Supplemental Data: The intra viral protein interaction network of hepatitis C virus.

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Supplemental Figure S1: Quantitative and statistical analyses of FRET signals in 293T and Huh7.5 cells.

Number of independent experiments (n), mean value % FRET+ cells (MF), and standard deviations (SD) are indicated. Analyses were performed for both combinations (YFP-protein A + CFP-protein B & CFP-protein A + YFP-protein B). For calculation of statistical significance, mean FRET signals of n experiments were compared to the negative control FRET signal (CFP-fusion with YFP-only) with the two-tailed unpaired Student's t test (Graph Pad Prism 5.0). Significance levels are indicated as follows: $p<0.05$ (*), $p<0.01$ (**) and $p<0.001$ (***). Grey boxes depict mean FRET values ≥10 % (numbers in red). This threshold was arbitrarily introduced as additional stringency criterion to define robust FRET of cotransfected fusion proteins. Some mean FRET values below 10 % also reached statistical significance. These values are shown in green. Primary data for transfection of 293T cells are accessible within the supplemental data set (see below).

A

B			Core		E1		E2		p7		NS2		NS3		NS4A		NS4B		NS5A		NS5B	
			293T	Huh7.5	293T	Huh7.5	293T	Huh7.5	293T	Huh7.5	293T	Huh7.5	293T	Huh7.5	293T	Huh7.5	293T	Huh7.5	293T	Huh7.5	293T	Huh7.5
Core	C-A + Y-B	n	9	8																		
		MF	**67.93**	**44.89**																		
		SD	14.88	25.25																		
			***	***																		
E1	C-A + Y-B	n	9	7	9	not tested																
		MF	3.91	**2.87**	**1.88**																	
		SD	3.33	3.53	2.35																	
			ns	*	*																	
	Y-A + C-B	n	12	1																		
		MF	1.84	0.90																		
		SD	2.84	.																		
			ns																			
E2	C-A + Y-B	n	9	3	11	not tested	18	13														
		MF	0.9	0.00	0.44		**62.29**	**47.12**														
		SD	1.91	0.00	0.45		28.53	34.40														
			ns	ns	ns		***	***														
	Y-A + C-B	n	23	6	17	7																
		MF	**21.11**	**3.00**	**74.76**	**42.21**																
		SD	17.46	3.71	17.68	10.45																
			***	*	***	***																
p7	C-A + Y-B	n	9	3	10	2	17	13	23	11												
		MF	6.43	0.00	0.18	0.35	**18.36**	**34.92**	**29.30**	**6.69**												
		SD	11.16	0.00	0.23	0.50	20.68	31.22	18.37	4.66												
			ns	ns	ns	.	***	**	***	***												
	Y-A + C-B	n	15	5	16	14	19	17														
		MF	**27.78**	0.62	**16.41**	**7.69**	**6.88**	4.67														
		SD	32.91	1.28	17.37	10.61	8.03	6.64														
			**	ns	***	*	**	ns														
NS2	C-A + Y-B	n	10	3	11	not tested	17	9	24	6	38	8										
		MF	0.09	0.00	0.34		**40.11**	**16.60**	**40.73**	6.18	**60.31**	**56.30**										
		SD	0.19	0.00	0.35		32.95	21.98	26.82	8.93	24.46	19.70										
			ns	ns	ns		***	*	***	ns	***	***										
	Y-A + C-B	n	35	3	34	9	39	2	32	6												
		MF	**15.54**	1.03	**17.78**	4.22	8.81	0.30	**22.54**	**14.05**												
		SD	21.20	1.79	14.09	8.47	18.43	0.42	24.18	11.29												
			**	ns	***	ns	ns	.	***	**												
NS3	C-A + Y-B	n	9	1	11	3	14	2	14	4	23	7	28	13								
		MF	0.28	0.00	1.88	0.00	**2.18**	7.05	**8.00**	0.40	2.90	4.84	**9.95**	**24.16**								
		SD	0.35	.	4.57	0.00	2.13	5.87	9.87	0.45	9.64	12.72	10.5	11.65								
			ns		ns	ns	**	.	**	ns	ns	ns	***	***								
	Y-A + C-B	n	13	not tested	13	6	14	3	15	2	14	1										
		MF	**1.7**		**5.96**	**9.30**	**1.43**	**2.13**	**3.61**	7.65	0.24	0.10										
		SD	2.01		5.51	7.54	1.93	0.61	7.07	1.49	0.42	.										
			**		***	**	**	***	*	.	ns											
NS4A	C-A + Y-B	n	8	not tested	10	not tested	14	2	10	not tested	22	not tested	20	8	10	not tested						
		MF	0.20		0.07		5.49	0.35	0.16		0.71		**2.99**	11.44	1.28							
		SD	0.49		0.09		17.50	0.50	0.21		1.34		4.31	18.44	1.48							
			ns		ns		ns	.	ns		ns		**	ns	ns							
	Y-A + C-B	n	9	not tested	9	not tested	10	not tested	10	not tested	9	not tested	20	14								
		MF	0.54		4.79		0.60		0.60		2.00		**27.97**	**2.46**								
		SD	0.48		9.36		1.05		0.90		3.38		23.88	2.65								
			ns		ns		ns		ns		ns		***	*								
NS4B	C-A + Y-B	n	8	not tested	10	not tested	14	not tested	13	not tested	29	2	11	not tested	10	not tested	13	6				
		MF	0.64		0.09		**5.66**		**8.15**		7.69	0.40	**1.01**		4.05		**36.18**	**7.68**				
		SD	1.27		0.14		6.32		15.58		11.32	0.00	1.52		8.08		16.68	7.74				
			ns		ns		***		*		ns	.	*		ns		***	*				
	Y-A + C-B	n	9	not tested	9	not tested	10	not tested	10	not tested	11	not tested	9	not tested	8	not tested						
		MF	1.03		1.08		0.09		0.62		0.36		0.23		0.30							
		SD	2.38		2.42		0.18		1.40		0.56		0.28		0.30							
			ns		ns		ns		ns		ns		ns		ns							
NS5A	C-A + Y-B	n	8	not tested	9	not tested	14	not tested	14	not tested	24	3	13	not tested	9	not tested	8	not tested	20	9		
		MF	2.15		0.28		**2.82**		**4.99**		3.68	0.17	0.47		1.69		0.93		**12.69**	**14.92**		
		SD	4.93		0.33		4.64		8.19		8.12	0.29	0.66		2.19		2.10		12.12	8.90		
			ns		ns		*		*		ns	ns	ns		ns		ns		***	**		
	Y-A + C-B	n	11	not tested	15	not tested	15	not tested	11	not tested	8	not tested	11	not tested	10	not tested	9	not tested				
		MF	0.30		0.65		0.92		**1.18**		0.14		0.08		0.15		**0.89**					
		SD	0.61		1.00		1.88		1.80		0.17		0.21		0.28		1.07					
			ns		ns		ns		*		ns		ns		ns		*					
NS5B	C-A + Y-B	n	8	2	11	3	16	3	8	not tested	21	not tested	13	not tested	9	not tested	8	not tested	8	not tested	15	not tested
		MF	7.91	0.00	0.22	0.00	2.09	0.00	**5.21**		0.91		0.15		0.59		0.44		0.15		**5.10**	
		SD	11.36	0.00	0.23	0.00	4.75	0.00	7.74		2.26		0.23		1.21		0.48		0.19		8.72	
			ns	.	ns	ns	ns	ns	*		ns		ns		ns		ns				*	
	Y-A + C-B	n	20	6	20	4	18	6	14	not tested	14	not tested	13	not tested	12	not tested	12	not tested	20	not tested		
		MF	**13.11**	3.90	**23.62**	**20.55**	**12.97**	**18.42**	4.07		1.34		0.91		0.19		1.10		**7.06**			
		SD	10.43	6.82	21.94	5.03	15.34	15.36	7.96		2.29		1.40		0.38		1.62		9.50			
			***	ns	***	***	***	**	ns		ns		ns		ns		ns		**			

