

## **Supplementary information**

### **Supplementary methods**

**Flow cytometry for the simultaneous detection of DNA synthesis and DNA content.** Cells were treated in culture with 10  $\mu$ M EdU for 30 min, harvested and fixed in with 70% EtOH/PBS. For staining cells were washed with PBS and then PBS with 1% BSA and resuspended and incubated in the dark in CLICK reaction mix (PBS containing 10  $\mu$ M 6-carboxyfluorescein TEG-azide, 10 mM sodium-L-ascorbate, 2 mM Copper-II-sulphate) for 30 min at room temperature. Cells were washed with PBS containing 1% BSA and 0.5% Tween-20, and then PBS before resuspension in PBS containing 1% BSA and 1  $\mu$ g/ml DAPI. Data was acquired using a FACS canto II (BD Biosciences) and analysed using FlowJo software.

### **Supplementary Figure S1. MS/MS spectra of TOP2A peptides phosphorylated by CDC7.**

MS/MS spectra of peptidepeptide 1205 TQmAEVLPsPR 1215 (A) and 1517 KPIKYLEEsDEDDLf 1531 (B) from human TOP2A and corresponding tables of fragmented ions.

### **Supplementary Figure S2. Overexpression of DBF4 does not obviously affect cell cycle progression and proliferation.**

(A) DNA content and EdU incorporation analysis by flow cytometry of T-REx-EV or T-REx-DBF4 cells overexpressing DBF4. (B) Growth curves of T-REx-EV and T-REx-DBF4 in the presence of doxycycline generated using Alamar blue cell viability

assay. (C) DNA content and EdU incorporation analysis by FACS of U2OS cells 24 hours after transfection with pAB1 vector or pAB1-DBF4. Cells showing high expression of DBF4 (Strep-positive) were selected and their DNA content and EdU incorporation is shown on the bottom right.

**Supplementary Figure S3. T-REx cells slowly progress through S-phase independently from DBF4 overexpression after release from mimosine cell cycle block.**

DNA content and EdU incorporation analysis flow cytometry of T-REx-EV or T-REx-DBF4 cells overexpressing DBF4 that were arrested with mimosine and released into fresh medium. Samples were taken at the indicated times after release as in Figure 3C. EdU was added to the medium 15 minutes before harvesting the cells.

**Supplementary Figure S4. Centromeric localisation of DBF4 is not affected by XL413.**

U2OS cells transiently transfected with pAB1-DBF4 were treated with the CDC7 inhibitor XL413 or DMSO control for 3 h. Nascent DNA was labeled with a 15 min pulse of 10  $\mu$ M EdU prior to pre-extraction, fixation, and immunofluorescence analysis. EdU containing DNA is shown in grey, DBF4 in green, and centromeres (ACA) in red. Early, mid or late S-phase cells were determined according to the pattern of EdU incorporation. Areas indicated in boxes are magnified next to the respective images.

**Supplementary Figure S5. DBF4 NMC domains are required for centromeric localisation.**

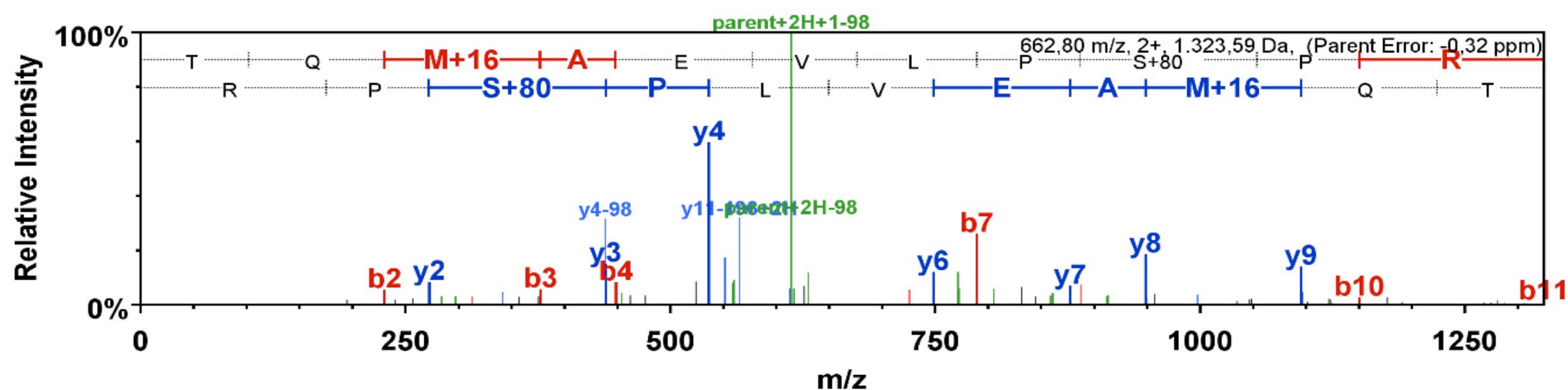
U2OS cells were transfected with expression plasmids for Strep-tagged DBF4 mutants containing deletions in their N (1-209 a.a.), MC (210-350 a.a.) or Tail (T; 351-674 a.a.) domains. Cells were fixed directly with 4% PFA, or salt- and detergent-extracted (HPEM/PFA) prior to fixation and immunofluorescence analysis. DBF4 is shown in green, centromeres (ACA) in red, and DAPI in blue. Images were collected with a (A) 40x or (B) 100x oil immersion objective. No positively stained cells were observed with the DBF4 MCT, NT, or T deletion mutants after pre-extraction.

