Supplemental Tables

Table 1 List of partial VGLL4 MS candidates

Gene names	Protein names	Peptides vgl14	Peptides IGG	Unique peptides vgl14	Unique peptides IGG	Unique sequence coverage [%]	Mol. weight [kDa]	Sequence length
VGLL4	Transcription cofactor vestigial-like protein 4	19	6	14	6	60.5	31.864	296
CTBP2	C-terminal-binding protein 2	5	0	3	0	9.7	56.101	513
CTBP1	C-terminal-binding protein 1	4	0	2	0	6.8	47.535	440
SLC25A5	ADP/ATP translocase 2	14	4	5	1	14.9	35.293	323
YWHAQ	14-3-3 protein theta	12	2	9	1	39.2	27.764	245
DNAJA1	DnaJ homolog subfamily A member 1	11	1	11	1	41.1	44.868	397
TUBB4B	Tubulin beta-4B chain	18	8	1	1	2.7	49.83	445
HSP90AB1	Heat shock protein HSP 90-beta	14	5	10	3	17.1	83.263	724
PKM2	Pyruvate kinase isozymes M1/M2;Pyruvate kinase	9	0	9	0	28.2	57.936	531
DNAJC7	DnaJ homolog subfamily C member 7	9	0	9	0	27.1	56.44	494
STUB1	E3 ubiquitin-protein ligase CHIP	9	0	9	0	33	34.856	303

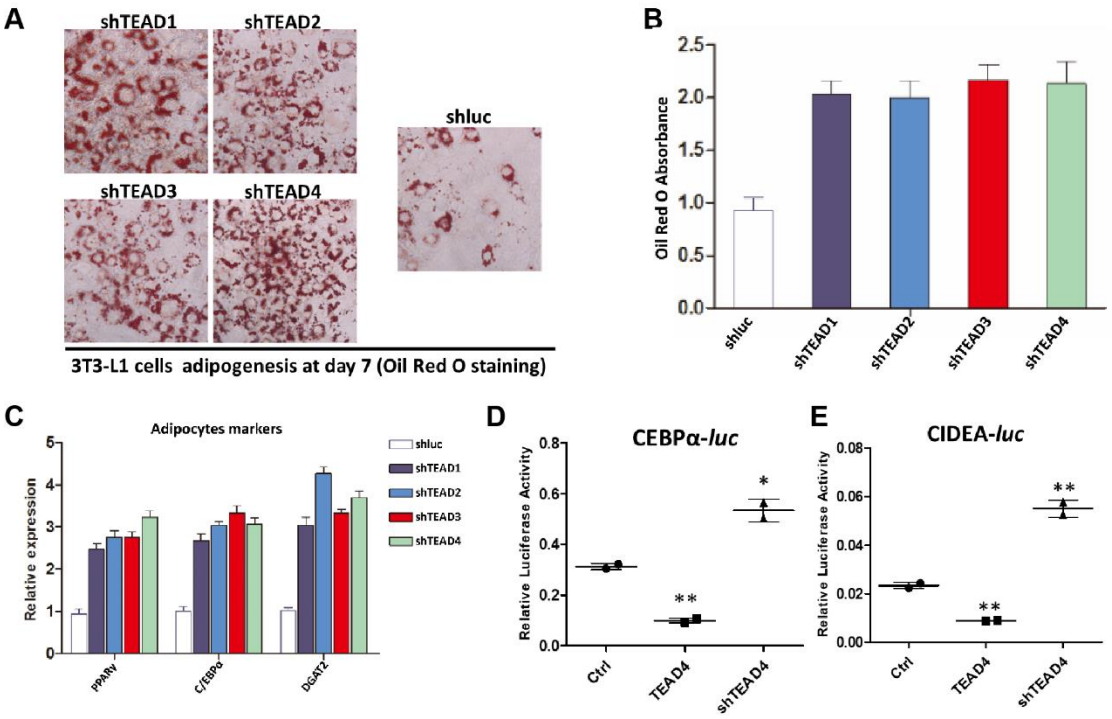
Table 2 List of all used shRNA sequences

