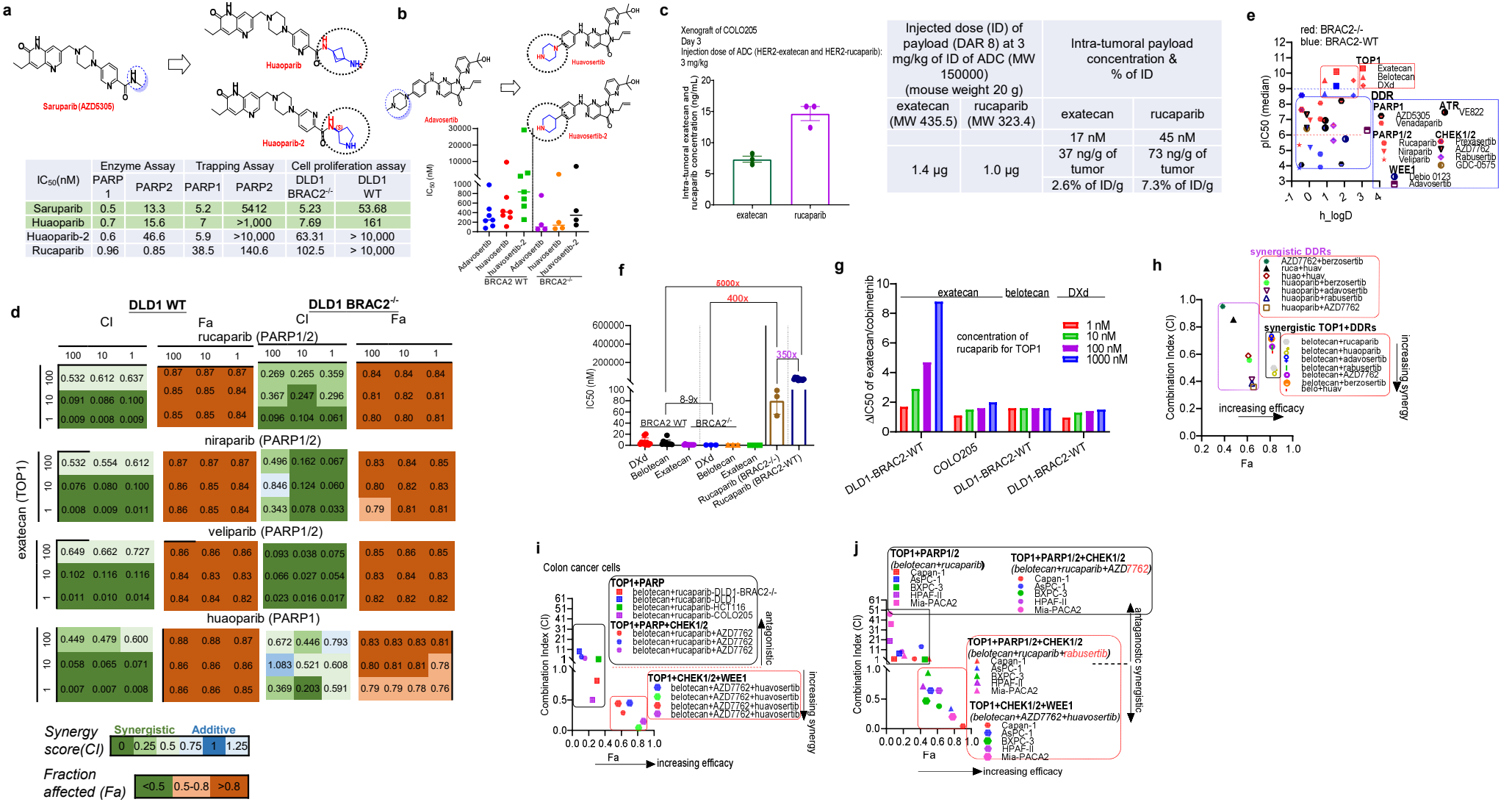


IC50(nM)	Enzyme Assay		Trapping Assay		Cell proliferation assay	
	PARP 1	PARP2	PARP1	PARP2	DLD1 BRAC2-/-	DLD1 WT
Saruparib	0.5	13.3	5.2	5412	5.23	53.68
Huaoparib	0.7	15.6	7	>1,000	7.69	161
Huaoparib-2	0.6	46.6	5.9	>10,000	63.31	> 10,000
Rucaparib	0.96	0.85	38.5	140.6	102.5	> 10,000

Injected dose (ID) of payload (DAR 8) at 3 mg/kg of ID of ADC (MW 150000) (mouse weight 20 g)		Intra-tumoral payload concentration & % of ID	
exatecan (MW 435.5)	rucaparib (MW 323.4)	exatecan	rucaparib
		17 nM	45 nM
1.4 μg	1.0 μg	37 ng/g of tumor	73 ng/g of tumor
		2.6% of ID/g	7.3% of ID/g

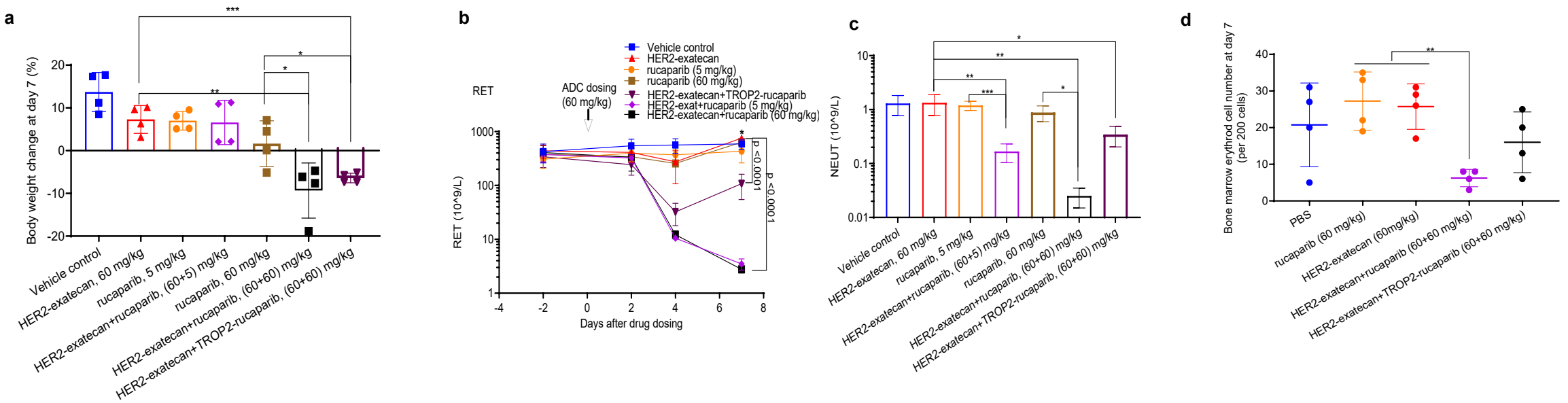
Extended Data Fig.1| Ratiometric synergy of T1000-ADC delivered TOP1 and DDR inhibitors.

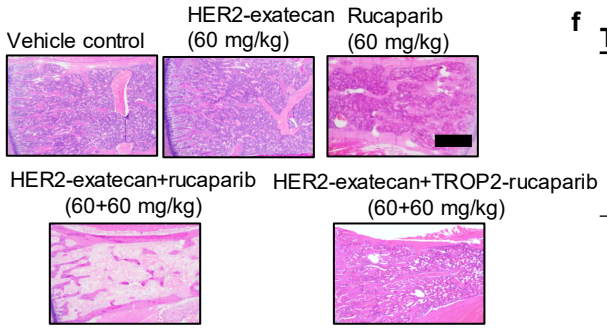
Extended Data Fig.1| Ratiometric synergy of T1000-ADC delivered TOP1 and DDR inhibitors.

a, b, Structural modifications (dotted circled) of PARP1-specific inhibitor Saruparib or WEE1 inhibitor Adavosertib to generate conjugatable Huaoparib (**a**) and Huavosertib (**b**), respectively. Enzymatic, PARP trapping (for Huaopraib) and in vitro cytotoxic assays were used to select Huaoparib and Huavosertib. **c,** Concentrations of exatecan and rucaparib in tumor samples harvested three days after dosing with 3 mg/kg (Injected dose, ID) of T-exatecan and T-rucaparib (DAR 8) ADCs in COLO205 xenograft. Intra-tumoral concentrations in ng/mL (left graph) or nM and percentage of ID (right table). Mean of three measurements. **d,** Synergy score and fraction affected (Fa) of TOP1 inhibitor exatecan with 3 PARP1/2 inhibitors and huaoparib (PARP1) in a 4x4 cytotoxicity combination assay. Combination index (CI) and Fa was calculated using Chou-Talalay method and score classification by color scheme is indicated below. **e,** Average potency (IC50) and hydrophobicity plot of TOP1 and DDR inhibitors. IC50 was measured in a panel of BRAC2-WT and BRAC2$^{-/-}$ cells. **f,** Relative in vitro cytotoxicity (IC50) of TOP1 inhibitors (exatecan/belotecan/DXd) and Rucaparib in isogenic DLD1-BRAC2 WT and DLD1-BRAC2$^{-/-}$ cells. Fold-difference of IC50 is indicated. **g,** In vitro drug ratio-dependent potentiation of TOP1 inhibitors by Rucaparib. Exatecan/Belotecan/DXd cytotoxicity (IC50, nM) was measured in the absence or presence of Rucaparib (1-1000 nM) and IC50 ratio (ΔIC50, mean from two independent measurements) was calculated. Cell lines are indicated. **h-j,** In vitro 2 or 3 drug combinations of TOP1 with DDR inhibitors and among DDR inhibitors in tumor cell lines. Drug concentration at 10 nM. Synergistic and antagonistic combinations were boxed in red and black squares, respectively. **h,** breast cancer cell line MDA-MB-436 (BRAC2-/-). **i,** five pancreatic cancer cell lines. **j,** four colon cancer cell lines.

TOP1, exatecan, belotecan, Dxd; PARP1/2, rucaparib, niraparib, veliparib; PARP1, saruparib, huaoparib; WEE1, adavosertib, huavosertib; T, Anti-HER2 Ab; RS7, anti-TROP2 Ab; DAR of ADCs are 8, unless otherwise indicated.

Unpaired two-sided t-test. *P<0.05, **P<0.01, ***P<0.001.





f

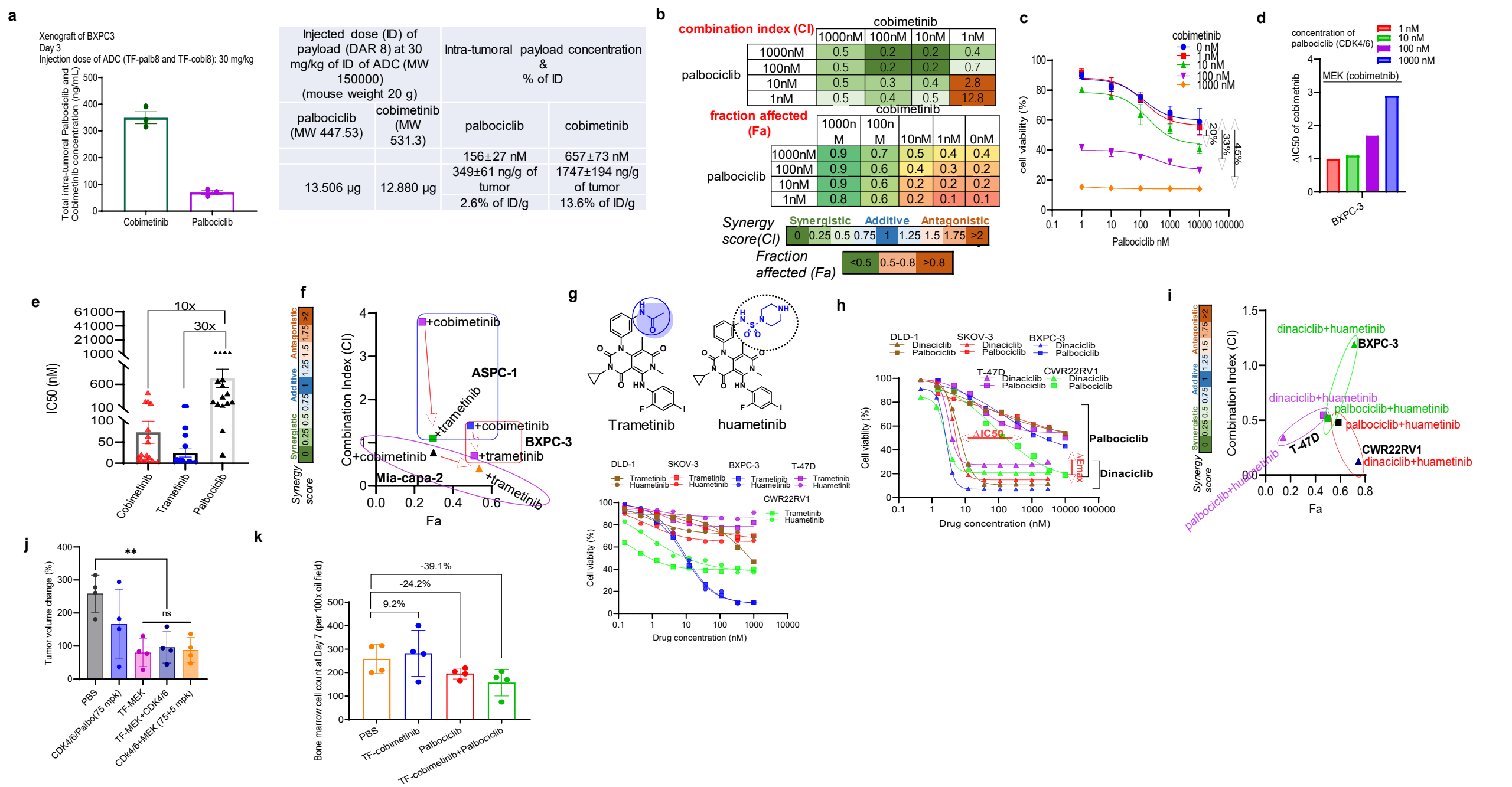
Table. Microscopic findings of decreased hematopoietic cellularity in rats

