Supplementary Information

G0/G1 switch gene 2 plays a critical role in adipocyte differentiation

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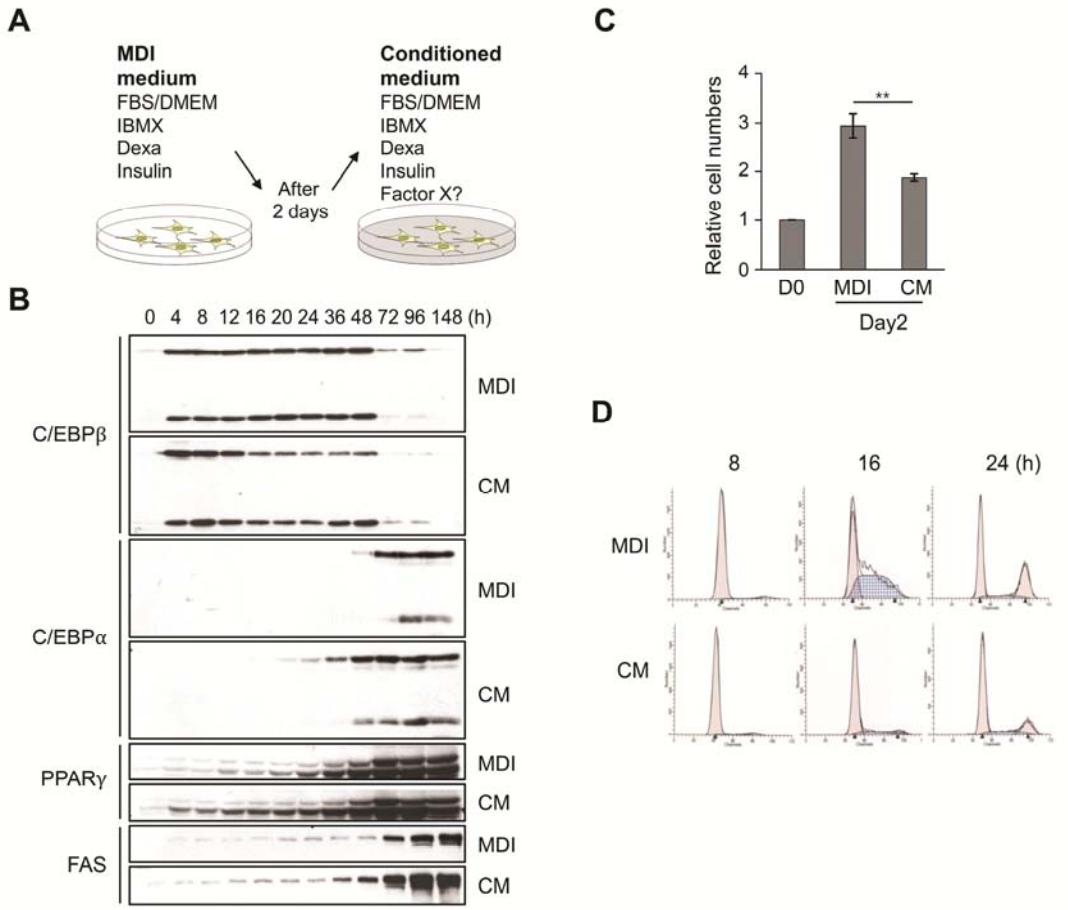
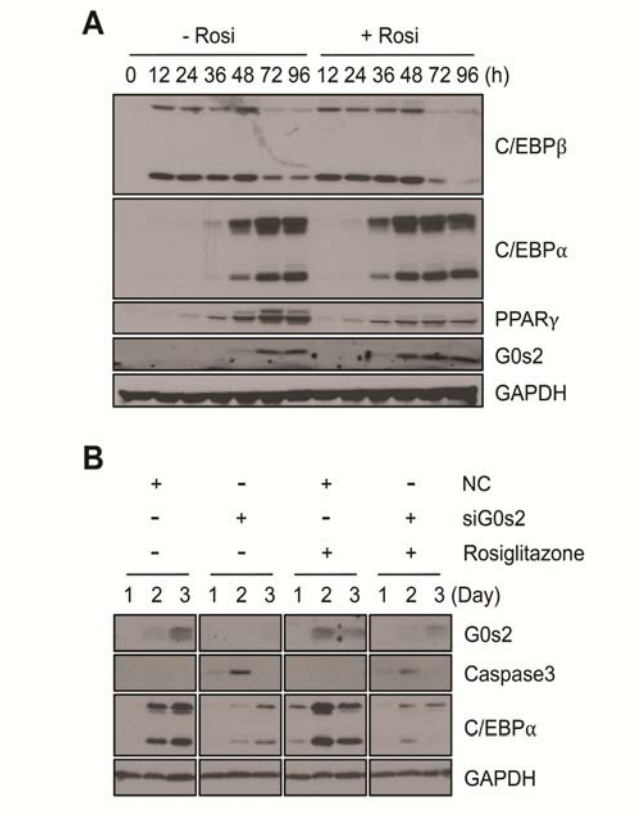
Supplementary Figure S1



