Supporting Information

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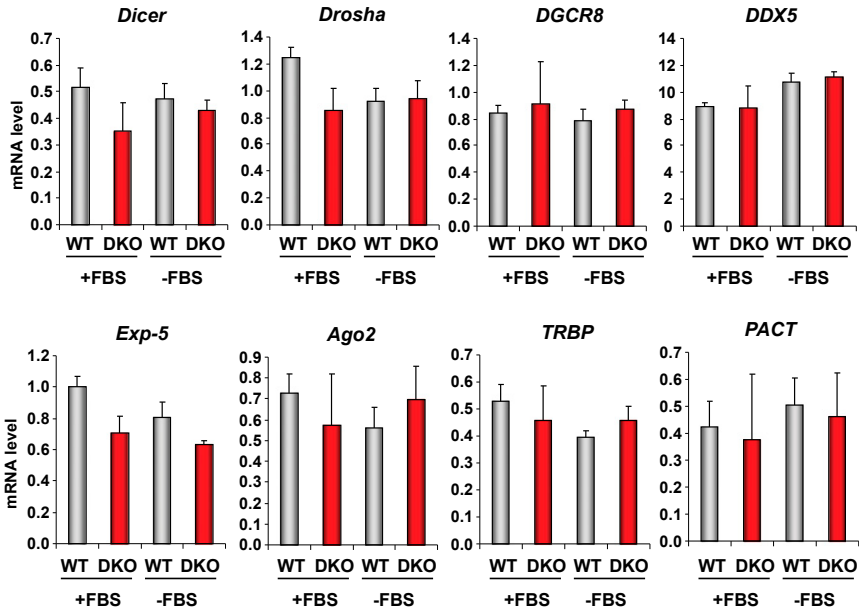

Fig. S1. Expression of proteins playing a role in microRNA (miRNA) formation or maturation. *Dicer, Drosha, DGCR8, Exp-5, Ago2, TRBP, PACT*, or *DDX5* mRNA levels were measured by real-time PCR in WT and double-knockout (DKO) cells in the presence (+FBS) or absence (−FBS) of serum for 6 h. Results represent the average ± SEM of six independent experiments.

