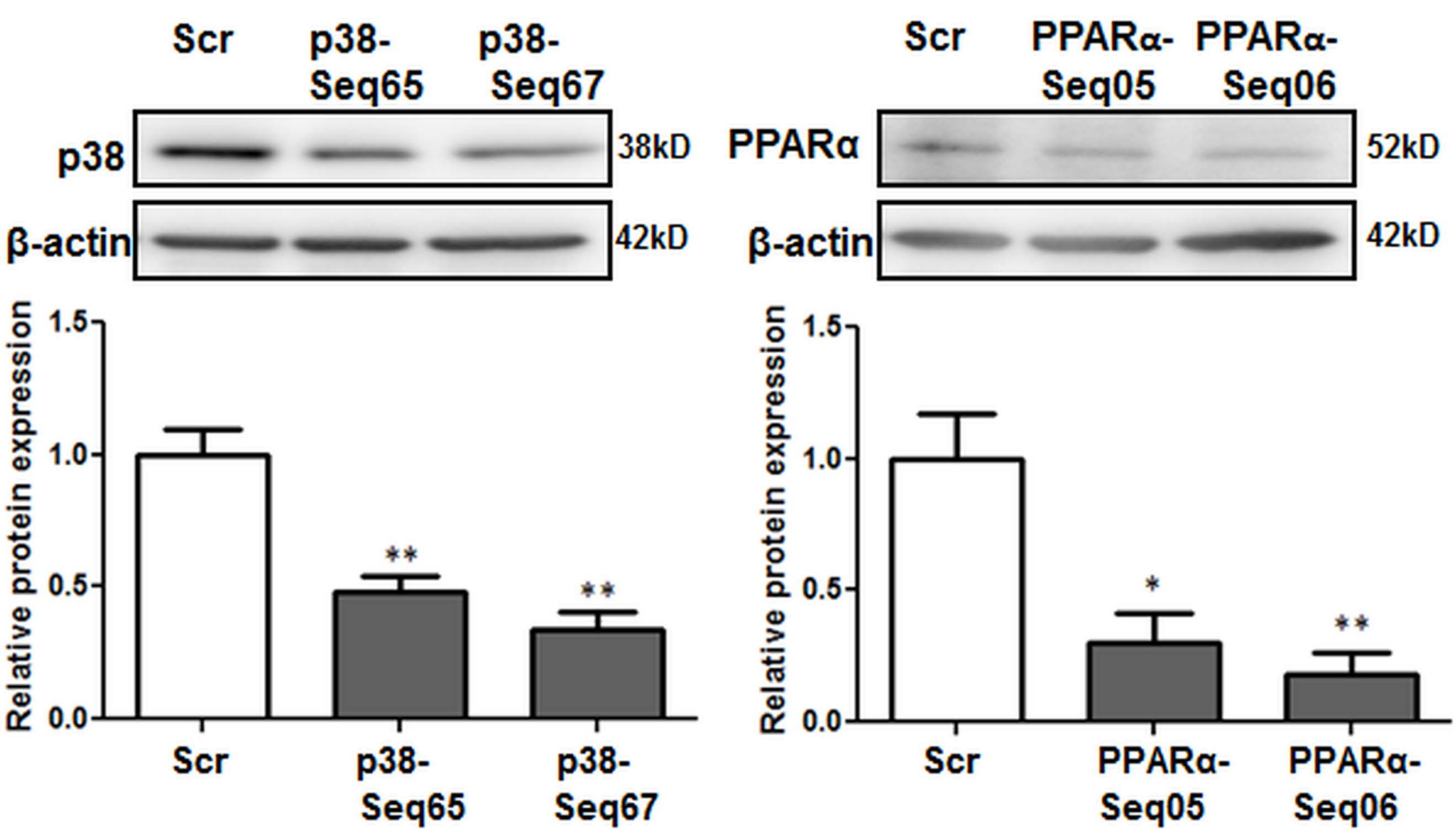
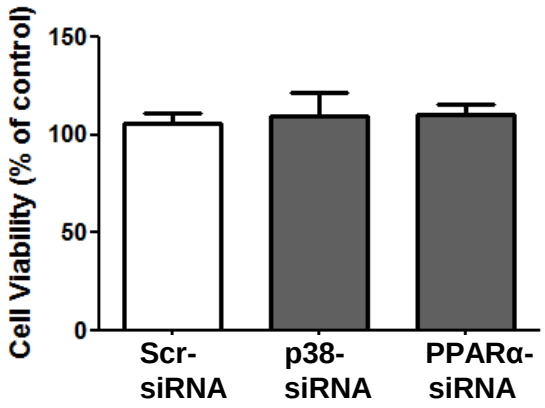
