

Supplemental Figure 1 A: Sequence of Herceptin CAR (4D5-28Z).

The sequence is composed of following parts as indicated below the sequence:

Ig kappa chain signal peptide; 4D5 light chain variable region (4D5 VL);

218 peptide linker; 4D5 heavy chain variable region (4D5 VH);

Hinge, transmembrane and cytoplasmic region of human CD28 (CD28);

and cytoplasmic region of human CD3 ζ chain (CD3 zeta).

MDFQVQIFSLLISASVIMSRGDIQMTQSPSSLSASVGDRVITICRASQDV
|← Ig kappa signal peptide →|-----
NTAWAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTLTISSLQP
-----4D5 VL-----
EDFATYYCQQHYTTPPTFGQGTKVEIKRTGSTSGSGKPGSGEGSEVQLV
-----218 Linker-----
ESGGGLVQPGGSLRLSCAASGFNIKDTYIHWVRQAPGKGLEWVARIYPT
-----4D5 VH-----
NGYTRYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAVYYCSRWGGD

GFYAMDVWGQGLVTVSSAAAIEVMYPPPYLDNEKSNGTIIHVKGKHL

CPSPLFPGPSKPFVVLVVVGGVLACYSLLVTVAFIIFWVRSKRSRLLHSD
-----CD28-----
YMNMTPRRPGPTRKHYPYAPPRDFAAYRSRVKFSRSADAPAYQQGQN

QLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNPQEGLYNELQKD
-----CD3 zeta-----
KMAEAYSEIGMKGERRRGKGGHDGLYQGLSTATKDTYDALHMQUALPPR.

Supplemental Figure 1 B: Sequence of KDR-2C6 scFV.

The sequence is composed of following parts as indicated under the sequence:

Ig kappa chain signal peptide; KDR-2C6 light chain variable region (2C6 VL);

218 linker; KDR-2C6 heavy chain variable region (2C6 VH).

The rest of the molecule is identical to 4D5-28Z (see supplemental figure 1A).

MDFQVQIFSLLISASVIMSRGEIVMTQSPATLSLSPGERATLSCRASQSV
|← Ig kappa signal peptide →|-----
SSYLAWYQQKPGQAPRLLIYDSSNRATGIPARFSGSGSGTDFTLTISSLEP
-----2C6 VL-----
EDFATYYCLQHNTFPSTFGQGTKVEIKGSTSGSGKPGSGEGSEVQLVQS
-----|←-----218 Linker-----|←-----
GGGLVKPGGSLRLSCAASGFTFSSYSMNWVRQAPGKGLEWVSSISSSSS
-----2C6 VH-----
YIYYADSVKGRFTISRDNKNSLYLQMNSLRAEDTAVYYCARVTDAFDI

WGQGMVTVSS
-----→|

Supplemental Figure 1 C.

