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Supplementary Materials for

GPRC5D is a target for the immunotherapy of multiple myeloma with rationally designed CAR T cells

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The PDF file includes:

Table S4. Multiplex immunostaining conditions.

Other Supplementary Material for this manuscript includes the following:

(available at www.sciencetranslationalmedicine.org/cgi/content/full/11/485/eaau7746/DC1)

Data file S1 (Microsoft Excel format). Original data.

Supplementary Materials

Materials and Methods

Cell lines. Human myeloma cell lines OPM2, NCI-H929, and L363 were obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), and MM.1S and RPMI-8226 from American Type Culture Collection (ATCC). Acute myeloid leukemia cell line SET2 was obtained from DSMZ. The Jurkat T cell leukemia line and cell lines used to generate artificial antigen-presenting cells (NIH-3T3 and K562) were obtained from ATCC. All cell lines were routinely tested for mycoplasma contamination and found negative.

Gene expression analysis. Datasets were accessed using OmicSoft Array Studio software, version 10 (Qiagen) and OmicSoft's accompanying OncoLand datasets. Cancer Cell Line Encyclopedia (CCLE, Broad Institute) are available at https://portals.broadinstitute.org/ccle, primary MM cell data are from the Blueprint Epigenome Project (http://dcc.blueprint-epigenome.eu/#/home, EGAD00001001471), and primary normal cell data are from GTEx (gtexportal.org/). CD138-sorted RNAseq expression data from 765 patients who participated in the Multiple Myeloma Research Foundation CoMMpass trial (NCT0145429) were accessed and analyzed through the web portal accessible at research.mmrf.org. Patients were stratified according to whether GPRC5D expression was above or below the median, and progression-free survival (PFS) was compared between the two groups. Kaplan-Meier curves for PFS with censoring are shown; significance was determined by log-rank test of equal hazards. For patients for whom such data were available, ISS score and common genetic abnormalities were compared between these two groups.

Single chromogenic IHC. Chromogenic IHC was performed on a Ventana Ultra instrument (Ventana Medical Systems). Formalin-fixed, paraffin-embedded (FFPE) sections were baked for 30 minutes at 65°C and then loaded onto the Ventana DISCOVERY ULTRA automated slide

stainer (table S2). Sections were antigen-retrieved and blocked with Inhibitor CM/Discovery inhibitor and/or goat Ig reagents. Then, sections were incubated with primary antibody followed by OmniMap anti-species horseradish peroxidase (HRP) multimer, and staining was visualized by brown 3,3' diaminobenzidine (DAB). For the HQ amplification system, a linking antibody and enzyme conjugate were used. Next, sections were counterstained with hematoxylin II and bluing reagents. Finally, sections were dehydrated in graded ethanol, cleared in xylene, and coverslipped. For negative controls, the staining procedures were the same except for primary antibodies, where mouse isotype controls were used. Antibodies are listed in table S3.

Multiplex fluorescence staining. Formalin-fixed, paraffin-embedded (FFPE) sections were baked for 30 minutes at 65°C and then deparaffinized and rehydrated through xylene, (2x 5 min), 100% ethanol (2x 3 min), 95% ethanol (2x 3 min), 80% ethanol (3 min), and deionized water (2x 5 min). Autofluorescence from red blood cells was quenched in 10% H_2O_2 solution for 10 min at room temperature (RT). The remaining staining procedures were performed using Discovery Ultra autostainer unless otherwise stated. Antigen was retrieved using Discovery CC1 reagent, then blocked with Discovery Inhibitor CM and serum-blocked with goat Ig. Sections were incubated with CD138 mouse mAb, followed by OmniMap anti-mouse HRP multimer, and then Discovery Rhodamine 6G. The primary-secondary antibody complex was heat-deactivated using Ultra CC2 reagent, unbound HRP enzyme was blocked with Discovery Inhibitor, and goat Ig block was applied. The same sequence of mouse mAb, HRP multimer, and secondary antibody was repeated for BCMA (10 µg/mL), with Discovery DCC as secondary, and then for GPRC5D (5 µg/mL), with Discovery CY5. Nuclei were counterstained offline with Hoechst 33342 solution, and sections were coverslipped with ProLong Diamond mounting medium. For negative controls, the staining procedures were the same except for primary antibodies, which consisted of mouse isotype controls. Staining conditions are described in table S4.

Immunofluorescence image analysis. Slides were scanned on the Pannoramic P250 (3DHistotech) with exposure optimized for the entire cohort. Images were analyzed on Halo (Indica Labs). Up to 10 fields of view were analyzed. We trained a random forest classifier to classify viable regions of interests, removing red blood cells, mast cells, and artifacts. A cutoff was selected for each marker under the guidance of a pathologist, and positive and negative cells were counted. Data analysis and visualization were performed using JMP (SAS). Positive cells were selected among $CD138^+$ tumor cells, and percentage positive was normalized to $CD138^+$ tumor cells. Prevalence of BCMA and GPRC5D was measured among $CD138^+$ tumor cells, with antigen positivity defined as expression in 1% or more of $CD138^+$ cells analyzed. Correlation of BCMA and GPRC5D expression was assessed by coefficient of determination (R^2) analysis.

RNA-ISH. RNAscope 2.5 LSx reagent kit – Brown (Advanced Cell Diagnostics) was used according to supplier instructions. Three adjacent sections were stained using probes for Hs-GPRC5D, Hs-PPIB (positive control), and DapB (negative control) (catalog numbers for all reagents are provided in table S2). In short, FFPE sections were loaded into a Bond Rx autostainer (Leica Biosystems) and subjected to antigen retrieval using ER2 solution followed by protease digestion and endogenous peroxidase blocking, hybridization with target probes, and sequential amplification with AMP1-6. Signals were detected with DAB and counterstained with hematoxylin and bluing solutions. Finally, sections were dehydrated using graded ethanol, cleared in xylene, and coverslipped. Staining was scored semi-quantitatively based on the vendor's recommendation; each punctate dot signal represents a single target RNA molecule.

qPCR. Total RNA was isolated from 2 to 4 curls (20 μm thickness) of FFPE cell pellets or tissue samples using the RNeasy FFPE kit (Qiagen), and cDNA was generated using the qScript cDNA Synthesis Kit (Quanto). GPRC5D (forward primer: 5'-ACTGCATCGAGTCCACTGGAGA-3', reverse: 5'-GGATCTTTCGCATGAGGAAGAG-3', 0.25 μM each) and the housekeeping gene β-actin (forward: 5'-AGCATCCCCCAAAGTTCAC-3', reverse: 5'-

AAGGGACTTCCTGTAACAACG-3', 0.25 μM each; all primers from Integrated DNA Technologies) were quantified. PCR reactions were carried out in duplicate using Power SYBR Green PCR Master Mix (Thermo Fisher Scientific). Samples were run in Hard-Shell 384-well PCR plates (Bio-Rad) in a CFX384 Real-Time System (Bio-Rad). Data were analyzed using CFX Manager software version 3.1 (Bio-Rad).

Flow cytometry. Expression of CARs was determined either via GFP expression (for bicistronic construct-transduced cells) or using anti-IgG4 spacer mAb (EP4420; Abcam). Cells were counted using 123count eBeads (Thermo Fisher). Mononuclear cells from primary bone marrow aspirates were stained for CD3 (7D6; Thermo Fisher), CD138 (MI15; Biolegend), and CD38 (HIT2, Biolegend). 7-aminoactinomycin D or DAPI (both from Thermo Fisher) staining was used to exclude non-viable cells. Proliferation was detected by pre-labeling T cells with CellTrace Violet (CTV) and monitoring for dilution of the dye; simultaneous CD25 staining with clone 2A3 (BD Horizon) was used as a surrogate for T cell activation. Flow cytometry was performed on a Gallios B43618 (Beckman Coulter) or BD FACSymphony (BD Biosciences), and data were analyzed using FlowJo (V10, FlowJo LLC).

In vitro cytotoxicity. Target human myeloma cell lines were stably transduced with ATP-dependent ffLuc and co-cultured at various ratios with CAR T cells (normalized for viability and CAR transduction efficiency) in U-bottom 96-well plates. After the indicated incubation time, D-luciferin (Millipore-Sigma) was added to each well, and bioluminescent signal was read using a TECAN plate reader. Statistical significance was evaluated by two-way ANOVA.

Primary MM sample co-culture. Samples were collected from patients consented to institutional biospecimen collection protocol MSK IRB# 06-107 or 09-141 and were accessed and investigated under MSK IRB# 17-268. Mononuclear cells were enriched from primary samples via Ficoll density gradient centrifugation and co-cultured 1:1 bone marrow mononuclear

cells: CAR T cells, as above. The $CD138^+$ fraction of $CD3^-$ cells was assessed by flow cytometry, as above.

Cytokine profiling. CAR T cells were co-cultured overnight with OPM2 human myeloma cells at a 1:1 ratio. Plates were centrifuged at 1500 rpm for 5 minutes, and supernatant was collected and stored at -80 °C until analysis. Cytokines were detected using the Milliplex MAP Human Cytokine/Chemokine, Premixed kit (Millipore-Sigma) and the Luminex 200 xMAP system (Luminex).

