FIGURE 1

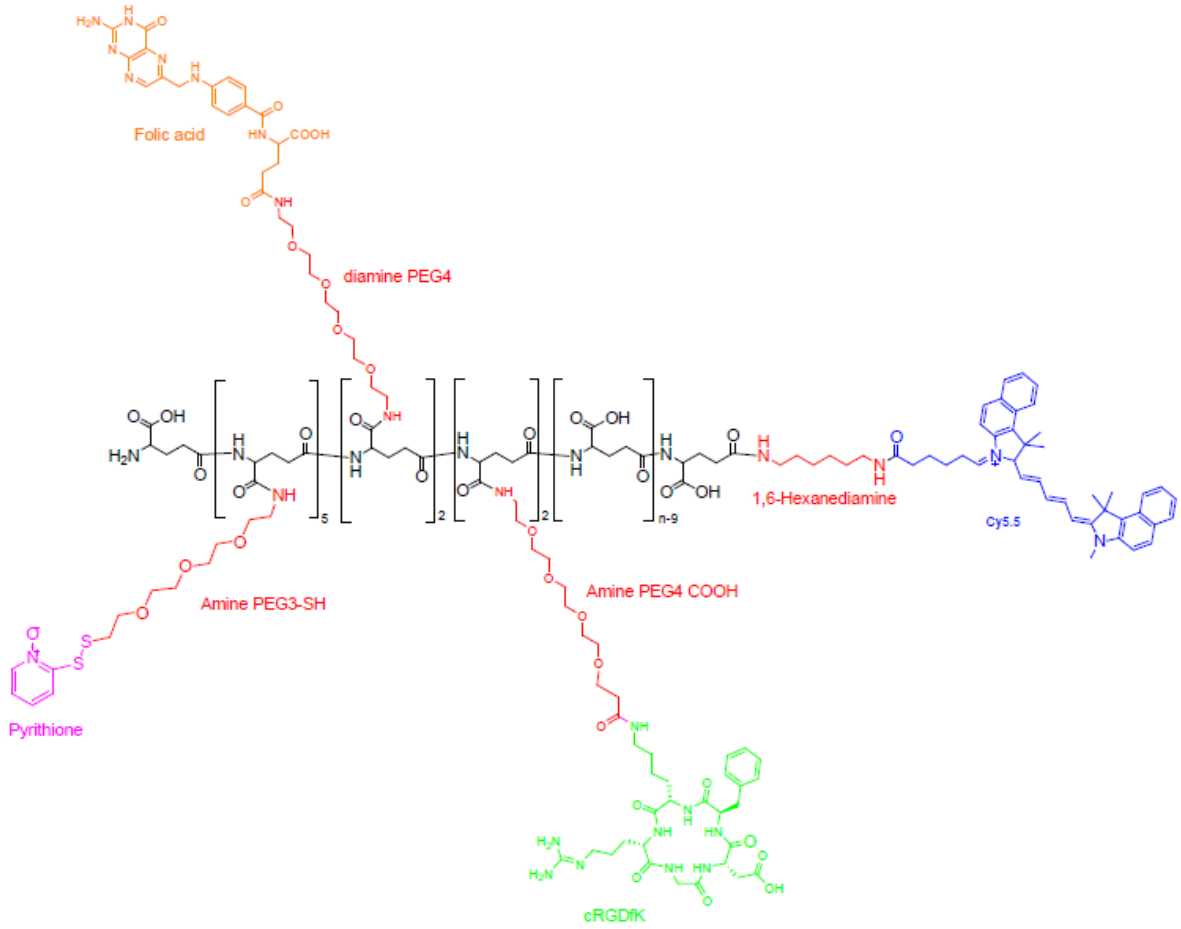

Folic acid
diamine PEG4
1,6-Hexanediamine
Cy5.5
Amine PEG3-SH
Pyrithione
Amine PEG4 COOH
cRGDfK

