SUPPLEMENTAL MATERIAL AND METHODS

Cell lines and reagents The murine 3T3-F442A pre-adipocyte cell line line (provided by Pr. P. Valet, I2MC, Toulouse, France) was cultured in DMEM medium supplemented with 10% of fetal calf serum (FCS). Differentiation was induced by incubating confluent cells in differentiation medium (DMEM supplemented with 10% FCS and 50nM insulin) for up to 14 days, as described previously (Meulle, Salles et al. 2008). Human mammary epithelial cells (HMEC) (provided by Dr J. Piette, IGMM, Montpellier, France) were grown in MEBM medium (Lonza, France), containing transferrin (5µg/ml) and isoproterenol (10^{-5}M). The human breast cancer cell line ZR-75-1 (Sigma, France) was cultured in DMEM medium supplemented with 10% FCS, and the SUM159PT cell line (provided by Dr J Piette, IGMM, Montpellier, France) was grown in Ham F12 (50:50) medium complemented with 5% FCS, 1μg/ml hydrocortisone (Sigma, Saint-Quentin les Ulysses, France) and 0.2UI /mL insulin. The murine breast cancer cell line TS/A (provided by Dr P. Valet, I2MC, Toulouse, France) was cultured in RPMI 1640 medium supplemented with 10% FCS. All cell lines were maintained at 37ºC in a humidified atmosphere with 5% CO_2 and used within 2 months after resuscitation of frozen aliquots. The suppliers, references and dilutions used for all antibodies are listed in table S3. Toluidine Blue O and Etomoxir (used at final concentrations from 1 to 30µM) were purchased from Sigma (France). DAPI (used at a final concentration of 1µM) was obtained from Invitrogen (France).

RNA interference studies To stably switch off *ATGL* (NM_020376) and *CPT1A* (NM_001876) gene expression in ZR-75-1 cells, we used replicative short hairpin (shRNA) expressing vectors (pEBVsiRNA) which impose a very strong and stable gene silencing in human cells even after several months in culture. siRNA design was performed using the DSIR program (Vert, Foveau et al. 2006). RNAi sequences against *ATGL* stretched from nucleotides 11-29 (with ATG as the first nucleotide, GCGAGAAGACGTGGAACAT; pBD2338) and from nucleotides 203-221 against *CPT1A* (GCGTGATGACAACGATGTA, pBD2405 or pBD2408). pEBVsiRNA vectors were transfected into ZR-75-1 cells using Lipofectamine and knocked down (KD) cell populations were selected with 30μg/mL of hygromycin B (Invitrogen, Life Technologies). ATGL and CPT1A silenced cells were termed as shATGL and shCPT1A cells. As a control, we used the pBD650 plasmid which expresses an inefficient shRNA sequence (shCtrl) (Despras, Pfeiffer et al. 2007).

Coculture, cell proliferation and invasion assays. Coculture and invasion assays were conducted as previously described (Dirat, Bochet et al. 2011). Briefly, $5x10^4$ (SUM159PT and TS/A) or $1x10^5$ (ZR-75-1) cells were seeded in the top chamber of a Transwell system (0.4μM pore size, Millipore) and cocultivated, or not, with mature adipocytes in the bottom chamber. Similar experiments were performed with shCtrl, shATGL or shCPT1A ZR-75-1 cells. In indicated experiments, cells were treated or not during coculture with Etomoxir for 3 days. For viable cell number evaluation, after coculture in the presence or not of adipocytes, tumor cells were fixed with methanol, stained with Toluidine Blue O buffer (1% supplemented with 0.1M Borax), washed several times with distilled water, air-dried and dissolved in lysis buffer (6.25mM Tris HCL pH 6.8, 10% glycerol, 2% SDS, 10% β-mercaptoethanol). The quantification of the absorbance (measured at 570 nm) was performed in duplicate. Matrigel invasion assays were conducted as previously described (Dirat, Bochet et al. 2011) except for TS/A cells for which invasive cell number was evaluated by quantification of the absorbance after dissolution in lysis buffer.

For cell cycle analysis, cells were harvested, washed in PBS and fixed for 30 minutes at 4°C in cold 70% ethanol. Cells were washed twice in PBS, centrifuged at 850 g and treated with 20 μg/ml of DNase free RNase for 30 minutes. Then cells were treated with 20 μg/ml of propidium iodide for 10 minutes at room temperature and analyzed with a FACScan flow cytometer (Becton Dickinson, Franklin Lanes, NJ). For each measurement, at least 15,000 cells were acquired.

For MTT assays, $4x10^3$ of ZR-75-1 shControl, shCPT1A, or shATGL cells were seeded in 96 well plates in quadruplicate and incubated for 24, 48 and 72 hours. Following this period, 100µl of Thiazolyl Blue Tetrazolium Bromide (0.05g dissolved in 50ml PBS) was added before 2 hours' further incubation. All medium was then removed, 100µl of DMSO was added and absorbance was read at a wavelength of 570nm.

Lipidomic analysis ZR-75-1 cells were cultivated alone or with mature adipocytes for 3 days. Cells were then were lysed in methanol: 5 mM EGTA (2:1, v/v), and lipids were extracted following the Bligh–Dyer method (Bligh and Dyer 1959) using chloroform:methanol:water (2.5:2.5:2.1, v/v/v). For neutral lipids analysis, extraction was performed in the presence of glyceryl trinonadecanoate, stigmasterol, and cholesteryl heptadecanoate (Sigma) as internal standards. TGs, free cholesterol, and cholesterol esters were analysed by gas-liquid chromatography as described previously (Montagner, Polizzi et al. 2016). For fatty acid analysis, extraction was performed in the presence of glyceryl triheptadecanoate (0.5 µg) as an internal standard. The lipid extract was transmethylated and fatty acid methyl esters were analyzed by gas-liquid chromatography as described previously (Montagner, Polizzi et al. 2016).

Immunofluorescence 3T3-F442A grown on coverslips were treated or not with HMEC, ZR-75-1 and SUM159PT conditioned medium for 3 days. The coverslips were fixed in 3.7% paraformaldehyde for 20 min at room temperature (RT) and then permeabilized with PBS 0.2% TritonX-100 for 5 min. After blocking with 10% FCS and 2% BSA in PBS for 1 hour, cells were incubated for 2 hours at RT with Phosphorylated Hormone-Sensitive Lipase (pHSL (1:1000). The secondary antibody, Alexa fluor 488-conjugated anti-rabbit (1:500) was used for 30 min at RT. For bodipy staining, cells were incubated for 30 min with Bodipy (5mg/ml) and then with DAPI for 5 min. Coverslips were examined using an Olympus FV-1000 with 60X oil PLAPON OSC objective or a Zeiss LSM 710 confocal microscope with 40× oil PlanApo objective (1.4 N.A.). A minimum of three independent experiments were performed. Images were processed to filter the noise with Fiji software and a similar filter was used to analyze all acquisitions for the experiment.

Measure of lipolysis ZR-75-1 and SUM159PT conditioned-medium (CM) containing 0.2% delipidated bovine serum albumin (Sigma, France) were collected after 12h in the absence of serum. Glycerol released into the medium of mature 3T3-F442A adipocytes, incubated or not with CM from the indicated breast cell lines and treated or not with propranolol (1μM), UK14304 (1μM) or an IL6 blocking antibody (10μg/ml) for 3 days, was measured using the Glycerol-Free Reagent Kit (Sigma, France).

