SUPPLEMENTAL INFORMATION

Inhibition of autophagy as a new means of improving chemotherapy efficiency in high-LC3B triple-negative breast cancers

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Running Title: Role of autophagy in tumorigenesis and chemosensitivity of triple-negative breast cancer patients

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Table S1. Main patient characteristics and clinico-pathological features of the five BC cohorts analyzed in the study. Patient characteristics and clinical features of the cohorts referred to as Curie, Stockholm, Marseille and TCGA have been previously described in refs. 32–34 and https://tcga-data.nci.nih.gov/tcga/ (see also Materials and Methods). BC subtypes were defined according to the ASCO's guidelines: LumA (ER^+ and/or PR^+ $HER2^-$), LumB (ER^+ and/or PR^+, $HER2^-$, high mitotic index), HER2 (ER^-, PR^-, $HER2^+$), TN (ER^-, PR^-, $HER2^-$). The five BC cohorts were examined for information on tumor size (pT), mean age at diagnosis, number of retrieved and metastatic axillary lymph nodes (pN), number of distant metastases (pM), hormonal receptor status, the date of relapse, the date and cause of death (only death from breast cancer has been considered), and the type of therapy used (hormono-, chimio-, radio-therapies). pN was considered as negative for N0 and positive for N1, N2 and N3. pM was considered as negative for M0 and positive for M1. The table summarizes the main clinical characteristics of the cohorts, helping in their comparison.

Table S2. *MAP1LC3B* mRNA levels in BC cell lines. *MAP1LC3B* mRNA levels in Lum, HER2 and TN BC-derived cell lines either determined from Affymetrix U133 plus 2.0 microarray analysis (*MAP1LC3B* log2 of probeset intensity, 208785_s_at) or quantified by qRT-PCR (Ct, threshold cycle). For qRT-PCR analysis (columns with Ct values), *MAP1LC3B* mRNA levels were normalized on the basis of the mean values of 3 housekeeping genes *TBP* (TATA box binding protein)*, RPLP0* (ribosomal protein, large, P0) and *PPIA* [peptidylprolyl isomerase A [cyclophilin A)]), as endogenous RNA controls for the total amount of RNA in each cell line. Ct means threshold cycle.

Figure S1. *MAP1LC3B* is not a prognostic marker in LumA, LumB and HER2 BC. **Top***,* Kaplan-Meier curves of OS for Curie/Stockholm cohorts with respect to *MAP1LC3B* mRNA levels, in LumA BC (*n* = 34 for low expression and *n* = 34 for high expression; cut-off value = -0.38, scatter plot shown in **Fig. 1C**), LumB BC (*n* = 26 for low expression and *n* = 27 for high expression; cut-off value = 0.27, scatter plot shown in **Fig. 1C**) and HER2 BC (*n* = 21 for low

expression and *n* = 24 for high expression; cut-off value = 0.36, scatter plot shown in **Fig. 1C**), as indicated. p-values are based on Log-rank test. **Bottom**, Scatter plots of *MAP1LC3B* mRNA levels in TN BC subtype from the TCGA cohort. *MAP1LC3B* mRNA level was evaluated from Agilent G4502A_07 array and processed by the TCGA as follows: Normalization using RMA algorithm and expression values were gene centered. Data are from https://tcga-data.nci.nih.gov/tcga/.

Figure S2. Verification of the specificity of the LC3B-directed antibody, used in this study. (**A**) MDA231 TN BC cells transfected with control- (si*Ctrl*) or *LC3B*-targeted (si*LC3B*) siRNA were fixed, embedded into paraffin, sectioned, and incubated with LC3B-specific antibody following the same protocol as the one used for IHC from BC patient samples. The specific LC3B staining is severely reduced in LC3B-depleted cells, confirming the specificity of the antibody in IHC experiments. (**B**) Bar graph showing reduced IHC staining in LC3B-depleted cells (si*LC3B*), compared to control (si*CTL*). (**C**) Western blots showing LC3B protein levels in MDA231 cells after transfection with control- (si*Ctrl*) or *LC3B*-targeted (si*LC3B*) siRNA. AP2A1/adaptin is used as an internal control for protein loading.

Figure S3. Automated quantification of autophagic vacuoles. (**A**) Representative confocal pictures from HeLa-GFP-LC3 cells cultured in growing normal medium (NM) or incubated with EBSS for 2 h with or without bafilomycin A_1 (BafA1; 100 nM). Scale bar = 10 μM. (**B**) Bar graph represents the average number of autophagosomes per cell based on the Fluofarma automated quantification algorithm. (**C-E**) Representative views of the same tumor sample following incubation with a mouse IGg1 antibody, used as a matched isotype control for LC3B staining (**C**), with LC3B antibody (**D, left**), or with LC3B antibody following deconvolution by Fluofarma algorithm, defining epithelial and stromal compartments (**D, right**). (**E**) Representative views of the same tumor sample before (**E, raw images, left**) and after (**E, computer-generated images, right**) segmentations achieved using Fluofarma algorithm are shown with low (**upper panels**) and high (**lower panels**) magnification images,

after computer-generated delimitations of nuclear and plasma membranes (green and red lines, respectively).

Figure S4. Quantification of autophagosomes in epithelial and stromal cells in human BC samples. (**A**) Scatter plot of the average number of autophagosomes per cell based on the automatized quantification algorithm (tumor samples from Curie cohort) in stromal versus epithelial cells (**A, left**), in stromal cells according to BC subtypes (**A, middle**) and in both epithelial and stromal cells according to BC subtypes (**A, right**). Horizontal bars represent the median values. 27 LumA, 37 LumB, 33 HER2 and 40 TN tumors were analyzed. (**B**) Correlation between the average number of autophagosomes per epithelial cells and the number of autophagosomes per epithelial and stromal cells. Correlation coefficient was computed using Pearson's test. (**C**) Box-and-whisker plot showing that the percentage (%) of autophagosome-positive epithelial cells (defined by the automated method) is significantly higher in TN BC than in LumA, LumB and HER2 BC, confirming classic histological LC3B scoring (Curie cohort) (**Fig. 2B**). (**D**) Each histogram represents, for a single TN tumor, the percentage distribution of epithelial cells according to the number of autophagosomes per cell. 5 TN tumors with a high number of autophagosomes per epithelial cell (**upper**) and 5 TN tumors with a low number of autophagosomes per epithelial cell (**lower**) are shown. (**E**) Positive correlation between automated quantification of LC3B immunofluorescence staining of TN and HER2 cell lines by ImageJ software or Fluofarma algorithm. (**F**) LC3B histological scoring (Hscore, see Methods) in the TN BC subtype from the St-Cloud cohort.

Figure S5. Levels of proteins involved in autophagy in human BC samples. (**A**) Western blots showing LC3B, ATG5, BECN1 and SQSTM1 expression levels in LumA and TN BC. Each line corresponds to individual BC patients. Ponceau staining was used as control for evaluation of protein loading. (**B**) Bar graphs represent the corresponding quantification of LC3B-II, BECN1, ATG5 and SQSTM1 protein levels. Data are expressed as means +/- sem

(n = at least 10 tumors per subtype). (**C**) Classification of the TN population of the Curie (**C, left**) and St-Cloud (**C, right**) cohorts by LC3B status, according to stage.

