Supplementary information

Antibodies. The rabbit polyclonal anti-vimentin, and anti-α tubulin (clone DM1A) mAbs were obtained from Neomarkers. The rabbit polyclonal antibody directed against Hormone-Sensitive Lipase (HSL) is a kind gift of Dr D. Langin (INSERM U858). The anti-β-catenin mAb (clone 14/beta-catenin) was from BD Biosciences. The Anti-E-cadherin mAb (clone HECD-1) was from Calbiochem. The mAb directed against N-Cadherin (3B9) was from Invitrogen as well as rhodamine-phalloidin used to detect polymerized actin. Anti-MMP-11 antibody (MAb 5ST-4A9) is a king gift of Dr MC Rio (IGBMC, Strasbourg, France). The mAb directed against human adiponectin (HADI 773) was from Alexis biochemicals, and the goat antibody directed against human IL-6 used in immunohistochemistry was from R&D. Neutralizing goat antibody directed against murine IL-6 was from R&D and the IgG from goat serum used as a control was from Sigma. Secondary antibodies (for immunofluorescence) were Goat anti-mouse or goat anti-rabbit IgG $F(ab')_2$ fragment AlexaFluor-488 conjugated (obtained from Invitrogen). TO-PRO-3 was used as a final 1µM concentration and was from obtained from Invitrogen.

Cell proliferation assay. Tumor cells (5 X 10^4 cells for SUM159PT or 67NR, 1.5 x 10^5 cells for 4T1 or 3 x 10^5 cells for ZR75.1 cells) were seeded in the upper chamber of the Transwell system in the culture medium of adipocytes and were cocultivated or not with adipocytes for 3 days. After 3 days, tumor cells were trypsinized and counted using a Mallassez counting chamber.

Immunofluorescence and confocal microscopy.
Cells seeded on glass coverslips were fixed with 3.7% paraformaldehyde (PFA) solution for 20 min at room temperature. PFA was quenched with 50 mM NH4Cl and cells were permeabilized with PBS-0.2% TritonX-100 for 5 min. After blocking with PBS containing 10% FCS for 60 min, cells were incubated with primary antibody in PBS-2% FCS for 2h. Cells were then washed three times with PBS-2% FCS and incubated with secondary antibody and TO-PRO-3 for 30 min. Actin filaments were stained with rhodamin-phalloidin for 30 min. After three washes with PBS, cells were mounted in Vectashield medium (Abcys). Fluorescence images were acquired with a confocal laser microscopy system (Leica SP2). The image resolution was 512 x 512 pixels and scan rate was 400 Hz. For each sample, over 100 cells were examined in at least three independent experiments.

RNA extraction and quantitative RT-PCR (qPCR). 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 et al., 2006) . Total RNAs (1 µg) 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 25 ng of cDNA and the indicated concentrations of both sense and antisense primers (presented in Table S3) in a final volume of 25 µl using the SYBR Green TaqMan Universal PCR master mix (Applied Biosystems, Foster City, CA). Fluorescence was monitored and analyzed in a GeneAmp 7300 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

(100 nM) or GAPDH RNA (100nM) to normalize gene expression, using geNorm Software. Analysis of hMMP-11, hE-cadherin and hN-Cadherin gene expression was performed using a TaqMan assay kit (Applied Biosystem). Analysis of oligonucleotide primers were designed using the Primer Express software (PerkinElmer Life Sciences) as previously described (Daviaud et al., 2006).

Coculture, migration and invasion assays. For coculture with preadipocytes, $3x10^5$ ZR 75.1 cells were seeded in the upper chamber of the Transwell system (0.4μM pore size, Millipore) in the culture medium of preadipocytes and were cocultivated or not for 3 days with preadipocytes (initially seeded at $6x10^4$ cells/wells). After three days, breast cancer cells were trypsinized and used for invasion assays (Monferran et al., 2004). In certain experiments, ZR 75.1 cells were cocultivated with mammary adipocytes obtained from the *ex vivo* differentiation of adipose progenitors present in the SVF fraction of normal mammary adipose tissue (see below). To evaluate the effect of tumor-conditioned medium on adipocyte phenotype (SUM159PT-cm), exponentially growing SUM159PT cells were incubated overnight in serum-free media in the presence of 0.5% delipidated BSA. The culture medium was collected and centrifuged at 1000 rpm for 10 minutes. After 3 days in culture alone, in the presence of SUM159PT cells or SUM159PT-cm (complemented with 10 % serum), mRNA were extracted from adipocytes to perform qPCR experiments.

Obtention of mammary adipocytes. Breast adipose tissue samples were collected from reduction mammoplasty according to the guidelines of the Ethical Committee of Toulouse-Rangueil. All subjects gave their informed consent to participate to the study, and investigations were performed in accordance with the declaration of Helsinki as revised in 2000. Adipose tissue pieces were immediately used for collagenase digestion as previously described (Daviaud et al., 2006) and centrifuged to separate adipocytes from the stroma-vascular fraction (pellet, SVF). The SVF fraction issued from mammoplasty reduction adipose tissue samples was used for *ex vivo* differentiation as previously described (Bour et al., 2007). After 10 to 14 days of culture in lipogenic medium, mammary adipocytes were used for coculture with tumor cells in the experimental conditions described for murine adipocytes in the Material and Methods Section of the manuscript.

References

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