Supplementary Data

Cell lines and primary cells

Human myeloma cell lines (HMCL) from ATCC were maintained in RPMI 1640 (Lonza)/10% fetal calf serum (FCS, Labtech).

Patients gave informed consent using a protocol approved by the UCL/UCLH Committee for Ethics of Human Research. Patients had a bone marrow (BM) biopsy taken prior to therapy. Mononuclear cells (MNCs) from BM aspirates were obtained by density gradient centrifugation (Ficoll Paque, GE lifesciences) and stained directly for BCMA and TACI. BM MNCs were also CD138 selected (Miltenyl Microbeads) for cytotoxicity and co-culture assays.

Cytogenetic analysis of primary MM cells

Patients had iFISH analysis on CD138-selected cells at the time of bone marrow (BM) sampling. Adverse genetics was defined as the presence of t(4;14), t(14;16), t(14;20), 1q gain, 1p loss and/or del(17p)(>50%).

Flow cytometry

BCMA/TACI quantification: 2.5x10^5 cells were incubated with rat serum (Sigma) and anti-CD16/CD32 (in-house hybridoma supernatant clone 2.4G2) to reduce background staining. CD138 APC (Clone MI15) was then added with one of murine IgG2a PE Isotype control (clone MOPC-173), rat IgG2a PE Isotype control (clone RTK2758), anti-BCMA PE (clone 19F2) or anti-TACI PE (clone 1A1) at 4^{O}C for 30 minutes before washing, resuspension in PBS/0.1%BSA/0.1%PFA (Santa Cruz) and analysis with BD QuantiBRITE^{TM} beads. All antibodies from BioLegend unless otherwise specified. BD Fortessa was used for cell acquisition and data was analysed using FlowJo_V10. Antibodies bound per cell (ABC) was then calculated by subtracting ABC of relevant isotype control. Calculated expression levels of greater than 100 ABC was taken as positive.

CAR T-cells: Transduced cells were stained with CD34 PE (clone QBEnd10, R&D) to detect RQR8 and APRIL biotin (clone 53E11, BioLegend) followed by Streptavidin APC (AbD Serotec) and transduction efficiency refer to RQR8+APRIL+ events.

FACS based cytotoxicity assays: Targets were stained with Efluor780 viability dye (eBioscience) and CD4 PE, CD8 PB and CD3 APC for SUPT1 targets or CD138 APC for myeloma cells with Flow-Check^{TM} beads (Beckman Coulter). Viable SUPT1 or myeloma cells determined as live/single/CD3-/CD4+/CD8+ or live/single/CD138+ events respectively. Effector proliferation was determined by staining for RQR8.

Real-time qPCR to quantitate BCMA and TACI transcript copies

Gene expression was performed by Asterand Bioscience (XpressWay® profile) testing 72 non-diseased tissue types, each from 3 different donors. Beta-actin cDNA quantification served as a control. Primers listed in Table S2.

Assessing BCMA and TACI binding to membrane bound APRIL

SUPT1 cells were transduced with RD114-pseudotyped viral supernatant to express truncated APRIL and CD34 separated by an autocatalytic 2A peptide (ie APRIL.2A.CD34)[1]. Then 0.5x10^6 SUPT1 cells were washed and incubated with 500µl of supernatant from 293T cells transfected with GeneJuice® (Novagen) to secrete BCMA-muFc or TACI-muFc. After 30 minutes at room temperature, cells were washed and stained with anti-muFc FITC (Jackson) and CD34 APC (clone QBend10 R&D).

Assessing BCMA and TACI binding to truncated APRIL by surface plasmon resonance

Protein production and purification: Recombinant APRIL containing an N terminal FLAG™ Tag and a C-terminal hinge was expressed in 293T cells. Both constructs contained a poly-histidine tag at the extreme C-terminus. Supernatant from transfected 293T cells were purified using TALON™ metal affinity chromatography. A Hi-trap crude TALON column was equilibrated in binding buffer, 300 mM NaCl, 50 mM sodium phosphate, pH 7.4. Supernatant was applied to the column using an Akta™ Purifier system at a flow rate of 1 ml/min^{-1}. Following application of supernatant, the column was washed with 10 column volumes of wash buffer 1, 5 mM imidazole, 300 mM NaCl, 50 mM sodium phosphate, followed by 10 column volumes of wash buffer 2, 10 mM imidazole, 300 mM NaCl, 50 mM sodium phosphate, pH 7.4. Bound protein was eluted using elution buffer, 150 mM imidazole, 300 mM NaCl, 50 mM sodium phosphate, pH 7.4. The desired fractions were pooled and dialysed against PBS (Sigma) overnight. All purification steps were carried out at 4^{o}C.

Surface plasmon resonance: Sensograms were obtained on a BIAcore™ T200 instrument by capturing His tagged APRIL on a CM5 Chip (GE Healthcare: 29104988) functionalised with an anti-His antibody (GE Healthcare). BCMA and TACI (Peprotech) were injected over the sensor surface at concentrations ranging from 1 – 300 nM. The sensor surface was regenerated between each injection with a short pulse of 3M MgCl2. In each experiment flow cell 1 was unmodified and used as a reference subtraction. A sensogram of buffer alone was used as a double reference subtraction to factor for drift. Data were fit to a 1:1 langmuir binding model.

Cytotoxicity assays

All effector cells were CD56 depleted (Miltenyl) prior to co-culture. Effector to target (E:T) ratios refer to ratio of ACAR+ T-cells to targets throughout. Four hour ^{51}Cr release assays were performed by incubating 5x10^3 chromium-labelled targets with ACAR T-cells at E:T ratios indicated.

With cell lines, FACS based cytotoxicity assays were performed by plating targets at 0.5x10^6/ml and adding equal volumes of ACAR T-cells at the relevant concentration. Percentage cytolysis was calculated relative to viable targets in media control. Interferon gamma (IFNG) release was assessed using a BioLegend IFNG ELISA Max kit. FACS analysis at 48 hours.

ACAR transduced PBMCs were labelled with 5uM Cell Trace Violet (Thermo Fisher) according to the manufacturer's instructions and co-cultured 1:1 ratio with SUPT1 targets. The cells were harvested at D+4 and stained with CD3, CD8 and RQR8 as well as 7-AAD before analysis by FACS. Percentage of ACAR cells proliferated determined gating on live/CD3+/RQR8+ cells compared to on co-culture with $SUPT1^{NT}$.

CD138-selected primary myeloma cells were cultured in RPMI 1640/20% pooled patient plasma (PPP)[2]. Percentage cytolysis was calculated relative to viable tumour cells on co-culture with PBMC NT. Blocking experiments were performed using anti-BCMA antibody S307118G03, muIgG2a Fc Protein (AcroBiosystems).

Engineering BCMA and TACI expressing SUPT1 targets

SUPT1 cells were transduced to express BCMA or TACI and green fluorescent protein (GFP) separated by an in-frame foot-and-mouth–like 2A peptide, TaV[1] (ie BCMA.2A.GFP or TACI.2A.GFP). $SUPT1^{BCMA}$ and $SUPT1^{TACI}$ were then sorted into discrete populations on a BD FACSAria Fusion. For the in vivo escape model, SUPT1-BCMA3 and SUPT1-TACI2 were the retrovirally transduced with SFG.FLuc.2A.RQR8 and SFG.FLuc.2A.HA in a second reaction respectively and then FACS sorted to a minimum of 90% FLuc purity.

Production of S307118G03

Variable Heavy and light domains of anti-BCMA antibody S307118G03 have been previously described[3]. Variable sequences were cloned into the AbVec-mIgG2a and AbVec-mIgK vectors[4]. ExpiCHO™ cells (Thermo Fisher) were split to a density of $6x10^6$ cells/ml in ExpiCHO medium four hours before transfection with Expifectamine reagent (Thermo Fisher). Secreted antibodies were purified by affinity chromatography, HiTRAP™ MabSelect SuRe™ (GE Healthcare), followed by size exclusion chromatography, Superdex S200 (GE Healthcare). Protein purity was confirmed by SDS PAGE electrophoresis.

Immunohistochemistry

Immunohistochemistry was performed on a Leica BOND-III platform and used the BOND Polymer Refine Detection kit. CD138 (Clone MI15, Dako) was diluted 1/200 in Primary Antibody Diluent BOND. The tissue was incubated in Epitope Retrieval Solution 1 (ER2) BOND for 20 minutes and stained using the 15, 8, 8 protocol. Sections were examined using an Olympus BX51 microscope and photographed using an Olympus DP20 microscope camera.

BM Sera levels of APRIL, BCMA and TACI

Sera from the BM aspirates from patients with myeloma were obtained. BM sera non-myeloma patients were obtained from 7 further patients. Four of these served as controls for sAPRIL and sTACI assays and were obtained from patients being investigated for myeloproliferative disease (n=2, JAK2 mutation V617F, calreticulin negative) or myelodysplasia (n=1) or mild neutropenia (n=1). A further 3 samples served as controls for the sBCMA assays and were BM aspirates obtained to investigate myeloproliferative disease (n=1) or myelodysplasia (n=2). These BM sera samples were then analysed by ELISA for sAPRIL (BioLegend), sTACI (Raybio) and sBCMA (R&D) as per manufacturer's instructions.

ACAR blocking or activation by soluble APRIL, BCMA and TACI

Blocking of ACAR mediated cytolysis assessed by performing FACS based cytolysis as described above in the presence or absence of soluble APRIL, BCMA or TACI (all from Peprotech). Activation was performed by incubating T-cells at a concentration of 2.5x10^5/ml in the presence of soluble APRIL/BCMA/TACI and measuring IFNG release at 24 hours by ELISA.

In vivo model

This work was performed under a United Kingdom Home Office-approved project license and in accordance with institutional policies. Six to eight-week-old, female, NSG mice (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ) were purchased from Charles River. 10x10^6 or 3.5x10^6 Firefly luciferase (Fluc) expressing MM.1s cells or SUPT1 cells (comprising a mix of SUPT1^{BCMA} and SUPT1^{TACI} at 4:1 ratio), respectively, were injected by tail vein injection at D0. Disease burden was assessed by bioluminescence imaging (BLI) where mice were anesthetized, and then imaged using a Xenogen IVIS Imaging System (Perkin Elmer Life Sciences) following intraperitoneal injection of D-luciferin.