FIGURE 2

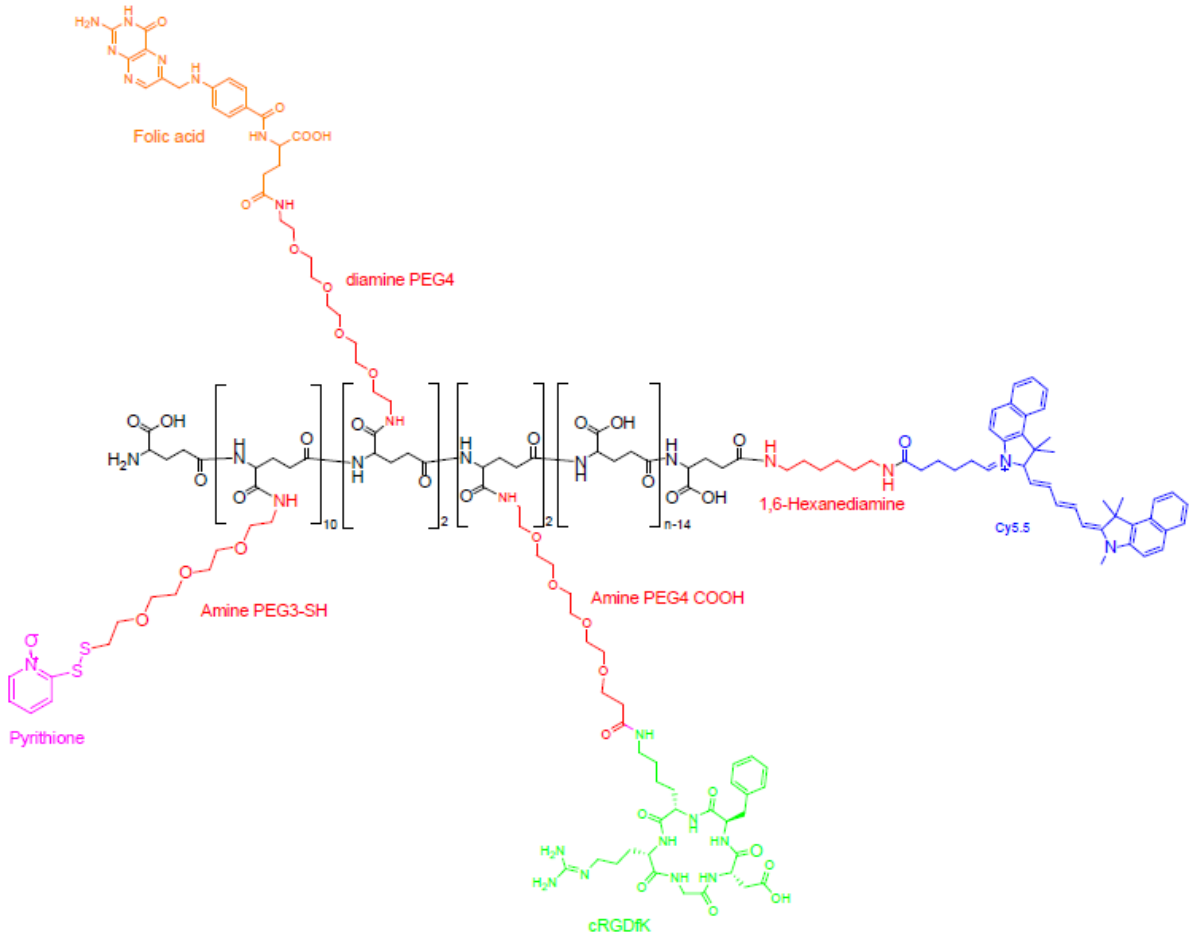

Folic acid
diamine PEG4
Amine PEG3-SH
Pyrithione
Amine PEG4 COOH
cRGDfK
1,6-Hexanediamine
Cy5.5

