

Supplementary Figure S3. Sequences of constructs and primers

1) Secretable EGFRxCD3 BiTE coding sequence

Human Serum Albumin Leader

atgaagtgggtcaccttcattctctctgctcttctctgttttcgagcgcctactcccgcggcggtgttccgcggt

M K W V T F I S L L F L F S S A Y S R G V F R R

Cetuximab VL

gacattctgctgacccagagccccgtgatcctgagcgtttcaccaggtgagcgcgtctccttctctctgccga

D I L L T Q S P V I L S V S P G E R V S F S C R

gcacccagtcctataggcacaacatccactggtagcagcgcaccaatggctccccgcgcctgctcatc

A S Q S I G T N I H W Y Q Q R T N G S P R L L I

aagtacgcgtccgagagcatttccggcatcccctctcgcttcagcgggagtggtatctggcaccgacttcact

K Y A S E S I S G I P S R F S G S G S G T D F T

ctgtctattaattctgtggagtctgaagacatcgcggtactactattgtcagagaacaacaactggcctacc

L S I N S V E S E D I A D Y Y C Q Q N N N W P T

Linker (G4S) x3

acgtttggtgccggcactaagctcgagctgaaaggaggcgaggagcagcgggcgggggcggtatctggcggcggt

T F G A G T K L E L K G G G G S G G G G S G G G

Cetuximab-VH

ggttctcaagtgcagttgaagcagtcgggacctggactgggtgcagcgcgtcccagagtctttccatcacctgc

G S Q V Q L K Q S G P G L V Q P S Q S L S I T C

accgtgtccggcttctcgctgaccaactacggcgtccactgggtccgccagtcgcccggcaagggcctggag

T V S G F S L T N Y G V H W V R Q S P G K G L E

tggctcggcgtgatttggtctggaggcaacactgattacaacacgcccttcacctcccgttgagcattaac

W L G V I W S G G N T D Y N T P F T S R L S I N

aaggacaactccaagagccaggtctttttcaagatgaattccttgagagcaacgacaccgccatctactat

K D N S K S Q V F F K M N S L Q S N D T A I Y Y

tgtgcccgcgcctcacttactacgactacgagttcgcctattggggccagggaacattgggtcacctgtgtcc

C A R A L T Y Y D Y E F A Y W G Q G T L V T V S

LINKER anti-CD3 VH

gctggcgggggcggttccgacatcaaactgcagcagtcggggggcgagcttgctcgcccggtgtcttcgtg

A G G G G S D I K L Q Q S G A E L A R P G A S V

aaaatgtcctgtaagaccagcggtatagcttcacacgctacaccatgcattgggtgaagcagaggcctgga

K M S C K T S G Y T F T R Y T M H W V K Q R P G

cagggcctcgaatggatcggttacatcaaccctctcggggctacaccaactacaaccagaagttaaagac

Q G L E W I G Y I N P S R G Y T N Y N Q K F K D

aaggccacgtgacaaccgacaaaagctcgctccactgcctacatgcagctgtcctcactgacctccgaagat

K A T L T T D K S S S T A Y M Q L S S L T S E D

tctgcggtgtaactactgcgctcggttactacgatgaccattactgcctggactattggggccagggcacaaca

S A V Y Y C A R Y Y D D H Y C L D Y W G Q G T T

LINKER (GGS) x4

anti-CD3VL

ctgaccgtctcctccgtggagggcggcagtgagggttctggtggatccggaggggtcaggtggggtggatgac
L T V S S V E G G S G G S G G S G G V D D

atccagcttacccagagtcctcgctatcatgtctgcctccccaggggagaagggttactatgacctgtcgtgct
I Q L T Q S P A I M S A S P G E K V T M T C R A

tccagctccgtatcttacatgaactggtaccaacagaagagcgggtacttctccgaagcgttggatctatgat
S S S V S Y M N W Y Q Q K S G T S P K R W I Y D

acttccaaggtggcgtccggcgctcccctatcgtttttagcgggagtggtccggcaccagctactcgctgacc
T S K V A S G V P Y R F S G S G S G T S Y S L T

atctcttctatggaggcggaggacgcagccacctactactgtcaacagtgggtctagcaacccccctgaccttc
I S S M E A E D A A T Y Y C Q Q W S S N P L T F

