Supporting Information

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Supporting Information Fig. S1

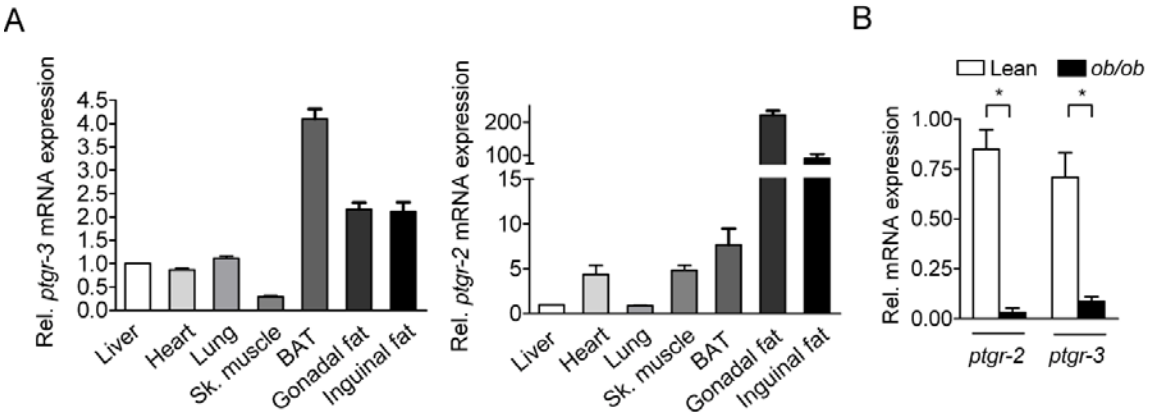

Fig. S1. The expression patterns of PTGR-2 and PTGR-3 mRNA in tissues and cells. (A) Tissue distribution of PTGR-2 and PTGR-3 mRNA from 12-wk-old mice C57BL/6 mice. (B) Expression of PTGR-2 and PTGR-3 mRNA from adipose tissue of 12-wk-old lean and *ob/ob* mice. * $P < 0.05$ versus Lean. The bars indicate the means ± SE for tissue or cells from three independent replicates (n = 3).

Supporting Information Fig. S2

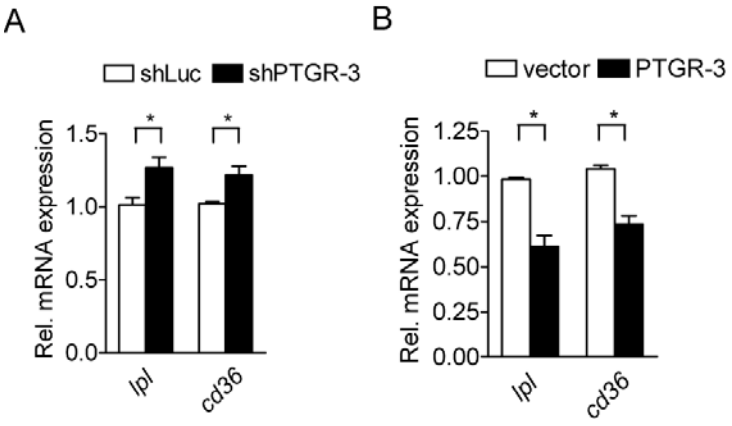

Fig. S2. Expression of lipid metabolism-related genes in genetically modified 3T3-L1 cells after 8 day hormonal induction. (A) Expression of adipogenic marker genes (*lpl* and *cd36*) in shRNA control and PTGR-3-knockdown cells. (B) Expression of adipogenic marker genes (*lpl* and *cd36*) in vector control and PTGR-3-overexpressing cells. The bars indicate the means ± SE for cells from three independent replicates (n = 3). * $P < 0.05$ versus shLuc.