Figure S6. *MAP1LC3B* mRNA and protein levels in human BC cell lines. (**A**) *MAP1LC3B* mRNA levels in LumA, HER2 and TN BC cell lines measured by qRT-PCR experiments (see also **Table S2** for detailed quantitative values). Horizontal bars represent the median values ($n \geq 6$ cell lines for each subtype). p-values are based on Student's t-test. (**B**) Bar graphs show the ratio of ATG5/AP2A1 (left panel) or BECN1/AP2A1 (right panel) protein levels, as assessed by densitometric analysis of the western blots shown in **Fig. 3B**. AP2A1 is used as an internal control for protein loading. Data are shown as means +/- sem (N = 3 independent experiments). p-values are based on Student's t-test. (**C**) Representative confocal pictures of MDA231 cells stably expressing the tandem probe RFP-GFP-LC3B cultivated in normal medium (NM) or treated with EBSS for 1 h, fixed and stained for DAPI (blue). This dual probe analysis enables a direct assessment of the level of autophagosome-lysosome fusion events and makes it possible to distinguish between autophagosomes (yellow) and autolysosomes (red). Scale bar = 10 µm. (**D**) The bar graph represents the number of autophagosomes (**yellow dots in C**, **green bar**) and autolysosomes (**red dots in C**, **red bar**) per cell, expressed as the percentage of total number of dots. (**E**) The indicated TN cell lines were treated or not with EBSS for 2 h with or without bafilomycin A_1 (BafA1; 100 nM). Western blots for AP2A1 and LC3B are shown. The graph represents the LC3B-II/AP2A1 ratios, quantified by densitometric analysis of the western blots shown above. AP2A1 is used as an internal control for protein loading. N=3 independent experiments.

Figure S7. Role of autophagy in invasion of high-LC3B TN BC cells. (**A-F**) MDA231 TN cells were transiently transfected with control- (Ctrl) or with two different siRNAs directed against *BECN1* (si*BECN1*(1), si*BECN1*(2)), or *ATG5* [si*ATG5*(1), si*ATG5*(2) as indicated] and maintained in 3D conditions for 3 days. (**A,D**) Western blots showing BECN1 (**A**) or ATG5

(**D**) protein levels in MDA231 cells following transfection with siRNAs, as indicated. AP2A1 is used as an internal control for protein loading. (**B,E**) Representative bright field images from MDA231 TN cells +/- siRNAs, as indicated. Scale bars = 100 µm. (**C,F**) Bar graphs represent the total area covered by the stellate structures per field (**left panel**; a total area of 0.85mm^2 has been evaluated; arbitrary unit, a.u) and the area per clone (**right panel**), as determined using Metamorph software. Data are from MDA231 TN cells +/- siRNAs, as indicated. Data shown are means +/- sem (N=3 independent experiments). (**G**) Representative photos from MDA231 TN cells transiently transfected with control- (*Ctrl*), *BECN1*-, *ATG5*-, *ATG7*-targeted siRNA and tested for invasion using a Transwell assay.

Figure S8. Role of autophagy in invasion of high-LC3B TN BC cells. **(A)** Western blots showing ATG7, ATG5, GAPDH and LC3B expression levels in MDA231 cells stably expressing the indicating shRNAs (**left panel**) TN BC without (-) or with (+) bafilmomycin A_1 (BafA1) treatment. Bar graphs represent the corresponding quantification of LC3B-II/GAPDH ratios (**right panel**). Data are expressed as means +/- sem (N = 2). (**B-E**) Results are from MDA231 (**B,C**) or BT549 (**D,E**) cell lines stably expressing 2 different shRNA directed against *ATG7*, referred to as sh*Ctrl*, sh*ATG7*(1) or sh*ATG7*(2), and cultured in 3D conditions. (**B**) Western blots show ATG7 and LC3B protein levels in MDA231 stable cell lines, following 3D culture. AP2A1 is used as an internal control for protein loading. (**C**) Representative bright field images from MDA231 TN cells stably expressing shRNAs, as indicated, following 3D culture. Scale bars = 100 µm (low magnification) and 50 µm (high magnification). Bar graphs represent the total area of the stellate structures per field (left panel) or the area per clone (middle panel) and the total number of cells extracted after 3 days of 3D culture (right panel). Data are shown as means +/- sem (N = 3 independent experiments). p-values are based on Student's t-test. (**D**) Western blots show ATG7 and LC3B protein levels in BT549 stable cell lines, following 3D culture. AP2A1 is used as an internal control for protein loading. (**E**) Representative bright field images from BT549 TN cell lines stably expressing shRNAs, as

indicated, following 3D culture. Bar graphs represent the total area of the stellate structures per field (left panel) or the area per clone (middle panel) and the total number of cells extracted after 3 days of 3D culture (right panel). Data are shown as means +/- sem (N=3 independent experiments). p-values are based on Student's t-test.

Figure S9. Inhibition of autophagy reduces YAP1 activity in high-LC3B TN BC cells (**A**) Proliferation curves of MDA231 TN BC stable cell lines expressing different shRNA (referred to as sh*Ctrl*, sh*ATG5* or sh*ATG7*) in 2D culture, over 9 days. (**B-D**) Results are from MDA231 stable cell lines expressing different shRNA (referred to as sh*Ctrl*, sh*ATG7*(1) or sh*ATG7*(2) and cultured in 3D conditions. Data are shown as means +/- sem (N=3 independent experiments). (**B**) Western blots showing the phosphorylated form of YAP1 on Ser127 residue (P-YAP1), YAP1 and AP2A1 proteins in stable cell lines following 3D culture. AP2A1 is used as internal control for protein loading. (**C**) The bar graph shows the corresponding quantification of P-YAP1/YAP1 protein level ratios. (**D**) mRNA levels of YAP1-target genes were monitored by RT-qPCR following 3D culture. GAPDH is used as an internal control for total mRNA levels. Data are shown as means +/- sem (N = 3 independent experiments). p-values are based on Student's t-test. (**E**) Results are from autophagy-proficient (si*Ctrl*) or autophagy-deficient (si*ATG5*) MDA231 cells, transfected with an empty vector (Ctl) or a vector expressing YAP-S127A, a non-phosphorylable mutant form of YAP, after 3 days of 3D culture. (**E, left**) Representative bright field images from ATG5-depleted cells expressing or not YAP-S127A, as indicated. Scale bars = 100 µm. Bar graph (**E, middle**) represents the total area covered by the stellate structures per field. (**E, right**) Bar graph shows the total number of cells extracted from the corresponding 3D culture after 3 days, as a percentage of si*Ctrl* cells transfected with an empty vector. Data are shown as means +/- sem (N = 2 independent experiments). p-values are based on Student's t-test.