In vivo screen for ACAR toxicity

After sacrifice of NSG mice (NOD.Cg-*Prkdc*^{scid} *Il2rg*^{tm1Wjl}/SzJ), organs were resected from 6/6 tumour only mice, 6/6 animals who received ACAR T-cells and 5 control animals (Charles River), formalin fixed and paraffin embedded before sections stained with haematoxylin and eosin and examined by an independent histopathologist. Tissues examined were eyes, optic nerves, harderian glands, skin, mammary gland, skeletal muscle, femur with BM and stifle joint, sternum with BM, sciatic nerves, liver, spleen, pancreas, mesenteric lymph nodes, stomach, duodenum, jejunum, ileum, gut-associated lymphoid tissue (GALT), caecum, colon, rectum, gall bladder, adrenal gland, kidney, ureters, urinary bladder, ovaries, uterus including cervix, oviducts, vagina, clitoral, salivary gland, mandibular lymph nodes, thymus, lungs with bronchi/bronchioles, heart, aorta, trachea, oesophagus, thyroids, parathyroids, larynx, tongue, pituitary gland, brain, spinal cord cervical, thoracic and lumbar, lacrimal glands.

Statistics

P values <0.05 were considered significant. Statistical tests were used as indicated and calculated using Prism version 6.0 (Graphpad Software).

References

1. Donnelly ML, Hughes LE, Luke G, et al. The 'cleavage' activities of foot-and-mouth disease virus 2A site-directed mutants and naturally occurring '2A-like' sequences. *J Gen Virol*. 2001;82(Pt 5):1027-1041.
2. Quinn J, Glassford J, Percy L, et al. APRIL promotes cell-cycle progression in primary multiple myeloma cells: influence of D-type cyclin group and translocation status. Vol. 117; 2011.
3. Tai YT, Mayes PA, Acharya C, et al. Novel anti-B-cell maturation antigen antibody-drug conjugate (GSK2857916) selectively induces killing of multiple myeloma. *Blood*. 2014;123(20):3128-3138.
4. Di Niro R, Mesin L, Raki M, et al. Rapid generation of rotavirus-specific human monoclonal antibodies from small-intestinal mucosa. *J Immunol*. 2010;185(9):5377-5383.
5. Hombach A, Hombach AA, Abken H. Adoptive immunotherapy with genetically engineered T cells: modification of the IgG1 Fc 'spacer' domain in the extracellular moiety of chimeric antigen receptors avoids 'off-target' activation and unintended initiation of an innate immune response. *Gene Ther*. 2010;17(10):1206-1213.
6. Carpenter RO, Evbuomwan MO, Pittaluga S, et al. B-cell maturation antigen is a promising target for adoptive T-cell therapy of multiple myeloma. *Clin Cancer Res*. 2013;19(8):2048-2060.

7. Ali SA, Shi V, Maric I, et al. T cells expressing an anti-B-cell-maturation-antigen chimeric antigen receptor cause remissions of multiple myeloma. *Blood*. 2016.

Supp Figure Legends

Table S1. BCMA and TACI expression levels on primary myeloma cells from 50 patients determined by FACS. Bone marrow mononuclear cells were stained with CD138 APC (displayed on y-axis) and BCMA PE, TACI PE or their respective isotype controls, mIgG2a PE and rIgG2a PE (x-axis). Antigen densities of BCMA and TACI on CD138+ tumour cells (gated) were then quantified using QuantiBRITE™ beads and subtracting antibodies bound per cell (ABC) of isotype controls.

Table S2. Primers for RT-PCR

S1. BCMA and TACI expression of primary myeloma cells (A) Surface Antigen expression levels on myeloma tumour cells was determined by staining BM MNCs with CD138 and BCMA PE, TACI PE or their respective isotype controls. Example histogram (Patient #7) showing fluorescence of CD138+ gated cells stained for **(i)**BCMA (blue) or **(ii)**TACI (red) compared to their relevant isotype controls (empty) superimposed onto QuantiBRITE™ beads (grey). **(B)**In a small number of patients, CD138+ tumour cells were stained with TACI streptavidin and BCMA PE to demonstrate co-expression of both receptors on myeloma cells. **(C)**Expression of BCMA and TACI on primary myeloma cells. The patient samples with a higher antigen density of TACI than BCMA highlighted as empty circles (n=50, r=0.27, p=0.054 by Spearman). **(D)**Tumour BCMA and TACI expression from patients at diagnosis (D, n=27) or relapsed and refractory (R, n=23) disease. **(E)**Tumour BCMA and TACI expression in patients with standard (S, n=17) and poor risk (P, n=25) genetic lesions. The following were included under the definition of poor genetic risk: t(4;14), t(14;16), t(14;20), 1q gain, 1p loss and/or del(17p)(>50%). Median shown, *=p<0.05 by Mann Whitney.

Figure S2. BCMA and TACI transcripts in normal tissue (A)qRT-PCR of BCMA and TACI transcripts in normal tissues displayed as mean tissue expression of BCMA and TACI (n=3). Mean±SD shown.

Figure S3. Membrane bound APRIL binds BCMA and TACI (A)SUPT1 cells were transduced with APRIL fused to a CD28 transmembrane (tm) domain via an IgG1 hinge, CD8 stalk or Fc spacer (as modified by Hombach[5]) as well as CD34 as a marker gene which was cloned downstream of an internal ribosome entry site (IRES) (ie CD34.IRES.APRIL). **(i)**These cells were then incubated for 30 mins with supernatant from 293T cells transfected to secrete BCMA-muFc or TACI-muFc. Cells were then washed and stained for anti-CD34 APC (marker

gene) and anti-muFc FITC. Membrane bound GD2 served as a negative control. Binding to **(ii)**BCMAmuFc and **(iii)**TACImuFc quantified by the MFI of the gated cells. **(B)**Kinetic affinity determination for the binding between truncated APRIL and **(i)**BCMA and **(ii)**TACI by surface plasmon resonance. A multi-cycle-kinetics approach was used for the determination of association and dissociation rates listed in inset table.

Figure S4. Proliferation of ACAR on co-culture with BCMA and TACI expressing SUPT1 cells. ACAR transduced PMNCs were labelled with Cell Trace Violet prior to co-culture (1:1) with $SUPT1^{NT}$, $SUPT1^{BCMA}$ or $SUPT1^{TACI}$ prior to FACS at D+4. Percentage of ACAR positive cells proliferated determined by gating on live/singlet/CD3+/RQR8+ events (ACAR transduced PBMCs from 2 donors shown).

Figure S5. ACAR mediated cytolysis of $SUPT1^{BCMA}$, $SUPT1^{TACI}$ and human myeloma cell lines. (A)ACAR-H and CD8 spacer variants were also tested against SUPT1 targets expressing a wide range of **(i)**BCMA and **(ii)**TACI targets in a 4 hour ^{51}Cr release assay. E:T ratio of 16:1 shown. Percentage specific cytolysis calculated by subtracting background kill observed on incubation with NT PBMC. **(B)** Tumour kill**(i)** was demonstrated on co-culture of ACAR-H and several human myeloma cell lines (1:1) expressing a range of BCMA and TACI levels**(ii). (C)** PBMCs transduced with ACAR(n=5) and a BCMA targeting CAR (differing to the ACAR in its exodomain consisting of the 11-D-5-3 ScFv[6,7] and CD8 spacer) were co-cultured and tested against **(i)**MM.1s and **(ii)**U266 at reducing E:T ratios and target cytolysis determined at 48 hours by FACS. There was no statistical significance between the ACAR and BCMA CAR constructs at any of the E:T ratios tested. Mean±SEM shown, *=p<0.05, **=p<0.01, ***=p<0.001 by *t* test.

Figure S6. ACAR mediated cytolysis and activation in the presence of soluble APRIL, BCMA and TACI and levels in BM sera: (A) Sera obtained from bone marrow aspirates from myeloma patients(n=10) and control donors (n=4 for sAPRIL and sTACI and n=3 for sBCMA) were assayed for soluble APRIL, BCMA and TACI. While BM levels of sBCMA and sTACI were raised in MM patients (median 294 vs 35ng/ml and 27 vs 1.2ng/ml in control BM respectively), levels of sAPRIL were reduced compared to controls (median 6.2 vs 18ng/ml respectively). Medians indicated on dot plots. **(B)**ACAR-H transduced T-cells from multiple donors were co-cultured with MM1.s cells at low E:T ratios indicated in the presence of soluble APRIL (n=3), BCMA(n=6) and TACI(n=3). Mean kill (at 48 hours)±SEM shown, **(C)** IFNG release by ACAR T-cells on incubation with soluble APRIL (50ng/ml), BCMA(1000ng/ml), TACI(200ng/ml) or co-culture with MM.1s cells (1:1) for 24 hours (n=3). Mean±SEM shown *=p<0.05, **=p<0.01, ***=p<0.001 by *t* test.

Figure S7. ACAR-H mediated cytolysis of SUPT1 cells expressing murine BCMA and TACI: (A)SUPT1 cells expressing human and murine isoforms of BCMA and TACI were co-cultured with ACAR-H transduced T-cells (E:T ratio 4:1, n=3) and cytolysis at 72 hours determined by FACS. Mean±SEM shown, ***=p<0.001 by *t* test. **(B)** Haematoxylin & eosin stained sections obtained from mice treated with ACAR failed to show ACAR mediated tissue cytotoxicity. Sections of organs indicated from single mouse from NT, EGFRvIII and ACAR treated cohorts shown.

Figure S8. T-cell persistence in murine model of antigen escape. Twenty-one NSG mice were injected with SUPT1 cells expressing either BCMA or TACI (at a ratio of 80% and 20%) and then treated with NT, ACAR or BCMA CAR T-cells (n=7 in each cohort). At D+13, the experiment was terminated and bone marrow T-cells defined as live/singlet/mCD11b-/CD2+/CD4+ or CD8+ events and normalised with Flow-Check™ beads.

