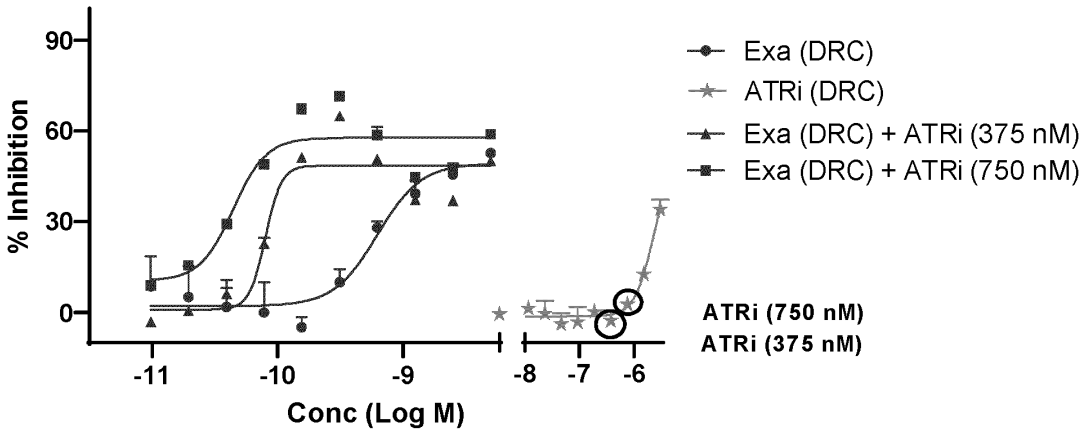

90
60
30
0
% Inhibition
-11
-10
-9
-8
-7
-6
Conc (Log M)
Exa (DRC)
ATRi (DRC)
Exa (DRC) + ATRi (375 nM)
Exa (DRC) + ATRi (750 nM)
ATRi (750 nM)
ATRi (375 nM)

Fig. 1A

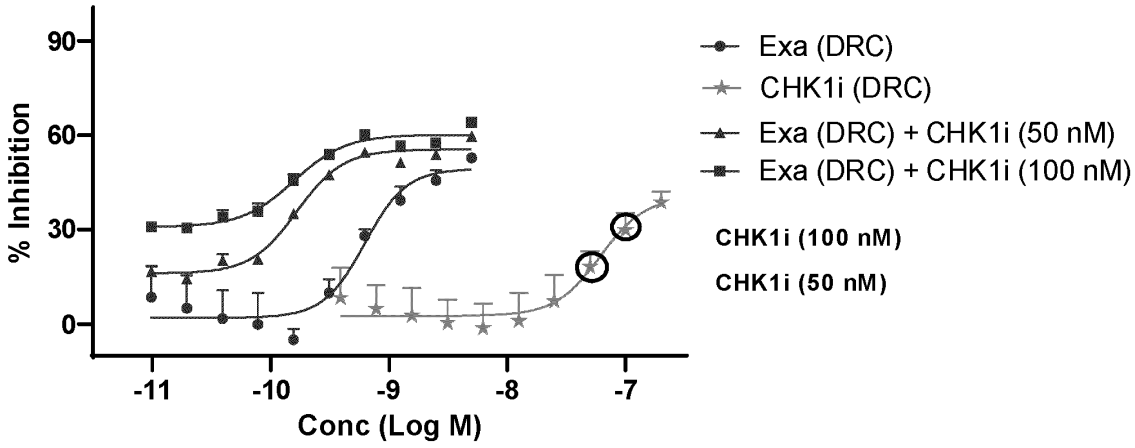

90
60
30
0
% Inhibition
-11
-10
-9
-8
-7
Conc (Log M)
Exa (DRC)
CHK1i (DRC)
Exa (DRC) + CHK1i (50 nM)
Exa (DRC) + CHK1i (100 nM)
CHK1i (100 nM)
CHK1i (50 nM)

Fig. 1B

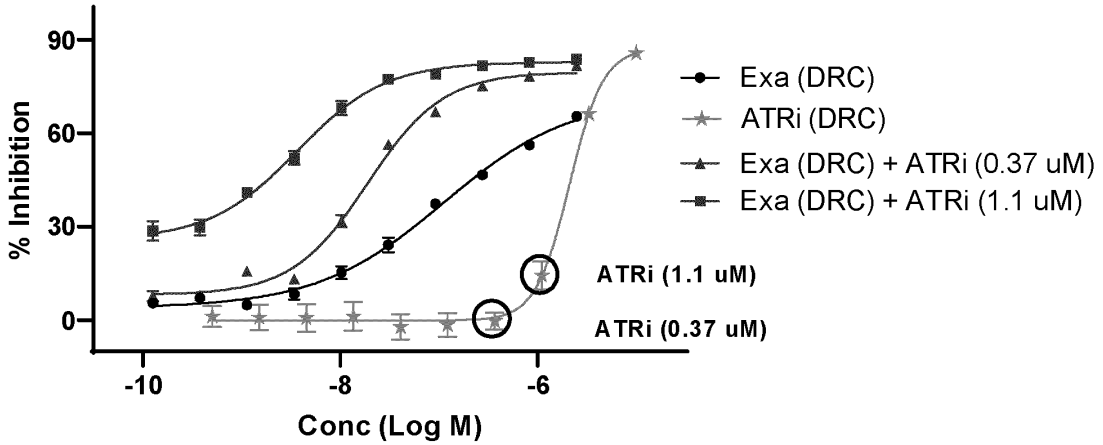

90
60
30
0
% Inhibition
-10
-8
-6
Conc (Log M)
Exa (DRC)
ATRi (DRC)
Exa (DRC) + ATRi (0.37 uM)
Exa (DRC) + ATRi (1.1 uM)
ATRi (1.1 uM)
ATRi (0.37 uM)

Fig. 1C

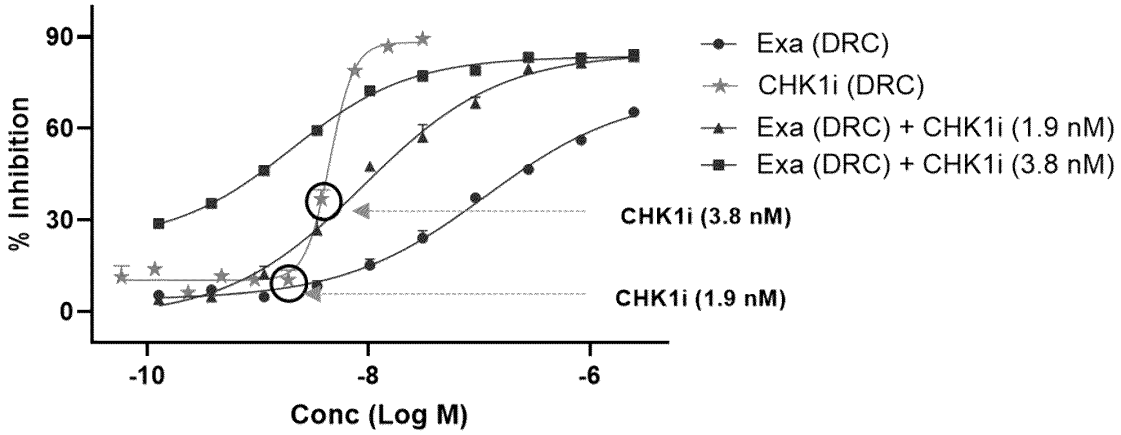

90
60
30
0
% Inhibition
-10
-8
-6
Conc (Log M)
Exa (DRC)
CHK1i (DRC)
Exa (DRC) + CHK1i (1.9 nM)
Exa (DRC) + CHK1i (3.8 nM)
CHK1i (3.8 nM)
CHK1i (1.9 nM)

Fig. 1D

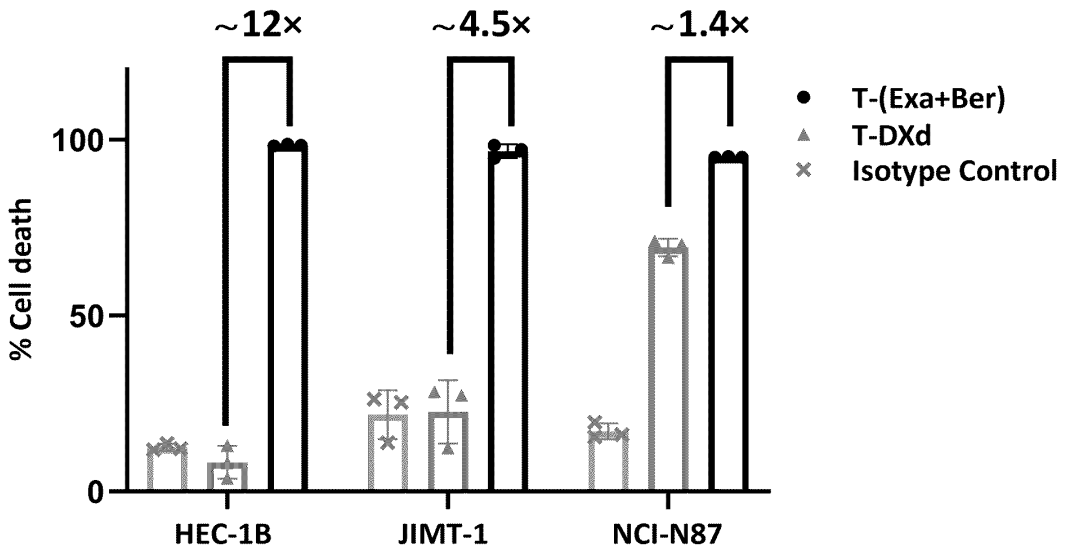

~12×
~4.5×
~1.4×
% Cell death
100
50
0
HEC-1B
JIMT-1
NCI-N87
T-(Exa+Ber)
T-DXd
Isotype Control

Fig. 2

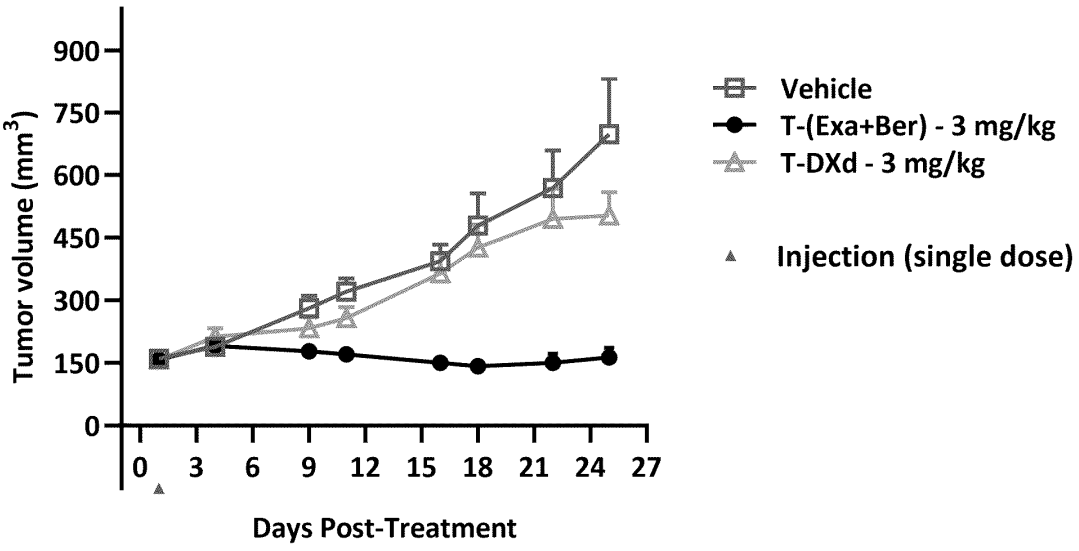

Tumor volume (mm3)
900
750
600
450
300
150
0
0 3 6 9 12 15 18 21 24 27
Days Post-Treatment
Vehicle
T-(Exa+Ber) - 3 mg/kg
T-DXd - 3 mg/kg
Injection (single dose)

Fig. 3

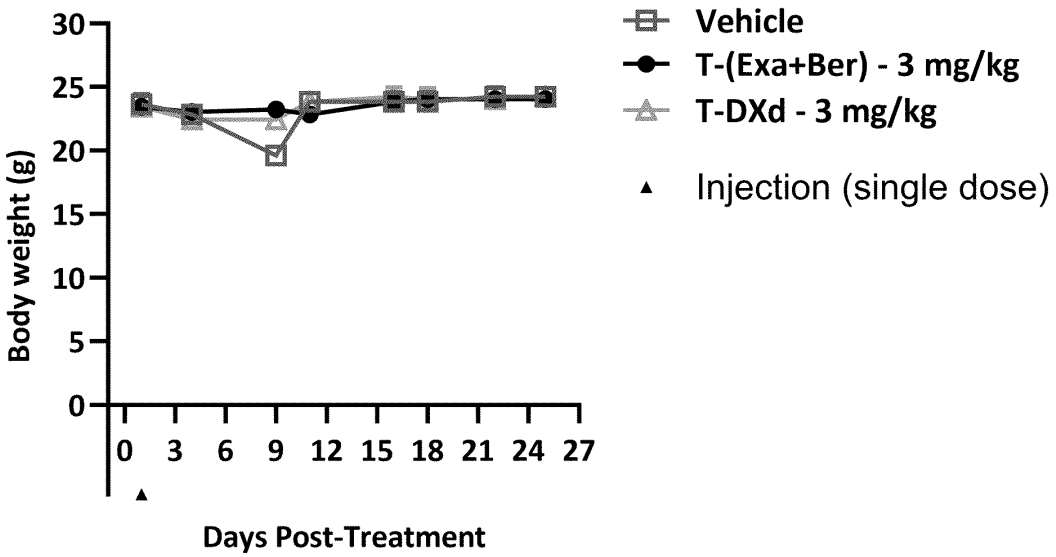

Vehicle
T-(Exa+Ber) - 3 mg/kg
T-DXd - 3 mg/kg
Injection (single dose)
Body weight (g)
30
25
20
15
10
5
0
0 3 6 9 12 15 18 21 24 27
Days Post-Treatment

