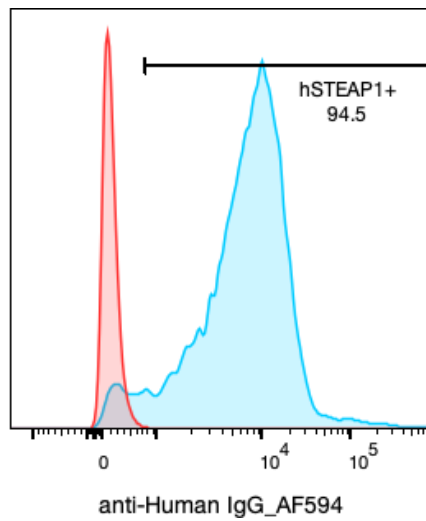
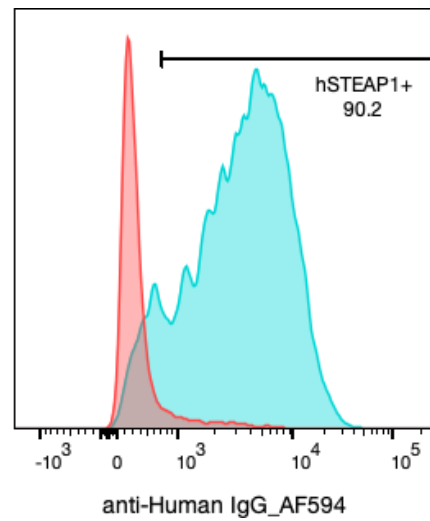


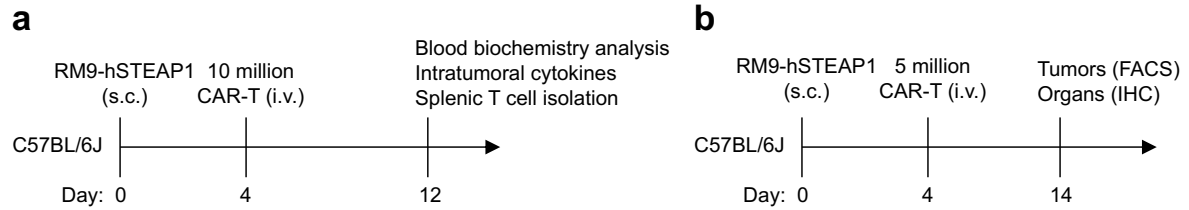
Collagen-binding IL-12-armoured STEAP1 CAR-T cells reduce toxicity and treat prostate cancer in mouse models

In the format provided by the
authors and unedited

a**RM9** **RM9-hSTEAP1****b****MyC-CaP** **MyC-CaP-hSTEAP1**

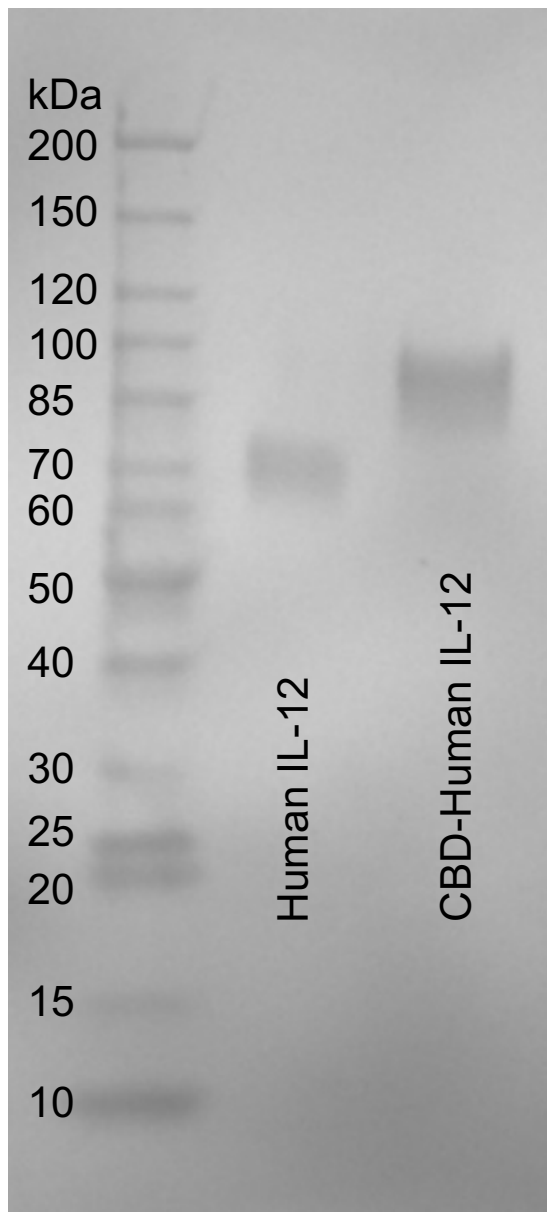
Supplementary Fig. 1 Flow cytometric analysis of human STEAP1 (hSTEAP1) expression in mouse prostate cancer cell lines.