FIGURE 3

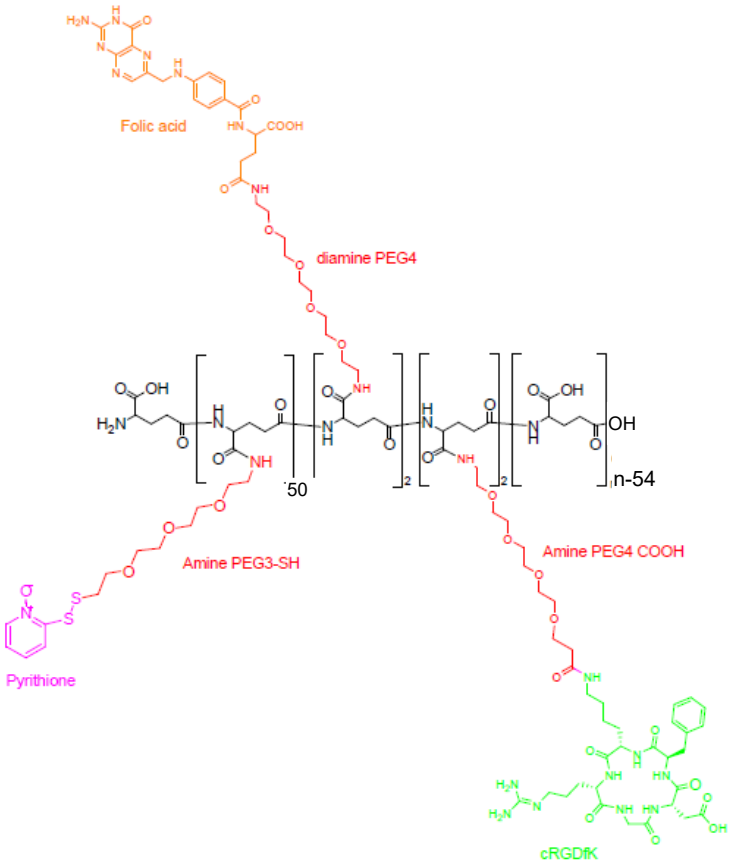

Folic acid
diamine PEG4
Amine PEG3-SH
Pyrithione
Amine PEG4 COOH
cRGDfK
50
n-54

FIGURE 4

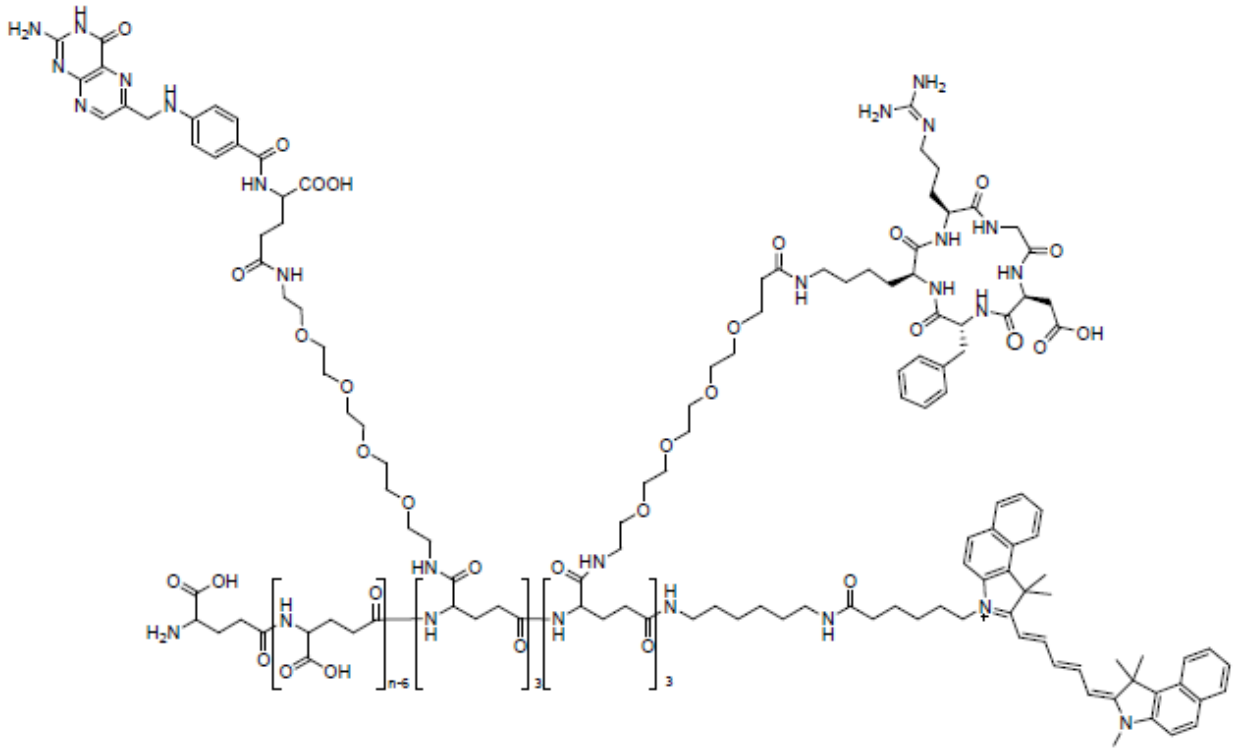


FIGURE 5A

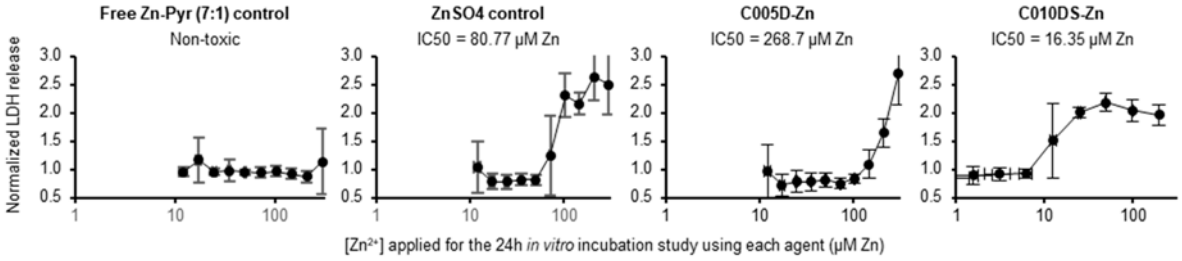


FIGURE 5B

				% Cells marked (Std deviation)			
		Cell marker		Incubation time			
Test Article	[Zn2+]	A5	PI	1.5h	3h	6h	8h
ZnSO4	450 µM	-	-	7 (0.2)	13 (2.0)	16 (1.0)	14 (1.5)
n=3		+	-	2 (0.5)	2 (0.2)	3 (0.7)	5 (0.2)
		-	+	24 (7.3)	18 (3.4)	9 (1.6)	18 (1.6)
		+	+	68 (6.9)	67 (4.6)	72 (2.0)	62 (2.3)
C005D-Zn	600 µM	-	-	72 (6.7)	8 (0.7)	16 (1.4)	13 (1.2)
n=3		+	-	1 (0.3)	4 (0.7)	6 (1.4)	5 (0.7)
		-	+	8 (4.0)	16 (8.6)	8 (1.8)	5 (0.5)
		+	+	19 (3.1)	72 (8.6)	70 (2.7)	77 (1.4)
C010DS-Zn	50 µM	-	-	16 (1.0)	7 (0.5)	5 (1.4)	5 (4.3)
n=3		+	-	4 (1.5)	4 (0.9)	7 (0.9)	6 (0.5)
		-	+	17 (4.1)	12 (1.4)	7 (1.7)	4 (0.4)
		+	+	63 (3.7)	77 (0.7)	81 (2.4)	86 (3.5)
Non-treated	0 µM	-	-	89	89	89	90
n=1		+	-	2	4	3	2
		-	+	4	3	3	3
		+	+	6	4	5	5

15% or greater value highlighted in grey

FIGURE 5C

E

Test Article	Top [Zn2+] Time	Cell marker A5	Cell marker PI	% Cells marked (Std deviation) Treatment strength (% top [Zn2+]) 25%	37.5%	50%	100%
ZnSO4	450 µM	-	-	92 (0.9)	91 (1.2)	93 (1.1)	78 (2.2)
n=3	3h	+	-	0.6 (0.2)	1 (0.2)	0.8 (0.3)	0.3 (0.1)
		-	+	6 (0.8)	5 (1.9)	6 (0.9)	18 (2.2)
		+	+	2 (0.3)	3 (0.5)	0.7 (0.3)	4 (1.1)
C005D-Zn	600 µM	-	-	77 (2.3)	65 (7.6)	23 (6.1)	23 (10.8)
n=3	3h	+	-	2 (0.3)	4 (2.2)	8 (3.7)	8 (1.4)
		-	+	12 (0.8)	14 (1.4)	30 (10.4)	29 (4.3)
		+	+	9 (2.2)	17 (6.6)	39 (12.9)	39 (13.5)
C010DS-Zn	50 µM	-	-	93 (1.0)	81 (1.7)	25 (9.5)	12 (4.3)
n=3	3h	+	-	1 (0.1)	1 (0.2)	6 (6.0)	1 (0.7)
		-	+	5 (0.6)	11 (0.9)	28 (11.4)	30 (5.5)
		+	+	1 (0.3)	7 (1.3)	41 (12.1)	57 (4.5)
Non-treated	0 µM	-	-	94 (1.0)	94 (1.0)	94 (1.0)	94 (1.0)
n=4	3h	+	-	1 (0.2)	1 (0.2)	1 (0.2)	1 (0.2)
		-	+	5 (0.8)	5 (0.8)	5 (0.8)	5 (0.8)
		+	+	0.5 (0.2)	0.5 (0.2)	0.5 (0.2)	0.5 (0.2)