Group	Vehicle	rucaparib		T-exatecan	T-exatecan+RS7-rucaparib	T-exatecan+rucaparib	
Dose level(mg/kg)	-	5 or 60		60	60+60	60+5	60+60
Dose method	QD*5, iv	QD*5, po			single, iv	single+QD*5, iv+po	
Animal No.					4		
Bone marrow(Sternum) Cellularity decreased, Hematopoietic Cells							
Minimal	0	0		0	2	0	0
Moderate	0	0		0	0	2	1
Severe	0	0		0	0	2	3
Number affected	0	0		0	2	4	4

Extended Data Fig.2.| Additive toxicities of exatecan and rucaparib.

a-f, Comparative rat toxicity study of T-exatecan and combinations with rucaparib or RS7-rucaparib. Single dose of conjugated drugs or 5 daily dose of rucaparib was administrated. **a,** Body weight change (%) from baseline at day 7. **b,** Time course of reticulocytes. **c,** Neutrophils at day 7. **d,** Bone marrow erythroblast cell counts at day 7. **e,** Representative haematoxylin and eosin (H&E) staining of sternal bone marrows. Scale bars, 50 μm. **f,** A summary of microscopic findings of decreased hematopoietic cellularity in rats.

Unpaired two-sided t-test. *P<0.05, **P<0.01, ***P<0.001.

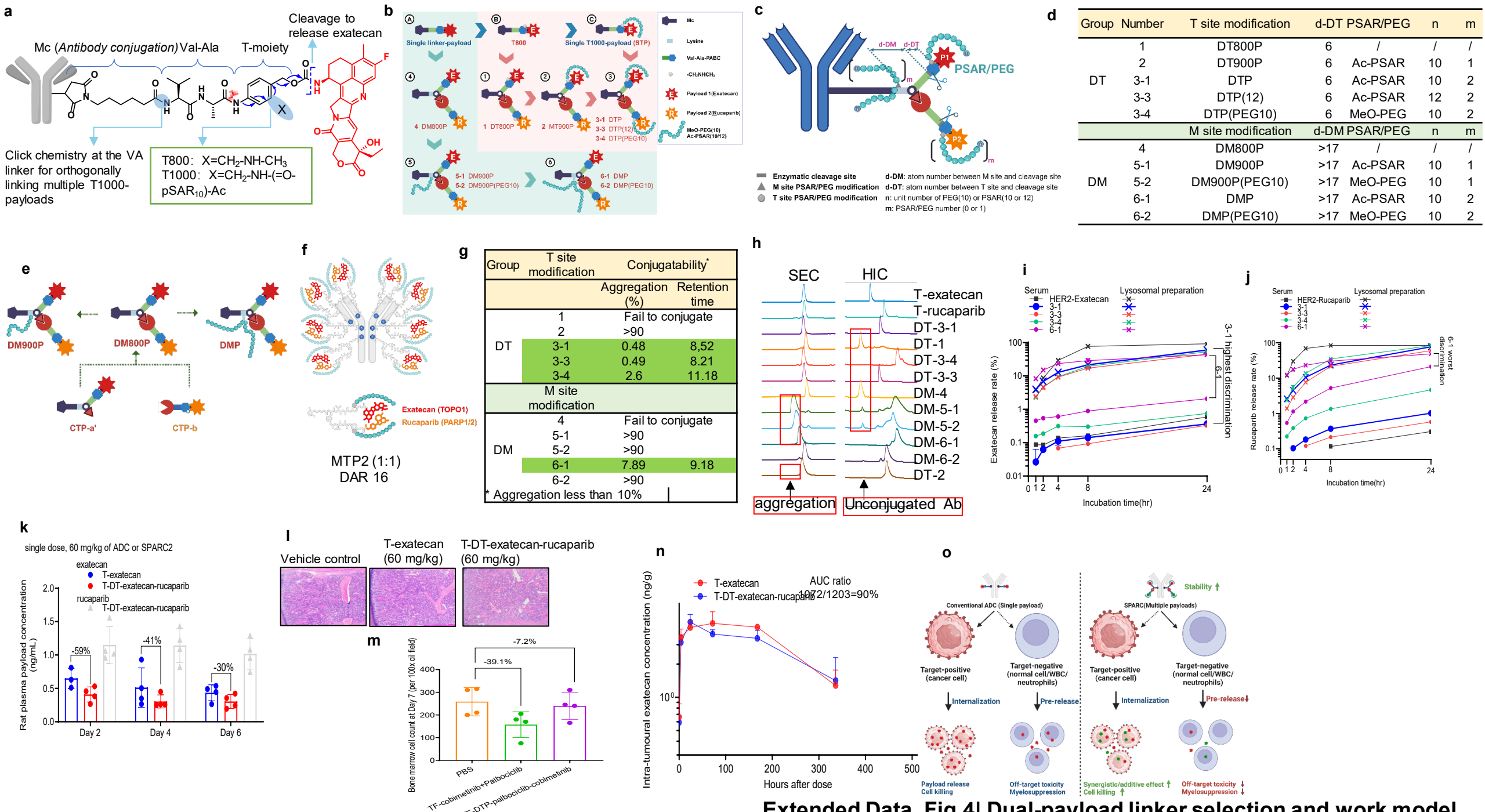


Extended Data Fig.3| Ratiometric synergy of T1000-ADC delivered CDKs and MEK inhibitors.

Extended Data Fig.3| Ratiometric synergy of T1000-ADC delivered CDKs and MEK inhibitors.

a, Concentration of Palbociclib (CDK4/6) and Cobimetinib (MEK) in BXPC3 tumor samples harvested three days after dosing with 30 mg/kg (Injected dose, ID) of Tissue factor (TF)-targeting T1000-ADCs, TF-Palbociclib and TF-Cobimetinib. Intra-tumoral concentration in ng/mL (left graph) or nM and percentage of ID (right table). Mean of three measurements. **b,** Synergy score and fraction affected (Fa) of Palbociclib and Cobimetinib in 4x4 cytotoxicity combination assay. Inhibitor concentration was 10-1000 nM. Combination index (CI) and Fa was calculated using Chou-Talalay method and score classification color scheme is indicated below. **c, d,** Mutual potentiation of Palbociclib and Cobimetinib. In vitro cytotoxicity of Palbociclib/Cobimetinib was measured in the presence of a concentration series (1-1000 nM) of Cobimetinib/Palbociclib. Palbociclib Emax improvement ($\Delta Emax$, mean from two independent measurements) relative to Palbociclib alone (**c**) or Cobimetinib IC50 ratio (ΔIC50, mean from two independent measurements) relative to Cobimetinib alone (**d**) was calculated, respectively. **e,** IC50 of Cobimetinib and Trametinib and Palbociclib in cancer cell lines. **f,** Palbociclib and Cobimetinib or Trametinib combination in pancreatic cancer cells. Red-arrowed line indicated the direction of synergy improvement. **g,** Structural modification (a dotted circle) of Trametinib to generate Huametinib. In vitro cytotoxic assays were used to select functional equivalent molecule of Trametinib. **h,** panCDK inhibitor dinaciclib has a higher cytotoxic potency than Palbociclib shown by $\Delta Emax$ and ΔIC50 on cell viability assay. **i,** In vitro (3 cell lines) combinations of Palbociclib and dinaciclib with Huametinib at an inhibitor concentration of 10 nM each. Red-arrowed line indicated the direction of synergy improvement. **j,** Tumor growth inhibition by combinations of TF-delivered MEK/free MEK with Palbociclib. BCPC-3 xenograft mice were intravenously administered with 30 mg/kg (QW*2) of ADC TF-cobimetinib. Each value represents the mean and SD (N=4 or 5 or otherwise indicated). **k,** Additive toxicity of combination of T1000-ADC delivered cobimetinib and Palbociclib. Mice bone marrow erythroblast cells at Day 7. TF-cobimetinib (30 mg/kg) was single-dose and Palbociclib for 5 days at 75 mg/kg in BXPC-3 xenograft.

Palbociclib, CDK4/6; Dinaciclib, panCDK; Cobimetinib, MEK; Trametinib and derivative huametinib, MEK; TF, Anti-Tissue Factor Ab. DAR of ADCs are 8, unless otherwise indicated. Unpaired two-sided t-test. *P<0.05, **P<0.01, ***P<0.001.
Synthesis chemistry is in Supplementary Information.



Group	Number	T site modification	d-DT	PSAR/PEG	n	m
DT	1	DT800P	6	/	/	/
	2	DT900P	6	Ac-PSAR	10	1
	3-1	DTP	6	Ac-PSAR	10	2
	3-3	DTP(12)	6	Ac-PSAR	12	2
	3-4	DTP(PEG10)	6	MeO-PEG	10	2
		M site modification	d-DM	PSAR/PEG	n	m
DM	4	DM800P	>17	/	/	/
	5-1	DM900P	>17	Ac-PSAR	10	1
	5-2	DM900P(PEG10)	>17	MeO-PEG	10	1
	6-1	DMP	>17	Ac-PSAR	10	2
	6-2	DMP(PEG10)	>17	MeO-PEG	10	2

Group	T site modification	Conjugatability*	
		Aggregation (%)	Retention time
DT	1	Fail to conjugate	
	2	>90	
	3-1	0.48	8,52
	3-3	0.49	8.21
	3-4	2.6	11.18
	M site modification		
DM	4	Fail to conjugate	
	5-1	>90	
	5-2	>90	
	6-1	7.89	9.18
	6-2	>90	

* Aggregation less than 10%

Extended Data Fig.4| Dual-payload linker selection and work model.

