



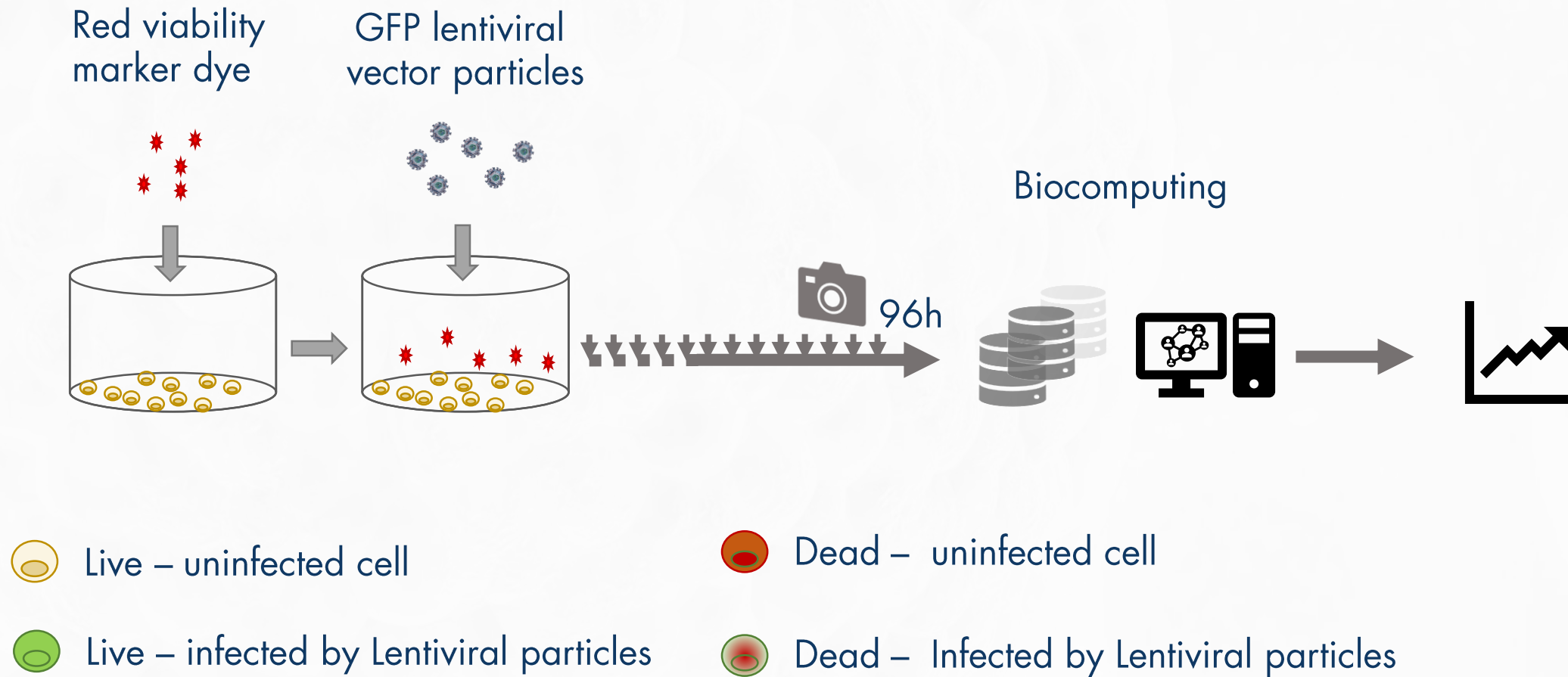
# Porsolt

Scientist-to-Scientist

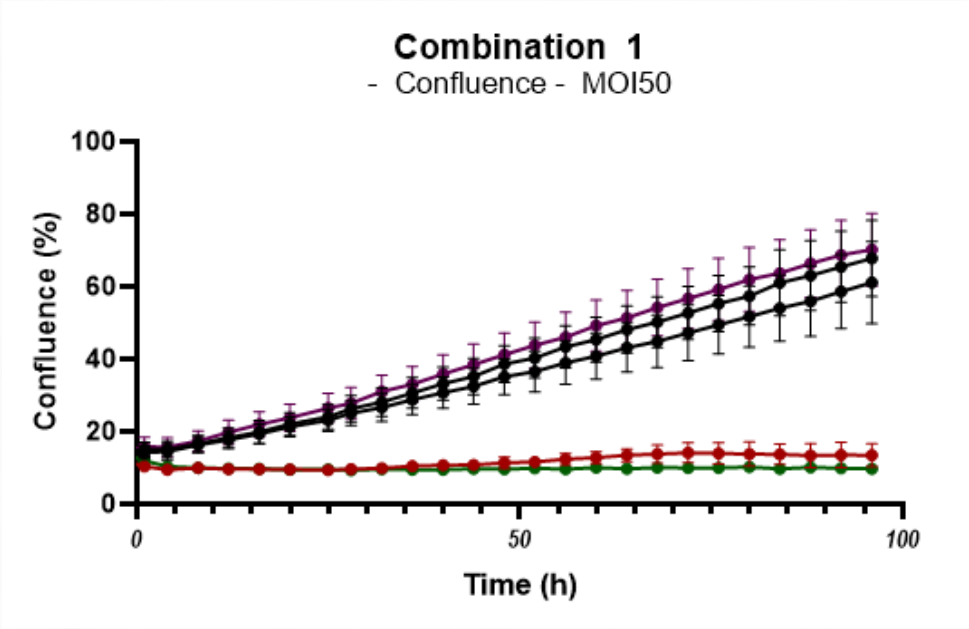
20.0451



[www.porsolt.com](http://www.porsolt.com)

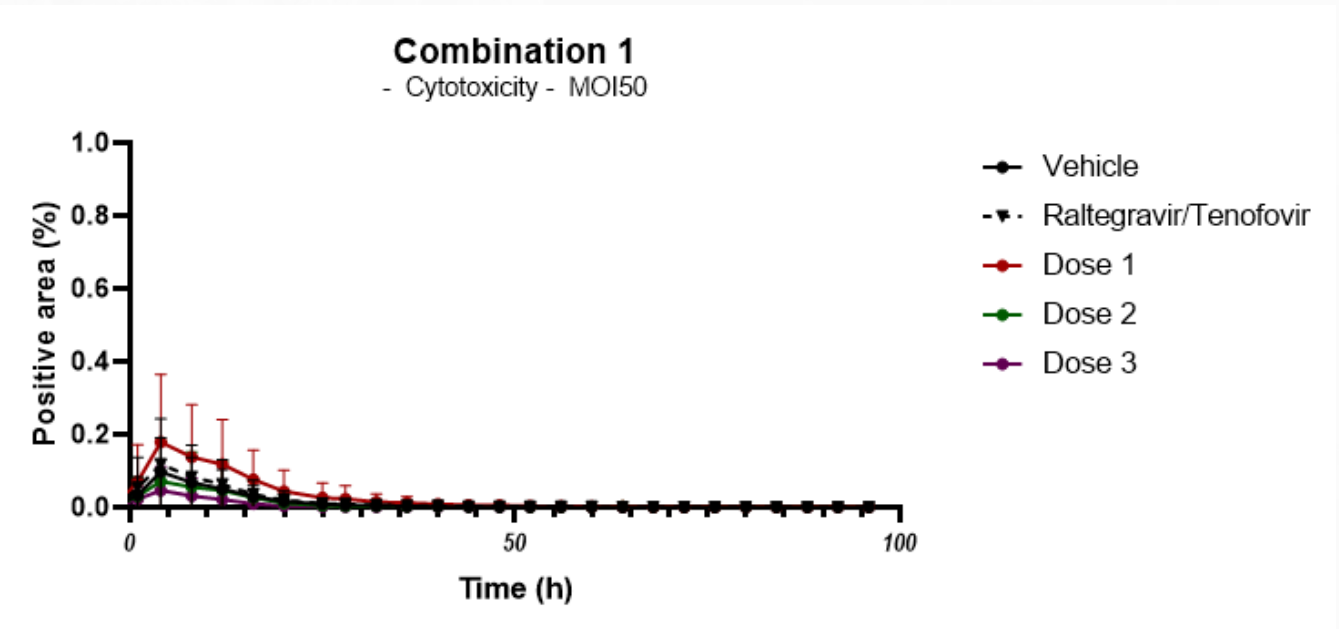


# Effect of test combination 1 MOI50



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

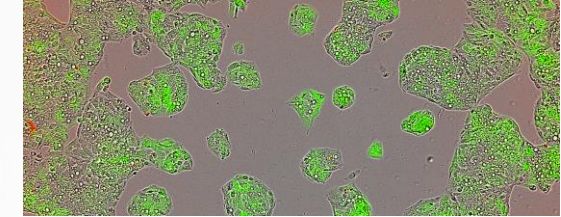
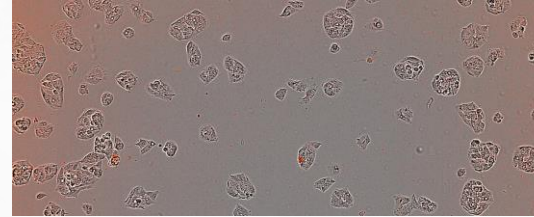
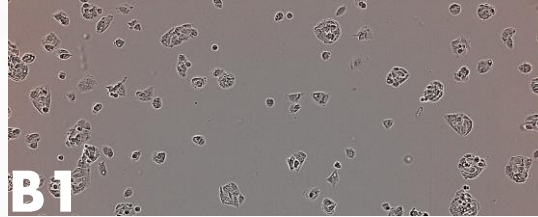
	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegrav	-0.02098	-0.1740 to 0.1320	No	ns	0.9855
Vehicle vs. Dose 1	-0.08192	-0.2929 to 0.1290	No	ns	0.644
Vehicle vs. Dose 2	0.02623	-0.1146 to 0.1670	No	ns	0.9586
Vehicle vs. Dose 3	0.05057	-0.06195 to 0.1631	No	ns	0.5527

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).