15% or greater value highlighted in grey

FIGURE 5D

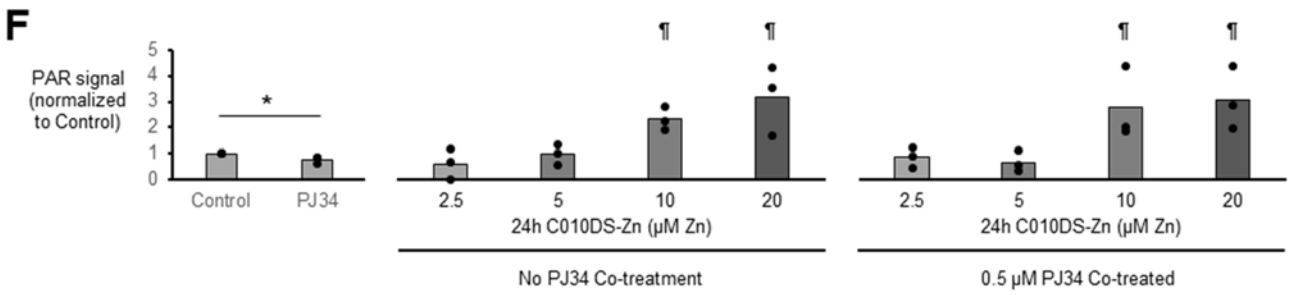


FIGURE 6A

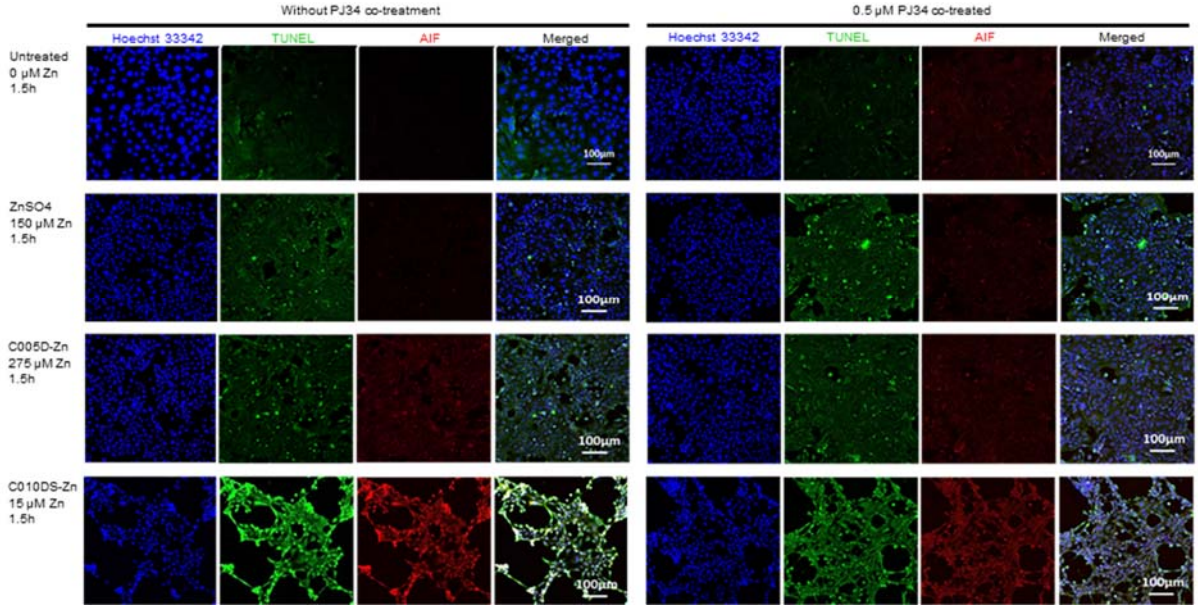


FIGURE 6B

