Supplementary Material

Table S1. Amino-acid sequence of BCMA-CD3 antibodies used in the study.

	PBM0012
Subunit 1	DVVMTQSPAFLSVTPGEKVTITCRASQSISDYLHWYQQKPDQAPKLLIKYASQSISGVPSRFSGS GSGTDFTFTISSLEAEDAATYYCQNGHSFPPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGEC
Subunit 2	Q A V V T Q E P S L T V S P G G T V T L T C G S S T G A V T T S N Y A N W V Q E K P G Q A F R G L I G G T N K R A P G T P A R F S G S L L G G K A A L T L S G A Q P E D E A E Y Y C A L W Y S N L W V F G G G T K L T V L S S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K K V E P K S C
Subunit 3	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYVMHWVRQAPGQGLEWMGYIIPYNDATKYN EKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARYNYDGYFDVWGQGTLVTVSSTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDGGGGSGGGGSEVQLLESGGGLVQPGGSLRLS CAASGFTFSTYAMNWVRQAPGKGLEWVSRIRSKYNNYATYYADSVKGRFTISRDDSKNTLYL QMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTVSSASVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGECDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSLWCLVKGFYPSDIAVEWE SNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS PGK
Subunit 4	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYVMHWVRQAPGQGLEWMGYIIPYNDATKYN EKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARYNYDGYFDVWGQGTLVTVSSTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALGAPIEKTISKAKGQPREPQV*C*TLPPSRDELTKNQVSL*SCA*VKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFL*V*SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK
	PBM0056
Subunit 1	DVVMTQSPAFLSVTPGEKVTITCRASQSISDYLHWYQQKPDQAPKLLIKYASQSISGVPSRFSGS GSGTDFTFTISSLEAEDAATYYCQNGHSFPPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGEC
Subunit 2	Q A V V T Q E P S L T V S P G G T V T L T C G S S T G A V T T S N Y A N W V Q E K P G Q A F R G L I G G T N K R A P G T P A R F S G S L L G G K A A L T L S G A Q P E D E A E Y Y C A L W Y S N L W V F G G G T K L T V L S S A S T K G P S V F P L A P S S K S T S G G T A A L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K K V E P K S C
Subunit 3	EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVSRIRSKYNNYATYY ADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTV SSASVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS

	KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGECDKTHTCPPCPAPEAAGG PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQ VSLWCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
Subunit 4	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYVMHWVRQAPGQGLEWMGYIIPYNDATKYN EKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARYNYDGYFDVWGQGTLVTVSSTKGPSV FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALGAPIEKTISKAKGQPREPQV*C*TLPPSRDELTKNQVSL*SCA*VKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFL*V*SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK
	PBM0060
Subunit 1	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYVMHWVRQAPGQGLEWMGYIIPYNDATKYN EKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARYNYDGYFDVWGQGTLVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP*C*RDELTKNQVSL*W*CLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
Subunit 2	EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYAMNWVRQAPGKGLEWVSRIRSKYNNYATYY ADSVKGRFTISRDDSKNTLYLQMNSLRAEDTAVYYCVRHGNFGNSYVSWFAYWGQGTLVTV SSGGGGSGGGGSGGGGSQAVVTQEPSLTVSPGGTVTLTCGSSTGAVTTSNYANWVQEKPGQA FRGLIGGTNKRAPGTPARFSGSLLGGKAALTLSGAQPEDEAEYYCALWYSNLWVFGGGTKLT VLGGGGSGGGGSGGGGSGGGGSGGGGSAAATHTCPPCPAPEAAGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK
Subunit 3	DVVMTQSPAFLSVTPGEKVTITCRASQSISDYLHWYQQKPDQAPKLLIKYASQSISGVPSRFSGS GSGTDFTFTISSLEAEDAATYYCQNGHSFPPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGEC
	PBM0055
Subunit 1	QVQLVQSGAEVKKPGSSVKVSCKASGYTFTSYVMHWVRQAPGQGLEWMGYIIPYNDATKYN EKFKGRVTITADKSTSTAYMELSSLRSEDTAVYYCARYNYDGYFDVWGQGTLVTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTV PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTL MISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD

	WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPCRDELTKNQVSL*W*CLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHY TQKSLSLSPGK
Subunit 2	EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATY YADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTLVT VSSGGGGSGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQ APRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKL TVLGGGGSGGGGSGGGGSGGGGSGGGGSAAATHTCPPCPAPEAAGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLSCAVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLVSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQK SLSLSPGK
Subunit 3	DVVMTQSPAFLSVTPGEKVTITCRASQSISDYLHWYQQKPDQAPKLLIKYASQSISGVPSRFSGS GSGTDFTFTISSLEAEDAATYYCQNGHSFPPTFGGGTKVEIKRTVAAPSVFIFPPSDEQLKSGTAS VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA CEVTHQGLSSPVTKSFNRGEC
	PBM0057
Subunit 1	E V Q L V E S G G G L V Q P G G S L R L S C A A S G F T F S D Y Y M T W V R Q A P G K G L E W V A F I R N R A R G Y T S D H N P S V K G R F T I S R D N A K N S L Y L Q M N S L R A E D T A V Y Y C A R D R P S Y Y V L D Y W G Q G T T V T V S S A S T K G P S V F P L A P C S R S T S E S T A A L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S N F G T Q T Y T C N V D H K P S N T K V D K T V E R K C R V R C P R C P A P P V A G P S V F L F P P K P K D T L M I S R T P E V T C V V V A V S H E D P E V Q F N W Y V D G V E V H N A K T K P R E E Q F N S T F R V V S V L T V V H Q D W L N G K E Y K C K V S N K G L P S S I E K T I S K T K G Q P R E P Q V Y T L P P S R E E M T K N Q V S L T C L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P M L D S D G S F F L Y S R L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L S P G K
Subunit 2	E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S S Y P M S W V R Q A P G K G L E W V S A I G G S G G S L P Y A D I V K G R F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A R Y W P M D I W G Q G T L V T V S S A S T K G P S V F P L A P C S R S T S E S T A A L G C L V K D Y F P E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S N F G T Q T Y T C N V D H K P S N T K V D K T V E R K C E V E C P E C P A P P V A G P S V F L F P P K P K D T L M I S R T P E V T C V V V A V S H E D P E V Q F N W Y V D G V E V H N A K T K P R E E Q F N S T F R V V S V L T V V H Q D W L N G K E Y K C K V S N K G L P S S I E K T I S K T K G Q P R E P Q V Y T L P P S R E

	EMTKNQVSLTCEVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSD GSFFL YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
Subunit 3	DIVMTQSPDSLAVSLGERATINCKSSQSLFNVRSRKNYLAWYQQKP GQPP KLLISWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCKQS YDL FTFGSGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPR EAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEK HKVYACEVTHQGLSSPVTKSFNRGEC
Subunit 4	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRL LMY DASIRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYQSWPL TFG QGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWK VDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE VTHQ GLSSPVTKSFNRGEC

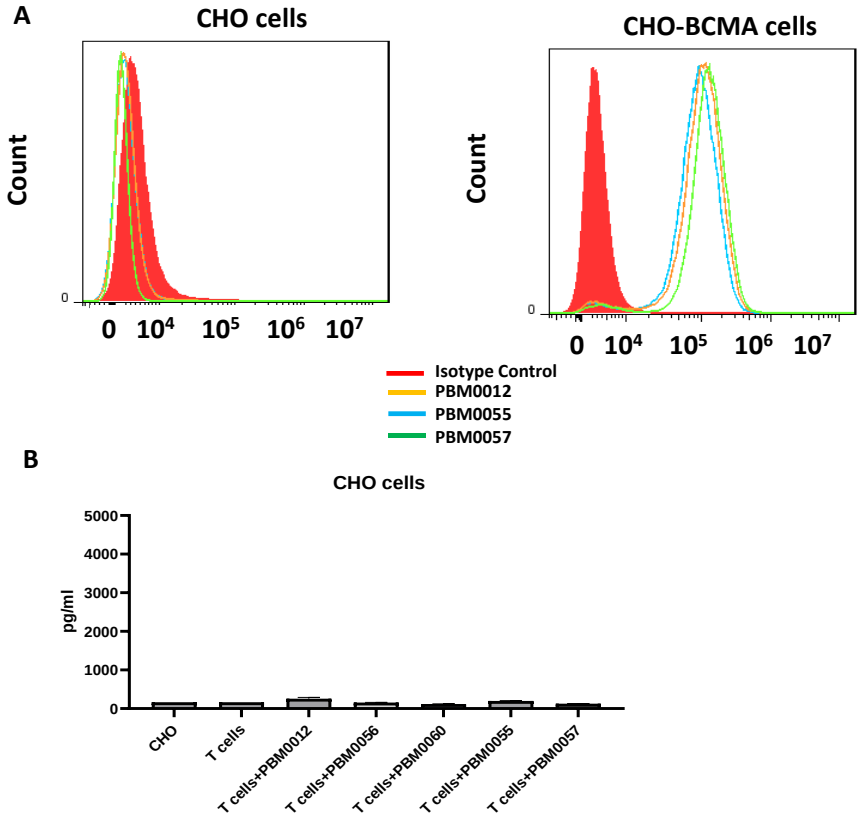
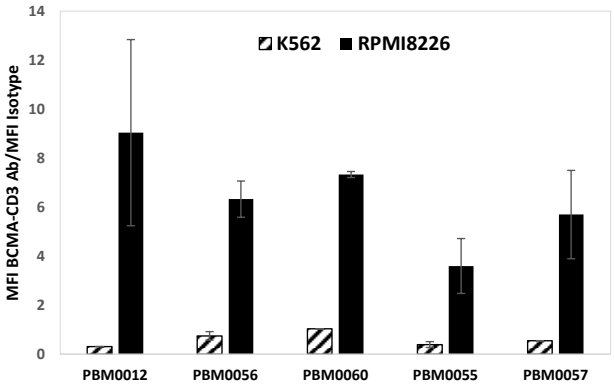
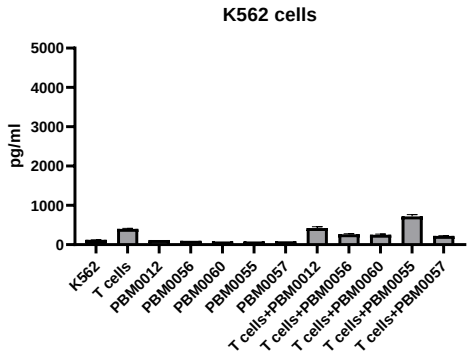