HIS-Tag

Stop

ggtgctggcaccaaactggagctgaagcaccaccaccaccaccatataa
G A G T K L E L K H H H H H H *

2) Coding sequences for anti-TNC-CARs

2A) CAR-TNC5

LEADER IgK Murine

VL ST2146

atggagaccgacaccctgctgctgtgggtgctgctgctgctgtgggtgccaggcagcaccggcgacatcgtgatg
M E T D T L L L W V L L L W V P G S T G D I V M

acacaagctgctcccagcgtgccagtgacacctggcgagctctgtgtccatctcttgccggagcagcaagagc
T Q A A P S V P V T P G E S V S I S C R S S K S

ctgctgcacagcaacggcaatacctacctgtactgggttcctgagaggcccgagcagctctcctcagctgctg
L L H S N G N T Y L Y W F L Q R P G Q S P Q L L

atctaccggatgagcaatctggccagcggcgtgcccgatagattttctggctctggcagcggcaccgccttc
I Y R M S N L A S G V P D R F S G S G S G T A F

acactgagaatctctagagtgggaagccgaggacgtgggcgtgtactactgtatgcagcacctggaatacct
T L R I S R V E A E D V G V Y Y C M Q H L E Y P

Linker (G4S)x4

ctgaccttcggagccggcaccaagctggaactgaaaaccaaggccggaggcggaggcctctggcggaggcggc
L T F G A G T K L E L K T K A G G G G S G G G G

VH ST2146

tctggcggaggcggcctctggcggaggcggcagcgaaaaagtgaagctgcagcagagcggccctgagctggtt
S G G G G S G G G G S E K V K L Q Q S G P E L V

aagcctggcgccctctgtgaaggtgtcctgtaaagccagcggcctacgcctttaccagctacaacatgtactgg
K P G A S V K V S C K A S G Y A F T S Y N M Y W

gtcaagcagagccacggcaagtccctggaatggatcggctacatcgacccctacaacggcgtgacctcctac
V K Q S H G K S L E W I G Y I D P Y N G V T S Y

aaccagaagttcaagggcaaagccacactgaccgtggacaagagcagctccaccgcctacatgcacctgaac
N Q K F K G K A T L T V D K S S S T A Y M H L N

agcctgaccagcagggacagcgccgtgtactattgtgctagaggcggcgatccatctactacgccatggat
S L T S E D S A V Y Y C A R G G G S I Y Y A M D

HINGE hIgG1

tattggggccagggcaccaccgtgacagtggtctagcgatcccgcggagcccaaatctcctgacaaaactcac
Y W G Q G T T V T V S S D P A E P K S P D K T H

CH2 hIgG1

acatgcccaccgtgcccagcacctgaactcctgggggggaccgtcagctcttctcttccccccaaaacccaag
T C P P C P A P E L L G G P S V F L F P P K P K

gacaccctcatgatctcccggaccctgaggtcacatgcgtgggtgggacgtgagccacgaagaccctgag
D T L M I S R T P E V T C V V V D V S H E D P E

gtcaagttcaactggtacgtggacggcgtggaggtgcataatgccaaagacaaagccgcgggaggagcagtac
V K F N W Y V D G V E V H N A K T K P R E E Q Y

aacagcacgtaccgtgtggtcagcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtacaag
N S T Y R V V S V L T V L H Q D W L N G K E Y K

CH3 hIgG1

tgcaaggtctccaacaaagccctcccagccccatcgagaaaaccatctccaaagccaaaagggcagccccga

C K V S N K A L P A P I E K T I S K A K G Q P R

gaaccacaggtgtacaccctgcccccatcccgggatgagctgaccaagaaccaggtcagcctgacctgcctg
E P Q V Y T L P P S R D E L T K N Q V S L T C L

gtcaaaggcttctatcccagcgacatcgccgtggagtgggagagcaatgggcaaccggagaacaactacaag
V K G F Y P S D I A V E W E S N G Q P E N N Y K

accacgcctcccggtgctggactccgacggctccttcttctctacagcaagctcaccgtggacaagagcagg
T T P P V L D S D G S F F L Y S K L T V D K S R

tggcagcaggggaacgtcttctcatgctccgtgatgcatgaggctctgcacaaccactacacgcagaagagc
W Q Q G N V F S C S V M H E A L H N H Y T Q K S

