Supporting Information

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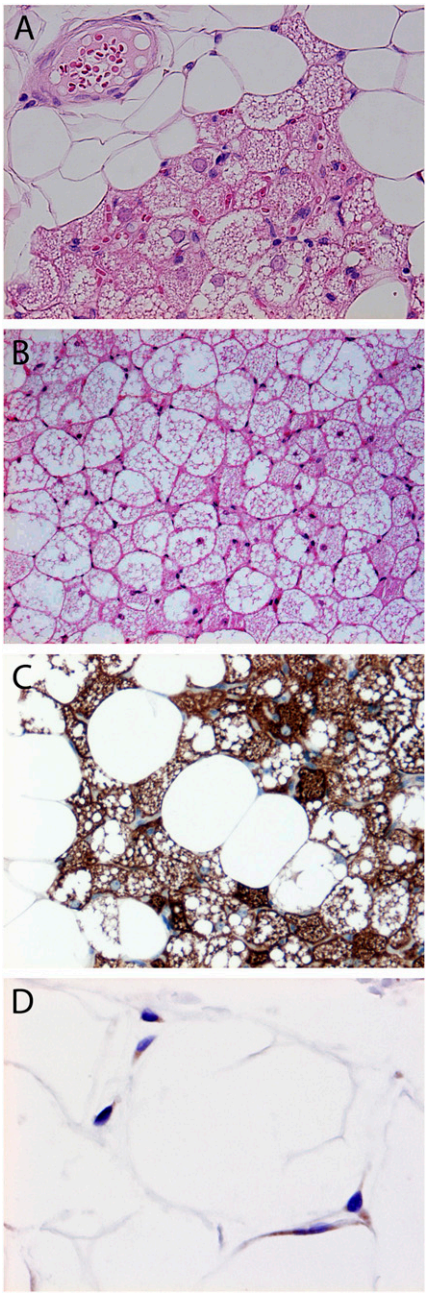

Fig. S1. Characteristics of normal and neoplastic adipose tissues. (*A*) Brown and white adipocytes are intermingled in normal brown fat from the parietal pleura of a healthy 18-y-old man. Whereas the white adipocytes have a single, large vacuole and a peripherally located nucleus, the brown adipocytes display a multivacuolated cytoplasm and a centrally located nucleus. (*B*) These features are very similar to the morphological characteristics of hibernoma. (*C*) UCP1 is highly expressed in multivacuolated cells of hibernomas, whereas univacuolated cells of hibernomas and (*D*) lipomas are only weakly positive or negative for UCP1.

