Curcumin Treated T-C1 Cell Proliferation (MTS)

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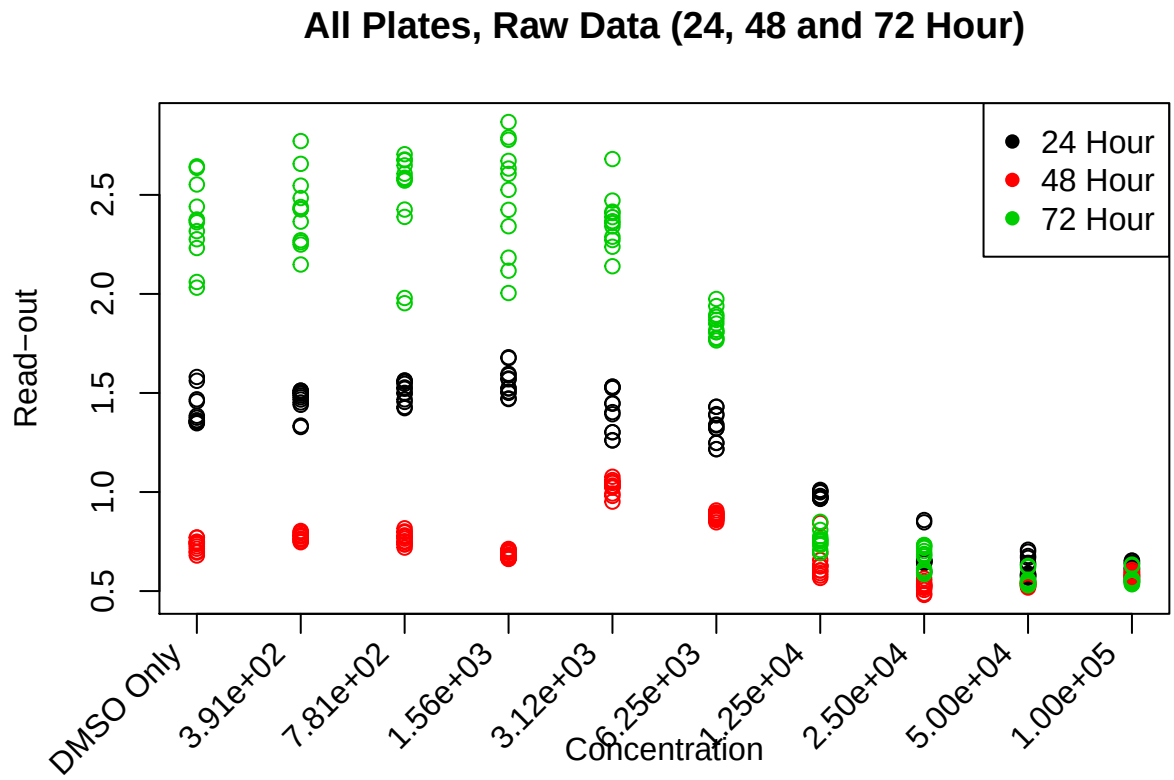
Design

T-C1 cells were dosed with serially diluted (. . . /2) Curcumin. Each of the 3 96-well plate contained 6 replicas for each of the 9 doses, plus DMSO control and medium control (background). Plates were analyzed at 24, 48 and 72 hours. Each plate was red twice. The results are presented below.

Analysis

Data was analyzed using R 3.2.1. The estimates and plots were obtained using my robust 4-parameter curve fitting R package 'ricf'.

Raw Data


All Plates, Raw Data (24, 48 and 72 Hour)
24 Hour
48 Hour
72 Hour
Read−out
0.5
1.0
1.5
2.0
2.5
DMSO Only
3.91e+02
7.81e+02
1.56e+03
3.12e+03
6.25e+03
1.25e+04
2.50e+04
5.00e+04
1.00e+05
Concentration

