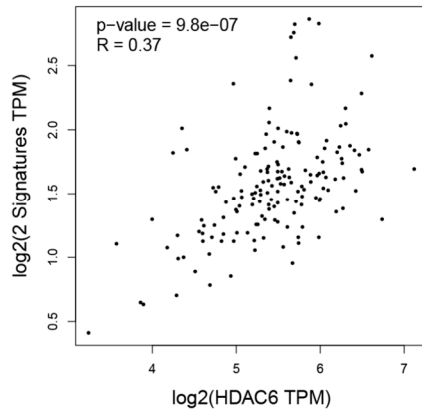
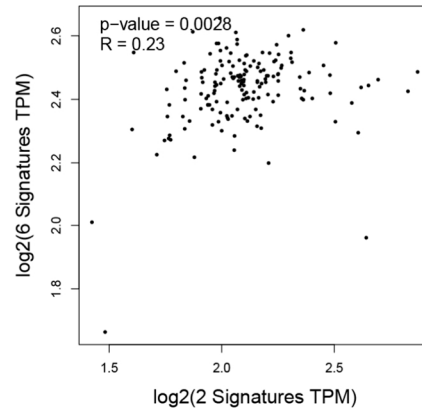
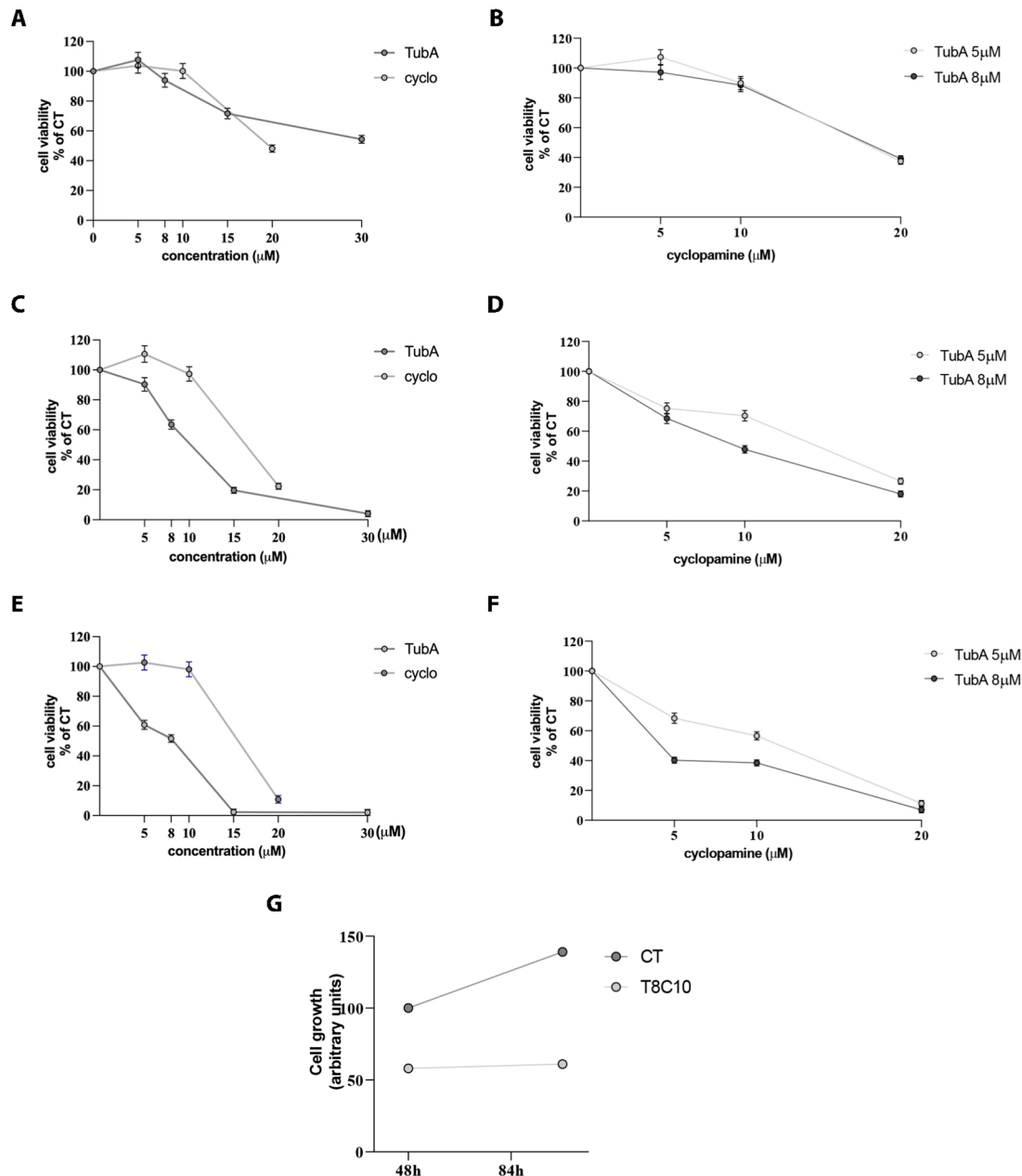


