

## Supplemental Information

### The Obesity-Susceptibility Gene *TMEM18* Promotes

### Adipogenesis through Activation of *PPARG*

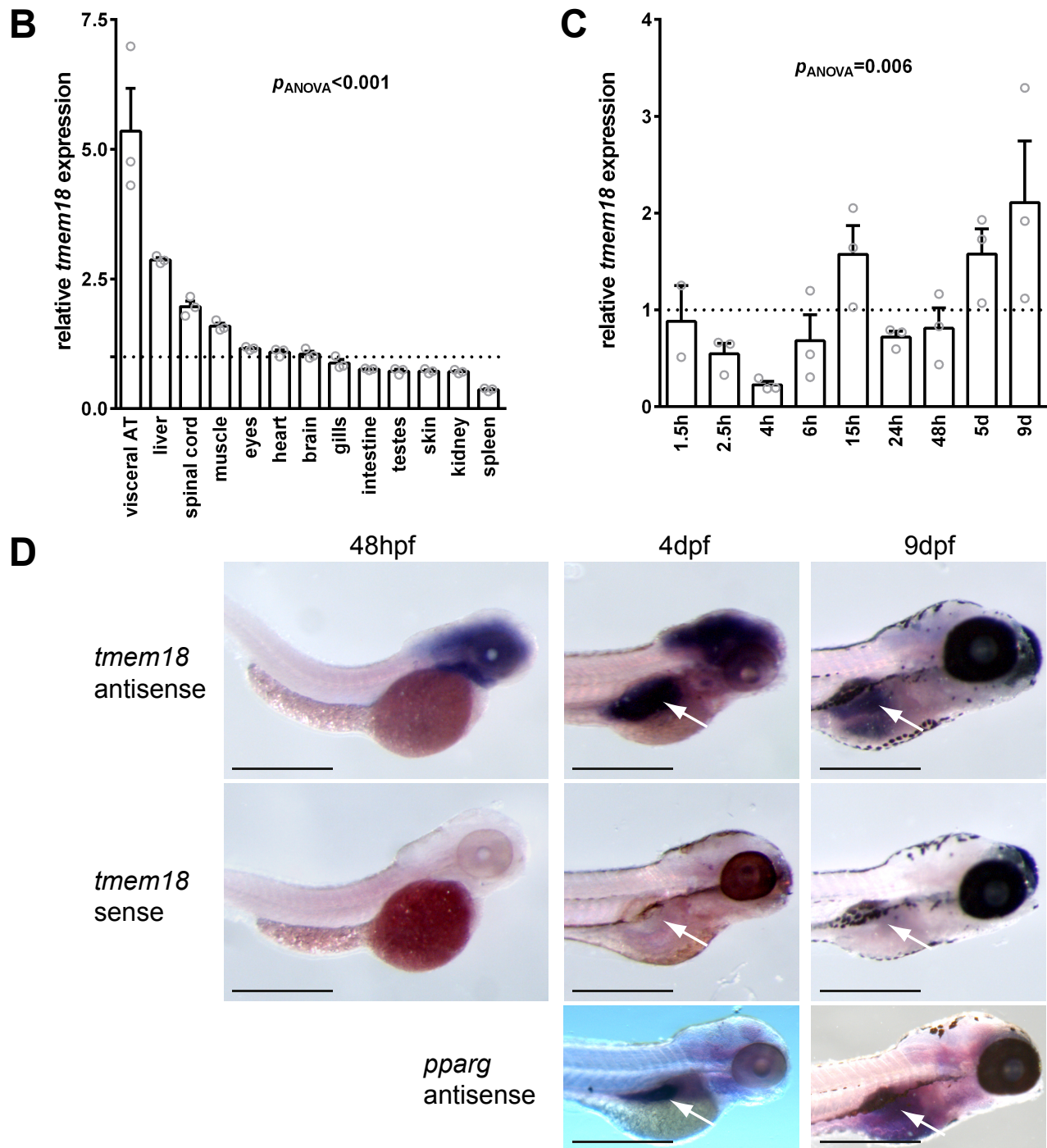
Kathrin Landgraf, Nora Klöting, Martin Gericke, Nitzan Maixner, Esther Guiu-Jurado, Markus Scholz, A. Veronica Witte, Frauke Beyer, Julian T. Schwartz, Martin Lacher, Arno Villringer, Peter Kovacs, Assaf Rudich, Matthias Blüher, Wieland Kiess, and Antje Körner

