Online material

Supplementary Figure 1

Lack of autophagosome degradation results in further increase of LC3-II levels in IM-treated cells.

Levels of LC3-II are higher in IM/CQ treated K562 than in Chloroquine (CQ) only treated cells (6 hours). Levels of LC3 and Actin were measured using relevant antibodies.

Supplementary Figure 2

Morphology of untreated K562 cells

(A and **B)** Multivesicular bodies are present in the cytoplasm of many untreated K562 cells. These organelles are characterised by the presence of numerous small vesicles, 25-100 nm in diameter, together with a few slightly larger, more electron-dense inclusions containing a lipofuscin-like material Bars = 1μm.

Supplementary Figure 3

Ultrastructural analysis of IM-treated K562 cells.

(**A** and **B**) Electron micrographs of IM-treated K562 cells (2 μM, 6 h). Many large vacuoles, most of which contained numerous vesicles and inclusions, were present in the cytoplasm. Similar, dense inclusions were present in small vacuoles distributed throughout the cytoplasm and also within the large vacuoles [arrows]. (**C** and **D**) Immunogold labelling of IM-treated eGFP-LC3- transduced K562 cells. Bars = 1μm.

Supplementary Figure 4

Effects of IM treatment on morphology and autophagy in K562 cells.

(A and **B)** Electron micrographs of K562 cells, treated with 2 μM IM for 6 hours. Small vacuoles, containing electron-dense inclusions, are distributed throughout the cytoplasm. Many of these vacuoles appear to be coalescing or fusing with the larger vacuoles (arrows) Bars = 1μm.

Supplementary Figure 5

Morphological changes and induction of autophagy in BV173 cells.

(**A**) IM induces cell shrinkage and vacuolization in BV173 cells. Light microscopy images of May-Grunwald-stained BV173 cells upon IM treatment. BV173 were cultured for 96 hours in the absence or presence of 4μM IM and processed for May-Grunwald staining. (**B**) Cell size is reduced in IM-treated CML cells. Size of untreated or IM-treated BV173 cells was analyzed using a cytofluorimeter. Representative histograms of the physical parameter FSC-H are shown. (**C)** Accumulation of LC3-II is readily detected in extracts from treated BV173 (12 hours; Ctrl, CQ, IM and CQ/IM). An anti-β-actin antibody was used as a loading control. (**D**) IM triggers the formation of LC3-positive vescicles in BV173 cells. Endogenous levels of LC3 in BV173 treated for 72 h with the indicated concentrations of IM and chloroquine (CQ). BV173 were stained with anti-LC3 antibody (red). Nuclei were counterstained with DAPI.

Supplementary Figure 6

Effects of IM and CQ treatment on morphology of BV173 cells.

(A) Untreated BV173 cells after incubation in medium alone for 48 h show a characteristically convoluted nuclear profile. Droplets of saturated lipid and a few dense lysosomes (arrows) are present in the cytoplasm. **(B)** Treatment with IM for 48 h resulted in a slight increase in the size of the lysosomes (arrows). **(C)** Combined treatment with IM and CQ for 48 h resulted in a significantly increased accumulation of material in phagolysosomes (arrows). Bars = 1μm.

Supplementary Figure 7

Morphological changes and induction of autophagy in 32Dcl3 cells.

(**A**) IL-3 withdrawal induces cell shrinkage and vacuolization. 32Dcl3 (32D) cells were grown in the presence or absence of IL-3 and zVADfmk (zVAD). Cells were stained with May-Grunwald and analyzed for morphological changes. (**B**) Cell size is reduced in starved 32D cells. Cell size was analyzed using a cytofluorimeter. Representative histograms of the physical parameter FSC-H are shown. (**C**) IL-3 deprivation induces accumulation of LC3-II in 32D cells. Western blot shows LC3-I and LC3-II levels in

protein extracts from 32D cells treated for 24 hours with the indicated concentrations of IL-3 and zVADFMK. β-actin was used to normalize protein levels in each sample.

Supplementary Figure 8

Imatinib (IM) does not induce autophagy in eGFP-LC3 HeLa cells.

