Supplementary Materials and Methods

Cell cycle analysis

To determine the effect of over-expression and/or ligand activation of PPARβ/δ on cell cycle, cell lines were cultured as described above until ~80% confluent and then cultured with or without the PPARβ/δ agonist GW0742 as described above for 24 hours. Cells were trypsinized and fixed in cold 70% ethanol for 10 minutes and then stained with propidium iodide (PI) solution (1 μg/μL PI and 0.125% RNaseA; Sigma Aldrich, St. Louis, MO) at room temperature for 15 minutes. Approximately 10,000/sample cells were analyzed using Cytomic FC500 flow cytometer (Beckman Coulter, Miami Lakes, FL) with excitation at 488 nm and emission at 617 nm. The percentage of cells in each phase of the cell cycle was determined using CXP System Software (Beckman Coulter, Miami Lakes, FL).

Staurosporine- and ultraviolet (UV)-induced apoptosis

Since it was suggested that ligand activation of PPARβ/δ can inhibit apoptosis (reviewed in (1)), the effect of over-expression and/or ligand activation of PPARβ/δ was examined using two different approaches to induce apoptosis: staurosporine and UV treatment.

Cells were cultured until ~80 confluent and then cultured with medium with or without 1 – 8 μM staurosporine for 24 h to determine that 4 μM staurosporine was sufficient to induce an apoptotic response as assessed by PARP cleavage.

Time course analysis over a 24 hour period showed that PARP cleavage occurred between 8 and 24 hours post-staurosporine treatment. Based on this analysis, cells were then cultured to ~80% confluency, treated in medium with or without GW0742 for 12 hours and then cultured for another 24 hour in medium containing 4 μM staurosporine. Soluble protein lysates were isolated and analyzed for PARP cleavage using western blot analysis as described above. The ratio of normalized cleaved PARP to normalized uncleaved PARP was used as a measure of relative apoptosis as previously described (2, 3).

Cells were cultured to ~80 confluency and then exposed to UVB (280-315 nm, 50 mJ/cm^2) using a CL-1000 Ultraviolet Crosslinker (Ultra-Violet Products, Upland, CA) and then cultured for up to 24 hours post-UVB exposure. This analysis determined that PARP cleavage began to occur in control MigR1 cells and hPPARβ/δ-overexpressing cells by 6 hours. Thus, cells were then cultured again until ~80% confluent and then cultured in medium with or without GW0742 for 12 hours, and then exposed to UVB and examined for PARP cleavage as described above 6 hours and 24 hours post-UVB treatment.

Histopathological analysis

Fixed tumors were embedded in paraffin, sections were cut at 5 μm and stained with hematoxylin and eosin. Tumor samples were examined by a pathologist to assess overt morphology and necrotic regions, which was the predominant phenotype observed.

Supplementary Figure Legends

Supplementary Fig. 1. The effect of over-expressing and/or ligand activation of PPARβ/δ on cell cycle progression in MDA-MB-231 and MCF7 cells. Cell cycle progression was analyzed in either (A) MDA-MB-231 cells or (B) MCF7 cells by flow cytometry. The parent cell line was used as one control as was the stable cell line with an integrated MigR1 vector expressing eGFP (MigR1) and compared with the cell line with an integrated MigR1 vector co-expressing PPARβ/δ (hPPARβ/δ). (C) MDA-MB-231 cells, MDA-MB-231-MigR1 (MigR1) or MDA-MB-231-hPPARβ/δ (hPPARβ/δ) were treated with the indicated concentration of GW0742 for 12 h and cell cycle progression was examined by flow cytometry. (D) MCF7 cells, MCF7-MigR1 (MigR1) or MCF7-hPPARβ/δ (hPPARβ/δ) were treated with the indicated concentration of GW0742 for 12 h and cell cycle progression was examined by flow cytometry. Values represent mean ± SEM.