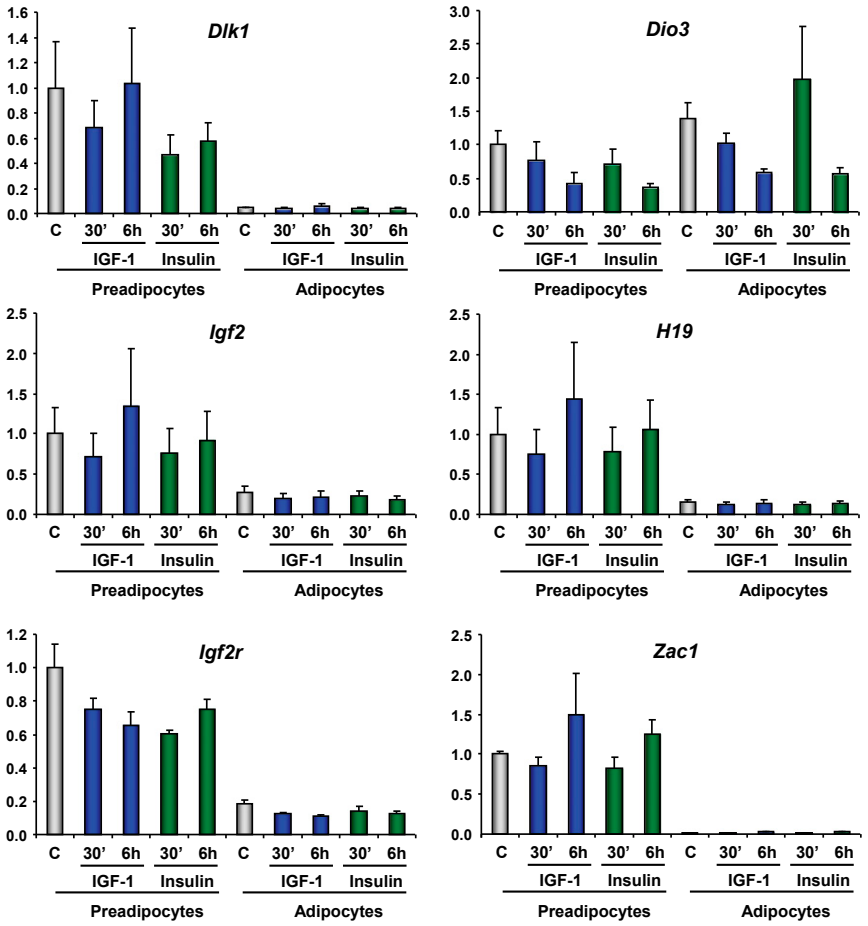

Fig. S2. Insulin and IGF1 gene regulation in nondifferentiated or differentiated brown preadipocytes. WT brown preadipocytes or differentiated adipocytes were serum-deprived for 1 h in DMEM plus 0.1% BSA and were treated with 1 nM insulin or IGF1 for 30 min or 6 h. Gene expression was measured by real-time PCR. Results represent the average ± SEM of three independent experiments.

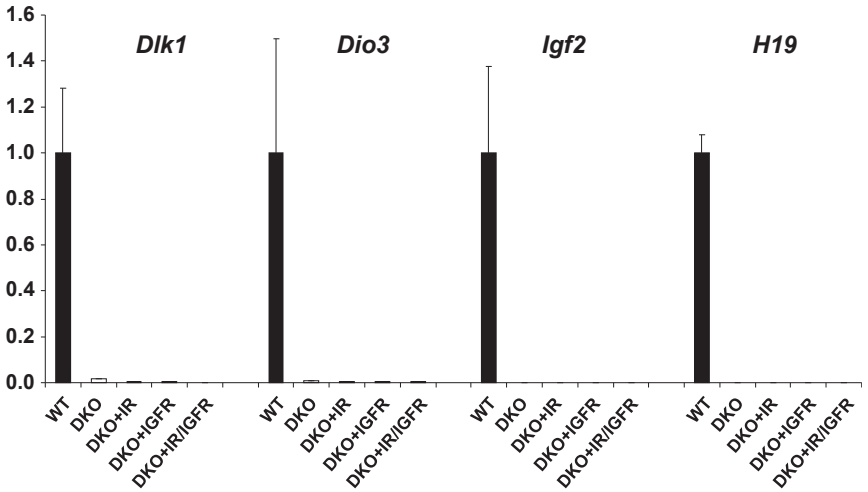

Fig. S3. Abundance of *Dlk1, Dio3, Igf2,* and *H19* mRNA was measured by real time PCR in WT, DKO, and DKO cells reexpressing hIR, hIGFR, or both, in the presence of serum. Results represent the average ± SEM of three independent experiments.

