SUPPLEMENTAL DATA

Estrogen receptors interact with the alpha catalytic subunit of AMP-activated protein kinase

Running title: Estrogen receptors interact with AMPK

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Figure S1.

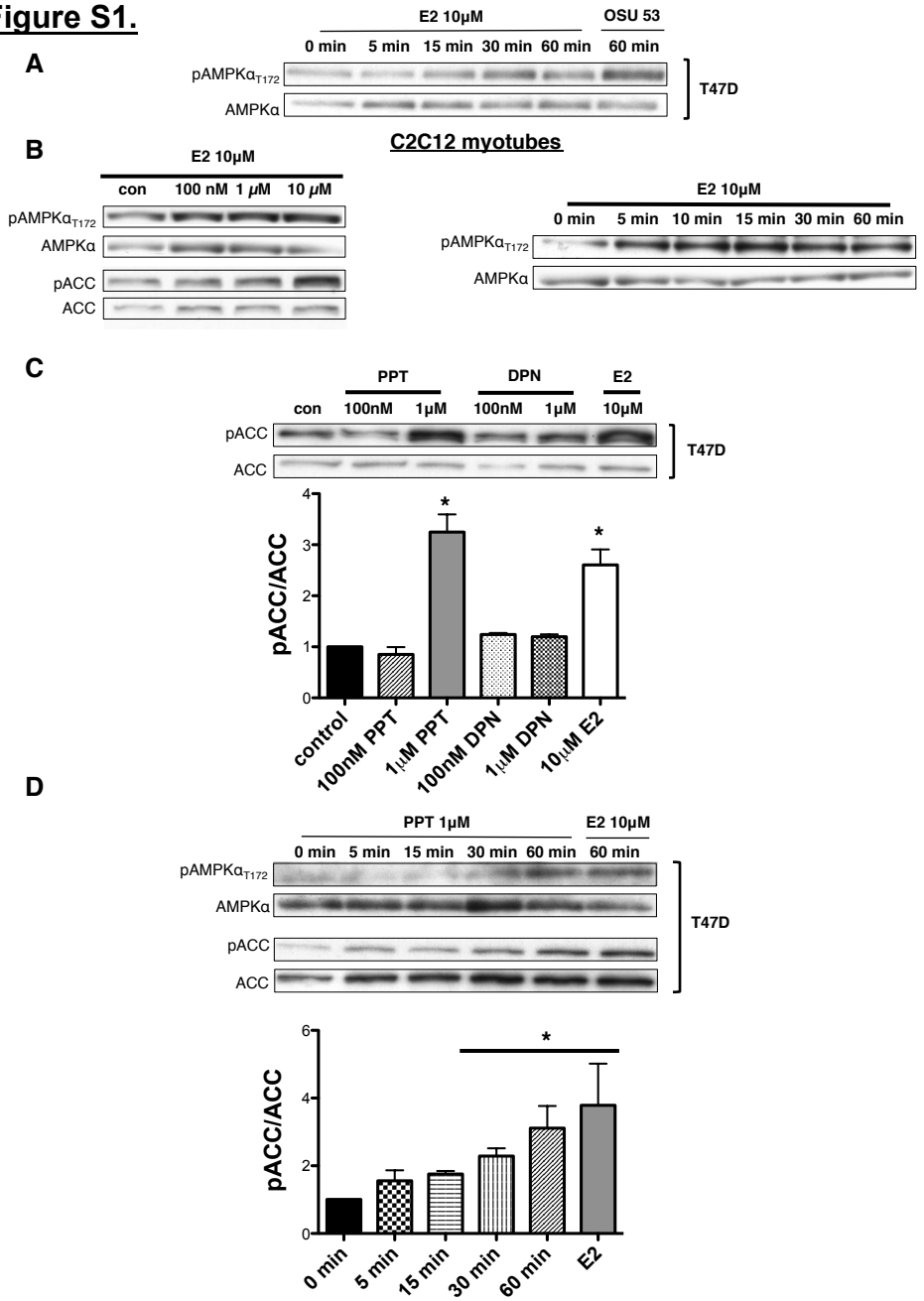

Figure S1: Estradiol activates AMPK. (A) T47D cells were treated with E2 concentrations (10μM) for 0, 5, 15, 30, and 60 minutes. An increase in pAMPKα$_{T172}$ was observed after 15 minutes of E2 exposure. OSU 53: AMPK activator. **(B)** Time and dose-dependent activation of AMPK in C2C12 myotubes as indicated by an increase in pAMPKα$_{T172}$ by Western blot analysis was performed on total cell lysate. **(C)** T47D cells were treated with 2 oncentrations (100 nm or 1μM) of PPT or DPN. Western blot (**top panel**) and bar graph representation (**bottom panel**) of pACC/ACC ratio. (**P* < 0.05 from control.) **(D)** T47D cells were treated with 1μM PPT for 5, 15, 30 and 60 minutes; a Western blot analysis was performed on total cell lysate. Western blot (**top panel**) and bar graph representation (**bottom panel**) of pACC/ACC ratio. (**P* < 0.05 from control.)

