Expanded View Figures

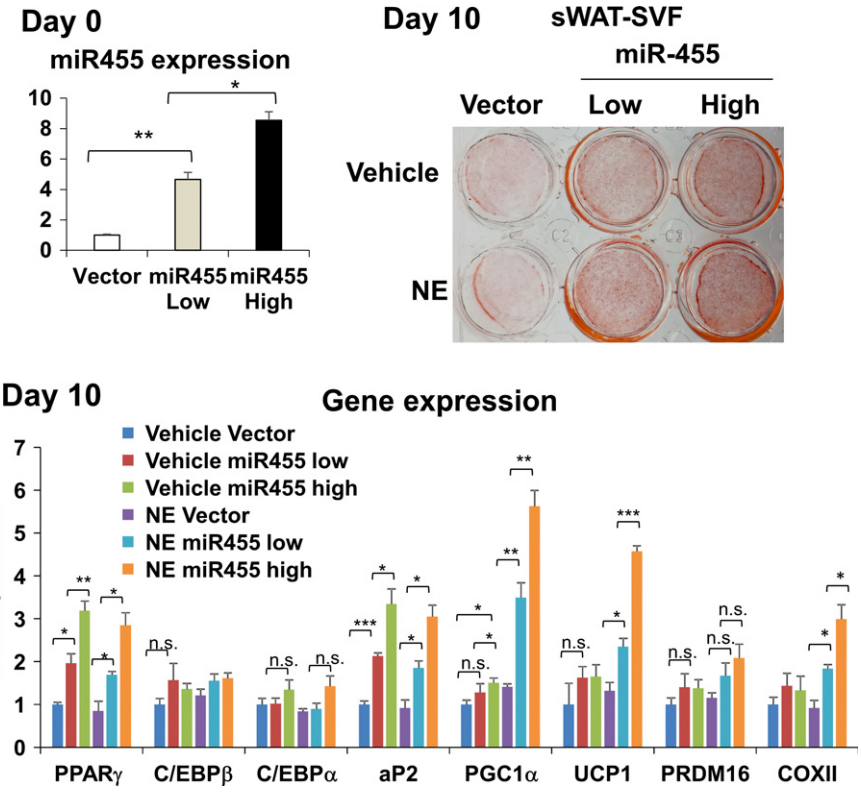

Figure EV1. miR-455 induced brown adipogenesis of primary sWAT-SVFs.
SVF was isolated from subcutaneous white adipose tissue (sWAT) of C57BL/6 mice, plated in a cell culture dish, and transduced with different dosages of miR-455 and control (vector) lentiviruses to achieve different levels (low or high) of miR-455 overexpression. At confluence, the cells were induced to differentiate by standard differentiation protocol (see Materials and Methods) with supplement of 1 mM rosiglitazone. On day 10, cells were treated with 100 mM norepinephrine (NE) for 4 h and stained with Oil Red O, and RNA was isolated for gene expression analysis by qRT–PCR. Data were analyzed with Student's *t*-test and are presented as mean ± SEM of a representative from three independent experiments each performed in quadruplicates (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$; n.s., non-significant).

