

Electronic Supplementary Information

2 Application of cell-based bioassays to evaluate treatment efficacy of conventional and intensified 3 constructed wetlands

4

5 Jaime Nivala,^{a§} Peta A. Neale,^{b§} Tobias Haasis,^c Stefanie Kahl,^d Maria König,^e Roland A. Müller,^a
6 Thorsten Reemtsma,^d Rita Schlichting,^e Beate I. Escher^{c,e*}

⁷ ^aUFZ – Helmholtz Centre for Environmental Research, Centre for Environmental Biotechnology,
⁸ 04318 Leipzig, Germany

⁹ ^bAustralian Rivers Institute, Griffith School of Environment, Griffith University, Southport QLD
¹⁰ 4222 Australia

11 ^cEberhard Karls University Tübingen, Environmental Toxicology, Center for Applied Geosciences,
12 72074 Tübingen, Germany

¹³ ^dUFZ – Helmholtz Centre for Environmental Research, Department of Analytical Chemistry, 04318 Leipzig, Germany

15 ^dUFZ – Helmholtz Centre for Environmental Research, Department Cell Toxicology, 04318
16 Leipzig, Germany

17

18 Table of Contents

19 Figure S1: Linear concentration-effect curves for positive reference compounds

20 Figure S2: Full concentration-effect curves for induction and cell viability in AhR CALUX with
21 cell viability assessed with both PrestoBlue and IncuCyte (July only).

22 Table S1: Conventional water quality parameters.

23 Table S2: Measured concentrations ($\mu\text{g/L}$) of indicator micropollutants at the studied sites.

24 Table S3: EC values \pm standard error in units of relative enrichment factor (REF) for all samples in
25 all bioassays. Cytotoxicity IC₁₀ values provided in brackets.

26 Figure S3: Full concentration-effect curves for induction (blue filled symbols) and cell viability
27 (empty symbols) in AhR CALUX (left plots), as well as linear concentration-effect curves for
28 induction for the linear portion of the concentration-effect curve (right plots).

29 Figure S4: Full concentration-effect curves for induction (blue filled symbols) and cell viability
30 (empty symbols) in PPAR γ -bla (left plots), as well as linear concentration-effect curves for
31 induction (right plots).

32 Figure S5: Full concentration-effect curves for induction (green filled symbols) and cell viability
33 (empty symbols) in ER α GeneBLAzer (agonist mode) (left plots), as well as linear concentration-
34 effect curves for induction (right plots).

35 Figure S6: Full concentration-effect curves for inhibition (green filled symbols) and cell viability
36 (empty symbols) in ER α GeneBLAzer (antagonist mode) (left plots), as well as linear
37 concentration-effect curves for suppression (right plots).

38 Figure S7: Full concentration-effect curves for induction (green filled symbols) and cell viability
39 (empty symbols) in AR GeneBLAzer (agonist mode) (left plots), as well as linear concentration-
40 effect curves for induction (right plots).

41 Figure S8: Full concentration-effect curves for inhibition (green filled symbols) and cell viability
42 (empty symbols) in AR GeneBLAzer (antagonist mode) (left plots), as well as linear concentration-
43 effect curves for suppression (right plots).

44 Figure S9: Full concentration-effect curves for induction (green filled symbols) and cell viability
45 (empty symbols) in GR GeneBLAzer (agonist mode) (left plots), as well as linear concentration-
46 effect curves for induction (right plots).

47 Figure S10: Full concentration-effect curves for inhibition (green filled symbols) and cell viability
48 (empty symbols) in GR GeneBLAzer (antagonist mode) (left plots), as well as linear concentration-
49 effect curves for suppression (right plots).

50 Figure S11: Full concentration-effect curves for induction (green filled symbols) and cell viability
51 (empty symbols) in PR GeneBLAzer (agonist mode) (left plots), as well as linear concentration-
52 effect curves for induction (right plots).

53 Figure S12: Full concentration-effect curves for inhibition (green filled symbols) and cell viability
54 (empty symbols) in PR GeneBLAzer (antagonist mode) (left plots), as well as linear concentration-
55 effect curves for suppression (right plots).

56 Figure S13: Full concentration-effect curves for induction (red filled symbols) and cell viability
57 (empty symbols) in AREc32 (left plots), as well as linear concentration-effect curves for induction
58 (right plots).

59 Figure S14: Full concentration-effect curves for induction (red filled symbols) and cell viability
60 (empty symbols) in NF- κ B-*bla* (left plots), as well as linear concentration-effect curves for
61 induction (right plots).

62 Figure S15: Removal of indicator chemicals after treatment in A) July and B) November.

63 Table S4: Removal efficacy of BEQ_{bio} by conventional and intensified treatment wetlands, as well
64 as conventional WWTP calculated using Equation 3.

65

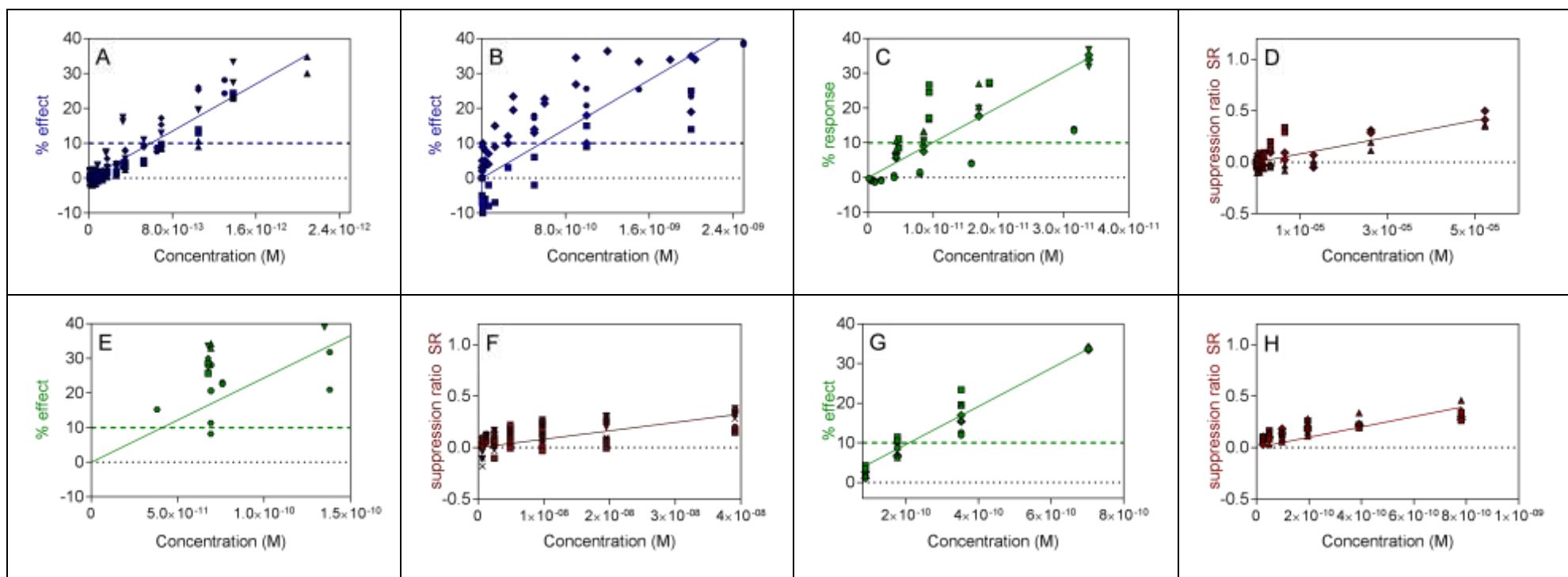
66

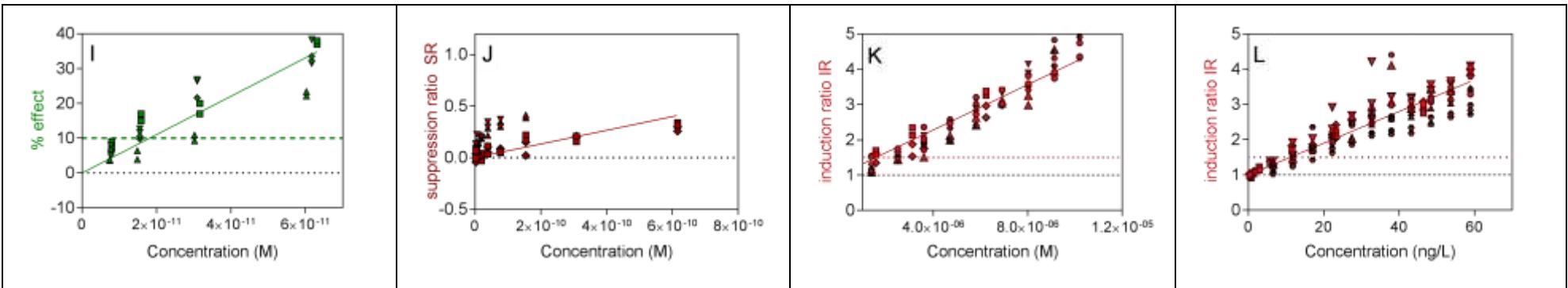
67

68

69

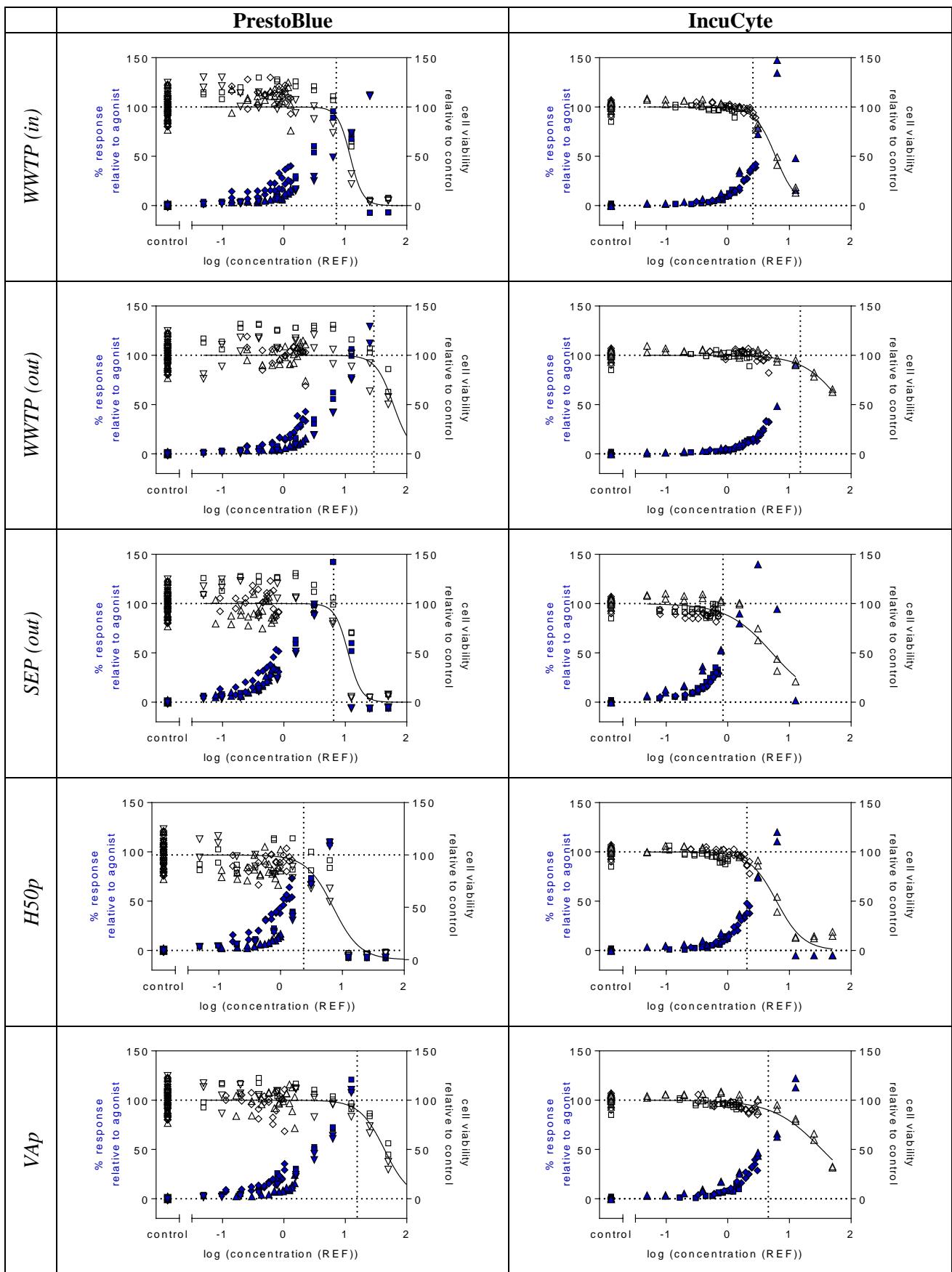
70 Figure S1: Linear concentration-effect curves for positive reference compounds A) 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) in AhR CALUX,
 71 B) Rosiglitazone in PPAR γ -bla, C) 17 β -Estradiol in ER α GeneBLAzer (agonist mode), D) Tamoxifen in ER α GeneBLAzer (antagonist mode),
 72 E) Metribolone (R1881) in AR GeneBLAzer (agonist mode), F) Cyproterone acetate in AR GeneBLAzer (antagonist mode), G) Dexamethasone in GR
 73 GeneBLAzer (agonist mode), H) Mifepristone (RU486) in GR GeneBLAzer (antagonist mode), I) Promegestone in PR GeneBLAzer (agonist mode),
 74 J) Mifepristone (RU486) in PR GeneBLAzer (antagonist mode), K) tert-Butylhydroquinone (tBHQ) in AREc32, L) Tumor necrosis factor alpha
 75 (TNF α) in NF- κ B-bla.

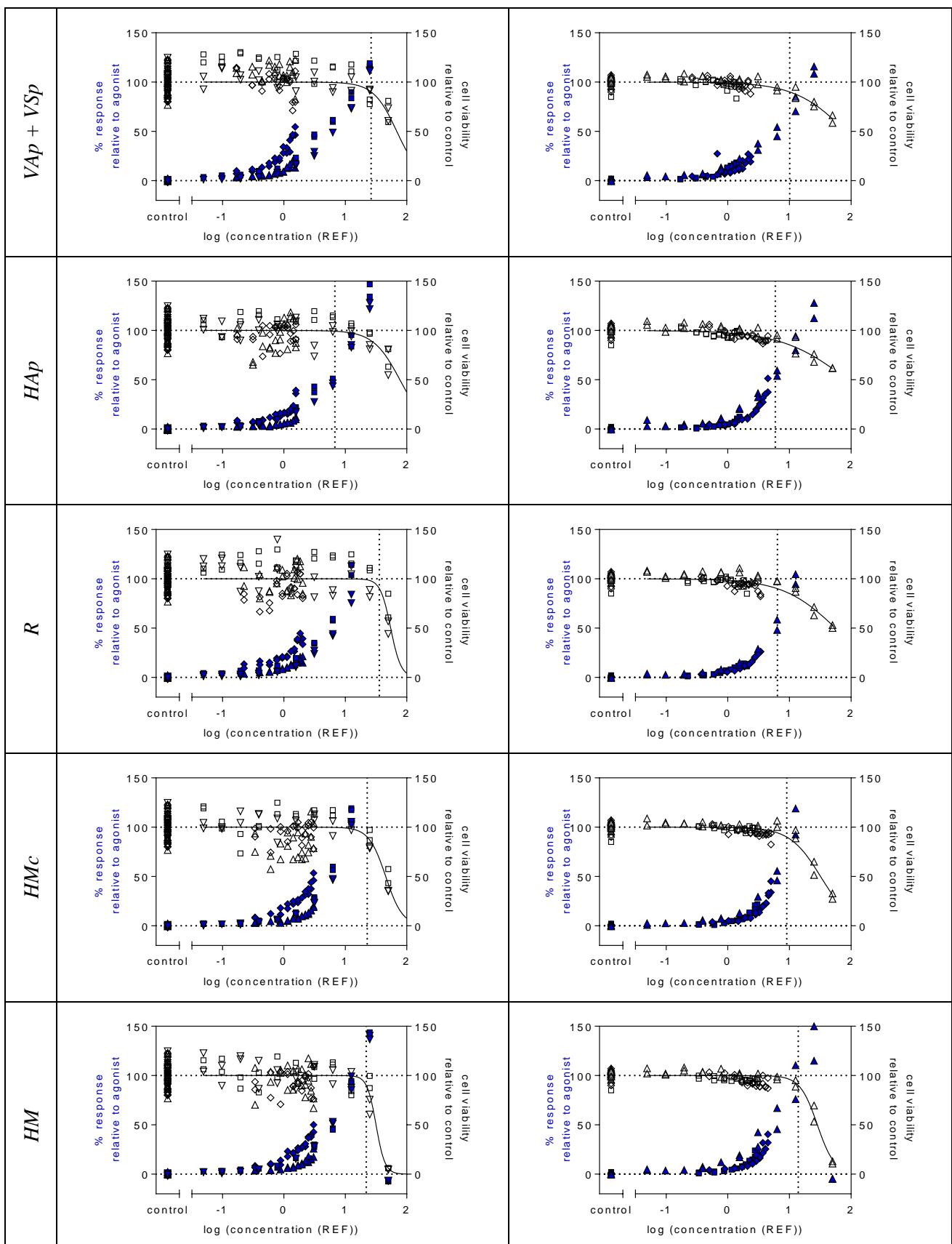


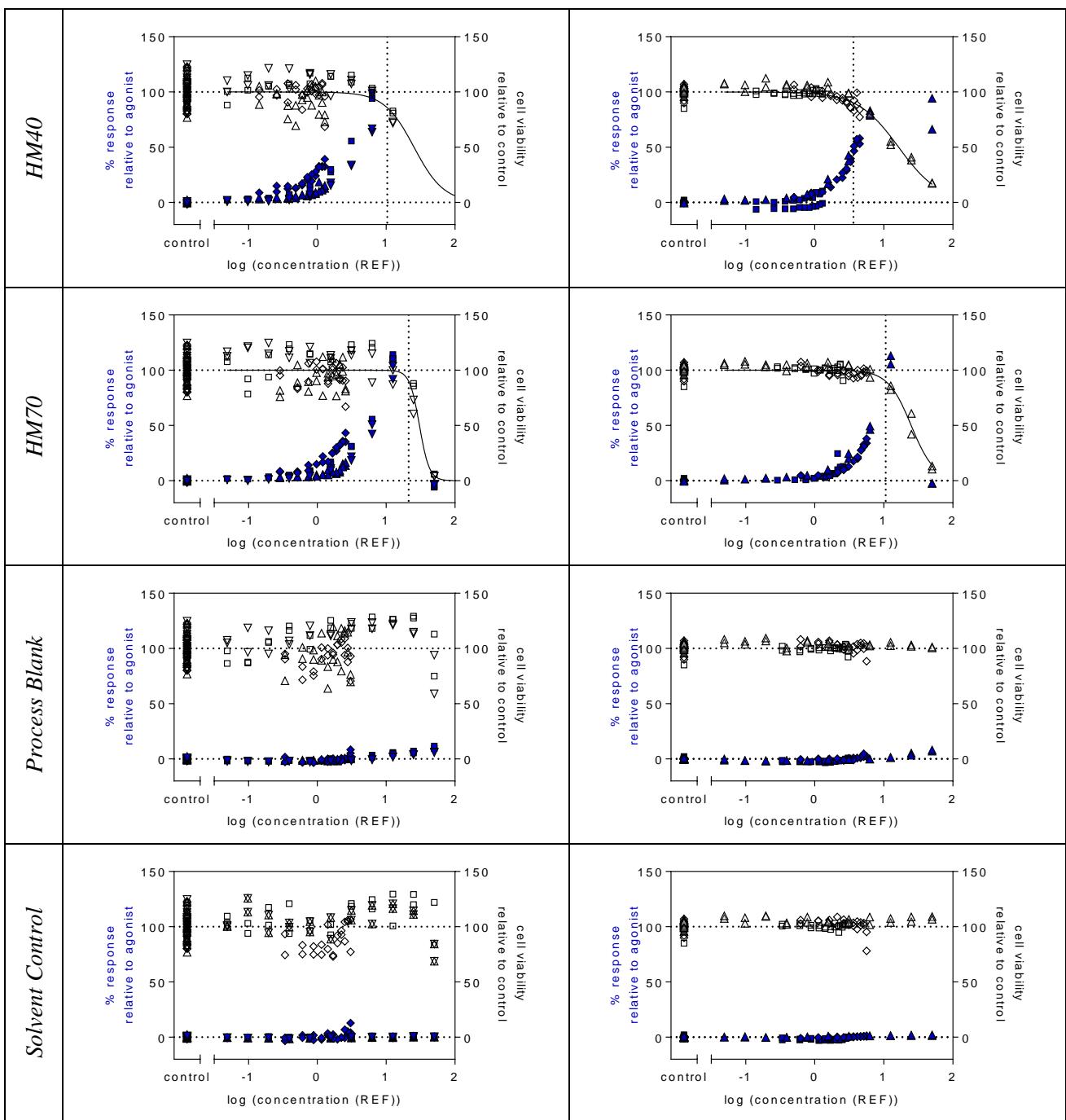


76

77 Figure S2: Full concentration-effect curves for induction and cell viability in AhR CALUX with
 78 cell viability assessed with both PrestoBlue and IncuCyte (July only).







80 Table S1: Conventional water quality parameters.

		WWTP (in)	WWTP (out)	SEP (out)	H50p	VAp	VAp + VSp	HAp	R	HM_c	HM	HM₄₀	HM₇₀
Temperature (°C)	<i>July</i>	N/A	N/A	N/A	21.1	20.5	19.3	20.9	22.7	21.3	21.2	N/A	N/A
	<i>November</i>	11.1	9.6	13.1	10.1	10.7	9.6	10.8	11	9.8	10	11.4	9.7
pH	<i>July</i>	7.40	7.23	6.99	7.00	7.42	7.42	7.50	7.10	7.91	7.90	6.96	7.67
	<i>November</i>	7.88	7.08	7.14	7.35	7.17	7.64	7.63	7.31	7.60	7.99	7.12	7.62
Electrical Conductivity (µS/cm)	<i>July</i>	1,594	1,271	1,965	2,420	1,556	1,723	1,627	1,244	1,363	1,329	1,355	1,343
	<i>November</i>	1,620	1,161	1,539	1,765	1,291	1,363	1,402	1,255	1,404	1,354	1,155	1,528
Dissolved Oxygen (mg/L)	<i>July</i>	0.08	4.42	0.11	1.83	5.54	7.99	9.05	2.02	9.02	9.21	5.21	7.91
	<i>November</i>	2.02	6.21	0.73	4.42	8.06	10.76	11.16	5.23	11.52	11.71	8.84	11.42
Redox Potential (mV)	<i>July</i>	-0.80	140	-248	-184	101	64.1	222	96.4	178	177	110	94.7
	<i>November</i>	29.9	85.2	-281	-209	201	209	194	149	208	256	130	131
5-Day carbonaceous biochemical oxygen demand (mg/L)	<i>July</i>	761	5.1	423	58.8	7.3	3.6	3.6	7.0	2.2	2.8	N/A	N/A
	<i>November</i>	304	2.2	245	30.0	1.4	<0.3	<0.3	<0.3	<0.3	<0.3	N/A	N/A
Total organic carbon (mg/L)	<i>July</i>	499	15.1	242	62.0	46.1	20.3	18.4	16.3	12.2	12.3	62.5	12.5
	<i>November</i>	242	34.8	125	27.0	10.3	8.24	16.9	8.50	11.7	8.60	182	17.0
Total nitrogen (mg/L)	<i>July</i>	98.5	5.90	97.4	86.6	27.1	19.6	50.9	12.1	39.7	35.4	48.1	36.0
	<i>November</i>	70.7	7.38	71.8	64.3	32.9	33.2	44.1	21.6	49.1	38.2	52.3	38.0
Ammonium-nitrogen (NH ₄ -N) (mg/L)	<i>July</i>	50.6	0.04	82.5	75.6	0.34	0.07	0.04	1.10	0.04	0.06	0.10	0.04
	<i>November</i>	42.6	0.16	61.5	57.4	1.41	0.02	0.02	1.41	0.02	0.02	0.05	0.20
Nitrate-nitrogen (NO ₃ -N) (mg/L)	<i>July</i>	0.18	4.13	<0.07	6.84	20.7	16.5	43.4	9.53	36.7	31.6	38.4	31.9
	<i>November</i>	0.88	5.11	<0.07	0.07	28.9	29.8	42.0	18.4	48.6	35.7	30.6	35.7
<i>E coli</i> (MPN/100 mL)	<i>July</i>	15,531,000	12,033	6,867,000	261,300	10,170	488	1,986	29,090	41	488	6,867	75
	<i>November</i>	8,664,000	27,500	2,282,000	248,100	28,510	133	144	7,710	74	128	160	95

81 N/A: not analysed; MPN/100 mL: most probable number per 100 mL.

82

83

84

85 Table S2: Measured concentrations ($\mu\text{g/L}$) of indicator micropollutants at the studied sites.

	$\log K_{ow}^{\text{a}}$	Limit of detection (LOD) $\mu\text{g/L}^{\text{l}}$		WWTP (in)	WWTP (out)	SEP (out)*	H50p	VAp	VAp + VSp	HAp	R	HM _c	HM
Acesulfame	-1.33	0.001	<i>July</i>	19.6	0.50	27.8	13.9	2.17	0.70	0.47	2.59	1.06	1.11
			<i>November</i>	12.4 ^b	0.57	14.6	17.7	1.29	0.55	0.27	1.00	0.22	0.54
Benzotriazole	1.44	0.11	<i>July</i>	8.70	3.14	15.1	6.81	2.40	0.39	0.61	3.16	1.46	1.72
			<i>November</i>	6.11 ^b	2.84	11.4	11.4	2.14	0.27	0.33	3.18	0.85	1.16
Caffeine	-0.07	0.19	<i>July</i>	47.3	<0.19	34.4	0.48	0.25	<0.19	<0.19	<0.19	<0.19	<0.19
			<i>November</i>	40.3 ^b	<0.19	4.30	0.84	0.44	<0.19	<0.19	0.26	0.29	<0.19
Carbamazepine	2.45	0.02	<i>July</i>	1.73	2.54	2.21	1.14	2.30	2.81	2.56	2.13	2.46	2.15
			<i>November</i>	1.39 ^b	1.74	1.25	1.63	1.60	1.64	1.70	1.49	1.93	1.76
Diclofenac	4.51	0.12	<i>July</i>	3.48	1.95	4.82	2.06	1.01	0.20	0.73	1.94	1.23	1.27
			<i>November</i>	2.93 ^b	1.63	3.23	3.68	0.91	0.38	0.78	2.06	1.23	1.42
Ibuprofen	3.97	0.81	<i>July</i>	14.3	<0.81	23.8	9.19	<0.81	<0.81	<0.81	<0.81	<0.81	<0.81
			<i>November</i>	13.8 ^b	<0.81	17.4	15.01	<0.81	<0.81	<0.81	<0.81	<0.81	<0.81
Naproxen	3.18	0.06	<i>July</i>	3.09	0.07	3.56	1.10	0.22	<0.06	<0.06	0.24	<0.06	0.07
			<i>November</i>	1.31 ^b	<0.06	1.85	1.54	0.11	<0.06	<0.06	0.11	<0.06	<0.06

86 ^aOctanol-water partition coefficient (US EPA); ^baverage of two duplicate samples.

87

88

89

90 Table S3: EC values \pm standard error in units of relative enrichment factor (REF) for all samples in all bioassays. Cytotoxicity IC₁₀ values provided in
 91 brackets.

