

## SUPPLEMENTARY DATA

### Supplementary methods: Transcriptomic analysis

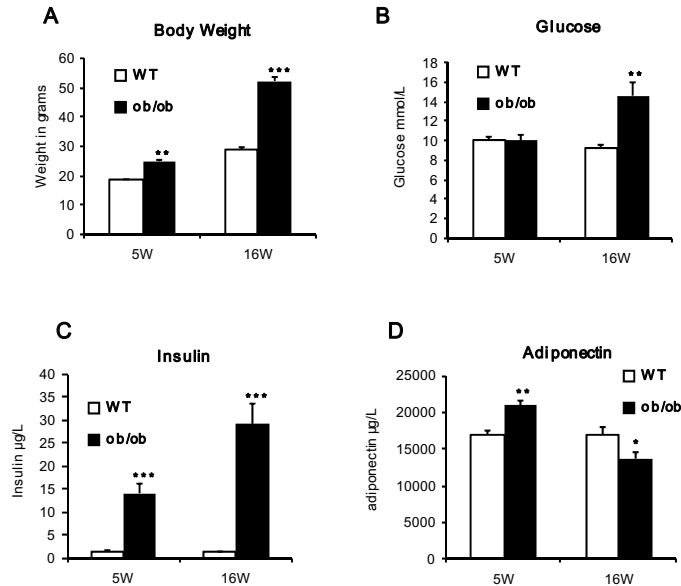
Differential gene expression was carried out using the *limma* (1) package from Bioconductor (<http://bioconductor.org/>). Limma methodology implements a moderated t-statistic which is computed for each gene; p-values and fold changes are also provided by the methodology. For each time point (5 weeks and 16 weeks) ob/ob mice were compared to WT; positive moderated t-statistics indicate overexpression of the gene in ob/ob mice; negative t-statistics indicate overexpression in the wild type animals. Multiple testing adjustment of p-values was done according to Benjamini and Hochberg (2). Genes were termed 'differentially expressed' if they had an adjusted p-value smaller than 0.05. Gene set analysis was carried out for the Gene Ontology Biological Process and KEGG pathways using logistic regression (3, 4) as implemented in Babelomics (<http://babelomics.org/>) (5). This methodology ranks genes according to their differential expression using the moderated t-statistic and searches for functional blocks (GOs or KEGGs) associated to the genes overexpressed in ob/ob mice or in the WT animals. For each functional block, a Log Odds Ratio indicating whether the function is enriched in ob/ob (positive LOR) or WT mice (negative LOR) is provided besides a p-value and a corrected p-value to estimate its significance. Functional blocks having a corrected p-value smaller than 0.05 were considered to be enriched in the conditions. GO annotation for the genes in the microarray were taken from Ensembl 55 release (<http://www.ensembl.org/>). The KEGG pathway annotation was directly retrieved from the KEGG web page (<http://www.genome.jp/kegg/>).