CD28 TM

ctctccctgtctccgggtaaa aaagatcccaaattttgggtgctggtggtggttgggtggagtcctggcttgc
L S L S P G K K D P K F W V L V V V G G V L A C

CD28 cytoplasmic

tatagcttgctagtaacagtggcctttattatcttctgggtgaggagtaagaggagcaggctcctgcacagt
Y S L L V T V A F I I F W V R S K R S R L L H S

gactacatgaacatgactccccgcgcggcccgggcccccgaagcattaccagccctatgccccaccacgc
D Y M N M T P R R P G P T R K H Y Q P Y A P P R

OX40

gacttcgcagcctatcgctccagggaccagaggctgccccccgatgcccaagccccctgggggaggcagt
D F A A Y R S R D Q R L P P D A H K P P G G G S

ttccggaccccccatccaagaggagcaggccgacgcccactccaccctggccaagatcagagtgaagttcagc
F R T P I Q E E Q A D A H S T L A K I R V K F S

CD3z

aggagcgcagacgccccgcgtaccagcagggccagaaccagctctataacgagctcaatctaggacgaaga
R S A D A P A Y Q Q G Q N Q L Y N E L N L G R R

gaggagtacgatgttttggacaagagacgtggccgggaccctgagatgggggggaaagccgagaaggaagaac
E E Y D V L D K R R G R D P E M G G K P R R K N

cctcaggaaggcctgtacaatgaactgcagaaagataagatggcggaggcctacagtgagattgggatgaaa
P Q E G L Y N E L Q K D K M A E A Y S E I G M K

ggcgagcgccggaggggcaaggggcacgatggcctttaccagggtctcagtacagccaccaaggacacctac
G E R R R G K G H D G L Y Q G L S T A T K D T Y

gacgcccttcacatgcaggccctgcctcctcgctaa
D A L H M Q A L P P R *

2B) CAR-TNC4

CD8 Leader

VL ST2146

atggccttaccagtgaccgccttgctcctgccgctggccttgctgctccacgccgcccaggccggagcagacatc
M A L P V T A L L L P L A L L L H A A R P D D I

gtgatgacacaagctgctcccagcgtgccagtgcacacctggcgagtctgtgtccatctcttgcgggagcagc
V M T Q A A P S V P V T P G E S V S I S C R S S

aagagcctgctgcacagcaacggcaatacctacctgtactggttcctgcagaggcccgacagtctcctcag
K S L L H S N G N T Y L Y W F L Q R P G Q S P Q

ctgctgatctaccggatgagcaatctggccagcggcgtgccgatagattttctggctctggcagcggcacc
L L I Y R M S N L A S G V P D R F S G S G S G T

gccttcacactgagaatctctagagtggaagccgaggacgtgggcgtgtactactgtatgcagcacctggaa
A F T L R I S R V E A E D V G V Y Y C M Q H L E

Linker (G4S)x3

taccctctgaccttcggagccggcaccaagctggaactgaaaggcgggcgagggaagcggaggcggaggatct
Y P L T F G A G T K L E L K G G G G S G G G G S

| VH ST2146

ggtggtggtggatctgaaaaagtgaagctgcagcagagcggccctgagctgggttaagcctggcgccctctgtg
G G G G S E K V K L Q Q S G P E L V K P G A S V

aaggtgtcctgtaaagccagcggctacgcctttaccagctacaacatgtactgggtcaagcagagccacggc
K V S C K A S G Y A F T S Y N M Y W V K Q S H G

aagtccctggaatggatcgggtacatcgacccttacaacggcggtgacctcctacaaccagaagttcaagggc
K S L E W I G Y I D P Y N G V T S Y N Q K F K G

aaagccacactgaccgtggacaagagcagctccaccgcctacatgcacctgaacagcctgaccagcggaggac
K A T L T V D K S S S T A Y M H L N S L T S E D

agcgccgtgtactattgtgctagaggcggcggatccatctactacgccatggattattggggccagggcacc
S A V Y Y C A R G G G S I Y Y A M D Y W G Q G T