CRISPR-mediated BCMA knockout. To generate the BCMA knockout, GFP/luciferase Cas9-expressing OPM2 cells (OPM2-GL-Cas9) were transduced with a BCMA single guide RNA (sgRNA) lentivirus. The BCMA sgRNA (CAATGGTCAGAGTCGACCTT) was cloned into the pLentiV2 plasmid (Addgene plasmid 49535) according to standard protocol at Addgene, and later co-transfected with packaging plasmids (Pax2 and Vsvg; Addgene 12259 and 8454, respectively) into 293T cells. Transduction was carried out in the presence of 10 μg/mL polybrene (EMD Millipore). Transduced cells were selected with 4 μg/mL puromycin (Thermo Fisher Scientific) for 10 days, and single cells were plated in a 96-well plate. Individual colonies were expanded and stained with BCMA antibody (Biolegend) to confirm BCMA knockout. Clones of confirmed BCMA knockout OPM2-GL-Cas9 cells were further expanded and used for experiments.

CRISPR-mediated GPRC5D knockout. CRISPR guide RNA was designed using Integrated DNA Technologies custom Alt-R CRISPR-Cas9 guide RNA program. Five guides were chosen proximal to and within GPRC5D exon 1 and ordered from IDT as crRNA.

GPRC5D_exon1_1 GGGUGUGGACCUCACCAGAA

GPRC5D_exon1_2 GAGAAGAAAAUAGUCUCCAG

GPRC5D_exon1_3 GUGACUCUCAUCAUGACCAG

GPRC5D_exon1_4 GACGACCGGGUCGUCCCACU

GPRC5D_exon1_5 GAGAACCAGGAGCUCUCCAG

crRNAs were complexed with Alt-R CRISPR-Cas9 tracrRNA at a 1:1 ratio to generate two-part gRNAs. All 5 gRNAs were pooled and incubated with Aldevron 2X-NLS Cas9 at a 2:1 ratio to generate ribonucleoprotein (RNP) complexes. OPM-2 cells were electroporated with Cas9 RNP targeting GPRC5D using the Neon Transfection System (Thermo Fisher Scientific). Briefly, 5e5 cells were washed once in 1X PBS and then resuspended in 100 µL R buffer with 10 µL of Cas9 RNP. Cells were loaded into a 100 µL Neon Tip and electroporated at 1050 V, 20 ms pulse width, 3 pulses and then transferred to 1 mL of medium. Cells were expanded and then subjected to cell cloning by serial dilution. Expanded clones were screened by PCR for deletions at the GPRC5D locus.

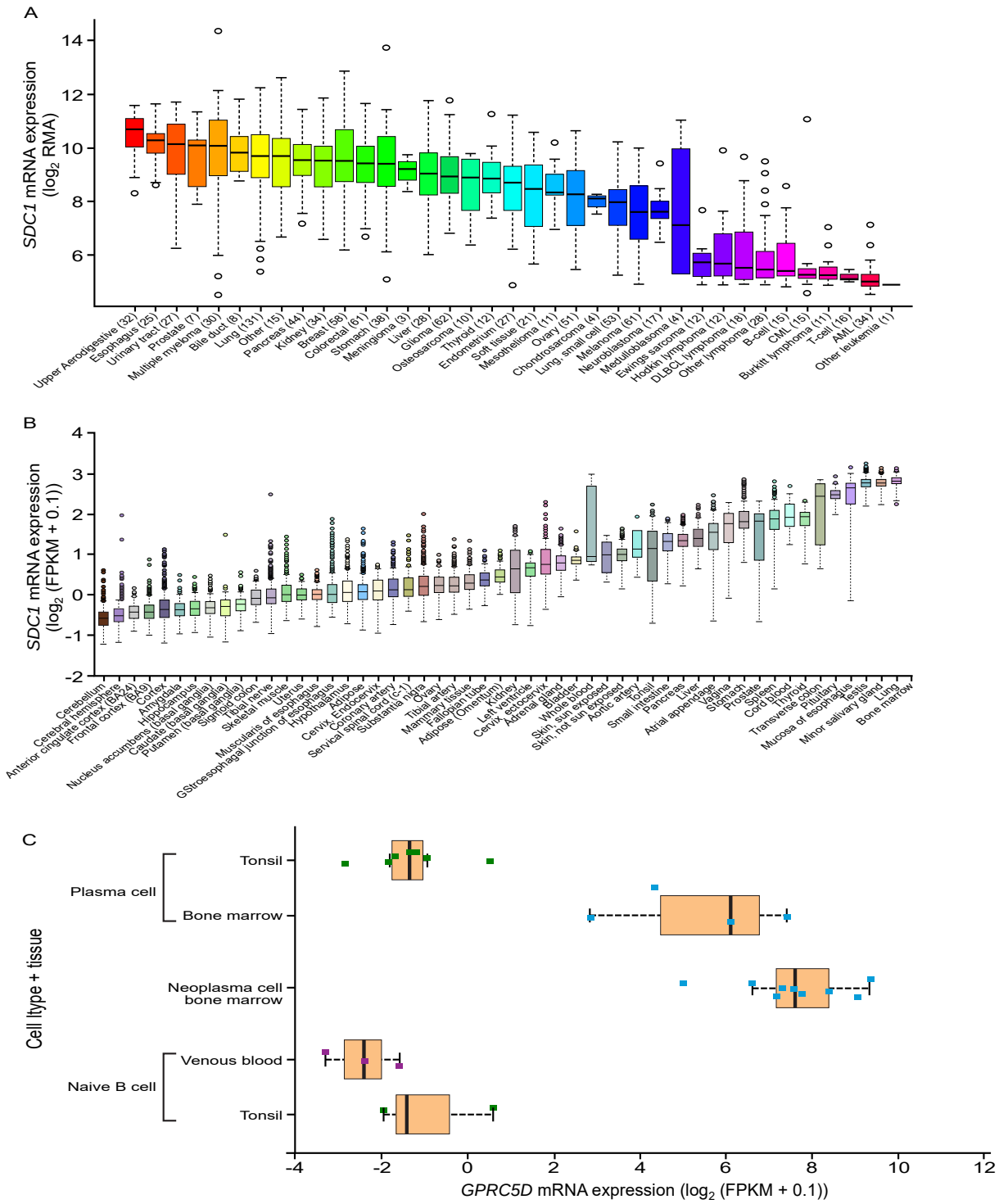


Fig. S1. *SDC1* (CD138) and *GPRC5D* mRNA expression. (A) Cancer Cell Line Encyclopedia *SDC1* mRNA expression data ($\log_2$ scale). **(B)** *SDC1* GTEx RNASeq expression data for various organs. **(C)** *GPRC5D* mRNA expression as reported by Blueprint RNAseq for primary human tissue cell types. Cancer Cell Line Encyclopedia (CCLE; Broad Institute) data are available at

https://portals.broadinstitute.org/ccle, data on primary MM cells from the Blueprint Epigenome Project are at http://dcc.blueprint-epigenome.eu/#/home, EGAD00001001471, and data on primary normal cells are from GTEx (gtexportal.org/). Datasets were accessed using OmicSoft Array Studio software, version 10 (Qiagen), and OmicSoft's accompanying OncoLand datasets.

