

Supplemental Figure 1. Map4k4 siRNA sequence targets both human and mouse Map4k4 mRNA. Human and mouse Map4k4 mRNA sequences as well as siRNA used throughout the study were aligned using MacVector.

Supplemental Figure 2. Map4k4 kinase inactivity results in increased Srebp-1 protein levels. (A) Mature 3T3-L1 adipocytes were infected with control virus or dead-kinase virus. 72 hrs post-infection, cells were serum starved for 2hr and treated with 1 μ M insulin added for 3.5 hr. (B) Quantification of A. Data is representative of three independent experiments.

Supplemental Figure 3. Increased Map4k4 expression and activity decreases lipid synthesis. (A) Srebp-1 protein immunoblots of control and Map4k4-overexpressing adipocytes treated with 1 μ M insulin for 3.4 hr (N=4). (B) RT-qPCR analysis of lipogenic gene expression in control and Map4k4-overexpressing adipocytes (N=4). (C) Quantification of A. Data are presented as average \pm SE and were compared between groups by Student's T-test. * P<. 01, ** P<. 001, *** P<. 0001.

Supplemental Figure 4. Ectopic JNK and Map4k4 expression enhances JNK signaling. (A) Protein immunoblots depicting JNK activation in response to 50ng/mL TNF α in HEK 293T cells transfected with equal amounts of empty vector, HA-Map4k4, Flag- JNK1 or Flag- JNK2 constructs (N=3). (B) Quantification of (A). (C) Map4k4 was depleted in HEK 293T cells using siRNA and cells were stimulated with 1 ng/mL and 50 ng/mL TNF α for 15 min. Protein immunoblots depicting JNK activation are shown (N=3).