Figure S1

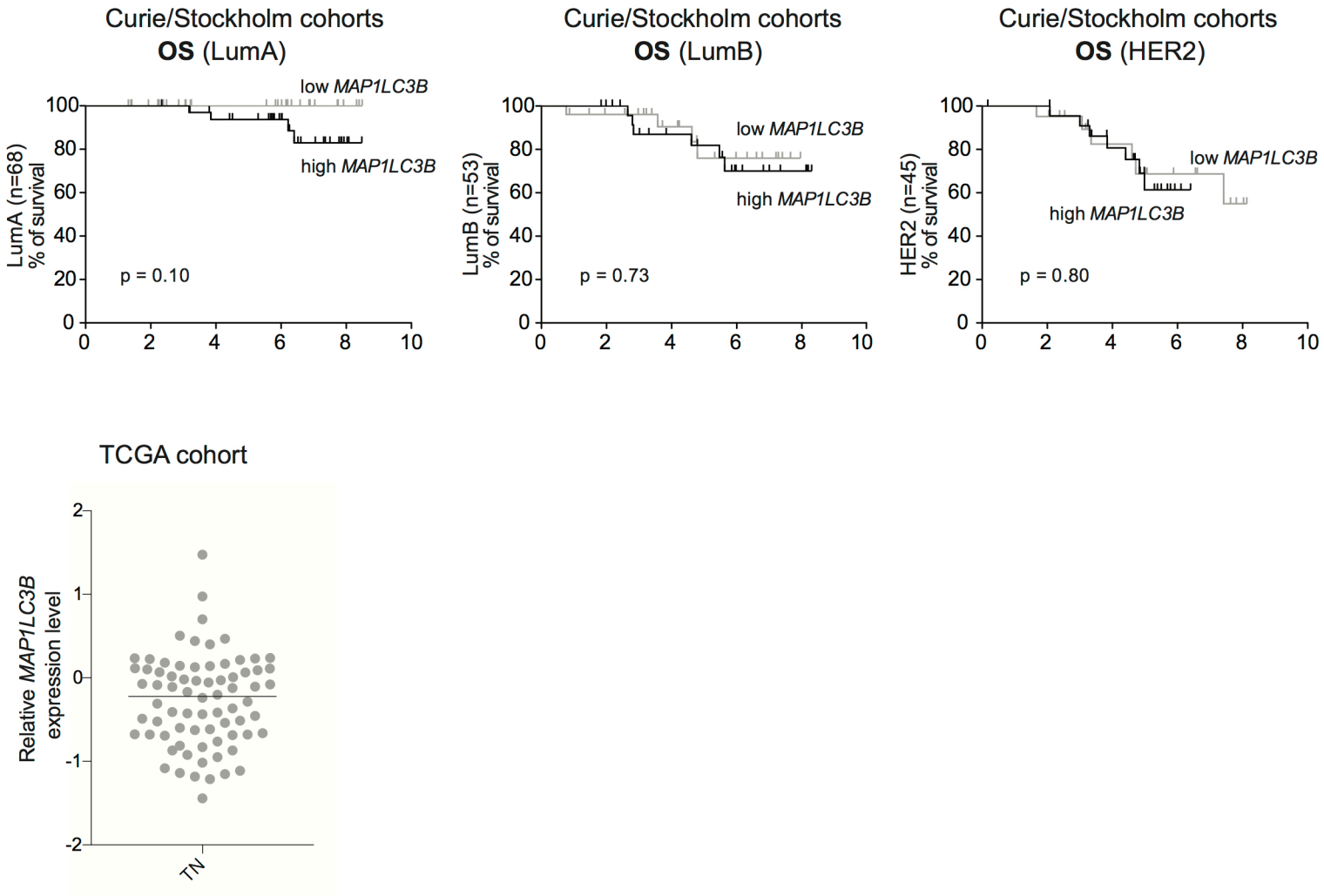
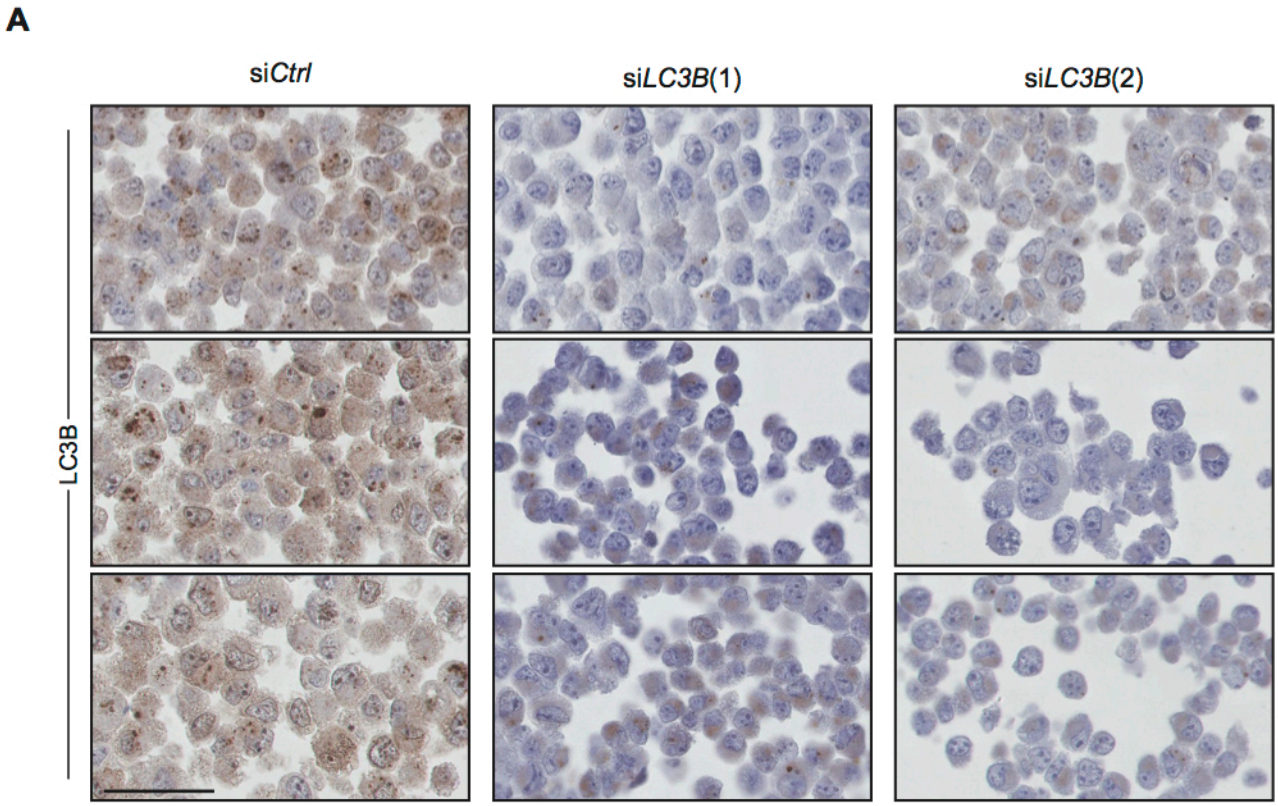
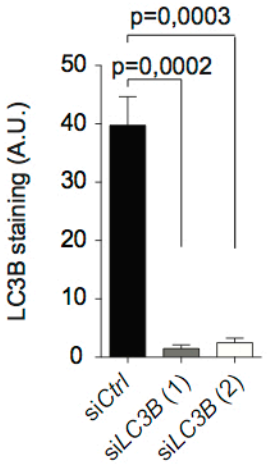

Curie/Stockholm cohorts
OS (LumA)
low MAP1LC3B
high MAP1LC3B
LumA (n=68)
% of survival
p = 0.10
Curie/Stockholm cohorts
OS (LumB)
low MAP1LC3B
high MAP1LC3B
LumB (n=53)
% of survival
p = 0.73
Curie/Stockholm cohorts
OS (HER2)
low MAP1LC3B
high MAP1LC3B
HER2 (n=45)
% of survival
p = 0.80
TCGA cohort
Relative MAP1LC3B expression level
TN

Figure S2


A
siCtrl
siLC3B(1)
siLC3B(2)
LC3B


B
p=0,0003
p=0,0002
LC3B staining (A.U.)
50
40
30
20
10
0
siCtrl
siLC3B (1)
siLC3B (2)