### Supplementary references

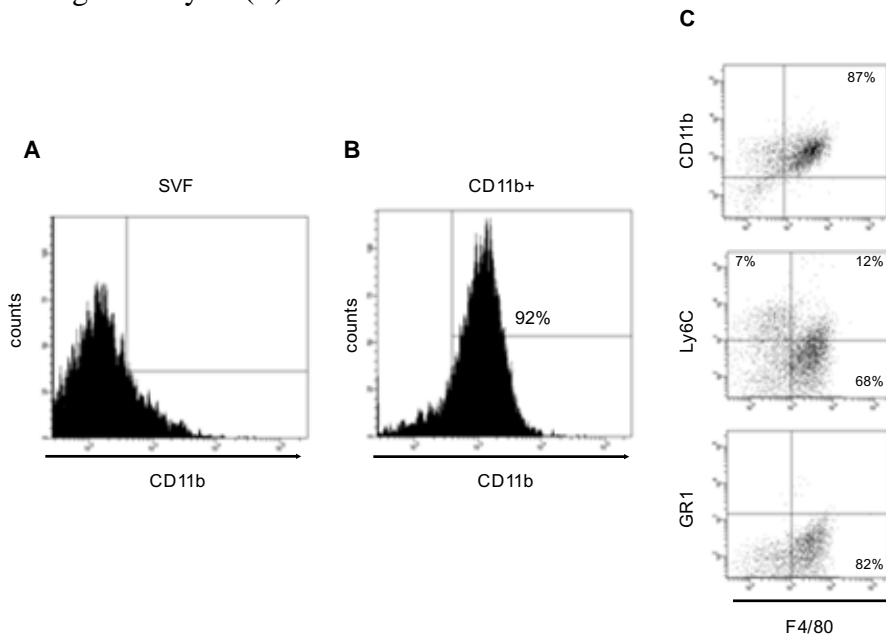
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2. Hochberg Y, Benjamini Y: More powerful procedures for multiple significance testing. *Stat Med* 9:811-818, 1990
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4. Sartor MA, Leikauf GD, Medvedovic M: LRpath: a logistic regression approach for identifying enriched biological groups in gene expression data. *Bioinformatics* 25:211-217, 2009
5. Medina I CJ, Pulido L, Madeira SC, Goetz S, Conesa A, Tárraga J, Pascual-Montano A, Nogales-Cadenas R, Santoyo J, García F, Marbà M, Montaner D, Dopazo J.: Babelomics: an integrative platform for the analysis of transcriptomics, proteomics and genomic data with advanced functional profiling. *Nucleic Acids Res.* 1:Suppl:W210-213. , 2010

SUPPLEMENTARY DATA

**Supplementary Figure 1. Metabolic characterization of 5 and 16 weeks ob/ob versus WT mice.** Body weight in grams (A), glucose levels in mmol/L (B), insulin levels in mg/L and adiponectin in mg/L are evaluated at the end of the experiment. Significant differences are as follows: \*,  $p < 0.05$ ; \*\*,  $p < 0.005$ ; \*\*\*,  $p < 0.001$ .

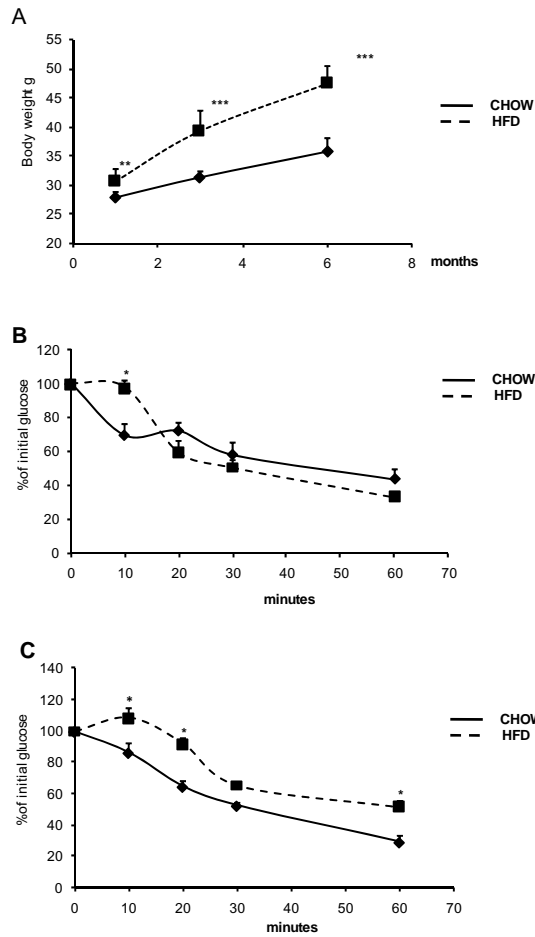


**Supplementary Figure 2. Characterisation of the ATMs fraction.** After isolation, as describe in methods section, ATM fraction were tested for purity with flow cytometry analysis. CD11b positive cells were only present in the positive fraction and not in SVF fraction (A and B). Cells positive for F4/80 were macrophages (C and D). Cells expressing high Ly6C levels were monocytes (D) and cells expressing GR1 were granulocytes (E).