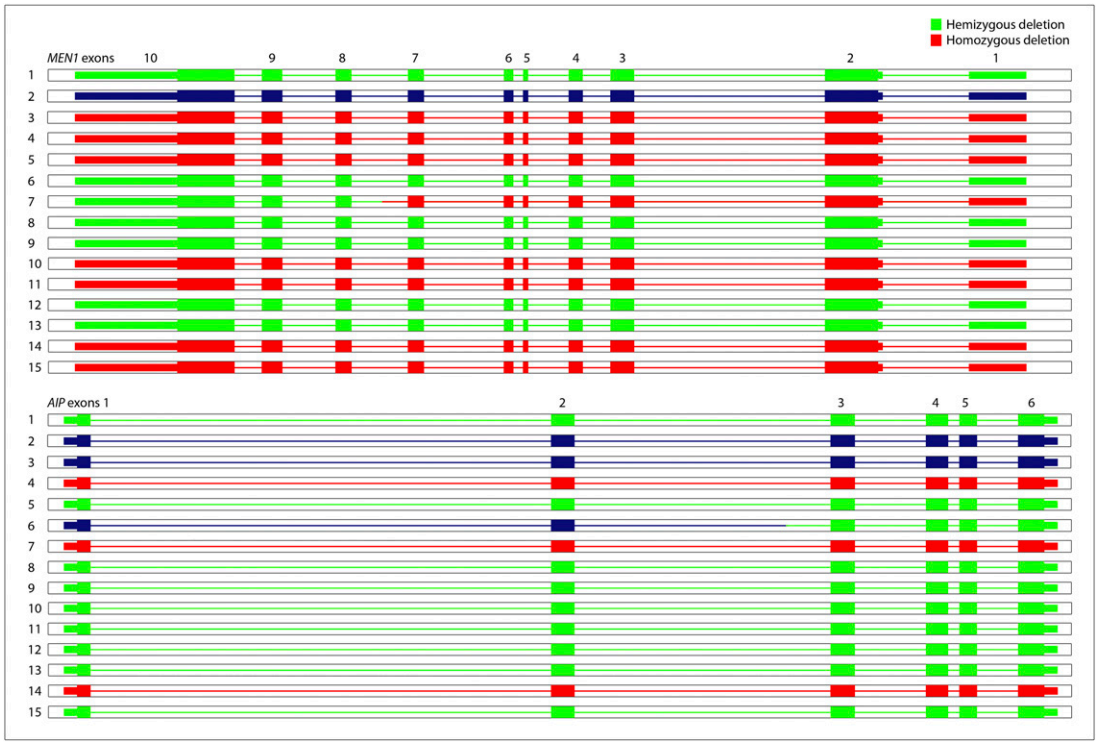

Fig. S2. Genomic aberrations of *MEN1* and *AIP* detected by SNP array. All cases with an aberrant SNP array profile displayed deletions in 11q13. In case 2, the genomic profile was normal. Eight tumors showed homozygous deletion of *MEN1* and three showed homozygous loss of *AIP*. In case 7, *MEN1* exons 1 to 7 were homozygously deleted and exons 8 to 10 were hemizygously lost. The remaining cases demonstrated hemizygous deletion of *MEN1* and all but one showed hemizygous loss of *AIP*. In case 6, *AIP* exons 1 and 2 displayed a normal copy number and exons 3 to 6 were hemizygously lost. The genomic positions of all alterations are available in Table S2.

