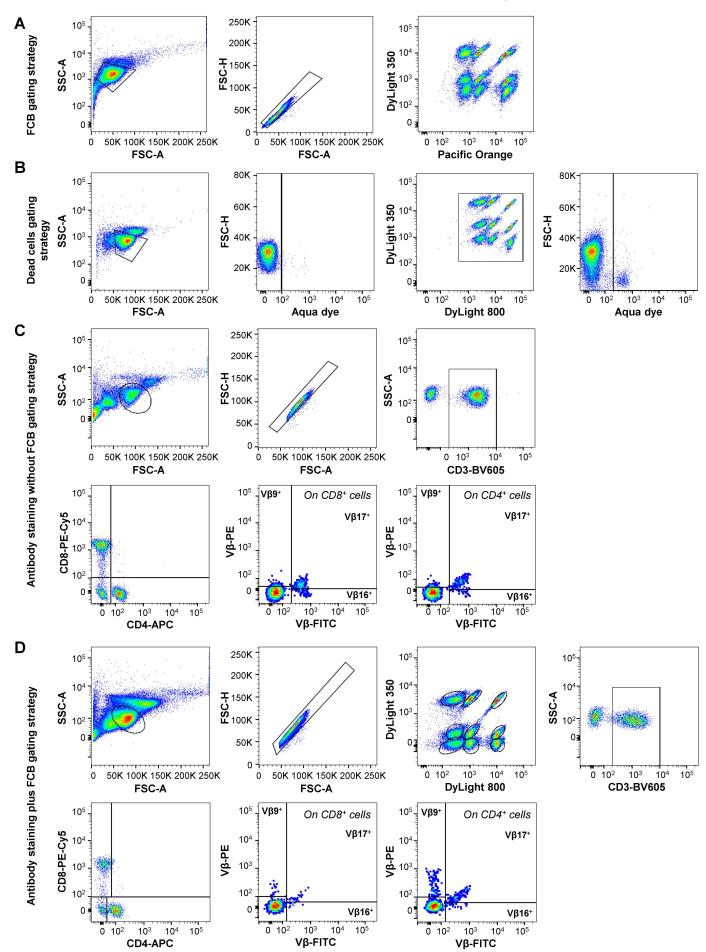


Resuspension in 300µl for acquisition

### Supplemental Figure 2. Giudice V. et al.

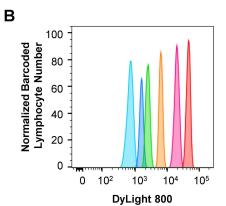


## Supplemental Figure 3. Giudice V. et al.

# Normalized Barcoded Parcoded P

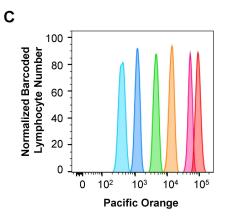
## DyLight 350

		<u>MFI</u>	<u>CV</u>	Fold increase
_	0	432.2	18.02	
ţi	1.56	770.4	12.34	1.68
Concentration (µg/ml)	13	2268.6	12.96	2.88
	50	7390.2	13.52	3.23
ůo;	250	29221.4	11.94	3.95
0	500	50796	12.72	1.74



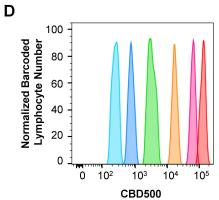
### DyLight 800

		<u>MFI</u>	<u>cv</u>	Fold increase
_	0	578.6	17.62	
Concentration (µg/ml)	1.56	1539.6	8.624	2.57
	13	2625.6	9.896	1.69
hg/	50	6148.6	10.192	2.33
Conc	250	18333.8	13.62	2.97
	500	43791.8	12.06	2.39



### **Pacific Orange**

		<u>MFI</u>	<u>cv</u>	Fold increase
_	0	649.6	18.54	
ţi	1.56	1660.6	14.16	2.46
Concentration (µg/ml)	13	4321.4	13.74	2.57
ba/	50	14057.6	14.8	3.24
ů )	250	51838.4	11.68	3.68
0	500	90700	11.666	1.75



