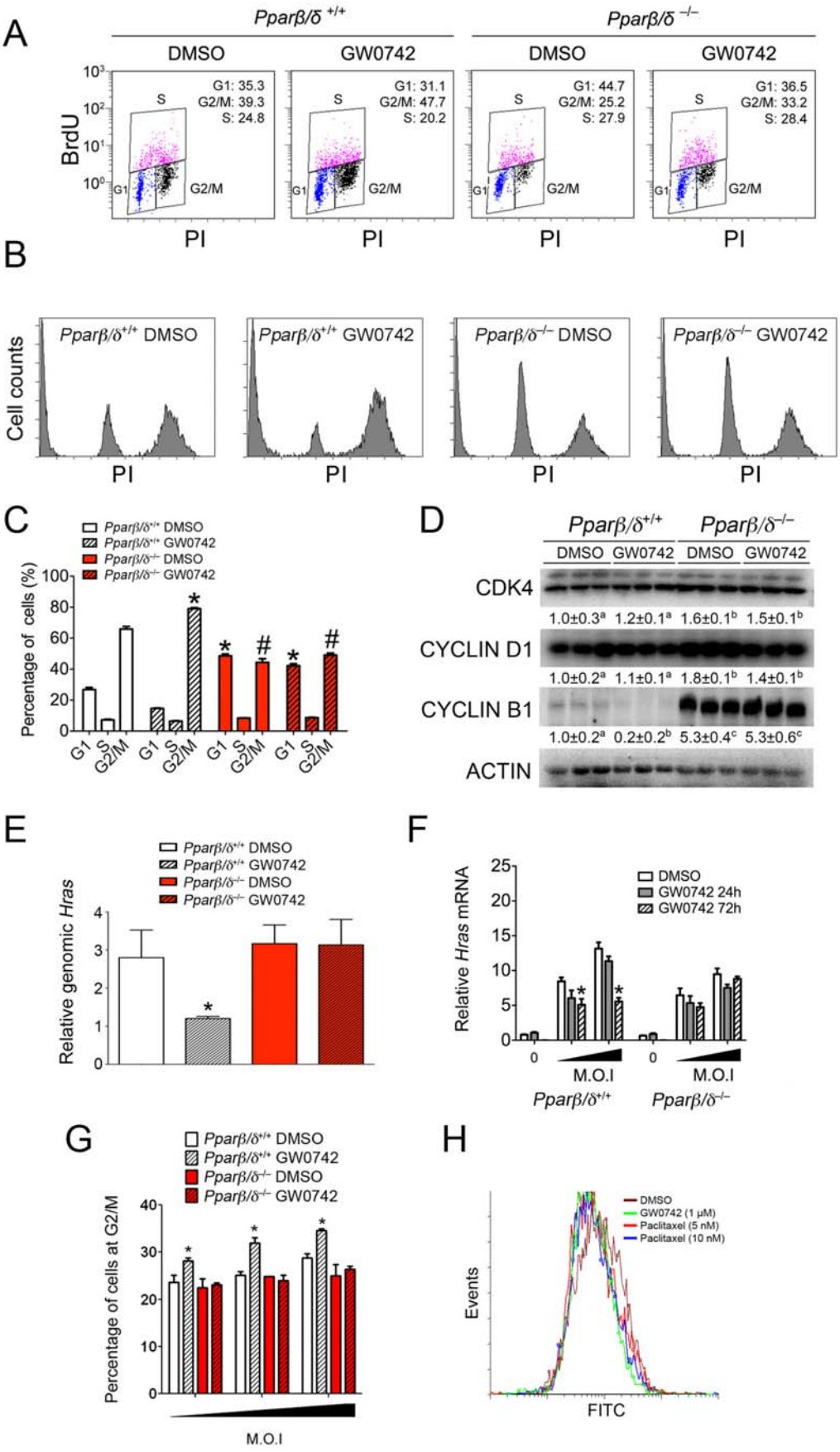