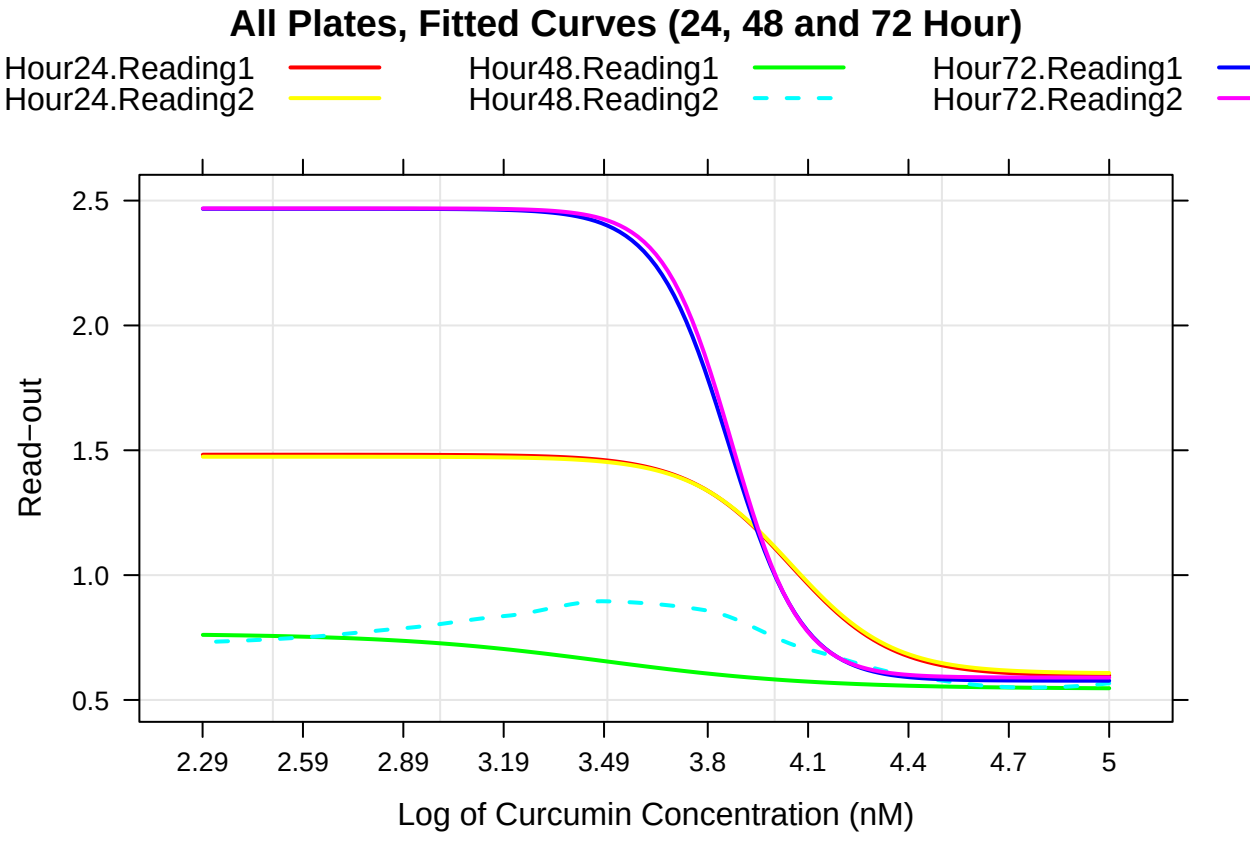

All Plates, Fitted Curves (24, 48 and 72 Hour)
Hour24.Reading1
Hour24.Reading2
Hour48.Reading1
Hour48.Reading2
Hour72.Reading1
Hour72.Reading2
Read−out
0.5
1.0
1.5
2.0
2.5
2.29
2.59
2.89
3.19
3.49
3.8
4.1
4.4
4.7
5
Log of Curcumin Concentration (nM)

Table 1: Hour24.Reading1

	Estimate	Std. Error	lb	ub
bot	31.020	2.688	25.621	36.418
top	106.544	2.031	102.465	110.623
lic50	4.048	0.032	3.984	4.111
scale	-2.762	0.513	-3.792	-1.732
Log10IC20	4.266	0.051	4.163	4.369
Log10IC80	3.830	0.051	3.727	3.933
IC20	18440.016	NA	14544.106	23379.519
IC80	6757.578	NA	5329.872	8567.721

NULL

Normalized Data

Percent DMSO = 100*(Treatment - Median(Plate Background))/(Row DMSO - Median(Plate Background))