		Activation of AhR	Binding to PPAR γ	Activation of ER	Inhibition of ER	Activation of AR	Inhibition of AR	Activation of GR	Inhibition of GR	Activation of PR	Inhibition of PR	Oxidative Stress Response	NF- κ B Response
		EC ₁₀	EC ₁₀	EC ₁₀	EC _{SRO.2}	EC ₁₀	EC _{SRO.2}	EC ₁₀	EC _{SRO.2}	EC ₁₀	EC _{SRO.2}	EC _{IR1.5}	EC _{IR1.5}
WWTP (in)	July	0.75 \pm 0.03	0.28 \pm 0.03	0.11 \pm 0.004	Cytotoxic (IC ₁₀ 0.75)	0.49 \pm 0.02	Cytotoxic (IC ₁₀ 2.51)	Cytotoxic (IC ₁₀ 1.74)	Cytotoxic (IC ₁₀ 0.66)	Cytotoxic (IC ₁₀ 2.00)	Cytotoxic (IC ₁₀ 2.34)	0.28 \pm 0.01	0.05 \pm 0.003
	November	0.71 \pm 0.02	0.22 \pm 0.01	0.25 \pm 0.02	Cytotoxic (IC ₁₀ 0.85)	0.82 \pm 0.06	Cytotoxic (IC ₁₀ 0.78)	Cytotoxic (IC ₁₀ 1.08)	Cytotoxic (IC ₁₀ 0.34)	Cytotoxic (IC ₁₀ 1.80)	Cytotoxic (IC ₁₀ 2.94)	0.30 \pm 0.01	0.36 \pm 0.01
WWTP (out)	July	1.62 \pm 0.05	2.45 \pm 0.11	6.39 \pm 0.21	Cytotoxic (IC ₁₀ 21.5)	Cytotoxic (IC ₁₀ 43.9)	Cytotoxic (IC ₁₀ 33.9)	4.78 \pm 0.13	Cytotoxic (IC ₁₀ 12.0)	>50	Cytotoxic (IC ₁₀ 42.7)	1.90 \pm 0.04	0.32 \pm 0.01
	November	1.46 \pm 0.04	1.52 \pm 0.12	3.81 \pm 0.17	Cytotoxic (IC ₁₀ 40.7)	Cytotoxic (IC ₁₀ 34.0)	Cytotoxic (IC ₁₀ 12.3)	4.22 \pm 0.16	Cytotoxic (IC ₁₀ 13.5)	Cytotoxic (IC ₁₀ 33.5)	Cytotoxic (IC ₁₀ 32.1)	1.54 \pm 0.03	0.09 \pm 0.01
SEP (out)	July	0.23 \pm 0.01	0.35 \pm 0.03	0.09 \pm 0.003	Cytotoxic (IC ₁₀ 3.59)	Cytotoxic (IC ₁₀ 2.49)	0.30 \pm 0.04	Cytotoxic (IC ₁₀ 1.63)	Cytotoxic (IC ₁₀ 1.13)	Cytotoxic (IC ₁₀ 2.26)	Cytotoxic (IC ₁₀ 2.62)	0.24 \pm 0.01	0.02 \pm 0.001
	November	0.43 \pm 0.01	0.27 \pm 0.01	0.14 \pm 0.01	Cytotoxic (IC ₁₀ 0.87)	Cytotoxic (IC ₁₀ 2.65)	0.36 \pm 0.05	Cytotoxic (IC ₁₀ 1.91)	Cytotoxic (IC ₁₀ 2.23)	Cytotoxic (IC ₁₀ 2.22)	Cytotoxic (IC ₁₀ 3.12)	0.31 \pm 0.01	0.02 \pm 0.001
H50p	July	0.65 \pm 0.02	0.44 \pm 0.03	0.10 \pm 0.01	Cytotoxic (IC ₁₀ 2.11)	Cytotoxic (IC ₁₀ 2.07)	Cytotoxic (IC ₁₀ 4.69)	Cytotoxic (IC ₁₀ 2.53)	Cytotoxic (IC ₁₀ 1.71)	Cytotoxic (IC ₁₀ 1.15)	Cytotoxic (IC ₁₀ 2.80)	0.52 \pm 0.01	0.02 \pm 0.001
	November	1.15 \pm 0.07	0.34 \pm 0.04	0.23 \pm 0.01	Cytotoxic (IC ₁₀ 0.84)	Cytotoxic (IC ₁₀ 2.56)	Cytotoxic (IC ₁₀ 4.01)	Cytotoxic (IC ₁₀ 2.74)	Cytotoxic (IC ₁₀ 2.30)	Cytotoxic (IC ₁₀ 2.66)	1.73 \pm 0.13	0.64 \pm 0.02	0.02 \pm 0.001
VAp	July	0.89 \pm 0.04	2.68 \pm 0.10	4.13 \pm 0.12	Cytotoxic (IC ₁₀ 27.5)	Cytotoxic (IC ₁₀ 29.4)	0.95 \pm 0.19	4.34 \pm 0.10	Cytotoxic (IC ₁₀ 4.86)	Cytotoxic (IC ₁₀ 24.1)	Cytotoxic (IC ₁₀ 28.1)	1.76 \pm 0.03	0.28 \pm 0.01
	November	1.86 \pm 0.08	3.11 \pm 0.17	5.83 \pm 0.15	>50	>50	2.19 \pm 0.05	6.99 \pm 0.14	Cytotoxic (IC ₁₀ 35.2)	Cytotoxic (IC ₁₀ 48.0)	21.8 \pm 2.19	2.17 \pm 0.04	0.27 \pm 0.01
VAp + VSp	July	0.91 \pm 0.04	5.57 \pm 0.23	19.0 \pm 0.53	>50	>50	6.45 \pm 1.42	17.9 \pm 0.33	Cytotoxic (IC ₁₀ 40.4)	>50	22.1 \pm 1.00	2.92 \pm 0.08	0.45 \pm 0.01
	November	2.47 \pm 0.09	5.74 \pm 0.36	18.2 \pm 0.54	>50	>50	5.19 \pm 0.95	41.1 \pm 1.29	>50	>50	28.0 \pm 1.49	4.88 \pm 0.14	1.81 \pm 0.07
HAp	July	1.53 \pm 0.08	3.95 \pm 0.17	12.9 \pm 0.49	>50	Cytotoxic (IC ₁₀ 42.8)	2.04 \pm 0.48	10.3 \pm 0.67	Cytotoxic (IC ₁₀ 39.2)	>50	Cytotoxic (IC ₁₀ 43.8)	2.85 \pm 0.07	0.50 \pm 0.01
	November	2.50 \pm 0.07	3.26 \pm 0.21	12.3 \pm 0.44	>50	>50	8.92 \pm 2.30	Cytotoxic (IC ₁₀ 17.7)	>50	>50	27.0 \pm 2.03	3.29 \pm 0.09	0.59 \pm 0.03
R	July	1.36 \pm 0.03	2.67 \pm 0.12	3.93 \pm 0.11	Cytotoxic (IC ₁₀ 43.8)	Cytotoxic (IC ₁₀ 48.4)	7.76 \pm 1.34	4.71 \pm 0.11	Cytotoxic (IC ₁₀ 49.6)	Cytotoxic (IC ₁₀ 41.4)	Cytotoxic (IC ₁₀ 35.8)	2.39 \pm 0.07	0.20 \pm 0.01
	November	1.90 \pm 0.01	2.05 \pm 0.09	4.78 \pm 0.16	>50	>50	15.5 \pm 1.47	4.57 \pm 0.09	>50	>50	18.3 \pm 0.77	2.98 \pm 0.07	0.29 \pm 0.03
HMc	July	1.73 \pm 0.07	1.55 \pm 0.05	7.14 \pm 0.32	Cytotoxic	2.49 \pm 0.05	0.73 \pm 0.03						

				$(IC_{10} 20.6)$	$(IC_{10} 5.28)$	$(IC_{10} 7.52)$	$(IC_{10} 8.74)$	$(IC_{10} 6.27)$	$(IC_{10} 10.6)$	$(IC_{10} 21.6)$			
	<i>November</i>	2.73 ± 0.09	1.79 ± 0.07	Cytotoxic $(IC_{10} 11.0)$	Cytotoxic $(IC_{10} 8.69)$	Cytotoxic $(IC_{10} 13.9)$	Cytotoxic $(IC_{10} 7.05)$	Cytotoxic $(IC_{10} 13.7)$	Cytotoxic $(IC_{10} 19.0)$	Cytotoxic $(IC_{10} 33.6)$	12.7 ± 1.18	4.23 ± 0.11	0.79 ± 0.05
HM	<i>July</i>	1.64 ± 0.07	2.13 ± 0.08	8.98 ± 0.38	Cytotoxic $(IC_{10} 14.0)$	Cytotoxic $(IC_{10} 9.13)$	Cytotoxic $(IC_{10} 7.38)$	9.11 ± 0.52	Cytotoxic $(IC_{10} 8.00)$	Cytotoxic $(IC_{10} 13.5)$	Cytotoxic $(IC_{10} 35.2)$	2.86 ± 0.08	0.45 ± 0.01
	<i>November</i>	2.28 ± 0.07	1.44 ± 0.05	5.21 ± 0.30	Cytotoxic $(IC_{10} 7.71)$	Cytotoxic $(IC_{10} 6.05)$	Cytotoxic $(IC_{10} 7.15)$	12.3 ± 0.53	Cytotoxic $(IC_{10} 6.47)$	Cytotoxic $(IC_{10} 25.9)$	Cytotoxic $(IC_{10} 23.2)$	6.78 ± 0.29	0.82 ± 0.06
HM40	<i>July</i>	1.06 ± 0.03	0.97 ± 0.04	Cytotoxic $(IC_{10} 3.59)$	Cytotoxic $(IC_{10} 2.19)$	Cytotoxic $(IC_{10} 7.64)$	Cytotoxic $(IC_{10} 4.32)$	3.87 ± 0.18	Cytotoxic $(IC_{10} 3.68)$	Cytotoxic $(IC_{10} 16.9)$	Cytotoxic $(IC_{10} 7.66)$	1.07 ± 0.05	0.32 ± 0.01
	<i>November</i>	1.53 ± 0.05	0.59 ± 0.03	Cytotoxic $(IC_{10} 2.30)$	Cytotoxic $(IC_{10} 3.03)$	Cytotoxic $(IC_{10} 1.50)$	Cytotoxic $(IC_{10} 1.70)$	Cytotoxic $(IC_{10} 2.73)$	Cytotoxic $(IC_{10} 2.04)$	Cytotoxic $(IC_{10} 12.4)$	9.24 ± 1.02	2.94 ± 0.24	1.05 ± 0.03
HM70	<i>July</i>	1.98 ± 0.08	1.49 ± 0.06	Cytotoxic $(IC_{10} 5.62)$	Cytotoxic $(IC_{10} 3.52)$	Cytotoxic $(IC_{10} 6.61)$	Cytotoxic $(IC_{10} 5.93)$	Cytotoxic $(IC_{10} 6.71)$	Cytotoxic $(IC_{10} 4.99)$	Cytotoxic $(IC_{10} 12.7)$	Cytotoxic $(IC_{10} 12.2)$	3.18 ± 0.11	0.41 ± 0.02
	<i>November</i>	2.51 ± 0.10	1.22 ± 0.06	Cytotoxic $(IC_{10} 5.83)$	Cytotoxic $(IC_{10} 9.94)$	Cytotoxic $(IC_{10} 7.17)$	Cytotoxic $(IC_{10} 4.07)$	Cytotoxic $(IC_{10} 4.68)$	Cytotoxic $(IC_{10} 4.94)$	Cytotoxic $(IC_{10} 26.7)$	5.95 ± 1.19	4.67 ± 0.16	0.58 ± 0.02
Process	<i>July</i>	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
Blank	<i>November</i>	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
Solvent	<i>July</i>	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50
Control	<i>November</i>	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50	>50

92

93

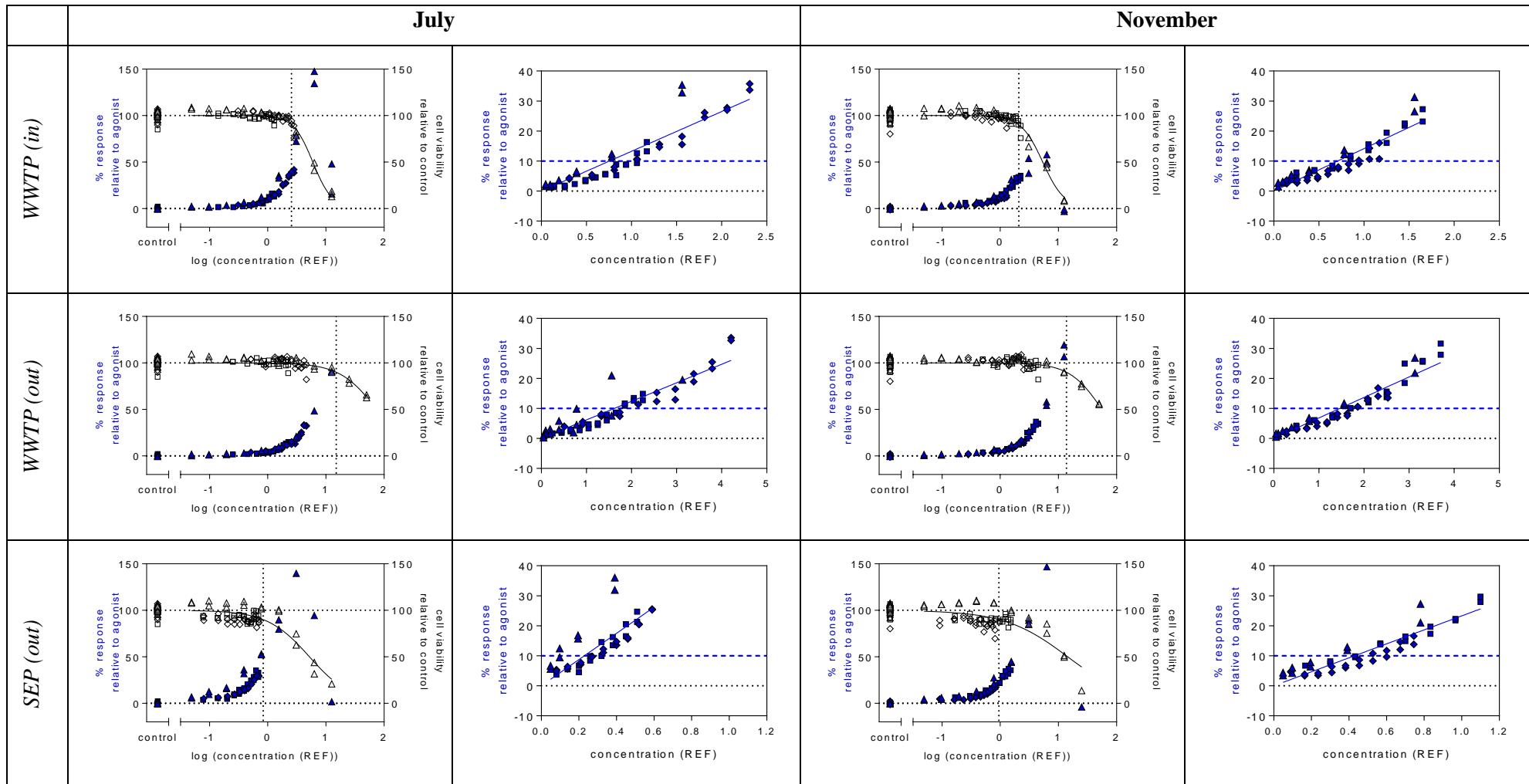
94

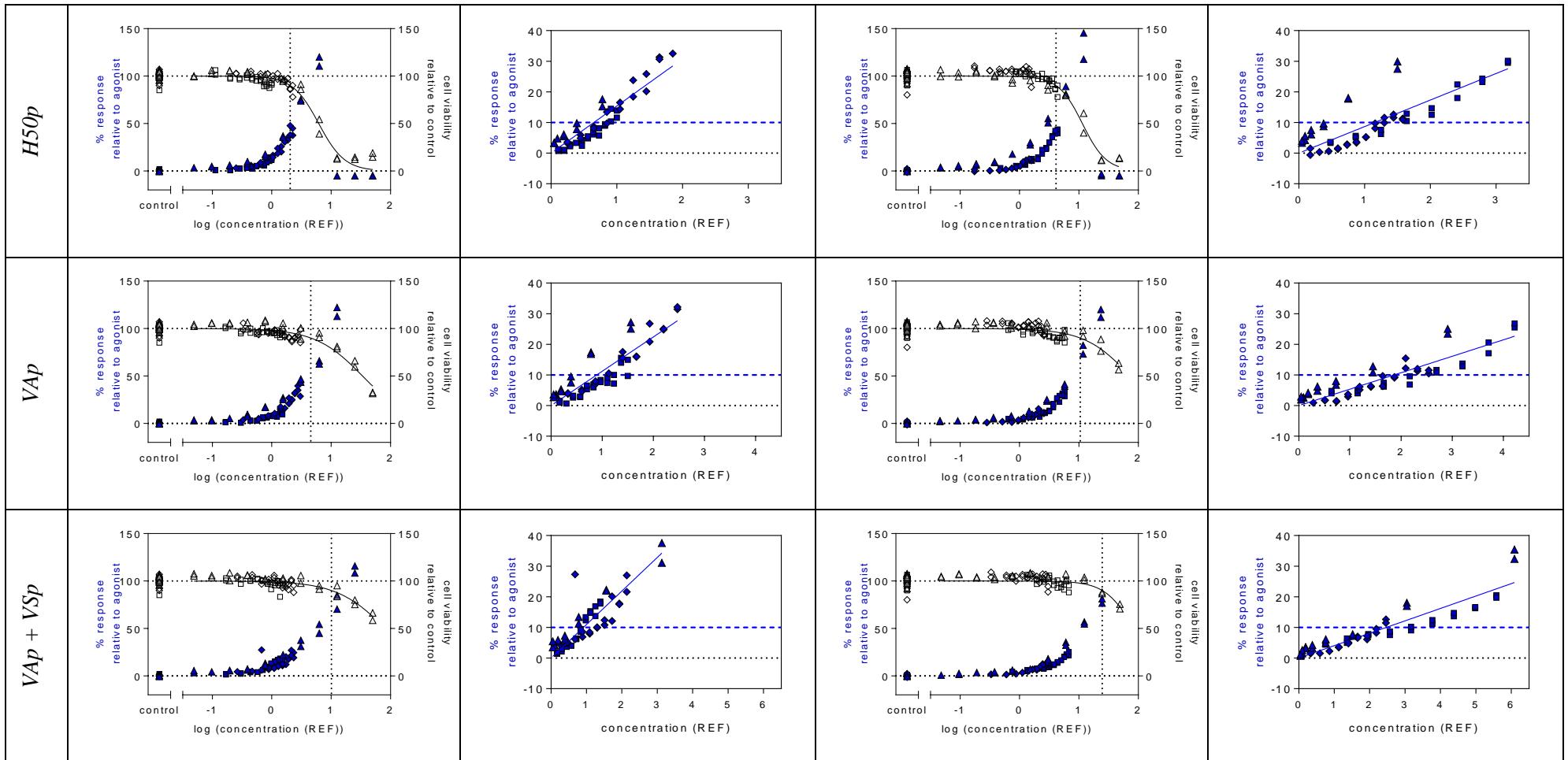
95

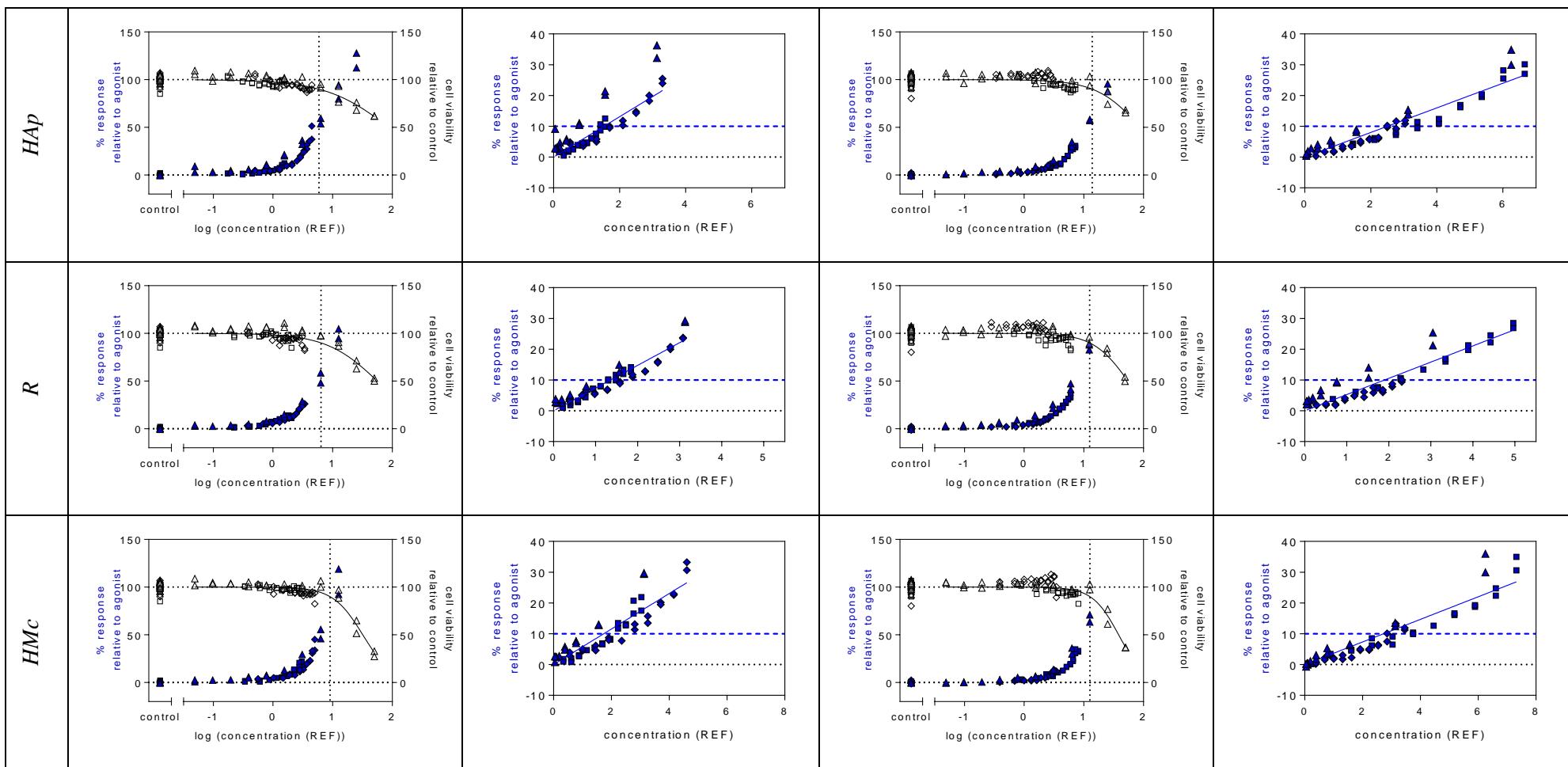
96

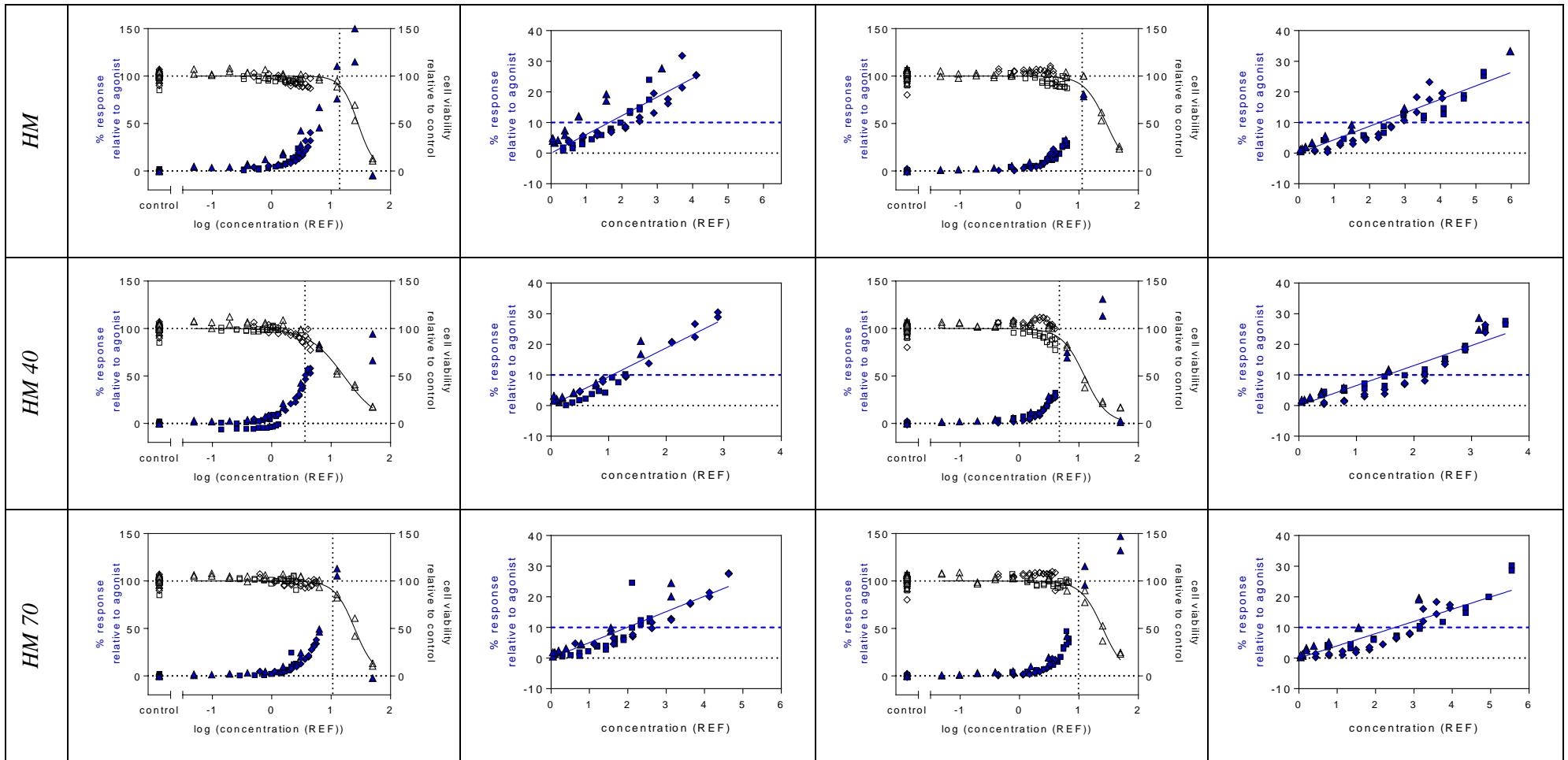
97

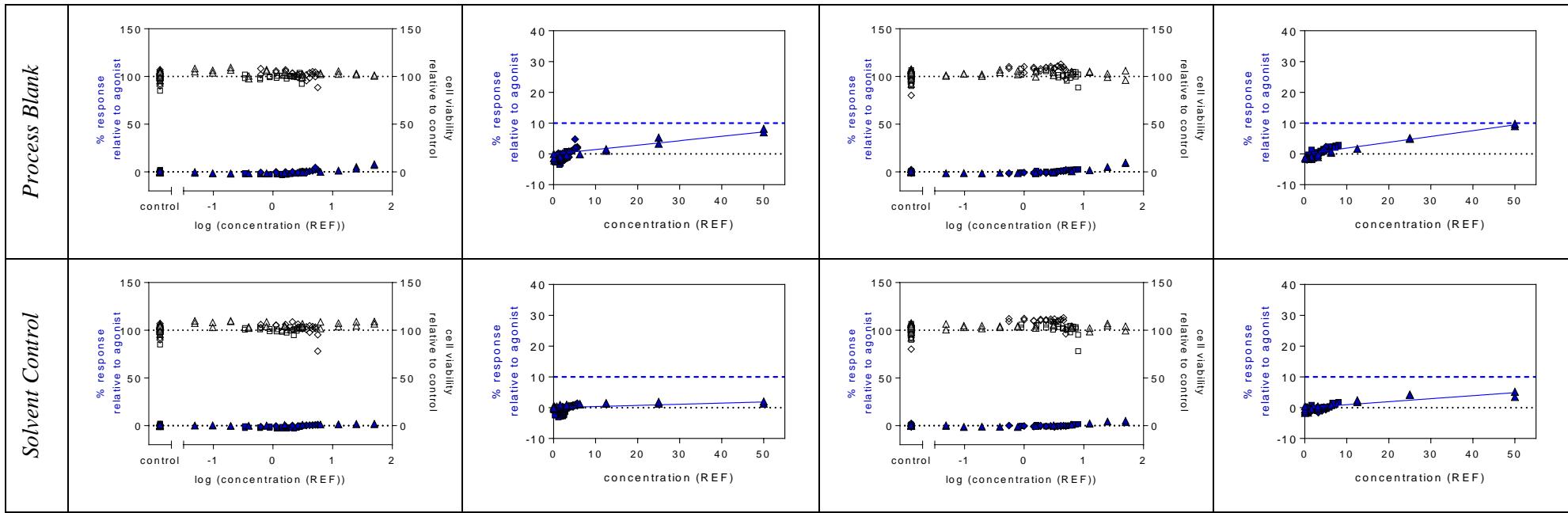
98 Figure S3: Full concentration-effect curves for induction (blue filled symbols) and cell viability (empty symbols) in AhR CALUX (left plots), as well
 99 as linear concentration-effect curves for induction for the linear portion of the concentration-effect curve (right plots).







