-IRES-puro or MSCV-*Myc*-ER^{TAM}-IRES-puro retroviruses. Puromycin-resistant cells were then expanded in culture and were expressing (HFF/*Myc*-ER) human fibroblasts were left untreated (Unt) or were treated with 4-HT (1 _M) +/- CQ (50μM) for 12h. Cells were fixed, washed and then stained with Geimsa. Magnifications shown are 60x. Note the numerous cytoplasmic vesicles in cells treated with CQ.

Supplemental Figure S6.

Chloroquine provokes hallmarks of macroautophagy in HeLa cells. (**A**) Chloroquine provokes the re-localization of LC3. HeLa cells were transfected with a rat GFP-LC3B expression construct and GFP^+ cells were left untreated or were treated with CQ (50μM) for 3h. Cells were fixed with 4% (w/v) paraformaldehyde, washed in PBS, and mounted in 50% glycerol. Fluorescent cells were visualized using Zeiss Axioplan fluorescence microscope. Images shown are 100x and representative of three independent experiments. The relocalization of GFP-LC3 is used as a marker of macroautophagy (36). (**B**) HeLa cells were left untreated or were treated with CQ (50μM) for 24h. Cells were then fixed with 4% (w/v) paraformaldehyde and then with 1% (v/v) glutaraldehyde and 1-μM sections were analyzed by transmission electron microscopy. Images shown are 5,000x. Magnified versions of the boxed regions containing lysosomes in CQ-treated cells are shown. **L**, lysosomes; **MVB**, Multi-vesicular bodies, thought to be products of late (acidic) endosomes, where membranes invaginate upon themselves, were extremely large in CQ-treated cells.

Supplemental Videos

The Supplementary Videos were performed using early-passage wild-type MEFs that were transduced with MSCV-GFP-LC3B-IRES-Puro retrovirus, and sorted for GFP expression. Prior to treatment with 50_M CQ, cells were labeled with 100nM Lysotracker™ Red for 30 minutes and washed extensively in cell culture medium. Both videos were generated by collecting images every 5 minutes for 4 hours and thus

contain 49 individual images. Supplementary Video 1 demonstrates the potent induction of autophagic vesiculation induced by CQ treatment, and its corresponding effects on lysosomal size and number. Supplementary Video 2 provides a demonstrates that GFP-LC3 positive vesicles form first, and then later become positive for Lysotracker™ staining indicating that autophagosomes are becoming acidic and/or have fused with lysosomal compartments. Images were collected on a Nikon inverted confocal microscope.