

# **Inactivation of class II PI3K-C2 $\alpha$ induces leptin resistance, age-dependent insulin resistance and obesity in male mice**

## **Electronic Supplementary Methods**

**Western blot analysis and antibodies** Proteins were extracted from tissues in lysis buffer containing 137 mM NaCl, 20 mM Tris.HCl pH7.4, 10% (vol/vol) glycerol, 1% (vol/vol) Nonidet P-40 (NP40), 10 mM EDTA, 1 mM EGTA, 1 mM  $\beta$ -glycerophosphate, 20  $\mu$ M leupeptin, 18  $\mu$ M pepstatin, 1 mM AEBSF, 4  $\mu$ g/ml aprotinin, 2 mM  $\text{Na}_3\text{VO}_4$ , 20 mM NaF and 1 mM DTT). The homogenate was cleared by centrifugation at 4°C for 20 min at 15,000g and the supernatant fraction recovered. Protein concentration was determined by colorimetric assay (BCA, Pierce). Homogenates were resolved by SDS-PAGE, transferred to nitrocellulose membranes and probed with specific antibodies overnight at 4°C. Antigen-specific binding of antibodies was visualized by ECL. Western blotting was performed using antibodies to PI3K-C2 $\alpha$  p170 (1:1000) from BD Biosciences (#611046); PI3K-C2 $\beta$  (1:1000) from BD Biosciences (#611342); p85 antibody was used as described previously [1]; p110 $\beta$  (1:500) and p110 $\delta$  (1:500) from Santa Cruz (SC #602 and #7176, respectively); vinculin (1:5000) from Sigma (#V9131), p110 $\alpha$  (1:1000), Vps34 (1:1000), phosphoTyr705 Stat3 (1:1000), phosphoS473 Akt (1:1000) from Cell Signaling Technology (CST #4249, #3811, #9145, #9271 respectively). Immunoprecipitation was performed using a homemade PI3K-C2 $\alpha$  p170 antibody (SK193, rabbit antibody).

## **Reference**

[1]. Shepherd PR, Nave BT, Rincon J, et al. (1997) Differential regulation of phosphoinositide 3-kinase adapter subunit variants by insulin in human skeletal muscle. *J Biol Chem* 272: 19000-19007