



Supplemental Material to:

**Paola Pellegrini, Angela Strambi, Chiara Zipoli,
Maria Hägg-Olofsson, Maria Buoncervello, Stig Linder,
and Angelo De Milito**

**Acidic extracellular pH neutralizes the autophagy-
inhibiting activity of chloroquine:
Implications for cancer therapies**

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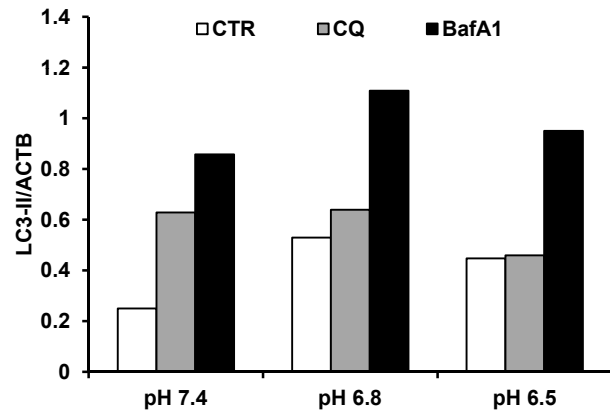
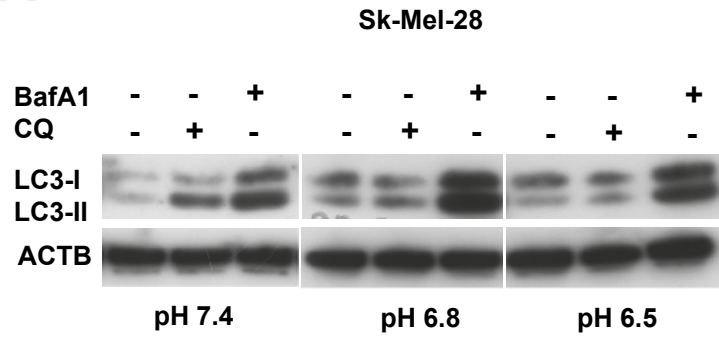
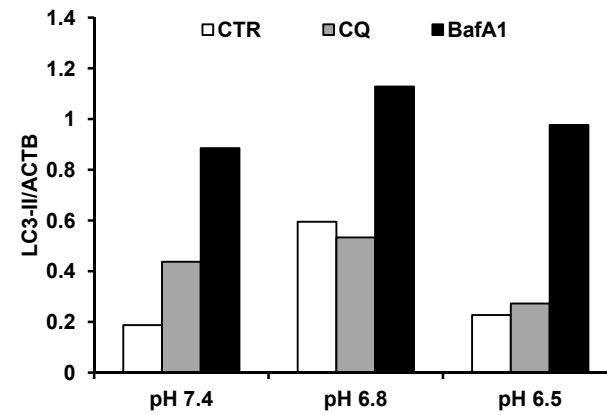
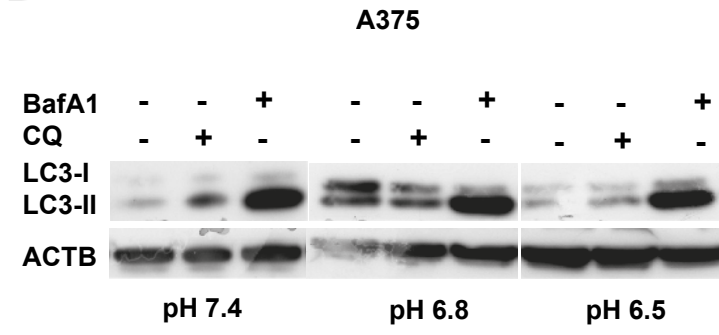
A**B**

