

Supplementary Materials for
Transcriptional determinants of lipid mobilization in human adipocytes

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Fig. S1.

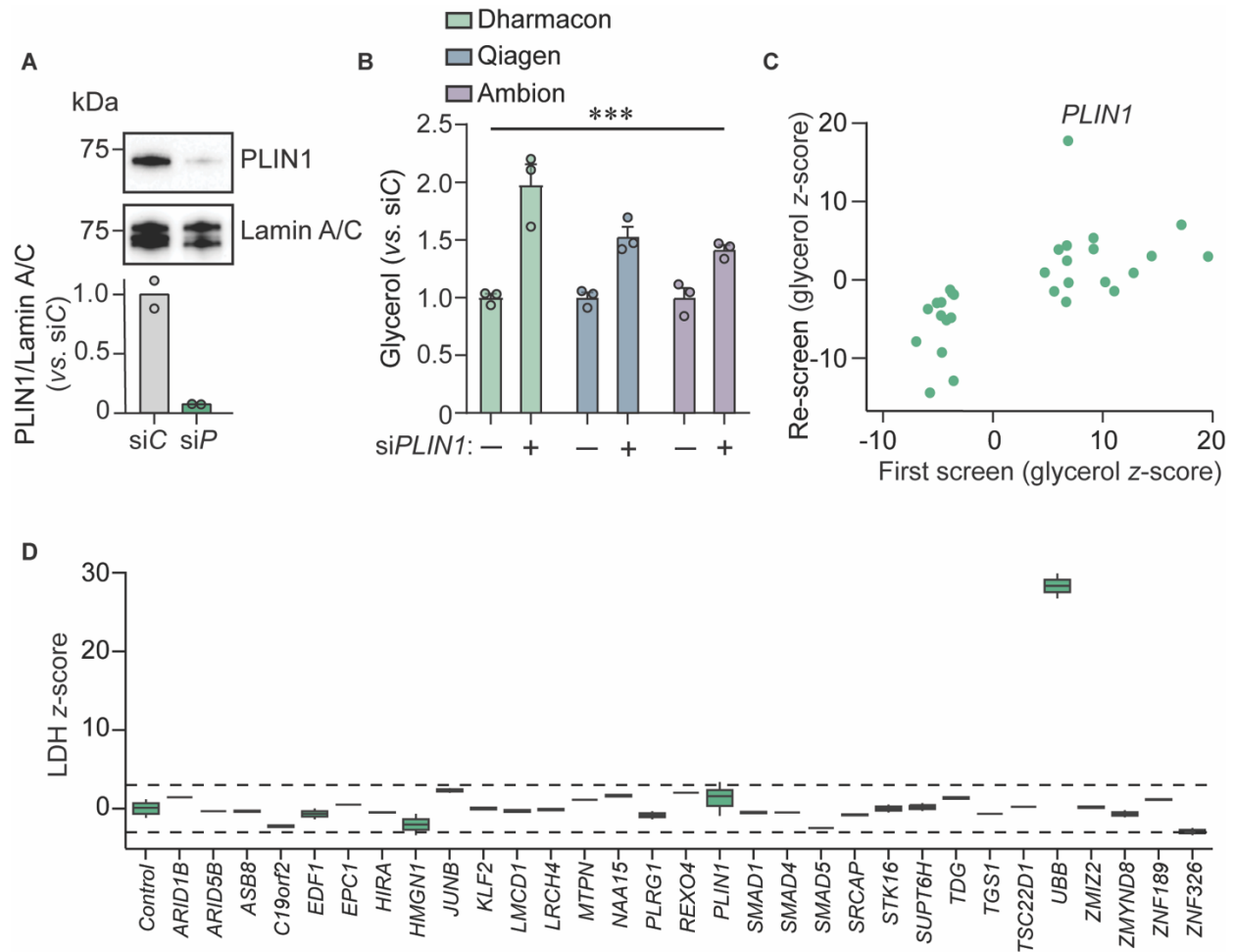
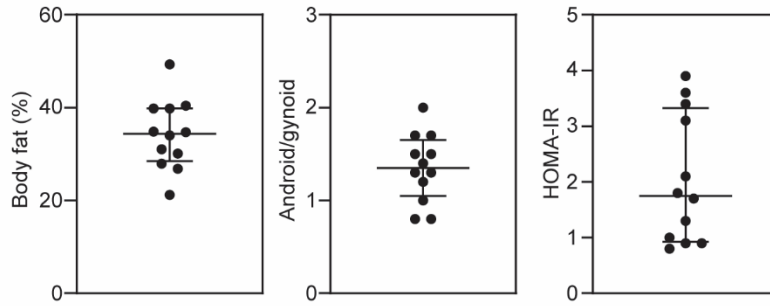