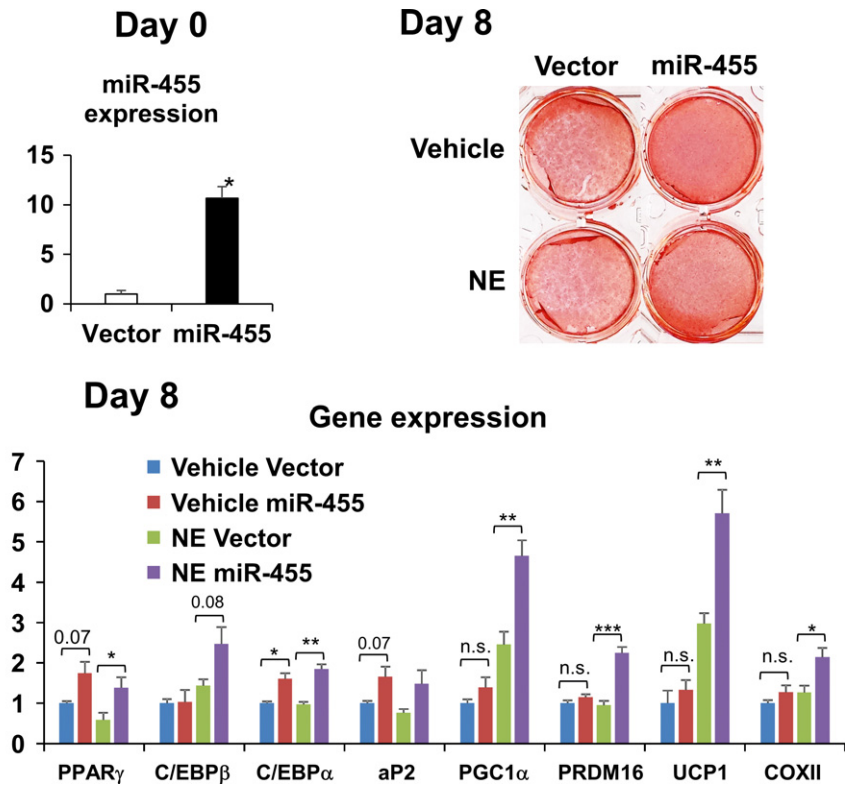

Figure EV2. miR-455 committed sWAT-ScaPCs to the brown adipogenic lineage.
ScaPCs isolated from subcutaneous adipose tissues as described previously [17] were transduced with miR-455 or control (vector) lentiviruses and induced to differentiate by standard differentiation protocol (see Materials and Methods). On day 8, cells were treated with 100 mM norepinephrine (NE) for 4 h and then stained with Oil Red O, and RNA was isolated for gene expression analysis by qRT–PCR. Data were analyzed with Student's *t*-test and are presented as mean ± SEM of a representative from three independent experiments each performed in quadruplicates (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$; n.s., non-significant).