Supporting Information Fig. S3

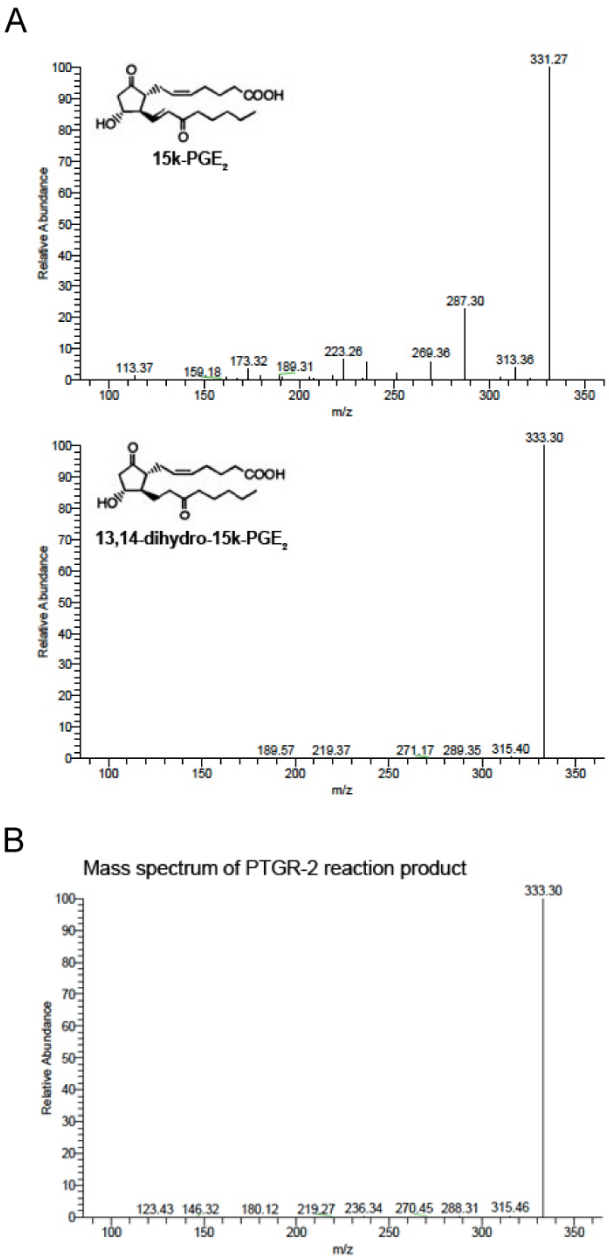

Fig. S3. Mass spectrum of 15-keto-PGE_2 and 13,14-dihydro-15-keto-PGE_2. (A) LC-MS/MS spectrum of standard molecule of 15-keto-PGE_2 (upper panel) and 13,14-dihydro-15-keto-PGE_2 (lower panel) were identified from fragmentation of the molecular anions of 15-keto-PGE_2 (m/z 349) and 13,14-dihydro-15-keto-PGE_2 (m/z 351). (B) LC-MS/MS spectrum of PTGR-2 enzymatic reaction product. 15-keto-PGE_2 was incubated with recombinant PTGR-2 and NADPH for 30 min at 37 °C. Mass spectrum of 13,14-

dihydro-15-keto-PGE_2 from enzymatic reaction product was identified by LC-MS/MS compared with standard molecule of 13,14-dihydro-15-keto-PGE_2.

Supporting Information Fig. S4

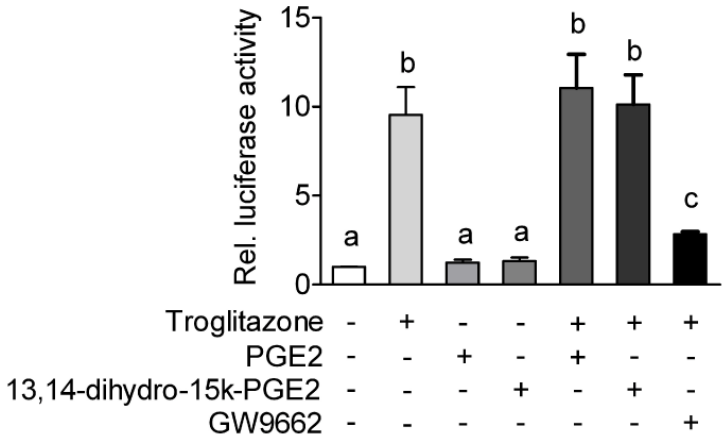

Fig. S4. The effect of transactivation of PGE_2 and 13,14-dihydro-15-keto-PGE_2 on ligand binding domain of PPARγ. 293 cells were transfected with expression vectors (CMX-GAL4 and CMX-GAL4-PPARγ) and UAS_G×4-TK-LUC reporter plasmid for 16 hour and then incubated cells in growth medium with or without treatments (1 μM troglitazone, 10 μM PGE_2, 10 μM 13,14-dihydro-15-keto-PGE_2 and 1 μM GW9662) for 24 hour. The activity of firefly luciferase was determined and normalized to the activity of renilla luciferase. The bars indicate the means ± SE for three independent replicates (n=3). Different superscripts indicate significant difference ($P \leq 0.05$).

Supporting Information Fig. S5

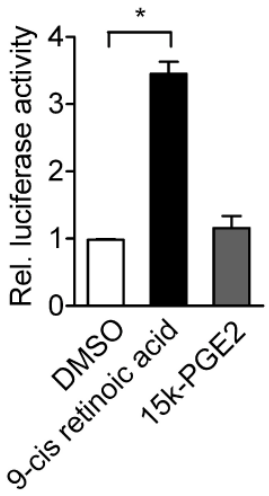

Fig. S5. The effect of 15-keto-PGE_2 on retinoid X receptor response element (RXRE)-driven luciferase activity. 293 cells were transfected with RXRE-LUC reporter plasmid for 16 hour and then incubated cells in growth medium with or without treatments (5 μM 9-cis retinoic acid and 10 μM 15-keto-PGE_2) for 24 hour. The activity of firefly luciferase was determined and normalized to the activity of renilla luciferase. The bars indicate the means ± SE for three independent replicates (n=3). * $P < 0.05$ versus DMSO.

