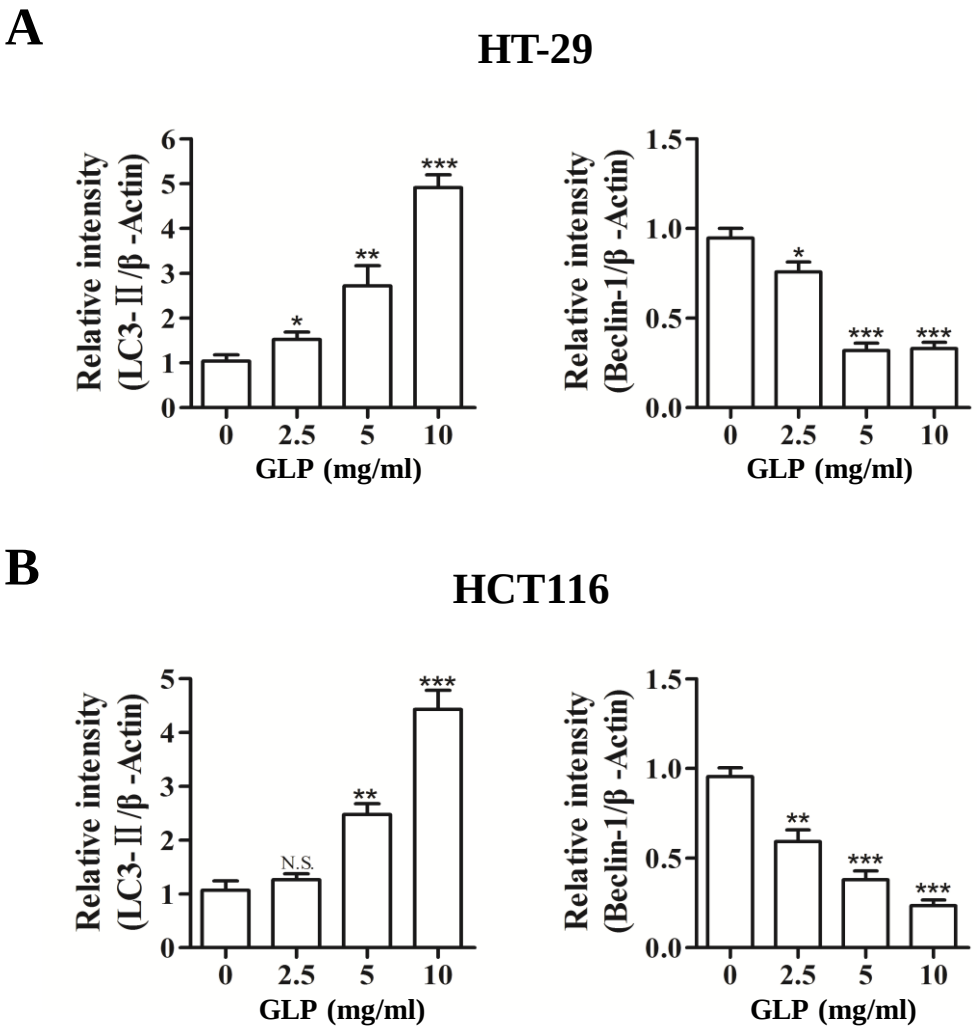
Supplementary Information

Supplementary method

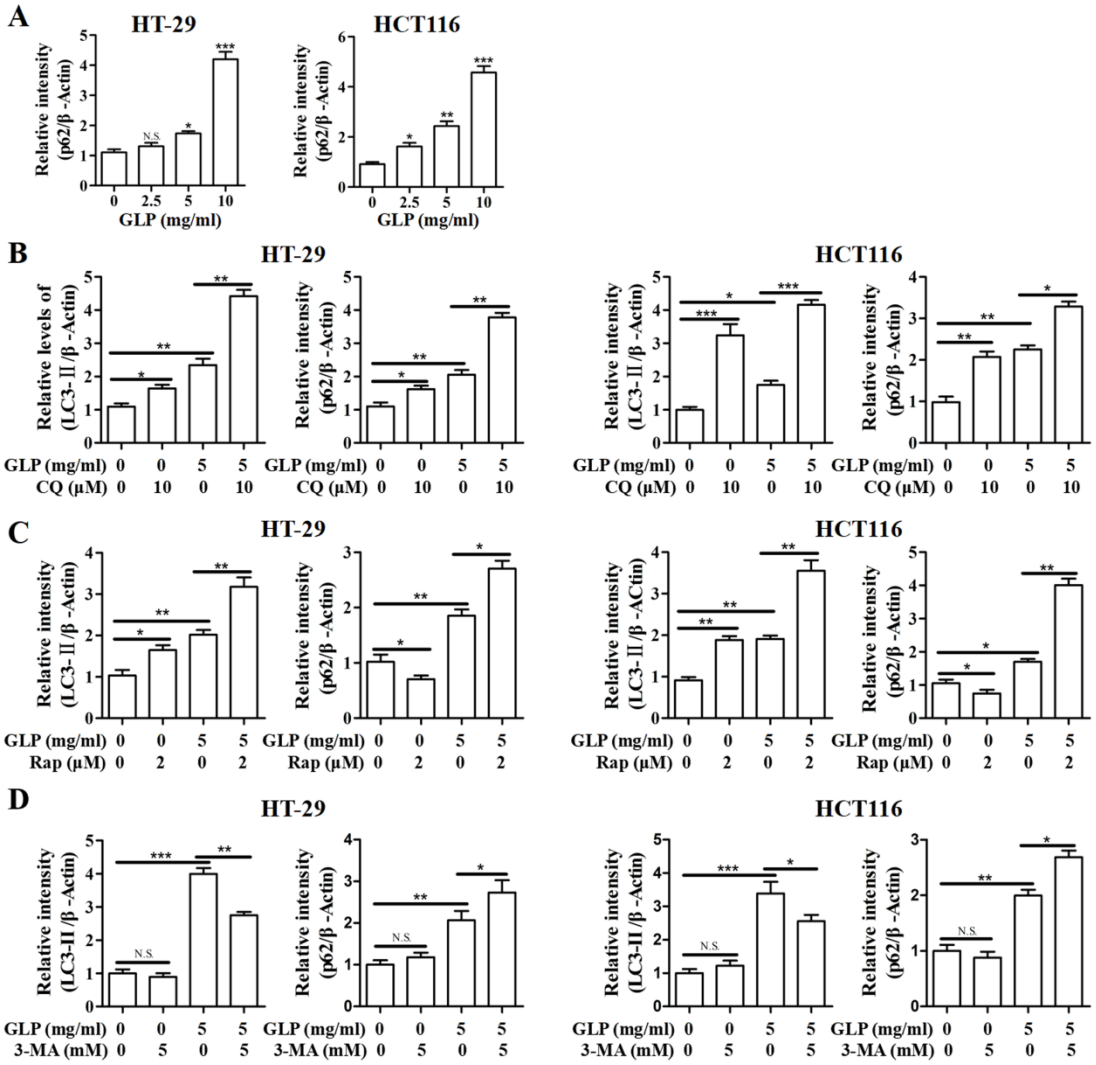
TUNEL staining and apoptotic index measurement

Apoptotic index was determined by TUNEL staining. Tumor samples were harvested and fixed in 10% formalin for 24 h. The fixed tumor tissues were embedded in paraffin, and 5-μm sections were cut for TUNEL staining using TUNEL assay kit (Roche Applied Sciences, Basel, Switzerland) according to manufacturer's instruction. Approximately 300 cells per field of each section and 3 fields of each section were counted. The number of TUNEL-positive cells detected in tumor samples were calculated to determine the apoptotic index.

