Supplementary figures and figure legends

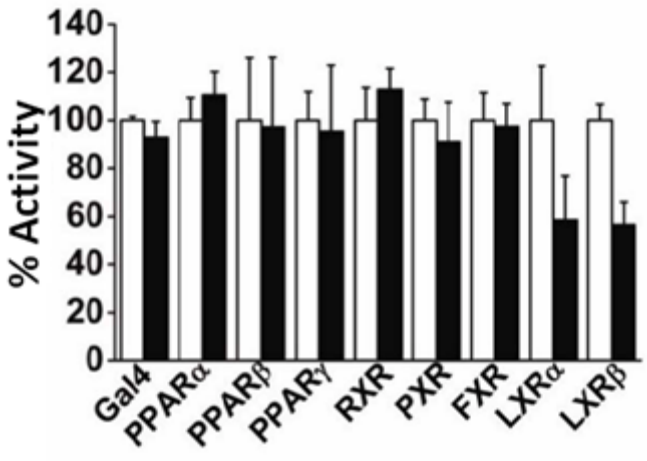

Figure S1 Rhein acts as an antagonist of liver X receptors (LXRs). 293T cells were transfected with a Gal4-responsive luciferase reporter and a series of chimeras in which the Gal4 DNA-binding domain was fused to the indicated nuclear hormone receptor ligand-binding domains (LBDs). Cells were treated with the appropriate agonist alone (white bars) or in combination with 12.5 μM rhein (black bars) for 24 h. Results are shown as the percent activities relative to the normalized luciferase activities in the presence of agonist alone (100%). The molecular targets and specific ligands used were as follows: peroxisome proliferator-activated receptor (PPAR) α, WY14643 (5 μM); PPARβ, GW0742 (1 μM); PPARγ, rosiglitazone (1 μM); retinoid X receptor (RXR), 9-cis-retinoic acid (1 μM); pregnane X receptor (PXR), PCN (10 μM); farnesoid X receptor (FXR), chenodeoxycholic acid (10 μM); and LXR, GW3965 (1μM).

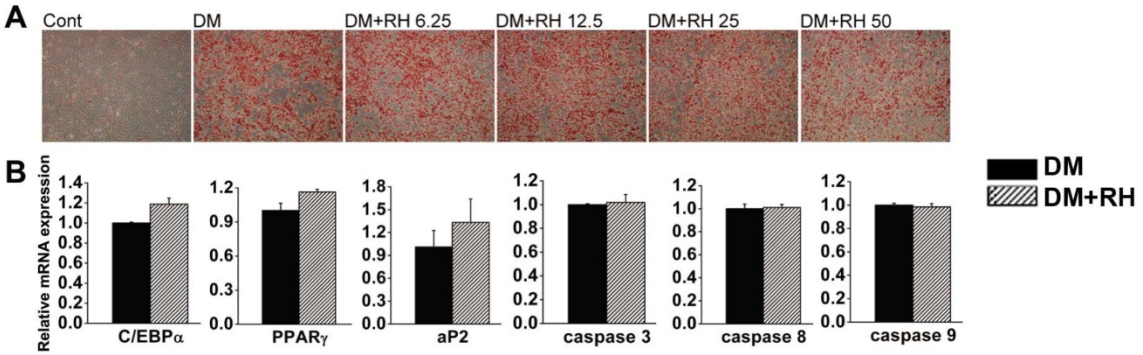

Figure S2 Effects of rhein on 3T3-L1 cell differentiation and apoptosis. Two days after reaching confluence, 3T3-L1 cells were induced by differentiation medium (DM) containing 10 μg/mL insulin, 1 μM dexamethasone and 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), followed by 6.25, 12.5, 25 or 50 μM rhein. Cells were then stained with Oil red O or collected for real-time PCR on day 7. Cont, undifferentiated 3T3-L1 cells. **(A)** Oil red O staining. **(B)** The mRNA expression levels of CCAAT/enhancer-binding protein (C/EBPα), activator protein-2 (aP2), peroxisome proliferator-activated receptor-γ (PPARγ), caspase 3, caspase 8 and caspase 9 were estimated by quantitative real-time RT-PCR. Black bars, DM; striped bars, DM + 25 μM rhein.