Oxygen consumption and extracellular acidification rate (OCR and ECAR respectively) OCR and ECAR were measured using the XF24 analyzer (Seahorse Bioscience, Billerica, MA, USA). Cells previously

cocultivated, or not, were seeded in the 24-well XF24 cell culture plate at a density of 20000 cells/well and incubated for a further 18h in their respective conditioned media. Media was then removed and wells were washed and incubated for 1h at 37°C without CO_2 in XF modified DMEM assay medium (Seahorse bioscience) pH 7.4, supplemented with 1mM glutamine for both glycolysis and mitochondrial stress tests and 2.5mM glucose, 1mM sodium pyruvate, 0.5mM carnitine and 1mM palmitate complexed with 0.2mM BSA for mitochondrial stress tests. For glycolytic tests, OCR and ECAR were measured at basal state (no glucose) or after injection of 10mM glucose, 5µM oligomycin and 50mM 2-deoxyglucose. For mitochondrial stress tests, OCR was measured at basal state (1mM palmitate complexed with 0.2mM BSA) or after injection of 5µM oligomycin, 1µM FCCP (2-[2-[4-(trifluoromethoxy)phenyl]hydrazinylidene]-propanedinitrile) and rotenone plus antimycin A (both at 5µM). After Seahorse experiments, proteins were quantified and all the results were normalized according to protein concentration.

Measure of mitochondrial membrane potential ($\Delta\Psi$) ZR-75-1 were cultivated alone or with mature adipocytes for 3 days. Non-cocultivated cells were treated or not with 30μM FCCP for 1h as a control for mitochondrial membrane depolarization. To analyze the $\Delta\Psi$, cells were incubated for 20 min with 2μg/ml JC-1 (Molecular Probes) in culture medium at 37°C and either directly observed using confocal microscopy (Zeiss LSM 710) in culture medium or subsequently collected, washed in PBS and processed for fluorescence analysis on a Clariostar Monochromator Microplate Reader (excitation 488 nm; emission 529 and 590 nm). The ratio of the fluorescence at 590 nm, representative of high mitochondrial polarization, versus that at 529 nm, representative of mitochondrial depolarization, was calculated to determine the $\Delta\Psi$.

Measure of fatty acid (FA) oxidation ZR-75-1 and TS/A were cultivated alone or with mature adipocytes for 3 days. Cells were then incubated with 2ml of warmed (37ºC), pre-gazed (95% O_2-5% CO_2, pH 7.4), modified Krebs-Henseleit buffer containing 1.5% FA-free BSA, 5mM glucose, 1mM palmitate and 0.5μCi/ml (^{14}C) palmitate (Perkin Elmer) for 60 min. At the end of the incubation, tumor cells were collected in 800μl of lysis buffer. A 0.5ml microtube containing 300μl of benzethonium hydroxide (Sigma) was placed in the vial to capture the $^{14}CO_2$. The incubation buffer was then acidified with 1ml of 1M H_2SO_4 and the vial was quickly sealed with parafilm. After 120 minutes, the microtube was removed, placed in a scintillation vial and the radioactivity, corresponding to complete oxidation, was counted (cytoscint. MP Biomedicals). A total of 500mL homogenate was placed into glass tubes to extract lipids with chloroform-ethanol (2:1) and 2M KCl-HCl. After centrifugation, the aqueous phase (500mL) was quantified by liquid scintillation to determine the acid-soluble metabolites production (incomplete oxidation).

Triglyceride (TG) content analysis Tumor cells were grown for 3 days in the presence or not of adipocytes. At the end of coculture, tumor cells were pelleted and resuspended in 15μl of TG buffer (10mM Tris HCL pH 7.5, 1mM EDTA). Adipocytes incubated or not in the presence of ZR-75-1-CM for 3 days were resuspended in 300μl of TG buffer. TG content was quantified using a colorimetric kit (Biomerieux, France) and results were read at a wavelength of 505nm.

Lactate dosage Lactate released into the medium by ZR-75-1 cells, grown alone or in the presence of mature 3T3-F442A adipocytes lines and treated or not with 2-deoxyglucose (2DG) (3mM) for 3 days, was measured in 5μl aliquots using the Lactate FS kit (Diagnostic system, France). TruCal U was used as calibrator.

RNA extraction and quantitative RT-PCR Total RNAs were extracted using the RNeasy mini kit (Qiagen GmbH, Hilden, Germany). Gene expression was analyzed using real time PCR as described previously (Daviaud, Boucher et al. 2006). Total RNAs (1μg) were reverse-transcribed for 60 min at 37°C using Superscript II reverse transcriptase (Invitrogen, Auckland, NZ) in the presence of a random hexamer. A minus reverse transcriptase reaction was performed in parallel to ensure the absence of genomic DNA contamination. Real time PCR was performed starting with 25ng of cDNA and 500nM of both forward and reverse primers (sequences are listed in table S4) in a final volume of 25µl using the SYBR Green TaqMan Universal PCR master mix (Applied Biosystems, Foster City, CA). Fluorescence was monitored and analyzed in a GeneAmp 7500 detection system instrument (Applied Biosystems, Foster City, CA). Analysis of 18S ribosomal RNA was performed in parallel using the ribosomal RNA control TaqMan assay kit (Applied Biosystem), or *HPRT* RNA or *GAPDH* RNA (table S4) to normalize gene expression. Oligonucleotide primers were designed using the Primer Express software (PerkinElmer Life Sciences) as previously described (Daviaud, Boucher et al. 2006).

Mitochondrial DNA analysis Total DNA was extracted (DNeasy, QIAGEN) and the quantity of mitochondrial DNA was evaluated by real time quantitative PCR as previously described (Attane, Foussal et al. 2012). Results were presented as a ratio between mitochondrial DNA (*COX1*) and genomic DNA (cyclophilin A and *YWHAZ*). Oligonucleotide sequences are listed in table S4.

Statistical analysis The statistical significance of differences between means was evaluated with unpaired Student t tests when comparing only two groups. Student's t test was 2 tailed. Comparisons between more than two groups were performed using Mann–Whitney U tests. The Benjamini-Hochberg procedure was applied for multiple comparisons. All reported p-values were two-sided. For all statistical tests, differences were considered significant at the 5% level. Statistical analysis was performed using R 3.2.2 software. p values below 0.05 (*), <0.01 (**) and <0.001 (***) were deemed as significant and "ns" was used to denote not significant.

In TMA analysis, we first performed a descriptive analysis with the clinical and biological features of patients. For the correlation between ATGL and MAGL expression with tumor grade (variable with more than two ordered classes regarding disease severity), the Spearman rank correlation test was used assuming a monotonic relation between considered variables. The Student's t-test was used to correlate ATGL and MAGL expression to all other variables which are non-ordered variable represented by a category (for example absence or expression of estrogen receptor).

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Despras, E., P. Pfeiffer, et al. (2007). "Long-term XPC silencing reduces DNA double-strand break repair." Cancer Res **67**(6): 2526-2534.

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Vert, J. P., N. Foveau, et al. (2006). "An accurate and interpretable model for siRNA efficacy prediction." BMC Bioinformatics **7**: 520.

		Number of patients	Percentage among the cohort
Size of the cohort		69	100
Age (Years), median (extent)		63 (49-76)	
Tumor size (cm), median (extent)		1.7 (2.5-3.5)	
Tumor grade, (number of patients)	Grade 1	9	13.0
	Grade 2	34	49.3
	Grade 3	26	37.7
Breast cancer subtype, (number of patients)	ER+	49	71.0
	PgR+	34	49.3
	HER2+	12	17.4
	Triple negative	14	20.3
Histology (number of patients)	Ductal invasive carcinoma	59	85.5
	Lobular invasive carcinoma	10	14.5
Lymph node invasion, (number of patients)	Invaded	30	43.5
	Not invaded	39	56.5

Supplementary Table 1: Histological and immunohistochemical characteristics of the cohort The abbreviations used are as follows: ER, estrogen receptor (ER positive when ≥ 1%); PgR, progesterone receptor (PgR positive when ≥ 10%); HER2, human epidermal growth factor receptor 2