Gene	Direction	Sequence
shmTEAD4-1	sense	CCGGGCTGAAACACTTACCCGAGAACTCGAGTTCTCGGGTAAGTGTTTCAGCTTTTTG
	antisense	AATTCAAAAAGCTGAAACACTTACCCGAGAACTCGAGTTCTCGGGTAAGTGTTTCAGC
shmTEAD4-2	sense	CCGGCCGCCAAATCTATGACAAGTTCTCGAGAACTTGTCATAGATTTGGCGGTTTTTG
	antisense	AATTCAAAAACCGCCAAATCTATGACAAGTTCTCGAGAACTTGTCATAGATTTGGCGG
shmVGLL4-1	sense	CCGGACACATGGCTTCAGATCAAAGCTCGAGCTTTGATCTGAAGCCATGTGTTTTTTG
	antisense	AATTCAAAAAACACATGGCTTCAGATCAAAGCTCGAGCTTTGATCTGAAGCCATGTGT
shmVGLL4-2	sense	CCGGGACAAGATGAACAACAATATCCTCGAGGATATTGTTGTTCATCTTGTCTTTTTG
	antisense	AATTCAAAAAGACAAGATGAACAACAATATCCTCGAGGATATTGTTGTTCATCTTGTC
shmYAP-1	sense	CCGGTGAGAACAATGACAACCAATACTCGAGTATTGGTTGTCATTGTTCTCATTTTTG
	antisense	AATTCAAAAATGAGAACAATGACAACCAATACTCGAGTATTGGTTGTCATTGTTCTCA
shmYAP-2	sense	CCGGGAAGCGCTGAGTTCCGAAATCCTCGAGGATTTCGGAACTCAGCGCTTCTTTTTG
	antisense	AATTCAAAAAGAAGCGCTGAGTTCCGAAATCCTCGAGGATTTCGGAACTCAGCGCTTC
shmCtBP2	sense	CCGGTACGAAACTGTGTCAACAAAGCTCGAGCTTTGTTGACACAGTTTCGTATTTTTG
	antisense	AATTCAAAAATACGAAACTGTGTCAACAAAGCTCGAGCTTTGTTGACACAGTTTCGTA

Table 3 List of the primers used for ChIP-PCR

Primer	Direction	Sequence	Length(bp)
PPARG-E	forward	5'-CAATATTGAACAATCTCTGCTCTG-3'	
	reverse	5'-ACAAGGAAAACGTTGCTACATT-3'	113
PPARG-A	forward	5'-TCCCGATGGTTCCTGAGCA-3'	
	reverse	5'-GGAGTGTAAAAAGATGAGAAATGG-3'	116
KLF8	forward	5'-AAATTCCTGCACTCACAAGACTCT-3'	
	reverse	5'-TCCCCTAGAACTGCAGTTACAGAC-3'	173
Adipo2-3	forward	5'-GATTGGGTTACCTCACTCAG-3'	
	reverse	5'-CCAGATGCCCCTCAACAG-3'	152
PLINB	forward	5'-AGAGACAGTGTTGGGAGGGTGGAG-3'	
	reverse	5'-GAGAGGGTTAGCTGAGGAGAAAGA-3'	104
CTGF	forward	5'-TGTGCCAGCTTTTTCAGACG-3'	
	reverse	5'-GAACTGAATGGAGTCCTACACA-3'	

