

2.4.3 SCREEN: Luminescence Normalization & Regression Analysis

Luminescence values are normalized to DMSO controls. Nonlinear regression analysis is performed using a four-parameter logistic dose-response model to calculate IC₁₀ and IC₅₀.

1. Raw Luminescence

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1		DMSO	1nM	3nM	10nM	30nM	100 nM	300 nM	1 uM	10 uM	10	11	12	
2	Replicate 1	18761	13796	6294	5664	3575	3148	2981	3601	91				Left
3	Replicate 2	21629	15749	6833	4330	3514	3308	3268	4166	141				Left
4	Replicate 3	21149	16263	5119	4219	3652	3289	3893	4327	133				Left
5														

2. Concentration Mapping

		X	Group A		
		Taxol conc. (uM)	Luminescence		
		X	A:Y1	A:Y2	A:Y3
1	Title	0.00001	18761	21626	21149
2	Title	0.00100	13796	15749	16263
3	Title	0.00300	6294	6833	5119
4	Title	0.01000	5664	4330	4219
5	Title	0.03000	3575	3514	3652
6	Title	0.10000	3148	3308	3289
7	Title	0.30000	2981	3268	3893
8	Title	1.00000	3601	4166	4327
9	Title	10.00000	91	141	133
10	Title				
11	Title				

3. Log Transformation

Transform		X	A		
		Taxol conc. (uM)	Luminescence		
		X	A:Y1	A:Y2	A:Y3
1		-5.000	18761.000	21626.000	21149.000
2		-3.000	13796.000	15749.000	16263.000
3		-2.523	6294.000	6833.000	5119.000
4		-2.000	5664.000	4330.000	4219.000
5		-1.523	3575.000	3514.000	3652.000
6		-1.000	3148.000	3308.000	3289.000
7		-0.523	2981.000	3268.000	3893.000
8		0.000	3601.000	4166.000	4327.000
9		1.000	91.000	141.000	133.000
10					

4. DMSO Normalization

Normalize		X	A		
		Taxol conc. (uM)	Luminescence		
		X	Mean	SEM	N
1		-5.000	100.000	4.321	3
2		-3.000	74.441	3.664	3
3		-2.523	29.651	2.467	3
4		-2.000	23.097	2.263	3
5		-1.523	17.455	0.195	3
6		-1.000	15.836	0.246	3
7		-0.523	16.481	1.313	3
8		0.000	19.654	1.073	3
9		1.000	0.593	0.076	3
10					

5. Nonlinear Fit Results (IC₅₀)

Table of results		Table of results	A
Nonlin fit		Luminescence	
Table of results		Y	
1	log(inhibitor) vs. response -- Variable slope (four parameters)		
2	Best-fit values		
3	Bottom		14.96
4	Top		100.3
5	LogIC50		-2.829
6	HillSlope		-2.021
7	IC50		0.001482
8	Span		85.32
9	Std. Error		
10	Bottom		1.722
11	Top		4.032
12	LogIC50		0.04770
13	HillSlope		0.3542
14	Span		4.410

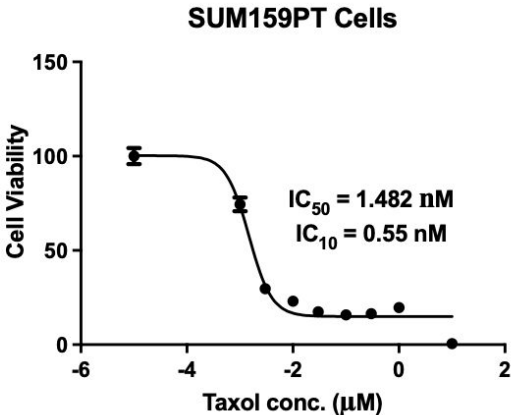
6. Four-Parameter Logistic Model

$$Y = Bottom + \frac{Top - Bottom}{1 + 10^{(X - LogIC_{50}) \times HillSlope}}$$

$Y = 90\% \text{ viability}$

$IC_{10} = 0.55 \text{ nM}$

7. Final Dose-Response Curve



3.6.1. SCREEN: Show cell viability analysis identifying the minimum puromycin concentration that results in 5% viability or less in the presence of verapamil.