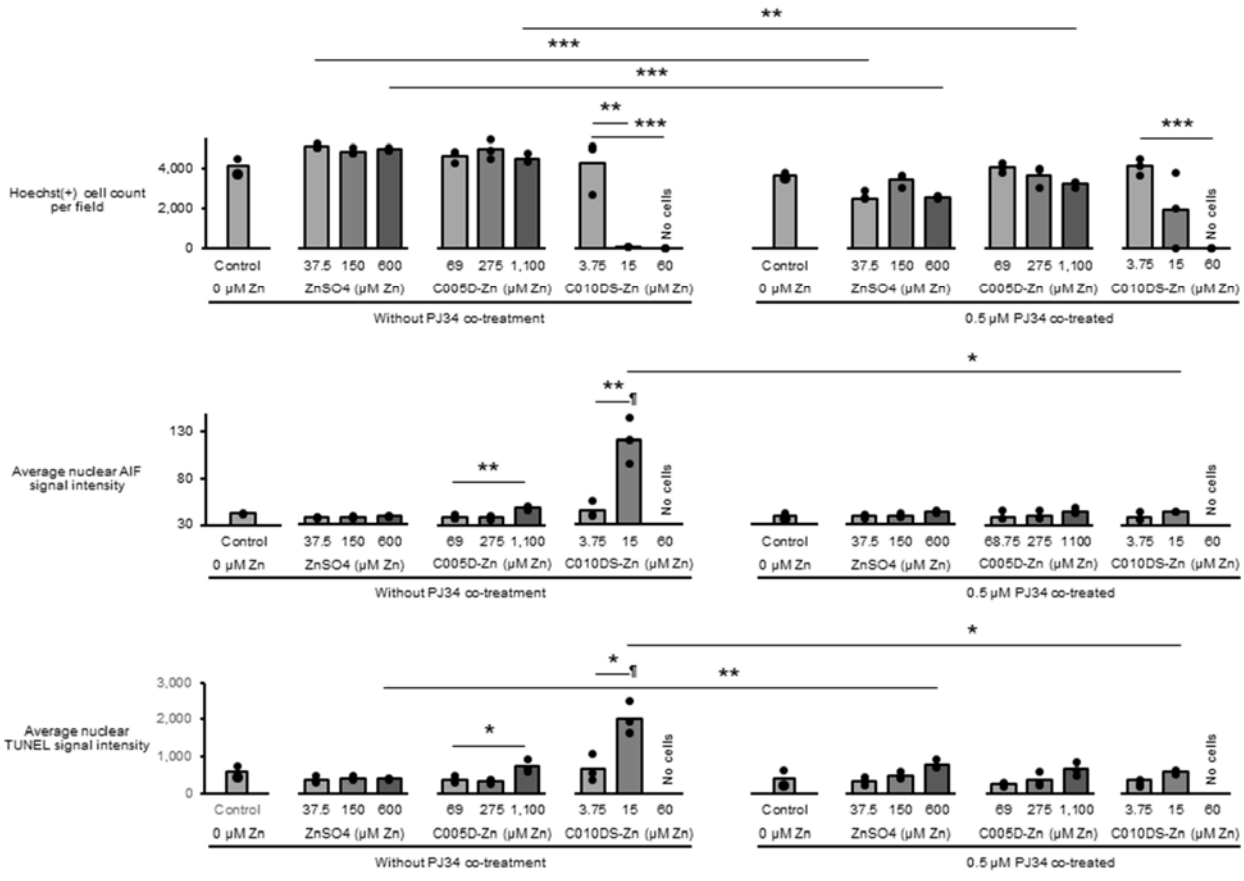


FIGURE 7A

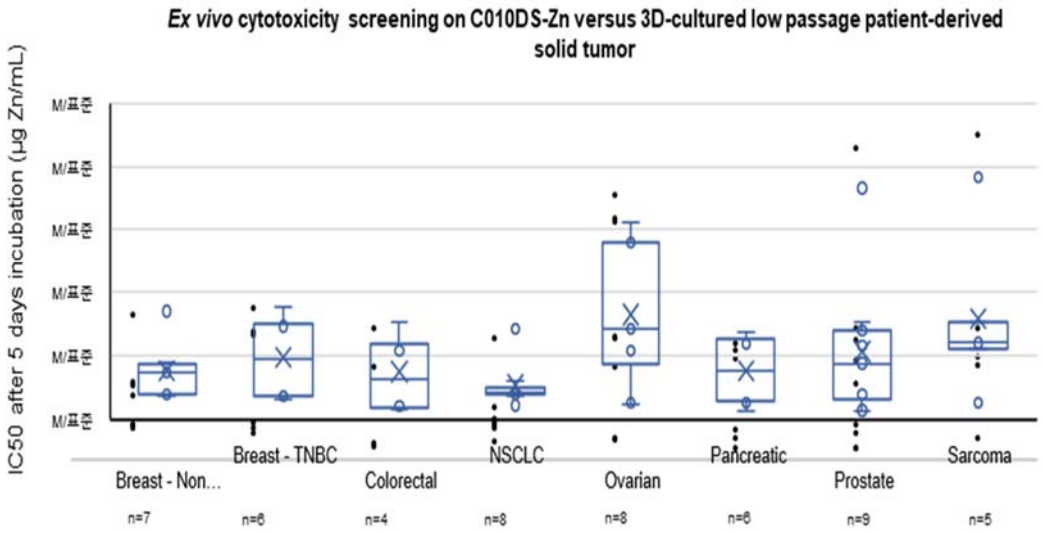


FIGURE 7B

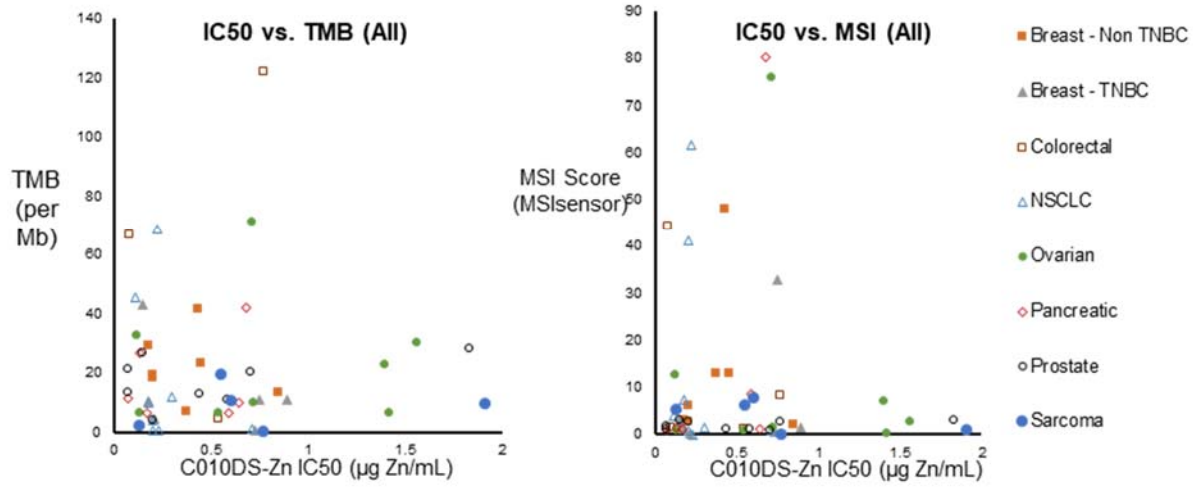
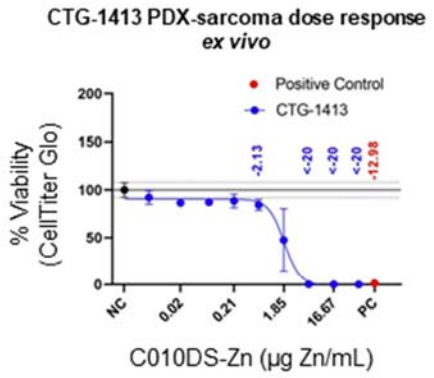