Supplemental Figures



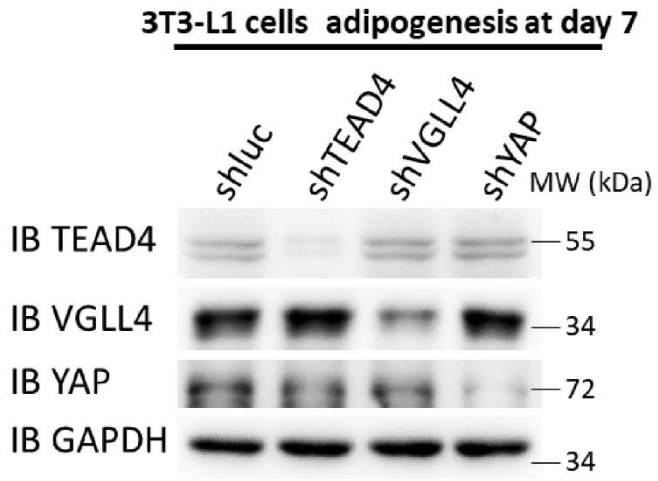
Supplemental Figure 1. Default repression of adipogenesis by TEADs in 3T3-L1 cells.

(A and B) Oil Red O staining of lentiviral-mediated knock-down of TEAD1, 2, 3, 4 3T3-L1 stable cells after adipogenic cocktails induced for 7 days (A), and quantified by measurement of the absorbance at 510 nm (B). Data were shown as mean ± SD (*n=3*). *, p<0.05 by Student's t test.

(C) qRT-PCR analysis of adipocytes markers expression in TEADs knock-down 3T3-L1 cells after adipogenic cocktails induced for 7 days.

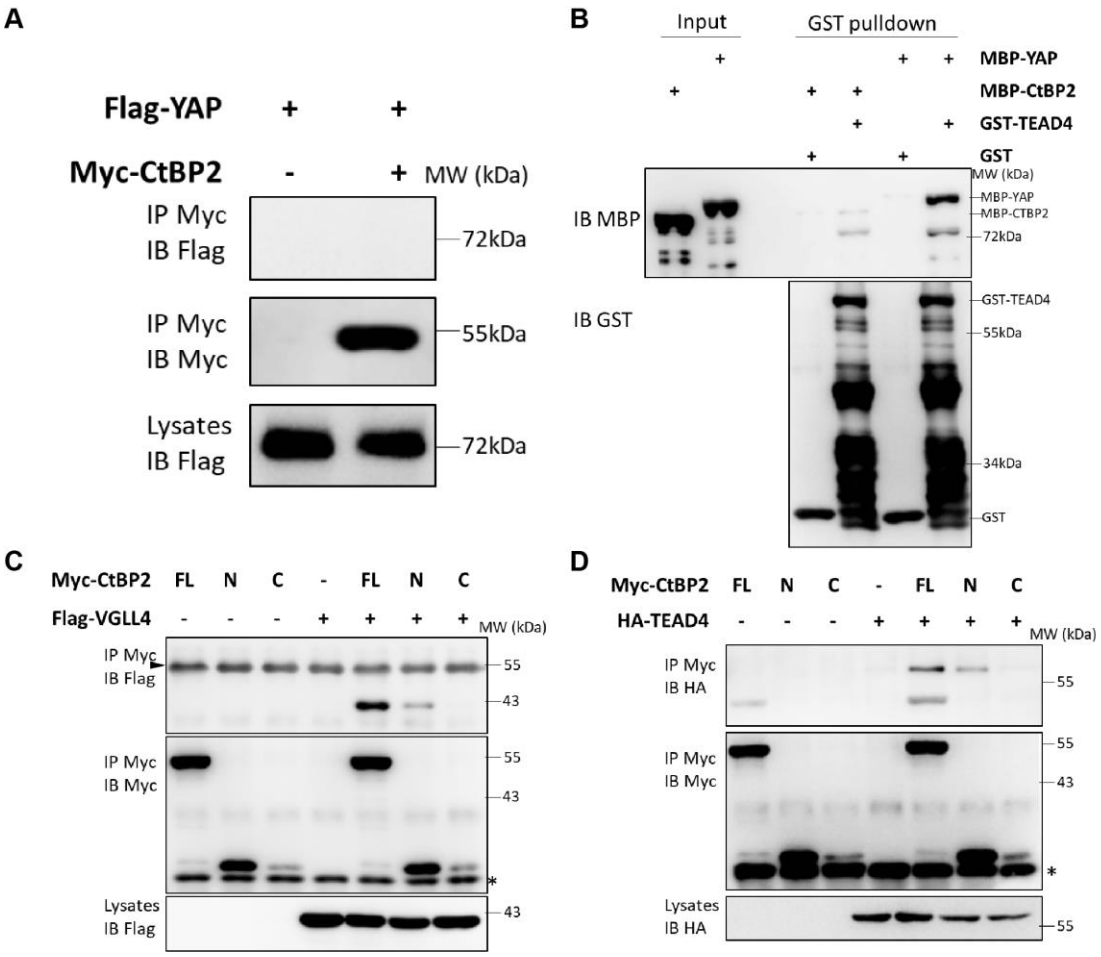
(D and E) Overexpression or knock-down of TEAD4 effect on C/EBPα-*Luc* (C), and CIDEA-*luc* (D) activity in 293T cells. Data were shown as mean ± SD (n=3). **, p＜0.01 by Student's t test. *, p＜0.05 by Student's t test.

