Supplementary information: supplementary figures and tables

Optimal design, anti-tumour efficacy and tolerability of anti-CXCR4 antibody drug conjugates

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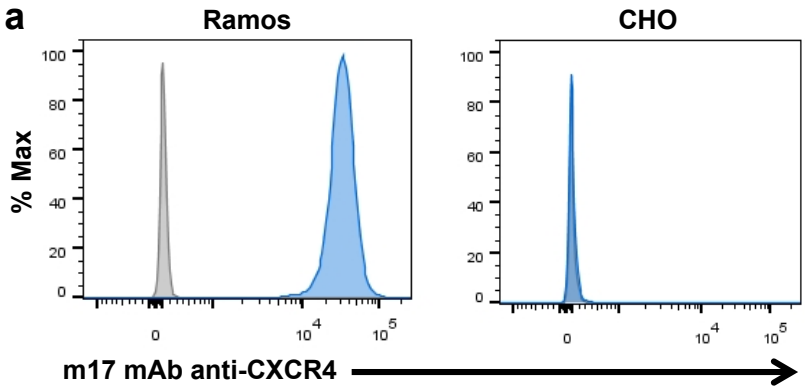
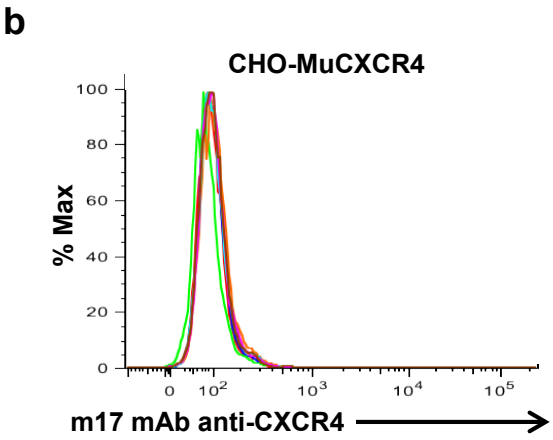
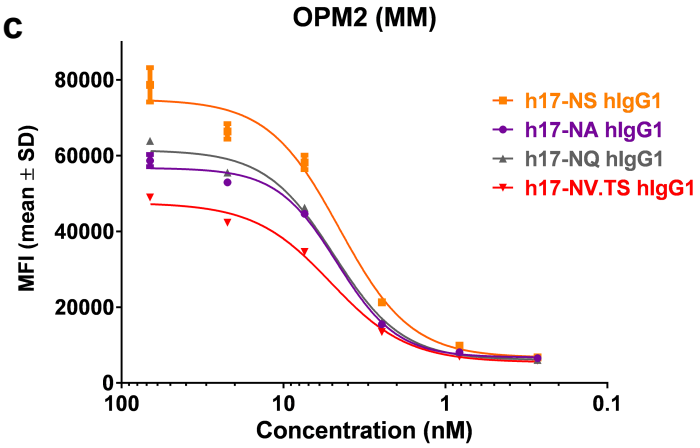
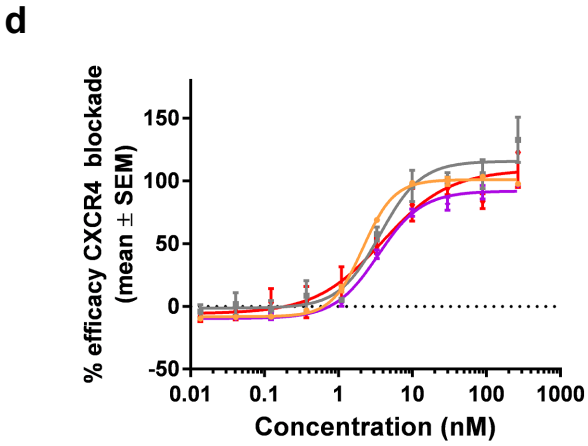








Antibody (hIgG1)	IC_{50} (nM)
h17-NS	2.1
h17-NQ	3.6
h17-NA	3.4
h17-NV.TS	3.9

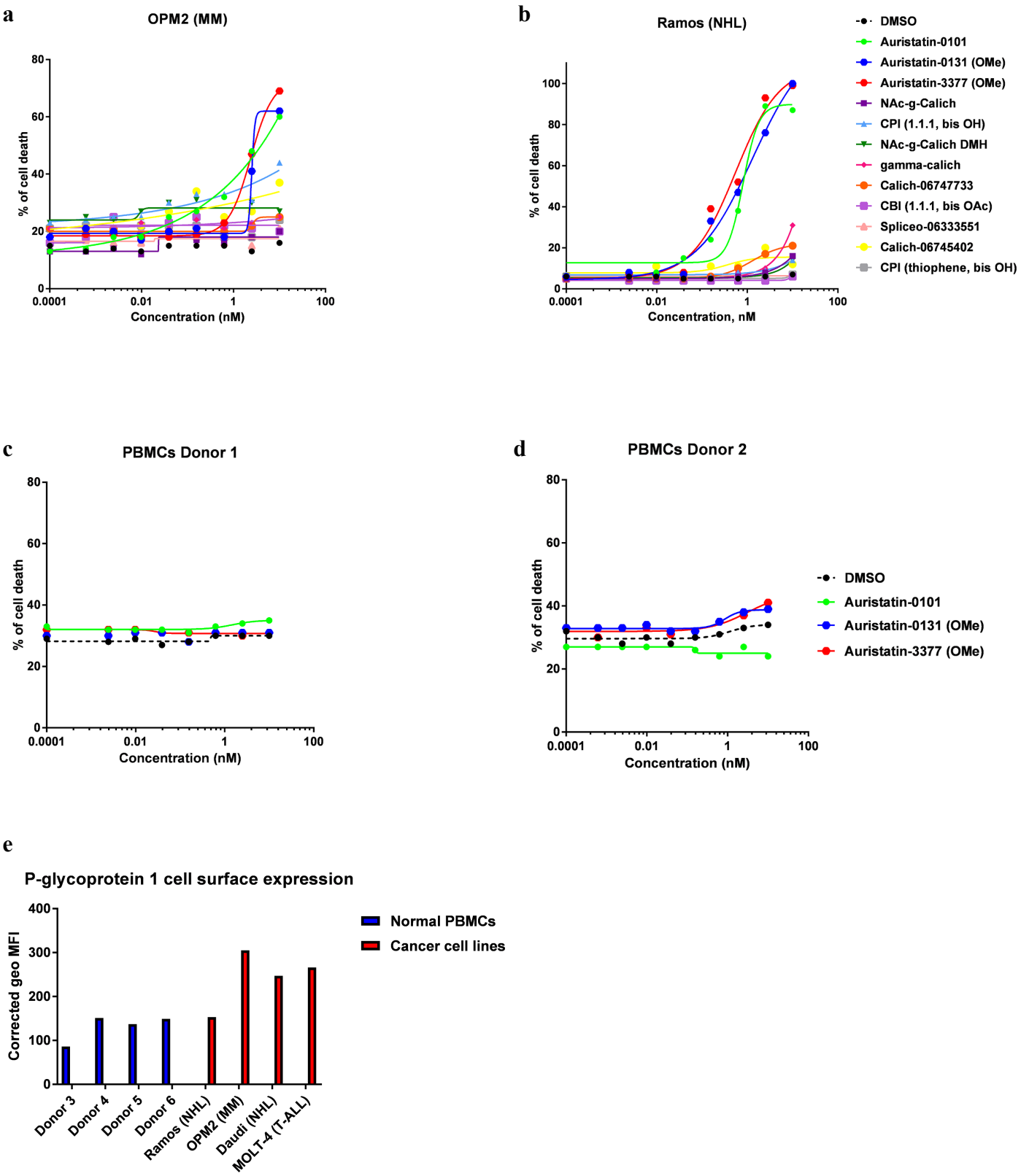
Supplementary Fig. 1 (related to Fig. 1). a, Flow cytometric analysis of m17 hIgG1 binding (blue histogram) on the $CXCR4^{High}$ Ramos cell line and on control CHO cells. Grey histogram is cells stained with secondary antibody alone. **b,** Flow cytometric analysis of m17 hIgG1 and its humanized variants binding on CHO expressing mouse CXCR4 (MuCXCR4). All histograms overlap with that of secondary antibody alone. **c,** Flow cytometric analysis: binding of serial diluted IgG on $CXCR4^{Low}$ MM-derived OPM2 cells, SD = standard deviation. **d,** Bioassay of CXCR4 blockade by h17 sequence variants on CHO-K1 cells over-expressing CXCR4, incubated in the presence of EC_{80} forskolin and EC_{80} CXCL12. SEM = standard error of the mean.

