Induction of retinoid X receptor activity and consequent up-regulation of p21$^{WAF1/CIP1}$ by indenoisoquinolines in MCF7 cells

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Supplementary Method S1.

3-Amino-6-(3-aminopropyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline dihydrochloride (3).

Method A. Triethyl phosphite (0.09 ml, 0.54 mmol) was added to a solution of **4** (75 mg, 0.217 mmol) in benzene (25 ml) and the reaction mixture was heated at reflux for 16 h. The reaction mixture was allowed to cool to room temperature, 3 M HCl in methanol (10 ml) was added, and the reaction mixture was heated at reflux for 2 h. The reaction mixture was allowed to cool to room temperature and the precipitate was filtered to provide a brick-red solid (0.075 g, 88%): mp 232°C (dec). IR (KBr, cm^{-1}) 3427, 2927, 1658, 1510; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.42 (d, J = 8.6 Hz, 1 H), 7.95 (bs, 3 H), 7.46 (d, J = 7.4 Hz, 1 H), 7.65 (d, J = 1.6 Hz, 1 H), 7.56−7.51 (m, 2 H), 7.46−7.41 (m, 1 H), 7.36−7.33 (m, 1 H), 4.55 (t, J = 7.7 Hz, 2 H), 2.97−2.96 (m, 2 H), 2.13−2.08 (m, 2 H); positive ESIMS *m/z* (rel intensity) 320 (MH^+, 100). Anal. Calcd for $C_{19}H_{19}Cl_2N_3O_2$: C, 58.17; H, 4.88; N, 10.71. Found: C, 58.38; H, 5.05; N, 10.38.

Method B. A solution of ammonium chloride (15 ml, 10% in water) was added at room temperature to a mixture of compound **2** (5.9 g, 15.7 mmol), iron powder (8.8 g, 0.16 mol), ethanol (150 ml) and water (50 ml). The resulting mixture was heated at reflux for 3 h. The mixture was allowed to cool to room temperature and a solution of potassium hydroxide (5 g) in ethanol (50 ml) was added. The resulting mixture was heated at reflux and filtered while hot. The precipitate was washed on a filter with hot ethanol (3 x 50 ml). The combined filtrates were concentrated to 100 ml under reduced pressure and diluted with water (300 ml). The resulting dark-purple solution was extracted with chloroform (7 x 100 ml). The combined extracts were washed with brine (100 ml), dried with sodium sulfate, and evaporated to dryness under reduced pressure. The crude dark-purple product was recrystalized four times from methanol. The solid obtained after recrystalization was suspended in benzene (200 ml) and 2 M HCl in methanol (20 ml) was added at room temperature. The resulting mixture was heated at reflux. The newly formed orange

precipitate was collected by filtration, washed with chloroform (3 x 30 ml), ether (50 ml) and dried to yield the desired product **3** (3.5 g, 60%): mp 234−236°C (dec). IR (KBr, cm^{-1}) 3430, 2927, 2584, 1659, 1511; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.39 (d, J = 9 Hz, 1 H), 7.94 (s, 3 H), 7.69−7.33 (m, 6 H), 4.52 (t, J = 6 Hz, 2 H), 2.95 (s, 2 H), 2.09 (pent, J = 6 Hz, 3 H); positive ESIMS *m/z* (rel intensity) 320 (MH^+, 100), 303 (64).

3-Amino-6-(3-azidopropyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (4). Sodium azide (86 mg, 1.328 mmol) and **19** (150 mg, 0.443 mmol) were diluted with dimethylsulfoxide (40 ml) and heated at 100˚C for 4 h. The reaction mixture was diluted with chloroform (100 ml), washed with water (3 x 30 ml), brine (30 ml), and dried over sodium sulfate. The solution was concentrated to provide a crude solid that was purified by flash column chromatography (silica gel), eluting with a gradient of chloroform-1% triethylamine to 3% methanol-chloroform-1% triethylamine, to provide a brown-purple solid (88 mg, 58%): mp 175−178°C (dec). IR (KBr, cm^{-1}) 3350, 2103, 1642, 1578, 1517; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.28 (d, J = 8.6 Hz, 1 H), 7.66 (d, J = 7.6 Hz, 1 H), 7.52−7.45 (m, 2 H), 7.39 (d, J = 7.4 Hz, 1 H), 7.34 (d, J = 2.3 Hz, 1 H), 7.11 (dd, J = 8.7 Hz and 2.5 Hz, 1 H), 5.78 (s, 2 H), 4.52−4.47 (m, 2 H), 3.62 (t, J = 7.5 Hz, 2 H), 2.02−1.98 (m, 2 H); negative ion ESIMS *m/z* (rel intensity) 344 [$(M–H)^-$, 55]. Anal. Calcd for $C_{19}H_{15}N_5O_2$: C, 66.08; H, 4.38; N, 20.28. Found: C, 65.89; H, 4.31; N, 20.00.