(a) RM9 and (b) MyC-CaP cells were engineered to express hSTEAP1 by lentiviral transduction. The cells were stained with Vandortuzumab (anti-hSTEAP1 human IgG1) followed by anti-human IgG-Alexa Fluor 594. Representative histograms from an experiment.



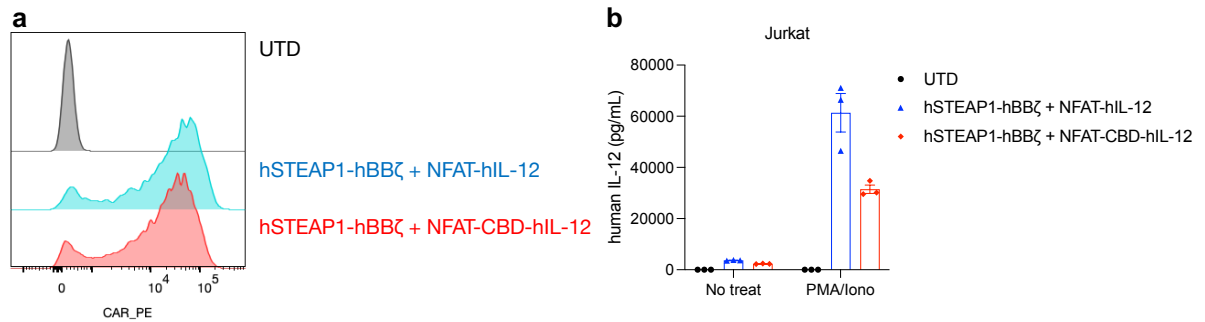
Supplementary Fig. 2 Experimental timelines, related to Figs. 4-6.

Male C57BL/6/J mice received subcutaneous injection of RM9-hSTEAP1 (5×10^5) on day 0. (a) The tumor-bearing mice received 10 million CAR-T cells (i.v.) on day 4. Blood samples, tumors and spleens were collected for biochemistry analysis (Fig. 4a, b), intratumoral cytokine analysis (Fig. 5a-c) and splenic T cell restimulation (Fig. 5m). (b) The tumor-bearing mice received 5 million CAR-T cells (i.v.) on day 4. Organs (liver, kidney, lung) and tumors were harvested on day 14. The organs were processed for histological analysis (Fig. 4c-e, Extended Data Fig. 5). Tumors were used for flow cytometric analysis of immune infiltrates (Fig. 5e-l, Extended Data Fig. 6, Extended Data Fig. 7a, Extended Data Fig. 8) and spatial transcriptomics (Fig. 6, Extended Data Fig. 7).