Figure S2.

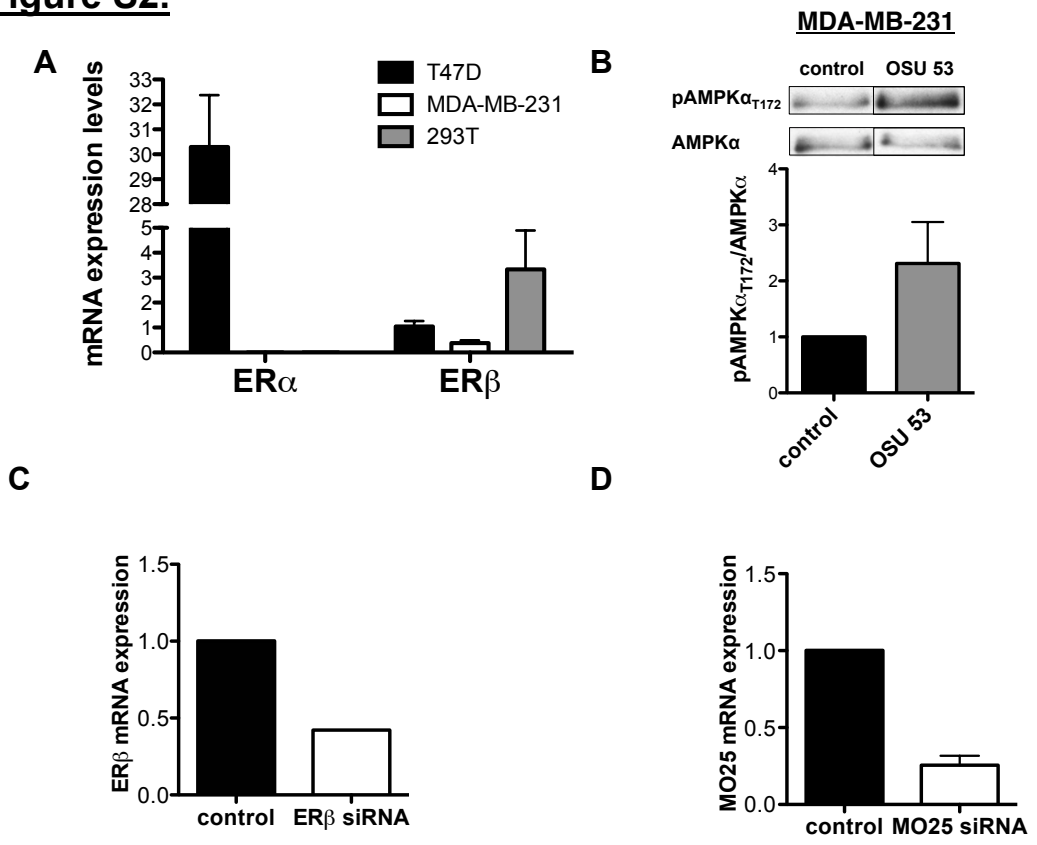

Figure S2: Interrogation of ER specificity underlying estradiol-dependent AMPK activation. (A) ERα and ERβ mRNA levels in T47D, MDA-MB-231 and 293T cells determined by RT-PCR. There was no detectable ERα expression in MDA-MB-231 and 293T cells. However, ERβ mRNA was detectable in all cell lines with 293T cells showing the highest experssion levels (3-fold over T47D) while MDA-MB-231 cells showed the lowest with expression leves about 50% that of T47D cells. **(B)** AMPK signaling remains intact in in MDA-MB-231 cells. MDA-MB-231 cells were treated with 1μM OSU-53 for 1 hour and then analyzed for AMPK activity. Western blot analysis of pAMPKα$_{T172}$ /AMPK showed a significant increase using this treatment strategy indicating, despite lacking ERα, AMPK signaling in MDA-MB-231 cells remains functional. **(C)** ERβ mRNA levels in T47D cells transfected with ERβ siRNA determined by RT-PCR. (**D**) MO25 mRNA levels in T47D cells transfected with MO25 siRNA determined by RT-PCR.

