Appendix A. Supplementary data

Activated macrophages control human adipocyte mitochondrial bioenergetics via secreted factors

Michaela Keuper*, Stephan Sachs, Ellen Walheim, Lucia Berti, Bernhard Raedle, Daniel Tews, Pamela Fischer-Posovszky, Martin Wabitsch, Martin Hrabe de Angelis, Gabi Kastenmüller, Matthias H. Tschöp, Martin Jastroch, Harald Staiger, Susanna M. Hofmann

Running title: Macrophages regulate adipocyte mitochondria

Content:

Figure S1

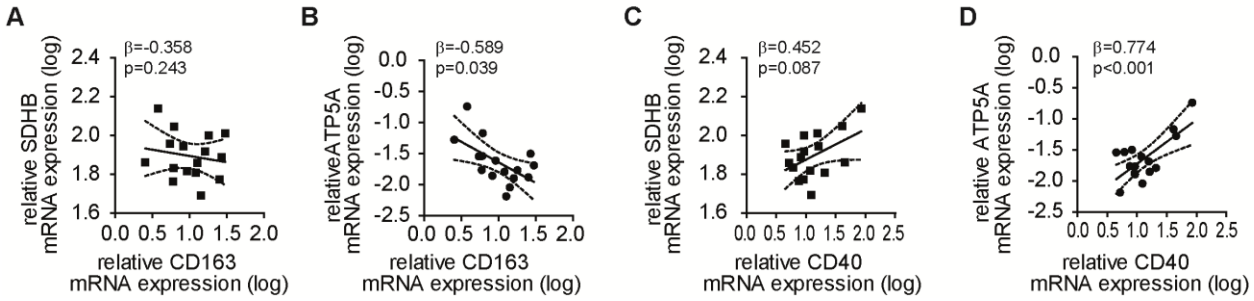


Figure S1: Correlations of CD40 and CD163 with SDHB and ATP5A expression in whole human WAT (Cohort A)

Total RNA of human subcutaneous adipose tissue from cohort A (n=16) was prepared and reversely transcribed. Expression levels of OXPHOS components (*SDHB*, *ATP5A)* and CD40 and CD163 were measured with qPCR and normalized to *HPRT* (ΔCt). Data have been log-transformed to meet assumption of normal distribution. Multiple linear regressions were performed for the relative mRNA expression of either *SDHB* **(A)** or *ATP5A* **(B)** with CD163 and for the relative mRNA expression of either *SDHB* **(C)** or *ATP5A* **(D**) with CD40. P-values and coefficient β for the multiple linear regression models including BMI as cofactor are presented in the graphs.