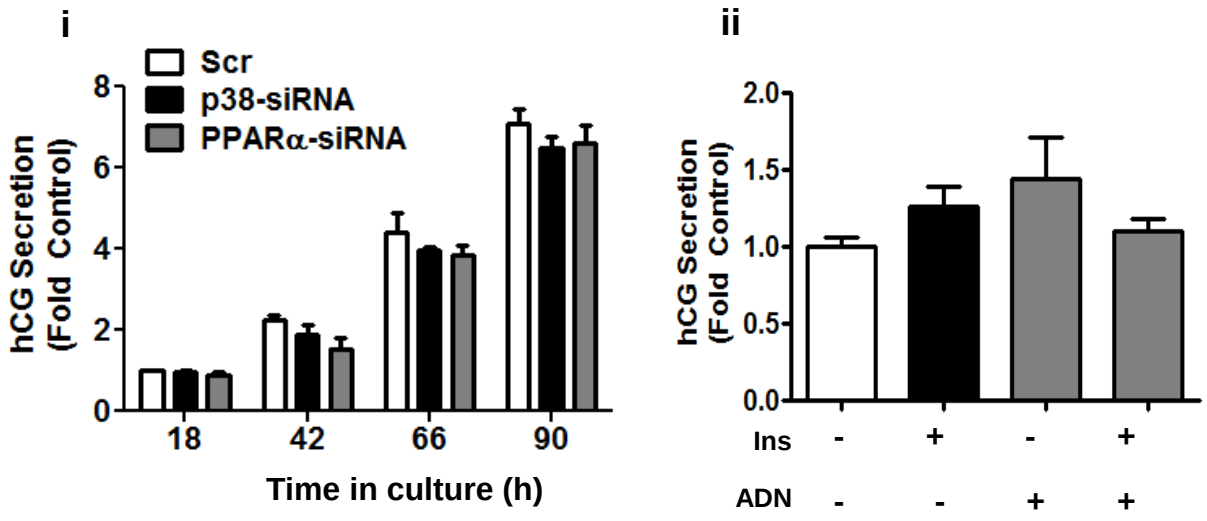