Fig. 4

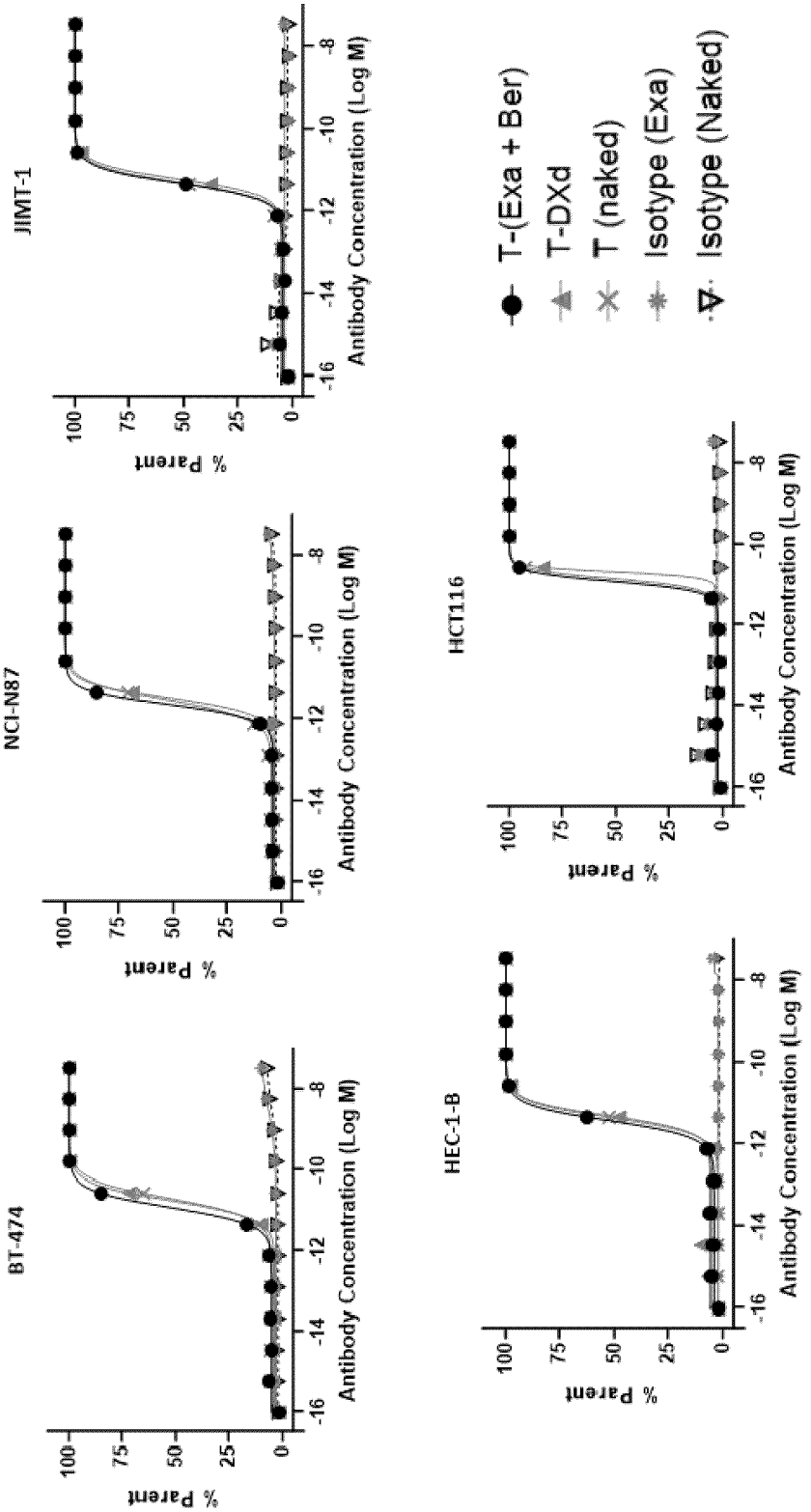

JIMT-1
NCI-N87
BT-474
HCT116
HEC-1-B
% Parent
Antibody Concentration (Log M)
T-(Exa + Ber)
T-DXd
T (naked)
Isotype (Exa)
Isotype (Naked)

Fig. 5A

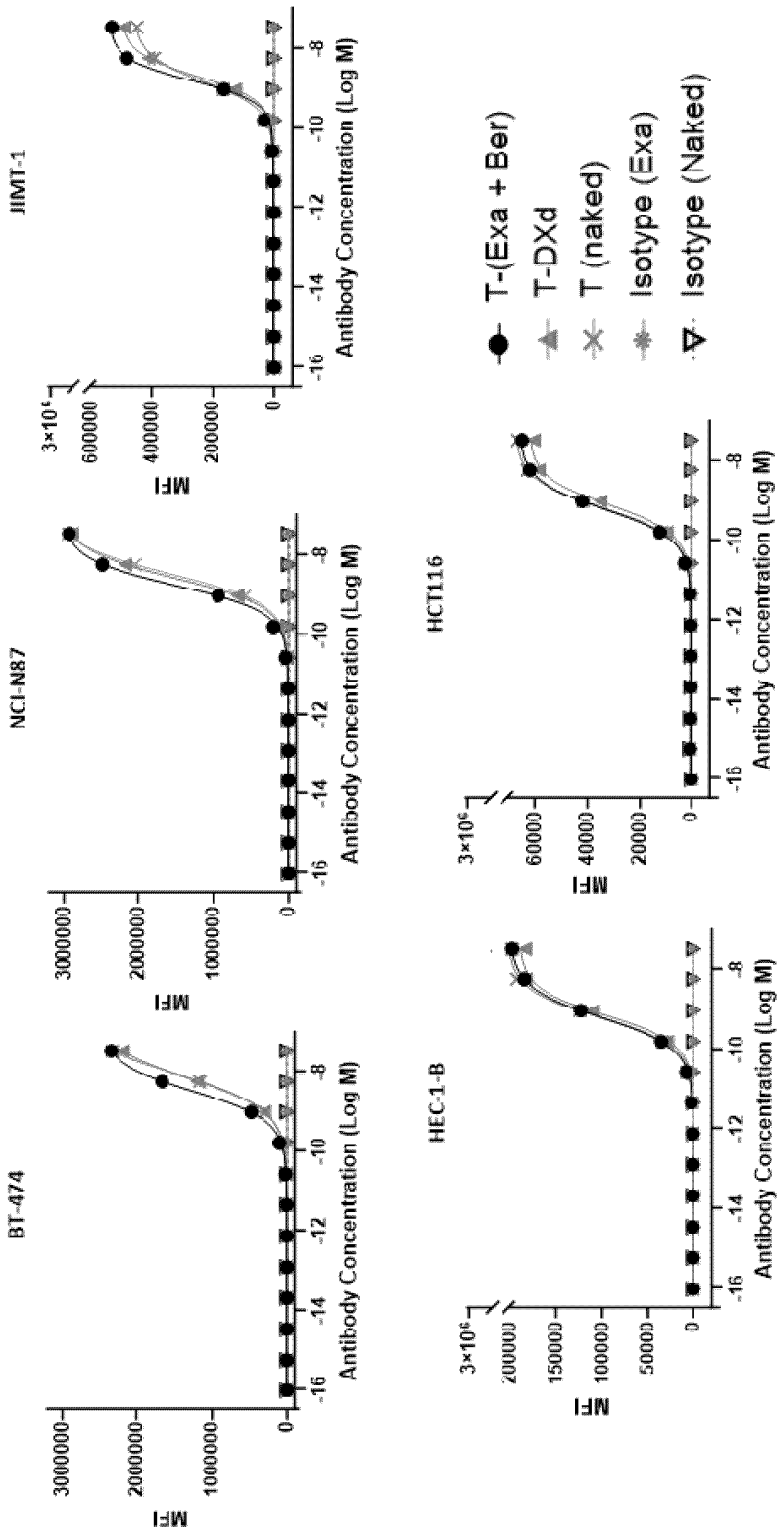

JIMT-1
NCI-N87
BT-474
HCT116
HEC-1-B
MFI
Antibody Concentration (Log M)
T-(Exa + Ber)
T-DXd
T (naked)
Isotype (Exa)
Isotype (Naked)

Fig. 5B

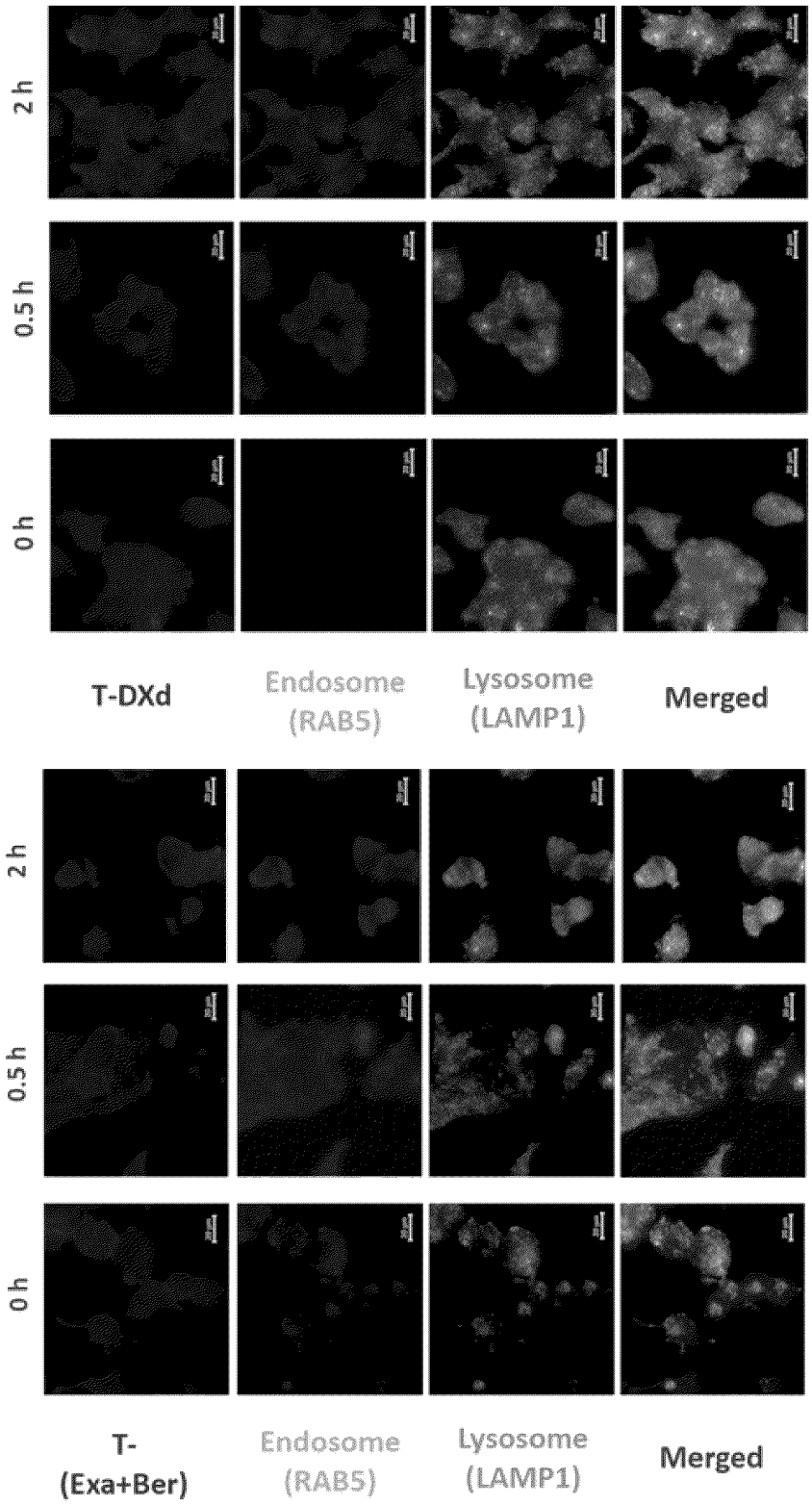

2 h
0.5 h
0 h
T-DXd
Endosome (RAB5)
Lysosome (LAMP1)
Merged
2 h
0.5 h
0 h
T- (Exa+Ber)
Endosome (RAB5)
Lysosome (LAMP1)
Merged