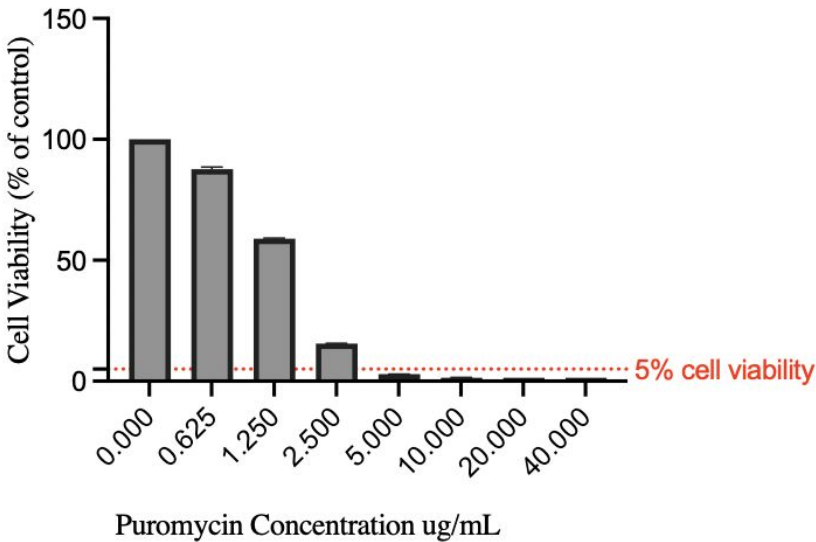
1. Raw Luminescence and Concentration Mapping

	Concetrations (ug/mL)	Luminescence		
⊗	X	A:Y1	A:Y2	A:Y3
Title	0.000	48500	46200	47100
Title	0.625	42100	40800	41500
Title	1.250	28500	27200	27900
Title	2.500	7600	7100	7350
Title	5.000	1450	1320	1380
Title	10.000	710	680	720
Title	20.000	690	660	700
Title	40.000	680	650	690

2. Normalization and Calculation as Percentages

Normalize		X	A		
	⊗	Concetrations (ug/mL)	Luminescence		
		X	A:Y1	A:Y2	A:Y3
1		0.000	100.000	100.000	100.000
2		0.625	86.804	88.312	88.110
3		1.250	58.763	58.874	59.236
4		2.500	15.670	15.368	15.605
5		5.000	2.990	2.857	2.930
6		10.000	1.464	1.472	1.529
7		20.000	1.423	1.429	1.486
8		40.000	1.402	1.407	1.465
9					

3. Visualization



The minimum concentration of puromycin that eliminates all the cells ($\leq 5\%$ viability) .