**A**

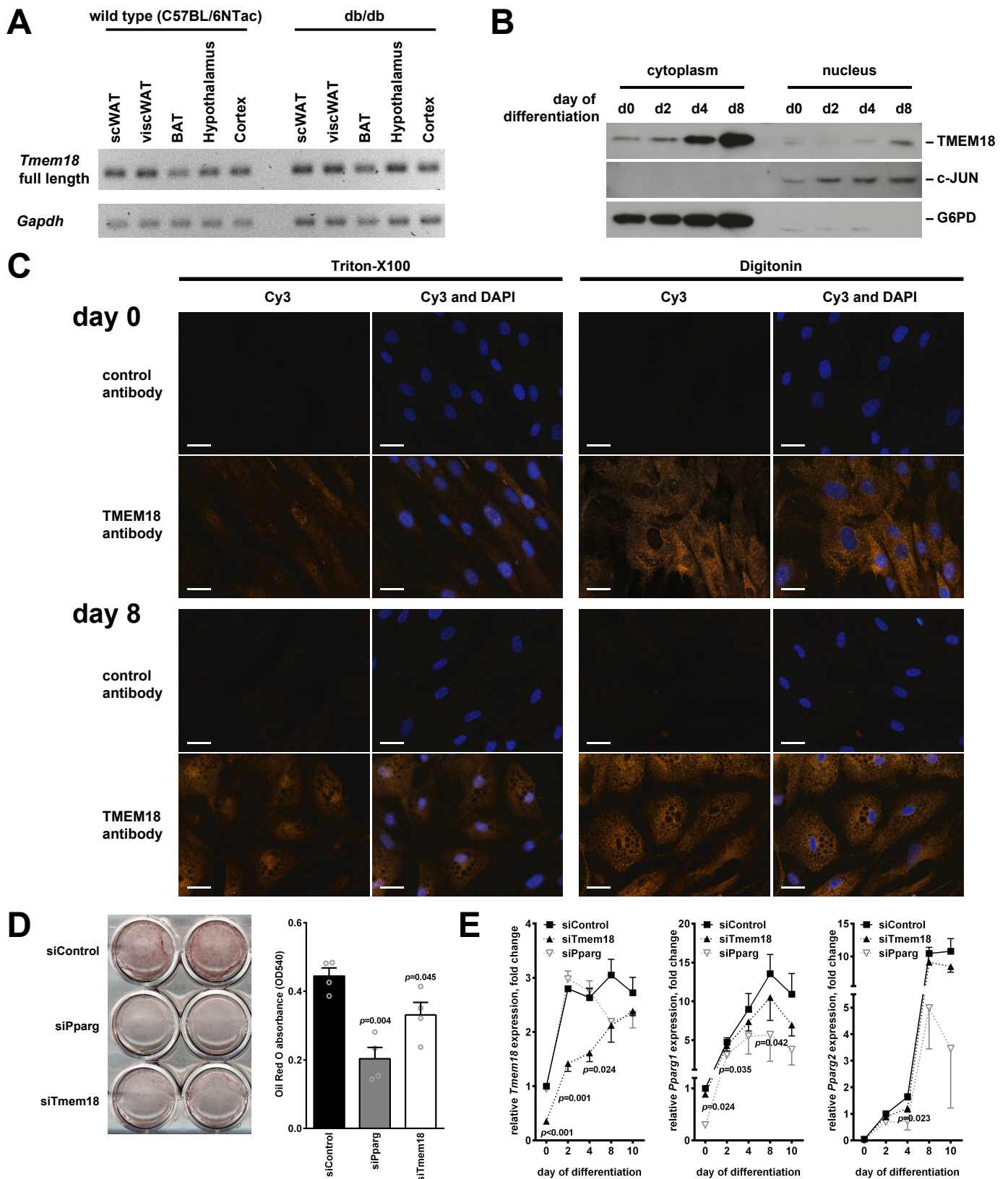
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 Mouse : -----MSAYSVRSEF-----VSIPAVIMETDWTEPWLLGLLAFHLLCLLLTQFSSORY : 49  
 Zebrafish : MTASNTKNASAIPTDKESNVRITSIWTFLLQSDWSEPWLMALLAFHVFCFAFTLLSCRY : 60

Human : RLQIGHFLCLVILVYCAEYINEAAMNWRLF SKYQYFDSRGMFISIVFSAPLLVNAMIIV : 109  
 Mouse : KLQIGHFLCLVILVYSAEYINEAAMNWRLF SKYQYFDSRGMFISLVFSAPLLFNAMLIV : 109  
 Zebrafish : RIQIGHFLMVAMVYSAEYLNEAAMNWRFSKFOYFDSKGMFISLVYSVPLLNTVIIV : 120

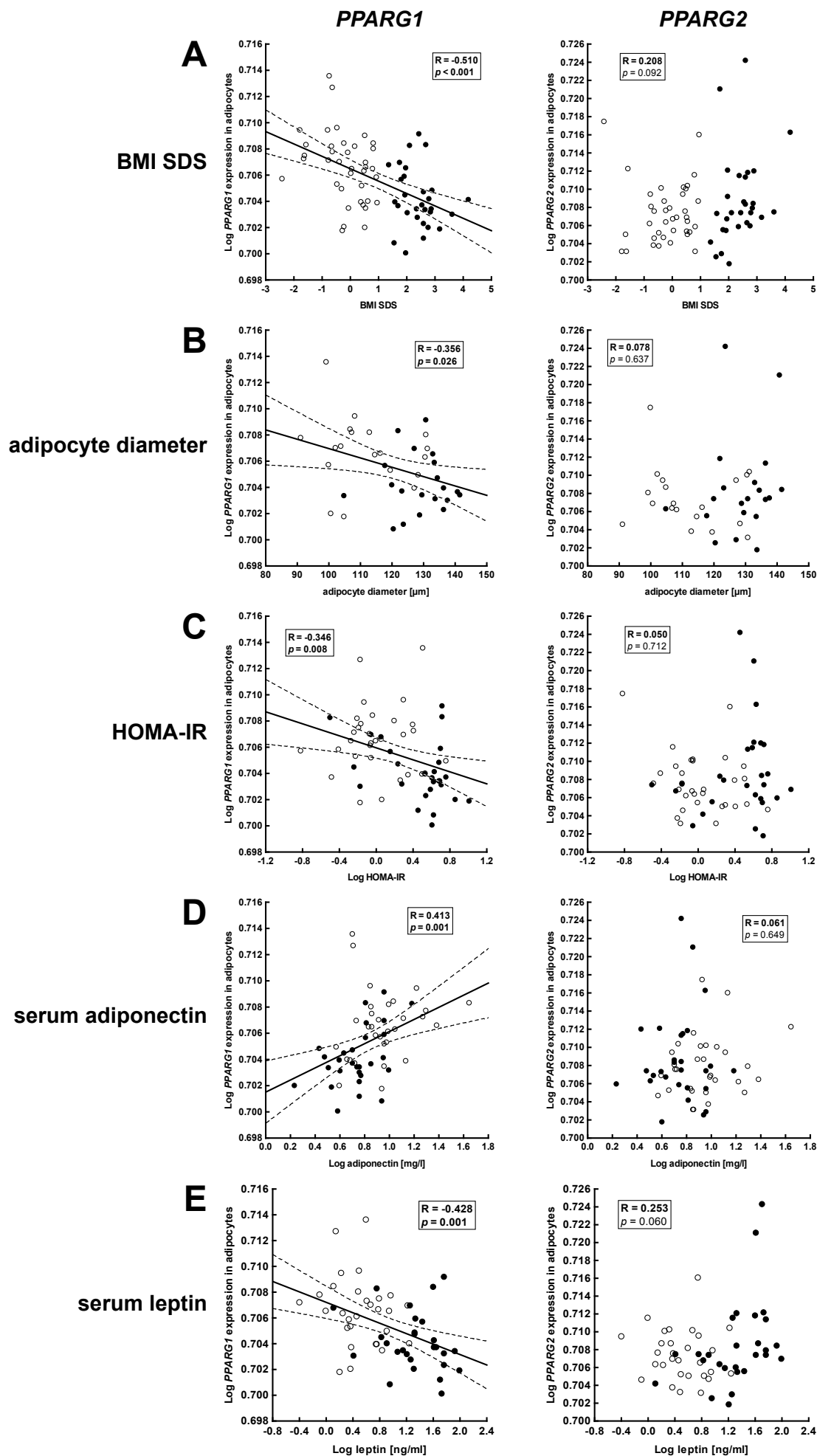
Human : VMWVWKTLLNMTDLKNAQERRKEKKRRRKED- : 140  
 Mouse : IMWVRKTLTVMTDLKTLQERRKERRRRRKEE- : 140  
 Zebrafish : AVWVWRTEFSLMTLKLILQLKRKAARENHKKTQ : 152



**Fig. S1. *TMEM18* is conserved in zebrafish and co-expressed with *pparg*. Related to Figure 2.** (A) Tmem18 protein sequences were aligned using the ClustalW algorithm. Zebrafish Tmem18 protein is highly similar compared to human (64%) and mouse (69%). (B) The expression profile of *tmem18* in different tissues of adult male zebrafish at 6mpf ( $n=3$ , pooled) was analyzed by quantitative *real-time* PCR and showed highest expression in visceral AT. Expression levels were quantified in triplicates and were standardized to the median expression across all tissues (dotted line). Data are presented as mean $\pm$ SEM. (C) During early zebrafish development overall *tmem18* mRNA levels were downregulated between 1.5hpf and 4hpf followed by a steady increase till 9dpf. Data were determined from 3 independent experiments (20 pooled embryos per time point) each measured in triplicates and are presented as mean $\pm$ SEM. (D) Localization of *tmem18* mRNA expression during early zebrafish development (48hpf, 4dpf, 9dpf) was analyzed by whole-mount *in situ* hybridization using gene-specific probes in antisense or sense orientation (scale bar 500 $\mu$ m). *Tmem18* was expressed in the brain and the region surrounding the swim bladder (white arrows), where *pparg* expression was also detected. mpf, months post fertilization; hpf, hours post fertilization; dpf, days post fertilization.

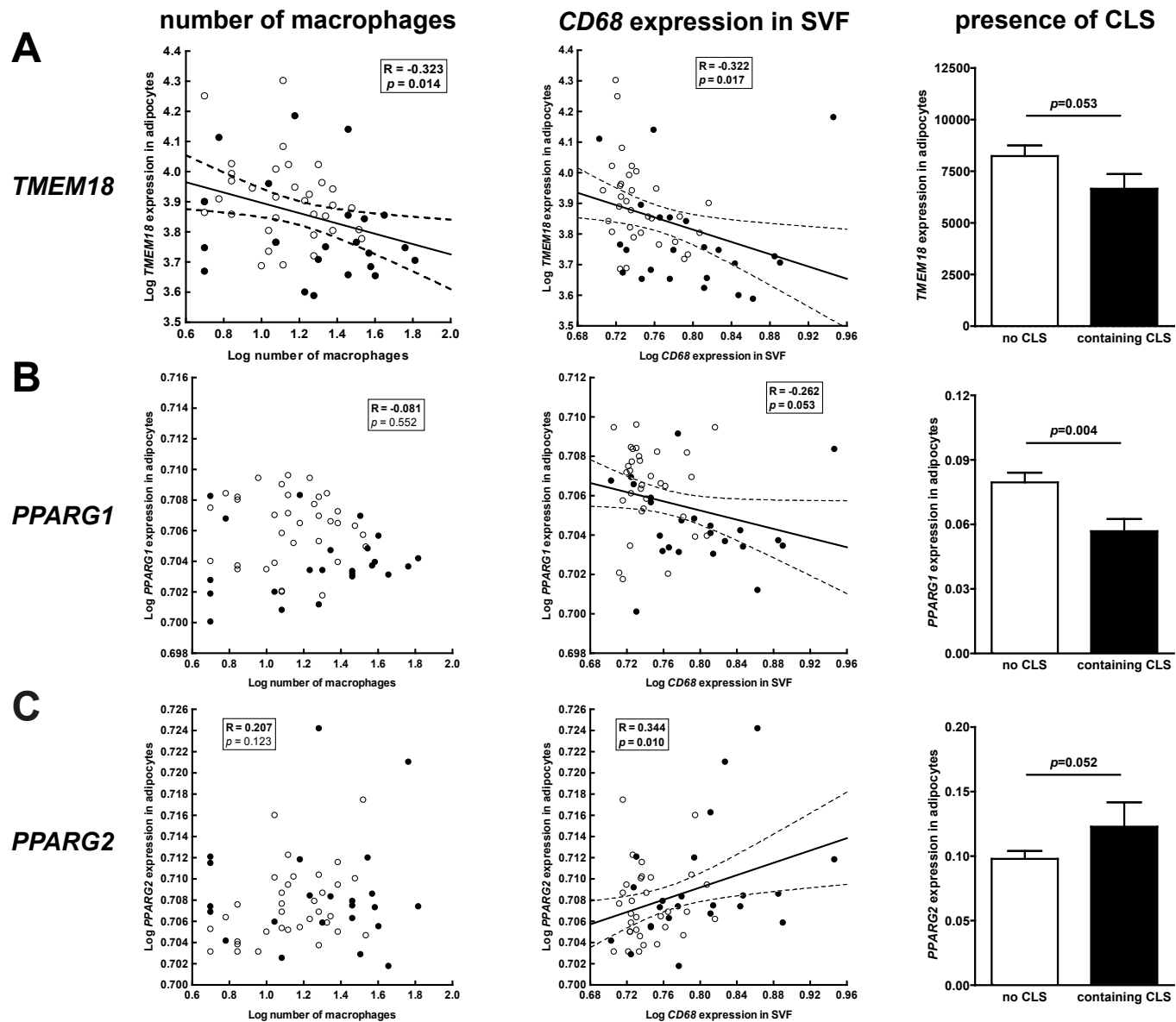


**Fig. S2. *Tmem18* is localized in both cytoplasm and nucleus of adipose progenitor cells and involved in adipocyte differentiation. Related to Figure 3.** (A) Expression of *Tmem18* was detected in adipose and brain tissues of wild type (C57BL/6NTac) and db/db mice using RT-PCR. Analysis of TMEM18 protein localization before adipogenic induction (=d0) and during adipocyte differentiation (d2, d4, d8 of differentiation) by cellular fractionation and immunoblot (B) or immunofluorescence (C) showed that TMEM18 protein is detectable in both cytoplasm and nucleus of human SGBS cells. Nuclear localization of TMEM18 was confirmed by comparing SGBS cells permeabilized with Triton-X100 (permeabilizes nucleus) and Digitonin (does not permeabilize nucleus) and TMEM18-specific immunofluorescence (scale bar 50µm). (D) Adipocyte differentiation of murine 3T3-L1 cells was reduced after siRNA-mediated knockdown of *Pparg* and *Tmem18* as indicated by Oil Red-O staining at day 8. (E) *Tmem18* knockdown reduced *Pparg1* expression during early and late adipocyte differentiation of 3T3-L1 cells and *Pparg2* expression during late adipocyte differentiation. Data are given as mean±SEM. Significant p-values are indicated for siTmem18 compared to siControl ( $p<0.05$ ). WAT, white adipose tissue; sc, subcutaneous; visc, visceral; BAT, brown adipose tissue.



**Fig. S3. *PPARG1* expression in adipocytes is associated with obesity and related parameters in children *in vivo*.** Related to Figure 3. Adipocyte *PPARG1* but not *PPARG2* expression was significantly correlated to BMI SDS (A), adipocyte diameter (B), HOMA-IR (C), and adiponectin (D) and leptin (E) serum levels in children. Pearson correlation coefficient R and p-value are given in each scatter plot. Significant p-values (p<0.05) are indicated in bold. Lean children are represented as open, children with obesity as closed circles.





**Fig. S4. Both adipocyte *TMEM18* and *PPARG1* expression are negatively associated with AT inflammation *in vivo*. Related to Figure 4.** Associations of *TMEM18* (A), *PPARG1* (B) and *PPARG2* (C) expression in adipocytes with parameters of AT inflammation are shown. Only adipocyte *TMEM18* expression was associated with the number of CD68-positive macrophages in subcutaneous AT samples of children, while there was no association of adipocyte *PPARG1* or *PPARG2* expression with macrophage infiltration. In line with this, adipocyte *TMEM18* expression negatively correlated with *CD68* expression in SVF cells. Similarly, *PPARG1* negatively correlated with SVF *CD68* expression by trend, while *PPARG2* showed a positive association. Pearson correlation coefficient *R* and *p*-value are given in each scatter plot. Lean children are represented as open, children with obesity as closed circles. In addition, both *TMEM18* and *PPARG1* expression in adipocytes were lower in AT samples containing CLS compared to samples without CLS, while *PPARG2* expression appeared to be increased in AT samples containing CLS. Data are given as mean±SEM and *p*-values are indicated.

**Table S1. Related to Fig. 1.** Characteristics of the Childhood Adipose Tissue Cohort (n=67)

	Lean			Obese			<i>p</i>
	<i>n</i>	Mean±SEM	Range	<i>n</i>	Mean±SEM	Range	
Male/Female (% male)		20/18 (52.6)			11/18 (37.9)		0.232
Age [years]	38	9.5±0.9	1.1–18.3	29	12.9±0.7	4.8–18.0	<b>0.004</b>
PH	33	2.3±0.3	1–6	27	3.4±0.3	1–6	<b>0.016</b>
BMI SDS	38	-0.2±0.1	-2.4–0.9	29	2.4±0.1	1.4–4.2	<b>&lt;0.001</b>
Adipocyte diameter [µm]	19	112.3±2.9	90.9–131.2	20	130.0±2.0	104–141.5	<b>&lt;0.001<sup>a</sup></b>
Macrophages per 100 adipocytes	34	10.4±1.3	0–29	23	20.2±3.6	0–60	<b>0.005</b>
<i>CD68</i> expression	32	0.6±0.1	0.1–1.6	23	1.3±0.2	0–3.8	<b>&lt;0.001<sup>a</sup></b>
Number of children with CLS (%)	34	3 (8.8%)		23	12 (52.2%)		<b>&lt;0.001</b>
Adiponectin [mg/l]	31	10.4±1.4	3.7–43.8	28	6.1±0.5	1.7–15.1	<b>0.007<sup>a</sup></b>
Leptin [ng/ml]	29	4.6±0.8	0.4–17.5	27	31.8±4.9	1.3–99.0	<b>&lt;0.001<sup>a</sup></b>
HOMA-IR	32	1.4±0.2	0.04–5.6	27	4.0±0.4	0.3–10.1	<b>&lt;0.001<sup>a</sup></b>

Data are given as mean ± SEM. For gender and occurrence of CLS, statistical significance was analysed by Chi-square test. Statistical significance for differences between groups was determined by Students *t* test. Significant *p* values are indicated in bold. PH, pubertal stage; BMI, body-mass index; SDS, standard deviation score; CLS, crown-like structures; HOMA-IR, homeostasis model assessment of insulin resistance. <sup>a</sup>Statistical analyses were performed for log-transformed parameters.

**Table S2. Related to Fig. 1.** Association of obesity-associated risk alleles with *TMEM18* mRNA levels in adipocytes and stroma vascular cells of lean and obese children (n=67).

variant	relative mRNA levels stratified by genotype				P
		0	1	2	
<b>rs7561317</b> <b>downstream</b>	genotype	AA	AG	GG	
	lean	6177±230 n=2	7061±538 n=9	9078±658 n=27	<b>0.030</b>
	adipocytes				
	obese	6940 n=1	6578±1133 n=8	6645±651 n=20	0.966
	lean	9724±610 n=2	12264±922 n=9	14107±815 n=27	0.106
	SVF				
	obese	9541 n=1	13415±848 n=8	12866±840 n=20	0.769
<b>rs17729501</b> <b>3'-UTR</b>	genotype	CC	CT	TT	
	lean	12076 n=1	10710±1653 n=6	7893±495 n=31	<b>0.014</b>
	adipocytes				
	obese	n=0	8392±2309 n=4	6645±651 n=25	0.212
	lean	15591 n=1	12114±1399 n=6	13627±737 n=31	0.563
	SVF				
	obese	n=0	11945±593 n=4	12866±840 n=25	0.714
<b>rs10168696</b> <b>intron3</b>	genotype	CC	CT	TT	
	lean	5408 n=1	7881±532 n=8	8709±641 n=29	0.436
	adipocytes				
	obese	5917±620 n=3	6673±1262 n=8	6737±688 n=19	0.832
	lean	9268 n=1	12565±1250 n=8	13825±757 n=29	0.273
	SVF				
	obese	15093±771 n=3	14280±1715 n=8	12049±670 n=19	0.094

Data are presented as mean±SEM. 0, homozygote for minor allele; 1, heterozygote; 2, homozygote for major allele. For statistical analyses minor allele carriers were combined into one group and compared to major allele carriers using Student's *t* test. Significant *p* values are indicated in bold.

**Table S3. Related to Methods.** Primer and probe sequences for quantitative *real-time* PCR

Target Gene	Forward Primer	Reverse Primer	Probe
<b>Zebrafish</b>			
<i>tmem18</i>	Predesigned Assay		Dr03147200_m1
<i>fabp10a</i> (Lin et al., 2014)	CACCTCCAAACTCCTGGAA	TTCTGCAGACCAGCTTTCCT	SYBR Green
<i>ifabp</i>	Predesigned Assay		DR03086825_g1
<i>cebpb</i> (Hall et al., 2012)	TCCGTCTGCCGCAAT	TAAAAACCGGCCACTTCCAT	SYBR Green
<i>cebpd</i>	Predesigned Assay		Dr03106022_s1
<i>cebpa</i>	Predesigned Assay		Dr03105987_s1
<i>pparg</i>	GCTGCACAGGCGCTTCA	CTCCAGCTCCTCCAGTTCCA	CAGAAAAGCTTCACTC TCCGCTGATATGGTG
<i>lpl-b</i>	CTGAGGGCTCTCGTTCATAAA GA	AATCCATCAAAGACTGTAAC TCAATACA	CTCTCAAACATACCC GTGACCGTCCATC
<i>fabp11a</i>	GGTTGACAAATTCGTAGGAAC GT	AACCCACACCTATAGCCTTCAT G	AATGACCACCAGCGA CAACTTTGACGA
<i>adipoq B</i>	GGATGGGCGGTGTTCCA	ACCTGCTTACCTTGATCTCCT T	CACACCTGGGCACAG TGGAAAACCTG
<i>lipe</i>	CGGCAAGGACAGGACAGT	GCATGGAGAAAAGAGGAGCT	SYBR Green
<i>actb</i>	TCCCCTTGTTACAATAACCTA CTAA	CATACCGGAGCCGTTGTCA	AGCGATTTCTCATCC ATGGCTGTGT
<i>tbp</i>	CCTGCGAATTATCGTTTACGTC TT	ACGGCATCATAGGACTGAAAA TG	TTGCTTCATAACCTGT CAGCATGGAGCA
<b>Mouse</b>			
<i>Tmem18</i>	GCTCTGTGAATGGCTTATCTG	CTCTGTCACCTCAAATCTCTAA AG	SYBR Green
<i>Pparg1</i>	TGAAAGAAGCGGTGAACCAC TG	TGGCATCTCTGTGTCAACCAT G	TAAAAACAAGACTA CCCTTTACTGAA
<i>Pparg2</i>	TGGCATCTCTGTGTCAACCAT G	GCATGGTGCCTTCGCTGA	SYBR Green
<i>Actb</i>	GCTCTGGCTCCTAGCACCAT	GCCACCGATCCACACCGCGT	TCAAGATCATTGCTC CTCCTGAGCGC
<i>Tbp</i>	AATCTTGGCTGTAACTTGAC CTAAAG	CGTGGCTCTCTATTCTCATGA TG	TCGTGCAAGAAATGC TGAATATAATCCAA GC
<b>Human: Analyses of SGBS cells and patient AT samples</b>			
<i>TMEM18</i>	GTCATCTTAGTCTACTGTGCTG AATACATC	CCCCCTGGAGTCGAAATACTG	CTCCAGTTCATCGCA GCCGCCT
<i>PPARG1</i> (Bruedigam et al., 2008)	GTGGCCGCAGATTTGAAAGA AG	TGTCAACCATGGTCATTTG	SYBR Green
<i>PPARG2</i> (Bruedigam et al., 2008)	CAAACCCCTATTCCATGCTGTT	AATGGCATCTCTGTGTCAACC	SYBR Green
<i>CEBPB</i>	Predesigned Assay		Hs00270923_s1
<i>CEBPD</i>	Predesigned Assay		Hs00270931_s1
<i>CEBPA</i>	Predesigned Assay		Hs00269972_s1
<i>SREBF1</i>	Predesigned Assay		Hs01088679_g1
<i>LPL</i>	Predesigned Assay		Hs00173425_m1
<i>FABP4</i>	Predesigned Assay		Hs01086177_m1
<i>PLIN1</i>	Predesigned Assay		Hs00160173_m1
<i>ADIPOQ</i>	GGCCGTGATGGCAGAGAT	CCTTCAGCCCCGGGTACT	CGATGTCTCCCTTAG GACCAATAAGACCTG G
<i>LIPE</i>	Predesigned Assay		Hs00943410_m1



<i>SLC2A4</i>	Predesigned Assay		Hs00943410_m1
<i>LEP</i>	Predesigned Assay		Hs00943410_m1
<i>ACTB</i>	TGAGCGCGGCTACAGCTT	CCTTAATGTCACGCACGATTT	ACCACCACGGCCGAGCGG
<i>TBP</i>	TTGTAAACTTGACCTAAGACCATTGC	TTCGTGGCTCTCTTATCCTCATG	AACGCCGAATATAATCCCAAGCGGTTTG
<i>HPRT</i>	GGCAGTATAATCCAAAGATGGTCAA	GTCTGGCTTATATCCAACACTTCGT	CAAGCTTGCTGGTGAAGGACCCC
<b>Human: Analyses of chubs-s7 cells</b>			
<i>TMEM18</i>	TCCGCCTTCTCTGTCAGC	GGCTCAGTCCAGTCCGTCT	UPL probe 89, Roche (04689143001)
<i>PPARG1</i>	GACAGGAAAGACAACAGACAATC	GGGGTGATGTGTTTGAACCTG	UPL probe 7, Roche (04685059001)
<i>PPARG2</i>	TCCATGCTGTTATGGGTGAA	TGTGTCAACCATGGTCATTTTC	UPL probe 14, Roche (04685130001)
<i>PPIA</i>	ATGCTGGACCCAACACAAAT	TCTTTCACCTTGCCAAACACC	UPL probe 48, Roche (04688082001)
<i>PGK1</i>	CTGTGGCTTCTGGCATACCT	CGAGTGACAGCCTCAGCATA	UPL probe 42, Roche (04688015001)

Primers and probes are given in 5'-3' direction. Predesigned assays were obtained from Life Technologies.