Cells were cultured in the presence of IM or were left untreated. LC3-I/II were detected using an anti-LC3 antibody. Asterisk indicates a non-specific band. Actin was detected as loading control.

Supplementary Figure 9

Inhibition of autophagy enhances IM-induced cell death in CML-lymphoid blast crisis BV173 cells.

(**A**) Effects of chloroquine (CQ) in IM-treated BV173 cells. Cells were cultured for 72 or 96 hours with 4 μM IM alone or in combination with 10 μM CQ and cell death was measured by Annexin V staining. (**B**) Effects of Bafilomycin A1 in IM-treated BV173 cells. Cells were treated as in (A) using 10 or 20 nM Bafilomycin A1 and cell death was measured by Annexin V staining. Values represent the means ± standard error of the mean (SEM) of 3 independent experiments. Data were analyzed by unpaired *t*-test.

Supplementary Figure 10

Inhibition of autophagy in CML cells augments IM-induced cell death.

(**A-D**) CQ potentiates the effect of IM in clonogenic assays of CML cells from four newly diagnosed CML-CP patients. Peripheral blood mononuclear cells were plated in the presence or absence of increasing doses of IM (0.5, 1.0 or 2.0 μM; Patient 1 panel A) or a single IM dose (1.0 μM, Patient 2-4, panels **B-D**) and 10 μM CQ. Colonies were counted 7 days later. Number of colonies in treated samples is expressed as percentage of control. (**E**) CQ potentiates the effect of IM on cells from a CML-BC patient carrying partially IM-resistant p210BCR/ABL mutants. Cells were plated as described above. Number of colonies in treated samples is expressed as percentage of control. Representative of two experiments. (**F**) CQ does not potentiate the effect of IM in clonogenic assays of marrow mononuclear cells from healthy donors. Number of colonies in treated samples is expressed as percentage of control. Representative of three

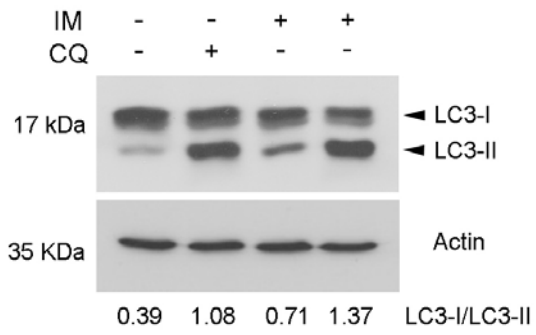
independent experiments using marrow mononuclear cells from three healthy donors. (**G**) CQ does not enhance the effect of IM in clonogenic assays of primary CD34+ cells. CD34+ cells were treated as described above. Number of colonies in treated samples was determined at day 10. Representative of two independent experiments.

Supplementary Figure 11

Chloroquine potentiates the effect of IM in primary CML derived from IM-treated CML-CP patients.

(**A**) CQ potentiates the effect of IM on cells from a newly diagnosed CML-CP patient under IM treatment (3 days/800mg/day; CML-CP-t-1). Number of colonies in treated samples is expressed as percentage of control. Values represent the means ± standard error of the mean (SEM) of two independent experiments (one using freshly- isolated cells, one using cells from a frozen aliquot); (**B**) CQ potentiates the effect of IM on cells from a a CML-CP patient undergoing IM treatment (10 days at 100mg/day; CML-CP-t-2; low IM doses were used due to development of bone marrow aplasia). Number of colonies in treated samples is expressed as percentage of control (one experiment from a frozen aliquot).