Figure S1. (**A**) FACS with BCMA-CD3 antibodies shows no binding with BCMA-negative CHO cells. CHO-BCMA cells show positive binding with BCMA-CD3 antibodies. Left panel FACS with CHO cells, right panel FACS with CHO-BCMA cells. Representative FACS with PBM0012, PBM0055 and PBM0057 BCMA-CD3 antibodies is shown. (**B**) IFN-gamma ELISA assay as described in Materials and Methods shows no secretion of IFN-gamma by T cells with BCMA-CD3 antibodies with CHO target cells. Bars show average ± standard deviations.



(A)



(B)

Figure S2. (**A**) FACS with BCMMA-CD3 antibodies shows no binding of BCMA-CD3 antibodies to K562 lymphoblast cells. Multiple myeloma RPMI8226 cells show positive binding with BCMA-CD3 antibodies. MFI BCMA Abs/MFI isotype antibody is shown on Y-axis. (**B**) T cells with BCMA-CD3 antibodies don't secrete high level IFN-gamma with K562 cells. IFN-gamma ELISA assay was performed as described in Materials and Methods. Bars show average ± standard deviations.

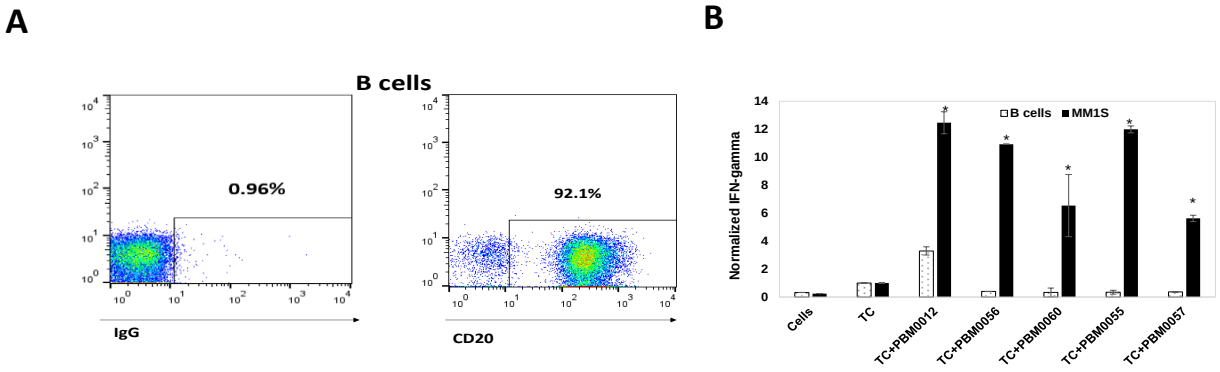


Figure S3. T cells with BCMA-CD3 antibodies secrete significantly less IFN-gamma with B cells than with MM1S multiple myeloma cells. (**A**) FACS with B cell marker, CD2O antibody shows >90% CD20-positive cells in expanded primary B cells. The binding was negative with BCMA-CD3 antibody (not shown). (**B**) Low level of IFN-gamma secreted by T cells and BCMA-CD3 antibodies with primary B cells. Bars show average ± standard deviations. IFN-gamma is normalized to the level of IFN-gamma secreted by T cells (TC) alone with target cells. * $p < 0.05$, IFN-gamma secreted by BCMA-CD3 antibodies with T cells with multiple myeloma MM1S target cells versus INF-gamma secreted with target B cells.