3-Amino-6-methyl-5*H*-indeno[1,2-*c*]isoquinoline-5,11(6*H*)-dione dihydrochloride (5). A solution of ammonium chloride (1 ml, 10% in water) was added at room temperature to a mixture of 5,6-dihydro-6-methyl-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (**27**) (487 mg, 1.6 mmol), iron powder (450 mg, 8 mol), ethanol (10 ml) and water (5 ml). The resulting mixture was heated at reflux for 3 h. The mixture was allowed to cool to room temperature and a solution of potassium hydroxide (0.5 g) in ethanol (5 ml) was added. The resulting mixture was heated at reflux and filtered while hot. The precipitate was washed on a filter with hot ethanol (3 x 10 ml). The combined filtrates were concentrated to 10 ml under reduced pressure and diluted with water (20 ml). The resulting dark-purple solution was extracted with chloroform (5 x 10 ml). The combined extracts were washed with brine (20 ml), dried with sodium sulfate and evaporated to dryness under reduced pressure. The crude dark-purple product was purified by column chromatography (silica gel), eluting with 3% methanol-chloroform to yield the desired product **5** (400 mg, 90%): mp 266−268°C. IR (KBr, cm^{-1}) 3452, 3362, 3247, 1693, 1578, 1517, 1455, 1429, 1319; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48 (d, J = 9 Hz, 1 H), 7.55 (d, J = 6 Hz, 3 H), 7.33–7.26 (m, 2 H), 7.10 (d, J = 6 Hz, 1 H), 4.03 (s, 3 H); positive ESIMS *m/z* (rel intensity) 277 (MH^+, 100); HPLC purity: 97.87% (C-18 reverse phase, MeOH/H_2O, 90:10); 97.07% (C-18 reverse phase, MeOH, 100).

3-(5,11-Dioxo-5*H*-indeno[1,2-*c*]isoquinolin-6(11*H*)-yl)propyl 4-methylbenzene-sulfonate (12). A solution of **21** (150 mg, 0.49 mmol), 4-methylbenzene-1-sulfonyl chloride (187 mg, 0.98 mmol), 4-dimethylaminopyridine (12 mg, 0.1 mmol), and triethylamine (0.1 ml, 0.74 mmol) in dichloromethane (10 ml) was stirred at room temperature for 14 h. The resulting mixture was further diluted with dichloromethane (50 ml), washed with saturated sodium bicarbonate (3 x 25 ml), brine (25 ml), dried with sodium sulfate and evaporated under reduced pressure. The residue was subjected to flash column chromatography (silica gel), eluting with chloroform, to obtain pure **12** (31 mg, 15%): mp 175−177°C. IR (KBr, cm^{-1}) 1696, 1671, 1505; ^1H NMR (300 MHz, $CDCl_3$) δ 8.39 (d, *J* = 7.8 Hz, 1 H), 8.28 (d, *J* = 7.8 Hz, 1 H), 7.81 (d, *J* = 8.4 Hz, 2 H), 7.76–7.62 (m, 3 H), 7.49−7.34 (m, 5 H) 4.58 (dd, *J* = 9.9, 5.7 Hz, 2 H), 4.28 (t, *J* = 6.0 Hz, 2 H), 2.46, (s, 3 H), 2.30 (m, 2 H); positive ESIMS *m/z* (rel intensity) 288 (100). Anal. Calcd for $C_{26}H_{21}NO_5S$: C, 67.96; H, 4.61; N, 3.05. Found: C, 67.65; H, 4.76; N, 7.07.

4-(5,11-Dioxo-5*H*-indeno[1,2-*c*]isoquinolin-6(11*H*)-yl)butyl 4-methylbenzene-sulfonate (13). A solution of **22** (109 mg, 0.34 mmol), 4-methylbenzene-1-sulfonyl chloride (130 mg, 0.68 mmol), 4-dimethylaminopyridine (8 mg, 0.07 mmol), and triethylamine (0.1 ml, 0.74 mmol) in dichloromethane (10 ml) was stirred at room temperature for 48 h. The resulting mixture was further diluted with chloroform (100 ml), washed with saturated sodium bicarbonate (3 x 25 ml), brine (25 ml), dried with sodium sulfate and evaporated under reduced pressure. The residue was subjected to flash column chromatography (silica gel), eluting with chloroform. The orange solid obtained after flash column chromatography was washed with ethyl ether to yield pure **13** (117 mg, 74%): mp 159−163°C. IR (KBr, cm^{-1}) 1694, 1665, 1612, 1504; ^1H NMR (300 MHz, $CDCl_3$) δ 8.70 (d, *J* = 8.1 Hz, 1 H), 8.30 (d, *J* = 8.1 Hz, 1 H), 7.78−7.70 (m, 3 H), 7.64 (d, *J* = 6.9 Hz, 1 H), 7.50−7.38 (m, 4 H), 7.31 (d, *J* = 8.1 Hz, 2 H), 4.52 (t, *J* = 6.9 Hz, 2 H), 4.14 (t, *J* = 6.0 Hz, 2 H), 2.42 (s, 3 H), 2.00−1.87 (m, 4 H); positive ESIMS *m/z* (rel intensity) 496 (MNa^+, 100), 302 (66). Anal. Calcd for $C_{27}H_{23}NO_5S$: C, 68.48; H, 4.908; N, 2.96. Found: C, 68.13; H, 4.93; N, 2.90.