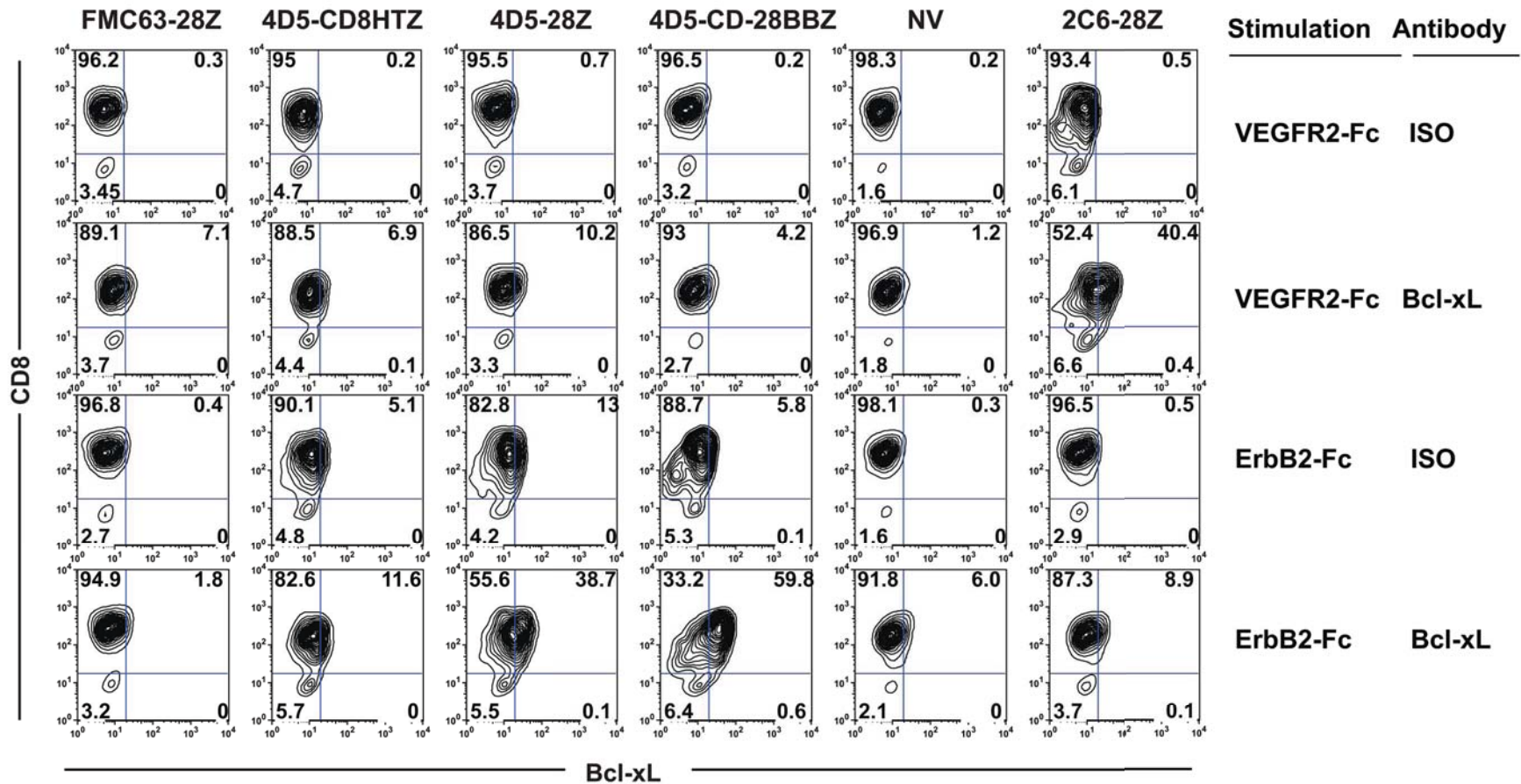
Primers used to synthesis 4D5 and KDR-2C6 scFv were as shown.

