

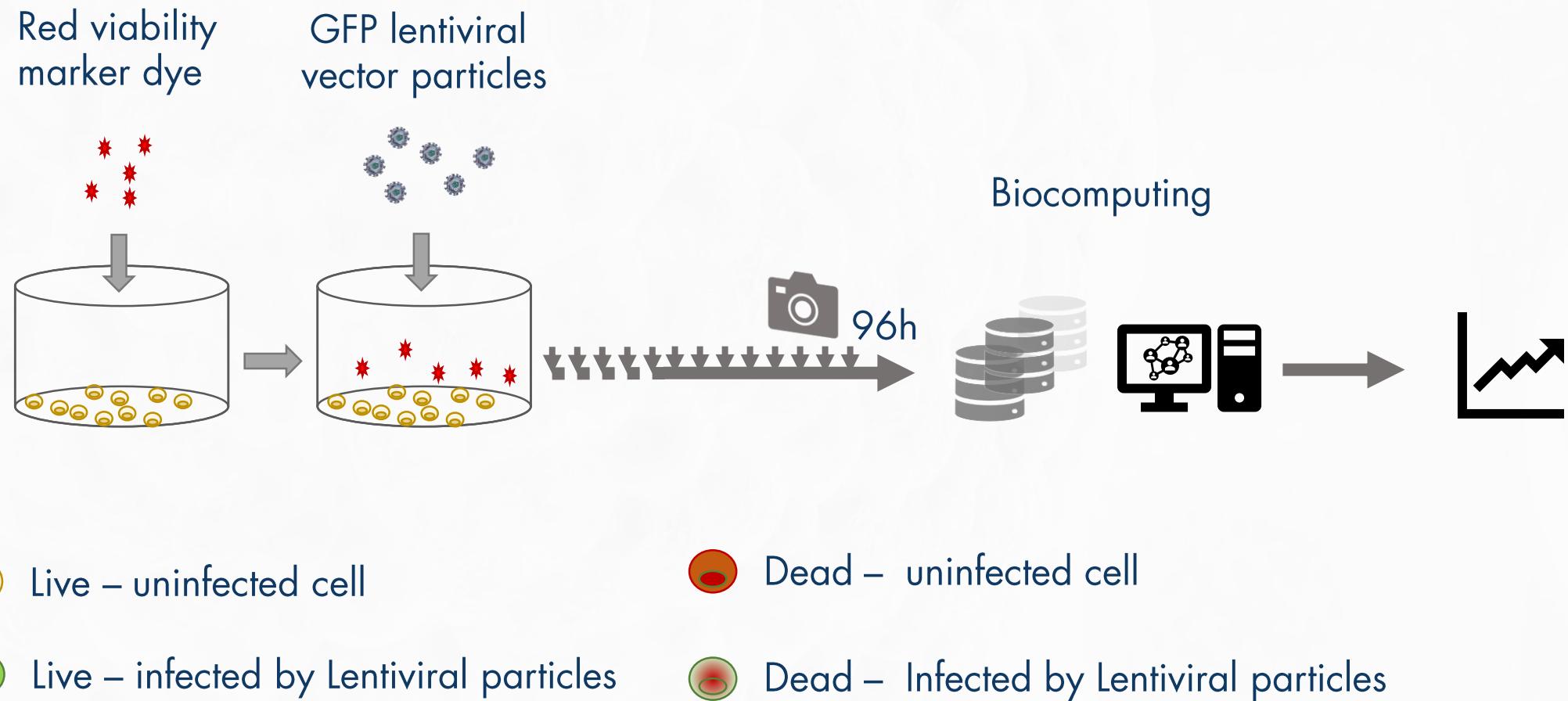
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Porsolt

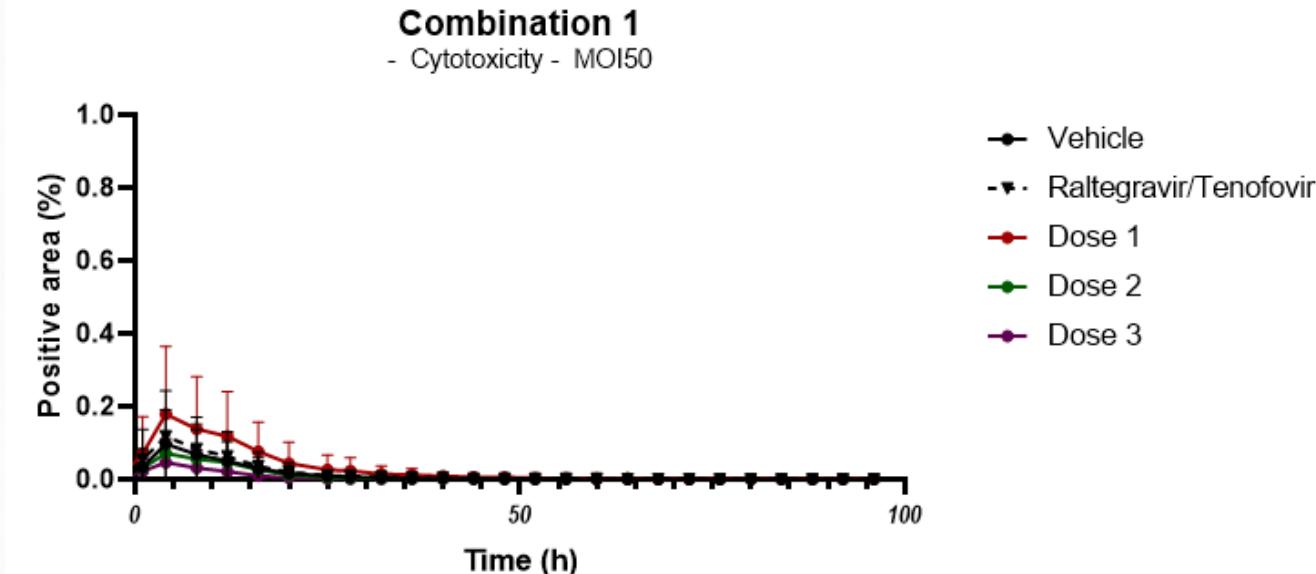
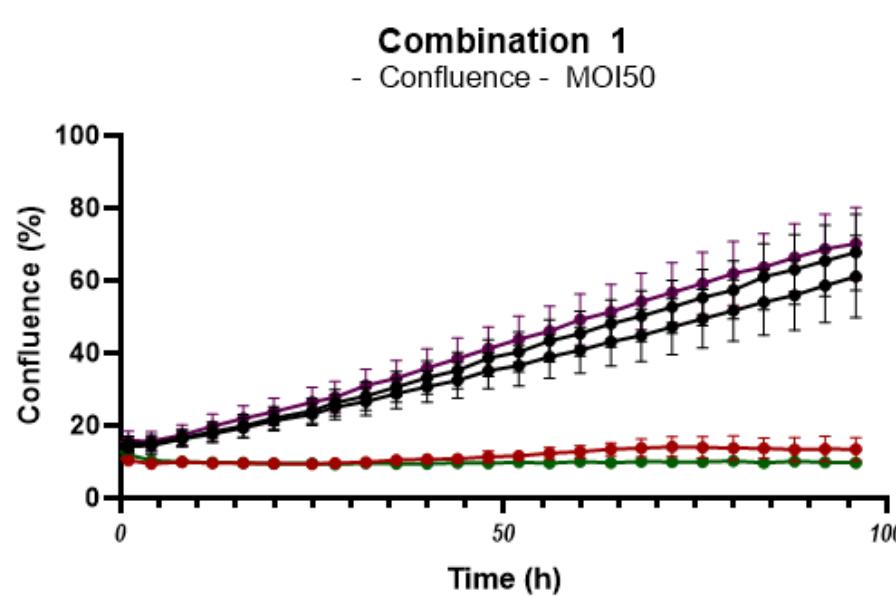
Scientist-to-Scientist

20.0451

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Effect of test combination 1 MOI50



Statistical comparison at 96hours stimulation

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegrav	6.686	-8.391 to 21.76	No	ns	0.5718
Vehicle vs. Dose 1	54.38	42.72 to 66.03	Yes	****	<0.0001
Vehicle vs. Dose 2	58.07	46.46 to 69.68	Yes	****	<0.0001
Vehicle vs. Dose 3	-2.404	-16.53 to 11.72	No	ns	0.9701

Statistical comparison at 4hours stimulation

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegrav	-0.02098	-0.1740 to 0.1320	No	ns	0.9855
Vehicle vs. Dose 1	-0.08192	-0.2929 to 0.1290	No	ns	0.644
Vehicle vs. Dose 2	0.02623	-0.1146 to 0.1670	No	ns	0.9586
Vehicle vs. Dose 3	0.05057	-0.06195 to 0.1631	No	ns	0.5527

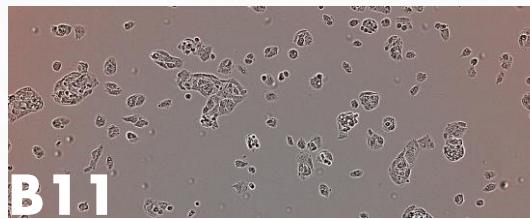
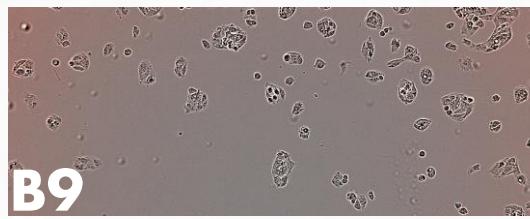
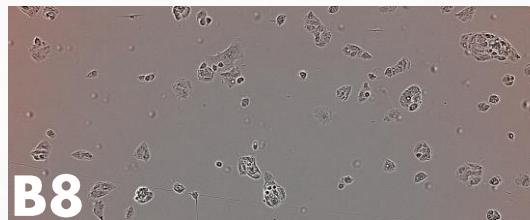
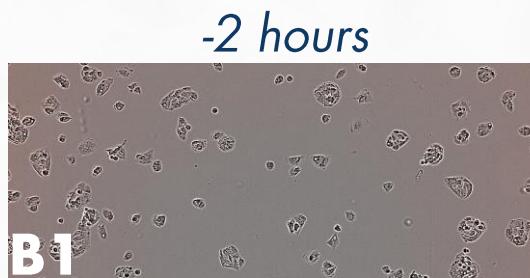
Figure: effect of the mentioned test substance at various concentrations on cell cytotoxicity (right) and confluence (left). Two ways ANOVA followed by a Dunnett's post hoc test has been used for statistical comparison. In table below, a summary of statistical comparison is shown at 96 hours and 4hours respectively for confluence and cytotoxicity (maximum effect).

