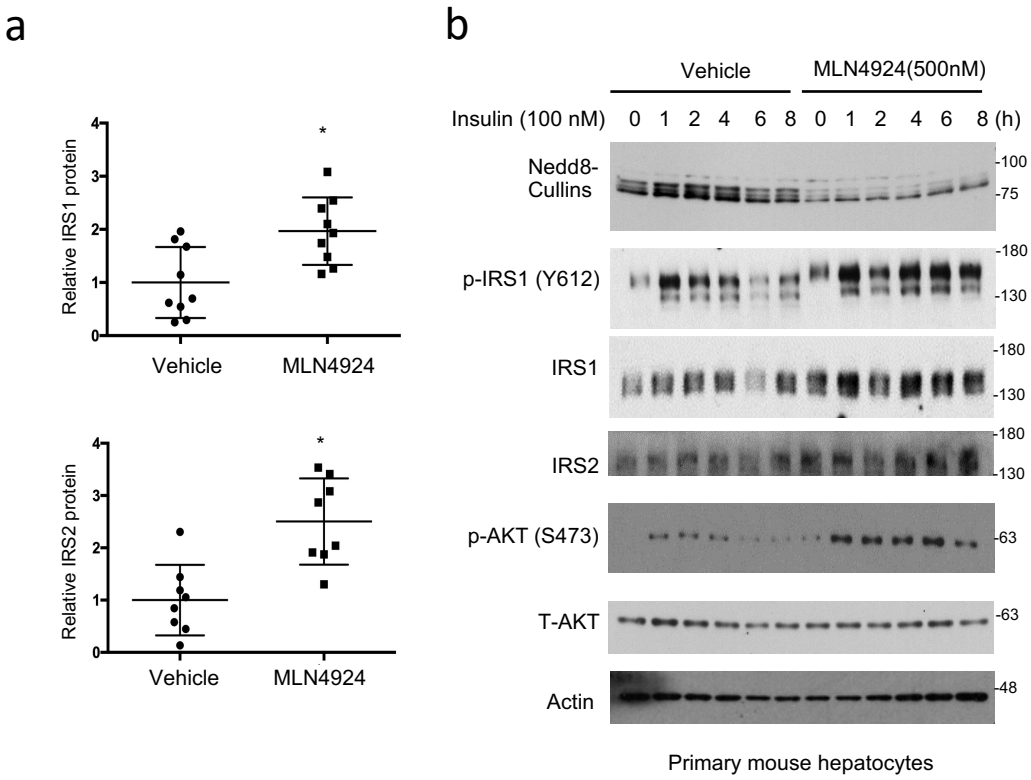
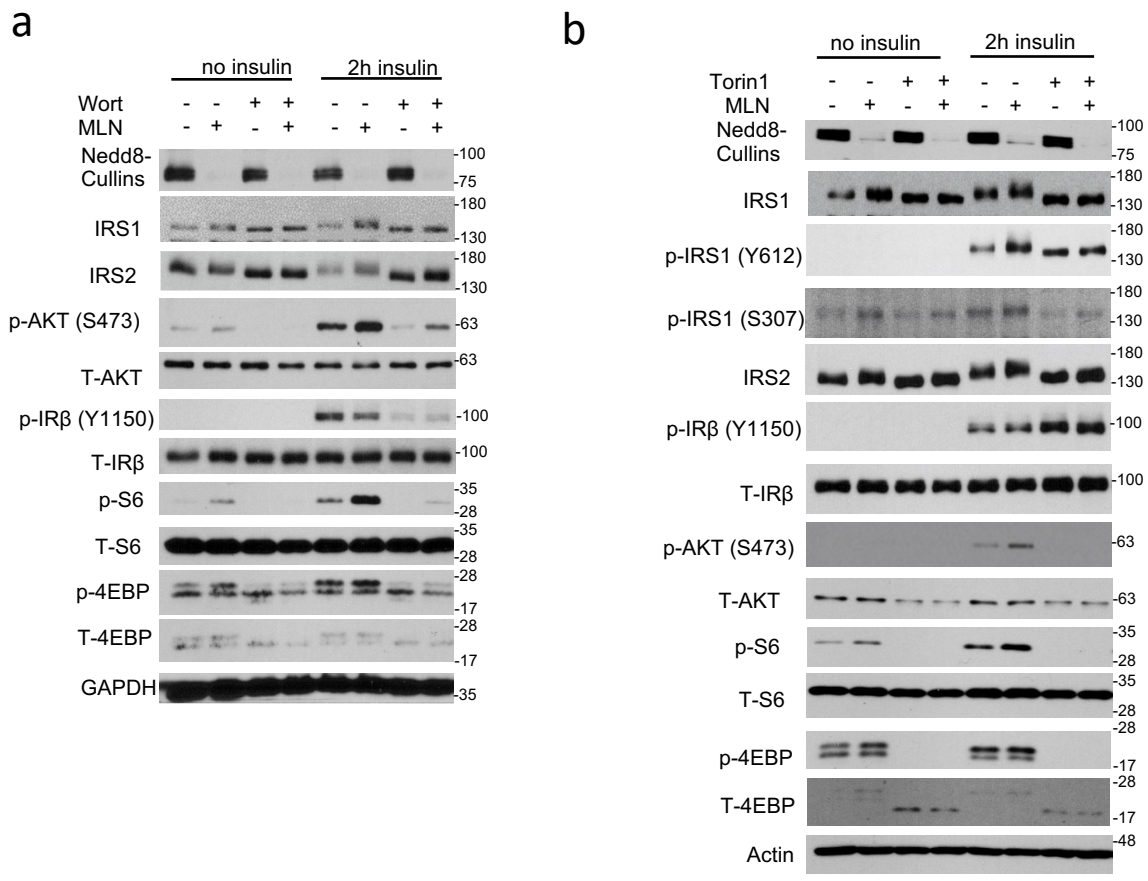
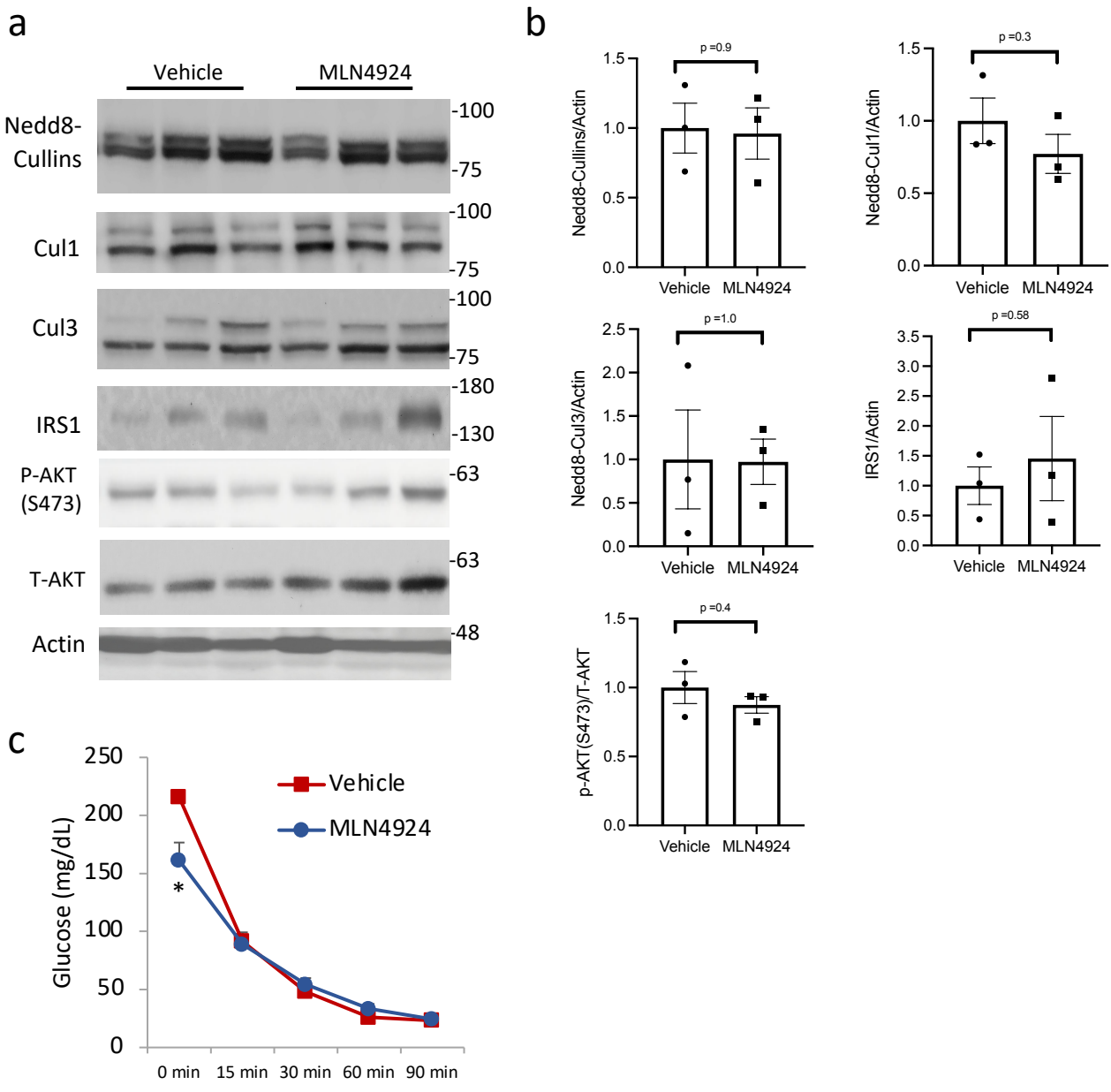


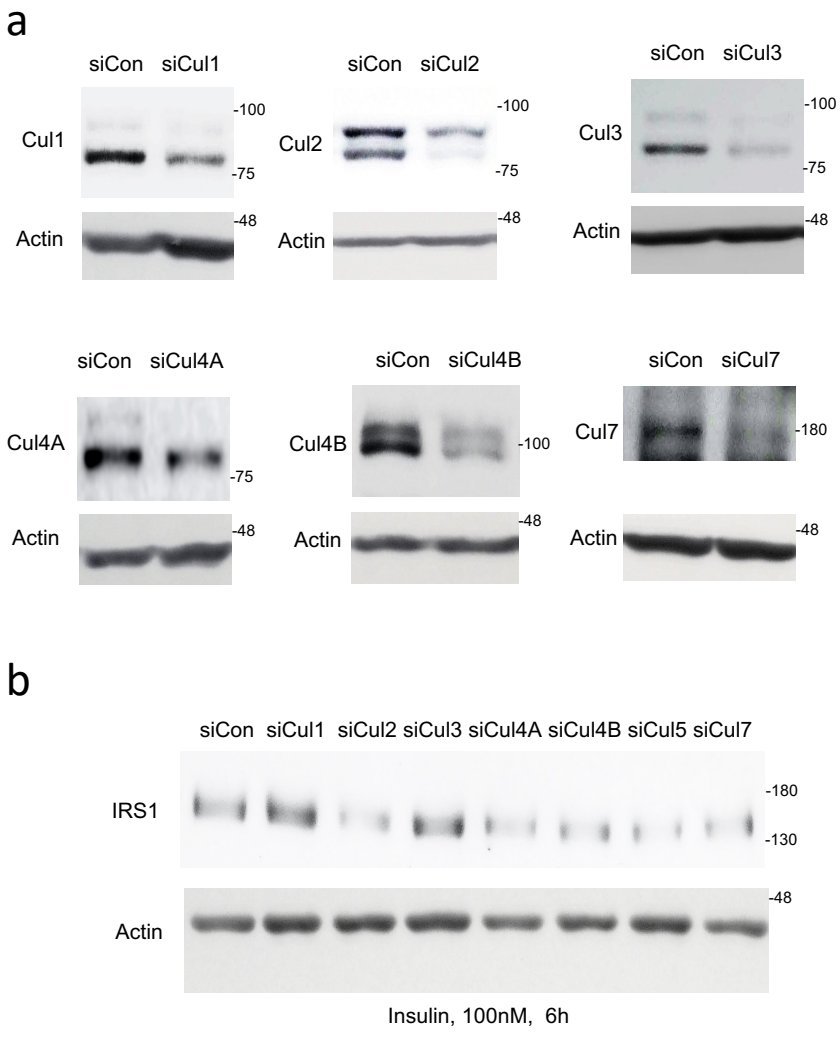
Supplemental Figure S1. MLN4924 enhances hepatocyte insulin responsiveness by stabilizing IRS protein. a. Western blot. IRS protein in different batches of primary human hepatocytes treated with vehicle (DMSO) or MLN4924 (500 nM) for 8 h. IRS1 and IRS2 band intensity was normalized to Actin band intensity. n=8-9. "*", $p<0.05$ (unpaired t-test), vs. Vehicle. **b.