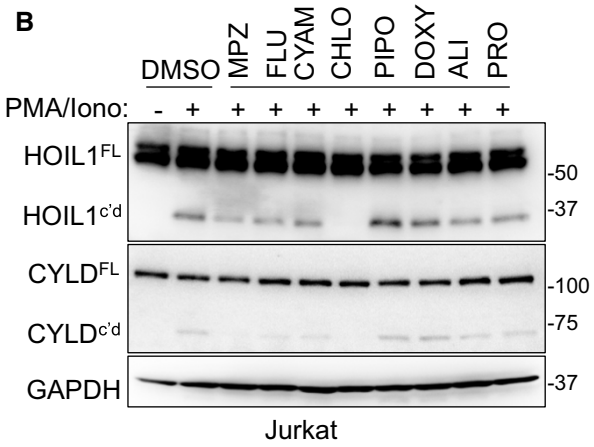
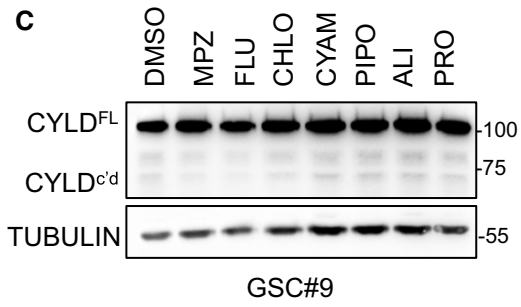
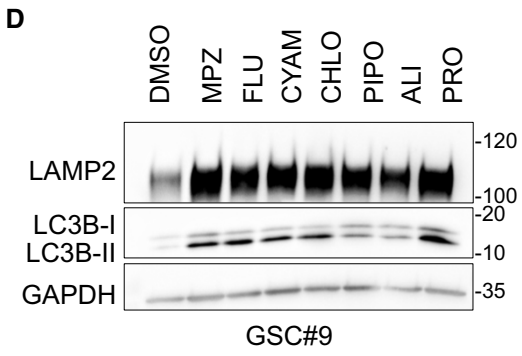
Expanded View Figures

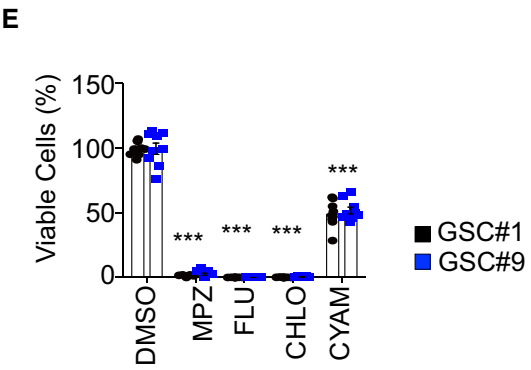
A

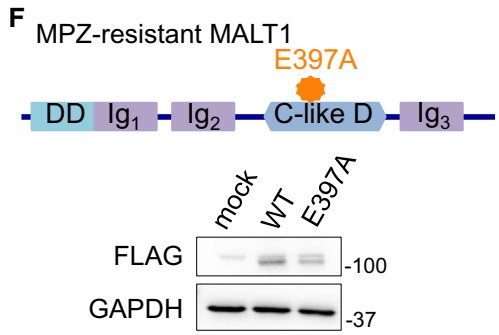
Class	Chemical Name (DRUG)	Structure
anti-psychotic	Mepazine (PACATAL)	
	Fluphenazine (MODECATE)	
	Chlorpromazine (LARGACTIL)	
	Cyamemazine (TERCIAN)	
	Pipotiazine (PIPORTIL)	
anti-histamine	Doxylamine (DONORMYL)	
	Alimemazine (THERALENE)	
	Promethazine (PHENERGAN)	











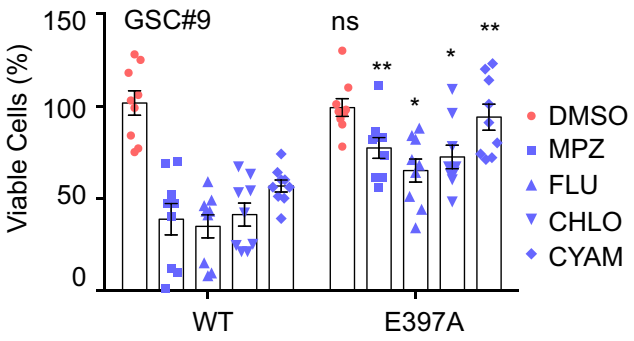


Figure EV1.