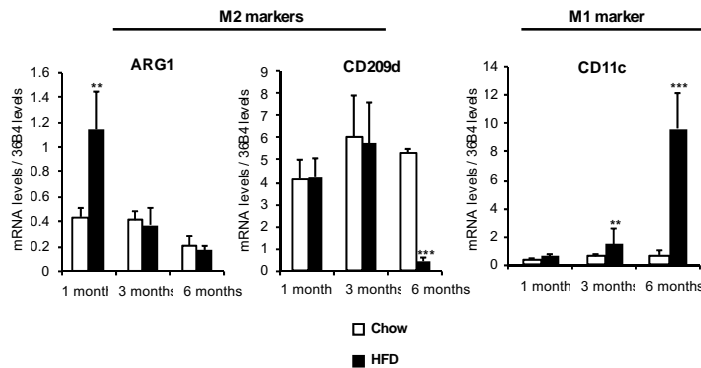


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**Supplementary Figure 3. Metabolic characterization of C57 bl6 mice fed chow or HFD for 1, 3 or 6 months.** Body weights (A) were evaluated at the end of the experiments. Insulin sensitivity was assessed by ITT one week before the end of the experiments, at 3 months (B) and 6 months (C) and represented as percentage of the initial basal glucose levels.

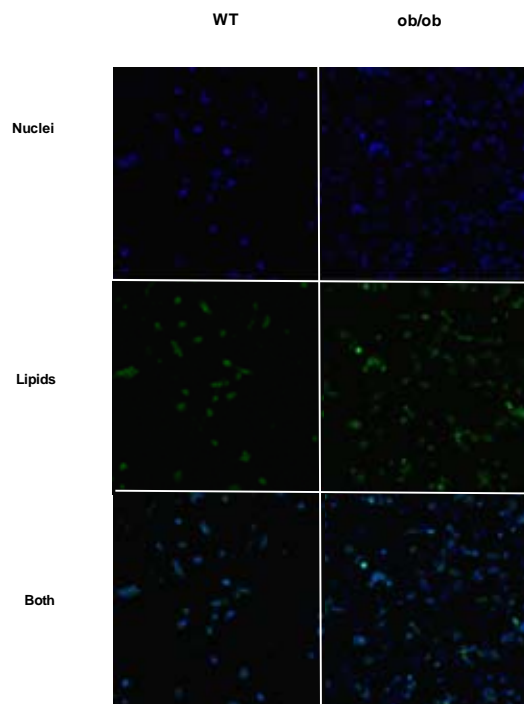


**Supplementary Figure 4. Polarisation profile of ATM from C57 bl6 mice fed chow or HFD for 1, 3 or 6 month.** Gonadal adipose tissue was digested by collagenase and macrophages were isolated from the SVF with CD11b beads. The expression of M2 and M1 markers were evaluated by quantitative PCR using the standard curves method. The expressions levels were normalised by the amount of 36B4. The bars represented the standards errors and significant differences were as follows: \*,  $p < 0.05$ ; \*\*,  $p < 0.005$ ; \*\*\*,  $p < 0.001$ .



