Supplemental Table 1. Kinetic results of the MPLSM monitoring of the uptake of fluorescent polyunsaturated fatty acids in L-cells.

PUFA	Cell Region	C_1 *(pool 1)*	k_1(min^{-1})	C_2 *(pool 2)*	k_2 (min^{-1})
Control L-cells					
A5c	nucleus	140 ± 30	0.80 ± 0.10	290 ± 10	0.11 ± 0.02
	nucleoplasm	127 ± 3	0.48 ± 0.04	-	-
	nucleus envelope	160 ± 40	0.70 ± 0.09	470 ± 20	0.11 ± 0.02
	cytoplasm	330 ± 70	0.44 ± 0.04	320 ± 60	0.13 ± 0.02
	cell	290 ± 50	0.48 ± 0.04	310 ± 30	0.11 ± 0.03
E6c	nucleus	128 ± 8	2.0 ± 0.4	414 ± 6	0.099 ± 0.006
	nucleoplasm	69 ± 7	2.24 ± 0.91	199 ± 5	0.11 ± 0.01
	nucleus envelope	143 ± 7	1.92 ± 0.31	500 ± 6	0.096 ± 0.005
	cytoplasm	220 ± 10	1.7 ± 0.2	810 ± 10	0.094 ± 0.006
	cell	200 ± 10	1.7 ± 0.2	700 ± 10	0.094 ± 0.006
D7c	nucleus	313 ± 3	0.503 ± 0.005	620 ± 68	0.019 ± 0.003
	nucleoplasm	249 ± 3	0.29 ± 0.01	-	-
	nucleus envelope	349 ± 3	0.590 ± 0.005	670± 20	0.029 ± 0.002
	cytoplasm	300 ± 20	0.81 ± 0.07	600 ± 70	0.046 ± 0.01
	cell	300 ± 20	0.71 ± 0.05	540 ± 70	0.043 ± 0.01
L-FABP expressing cells					
A5c	nucleus	280 ± 50**	0.58 ± 0.09	700 ± 60****	0.06 ± 0.02
	nucleoplasm	-	-	364 ± 7[a]	0.143 ± 0.007[a]
	nucleus envelope	320 ± 20***	0.96 ± 0.09*	890 ± 50****	0.063 ± 0.009*
	cytoplasm	430 ± 20	0.85 ± 0.05****	590 ± 20****	0.07 ± 0.01**
	cell	400 ± 20*	0.75 ± 0.04****	600 ± 20****	0.05 ± 0.01*
E6c	nucleus	231 ± 6****	1.43 ± 0.08	440 ± 80	0.04 ± 0.01****
	nucleoplasm	101 ± 4****	1.8 ± 0.2	170 ± 30	0.04 ± 0.01****
	nucleus envelope	262 ± 6****	1.48 ± 0.08	470 ± 60	0.045 ± 0.009****
	cytoplasm	480 ± 10****	1.6 ± 0.1	1120 ± 70	0.040 ± 0.005****
	cell	425 ± 8****	1.57 ± 0.09	940 ± 60***	0.040 ± 0.004****
D7c	nucleus	120 ± 10****	1.6 ± 0.3***	650 ± 8	0.129 ± 0.006****
	nucleoplasm	-	-	377 ± 5[a]	0.169 ± 0.006[a]
	nucleus envelope	160 ± 10****	2.1 ± 0.3****	700 ± 8	0.123 ± 0.005****
	cytoplasm	300 ± 10	1.4 ± 0.1****	850 ± 10***	0.106 ± 0.006****
	cell	260 ± 10	1.4 ± 0.1****	810 ± 9****	0.108 ± 0.005****

Values represent fitted parameters ± standard error from 300-500 cells.
*P< 0.05; ** P<0.02; ***P<0.001, ****P<0.0001
[a]Compared to fitted parameters 1 with 2, P<0.0001