Table S1

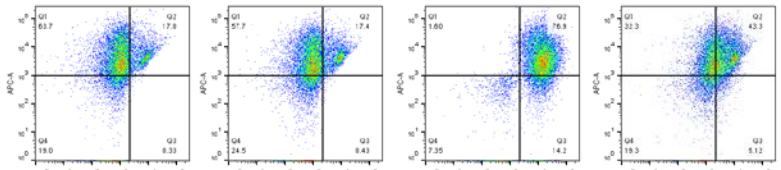
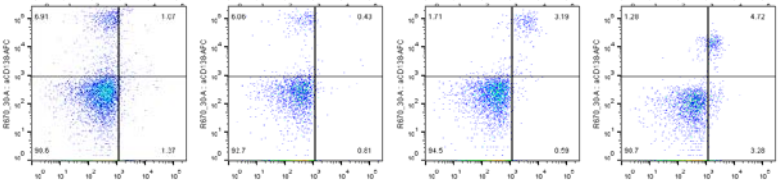
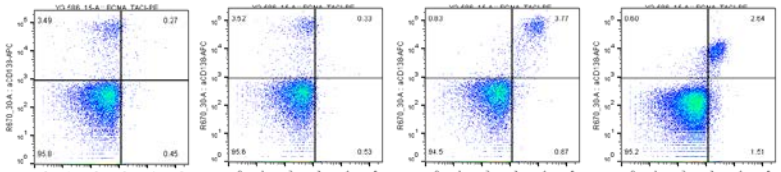
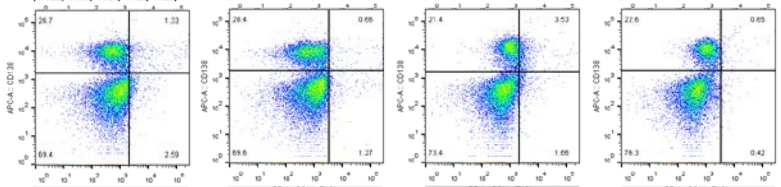
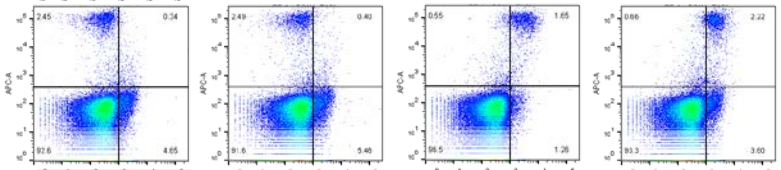
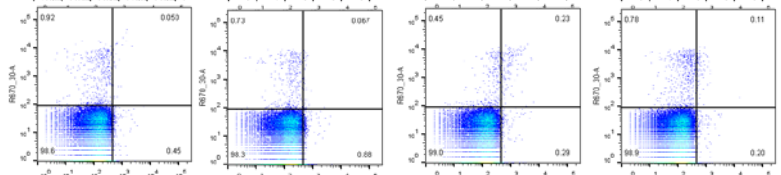
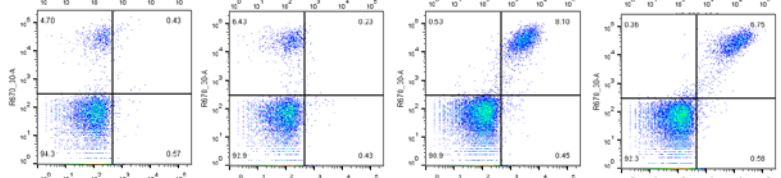
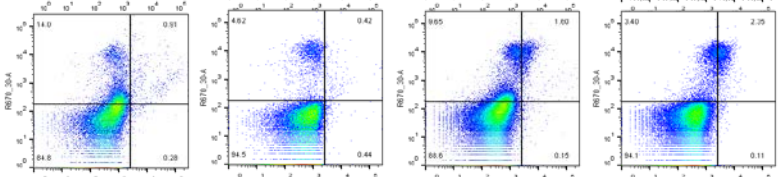
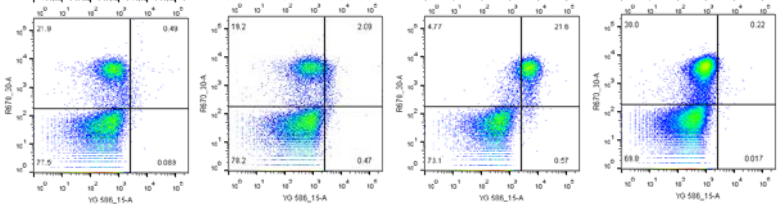
Patient	mIgG2a	rIgG2a	BCMA	TACI	Antigen density
1					BCMA: 7728 TACI: 1213
2					BCMA: 1224 TACI: 563
3					BCMA: 2831 TACI: 830
4					BCMA: 344 TACI: 251
5					BCMA: 1962 TACI: 1391
6					BCMA: 379 TACI: 36
7					BCMA: 5099 TACI: 21301
8					BCMA: 1886 TACI: 2331
9					BCMA: 5538 TACI: 107

Table S1 Cont'd

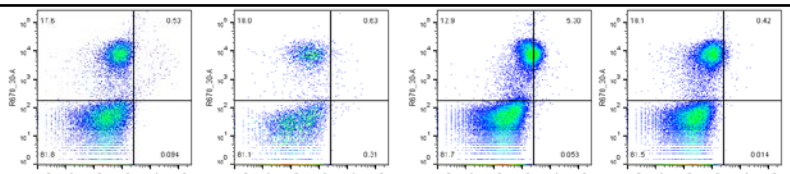
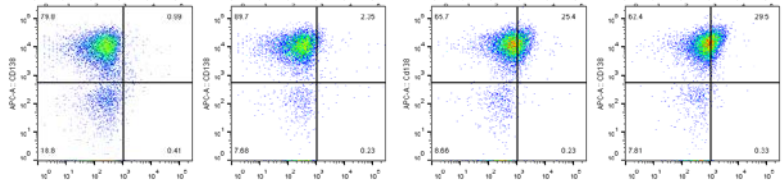
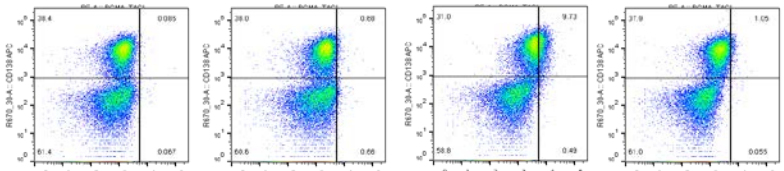
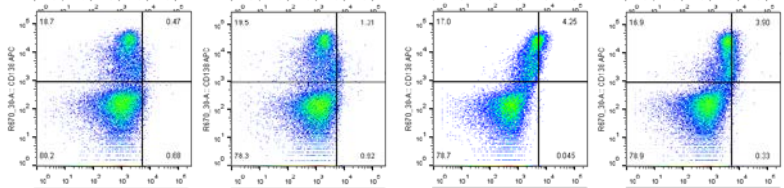
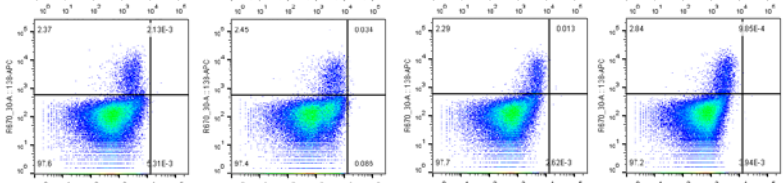
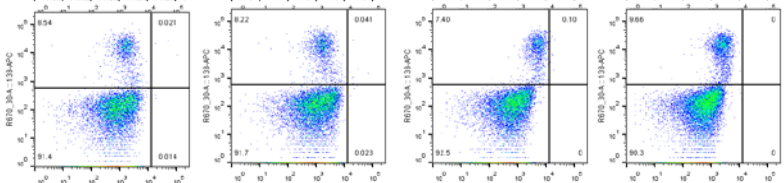
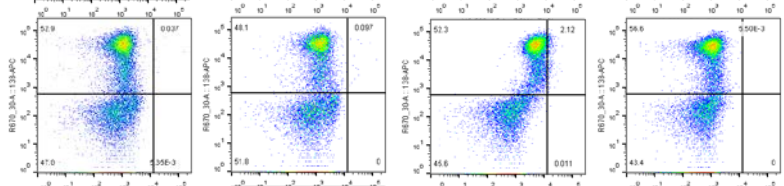
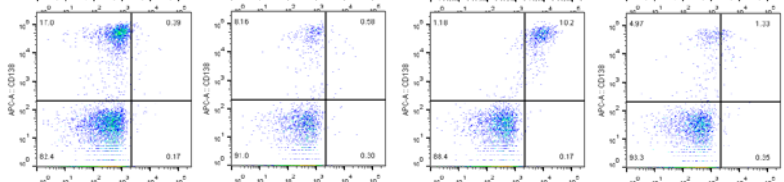
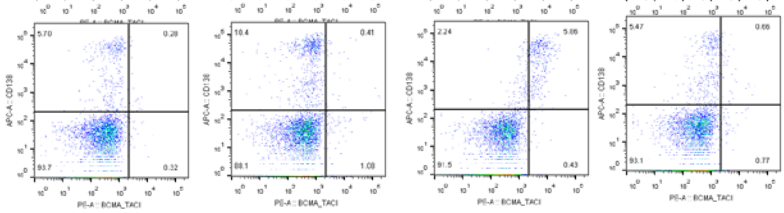
Patient	mIgG2a	rIgG2a	BCMA	TACI	Antigen density
10					BCMA: 1652 TACI: 512
11					BCMA: 413 TACI: 432
12					BCMA: 1051 TACI: 64
13					BCMA: 1226 TACI: 997
14					BCMA: 691 TACI: 0
15					BCMA: 1426 TACI: 333
16					BCMA: 2006 TACI:0
17					BCMA: 3051 TACI: 192
18					BCMA: 1903 TACI: 264