Fig. 6A

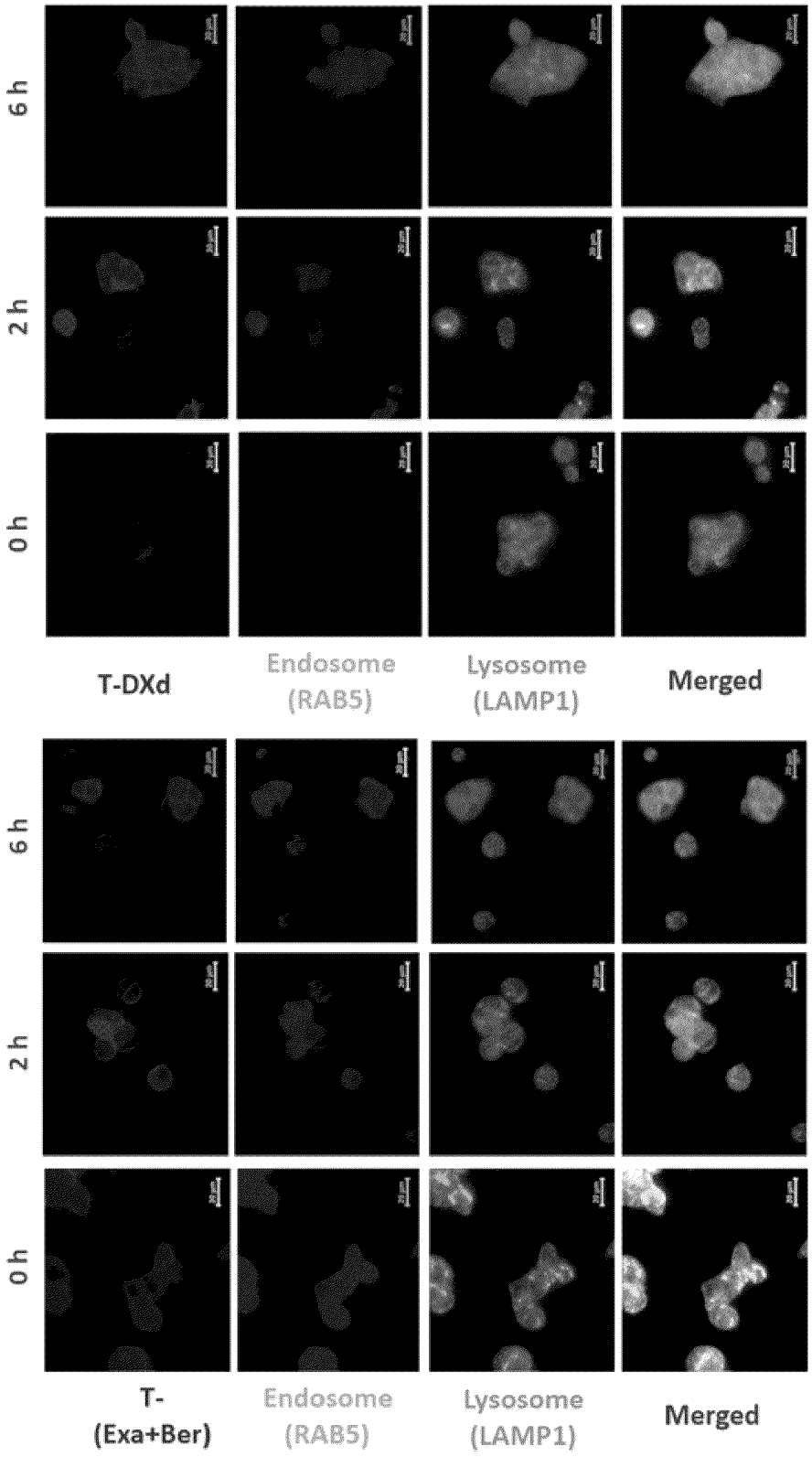

6 h
2 h
0 h
T-DXd
Endosome (RAB5)
Lysosome (LAMP1)
Merged
6 h
2 h
0 h
T- (Exa+Ber)
Endosome (RAB5)
Lysosome (LAMP1)
Merged