5-(5,11-Dioxo-5*H*-indeno[1,2-*c*]isoquinolin-6(11*H*)-yl)pentyl 4-methylbenzene-sulfonate (14). A solution of **23** (125 mg, 0.38 mmol), 4-methylbenzene-1-sulfonyl chloride (143 mg, 0.75 mmol), 4-dimethylaminopyridine (9 mg, 0.075 mmol), and triethylamine (0.1 ml, 0.74 mmol) in dichloromethane (10 ml) was stirred at room temperature for 48 h. The resulting mixture was further diluted with chloroform (100 ml), washed with saturated sodium bicarbonate (3 x 25 ml), brine (25 ml), dried with sodium sulfate and evaporated under reduced pressure. The residue was subjected to flash column chromatography (silica gel) eluting with chloroform. The orange solid obtained after flash column chromatography was washed with ethyl ether to yield pure **14** (110 mg, 60%): mp 164−166°C. IR (KBr, cm^{-1})

1693, 1655, 1611, 1549, 1503; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.70 (d, *J* = 8.1 Hz, 1 H), 8.31 (d, *J* = 8.1 Hz, 1 H), 7.79–7.72 (m, 3 H), 7.49–7.38 (m, 4 H), 7.32 (d, *J* = 6.0 Hz, 1 H), 4.48 (t, *J* = 6.9 Hz, 2 H), 4.07 (t, *J* = 6.0 Hz, 2 H), 2.43 (s, 3 H), 1.90–1.74 (m, 4 H), 1.64–1.59 (m, 2 H); positive ESIMS *m/z* (rel intensity) 488 (MH^+,100). Anal. Calcd for $C_{28}H_{25}NO_5S$: C, 68.98; H, 5.17; N, 2.87. Found: C, 68.71; H, 5.00; N, 2.77.

5-(5,11-Dioxo-5*H*-indeno[1,2-*c*]isoquinolin-6(11*H*)-yl)hexyl 4-methylbenzene-sulfonate (15). A solution of **16** (100 mg, 0.38 mmol), 4-methylbenzene-1-sulfonyl chloride (110 mg, 0.58 mmol), 4-dimethylaminopyridine (7 mg, 0.058 mmol), and triethylamine (0.08 ml, 0.58 mmol) in dichloromethane (10 ml) was stirred at room temperature for 24 h. The resulting mixture was further diluted with chloroform (50 ml), washed with saturated sodium bicarbonate (3 x 25 ml), brine (25 ml), dried with sodium sulfate and evaporated under reduced pressure. The residue was subjected to flash column chromatography (silica gel), eluting with chloroform to yield pure **15** (110 mg, 60%): mp 145−147°C. IR (KBr, cm^{-1}) 1689, 1660, 1611, 1550, 1503; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.70 (d, *J* = 8.1 Hz, 1 H), 8.32 (dd, *J* = 8.1, 0.6 Hz, 1 H), 7.80–7.70 (m, 3 H), 7.63 (d, *J* = 6.6 Hz, 1 H), 7.49–7.32 (m, 6 H), 4.48 (t, *J* = 7.8 Hz, 2 H), 4.05 (t, *J* = 6.3 Hz, 2 H), 2.44 (s, 3 H), 1.87 (m, 2 H), 1.70 (m, 2 H), 1.50–1.47 (m, 4 H); positive ESIMS *m/z* (rel intensity) 502 (MH^+, 100). Anal. Calcd for $C_{22}H_{21}NO_3$: C, 69.44; H, 5.43; N, 2.79. Found: C, 68.71; H, 5.34; N, 2.78.

6-(6-Hydroxyhexyl)-5*H*-indeno[1,2-*c*]isoquinoline-5,11(6*H*)-dione (16). A mixture of **20** (300 mg, 0.81 mmol) and 6-hydroxyhexylamine (283 mg, 2.42 mmol) in chloroform (50 ml) was heated to reflux for 6 h. The resulting mixture was cooled to room temperature, diluted with chloroform (50 ml), washed with saturated sodium bicarbonate (3 x 30 ml), brine (30 ml), dried with sodium sulfate and evaporated to dryness. The resulting orange solid was washed with ethyl ether (50 ml) to provide the pure product as a red solid (238 mg, 85%): mp 140−142°C. IR (KBr, cm^{-1}) 1692, 1641, 1612, 1506; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.70 (d, *J* = 8.1 Hz, 1 H), 8.33 (dd, *J* = 8.1, 0.6 Hz, 1 H), 7.72 (m, 1 H), 7.63 (d, *J* = 6.3 Hz, 1 H), 7.49 – 7.37 (m, 4 H), 4.53 (t, *J* = 7.8 Hz, 2 H), 3.67 (t, *J* = 6.0 Hz, 2 H), 1.93 (m, 2 H), 1.61 (m, 6 H); positive ESIMS *m/z* (rel intensity) 348 488 (MH^+, 100). Anal. Calcd for $C_{22}H_{21}NO_3$: C, 76.06; H, 6.09; N, 4.03. Found: C, 76.06; H, 6.01; N, 4.04.

6-Methyl-5*H*-indeno[1,2-*c*]isoquinoline-5,11(6*H*)-dione (17) and 3-bromo-6-methyl-5*H*-indeno[1,2-*c*]isoquinoline-5,11(6*H*)-dione (28). 3-Amino-6-methyl-5*H*-indeno[1,2-*c*]isoquinoline-5,11(6*H*)-dione (**5**) (870 mg, 3.14 mmol) was dissolved in water (6 ml) and 1,4-dioxane (3 ml). Hydrobromic acid (48% in water, 1.8 ml) was added and the reaction mixture was heated at reflux for 20 min. The reaction mixture was cooled to 0°C and a solution of sodium nitrite (280 mg) in water (10 ml) and 1,4-dioxane (20 ml) was added slowly. The reaction mixture was stirred for 2 h at 0°C. This solution was added dropwise