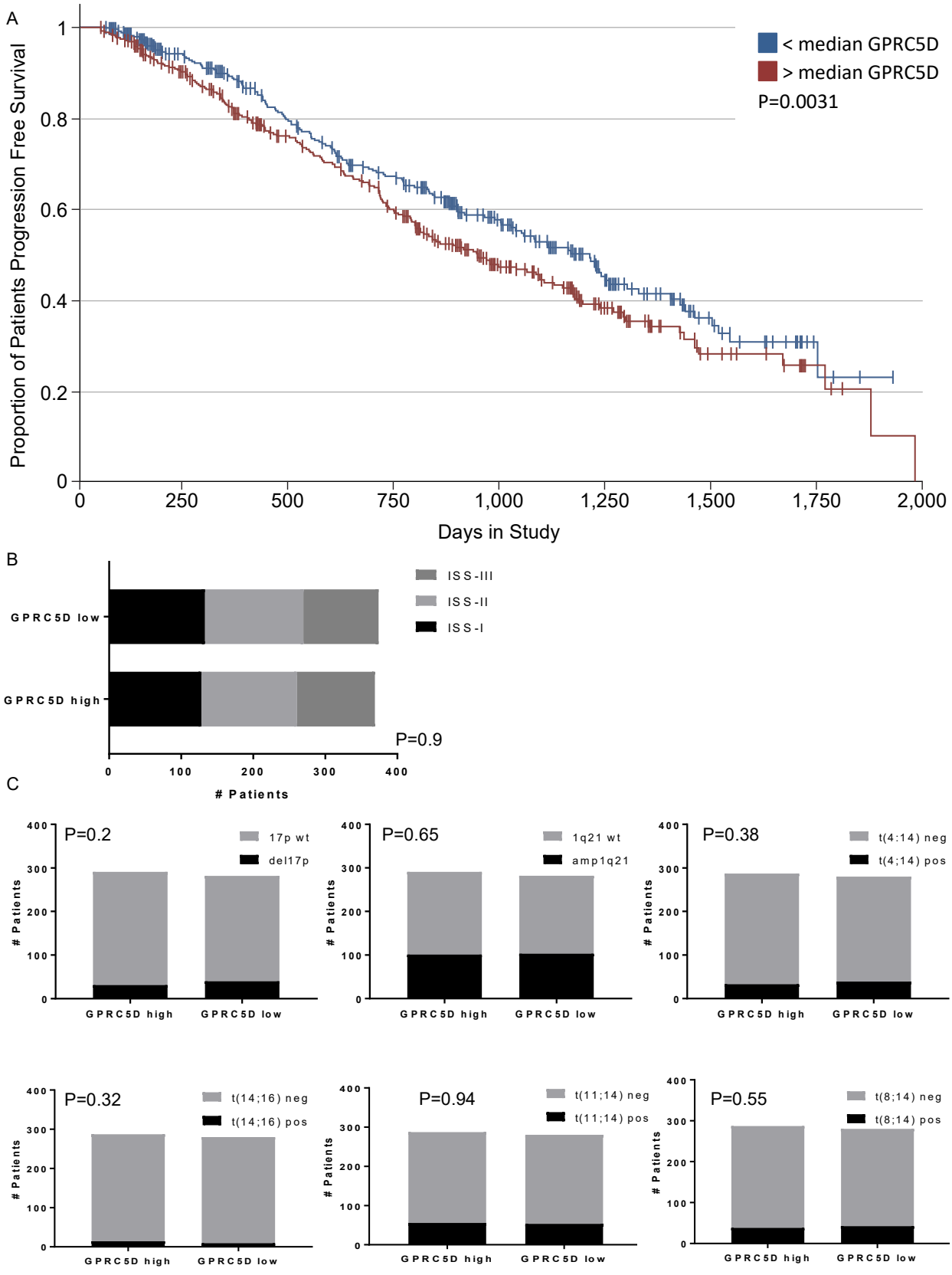

A
Proportion of Patients Progression Free Survival
1
0.8
0.6
0.4
0.2
0
0
250
500
750
1,000
1,250
1,500
1,750
2,000
Days in Study
< median GPRC5D
> median GPRC5D
P=0.0031
B
GPRC5D low
GPRC5D high
ISS-III
ISS-II
ISS-I
P=0.9
0
100
200
300
400
Patients
C
P=0.2
17p wt
del17p
P=0.65
1q21 wt
amp1q21
P=0.38
t(4;14) neg
t(4;14) pos
P=0.32
t(14;16) neg
t(14;16) pos
P=0.94
t(11;14) neg
t(11;14) pos
P=0.55
t(8;14) neg
t(8;14) pos
Patients
GPRC5D high
GPRC5D low

Fig. S2. Correlation of higher GPRC5D expression by MM cells with shorter progression-free survival. The Multiple Myeloma Research Foundation (MMRF) CoMMpass trial (NCT0145429) is a longitudinal study of newly diagnosed MM patients. Anonymized clinical outcomes and accompanying CD138-sorted RNAseq expression data from 765 CoMMpass patients are publicly available (research.mmrf.org; version IA13). **(A)** These 765 patients were stratified according to whether GPRC5D expression was above or below the median, and progression-free survival (PFS) was compared between the two groups. Kaplan-Meier curves for PFS with censoring are shown; significance was determined by log-rank test of equal hazards ($p=0.0031$). **(B)** International staging system (ISS) score was available for 369 of patients with above-median GPRC5D expression and 374 with below-median GPRC5D expression. **(C)** Frequency of gene amplification, deletion, and translocations in the 287-291 patients with above-median GPRC5D expression and the 280-282 patients with below-median GPRC5D expression for whom these data were available.

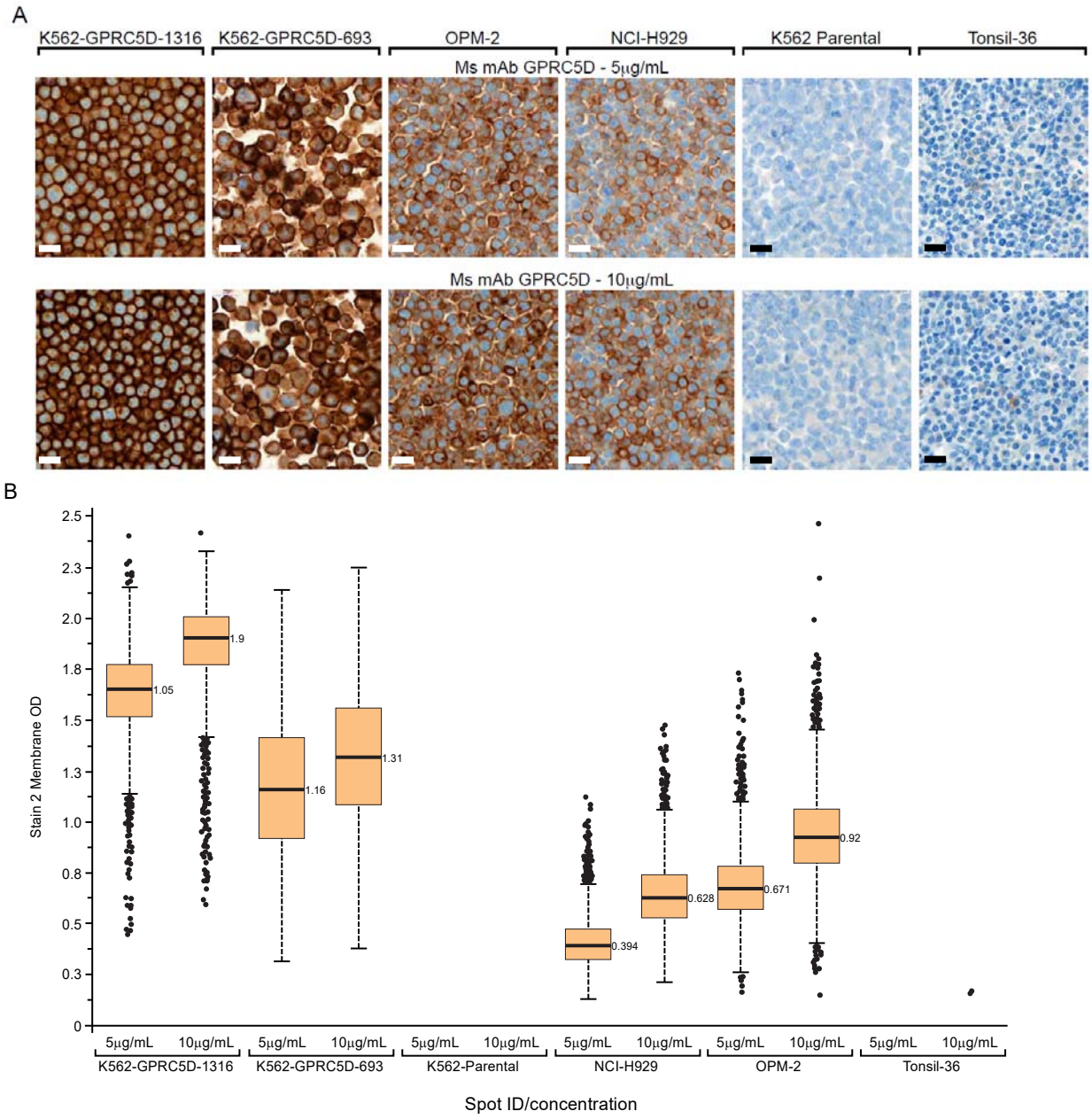


Fig. S3. GPRC5D protein expression on control cells. (A) Representative IHC staining of indicated cell lines. Scale bar = 20 µm. **(B)** Quantification by digital image analysis (Halo) of membrane optical density (median ± SD) to identify mAb concentration allowing the greatest dynamic range.