Bellodi et al Suppl. Fig. 1



Bellodi et al. Suppl. Fig. 2

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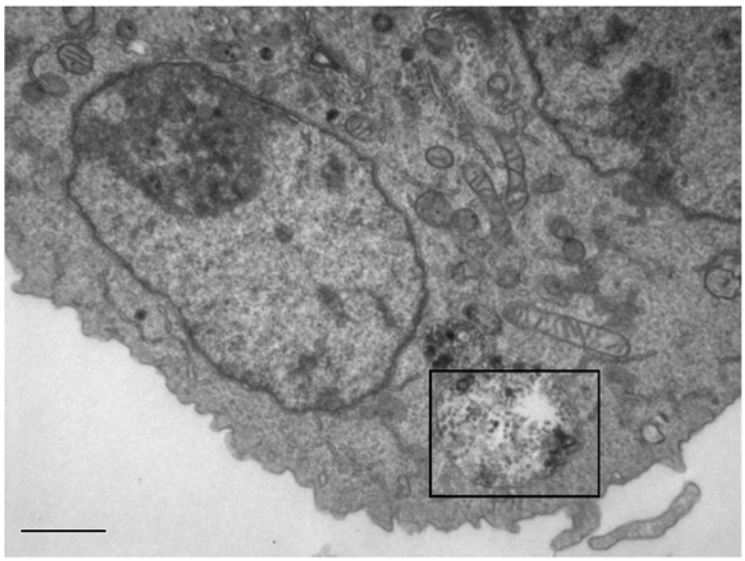

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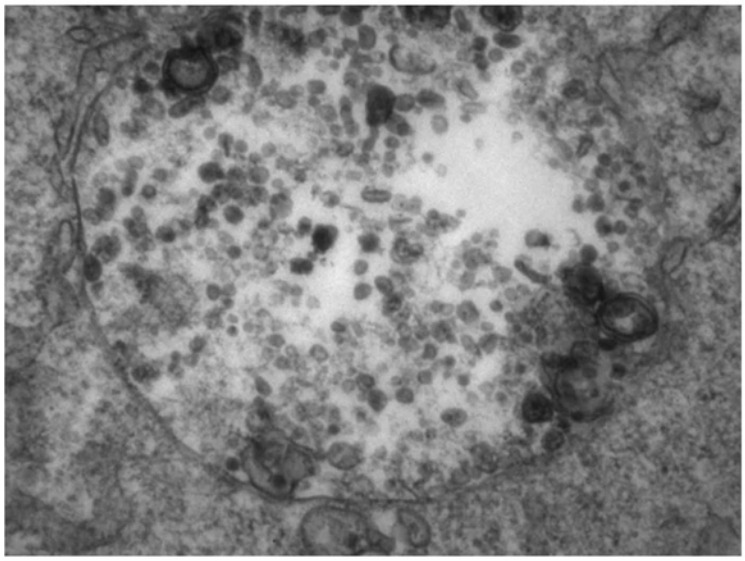