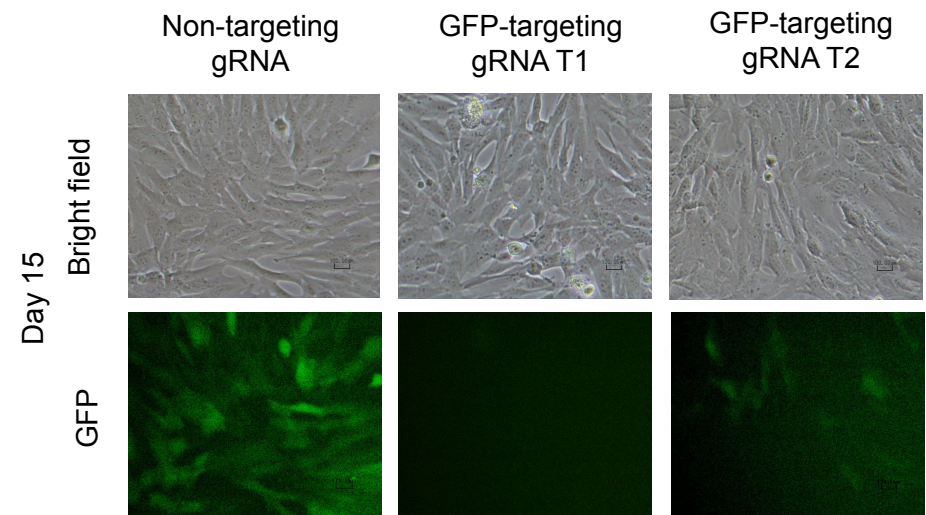
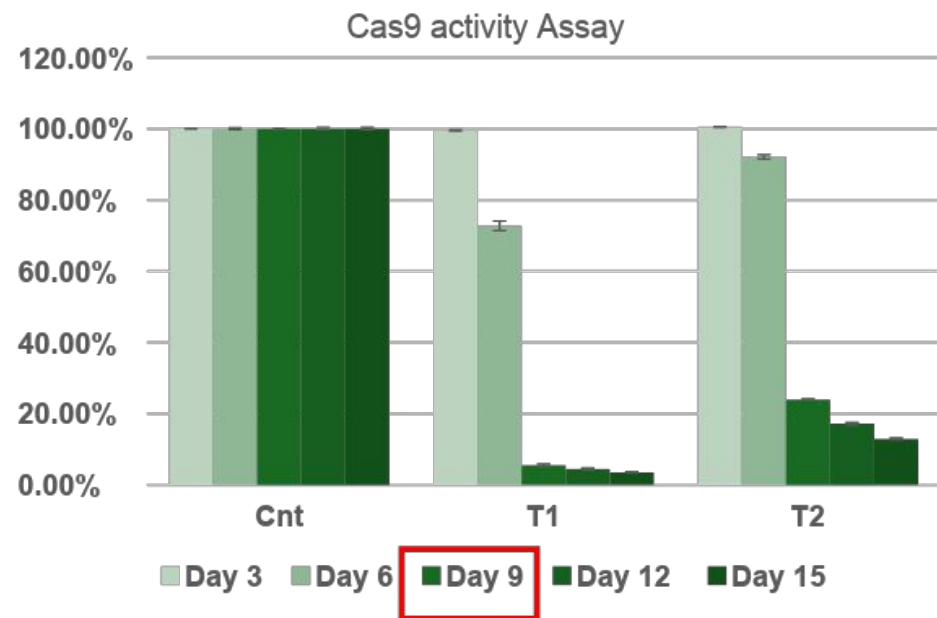
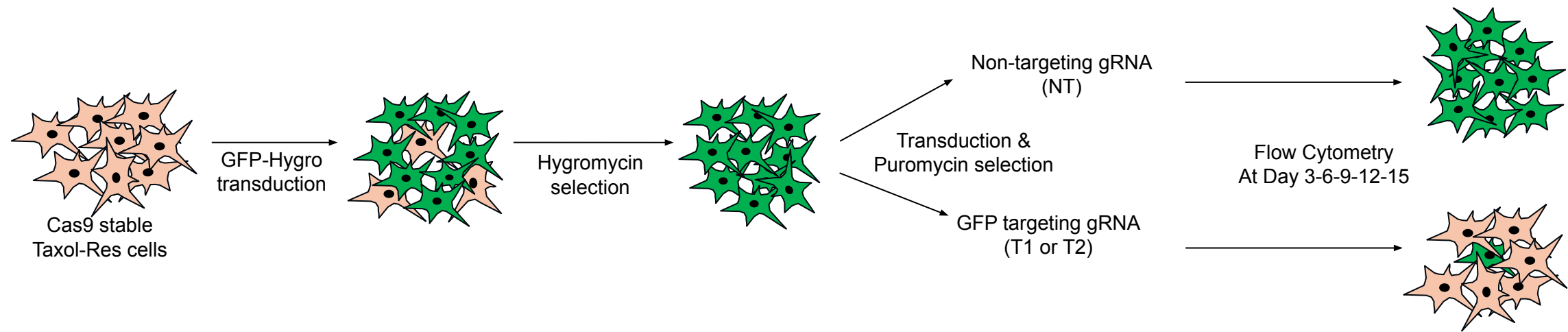
4.2.1. SCREEN: Show calculation of the virus amount required to achieve a multiplicity of infection of 10.

