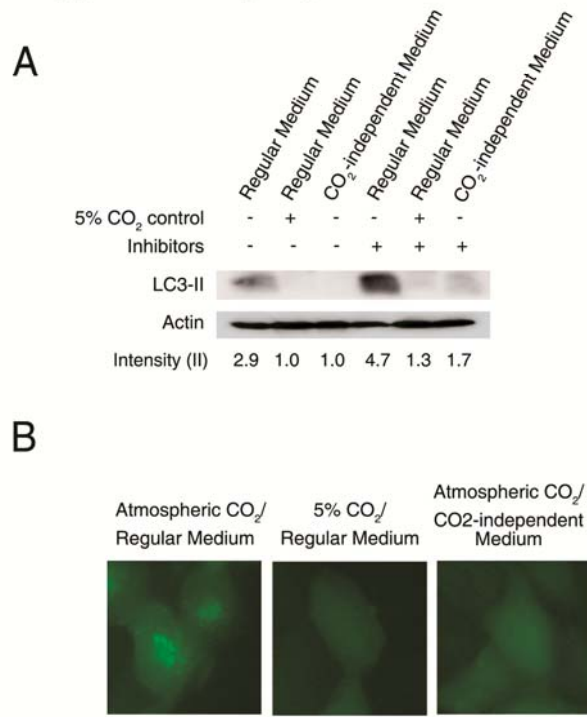
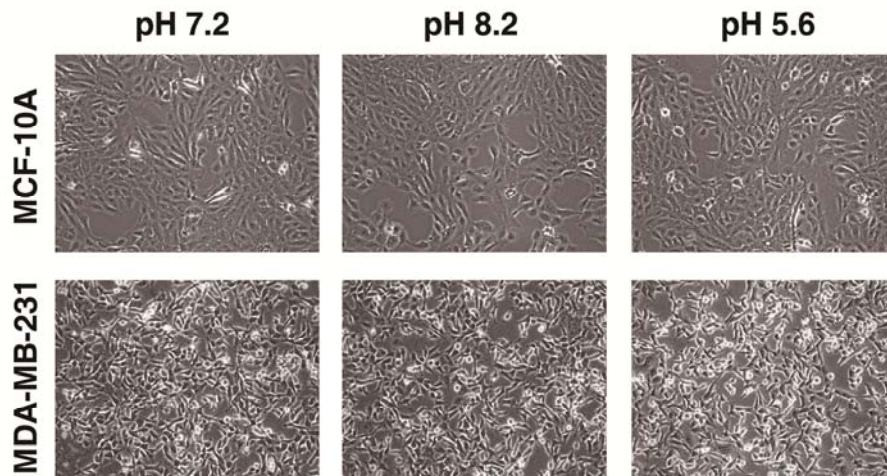


Supplementary Figure 1



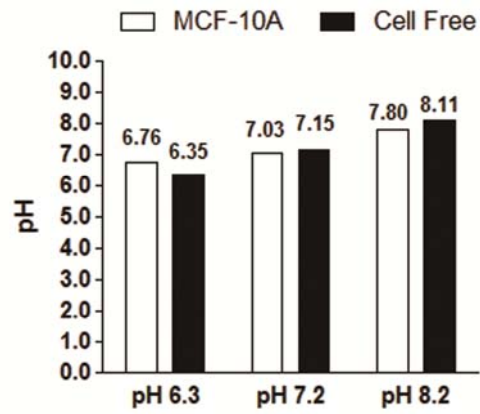
Supplementary Fig 1. Autophagic activity under different culture conditions. MCF-10A cells (A) or MCF-10A cells stably expressing GFP-LC3 (B) were cultured in CO₂-dependent DMEM-F12 medium (*Regular Medium*). During the experiment, cells were incubated either in the regular medium or in CO₂-independent medium for 6 h in the presence or absence of lysosomal inhibitors as indicated. 5% CO₂ control was achieved using a regular cell culture incubator to maintain the pH of the regular medium. Autophagic activity was assessed by western blot for LC3 (A) and GFP-LC3 punctate formation assay (B).