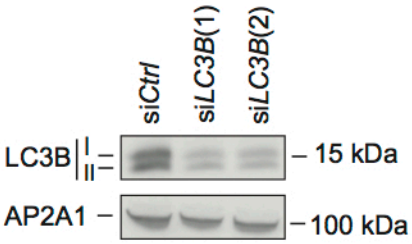

C
siCtrl
siLC3B(1)
siLC3B(2)
LC3B
I
II
15 kDa
AP2A1
100 kDa

Figure S3

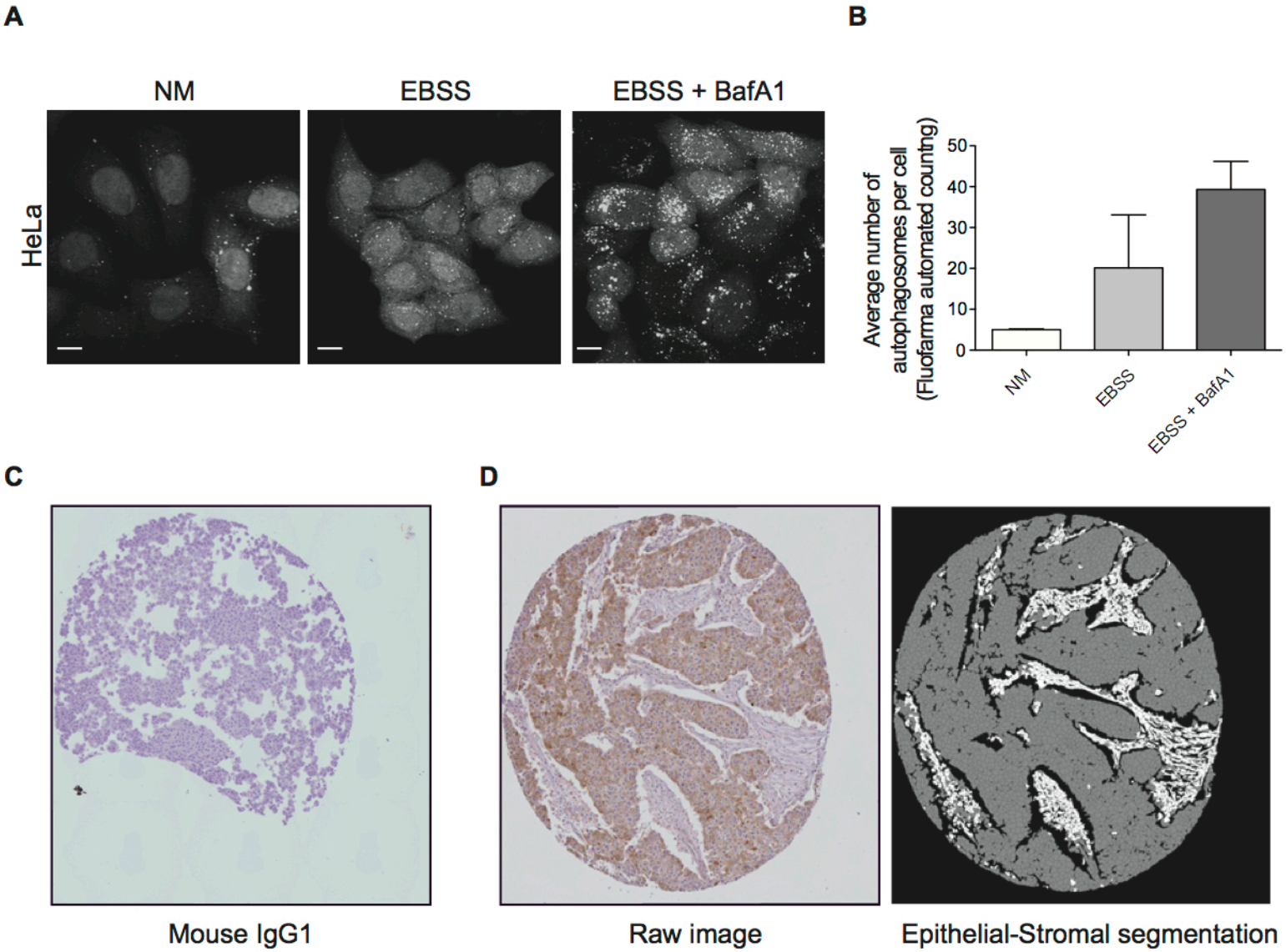

A
NM
EBSS
EBSS + BafA1
HeLa
B
Average number of autophagosomes per cell (Fluofarma automated counting)
50
40
30
20
10
0
NM
EBSS
EBSS + BafA1
C
D
Mouse IgG1
Raw image
Epithelial-Stromal segmentation
E
Low magnification
Raw image
Computer-generated lines and dots
High magnification
Raw image
Computer-generated lines and dots

Figure S4

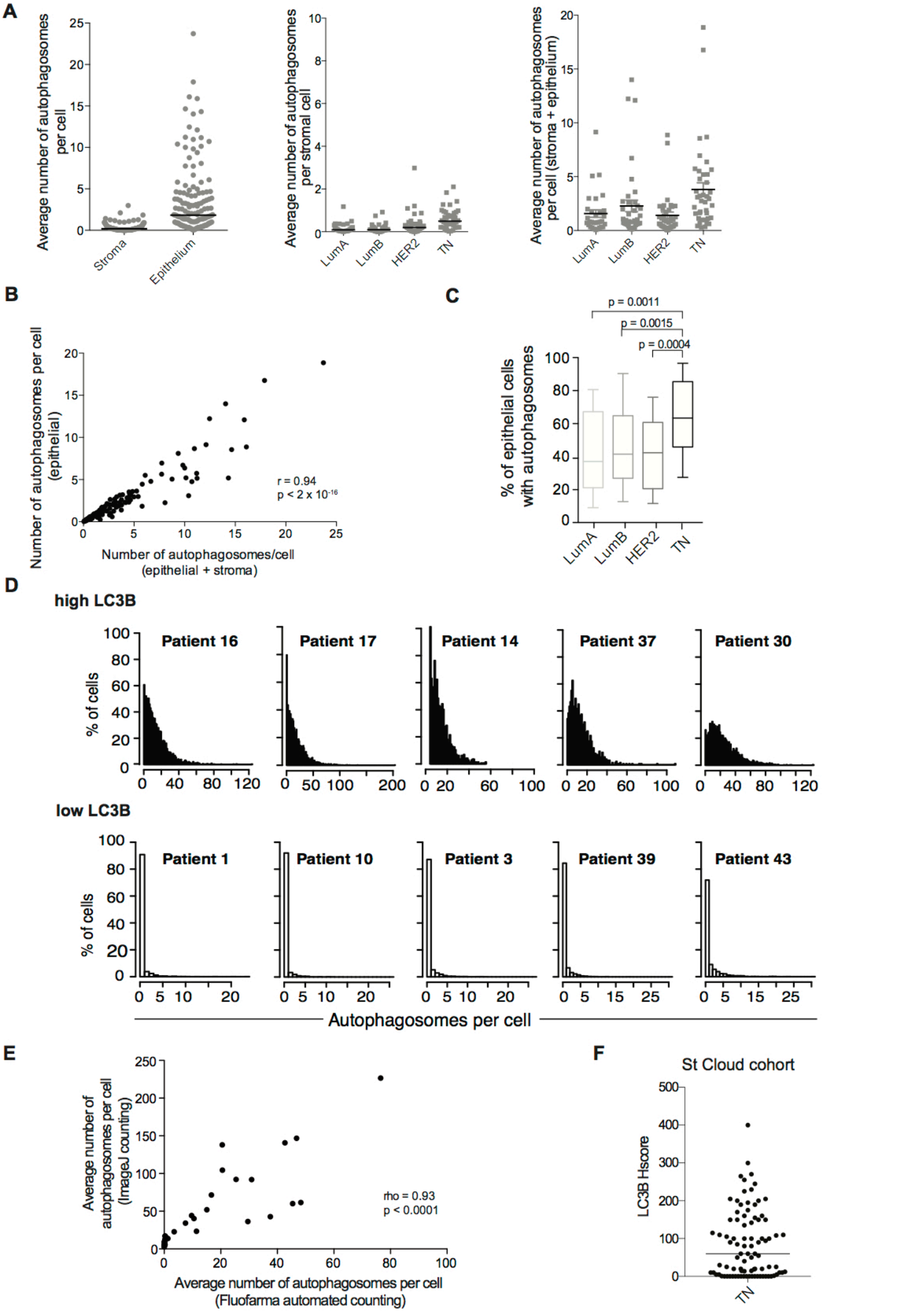

A
Average number of autophagosomes per cell
Stroma
Epithelium
Average number of autophagosomes per stromal cell
LumA
LumB
HER2
TN
Average number of autophagosomes per cell (stroma + epithelium)
B
Number of autophagosomes per cell (epithelial)
r = 0.94
p < 2 x 10-16
Number of autophagosomes/cell (epithelial + stroma)
C
p = 0.0011
p = 0.0015
p = 0.0004
% of epithelial cells with autophagosomes
D
high LC3B
Patient 16
Patient 17
Patient 14
Patient 37
Patient 30
% of cells
low LC3B
Patient 1
Patient 10
Patient 3
Patient 39
Patient 43
Autophagosomes per cell
E
Average number of autophagosomes per cell (ImageJ counting)
rho = 0.93
p < 0.0001
Average number of autophagosomes per cell (Fluofarma automated counting)
F
St Cloud cohort
LC3B Hscore
TN