to a solution of copper(I) bromide in water (10 ml) and 1,4-dioxane (10 ml) that was maintained at 0°C. The reaction mixture was stirred at 0°C for 30 min and then heated at reflux for 6 h. The solution was cooled down to room temperature and extracted with chloroform (3 x 200 ml). The organic extracts were combined and concentrated in vacuo. The products were separated by preparative TLC using chloroform as the eluent. Compound **28** was obtained as an orange solid (120 mg, 0.35 mmol, 11%): mp 269−271°C. IR (KBr, cm^{-1}) 3066, 2917, 1693, 1669, 1657, 1571, 1498, 1312; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.54 (d, *J* = 8.6 Hz, 1 H), 8.47 (d, *J* = 2.0 Hz, 1 H), 7.78 (dd, *J* = 8.6, 2.1 Hz, 1 H), 7.65 (d, *J* = 6.7 Hz, 1 H), 7.63 (d, *J* = 7.0 Hz, 1 H), 7.43 (m, 2 H), 4.05 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.0, 162.2, 156.1, 137.4, 136.9, 134.9, 133.1, 131.2, 131.0, 130.7, 128.5, 125.1, 124.6, 123.4, 122.9, 120.8, 107.8, 33.1; EIMS *m/z* 339 (M^+), CIMS *m/z* 340 (MH^+); HPLC purity: 95.10% (C-18 reverse phase, MeOH/H_2O, 90:10); 95.72% (C-18 reverse phase, MeOH, 100). Compound **17** was obtained as a light red solid (280 mg, 1.07 mmol, 34.1%): mp 223-225°C. IR (KBr, cm^{-1}) 2922, 1693, 1668, 1613, 1506, 1432; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.68 (d, *J* = 8.1 Hz, 1 H), 8.34 (d, *J* = 8.0 Hz, 1 H), 7.65 (m, 3 H), 7.41 (m, 3 H), 4.09 (s, 3 H); EIMS *m/z* 261 (M^+), CIMS *m/z* 262 (MH^+); HPLC purity: 96.75% (C-18 reverse phase, MeOH/H_2O, 90:10); 95.36% (C-18 reverse phase, MeOH, 100).

3-Iodo-6-methyl-5*H*-indeno[1,2-*c*]isoquinoline-5,11(6*H*)-dione (18). 3-Amino-6-methyl-5*H*-indeno[1,2-*c*]isoquinoline-5,11(6*H*)-dione (**5**) (3.15 g, 11.3 mmol,) was dissolved in water (39 ml) and 1,4-dioxane (19 ml). Hydrochloric acid (36% in water, 5.5 ml) was added dropwise and the reaction mixture was heated to reflux for 10 min. The reaction mixture was cooled to 0°C and a solution of sodium nitrite (1.15 g) in water (10 ml) was added slowly. The reaction mixture was stirred for 2 h at 0°C. This solution was transferred to a funnel and added dropwise to a solution of potassium iodide (3.20 g) in water (15 ml) and 1,4-dioxane (5 ml) that was maintained at 0°C. The reaction mixture was stirred at 0°C for 30 min and then heated to reflux for 1 h. The reaction mixture was stirred overnight at room temperature and extracted with chloroform (3 x 200 ml). The organic extracts were combined and concentrated in vacuo. The solid was purified by flash column chromatography (silica gel), eluting with dichloromethane to provide a red solid (405 mg, 1.05 mmol, 9.30%): mp 262−264°C. IR (KBr, cm^{-1}) 3065, 1690, 1664, 1602, 1571, 1536, 1497, 1426, 1310; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.67 (d, *J* = 1.8 Hz, 1 H), 8.39 (d, *J* = 8.5 Hz, 1 H), 7.98 (dd, *J* = 8.4, 2.0 Hz, 1 H), 7.65(m, 2 H), 7.43 (m, 2 H), 4.05 (s, 3 H); EIMS *m/z* 387 (M^+), CIMS *m/z* 388 (MH^+); HPLC purity: 97.57% (C-18 reverse phase, MeOH/H_2O, 90:10); 97.66 (C-18 reverse phase, MeOH, 100).

3-Amino-6-(3-chloropropyl)-5,6-dihydro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (19). 6-(3-Chloropropyl)-5,6-dihydro-3-nitro-5,11-dioxo-11*H*-indeno[1,2-

c]isoquinoline (1) (0.200 g, 0.542 mmol) and 5% Pd/C (0.150 g) were diluted with THF (50 ml). The solution was degassed and allowed to stir at room temperature under a hydrogen atmosphere for 3 h. The solution was filtered, the filterpad was washed with chloroform-methanol 1:1 (100 ml), and the filtrate was concentrated to provide a crude purple solid. The obtained solid was purified by flash column chromatography (silica gel), eluting with a gradient of chloroform-1%-triethylamine to methanol-4%-chloroform-1% triethylamine to provide a brown solid (0.065 g, 35%): mp 192−196°C (dec). IR (KBr, cm^{-1}) 3358, 1703, 1641, 1577, 1517; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.40 (d, J = 8.6 Hz, 1 H), 7.74 (d, J = 7.6 Hz, 1 H), 7.63 (s, 1 H), 7.55−7.32 (m, 4 H), 4.59 (t, J = 6.8 Hz, 2 H), 3.90 (t, J = 6.4 Hz, 2 H), 2.27 (m, 2 H); EIMS *m/z* (rel intensity) 338/340 (M^+, 100/36). Anal. Calcd for $C_{19}H_{15}ClN_2O_2 \cdot 0.3\ H_2O$: C, 66.30; H, 4.57; N, 8.14. Found: C, 66.20; H, 4.59; N, 7.86.