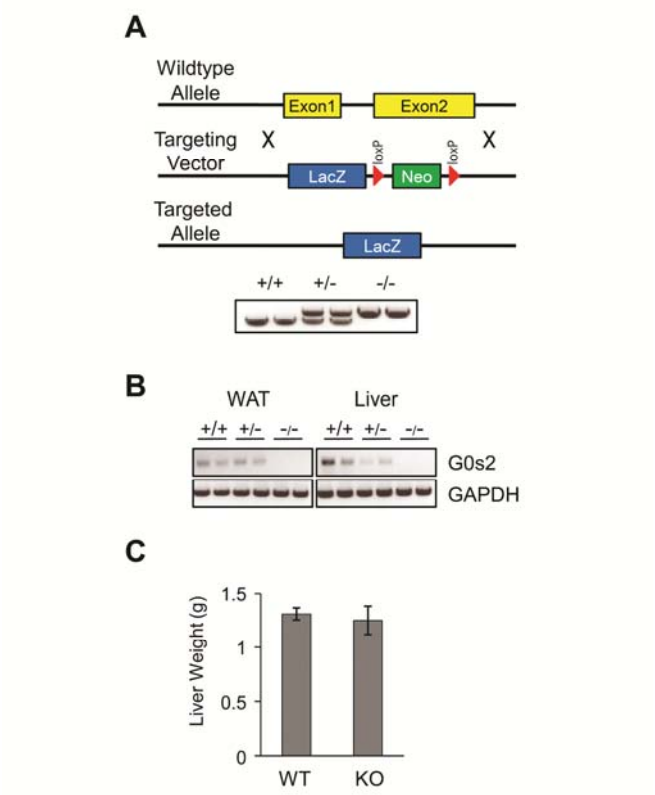
Supplementary Figure S1. Effect of conditioned medium on the 3T3-L1 differentiation. **(A)** Design of collecting conditioned medium using a preadipocyte differentiation system. 3T3-L1 preadipocytes were induced to differentiate into adipocytes with a mixture of hormonal agents (MDI medium), and after 2 days, cell-exposed medium was collected and designated as conditioned medium (CM). **(B)** 3T3-L1 cells were differentiated either in MDI or CM and then cell lysates were prepared at various times after adipogenic induction, subjected to SDS-PAGE and transfer to immunoblots, and these were hybridized with antibody against C/EBPβ, C/EBPα, PPARγ, or FAS. **(C)** Cell counts at day 2 after induction. Cells were induced with MDI or CM, and they were counted at day 2. **(D)** FACS analysis of DNA content of 3T3-L1 preadipocytes induced with MDI or CM. After induction, cells were harvested at 8, 16, or 24 h for FACS analysis using a FACS Calibur flow cytometry system. The data were analyzed by ModFit software.

Supplementary Figure S2



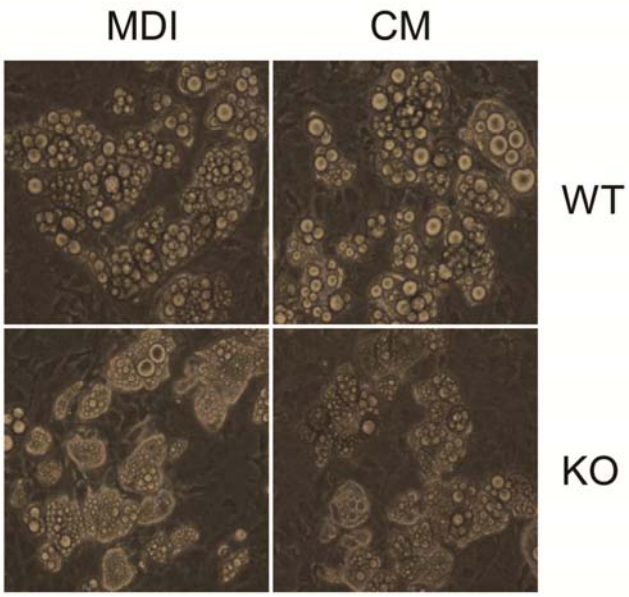
Supplementary Figure S2. Rosiglitazone enhances G0s2 expression and promotes fat accumulation. **(A)** 3T3-L1 cells were harvested at the indicated times after treatment with rosiglitazone, and western blot analysis of C/EBPβ, C/EBPα, PPARγ, and G0s2 expression was carried out, with GAPDH as loading control. **(B)** Western blot analysis showing expression of G0s2, caspase 3, C/EBPα, and GAPDH in 3T3-L1 cells transfected with negative control or G0s2 siRNA, in the presence or absence of 2 μM rosiglitazone.