Supplementary Table 1:

Dissociation rate constants for antibody F(ab) at 37 ºC determined by SPR

mAb	k_d (1/s)	$t_{1/2}$ (min)	Binding on CXCR4$^+$ cells
h17-NV.TS	5.10E-03	2.3	Low
h17-NA	4.20E-03	2.8	Medium
h17-NQ	2.20E-03	5.2	Medium
h17-NS	4.30E-04	26.8	High
m17	1.30E-03	8.7	High

k_d = dissociation rate constant. $t_{1/2}$ = dissociation half life (calculated from k_d).



Supplementary Fig. 2. Auristatins cause cytotoxicity on cancer cell lines, but not on normal PBMCs. Cells were incubated with serial diluted payloads as free drugs (or as a modified cell membrane permeable form -OMe) or with vehicle control for 48 hours. Cumulative cell death was measured at endpoint using CellToxGreen Assay (Promega). Data was plotted in GraphPad Prism 7 as non-linear fit of log(concentration) vs. response. **a-b,** Screening of free payloads on tumor cell lines. **c-d,** Cytotoxicity of auristatins on normal PMBCs. **e**, Expression of P-glycoprotein 1/ABCB1 in normal PBMCs and cancer cell lines. Cells were incubated with APC-conjugated anti-P-glycoprotein 1 antibody (clone UIC2) and analyzed by flow cytometry. Geo MFI of the respective cell type unstained control was subtracted from the geo MFI of antibody stained samples (corrected geo MFI) to allow for comparison of P-glycoprotein expression across cell types with different levels of auto-fluorescence.

Supplementary Table 2. Relative CXCR4 density on plasma membrane of haematological cancer-derived cell lines and normal hematopoietic cells. Number of 12G5 mAb bound/cell estimated based on MFI of total population in up to 4 independent experiments per cell type.

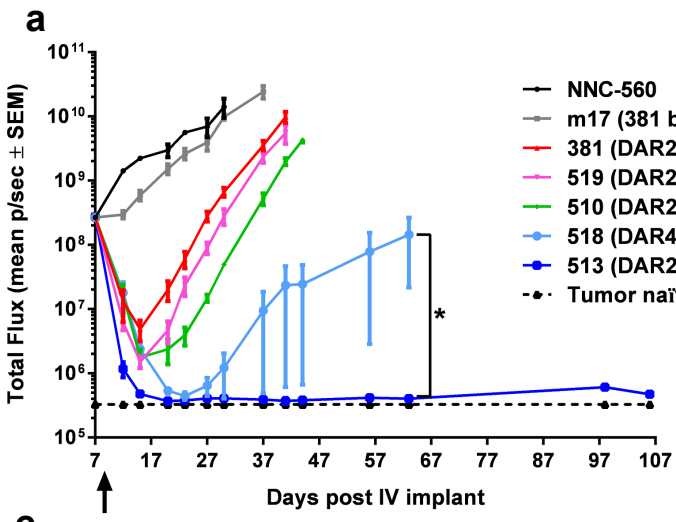
Cell type	Cancer type	12G5 mAb/Cell				Average 12G5 mAb/Cell	Relative surface CXCR4 density level
Ramos	**NHL**	30598	201774			116186	**High CXCR4 (++++)**
HPB-ALL	**T-ALL**	30839				30839	
NALM6	**B-ALL**	28576				28576	
BV173-GFP-luc	**B-ALL**	28287				28287	
Daudi	**NHL**	28480	3270			15875	
Jurkat	**T-ALL**	7523	18414	13788		13242	
JEKO-1-GFP-luc	**NHL**	11914				11914	
Molp8-GFP-luc	**MM**	9214	9041			9128	**Medium-high CXCR4 (+++)**
REH	**ALL**	4201				4201	
Raji-GFP-luc	**NHL**	3841				3841	
H929-VR20-GFP-luc	**MM**	4038	3184			3611	
U937	**AML**	3439	3142			3291	
U266-GFP-luc	**MM**	2417	2062			2240	
697	**B-ALL**	1944				1944	**Medium-low CXCR4 (++)**
KMS12BM-GFP-luc	**MM**	1108	1681			1394	
OPM2-GFP-luc	**MM**	966	2154	693	1011	1206	
HL60	**AML**	516	1771			1144	
MOLM13	**AML**	984				984	**Low CXCR4 (+)**
MM1S-GFP-luc	**MM**	675	1165			920	
MV-4-11-luc	**AML**	499	1260			880	
Normal BM $CD45^{low}CD34^{+}$	**N/A**	519	534			527	
Normal BM $CD45^{+}CD34^{-}$	**N/A**	324	450			387	
EOL1	**AML**	358				358	
ML2-GFP-luc	**AML**	340				340	
Kasumi3	**AML**	146				146	**CXCR4 level too low, no *in vitro* cytotoxicity of anti-CXCR4 ADCs observed**
Kasumi1	**AML**	94				94	
K562	**CML**	59	67			63	
H929	**MM**	47				47	
TF1	**AML**	14				14	

Normal human bone marrow (BM) data is from two different donors. GFP-luc = cells transduced and selected to express high levels of GFP-luciferase for *in vivo* tracing of tumor burden.

Supplementary Table 3. *In vitro* cytotoxicity screening of m17-derived ADCs on cell lines derived from haematological cancers.
See also Table 1 for ADC configuration and properties and Supplementary Table 2 for estimated relative density of CXCR4 on cell surface. Data for the most efficacious ADC for each cell line is highlighted in bold.

	IC_{50} (nM)																			
	Ramos (NHL)	Raji (NHL)	Jeko1 (NHL)	REH (NHL)	H929-VR20 (MM)	Molp8 (MM)	OPM2 (MM)	KMS12BM (MM)	U266 (MM)	BV173 (B-ALL)	Nalm6 (B-ALL)	697 (B-ALL)	Jurkat (T-ALL)	HPB-ALL (T-ALL)	U937 (AML)	EOL-1 (AML)	HL-60 (AML)	Molm13 (AML)	MV4-11 (AML)	K562* (AML)	Kasumi-1* (AML)
NNC-358	180.900	>267	139.500	>267	>267	165.533	121.500	127.889	70.793	57.91	>267	>267	>267	>267	151.700	>267	>267	>267	220.600	>267	161.700
Aur0101	0.208	0.411	0.031	0.042	2.035	0.920	0.210	0.042	0.029	0.0004	0.079	0.104	0.053	0.076	1.016	4.440	3.367	1.681	5.297	3.366	3.247
381	0.268	77.800	0.269	0.225	6.256	1.119	0.480	2.246	0.042	0.068	0.230	88.267	0.135	0.849	0.397	3.153	2.446	1.860	3.483	101.9	52.520
519	0.312	248.467	0.275	0.797	>267	0.900	0.314	7.812	0.039	0.059	0.222	140.333	0.187	1.383	0.456	1.097	3.828	2.003	2.432	>267	164.400
518	0.291	18.233	0.172	0.122	17.587	0.268	0.184	**0.340**	0.041	0.057	0.172	26.420	0.098	0.332	**0.276**	**0.428**	**1.126**	0.643	**0.598**	255.5	77.790
Aur0131 (OMe)	0.094	0.755	0.035	0.011	0.847	0.750	0.132	0.037	0.024	0.0004	0.094	0.064	0.095	0.070	1.377	4.438	3.232	1.809	7.396	4.334	3.448
510	0.217	142.600	0.167	0.052	0.662	0.201	0.126	176.198	0.018	0.038	0.232	214.400	**0.037**	0.237	4.453	0.785	6.726	0.461	1.129	>267	>267
513	**0.128**	**7.760**	**0.104**	**0.005**	**0.228**	**0.108**	**0.038**	0.347	**0.008**	**0.028**	**0.113**	**0.814**	0.043	**0.105**	0.997	0.856	1.592	**0.262**	1.040	>267	>267