6-(4-Hydroxybutyl)-5*H*-indeno[1,2-*c*]isoquinoline-5,11(6*H*)-dione (22). A mixture of **20** (100 mg, 0.40 mmol) and 4-hydroxybutylamine (0.11 ml, 1.2 mmol) in chloroform (20 ml) was heated at reflux for 2 h. The resulting mixture was cooled to room temperature, diluted with chloroform (100 ml), washed with saturated sodium bicarbonate (3 x 30 ml), dried with sodium sulfate and concentrated to provide a product that was introduced into the next step without additional purification.

6-(5-Hydroxypentyl)-5*H*-indeno[1,2-c]isoquinoline-5,11(6*H*)-dione (23). A mixture of **20** (100 mg, 0.40 mmol) and 5-hydroxypenylamine (125 mg, 1.21 mmol) in chloroform (30 ml) was heated at reflux for 2 h. The resulting mixture was cooled to room temperature, diluted with chloroform (100 ml), washed with saturated sodium bicarbonate (3 x 30 ml), dried with sodium sulfate and concentrated to provide the product as a red solid (125 mg, 93%) that was introduced into the next step without additional purification.

***cis*-4-Carboxy-3,4-dihydro-*N*-(methyl)-3-(phenyl)-1(2*H*)isoquinolone (26).** 5-Nitrohomophthalic anhydride (3.466 g, 16.73 mmol) was added to a chloroform (100 ml) solution of benzylidenemethylamine (**25**) (1.994 g, 16.73 mmol) and the mixture was stirred at room temperature for 2 h. The precipitate was filtered, washed with chloroform (100 ml), and dried to provide a yellow solid (4.192 g, 77%): mp 140−142°C. ^{1}H NMR (300 MHz, CD_3OD) δ 8.90 (d, J = 2.51 Hz, 1 H), 8.38 (dd, J = 8.61, 2.55 Hz, 1 H), 7.95 (dd, J = 8.63 Hz and 0.99 Hz, 1 H), 7.26 (m, 3 H), 7.07 (m, 2 H), 5.26 (d, J = 6.49 Hz, 1 H), 4.92 (d, J = 6.53 Hz, 1 H), 3.08 (s, 3 H).

5,6-Dihydro-6-methyl-3-nitro-5,11-dioxo-11*H*-indeno[1,2-*c*]isoquinoline (27). Thionyl chloride (5 ml) was added to a solution **26** (1.000 g, 3.064 mmol) in benzene (50 ml). The solution was heated at reflux for 30 min, cooled to room temperature, and concentrated. The residue was diluted with nitrobenzene (30 ml), cooled in an ice bath, and aluminum chloride (0.817 g, 6.129 mmol) was added. The solution was removed from the bath and

heated at 100ºC for 1 h. Ice water (100 ml) was added and the solution was extracted with $CHCl_3$ (3 x 500 ml). The combined organic layer was washed with sat $NaHCO_3$ (3 x 250 ml), sat NaCl (250 ml), and dried over sodium sulfate. The solution was concentrated, hexanes (500 ml) were added and liquid was decanted. The solid was washed with hexanes (100 ml) and the liquid was again decanted. The solid was purified by precipitation from chloroform-hexanes to provide a red-orange solid (0.450 g, 48%): mp 344−345°C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.23 (d, *J* = 2.47 Hz, 1 H), 8.86 (d, *J* = 8.82 Hz, 1 H), 8.51 (dd, *J* = 8.29 Hz and 2.45 Hz, 1 H), 7.77 (m, 2 H), 7.55 (m, 2 H), 4.13 (s, 3 H).

Supplementary Method S2. **Preparation of nuclear extracts and the electrophoretic mobility shift assay (EMSA).** 5'IRDye®700-labelled RXRE oligonucleotides (5'-AGGTTCAGGTCAGAGGTCAGAGAGCT-3') (1) were synthesized by Integrated DNA Technologies Inc. (Coralville, IA). Nuclear extracts were prepared as previously reported (2). Briefly, cells were washed with PBS and incubated in lysis buffer (10 mM Tris–HCl, pH 8.0, 60 mM KCl, 1 mM EDTA, 1 mM dithiothreitol, 100 mM PMSF, and 0.2% NP-40) for 5 min on ice. Following centrifugation at 2500 rpm at 4°C for 4 min, the pellet was washed with lysis buffer without NP-40. The nuclear proteins were extracted from the pellet after 10 min incubation with nuclear extract buffer (20 mM Tris–HCl, pH 8.0, 420 mM NaCl, 1.5 mM $MgCl_2$, 0.2 mM EDTA and 25% glycerol) and centrifugation at 14000 rpm at 4°C for 15 min. Binding reactions were performed by incubation of 5 μg of nuclear protein extracts, 2.5 mM dithiothreitol, 1 μg of poly (dI.dC), 2.5% glycerol, 50 mM KCl, 10 mM EDTA, and 50 nM DR-1 with IRDye™ 700 infrared dye-5'-end-labelled for 30 min at room temperature. Protein-DNA complexes were resolved by electrophoresis on 5% polyacrylamide gels in 0.5x TBE buffer at 100 V for 1 h 30 min. The gel was visualized by Odyssey® Infrared Imaging System (LI-COR Biosciences, Lincoln, NE).

References

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Supplementary Table S1. Cell cycle-related genes altered by bexarotene or AM6-36 in MCF7 cells (blue background: siginificantly down-regulated genes; red background: significanlty up-regulated genes).

