Supplementary Material

**Supplemental Table 1. Sequences of the primers and TaqMan® probes.**

|  |  |
| --- | --- |
| **Gene (GenBank accession)** | **Oligonucleotide sequence (5’-3’)** |
| *CCL2* (NM\_002982) |  |
| Forward | GCTCATAGCAGCCACCTTCATT |
| Reverse | TCTGCACTGAGATCTTCCTATTGGT |
| TaqMan® Probe | FAM-TCGCTCAGCCAGATGCAATCAATGC-TAMRA |
| *COL1A1* (NM\_000088.3)  |  |
| Forward | CTCCCGGGCCTCAAGGTAT |
| Reverse | TTGCTCCAGAGGGACCTTGTT |
| TaqMan® Probe | FAM-TCTTCCTGGCCCCTCTGGTGAACCT-TAMRA |
| *COL4A3* (NM\_000091.4) |  |
| Forward | AGTGGATTGCCAGGATTTTCTG |
| Reverse | TGGTACACCGACCAGTCCGTAA |
| TaqMan® Probe | FAM-CCAGGCACCCCAGGCAATACCG-TAMRA |
| *COL6A3* (NM\_004369.3) |  |
| Forward | GACGGAGATCTGGCTGATTTACA |
| Reverse | AGATGCATTAGCCGCTCCAA |
| TaqMan® Probe | FAM-AGAACCTCCGCCAAGAAGGAGTCCGT-TAMRA |
| *CTSG* (NM\_000501.4) |  |
| Forward | ATGTTATTGCAGCTGAGCAGAAGA |
| Reverse | TCAGTCCCTCCTGGGCTCTAG |
| TaqMan® Probe | FAM-TCAGACGGAATCGAAACGTGAACCCA-TAMRA |
| *ELN* (NM\_000501.4) |  |
| Forward | TGGAGGAGTGGCAGCAAGA |
| Reverse | CTTCCGGCCACAAGCTTTC |
| TaqMan® Probe | FAM-TCGGATTGTCTCCCATTTTCCCAGGT-TAMRA |
| *HMGB1* (NM\_001313893.1) |  |
| Forward | GAAGTTCAAGGATCCCAATGCA |
| Reverse | CTCCTTTGATTTTTGGGCGATAC |
| TaqMan® Probe | FAM-CTCCTTCGGCCTTCTTCCTCTTCTGCTC-TAMRA |
| *IL1A* (NM\_000575) |  |
| Forward | GTTCTGAAGAAGAGACGGTTGAGTTT |
| Reverse | AAGTTGTATTTCACATTGCTCAGGAA |
| TaqMan® Probe | FAM-CATCGCCAATGACTCAGAGGAAGAAATCA-TAMRA |
| *IL1B* (NM\_000576) |  |
| Forward | CAGTGGCAATGAGGATGACTTG |
| Reverse | GTAGTGGTGGTCGGAGATTCGTA |
| TaqMan® Probe | FAM-TGGCCCTAAACAGATGAAGTGCTCCTTCC-TAMRA |
| *IL6* (NM\_000600) |  |
| Forward | GCCCTGAGAAAGGAGACATGTAAC |
| Reverse | ATCCATCTTTTTCAGCCATCTTTG |
| TaqMan® Probe | FAM-AGGCACTGGCAGAAAACAACCTGAACC-TAMRA |
| *IL8* (NM\_000584.3) |  |
| Forward | ACCTTTCCACCCCAAATTTATCA |
| Reverse | TTCTCAGCCCTCTTCAAAAACTTC |
| TaqMan® Probe | FAM-CCACACTGCGCCAACACAGAAATTATTGTA-TAMRA |
| *IL17* (NM\_002190) |  |
| Forward | ACAACCGATCCACCTCACCTT |
| Reverse | CCTCCCAGATCACAGAGGGATAT |
| TaqMan® Probe | FAM-ATCTCCACCGCAATGAGGACCCTGA-TAMRA |
| *IL32* (NM\_001012631) |  |
| Forward | GAGACAGTGGCGGCTTATTATGA |
| Reverse | GGCACCGTAATCCATCTCTTTCT |
| TaqMan® Probe | FAM-CAGCACCCAGAGCTCACTCCTCTACTTGAA-TAMRA |
| *IL36* (NM\_004530) |  |
| Forward | TGTGGGACTTCCACGAAGTG |
| Reverse | CTTGCTCAAGAGCCTCTGGATAC |
| TaqMan® Probe | FAM-ACCCCAGTCACTGTTGCTGTTATCACATGC-TAMRA |
| *IL36R* (NM\_004530) |  |
| Forward | AGATTATGGCCTTCCTTTCATGT |
| Reverse | GGCGATAAGCCCTCCTATCAA |
| TaqMan® Probe | FAM-GACCTCCCAGCTCCGGA-TAMRA |
| *MMP2* (NM\_004530) |  |
| Forward | CCATTTTGATGACGATGAGCTATG |
| Reverse | GTTGTACTCCTTGCCATTGAACAA |
| TaqMan® Probe | FAM-CTTGGGAGAAGGCCAAGTGGTCCGT-TAMRA |
| *MMP9* (NM\_004994) |  |
| Forward | GCCCGGACCAAGGATACAGT |
| Reverse | CCCCTCAGTGAAGCGGTACA |
| TaqMan® Probe | FAM-ACGCGCTGGGCTTAGATCATTCCTCA-TAMRA |
| *NGAL* (NM\_005564) |  |
| Forward | CCCAGCCCCACCTCTGA |
| Reverse | CTTCCCCTGGAATTGGTTGTC |
| TaqMan® Probe | FAM-CAAGGTCCCTCTGCAGCAGAACTTCCA-TAMRA |
| *S100A9* (NM\_002965) |  |
| Forward | CTCAAGAAGGAGAATAAGAATGAAAAGG |
| Reverse | TCAGCTGCTTGTCTGCATTTG |
| TaqMan® Probe | FAM-CATAGAACACATCATGGAGGACCTGGAC-TAMRA |
| *SPP1* (NM\_000582) |  |
| Forward | CATCCAGTACCCTGATGCTACAGA |
| Reverse | GGCCTTGTATGCACCATTCAA |
| TaqMan® Probe | FAM-ACATCACCTCACACATGGAAAGCGAGGA-TAMRA |
| *TGFB* (NM\_000660) |  |
| Forward | GCCCAGCATCTGCAAAGC |
| Reverse | TCCTTGCGGAAGTCAATGTACA |
| TaqMan® Probe | FAM-CACCAACTATTGCTTCAGCTCCACGGA-TAMRA |
| *TNF* (NM\_000594) |  |
| Forward | CCCCAGGGACCTCTCTCTAATC |
| Reverse | ACATGGGCTACAGGCTTGTCA |
| TaqMan® Probe | FAM-CCTCTGGCCCAGGCAGTCAGATCAT-TAMRA |
| Mouse *Il36g* (NM\_000594) |  |
| Forward | AGGCCCTTGTGACAGTTCCA |
| Reverse | AATCCCTTTGTCCTGTTCAAGAGA |
| TaqMan® Probe | FAM-AGCCACAGAGTAACCCCAGTCAGCGTG-TAMRA |