Supplementary Fig. 2. The effect of over-expressing PPARβ/δ and/or ligand activation of PPARβ/δ on staurosporine-induced PARP cleavage in MDA-MB-231 and MCF7 cells. (A) MDA-MB-231 cells, MDA-MB-231-MigR1 (MigR1) or MDA-MB-231-hPPARβ/δ (hPPARβ/δ; upper panels) or MCF7 cells, MCF7-MigR1 (MigR1) or MCF7-hPPARβ/δ (hPPARβ/δ; lower panels) were treated with the indicated concentration of staurosporine for 24 hours and examined by western blot analysis for PARP cleavage. (B) MDA-MB-231 cells, MDA-MB-231-MigR1

(MigR1) or MDA-MB-231-hPPARβ/δ (hPPARβ/δ; upper panels) or MCF7 cells, MCF7-MigR1 (MigR1) or MCF7-hPPARβ/δ (hPPARβ/δ; lower panels) were treated with staurosporine (4 μM) for up to 24 hours and examined by western blot analysis for PARP cleavage. (C) MDA-MB-231 cells, MDA-MB-231-MigR1 (MigR1) or MDA-MB-231-hPPARβ/δ (hPPARβ/δ; upper panels) or MCF7 cells, MCF7-MigR1 (MigR1) or MCF7-hPPARβ/δ (hPPARβ/δ; lower panels) were cultured in medium with or without the indicated concentration of GW0742 for 6 hours and then cultured in medium also containing 4 μM staurosporine and examined by western blot analysis for PARP cleavage. The ratio of cleaved to uncleaved PARP was calculated using values for normalized (to ACTIN) for cleaved (C) and uncleaved (U) PARP and are shown below each blot. Each experiment was performed 3 times and only a representative blot is shown for each treatment. Values represent the mean from the 3 experiments.

Supplementary Fig. 3. The effect of over-expressing PPARβ/δ and/or ligand activation of PPARβ/δ on UVB-induced PARP cleavage in MDA-MB-231 and MCF7 cells. (A) MDA-MB-231 cells, MDA-MB-231-MigR1 (MigR1) or MDA-MB-231-hPPARβ/δ (hPPARβ/δ; upper panels) or MCF7 cells, MCF7-MigR1 (MigR1) or MCF7-hPPARβ/δ (hPPARβ/δ; lower panels) were irradiated with UVB (50 mJ/cm^2) and examined by western blot analysis for PARP cleavage up to 24 hours post-UVB. (B) MDA-MB-231 cells, MDA-MB-231-MigR1 (MigR1) or MDA-MB-231-hPPARβ/δ (hPPARβ/δ; upper panels) or MCF7 cells, MCF7-MigR1 (MigR1) or MCF7-hPPARβ/δ (hPPARβ/δ; lower panels) were cultured in medium