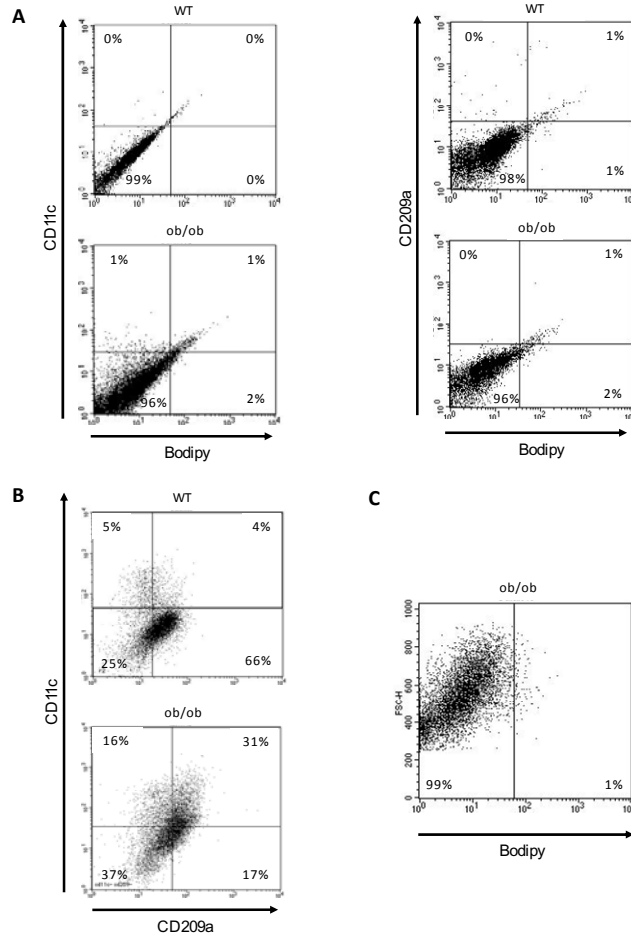
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**Supplementary Figure 5. Analysis of the ATMs lipid content at 5 weeks.** Representative image taken with confocal microscope of ATM isolated from 5 weeks WT or ob/ob. Bodipy (green) detect neutral lipids and Dapi (blue) dye nucleus. The image used a 10X amplification.



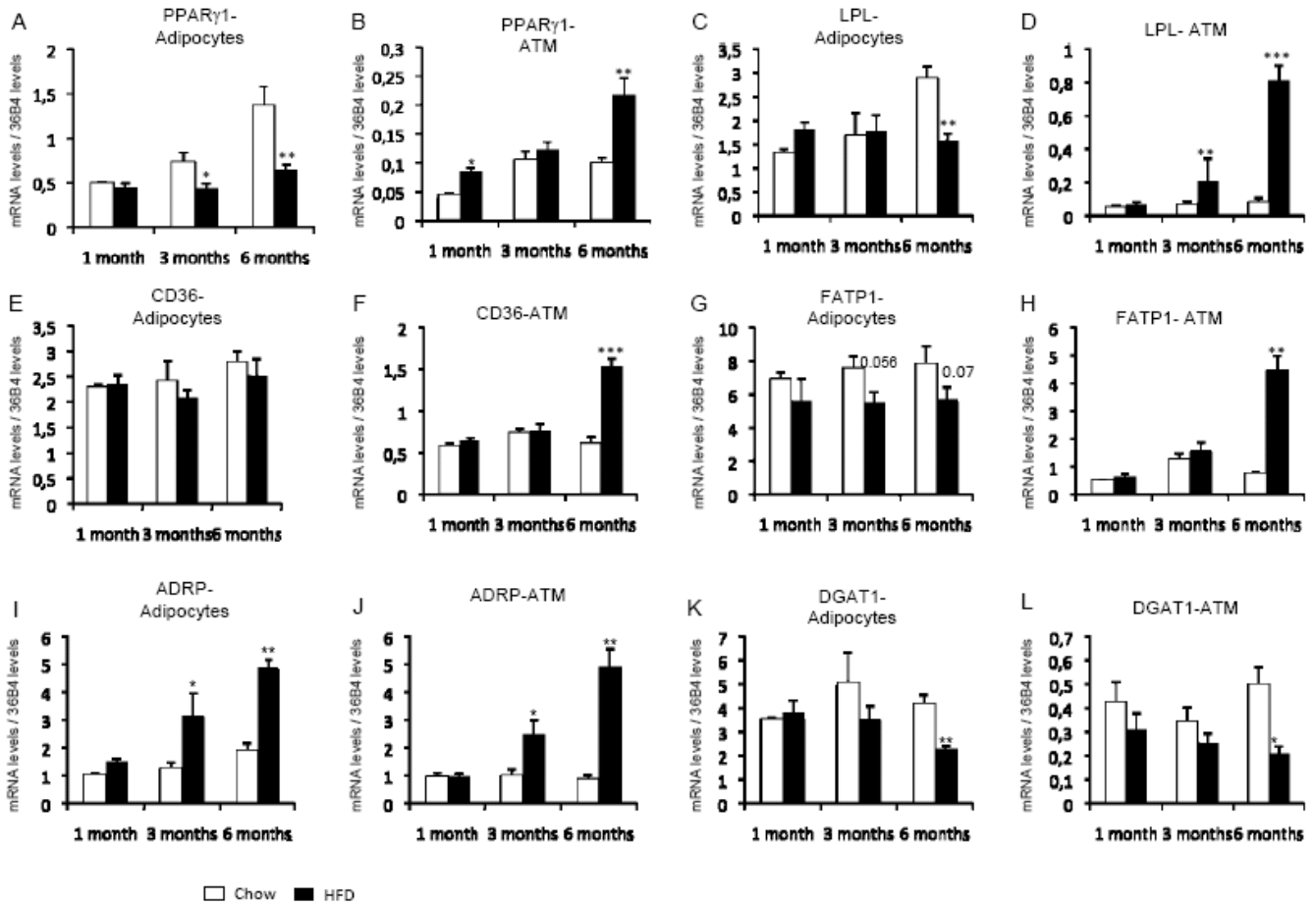
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**Supplementary Figure 6. ATM polarisation evaluated with flow cytometry.** A, Flow cytometry analysis of ATMs pre-incubated with corresponding PE- or APC- isotype control, showing how cutoffs for bodipyhigh, CD11c + and CD209a were set in Fig 3F. B, Flow cytometry analysis of WT and ob/ob ATMs for CD209a and CD11c. C, Flow cytometry analysis of ATMs from obese mice showing how cutoff for bodipyhigh was set in Fig 3G.