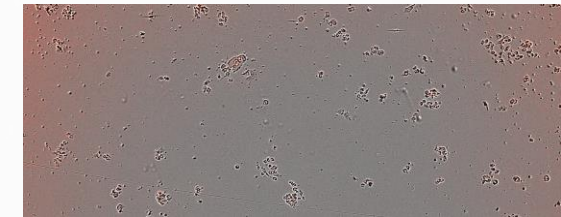
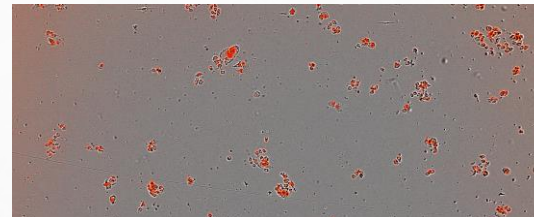
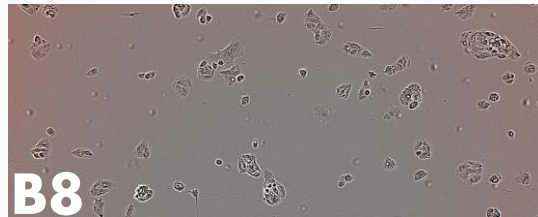


*-2 hours**16 hours**84 hours*

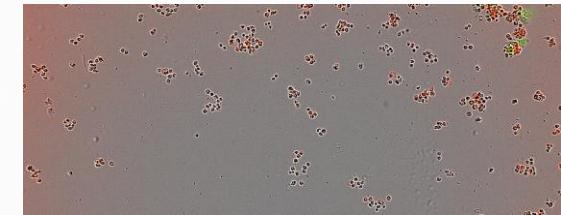
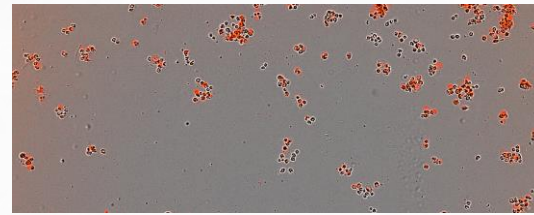
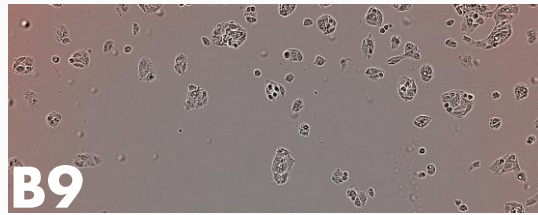
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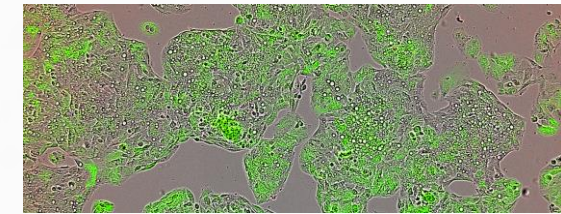
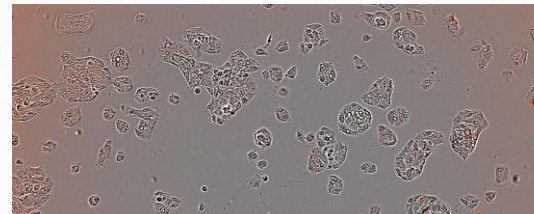
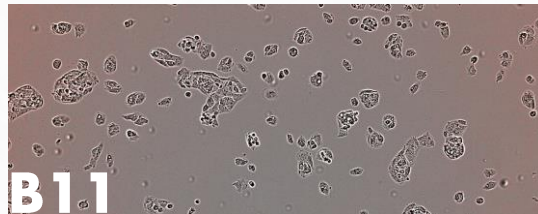
Dose 1