### CBD500

		<u>MFI</u>	<u>CV</u>	Fold increase
_	0	321.4	17.5	
ţi	1.56	939.4	16.34	2.72
itra m	13	4285.4	16.96	4.47
Concentration (µg/ml)	50	13182.4	13.88	3.06
uo;	250	66927.2	13.5	5.07
O	500	138229	14	2.06

Α

				Pacific Ora	inge		DyLight 8	00
			<u>MFI</u>	<u>CV</u>	Fold increase	<u>MFI</u>	<u>CV</u>	Fold increase
Concentration (µg/ml)	-	0	732	20		1242	33.3	
	Combo	13	2655	20.5	3.5	5649	27.7	4.4
ion	ပိ	250	28258	27.5	10.6	31628	23.2	5.6
ıtrat	22	1.56	1601	19.7		3750	24.2	
ncer	Combo	50	6390	19.5	3.9	13995	19.2	3.7
ပိ	ပိ	500	38141	20.1	5.9	33778	22.5	2.4

В

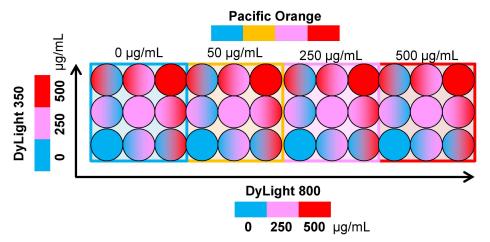
				Pacific Ora	<u>inge</u>		DyLight 3	<u>50</u>
			<u>MFI</u>	CV Fold increase		<u>MFI</u>	<u>cv</u>	Fold increase
<u>E</u>	7	0	591	23.2		389	22.9	
Concentration (µg/ml)	Combo	13	1990	21.4	3.2	912	23.4	2.2
ioi	ပိ	250	23076	25.2	11.5	9269	25.4	9.9
ıtrat	2 2	1.56	1433	22.5		528	25.3	
ncer	Combo	50	6276	24.4	4.3	2366	28.2	4.2
క	ပိ	500	45679	22.2	7.2	18302	26.1	7.6

С

				DyLight 8	<u>:00</u>	DyLight 350				
			<u>MFI</u>	<u>CV</u>	Fold increase	<u>MFI</u>	<u>CV</u>	Fold increase		
Concentration (µg/ml)	7	0	639	29.3		241	26.4			
	Combo	13	2326	18.8	3.5	911	21.7	3.3		
<u>io</u>	ပိ	250	22868	16.6	9.7	10678	19.4	11.4		
ıtrat	0.2	1.56	2615	22.4		674	27.1			
ncer	Combo	50	7753	16.2	2.9	2489	22.1	3.5		
Ŝ	ပိ	500	37017	15.8	4.8	17801	18.8	7.1		

Α

# 4x(3x3) matrix – 36 samples Pacific Orange vs DyLight 800 vs DyLight 350



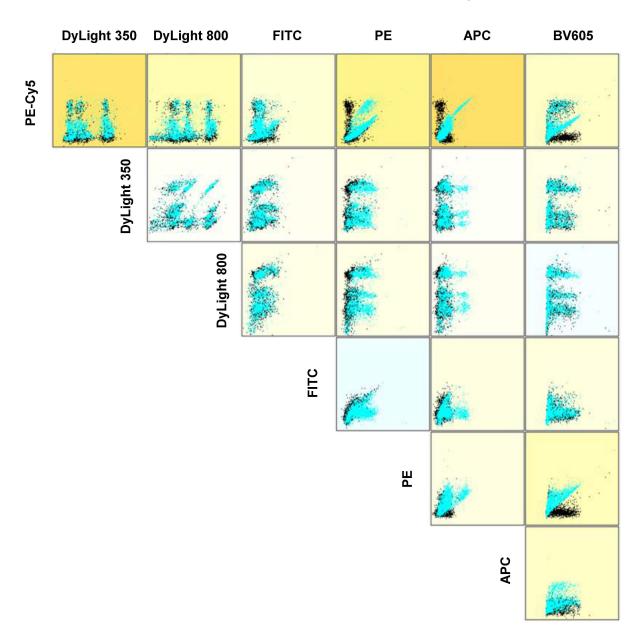
В

		Ţ	OyLight 350	<u>)</u>		DyLight 800	<u>)</u>	Pacific Orange		
		<u>MFI</u>	<u>CV</u>	<u>Fold</u> <u>increase</u>	<u>MFI</u>	<u>cv</u>	<u>Fold</u> <u>increase</u>	<u>MFI</u>	<u>cv</u>	<u>Fold</u> <u>increase</u>
	0	482	27.4		3920	18.4		1767	18.9	
(mg/mı)	50							9606	16.3	5.4
<u> </u>	250	2518	18.3	4.9	10157	18.1	2.6	37445	17.1	3.9
(lm/grl)	500	20131	16.1	7.9	39532	13.2	3.9	67078	18.5	1.8