*Cell lines with undetectable cell surface CXCR4 expression.



b

Treatment	Survival (days)
NNC-560	30
m17	37
381	44
510	44
519	48
518	>98
513	>98

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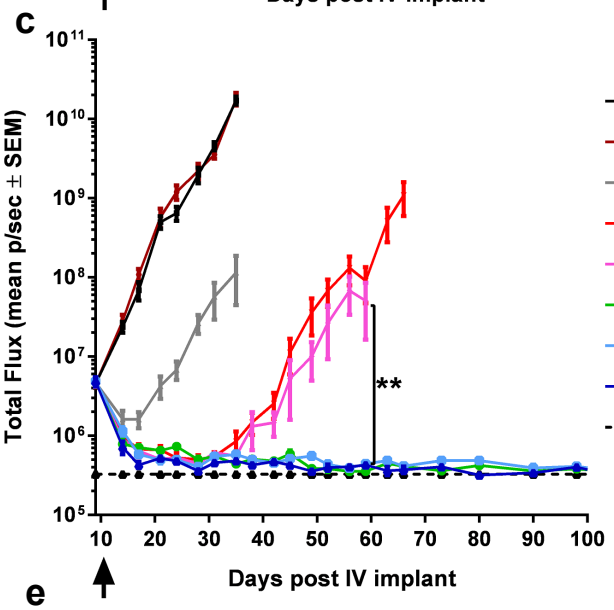

d

Treatment	Survival (days)
PBS	34
NNC-560	34
381	78
519	90
510	>210
518	>210
513	>210

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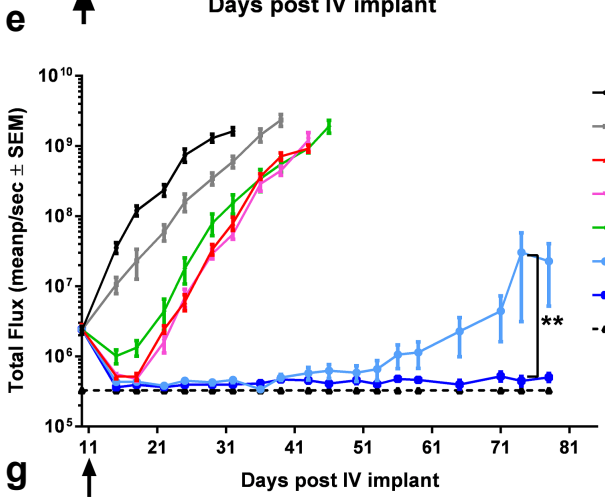

f

Treatment	Survival (days)
NNC-358	36
m17	43
381	50
519	50
510	50
518	120
513	>135

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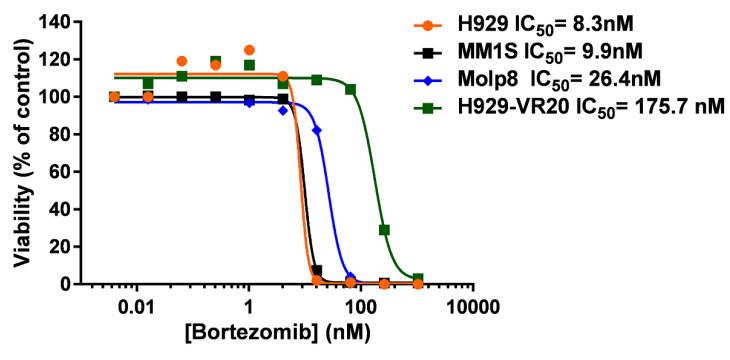
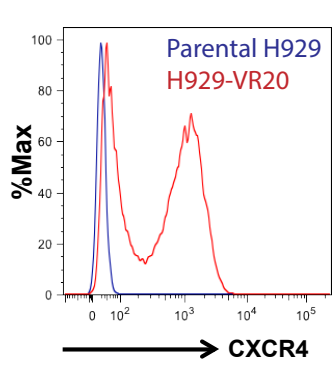
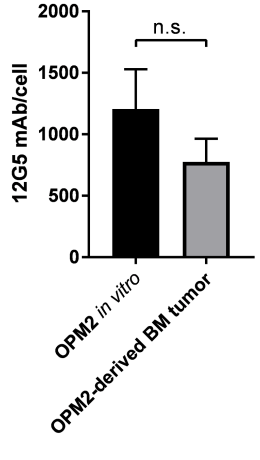
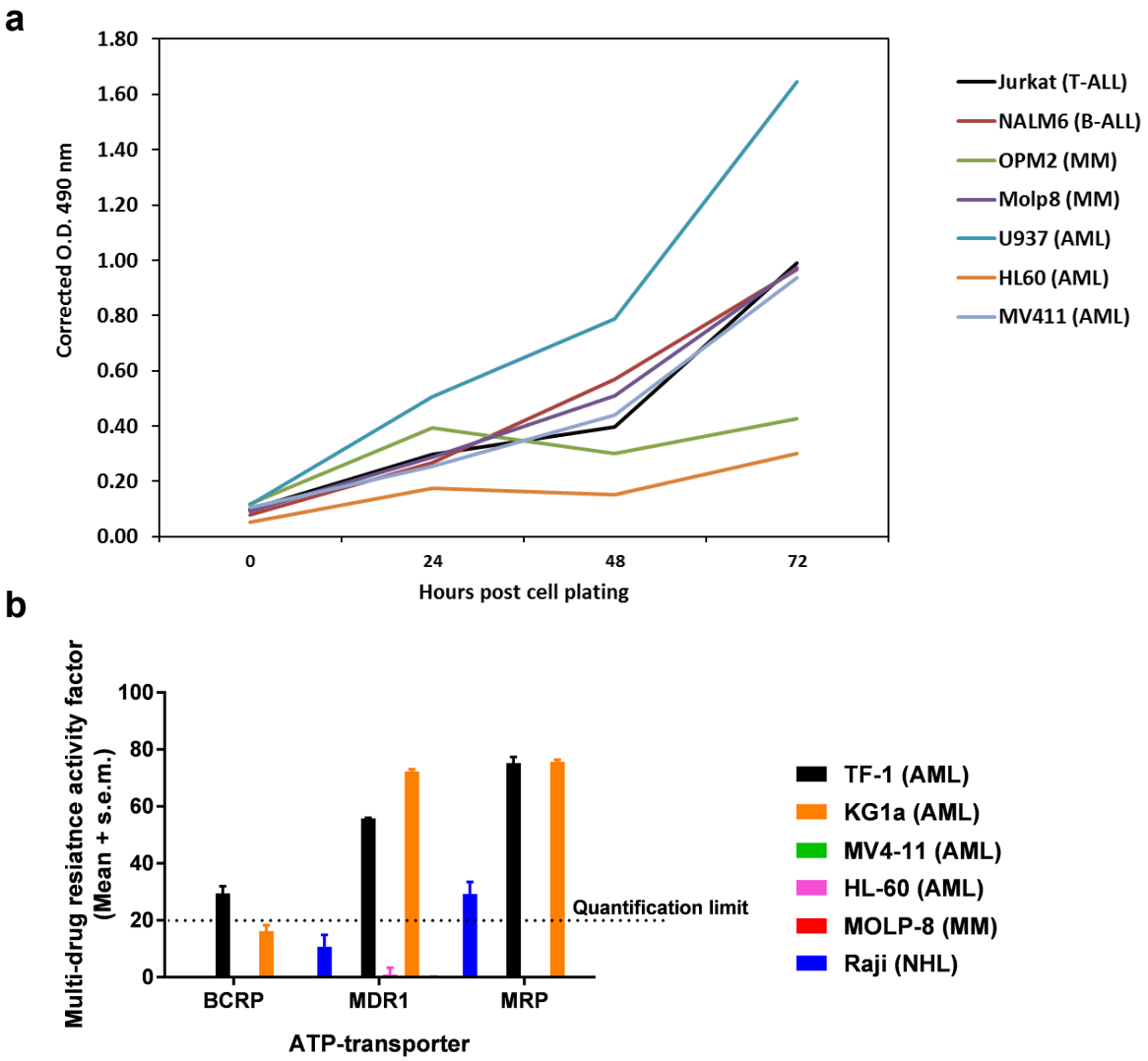




