4-Hexylresorcinol exhibits different characteristics to estrogen

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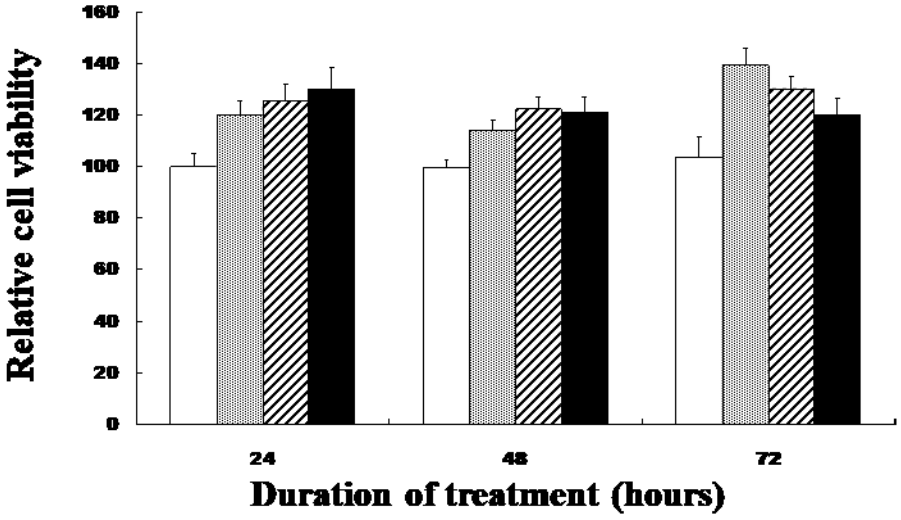
Supplementary Data for normal human dermal fibroblast (NHDF)

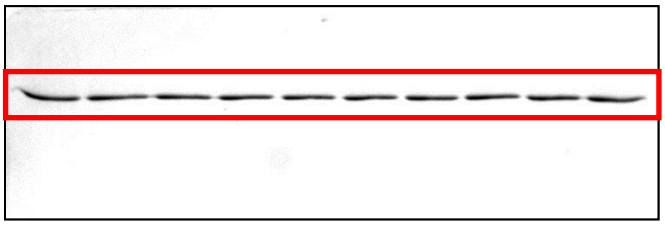


MTT assay results demonstrated that the application of 4HR on NHDF did not show any inhibition in the tested range of concentration (1-100 μM). Untreated control (blank) showed mild increase from 24 to 72 h. Though 4HR treated groups showed generally higher value than untreated control, there was no statistically significant difference ($P>0.05$) (gray: 1 μM, hatched: 10 μM, black: 100 μM). However, NHDF also showed decreased value over 1 mM concentration of 4HR administration (data not shown).

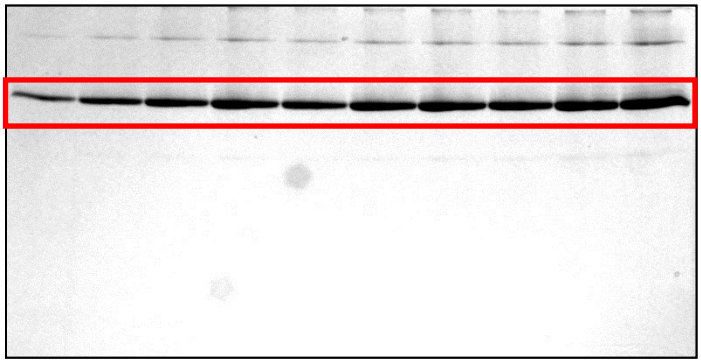
Supplementary Figure 1. Full length blot of Figure 2A