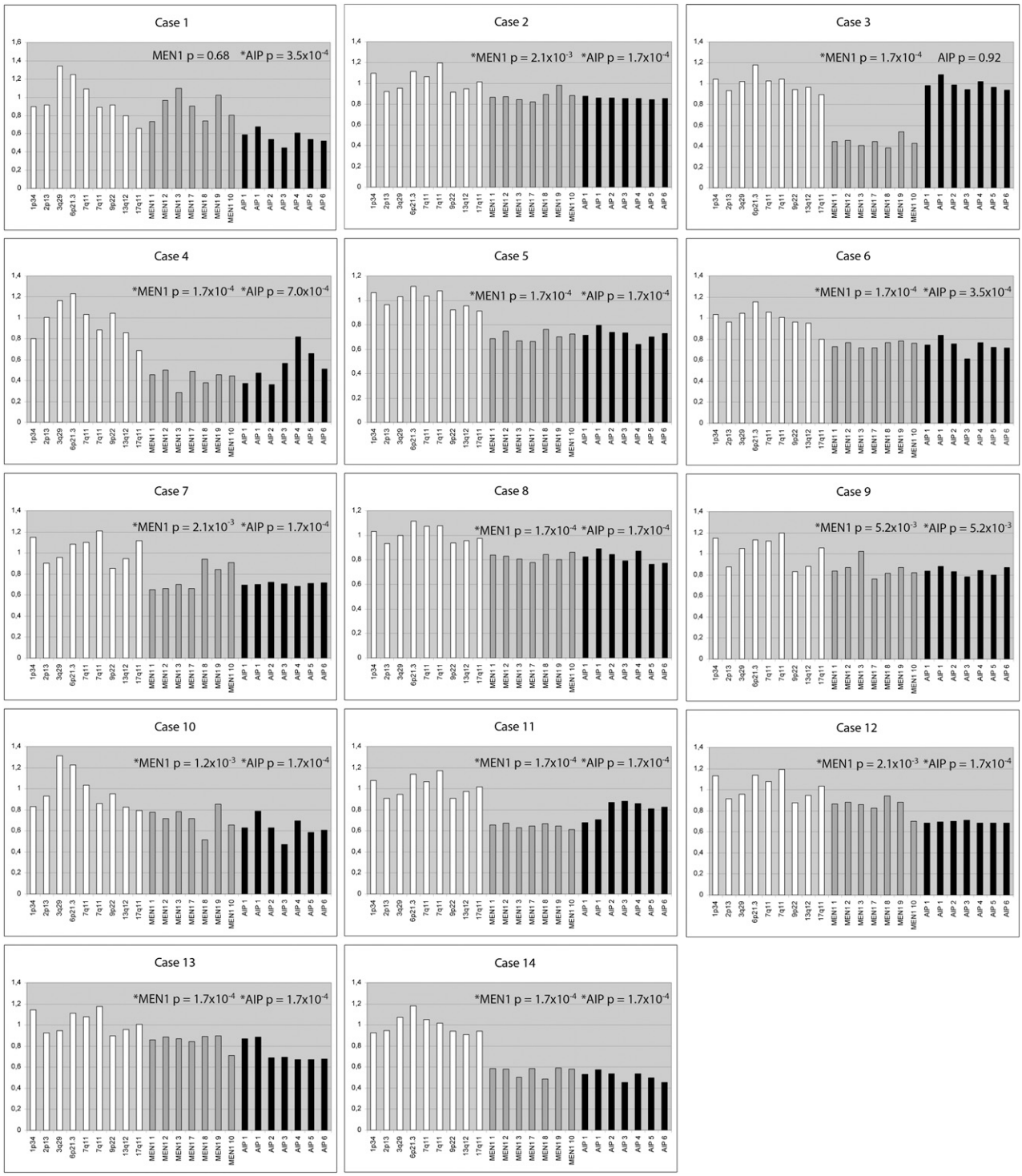

Fig. S3. Deletions of *MEN1* and *AIP* were confirmed by MLPA. Using SNP array, *MEN1* and *AIP* were found to be hemizygously deleted in six and 10 cases and homozygously lost in eight and three cases, respectively. The results were confirmed by MLPA analysis in cases 1 to 14, with two exceptions. A hemizygous deletion of *MEN1* detected in case 1 by SNP array was not statistically significant by MLPA analysis. Case 2 displayed no alterations by SNP array whereas MLPA showed deletions of both genes. Discrimination between hemi- and homozygous deletions by using MLPA was difficult as a result of normal cell contamination. Use of peak area or height in the calculations gave the same results. In the figure, calculations based on peak height are shown. Statistical analyses were performed using the Mann–Whitney *U* test and *P* values <0.05 were considered significant, indicated by asterisks.

Table S1. Clinical and cytogenetic data

Case	Case no. in previous publication*	Sex/age at diagnosis, y	Site	Size (largest diameter), cm	Karyotype†
1	12980:52	F/51	Thigh	20	46,XX,t(**11**;17;12)(**q13**;q12;p13)
2	12980:53	M/38	Arm	15	46,XY,t(2;**11**)(p21;**q13**),ins(17;12)(q21;p11p13)/46,XY, der(11)t(2;**11**)(p21;**q13**),del(12)(p11),add(17)(q21)
3	12980:50	F/25	Thigh	14	46,XX,t(6;14;**11**)(p21;q11;**q13**)
4	5798:1	M/36	Thigh	13	46,XY,inv(**11**)(p15**q21**),ins(12;?)(p12;?)/46,idem, t(5;9)(q12;q34)
5	9210:1	M/22	Thigh	12	46,XY,del(**11**)(**q13q13**),der(11)t(**11**;17)(**q13**;p13), der(17)t(**11**;17)(**q13**;p13)del(**11**)(**q13q13**)
6	9210:5	M/47	Buttock	18	46,XY,der(5)t(5;**11**)(q11;**q13**),der(11)del(11)(p11) del(**11**)(**q13q13**)dup(**11**)(**q13q13**)t(5;**11**),der(11) del(**11**)(**q13q13**)del(11)(q?)dup(**11**)(**q13q?**)
7	—	F/41	Thigh	6	46,XX,del(**11**)(**q13q13**),der(11)t(11;14)(p15;q11) t(**11**;16)(**q13**;p11),der(14)t(**11**;14)(**q13**;q11),del(16) (p11)/47,idem,+del(16)(p11)
8	—	F/32	Thigh	8	No karyotype
9	—	F/40	Shoulder	7	No karyotype
10	—	M/73	Buttock	>10	No karyotype
11	—	F/47	Groin	7	No karyotype
12	—	M/43	Thigh	7	No karyotype
13	—	M/74	Thigh	22	No karyotype
14	12980:51	F/16	Thigh	9	No karyotype
15	—	F/42	Back	6	45,XX,-11,der(12)inv(12)(q13q24)t(**11**;12)(**q13**;q13) inv(11)(q14q23)

*Reference numbers are from the Mitelman Database of Chromosome Aberrations in Cancer (http://cgap.nci.nih.gov/Chromosomes/Mitelman).
†Karyotypes are based on G-banding and FISH analysis. Rearrangements of chromosome band 11q13, or the neighboring 11q21, are indicated in bold type.