Supplemental Figure Legends

Supplemental Fig. 1. M**etabolism of the fluorescent E6c and non-fluorescent ^{14}C-20:5n-3 VLC-PUFA to esterified lipids.** L-cells were incubated with E6c (for 5, 15, 30 minutes and overnight) or non-fluorescent ^{14}C-20:5n-3 (for 5min and overnight), lipids were extracted, and analyzed as described in the Experimental Procedures. **A.** The amount in nmol/mg protein of fluorescent E6c VLC-PUFA appearing in cholesterol esters (CE), triacylglycerides (TG), free fatty acid (FFA), phosphatidyl ethanolamine (PE), phosphatidyl choline (PC) are shown. **B.** The % distribution of each of the fluorescent E6c lipid classes in A is shown. **C.** The amount in nmol/mg protein of non-fluorescent 20:5n-3 appearing in cholesterol ester (CE), triacylglycerides (TG), free fatty acid (FFA), phosphatic acid (PA), phosphatidyl ethanolamine (PE), phosphainositol (PI), phosphatidylserine (PS), and phosphatidyl choline (PC) at 5min are shown. **D.** The amount in nmol/mg protein of non-fluorescent 20:5n-3 appearing in each of the lipid classes in C is shown. Values represent the mean ± SE (n=4-6).

Supplemental Fig. 2. M**etabolism of the fluorescent D7c and non-fluorescent ^{14}C-22:6n-3 VLC-PUFA to esterified lipids.** L-cells were incubated with D7c (for 5, 15, 30 minutes and overnight) or non-fluorescent ^{14}C-22:6n-3 (for 5min and overnight), lipids were extracted, and analyzed as described in the Experimental Procedures. **A.** The amount in nmol/mg protein of fluorescent D7c VLC-PUFA appearing in cholesterol esters (CE), triacylglycerides (TG), free fatty acid (FFA), phosphatidyl ethanolamine (PE), phosphatidyl choline (PC) are shown. **B.** The % distribution of each of the fluorescent D7c lipid classes in A is shown. **C.** The amount in nmol/mg protein of non-fluorescent 22:6n-3 appearing in cholesterol ester (CE), triacylglycerides (TG), free fatty acid (FFA), phosphatic acid (PA), phosphatidyl ethanolamine (PE), phosphainositol (PI), phosphatidylserine (PS), and phosphatidyl choline (PC) at 5min are shown. **D.** The amount in nmol/mg protein of non-fluorescent 22:6n-3 appearing in each of the lipid classes in C is shown. Values represent the mean ± SE (n=4-6).