		ATGL expression				
		Not expressed	Low	Moderate	High	P-value
Tumor grade, n (percentage among the event)	Grade 1	8 (88.9)	1 (11.1)	0 (0)	0 (0)	<0.001 *
	Grade 2	31 (91.2)	2 (5.9)	1 (2.9)	0 (0)	
	Grade 3	13 (50)	4 (15.4)	5 (19.2)	4 (15.4)	
PgR expression, n (percentage among the event)	Not expressed	23 (65.7)	5 (14.3)	4 (11.4)	3 (8.6)	0.311
	Expressed	29 (85.3)	2 (5.9)	2 (5.9)	1 (2.9)	
ER expression, n (percentage among the event)	Not expressed	12 (60)	3 (15)	3 (15)	2 (10)	0.305
	Expressed	40 (81.6)	4 (8.2)	3 (6.1)	2 (4.1)	
HER2 expression, n (percentage among the event)	Not expressed	47 (82.5)	6 (10.5)	2 (3.5)	2 (3.5)	0.002
	Expressed	5 (41.7)	1 (8.3)	4 (33.3)	2 (16.7)	
Triple negative, n (percentage among the event)	Not triple neg.	45 (75)	6 (10)	5 (8.3)	4 (6.7)	0.879
	Triple negative	7 (77.8)	1 (11.1)	1 (11.1)	0 (0)	
Lymph Node invasion, n (percentage among the event)	Not invaded	31 (79.5)	5 (12.8)	2 (5.1)	1 (2.6)	0.358
	Invaded	21 (70)	2 (6.7)	4 (13.3)	3 (10)	

Supplementary Table 2: Immunohistochemical staining of ATGL in breast cancer TMA ATGL expression was evaluated by immunohistochemistry on a TMA containing 69 tumors in duplicate. For the correlation between ATGL expression with tumor grade (more than two ordered classes regarding disease severity), Spearman correlation test (notified by *) was used assuming a monotonic relation between considerable variables. Student's t-test was used to correlate ATGL expression to all the other non-ordered categorical variables. Abbreviations used: TMA, Tissue microarray; ER, estrogen receptor (ER positive when ≥ 1%); PgR, progesterone receptor (PgR positive when ≥ 10%); HER2, human epidermal growth factor receptor 2

		MAGL expression				
		Not expressed	Low	Moderate	High	P-value
Tumor grade, n (percentage among the event)	Grade 1	3 (33.3)	2 (22.2)	1 (11.2)	3 (33.3)	0.634 *
	Grade 2	3 (8.8)	7 (20.6)	11 (32.4)	13 (38.2)	
	Grade 3	6 (23.1)	4 (15.4)	10 (38.4)	6 (23.1)	
PgR expression, n (percentage among the event)	Not expressed	6 (17.1)	6 (17.1)	13 (37.2)	10 (28.6)	0.811
	Expressed	6 (17.6)	7 (20.6)	9 (26.5)	12 (35.3)	
ER expression, n (percentage among the event)	Not expressed	4 (20)	2 (10)	8 (40)	6 (30)	0.563
	Expressed	8 (16.3)	11 (22.4)	14 (28.6)	16 (32.7)	
HER2 expression, n (percentage among the event)	Not expressed	12 (21.1)	10 (17.5)	18 (31.6)	17 (29.8)	0.359
	Expressed	0 (0)	3 (25)	4 (33.3)	5 (41.7)	
Triple negative, n (percentage among the event)	Not triple neg.	8 (14.8)	11 (20.4)	18 (33.3)	17 (31.5)	0.867
	Triple negative	4 (26.7)	2 (13.3)	4 (26.7)	5 (33.3)	
Lymph Node invasion, n (percentage among the event)	Not invaded	8 (20.5)	8 (20.5)	14 (35.9)	9 (23.1)	0.358
	Invaded	4 (13.3)	5 (16.7)	8 (26.7)	13 (43.3)	

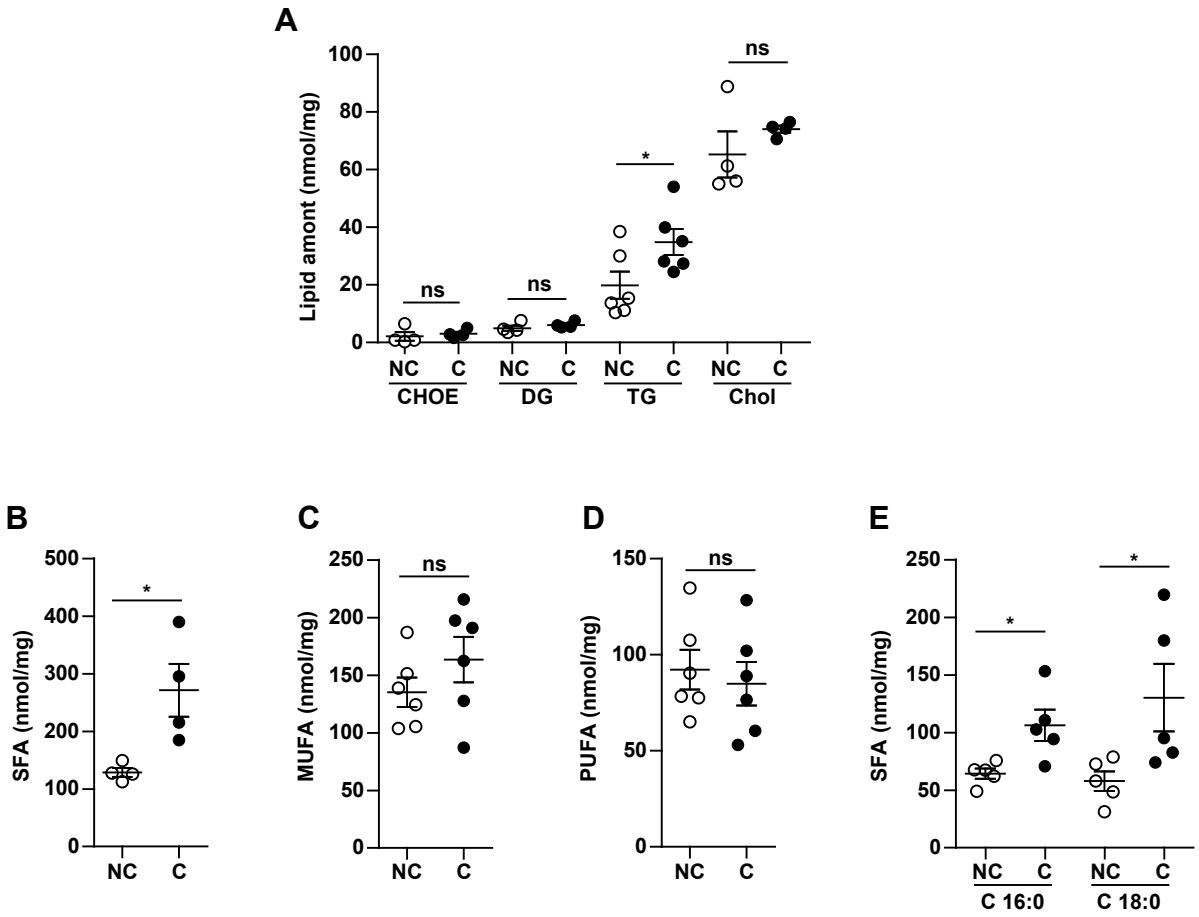
Supplementary Table 3: Immunohistochemical staining of MAGL in breast cancer TMA MAGL expression was evaluated by immunohistochemistry on a TMA containing 69 tumors in duplicate. For the correlation between MAGL expression with tumor grade (more than two ordered classes regarding disease severity), Spearman correlation test (notified by *) was used assuming a monotonic relation between considerable variables. Student's t-test was used to correlate MAGL expression to all the other non-ordered categorical variables. Abbreviations used: TMA, Tissue microarray; ER, estrogen receptor (ER positive when ≥ 1%); PgR, progesterone receptor (PgR positive when ≥ 10%); HER2, human epidermal growth factor receptor 2