Supplementary Figure 2



Supplementary Fig 2. Cell morphology after incubation in media of different pHs. MCF-10A (A) and MDA-MB-231 (B) cells were incubated in media of different pHs as indicated for 6 h. After that, cell morphology was examined by phase-contrast microscopy.

Supplementary Figure 3



Supplementary Fig 3. pH of culture medium before and after the experiment (6 h) was measured with three different initial pHs (6.3, 7.2 and 8.2). Experiments were performed either with the culture of MCF-10A cells or in a cell-free setting. X-axis indicates the initial pHs and Y-axis indicates the pH after the course of experiment (6 h).

	Relative Intensity (LC3-II)
pH 7.2	1.0
pH 7.2 +Inhibitor	1.4
pH 8.2	1.4
pH 8.2 +Inhibitor	4.7
pH 8.0	1.2
pH 8.0 +Inhibitor	4.7
pH 7.8	1.1
pH 7.8 +Inhibitor	3.7
pH 7.6	1.0
pH 7.6 +Inhibitor	1.4

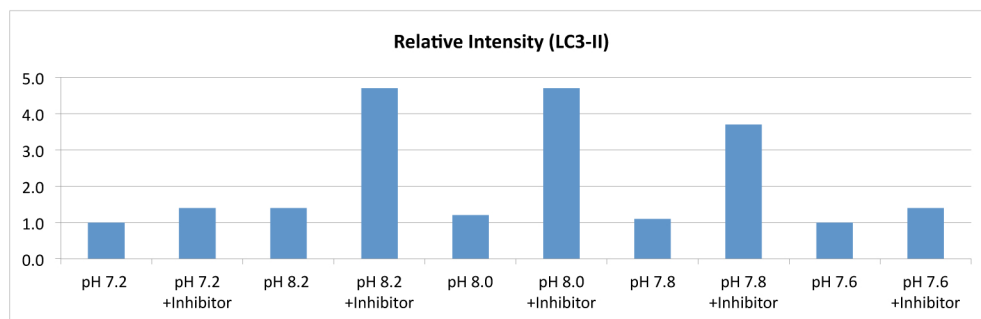
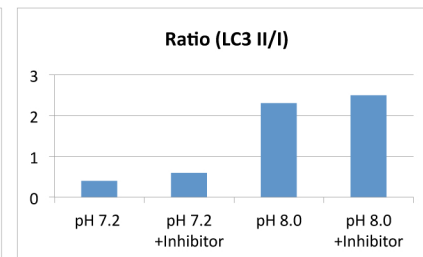
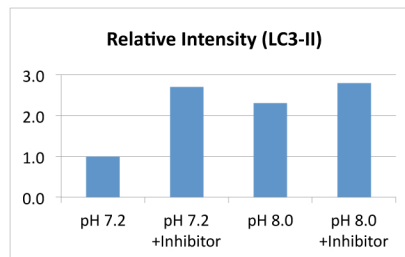


Figure 2B

MCF-7		Ratio (LC3 II/I)	
	Relative Intensity (LC3-II)		
pH 7.2	1.0	pH 7.2	0.4
pH 7.2 +Inhibitor	2.7	pH 7.2 +Inhibitor	0.6
pH 8.0	2.3	pH 8.0	2.3
pH 8.0 +Inhibitor	2.8	pH 8.0 +Inhibitor	2.5



Hela		Ratio (LC3 II/I)	
	Relative Intensity (LC3-II)		
pH 7.2	1.0	pH 7.2	0.9
pH 7.2 +Inhibitor	2.2	pH 7.2 +Inhibitor	1.5
pH 8.0	2.0	pH 8.0	1.8
pH 8.0 +Inhibitor	2.4	pH 8.0 +Inhibitor	3

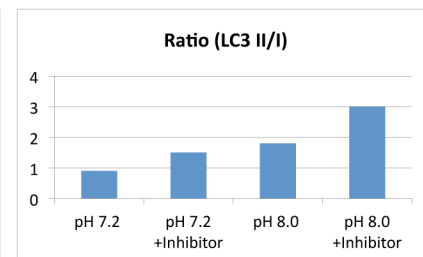
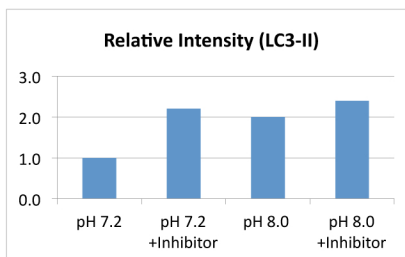


Figure 2C

	Relative Intensity (LC3-II)
pH 7.2	1.0
pH 7.2 +Inhibitor	2.7
pH 5.6	0.6
pH 5.6 +Inhibitor	0.6
pH 6.3	0.6
pH 6.3 +Inhibitor	1.3
pH 6.6	0.8
pH 6.6 +Inhibitor	1.5
pH 6.8	1.1
pH 6.8 +Inhibitor	2.2

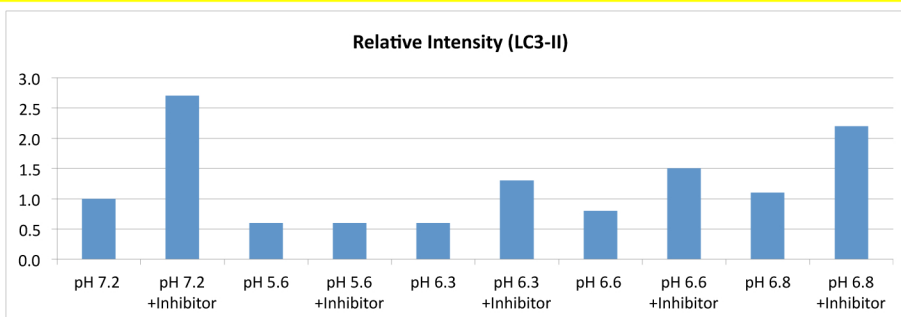


Figure 2D

	Relative Intensity (LC3-II)
pH 7.2	1.0
pH 7.2 +Inhibitor	2.5
pH 8.0	2.4
pH 8.0 +Inhibitor	5.1
HAc	0.5
HAc +Inhibitor	0.5

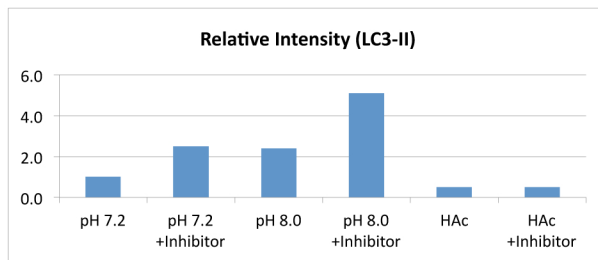


Figure 2E

	Relative Intensity (LC3-II)
pH 7.2	1.0
EBSS (pH 7.2)	1.7
EBSS (pH 7.0)	1.6
EBSS (pH 6.7)	1.6
EBSS (pH 6.3)	1.5
EBSS (pH 5.6)	1.2

	Ratio (LC3 II/I)
pH 7.2	1.2
EBSS (pH 7.2)	3.2
EBSS (pH 7.0)	2.8
EBSS (pH 6.7)	2.6
EBSS (pH 6.3)	2.5
EBSS (pH 5.6)	1.6