Dose 2

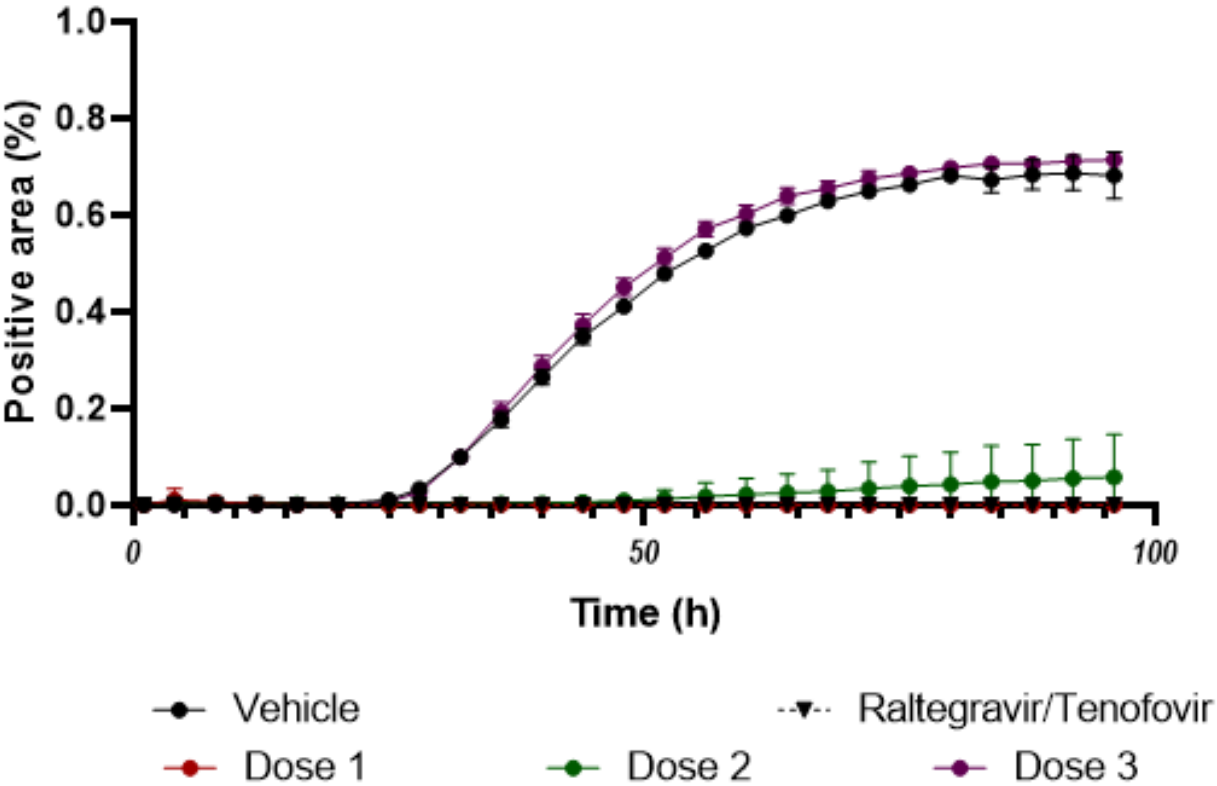


Dose 3



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

**Combination 1**  
Transduction efficiency - MOI 50



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

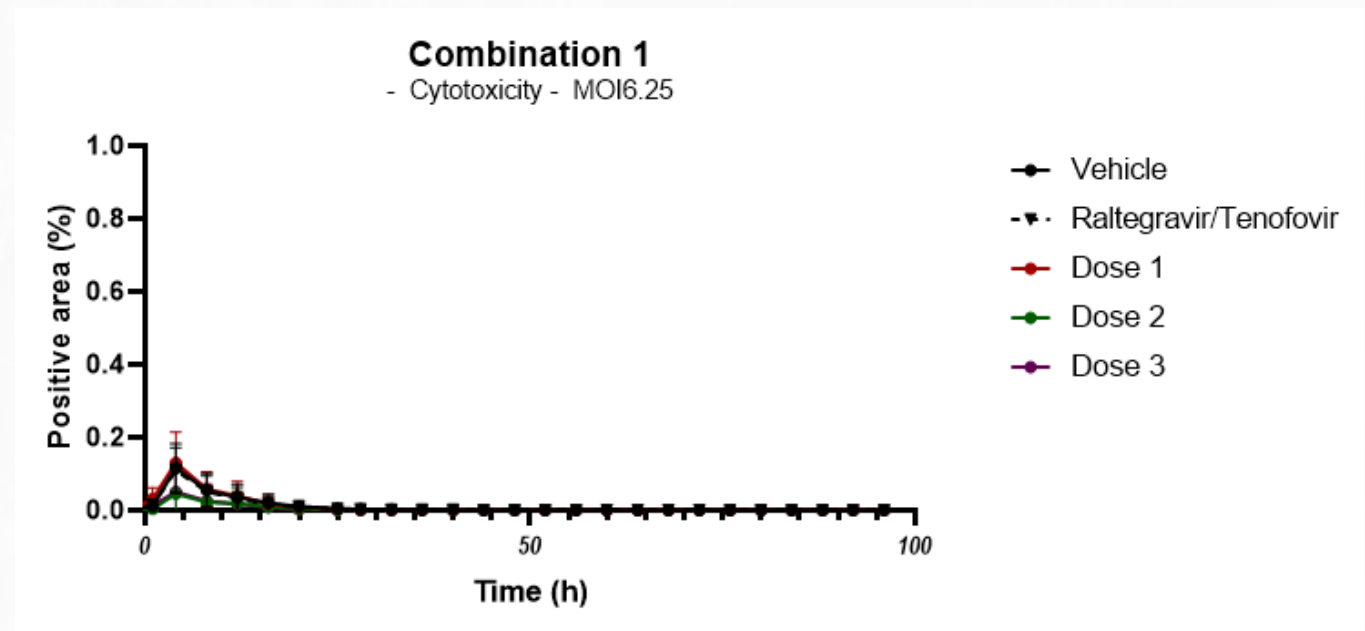
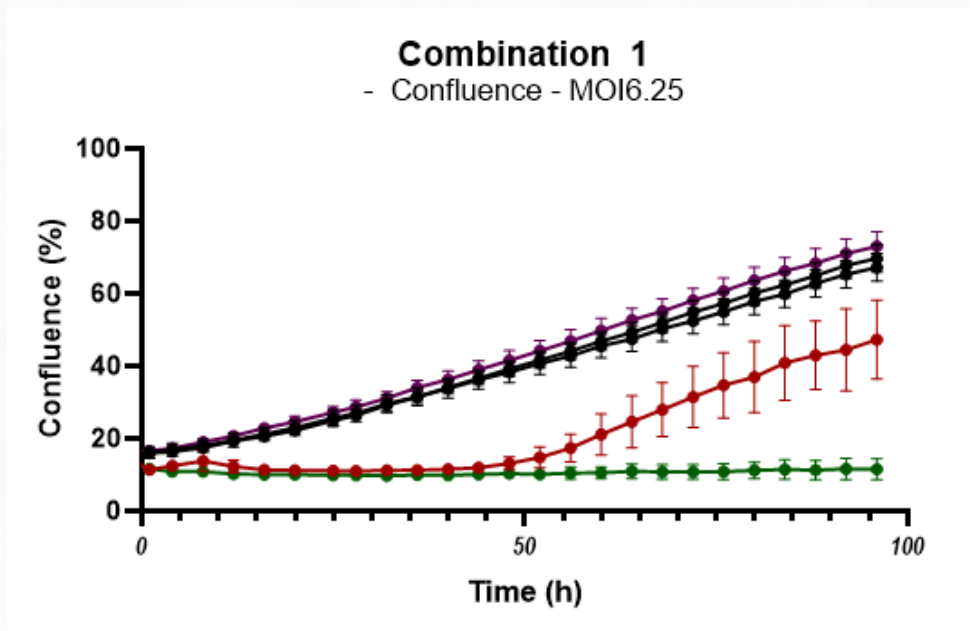
Figure: Effect of test combination on the transduction efficiency over time (0-96hours). The percentage of positive area (positive area over total cell area) is reported in kinetics. Two ways ANOVA followed by Tukey's post-hoc tests has been used for statistical comparison.

- ✦ 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

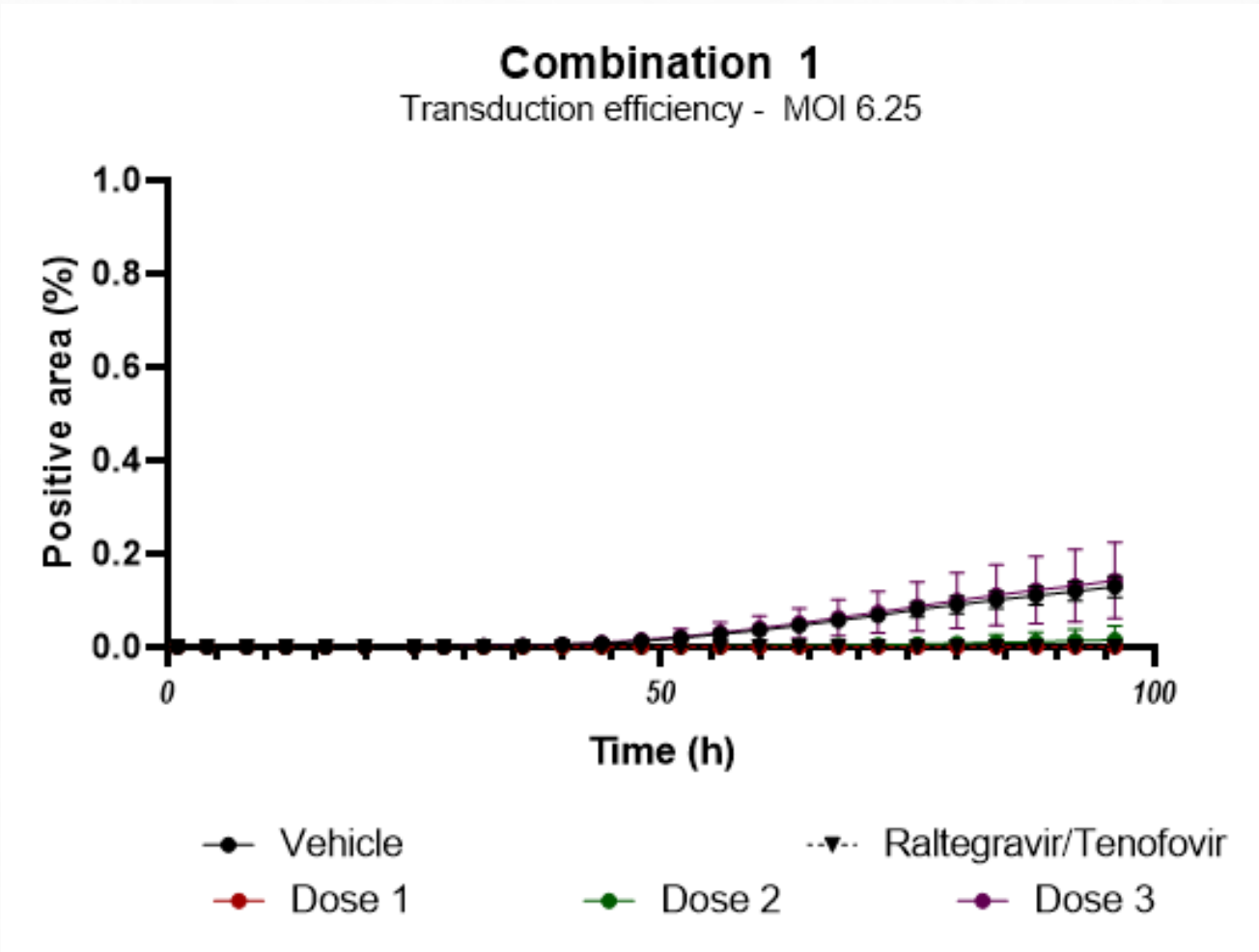
	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegrav	2.53	-2.686 to 7.746	No	ns	0.4998
Vehicle vs. Dose 1	22.46	10.33 to 34.59	Yes	**	0.0015
Vehicle vs. Dose 2	58.2	53.53 to 62.87	Yes	****	<0.0001
Vehicle vs. Dose 3	-3.336	-8.685 to 2.013	No	ns	0.2955

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 0.09591	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).