Fig. 6B

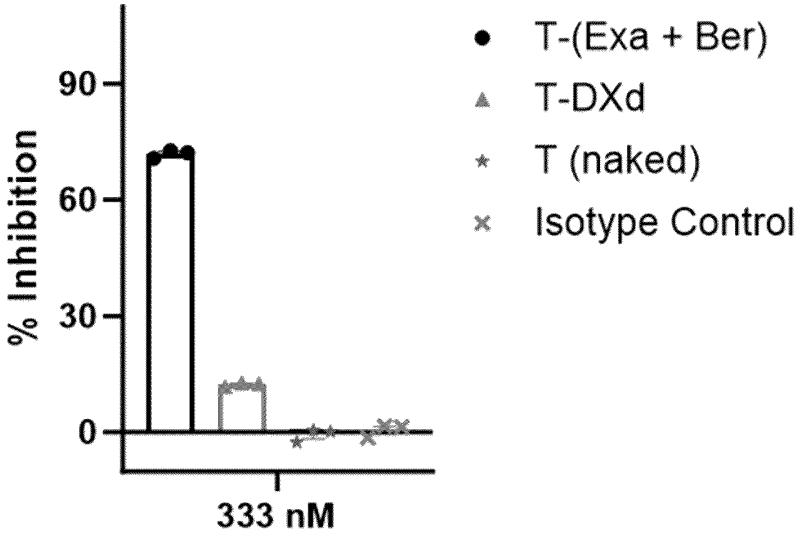

% Inhibition
90
60
30
0
333 nM
T-(Exa + Ber)
T-DXd
T (naked)
Isotype Control

Fig. 7A

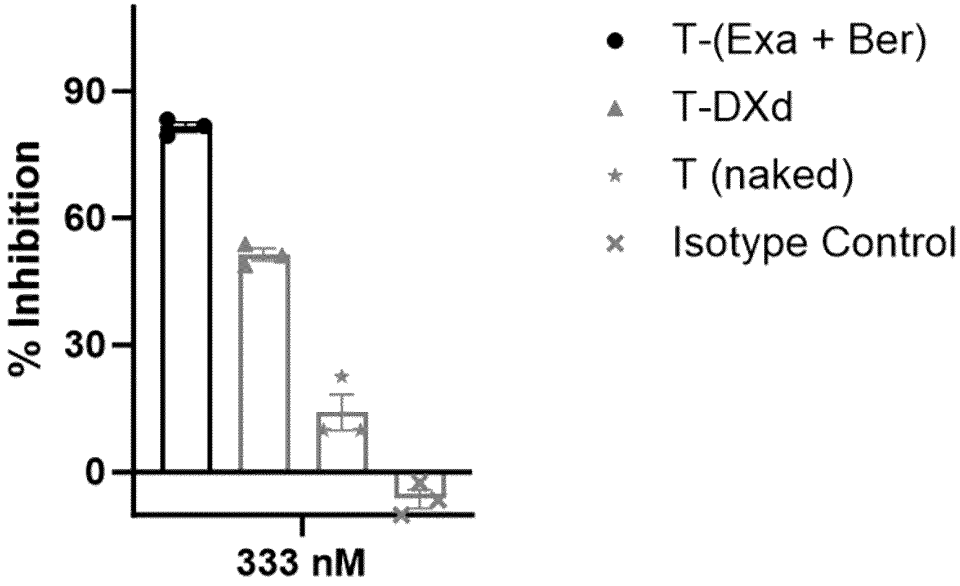

% Inhibition
90
60
30
0
333 nM
T-(Exa + Ber)
T-DXd
T (naked)
Isotype Control

Fig. 7B

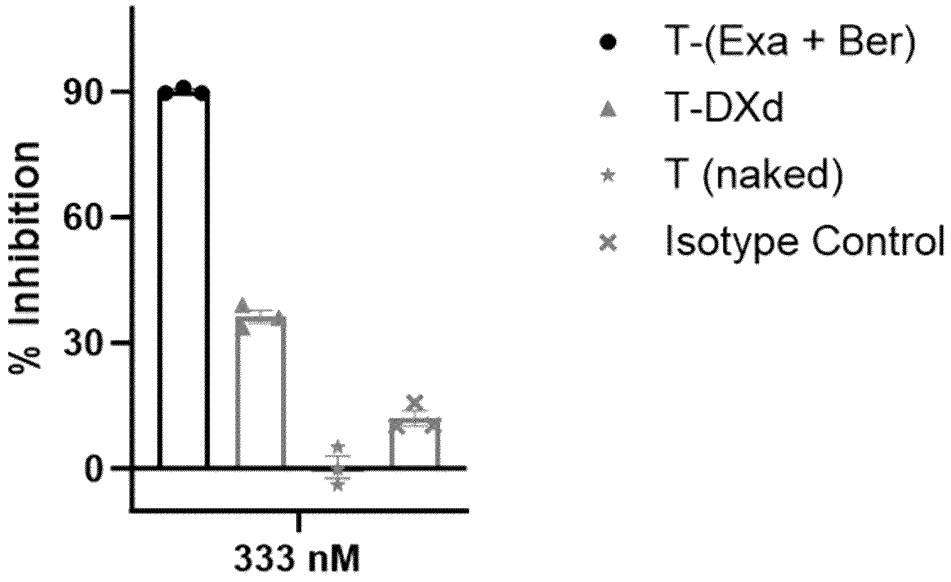

% Inhibition
90
60
30
0
333 nM
T-(Exa + Ber)
T-DXd
T (naked)
Isotype Control

Fig. 7C

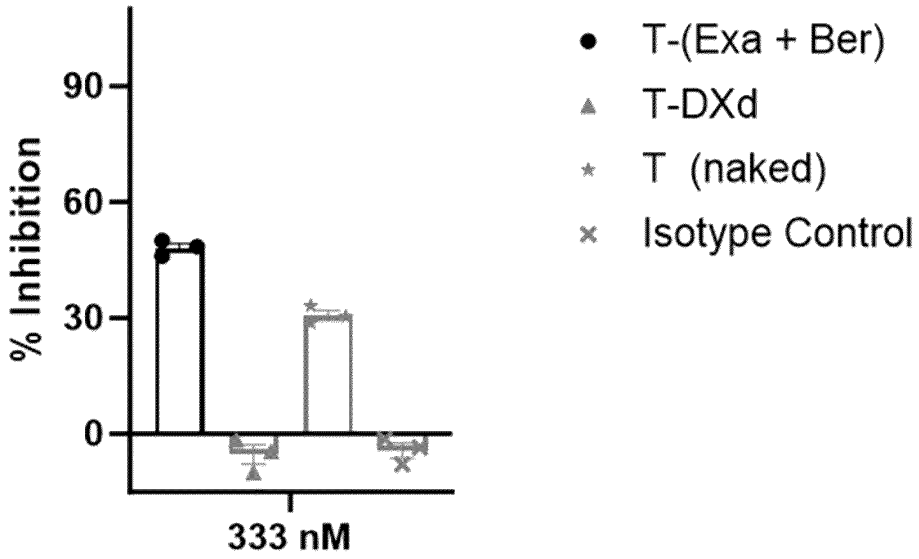

% Inhibition
90
60
30
0
333 nM
T-(Exa + Ber)
T-DXd
T (naked)
Isotype Control

Fig. 7D

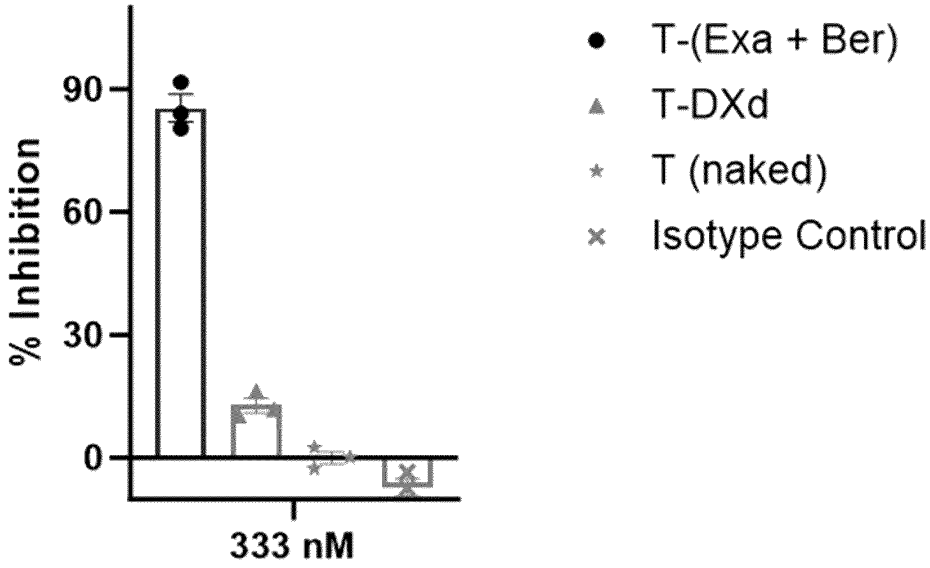

% Inhibition
90
60
30
0
333 nM
T-(Exa + Ber)
T-DXd
T (naked)
Isotype Control

Fig. 7E

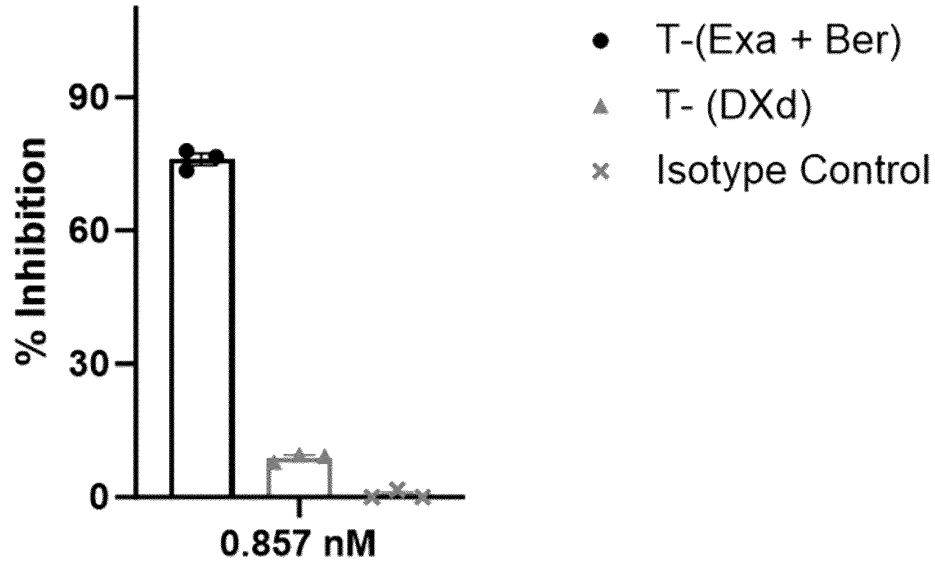

% Inhibition
90
60
30
0
0.857 nM
T-(Exa + Ber)
T- (DXd)
Isotype Control

Fig. 7F

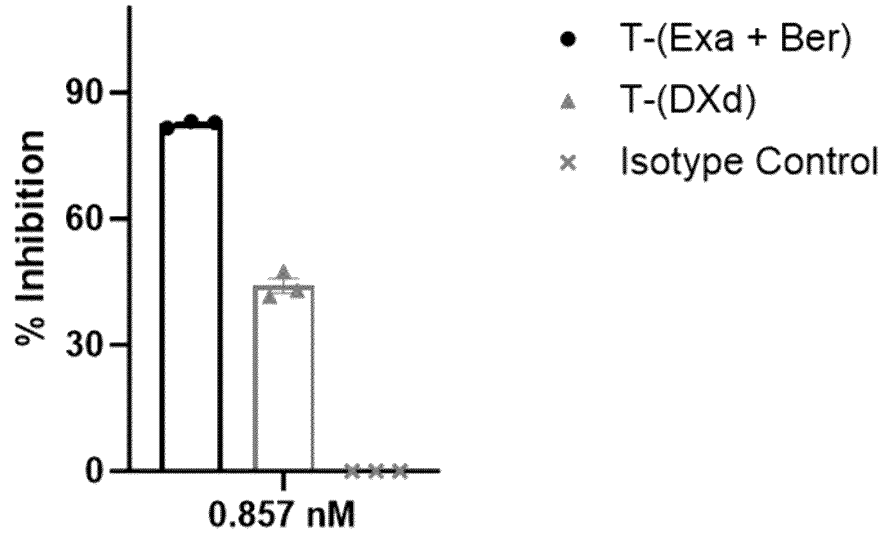

% Inhibition
90
60
30
0
0.857 nM
T-(Exa + Ber)
T-(DXd)
Isotype Control

Fig. 7G

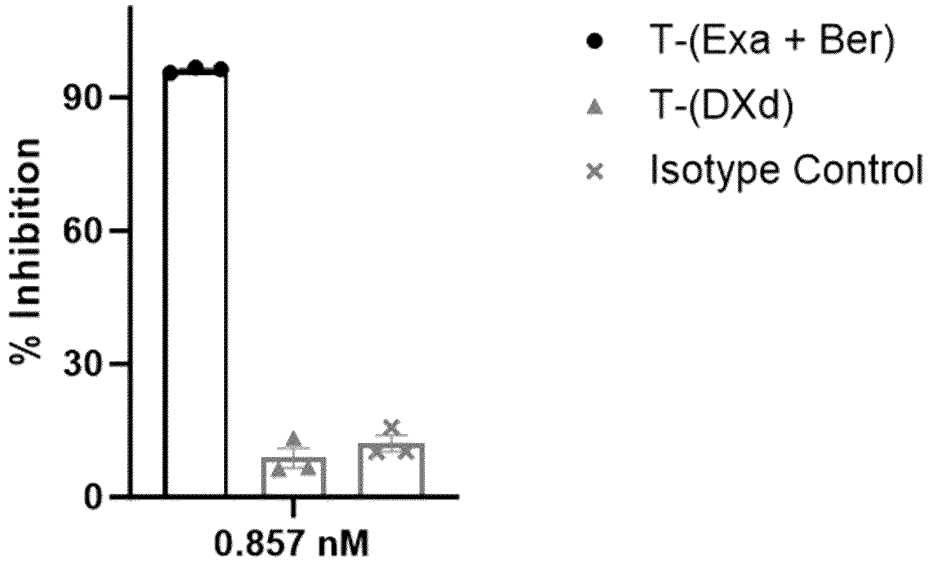

% Inhibition
90
60
30
0
0.857 nM
T-(Exa + Ber)
T-(DXd)
Isotype Control

Fig. 7H

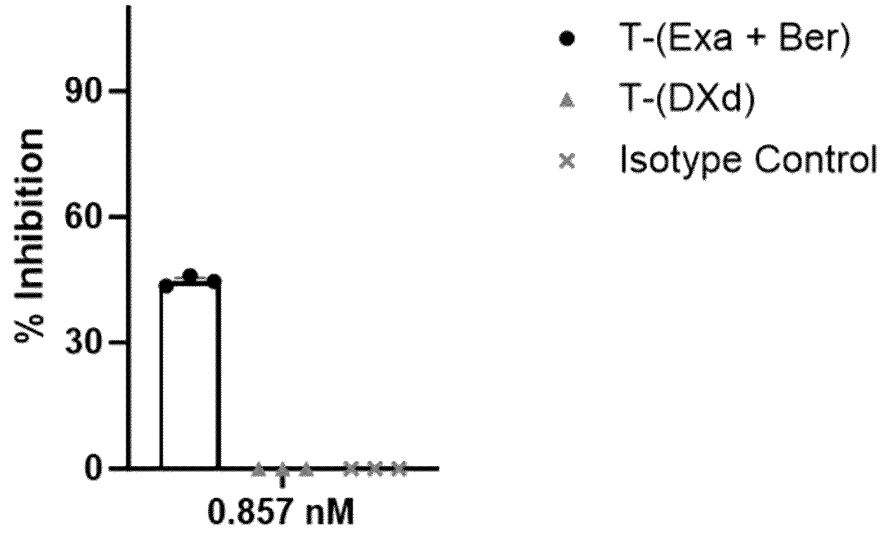

% Inhibition
90
60
30
0
0.857 nM
T-(Exa + Ber)
T-(DXd)
Isotype Control

Fig. 7I

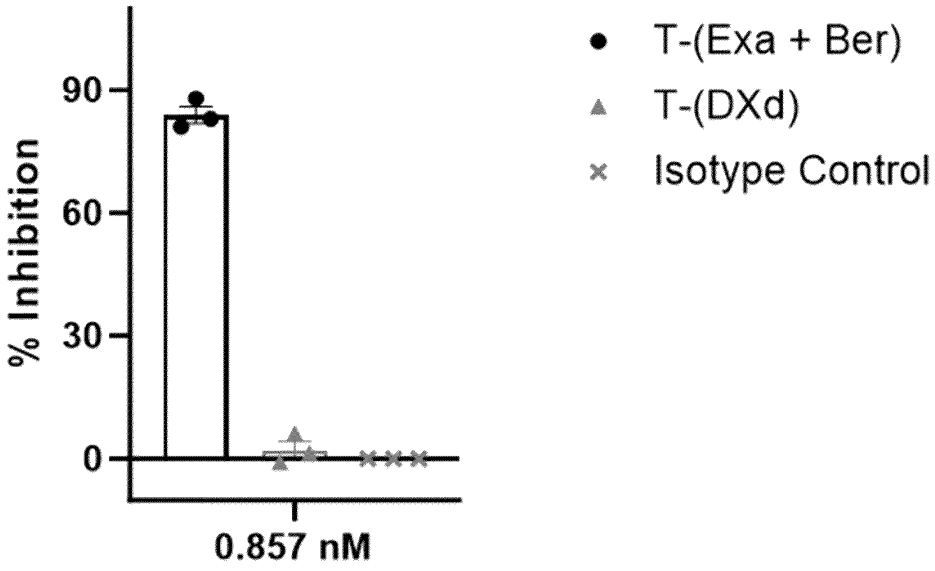

% Inhibition
90
60
30
0
0.857 nM
T-(Exa + Ber)
T-(DXd)
Isotype Control

Fig. 7J

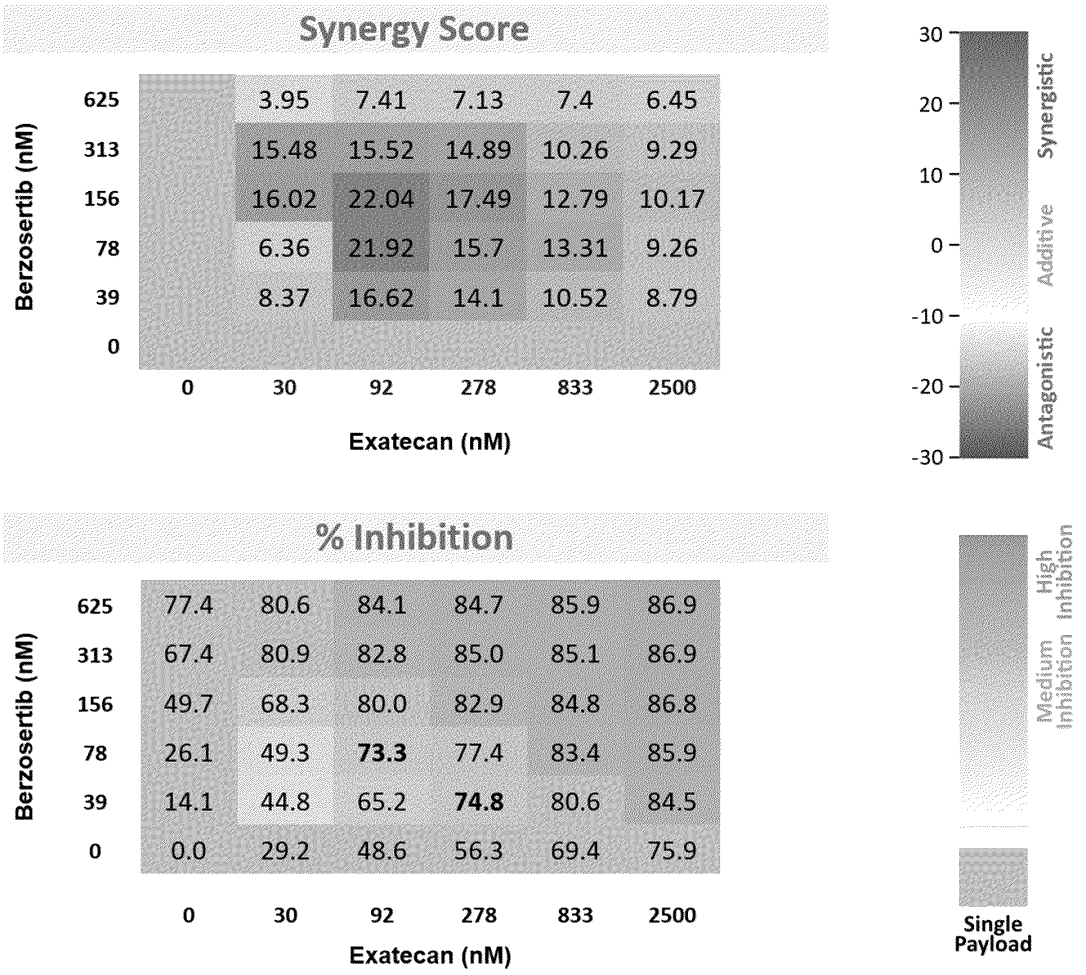

Synergy Score
Berzosertib (nM)
625 3.95 7.41 7.13 7.4 6.45
313 15.48 15.52 14.89 10.26 9.29
156 16.02 22.04 17.49 12.79 10.17
78 6.36 21.92 15.7 13.31 9.26
39 8.37 16.62 14.1 10.52 8.79
0
0 30 92 278 833 2500
Exatecan (nM)
30
20
10
0
-10
-20
-30
Synergistic
Additive
Antagonistic
% Inhibition
Berzosertib (nM)
625 77.4 80.6 84.1 84.7 85.9 86.9
313 67.4 80.9 82.8 85.0 85.1 86.9
156 49.7 68.3 80.0 82.9 84.8 86.8
78 26.1 49.3 73.3 77.4 83.4 85.9
39 14.1 44.8 65.2 74.8 80.6 84.5
0 0.0 29.2 48.6 56.3 69.4 75.9
0 30 92 278 833 2500
Exatecan (nM)
High Inhibition
Medium Inhibition
Single Payload