A



Supplemental Figure 2. TEAD4 modulates adipogenesis-related pathways.

(A) Immunoblot analysis of TEAD4, VGLL4 and YAP in lentiviral-mediated expression of shluc/shTEAD4/shVGLL4/shYAP 3T3-L1 stable cell lines after adipogenic cocktails induced for 7 days. GAPDH was used as an internal control. Positions of protein molecular mass marker are indicated on the right.

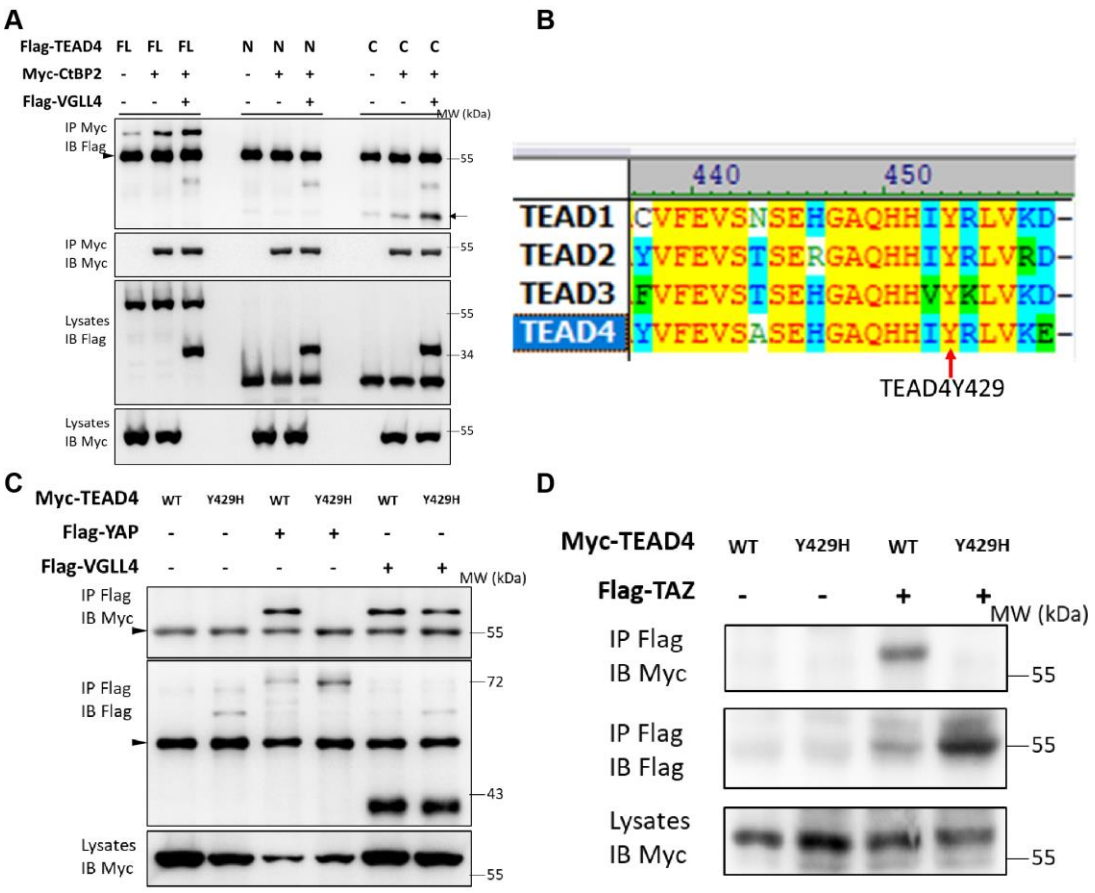


Supplemental Figure 3. CtBP2 binds to both TEAD4 and VGLL4.

(A) Co-IP assay detected no interaction between YAP and CtBP2. The indicated plasmids were transfected into 293T cells and analyzed by Co-IP.