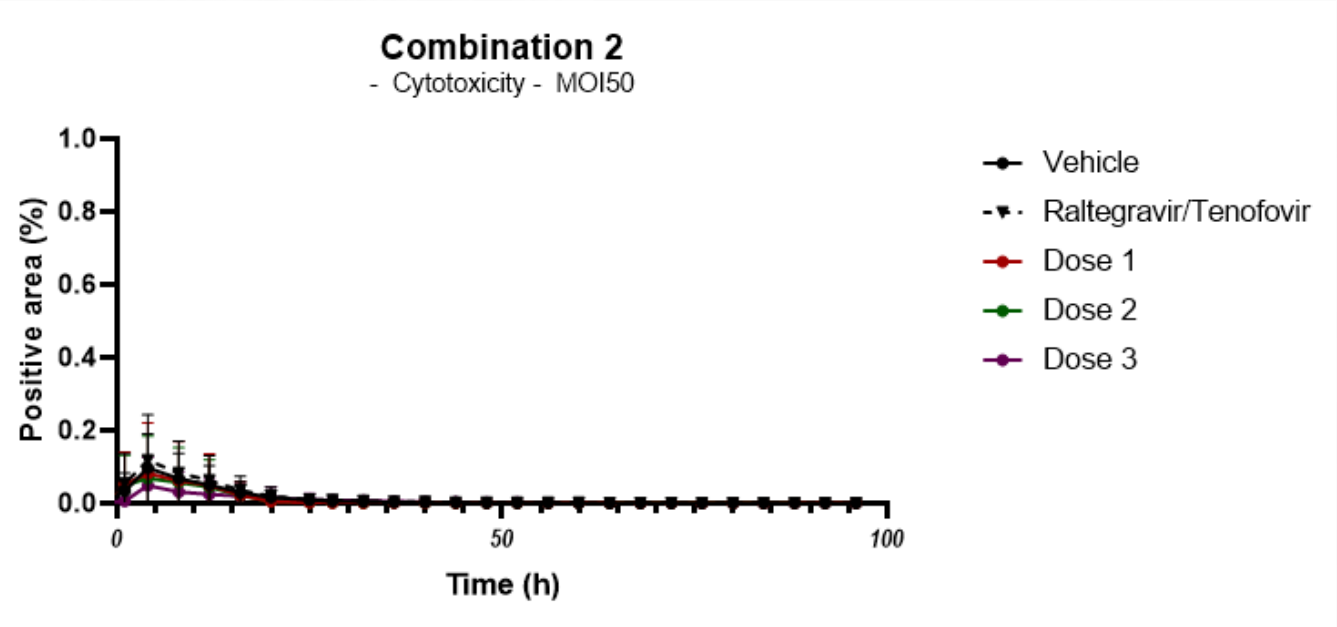
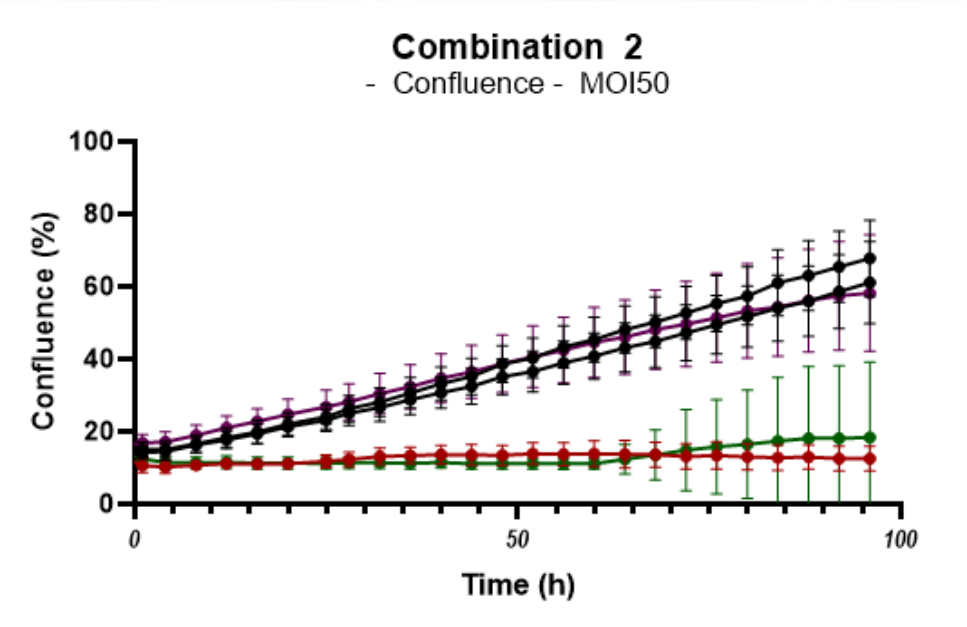


Statistical comparison at 60hours stimulation						
	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value	
Vehicle vs. Raltegrav	0.03666	0.02642 to 0.04689	Yes	****	<0.0001	
Vehicle vs. Dose 1	0.03656	0.02633 to 0.04680	Yes	****	<0.0001	
Vehicle vs. Dose 2	0.03465	0.02431 to 0.04499	Yes	****	<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. Raltegravir	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

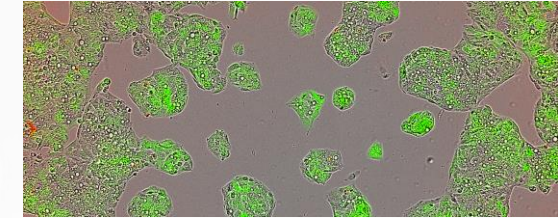
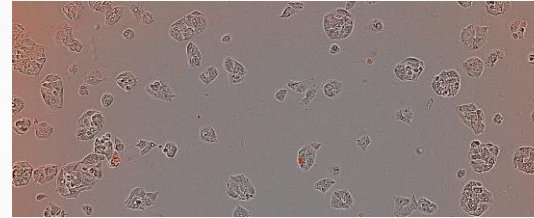
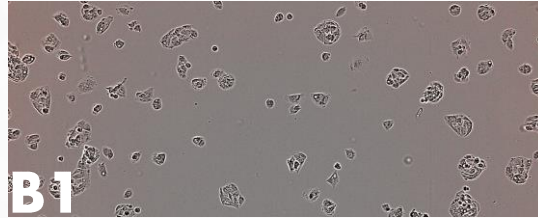
Statistical comparison at 4hours stimulation					
	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegravir	-0.02098	-0.1740 to 0.1320	No	ns	0.9855
Vehicle vs. Dose 1	0.0151	-0.1497 to 0.1799	No	ns	0.9967
Vehicle vs. Dose 2	0.02868	-0.1163 to 0.1737	No	ns	0.9491
Vehicle 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).

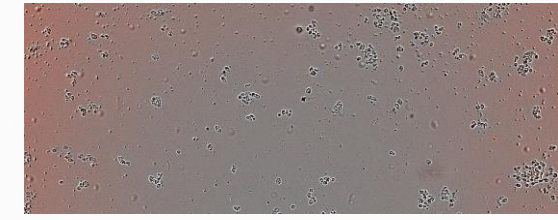
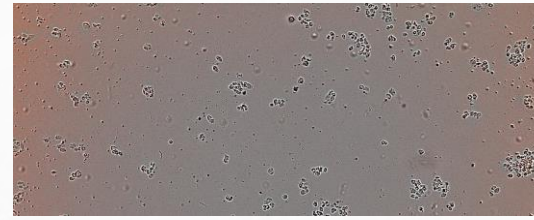
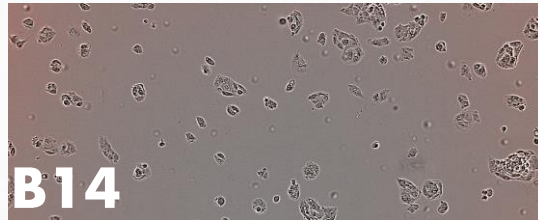


*-2 hours**16 hours**84 hours*

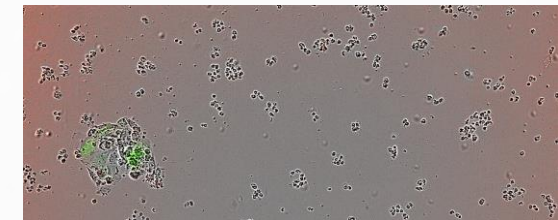
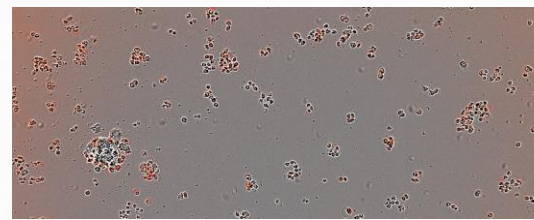
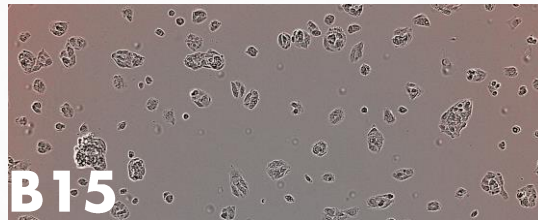
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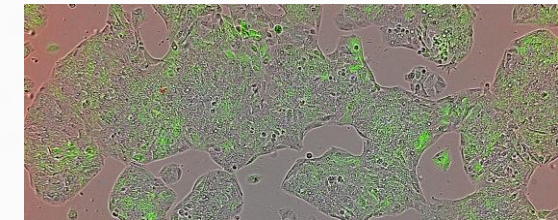
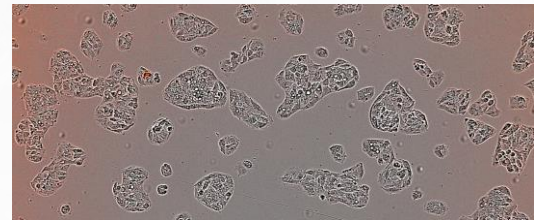
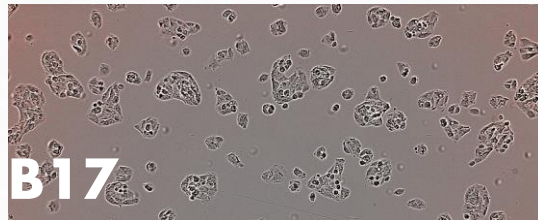
Dose 1