p < 0.05 *
p < 0.01 **
p < 0.001 ***

p > 0.05 ns

mean above 10 % threshold

mean below 10 % threshold

not significant

n number of experiments
MF mean value % FRET+ cells
SD standard deviation

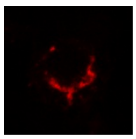
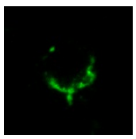
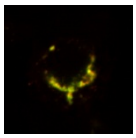
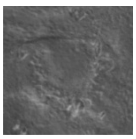
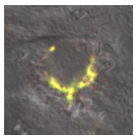
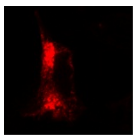
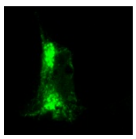
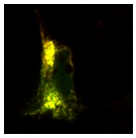
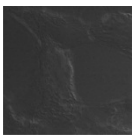
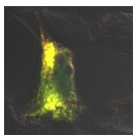
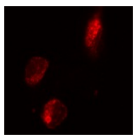
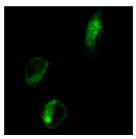
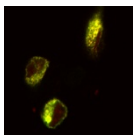
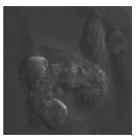
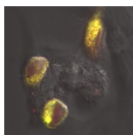
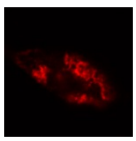
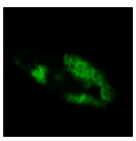
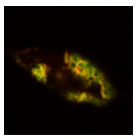
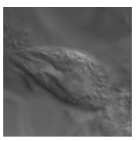
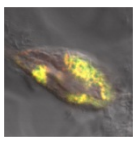
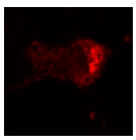
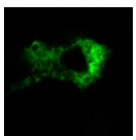
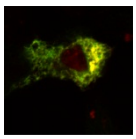
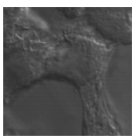
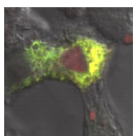
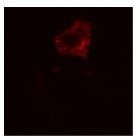
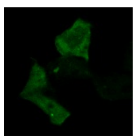
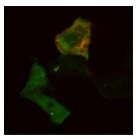
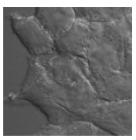
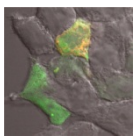
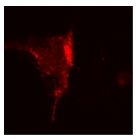
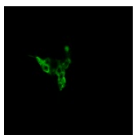
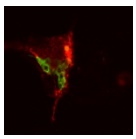
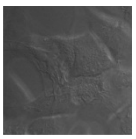
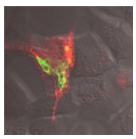
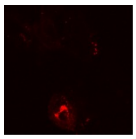
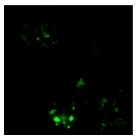
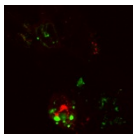
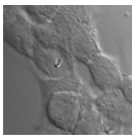
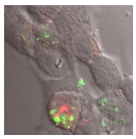
Supplemental Dataset: FRET results of HCV CFP and YFP fusion proteins transfected in 293T cells.