Protein name	Clone	Furnishers and product reference	WB dilution	IF or IHC dilution
α-Tubulin	Ab-2	Thermo Scientific (MS-581)	1/1000	
β-Actin	Ab-5	Thermo Scientific (MS-1295)	1/1000	
ACC		Cell signaling (3662)	1/1000	
P-ACC (Ser79)		Cell signaling (3661)	1/1000	
AMPK		Cell signaling (2603)	1/1000	
P-AMPK (thr172)		Cell signaling (2535)	1/1000	
ATGL		Cell signaling (2138)	1/1000	1/100
CPT1A		Abcam (ab53532)	1/500	
E-cadherin	HECD-1	Calbiochem (205601)		1/50
F0F1-ATP synthase subunit IF1	5E2D7	life technologies (A-21355)	1/1000	
HSL		Cell signaling (4107)	1/1000	
p-HSL (ser660)		Cell signaling (4126)		1/150
MAGL	H-300	Santa Cruz (sc-15324)	1/200	1/100
OXPHOS		Abcam (ab110413)	1/1000	
SNAI1		Cell signaling (3879)	1/1000	
UCP2	C-20	Santa Cruz (sc-6525)	1/200	

Supplementary Table 4: Characterization of the antibodies used in the study

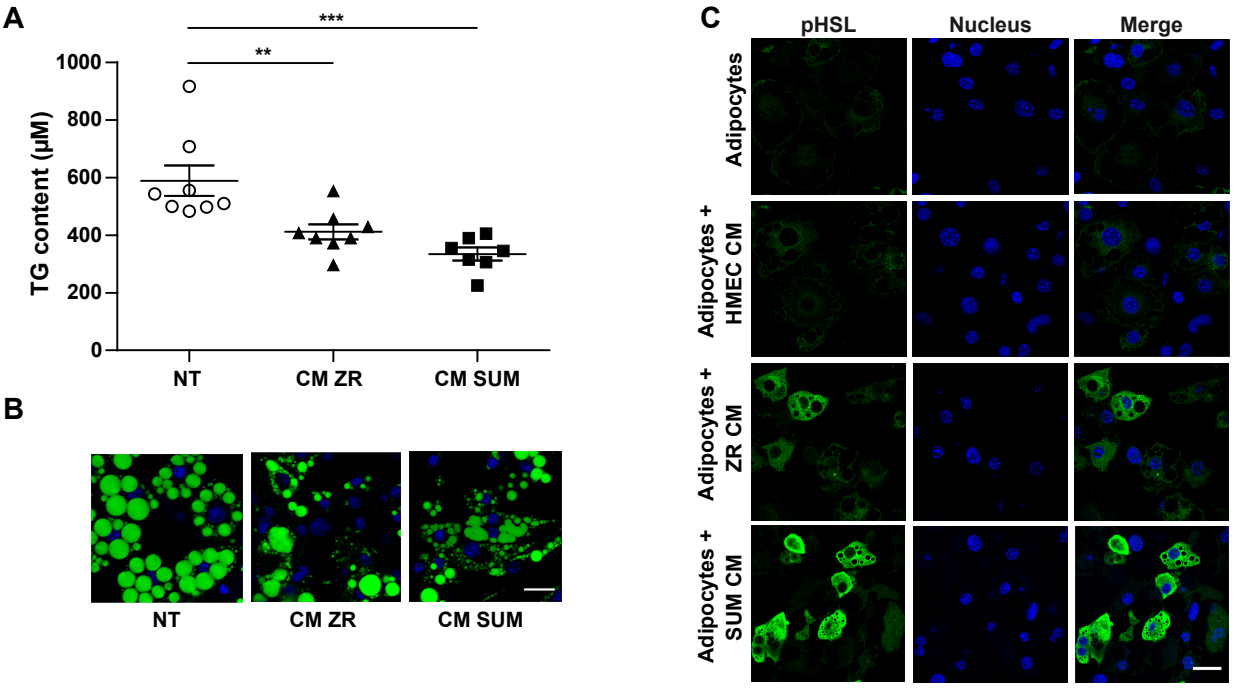
Experiment	Gene Name	Sequences
RT-qPCR	*SNAI1*	Forward: 5’ TCCCAGATGAGCATTGGCAG
		Reverse: 5’ CGCGCTCTTTCCTCGTCAG
RT-qPCR	*CPT1A*	Forward: 5’ TGTGCTGGATGGTGTCTGTCTC
		Reverse: 5’ CGTCTTTTGGGATCCACGATT
RT-qPCR	*PGC1A*	Forward: 5’ AACAGCAGCAGAGACAAATGCACC
		Reverse: 5’ TGCAGTTCCAGAGAGTTCCACACT
RT-qPCR	*PPARA*	Forward: 5’ CTATCATTTGCTGTGGAGATCG
		Reverse: 5’ AAGATATCGTCCGGGTGGTT
RT-qPCR	*PPARD*	Forward: 5’ GTCACACAACGCTATCCGTTT
		Reverse: 5’ AGGCATTGTAGATGTGCTTGG
RT-qPCR	*HPRT*	Forward: 5’ TGACACTGGCAAAACAATGCA
		Reverse: 5’ GCTTGCGACCTTGACCATCT
RT-qPCR	*GAPDH*	Forward: 5’ TGCACCACCAACTGCTTAGC
		Reverse: 5’ GGCATGGACTGTGGTCATGAG
RT-qPCR	*UCP2*	Forward: 5’ ATTGTCTCAGTGCTGGTGGA
		Reverse: 5’ TGGATGACAAGTGGGCTAGG
qPCR	cyclophilin A	Forward: 5′-ACACGCCATAATGGCACTGG
		Reverse: 5′-CAGTCTTGGCAGTGCAGAT
qPCR	*YWHAZ*	Forward: 5′- ACTTTTGGTACATTGTGGCTTCAA
		Reverse: 5’- CCGCCAGGACAAACCAGTAT
qPCR	*COX1*	Forward: 5′-ACTATACTACTACTAACAGACCG
		Reverse : 5′-GGTTCTTTTTTTCCGGAGTA

Supplementary Table 5: Sequences of the primer used in the study

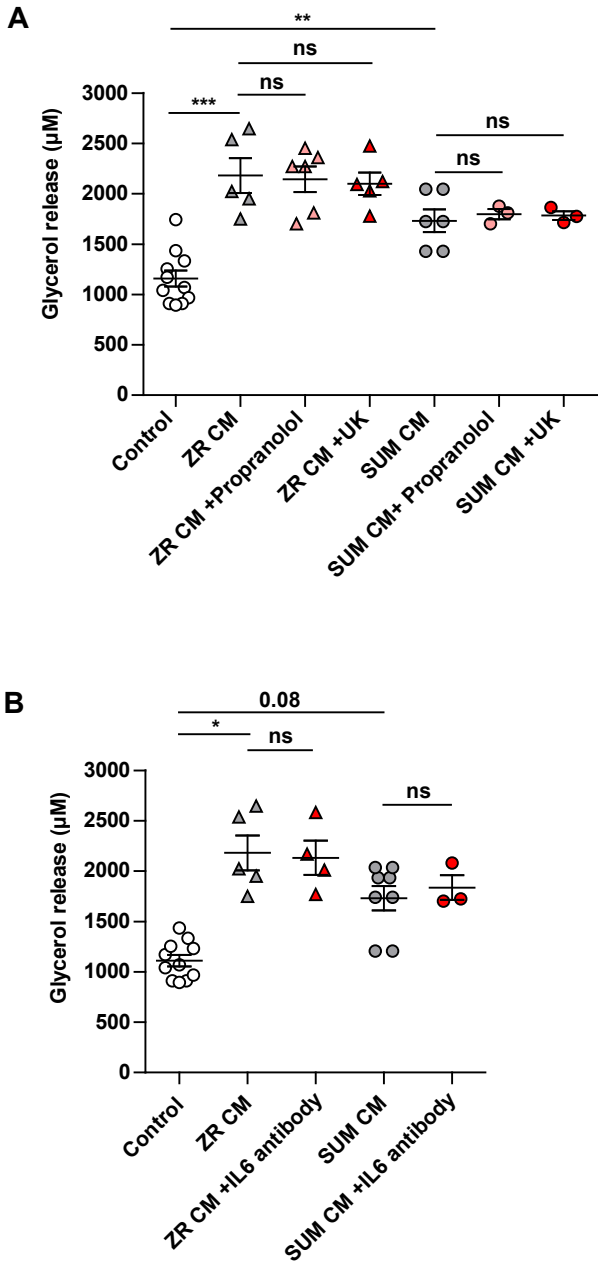


Supplementary Figure 1. Cocultivated tumor cells exhibit increases in triglycerides (TG) and saturated free fatty acid (FFA) content ZR-75-1 cells were cocultivated (C) or not (NC) in the presence of 3T3-F442A mature adipocytes. Total lipids were extracted and analyzed by high performance thin layer chromatography (HPTLC) and electrospray ionization mass spectroscopy (ESI-MS). **(A)** Dosage of the different lipid species (n=4-6). **(B, C, D and E)** Dosage of FFAs.

