

Supplementary Figure Legends

Figure S1. 3T3-F442A subline isolation strategy. FBS = Fetal Bovine Serum. CS = Calf Serum. Insulin was used at 5 $\mu\text{g}/\text{mL}$.

Figure S2. Heat map depicting differentially regulated genes identified in isolated sublines of 3T3-F442A pre-adipocytes using whole mouse genome arrays. Cells were grown in 10% FBS with insulin (5 $\mu\text{g}/\text{mL}$). RNA was isolated 48 h post-confluence.

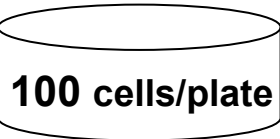
Figure S3. Correlation of mouse adipose Vgl13 mRNA expression with total fat, mesenteric, retroperitoneal, and subcutaneous fat content. N \sim 300 (16).

Figure S4. Analysis of the activation of -5.4 kB aP2 promoter co-expressing PPAR γ 2/RXR and Vgl13 in undifferentiated 3T3-L1 pre-adipocytes in 10% FBS with insulin (5 $\mu\text{g}/\text{mL}$) with or without PPAR γ ligand GW7845 (100 nM). N=4.

Figure S5. Expression of osteogenic-associated genes in C310T1/2 cells overexpressing Vgl13 or pBABE control grown in 10% FBS + β -glycerophosphate (1M) + Ascorbic Acid (50 mg/mL) at Day 0 and Day 30. Gene expression was analyzed by real-time PCR and normalized to 36B4 control.

1. Picking Single Colonies

Incubation time:
~ 72 hours



cloning discs



Grow each clone to
~ 80% confluence in 10% CS
~100 colonies picked.

2. Selecting Clones



~60 clones grown to confluence in 10% Fetal Bovine Serum (FBS) +/- insulin for 8 days.

~60 clones grown to nearly confluence in 10% Calf Serum (CS).

3. Scoring and Initial Freezing



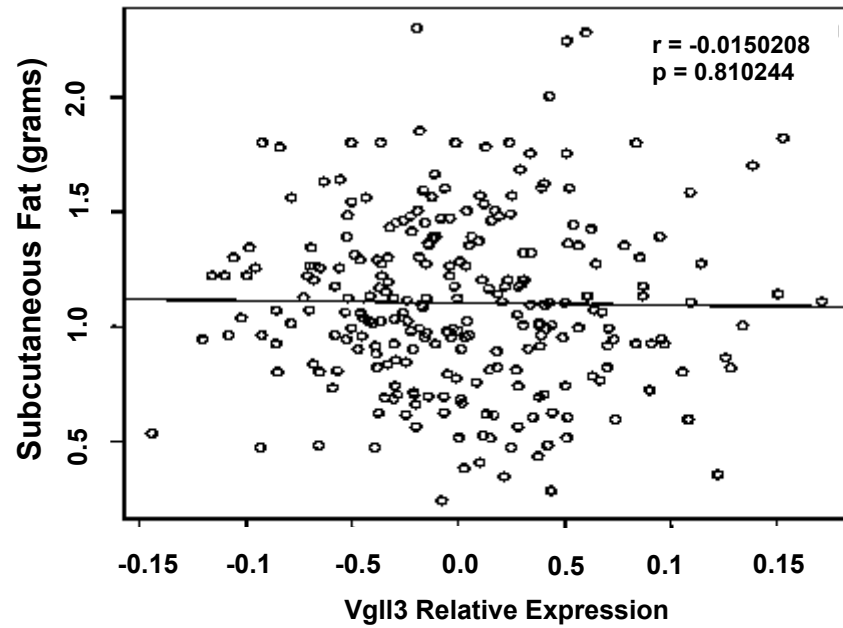
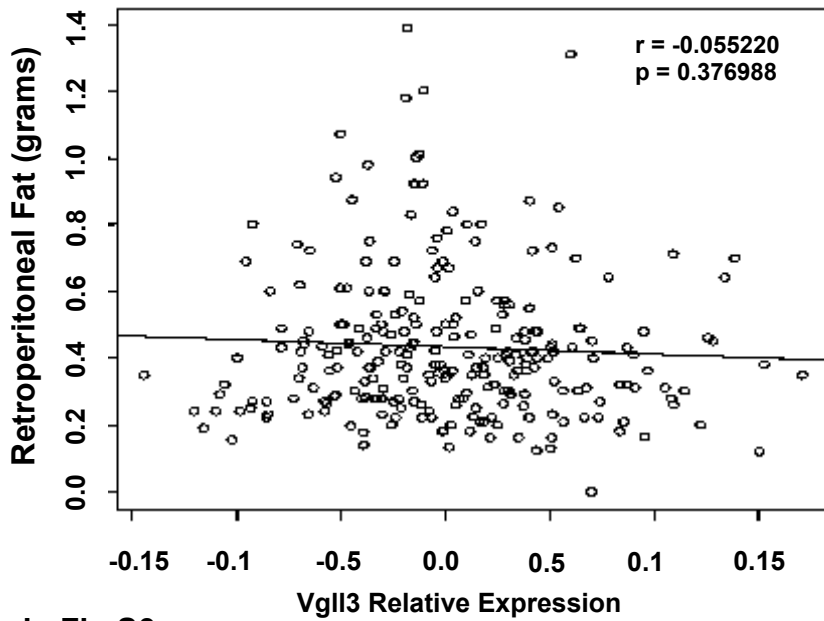
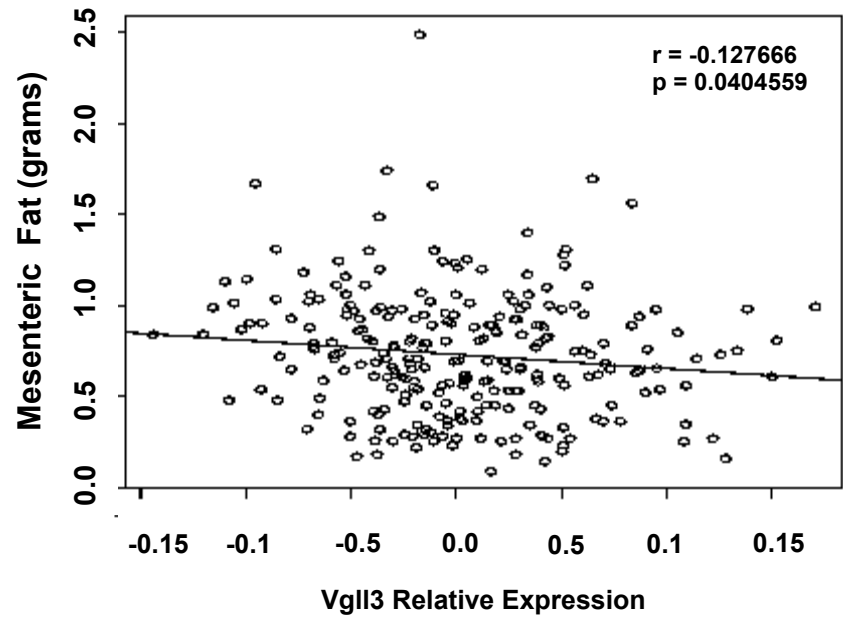
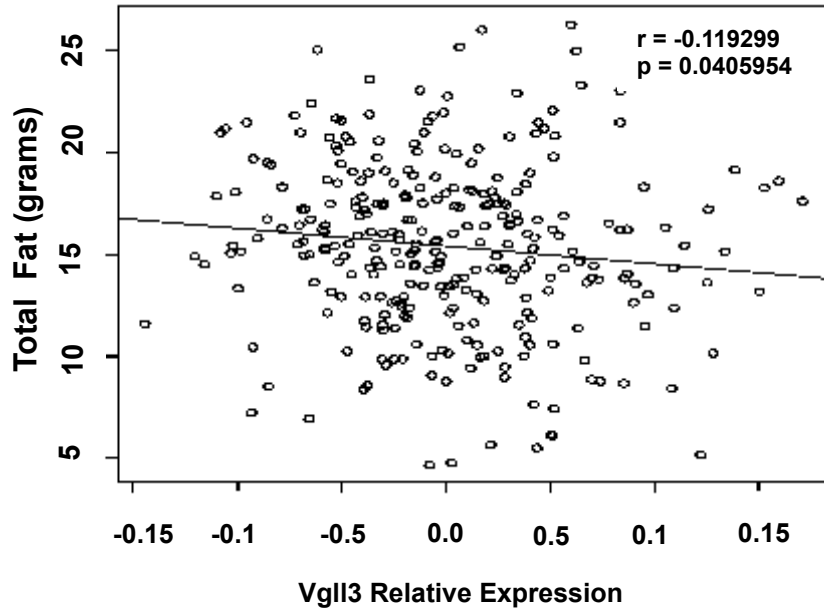
10% FBS