Figure S2

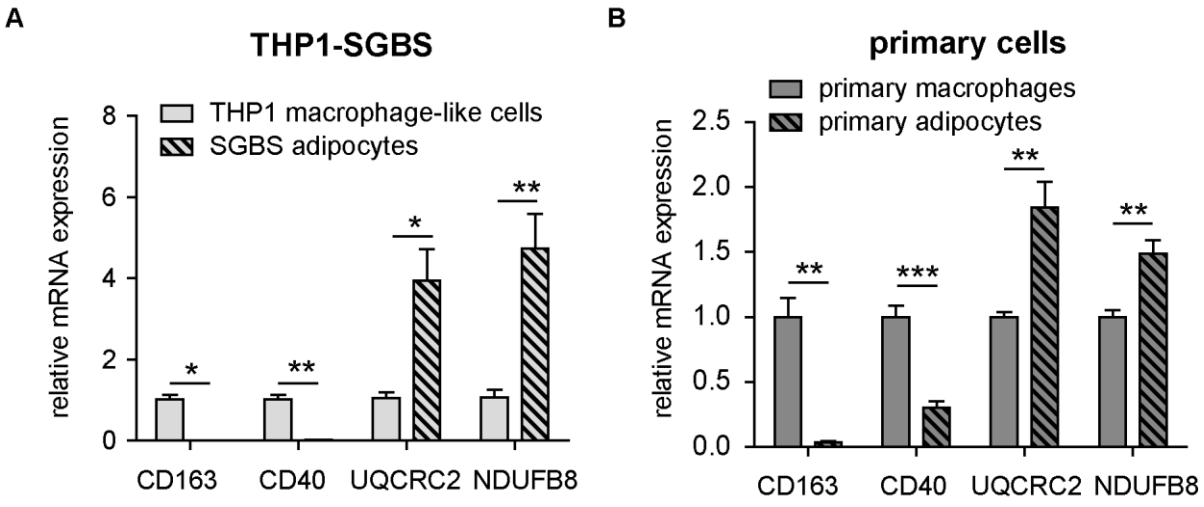


Figure S2: The comparative qPCR analysis of CD40, CD163, UQCRC2 and NDUFB8 in adipocytes (SGBS and primary) and macrophages (THP1 and primary)
To study the contribution of macrophages and adipocytes to the associations of CD40/CD163 with UQCRC2 and NDUFB8 in whole WAT samples, we determined the mRNA levels of these genes in cultures of THP1, primary (blood-derived) macrophages, SGBS adipocytes and primary subcutaneous adipocytes via qPCR. The same data as in main Figure 1 are presented, but in contrast to the main figure, Ct values were normalized to THP1 (A) or primary macrophages (B) and set as 1, without normalizing to housekeeping gene.

Figure S3
A

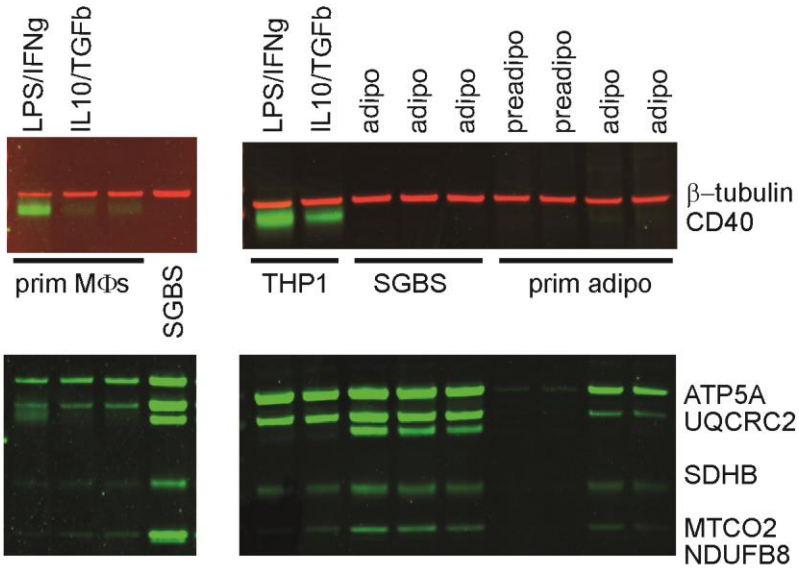


Figure S3: The comparative western blot analysis of CD40, UQCRC2 and NDUFB8 in adipocytes (SGBS and primary) and macrophages (THP1 and primary)
To study the contribution of macrophages and adipocytes to the associations of CD40 with UQCRC2 and NDUFB8 in whole WAT samples, we determined their protein levels in cultures of THP1, primary (blood-derived) macrophages, SGBS adipocytes and primary subcutaneous adipocytes via western blot.

