

SUPPLEMENTARY DATA

β,β -Carotene Decreases Peroxisome Proliferator Receptor γ Activity and Reduces Lipid Storage Capacity of Adipocytes in a β,β -Carotene-Oxygenase 1-Dependent Manner

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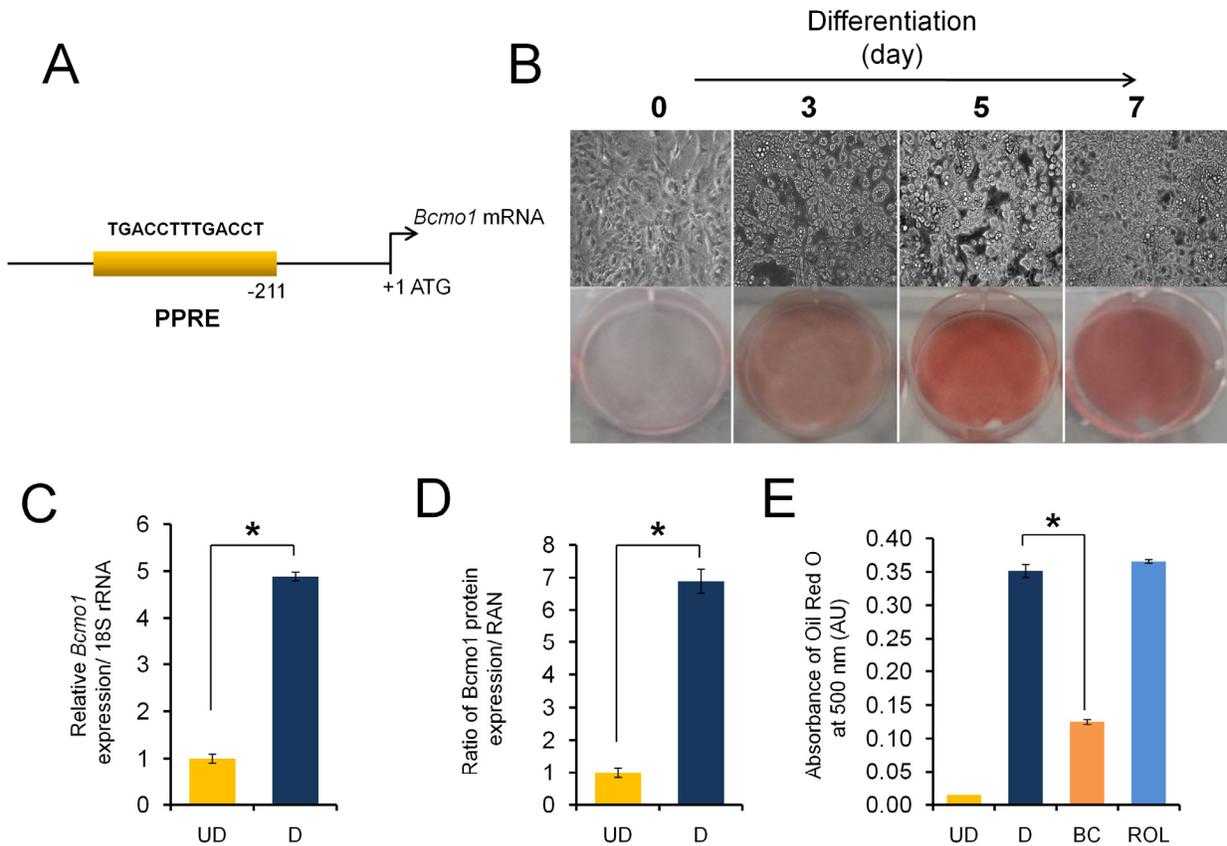


Figure S1. *Bcm1* expression is induced during adipogenesis. **(A)** Schematic representation of the murine *Bcm1* promoter showing the position of the DR1 peroxisome proliferator response element (PPRE). **(B)** Oil red O staining of differentiating NIH 3T3-L1 cells (bottom panel). Top panel shows representative phase contrast images acquired at 10x magnification. **(C)** *Bcm1* expression as determined by qRT-PCR relative to that of endogenous 18S rRNA. **(D)** Quantification of *Bcm1* protein levels by densitometry of immunoblots, expressed as the ratio of *Bcm1* to Ran (Ras related nuclear protein, used as protein loading control) levels. **(E)** Quantification of Oil Red O stain extracted from stained cells was performed by eluting the stain from cells in 1 mL of 100% isopropyl alcohol and then measuring the absorbance of the stain against a blank (100% isopropyl alcohol) at 500 nm. Error bars represent S.D., n = 3 per condition. Two tailed Student's *t*-test * $P < 0.001$. UD, undifferentiated preadipocytes; D, differentiated adipocytes at day 5; BC, β,β -carotene; ROL, all-*trans*-retinol.