** Western blot analysis of insulin signaling in primary mouse hepatocytes. Cells were serum starved for 16 h. Cells were then pre-treated with 500 nM MLN4924 for 1 h followed by 100 nM insulin stimulation in time course.



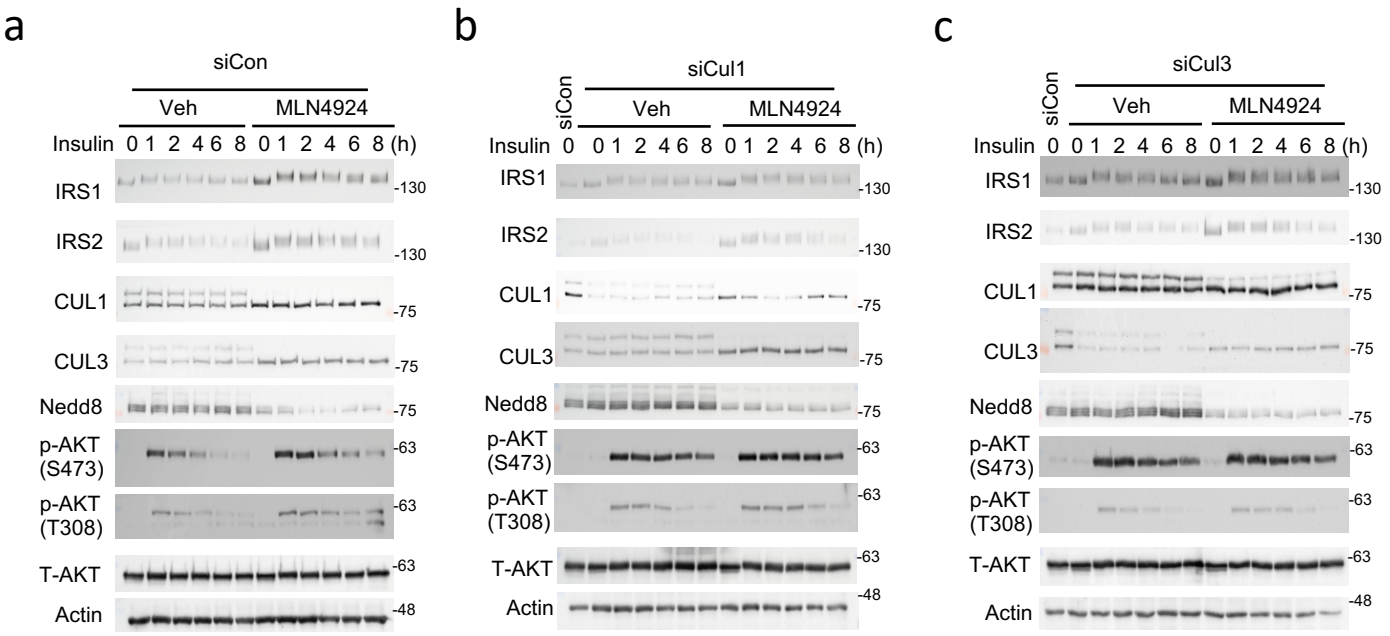
Supplemental Figure S2. MLN4924 enhances hepatocyte insulin responsiveness by delaying feedback IRS degradation. a. AML12 cells