

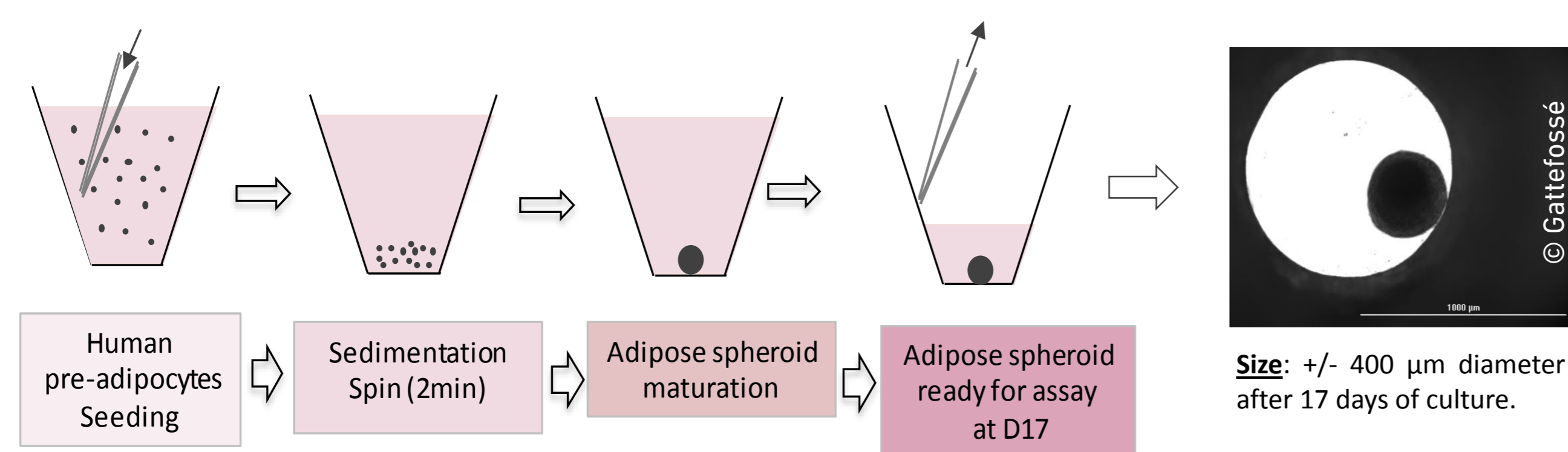
INTRODUCTION

Adipose tissue plays a critical role in energy balance and lipid metabolism. Disorders of adipose function can lead, amongst others, to type II diabetes, obesity and unsightly conditions such as cellulite. Monolayer cell culture systems have proven useful to understand many aspects of fat biology, but these 2D *in vitro* systems do not reflect the complexity of human adipose tissue.

To overcome this limitation, we took advantage of the ability of pre-adipocytes to form spheroids when cultured in ultra-low attachment systems. Therefore, the goal of our study was first to develop adipose spheroids, and then to validate their lipolysis responsiveness, when stimulated with reference molecules.