Table S1 Cont'd

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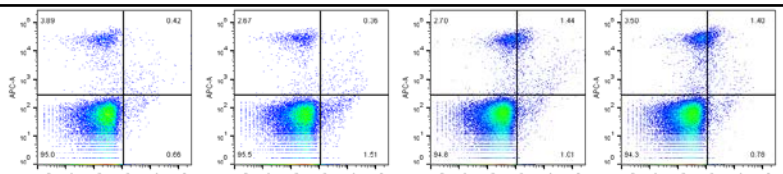
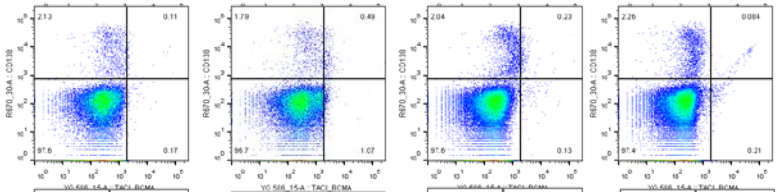
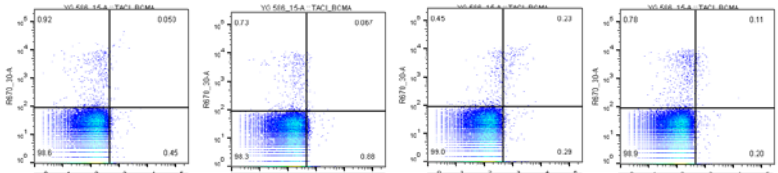
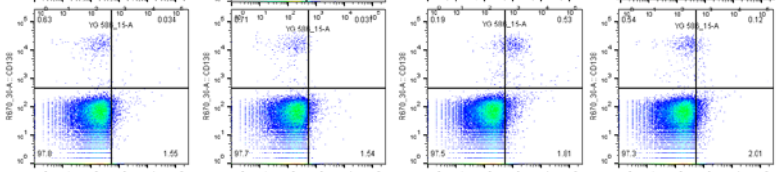
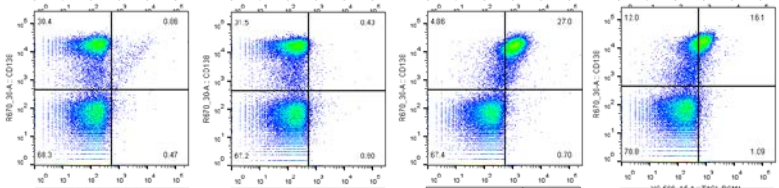
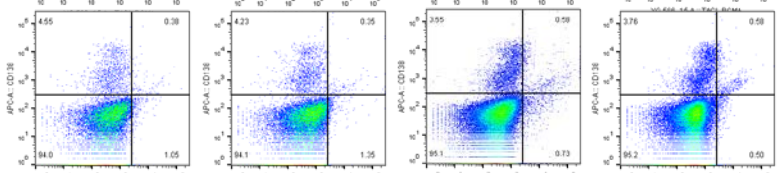
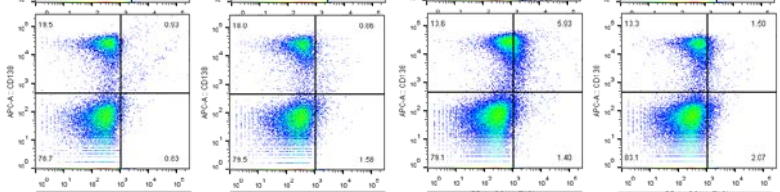
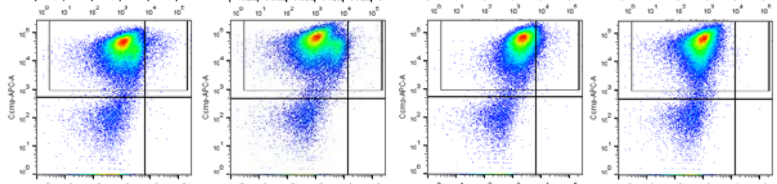
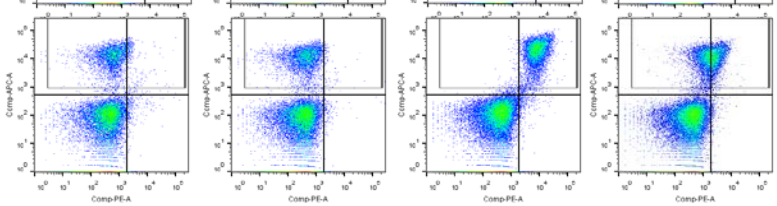
19					BCMA: 499 TACI: 484
20					BCMA: 452 TACI:0
21					BCMA: 279 TACI:0
22					BCMA: 1235 TACI: 205
23					BCMA: 1592 TACI: 769
24					BCMA: 531 TACI: 655
25					BCMA: 365 TACI: 135
26					BCMA: 520 TACI: 0
27					BCMA: 4221 TACI: 742

Table S1 Cont'd

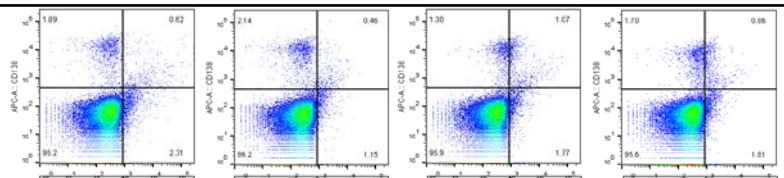
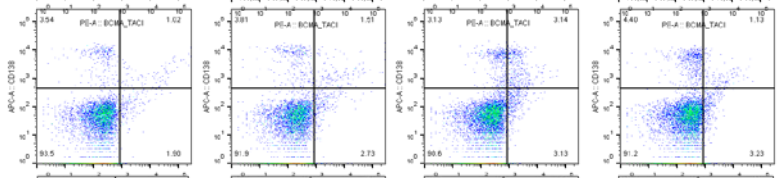
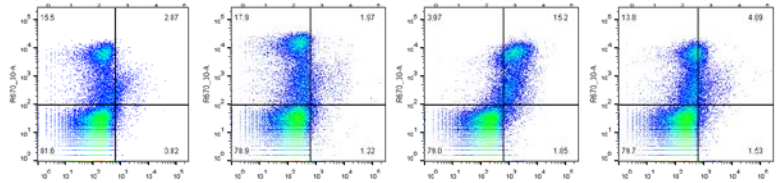
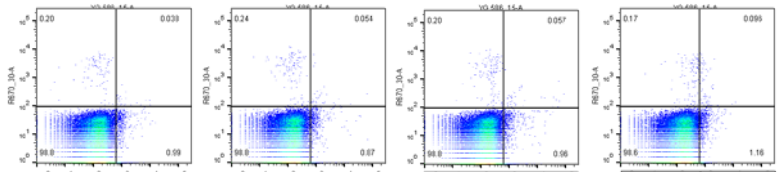
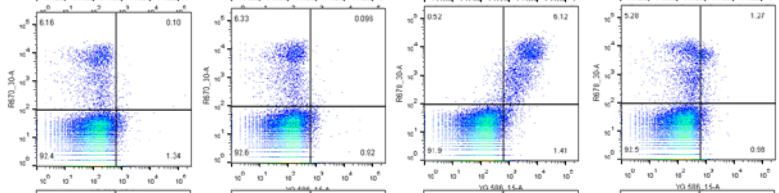
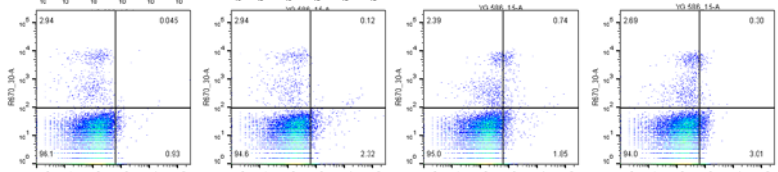
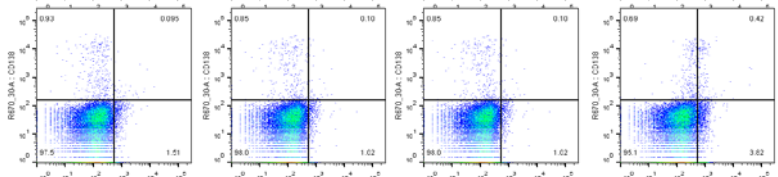
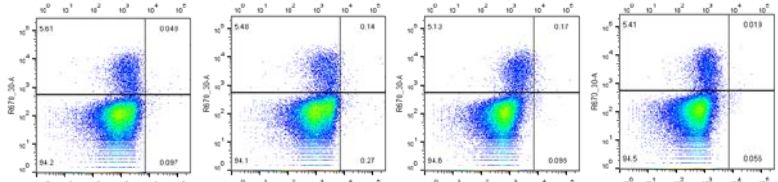
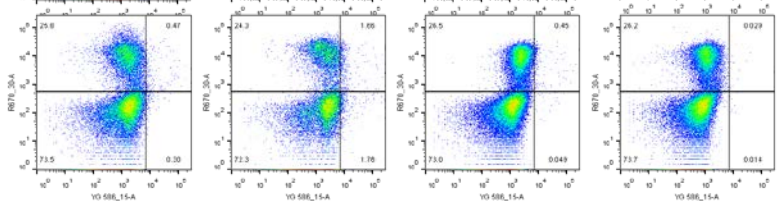
Patient	mIgG2a	rIgG2a	BCMA	TACI	Antigen density
28					BCMA: 361.8 TACI: 262
29					BCMA: 413 TACI: 170
30					BCMA: 2390 TACI: 386
31					BCMA: 118 TACI: 467
32					BCMA: 8323 TACI: 345
33					BCMA: 783 TACI: 364
34					BCMA: 203 TACI: 571
35					BCMA: 403 TACI: 0
36					BCMA: 745 TACI:0

