SUPPLEMENTARY APPENDIX

Targeting the 5T4 oncofetal glycoprotein with an antibody drug conjugate (A1mcMMAF) improves survival in patient-derived xenograft models of acute lymphoblastic leukemia

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Supplemental Table 1

UPN	MRD Risk	Cytogenetics	Sex	Age (Years)
SR03	SR	NA	F	3.4
SR14	SR	ETV6-RUNX1	M	5.8
SR_M1	SR	ETV6-RUNX1	M	11.6
SR_M2	SR	High Hyperdiploid	M	6.1
VHR03	HR	NA	M	17.1
HR08	HR	MLL	F	0.3
HR_M1	HR	High Hyperdiploid	M	11.5
HR_M2	HR	t(1;9)	F	15

List of patient samples engrafted into NSG mice to create PDX models. MRD Risk, SR = MRD<10^{-4}; HR = MRD ≥10^{-4} detected post induction. Details on SR03, SR14, VHR03 and HR08 have been previously reported[1].

1. Schmitz M, Breithaupt P, Scheidegger N, Cario G, Bonapace L, Meissner B, Mirkowska P, Tchinda J, Niggli FK, Stanulla M, Schrappe M, Schrauder A, Bornhauser BC, Bourquin JP. Xenografts of highly resistant leukemia recapitulate the clonal composition of the leukemogenic compartment. *Blood*. 2011 Aug 18;118(7):1854-64.

Supplemental Table 2

Dosing schedules for immunotherapy experiments

Figure	Tumour	Dose/ Route Day 0	Chemo	ADC (start)	**ADC Doses per Cycle	No. of cycles	Monitoring
5	Sup5T4	$5x10^6$ ip	- - -	Untreated 5T4-ADC (day 7) Control-ADC (day 7)	- 3 3	- 2 2	IVIS weekly
6	HR08	$2x10^6$ iv & $2x10^3$ iv	- - -	Untreated 5T4-ADC (day 7) Control-ADC (day 7)	- 4 4	- 3 3	PBL flow cytometry
S1	VHR08	$2x10^6$ iv	- - -	Untreated 5T4-ADC (day 7) Control-ADC (day 7)	- 4 4	- 2 2	PBL flow cytometry
7	HR08	$1x10^6$ iv	- VXL* VXL VXL	Untreated Untreated 5T4-ADC (day 21) Control-ADC (day 21)	- - 4 4	- - 3 3	PBL flow cytometry
8	HR08	$2x10^3$ iv	- DEX*** DEX DEX	Untreated Untreated 5T4-ADC (day 21) Control-ADC (day 21)	- - 4 4	- - 4 4	PBL flow cytometry

***VXL given on weekdays of weeks 2 and 3 after tumour initiation**
**** ADC given 3 or 4 times at 4 day intervals and cycle repeated after a week.**
***** DEX given on weekdays of weeks 2 and 3 after tumour initiation**

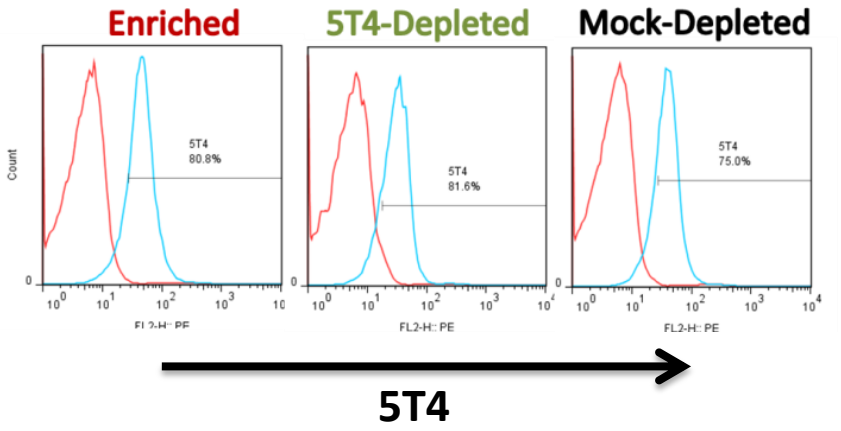
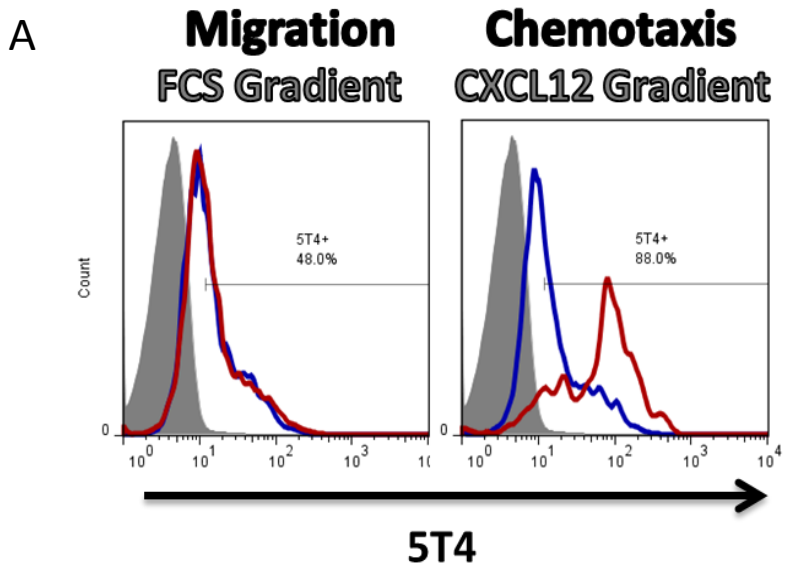


