Legends to Supplemental Figures

Supplemental Figure 1: Combined treatment with sorafenib and obatoclax kills human leukemia cells through a caspase-dependent mechanism and markedly reduces their viability. (A) U937 cells were exposed to sorafenib (7.5 μM) and obatoclax (1.5 μM) alone or in combination for 30 h after which loss of mitochondrial membrane potential was assessed by monitoring $DiOC_6$ uptake. * = significantly greater than values for obatoclax or sorafenib alone; $P < 0.01$. **(B)** U937 cells were exposed to 7.5 μM sorafenib ± 1.5 μM obatoclax in the presence or absence of the pan-caspase inhibitor Q-VD-OPH (Ci; 15 μM) for 48 h, after which the percentage of dead cells was determined using the 7-AAD assay. * = significantly less than values for Sor/Ob-treated cells; $P < 0.01$. **(C)** U937 cells were treated with sorafenib (7.5 μM) ± obatoclax (1.5 μM) for 48 h, after which cell growth and viability was assessed by the CellTiter-Glo® Luminescent Assay (Promega).

Supplemental Figure 2: Combined exposure of human leukemia cells to sorafenib and HA14-1 results in the pronounced induction of cell death in association with marked mitochondrial injury and caspase activation. (**A**) U937 cells were exposed to 7.5 μM sorafenib, 10 μM HA14-1 alone or in combination for 24 h after which the percentage of apoptotic cells was determined using the Annexin V/PI staining assay. Alternatively mitochondria-free cytosolic fractions were isolated as described in Methods, and subjected to Western blot analysis to monitor the release of cytochrome c and AIF into the cytosol (**B**). (**C**) U937 cells were exposed to sorafenib (7.5 μM) and HA14-1 (10 μM) alone or in combination for 6 h or 24 h after which protein lysates were prepared and subjected to Western blot analysis. (**D**) Median Dose Effect analysis of cell death induction by sorafenib and HA14-1. U937 cells were exposed to varying concentrations of sorafenib and HA14-1 at a fixed ratio (1:1), for 24 h after which the extent of cell death was monitored with the annexin V/PI assay. Combination Index (C.I.) values were determined in relation to the Fractional Effect using a commercially available software program as described in Methods. C.I. values < 1.0 correspond to a synergistic interaction. (**E**) U937 cells were exposed to 7.5 μM sorafenib and 7.5 μM gossypol alone or in combination for 24 h after which the percentage of apoptotic cells was determined by the

Annexin V/PI assay. * = significantly greater than values for obatoclax or gossypol alone; P < 0.02.

Supplemental Figure 3: Knock-down of Bax or Bak, or over-expression of Mcl-1 significantly protects cells against sorafenib and obatoclax lethality. U937 cells in which Bak (shBak) or Bax (shBax) were stably knocked down using shRNA and their control counterpart GFP (shGFP) cells were exposed to the designated concentrations of sorafenib **(A)** or obatoclax **(B)** for 48 h, after which the extent of cell death was determined using the 7-AAD assay. * = significantly less than values for shGFP controls; P < 0.05. U937 cells ectopically expressing Mcl-1 and their empty vector control cells (pCEP4) were treated with the designated concentrations of sorafenib **(C)** or obatoclax **(D)** for 48 h, after which the extent of cell death was determined using the 7-AAD staining assay. * = significantly less than values for pCEP4 controls; P < 0.05; ** = P < 0.02.

Supplemental Figure 4: Bim knock down significantly protects and Bim overexpression markedly enhances sorafenib/obatoclax lethality. (A) MV4-11 cells were infected with lentiviruses carrying pLK01 Bim-shRNA constructs (cat# RHS3979-9569482; open Biosystems) and selected in the presence of puromycin for 1 week, after which Bim expression was monitored by western blot analysis and compared with negative control cells (NC) infected with a scrambled shRNA construct. Alternatively, these cells were exposed to sorafenib and obatoclax alone or in combination for 24 h after which cell death was assessed by 7-AAD assay **(B)**. * = significantly less than values for NC cells; P < 0.02. **(C)** U937 cells ectopically expressing wild type Bim (Bim wt) or their empty vector counterparts (pcDNA3.1) were treated with sorafenib (7.5 μM) and obatoclax (1.5 μM) alone and in combination for 24 h, after which protein lysates were prepared and subjected to Western blot analysis to monitor caspase-3 and PARP cleavage. Blots were stripped and reprobed with antibodies directed against tubulin to ensure equivalent loading and transfer.

