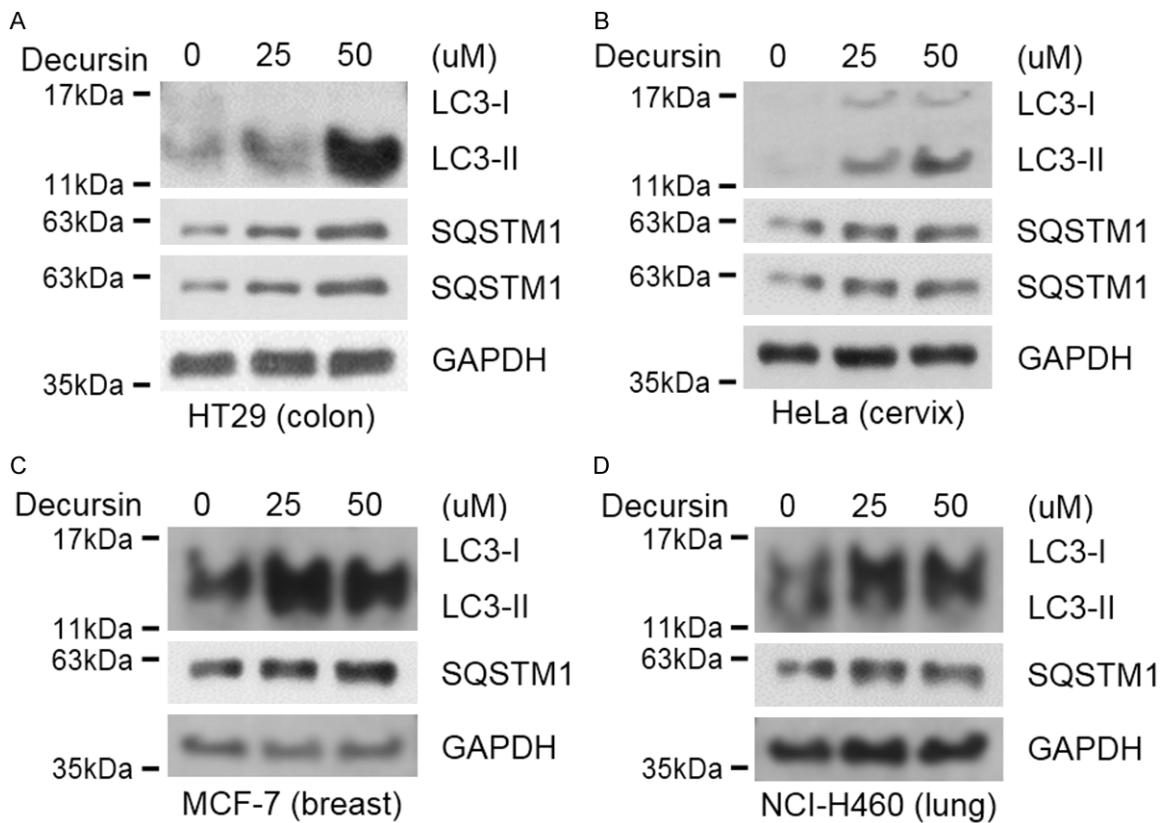


Decursin and autophagy in gastric cancer

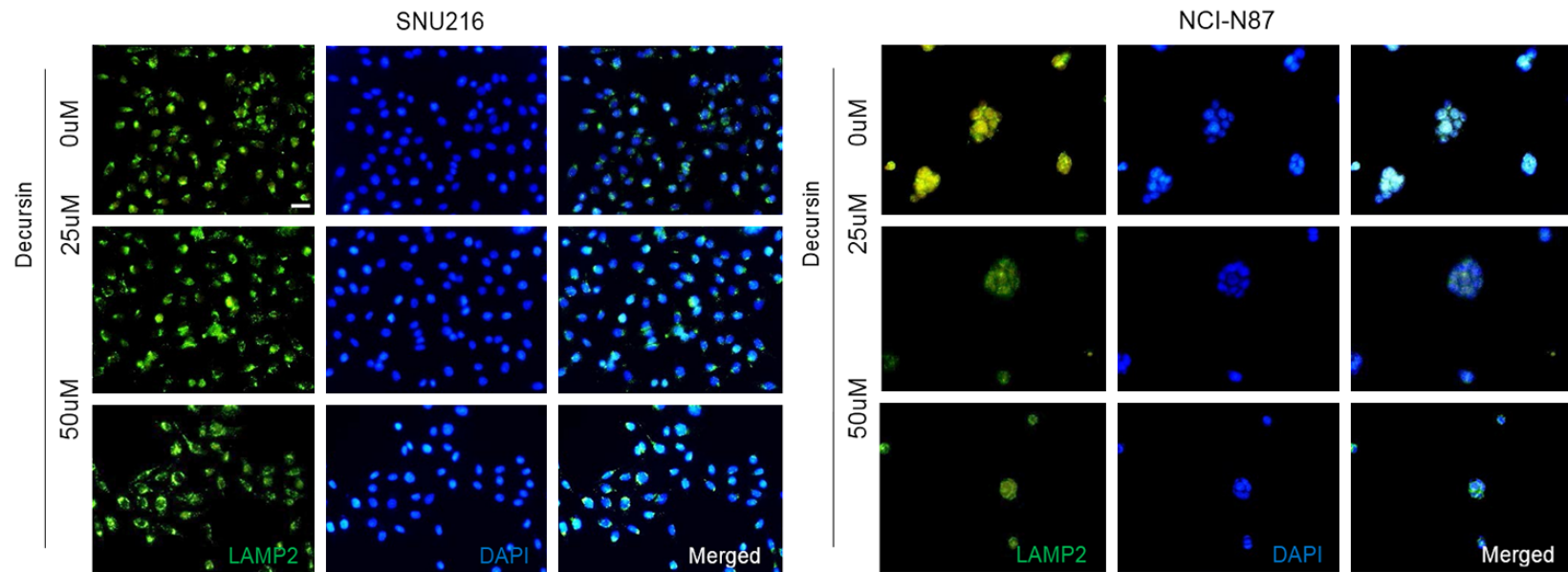
Supplementary Table 1. The primers used in this study

Gene name	Primer sequence
<i>CDK4</i>	sense: 5'-GAGTGTGAGAGTCCCAATG-3'
	antisense: 5'-GGTCCTGGTCTACATGCTCAA-3'
<i>CDK6</i>	sense: 5'-GGATAAAGTCCAGAGCCTGG-3'
	antisense: 5'-GGCCGAAGTCAGCGAGTTTT-3'
<i>E2F1</i>	sense: 5'-CCTGGCCTACGTGACGTGTC-3'
	antisense: 5'-CGGCTTGGAGCTGGGTCT-3'
<i>E2F2</i>	sense: 5'-GAGGACAAGGCCAACAAGAGG-3'