**Supplementary Figure S6. TOP2A is detected along chromosome arms and centromeres in mitosis with the DRT assay.**

Asynchronously growing U2OS cells were treated with 50 µg/ml ICRF-187 for 15 min prior to pre-extraction and fixation. TOP2A is shown in green. Centromeres were labelled with an anti-centromere antibody (Red; ACA). Scale bar = 10 µm.

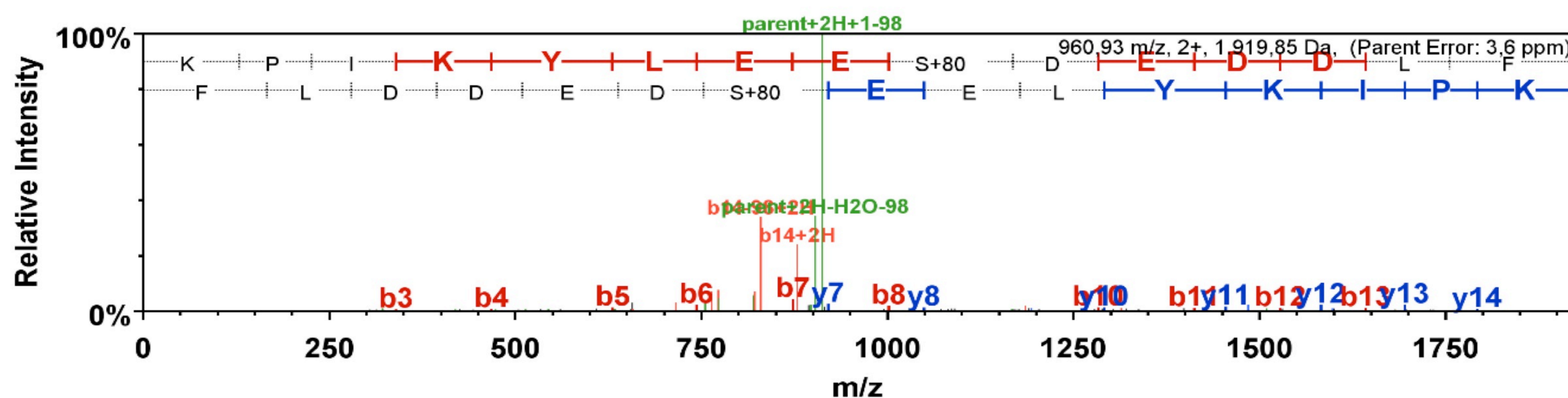


### A) TOP2A - peptide 1205-1215 - (K)TQmAEVLPsPR(G)



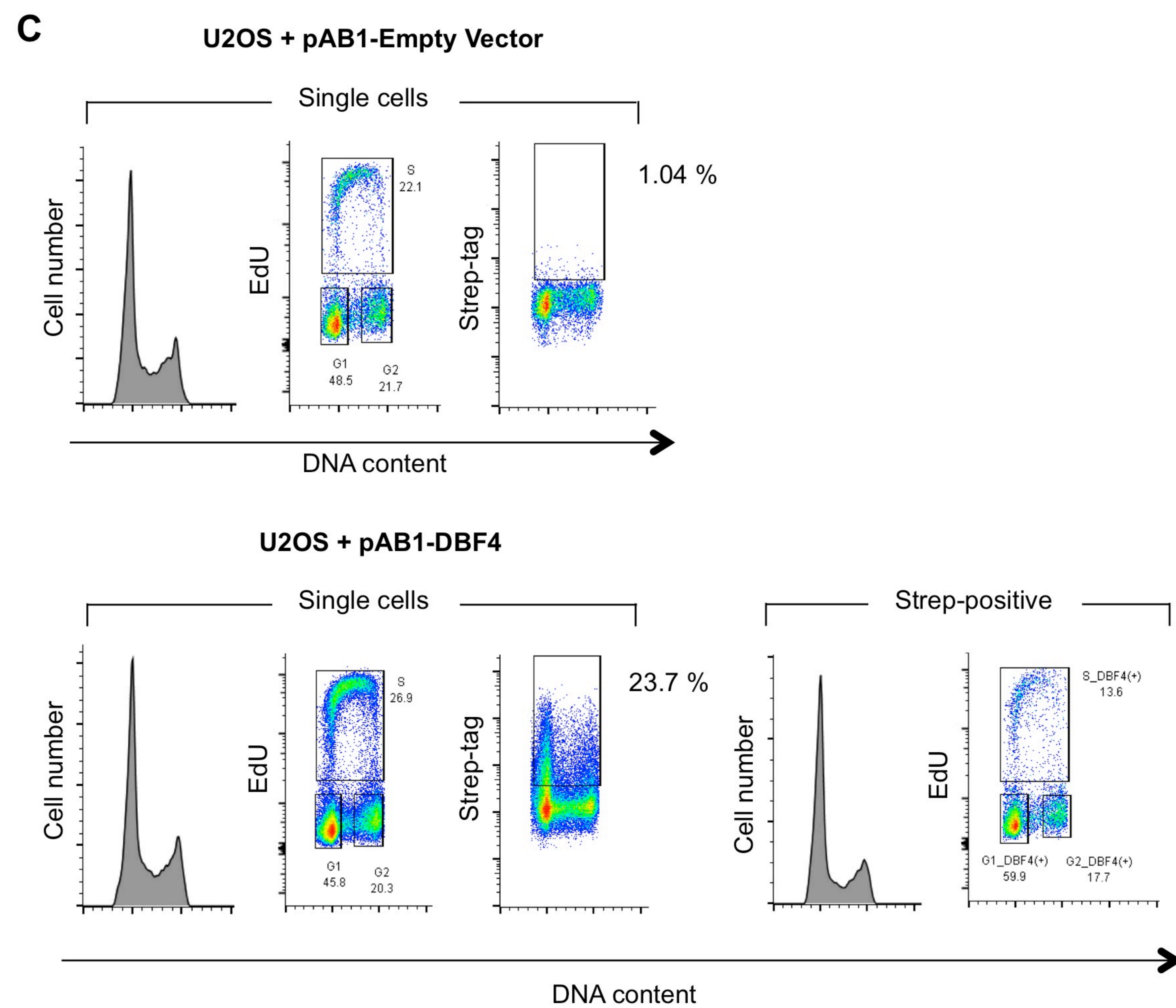
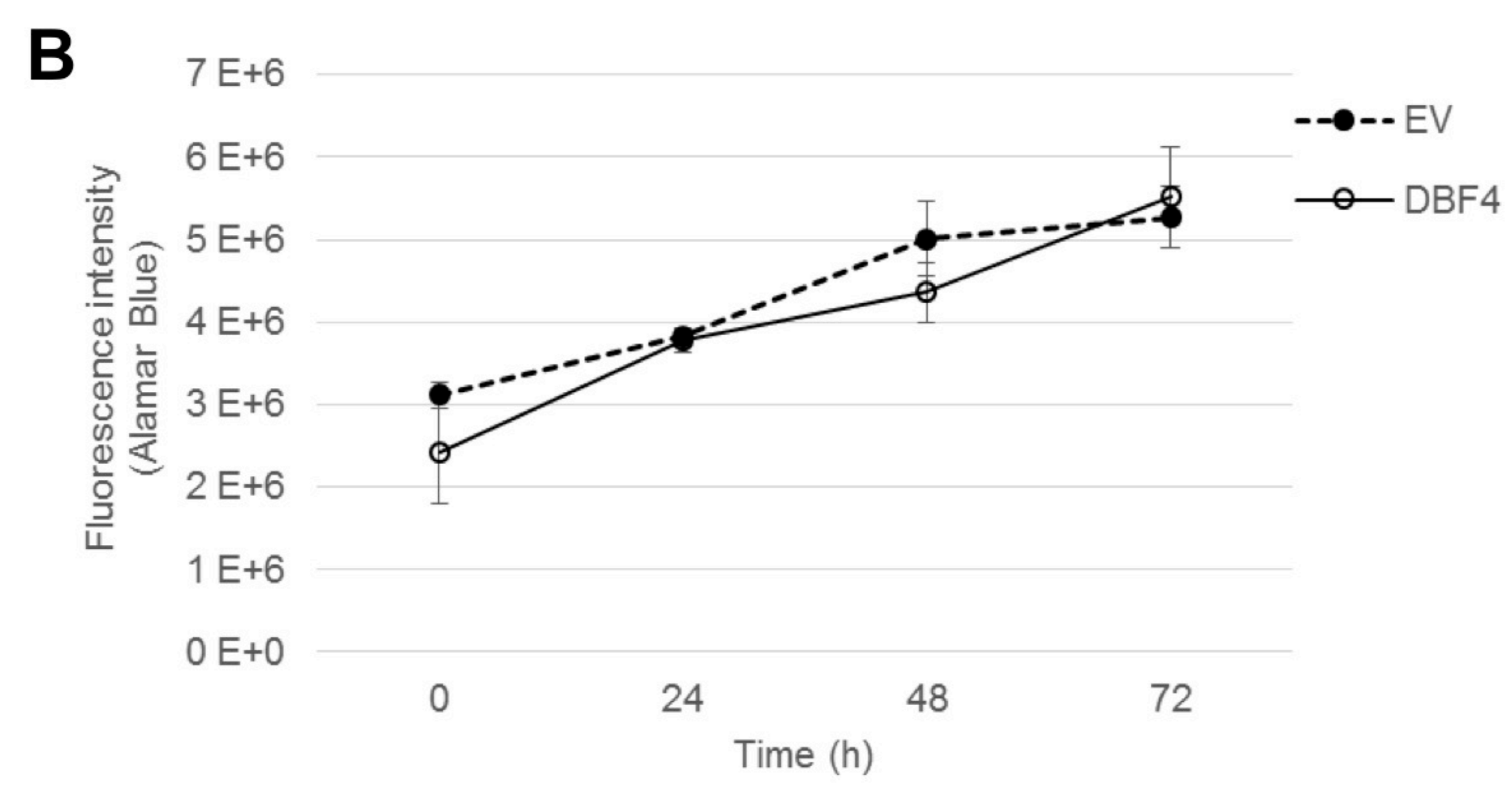
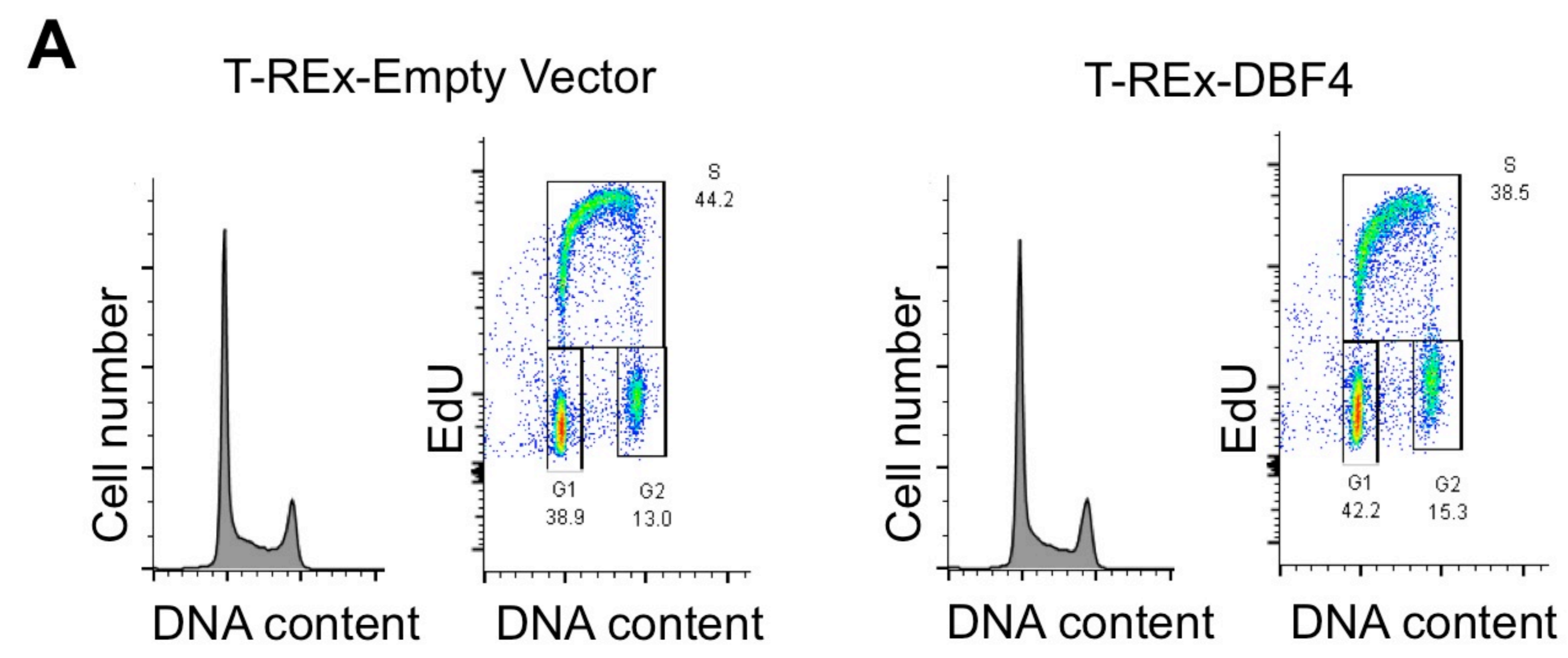
B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	102,1			84,0	T	1.324,6	662,8	1.307,6	1.306,6	11
2	230,1		213,1	212,1	Q	1.223,5	612,3	1.206,5	1.205,5	10
3	377,1		360,1	359,1	M+16	1.095,5	548,2	1.078,5	1.077,5	9
4	448,2		431,2	430,2	A	948,5	474,7	931,4	930,4	8
5	577,2		560,2	559,2	E	877,4	439,2	860,4	859,4	7
6	676,3	338,7	659,3	658,3	V	748,4	374,7	731,3	730,4	6
7	789,4	395,2	772,4	771,4	L	649,3		632,3	631,3	5
8	886,4	443,7	869,4	868,4	P	536,2		519,2	518,2	4
9	1.053,4	527,2	1.036,4	1.035,4	S+80	439,2		422,1	421,2	3
10	1.150,5	575,7	1.133,5	1.132,5	P	272,2		255,1		2
11	1.324,6	662,8	1.307,6	1.306,6	R	175,1		158,1		1