Primers for 4D5 scFv

Primer	sequences
4D5S1	ACTCTCGAGGCCACCATGGATTTTCAG
4D5S2	GTGCAGATTTTCAGCTTCTGCTAATCAGTGCCTCAGT
4D5S3	CATAATGTCCAGAGGAGATATCCAGATGACCCAGTCCC
4D5S4	CGAGCTCCCTGTCCGCCTCTGTGGGCGATAGGGTCACC
4D5S5	ATCACCTGCCGTGCCAGTCAAGATGTGAATACTGCTGT
4D5S6	AGCCTGGTATCAACAGAAACCAGGAAAAGCTCCGAAAC
4D5S7	TACTGATTACTCGGCATCCTTCTTATTCTGGAGTC
4D5S8	CCTTCTCGCTTCTCTGATCTAGATCTGGGACGGATT
4D5S9	CACTCTGACCATCAGCAGTCTGCAGCCGGAAGACTTCG
4D5S10	CAACTTATTACTGTGACAAACATTATACTACTCCTCCC
4D5S11	ACGTTTCGACAGGGTACCAAGGTGGAGATCAAAACGCAC
4D5S12	TGGGTCTACATCTGGATCTGGGAAGCCGGGTTCTGGTG
4D5S13	AGGGTCTGAGGTTCACTGGTGGAGTCTGGCGGTGGC
4D5S14	CTGGTGCAGCCAGGGGGCTCACTCCGTTTGTCTGTGC
4D5S15	AGCTTCTGGCTTCAACATTAAGACACCTATATACT
4D5S16	GGGTGCGTCAGGCCCGGGTAAAGGCCTGGAATGGGT
4D5S17	GCAAGGATTTATCCTACGAATGGTTATACTAGATATGC
4D5S18	CGATAGCGTCAAGGGCCGTTTACTATAAGCGCAGACA
4D5S19	CATCCAAAACACAGCCTACCTGCAGATGAACAGCCTG
4D5S20	CGTGTGAGGACACTGCCGTCTATTATTGTTCTAGATG
4D5S21	GGGAGGGGACGGCTTCTATGCTATGACGCTGTGGGGTC
4D5S22	AAGGAACCTGTGACCCAGTCTCCTCGGCGGCCGCTAA
4D5AS1	ATAGGCGGCCCGCAGGAG
4D5AS2	ACGGTGACCAGGGTTCCTTGACCCACACGTCCATAGC
4D5AS3	ATAGAAGCCGTCCCTCCCATCTAGAACAATAATAGA
4D5AS4	CGGCAGTGTCTCAGCACGCAGGCTGTTCACTGCAGG
4D5AS5	TAGGCTGTGTTTTGGATGTGTCTGCGCTTATAGTGAA
4D5AS6	ACGGCCCTTGACGCTATCGGCATATCTAGTATAACCAT
4D5AS7	TCGTAGGATAAATCCTTGCAACCCATTCCAGGCCCTTA
4D5AS8	CCCGGGCCTGACGCACCCAGTGTATATAGGTGTCTTT
4D5AS9	AATGTTGAAGCCAGAAGTGCACAGGACAAACGGAGTG
4D5AS10	AGCCCCCTGGCTGCACCAGGCCACCCGAGACTCCACC
4D5AS11	AGCTGAACCTCAGAACCCTCACCAGAACCCTCCCTCCC
4D5AS12	AGATCCAGATGAGACCCAGTGCCTTTGATCTCCACT
4D5AS13	TGGTACCCTGTCCGAACGTGGGAGGAGTAGTATAATGT
4D5AS14	TGCTGACAGTAATAAGTTGCGAAGTCTTCCGGCTGCAG
4D5AS15	ACTGCTGATGGTCAAGTGAATCCGTCAGATCTAG
4D5AS16	ATCCAGAGAAGCGAGAGTGTGACCCATATCGCCACAC
4D5AS17	GATGCCGAGTAAATCAGTAGTTTCCGGAGCTTTCTCTGG
4D5AS18	TTTCTGTTGATACCAGGCTACAGCAGTATTACATCCT
4D5AS19	GACTGGCACGGCAGGTGATGGTACCCATATCGCCACAC
4D5AS20	GAGGCGGACAGGAGCTCGGGGACTGGGTCACTGGAT
4D5AS21	ATCTCTCTGGACATTATGACTGAGGCACTGATTAGCA
4D5AS22	GGAAGCTGAAAATCTGCACCTGAAAATCCATGGTGGCT