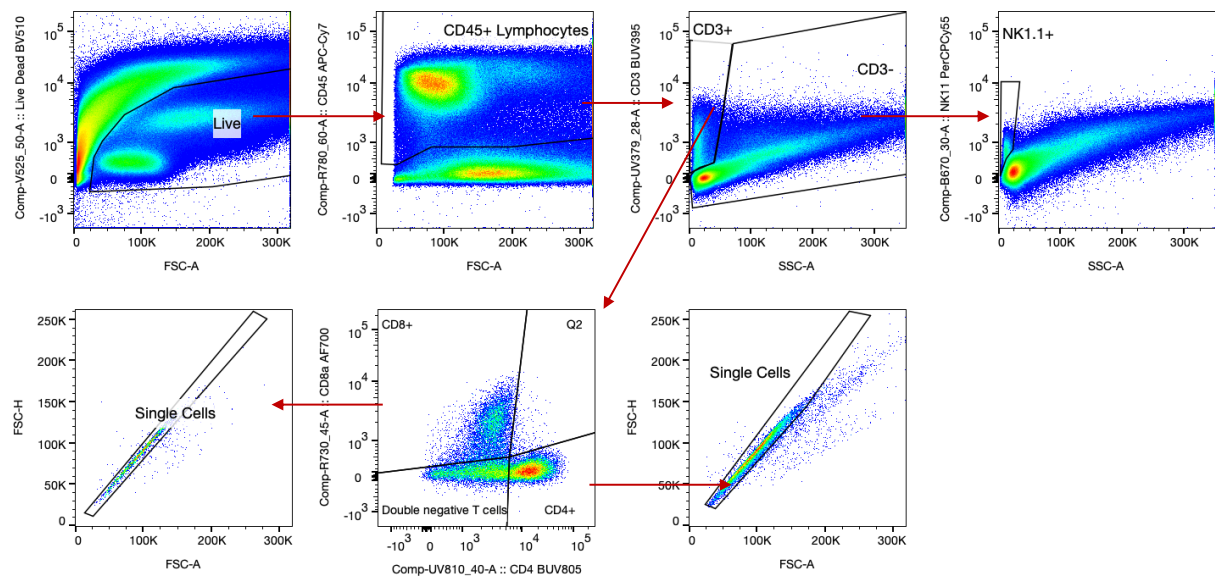
Supplementary Fig. 3 SDS-PAGE for recombinant human scIL-12 and CBD-human scIL-12.

Human scIL-12 variants were produced by transient expression in HEK293F cells, purified and analyzed by SDS-PAGE under reducing conditions with Coomassie blue staining.