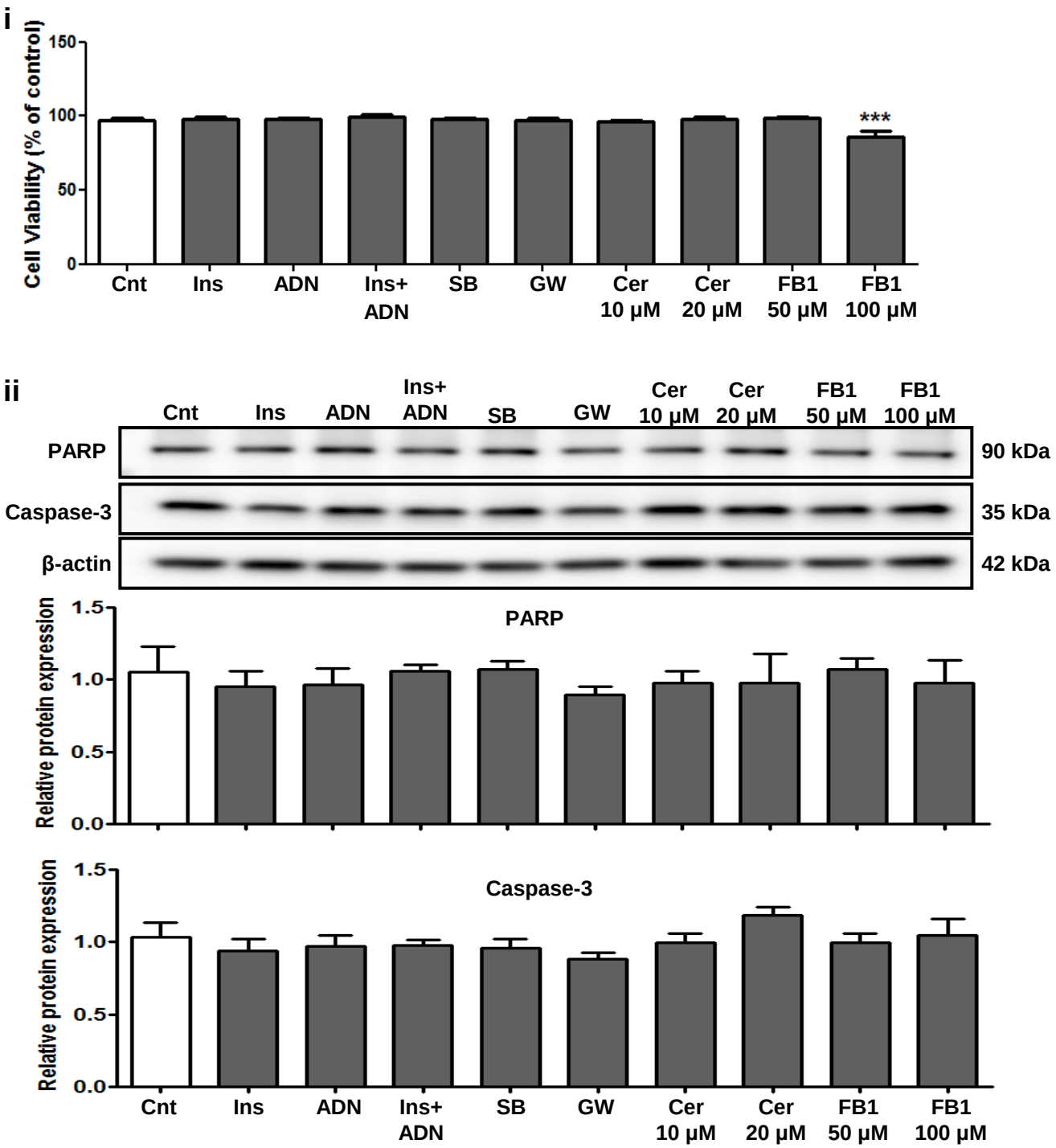
Supplementary Figure A. Transfection efficiency of p38 and PPARα siRNA sequences. PHTs after 18 h of culture were transfected with siRNA sequences as described in the methods. Western blots were performed to determine protein expression of p38 and PPARα. p38 siRNA Seq-67 and PPARα siRNA Seq-06 were selected for all siRNA experiments described in this study. Mean + sem, n = 3; One-Way ANOVA *P<0.05; **P<0.01. Scr, scramble siRNA (SIC001); p38-Seq65, p38 MAPK siRNA sequence (SASI_Hs01_00018**65**); p38-Seq67, p38 MAPK siRNA sequence (SASI_Hs01_00018**67**); PPARα-Seq05, PPARα siRNA sequence (SASI_Hs02_00304**05**); PPARα-Seq06, PPARα siRNA sequence (SASI_Hs02_00304**06**).