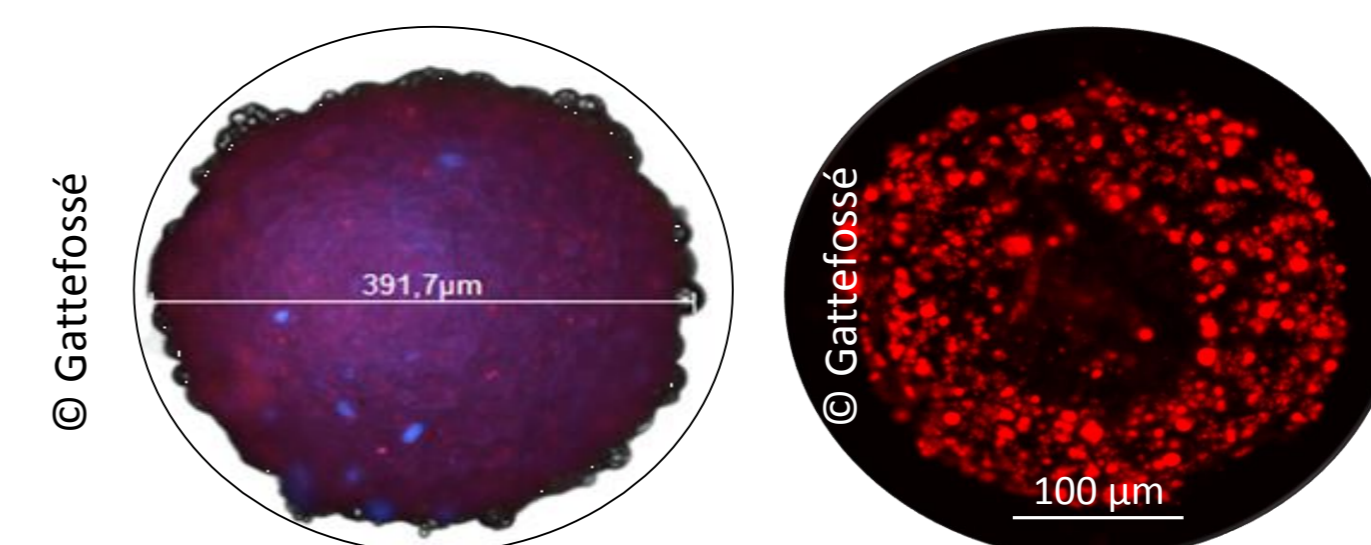
3D ADIPOSE SPHEROIDS FORMATION

GravityTRAP ULA™ 96-well plate (Microtissue Culture and Assay Platform, PerkinElmer)



Gattefossé has developed its own **in-house method** to reconstruct **3D adipose spheroids** from human pre-adipocytes cultured for 17 days:

- Spheroids **grow in size** as the pre-adipocytes mature into adipocytes
- Spheroids **accumulate large lipid droplets** within the thickness of the microtissue
- Spheroids **respond to caffeine**, a gold standard having slimming effects



SLIMMING EFFECT IN ADIPOSE MICROTISSUE

Development of the adipose spheroids model: RESPONSE TO TNF- α – Oil red O staining