Table S1. List of antibodies and their relative dilution. Wester-blot (WB), immunohistochemistry (IHC) and immunofluorescence (IF) analyses.

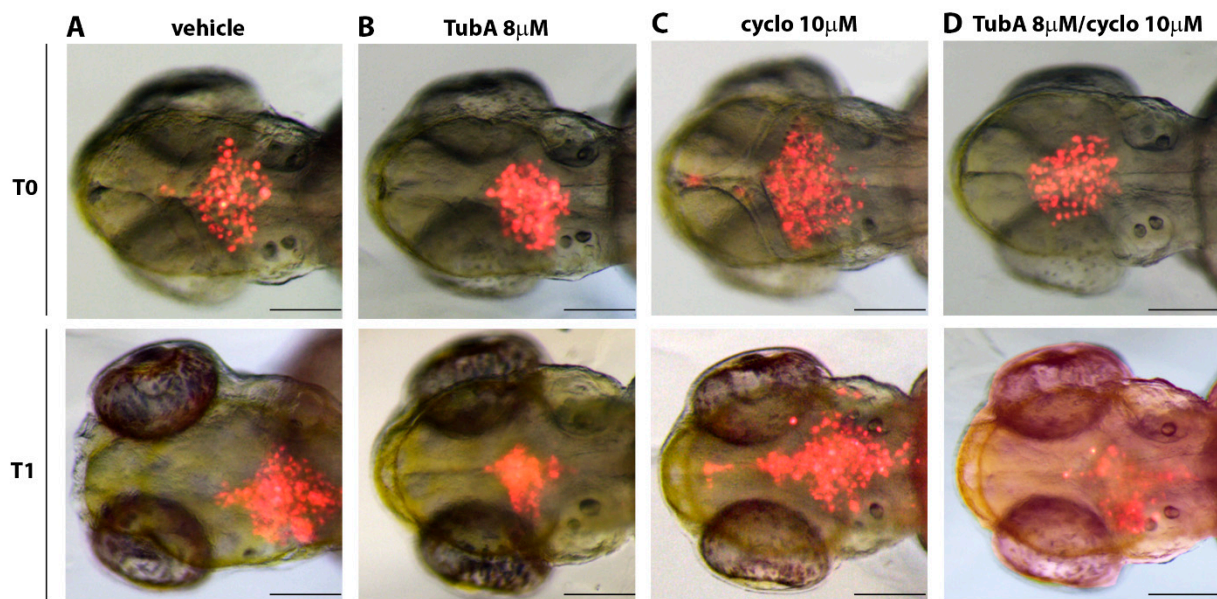
Antibody	Brand	Protocol	Dilution	Model
Rabbit anti LC3A/LC3B	Immunological Sciences (Roma, Italy)	WB IHC	1:500 1:60	U87-MG
Rabbit anti LAMP1	Immunological Sciences (Roma, Italy)	WB IHC	1:100 1:500	U87-MG
Rabbit anti GAPDH	Santa Cruz Biotechnology (Santa Cruz, CA, USA)	WB	1:1000	U87-MG
Mouse anti p-AKT	Cell Signaling (Danvers, MA, USA)	WB	1:1000	U87-MG
Rabbit anti AKT	Cell Signaling (Danvers, MA, USA)	WB	1:1000	U87-MG
Mouse anti p-ERK 1/2	Cell Signaling (Danvers, MA, USA)	WB	1:1000	U87-MG
Rabbit anti ERK 1/2	Cell Signaling (Danvers, MA, USA)	WB	1:1000	U87-MG
Goat anti-rabbit IgG-HRP	Immunological Sciences (Roma, Italy)	WB	1:200	U87-MG
Goat anti-mouse IgG-HRP	Immunological Sciences (Roma, Italy)	WB	1:5000	U87-MG
IgG anti-rabbit HiLyte Flour 488	Thermo Fisher Scientific Inc. (Waltham, MA, USA)	IF	1:500	U87-MG
IgG anti-mouse Alexa Fluor 546	Thermo Fisher Scientific Inc. (Waltham, MA, USA)	IF	1:7000	U87-MG
Mouse anti-GFP	Sigma-Aldrich (Saint Louis, MO)	IF	1:1000	Zebrafish
Rabbit anti-SQSTM1/p62	Immunological Sciences (Roma, Italy)	IF WB	1:200 1:1000	Zebrafish U87-MG
Alexa 488-conjugated goat anti-mouse IgG	Invitrogen Life Technologies (Carlsbad, CA, USA)	IF	1:400	Zebrafish
Alexa 546-conjugated goat anti-rabbit IgG	Invitrogen Life Technologies (Carlsbad, CA, USA)	IF	1:400	Zebrafish