Supplementary Figure B. Influence of p38 and PPARα siRNA transfections on PHT cell viability. PHTs after 18 h of culture were transfected with siRNA sequences as described in the methods. At 90 h of culture, 1 mg/ml of 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent was added. After 4 h incubation at 37 °C, cells were lysed with 10 % SDS and absorbance read at 570 nm. Cell viability was presented as a percentage of Scr-siRNA controls. Mean + sem, n = 3; One-Way ANOVA.



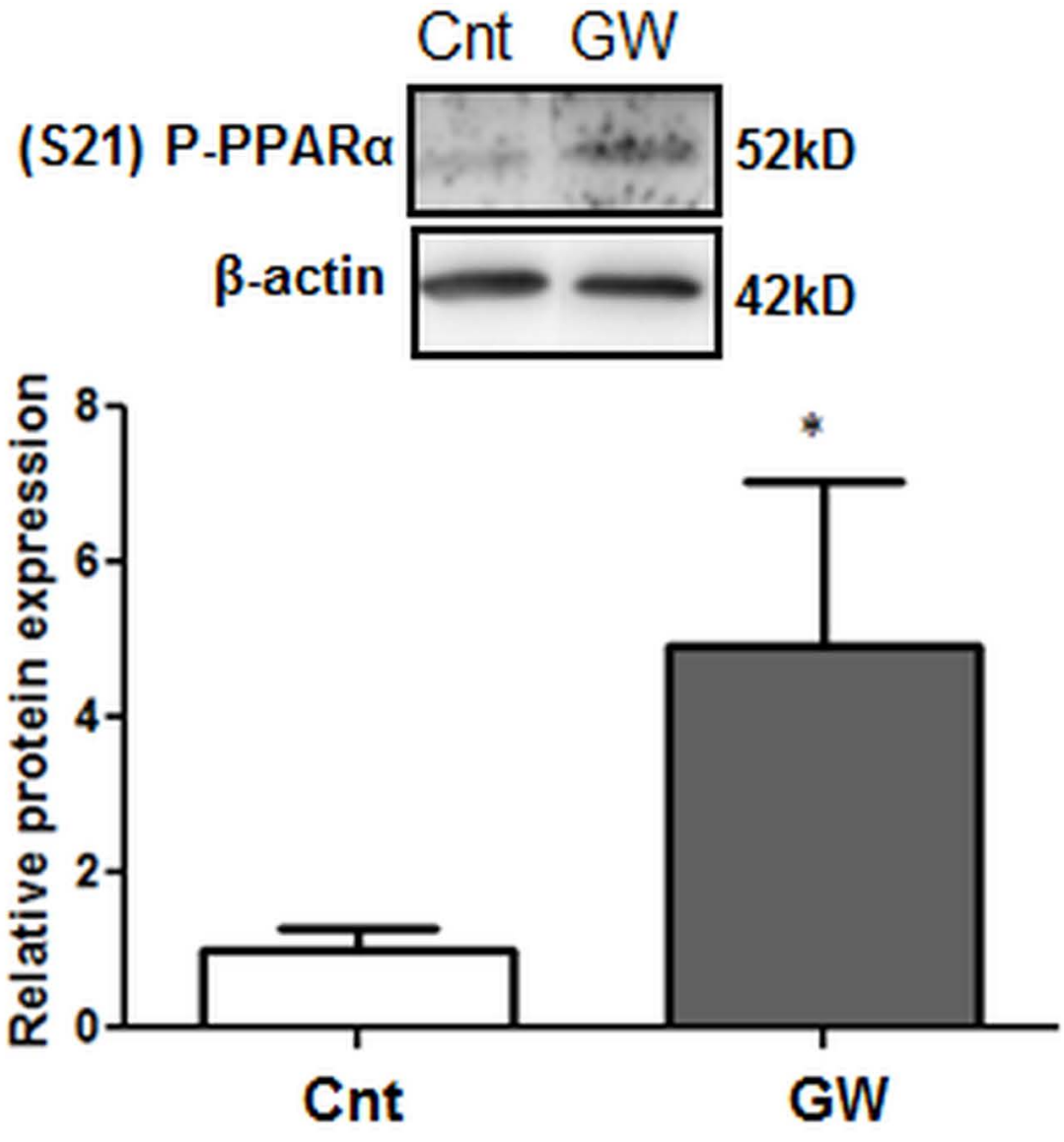
Supplementary Figure C. Effect of transfections and treatments on hCG secretion in PHT cells. **(i)** PHTs were transfected with Scr, p38 or PPARα siRNA as described in the methods. Conditioned media was collected at time points indicated for hCG ELISA. hCG levels were normalized against Scr-siRNA controls at 18 h and reported as fold change. Mean + sem, n = 4; Two-Way ANOVA. hCG secretion was significantly different from 18 h to 42 h, 66 h and 90 h ($P<0.0001$). p38-siRNA or PPARα-siRNA transfections did not significantly alter hCG secretion from Scr-siRNA transfected controls. **(ii)** PHTs were isolated and cultured for 66 h prior to treatment with insulin (5.8 ng/ml) and/or adiponectin (5 μg/ml) and cell culture media collected for hCG ELISA at 90 h. Culture media hCG levels were normalized against vehicle control and reported as fold change. Mean + sem, n = 4; One-Way ANOVA.