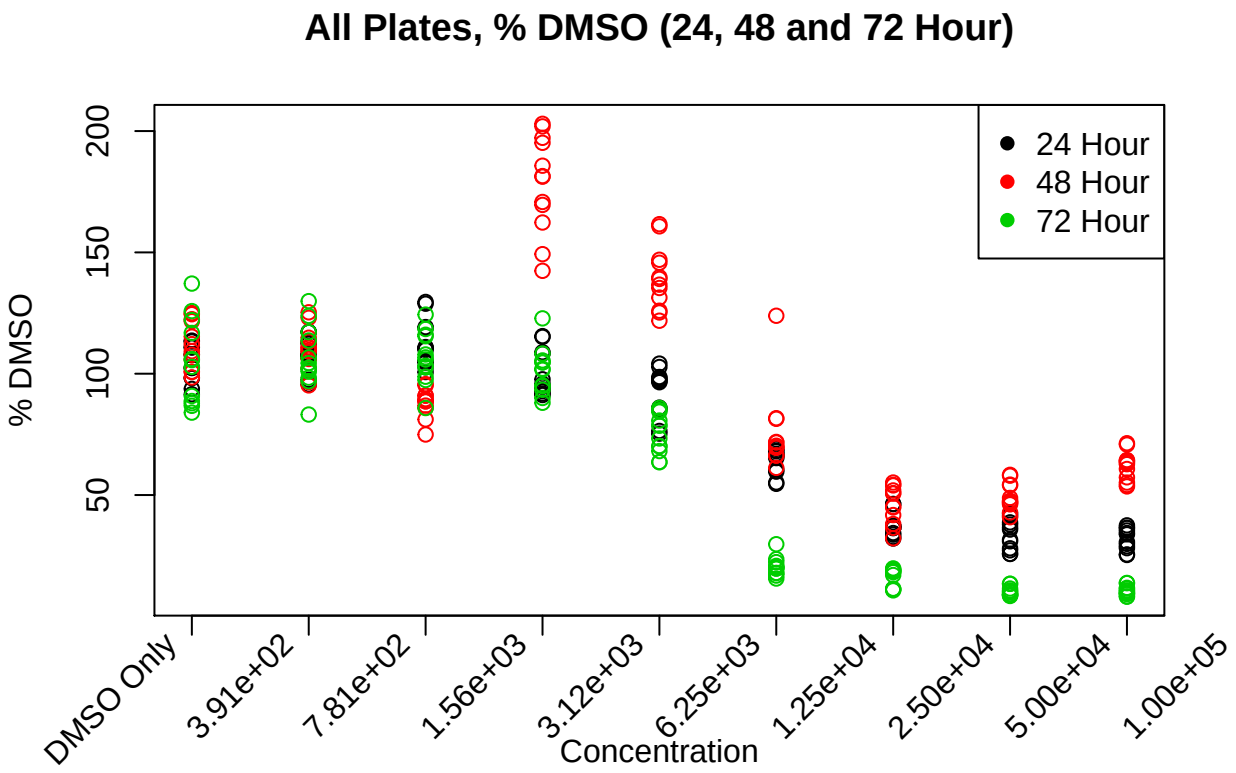


Table 2: Hour24.Reading2

	Estimate	Std. Error	lb	ub
bot	31.821	2.819	26.159	37.484
top	106.499	2.132	102.217	110.781
lic50	4.051	0.033	3.985	4.118
scale	-2.812	0.562	-3.941	-1.683
Log10IC20	4.265	0.054	4.157	4.374
Log10IC80	3.837	0.054	3.728	3.946
IC20	18428.767	NA	14345.343	23674.542
IC80	6875.072	NA	5351.702	8832.072