### B) TOP2A - peptide 1517-1531 - (K)KPIKYLEEsDEDDLf(-)

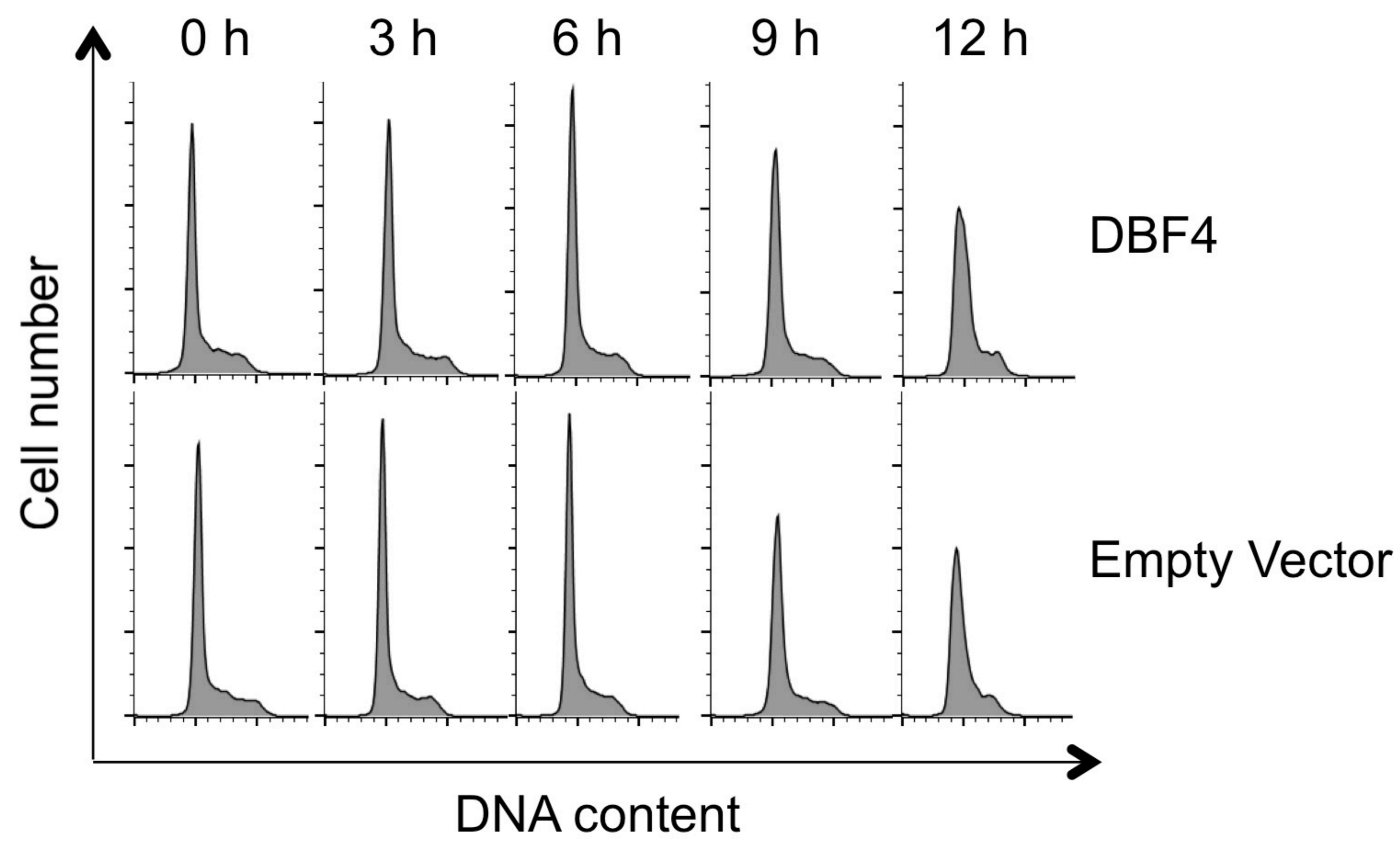
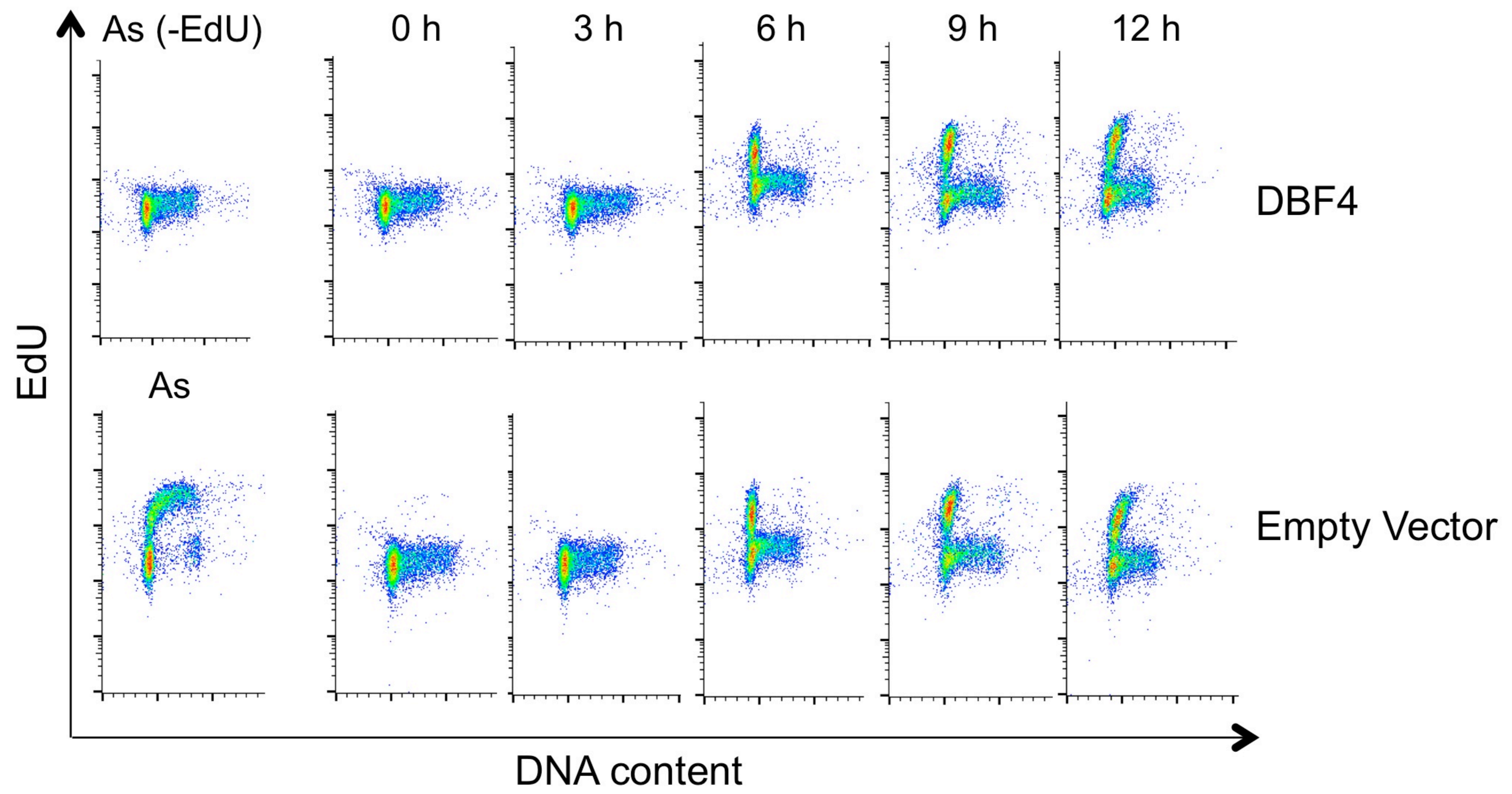