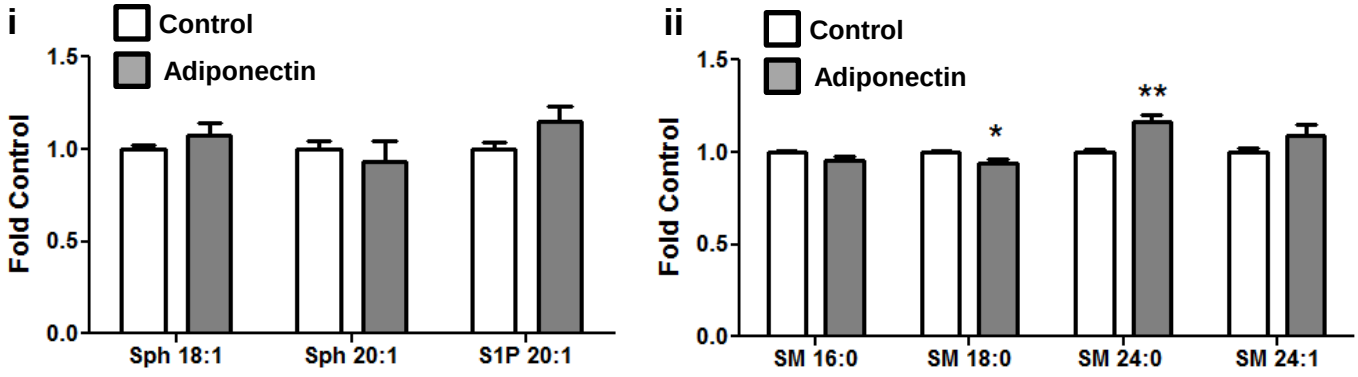
Supplementary Figure D. Effect of cell culture treatments on PHT cell viability and apoptosis. PHTs were treated with insulin (5.8 ng/ml), adiponectin (5 µg/ml), insulin + adiponectin, SB203580 (20 µM), GW7647 (0.1 µM), C2-ceramide (10 µM or 20 µM) and fumonisin B1 (50 µM or 100 µM) as described in methods. (i) Cell viability presented as a percentage of vehicle control. (ii) PARP and Caspase-3 mediated apoptosis was measured by western blot analyses as described in methods. C_2-Cer (10 µM) and FB1 (50 µM) were chosen for all experiments described in this study. Mean + sem, n = 6; One-Way ANOVA ***P <0.001. Cnt, control; Ins, insulin; ADN, adiponectin; SB, SB203580; GW, GW7647; Cer, C_2-ceramide; FB1, fumonisinB1.

Gene	Oligonucleotide sequence	GeneBank Accession Number
Ceramide Synthase 1	Forward 5'-TGGTTCCTGTACATCGTGGC-3' Reverse 5'-CTCAGTGGCTTCTCGGCTTT-3'	NM_021267.3
Ceramide Synthase 2	Forward 5'-GCTGGAGTCAGCCAAGATGT-3' Reverse 5'-AGGATCCAGAAGGGCAGGAT-3'	NM_181746.3
Ceramide Synthase 3	Forward 5'-GGAGGAGGTGGTGAAACAGG-3' Reverse 5'-TCCAACCAGCTTCGTTCTCC-3'	NM_178842.3
Ceramide Synthase 4	Forward 5'-CTGCTGGAGGCCTGTAAGAT-3' Reverse 5'-GCAACATCAGAAGCCCGTTG-3'	NM_024552.2

Supplementary Figure E. Primer sequences used in this study.
Primers were designed using the NCBI primer designer tool http://www.ncbi.nlm.nih.gov/tools/primer-blast/



Supplementary Figure F. PPARα agonist GW7647 stimulates PPARα phosphorylation. PHTs were treated with GW7647 (0.1 μM) or vehicle control as described in methods. Western blots were performed to determine the expression of phospho-PPARα (S21). Mean + sem, n = 3; One-Way ANOVA *P<0.05. Cnt, control; GW, GW7647.