GeneBank	Symbol	Description	Gene Name	AM6-36		Bexarotene	
				Fold change	*P*-value	Fold change	*P*-value
NM_005157	*ABL1*	C-abl oncogene 1, receptor tyrosine kinase	ABL/JTK7	0.88	0.58	1.32	0.63
NM_013366	*ANAPC2*	Anaphase promoting complex subunit 2	APC2	1.56	0.30	0.98	0.77
NM_013367	*ANAPC4*	Anaphase promoting complex subunit 4	APC4	1.08	0.98	0.90	0.60
NM_004675	*DIRAS3*	DIRAS family, GTP-binding RAS-like 3	ARHI/NOEY2	0.80	0.53	1.83	0.17
NM_000051	*ATM*	Ataxia telangiectasia mutated	AT1/ATA	0.55	0.14	1.26	0.67
NM_001184	*ATR*	Ataxia telangiectasia and Rad3 related	FRP1/MEC1	0.65	0.21	0.98	0.80
NM_004324	*BAX*	BCL2-associated X protein	BCL2L4	1.69	0.19	1.14	0.90
NM_016567	*BCCIP*	BRCA2 and CDKN1A interacting protein	TOK-1/TOK1	1.11	0.92	0.78	0.38
NM_000633	*BCL2*	B-cell CLL/lymphoma 2	Bcl-2	0.22	0.06	0.13	0.04
NM_001168	*BIRC5*	Baculoviral IAP repeat-containing 5	API4/EPR-1	0.07	0.02	0.26	0.04
NM_007294	*BRCA1*	Breast cancer 1, early onset	BRCAI/BRCC1	0.18	0.06	0.35	0.11
NM_000059	*BRCA2*	Breast cancer 2, early onset	BRCC2/BROVCA2	0.09	0.02	0.36	0.06
NM_031966	*CCNB1*	Cyclin B1	CCNB	0.37	0.07	0.60	0.19
NM_004701	*CCNB2*	Cyclin B2	HsT17299	0.18	0.04	0.50	0.14
NM_005190	*CCNC*	Cyclin C	CycC	0.98	0.75	1.18	0.87
NM_053056	*CCND1*	Cyclin D1	BCL1/D11S287E	1.30	0.67	0.33	0.08
NM_001759	*CCND2*	Cyclin D2	KIAK0002	0.96	0.72	0.24	0.10
NM_001238	*CCNE1*	Cyclin E1	CCNE	0.58	0.20	0.63	0.25
NM_001761	*CCNF*	Cyclin F	FBX1/FBXO1	0.19	0.04	0.40	0.11
NM_004060	*CCNG1*	Cyclin G1	CCNG	1.70	0.16	1.12	0.94
NM_004354	*CCNG2*	Cyclin G2	Cyclin G2	2.81	0.01	1.88	0.12
NM_001239	*CCNH*	Cyclin H	CAK/p34	0.96	0.69	1.21	0.80
NM_001240	*CCNT1*	Cyclin T1	CCNT/CYCT1	0.99	0.69	1.14	0.97
NM_001241	*CCNT2*	Cyclin T2	FLJ90560	0.94	0.62	1.24	0.75
NM_003903	*CDC16*	Cell division cycle 16 homolog (*S. cerevisiae*)	APC6	1.03	0.81	1.27	0.68
NM_001786	*CDC2*	Cell division cycle 2, G1 to S and G2 to M	CDC28A/CDK1	0.09	0.04	0.32	0.08
NM_001255	*CDC20*	Cell division cycle 20 homolog (*S. cerevisiae*)	CDC20A/p55CDC	0.11	0.04	0.54	0.20
NM_004359	*CDC34*	Cell division cycle 34 homolog (*S. cerevisiae*)	E2-CDC34/UBC3	2.22	0.03	1.56	0.30
NM_001798	*CDK2*	Cyclin-dependent kinase 2	p33(CDK2)	0.28	0.05	0.28	0.05
NM_000075	*CDK4*	Cyclin-dependent kinase 4	CMM3/PSK-J3	0.53	0.14	1.19	0.79
NM_003885	*CDK5R1*	Cyclin-dependent kinase 5, regulatory subunit 1 (p35)	CDK5P35/CDK5R	0.63	0.24	0.70	0.31
NM_016408	*CDK5RAP1*	CDK5 regulatory subunit associated protein 1	C20orf34/C42	1.95	0.04	0.99	0.82
NM_001259	*CDK6*	Cyclin-dependent kinase 6	PLSTIRE	0.51	0.13	0.47	0.12
NM_001799	*CDK7*	Cyclin-dependent kinase 7	CAK1/CDKN7	1.61	0.24	1.98	0.09
NM_001260	*CDK8*	Cyclin-dependent kinase 8	K35	0.80	0.42	1.40	0.59
NM_000389	*CDKN1A*	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	CAP20/CDKN1	34.55	0.00	5.46	0.00
NM_004064	*CDKN1B*	Cyclin-dependent kinase inhibitor 1B (p27, Kip1)	CDKN4/KIP1	0.92	0.60	0.83	0.50
NM_000077	*CDKN2A*	Cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)	ARF/CDK4I	1.68	0.48	1.07	0.85
NM_004936	*CDKN2B*	Cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)	CDK4I/INK4B	1.90	0.10	2.58	0.03
NM_005192	*CDKN3*	Cyclin-dependent kinase inhibitor 3	CDI1/CIP2	0.16	0.04	0.52	0.16
NM_001274	*CHEK1*	CHK1 checkpoint homolog (*S. pombe*)	CHK1	0.04	0.03	0.36	0.08
NM_007194	*CHEK2*	CHK2 checkpoint homolog (*S. pombe*)	CDS1/CHK2	0.32	0.07	0.31	0.07