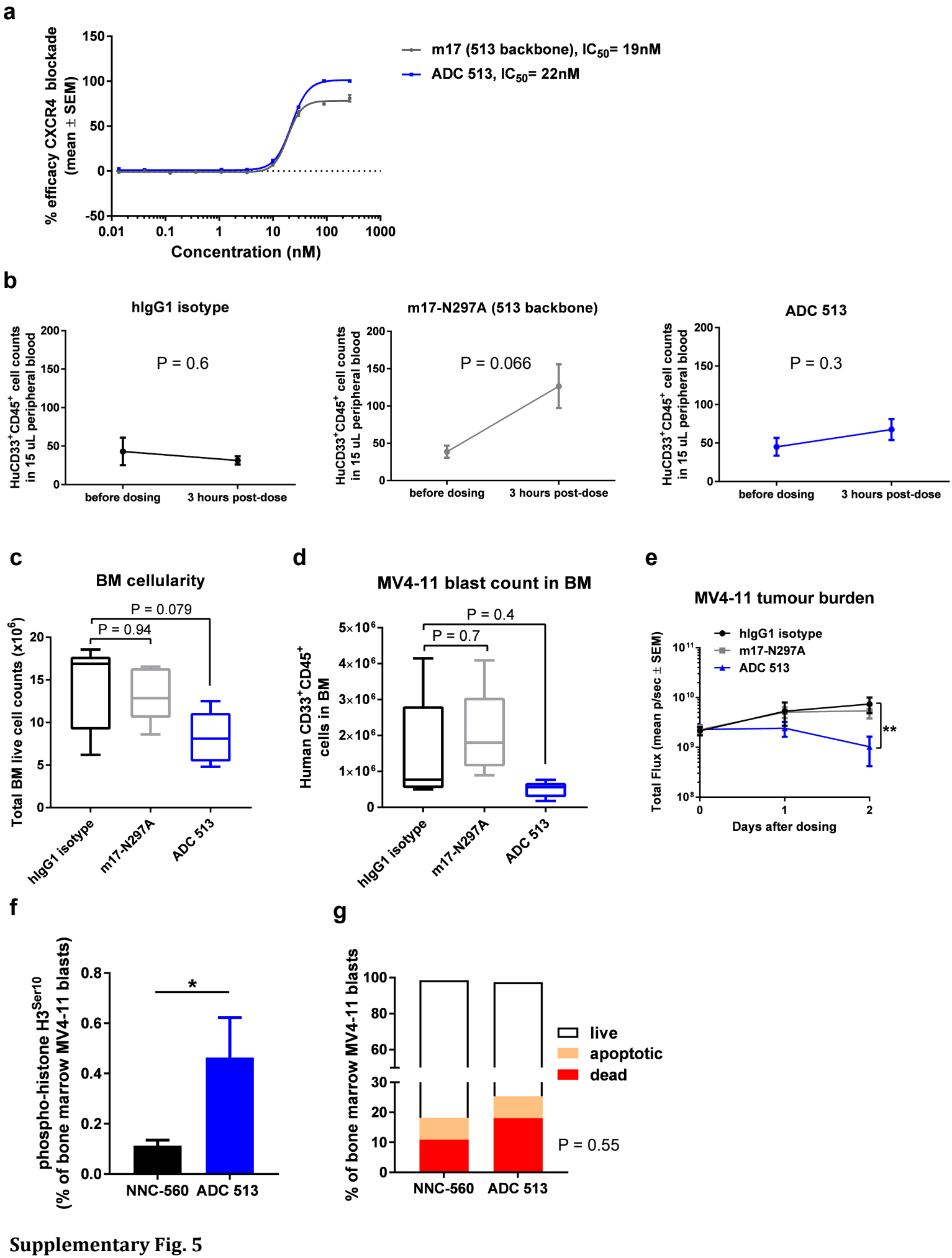


Supplementary Fig. 3 (related to Fig. 3)

Supplementary Fig. 3 (related to Fig. 3). a, c, e, Kinetics of orthothopic tumor growth (whole body tumour burden) after single dose at 3 mg/kg (arrows) of various m17-derived ADC configurations, p/sec = photos/second, Unconjugated m17 (mAb backbone for ADC 381) bearing active Fc-mediated effector function was included as a control. LP = linker-payload, Fc (+) = active Fc-mediated effector function, Fc (-) = reduced Fc-mediated effector function, SEM = standard error of the mean, (**a**) *P=0.016, (**c**) **P=0.004, (**e**) **P=0.002, all comparisons: two-way ANOVA with Sidak's multiple comparisons test. **b, d, f,** Kaplan-Meier analysis of median survival of mice in each treatment group, (**b**) **P=0.003, (**d**) *P=0.02, (**f**) **P=0.04. **a-f,** N=5 mice/group. **g,** Concentration-response curves showing relative sensitivity of MM-derived cell lines to SoC Velcade/bortezomib *in vitro*. H929-VR20 is derived from H929 cell line upon selection with 20 nM Velcade/bortezomib treatment *in vitro*. **h,** Expression of CXCR4 in the MM cell line H929 (parental H929) and in its subpopulation H929-VR20. Expression was measured using 12G5 anti-CXCR4 antibody by flow cytometry. **i,** Quantification of CXCR4 surface density (12G5 antibody binding) in the MM-derived cell line OPM2 (N=4 independent measurements) and in tumours isolated from bone marrow (BM) 36 days after i.v. OPM2 cell inoculation in tail vein of NSG mice (N=3 mice). Error bars = standard error of the mean, n.s. = non-significant (unpaired t test).



Supplementary Fig. 4. AML cell lines used in the *in vitro* ADC cytotoxicity screen do not show different *in vitro* proliferation rates or increased activity of ATP transporters associated with multi-drug resistance, as compared to cell lines derived from other cancers. a, Growth kinetics of human cell lines determined by XTT assay upon plating of 10,000 cells/well in 96-well plates. XTT was added to cells during the last 4 hours of incubation for each time point. O.D. = optical density. **b,** Activity of ATP transporters associated with multi-drug resistance. The AML cell lines KG1a and TF-1 were used as positive controls in this assay. BCRP = breast cancer resistance protein, MDR1 = multidrug resistance protein 1 (P-glycoprotein 1), MRP = Multidrug Related Protein, s.e.m. = standard error of the mean.