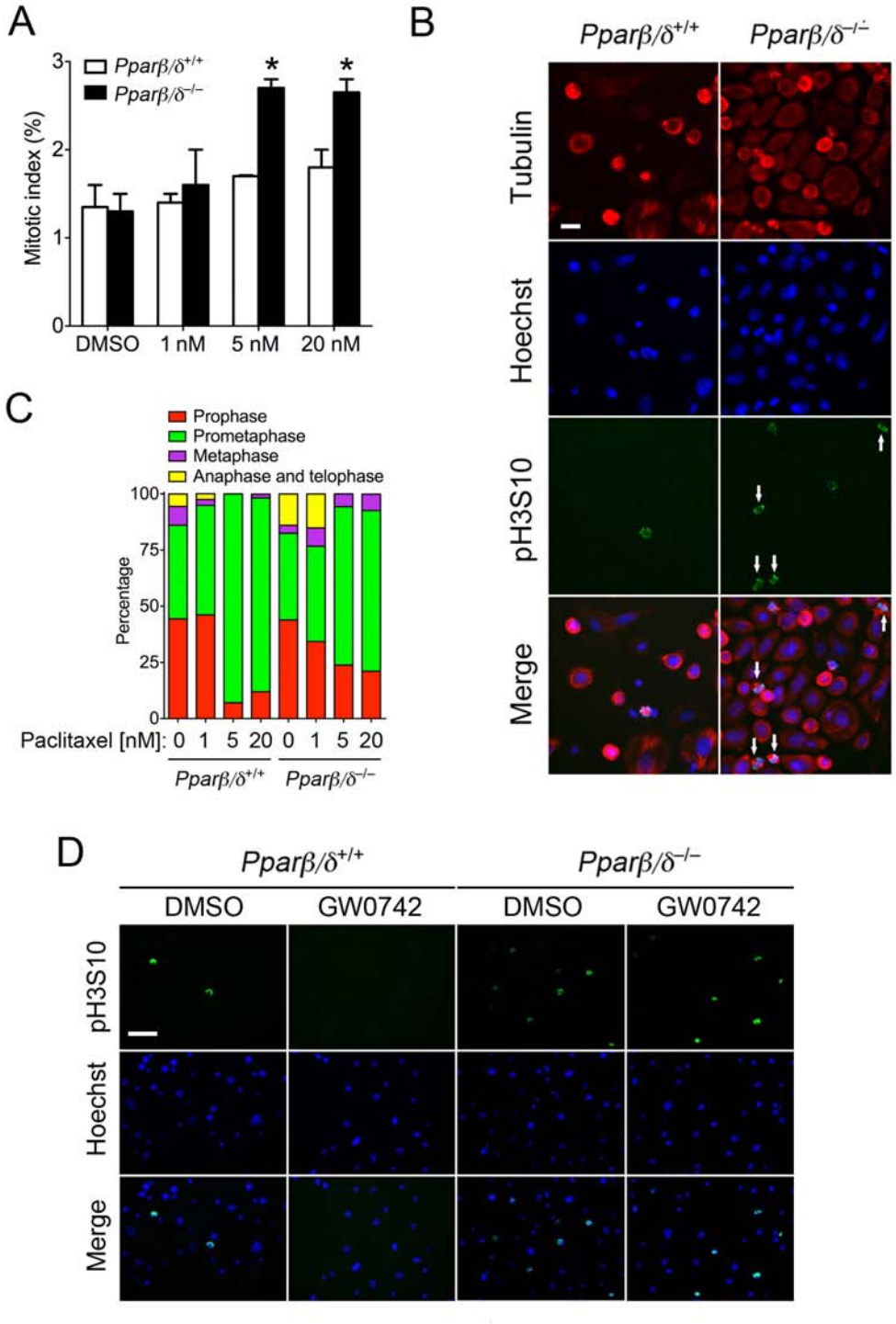
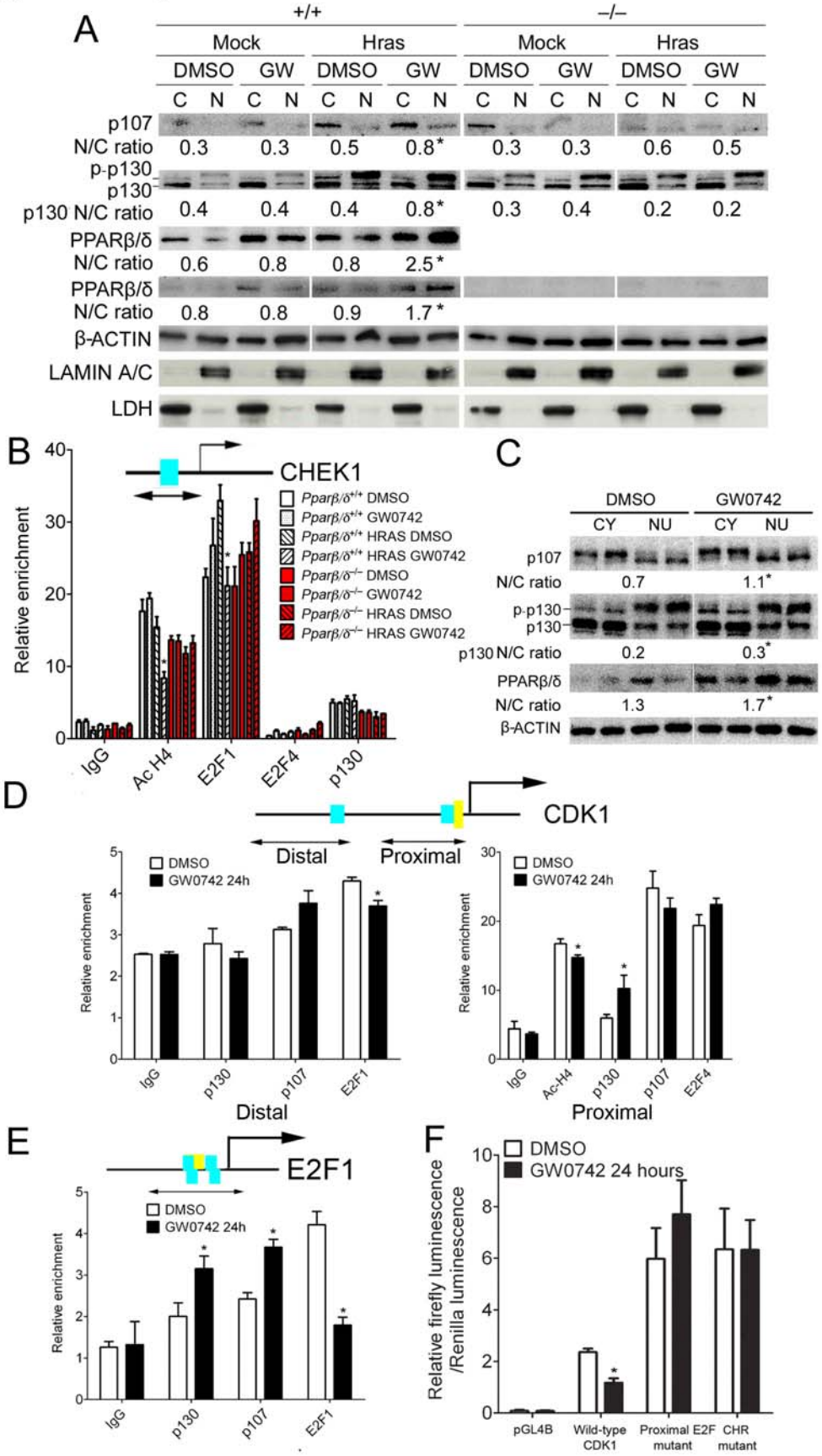
Supplemental Fig. 1, Zhu et al