(B) GST pull-down assay to detect the interaction between TEAD4 and CtBP2, using YAP as a positive control.

(C and D) Co-IP assay to map the interaction between CtBP2 and VGLL4 (C), and TEAD4 (D), respectively. The plasmids of CtBP2 full-length (FL), CtBP2 N-terminal, CtBP2 C-terminal, VGLL4 and TEAD4 were transfected into 293T cells and analyzed by Co-IP. The arrowhead indicates the IgG heavy chain and the asterisk denotes the nonspecific band.



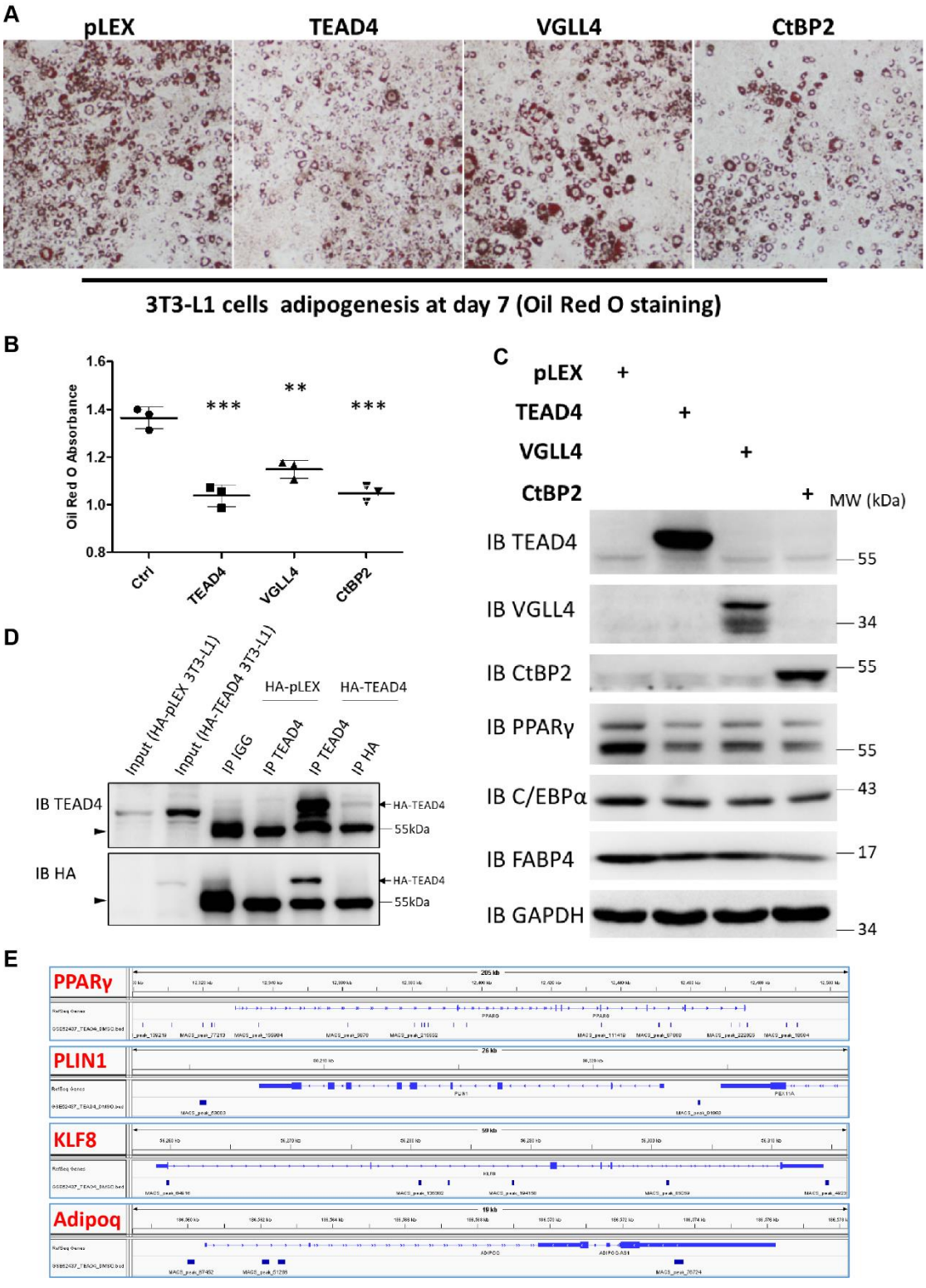
Supplemental Figure 4. VGLL4 mediates the interaction between TEAD4 and CtBP2.