Figure S3.

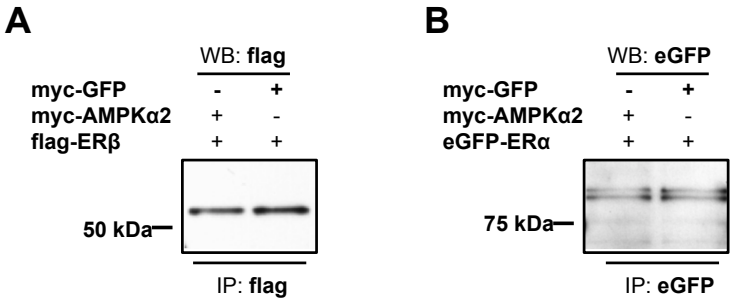

Figure S3: Validation of co-immunoprecipitation. 293-T cells were co-transfected with constructs expressing flag-ERβ, eGFP-ERα, myc-AMPKα2 and myc-GFP. Cell lysates were immunoprecipitated with anti-flag (IP: **flag**) or anti-eGFP (IP: **eGFP**) antibody, followed by Western blot analysis using anti-flag (**A**, WB: **flag**) or anti-eGFP (**B**, WB: **eGFP**) antibody.

Figure S4.

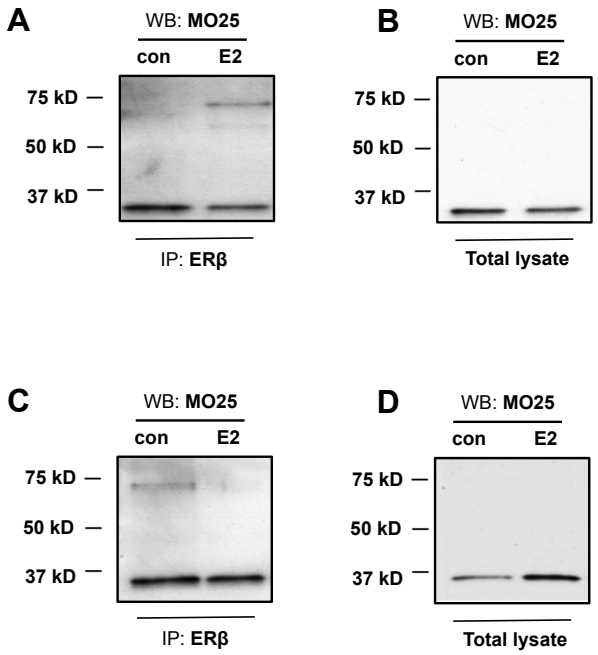

Figure S4: ERβ interacts with MO25 in C2C12 cells and neonatal rat cardiomyocytes (NRCM). (**A**) C2C12 cells were treated with 10μM E2 for 1 hour. Cell lysates were immunoprecipitated with anti-ERβ (IP: **ERβ**) antibody, followed by Western blot analysis using anti-MO25 (WB: **MO25**) antibody. (**B**) The same lysates were immunoblotted directly using anti-MO25 (WB: **MO25**) antibody. (**C**) NRCM were treated with 10μM E2 for 1 hour. Cell lysates were immunoprecipitated with anti-ERβ (IP: **ERβ**) antibody, followed by Western blot analysis using anti-MO25 (WB: **MO25**) antibody. (**D**) The same lysates were immunoblotted directly using anti-MO25 (WB: **MO25**) antibody.

Figure S5.