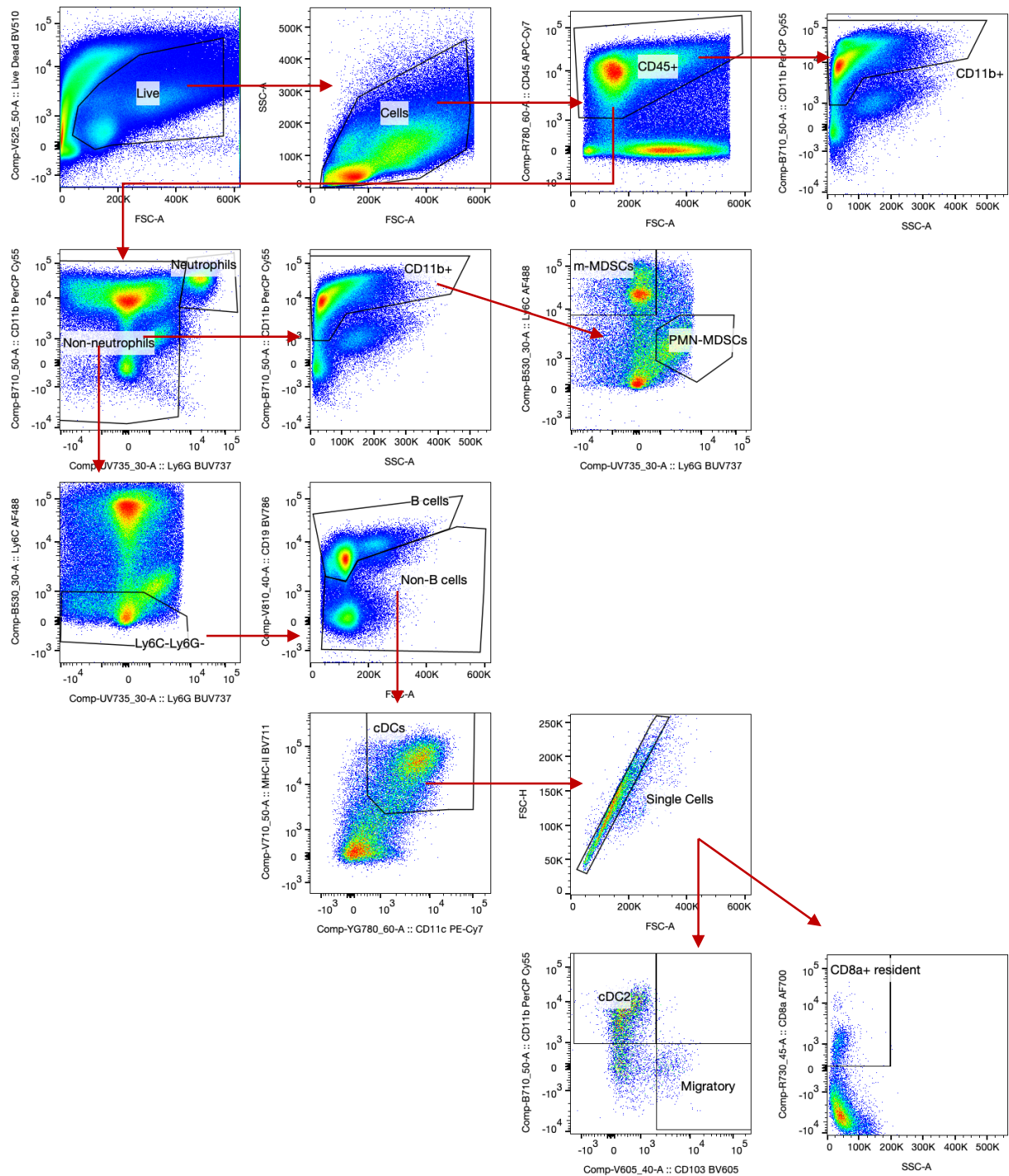
Supplementary Fig. 4 Jurkat T cells engineered to express hSTEAP1 CAR and CBD-human IL-12.

(a) Jurkat T cells were transduced to express the indicated transgenes. Cells were stained with Biotin-goat anti-Human IgG, and PE Streptavidin. Representative histograms of two experiments. (b) 50000 CAR⁺ Jurkat T cells were stimulated with phorbol 12-myristate 13-acetate and ionomycin (PMA/Iono) for 24 h. Secreted IL-12 was quantified by ELISA (technical replicates, mean $\pm$ SEM). Human scIL-12-His and CBD-human scIL-12-His were used to draw standard curves.



Supplementary Fig. 5 Gating strategy for characterizing T/NK cells.

Cells were first gated based on BD Horizon Fixable Viability Stain 510 followed by staining with indicated markers.



Supplementary Fig. 6 Gating strategy for characterizing B cells and myeloid cells.

Cells were first gated based on BD Horizon Fixable Viability Stain 510 followed by staining with indicated markers.

Supplementary Table 1. Amino acid sequences of the scIL-12 variants

Mouse IL-12 (for production in HEK293F):

MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSDQRHGVIGSGKTLTIT
VKEFLDAGQYTCHKGGETLSHSHLLLHKKENGIWSTEILKNFKNKTKFLKCEAPNYS
GRFTCSWLVRNMDLKFNIKSSSSSPDSRAVTCGMASLSAEKVTLDQRDYEKYSV
SCQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKPDPPKNLQMKPLKNS
QVEVSWEYPDSWSTPHSYFSLKFFVRIQRKKEKMKETEEGCNQKGAFLVEKTSTE
VQCKGGNVCVQAQDRYYNSSCSKWACVPCRVRSGGSGGGSGGGSGGGSGRVIPV
SGPARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITRDQTSTLKTCLPL
ELHKNESCLATRETSSTTRGSCLPQKTSMMTLCLGSIYEDLKMYQTEFQAINAAL
QNHNHQQIILDKGMLVAIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAF
STRVVTINRVMGYLSSAHHHHHH*

Mouse IL-12 (as a payload for CAR-T):

MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSDQRHGVIGSGKTLTIT
VKEFLDAGQYTCHKGGETLSHSHLLLHKKENGIWSTEILKNFKNKTKFLKCEAPNYS
GRFTCSWLVRNMDLKFNIKSSSSSPDSRAVTCGMASLSAEKVTLDQRDYEKYSV
SCQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKPDPPKNLQMKPLKNS
QVEVSWEYPDSWSTPHSYFSLKFFVRIQRKKEKMKETEEGCNQKGAFLVEKTSTE
VQCKGGNVCVQAQDRYYNSSCSKWACVPCRVRSGGSGGGSGGGSGGGSGRVIPV
SGPARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITRDQTSTLKTCLPL
ELHKNESCLATRETSSTTRGSCLPQKTSMMTLCLGSIYEDLKMYQTEFQAINAAL
QNHNHQQIILDKGMLVAIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAF
STRVVTINRVMGYLSSA*

CBD-mouse IL-12 (for production in HEK293F):

CSQPLDVILLLDGSSSFASYFDEMKSFAKAFISKANIGPRLTQVSVLQYGSITTIDVP
WNVVPKHAHLLSLVDVMQREGGPSQIGDALGFAVRYLTSEMHGARGASKAVVILV
TDVSVDSVDAAADAARSNRVTVPFPIGIGDRYDAAQLRILAGPAGDSNVVKLQRIEDL
PTMVTLGNSFLHKLCSGFVRIGGGSGGGSGMWELEKDVYVVEVDWTPDAPGETVN
LTCDTPEEDDITWTSDQRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLL
HKKENGIWSTEILKNFKNKTKFLKCEAPNYSGRFTCSWLVRNMDLKFNIKSSSSSP
DSRAVTCGMASLSAEKVTLDQRDYEKYSVSCQEDVTCPTAEETLPIELALEARQQN
KYENYSTSFFIRDIKPDPPKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVR
IQRKKEKMKETEEGCNQKGAFLVEKTSTEVQCKGGNVCVQAQDRYYNSSCSKWA
CVPCRVRSGGSGGGSGGGSGGGSGRVIPVSGPARCLSQSRNLLKTTDDMVKTARE
KLKHYSCTAEDIDHEDITRDQTSTLKTCLPLELHKNESCLATRETSSTTRGSCLPQ
KTSMMTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGMLVAIDELMQSLN
HNGETLRQKPPVGEADPYRVKMKLCILLHAFSTRVVTINRVMGYLSSAHHHHHH*

CBD-mouse IL-12 (as a payload for CAR-T):

CSQPLDVILLLDGSSSFPA SYFDEMKSFAKAFISKANIGPRLTQVSVLQYGSITTIDVP
WNVVPEKAHLLSLVDVMQREGGPSQIGDALGFAVRYLTSEMHGARPGASKAVVILV
TDVSVDSVDAAADAARSNRVTVFPIGIGDRYDAAQLRILAGPAGDSNVVKLQRIEDL
PTMVTLGNSFLHKLCSGFVRIGGGSGGGSGMWELEKDVYVVEVDWTPDAPGETVN
LTCDTPEEDDITWTSDQRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLL
HKKENGIWSTEILKNFKNKTKFLKCEAPNYSGRFTCSWLVRNMDLKFNIKSSSSSP
DSRAVTCGMASLSAEKVTL DQRDYEKYSVSCQEDVTCPTAEETLPIELALEARQQN
KYENYSTSFFIRDIKPDPPKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVR
IQRKKEKMKETE EGCNQKGAFLVEKTSTE VQCKGGNVCVQAQDRYYNSSCSKWA
CVPCRVRSGGSGGGSGGGSGGGSRVIPVSGPARCLSQSRNLLKTTDDMVKTARE
KLKHYSCTAEDIDHEDITRDQTSTLKTCLPLELHKNESCLATRETSSTTRGSC LPPQ
KTS LMMTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGMLVAIDELMQSLN
HNGETLRQKPPVGEADPYRVKMKLCILLHAFSTRVVTINRVMGYLSSA*

Mouse IL-12-CBD (for production in HEK293F):

MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSDQRHGVIGSGKTLTIT
VKEFLDAGQYTCHKGGETLSHSHLLLHKKENGIWSTEILKNFKNKTKFLKCEAPNYS
GRFTCSWLVRNMDLKFNIKSSSSSPDSRAVTCGMASLSAEKVTL DQRDYEKYSV
SCQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKPDPPKNLQMKPLKNS
QVEVSWEYPDSWSTPHSYFSLKFFVRIQRKKEKMKETE EGCNQKGAFLVEKTSTE
VQCKGGNVCVQAQDRYYNSSCSKWACVPCRVRSGGSGGGSGGGSGGGSRVIPV
SGPARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITRDQTSTLKTCLPL
ELHKNESCLATRETSSTTRGSC LPPQKTS LMMTLCLGSIYEDLKMYQTEFQAINAAL
QNHNHQQIILDKGMLVAIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAF
STRVVTINRVMGYLSSACSQPLDVILLLDGSSSFPA SYFDEMKSFAKAFISKANIGPR
LTQVSVLQYGSITTIDVPWNVVPEKAHLLSLVDVMQREGGPSQIGDALGFAVRYLTS
EMHGARPGASKAVVILVTDVSVDSVDAAADAARSNRVTVFPIGIGDRYDAAQLRILA
GPAGDSNVVKLQRIEDLPTMVTLGNSFLHKLCSGFVRIGGGSGGGSGGGSHHHHHH*

Mouse IL-12-CBD (as a payload for CAR-T):

MWELEKDVYVVEVDWTPDAPGETVNLTCDTPEEDDITWTSDQRHGVIGSGKTLTIT
VKEFLDAGQYTCHKGGETLSHSHLLLHKKENGIWSTEILKNFKNKTKFLKCEAPNYS
GRFTCSWLVRNMDLKFNIKSSSSSPDSRAVTCGMASLSAEKVTL DQRDYEKYSV
SCQEDVTCPTAEETLPIELALEARQQNKYENYSTSFFIRDIKPDPPKNLQMKPLKNS
QVEVSWEYPDSWSTPHSYFSLKFFVRIQRKKEKMKETE EGCNQKGAFLVEKTSTE
VQCKGGNVCVQAQDRYYNSSCSKWACVPCRVRSGGSGGGSGGGSGGGSRVIPV
SGPARCLSQSRNLLKTTDDMVKTAREKLKHYSCTAEDIDHEDITRDQTSTLKTCLPL

ELHKNESCLATRETSSTTRGSCCLPPQKTSMMTLCLGSIYEDLKMYQTEFQAINAAL
QNHNHQQIILDKGMLVAIDELMQSLNHNGETLRQKPPVGEADPYRVKMKLCILLHAF
STRVVTINRVMGYLSSACSQPLDVILLLDGSSSFASYFDEMKSFAKAFISKANIGPR
LTQVSVLQYGSITTIDVPWNVVPEKAHLLSLVDVMQREGGPSQIGDALGFAVRYLTS
EMHGARPGASKAVVILVTDVSVDSVDAAADAARSNRVTVPFPIGIGDRYDAAQLRILA
GPAGDSNVVKLQRIEDLPTMVTLGNSFLHKLCSGFVRI*

CBD-mouse IL-12-CBD (for production in HEK293F):