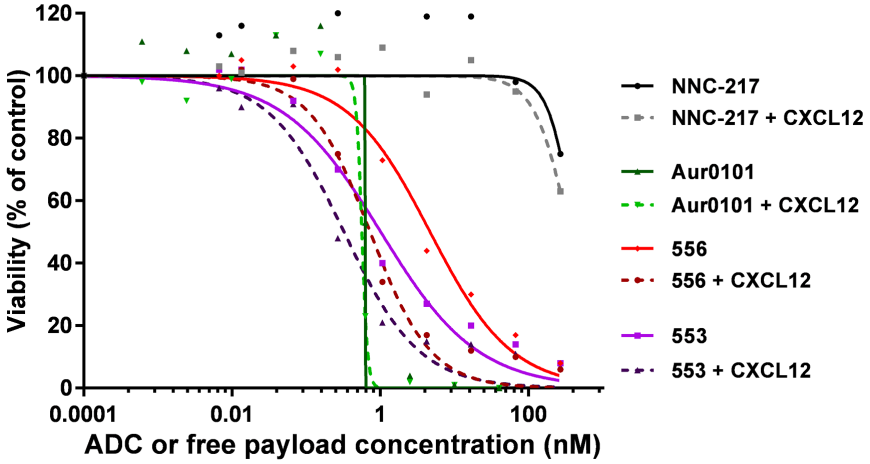
Supplementary Fig. 5

Supplementary Fig. 5. MoA of anti-CXCR4 ADC-mediated cytotoxicity *in vivo*. a, Bioassay of CXCR4 blockade by ADC 513 and respective unconjugated antibody in the presence of EC_{80} forskolin and EC_{80} CXCL12, SEM = standard error of the mean. **b-d,** Peripheral blood (**b**) and femur bone marrow (**c-d**) and were harvested 3 hours post-dose from same MV4-11 xenografts, N=5 mice/group. **b-g,** all compounds were single dosed at 3 mg/kg. **b,** Tumour leucocytosis assay in MV4-11 orthotopic xenografts with similar tumour burden as that of efficacy experiment (see Fig. 3g), N=5 mice/group, error bars = standard error of the mean, P values calculated using a paired t-test. **c,** Bone marrow (BM) cellularity. **d,** MV4-11 blast count in bone marrow (BM) of same mice as in b. **c-d**, P value calculated with ANOVA with Tukey's multiple comparisons test. **e**, Kinetics of MV4-11 orthotopic tumour burden, mice were dosed in parallel cohort (and with same tumour burden) as mice from b-d, **P = 0.004, two-way ANOVA with Dunnett's multiple comparisons test. **a-e,** m17-N297A corresponds to unconjugated mAb backbone of ADC 513. **f-g,** Mice with high MV4-11 orthotopic tumour burden were dosed with ADC 513 (N=3) or control ADC (N=4) and sacrificed 1.5 hours later for flow cytometric analysis of AML blasts in bone marrow. **f,** Phosphorylation levels of histone-$H3^{Ser10}$ in MV4-11 blasts, error bars = standard error of the mean, *P < 0.05 (unpaired t test). **g**, Proportion of viable, apoptotic and dead MV4-11 blasts by AnnexinV/propidium iodide staining (same samples as in f), P value (for proportion of dead cells) calculated by ANOVA with Sidak's multiple comparisons test.

Supplementary Table 4. ***In vitro*** **cytotoxicity screening of h17 variants-derived ADCs on cell lines from haematological cancers.** See also Table 1 for ADC configuration and properties and Supplementary Table 2 for CXCR4 surface density. Data for the most efficacious ADC for each cell line is highlighted in bold.

	Binding on CXCR4$^+$ cells	IC_{50} (nM)				
		Ramos (NHL)	Daudi (NHL)	U266 (MM)	OPM2 (MM)	MOLP-8 (MM)
NNC-217	**N/A**	>267	>267	69.533	>267	>267
Aur0101	**N/A**	0.082	0.198	0.108	0.137	0.306
555	**Medium**	0.206	8.053	0.117	0.400	0.879
556	**Low**	0.176	**3.775**	0.118	0.756	1.027
554	**High**	**0.111**	10.320	**0.013**	**0.097**	**0.749**
553	**Medium**	0.229	10.687	0.016	0.360	0.983

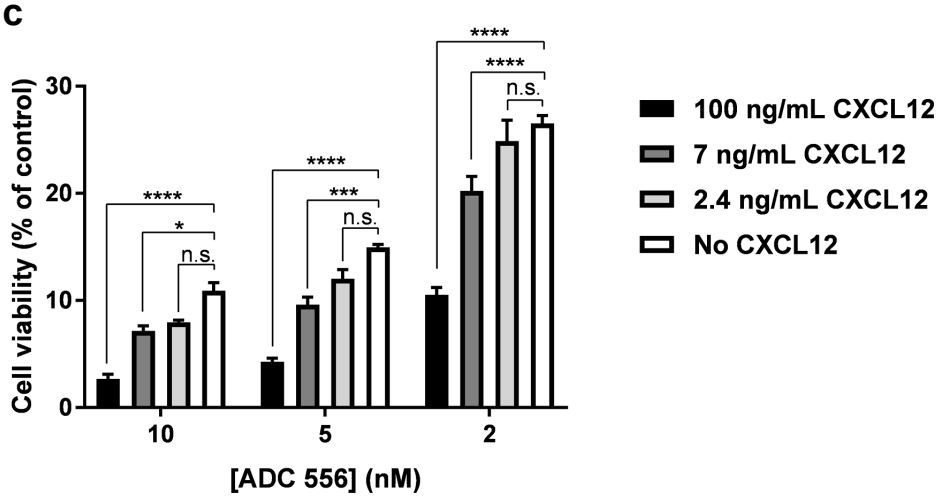
a



b

	IC_{50} (nM)
NNC-217	>267
NNC-217 + CXCL12	>267
Aur0101	0.625
Aur0101+ CXCL12	0.562
556	4.677
556 + CXCL12	0.726
553	1.011
553 + CXCL12	0.323

c



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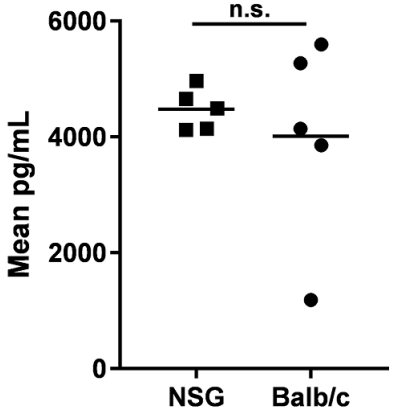
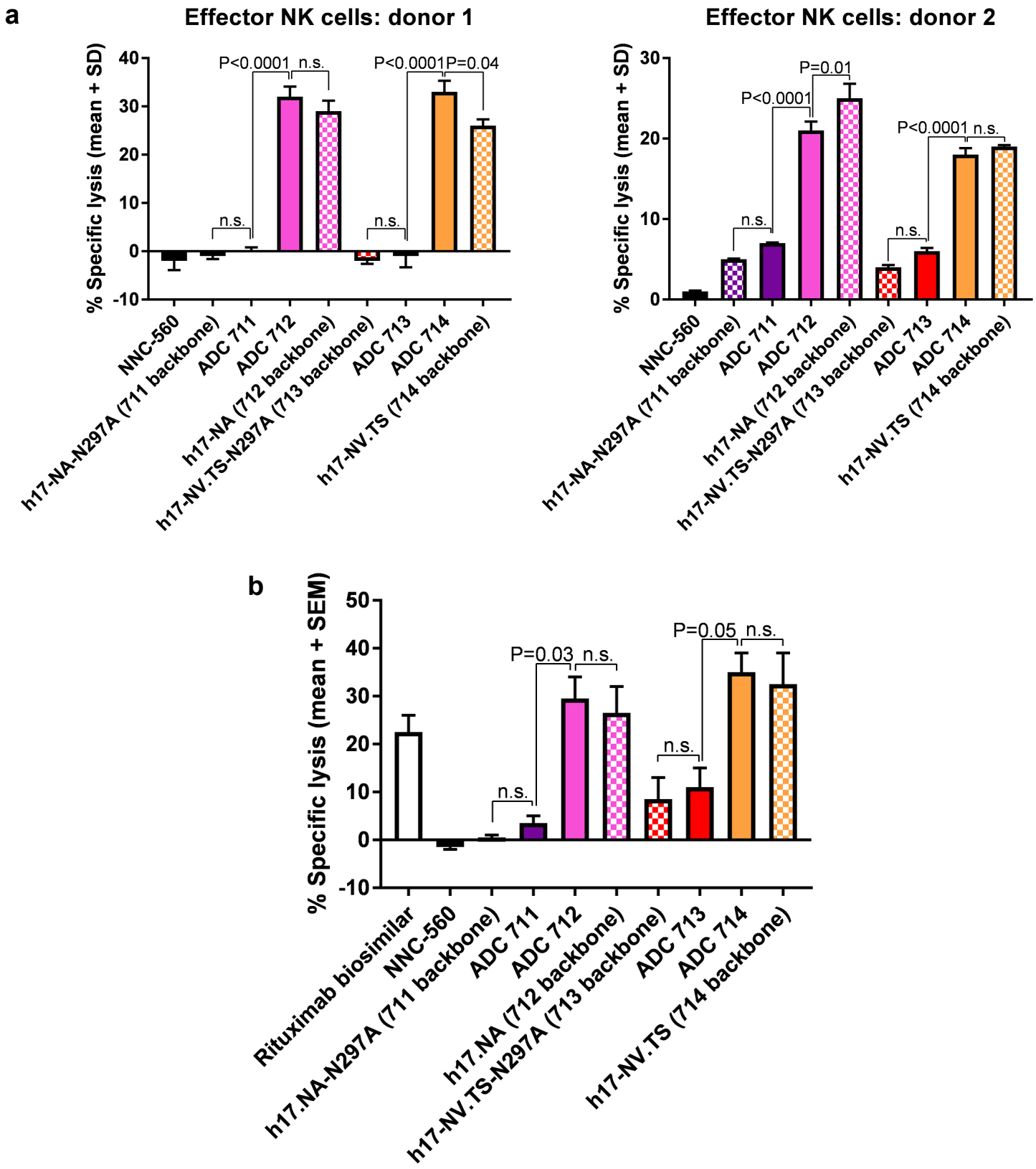


