

SUPPLEMENTARY DATA

Comprehensive assessment of NR ligand polypharmacology by a multiplex reporter NR assay.

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#	NR	Nomengl.	Inducer	GAL4-NR Activity (fold)	Assay
1	AR	NR3C4	GAL4-AR	0.87 ± 0.04	one-hybrid assay
			6a-Fluorotestosterone	77.45 ± 11.4	agonist
2	CAR	NR1I3	GAL4-CAR	193.07 ± 7.6	one-hybrid assay
			PK 11195	0.11 ± 0.01	antagonist
3	COUP-TFI	NR2F1	GAL4-COUP-TFI	0.41 ± 0.01	one-hybrid assay
			RIP140-VP16	109 ± 3	two hybrid assay
4	COUP-TFII	NR2F2	GAL4-COUP-TFII	0.39 ± 0.01	one-hybrid assay
			RIP140-VP16	58.3 ± 2.0	two hybrid assay
5	DAX-1	NR0B1	GAL4-DAX-1	0.3 ± 0.01	one-hybrid assay
			RIP140-VP16	8.6 ± 0.2	two hybrid assay
6	EAR2	NR2F6	GAL4-EAR2	0.3 ± 0.02	one-hybrid assay
			RIP140-VP16	161 ± 9	two hybrid assay
7	ERR α	NR3B1	GAL4-ERR α	5.02 ± 0.27	one-hybrid assay
			XCT790	0.15 ± 0.01	antagonist
8	ERR β	NR3B2	GAL4-ERR β	21.55 ± 0.36	one-hybrid assay
			DY131	2.6 ± 0.1	agonist
9	ERR γ	NR3B3	GAL4-ERR γ	33.21 ± 0.61	one-hybrid assay
			4-Hydroxytamoxifen	0.21 ± 0.01	antagonist
10	ER α	NR3A1	GAL4-ER α	0.47 ± 0.03	one-hybrid assay
			Estradiol	142.18 ± 14.66	agonist
			4-Hydroxytamoxifen	0.21 ± 0.01	antagonist
11	ER β	NR3A2	GAL4-ER β	0.51 ± 0.02	one-hybrid assay
			Estradiol	49.61 ± 0.79	agonist
12	FXR	NR1H4	GAL4-FXR	0.8 ± 0.05	one-hybrid assay
			CDCA	176.51 ± 9.2	agonist
13	GCNF	NR6A1	GAL4-GCNF	0.21 ± 0.01	one-hybrid assay
			SMRT-VP16	16.9 ± 1.8	two hybrid assay
14	GR	NR3C1	GAL4-GR	1.57 ± 0.05	one-hybrid assay
			Dexamethasone	67.58 ± 0.55	agonist
15	HNF4 α	NR2A1	GAL4-HNF4 α	14.1 ± 0.86	one-hybrid assay
			RIP140-VP16	31.2 ± 3.6	two hybrid assay
16	HNF4 γ	NR2A2	GAL4-HNF4 γ	6.8 ± 0.49	one-hybrid assay
			RIP140-VP16	18.1 ± 2.1	two hybrid assay
17	LRH-1	NR5A2	GAL4-LRH-1	3.45 ± 0.59	one-hybrid assay
			RIP140-VP16	43.9 ± 8.1	two hybrid assay
18	LXR α	NR1H3	GAL4-LXR α	2.38 ± 0.1	one-hybrid assay
			T0901317	112.32 ± 2.78	agonist
19	LXR β	NR1H2	GAL4-LXR β	0.92 ± 0.06	one-hybrid assay
			T0901317	313.93 ± 7.09	agonist
20	MR	NR3C2	GAL4-MR	1.44 ± 0.04	one-hybrid assay
			Aldosterone	12.27 ± 0.36	agonist
21	NOR1	NR4A3	GAL4-NOR1	36.92 ± 0.69	one-hybrid assay
			RIP140-VP16	1.93 ± 0.08	two hybrid assay
22	NUR77	NR4A1	GAL4-NUR77	1.6 ± 0.02	one-hybrid assay
			9-cis-Retinoic acid	9.29 ± 0.11	agonist
23	NURR1	NR4A2	GAL4-NURR1	105.64 ± 4.41	one-hybrid assay
			9-cis-Retinoic acid	54.97 ± 0.75	agonist
24	PNR	NR2E3	GAL4-PNR	0.34 ± 0.01	one-hybrid assay
			RIP140-VP16	3.1 ± 0.2	two hybrid assay

Table S1

25	PPARα	NR1C1	GAL4-PPAR α GW-7647	6.02 $\pm$ 0.44 21.11 $\pm$ 1.2	one-hybrid assay agonist
26	PPARγ	NR1C3	GAL4-PPAR γ Rosiglitazone	5.29 $\pm$ 0.19 43.49 $\pm$ 0.76	one-hybrid assay agonist
27	PPARδ	NR1C2	GAL4-PPAR δ GW-0742	0.2 $\pm$ 0.01 37.2 $\pm$ 0.73	one-hybrid assay agonist
28	PR	NR3C3	GAL4-PR Progesterone	1.04 $\pm$ 0.04 14.78 $\pm$ 0.72	one-hybrid assay agonist
29	PXR	NR1I2	GAL4-PXR Rifampicin	0.33 $\pm$ 0.01 43.9 $\pm$ 1.74	one-hybrid assay agonist
30	RARα	NR1B1	GAL4-RAR α Retinoic acid	42.05 $\pm$ 0.19 9.02 $\pm$ 0.19	one-hybrid assay agonist
31	RARβ	NR1B2	GAL4-RAR β Retinoic acid	45.24 $\pm$ 1.21 4.92 $\pm$ 0.04	one-hybrid assay agonist
32	RARγ	NR1B3	GAL4-RAR γ Retinoic acid	6.77 $\pm$ 0.3 12.61 $\pm$ 0.49	one-hybrid assay agonist
33	Rev-erbα	NR1D1	GAL4-Rev-erb α SMRT-VP16	0.67 $\pm$ 0.01 703 $\pm$ 85	one-hybrid assay two hybrid assay
34	Rev-erbβ	NR1D2	GAL4-Rev-erb β NCOR-VP16	0.7 $\pm$ 0.02 65 $\pm$ 6	one-hybrid assay two hybrid assay
35	RORα	NR1F1	GAL4-ROR α RIP140-VP16	79.03 $\pm$ 6.77 1.11 $\pm$ 0.12	one-hybrid assay two hybrid assay
36	RORβ	NR1F2	GAL4-ROR β RIP140-VP16	375.5 $\pm$ 1.33 1.37 $\pm$ 0.17	one-hybrid assay two hybrid assay
37	RORγ	NR1F3	GAL4-ROR γ GSK805	238.07 $\pm$ 10.34 0.22 $\pm$ 0.01	one-hybrid assay antagonist
38	RXRα	NR2B1	GAL4-RXR α 9-cis-Retinoic acid	0.54 $\pm$ 0.02 32.22 $\pm$ 1.27	one-hybrid assay agonist
39	RXRβ	NR2B2	GAL4-RXR β 9-cis-Retinoic acid	0.89 $\pm$ 0.02 28.43 $\pm$ 2.82	one-hybrid assay agonist
40	RXRγ	NR2B3	GAL4-RXR γ 9-cis-Retinoic acid	0.79 $\pm$ 0.02 21.84 $\pm$ 1.33	one-hybrid assay agonist
41	SF1	NR5A1	GAL4-SF1 RIP140-VP16	98.49 $\pm$ 9.45 2.5 $\pm$ 0.05	one-hybrid assay two hybrid assay
42	SHP	NR0B2	GAL4-SHP N/A	0.31 $\pm$ 0.01 n/a	one-hybrid assay RNA expression
43	TLX	NR2E2	GAL4-TLX RIP140-VP16	0.23 $\pm$ 0.01 8.9 $\pm$ 0.7	one-hybrid assay two hybrid assay
44	TR2	NR2C1	GAL4-TR2 RIP140-VP16	1.19 $\pm$ 0.02 3.2 $\pm$ 0.2	one-hybrid assay two hybrid assay
45	TR4	NR2C2	GAL4-TR4 RIP140-VP16	1.76 $\pm$ 0.12 2.9 $\pm$ 0.06	one-hybrid assay two hybrid assay
46	THRα	NR1A1	GAL4-THR α Thyroid hormone T-3	0.97 $\pm$ 0.13 179.14 $\pm$ 7.84	one-hybrid assay agonist
47	THRβ	NR1A2	GAL4-THR β Thyroid hormone T-3	0.19 $\pm$ 0.02 218.58 $\pm$ 5.06	one-hybrid assay agonist
48	VDR	NR1I1	GAL4-VDR 1,25-dihydroxyvitamin D3	0.78 $\pm$ 0.03 606.99 $\pm$ 22.31	one-hybrid assay agonist

Table S1 cont'd

Table S1. The basal activity and responsiveness of GAL4-NR vectors. The individual constructs were assessed in the SEAP reporter gene assay. The GAL4-NR vectors were cotransfected with the GAL4 SEAP reporter plasmid and the SEAP activity was assessed in cell growth medium. The data show the activity of GAL4-NR vs. that of the GAL4 expression vector in unstimulated cells or in the presence of saturating concentrations of indicated NR agonists or antagonists. For NRs with unknown ligands, we used a two-hybrid assay, co-transfecting the GAL4-NR vectors with indicated corepressor (SMRT-VP16, NCOR-VP16) or coactivator (RIP140-VP16) plasmids. The maximal fold change activity values are shown. The data are average values $\pm$ SD of at least three independent assays.