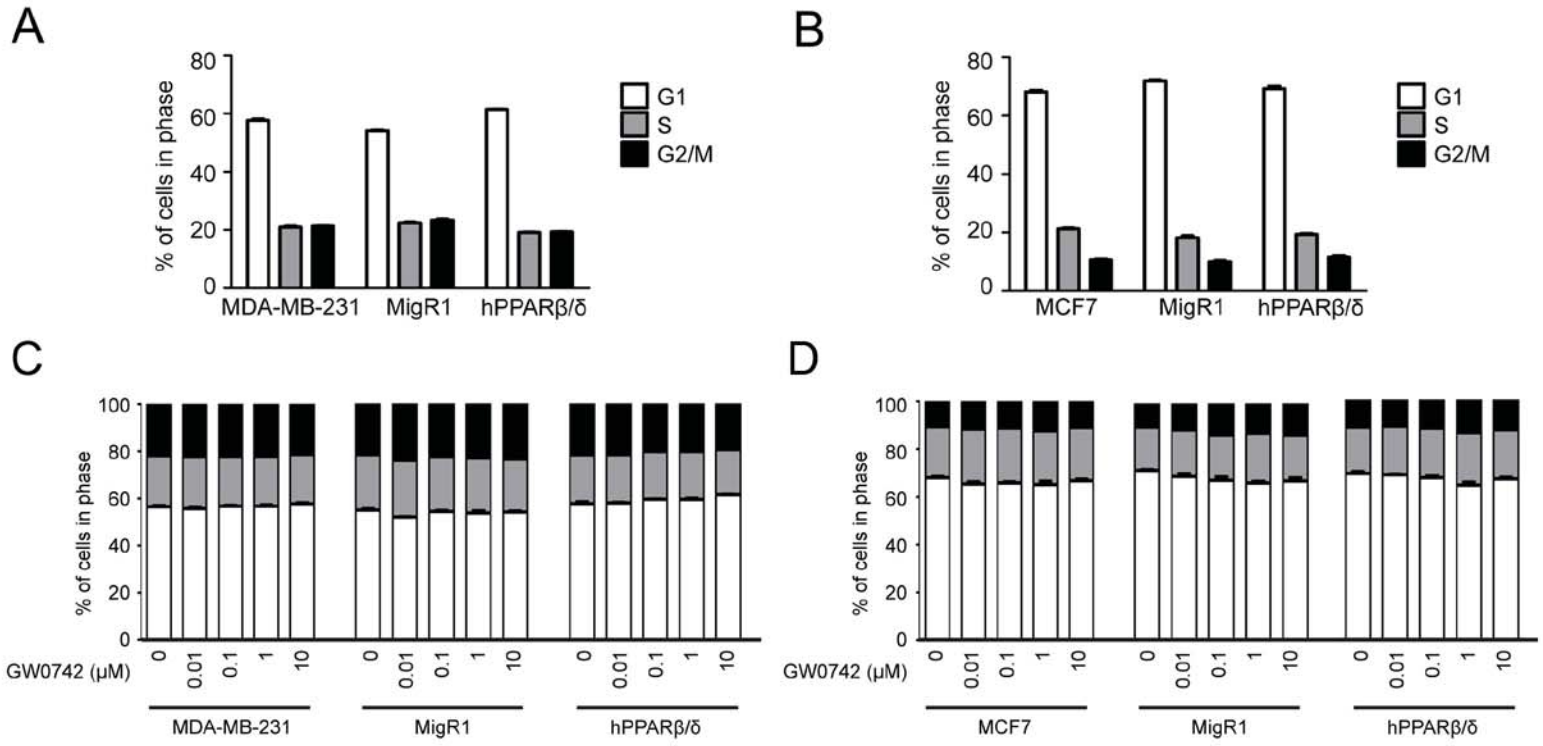
with or without the indicated concentration of GW0742 for 6 hours and then were irradiated with UVB (50 mJ/cm^2) and examined by western blot analysis for PARP cleavage after 6 or 24 hours post-UVB. The ratio of cleaved to uncleaved PARP was calculated using values for normalized (to ACTIN) for cleaved (C) and uncleaved (U) PARP and are shown below each blot. Each experiment was performed 3 times and only a representative blot is shown for each treatment. Values represent the mean from the 3 experiments.

Supplementary Fig. 4. The effect of over-expressing PPARβ/δ and/or ligand activation of PPARβ/δ on xenograft histopathology and necrosis. Xenograft tumors from (A) MDA-MB-231-MigR1 (MigR1) or MDA-MB-231-hPPARβ/δ (hPPARβ/δ) treated with or without GW0742 (2.5 mg/kg/d) were stained with H&E. In the lower magnification photomicrographs, note large regions of necrosis (pale and eosinophilic regions with dead cells showing pyknosis, karyorrhexis and/or karyolysisin) in MDA-MB-231 xenografts from mice treated with GW0742 and/or MDA-MB-231-hPPARβ/δ xenografts from mice treated with or without GW0742. (B) MCF7-MigR1 (MigR1) or MCF7-hPPARβ/δ (hPPARβ/δ) treated with or without GW0742 (2.5 mg/kg/d) were stained with H&E. Magnification = 12.5X for left panels and 400X for right panels. Bar = 500 μm for left panels and 100 μm for right panels. Yellow arrows indicate mitotic figures indicative of malignant tumor cells. (C) The necrotic index represents the relative increase in necrosis observed by light microscopy in the MDA-MB-231 cell xenografts. (D) The area of necrosis in the MDA-MB-231 cell xenografts. (E) The relative

percentage of total tumor area exhibiting necrosis in the MDA-MB-231 cell xenografts. Five samples were examined per group. Values represent the mean ± S.E.M..