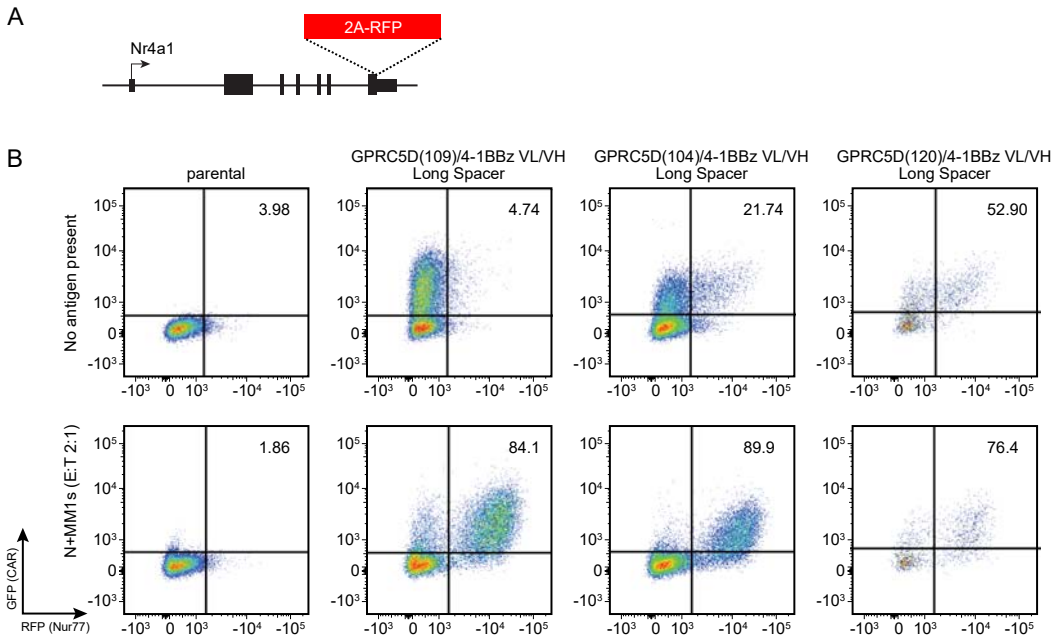


Fig. S4. Representative flow cytometric analyses of antigen-independent and antigen-specific activation using the Jurkat Nur77-RFP reporter line. (A) *RFP* reporter cDNA, separated from the last exon of the *NR4A1* gene by a "self-cleaving" 2A element, was placed in-frame under control of the Nur77 promoter via homologous recombination in a Jurkat T cell leukemia cell line. Cells were then transduced with the indicated CAR in a bicistronic construct with GFP. **(B)** CAR-transduced cells are indicated as GFP^+ along the y-axis. RFP expression, a surrogate for Nur77 expression, is shown along the x-axis. Percentages show the proportion of transduced GFP^+ cells (top quadrants) that are RFP^+.

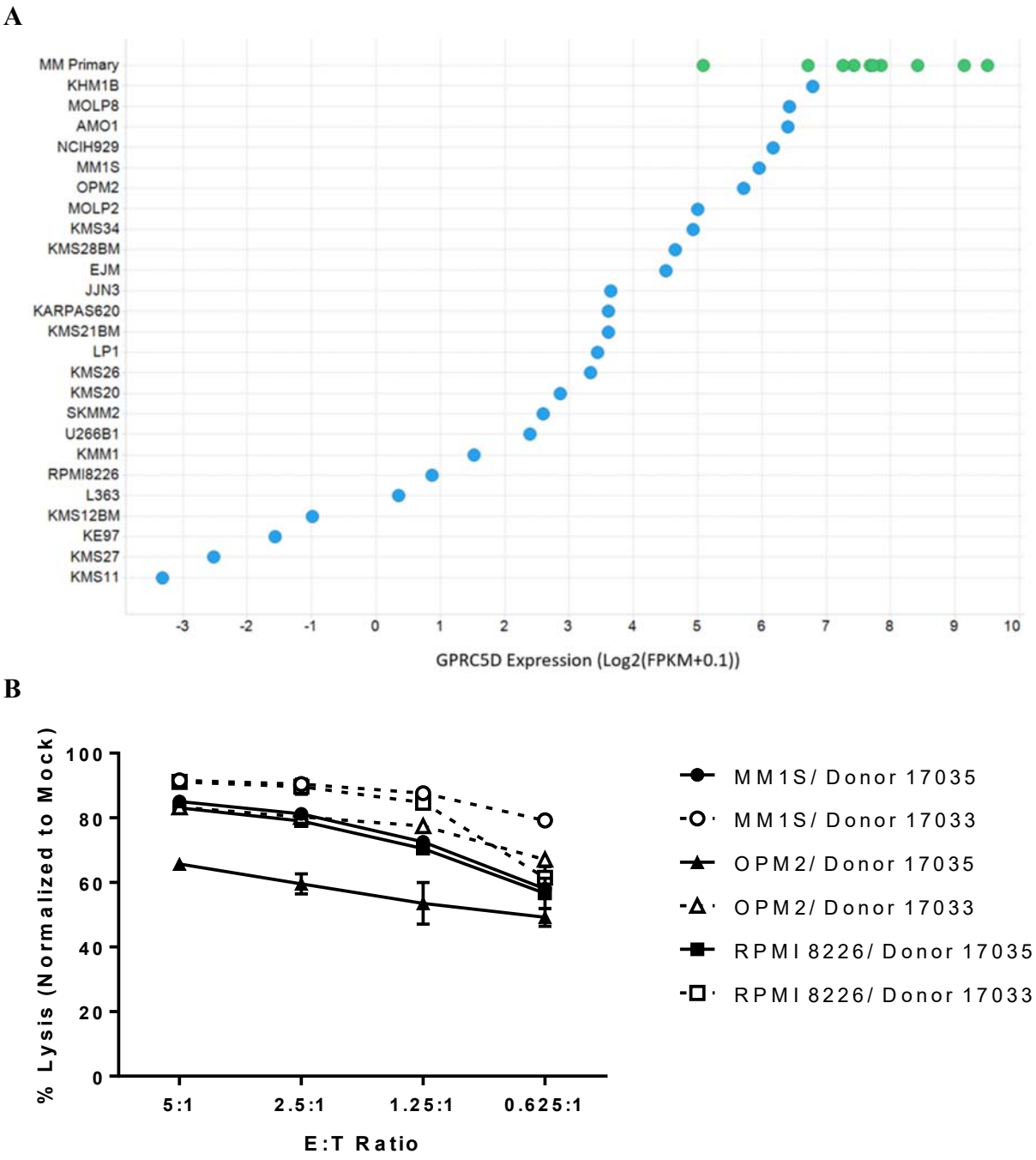


Fig. S5. GPRC5D(109) CAR T cell–mediated cytotoxicity of MM cell lines with varying GPRC5D expression. (A) *GPRC5D* mRNA expression across MM cell lines (CCLE RNAseq, blue dots) and primary MM cells (Blueprint RNAseq, green dots). **(B)** GPRC5D(109) CAR T cell 24-h cytotoxicity co-culture assay against MM cell lines RPMI-8226, MM1.S, and OPM2, normalized to donor-matched, mock-transduced T cells; technical triplicates in each of 2 donors (donor numbers 17033 and 17035) (mean ± SD).

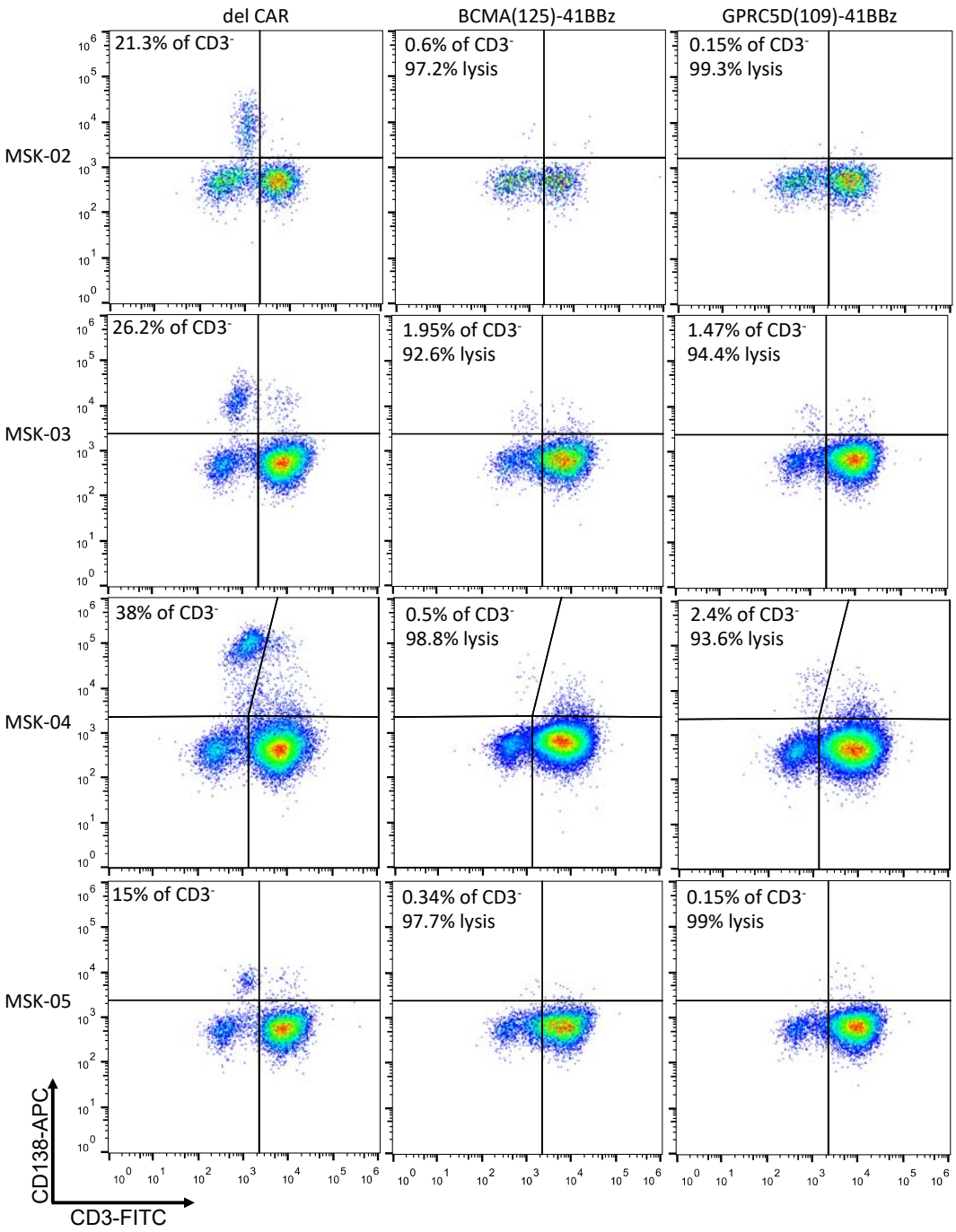


Fig. S6. Lysis of primary bone marrow aspirate MM cells by GPRC5D-targeted CAR T cells. Flow cytometry of primary bone marrow mononuclear cells (BMMCs) from patients with MM after 24-48 h co-culture with CAR T cells incorporating either BCMA-targeted scFv with no signaling domains (del CAR) or the indicated BCMA- or GPRC5D-targeted scFv with 4-1BB and CD3z signaling domains at a 1:1 ratio of CAR^+ T cells: BMMCs. To avoid contribution by T cell expansion or transduction efficiency, percentage of $CD3^-$ cells is reported.