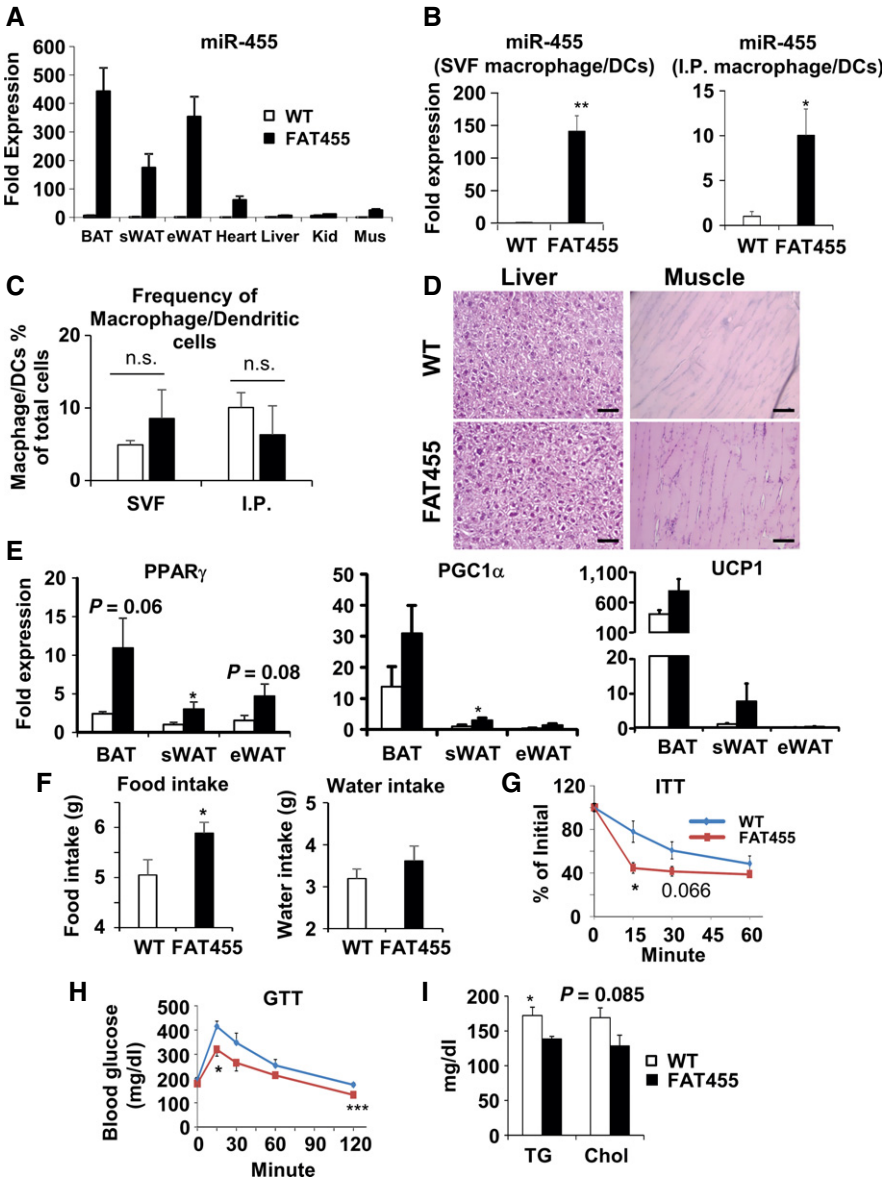

Figure EV3. Characterization of FAT455 transgenic mice. FAT455 and WT mice aged 7–9 weeks were analyzed (n = 5–6/group).

A miR-455 expression in tissues of FAT455 transgenic mice analyzed by qRT–PCR.

B, C Macrophage/dendritic cells were isolated from SVF (white adipose tissue) and intraperitoneal (i.p.) fluid by FACS using anti-F4/80 and CD11b antibodies. (B) miR-455 expression quantified by qRT–PCR. (C) The frequency of sorted macrophage/DC cells within total parental SVF or i.p. cells.

D H&E staining of tissues from FAT455 mice or WT littermates, showing no visible effect of miR-455 in liver and muscle. Scale bar, 50 μm.

E qRT–PCR of brown adipogenic marker gene expression in FAT455 and WT littermates kept chronically at room temperature.

F Food and water intake of FAT455 and WT littermates during a 36-h period.

G IP-ITT assay.

H IP-GTT assay.

I Blood triglyceride (TG) and cholesterol (Chol) quantified by ELISA.

Data information: FAT455 and WT mice aged 7–9 weeks were analyzed (n = 5–6/group). Data were analyzed with Student's t-test and are presented as mean ± SEM (*P < 0.05, **P < 0.01, and ***P < 0.001; n.s., non-significant).

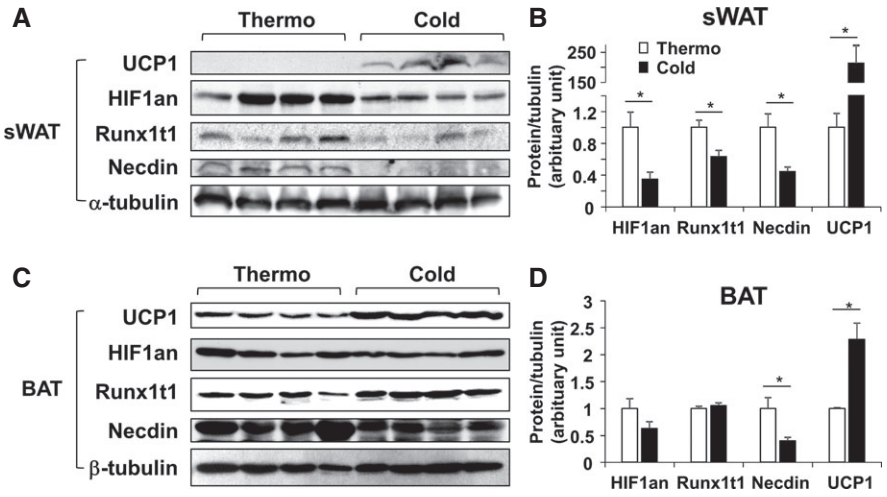

Figure EV4. Cold exposure suppressed the miR-455 target proteins in adipose tissues.