Table S2. DNA copy number aberrations detected by SNP array analysis

Case	Chromosome band	Base pair position, Mb*	Aberration	Comment†
1	11q13	64,239–64,336	Deletion	*MEN1*
1	11q13	64,341–64,512	Homozygous deletion	
1	11q13	64,512–65,035	Deletion	
1	11q13	65,546–65,679	Deletion	
1	11q13	66,964–67,023	Deletion	*AIP*
1	11q13	67,025–67,068	Homozygous deletion	
1	11q13	67,069–67,109	Deletion	
1	17q11-21	24,936–40,604	Deletion	
2				No aberration
3	6p21	34,324–34,648	Deletion	Bp in 6p21
3	8p12-p22	16,116–33,183	Deletion	
3	8p11-p12	36,765–38,738	Deletion	
3	11q13	64,222–64,314	Deletion	
3	11q13	64,316–64,363	Homozygous deletion	*MEN1*
3	11q13	64,367–64,485	Deletion	
3	14q11-12	23,830–26,464	Deletion	Bp in 14q11
3	14q12	26,857–26,930	Deletion	
4	11p15	20,047–20,076	Deletion	Bp in 11p15
4	11q12	60,999–61,277	Deletion	
4	11q13	64,236–64,318	Deletion	
4	11q13	64,320–64,505	Homozygous deletion	*MEN1*
4	11q13	64,510–64,936	Deletion	
4	11q13	65,550–65,880	Deletion	
4	11q13	66,457–66,997	Deletion	
4	11q13	67,003–67,111	Homozygous deletion	*AIP*
4	11q13	67,111–68,225	Deletion	
4	11q13	71,170–72,008	Deletion	
4	11q23	112,909–113,228	Deletion	
4	11q24	121,589–121,824	Deletion	
4	12p13	0,515–0,923	Deletion	
4	12p13	1,983–2,292	Deletion	
4	12p13	3,660–3,825	Deletion	
4	12p13	8,584–8,742	Deletion	
4	12p12	23,682–23,862	Deletion	Bp in 12p12
5	11q13	64,297–64,314	Deletion	
5	11q13	64,316–64,377	Homozygous deletion	*MEN1*
5	11q13	64,377–64,521	Deletion	
5	11q13	66,827–67,106	Deletion	*AIP*
5	11q13	67,505–67,635	Deletion	
6	5q11	55,308–55,909	Deletion	Bp in 5q11
6	11p11	45,507–45,647	Deletion	Bp in 11p11
6	11q13	63,465–63,714	Deletion	
6	11q13	64,015–64,471	Deletion	*MEN1*
6	11q13	67,013–67,148	Deletion	*AIP* exons 3–6
6	11q13	67,897–68,074	Deletion	
7	11q13	61,918–62,100	Deletion	
7	11q13	64,308–64,329	Deletion	
7	11q13	64,330–64,465	Homozygous deletion	*MEN1* exons *1–7*
7	11q13	64,466–64,897	Deletion	
7	11q13	66,827–67,006	Deletion	
7	11q13	67,007–67,097	Homozygous deletion	*AIP*
7	14q11	24,417–24,558	Deletion	Bp in 14q11
7	16p11	29,814–29,915	Deletion	Bp in 16p11
8	11q13	64,314–64,465	Deletion	*MEN1*
8	11q13	66,928–67,097	Deletion	*AIP*
9	4q28-31	131,320–140,332	Deletion	
9	11q13	63,521–64,516	Deletion	*MEN1*
9	11q13	66,922–67,157	Deletion	*AIP*
9	11q13	71,392–71,628	Deletion	
10	11q13	63,901–64,310	Deletion	
10	11q13	64,312–64,431	Homozygous deletion	*MEN1*
10	11q13	64,432–64,471	Deletion	
10	11q13	65,035–65,185	Deletion	

Table S2. Cont.

