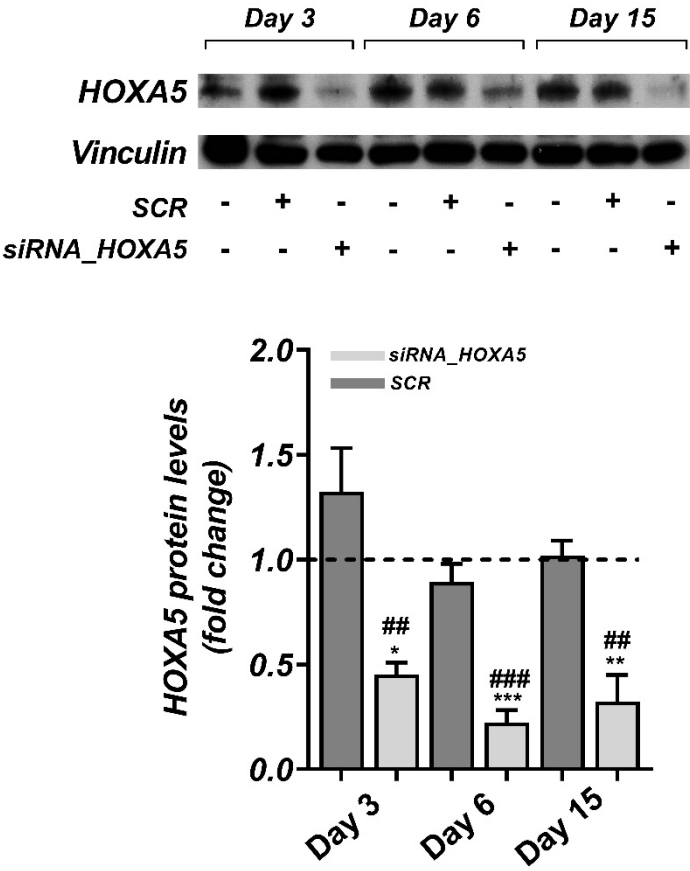


Supplementary Table S1. List of primers used in this study.

<i>GENE</i>	<i>Forward/Reverse</i>	<i>Primer (5' to 3')</i>
<i>HOXA5</i> <i>Bisulphite</i>	<i>Forward</i> <i>Reverse</i>	TTTTGTATAATTTATGATGAATTATGGAAA ACAAAAATAAAAAAAAAACTACAAAAC
<i>HOXA5</i> <i>mRNA</i>	<i>Forward</i> <i>Reverse</i>	GCGCAAGCTGCACATAAG CGGTTGAAGTGGAACCTCTT
<i>GLUT4</i> <i>mRNA</i>	<i>Forward</i> <i>Reverse</i>	TCTGGCATCAATGCTGTTTTCTAT ACCAACAACACCGAGACCAAG
<i>FABP4</i> <i>mRNA</i>	<i>Forward</i> <i>Reverse</i>	GACAGGAAAGTCAAGAGCACCATA GACGCATTCCACCACCAGTT
<i>RPL13A</i> <i>mRNA</i>	<i>Forward</i> <i>Reverse</i>	CTTTCCGCTCGGCTGTTTTTC GCCTTACGTCTGCGGATCTT
<i>ADIPONECTIN</i> <i>mRNA</i>	<i>Forward</i> <i>Reverse</i>	CCGGGCCATAATGGGG CCCTTAGGACCAATAAGACCTGG
<i>WNT2B</i> <i>mRNA</i>	<i>Forward</i> <i>Reverse</i>	CGAGAGTGTCAGCACCAATTC AAGCTGCCTCTCGGCTACT
<i>FZD8</i> <i>mRNA</i>	<i>Forward</i> <i>Reverse</i>	CACCGTCTCCACCTTCCTTA GACACGAAGAGGTAGCAGGC
<i>CSNK2A1</i> <i>mRNA</i>	<i>Forward</i> <i>Reverse</i>	GAACGCTTTGTCCACAGTGA TATCGCAGCAGTTTGTCCAG
<i>NFATC1</i> <i>mRNA</i>	<i>Forward</i> <i>Reverse</i>	CACCGCATCACAGGGAAGAC GCACAGTCAATGACGGCTC
<i>ROHA</i> <i>mRNA</i>	<i>Forward</i> <i>Reverse</i>	AGCCTGTGGAAAGACATGCTT TCAAACACTGTGGGCACATAC
<i>FOSL1</i> <i>mRNA</i>	<i>Forward</i> <i>Reverse</i>	CAGTGGATGGTACAGCCTCA CAGTTTGTGAGTCTCCTGTTTCA
<i>GATA2</i> <i>mRNA</i>	<i>Forward</i> <i>Reverse</i>	CAGCAAGGCTCGTTCTCTGTTCA ATGAGTGGTCGGTTCTGCCCCAT
<i>GATA3</i> <i>mRNA</i>	<i>Forward</i> <i>Reverse</i>	TCATTAAGCCCAAGCGAAGG GTCCCCATTGGCATTCTCTC
<i>FAS</i> <i>mRNA</i>	<i>Forward</i> <i>Reverse</i>	ACCCTTGACCAAATGTGAAC AAGAAGACAAAGCCACCCCA
<i>HOXA5</i> <i>Cloning</i>	<i>Forward</i> <i>Reverse</i>	CGCCCTAGGGTCTATAGCACCTTGCACA CGCGGATCCGCTGCAAAACGGGGGAA