	antisense: 5'-TGTCGGGCACTTCCAGTCTC-3'
<i>E2F3</i>	sense: 5'-CACTTCCACCACCTCCTGTT-3'
	antisense: 5'-TGACCGCTTCTCCTAGCTC-3'
<i>GAPDH</i>	sense: 5'-GAGTCAACGGATTGGTCGT-3'
	antisense: 5'-TTGATTTGGAGGGATCTCG-3'



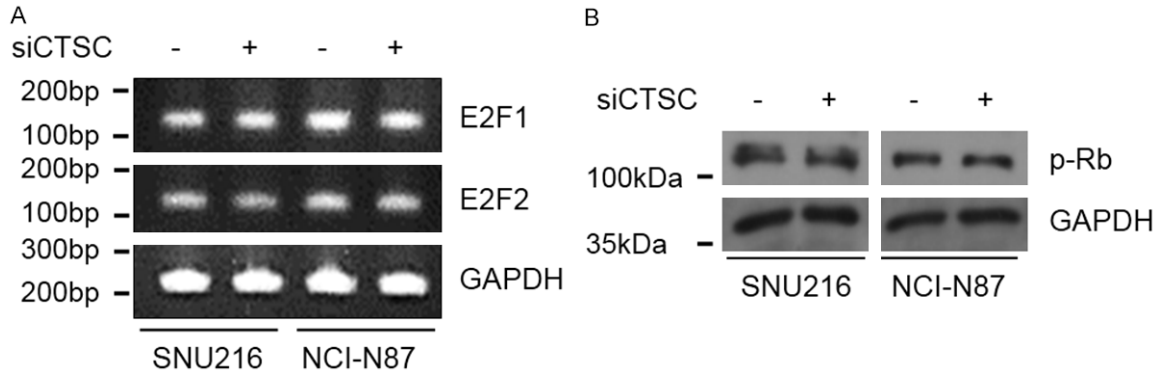
Supplementary Figure 1. Decursin acts as an autophagy inhibitor in other cancer cell lines. A-D. Each cell line was treated with decursin for 24 h; expression levels of LC3 and SQSTM1 were assessed by western blotting. Full-length blots are presented in [Supplementary Figure 6](#).