serum starved for 16 h. Cells were then pre-treated with 1 μM wortmannin (Wort) and 500 nM MLN4924 as indicated for 1 h followed by additional 2 h incubation in the presence or absence of 100 nM insulin. **b.** AML12 cells serum starved for 16 h. Cells were then pre-treated with 250 nM Torin1 and 500 nM MLN4924 as indicated for 1 h followed by additional 2 h incubation in the presence or absence of 100 nM insulin.



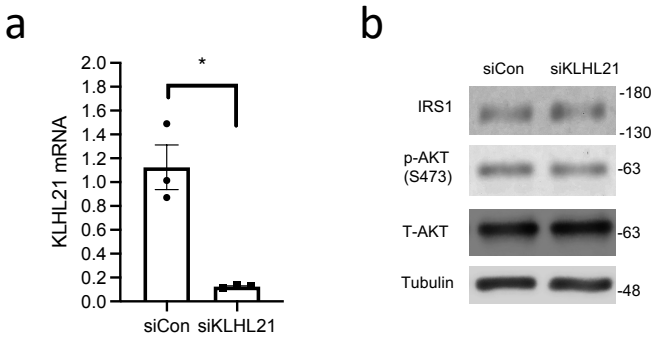
Supplemental Fig S3. MLN4924 administration does not affect skeletal muscle cullin neddylation or AKT phosphorylation in mice. a-b. Male C57BL/6J mice on chow diet were treated with 60 mg/kg MLN4924 via subcutaneous (SQ) injection at 5 pm on day 1 and 9 am on day 2. Mice were fasted from 9 am to 3 pm on day 2 and sacrificed. Effect of MLN4924 on gastrocnemius muscle cullin neddylation and AKT phosphorylation was measured by Western blotting. Densitometry is shown in "b". **c.** Male C57BL/6J mice were first fed WD for 6 weeks, and then treated with 60 mg/kg MLN4924 via subcutaneous (SQ) injection at 5 pm on day 1 and 9 am on day 2. Mice were fasted from 9 am to 3 pm on day 2 and insulin tolerance test was performed with 0.5 U/kg insulin i.p. injection (n=5). All results are expressed as mean ± SEM. "*", $p<0.05$, vs. Vehicle group at the same time point. Unpaired Student's t-test was used to calculate the p value.