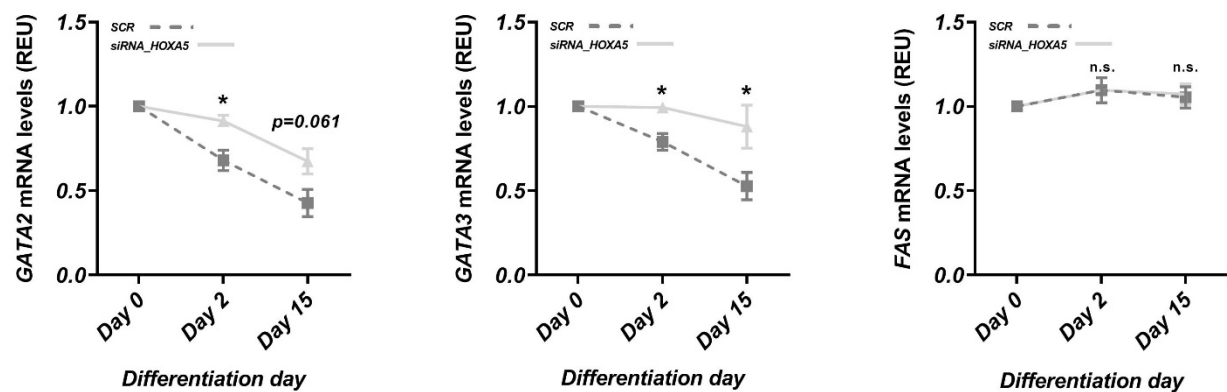
Supplementary Figure S1

HOXA5 silencing during *in vitro* adipogenesis of human SAT preadipocytes. HOXA5 protein levels were measured by western blot analysis at different time points during differentiation in cells treated with HOXA5-specific siRNA, Scramble siRNA (SCR) or the control (CTRL). Vinculin served as a loading control. The upper figure shows representative blots; the lower figure presents the results quantification, where the black dotted line represents control un-transfected cells. The results are the mean \pm SD of three independent experiments. The statistical significance was tested by one-way ANOVA followed by Tukey's multi-comparison test at each indicated time point: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for comparison *versus* CTRL; ## $p < 0.01$, ### $p < 0.001$ *versus* SCR.



Supplementary Figure S2

The effect of *HOXA5* silencing on the expression of preadipocyte and apoptosis markers. The expression levels of *GATA2*, *GATA3* and *FAS* were assessed by qPCR at different time points during differentiation in the cells treated with scramble (SCR) or *HOXA5*-specific siRNA (*siRNA_HOXA5*). All data are presented as means \pm SD of 3 independent experiments. Statistical significance was tested by one-way ANOVA followed by Tukey's multi-comparison test at each indicated time. * $p < 0.05$ versus SCR. N.S., no significant difference.