Fig. S1. Validation of the adipocyte screening system. (A) Representative Western blots of PLIN1 and Lamin A/C protein content for differentiated adipocytes transfected with non-targeting control (siC) or *PLIN1* siRNA (siP) (n = 2). (B) Glycerol release from siP vs. siC cells. *** $P < 0.001$ for a main effect for siRNA from a two-way ANOVA (n = 3). Bar plots in panel B are presented as mean \pm SEM. (C) Hits from the siRNA screen were re-screened on a separate adipose tissue biopsy (“Re-screen”). (D) LDH activity z-scores for screen hits. The dashed line marks a z-score ± 3 .

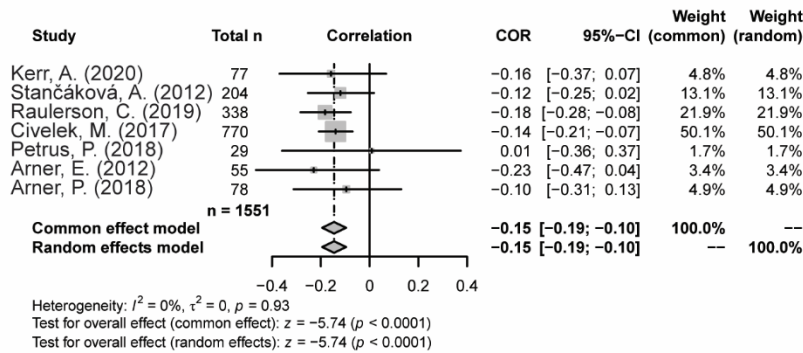
Fig. S2.

A



B

Waist-to-hip ratio



HOMA-IR

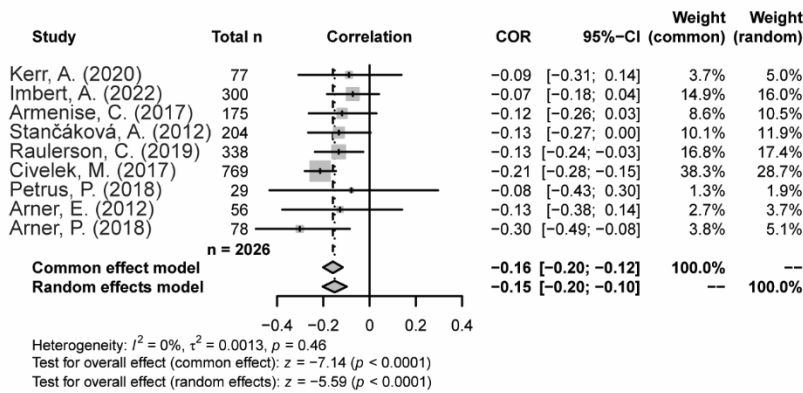


Fig. S2. Clinical parameters. (A) Subject characteristics for the clinical cohort for lipolysis measurements with median and interquartile range ($n = 12$). **(B)** Forest plot displaying correlations and effects for meta-analysis of *ZNF189* expression in subcutaneous adipose tissue from 7-9 published human cohorts.

Fig. S3.