The two-hybrid SEAP assay

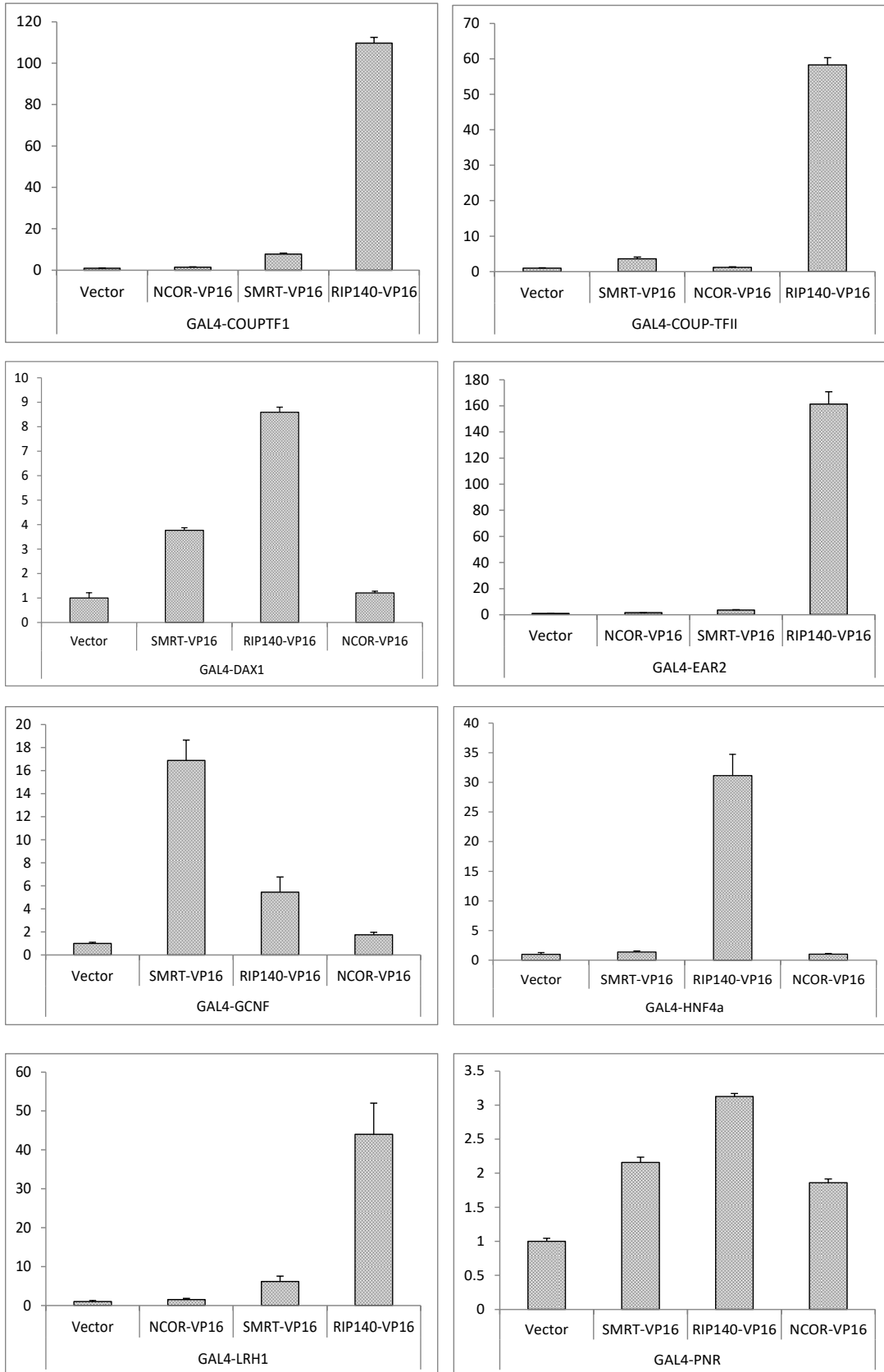


Figure S1

The two-hybrid SEAP assay

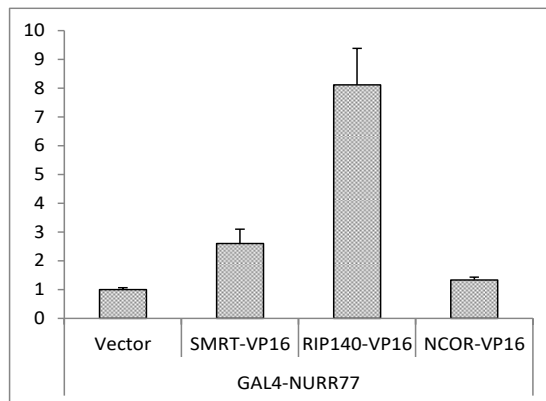
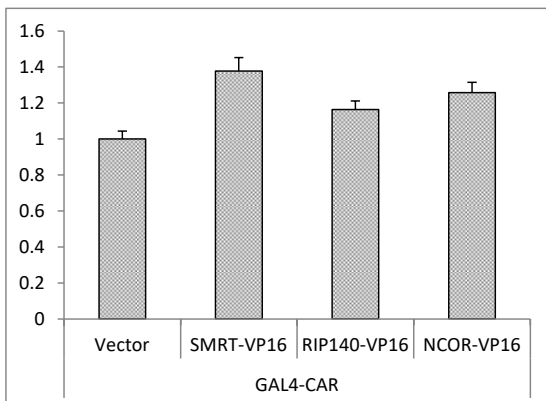
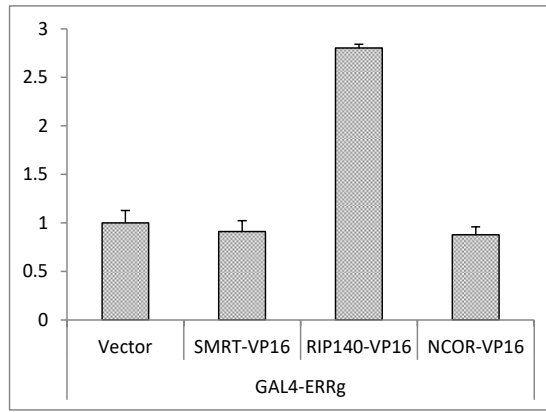
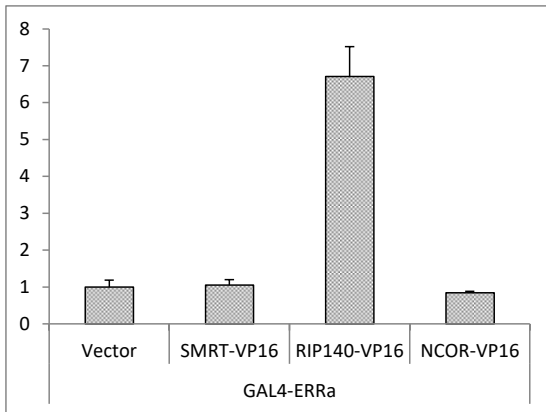
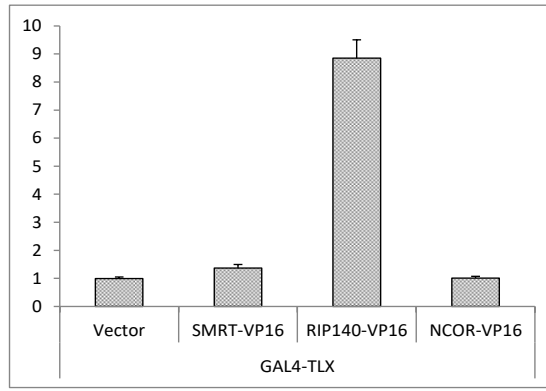
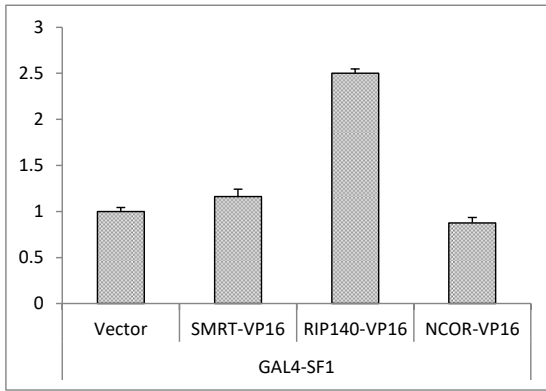
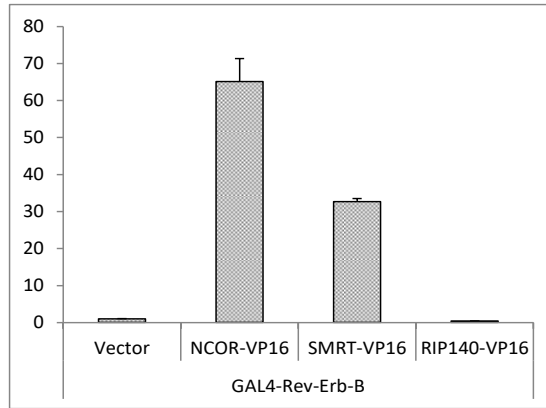
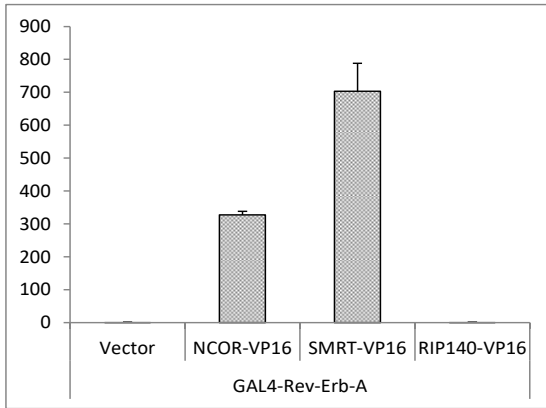


Figure S1 cont'd.