20.0451

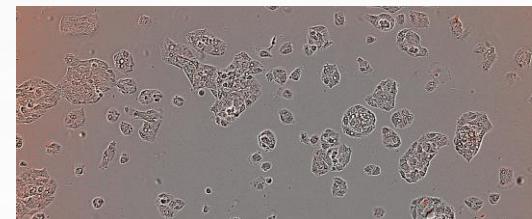
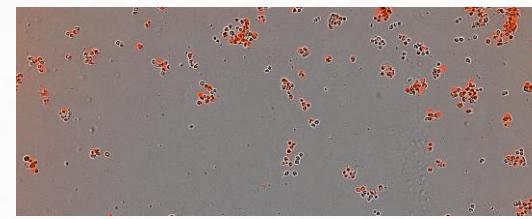
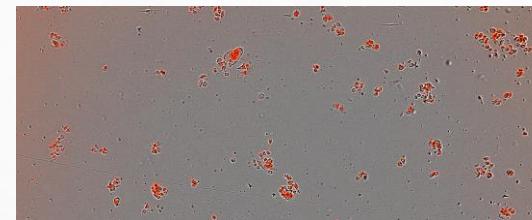
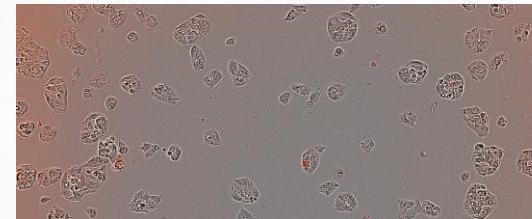
Combination 1 : Cytotoxicity and cell growth at MOI50

Pictures

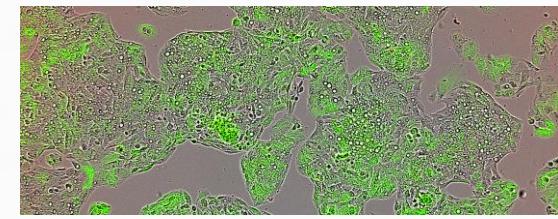
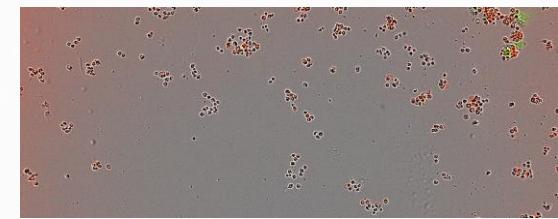
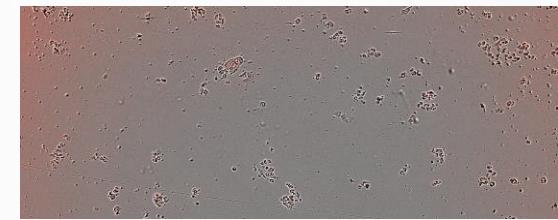
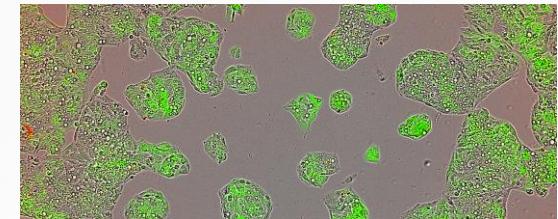
Vehicle



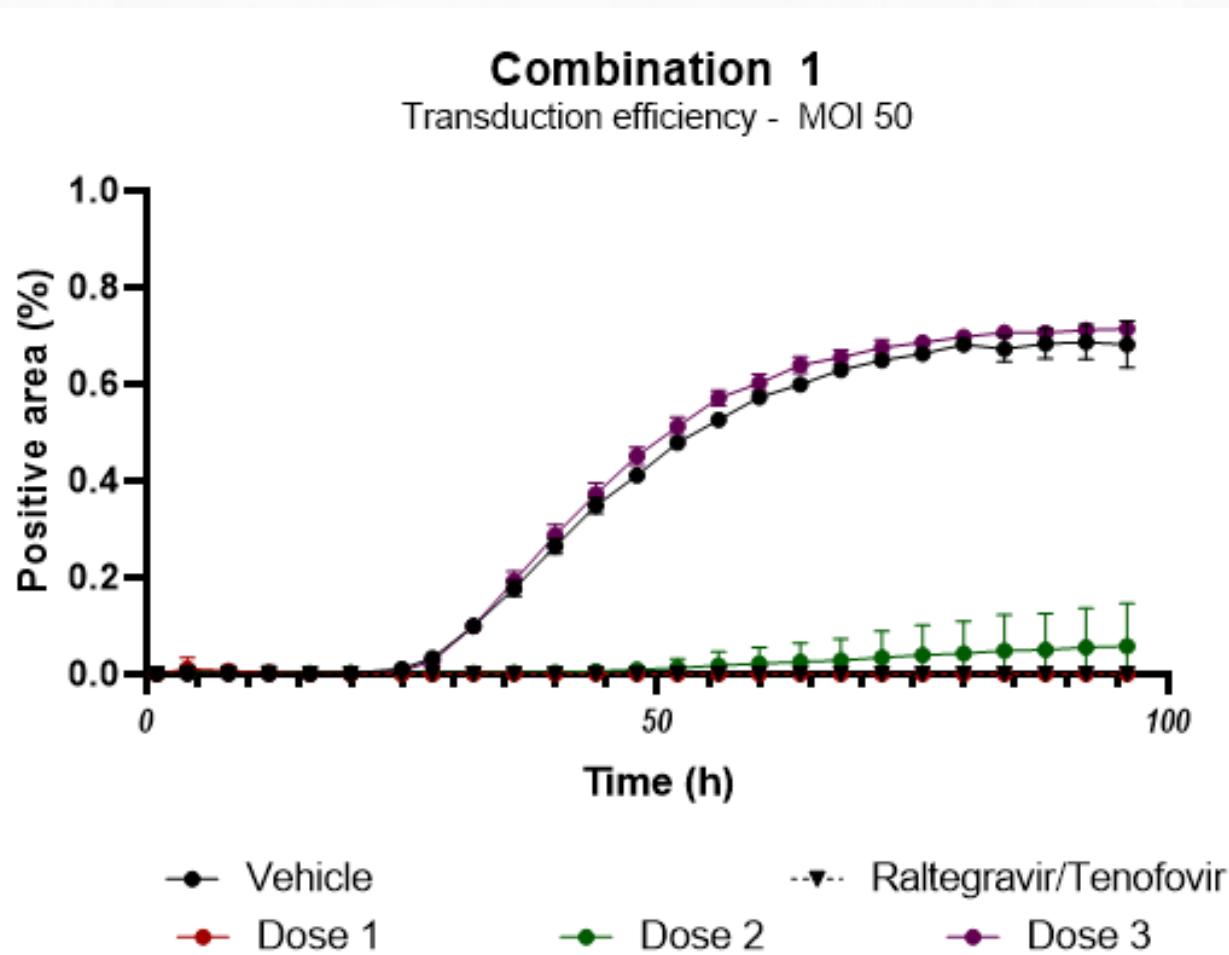
16 hours



84 hours



- Cells die at doses 1 and 2. Infectivity could only be analyzed with dose 3.

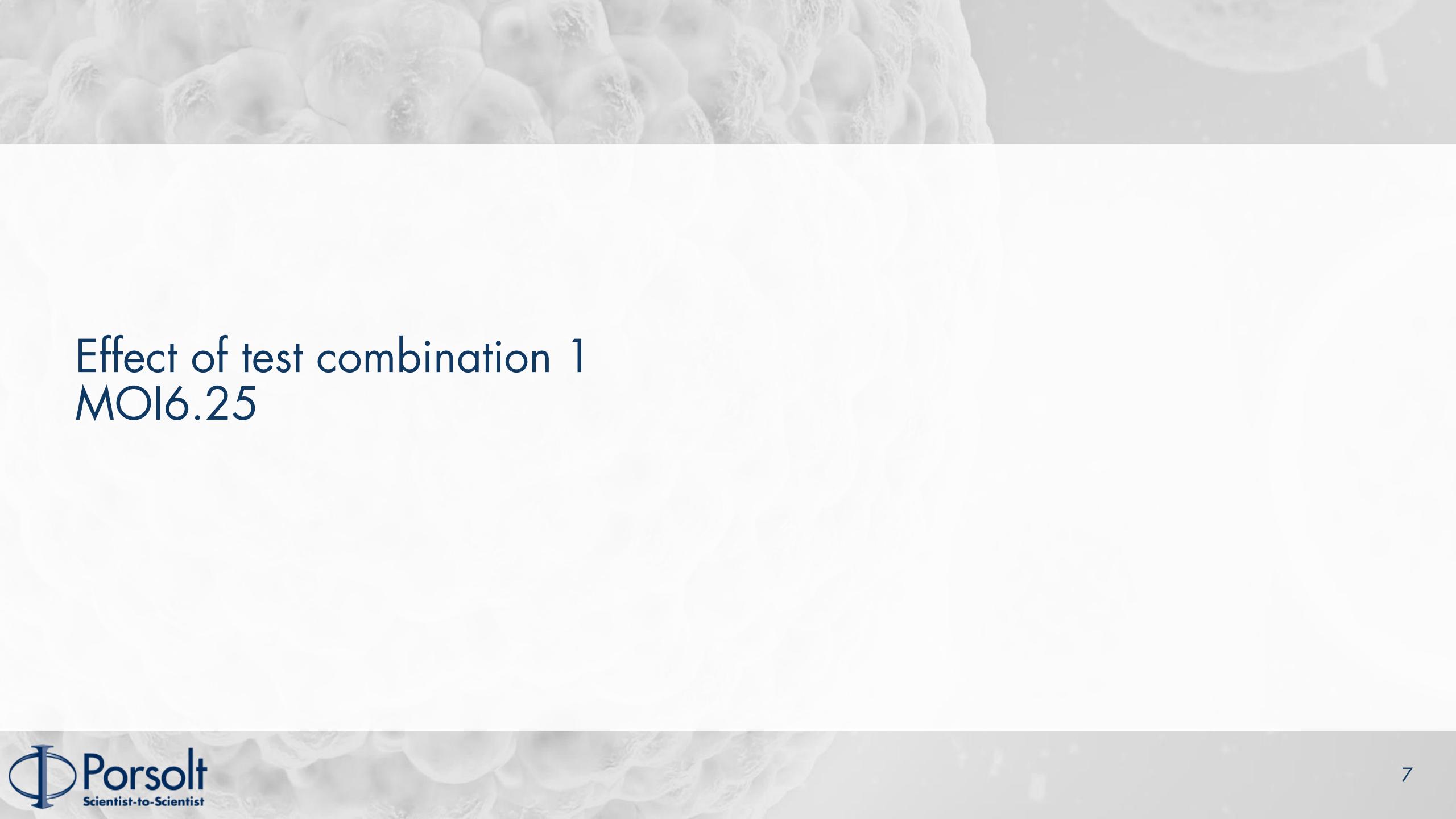


Statistical comparison at 60hours stimulation

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegrav	0.5722	0.5589 to 0.5854	Yes	****	<0.0001
Vehicle vs. Dose 1	0.5722	0.5589 to 0.5854	Yes	****	<0.0001
Vehicle vs. Dose 2	0.5507	0.5141 to 0.5874	Yes	****	<0.0001
Vehicle vs. Dose 3	-0.02938	0.05151 to -0.00726	Yes	**	0.0097