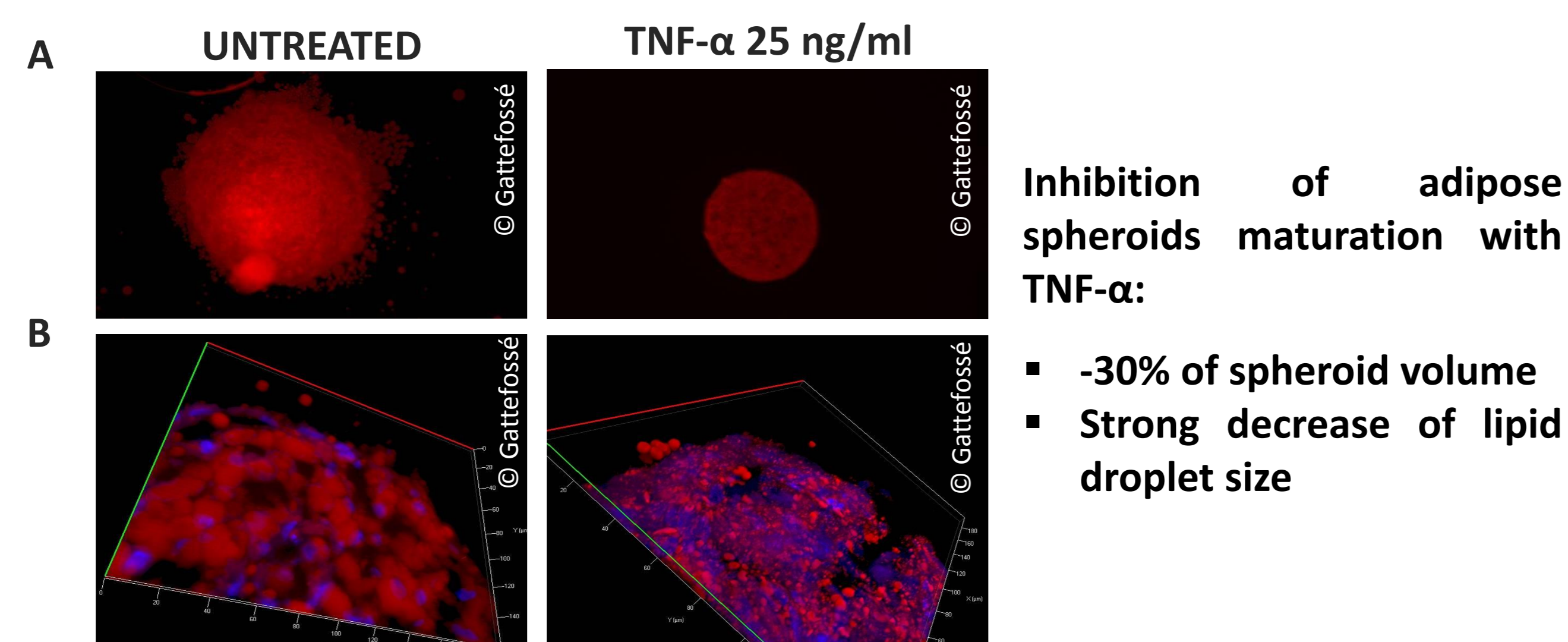


Figure 1: Oil Red O staining of spheroids treated with TNF- α 25 ng/ml during microtissues differentiation and maturation steps, i.e. 10 days of treatment. A. Analysis of whole-spheroids volume, objective x10. B. Analysis of lipid droplet size on spheroids sections, 3D view, objective x63.