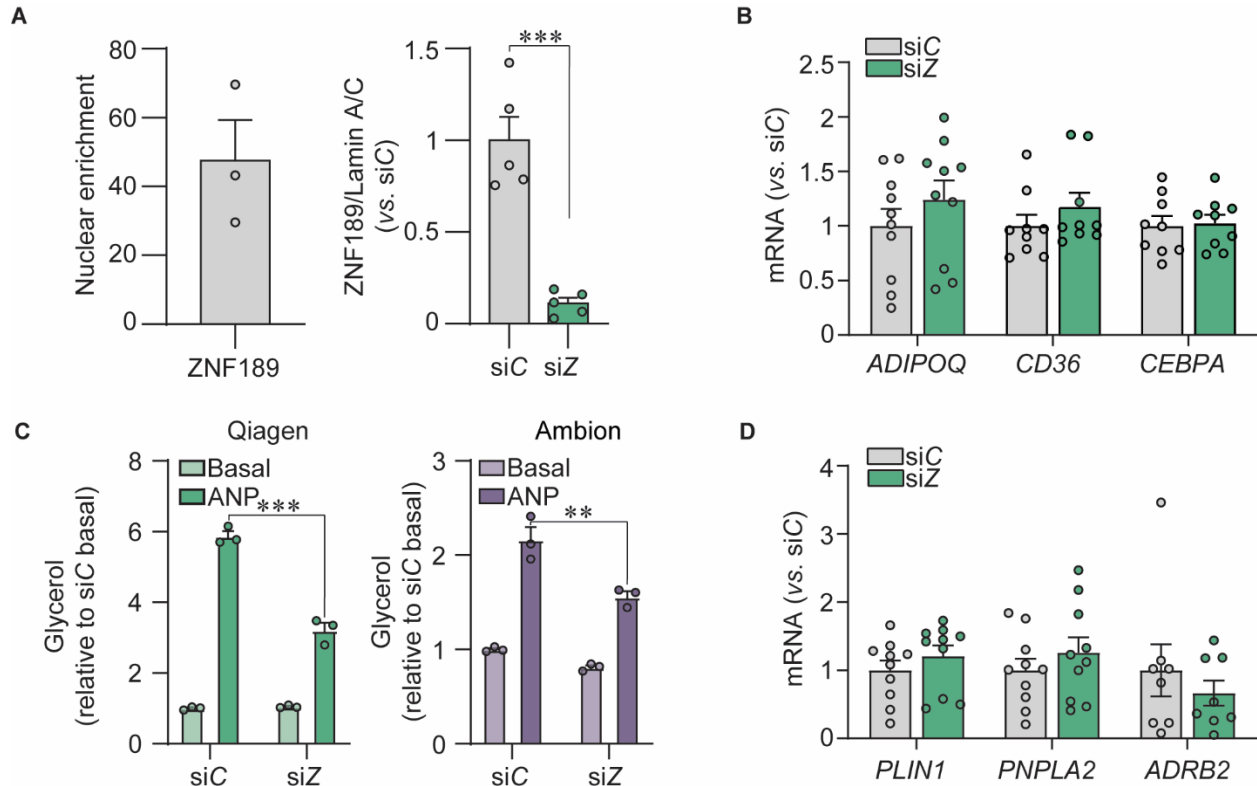