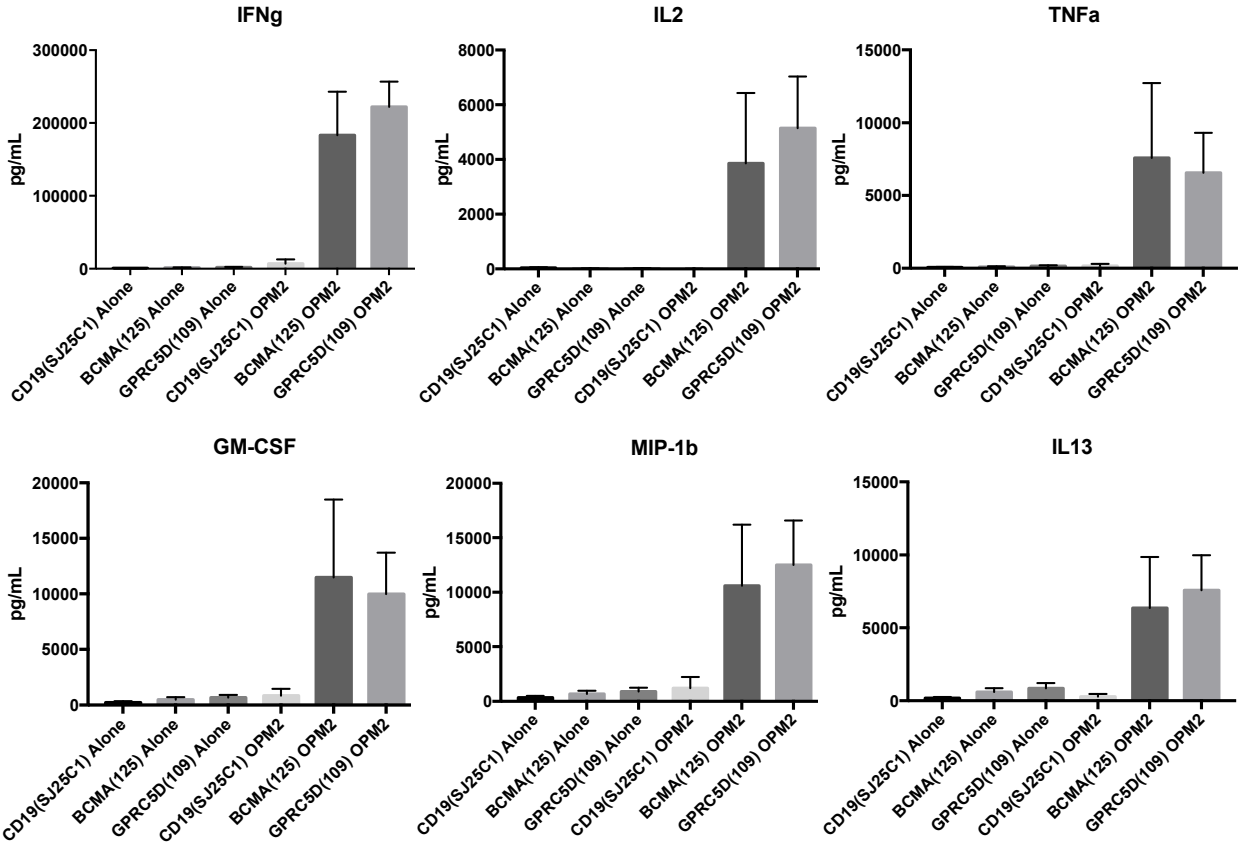


Fig. S7. Cytokine secretion by GPRC5D-targeted CAR T cells upon coculture with GPRC5D-expressing cells. Cytokines produced by CAR T cells incorporating the indicated scFv after 1:1 co-culture with OPM2 MM cells or alone for 24 h, measured in the supernatant by multiplex luminex assay as in Fig. 4C. In all examples above, BCMA and GPRC5D co-cultured with OPM2 cells were not significantly different from each other, whereas $p<0.05$ for all comparisons to matched CAR T cells in the absence of antigen or to irrelevantly CD19-targeted CAR T cells co-cultured with OPM2 MM cell line.

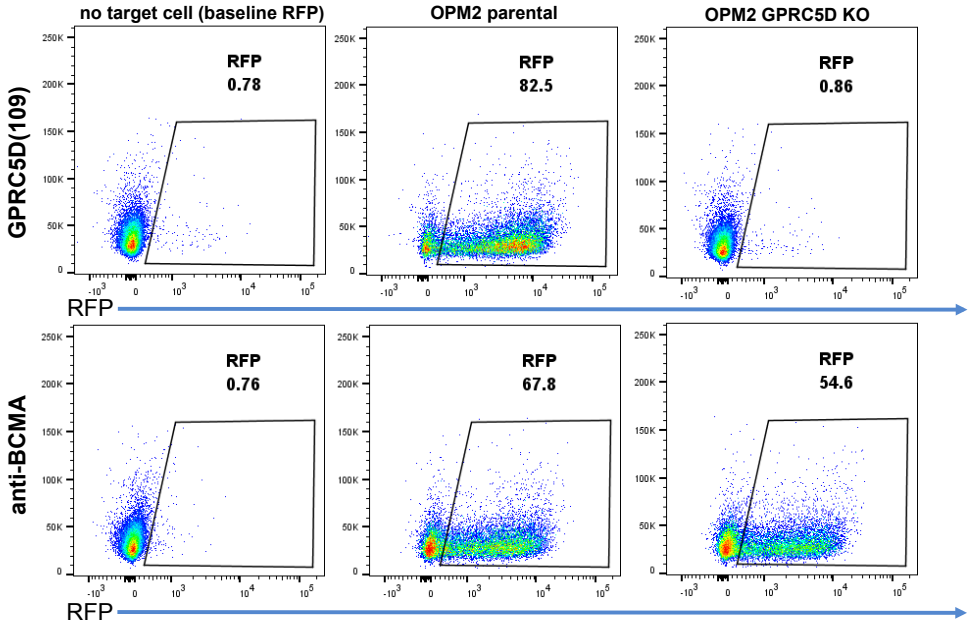


Fig. S8. Requirement of GPRC5D expression for activation through GPRC5D(109) CAR. Jurkat Nur77-RFP cells (fig. S4) engineered to express GPRC5D(109) or an anti-BCMA CAR were cultured 1:1 with OPM2 parental or GPRC5D-knockout cells for 20 h. Activation of the CAR was assessed by measuring changes in RFP expression by flow cytometry as in Fig. 3D.

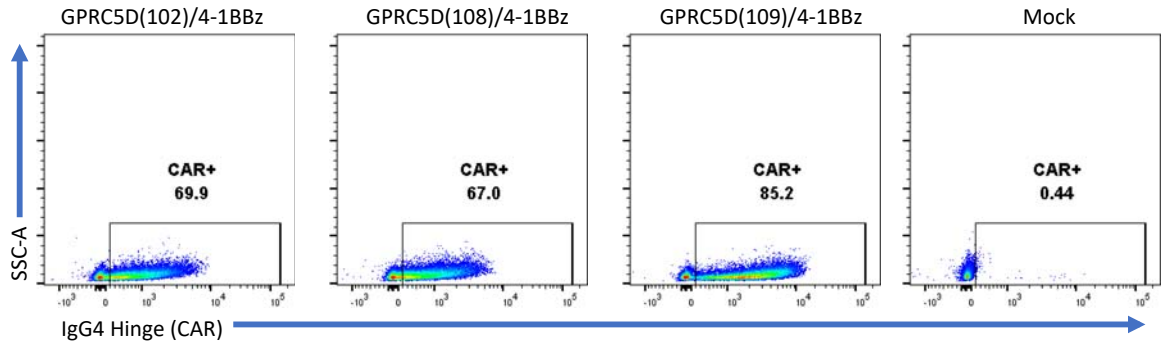


Fig. S9. Comparable surface expression of different CAR vectors on primary T cells. Representative FACS analysis of CAR expression in the CAR T cells used in the experiment presented in Fig. 6A, measured using anti-IgG4 spacer.