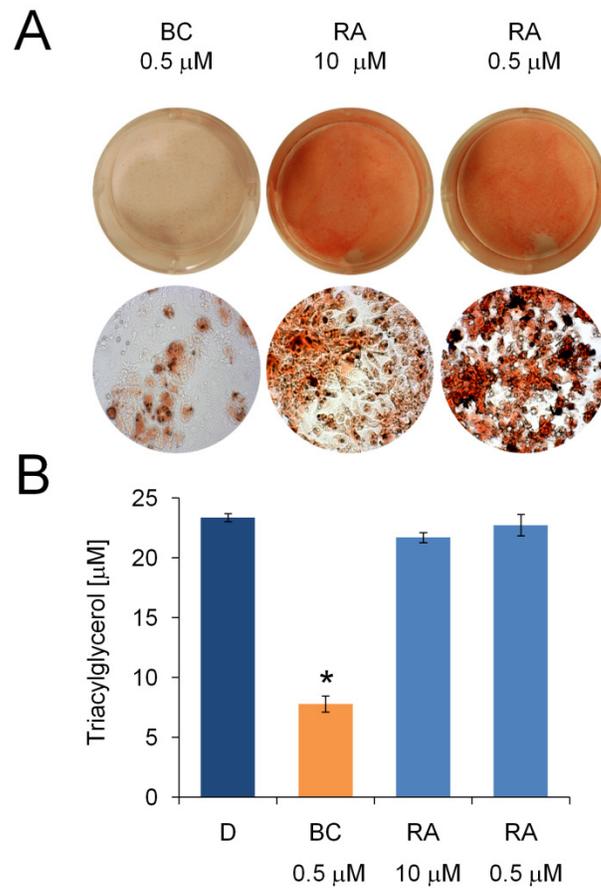


Figure S2. Effects of different concentration of β,β -carotene and RA on adipocytes. Differentiated NIH 3T3-L1 adipocytes (day 5) were treated as indicated. **(A)** Top panels show representative images of cells stained with Oil Red O; bottom panel are images from Oil Red O stained cells taken at 20x magnification. **(B)** Effects of different treatments on the TG content of mature NIH 3T3-L1 adipocytes. Error bars represent \pm S.D., $n = 3$ per condition, Two tailed Student's t -test, * $P < 0.001$. D, differentiated mature adipocytes at day 5; BC, β,β -carotene; RA, all-*trans*-retinoic acid.