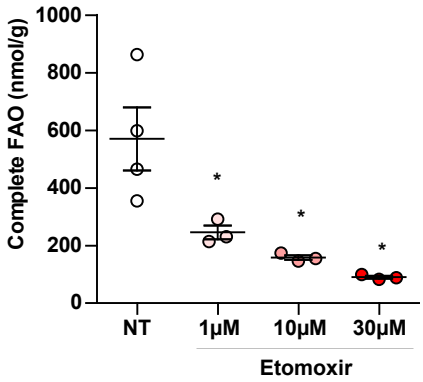
CHOE, esterified cholesterol; DG, diglycerides; TG, triglycerides; Chol, cholesterol; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids, C 16:0, palmitic acid; C 18:0, stearic acid (n=4-6). Bars and error flags represent means ± SEM; statistically significant by Mann-whitney U test **(A and E)** and Student's t-test **(B-D)**, * $p < 0.05$, ns: not-significant.



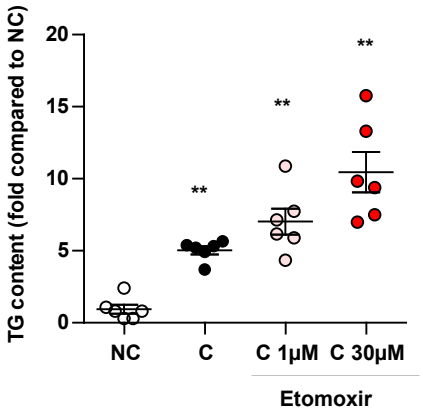
Supplementary Figure 2. Conditioned-medium from tumor cells but not normal epithelial cells induce lipolysis in adipocytes 3T3-F442A adipocytes were treated or not with human mammary epithelial cells (HMEC), ZR-75-1 and SUM159PT conditioned medium (respectively HMEC CM, ZR CM and SUM CM) between 3 and 5 days. Lipid content was evaluated in adipocytes, shown by measure of triglyceride (TG) content (n=7-8) **(A)** and after staining with Bodipy (in green) **(B)**. **(C)** The presence of Phosphorylated Hormone-Sensitive Lipase (p-HSL) was then assayed by immunofluorescence followed by confocal microscopy analysis (in green). Nuclei were labeled with DAPI (in blue). Scale bar: 30µm. **B-C** At least 3 experiments were conducted and representative images are shown. Bars and error flags represent means ± SEM; statistically significant by Mann-whitney U test, ** $p < 0.01$, *** $p < 0.001$.



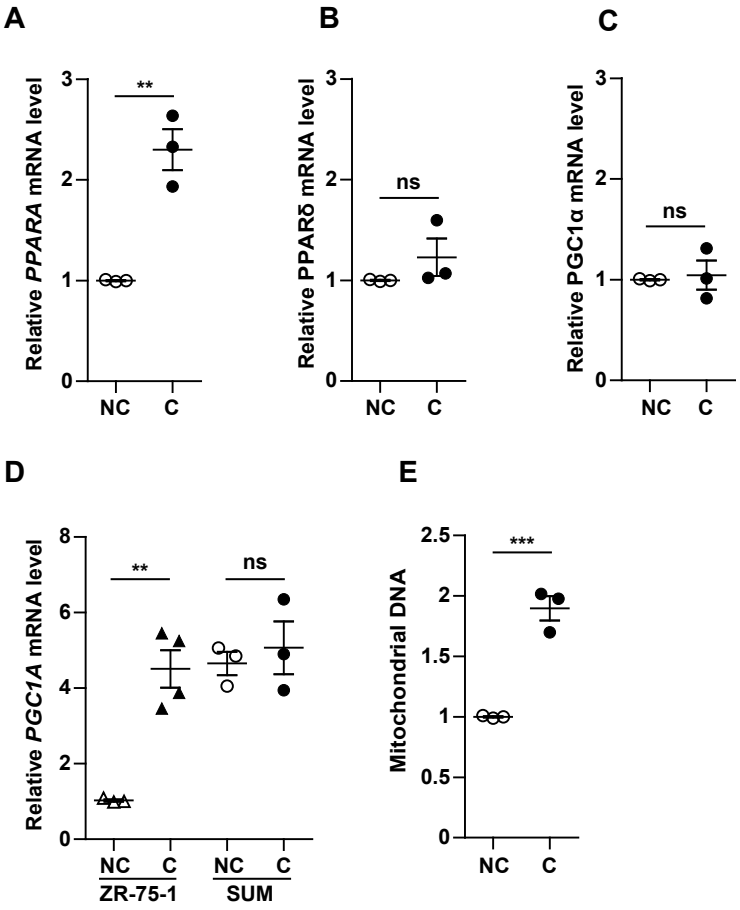
Supplementary Figure 3. Induction of lipolysis in adipocytes by tumor secretions is not controlled by adrenergic receptors, nor IL6 3T3-F442A adipocytes were treated or not with ZR-75-1 and SUM159PT conditioned medium (respectively ZR CM and SUM CM) in the presence of **(A)** propranolol (1µM) or UK14304 (1µM) or **(B)** IL6-bloking Ab for 3 days. Cell supernatants were collected and free glycerol release was measured (n=3-11). Bars and error flags represent means ± SEM; statistically significant by Mann-whitney U test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns: not significant.