Figure S5

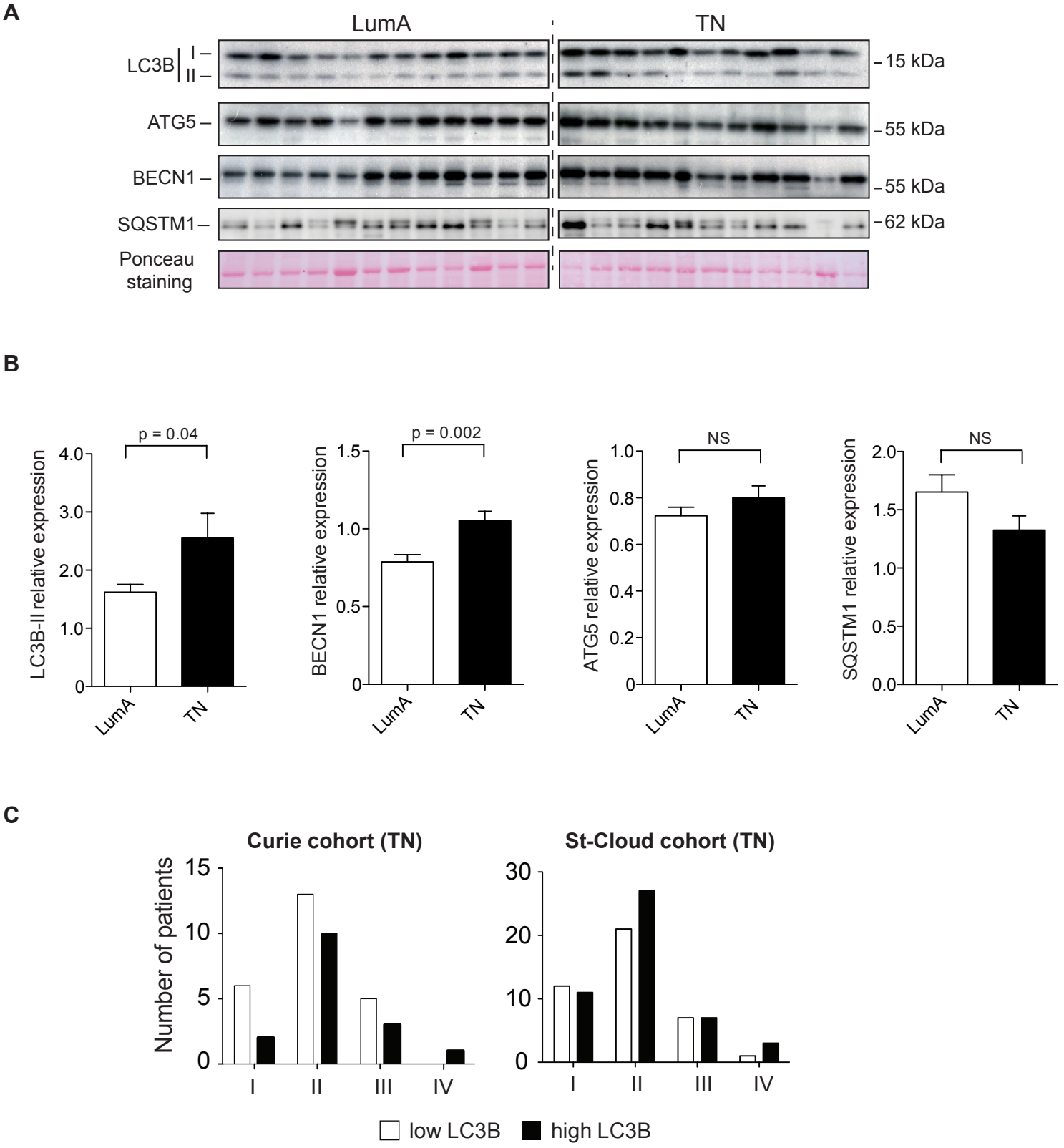

A
LumA
TN
LC3B I
II
ATG5
BECN1
SQSTM1
Ponceau staining
15 kDa
55 kDa
55 kDa
62 kDa
B
p = 0.04
LC3B-II relative expression
p = 0.002
BECN1 relative expression
NS
ATG5 relative expression
NS
SQSTM1 relative expression
LumA
TN
C
Curie cohort (TN)
St-Cloud cohort (TN)
Number of patients
I
II
III
IV
low LC3B
high LC3B

Figure S6

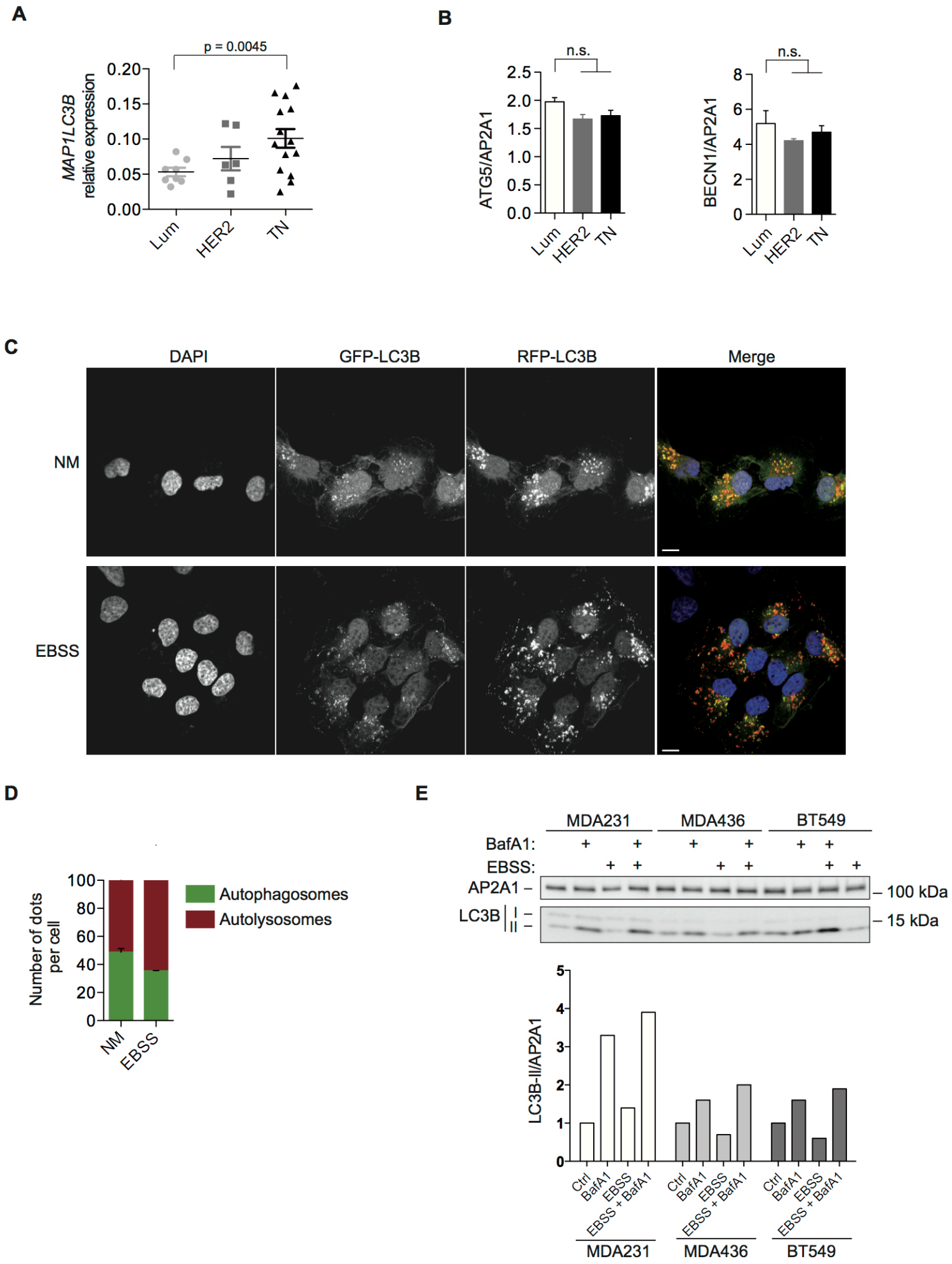

A
p = 0.0045
MAP1LC3B relative expression
0.20
0.15
0.10
0.05
0.00
Lum
HER2
TN
B
n.s.
ATG5/AP2A1
2.5
2.0
1.5
1.0
0.5
0.0
n.s.
BECN1/AP2A1
8
6
4
2
0
C
DAPI
GFP-LC3B
RFP-LC3B
Merge
NM
EBSS
D
Number of dots per cell
100
80
60
40
20
0
Autophagosomes
Autolysosomes
NM
EBSS
E
MDA231
MDA436
BT549
BafA1:
EBSS:
AP2A1
LC3B
I
II
100 kDa
15 kDa
LC3B-II/AP2A1
5
4
3
2
1
0
Ctrl
BafA1
EBSS
EBSS + BafA1