Extended Data Fig.4| Dual-payload linker selection and work model.

a, Structure of T1000 and T800 modification on the self-immolative pAb. Orthogonal attachment of second linker-payload structure on the VA linker site generates multi-payload SPARCs. Exatecan as payload is shown. **b**, Schematic of dual-payload linker design using exatecan (E) and rucaparib (R). A, B and C are single-payload, 1-3 are DT (PEG/PSAR on pAb) and 4-6 are DM (PEG/PSAR near VA site) structures. A, Single linker-payload with unmodified VA-pAb-payload. B, T800 with a *m*-methylaminomethyl group on pAb. C, Single T1000-payload with a polysarcosine10 (PSAR10) modification on structure B. 1, DT800P with two orthogonally linked T800-payloads. 2, DT900P with an addition of a single T1000 on DT800P (1). 3, Three DT structures with two paired T1000-payload but with different hydrophilic sidechain modifications, 3-1, 3-3 are modified with PSAR of different length (10, 12). 3-4 uses PEG10. 4, DM800P with an additional lysine between the Mc and VA group. 5, Two structures, DM900P (5-1) and DM900P(PEG10) (5-2) with a single PSAR10 or PEG10 addition to DM800P (4). 6, Two structures, DMP (6-1) and DMP(PEG10) (6-2) with two paired PSAR10/PEG10 modification-payload. Shape legend is in the side box. **c,** Variables for measuring steric hindrance and hydrophilic shield around the cleavage site. d-DM = number of atoms between M site and cleavage site; d-DT = number of atoms between T site and cleavage site. n = length of the hydrophilic PSAR/PEG sidechain (10, 12) and m = the number of sidechain (0, 1, 2). **d,** List of DT and DM structures and their structural variables defined in **b** and **c**. **e,** Schematic of DM900P and DMP synthesis. CTP-a' was synthesized by incorporating an additional L-lysine and L-propargylglycine between the Mc and VA groups. Copper-catalyzed azide-alkyne cycloaddition (CuAAC) of CTP-a' and CTP-b produced DM800P. Addition of one or two PSAR on DM800P yielded DM900P and DMP, respectively. **f,** Schematic of a dual-payload HER2 conjugate using DTP (3-1) linker-payload structure. DAR is 16. **g, h,** Conjugatability of dual-payload linker structures. Conjugates were analyzed by Hydrophobic Interaction Chromatograms (HIC) and Size Exclusion Chromatograms (SEC). **g,** Aggregation and hydrophobicity (retention time) of HER2-conjugates. Four (labeled green) were selected for in vitro payload release assay in **i**. **h,** HIC and SEC. Red boxed was aggregation on SEC and unconjugated antibody on HIC. T, Anti-HER2 Ab. **i, j,** In vitro payload release of representative HER2-dual-payload-linker conjugates. Conjugates were incubated with lysosomal preparation or mouse serum at 37 °C for 24 hours and payload release was measured by LC-MS. Payload release is presented as % of conjugates in PBS. **i,** exatecan release. **j,** rucaparib release. **k,** Rat plasma exatecan concentration at day 2, 4 and 6 after a single–dose of T-DT-exatecan-rucaparib or T-exatecan at 60 mg/kg. **l,** Comparative rat toxicity profile of T-DT-exatecan-rucaparib and T-exatecan. Representative haematoxylin and eosin (H&E) staining of sternal bone marrows. Scale bars, 50 μm. **m,** Comparative toxicity of dual-payload versus combination of T1000-ADC delivered cobimetinib and Palbociclib. Mice bone marrow erythroblast cells at Day 7. Dual-payload TF-DTP-palbociclib-cobimetinib (30 mg/kg) and TF-cobimetinib (30 mg/kg) was single-dose and palbociclib for 5 days at 75 mg/kg in BXPC-3 xenograft. **n,** Intra-tumoral exatecan concentration after a single-dose of T-DT-exatecan-rucaparib or T-exatecan at 3 mg/kg in COLO205 xenograft. **o,** Proposed work model of dual-payload linker structure DTP (3-1) in comparison to single-T1000 ADC. In target-negative cells, reduced payload release from DTP (3-1) mitigate additive toxicities of two payloads, resulting in a comparable systemic toxicity profile of DTP-conjugate and ADC. However, in target-positive cells, reduced payload is likely more than compensated by synergistic/additive interactions of co-internalized and synchronized (see Extended Data Fig. 7g-7k). This on-target and off-target pharmacological discrimination forms the basis of enhanced therapeutic window for SPARCs.

a

Suggested SPARC name/abbreviation rules

1) starting with SPARCn (n=2-6, number of payloads)
2) followed by target/antibody name (HER2 or T; TROP2 or hRS7)

3a) followed by concatenated payload name in parentheses and its DAR number: (exatecan-rucaparib-berzosertib)8

3b) alternatively, use target name: TOP1 or PARP1/2 or CDK4/6

Example 1: a HER2-targeting SPARC with exatecan (DAR 8), rucaparib (DAR 8) and VE-822 (DAR 8) is named:

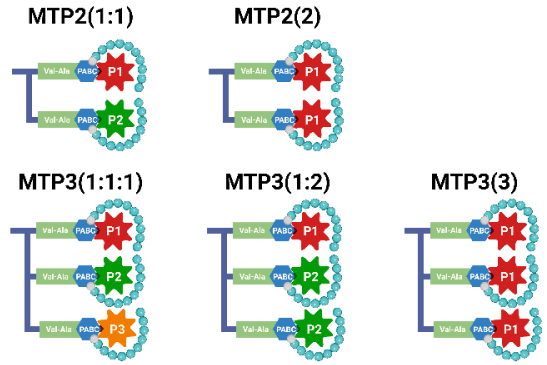
SPARC3-T-(exatecan-rucaparib-berzosertib)8 OR
SPARC3-T-(TOP1-PARP1/2-ATR)8

Example2: for THIOMAB, a HER2-targeting SPARC5 with exatecan, rucaparib and berzosertib at the DAR 2 conjugation and palbociclib and cobimetinib at DAR 8 conjugation is named:

SPARC5-T-(TOP1-PARP1/2-ATR)2-(CDK4/6-MEK)8

SPARCs in this study are numbered staring S1 to S120 in Supplement Information

b



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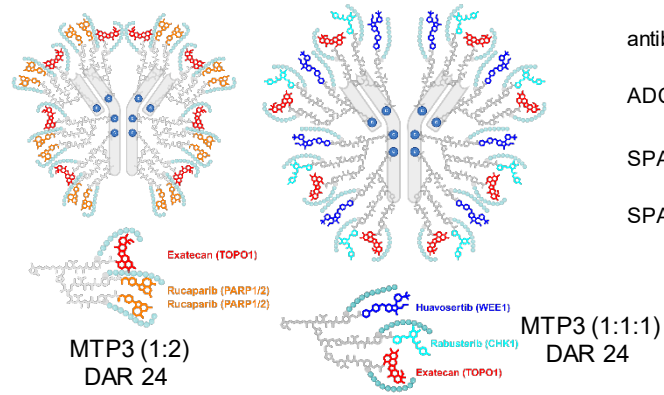

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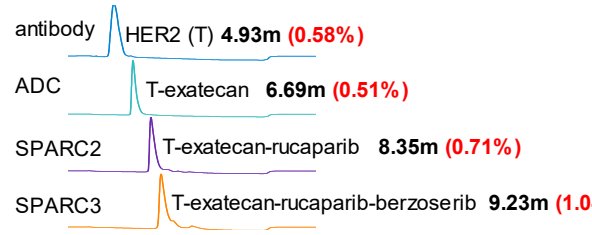

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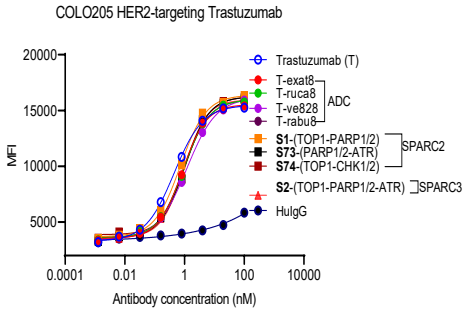

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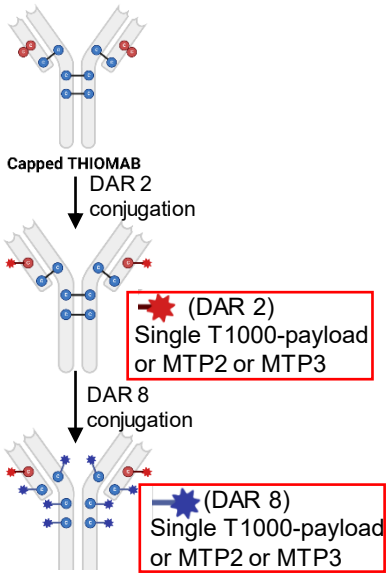

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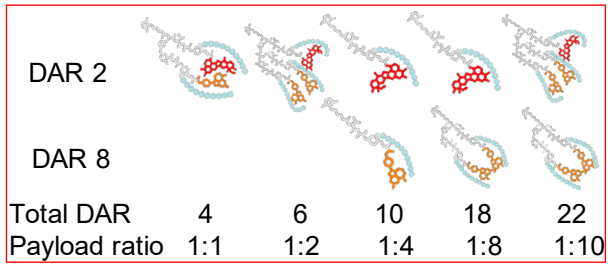

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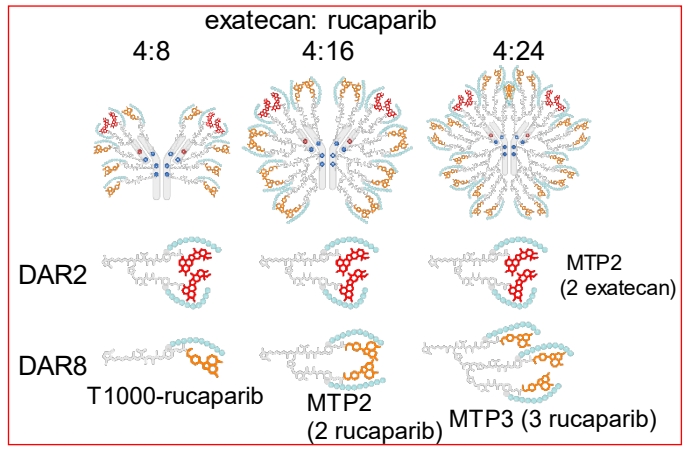

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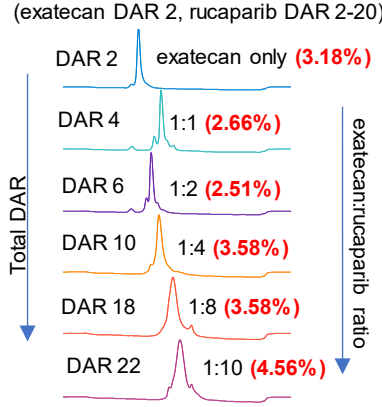
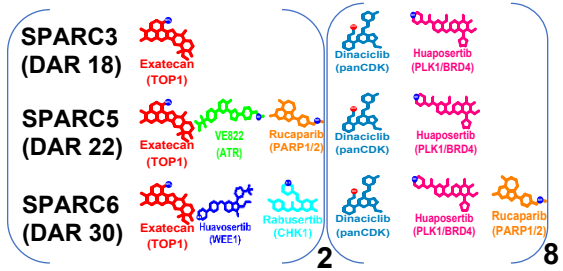


j

MTP for engineered cysteine conjugation	DAR (MS) (expected DAR 2)	MTP for native cysteine conjugation	DAR (MS) (expected DAR 8)
exatecan	1.82	/	-
Exatecan-rucaparib	1.76	/	-
exatecan-rucaparib-rucaparib	1.66	/	-
exatecan	1.67	rucaparib	8.25
exatecan	1.94	rucaparib-rucaparib	7.86
exatecan-rucaparib-rucaparib	1.85	rucaparib-rucaparib	6.61

k



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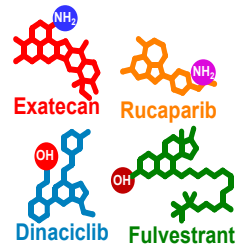

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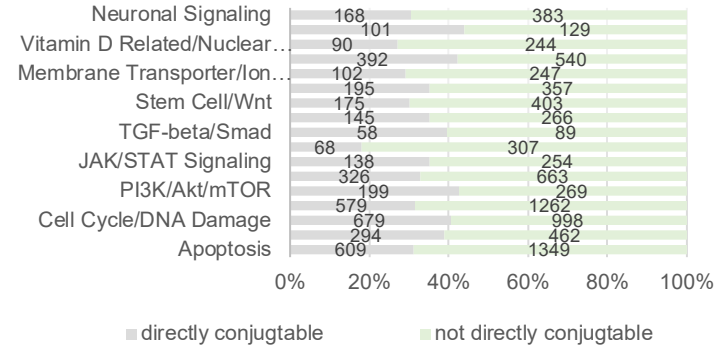


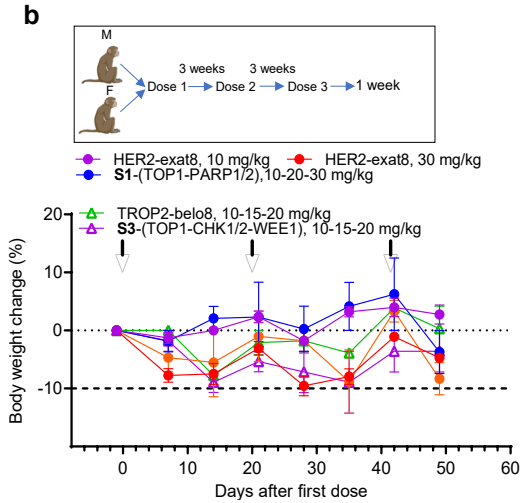
Extended Data Fig.5| Programmable SPARCs with diverse payloads.