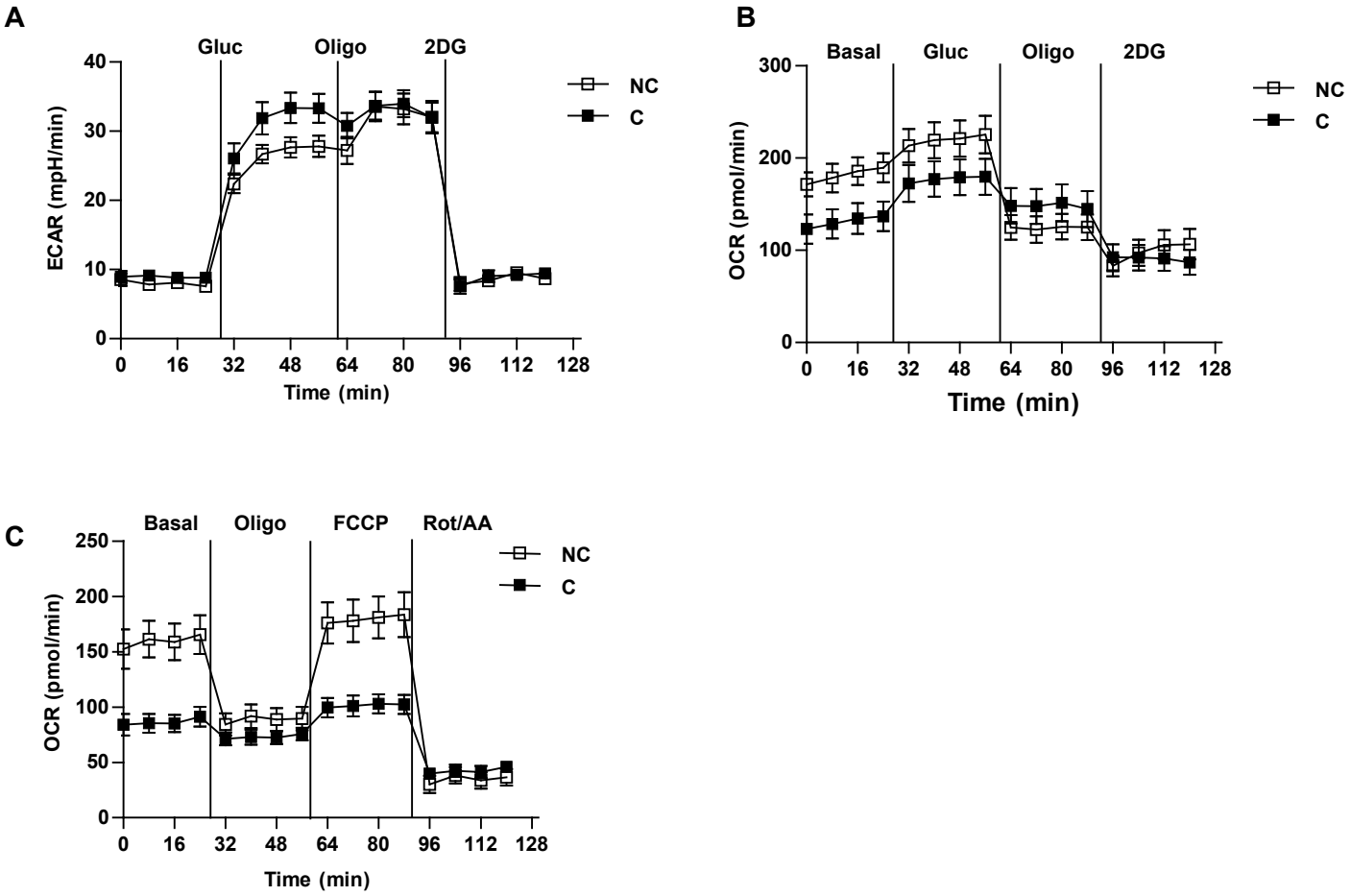
Supplementary Figure 4. Etomoxir efficiently inhibits fatty acid oxidation (FAO) in the ZR-75-1 cell line ZR-75-1 cells were treated or not with increasing doses of Etomoxir (1 to 30µM). Complete FAO was assayed as described in Material and Methods (n=3-4). Bars and error flags represent means ± SEM; the statistical significance of differences between means of complete FAO in treated versus untreated (NT) was evaluated with Mann-whitney U test, * $p < 0.05$.



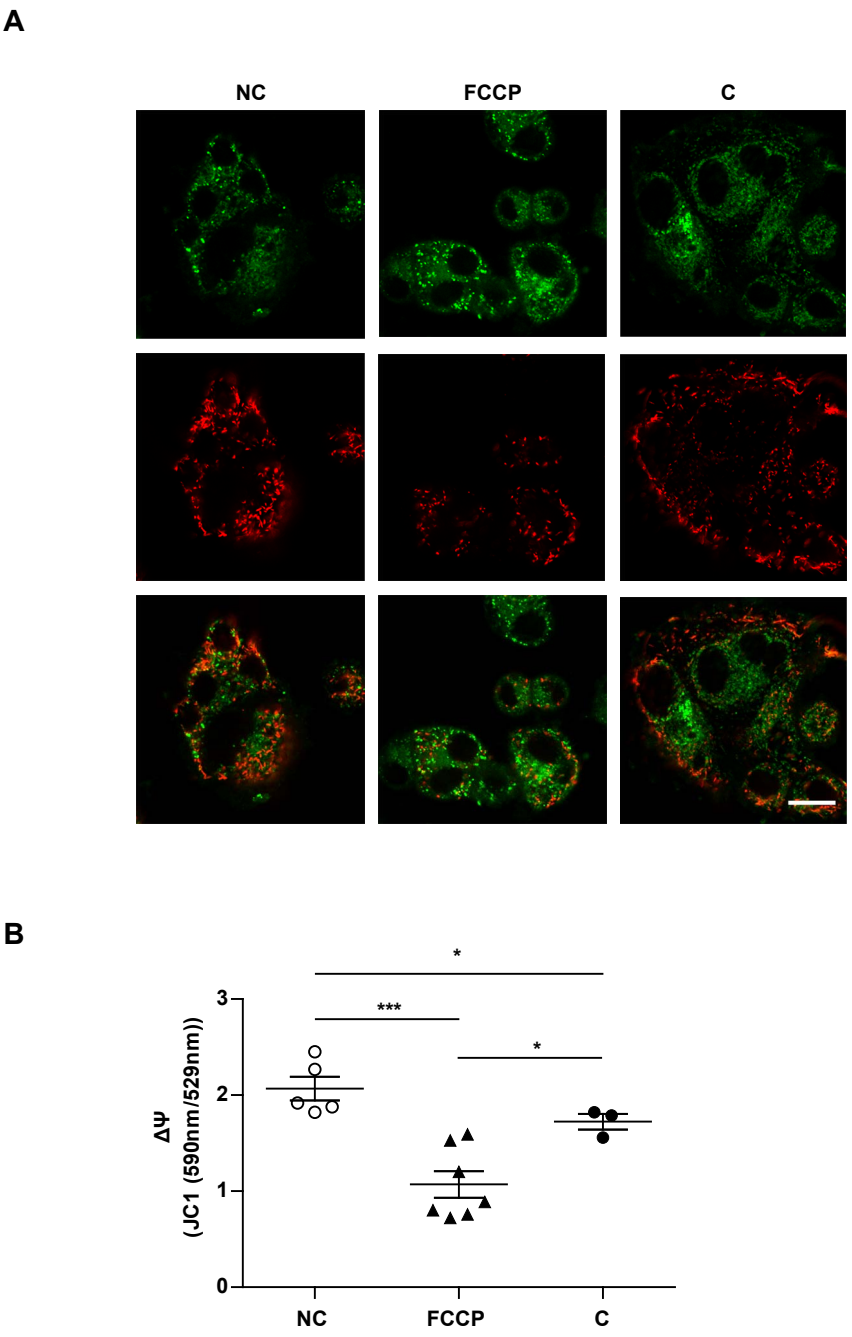
Supplementary Figure 5. Etomoxir increases triglyceride (TG) content in cocultivated SUM159PT tumor cells SUM159PT cells were cocultivated (C) or not (NC) in the presence of adipocytes, and cocultivated cells were treated or not with Etomoxir (1 or 30µM) for 3 days. After this incubation period, TG content was assayed in tumor cells (n=6). Bars and error flags represent means ± SEM; the statistical significance of difference between means of TG content in cocultivated treated or not versus non-cocultivated was evaluated with Mann-whitney U test, ** $p < 0.01$.