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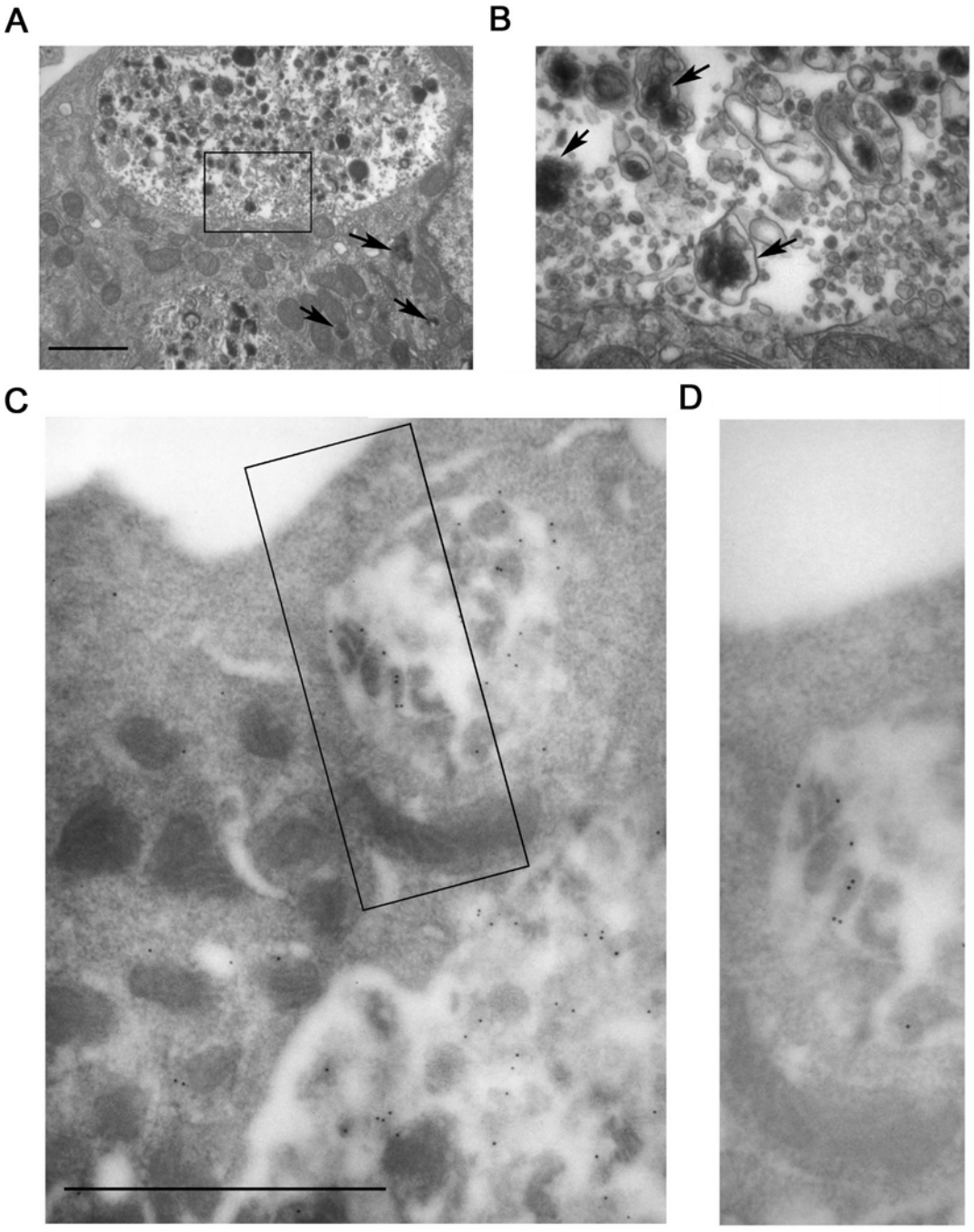

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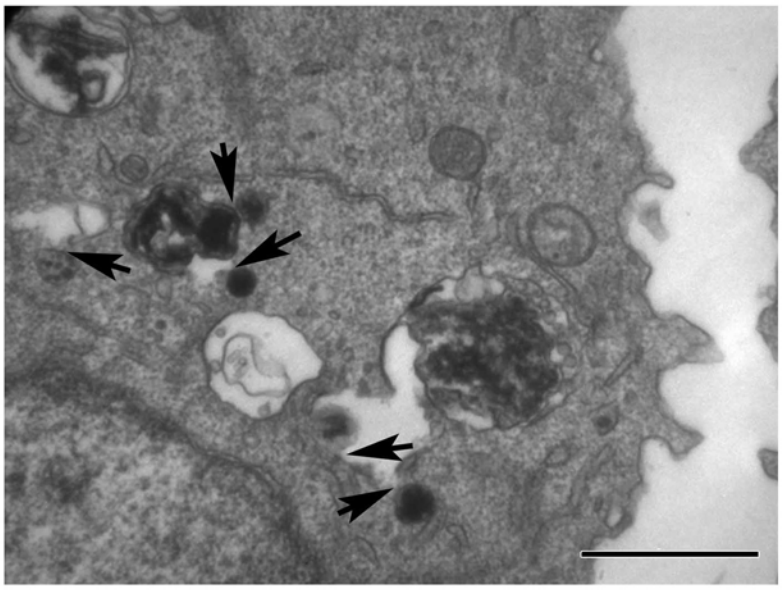