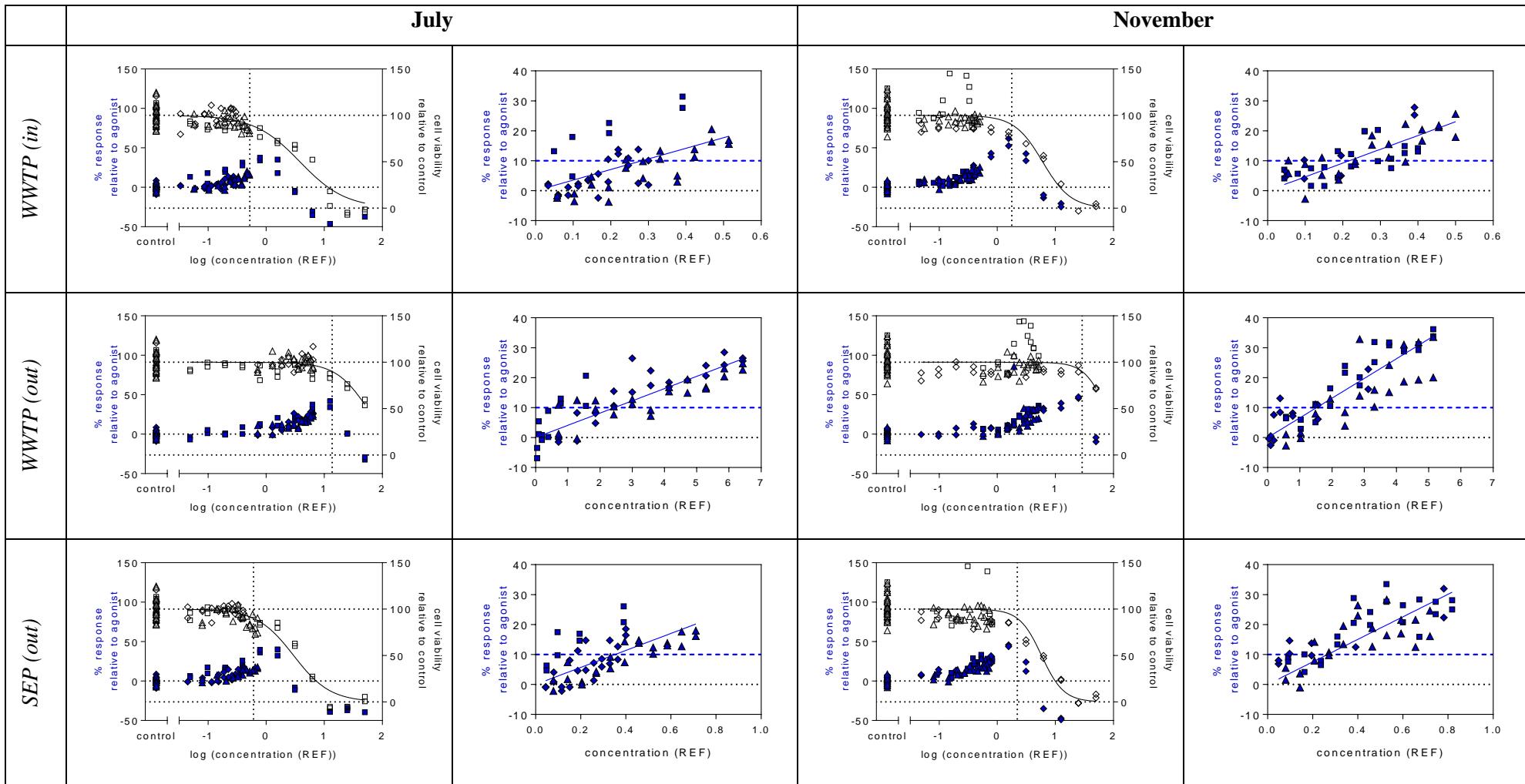


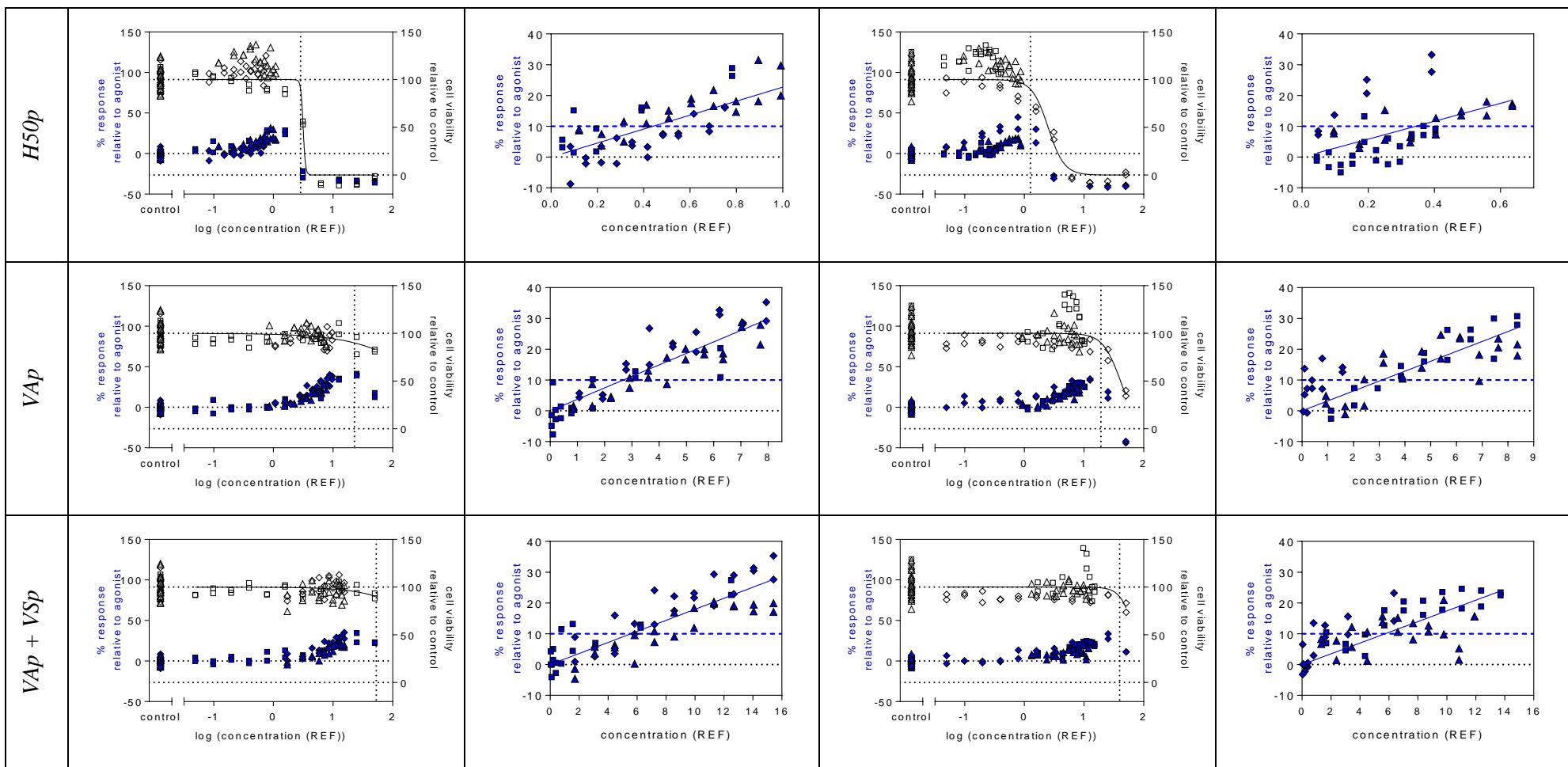


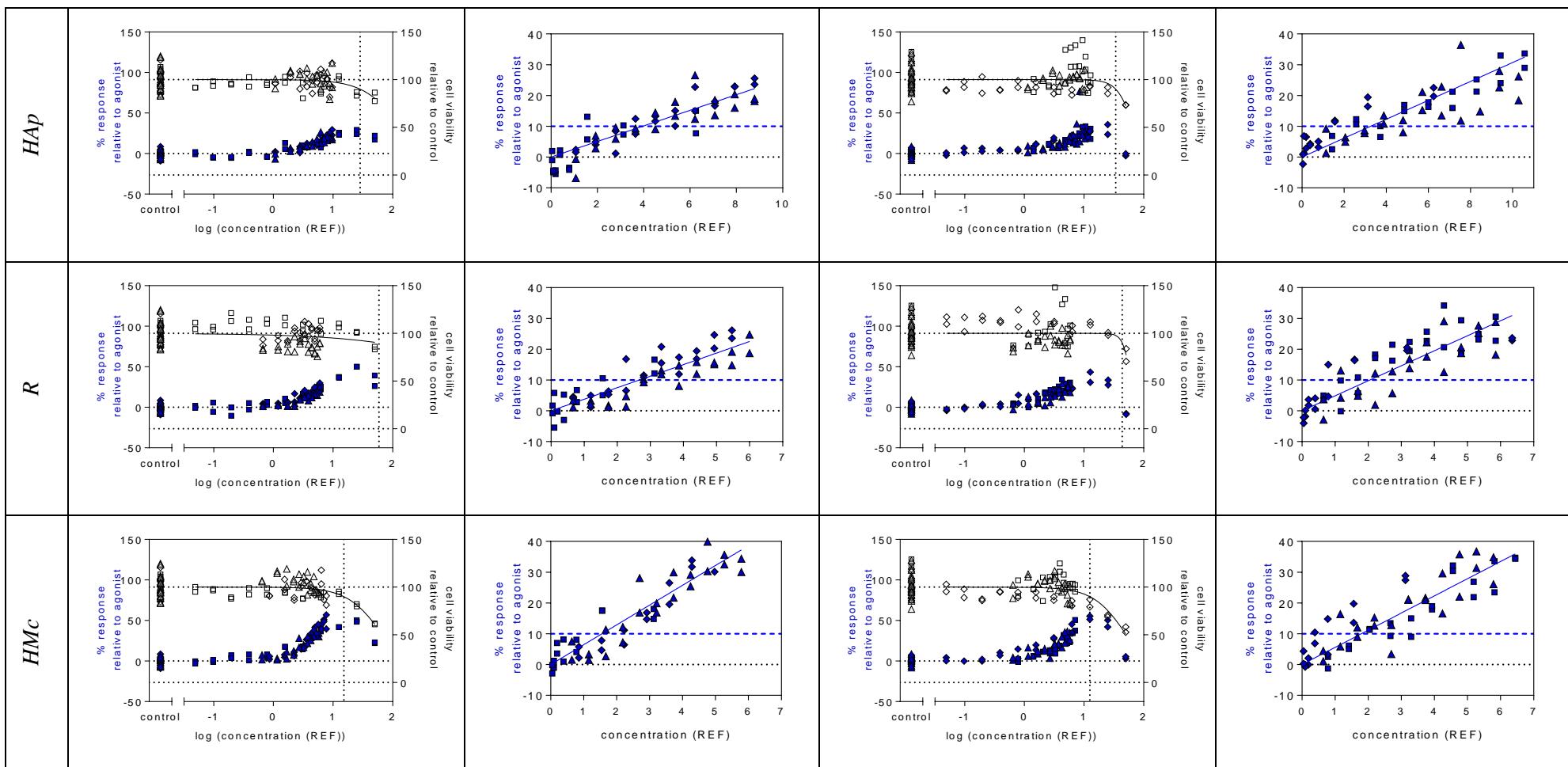
100

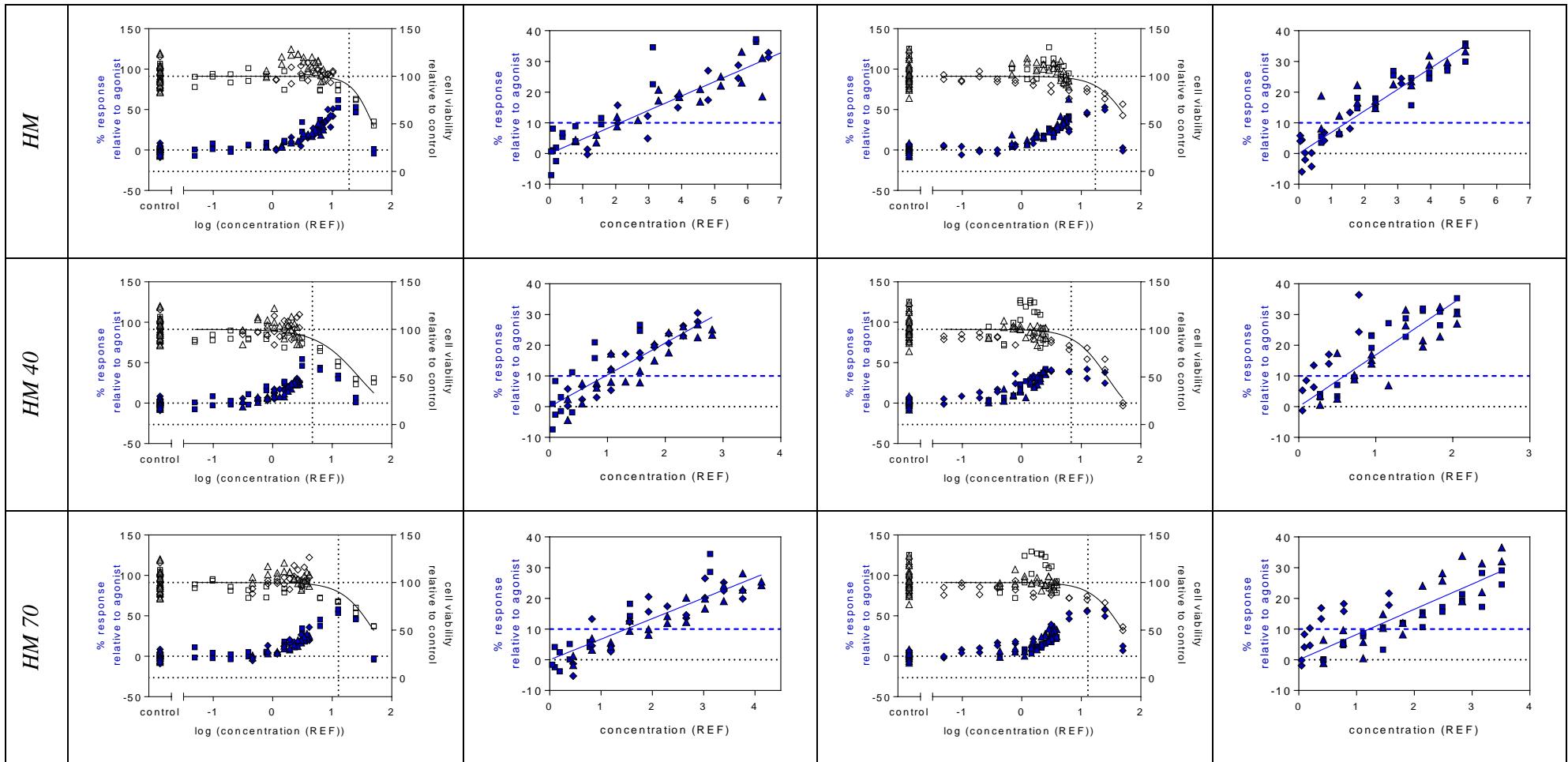
101

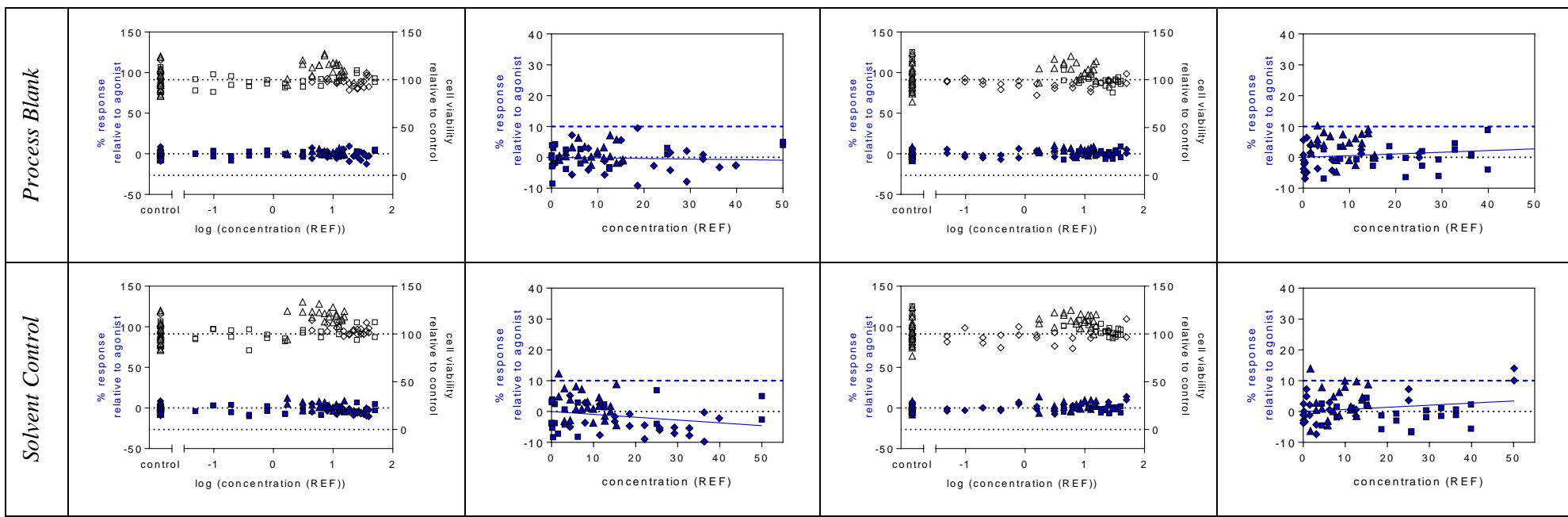
102 Figure S4: Full concentration-effect curves for induction (blue filled symbols) and cell viability (empty symbols) in PPAR γ -bla (left plots), as well as
 103 linear concentration-effect curves for induction (right plots).







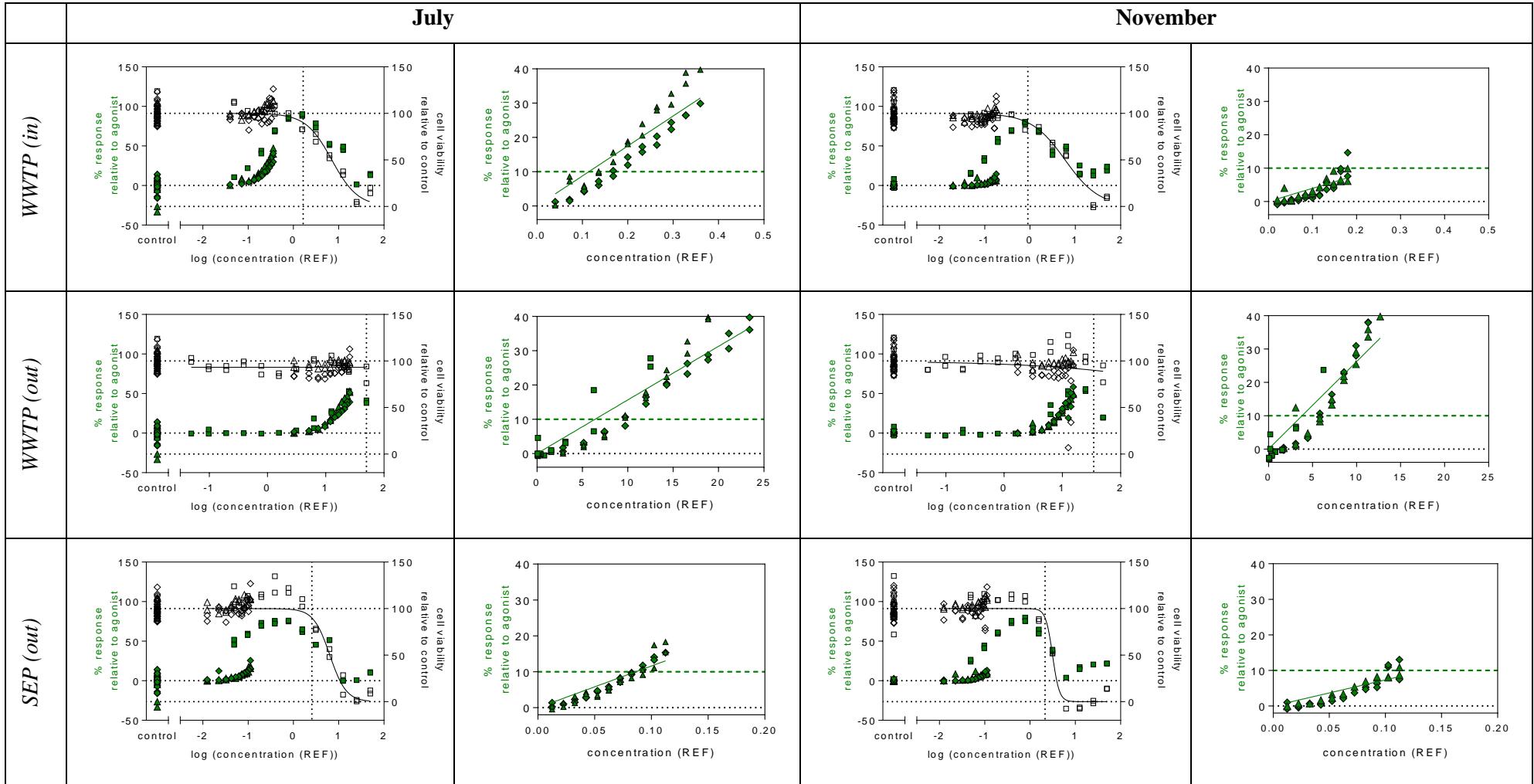


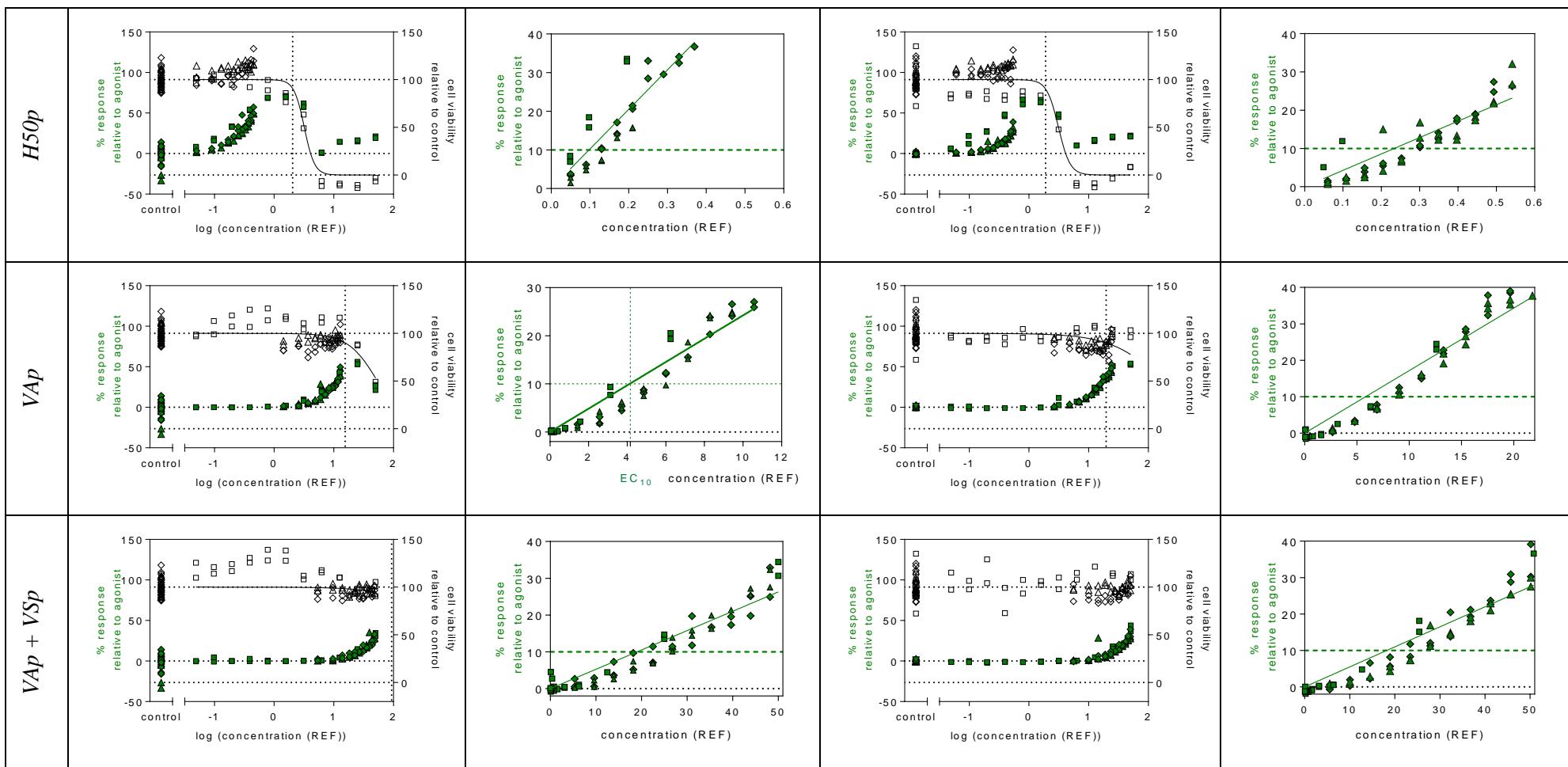


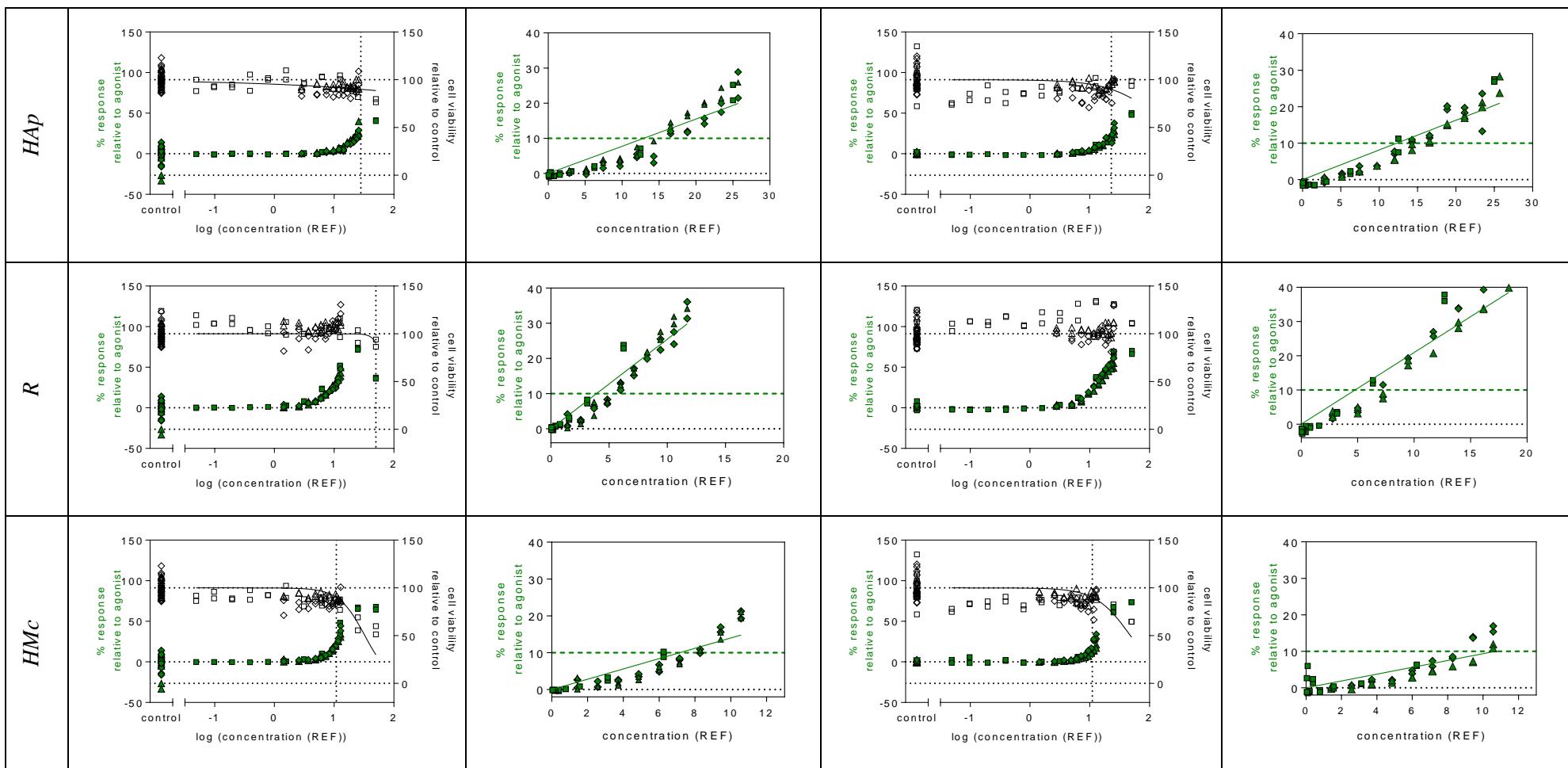
104

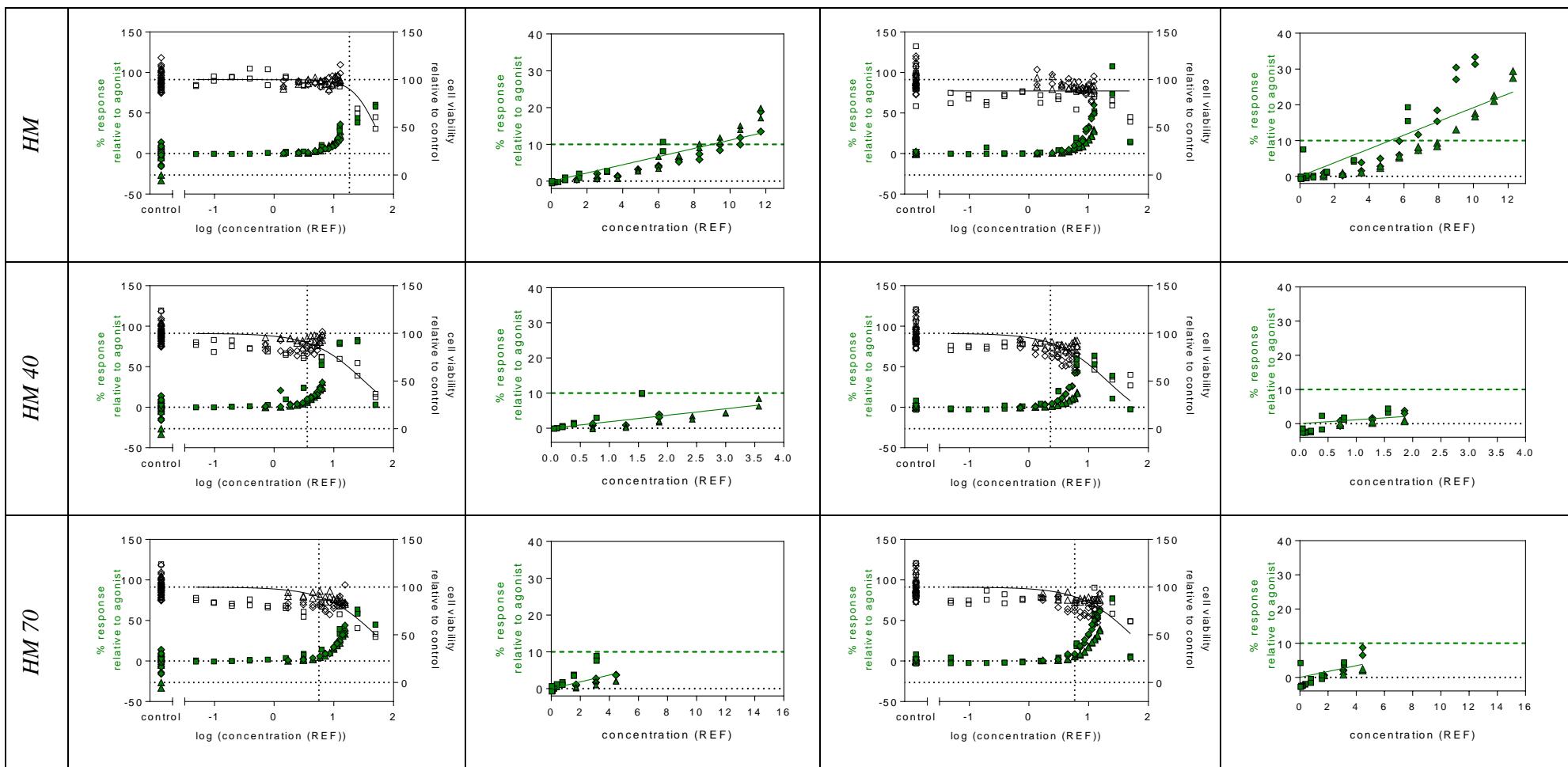
105

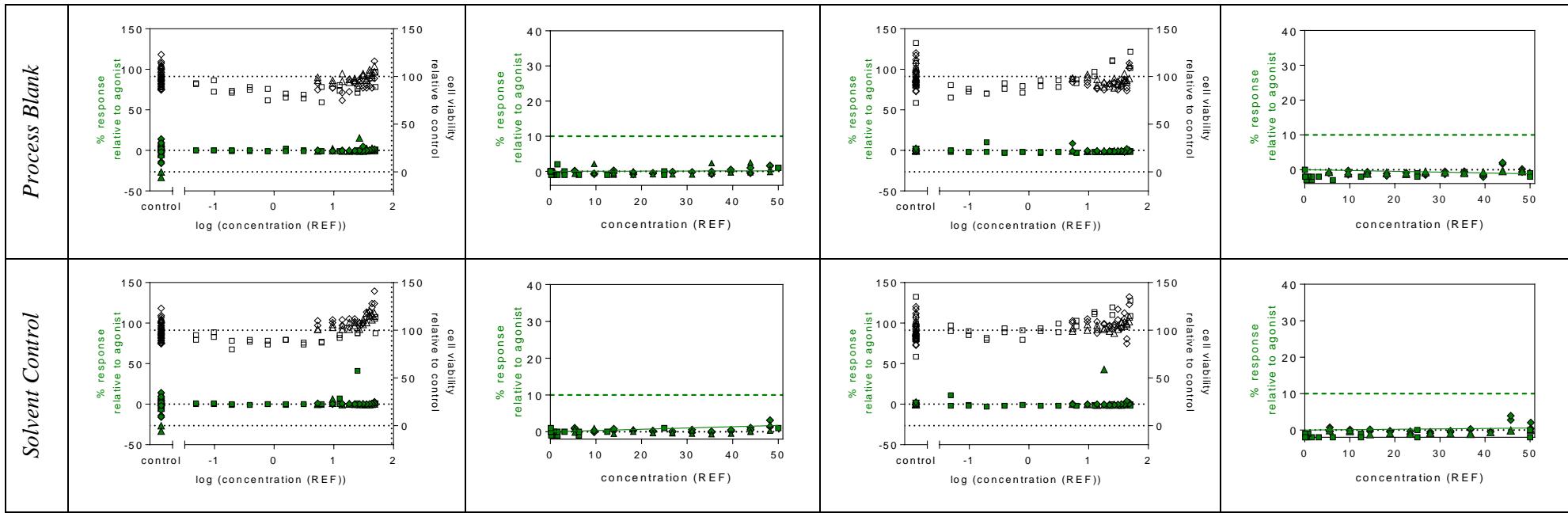
106 Figure S5: Full concentration-effect curves for induction (green filled symbols) and cell viability (empty symbols) in ER α GeneBLAzer (agonist mode)
 107 (left plots), as well as linear concentration-effect curves for induction (right plots).