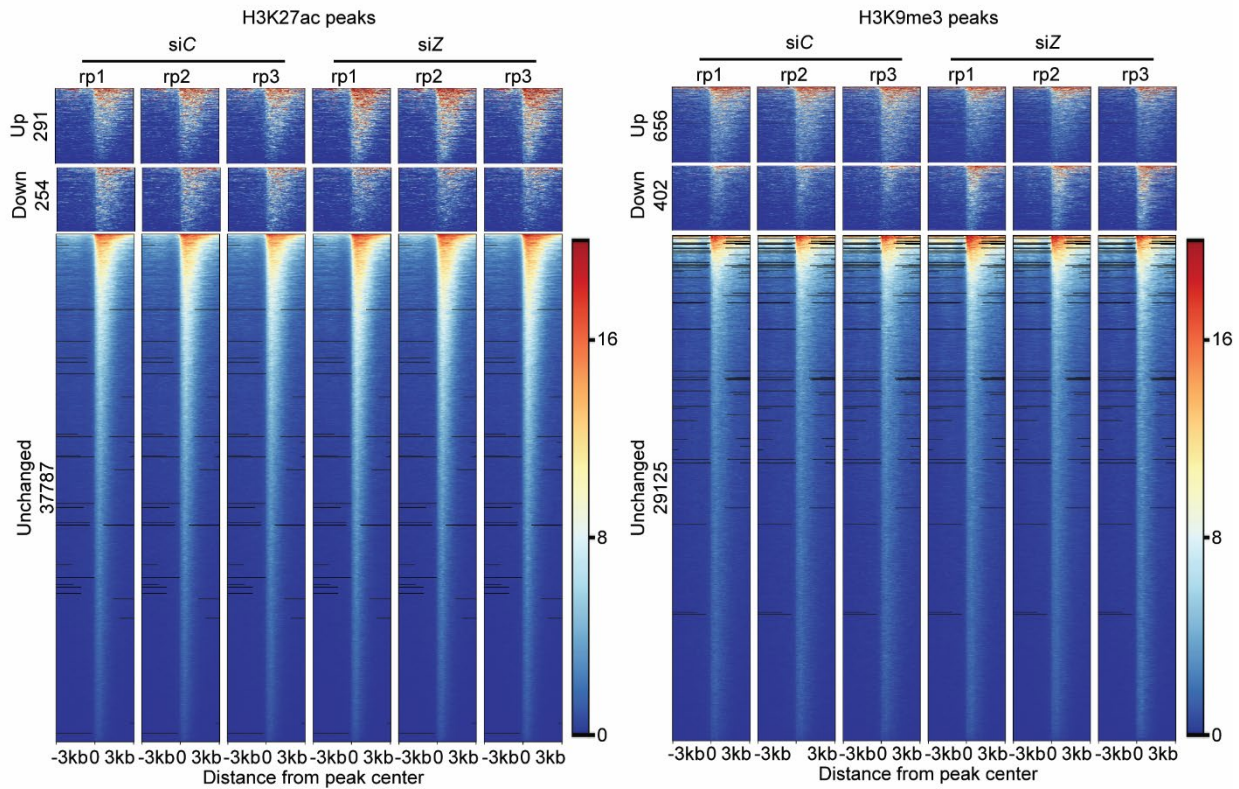