Fig. 8

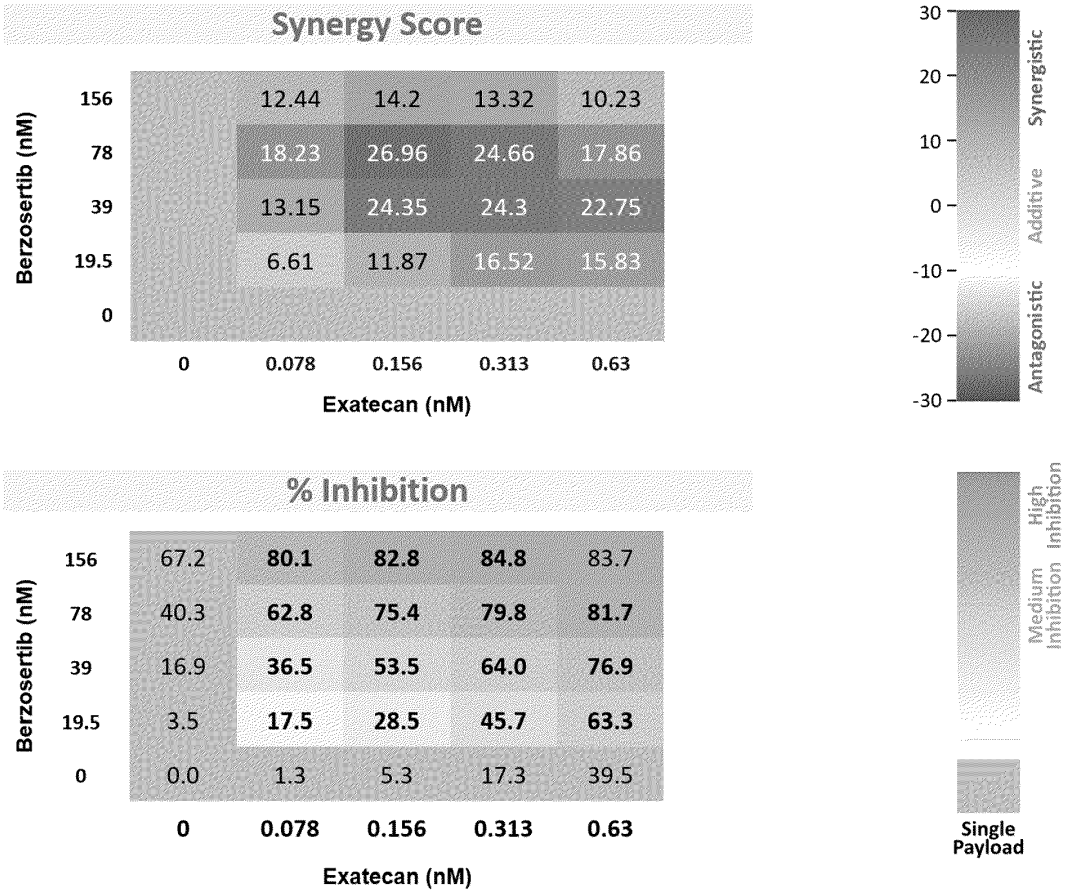

Synergy Score
Berzosertib (nM)
156
78
39
19.5
0
12.44
14.2
13.32
10.23
18.23
26.96
24.66
17.86
13.15
24.35
24.3
22.75
6.61
11.87
16.52
15.83
0
0.078
0.156
0.313
0.63
Exatecan (nM)
30
20
10
0
-10
-20
-30
Synergistic
Additive
Antagonistic
% Inhibition
Berzosertib (nM)
156
78
39
19.5
0
67.2
80.1
82.8
84.8
83.7
40.3
62.8
75.4
79.8
81.7
16.9
36.5
53.5
64.0
76.9
3.5
17.5
28.5
45.7
63.3
0.0
1.3
5.3
17.3
39.5
0
0.078
0.156
0.313
0.63
Exatecan (nM)
High Inhibition
Medium Inhibition
Single Payload

Fig.9

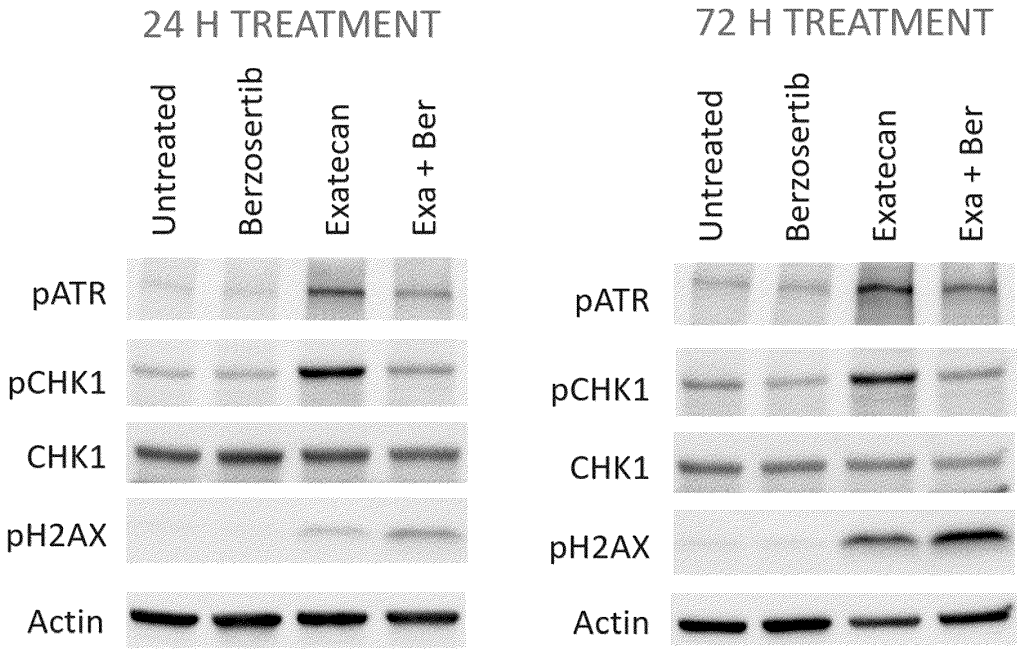

24 H TREATMENT
72 H TREATMENT
Untreated
Berzosertib
Exatecan
Exa + Ber
pATR
pCHK1
CHK1
pH2AX
Actin

Fig. 10

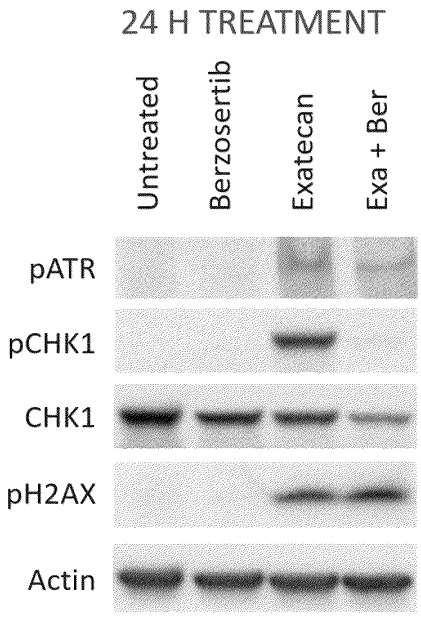

24 H TREATMENT
Untreated
Berzosertib
Exatecan
Exa + Ber
pATR
pCHK1
CHK1
pH2AX
Actin

Fig. 11

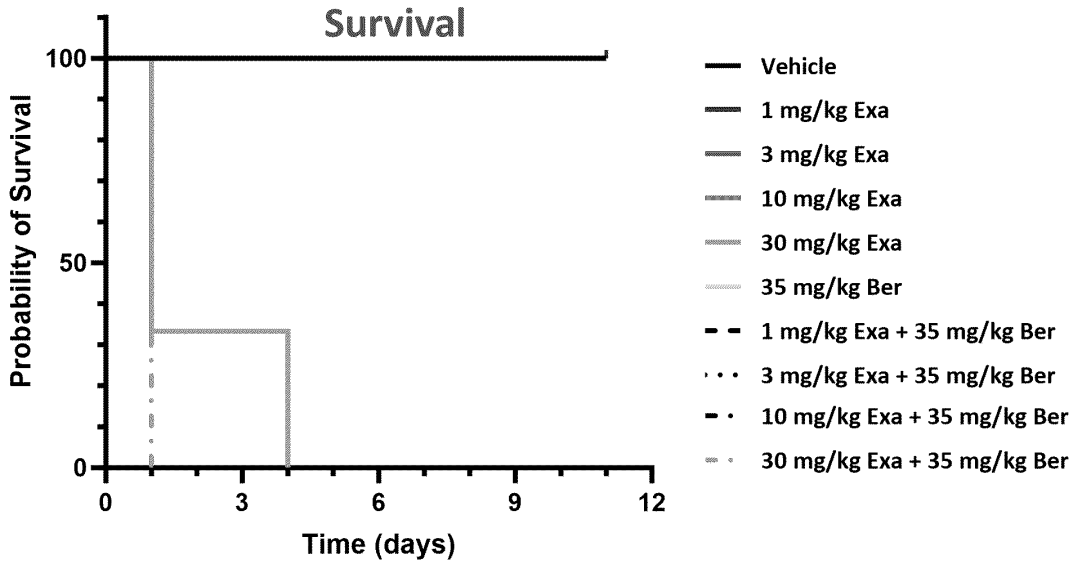

Survival
Probability of Survival
100
50
0
0
3
6
9
12
Time (days)
Vehicle
1 mg/kg Exa
3 mg/kg Exa
10 mg/kg Exa
30 mg/kg Exa
35 mg/kg Ber
1 mg/kg Exa + 35 mg/kg Ber
3 mg/kg Exa + 35 mg/kg Ber
10 mg/kg Exa + 35 mg/kg Ber
30 mg/kg Exa + 35 mg/kg Ber

Fig. 12

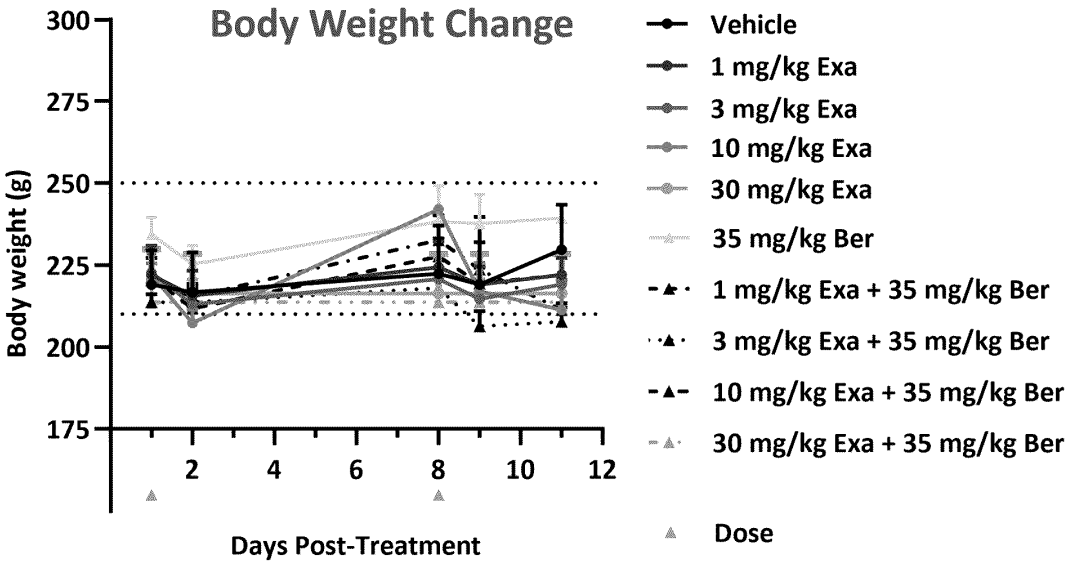

Body Weight Change
Body weight (g)
300
275
250
225
200
175
2
4
6
8
10
12
Days Post-Treatment
Vehicle
1 mg/kg Exa
3 mg/kg Exa
10 mg/kg Exa
30 mg/kg Exa
35 mg/kg Ber
1 mg/kg Exa + 35 mg/kg Ber
3 mg/kg Exa + 35 mg/kg Ber
10 mg/kg Exa + 35 mg/kg Ber
30 mg/kg Exa + 35 mg/kg Ber
Dose