B	B Ions	B+2H	B-NH3	B-H2O	AA	Y Ions	Y+2H	Y-NH3	Y-H2O	Y
1	129,1	65,1	112,1		K	1.920,9	960,9	1.903,8	1.902,8	15
2	226,2	113,6	209,1		P	1.792,8	896,9	1.775,7	1.774,7	14
3	339,2	170,1	322,2		I	1.695,7	848,4	1.678,7	1.677,7	13
4	467,3	234,2	450,3		K	1.582,6	791,8	1.565,6	1.564,6	12
5	630,4	315,7	613,4	612,4	Y	1.454,5			1.436,5	11
6	743,5	372,2	726,5	725,5	L	1.291,5			1.273,5	10
7	872,5	436,8	855,5	854,5	E	1.178,4			1.160,4	9
8	1.001,6	501,3	984,5	983,6	E	1.049,3			1.031,3	8
9	1.168,6	584,8	1.151,5	1.150,6	S+80	920,3			902,3	7
10	1.283,6	642,3	1.266,6	1.265,6	D	753,3			735,3	6
11	1.412,6	706,8	1.395,6	1.394,6	E	638,3			620,3	5
12	1.527,7	764,3	1.510,6	1.509,7	D	509,2			491,2	4
13	1.642,7	821,8	1.625,7	1.624,7	D	394,2			376,2	3
14	1.755,8	878,4	1.738,7	1.737,8	L	279,2				2
15	1.920,9	960,9	1.903,8	1.902,8	F	166,1				1