Supplementary Figure G. Influence of adiponectin on PHT sphingolipid levels.
PHTs were treated with adiponectin (5 μg/ml), lipids extracted and sphingolipid levels determined by LC/MS-MS as described in methods. Adiponectin did not affect cellular Sph or S1P levels. SM18:0 was decreased whereas SM 24:0 was increased with adiponectin, therefore total SM levels were unlikely to be significantly altered. Sphingolipid species identified with respective standards were normalized against vehicle controls and reported as fold change. Mean + sem, n = 5; One-Way ANOVA *P<0.05, **P<0.01. Sph, sphingosine; S1P, sphingosine-1-phosphate; SM, sphingomyelin.

Supplementary Table A. Mass values and HPLC retention times of quantified sphigolipids

		$[M+H]^+$ (*m/z*)			
Name	Formula	Calculated	Observed	Error (ppm)	rt (min)[1]
Sphinganine (d18:0)	$C_{18}H_{39}NO_2$	302.3059	302.3062	1.0	12.6
Sphinganine (d20:0)	$C_{20}H_{43}NO_2$	330.3372	330.3376	1.2	13.9
Sphingosine (d18:1)	$C_{18}H_{37}NO_2$	300.2902	300.2903	0.2	12.2
Sphingosine (d20:1)	$C_{20}H_{41}NO_2$	328.3215	328.3218	0.8	12.3
Sphingosine-1-phosphate (d20:1)	$C_{20}H_{42}NO_5P$	408.2879	408.2885	1.5	12.9
Ceramide (d18:1/14:0)	$C_{32}H_{63}NO_3$	510.4886	510.4893	1.4	26.3
Ceramide (d18:1/16:0)	$C_{34}H_{67}NO_3$	538.5199	538.5190	-1.6	28.9
Ceramide (d18:1/18:1)	$C_{36}H_{69}NO_3$	564.5355	564.5355	-0.1	29.6
Ceramide (d18:1/18:0)	$C_{36}H_{71}NO_3$	566.5512	566.5515	0.5	31.3
Ceramide (d18:1/20:0)	$C_{38}H_{75}NO_3$	594.5825	594.5829	0.7	33.6
Ceramide (d18:1/22:0)	$C_{40}H_{79}NO_3$	622.6138	622.6137	-0.1	35.7
Ceramide (d18:1/24:1)	$C_{42}H_{81}NO_3$	648.6294	648.6299	0.8	35.7
Ceramide (d18:1/24:0)	$C_{42}H_{83}NO_3$	650.6451	650.6440	-1.7	37.4
Dihydroceramide (d18:0/16:0)	$C_{34}H_{69}NO_3$	540.5355	540.5360	0.9	29.7
Dihydroceramide (d18:0/18:0)	$C_{36}H_{73}NO_3$	568.5668	568.5663	-1.0	32.1
Sphingomyelin (d18:1/16:0)	$C_{39}H_{79}N_2O_6P$	703.5754	703.5762	1.2	26.2
Sphingomyelin (d18:1/18:0)	$C_{41}H_{83}N_2O_6P$	731.6067	731.6072	0.7	29.8
Sphingomyelin (d18:1/24:0)	$C_{47}H_{95}N_2O_6P$	815.7006	815.7013	1.0	41.5
Sphingomyelin (d18:1/24:1)	$C_{47}H_{93}N_2O_6P$	813.6849	813.6858	1.1	36.5

[1]rt, retention time; see Methods section for HPLC conditions.