Figure EV1. Impact of phenothiazines on MALT1 protease activity and lysosomes.

A Table summarizing eight phenothiazines used in clinics as either anti-psychotic or anti-histaminic, along with their generic and brand names (cap letters), and chemical structures.

B Western blot analysis of two MALT1 substrates, HOIL1 and CYLD, either full length (FL) or cleaved (c'd) in Jurkat T cells treated with vehicle (DMSO) or phenothiazines, as follows: 20 μM CYAM (cyamemazine), CHLO (chlorpromazine), PIPO (pipotiazine), DOXY (doxylamine), ALI (alimemazine), and PRO (promethazine), and 10 μM MPZ (mepazine) and FLU (fluphenazine) for 30 min and stimulated for 30 min more with PMA (20 ng/ml) and Ionomycin (Iono, 300 ng/ml). TUBULIN served as a loading control.

C Western blot analysis of CYLD processing in GSC#9 treated with vehicle (DMSO) or phenothiazines (20 μM CYAM, CHLO, PIPO, DOXY, ALI, and PRO, 10 μM MPZ and FLU) for 60 min. GAPDH served as a loading control.

D Western blot analysis of LAMP2 and LC3B in equal amount of total protein lysates from GSC#9 treated for 6 h with vehicle (DMSO) or 20 μM phenothiazines (MPZ, FLU, CYAM, CHLO, ALI, PRO). GAPDH served as a loading control.

E Cell viability of GSC#1 and GSC#9 using 20 μM of MPZ, FLU, CHLO, and CYAM, using Cell TiterGlo assays. Data were normalized to their respective DMSO-treated controls and are presented as the mean ± SEM of three independent experiments in triplicate.

F Schematic drawing of MALT1 structures highlighting the E397A substitution in the mepazine-resistant version. DD: death domain, C-like D: caspase-like domain, Ig: immunoglobulin domain. Western blot analysis of FLAG in equal amount of total protein lysates from HEK-293T cells transfected with empty vector (mock), MALT-WT, or MALT1-E397A. GAPDH serves as a loading control. GSC#9 were transduced with MALT-WT or MALT1-E397A and treated with phenothiazines (10 μM of MPZ, FLU, CYAM, CHLO) for 24 h. Cell Viability was analyzed using Cell TiterGlo assay. Data were normalized to their respective DMSO-treated controls and are presented as the mean ± SEM of three independent experiments in triplicate.

Data information: All data were repeated in three independent experiments. Statistics were performed using a one-way ANOVA with a 95% confidence interval for all experiments with *P*-values stated. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Source data are available online for this figure.