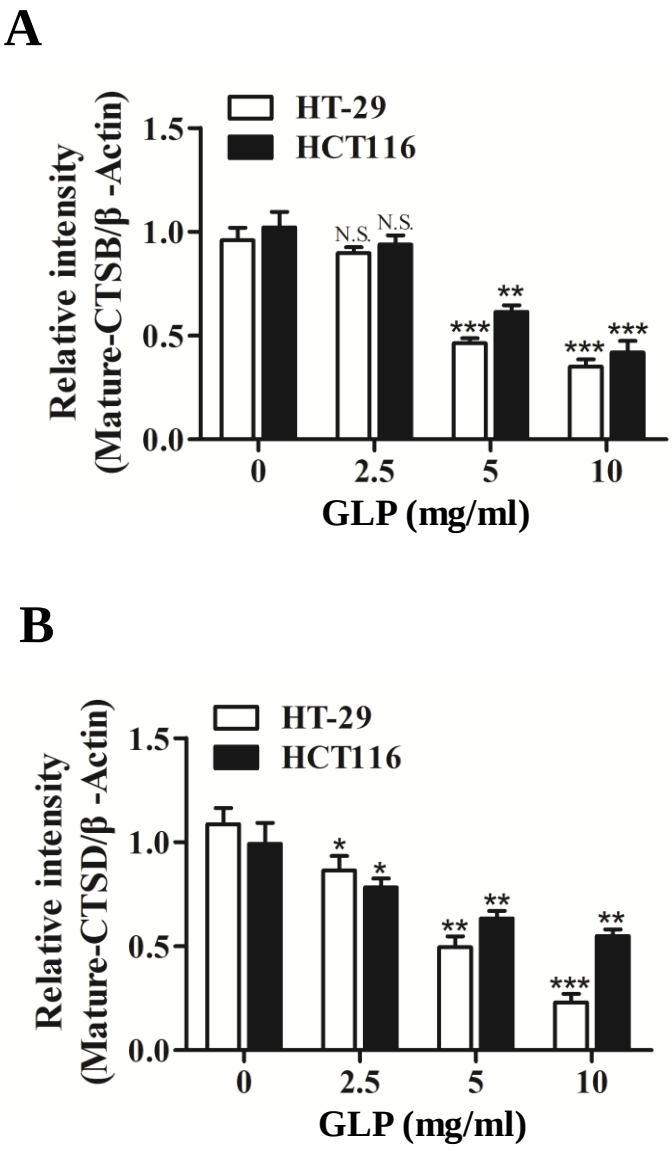
Supplementary Figure 1



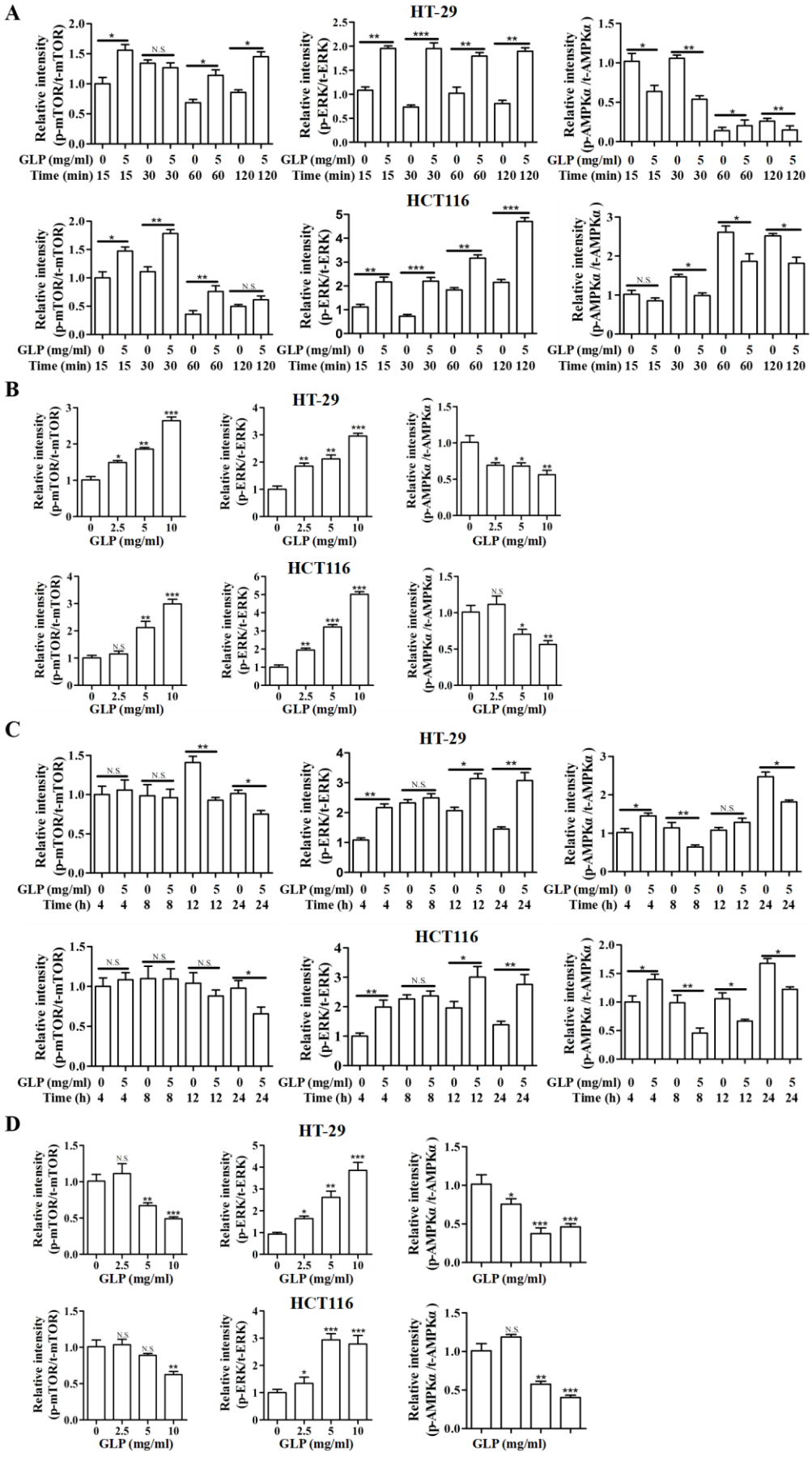
Supplementary Figure 2



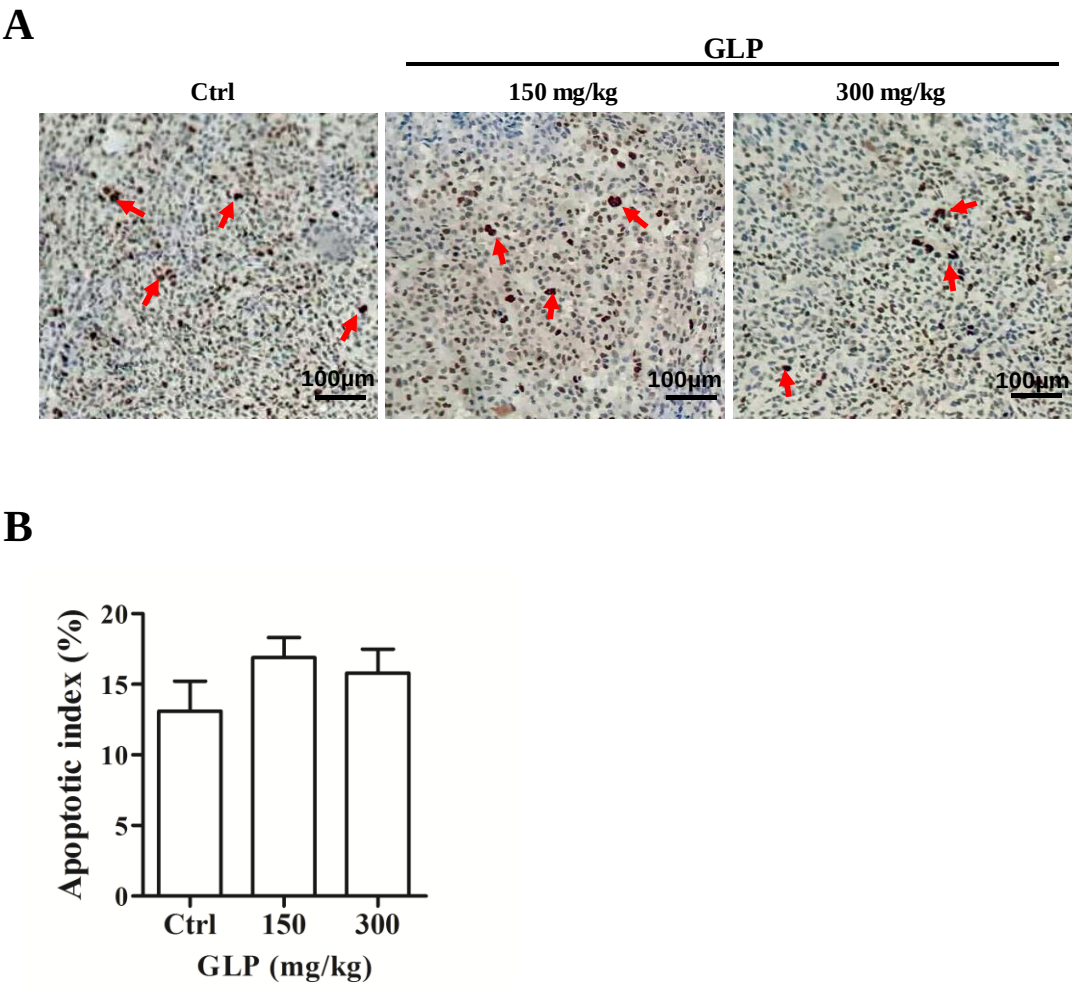
Supplementary Figure 3



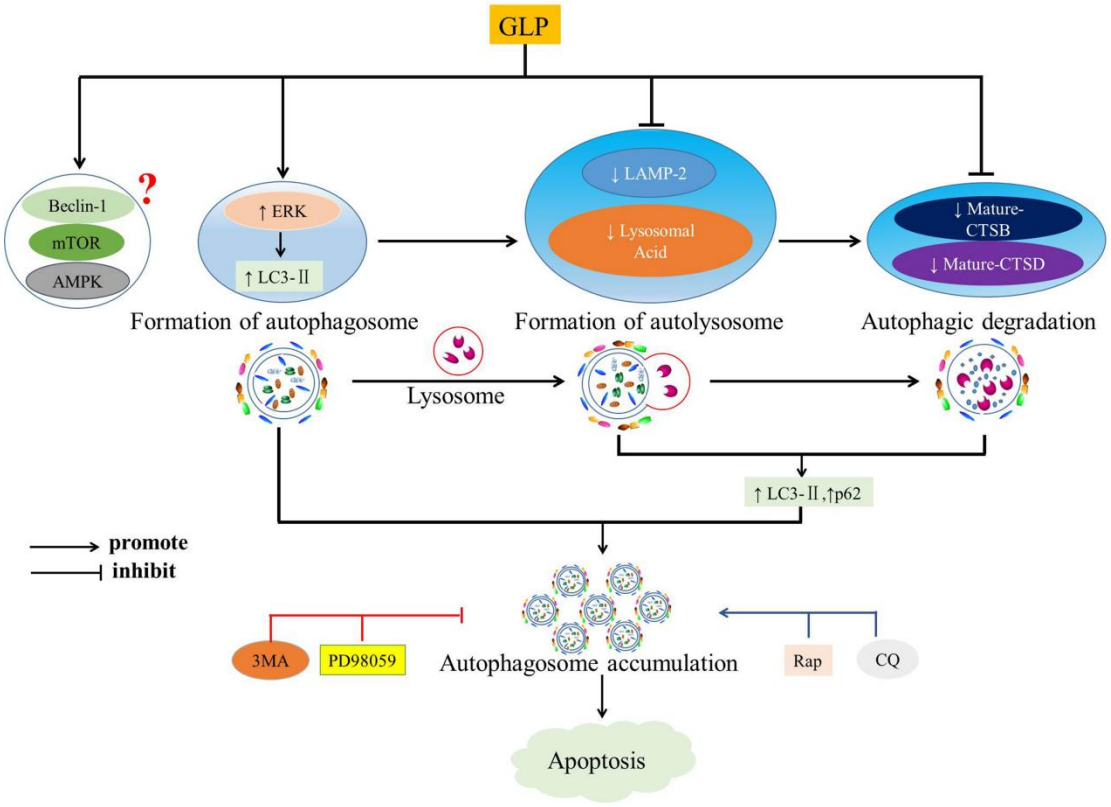
Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6



Spplementary Table 1 Primer sequence of qRT-PCR reactions

Primers		Sequences
LC3-II	Forward	GATGTCCGACTTATTCGAGAGC
	Reverse	TTGAGCTGTAAGCGCCTTCTA
Beclin-1	Forward	AGCTGCCGTTATACTGTTCTG
	Reverse	ACTGCCTCCTGTGTCTTCAATCTT
p62	Forward	GAACTCCAGTCCCTACAGATGCC
	Reverse	CGGGAGATGTGGGTACAAGG
CTSB	Forward	AACACGTCACCGGAGAGATGA
	Reverse	CCCAGTCAGTGTTCCAGGAGTT
CTSD	Forward	GGCTCTGTGGAGGACCTGATTG
	Reverse	CGATGCCAATCTCCCCGTGTA
LAMP-2	Forward	ACAACAGTGGATCAGACAGTACG
	Reverse	AGCAGCAAGCATCAGTTCTTC
β-Actin	Forward	CTGGAACGGTGAAGGTGACA
	Reverse	AAGGAACTTCCTTGAACAATGCA