-Oil Red O stained; Day 8
-sub-clones validated by PPAR γ 2 & aP2 gene expression.



10% CS

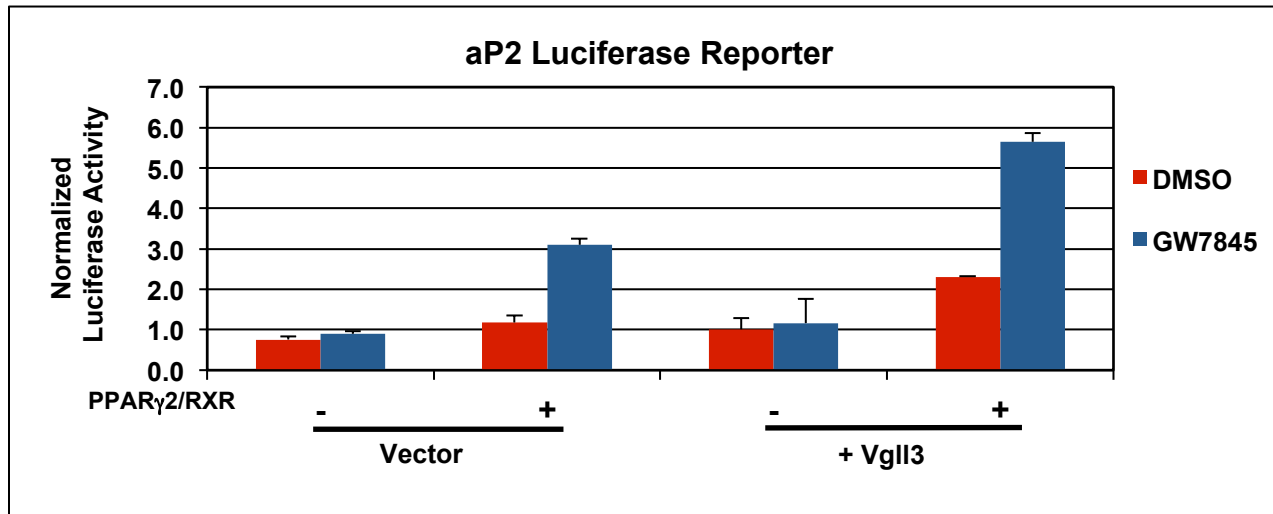
Cell trypsinized and frozen down.
All clones passed to small flasks.



Halperin Fig.S3

5.4 Kb aP2 Promoter

Luciferase Reporter Gene



Halperin Fig.S4