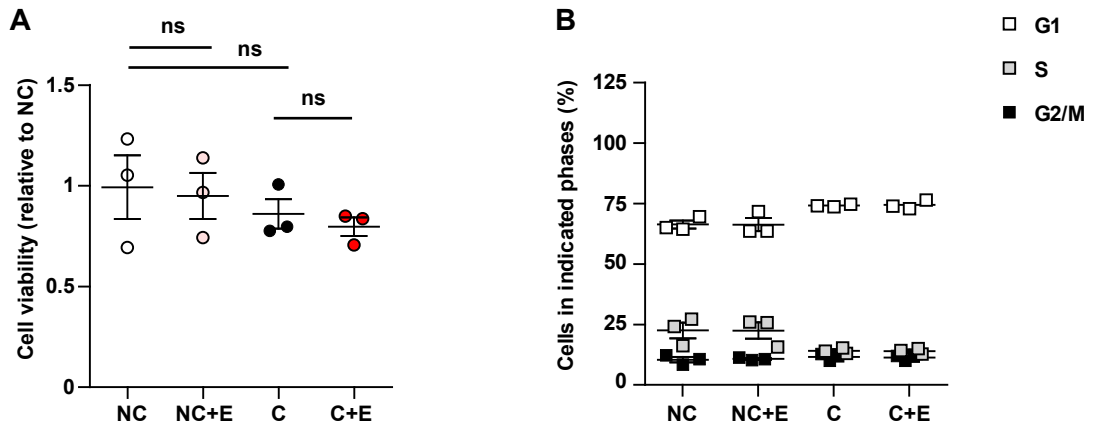
Supplementary Figure 6. Pathways controlling mitochondrial number and activity are upregulated in cocultivated SUM195PT cells SUM159PT cells were cocultivated (C) or not (NC) in the presence of adipocytes for 3 days. **(A-C)** Quantification of the mRNA levels of indicated genes. The level of expression of NC cells was arbitrarily set at 1 (n=3). **(D)** The expression of the PPARγ coactivator 1α (*PGC1*A) in ZR-75-1 and SUM159PT (SUM) cells. The level of *PGC1*A expression in non-cocultivated ZR-75-1 cells was arbitrarily set at 1 (n=3-4). **(E)** The quantity of mitochondrial DNA, measured by qPCR, in SUM159PT cells is expressed as the ratio of mitochondrial *COX1* (cytochrome c sub-unit 1) to genomic (Cyclophilin 1 and *YWHAZ*) DNA levels (n=3). Bars and error flags represent means ± SEM; statistically significant by Student's t-test **(A-C and E)** and Mann-whitney U test **(D)**, ** $p < 0.01$, *** $p < 0.001$, ns: not significant.



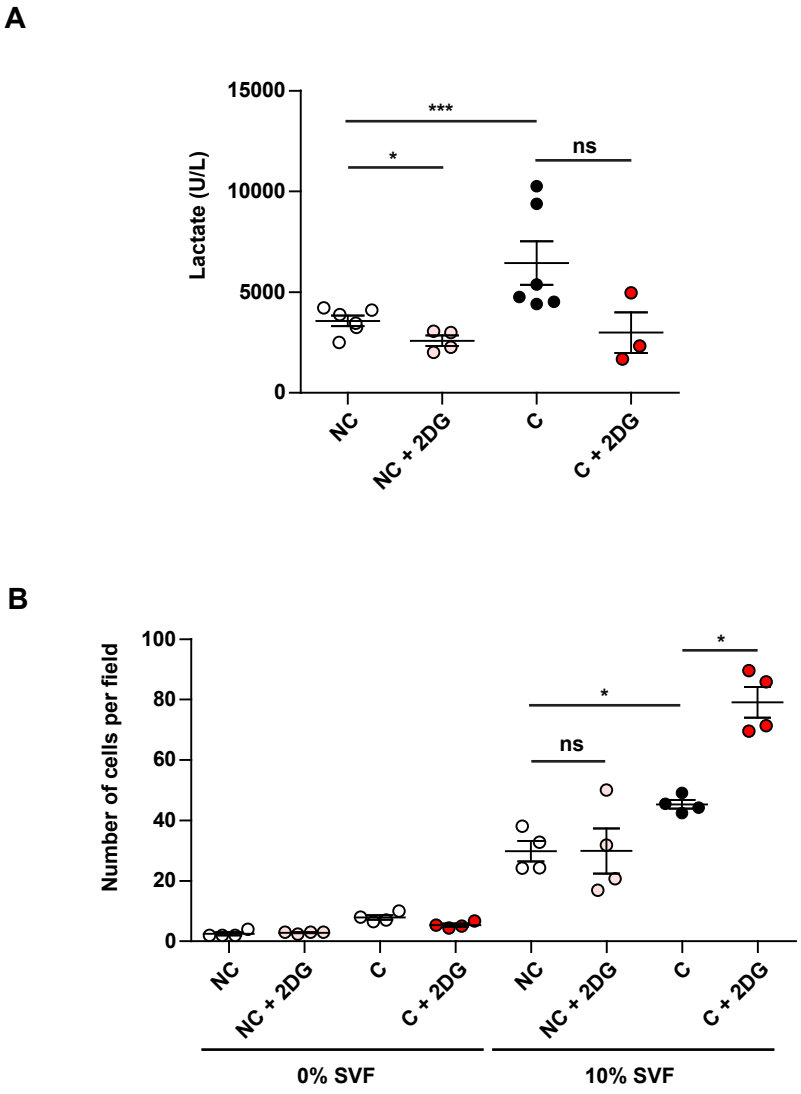
Supplementary Figure 7. Cocultivated ZR-75-1 cells display increased anaerobic glycolysis and uncoupled FAO (A) Extracellular acidification rate (ECAR) was measured in non-cocultivated (NC) and co-cultivated (C) ZR cells before and after addition of 10mM glucose (gluc), 5µM oligomycin (oligo) and 50mM 2-deoxyglucose (2DG) (n=10-11). **(B)** Oxygen consumption rate (OCR) was measured in the absence of glucose (basal condition) and after the addition 10mM glucose (gluc), 5µM oligomycin (Oligo) and 50mM 2-deoxyglucose (2DG) in NC and C ZR cells (n=10-11). **(C)** Oxygen consumption rate (OCR) was measured in the basal state (1mM palmitate complexed with 0.2mM BSA) or after injection of 5µM oligomycin (oligo), 1µM (2-[2-[4-(trifluoromethoxy)phenyl]hydrazinylidene]-propanedinitrile) (FCCP) and 5µM rotenone plus 5µM antimycin A (Rot/AA) in NC and C ZR cells (n=9-11). Bars and error flags represent means ± SEM.



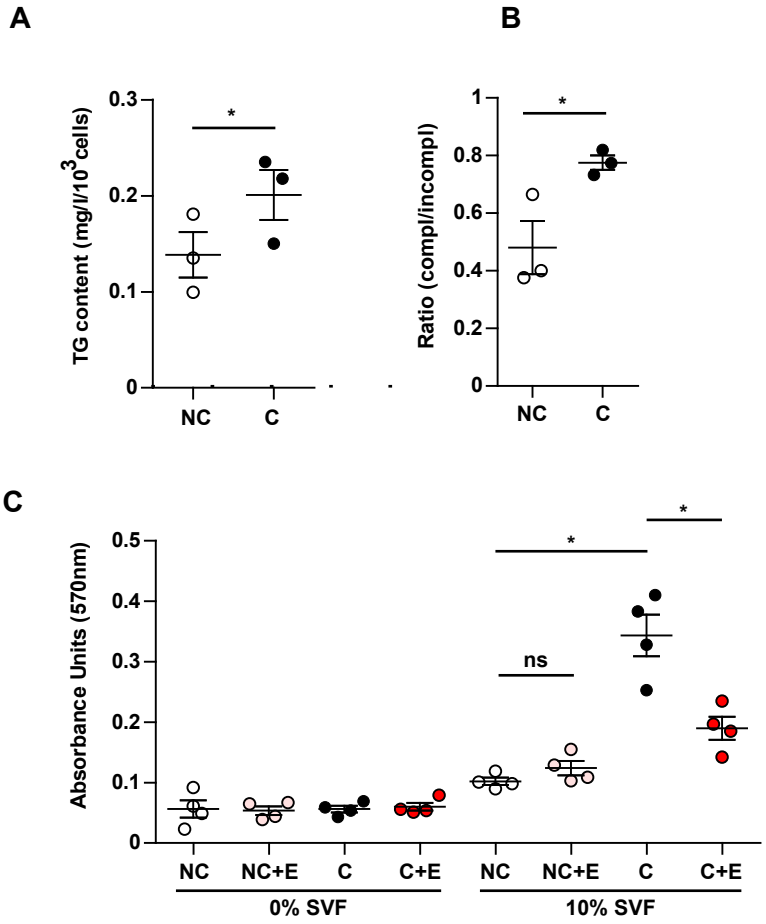
Supplementary Figure 8. Cocultivated ZR-75-1 cells exhibit decreased mitochondrial membrane potential (ΔΨ) ZR-75-1 cells were cocultivated (C) or not (NC) in the presence of 3T3-F442A mature adipocytes. 30µM FCCP (2-[2-[4-(trifluoromethoxy)phenyl]hydrazinylidene]-propanedinitrile) was used as a control of decreased ΔΨ. Cells were stained with the mitochondrial membrane potential probe, JC-1, and fluorescence was analyzed by confocal microscopy (scale bar: 20µm) **(A)** and with the Clariostar Monochromator Microplate Reader (excitation 488 nm; emission 529 and 590 nm). The ratio of fluorescence at 590 nm (in red), representative of high mitochondrial polarization, versus that at 529 nm (in green), representative of mitochondrial depolarization, was calculated to determine the ΔΨ (n=3-7) **(B)**. **(A)** At least 3 experiments were conducted and representative images are shown. **(B)** Bars and error flags represent means ± SEM; statistically significant by Mann-whitney U test, * $p < 0.05$, *** $p < 0.001$.