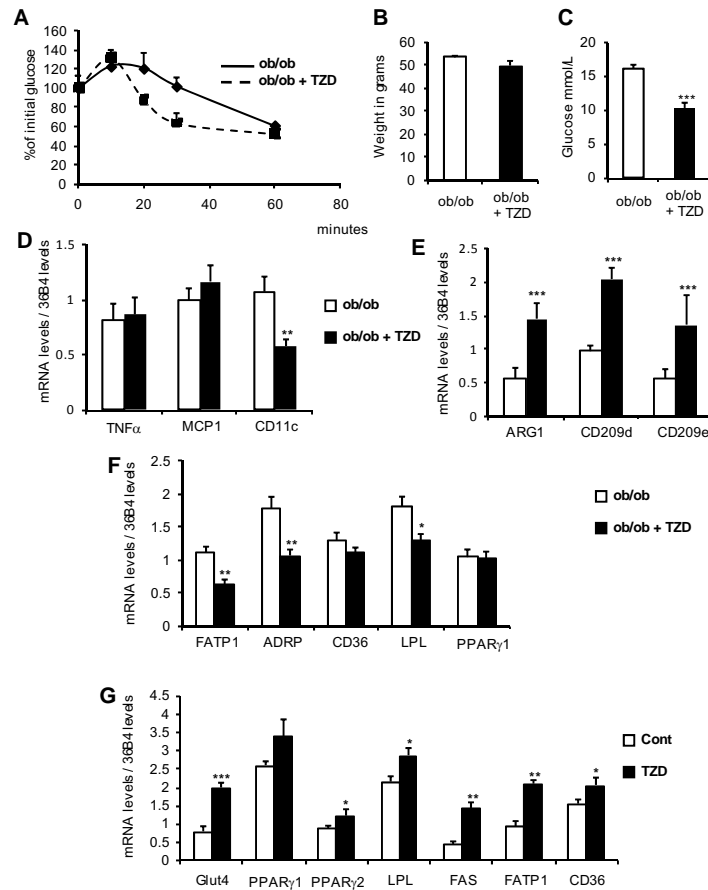
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**Supplementary Figure 7. ATM expression profile of key lipid metabolic genes during HFD time course.** Expression profile of key genes of lipid metabolism in ATM and in adipocytes normalised by the amount of 36B4. The bars represent the standard errors and significant differences were as follows: \*,  $p < 0.05$ ; \*\*,  $p < 0.005$ ; \*\*\*,  $p < 0.001$ .