Dose 2

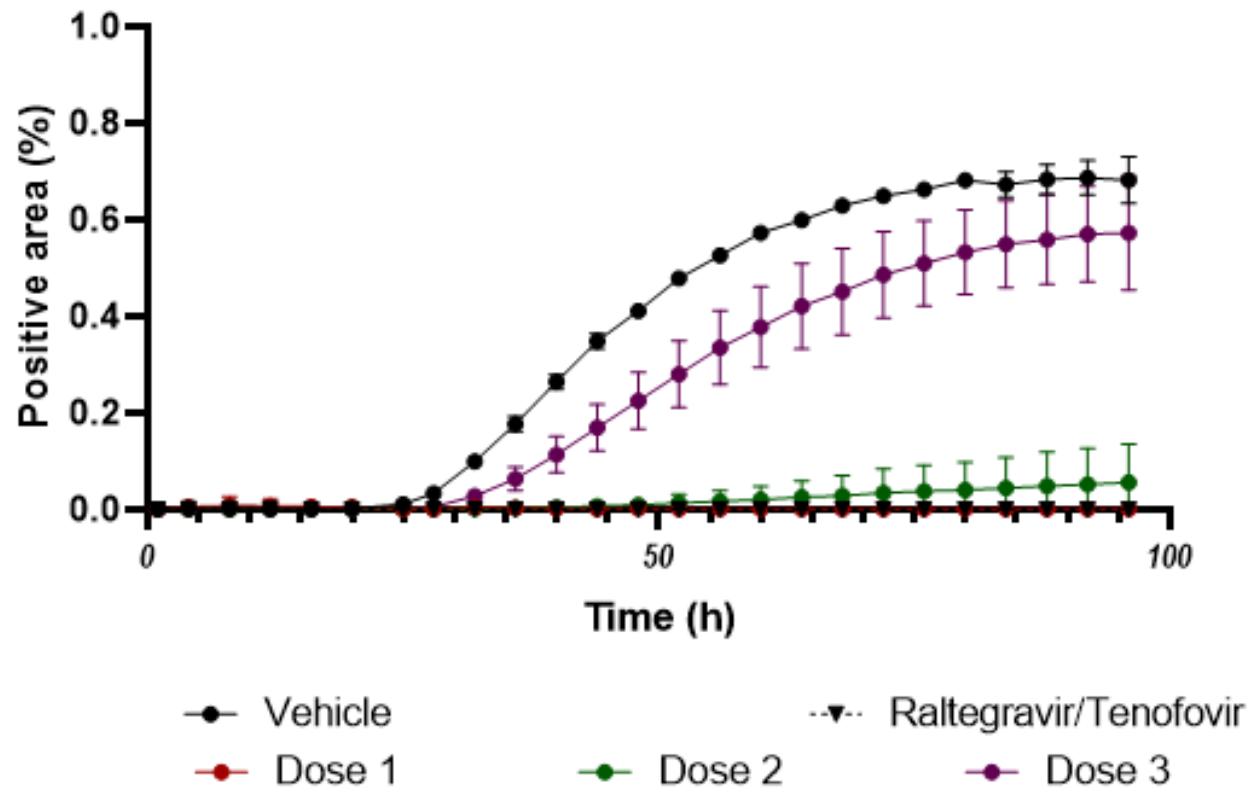


Dose 3



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

**Combination 2**  
Transduction efficiency - MOI 50



Statistical comparison at 60hours stimulation

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegravir	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

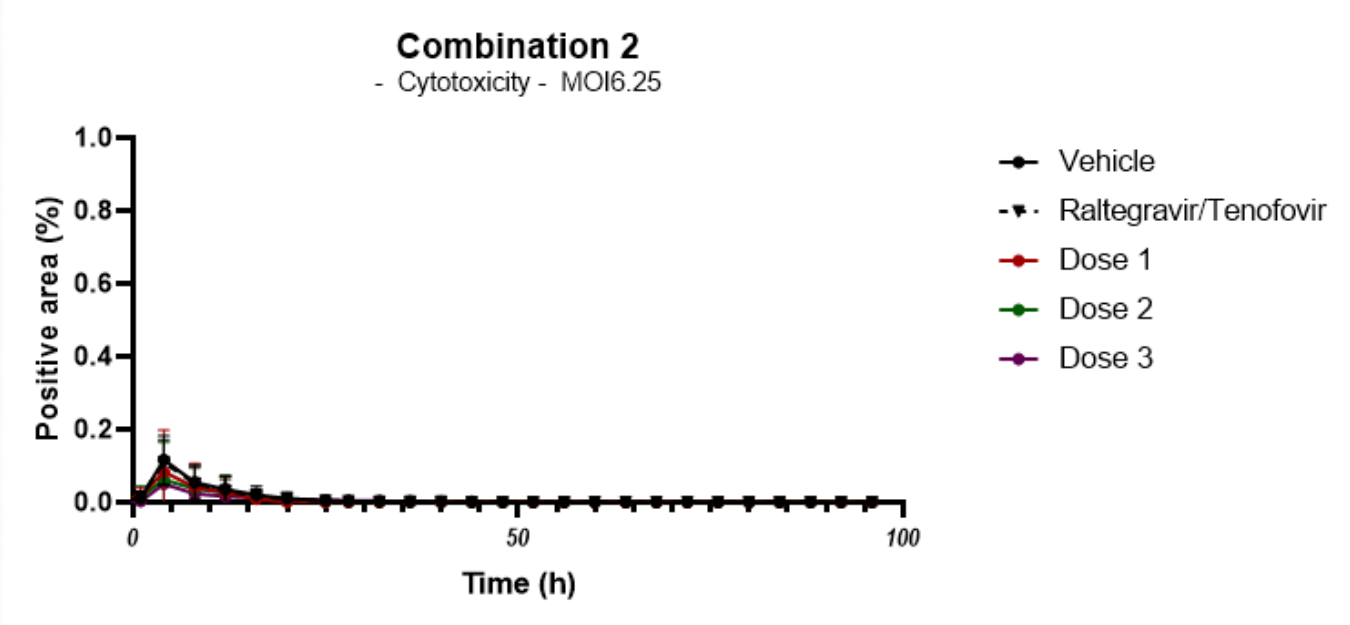
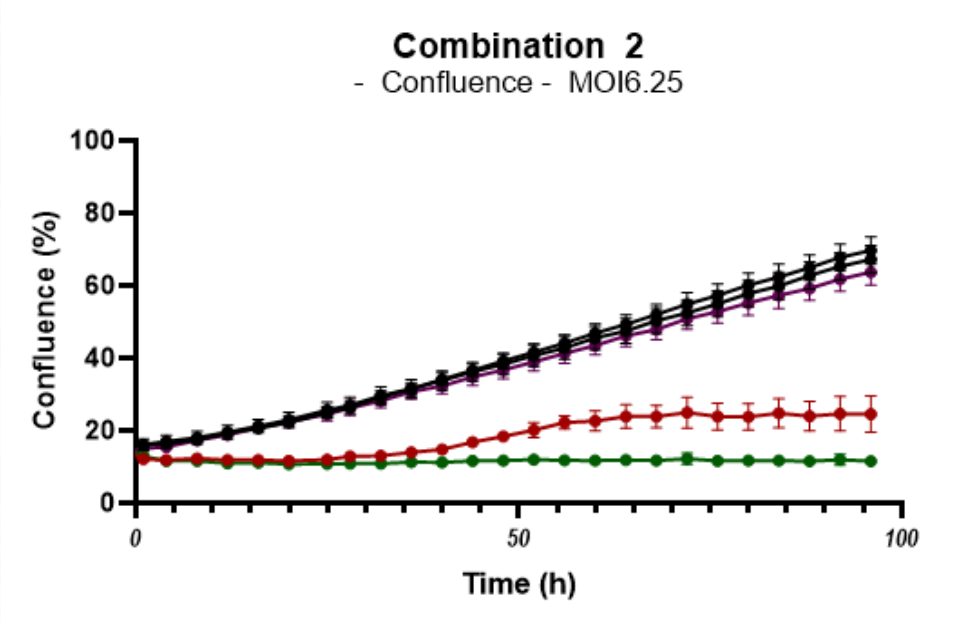
Figure: Effect of test combination on the transduction efficiency over time (0-96hours). The percentage of positive area (positive area over total cell area) is reported in kinetics. Two ways ANOVA followed by Tukey's post-hoc tests has been used for statistical comparison.