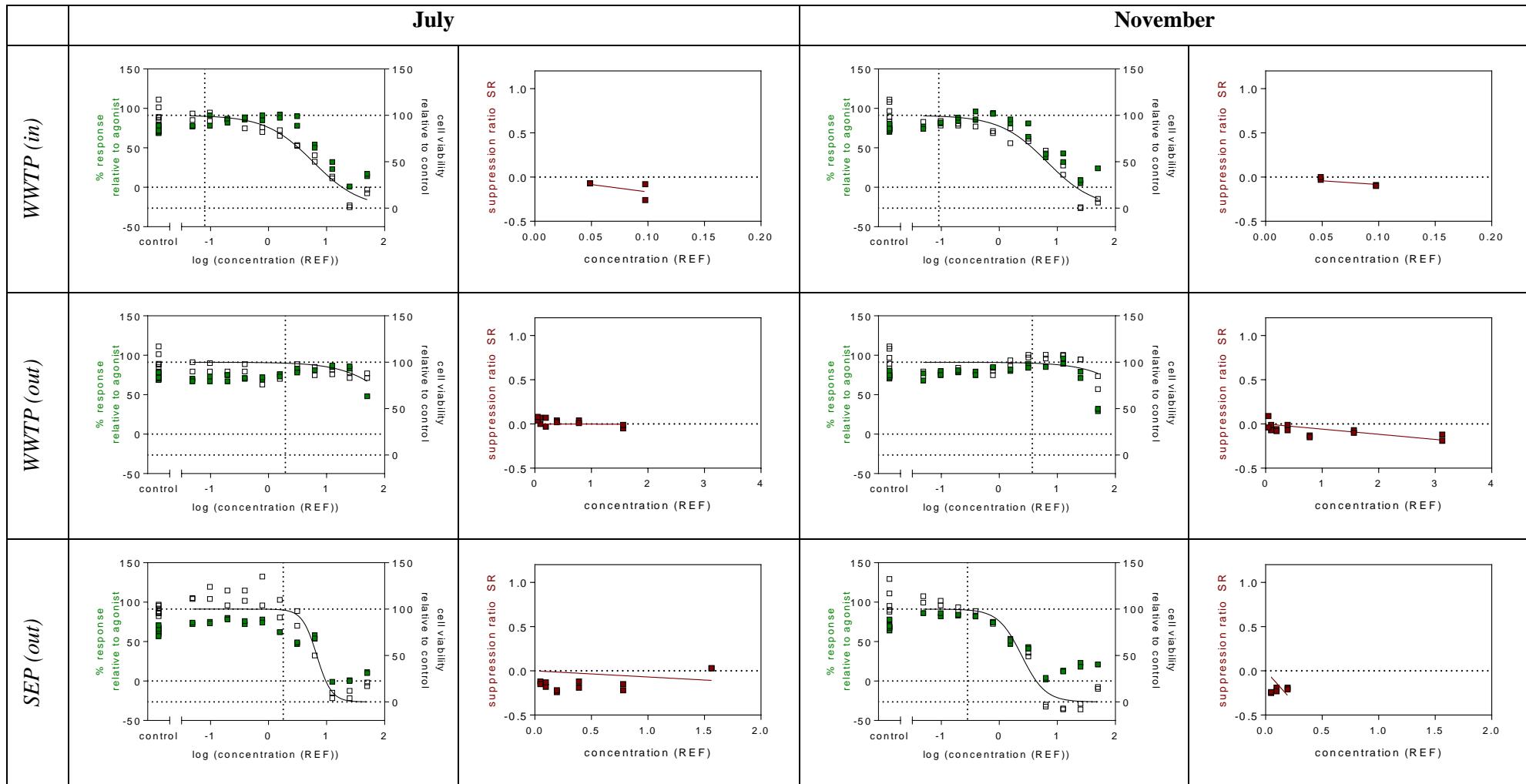


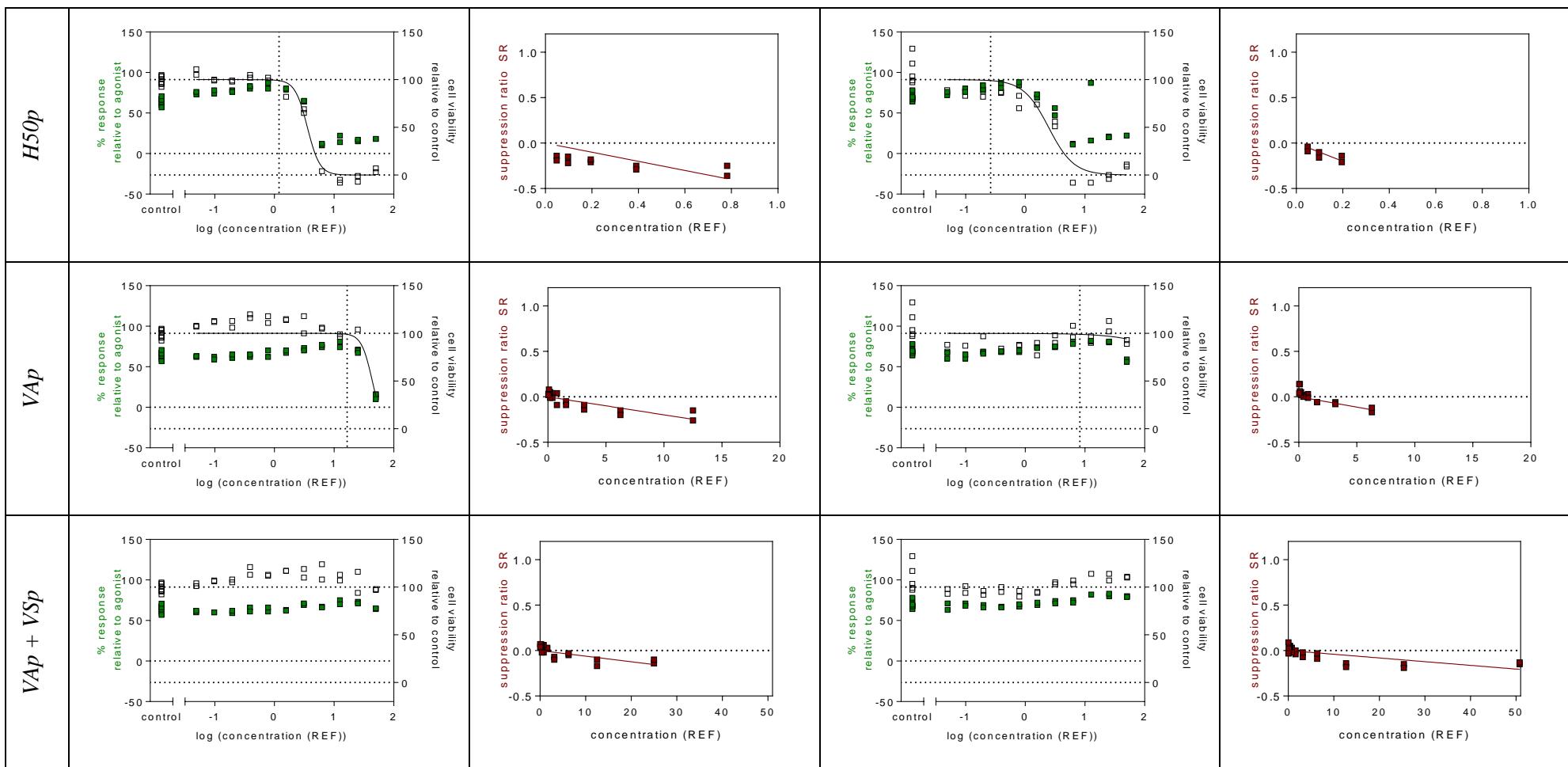


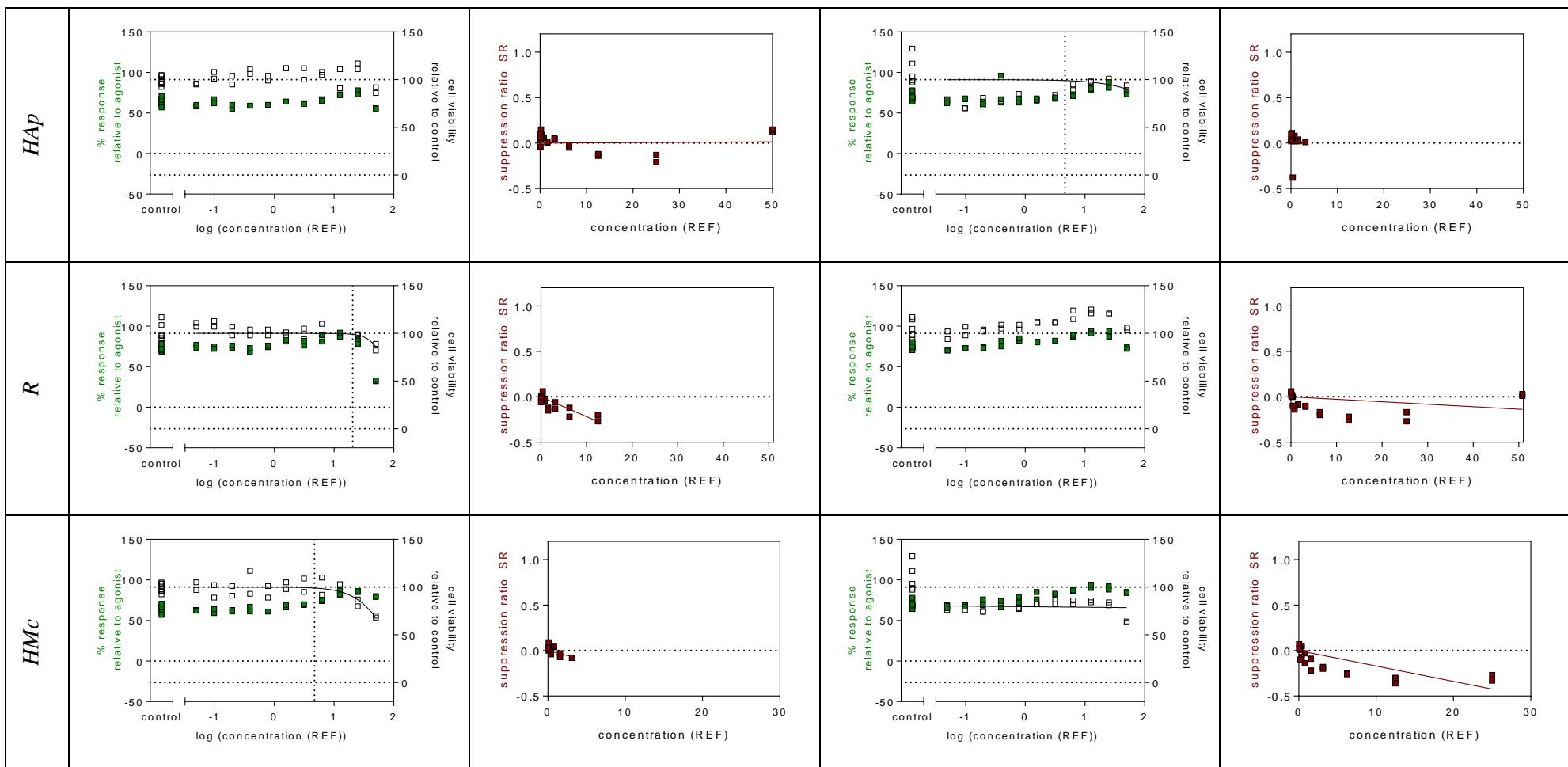
108

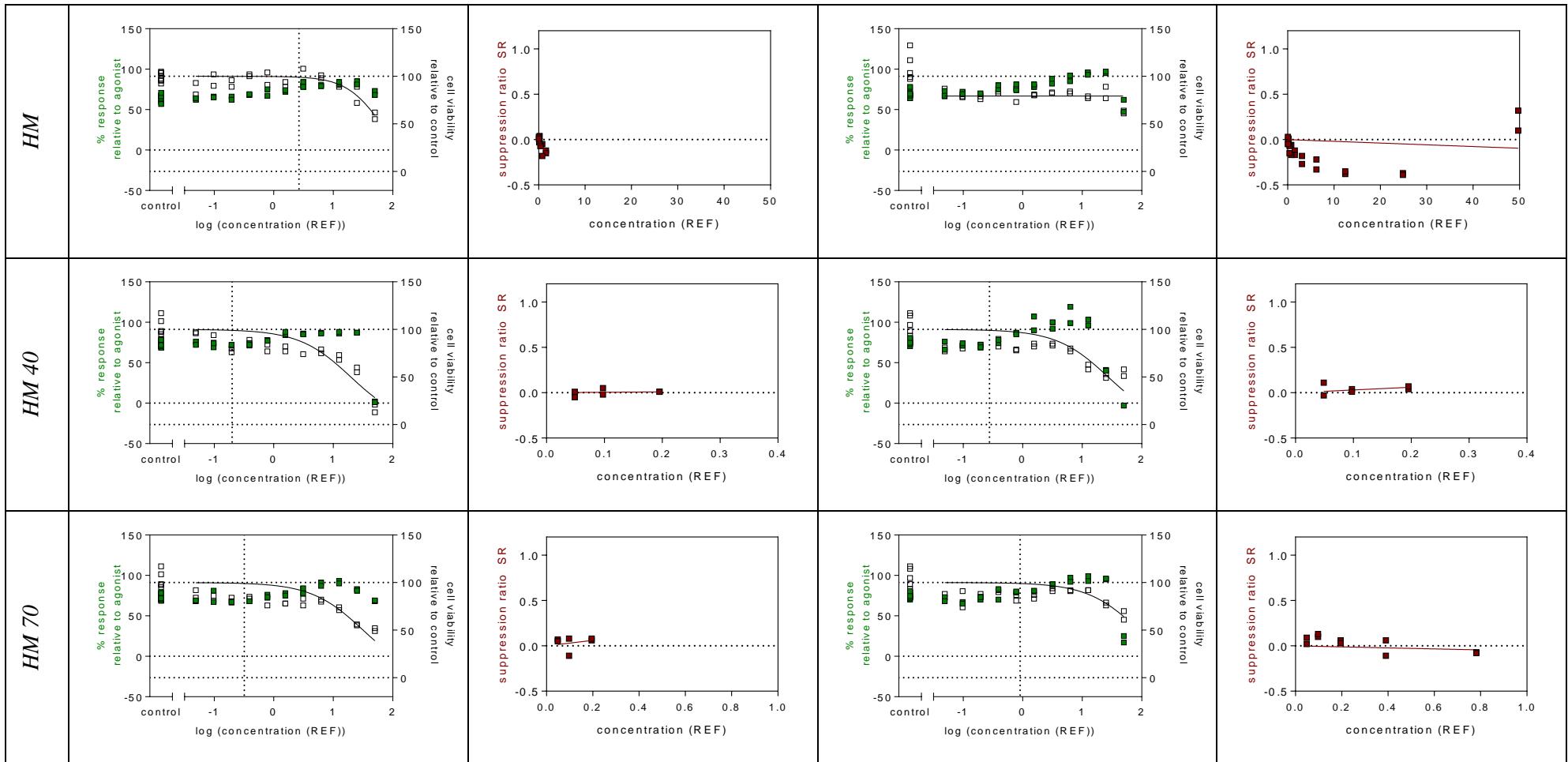
109

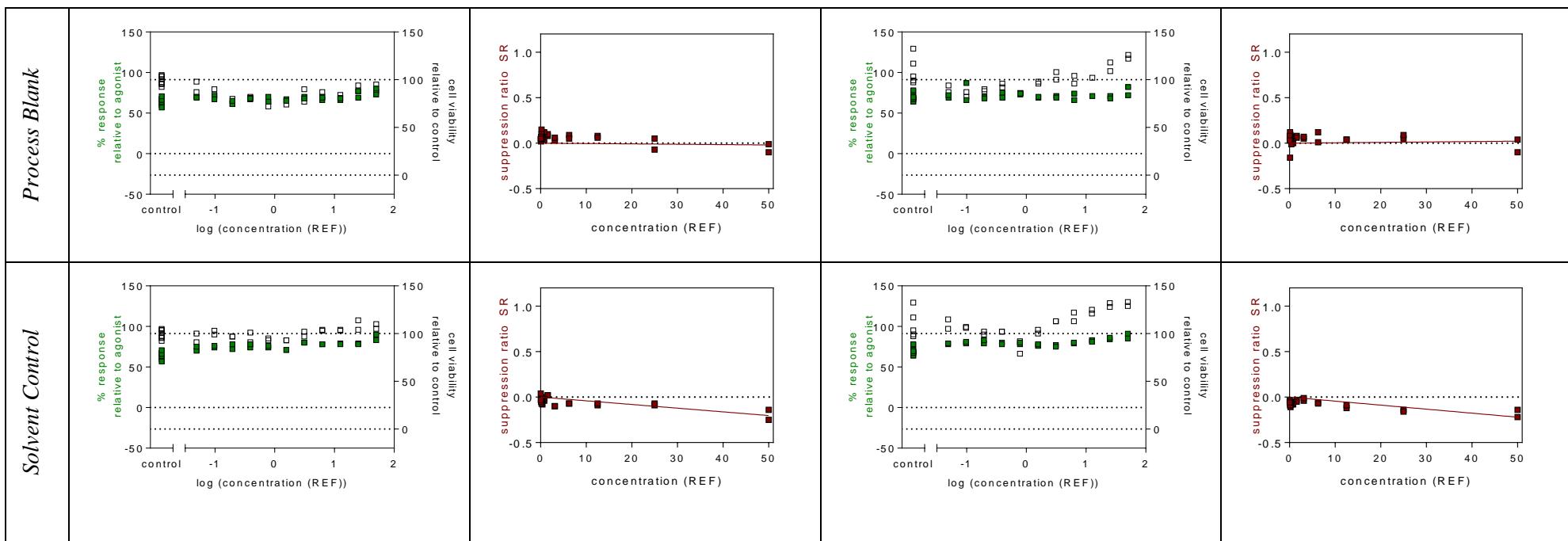
110 Figure S6: Full concentration-effect curves for inhibition (green filled symbols) and cell viability (empty symbols) in ER α GeneBLAzer (antagonist
 111 mode) (left plots), as well as linear concentration-effect curves for suppression (right plots).







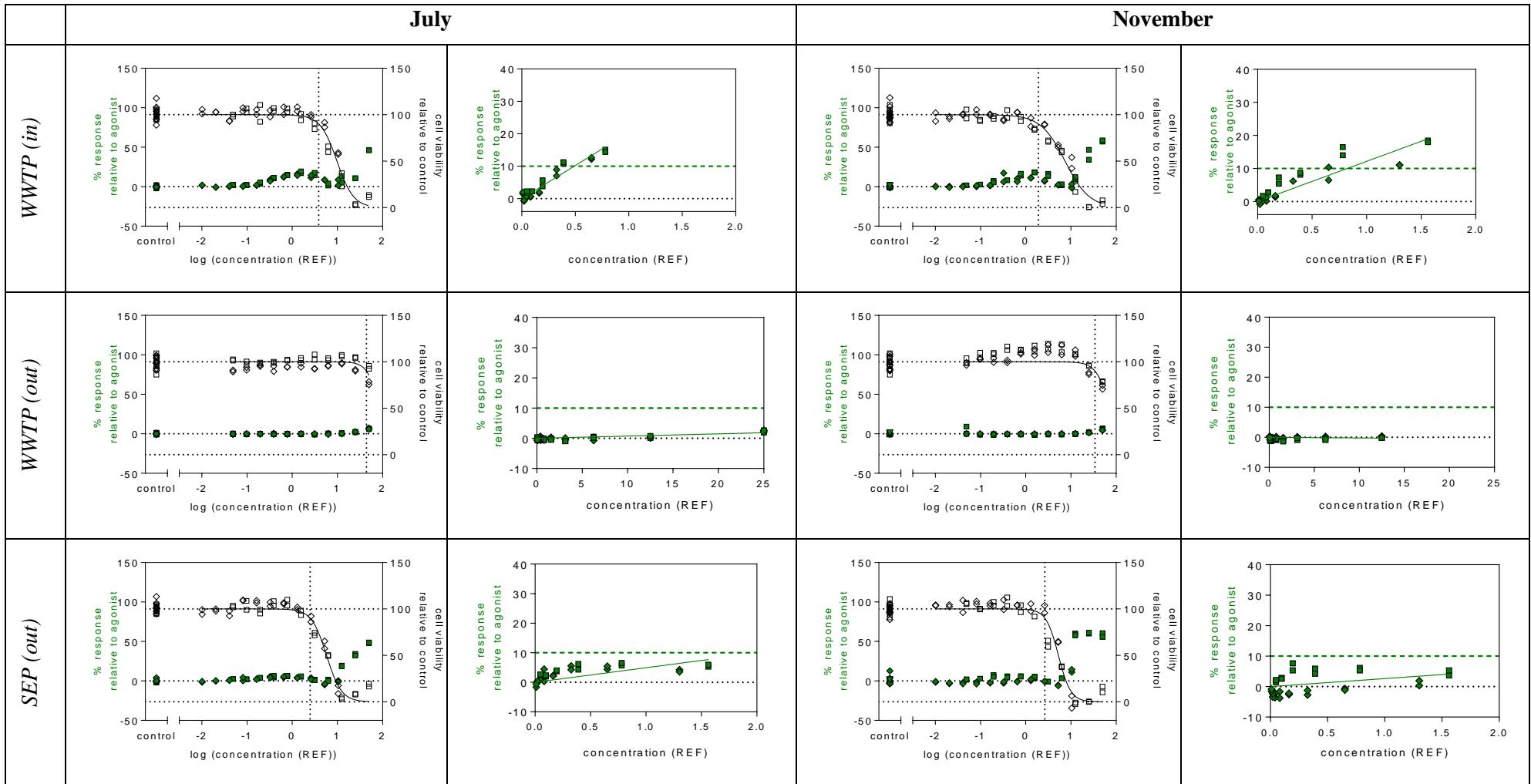


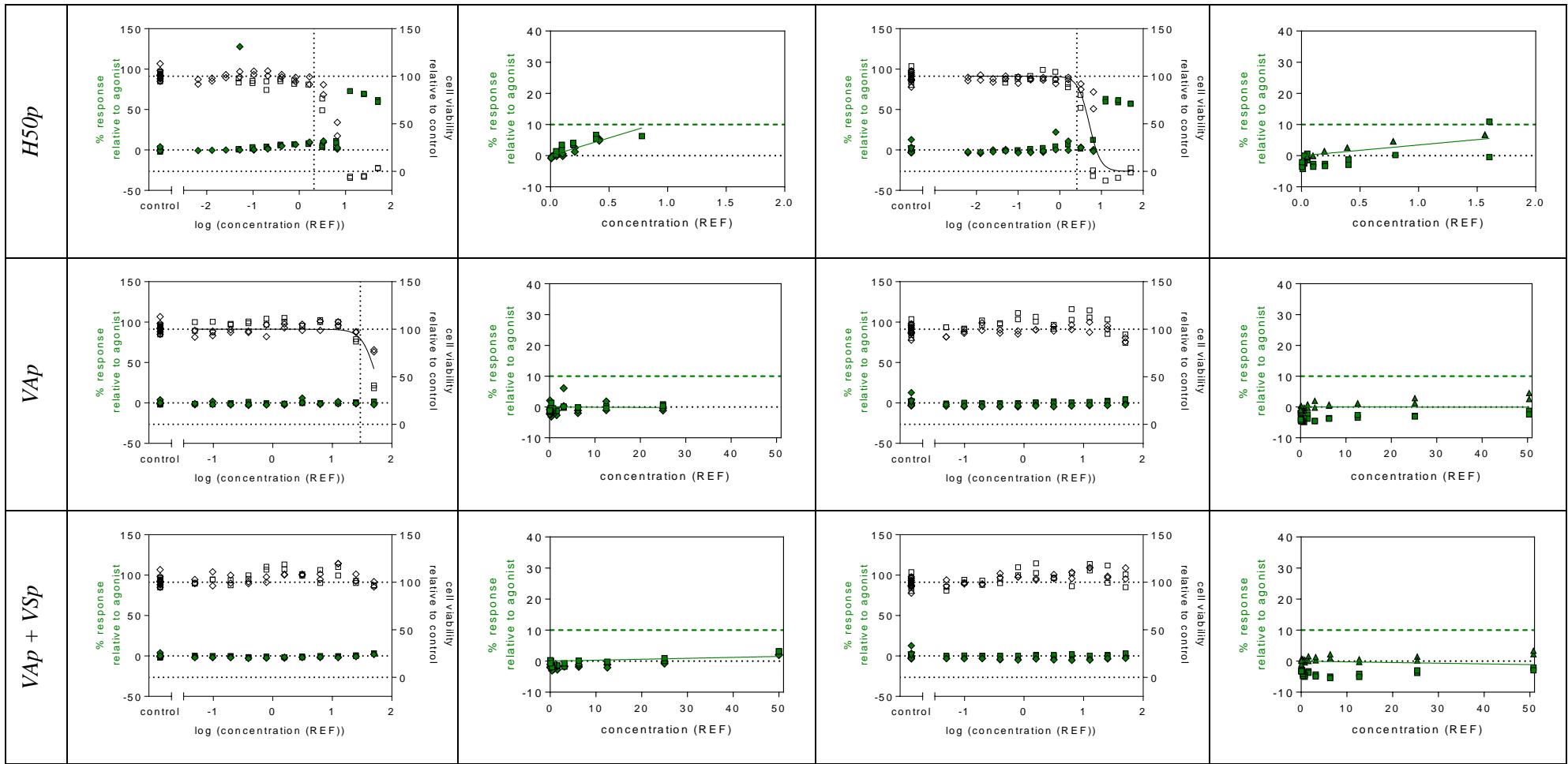


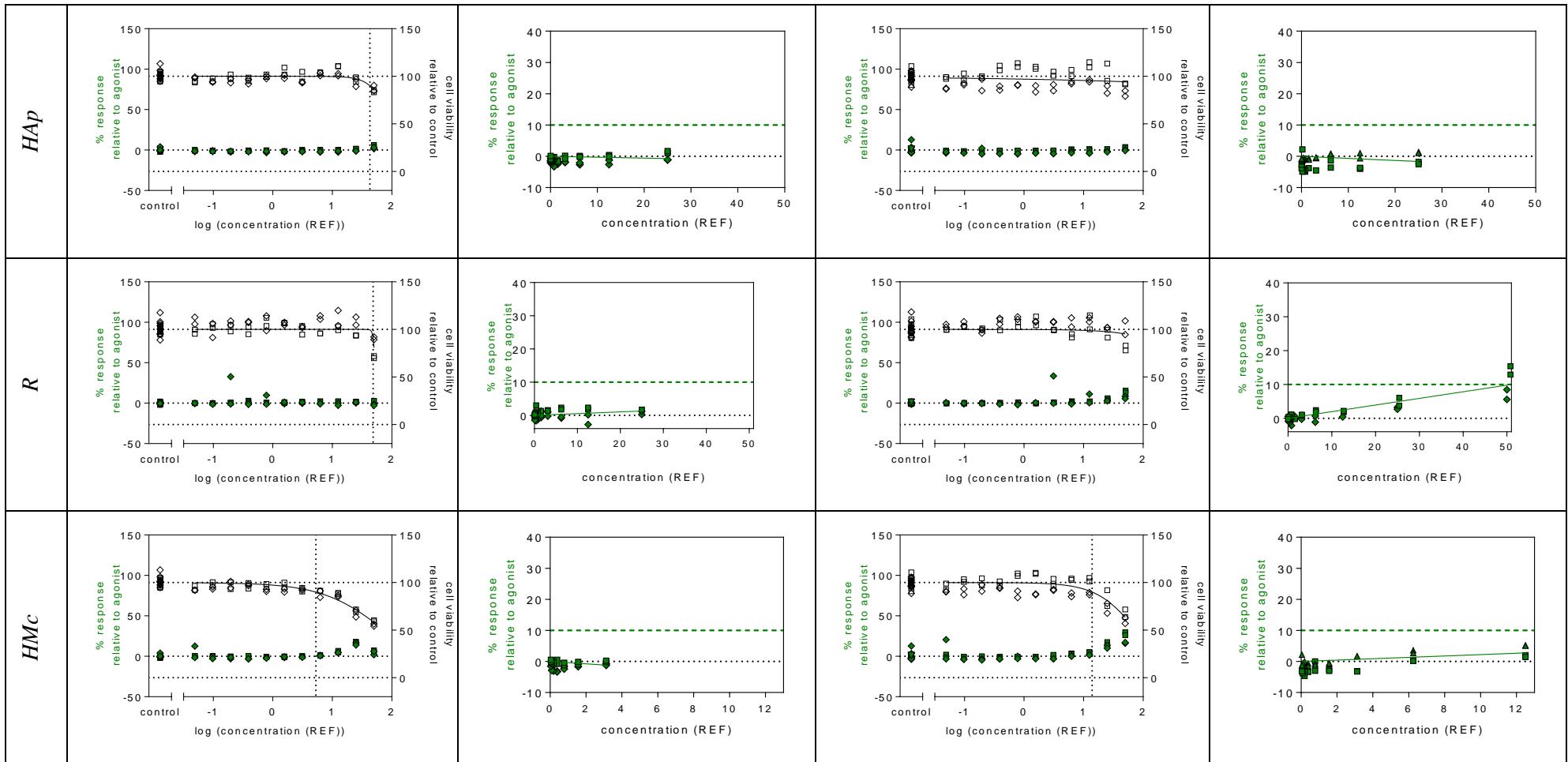
112

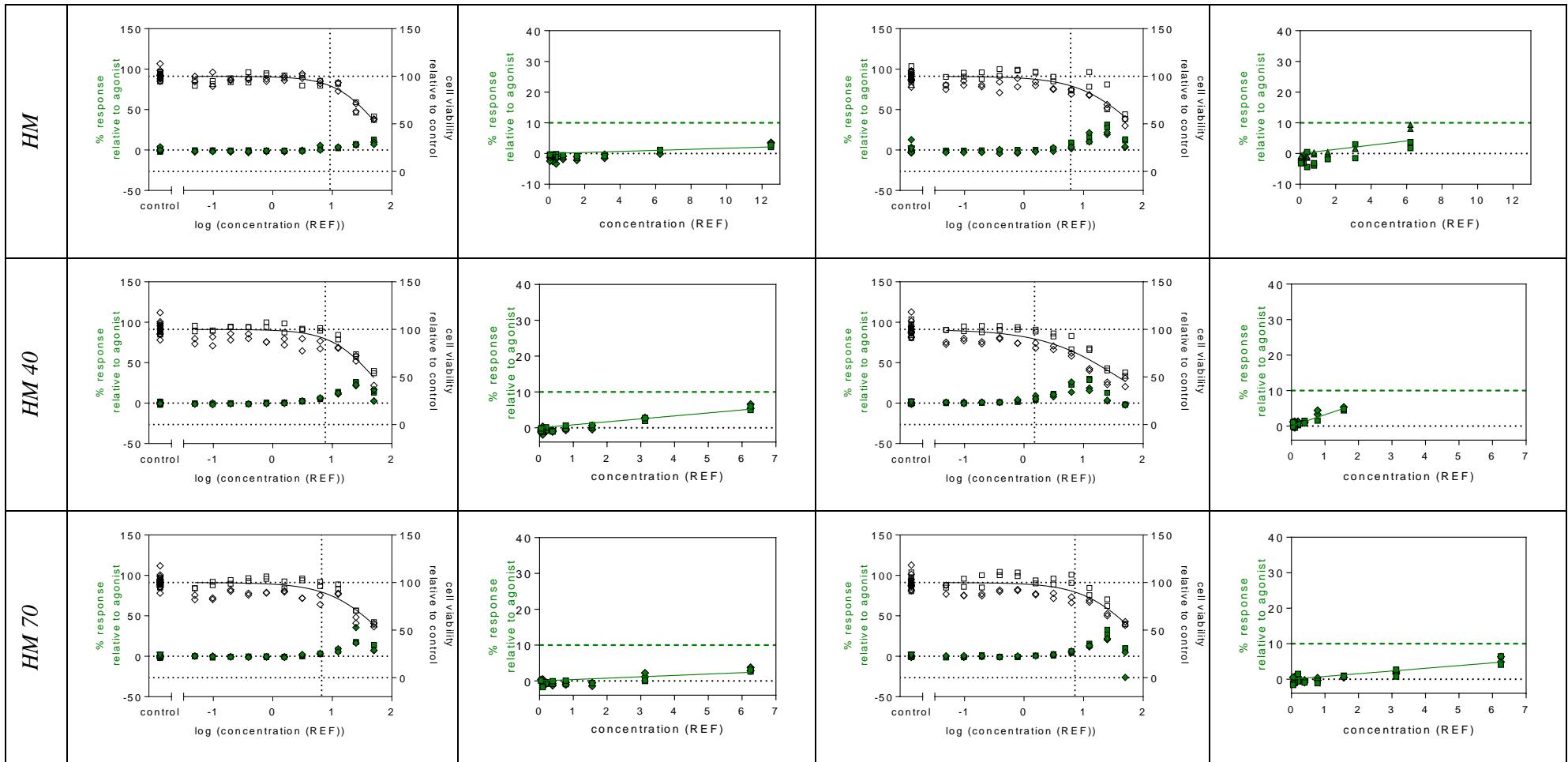
113

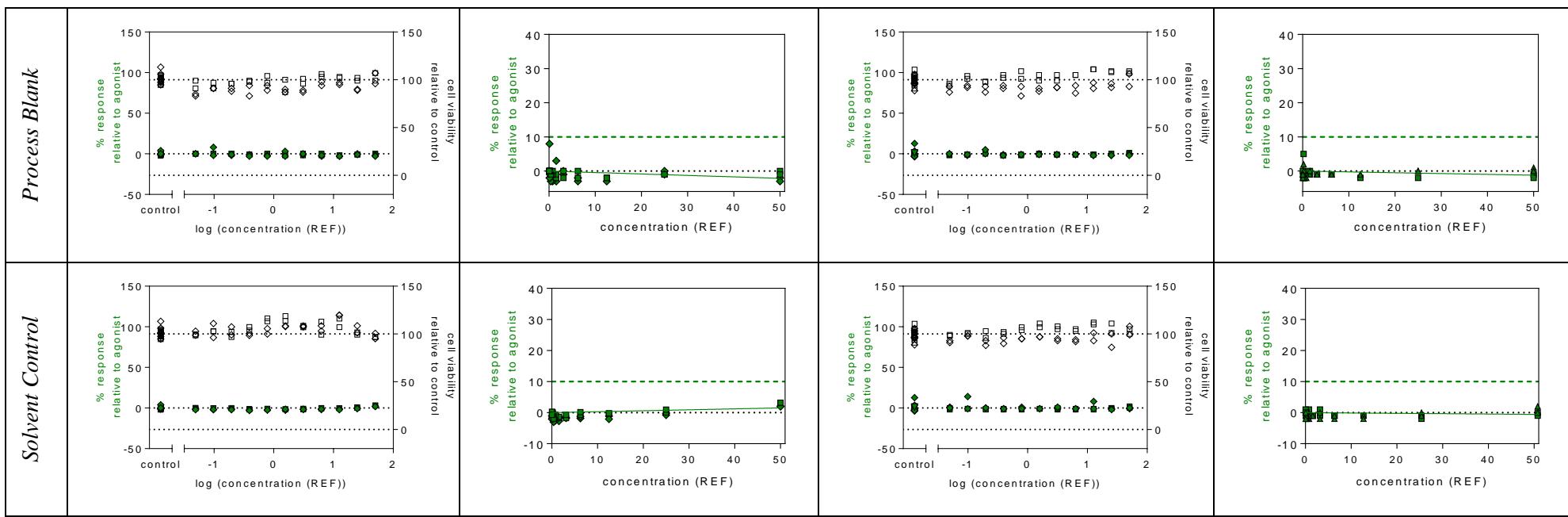
114 Figure S7: Full concentration-effect curves for induction (green filled symbols) and cell viability (empty symbols) in AR GeneBLAzer (agonist mode)
 115 (left plots), as well as linear concentration-effect curves for induction (right plots).