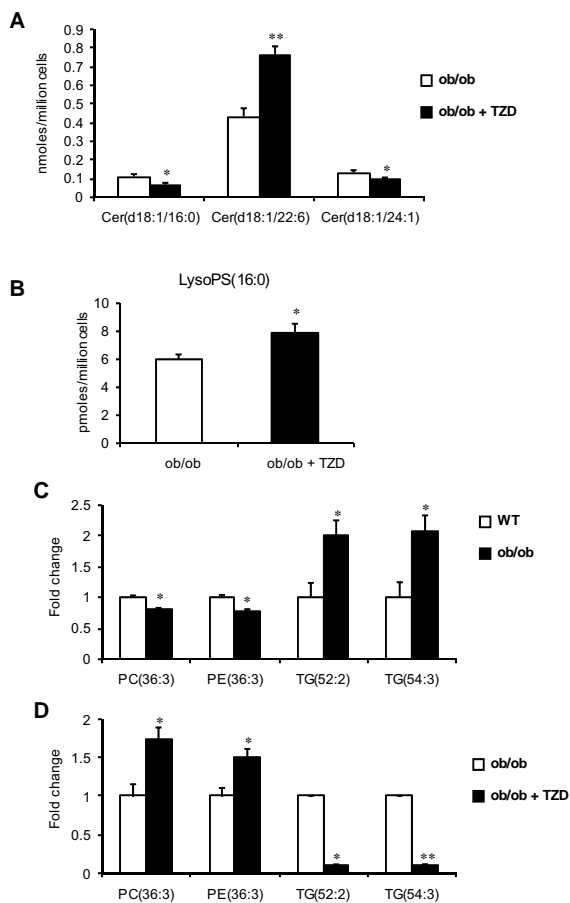
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**Supplementary Figure 8. Phenotypic characterization and gene expression of ob/ob treated with rosiglitazone.** Four weeks old males ob/ob (n=7 in each groups) were fed chow diet with (ob/ob+TZD) or without (ob/ob) 10 ppm rosiglitazone for 12 weeks. ITT was performed using 2 mU of insulin/g (A). Body weight (B) and glucose levels in mmol/L (C) are measured at the end of the experiment. ATMs from subcutaneous white adipose tissue are isolated and the mRNA levels of pro-inflammatory (M1) markers (D) and M2 markers (E) as well as key genes of lipid metabolism (F) are evaluated by quantitative PCR. Similarly, the expression of profile of adipocyte from the same subcutaneous fat pad are measured (G). Significant differences are as follows: \*,  $p < 0.05$ ; \*\*,  $p < 0.005$ ; \*\*\*,  $p < 0.001$ .



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**Supplementary Figure 9. lipids analysis of ATMs from TZD treated ob/ob mice.** Lipid content in ceramides (A) and LysoPS (B) of ATMs isolated from control or TZD treated ob/ob mice. Ratio representation of fold change of lipid species in WT versus ob/ob (C) and in control versus TZD treated ob/ob (D).





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**Supplementary Figure 10. Lipidomics analysis of adipocytes.** Heat map representation of lipidomics analysis of adipocytes from 16 weeks old WT, ob/ob, control and TZD treated mice.