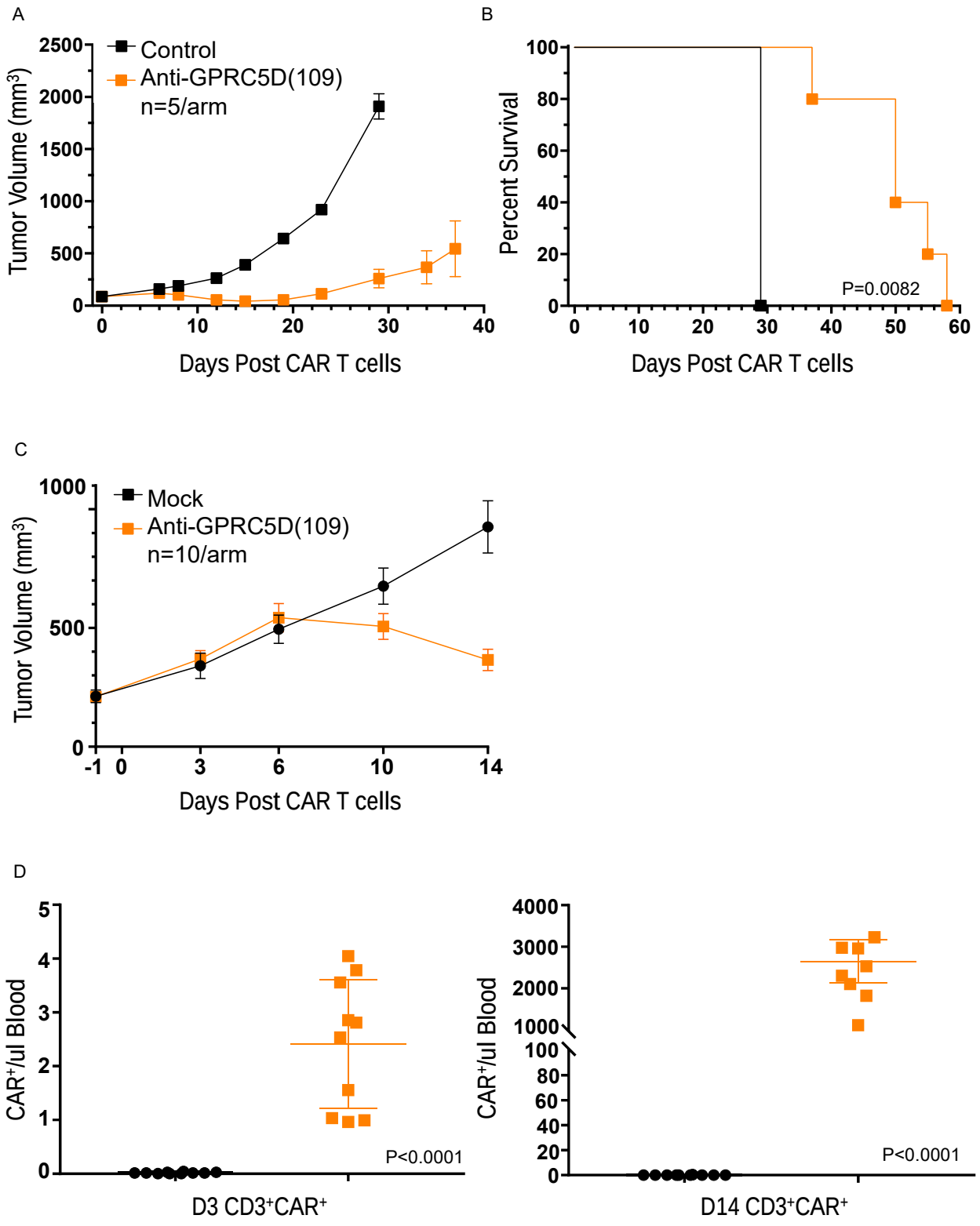


Fig. S10. In vivo expansion and antitumor activity of GPRC5D-targeted CAR T cells in an RPMI-8226 MM xenograft model. NSG mice were injected subcutaneously with RPMI-8226 cells. When tumors were palpable (day 0), mice were stratified into treatment and control groups

with comparable tumor volumes and treated with a single IV injection of 3×10^6 CAR^+ T cells. **(A)** Tumor volume. **(B)** Survival of mice in A; median 29 vs. 50 days ($p<0.05$; n=5 per arm, representative of 2 experiments). **(C-D)** In a separate group of mice, early anti-tumor efficacy was monitored by tumor volume (**C**), and CAR T cell expansion was monitored by flow cytometry of peripheral blood using an antibody to the IgG4-derived long spacer to detect the CAR (**D**) (n=10 per arm; both timepoints; $p<0.001$).

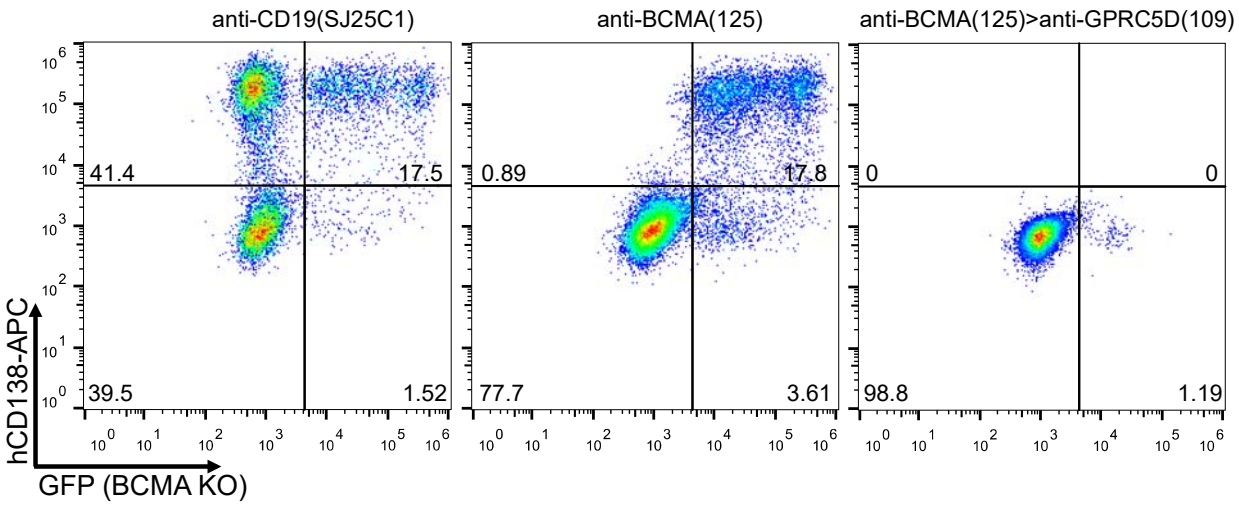


Fig. S11. Eradication of OPM2$^{BCMA\text{-}KO}$ MM cells by GPRC5D-targeted CAR T cells. OPM2 (hCD138^{+}) composition (WT: GFP^{-}; BCMA-KO: GFP^{+}) was assessed in flushed bone marrows from mice treated with the indicated CAR T cells as in Fig. 6G. Representative of 3 mice per arm (two replicate experiments with comparable results). Live/dead gating was performed.