Fig. S3. Quality control of the *ZNF189* knockdown in fat cells. (A) Quantitation of the Western blots presented in Figure 3A-B. *Nuclear* enrichment is calculated vs. cytosolic fraction. (B) Gene expression of adipocyte marker genes. (C) Stimulated lipolysis validation experiments for *ZNF189* depletion using siRNA from two independent vendors. (D) Gene expression of lipolytic regulator genes. *siC* = control cells, *siZ* = *ZNF189* depleted cells. Data are mean \pm SEM. In all panels, replicates are highlighted by dots and are based on at least three independent experiments. $**P < 0.01$, $***P < 0.001$ for Welch's t-test assuming unequal variance (A), or Tukey's multiple comparison's (C).

Fig. S4.

A



B

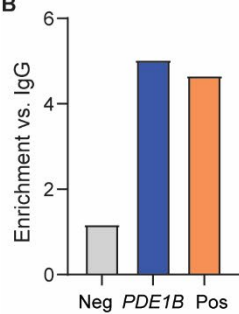


Fig. S4. Quality control of the functional genomics assays. (A) Heatmap for CUT&Tag peaks from siControl (siC) and siZNF189 (siZ) cells analyzed by edgeR with an adjusted P value < 0.05 set as the threshold for up- and down-regulated peaks ($n = 3$). **(B)** ChIP qPCR validation of the ChIP sequencing experiment ($n = 1$). Neg = negative control locus, 3kb downstream of ZNF189 ChIPseq *PDE1B* peak. Pos = positive control region from the ZNF189 ChIPseq.

