# Activation of prostaglandin $E_2$-EP4 signaling reduces chemokine production in adipose tissue

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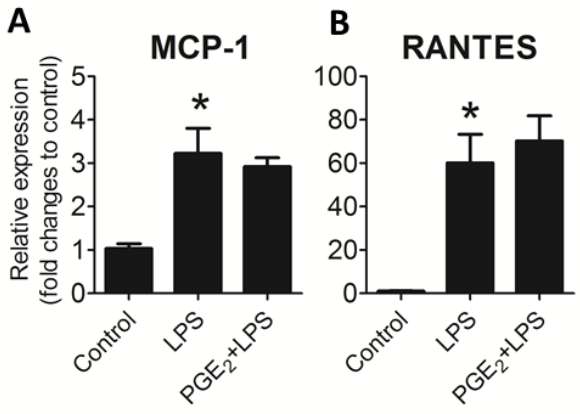
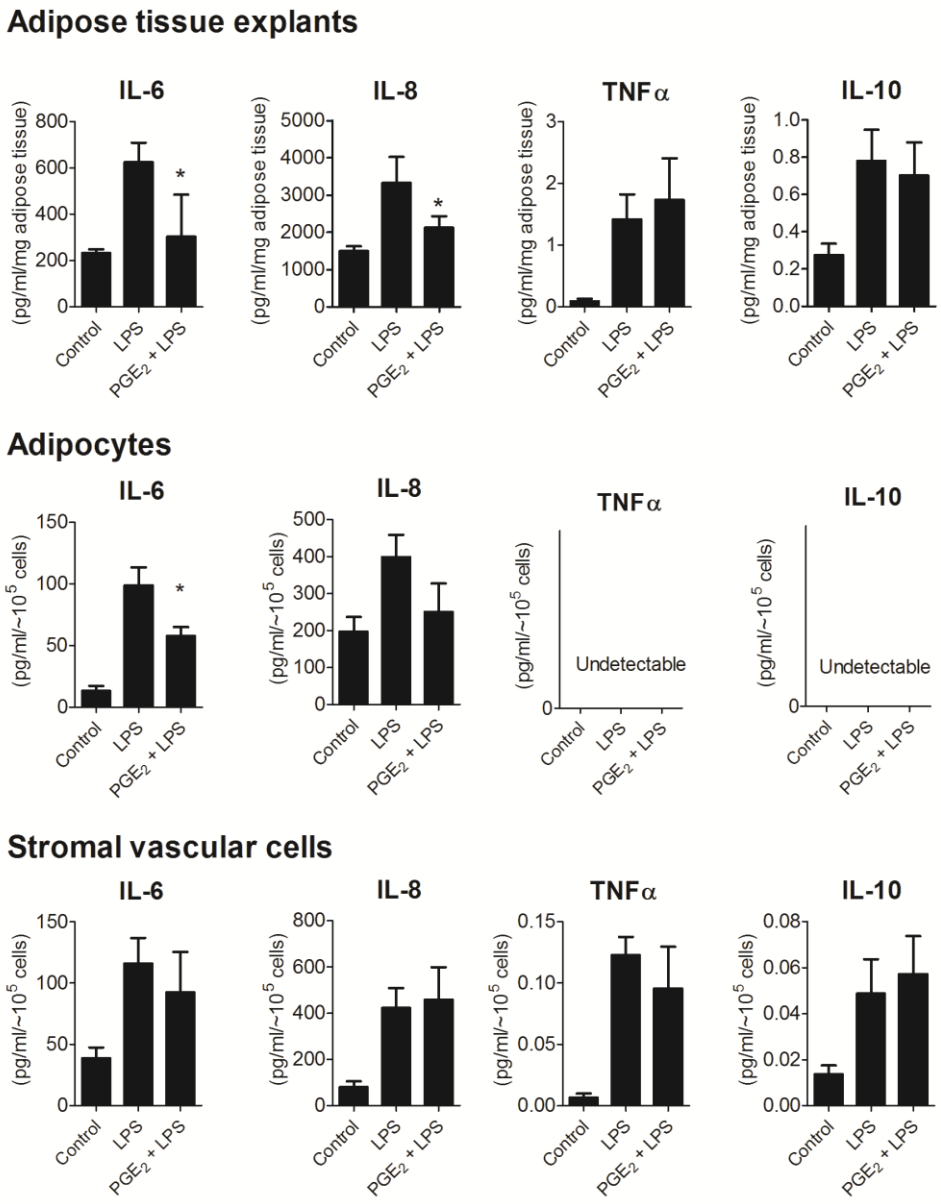
**ONLINE SUPPLEMENTAL MATERIAL**