Supplementary Fig. 6 (related to Fig.4). CXCL12 enhances anti-tumour activity of anti-CXCR4 ADCs in CXCR4^{Low} tumour cells. a-b, Effect of 100 ng/mL CXCL12 on the cytotoxicity (dose-response) of medium (ADC 553) and low (ADC 556) cell binding ADCs on the CXCR4^{Low} MM-derived cell line OPM2. Data points are mean from triplicates of one experiment, with similar results observed in 2 other independent experiments. **c,** Effects of pathophysiologically relevant CXCL12 levels on the cytotoxicity of low cell binding ADC 556 on the CXCR4^{Low} MM-derived cell line OPM2. High concentration (100 ng/mL) of CXCL12 was used as a positive control (based on results of experiments illustrated in panels a-b), 7 ng/mL is the average CXCL12 concentration reported in bone marrow samples of MM patients and 2.4 ng/mL is the average CXCL12 concentration reported in peripheral blood of same patients. Error bars = standard error of the mean, n.s. = non-significant, *P=0.01, ***P=0.0006, ****P<0.0001 (two-way ANOVA with Dunnett's multiple comparisons test). **d,** ELISA determination of CXCL12 levels in the serum of immuno-deficient (NSG) and immuno-competent (Balb/c) tumour-naïve mice. Each data point represents result from one animal, n.s. = non-significant (unpaired t test).

Supplementary Table 5. ***In vitro*** **cytotoxicity and specificity of h17-NV.TS- and h17-NA- derived, AmPEG6C2-Aur0131-conjugated (DAR4) ADCs.** See also Table 1 for ADC configuration and properties.

	Binding on CXCR4+ cells	IC_{50} (nM)	
		Jurkat (CXCR4++++)	CHO (CXCR4-)
NNC-715	N/A	>267	>267
NNC-560	N/A	>267	>267
Aur0131 (Ome)	N/A	1.675	3.997
711	Medium	0.245	>267
712	Medium	0.262	>267
713	Low	0.294	>267
714	Low	0.282	>267



Supplementary Fig. 7 (related to Fig.5). Fc-mediated effector function in humanized ADCs is equivalent to that of respective unconjugated antibodies. a, Result of *in vitro* ADCC assay on MOLT-4 cell line (T-ALL), using 5 µg/mL h17-derived ADCs, or their respective unconjugated antibody and human NK cells as effectors. Effector-to-target cells ratio = 1:10. Data shown are mean + standard deviation (SD) of duplicates per condition, with 2 independent experiments using different NK cells donors. One-way ANOVA with Tukey's multiple comparisons test used for statistical analysis, n.s. = non significant). **b**, Result of *in vitro* CDC assay on Daudi cell line (NHL), using 7 µg/mL h17-derived ADCs, or their respective unconjugated antibody backbones and 2.5% human serum. A rituximab biosimilar was included as a positive control. Data shown are mean of 2 independent experiments. One-way ANOVA with Tukey's multiple comparisons test used for statistical analysis, n.s. = non significant, SEM= standard error of the mean.

Supplementary Table 6. Pharmacokinetic parameters of h17-NV.TS- and h17-NA- derived, AmPEG6C2-Aur0131-conjugated (DAR4) ADCs in MOLP-8 xenografts and HuCXCR4KI mice. Mice were single dosed with indicated ADCs through the tail vein. MOLP-8 tumour burden is similar to that of efficacy studies at day of dosing. See also Table 1 for ADC configuration and properties.

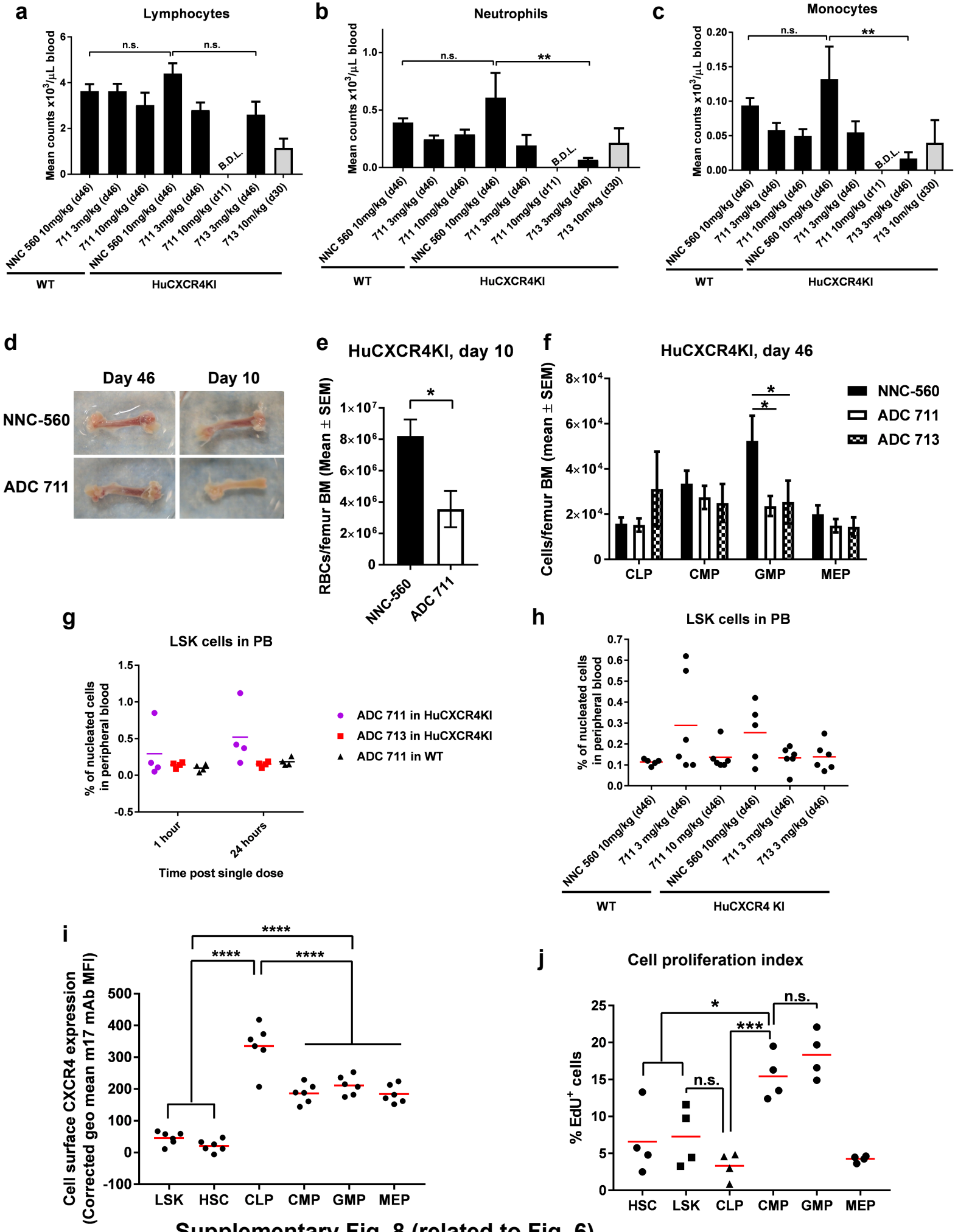
Mouse Strain/Model	ADC	Cell binding	Fc	Dose (mg/kg)	Assay	Cmax (mg/mL)	Tmax (hr)	AUC_{0-x} (hr*mg/mL)	AUC interval (hr)	T1/2 (hr)
HuCXCR4KI	711	Medium	(-)	3.0	Conjugated mAb	23.1	6	1208	0-504	48
HuCXCR4KI	711	Medium	(-)	3.0	Total mAb	23.2	6	1243	0-504	24
HuCXCR4KI	713	Low	(-)	3.0	Conjugated mAb	21.5	6	1564	0-504	48
HuCXCR4KI	713	Low	(-)	3.0	Total antibody	21.5	6	1658	0-504	35
HuCXCR4KI	711	Medium	(-)	10.0	Conjugated mAb	281.0	0.5	10600	0-168	73
HuCXCR4KI	711	Medium	(-)	10.0	Total antibody	292.0	0.5	11000	0-168	71
HuCXCR4KI	713	Low	(-)	10.0	Conjugated mAb	153.0	0.5	8090	0-168	78
HuCXCR4KI	713	Low	(-)	10.0	Total antibody	169.0	0.5	9200	0-168	62
MOLP-8 Xenograft	711	Medium	(-)	3.0	Conjugated mAb	40.5	6	10575	0-504	233
MOLP-8 Xenograft	711	Medium	(-)	3.0	Total antibody	40.6	6	11044	0-504	251
MOLP-8 Xenograft	712	Medium	(+)	3.0	Conjugated mAb	42.5	6	3828	0-504	39
MOLP-8 Xenograft	712	Medium	(+)	3.0	Total antibody	42.8	6	4074	0-504	31
MOLP-8 Xenograft	713	Low	(-)	3.0	Conjugated mAb	37.8	6	10230	0-504	321
MOLP-8 Xenograft	713	Low	(-)	3.0	Total antibody	37.3	6	10510	0-504	340
MOLP-8 Xenograft	714	Low	(+)	3.0	Conjugated mAb	33.3	6	2189	0-504	42
MOLP-8 Xenograft	714	Low	(+)	3.0	Total antibody	30.5	6	2161	0-504	30
MOLP-8 Xenograft	711	Medium	(-)	0.03	Conjugated mAb	0.092	6	18	0-336	107
MOLP-8 Xenograft	711	Medium	(-)	0.03	Total antibody	0.090	6	14	0-336	186
MOLP-8 Xenograft	711	Medium	(-)	0.1	Conjugated mAb	0.41	6	88	0-504	284
MOLP-8 Xenograft	711	Medium	(-)	0.1	Total antibody	0.43	6	81	0-504	268
MOLP-8 Xenograft	711	Medium	(-)	0.3	Conjugated mAb	1.33	6	265	0-504	335
MOLP-8 Xenograft	711	Medium	(-)	0.3	Total antibody	1.51	6	270	0-504	278
MOLP-8 Xenograft	713	Low	(-)	0.05	Conjugated mAb	0.50	6	64	0-336	209
MOLP-8 Xenograft	713	Low	(-)	0.05	Total antibody	0.46	6	66	0-336	186
MOLP-8 Xenograft	713	Low	(-)	0.15	Conjugated mAb	1.82	6	329	0-504	243
MOLP-8 Xenograft	713	Low	(-)	0.15	Total antibody	1.58	6	291	0-504	267
MOLP-8 Xenograft	713	Low	(-)	0.5	Conjugated mAb	6.82	6	1272	0-504	237
MOLP-8 Xenograft	713	Low	(-)	0.5	Total antibody	5.83	6	1075	0-504	247