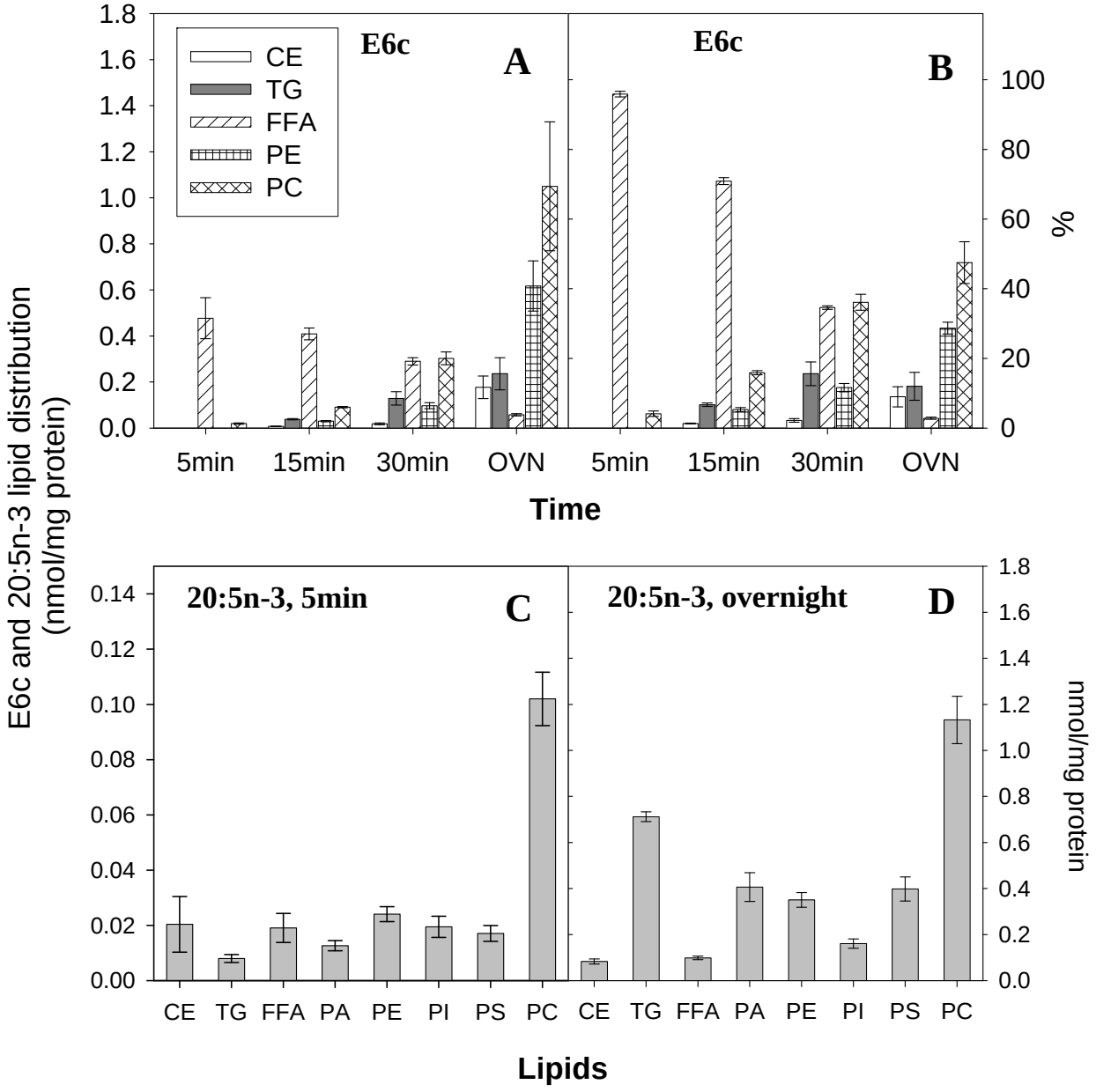
Supplemental Fig. 3. Effect of L-FABP expression upon metabolism of the fluorescent and non-fluorescent VLC-PUFA at 5min. L-cells and L-cells expressing L-FABP were incubated with (Panel A) A5c, E6c, and D7c or with (Panels B,C,D) radiolabeled 20:4n-6, 20:5n-3, and 22:6n-3 for five minutes. The lipids were extracted and analyzed described in the Experimental Procedures. The % distribution of each VLC-PUFA appearing in each of the lipid classes are shown. Values represent the mean $\pm$ SE (n=4-6). *, $p<0.05$; **, $p<0.01$, significantly different in L-FABP expressing cells (solid bars) compared to control L-cells (open bars).

Supplemental Fig. 4. **Real-time MPLSM of fluorescent n-3 and n-6 VLC-PUFAs in living L-FABP expressing cells at 5 minutes.** MPLSM with non-descanned 2 channel fluorescence emission detection was used to monitor the distribution of the fluorescent n-3 and n-6 VLC-PUFA analogues A5c, E6c, and D7c (2.5 μM) in L-FABP expressing cells wherein very little of the PUFA analogues have been metabolized. The PUFA labeled cells (red) were subsequently incubated with 5μM SYTO 11 (green) in order to stain the nuclei (see Experimental Procedures). Images of the distribution of the fluorescent PUFAs, A5c **(A)**, E6c **(D)**, and D7c **(G)**; the corresponding images **(B)**, **(E)**, and **(H)** show only the SYTO 11 fluorescence distribution in the nuclei; combined images showing the colocalization of A5c with SYTO 11 **(C)**, E6c with SYTO 11 **(F)**, and D7c with SYTO 11 **(I)**.