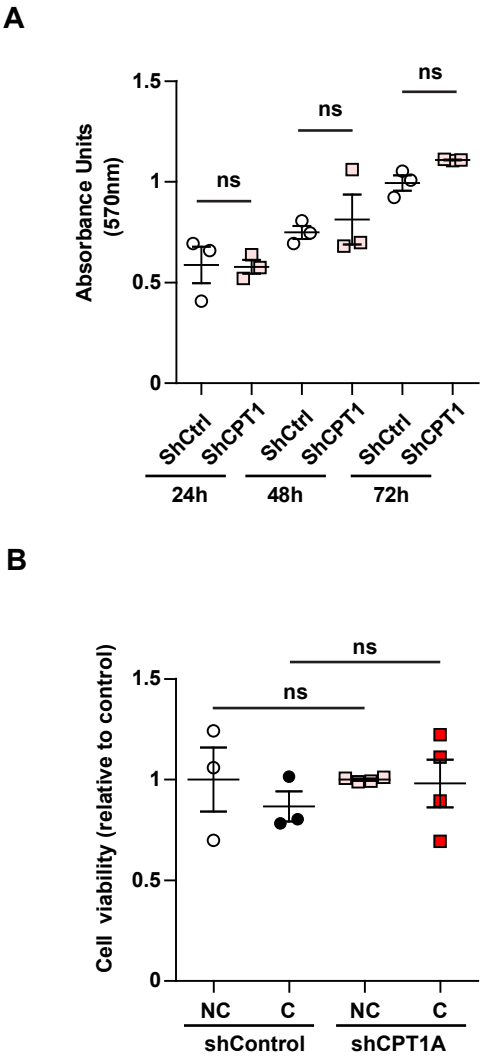
Supplementary Figure 9. Etomoxir has no effect on breast tumor cell growth $1x10^5$ ZR-75-1 cells were grown for 3 days on Transwells in the presence (C) or not (NC) of mature adipocytes and treated (+E) or not with Etomoxir (30µM). **(A)** Viable cells were stained by toluidine blue, dissolved with lysis buffer and the quantity of viable cells was evaluated by colorimetric absorbance (570nm). Results were arbitrarily set at 1 for untreated NC cells (n=3). **(B)** Cells were stained with propidium iodide and the distribution of cells in each cell cycle phase was analyzed by flow cytometry (n=3). Bars and error flags represent means ± SEM; statistically not significant by Mann-whitney U test (ns).



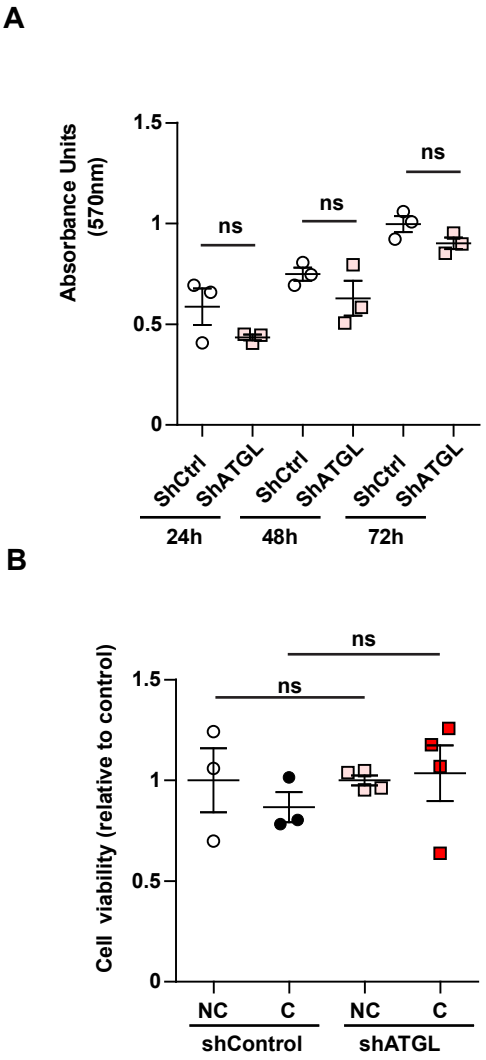
Supplementary Figure 10. 2-deoxyglucose (2DG) stimulates the invasive properties of breast tumor cells cocultivated with adipocytes 1x10^5 ZR-75-1 cells were grown for 3 days on Transwells in the presence (C) or not (NC) of mature adipocytes and treated (+2DG, 3mM) or not with 2DG. **(A)** Cell supernatants were collected and lactate release was measured (n=3-6). **(B)** Cells were used for Matrigel invasion assays against medium containing either 0% or 10% FCS (n=4). Bars and error flags represent means ± SEM; statistically significant by Mann-whitney U test, * $p < 0.05$, *** $p < 0.001$, ns: not significant.



Supplementary Figure 11. In the presence of adipocytes, the murine TS/A cell line exhibits metabolic remodeling and increased invasive capacities (A) Triglyceride (TG) content (n=3) and **(B)** the complete to incomplete ratio of fatty acid oxidation (FAO) in TS/A cells cocultivated (C) or not (NC) with adipocytes (n=3). **(C)** Invasion assays were performed in cells cocultivated (C) or not (NC) with adipocytes and treated (+E) or not with Etomoxir (n=4). Bars and error flags represent means ± SEM; statistically significant by Student's t-test **(A and B)** and Mann-whitney U test **(C)**, * $p < 0.05$, ns: not significant.



Supplementary Figure 12. Down-regulation of carnitine palmitoyltransferase 1A (CPT1A) expression in breast cancer cells does not affect cell proliferation and viability (A) $4x10^3$ ZR-75-1 cells transfected with either a control vector (shCtrl) or shRNA directed against *CPT1A* (shCPT1A) were plated in quadruplicate for each time point in 96 well plates. The number of viable cells was measured by MTT assay at indicated time points. The results are expressed as mean optical density obtained (n=3). **(B)** $1x10^5$ ZR-75-1 stably transfected with shControl or shCPT1A vectors were grown on Transwells in the presence (C) or not (NC) of mature adipocytes and the cell number was evaluated after 3 days as described in material and methods. Results were arbitrarily set at 1 for control cells (non cocultivated shControl cells) (n=3-4). Bars and error flags represent means ± SEM; statistically not significant by Mann-whitney U test (ns).



Supplementary Figure 13. Down-regulation of adipose triglyceride lipase (ATGL) expression in breast cancer cells does not affect cell proliferation and viability (A) $4x10^3$ ZR-75-1 cells transfected with either a control vector (shCtrl) or a small hairpin directed against *ATGL* (shATGL) were plated in quadruplicate for each time point in 96 well plates. The number of viable cells was measured by MTT assay at indicated time points. The results are expressed as mean optical density obtained (n=3). **(B)** $1x10^5$ ZR-75-1 stably transfected with shControl or shATGL vectors were grown on Transwells in the presence (C) or not (NC) of mature adipocytes and the cell number was evaluated after 3 days as described in material and methods. Results were arbitrarily set at 1 for control cells (non cocultivated shControl cells) (n=3-4). Bars and error flags represent means ± SEM; statistically not significant by Mann-whitney U test (ns).