BPA induced ERα and ERβ expression

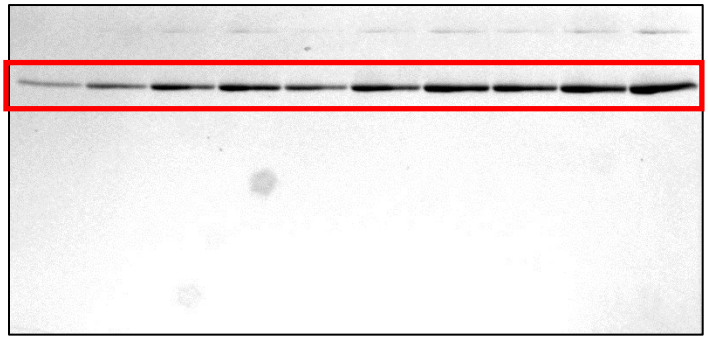
A. β-actin (from left lane to right, **1:** no-treatment, **2:** 1 μM BPA at 2 h, **3:** 10 μM BPA at 2 h, **4:** 100 μM BPA at 2 h, **5:** 1 μM BPA at 8 h, **6:** 10 μM BPA at 8 h, **7:** 100 μM BPA at 8 h, **8:** 1 μM BPA at 24 h, **9:** 10 μM BPA at 24 h, **10:** 100 μM BPA at 24 h)



B. Estrogen receptor-α (from left lane to right, **1:** no-treatment, **2:** 1 μM BPA at 2 h, **3:** 10 μM BPA at 2 h, **4:** 100 μM BPA at 2 h, **5:** 1 μM BPA at 8 h, **6:** 10 μM BPA at 8 h, **7:** 100 μM BPA at 8 h, **8:** 1 μM BPA at 24 h, **9:** 10 μM BPA at 24 h, **10:** 100 μM BPA at 24 h)



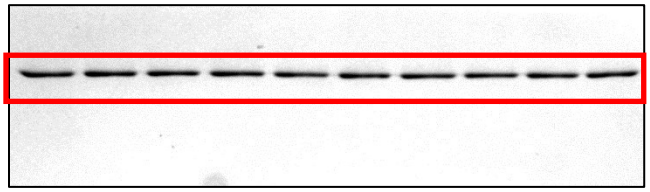
C. Estrogen receptor-β (from left lane to right, **1:** no-treatment, **2:** 1 μM BPA at 2 h, **3:** 10 μM BPA at 2 h, **4:** 100 μM BPA at 2 h, **5:** 1 μM BPA at 8 h, **6:** 10 μM BPA at 8 h, **7:** 100 μM BPA at 8 h, **8:** 1 μM BPA at 24 h, **9:** 10 μM BPA at 24 h, **10:** 100 μM BPA at 24 h)



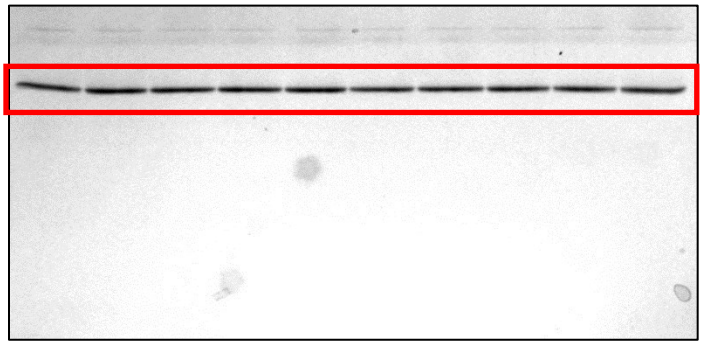
Supplementary Figure 2. Full length blot of Figure 2B

4HR did not induce ERα and ERβ expression

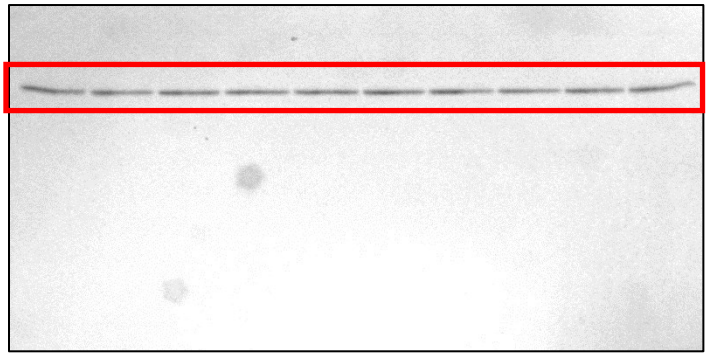
A. β-actin (from left lane to right, **1:** no-treatment, **2:** 1 μM 4HR at 2 h, **3:** 10 μM 4HR at 2 h, **4:** 100 μM 4HR at 2 h, **5:** 1 μM 4HR at 8 h, **6:** 10 μM 4HR at 8 h, **7:** 100 μM 4HR at 8 h, **8:** 1 μM 4HR at 24 h, **9:** 10 μM 4HR at 24 h, **10:** 100 μM 4HR at 24 h)