FIGURE 7C



Model ID	Test Agent	IC50 (ug Zn/mL)	R2	Max inhibition (%)
CTG-1413	C010DS-Zn	1.91	0.99	99.91%

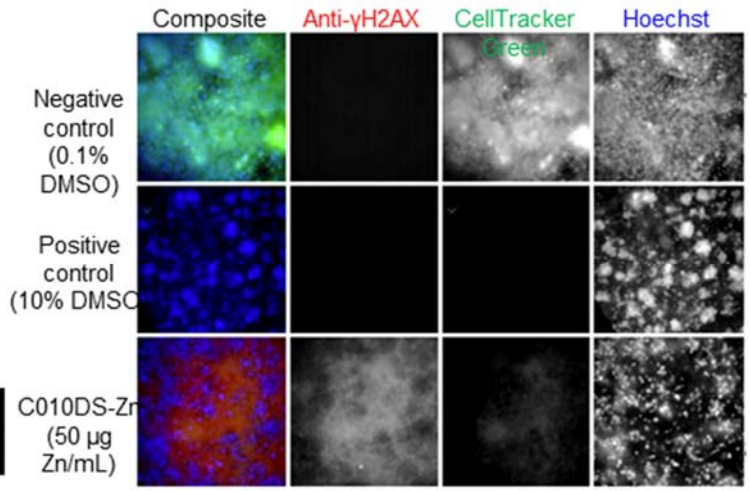


FIGURE 8

Fluorescence imaged anti- γH2AX uptake in the PDX tumor fragments upon ex vivo treatment with 10% DMSO (PC) or C010DS-Zn at 50 µg Zn/mL for 5 days



FIGURE 9A

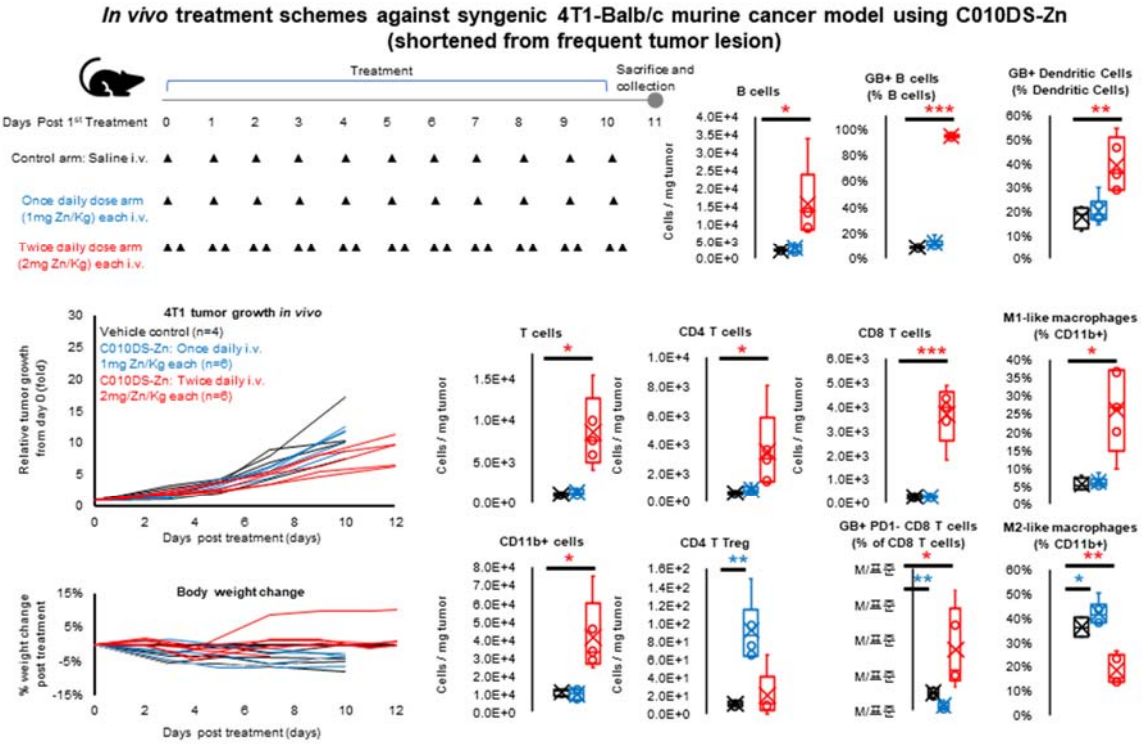

In vivo treatment schemes against syngenic 4T1-Balb/c murine cancer model using C010DS-Zn (shortened from frequent tumor lesion)
Treatment
Sacrifice and collection
Days Post 1st Treatment
Control arm: Saline i.v.
Once daily dose arm (1mg Zn/Kg) each i.v.
Twice daily dose arm (2mg Zn/Kg) each i.v.
B cells
GB+ B cells (% B cells)
GB+ Dendritic Cells (% Dendritic Cells)
Cells / mg tumor
4T1 tumor growth *in vivo*
Vehicle control (n=4)
C010DS-Zn: Once daily i.v. 1mg Zn/Kg each (n=6)
C010DS-Zn: Twice daily i.v. 2mg/Zn/Kg each (n=6)
Relative tumor growth from day 0 (fold)
Days post treatment (days)
Body weight change
% weight change post treatment
T cells
CD4 T cells
CD8 T cells
M1-like macrophages (% CD11b+)
CD11b+ cells
CD4 T Treg
GB+ PD1- CD8 T cells (% of CD8 T cells)
M2-like macrophages (% CD11b+)