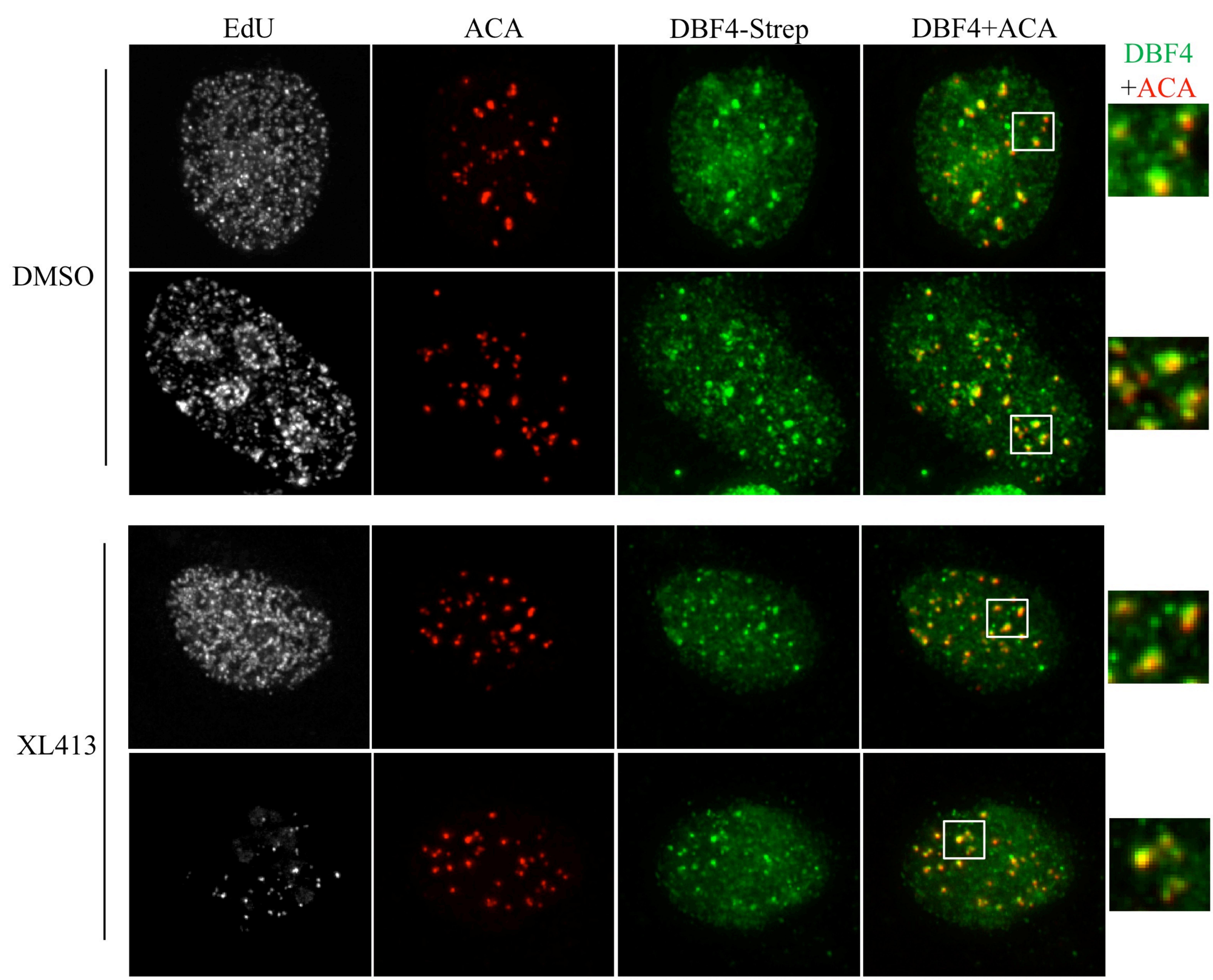






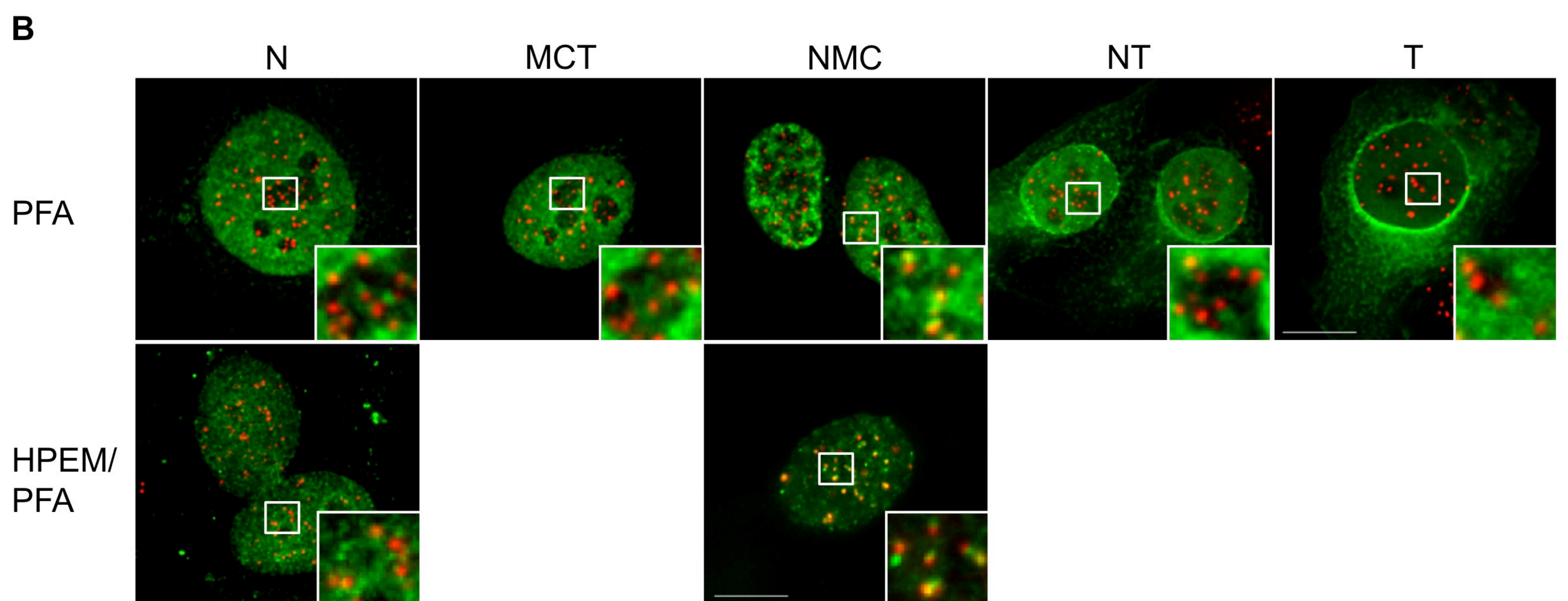