| Gene | Forward sequence | Reverse sequence |
|---|---|---|
| IP-10 | GCCGTCATTTTCTGCCTCA | CGTCCTTGCGAGAGGGATC |
| MIP1α | CAGCCAGGTGTCATTTTCCT | AGGCATTCAGTTCCAGGTCA |
| MIP1β | TCT GCG TGT CTG CCC TCT C | TGC TGA GAA CCC TGG AGC A |
| MCP-1 | GCTGGAGCATCCACGTGTT | ATCTTGCTGGTGAATGAGTAGCA |
| RANTES | GCAAGTCTCCAATCTTGCA | CTTCTCTGGGTTGGCACACA |
| EP1 | CTGGGCCTAACCAAGAGTGC | CCGGGAACTACGCAGTGAAC |
| EP2 | ATGCTCCTGCTGCTTATCGT | TAATGGCCAGGAGAATGAGG |
| EP3 | ATGGGAAAGGAGAAGGAGTGC | AGCCAGGCGAACTGCAATTA |
| EP4 | CCATCGCCACATACATGAAG | TGCATAGATGGCGAAGAGTG |
| mPGES-1 | CTGCTGGTCATCAAGATGTACG | CCCAGGTAGGCCACGTGTGT |
| mPGES-2 | AAGACATGTCCCTTCTGC | CCAAGATGGGCACTTTCC |
| cPGES | AGTCATGGCCTAGGTTAAC | TGTGAATCATCATCTGCTCC |
| β-actin | CCTGAGCGCAAGTACTCTGTGT | GCTGATCCACATCTGCTGGAA |

**Supplementary Table 1: Primer sequences used in quantitative real-time PCR.**

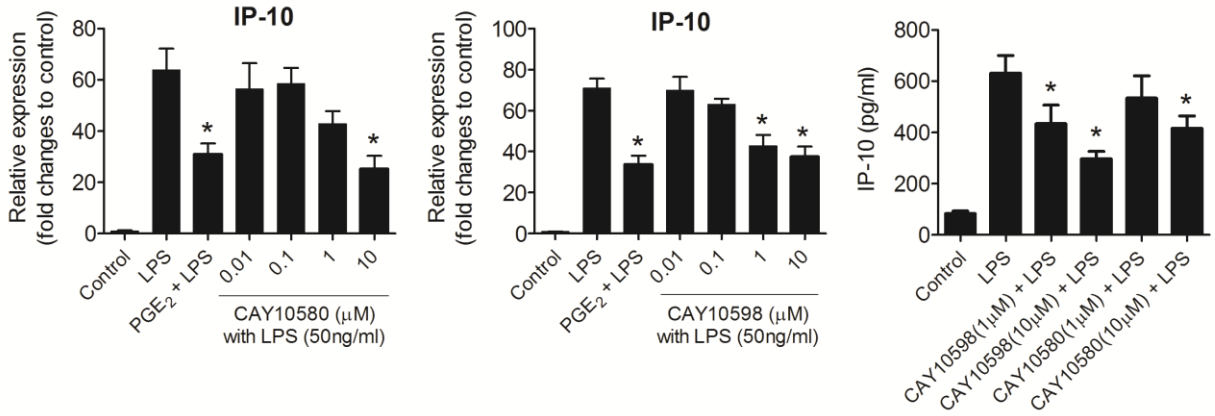



**Supplementary Figure 1: $PGE_2$ does not alter LPS-induced MCP-1 and RANTES expression.** RNA was isolated from $EP4^{+/+}$ adipose tissue explants pretreated with $PGE_2$ (50nM for 1.5 hours) before exposure to LPS (5ng/ml for nine hours). The mRNA expression of MCP-1 *(A)* and RANTES *(B)* are expressed as fold changes to control. N=5; $p<0.05$ vs. control. Data are expressed as means $\pm$ SEM.

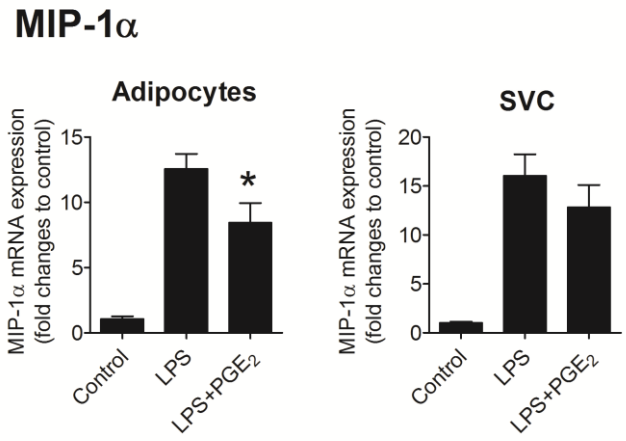



**Supplementary Figure 2: $PGE_2$ lowers LPS-stimulated cytokine production (IL-6 and IL-8) in whole adipose tissue, adipocytes but not stromal vascular cells.** Adipose tissue explants, adipocytes or stromal vascular cells were cultured in the presence or absence of $PGE_2$ (50 nM for 1.5 hours), and then stimulated with LPS (5 ng/ml for nine hours). Supernatant medium was collected for ELISA. TNFα and IL-10 production are undetectable in adipocytes and their production is unaffected by