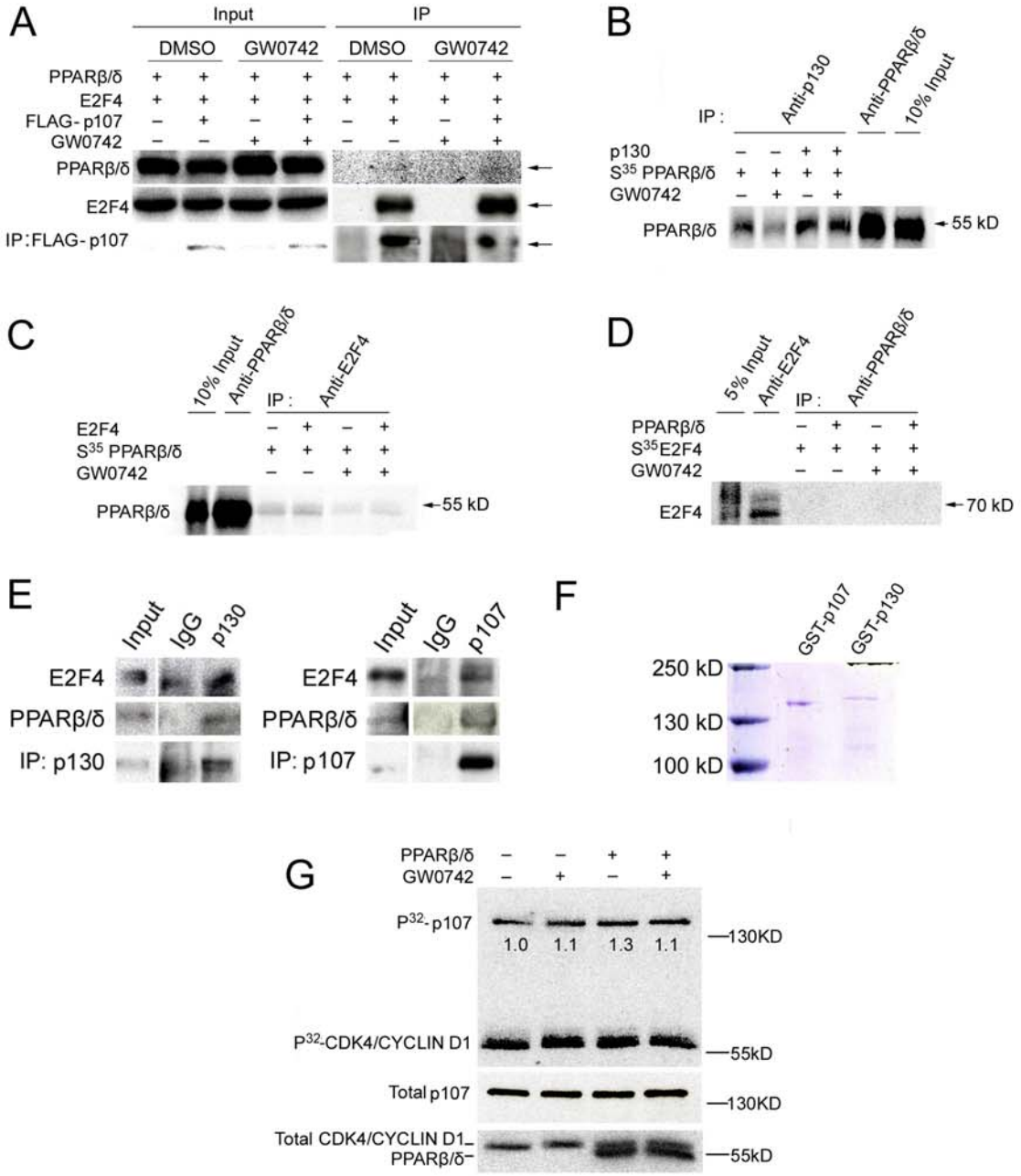
Supplemental Fig. 2, Zhu et al



Supplemental Fig. 3, Zhu et al



Supplemental Fig. 4, Zhu et al



Supplemental Figure legends

FIG. S1. Ligand activation of PPARβ/δ inhibits HRAS-expressing keratinocyte proliferation by inducing G2/M arrest and selects against highly expressing HRAS-expressing keratinocytes. HRAS-expressing wild-type and *Pparβ/δ*-null keratinocytes were treated with or without 1 μM GW0742 for 3 d (A) Cell cycle analysis was performed with bromodeoxyuridine/propidium iodide (PI) labeling followed by flow cytometry. (B) Representative DNA histograms from flow cytometric analysis of PI in HRAS-expressing keratinocytes treated with or without 1 μM GW0742 for 9 d. (C) Quantified distribution of cells in the different phases of the cell cycle from samples in (B). (D) Western blot analysis of CDK4, CYCLIN D1, and CYCLIN B1 from HRAS-expressing keratinocytes treated with or without 1 μM GW0742 for 9 d. Hybridization signals for each protein was normalized to that of ACTIN and are presented as fold change relative to control DMSO. (E) HRAS-expressing wild-type and *Pparβ/δ*-null keratinocytes were treated with or without 1 μM GW0742 for 3 d and qPCR was performed to quantify copy number of genomic *Hras* DNA. (F) Mock-infected or HRAS-expressing keratinocytes with increasing M.O.I. were treated with or without 1 μM GW0742 for 24 or 72 h and *Hras* mRNA expression was measured by qPCR. (G) HRAS-expressing keratinocytes with increasing M.O.I. were treated with or without 1 μM GW0742 for 3 d and the percentage of cells in different phases of the cell cycle was determined by flow cytometry after PI staining. (H) HRAS-expressing keratinocytes were treated with or without 1 μM GW0742, 5 nM paclitaxel or 10 nM paclitaxel for 3 d. Representative flow cytometric analysis after labeling with an anti-HRAS antibody is

shown for each treatment. For all datasets, N = 3-4 independent samples per treatment group. Values represent the mean ± SEM. *significantly different than wild-type vehicle control (DMSO) group, $P \leq 0.05$. #significantly less than wild-type control, $P \leq 0.05$. Values with different letters are significantly different, $P \leq 0.05$.