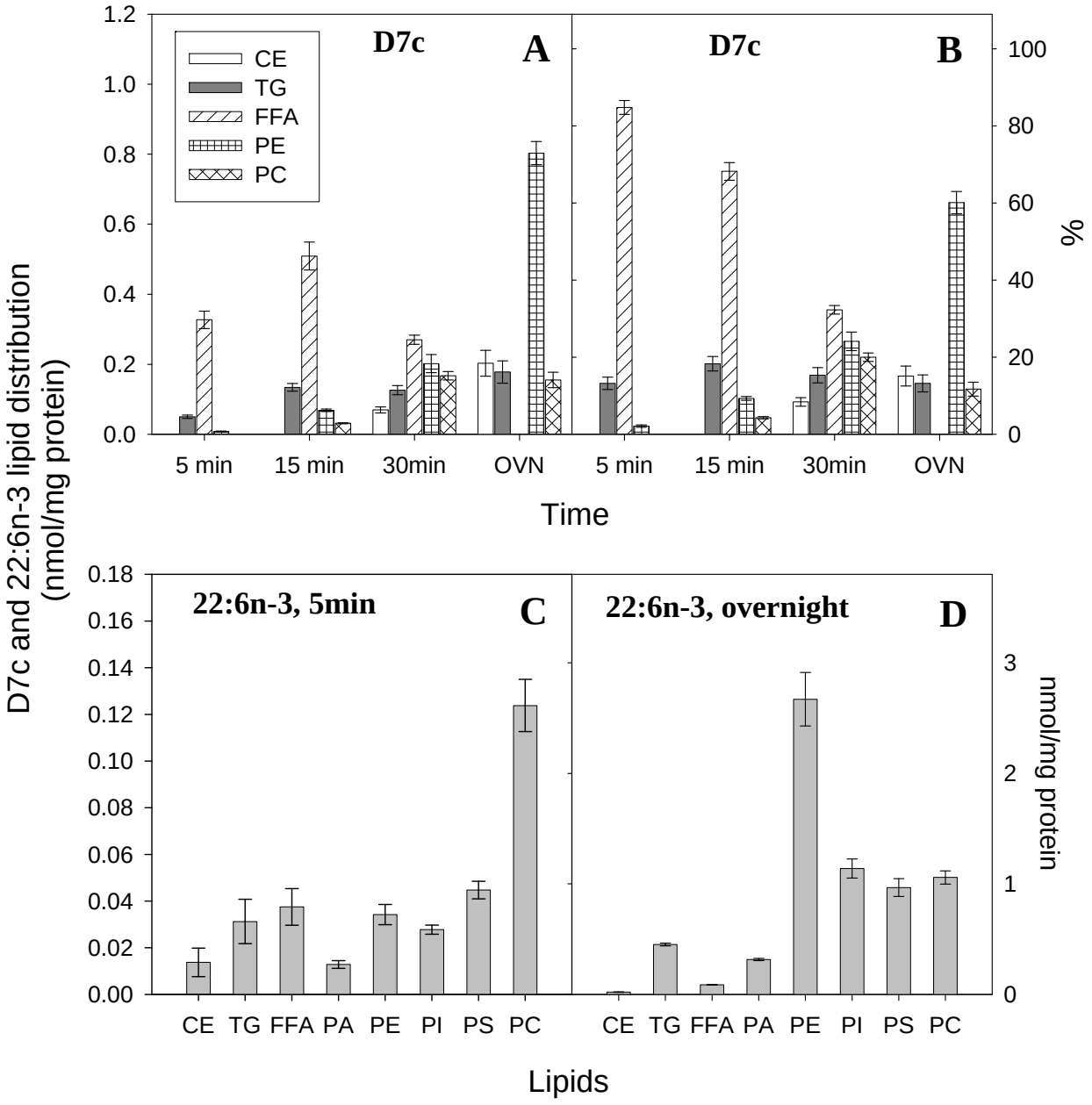
Supplemental Fig. 5. The effect of L-FABP expression on the uptake kinetics of the fluorescent PUFAs in living cells. L-cells expressing L-FABP grown to confluency on chambered coverglass were labeled with 2.5 μM A5c, E6c, and D7c and the fluorescent intensities were measured using MPLSM while the cells were maintained at 37 ºC during the time course (see Methods) as in the case of the control L-cells. Morphometric analysis of the acquired images was performed in MetaMorph. The emission density was converted to concentration by normalization of whole cell area to 1 and calibration at the 5