Figure S7

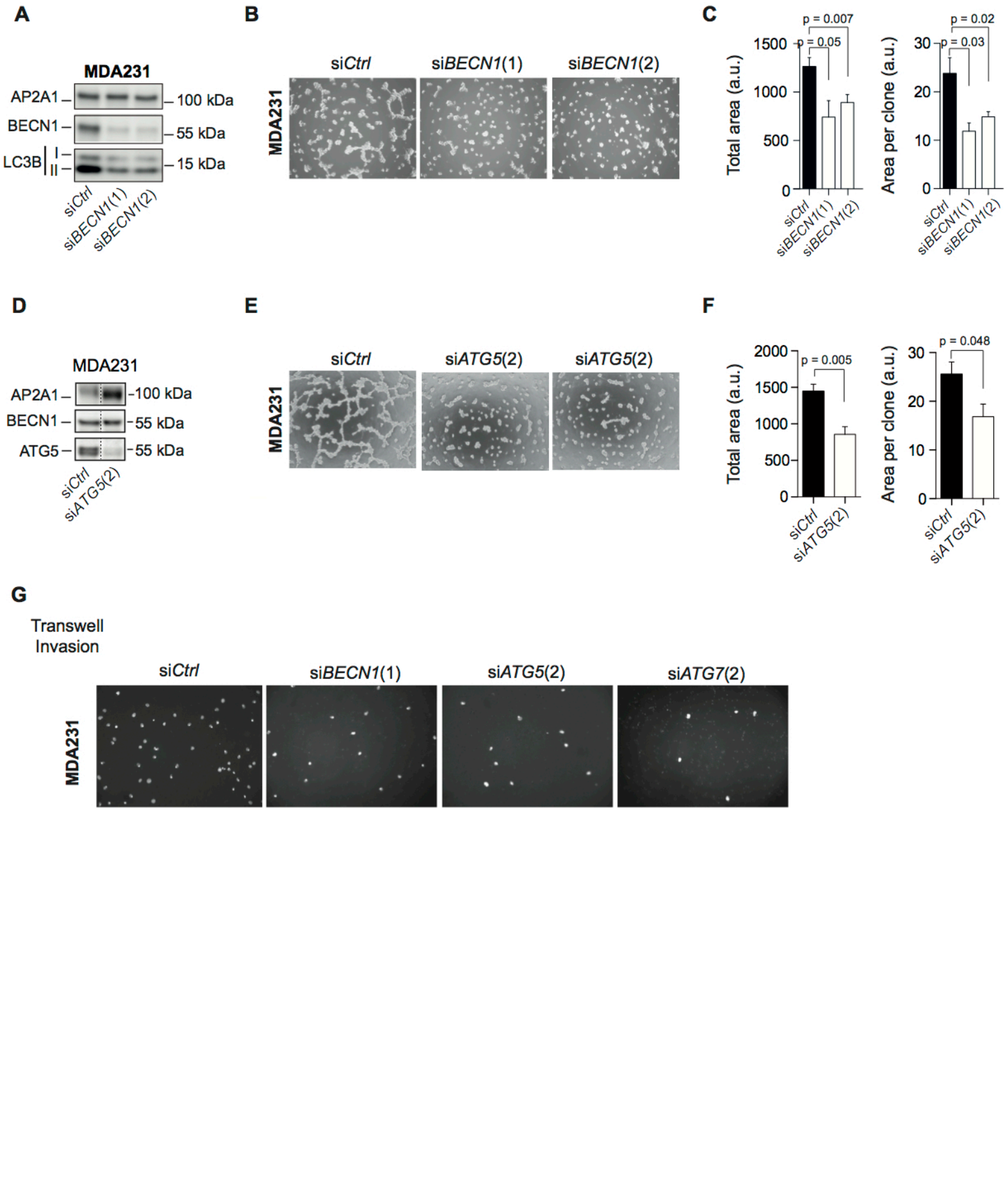

A
MDA231
AP2A1
BECN1
LC3B
I
II
100 kDa
55 kDa
15 kDa
siCtrl
siBECN1(1)
siBECN1(2)
B
siCtrl
siBECN1(1)
siBECN1(2)
MDA231
C
p = 0.007
p = 0.05
p = 0.02
p = 0.03
Total area (a.u.)
Area per clone (a.u.)
1500
1000
500
0
30
20
10
0
siCtrl
siBECN1(1)
siBECN1(2)
D
MDA231
AP2A1
BECN1
ATG5
100 kDa
55 kDa
55 kDa
siCtrl
siATG5(2)
E
siCtrl
siATG5(2)
siATG5(2)
MDA231
F
p = 0.005
p = 0.048
Total area (a.u.)
Area per clone (a.u.)
2000
1500
1000
500
0
30
20
10
0
siCtrl
siATG5(2)
G
Transwell
Invasion
siCtrl
siBECN1(1)
siATG5(2)
siATG7(2)
MDA231