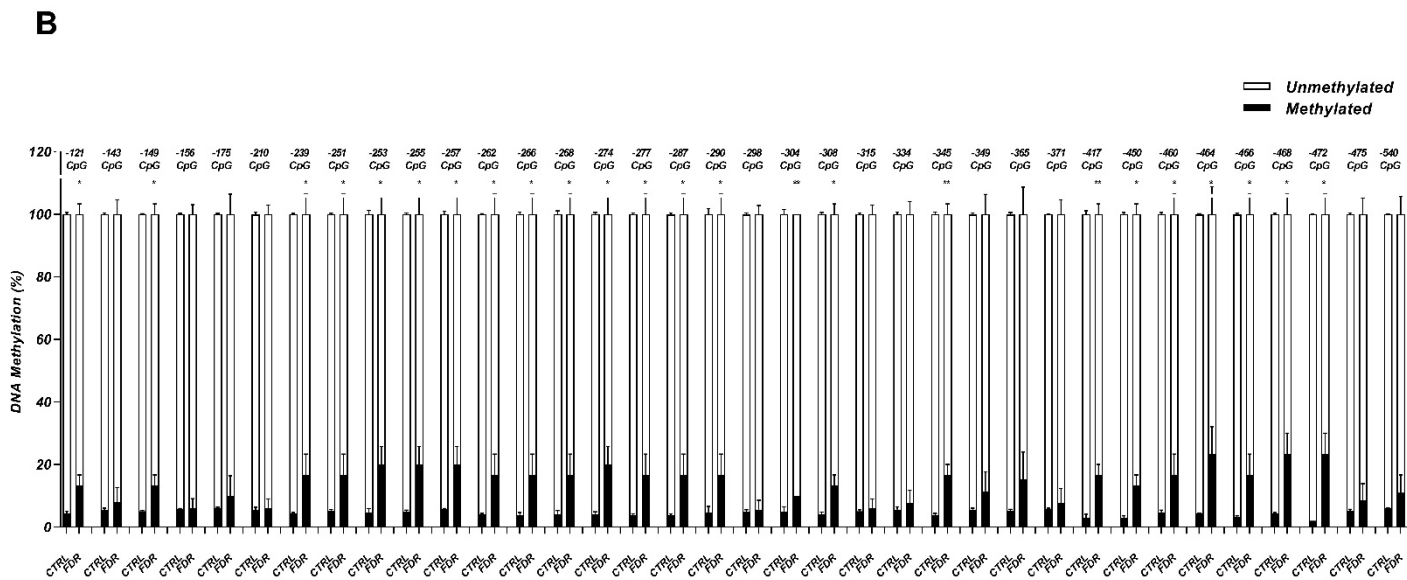
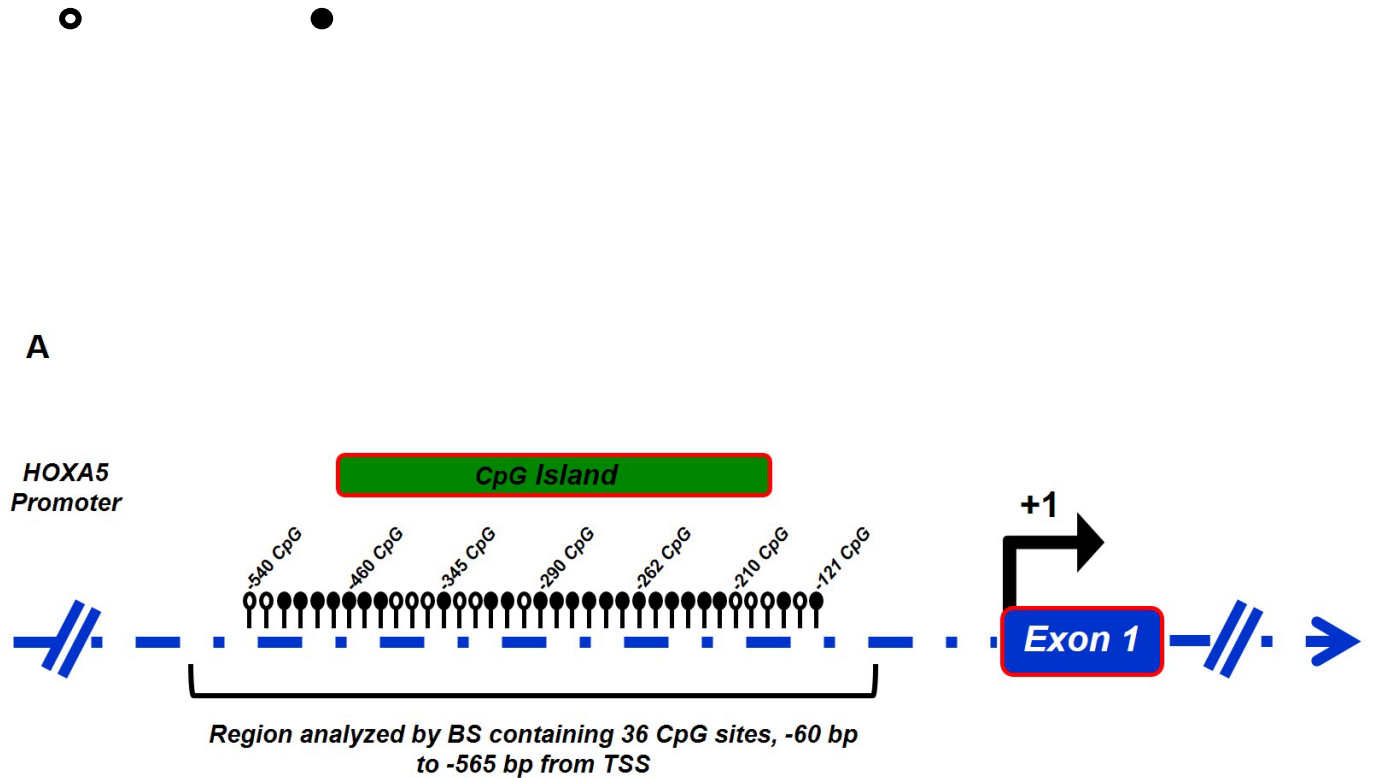


