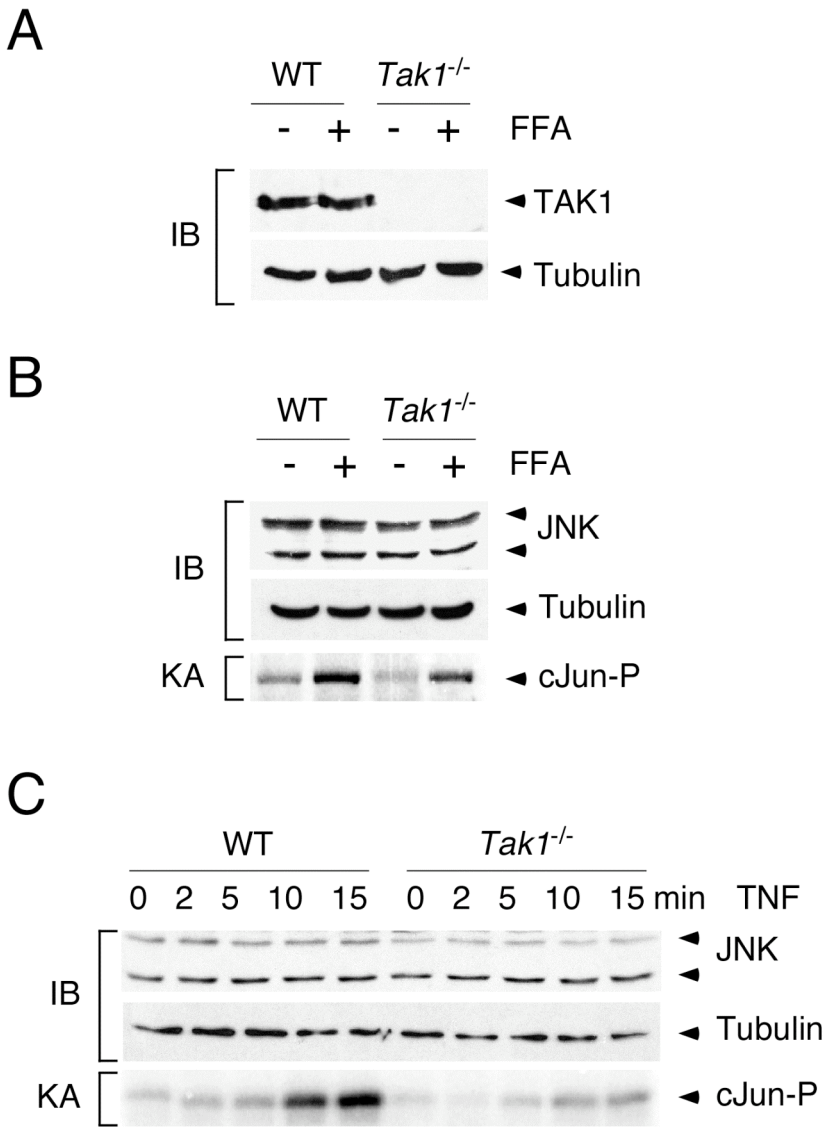
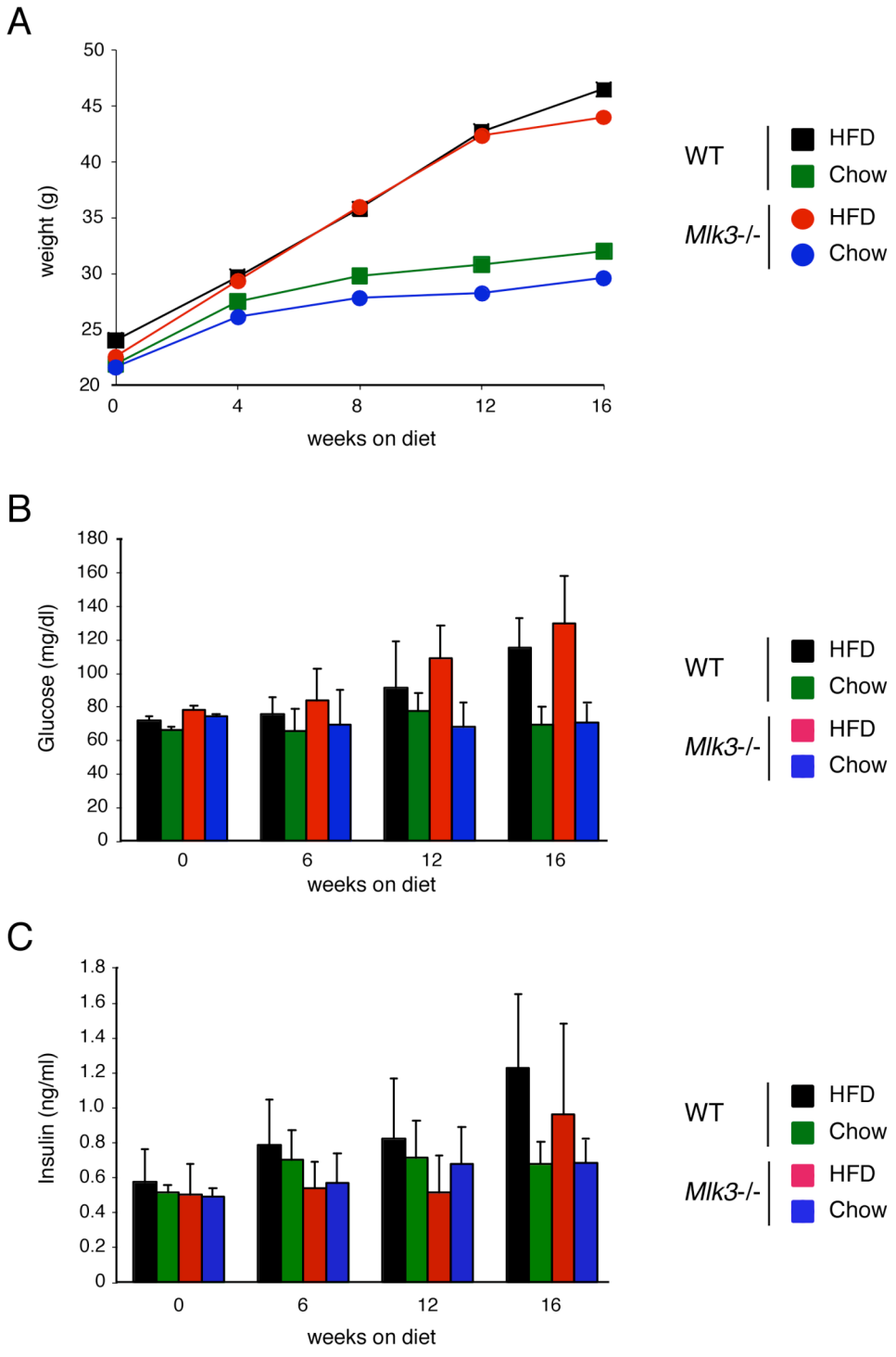