Hr = hours. AUC = Area under the concentration curve. Cmax = peak serum concentration. Tmax = time taken to reach the maximum concentration. T1/2 = time taken for Cmax to drop in half. (-) = Reduced Fc-mediated effector function. (+) = Active Fc-mediated effector function.

Supplementary Table 7. Tolerability of ADCs 711 and 713 in HuCXCR4KI mice. See also Table 1 for ADC configuration and properties and Fig.6a for dosing schedule.

ADC and mouse genotype	Dose (mg/kg/dose)	Tolerability after 1st dose		Tolerability after 2nd dose		Tolerability after 3rd dose	
		males	females	males	females	males	females
711 (Medium cell binding) in HuCXCR4KI	10.0	3/6 (50%)	0/6 (0%)				
	8.0						
	6.0	0/5 (0%)	4/5 (80%)		4/5 (80%)		
	4.5	4/5 (80%)					
	3.0	9/10 (90%)*	6/6 (100%)	9/10 (90%)	6/6 (100%)	9/10 (90%)	6/6 (100%)
713 (Low cell binding) in HuCXCR4KI	10.0	6/6 (100%)	4/6 (67%)	4/6 (67%)	3/6 (50%)		
	8.0	4/5 (80%)		4/5 (80%)			
	6.0	5/5 (100%)	4/5 (80%)	5/5 (100%)	2/5 (40%)	5/5 (100%)	
	4.5	5/5 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)
	3.0	10/10 (100%)	6/6 (100%)	10/10 (100%)	6/6 (100%)	10/10 (100%)	6/6 (100%)
711 (Medium cell binding) in WT	10.0	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)	6/6 (100%)

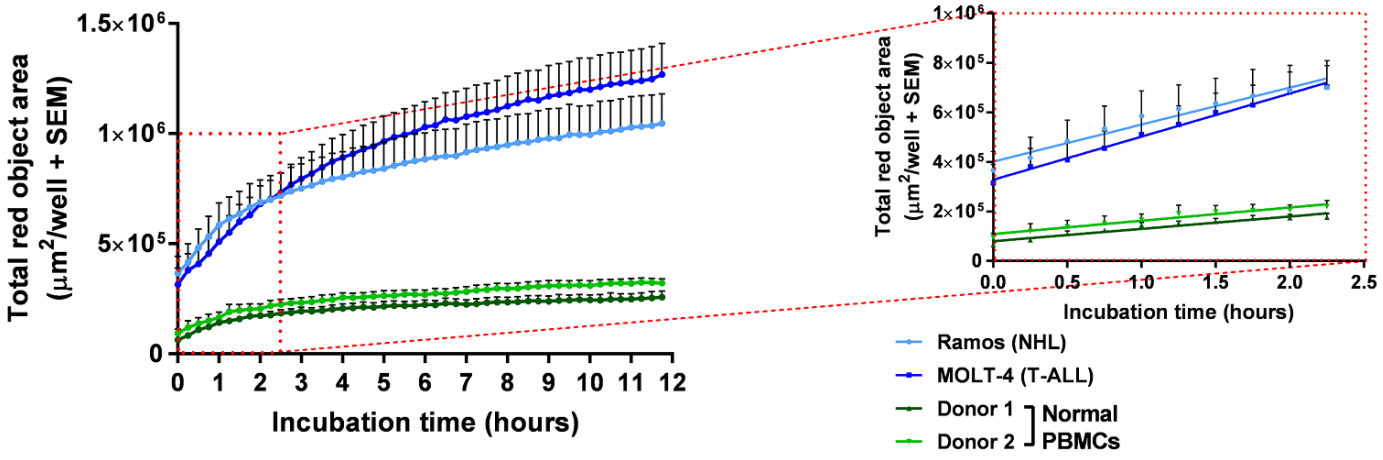
*1/10 males died after first dose. We presume the death is likely not test article-related because all other mice of both genders tolerated the multiple dose study.



Supplementary Fig. 8 (related to Fig. 6)

Supplementary Fig. 8 (related to Fig. 6). a-c, Hematology data from tolerability study. The necropsy day is indicated in parenthesis, in x-axis. Black bars are mean from scheduled necropsy on day 46 (N=6/group), grey bars are mean from unscheduled necropsies on days 11 and 30 (N=3/group). B.D.L. = below detection limit, error bars = standard error of the mean. One-way ANOVA with Tukey's multiple comparisons test for data collected at day 46, n.s. = non-significant (**b**) **P=0.004 (**c**) **P=0.005. **d-e,** Red blood cells in femur bone marrow decrease after single 3 mg/kg dose of ADC711 (day 10), but recovery is observed at day 46, in spite of HuCXCR4KI receiving two additional doses (day 46). See also Figs. 6a and 6f. **e,** Red blood cell (RBCs) quantification in femur bone marrow at day 10, SEM = standard error of the mean, *P=0.02, unpaired t test. **f**, Enumeration of specific hematopoietic progenitor populations in femur bone marrow (BM) of HuCXCR4KI mice by flow cytometry at day 46 of the tolerability study (dose level 3 mg/kg/dose), N=5/group, SEM = standard error of the mean, *P <0.03, two-way ANOVA with Dunnett's multiple comparisons test. **g-j,** Each symbol represents data from an individual mouse. **g-h,** Frequency of hematopoietic stem cells and progenitors - $Lin^{-}Sca1^{+}c\text{-}kit^{+}$ (LSK) - measured by flow cytometry in peripheral blood (PB) after either single 3 mg/kg dose (**g**) or repeat dosing at necropsy day 46 (**h**). No statistically significant differences were found in either experiment by two-way ANOVA with Tukey's multiple comparisons test (**g**) and one-way ANOVA with Dunnett's multiple comparisons test. **g-h,** HSC = hematopoietic stem cells, CLP = common lymphoid progenitor, CMP = common myeloid progenitor, GMP = granulocyte-monocyte progenitor, MEP = megakaryocyte-erythroid progenitor. **i,** Cell surface CXCR4 expression levels in hematopoietic progenitor populations from femur bone marrow of HuCXCR4KI mice in homeostatic conditions, measured with m17 mAb directly conjugated to AlexaFluor647, ****P<0.0001 (one-way ANOVA with Tukey's multiple comparisons test). **j,** Cell proliferation index of hematopoietic progenitors in femur bone marrow of HuCXCR4KI mice in homeostatic conditions (non-treated), n.s. = non-significant, *P<0.02, ***P=0.0008 (one-way ANOVA with Tukey's multiple comparisons test).