Supplemental Figure 5: Autophagy plays a cytoprotective role against sorafenib/obatoclax lethality (A) MV4-11 cells were stably transfected with LC3-EGFP using lentiviral transduction. Cells were then exposed to sorafenib and obatoclax alone or in combination for 6 h, after which

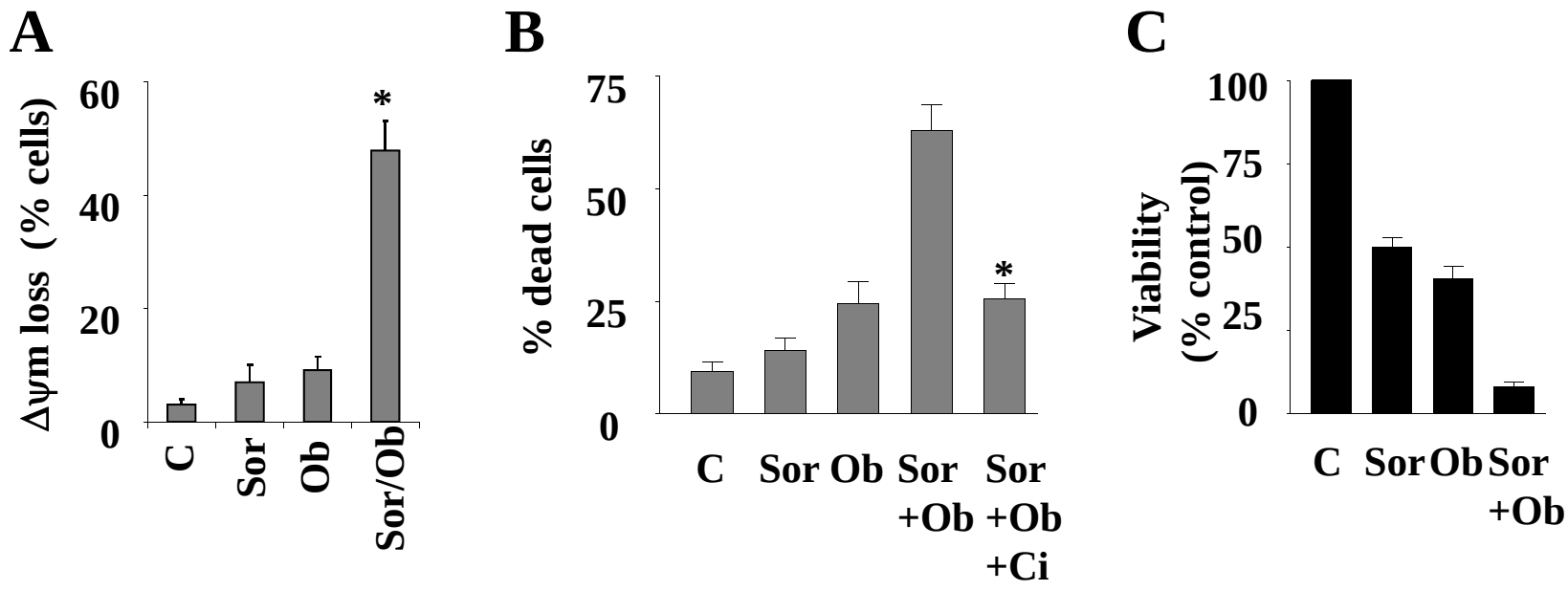
cells were fixed and subjected to confocal microscopy. (**B)** Enlarged images of outlined areas of the Figure 6C illustrating co-localization of LC3-GFP (Green) and LAMP1 (red) in U937 cells following 6-h treatment with sorafenib (7.5 μM) and obatoclax (1.5 μM). **(C)** U937 cells were treated with sorafenib (7.5 μM) and obatoclax (1.5 μM) alone or in combination for 6-24 h, after which protein lysates were prepared and subjected to Western blot analysis to monitor p62 protein levels. **(D)** U937 cells were pretreated with 3-MA (2.5 mM) or Bafilomycin A1 (Baf; 75 nM) for 30 min, and exposed to sorafenib (7.5 μM) or obatoclax (1.5 μM) alone for an additional 16 h, after which the extent of cell death was monitored by the 7-AAD assay. **(E)** HL-60 cells were pretreated with 3-MA (4 mM), chloroquine (CQ; 50 μM), or Bafilomycin A1 (100 nM) for 30 min, and exposed to sorafenib (7.5 μM) and obatoclax (2 μM) alone or in combination for an additional 28 h, after which the extent of cell death was monitored by the 7-AAD assay. Values represent the means ± S.D. for triplicate determinations performed on three separate occasions. * = significantly greater than values obtained in the absence of CQ, Baf, or 3MA; $P < 0.02$. Alternatively, after treatment as in **(E)**, cells were lysed and Western blot analysis performed to monitor expression of cleaved caspase-3, PARP degradation, and expression of LC3-II **(F)**. (**G**) U937 cells were exposed to 7.5 μM sorafenib ± 1.5 μM obatoclax in the presence or absence of 50 μM chloroquine for 6 h, after which cells were lysed and subjected to Western blot analysis to monitor LC3-II and PARP cleavage.