Table S1 Cont'd

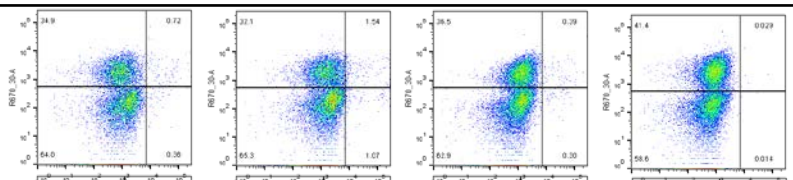
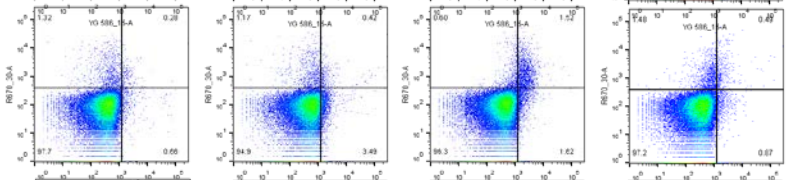
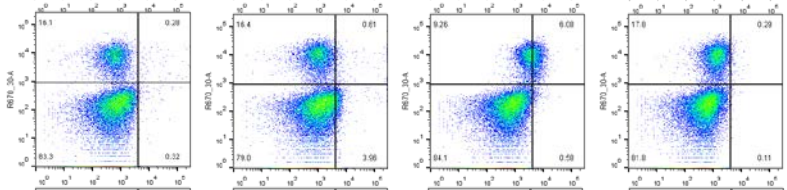
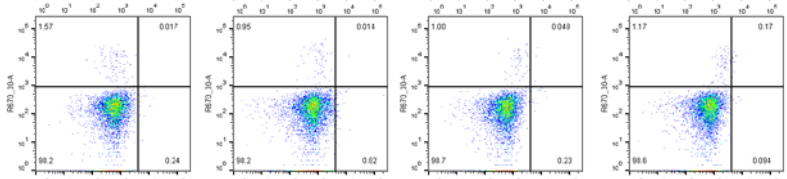
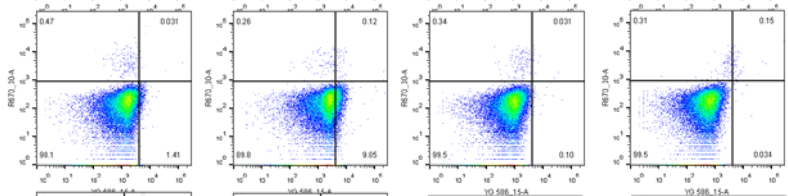
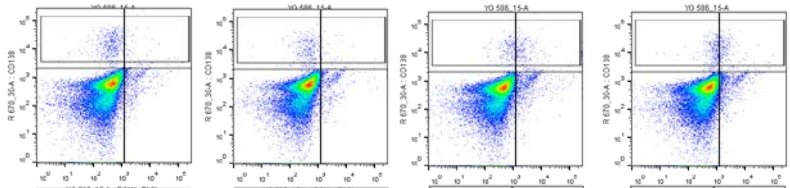
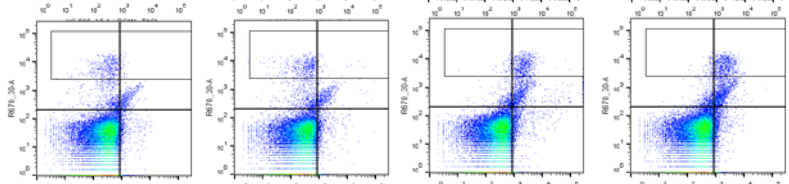
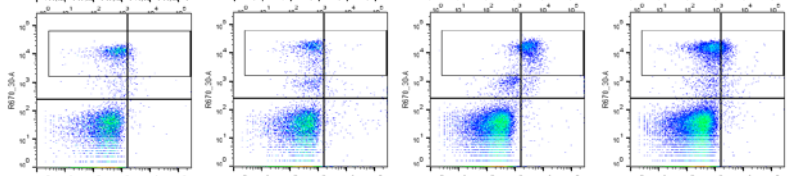
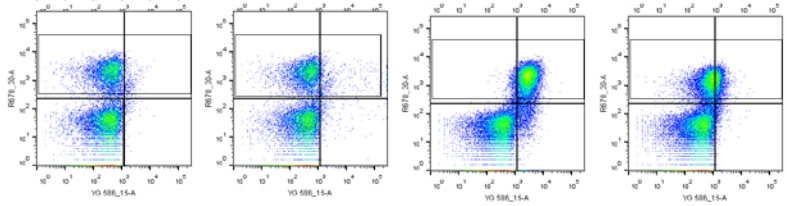
Patient	mIgG2a	rIgG2a	BCMA	TACI	Antigen density
37					BCMA: 420 TACI:0
38					BCMA: 1071 TACI: 101
39					BCMA: 2651 TACI: 234
40					BCMA: 888 TACI: 1081
41					BCMA: 105 TACI: 647
42					BCMA: 345 TACI: 265
43					BCMA: 1953 TACI: 1747
44					BCMA: 2088 TACI:159
45					BCMA: 2208 TACI: 396

Table S1 Cont'd

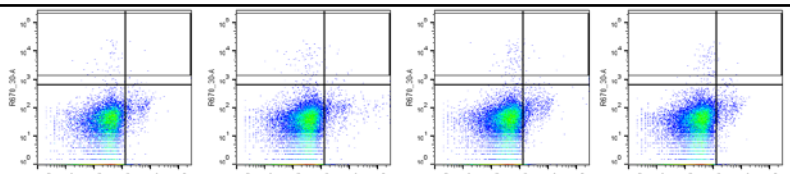
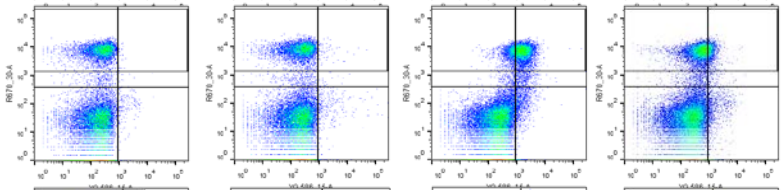
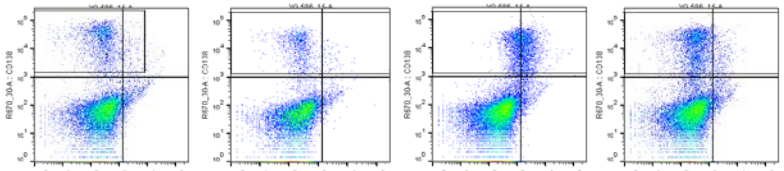
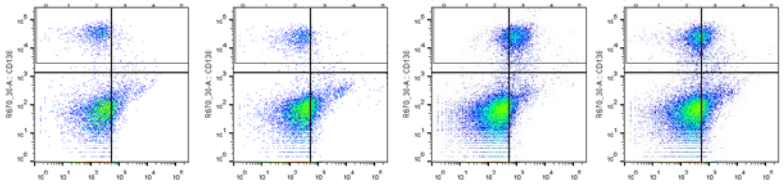
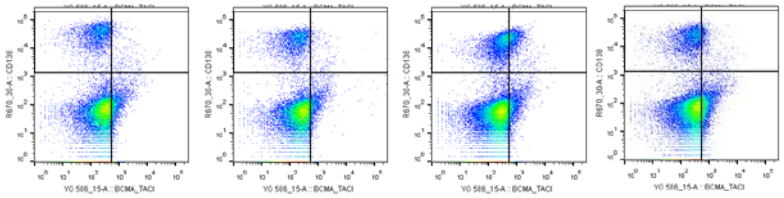
Patient	mIgG2a	rIgG2a	BCMA	TACI	Antigen density
46					BCMA: 305 TACI: 566
47					BCMA: 1220 TACI: 334
48					BCMA: 1883 TACI: 152
49					BCMA: 867 TACI: 259
50					BCMA: 202 TACI: 154

Table S2

	TACI	**BCMA**
Protein Accession Number	O14836	Q02223
Nucleotide Accession Number	NM_012452	NM_001192
Forward primer	GCAAGGAGCAAGGCAAGTTCT	AAGAGCAAACCGAAGGTCGA
Reverse primer	TGAGCTCTGGTGGAAGGTTCAC	GGTTGCGCCTTCCTCCAT
Probe	CTGCATCAGCTGTGCCTCCATCTGTG	TCTGACCATTGCTTTCCACTCCCAGC
SwissProt Name	TNFRSF13B. Synonyms: TACI	TNFRSF17. Synonyms: BCM, BCMA.
SwissProt Description	Tumor necrosis factor receptor superfamily member 13B	Tumor necrosis factor receptor superfamily member 17

Figure S1

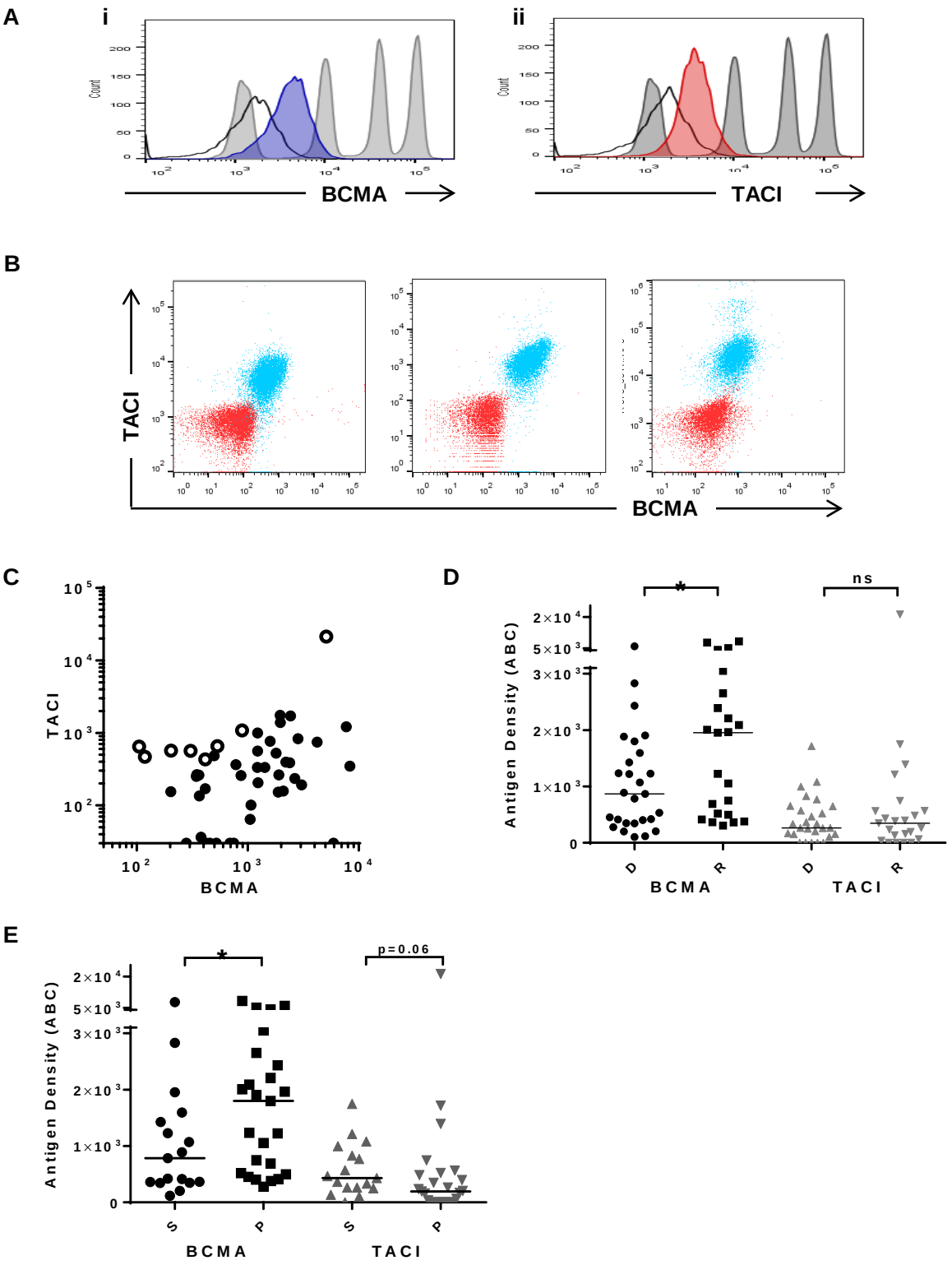

A
i
ii
Count
BCMA
TACI
B
TACI
BCMA
C
TACI
BCMA
D
Antigen Density (ABC)
BCMA
TACI
ns
E
Antigen Density (ABC)
p=0.06
BCMA
TACI

