

SUPPLEMENTAL INFORMATION:

**The citrus flavonoid nobiletin confers protection from metabolic dysregulation in high-fat fed mice independent of AMPK**

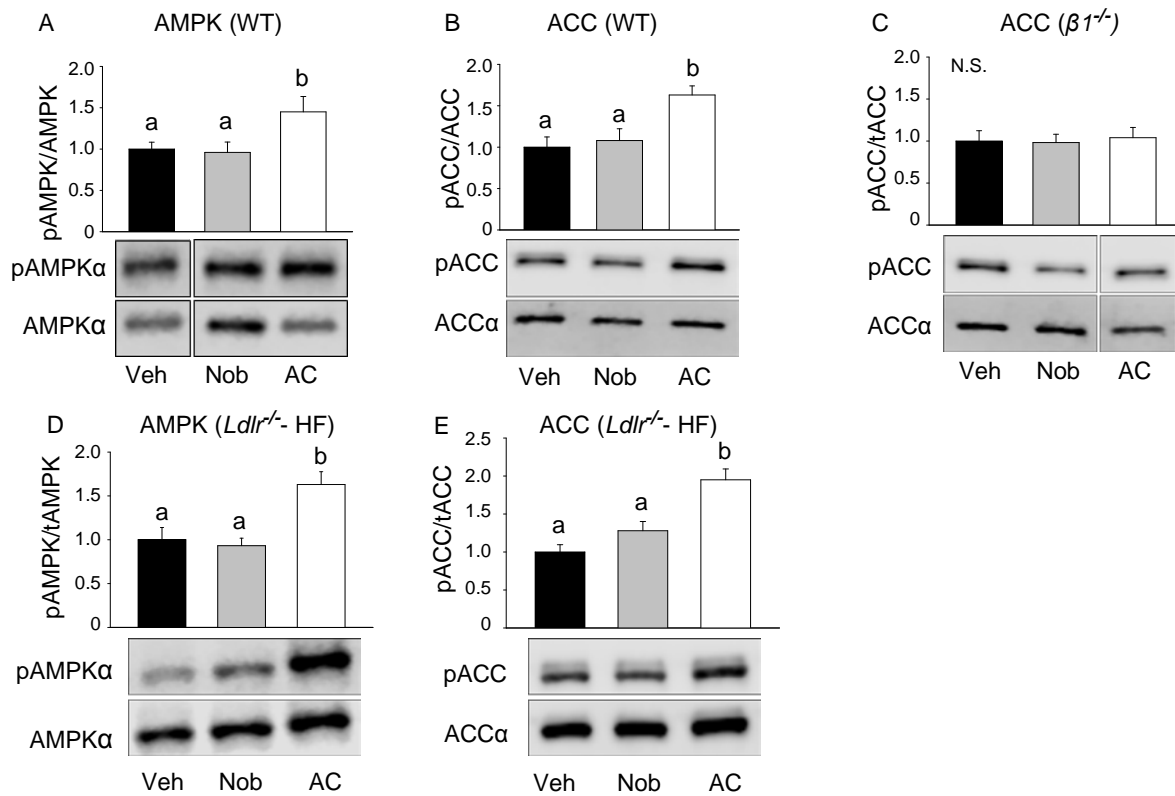
Nadya M. Morrow<sup>\*,†</sup>, Amy C. Burke<sup>\*,†</sup>, Joshua P. Samsoumar<sup>\*,†,§</sup>, Kyle E. Seigel<sup>\*,†</sup>, Andrew Wang<sup>\*,†</sup>, Dawn E. Telford<sup>\*,§</sup>, Brian G. Sutherland<sup>\*</sup>, Conor O'Dwyer<sup>\*\*</sup>, Gregory R. Steinberg<sup>§§</sup>, Morgan D. Fullerton<sup>\*\*</sup>, Murray W. Huff<sup>\*,†,§</sup>

Molecular Medicine<sup>\*</sup>, Robarts Research Institute, Departments of Biochemistry<sup>†</sup> and Medicine<sup>§</sup>, The University of Western Ontario, London, Ontario, Canada N6A 5B7