Table 3: Hour48.Reading1

Table 4: Hour48.Reading2

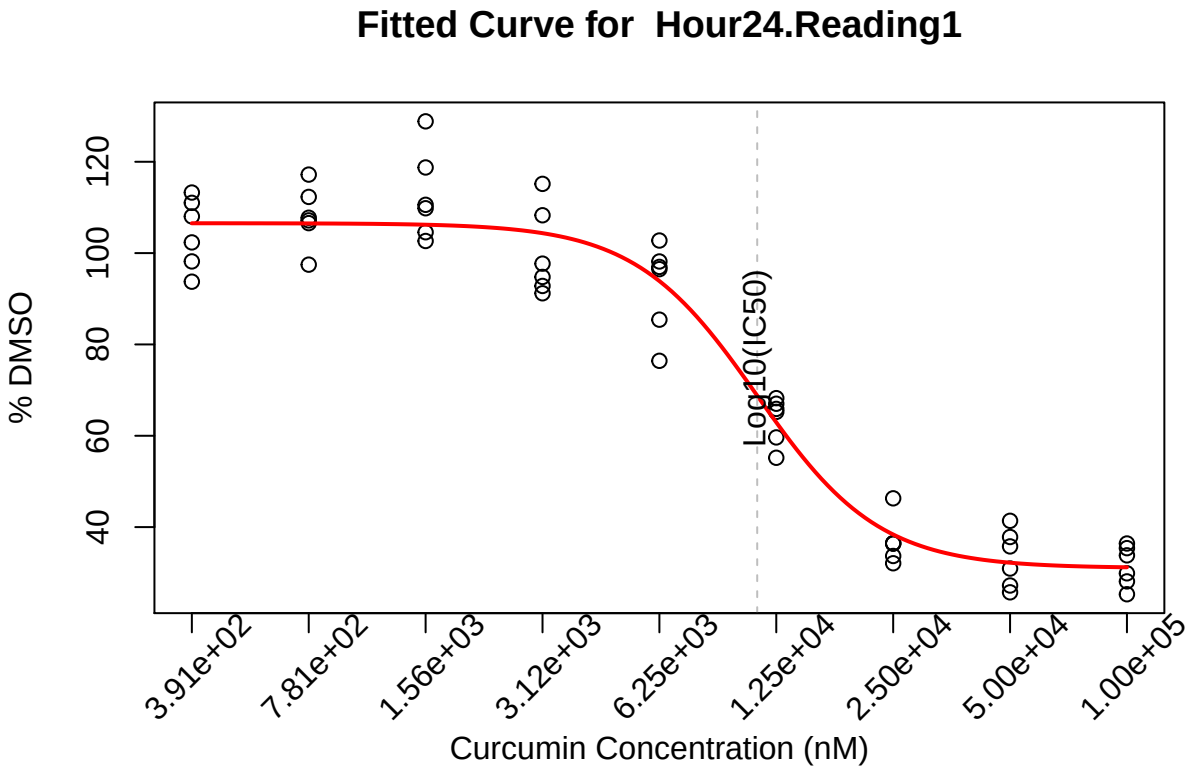
	Estimate	Std. Error	lb	ub
bot	54.826	6.762	41.124	68.527
top	124.146	22.638	78.277	170.014
lic50	3.276	0.270	2.729	3.823
scale	-1.219	0.958	-3.161	0.723
Log10IC20	3.770	0.473	2.812	4.728
Log10IC80	2.782	0.473	1.824	3.740
IC20	5885.020	NA	648.150	53434.361
IC80	605.361	NA	66.672	5496.508