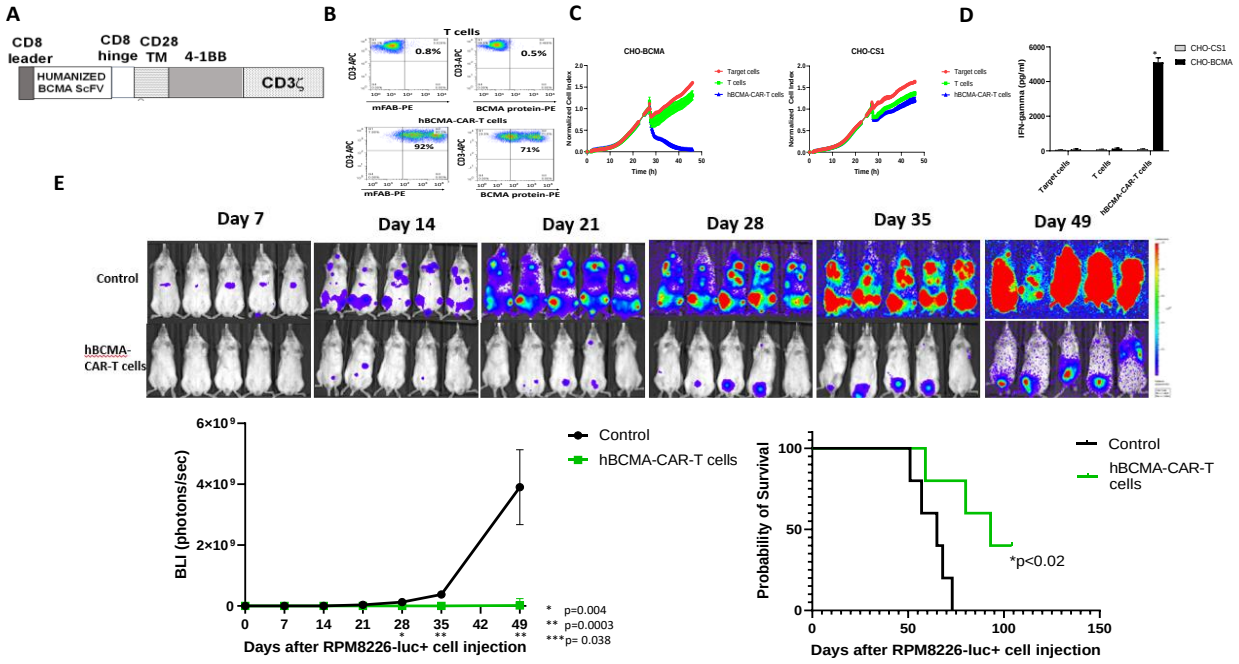


Figure S4. Humanized BCMA-CAR-T cells significantly block RPMI8226-luciferase+ multiple myeloma xenograft tumor growth. (**A**) The structure of humanized hBCMA-CAR construct. BCMA ScFv contains VH and VL (Table S1) linked with G4S linker. CAR structure contains CD8 alpha hinge, CD28 transmembrane, 4-1BB co-stimulatory and CD3 activation domains. (**B**) FACS detects CAR-positive T cells after transduction of T cells with hBCMA-CAR lentivirus. FACS was performed on BCMA-CAR-T cells with anti-mouse-F(ab)2 antibody (left panel) and with recombinant BCMA protein (right panel). (**C**) hBCMA-CAR-T cells kill CHO-BCMA cells (left) and don't kill CHO-CS1 target cells (right). RTCA assay was performed as described in Materials and Methods. (**D**) hBCMA-CAR-T cells secrete high level of IFN-gamma with CHO-BCMA cells. $p < 0.0004$, Student's t-test. IFN-gamma secreted by hBCMA-CAR-T cells with CHO-BCMA cells compared with CHO-CS1 target cells. (**E**) hBCMA-CD3 CAR-T cells significantly decreased RPMI8226-luc$^+$ xenograft tumor growth. Upper panel shows imaging of BCMA-CAR-T cell-treated mice (n = 5 mice/group). Lower left panel shows quantification of imaging BLI (photons/sec). * $p < 0.004$, BLI of BCMA-treated mice at day 28; ** $p < 0.0003$ at day 35; *** $p < 0.04$ at day 49 versus control group by Student's t-test. Lower right panel. BCMA-CAR-T cells significantly prolong survival of RPMI8226 xenograft NSG mice. * $p < 0.02$ of BCMA-CAR-T cell treated versus control mice by Log-rank Mantel-Cox test.