Supplementary Figure S3



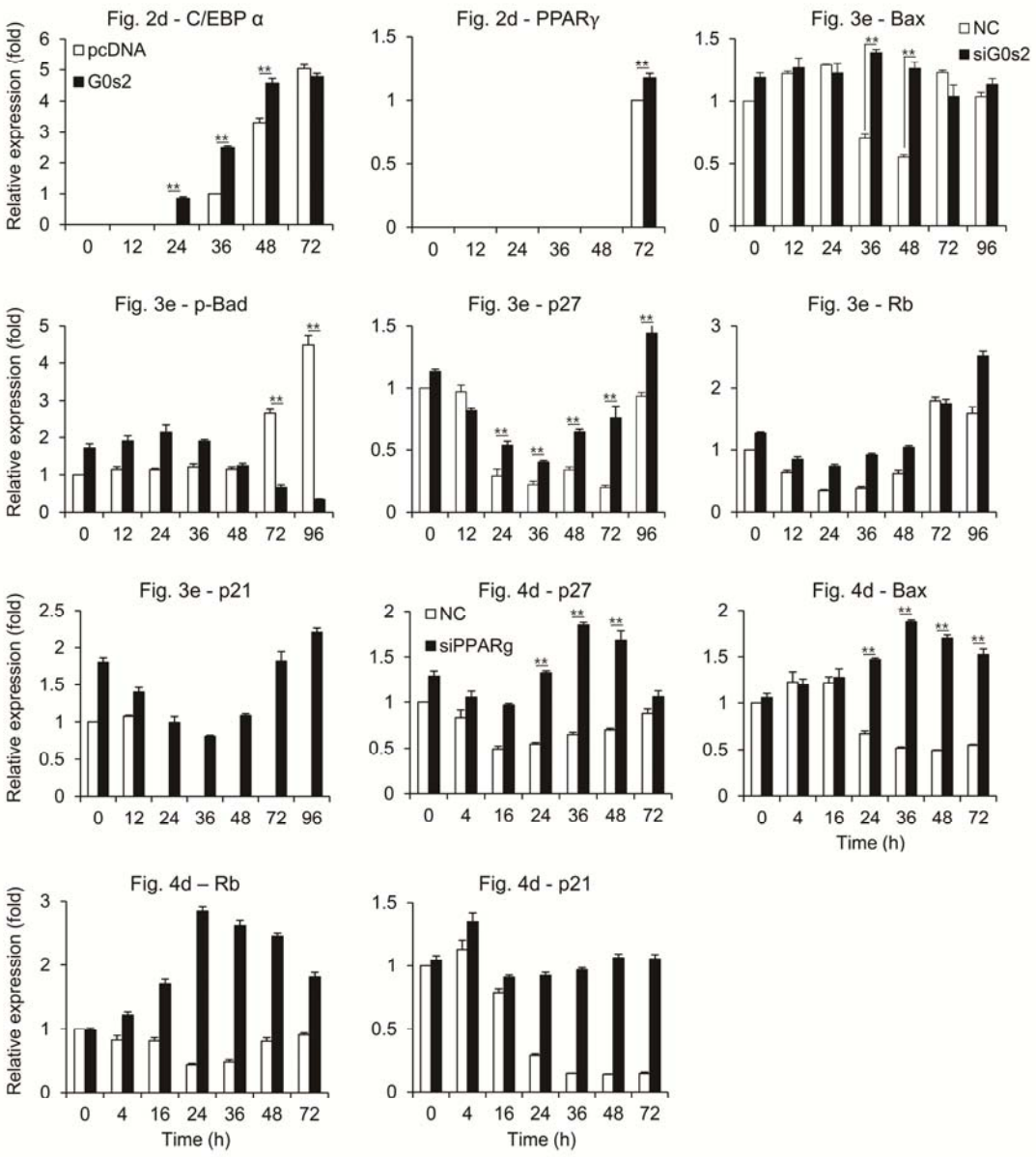
Supplementary Figure S3. Generation and characterization of G0s2-knockout mice. **(A)** The G0s2 gene was replaced with a LacZ-neo cassette by homologous recombination. Genotyping of mice was performed on genomic DNA by PCR. Expected band sizes for G0s2 during gel analysis were 526 bp for the wild-type and 610 bp for the knockout allele. Results shown for G0s2 wild-type (+/+), heterozygote (+/–), or homozygote (–/–). **(B)** RT-PCR analysis of genes of white adipose tissue and liver from control and G0s2-knockout mice. **(C)** Liver weight was evaluated in G0s2-knockout and wild-type control mice at the age of 12 weeks.