С

		DyLight 350	<u>)</u>		DyLight 80	<u>o</u>		CBD500	
	<u>MFI</u>	<u>CV</u>	<u>Fold</u> <u>increase</u>	<u>MFI</u>	<u>CV</u>	<u>Fold</u> <u>increase</u>	<u>MFI</u>	CV	<u>Fold</u> <u>increase</u>
0	732	20.3		4737	14.2		382	14.7	
50							1079	13.9	2.7
250	2971	19.7	3.9	9681	16.6	2.0	3821	15.4	3.5
500	21256	18.8	7.1	38489	14.2	4.0	7604	13.1	2.0

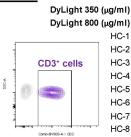
# Supplemental Figure 6. Giudice V. et al.



## Supplemental Figure 7. Giudice V. et al.

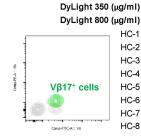
## Percentage of positive cells

### CD3-BV605



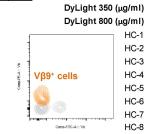
) [		0			13			250		Control w/o	Variability	Ratio
) [	0	13	250	0	13	250	0	13	250	FCB	variability	Rauo
1 [	48.6	44	43.2	41.3	40.7	40.1	48.2	47.2	50.3	46.2	LOW	0.97
2	50.7	55.2	57.3	53.3		56.2	65.7	67	70.6	61.8	LOW	0.96
3	67.5	71.1	71.6	71.4	71.1	72.2	72.7	75.9	76.7	76	LOW	0.95
4 [	70.7	71.6	72.8	69.9	71.8	75.6	78.2	77.2	79.4	74.4	LOW	1.00
5 [	65	61.9	67.8	57.8	57.1	62.3	77.7	78.1	79.1	56.9	LOW	1.18
6	78.8	76	78.7	71.3	70	68.3	83.3	82.2	82.5	77	LOW	1.00
7 [	51.9		54.8	46	49.8	48.3	67.5	68.7	68.4	44.1	LOW	1.29
8 [	70.1	66.3	75.2	62.5	63.1	68.3	77.5	76	78.3	64.1	LOW	1.10

### Vβ-FITC/PE



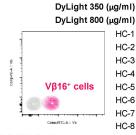
	0			13			250		Control w/o	Variability	Ratio
0	13	250	0	13	250	0	13	250	FCB	variability	Ratio
2.67	2.81	3.28	1.53	2.11	4.39	3.09	3.5	3.42	3.8	LOW	0.78
2.8	1.6	3.23	2.93		3.8	3.5	3.01	4.63	3.82	LOW	0.83
3.78	5.19	7.18	4.02	4.06	6.06	5.83	4.24	8.14	6.65	LOW	0.81
10.4	11.5	12.5	11	12.9	22.7	12.7	13.7	16	10.9	LOW	1.26
2.06	3.28	3.09	1.23	2.15	2.02	2.53	1.85	3.03	2.52	LOW	0.94
2.91	2.26	3.1	2.32	2.16	3.09	2.11	2.33	2.47	2.47	LOW	1.02
2.38		2.7	3.36	2.15	3.69	2.37	1.73	2.6	2.93	LOW	0.90
0.64	0.47	1.36	0.58	0.46	0.59	0.66	0.85	0.86	0.85	LOW	0.85