Figure S1. Engraftment of 5T4 depleted and enriched HR08 blasts in NSG mice

At termination, spleens were harvested from all animals. Flow cytometry of splenic cells showed recapitulation of the parental phenotype, ~75% 5T4 positive blasts, in all groups independent of fractionation.



Pre-analysis in blue, post-analysis of lower chamber cells in red

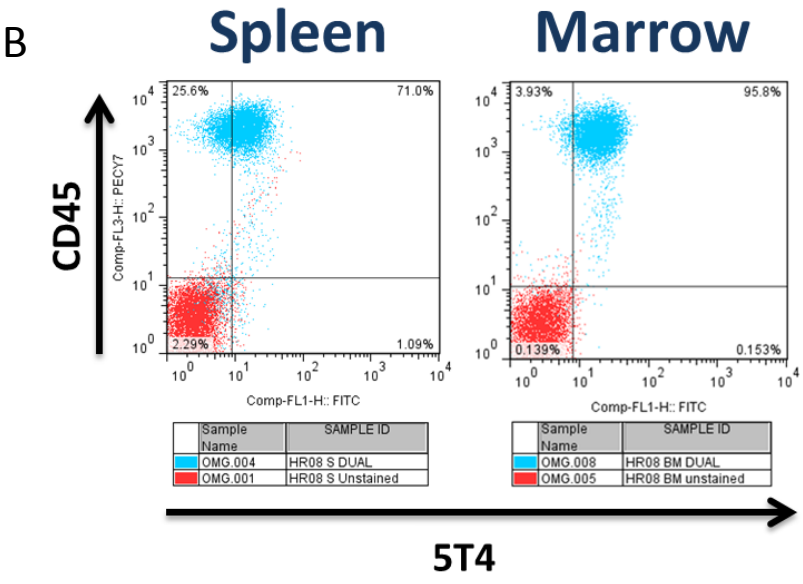


Figure S2. CXCL12 chemotaxis of BCP-ALL PDX cells.

(A) 5T4 positive blasts are the predominant responders to the CXCL12 gradient as seen by preferential accumulation of 5T4 expressing PDX cells in the chemoattractant chamber in response to CXCL12 but not FCS.

(B) Enrichment of 5T4 positive blasts seen in HR08 blasts in the bone marrow of NSG hind limb femurs. Leukaemic blasts in BM are >95% 5T4 positive compared to spleen levels (71%).