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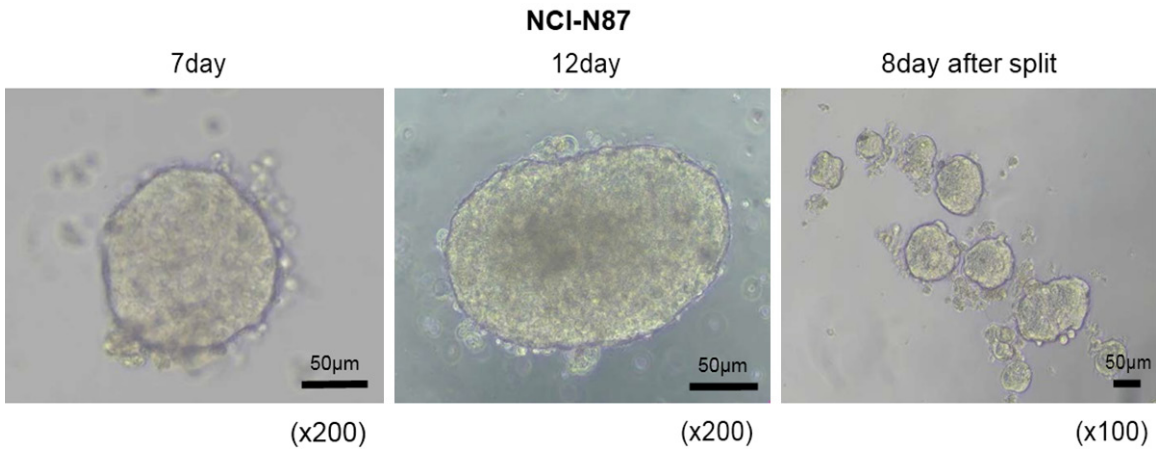


Supplementary Figure 2. Expression of LAMP2 after decursin treatment of GC cells. Immunocytochemistry revealed that LAMP2 in SNU216 and NCI-N87 cells did not change, regardless of decursin concentration ($\times 100$). Scale bar: 50 μm .

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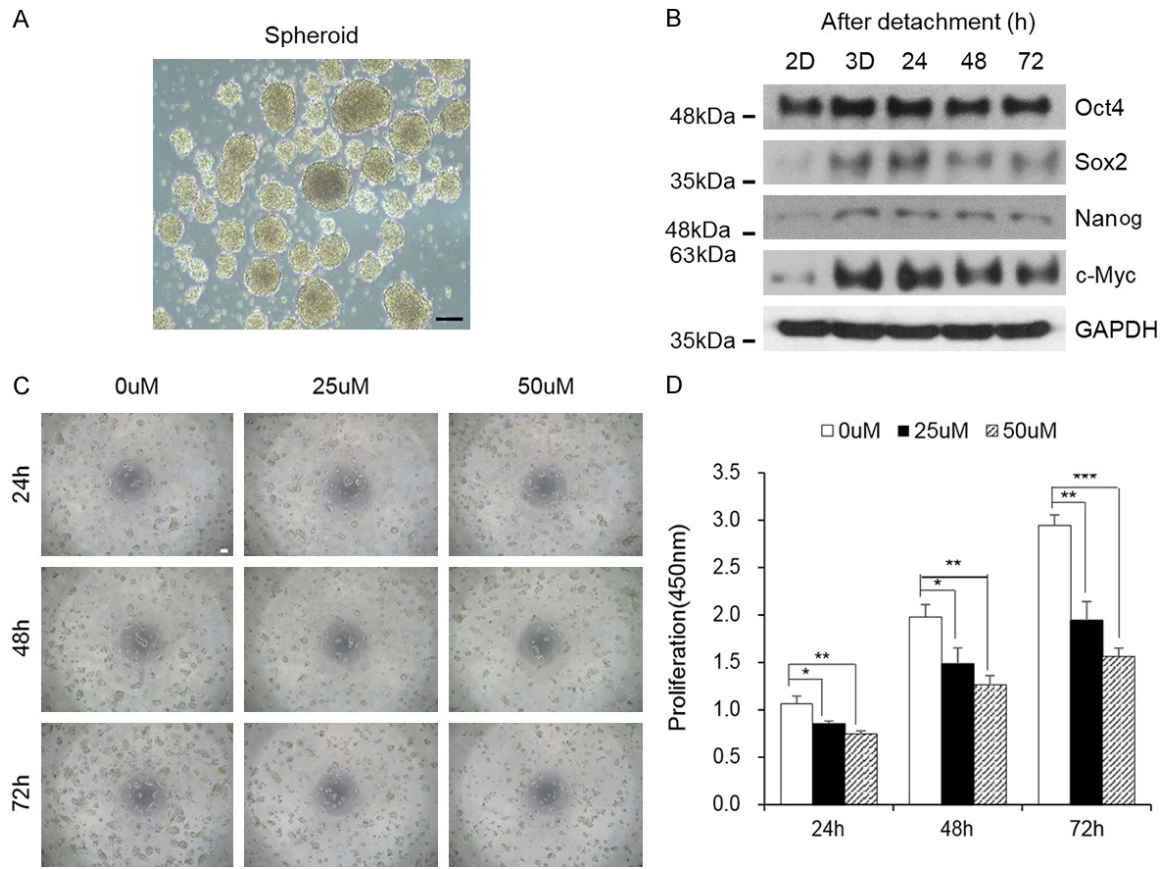