CD8 Stalk

accgtgacagtgtctagcaccacaacacccgctccttagacctccaacaccagctccaacaatcgccagccag
T V T V S S T T T P A P R P P T P A P T I A S Q

cctctgtctctgaggccagaagcttgtagacctgctgctggcggagccgtgcatacaagaggactggacttc
P L S L R P E A C R P A A G G A V H T R G L D F

CD8 TM

gcctgcgacatctacatctgggctcctctggctggcacatgcggagtgttgctgctgagcctgggtcacc
A C D I Y I W A P L A G T C G V L L L S L V I T

CD8cyt 4-1BB

ctgtactgcaagcggggcagaaagaaactgctctacatcttcaagcagcccttcatgcggcccgtgcagacc
L Y C K R G R K K L L Y I F K Q P F M R P V Q T

CD3z

acacaagaggaagatggctgctcctgcagattccccgaggaagaagaaggcggctgcgagctgagagtgaag
T Q E E D G C S C R F P E E E E G G C E L R V K

ttcagcagatccgccgacgctcctgcctatcagcagggacagaaccagctgtacaacgagctgaacctgggg

F S R S A D A P A Y Q Q G Q N Q L Y N E L N L G

agaagagaagagtacgacgtgctggacaagcggagaggcagagatcctgagatgggcggcaagcccagacgg
R R E E Y D V L D K R R G R D P E M G G K P R R

aagaatcctcaagagggcctgtataatgagctgcagaaagacaagatggccgaggcctacagcgagatcgga
K N P Q E G L Y N E L Q K D K M A E A Y S E I G

atgaagggcgagcgcagaagaggcaagggacacgatggactgtaccagggcctgagcaccgccaccaaggat
M K G E R R R G K G H D G L Y Q G L S T A T K D

acctatgatgccctgcacatgcaggccctgcctccaagataa
T Y D A L H M Q A L P P R *

3) Sequences of furin-T2A linkers

3A: in EGFRxCD3/TNC5

Furin site linker T2A

cgccgcaagcgcggcagcggtgagggccgtgggtctctgctaacctgcggggatgtagaggagaacccgggc
R R K R G S G E G R G S L L T C G D V E E N P G

cct
P

3B: in TNC5/EGFRxCD3

Furin site linker T2A

cgccggaagcgtggttcgggcgagggccggggctcgctgctgacctgcggggacgtggaggagaatcccggg
R R K R G S G E G R G S L L T C G D V E E N P G

cca
P

4) Coding sequence of TNC-TM

HB-EGF Signal Peptide

atgaagctgctgccatctgtggtgctgaagctgtttctggccgcctgctgtctgcactggttacaggcgag
M K L L P S V V L K L F L A A V L S A L V T G E

HB-EGF N-Terminal

agcctggaacggctgagaagaggacttgccgcggaaccagcaatcccgatcctcctaccgtgtccaccgat
S L E R L R R G L A A G T S N P D P P T V S T D

cagttgctgcctcttggcggcggaacgggatagaaaagtgcgggatctgcaagaggccgacctggacctgctg
Q L L P L G G G R D R K V R D L Q E A D L D L L

agagtgcactgtctagcaagccccaggctctggccacacctaacaaagagggaacacgggcaagcgcaagaag
R V T L S S K P Q A L A T P N K E E H G K R K K