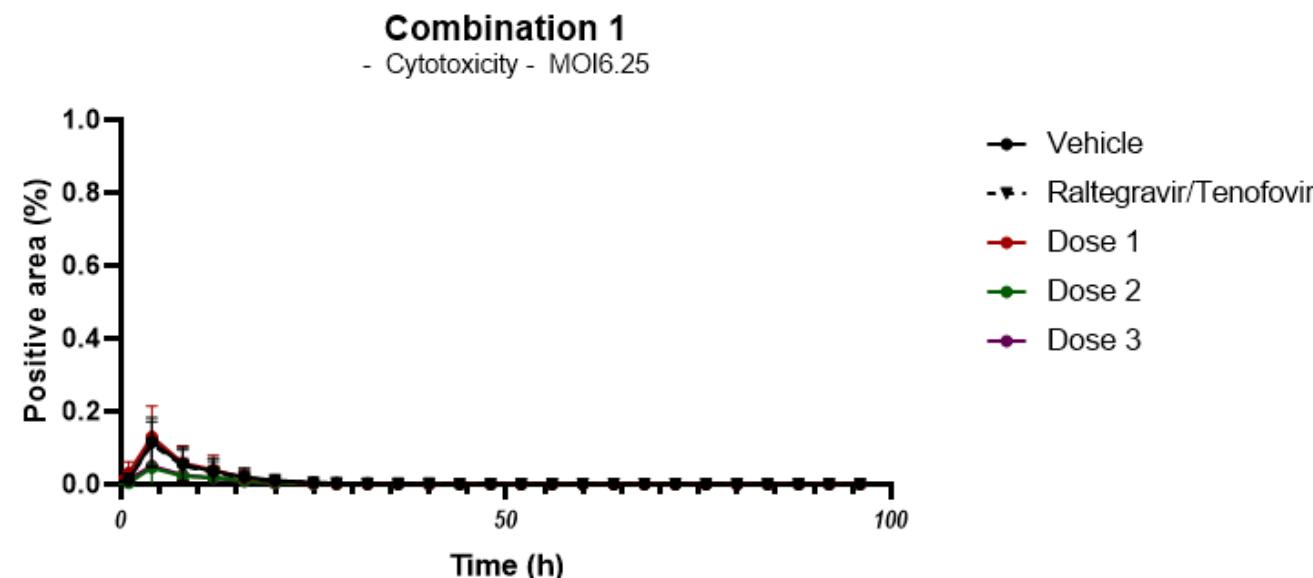
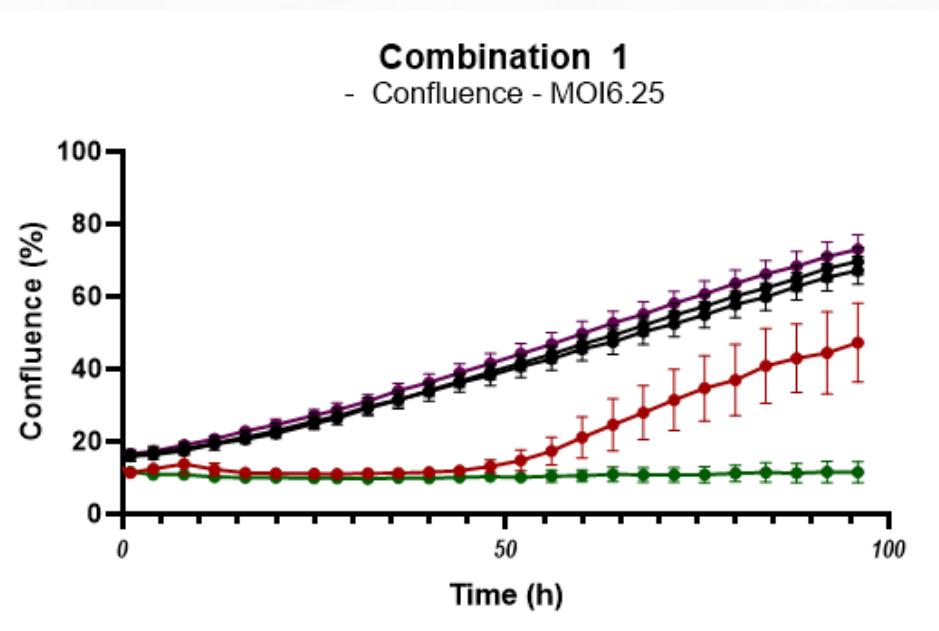
Figure: Effect of test combination on the transduction efficiency over time (0-96hours). The percentage of positive area (positive area over total cell area) is reported in kinetics. Two ways ANOVA followed by Tukey's post-hoc tests has been used for statistical comparison.

- ↗ Significant effect of Raltegravir/Tenofovir (Positive control)
- ↗ Uninterpretable results for doses 1 and 2
- ↗ Slight, but Significant impact of Combination 1 at dose 3 at 60 hours



Effect of test combination 1

MOI6.25



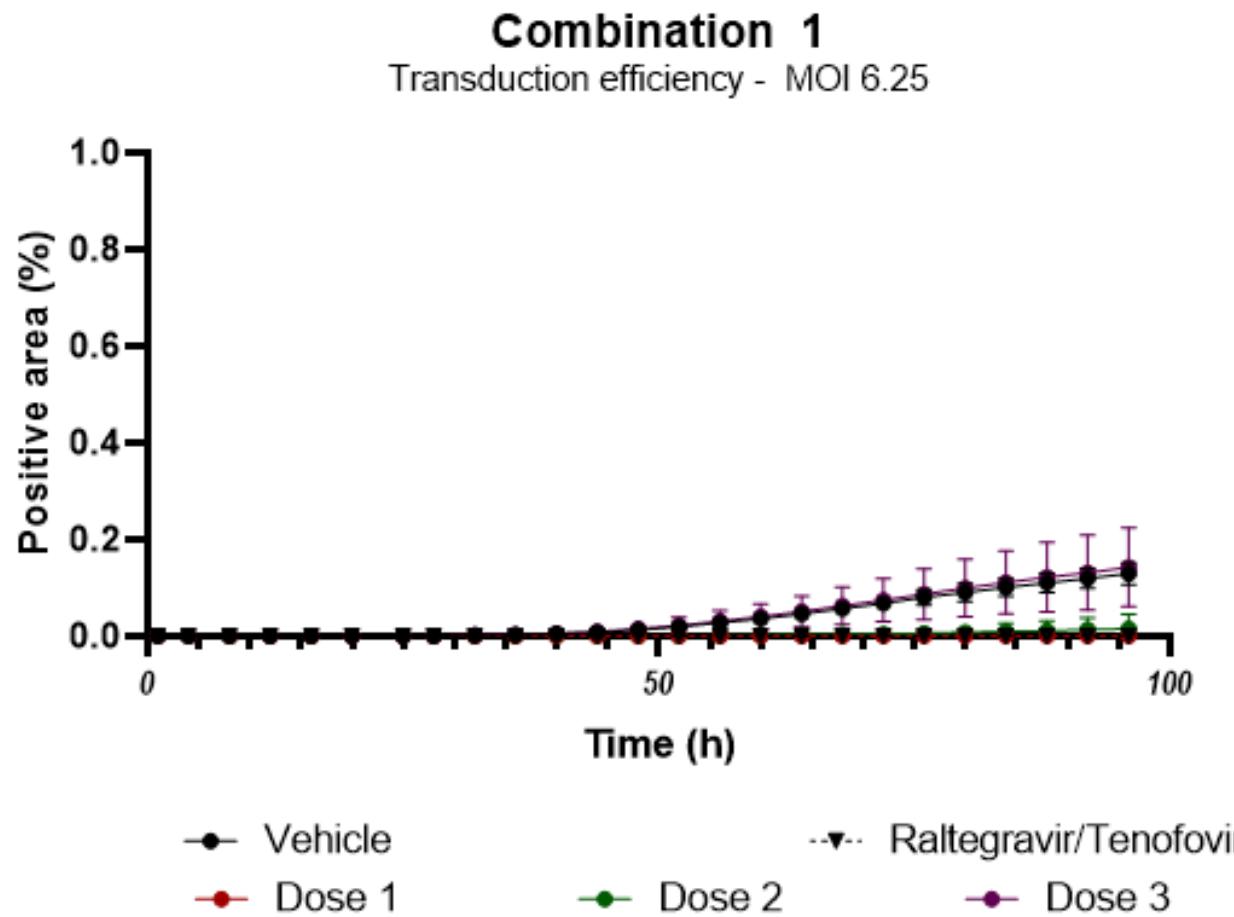
Statistical comparison at 96hours stimulation

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegrav	2.53	-2.686 to 7.746	No	ns	0.4998
Vehicle vs. Dose 1	22.46	10.33 to 34.59	Yes	**	0.0015
Vehicle vs. Dose 2	58.2	53.53 to 62.87	Yes	****	<0.0001
Vehicle vs. Dose 3	-3.336	-8.685 to 2.013	No	ns	0.2955

Statistical comparison at 4hours stimulation

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegrav	0.006888	0.08215 to 0.09593	No	ns	0.9983
Vehicle vs. Dose 1	-0.01375	-0.1189 to 0.09136	No	ns	0.988
Vehicle vs. Dose 2	0.0709	-0.02933 to 0.1711	No	ns	0.2054
Vehicle vs. Dose 3	0.0662	-0.03412 to 0.1665	No	ns	0.2526

Figure: effect of the mentioned test substance at various concentrations on cell cytotoxicity (right) and confluence (left). Two ways ANOVA followed by a Dunnett's post hoc test has been used for statistical comparison. In table below, a summary of statistical comparison is shown at 96 hours and 4hours respectively for confluence and cytotoxicity (maximum effect).