FIGURE 9B

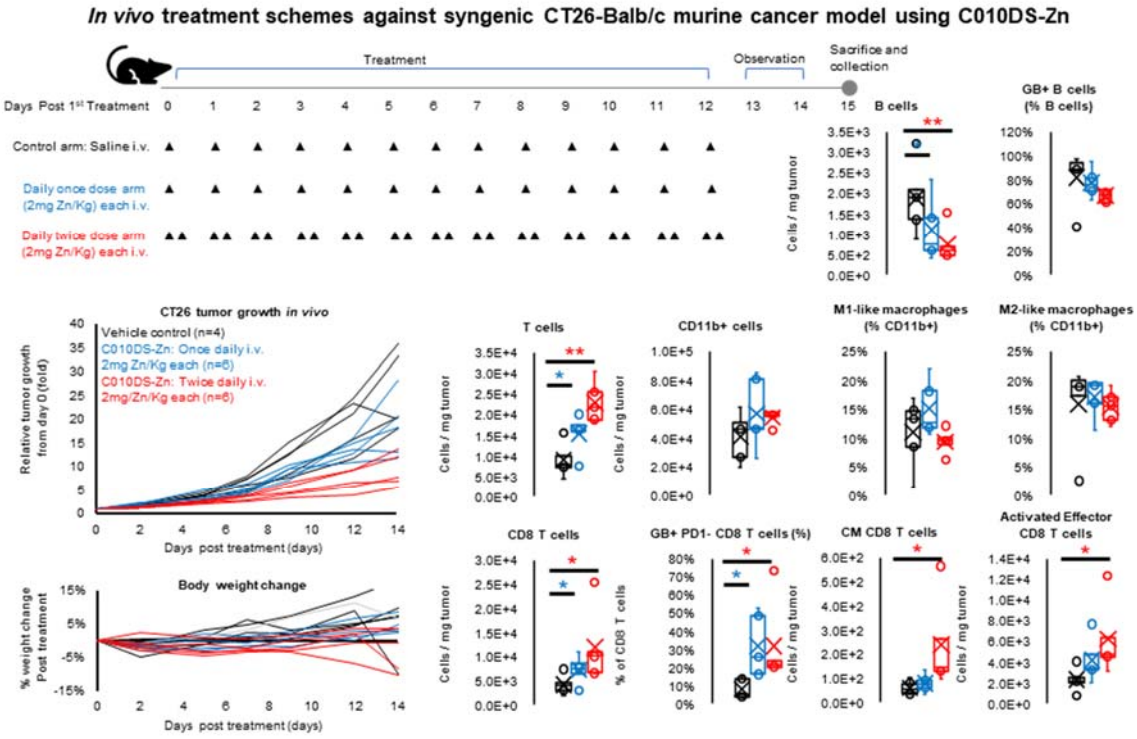
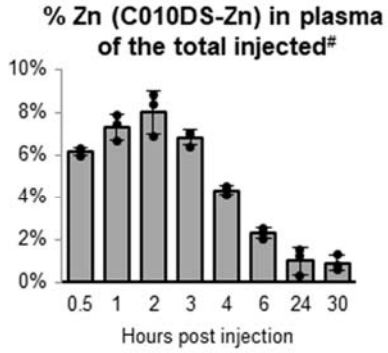

In vivo treatment schemes against syngenic CT26-Balb/c murine cancer model using C010DS-Zn
Treatment
Observation
Sacrifice and collection
Days Post 1st Treatment
Control arm: Saline i.v.
Daily once dose arm (2mg Zn/Kg) each i.v.
Daily twice dose arm (2mg Zn/Kg) each i.v.
B cells
GB+ B cells (% B cells)
CT26 tumor growth *in vivo*
Vehicle control (n=4)
C010DS-Zn: Once daily i.v. 2mg Zn/Kg each (n=6)
C010DS-Zn: Twice daily i.v. 2mg/Zn/Kg each (n=6)
Relative tumor growth from day 0 (fold)
Days post treatment (days)
Body weight change
% weight change Post treatment
T cells
CD11b+ cells
M1-like macrophages (% CD11b+)
M2-like macrophages (% CD11b+)
CD8 T cells
GB+ PD1- CD8 T cells (%)
CM CD8 T cells
Activated Effector CD8 T cells
Cells / mg tumor
% of CD8 T cells

FIGURE 10A

C010DS-Zn single-bolus i.v. PK parameters	Observed in-plasma values		
	C010DS (Cy5.5)	Zn (total)	Zn (total - background)
Injected dose (mg/Kg)	25.000	2.500	2.500
T1/2(h)	2.250	47.108	19.021
Tmax(h)	0.500	2.000	2.000
Cmax (µg/mL)	476.965	6.624	5.073
AUClast (h*µg/mL)	1389.528	89.459	42.926



#Value calculated based on the rat plasma volume assumption of 39.6 mL/Kg by Gillen et al.