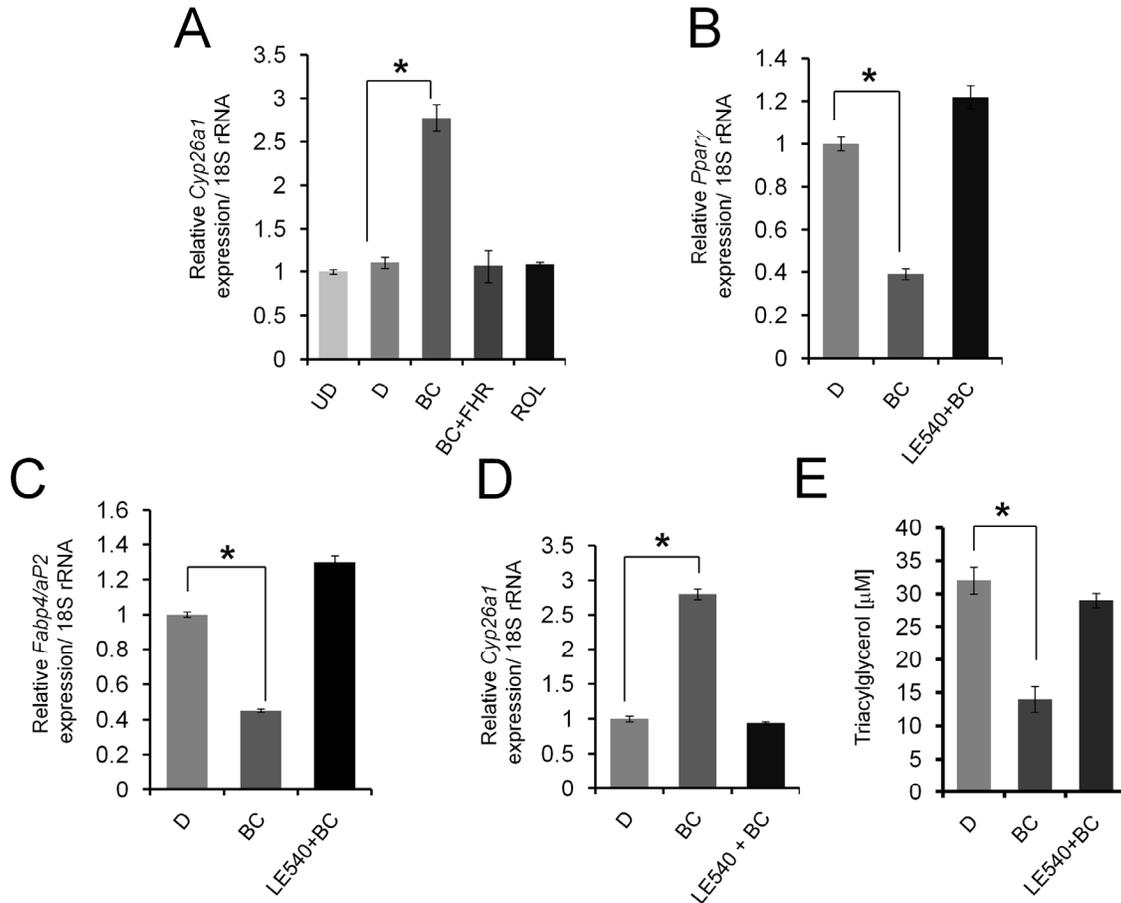


Figure S3. Effects of β,β -carotene on adipocytes are Rar-dependent. Differentiated NIH 3T3-L1 adipocytes (day 5) were treated as indicated. After 24 h, total RNA was extracted, reverse transcribed and qRT-PCR was performed. (A) *Cyp26a1* expression is induced by β,β -carotene. (B-D) Effects of BC on mRNA expression of marker genes, (B) *Ppar γ* (C) *Fabp4/aP2* and (D) *Cyp26a1*, in mature NIH 3T3-L1 adipocytes with and without pretreatment with the Rar pan-antagonist LE540. (E) Effects of BC on the TG content of mature NIH 3T3-L1 adipocytes with and without pretreatment with the Rar pan-antagonist LE540. Error bars represent \pm S.D., $n = 3$ per condition, Two tailed Student's *t*-test, * $P < 0.001$. UD, undifferentiated NIH 3T3-L1 preadipocytes; D, differentiated mature adipocytes at day 5; BC, β,β -carotene; FHR, fenretinide; ROL, all-*trans*-retinol.

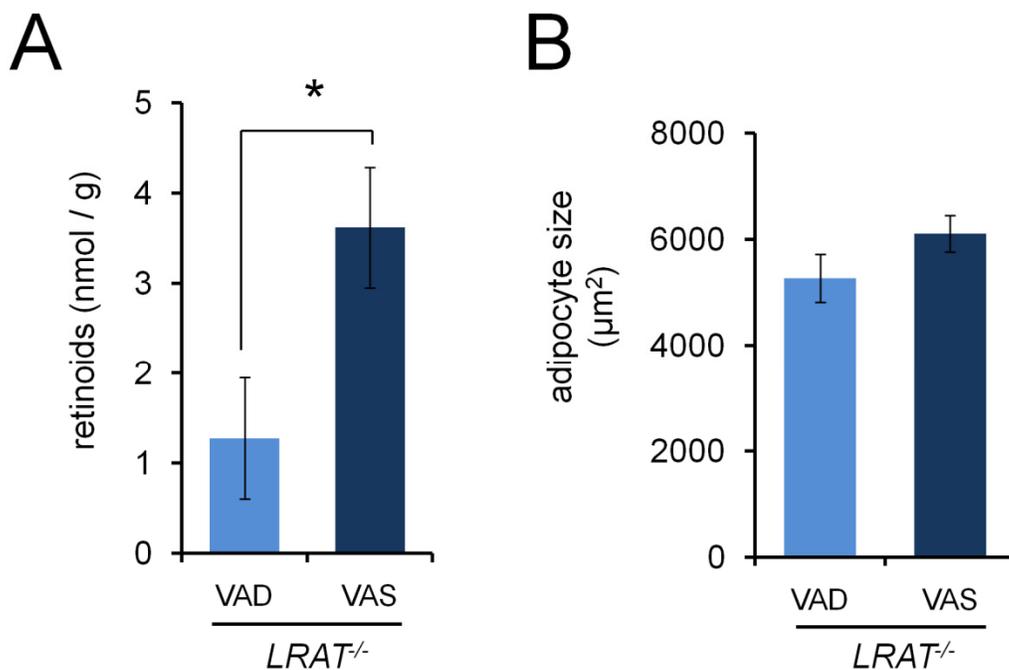


Figure S4. Dietary restriction of preformed vitamin A reduces vitamin A levels in adipose tissue but does not affect adipocyte size in $LRAT^{-/-}$ mice. $LRAT^{-/-}$ mice were maintained either on a vitamin A deficient diet (VAD) or a vitamin A sufficient diet (VAS). **(A)** HPLC analysis of vitamin A concentrations in adipose tissue of mice on either VAD or VAS diets **(B)** Measurements of iWAT adipocyte size in square microns (Representative images are shown in Figure 6 B in the main text part). Images were analyzed with Metamorph Imaging Software (about 600 adipocytes were counted and sized per condition). Error bars represent \pm S.D., n = 5 per group. Two tailed student's *t*-test, * $P < 0.001$.