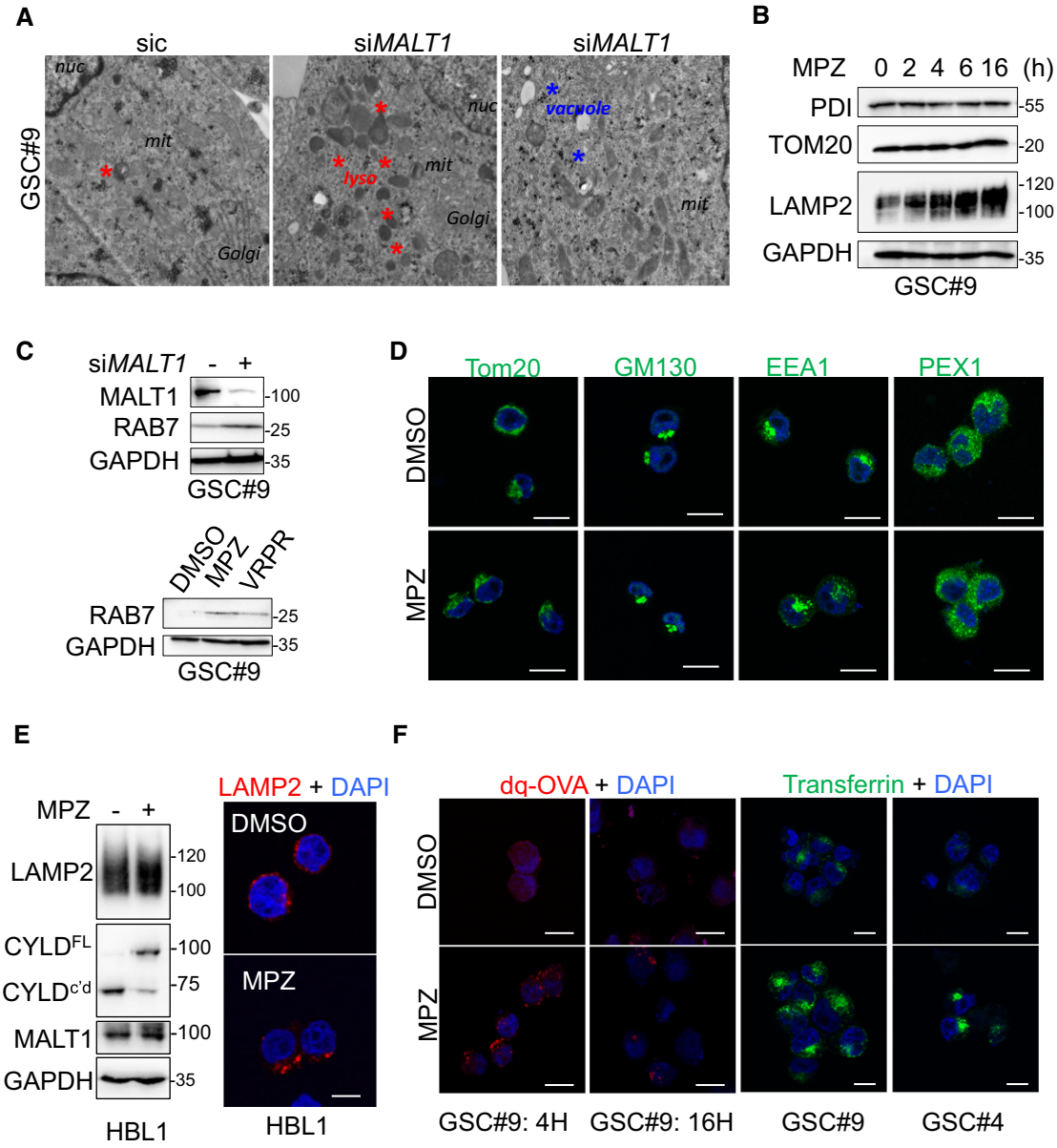


Figure EV2. Impact of MALT1 inhibition on intracellular organelles.

A Transmission electron microscopy images from GSC#9 transfected with non-silencing duplexes (sic) or siRNA duplexes targeting *MALT1* (si*MALT1*). Multiple images and sections from one experiment were analyzed. Red stars denote lysosomes; blue stars vacuoles.

B Western blot analysis of PDI, TOM20, and LAMP2 in total protein lysates from GSC#9 treated vehicle (DMSO) or MPZ (20 μM) for the indicated times. GAPDH serves as a loading control.

C Western blot analysis of RAB7 and MALT1 in GSC#9 in total protein lysates from GSC#9 transfected with non-silencing duplexes (sic) or siRNA duplexes targeting *MALT1* (si*MALT1*). Alternatively, GSC#9 received Z-VRPR-FMK (VRPR, 75 μM, 16 h) and mepazine (MPZ, 20 μM, 16 h). GAPDH serves as a loading control.

D Confocal analysis of TOM20, GM130, EEA1, and PEX1 immunostaining (green) in GSC#9 treated with vehicle (DMSO) or MPZ (20 μM) for 4 h. Nuclei (DAPI) are shown in blue. Scale bars: 10 μm.