Figure S2

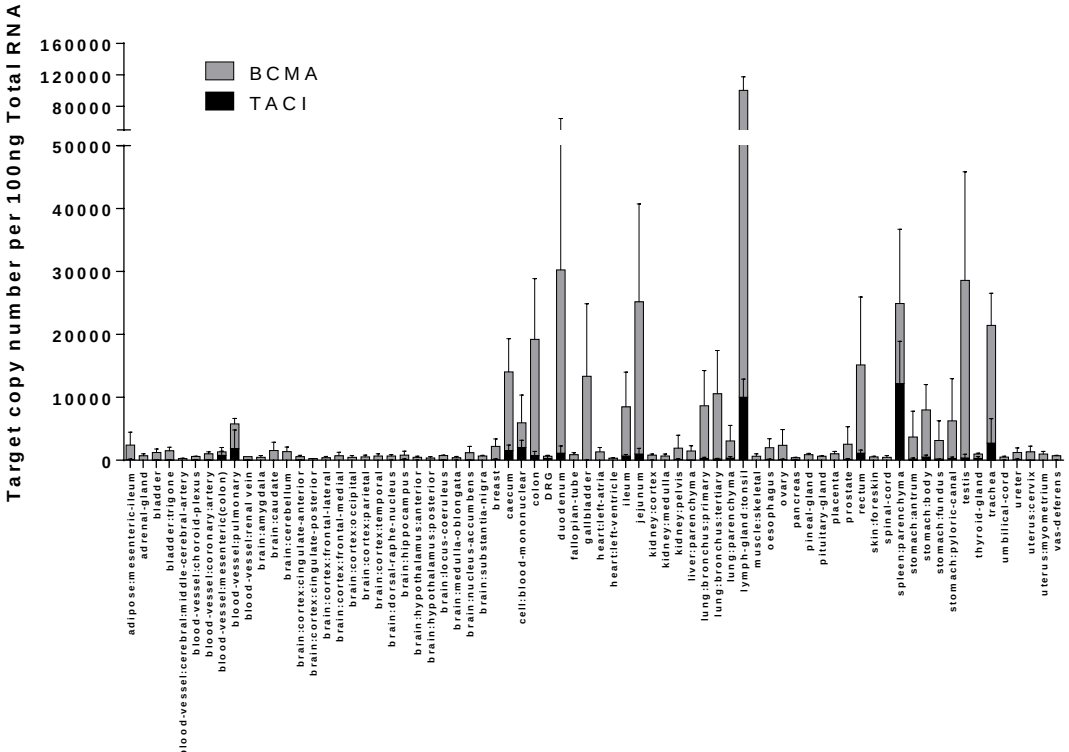
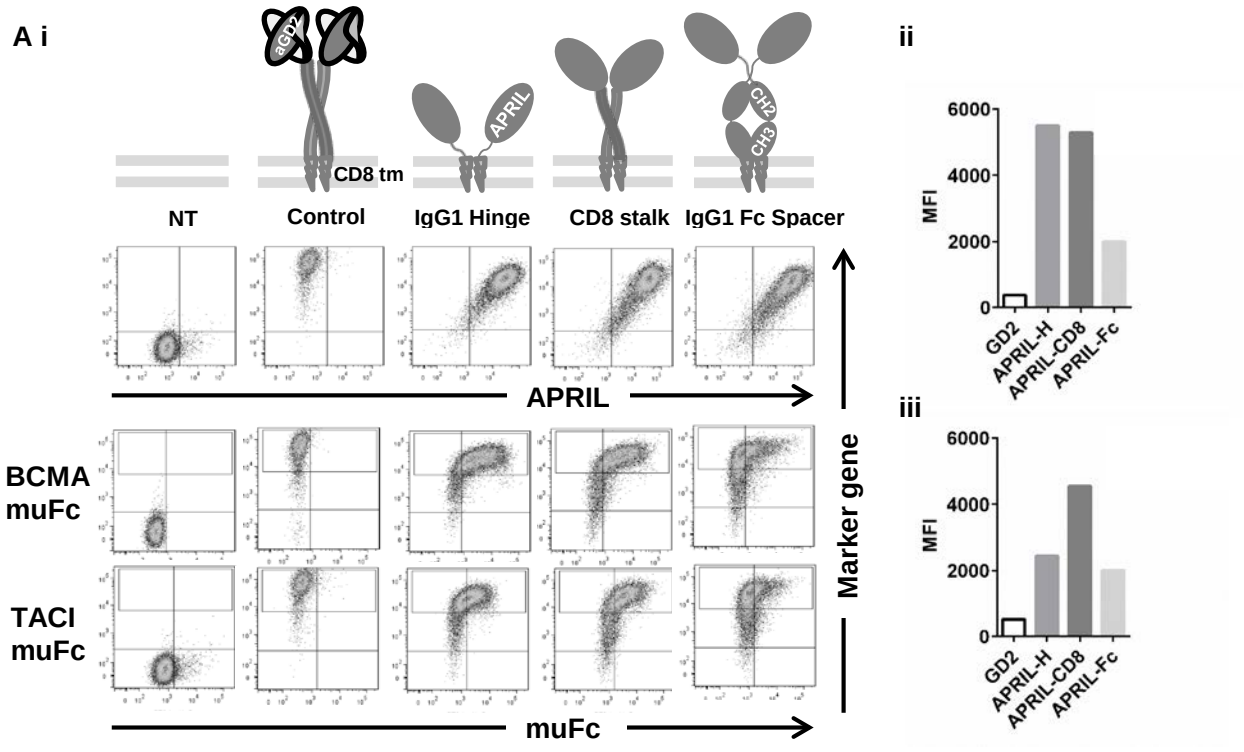
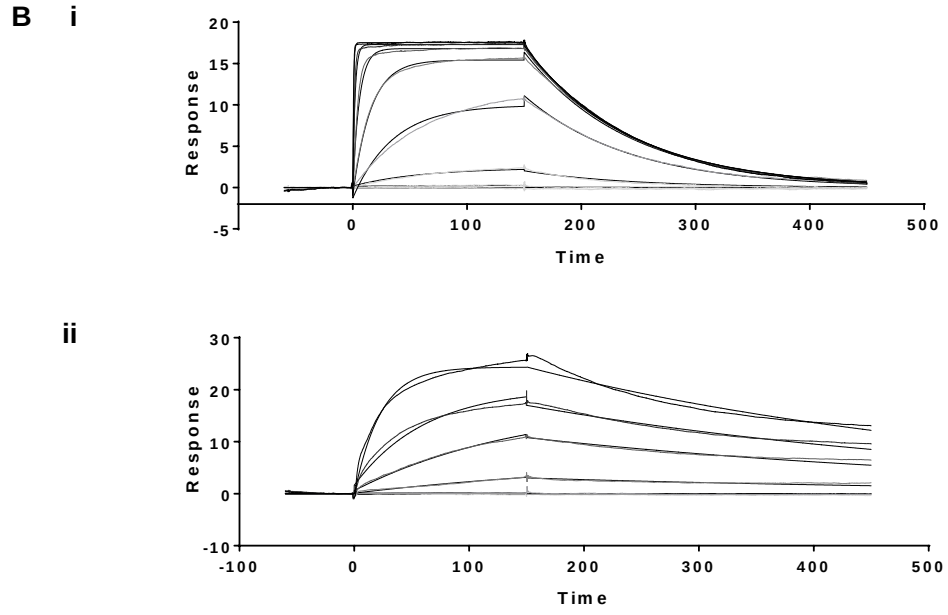

Target copy number per 100ng Total RNA
160000
120000
80000
50000
40000
30000
20000
10000
0
BCMA
TACI
adipose:mesenteric-ileum
adrenal-gland
bladder
bladder:trigone
blood-vessel:cerebral:middle-cerebral-artery
blood-vessel:choroid-plexus
blood-vessel:coronary:artery
blood-vessel:mesenteric(colon)
blood-vessel:pulmonary
blood-vessel:renal vein
brain:amygdala
brain:caudate
brain:cerebellum
brain:cortex:cingulate-anterior
brain:cortex:cingulate-posterior
brain:cortex:frontal-lateral
brain:cortex:frontal-medial
brain:cortex:occipital
brain:cortex:parietal
brain:cortex:temporal
brain:dorsal-raphe-nucleus
brain:hippocampus
brain:hypothalamus:anterior
brain:hypothalamus:posterior
brain:locus-coeruleus
brain:medulla-oblongata
brain:nucleus:accumbens
brain:substantia-nigra
breast
caecum
cell:blood-mononuclear
colon
DRG
duodenum
fallopian-tube
gallbladder
heart:left-atria
heart:left-ventricle
ileum
jejunum
kidney:cortex
kidney:medulla
kidney:pelvis
liver:parenchyma
lung:bronchus:primary
lung:bronchus:tertiary
lung:parenchyma
lymph-gland:tonsil
muscle:skeletal
oesophagus
ovary
pancreas
pineal-gland
pituitary-gland
placenta
prostate
rectum
skin:foreskin
spinal-cord
spleen:parenchyma
stomach:antrum
stomach:body
stomach:fundus
stomach:pyloric-canal
testis
thyroid-gland
tonsil
umbilical-cord
ureter
uterus:cervix
uterus:myometrium
vas-deferens

Figure S3





Ligand	K_D (nM)	K_{on} (Ms^{-1})	K_{off} (s^{-1})
BCMA	2.99	4.14E+06	0.0123
TACI	5.63	3.87E+05	0.0020