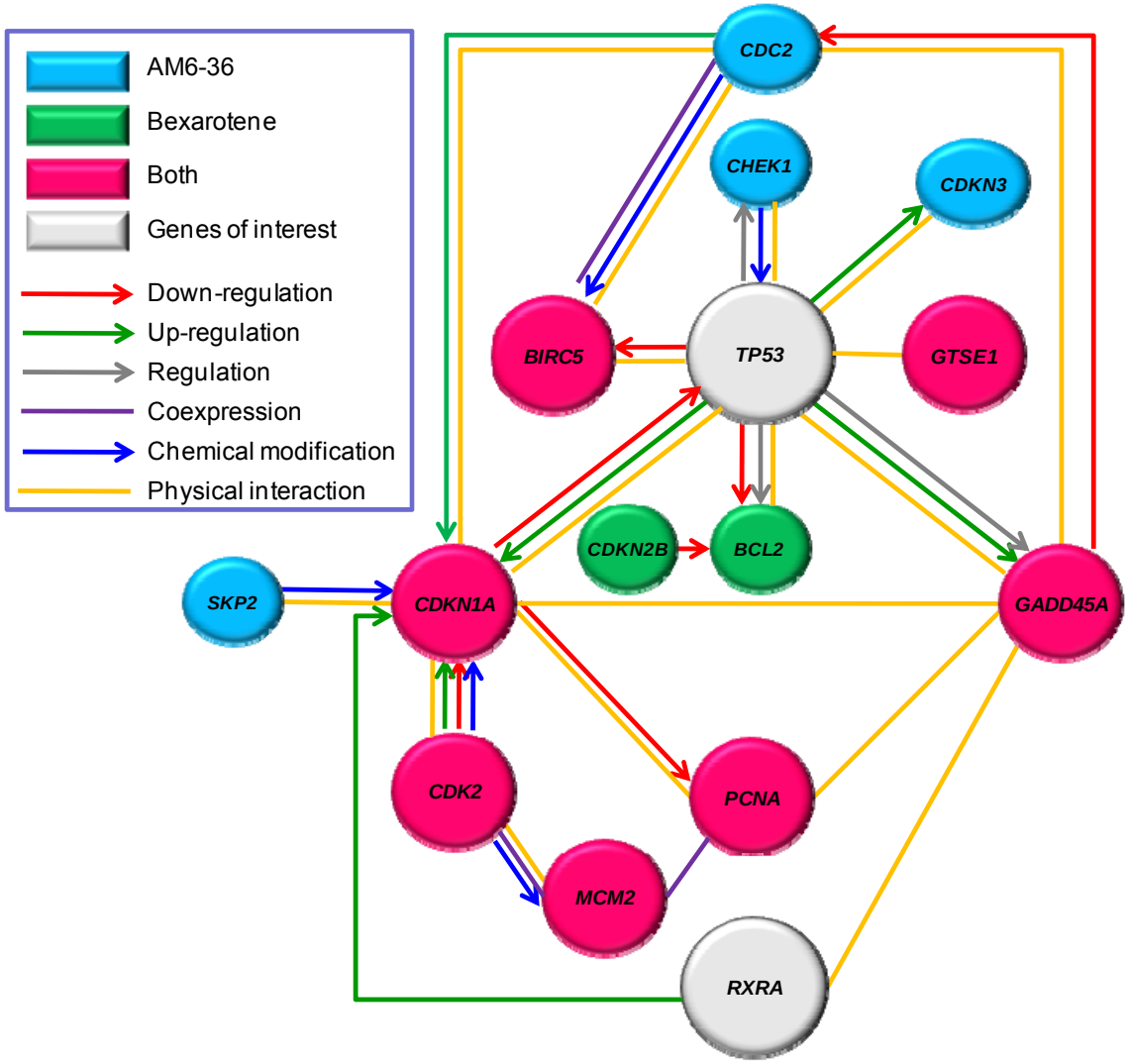
NM_001826	*CKS1B*	CDC28 protein kinase regulatory subunit 1B	CKS1/PNAS-16	0.60	0.19	0.93	0.65
NM_001827	*CKS2*	CDC28 protein kinase regulatory subunit 2	CKSHS2	1.34	0.44	0.85	0.45
NM_003592	*CUL1*	Cullin 1	MGC149834	1.44	0.46	1.25	0.75
NM_003591	*CUL2*	Cullin 2	MGC131970	1.65	0.22	0.82	0.48
NM_003590	*CUL3*	Cullin 3	Cullin-Cul3	1.18	0.84	0.99	0.80
NM_004399	*DDX11*	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11 (CHL1-like helicase homolog, *S. cerevisiae*)	CHL1/CHLR1	0.17	0.05	0.44	0.13
NM_004945	*DNM2*	Dynamin 2	CMTDI1/CMTDIB	0.81	0.44	1.08	0.88
NM_001950	*E2F4*	E2F transcription factor 4, p107/p130-binding	E2F-4	1.11	0.95	1.04	0.83
NM_001924	*GADD45A*	Growth arrest and DNA-damage-inducible, alpha	DDIT1/GADD45	12.09	0.00	13.68	0.00
NM_005316	*GTF2H1*	General transcription factor IIH, polypeptide 1, 62kDa	BTF2/TFB1	1.41	0.46	1.57	0.28
NM_016426	*GTSE1*	G-2 and S-phase expressed 1	B99	0.09	0.01	0.30	0.04
NM_016323	*HERC5*	Hect domain and RLD 5	CEB1/CEBP1	2.36	0.06	1.37	0.53
NM_004507	*HUS1*	HUS1 checkpoint homolog (*S. pombe*)	Hus1	0.46	0.14	1.18	0.85
NM_014708	*KNTC1*	Kinetochore associated 1	ROD	0.29	0.06	0.31	0.07
NM_002266	*KPNA2*	Karyopherin alpha 2 (RAG cohort 1, importin alpha 1)	IPOA1/QIP2	0.43	0.10	0.56	0.17
NM_002358	*MAD2L1*	MAD2 mitotic arrest deficient-like 1 (yeast)	HSMAD2/MAD2	0.11	0.03	0.35	0.08
NM_006341	*MAD2L2*	MAD2 mitotic arrest deficient-like 2 (yeast)	MAD2B/REV7	1.21	0.75	0.85	0.52
NM_004526	*MCM2*	Minichromosome maintenance complex component 2	BM28/CCNL1	0.15	0.04	0.20	0.05
NM_002388	*MCM3*	Minichromosome maintenance complex component 3	HCC5/P1-MCM3	0.17	0.04	0.27	0.06
NM_005914	*MCM4*	Minichromosome maintenance complex component 4	CDC21/CDC54	0.28	0.07	0.24	0.06
NM_006739	*MCM5*	Minichromosome maintenance complex component 5	CDC46/P1-CDC46	0.20	0.04	0.31	0.07
NM_002417	*MKI67*	Antigen identified by monoclonal antibody Ki-67	KIA	0.03	0.04	0.23	0.07
NM_002431	*MNAT1*	Menage a trois homolog 1, cyclin H assembly factor (*Xenopus laevis*)	MAT1/RNF66	1.05	0.85	1.05	0.91
NM_005590	*MRE11A*	MRE11 meiotic recombination 11 homolog A (*S. cerevisiae*)	ATLD/HNGS1	0.18	0.10	0.33	0.08
NM_002485	*NBN*	Nibrin	AT-V1/AT-V2	0.62	0.21	0.80	0.40
NM_182649	*PCNA*	Proliferating cell nuclear antigen	MGC8367	0.20	0.05	0.20	0.05