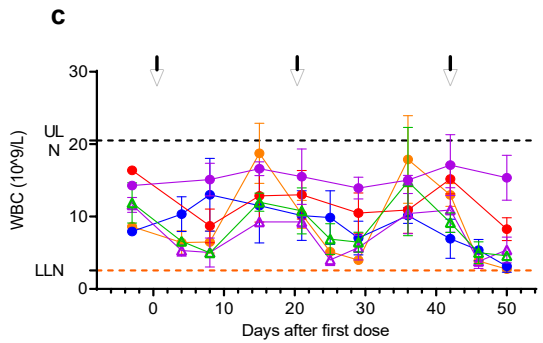
Extended Data Fig.5| Programmable SPARCs with diverse payloads.

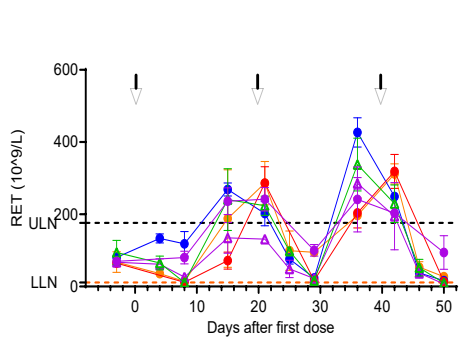
a, Suggested nomenclature/abbreviation for SPARCs. **b,** Multi-T1000 payload (MTP) structures. MTPs are orthogonally linked single T1000-payload units through click chemistry on the VA site. MTP2 and MTP3 contains 2 and 3 payloads (P1-P3). Payload ratio (when payloads are different) or total payload number (same payloads) in MTP2 or MTP3 is shown in parentheses. T1000 is shown as beaded strings. **c,** Representative SPARC2 and SPARC3 of TOP1 and DDR inhibitors by direct conjugation with a native antibody. MTPs used are shown. **d,** Representative HIC of HER2-targeting SPARC2 and SPARC3 (payload: exatecan, rucaparib and berzosertib), T-exateca, T-exatecan-rucaparib, and T-exatecan-rucaparib-berzosertib. Retention time (minutes/m) for each molecule is indicated in bold and percentage of aggregation measured by SEC is shown in parentheses. **e,** Flow cytometry of SPARCs and ADCs binding to cell surface targets relative to unconjugated antibody and isotype control (IgG). HER2-targeting molecules on COLO205. **f,** Two-step conjugation with a THIOLMAB. A single-T1000 payload or a MTP2/3 is conjugated to engineered cysteine residues at V205C on each antibody light chain (DAR 2) followed by a MTP2/3 to antibody's native cysteine residues (DAR 8). **g,** Building blocks for a SPARC2 (exatecan DAR 2) series of exatecan and rucaparib shown in **Fig.1h**. A combinatorial conjugation of shown payload structures with a THIOLMAB produced SPARC2s with an exatecan:rucaparib ratio from 1:1 to 1:10 . **h,** Three SPARC2s of exatecan (DAR 4) and rucaparib with an exatecan:rucaparib ratio 1:2, 1:4 and 1:6. **i,** HIC of PSMA-targeting SPARC2 series with exatecan and rucaparib ratio from 1 to 10. Retention time and percentage of aggregation is shown as in **d**. **j,** DAR measurement by Mass Spectrometry (MS) for SPARC2 series in **g**. Payload compositions of MTPs for DAR2 and DAR 8 conjugation are shown. MS only measured the number of cysteines (2 or 8) with conjugated MTPs or Conjugated DAR, not the total number of individual payloads (Total DAR for a SPARC). **k,** Structures of three SPARCs consisting of 3-6 payloads and DAR from 18 to 30. **l,** Representative nucleophilic amine (primary amine in exatecan and secondary amine in rucaparib) or hydroxyl (in dinaciclib)/phenolic (in Fulvestrant) group required for SPARC chemistry. **m,** An analysis of a 8,000-compound MedChemExpress anticancer library for directly conjugatable molecules. Drugs are classified by biology function/pathways.

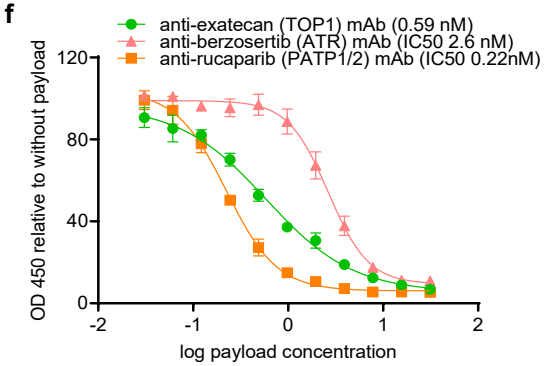
a

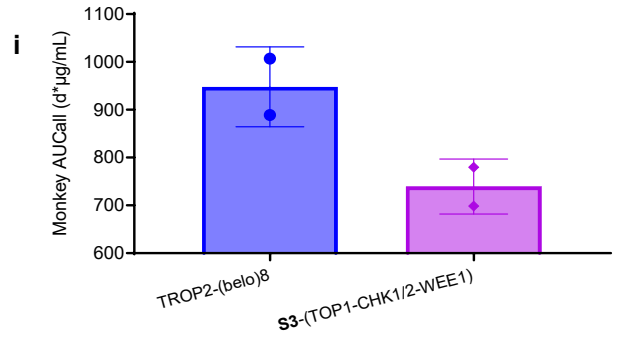
Species	Cynomolgus monkey				
Regimen	Q3W*2-3				
	HER2-targeting ADC/SPARCs			TROP2-targeting ADC/SPARCs	
Compound	ADC	**S1**-(TOP1-PARP1/2)	**S2**-(TOP1-PARP1/2-ATR)	ADC	**S3**-(TOP1-CHK1/2-WEE1)
	(exatecan)8	(exatecan-rucaparib)8	(exatecan-rucaparib-ve822)8	(belotecan)8	(beloletcan-rabusertib-huavosertib)8
Dose(mg/kg)	10-30	10-20-30	20-25-30	10-15-20	10-15-20
Dead/moribund	0	0	0	0	0
Clinical signs	-	-	skin discoloration, black	skin discoloration, black	skin discoloration, black
BW/FC	-	-	-	-	-
Hematology	RET ↓	RET ↓ RBC ↓	RET ↓ RBC ↓	-	RET ↓
Serum chemistry	-	-	-	ALT ↑	ALT ↑ AST ↑
Gross Pathology	small-sized thymuses	-	-	small-sized thymuses	small-sized thymuses
Histopathology	lymphocyte moderate reduction	thymus, kidney, bone marrow, stomach, intestines,	thymus, tonsil, adrenal glands, liver, bone marrow, intestines	thymus, kidney, tonsil, stomach, esophageal, skin	thymus, kidney, tonsil, stomach, esophageal, skin
HNSTD (mg/kg)	30	30	30	20	20

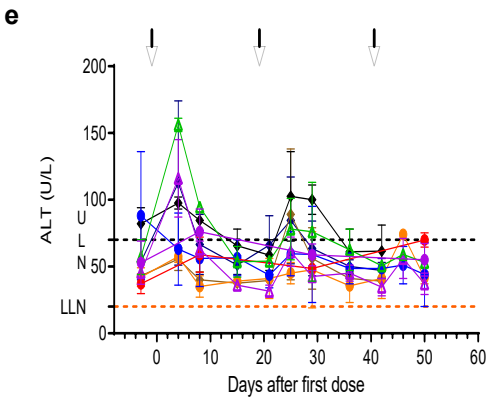


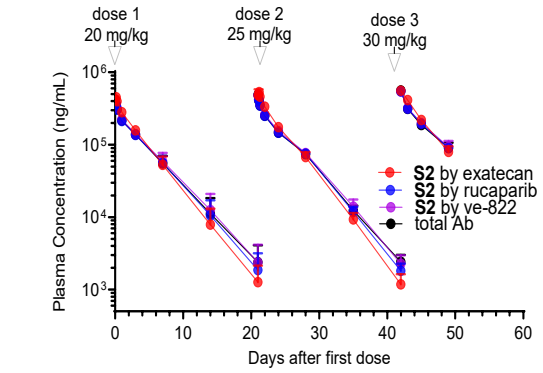


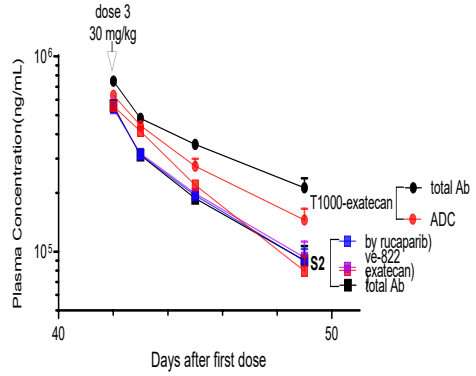


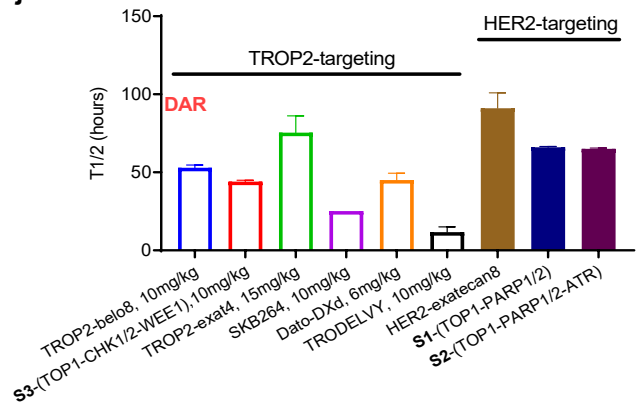








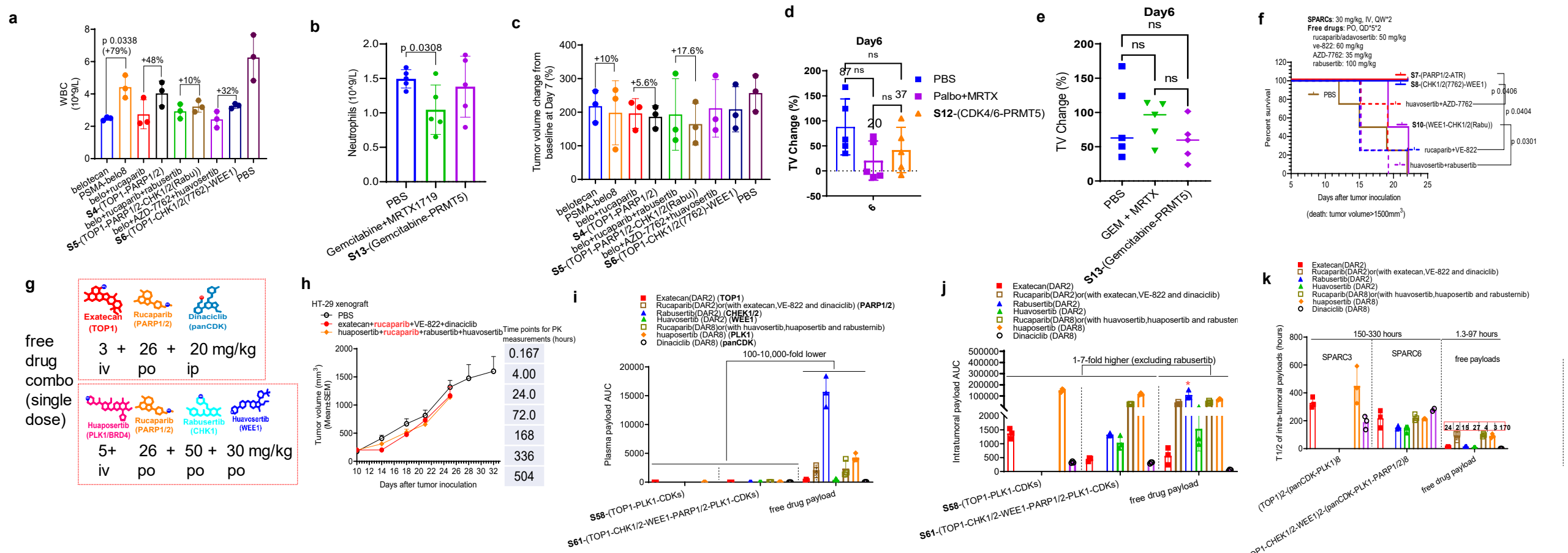




Extended Data Fig.6.| Comparative monkey tolerability and PK.