Efficacy test (1): SIZE of adipose spheroids - Diameter measurement

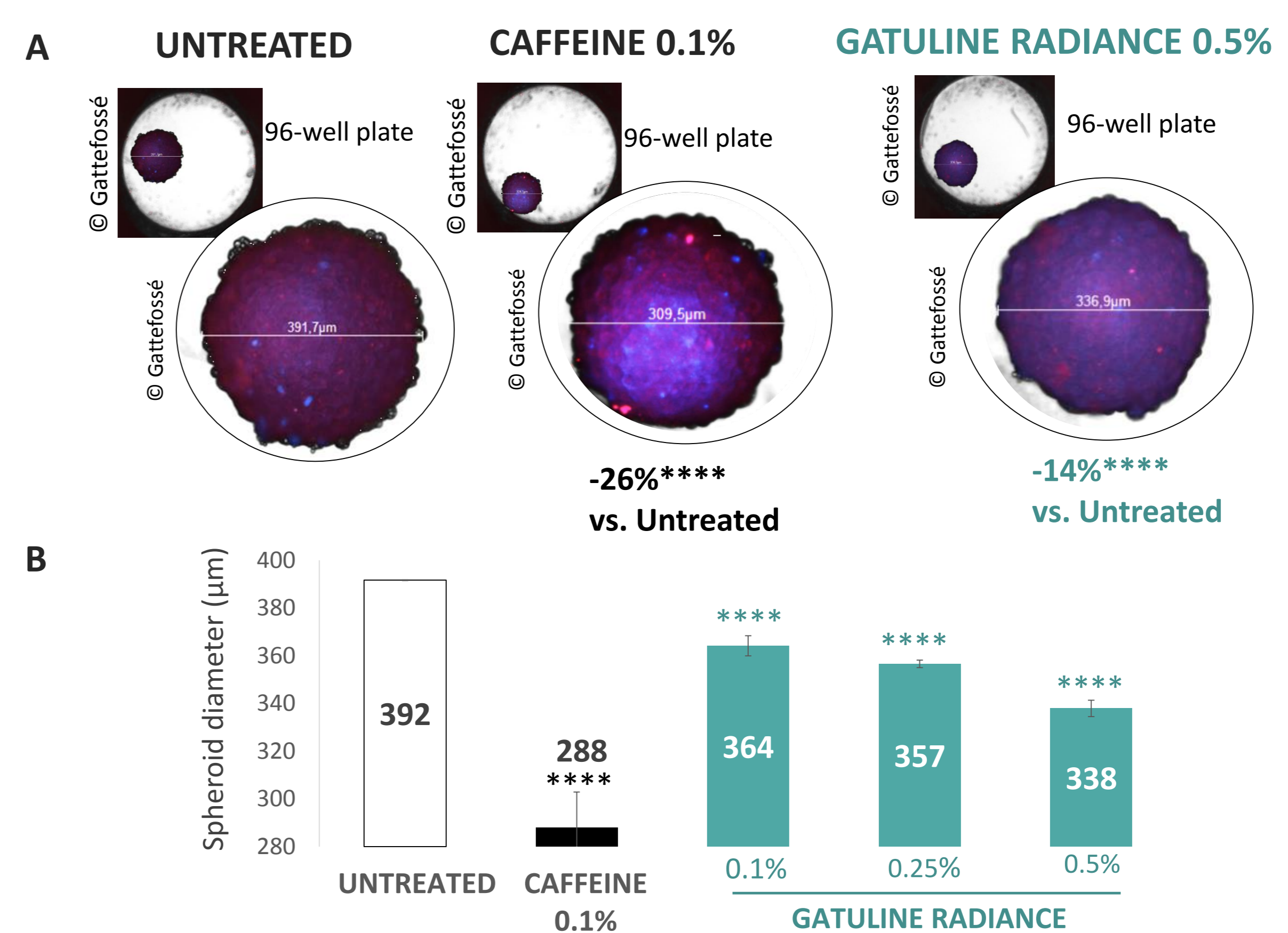
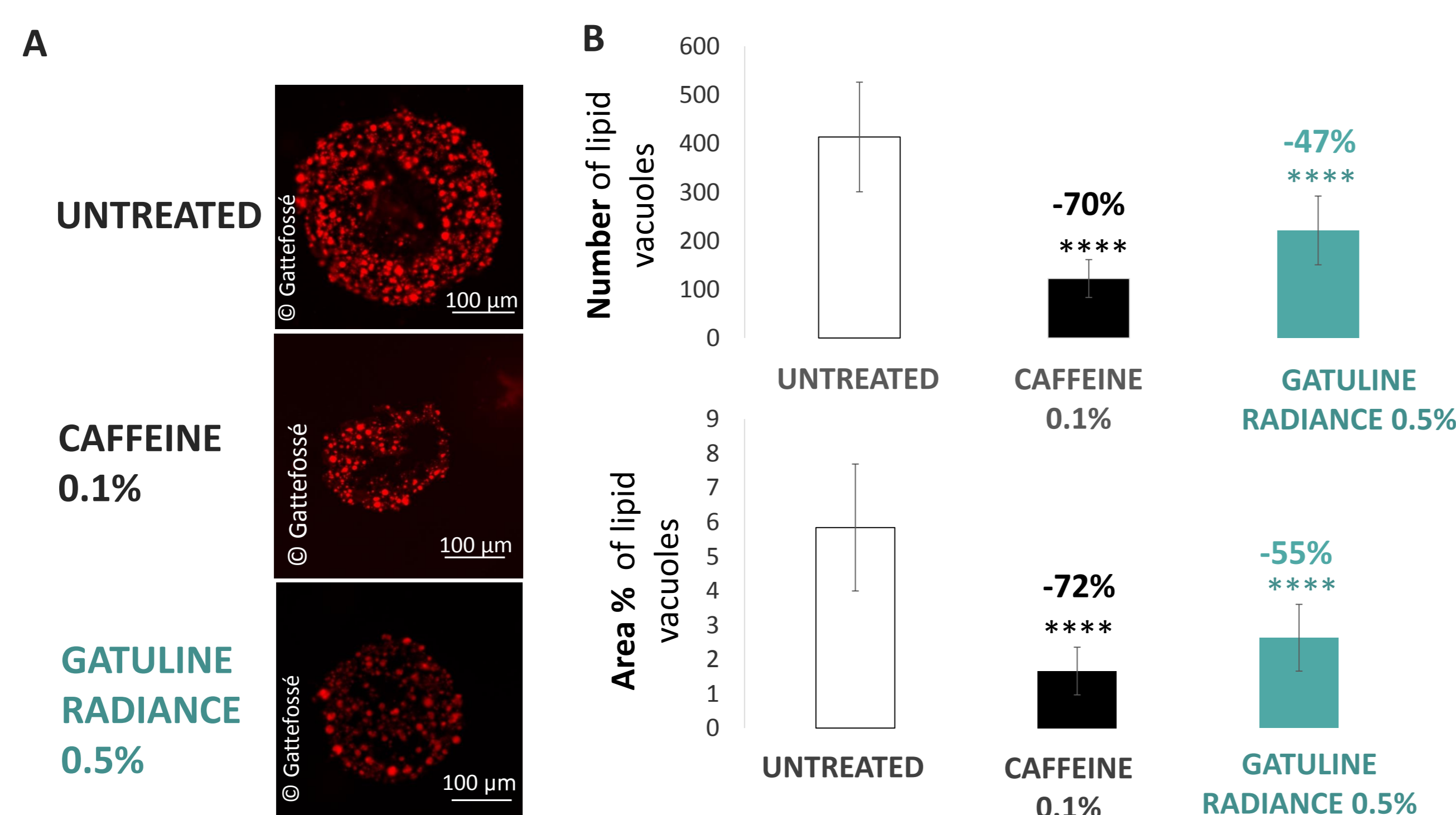