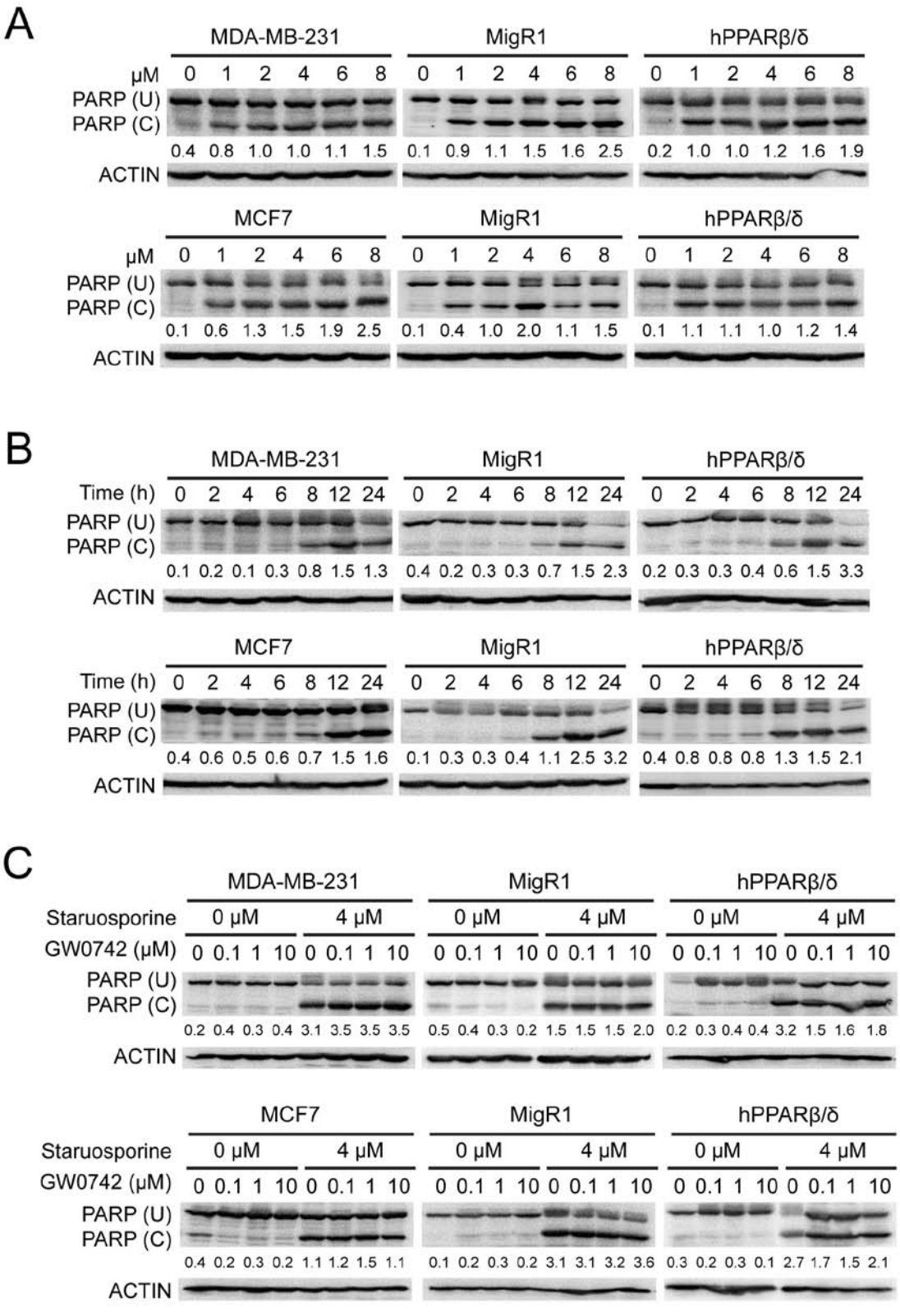
References

1. Peters JM, Shah YM, Gonzales FJ. The role of peroxisome proliferator-activated receptors in carcinogenesis and chemoprevention. Nat Rev Cancer 2012;12:181-95.
2. Borland MG, Khozoie C, Albrecht PP, et al. Stable over-expression of PPARβ/δ and PPARγ to examine receptor signaling in human HaCaT keratinocytes. Cell Signal 2011;23:2039-50.
3. Foreman JE, Chang WC, Palkar PS, et al. Functional characterization of peroxisome proliferator-activated receptor-β/δ expression in colon cancer. Mol Carcinog 2011;50:884-900.