(A) Co-IP assay to detect exogenous VGLL4 effect on the interaction between TEAD4 full-length, TEAD4 N-terminal or TEAD4 C-terminal and CtBP2. The indicated plasmids were transfected into 293T cells and analyzed by Co-IP. The arrowhead indicates the IgG heavy chain.

(B) Sequence alignment of TEADs YAP interactions.

(C) Co-IP assay to detect TEAD4Y429 site function on its interaction with YAP or VGLL4, respectively. The indicated plasmids were transfected into 293T cells and analyzed by Co-IP. The arrowheads indicates the IgG heavy chains.

(D) Co-IP assay to detect TEAD4Y429 site function on its interaction with TAZ. The indicated plasmids were transfected into 293T cells and analyzed by Co-IP.



Supplemental Figure 5. TEAD4 directly regulates adipogenesis and targets PPARγ, PLIN1, KLF8 and Adipoq.

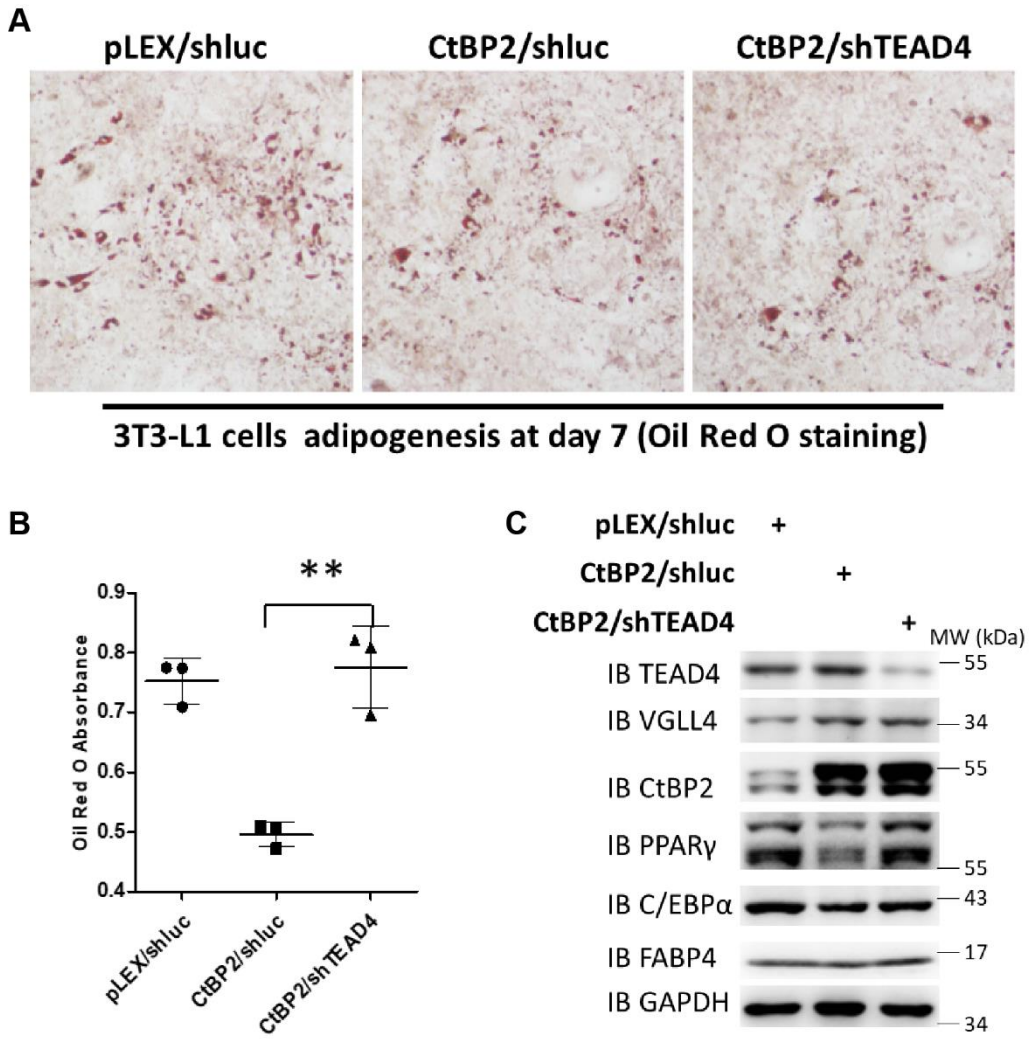
(A and B) Oil Red O staining of lentiviral-mediated overexpression of pLEX, TEAD4, VGLL4 and CtBP2 3T3-L1 stable cell lines after adipogenic cocktails induced for 7 days

(A) and quantified by measurement of the absorbance at 510 nm (B).