A**B**

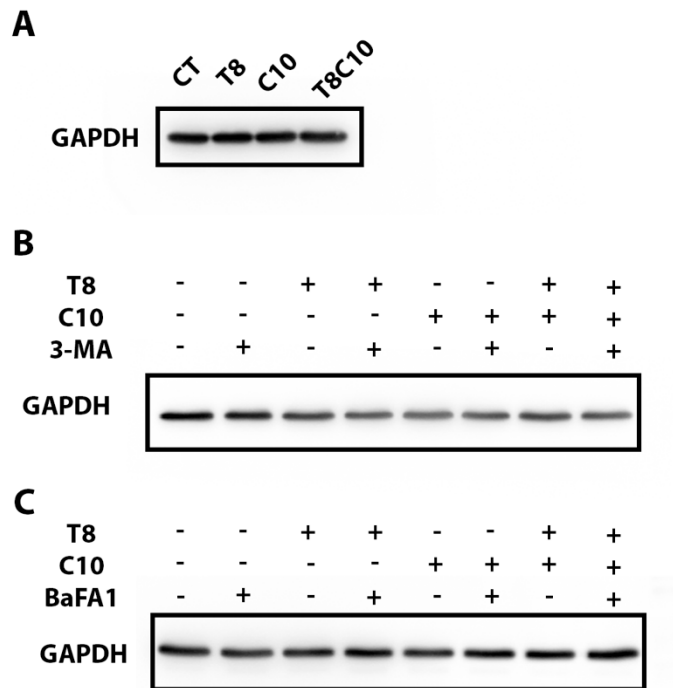
Supplementary Figure S1. *Hh*, *HDAC6* and sphingolipids enzyme correlation in GBM datasets and U87-MG. A) GEPIA-2 correlation analyses between the expression levels of *HDAC6* (HDAC6 TPM) and *GLI1* and *PTCH1* (2 signature TPM). B) correlation analyses between the expression levels of *HDAC6* and *GLI1* (2 signature TPM) and expression of the genes of sphingolipid metabolism (6 signature TPM; *CERS1*, *SGMS1*; *SPHK1*; *SPTLC1*; *UGCG*; *SGPP1*). GBM: Glioblastoma; TPM: transcript per millions. Correlation analyses: Spearman correlation. *** $p < 0.001$.