The two-hybrid SEAP assay

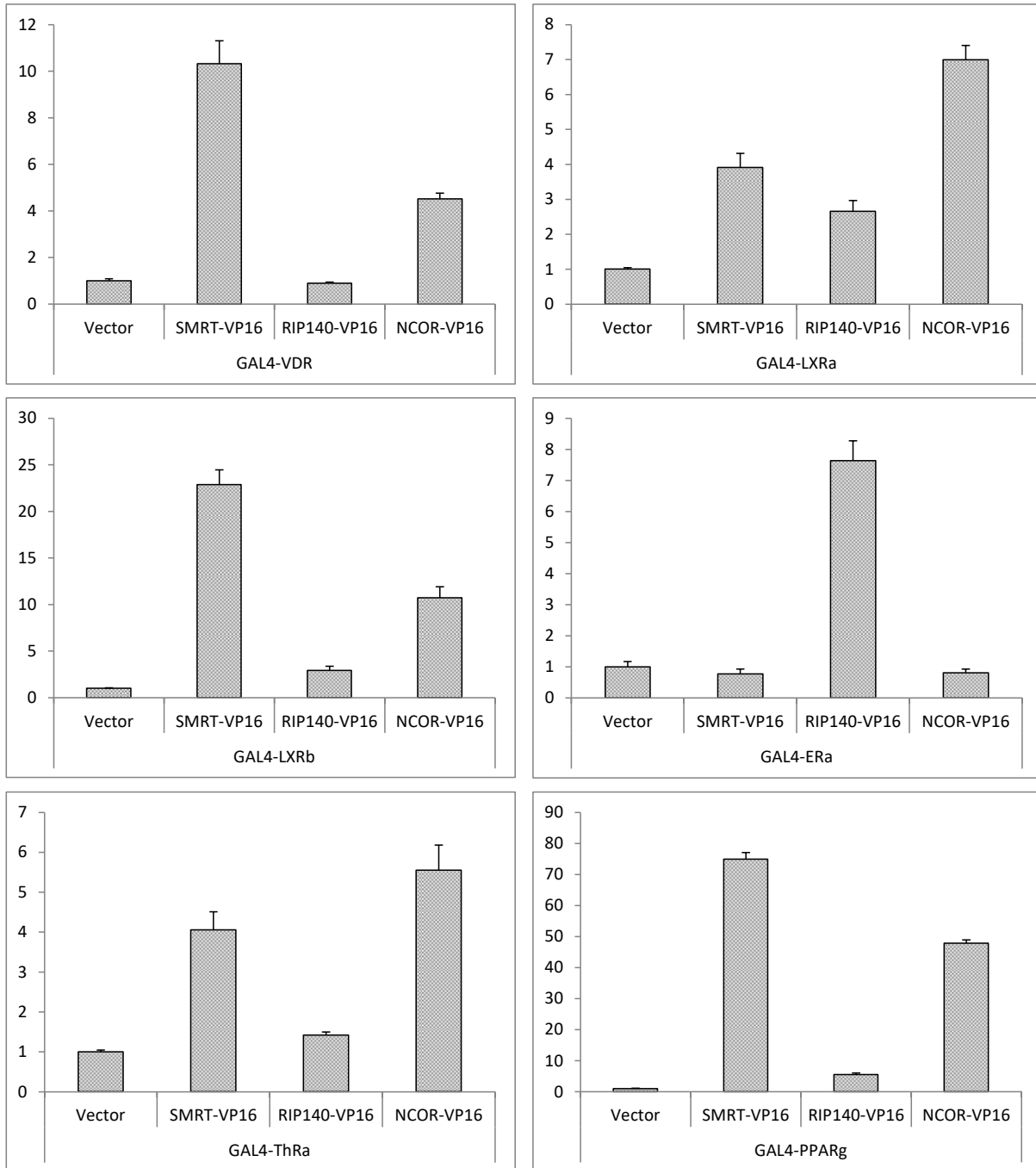


Figure S1. Assessing the functionality of the FACTORIAL NR GAL4-NR proteins by two-hybrid SEAP reporter gene assay. The GAL4-SEAP reporter was co-transfected with expression vectors for tested GAL4-NRs and the indicated NR co-factors (SMRT-VP16, NCOR-VP16, RIP140-VP16, or the VP16 (vector)). The SEAP induction was normalized to that in VP16-expressing cells. Average values of three independent SEAP assays $\pm$ SD are shown.

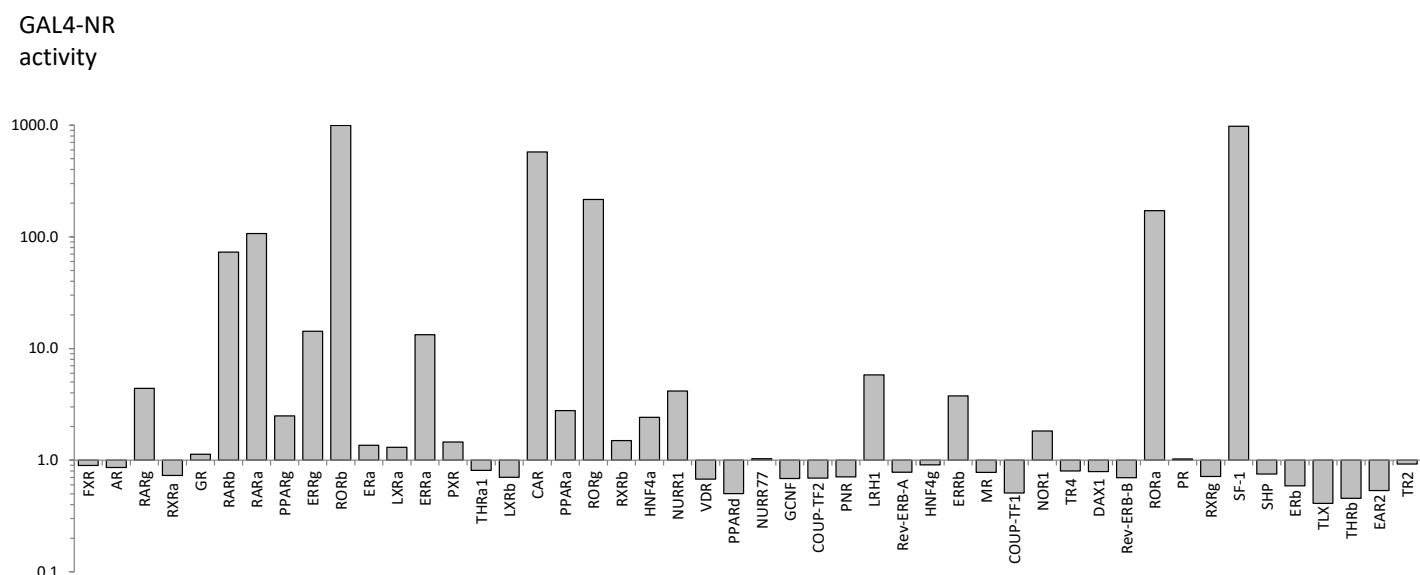
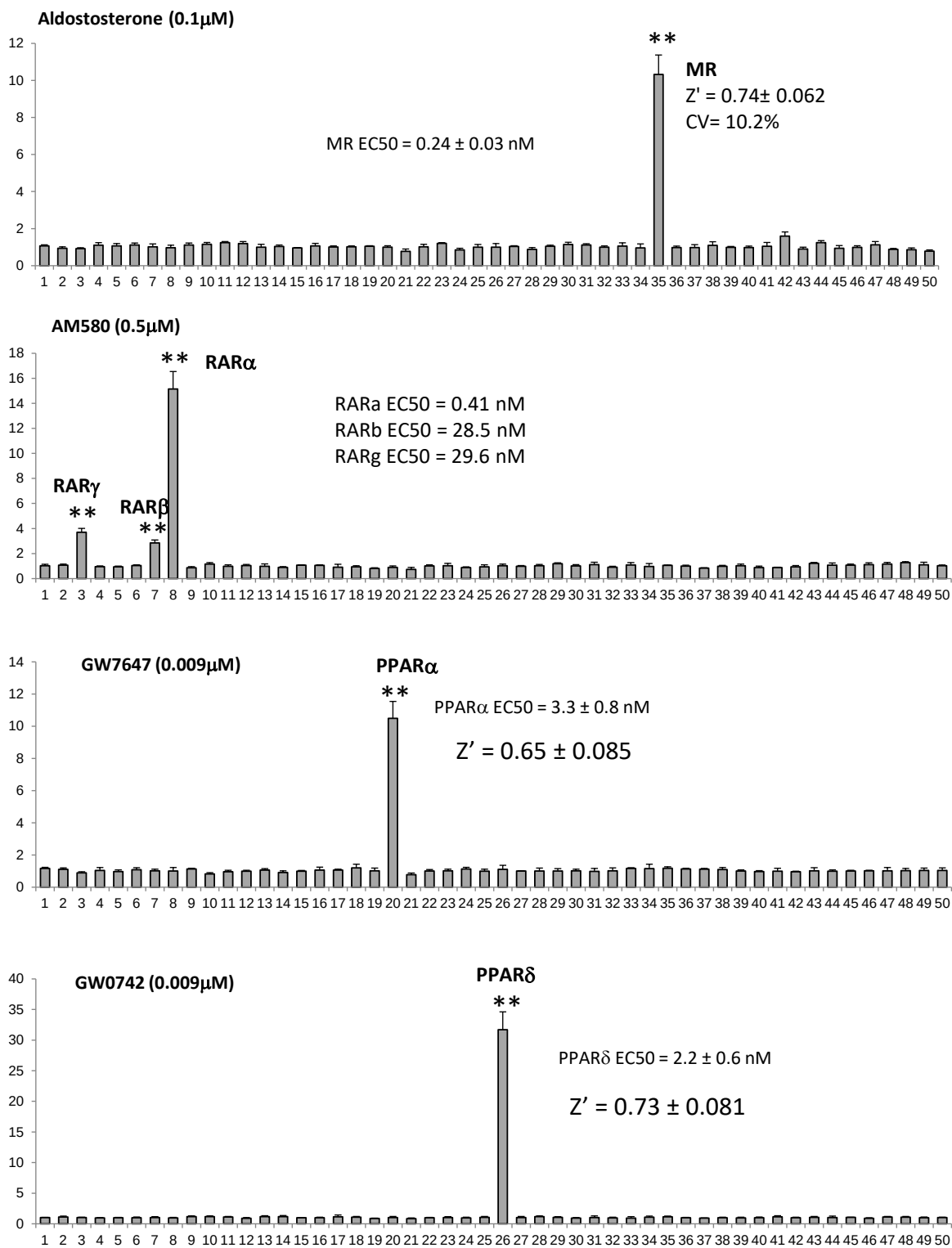