Supplementary Table S2. Clinical characteristics of FDR and CTRL subjects.

<i>Measure</i>	<i>FDR</i>	<i>CTRL</i>
<i>N (female/male)</i>	12 (6/6)	12 (6/6)
<i>Age, years</i>	42.3 ± 2.9	38.7 ± 2.3
<i>BMI, Kg/m²</i>	25.4 ± 0.5	24.5 ± 0.7
<i>Fat percent, %</i>	26.9 ± 2.4	24.0 ± 1.9
<i>Waist to Hip Ratio (WHR)</i>	0.90 ± 0.02**	0.81 ± 0.02
<i>Total triglycerides, mmol/L</i>	1.5 ± 0.6**	0.8 ± 0.2
<i>HDL cholesterol, mmol/L</i>	1.3 ± 0.2*	1.6 ± 0.3
<i>Cell size, μm</i>	100.2 ± 1.7***	89.4 ± 1.9
<i>GIR/bw, mg/Kg/min</i>	7.9 ± 0.6**	11.5 ± 0.8
<i>f-insulin, pmol/L</i>	60.1 ± 8.0**	35.4 ± 3.9
<i>fb-glucose, mmol/L</i>	4.8 ± 0.1**	4.4 ± 0.1
<i>OGTT p-glucose 2h, mmol/L</i>	6.6 ± 0.6*	4.8 ± 0.4
<i>HOMA-IR index</i>	2.0 ± 0.8***	1.1 ± 0.4

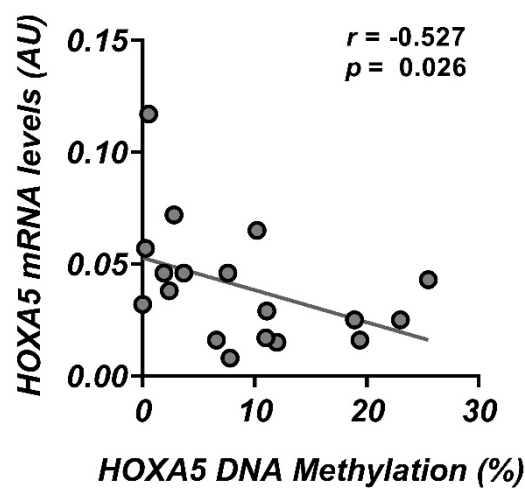
Clinical characteristics of FDR and CTRL subjects. The study group has been described under Materials and Methods. All data are expressed as means ± SD. Statistical significance was tested by a two-tailed Mann-Whitney U-test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, FDR *vs.* CTRL. BMI, body mass index; GIR/bw, glucose infusion rate/body weight; OGTT, oral glucose tolerance test; HDL, high density lipoprotein; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance.

Supplementary Figure S3



Supplementary Figure S4

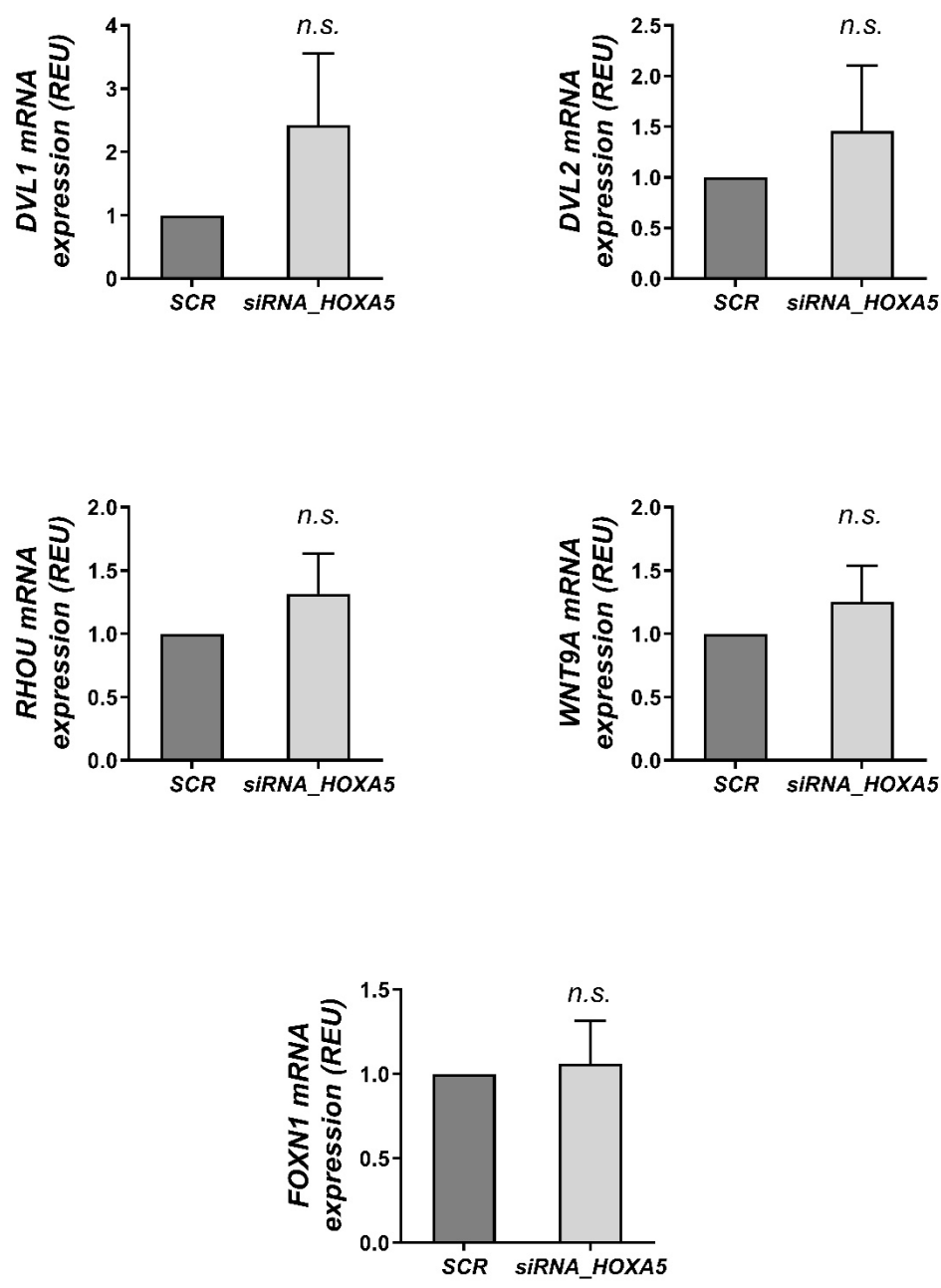
The correlation between *HOXA5* DNA methylation and its mRNA levels. The correlation between *HOXA5* DNA methylation and *its* mRNA levels in preadipocytes from FDR (n=9) and CTRL (n=9) subjects available from the study group. The r correlation coefficient and p-value are indicated in the graph.



Supplementary Figure S5

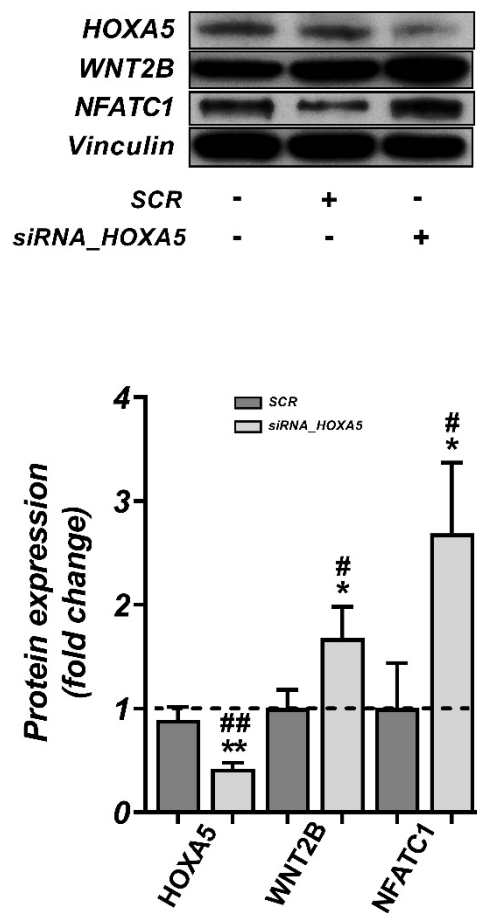
Validation of the WNT-Signalling Pathway array results between the HOXA5-silenced and scrambled cells.

The 11 differentially expressed genes, identified by the RT2 Profiler™ PCR array, were individually analysed by qPCR in the same samples also tested in the PCR array (n = 3 per group/condition; please also refer to Figure 3 of the main text). Five of the 11 differentially expressed genes were not confirmed by qPCR assay. All the data are expressed as means \pm SD. N.S., no significant difference.