B. Estrogen receptor-α (from left lane to right, **1:** no-treatment, **2:** 1 μM 4HR at 2 h, **3:** 10 μM 4HR at 2 h, **4:** 100 μM 4HR at 2 h, **5:** 1 μM 4HR at 8 h, **6:** 10 μM 4HR at 8 h, **7:** 100 μM 4HR at 8 h, **8:** 1 μM 4HR at 24 h, **9:** 10 μM 4HR at 24 h, **10:** 100 μM 4HR at 24 h)



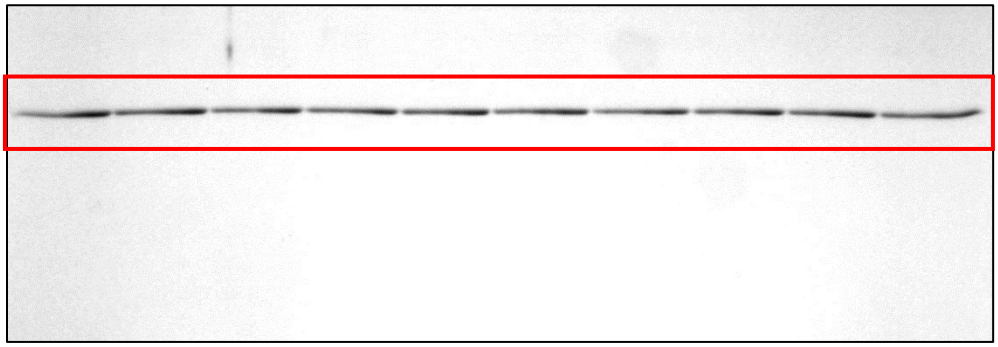
C. Estrogen receptor-β (from left lane to right, **1:** no-treatment, **2:** 1 μM 4HR at 2 h, **3:** 10 μM 4HR at 2 h, **4:** 100 μM 4HR at 2 h, **5:** 1 μM 4HR at 8 h, **6:** 10 μM 4HR at 8 h, **7:** 100 μM 4HR at 8 h, **8:** 1 μM 4HR at 24 h, **9:** 10 μM 4HR at 24 h, **10:** 100 μM 4HR at 24 h)



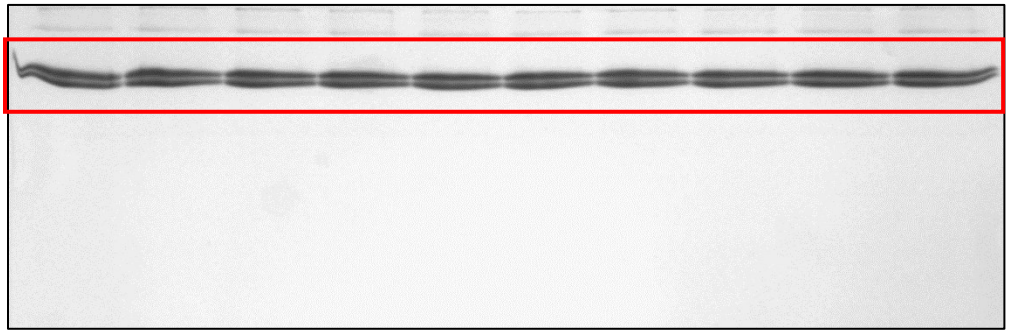
Supplementary Figure 3 Full length blot of Figure 3A

The expression level of extracellular signal regulated kinase (Erk) and phosphorylated Erk (p-Erk) after BPA treatment