Primers for 2C6 scFv

Primer	Sequences
2C6-S1	TTTGGCAAACCTCGAGGCCA
2C6-S2	CCATGGACTTTCAGGTCCAGATCTTCTCTCTCTGCTC
2C6-S3	ATTAGTGCCAGCGTTATTATGTGCGGAGGGGAAATCGT
2C6-S4	GATGACCCAATCACCGGCCACTCTCTCGTTGCCCCCG
2C6-S5	GAGAGCGGCCACACTCTCATGTAGAGTTCCTCCAGTCC
2C6-S6	GTTTCGTCTACCTCGCGTGGTACCAGGAAAAGCCCGG
2C6-S7	GCAGGCTCCCGGCTCTGATCTACGACAGTCTGAACA
2C6-S8	GGGCCACCGGTATCCAGCTCGCTTCTCCGGGTCGGGG
2C6-S9	TCCGGCACAGACTTCACCCGTGACAATCTCTCGTGGGA
2C6-S10	GCCCGAGGACTTCGCCACTTACTACTGTCTGCAGCATA
2C6-S11	ACACATTCCCGTCGACGTTTCGGCCAGGGCACTAAGGTG
2C6-S12	GAAATTAAGGGTCTACCTCCGGTCTGGGAAGCCCTGG
2C6-S13	CTCAGGCGAGGGCTCCGAGGTACAACCTCGTGCAGAGTG
2C6-S14	GCGGAGGTCTGGTCAAGCCCGGGGCTCCCTGAGGCTG
2C6-S15	TCCTGTGCAGCCAGCGGGTTCACGTTCTCGTCTTACAG
2C6-S16	TATGAACTGGGTGCGTCAGGCGCCTGGGAAGGGACTTG
2C6-S17	AGTGGGTGTCTCCATCTCCAGCTTCTCCATACATA
2C6-S18	TACTACGCGGACAGTGTCAAGGGGAGGTTTACAATCTC
2C6-S19	GCGGGATAACGCCAAGAATTCAGTACTTGCAGATGA
2C6-S20	ACTCCCTACGAGCCGAAGACACCGCCGTGATTACTGC
2C6-S21	GCCCGCGTGACAGACGCCCTTTGACATTTGGGGCCAGGG
2C6-S22	CAGGATGGTGACAGTGTCTCCGCGGGCCGAACAGTCT
2C6-AS1	AGACTGTTGCGGCCGCGGA
2C6-AS2	GGACTGTCCACATCGTGCCTGGCCCAAATGTCAA
2C6-AS3	AGGCGTCTGTACGCGGGGCGAGTAATACAGCGCGGTG
2C6-AS4	TCTTCGGCTCGTAGGGAGTTCATCTGCAAGTACAGTGA
2C6-AS5	ATTCTTGGCGTTATCCCGGAGATTGTAACCTCCCT
2C6-AS6	TGACTGTCCGCGTAGTATATGTATGAGGAAGAGCTG
2C6-AS7	GAGATGGAAGACACCCACTCAAGTCCCTCCAGGCCG
2C6-AS8	CTGACGCACCCAGTTCATACTGTAAGACGAGAACGTGA
2C6-AS9	ACCCGCTGGCTGCACAGGACAGCCTCAGGGAGCCGCC
2C6-AS10	GGCTTGACCAGACCTCCGCCACTTGCACAGGATTTGAC
2C6-AS11	CTCGGAGCCCTCGCTGAGCCAGGCTTCCAGAACCCG
2C6-AS12	AGGTAGAACCCTTAATTTCCACTTAGTGCCCTGGCCG
2C6-AS13	AACGTCGACGGGAATGTGTTATGCTGCAGACAGTAGTA
2C6-AS14	AGTGGCGAAGTCTCTCGGGCTCCAGCGAGGAGATTGTCA
2C6-AS15	GGGTGAAGTCTGTGCCGGACCCCGACCCGGAAGCGGA
2C6-AS16	GCTGGGATACCGGTGGCCCTGTTGAGCTGTCTAGAT
2C6-AS17	CAGGAGCCGGGAGCCTGCCCGGGCTTTGCTGGTACC
2C6-AS18	ACGCGAGGTAGGACGAAACGGACTGGGAAGCTCTACAT
2C6-AS19	GAGAGTGTGGCGCTCTCCGGGGGACAACGAGAGAGT
2C6-AS20	GGCCGGTGATTGGGTCACTACGATTTCCCTCGCGACA
2C6-AS21	TAATAACGCTGGCACTAATGAGCAGGAAGGAGAAGATC
2C6-AS22	TGGACTGAAAGTCCATGGTGGCCTCGAGTTTCCAAA



Supplemental figure 3. PBLs transduced with 4-1BB upregulate Bcl-xL.

PBLs transduced with 4D5 CARs with signaling moiety of CD3 ζ alone (4D5-CD8HTZ), CD28 and CD3 ζ (4D5-28Z) or CD28, 4-1BB and CD3 ζ (4D5-CD8-28BBZ) were sorted using ErbB2-Fc to over 98% purity. Controls were; PBLs expressing the CD19 CAR (FMC63-28Z), sorted using anti-mouse IgG Ab, and PBL expressing the anti-VEGFR2 CAR (2C6-28Z) sorted using VEGFR2-Fc, non-transduced PBLs (NV) were also used as control. T cells were stimulated with ErbB2-Fc or VEGFR2-Fc coated plate for 17h, and subjected to intracellular staining for Bcl-xL and CD8.