Figure S1

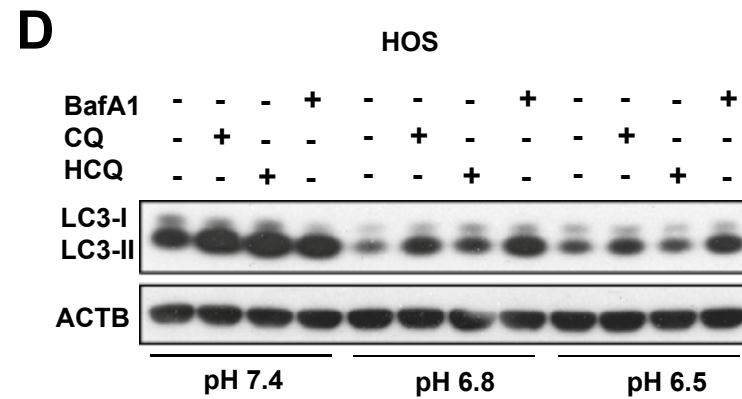
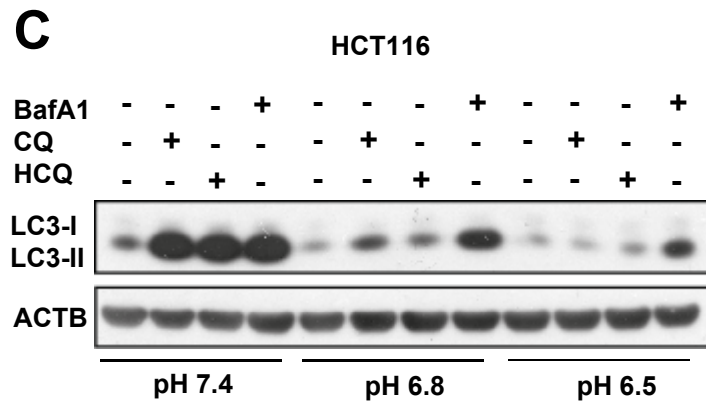
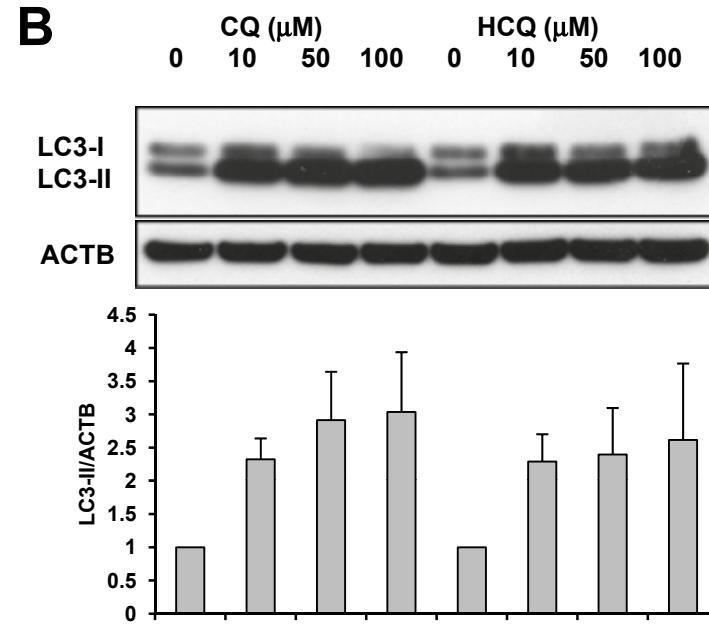
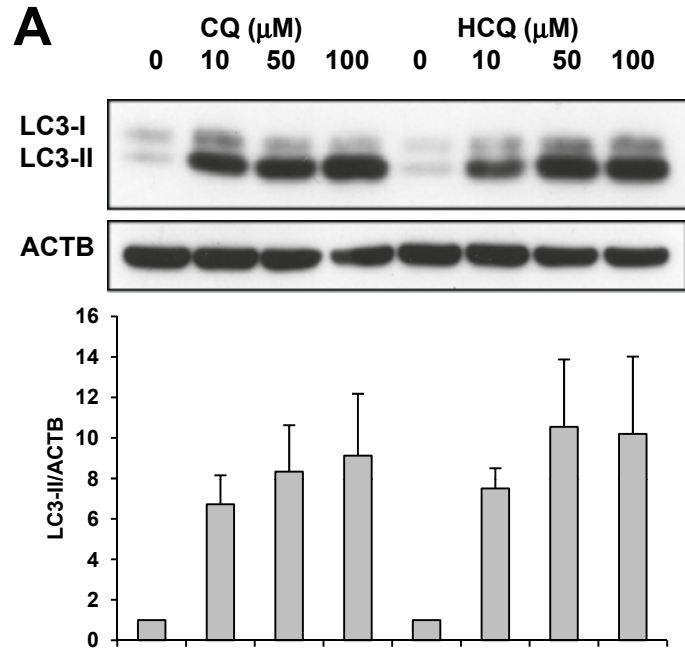