FIG. S2. PPARβ/δ delays entry into mitosis of HRAS-expressing keratinocytes. (A-C) HRAS-expressing wild-type and *Pparβ/δ*-null keratinocytes were cultured for 4 d and then treated with paclitaxel for 24 h. (A) Cells were immunostained to determine the mitotic index. (B) Representative photomicrographs of immunostained keratinocytes. Arrowheads indicate cells with a metaphase plate on a bipolar spindle. A significant number of HRAS-expressing *Pparβ/δ*-null keratinocytes showed fully aligned chromosomes at metaphase plate after 20 nM paclitaxel treatment. Scale bar = 10 μm. (C) The distribution of cells in different mitotic phases was determined as described in Materials and methods. (D) Representative photomicrographs of HRAS-expressing keratinocytes synchronized at the G2 phase by treatment with 15 μM RO-3306 (CDK1 inhibitor) and then released into nocodazole (to block the cells at prometaphase) with or without 1 μM GW0742 for 12 hours. Note the decrease in the number of pH3S10-positive cells in GW0742-treated wild-type keratinocytes. Scale bar = 50 μm. For all datasets, N ≥ 3 independent samples per treatment group. Values represent the mean ± SEM. *significantly greater than control, $P \leq 0.05$.

FIG. S3. Effect of ligand activation of PPARβ/δ on nuclear accumulation and promoter occupancy of p130/p107 and/or E2F in keratinocytes and 308 cells. Wild-type and

Pparβ/δ-null keratinocytes were mock infected or infected with an *Hras* encoding retrovirus for 2 d. Cells were cultured with or without 1 μM GW0742 for 24 h. (A) Western blot analysis of p130, p107, E2F4 and PPARβ/δ in cytosol (C) and nuclear (N) extracts. Expression levels were normalized to β-ACTIN. The average ratio of nuclear to cytoplasmic protein (N/C) is shown. Promoter occupancy of acetylated histone 4 (AC-H4), E2F1, E2F4 and p130 was examined by ChIP analysis of the mouse (B) CHEK1 promoter. For the CHEK1 promoter, the E2F1 activator binding site is depicted as a blue box. The relative position of the PCR products used for ChIP analysis is shown by the lines with double arrows. (C) 308 cells were cultured to near confluency and then treated with DMSO or 1 μM GW0742 for 24 hours. Western blot analysis of p107, p130 and PPARβ/δ in cytosol (C) and nuclear (N) extracts. The average ratio of nuclear to cytoplasmic protein (N/C) is shown. Promoter occupancy of AC-H4, E2F1, p130, p107 and E2F4 was examined by ChIP analysis of the mouse (D) CDK1 or (E) E2F1 promoter in 308 cells. For the CDK1 and E2F1 promoter, the distal E2F1 activator binding site and the proximal E2F4 repressor binding site is depicted as two blue boxes and the CHR binding site is depicted as the yellow box. The relative position of the PCR products used for ChIP analysis is shown by the lines with double arrows. (F) Promoter analysis of the mouse CDK1 promoter. Mutations in the proximal repressor E2F4 binding site and the proximal CHR are described in the Materials and methods. For all datasets, N = 3 independent samples. Values represent the mean ± S.E.M..
*significantly different than DMSO control, $P \leq 0.05$.

FIG. S4. PPARβ/δ binds with p107/p130. (A) Co-immunoprecipitations using HEK293T cells transiently transfected with FLAG-p107, pCMV-E2F4 and pSG5-PPARβ/δ, treated with DMSO or 1μM GW0742 for 24 hours. (B) Co-immunoprecipitations of *in vitro* translated p130 and ^{35}S-labeled PPARβ/δ in the absence or presence of 1μM GW0742. (C, D) Co-immunoprecipitations of *in vitro* translated E2F4 and ^{35}S-labeled PPARβ/δ (C) or ^{35}S-labeled E2F4 and PPARβ/δ (D) in the absence or presence of 1 μM GW0742. (E) Interaction between endogenous p130/p107 and PPARβ/δ in HEK293T cells. (F) Commassie staining of affinity purified recombinant GST-p107 and GST-p130 protein isolated from *E. coli*. (G) *In vitro* kinase assay with recombinant GST-p107. GST-p107 was pre-incubated with dilution buffer or recombinant human PPARβ/δ in the presence of DMSO or 1 μM GW0742 before ^{32}γ-ATP and a CDK4/CYCLIN D1 complex were added. The reaction was stopped by adding SDS loading buffer and resolved by SDS-PAGE and the amount of phosphorylated p107 was determined by autoradiography. The ratio of the phosphorylated p107 relative to total p107 is shown below each band.