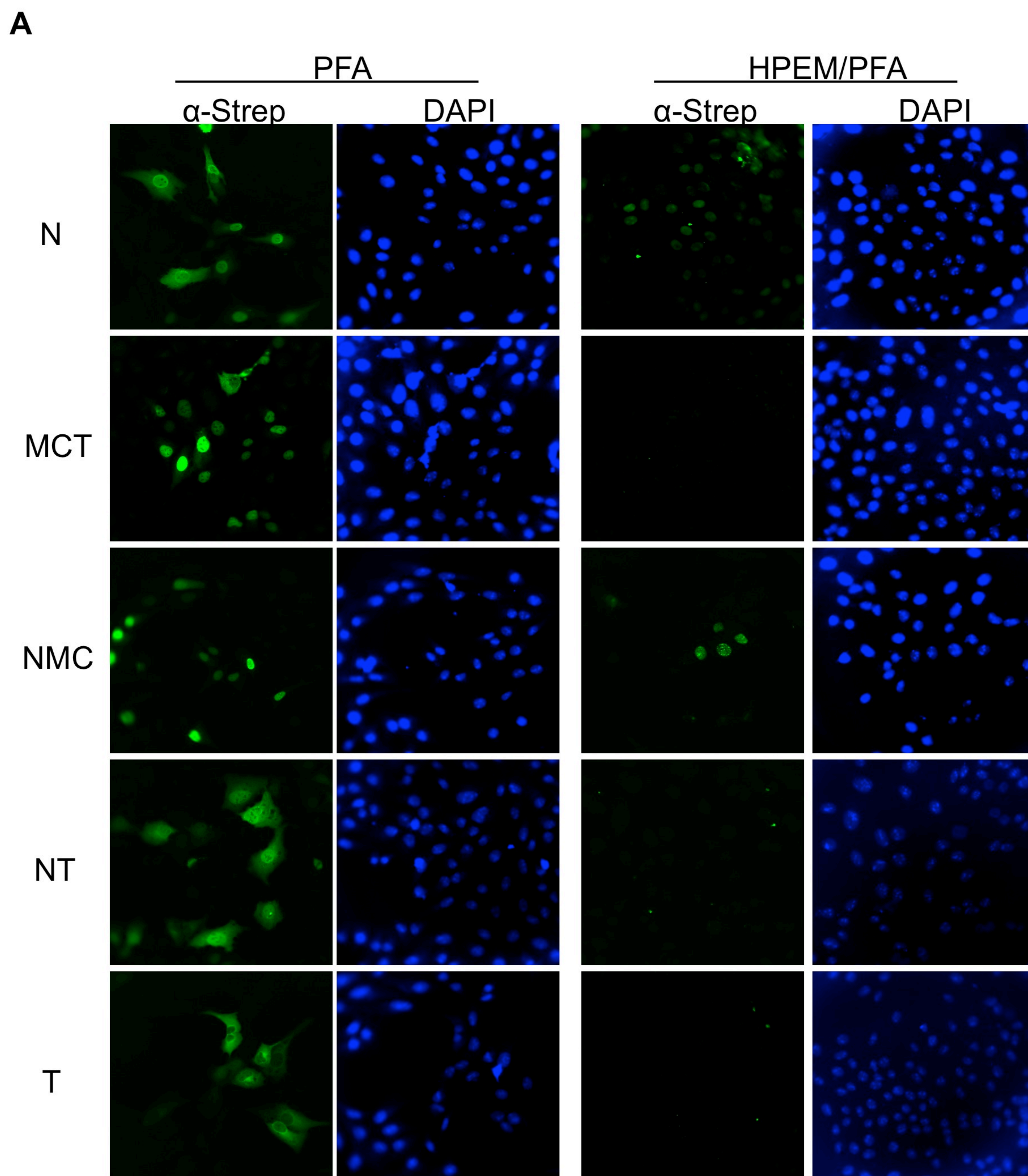
**A****B**