Supplemental Figure 6: Bim knock-down is associated with increased LC3 processing. U937 cells in which Bim was stably knocked down using lentivirus-mediated shRNA as in Supplemental Figure 4A and their control counterparts (NC) were exposed to sorafenib and obatoclax for 6 h, after which proteins were extracted and subjected to Western blot analysis to monitor expression of LC3-II and Bim.

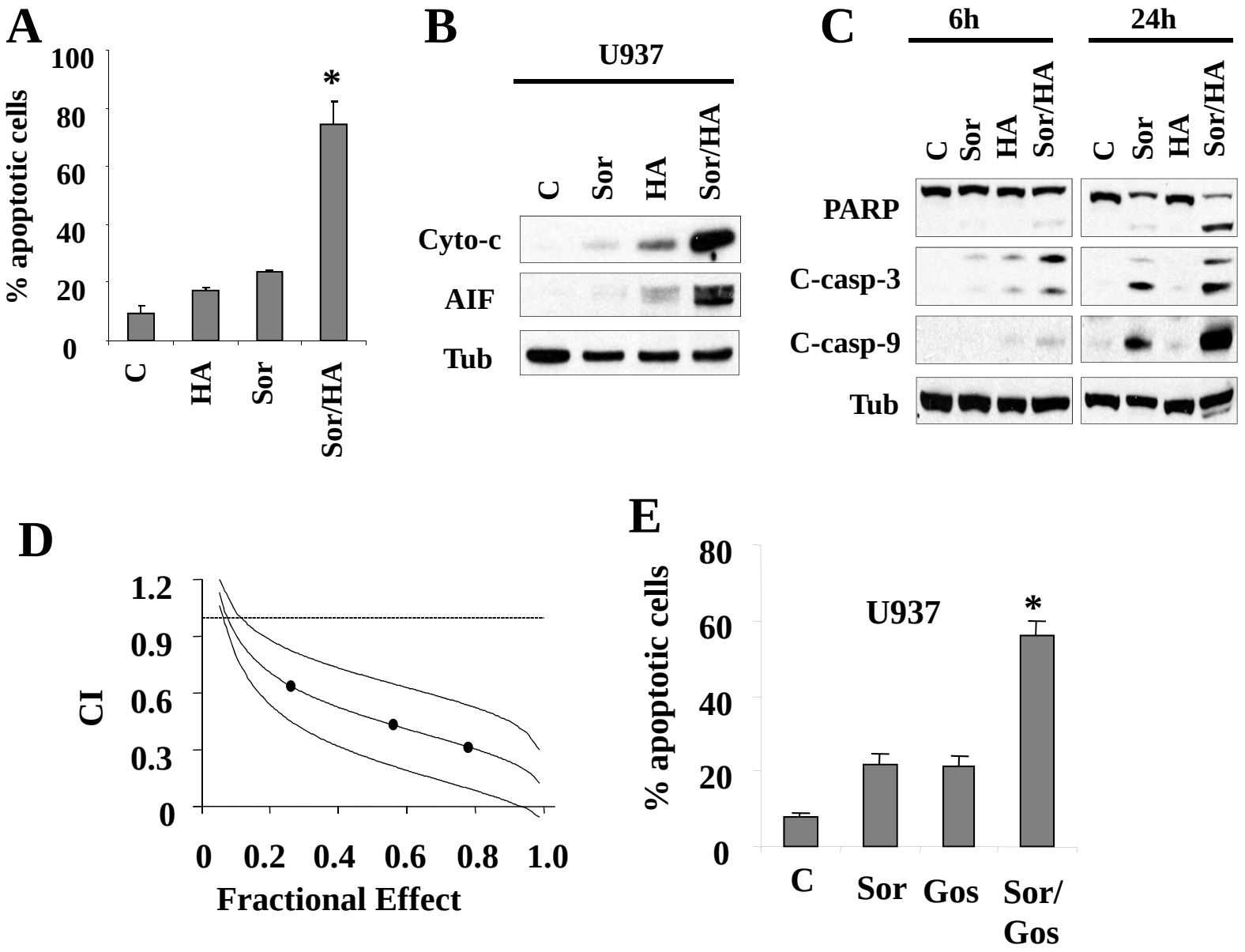
Supplemental Figure 7: Exposure to sorafenib/obatoclax does not result in weight loss and minimally reduces white and red blood cell counts in xenograft-bearing mice. **(A)** Nude mice were injected with luciferase-expressing U937 cells, treated with sorafenib (80 mg/kg) and obatoclax (3.5 mg/kg) alone or together as described in Methods for the designated intervals, and imaged using the IVIS 200 imaging system. X marks correspond to deceased mice. **(B)** U937 xenograft-bearing nude mice were treated with sorafenib and obatoclax alone or in combination