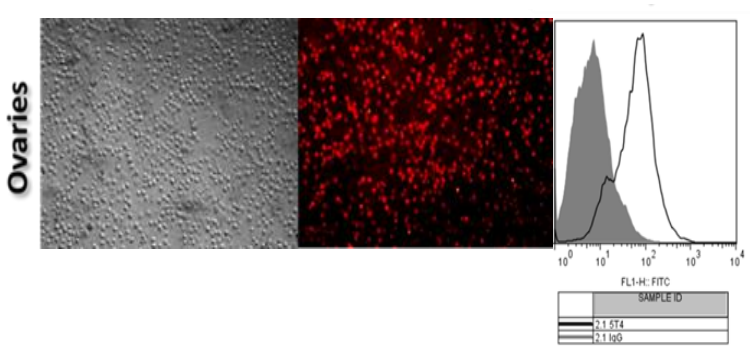


Figure S3. A1mcMMAF monotherapy of Sup5T4 cells *in vivo*. Ovaries were disaggregated at end of the experiment to identify Sup5T4 cells (mCherry) and then analysed by flow cytometry. Recurrent leukaemia, post A1mcMMAF treatment, retains a 5T4 positive phenotype.

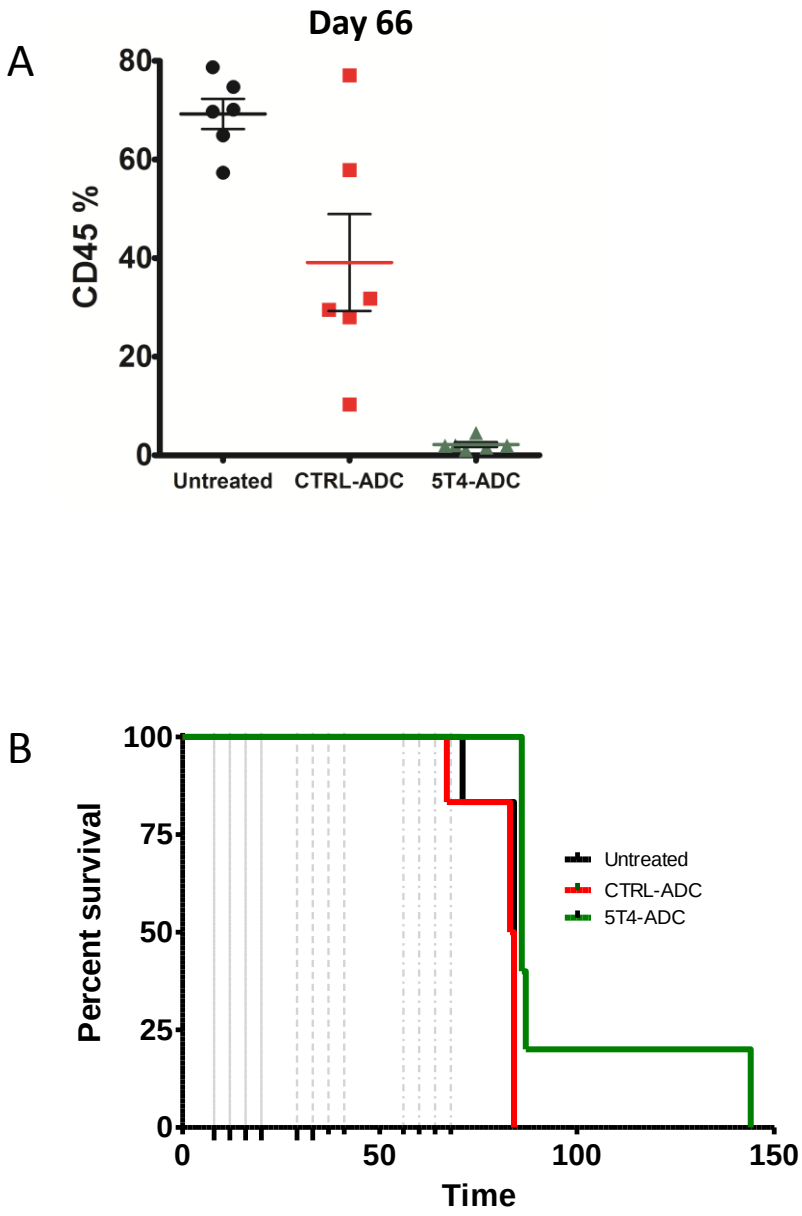


Figure S4. A1mcMMAF monotherapy of HR08 B-ALL PDX challenge (A) A1mcMMAF significantly reduces the engraftment of 1x10 ^{6} HR08 cells in NSG mice at day 66 (ANOVA/Tukey; $p<0.0001$) but (B) had no impact on the overall survival. Dotted vertical lines represent timing of doses of ADC therapy (see supplemental Table 2)

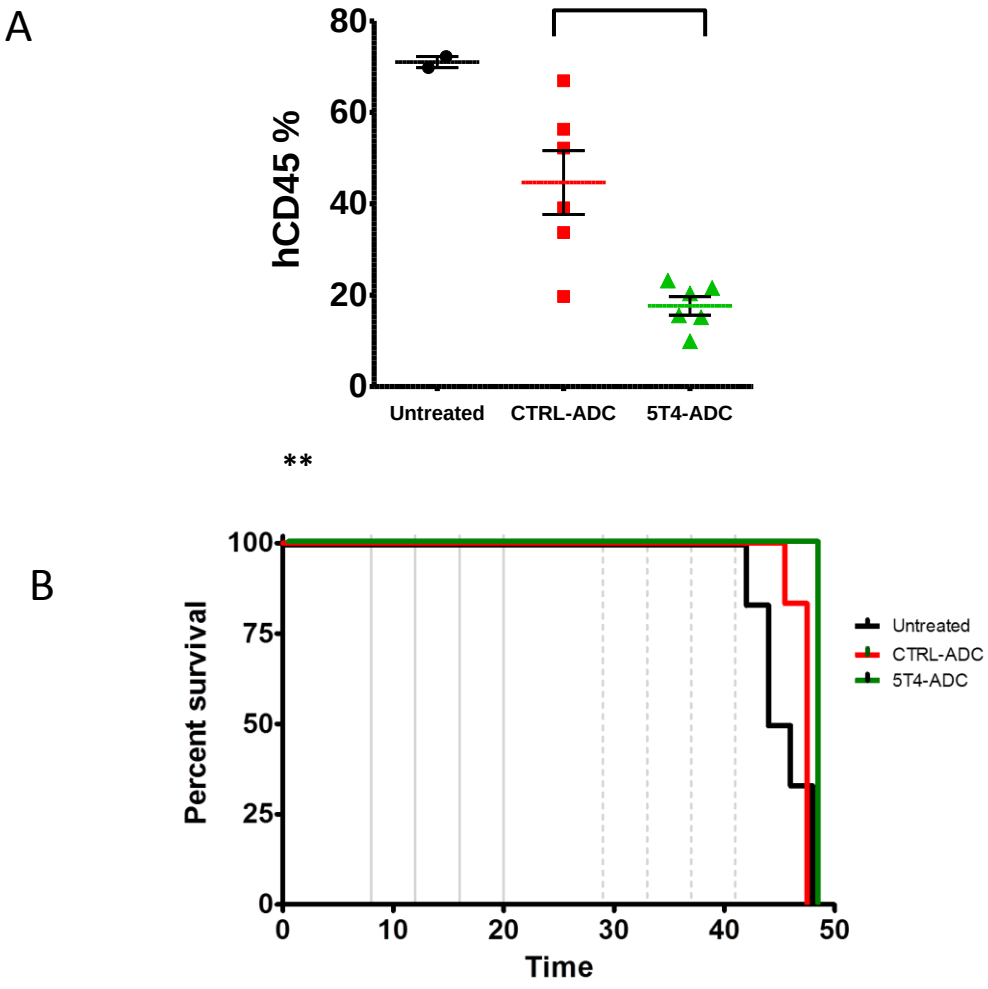


Figure S5: Engraftment and survival of VHR03 primagrafts given ADC therapy

(A) Compared to control therapy, 5T4-ADC significantly reduces the engraftment of $2x10^6$ VHR03 cells in NSG mice at day 42 (unpaired t test; p=0.004) but (B) had no impact on the overall survival

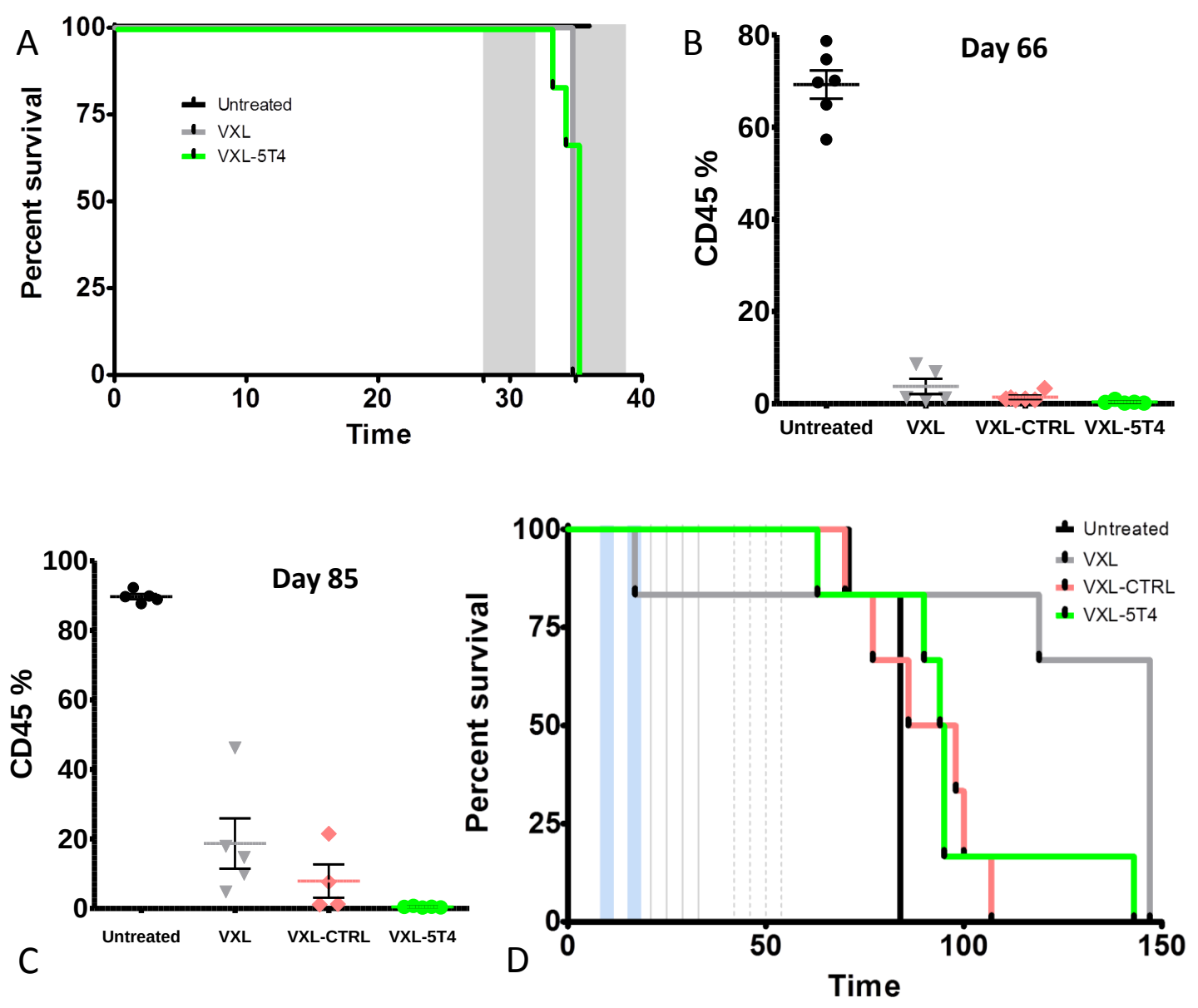


Figure S6: Combination VXL chemotherapy and ADC treatment of HR08 PDX

(A) Kaplan-Meier plots show that when VXL therapy is administered 4 weeks after transplantation of $1x10^6$ HR08 cells all animals reached morbidity due to therapeutic challenge, within the first VXL cycle of drugs and before A1mcMMAF administration. (B) VXL therapy administered 1 week after transplantation of $1x10^6$ HR08 cells significantly reduced engraftment for all groups in NSG mice at day 66 (ANOVA-Tukey; $p<0.0001$). (C) At day 85 engraftment of HR08 blasts had increased in the VXL and VXL-control treated groups, while the A1mcMMAF combination therapy group were significantly less engrafted ANOVA-Tukey; $p<0.05$. (D) Kaplan-Meier plots show no significant impact in overall survival between treatment groups. Hatched areas and dotted vertical lines represent timing of chemotherapy and doses of ADC therapy respectively (see supplemental Table 2)

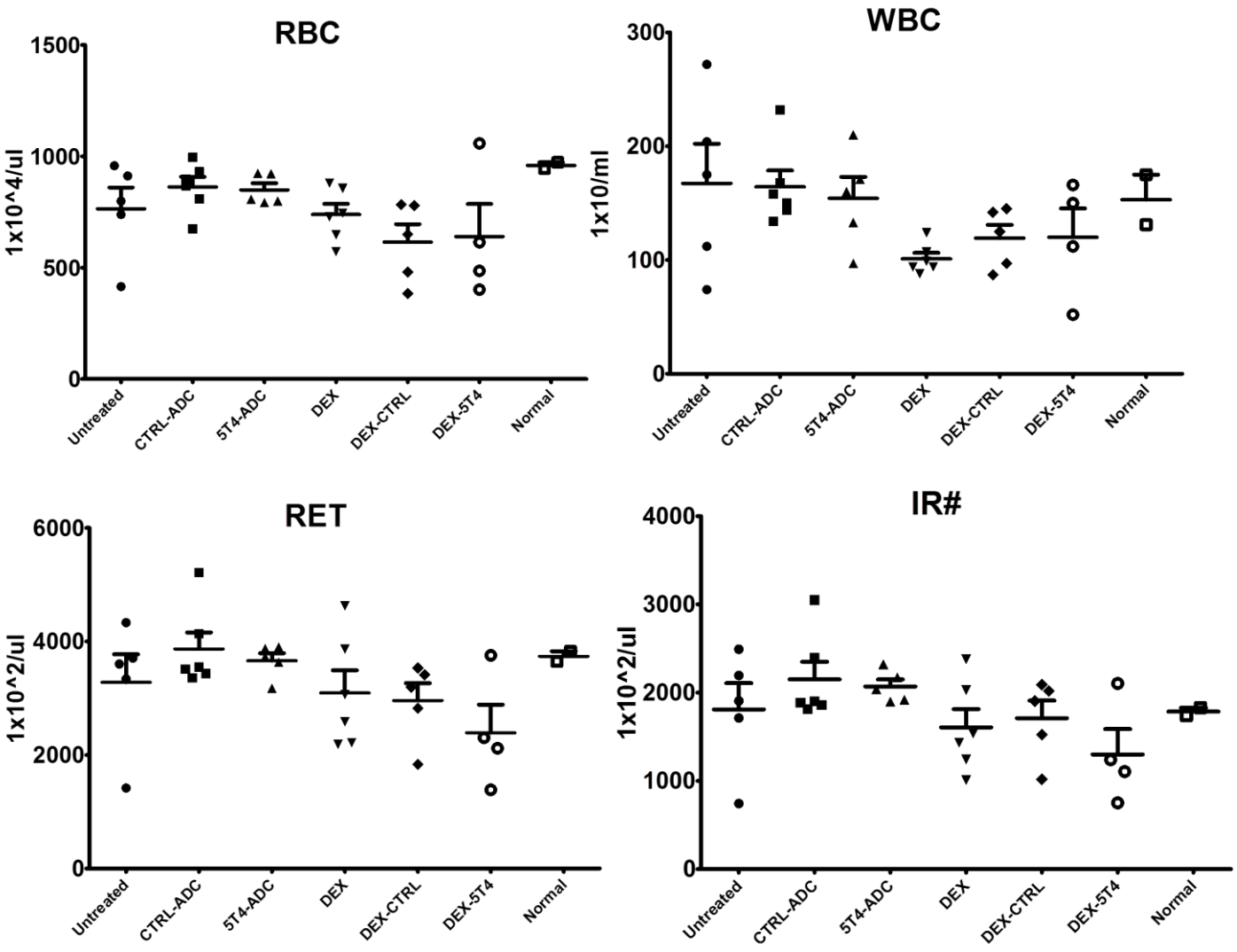


Figure S7: Monitoring in vivo hematopoiesis

Anaemia is one of the most frequent side effects of anticancer treatment, it is also caused by disease itself. Effective erythropoiesis can be monitored by quantitative measurement of reticulocytes, the amount of RNA in these cells can be assessed by flow cytometry and divided into low- (LFR), middle- (MFR) and high-fluorescence reticulocytes (HFR), this distribution is correlated with their maturation (Luczynski et al 2006; Adv. Med Sci 51: 188-90). To assess this we used the Sysmex XT-2000iV system. Analyses of murine peripheral blood from day 42 of 5T4-ADC monotherapy and combination therapy with DEX. No significant differences were observed across any groups, including additional controls of un-transplanted mice, in red (RBC) or white blood cell (WBC) count, overall reticulocyte number (RET) or immature reticulocyte fraction (IR#).