FIGURE 10B

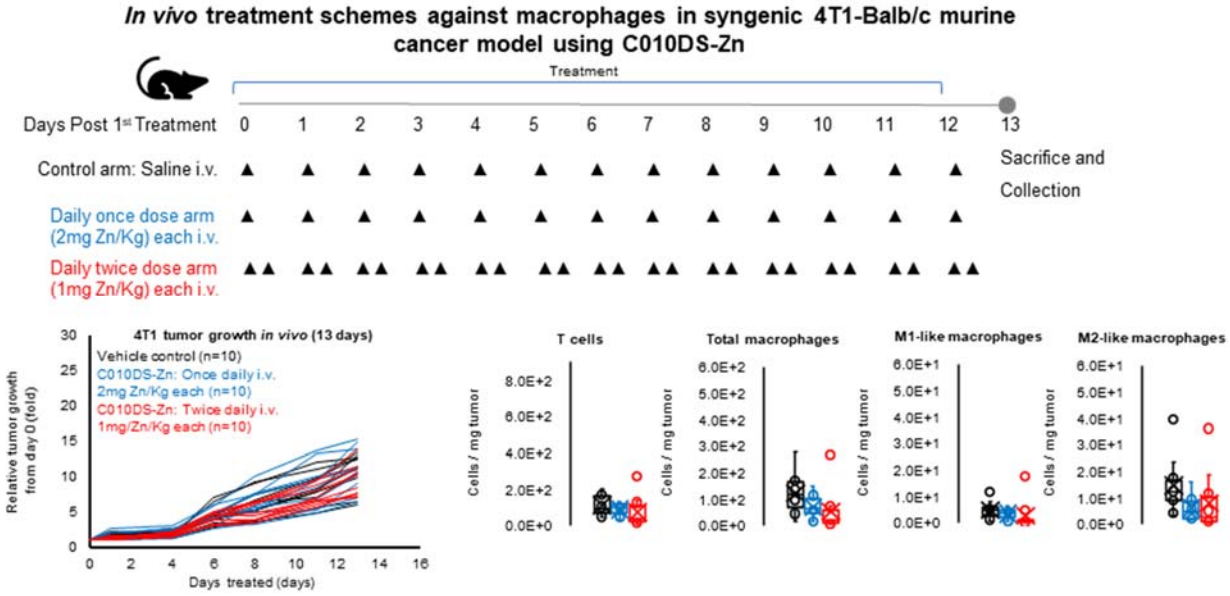
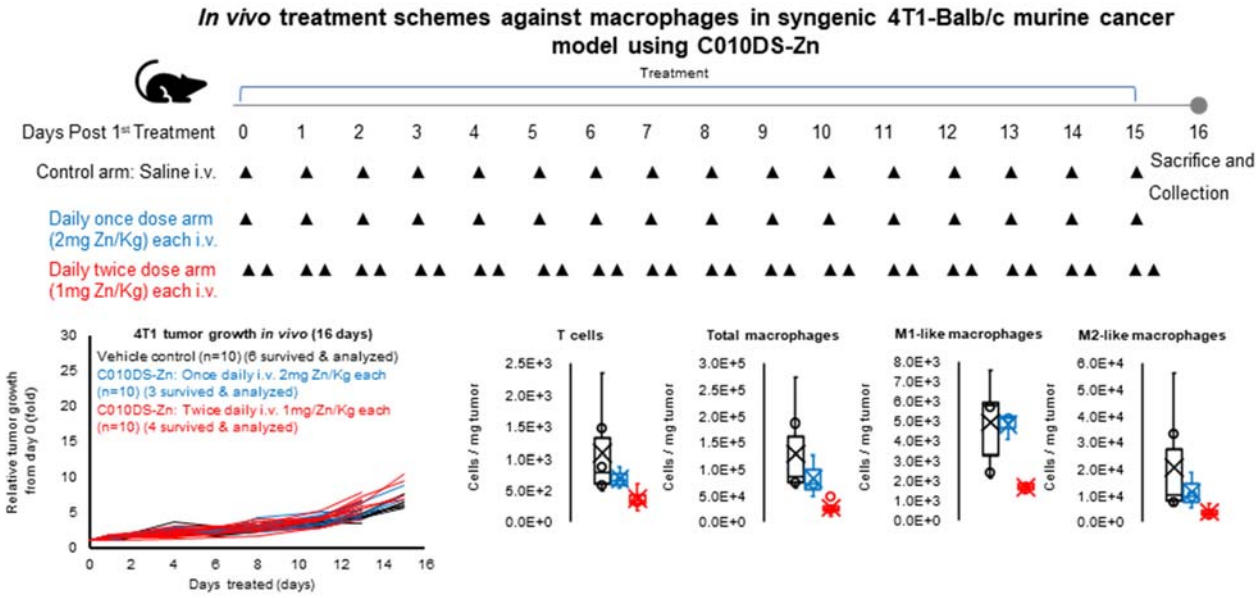


FIGURE 10C


In vivo treatment schemes against macrophages in syngenic 4T1-Balb/c murine cancer model using C010DS-Zn
Treatment
Days Post 1st Treatment
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Control arm: Saline i.v.
Sacrifice and Collection
Daily once dose arm (2mg Zn/Kg) each i.v.
Daily twice dose arm (1mg Zn/Kg) each i.v.
4T1 tumor growth *in vivo* (16 days)
Vehicle control (n=10) (6 survived & analyzed)
C010DS-Zn: Once daily i.v. 2mg Zn/Kg each (n=10) (3 survived & analyzed)
C010DS-Zn: Twice daily i.v. 1mg/Zn/Kg each (n=10) (4 survived & analyzed)
Relative tumor growth from day 0 (fold)
Days treated (days)
T cells
Total macrophages
M1-like macrophages
M2-like macrophages
Cells / mg tumor