for the designated intervals after which animal body weights were measured and represented as a percentage of animal weight before treatment. Alternatively white and red blood cells counts were determined after 1 week of treatment using a Coulter counter and presented as a percentage of control values **(C)**.

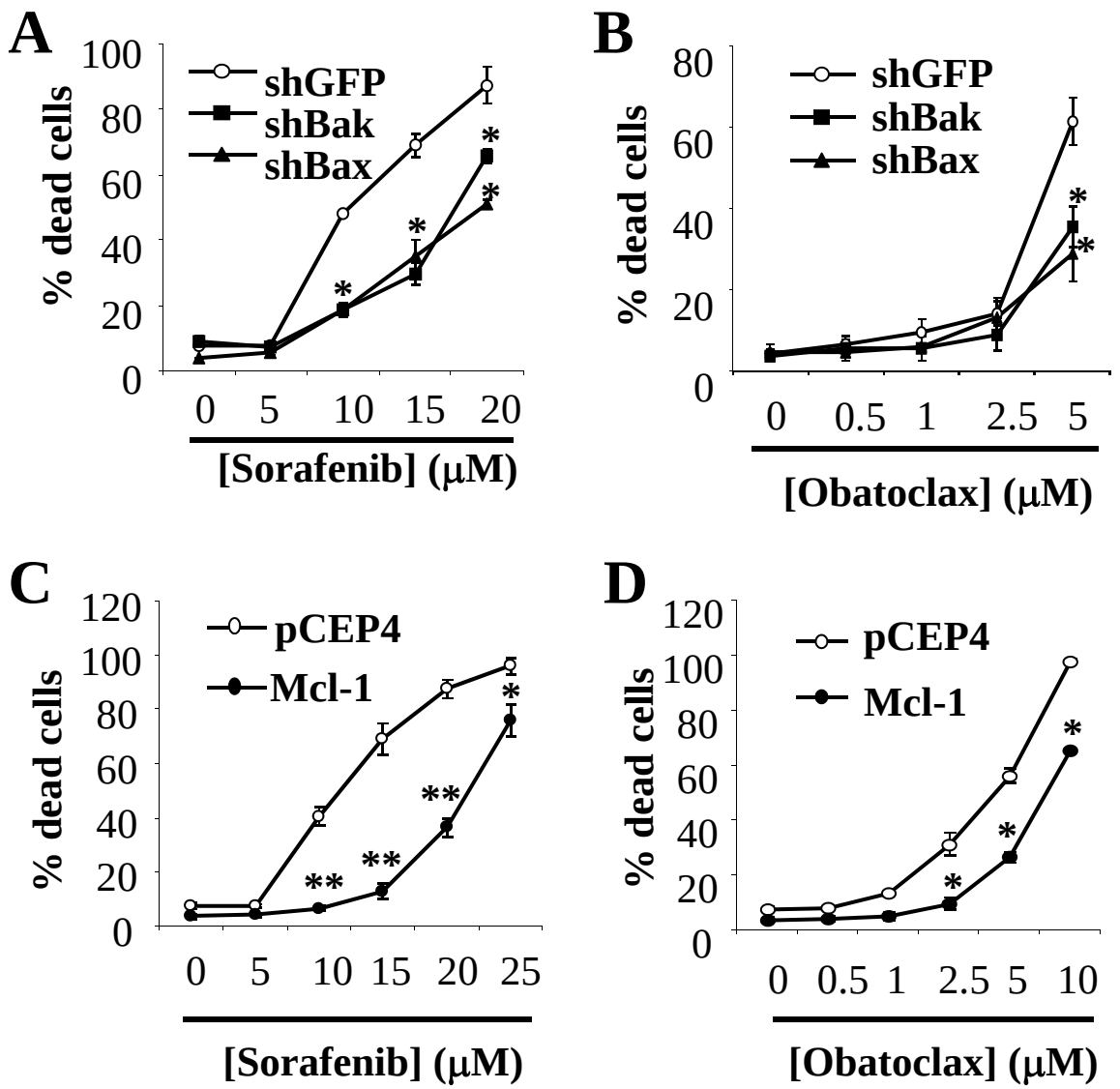