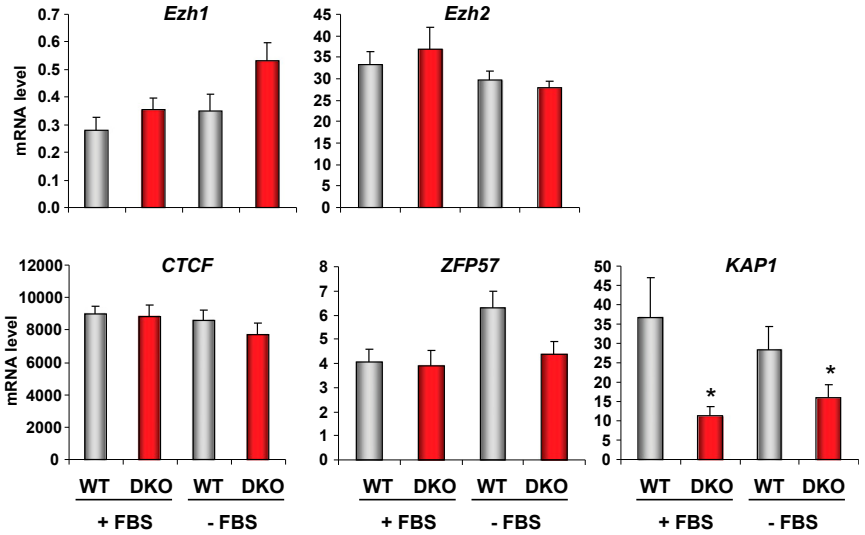

Fig. S4. mRNA levels in WT and DKO cells. mRNA levels *of Ezh1, Ezh2, CTCF, ZFP57*, and *KAP1* were measured by real-time PCR in WT and DKO cells in the presence (+FBS) or absence (−FBS) of serum for 6 h. Results represent the average ± SEM of six independent experiments. *$P < 0.05$ compared with WT cells.

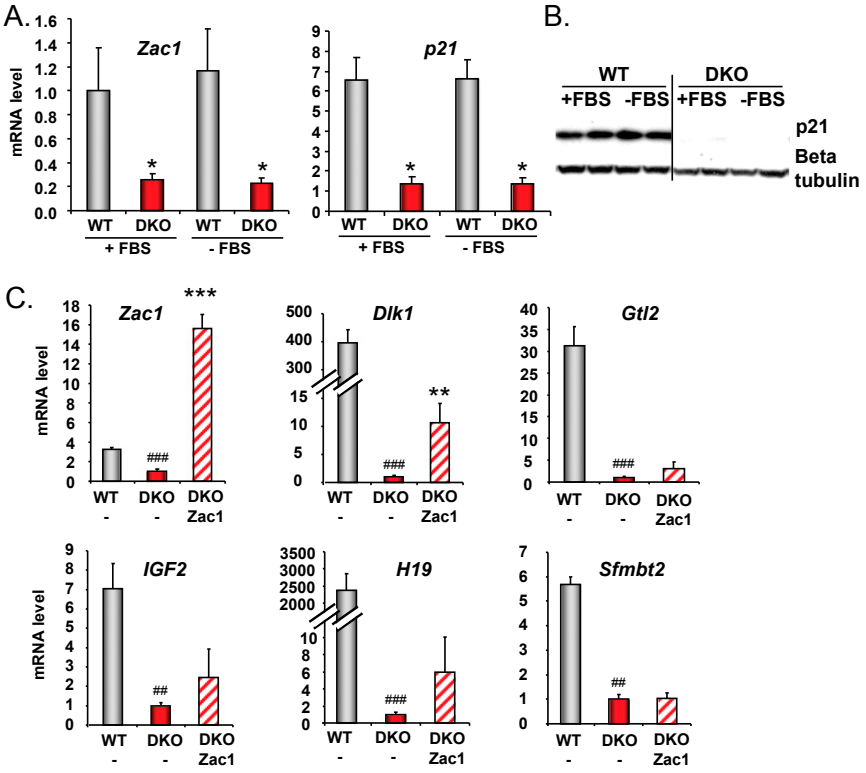

Fig. S5. Zac1 levels and overexpression in DKO cells. (*A*) mRNA levels of Zac1 and p21 were measured by real-time PCR in WT and DKO cells in the presence (+FBS) or absence (−FBS) of serum for 6 h. Results represent the average ± SEM of six independent experiments. (*B*) p21 and beta-tubulin protein levels were quantified in WT and DKO confluent cells maintained in the presence or absence of serum for 6 h. One representative Western blot from three independent experiments is shown. (*C*) Expression of imprinted genes in DKO cells stably overexpressing Zac1 and WT and DKO cells stably expressing the empty control pBabe vector were measured by real-time PCR. Results represent the average ± SEM of six independent experiments. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ (compared with DKO cells expressing the control vector); $^{\#}P < 0.05$; $^{\#\#}P < 0.01$; $^{\#\#\#}P < 0.001$ (compared with WT cells expressing the control vector).