minute time point using the direct quantitation by HPLC as described in Experimental Procedures. The uptake curves are shown as follows: **A.** The cytoplasmic and nuclear uptake kinetics of A5c in L-FABP expressing cells, **B.** Nucleoplasmic and nuclear envelope uptake kinetics of A5c in L-FABP expressing cells; **C.** The cytoplasmic and nuclear uptake kinetics of E6c in L-FABP expressing cells, **D.** Nucleoplasmic and nuclear envelope uptake kinetics of E6c in L-FABP expressing cells; **E.** The cytoplasmic and nuclear uptake kinetics of D7c in L-FABP expressing cells, F**.** Nucleoplasmic and nuclear envelope uptake kinetics of D7c in L-FABP expressing cells. Values represent the mean $\pm$ SE (n=300-500).

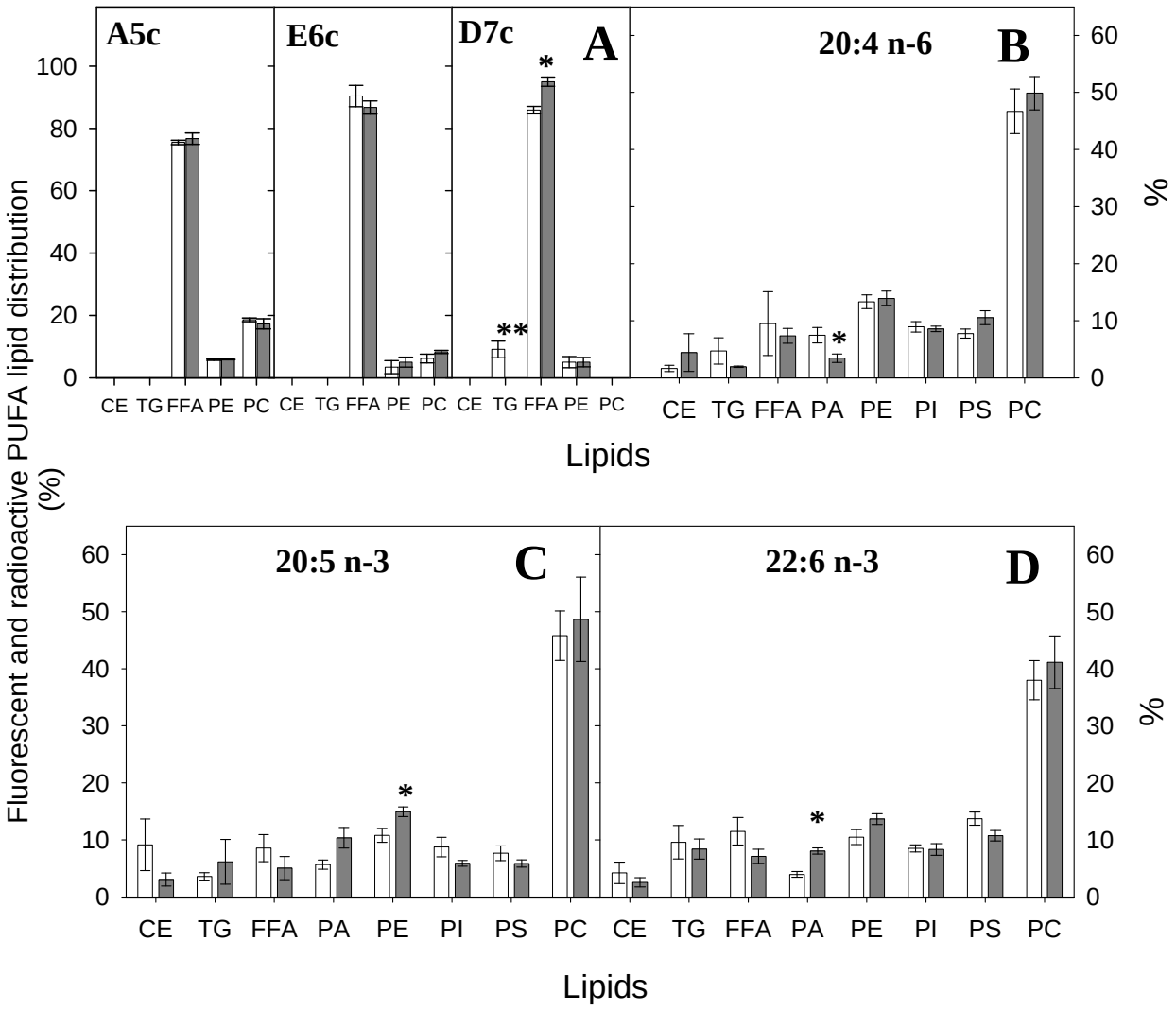
Supplemental Figure 1



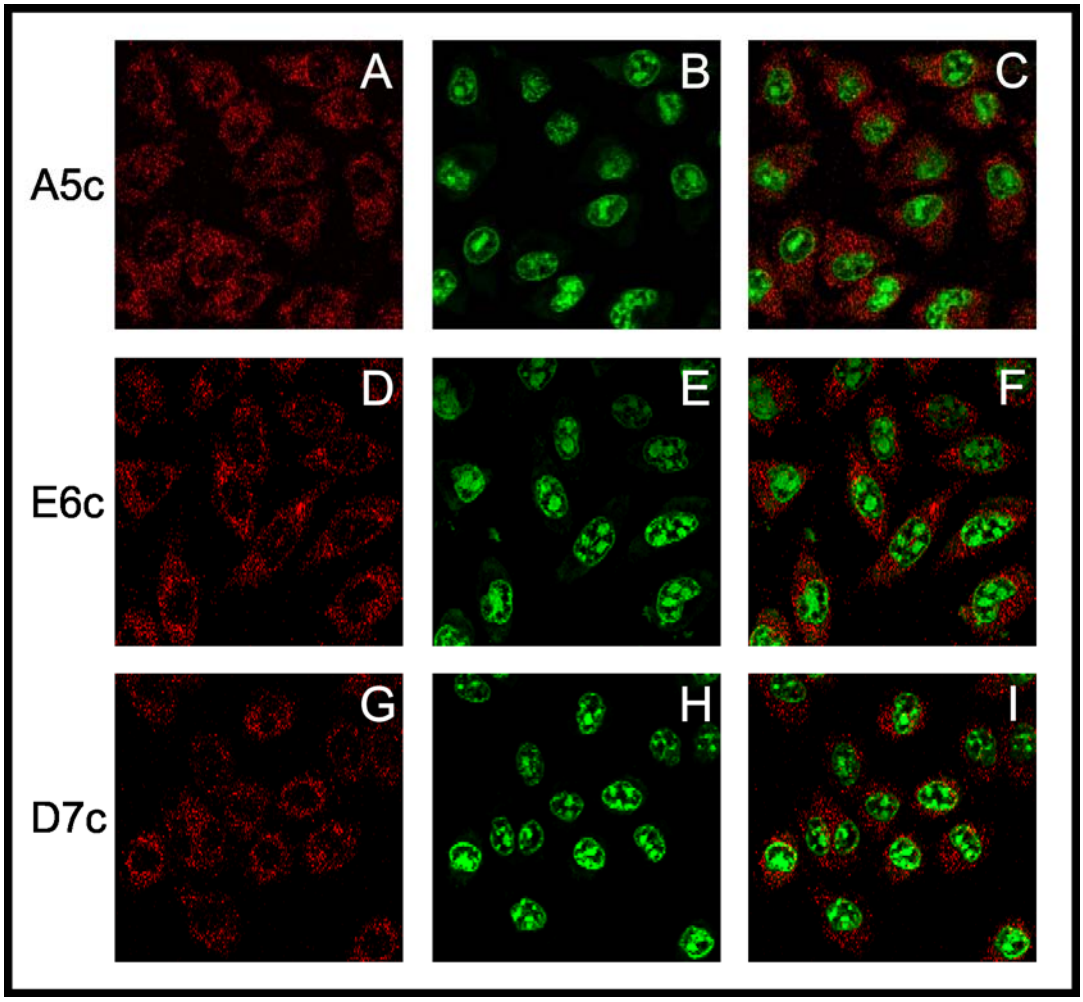
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5