Supplemental Figure 1



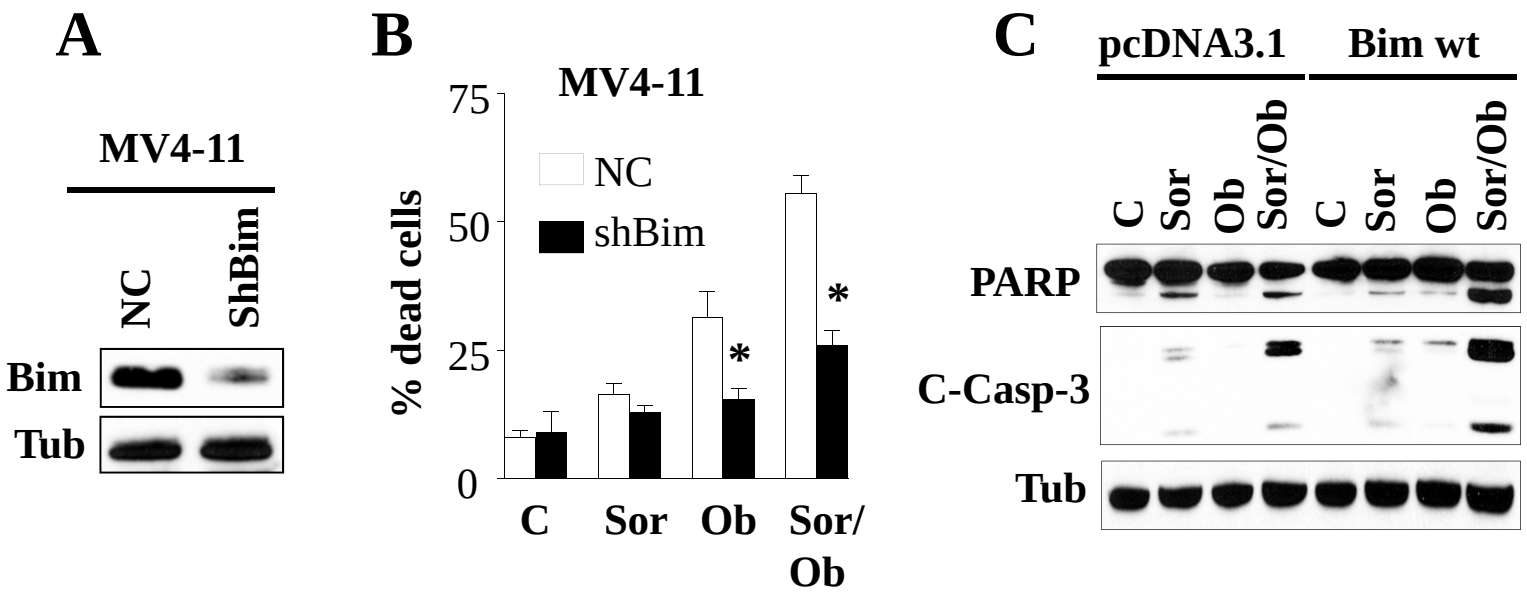
Supplemental Figure 2



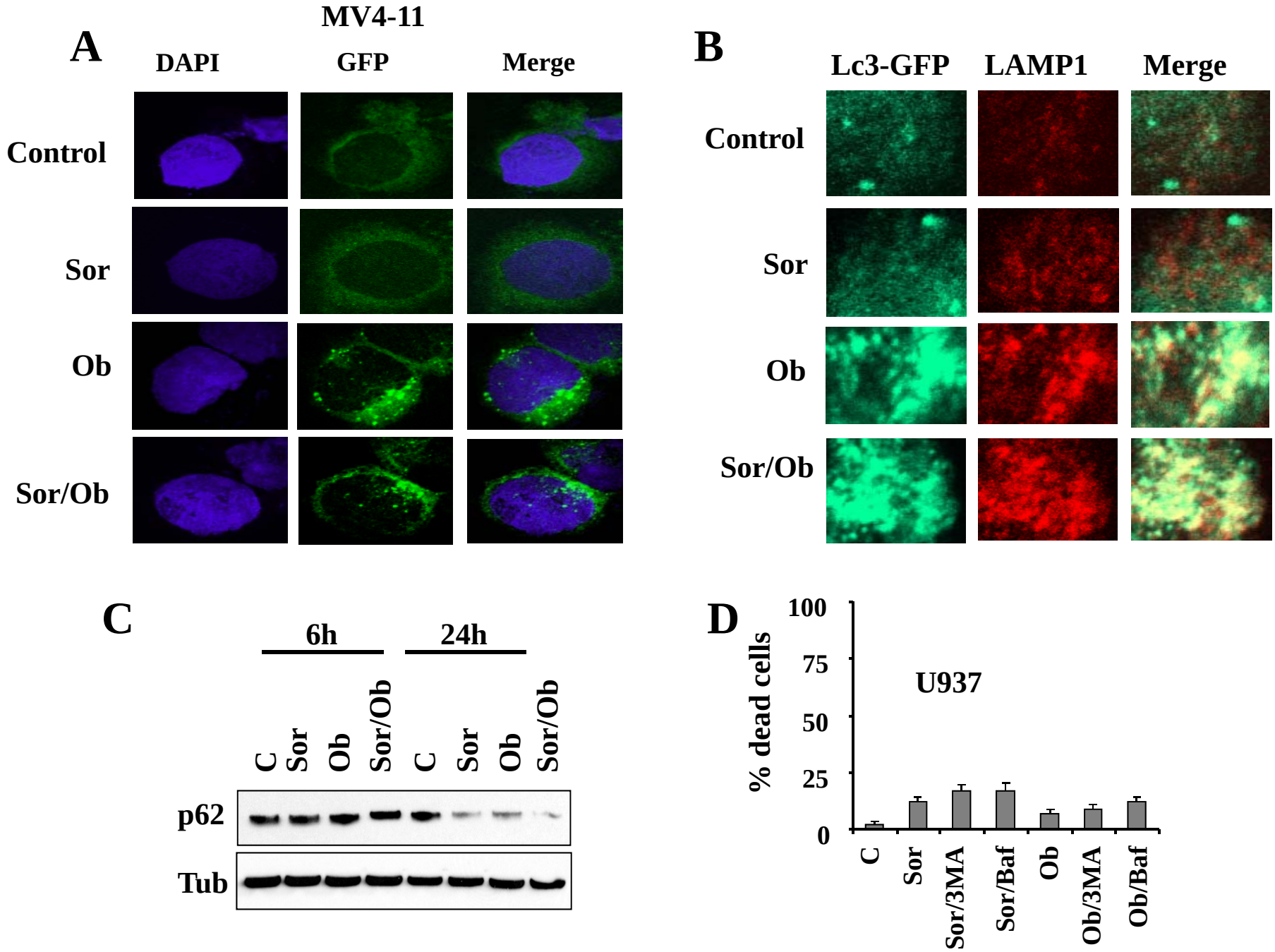
Supplemental Figure 3



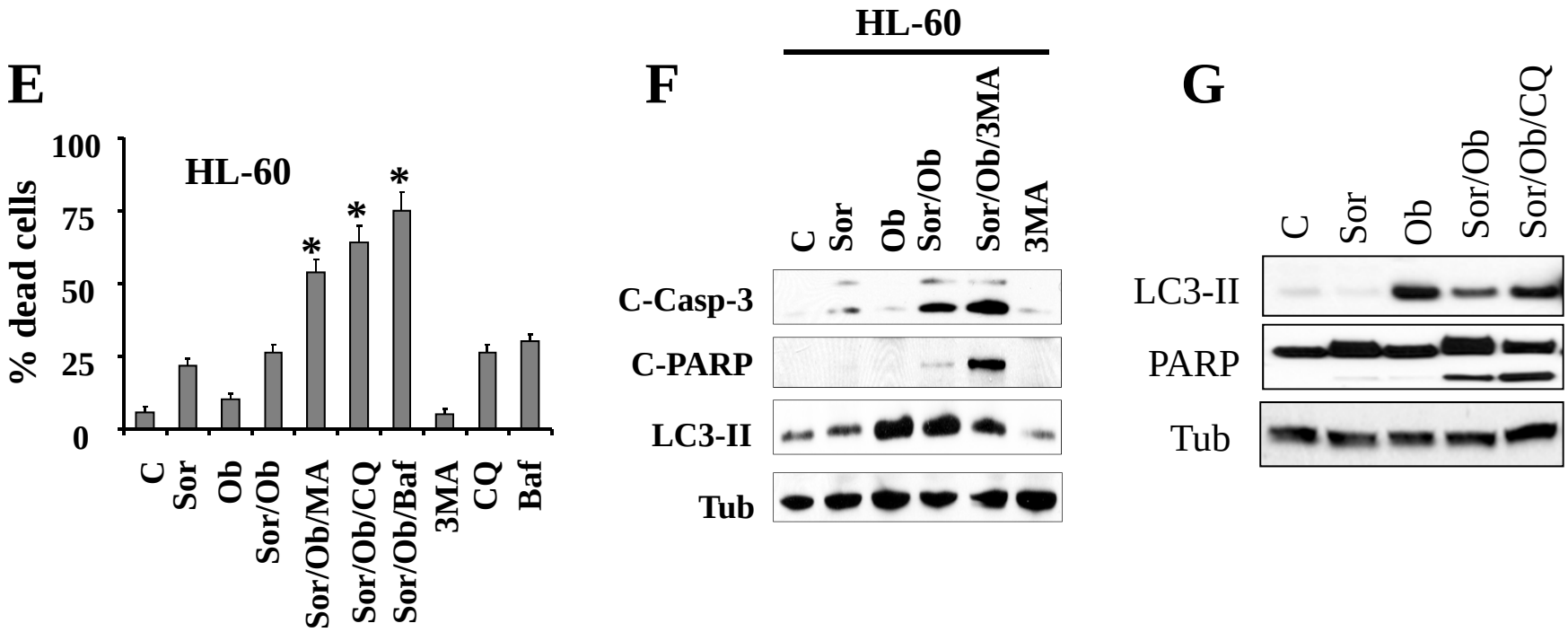