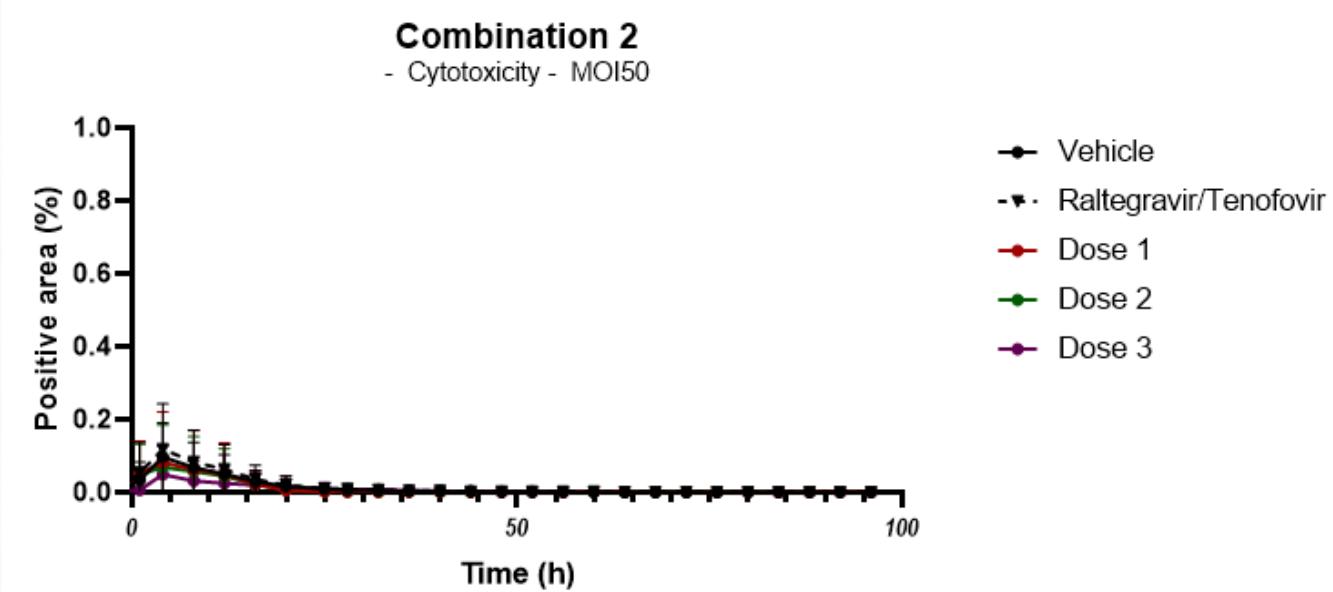
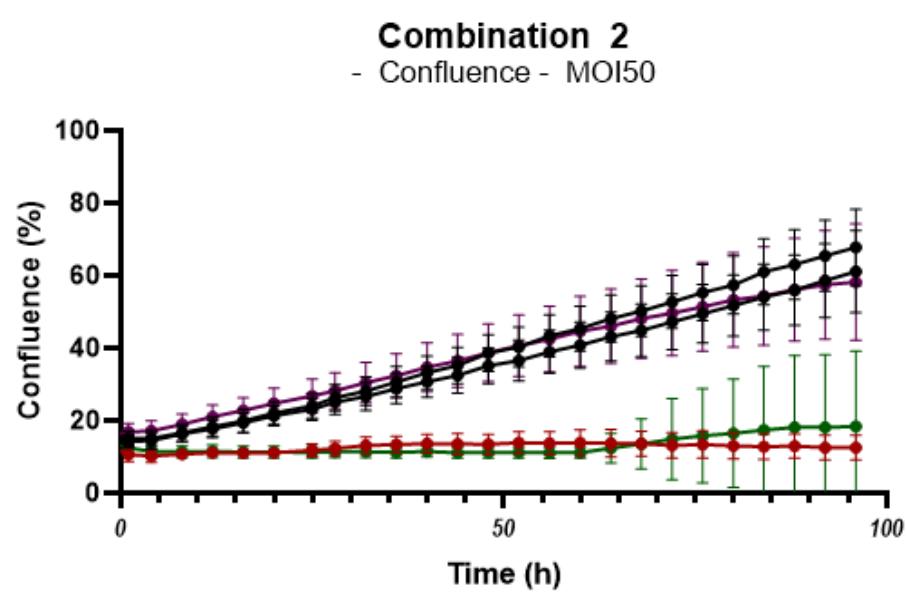
Statistical comparison at 60hours stimulation

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegrav	0.03666	0.02642 to 0.04689	Yes	****	<0.0001
Vehicle vs. Dose 1	0.03656	0.02633 to 0.04680	Yes	****	<0.0001
Vehicle vs. Dose 2	0.03465	0.02431 to 0.04499	Yes	****	<0.0001
Vehicle vs. Dose 3	-0.004144	-0.03385 to 0.02556	No	ns	0.9796

Figure: Effect of test combination on the transduction efficiency over time (0-96hours). The percentage of positive area (positive area over total cell area) is reported in kinetics. Two ways ANOVA followed by Tukey's post-hoc tests has been used for statistical comparison.

- ↗ Significant effect of Raltegravir/Tenofovir (Positive control)
- ↗ Uninterpretable results for doses 1 and 2
- ↗ No significant effect of Combination 1 at dose 3

Effect of test combination 2 MOI50



Statistical comparison at 96hours stimulation

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegrav	6.686	-8.391 to 21.76	No	ns	0.5718
Vehicle vs. Dose 1	55.28	43.61 to 66.96	Yes	****	<0.0001
Vehicle vs. Dose 2	49.4	25.84 to 72.96	Yes	***	0.0004
Vehicle vs. Dose 3	9.563	-9.496 to 28.62	No	ns	0.4596

Statistical comparison at 4hours stimulation

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegrav	-0.02098	-0.1740 to 0.1320	No	ns	0.9855
Vehicle vs. Dose 1	0.0151	-0.1497 to 0.1799	No	ns	0.9967
Vehicle vs. Dose 2	0.02868	-0.1163 to 0.1737	No	ns	0.9491
Vehicle vs. Dose 3	0.04843	-0.07021 to 0.1671	No	ns	0.6335

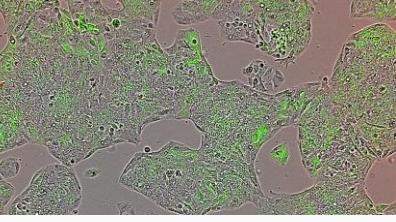
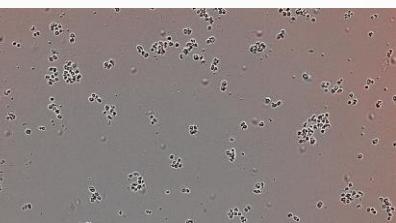
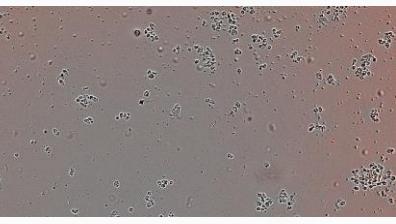
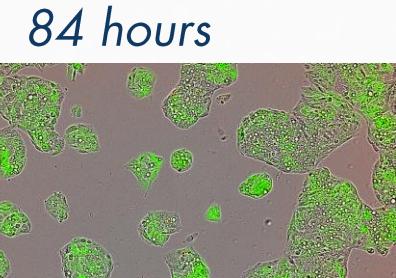
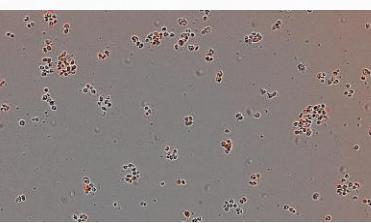
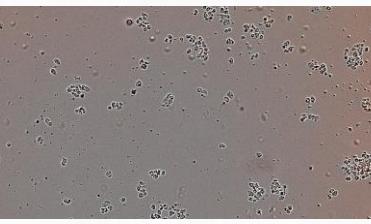
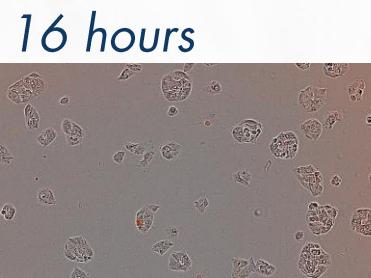
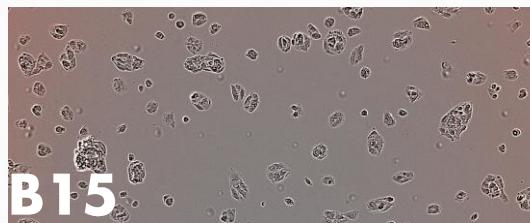
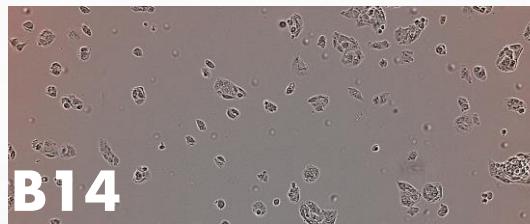
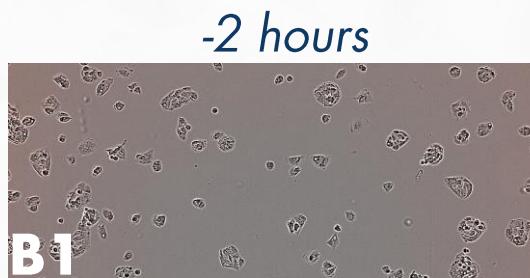
Figure: effect of the mentioned test substance at various concentrations on cell cytotoxicity (right) and confluence (left). Two ways ANOVA followed by a Dunnett's post hoc test has been used for statistical comparison. In table below, a summary of statistical comparison is shown at 96 hours and 4hours respectively for confluence and cytotoxicity (maximum effect).