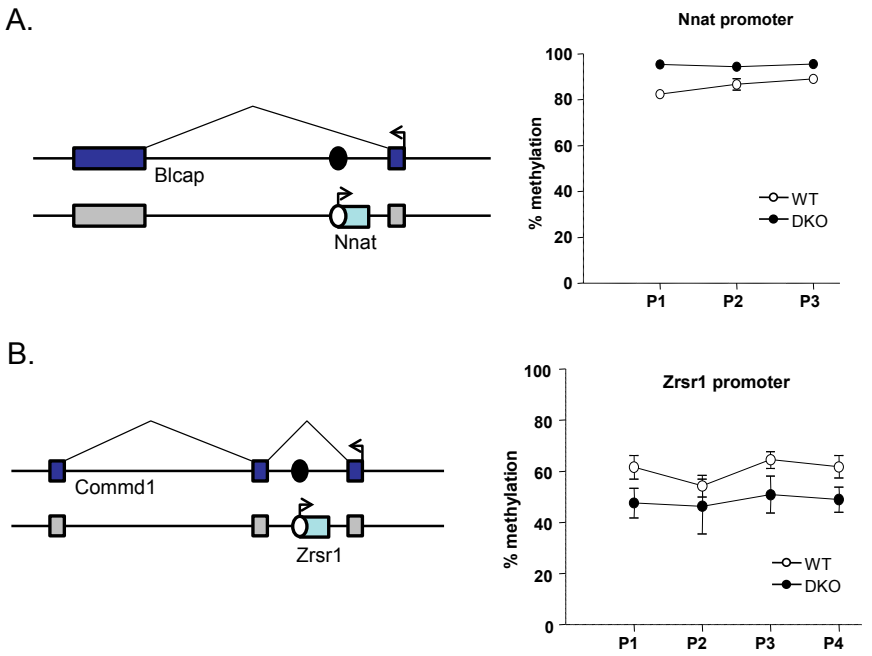

Fig. S6. Methylation analysis of the Nnat and Zrsr1 promoters. Schematic representation of imprinted loci and methylation analysis of Nnat (*A*) and Zrsr1 (*B*) promoters by pyrosequencing after bisulfite treatment of DNA from confluent WT and DKO cells.

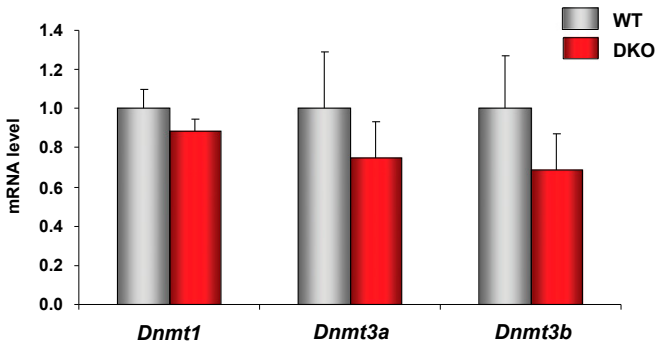

Fig. S7. DNA methyl transferase mRNA expression. mRNA levels of DNA methyltransferase enzymes were measured by real-time PCR in WT and DKO cells in the presence of serum. Results represent the average ± SEM of four independent experiments.