Figure S8

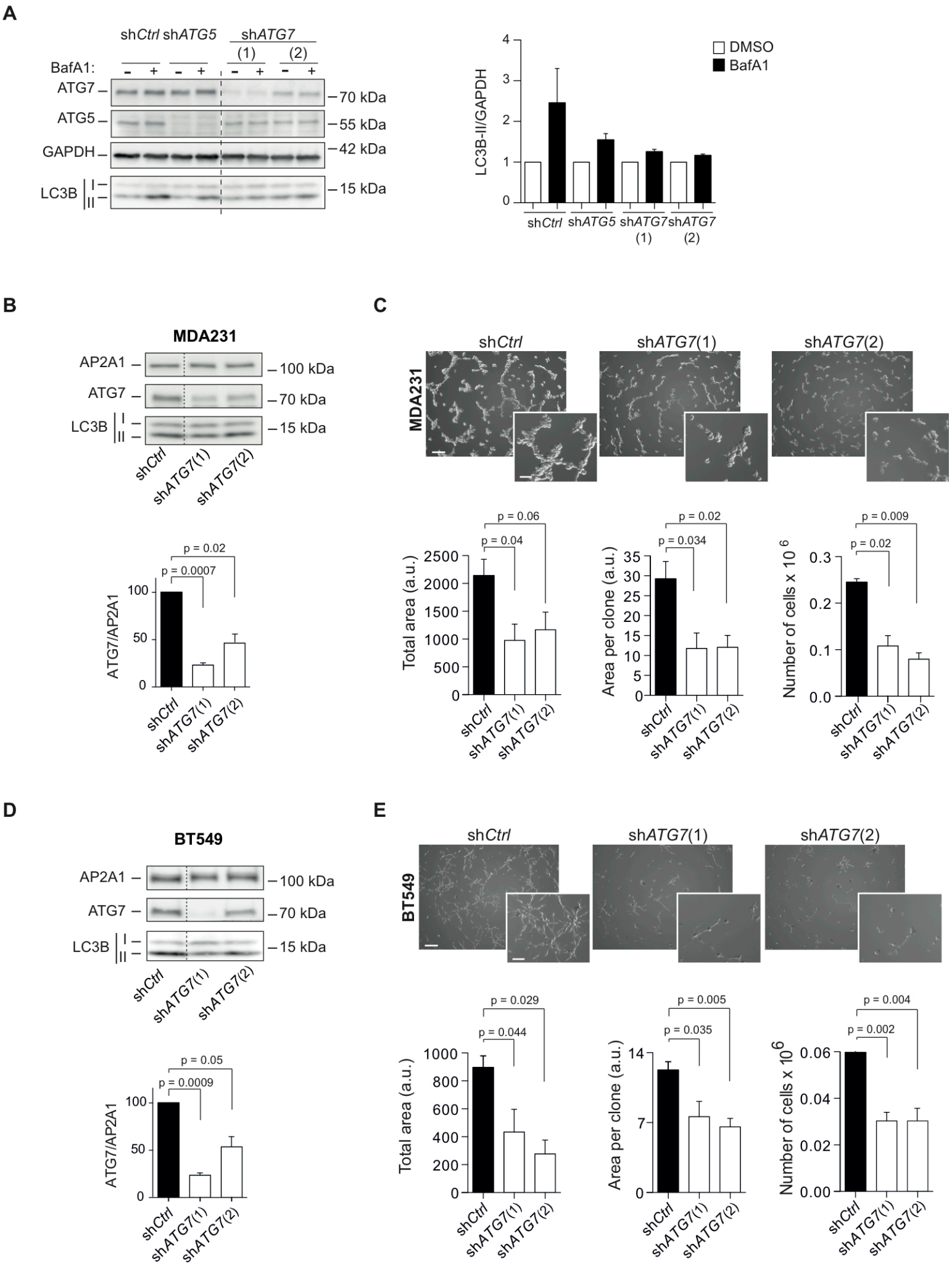

A
shCtrl shATG5 shATG7
(1) (2)
BafA1: - + - + - + - +
ATG7
ATG5
GAPDH
LC3B I II
70 kDa
55 kDa
42 kDa
15 kDa
DMSO
BafA1
LC3B-II/GAPDH
shCtrl shATG5 shATG7 (1) shATG7 (2)
B
MDA231
AP2A1
ATG7
LC3B I II
100 kDa
70 kDa
15 kDa
shCtrl shATG7(1) shATG7(2)
ATG7/AP2A1
p = 0.02
p = 0.0007
C
shCtrl shATG7(1) shATG7(2)
MDA231
Total area (a.u.)
p = 0.06
p = 0.04
Area per clone (a.u.)
p = 0.02
p = 0.034
Number of cells x 10 6
p = 0.009
p = 0.02
D
BT549
AP2A1
ATG7
LC3B I II
100 kDa
70 kDa
15 kDa
shCtrl shATG7(1) shATG7(2)
ATG7/AP2A1
p = 0.05
p = 0.0009
E
shCtrl shATG7(1) shATG7(2)
BT549
Total area (a.u.)
p = 0.029
p = 0.044
Area per clone (a.u.)
p = 0.005
p = 0.035
Number of cells x 10 6
p = 0.004
p = 0.002

Figure S9

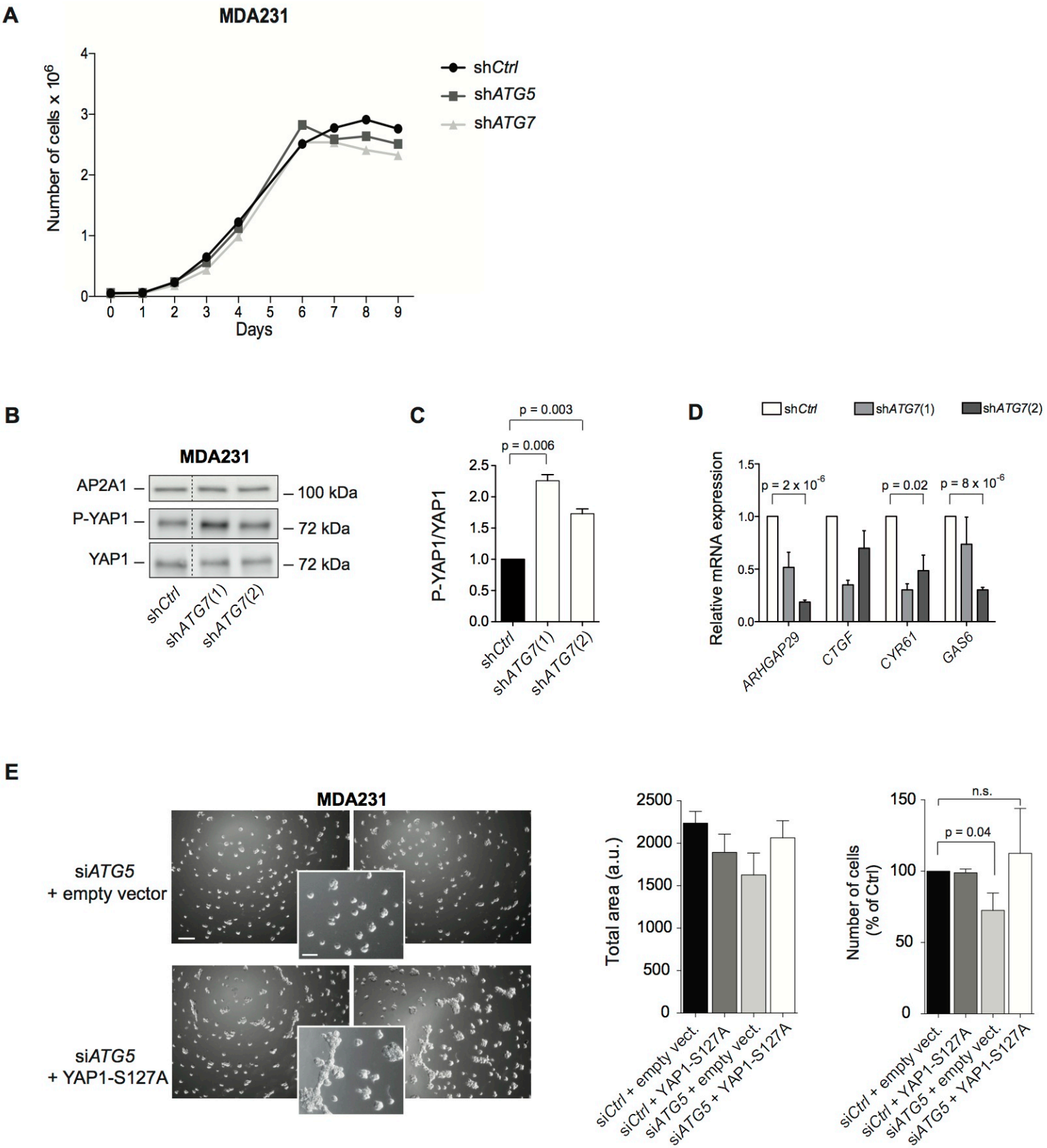

A
MDA231
Number of cells x 10^6
Days
shCtrl
shATG5
shATG7
B
MDA231
AP2A1
P-YAP1
YAP1
100 kDa
72 kDa
72 kDa
shCtrl
shATG7(1)
shATG7(2)
C
p = 0.003
p = 0.006
P-YAP1/YAP1
shCtrl
shATG7(1)
shATG7(2)
D
shCtrl
shATG7(1)
shATG7(2)
Relative mRNA expression
p = 2 x 10^-6
p = 0.02
p = 8 x 10^-6
ARHGAP29
CTGF
CYR61
GAS6
E
MDA231
siATG5 + empty vector
siATG5 + YAP1-S127A
Total area (a.u.)
siCtrl + empty vect.
siCtrl + YAP1-S127A
siATG5 + empty vect.
siATG5 + YAP1-S127A
n.s.
p = 0.04
Number of cells (% of Ctrl)
siCtrl + empty vect.
siCtrl + YAP1-S127A
siATG5 + empty vect.
siATG5 + YAP1-S127A

Table S1. Main patient characteristics and clinico-pathological features of the five BC cohorts analyzed in the study