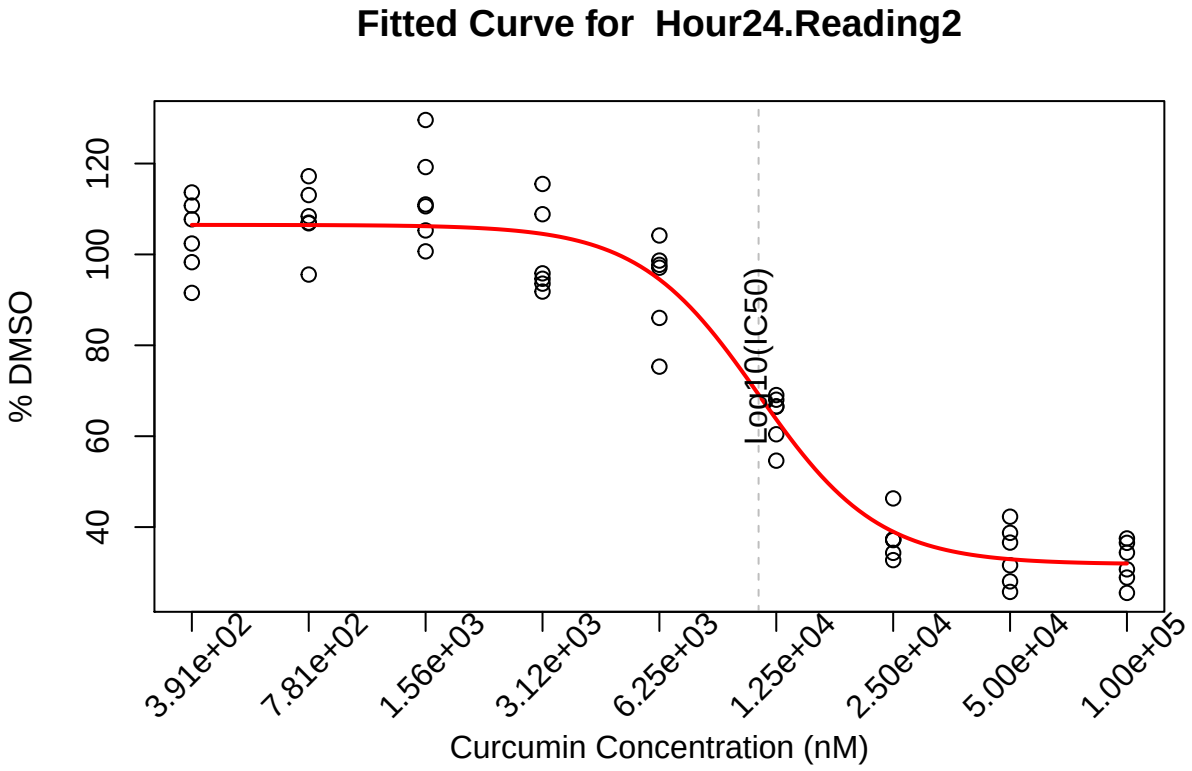
Table 5: Hour72.Reading1

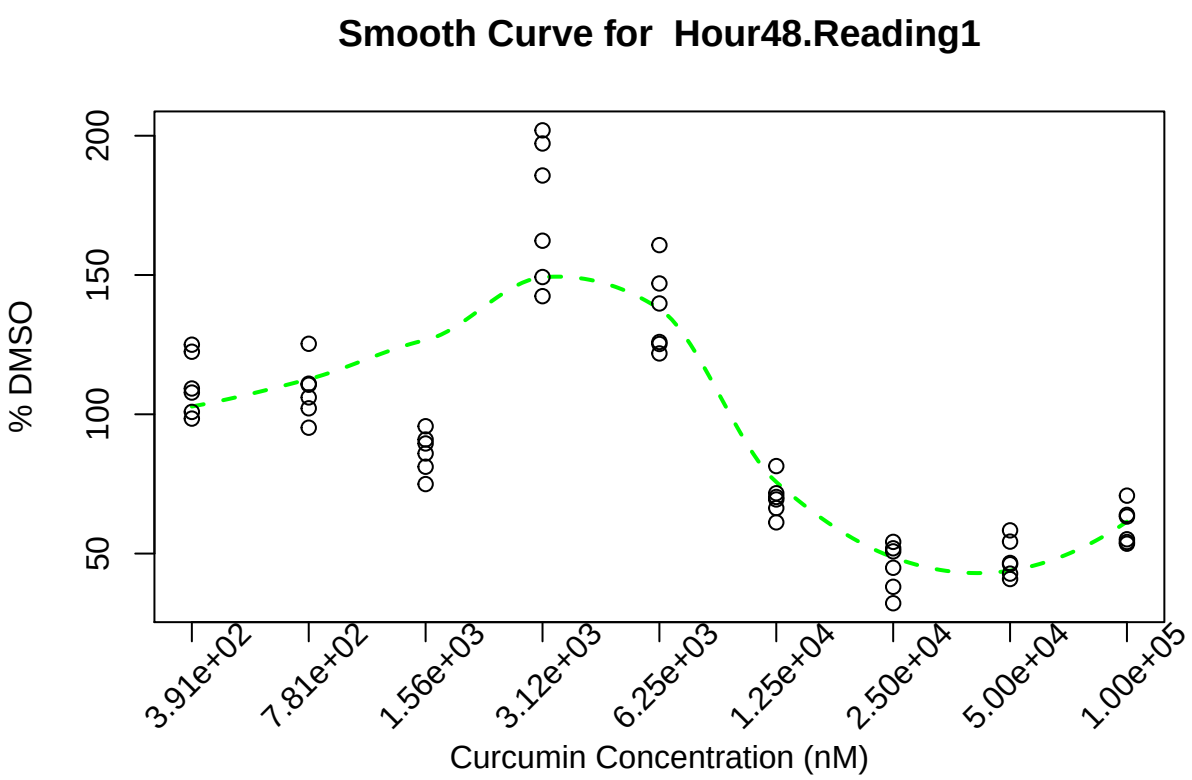
	Estimate	Std. Error	lb	ub
bot	11.909	2.005	7.878	15.939
top	102.862	2.052	98.736	106.989
lic50	3.877	0.021	3.835	3.918
scale	-4.258	0.695	-5.655	-2.861
Log10IC20	4.018	0.031	3.956	4.080
Log10IC80	3.735	0.031	3.673	3.797
IC20	10422.536	NA	9032.664	12026.269
IC80	5434.819	NA	4710.073	6271.084