- ✦ 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



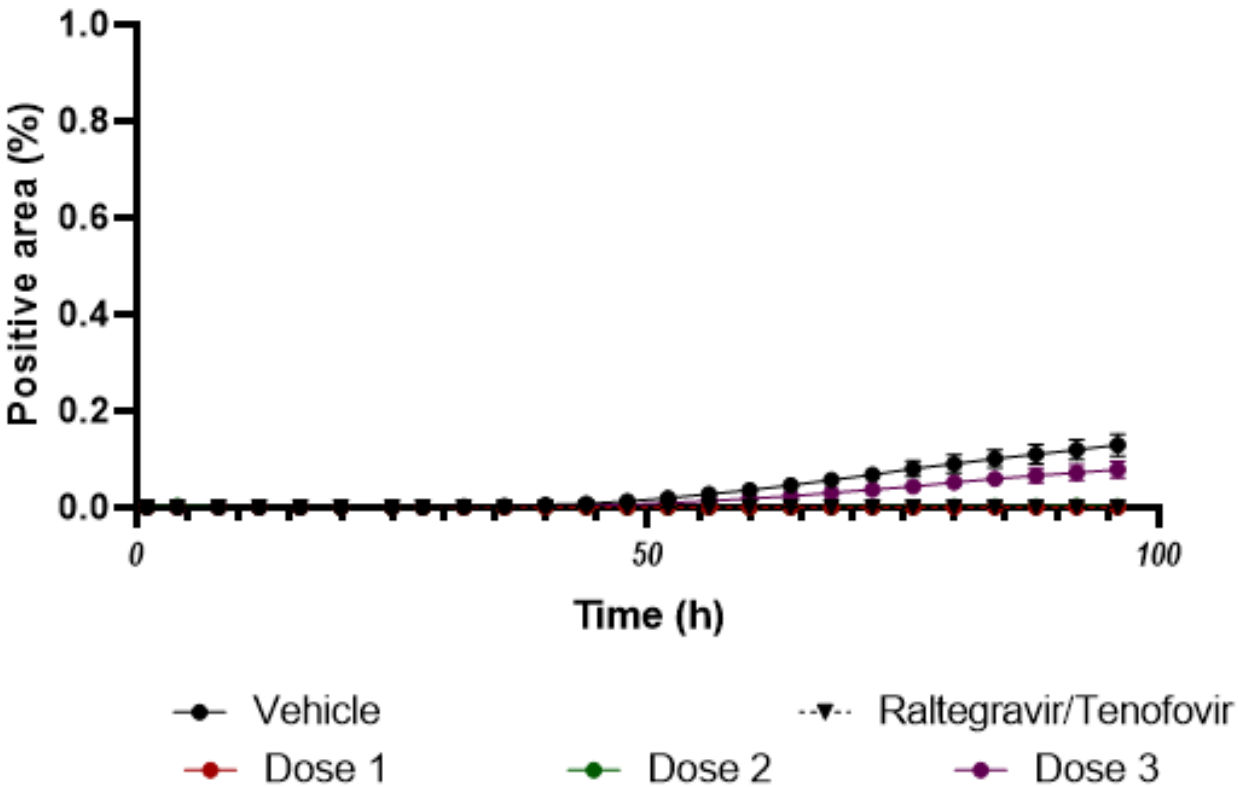


Statistical comparison at 96hours stimulation					
	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegrav	2.53	-2.698 to 7.758	No	ns	0.5046
Vehicle vs. Dose 1	45.22	38.01 to 52.43	Yes	****	<0.0001
Vehicle vs. Dose 2	58.17	53.94 to 62.39	Yes	****	<0.0001
Vehicle vs. 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).

**Combination 2**  
Transduction efficiency - MOI 6.25



Statistical comparison at 60hours stimulation

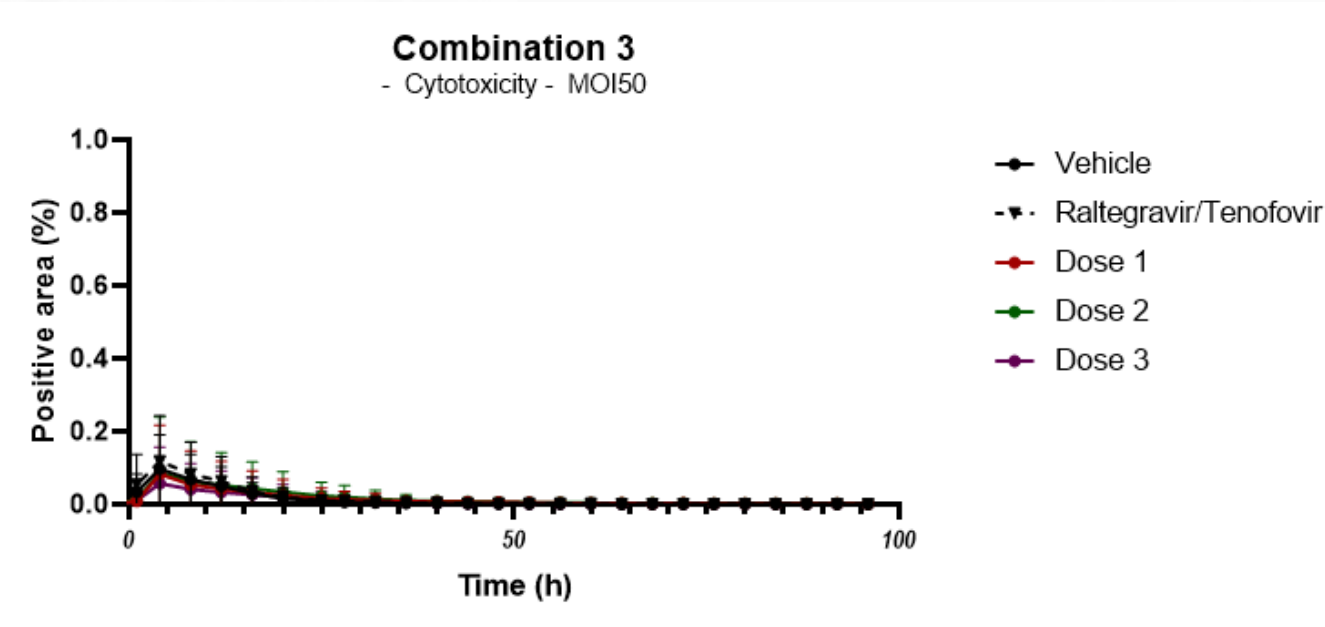
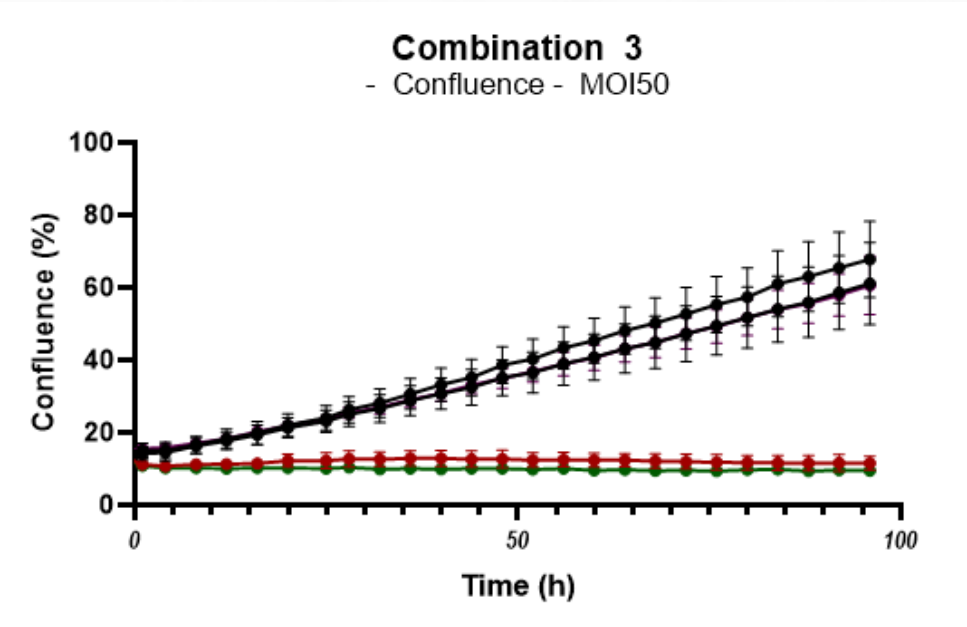
	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegrav	0.03666	0.02639 to 0.04692	Yes	****	<0.0001
Vehicle vs. Dose 1	0.03655	0.02629 to 0.04681	Yes	****	<0.0001
Vehicle vs. Dose 2	0.0361	0.02583 to 0.04636	Yes	****	<0.0001
Vehicle vs. Dose 3	0.01767	0.006792 to 0.02855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