		Curie	Stockholm	Marseille	TCGA	St-Cloud
Total		130	159	252	74	89
Inclusion		2000-2007	1994-1996	1992-2004	1988-2010	2002-2007
Average follow-up (years)		4,1	6,1	5	3.2	5.8
Mean age at diagnosis		54.1 (27 - 89)	58	54.8 (24 - 85)	52 (26-82)	-
Histological Grade	I	29 (23%)	28 (18%)	44 (17%)	-	4 (4%)
	II	3 (2%)	58 (36%)	87 (35%)	-	7 (8%)
	III	98 (75%)	61 (38%)	114 (45%)	-	78 (88%)
	NA	-	12 (8%)	7 (3%)	74 (100%)	-
Pathological tumor Size pT	pT1	59 (45%)	99 (62%)	57 (23%)	15 (20%)	25 (28%)
	pT2	55 (42%)	-	121 (48%)	50 (67%)	46 (52%)
	pT3	10 (8%)	-	62 (25%)	5 (7%)	14 (16%)
	pT4	6 (5%)	-	-	2 (3%)	4 (4%)
	NA	-	60 (38%)	12 (4%)	2 (3%)	-
Pathological lymph node status pN	Negative	58 (45%)	99 (62%)	111 (44%)	46 (62%)	70 (79%)
	Positive	70 (54%)	60 (38%)	135 (54%)	28 (38%)	19 (21%)
	NA	2 (1%)	-	6 (2%)	-	-
Metastasis status pM	Negative	126 (97%)	-	-	72 (97%)	-
	Positive	4 (3%)	-	-	2 (3%)	-
	NA	-	159 (100%)	252 (100%)	-	89 (100%)
Mean tumor size (mm)		25,9	22	-	-	-
Subtype	LumA	29 (22%)	39 (25%)	89 (35%)	-	-
	LumB	30 (23%)	23 (15%)	49 (19%)	-	-
	Her2	30 (23%)	15 (9%)	24 (10%)	-	-
	TN	41 (32%)	25 (16%)	61 (24%)	74 (100%)	89 (100%)
	No Subtype	-	20 (13%)	-	-	-
	Normal-like	-	37 (22%)	29 (12%)	-	-
Hormonotherapy	Yes	50 (38%)	104 (65%)	90 (36%)	-	-
	No	-	55 (35%)	117 (46%)	-	-
	NA	80 (62%)	-	45 (18%)	74 (100%)	89 (100%)
Radiotherapy	Yes	121 (93%)	-	188 (75%)	24 (33%)	79 (89%)
	No	9 (7%)	-	10 (4%)	-	10 (11%)
	NA	-	159 (100%)	54 (21%)	50 (67%)	-
Chemotherapy	Yes	100 (77%)	126 (79%)	155 (62%)	27 (36%)	77 (87%)
	No	30 (23%)	33 (21%)	45 (18%)	6 (8%)	12 (13%)
	NA	-	-	52 (20%)	41 (56%)	-
TN Chemotherapy	AC	32 (78%)	-	-	-	59 (66%)
	Taxanes	2 (5%)	-	-	-	5 (6%)
	AC + taxanes	1 (2%)	-	-	-	9 (10%)
	NA	6 (15%)	25 (100%)	61 (100%)	74 (100%)	16 (18%)

Patient characteristics and clinical features of the cohorts referred to as Curie, Stockholm, Marseille and TCGA have been previously described in refs. 32–34 and https://tcga-data.nci.nih.gov/tcga/ (see also Materials and Methods). BC subtypes were defined according to the ASCO's guidelines: LumA (ER+ and/or PR+ HER2-), LumB (ER+ and/or PR+, HER2-, high mitotic index), HER2 (ER-, PR-, HER2+), TN (ER-, PR-, HER2-). The five BC cohorts were examined for information on tumor size (pT), mean age at diagnosis, number of retrieved and metastatic axillary lymph nodes (pN), number of distant metastases (pM), hormonal receptor status, the date of relapse, the date and cause of death (only death from breast cancer has been considered), and the type of therapy used (hormono-, chimio-, radio-therapies). pN was considered as negative for N0 and positive for N1, N2 and N3. pM was considered as negative for M0 and positive for M1. The table summarizes the main clinical characteristics of the cohorts, helping in their comparison.

Table S2. MAP1LC3B mRNA levels in BC cell lines

Subtype	Cell line	*MAP1LC3B* (log2 of probeset intensity)	*MAP1LC3B* (Ct)	*TBP* (Ct)	*RPLP0* (Ct)	*PPIA* (Ct)	Normalized *MAP1LC3B*
Lum	BT-483	9.82922	25.46	28.97	20.7	22.22	0.08163
	CAL-148	8.15649					
	CAMA-1	9.56485	27.1	27.91	21.27	23.26	0.04168
	EFM-19	8.65158					
	HCC1428	8.83568	25.76	28.48	19.61	21.16	0.03152
	KPL-1	10.1164					
	MCF7	9.87316	25.88	29.13	20.86	22.6	0.07103
	MDA-MB-134-VI	8.63808	26.27	27.51	21.08	22.28	0.0568
	MDA-MB-175-VII	9.90964					
	MDA-MB-415	9.74844	25.79	29.05	20.49	22.09	0.05724
	T-47D	9.8821	25.49	28.47	19.92	21.09	0.04368
	UACC-812	8.57292					
	ZR-75-1	9.42224	26.41	28.19	20.41	22.92	0.03979
HER2	AU565	8.44571					
	BT-474	9.56742	27.14	29.05	20.73	21.45	0.02197
	EFM-192A	9.37299					
	HCC1419	9.02279					
	HCC1569	8.41577	25.85	28.17	20.9	21.99	0.06551
	HCC1954	10.0596	24.7	27.91	20.87	21.36	0.12233
	HCC202	9.59094					
	HCC2218	8.27281					
	JIMT-1	11.7046					
	MDA-MB-361	10.4209	25.39	28.31	21.16	22.7	0.11829
	MDA-MB-453	8.29676	26.28	28.16	21.28	22.34	0.06303
	SK-BR-3	8.4241	26.28	28.21	21.03	21.21	0.04158
	ZR-75-30	8.59943					
TN	BT-20	10.3084	24.18	27.68	20.67	20.85	0.13932
	BT-549	10.7133	24.69	28.18	21.33	21.95	0.17579
	CAL-120	10.6969					
	CAL-51	9.41878					
	CAL-85-1	10.074					
	DU4475	9.54485					
	HCC1143	8.97916	25.58	26.71	20.15	20.53	0.03916
	HCC1187	10.1825	25.41	27.54	20.09	21.65	0.05585
	HCC1395	11.1506					
	HCC1500	10.562	23.52	26.22	20.27	20.39	0.16273
	HCC1599	8.03435	27.34	28.51	20.68	23.12	0.02487
	HCC1806	9.40838					
	HCC1937	10.296	24.22	27.84	20.54	21.87	0.16609
	HCC2157	9.38436					
	HCC38	10.3581	24.31	28.73	19.38	21.55	0.08019
	HCC70	10.1764	24.15	27.65	20.19	20.11	0.0935
	HDQ-P1	9.46084					
	Hs 578T	10.9184	24.8	28.65	21.08	21.86	0.1435
	MDA-MB-157	8.10773	25.76	27.26	20.46	21.25	0.048
	MDA-MB-231	9.54688	24.83	26.92	20.04	21.41	0.07775
	MDA-MB-436	10.6143	24.41	27.55	20.1	21.61	0.11131
	MDA-MB-468	10.3774	24	26.95	20.23	19.92	0.09767
	UACC-893	9.96325					
	YMB-1	9.29721					

MAP1LC3B mRNA levels in Lum, HER2 and TN BC-derived cell lines either determined from Affymetrix U133 plus 2.0 microarray analysis (MAP1LC3B log2 of probeset intensity, 208785_s_at) or quantified by qRT-PCR (Ct, threshold cycle). For qRT-PCR analysis (columns with Ct values), MAP1LC3B mRNA levels were normalized on the basis of the mean values of 3 housekeeping genes TBP (TATA box binding protein), RPLP0 (ribosomal protein, large, P0) and PPIA (peptidylprolyl isomerase A [cyclophilin A]), as endogenous RNA controls for the total amount of RNA in each cell line. Ct means threshold cycle.