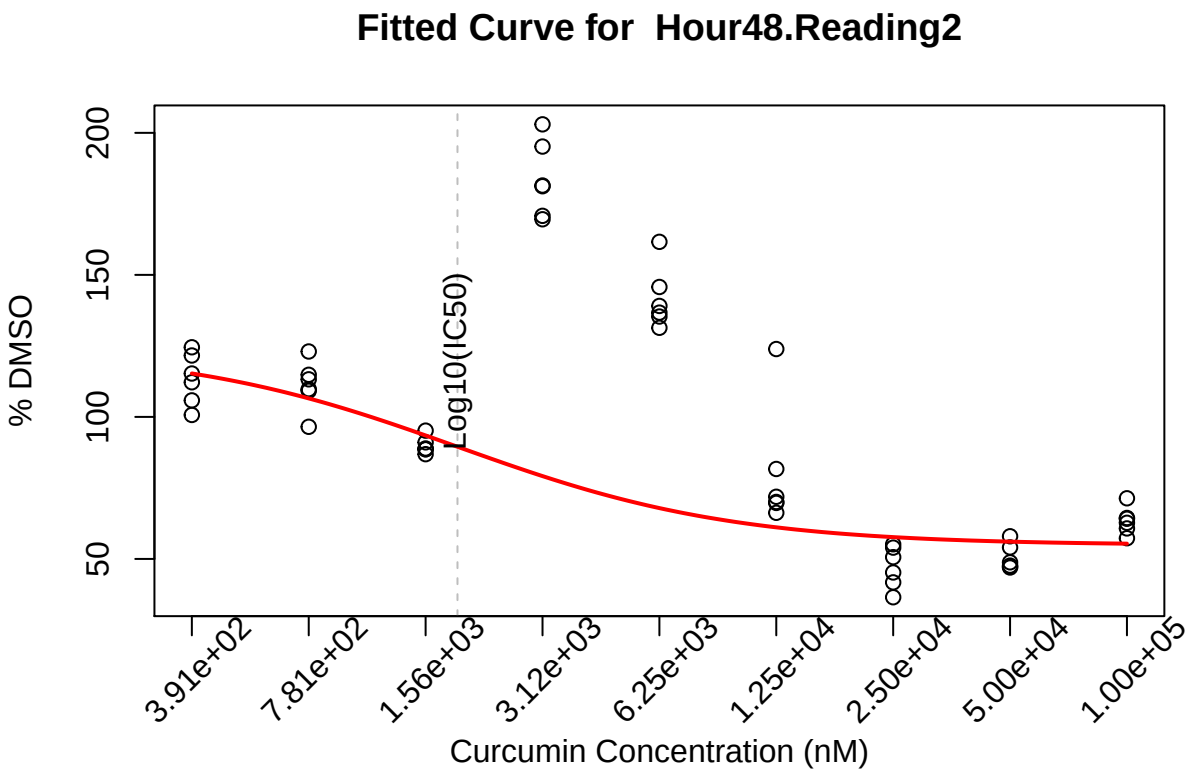
Table 6: Hour72.Reading2

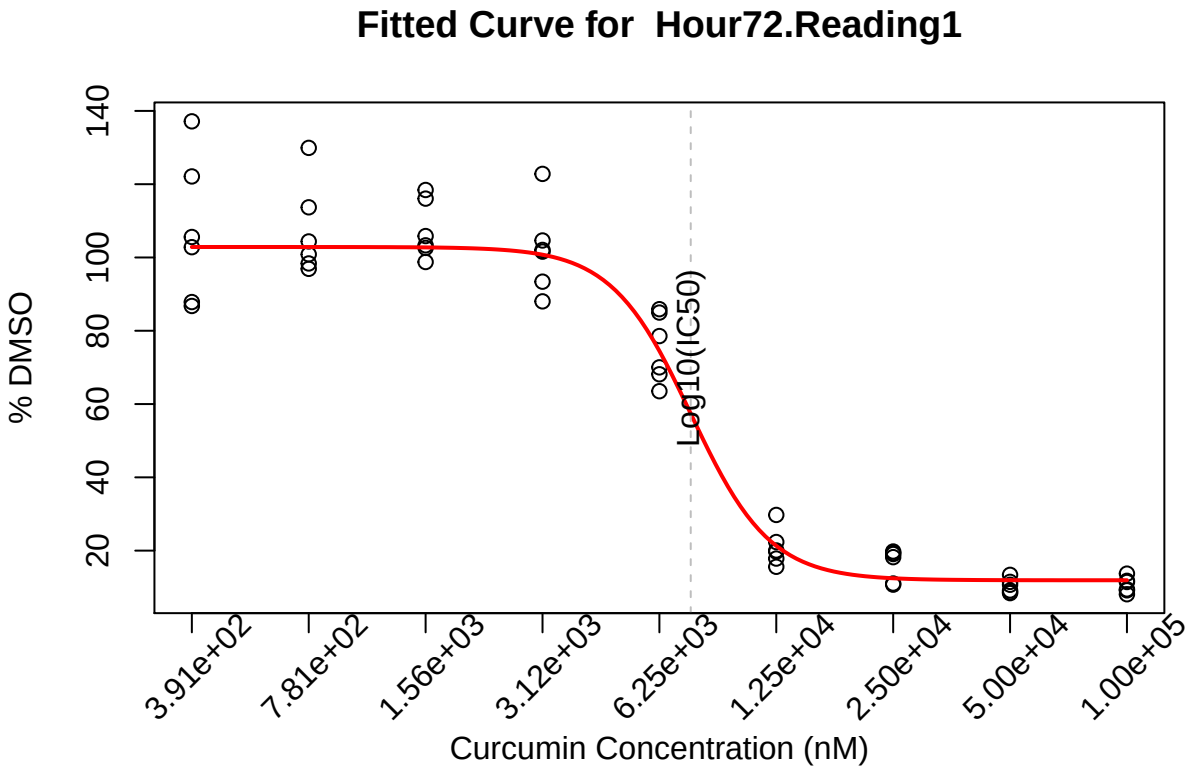
	Estimate	Std. Error	lb	ub
bot	11.572	1.980	7.596	15.548
top	104.936	2.255	100.407	109.464
lic50	3.872	0.020	3.833	3.912
scale	-4.309	0.693	-5.700	-2.917
Log10IC20	4.012	0.030	3.952	4.072
Log10IC80	3.732	0.030	3.673	3.792
IC20	10278.436	NA	8955.030	11797.420
IC80	5400.841	NA	4705.452	6198.997