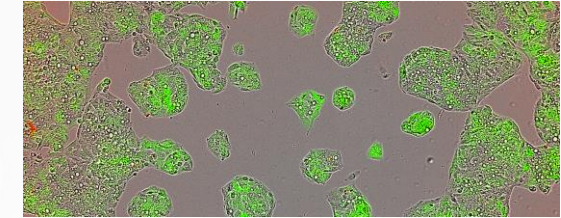
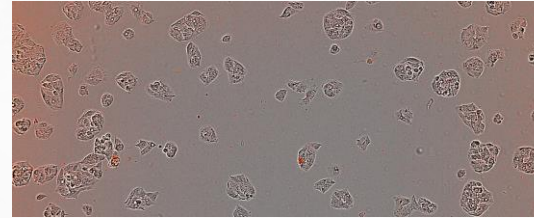
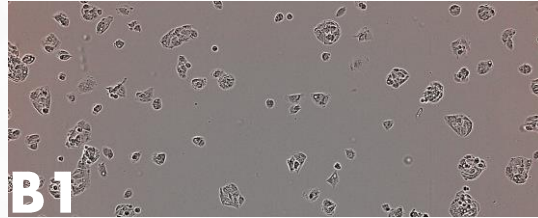
Statistical comparison at 4hours stimulation

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegravir	-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

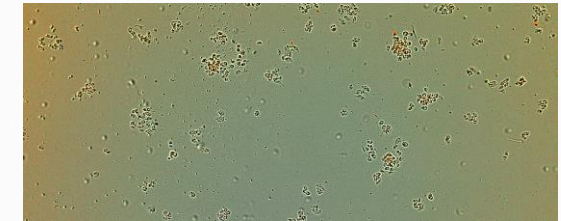
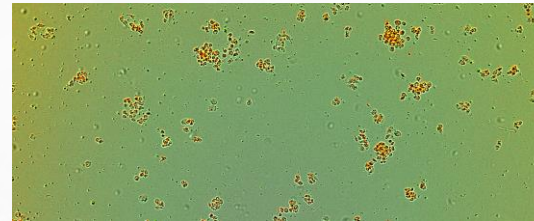
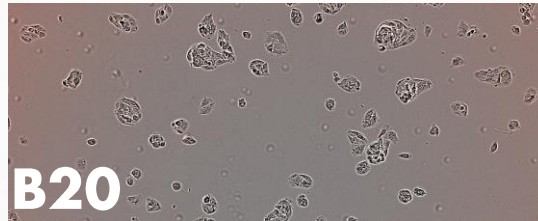
Figure: effect of the mentioned test substance at various concentrations on cell cytotoxicity (right) and confluence (left). Two ways ANOVA followed by a Dunnett’s post hoc test has been used for statistical comparison. In table below, a summary of statistical comparison is shown at 96 hours and 4hours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

*-2 hours**16 hours**84 hours*

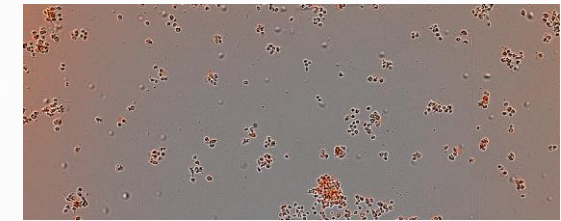
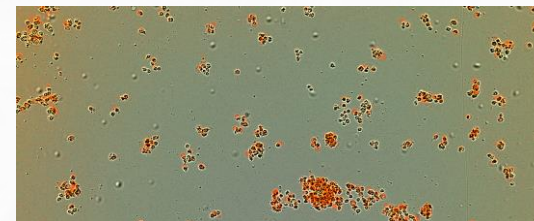
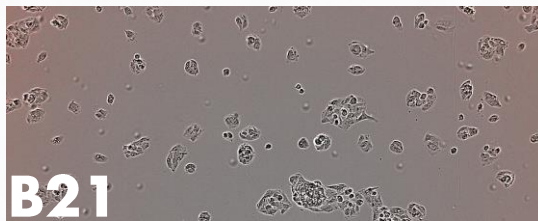
Vehicle



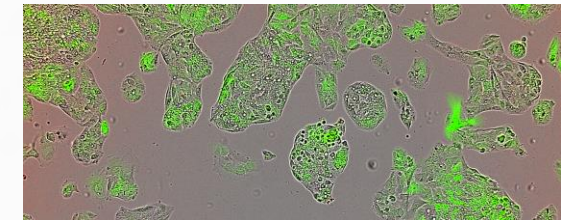
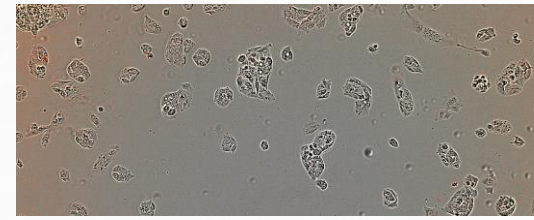
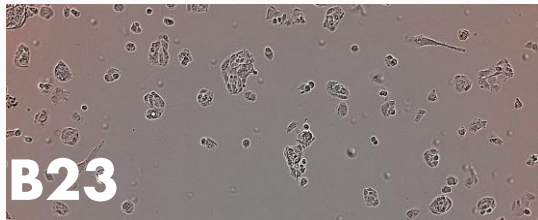
Dose 1