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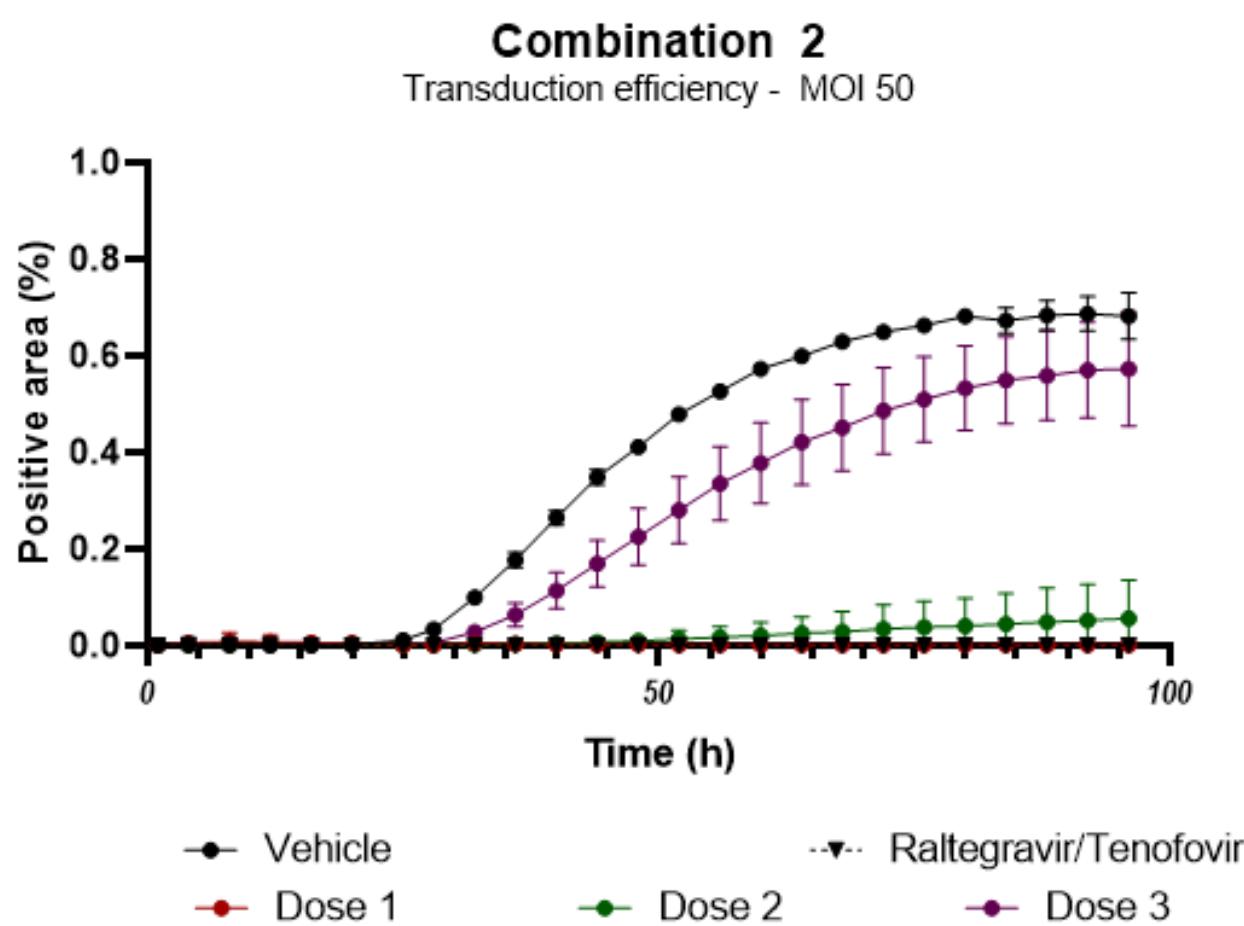
Combination 2 : Cytotoxicity and cell growth at MOI50

Pictures

Vehicle



- Cells die at doses 1 and 2. Infectivity could only be analyzed with dose 3.

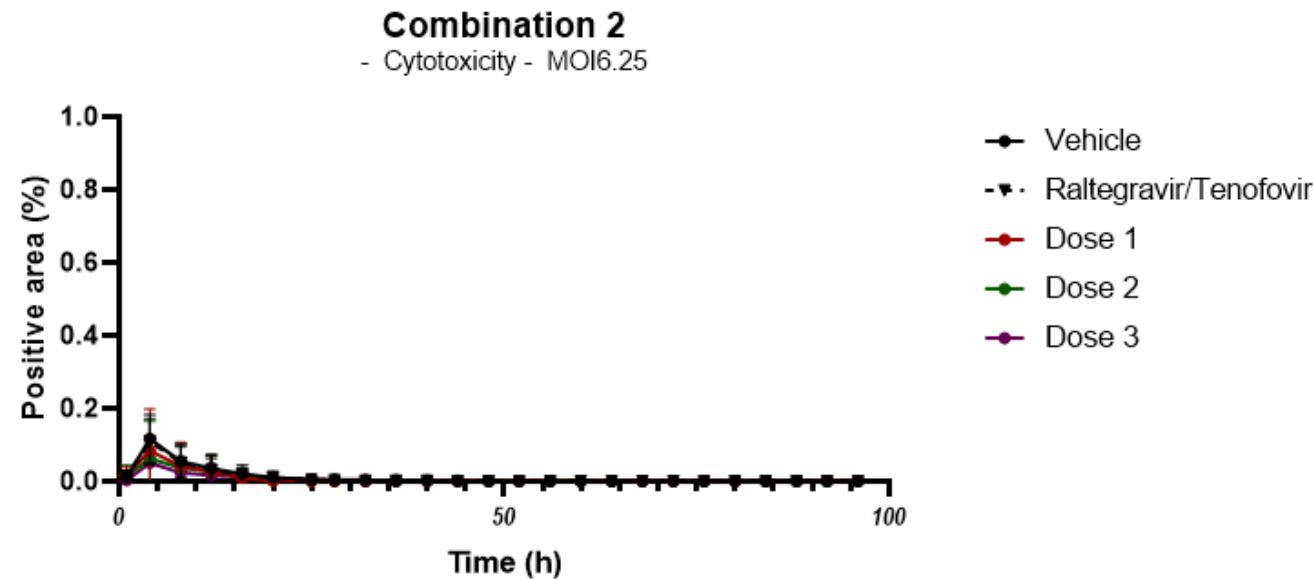
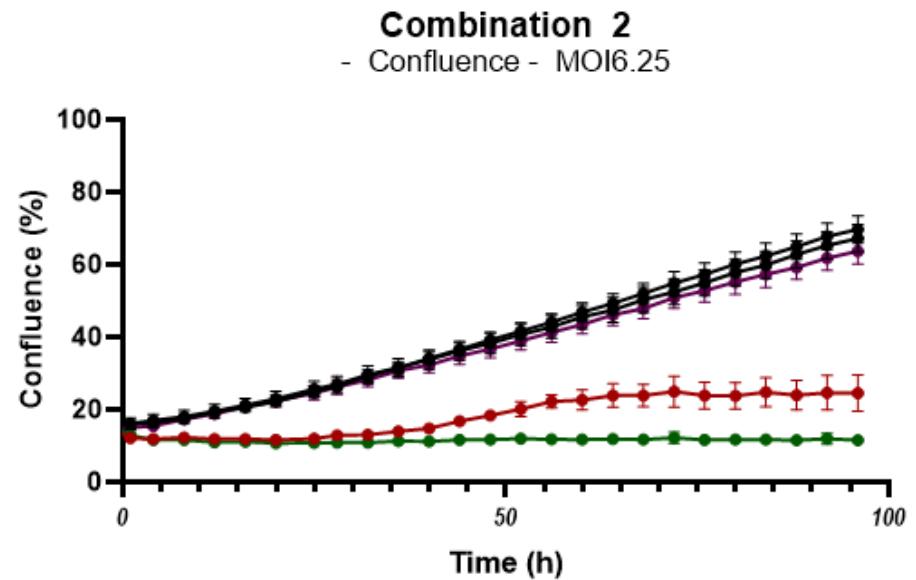


Statistical comparison at 60hours stimulation					
	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegrav	0.5722	0.5589 to 0.5854	Yes	***	<0.0001
Vehicle vs. Dose 1	0.5716	0.5583 to 0.5848	Yes	***	<0.0001
Vehicle vs. Dose 2	0.5525	0.5212 to 0.5837	Yes	***	<0.0001
Vehicle vs. Dose 3	0.1951	0.1032 to 0.2870	Yes	***	0.0009

Figure: Effect of test combination on the transduction efficiency over time (0-96hours). The percentage of positive area (positive area over total cell area) is reported in kinetics. Two ways ANOVA followed by Tukey's post-hoc tests has been used for statistical comparison.