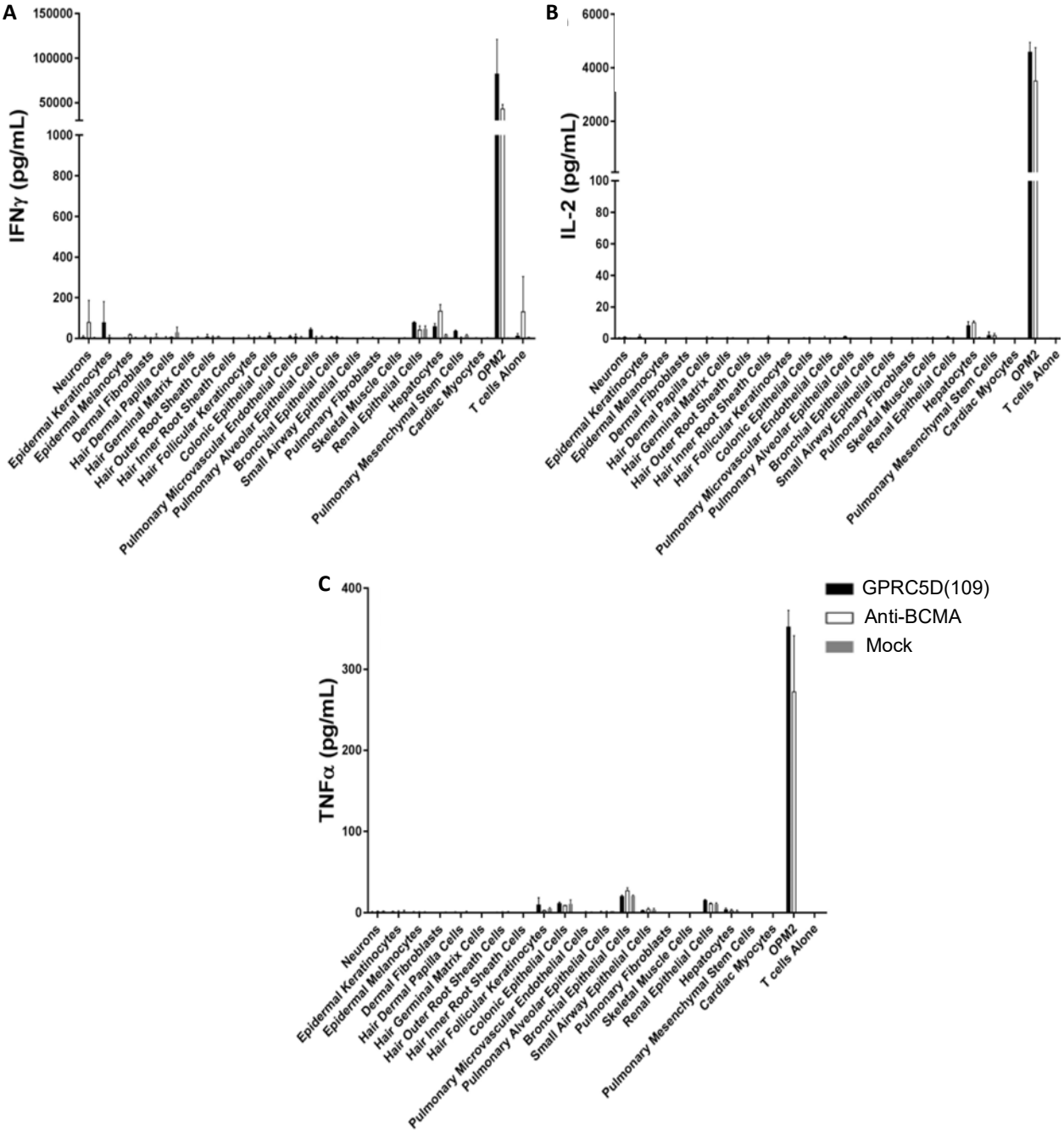


Fig. S12. Minimal cytokine release by GPRC5D-targeted CAR T cells upon coculture with primary human cell types isolated from normal tissues. GPRC5D(109) CAR, anti-BCMA CAR, or mock-processed T cells were co-cultured with one of 20 different isolated normal primary human cell types or OPM2 tumor cells. The following secreted cytokines were measured in supernatants after 20 h co-incubation: **(A)** interferon-γ (IFNγ), **(B)** interleukin-2 (IL2), **(C)** tumor necrosis factor alpha (TNFα). Graphs show mean concentrations ± SD.

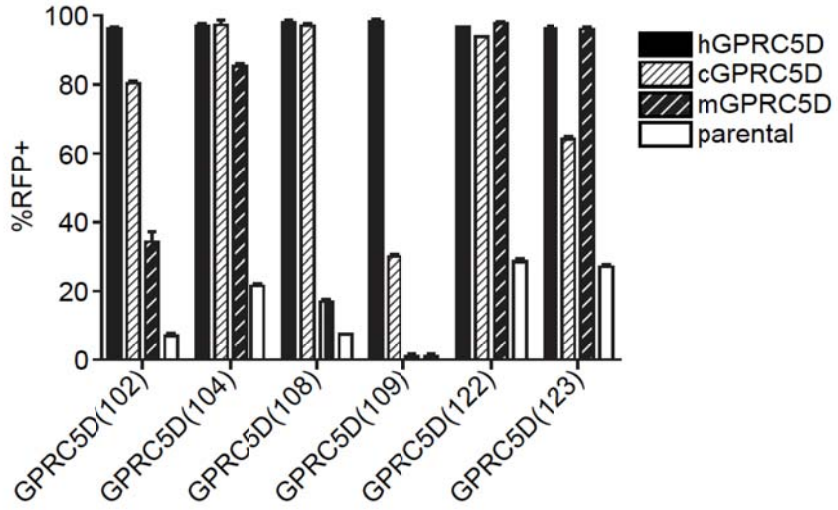


Fig. S13. Screening for murine and cynomolgus cross-reactive scFv clones. K562 cells were transduced to express human, cynomolgus, or murine GPRC5D. Jurkat Nur77-RFP reporter cells were transduced with a bi-cistronic plasmid containing anti-GPRC5D CAR and GFP to identify transduced cells. %RFP^{+} indicates activation after co-culture at an effector: target ratio of 1:1 (relative to GFP^{+} CAR-transduced cells).

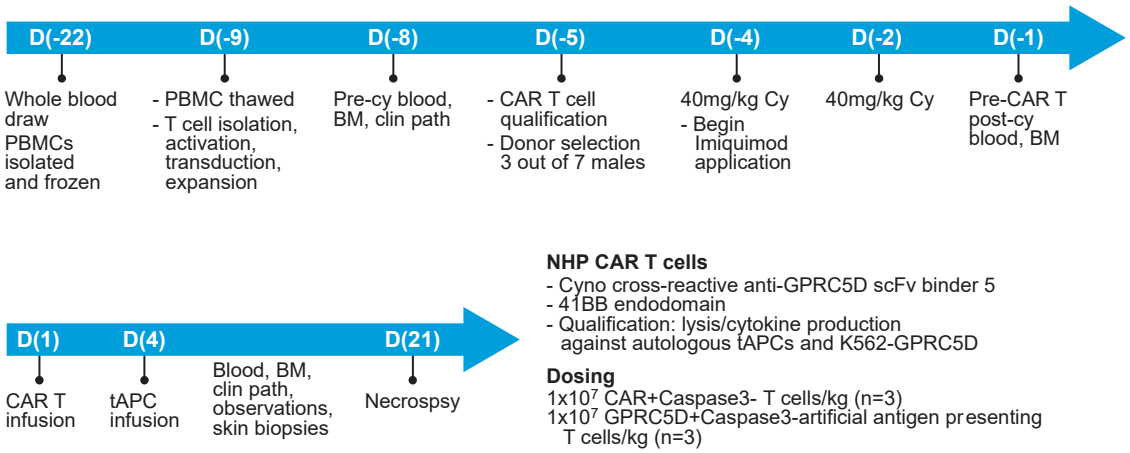
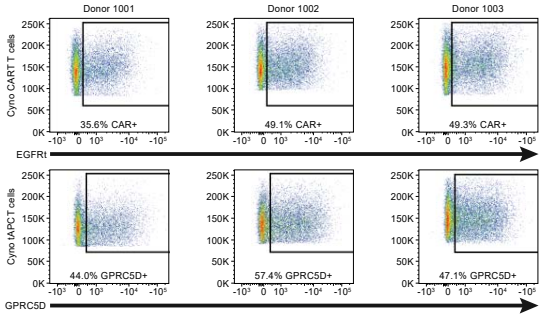


Fig. S14. Schematic of the NHP study protocol.

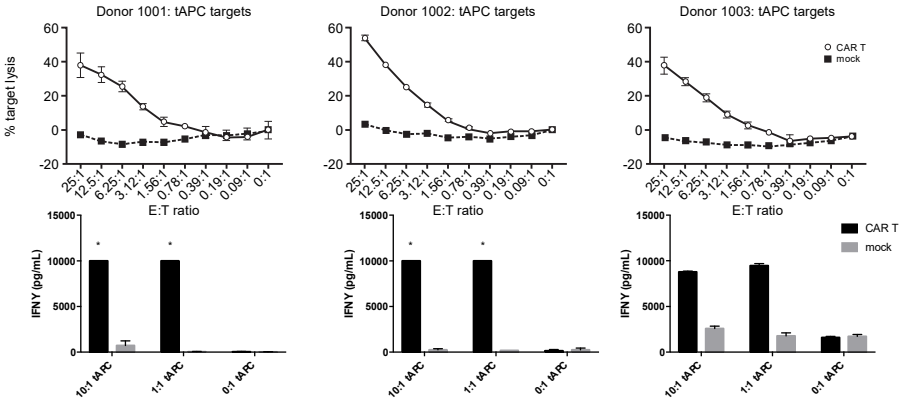
A

Donor	Construct	Viability	%TE	%CD4+	%CD8+	%T_{cm}	%T_{eff}	%Cas3+ or AnnV+	Projected CAR/tAPC+, Caspase3-cells/kg dose
1001	CAR	80.1	35.6	44.8	50.4	90.0	7.8	9.4	1.82e7
	tAPC	75.0	44.0	60.8	34.9	86.6	10.7	8.7	5.25e6
1002	CAR	92.1	49.1	48.5	60.5	93.8	3.8	9.2	1.37e7
	tAPC	91.4	54.4	41.3	55.4	95.2	2.7	8.9	1.19e7
1003	CAR	91.2	49.3	41.7	55.4	89.2	7.5	8.1	1.25e7
	tAPC	87.9	47.1	38.2	55.7	90.9	6.2	11.7	1.66e7