Figure S2

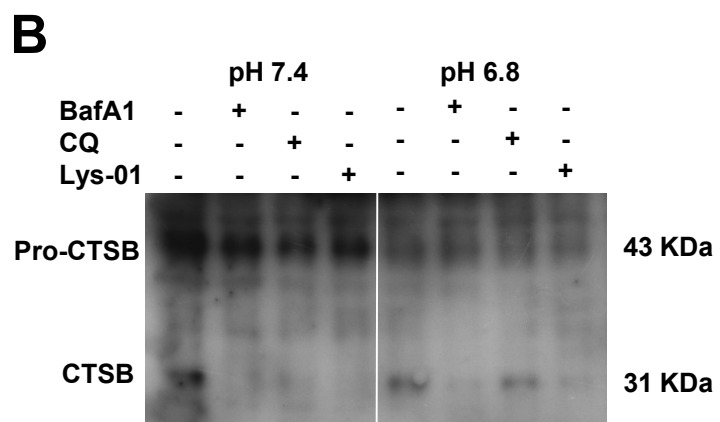
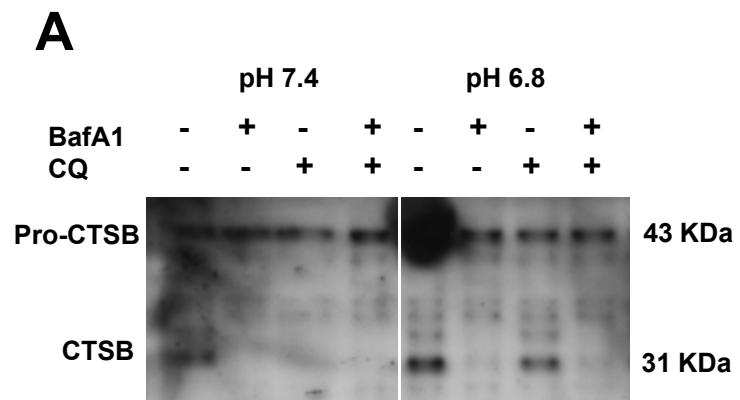