Extended Data Fig 6.| Comparative monkey tolerability and PK.

a-e, Comparative monkey toxicity study. For each molecule, 2 monkeys were given vehicles and indicated doses of drugs on day 1, 22, 43 and with a 1-week recovery period (shown in **b**). **a,** Summary of major toxicity findings of 3 HER2-targeting and 2 TROP2-targeting ADCs/SPARCs. HNSTD for each molecule is indicated. **b-e,** Representative monkey parameters. Groups are only labeled in **b**. Arrows indicated drug dosing time points. **b,** Body weight change. **c, d,** Representative hematology. **c,** Count of white blood cells. **d,** Count of reticulocytes. **e,** Representative serum chemistry. **f,** Affinity measurement of three anti-payload mAbs by payload competition binding assay. SPARC3-HER2-(exatecan-rucaparib-berzosertib)8 (**S2**-(TOP1-PARP1/2-ATR)) was used to coat ELISA plate and anti-payload mAb (10 ng/mL) was added together with a concentration series of payload molecules. IC50 for each antibody-payload binding is indicated. **g,** Monkey plasma concentrations of total antibody and **S2**-(TOP1-PARP1/2-ATR) measured independently by anti-payload antibodies. **h,** Comparative monkey payload PK of HER2-exatecan and SPARC3-HER2-exatecan-rucaparib-berzosertib (**S2**-(TOP1-PARP1/2-ATR)) at the third dose of 30 mg/kg. **i,** Monkey plasma exatecan AUC of TROP2-belotecan8 and SPARC3-TROP2-(belotecan-huavosertib-rabusertib)8 (**S3**-(TOP1-CHK1/2-WEE1)) at the dose of 10 mg/kg. **j,** Compiled $t_{1/2}$ in monkey of TROP2- and HER2-targeting ADCs and SPARCs. Drug dose is indicated for each measurement. Data source, 1. SKB264, Front Oncol. 12:951589 (2022); 2. TROP2-exatecan4, internal data; 3. Dato-DXd and Trodelvy, Mol Cancer Ther. 20, 2329-2340 (2021).

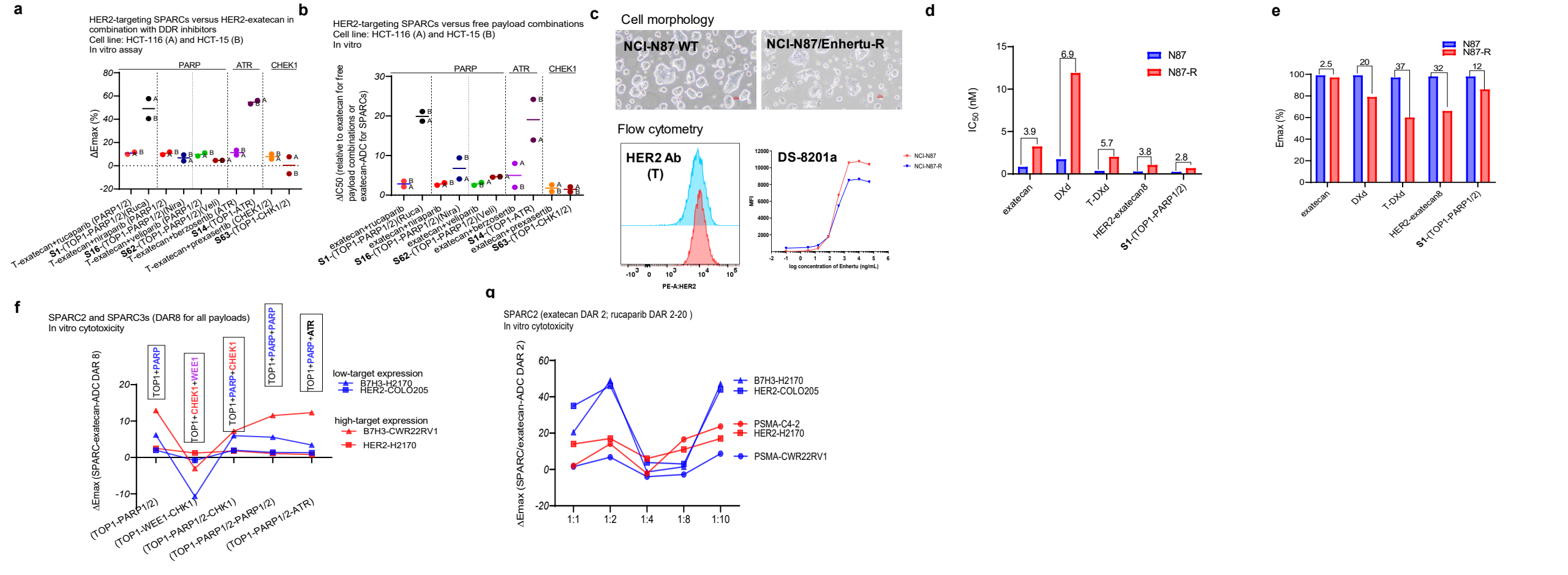


Extended Data Fig.7.| Toxicity and payload PK of SPARCs versus free drug combinations.

Extended Data Fig.7.| Toxicity and payload PK of SPARCs versus free drug combinations.

a, White blood cell counts of combinations of TOP1 and DDR or delivered by PSMA-targeting ADC/SPARCs in CWR22RV xenograft. **b,** Neutrophil counts of Gemcitabine and PRMT5 inhibitor combination and their TF-targeting SPARC in BXPC-3 xenograft. **c,** Tumor volume change from baseline at day 7 in CWR22RV1 xenograft. **d, e,** Tumor volume change from baseline at day 6 for CDK4/6 and PRMT5 (**d**) or of Gemcitabine and PRMT5 (**e**) and their TF-targeting SPARCs in BXPC-3 xenograft. **f,** Kaplan–Meier survival curves of DDR combinations and SPARCs in DLD1-BRAC2$^{-/-}$. Statistical analysis was performed using the log-rank Mantle–Cox test. **g, h.** On- and off-target PK of free drug combination versus SPARCs in HT-29 xenograft. **g,** Free 3- and 4-drug combinations. Dose and administration route for each drug is indicated. Rucaparib is shared by two groups. **h,** Tumor growth curve for HT-29. Time points for tumor and plasma payload PK measurements are indicated. **i,** Mice plasma AUC. The range of fold-difference of payload AUC between SPARCs and free drug combinations is indicated. **j,** Mice intra-tumoral payload AUC. **k,** Intra-tumoral half-life ($t_{1/2}$) of individual drugs of SPARCs or free drug combinations. Fold increase of SPARC payloads $t_{1/2}$ versus free drugs was indicated for each payload (inside red rectangle). Doses for **a, c**: SPARCs (5mg/kg, single dose); belotecan (5mg/kg, single dose); rucaparib (43 mg/kd, QD*5); rabusertib (50 mg/kg, QD*5); AZD-7762(35 mg/kg, QD*5). Doses for **b,d,e**: SPARCs (25mg/kg, single dose); gemcitabine (10mg/kg, single dose); MRTX1719 (25 mg/kg, QD*5); palbociclib (75 mg/kg, QD*5).

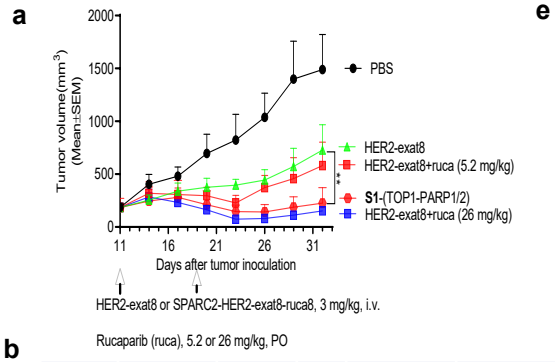
In all in vivo studies, xenograft mice were intravenously administered with indicated doses of ADCs/SPARCs (QW*2) or free payloads (QD*5*2), unless otherwise indicated. Each value represents the mean and SD (N=3-5 or otherwise indicated). Unpaired two-sided t-test. *P<0.05, **P<0.01, ***P<0.001.

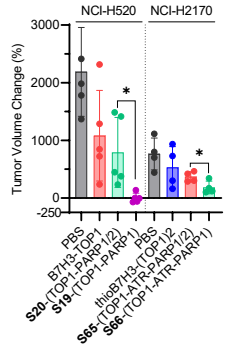


Extended Data Fig.8| In vitro cytotoxicity of SPARCs.

Extended Data Fig.8.| In vitro cytotoxicity of SPARCs.

a-d, In vitro synergy improvement of TOP1-DDR inhibitor SPARCs relative to combinations of TOP1-ADC/free TOP1 with free DDR. Cells were incubated with a concentration series of drug groups for 5 days and cell viability was measured by CCK8 and IC50/Emax for each drug/cell line was calculated from cell viability-concentration plot. Data represent mean of 3 independent measurements. For ADC combined with free DDR, ΔIC50 is the ratio of (TOP1-ADC)/(TOP1-ADC+DDR inhibitor) or (TOP1-ADC)/(SPARC). ΔEmax is Emax of (TOP1-ADC+DDR)-(TOP1-ADC) or (SPARC)-(TOP1-ADC). For free drug combinations, ΔIC50 and ΔEmax is the difference between TOP1 and (TOP1+DDR). **a, b,** Efficacy (**a**) and potency (**b**) of HER2-targeting SPARCs in HCT-116 and HCT-15 in comparison to TOP1-ADC+DDR (**a**) or free TOP1+DDR (**b**). **c-e,** Cytotoxicity of HER2-ADCs and SPARC2-HER2-(exatecan-rucaparib)8 (**S1**-(TOP1-PARP1/2)) in a cell line resistant to DS-8201a. **c,** Characterization of cell line N87-R by growth morphology and HER2- and DS-8201a-binding by flow cytometry. **d, e,** IC50 and Emax difference of exatecan and DXd (free payload), HER2-exatecan and DS-8201a (ADCs) and SPARC2 in N87 and N87R. Fold-difference is indicated. **f, g,** In vitro efficacy improvement of SPARCs relative to single-payload ADC measured by ΔEmax. ADCs/SPARCs targeting HER2 and B7H3 were assayed. ΔEmax is the difference between Emax of ADC and SPARCs. Data represent mean of 3 independent measurements. Target expression level for each cell line was indicated. **f,** SPARC2s of exatecan and rucaparib and SPARC3s of exatecan and two DDR inhibitors. All payloads were DAR 8. **g**, SPARC2 series of exatecan and rucaparib at 1:1 to 1:10 ratio. Exatecan DAR was 2.





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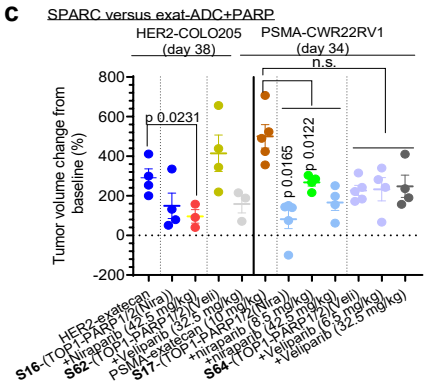
Tumor volume change from baseline (%)			Exat+								Belo+			Ruca+
				PARP/PARP1				CHEK1		ATR	PARP			
Tumor type	Cell line	target		rucaparib	niraparib	huaoparib	veliparib	rabusertib	prexasertib	berzosertib	rucaparib	niraparib	huaoparib	berzosertib
Colon	COLO205	HER2	567	107	270	105	409	193		193	58		358	769
	SW837	HER3	78	1	2	32					22	13	33	
	HT-29	HER2		-57	-4	-77					-48	-72	115	
Breast	MDA-MB-436	B7H3	-33	-31	-29	-30					-25			94
Pancreatic	BXPC-3	Tissue	13	-45	-47	-44					-14		-55	
	HPAF-II	Factor	-65.3	-90.7	-83.2	-70.3					-83.6	-46.6	-70.0	
Prostate	CWR22RV1	PSMA	549	310	429	551	220		406	148	363		603	
Ovarian	SKOV-3	CDH6	592	316	365					429				
		FOLR	551	229	311					355				
Mean			341.3	94.0	158.7	89.4	281.3	193.0			59.5	-29.1	210.9	431.6

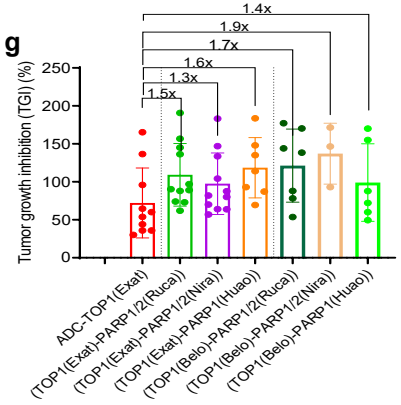
b

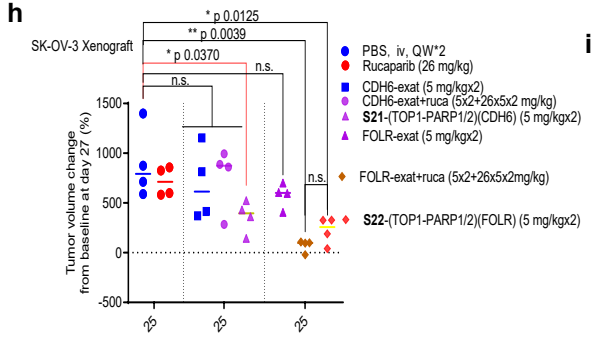
Target of payload	Payload	Molecular weight	DAR	Total ADC dose (mg/kg)*	Equivalent free payload dose (mg/kg)	500x payload dose (mg/kg/day)#	2500x payload dose (mg/kg/day)
TOP1	Exatecan	435.5	8	10	0.232	11.6	58
PARP	Rucaparib	323.4	8	10	0.172	8.6	43
PARP	Niraparib	320.4	8	10	0.17	8.5	42.5
ATR	Berzosertib	463.6	8	10	0.248	12.4	62
CHK1	Prexasertib	365.4	8	10	0.194	9.7	48.5