Case	Chromosome band	Base pair position, Mb*	Aberration	Comment†
10	11q13	66,034–66,225	Deletion	
10	11q13	66,933–67,104	Deletion	*AIP*
10	11q13	67,653–68,285	Deletion	
11	5p14	26,587–26,773	Gain	
11	7q21	81,686–81,853	Deletion	
11	7q31	116,515–116,742	Deletion	
11	11q13	64,308–64,483	Homozygous deletion	*MEN1*
11	11q13	64,484–67,199	Deletion	*AIP*
12	4q35	185,802–185,858	Deletion	
12	11q13	64,228–64,413	Deletion	*MEN1*
12	11q13	65,560–65,584	Deletion	
12	11q13	65,713–66,105	Deletion	
12	11q13	66,372–66,590	Deletion	
12	11q13	66,922–66,956	Deletion	
12	11q13	66,956–66,982	Homozygous deletion	
12	11q13	66,982–67,068	Deletion	*AIP*
13	6q23-26	134,529–164,298	Deletion	
13	11q13	63,031–71,227	Deletion	*MEN1*, *AIP*
14	11q13	64,260–64,318	Deletion	
14	11q13	64,320–64,494	Homozygous deletion	*MEN1*
14	11q13	64,495–64,569	Deletion	
14	11q13	65,106–65,232	Deletion	
14	11q13	65,722–65,840	Deletion	
14	11q13	66,120–66,347	Deletion	
14	11q13	66,928–67,025	Homozygous deletion	*AIP*
14	11q13	67,031–67,097	Deletion	
14	12q13	48,445–48,733	Deletion	
15	6q12-q16	66,979–97,751	Deletion	
15	11p15	0,061–3,063	Deletion	
15	11p15	10,372–11,017	Deletion	
15	11p15	11,928–12,165	Deletion	
15	11p15	12,522–13,089	Deletion	
15	11p11-q13	50,950–63,728	Deletion	
15	11q13	63,730–63,783	Homozygous deletion	
15	11q13	63,791–64,013	Deletion	
15	11q13	64,316–64,570	Homozygous deletion	*MEN1*
15	11q13	65,220–65,718	Deletion	
15	11q13	66,926–67,710	Deletion	*AIP*
15	11q23-q25	117,156–134,444	Deletion	Bp in 11q23
Normal blood DNA from case 1				No aberration
Normal blood DNA from case 2				No aberration
Normal blood DNA from case 4				No aberration
Normal blood DNA from case 6				No aberration

*Base pair positions are indicated according to the NCBI build 36 (hg18).

†Deletions in chromosome bands involved in translocations, inversions, or insertions are noted as potential breakpoints (Bp) for the rearrangements.