### <u> Vβ-PE</u>



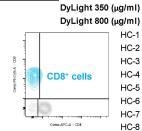
I)		0			13		250			Control w/o	Variabilit.	Ratio
I)	0	13	250	0	13	250	0	13	250	FCB	Variability	Ratio
-1	1.53	1.2	1.82	2.45	0.9	1.88	0.62	0.27	1.14	1.37	LOW	0.96
-2	1.75	1.33	0.25	1.02		0.84	1.23	1.42	1.6	0.99	LOW	1.19
-3	2.77	3.8	2.71	2.57	2.2	3.24	2.35	3.33	3.76	1.97	LOW	1.51
-4	1.52	0.85	1.39	0.27	0.26	0.45	1.6	1.83	0.81	1.44	LOW	0.69
-5	1.52	0.99	1.34	1.7	1.08	0.97	1.06	1.37	1.56	1.75	LOW	0.74
-6	4.06	2.63	2.94	4.21	3.39	3.51	3.17	2.91	2.4	3.07	LOW	1.06
-7	0.32		0.35	0.19	0.41	0.74	0.17	0.23	0.28	0.66	LOW	0.51
-8	0.78	0.37	0.9	0.35	0.66	0.39	0.33	0.17	0.38	0.48	LOW	1.00

### <u>Vβ-FITC</u>



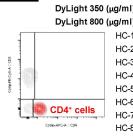
I)		0			13			250		Control w/o	Variability	Ratio	
I)	0	13	250	0	13	250	0	13	250	FCB	variability	Ratio	
1	1.53	0.4	1.09	1.83	2.41	1.57	2.15	1.62	2.56	2.43	LOW	0.69	
2	5.94	2.93	2.49	3.57		2.95	1.44	2.48	1.25	2.36	LOW	1.22	
3	4.91	5.06	2.24	4.8	4.29	2.19	4.37	4.04	2.14	2.45	LOW	1.54	
4	5.81	4.7	6.71	7.24	6.04	0.89	5.5	5.25	1.22	4.89	LOW	0.99	
5	0.98	0.89	0.58	0.94	0.88	0.61	0.98	1.05	0.62	0.98	LOW	0.85	
6	2.2	1.88	0.4	1.74	1.39	1.17	1.06	1.29	0.47	1.12	LOW	1.15	
7	0.53		0.24	0.75	1.23	0.46	1.07	1.05	0.49	1.2	LOW	0.61	
8	20.4	23.2	23.5	22.5	23	21.8	22.8	20.5	20.4	13.6	HIGH	1.62	

### CD8-PE-Cy5



	0			13			250		Control w/o	Variability	Ratio	
0	13	250	0	13	250	0	13	250	FCB	variability	Nauo	
11.7	12.7	13.4	14.7	13.6	14.6	13.3	13.7	12.2	15.5	HIGH	0.86	
37.9	41.1	41.9	40.7		43.7	40	36.9	36.9	42.6	LOW	0.94	
39.1	41.4	38.8	41.7	40.2	39.6	38.4	39.6	37.5	44.5	HIGH	0.89	
19.5	23.1	21	22.3	22.6	22.1	20	20.2	22.2	23.8	LOW	0.90	
29.4	32.2	30.6	36.5	35.2	35.8	29	27.6	28.5	35.4	LOW	0.89	
23.9	26.2	25.8	37.2	38.1	39.6	30.4	31.3	31.4	35.2	LOW	0.90	
39.5	•	35.4	43.2	40.6	41.7	31.5	29.8	29.3	39	LOW	0.93	
37.1	40.1	37.3	47.4	47.3	48.6	41	38.4	40.9	44	LOW	0.95	

### CD4-APC



il)		0			13			250		Control w/o	Variability	Ratio	
I)	0	13	250	0	13	250	0	13	250	FCB	variability	Italio	
-1	85.5	84.1	81.3	82.7	83.1	80.3	75	73.9	70.5	82.2	LOW	0.97	
-2	52	47.1	42.6	49.3		42	41	42.7	36.5	49.6	LOW	0.89	
-3	33.6	35	31.4	34.3	35.1	30	36.4	35.4	31	36.7	LOW	0.91	
-4	66	66.3	63.6	64.1	63.5	59.8	64.1	62.3	58.6	64.5	LOW	0.98	
-5	54.7	50.2	49.2	44.3	45.7	37.9	38.6	38.2	35.3	45.1	LOW	0.97	
-6	71.4	68.3	68	55.8	54.2	50.1	51.6	52	50.6	56.6	LOW	1.02	
-7	54.7		55.2	50.7	53.5	41.7	38.1	37.3	35.7	52.2	LOW	0.88	
-8	54.2	50.5	47.4	40.2	41	30.8	35.5	36.9	36.5	18.6	HIGH	2.23	