CSQPLDVILLLDGSSSFASYFDEMKSFAKAFISKANIGPRLTQVSVLQYGSITTIDVP
WNVVPEKAHLLSLVDVMQREGGPSQIGDALGFAVRYLTSEMHGARPGASKAVVILV
TDVSVDSVDAAADAARSNRVTVPFPIGIGDRYDAAQLRILAGPAGDSNVVKLQRIEDL
PTMVTLGNSFLHKLCSGFVRIGGGSGGGSMWELEKDVYVVEVDWTPDAPGETVN
LTCDTPEEDDITWTSDQRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLL
HKKENGIWSTEILKNFKNKTKFLKCEAPNYSGRFTCSWLVQRNMDLKFNKSSSSSP
DSRAVTCGMASLSAEKVTLDQRDYEKYSVSCQEDVTCPTAEETLPIELALEARQQN
KYENYSTSFFIRDIKPDPPKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVR
IQRKKEKMKETEEGCNQKGAFLVEKTSTEVQCKGGNVCVQAQDRYYNSSCSKWA
CVPCRVRSGGSGGGSGGGSGGGSRVIPVSGPARCLSQSRNLLKTTDDMVKTARE
KLKHYSCTAEDIDHEDITRDQTSTLKTCLPLELHKNESCLATRETSSTTRGSCCLPPQ
KTSMMTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGMLVAIDELMQSLN
HNGETLRQKPPVGEADPYRVKMKLCILLHAFSTRVVTINRVMGYLSSACSQPLDVIL
LLDGSSSFASYFDEMKSFAKAFISKANIGPRLTQVSVLQYGSITTIDVPWNVVPEKA
HLLSLVDVMQREGGPSQIGDALGFAVRYLTSEMHGARPGASKAVVILVTDVSVDSV
DAAADAARSNRVTVPFPIGIGDRYDAAQLRILAGPAGDSNVVKLQRIEDLPTMVTLG
NSFLHKLCSGFVRIGGGSGGGSHHHHHH*

CBD-mouse IL-12-CBD (as a payload for CAR-T):

CSQPLDVILLLDGSSSFASYFDEMKSFAKAFISKANIGPRLTQVSVLQYGSITTIDVP
WNVVPEKAHLLSLVDVMQREGGPSQIGDALGFAVRYLTSEMHGARPGASKAVVILV
TDVSVDSVDAAADAARSNRVTVPFPIGIGDRYDAAQLRILAGPAGDSNVVKLQRIEDL
PTMVTLGNSFLHKLCSGFVRIGGGSGGGSMWELEKDVYVVEVDWTPDAPGETVN
LTCDTPEEDDITWTSDQRHGVIGSGKTLTITVKEFLDAGQYTCHKGGETLSHSHLLL
HKKENGIWSTEILKNFKNKTKFLKCEAPNYSGRFTCSWLVQRNMDLKFNKSSSSSP
DSRAVTCGMASLSAEKVTLDQRDYEKYSVSCQEDVTCPTAEETLPIELALEARQQN
KYENYSTSFFIRDIKPDPPKNLQMKPLKNSQVEVSWEYPDSWSTPHSYFSLKFFVR
IQRKKEKMKETEEGCNQKGAFLVEKTSTEVQCKGGNVCVQAQDRYYNSSCSKWA
CVPCRVRSGGSGGGSGGGSGGGSRVIPVSGPARCLSQSRNLLKTTDDMVKTARE
KLKHYSCTAEDIDHEDITRDQTSTLKTCLPLELHKNESCLATRETSSTTRGSCCLPPQ
KTSMMTLCLGSIYEDLKMYQTEFQAINAALQNHNHQQIILDKGMLVAIDELMQSLN
HNGETLRQKPPVGEADPYRVKMKLCILLHAFSTRVVTINRVMGYLSSACSQPLDVIL

LLDGSSSFASYFDEMKSFAKAFISKANIGPRLTQVSVLQYGSITTIDVPWNVPEKA
HLLSLVDVMQREGGPSQIGDALGFAVRYLTSEMHGARPGASKAVVILVTDVSVDSV
DAAADAARSNRVTVPFIGIGDRYDAAQLRILAGPAGDSNVVKLQRIEDLPTMVTLGN
SFLHKLCSGFVRI*

Human IL-12 (for production in HEK293F):

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTIQ
VKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTLRCEAK
NYSGRFTCWWLTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEY
SVEQCEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLKPLK
NSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRK
NASISVRAQDRYYSSSWSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGM
FPCLHHSQNLLRAVSNNMLQKARQTLEFYPTCTSEEIDHEDITKDKTSTVEACLPLELT
KNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIEDLKMYQVEFKTMNAKLLM
DPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRA
VTIDRVMSYLNASHHHHHH*

Human IL-12 (as a payload for CAR-T):