Supplemental Figure 4



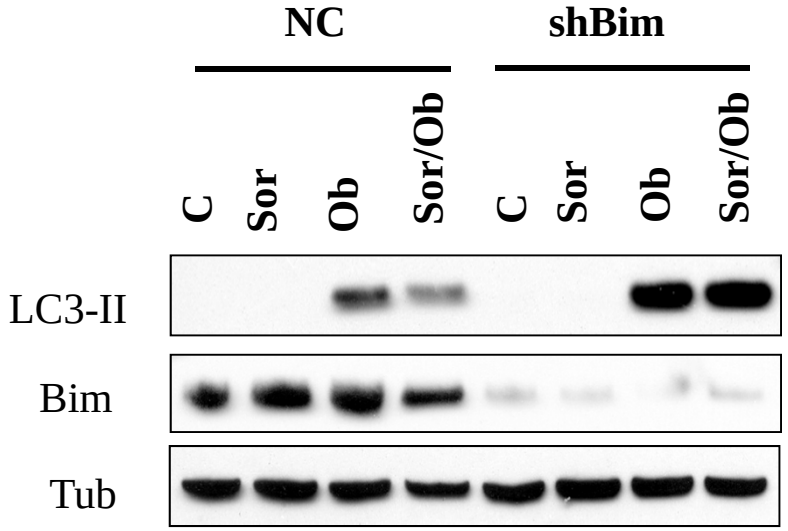
Supplemental Figure 5



Supplemental Figure 5 (continued)



Supplemental Figure 6



Supplemental Figure 7