See **Supplementary Table 2** for titer and moi calculation template

Example Viral Titer and MOI Calculation						
Starting cell number (Nstarting)	Virus Volume (µl)	Total cell number after selection (Nselected)	Transduction rate (p = Nselected/Ncontrol)	MOI (MOI = -ln (1-p))	Titer (ip/mL) (MOI x Nstarting)/Volume of virus	Final Titer (ip/mL) (average of two closest results)
200000	1	2800000	0.9333333333	2.708050201	541610040	2226567230 (round up 2x10^9)
200000	0.1	2000000	0.6666666667	1.098612289	2197224577	
200000	0.01	320000	0.1066666667	0.112795494	2255909883	
200000	0.001	55000	0.0183333333	0.018503472	3700694313	
200000	No virus (Ncontrol)	3000000	-	-		

Calculation of the amount of virus for Cas9 infection				
Infected cell number (N infected)	Desired MOI	Transduction rate (p = 1- e^(-MOI))	Cell number to be seeded (N starting = N infected / p)	Required viral volume (mL) (V=(N starting x MOI)/ Final titer*)
200000	10	1	200000	~0.001 mL
*Final titer assumed as 2x10^9				

4.5.1. SCREEN: Images of Cas9 validation results on the computer.



5.3.1 SCREEN: Show calculation of the required viral volume per replicate.

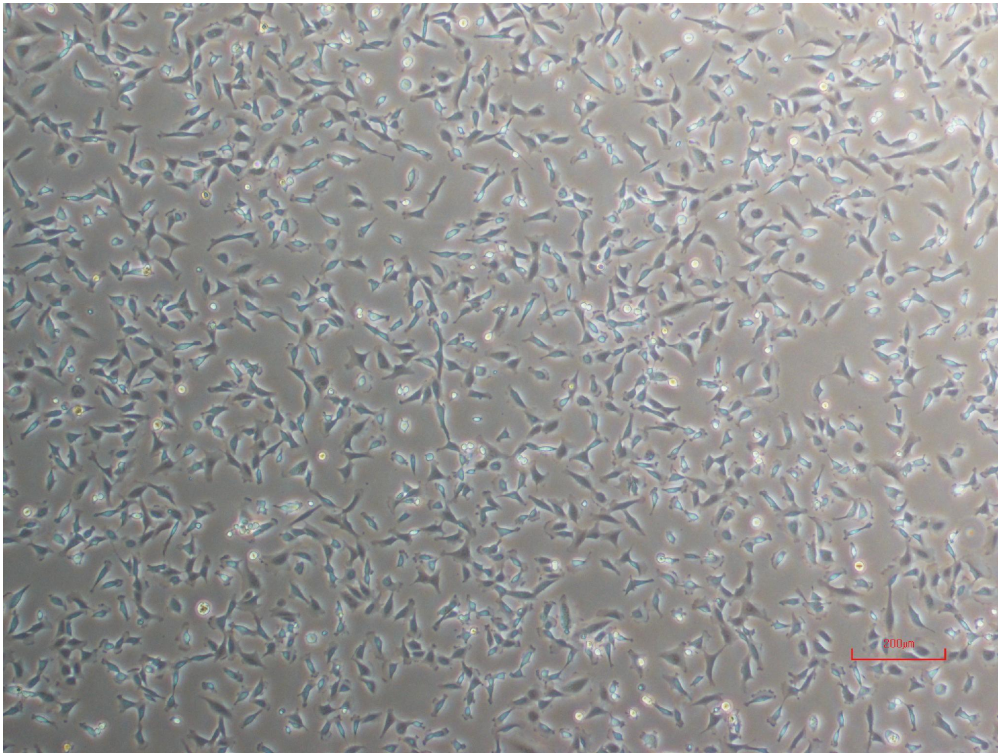
Calculation of the amount of virus for EPIKOL infection						
Number of sgRNAs in the library ^a	Desired Library Coverage	Infected cell number (N infected = number of sgRNAs x target coverage)	Desired MOI	Transduction rate (at least 1 sgRNA) (p = 1- e[^](-MOI))	Cell number to be seeded (N starting = N infected / p)	Required viral volume per replicate (mL) (V=(N starting x MOI)/ Final titer^b)
8000	1000	8000000	0.3	0.26	30866367	~0.046 mL
8000	1000	8000000	0.4	0.33	24265958	~0.048 mL

^aRound up numbers; ^bFinal titer assumed as 2x10[^]8

5.10.1. SCOPE

The cell viability and growth kinetics is being checked during routine passaging.

Parental Cell Line



Taxol-Res Cell Line