Table S3. Primer sequences

Designation	Sequence (5′-3′)	Position	Gene (accession no.)
MEN1-1752F	TTGCCTTGCAGGCCGCCGCC	1752–1771	MEN1 (ENSG00000133895)
MEN1-2091R	CTCGAGGATAGAGGGACAGG	2091–2110	MEN1 (ENSG00000133895)
MEN1-1909F	GGCTTCGTGGAGCATTTTCT	1909–1928	MEN1 (ENSG00000133895)
MEN1-2239R	CATGGATAAGATTCCCACCTACTGG	2239–2263	MEN1 (ENSG00000133895)
MEN1-3758F	CACAGAGGACCCTCTTTCATTAC	3758–3780	MEN1 (ENSG00000133895)
MEN1-3936R	CTTGCCGTGCCAGGTGAC	3936–3953	MEN1 (ENSG00000133895)
MEN1-3873F	CTCGCCCTGTCTGAGGATCATG	3873–3894	MEN1 (ENSG00000133895)
MEN1-4041R	TGGGTGGCTTGGGCTACTACAG	4041–4062	MEN1 (ENSG00000133895)
MEN1-4176F	GGGCCATCATGAGACATAATG	4176–4196	MEN1 (ENSG00000133895)
MEN1-4351R	CTGCCCCATTGGCTCAG	4351–4367	MEN1 (ENSG00000133895)
MEN1-4637F	CCTGTTCCGTGGCTCATAACTC	4637–4658	MEN1 (ENSG00000133895)
MEN1-4913R	CTCAGCCACTGTTAGGGTCTCC	4913–4934	MEN1 (ENSG00000133895)
MEN1-5487F	GGCTGCCTCCCTGAGGATC	5487–5505	MEN1 (ENSG00000133895)
MEN1-5720R	CTGGACGAGGGTGGTTGG	5720–5737	MEN1 (ENSG00000133895)
MEN1-6081F	GTGAGACCCCTTCAGACCCTAC	6081–6102	MEN1 (ENSG00000133895)
MEN1-6281R	TGGGAGGCTGGACACAGG	6281–6298	MEN1 (ENSG00000133895)
MEN1-6651F	GGGTGAGTAAGAGACTGATCTGTGC	6651–6675	MEN1 (ENSG00000133895)
MEN1-6876R	TGTAGTGCCCAGACCTCTGTG	6876–6896	MEN1 (ENSG00000133895)
MEN1-7053F	TCACCTTGCTCTCCCCACTG	7053–7072	MEN1 (ENSG00000133895)
MEN1-7375R	CACTCTGGAAAGTGAGCACT	7375–7394	MEN1 (ENSG00000133895)
MEN1-7212F	CCAAGAAGCCAGCACTGGAC	7212–7231	MEN1 (ENSG00000133895)
MEN1-7570R	CCCCACAAGCGGTCCGAAGTCC	7570–7591	MEN1 (ENSG00000133895)
AIP-ex1F	CCGAGACATTCCTAGGCTCC	504–523	AIP (ENSG00000110711)
AIP-ex1R	CTCTCGCCTAAGGCCTCC	883–900	AIP (ENSG00000110711)
AIP-ex2F	GGACTGGACTTCTCCTTGGG	4503–4522	AIP (ENSG00000110711)
AIP-ex2R	GTCTAGCAGAGGGTGGAGGG	4829–4848	AIP (ENSG00000110711)
AIP-ex3F	GATGGTGGTGGGGAAGG	6743–6759	AIP (ENSG00000110711)
AIP-ex3R	ACCCCTGGGTGGACAGG	7085–7101	AIP (ENSG00000110711)
AIP-ex4-5F	ATGTGGGTCAGGTCTGCTG	7527–7545	AIP (ENSG00000110711)
AIP-ex4-5R	AAAGGCTAGGTCTTGACCCC	8094–8113	AIP (ENSG00000110711)
AIP-ex6F	AGGAGACATGAGGGCAGGC	8275–8293	AIP (ENSG00000110711)
AIP-ex6R	AACAGCCACCCAAGTACCAG	8724–8743	AIP (ENSG00000110711)

Table S4. *TBP* gene expression levels

Sample	TBP*
Hibernoma	8.30
Hibernoma	8.32
Hibernoma	8.14
Hibernoma	8.41
Hibernoma	8.47
Hibernoma	8.43
Hibernoma	8.23
Hibernoma	8.14
Hibernoma	8.26
Hibernoma	8.41
Hibernoma	8.31
Hibernoma	8.44
Hibernoma	8.54
Lipoma	8.40
Lipoma	8.09
Lipoma	8.18
Lipoma	8.37
Lipoma	7.93
Lipoma	8.17
WAT	8.40
WAT	8.12
WAT	8.28
SM	8.21
SM	8.37

*RMA normalized $\log_2$ expression level.

Dataset S1. Expression data for deleted genes in 11q13 and genes up-regulated in brown fat

Dataset S1 (XLS)

By using Affymetrix Human Gene 1.0 ST arrays, the expression levels of the 132 genes located to the most frequently deleted region in 11q13 were evaluated in 14 hibernomas, 22 lipomas, and three normal WAT samples. Thirteen genes (marked in red) displayed a significantly lower expression level in hibernomas compared with lipomas and WAT (Mann–Whitney *U* test, Bonferroni corrected $P < 3.7 \times 10^{-4}$). The expression levels of 11 of these 13 genes could be evaluated in white and brown preadipocytes of mouse (GEO accession no. GSE7032). Sf1 and Ehd1 displayed a lower expression in brown compared with white preadipocytes. The remaining genes, including Men1, Aip, and Cdk2ap2, were similarly expressed in brown and white preadipocytes. The expression levels of *UCP1*, *PPARGC1A*, *PPARA*, and *PPARG* were also evaluated and these genes showed higher expression levels in hibernomas compared with lipomas and WAT (Mann–Whitney *U* test, Bonferroni corrected $P < 3.7 \times 10^{-4}$).