- ↗ Significant effect of Raltegravir/Tenofovir (Positive control)
- ↗ Uninterpretable results for doses 1 and 2
- ↗ Significant and positive effect of Combination 2 at dose 3

Effect of test combination 2 MOI6.25



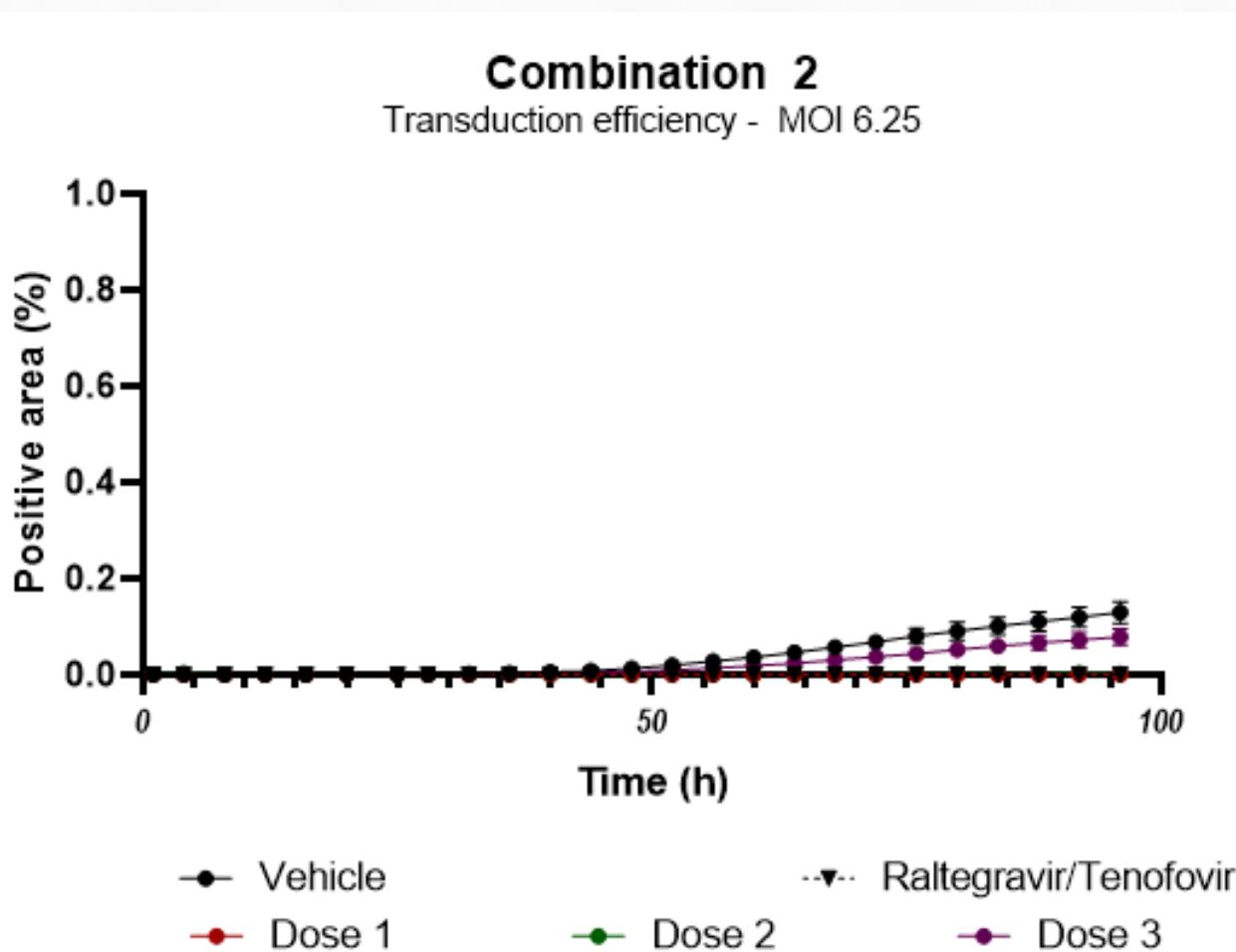
Statistical comparison at 96hours stimulation

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegrav	2.53	-2.698 to 7.758	No	ns	0.5046
Vehicle vs. Dose 1	45.22	38.01 to 52.43	Yes	****	<0.0001
Vehicle vs. Dose 2	58.17	53.94 to 62.39	Yes	****	<0.0001
Vehicle vs. Dose 3	6.045	0.9601 to 11.13	Yes	*	0.0186

Statistical comparison at 4hours stimulation

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegrav	0.006888	0.08235 to 0.09612	No	ns	0.9984
Vehicle vs. Dose 1	0.03246	-0.1280 to 0.1929	No	ns	0.9226
Vehicle vs. Dose 2	0.05386	-0.06735 to 0.1751	No	ns	0.5608
Vehicle vs. Dose 3	0.06623	-0.02509 to 0.1576	No	ns	0.1913

Figure: effect of the mentioned test substance at various concentrations on cell cytotoxicity (right) and confluence (left). Two ways ANOVA followed by a Dunnett's post hoc test has been used for statistical comparison. In table below, a summary of statistical comparison is shown at 96 hours and 4hours respectively for confluence and cytotoxicity (maximum effect). Two wells have been excluded due to technical reason (Dose 1).

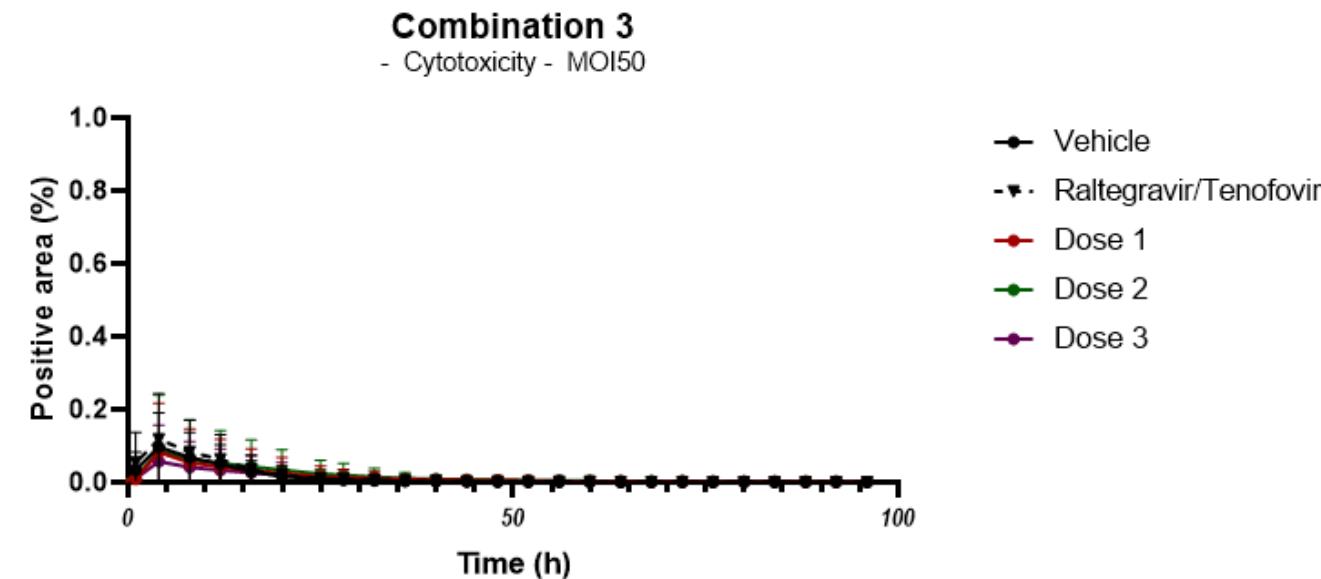
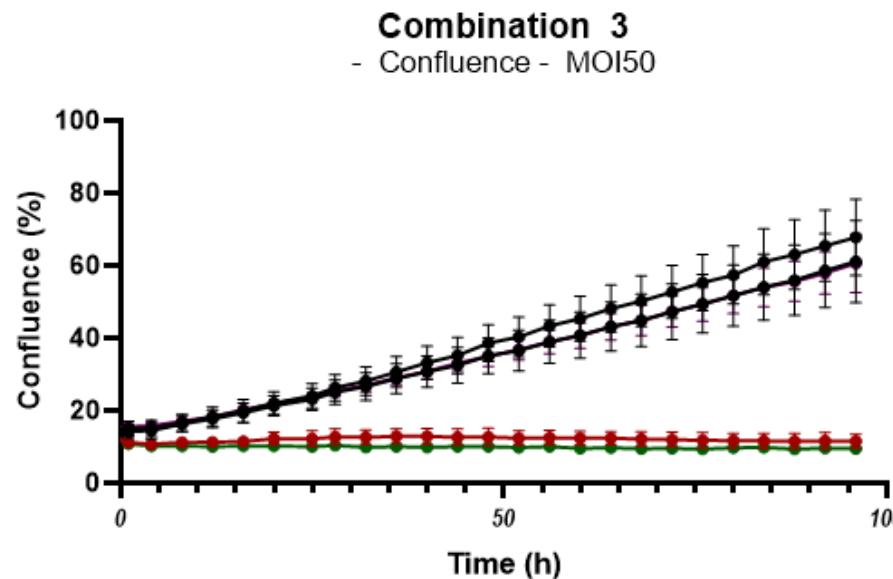


Statistical comparison at 60hours stimulation					
	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegravir	0.03666	0.02639 to 0.04692	Yes	***	<0.0001
Vehicle vs. Dose 1	0.03655	0.02629 to 0.04681	Yes	***	<0.0001
Vehicle vs. Dose 2	0.0361	0.02583 to 0.04636	Yes	***	<0.0001
Vehicle vs. Dose 3	0.01767	0.006792 to 0.02855	Yes	**	0.0023

Figure: Effect of test combination on the transduction efficiency over time (0-96hours). The percentage of positive area (positive area over total cell area) is reported in kinetics. Two ways ANOVA followed by Tukey's post-hoc tests has been used for statistical comparisons. Two wells have been excluded due to technical reason (Dose 1).