E ABC DLBCL lymphoma cells HBL1 treated with vehicle (DMSO) or MPZ (20 μM) for 4 h. (Left) Western blot analysis of LAMP2 and CYLD (full length, FL, or cleaved, c'd) in total protein lysates. MALT1 and GAPDH serve as loading controls. (Right) Confocal analysis of LAMP2 (red). Nuclei (DAPI) are shown in blue. Scale bars: 10 μm.

F Confocal analysis of dq-ovalbumin (dq-OVA, red) in GSC#9 treated with vehicle (DMSO) or MPZ (20 μM) for 4 or 16 h. Nuclei (DAPI) are shown in blue. Alternatively, confocal analysis of transferrin uptake (green) in GSC#9 and GSC#4 treated with vehicle (DMSO) or MPZ (20 μM) for 4 h. Nuclei (DAPI) are shown in blue. Scale bars: 10 μm.

Data information: All data were repeated in three independent experiments, unless specified.

Source data are available online for this figure.

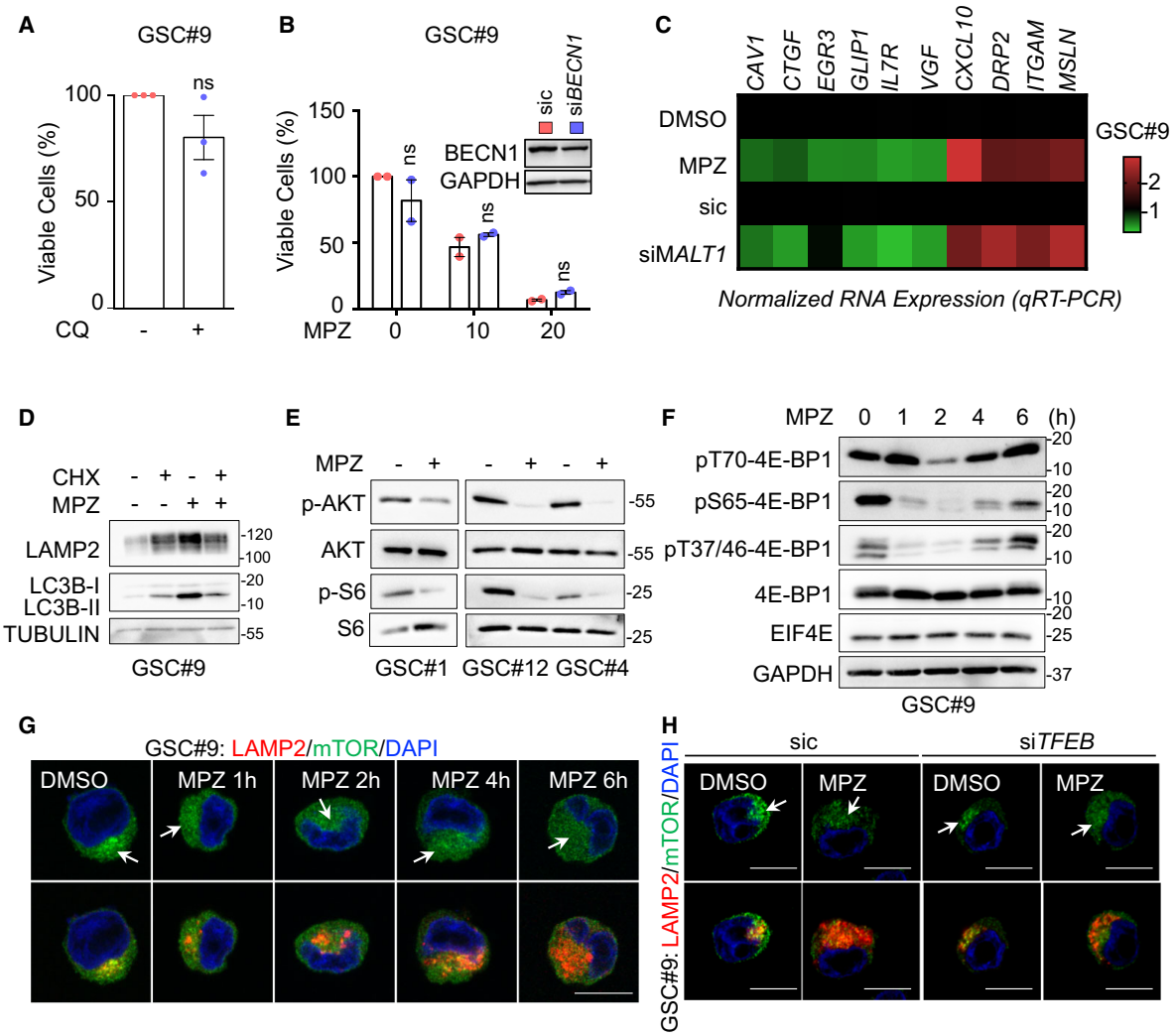


Figure EV3. Impact of MALT1 inhibition on cell death and mTOR signaling.

A Cell viability was measured using Cell TiterGlo luminescent assay in GSC#9 treated for 72 h with vehicle (DMSO) or chloroquine (CQ, 20 μM). Data were normalized to the vehicle-treated controls and are presented as the mean ± SEM of three independent experiments in triplicate.

B Cell viability was measured using Cell TiterGlo luminescent assay in GSC#9 transfected with non-silencing duplexes (sic, red) or siRNA duplexes targeting *BECLIN1* (si*BECN1*, blue) and further treated with vehicle (DMSO) and MPZ (10 and 20 μM) for 72 h. Data were normalized to the vehicle-treated controls and are presented as the mean ± SEM of two independent experiments in triplicate. Knockdown efficiency was checked at the end point by Western blot. GAPDH serves as a loading control.