Figure S2. The basal NR activity profile of the FACTORIAL NR assay in HepG2 cells. The NR activity profile in unstimulated HepG2 cells was obtained according to FACTORIAL detection protocol (see Methods). The profile was calculated by dividing the activity of GAL4-NR modules by that of the GAL4 module. The NR activities are shown on a log scale. An average profile of three independent FACTORIAL NR assays is shown.



#	NR LBD
1	FXR
2	AR
3	RAR γ
4	GAL4
5	RXR α
6	GR
7	RAR β
8	RAR α
9	PPAR γ
10	ERR α
11	ROR β
12	ER α
13	LXR α
14	ERR α
15	TATA
16	PXR
17	THR α
18	LXR β
19	CAR
20	PPAR α
21	ROR α
22	RXR β
23	HNF4 α
24	NURR1
25	VDR
26	PPAR δ
27	NUR77
28	GCNF
29	COUP-TFII
30	PNR
31	LRH1
32	Rev-Erb α
33	HNF4
34	ERR β
35	MR
36	COUP-TFI
37	NOR1
38	TR4
39	DAX1
40	Rev-Erb β
41	ROR α
42	PR
43	RXR γ
44	SF-1
45	SHF
46	ER β
47	TLX
48	THR β
49	EAR2
50	TR2

Fig. S3. The NR activity profiles of physiological and synthetic NR ligands. The NR activity profiles were obtained as described by figure legends of Fig. 1. Significant NR responses are marked (**P<0.01; *P<0.05).

