Legends to supplementary figures

Fig. S1. Titration of B10 cytotoxic activity on glioblastoma cell lines. U87MG, U118, U138, A172 and LN18 were incubated with increasing concentrations of B10 for 24h (U87MG, U118, U138) or 48h (A172, LN18). Cell viability was measured by MTT assay. Mean + SD of three independent experiments are shown.

Fig. S2. Bcl-2 is not involved in B10 cytotoxic activity. U87MG cells overexpressing murine Bcl-2 (Bcl-2) or empty vector (EV) were treated with B10 (18 μM) for 21h or TRAIL (20 ng/ml)/CHX (1 μg/ml) for 15h. Cell viability was measured by crystal violet staining (a) and caspase-3 activity was determined using a fluorogenic substrate (b). TRAIL/CHX combination treatment was used as a positive control. Mean + SD of three independent experiments are shown, *P<0.05, #P>0.05.

Fig. S3. α-tocopherol reduces B10 cytotoxicity, LC3 lipidation and lysosomal permeabilization. A) Expression and phosphorylation status of Akt and S6 ribosomal protein as well as LC3 lipidation was determined in U87MG cells treated with 18 μM B10 for 24h in the presence or absence of α-tocopherol. B) Cell viability was measured in the presence or absence of α-tocopherol in U87MG cells treated with 18 μM B10 for 24h. The percentage of cell density normalized to cell density prior to stimulation is shown with mean + SD of four independent experiments, *P<0.01. C) Low LTR population was quantified in cells U87MG treated with 18 μM B10 for 21h in the presence or absence of α-tocopherol. Mean + SD of three independent experiments are shown, *P<0.05.

Fig. S4. Effect of TSC2 knockdown on B10-induced cytotoxicity. U87MG cells transduced with control or TSC2 shRNA vectors were treated for 24h with 18 μM B10 or DMSO. TSC2

expression (A) and LC3 lipidation as well as phosphorylation and expression of Akt and 4EBP1 (B) were analyzed by immunoblot. (C) Cell viability was determined by crystal violet staining after treatment for 24h with 18 μM B10 or DMSO; white bars: control shRNA, black bars: TSC2 shRNA. Mean + SD of three independent experiments in triplicate are presented; *P<0.05, #P>0.05.

Fig. S5. Effect of ATG5 knockdown on B10-induced cytotoxicity. U87MG cells transduced with control or ATG5 shRNA vectors were treated for 24h with 18 μM B10 or DMSO. ATG5 expression (A) and LC3 lipidation (B) were analyzed by immunoblot. (C) Cell viability was determined by crystal violet staining after treatment for 24h with 18 μM B10 or DMSO; white bars: control shRNA, black bars: ATG5 shRNA. Mean + SD of three independent experiments in triplicate are presented; *P<0.05, #P>0.05.

Fig. S6. Effect of combined inhibition of autophagy and lysosomal enzymes on B10-induced cell death. U87MG cells transfected with control or ATG7 siRNA were treated for 24h with 18 μM B10 or DMSO in the presence or absence of 5 μM Ca074Me. Cell viability was determined by crystal violet staining. Mean + SD of three independent experiments in triplicate are presented.