C GSC#9 were treated with vehicle (DMSO) and mepazine (MPZ, 20 μM) for 16 h. Alternatively, GSC#9 were transfected with non-silencing duplexes (sic) or siRNA duplexes targeting *MALT1* (si*MALT1*). RNAs were processed for qRT–PCR on 10 gene candidates from RNAseq data (Table EV1). Data are represented as heatmap representation of RNA expression, normalized to two housekeeping genes (*HPRT1* and *ACTB*).

D Western blot analysis of LAMP2 and LC3B in total protein lysates from GSC#9 treated with vehicle (DMSO) and mepazine (MPZ, 20 μM) in the presence of cycloheximide (CHX, 50 μg/ml) for 16 h. TUBULIN served as a loading control.

E Western blot analysis of indicated antibodies in total protein lysates from GSC#1, GSC#12, and GSC#4 that received vehicle (DMSO, –) or mepazine (MPZ, 20 μM, 1 h).

F Western blot analysis of indicated antibodies in total protein lysates from GSC#9 treated vehicle (DMSO) or mepazine (MPZ, 20 μM) for the indicated times. GAPDH serves as a loading control.

G Confocal analysis of LAMP2 (red) and mTOR (green) staining in GSC#9 treated vehicle (DMSO) or MPZ (20 μM) for the indicated times. Arrows point to LAMP2-positive area. Nuclei (DAPI) are shown in blue. Scale bars: 10 μm.

H GSC#9 were transfected with sic or si*TFEB* and treated with vehicle (DMSO) or MPZ (20 μM) for 16 h. Samples were analyzed as described in (G). Arrows point to LAMP2-positive area. Nuclei (DAPI) are shown in blue. Scale bars: 10 μm.

Data information: All data were repeated in three independent experiments, unless specified.

Source data are available online for this figure.