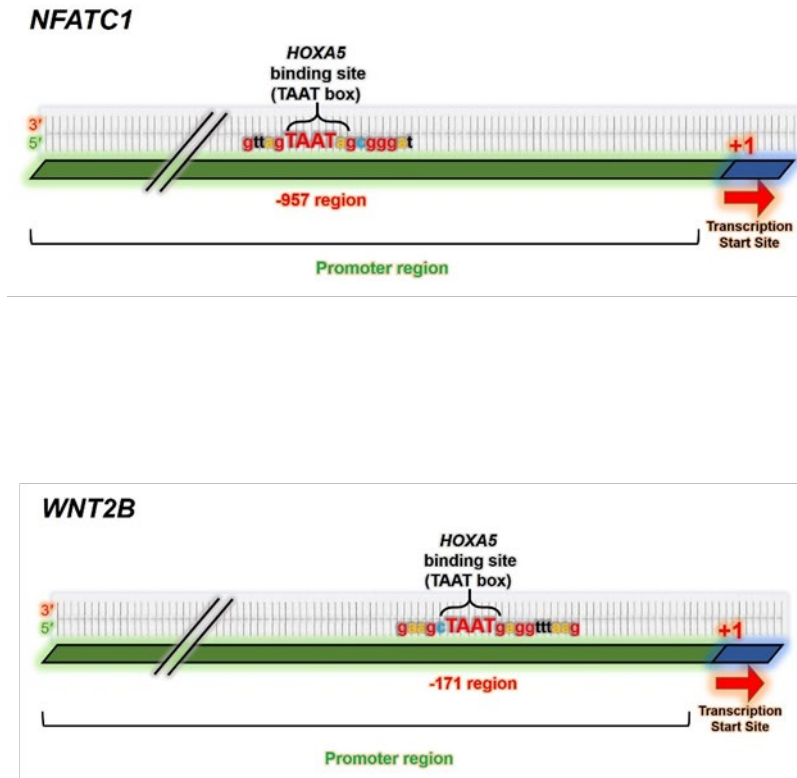
Supplementary Figure S6

The effect of *HOXA5* silencing on the protein levels of WNT-Signalling factors. The *HOXA5*, *WNT2B* and *NFATC1* protein levels were measured by western blot analysis in preadipocytes treated with *HOXA5*-specific siRNA (*siRNA_HOXA5*), Scramble siRNA (SCR) and in control un-transfected cells. Vinculin served as a loading control. The upper figure shows representative blots; the lower figure presents the results quantification, where the black dotted line represents control un-transfected cells. The results are the mean \pm SD of three independent experiments. The statistical significance was tested by one-way ANOVA followed by Tukey's multi-comparison test: * $p < 0.05$ and ** $p < 0.01$ versus control un-transfected cells; # $p < 0.05$ and ## $p < 0.01$ versus SCR.



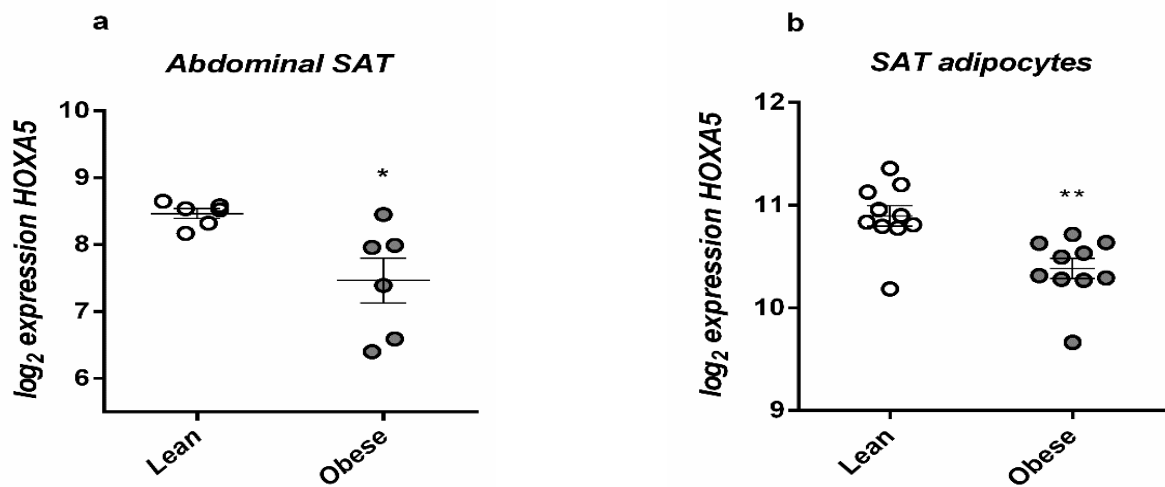
Supplementary Figure S7

In silico identification of putative *HOXA5* binding sites across the promoter regions of *NFATC1* and *WNT2B* genes. The promoter region of both the *NFATC1* and *WNT2B* genes (1500 bp upstream the transcription start site; TSS) was analyzed in the *Genomatrix Database* (<https://www.genomatrix.de/>) using the *MatInspector* platform. One putative *HOXA5* binding motif was identified in the *NFATC1* promoter at -957 bp upstream the TSS one further motif in the *WNT2B* promoter at -171 bp upstream the TSS.