Fig. 13

Treatment Arm	Dose (mg/kg)	Time point	Hematological parameters										
			Red Blood Cell			Anemia Parameters			White Blood Cell				
			RBC Count (10^12/L) Average	Hemoglobin (g/dl) Average	Hematocrit (%) Average	MCV (fl) Average	MCH (pg) Average	MCHC (g/dl) Average	WBC Count (10^9/ L) Average	Lymphocyte Count (10^9/ L) Average	Monocyte Count (10^9/ L) Average	Neutrophil Count (10^9/ L) Average	Platelets (10^9/ L) Average
Vehicle	0	Day 2	7.47	13.77	45.12	60.33	18.37	30.47	7.80	7.88	0.25	1.04	275.67
		Day 7	8.43	15.13	48.93	58.33	18.00	31.00	10.36	8.55	0.55	1.26	721.67
		Day 9	8.52	15.53	47.53	55.67	18.27	32.73	10.82	9.07	0.31	1.44	532.33
		Day 11	8.32	15.13	45.21	54.33	18.20	33.53	10.82	9.25	0.17	1.39	447.00
Berzosertib	35	Day 2	7.01	15.50	41.58	59.33	22.27	38.07	5.84	5.10	0.24	0.50	728.33
		Day 7	8.38	14.83	45.73	54.67	17.73	32.47	9.05	7.75	0.12	1.18	347.67
		Day 9	6.70	11.80	38.11	57.00	17.40	30.60	6.10	5.33	0.11	0.67	319.67
		Day 11	7.22	12.73	39.54	54.67	17.63	32.10	9.66	6.89	0.32	2.45	376.67
Exatecan	1	Day 2	8.37	14.47	44.74	53.67	17.27	32.33	5.51	4.85	0.18	0.48	529.33
		Day 7	8.52	14.57	47.05	55.00	17.13	31.00	10.03	8.52	0.54	0.97	458.33
		Day 9	8.24	13.90	44.25	53.67	16.87	31.40	6.49	5.94	0.05	0.51	606.00
		Day 11	8.03	13.43	33.30	53.00	16.73	31.77	8.91	7.84	0.16	0.92	543.67
Exatecan	3	Day 2	8.41	15.43	49.87	59.33	18.37	30.97	7.49	5.32	0.25	1.91	356.67
		Day 7	8.16	14.30	45.51	56.00	17.57	31.33	9.98	8.31	0.28	1.39	547.33
		Day 9	7.58	12.97	42.50	56.00	17.10	30.50	6.19	5.10	0.15	0.94	673.33
		Day 11	6.99	12.03	38.36	55.00	17.23	31.40	6.32	5.65	0.07	0.60	490.00
Exatecan	10	Day 2	9.16	16.50	51.61	56.67	18.07	32.03	4.73	3.91	0.11	0.70	569.33
		Day 7	8.05	14.27	43.79	54.00	17.73	32.63	7.18	6.42	0.18	0.58	417.00
		Day 9	7.04	13.23	38.04	54.33	19.50	36.03	3.36	2.85	0.07	0.44	396.33
		Day 11	7.03	11.87	37.94	54.00	16.83	31.23	3.20	2.81	0.10	0.29	143.00
Exatecan + Berzosertib	1 + 35	Day 2	5.50	17.73	31.02	56.67	32.73	57.50	4.08	3.30	0.21	0.57	645.33
		Day 7	8.26	14.83	46.71	56.67	17.97	31.70	9.54	7.17	0.53	1.84	822.00
		Day 9	8.63	15.47	48.24	55.67	17.93	32.03	10.05	6.83	0.44	2.77	946.67
		Day 11	6.81	11.93	38.04	56.00	17.50	31.30	8.56	6.35	0.30	1.91	742.33
Exatecan + Berzosertib	3 + 35	Day 2	6.48	14.53	37.70	59.00	23.90	40.23	4.41	3.58	0.11	0.72	707.33
		Day 7	8.82	15.60	49.12	55.50	17.70	31.80	11.46	6.90	0.36	4.21	664.50
		Day 9	7.28	12.57	39.62	54.33	17.23	31.67	8.10	5.04	0.39	2.67	577.33
		Day 11	7.65	13.03	41.24	54.00	17.03	31.60	7.55	6.49	0.24	0.82	349.33
Exatecan + Berzosertib	10 + 35	Day 2	8.53	15.70	47.62	56.00	18.40	32.93	9.24	7.35	0.27	1.62	505.67
		Day 7	7.93	14.10	45.00	57.00	17.83	31.33	9.57	7.91	0.31	1.36	762.00
		Day 9	7.52	13.23	43.10	57.33	17.57	30.63	6.58	4.83	0.30	1.45	497.33
		Day 11	7.63	13.53	42.50	56.00	17.73	31.87	4.34	4.00	0.04	0.30	227.67

Fig. 14

Treatment Arm	Dose (mg/kg)	Time point	Biochemistry												
			Liver				Kidney (mg/dL)		Pancreas		Electrolytes				
			ALT (U/L) Average	ALP (U/L) Average	AlB (G/DL) Average	TP (g/dL) Average	CREA Average	BUN Average	GLU (mg/dL) Average	AMY (U/L) Average	NA+ (mmol/L) Average	K+ (mmol/L) Average	P (mg/dL) Average	CA (mg/dL) Average
Vehicle	0	Day 2	50.33	229.33	4.47	6.37	0.27	14.67	172.00	636.00	138.00	6.63	8.57	11.03
		Day 7	33.00	263.67	4.50	6.17	0.20	16.00	166.33	641.67	137.33	5.97	7.50	10.70
		Day 9	45.00	254.33	4.23	6.30	0.30	13.00	195.00	633.00	137.00	6.63	7.80	10.77
		Day 11	41.67	284.00	4.27	6.27	0.20	14.33	189.67	650.00	137.67	7.07	8.43	10.73
Berzosertib	35	Day 2	40.00	169.33	3.77	6.10	0.07	17.00	183.00	658.67	139.00	6.80	8.77	10.53
		Day 7	36.67	258.00	4.03	5.93	0.07	13.33	241.67	551.33	135.67	7.47	8.67	11.07
		Day 9	43.00	226.33	3.53	5.60	0.20	12.00	183.00	563.00	137.00	7.07	8.27	10.40
		Day 11	41.00	201.00	3.67	5.83	0.13	12.00	193.67	529.33	139.33	6.50	9.03	10.83
Exatecan	1	Day 2	31.00	239.33	4.70	6.03	0.23	16.33	190.00	657.00	138.00	5.85	8.80	11.07
		Day 7	33.33	268.67	4.67	5.97	0.17	15.33	150.33	613.33	135.67	6.53	8.47	10.87
		Day 9	42.33	207.00	4.07	5.37	0.00	14.00	175.67	635.00	137.00	6.80	8.37	10.27
		Day 11	27.67	190.33	4.00	5.77	0.10	12.67	183.67	607.00	138.33	6.90	8.43	10.33
Exatecan	3	Day 2	49.00	215.00	4.43	6.37	0.13	16.67	152.67	744.33	137.33	5.90	8.87	11.13
		Day 7	36.33	223.67	4.10	5.93	0.07	15.33	181.67	590.33	135.00	4.13	8.40	10.80
		Day 9	40.33	198.00	3.97	5.60	0.13	13.67	165.67	705.00	138.00	6.67	9.50	10.73
		Day 11	27.67	151.33	3.67	5.93	0.00	12.33	179.67	526.33	139.00	6.30	8.23	10.80
Exatecan	10	Day 2	144.33	212.67	4.80	5.97	0.17	17.33	167.33	731.33	136.33	4.33	8.97	10.73
		Day 7	48.33	230.00	4.73	5.80	0.30	15.00	207.33	776.50	134.67	6.57	8.77	10.80
		Day 9	51.00	162.00	4.53	5.37	0.20	14.67	183.33	700.00	135.00	7.13	8.03	10.23
		Day 11	27.00	86.67	3.97	5.43	0.27	12.00	212.33	644.33	136.67	6.27	8.37	10.70
Exatecan + Berzosertib	1 + 35	Day 2	44.00	101.00	4.30	6.05	0.00	11.50	144.50	541.33	139.00	4.00	8.50	10.85
		Day 7	34.00	188.00	3.90	5.80	0.10	12.00	194.33	492.67	136.00	7.70	9.10	10.80
		Day 9	39.33	141.33	3.27	5.97	0.27	11.00	153.00	512.00	139.67	7.27	8.37	10.50
		Day 11	34.33	135.67	3.17	6.10	0.00	10.67	164.67	410.33	138.00	6.70	8.50	10.93
Exatecan + Berzosertib	3 + 35	Day 2	45.33	157.67	4.17	5.50	0.17	14.00	206.67	681.00	136.33	7.37	8.37	10.20
		Day 7	36.00	202.33	3.97	5.83	0.10	12.33	205.67	537.00	136.33	7.43	8.63	10.60
		Day 9	46.33	144.00	3.77	5.33	0.27	15.67	171.67	827.33	135.00	7.27	8.00	9.77
		Day 11	20.67	74.33	3.30	5.70	0.37	14.33	172.33	529.67	138.67	6.53	8.00	10.30
Exatecan + Berzosertib	10 + 35	Day 2	60.33	136.00	4.57	5.67	0.17	18.33	175.33	704.33	136.67	8.27	7.97	10.43
		Day 7	53.67	173.67	4.57	5.77	0.47	12.67	206.67	648.00	136.00	6.67	9.07	10.90
		Day 9	66.00	131.33	4.13	5.37	0.13	16.00	175.00	870.33	136.33	7.37	7.93	10.23
		Day 11	23.00	38.00	3.67	5.17	0.17	14.67	215.00	688.67	134.33	4.47	8.40	10.23

Fig.15

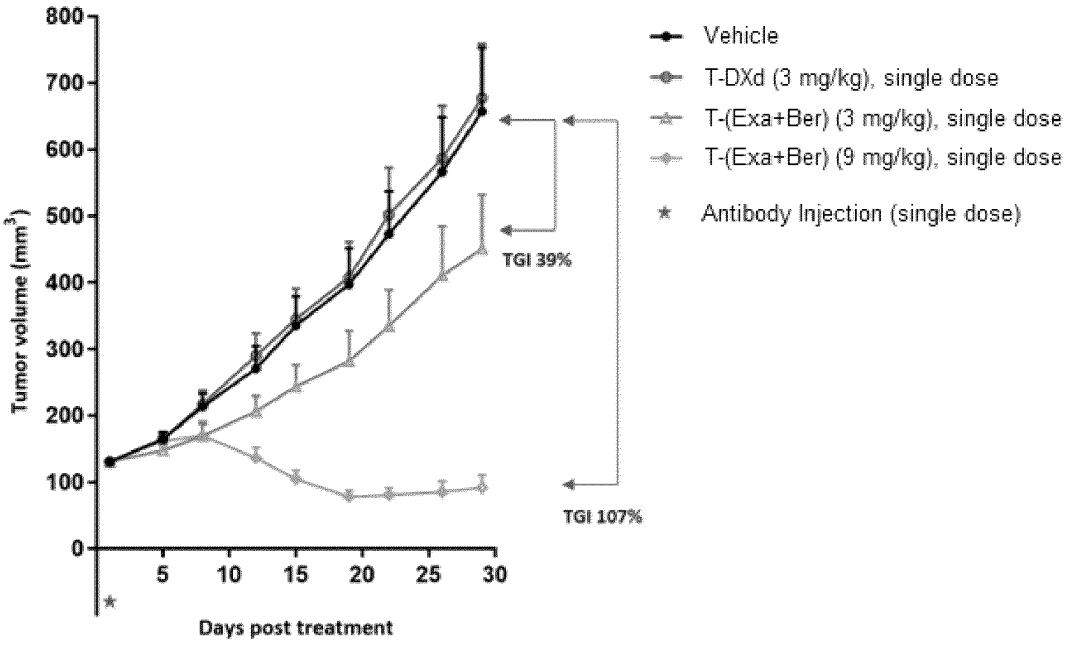
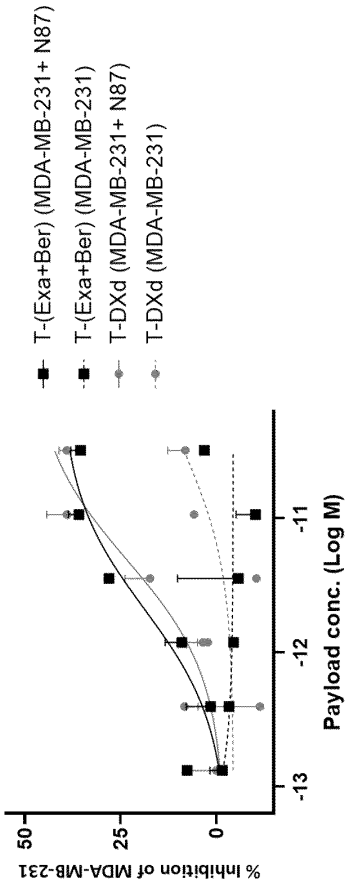
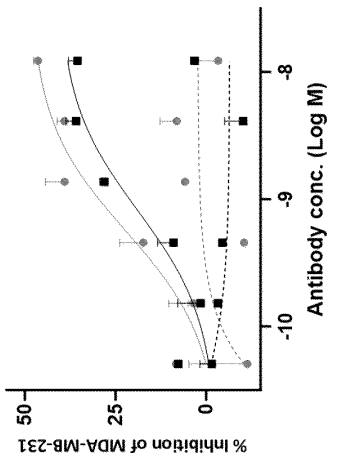