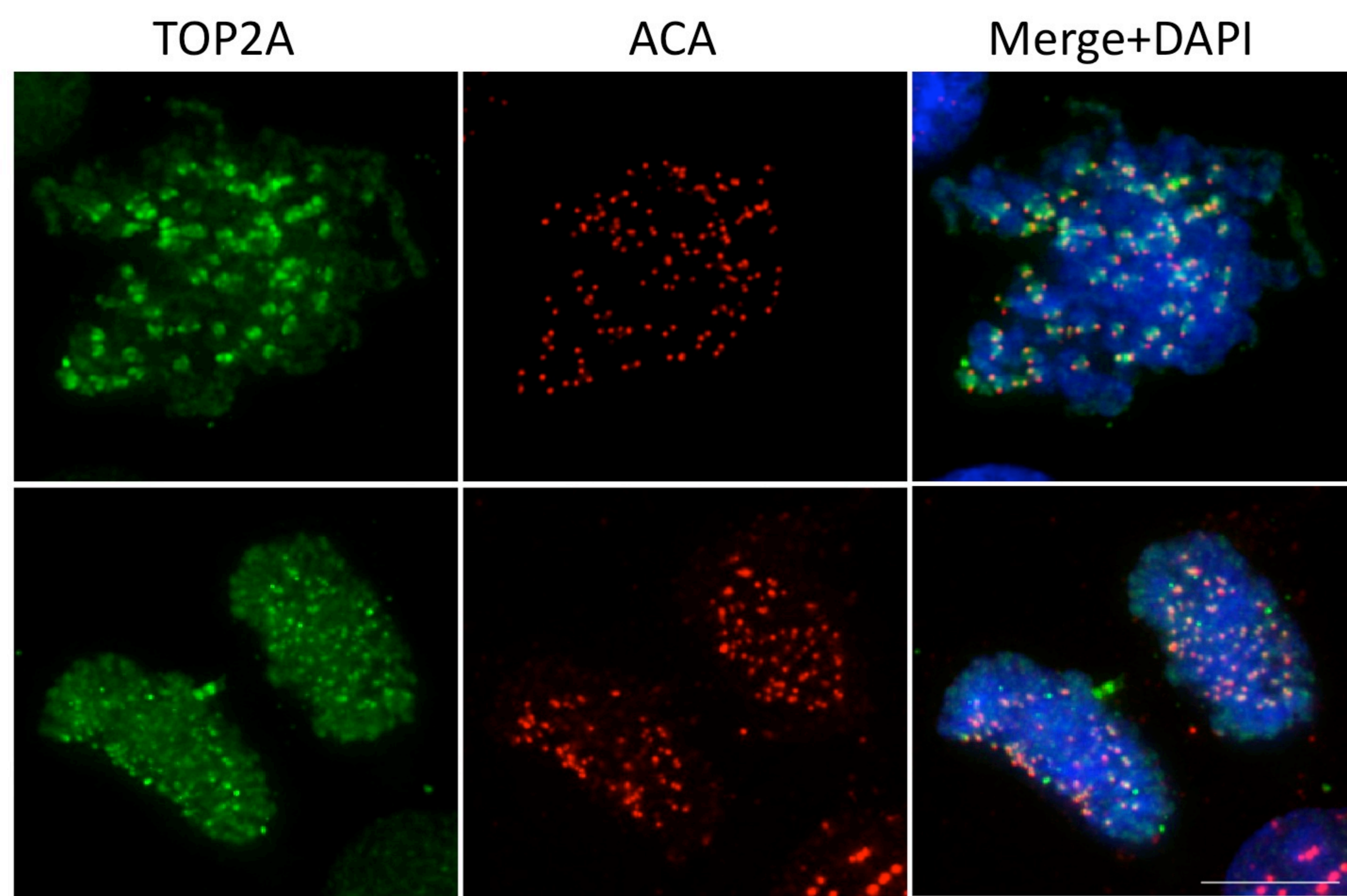
Supplementary Fig. S4





Supplementary Fig. S5





Supplementary Fig. S6