- ↗ Significant effect of Raltegravir/Tenofovir (Positive control)
- ↗ Uninterpretable results for doses 1 and 2
- ↗ Significant and positive effect of Combination 2 at dose 3

Effect of test combination 3 MOI50



Statistical comparison at 96hours stimulation

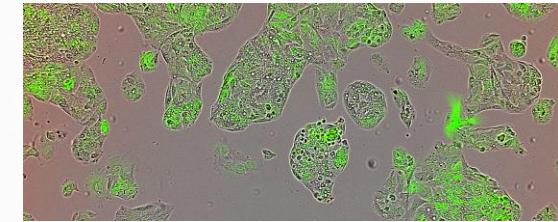
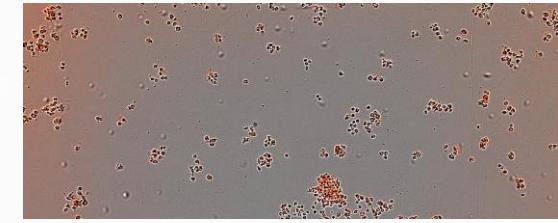
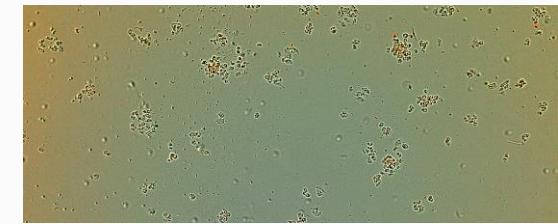
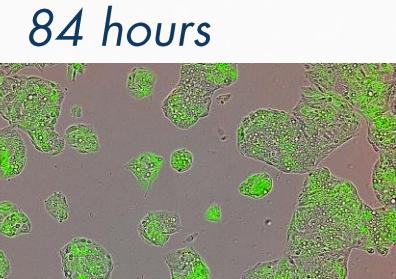
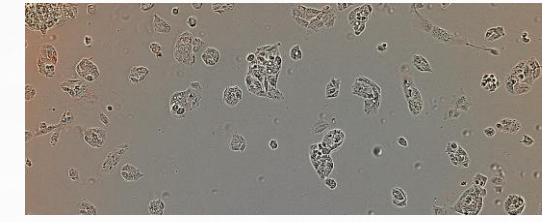
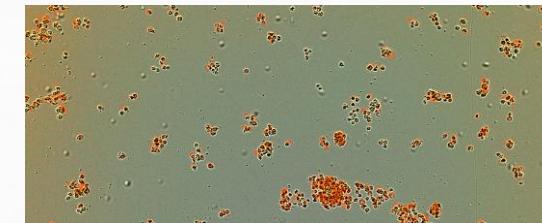
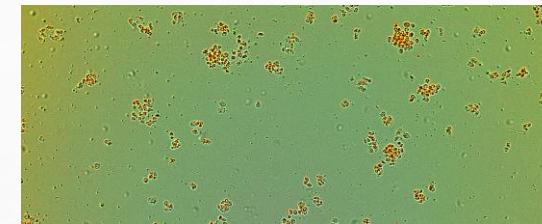
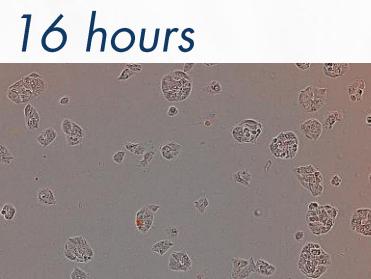
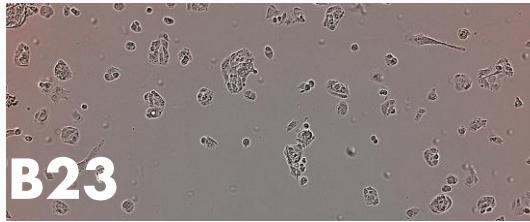
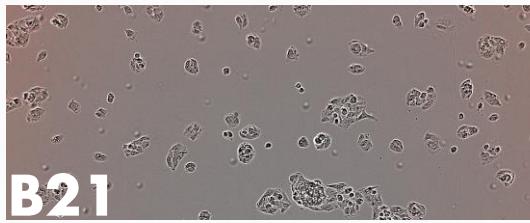
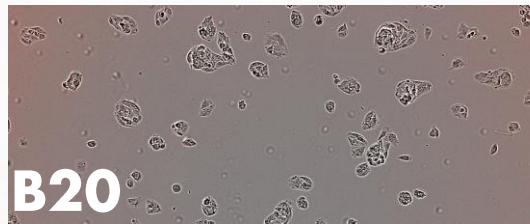
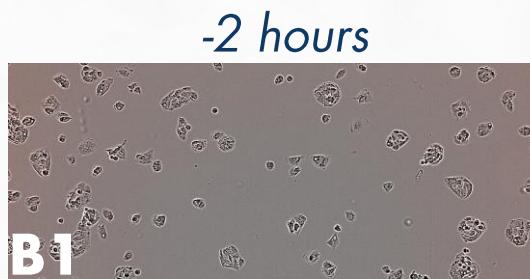
	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegrav	6.686	-8.391 to 21.76	No	ns	0.5718
Vehicle vs. Dose 1	56.24	44.63 to 67.85	Yes	****	<0.0001
Vehicle vs. Dose 2	58.35	46.74 to 69.96	Yes	****	<0.0001
Vehicle vs. Dose 3	7.184	-5.809 to 20.18	No	ns	0.3867

Statistical comparison at 4hours stimulation

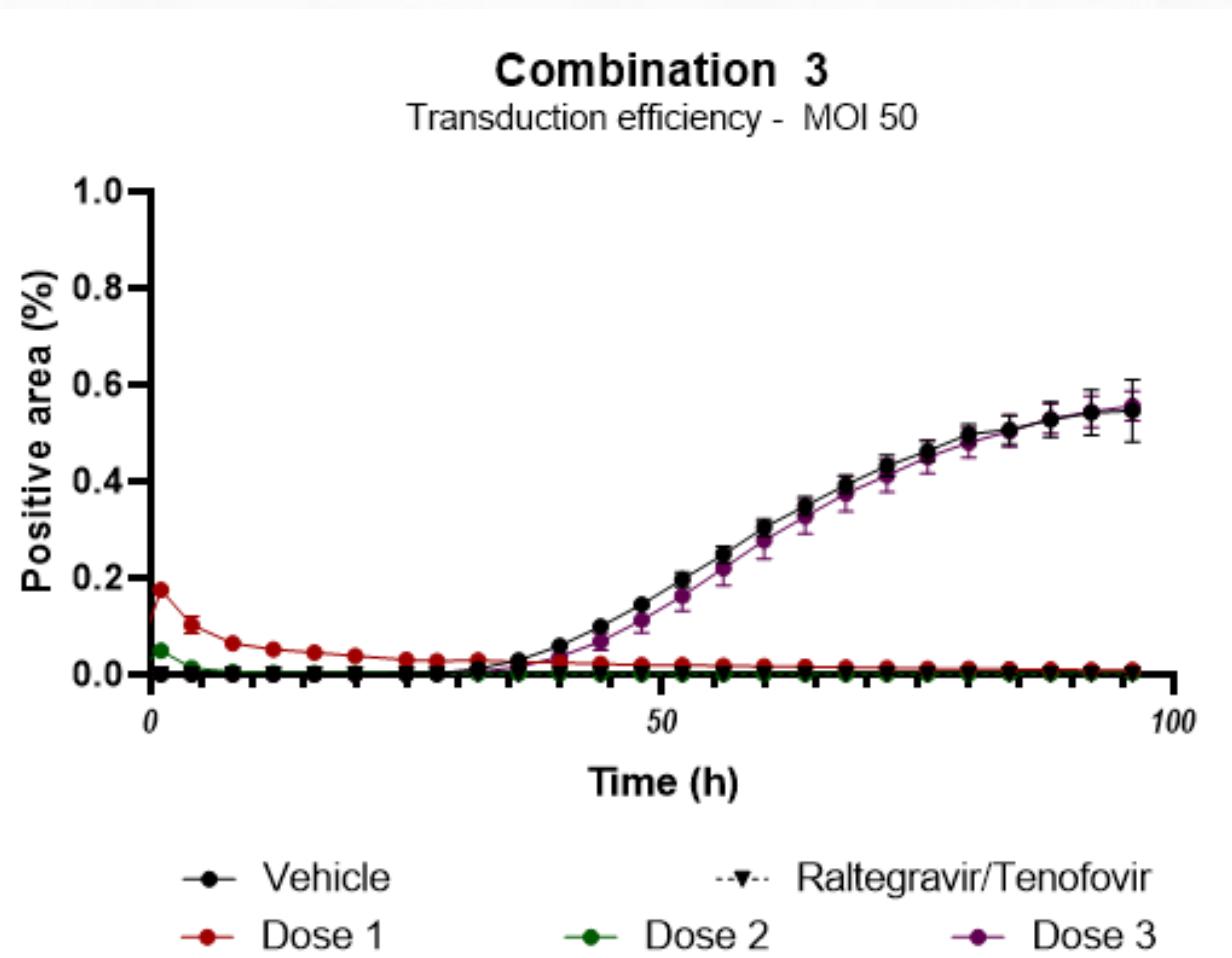
	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegrav	-0.02098	-0.1740 to 0.1320	No	ns	0.9855
Vehicle vs. Dose 1	0.01419	-0.1470 to 0.1754	No	ns	0.9971
Vehicle vs. Dose 2	0.01014	-0.1684 to 0.1887	No	ns	0.9993
Vehicle vs. Dose 3	0.03963	-0.09287 to 0.1721	No	ns	0.8256

Figure: effect of the mentioned test substance at various concentrations on cell cytotoxicity (right) and confluence (left). Two ways ANOVA followed by a Dunnett's post hoc test has been used for statistical comparison. In table below, a summary of statistical comparison is shown at 96 hours and 4hours respectively for confluence and cytotoxicity (maximum effect).

Vehicle



- Cells die at doses 1 and 2. Infectivity could only be analyzed with dose 3.