TNC

aaaggcaaaggcctggggaagtgcgtgtgcgagcctggatggaagggccctaattgcagcgcagcctgagtg
K G K G L G K C V C E P G W K G P N C S E P E C

cctggcaactgtcacctgagaggcagatgcatcgacggccagtgcattctgcgacgatggattcacaggcgag
P G N C H L R G R C I D G Q C I C D D G F T G E

gactgtcccagctggcctgtcctagcgactgcaacgatcagggcaaattgcgtgaacggcgtgtgcatctgt
D C S Q L A C P S D C N D Q G K C V N G V C I C

ttcgagggatacgttggcgccgactgcagcagagaaattctgccctgtgccatgcagcagaggaacatggcaca
F E G Y A G A D C S R E I C P V P C S E E H G T

tgtgtggatggcctgtgctgtgtgtcacgatggcttttgcctggcgacgattgcaacaagcccctgtgcctgaac
C V D G L C V C H D G F A G D D C N K P L C L N

aactgctacaaccgggggcagatgcgtggaaaacgagtgtgtgtgtgcgacgagggctttaccggggaagattgc
N C Y N R G R C V E N E C V C D E G F T G E D C

tccgagctgatctgccccaacgactgcttcgacagaggccggtgcattcaacggcacctgttactgcgaggaa
S E L I C P N D C F D R G R C I N G T C Y C E E

ggcttcactggcgaagattgtggcaagcccacatgtccccacgcctgtcacacacaaggcagatgtgaagag
G F T G E D C G K P T C P H A C H T Q G R C E E

ggccagtgtgtctgtgatgagggatttgcggcgctggactgttagcgagaagagatgccctgcgcactgccac
G Q C V C D E G F A G V D C S E K R C P A D C H

aatagaggacgctgtgtgtggacggaagatgcgagtgtgacgacgggtttacaggcgccgattgtggcgagctg
N R G R C V D G R C E C D D G F T G A D C G E L

aagtgcccaaattggctgttctggccatggaagatgtgtgaacggacagtgcgttttgtgatgaaggctacacc
K C P N G C S G H G R C V N G Q C V C D E G Y T

ggcgaggattgcagccagctgagatgtcccaacgattgccacagccgtgggagatgtgtggaaggcaaattgt
G E D C S Q L R C P N D C H S R G R C V E G K C

gtctgcgagcagggcttcaagggtacgactgctccgatatgagctgccctaattgattgccaccagcacggt
V C E Q G F K G Y D C S D M S C P N D C H Q H G

cgctgtgtgaatgggatgtgcgttttgcgacgacggatacacaggggaagattgccgggacagacagtgcccc
R C V N G M C V C D D G Y T G E D C R D R Q C P

agagactgtagcaataggggcctgtgtgtcgatgggcagtgtgttttgcgaagatgggttcaccggacctgac
R D C S N R G L C V D G Q C V C E D G F T G P D

tgtgccgagctgtcctgtccaaattgactgccacggacaggggagatgcgtcaacgggtcaatgcgtgtgccat
C A E L S C P N D C H G Q G R C V N G Q C V C H

gagggcttcattgggaaaagactgcaaagaacagcgggtgccctccgactgtcatggccagggacgttgtgtt
E G F M G K D C K E Q R C P S D C H G Q G R C V

gatggccaatgtatctgccacgaggggttcaactggcctggattgtggccagcacagctgccaagcgactgt
D G Q C I C H E G F T G L D C G Q H S C P S D C

aacaacctgggacagtgtgtgtccggccgctgcatctgtaatgagggctatagcggagaggactgttccggc
N N L G Q C V S G R C I C N E G Y S G E D C S G
HB-EGF like domain **HB-EGF TM**
ctgtctggccctgtggaaaaccggctgtacacctacgaccacaccaccatcctggctgtggtggcagtgggtg
L S G P V E N R L Y T Y D H T T I L A V V A V V
HB-EGF Cytoplasmatic
ctgtctagcgtgtgtctgctggttatcgtgggcctgctgatgttcagataccatcggagaggcggctacgac
L S S V C L L V I V G L L M F R Y H R R G G Y D
gtggaaaatgaggaaaagggtggccctgggctga
V E N E E K V A L G *

5) *Primer sequences used for qRT-PCR of human TNC mRNA*

a) **Forward primer**

5' - ACTGTGGACGGAACCAAGAC - 3'

b) **Reverse Primer**

5' - TGATGTTGGCTGTCACCAGG - 3'