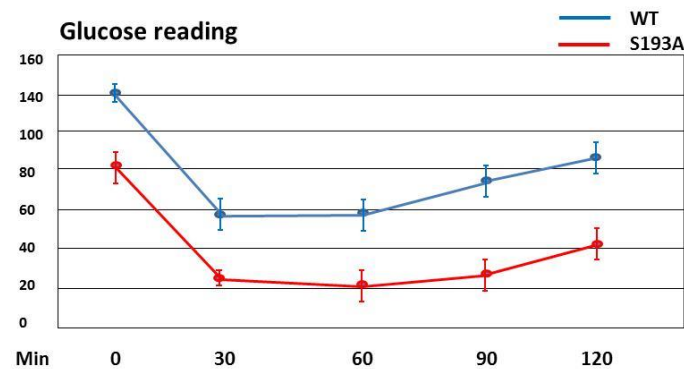


**Jin et al. Activation of CDK4 triggers development of Non-Alcoholic Fatty Liver Diseases.**

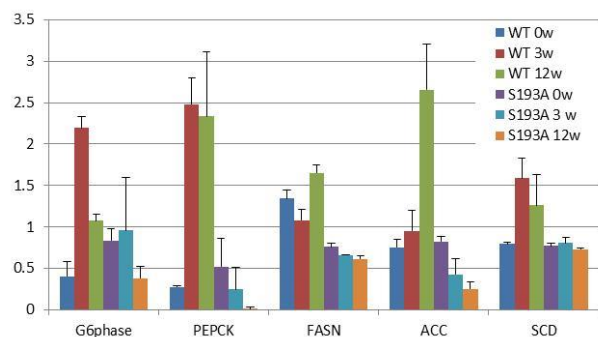
**Supplemental Materials.**

This supplemental material file contains three supplemental figures and description of experimental procedure for Insulin Tolerance Test.

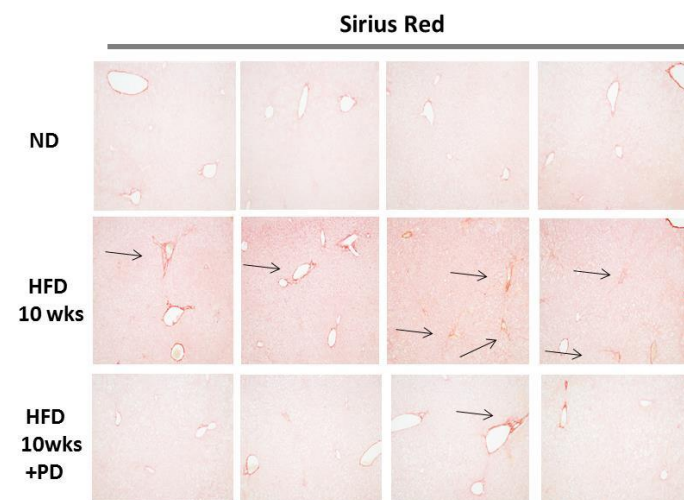
**Supplemental Figures and Legends:**



**Supplemental Figure 1 (related to Figure 2B). Insulin Tolerance Test: glucose reading.** Four animals of each genotype were used. Mice were fasted for 5 hours before injection of insulin. Insulin injection was done intraperitoneally; 0.75 U/kg body weight. Glucose levels were determined in the same mice at 0, 30, 60, 90 and 120 min. Basal levels of glucose are lower in S193A mice and remain lower within duration of the experiment perhaps due to low levels of enzymes of glucose synthesis PEPCK and G6-phase (see Fig 2 in the manuscript); however, insulin-dependent glucose lowering was identical in S193A and WT mice.



**Supplemental Figure 2 (related to Figure 2D).** Levels of enzymes of glucose synthesis and lipogenic pathways in WT and S193A mice at 3 and 12 weeks after initiation of HFD protocol. Bar graphs show the levels of PEPCK, G6-phase, FASN, ACC and SCD as ratios to  $\beta$ -actin. Western blotting for these proteins is shown in Fig 2D.



**Supplemental Figure 3 (related to Figure 7). Inhibition of cdk4 by PD-0332991 prevents development of early stages of liver injury.**

Livers of mice with normal diet (ND), HFD 10 weeks and HFD 10 weeks treated with PD were stained with Sirius Red. Within this duration of HFD (10 weeks), only small Sirius Red positive regions are detected (shown by arrows) in HFD-treated mice. These regions are dramatically reduced or not detectable in livers of control mice (normal diet) and in mice treated with HFD + PD-0332991.

## **Experimental Procedures:**

### **Insulin Tolerance Test**

WT and S193A mice were fasted for four hours, weighed and then given an insulin injection intraperitoneally of 0.75 U/kg body weight. Blood glucose levels were measured using a Freestyle Lite blood glucometer (Abbott) and blood from the tail vein. The tail tips were anesthetized by dipping into Bupivacaine (0.25%) to reduce pain prior to tail snipping. Blood glucose levels were measured at 0, 30, 60, 90 and 120 minutes post-insulin injection. Four animals of each genotype were used.