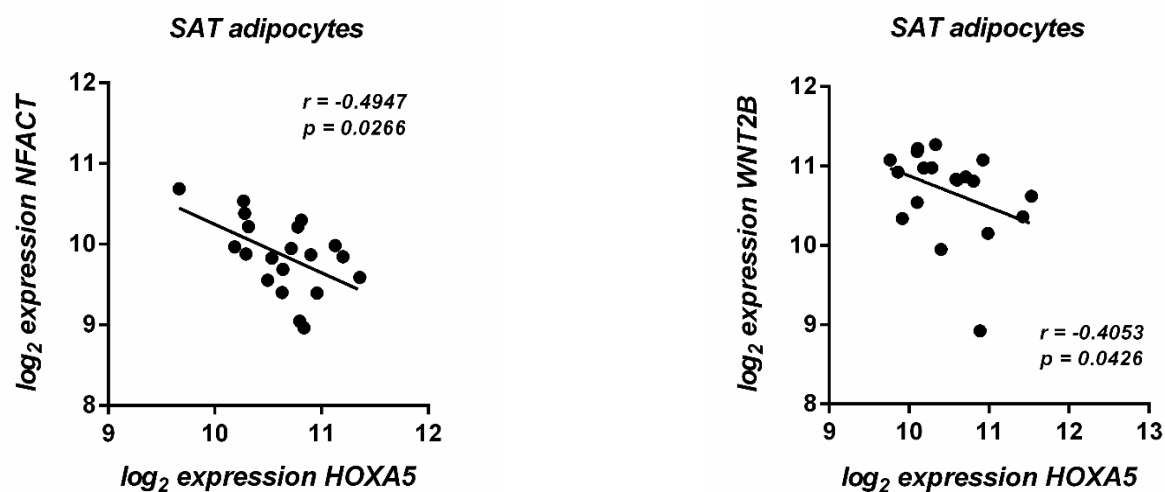
Supplementary Figure S8

***HOXA5* levels in whole abdominal SAT and in isolated SAT adipocytes from obese individuals.** Normalized mRNA expression of *HOXA5* in human abdominal SAT (a) and isolated SAT adipocytes (b) from obese and lean individuals. The data were obtained from large-scale human obesity expression datasets published in Gene Expression Omnibus (GEO). GEO accession number: GSE27951, (a), and GEO: GSE2508, (b). * $p < 0.05$, ** $p < 0.01$ in a two-tailed Mann–Whitney U-test.



Supplementary Figure S9

Correlation between *NFATC1* and *WNT2B* expression and *HOXA5* expression in isolated SAT adipocytes from obese individuals. The data were obtained from large-scale human obesity expression datasets published in Gene Expression Omnibus (GEO). GEO accession number: GSE2508. The *r* correlation coefficients and *p*-values are indicated in the graph.



Supplementary Table S3. Clinical characteristics of Obese and Lean subjects.

<i>Measure</i>	<i>Obese</i>	<i>Lean</i>
<i>N (female/male)</i>	8 (4/4)	8 (4/4)
<i>Age, years</i>	36.2 ± 5.3*	31.3 ± 2.1
<i>BMI, Kg/m²</i>	43.0 ± 6.9***	22.2 ± 2.9
<i>Total triglycerides, mmol/L</i>	1.6 ± 0.6***	0.7 ± 0.2
<i>HDL cholesterol, mmol/L</i>	1.3 ± 0.3*	1.8 ± 0.5
<i>fb-glucose, mmol/L</i>	4.7 ± 0.3	4.9 ± 0.4

Clinical characteristics of Obese and Lean subjects. All data are expressed as means ± SD. Statistical significance was tested by a two-tailed Mann-Whitney U-test: * $p < 0.05$, *** $p < 0.001$, Obese *vs.* Lean. BMI, body mass index; HDL, high density lipoprotein.