Figure S4

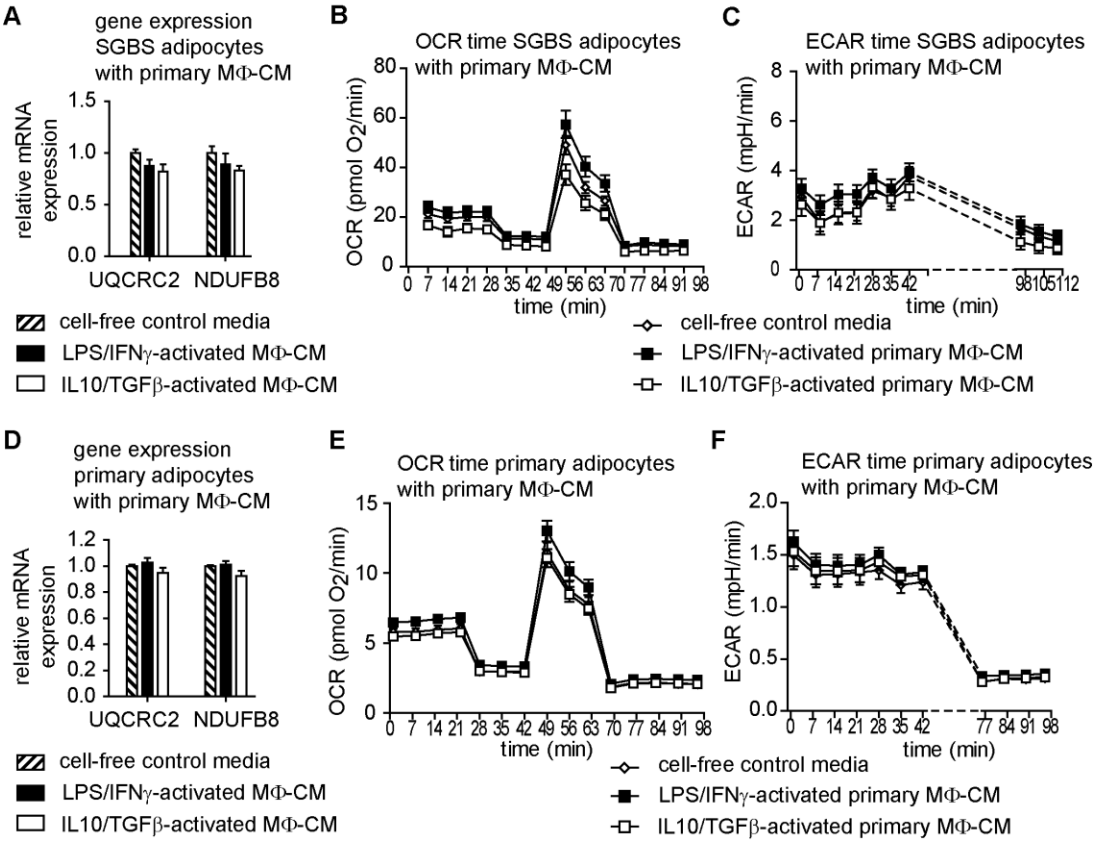


Figure S4: Gene expression of UQCRC2 and NDUFB8 as well as time traces of OCR and ECAR measurements of SGBS and primary adipocytes treated with primary MΦ-CM
(A-C) Differentiated SGBS cells were treated with either cell-free control media, LPS/IFNγ- or IL10/TGFβ-activated MΦ-CM for 48h as described in Methods. **(A)** mRNA expression of UQCRC2 and NDUFB8 were analyzed with ΔΔCT using SGBS cells exposed to cell-free control media as Calibrator. Data are the mean + SEM of 3 independent experiments. Two-way ANOVA (post-hoc: Bonferroni) revealed a trend for the global effect of the CM between SGBS adipocytes treated with cell-free control vs. IL10/TGFβ-activated MΦ-CM (p=0.058). **(B)** Oxygen consumption rates over time (OCR) of SGBS adipocytes treated for 48h with indicated MΦ-CM using a XF96 extracellular flux analyzer**. (C)** Extracellular acidification rates over time (ECAR) of SGBS adipocytes treated for 48h with indicated MΦ-CM.
(D-F) Differentiated primary adipocytes were treated with either cell-free control media, LPS/IFNγ- or IL10/TGFβ-activated MΦ-CM for 48h as described in Methods. **(D)** mRNA expression of UQCRC2 and NDUFB8 were analyzed with ΔΔCT using cells exposed to cell-free control media from each donor as Calibrator. Data are the mean + SEM of 3 donors performed in duplicates. Two-way ANOVA (post-hoc: Bonferroni) revealed significant (p=0.031) effect of the CM between adipocytes treated with LPS/IFNγ- vs. IL10/TGFβ-activated MΦ-CM. **(E)** Oxygen consumption rates over time (OCR) of adipocytes from three donors treated for 48h with indicated MΦ-CM using a XF96 extracellular flux analyzer**. (F)** Extracellular acidification rates over time (ECAR) of adipocytes from 3 donors treated for 48h with indicated MΦ-CM.