A–D C57BL/6 mice (4–7 mice/group) were maintained at thermoneutral (30°C) or cold (5°C) temperature for 7 days, and target gene expression was analyzed by Western blots (A, C) and quantified by densitometry (target protein levels normalized tubulin level) (B, D). Data were analyzed with Student's *t*-test and are presented as mean ± SEM (n = 4/group, $^*P < 0.05$).

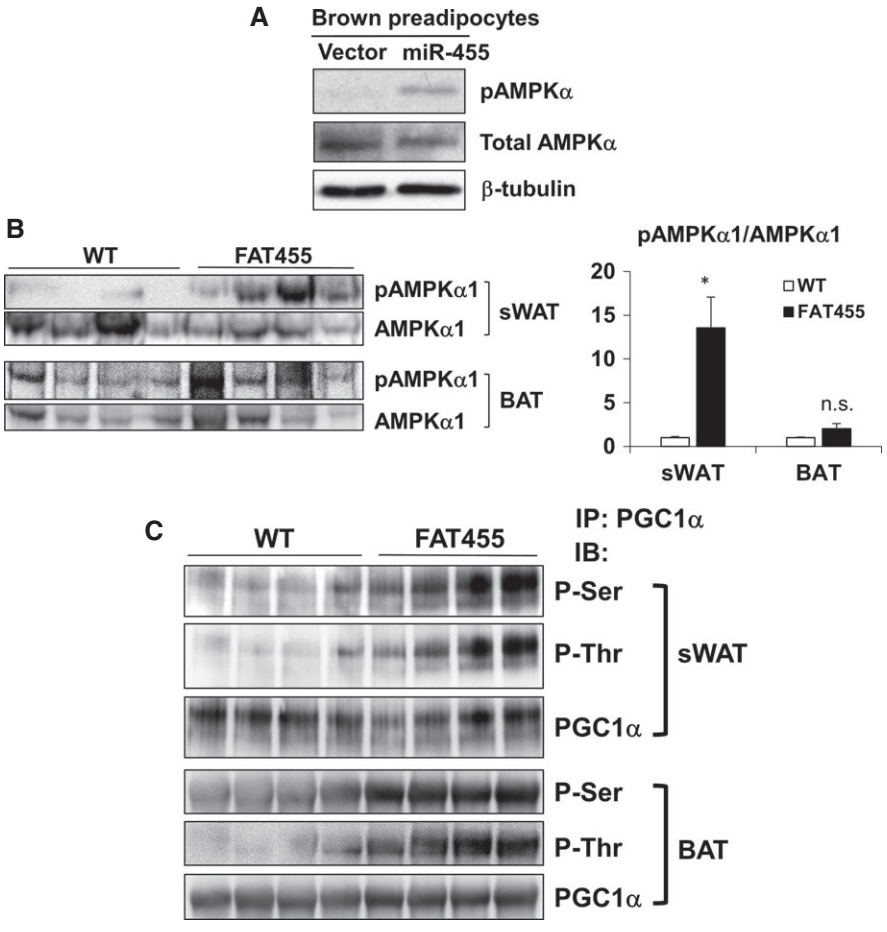

Figure EV5. miR-455 induced phosphorylation of AMPKalpha1 and PGC1α.

A miR-455 induced AMPKα1 phosphorylation without affecting total AMPKα1. Western blot analysis of AMPKα in brown preadipocytes (day 0, prior to the induction of differentiation) transduced by vector or miR-455 lentivirus.

B miR-455 induced AMPKα1 phosphorylation *in vivo*. FAT455 and WT mice aged 5 weeks (n = 4) were exposed to cold (5°C) for 10 days and then sacrificed. sWAT and BAT were isolated. Western blot analysis was performed to examine AMPKα1 phosphorylation using phospho-AMPKα1 (Thr172)-specific antibody and AMPKα1 antibody, and densitometry quantification of the Western blots was performed using ImageQuant, presented as mean ± SEM. Student's *t*-test was used for statistical analysis ($^*P < 0.05$; n.s., non-significant).

C miR-455 enhanced PGC1α phosphorylation *in vivo*. PGC1α was immunoprecipitated from sWAT and FAT isolated from FAT455 and WT mice aged 5 weeks (n = 4) using anti-PGC1α antibody and then probed with anti-phospho-serine and anti-phospho-threonine antibodies.