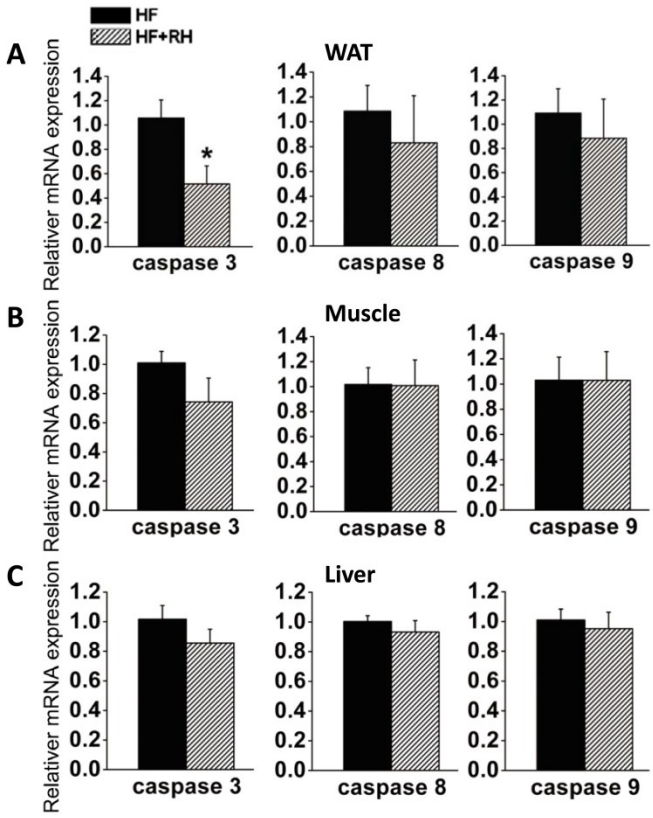

Figure S3 Effects of rhein on markers of apoptosis in white adipose tissue (A), muscle (B) and liver (C). After administration of rhein or water for 4 weeks to high-fat diet-induced obese mice, total RNA was isolated from WAT, muscle and liver, and was then subjected to quantitative real-time RT-PCR. Rhein significantly decreased the mRNA expression of caspase 3 in WAT, but not caspase 8 or caspase 9. Rhein did not significantly affect the expression of caspase 3, 8 or 9 in muscle and liver. mRNA expression levels are shown as values in rhein-treated groups (striped bars, HF + RH) relative to the control (water) groups (black bars, HF). Values are means ± standard error of the mean for five mice per group. *$P < 0.05$.

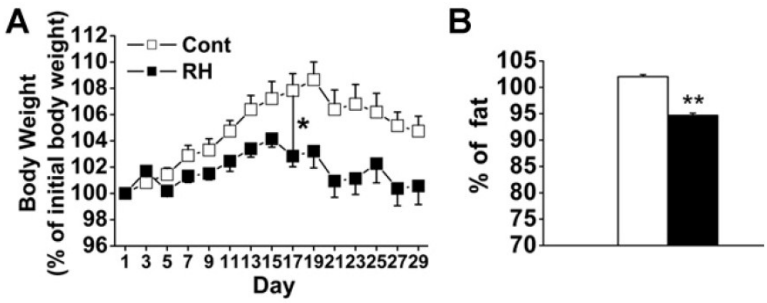

Figure S4 Rhein decreases body weight and fat content of *db/db* mice. Female *db/db* mice (8–10 weeks old) were fed a normal diet and were treated with rhein (RH, 150 mg/kg) or water (control) for 4 weeks. **(A)** Body weight gain as a percentage of the initial body weight. White squares, control; black squares, rhein. **(B)** Body fat content as a percentage of total body mass analyzed by nuclear magnetic resonance. White bar, control; black bar, rhein. Values are means ± SEM for five mice per group. *$P < 0.05$, **$P < 0.01$.

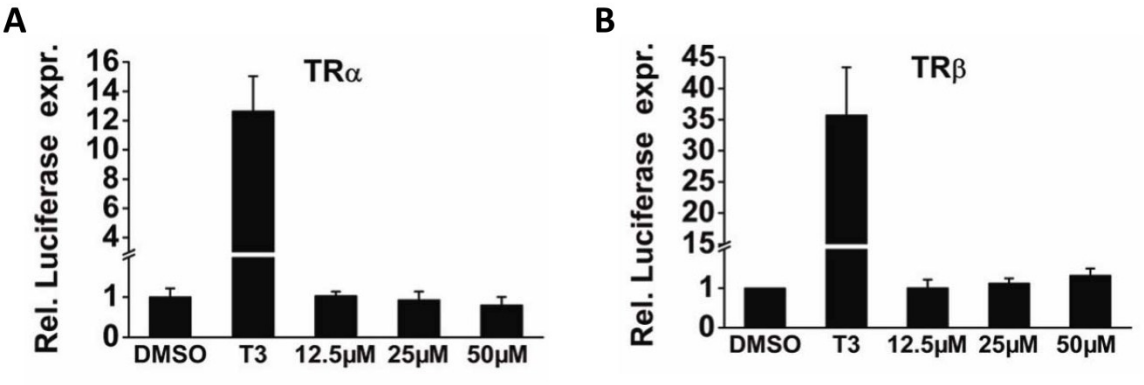

Figure S5 Rhein did not activate or inhibit the transcriptional activity of thyroid hormone receptors (TRs). (A) and **(B)** 293T cells were cotransfected with TRE-pal-luc, renilla luciferase plasmid and expression plasmid (pcDNA3.1-TRα1 or pcDNA3.1-TRβ1) using Lipofectamine 2000 for 24 h, and were then treated with DMSO, T3 (100 nM) or rhein (12.5, 25 or 50 μM) for another 24 h. Luciferase activities were analyzed using Dual-Luciferase Reporter Assay System (Promega). Values are means ± standard deviation of three independent experiments.