Supplementary Figure S4

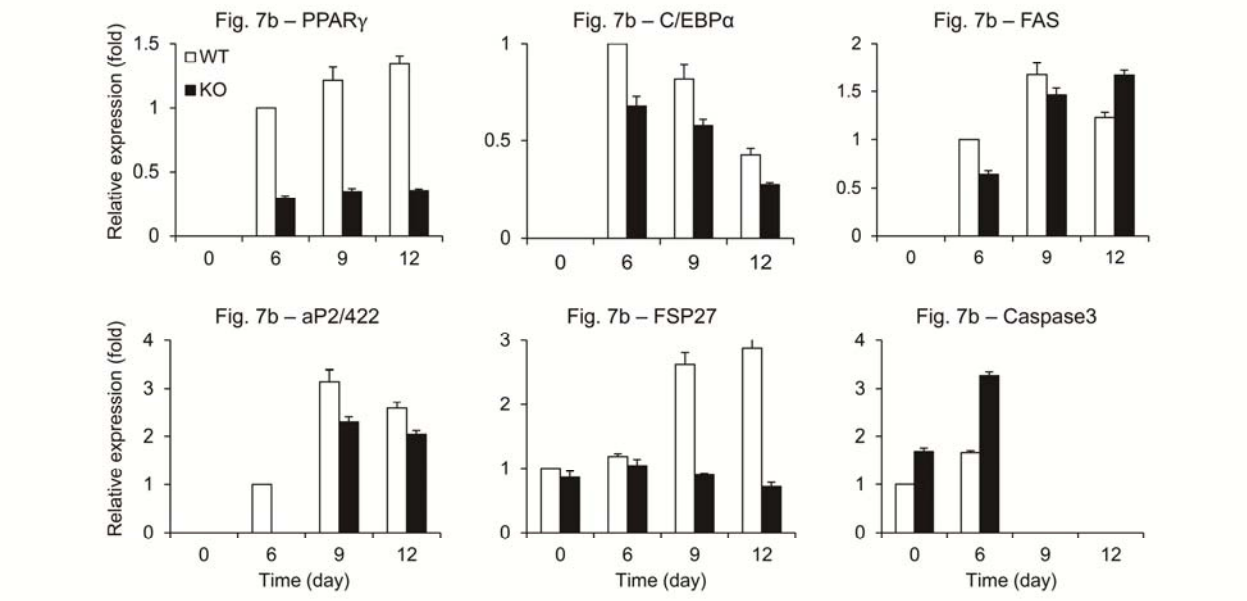


Supplementary Figure S4. CM did not affect the differentiation pattern in G0s2-knockout mouse embryo fibroblast (MEF) cells. MEFs from wild-type (WT) or G0s2-knockout (KO) mice were induced for adipocyte differentiation, either by MDI or CM supplemented with rosiglitazone, and microscopy was done at day 12.

Supplementary Figure S5



Supplementary Figure S5. Quantification analysis of the western blot data. Western blot results were analyzed to quantify signal intensities using ImageJ after normalization to GAPDH levels.



Supplementary Figure S5. Continued

Supplementary Table S1

Supplementary Table S1. Sequences of oligonucleotide primers used for quantitative real-time PCR (qPCR)

Oligonucleotide		Sequence (5'→3')
Gas1	Sense	GAATCGGTCAAAGAGAACAT
	Antisense	GTCGTCATATTCTTCGTCGT
Gas2	Sense	CACTGAAGAGAGTTCCTTGC
	Antisense	TTCATCAACTCCCAAATCTC
Gas5	Sense	ATGAAGGCTTACGAG
	Antisense	TTAAAGCTATCGTCACCCCA
Gas6	Sense	CTGCCAAGATATCGATGAAT
	Antisense	TTCTCCTTGGAGCTGTATGT
G0s2	Sense	AAAGTGTGCAGGAGCTGAT
	Antisense	CCAGCACGTATAGCTTCACT
C/EBPα	Sense	TGGACAAGAACAGCAACGAG
	Antisense	TCACTGGTCAACTCCAGCAC
PPARγ2	Sense	TATGGGTGAAACTCTGGGAG
	Antisense	GCTGGAGAAATCAACTGTGG
aP2/422	Sense	TCTCCAGTGAAAACTTCGAT
	Antisense	TTACGCTGATGATCATGTTG
L32	Sense	GCCTCTGGTGAAGCCCAAGATCG
	Antisense	CTCTGGGTTTCCGCCAGTTTCGC