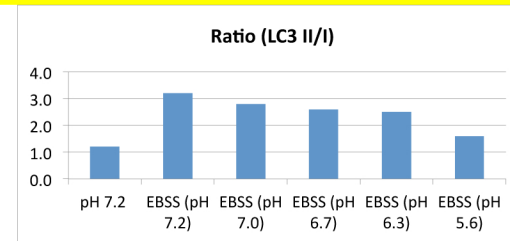
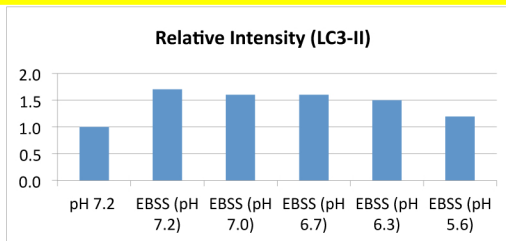
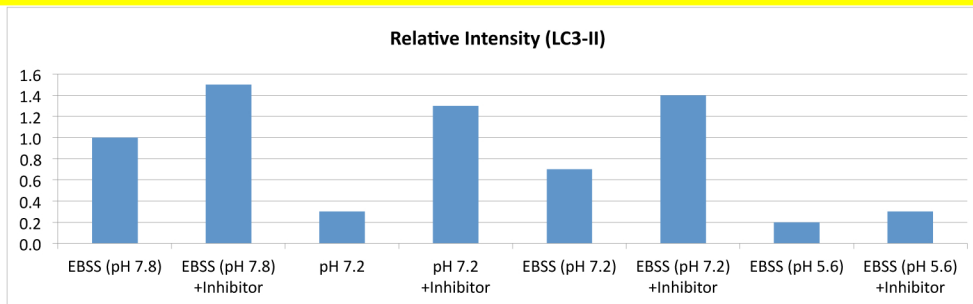


Figure 2F

	Relative Intensity (LC3-II)
EBSS (pH 7.8)	1.0
EBSS (pH 7.8) +Inhibitor	1.5
pH 7.2	0.3
pH 7.2 +Inhibitor	1.3
EBSS (pH 7.2)	0.7
EBSS (pH 7.2) +Inhibitor	1.4
EBSS (pH 5.6)	0.2
EBSS (pH 5.6) +Inhibitor	0.3



	Ratio (LC3 II/I)
EBSS (pH 7.8)	2.0
EBSS (pH 7.8) +Inhibitor	2.8
pH 7.2	0.4
pH 7.2 +Inhibitor	1.6
EBSS (pH 7.2)	1.5
EBSS (pH 7.2) +Inhibitor	3.2
EBSS (pH 5.6)	0.4
EBSS (pH 5.6) +Inhibitor	0.8

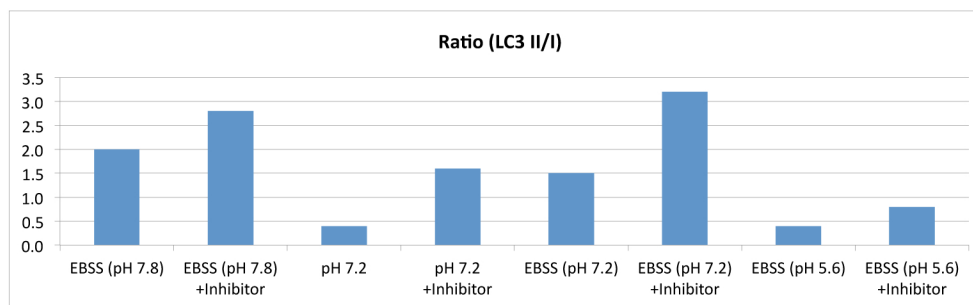


Figure 3B

	Relative Intensity (LC3-II)
pH 7.2	1.0
0 min	6.3
30 min	5.8
60 min	2.0
90 min	1.4

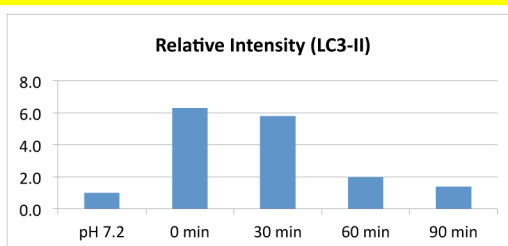
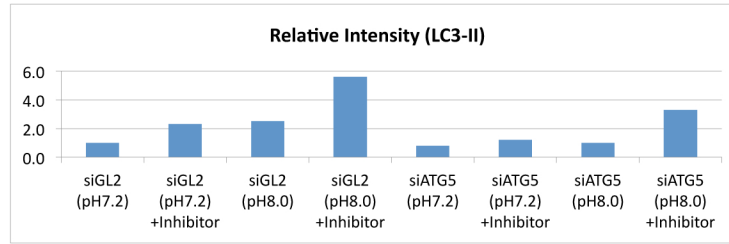