Tumor volume (mm3)
800
700
600
500
400
300
200
100
0
5
10
15
20
25
30
Days post treatment
TGI 39%
TGI 107%
Vehicle
T-DXd (3 mg/kg), single dose
T-(Exa+Ber) (3 mg/kg), single dose
T-(Exa+Ber) (9 mg/kg), single dose
Antibody Injection (single dose)

Fig. 16



	IC_{50} (M) at matched payload conc.	
	T-(Exa+Ber)	T-DXd (Enhertu)
MDA-MB-231+ N87	2.099e-012	4.878e-012
MDA-MB-231	-	-



	IC_{50} (M) at matched antibody conc.	
	T-(Exa+Ber)	T-DXd (Enhertu)
MDA-MB-231+ N87	8.136e-010	5.652e-010
MDA-MB-231	-	-

Fig. 17

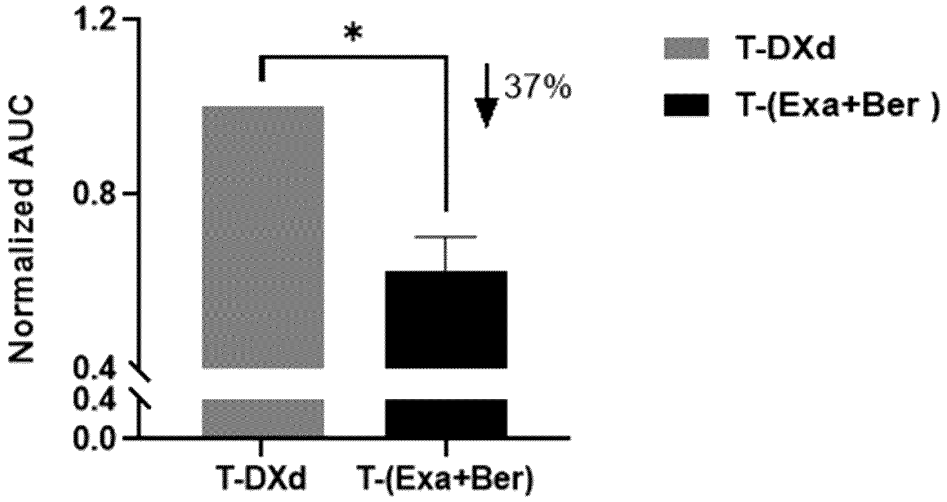

1.2
0.8
0.4
0.4
0.0
Normalized AUC
*
37%
T-DXd
T-(Exa+Ber)
T-DXd
T-(Exa+Ber)

Fig. 18

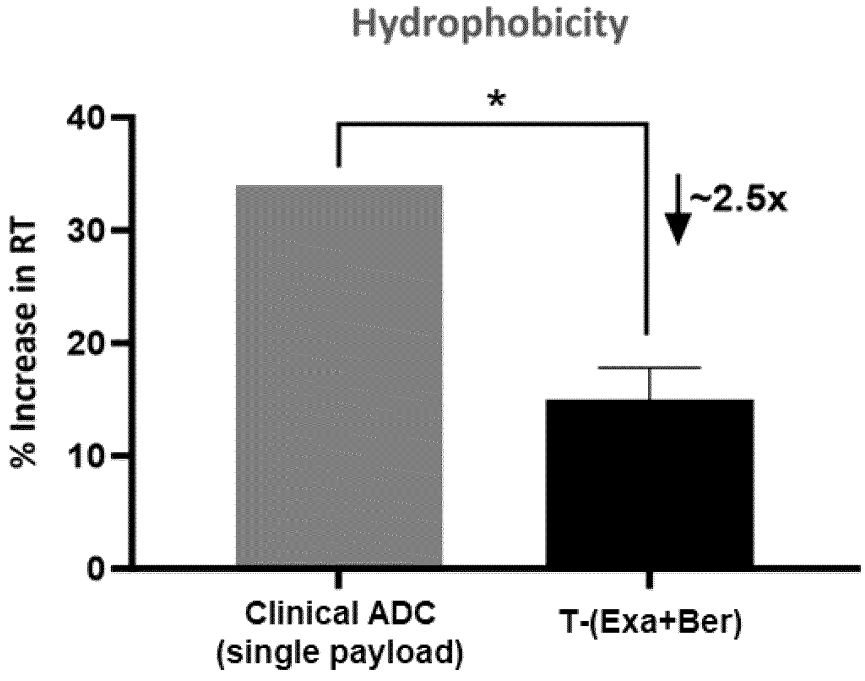

Hydrophobicity
40
30
20
10
0
% Increase in RT
*
~2.5x
Clinical ADC
(single payload)
T-(Exa+Ber)

Fig. 19

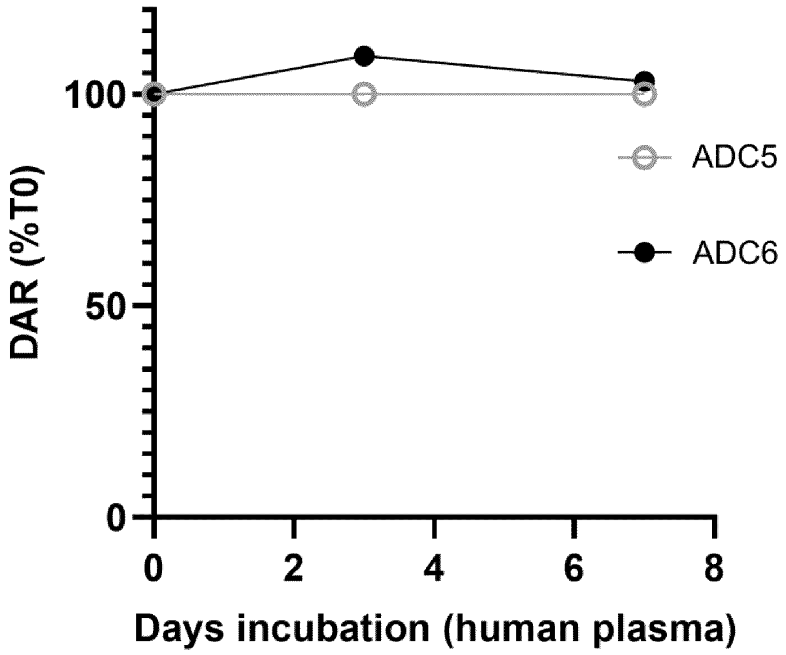
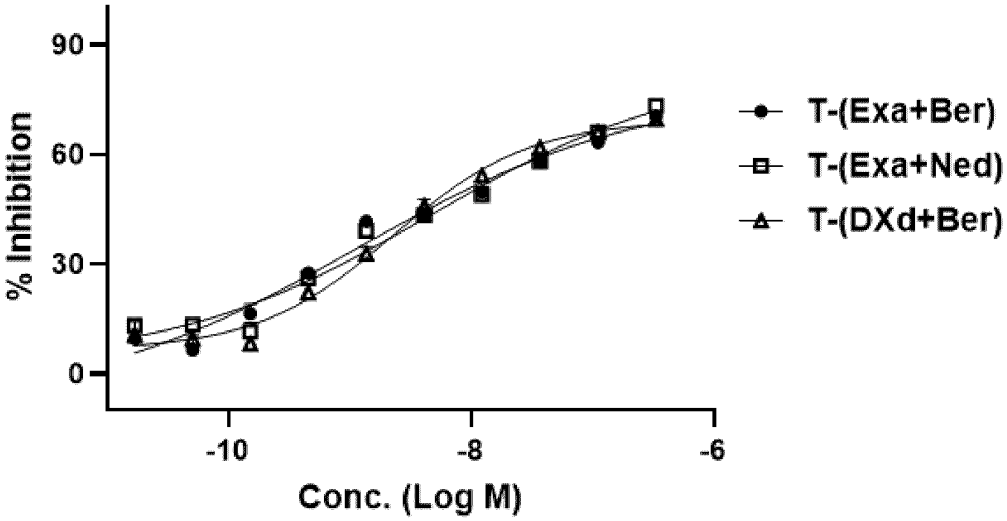


Fig. 20



IC_{50} (M)		
T-(Exa+Ber)	T-(Exa+Ned)	T-(DXd+Ber)
1.144e-009	4.549e-009	2.288e-009

Fig. 21

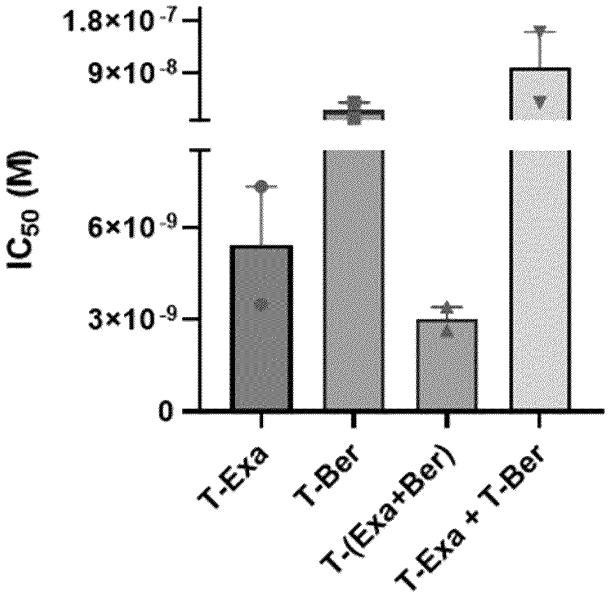

1.8×10-7
9×10-8
6×10-9
3×10-9
0
IC50 (M)
T-Exa
T-Ber
T-(Exa+Ber)
T-Exa + T-Ber

Fig. 22

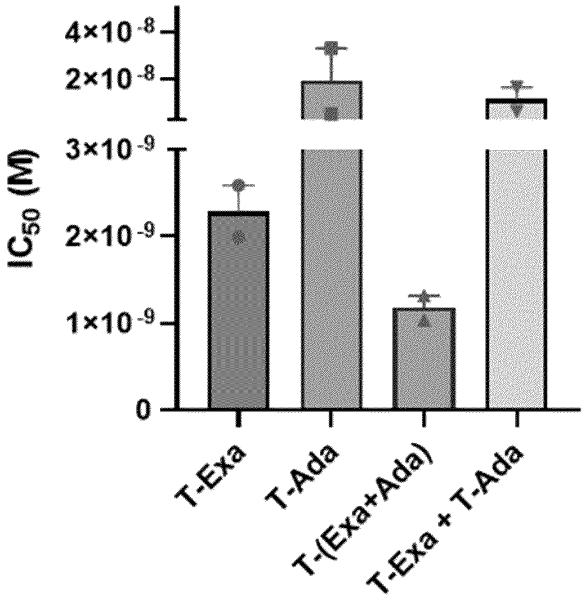
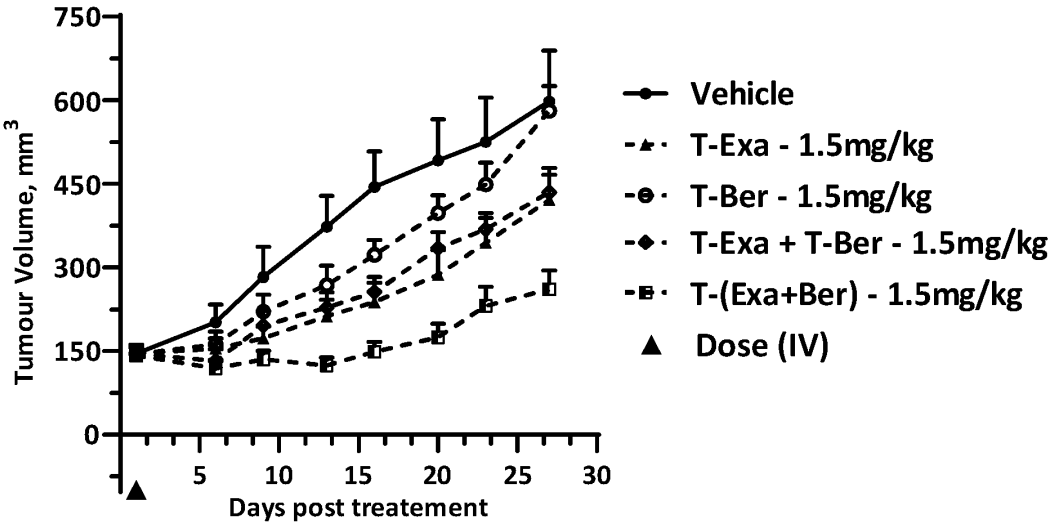
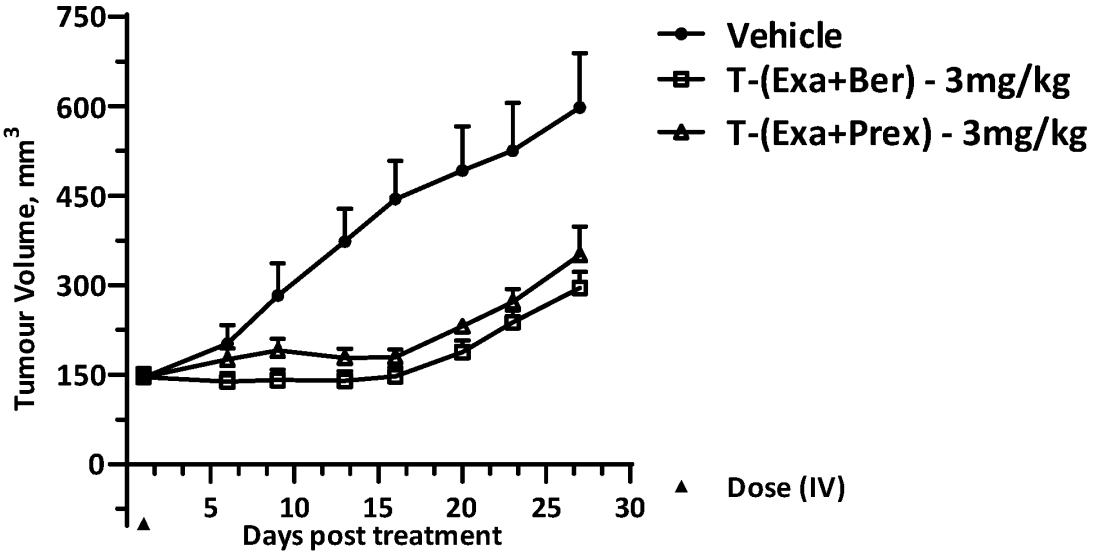