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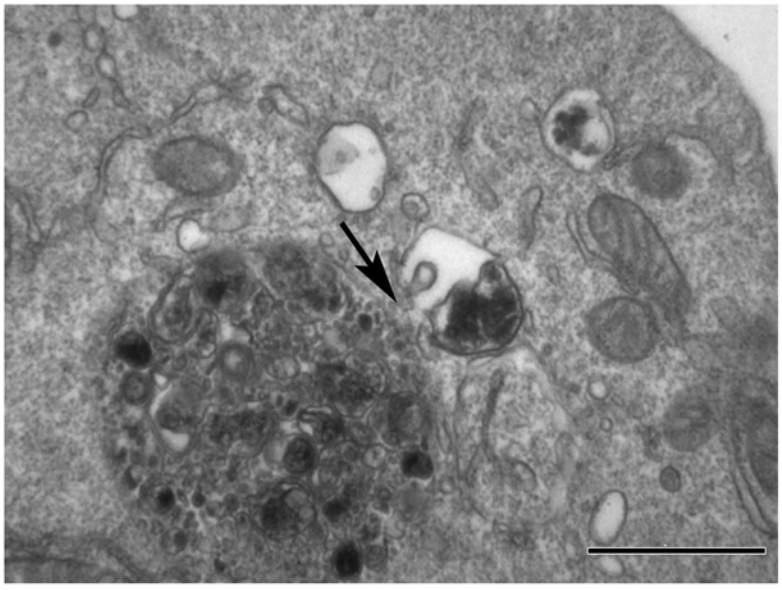

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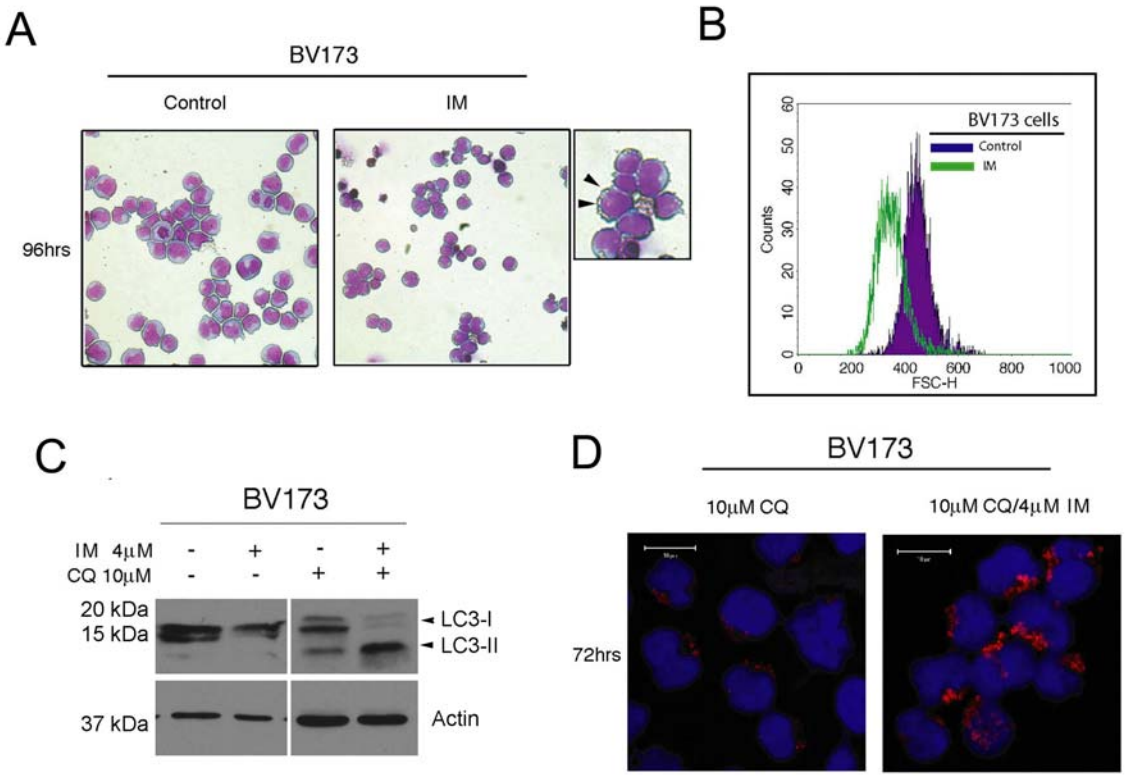