Figure 4B

Upper Panel

	Relative Intensity (LC3-II)
siGL2 (pH7.2)	1.0
siGL2 (pH7.2) +Inhibitor	2.3
siGL2 (pH8.0)	2.5
siGL2 (pH8.0) +Inhibitor	5.6
siATG5 (pH7.2)	0.8
siATG5 (pH7.2) +Inhibitor	1.2
siATG5 (pH8.0)	1.0
siATG5 (pH8.0) +Inhibitor	3.3



Lower Panel

	Relative Intensity (LC3-II)
siVps34 (pH7.2)	1.0
siVps34 (pH7.2) +Inhibitor	1.1
siVps34 (pH8.0)	1.3
siVps34 (pH8.0) +Inhibitor	3.4
siGL2 (pH7.2)	1.0
siGL2 (pH7.2) +Inhibitor	2.3
siGL2 (pH8.0)	2.6
siGL2 (pH8.0) +Inhibitor	7.2
siBeclin1 (pH7.2)	1.2
siBeclin1 (pH7.2) +Inhibitor	1.3
siBeclin1 (pH8.0)	1.2
siBeclin1 (pH8.0) +Inhibitor	3.4

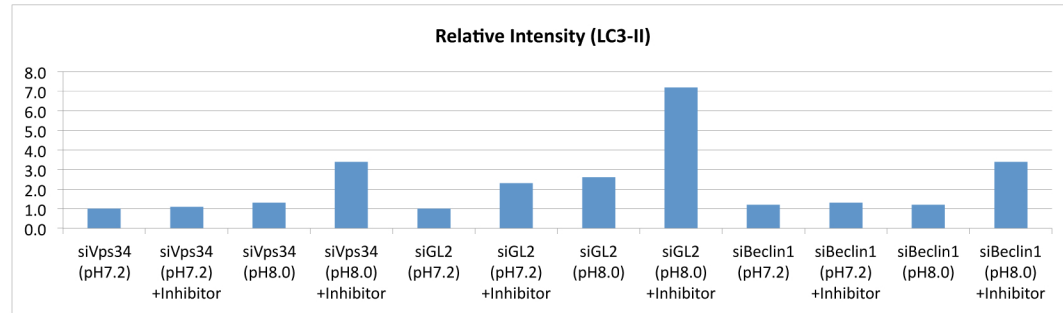
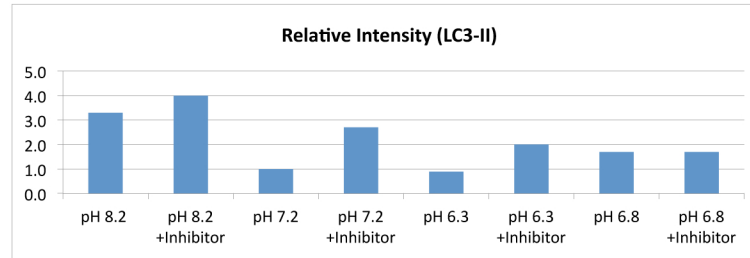


Figure 5B

	Relative Intensity (LC3-II)
pH 8.2	3.3
pH 8.2 +Inhibitor	4.0
pH 7.2	1.0
pH 7.2 +Inhibitor	2.7
pH 6.3	0.9
pH 6.3 +Inhibitor	2.0
pH 6.8	1.7
pH 6.8 +Inhibitor	1.7



	Ratio (LC3 II/I)
pH 8.2	2.2
pH 8.2 +Inhibitor	4.5
pH 7.2	1.8
pH 7.2 +Inhibitor	2.9
pH 6.3	0.5
pH 6.3 +Inhibitor	0.9
pH 6.8	0.9
pH 6.8 +Inhibitor	1.9