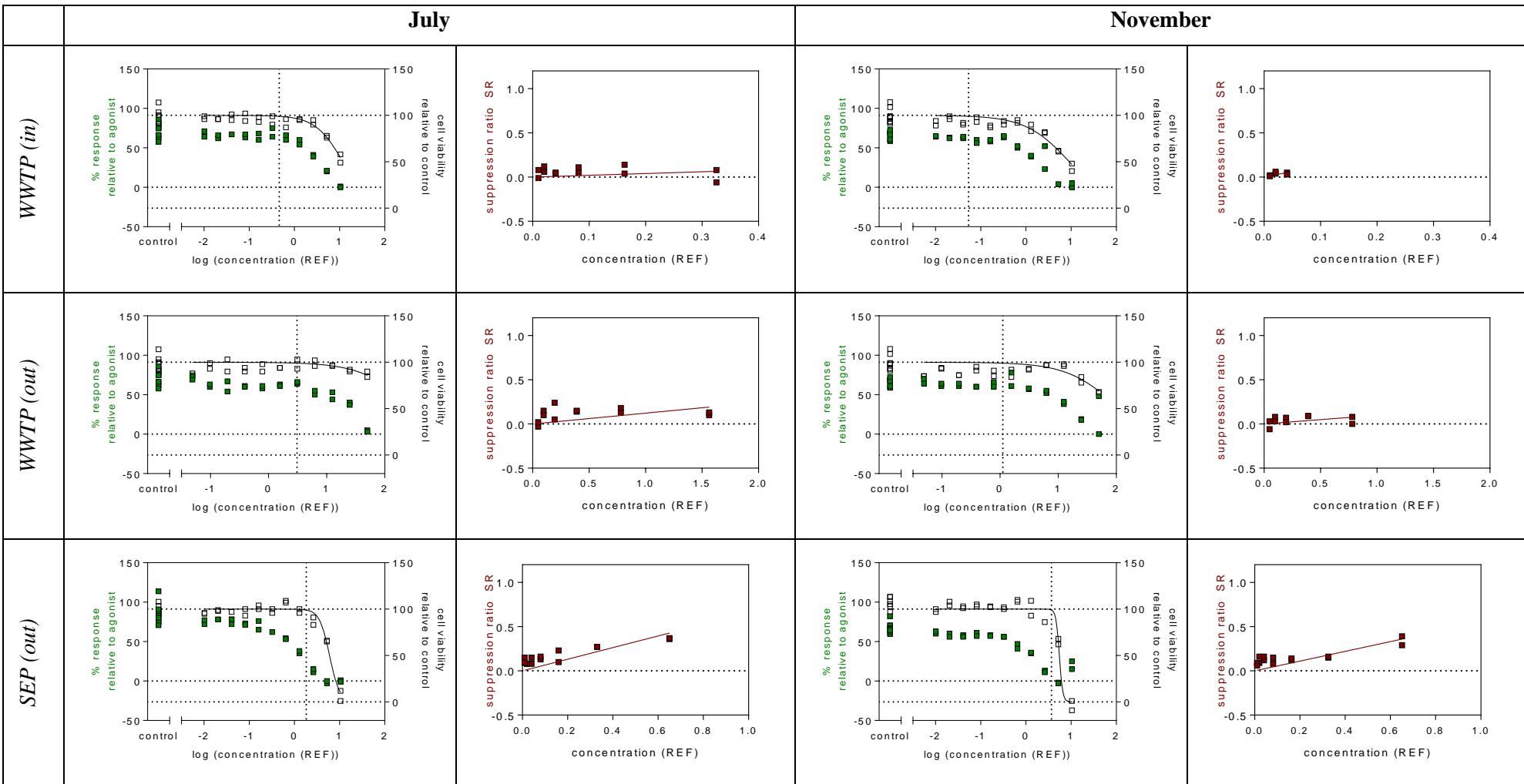


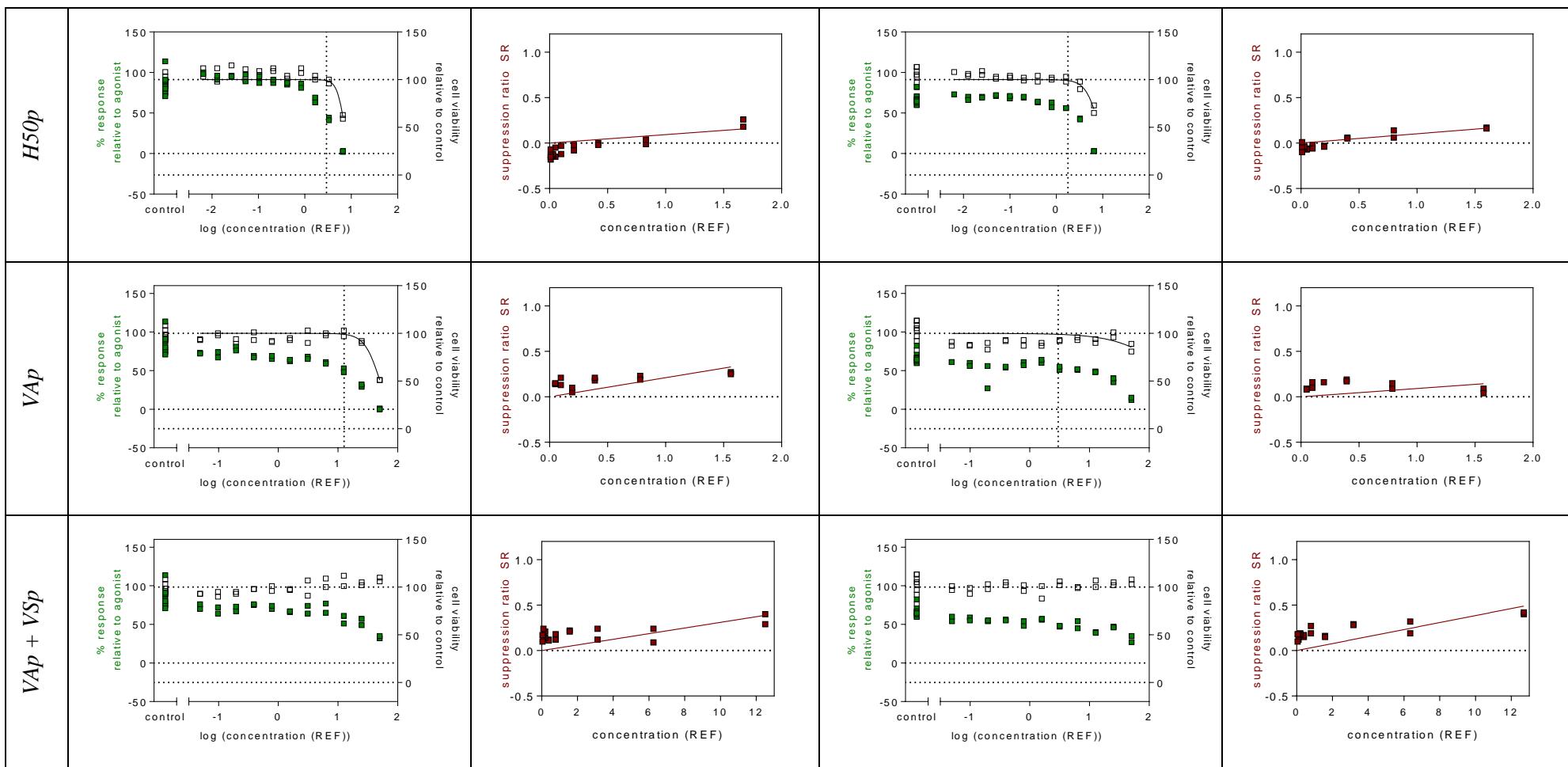


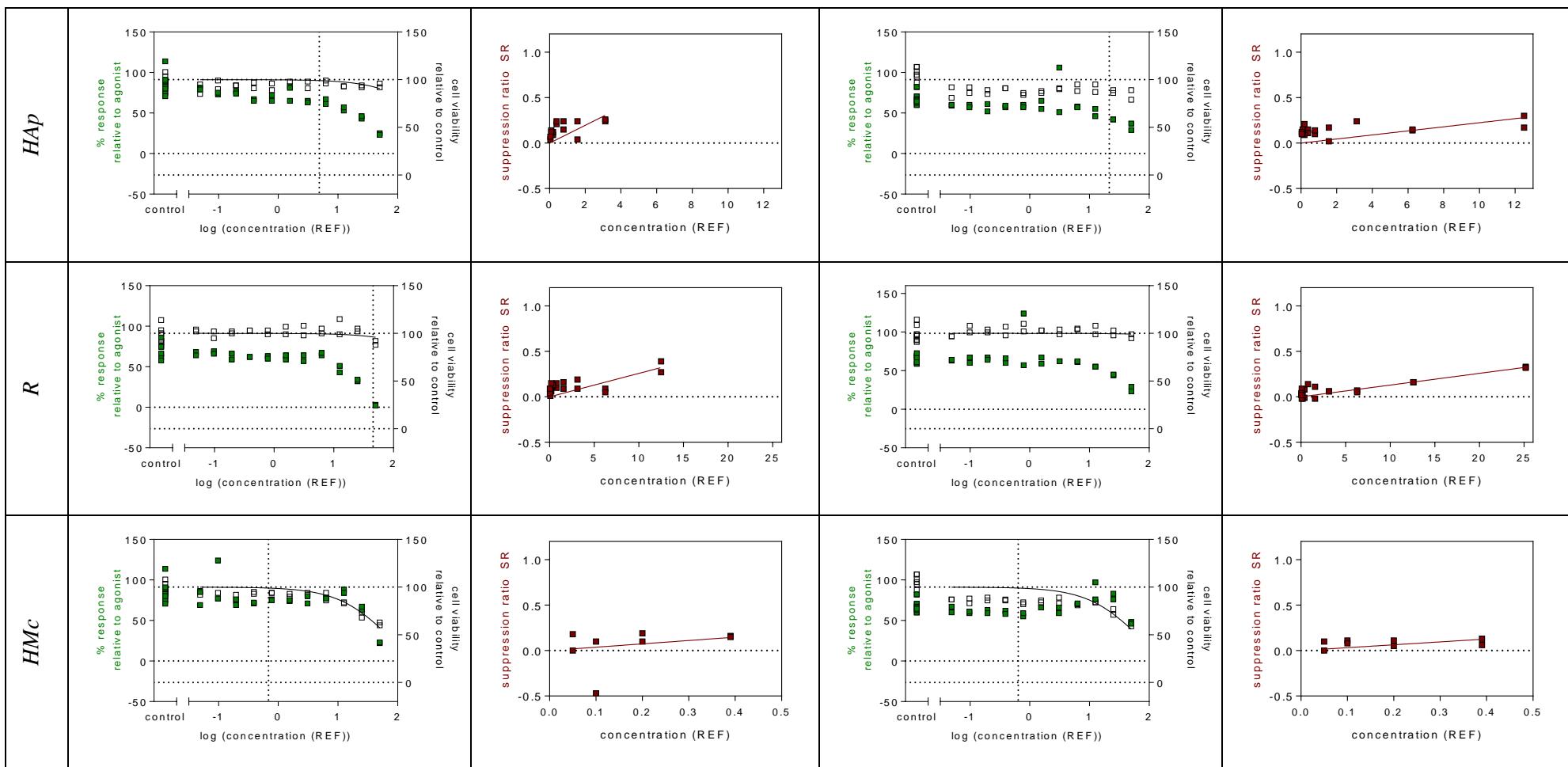
116

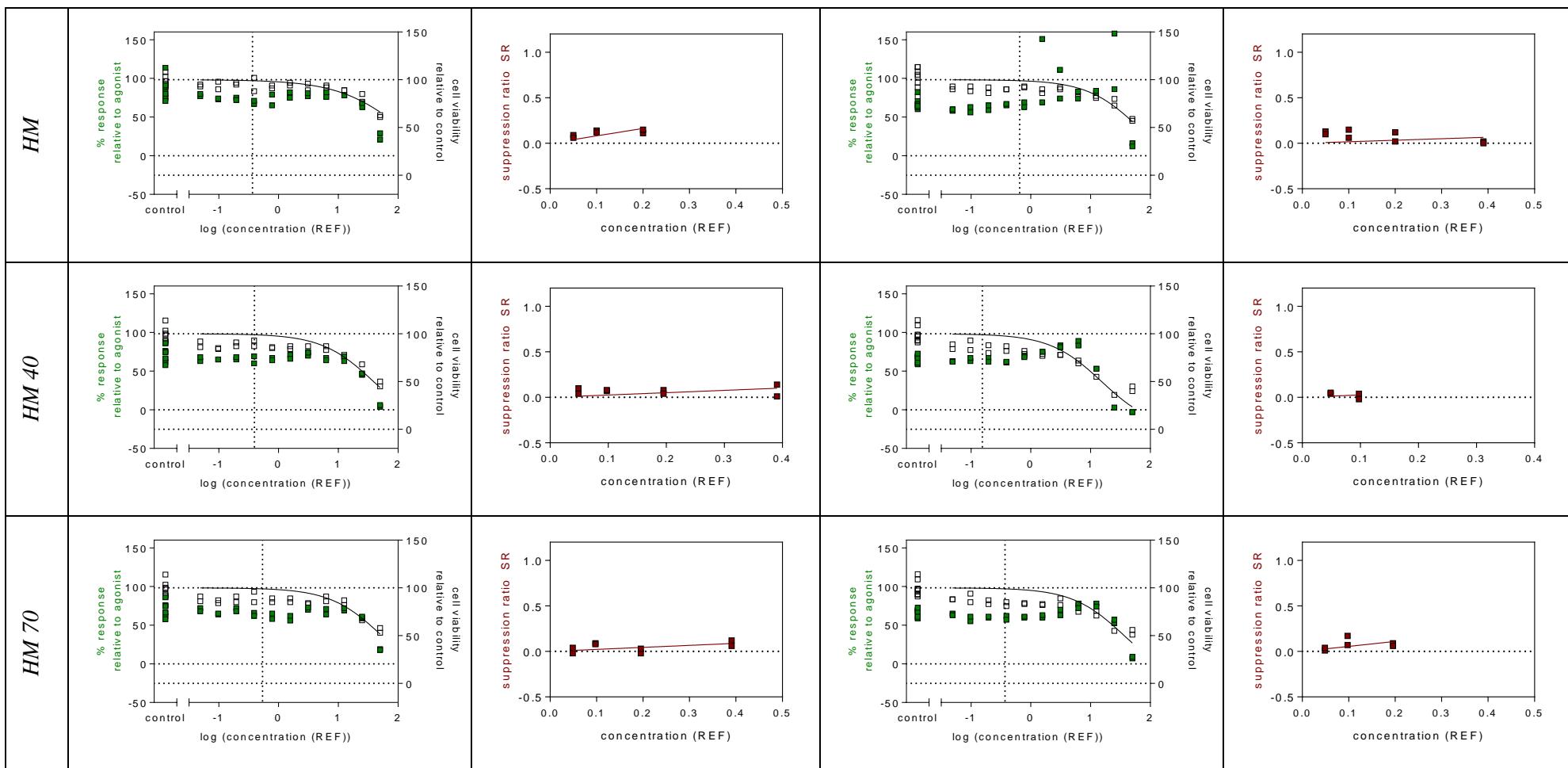
117

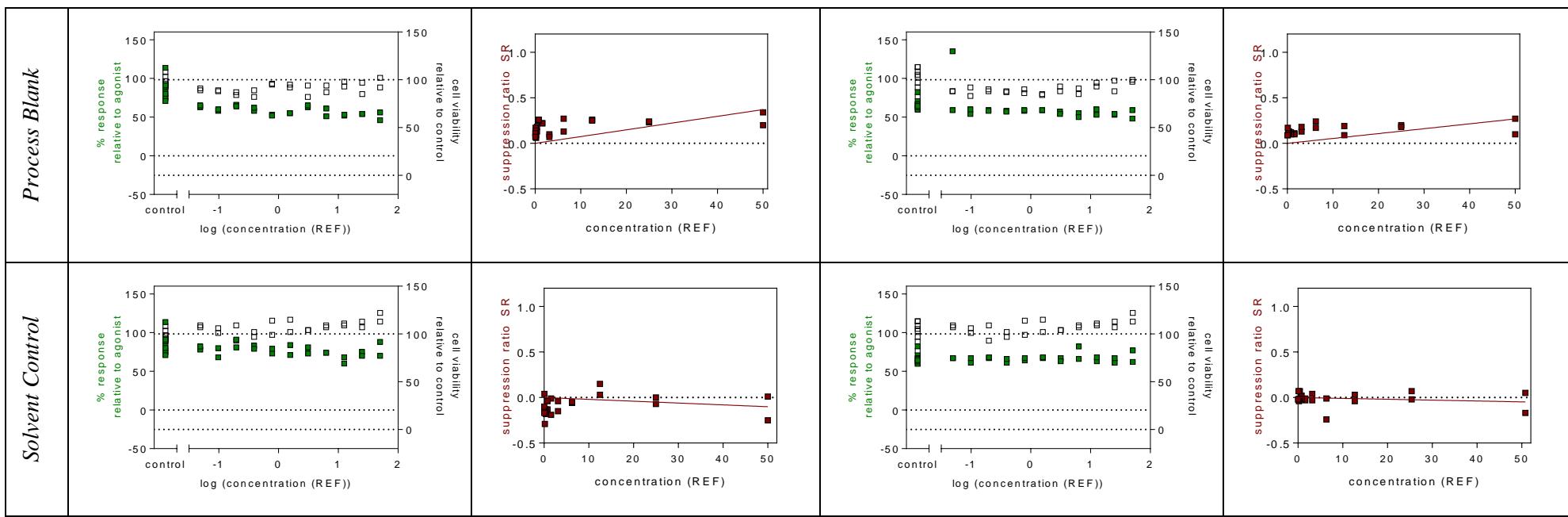
118 Figure S8: Full concentration-effect curves for inhibition (green filled symbols) and cell viability (empty symbols) in AR GeneBLAzer (antagonist
 119 mode) (left plots), as well as linear concentration-effect curves for suppression (right plots).







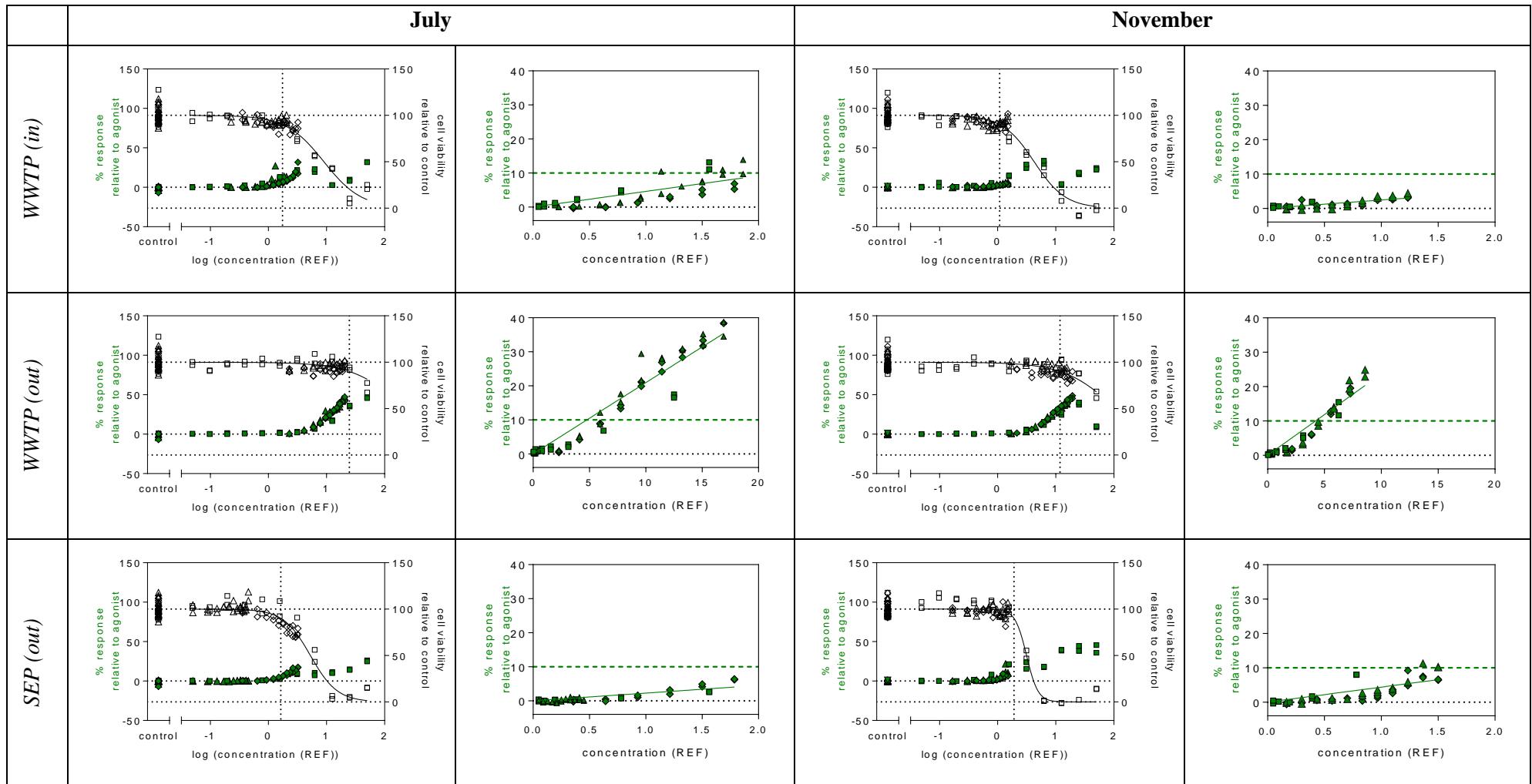


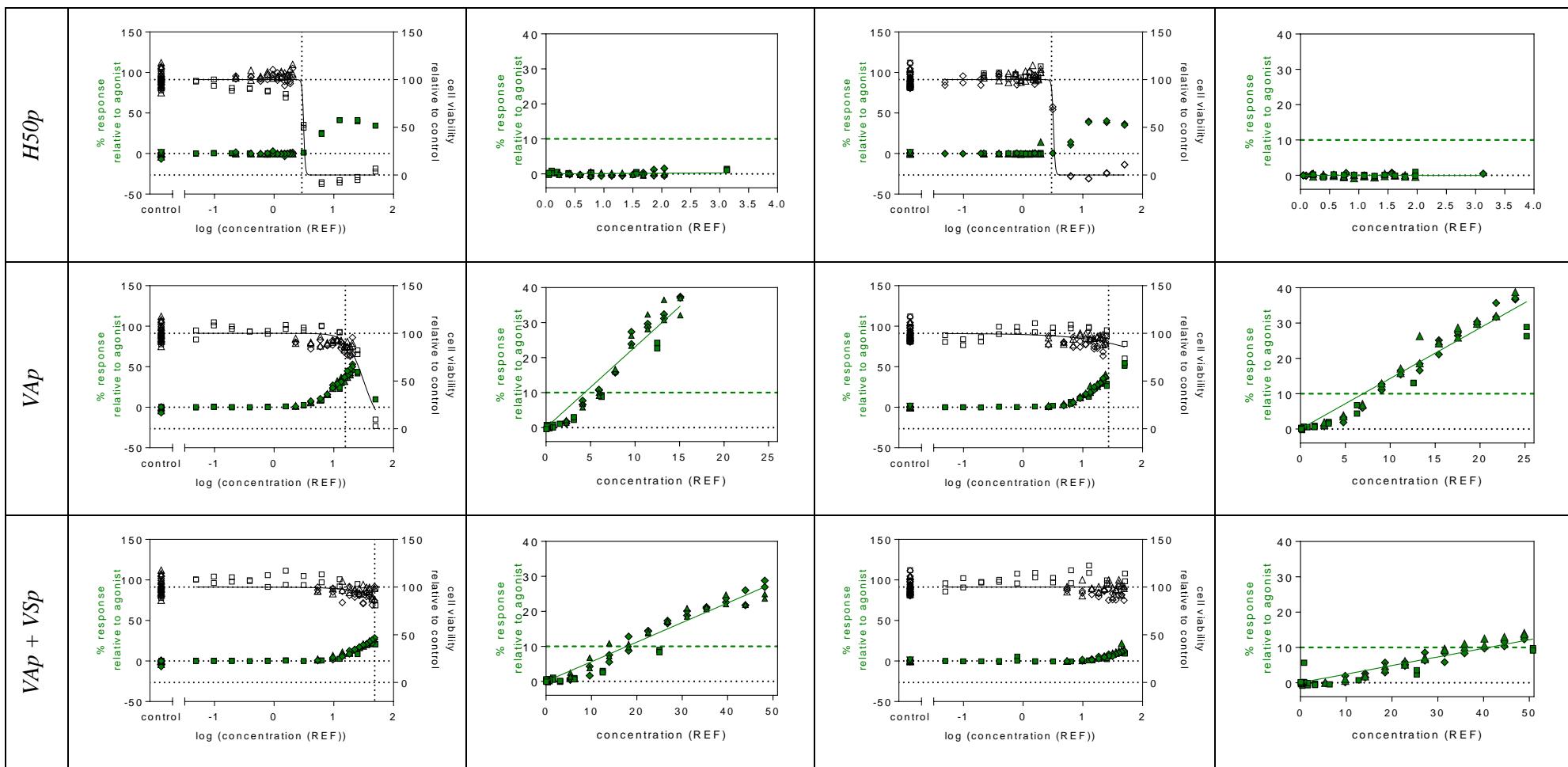


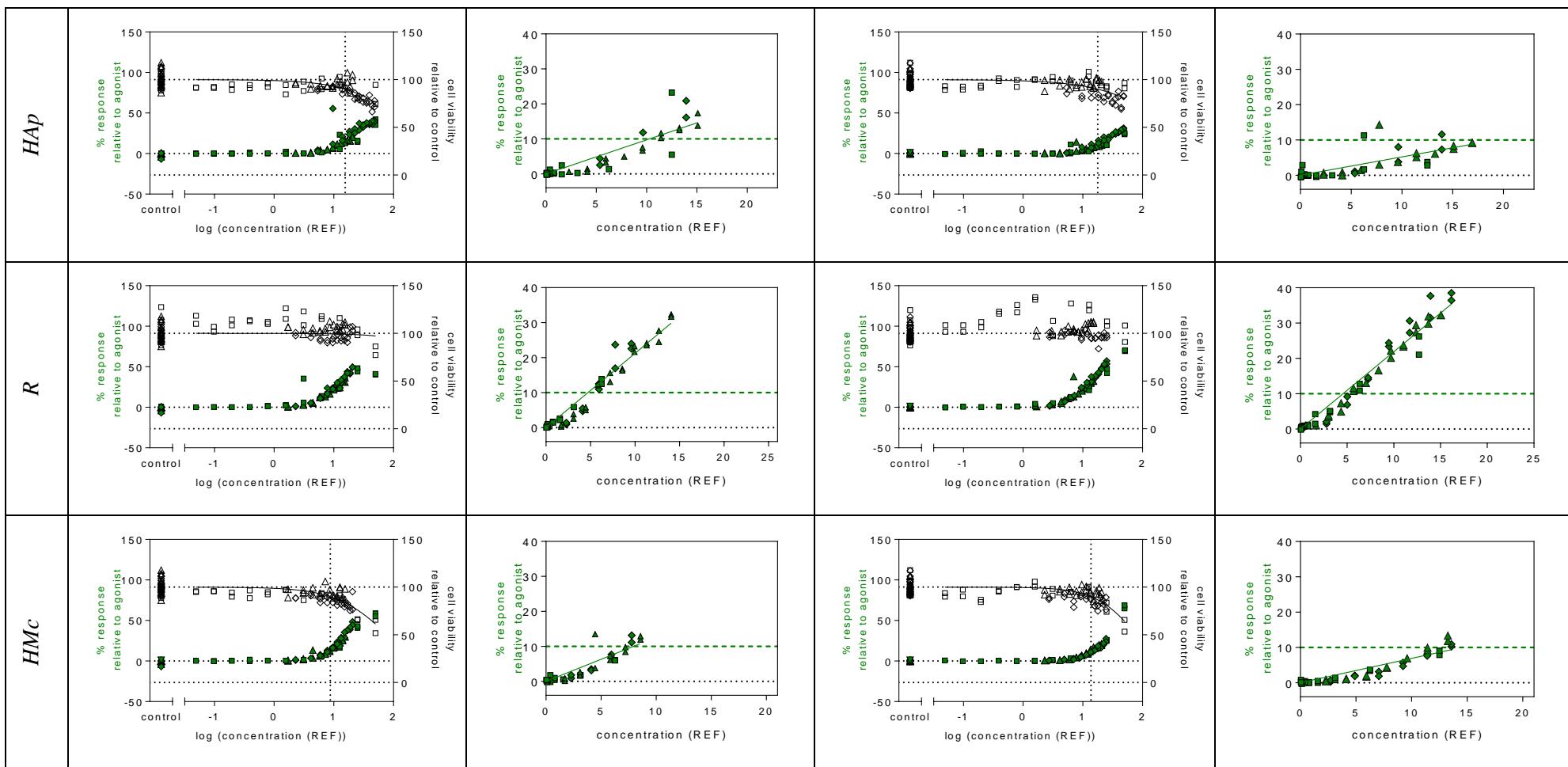
120

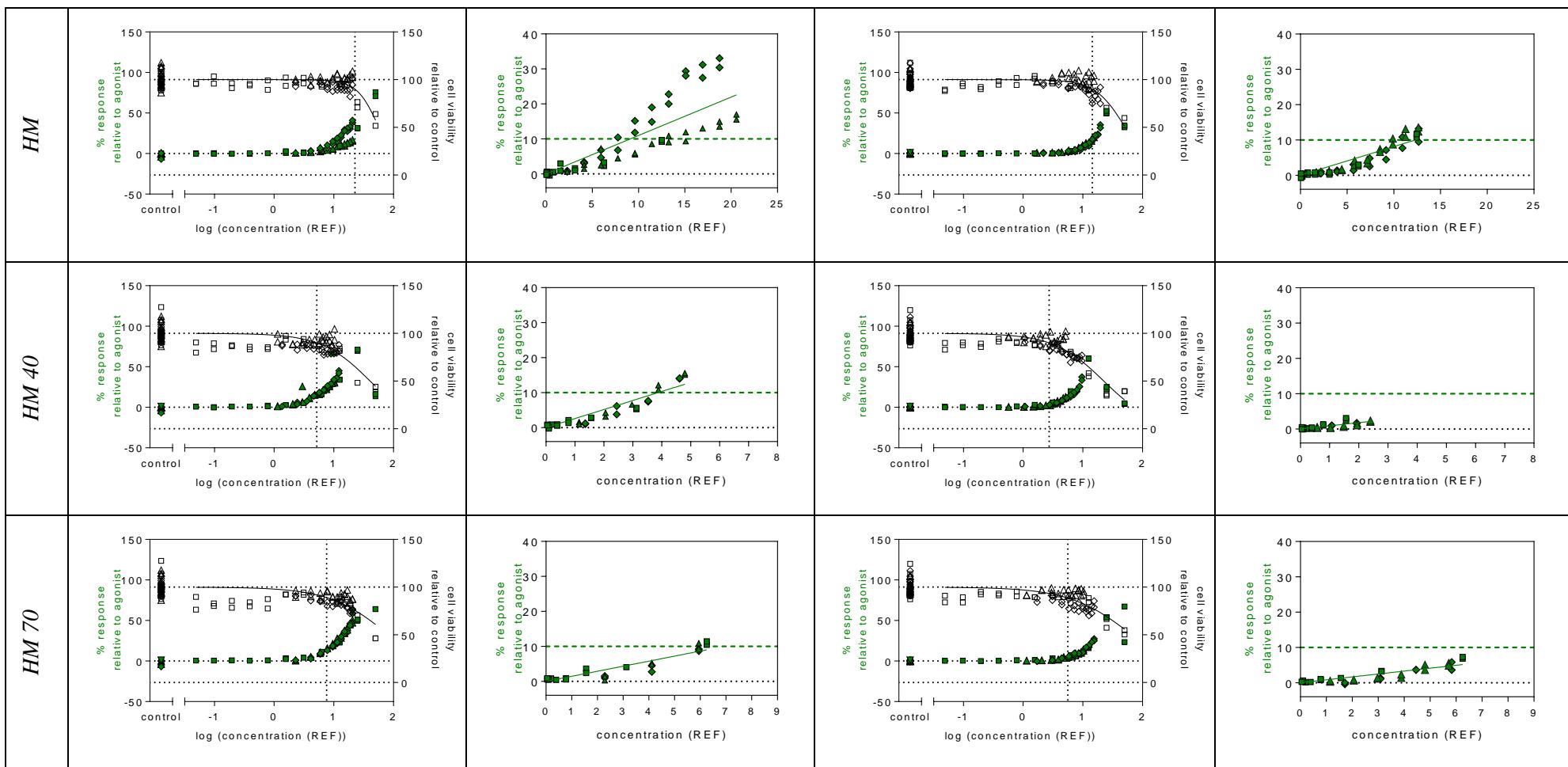
121

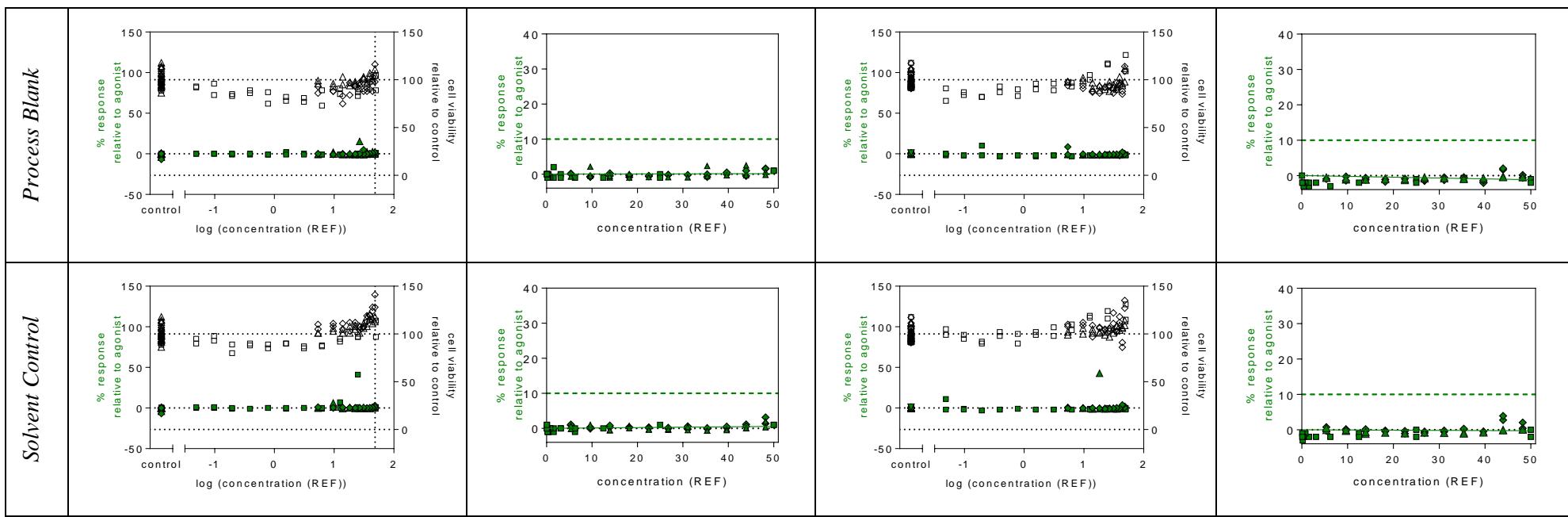
122 Figure S9: Full concentration-effect curves for induction (green filled symbols) and cell viability (empty symbols) in GR GeneBLAzer (agonist mode)
 123 (left plots), as well as linear concentration-effect curves for induction (right plots).