(C) Immunoblot analysis of TEAD4, VGLL4, CtBP2, PPARγ, C/EBPα and FABP4 expression in the above cell samples. GAPDH was used as an internal control. Positions of protein molecular mass marker are indicated on the right.

(D) Co-IP assay to verify TEAD4 antibody immunoprecipitation efficiency in 3T3-L1 cells. TEAD4 or IgG antibodies were incubated with cell lysates stable-expressing TEAD4 and analyzed by Co-IP. The arrowheads indicate the IgG heavy chains.

(E) Positive binding peaks of TEAD4 in PPARγ, PLIN1, KLF8 and Adipoq promoter regions.



Supplemental Figure 6. Ectopic expression of CtBP2 in the depletion of TEAD4 inhibits adipogenesis.

(A and B) Oil Red O staining of lentiviral-mediated overexpression of CtBP2 in TEAD4-depletion 3T3-L1 stable cell lines after adipogenic cocktails induced for 7 days (A) and quantified by measurement of the absorbance at 510 nm (B). **, $p < 0.01$ by Student's t test.

(C) Immunoblot analysis of TEAD4, VGLL4, CtBP2, PPARγ, C/EBPα and FABP4 expression in the above cell samples. GAPDH was used as an internal control. Positions of protein molecular mass marker are indicated on the right.