The percentage of FRET+ cells (mean) from the indicated number of experiments (n) as well as standard deviation (SD) is presented in every graph (left panel). We furthermore show a representative example of the cotransfection efficiency (% cotransfected cells) and the percentage of FRET+ cells according to the gating strategy previously established (Banning et al., 2010 PLoS ONE 5(2): e9344). Furthermore, the subcellular localization of the respective fusion proteins was analysed by confocal microscopy (right panel; CFP is false colored in red and YFP in green).

CFP-Core tested with:

Interaction via FACS-FRET

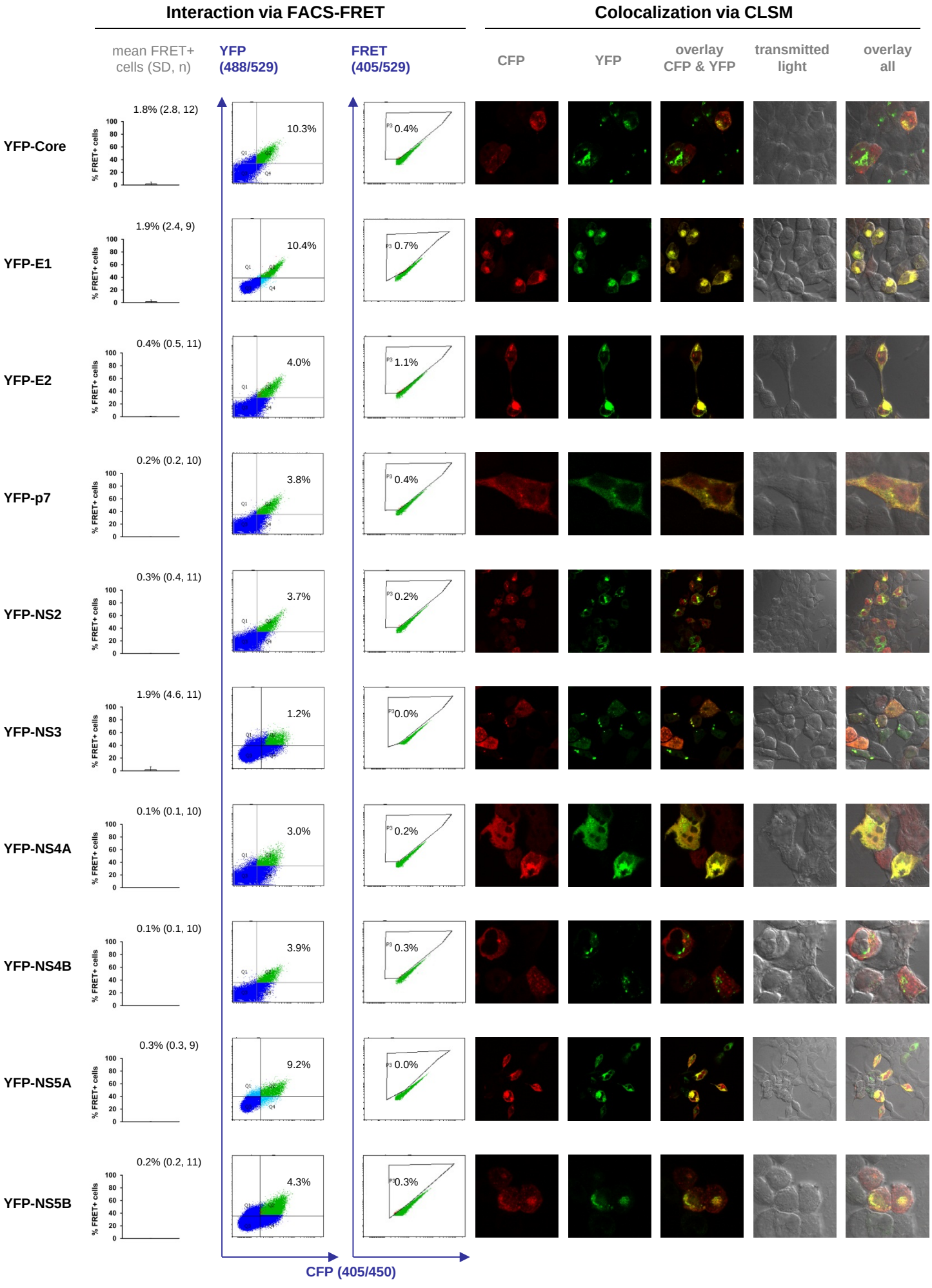
Colocalization via CLSM

	mean FRET+ cells (SD, n)	YFP (488/529)	FRET (405/529)	CFP	YFP	overlay CFP & YFP	transmitted light	overlay all
YFP-Core	67.9% (14.9, 9)	6.3%	73.8%					
YFP-E1	3.9% (3.3, 9)	6.1%	0.4%					
YFP-E2	0.9% (0.9, 9)	2.3%	0.3%					
YFP-p7	6.4% (11.2, 9)	1.2%	0.1%					
YFP-NS2	0.1% (0.2, 10)	2.2%	0.0%					
YFP-NS3	0.3% (0.4, 9)	7.8%	0.1%					
YFP-NS4A	0.2% (0.5, 8)	9.0%	0.0%					
YFP-NS4B	0.6% (1.3, 8)	11.0%	0.1%					
YFP-NS5A	2.2% (4.9, 8)	15.7%	0.0%					
YFP-NS5B	7.91% (11.4, 8)	2.2%	6.0%					

% FRET+ cells

CFP (405/450)