Figure 2: Analyses of adipose spheroids volume treated with Gatuline Radiance 0.5% and caffeine 0.1% for 7 days (from D7 to D14). A. Nile Red Fluorescence imaging of whole-spheroids cultivated in 96-well plate. Diameter of spheroids were measured for each condition. B. Analysis of spheroids diameter from the fluorescent images. Data represents Mean \pm SEM of 2 independent experiments (n=6). Non parametric statistical analyses were performed with the two-way ANOVA test followed Bonferroni multiple comparison test. The level of significance is given by the p-value:****p \leq 0,0001.

Efficacy test (2): LIPID VACUOLES in adipose spheroids – Nile red staining



Gatuline Radiance 0.5% decreased the number and the area of lipid vacuoles in adipose spheroids.

Figure 3: Analyses of lipid vacuoles in adipose spheroids treated with Gatuline Radiance 0.5% and caffeine 0.1% for 6 days (from D7 to D13). A. Analysis of lipid vacuoles by Nile Red solution on spheroids sections. Scale bar: 100µm. B. Evaluation of lipid vacuoles number and area from images of spheroids sections. Data represents Mean \pm SEM of 2 independent experiments (n=6). Parametric statistical analyses were performed with the one-way ANOVA test followed by Dunnett multiple comparison test. The level of significance is given by the p-value:****p \leq 0,0001.

Efficacy test (3): LIPOLYSIS in adipose spheroids – Non-esterified Free Fatty Acids release