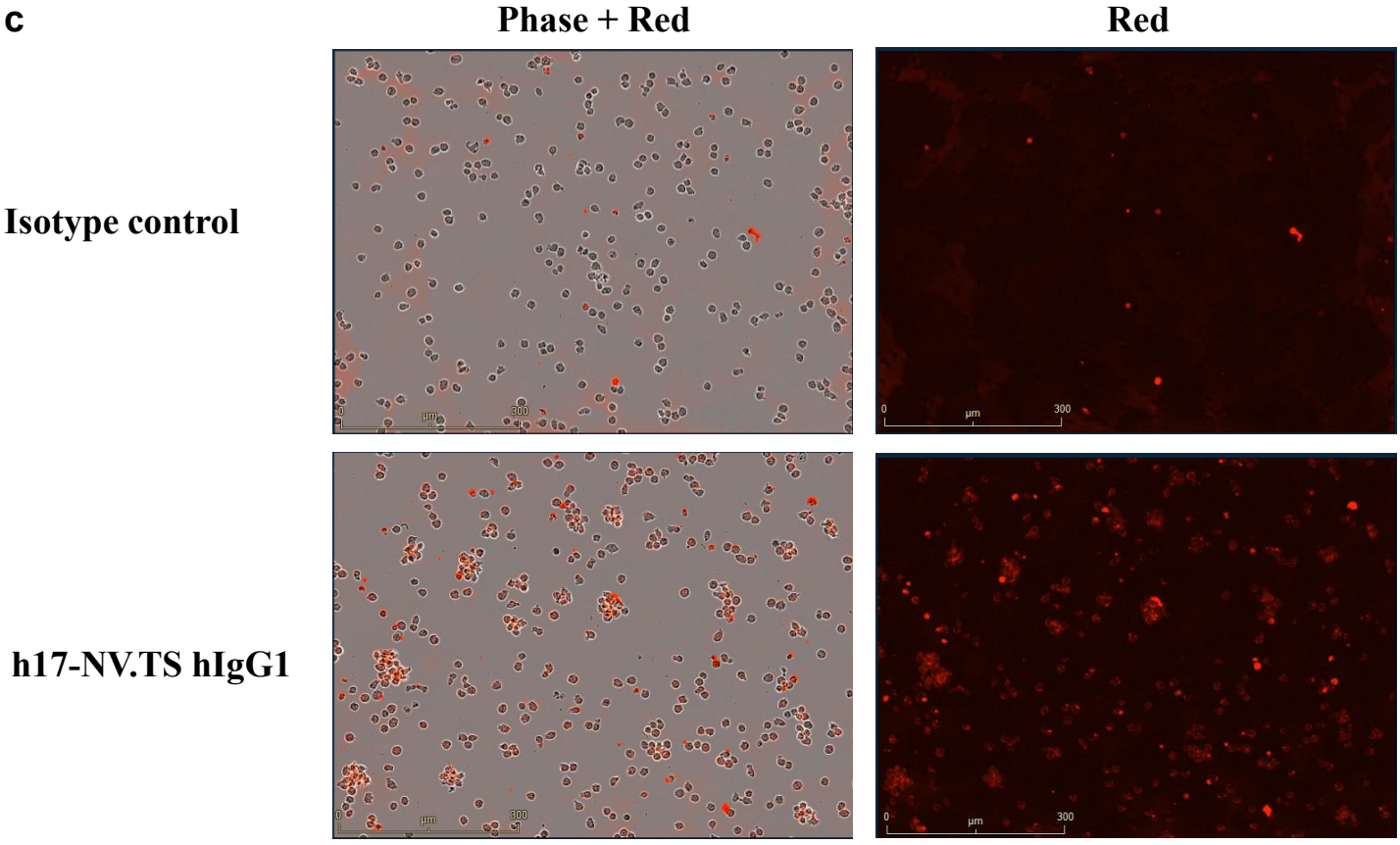
a



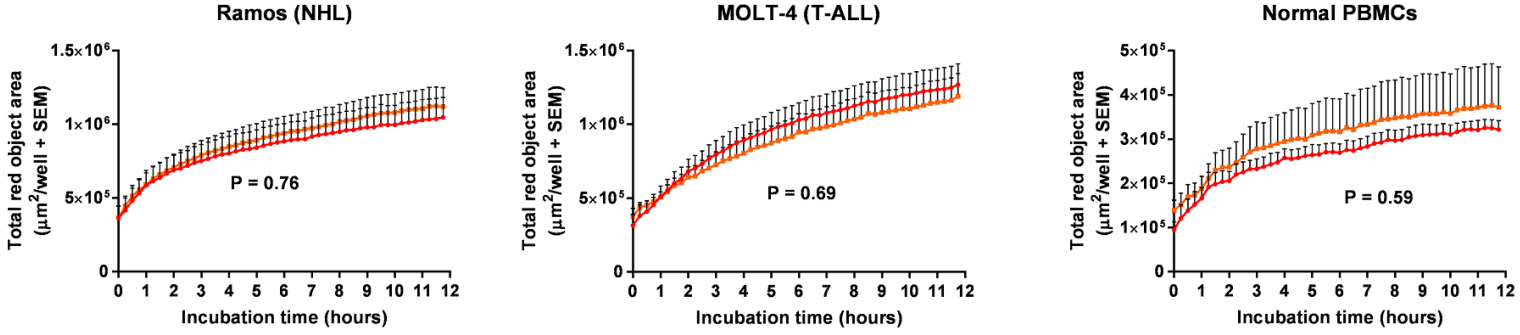
b

h17-NV.TS internalization rate during first 2.5 hours incubation (Best-fit slope ± SE)			
Ramos	MOLT-4	PBMC Donor 1	PBMC Donor 2
149,379 ± 11,684	174,310 ± 4,467	49,933 ± 4,820	53,554 ± 4,260

c



d



Supplementary Fig. 9

Supplementary Fig. 9. Kinetics of internalization of CXCR4:antibody complexes is faster in human cancer cells than in normal PBMCs. a, d, Recorded fluorescent signal of pH-sensitive fluorophore-labeled antibodies upon addition to cells (3 μg/mL antibody) and start of incubation at 37 °C, SEM = standard error of the mean (from triplicate wells). **a,** Internalization rates of CXCR4:h17-NV.TS antibody complexes in cancer cells and normal PBMCs. Linear regression analysis was applied to the data from up to 2.5 hours incubation, at which time point fluorescent probe signal in normal PBMCs reaches plateau. **b,** Internalization rates: linear regression of variation in total red object area over the first 2.5 hours of incubation time (from a, right panel), SE = standard error. **c,** Micrographs of MOLT-4 cells at 6 hours post incubation with 3 μg/mL h17-NV.TS antibody show high intracellular (red) signal resultant from fluorescent probe exposure to low pH in lysosome. No specific signal is detected with isotype control at the same concentration and time point. **d,** Internalization rates of high (h17-NS) and low (h17-NV.TS) cell binding anti-CXCR4 antibody variants in cancer cell lines and normal PBMCs. P values calculated using two-way ANOVA with Sidak's multiple comparisons test.