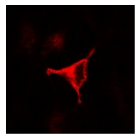
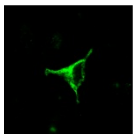
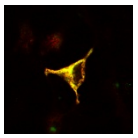
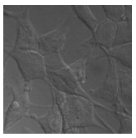
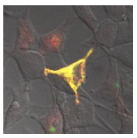
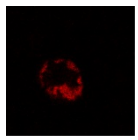
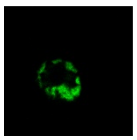
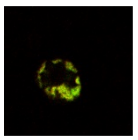
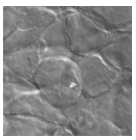
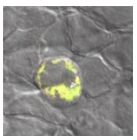
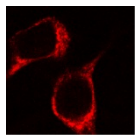
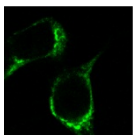
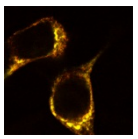
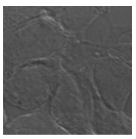
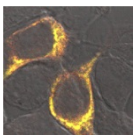
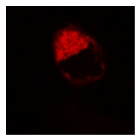
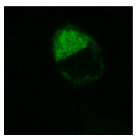
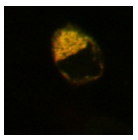
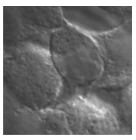
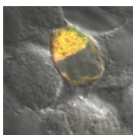
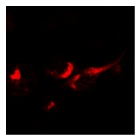
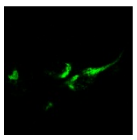
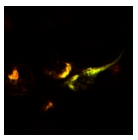
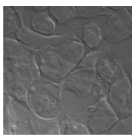
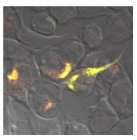
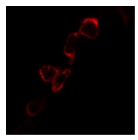
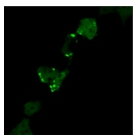
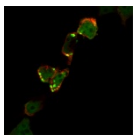
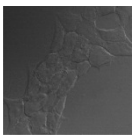
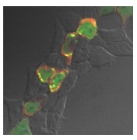
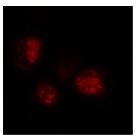
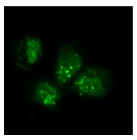
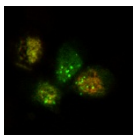
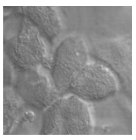
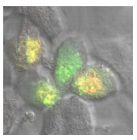
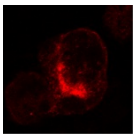
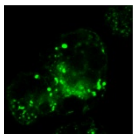
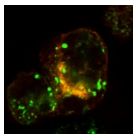
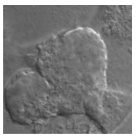
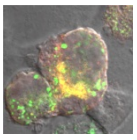
CFP-E1 tested with:



CFP-E2 tested with:

Interaction via FACS-FRET

Colocalization via CLSM

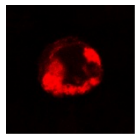
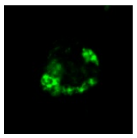
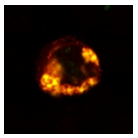
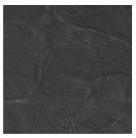
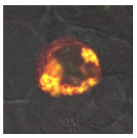
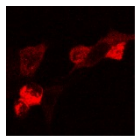
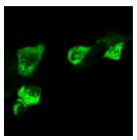
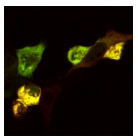
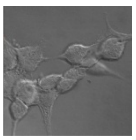
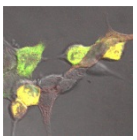
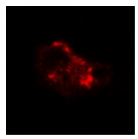
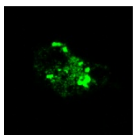
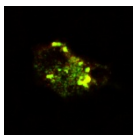
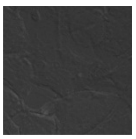
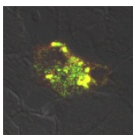
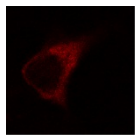
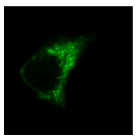
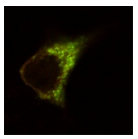
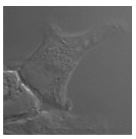
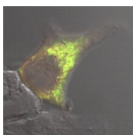
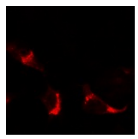
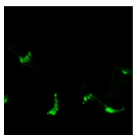
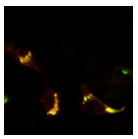
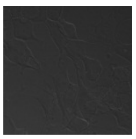
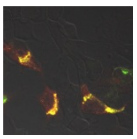
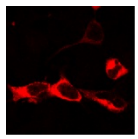
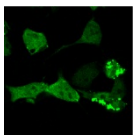
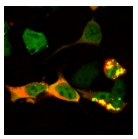
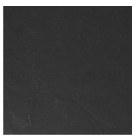
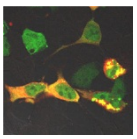
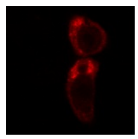
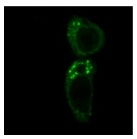
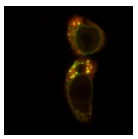
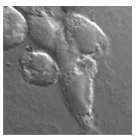
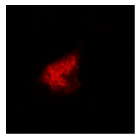
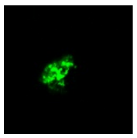
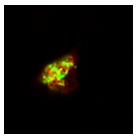
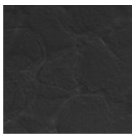
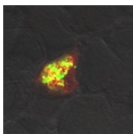
	mean FRET+ cells (SD, n)	YFP (488/529)	FRET (405/529)	CFP	YFP	overlay CFP & YFP	transmitted light	overlay all
YFP-Core	21.1% (17.5, 23)	1.9%	17.9%					
YFP-E1	74.8% (17.7, 17)	1.8%	64.7%					
YFP-E2	62.3% (28.5, 18)	14.5%	79.1%					
YFP-p7	18.4% (20.7, 17)	10.9%	16.0%					
YFP-NS2	40.1% (33.0, 17)	5.3%	41.8%					
YFP-NS3	2.2% (2.1, 14)	10.4%	1.7%					
YFP-NS4A	5.5% (17.5, 14)	5.0%	5.4%					
YFP-NS4B	5.7% (6.3, 14)	4.4%	3.2%					
YFP-NS5A	2.8% (4.6, 14)	7.4%	2.5%					
YFP-NS5B	2.1% (4.8, 16)	0.7%	3.3%					