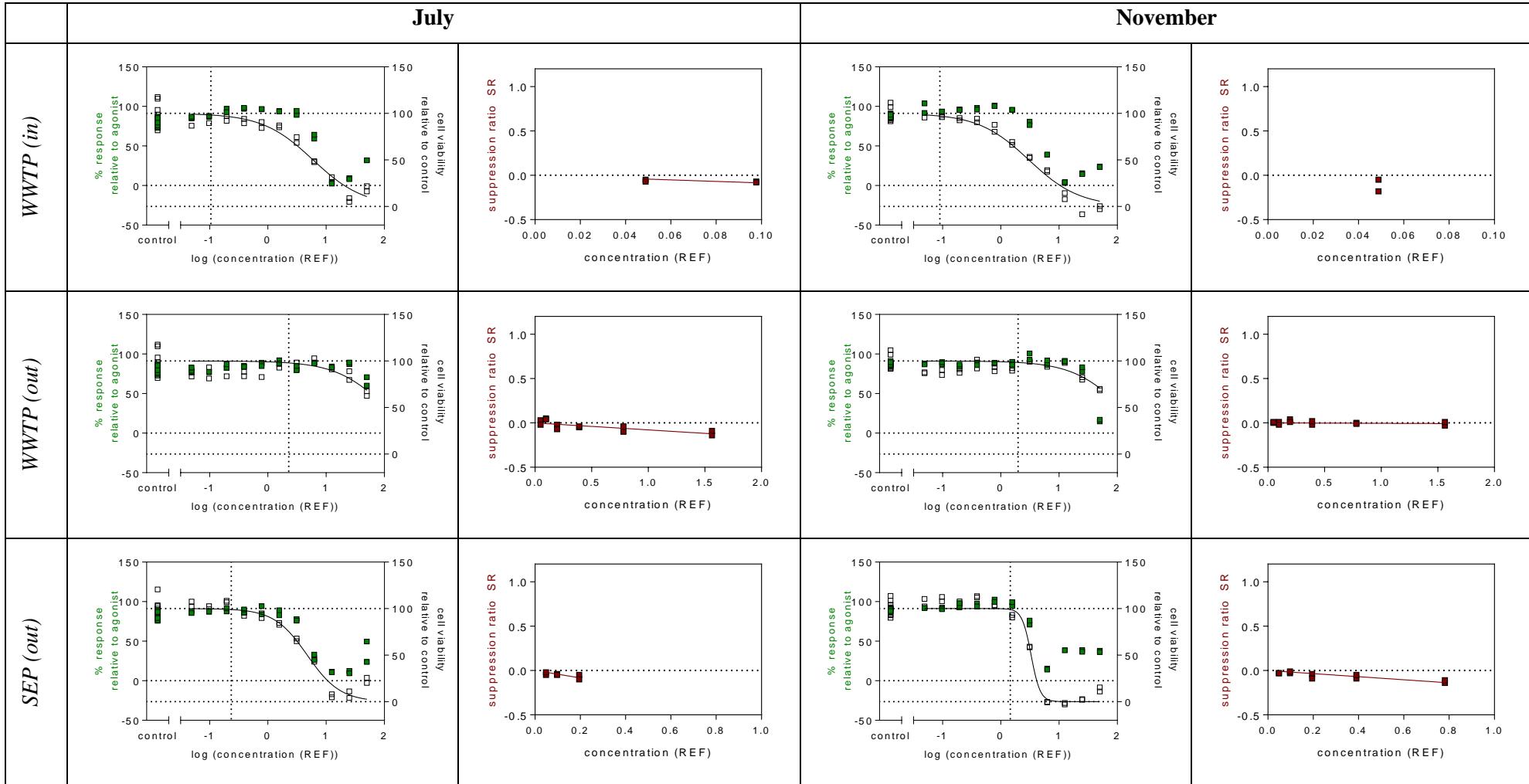


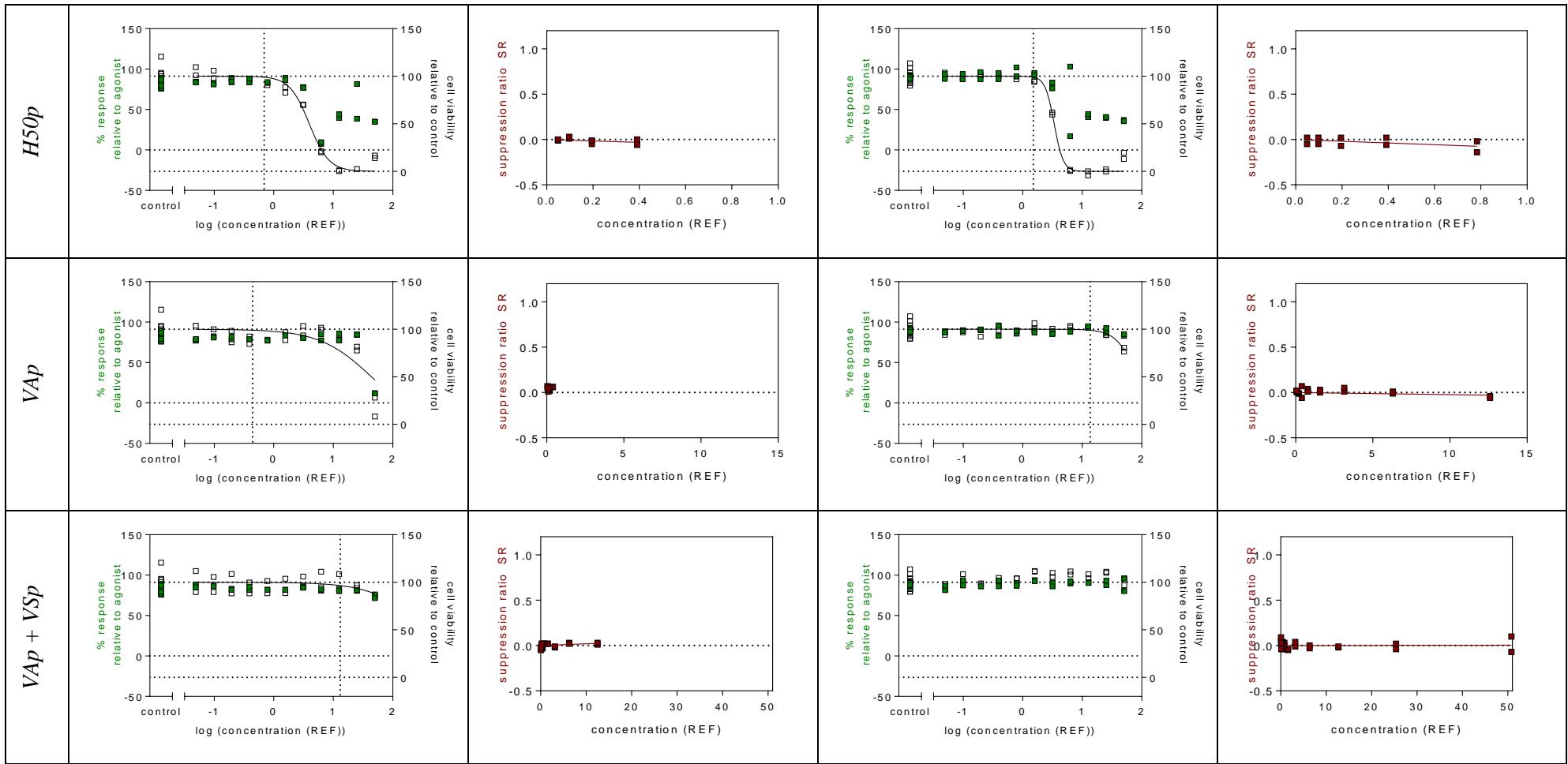


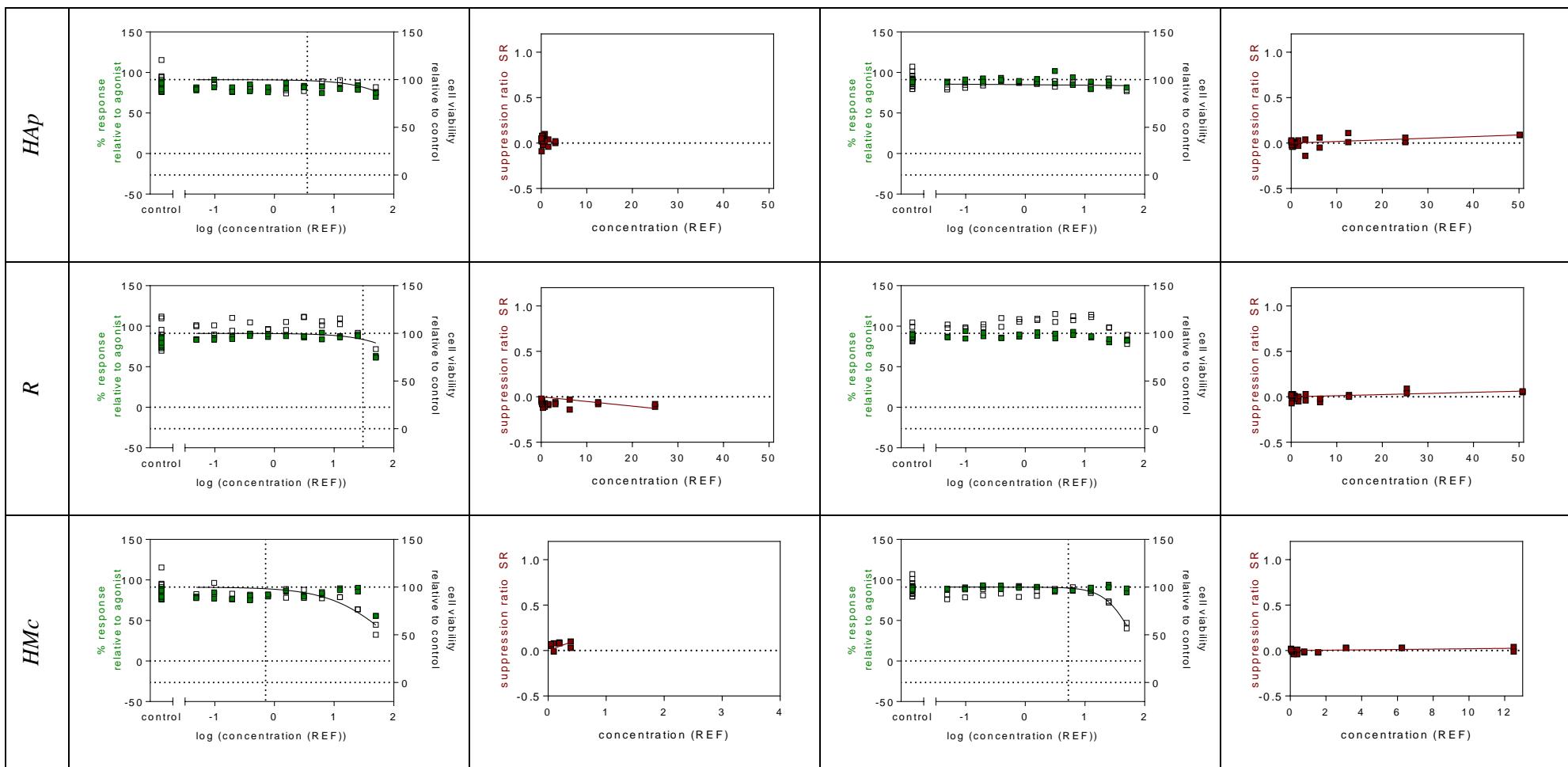
124

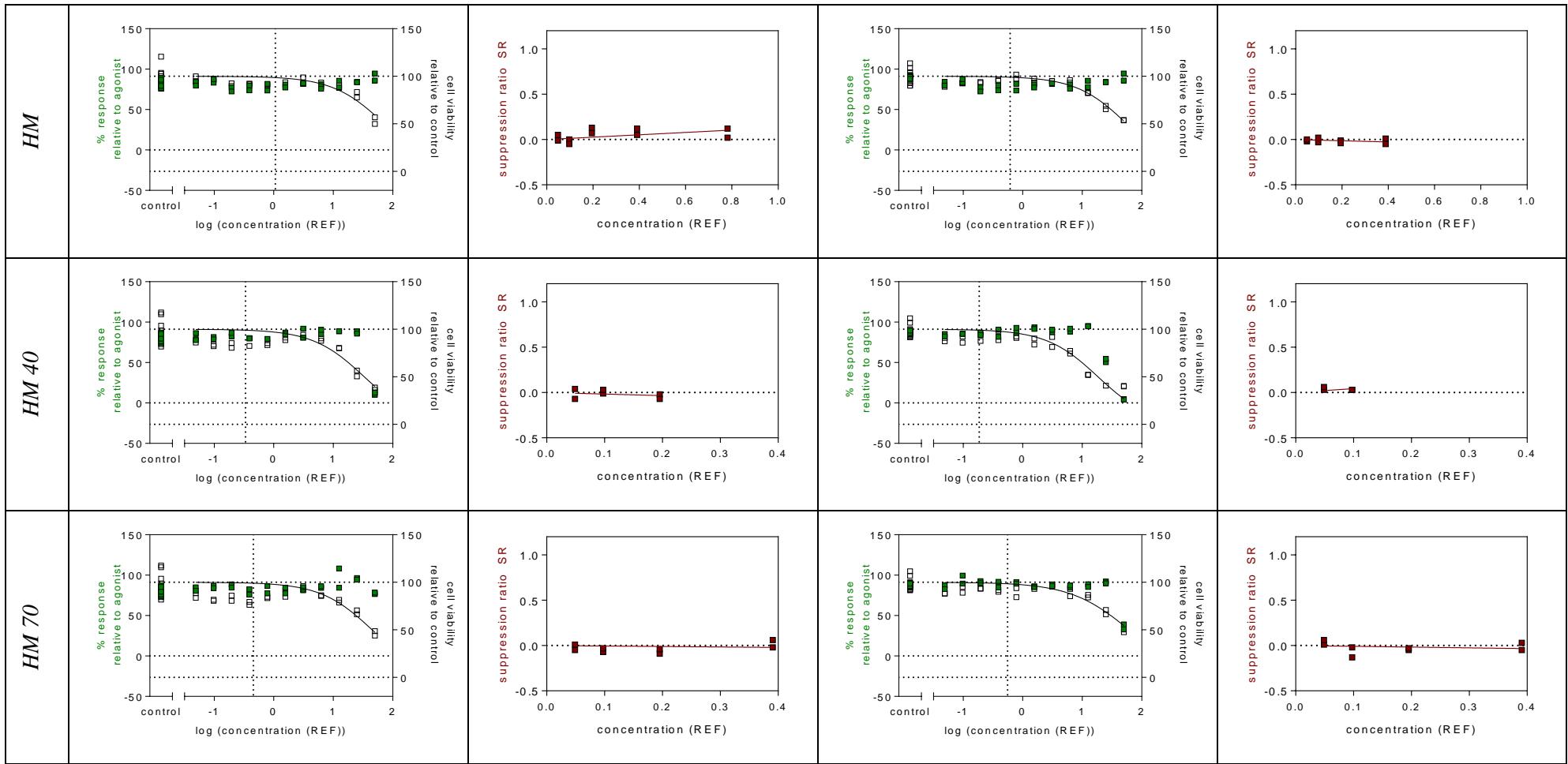
125

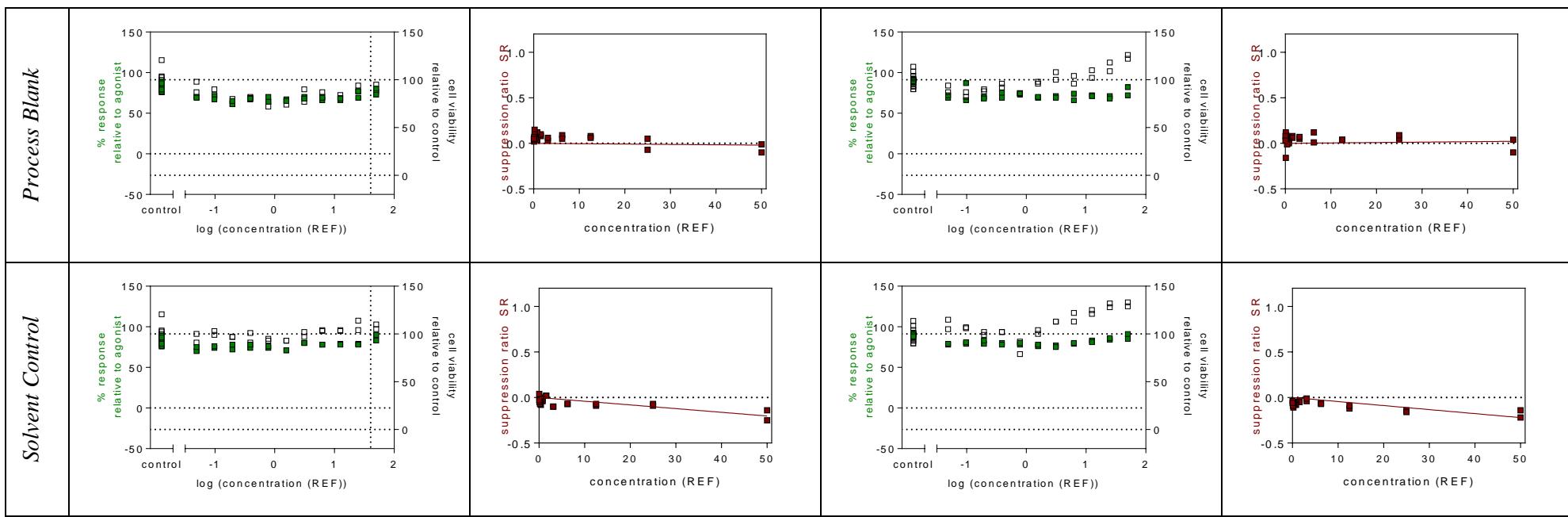
126 Figure S10: Full concentration-effect curves for inhibition (green filled symbols) and cell viability (empty symbols) in GR GeneBLAzer (antagonist
 127 mode) (left plots), as well as linear concentration-effect curves for suppression (right plots).