Supporting Information Table I. Primer sequences for quantitative reverse transcription-PCR

Gene	Sequence	
ucp1	Sense	5'-CGTACCAAGCTGTGCGATGT-3'
	Anti-sense	5'-TAGAAGCCCAATGATGTTCAGT-3'
ucp2	Sense	5'-CCGGGCTGGTGGTGGTC-3'
	Anti-sense	5'-ACACCTTTCCAGAGGCCC-3'
ucp3	Sense	5'-CATCGCCAGGGAGGAAGGA-3'
	Anti-sense	5'-GTTGACAATGGCATTTCTTGTGA-3'
pparα	Sense	5'-TGCCAAGGAGTCGAGGATGT-3'
	Anti-sense	5'-TCGGCACCAGGAACCAA-3'
pparβ/δ	Sense	5'-AATGCGCTGGAGCTCGATGAC-3'
	Anti-sense	5'-ACTGGCTGTCAGGGTGGTTG-3'
pparγ	Sense	5'-CAAGAATACCAAAGTGCGATCAA-3'
	Anti-sense	5'-GAGCTGGGTCTTTTCAGAATAATAAG-3'
atgl	Sense	5'-TGTGGCCTCATTCCTCCTAC-3'
	Anti-sense	5'-TCGTGGATGTTGGTGGAGCT-3'
hsl	Sense	5'-GGCTTACTGGGCACAGATACCT-3'
	Anti-sense	5'-CTGAAGGCTCTGAGTTGCTCAA-3'
ptgr-2	Sense	5'-CAGGGTGGAAGAGTTCAGTTT-3'
	Anti-sense	5'-CCAGTGTCCTCGTTCATCTTAC-3'
ptgr-3	Sense	5'-GGAACTGTCAGAAGGGAAGAAA-3'
	Anti-sense	5'-TAACGTGGCACTTGGCTATC-3'
lpl	Sense	5'-GGATGGACGGTAAGAGTGATTC-3'
	Anti-sense	5'-ATCCAAGGGTAGCAGACAGGT-3'
cd36	Sense	5'-CCCCGTGCCTCCTCCCAGAA-3'
	Anti-sense	5'-GGCTAGGAAACCATCCACCAGTTGC-3'
fas	Sense	5'-CCCTTGATGAAGAGGGATCA-3'
	Anti-sense	5'-GAACAAGGCGTTAGGGTTGA-3'
acc	Sense	5'-AACATCCCCACGCTAAACAG-3'
	Anti-sense	5'-CTGACAAGGTGGCGTGAAG-3'
srebp-1c	Sense	5'-GGAGCCATGGATTGCACATT-3'
	Anti-sense	5'-GCTTCCAGAGAGGAGGCCAG-3'
gapdh	Sense	5'-GGTGAAGGTCGGTGTGAACG-3'
	Anti-sense	5'-CTCGCTCCTGGAAGATGGTG-3'

Abbreviations used: uncoupling protein 1, UCP1; uncoupling protein 2, UCP2; uncoupling protein 3, UCP3; peroxisome proliferator-activated receptor α, PPARα; peroxisome proliferator-activated receptor β/δ, PPARβ/δ; peroxisome proliferator-activated receptor γ, PPARγ; adipose triglyceride lipase, ATGL; hormone sensitive lipase, HSL; sterol regulatory element-binding transcription factor-1c, SREBP-1c; acetyl-CoA carboxylase, ACC; fatty acid synthase, FAS; glycerol-3-phosphate dehydrogenase, GPDH; fatty acid translocase, CD36; lipoprotein lipase, LPL; PTGR-2, prostaglandin

reductase-2; PTGR-3 prostaglandin reductase-3; glyceraldehyde-3-phosphate dehydrogenase, GAPDH.

Supporting information methods

Quantitative reverse transcription-PCR. Tissue and cellular RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA). Reverse transcription was performed with a Transcriptor Reverse Transcriptase kit (Roche Applied Science, Indianapolis, IN, USA). Quantitative reverse transcriptase-PCR was performed using ABI PRISM 7000 sequence detector system (Applied Biosystems, Foster City, CA, USA) and KAPA SYBR® FAST qPCR Kit (Kapa Biosystems, Inc., Boston, MA, USA). PCR was performed by 40 cycles of 95°C for 30 s, 58–60°C for 60 s, and 72°C for 30 s. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression was determined as the internal control gene. The sequence of primers for quantitative reverse transcription-PCR was listed in Supporting Information Table I. The mRNA expression of each gene was normalized to its GAPDH mRNA expression in the same sample. Threshold cycle (C_t) values were obtained and relative gene expression was calculated using the formula $(1/2)^{\text{Ct target genes}-\text{Ct GAPDH}}$.

Retinoid X receptor activity assay. Confluent 293T cell were incubated with reporter plasmids (retinoid X receptor response element-driven luciferase and CMV-driven renilla luciferase vectors) and lipofectamine. After 16 hour of transfection, media were changed to growth media without or with chemicals (5 μM 9-cis retinoic acid and 10 μM 15-keto-PGE_2). Twenty-four hours after treatment, cells were harvested for determination of luciferase activity (Dual-Glo luciferase assay system, Promega).