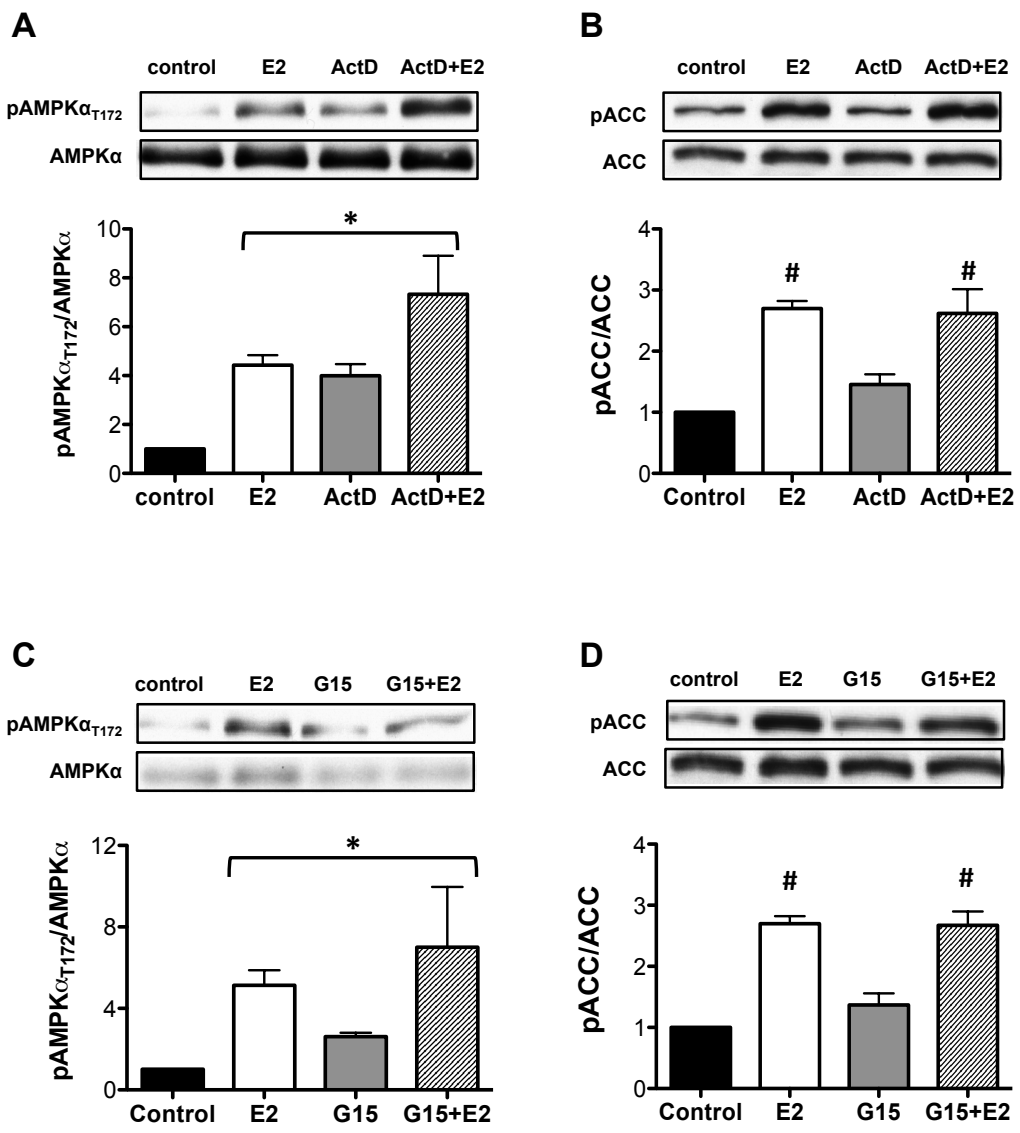

Figure S5: Estradiol activates AMPK through non-genomic pathway and independent of GPER. T47D cells were treated with 10μM E2 in the presence or absence of 1μM Actinomycin D (ActD); Western blot analysis was performed on total cell lysate. **(A)** Western blot (**top panel**) and bar graph representation (**bottom panel**) of pAMPKα$_{T172}$/AMPKα ratio and **(B)** pACC/ACC ratio. T47D cells were pre-treated with 25nM G15, a GPER antagonist for 45 minutes and then treated with 10μM E2 for 1 hour; a Western blot analysis was performed on total cell lysate. (**C**) Western blot (**top panel**) and bar graph representation (**bottom panel**) of pAMPKα$_{T172}$/AMPKα ratio and **(D)** pACC/ACC ratio. (*$P < 0.05$ from control group; #$P < 0.05$ from control and G15- or ActD-treated group.)

Figure S6.

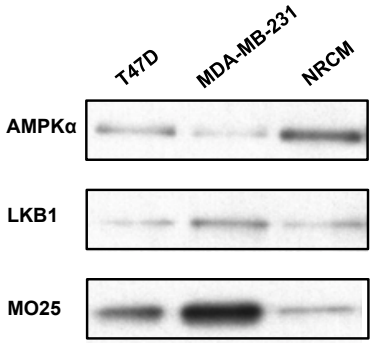

Figure S6: AMPK pathway protein expression levels in T47D, MDA-MB-231 and NRCM cells. Western blot analysis was performed on total cell lysates using anti-AMPKα, LKB1 and MO25 antibodies.