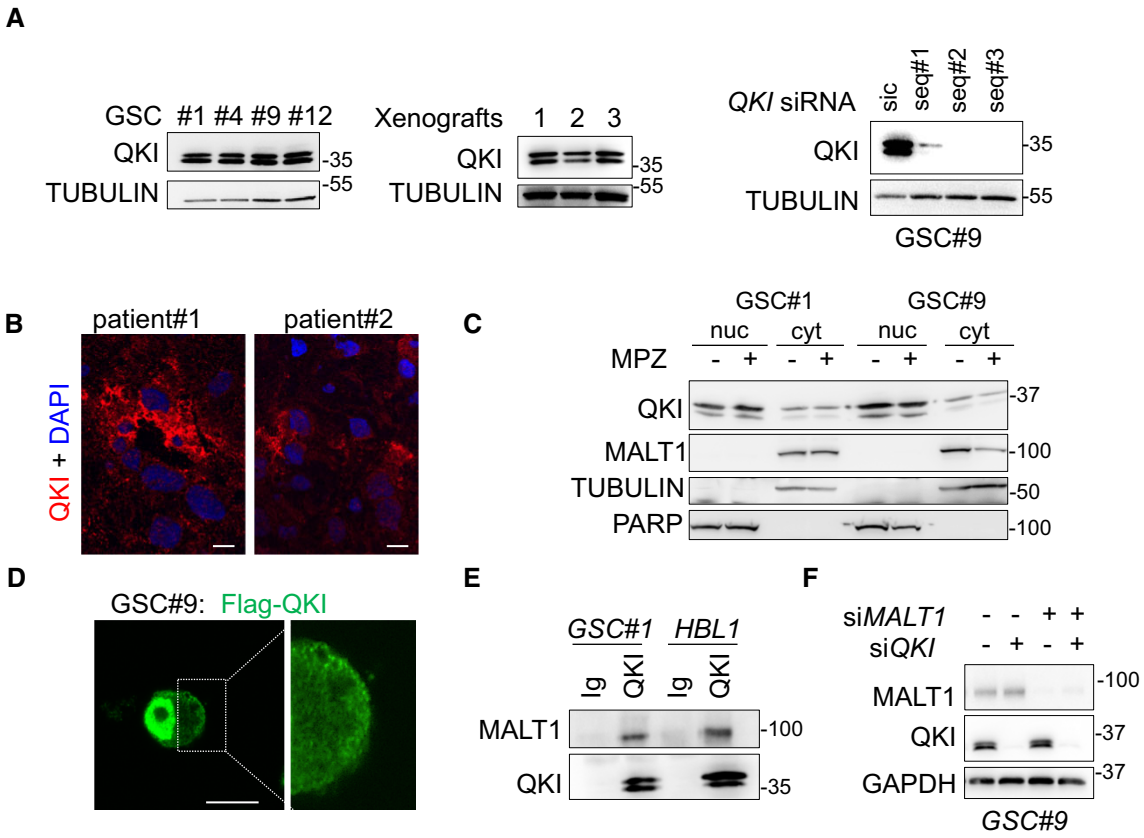


Figure EV4. Characterization of the RNA-binding protein QKI in glioblastoma cells.

A Western blot analysis of QKI in total protein lysates from GSC #1, #4, #9, #12, and from GSC-xenografted tumors. Alternatively, GSC#9 were transfected with sic or si*QKI* using three different duplexes. TUBULIN served as a loading control.

B Confocal analysis of QKI immunostaining (red) in glioblastoma tissue sections from two patients. Nuclei (DAPI) are shown in blue. Scale bars: 10 μm.

C Western blot analysis of QKI in cytosolic (cyt.) and nuclear (nuc.) cell fractionation from GSC#1 and GSC#9, treated with vehicle (–) and mepazine (MPZ, 20 mM, 1 h). TUBULIN and PARP served as loading controls for each fraction. Each panel was replicated at least twice.

D Confocal analysis of FLAG-QKI (green) localization in transfected GSC#9. Scale bars: 10 μm.

E GSC#1 and HBL1 protein lysates were processed for immunoprecipitation using control immunoglobulins (Ig) and anti-QKI antibodies. Western blots were performed using anti-MALT1 and anti-QKI, as specified.

F GSC#9 were transfected with non-silencing RNA duplexes (sic), *QKI* targeting siRNA duplexes (si*QKI*), *MALT1* targeting siRNA duplexes (si*MALT1*), or double-transfected with si*QKI* and si*MALT1* and analyzed 72 h later. Knockdown efficiency was checked by Western blot analysis using the indicated antibodies.

Source data are available online for this figure.