Figure S3

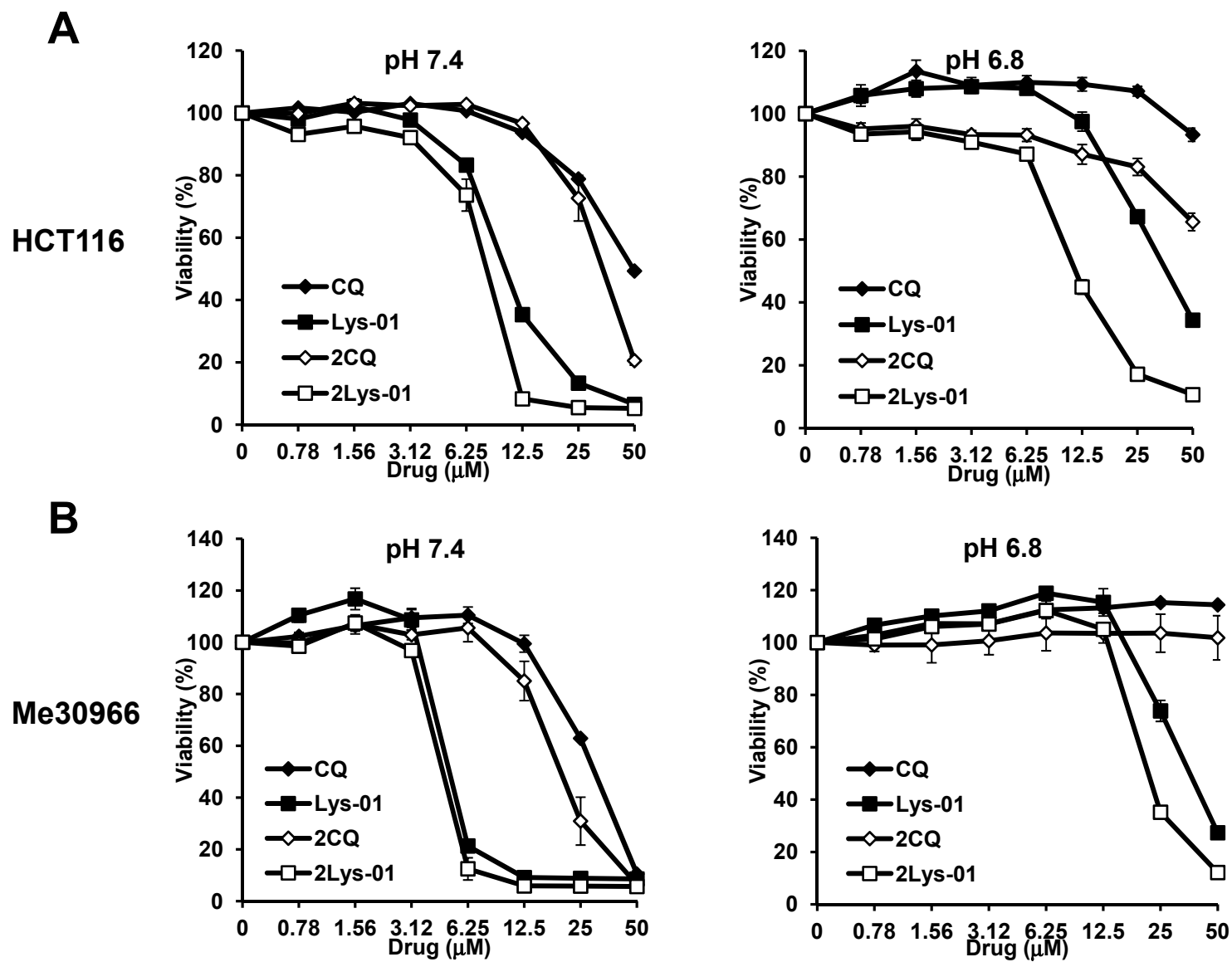


Figure S4

Figure S1. Autophagic flux in melanoma cells at different pH conditions. SK-Mel-28 (A) and A375 (B) cells were cultured for 8 h at pH 7.4, 6.8 and 6.5 in the presence of BafA1 (40 nM) or CQ (30 μ M) and WB analysis of LC3 expression was performed. The WB shows that while CQ blocks degradation of LC3-II at pH 7.4 it does not maintain this activity at lower pH conditions. The quantification of LC3-II signal was normalized to ACTB by densitometric analysis.

Figure S2. Autophagy-inhibiting activity of CQ and HCQ. HCT116 (A) and HOS (B) cells were cultured in the presence of increasing concentrations of CQ and HCQ for 4 h and the autophagic flux was evaluated by WB. The data show that the inhibition of autophagy reached a plateau when 50 μ M of both compounds were used. Data are presented as means \pm standard deviations. Panels (C) and (D) show the lack of activity of CQ and HCQ as autophagy blockers in acidic conditions in HCT116 and HOS cells, respectively.

Figure S3. CTSB maturation at different pH conditions. (A) WB analysis of pro-CTSB and mature CTSB in HCT116 cells cultured at pH 7.4 and 6.8 in presence of BafA1 (50 nM), CQ (50 μ M) and a combination of BafA1 + CQ (as in Fig. 2B). (B) WB analysis of pro-CTSB and mature CTSB in HCT116 cells cultured at pH 7.4 and 6.8 in presence of BafA1 (50 nM), CQ (50 μ M) or Lys-01 (as in Fig. 3A).

Figure S4. Cell viability after repeated exposure to CQ and Lys-01. HCT116 and Me30966 cells were cultured at pH 7.4 or 6.8 and treated twice with CQ or Lys-01 with a 1-day interval. Cell viability was measured the day after the second exposure. Data are from 1 experiment run in triplicate wells.

Table S1. IC₅₀ (μ M) of CQ and Lys-01. HCT116, HCT116_{pH6.8}, Me30966 and Me30966_{pH6.8} cells were exposed for 48 h to CQ or Lys-01. Data are expressed as means \pm standard deviations.

	HCT116	HCT116 _{pH6.8}	Me30966	Me30966 _{pH6.8}
CQ	37 \pm 13	>50	27 \pm 2	>50
Lys-01	8 \pm 2	37 \pm 7	6 \pm 2	32 \pm 7