IWELKKDVYVVELDWYPDAPGEMVVLTCDTPEEDGITWTLDQSSEVLGSGKTLTIQ
VKEFGDAGQYTCHKGGEVLSHSLLLLHKKEDGIWSTDILKDQKEPKNKTLRCEAK
NYSGRFTCWWLTISTDLTFSVKSSRGSSDPQGVTCGAATLSAERVRGDNKEYEY
SVEQCEDSACPAAEESLPIEVMVDAVHKLKYENYTSSFFIRDIIKPDPPKNLQLKPLK
NSRQVEVSWEYPDTWSTPHSYFSLTFCVQVQGKSKREKKDRVFTDKTSATVICRK
NASISVRAQDRYYSSSWSEWASVPCSGGGGSGGGGSGGGGSRNLPVATPDPGM
FPCLHHSQNLLRAVSNNMLQKARQTLEFYPTCTSEEIDHEDITKDKTSTVEACLPLELT
KNESCLNSRETSFITNGSCLASRKTSFMMALCLSSIEDLKMYQVEFKTMNAKLLM
DPKRQIFLDQNMLAVIDELMQALNFNSETVPQKSSLEEPDFYKTKIKLCILLHAFRIRA
VTIDRVMSYLNAS*

CBD-human IL-12 (for production in HEK293F):

CSQPLDVILLLDGSSSFASYFDEMKSFAKAFISKANIGPRLTQVSVLQYGSITTIDVP
WNVPEKAHLLSLVDVMQREGGPSQIGDALGFAVRYLTSEMHGARPGASKAVVILV
TDVSVDSVDAAADAARSNRVTVPFIGIGDRYDAAQLRILAGPAGDSNVVKLQRIEDL
PTMVTLGNLSFLHKLCSGFVRIGGGSGGGGSIWELKKDVYVVELDWYPDAPGEMVVL
TCDTPEEDGITWTLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSHSLLLL
HKKEDGIWSTDILKDQKEPKNKTLRCEAKNYSGRFTCWWLTISTDLTFSVKSSR
GSSDPQGVTCGAATLSAERVRGDNKEYEYSVEQCEDSACPAAEESLPIEVMVDAV
HKLKYENYTSSFFIRDIIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLT
FCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPC

SGGGGSGGGGSGGGGSRNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTL
EFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTS
FMMALCLSSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFN
SETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNASHHHHHH*

CBD-human IL-12 (as a payload for CAR-T):

CSQPLDVILLLDGSSSFPAASYFDEMKSFAKAFISKANIGPRLTQVSVLQYGSITTIDVP
WNVVPEKAHLLSLVDVMQREGGPSQIGDALGFAVRYLTSEMHGARPGASKAVVILV
TDVSVDSVDAAADAARSNRVTVPFIGIGDRYDAAQLRILAGPAGDSNVVKLQRIEDL
PTMVTLGNSFLHKLCSGFVRIGGGSGGGSIWELKKDVYVVELDWYPDAPGEMVVL
TCDTPEEDGITWTLDQSSEVLGSGKTLTIQVKEFGDAGQYTCHKGGEVLSSHLLLL
HKKEDGIWSTDILKDQKEPKNKTFLRCEAKNYSGRFTCWWLTTISTDLTFSVKSSR
GSSDPQGVTGGAATLSAERVRGDNKEYEYSVEQCEDSACPAAEESLPIEVMVDAV
HKLKYENYTSSFFIRDIKPDPPKNLQLKPLKNSRQVEVSWEYPDTWSTPHSYFSLT
FCVQVQGKSKREKKDRVFTDKTSATVICRKNASISVRAQDRYYSSSWSEWASVPC
SGGGGSGGGGSGGGGSRNLPVATPDPGMFPCLHHSQNLLRAVSNMLQKARQTL
EFYPCTSEEIDHEDITKDKTSTVEACLPLELTKNESCLNSRETSFITNGSCLASRKTS
FMMALCLSSIIYEDLKMYQVEFKTMNAKLLMDPKRQIFLDQNMLAVIDELMQALNFN
SETVPQKSSLEEPDFYKTKIKLCILLHAFRIRAVTIDRVMSYLNAS*