4×10-8
2×10-8
3×10-9
2×10-9
1×10-9
0
IC50 (M)
T-Exa
T-Ada
T-(Exa+Ada)
T-Exa + T-Ada

Fig. 23



Arms	TGI (%)
T-Exa	39
T-Ber	3.6
T-Exa + T-Ber	35.8
T-(Exa+Ber)	73.7

Fig. 24



Arms	HMBD ADC ID	TGI (%)
T-(Exa+Ber)	22	67.0
T-(Exa+Prex)	43	54.9

Fig. 25

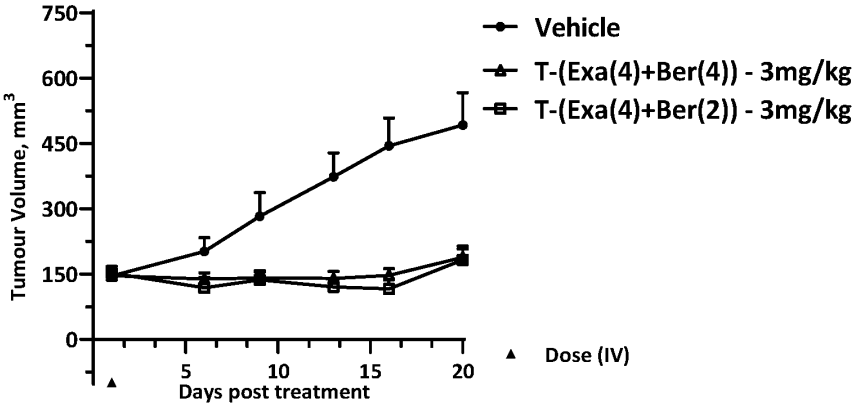


Fig. 26

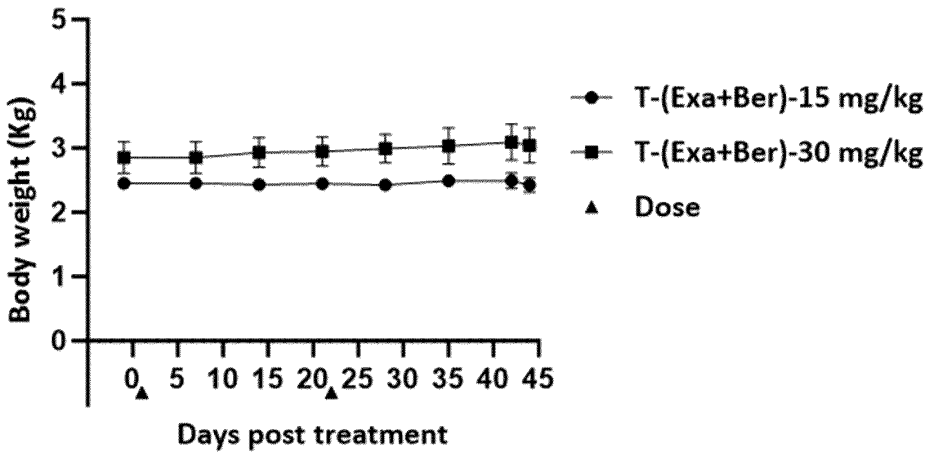
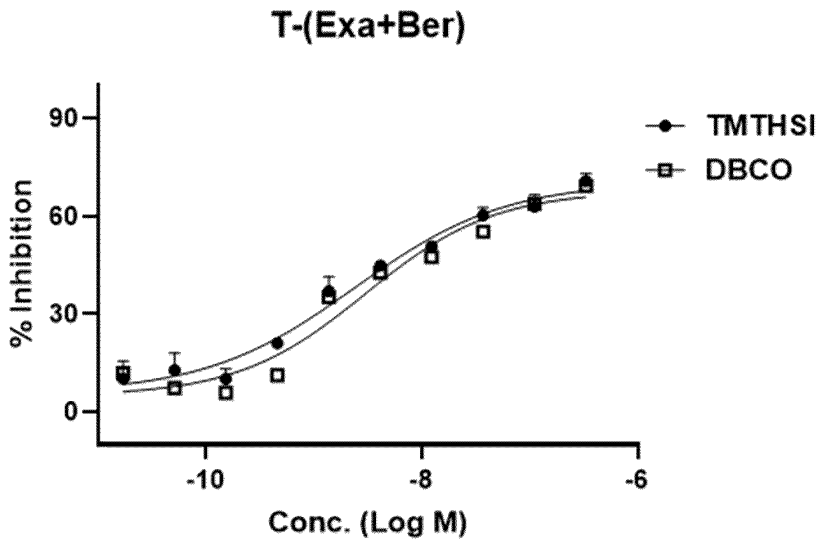


Fig. 27

Dose (mg/kg)	Time point	Biochemistry				Hematology									
		ALT (U/L)		ALP (U/L)		Neutrophils (10^3/uL)		Platelets (10^3/uL)		RBC (10^6/uL)		WBC (10^3/uL)		Reticulocytes (10^9/L)	
	Gender	M	F	M	F	M	F	M	F	M	F	M	F	M	F
15	Day -5	53	28	682	346	1.02	1.22	355	399	6.15	6.06	5.73	7.43	41.3	93.0
	Day 4	65	43	668	305	1.59	1.71	444	533	5.94	5.59	6.71	5.27	66.0	144.5
	Day 7	63	45	663	315	1.39	1.55	409	481	5.79	5.64	5.51	5.54	43.0	195.6
	Day 15	50	27	569	302	3.13	1.49	402	537	5.65	5.39	7.53	4.64	48.2	153.9
	Day 22	64	32	545	307	0.98	1.79	469	466	5.79	5.88	4.68	6.25	51.6	135.9
	Day 26	60	30	452	279	0.96	1.47	427	481	5.35	5.75	3.96	5.34	46.8	103
	Day 29	68	28	468	270	0.39	1.15	445	483	5.34	5.49	3.19	4.27	76.9	150.9
	Day 44	66	44	500	278	0.67	2.23	389	428	5.53	6.07	5.6	7.83	56.6	90.9
30	Day -5	79	36	917	670	2.25	2.11	378	516	6.26	6.27	9.72	8.18	74.5	43.0
	Day 4	111	61	920	667	3.13	2.20	390	473	6.10	5.57	9.97	7.74	117.9	88.5
	Day 7	102	52	990	678	2.83	1.57	366	457	6.18	5.60	9.41	7.07	107.0	82.2
	Day 15	63	33	1068	660	4.47	4.11	350	500	5.17	5.32	11.97	10.80	126.8	118.4
	Day 22	113	39	987	675	3.43	2.78	378	456	6.06	5.71	9.14	9.97	112	92.4
	Day 26	82	59	872	635	2.22	1.31	381	526	5.69	5.53	8.97	8.09	93.1	110.4
	Day 29	111	49	1046	615	3.91	3.28	432	467	5.93	5.47	10.09	8.39	146	95.8
	Day 44	124	30	1300	653	3.07	2.91	429	473	6.24	5.44	12.68	9.74	142.6	105.5
Ref range	M	18.04 – 81.04		385.90 – 1378.40		1.93 – 22.74		181.80 – 479.00		4.64 – 6.51		6.19 – 26.93		16.50 – 154.40	
	F	11.80 – 77.60		185.30 – 1326.70		2.10 – 21.01		208.30 – 584.00		4.59 – 6.60		6.27 – 25.48		15.20 – 158.80	

Fig. 28



IC_{50} (M)	
TMTHSI	DBCO
2.407e-009	3.019e-009

Fig. 29

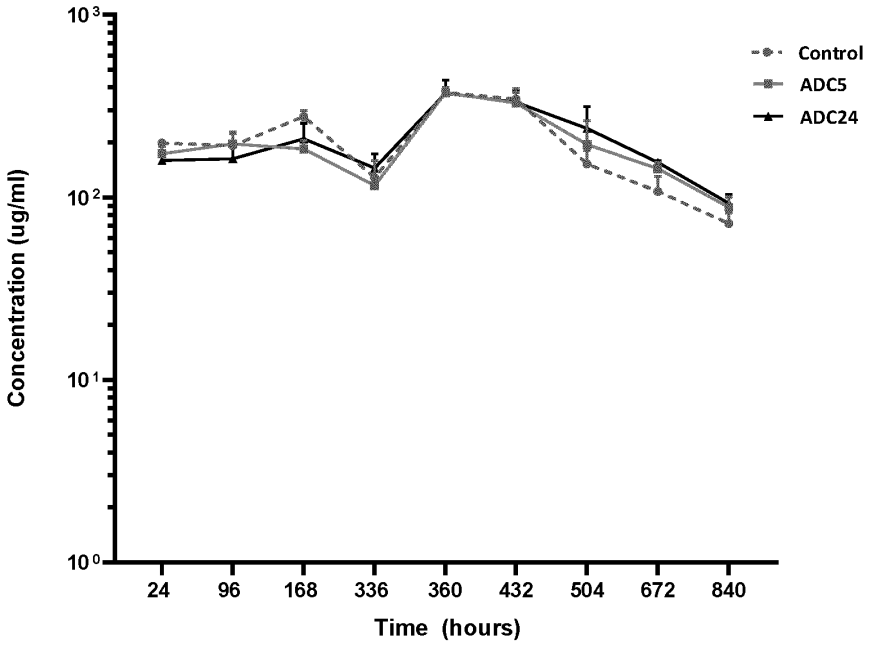


Fig. 30

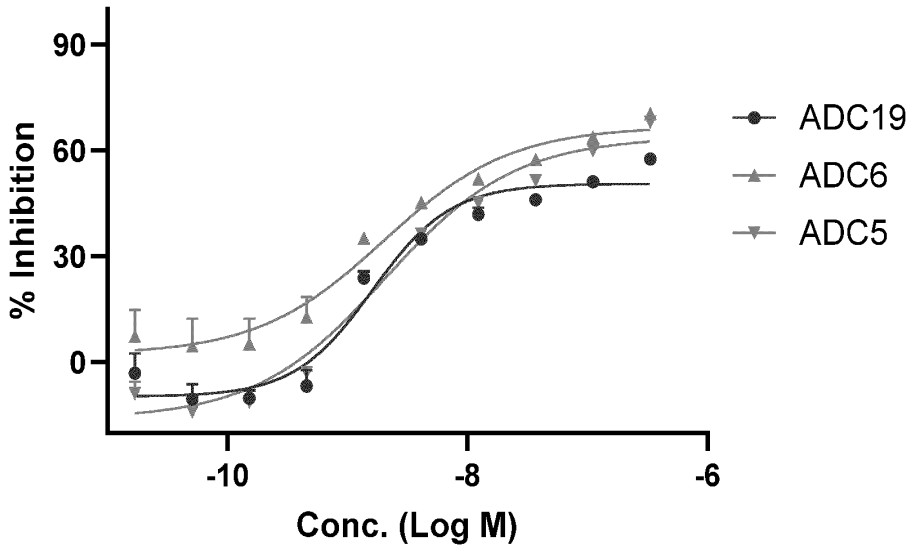


Fig. 31