Fig. S5.

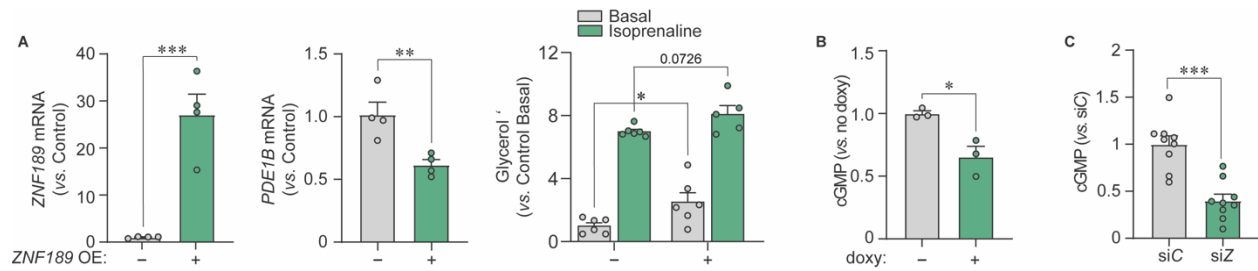


Fig. S5. *PDE1B* regulation in human adipocytes. (A) Gene expression (left and middle plots) and glycerol release (right plot) measures from control cells and *ZNF189* overexpressing (OE) adipocytes. Glycerol release was measured over three hours under basal (non-stimulated) conditions as well as following incubation with isoprenaline. (B) cGMP levels in cells overexpressing *PDE1B2* under a doxycycline (doxy)-inducible promoter. (C) cGMP levels in adipocytes treated with nontargeting control (siC) or *ZNF189* siRNA (siZ). Isoprenaline concentrations were 1-10 μ M. Data are mean \pm SEM. In all panels, replicates are highlighted by dots and are based on at least three independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for a one-tailed (A) or two-tailed (B) t-test. Glycerol data in (A) were analyzed by two-way ANOVA, results from post-hoc statistical analyses are indicated.

Table S1.

Parameter 1	Parameter 2	r	P
Replicate 1	Replicate 2	0.88	1.16E-243
Replicate 1	Replicate 3	0.89	7.15E-250
Replicate 2	Replicate 3	0.89	8.43E-251

Pearson correlation statistics for lipolysis screen replicates.

Table S2.

Gene ID	Glycerol (z-score)	Adj. <i>P</i>
<i>UBB</i>	25.48	1.00E-13
<i>STK16</i>	19.59	1.00E-13
<i>NAA15</i>	17.16	1.00E-13
<i>PLRG1</i>	14.45	1.00E-13
<i>SUPT6H</i>	12.80	1.00E-13
<i>KLF2</i>	11.05	1.00E-13
<i>LRCH4</i>	10.22	1.00E-13
<i>ARID5B</i>	9.26	1.00E-13
<i>ZMIZ2</i>	9.16	1.00E-13
<i>ARID1B</i>	9.15	1.00E-13
<i>KLF7</i>	7.03	1.07E-05
<i>JUNB</i>	6.89	2.05E-13
<i>PLIN1</i>	6.85	1.00E-13
<i>MTPN</i>	6.74	1.61E-11
<i>HMGNI</i>	6.73	2.52E-11
<i>SMAD4</i>	6.67	1.27E-10
<i>TGSI</i>	5.98	9.28E-08
<i>ASB8</i>	5.58	1.45E-10
<i>JUN</i>	4.90	2.74E-05
<i>EPC1</i>	4.72	1.13E-05
<i>TCERG1</i>	4.69	1.70E-05
<i>CLPB</i>	4.51	5.66E-05
<i>ANKRD54</i>	4.26	9.67E-09
<i>PTRF</i>	4.23	3.72E-05
<i>HDAC7</i>	2.07	4.26E-05
<i>TDG</i>	-3.54	1.35E-02
<i>ZMYND8</i>	-3.58	6.51E-04
<i>HIRA</i>	-3.73	4.03E-02
<i>REXO4</i>	-3.81	1.77E-05
<i>SRCAP</i>	-3.89	7.99E-04
<i>LASS4</i>	-4.01	2.37E-02
<i>C19orf2</i>	-4.23	9.80E-04
<i>ZNF326</i>	-4.64	1.44E-02
<i>EDF1</i>	-4.70	9.35E-04
<i>TSC22D1</i>	-4.72	3.13E-04
<i>ZNF189</i>	-5.09	2.12E-05
<i>SMAD5</i>	-5.73	2.34E-12
<i>LMCD1</i>	-5.92	2.85E-04
<i>SMADI</i>	-6.98	1.61E-09