Figure S4

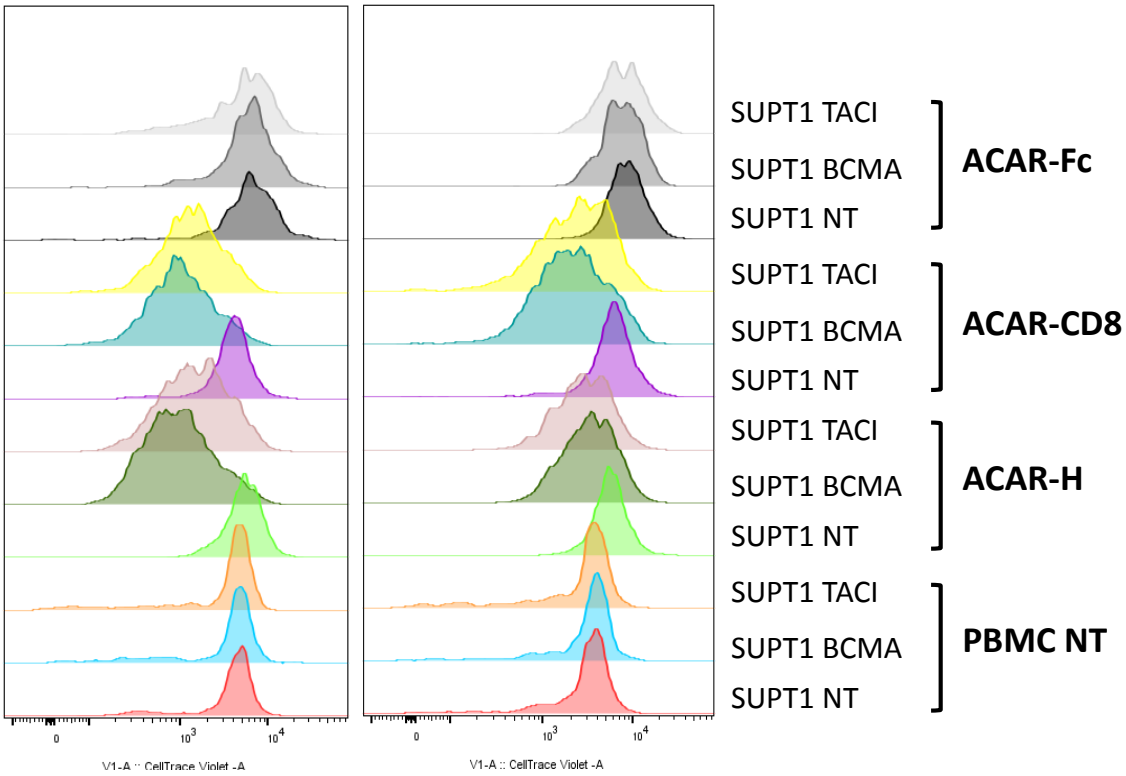

SUPT1 TACI
SUPT1 BCMA
SUPT1 NT
ACAR-Fc
SUPT1 TACI
SUPT1 BCMA
SUPT1 NT
ACAR-CD8
SUPT1 TACI
SUPT1 BCMA
SUPT1 NT
ACAR-H
SUPT1 TACI
SUPT1 BCMA
SUPT1 NT
PBMC NT
0
10^3
10^4
V1-A :: CellTrace Violet -A

Figure S5

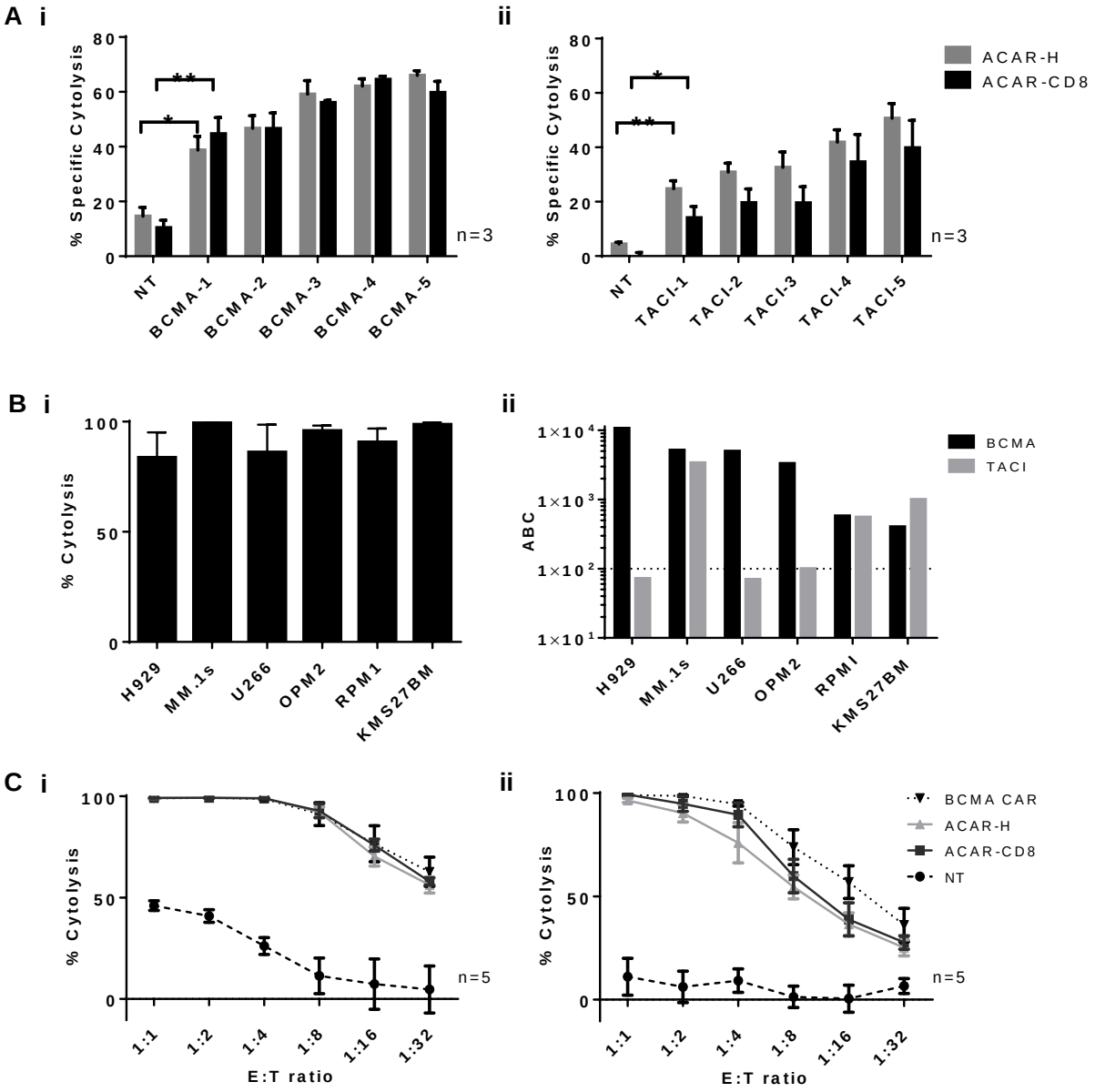

A i
% Specific Cytolysis
80
60
40
20
0
*
**
n=3
NT
BCMA-1
BCMA-2
BCMA-3
BCMA-4
BCMA-5
ii
% Specific Cytolysis
*
**
ACAR-H
ACAR-CD8
n=3
NT
TACI-1
TACI-2
TACI-3
TACI-4
TACI-5
B i
% Cytolysis
100
50
0
H929
MM.1s
U266
OPM2
RPM1
KMS27BM
ii
ABC
1×10⁴
1×10³
1×10²
1×10¹
BCMA
TACI
H929
MM.1s
U266
OPM2
RPMI
KMS27BM
C i
% Cytolysis
100
50
0
n=5
1:1
1:2
1:4
1:8
1:16
1:32
E:T ratio
ii
% Cytolysis
100
50
0
BCMA CAR
ACAR-H
ACAR-CD8
NT
n=5
1:1
1:2
1:4
1:8
1:16
1:32
E:T ratio

Figure S6

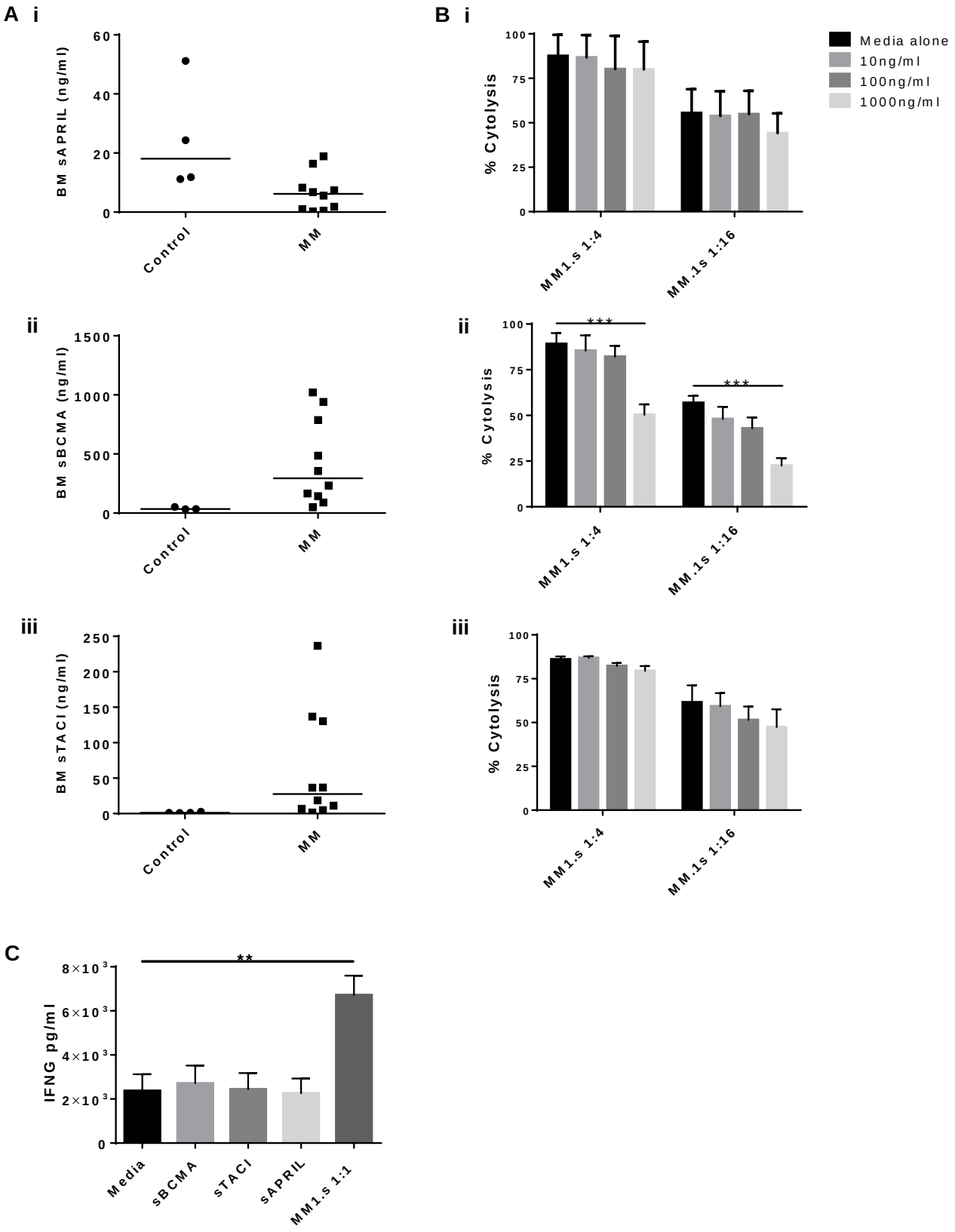
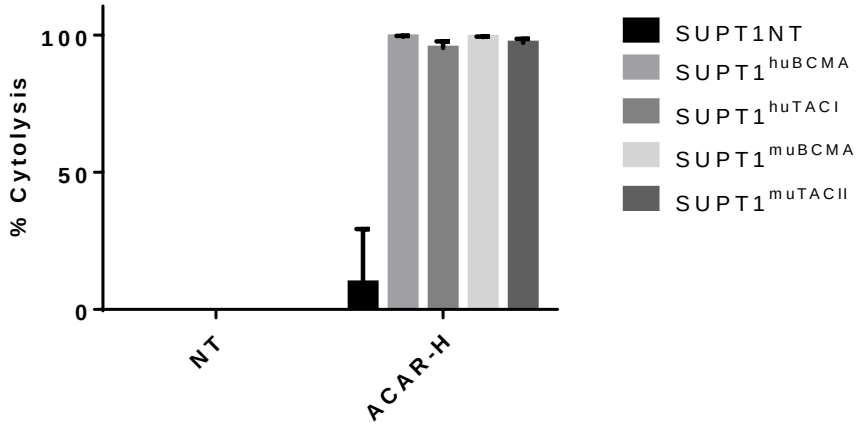