Supplementary Figure 3. Cell cycle-related gene expression after cathepsin C knockdown. A. mRNA expression levels of E2F1, E2F2, and E2F3, were confirmed. E2F3 exhibited a change after transfection with siCTSC, whereas E2F1 and E2F2 did not. B. Phosphorylation of Rb, which binds E2F3 and regulates its expression, was confirmed; however, its phosphorylation levels did not change. Full-length blots are presented in [Supplementary Figure 6](#).



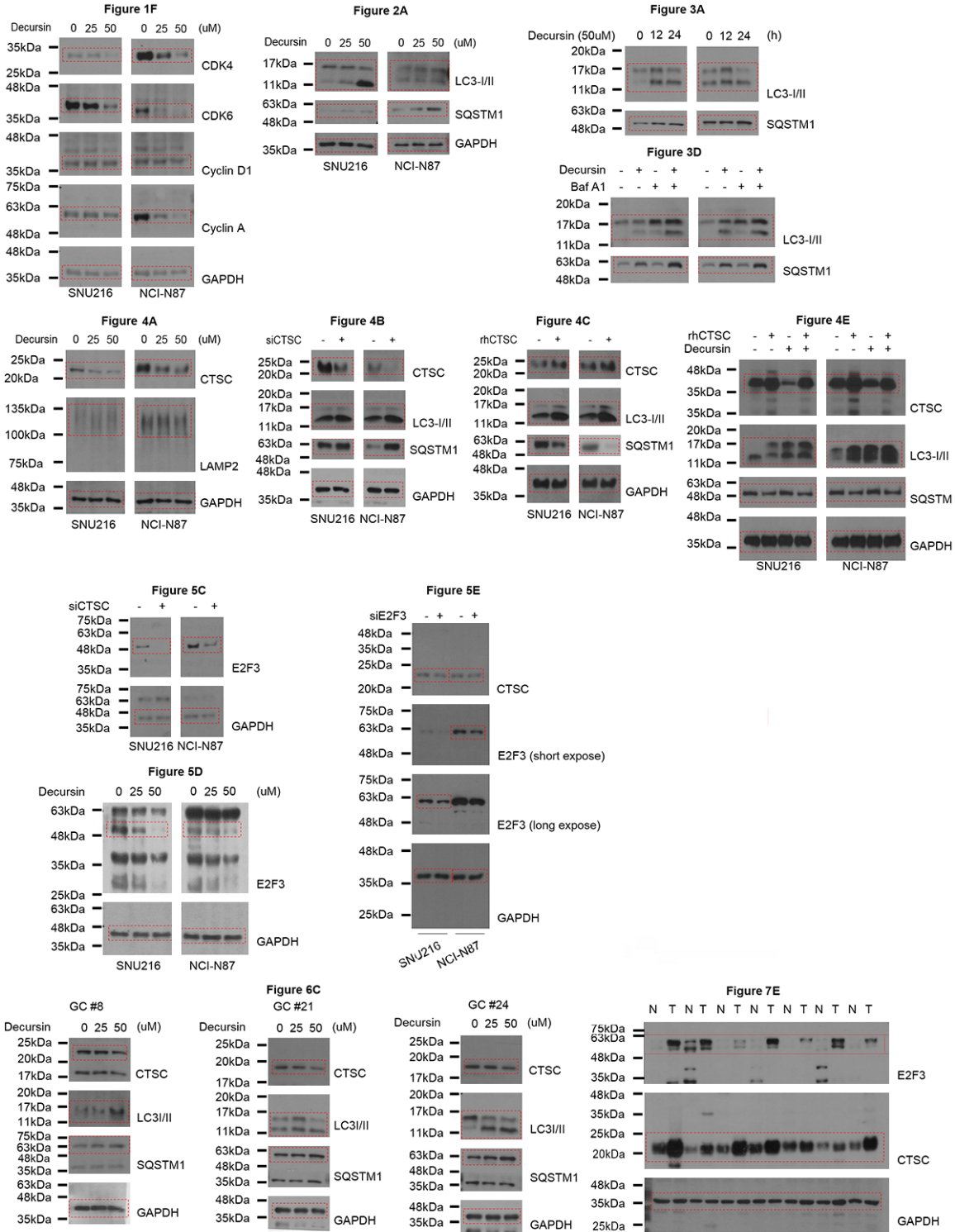
Supplementary Figure 4. Gastric tumouroid formation. Generation of a spheroid from NCI-N87 cells after 7 and 12 days, as well as 8 days after split into 6-well low attachment dishes (×200). Scale bar: 50 µm.