Lipid mobilization screen hits. Adj. *P* from Dunnett's *posthoc* test after a One-way ANOVA.

Table S3.

Protein	Normalized intensity (median)	Enrichment (intensity in ZNF189 IP/intensity in IgG)
ZNF189	3.97	15.67
SUMO2	2.94	7.13
CCAR2	2.00	4.01
XRCC6	1.75	5.94
TRIM28	1.63	6.00
ZMYM4	1.52	2.86
SUMO1	1.29	3.08
GTF2I	1.01	2.02
AHNAK	0.85	16.37
ALDH18A1	0.69	1.61
ADNP	0.67	1.59
SLC25A3	0.38	1.30
ZBED3	0.29	1.22
EML1	-0.27	0.83
SAMHD1	-0.28	3.14
PC	-0.45	6.26
RPL7	-0.61	4.44
BLMH	-2.63	0.16

ZNF189 interacting proteins identified by Immunoprecipitation-Mass Spectrometry. Interacting proteins were selected after quantification by filtering to include proteins with no detection in IgG samples or enrichment (fold change *versus* IgG) > 3 (n = 4).

Table S4.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
<i>18S</i>	TGACTCAACACGGGAAACC	TCGCTCCACCAACTAAGAAC
<i>ADIPOQ</i>	GGTCTCGAACTCCTGGCCTA	TGAGATATCGACTGGGCATGGT
<i>ANKRD54</i>	AGATCATCCAT ATGCTGAGG	TCTTTGGTACTGGTCATCTG
<i>ARID1B</i>	ATGGAACTAGATCTCAACCTC	CTGGTAAACTTGATGGTCTTTC
<i>ASB8</i>	TTCTAGTCCGGTAAACAGAG	CTCCAAACTGCCGAATTG
<i>B2M</i>	AAGGACTGGTCTTTCTATCTC	GATCCCCTTAACTATCTTGG
<i>CEBPA</i>	CCCGGCAACTCTAGTATTTAG	AAATGACAAGGCACGATTG
<i>CD36</i>	CAAGTTGTCCTCGAAGAAGG	CATCATCACTTCCTGTGGATT
<i>EDF1</i>	CATTCTATTACCAAGAACACGG	TGGCTTCTCATTGATTTTCG
<i>EPC1</i>	CAAAGTCTTACCATCGTCTG	TTTCTACGGAAAGCAAAAG
<i>HIRA</i>	AGGAAGTGATATGACGGTATC	GTGGAAAGTGACGGATTAAG
<i>JUN</i>	AAAGGATAGTGCATGTTTC	TAAAATCTGCCACCAATTCC
<i>JUNB</i>	TACTGTGGAAAAGAAACACG	GAACAAACACACACAAACAC
<i>KLF2</i>	AAGGGCCTT AATTTGTACTG	ATAAAAACGAACCAGGTAGC
<i>KLF7</i>	AAAACAAGAAGAGGGTTCAC	ACTTATAAGGCTTCTCACCTG
<i>LMCD1</i>	TGTCAAGCAATATAAGAGCG	CAGACGTATTCCACTTCTTTG
<i>LRCH4</i>	ATTTTCAGAGCTGTCATTCC	CTGTTCGATGAAGTCAATCTG
<i>LRP10</i>	GATGGAGGCTGAGATTGTG	GAGTCATATCCTGGCGTAAG
<i>NAA15</i>	AAGGATGAATTTAGCAAGG	CCACCTTTTCTTTGTCTTTG
Neg ctrl-ChIP qPCR	CAACCTGACTGAGATGGATGAG	AAGCTCCCTAACAGAGGAAATG
<i>PDE1B1</i>	GTCCTCCGGAGATGCTG	AGCAAGAAGATGTGGATTAAGC
<i>PDE1B</i> -all variants	CTTCAAGATTCCCCTGTGTTT	GAATCCTTACCACAACCAGATC
<i>PDE1B</i> -ChIP qPCR	CTTTCTGCTGACATACTCAACC	GGGATCTGCAATCTCTTAGATG
<i>PLRG1</i>	ACTATAAAGATCTGGGACTTGG	CAGCATTTCCTTGTGTTGTC
Pos ctrl-ChIP qPCR	AGTCCAGCTCTCTTTGCCTC	GCAGTTCTGAGACGTTTAAGCA
<i>PPIA</i>	CCCACCGTGTTCTTCGACATT	GGACCCGTATGCTTTAGGATGA
<i>PTRF</i>	TCATGATCTACCAGGATGAAG	TCCTCAATAACCTCCTCAAC
<i>REXO4</i>	TGAAGTTGTTGAGAAGGAAG	GTACCTTTAGGTCATTATGCAG
<i>SMAD1</i>	GGCATATTGAAAAGGAGTTC	AGATGCTACTGTCCTAAGG
<i>SMAD5</i>	CCAGTCTTACCTCCAGTATTAG	TCCTAAACTGAACCAGAAGG
<i>SRCAP</i>	CATCTCATGATAGTGACACC	CTTTATCTCCAACACCTGAG
<i>STK16</i>	GAATGAGATAGAAAGGCTGAAG	ACCCAAGTCCATTAAACTG
<i>SUPT6H</i>	GAACTAGAAGAAGAAGCTGAC	CACCTCAAAATGCTGATTTC
<i>TCERG1</i>	AGAGGAGATGACTGAAGAAG	CCATGGAGTACCAGGAATAG
<i>TDG</i>	TCAAATGGGCTAATTGAGAG	TAAGCATGGCTTTCTTCTTC
<i>TGS1</i>	GAACAAAAAGGTGAATGGTC	TCTCTGTCCAACCTTAATCCC
<i>UBB</i>	CCGCTAACAGGTCAAAATG	TAGTCAGAAAGAGTACGGC
<i>UR11</i>	CTTGATAGTAAGCCTGATACTG	GTGTTACGATCTTCTTTTCC
<i>ZMIZ2</i>	AGGAATGACACCCTTGTATG	ATACACCTCAGAGTAGGTTC
<i>ZMYND8</i>	ACTATATGAAGCAGCCACTC	TCTCACATTCAGGACAAAAC
<i>ZNF189</i>	TCTCACTGGATGTTTTGAAC	GAAATTGAGGAAGAAGTGGA
<i>ZNF326</i>	CTGCATCTGGAAAGTTCTTC	TACTCATGCAAAAACCTCC

ZNF189 DNA gblock sequence:

ctgtctgtagctagegccaccATGGCATCACCATCTCCTCCACCTGAATCAAAGGACTGTTGA
CTTTTGAGGACGTAGCGGTCTTCTTTACTCAAGAAGAATGGGACTATTTGGACCCAG
CTCAAAGGTCTCTTTACAAAGATGTGATGATGGAAAACCTATGGGAACCTCGTTTCAC
TTGACGTAACAGGGATAAAGATGAGGAGCCTACCGTTAAACAGGAGATCGAG
GAGATTGAAGAGGAGGTAGAACCACAGGGTGTAAATTGTAACACGCATCAAAGCGA
AATCGACCAGGACCCCATGGGTCGCGAGACATTTGAACTCGTGGGAAGACTCGACA
AACAAAGAGGGATTTTCTTGTGGGAAATCCCCGGGAAAGCCTGACTCAGGAACAG
CGAATGTTTAGGGAGAATACAAATATCATTAGGAAGAGGCCTAATAGTGAGGAGAA
GTGTCATAAATGCGAGGAATGTGGGAAAGGATTCGTAAGAAAAGCGCATTTTATCC
AGCATCAGAGGGTCCACACCGGAGAAAAACCTTTTCAGTGTAACGAATGCGGAAAA
TCCTTTTCACGGAGTTCATTCGTAATAGAACACCAACGCATTCATACCGGGGAGAGG
CCATATGAGTGTAATTATTGCGGCAAGACGTTTTTCTGTTTCTTCAACATTGATACGGC
ACCAAAGGATTCACACCGGCGAGCGGCCCTATCAATGTAACCAATGTAAGCAGTCA
TTTTCTCAGCGCAGGAGCCTTGTTAAGCATCAGAGAATACACACCGGCGAAAAACC
GCATAAATGTTTCAGACTGTGGAAAGGCTTTCAGTTGGAAGTCCCATCTTATCGAACA
TCAGCGGACTCACACGGGCGAGAAGCCGTACCACTGCACGAAGTGCAAGAAGAGCT
TTTCTCGCAACAGTTTGTCTCGTGGAGCATCAACGAATCCACACAGGTGAGCGGCCCC
ACAAATGTGGGAATGCGGGAAAGCTTTCGGGCTTAGCACGTATCTCATTACAGCACC
AAAAAATCCATACGGGAGAGAAACCCTTCTTGTGCATAGAATGCGGAAAGTCCTTT
AGCCGGTCCAGTTTTCTGATAGAGCATCAGCGGATACATACTGGTGAAAGGCCTTAC
CAATGCAAGGAGTGTGGAAAAAGCTTTAGTCAACTGTGCAACTTGACCCGACACCA
GAGGATTCACACAGGTGATAAACCACATAAGTGCGAAGAATGCGGAAAAGCTTTTA
GCCGATCTTCCGGCCTGATCCAACATCAGAGAATCCACACGCGAGAGAAGACATAC
CCGTACAATGAACTAAAGAATCTTTTGATCCGAATTGTTCCCTCGTTATCCAACAA
GAGGTGTACCCCAAGGAGAAGTCTTATAAGTGCGATGAGTGTGGAAAGACATTTAG
TGTATCTGCTCATCTCGTACAGCACCAACGAATCCACACTGGGGAAAAACCCTATCT
CTGCACTGTCTGCGGGAAGTCATTCTCACGATCCTCTTTTCTTATTGAACACCAGCGC
ATTCACACGGGTGAGCGGCCGTATTTGTGCCGGCAGTGCGGGAAGAGCTTCTCCAG
TTGTGCAATCTTATTCGGCATCAGGGCGTGCACACGGGGAATAAGCCCCACAAGTGT
GACGAATGTGGAAAAGCGTTCAGTCGAACTCAGGCCTCATTCAACACCAGCGAAT
CCATACAGGAGAGAAACCGTATAAGTGTGAGAAATGCGATAAATCCTTCAGCCAAC
AACGCAGCCTGGTTAATCATCAGAAGATACACGCAGAAGTGAAGACCCAAGAAACG
CATGAATGTGACGCTTGCGGGGAGGCCTTTAACTGTAGGATATCTTTGATTACGCAT
CAAAAATTGCATACAGCTTGGATGCAATAGggatcctgtctgata

T7 ARCA forward primer:

TAATACGACTCACTATAGGGTAGCGCCACCATGGCATC

Reverse primer:

GGATCCCTATTGCATCCAAGCTG

Primer sequences for qPCR assays and gblock sequence/primers for mRNA overexpression.