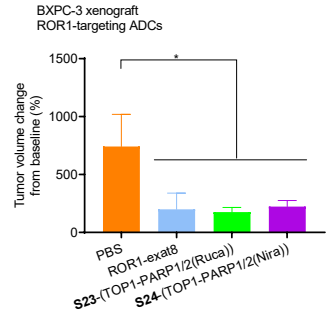
** ADC was given twice at day 14 and day 21 after tumor cell inoculation, each at 5 mg/kg*

 # Free payload drug was given at indicated dose for two consecutive 5 days (days 14-18 and days 21-25)









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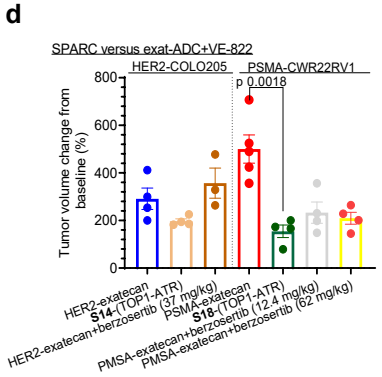
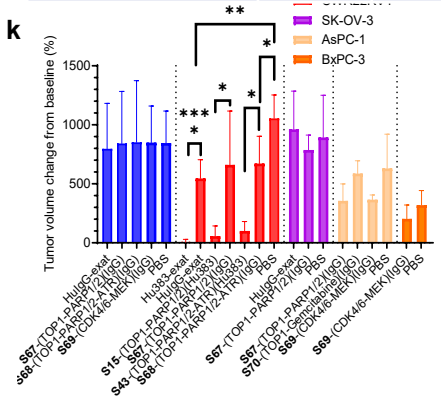
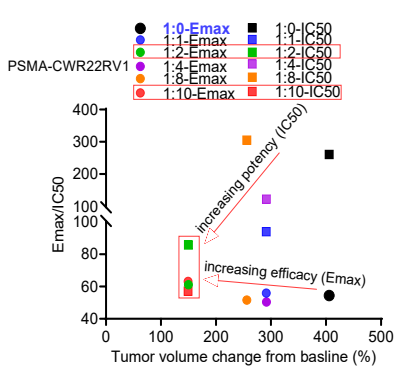
Days after TROP2-targeting ADC dosing	Number of mice with a Complete Response (Tumor volume 0)			
BXPC3 Xenograft	exatecan	belotecan	belotecan-rucaparib (**S26**)	belotecan-rucaparib-rabusertib (**S25**)
16	1/4	1/4	1/4	2/4
19	0/4	0/4	1/4	3/4
22	1/4	1/4	2/4	4/4

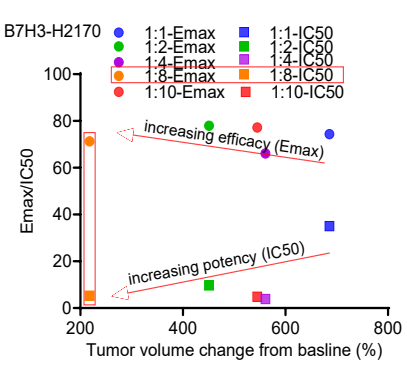
n

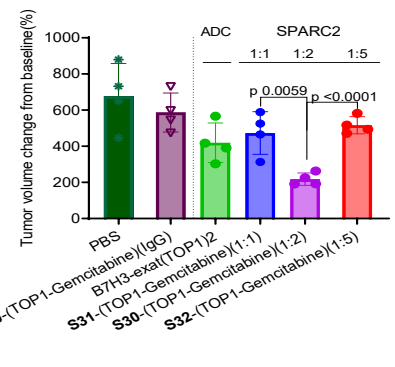
	exatecan/ gemcitabine ratio	dose	
ADC-exatecan2		20 mg/kg	all have equal exatecan dose at 40 (DAR*dose)
S31-(TOP1-Gemcitabine)	1:1	20 mg/kg	
S30-(TOP1-Gemcitabine)	1:2	10 mg/kg	
S32-(TOP1-Gemcitabine)	1:5	20 mg/kg	









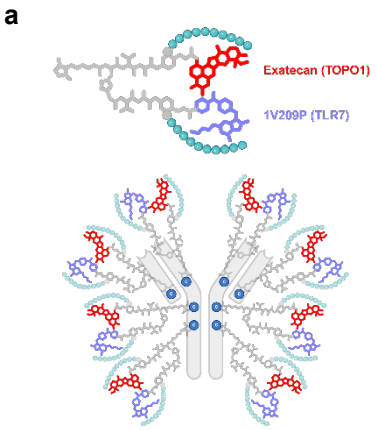


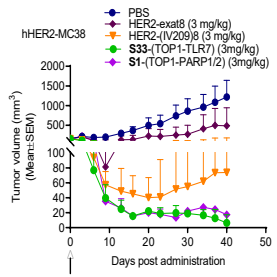
Extended Data Fig.9| In vivo synergy of SPARCs.

Extended Data Fig.9.| In vivo synergy of SPARCs.

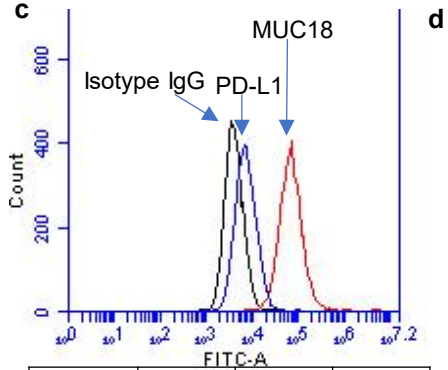
a, In vivo tumor growth and inhibition by HER2-targeting exatecan-ADC, SPARC2-(exatecan-rucaparib)8 (**S1**-(TOP1-PARP1/2)) and exatecan-ADC plus rucaparib. Doses and schedule are labeled. **b,** Tabulation of injected dose (ID) of free drugs equivalent to those delivered by ADCs at a dose of 5 mg/kgx2 (QW*2). The daily dosing (for 10 ten days) of free drugs at 500- and 2500-fold of payloads delivered by ADC was calculated. **c, d,** In vivo efficacy of SPARC2 (exatecan-DDR inhibitors) versus exatecan-ADC in combination with different dose of DDR inhibitors measured by tumor volume change from baseline. HER2-targeting and PSMA-targeting molecules were assayed in COLO205 and CWR22RV1, respectively. Doses for ADCs/SPARCs were 5mg/kg. **c,** PARP1/2 inhibitors. **d,** ATR and CHEK1/2 inhibitors. **e,** Comparison of PARP1-specific huaoparib and rucaparib in squamous cell carcinoma models. Doses for ADCs/SPARCs were 10mg/kg .**f, g,** Summary of in vivo efficacy of seven targets in eight cell lines representative of five tumor types. TOP1 inhibitor (exatecan/belotecan)-ADCs, SPARC2 of TOP1 and DDR inhibitors, or SPARC2 of two DDR inhibitors were assayed. SPARC2s were dosed at 3 or 5 mg/kg (single dose or QW*2). **f,** Efficacy was measured by tumor volume change from baseline. **g,** Tumor growth inhibition by payload combination. Fold improvement of SPARC2s of (exatecan/belotecan-indicated PARP1/2 or PARP1 inhibitor) was calculated relative to exatecan-ADC. **h,** Ineffectiveness of two exatecan-ADCs against low-expression targets was overcome by respective SPARC2 of exatecan and rucaparib. For CDH6, the combination of exatecan-ADC with high-dose free rucaparib was ineffective but SPARC2 was. **i,** ROR1-targeting SPARC2s of exatecan and rucaparib/niraparib effectively lowered DAR requirement for exatecan-ADC. Doses for ADCs/SPARCs were 10 mg/kg. **j,** In vivo efficacy of TROP2-targeting SPARCs in a BXPC-3 xenograft model measured by number of mice with a complete response defined as tumor volume reaches zero. TROP2-exatecan/belotecan ADC was compared to a single dose of 3 mg/kg of SPARC2-exatecan-rucaparib (**S26**-(TOP1-PARP1/2)) and SPARC3-exatecan-rucaparib-rabusternib (**S25**-(TOP1-PARP1/2-ATR). **k,** Summary of efficacy of control SPARCs of isotype IgG in different tumor models. In CWR22RV1, SPARCs of isotype IgG showed significant antitumor efficacy relative to PBS. However, PSMA-targeting SPARCs were significantly more effective than SPARCs of isotype IgG. Doses: (CDK4/6-MEK) SPARCs were dosed at 30mg/kg; exatecan-ADCs were dosed at 20mg/kg in CWR22RV1; ADCs/SPARCs were dosed at 5mg/kg in SK-OV-3; Other ADCs/SPARCs were dosed at 10mg/kg in the rest models. **l, m,** Correlation of SPARC in vivo antitumor efficacy with in vitro synergy. Tumor volume change from baseline (%) of PSMA(**l**) and B7H3 (**m**) -targeting SPARC2 series of exatecan-rucaparib with a ratio of 1:1 to 1:10 in CWR22RV1 and H2170 was plotted against their in vitro potency (IC50) and efficacy (Emax) data, respectively. SPARC2s with best in vivo efficacy are boxed. SPARC2s were dosed at 10mg/kg in H2170, and 20 mg/kg in CWR22RV1. **n,** ADC and SPARCs of exatecan and gemcitabine for in vivo efficacy study in **o**. Drugs were dosed to yield same injected dose for exatecan. **o,** Tumor growth inhibition by B7H3-targeting ADC/SPARCs from **n** in ASPC-1 xenograft.

In all in vivo studies, xenograft mice were intravenously administered with indicated doses of ADCs/SPARCs (QW*2) or free payloads (QD*5*2), unless otherwise indicated. Each value represents the mean and SD (N=3-5 or otherwise indicated). Unpaired two-sided t-test. *P<0.05, **P<0.01, ***P<0.001.

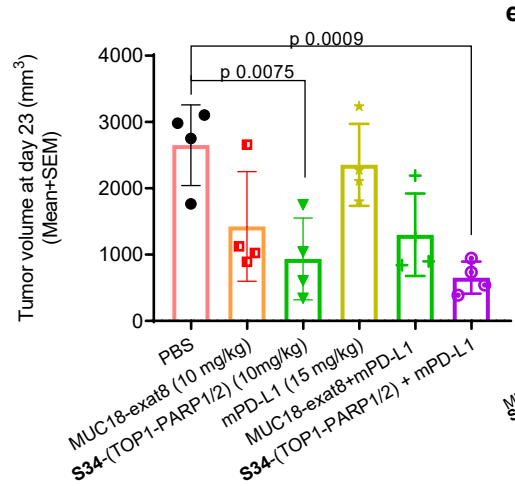


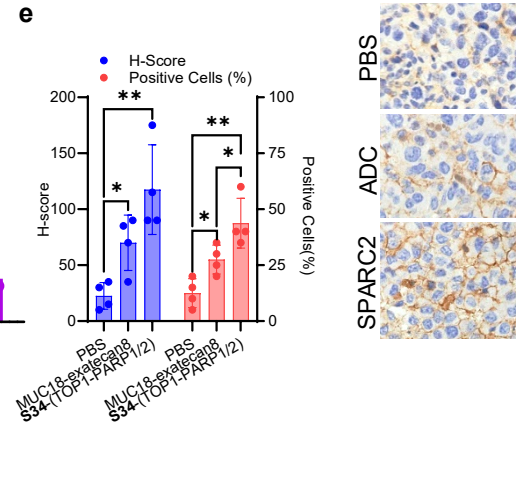


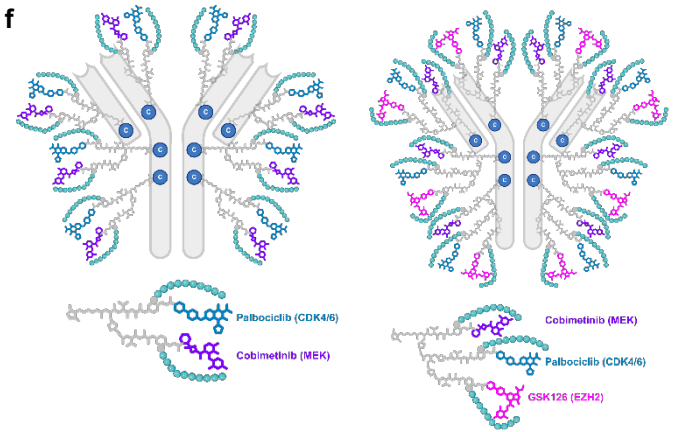
Days after HER2-targeting ADC/SPARC dosing	Number of mice with a Complete Response (Tumor volume 0)			
hHER-MC38 Xenograft	exat	TLR8	exat-ruca	exat-TLR8
37	0	1	0	1
40	0	1	0	2