Supplementary Table 2 Sources and dilutions of primary antibodies for WB

Antibody	Supplier and catalog no.	Dilution
LC3-I/II	Cell signaling, 4108	1:1000
p62	Cell signaling, 8025	1:1000
Beclin-1	Cell signaling, 3495	1:1000
p-ERK	Cell signaling, 9101s	1:1000
ERK	Cell signaling, 9102s	1:1000
p-mTOR	Cell signaling, 5536p	1:1000
mTOR	Cell signaling, 2983p	1:1000
p-AMPKα	Cell signaling, 2537	1:1000
AMPKα	Cell signaling, 4184	1:1000
CTSB	Cell signaling, 31718	1:1000
CTSD	Abcam, ab75852	1:5000
LAMP-2	Abcam, ab199946	1:2000
PARP	Cell signaling, 9542	1:1000
β-Actin	Cell signaling, 4967	1:1000

WB Western Blotting

Supplementary figure legends

Supplementary Fig. 1 Densitometric analysis of western blots for Fig. 1e. a Quantitative analysis of LC3-II and Beclin-1 expression in HT-29 cells. **b** Quantitative analysis of LC3-II and Beclin-1 expression in HCT116 cells. The relative intensities of LC3-II and Beclin-1 were calculated after normalization against β-Actin. Data are presented as the mean ± SE from three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001 compared with untreated cells. N.S., No significance.

Supplementary Fig. 2 Densitometric analysis of western blots for Fig. 2a, 2c, 2d, and 2e. a Quantitative analysis of p62 expression in HT-29 and HCT116 cells. **b** Quantitative analysis of LC3-II and p62 expression in HT-29 and HCT116 cells upon CQ treatment. **c** Quantitative analysis of LC3-II and p62 expression in HT-29 and HCT116 cells upon Rap treatment. **d** Quantitative analysis of LC3-II and p62 expression in HT-29 and HCT116 cells upon 3-MA treatment. The relative intensities of LC3-II and p62 were calculated after normalization against β-Actin signal intensity. Data are presented as the mean ± SE from three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001 compared with indicated samples. N.S., No significance.

Supplementary Fig. 3 Densitometric analysis of western blots for Fig. 4c. a Quantitative analysis of Mature-CSTB expression in HT-29 and HCT116 cells. **b** Quantitative analysis of Mature-CSTD expression in HT-29 and HCT116 cells. The relative intensity of mature-CSTB and mature-CSTD were calculated after normalization against β-Actin. Data are presented as the mean ± SE from three independent experiments. *P < 0.05; **P < 0.01; ***P < 0.001 compared with untreated

cells. N.S, No significance.

Supplementary Fig. 4 Densitometric analysis of western blots for Fig. 5a, 5b, 5c and 5d. a Quantitative analysis of p-mTOR, p-MAPK/ERK and p-AMPKα expression in HT-29 and HCT116 cells upon treatment with GLP (5 mg/ml) for 15, 30, 60, and 120 min. **b** Quantitative analysis of p-mTOR, p-MAPK/ERK and p-AMPKα expression in HT-29 and HCT116 cells upon treatment with indicated concentrations of GLP for 1 h. **c** Quantitative analysis of p-mTOR, p-MAPK/ERK and p-AMPKα expression in HT-29 and HCT116 cells upon treatment with GLP (5 mg/ml) for 4, 8, 12, and 24 h. **d** Quantitative analysis of p-mTOR, p-MAPK/ERK and p-AMPKα expression in HT-29 and HCT116 cells upon treatment with indicated concentrations of GLP for 24 h. The relative intensities of p-mTOR, p-MAPK/ERK and p-AMPKα were calculated after normalization against p-mTOR, p-MAPK/ERK and p-AMPKα, respectively. Data are presented as the Mean ± SE from three independent experiments. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ compared with untreated cells. N.S., No significance.

Supplementary Fig. 5 TUNEL detection of apoptosis index in xenograft tumor samples after treating with GLP. a TUNEL staining of xenograft tumor sections. The arrows indicate representative, but not all, apoptotic cells. Images are representative of 3 samples per treatment group. Scale bar: 100μm. **b** Apoptosis index as calculated by % TUNEL positive cells in tumor samples. Data are represented as Mean ± SE from 3 fields from each sample per group (n=9).

Supplementary Fig. 6 Working model of molecular mechanisms by which GLP exerts its anticancer activity and regulate autophagy in CRC HT-29 and HCT116 cells.