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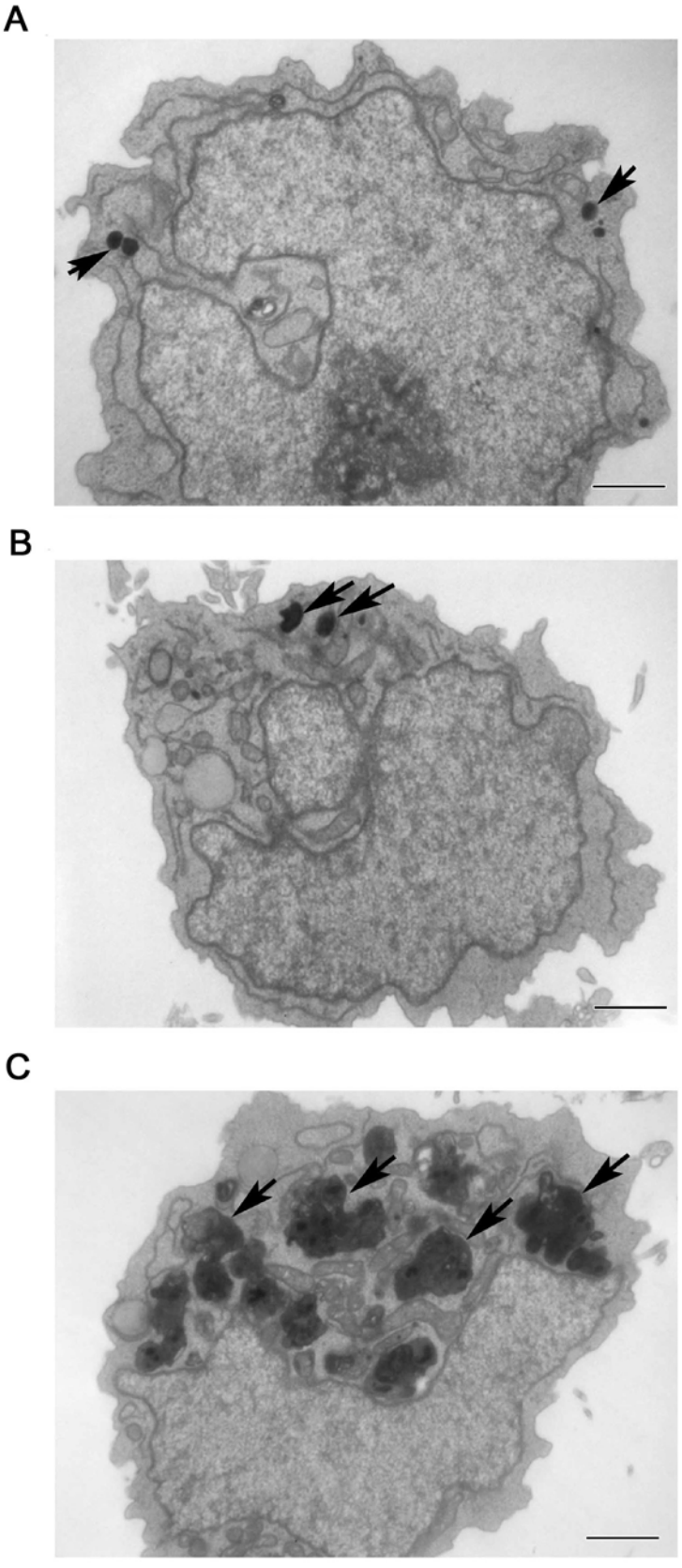