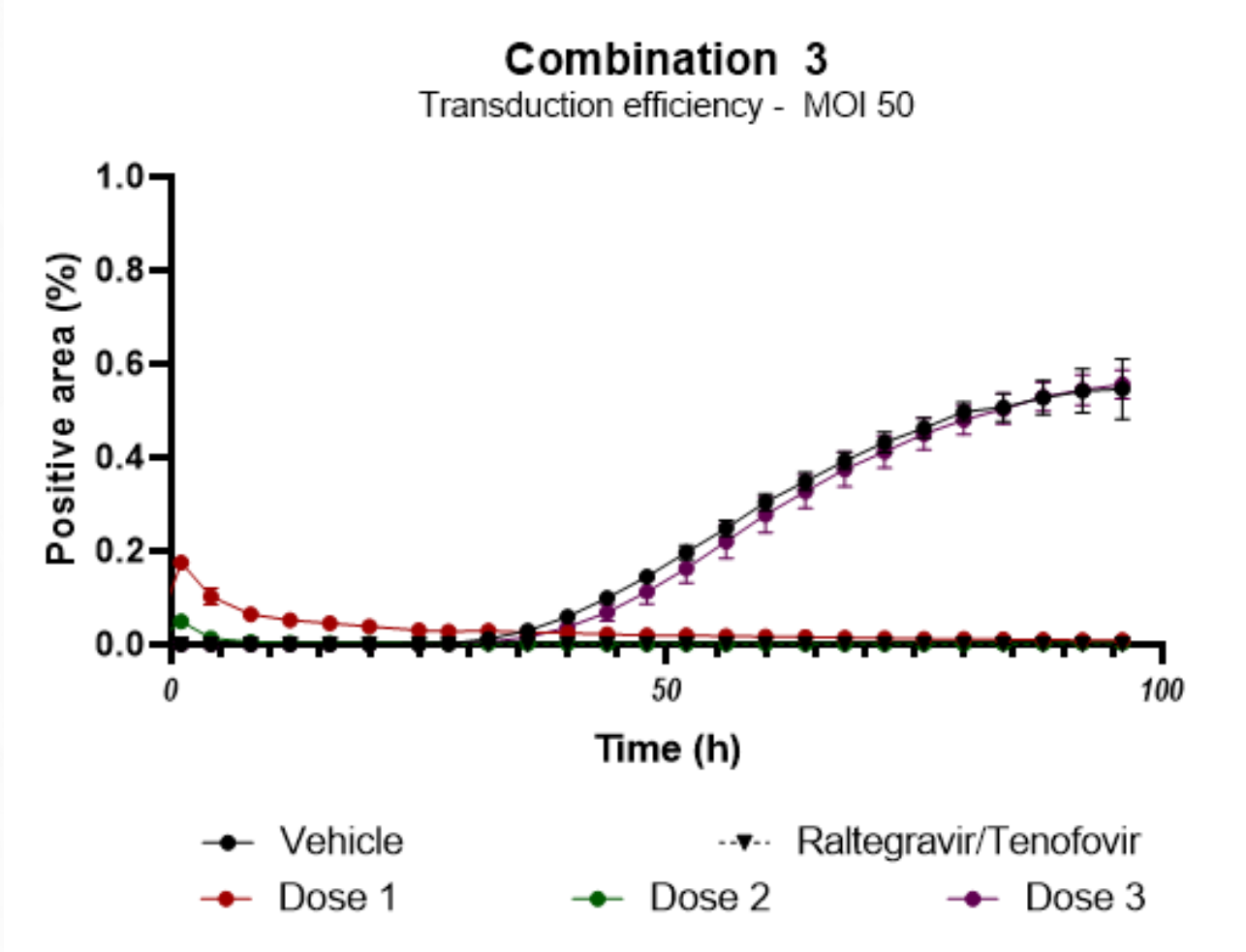
Dose 2



Dose 3



✦ 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

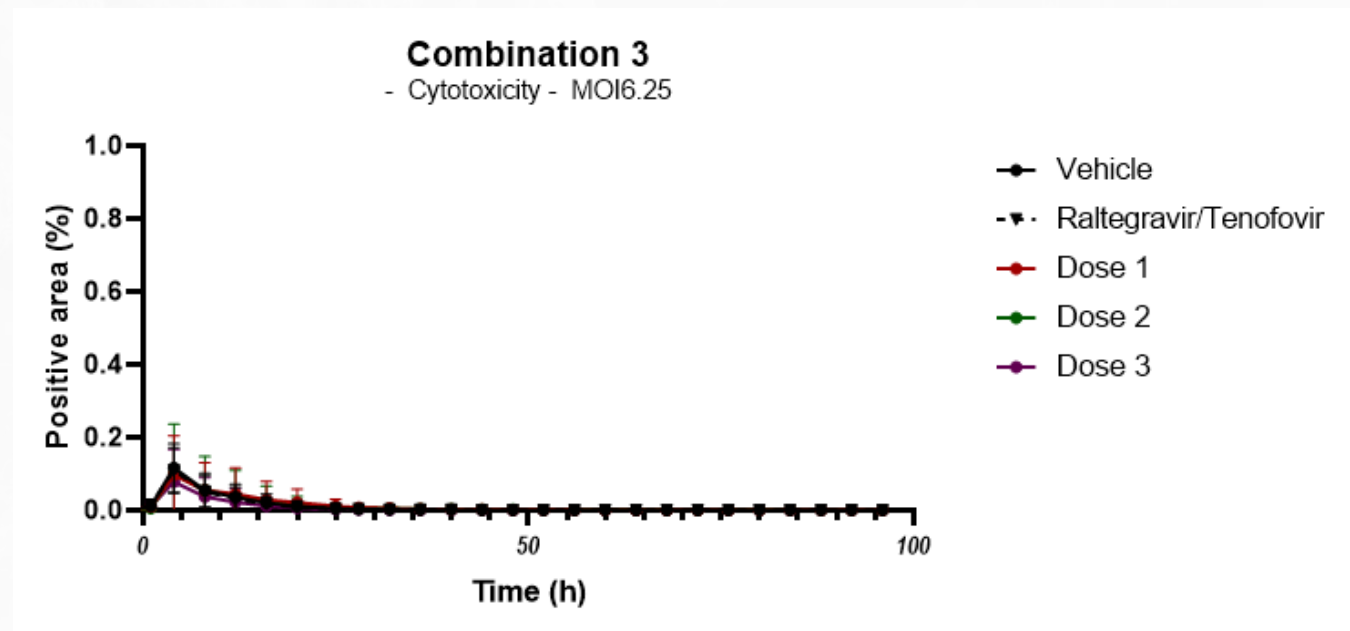
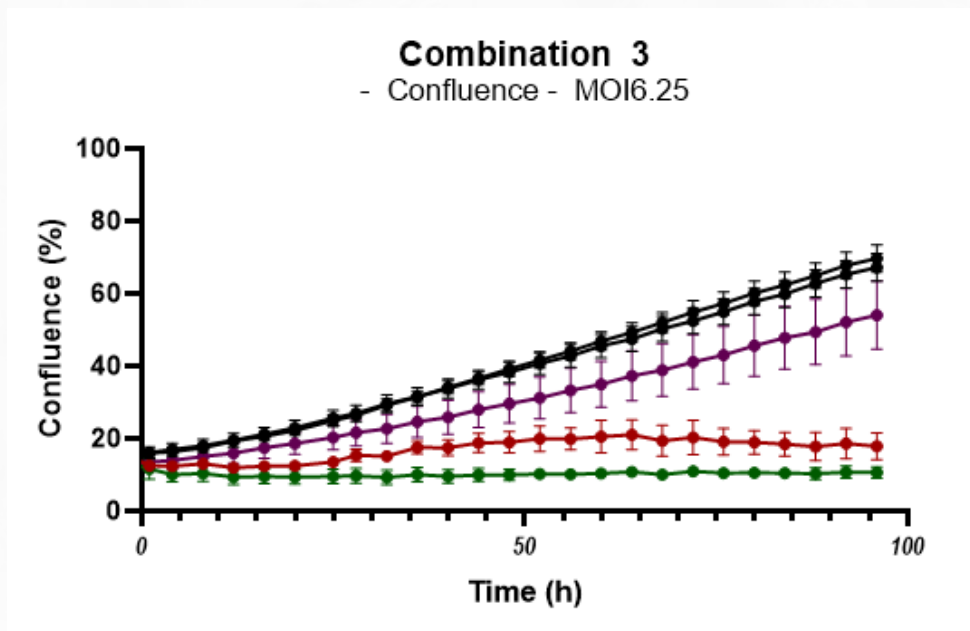
Figure: Effect of test combination on the transduction efficiency over time (0-96hours). The percentage of positive area (positive area over total cell area) is reported in kinetics. Two ways ANOVA followed by Tukey's post-hoc tests has been used for statistical comparison. Due to some autofluorescence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

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegravir	2.53	-2.698 to 7.758	No	ns	0.5046
Vehicle vs. Dose 1	51.82	46.03 to 57.60	Yes	****	<0.0001
Vehicle vs. Dose 2	59.09	54.84 to 63.33	Yes	****	<0.0001
Vehicle vs. Dose 3	15.72	5.191 to 26.26	Yes	**	0.0055

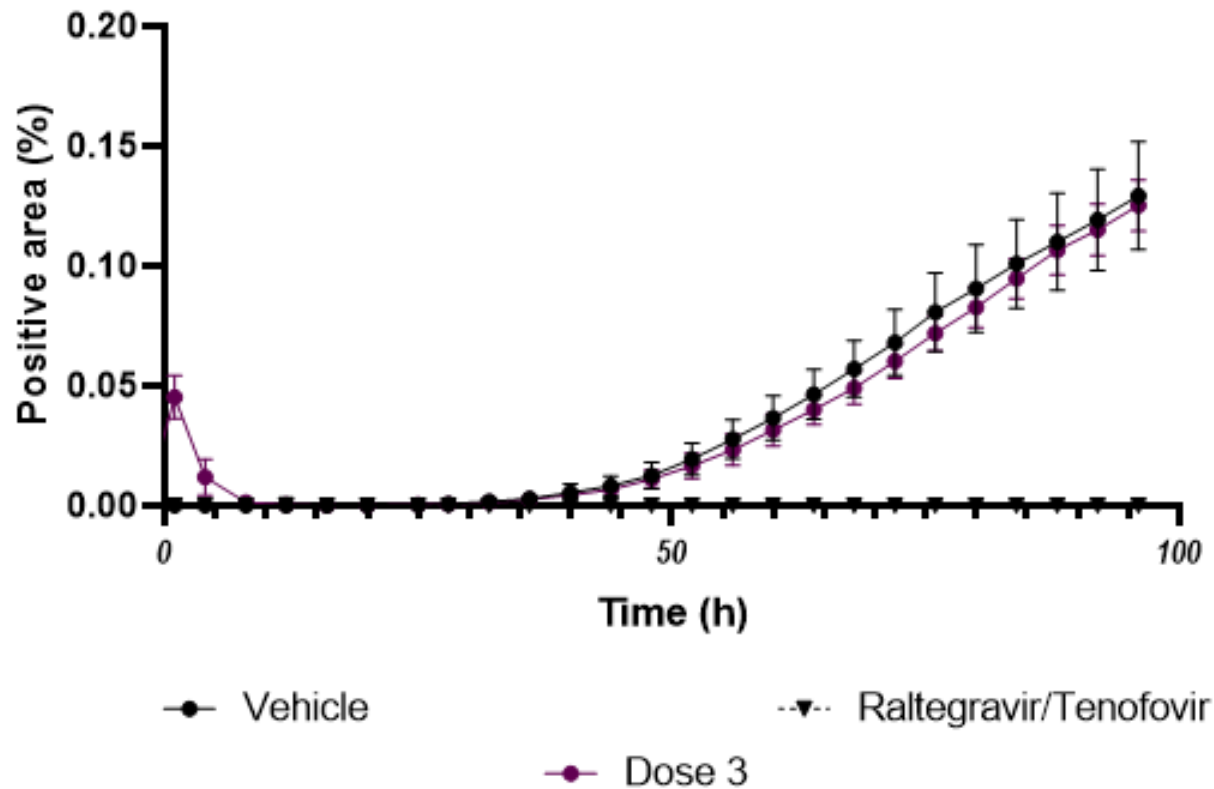
Statistical comparison at 4hours stimulation

	Mean Diff.	95.00% CI of diff.	Significant?	Summary	Adjusted P Value
Vehicle vs. Raltegravir	0.006888	0.08215 to 0.09595	No	ns	0.9983
Vehicle vs. Dose 1	0.0193	-0.1065 to 0.1451	No	ns	0.977
Vehicle vs. Dose 2	0.01583	-0.1382 to 0.1699	No	ns	0.9943
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).

**Combination 3**

Transduction efficiency - MOI 6.25



## 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