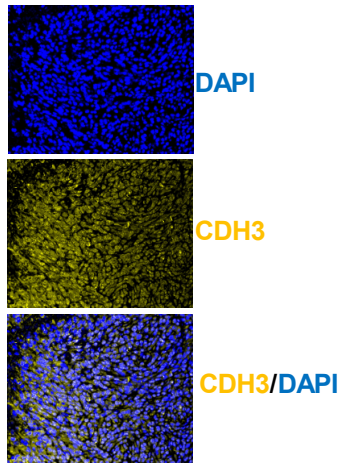


	MFI	% Pos.	rMFI
PD-L1	2,463.0	11.78%	1.50
MUC18	71,063.5	99.88%	17.81







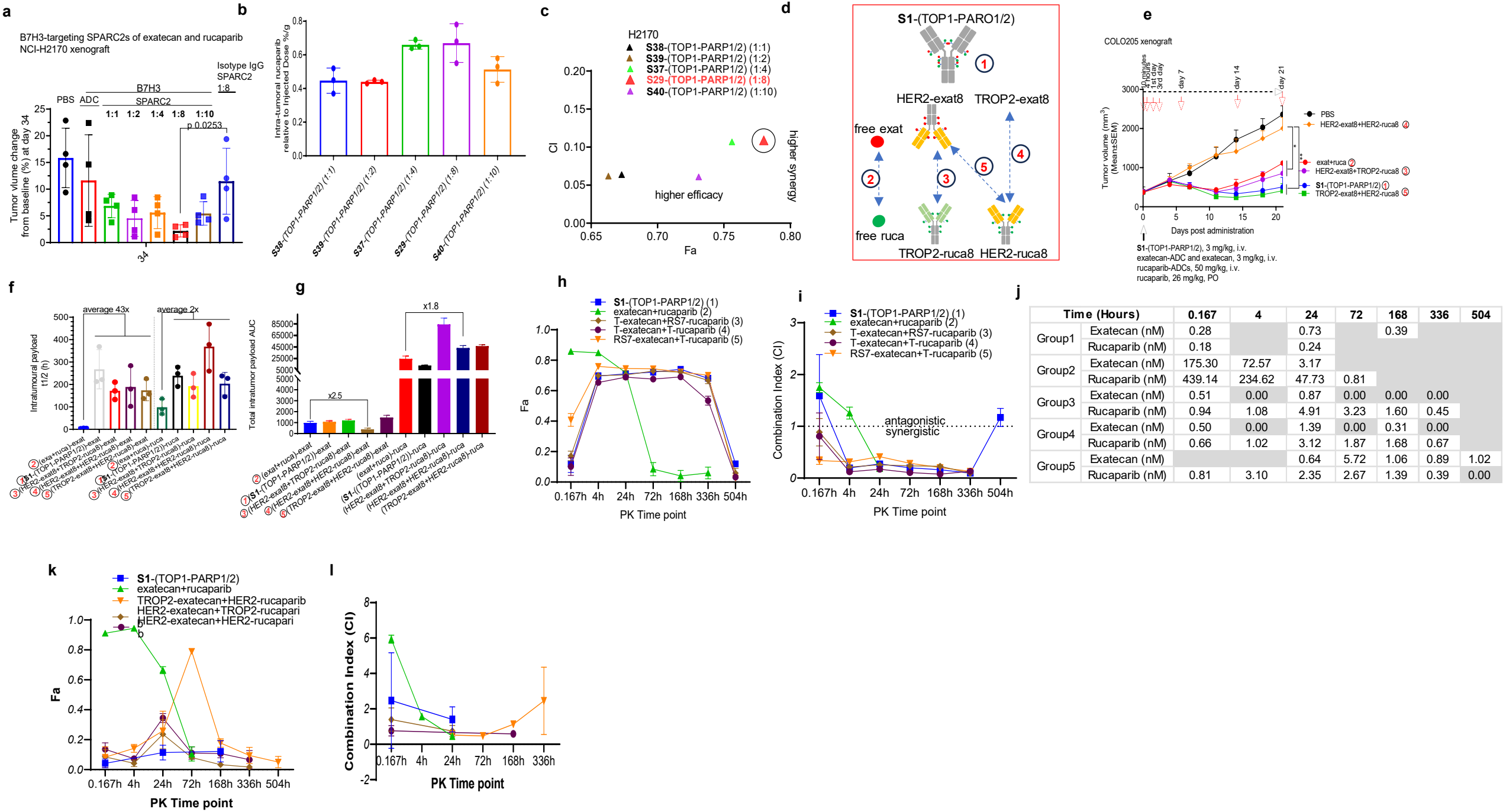


Extended Data Fig.10| SPARCs of targeted and immune-stimulating molecules.

Extended Data Fig.10| SPARCs of targeted and immune-stimulating molecules.

a, Structure of SPARC2-(exatecan-1V209)8 (TOP1-TLR7) (**S33**-TOP1-TLR7). **b,** In vivo efficacy of a single-dose ADCs/SPARCs in a synergic HER2-M38 xenograft. Number of mice with a complete response (tumor volume zero) is tabulated. **c,** Flow cytometry of PD-L1 and MUC18 expression in M3 cell line. **d,** MUC18-targeting ADC and SPARC in combination with mPD-L1 in M3 xenograft. **e,** PD-L1 expression in tumor tissues of M3 xenograft. Left shows H-score and % of positive cells. Right shows representative IHC images. **f,** Structure of CDH3-targeting SPARC2-(palbociclib-cobimetinib)8) (**S35**-(CDK4/6-MEK/cobi) (CDK4/6-MEK) and SPARC3-(palbociclib-cobimetinib-GSK126) (**S36**-(CDK4/6-MEK/cobi-EZH2/GSK126)) (CDK4/6-MEK-EZH2). **g,** Immunofluorescence staining of frozen sections from KPC tumor with a CDH3-specific antibody. KPC tumor is CDH3-positive.

In all in vivo studies, xenograft mice were intravenously administered with indicated doses of ADCs/SPARCs (QW*2). Each value represents the mean and SD (N=3-5). Unpaired two-sided t-test. *P<0.05, **P<0.01, ***P<0.001.



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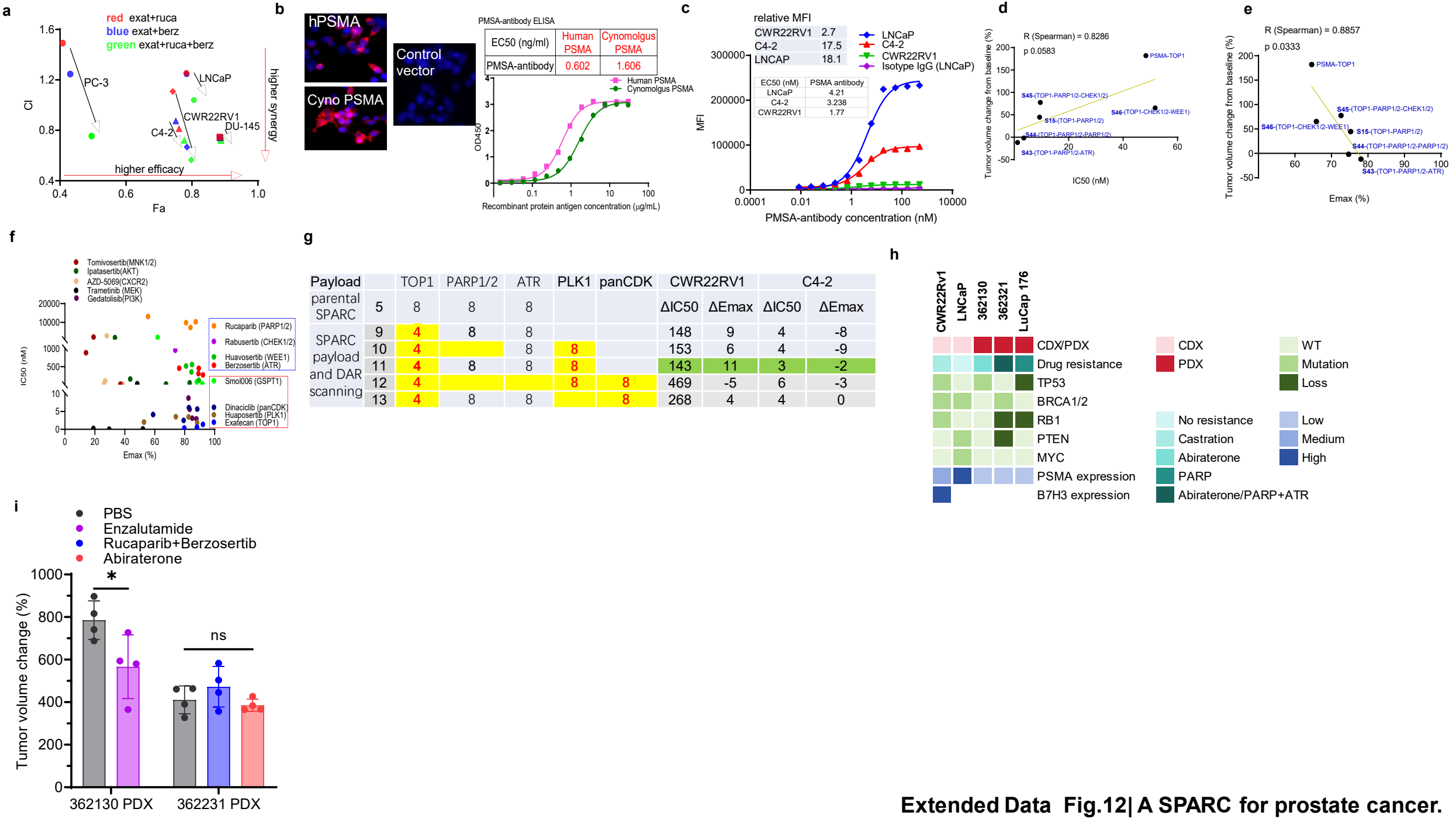
Time (Hours)		0.167	4	24	72	168	336	504
Group1	Exatecan (nM)	0.28		0.73		0.39		
	Rucaparib (nM)	0.18		0.24				
Group2	Exatecan (nM)	175.30	72.57	3.17				
	Rucaparib (nM)	439.14	234.62	47.73	0.81			
Group3	Exatecan (nM)	0.51	0.00	0.87	0.00	0.00	0.00	
	Rucaparib (nM)	0.94	1.08	4.91	3.23	1.60	0.45	
Group4	Exatecan (nM)	0.50	0.00	1.39	0.00	0.31	0.00	
	Rucaparib (nM)	0.66	1.02	3.12	1.87	1.68	0.67	
Group5	Exatecan (nM)			0.64	5.72	1.06	0.89	1.02
	Rucaparib (nM)	0.81	3.10	2.35	2.67	1.39	0.39	0.00

Extended Data Fig.11| SPARC pharmacology and toxicology.