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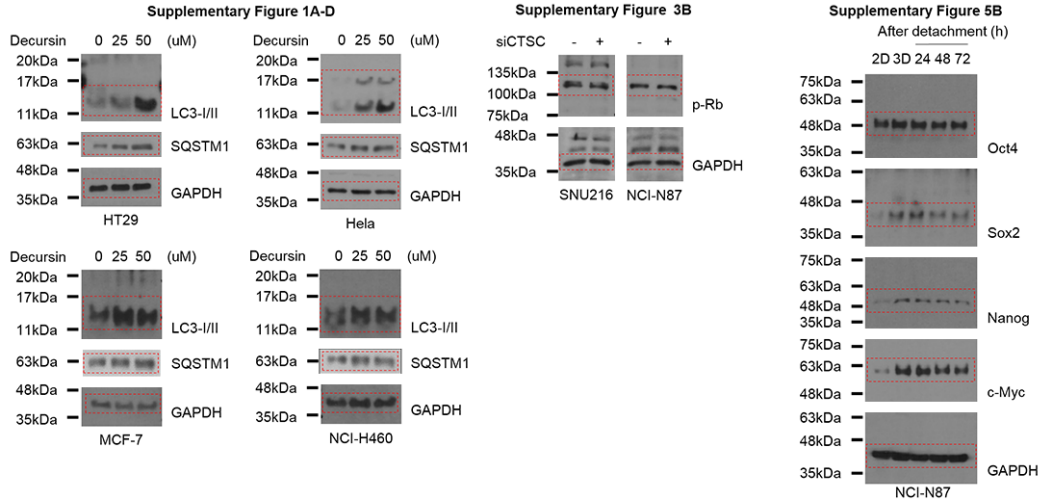


Supplementary Figure 5. Decursin treatment after 3D culture of NCI-N87 cells. A. Representative images are well-formed spheroids using NCI-N87 cell lines ($\times 100$). Scale bar: 50 μm . B. Comparison of stem cell marker expression between 2D and 3D cultured cells. Stem cell markers were maintained until 72 h after dissociation of 3D cultured cells. Full-length blots are presented in [Supplementary Figure 6](#). C, D. 3D cultured cells were dissociated again in 2D and re-seeded in 96-well plates. Then, cells were treated with decursin and proliferation was measured at indicated times ($\times 40$). Scale bar: 100 μm . * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

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Supplementary Figure 6. Uncropped scans of western blots displayed in **Figures 1F, 2A, 3A, 3D, 4A-4E, 5C-E, 6C, 7E, Supplementary Figures 1A-D, 3B and 5B.**

Supplementary Table 2. Patient information on patient-derived gastric cancer organoids

No	Gender	Age	WHO	Lauren	pStage
GC #8	M	72	Poorly cohesive carcinoma	Diffuse	pT2N2M0
GC #21	F	44	Poorly cohesive carcinoma	Diffuse	pT3N0M0
GC #24	M	81	Tubular adenocarcinoma	Intestinal	pT2N0M0

WHO, World Health Organization classification; Lauren, Lauren classification; pStage, pathologic stage.