Supporting Information

Hop (*Humulus lupulus L.*) extract and 6-prenylnaringenin induce P450 1A1 catalyzed estrogen 2-hydroxylation

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Table S1. Inhibition of recombinant P450 1A1/1B1 enzymes with hop compounds.[a]

	apparent Ki (μM)			
	6-PN	**8-PN**	**IX**	**XH**
P450 1A1	0.59 ± 0.10	0.20 ± 0.06	1.6 ± 0.53	0.11 ± 0.04
P450 1B1	0.23 ± 0.02	0.17 ± 0.04	0.54 ± 0.11	0.17 ± 0.02

[a]The apparent Ki (Ki') values are expressed as means ± SD from three independent dose-responsive curves using recombinant P450 1A1 and P450 1B1 enzymes.

Figure Legends

Figure S1. Hop compounds partially contribute to the activity of hops extract for up-regulation of P450 1A1/1B1 activity. MCF-7 cells were treated with hops extract (2.5 μg/ml) as well as the four marker hop compounds at the equivalent amount to those present in the extract, individually and as a mixture (6-PN 0.084 μM, 8-PN 0.024 μM, IX 0.070 μM, and XH 2.3 μM) for 48 h. Cells were then measured for EROD activity as previously described and the results were analyzed by one-way ANOVA for comparison of treatment groups with control, * $p < 0.05$.

Figure S2. Hop compounds inhibited human recombinant P450 1A1 and 1B1 activity. Human recombinant P450 1A1 and P450 1B1 protein with reductase were incubated with 7-ethoxyresorufin, NADPH and different doses of A) XH, B) IX, C) 8-PN and D) 6-PN at 37°C for 20 min. EROD activity was measured and data were plotted as percentage of activity against vehicle control.

Figure S3. Hop compounds moderately inhibited CYP1 activity in cells. MCF-7 cells were pretreated with TCDD (10 nM) for 48 h and pre-incubated with 6-PN/8-PN/IX/XH (0.1 μM, 1 μM, and 10 μM) for 5 min at 37°C before ethoxyresorufin and NADPH was added for 20 min. EROD activity was measured and results were analyzed by one-way ANOVA with Dunnett's multiple comparison post-test, * $p < 0.05$.

Figure S4. COMT mRNA expression was not affected by hop compounds in MCF-10A cells. COMT mRNA expression were analyzed after 24 h via qPCR in MCF-10A cells treated with DMSO control, 6-PN, 8-PN, IX, XH (1 μM), and TCDD (10 nM). Results are the means ± SEM of three independent experiments.

Figure S5. 6-PN dose-responsively and preferentially increased estrogen 2-hydroxylation in MCF-10A cells. MCF-10A cells were treated with the hop marker

compounds in the presence of E_2 (1 μM) for 2 days and media were analyzed for 2-$MeOE_1$ and 4-$MeOE_1$ metabolites. Results were normalized to fold induction against estradiol treated cells. Data were plotted as the means ± SEM and analyzed by one-way ANOVA with Dunnett's multiple comparison post-test to compare treatment groups with control group, *$p < 0.05$.

Figure S6. MCF-7 cells had significant higher response than MCF-10A cells to TCDD induced EROD activity. MCF-10A and MCF-7 cells were treated with TCDD (10 nM) for 48 h. Cells were then measured for EROD activity as previously described.

Figure S7. HepG2 cells had significantly higher response than MCF-7 cells to TCDD induced XRE-luciferase activity. MCF-7 cells and HepG2 cells were treated with TCDD (10 nM) after transfection for 24 h. Cells were then lysed and measured for luciferase as previously described.

Figure S8. TCDD induced EROD activity was highest after 2 days of treatment. MCF-7 cells were treated with TCDD (10 nM) for 1 day, 2 day and 3 days. Cells were then measured for EROD activity as previously described.

Figures

Figure S1

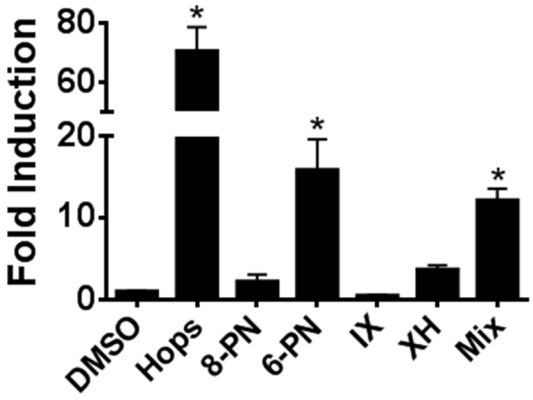

Figure S2

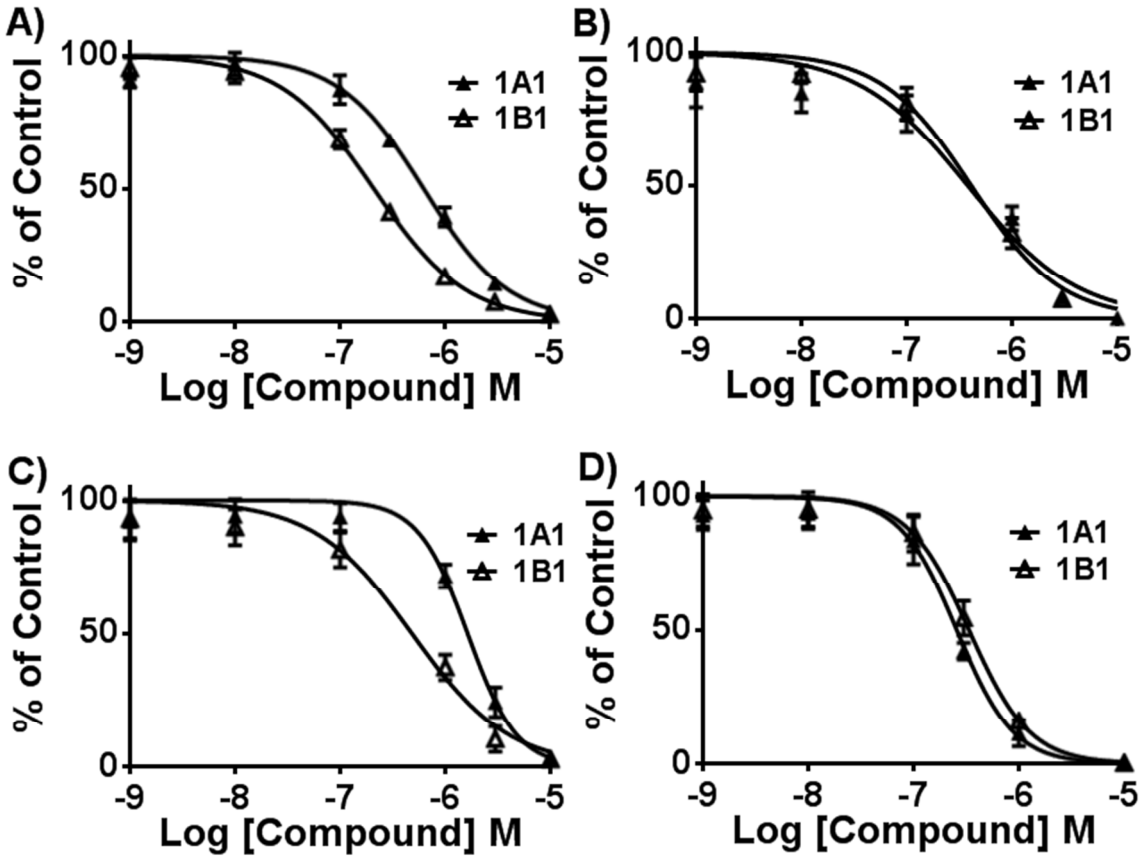

Figure S3

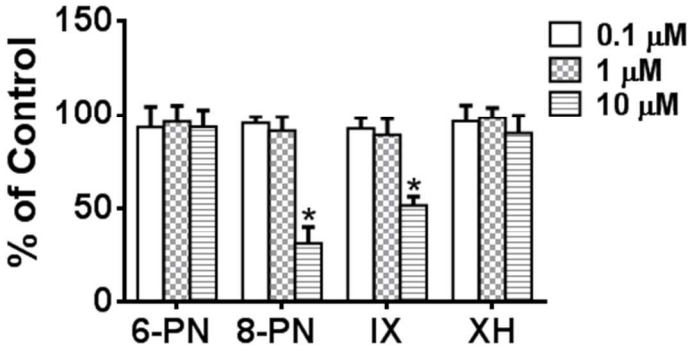

Figure S4

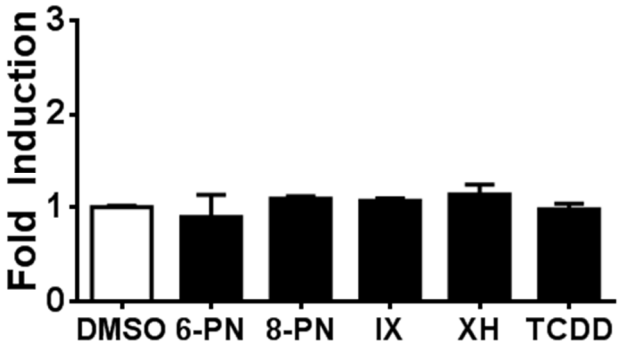

Figure S5

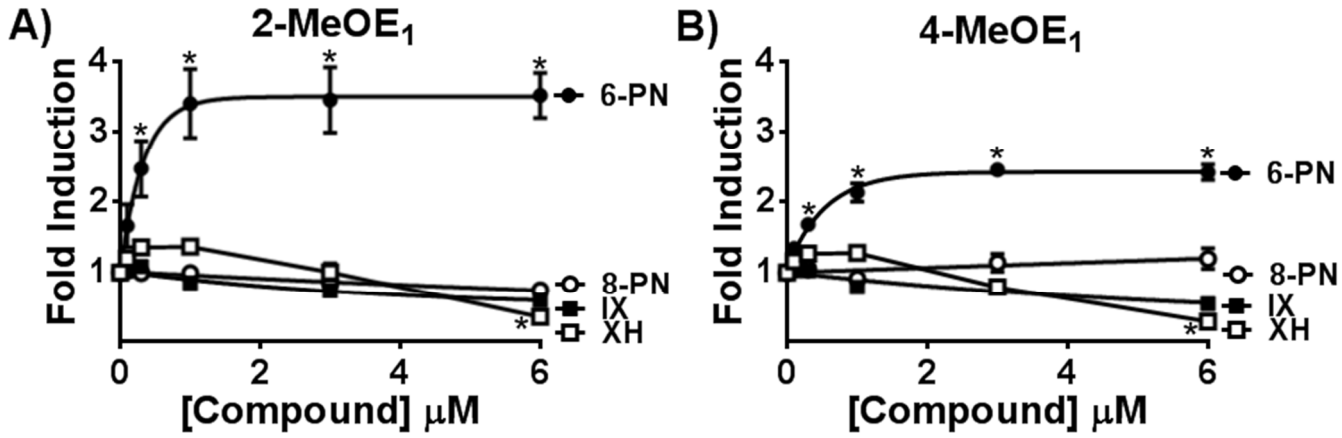

Figure S6

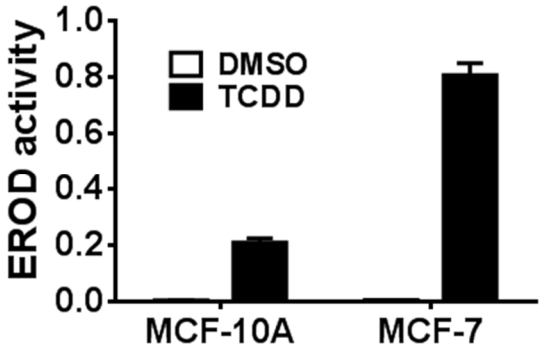

Figure S7

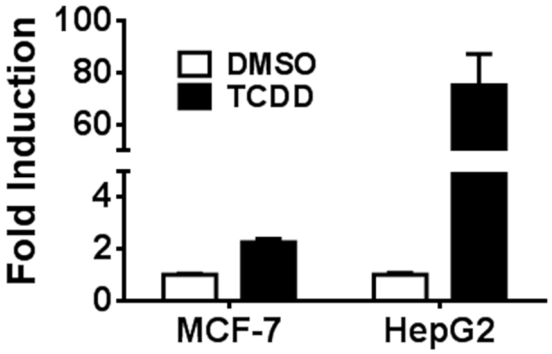

Figure S8