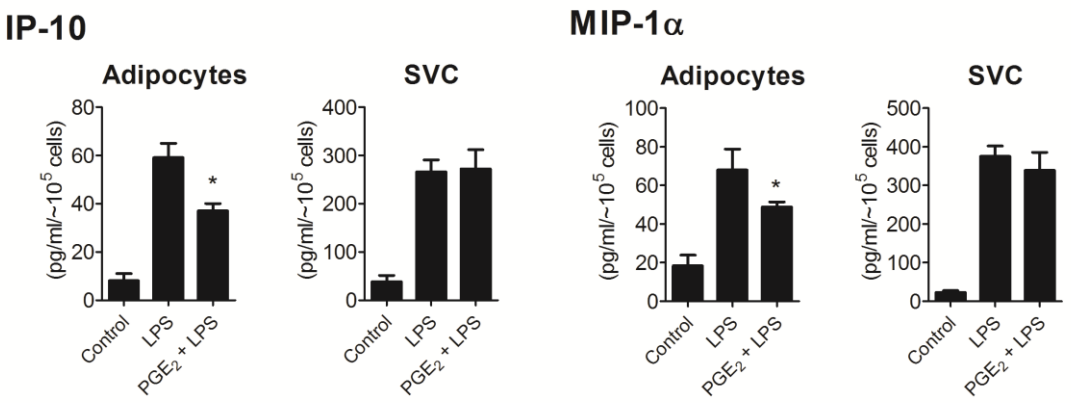
$PGE_2$ in LPS-activated adipose tissue explants and stromal vascular cells. bN=6; *$p<0.05$ vs. LPS. Data are expressed as means $\pm$ SEM.



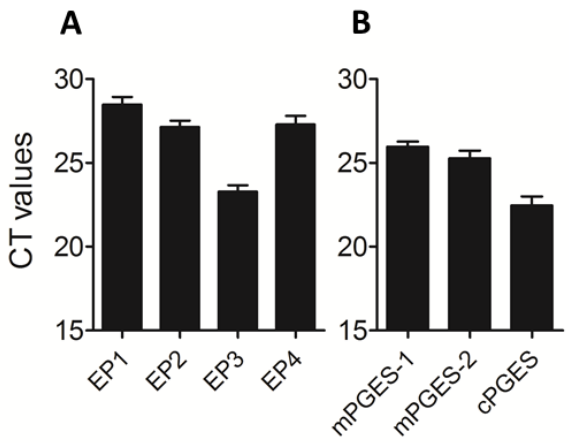

**Supplementary Figure 3:** Effect of different EP4 agonists on LPS-stimulated IP-10 production in eWAT derived from C57BL/6 mice. Pre-treatment with CAY10580 and CAY10598 at various concentrations on LPS-stimulated IP-10 mRNA and protein levels. All mRNA data are expressed as fold changes from control. Protein changes are expressed as absolute amount produced (pg/ml). N=6; *$p<0.05$ vs. LPS. Data are expressed as means $\pm$ SEM.



