Supplementary information

Cell Culture. The breast cancer cell line ZR 75.1 (gift of Pr K. Bystricky, LBME, Toulouse, France) was cultured in DMEM medium supplemented with 10% FCS. HMT-3522-T4-2 mammary epithelial cells (gift of Dr E. Liaudet-Coopman, IRCM, Montpellier, France) were cultured in H14 medium (Weaver et al, 1997) consisting in DMEM/F12 with 250 ng/ml insulin, 10 mg/ml transferrin, 2.6 ng/ml sodium selenite, 10^{-10} M estradiol and 1.4 $x10^{-6}$ M hydrocortisone. The breast cancer cell line MDA-MB231 (gift of Pr K. Bystricky, LBME, Toulouse, France) was cultured in RPMI medium supplemented with 10% FCS.

Antibodies. The polyclonal Ab directed against Adiponectin (APN) was obtained from Thermoscientic. The polyclonal Ab directed against Horomono-Sesitive Lipase (HSL) was from Cell Signaling. Both the polyclonal Ab that recognized CEBP/α and the monoclonal Ab against PPARγ (clone E-8) were from Santa-Cruz.

Ex-vivo differentiation of human 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 oh 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). Briefly, the stromal vascular pellets were incubated overnight with DMEM/Ham F12 medium supplemented with 10% FCS. Then, the medium was replaced by an adipogenic induction medium (DMEM/Ham's F12 (1:1) medium supplemented with 1µg/ml ciglitazone, 10 mg/ml transferrin, 33 mM biotin, 66 mM insulin, 1 nM triiodothyronine, and 17 mM pantothenate). Cells were cultivated in this condition during 3 days before the ciglitazone was removed. Cells are differentiated after 10 days of culture.

Cell cycle. Adipocytes were cocultivated or not with SUM159PT cell lines during 3 or 5 days. Cells were harvested by trypsinization and pelleted by centrifugation. The pellets were incubated 10 minutes on ice, then resuspended in 300 µl phosphate-buffered saline containing 25 µg/ml propidium iodide (PI), 100 µg/ml RNaseA and 0.01 % triton X-100. After staining, 10 000 cells were analysed on a FACScan flow cytometer utilizing CellQuest software (Becton Dickinson) to determine the cell cycle distribution.

References

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