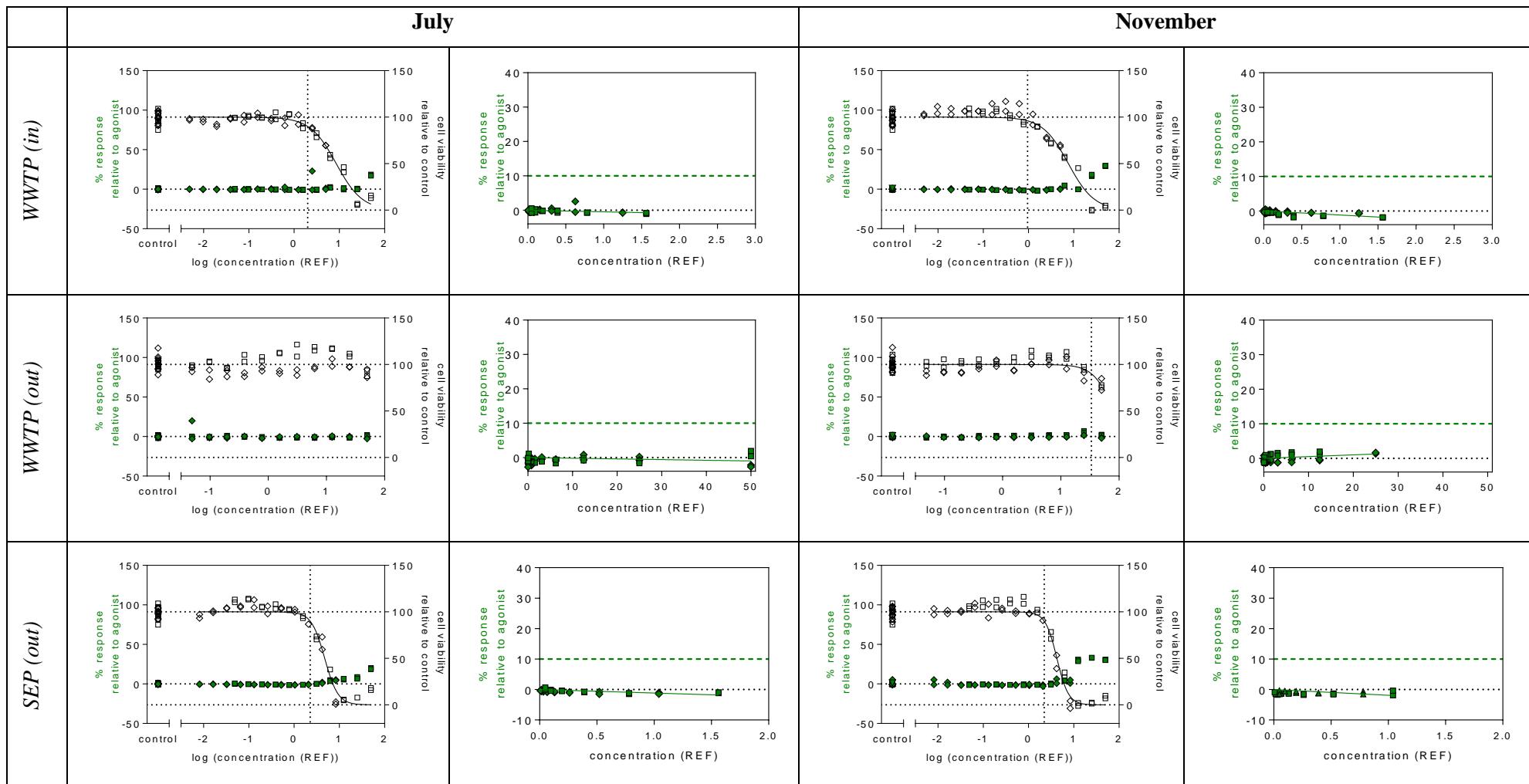


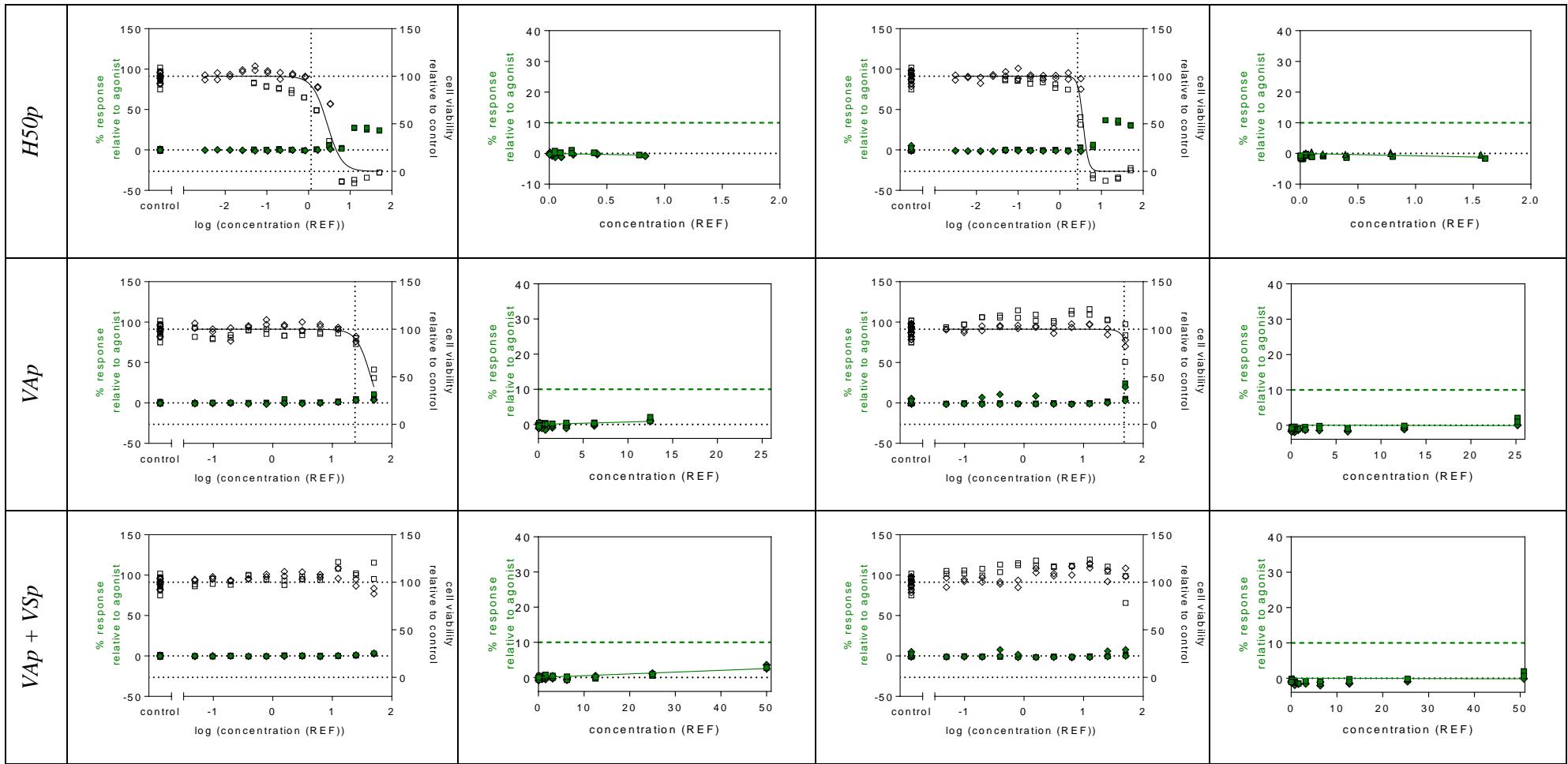


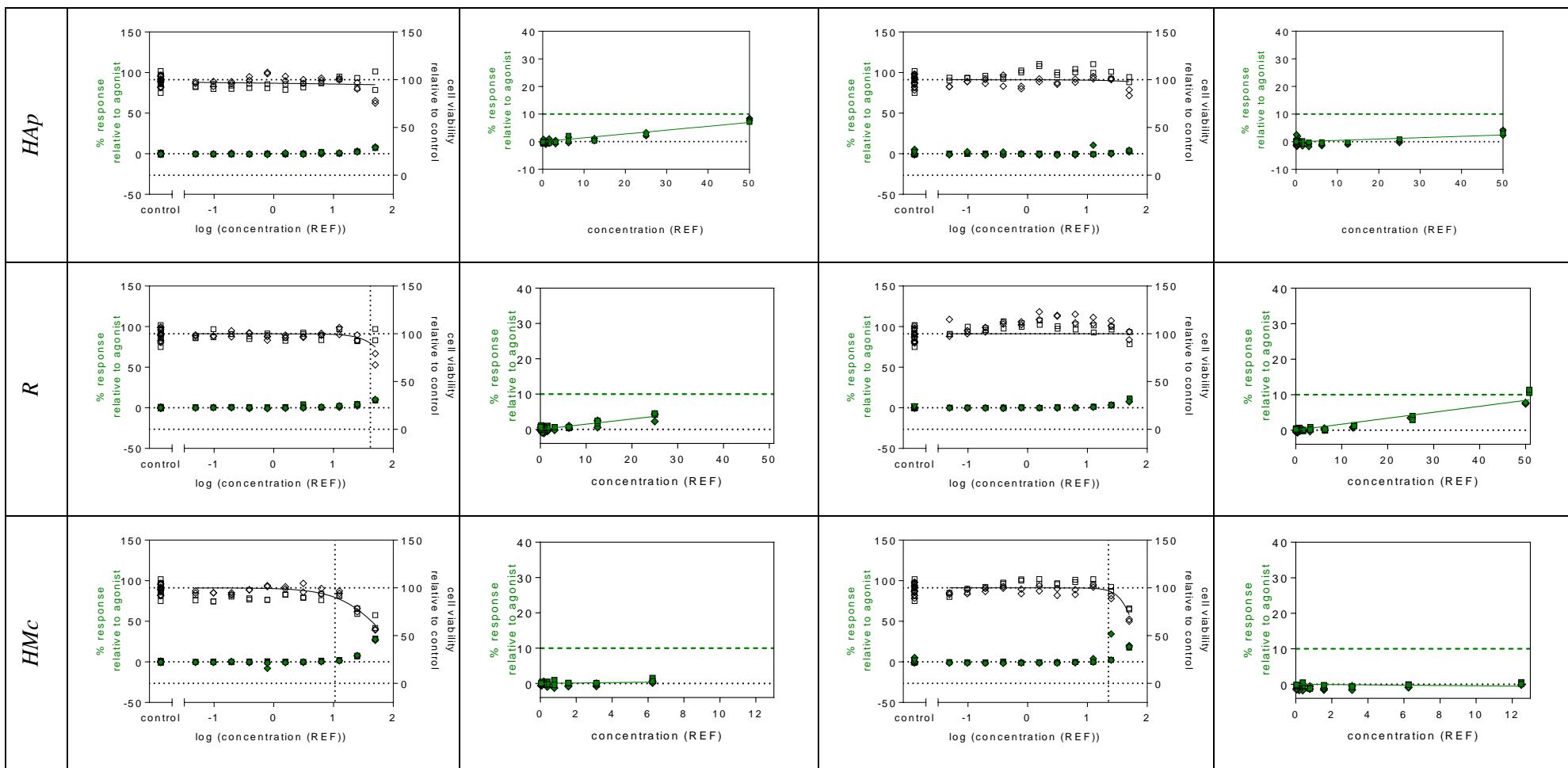
128

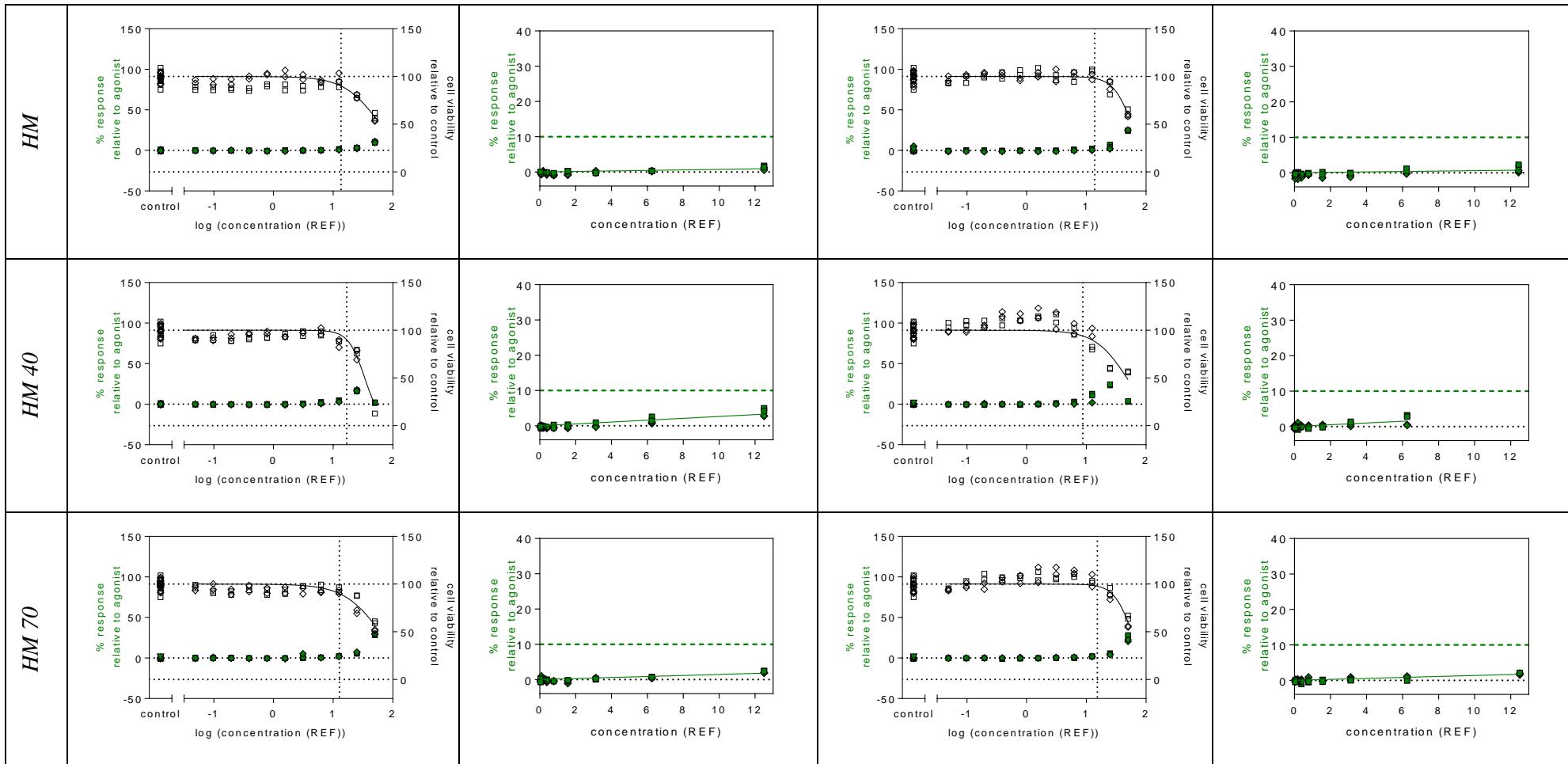
129

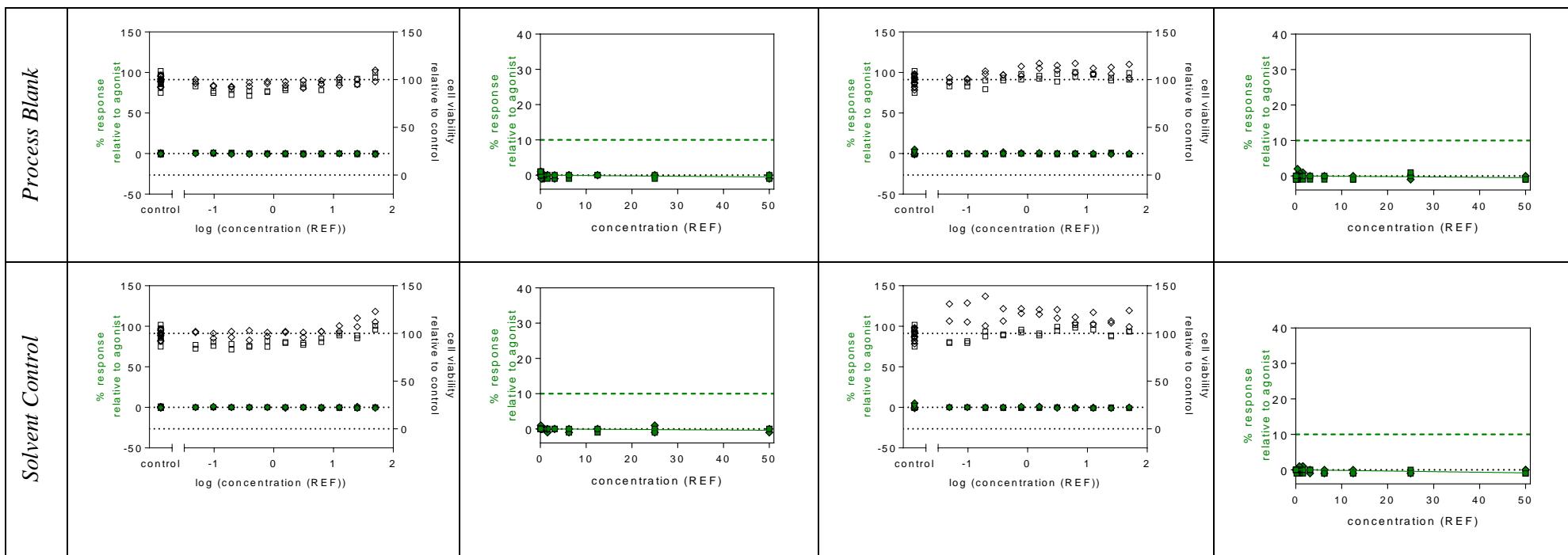
130 Figure S11: Full concentration-effect curves for induction (green filled symbols) and cell viability (empty symbols) in PR GeneBLAzer (agonist mode)
 131 (left plots), as well as linear concentration-effect curves for induction (right plots).







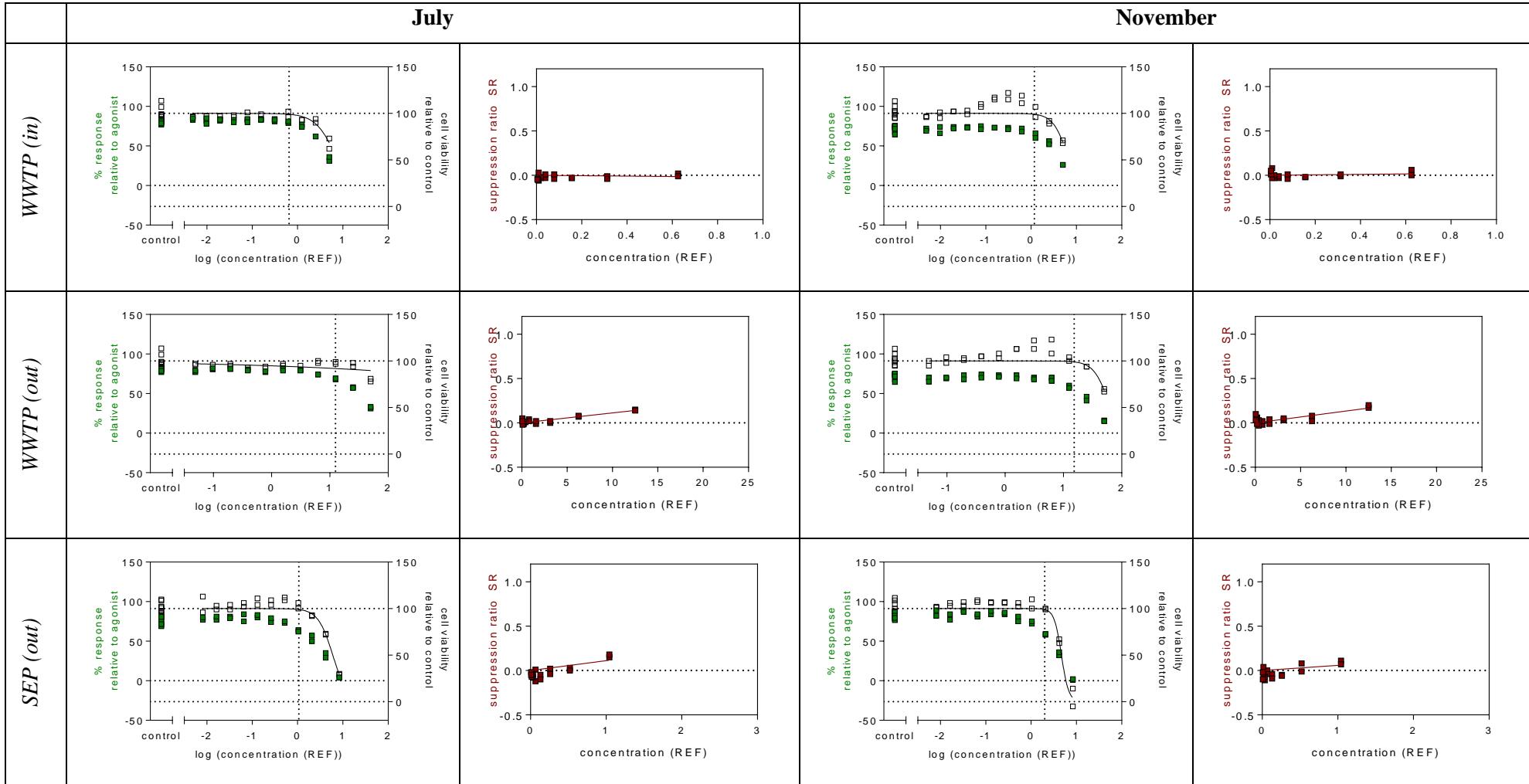


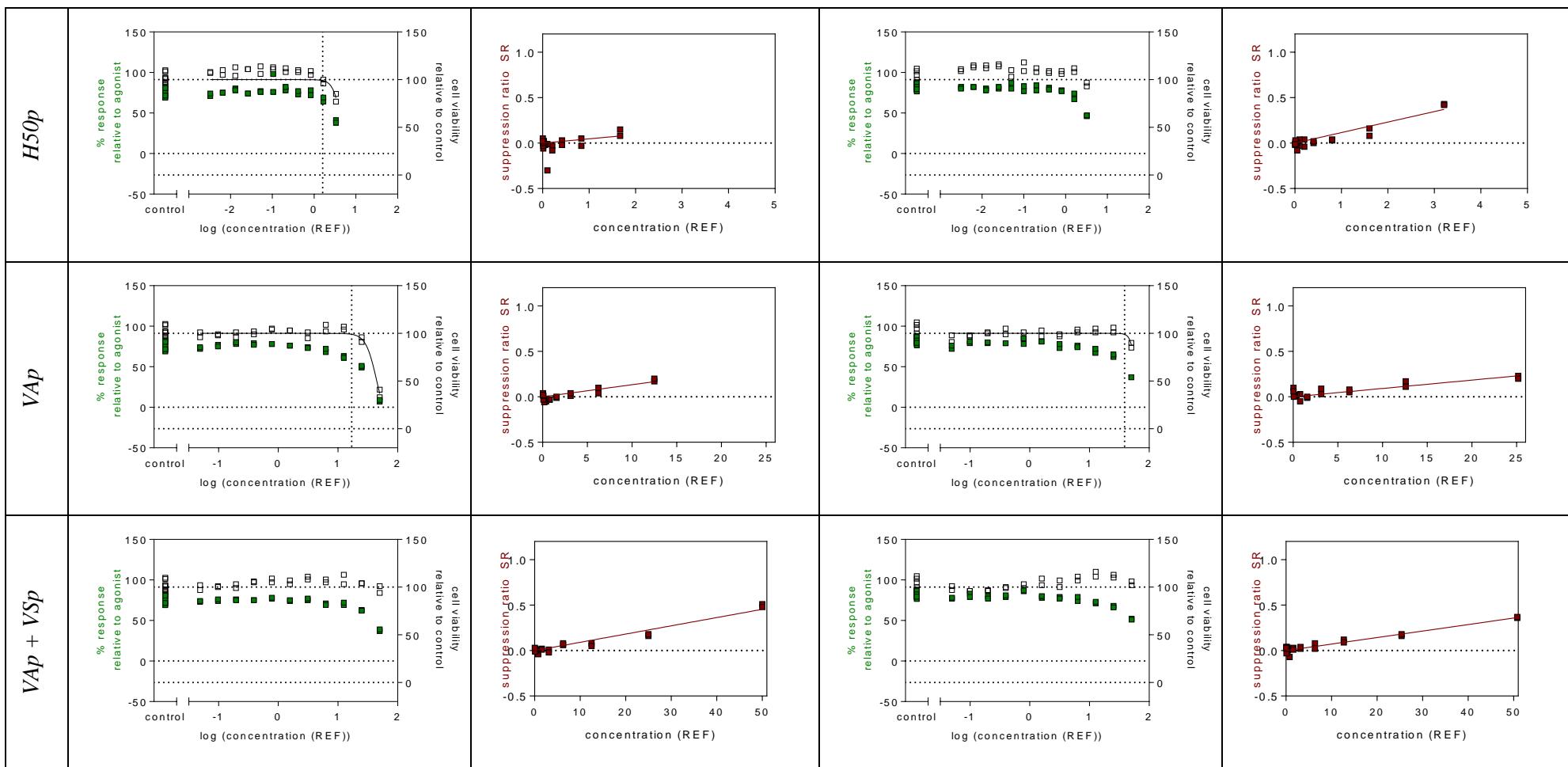


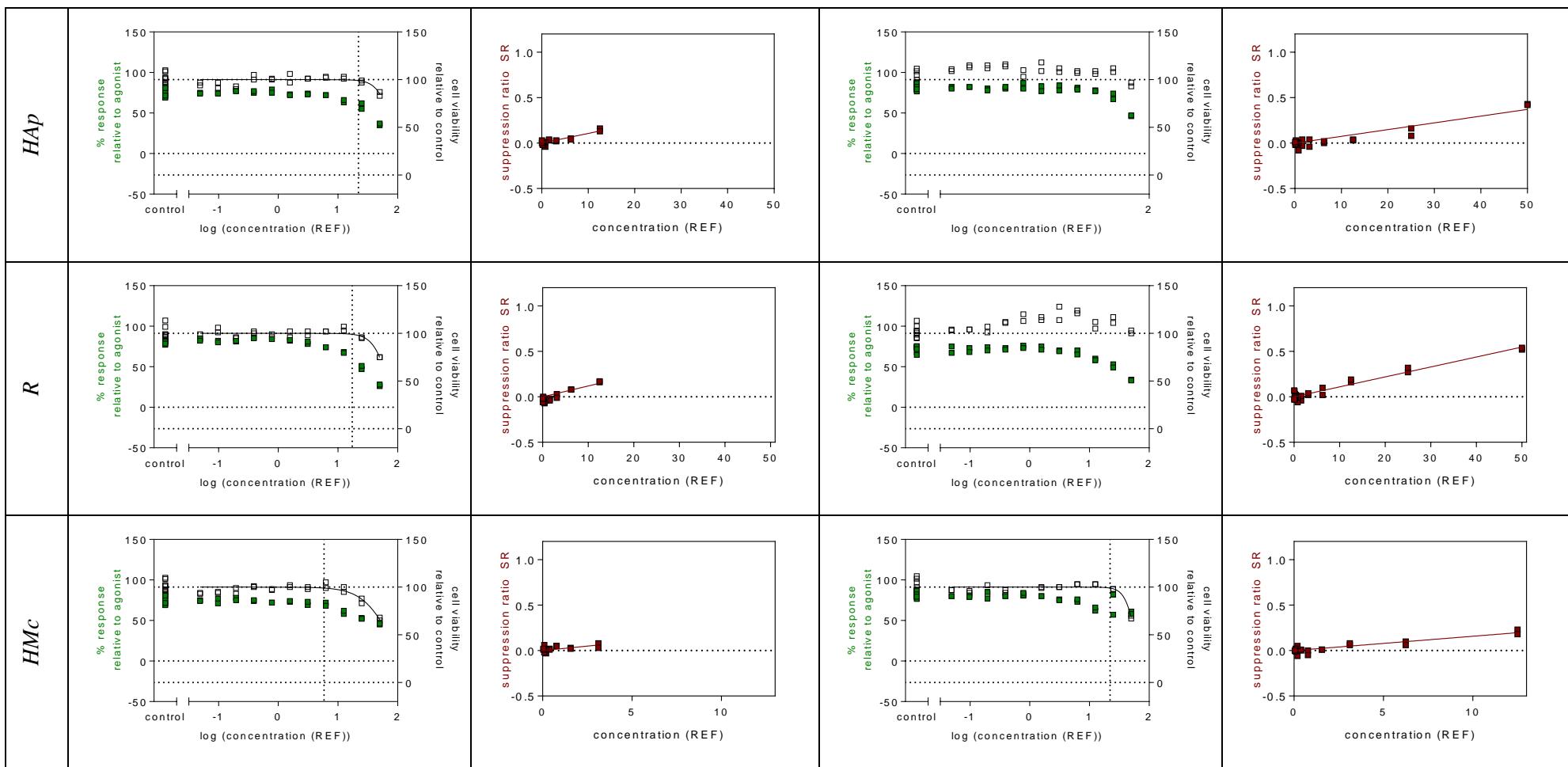
132

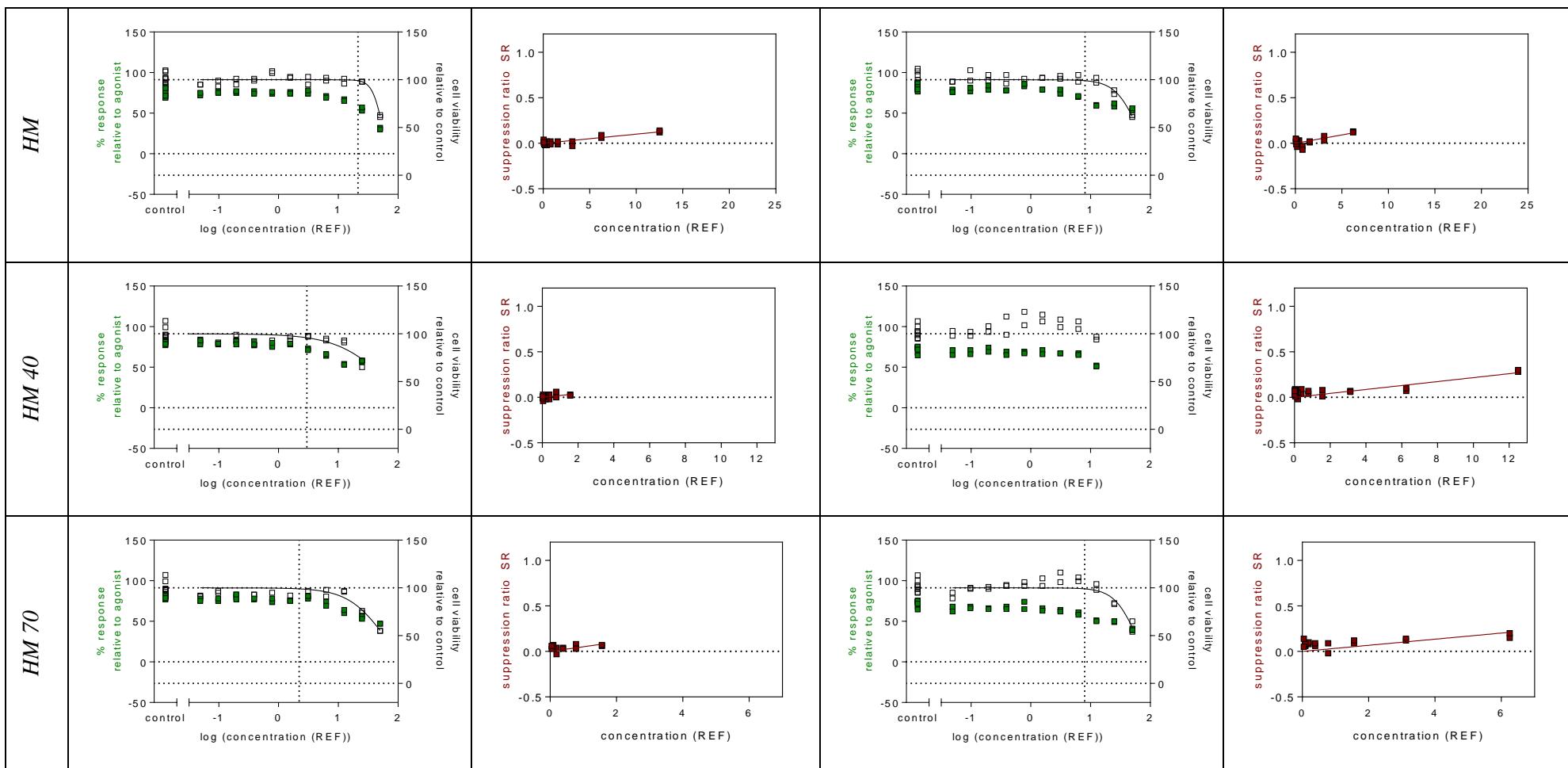
133

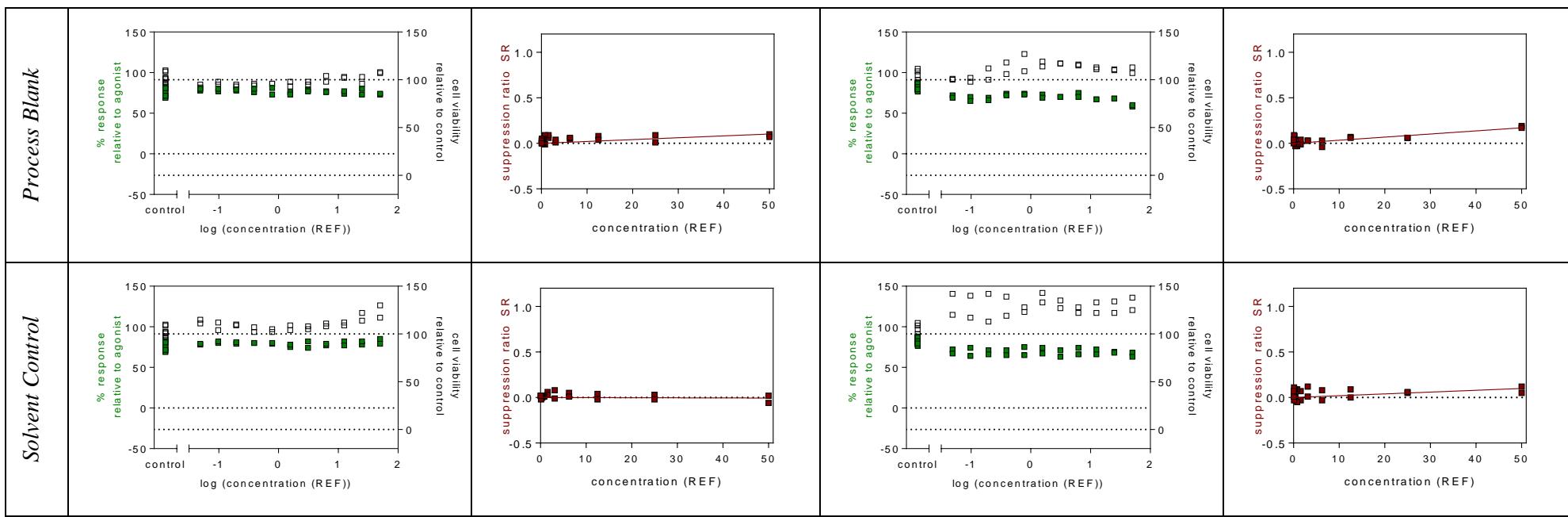
134 Figure S12: Full concentration-effect curves for inhibition (green filled symbols) and cell viability (empty symbols) in PR GeneBLAzer (antagonist
 135 mode) (left plots), as well as linear concentration-effect curves for suppression (right plots).