*CCL2*,monocyte chemoattractant protein-1; *COL*,collagen; *CTSG*, cathepsin G; *HMGB1*, high mobility group box 1; *IL*, interleukin; *MMP*, matrix metalloproteinase; *NGAL*, lipocalin 2; S100A9, S100 calcium-binding A9; *SPP1*,osteopontin; *TGFB*, transforming growth factor-; *TNF*, tumor necrosis factor-.

**Supplemental Table 2. Effects of weight loss in obese patients after Roux-en-Y gastric bypass (RYGB).**

|  |  |  |
| --- | --- | --- |
|  | **Before WL** | **After WL** |
| **n (male, female)** | 31 (8, 23) | 31 (8, 23) |
| **Age (years)** | 47  2 | 48  2 |
| **BMI (kg/m2)** | 42.0  1.2 | 29.3  0.8\*\*\* |
| **Body fat (%)** | 52.3  1.2 | 35.5  1.7\*\*\* |
| **Waist-to-hip ratio** | 0.94  0.02 | 0.88  0.02\*\* |
| **Fasting glucose (mg/dL)** | 104  4 | 86  2\*\* |
| **Fasting insulin (U/mL)** | 20.6  2.7 | 6.9  0.8\* |
| **HOMA** | 5.3  0.8 | 1.4  0.2\*\*\* |
| **QUICKI** | 0.313  0.008 | 0.377  0.010\*\*\* |
| **Triglycerides (mg/dL)** | 127  18 | 100  31\*\*\* |
| **Cholesterol (mg/dL)** | 191  7 | 157  7\*\*\* |
| **LDL-cholesterol (mg/dL)** | 116  6 | 87  6\*\*\* |
| **HDL-cholesterol (mg/dL)** | 49  3 | 54  2\* |
| **Leptin (ng/mL)**  | 50.6  3.6 | 12.8  1.7\*\*\* |

BMI, body mass index; HOMA, homeostatic model assessment; QUICKI, quantitative insulin sensitivity check index; WL, weight loss. Data are mean ± SEM. Differences between groups were analyzed by paired two-tailed Student’s *t* tests. \**P*<0.05, \*\**P*<0.01 and \*\*\**P*<0.001 *vs* before WL.

**Supplemental Table 3. Impact of IL-36 on ECM remodelling genes in visceral adipocytes.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene** | **CTL** | **100 ng/mL** | **200 ng/mL** |
| ***COL4A3*** | 1.00  0.14 | 1.01  0.14 | 1.07  0.19 |
| ***COL6A3*** | 1.00  0.11 | 0.67  0.08 | 0.66  0.05 |
| ***ELN*** | 1.00  0.12 | 1.02  0.14 | 1.04  0.12 |
| ***MMP2*** | 1.00  0.10 | 1.30  0.11 | 1.35  0.04 |
| ***MMP9*** | 1.00  0.19 | 1.70  0.08 | 1.69  0.99 |

Analysis of mRNA levels in human visceral adipocytes after IL-36 treatment. Data represent the mean  SEM of the ratio between the gene expressionto *18S* rRNA. Differences between groups were analyzed by one-way ANOVA followed by Dunnett’s *post hoc* test. *COL*, collagen, ECN, extracellular matrix; *ELN*, elastin; MMP, matrix metalloproteinase.



**Supplemental Figure 1**. Gene expression levels of *Il36g* in A) epididymal white adipose tissue (EWAT) and B) liver (LV) from mice submitted to normal (ND) and high-fat (HFD) diet. Bars represent the mean  SEM. Differences between groups were analyzed by unpaired two-tailed Student’s *t* test. \**P*<0.05.



**Supplemental Figure 2.** Secreted levels of IL-36 after LPS treatment in A) human visceral adipocytes and B) monocyte-derived macrophages. Bars represent the mean  SEM. Differences between groups were analyzed by one-way ANOVA followed by Dunnetts’s *post hoc* test.

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