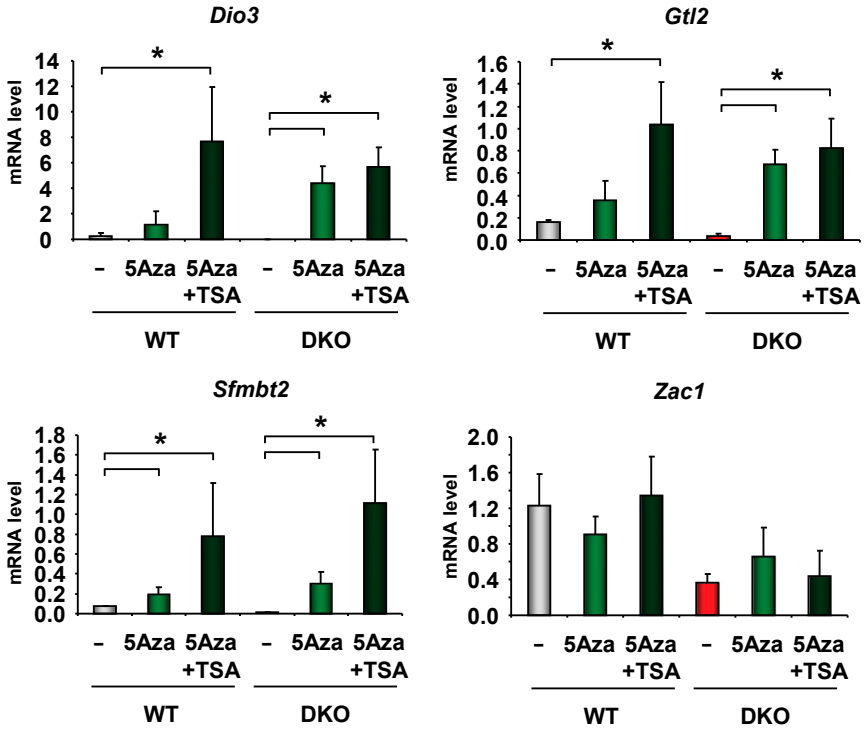

Fig. S8. Reactivation of imprinted genes by 5-Azacytidine (5Aza) and trichostatin A (TSA) treatment. Proliferating WT and DKO cells were treated with 0.2 μM 5Aza for 6 d, with or without 1 μM TSA for the last 18 h. Gene expression was measured by real-time PCR. Results represent the average ± SEM of three independent experiments. *$P < 0.05$ compared with untreated cells.

Table S1. List of imprinted genes expressed in either WT or DKO cells

Gene symbol	Average WT	SEM WT	Average DKO	SEM DKO	*P* value	Ratio DKO/WT
Zrsr1	1,167	138	1,993	64	0.002	1.71
Kcnq1ot1	308	74	342	79	0.763	1.11
Copg2	3,198	335	3,047	218	0.717	0.95
Ube3a	6,232	1,416	5,720	755	0.760	0.92
Commd1	15,331	2,259	13,145	2,153	0.504	0.86
Sgce	7,766	1,015	6,579	952	0.419	0.85
Mcts2	3,823	588	3,138	272	0.333	0.82
Airn	439	77	351	51	0.370	0.80
Tssc4	4,536	341	3,100	53	0.013	0.68
Dhcr7	2,817	401	1,836	222	0.074	0.65
H13	8,945	1,687	5,786	360	0.135	0.65
Blcap	2,767	384	1,663	143	0.042	0.60
Cd81	32,596	3,552	19,236	1,410	0.016	0.59
Osbpl5	2,276	407	1,185	115	0.053	0.52
Grb10	303	59	149	19	0.059	0.49
Ddc	380	110	150	40	0.105	0.39
Plagl1	14,886	4,910	5,560	1,362	0.132	0.37
Igf2r	2,018	549	592	140	0.059	0.29
Slc38a4	5,675	608	1,218	253	0.001	0.21
Nnat	421	157	64	15	0.086	0.15
Tfpi2	561	169	41	21	0.037	0.07
Mirg	1,012	259	70	18	0.022	0.07
Mest	10,179	3,292	680	193	0.045	0.07
Ndn	2,460	1,135	61	27	0.102	0.02
Igf2	5,163	3,799	84	32	0.252	0.02
Cdkn1c	4,444	2,089	31	12	0.102	0.01
Dlk1	25,160	8,707	136	16	0.045	0.01
H19	65,694	16,620	162	18	0.017	0.00

Averages of Affymetrix values for imprinted genes expressed either in WT or DKO cells are listed (all genes for which expression value were <300 have been eliminated. For genes with two or more probe sets, only the probe set with the highest Affymetrix value was kept). $n = 5$ per group.