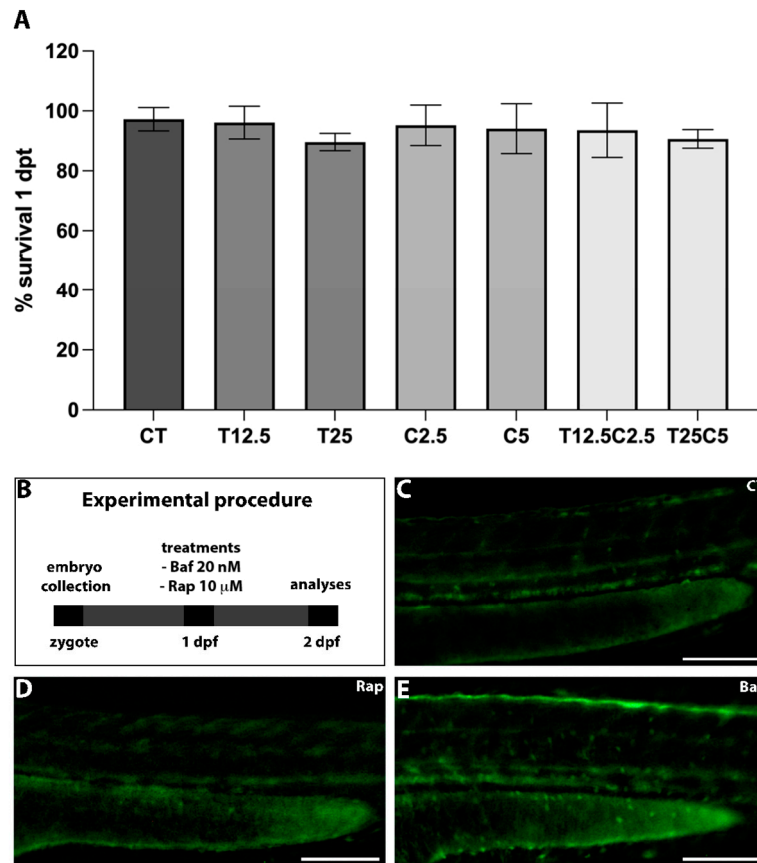
Supplementary Figure S2. Setting conditions for TubA and cyclo administration in U87-MG cells: Effects of TubA and cyclo on U87-MG cells viability alone (**A**, **C**, **E**) and in combination (**B**, **D**, **F**) after 24 h (**A** and **B**), 48h (**C** and **D**) and 72h (**E** and **F**) of treatment. **G**) Effect of 48h combined treatment with 8 μM TubA and 10 μM cyclo following 24h treatment withdrawal on cell viability. Data are presented as cell growth (arbitrary units, A.U.) \pm standard deviation, at each time point. TubA: TubastatinA; cyclo:cyclopamine.



Supplementary Figure S3. Xenotransplantation of U87 GBM cells treated with *Hh* and HDAC6 inhibitors in zebrafish hindbrain A) Representative images of the head region of zebrafish embryos xenotransplanted with la-belled U87-MG cells pre-treated with B) CT, C) T8, D) C10 and E) T8C10 a T0 or T1 (24 hpi). Scale bare indicates 100 μ m.



Supplementary Figure S4. Western blot analyses of GAPDH in the U87-MG cell lines. A) GAPDH levels of U87-MG cell line used in the experiment for the analyses of pERK and pAKT (Figure 1G) and GAPDH levels of U87-MG cell line in the experiments with the treatment with B) 3-MA (Figure 2A) and C) BafA1 (Figure 2B). CT: vehicle; TubA: TubastatinA; cyclo: cyclopamine; 3-MA: 3-methyladenine; BafA1: Bafilomycin A1. + and – indicate the presence or the absence of the chemical in the cell medium respectively.



Supplementary Figure S5. Setting doses for pharmacological treatment and effect of autophagy modulators in zebrafish embryos **A)** Survival analyses of embryos treated with different concentration of TubA and cyclo, alone or in combination setting. **B)** Schematic representation of the pharmacological treatment with autophagy modulators in the Tg(*CMV-lc3b:GFP*) line. **C-E)** Representative images of the trunk region of embryos treated with **B)** CT; **C)** 1 μ M Rap; **D)** 20 nM BafA1. Scale bar indicates 100 μ m. CT: control; T: TubastatinA; C: cyclopamine; Rap: rapamycin; BafA1: Bafilomycin A1.