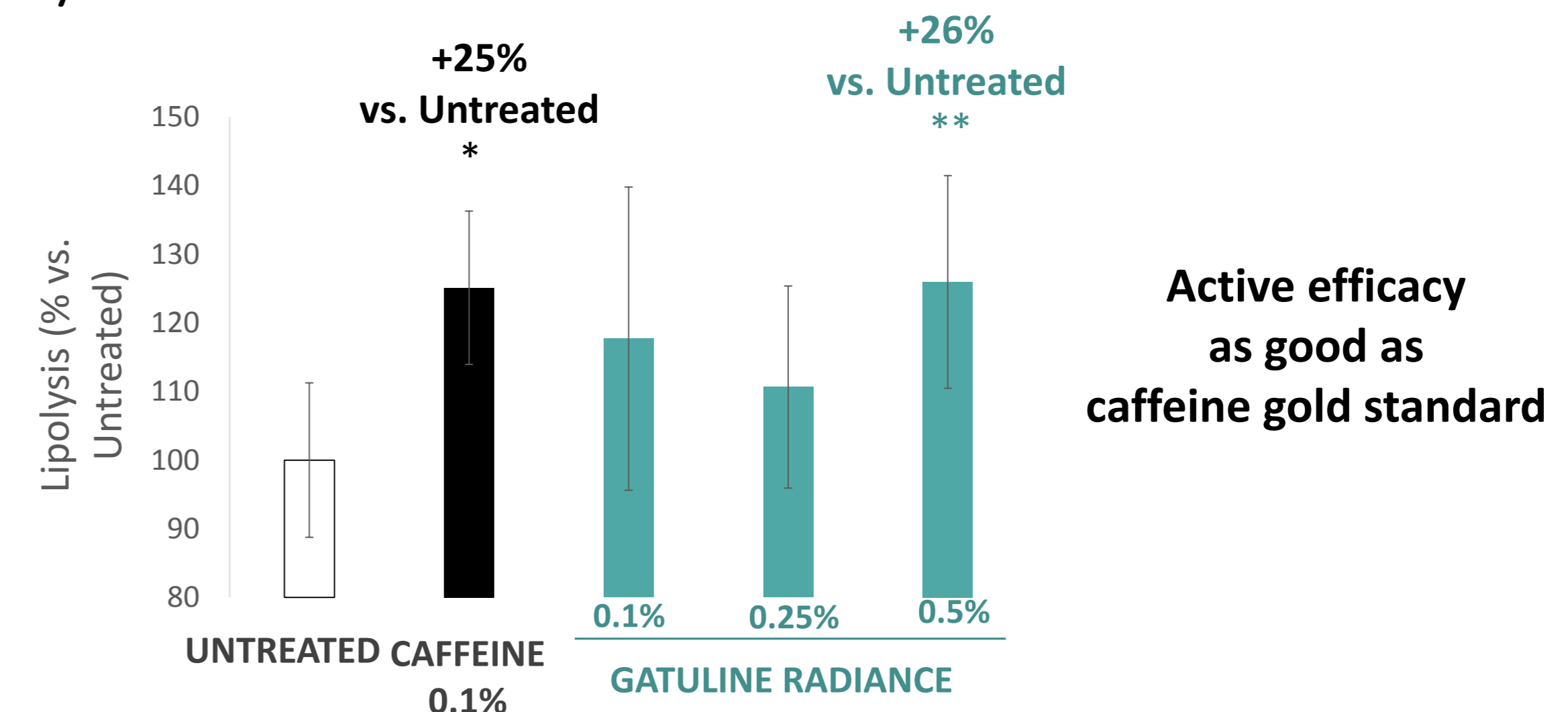


Figure 4: Quantitative determination of non-esterified fatty acids in supernatants of adipose spheroids cultures treated with Gatuline Radiance (0.1-0.5%) and caffeine 0.1% for 5h. Data represents Mean \pm SEM of 2 independent experiments (n=6). Parametric statistical analyses were performed with the one-way ANOVA test followed by Bonferroni multiple comparison test. The level of significance is given by the p-value: *p \leq 0,1; **p \leq 0,01.

CONCLUSION

Adipose spheroids provide an interesting *in vitro* model of pre-adipocyte maturation and adipocyte expansion. They represent relevant *in vitro* assays to study adipogenesis, adipose tissue dysfunction and evaluate pharmacological agents.

Gatuline Radiance displays a global slimming properties on these 3D advanced models of adipose microtissue, highlighted at different levels: tissue (decreasing size of the adipose microtissue), cellular (decreasing number and size of lipid vacuoles) and molecular (increasing release of non-esterified free fatty acid).