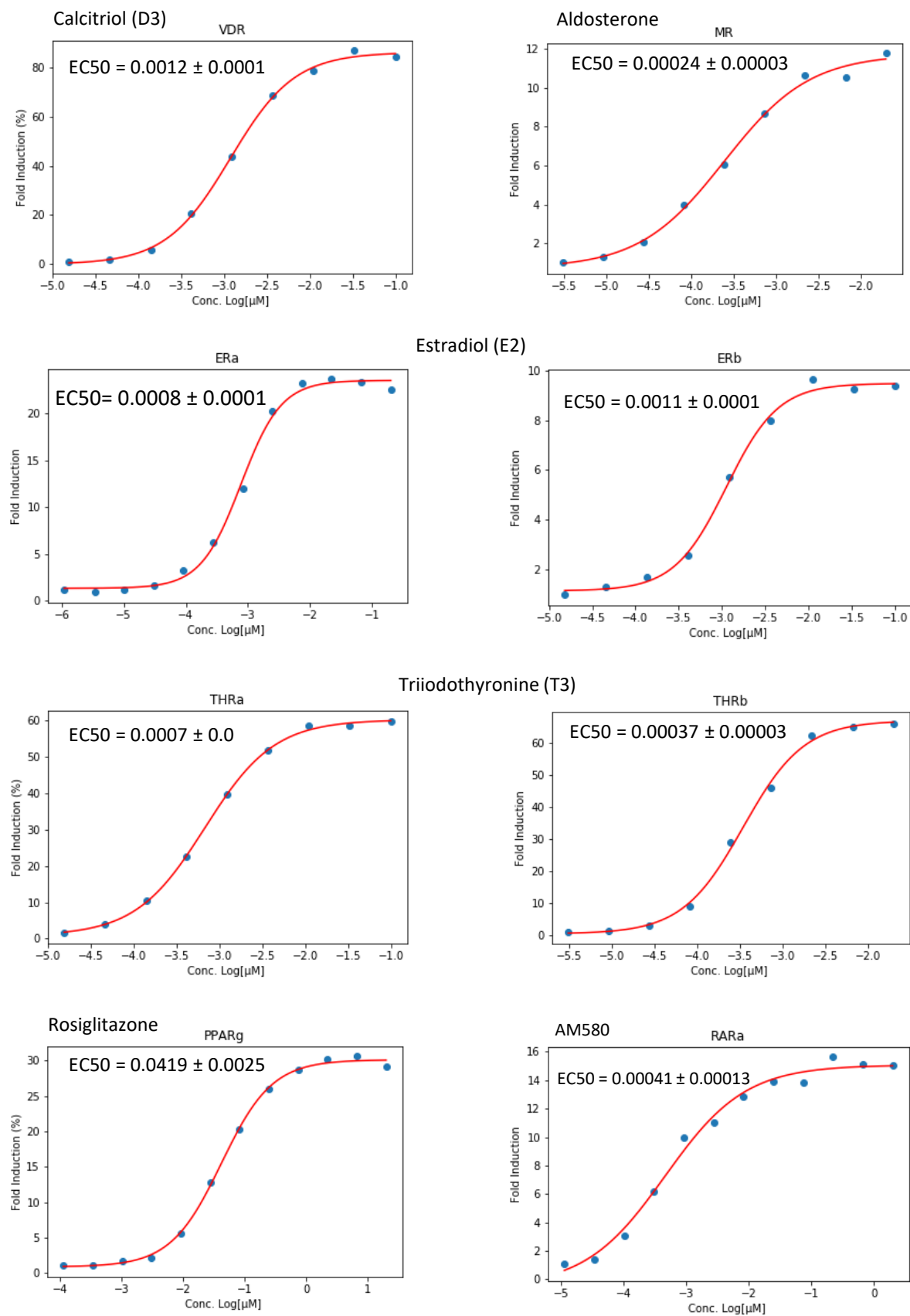


Figure S4

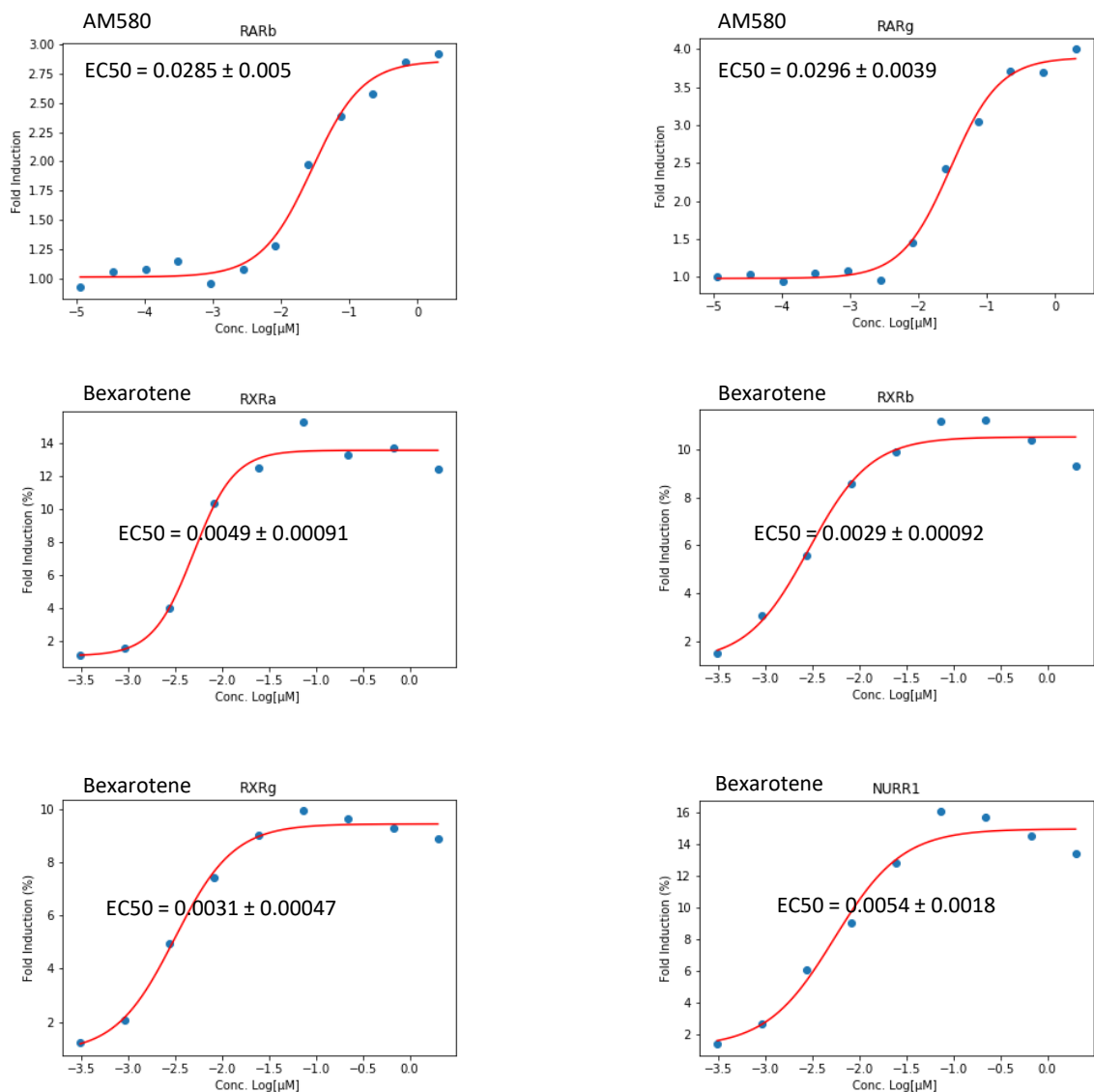
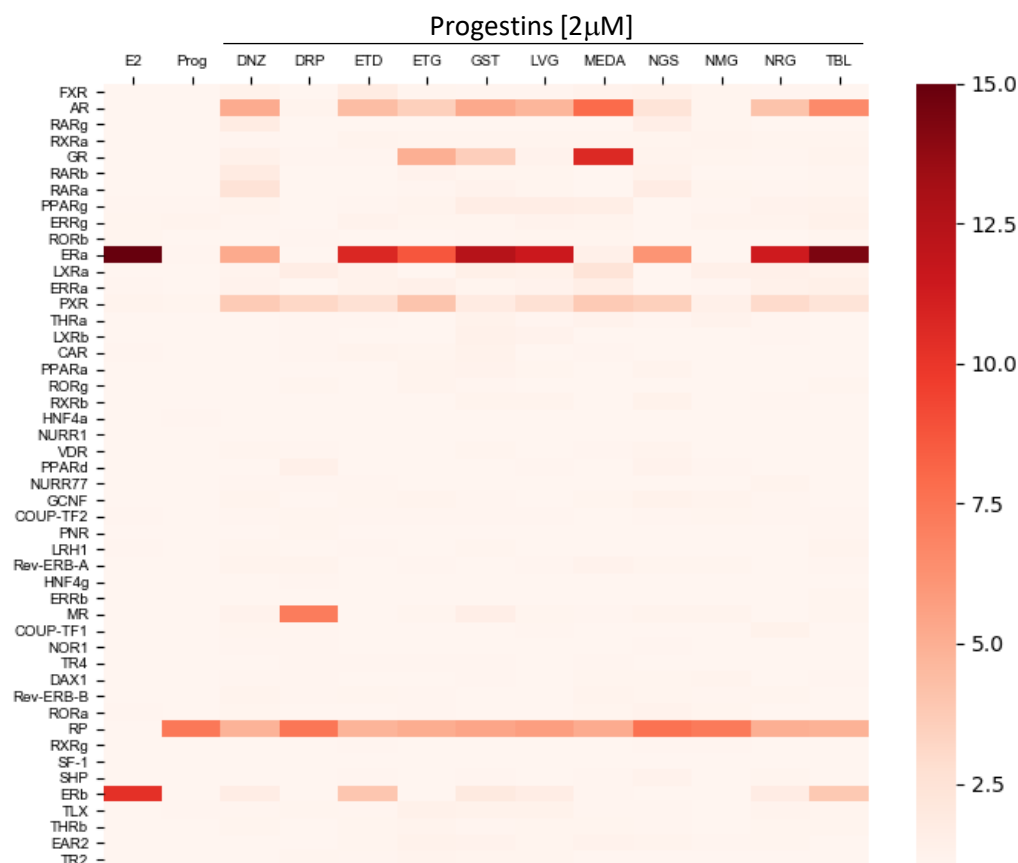


Fig. S4. Assessing the EC_{50} values by the FACTORIAL NR assay. Concentration-responses of FACTORIAL NR endpoints were assessed as described by the figure legend of Fig. 3. Average data of three independent FACTORIAL NR assays are shown.

A



B

Relative Emax vs. hormones

	AR	ER α	MR	PR	ER β
DNZ	65.31	54.81		68.33	19.06
DRP	19.43		80.44	132.80	
ETD	42.13	74.65	13.67	124.03	73.61
ETG	55.41	56.68		94.26	14.70
GST	79.92	84.22		129.75	40.34
LVG	60.71	77.75	16.01	134.60	31.16
MEDA	97.24	10.03		95.82	23.82
NGS	29.07	66.39		104.44	
NMG				118.81	
NRG	54.57	67.53	13.11	118.41	35.11
TBL	75.77	99.99		107.61	78.93

Legend:

E2 - Estradiol
 Prog - Progesterone
 DNZ - Danazol
 DRP - Drospirenone
 ETD - Ethynodiol diacetate
 ETG - Etonogestrel
 GST - Gestodene
 LVG - Levonorgestrel
 MEDA - Medroxyprogesterone Acetate
 NGS - Norgestimate
 NMG - Nomegestrol
 NRG - Norgestrel
 TBL - Tibolone