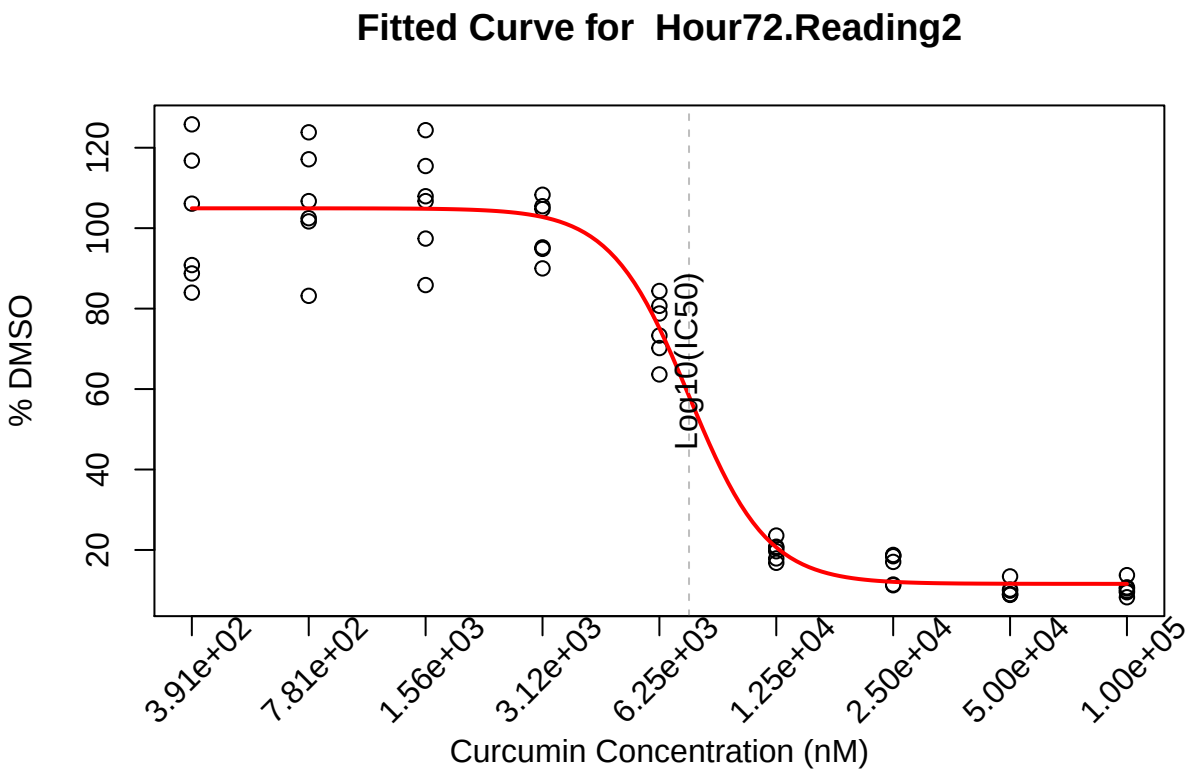

Fitted Curve for Hour24.Reading1
% DMSO
40
60
80
100
120
L.10(IC50)
3.91e+02
7.81e+02
1.56e+03
3.12e+03
6.25e+03
1.25e+04
2.50e+04
5.00e+04
1.00e+05
Curcumin Concentration (nM)