NM_002853	*RAD1*	RAD1 homolog (*S. pombe*)	HRAD1/REC1	2.40	0.04	0.55	0.17
NM_002873	*RAD17*	RAD17 homolog (*S. pombe*)	CCYC/HRAD17	1.64	0.26	1.55	0.33
NM_002875	*RAD51*	RAD51 homolog (RecA homolog, *E. coli*) (*S. cerevisiae*)	BRCC5/HRAD51	0.33	0.08	0.31	0.06
NM_004584	*RAD9A*	RAD9 homolog A (*S. pombe*)	RAD9	0.72	0.32	1.02	0.85
NM_000321	*RB1*	Retinoblastoma 1	OSRC/RB	0.91	0.58	0.71	0.35
NM_002894	*RBBP8*	Retinoblastoma binding protein 8	CTIP/RIM	0.38	0.08	0.96	0.72
NM_002895	*RBL1*	Retinoblastoma-like 1 (p107)	CP107/PRB1	0.12	0.04	0.27	0.08
NM_005611	*RBL2*	Retinoblastoma-like 2 (p130)	P130/Rb2	1.44	0.41	0.69	0.29
NM_002947	*RPA3*	Replication protein A3, 14kDa	REPA3	0.64	0.21	0.51	0.13
NM_013376	*SERTAD1*	SERTA domain containing 1	SEI1/TRIP-Br1	3.27	0.01	1.47	0.37
NM_005983	*SKP2*	S-phase kinase-associated protein 2 (p45)	FBL1/FBXL1	0.06	0.03	0.24	0.06
NM_003352	*SUMO1*	SMT3 suppressor of mif two 3 homolog 1 (*S. cerevisiae*)	DAP-1/GMP1	1.35	0.48	0.83	0.47
NM_007111	*TFDP1*	Transcription factor Dp-1	DP1/DRTF1	0.44	0.12	0.44	0.12
NM_006286	*TFDP2*	Transcription factor Dp-2 (E2F dimerization partner 2)	DP2/Dp-2	0.94	0.70	1.14	0.91
NM_000546	*TP53*	Tumor protein p53	LFS1/TRP53	0.83	0.45	1.37	0.60
NM_003334	*UBA1*	Ubiquitin-like modifier activating enzyme 1	A1S9/A1S9T	1.57	0.30	0.95	0.69

Supplementary Figure legends

Supplementary Figure S1. Web of genes affected by bexarotene or AM6-36 in this array are shown using database supported by SAbiosciences (derived with some modification from http://www.sabiosciences.com/genenetwork/). Data are shown for $p \leq 0.05$. Blue circles: genes altered by AM6-36; green circles: genes altered by bexarotene; red circles: genes altered by both AM6-36 and bexarotene; empty circles: genes of interest). With the exception of *CDKN2B, and CDKN3* every gene shown in the chart is altered by both AM6-36 and bexarotene at $p \leq 0.083$.

Supplementary Figure S2. Effect of AM6-36 on DNA binding activity of RXR in MCF7 cells. MCF7 cells (10×10^4 cells/ml) were incubated in 6 cm dishes for 24 h, and then treated samples as indicated for an additional 24 h. Cells were lysed, nuclear extracts were prepared, and used in the EMSA (5 µg each). DNA-protein complexes were separated on 5% polyacrylamide gel.

Park, Supplementary Figure S1.



Park, Supplementary Figure S2.