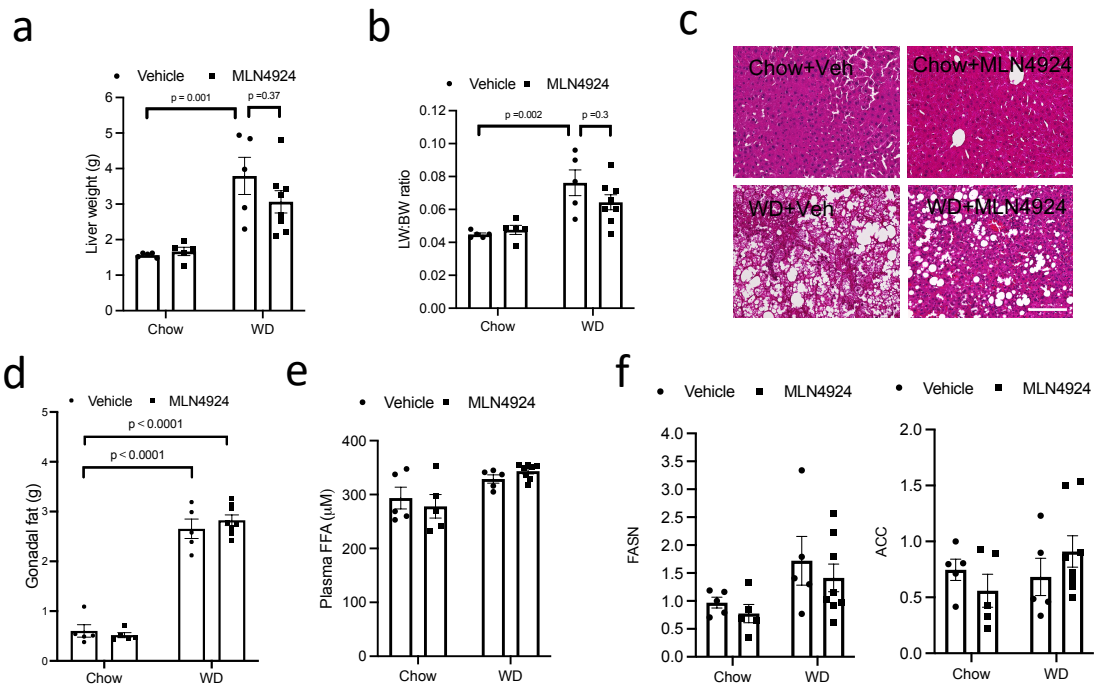
Supplemental Figure S4. Knockdown of Cul1 or Cul3 increases IRS protein abundance. a. Western blot. AML12 cells were transfected with siControl (siCon) or siRNA against individual Cullin. Knockdown of Cullin protein was confirmed 48 h later by immunoblotting except Cul5 due to the lack of a working antibody from commercial sources. **b.** Western blot. AML12 cells were transfected with siControl (siCon) or siRNA against each Cullin. After 16 h serum starvation, cells were treated with 100 nM insulin for 6 h to stimulate IRS protein degradation.



Supplemental Figure S5. Knockdown of Cul1 or Cul3 partially abolishes further MLN4924-mediated insulin sensitization. AML12 cells were transfected with siControl, siCul1 or siCul3 as indicated. After 16 h serum starvation, cells were pre-treated with 500 nM MLN4926 followed by 100 nM insulin stimulation in time course. In “b” and “c”, an siCon (left lane) is included for comparison to Cul1 or Cul3 knockdown cells.



Supplemental Figure S6. Knockdown of KLHL21 does not stabilize IRS protein. AML12 cells were transfected with siControl (siCon) or siKLHL21 as indicated for 24 h. Then, after 16 h serum starvation, cells were treated with 100 nM insulin for 6 h to stimulate IRS degradation in the presence of 100 ug/ml cycloheximide to block protein synthesis. **a.** KLHL21 mRNA. **b.** Western blotting.



Supplemental Figure S7. Chronic MLN4924 treatment attenuates hepatic steatosis in Western diet-fed mice. Male C57BL/6J mice were fed Chow (C) or Western diet (WD) for 16 weeks. MLN4924 treatment (60 mg/kg, SQ, every other day) was initiated after mice were fed WD for 7 weeks. Control mice were injected with vehicle. After 16 weeks of feeding, mice were fasted from 9 am to 3 pm and euthanized. **a**. Liver weight. **b.** Liver weight (LW): body weight (BW) ratio. **c.** Representative liver H&E staining. Scale bar = 125 μm. **d.** Gonadal fat pad weight. **e.** Serum free fatty acids (FFA). **f.** Liver mRNA expression. Results are expressed as mean ± SEM (n=5-8). Two-way ANOVA and Tukey post hoc test were used to calculate the p values.