CFP (405/450)

CFP-p7 tested with:

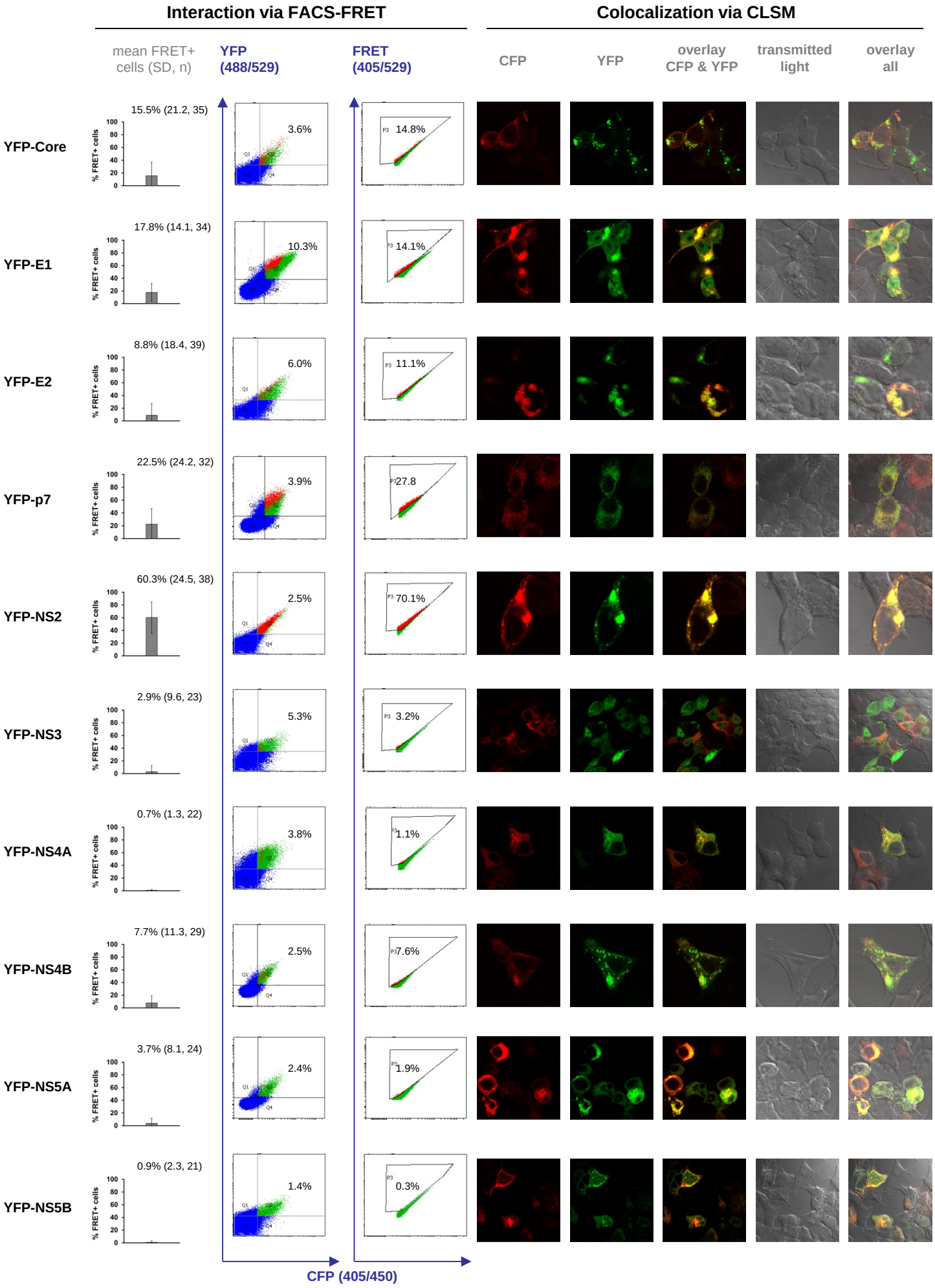
Interaction via FACS-FRET

Colocalization via CLSM

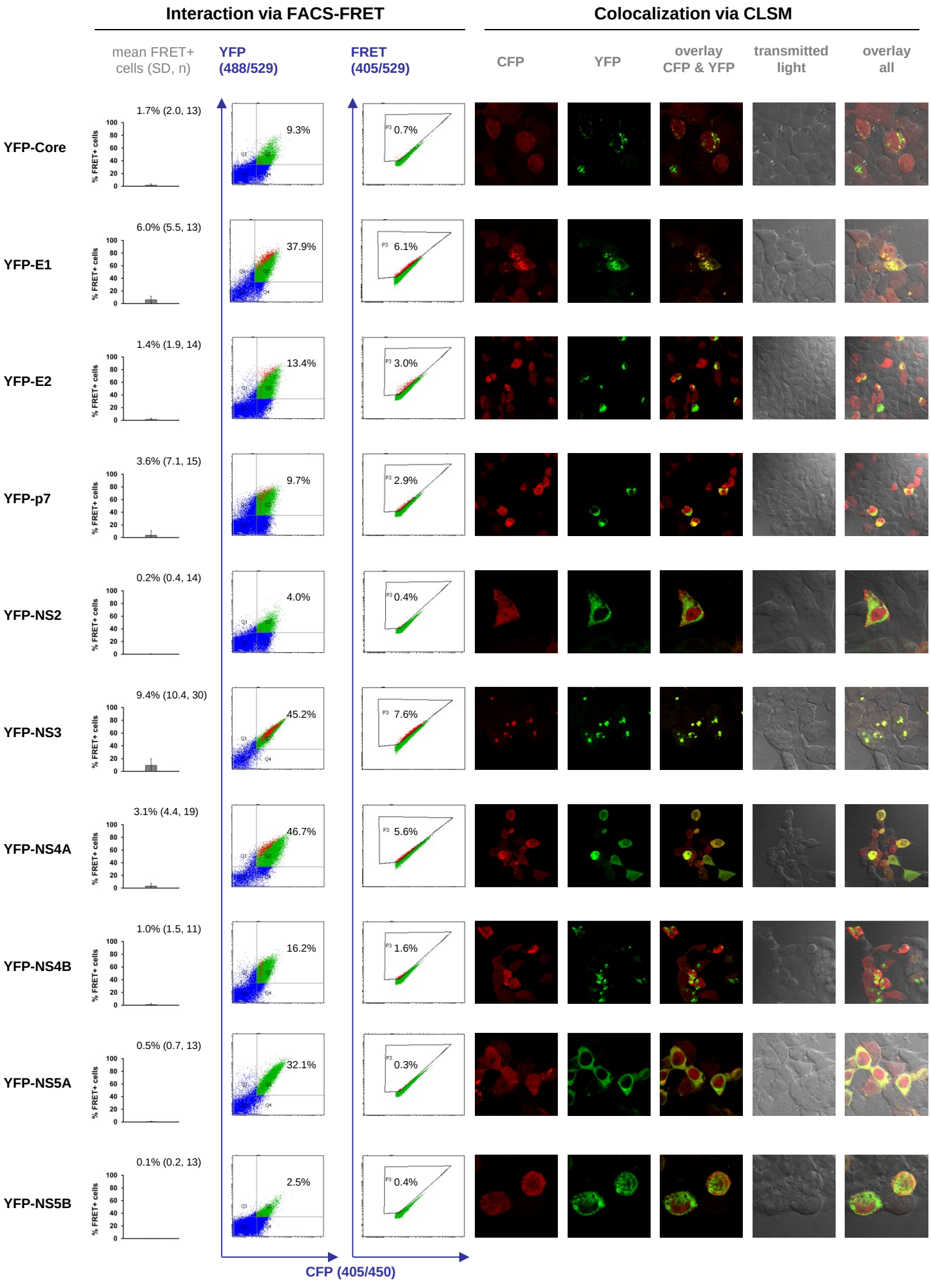
	mean FRET+ cells (SD, n)	YFP (488/529)	FRET (405/529)	CFP	YFP	overlay CFP & YFP	transmitted light	overlay all
YFP-Core	27.8% (32.9, 15)	3.4%	24.4%					
YFP-E1	16.4% (17.4, 16)	3.2%	18.8%					
YFP-E2	6.9% (8.0, 19)	10.9%	16.3%					
YFP-p7	29.3% (18.4, 23)	6.5%	52.3%					
YFP-NS2	40.7% (26.8, 24)	3.2%	31.5%					
YFP-NS3	8.0% (9.9, 14)	1.3%	1.5%					
YFP-NS4A	0.2% (0.2, 10)	2.9%	0.4%					
YFP-NS4B	8.2% (15.6, 13)	0.5%	2.3%					
YFP-NS5A	5.0% (8.2, 14)	8.3%	1.3%					
YFP-NS5B	5.2% (7.7, 8)	3.4%	5.7%					

CFP (405/450)