Figure S5A,B

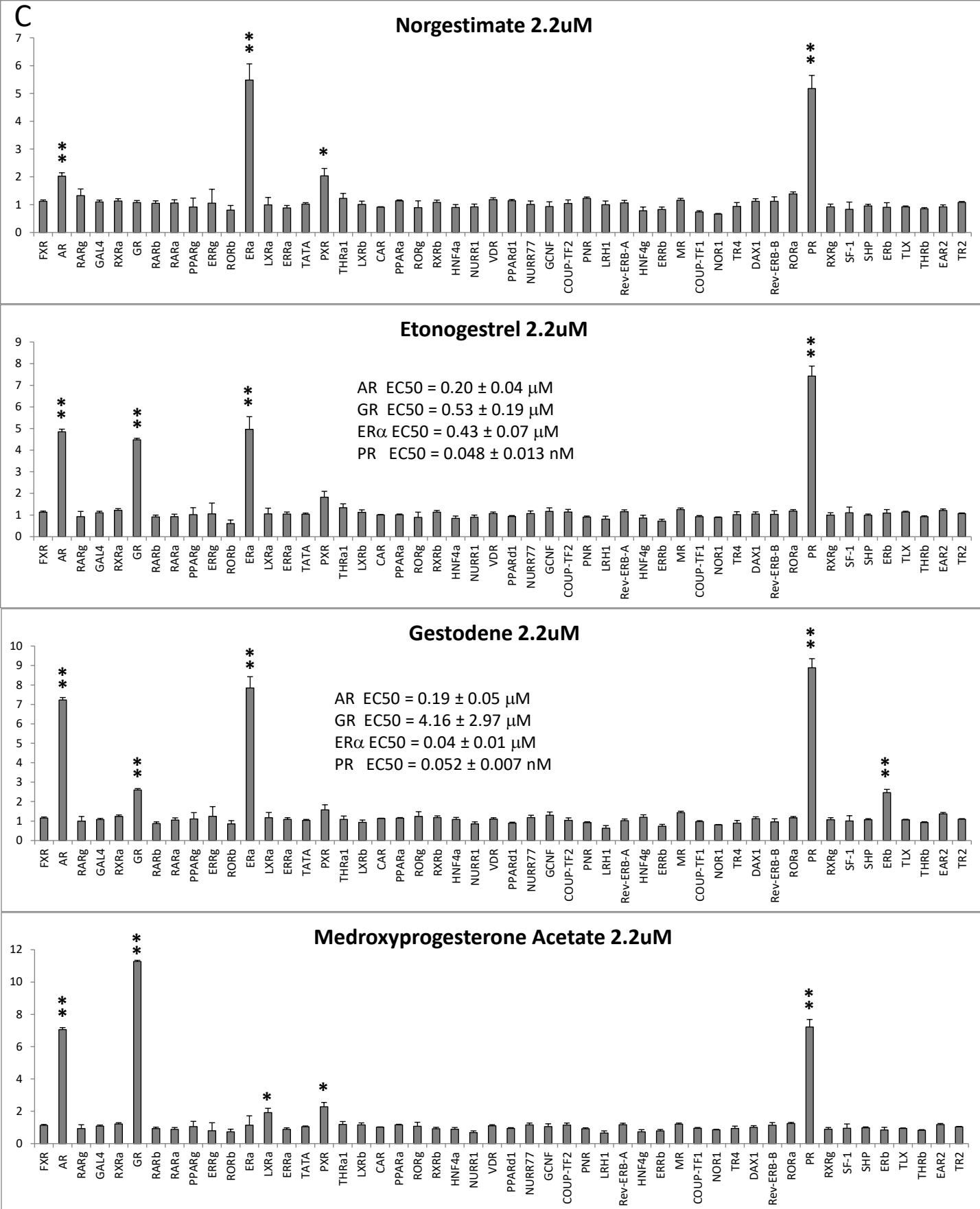


Figure S5C

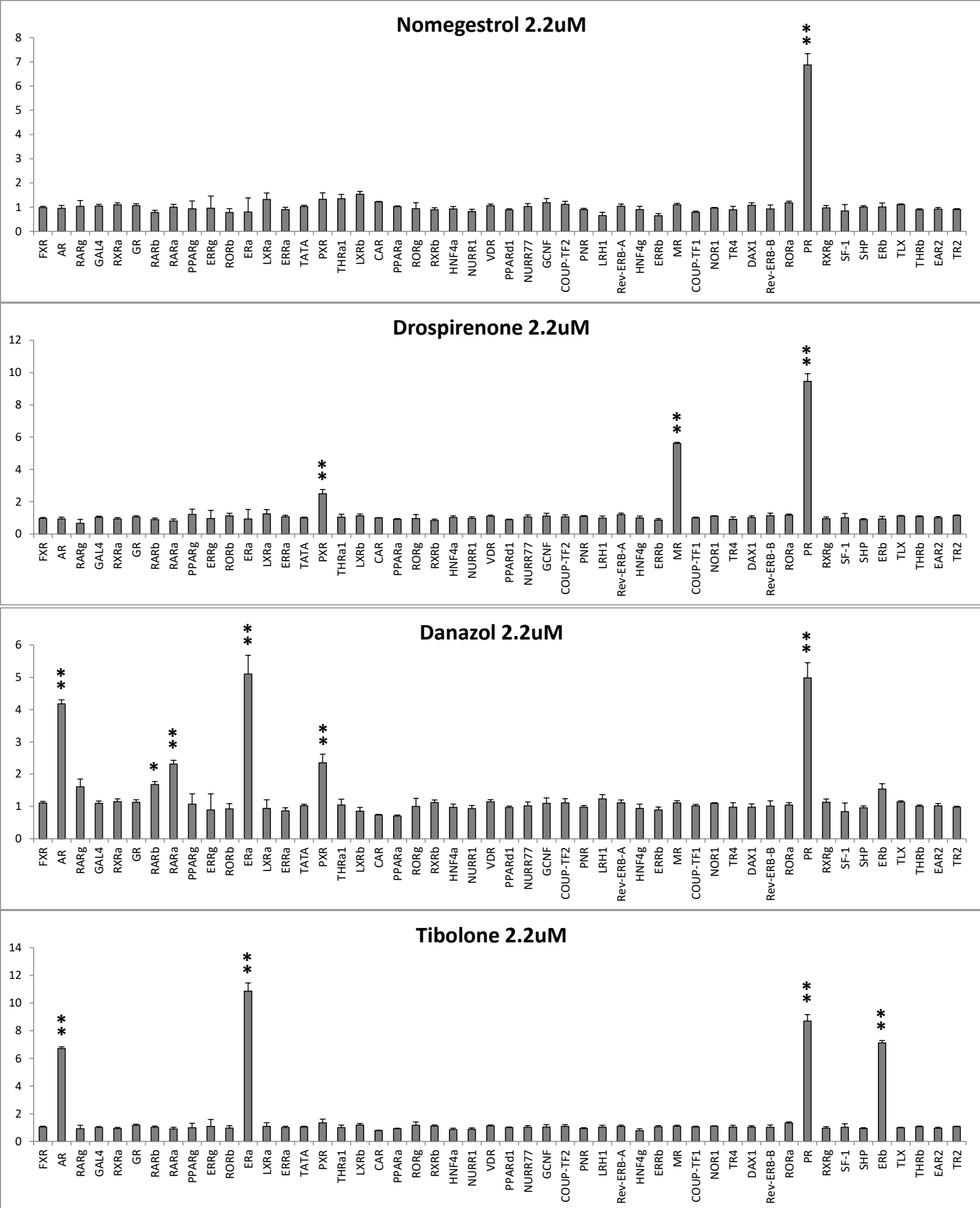


Figure S5C cont'd

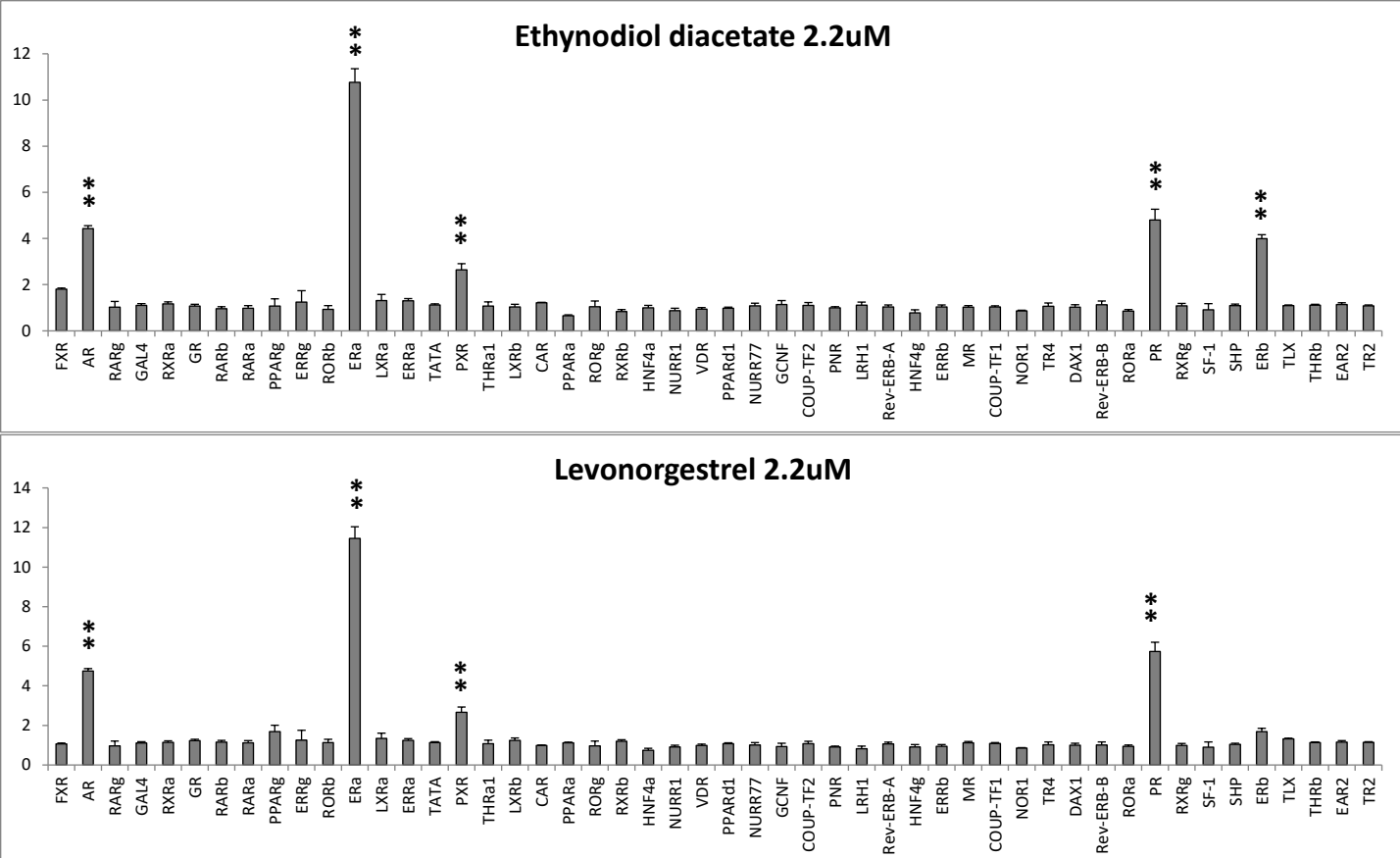


Fig. S5. The NR activity profiles of progestins. The NR activity profiles for progestins show the GAL4-NR activity in stimulated cells normalized to that in vehicle-treated cells. **A.** The heatmap shows NR responses to progestins at 2.2μM (see figure legend for Fig. 5). **B.** Table summarizes relative Emax activity of synthetic progestins at 20μM (or lower) as percentage of corresponding native hormones (testosterone (AR), estradiol (ERα, ERβ), progesterone (PR) and aldosterone (MR)). **C.** The NR activity profiles are shown as the bar graphs at 2.2μM concentration with the error bars showing standard deviation. The profiles are average data of three independent FACTORIAL NR assays. Significant NR responses are marked (**P<0.01; *P<0.05).

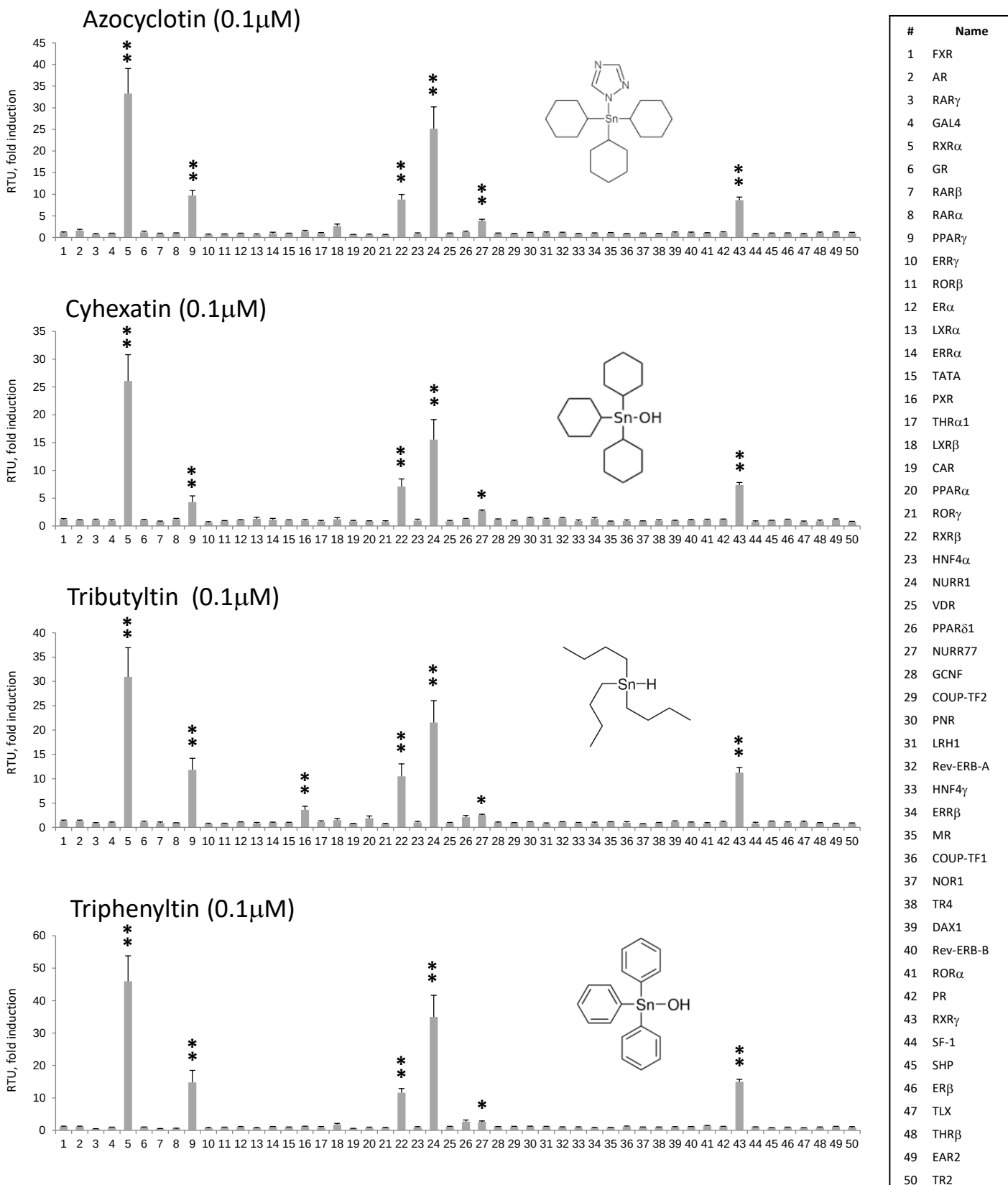


Fig. S6. The NR activity profiles of organotins. The NR activity profiles show NR responses in stimulated cells normalized to those in vehicle-treated cells. The bar graphs show average data of three independent FACTORIAL NR assays $\pm$ SD values. Significant NR responses are marked (**P<0.01; *P<0.05).

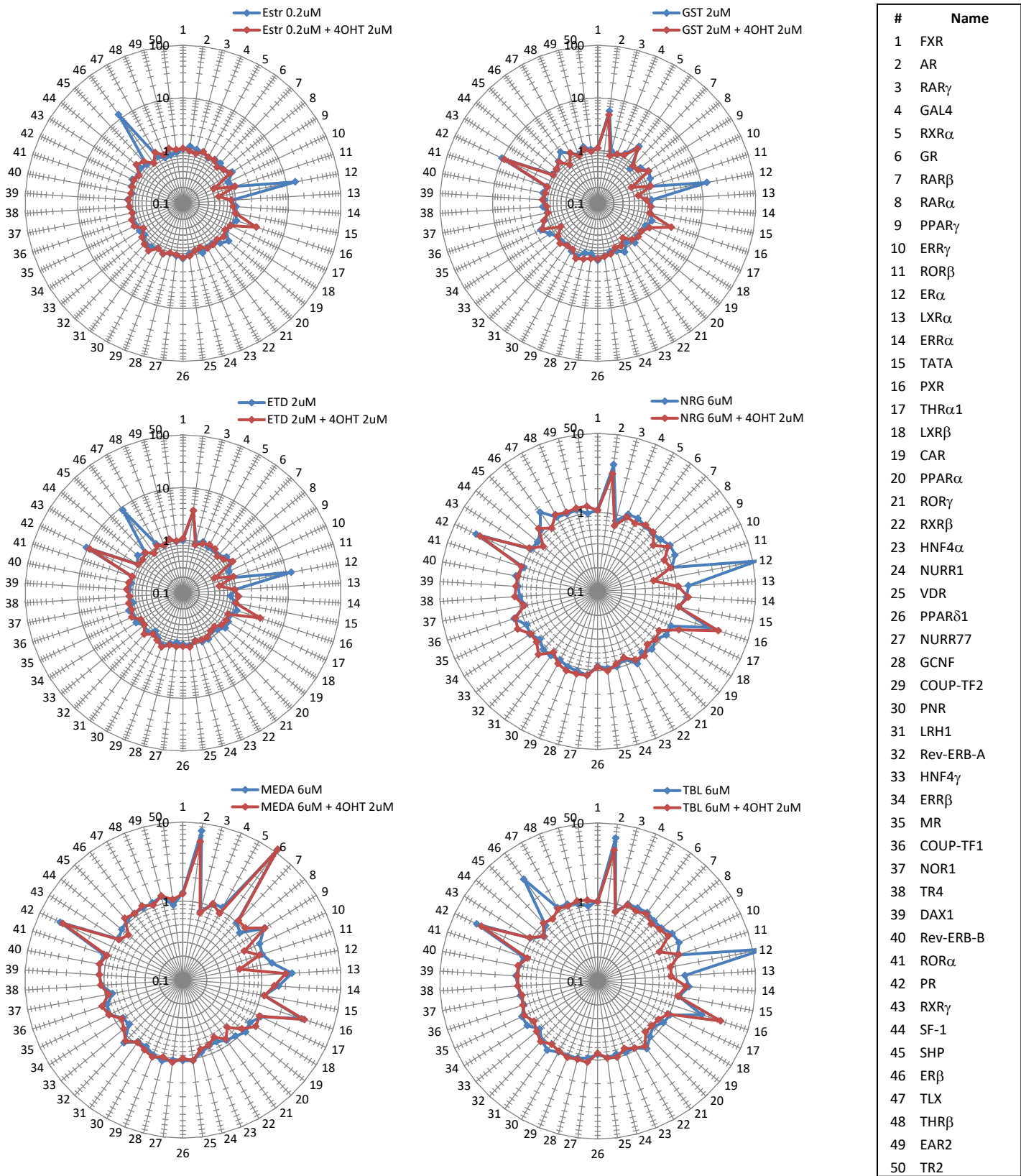
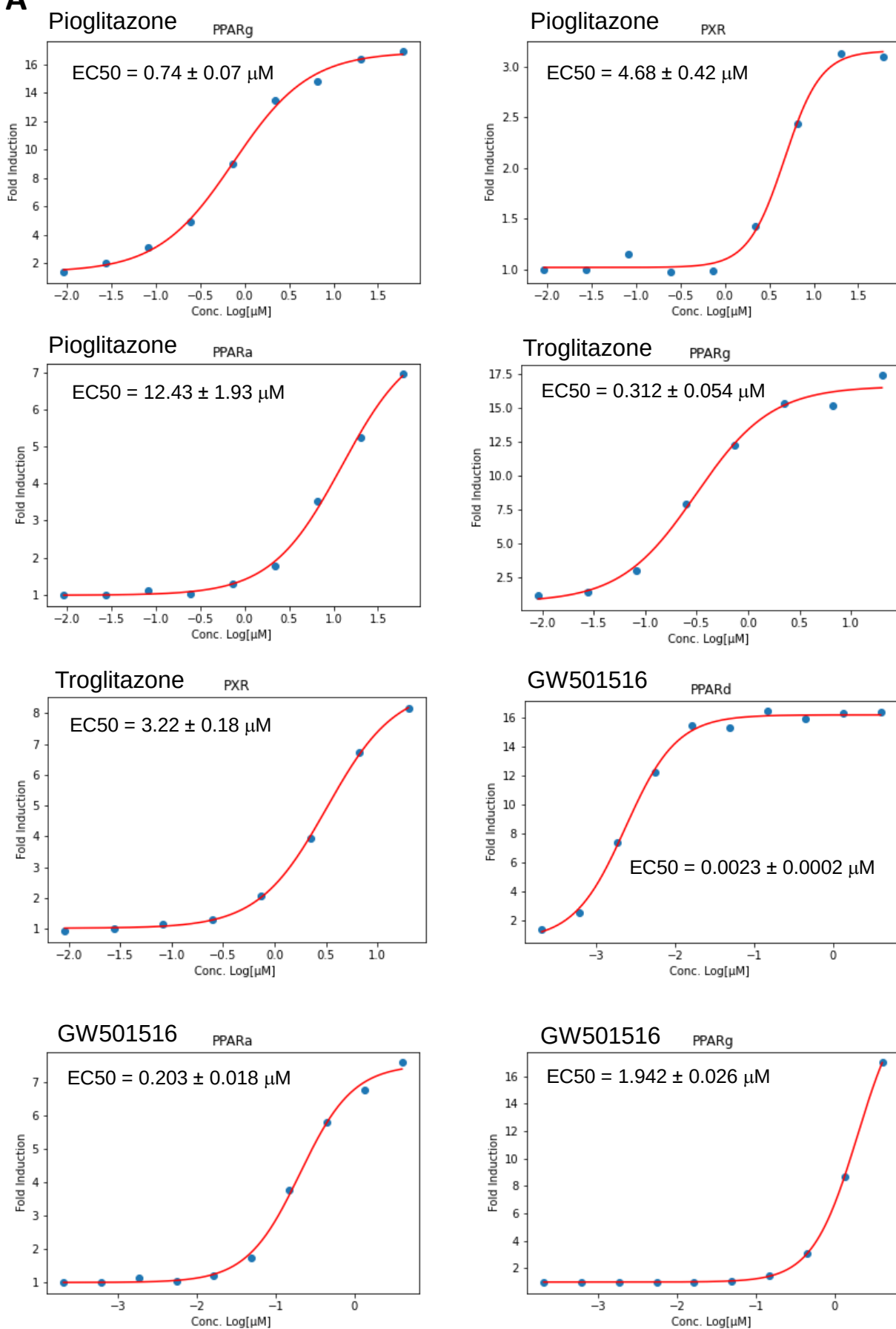
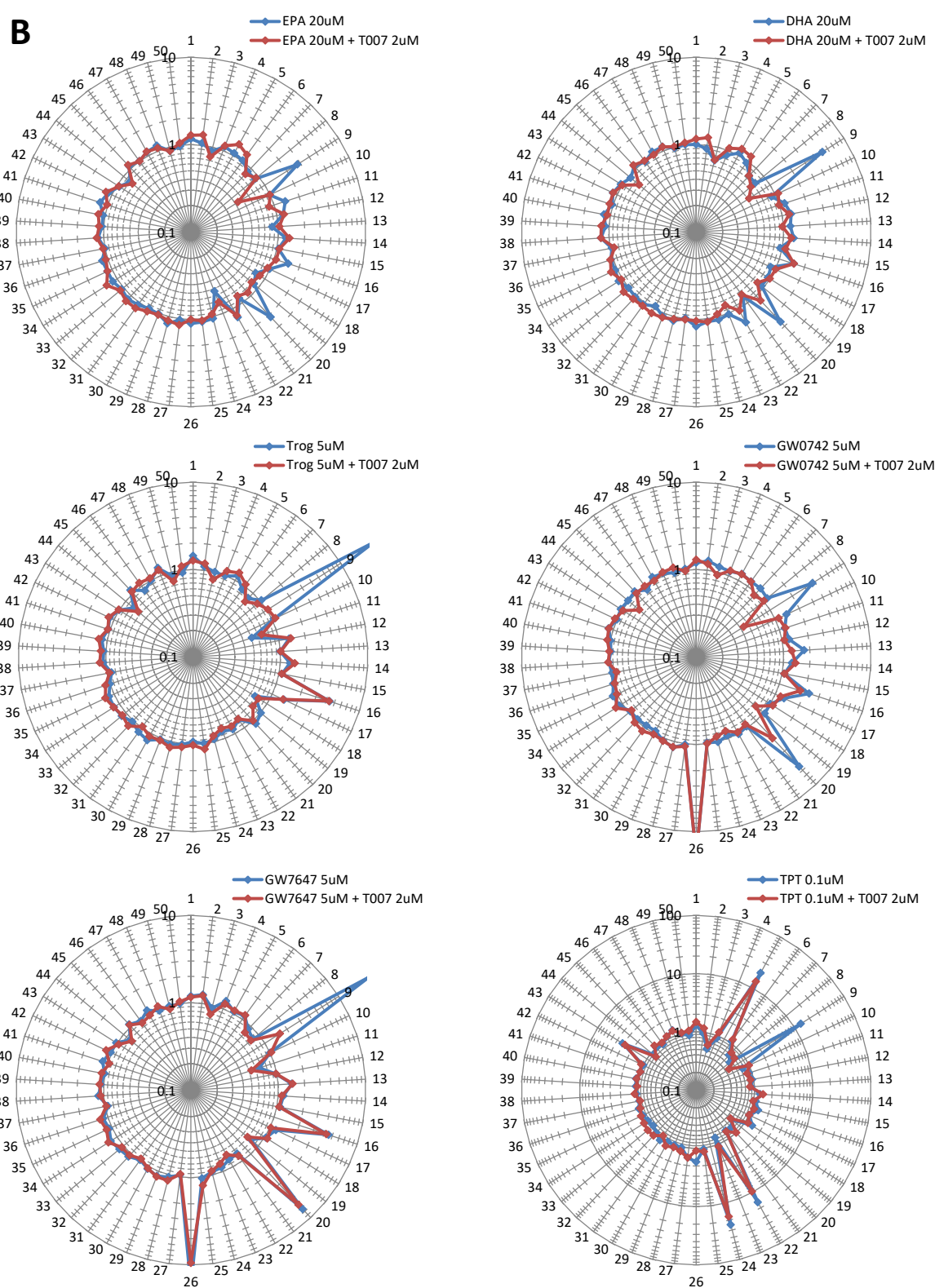


Fig. S7. Examining mechanisms for the off-target progestin activity. The NR activity profiles were obtained using the FACTORIAL NR in a competitive mode, as described by figure legend for Fig. 7C. The blue line graphs show NR activity fold-changes in progestin- vs. vehicle-treated cells after a 24-h treatment. The red line graphs show the NR responses to progestins in the presence of ER inhibitor 4-Hydroxytamoxifen (4-HT). Average profiles of three independent replicate FACTORIAL NR assays are shown.

A**Fig. S8A**

B



#	Name
1	FXR
2	AR
3	RAR γ
4	GAL4
5	RXR α
6	GR
7	RAR β
8	RAR α
9	PPAR γ
10	ERR γ
11	ROR β
12	ER α
13	LXR α
14	ERR α
15	TATA
16	PXR
17	THR α 1
18	LXR β
19	CAR
20	PPAR α
21	ROR γ
22	RXR β
23	HNF4 α
24	NURR1
25	VDR
26	PPAR δ 1
27	NURR77
28	GCNF
29	COUP-TF2
30	PNR
31	LRH1
32	Rev-ERB-A
33	HNF4 γ
34	ERR β
35	MR
36	COUP-TF1
37	NOR1
38	TR4
39	DAX1
40	Rev-ERB-B
41	ROR α
42	PR
43	RXR γ
44	SF-1
45	SHF
46	ER β
47	TLX
48	THR β
49	EAR2
50	TR2

Fig. S8. Examining mechanisms for the off-target activity of PPAR agonists. The NR activity profiles were obtained after a 24 h incubation with indicated concentrations of PPAR ligands. **A.** Concentration-response curves of on-target and off-target effects of PPAR agonists. **B.** The blue line graphs show NR activity fold changes in the stimulated vs. vehicle-treated cells. The red line graphs show NR responses to the PPAR ligands in the presence of a PPAR inhibitor T0070907 (T007). Average profiles of three independent replicate FACTORIAL NR assays are shown. *Abbreviations:* EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; Trog, troglitazone; TPT, triphenyltin.