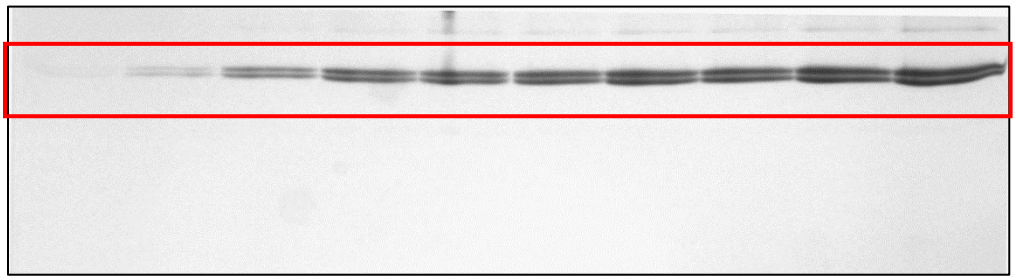
A. β-actin (from left lane to right, **1:** no-treatment, **2:** 1 μM BPA at 2 h, **3:** 10 μM BPA at 2 h, **4:** 100 μM BPA at 2 h, **5:** 1 μM BPA at 8 h, **6:** 10 μM BPA at 8 h, **7:** 100 μM BPA at 8 h, **8:** 1 μM BPA at 24 h, **9:** 10 μM BPA at 24 h, **10:** 100 μM BPA at 24 h)



B. Erk (from left lane to right, **1:** no-treatment, **2:** 1 μM BPA at 2 h, **3:** 10 μM BPA at 2 h, **4:** 100 μM BPA at 2 h, **5:** 1 μM BPA at 8 h, **6:** 10 μM BPA at 8 h, **7:** 100 μM BPA at 8 h, **8:** 1 μM BPA at 24 h, **9:** 10 μM BPA at 24 h, **10:** 100 μM BPA at 24 h)



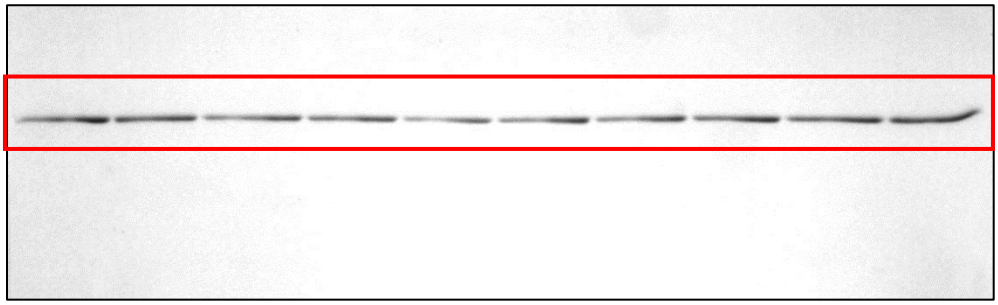
C. p-Erk (from left lane to right, **1:** no-treatment, **2:** 1 μM BPA at 2 h, **3:** 10 μM BPA at 2 h, **4:** 100 μM BPA at 2 h, **5:** 1 μM BPA at 8 h, **6:** 10 μM BPA at 8 h, **7:** 100 μM BPA at 8 h, **8:** 1 μM BPA at 24 h, **9:** 10 μM BPA at 24 h, **10:** 100 μM BPA at 24 h)



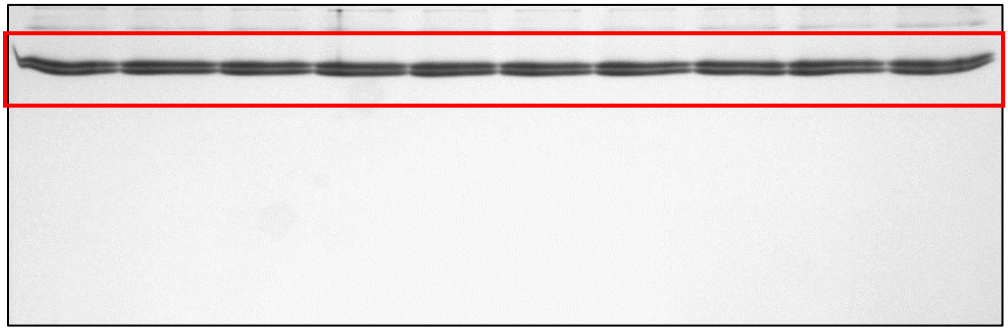
Supplementary Figure 4. Full length blot of Figure 3B

The expression level of extracellular signal regulated kinase (Erk) and phosphorylated Erk (p-Erk) after 4HR treatment