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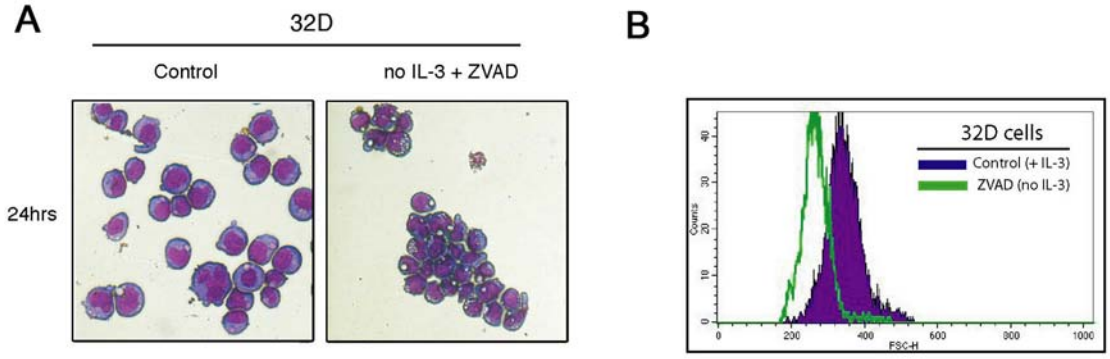

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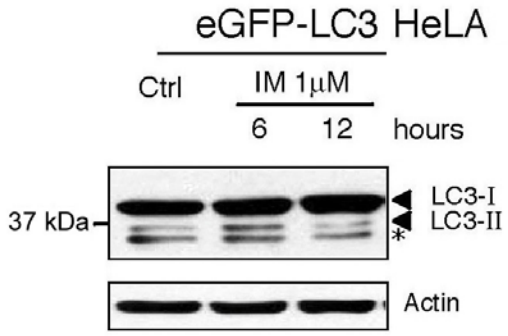

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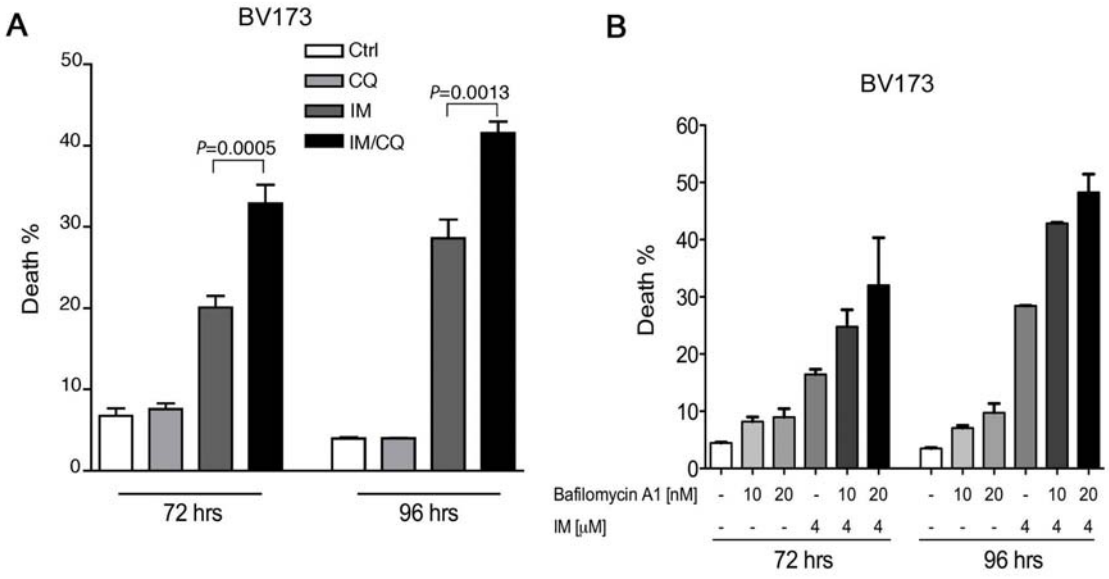