A i
BM sAPRIL (ng/ml)
60
40
20
0
Control
MM
B i
% Cytolysis
100
75
50
25
0
MM1.s 1:4
MM.1s 1:16
Media alone
10ng/ml
100ng/ml
1000ng/ml
ii
BM sBCMA (ng/ml)
1500
1000
500
0
Control
MM
ii
% Cytolysis
***
***
100
75
50
25
0
MM1.s 1:4
MM.1s 1:16
iii
BM sTACI (ng/ml)
250
200
150
100
50
0
Control
MM
iii
% Cytolysis
100
75
50
25
0
MM1.s 1:4
MM.1s 1:16
C
IFNG pg/ml
**
8×10³
6×10³
4×10³
2×10³
0
Media
sBCMA
sTACI
sAPRIL
MM1.s 1:1

Figure S7

A


100
50
0
% Cytolysis
NT
ACAR-H
SUPT1NT
SUPT1huBCMA
SUPT1huTACI
SUPT1muBCMA
SUPT1muTACII

B

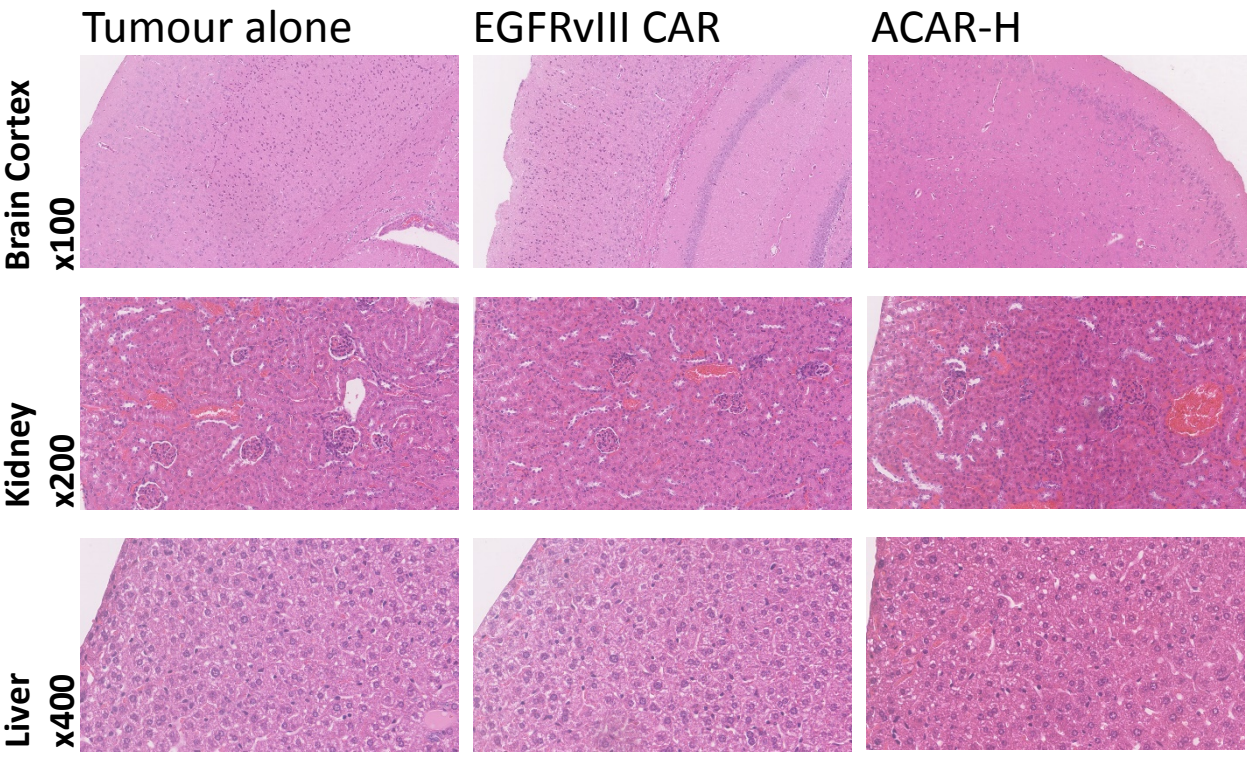

Tumour alone
EGFRvIII CAR
ACAR-H
Brain Cortex x100
Kidney x200
Liver x400

Figure S7B cont'd

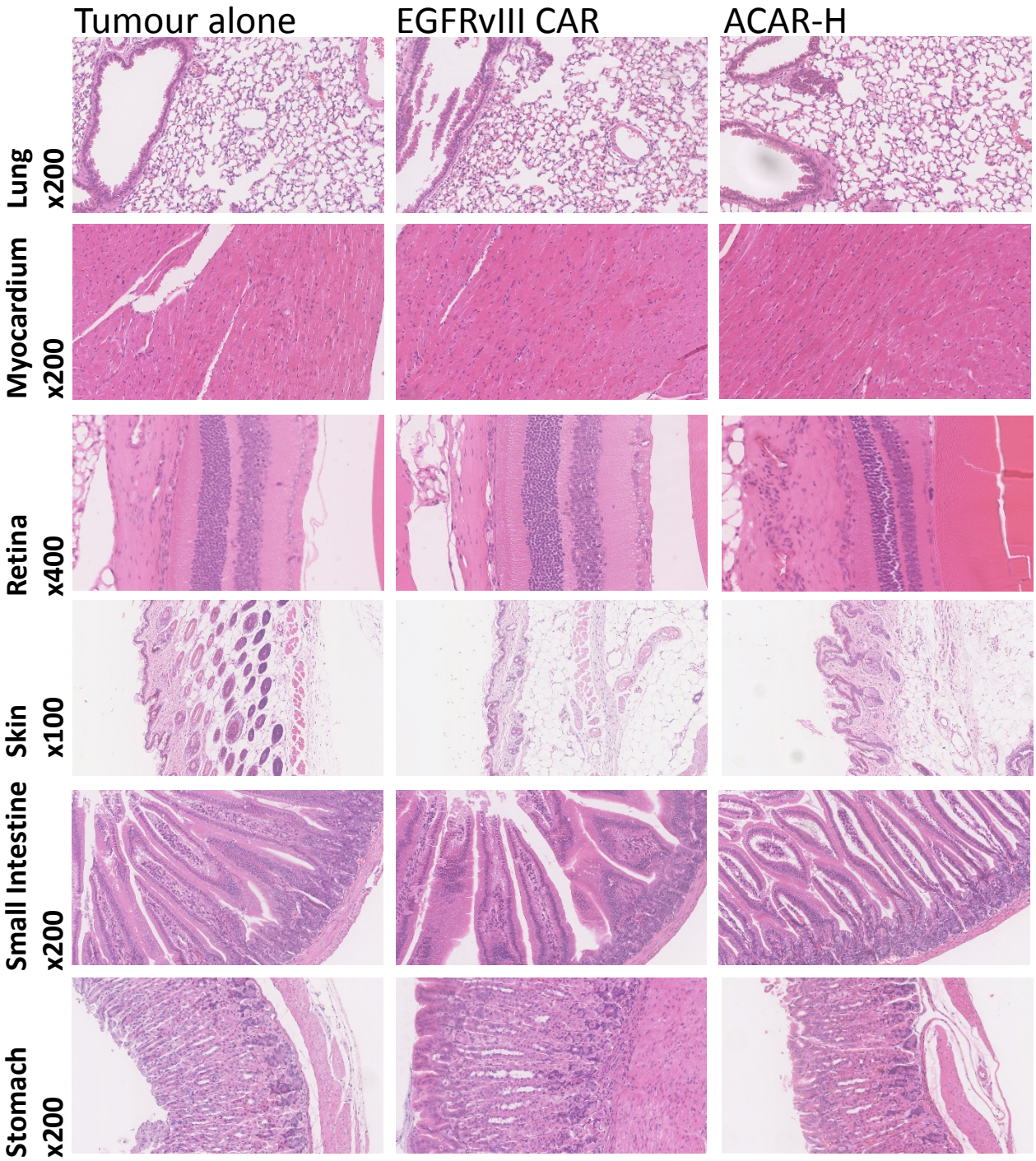


Figure S8