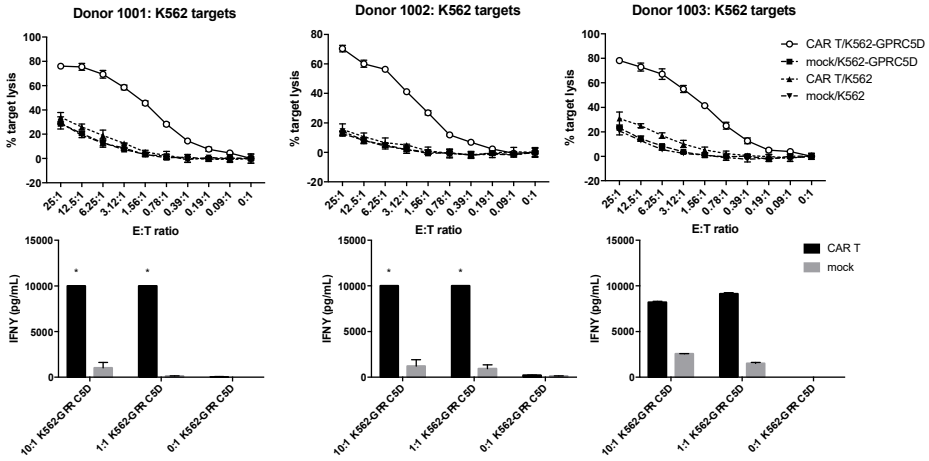
B



C



D



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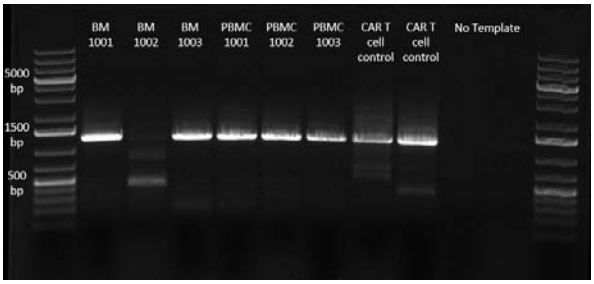

Fig. S15. Viability and functionality of cynomolgus GPRC5D(108) CAR T cells. NHP T cells were transduced to express either the cynomolgus cross-reactive GPRC5D CAR or cynomolgus GPRC5D antigen (tAPCs). **(A)** Summary of both cell types' properties after co-culture. %TE, % transduced; % Tem and % Tcm, %$CD95^{+}CD28^{-}$ (T effector memory) and %$CD95^{+}CD28^{+}$ (T central memory), respectively. **(B)** Representative FACS analysis of CAR expression measured using the surrogate marker EGFRt. **(C)** Target lysis and IFNγ production using tAPC targets. **(D)** Target lysis and IFNγ production using K562-GPRC5D targets. **(E)** CAR T cell persistence at day 21 after infusion in peripheral blood and bone marrow, as measured by PCR for the CAR. CAR-transduced NHP T cells were used as a positive control.

Table S1. GPRC5D expression in normal tissue. Thirty (30) normal tissue types from 3 donors were screened by IHC. **(A)** Twenty-four (24) tissue types with no GPRC5D expression by IHC. **(B)** In the 6 tissues found to express GPRC5D by IHC, expression was validated by RNA-ISH (RNAscope) and in some cases qPCR across additional species. *apocrine glands not present in murine tissue assessed for RNA-ISH.

A

Adrenal	Bone marrow	*Breast (n=5)
Brain, cerebellum	Brain, cerebellum	Brain, pituitary
Esophagus	Heart	Liver
Lung (no peri-bronchial glands)	Mesothelial cell	Ovary
Peripheral nerve	Placenta	Prostate
Salivary gland	Spleen	Skeletal muscle
Testis	Thymus	Thyroid
Tonsil	Uterus	Uterus, cervix

B

Tissue	Cell Types	Human			Cynomolgus Monkey		Mouse	
		IHC	RNA-ISH	qPCR	IHC	RNA-ISH	IHC	RNA-ISH
Skin	Hair shafts	High	High	Very weak	High	High	–	High
	Apocrine glands	Moderate-high	Negative		Low-Moderate	Negative	–	?*
Bronchus	Peri-bronchial glands	Low-high	Negative	Negative	–	–	–	Negative
Lung	Lung parenchyma	Negative	Negative	–	Negative	Negative	–	–
Duodenum	Intestinal epithelium; immune cells	Low-moderate	Negative	Negative	–	–	–	–
Tongue	Mast cells and eosinophils	–	–	–	High	Negative	–	–
Nail bed	Glands	–	–	–	Moderate-high	Negative	–	–

Table S2. Single chromogenic immunostaining conditions. HQ, Ventana Discovery HQ hapten.

	Anti-GPRC5D (OmniMap)	Anti-BCMA (HQ)	Anti-CD138 (OmniMap)
Antigen Retrieval with Discovery CC1 reagent (EDTA)	56 min at 99°C	92 min at 99°C	32 min at 99°C
Inhibitor CM	8 min	12 min	12 min
Goat IgG	4 min	N/A	N/A
Primary Antibody or isotype control	Test conc: 10 µg/mL 60 min, no heat	Test conc: 10 µg/mL 32 min, 37°C	Test conc: 0.198 µg/mL 32 min, 37°C
Linking antibody	N/A	DISCOVERY anti-mouse HQ: 12 min	N/A
Multimer HRP	OmniMap anti-mouse: 16 minutes	N/A	OmniMap anti-rabbit 16 minutes
Enzyme Conjugate	N/A	Anti-HQ HRP: 12 min	N/A
Chromogen	DAB	DAB	DAB
Counterstain	Hematoxylin II: 8-12 min	Hematoxylin II: 8-12 min	Hematoxylin II: 8-12 min
Bluing Reagent	4 min	4 min	4 min

Table S3. Antibodies and reagents for IHC and RNA-ISH.

Material	Vendor	Catalog #
Mouse mAb to GPCR (GPRC5D)	Abcam	ab55044
Mouse mAb to BCMA (D-6)	Santa Cruz Biotechnology	sc-390147
Mouse mAb to CD138 (B-A38)	Ventana	760-4248
Rabbit mAb CD138 (EP201)	Cell Marque	138R-26
Mouse IgG1K isotype control	BD Pharmingen	550878
Mouse IgG2a isotype control	BD Pharmingen	550339
Mouse IgG2b isotype control	BD Pharmingen	557351
Rabbit mAb IgG isotype control (DA1E)	Cell Signaling Technology	3900S
DISCOVERY OmniMap anti-mouse HRP	Ventana	760-4310
DISCOVERY OmniMap anti-rabbit HRP	Ventana	760-4311
Anti-Mouse HQ	Ventana	760-4814
Anti-HQ HRP	Ventana	760-4820
DISCOVERY Ab diluent	Ventana	760-108
DISCOVERY Rhodamine 6G kit	Ventana	760-244
DISCOVERY DCC kit	Ventana	760-240
DISCOVERY CY5 kit	Ventana	760-238
DISCOVERY purple kit	Ventana	760-229
DISCOVERY ChromoMap DAB kit	Ventana	760-159
DISCOVERY inhibitor	Ventana	760-4840
DISCOVERY CC1	Ventana	950-500
ULTRA CC2	Ventana	950-223
DISCOVERY goat Ig block	Ventana	760-6008
DISCOVERY Ab diluent	Ventana	760-108
Bluing reagent	Ventana	760-2037
Hematoxylin II	Ventana	760-2208
ProLong Diamond anti-fade mountant	Invitrogen	P36961
Hoechst 33342	Invitrogen	H3570
Hydrogen peroxide, 30%	Fisher Scientific	H325-500
RNAscope 2.5 LSx reagent kit, brown	Advanced Cell Diagnostics	322700
Hs-GPRC5D	Advanced Cell Diagnostics	489698
Hs-PPIB positive control probe	Advanced Cell Diagnostics	313908
DapB negative control probe	Advanced Cell Diagnostics	312038
Hs-PPIB positive control probe	Advanced Cell Diagnostics	313908
DapB negative control probe	Advanced Cell Diagnostics	312038

Table S4. Multiplex immunostaining conditions. HQ, Ventana Discovery HQ hapten.

	CD138 rabbit mAb (OmniMap)	**BCMA (HQ)**	**GPRC5D (OmniMap)**	**GPRC5D (HQ)**
Deparaffinization	24 min at 70 °C	24 min at 70 °C	24 min at 70 °C	24 min at 70 °C
Pretreatment with Ventana Discovery CCl reagent	32 min at 99 °C	92 min at 99 °C	56 min at 99 °C	56 min at 99 °C
Blocking with Ventana Discovery Inhibitor CM	12 min	12 min	8 min	12 min
Antibody blocking: goat Ig	N/A	N/A	4 min	4 min
Primary antibody	32 min at 37 °C; test dilution: 1/200	32 min at 37 °C; test conc.: 10 µg/mL	60 min at RT; test conc.: 10 µg/mL	60 min at RT; test conc.: 10 µg/mL
Linking antibody	N/A	anti-mouse HQ 12 min	N/A	anti-mouse HQ 12 min
Multimer HRP	OmniMap anti-rabbit HRP: 16 min	N/A	OmniMap anti-mouse HRP: 16 min	N/A
Enzyme conjugate	N/A	anti-HQ HRP 12 min	N/A	anti-HQ HRP 12 min
Chromogen	DAB	DAB	DAB	DAB or purple (32 min)