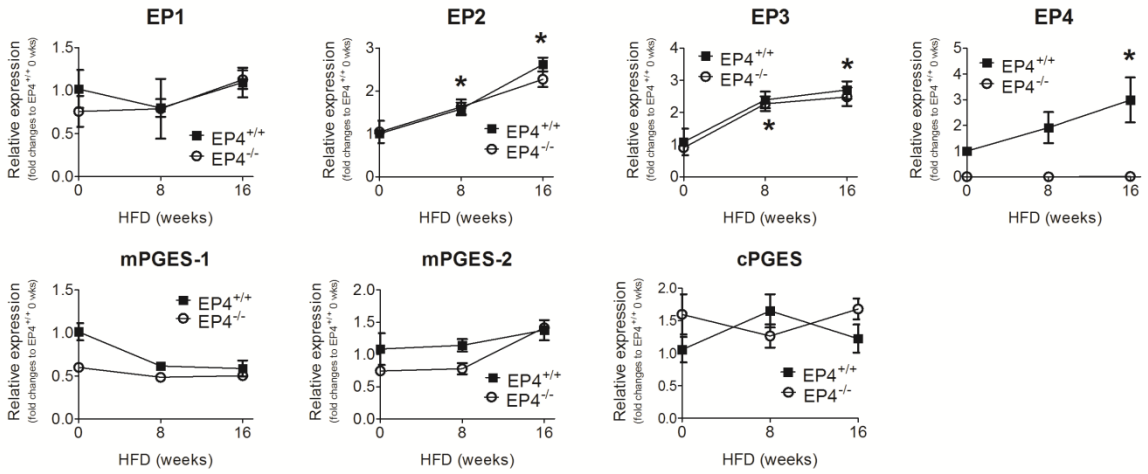

**Supplementary Figure 4: $PGE_2$ lowers MIP-1α mRNA in LPS-activated adipocytes but not in stromal vascular cells (SVC).** Adipocytes and SVC separated by collagenase digestion of $EP4^{+/+}$ adipose tissue were cultured in the presence or absence of $PGE_2$ (50 nM for 1.5 hours), and then stimulated with LPS (5 ng/ml for nine hours). RNA then was isolated and MIP-1α mRNA was quantified. N=6; *$p<0.05$ vs. LPS. Data are expressed as means $\pm$ SEM.



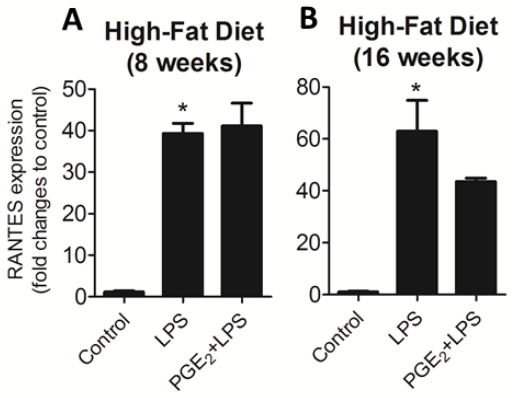

**Supplementary Figure 5: $PGE_2$ lowers IP-10 and MIP-1α protein levels in LPS-activated adipocytes, but not in stromal vascular cells (SVC).** Adipocytes and SVC separated by collagenase digestion of $EP4^{+/+}$ adipose tissue were cultured in the presence or absence of $PGE_2$ (50 nM for 1.5 hours), and then stimulated with LPS (5 ng/ml for nine hours). For protein analysis, approximately, 10,000 cells were seeded into each well and treated accordingly. Supernatant medium was collected for ELISA. N=6; *$p<0.05$ vs. LPS. Data are expressed as means $\pm$ SEM.



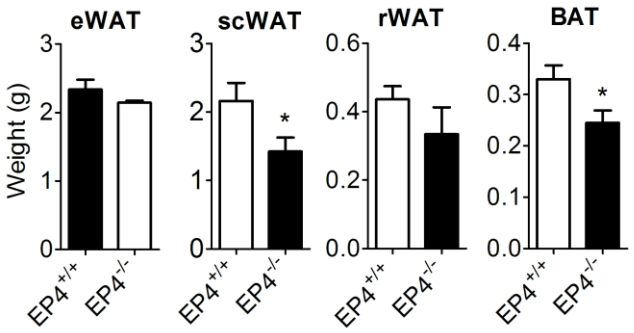

**Supplementary Figure 6: Expression of EP receptors and $PGE_2$ synthases in eWAT of $EP4^{+/+}$ mice.** Critical Threshold (CT) values for EP1, EP2, EP3, EP4 *(A)* and mPGES-1, mPGES-2, cPGES *(B)* expression. Values are obtained from the same eWAT sample run in the same PCR reaction. Lower CT values indicate higher abundance. N=6. Data are expressed as means + SEM.




**Supplementary Figure 7: Expression of EP receptors and $PGE_2$ synthases during consumption of high-fat diet.** mRNA expression of EP1, EP2, EP3, EP4, mPGES-1, mPGES-2, cPGES in eWAT of $EP4^{+/+}$ and $EP4^{-/-}$ mice. N=6; *$p<0.05$ vs. 0 weeks (mice of the same genotype). Data are expressed as means $\pm$ SEM.




**Supplementary Figure 8: $PGE_2$ does not reduce LPS-induced RANTES expression in adipose tissue derived from high-fat fed mice.** RNA was isolated from eWAT of $EP4^{+/+}$ mice fed a high-fat diet for 8- *(A)* or 16-*(B)* weeks. The eWAT was pretreated with $PGE_2$ (50nM for 1.5 hours) prior to exposure to LPS (5ng/ml for nine hours). N=5; $p<0.05$ vs. control. Data are expressed as means $\pm$ SEM.




**Supplementary Figure 9: EP4$^{-/-}$ mice have reduced adipose tissue depots.** Weight of epididymal (eWAT), subcutaneous (scWAT), peri-renal (rWAT) white adipose tissue and brown adipose tissue (BAT) in EP4$^{+/+}$ and EP4$^{-/-}$ after 16 weeks of high-fat diet. N=5; *$p<0.05$ EP4$^{+/+}$ vs. EP4$^{-/-}$. Data are expressed as means $\pm$ SEM.