Figure S5

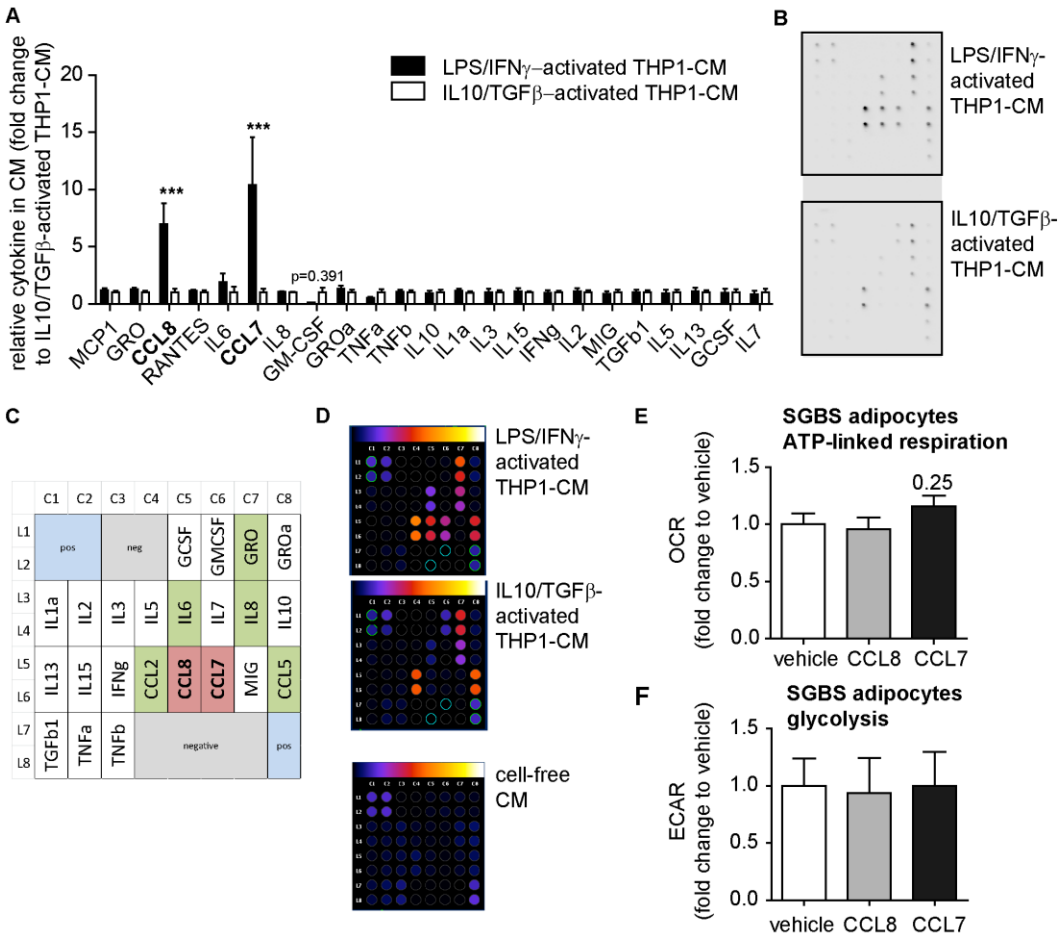


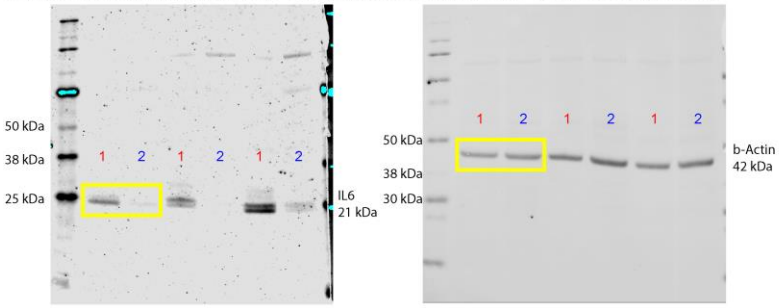
Figure S5: The relative abundance of cytokines in LPS/IFNγ- or IL10/TGFβ-activated THP1-CM and the effect of CCL7 and CCL8 on ATP-linked respiration and glycolysis of SGBS adipocytes