Supplemental Figure S1. Effect of TAK1-deficiency on FFA-stimulated JNK activation.

(**A**) The expression of TAK1 in wild-type and *Tak1*-/- MEF was examined by immunoblot analysis (IB). Tubulin was examined as a loading control. The cells were treated without and with 0.5 mM palmitic acid (16 h.).

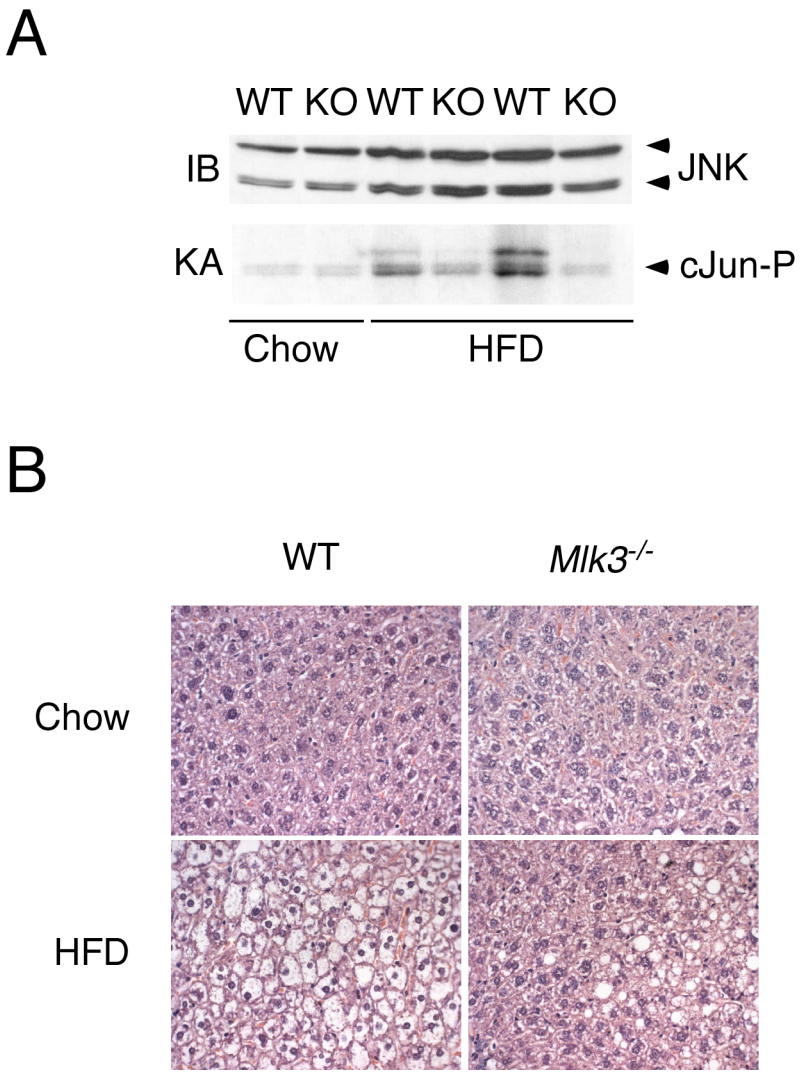
(**B**) JNK activity was examined by immunecomplex kinase assay (KA) using [γ-^{32}P]ATP and cJun as substrates. The amount of phosphorylated c-Jun (cJun-P) was detected by autoradiography. The amount of JNK and Tubulin was examined by immunoblot analysis (IB). The effect of treating the cells without and with 0.5 mM palmitic acid (16 h.) is presented.