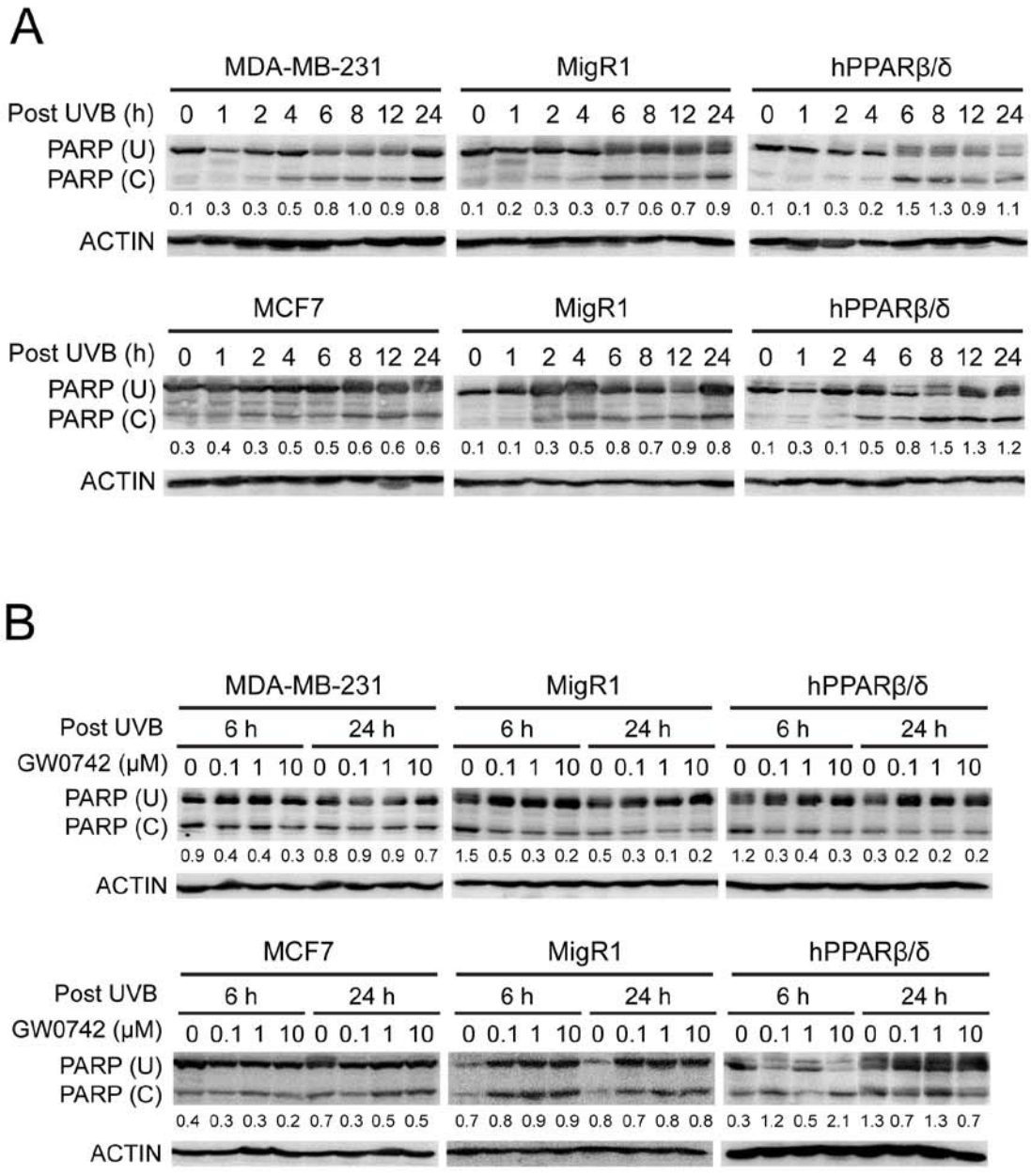


Suppl. Fig. 1, Yao et al

Suppl. Fig. 2, Yao et al



Suppl. Fig. 3, Yao et al



Supple. Fig. 4, Yao et al