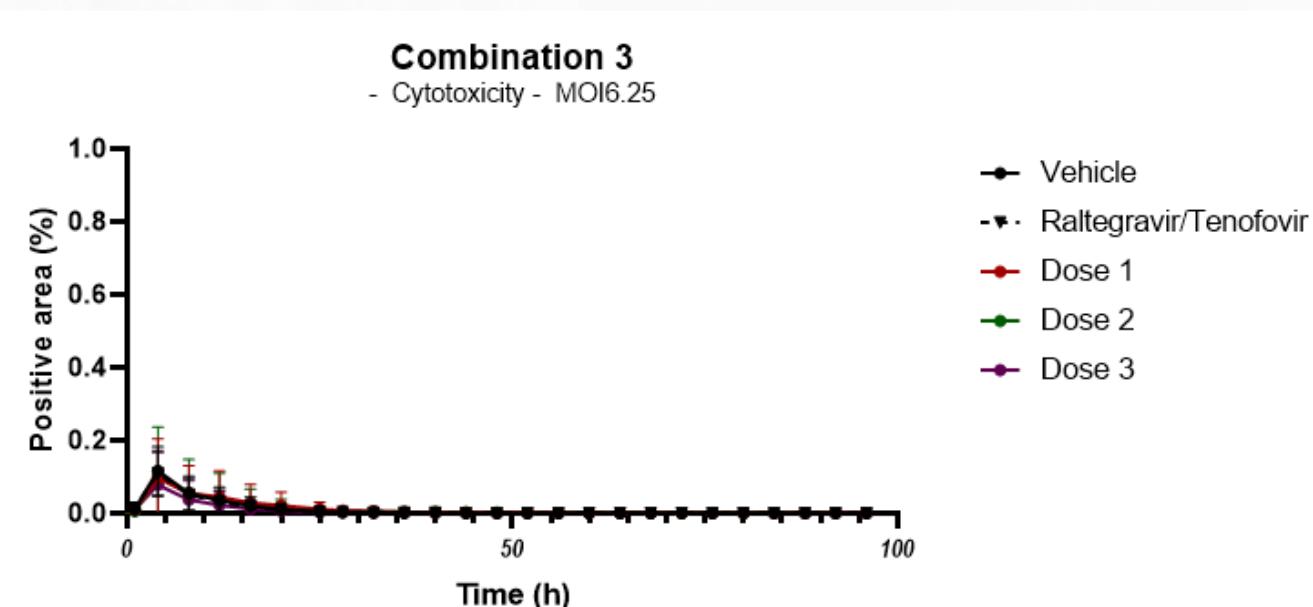
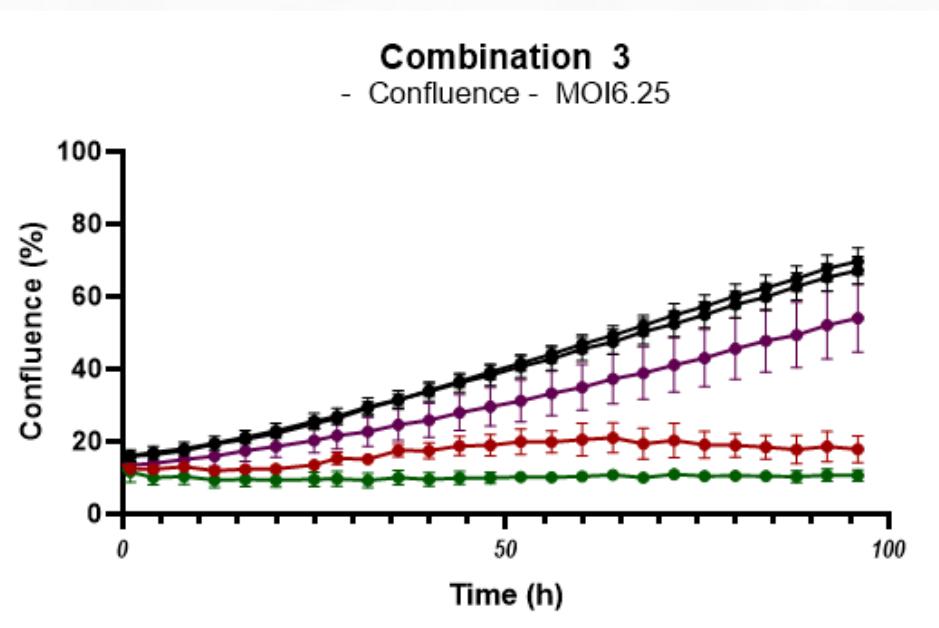
Statistical comparison at 60hours stimulation

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegrav	0.3045	0.2853 to 0.3236	Yes	****	<0.0001
Vehicle vs. Dose 1	0.2873	0.2682 to 0.3065	Yes	****	<0.0001
Vehicle vs. Dose 2	0.3033	0.2842 to 0.3224	Yes	****	<0.0001
Vehicle vs. Dose 3	0.0264	-0.01722 to 0.07002	No	ns	0.2939

Figure: Effect of test combination on the transduction efficiency over time (0-96hours). The percentage of positive area (positive area over total cell area) is reported in kinetics. Two ways ANOVA followed by Tukey's post-hoc tests has been used for statistical comparison. Due to some autofluorescence of curcumin, a different threshold have been applied to analyse transduction efficiency for this combination (vehicle and Raltegravir/Tenofovir have been analysed with the same parameters)

- ↗ Significant effect of Raltegravir/Tenofovir (Positive control)
- ↗ Uninterpretable results for doses 1 and 2
- ↗ No significant effect of Combination 3 at dose 3

Effect of test combination 3 MOI6.25

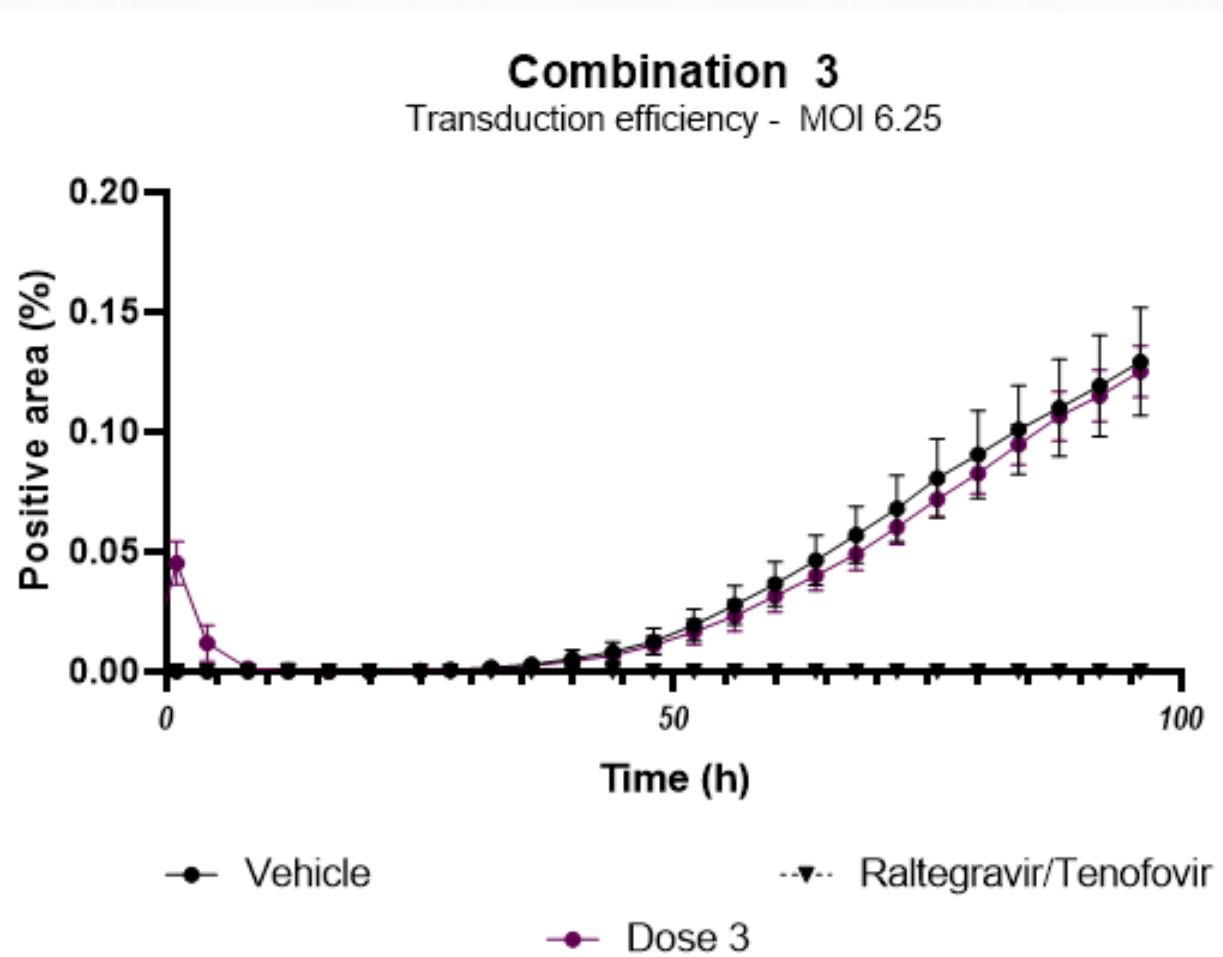


Statistical comparison at 96hours stimulation

Statistical comparison at 4hours stimulation

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value		Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegrav	2.53	-2.698 to 7.758	No	ns	0.5046	Vehicle vs. Raltegrav	0.006888	0.08215 to 0.09593	No	ns	0.9983
Vehicle vs. Dose 1	51.82	46.03 to 57.60	Yes	****	<0.0001	Vehicle vs. Dose 1	0.0193	-0.1065 to 0.1451	No	ns	0.977
Vehicle vs. Dose 2	59.09	54.84 to 63.33	Yes	****	<0.0001	Vehicle vs. Dose 2	0.01583	-0.1382 to 0.1699	No	ns	0.9943
Vehicle vs. Dose 3	15.72	5.191 to 26.26	Yes	**	0.0055	Vehicle vs. Dose 3	0.0385	-0.07035 to 0.1473	No	ns	0.7286

Figure: effect of the mentioned test substance at various concentrations on cell cytotoxicity (right) and confluence (left). Two ways ANOVA followed by a Dunnett's post hoc test has been used for statistical comparison. In table below, a summary of statistical comparison is shown at 96 hours and 4hours respectively for confluence and cytotoxicity (maximum effect). Two wells have been excluded due to technical reason (Dose 1).



Statistical comparison at 60hours stimulation					
	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegrav	0.03666	0.02642 to 0.04689	Yes	***	<0.0001
Vehicle vs. Dose 1	-0.06366	-0.1111 to -0.01616	Yes	*	0.0121
Vehicle vs. Dose 2	0.02764	0.01739 to 0.03790	Yes	***	0.0002
Vehicle vs. Dose 3	0.005092	-0.006163 to 0.01635	No	ns	0.5483

Figure: Effect of test combination on the transduction efficiency over time (0-96hours). The percentage of positive area (positive area over total cell area) is reported in kinetics. Two ways ANOVA followed by Tukey's post-hoc tests has been used for statistical comparisons.

- ↗ Significant effect of Raltegravir/Tenofovir (Positive control)
- ↗ Uninterpretable results for doses 1 and 2, lower cell growth for dose 3 (remain in acceptable range).
- ↗ No significant effect of Combination 3 at dose 3

- ❖ Doses 1 and 2 of all test combinations show toxicity and inhibition of cell growth
- ❖ For each combination, effect of dose 3 could be interpreted either at MOI50 or at MOI6.25
- ❖ No significant effect has been observed for combination 1 and 3 (dose 3).
- ❖ Significant and positive effect has been observed for combination 2 (dose 3). These effects have been observed at MOI50 and MOI6.25