(**C**) The effect of TNF (10 ng/ml) on JNK activity was examined by immunecomplex kinase assay (KA) using [γ-^{32}P]ATP and cJun as substrates. The amount of phosphorylated c-Jun (cJun-P) was detected by autoradiography. The amount of JNK and Tubulin was examined by immunoblot analysis.



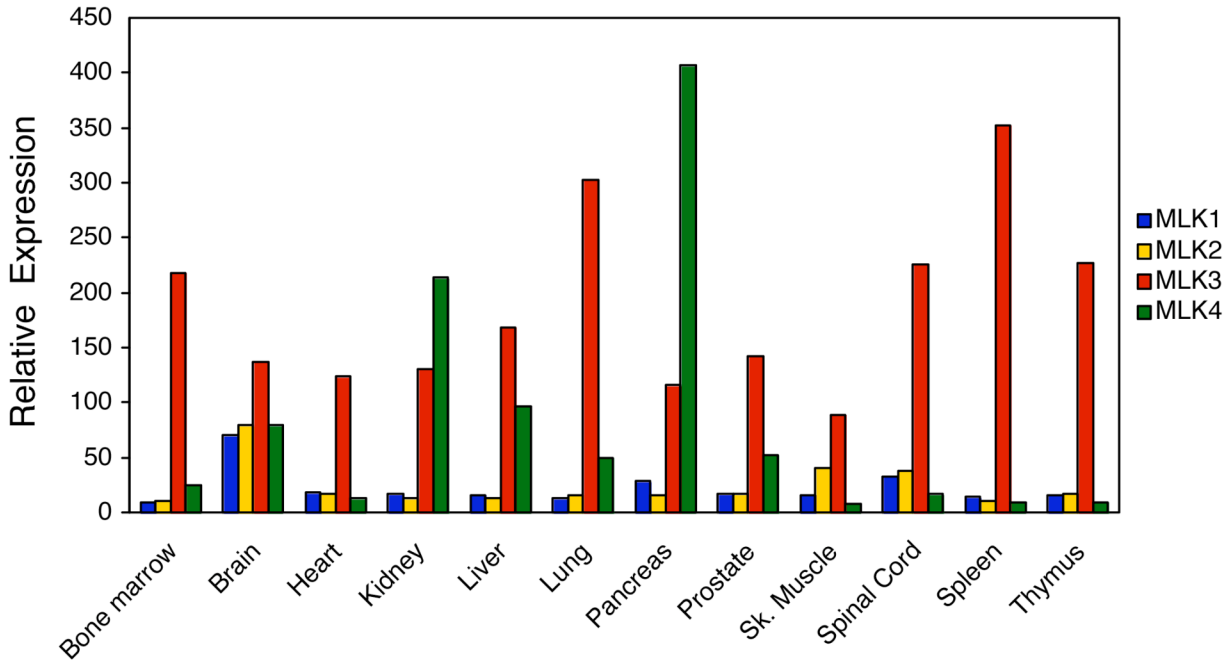
Supplemental Figure S2. Metabolic effects of MLK3-deficiency.

The body weight (**A**), fasting blood glucose concentration (**B**), and fasting plasma insulin concentration (**C**) of wild-type and *Mlk3*$^{-/-}$ male mice maintained on a standard (chow) or a high fat diet (HFD) is presented (mean ± SEM, n=10 per group).



Supplemental Figure S3. MLK3 is required for obesity-induced JNK activation in liver.

Wild-type mice (WT) and *Mlk3*$^{-/-}$ mice (KO) maintained (16 weeks) on a standard diet (chow) or on a high fat diet (HFD). **A)** JNK expression in the liver was examined by immunoblot (IB) analysis. JNK activity was measured in a kinase assay (KA) using [γ-^{32}P]ATP and cJun as substrates. **B)** Representative histological sections of liver stained with hematoxylin and eosin from wild-type and *Mlk3*$^{-/-}$ mice.



Supplemental Figure S4. Tissue distribution of expression of MLK isoforms.

The relative expression of MLK isoforms in different tissues measured by Affymetrix microarray analysis (http://www.genecards.org) is presented. This analysis demonstrates that MLK3 is ubiquitously expressed and that the MLK1, MLK2, and MLK4 are expressed in a more limited number of tissues.