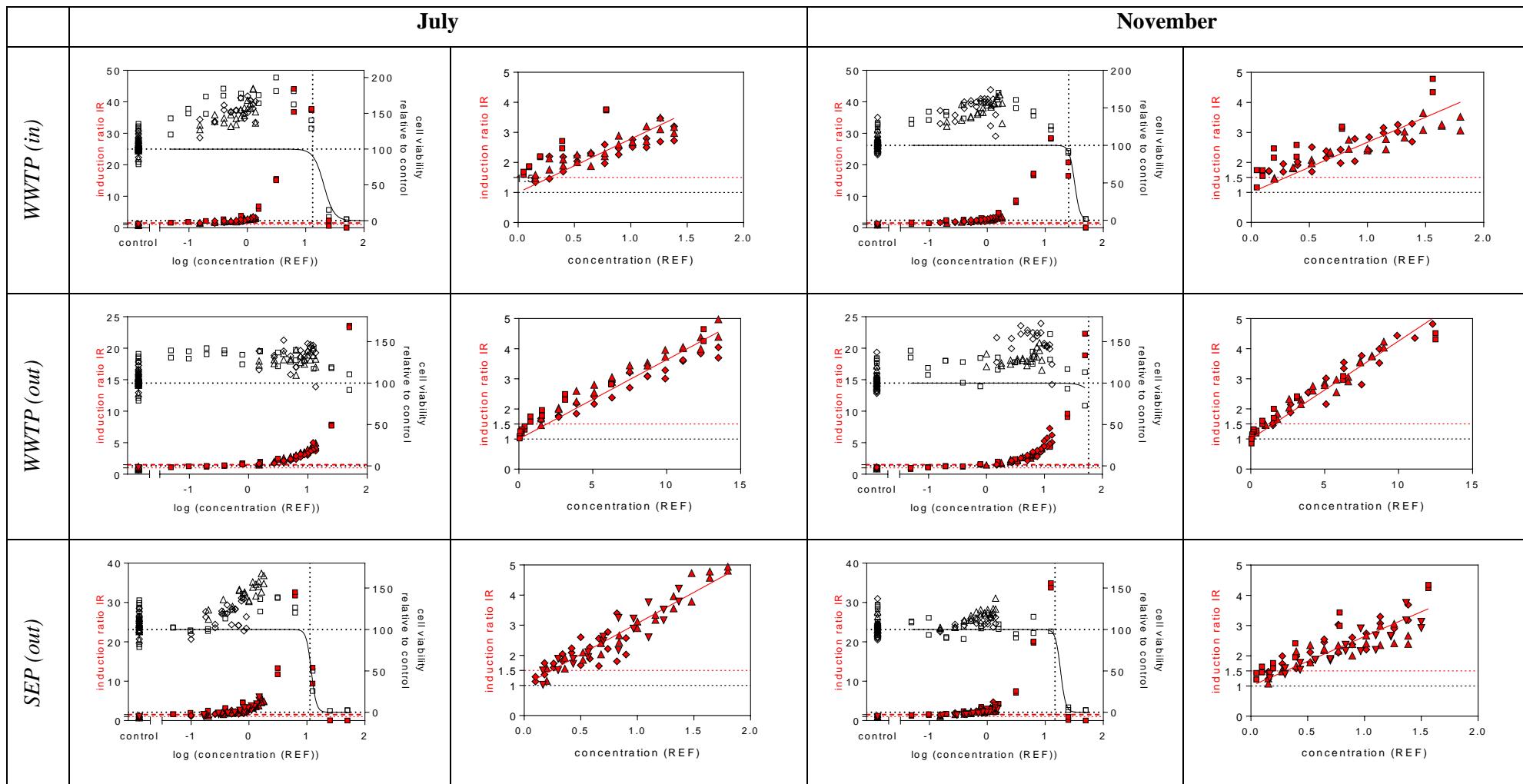


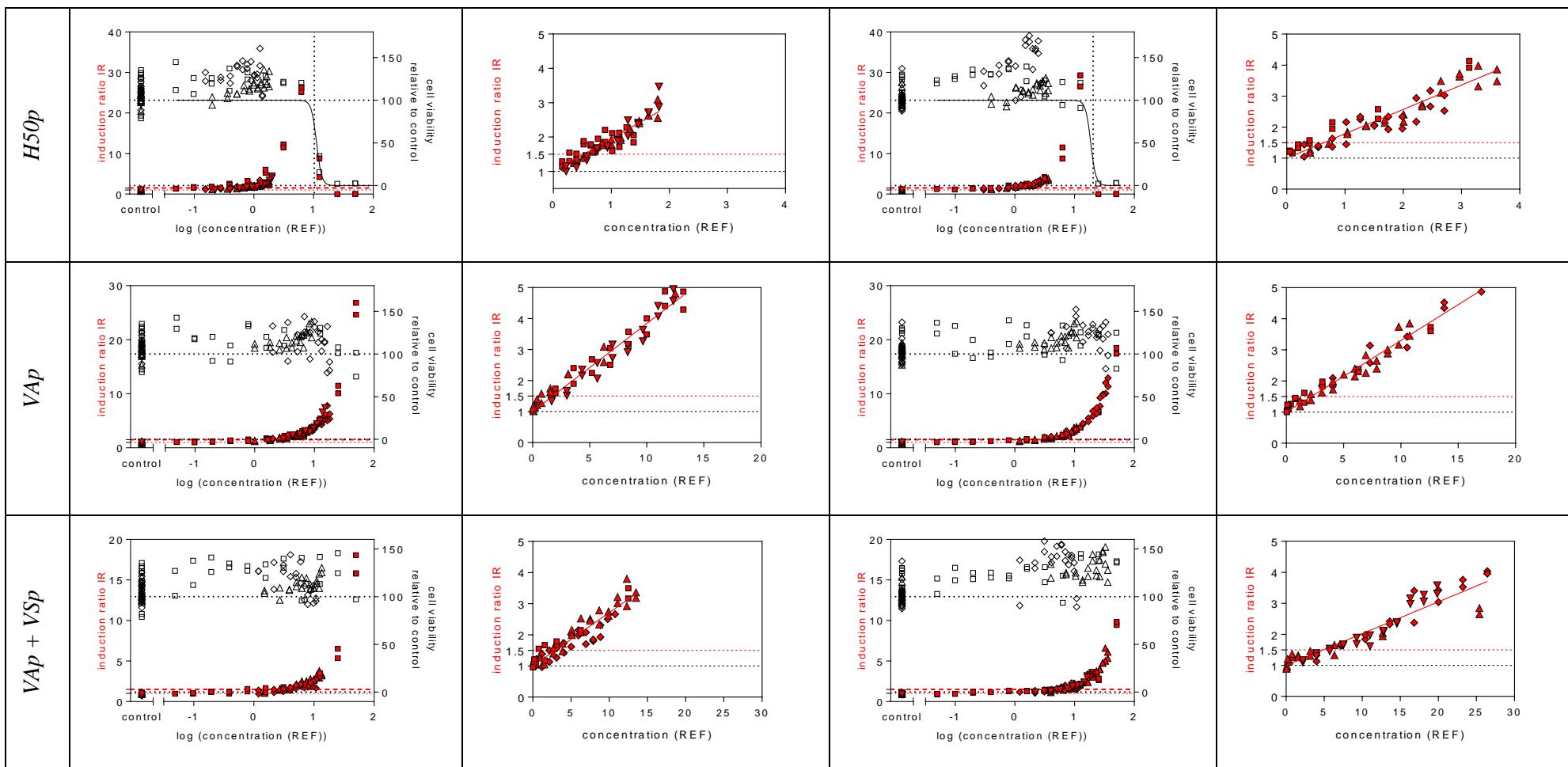


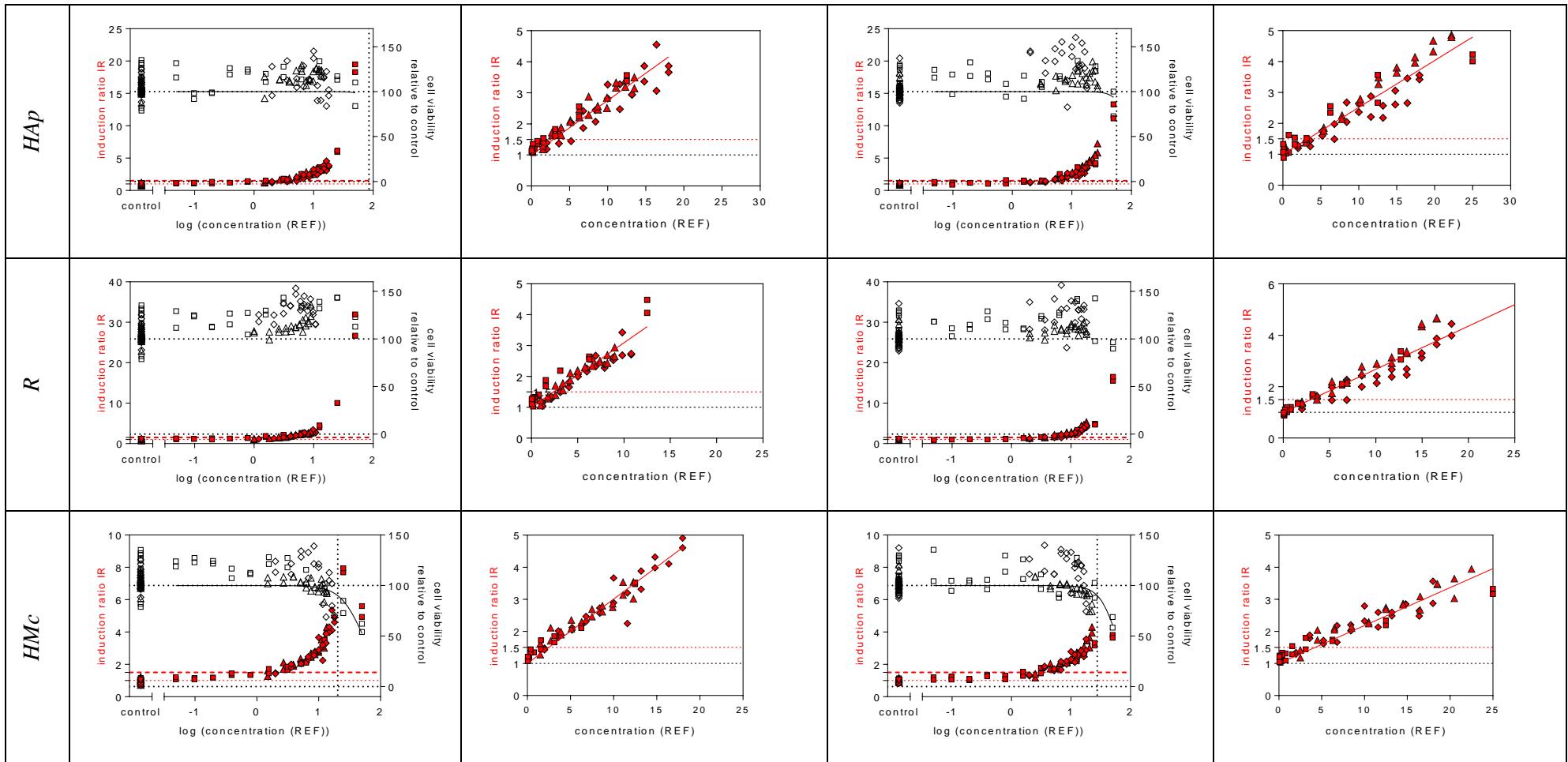
136

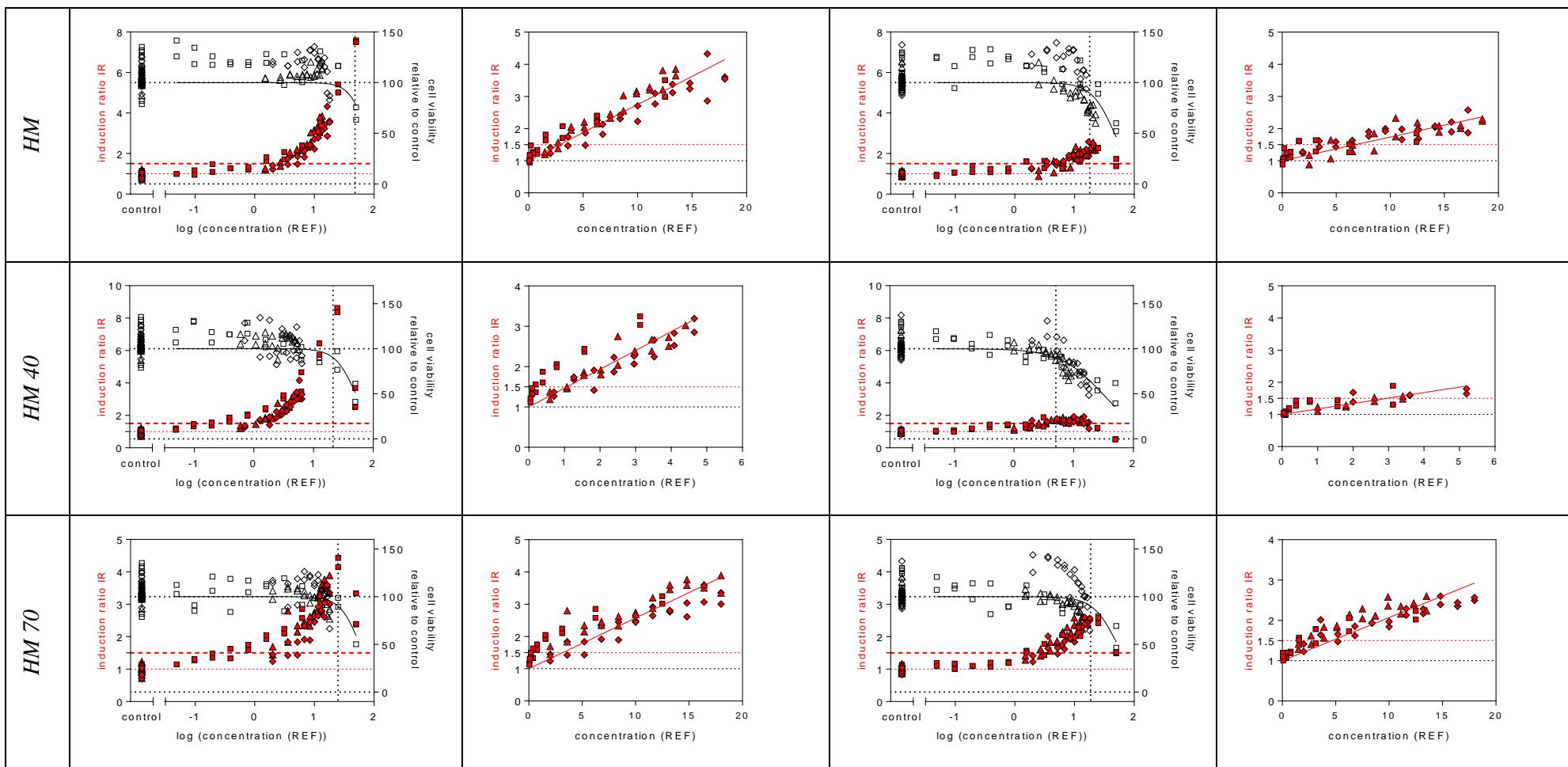
137

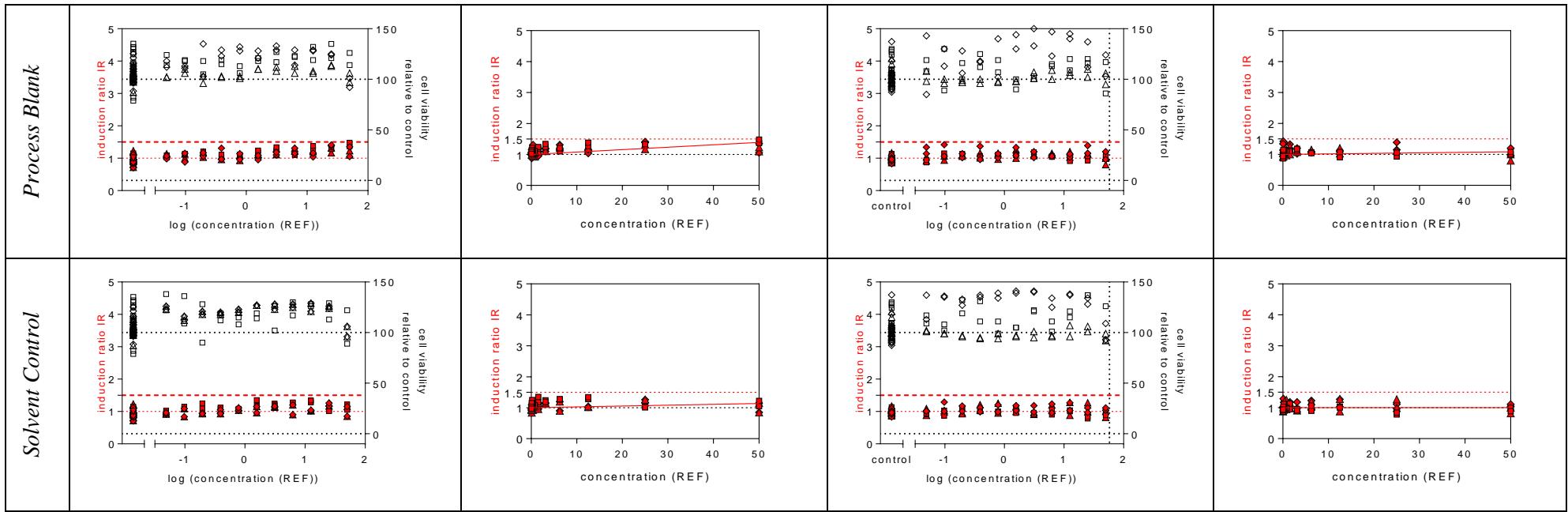
138 Figure S13: Full concentration-effect curves for induction (red filled symbols) and cell viability (empty symbols) in AREc32 (left plots), as well as
 139 linear concentration-effect curves for induction (right plots).









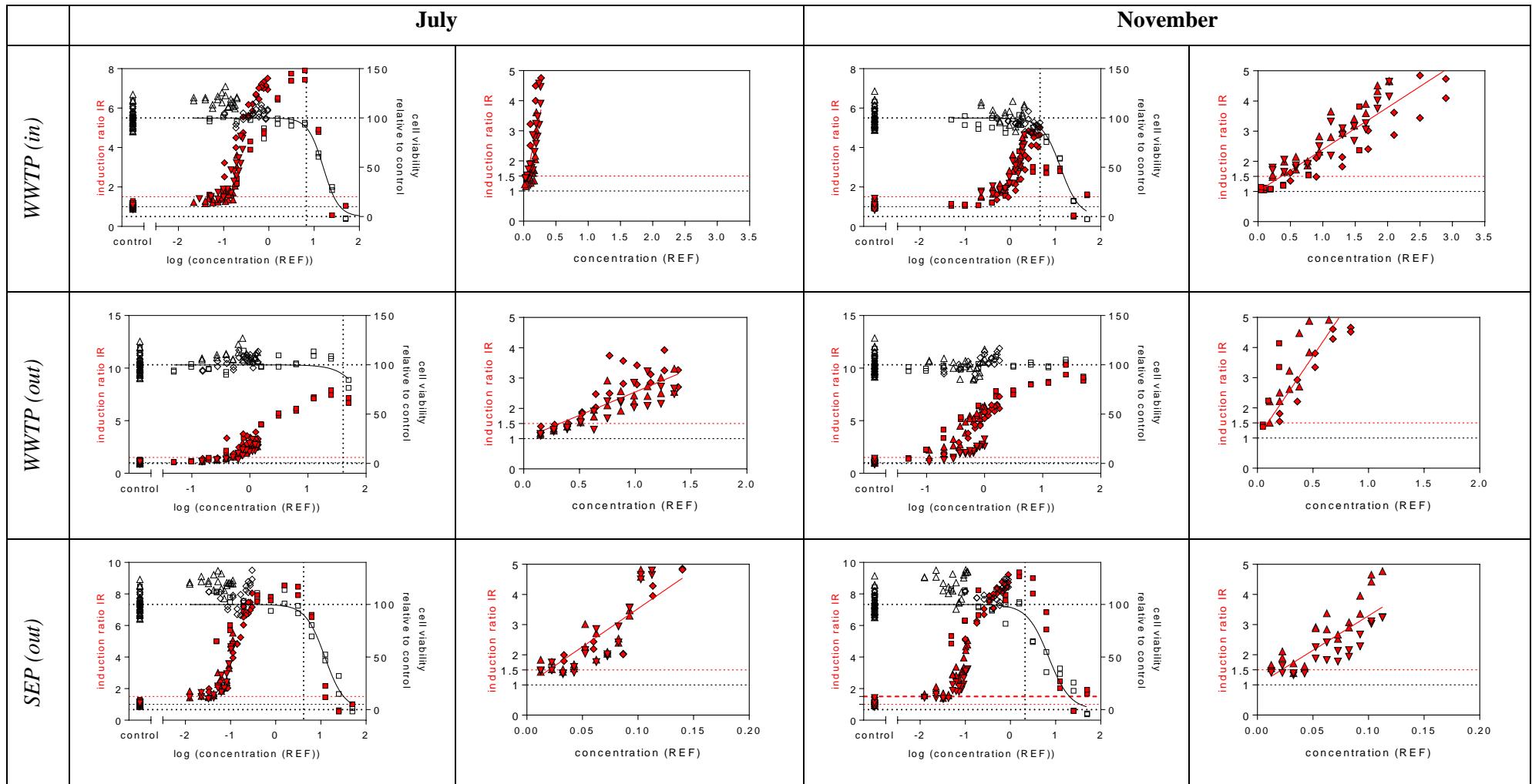


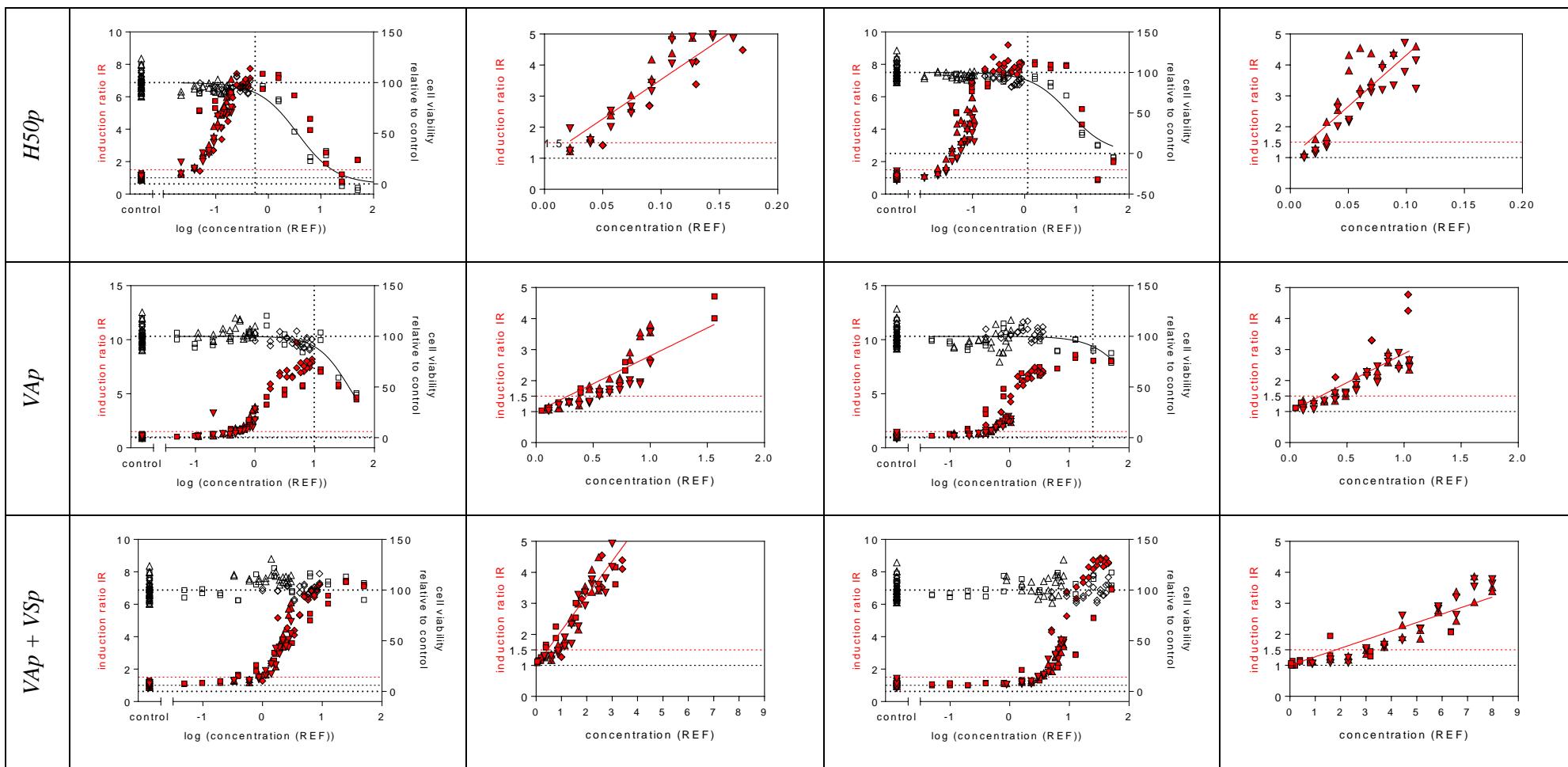
140

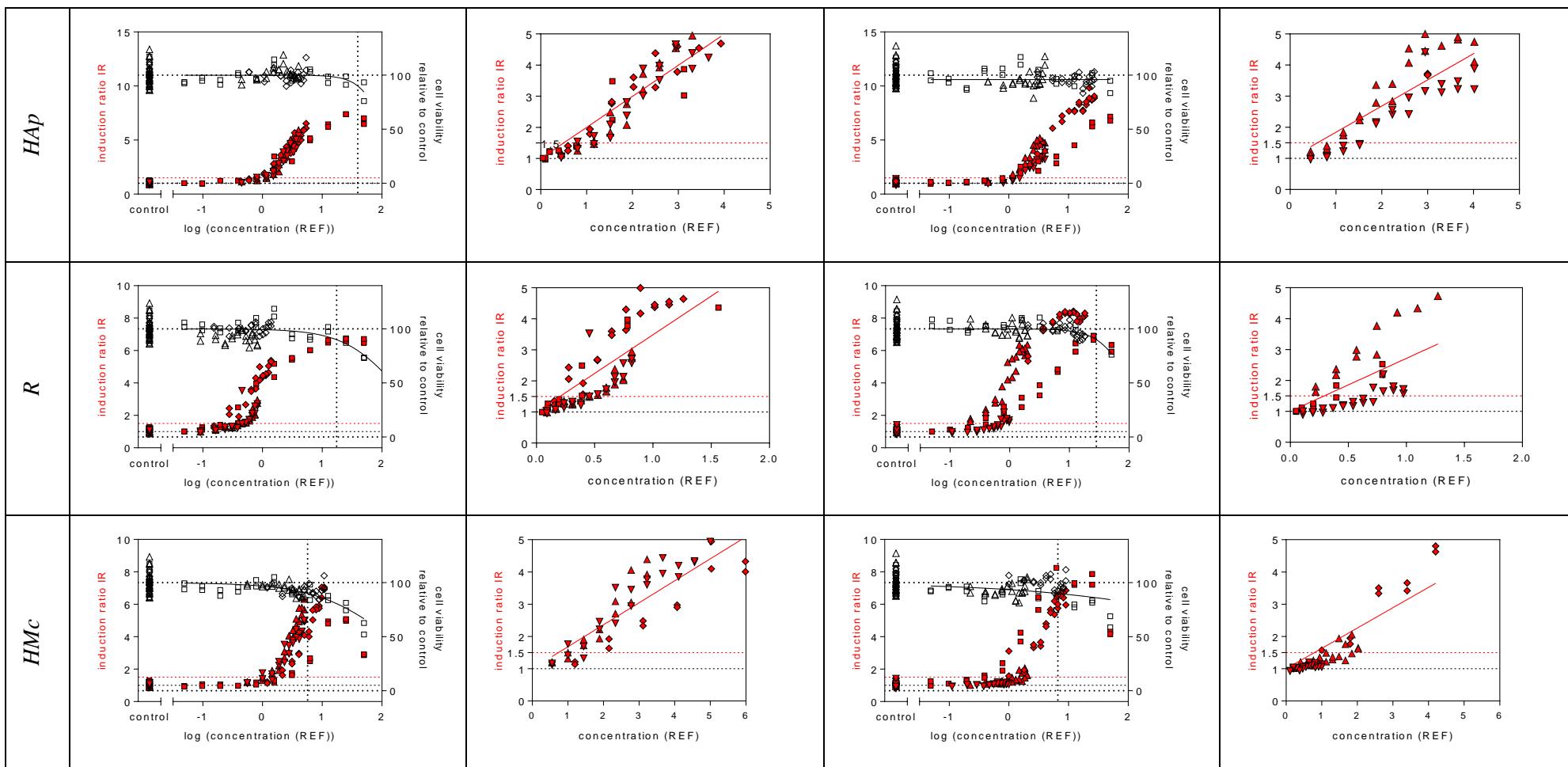
141

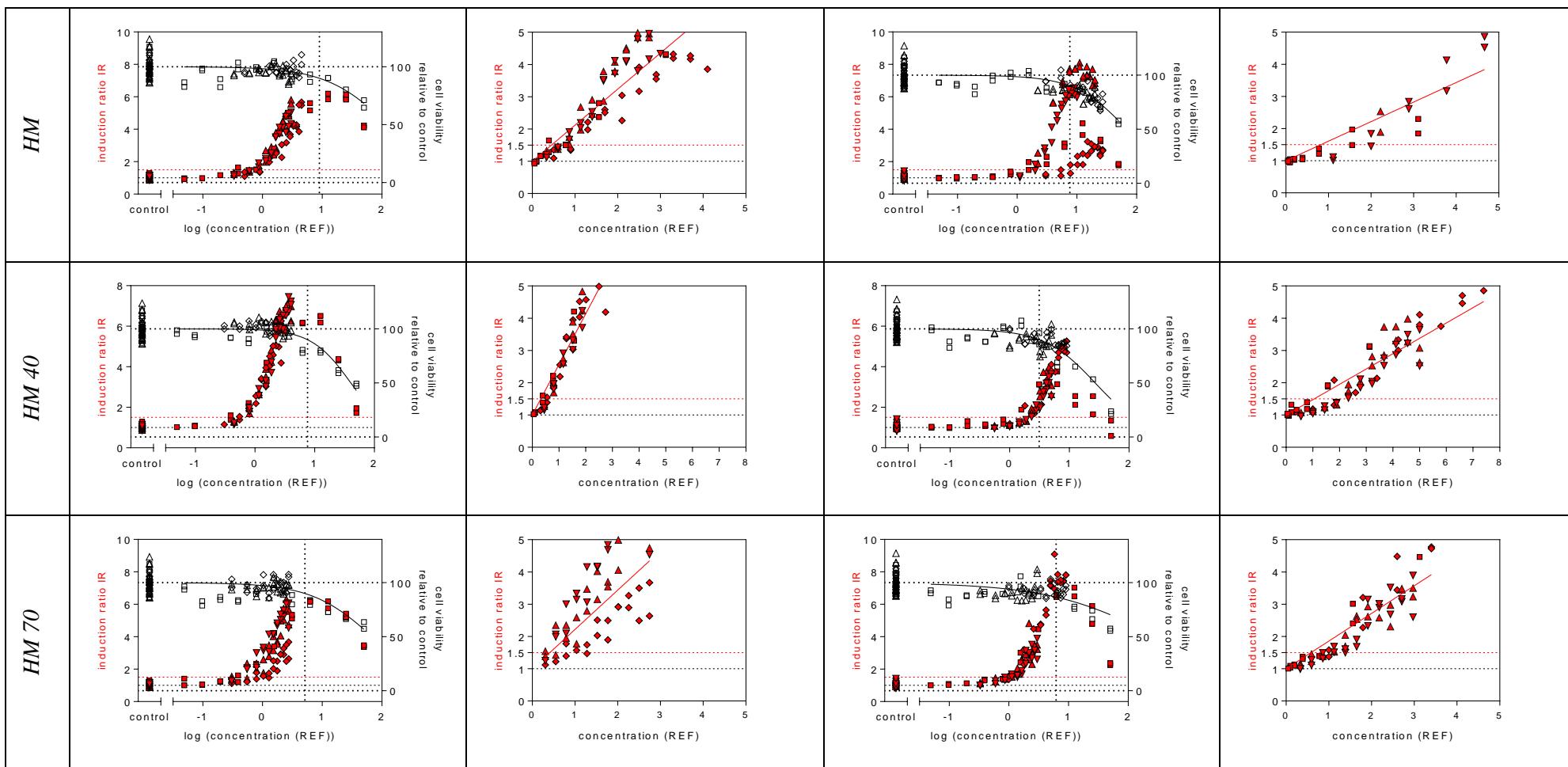
142 Figure S14: Full concentration-effect curves for induction (red filled symbols) and cell viability (empty symbols) in NF- κ B-*bla* (left plots), as well as
 143 linear concentration-effect curves for induction (right plots)

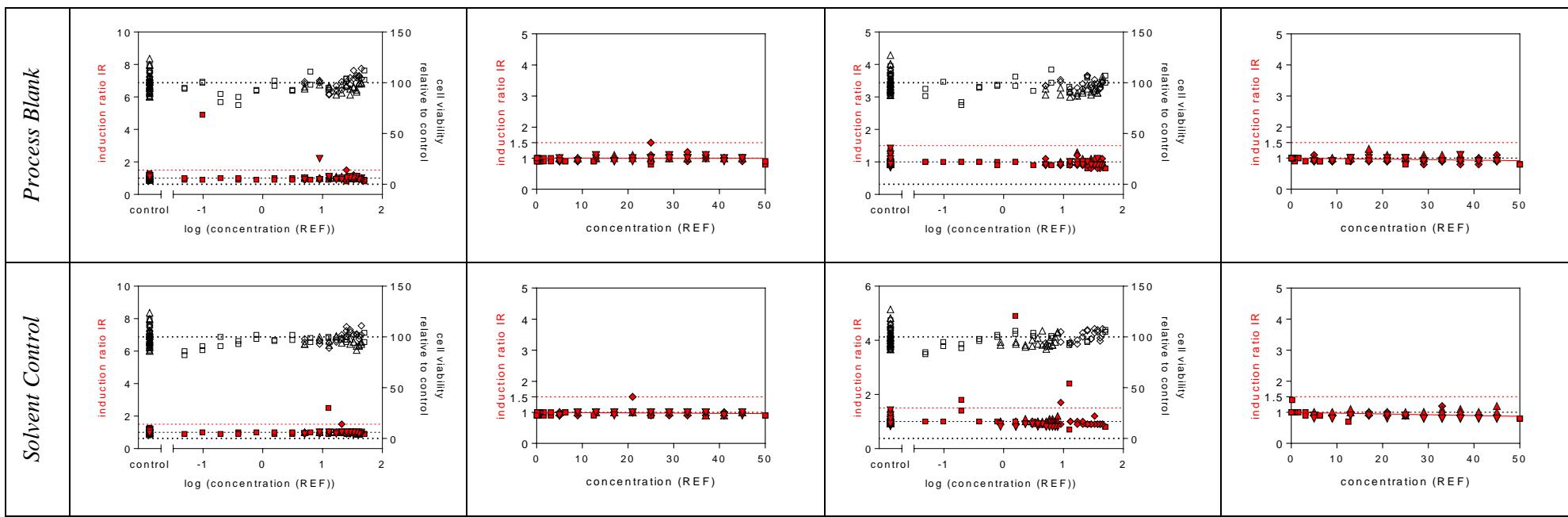
144 .







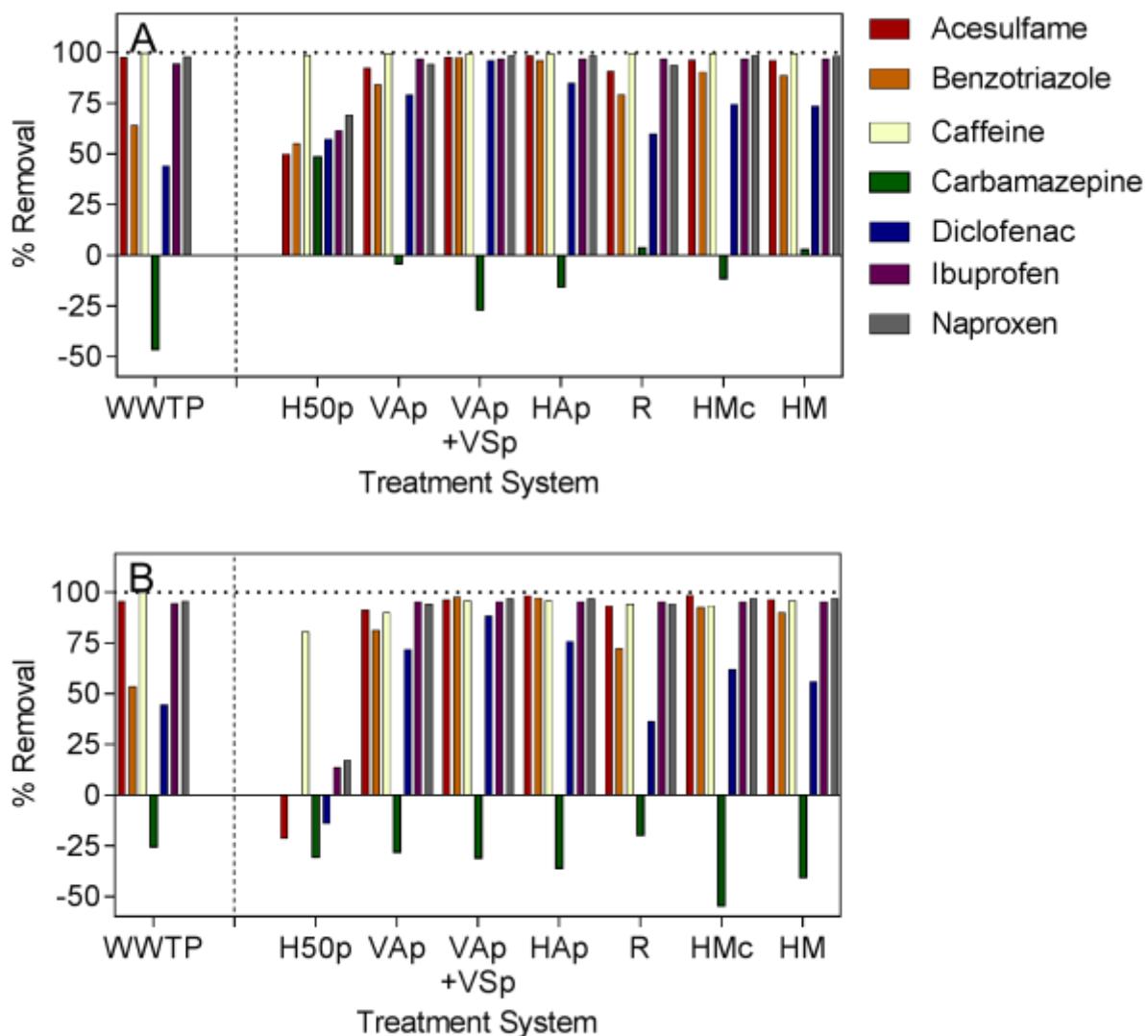




145

146

147 Figure S15: Removal of indicator chemicals after treatment in A) July and B) November.



148

149 Table S4: Removal efficacy of BEQ_{bio} by conventional and intensified treatment wetlands, as well as conventional WWTP calculated using Equation
 150 3.

		WWTP (out)	H50p	VAp	VAp + VSp	HAp	R	HMc	HM
Activation of AhR	<i>July</i>	53%	65%	74%	75%	85%	83%	87%	86%
	<i>November</i>	52%	63%	77%	83%	83%	77%	84%	81%
Binding to PPAR γ	<i>July</i>	88%	20%	87%	94%	91%	87%	77%	84%
	<i>November</i>	86%	23%	91%	95%	92%	87%	85%	81%
Activation of ER	<i>July</i>	98%	12%	98%	100%	99%	98%	99%	99%
	<i>November</i>	93%	42%	98%	99%	99%	97%	-*	97%
Oxidative Stress Response	<i>July</i>	85%	54%	86%	92%	92%	90%	90%	92%
	<i>November</i>	81%	52%	86%	94%	91%	90%	93%	95%
NF- κ B Reponse	<i>July</i>	85%	0%	93%	96%	96%	90%	97%	96%
	<i>November</i>	-290%	-45%	92%	99%	96%	93%	97%	97%

151 *cytotoxic

152

153

154

155

References

1. S. Kahl, J. Nivala, M. van Afferden, R. A. Müller and T. Reemtsma, *Water Res.*, 2017, **125**, 490-500.