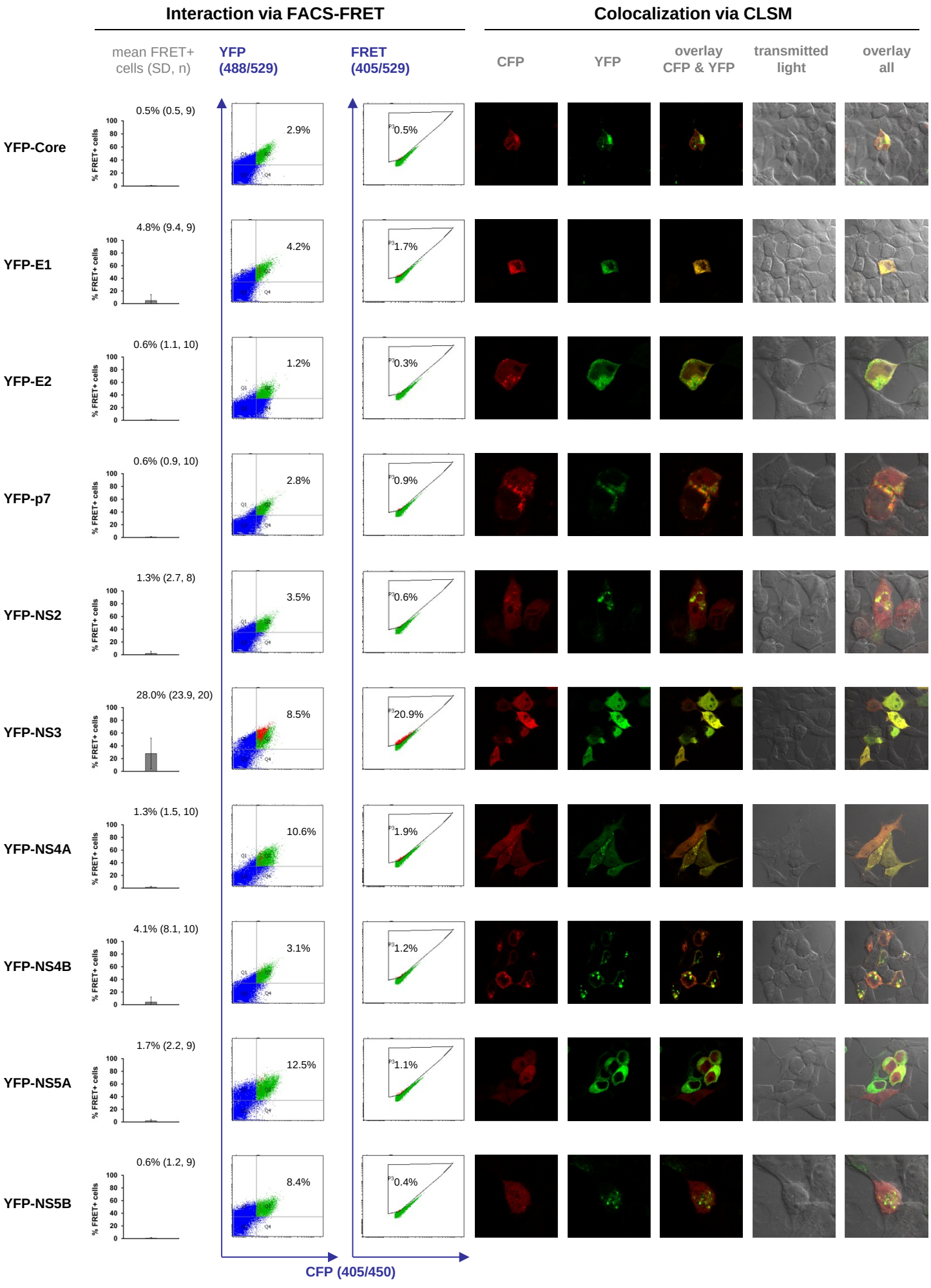
CFP-NS2 tested with:

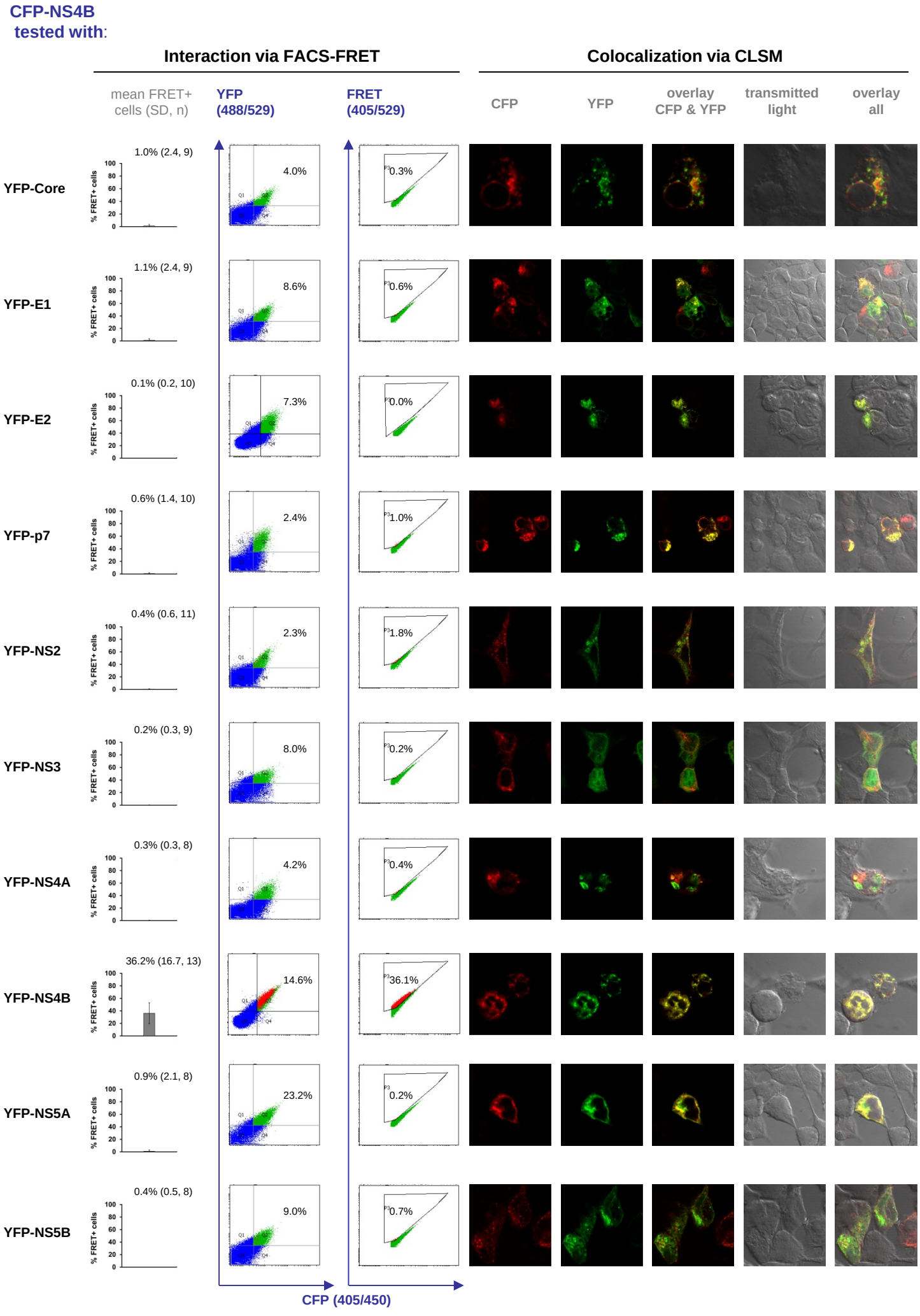


CFP-NS3 tested with:



CFP-NS4A tested with:

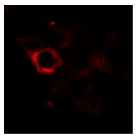
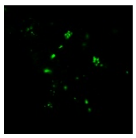
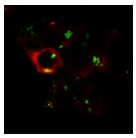
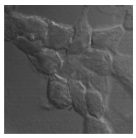
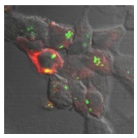
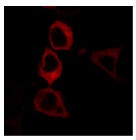
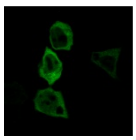
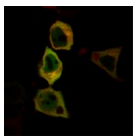
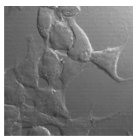
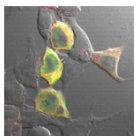
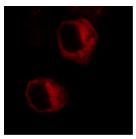
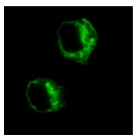
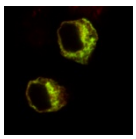
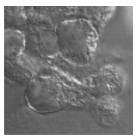
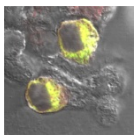
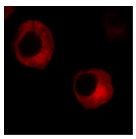
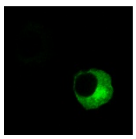
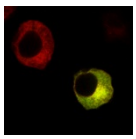
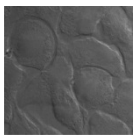
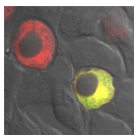
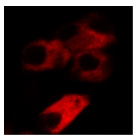
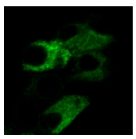
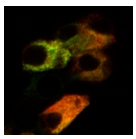
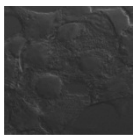
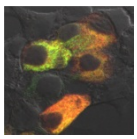
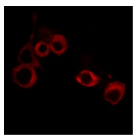
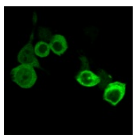
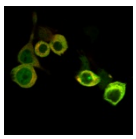
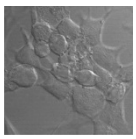
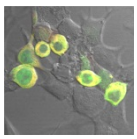
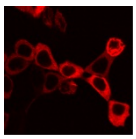
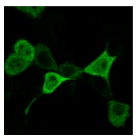
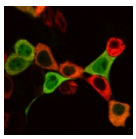
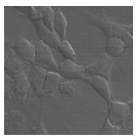
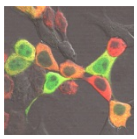
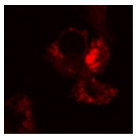
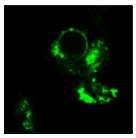
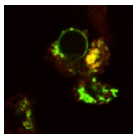
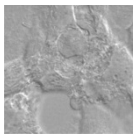
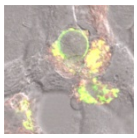



CFP-NS4B
tested with:
Interaction via FACS-FRET
Colocalization via CLSM
mean FRET+ cells (SD, n)
YFP (488/529)
FRET (405/529)
CFP
YFP
overlay CFP & YFP
transmitted light
overlay all
YFP-Core
1.0% (2.4, 9)
4.0%
0.3%
YFP-E1
1.1% (2.4, 9)
8.6%
0.6%
YFP-E2
0.1% (0.2, 10)
7.3%
0.0%
YFP-p7
0.6% (1.4, 10)
2.4%
1.0%
YFP-NS2
0.4% (0.6, 11)
2.3%
1.8%
YFP-NS3
0.2% (0.3, 9)
8.0%
0.2%
YFP-NS4A
0.3% (0.3, 8)
4.2%
0.4%
YFP-NS4B
36.2% (16.7, 13)
14.6%
36.1%
YFP-NS5A
0.9% (2.1, 8)
23.2%
0.2%
YFP-NS5B
0.4% (0.5, 8)
9.0%
0.7%
% FRET+ cells
CFP (405/450)

CFP-NS5A tested with:

Interaction via FACS-FRET

Colocalization via CLSM

	mean FRET+ cells (SD, n)	YFP (488/529)	FRET (405/529)	CFP	YFP	overlay CFP & YFP	transmitted light	overlay all
YFP-Core	0.3% (0.6, 11)	21.5%	0.1%					
YFP-E1	0.7% (1.0, 15)	21.4%	1.7%					
YFP-E2	0.9% (1.9, 15)	16.3%	0.0%					
YFP-p7	1.2% (1.8, 11)	9.0%	0.3%					
YFP-NS2	0.1% (0.2, 8)	20.1%	0.2%					
YFP-NS3	0.1% (0.2, 11)	31.1%	0.0%					
YFP-NS4A	0.2% (0.3, 10)	10.5%	0.9%					
YFP-NS4B	0.9% (1.1, 9)	17.1%	2.5%					
YFP-NS5A	12.7% (12.1, 20)	24.0%	11.4%					
YFP-NS5B	0.2% (0.2, 8)	16.6%	0.5%					

% FRET+ cells

CFP (405/450)

CFP-NS5B tested with: