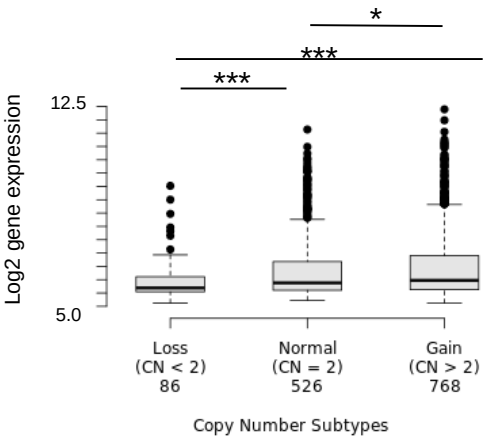
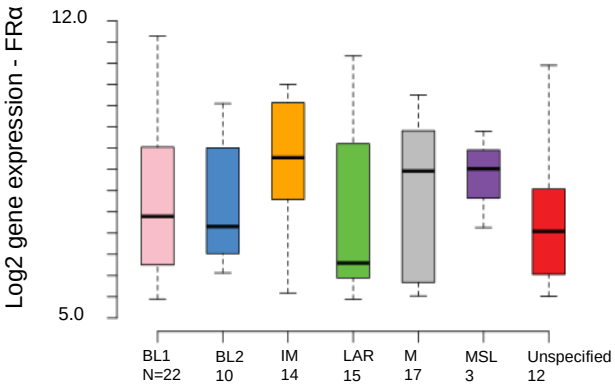
Supplementary figure 1

(A) **METABRIC Breast Cancer Cohort**
FRα copy number



(B) **Lehman- Pietenpol TNBC subtype**

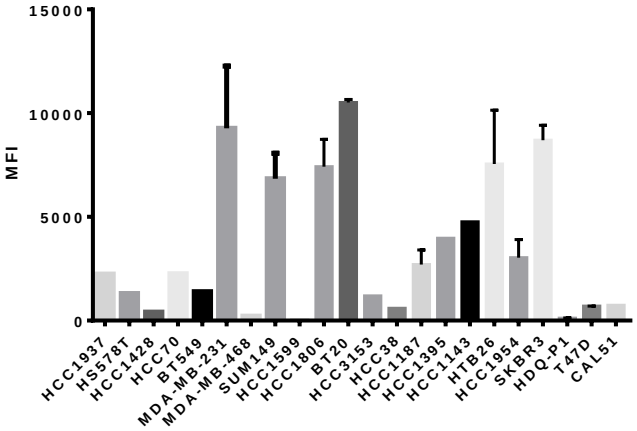


Supplementary figure 2

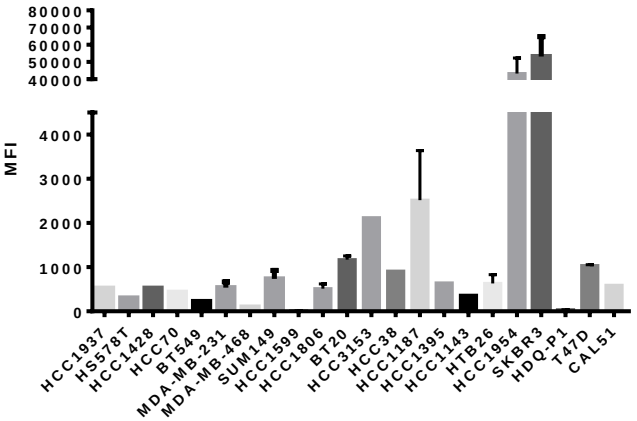
(A) Breast cancer histopathological subtype

Cell line	Breast cancer histopathological subtype	Cell line	Breast cancer histopathological subtype
HCC1937	TNBC	HCC3153	TNBC
HS578T	TNBC	HCC38	TNBC
HCC1428	TNBC	HCC1187	TNBC
HCC70	TNBC	HCC1395	TNBC
BT549	TNBC	HCC1143	TNBC
MDA-MB-231	TNBC	HTB26	TNBC
MDA-MB-468	TNBC	HCC1954	HER2+
SUM149	TNBC	SKBR3	HER2+
HCC1599	TNBC	HDQ-P1	TNBC
HCC1806	TNBC	T47D	ER+
BT20	TNBC	CAL51	TNBC

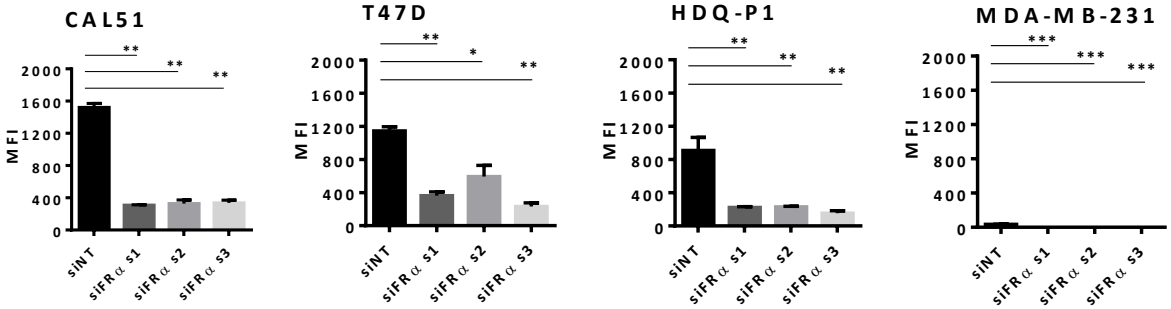
(B) EGFR protein expression by FACS



(C) HER2 protein expression by FACS



(D) siRNA knockdown - FRα expression



(E) Cancer cell viability after reduction of FRα

	% cell viability ± S.E.M. compared with scrambled siRNA-treated cells (N=3)								
	siFRα1	S.E.M.	*P* value	siFRα2	S.E.M.	*P* value	siFRα3	S.E.M.	*P* value
CAL51	67.8	3.3	0.0005	75.6	4.0	0.0005	80.1	2.0	0.0005
T47D	74.3	3.2	0.0005	74.2	4.4	0.0005	74.2	3.1	0.0005
HDQ-P1	85.9	3.3	0.0005	41.1	3.1	0.0005	31.5	2.4	0.0005

(F) Colony formation after reduction of FRα

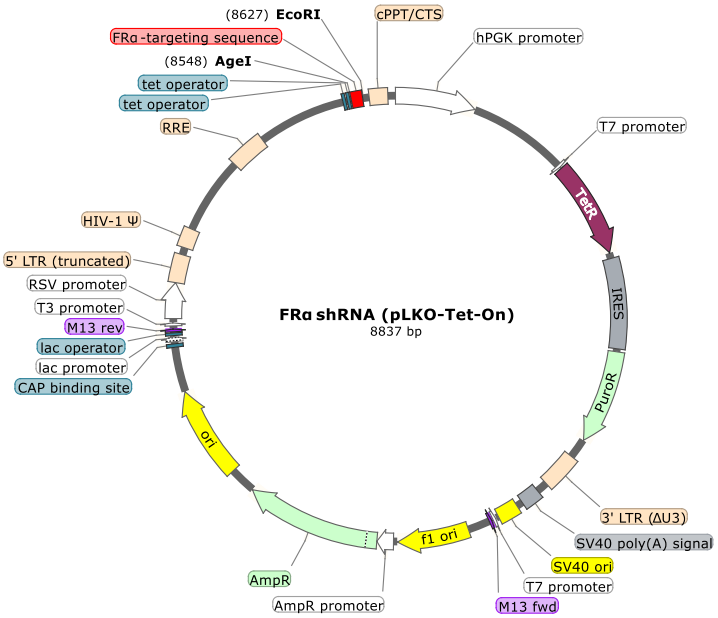
	% surviving colony formation ± S.E.M. compared with scrambled siRNA-treated cells (N=3)								
	siFRα1	S.E.M.	*P* value	siFRα2	S.E.M.	*P* value	siFRα3	S.E.M.	*P* value
CAL51	61.7	3.1	0.0005	74.7	2.9	0.0005	73.3	2.1	0.0005
T47D	71.6	2.7	0.0005	78.1	4.0	0.0005	82.0	3.0	0.0005
HDQ-P1	88.6	7.6	n/s	29.1	2.5	0.0005	56.0	6.1	0.005

Supplementary figure 3

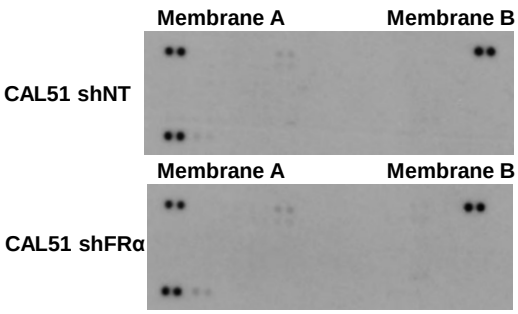
(A) shRNA knockdown in CAL51 cells

5' AgeI- FRα target sequence - shRNA loop- FRα target sequence (rev. comp.) - terminator -EcoRI 3'

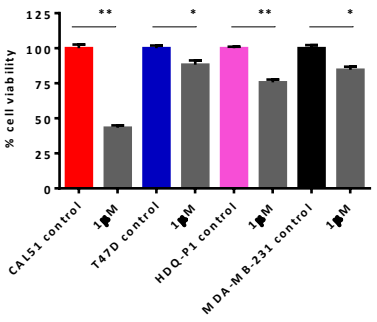
5'CCGGTGGATGTTTCCTACCTATATAGATTCAGTGAAGCCACAGATGTGAATCTATATAGGTAGGAAACATCCTTTTTTG 3'
3' ACCTACAAAGGATGGATATATCTAAGTCACTTCGGTGTCTAGACTTAGATATATCCATCCTTTGTAGGAAAAAACTTAA 5'



(B) Human Phospho-Kinase Array reference points



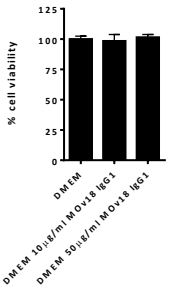
(C) Src kinase inhibitor AZM475271



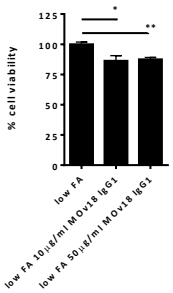
(D) Inhibitory effect of MOv18 on cell proliferation in medium acutely depleted of folate

Physiological folate concentration	20 nM
DMEM folate concentration	9.2 mM
FBS folate concentration	4 nM
Folate-depleted DMEM with 10% FCS	0.4 nM

Normal media

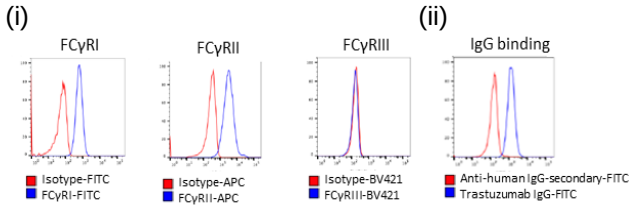


Folate- depleted media

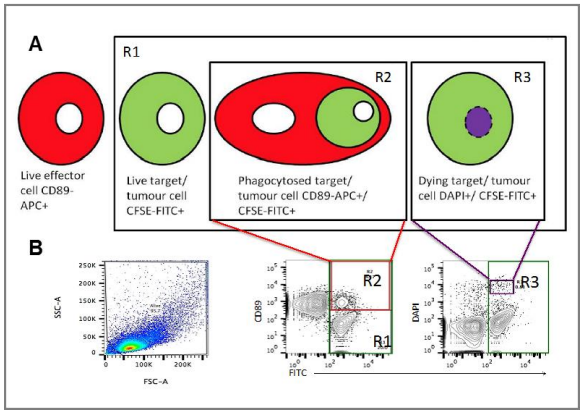


Supplementary figure 4

(A) Fc receptor expression profile and binding of IgG1



(B) Calculation of % tumor cell killing



(C) % tumor cell death with U937 human monocytes as effector cells (refers to data from Figure 5B)

	MOv18 IgG1				Isotype IgG1					No antibody				
	ADCC	S.E.M.	ADCP	S.E.M.	ADCC	S.E.M.	ADCP	S.E.M.	*P* value	ADCC	S.E.M.	ADCP	S.E.M.	*P* value
CAL51	20.5	7.8	13.2	3.3	9.1	2.7	3.6	0.8	0.05	11.3	3.0	3.5	0.5	0.05
T47D	8.5	6.7	23.0	7.0	3.9	1.7	11.1	4.7	0.05	3.7	0.8	10.3	2.3	0.05
HDQ-P1	14.6	4.7	29.2	8.8	9.3	4.1	14.7	6.9	0.0005	12.2	4.0	7.2	0.4	0.0005
SKBR3	2.2	0.9	7.7	0.6	3.7	3.3	5.9	1.2	n/s	4.8	2.6	5.9	0.4	n/s
MDA-MB-231	7.1	3.2	17.6	2.3	4.7	2.7	14.8	0.4	n/s	9.9	2.9	13.2	0.9	n/s
HCC1428	4.4	0.7	9.7	1.8	11.3	2.2	5.5	0.5	n/s	6.5	3.2	6.1	0.9	n/s

(D) Patient characteristics (refers to data from Figure 5C)

Patient	Type	Age	Grade	Stage	Metastatic disease	Chemotherapy
1	TNBC	43	2	n/a	Yes	Yes
2	TNBC	44	3	2	No	Yes
3	TNBC	61	3	n/a	Yes	Yes
4	TNBC	31	3	n/a	Yes	No
5	TNBC	28	3	2	No	Yes
6	TNBC	47	3	2	Yes	Yes
7	TNBC	59	3	2	No	Yes
8	TNBC	51	3	4	Yes	Yes
9	TNBC	55	n/a	n/a	Yes	Yes

(E) % tumor cell death with healthy volunteers' PBMC as effector cells (refers to data from Figure 5C)

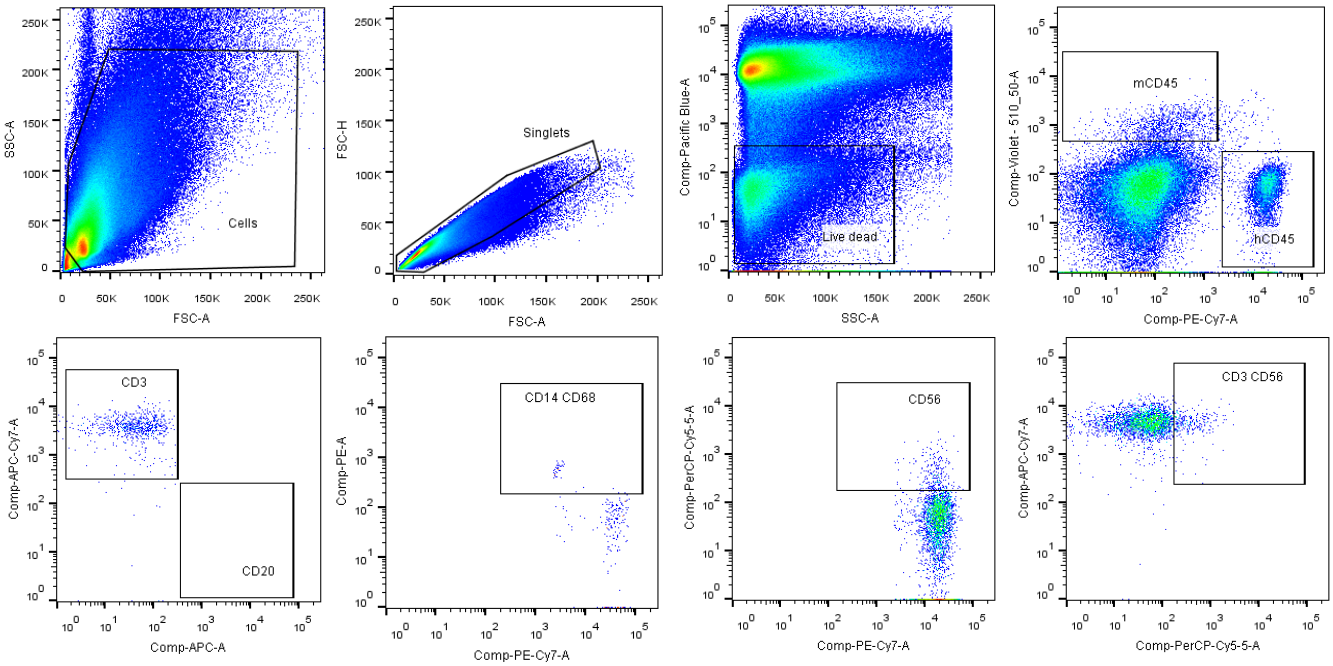
	MOv18 IgG1				Isotype IgG1					No antibody				
	ADCC	S.E.M.	ADCP	S.E.M.	ADCC	S.E.M.	ADCP	S.E.M.	*P* value	ADCC	S.E.M.	ADCP	S.E.M.	*P* value
CAL51	12.9	6.0	19.4	6.8	10.6	4.6	8.1	4.1	0.005	10.4	3.4	8.1	4.0	0.005

(F) % tumor cell death with TNBC patients' PBMC as effector cells (refers to data from Figure 5C)

	MOv18 IgG1				Isotype IgG1					No antibody				
	ADCC	S.E.M.	ADCP	S.E.M.	ADCC	S.E.M.	ADCP	S.E.M.	*P* value	ADCC	S.E.M.	ADCP	S.E.M.	*P* value
CAL51	17.1	8.2	21.9	11.2	11.7	3.0	7.0	3.6	0.005	12.5	3.8	5.7	2.6	0.005

Supplementary figure 5

(A) Phenotyping of engrafted immune cells



(B) Antibody panel

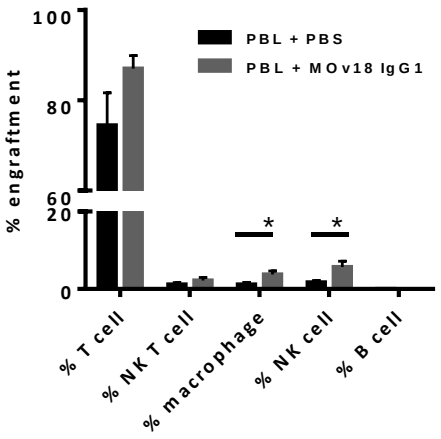
Reagent	Colour
Anti-mouse CD45	V500
Anti-human CD45	PE-Cy7
Anti-human CD20	APC
Anti-human CD3	APC-Cy7
Anti-human CD14	PE
Anti-human CD68	PE
Anti-human CD56	PerCP-Cy5.5
Dead cell	DAPI

Marker	Cell type
CD45	Immune cells
CD3	T cells
CD20	B cells
CD14 CD68	Macrophages
$CD3^-$ CD56	NK cells
$CD3^+$ CD56	NK T cells

(C)

WHIM02

CD45+ FACS staining in tumor tissue



Supplementary Table 1

King's College London TMA

Sample type	Number of sample	FRα positive sample	Positive [%]
Grade			
Grade 1	24	0	0.0
Grade 2	78	6	7.7
Grade 3	186	46	24.7
	(288)		
Defined by IHC			
TNBC	76	28	36.8
ER negative	10	3	30.0
ER positive	67	5	7.5
HER2	45	7	15.6
Luminal	107	7	6.5
(Total)	*(305)*		
Defined by PAM50			
Basal-like	72	24	33.3
HER2	28	3	10.7
Luminal A	14	1	7.1
Luminal B	4	1	25.0
Normal-like	7	2	28.6
(Total)	*(125)*		