Relative abundance of 23 cytokines in CM from activated THP1 cells were analyzed with human cytokine antibody array and ImageJ as described in Methods. **(A)** Signal intensity of all spotted cytokine antibodies presented as fold change to IL10/TGFβ-activated THP1-CM and mean + SEM (n=5). ** $p<0.01$ vs. IL10/TGFβ-activated THP1-CM. **(B)** One representative membrane probed with LPS/IFNγ- or IL10/TGFβ-activated THP1-CM is shown. **(C)** Array layout with blank/negative (grey) and positive spots (blue) and spot IDs for cytokines with strong (not significant: green, significantly different: red). **(D)** Analysis (background correction/ normalization) was performed with ImageJ for five independent experiments in duplicates for THP1-CM and one experiment for cell-free control media, the latter showing no specific signal for all 23 spotted cytokine antibodies. **(E,F)** SGBS adipocytes were treated with either vehicle (PBS+0.1%BSA), CCL7 (10 ng/ml) or CCL8 (10 ng/ml) for 48h and bioenergetic profile was performed and analyzed as described for Fig 4 and 5. Data are fold change to vehicle control and the mean + SEM of 4 independent experiments each performed in 3-13 wells/condition. **(E)** ATP-linked respiration of SGBS adipocytes after treatment for 48h with indicated condition. P value for t test CCL7 vs vehicle is given. **(F)** ECARs due to glycolysis of SGBS adipocytes after treatment for 48h with indicated condition.

original blot Figure 2I

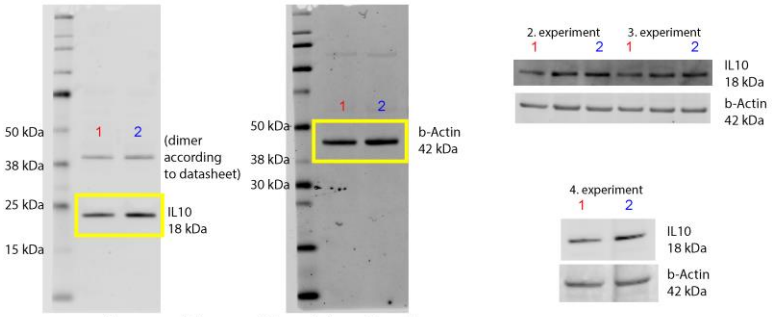
1 = LPS/IFNγ-activated THP1
2 = IL10/TGFβ-activated THP1

membrane probed with IL6 (rabbit 790) -> reprobed with b-Actin (goat 680) -> 3 independent experiments



yellow rectangle illustrate which part is shown in Figure 2I

membrane probed with IL10 (rabbit 790) -> reprobed with b-Actin (goat 680)



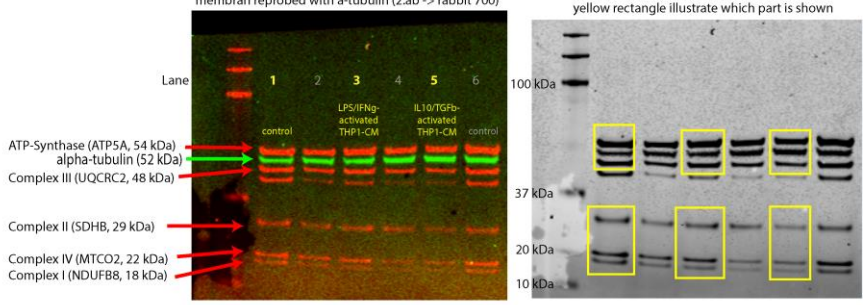
yellow rectangle illustrate which part is shown FIgure 2I

original blot Figure 3B

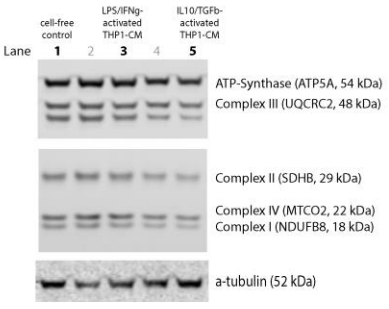
(Lane 1, 3 and 5 are shown in Figure 3B)

membrane probed with OXPHOS antibody cocktail (2. ab -> mouse 800)
membran reprobed with a-tubulin (2.ab -> rabbit 700)

black and white version as shown in Figure 3B
yellow rectangle illustrate which part is shown



second experiment



third experiment