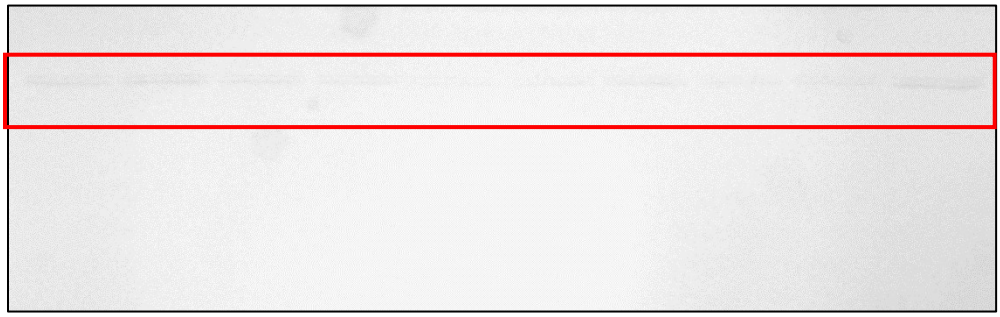
A. β-actin (from left lane to right, **1:** no-treatment, **2:** 1 μM 4HR at 2 h, **3:** 10 μM 4HR at 2 h, **4:** 100 μM 4HR at 2 h, **5:** 1 μM 4HR at 8 h, **6:** 10 μM 4HR at 8 h, **7:** 100 μM 4HR at 8 h, **8:** 1 μM 4HR at 24 h, **9:** 10 μM 4HR at 24 h, **10:** 100 μM 4HR at 24 h)



B. Erk (from left lane to right, **1:** no-treatment, **2:** 1 μM 4HR at 2 h, **3:** 10 μM 4HR at 2 h, **4:** 100 μM 4HR at 2 h, **5:** 1 μM 4HR at 8 h, **6:** 10 μM 4HR at 8 h, **7:** 100 μM 4HR at 8 h, **8:** 1 μM 4HR at 24 h, **9:** 10 μM 4HR at 24 h, **10:** 100 μM 4HR at 24 h)



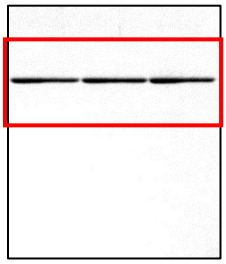
C. p-Erk (from left lane to right, **1:** no-treatment, **2:** 1 μM 4HR at 2 h, **3:** 10 μM 4HR at 2 h, **4:** 100 μM 4HR at 2 h, **5:** 1 μM 4HR at 8 h, **6:** 10 μM 4HR at 8 h, **7:** 100 μM 4HR at 8 h, **8:** 1 μM 4HR at 24 h, **9:** 10 μM 4HR at 24 h, **10:** 100 μM 4HR at 24 h)



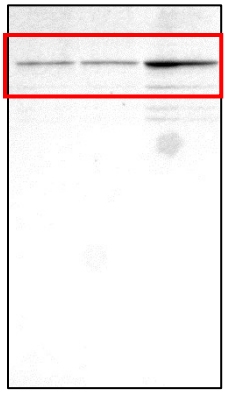
Supplementary Figure 5. Full length blot of Figure 4C

Western blot for pituitary gland tissue samples. Prolactin, ERα, and ERβ expression were increased in estradiol group.

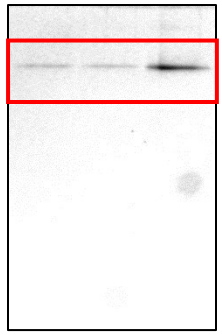
A. β-actin (from left lane to right, 1: solvent only, 2: 125 mg/kg 4HR daily subcutaneous injection for 7 days, 3: 1 μg/kg 17-β-estradiol daily subcutaneous injection for 7 days)



B. Estrogen receptor-α (from left lane to right, 1: solvent only, 2: 125 mg/kg 4HR daily subcutaneous injection for 7 days, 3: 1 μg/kg 17-β-estradiol daily subcutaneous injection for 7 days)



C. Estrogen receptor-β (from left lane to right, 1: solvent only, 2: 125 mg/kg 4HR daily subcutaneous injection for 7 days, 3: 1 μg/kg 17-β-estradiol daily subcutaneous injection for 7 days)



D. Prolactin (from left lane to right, 1: solvent only, 2: 125 mg/kg 4HR daily subcutaneous injection for 7 days, 3: 1 μg/kg 17-β-estradiol daily subcutaneous injection for 7 days)