### **Median Fluorescence Intensity** CD3-BV605 DyLight 350 (µg/ml) 0 μg/ml Variability Ratio population DyLight 800 (µg/ml) HIGH 1.11 HC-2 LOW 1.15 HC-3 LOW 1.12 HC-4 LOW 1.17 HC-5 LOW 1.02 HC-6 LOW 1.01 HC-7 LOW 1.18 LOW 1.04 Vβ-FITC/PE PE MFI 0 μg/ml DyLight 350 (µg/ml) Variability Ratio population DyLight 800 (µg/ml) HC-1 76.7 55.6 94.2 76.7 86.7 94.8 65.9 76.7 LOW 1 09 82.8 74.4 64.2 95.5 LOW 0.97 HC-2 75.8 75.8 82.8 HC-3 74.4 68.4 80.5 69.8 81.4 85.8 69.8 66.4 92. 74.4 LOW 1.03 HC-4 79.3 81.4 86.7 79.3 LOW 1.23 85.2 HC-5 83.1 82.2 92. 93.6 85.8 83.1 LOW 1.09 HC-6 89.4 76.4 95.2 83.1 85.5 74.4 80.8 89.4 LOW 0.98 93.6 HC-7 93.9 71.5 82.2 89.3 82.8 HIGH 0.69 HC-8 LOW 1.12 Vβ-FITC/PE FITC MFI DvLight 350 (µg/ml) 0 μg/ml Variability Ratio DyLight 800 (μg/ml) population HC-1 LOW 0.95 HC-2 LOW 1.11 0.90 HC-3 LOW HC-4 LOW 0.99 LOW 1.00 HC-5 HC-6 LOW 0.82 HC-7 HIGH 0.72 HC-8 LOW 0.95 Vβ-FITC DyLight 350 (μg/ml) 0 μg/ml Variability Ratio population DyLight 800 (µg/ml) LOW 0.92 HC-1 HC-2 LOW 1.08 HC-3 LOW 0.97 HC-4 LOW 1.18 HC-5 0.98 LOW HC-6 LOW 1 04 HC-7 LOW 0.85 LOW 1.01 HC-8 **V**β-PE DyLight 350 (µg/ml) 0 μg/ml Variability Ratio population DyLight 800 (µg/ml) LOW 0.82 12: 91.5 HC-2 LOW 0.99 HC-3 LOW 1.00 HC-4 LOW 1.30 HC-5 19: LOW 0.98 HC-6 LOW 0.94 HC-7 13! LOW 1.13 LOW CD8-PE-Cy5 DvLight 350 (µg/ml) 0 μg/ml Variability Ratio DyLight 800 (μg/ml) population HC-1 LOW 0.91 HC-2 LOW 0.91 HC-3 LOW 0.92 HC-4 LOW 0.95 HC-5 LOW 0.88 HC-6 LOW 0.91 HC-7 LOW 0.90 HC-8 LOW 0.98 CD4-APC

DyLight 350 (μg/ml)	0			13				250		0 μg/ml	Variability	Ratio	
DyLight 800 (μg/ml)	0	13	250	0	13	250	0	13	250	population	variability	Natio	
HC-1	108	93.3	78.9	98.5	95.6	78.9	92	91.2	74.2	108	LOW	0.83	
HC-2	78.9	70.8	63.2	81.1		62.3	80.8	85	70.3	78.9	LOW	0.94	
HC-3	55.7	58.2	53.8	61.5	61.7	54	64.1	61.9	54.9	55.7	LOW	1.05	
HC-4	80.6	84	74	82.5	84	72.6	83.5	83.5	74.7	80.6	LOW	0.99	
HC-5	101	90.7	77.5	97.2	90.5	76.1	95.1	87.7	75.6	101	LOW	0.87	
HC-6	115	110	92.5	109	104	89.2	105	103	91.2	115	LOW	0.89	
HC-7	82		72.6	80.1	82	70.8	81.3	83	73.8	82	LOW	0.95	
HC-8	127	128	120	130	127	108	129	130	118	127	LOW	0.98	