Extended Data Fig.11| SPARC pharmacology and toxicology.

a-c, Structure-PK-efficacy relationship of SPARC2-B7H3-(exatecan)2-(rucaparib)2-20 series dosed at 10 mg/kg in H2170 xenograft. Antitumor efficacy (day 34) and intra-tumoral exatecan/rucaparib concentrations (day 7) of SPARC2s from B7H3-(exatecan)2-(rucaparib)2-20 series were measured in H2170 xenograft. **a,** Tumor growth inhibition. **b,** Intra-tumoral rucaparib concentration normalized to injected dose (ID). **c,** In vitro synergy and Fa of exatecan and rucaparib at intra-tumoral concentrations. exatecan:rucaparib at 1:8 data point is circled. **d,** Drug groups for plasma and intra-tumoral payload PK study. Different combinations of two free payloads, an ADC and a payload and two ADCs were numerically labeled. **e,** Mouse efficacy and PK study design. COLO205 tumors were dosed when reached at 200 mm^3 and tumor/plasma samples were taken at indicated time points. Exatecan and rucaparib concentration was measured by an established method using LC-MS. Dose: ADCs/SPARC (3 mg/kg for TOP1-ADCs and SPARC; 50 mg/kg for PARP1/2-ADCs); drug combination groups (3 mg/kg for exatecan; 26 mg/kg for rucaparib). Unpaired two-sided t-test. *P<0.05, **P<0.01. **f,** Payload $t_{1/2}$ comparison. **g,** Total intra-tumoral payload ADC. **h, i,** In vitro exatecan and rucaparib combination assays at intra-tumoral concentrations. **h,** Time course of Fa. **i,** Time course of CI. **j-l,** Measured plasma payload concentrations and in vitro combination assay. **j,** Plasma exatecan and rucaparib concentrations measured for different groups. Payloads at grayed time points were undetectable. **k, l,** Fa and CI of in vitro combination assay using concentrations from j in $CD34^+$bone marrow cells. **k,** Fa. **l,** CI.



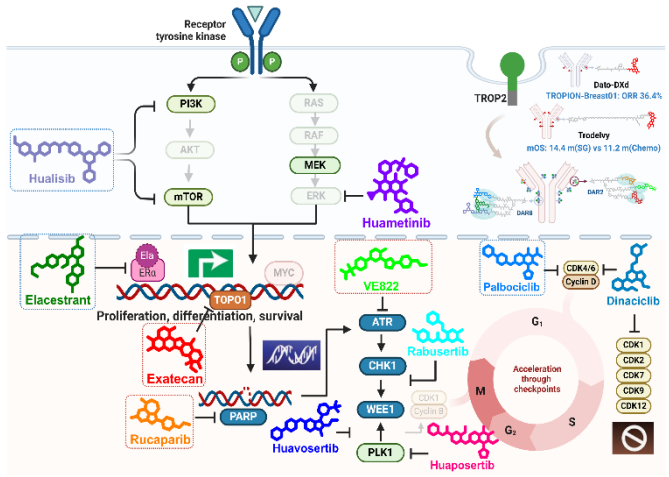
Extended Data Fig.12| A SPARC for prostate cancer.

Extended Data Fig.12| A SPARC for prostate cancer.

a, TOP1, PARP1/2 and ATR combinations in prostate cancer cell lines. Payload concentration at 10 nM and Fa and CI was plotted. Arrow-head lines indicate increasing synergy. **b, c,** Characterization of a humanized anti-PSMA antibody for SPARC development. **b,** binding affinity measured by its binding to prostate cancer cell lines with different level of PSMA expression. **c,** Specificity of PSMA-antibody and relative binding affinity to human and monkey target. **d, e,** Correlation of in vitro potency (IC50 in **d**) and efficacy (Emax in **e**) with in vivo antitumor activity of representative SPARCs. Antitumor activity was measured by tumor volume change from the baseline (%) in CWR22RV1 model**.** ADCs/SPARCs were dosed at 10 mg/kg. **f,** In vitro cytotoxicity of molecularly targeted agents in prostate cancer cell lines. Cells were incubated with a concentration series of drugs for 5 days. Cell viability was measured by CCK8 and IC50 for each drug/cell line was calculated from cell viability-concentration plot. Data represent mean of 2-3 independent measurements. **g,** SPARC variants from payload and DAR scanning of SPARC3-TOP1-PARP1/2-ATR (all DAR 8). DAR for each payload is labeled and difference between base molecule and each variant is highlighted with yellow. In vitro potency and efficacy difference between base molecule and each variant was measured by ΔIC50 and ΔEmax in CWR22RV1 (PSMA low expression) and C4-2 (PSMA high expression). **h,** Prostate cancer model information. **i,** Tumor growth of drug-resistant models. Dose: enzalutamide (10mg/kg, QD*28); Rucaparib (50mg/kg, QD*5*2); berzosertib (25mg/kg, QD*5*2); abiraterone (25mg/kg, QD*5*2).

In all in vivo studies, xenograft mice were intravenously administered with indicated doses of ADCs/SPARCs (QW*2) or free drugs (QD*5*2), unless otherwise indicated. Each value represents the mean and SD (N=3-5). Unpaired two-sided t-test. ns, not significant; *P<0.05.

a



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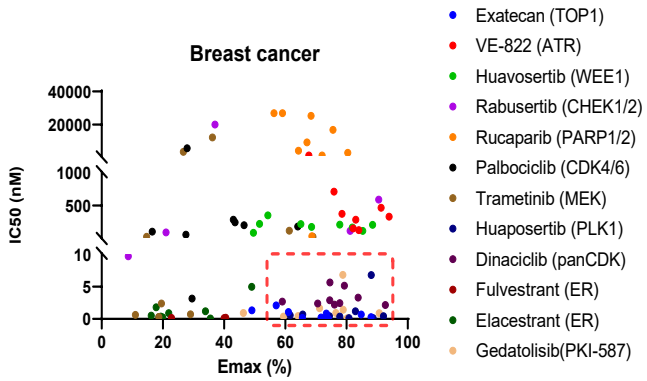

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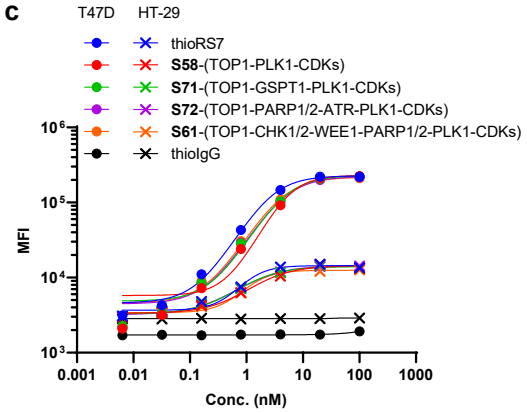
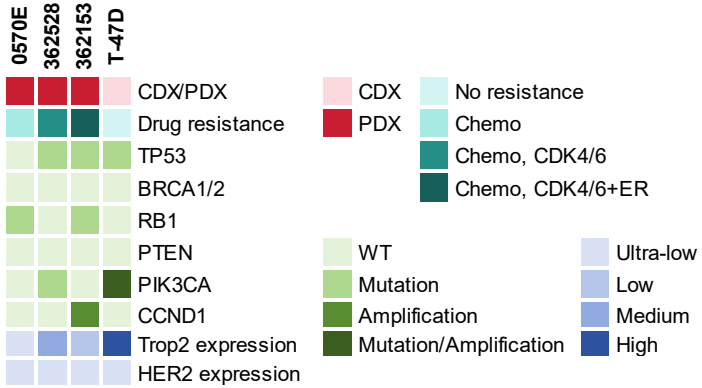


d

		TROP2-Exat(DAR4)	S58-(TOP1-PLK1-CDKs)	S54-(TOP1-PARP1/2-PLK1-CDKs)	S55-(TOP1-PARP1/2-PLK1-CDKs)
		DAR4	DAR2	DAR2	DAR2
			DAR8	DAR8	DAR8
TOP1:PARP:PLK1:CDKs ratio		1 : 0 : 0 : 0	1 : 0 : 4 : 4	1 : 1 : 4 : 4	1 : 4 : 4 : 4
Digestive System	Liver				
	Gallbladder				
	Jejunum				
	Ileum				
	Cecum				
	Colon				
	Esophagus				
Immune& Hematopoietic	Bone marrow				
	Thymus				
	Tonsil				
	Kidney				
	Adrenal glands				
	Skin				
		minimal	mild	moderate	marked

e



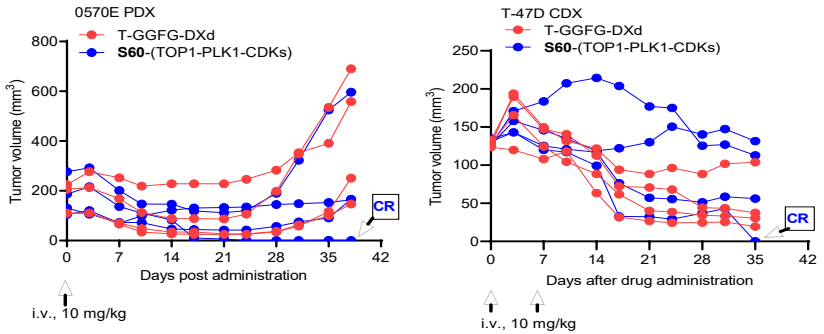
i

Tumor model	362528	362153	T-47D
CCND1 (FPKM)	90.68	409.86	55.45
TROP2 (IHC)	125	36	240
TV change (%)	32%	-25%	-12%

f

Target	Model	ADCs/SPARCs	MED (mg/kg)	HNSTD (mg/kg)	TI	TI improvement ratio
TROP2	T-47D CDX	RS7-Exatecan(D4)	7	30	17.1	2.14
		Dato-DXd(D4)	7	10	5.7	0.71
		TRODELVY	25	50	8.0	1.00
		S55-(TOP1-PARP1/2-PLK1-CDKs)	7	30	17.1	2.14
		S54-(TOP1-PARP1/2-PLK1-CDKs)	7	50	28.6	3.57
		S58-(TOP1-PLK1-CDKs)	3	50	66.7	8.33
	362528 PDX	**S58**-(TOP1-PLK1-CDKs)	10	50	20.0	2.50
		S54-(TOP1-PARP1/2-PLK1-CDKs)	>10	50	NA	NA
		TRODELVY	25	50	8.0	1.00
	362153 PDX	**S58**-(TOP1-PLK1-CDKs)	10	50	20.0	2.50
		TRODELVY	25	50	8.0	1.00

g



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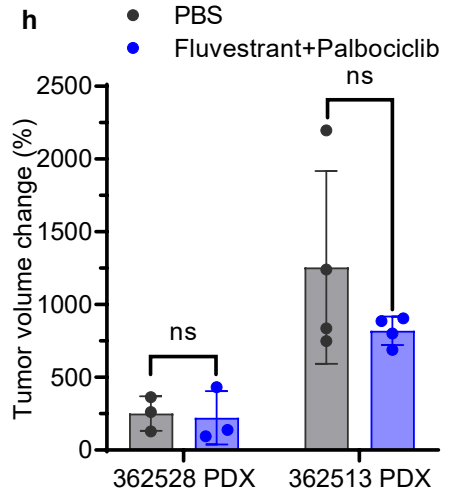

Extended Data Fig.13| A SPARC for breast cancer.

Extended Data Fig.13| A SPARC for breast cancer.

a, Selective oncogenic pathways and targets in $HR^+/HER2^-$ breast cancer to overcome resistance post CDK4/6 inhibition. Two clinical-stage TROP2-targeting ADCs, Trodelvy (SN38, DAR 8) and Dato (DXd, DAR 4), and a representative SPARC6 are shown. **b,** In vitro cytotoxicity of molecularly targeted agents in breast cancer cell lines. Cells were incubated with a concentration series of drugs for 5 days. Cell viability was measured by CCK8 and IC50 for each drug/cell line was calculated from cell viability-concentration plot. Data represent mean of 2-3 independent measurements. **c,** Flow cytometry of breast cancer SPARCs binding to breast cancer cell T47D relative to unconjugated antibody and isotype control (IgG). **d,** Summary of major toxicity findings of 3 TROP2-targeting SPARCs and TROP2-exat4 ADC. HNSTD for each molecule is indicated. Payload ratio is highlighted for three SPARCs. **e,** breast cancer model information. **f,** Calculation of therapeutic index by (HNSTD*4)/MED for each molecule. MED is defined as minimal dose for tumor regression. **g**, Tumor growth spider plots for HER2-targeting GGFG-DXd ADC (DS-8201a equivalent) and SPARC3 of HER2-TROP2-PLK1/BRD4-CDKs (**S60**-(TOP1-PLK1-CDKs)) in T47D and a PDX with ultra-low HER2 expression. Two CR mice in SPARC3 groups are indicated. Conjugates were dosed at 10 mg/kg (single dose for 0570E PDX; QW*2 for T-47D CDX). **h**, Tumor growth of drug-resistant models. Dose: fluvestrant (5 mg/kg, QD*5*2); palbociclib (75 mg/kg, QD*5*2). Unpaired two-sided t-test. ns, not significant. i, Antitumor efficacy of SPARC3 **S58**-(TOP1-PLK1-CDKs), TROP2 expression (IHC Score), and CCND1 expression (FPKM by RNAseq from PDX samples) in 362528 PDX, 362153 PDX, and T-47D CDX. A single dose of **S58** was dosed at 10 mg/kg for the two PDXs and at 5mg/kg for T47D CDX.