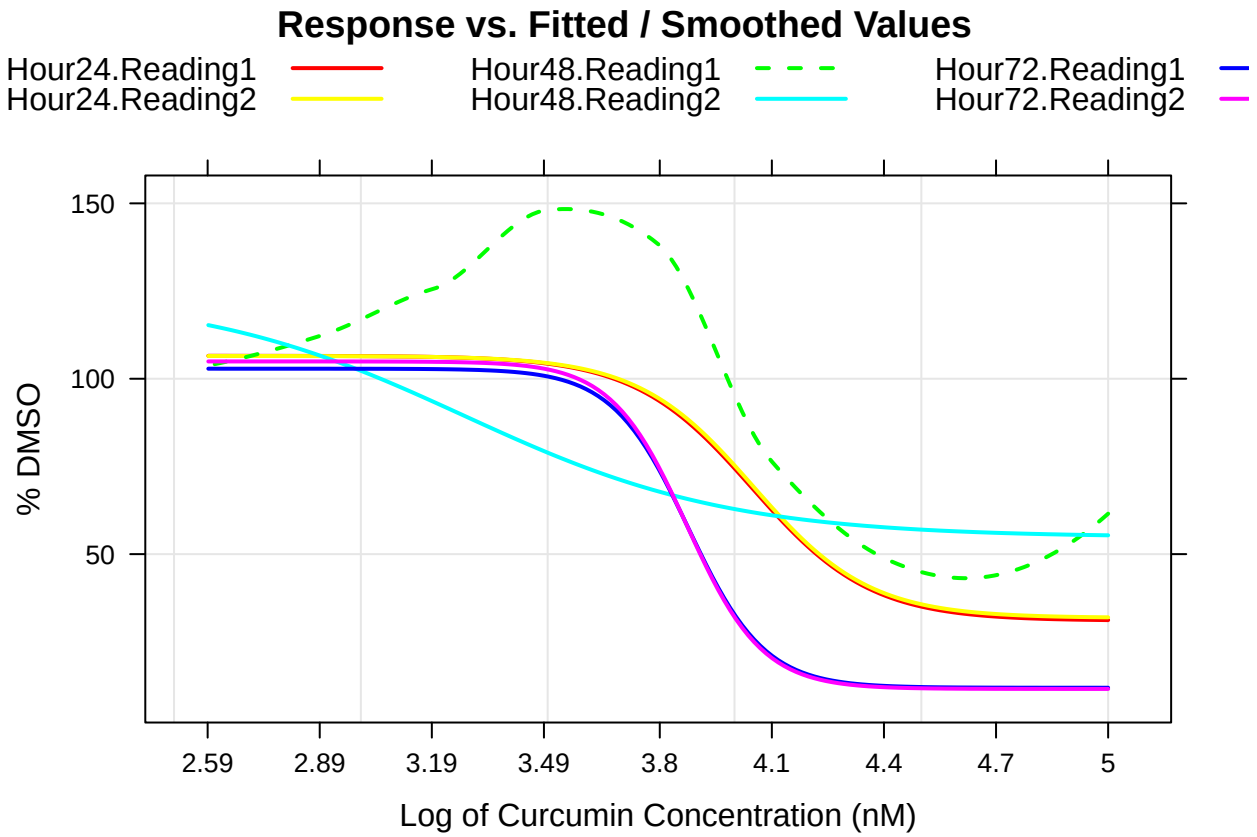

Fitted Curve for Hour24.Reading2
% DMSO
40
60
80
100
120
L.10(IC50)
3.91e+02
7.81e+02
1.56e+03
3.12e+03
6.25e+03
1.25e+04
2.50e+04
5.00e+04
1.00e+05
Curcumin Concentration (nM)


Smooth Curve for Hour48.Reading1
% DMSO
50
100
150
200
3.91e+02
7.81e+02
1.56e+03
3.12e+03
6.25e+03
1.25e+04
2.50e+04
5.00e+04
1.00e+05
Curcumin Concentration (nM)


Fitted Curve for Hour48.Reading2
% DMSO
50
100
150
200
Log10(IC50)
3.91e+02
7.81e+02
1.56e+03
3.12e+03
6.25e+03
1.25e+04
2.50e+04
5.00e+04
1.00e+05
Curcumin Concentration (nM)


Fitted Curve for Hour72.Reading1
% DMSO
20
40
60
80
100
140
Log10(IC50)
3.91e+02
7.81e+02
1.56e+03
3.12e+03
6.25e+03
1.25e+04
2.50e+04
5.00e+04
1.00e+05
Curcumin Concentration (nM)


Fitted Curve for Hour72.Reading2
% DMSO
20
40
60
80
100
120
Log10(IC50)
3.91e+02
7.81e+02
1.56e+03
3.12e+03
6.25e+03
1.25e+04
2.50e+04
5.00e+04
1.00e+05
Curcumin Concentration (nM)



NULL
NULL

Discussion

1. Although data followed 4-parameter model well for 24 and 72 hour read-outs, 48 hour read-out had major issues.
2. Variance decreased from lowest to highest doses at 72 hours but the robust model assumed homogeneity of variance across concentrations. Although this might not have influenced the estimates in this case, generally a transformation of the response might be needed to stabilize variance.
3. Hook effect was observed at low doses at 24 hours. A model that can describe the hook might be more appropriate, especially if the hook effect becomes more pronounced.
4. Smaller intervals between the doses can be beneficial as we can get smoother curves around IC50 values and decrease standard errors of the estimates.