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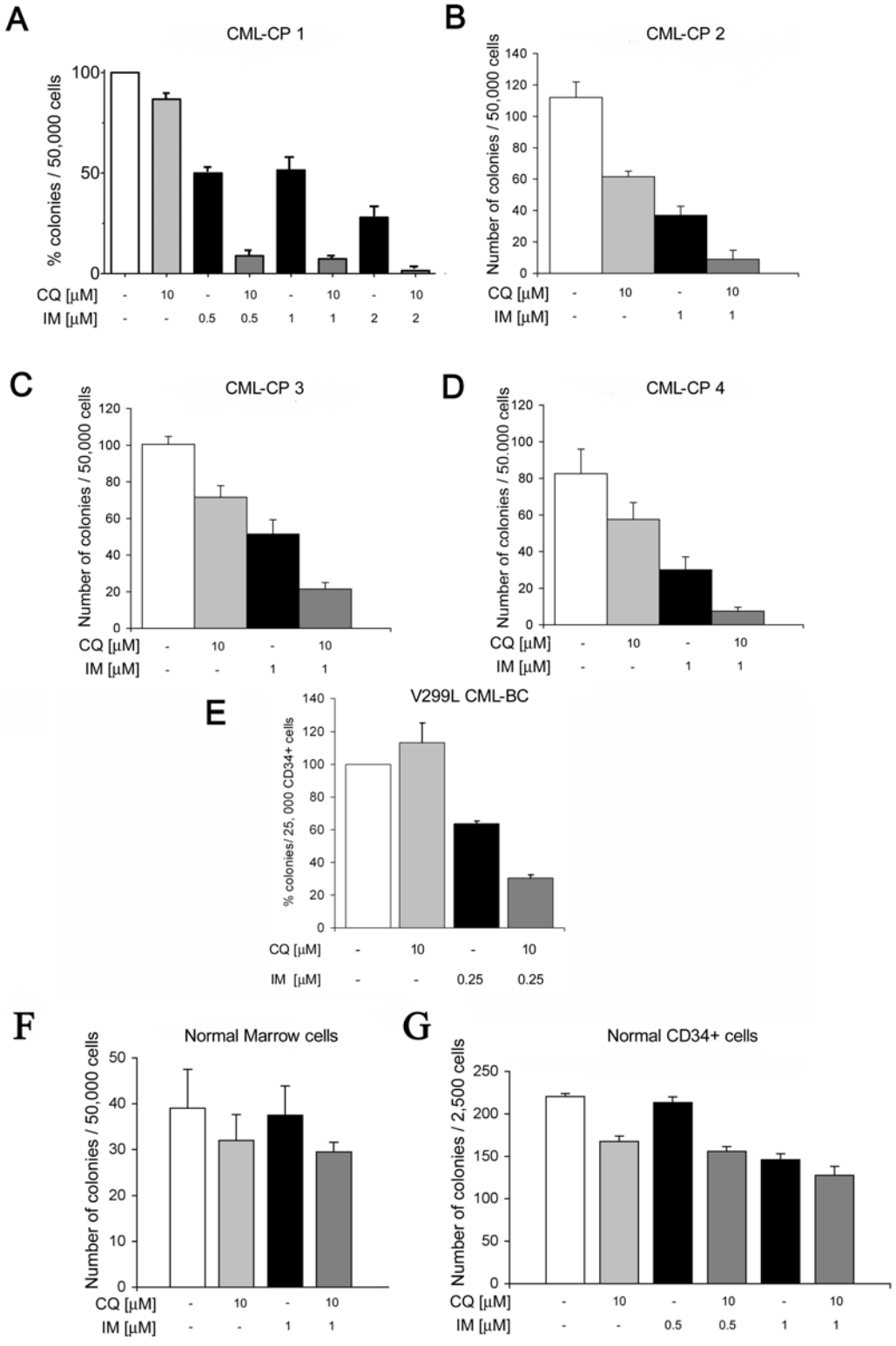

Bellodi et al. Suppl. Fig. 11

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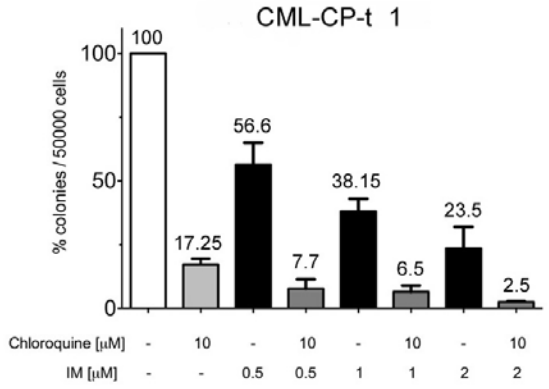

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