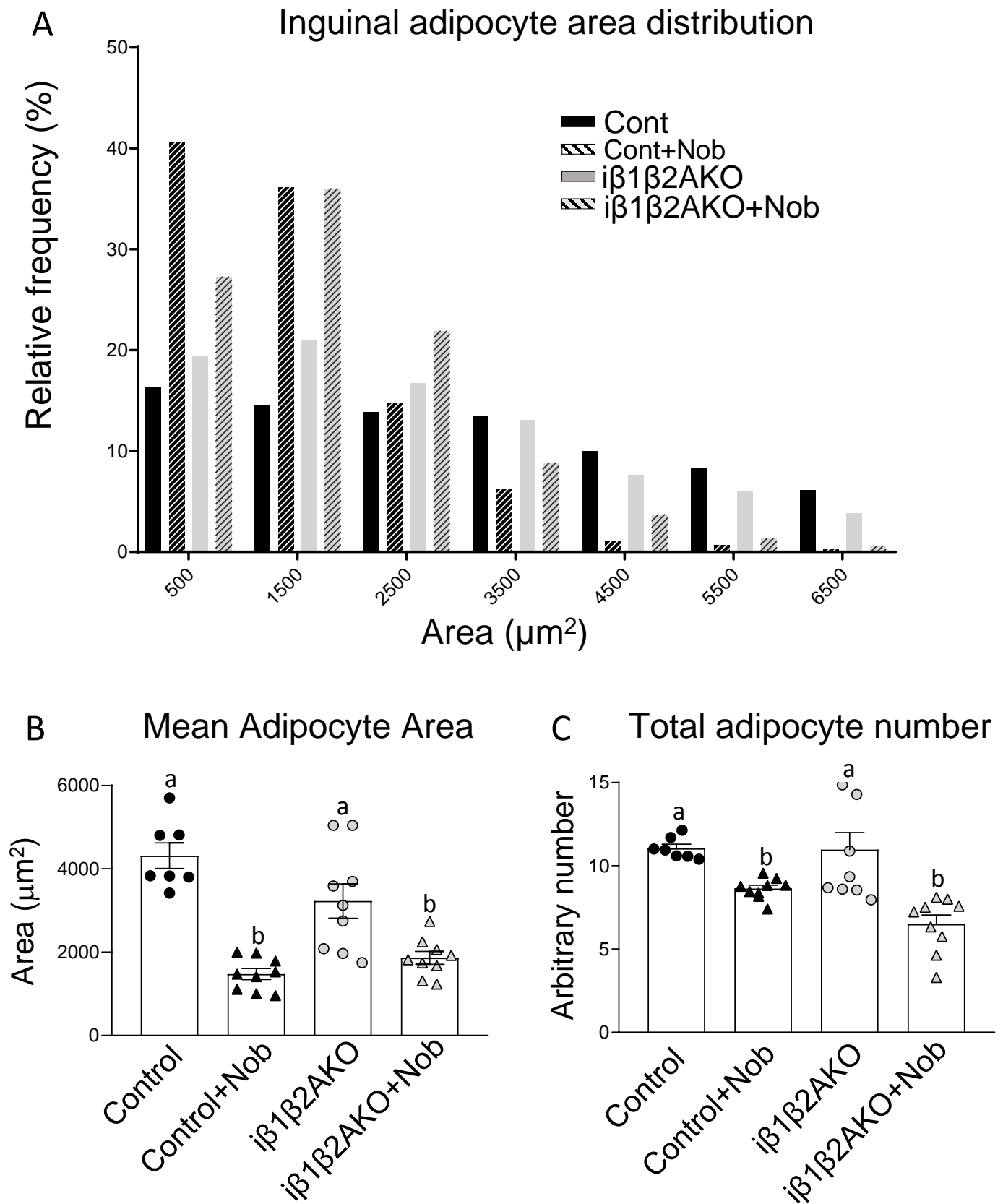
Department of Biochemistry, Microbiology and Immunology<sup>\*\*</sup>, Ottawa Institute of Systems Biology, University of Ottawa, Ottawa, Ontario, Canada, K1H 8M5

Division of Endocrinology and Metabolism, Department of Medicine<sup>§§</sup>, McMaster University, Hamilton, Ontario, Canada, L8S 4K1

Supplemental Figure S1



**Supplemental Figure S1:** Acute nobiletin treatment of mice does not increase phosphorylated hepatic AMPK or ACC. A-E: Chow fed-WT and *-Ampk $\beta 1^{-/-}$*  mice and HFHC diet-fed *Ldlr*<sup>-/-</sup> mice were fasted overnight, fed at 0:700 h for 2h and re-fasted at 09:00 h. Intraperitoneal injection of vehicle, nobiletin (50 mg/kg), or A-769662 (30 mg/kg) occurred at the beginning of the fasting period. Representative immunoblots and quantitation of pAMPK, AMPK, pACC and ACC in freeze-clamped liver lysates 90 min post-injection. A,B: pAMPK and AMPK (A) and pACC and ACC (B) in liver from chow-fed WT mice (n=4/treatment). C: pACC and ACC in liver from chow-fed *Ampk $\beta 1^{-/-}$*  mice (n=4/treatment). D,E: pAMPK and AMPK (D) and pACC and ACC (E) in liver from HFHC diet-fed *Ldlr*<sup>-/-</sup> mice (n=4/treatment). The immunoblots shown are from the same gel; for some blots, lanes have been reordered for consistency. Data represent the mean  $\pm$  SEM. Different letters indicate statistical difference by ANOVA with post-hoc Tukey's test ( $P < 0.05$ ). N.S. indicates no significant difference.



**Supplemental Figure S2:** Nobiletin normalizes inguinal adipocyte size and number in both HFHC-fed  $i\beta 1\beta 2\text{AKO}$  and wild type mice. Wild type (WT) and  $i\beta 1\beta 2\text{AKO}$  mice were fed a HFHC diet (HF) alone or HFHC plus nobiletin for 12 weeks,  $n=8-9$  per group. A: Frequency distribution of adipocyte area in iWAT. B: Mean adipocyte area in iWAT. C: Total adipocyte number in iWAT calculated as number of cells per field of view X weight of iWAT. Data represent the mean  $\pm$  SEM. Different letters are statistically different by ANOVA with post-hoc Tukey's test ( $P<0.05$ ).